School of Pharmacy and Pharmaceutical Sciences

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Antiviral Drug Design, Synthesis and Biological Evaluation For Treatment Of Hepatitis C Virus

A thesis submitted *in accordance with the conditions governing candidates* for the degree of

Philosophiae Doctor in Cardiff University

by

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In collaboration with Inhibitex *Inc.* and Bristol-Myers Squibb

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Abstract

Hepatitis C virus is an infectious disease affecting millions of people worldwide and causing chronic liver disease. The current standard of care is not only long but causes numerous side effects. Due to incomplete virological response and poor tolerability, only 50% of the patients are cured, with variability by genotype. Despite the development of non-enzymatic viral protein inhibitors, new therapies target mainly enzymes responsible for viral replication or translation. Being commonly used for antiviral and anticancer therapy, nucleosides analogues have played an important role as anti-HCV agents.

Despite their potency and selectivity, nucleoside analogues appear to be poor substrates for metabolic enzymes. In particular, the first essential phosphorylation step is often rate-limiting thus, resulting in poor bioactivation to the active triphosphate form. Hence, monophosphate prodrug strategies have been applied to efficiently deliver intracellularly the key monophosphate derivatives. Such strategies have been successfully used for anti-HCV therapy and the phosphoramidate ProTide INX-08189, discovered in our lab, is one such example.

Aiming at developing back-up molecules of INX-08189, we report in the present work, the synthetic strategies to obtain several modified *β*-2'-*C*-methyl-6-*O*-methyl guanosine and other modified *β*-2'-*C*-methyl purine nucleoside analogues. The phosphoramidate ProTide approach and the phosphorodiamidate approach were applied to these modified nucleosides. In-*vitro*, and sometimes in-*vivo* evaluation against HCV replication is reported, and the mechanism of bioactivation to their corresponding monophosphate species is discussed. Enzymatic experiments using carboxypeptidase Y and Huh-7 cell lysates were carried out to investigate the release of the monophosphate forms. We also investigated the hydrolysis of the 6-*O*-methyl group at the nucleoside level with adenosine deaminase enzyme, and at the monophosphate level using molecular docking in adenosine deaminase like protein-1. Eventually, the intracellular putative mechanism of activation of the ProTides was studied using molecular modeling with cathepsin A enzyme and human Hint-1 phosphoramidase.

Publications

A part of the work presented in this thesis has been published in the following journals:

McGuigan C., Madela K., Aljarah M., Bourdin C., Arrica M., Barrett E., Jones S., Kolykhalov A., Bleiman B., Bryant K.D., Ganguly B., Gorovits E., Henson G., Hunley D.,Hutchins J., Muhammad J., Obikhod A., Patti J., Walters CR., Wang J., Vernachio J., Ramamurty C.V., Battina S.K., Chamberlain S., **Phosphorodiamidates as a promising new phosphate prodrug motif for antiviral drug discovery: application to anti-HCV agents.**, *J. Med. Chem.*, **2011**, *54*, 8632-8645.

Bourdin C., McGuigan C., Brancale A., Chamberlain S., Vernachio J., Hutchins J., Gorovits E., Kolykhalov A., Muhammad J., Patti J., Henson G., Bleiman B., Bryant K.D., Ganguly B., Hunley D., Obikhod A., Walters C.R., Wang J., Ramamurty C.V.S., Battina S.K., Rao S.C., **Synthesis and evaluation against Hepatitis C virus of 7-deaza analogues of 2'-*C*-methyl-6-*O*-methyl guanosine nucleoside and *L*-Alanine ester phosphoramidates**, *Bioorg. Med. Chem. Lett*., **2013**, *23*, 2260-2264.

The full articles are referred in the Appendix section.

Table of Contents

Abbreviations and Acronyms

Ac	Acetyl
ACN	Acetonitrile
ACV	Acyclovir
ADA	Adenosine deaminase
AMP	Adenosine monophosphate
Ar	Aryl
ATP	Adenosine triphosphate
BAIB	([bis(aceoxy)-iodo]benzene
Bn	Benzyl
Boc	*Tert*-Butoxycarbonyl
BTEA-Cl	Benzyl triethylammonium chloride
Bu	Butyl
°C	degree Celsius
cHex	Cyclohexyl
ClogP	Calculated logP
cPent	Cyclopentyl
d	deuterated
DCC	*N*,*N*'-Dicyclohexylcarbodiimide
DCM	Dichloromethane
DIAD	Diisopropyl azodicarboxylate
DIBAL-H	Diisobutylaluminium hydride
DIPEA	*N*,*N*-diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	Dimethylformamide
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
DTBS	di-*tert*-butylsilanedyil
eq	equivalent
Et	Ethyl
Et_2O	Diethyl ether

EtOAc	Ethyl acetate
EtOH	Ethanol
FDA	Food and Drug administration
Fmoc	Fluorenylmethoxycarbonyl
g	gram
HAOS	hydroxylamine-O-sulfonic acid
HBV	Hepatitis B virus
HCMV	Human cytomegalovirus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HOBt	Hydroxybenzotriazole
HMDS	Hexamethyldisilazane
HSV	Herpes simplex virus
IMP	Inosine monophosphate
IMPDH	Inosine monophosphate dehydrogenase enzyme
*i*Pr	isopropyl
L-Ala	*L*-Alanine
L-Ile	*L*-Isoleucine
L-Leu	*L*-Leucine
L-Met	*L*-Methionine
L-Phe	*L*-Phenylalanine
L-PhGly	*L*-Phenylglycine
L-Val	*L*-Valine
M	molar
MDPS	Methylene-bis-(diisopropylsilyl)
Me	Methyl
MeOH	Methanol
MeMgBr	Methyl magnesium bromide
min	minute
mL	millilitre
mol	mole
mmol	millimole
$MgSO_4$	Magnesium sulfate

NA	Nucleoside analogue
NaH	Sodium hydride
$NaHCO_3$	Sodium bicarbonate
NaHMDS	sodium hexamethyldisilazide
NaOH	Sodium hydroxide
Na_2SO_4	Sodium sulfate
NBS	*N*-bromosuccinimide
NCS	*N*-chlorosuccinimide
NIS	*N*-iodosuccinimide
neopentyl	2,2-dimethylpropyl
NEt_3	Triethylamine
$NEt_3.HF_3$	Triethylamine trihydrofluoride
NMI	N-methylimidazole
Ph	Phenyl
$POCl_3$	Phosphorus oxychloride
PPh_3	Triphenyl phosphine
Pr	Propyl
ProTide	Nucleotide prodrug
pTSA	para-Toluenesulfonic acid
PyBrop	Bromo-tris-pyrrolidino phosphoniumhexafluorophosphate
RNA	Ribonucleic acid
SATE	S-acyl-2-thioethyl
SI	Selectivity index
TBAF	Tetrabutylammonium fluoride
TBDMS	*Tert*-butyldimethylsilyl
*t*Bu	*Tert*-butyl
*t*BuMgCl	*Tert*-butylmagnesium chlroride
TDA-1	Tris(2-(2-ethoxymethoxy)ethyl)amine
TCC	Trichlorocyanuric acid
TEMPO	2,2,6,6-tetramethyl-1-piperidinyl oxide
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran

TI	Therapeutic index
TIPDS	1,1,3,3,-tetraisopropylsilyl
TMSCl	Trimethylsilyl chloride
Ts	Tosylate
VZV	Varicella zoster virus

Chapter One: Introduction

1. Hepatitis C Virus

About 130 to 170 million[1] people worldwide are infected by hepatitis C virus (HCV). This spherical[2] small-enveloped single-stranded positive-sense RNA hepacivirus (Figure 1.1) belongs to the *Flaviviridae* family[3] and affects only humans.[1] It spreads by blood-to-blood contact[4] and causes liver disease in 60-70%[5] of cases, often resulting in liver failure or cancer[6] and transplantation need.[7]

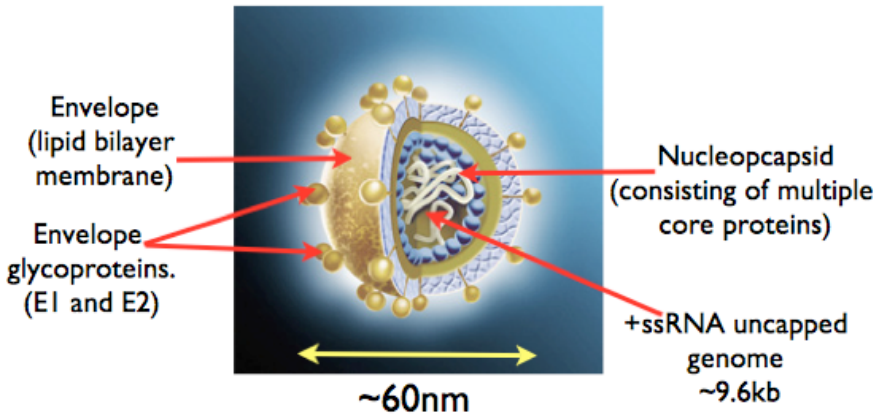


Figure 1.1. HCV structure.[3]

1.1. History and Epidemiology

Following the differentiation of hepatitis A and hepatitis B viruses, a "non-A, non-B" entity was discovered during the second part of the 20th century.[8] After Micheal Houghton's group developed new molecular biological techniques to characterise the viral genome,[9] it became known as hepatitis C virus.[10] The viral pandemia has mainly been influenced by inadequately screened blood used for transfusion, unsafe injection equipment or illegal injection of drugs[11] affecting 3 to 4 million people each year.[5] The HCV infection prevalence varies geographically with the highest rate for Egypt.[12] In each geographic region predominates one of the six major genotypes of HCV[13] (Figure 1.2), among which genotype 1a being the most widespread.

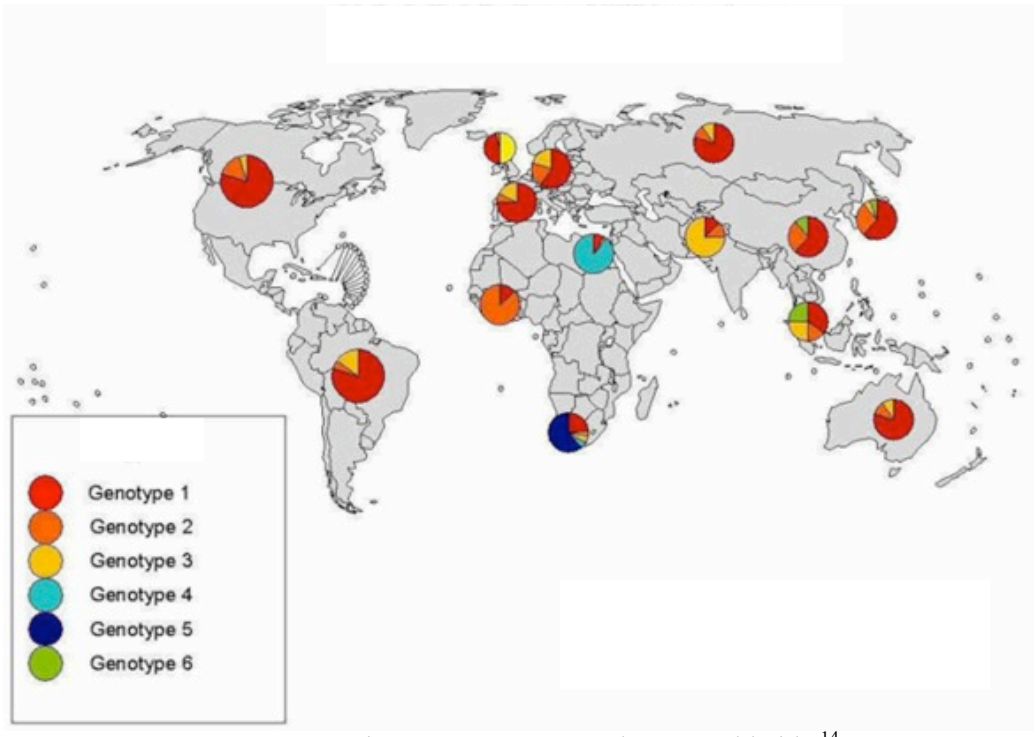


Figure 1.2. HCV prevalence worldwide.[14]

1.2. HCV infection

After exposure to the virus, the disease develops slowly through different stages. The acute phase corresponds to the first 6 months after infection[15] and is difficult to diagnose because of the lack of symptoms[16] or specific diagnostic tests.[17] However, non-specific symptoms might occur during this phase, such as loss of appetite, abdominal pain, fatigue, nausea and jaundice[18] and HCV antibodies can only be detected after 15 weeks of exposure in 80% of cases.[16] Spontaneous viral clearance is still likely during this phase,[17] with variability from 10% to 50% depending on the genotype,[19] whereas 60% to 80% of infected individuals develop persistent infection.[20] The natural evolution of acute HCV infection depends on the transmission pattern, the viral factors and the ability of developing a strong immune response to eliminate the virus.[3] During the chronic phase of the disease the clinical symptoms are still minor[3] and symptoms of liver failure such as accumulation of fluid in the abdomen, bruises and bleeding only develop at a later stage, sometimes decades after the infection, once the disease has progressed to cirrhosis or liver cancer.[3]

1.3. HCV genome

The HCV genome is approximately 9600 base pairs long[21] containing a single open reading frame (ORF) encoding for a polyprotein of 3011 amino acids,[22] and is divided into two non-translated regions and three functional regions (Figure 1.3).

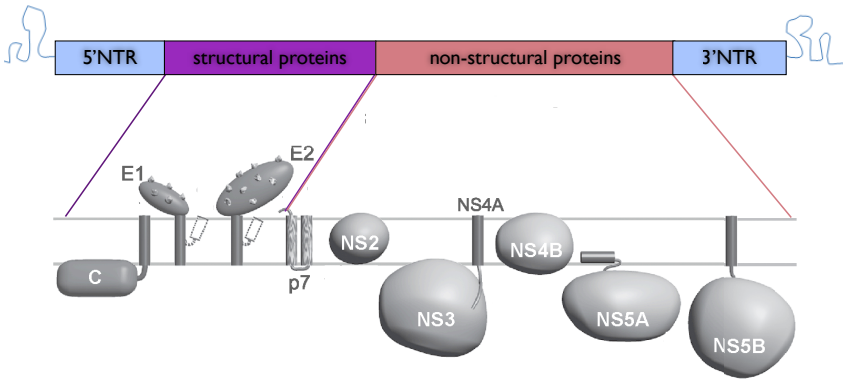


Figure1.3. HCV genome.[3, 23]

The most conserved segment of the HCV genome is the 5' non-translated region containing the structured internal ribosomal entry site (IRES) at which binding initiates the first step of viral RNA polyprotein translation.[24]

The 5'-terminal region contains the structural proteins C,[23] E1[25] and E2,[26] which form respectively the viral capsid[27] and envelope essential for viral entry[28] and fusion.[29]

Seven non-structural proteins[30] complete the HCV genome: NS2,[31, 32] zinc-metalloprotease, and p7 (NS1),[33] integral membrane protein, are not essential for replication but play an essential role in virion formation and membrane permeability.[34] The 3'-terminal segment comprises the proteins NS3, NS4A, NS4B, NS5A and NS5B assembling as a replicase complex required for replication of the viral genome. While NS3-NS4A protease catalyses HCV polyprotein cleavage,[35, 36, 37] NS4B induces a special membranous web-like compartment in which replication occurs.[38] Interactions between the multi-functional protein NS5A and host proteins regulate viral genome replication and assembly,[39, 40] whereas the error-prone NS5B RNA-dependant-RNA-polymerase (RdRp) enables the synthesis of positive- and negative- strand HCV RNAs,[41] hence resulting in the genetic variability of the virus.[42, 43]

The sequence of the 3' non-translated region is genotype-variable[44] and takes part in RNA replication.[45, 46]

1.4. HCV life cycle

Common to all flaviviruses, six key steps (Figure 1.4) taking place outside the host cell nucleus are required for HCV life cycle: binding to receptors and entry into the host cell (1; Figure 1.4), uncoating of the viral genome (2; Figure 1.4), translation (3; Figure 1.4) followed by replication (4; Figure 1.4), viron assembly (5; Figure 1.4) and eventually release of the newly formed virions (6; Figure 1.4).[47]

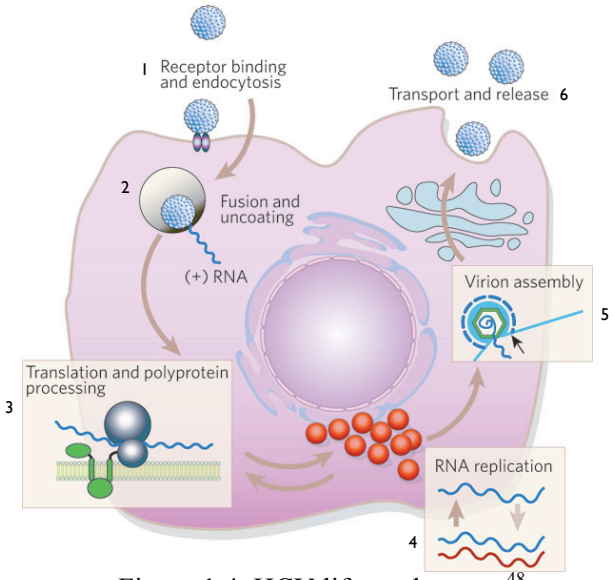


Figure 1.4. HCV life-cycle steps.[48]

Molecular recognition between glycoproteins at the surface of the virus and specific host cell receptors[49] triggers the virus attachment, and allows its entry into cells *via* endocytosis (Figure 1.4, step 1).[46, 50]

Fusion of the viral envelope with the cellular membrane is pH-dependant,[51] enabling the removal of the nucleocapsid and release of the positive-stranded genomic RNA into the cytosol (Figure 1.4, step 2).[52]

Acting as messenger RNA (mRNA), it then undergoes translation *via* binding of the IRES of the 5' non-translated region and 40S ribosome subunit containing the initiation codon of the host system (Figure 1.4, step 3).[25] A single polyprotein of approximately 3000 amino acids is produced and cleaved by the viral protease complex NS3-NS4B releasing ten viral proteins, essential for the viral replication machinery.[30, 53]

Semi-conservative and asymmetric, HCV replication takes place in a membranous web induced by NS4B[23] and occurs in two steps catalysed by NS5B RdRp. Firstly, the

positive strand RNA serves as a template for the synthesis of negative-strand RNA, which then becomes a template for several nascent positive-strand viral RNAs (Figure 1.5) ready to be encapsulated.[54]

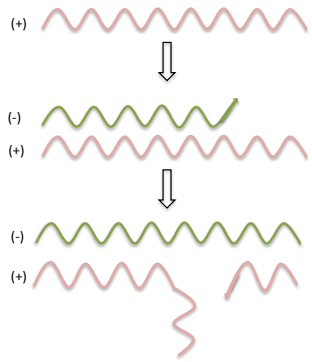


Figure 1.5. Mechanism of RNA replication.[23]

Virion assembly involves interactions between the 5' non-translated region of new positive-strand RNAs and the core protein (C)[55, 56] enabling the formation of a viral internal coat with subsequent encapsidation of the viral genome (Figure 1.4, step 5).[54]

Further interactions between structural proteins and NS2 control excretion of nascent viral particles outside the host cell by exocytosis (Figure 1.4, step 6).[23]

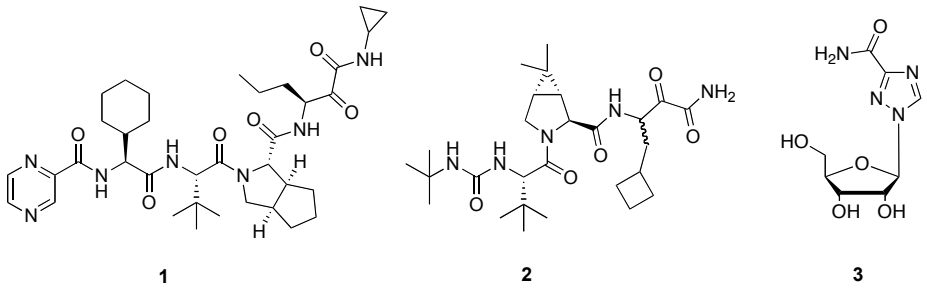
1.5. Current treatment

As mentioned earlier, acute HCV infection evolves to either spontaneous viral clearance or to chronic infection leading to cirrhosis, liver failure or cancer at a later stage. Neither a protective vaccination currently exists, nor a highly effective antiviral agent allows total eradication of the virus. Thus the use of combination therapies, which are not always efficient in all patients at long term and often cause side effects.

Three responses to anti-HCV therapy are described: lack of detectable HCV RNA during and six months after the end of the treatment known as sustained virological response (SVR), end of treatment response and relapse or non-response to the therapy.

The recent discovery and FDA approval of two NS3 protease inhibitors, telaprevir[57] (Vertex, **1**) and boceprevir (Merck, **2**) (Figure 1.6),[58] has slightly changed the optimal current therapy. To avoid resistance if these protease inhibitors were dosed alone,[59] the new standard of care comprises a triple combination therapy with one protease inhibitor

and the two non-specific antiviral agents pegylated interferon-α and ribavirin (**3**, Figure 1.6).[60]



1 **2** **3**

Figure 1.6. Structures of telaprevir (**1**), boceprevir (**2**) and ribavirin (**3**).

The multi-functional protein pegylated interferon-α is involved in cell regulation, antiviral defense and activation of the immune mechanism.[59] Pegylated interferon-α is slowly absorbed thus reducing the dosing from three to one sub-cutaneous injection per week during the treatment.[61]

1-β-*D*-ribofuranosyl-1,2,4-triazole-3-carboxamide, also known as ribavirin (**3**, Figure 1.6), has a broad spectrum of antiviral activities against DNA and RNA viruses.[62] First, the inhibitory effect of the ribavirin 5'-monophosphate towards the inosine 5'-monophosphate dehydrogenase (IMPDH) has an indirect effect on the synthesis of the viral RNA.[63] However the antiviral mechanism has not yet been entirely understood and recently ribavirin has shown enhancement of antiviral response of lymphocytes Th1, responsible for the activation of B and T cells and for immune cell response, and suppression of lymphocytes Th2, responsible for hypersensitive immune reaction.[64] In addition to its mutagenic action leading to virion mutations,[65] the misincorporation of ribavirin monophosphate into viral RNA increases the frequency of mutations, and eventually leads to a reduction of the virus replication.[61]

The new triple combination therapy applied to genotype 1 infected patients improved SVR to 70%[66] in comparison with the 55%[67] obtained with dual combination pegylated interferon-α and ribavirin. However the treatment still lasts 24 to 48 weeks and is not only high-dosing (750-800 mg tablets, three times a day)[60] but also causes side effects such as anaemia, severe rash and gastrointestinal symptoms.[59] To face the need for more effective HCV therapy, the development of potent and selective virus inhibitors suitable for clinical application is essential.

1.6. Clinical candidates for HCV treatment

Every steps of the HCV life cycle is a potential target for HCV treatment. Many molecules targeting the viral proteins have been designed and synthesised,[68, 69] and several are currently in clinical trial or under clinical investigation. Despite the recent development of non-enzymatic viral protein inhibitors,[38] the two favoured viral targets are the HCV NS3 protease and the NS5B polymerase, since biochemical assays are readily available.[68] Currently, approximately 100 potential drugs have been advanced to clinical development and aim at targeting directly HCV; some of them are reported Table 1.1, while others are tested in drug combination to improve HCV antiviral therapy.[69] These enzymatic viral protein inhibitors are either nucleoside analogues or non-nucleosides agents.

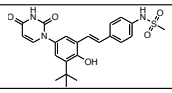
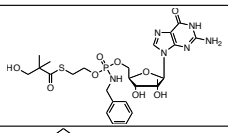
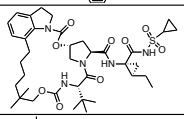
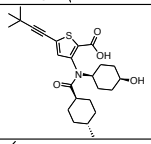
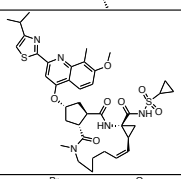
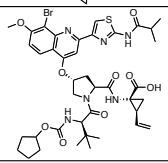
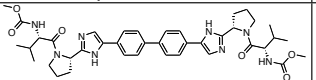
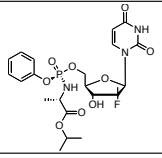
name	structure	inhibitor class	viral protein targeted	clinical trial phase
ALS-2200	not available	nucleotide prodrugs	NS5B polymerase	1
TMC649128	not available	nucleotide prodrugs	NS5B polymerase	1
ABT-072		non-nucleoside	NS5B polymerase	1
IDX-719	not available	nucleotide prodrugs	NS5A	2a
IDX-184		nucleotide prodrugs	NS5B polymerase	2b (on hold)
MK-7009		non-nucleoside	NS3/4A protease	2a
VX-222		non-nucleoside	NS3/4A protease	2a
TMC435		non-nucleoside	NS3/4A protease	3
BI 201335		non-nucleoside	NS3/4A protease	3
BMS-790052		non-nucleoside	NS5A	3
GS-7977		nucleotide prodrug	NS5B polymerase	3

Table 1.1. Non-nucleosides and nucleosides inhibitors of HCV polymerase and protease currently in clinical trials.[69]

2. Classes of inhibitors for HCV therapy

Despite their potent antiviral activity, respectable safety profile and good tolerability in HCV-treated patients, nucleos(t)ide analogues and non-nucleoside inhibitors differ by their mode of action, efficacy and resistance profile as well as their activity against the different genotypes.

2.1. Non-nucleoside inhibitors

Non-nucleoside analogues, such as telaprevir (**1**, Figure 1.6) or BMS-79005 (Table 1.1), target specifically the allosteric sites of the enzyme.[70] Their properties define the area of binding, altering the conformation of the enzyme active site and disrupting the steps prior to or during the initiation phase of the viral RNA synthesis.[71]

As mentioned earlier, mutations occur easily and result in genetic variability,[71] therefore the rapid emergence of resistance constitutes the main drawback of non-nucleoside inhibitors. Hence numerous mutations at allosteric sites happen without impairment of the enzyme function[72] but conferring resistance to the inhibitor. This is the main reason for the failure of some clinical developments and for the limited activity across genotypes.[73] However, resistance mutations at one allosteric site do not usually show cross-resistance to other inhibitors binding at other allosteric sites of the enzyme, thus the importance of combination therapy[74] for HCV treatment is evident.

2.2. Nucleoside and nucleotide analogues

Nucleoside analogues are widely used in antiviral and anticancer therapies as they mimick the role of natural nucleosides essential for genome replication and transcription.[75] Upon cellular uptake *via* specific nucleoside transporters, nucleoside analogues undergo bioactivation in the presence of three kinases to release the active triphosphate form (Figure 1.7).[75, 76, 77] The latter binds to the enzyme active site and competes with natural nucleotides for incorporation into growing DNA or RNA strands,[70] resulting in chain termination or inhibition of the viral polymerase.

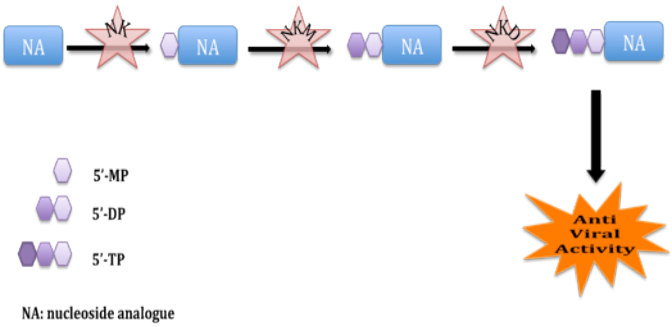


Figure 1.7. Activation pathway of nucleoside analogues (NK: nucleoside kinase, NKM: nucleoside monophosphate kinase, NKD: nucleoside diphosphate kinase).

Over the past decades, several modified ribonucleosides such as 4'-azidocytidine (R1479)[78] and 2'-C-methylcytidine (NM107)[79] have been reported as potent inhibitors of HCV RNA replication in replicon assays. Acting as non-obligate chain terminators, their lack of bioavailability and enzymatic stability, together with their poor conversion to the bioactive triphosphate, as for the majority of nucleoside analogues, led to the investigation of prodrug strategies.

2.2.1. Prodrugs

An inactive pharmacological substance, undergoing physico-chemical or enzymatic transformations *in vivo*, to afford the active metabolite was defined in 1951 by Adrien Albert[80] as a prodrug.

Such a prodrug should not only display a good stability towards enzymes or other chemical reactions occurring during the absorption, sufficient solubility, good membrane permeability and good distribution, but also be less toxic than the related drug, stable in plasma and blood, and eventually intravenously or orally dosed.[81] After cell permeation, efficient and selective activation[82, 83] should generate the desired metabolite at the site of action, allowing higher exposure of the drug, thus enhancing therapeutical effects towards the target.[84] Optimisation of the drug physico-chemical properties should enable better selectivity towards its target.[85]

The site of conversion of prodrug into its active form defines the two different classes. In some cases, such as antiviral nucleotide analogues, the conversion takes place intracellularly; whereas others are converted outside the cells and will not further discussed in this thesis.

Many issues encountered with nucleos(t)ide analogues can be overcome by application of the prodrug strategy.

2.2.2. Nucleoside prodrugs

Many pharmaceutical companies have investigated nucleoside prodrugs as anti-HCV agents targeting essentially the enzyme for viral replication, with consequent inhibition of the NS5B polymerase to stop the synthesis of viral RNA (Figure 1.8).

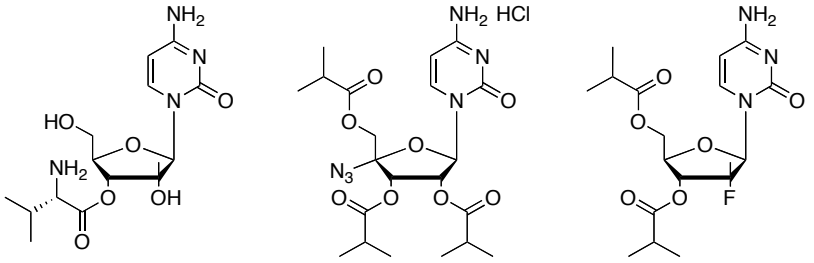


Figure 1.8. Nucleoside prodrugs as NS5B RdRp inhibitors.

Despite their potency and selectivity, nucleoside prodrugs appear to be poor substrates for metabolic enzymes.[86] Resistance is most likely to develop due to poor membrane permeability, lack of transporters, poor affinity towards targeted enzyme and thus poor bioactivation.[87] Moreover, the first phosphorylation step is often the rate limiting,[88] preventing sufficient exposure of triphosphate at the active site to compete with natural substrates.[71] Research on nucleotide analogues is currently heavily conducted to overcome these major issues, by enhancing the formation of the active triphosphate form, and therefore potentially increasing antiviral activity.[89]

2.2.3. Nucleotide prodrugs

Despite their interesting biological activities in a variety of assays and the discovery of potential drug candidates, nucleotides bear negative charges responsible for high hydrophilicity, resulting in poor membrane permeability and instability in cell media.[90]

Nucleotide prodrug technology, aiming at delivery of the monophosphate directly inside the cells following passive diffusion and hydrolytic or enzymatic metabolism,[80, 91] was successfully applied in anticancer and antiviral therapies.[92, 93, 94] Once the highly polar and charged monophosphate is trapped inside the cell, it either acts as the bioactive

form or it undergoes enzymatic conversion to its corresponding di- and tri-phosphate forms. This technology may improve potency as well as selectivity, while decreasing toxicity.

Several nucleotide prodrug methodologies have been developed based on enhancement of cell permeability and enzymatic stability by introducing neutral lipophilic moieties at the phosphorus center.[81] The most interesting approaches are described below and combine ester, ether or amino acid masking groups.

2.2.3.1. Phosphonate and tri-esters approaches

Farquhar and co-workers were the first to report a phosphotriester approach by developing mono(POM) and later on bis(POM) derivatives (**4**, Figure 1.9).[95] The latter enhances cellular delivery of the 5'-monophosphate species inside the cells after two enzymatic hydrolysis by carboxyesterase and/or phosphodiesterase[96] resulting in release of potentially cytotoxic formaldehyde.

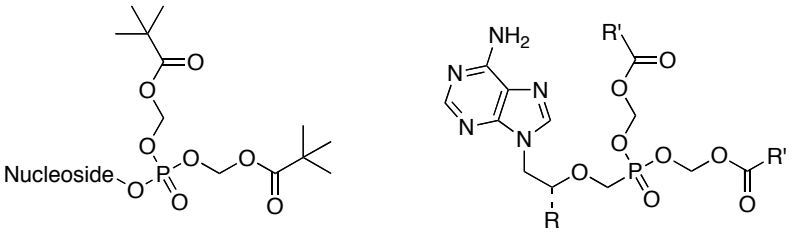
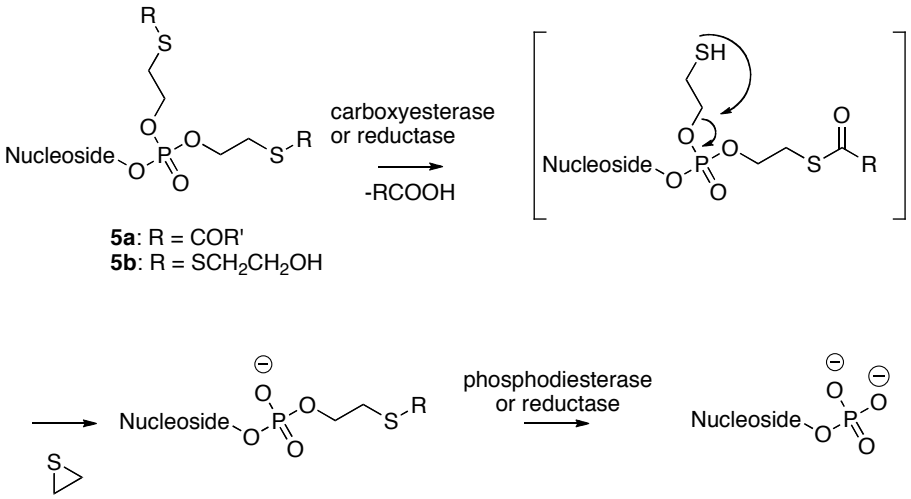


Figure 1.9. Structure of bis(POM) **4** and prodrugs: adefovir dipivoxil **4a** and tenofovir disoproxil **4b**.

With the exception of marketed adefovir dipivoxil **4a** and tenofovir disoproxil **4b** for the treatment of hepatitis B (HBV) (Figure 1.9), the poor stability of such prodrugs in plasma has mainly limited their development to in *vitro* assessments.[97]

Undergoing similar enzymatic mediated hydrolysis *via* the action of a reductive enzyme, Imbach and co-workers developed bis(*S*-acyclic-2-thioethyl) phosphotriester (SATE) **5a**, and later on bis(dithioethyl) phosphotriester (DTE) **5b** (Scheme 1.1). The thioethyl intermediate formed by release of the *S*-acyl-2-thioethanol or thioethanol masking groups,[98] liberates the highly toxic ethylene sulfide. Thus limiting their advancement into in-*vivo* studies.

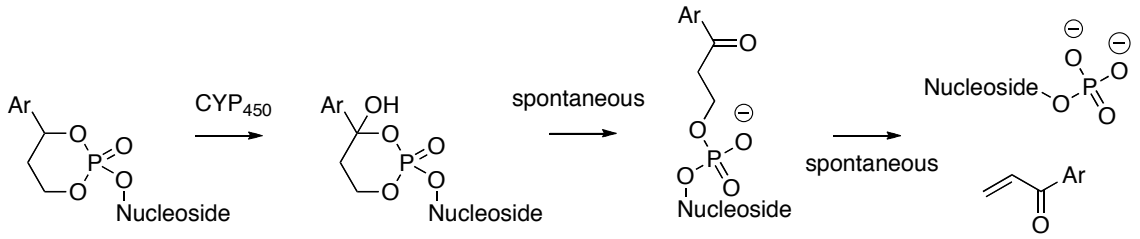


Scheme 1.1. Mechanism of activation of bis(SATE) **5a** or bis (DTE) **5b**.

2.2.3.2. Cyclic phosphotriester approaches

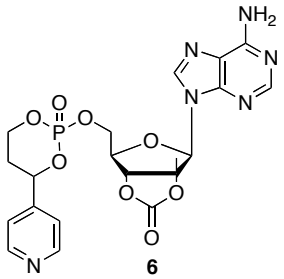
Because of early cleavage of phosphotriester prodrugs in plasma, cyclic analogues were designed in order to enhance their stability in plasma and avoid the release of toxic metabolites.

HepDirect prodrugs are designed to undergo specific activation within the liver[99] *via* oxidation triggered by a single enzyme, cytochrome P_{450} (CYP_{450}), affording the cyclic hemiketal (Scheme 1.2). The later undergoes a spontaneous ring opening releasing the free monophosphate and an aryl vinyl ketone.



Scheme 1.2. HepDirect phosphotriester and mechanism of activation.

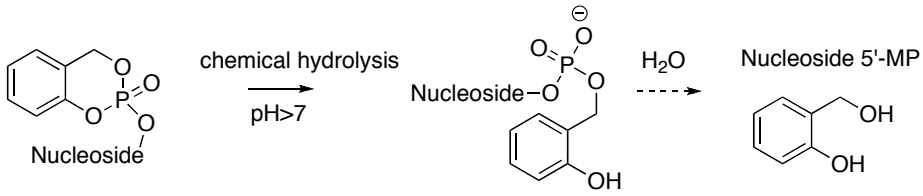
Several agents are based on this strategy, for example the HepDirect prodrug of 2'-C-methyladenosine **6** (Figure 1.10) improved dramatically the triphosphate exposure.[100] Nevertheless, these agents have not progressed to clinical trials for HCV treatment but only for HBV.[101]



6

Figure 1.10. HepDirect prodrug of 2'-*C*-methyladenosine.

In contrast with the use of enzymatic metabolism, Meier developed an approach describing the release of 5'-monophosphates from cyclic phosphotriesters *via* a controlled and selective chemical hydrolysis mechanism of the benzylic and phenolic phosphate ester bonds (Scheme 1.3).[100]

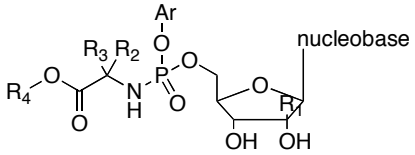


Scheme 1.3. CycloSal phosphotriesters and mechanism of activation.

This approach was applied successfully to different antiviral and antiviral nucleosides,[102, 103] however it was less efficient than strategies involving enzymatic activation.[104]

2.2.3.3. Phosphoramidate di- and mono-ester approaches

The first aryl phosphoramidate prodrug was designed by McGuigan.[105] The introduction of an amino acid ester moiety and an aryl group attached to the phosphorus center was applied to a large number of nucleoside monophosphate analogues. Later on, Wagner and co-workers designed an achiral phosphorus center by masking only one negative charge of the monophosphate (phosphoramidate monoester).[106] Despite improvement of hydrophilicity and stability in plasma, the later displayed poor oral bioavailability compared to the aryloxyphosphoramidate (ProTides),[107] whose general structure is described below (Figure 1.11).



R_1 = alkyl, H
R_2, R_3 = alkyl, H
R_4 = alkyl
Ar = aryl

Figure 1.11. General structure of ProTide.

Despite the obtention of diastereoisomeric mixtures at the phosphorus center, the advantage of this strategy is the release of natural or metabolites with relatively low toxicity.

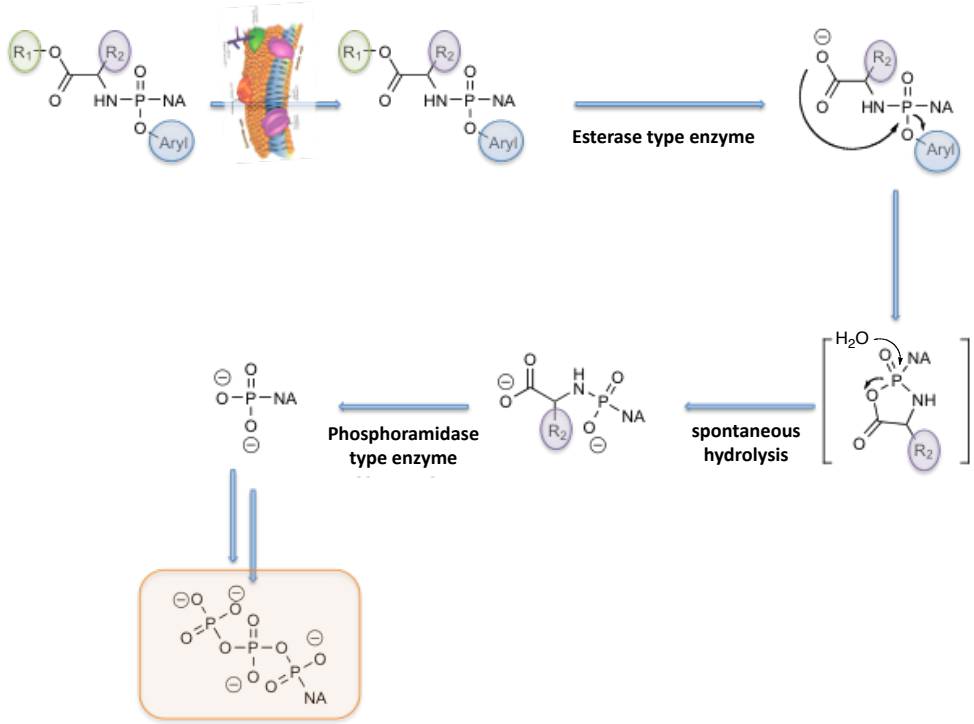
2.2.3.3.1. Mechanism of ProTide activation

Valette and co-workers studied extensively the enzymatic decomposition of aryl phosphoramidate prodrugs.[108, 109] Once inside the cells, the first step of activation consists of the ester cleavage by an esterase or carboxypeptidase type enzyme (Scheme 1.4).

Interactions between the ester moiety and the three catalytic residues of human lysosomal carboxypeptidase (cathepsin A)[109] trigger the initial ester cleavage in the activation of the ProTide.[110] While Ser146 attacks the ester carbonyl group, the two glycines (Gly53 and Gly52) stabilise the resulting intermediate.[110] Conversion to the phosphoramidate monoester intermediate (or amino-acyl intermediate) occurs *via* an intra-molecular attack of the nucleophilic oxygen at the phosphorus atom, releasing the aryl moiety and followed by spontaneous hydrolysis of the putative unstable cyclic anhydride intermediate.[110]

The discovery of adenosine monophosphate-bound amines as substrates[111] of histidine triad nucleotide-binding proteins family (Hint-1),[112, 113] indicates its possible role in the convertion of the phosphoramidate monoester into its corresponding nucleoside monophosphate.[114] Interactions between the key residues Ser107, His112 and His114[115] of these phosphoramidase-type enzymes and the phosphorus center, catalyse the hydrolysis of the P-N bond of the phosphoramidate monoester intermediate.

The resulting 5'-monophosphate can then undergo two phosphorylation steps to afford the bioactive 5'-triphosphate species.



Scheme 1.4. Putative mechanism of ProTide activation.

2.2.3.3.2. Structure activity relationship

Numerous aryl phosphoramidates have been synthesised on various nucleosides. An extensive SAR was built varying the amino acid, ester or aryl moieties, and results from *in vitro* evaluation suggest that the efficacy of ProTides varies significantly depending on the nucleoside. However from these studies resulted a general trend usually considered for designing new ProTide families.

The first part considered for the ProTide activation is the ester moiety. Linear, branched, cyclic and aromatic esters have been considered as substrates for esterases. In general, tertiary ester such as the *tert*-butyl group is not cleaved, whereas ProTides bearing the benzyl ester proved to be highly potent.[110, 116]

The investigation of the aryl moiety resulted in enhanced activity for aryl phosphoramidates bearing more lipophilic moieties such as naphthyl instead of phenyl. Variety of aryl group can generally be used to enhance lipophilicity, hence better cell permeation, and eventually activity of the ProTides.[116]

Natural amino acids tend to be preferred than unnatural amino acids, with the exception of α,α-dimethylglycine that showed interesting efficacy.[117] Only *α*-amino acids deliver

the 5'-monophosphate,[110] and in terms of stereochemistry the *L*-series is often more active than the *D*-series.[118] These results are probably due to the substrate specificity of the carboxypeptidase/esterase and phosphoramidase/human Hint enzymes responsible for bioactivation. *L*-Alanine usually gives the best results in terms of activity compared to other amino acids.

2.2.3.3.3. Stereochemistry of ProTide

The chirality issue at the phosphorus center is the one of the limitation of this strategy. The diastereoisomeric ratio is usually 1:1, but often varies depending on the SAR considered, making the separation of both diastereoisomers difficult. Since cellular targets are chiral, either only one isomer may fit in the pocket,[98] or if both fit, one may be processed faster than the other, thus resulting in difference of activity.[119] However, because the chirality is lost during the activation of ProTide, even diastereoisomeric mixtures can be highly potent, and progress into clinical trials such as the anti-HCV BMS-986094 (known as INX-08189, Figure 1.12) from Inhibitex *Inc.*.[120] In comparison, Pharmasset recently developed a separation technique to pursue the clinical trials with only one isomer GS-7977 (known as PSI-7977, Table 1.1).[121]

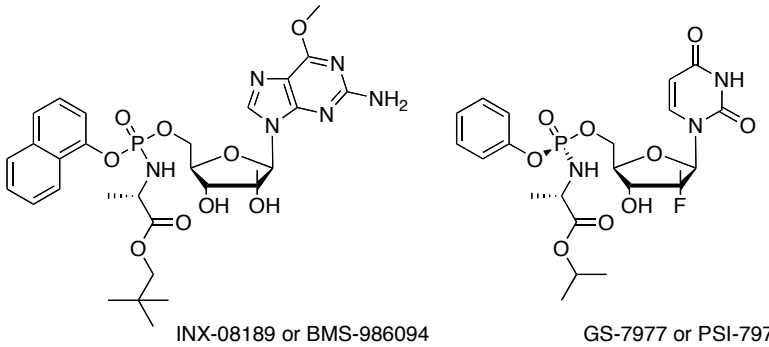


Figure 1.12. Structures of ProTides progressed in clinical trials for HCV treatment.

2.2.3.4. Phosphorodiamidate approach

To overcome the problems encountered due to chirality, another prodrug approach was developed and consisted in attaching two identical amino acid moieties at the phosphorus center.

This approach was first reported by McGuigan and co-workers,[122] but was not further explored until recently. Similarly to the phosphodiester approach, such

phosphorodiamidate prodrugs bear an achiral phosphorus center. The lack of electronic charges enables passive diffusion as well as increases hydrophilicity.

Recently, phosphonic diamidate prodrugs have been reported with good activity against various viruses (Figure 1.13) and further studies indicate delivery of the parent molecule into the cells.[98]

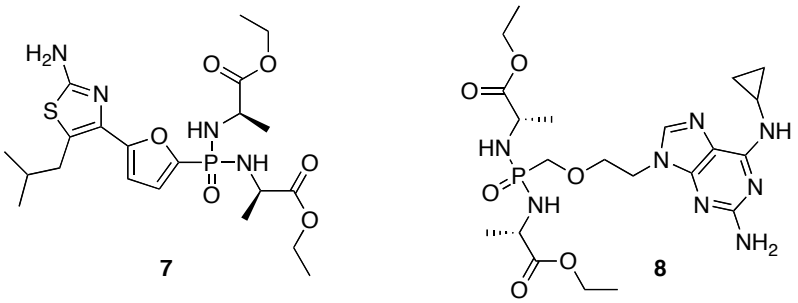


Figure 1.13. Structures of phosphonic diamide against type 2 diabetes (**7**) or orthopox virus (**8**).[98]

The mechanism of bioactivation is similar to the aryl phosphoramidate prodrugs, and non-toxic amino acids by-products are released.

2.2.3.5. Summary of nucleotide prodrug approaches

To summarize and facilitate the comparison, Table 1.2 reports the key attributes of the different nucleotide prodrug technologies discussed in this section, and whether they reached clinical trials in humans.

Prodrug approach	Prodrug class	Bioactivation	By-product	human trials
Phosphotriester	Bis(POM)	Esterase	Formaldehyde	Yes
	Bis(SATE)/Bis(DTE)	Esterase	Episulfide	No
	HepDirect	Cytochrome P_{450}	Aryl vinyl ketone	Yes
	CycloSal	Chemical	Quinone methide	No
Phosphoramidate	Aryloxyphosphoramidate (ProTide)	Esterase, amidase	Phenol, naphtol, amino acid	Yes
	Phosphoramidate monoester (Wagner)	Amidase	Amino acid	No
Phosphorodiamidate	Phosphorodiamidate	Esterase, amidase	Amino acid	Yes

Table 1.2. Comparison of prodrug approaches.

2.3. Combination for HCV therapy

In the near future, the therapy of HCV is likely to involve regimens comprising both nucleoside analogues and non-nucleoside inhibitors. As an example, the combination of GS-7977 and TMC435 (Table 1.1) has been advanced into clinical phase 2 to investigate the safety during 12 and 24 weeks treatment.[69]

Such therapy would alleviate resistance issues, minimise side effects and enhance tolerability in patients while potentially boosting antiviral potency, compared to either mono-therapy. Nevertheless, if not carefully applied, such dual combination would increase the risk of multi-drug resistance.

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Chapter Two: Background and aim of the work

1. Background relevant to the present work

1.1. *β*-2'-*C*-methyl purines: nucleoside analogues and ProTide strategy

Among anti-HCV nucleoside analogues, *β*-2'-*C*-methyl adenosine (**9**, Figure 2.1) and *β*-2'-*C*-methyl guanosine (**10**, Figure 2.1) have shown to inhibit HCV at micromolar concentrations, respectively 0.3 μM and 3.5 μM in subgenomic replicon assays.[1]

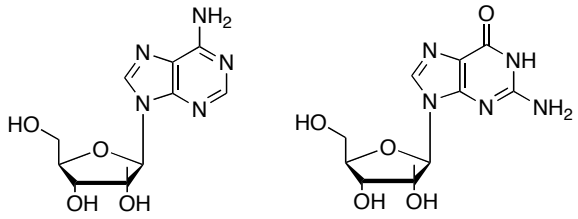


Figure 2.1. Structures of *β*-2'-*C*-methyl adenosine (**9**) and *β*-2'-*C*-methyl guanosine (**10**).

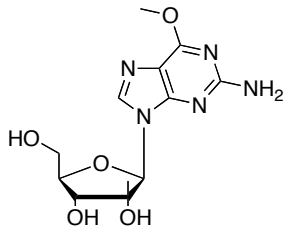
However, *β*-2'-*C*-methyl guanosine 5'-triphosphate intracellular levels were rather poor compared to *β*-2'-*C*-methyl adenosine 5'-triphoshate.[2] The ProTide approach applied to these two nucleoside analogues proved that this difference was related to the *β*-2'-*C*-methyl guanosine not being a good substrate for nucleoside kinases, and thus limiting antiviral efficacy.[3] While ProTides of *β*-2'-*C*-methyl adenosine showed similar efficiency to the parent nucleoside in delivering the 5'-monophosphate, ProTides of *β*-2'-*C*-methyl guanosine were 10-30 fold more active in HCV replicon assays than *β*-2'-*C*-methyl guanosine.[3]

An extensive structure activity relationship (SAR) on *β*-2'-*C*-methyl guanosine ProTides was then built within our group.[4] Resulting from this work, *α*-naphthyl as aryl moiety increased anti-HCV potency independently from the ester and amino acid moieties. The favoured amino acid moiety was the *L*-Alanine, showing activity against HCV replicon in nanomolar range, depending on the ester moiety. ProTides bearing *L*-Valine and *L*-Phenylalanine were more potent than the parent *β*-2'-*C*-methyl guanosine, but giving molecules more cytotoxic and ten times less potent than *L*-Alanine ProTides. Branched amino acids such as *L*-Leucine, *L*-Dimethylglycine or *L*-Isoleucine were slightly or similarly active than the parent nucleoside but more cytotoxic. The ester

variations led to the following conclusions: small such as methyl, branched such as neopentyl or bigger cyclic ester groups such as benzyl and cyclohexyl, are tolerated in the pocket of esterase-type enzymes and relatively quickly processed. Hence, resulting in highly potent molecules stable in plasma, delivering efficiently the 5'-monophosphate species inside the cells and further inhibiting at sub-micromolar concentrations the HCV RNA polymerase.[4]

1.2. *β*-2'-*C*-methyl-6-*O*-modified guanosine analogues

Following the discovery of the *β*-2'-*C*-methyl modified guanosine analogue as an inhibitor of the HCV NS5B polymerase,[1] and the reported low triphosphate levels after dosing,[2] further modifications of the nucleobase were applied to boost the lipophilicity and increase the cellular uptake. Among other 6-modified nucleosides,[5] the *β*-2'-*C*-methyl-6-*O*-methyl guanosine (**11**, Figure 2.2) derivative resulted from this work, and showed poor replicon activity (EC_{50} = 3.0 μM) as predicted by the modelling.[5]



11

Figure 2.2. Structure of *β*-2'-*C*-methyl-6-*O*-methyl guanosine **11**.

The poor anti-HCV potency observed in general for *β*-2'-*C*-methyl-6-*O*-modified analogues can be explained by slow intracellular conversion at a nucleoside or nucleotide level, to the *β*-2'-*C*-methyl guanosine.[5]

2. Aim of the work

The discovery of the poorly active *β*-2'-*C*-methyl-6-*O*-methyl guanosine[5] and the prediction that its triphosphate binds poorly into the NS5B active site,[5] led us to investigate prodrug strategies in order to improve cellular uptake and to bypass the first phosphorylation step, thus eventually resulting in efficient conversion to the *β*-2'-*C*-methyl guanosine triphosphate.

Moreover, the need of more efficient therapy and the use of nucleoside analogues to target the essential enzyme responsible for replication of the virus (NS5B RdRp), led us to modify several moieties of *β*-2'-*C*-methyl-6-*O*-methyl guanosine **11** depicted in Figure 2.3, in order to explore the SAR and understand better the metabolism pathway of such nucleoside analogues.

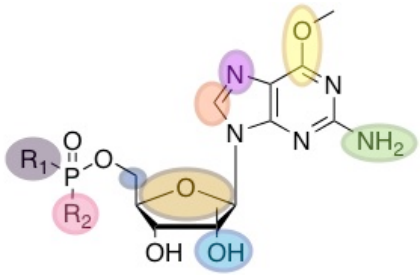


Figure 2.3. Modifications of 2'-*C*-methyl-6-*O*-methyl guanosine.

Aiming at delivering efficiently inside the cells the desired bioactive triphosphate form after metabolism, two prodrugs technologies, the ProTide and phosphorodiamidate approaches, were applied during this project, as a tool for potential inhibition of HCV replication, improvement of potency and selectivity of these modified nucleosides.

The following chapters describe the design and synthesis of *β*-2'-*C*-methyl-6-*O*-methyl guanosine prodrugs and modified analogues as well as novel acyclic derivatives, a new concept in the HCV field. The biological results are reported and correlated with mechanistic analysis and enzymatic assays to provide better understanding of the metabolic pathway, and to explore the moieties essential for the inhibition of NS5B RdRp.

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Chapter Three: Phosphate moiety modifications of *β*-2'-*C*-methyl-6-*O*-methyl guanosine prodrugs

1. ProTide technology applied to *β*-2'-*C*-methyl-6-*O*-methyl guanosine

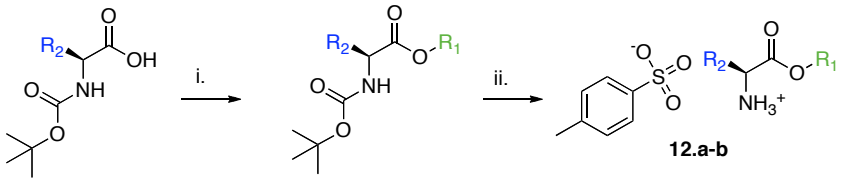
Aiming at boosting the anti-HCV activity, the ProTide technology was applied to *β*-2'-*C*-methyl-6-*O*-methyl guanosine **11**, prodrug of *β*-2'-*C*-methyl guanosine **10**. Aiming at enhancing passive cellular uptake and investigating the effect on potency of bypassing the first phosphorylation step,[1] several ProTides were prepared, with variation at the amino acid and the ester moieties.

1.1. Procedure

1.1.1. Preparation of amino acid tosylate salts

Several amino acid esters were commercially available, whilst some others were prepared as para-toluene sulfonic acid salts (Scheme 3.1).

Following the path described in Scheme 3.1, tosylate salts of *L*-phenylglycine neopentyl ester (**12.a**) and *L*-phenylglycine benzyl ester (**12.b**) were prepared respectively in 77% and 80% yield, over two steps. The last step enabled recovery of a white precipitate in both case, while the Boc protected intermediate required flash chromatography. *L*-Alanine cyclopropyl methyl ester was provided by *Inhibitex Inc*.

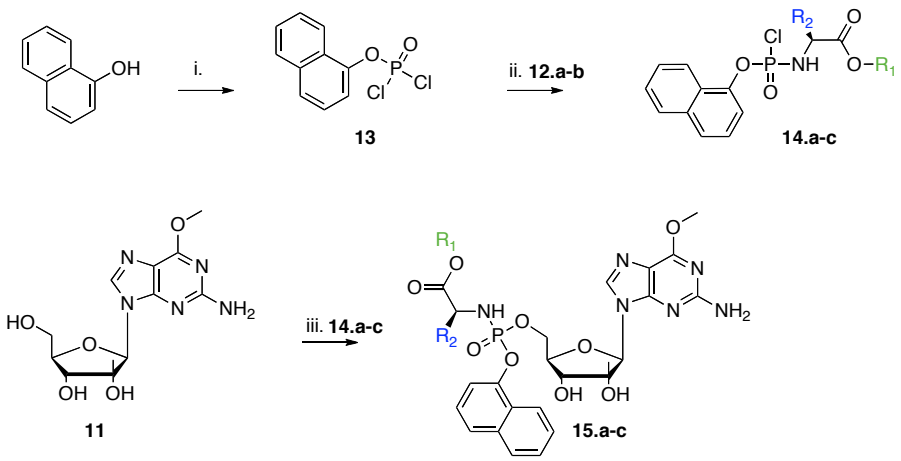


(i. anh.DCM, DCC (1.0 eq), DMAP (0.1 eq), R_1OH (1.4 eq), overnight, rt; ii. EtOAc, pTSA (1.0 eq), overnight, 65 °C.)

Scheme 3.1. General synthesis of amino acid ester tosylate salts.

1.1.2. Preparation of *β*-2'-*C*-methyl-6-*O*-methyl guanosine ProTides

The naphthyloxy phosphodichloridate (**13**, Scheme 3.2) was formed in 51% yield by addition of *α*-naphthol to phosphorus oxychloride ($POCl_3$). The ^{31}P-NMR appeared as one peak at 3.74 ppm.



(i. anh. Et_2O, $POCl_3$ (1.0 eq), anh. NEt_3 (1.0 eq), 30 min, -78 °C, 1hr, rt; ii. anh. DCM, anh. NEt_3 (2.0 eq), ***12.a-b*** *(1.0 eq), 1 hr, -78 °C, rt, 30 min; iii. anh. THF, tBuMgCl (2.0 eq), 20 min, RT,* ***14.a-c*** *(2.0 eq), overnight, rt.)*

Scheme 3.2. Route to *β*-2'-*C*-methyl-6-*O*-methyl guanosine ProTides.

Addition of **13** with **12.a**, **12.b** and *L*-alanine-cyclopropylmethyl ester tosylate salt, provided by Inhibitex *Inc.*, gave respectively the *α*-naphtyl amino acid ester phosphochloridates **14.a**, **14.b** and **14.c**, all as a mixture of two diastereoisomers at the phosphorus center. This was confirmed by ^{31}P-NMR with the apparition of two peaks around 7-8 ppm. Without further protection of the nucleoside hydroxyl groups, phosphorochloridates **14.a-c** were reacted with *β*-2'-*C*-methyl-6-*O*-methyl guanosine **11** following the Uchiyama procedure,[2] using the Grignard reagent *tert*-butyl magnesium chloride (*t*BuMgCl) as a base. The desired ProTides (**15.a-c**, Scheme 3.2) were formed as mixtures of unseparable diastereoisomers in different ratio (Table 3.1). Substitution of the remaining chlorine of **14** by the 5'-oxigen results in an upfield ^{31}P-NMR peaks, shifting from 7-8 (**14**) to 4 ppm (**15**).

	Amino acid	Ester	Yield	^{31}P (ppm) in $CDCl_3$ / ratio
15.a	*L*-PhGly	*t*BuCH_2	46%	3.97, 3.60 (62/38)
15.b	*L*-PhGly	Bn	32%	3.82, 3.41 (20/80)
15.c	*L*-Ala	cProp-CH_2	32%	4.07, 3.90 (43/57)

Table 3.1. Yields, ^{31}P NMR and diastereoisomer ratio of *β*-2'-*C*-methyl-6-*O*-methyl guanosine ProTides.

1.2. Structure activity relationship

Upon completion of the family of *β*-2'-*C*-methyl-6-*O*-methyl guanosine ProTides, which majority were synthesised previously, three additional ProTides were synthesised. Bearing respectively *L*-phenylglycine neopentyl ester (**15.a**), *L*-phenylglycine benzyl ester (**15.b**) and *L*-Alanine cyclopropyl methyl ester (**15.c**) moieties. The most important results of the SAR are reported in Table 3.2.

	Amino acid	Ester	EC_{50} (μM)	CC_{50} (μM)	ClogP**
10*	-	-	3.5	>100	
11*	-	-	5.2	>100	
10a*	*L*-Ala	*t*BuCH$_2$	0.057	>100	1.3
15.a	*L*-PhGly	*t*BuCH$_2$	0.032	n.d	4.5
15.b	*L*-PhGly	Bn	0.028	n.d	4.3
15.c	*L*-Ala	cProp-CH$_2$	n.d	n.d	2.40
15.d*	*L*-Ala	*t*BuCH$_2$	0.01	7	3.3
15.e*	*L*-Ala	Bn	0.03	12	3.1
15.f*	*D*-Ala	*t*BuCH$_2$	0.24	51	3.3
15.g*	*L*-Met	*t*BuCH$_2$	0.06	28	3.4
15.h*	*L*-Leu	*t*BuCH$_2$	0.07	14	4.7
15.i*	*L*-Ile	*t*BuCH$_2$	0.86	18	4.7
15.j*	*L*-Val	*t*BuCH$_2$	0.19	33	4.2
15.k*	*L*-Ala	cHex	0.03	6	3.5

* synthesised by previously.[1, 3, 4]
** ClogP from CS ChemDraw Ultra 11.0 software.

Table 3.2. Activity (EC_{50}) in HCV replicon assays and cytotoxicity (CC_{50}) in Huh-7 cells of *β*-2'-*C*-methyl-6-*O*-methyl guanosine ProTides.

Considering the *L*-Alanine amino acid moiety, the neopentyl ester (**15.d**) not only exhibits a 500-fold boost in potency compared to the parent nucleoside *β*-2'-*C*-methyl-6-*O*-methyl guanosine (**11**), but also enhances activity and has greater lipophilicity than its *β*-2'-*C*-methyl guanosine analogue (**10.a**), facilitating cellular uptake.[1] Similar trends were observed when the lipophilic benzyl ester (**15.e**) was used.[1]

Variation of the amino acid was studied while retaining the neopentyl ester moiety. Exhibiting a 24-fold reduction of activity compared to **15.d**, the *D*-Ala ProTide (**15.f**) proves the importance of intracellular metabolism for potency.[1] Bigger or branched

amino acid moieties (**15.a-b**, **15.g-j**) decrease anti-HCV activity compared to **15.d**, but in general are more potent than their *β*-2'-*C*-methyl guanosine analogues.[1]

1.3. From bench to clinical trials

From this study, **15.d** (known as INX-08189 or BMS-986094) emerged as the most promising candidate. Separated diastereoisomers showed similar activity in replicon assays.[1] In addition to excellent properties, such as cell permeability and good bioavailability,[5] good metabolism and high triphosphate levels detected in plasma,[5] **15.d** is potent against multiple HCV genotypes,[1] exhibiting IC_{50}s in replicon assays of 12, 10 and 0.9 nM *versus* genotype 1a, 1b and 2 respectively.[5] Moreover, in combination with the standard of care therapy, **15.d** showed high synergy with ribavirin.[5, 6] These data made **15.d** one of the most potent NS5B polymerase inhibitors ever reported. Unfortunately, its clinical development has recently been discontinued due to cardiotoxicity developed by patients during phase 2b clinical.[7]

1.4. Conclusion of the ProTide approach

The ProTide technology was successfully applied for anti-HCV therapy, to the poorly active *β*-2'-*C*-methyl-6-*O*-methyl guanosine **11**, affording mixtures of diastereoisomers at the phosphorus center, releasing the same metabolite and showing submicromolar potency against HCV.

The only disadvantage of the ProTide approach is the difficulty of separating the two diastereoisomers using common separation techniques. As a result, only the mixture was tested. Because metabolic enzymes are substrate specific, one diastereoisomer might not be processed or processed slower than the other, thus affecting the overall potency.

Hence to avoid isolation of a diastereoisomeric mixture, the notion of achiral phosphate prodrugs was investigated by application of the phosphorodiamidate approach.

2. Phosphorodiamidates of *β*-2'-*C*-methyl-6-*O*-methyl guanosine

A new motif was designed in order to investigate the effect of an achiral phosphate prodrug on delivering the *β*-2'-*C*-methyl guanosine 5'-triphosphate, and eventual inhibition of HCV replication. The phosphoramidate core was retained and preferably non-toxic natural moieties such as amines and amino acids were considered, affording phosphorodiamidates[8] which general structure is depicted in Figure 3.1.

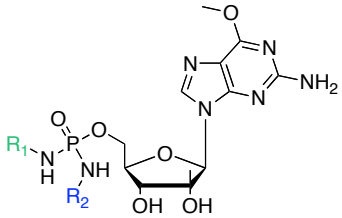


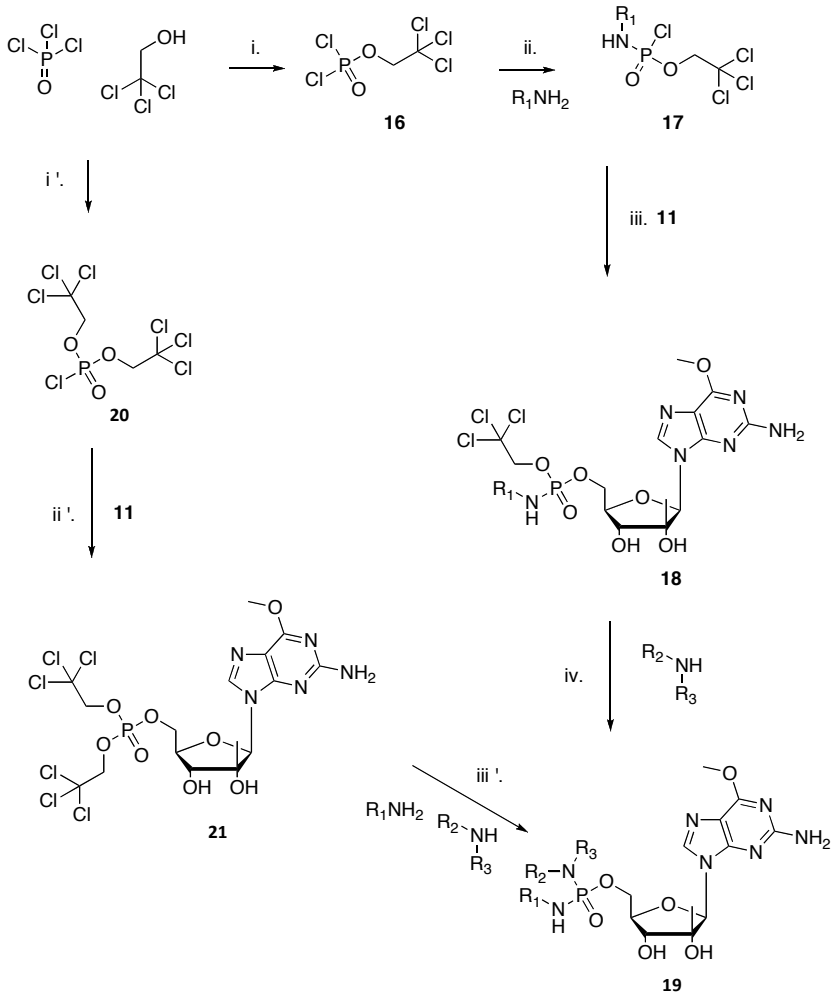
Figure 3.1. General structure of phosphorodiamidate prodrug of *β*-2'-*C*-methyl-6-*O*-methyl guanosine (R_1: amine 1 or amino acid 1, R_2: amine 2 or amino acid 2).

2.1. Synthetic route

Little is reported in the literature about phosphorodiamidate motif on nucleosides, however our group was among the first to explore synthetic pathways of such prodrugs.[9, 10] Nevertheless a new synthetic route had to be adapted for *β*-2'-*C*-methyl-6-*O*-methyl guanosine **11**.

2.1.1. 2,2,2-trichloroethyl approach

Described by Sheeka,[11] the first approach considered aimed at forming either the 2,2,2-trichloroethyl (2,2,2-TCE) phosphorochloridate or dichloridate, followed by sequential displacement of TCE with amines, as shown in Scheme 3.3.



(i. anh. Et_2O, anh. NEt_3, -80 °C then rt, 6hrs 30min; ii. anh. DCM, anh. NEt_3, -80 °C then rt, 1hr 30min; iii. anh. THF, NMI, rt, 8hrs 30min; iv. CsF, rt / i'. anh. Et_2O, anh. NEt_3, -80 °C then rt, overnight; ii'. pyridine, rt, 2 days; iii'. CsF, rt, 2 weeks.)

Scheme 3.3. Synthetic routes to phosphorodiamdates using TCE.

Both the 2,2,2-TCE phosphodichloridate (**16**) and phosphochloridate (**20**) were synthesized from phosphorus oxychloride ($POCl_3$) and 2,2,2-TCE, and were obtained in relatively low yields (2 to 6%) after distillation. Their formation was monitored by ^{31}P-NMR showing a phosphorus chemical shift recorded was in accordance with the substituents and the number of substitutions at the phosphorus center. Bis-substitution of TCE shields the phosphorus, hence lowers the chemical shift recorded for **20** (3.10 ppm) compared to the phosphorodichloridate **16** (7.70 ppm).

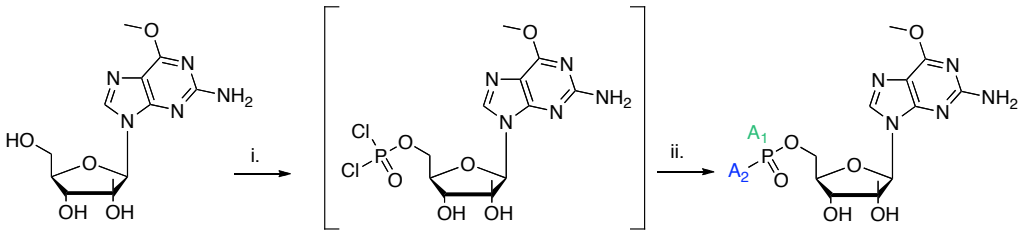
Following the substitution of **16** with *L*-Alanine methyl ester, **17** (R_1: $CH(CH_3)COOCH_3$) was coupled with *β*-2'-*C*-metyl-6-*O*-methyl guanosine **11** provided by Inhibitex *Inc*. While the ^{1}H-NMR and mass spectrum proved formation of the desired molecule **18.a** (R_1: $CH(CH_3)COOCH_3$), ^{31}P-NMR recorded a single peak instead of the two expected due to the presence of a chiral phosphorus center. Either the

signals are overlapping each other or less likely only one isomer has been formed. However, too little amount was recovered to be able to conclude on the formation of both diastereoisomers by HPLC or for further experiments leading to **19.a** (R_1: $CH(CH_3)COOCH_3$) or to the phosphoramidate monoester intermediate.
Coupling of **20** with **11** afforded **21**, which was sent for biological evaluation in HCV replicon. Unfortunately, displacement of the TCE groups by *N*-butylamine to afford **19.b** (R_1=R_2: $CH_2CH_2CH_2CH_3$, R_3: H) failed.

Due to the lack of reaction and material recovered, and the poor properties of TCE as a leaving group, this approach was eventually abandoned. The next approach considered was based on the lability of the P-Cl bond towards substitution.

2.1.2. Phosphorus oxychloride approach

Considering the lability of the P-Cl bond and reported synthetic routes on phosphorodiamidates of AZT,[10] the unprotected nucleoside was treated with phosphorus oxychloride ($POCl_3$) to generate in-*situ* the phosphorodichloridate (**22**, Scheme 3.4), which formation was monitored by ^{31}P-NMR (~8.00 ppm). Subsequently various amines or amino acid esters were added to obtained either symmetrical or asymmetrical phosphorodiamidates (12 ppm < ^{31}P NMR < 18 ppm).



(i. anh. THF, anh. NEt_3 (1.2 eq), RT, 30 min, $POCl_3$ (1.2 eq), -78 °C, 30 min, rt, 30 min; ii. anh. DCM, A_1, A_2 (5.0 eq), NEt_3 (10.0 eq), -78 °C to rt, overnight.)

Scheme 3.4. Phosphorus oxychloride approach to phosphorodiamidates.
(A_1 = A_2 or $A_1 \neq A_2$, A_1: amine 1 or amino acid 1, A_2: amine 2 or amino acid 2)

The formation of the intermediate **22** usually takes place under Yoshikawa conditions[12] to give the regioselectivity of the reaction at the 5'-hydroxyl. However here the regioselectivity is obtained using triethylamine as a base and only 1.2 equivalents of phosphorus oxychloride in tetrahydrofuran. To prevent degradation and hydrolysis,

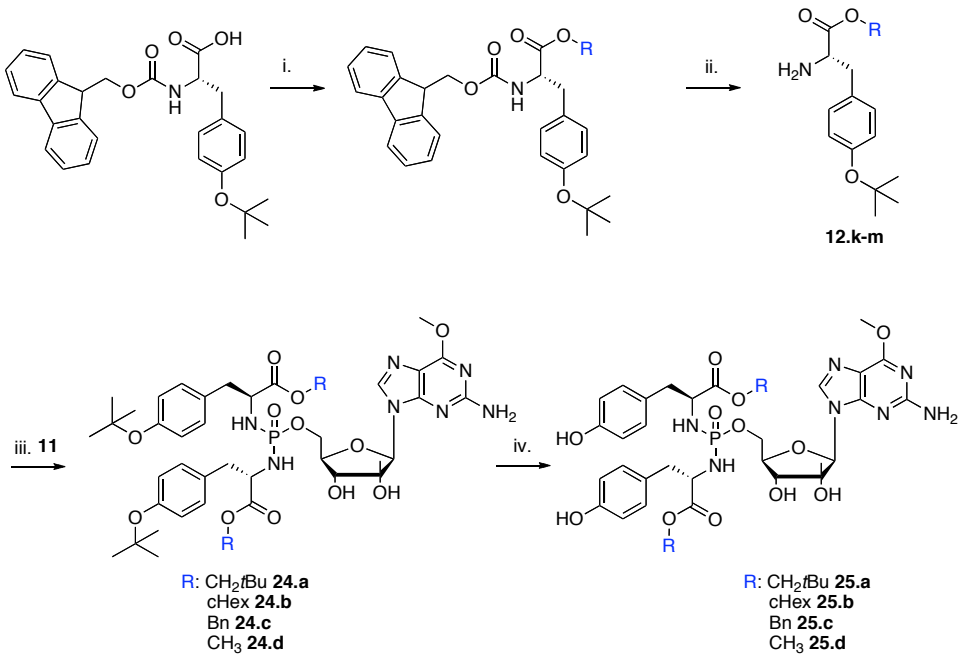
formation of **22** is monitored by ^{31}P-NMR after an average optimum reaction time of 30 min. Symmetrical phosphorodiamidates required the addition of 5.0 eq of amino acid ester or amine. The only asymmetrical phosphorodiamidate **23** synthesised, was obtained by addition of 1.0 eq of the *L*-Alanine cyclohexyl ester para-toluene sulfonic acid salt affording the phosphochloridate seen by NMR, and then addition of 5.0 eq of *L*-Alanine cyclopentyl ester para-toluene sulfonic acid salt.

A wide structure activity relationship (SAR) was built following this synthetic route. The benzyl, cyclohexyl, neopentyl and methyl esters were selected from the ProTide SAR results.

The tosylate salts of *L*-Leucine benzyl ester, *L*-Phenylalanine benzyl ester, and both *L*-Alanine cyclopentyl ester and *L*-Alanine cyclopropyl methyl ester, were supplied by Inhibitex *Inc*. Except *L*-Tyrosine and *S*-methyl-*L*-Cysteine, all amino acid ester tosylate salts (**12.c-f, 12.j**) were synthesised in relatively good yields (44-94%) from the Boc protected amino acid as described in Scheme 3.1. *S*-methyl-*L*-Cysteine amino acid ester tosylate salts (**12.g-i**) were obtained by reaction of the free *S*-methyl-*L*-Cysteine with an excess of the desired alcohol (13 to 15 eq) using the Dean-Stark apparatus.

Synthesis of *L*-Tyrosine phosphorodiamidates (**25 a-d**) was first undertaken with commercially available *L*-Tyrosine–*O*-methyl chloridate salt, and afforded *L*-Tyrosine and an achiral specie different from the one desired. Because of the non-protected phenol, competitive substitution on the phosphorus can occur, hence resulting in the formation of three products: the phosphorodiamidate desired, the phosphotriester and the mixed phosphoramidate. To prevent such competition, the commercially available phenolic protected Boc-*L*-Tyrosine(*t*Bu) amino acid was used to yield the corresponding amino acid esters as tosylate salts, with the exception of the commercially available *L*-Tyrosine(*t*Bu)-O-methyl chloridate salt. The standard procedure used for the formation of amino acid ester tosylate salts (Scheme 3.1) resulted in the cleavage of both the Boc and *t*Bu protecting groups. Attempt of re-protection of the free phenolic alcohol by refluxing overnight using potassium hydrogencarbonate and methyl iodide[13] failed, and the starting material was recovered. To enable orthogonal deprotection, Fmoc instead of Boc protecting group appeared to be a wise choice since it is cleaved under mild basic conditions,[14] which would not affect the phenolic *t*Bu ether. Scheme 3.5 describes the route to *L*-Tyrosine phosphorodiamidates, starting from commercially available Fmoc-*L*-Tyrosine(*t*Bu)-OH

to obtained desired amino acid esters, followed by selective deprotection of Fmoc using morpholine/DMF to afford the free amino acid esters (**12**.**k-m**, Scheme 3.5). Successful coupling with *β*-2'-*C*-methyl-6-*O*-methyl guanosine **11**, as described in Scheme 3.5, afforded **24**.**a-d** in moderate yields (18-31%). Eventually the *t*Bu ether group was cleaved with trifluoroacetic acid (TFA)[15] to afford the desired phosphorodiamidates (**25.a-d**, 24-49%).



(i. anh DCM, DCC (1.0 eq), DMAP (0.1 eq), ROH (1.4 eq), overnight, rt; ii. anh. DMF/morpholine (1:1), 30 min, rt; iii. a) anh. DMF, anh. NEt_3, 30 min, rt, $POCl_3$, -78 °C, 30 min to rt, 30 min; b) anh. DCM, anh. NEt_3, -78 °C to rt, overnight; iv. anh. DCM, TFA, 30 min, rt.)

Scheme 3.5. Route to *L*-Tyrosine ester phosphorodiamidates.

The synthesis of *L*-Isoleucine benzyl ester (**26.c**), dimethylglycine neopentyl and benzyl ester phosphorodiamidates failed. The non-natural amino acid is probably too hindered to allow two consecutive substitutions at the phosphorus center, while the reaction with *L*-Isoleucine benzyl ester was carried out with too little material to enable the coupling.

The poor solubility of *L*-Asparagine did not allow formation of amino acid ester chloridate salts using thionyl chloride, as decribed by Turner[16], but free amino acid esters along with the starting material were obtained instead. Second reaction to obtain tosylate salt by reaction with pTSA was attempted in vain.

The phosphorus oxychloride route enabled the recovery of one asymmetrical **23** and twenty-two symmetrical phosphorodiamidates (**19.b**, **24.a-d**, **25.a-d**, **27.e-f**, **29.a-c**, **32.a-c**, **34.a**, **35.a-c**, **36**).

2.2. Biological evaluation

2.2.1. In-*vitro* results

2.2.1.1. Replicon assays

The potency and cytotoxicity of phosphorodiamidates synthesised were tested in HCV replicon assays at Inhibitex *Inc*. and are reported in Table 3.3.

	EC_{50} (µM) in HCV replicon						CC_{50} (µM)
10	2.2						>100
11	4.4						>100
15.d	0.01						7.01
21	11.3						n.d
amine							
butylamine **19.b**	37.8						>100
morpholine **36**	>100						>100
amino acid	ester						
	neopentyl a	cyclohexyl b	benzyl c	methyl d	cyclopentyl e	CH_2-cyclopropyl f	
L-Alanine **27**	0.06*	0.05*	0.49*	5.9*	0.06	0.21	>100
L-Valine **28***	0.72	2.5	0.12				20-49
L-Leucine **29**	0.47	0.45	0.38				20-39
L-Isoleucine **26***	2.9	4.0	0.40	5.17			14-24
L-Methionine **30***	2.2	0.60	0.25				>51
L-Proline **31***	0.81		0.52				>56
L-Phenylalanine **32**	0.05	0.50	0.32				24-67
L-Phenylglycine **33***	0.27	0.32					18-24
D-Alanine **34****	0.11						>100
L-Tyrosine **25**	0.91	0.68	1.3	1.2			>100
L-Tyrosine (tBu) **24**	2.1	3.0	0.17	0.11			>89
S-Me-*L*-Cysteine **35**	0.097	0.20	0.23				>69
L-Ala-cHex/*L*-Ala-cPent **23**	0.04						>100

* synthesised by co-workers.[8]
** scaled up for in-*vivo* studies.[8]

Table 3.3. Biological activity (EC_{50}) and cytotoxicity (CC_{50}) of phosphorodiamidates in replicon-based assays.[8]

The biological results clearly indicate that phosphotriester **21** and phosphorodiamidate prodrugs bearing simple amines (**19.b** and **36**) are, at best, poorly active in HCV replicon. The inactivity of **36** is likely to result from non-permeation of the host cell

membrane since its ClogP is low (0.85), while **19.b** (ClogP~2.31) is probably not a good substrate for intracellular metabolism. The latter suggestion will be studied later in this chapter.

Switching from simple amines to amino acid ester moieties (**23**-**35**) has a significant impact on the activity against HCV, however cellular toxicity is observed for some amino acids different than the *L*-Alanine.

The SAR of the ester moiety shows that the benzyl and methyl esters (**c** and **d**) give similar activities for all amino acid moieties, while the cyclohexyl and neopentyl esters (**b** and **a**) allow discrimination. In general, bigger (**32-33** and **24-25**) or more branched (**26**, **28-31** and **35**) amino acid moieties result in poor activity, with the exception of *L*-Phenylalanine neopentyl ester diamidate **32.a**, *L*-Tyrosine(*t*Bu)-methyl ester and *S*-methyl-*L*-Cysteine neopentyl ester diamidates (**24.d** and **35.a**). The extra methylene group present in *L*-Methionine phosphorodiamidates (**30.a-b**) results in a significant loss of potency in comparison with *S*-methyl-*L*-Cysteine phosphorodiamidates (**35.a-b**). Results from phosphoramidates **24.a-d**, **25.a-d** and **32.a-c** indicate that substitution of the phenyl group has a negative impact on HCV potency, with the *t*Bu group being the less tolerated, with the exception of **24.d** which has a ClogP value similar to phosphoramidates **25.a-c** (5.45 < ClogP < 6.0). The low potency exhibited by such molecules is probably due to poor cell permeation resulting from their high liphophilicity (5.45 < ClogP < 9.8). However, with the lowest ClogP value (2.02), phosphoramidate **25.d** is also poorly active in replicon. This biological evaluation suggests that the pocket where the amino acid moiety lies in the metabolic enzymes accommodates preferentially small groups, such as methyl. Among the *L*-Alanine family, phosphorodiamidates bearing the neopentyl (**27.a**), cyclohexyl (**27.b**) and cyclopentyl (**27.e**) ester moieties are potent inhibitors of HCV replication, hence well tolerated by the metabolic esterase-type enzymes. Bigger ester moieties (**27.c**) decrease significantly the anti-HCV activity and exhibit 5 to 10-times loss of potency compared to alkyl or cyclic esters.

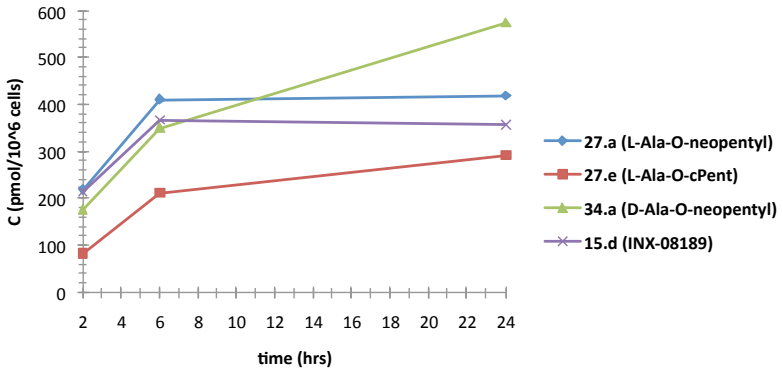
Surprisingly, the unnatural *D*-Alanine neopentyl ester diamidate (**34.a**) retains the selectivity (TI = 909) and exhibits only a 2-fold lost in potency compared to its analogue in the *L*-series **27.a** (TI = 1666). Similar results were not expected since the *D*-

Alanine neopentyl ester ProTide was 24-fold weaker than **15.d** in HCV replicon assays.[1]

From this study, **27.a**, **27.e** and **34.a** were selected for further in-*vitro* and in-*vivo* studies performed at Inhibitex *Inc*.

2.2.1.2. Intracellular triphosphate level

Phosphorodiamidates **27.a**, **27.e** and **34.a** were incubated at a concentration of 10 µM in human hepatocytes, and levels of triphosphate were measured after 2, 6 and 24 hours using LC-MS/MS spectroscopy (Graph 3.1). ProTide **15.d** was also incubated for comparison. This work was done by Inhibitex *Inc*.



Graph 3.1. Intracellular triphosphate concentrations (C) in human hepatocytes.

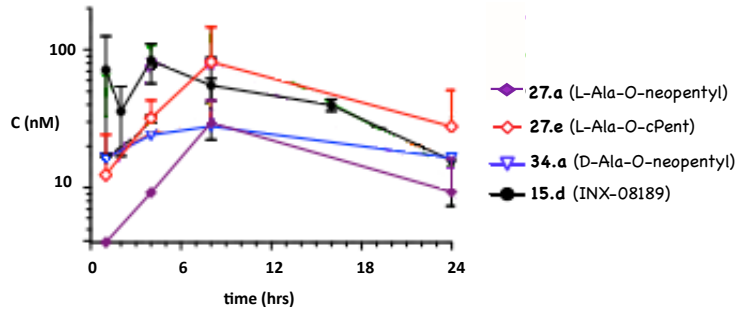
Similarly to the lead compound **15.d**, phosphorodiamidates deliver efficiently the bioactive triphosphate form inside the cells, with **27.e** being the less efficient. While the maximum concentration of triphosphate is achieved after 6 hours of incubation in the case of **15.d**, both its diamidate derivatives **27.e** and **34.a** produce triphosphate levels up to 24 hours after incubation, thus increasing its bioavailability.

2.2.2. In-*vivo* results

The three phosphorodiamidates selected (**27.a**, **27.e** and **34.a**) were formulated (95% Capmul MCM / 5% Tween 80) and orally dosed to rats. Concentrations were collected up to 24 hours post administration using LC-MS/MS spectroscopy. This work was done by Inhibitex *Inc*.

2.2.2.1. Pharmacokinetics

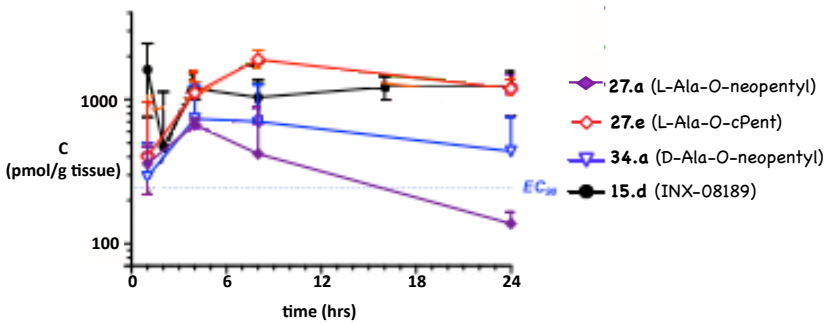
The pharmacokinetic profiles of the selected phosphorodiamidates together with **15.d** were evaluated after dosing 10 mg/kg orally to rats (Graph 3.2 and Graph 3.3).



Graph 3.2. Concentrations of *β*-2'-*C*-methyl guanosine **10** in rat plasma.

The exposure of parent nucleoside *β*-2'-*C*-methyl guanosine **10** in rat plasma was measured as a biomarker to monitor liver pharmacokinetics of *β*-2'-*C*-methyl guanosine triphosphate in-*vivo*.[5] The three phosphorodiamidates selected (**27.a**, **27.e** and **34.a**) release similar or lower maximum concentrations of the parent nucleoside *β*-2'-*C*-methyl guanosine **10** in comparison with **15.d**, hence suggesting relatively good stability of *β*-2'-*C*-methyl guanosine triphosphate in the liver.

The liver concentrations of *β*-2'-*C*-methyl guanosine triphosphate were also determined (Graph 3.3) at Inhibitex *Inc*.



Graph 3.3. Concentrations of *β*-2'-*C*-methyl guanosine triphosphate in rat liver.

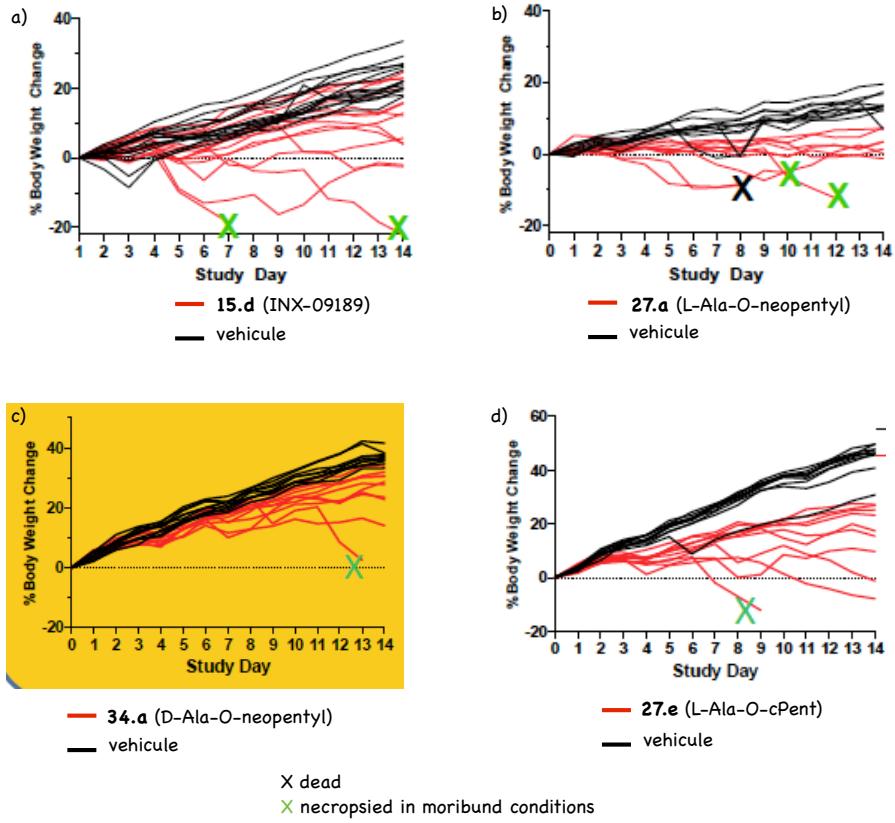
After 24 hours, all phosphorodiamidates but **27.a** show a concentration of 2'-*C*-methyl guanosine triphosphate in liver similar or higher than **15.d**, and most importantly above

the concentration necessary to achieve 90% of inhibition of HCV replication (EC_{90} ~ 243 pmol/g liver).[8]

These combined results prove that phosphorodiamidates **27.a**, **27.e** and **34.a**, are effective prodrugs delivering the desired 2'-*C*-methyl guanosine triphosphate in high concentrations and inhibiting effectively HCV replication.

2.2.2.2. Toxicology studies

Preliminary 14 days toxicity studies were performed by Inhibitex *Inc*. Rats were administered orally a 30 mg/kg formulation containing each of the three most potent phosphorodiamidates (**27.a**, **27.e** and **34.a**). The body weight was measured daily pre-dosing. All three diamidates exhibit a more favourable toxicity profile than **15.d** (Graph 3.4 b-d), with **34.a** showing the best profile (Graph 3.4 c), with less variation in body mass compared with the vehicule group. Its analogue in the *L*-series (**27.a**) exhibits high gastrointestinal tract toxicity leading to morbidity in both groups of rats.

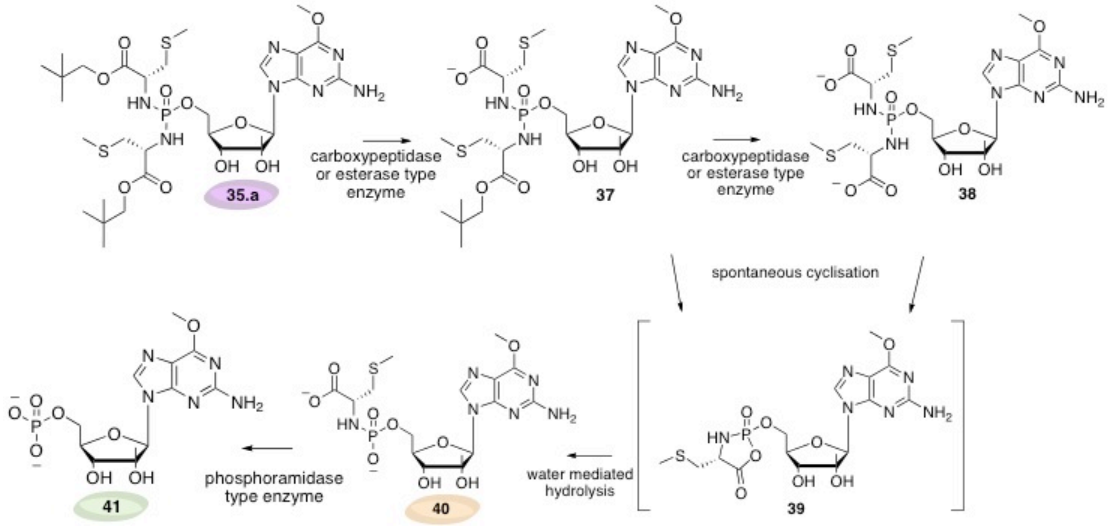


Graph 3.4. Toxicity studies in rats (30 mg/kg dose).

2.3. Mechanistic studies

To probe and better understand the mechanism of activation of phosphorodiamidate prodrugs inside the host cells, enzymatic studies were performed and metabolic steps were monitored by ^{31}P-NMR.

The putative mechanism of activation is described in Scheme 3.6.[8] The first step consists of the cleavage of the ester moiety by esterase-type enzymes to afford **37** or **38**, followed by intramolecular attack of the charged oxygen onto the phosphorus center forming a putative five-membered ring intermediate **39**. The latter opens by attack of a water molecule releasing the amino-acyl intermediate **40** (also called phosphoramidate monoester), which P-N bond is then cleaved by phosphoramidase-type enzymes to afford the desired 5'-monophosphate **41**.



Scheme 3.6. Putative mechanism of activation of phosphorodiamidates.

2.3.1. Carboxypeptidase Y assays

The commercially available carboxypeptidase Y (CPY) is an esterase type enzyme known to carry out the initial cleavage of the ester moiety. This assay was performed incubating **35.a** (3.5 mg in 0.140 mL d_6-acetone) with carboxypeptidase Y (0.1 mg) in Trizma buffer (pH~7, 0.420 mL), and ^{31}P-NMR experiments were run every 7 minutes at room temperature (25 °C) (Figure 3.2). While the peak corresponding to **35.a** (purple) slowly disappears with time, one new species is formed (peak at 6.45 ppm, orange). According to the literature,[17] this species corresponds to the amino-acyl intermediate **40**. Due to its putative transient nature, the ^{31}P-NMR peak corresponding to the 5-

membered ring intermediate **39** is not detectable. **37** and **38** are also not detected by ^{31}P-NMR suggesting instability of **37**, which cyclises and undergoes fast hydrolysis following the cleavage of one or both esters. This experiment does not discriminate if both esters need to be cleaved to afford **40**, however based on the ProTide mechanism (Scheme 1.4, Chapter 1) the cleavage of only one ester should be sufficient for delivering the amino-acyl intermediate.

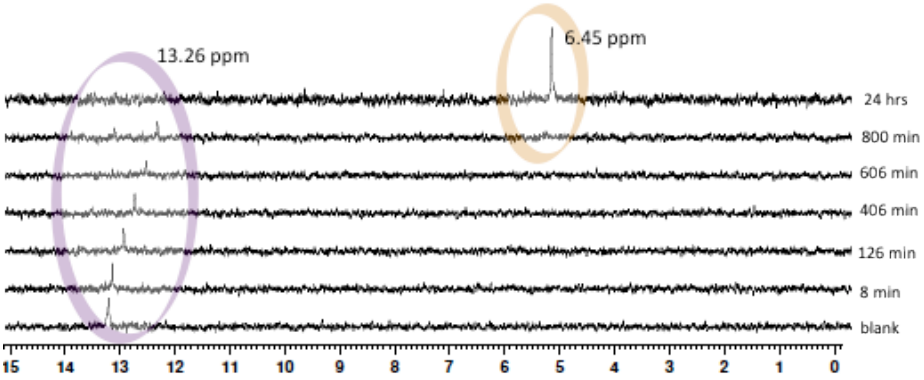


Figure 3.2. Carboxypeptidase Y catalysed ester hydrolysis of phosphorodiamidate **35.a** (purple: **35.a**, orange: hydrolysed specie **40**).

Results from the carboxypeptidase Y assay (0.1 mg) with the morpholine phosphorodiamidate **36** (5.0 mg in 0.200 mL deuterated water) in Trizma buffer (pH~7, 0.600 mL) are reported Figure 3.3. The peak of **36** remains stable overnight, suggesting no metabolism by carboxypeptidase Y to the key intermediate. This data correlates with the biological results observed in replicon for both **19.b** and **36** (Table 3.3) and indicates that at least one ester moiety is required for activation of phosphorodiamidates to the key intermediate, essential for delivery of the 5'-monophosphate.

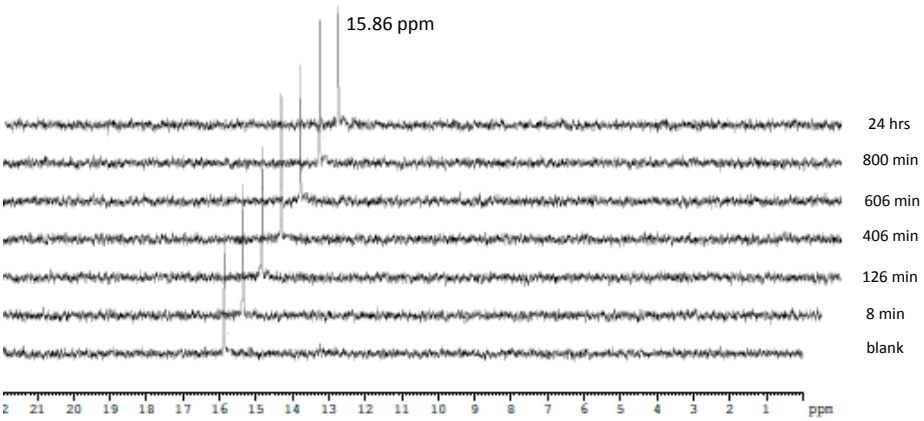
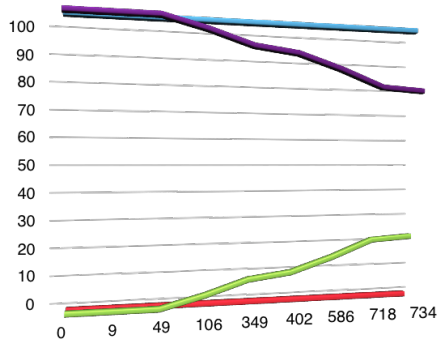


Figure 3.3. Carboxypeptidase Y catalysed hydrolysis of phosphorodiamidate **36**.

Similar assays were performed with phosphorodiamidates **24.c** and **25.c**, bearing *L*-Tyrosine as amino acid and benzyl as ester moiety, only differing by the presence of the *tert*-butyl group. **25.c** (3.1 mg) was dissolved in deuterated acetone (0.124 mL) and incubated with CPY (0.1 mg) in Trizma buffer (pH~7, 0.372 mL). Similarly, **24.c** (1.6 mg) was dissolved in deuterated acetone (0.064 mL) and incubated with CPY (0.1 mg) in Trizma buffer (pH~7, 0.192 mL). ^{31}P-NMR experiments were recorded every 7 minutes for 14 hours. The data are represented in Graph 3.5.



Graph 3.5. Graphic representation of carboxypeptidase Y assay experiments of **24.c** (blue) and **25.c** (purple) (green: amino acyl intermediate of **25.c**, red: amino acyl intermediate of **24.c**, x: time in min, y: % of molecule present during the experiment).

This graph indicates slow ester cleavage of **25.c** to its amino-acyl intermediate, whereas **24.c** is not processed. *L*-Tyrosine amino acid is probably too big to fit properly in the esterase enzymatic pocket, hence poor hydrolysis to its amino-acyl intermediate.

2.3.2. Cell lysate

A study of the second metabolic step was performed at 37 °C in deuterated water (0.200 mL) incubating Huh-7 cell lysate with **35.a** (1mg in 0.05 mL of DMSO) and was monitored by ^{31}P-NMR overnight (Figure 3.4). This experiment was not conclusive on the delivery of the 5'-monophosphate **41** (green), since the ^{31}P-NMR peak at 2.54 ppm corresponds to the cell lysate and not the desired metabolite, which ^{31}P-NMR peak should appear more upfield (~0.9 ppm).[18] However, if conversion to the 5'-monophosphate occurs, it is relatively slow since **35.a** remains after 24 hours.

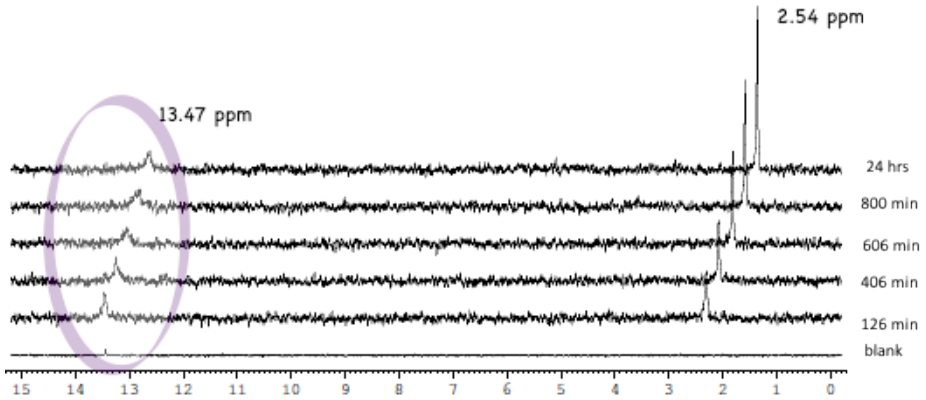


Figure 3.4. Huh-7 cell lysate assay of **35.a** (purple: **35.a**).

2.3.3. Molecular modelling

2.3.3.1. Docking with cathepsin A enzyme

Cathepsin A is an esterase-type enzyme sharing similar structural homology and substrate specificity with carboxypeptidase Y.[19, 20] The crystal structure of cathepsin, available in the protein data bank (PNB 1YSC),[21] was used for docking studies. Interactions between the carbonyl group of the ester moiety and Ser146, Gly53, and Gly52 are essential for ester catalytic cleavage.

In order to investigate the ester cleavage step, phosphoramidates **35.a**, **24.c** and **25.c** were docked into the catalytic site of cathepsin A. Figure 3.5 and Figure 3.6 report the molecular modelling predictions. Docking of **35.a** (Figure 3.5) suggests that one carbonyl group of the *S*-methyl-Cysteine neopentyl ester moiety is in the right position for ester cleavage (in comparison with the *L*-Alanine-neopentyl ester *β*-2'-*C*-methyl-6-*O*-methyl guanosine ProTide **15.d**). It correlates with the formation of the amino-acyl intermediate **40** observed in the carboxypeptidase Y experiment (Figure 3.2).

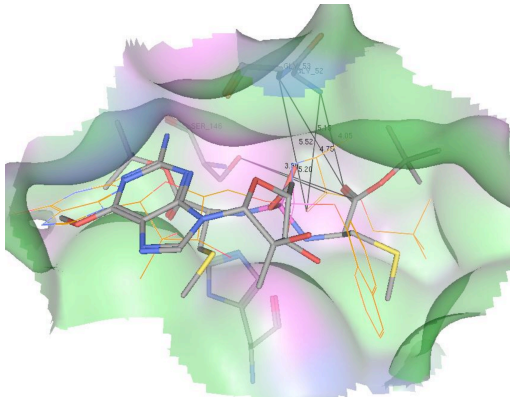


Figure 3.5. Docking of *S*-methyl-Cysteine neopentyl ester phosphorodiamidate **35.a** (grey) and **15.d** (orange line) in the catalytic site of cathepsin A enzyme (red: oxygen, blue: nitrogen, pink: phosphorus, yellow: sulfur).

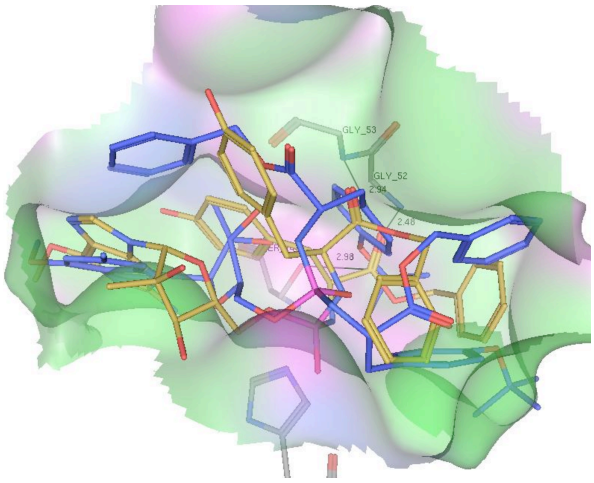


Figure 3.6. Docking of *L*-Tyrosine benzyl ester phosphoramidate **25.c** (yellow) and *L*-Tyrosine-O-*tert*-butyl benzyl ester phosphoramidate **24.c** (blue) in the catalytic site of cathepsin A enzyme (red: oxygen, blue: nitrogen, pink: phosphorus).

Docking of **24.c** and **25.c** predicts that the combination of the bulky *L*-Tyrosine amino acid together with the benzyl ester moiety does not accommodate easily in the catalytic pocket of cathepsin A. The *tert*-butyl group of **24.c** makes it even worse and provokes a clash with the surface of the pocket preventing good fit for catalytic mechanism; hence no delivery of the amino acyl intermediate is predicted and correlates with the observations of the carboxypetidase Y assay (Graph 3.5). Whereas **25.c** accommodates tightly in the pocket, enabling the ester carbonyl group to lie in proximity to the catalytic residues. This prediction may explain the extremely slow conversion to the amino-acyl intermediate (Graph 3.5) and the low antiviral activity exhibited in replicon-based assays.

2.3.3.2. Docking with Hint-1 enzyme

Human histidine triad nucleotide-binding proteins (Hint) are a superfamily of nucleotide transferases and hydrolases which are thought to be responsible for the P-N bond cleavage of phosphoramidate monoesters into nucleoside monophosphates.[22, 23] Adenosine monophosphate-bound amines are the natural substrates of Hint, and hydrolysis of the P-N bond occurs *via* interaction of the key residue Ser107, His112 and His114.[24] Interactions between the substrate and the histidines are key for catalytic hydrolysis (Figure 3.7).[25] One of the three histidines (blue) attacks the phosphorus center resulting in release of the amino acid moiety, which is subsequently protonated by the serine (green). Attack of a molecule of water triggers the hydrolysis to the 5'-monophosphate and release the histidine (blue).

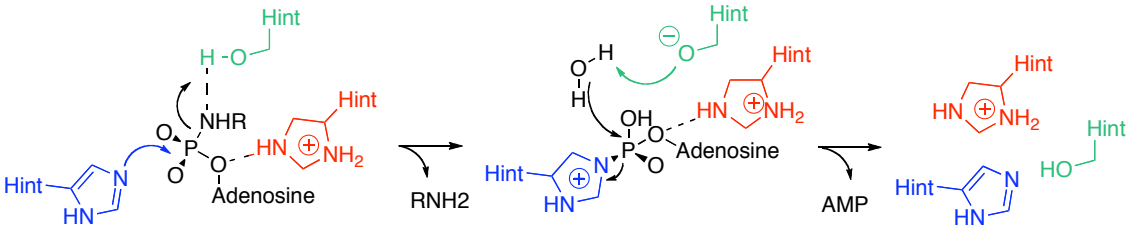


Figure 3.7. Mechanism of action of Hint enzyme.

Docking studies with the amino acyl intermediate **40** of phosphorodiamidate **35.a** were carried out with human Hint-1 enzyme (PNB 1KPF)[20] and the co-crystallised structure of adenosine monophosphate (AMP, orange line), in order to identify the active site of this enzyme (Figure 3.8).[23]

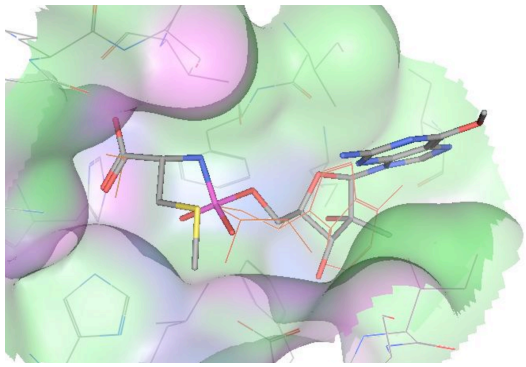


Figure 3.8. Docking of *S*-methyl-Cysteine amino acyl intermediate **40** within the catalytic pocket of human Hint-1 enzyme.

This docking predicts that the phosphate moiety lies far from the catalytic residues. In

comparison with AMP, the carbonyl group of the amino acyl intermediate **40** lies where the phosphorus moiety should be accommodated for efficient hydrolysis. This might be the reason of poor intracellular conversion of **35.a** to its 5'-monophosphate, as suggested by the cell lysate assay (Figure 3.4), and thus, might explain the biological activity reported against HCV (Table 3.3).

2.4. Summary and conclusions

The phosphorodiamidate technology applied to *β*-2'-*C*-methyl-6-*O*-methyl guanosine **11** was successful and resulted in *L*-Alanine neopentyl ester ProTide as a mixture of diastereoisomers (**15.d**). The diastereoisomers being hardly separable by flash chromatography, the mixture was advanced up to phase 2 clinical trials for hepatitis C treatment, at which stage severe cardiac and liver toxicity were found and halted its progression. As a back-up project, new achiral phosphate prodrugs were investigated for delivery of the same bioactive form, *β*-2'-*C*-methyl guanosine triphosphate, inside the cells. Novel phosphorodiamidates were designed and synthesised successfully and exhibited good inhibitory potency in HCV replicon assays. A large SAR was built and enzymatic assays showed that at least one amino acid ester moiety is essential for activity. Biological results showed that alkyl ester moieties and small amino acid moieties were preferred. Enzymatic assays and molecular docking experiments predict that long chains or bigger groups at the amino acid C_α may not only slow down or prevent hydrolysis to the amino acyl intermediate (CPY and cathepsin A), but also may lead to incomplete or relatively poor metabolism in Huh-7 cells (cell lysate and Hint-1). These data reflect the reported anti-HCV activity in replicon assays.

From this study, three of the most potent molecules in-*vitro* were selected and dosed to rats. High *β*-2'-*C*-methyl guanosine triphosphate levels were measured in-*vivo* and good pharmacokinetics profiles were obtained. In particular, the unexpected promising results of the *D*-Alanine neopentyl ester phosphorodiamidate **34.a**, which appeared as the best back-up molecule for **15.d**.

To conclude, phosphorodiamidate is a new and effective phosphate prodrug motif, which may enhance the properties and selectivity index of ProTide analogues.

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Chapter Four: Nucleobase modifications of *β*-2'-*C*-methyl-6-*O*-methyl guanosine

1. Background and Objectives

Numerous heterobase modifications of nucleoside analogues have resulted in potent antiviral agents, such as anti-HCMV acyclic uracil analogues[1] or the anti-HCV *N*-1-hydroxy-inosine (**42**, Figure 4.1)[2] and *β*-*D*-*N*-4-hydroxycytidine (**43**, Figure 4.1).[3] Within the *β*-2'-*C*-methyl ribonucleoside family, modifications are mainly reported at positions *C*-2,[4] *C*-6[4,5,6] and *C*-7 of the nucleobase.[7,8] While *C*-6 modifications increase significantly the lipophilicity and eventually result in retention or enhancement of potency against HCV,[6] the 7-deaza modified adenosine analogue (**44**, Figure 4.1) exhibits significant in-*vivo* potency against HCV.[9] Whereas the *C*-8 position has never been explored on the 2'-*C*-methyl series, it has been extensively studied on other nucleoside analogues[10] such as guanosine and its 2'-deoxy derivatives as anti-proliferative agents.[11]

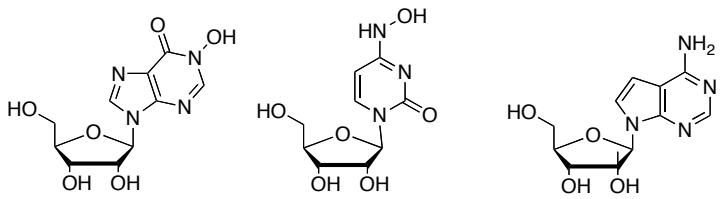

Figure 4.1. Structures of anti-HCV base modified purine and pyrimidine nucleosides.

Considering the antiviral success of these nucleobase modifications, *C*-8 and 7-deaza modifications were investigated on *β*-2'-*C*-methyl-6-*O*-methyl guanosine **11** in order to explore the impact of steric, electronic and hydrogen bonding changes on the anti-HCV activity.

2. 8-modifications of *β*-2'-*C*-methyl-6-*O*-methyl guanosine

2.1. Introduction

Since Holmes and *al*. reported the preparation of the active 8-bromoguanosine (**45**, Figure 4.2),[12] numerous *C*-8 modified purine nucleosides have been designed and synthesised as anticancer[13] and antiviral agents,[13-16] and have also been used for the study of DNA modifications caused by carcinogenic agents.[17-21] The introduction of *C*-8 substituents do not affect the base pairing with pyrimidines, thus the ability to form nucleic acid duplexes is retained. Pointing outside the DNA minor groove, 8-substituents only modulate the interaction within proteins.[22]

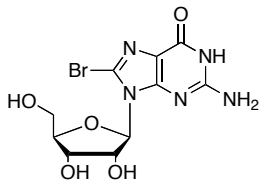

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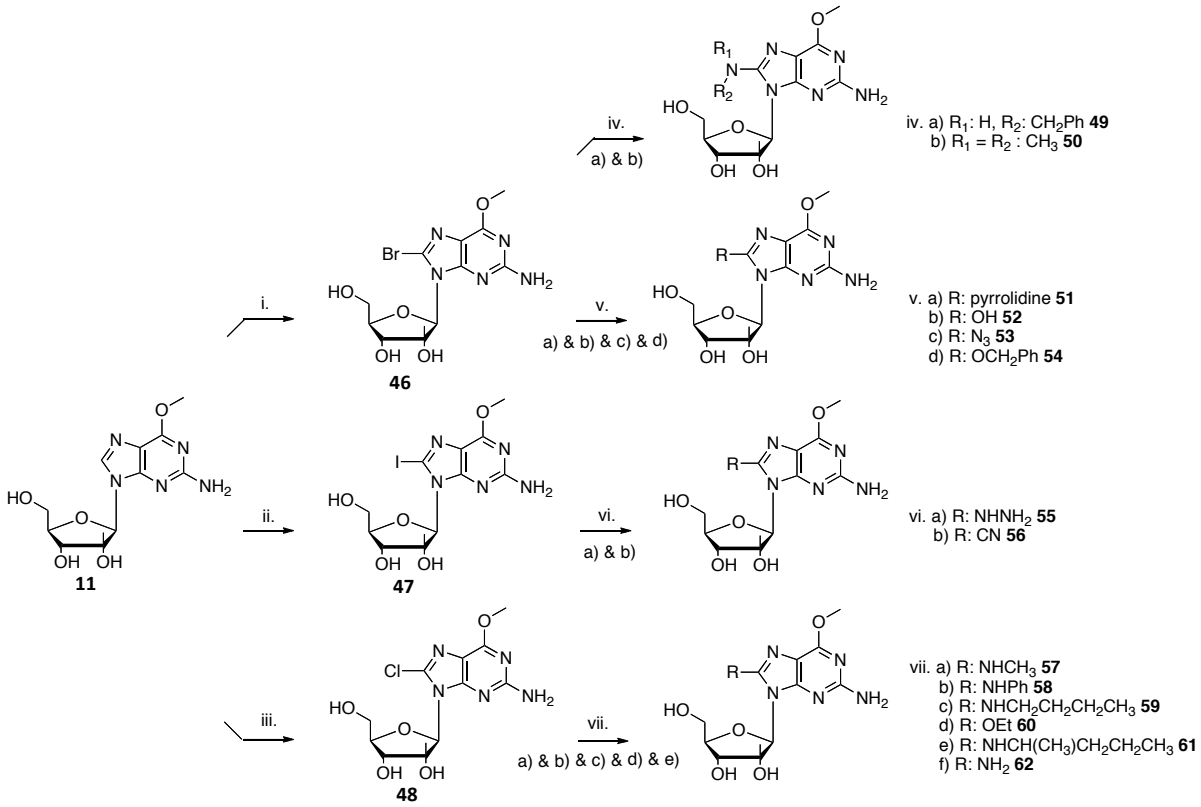
Figure 4.2. Structure of 8-bromoguanosine.

Modifications at the *C*-8 position of guanosine have been extensively investigated and reactions such as halogenation, oxidation and nucleophilic addition of various amines or aryl groups are largely reported in the literature. However, as mentioned previously, these modifications have never been explored in the HCV field and it was decided to address the effect of *C*-8 steric hindrance on HCV activity when applied to *β*-2'-*C*-methyl-6-*O*-methyl guanosine **11**.

2.2. Synthesis of 8-modified nucleosides

Modifications including amino, ether and cyano groups, were considered for substitution at the 8-position of the *β*-2'-*C*-methyl-6-*O*-methyl guanosine **11** (Scheme 4.1). These were obtained starting from the halogenated derivatives previously prepared by electrophilic addition[23] at *C*-8 of *β*-2'-*C*-methyl-6-*O*-methyl guanosine **11**, provided by Inhibitex *Inc*.



(i. anh. MeOH, NBS (1.2 eq), rt, 90 min, 96%; ii. anh. THF, NIS (2.6 eq), rt, 3 days, in the dark, 25%; iii. anh. THF, NCS (1.2 eq), rt, 3 days, in the dark, 66%; iv. a) anh. MeOH, $BnNH_2$ (2.5 eq), sealed vessel, 115 °C, 3 days, 6.6%; b) anh. DMF, $NH(CH_3)_2$ (1.2 eq), anh. NEt_3 (2.6 eq), 115 °C, 3 days, 51%; v. a) anh. DMF, pyrrolidine (1.2 eq), anh. NEt_3 (2.6 eq), 115 °C, 3 days, 9%; b) anh. pyridine, NaOAc (10 eq), NMI (0.38 eq), Ac_2O (10.6 eq), $(NH_4)HCO_3$, relux, overnight; c) DMSO, NaN_3 (3.0 eq), 75 °C, overnight; d) anh. BnOH, NaOBn (3.0 eq), 85 °C, 48 hrs; vi. a) hydrazine hydrate, 100 °C, 42 hrs; b) anh. DMF, tributyltin cyanide (1.1 eq), Pd Tetrakis (0.05 eq), 95 °C, 3 days; vii. a) anh. MeOH, anh. NHMe (1.3 eq), 115 °C, 18hrs, 37%; b) anh. MeOH, anh. aniline (2.0 eq), 115 °C, 3 days, 32%; c) anh. MeOH, N-butylamine (2.0 eq), sealed vessel, 115 °C, 3 days, 27%; d) anh. EtOH, NaOEt (3.0 eq), reflux, overnight; e) anh. MeOH, 2-aminopentane (2.0 eq), sealed vessel, 115 °C, 3 days; f) H_2O, hydrazine dihydrate, reflux, 2 hrs)

Scheme 4.1. Synthetic pathway to
8-modified *β*-2'-*C*-methyl-6-*O*-methyl guanosine nucleosides.

2.2.1. Preparation of *β*-2'-*C*-methyl-6-*O*-methyl-8-halogeno guanosine

Halogenation at *C*-8 postion of purine analogues is extensively reported in the literature.[14, 24, 25] The most common reagent for iodination, chlorination or bromination is the corresponding *N*-halogeno succinimide, known as a source of halogens in various electrophilic additions.[26] *N*-halogeno succinimide reagents were used either in methanol or tetrahydrofuran[27] for the preparation of molecules **46**, **47** and **48** (Scheme 4.1) in moderate to good yields (25 to 96%). The electron rich heterobase acts as nucleophile by total delocalization of the π-electrons, while the *N*-succinimide releases the corresponding electrophilic halogen at a low concentration in the media. Since *β*-2'-*C*-

methyl-6-*O*-methyl guanosine **11** is soluble in both methanol and tetrahydrofuran, protection of the hydroxyl groups on the sugar was not required.
Bromination at the 8-position was fast (one hour at room temperature) in comparison with the chlorination or iodination reactions. Moreover, the later was kept in the dark to avoid decomposition due to the presence of the photolabile iodine atom. All novel 8-halogenated nucleosides **46**, **47** and **48** were then subject to various aromatic nucleophilic substitutions.

2.2.2. Substitutions of *β*-2'-*C*-methyl-6-*O*-methyl-8-halogeno guanosine

The electronic effect of the halogen has an impact on the stability of the carbon-halogen bond, hence it is expected that 8-iodo derivative (**47**) would react quicker than the 8-bromo derivative (**46**), which should be more reactive than the 8-chloro derivative (**48**).
In addition, substitution of halogens with various groups at the *C*-8 of the heterobase is not usually regioselective and depending on the electron density of the ring the attack can be favoured in position *C*-6 of the purine ring.[28] However, in the case of **46**, **47** and **48**, the methoxy group at *C*-6 is much less reactive than the halogen at *C*-8 towards substitutions. Furthermore, even in case of 6,8-dihalogenopurine the nucleophilic displacement occurs primarily at *C*-8 even in the case of weakly basic nucleophilic reagent.[28] Hence, explaining the regioselectivity observed at *C*-8 with our nucleoside during the nucleophilic aromatic substitution.

2.2.2.1. 8-*N*-modified *β*-2'-*C*-methyl-6-*O*-methyl guanosine

Substitution of *C*-8 halogens was first performed with primary and secondary alkyl or aryl amines such as methylamine, dimethylamine, aniline, benzylamine, pyrrolidine, *N*-butylamine, 2-aminopentane or hydrazine hydrate; as already reported on 2'-deoxyguanosine, guanosine or adenosine.[29]
High temperature and pressure were necessary to achieve most of these aromatic aminations, while the same reaction at room temperature led to the recovery of the unreacted 8-halogeno *β*-2'-*C*-methyl-6-*O*-methyl guanosine (**46**-**48**).

2.2.2.1.1. *C*-8 substitution by primary amines

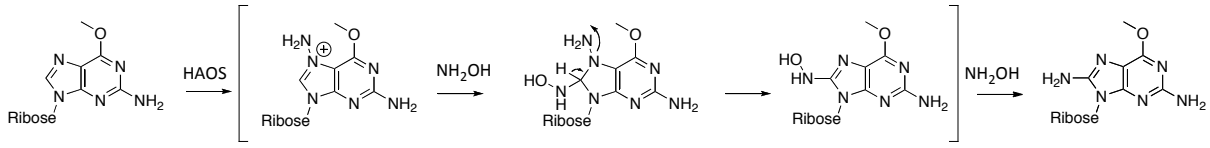
Substitution of chlorine (**48**) or bromine (**46**) in 8-position with primary amines was relatively easy, as previously reported in the literature in the case of several other purine analogues.[29] Nucleosides **49**, **57** and **58** were obtained in low to moderate yields (7 to 37%), only changing the equivalent of primary amine to reduce the eventual double substitution (*C*-8 and *C*-6). However, even addition of 2.0 equivalents of primary amine did not substitute the 6-*O*-methyl group. The use of anhydrous solvent with liquefied amines was required to avoid side reactions and degradation.[30]

Aromatic groups being synthetically tolerated at *C*-8, substitutions of longer alkyl amine and branched alkyl amine were then investigated. Reaction of **48** with *N*-butylamine afforded **59** in 27% yield, following the experimental conditions previously mentioned. In contrast, substitution with 2-aminopentane did not occur. More reactive 8-bromo analogue **46,** was subject to similar conditions. However, introducing triethylamine or switching from polar protic solvent (methanol) to polar aprotic solvent (dimethyl formamide and tetrahydrofuran) did not allow the formation of the desired molecule **61**, but only degradation and recovery of unreacted starting material. This behaviour would suggest that branched alkyl amines are not tolerated at position 8 of the nucleobase in this reaction. This result is contradictory to the successful substitution of more hindered phenylamine (**58**) and benzyl amine (**49**).

Following the conditions reported in the literature for the synthesis of 8-hydrazino guanosine,[31] reaction between compound **47** and hydrazine hydrate led to total degradation. The synthetic conditions reported[31] to form 8-hydrazino guanosine uses hydrazine 60% instead of hydrazine 25%, at high temperature (90-100 °C). When the procedure described by Holmes & *al.*[32] was followed, only the parent nucleoside **11** was recovered. Several papers report degradation and also cleavage of the glycosidic bond when using aqueous hydrazine.[30, 32] Unfortunately, the degradation products were not successfully isolated and characterised.

Another route for the synthesis of **62** was explored by treating *β*-2'-*C*-methyl-6-*O*-methyl guanosine (**11**) with hydroxylamine-O-sulfonic acid (HAOS) in water and maintaining acidic conditions.[33, 34] The mechanism (Scheme 4.2) of this reaction is described to go through the formation of the 7-amino derivative as a result of an electrophilic addition at the nucleophilic position *N*-7 by HAOS. This 7-amino

intermediate is then converted to 7,8-dihydro-7-amino-8-hydroxyamino intermediate by nucleophilic attack of hydroxylamine, which then undergoes aromatisation to 8-hydroxyamino derivative, which is eventually reduced to its 8-amino derivative.[34, 35] Unfortunately, the reaction did not allow the formation of **62** in our case.



Scheme 4.2. Mechanism of formation of 8-NH_2 derivatives using HAOS.

2.2.2.1.2. *C*-8 substitution by secondary amines

Substitutions by secondary amines (dimethylamine and pyrrolidine) proved to be more difficult than with the primary amines. The first attempt of substitution of **48** with dimethylamine was performed under similar conditions used in the case of methylamine. Too little crude material was recovered that no pure desired material could be isolated in significant quantity to be able to carry out a complete NMR characterisation. No improvement was observed when starting from the 8-bromo derivative **46**. Similar conditions were applied to the 8-iodo derivative **47**, but the reaction did not take place and only unreacted **47** was recovered. More recent literature describing substitution of proline on protected 8-bromoguanosine suggests the use of triethylamine in the mixture.[36] Acting as a base, triethylamine enhances the nucleophilicity of the secondary amine by partially removing the proton, enabling better reactivity towards substitution. As last attempt, **46** in anhydrous dimethylformamide was treated with 2.6 equivalents of triethylamine followed by 1.2 equivalents of dimethylamine. Under these conditions, nucleosides **50** and **51** were obtained in 51% and 9% yields respectively, along with unreacted **46**.

2.2.2.2. 8-*O*-modified *β*-2'-methyl-6-*O*-methyl guanosine

Previous work in our group enabled the formation of 8-*O*-methyl modified *β*-2'-methyl-6-*O*-methyl guanosine. Further work was undertaken to synthesise other 8-*O*-alkyl and 8-*O*-aryl modified nucleosides as well as 8-oxo derivatives.

The same experimental conditions reported in the literature for the synthesis of 8-*O*-ethyl derivatives,[32] were applied to **48** with an unsuccessful outcome. Neither increasing the number of equivalents of sodium ethoxide, nor changes on reaction or temperature enabled formation of desired molecule **60**. Similarly to the formation of 8-benzyloxyguanosine,[11, 12] **46** was treated with commercially available sodium benzyloxide in dimethyl sufloxide. Unfortunately only unreacted starting material **46** was recovered. Another attempt was carried out similarly to the introduction of ether modifications at the 6-position,[32] ie. using benzyl alcohol (BnOH) as solvent instead of dimethyl sulfoxide (DMSO), with excess of sodium benzyloxide (NaOBn, 3.0 equivalents). Neither the desired material **60** nor the 6-substitued derivative were formed, and degradation products were recovered.
In contrast with guanosine but similarly to adenosine, the basic conditions applied to 8-bromo derivative **46**, ie. sodium ethoxide or sodium benzyloxide, should not affect the nucleophilic attack at the 8-position since *N*-1 does not bear any acidic proton, which could form an anion and would then resist to the introduction of another negatively charged nucleophile.[12, 28] Hence, in our case the experimental conditions to form 8-O-alkyl modified nucleosides would have needed further optimisation.

8-oxo modification on *β*-2'-*C*-methyl-6-*O*-methyl guanosine **11** was also of interest as a biological marker. In the early 60s, 8-hydroxynucleosides were synthesised not only as intermediates for the synthesis of cyclonucleosides[37] but also for their biological interest.[38] Numerous publications report the work on 8-oxo-guanosine, and activation of humoral immune response was detected.[10, 39-41] The 8-oxo is one of the only 8-modifications on nucleoside to be enzymatically tolerated by adenosine deaminase (ADA).[37]

The first route towards the synthesis of 2'-*C*-methyl-8-oxo guanosine **63**, was to react **46** with sodium acetate (NaOAc) and glacial acetic acid under reflux,[42] but only degradation was observed. Recent literature published formation of 2'-deoxy-8-oxo guanosine *via* Fenton reaction, a radical reaction using hydrogen peroxide in water.[42] The conditions of Fenton carbonylation were applied to *β*-2'-*C*-methyl guanosine **10** without any success. Another attempt consisted of treatment of **46** with sodium acetate, silver acetate and 1-methylimidazole (NMI) in acetic anhydride.[42] The residue was purified on Sephadex G-10,[43] and two fractions were recovered. The NMR spectra were

analysed to confirm the formation of corresponding to *β*-2'-*C*-methyl-8-oxo guanosine **63** and the *β*-2'-*C*-methyl-6-*O*-methyl-8-oxo guanosine protected by the acetate group on the exocyclic amine **64** (Figure 4.3). They were obtained respectively in 2% and 5% yield. Pendant ^{13}C-NMR in deuterated dimethylsulfoxide confirmed the presence of quaternary carbons, appearing at 155.3 ppm (*C*-6), 149.6 ppm (*C*-8) for **63**, and at 166.6 ppm ($NH\underline{C}OCH_3$), 158.4 ppm (*C*-6), 149.9 ppm (*C*-8) for **64**. The latter structure was also confirmed by ^{1}H-NMR. The spectra revealed a broad peak corresponding to a proton next to *N*-7 appearing at 11.43 ppm, which type of proton was not seen for **63**. Hence, the most stable form probably depends on the substituent at the 6-position of the nucleobase.

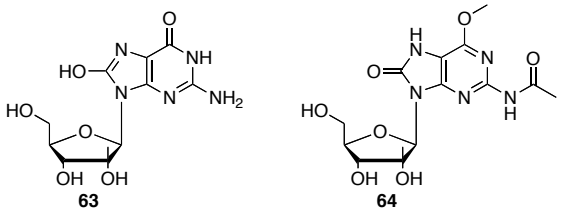


Figure 4.3. Structures of 8-oxo derivatives **63** and **64**.

More recent literature describes the oxidation of guanidinium iodide by hydrogen peroxide.[44] The preparation of such halide should also have been an easy way of oxidation at *C*-8.

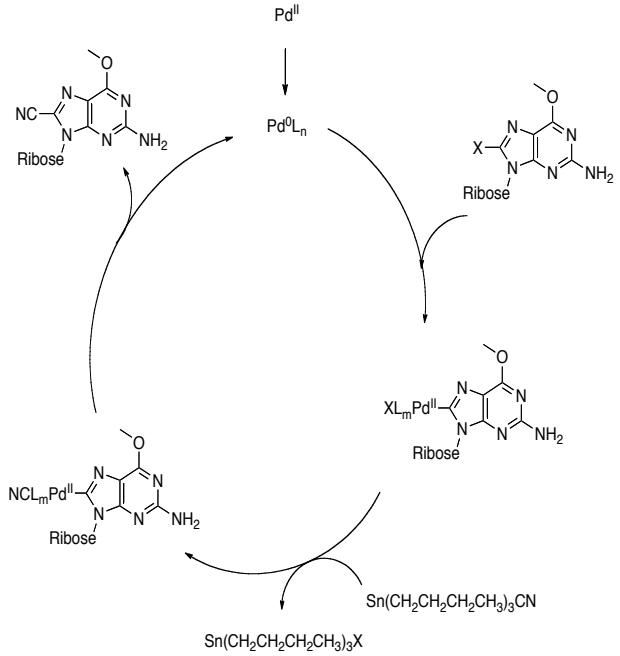
2.2.2.3. Others 8-modifications of *β*-2'-*C*-methyl-6-*O*-methyl guanosine

In addition to the 8-amino family, other substituents were considered such as 8-azido and 8-cyano however none of the reaction performed was successful.

Substitution of the azido group at position 8 of the nucleobase was performed under reflux in dimethylsulfoxide using sodium azide (NaN_3) as nucleophilic reagent.[12] Being light sensitive, the azido group tends to be very unstable. In fact only the starting material **46** was recovered from the reaction together with degradation products.

Based on palladium-catalysed chemistry, attempts for substitution of the cyano group at *C*-8 to form compound **56** from the 8-iodo derivative **47**, failed. The mechanism of Stille coupling is described in Scheme 4.3 and is divided in four steps: first the reduction of the palladium (II) to the active specie, palladium (0), then oxidative addition of the palladium with the halide, followed by transmetallation with the

tributyltin cyanide to afford an intermediate, which collapses to the desired molecule after reductive elimination of the palladium (II) to palladium (0).



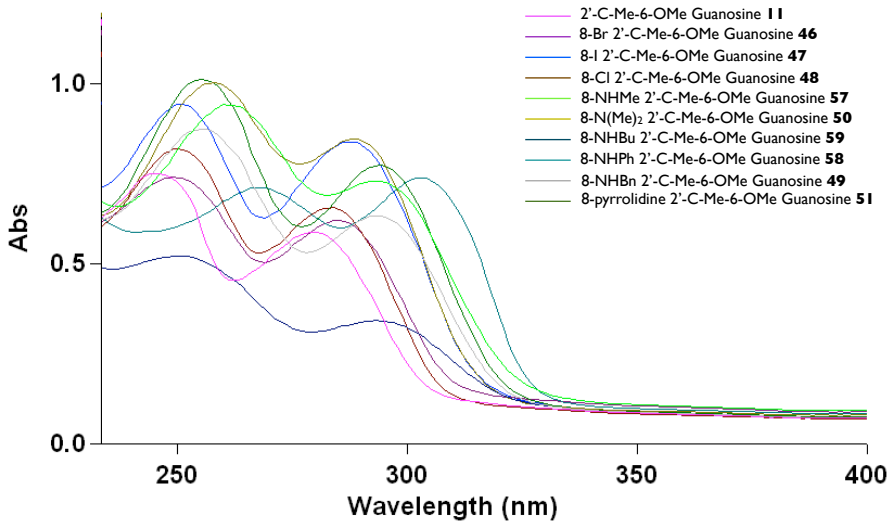
Scheme 4.3. Mechanism of Stille coupling.

A first reaction was carried out with palladium-Tetrakis, as described by Shen and co-workers,[45] but only *β*-2'-*C*-methyl-6-*O*-methyl guanosine **11** was recovered. The importance of the palladium catalyst and its ligands in such coupling is well known[46] and the key step in the mechanism is the oxidative addition, which occurs faster if the palladium (0) complex is electron-rich, hence the importance of strong donating ligands. Taking into consideration this view, sterically hindered hydrophilic and electron rich TPPTS (tris(3-sulfonatophenyl)phosphine)[47] was used as a ligand with palladium acetate in order to form a strong palladium catalyst.[17] This palladium-ligand complex is mainly used in aqueous phase for cross-coupling reaction.[17] However, Wolf and co-workers showed that such catalyst system could be used for Stille coupling in aqueous phase.[48] Once again the starting material was degraded to *β*-2'-*C*-methyl-6-*O*-methyl guanosine **11** and no further attempts were performed; however further investigations on the type of palladium catalyst to use for this specific reaction would be necessary.

2.2.3. Conclusion and characterisation of 8-modified nucleosides

High temperature and pressure were needed for aromatic substitutions of 8-halogeno nucleosides, and the reactions gave low to moderate yields (7 to 51%). Introduction of triethylamine seemed essential for substitutions of secondary amines. Substitutions of halogens by electron-withdrawing O-alkyl/aryl or cyano groups were unsuccessful, as well as electron-donating azido group. However, further investigation of palladium catalyst or other routes[49] for the synthesis of 8-modified nucleosides maybe interesting. Eleven 8-modified nucleosides were successfully synthesised, purified, characterised and were sent for biological evaluation in HCV replicon assays.

8-amino modified nucleosides (**46-51**, **54**, **57-59**) together with *β*-2'-*C*-methyl-6-*O*-methyl guanosine **11**, were dissolved in phosphate buffer (0.05M, pH~7.4) to reach a concentration of approximatively 50 µM, and characterised by ultra-violet spectroscopy (Graph 4.1).



Graph 4.1. UV characterisation of 8-modified nucleosides.

Aromatic groups at *C*-8 (**58** and **49**) tend to have bathochromic properties (increase λ_{max}), while 8-alkyl amino modified nucleosides (**57** and **59**) tend to lower the wavelenghth to reach a λ_{max} comparable to 8-halogeno nucleosides (**46-48**). Secondary amino groups at *C*-8 (**50** and **51**) blue-shift the λ_{max} (hypsochromic effect).

From this graph, the extinction coefficient (ε) of each nucleoside was extrapolated applying the Beer-Lambert law (Table 4.1) with the length of the cuve being 1 cm (l = 1 cm).

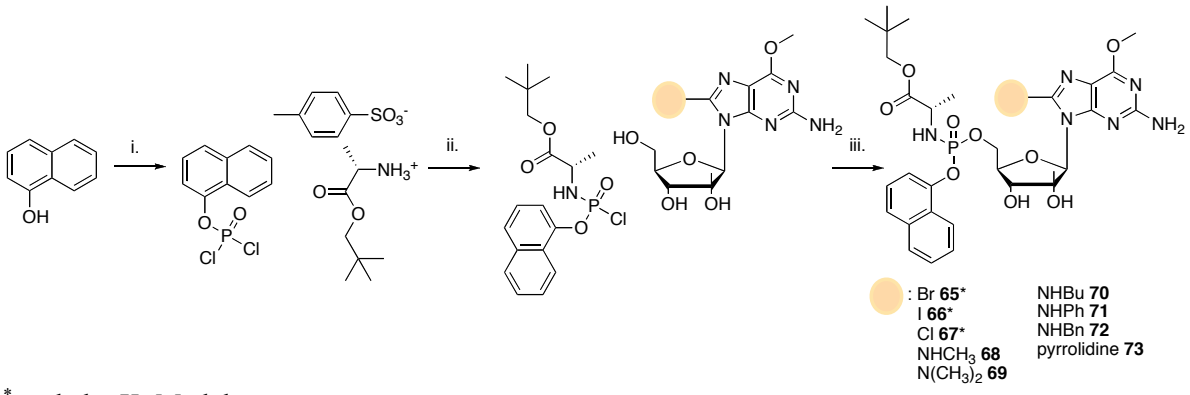
Cpd	8-R	λ_{max} (nm)	A_{max}	ε ($L.mol^{-1}.cm^{-1}$)
11	-	245	0.75	15000
46	Br	249	0.74	14800
47	I	251	0.94	18800
48	Cl	249	0.82	16400
57	NHMe	261	0.93	18680
50	$N(Me)_2$	257	0.99	19960
59	NHBu	250	0.52	10400
58	NHPh	302	0.74	14800
49	NHBn	255	0.87	17400
51	Pyrrolidine	255	1.01	20200

Table 4.1. Maximum length-wave (λ_{max}), maximal absorbance (A_{max}) and calculated extinction coefficient (ε) of 8-modified nucleosides.

Secondary amino groups at *C*-8 (**50** and **51**) have the highest extinction coefficient (hyperchromic effect), thus are more efficient in absorbing UV light compared to 8-modified nucleosides bearing primary amines. The 8-bromo modification (**46**) does not have important impact effect on light absorption compared to the parent nucleoside **11**, whereas both chlorine (**48**) and iodine (**47**) atoms exhibit higher extinction coefficients. It is also notable that **59**, bearing 8-butyl amine, absorbs the least UV-light.

2.3. ProTide technology applied to 8-modified nucleosides

Except for 8-oxo derivatives (**63** and **64**), *α*-naphthyl *L*-alanine neopentyl phosphoramidates of 8-modified nucleosides were synthesised (Scheme 4.4) according to the Uchiyama procedure previously mentioned.



*made by K. Madela.

(i. anh. Et_2O, anh. NEt_3 (1.0 eq), 30 min, -78 °C to rt, 51%; ii. anh. DCM, anh. NEt_3 (2.0 eq), 1 hr, -78 °C to rt, 78%; iii. anh. THF, tBuMgCl (2.0 eq), rt, overnight.)

Scheme 4.4. Synthetic scheme to 8-modified ProTides.

The reactions were relatively low yielding (2% to 4%) probably due to the steric hindrance resulting from the 8-modifications. Interestingly, 8-modified ProTides revealed a clear separation of the two diastereoisomers by NMR (0.20 ppm separation in ^{31}P NMR), however flash chromatography was unsuccessful at separating them and after purity check, they were sent as a mixture of two diastereoisomers for biological evaluation against HCV replication.

2.4. Biological evaluation

The evaluation of 8-modified *β*-2'-*C*-methyl-6-*O*-methyl guanosine nucleosides and their corresponding phosphoramidates in replicon assays using Huh-7 cells was performed at Inhibitex *Inc.*, and the potency and toxicity are reported in Table 4.2.

	nucleosides				*α*-naphthyl *L*-Ala neopentyl Protide			
8-position	Cpd	ClogP	EC_{50} (μM)	CC_{50} (μM)	Cpd	ClogP	EC_{50} (μM)	CC_{50} (μM)
	11	-0.66	5.2	>100	**15.d**	3.30	0.01	7
8-Cl	**48**	0.05	>100	>100	**67**	4.00	>40	60
8-Br	**46**	0.20	>100	>100	**65**	4.15	15	60
8-I	**47**	0.16	6	>100	**66**	5.11	0.02	14
8-NHMe	**57**	0.15	>100	>100	**68**	4.19	9.4	>100
8-N(Me)$_2$	**50**	0.24	96	>100	**69**	4.20	>40	79
8-NHBu	**59**	1.74	>100	>100	**70**	5.69	16	33
8-NHPh	**58**	2.13	42	>100	**71**	6.09	11	16
8-NHBn	**49**	1.60	>100	>100	**72**	5.55	4.6	23
8-Pyrrolidine	**51**	0.35	65	>100	**73**	4.31	23	58

	Cpd	ClogP	EC_{50} (μM)	CC_{50} (μM)
8-oxo-2'-*C*-MeG	**63**	-0.47	>100	>100
8-oxo-6-*O*-Me-*N*2-Ac-2'-*C*-MeG	**64**	-2.03	41	>100

ClogP from CS ChemDraw Ultra 11.0 software.

Table 4.2. Activity (EC_{50}) and cytotoxicity (CC_{50}) in HCV replicon assays of 8-modified nucleosides and *α*-naphthyl *L*-Alanine neopentyl phosphoramidates.

Only the 8-iodo nucleoside **47** exhibits micromolar potency activity (EC_{50} = 6 μM). Other 8-modified nucleosides are barely inactive against HCV replication and no cellular toxicity is detected up to 100 μM.

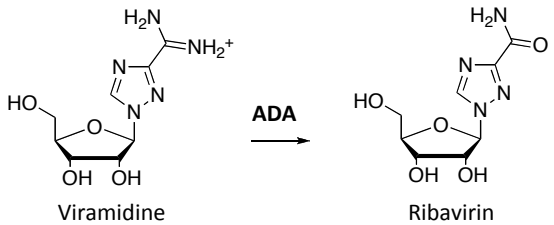
8-modified ProTides improve anti-HCV potency compared to their parent nucleosides but are still poor inhibitors. This boost of activity is probably related to greater lipophilicity, with ClogP in a range between 4 and 6, optimising the passive cellular uptake. The 8-iodo Protide (**66**) appears to be 300 fold more active against HCV than its parent nucleoside (**47**), while others improve the potency by only 7-fold. Nevertheless, further metabolism studies done at Inhibitex *Inc.* suggested quick de-iodination inside the cells, resulting in 2'-*C*-methyl guanosine analogues. None of the 8-oxo derivatives displays activity in HCV replicon assays.

2.5. Enzymatic hydrolysis study

The general lack of activity of the 8-modified nucleosides, reported by in-*vitro* evaluation, and the recent discovery of rapid cleavage of the 8-iodo guanosine derivatives (**47** and **66**) encouraged us to further investigate the enzymatic stability of 8-modified nucleosides.

2.5.1. Hydrolysis study at the nucleoside level

Adenosine deaminase (ADA) is a key enzyme found widely in cells and in human spleen, involved in purine metabolism, playing a critical role in immune competence and acting in the metabolism of nucleic acid components,[50] particularly the irreversible conversion of adenosine into inosine and ammonia.[51] While viramidine is enzymatically deaminated into ribavirin by adenosine deaminase (Scheme 4.5),[52] 8-modified adenosines bearing a group bigger than a fluorine atom are not reported as substrates for ADA.[50]

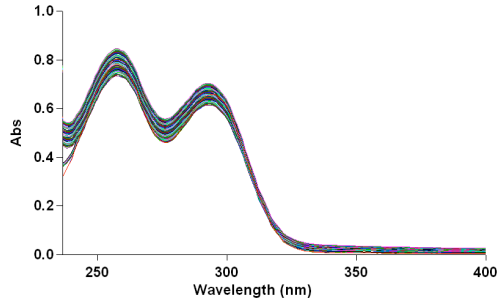


Viramidine Ribavirin

Scheme 4.5. Deamination of viramidine to ribavirin by ADA.[52]

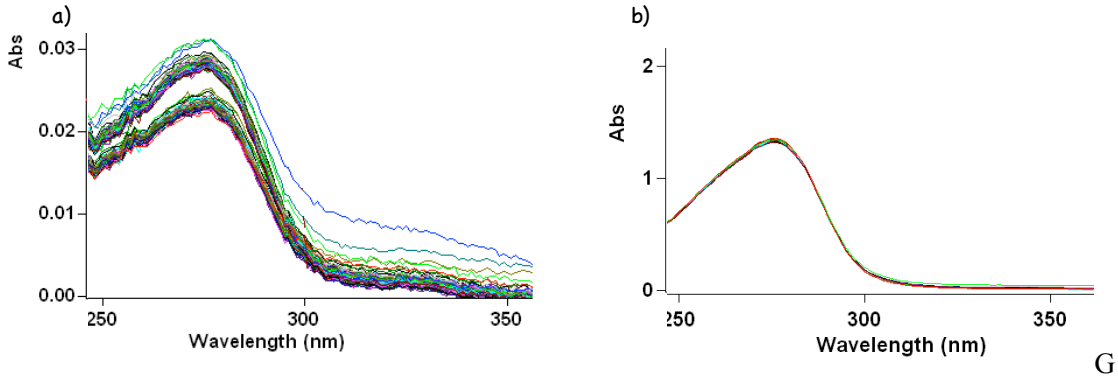
To prove the enzymatic stability of 8-amino modified nucleosides towards ADA, an enzymatic experiment[50] was performed with nucleoside **57** (8-methylamine) bearing the smallest substituent at *C*-8. The metabolism was monitored by UV spectrometry and the

UV spectra were recorded every minute for 30 min and then every 20 min for 20 hours (Graph 4.2). Despite addition of 100 µL of ADA after 3 hours 45 minutes, **57** remained stable to ADA. Therefore, as predicted by the literature, none of 8-modified analogues synthesised should undergo hydrolysis of the *C*-6 position by liver enzymes at a nucleoside level, especially bulky groups.[53] This result correlates with in-*vitro* assays since none of them are potent against HCV replication.



Graph 4.2. Study of enzymatic stability of **57** (0.05 µM in phosphate buffer pH-7) in the presence of ADA (50 µL).

Only nucleosides bearing small substituents at *C*-8 are reported to be substrate of ADA,[50] hence the 8-oxo nucleoside **64** may be enzymatically converted into the *N*-2-acetylated derivative of **63**. However, the stability study proved that **64** is not substrate of ADA (Graph 4.3), therefore the *N*-2 acetyl group may prevent **64** to fit in the catalytic pocket of ADA.



Graph 4.3. Stability study of **64** (a) and **63** (b) (0.05 µM in phosphate buffer pH-7) in the presence of ADA (20 µL).

2.5.2. Hydrolysis study at the 5'-monophosphate level

As for many 6-*O*-subtituted prodrugs in clinical development,[6, 54, 55] metabolism to the bioactive form requires hydrolysis of the 6-*O*-substituent of the purine base. Adenosine

deaminase-like protein-1 (ADAL-1) has recently been reported as the enzyme responsible for effective metabolism of 6-*O*/*N* substituted purine, and acts specifically at the 5'-monophosphate level.[56]

Docking of 5'-monophosphate of **57** (8-methylamine 2'-*C*-methyl-6-*O*-methyl guanosine) was performed using an homology model of ADAL-1 with its natural substrate the 5'-monophosphate of adenosine (AMP).[57] The prediction is represented in Figure 4.4.

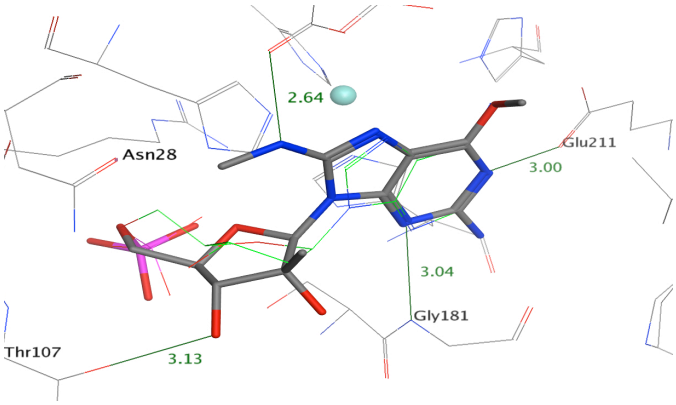


Figure 4.4. 5'-monophosphate of **57** docked in the catalytic site of ADAL-1.
(grey stick: 5'-monophosphate of **57**, light green line: AMP)

The docking suggests that the 5'-monophosphate of **57** would fit in the catalytic pocket of ADAL-1. However the 3'-hydroxyl group of AMP usually interacts with the Asparagine 28 (Asn28), and in our case it is the Threonine 107 (Thr107) involved in the hydrogen bonding interaction. This observation results from the position of the sugar moiety, which does not lie in optimal position in the pocket (in comparison with AMP). Thus, the hydrolysis of the 6-position of the nucleobase might happen, and the 5'-monophosphate species of 8-methylamine-2'-*C*-methyl guanosine might be slowly deliver intracellularly. Therefore, if 8-modified 2'-*C*-methyl-6-*O*-methylguanosine phosphoramidates are efficiently converted to their corresponding 5'-monophosphate species, hydrolysis of the 6-*O*-methyl might take place.

2.6. Mechanistic studies

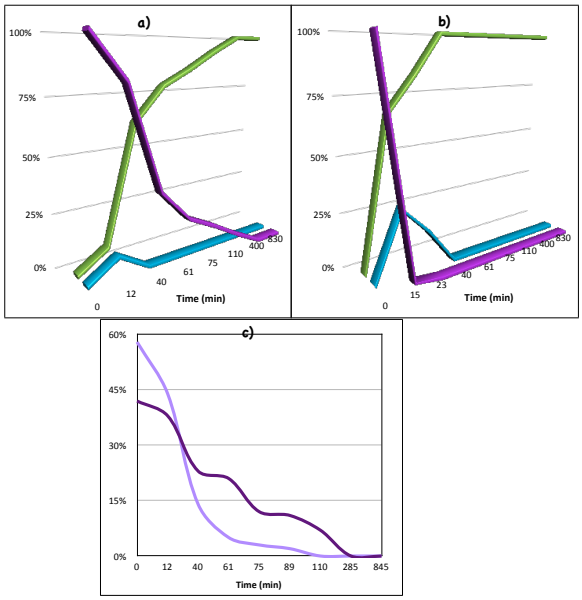
In-*vitro* evaluation reported the 8-modified *β*-2'-*C*-methyl-6-*O*-methyl guanosine phosphoramidates as poor inhibitors of HCV replication. Therefore, enzymatic assays were carried out with the intention of studying their putative mechanism of activation, already described Chapter 3. Further modelling studies were performed not only to

support the carboxypeptidase Y assays but also to study the last step of activation to the 5'-monophosphate.

2.6.1. Carboxypeptidase Y assays

Carboxypeptidase Y (CPY) is an esterase type enzyme known to be responsible for the initial ester cleavage of ProTide. As previously reported in our group,[58] the CPY-mediated ester cleavage of 8-amino modified ProTides can be monitored using ^{31}P-NMR.

Hydrolysis of *α*-naphthyl *L*-Alanine-neopentyl ester ProTides of 8-methylamine *β*-2'-*C*-methyl-6-*O*-methyl guanosine (**68**) and 8-butylamine *β*-2'-*C*-methyl-6-*O*-methyl guanosine (**70**) are represented in Graph 4.4 (a and b). ProTide **70** was more unstable to CPY than **68**, hence converted faster to its phosphoramidate monoester. In both case, the ester-hydrolysed species were visible by ^{31}P-NMR. The experiment also suggested that the conversion rates of the two diastereoisomers differ slightly (Graph 4.4 c).



Graph 4.4. Time course of disappearance of prodrug and appearance of metabolites in CPY catalyzed hydrolysis of **68** (a) and **70** (b) (purple: ProTide, blue: ester hydrolysed species, green: phosphoramidate monoester).
Time course of conversion of the two diastereoisomers of **68** (c) (light purple: diastereoisomer at ^{31}P of 4.19 ppm, dark purple: diastereoisomer at ^{31}P of 3.69 ppm).
The percentage of prodrug remaining and metabolites detected at given time is plotted against time.

All but phosphoramidate **69**, bearing 8-dimethylamine at *C*-8, were easily converted to their amino-acyl intermediate, while **69** remains stable to CPY (Figure 4.5). This result correlates with in-*vitro* data, and is even more surprising since **73**, bearing a secondary cyclic amine at *C*-8 (8-pyrrolidine), is hydrolysed under similar conditions (Figure 4.6).

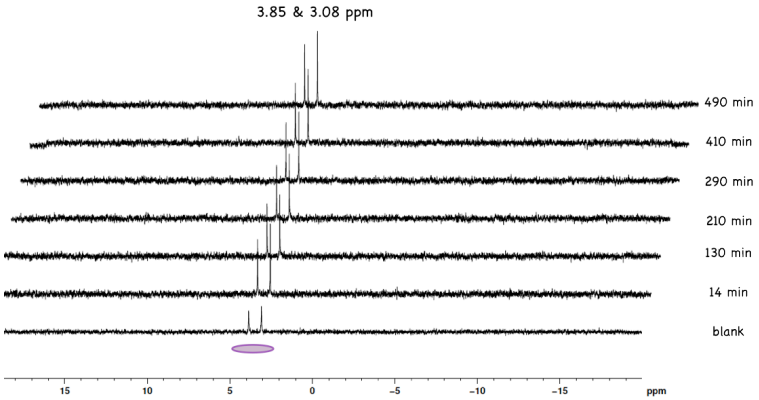


Figure 4.5. CPY catalysed ester hydrolysis of **69** monitored by ^{31}P NMR (pink: ProTide peaks).

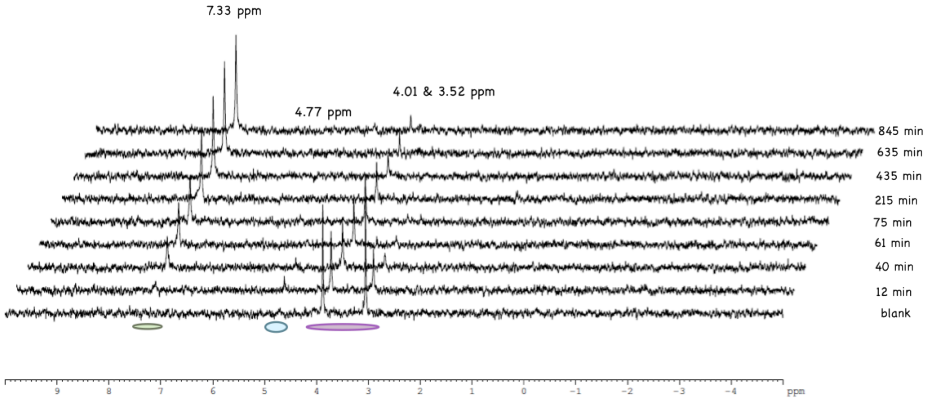
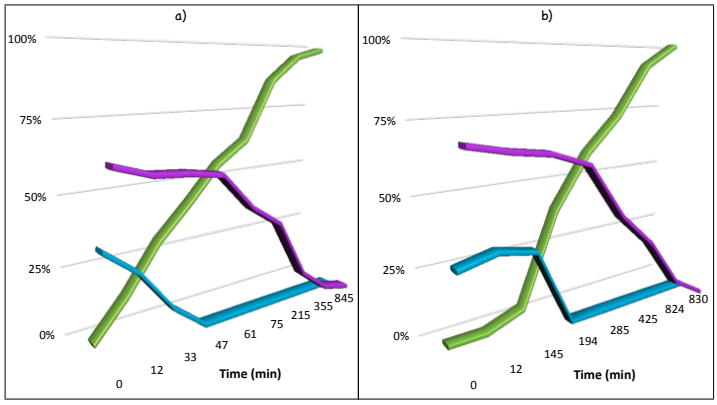


Figure 4.6. CPY catalysed ester hydrolysis of **73** monitored by ^{31}P NMR (pink: ProTide peaks, blue: ester hydrolysed species, green: phosphoramidate monoester).

Aromatic substitutions at *C*-8 (**71** and **72**) appear to slow down the enzymatic cleavage of the ester (Graph 4.5). Formation of the ester hydrolysed species was not detectable during these assays, suggesting quick conversion into the phosphoramidate monoester. However, the introduction of a methylene group between the amine and the phenyl ring (**72**, 8-NHBn) tends to slow down the conversion of the ProTide by about four fold compared to **71** (8-NHPh).



Graph 4.5. Time course of disappearance of prodrug and appearance of metabolites in CPY catalysed hydrolysis of **71** (a) and **72** (b)
(pink: diastereoisomer 1, blue: diastereoisomer 2, green: phosphoramidate monoester).

2.6.2. Molecular modelling

Molecular modelling was performed to gain deeper understanding of the ester hydrolysis step, and to predict the conversion of phosphoramidate monoesters by human Hint-1 enzyme into their 5'-monophosphate.

2.6.2.1. Docking with cathepsin A enzyme

To investigate the unexpected stability of **69** (*β*-2'-*C*-methyl-6-*O*-methyl-8-dimethylamine guanosine *L*-Alanine-neopentyl ester ProTide) towards CPY, molecular modelling was carried out, docking both **68** (bearing 8-methylamino group) and **69** in the catalytic pocket of cathepsin A (Figure 4.7). While the carbonyl group of **68** lies close to the catalytic residues (Ser146, Gly53 and Gly52), an extra methyl group seem to affect significantly the fitting of **69** in cathepsin, placing the ester carbonyl group outside the catalytic pocket. Thus, predicting no conversion into its phosphoramidate monoester essential for delivery of the 5'-monophosphate species.

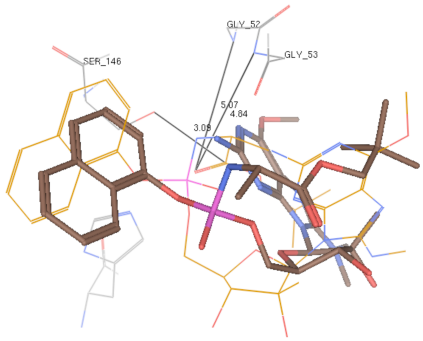


Figure 4.7. Docking of S_P isomers of **68** (orange line) and **69** (brown stick) in cathepsin A (grey sticks: catalytic residues).

In contrast but in correlation with the CPY assays, a secondary cyclic amine at *C*-8 (**73**) enables better fitting in the enzyme placing the carbonyl group of both isomers far from the catalytic residues, but still inside the pocket (Figure 4.8). This might explain the slow conversion rate into its corresponding phosphoramidate monoester species, with a slight preference for the S_P isomer.

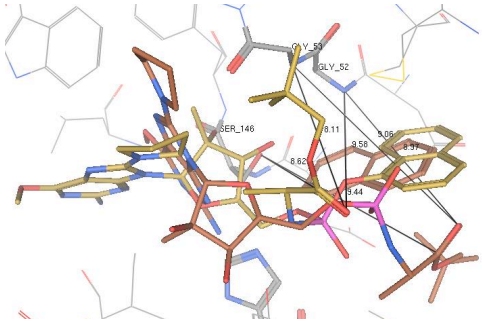


Figure 4.8. Docking of S_P (yellow stick) and R_P (brown stick) isomers of **73** in cathepsin (grey lines: catalytic residues).

Longer alkyl chains at *C*-8 (**70**) allow a good fit into the catalytic pocket of cathepsin A, especially for the S_P diastereoisomer, for which the distance to the catalytic residue is similar to the S_P isomer of **68** (Figure 4.9). The proximity of the Gly52 (4.54 Å) and Gly53 (3.34 Å) seems to have an influence on the rapidity of the processing of phosphoramidate diester to phosphoramidate monoester. The more the intermediate formed is stablilized by the glycine residues, the quicker is the phosphoramidate monoester released. Thus, this might explain the different conversion rate of the two diastereoisomers observed during CPY assays.

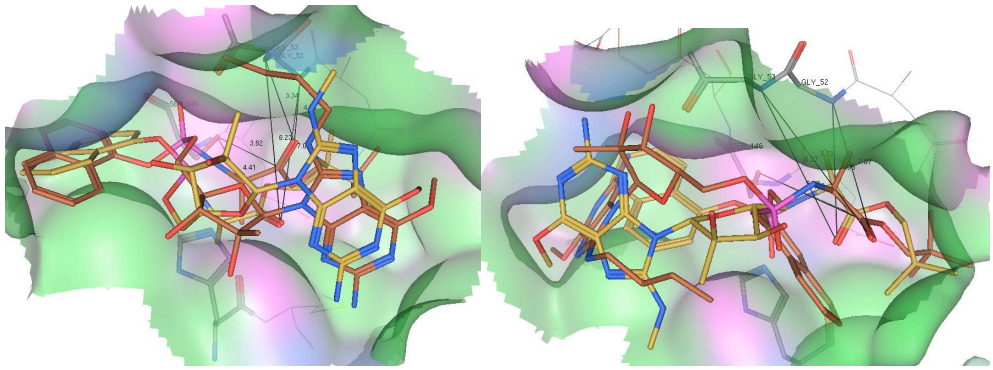


Figure 4.9. Docking of S_P (left) and R_P (right) diastereoisomers of **68** (brown stick) and **70** (yellow stick)**.**

In contrast, bulky aromatic amino groups at *C*-8 (**71** and **72**) do not allow the molecules to fit within the catalytic pocket, thus supporting the results observed during the CPY

experiments (Graph 4.5). In these cases, the ester carbonyl group of the R_P diastereoisomer lies in a better position for enzymatic hydrolysis than the S_P diastereoisomer.

2.6.2.2. Docking with human Hint-1 enzyme

As already mentioned in Chapter 3, human Hint enzymes are known to hydrolyse the P-N bond of phosphoramidate monoesters. Phosphoramidate monoesters of 8-amino modified ProTides together with the natural substrate adenosine 5'-monophosphate (AMP), were docked within the active site of Hint-1 enzyme with the intention to investigate the step of intracellular release of the corresponding 5'-monophosphates.
While the phosphorus center of aminoacyl intermediate of **68** (ProTide of *β*-2'-*C*-methyl-6-*O*-methyl-8-methylamino guanosine) lies far from the catalytic triad, longer alkyl chain at *C*-8, such as **70** (ProTide of 8-butylamine *β*-2'-*C*-methyl-6-*O*-methyl guanosine), might favour the P-N bond cleavage position of phosphoramidate monoester (in comparison with the phosphate of the reference AMP) (Figure 4.10). As a result, the intracellular delivery of the 5'-monophosphate of **70** may be quicker than **68**, and therefore may result in higher anti-HCV activity in in-*vitro* assays. Thus, molecular modelling in both cathepsin A and Hint-1 does not offer a clear correlation between the observed higher potency of **68** and its enzymatic bioactivation.

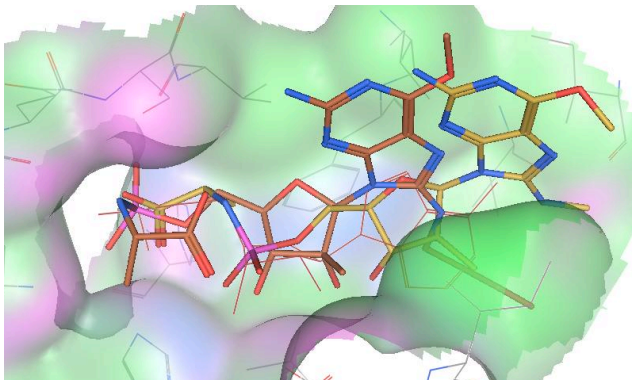


Figure 4.10. Docking of phosphoramidate monoester of **68** (yellow stick) and **70** (brown stick) in the catalytic pocket of human Hint-1 enzyme (AMP in red line).

Similarly, phosphoramidate monoesters of **69** and **73**, bearing respectively secondary alkyl and cyclic amine at *C*-8, are well positioned in the catalytic pocket (Figure 4.11). Nevertheless, as suggested by the CPY assays and predicted by cathepsin docking, the phosphoramidate monoester of **69** may not be formed, so unless other liver enzymes

hydrolyse the latter, **69** may not exhibit activity against HCV replication, as supported by biological testing. Predicted slow ester hydrolysis of phosphoramidate monoester of **73** may result in a slow conversion into the 5'-monophosphate by Hint-1 enzyme. In this case molecular modelling offers a prediction of the slightly higher in-*vitro* potency of **73** compared to **69**.

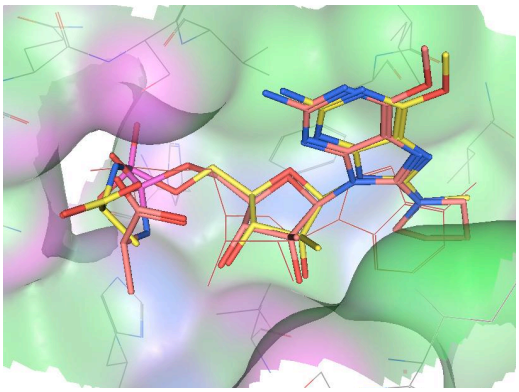


Figure 4.11. Docking of phosphoramidate monoester of **69** (yellow stick) and **73** (pink stick) in the catalytic pocket of human Hint-1 enzyme (AMP in red line).

Docking of 8-aryl amine modified phosphoramidate monoesters of **71** (8-phenylamino) and **72** (8-benzylamino) into human Hint-1 enzyme predicts faster P-N bond hydrolysis for **72** than **71** (Figure 4.12). Therefore, long and bulky groups at *C*-8 might position the phosphorus center in optimal position within the catalytic site for release of 5'-monophosphate (in comparison with the phosphate group of AMP). This prediction supports the in-*vitro* data, suggesting that **72** is a stronger inhibitor of HCV replication than **71**.

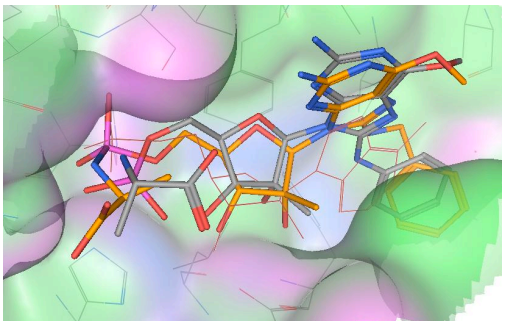


Figure 4.12. Docking of phosphoramidate monoester of **71** (grey stick) and **72** (orange stick) in the catalytic pocket of human Hint-1 enzyme (AMP in red line).

2.7. Conclusion and perspectives

Before initiating this project, modifications at *C*-8 position of the nucleobase of 2'-*C*-methyl nucleosides had never been investigated and were totally unknown in the HCV field. A novel synthetic approach to synthesise 8-amino modified *β*-2'-*C*-methyl-6-*O*-methyl guanosine analogues was developed, and the ProTide technology was successfully applied to the new nucleoside analogues in order to boost the anti-HCV activity.

Despite a boost of potency exhibited by 8-modified ProTides in comparison with their corresponding nucleosides, none appears as strong inhibitor of HCV replication as **15.d** (*α*-naphthyl *L*-Alanine-neopentyl ester Protide of *β*-2'-*C*-methyl-6-*O*-methyl guanosine).

Enzymatic experiments performed with adenosine deaminase together with the molecular docking using a model of ADAL-1, suggest that the 6-O-methyl might be hydrolysed at the monophosphate level only. Therefore, 8-modified 2'-*C*-methyl guanosine phosphorylated species might be delivered inside the cells, if their corresponding 6-*O*-methyl monophosphate analogues are released after metabolism of the ProTides.

Molecular modelling supports the results of both carboxypeptidase Y assays and anti-HCV in-*vitro* results. While small groups at *C*-8 may enable quick ester hydrolysis (**68**), long and bulky group may favour efficient P-N bond cleavage (**72**). However, 8-modified primary amines phosphoramidates (**68**, **70**, **71**, **72**) are stronger inhibitors of HCV replication than 8-modified analogues bearing secondary amines (**69**, **73**). While cyclic secondary amines at *C*-8 might result in relatively slow bioactivation of the ProTide (**73**) to its 5'-monophosphate species, the ester hydrolysis might be the limiting step for phosphoramidates bearing secondary alkyl amine at *C*-8 (**69**).

Future expansion of 8-modified nucleosides should focus on synthesis and evaluation of 8-*O*-alkyl/aryl and 8-*C*-alkyl/aryl analogues. The latter would be of particular interest to understand the effect on HCV replication of bigger and smaller atoms directly connected to *C*-8.

3. 7-deaza modifications

3.1. Introduction

Natural 7-deaza purine nucleosides, such as the antibiotics tubercidin (**74**) and toyocamicin (**75**) (Figure 4.13),[59-62] possess broad biological activity. 7-deaza modified purines have been extensively studied for their antiviral[63, 64] and cytostatic properties.[65, 66]

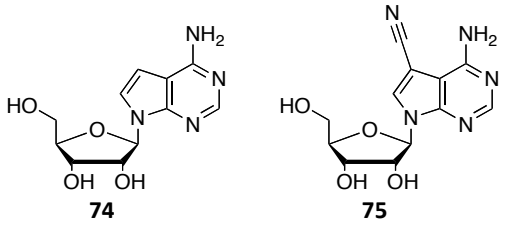


Figure 4.13. Structures of antibiotics tubercidin **74** and toyocamicin **75.**

Numerous 7-modified 7-deaza nucleosides are reported in the literature,[67-69] among which 7-deaza-2'-*C*-methyl adenosine **44**[9] and its 7-fluorinated derivative **76**[8] (Figure 4.14) were identified as potent inhibitors of HCV replication. In contrast, the 7-deaza-2'-*C*-methyl guanosine analogue **77** (Figure 4.14) did not exhibit anti-HCV activity in replicon-based assays, however its 5'-triphosphate inhibits HCV NS5B-mediated RNA synthesis at submicromolar concentrations.[8]

Since the 6-*O*-methyl modification has previously been reported as a potential prodrug of its guanosine analogue,[6] the application of 7-deaza modification on *β*-2'-*C*-methyl-6-*O*-methyl guanosine **11** should overcome the eventual poor cellular uptake of **77** and boost the inhibitory activity in replicon assays.

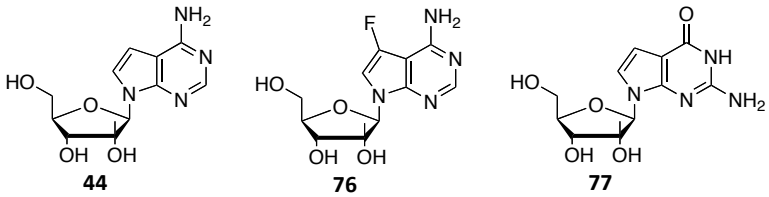


Figure 4.14. Inhibitory activity of 7-deaza-2'-*C*-methyl adenosine **44**, 7-deaza-7-fluoro-2'-*C*-methyl adenosine **76** and 7-deaza-2'-*C*-methyl guanosine **77,** inhibitory activity of nucleosides in replicon assays (EC_{50}) and inhibitory activity of nucleoside 5'-triphosphates on HCV NS5B-mediated RNA replication (IC_{50}), cellular toxicity (CC_{50}).

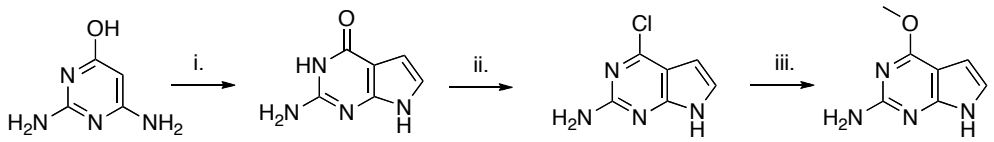
3.2. 7-deaza-6-*O*-methyl-2'-*C*-methyl guanosine

3.2.1. Synthesis of 7-deaza-6-*O*-methyl-2'-*C*-methyl guanosine and prodrugs

Several synthetic approaches for the formation of 7-deazapurine ribonucleosides have been developed,[70, 71] based on the preparation of the 7-deaza modified nucleobase followed by a glycosylation step.

3.2.1.1. Preparation of 7-deaza-6-*O*-methyl guanine

En route to 7-deaza-6-*O*-methyl guanine **81** (Scheme 4.6), 7-deaza-6-chloro guanine **80** was formed in moderate yield over two steps as reported in the literature.[69, 72] Addition of α-chloroacetaldehyde to the commercially available 2,4-diamino-6-hydroxypyrimidine **78** in water enabled the formation of the pyrrole ring *via* Bischler reaction and afforded 7-deazaguanine **79** (Scheme 4.6). The later was treated with phosphorus oxychloride ($POCl_3$) under reflux[73] over two hours to yield 23% of **80**. The reflux conditions were preferred since the yield of the same reaction performed over two days at room temperature[74] was only 16%.



78 **79** **80** **81**

(i. NaOAc (1.0 eq), α-chloroacetaldehyde (50% aq., 1.0 eq), DMF/H_2O, rt, overnight, 23%; ii. $POCl_3$, reflux, 2 hrs, 64%; iii. NaOMe (3.0 eq), anh. MeOH, reflux, 5 days, 3%)

Scheme 4.6. Route to 7-deaza-6-*O*-methyl guanine.

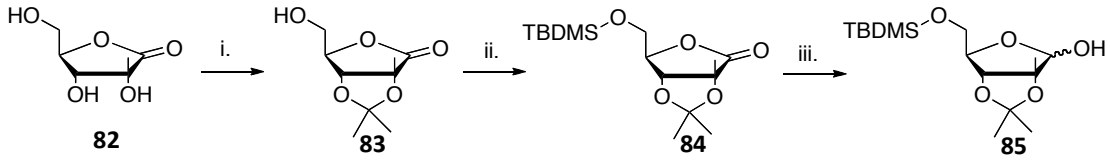
Displacement of the 6-chlorine was carried out refluxing **80** with sodium methoxide (NaOMe) for five days. Due to similar polarity of **80** and **81**, completion of the reaction was monitored by ^{1}H-NMR and the presence of a peak around 4.00 ppm in deuterated methanol indicated the formation of the 6-*O*-methyl group. But **81** was formed in very poor yield (3%), therefore we decided to change the strategy and perform the aromatic nucleophilic substitution directly at the nucleoside level.

3.2.1.2. Synthesis of 7-deaza-6-*O*-methyl-2'-*C*-methyl guanosine

Several publications report the synthesis of 2'-*C*-methylribonucleoside in 'one-pot', reacting the protected 2'-*C*-methyl ribofuranose with the heterobase.[75, 76] However, electrophilic glycosylation is not recommended since the nitrogen of the 7-deaza pyrrole ring is part of the aromatic π-system and thus, is inert towards glycosylation; hence resulting in either *C*-nucleosides or reaction of the pyrimidine ring.[59, 77, 78] Subsequently, nucleobase-anion glycosylation appears as the best step towards the synthesis of 7-deaza-6-*O*-methyl-2'-*C*-methyl guanosine.[68]

3.2.1.2.1. Glycosylation with fully protected 2'-*C*-methyl ribofuranose

Preparation of the fully protected 2'-*C*-methyl ribofuranose was required before the glycosylation step (Scheme 4.7).



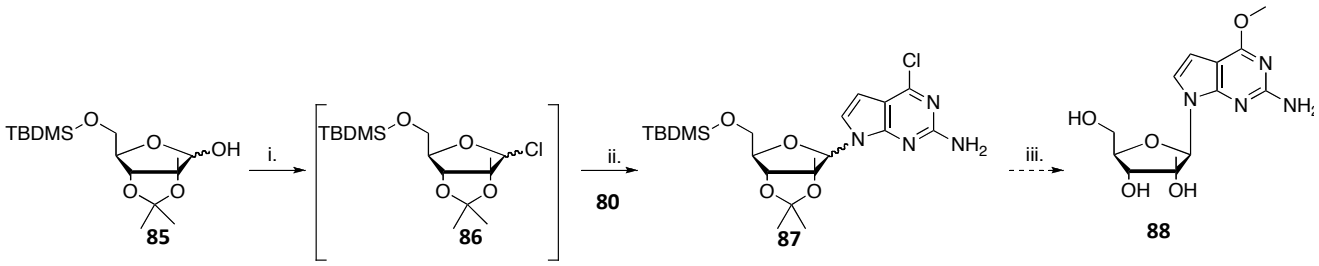
(i. anh. acetone, $HClO_4$ (60% aq., 1.6 eq), rt, overnight, 76%; ii. anh. DMF, imidazole (2.0 eq), TBDMSCl (2.0 eq), rt, overnight, 28%; iii. anh. THF, DIBAL-H (1M in hexanes, 3.0 eq), -78 °C, 1hr, -15 °C, 3-4hrs, 28%.)

Scheme 4.7. Synthetic route to fully protected 2'-C-methyl ribofuranose **85**.

Glycosylation with a heterobase bearing a non-protected exocyclic amine is reported to be more efficient when using the isopropylidene protected ribofuranose,[59] subsequently this group was chosen as protecting group for 2'- and 3'-hydroxyls starting from the commercially available 2'-*C*-methyl-1-ribonolactone **82**, to obtain compound **83**. The latter was further protected at the 5'-hydroxyl with *tert*-butyl-dimethyl-silyl chloride (TBDMSCl) to form **84**. The protected lactone was then reduced to a primary alcohol using diisobutylaluminium hydride (DIBAL-H) as electrophilic reducing reagent to afford both anomers of the fully protected 2'-*C*-methyl ribofuranose **85**. The mixture of both anomers was fully characterised by ^{1}H-NMR and ^{13}C-NMR. It is important to notice that the signals of H-3' protons differs, one appearing as a broad singlet and the other as a doublet. Calculation of the coupling constant $J_{3',4'}$ gave a value of 10.5 Hz, reported to be characteristic of the *β*-anomer,[79, 80] hence assuming that the broad singlet corresponds to the *α*-anomer. The ^{13}C-NMR has also significant difference in chemical

shifts and the α-anomer peaks appear more downfield.

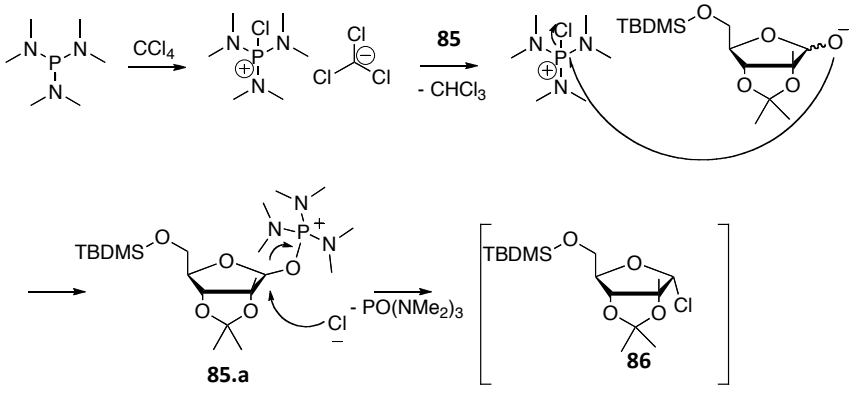
With **85** in hand, the glycosylation step was performed as described in Scheme 4.8.



(i. anh. toluene, CCl_4 (15 eq), $P(NMe_2)_3$ (1.3 eq), -35 °C to 0 °C, 1hr 30min; ii. anh. toluene, KOH (2.3 eq), TDA-1 (0.5 eq), rt, overnight; iii. anh. MeOH, NaOMe (3.3 eq), rt, overnight.)

Scheme 4.8. Synthetic scheme of glycosylation with fully protected 2'-*C*-methyl ribofuranose **85**.

The chloro derivative **86** was formed *in situ* using the mild conditions of Appel's chlorination (Scheme 4.9).[65] Activation using carbon tetrachloride (CCl_4) and tris(dimethylamino)phosphine ($P(NMe_2)_3$) enables deprotection of the anomeric hydroxyl of **85** and formation of the 1'-hexamethyl phosphorus triamide intermediate **85.a**. The later is then displaced under nucleophilic substitution conditions and should yield the only α-anomer of **86** in the presence of tris(dimethylamino)phosphine oxide.[81]



Scheme 4.9. Mechanism of Appel's chlorination.

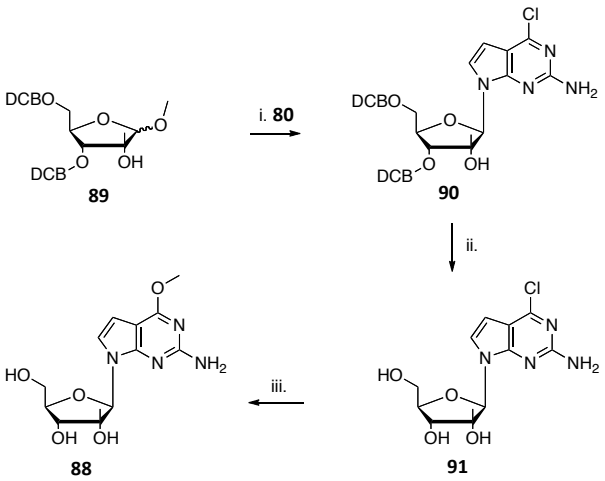
The conformation of compound **86** was not confirmed because of its reported instability,[65] however it is known to be a successful reagent for the synthesis of anomerically pure enantiomers.[81] **86** in toluene was then directly added into a well-stirred mixture of **80**, tris(2-(2-methoxyethoxy)ethylamine (TDA-1) and potassium hydroxide (KOH). These conditions, known as Ugarkar conditions,[65, 82] are reported to yield the only β-anomer after direct Walden inversion occurring during nucleophilic substitution type 2 (SN_2) of the chlorine atom with the entry of nitrogen *N*-9 of the

potassium salt of **80** formed in *situ.* In our case, ^{1}H-NMR of the crude product clearly indicated that the coupling did not happen. Similar findings were reported in the literature for standard 'one-pot' glycosylations using 7-deaza-6-chloro[83] in a series of ribonucleosides[70, 86] among which 2'-*C*-methyl sugar donor.[75] Thus, this route was then abandoned and the nucleobase-anion glycosylation procedure developed by Merck was tested.[8]

3.2.1.2.2. Glycosylation with 3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2'-*C*-methyl-1-*O*-methyl-*α*-*D*-ribofuranose

Merck laboratories have reported the synthesis of 7-nonhalogenated-7-deaza-6-chloro purines[8] *via* the formation of an α-epoxide intermediate as ribonucleoside donor.[83]

Starting from the commercially available 3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2'-*C*-methyl-1-*O*-methyl-*α*-*D*-ribofuranose **89**, condensation was performed in acetonitrile by in-*situ* conversion to the 1-bromo derivative followed by subsequent reaction with the potassium salt of **80** formed in-*situ*, to yield **90** (Scheme 4.10). ^{1}H-NMR suggested the presence of only one anomer of **90** whose peaks are in accordance with the β-anomer reported in the literature (H-7, 6.34 ppm, d, $J_{7\text{-}8}$ = 3.80 Hz; H-1', 6.23 ppm, s; H-5', 3.97 & 3.76 ppm, 2dd, J = 10.5 Hz, 2.0 Hz).[8, 85] A NOESY experiment was also performed to confirm the conformation of the glycosidic bond, however the presence of 2,4-dichlorophenylmethyl protecting group affected the spectra and did not allow full characterisation. It is also important to note that the use of potassium hydroxide powder instead of pellets improved the solubility of **80** and improved the yield of the glycosylation reaction by 3-fold.



(i. anh. DCM, 0 °C, HBr (33% in acetic acid, 6.7 eq), rt, 2hrs, anh. ACN, KOH (85% powdered, 3.0 eq), TDA-1 (0.2 eq), rt, 1hr, 31%; ii. anh. DCM, , 0 °C, BCl_3 (10 eq), -78 °C, 2hrs, -20 °C, 2hrs 30min, 66%; iii. anh. MeOH, NaOMe (3.0 eq), reflux, overnight, 49%.)

Scheme 4.10. Merck's route to 7-deaza-6-*O*-methyl-2'-C-methyl guanosine **88**.

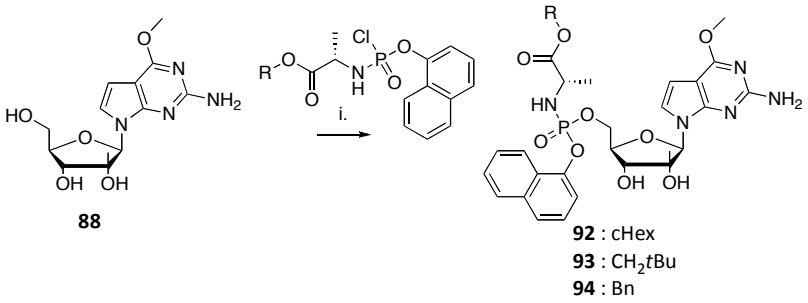
Treatment of **90** with the Lewis acid boron trichloride (BCl_3) in dichloromethane, afforded the deprotected 2'-*C*-methyl-6-chloro-7-deazaguanosine **91**. Chemical shifts and coupling constant ($J_{7,8}$ = 3.55 Hz) of the ^{1}H-NMR of **91** in deuterated methanol corresponded to the assignment of the *β*-anomer reported by Merck.[8] Substitution of **91** with sodium methoxide (NaOMe) in methanol under reflux afforded the 6-*O*-methyl analogue **88** in 50-60% yield after purification by flash chromatography. Standard conditions of substitution at the 6-position used for the guanosine derivative[6] were unsuccessful in the case of 7-deaza guanosine, and reflux was essential to form **88**.[86] The nucleophilic aromatic substitution was more difficult due to the removal of the 7-nitrogen lone pair, which is part of the π-system and favours resonance stabilisation of the intermediate. A singlet at 4.01 ppm in deuterated chloroform proved the presence of the methyl ether group at *C*-6. The *β*-configuration has previously been reported by Merck,[8] however since the Baker *trans* rule does not apply in this case, NOESY experiment was performed. Selective irradiation of the signal of H-1' increased the intensity of the signal of H-4', thus, indicating that H-1' and H-4' are located on the same face of the ribofuranose ring.[87] Moreover the signal of H-8 was not enhanced when H-1' was irradiated. This observation confirmed the anti orientation of the glycosidic bond[68] as reported for **44**.[28]

7-deaza-6-*O*-methyl-2'-*C*-methyl guanosine **88** was sent to Inhibitex *Inc.* for evaluation in HCV replicon-based assays. In the meantime, knowing that the phosphorylation of

nucleoside analogues is often the limiting step towards delivery of the 5'-monophosphate, the ProTide approach was anticipated and applied to **88**.

3.2.1.3. ProTide technology applied to 7-deaza-6-*O*-methyl-2'-*C*-methyl guanosine

Three *α*-naphthyl *L*-Alanine phosphoramidates with variation at the ester moiety (**92**, **93**, **94**) were synthesised following the standard Uchiyama procedure (Scheme 4.11), without requiring protection of the 2'- and 3'- hydroxyl groups of **88**.



(i. anh. THF, tBuMgCl (2.0 eq), rt, overnight.)

Scheme 4.11. Synthesis of 7-deaza-6-*O*-methyl-2'-*C*-methyl guanosine ProTides.

After flash chromatography and preparative chromatography, the phosphoramidates **92**, **93** and **94** were isolated and characterised by NMR, and analytical HPLC. All the compounds were obtained as a mixture of diastereoisomers in 12-13% yield. The two diastereoisomers of **92** were separated (**92.a** and **92.b**) by flash chromatography. These ProTides were tested for their ability to inhibit HCV replication by Inhibitex *Inc*.

3.2.2. Biological evaluation

Cytotoxicity and inhibitory potency of **88**, **92.a**, **92.b**, **93** and **94** were evaluated in replicon assays by Inhibitex *Inc.* The results are reported in Table 4.3 and are compared with the *β*-2'-*C*-methyl-6-*O*-methyl guanosine (7-aza) series.

R	7-deaza			7-aza		
	Cpd	EC_{50} (μM)	CC_{50} (μM)	Cpd	EC_{50} (μM)	CC_{50} (μM)
-	**88**	>100	>100	**11**	3	>100
cHex	**92.a**	13.3	18	**15.k**	0.03	6
cHex	**92.b**	8.9	13	-	-	-
CH2*t*Bu	**93**	12.9	27	**15.d**	0.01	6
Bn	**94**	27.3	27	**15.e**	0.02	11

Table 4.3. Anti-HCV potency (EC_{50}) and cellular cytotoxicity (CC_{50}) of 7-deaza-6-*O*-methyl guanosine derivatives and their 7-aza analogues.

The key observation resulting from these biological tests is that the absence of the 7-nitrogen on the nucleobase is critical for anti-HCV activity, resulting in a total loss of potency at the nucleoside level (**88** vs **11**) and poor selectivity at the ProTide level. The 7-deaza phosphoramidates (**92.a**, **92.b**, **93** and **94**) somehow restore the antiviral activity from 3- to 10-fold. Hence, the ProTide approach applied to 7-deaza-6-*O*-methyl guanosine is to some extend effective, whereas **88** is either a poor substrate for nucleoside kinases or lacks of cellular permeation, hence preventing intracellular delivery of the bioactive 5'-triphosphate form. **92.a** and **92.b** exhibit similar potency, thus, the phosphorus stereochemistry does not influence the bioactivation. Moreover, it is remarkable that the removal of the 7-nitrogen lead to a loss of efficacy over 1000-fold when the neopentyl (**93** vs **15.d**) and the benzyl (**94** vs **15.e**) ester moieties are considered. With a range of ClogP varying from 3.14 to 4.00, poor cell uptake can not be considered as the limiting step.

Phosphoramidates **92.a**, **92.b**, **93** and **94** exhibit cellular toxicity in Huh-7 cells in comparison to their 7-aza derivatives (**15.d**, **15.e** and **15.k**). The 2'-C-methyl modification introduced on 7-deaza adenosine has resulted in loss of cytotoxicity.[9] Moreover neither 2'-*C*-methyl guanosine nor its 7-deaza derivative exhibited cytotoxicity in Huh-7 cells.[8] In addition the potency of 7-deaza guanosine when administered orally in-*vivo* has been reported against numerous RNA viruses without high toxicity despite its lack of antiviral properties in cell cultures.[68] Therefore, the cytotoxicity exhibited by 7-deaza-6-*O*-methyl guanosine phosphoramidates may result from the 6-*O*-methyl modification. This observation has previously been made by our group,[6] but ideally, the latter modification would be metabolised to its guanosine analogue, hence, would result in the intracellular delivery of 2'-*C*-methyl-7-deaza guanine 5'-triphosphate form (IC_{50} = 0.12 μM).[8]

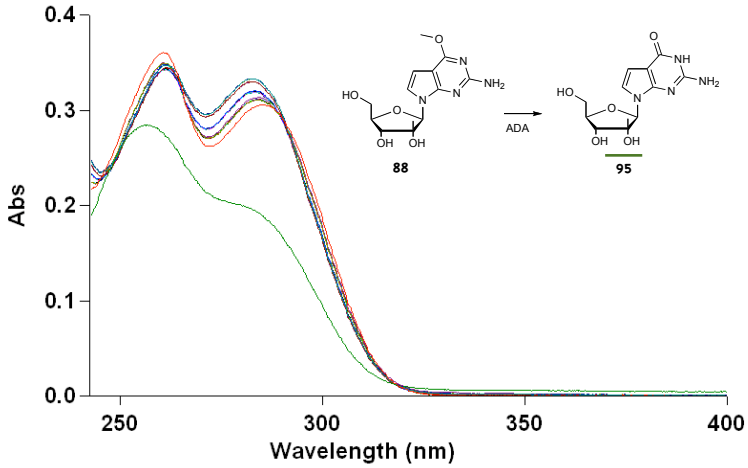
To further understand the impact of replacing the 7-nitrogen of the heterobase by a methine group in the bioactivation pathway, enzymatic and modelling studies were performed to assess the stability of 7-deaza derivatives.

3.2.3. Metabolic studies

Eventual metabolism of 7-deaza-6-*O*-methyl-2'-*C*-methyl guanosine **88** and its *L*-Alanine neopentyl phosphoramidate **93** to their corresponding 7-deaza guanosine analogues was investigated.

3.2.3.1. Adenosine deaminase assay

88 (44 µM) was incubated with commercially available adenosine deaminase (ADA, 80 µL, 0.05 mg/mL) in phosphate buffer (0.05M, pH~7.4) at room temperature. The reaction was followed by UV spectrometry for four hours and spectra were recorded every 15 minutes for one hour, and then at 30 minutes interval (Graph 4.6). Every hour 80 µL of ADA was added, and at the end of the run 2'-*C*-methyl-7-deaza guanosine **95** was added as a reference.



Graph 4.6. UV spectra of conversion of 7-deaza-6-*O*-methyl-2'-*C*-methyl guanosine **88** into 7-deaza-2'-*C*-methyl guanosine **95** by ADA.

In contrast with 2'-*C*-methyl adenosine but similarly to 7-deaza-2'-*C*-methyl adenosine,[8] **88** is not a substrate for adenosine deaminase. Hence, the combination of a 7-deaza and 6-O-methyl substituent increases significantly the stability to metabolic enzymes responsible for deamination of the 6-position of the nucleobase.

3.2.3.2. Docking study with adenosine deaminase-like protein-1

Docking studies of 7-deaza-6-*O*-methyl-2'-*C*-methyl guanosine 5'-monophosphate were performed with an homology model of adenosine deaminase-like protein-1

(ADAL-1).[57] As previously mentioned, this enzyme is responsible for the hydrolysis of the 6-position of guanine on the 5'-monophosphate.[56] This study predicted that the 7-deaza-6-*O*-methyl-2'-*C*-methyl guanosine 5'-monophosphate might be a relatively good substrate for ADAL-1 (Figure 4.14), in comparison with the natural substrate AMP. Thus, as long as the 7-deaza-2'-*C*-methyl guanosine 5'-monophosphate is released from the metabolism of the ProTide, 7-deaza-2'-*C*-methyl guanosine 5'-monophosphate might be delivered intracellularly.

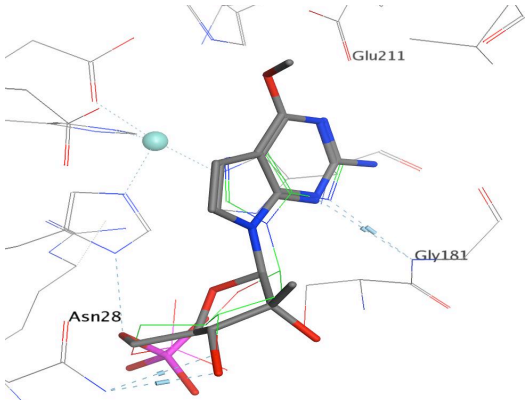
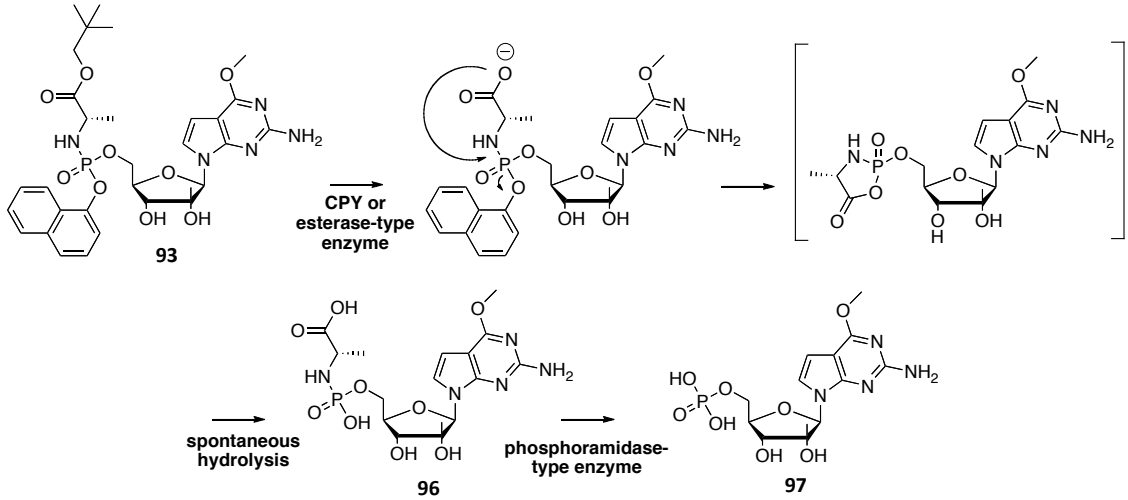


Figure 4.14. Docking of 7-deaza-6-*O*-methyl-2'-*C*-methyl guanosine 5'-monophosphate (grey stick) into the catalytic pocket of ADAL-1 (green line: AMP).

3.2.4. Mechanistic studies

Aiming at understanding better the bioactivation pathway resulting in the intracellular release of 5'-monophosphate (**97**), enzymatic and docking experiments were performed to probe the two steps of activation of phosphoramidate **93**, analogue of **15.d** (Scheme 4.12).



Scheme 4.12. Putative mechanism of activation of α-naphthyl *L*-Alanine neopentyl/benzyl ester 7-deaza-6-*O*-methyl-2'-*C*-methyl guanosine ProTide **93**.

3.2.4.1. Ester hydrolysis

Phosphoramidate **93** (2.3 mg) was dissolved in a mixture of deuterated acetone (92 µL) and Trizma buffer (184 µL), and was incubated with commercially available carboxypeptidase Y (CPY) (0.1 mg) in Trizma buffer (92 µL). The enzyme-mediated ester hydrolysis was monitored by ^{31}P-NMR, overnight, at room temperature at regular intervals, and selected spectra are shown in Figure 4.15.

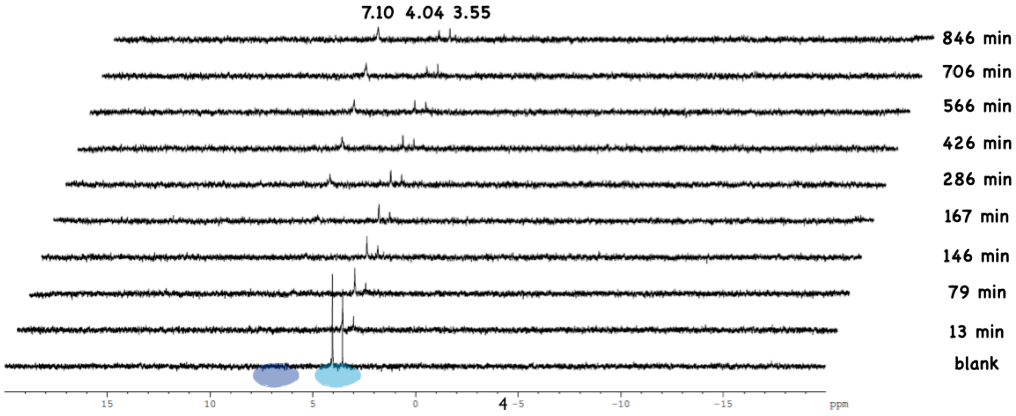


Figure 4.15. Carboxypeptidase Y assays with phosphoramidate **93**.
(light blue: diastereoisomers of **93**, dark blue: phosphoramidate monoester species **96**)

One diastereoisomer of **93** shows to be less stable towards carboxypeptidase Y than the other, suggesting better fitting in the enzymatic catalytic site. However, both phosphorus signals decrease over time while achiral **96** is formed ($\delta_P \sim 7.10$ ppm). The 7-aza analogue **15.d** has a metabolic conversion rate 20-times faster than **93**.[6] It is surprising that the remote 7-deaza modification has such an impact on the ester hydrolysis step and would explain the significant loss of potency against HCV RNA replication.

This notable difference of reactivity towards carboxypeptidase-type enzymes was investigated using molecular docking in cathepsin A. Preliminary docking of **93** suggested its S_P isomer to fit better in the catalytic pocket (Figure 4.16 a); hence, S_P isomers of both **93** and **15.d** were docked into the catalytic site of cathepsin A (Figure 4.16 b). While the carbonyl group of **15.d** S_P isomer faces towards the key residues (Gly52, Gly53 and Ser146) of the catalytic site, replacement of the 7-nitrogen by a methine group provokes an undesirable effect affecting the position of the ester carbonyl group, now lying towards the front of the pocket. This observation predicts the

slow release of the phosphoramidate monoester **96** and might suggest that hydrogen bond acceptor at C-7 of the nucleobase is required for locking the configuration of the nucleobase so that the ester carbonyl group fits properly into the catalytic site of carboxypeptidase-type enzymes.

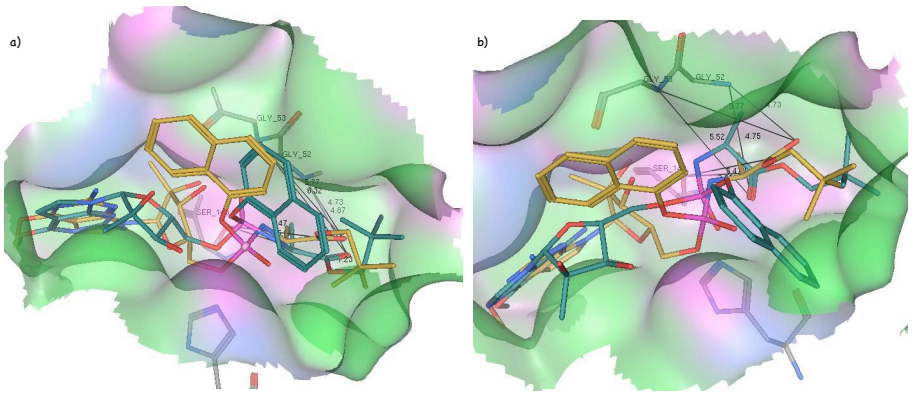


Figure 4.16. a) Docking of S_P (yellow stick) and R_P (blue sticks) isomers of **93** in the catalytic site of cathepsin. b) Docking of S_P isomers of **93** (yellow sticks) and **15.d** (blue sticks) into cathepsin. (red: oxygen, pink: pphosphorus)

3.2.4.2. P-N bond cleavage

The release of the 5'-monophosphate **97** was investigated performing metabolic experiment with Huh-7 cell lysate. **93** was incubated with Huh-7 cell lysate at 37 °C, and ^{31}P-NMR spectra were recorded overnight. However the phosphorus spectra were not conclusive due to a peak from the cell lysate, which was probably covering the peak of the eventual 5'-monoposphate released. After centrifugation, mass spectra of the aliquot suggested the formation of desired **97** (pink circle, M+Na$^+$ = 413). Nevertheless conversion of **93** is extremely slow and even after 48 hours, **93** remains the main species present in the medium (blue circle, M+H$^+$ = 658, M+Na$^+$ = 680, M+K$^+$ = 696) (Figure 4.17).

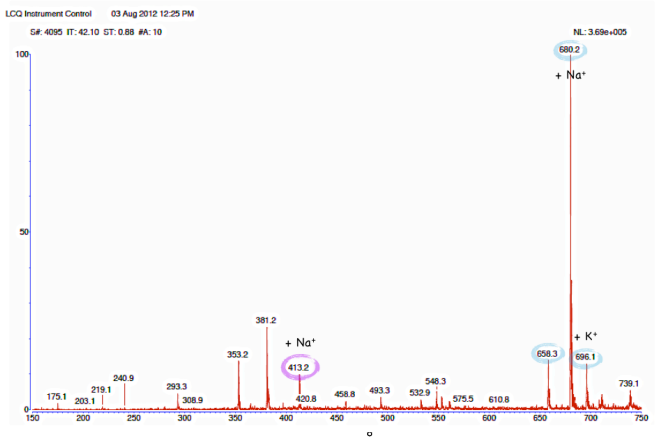


Figure 4.17. Mass spectra of Huh-7 cell lysate experiment.

This observation is supported by the docking of **96** in human Hint-1 enzyme (Figure 4.18), known to be responsible for P-N bond cleavage. The docking predicts that the phosphorus center of **96** lies far from the catalytic residues and is not in the optimal position for efficient P-N bond hydrolysis, in comparison with the natural substrate adenosine monophosphate (AMP). It is likely that an inefficient P-N bond cleavage results in a poor inhibitory activity in replicon-based assays.

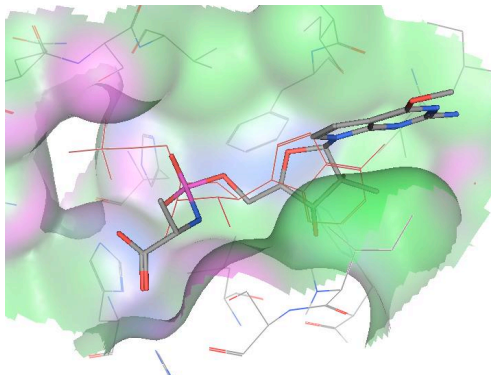


Figure 4.18. Docking of phosphoramidate monoester **96** (grey stick) and AMP (red line) in the catalytic pocket of human Hint-1 enzyme.

3.2.5. Conclusion: 2'-*C*-methyl-6-*O*-methyl-7-deaza guanosine analogues

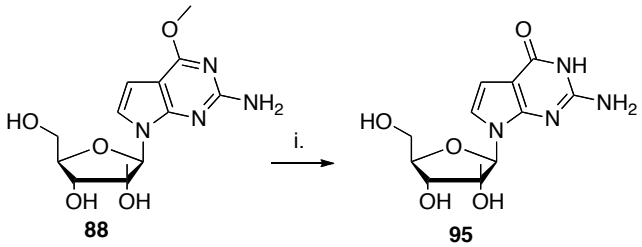
7-deaza analogues of 2'-*C*-methyl-6-*O*-methyl guanosine (nucleoside and phosphoramidates) were synthesised and tested against HCV RNA replication. Despite the efficiency of the ProTide technology, the loss of the 7-aza of the nucleobase leads to dramatic loss of potency and antiviral selectivity. Docking predictions suggest that the combination of 6-*O*-methyl substituent and 7-deaza moiety is acceptable for intracellular release of 7-deaza-2'-*C*-methyl guanosine 5'-monophosphorylated species. However, enzymatic experiments support the docking studies, and suggest that the release of the 2'-*C*-methyl-6-*O*-methyl-7-deaza guanosine 5'-monophosphate is likely to be the limiting step. Hence, the poor anti-HCV activity exhibited in-*vitro*.

To address this problem and investigate the effect of the removal of the 7-nitrogen on anti-HCV activity, 2'-*C*-methyl-7-deaza guanosine analogues were synthesised and evaluated in HCV replicon assays.

3.3. 7-deaza-2'-*C*-methyl guanosine analogues

3.3.1. Preparation of nucleoside and ProTides

7-deaza-2'-*C*-methyl guanosine nucleoside **95** was prepared following the Merck's route (Scheme 4.10) using 7-deaza guanine **79** (Scheme 4.6) for the glycosylation step. However the poor solubility of **79** in dichloromethane resulted in only 9% yield of the 3',5'-DCB protected guanosine, followed by deprotection to yield **95**. To overcome this solubility issue and as an attempt of increasing the yield, **88** was treated with sodium iodide (NaI) and trimethylsilyl chloride[88] (TMSCl) in acetonitrile (Scheme 4.13) to yield 21% of **95**. ^{1}H-NMR and ^{13}C-NMR spectra of **95** obtained from both routes were similar, and comparison with literature ($J_{7,8}$ = 3.60 Hz vs 3.70 Hz)[89] confirmed the β-configuration of the nucleobase.



(i. anh. ACN, NaI (1.5 eq), TMSCl (1.5 eq), rt, 1hr, 21%)

Scheme 4.13. Route to 7-deaza-2'-*C*-methyl guanosine **95**.

The α-naphthyl *L*-Alanine cyclohexyl ester phosphoramidate of **95** (**98**) was prepared in 34% yield using the conditions described above (Scheme 4.13) starting from its 7-deaza-6-*O*-methyl ProTide derivative **92**. Both **95** and **98** were sent for biological evaluation in HCV cell-based replicon assays.

3.3.2. Biological evaluation

Bristol-Myers Squibb evaluated the cellular toxicity (CC_{50}) and HCV RNA inhibition potency (EC_{50}) of **95** and **98** in cell-based replicon assays. The results reported in Table 4.4 suggest that the removal of the 6-*O*-methyl does not enhance in-*vitro* anti-HCV activity, however its presence is responsible for the cytotoxicity reported for 7-deaza-6-*O*-methyl-2'-*C*-methyl derivatives. Hence the 6-*O*-methyl group is not responsible for the dramatic loss of antiviral activity.

R	7-deaza-6-O-methyl-2'-C-methyl guanosine			7-deaza-2'-C-methyl guanosine			2'-C-methyl-6-O-methyl guanosine		
	Cpd	EC_{50} (μM)	CC_{50} (μM)	Cpd	EC_{50} (μM)	CC_{50} (μM)	Cpd	EC_{50} (μM)	CC_{50} (μM)
-	**88**	>100	>100	**95**	>10	>100	**11**	3	>100
cHex	**92**	8.9	13	**98**	>10	>100	**15.k**	0.03	6

Table 4.4. Evaluation of anti-HCV activity (EC_{50}) and cytotoxicity (CC_{50}) of **95** and **98** in replicon-based assays compared to their 6-*O*-methyl (**88** and **92**) and 7-aza (**11** and **15.k**) analogues.

From these results, **98** appears to be a poor inhibitor of HCV replication as well as its parent nucleoside **95**, hence in this case the ProTide technology seems to be inefficient. This result suggests that the first phosphorylation step is not the only limiting step of activation to the 5'-monophosphate, and that the removal of the hydrogen bond donor at *C*-7 of the nucleobase might be critical for anti-HCV activity. Not enough material of **98** was recovered to perform enzymatic experiments, however its metabolism was predicted by molecular docking in cathepsin A and Hint-1 enzymes to understand whether it was converted to its 5'-monophosphorylated species.

3.3.3. Mechanistic studies

In first instance, **98** was docked in cathepsin A enzyme (Figure 4.19 a), an esterase type enzyme responsible for ester cleavage, followed by docking in Hint-1 enzyme (Figure 4.19 b) known to be responsible for the last step of ProTide activation to release the 5'-monophosphate.

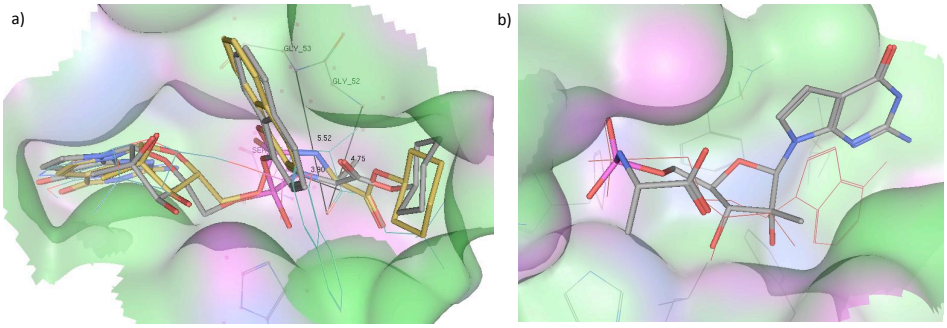


Figure 4.19. a) Docking of diastereoisomers of **98** in cathepsin A enzyme (yellow stick: R_P isomer, grey stick: S_P isomer, blue line: **15.d**).
b) Docking of phosphoramidate monoester of **98** in human Hint-1 enzyme (grey stick: phosphoramidate monoester specie, red line: AMP).
(red: oxygen, pink: phosphorus, blue: nitrogen)

Molecular docking predicts that ester carbonyl groups of either isomers of **98** lie far and point opposite direction of the catalytic residues in comparison with **15.d**. This suggests that the conversion to the phosphoramidate monoester derivative will be slow if

happening at all. The latter fits well into human Hint-1 enzyme catalytic pocket, with its phosphorus center lying close to the optimal position for P-N bond cleavage (by comparison to the phosphorus center of AMP).
Eventually, the 7-deaza-2'-C-methyl guanosine 5'-monophosphate species might be poorly released due to slow ester cleavage. Hence, resulting in poor intracellular concentrations of 7-deaza-2'-*C*-methyl guanosine 5'-triphosphate.

3.3.4. Conclusion: 7-deaza-2'-*C*-methyl guanosine analogues

The 6-*O*-methyl substituent is not a bad prodrug moiety for 7-deaza derivatives since the corresponding 5'-monophosphate species might be metabolised to their guanosine derivatives, as predicted by the modeling docking with ADAL-1. However its removal does not boost the inhibitory potency at either both nucleoside and ProTide levels. As a result, the remarkable loss of potency of 7-deaza species is due to the replacement of the nitrogen at *C*-7 of the nucleobase by a hydrogen donor methine group. The presence of a hydrogen bond acceptor at *C*-7 is apparently required for conversion to the active guanosine species (**88**, **95** and **11**). Even the ProTide motif does not salvage the loss of selectivity and potency (**92** and **98** vs **15.k**), and it shows the need to tune the ProTide motif on each occasion.
Molecular docking, biological results and enzymatic experiments of 7-deaza analogues predict and confirm that the 7-nitrogen is essential for substrate recognition by adenosine deaminase, as previously reported.[90] Absence of the hydrogen bond acceptor at *C*-7 contributes significantly to stability towards metabolic enzymes[9] involved at nucleoside and phosphate levels.

2'-*C*-methyl-6-*O*-methyl and 2'-*C*-methyl guanosine phosphoramidates are very potent[6] against HCV replication, but their 7-deaza analogues display weak potency against HCV RNA replication due to very slow enzyme-mediated metabolism to their active phosphorylated species. This led us to investigate the release of the potent 7-deaza-2'-*C*-methyl guanosine 5'-triphosphate after metabolic conversion of 7-deaza-2'-*C*-methyl inosine analogues.

3.4. 7-deaza-2'-*C*-methyl inosine analogues

3.4.1. Introduction

While 7-deaza inosine **99** (Figure 4.20) is known to be an effective growth inhibitor and exhibits better tissue selectivity than tubercidin **74**,[91] 2'-*C*-methyl inosine **100** is a moderate inhibitors of HCV RNA replication (EC_{50} = 8 μM, CC_{50} = 300 μM)[74] and its corresponding 5'-triphospate inhibits more efficiently HCV NS5B polymerase (IC_{50} = 4.0 μM).[92]

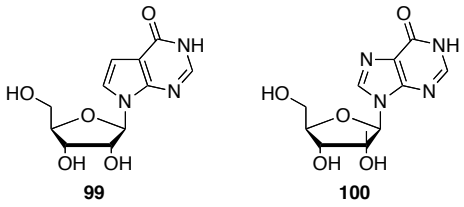
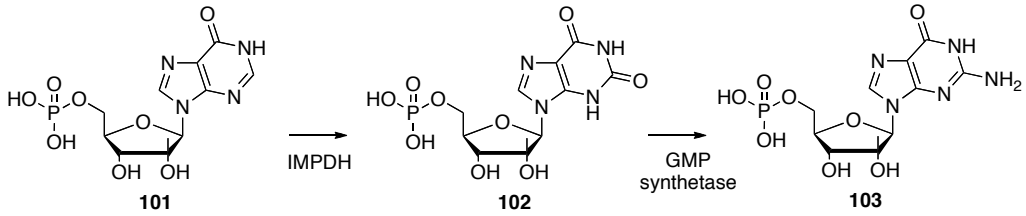


Figure 4.20. Structures of 7-deaza inosine **99** and 2'-*C*-methyl inosine **100**.

Action of inosine monophosphate dehydrogenase (IMPDH) is responsible for the oxidation of 2'-*C*-methyl inosine 5'-monophosphate (**101**) to its xanthine (**102**) derivative. As substrate of guanylate monophosphate synthetase (GMP synthetase), the later releases 2'-*C*-methyl guanosine 5'-monophosphate (**103**)[93] (Scheme 4.14).

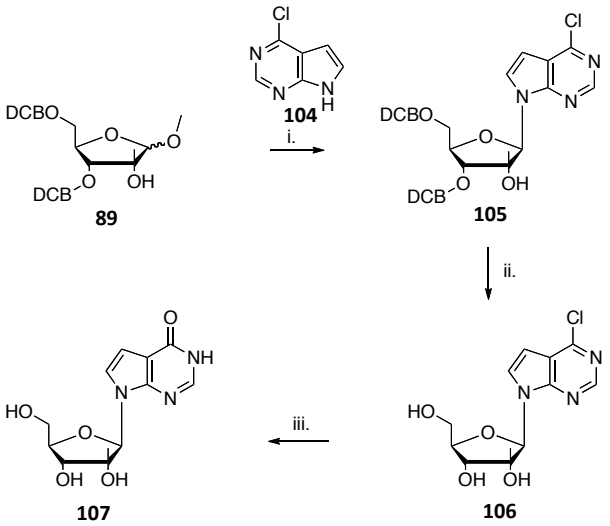


Scheme 4.14. Role of IMPDH in *de novo* biosynthesis of guanine nucleotides.

Aiming at investigating the release of 7-deaza-2'-*C*-methyl guanosine phosphorylated species after IMPDH-mediated metabolism of their inosine analogues, 7-deaza-2'-*C*-methyl inosine derivatives were synthesised and evaluated against HCV replication.

3.4.2. Preparation of nucleoside and ProTides

7-deaza-2'-*C*-methyl inosine **107** was prepared according to the Merck's route using the nucleobase-anion glycosylation conditions as described in Scheme 4.15. Starting from commercially available 7-deaza-6-chloro pyrimidine **104**, **107** was recovered in 47% over 3 steps, and ^{1}H-NMR was in correlation with data reported by Merck.[81]

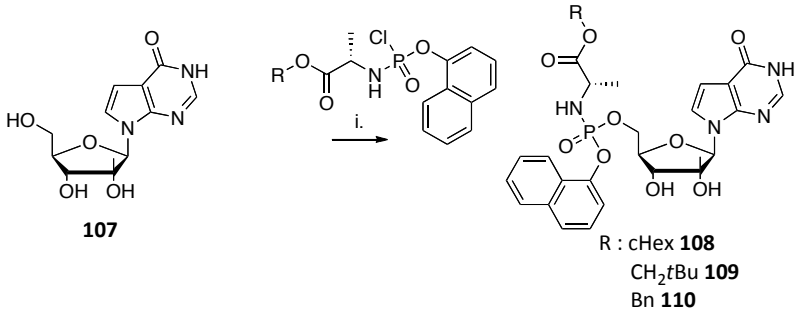


(i. anh. DCM, 0 °C, HBr (33% in acetic acid, 6.7 eq), RT, 2hrs, anh. ACN, KOH (85% powdered, 3.0 eq), TDA-1 (0.2 eq), RT, 1hr, 60%; ii. anh. DCM, 0 °C, BCl_3 (10 eq), -78 °C, 2hrs, -20 °C, 2hrs 30min, 37%; iii. anh. MeOH, aq. NaOH (1M), reflux, 1hr, 43%.)

Scheme 4.15. Synthetic route toward 7-deaza-2'-*C*-methyl inosine **107**.

Also in this case, the *β*-configuration of the glycosidic bond reported by Merck[85, 92] was determined by comparison of ^{1}H-NMRs ($J_{7,8}$ = 3.55 Hz vs 3.60 Hz).

ProTides of **107** were prepared following the standard Uchiyama procedure previously described. Coupling with the *α*-naphthyl *L*-Alanine cyclohexyl (**14.c**) or neopentyl (**14.d**) or benzyl ester (**14.e**) phosphorochloridate yielded respectively **108**, **109** and **110** (1-2%), all as mixtures of two diastereoisomers (Scheme 4.16).



(i. anh. THF, tBuMgCl (2.0 eq), rt, overnight.)

Scheme 4.16. Synthesis of 7-deaza-2'-*C*-methyl inosine phosphoramidates.

107, and its ProTides **108**, **109** and **110**, were sent for biological evaluation in HCV replicon-based assays.

3.4.3. Biological evaluation

7-deaza-2'-*C*-methyl inosine derivatives were tested to evaluate their eventual inhibitory activity in HCV replicon assays. This work was carried out by Bristol-Myers

Squibb and results of cell-based assays are reported in Table 4.5.

R	7-deaza-2'-C-methyl inosine		
	Cpd	EC_{50} (μM)	CC_{50} (μM)
-	**107**	>10	>100
cHex	**108**	>10	>100
CH_2tBu	**109**	13.9	>10
Bn	**110**	>10	>100

Table 4.5. Inhibitor activity (EC_{50}) and cytotoxicity (CC_{50}) of 7-deaza-2'-*C*-methyl inosine derivatives against HCV replication.

These results indicate that neither the nucleoside nor its prodrugs are inhibitors of HCV replication and they do not exhibit cellular toxicity. As seen with 7-deaza guanosine analogues, the replacement of the 7-nitrogen by a methine group results in loss of potency in replicon-based assays. The ProTide technology is inefficient for 7-deaza-inosine derivatives, hence either the 5'-monophosphate of 7-deaza-2'-*C*-methyl inosine is not delivered due to poor metabolism or it is likely that its triphosphate form is not an inhibitor of HCV replication in contrast with its 7-aza derivative (IC_{50} = 4.0 μM).[88] These results might also suggest that 7-deaza-2'-*C*-methyl inosine 5'-monophosphate is not metabolised by IMPDH and GMP synthetase to its corresponding 7-deaza guanosine analogue.

Because of poor yielding reactions, enzymatic experiments could not be performed. Nevertheless, the metabolism of activation of 7-deaza-2'-*C*-methyl inosine ProTide **108** was investigated using molecular docking.

3.4.4. Molecular modelling

3.4.4.1. Ester hydrolysis and P-N bond cleavage

Docking in the esterase-type enzyme cathepsin A predicts that both diastereoisomers of **108** might fit similarly within the catalytic pocket (Figure 4.21 a), with the ester carbonyl group lying far from the catalytic residues. This observation predicts slow release of the phosphoramidate monoester species. On the other hand, docking of the latter in phosphoramidase-type enzyme Hint-1 resulted in poor fit within the catalytic site (Figure 4.21 b), with the P-N bond lying far from the optimal position (shown by phosphorus center of the natural substrate AMP).

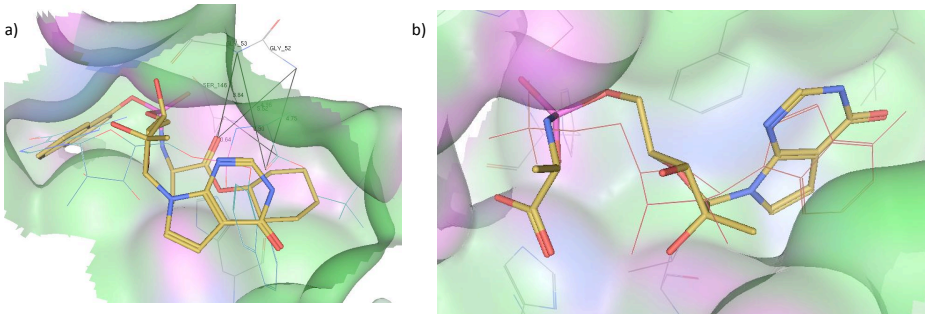
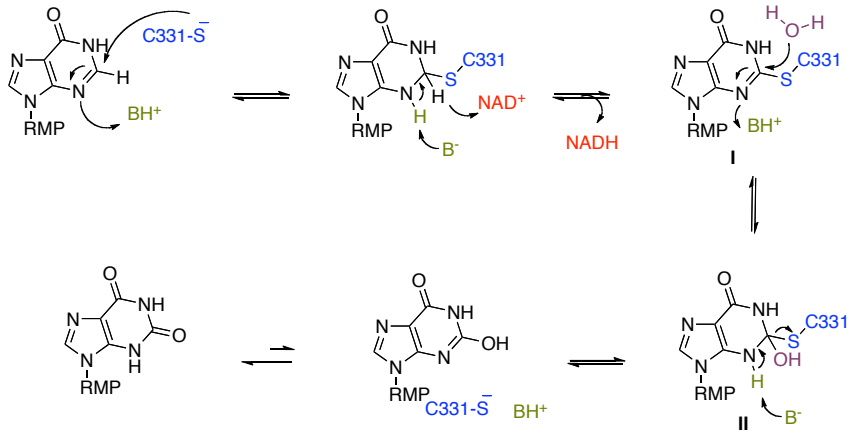


Figure 4.21. a) Docking of S_p isomer of phosphoramidate **108** (yellow stick) in the catalytic pocket of cathepsin A (blue line: S_P isomer of **15.d**)
b) Docking of phosphoramidate monoester specie of **108** (yellow stick) in human Hint-1 enzyme (red lime: AMP).
(red: oxygen, pink: phosphorus, blue: nitrogen)

Eventually, docking predictions suggest that metabolism of 7-deaza-2'-*C*-methyl inosine phosphoramidates to their 5'-monophosphate species may not be efficient, if at all happening. These predictions correlate with the biological results, since no prodrugs of 7-deaza-2'-*C*-methyl inosine exhibit anti-HCV potency in cell-based assays. However, evaluation of the inhibitory potency of the 7-deaza-2'-*C*-methyl inosine 5'-triphosphate (IC_{50}) against HCV NS5B-mediated replication would have distinguished clearly between poor metabolism and inefficacy of the 5'-triphosphate bioactive form.

3.4.4.2. Molecular docking in IMPDH

IMPDH is a NAD (nicotinamide adenine dinucleotide)-dependant enzyme that controls the purine nucleotides biosynthetic pathway.[94] The catalysis of the oxidation of inosine 5'-monophosphate (IMP) to xanthine 5'-monophosphate (XMP)[93] is reported as the rate limiting step in the *de novo* synthesis of guanine nucleotides.[95] The mechanism of conversion (Scheme 4.17) is triggered by nucleophilic attack of Cys331 on IMP. A hydrogen atom is then trapped by NAD^+ to release NADH and intermediate **I**, which is then hydrolysed to liberate XMP via a tetrahedral intermediate **II**.[93]



Scheme 4.17. Mechanism of biochemical conversion of IMP to XPM by IMPDH. (RMP: ribose 5'-monophosphate, B: base, could be water or amino acid residue)

The rationale behind designing 7-deaza-2'-*C*-methyl inosine ProTides being the conversion of their 5'-mophosphate by IMPDH and GMP synthetase to afford the 7-deaza guanine phosphorylated species. Molecular modelling was carried out using human type II IMPDH crystal structure (PNB 1B30)[95] to investigate the first metabolic step towards the release of 5'-monophosphate 7-deaza-2'-*C*-methyl xanthine. The result of the docking is represented in Figure 4.22.

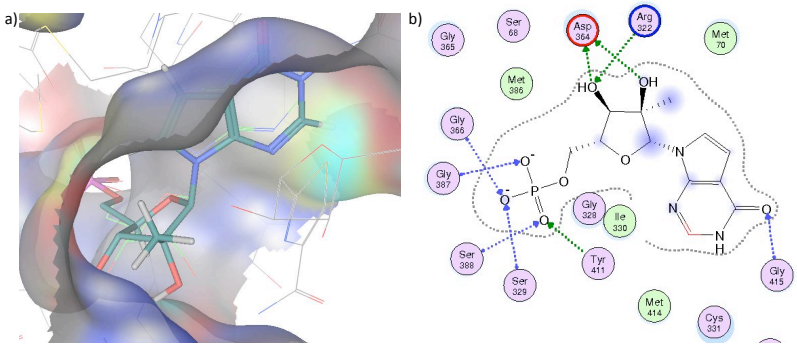


Figure 4.22. a) Docking of 7-deaza-2'-*C*-methyl inosine 5'-monophosphate (blue stick) in human type II IMPDH catalytic pocket. b) Ligand interactions with catalytic residues. (red circle: acidic, blue circle: basic, pink: polar, green: greasy, dotted blue arrow: backbone acceptor/donor, dotted green arrow: side chain acceptor/donor)

7-deaza-2'-*C*-methyl inosine 5'-monophosphate fits in a proper direction within the IMPDH catalytic site. The ribose and the phosphate moieties lie in the right pockets, however, both hydrogen at *C*-7 and the one at *C*-2 of the nucleobase provoke a steric clash with the pocket as predicted by the ligand interactions diagram (Figure 4.22 b). Nevertheless, the key catalytic atom Cys331 lies close to the *C*-2 position, as expected for efficient oxidation to the xanthine derivative. In addition, the ligand interactions diagram predicts that the hydrophobic 2'-*C*-methyl group lies in a rather hydrophilic

pocket, next to an Arginine residue (Arg322), hence this might also affect the affinity of 7-deaza-2'-*C*-methyl inosine 5'-monophosphate with IMPDH.
These observations lead to predict that catalytic oxidation of 7-deaza-2'-*C*-methyl inosine 5'-monophosphate might take place, however may not probably be efficient.

3.4.5. Conclusion: 7-deaza-2'-*C*-methyl inosine analogues

7-deaza-2'-*C*-methyl inosine **108** and three phosphoramidates were synthesised and evaluated against HCV RNA replication in cell-based assays. None of these molecules exhibited inhibitory activity, and this might result from either a poor bioactivation, as predicted by molecular docking, or from inactivity of the triphosphate form. Thus, it is unlikely that 7-deaza-2'-*C*-methyl inosine phosphorylated species are delivered intracellularly. As previously seen with guanosine derivatives, the subtle alterations from 7-aza to 7-deaza seems to be responsible for poor intracellular metabolism of 7-deaza-2'-*C*-methyl inosine analogues.
An enzyme inhibitory assay with the 5'-triphosphate of 7-deaza-2'-*C*-methyl inosine would provide the information of eventual inhibitory activity of 5'-triphosphate of 7-deaza-2'-*C*-methyl inosine against HCV NS5B-mediated RNA replication.
Despite, the molecular docking with IMPDH predicting slow catalytic oxidation, due to steric and spatial arrangement that would affect the affinity of 7-deaza-2'-*C*-methyl inosine monophosphate with the enzyme, replicon-based assays suggest that 7-deaza-2'-*C*-methyl inosine analogues do not undergo metabolism by IMPDH to the desired 7-deaza-2'-*C*-methyl guanosine phosphorylated species.

3.5. General conclusion

The replacement of the nitrogen at *C*-7 of the heterobase by a methine group affects greatly the inhibitory activity of parent 2'-*C*-methyl-6-*O*-methyl guanosine **11** against HCV RNA replication, leading to significant loss of activity and reducing selectivity.
The ProTide technology applied to the 7-deaza-2'-*C*-methyl guanosine derivatives does not reinstate potency in cell-based assays. Enzymatic experiments and molecular modelling prediction performed with both families, conclude on inefficient metabolism of the phosphoramidate prodrugs to their corresponding phosphorylated species, in marked contrast with 7-aza analogues. Similarly to 7-deaza-2'-*C*-methyl guanosine analogues, more disappointing results were obtained for 7-deaza-2'-*C*-methyl inosine

analogues, which are unlikely to be processed to their 5'-phosphorylated species, hence losing the possibility of delivering potent 7-deaza-2'-*C*-methyl guanosine 5'-triphosphate after metabolism by IMPDH and further phosphorylation. Nevertheless, even if 7-deaza-2'-*C*-methyl inosine 5'-monophosphate species were delivered efficiently, modelling predicts rather poor affinity with IMPDH catalytic site, hence expecting rather slow conversion to their xanthine analogues.

This surprising outcome suggests that the removal of the 7-nitrogen contributes to enzymatic stability.[8] The presence of a hydrogen-bond acceptor at *C*-7 of the nucleobase seems essential for the substrate recognition for metabolic enzymes responsible for the delivery of the 5'-monophosphate species.

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Chapter Five: Ribonucleoside modifications

1. Background and Objectives

Among the strategies to design potent antiviral or antitumour nucleoside analogues, modifications of the carbohydrate moiety have been extensively investigated to alter the physico-chemical and biological properties.

Following the discovery of oxetanocin A (**111**, Figure 5.1) as an effective anti-HIV1 and anti-HSV agent, hydroxymethyl alterations were introduced in nucleoside analogues.[1] Later, other modifications consisted in adding other heteroatoms or substituting varied functional groups at the different positions of the nucleoside sugar ring.[1] Among many, Roche reported 4'-azidocytdine (also known as **R1479**, Figure 5.1) as a potent and selective inhibitor for HCV replication in cell-based assays.[2]

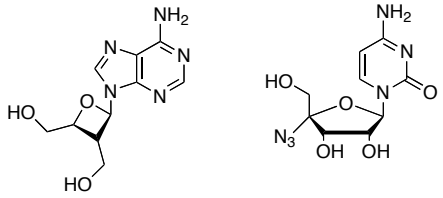


Figure 5.1. Structures of oxetanocin A (**111**) and 4'-azidocytidine (**R1479**).

Moreover, sugar-modified nucleosides are also commonly used in oligonucleotides for antisense therapy.[3, 4] In particular, 2'-*O*-alkyl oligonucleotides[5, 6] and 5'-*C*-branched oligodeoxynucleotides[7, 8] enhance stability towards nuclease enzymes.[9, 10] Despite the reported synthetic procedures to access the monomers, little literature is reported on 2'-*O*-alkyl and 5'-*C*-branched modified nucleosides as inhibitors of HCV RNA replication. Aiming at developing potential selective and non-toxic anti-HCV agents, we investigated the synthetic access to 2'-*O*-alkyl and 5'-*C*-methyl substituted analogues of 2'-*C*-methyl guanosine **10** and its 6-*O*-methyl derivative **11**.

2. Modifications at *C*-2' of 6-*O*-methyl guanosine derivatives

2.1. Introduction

2.1.1. 2'-*O*-alkylated nucleosides and hepatitis C virus

Following the isolation of natural 2'-*O*-methyladenosine (**112**, Figure 5.2),[11] numerous compounds of this class have been prepared and used in the field of anti-sense oligonucleotides[12] and their application to treatment of inflammatory, infectious and cardiovascular diseases.[13]

Despite the modelling prediction that HCV NS5B should accommodate 2'-*O*-alkylated nucleoside triphosphates, due to space available in the proximity of the *C*-2' position, only few examples have been investigated as inhibitors of HCV replication.[14] 2'-*O*-methyl modified cytidine **113**,[15] guanosine **114** and adenosine **112**[16] (Figure 5.2) have been prepared via glycosylation of the carbohydrate precursor by Merck laboratories, and exhibited low potency (EC_{50}) in HCV cell-based assays, while their triphosphates acted as relatively potent chain terminators (IC_{50}) with the exception of the adenosine derivative.[16]

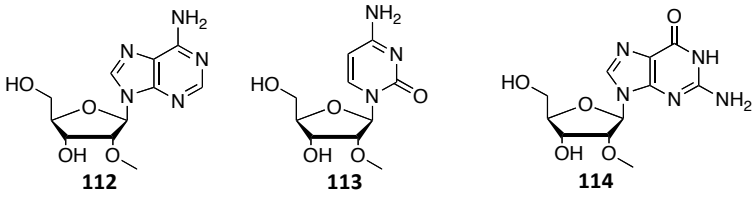


Figure 5.2. Inhibitory activity against HCV RNA replication of 2'-*O*-methyl nucleosides in replicon (EC_{50}) and their corresponding triphosphates (IC_{50}).

The lack of antiviral potency of the nucleosides **112-114** is likely to be due to inefficient cellular uptake and poor intracellular metabolism. On the contrary, incorporation of 2'-*O*-modified phosphorylated species may provoke interference with either the polymerase or the incoming nucleoside triphosphate, hence resulting in inhibition of NS5B-mediated RNA replication.[13]

In order to investigate the allowance of steric bulkiness around *C*-2' and aiming at boosting the inhibitory activity of nucleosides in replicon-based assays, the 2'-*O*-alkyl

modification strategy was applied to 6-*O*-methyl guanosine analogues. The alkylation was performed at a nucleoside level using methyl-, ethyl-, propyl- or isopropyl- iodides.

2.1.2. Synthetic approach to 2'-*O*-alkylated nucleosides

Synthetic efforts have been largely stimulated to prepare 2'-*O*-alkyl nucleosides in a selective and cost-effective way for oligonucleotide synthesis. Table 5.1 summarises the different approaches towards 2'-*O*-methylated and other 2'-*O*-alkylated nucleosides, their advantages and drawbacks.[14, 17]

Despite numerous approaches described in the literature,[14, 17] most direct alkylation procedures are not applicable to purine nucleosides, and particularly to guanosine derivatives. Selective alkylation of the 2'-position usually requires protecting groups both at the ribose and nucleobase moieties to avoid side products.

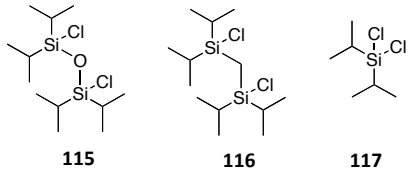
	method	***drawbacks***	***advantages***
From carbohydrate precursor	Glycosylation	No stereoselectivity. Glycosylation: limiting step. Poor yield for purines.	Large scale synthesis.
Direct methylation of ribonucleosides	Diazomethane / Dimethoxyethane	Low yield.	First practical synthesis.
	Methyl(ethyl) sulfate /DMF	Low yield. For cytosine and adenosine only.	Different alkylations possible.
	NaH/MeI	Undesired base methylation. Requires *N*-2 and *O*-6 protection for uridine and guanosine. Requires separation of 2‘- and 3’-isomers.	Applicable for adenosine and phosphoramidite.
	Ag_2O/MeI	Seldom use for purines.	Exclusive 2’-*O*-alkylation on pyrimidine.
Metal-directed mehylation of *cis*-diol	$SnCl_2.2H_2O$	Poor yield for guanosine. Not suitable for any other alkyl groups.	For all four 2’-*O*-Me nucleosides. Absence of base.
Methylation of protected nucleosides	3’,5’-TIPDSi + MeI/Ag_2O	Not suitable for guanosine.	Direct 2’-O-alkylation.
	3’,5’-TIPDSi-6-*O*-TIPSDSi + MeI/BEMP	High cost of BEMP. Moderate yield.	
	5’-*O*-Trityl + KOH	Moderate yield.	No alkylation on nucleobase.
	BEMP	High cost of BEMP. Multistep synthesis.	High yielding. Formation of single products.
	Dialkyl sulfate + alkali metal hydroxide	No nucleobase alkylation only at pH>13.	No side alkylation.
Other methods	2,2’-anhydro nucleoside		Ease of synthesis.

BEMP: 2-*tert*-butylimino-2-diethylamino-1,3-dimethyl-perhydro-1,3,2 -diazaphosphorine

Table 5.1. Methods for the synthesis of 2’-*O*-alkylated nucleosides.

2.2. 2'-*O*-alkyl modifications of 2'-*C*-methyl-6-*O*-methyl guanosine

Aiming at developing a selective 2'-*O*-alkylation of 2'-*C*-methyl-6-*O*-methyl guanosine **11**, 3'- and 5'- hydroxyls were protected using silicon-based protecting groups. The literature reports use of such protecting groups for a variety of applications thanks to their ease of introduction.[18, 19] The most commonly used are the 1,1,3,3,-tetraisopropyl-1,3-dichlorodisilyloxane ($TIPDSCl_2$, **115**, Figure 5.3) also known as the Markiewicz reagent,[20] the methylene-bis-(diisopropylsilyl) chloride ($MDPSCl_2$, **116**)[16] and the di-*tert*-butylsilanedyil (DTBS, **117**).[20] Similarly hindered, they enable direct alkylation of the 2'-hydroxyl. Nevertheless, **115** is less stable under basic conditions required for the alkylation step than **116**, due to the inductive effect of the oxygen atom connecting the two silicon atoms.[21]

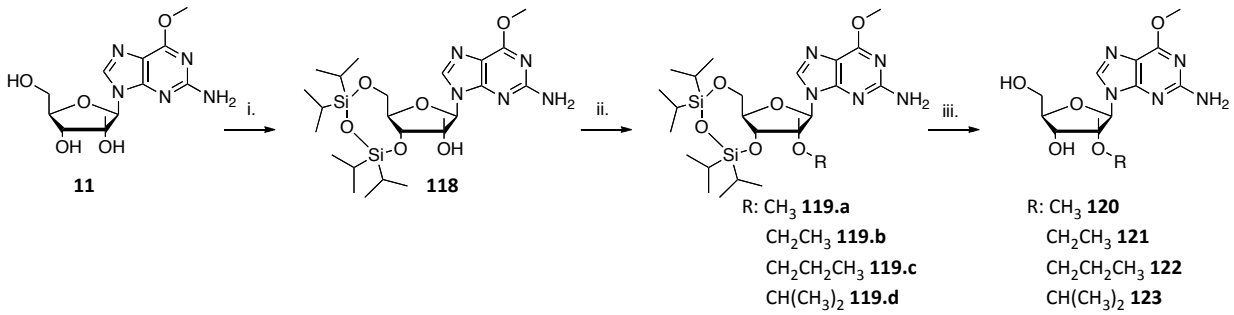


115 **116** **117**

Figure 5.3. Structures of silicon-based protecting groups.

2.2.1. Use of Markiewicz protecting group

The Markiewicz's reagent **115** was first used as protecting group, previous to the 2'-*O*-alkylation, since its isostere **116** is not commercially available. The three-step synthesis, consisted of protection-, alkylation- and deprotection- steps, is described in Scheme 5.1.



11 **118**

R: CH_3 **119.a**
CH_2CH_3 **119.b**
$CH_2CH_2CH_3$ **119.c**
$CH(CH_3)_2$ **119.d**

R: CH_3 **120**
CH_2CH_3 **121**
$CH_2CH_2CH_3$ **122**
$CH(CH_3)_2$ **123**

(i. anh. pyridine, $TPDSCl_2$ (1.2 eq), 0 °C, 30 min, rt, overnight, 45%; ii. anh. DMF, molecular sieves 3Å, RI (1.2 eq), NaH (60% dispersion in mineral oil, 1.2 eq), 0 °C, 1hr 30 min; iii. anh. THF, TBAF (2.2 eq), 2hr 30 min, rt.)

Scheme 5.1. Synthetic route to 2'-*O*-alkyl 2'-*C*-methyl-6-*O*-methyl guanosine derivatives.

Protection of **11** was accomplished in moderate yield (45%), and **118** was recovered after purification on silica gel chromatography. Alkylation of the unprotected 2'-hydroxyl group was performed accordingly to the literature,[22] via Williamson synthesis, using the desired alkyl iodide and sodium hydride as a strong base.[21] We were confident that thanks to the inductive effect of the 6-*O*-methyl of **118**, the *N*-1 nitrogen would be not undergo methylation under such basic conditions.

Similarly to guanosine derivatives,[21] only 2'-*O*-alkylation occurred without obtaining the *N*-2-alkylated side products. 2'-*O*-methyl (**119.a**), 2'-*O*-ethyl (**119.b**) and 2'-*O*-propyl (**119.c**) intermediates were obtained in poor to moderate yields after purification (5% to 57%, Table 5.2), whereas the 2'-*O*-isopropyl intermediate (**119.d**) was not formed and only the starting material **118** was recovered. This observation suggests that the 2'-position is probably already too hindered and thus, might not favour the substitution of a branched alkyl group on the *α*-phase. The poor yield observed for **119.a** results from the migration of the Markiewicz protecting group from 3', 5'- to 2', 3'- positions or its partial cleavage.[23]

Deprotection of intermediates **119.a-c** was performed using tetrabutyl ammonium fluoride (TBAF) as desilylating agent, which releases the nucleophilic fluorine ion in the medium. Desired molecules **120**, **121** and **122** were recovered, but only the last two were pure enough to be sent for biological evaluation in HCV replicon cells.

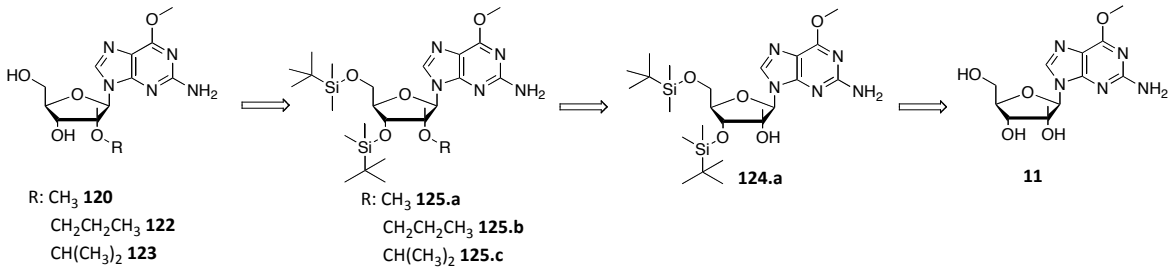
Step / R	CH_3	CH_2CH_3	$CH_2CH_2CH_3$	$CH(CH_3)_2$
ii.	5%	44%	57%	0%
iii.	20%	55%	82%	-

Table 5.2. Yields of alkylation (ii.) and deprotection (iii.) steps.

The results of this first approach were quite disappointing; either low yields, due to the instability of the Markiewicz protecting group, or no reaction was achieved. Thus, since higher quantities of 2'-*O*-methyl, 2'-*O*-propyl and 2'-*O*-isopropyl nucleosides were needed, in order to prepare a ProTide for each 2'-*O*-modified nucleoside, another approach using *tert*-butyl dimethyl silyl group (TBDMS) at 3'- and 5'- position was considered.

2.2.2. Use of *tert*-butyl dimethyl silyl protecting group

The stability in basic conditions, the ease of introduction and the high yields reported for the protection of ribose hydroxyls[24] with the *tert*-butyl dimethyl silyl (TBDMS) group, led us to investigate the latter for selective 2'-*O*-alkylation of 2'-*C*-methyl-6-*O*-methyl guanosine **11**. The retrosynthetic pathway is described in Scheme 5.2.



Scheme 5.2. Retrosynthetic pathway to 2'-*O*-alkylated 2'-*C*-methyl-6-*O*-methyl guanosine derivatives.

Contrary to the Markiewicz reagent, *tert*-butyl dimethyl silyl is not a regiospecific group, however the methyl at *C*-2' hinders that position, thus allowing the 3'- and 5'-hydroxyls to be selectively protected. In this case, the nucleophilic exocyclic amine of the heterobase reacted equally with the 4.3 equivalents of *tert*-butyl dimethyl silyl introduced, affording a mixture of 3',5'-TBDMS protected (**124.a**) and 3',5'-TBDMS-*N*-2-TBDMS protected (**124.b**, Figure 5.4) nucleosides, in respectively 45% and 55% yield.

The treatment of **124.a** with sodium hydride (NaH) and 3.0 equivalents of isopropyl- or propyl-iodide was selective at the 2'-position, affording **125.b** and **125.c**. Deprotection step afforded **122** and **123** in respectively 49% and 20% over two steps. These yields support the outcome previously mentioned concerning the difficulty of introducing branched group at the 2'-position already hindered by the 2'-*C*-methyl group and the 3'-protected hydroxyl.

As reported in the literature,[21] alkylation conditions with methyl iodide (MeI) resulted in double alkylation at the *N*-2 and *C*-2' (**125.d**), however, in extremely low yield (Table 5.3). Decreasing the number of equivalents of MeI from 4.3 to 1.2, introduced at low temperature[25] was also unsuccessful. Other methylation reactions performed at low temperature with electrophilic gaseous methyl chloride (MeCl) and sodium hexamethyldisilazane (NaHMDS)[21] resulted in no methylation and in both full and partial deprotection at 3'- and 5'- positions of 2'-*C*-methyl-6-*O*-methyl guanosine.

	Attempt 1	*Attempt 2*	*Attempt 3*	*Attempt 4*
SM	**124.a**	**124.a**	**124.a**	**124.b**
conditions	MeI (3.0 eq), NaH, 1 hr, 0 °C	1.2 eq MeI, 4 hrs, - 50 °C to - 15 °C	MeCl (g), NaHMDS (3.0 eq), 4 hrs, - 20 °C to RT	MeI (3.0 eq), NaH, 1 hr, 0 °C
results and comments	**125.d** 3-4%	**124.a**	Mixture of partially and fully deprotected SM	**125.e** 5%

Table 5.3. Conditions attempted for selective 2'-*O*-methylation.

A last experiment was attempted starting from **124.b** and using standard alkylation conditions described previously. In this case as well, fully alkylated product **125.e** (Figure 5.5) was recovered. Deprotection of **125.e** provided *C*-2' and *N*-2 methylated 2'-*C*-methyl-6-*O*-methyl guanosine **126** (Figure 5.4).

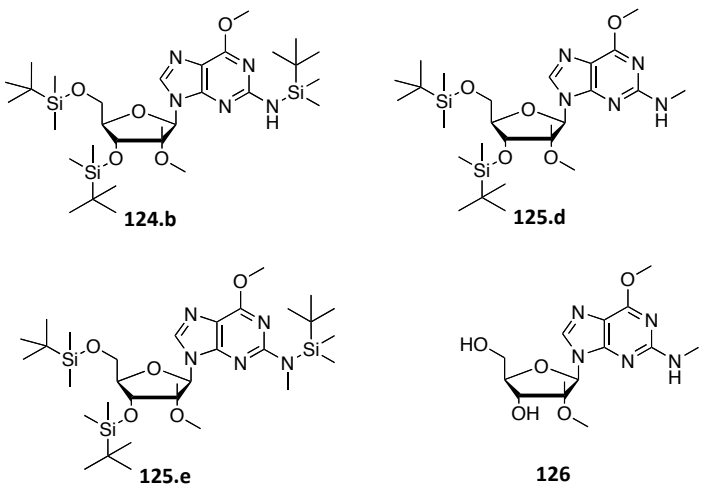
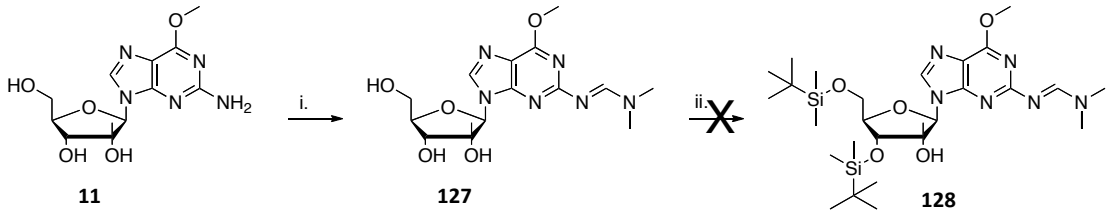


Figure 5.5. Structures of **124.b**, **125.d**, **125.e** and **126**.

From these results, it proved to be necessary to protect the acidic exocyclic amine of the heterobase to avoid its inherent reactivity towards methylation.[21, 26] As *N,N*-dialkyl formamidines are extensively described in the literature and widely used as a protecting group for the exocyclic primary amine of nucleosides,[27, 28] 2'-*C*-methyl-6-*O*-methyl guanosine **11** was protected with dimethyl formamide dimethyl acetal[29] at room temperature overnight to yield **127** (Scheme 5.3).



(i. anh. DMF, dimethylformamide dimethyl acetate (5.0 eq), rt, overnight, 14%; ii. anh. DMF, imidazole (8.1 eq), TBDMSCl (4.3 eq), rt, overnight.)

Scheme 5.3. Pathway to 3', 5'-TBDMS and *N*-2-formamidine protected 2'-*C*-methyl-6-*O*-methyl guanosine.

Then 3'- and 5'- hydroxyls were protected with TBDMS protecting group using the previous conditions reported. However, residues recovered from the flash chromatography did not correspond to **128**, but only to **124.a** (Scheme 5.2) along with **129** (Figure 5.6) were isolated.

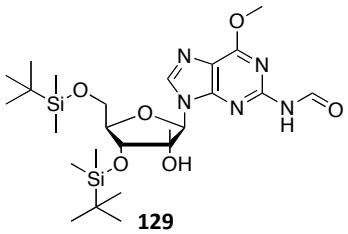
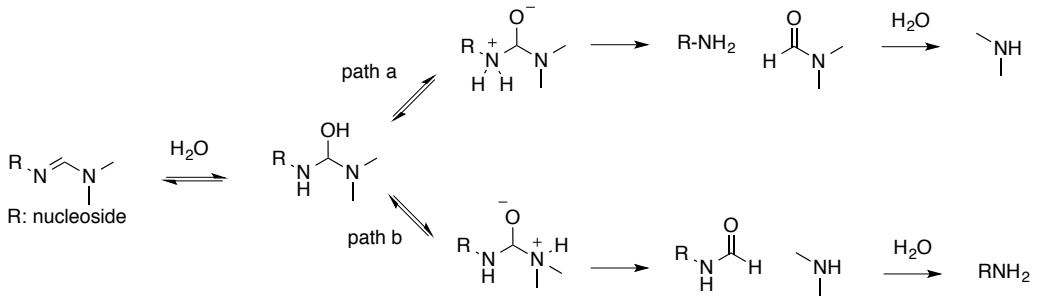


Figure 5.6. Structure of partially hydrolysed *N*-2-formamidine intermediate.

The structure of **129** was confirmed by mass ($M+Na^+$: 590) and ^{1}H-NMR. The latter showed indeed signals characteristic of the partially cleaved formamidine (doublets at 9.54 ppm (NHCHO) and 8.19 ppm (NHCHO), with a coupling constant J^3 of 10.5 Hz). This intermediate was not useful for selective 2'-*O*-methylation, due to the acidic proton remaining on the *N*-2. A second attempt was performed by protecting the exocylic amine of 3',5'-TBDMS protected 2'-*C*-methyl-6-*O*-methyl guanosine **124.a** with dimethyl acetate dimethylformamide. Unfortunately, similar results were obtained.

Hydrolysis of the formamidine protecting group has been reported in the literature, and its mechanism has been extensively studied.[29, 30] Governed by the acidity of each nitrogen atom of the hemiorthoamide intermediate and by the electron-donating character of the methyl groups, the hydrolysis is fast and should evolve towards path a (Scheme 5.4) as for guanosine nucleosides.[27] However in this case, the electron donating character and inductive effect of the 6-methoxy group of the heterobase influences the basicity of the formamidine nitrogens and as a result, proceeds through

path b (Scheme 5.4). Nevertheless the total hydrolysis of the exocyclic amine still requires basic conditions.[27]

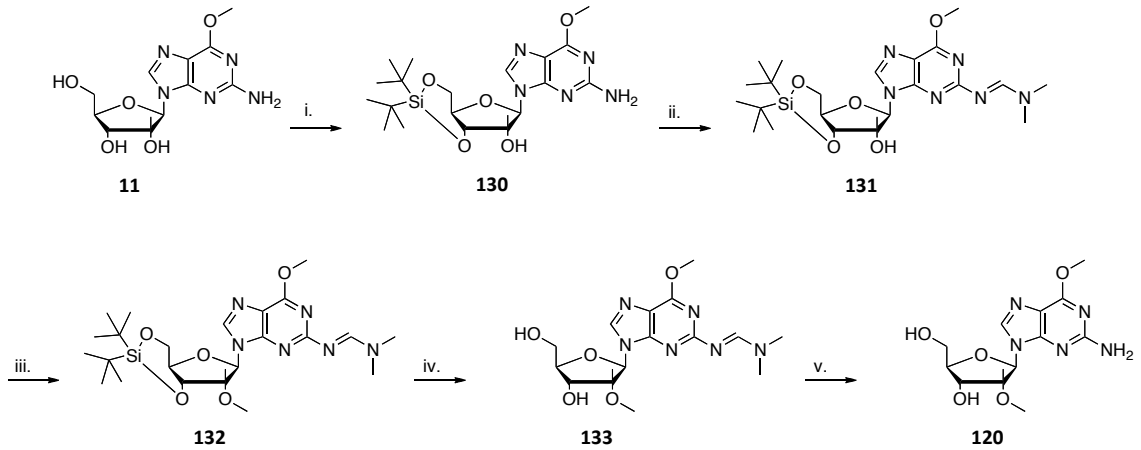


Scheme 5.4. Hydrolysis of N-2 formamidine protecting group.[27]

Drier conditions were then required to enable stability of the required *N*-2-formamidine protecting group.

2.2.3. Use of di-*tert*-butylsilane and *N*-2 formamidine protecting group

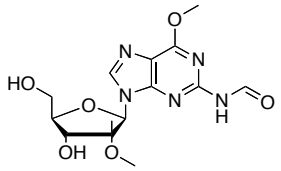
Recently, Mukobata *et al.* published the synthesis of *N*-2-formamidine-2'-*O*-methyl guanosine combining the use of di-*tert*-butylsilane (DTBS) for 3',5'-hydroxyl protection and N-2 formamidine.[27] This approach was applied to 2'-*C*-methyl-6-*O*-methyl guanosine **11** as described in Scheme 5.5.



(i. anh DMF, di-tert-butylsilyl-ditriflate (1.1 eq), 0 °C, 1hr 30min, 26%; ii. anh. DMF, N,N-dimethyl formamide dimethyl acetal (4.0 eq), rt, overnight, 94%; iii. anh. DMF, molecular sieves, NaH (60% dispersion in oil, 1.2 eq), MeI (6.0eq), 0 °C, 1hr, 75%; iv. anh. THF, anh. NEt_3 (1.8 eq), $NEt_3.3HF$ (3.5 eq), rt, 1 hr, 35%; v. THF/H_2O (1:1), KOH (powdered, 4.0 eq), rt, 3-4 hrs, 98%.)

Scheme 5.5. Towards the synthesis of 2'-*O*-methyl-2'-*C*-methyl-6-*O*-methyl guanosine **120** using DTBS and formamidine as protecting groups.

The 5'- and 3'-hydroxyl groups of the ribose were protected using DTBS to afford **130** after precipitation in water. Protection of the *N*-2 exocyclic amine was carried out with dimethylformamide dimethyl acetal as previously mentioned, and after basic aqueous work up, **131** was directly used crude for the next step. Methylation using standard alkylation conditions was performed in 75% yield (step iii, Scheme 5.4), followed by subsequent deprotection of the DTBS group of **132** with triethylamine trihydrofluoride ($NEt_3{\cdot}3HF$). The latter being quite hygroscopic, partial hydrolysis of the formamidine occurred along with deprotection of the hydroxyls groups (**133.a**, Figure 5.7) in moderate yield after purification. Once again, in this case, the partial hydrolysis of the formamidine group tends to proceed *via* path b (Scheme 5.4), in contrast with path a described in the literature for guanosine.[27] Contrary to silicon-based protecting groups, the *N*-2-formamidine group has never been reported as unstable during flash chromatography purification. Subsequently, the presence of the 6-*O*-methyl group of the heterobase influences the hydrolysis pathway.



133.a

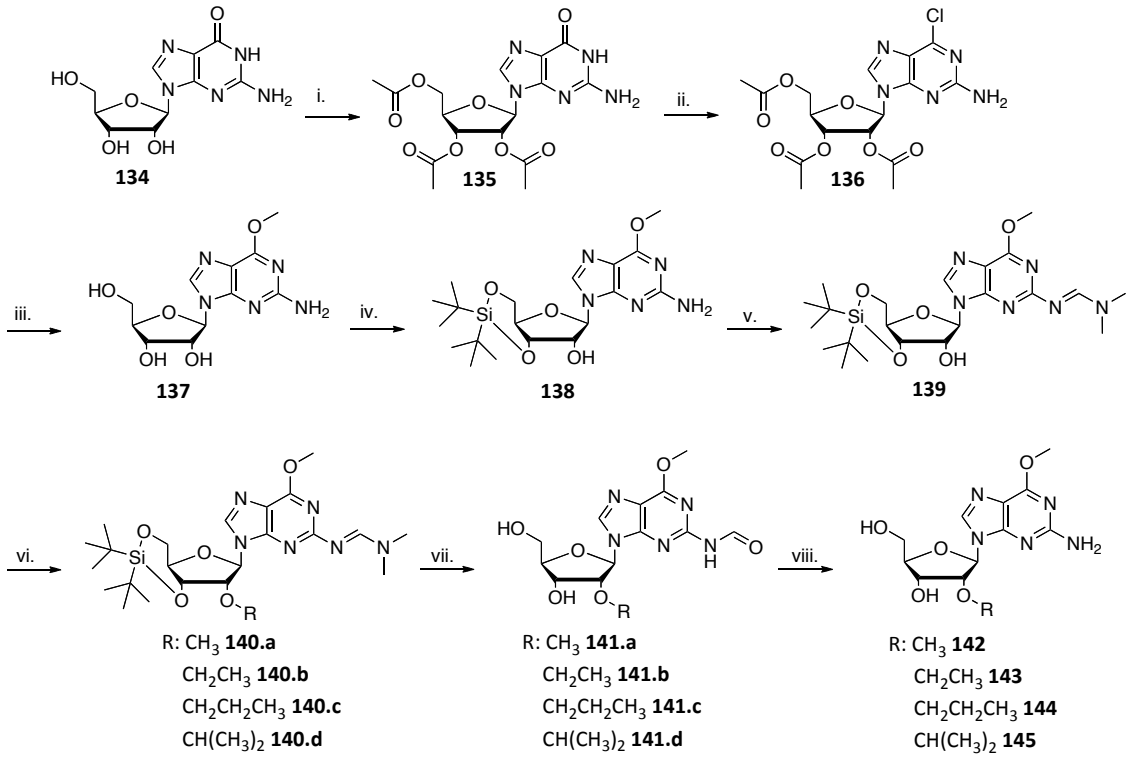
Figure 5.7. Structure of *N*-2 partially hydrolysed formamidine group of **133.a**.

Total hydrolysis of **133.a** to recover the exocyclic amine, required strong basic conditions, such as potassium hydroxide (KOH),[27] to recover almost quantitatively (98%) the desired 2'-*O*-methyl-2'-*C*-methyl-6-*O*-methyl guanosine **120**.

This new route was relatively easy and high yielding compared to previous methods, and only two purifications were necessary to recover 2'-*O*-alkylated-2'-*C*-methyl-6-*O*-methyl guanosine derivatives. Despite the partial hydrolysis occurring during the deprotection of di-*tert*-butylsilane (DTBS) group and regeneration of the acidity at the *N*-2 nitrogen after the alkylation, the selectivity at the 2'-position is maintained.
2'-*O*-alkylated-2'-*C*-methyl guanosine derivatives **120**, **121**, **122**, **123** and fully methylated **126** were sent to Bristol-Myers Squibb for biological evaluation in HCV cell-based assays.

2.3. 2'-*O*-alkyl modifications of 6-*O*-methyl guanosine

2'-*O*-alkylated derivatives of 6-*O*-methyl guanosine were synthesised with the purpose to evaluate the steric hindrance allowed around the 2'-position in the pocket of the HCV NS5B polymerase. The synthetic approach required first the formation of 6-*O*-methyl guanosine **137** in a simple three-step synthesis (Scheme 5.6), followed by the protection of both 3',5'-hydroxyls (step iv.) and exocyclic amine (step v.), then alkylation (step vi.) and deprotection steps (vii. and viii.) as reported previously.



(i. anh. ACN, DMAP (0.1 eq), anh. NEt_3 (4.0 eq), Ac_2O (3.6 eq), rt, overnight, 87%; ii. anh. ACN, BTEA-Cl (2.0 eq), N,N-dimethylaniline (1.1 eq), $POCl_3$ (5 eq), reflux, 2hrs, 72%; iii. anh. MeOH, NaOMe (6.6 eq), rt, overnight, 95%; iv. anh DMF, di-tert butylsilyl-ditriflate (1.1 eq), 0 °C, 1hr 30min, 73%; v. anh. DMF, N,N-dimethyl formamide dimethyl acetal (4.0 eq), rt, overnight, 84%; vi. anh. DMF, molecular sieves, NaH (60% dispersion in oil, 1.2 eq), RI (6.0eq), 0 °C, 1-2 hrs; vii. anh.THF, NEt_3 (1.8 eq), triethylamine trihydrofluoride (3.5 eq), rt, 1 hr; viii. KOH (4.0 eq), H_2O/THF, rt, 3hrs.)

Scheme 5.6. Pathway to 2'-*O*-alkylated 6-*O*-methyl guanosine derivatives.

Starting from guanosine **134**, protection of the ribose hydroxyls (**135**) was required before chlorinating the 6-position of the nucleobase to afford **136**. Displacement of the 6-chlorine was performed simultaneously with deprotection of the hydroxyl groups using 6.6 equivalents of sodium methoxide (NaOMe). 6-*O*-Methyl guanosine **137** was obtained in 84% over three steps.[31] Then the approach using DTBS and *N*-2

formamidine protecting groups, as described previously for alkylation of 2'-*C*-methyl-6-*O*-methyl guanosine, was successfully applied for recovery of 2'-*O*-alkylated-6-*O*-methyl guanosine derivatives **142-145**.

The protection steps were higher yielding than in the case of 2'-*C*-methyl-6-*O*-methyl guanosine, affording compounds **138** and **139** in respectively 73% and 84% yield. While the alkylation steps (step vi., Scheme 5.6) were successful and rather high yielding (**140.a-d**, Table 5.4), partially *N*-2-hydrolysed species (**141.a-d**) resulting from the deprotection of di-*tert*-butylsilane group were recovered in moderate yield (step vii., Table 5.4). Formation of similar species have previously been described with 2'-*O*-alkyl-2'-*C*-alkyl-6-*O*-methyl guanosine derivatives. Hence, the need of extremely dry conditions to keep the *N*-2-formamidine intact or the necessity of more basic conditions to obtained totally hydrolysed species **142.a-d**.

Step / R	CH_3	CH_2CH_3	$CH_2CH_2CH_3$	$CH(CH_3)_2$
vi.	70%	67%	80%	61%
vii.	10%	37%	37%	25%
viii.	90%	88%	88%	60%

Table 5.4. Yields of alkylation step (vi.) and deprotection steps (vii. and viii.).

Characteristic ^{13}C-NMR and ^{1}H-NMR peaks of **140.a-d** and **141.a-d** in deuterated chloroform are reported in Table 5.5 (chemical shift in ppm). At first glance, it appears that the hindrance of the alkylating group at *C*-2' provokes an upfield shift of the peaks corresponding to the methyl of the formamidine group of **140.a-d** (NCHN($\underline{CH_3}$)$_2$) and the formaldehydic group of **141.a-d** (NH$\underline{CH}$O). It is quite surprising that such remote groups are affected by the steric bulk of the 2'-*O*-alkyl substituents. The coupling constant J^3 corresponding to the partially hydrolysed formamidine group in molecules **141.a-d** varied between 9.5 and 10 Hz, which is in correlation with the data obtained for the 2'-*C*-methyl derivative **129**.

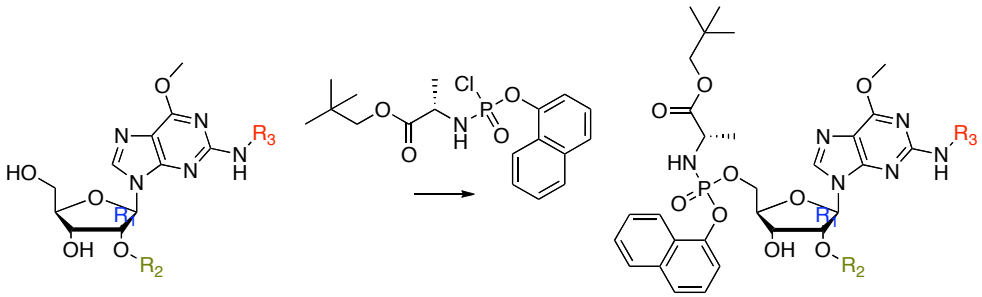
6-*O*-Methyl guanosine **137**, intermediate **141.a** and 2'-*O*-alkylated-6-*O*-methyl guanosine derivatives **142**, **143**, **144** and **145** were sent for biological testing in HCV replicon assays.

		140		141	
		$NCHN(\underline{CH_3})_2$	$NCHN(CH_3)_2$	NHCHO	NHCHO
a	^{1}H	3.18, 3.16	8.64	9.54	9.49
	^{13}C	41.23, 40.94	158.39	-	165.58
b	^{1}H	3.16, 3.13	8.64	9.50	9.51
	^{13}C	41.09, 39.18	158.38	-	163.02
c	^{1}H	3.02, 3.00	8.50	8.67	9.49
	^{13}C	36.18, 34.74	158.19	-	163.17
d	^{1}H	2.89, 2.81	8.57	8.39	9.51
	^{13}C	35.55, 35.18	158.83	-	162.88

Table 5.5. Chemical shifts δ (ppm) of characteristic peaks of *N*-2-group.

2.4. 2'-*O*-alkyl modified ProTides

In order to overcome eventual poor cell permeation or poor phosphorylation, *α*-naphthyl *L*-alanine neopentyl ester phosphoramidates of 2'-*O*-alkylated-6-*O*-methyl guanosine derivatives were synthesised following the Uchiyama procedure previously mentioned (Scheme 5.7).



*R_1: CH_3, R_3: H, R_2: CH_3 **120**
CH_2CH_3 **121**
$CH_2CH_2CH_3$ **122**
$CH(CH_3)_2$ **123**
*R_1 = R_2 = R_3: CH_3 **126**
*R_1 = R_3: H, R_2: CH_3 **142**
CH_2CH_3 **143**
$CH_2CH_2CH_3$ **144**
$CH(CH_3)_2$ **145**
H **137**
*R_1: H, R_2: CH_3, R_3: CHO **141.a**

*R_1: CH_3, R_3: H, R_2: CH_3 **146**
CH_2CH_3 **147**
$CH_2CH_2CH_3$ **148**
$CH(CH_3)_2$ **149**
*R_1 = R_2 = R_3: CH_3 **150**
*R_1 = R_3: H, R_2: CH_3 **151**
CH_2CH_3 **152**
$CH_2CH_2CH_3$ **153**
$CH(CH_3)_2$ **154**
H **155**
*R_1: H, R_2: CH_3, R_3: CHO **156**

(anh. THF, tBuMgCl (1.2 eq), α-naphthyl L-Alanine neopentyl phosphochloridate (2.0 eq), rt, overnight.)

Scheme 5.7. Synthetic route to 2'-*O*-alkylated *α*-naphthyl *L*-alanine neopentyl ester ProTides.

Compounds **146-156** were isolated as mixtures of diastereoisomers (1% to 6%) except in the case of the more lipophilic **150** (20%). The presence of a methyl at *C*-2' substituent did not improve the solubility or the yield. 6-*O*-methyl guanosine **137** was extremely insoluble in tetrahydrofuran and the addition of pyridine in the reaction mixture enable better solubilisation. Nevertheless **155** was obtained in 1% yield. The low yields of purified products here may also reflect the need for repeated chromatographic purification, hence the necessity of finding better reaction conditions for such polar molecules.

Surprisingly no 3'-ProTide was isolated from nucleosides **137**, **142-145** under these reaction conditions. Usually, the use of *tert*-butyl magnesium chloride does not provide regioselective phosphorylation at the 5'-position,[32] which can instead be achieved with *N*-methylimidazole (NMI).[33]

With the exception of **154** whose purity was not satisfactory after several purifications, 2'-*O*-alkylated ProTides of the 2'-*C*-methyl-6-*O*-methyl guanosine and 6-*O*-methyl guanosine families were sent for HCV replication activity.

2.5. Biological testing in HCV replicon-based assays

Bristol-Myers Squibb performed the biological evaluation of 2'-*O*-alkylated nucleosides and phosphoramidates. The inhibitory activity of HCV replication (EC_{50}) and cellular toxicity (CC_{50}) were evaluated in replicon-based assays, and the results are reported Table 5.6.

2'-C-methyl-6-O-methyl guanosine ($R_1 = CH_3$)

R_2	*nucleosides*				*ProTides*			
	Cpd	ClogP*	EC_{50} (µM)	CC_{50} (µM)	**Cpd**	ClogP*	EC_{50} (µM)	CC_{50} (µM)
CH_3	**120**	0.13	>10	>100	**146**	4.09	2.37	47.38
	126	0.97	>40	>100	**150**	4.92	12.05	14.44
CH_2CH_3	**121**	0.52	42.6	>100	**147**	4.48	>2.5	>100
$CH_2CH_2CH_3$	**122**	1.05	>100	>100	**148**	5.01	20.87	30.23
$C(CH_3)_3$	**123**	0.83	>10	>100	**149**	4.79	9-10	>100

6-O-methyl guanosine ($R_1 = H$)

R_2	*nucleosides*				*ProTides*			
	Cpd	ClogP	EC_{50} (µM)	CC_{50} (µM)	**Cpd**	ClogP*	EC_{50} (µM)	CC_{50} (µM)
H	**137**	-1.19	>10	>100	**155**	2.76	>10	N.D
CH_3	**142**	-0.38	>10	>100	**151**	3.57	>10	N.D
	141.a	-0.03	>10	>100	**156**	3.92	2.02	31
CH_2CH_3	**143**	0.005	>10	>100	**152**	3.96	2.22	33
$CH_2CH_2CH_3$	**144**	0.53	>10	>100	**153**	4.49	2.92	37
$C(CH_3)_3$	**145**	0.31	>10	n.d	**154**	4.27	**	**

* calculated ClogP from ChemDrawUltra 11.0.
** purity not satisfactory for biological testing.
Table 5.6. Inhibitory activity and cytotoxicity of 2'-*O*-alkylated nucleosides and ProTides.

The data suggest that no nucleoside (**120-123**, **126**, **137**, **141-145**) is inhibitor of HCV RNA replication or toxic towards cells. 2'-*O*-alkylated nucleosides of both 2'-*C*-methyl-6-*O*-methyl and 6-*O*-methyl guanosine families are likely to be too hydrophilic to permeate cell membranes since their calculated ClogP lies between -1.19 and 1.05. Despite increased liphophilicity (2.76 < ClogP < 5.01), the poor potency displayed by the ProTides of both families indicates that the bioactivation to their corresponding 5'-monophosphate is probably not efficient, or the 5'-triphosphates of 2'-*O*-alkylated derivatives might not be efficient inhibitors of HCV RNA replication. In general, the 2'-*O*-alkyl substitution was better tolerated without the 2'-*C*-methyl substituent, and however less lipophilic, 2'-*O*-alkyl-6-*O*-methyl guanosine ProTides appeared slightly more efficient in inhibiting HCV RNA replication than their 2'-*O*-alkyl-2'-*C*-methyl-6-*O*-methyl guanosine analogues.

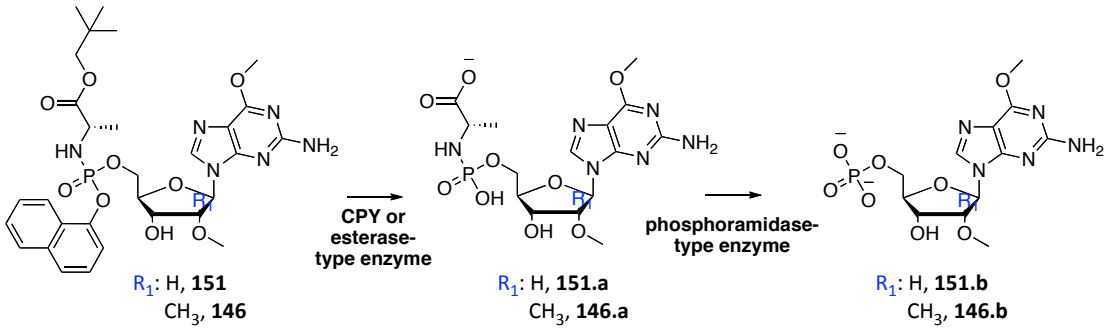
While increasing the hindrance of the 2'-*O*-alkyl substituent tends to decrease the ProTide inhibitory activity against HCV RNA replication (**146-149**) within the 2'-*C*-methyl-6-*O*-methyl guanosine family, it does not seem to affect the activity of 6-*O*-methyl guanosine ProTides (**151-155**) since similar values of EC_{50} and CC_{50} are

reported. Hence, among the 2'-*C*-methyl family, there is an optimal hindrance tolerated at the α-face, since nucleosides (**122** and **123**) and ProTides (**148** and **149**) bearing 2'-*O*-alkyl bigger than ethyl (**120/121** and **146**/**147**) are relatively less potent in HCV replicon assays.

O-2, *N*-2 dimethylated ProTide **150** displayed high cellular toxicity with a selectivity index of 1. The ProTide bearing partially hydrolysed formamidine group (**156**) neither boosted the activity nor decreased the cytotoxicity seen among the 6-*O*-methyl guanosine family. As a result, the modifications on the exocyclic *N*-2 do not favour efficient metabolism, even if the cellular permeation may be enhanced.
In order to understand better these results, the mechanism of activation of the 2'-*O*-alkylated ProTides was investigated.

2.6. Mechanistic studies

The yield of 2'-*O*-alkyl ProTide synthesis being extremely low and the quantity recovered being just enough for biological evaluation, enzymatic experiments could not be performed. However, docking experiments were carried out with 2'-*O*-methyl Protides of both families (**146** and **151**), bearing the smallest group introduced at *C*-2', to investigate the potential intracellular release of their corresponding monophosphate form (**146.b** and **151.b**, Scheme 5.8) which would then undergo several phosphorylation steps to afford the corresponding 5'-triphosphate species.



Scheme 5.8. Putative activation of 2'-*O*-methylated ProTides (**151** and **146**) into their 5'-monophosphate form (**151.b** and **146.b**).

2.6.1. Molecular docking with cathepsin A enzyme

As mentioned earlier, cathepsin A is an esterase-type enzyme, which may be responsible for the cleavage of the amino acid ester, during the first step of bioactivation of the phosphoramidates.

Molecular modelling docking was performed with compounds **146** and **151** in order to investigate the formation of their corresponding phosphoramidate monoesters **146.a** and **151.a** (Figure 5.8).

Both S_P and R_P isomers of **146** and **151** were docked into cathepsin A, however the R_P isomers did not fit into the catalytic site without provoking steric clash with the surface of the pocket (results not shown), suggesting that only the S_P isomers may be processed and are eventually responsible for the observed poor activity in HCV replicon assays.

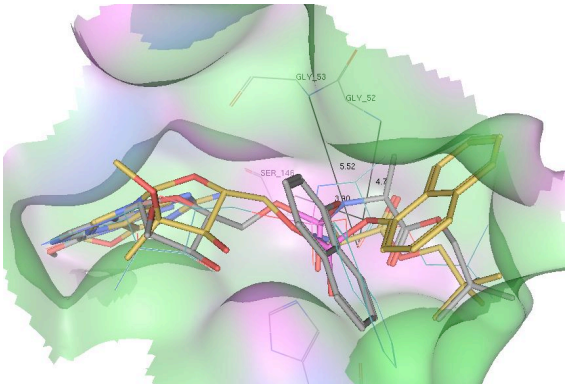


Figure 5.8. S_P isomers of **146** and **151** docked into cathepsin A.
(blue line: S_P isomer of **15.d**, grey stick: S_P isomer of **151**, yellow stick: S_P isomer of **146**)

S_P isomers of **146** and **151** fit in the active site of cathepsin A enzyme (Figure 5.8), however in both case the carbonyl group of the ester moiety lies far from the catalytic residues Ser146, Gly52 and Gly53 in comparison with **15.d** (*α*-napthyl *L*-Alanine neopentyl 2'-*C*-methyl-6-*O*-methyl guanosine ProTide). This observation predicts slow cleavage of the ester group, resulting in slow release of the phosphoramidate monoester, and only from the S_P isomers. Comparing the 2 compounds, it appears that the carbonyl group of **151** S_P isomer lies further outside the catalytic pocket than **146** S_P isomer, hence in this case the 2'-*C*-methyl substituent seems to have a positive effect and may result in relatively faster delivery of the phosphoramidate monoester **146.a**.

2.6.2. Molecular docking with human Hint-1 enzyme

The second enzymatic step of ProTide activation was investigated and the P-N bond cleavage of **146.a** and **151.a** was predicted by molecular docking studies using human Hint-1 enzyme (Figure 5.9).

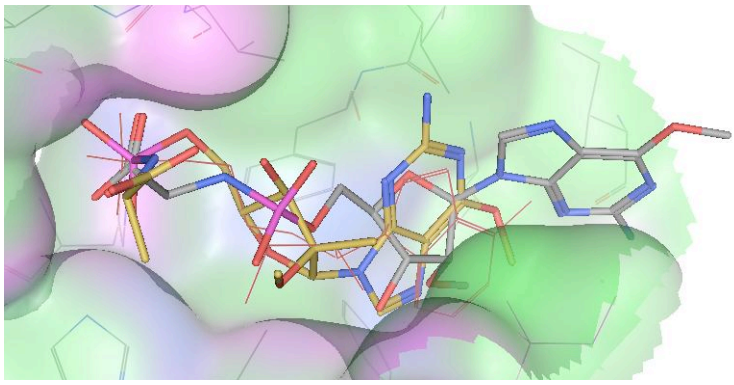


Figure 5.9. Docking of phosphoramidate monoesters **146.a** and **151.a** in human Hint-1 enzyme (yellow: **146.a**, grey: **151.a**, red line: AMP).

In this case, the docking suggests that the 2'-*C*-methyl substituent is once again responsible for the better fitting of the P-N bond inside the catalytic pocket, in comparison with the natural substrate AMP. As a result, the 2'-*O*-methyl-6-*O*-methyl guanosine derivatives should deliver the corresponding 5'-monophosphate species (**151.b**, Figure 5.9) in a slower rate compared to their 2'-*C*-methyl analogues (**146.b**). This observation correlates with the anti-HCV activity in replicon assays, since the 2'-*C*-methyl derivative **146** is more potent than **151**.

Taking into consideration the fact that the hindrance of the 2'-*O*-alkyl group does not affect much the activity of 6-*O*-methyl guanosine analogues, it is likely that the presence of the methyl on the *β*-face provides faster metabolism to the 5'-triphosphate.

2.7. Conclusion and perspectives

The use of 2'-*O*-alkylated nucleosides has been widely used in medicinal chemistry, however 2'-*O*-alkylation has never been extensively investigated for HCV therapy.[12, 13] Despite numerous synthetic approaches described in the literature,[11] optimisation of 2'-*O*-alkylation appeared to be difficult, due to side reactions at the exocyclic *N*-2 and 3'-hydroxyl.[27] Aiming at investigating the allowance of hindrance at the 2'-position in the NS5B polymerase, a new approach was developed to synthesise 2'-*O*-alkylated derivatives of 2'-*C*-methyl-6-*O*-methyl guanosine **11** and 6-*O*-methyl guanosine **137**.

Despite the modification at *C*-2', the molecules synthesised were extremely polar making the purification steps difficult and resulting in low yields during the formation of corresponding phosphoramidates. While 2'-*O*-alkylated nucleosides do not exhibit activity in replicon assays, the ProTides slightly boost the potency though increase the cellular toxicity.

Varying the bulkiness of the alkyl group does not affect much the potency, however in the case of the 2'-*C*-methyl-6-*O*-methyl family, the ethoxy at the 2'-position appears to be the biggest group tolerated in term of potency boost.

Molecular modelling using cathepsin A predicts that in both families, the S_P isomers are more likely to be processed than R_P isomers, and might deliver slowly the phosphoramidate monoester. The 2'-*C*-methyl-2'-*O*-methyl-6-*O*-methyl guanosine phosphoramidate monoester species **146.a** also lie in a good position within the active site of human Hint-1 enzyme for an efficient P-N bond cleavage compared to the 2'-*O*-methyl-6-*O*-methyl guanosine phosphoramidate monoester analogues. The slow delivery of the 2'-*O*-alkylated 5'-monophosphates might correlates with the poor inhibitory activity and poor selectivity index displayed during replicon assays.

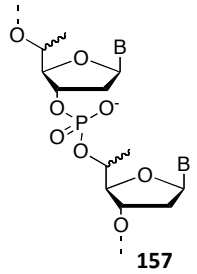
In conclusion, it is likely the 2'-*O*-alkyl substitutions would affect greatly the metabolism and might not allow an efficient intracellular delivery of the potentially active 2'-*O*-alkylated triphosphates.[17] Future expansion of 2'-*O*-alkyl modifications to other family of nucleosides, that have previously shown activity in HCV replicon, would be of interest in order to define the bulkiness allowed in the catalytic pocket of HCV NS5B polymerase. It may be also of interest to tune the ProTide, varying the amino acid and ester moieties, to eventually achieve more efficient metabolism.

3. Modifications at *C*-5' of 6-*O*-methyl guanosine derivatives

3.1. Introduction

Modifications at *C*-5' of nucleosides have mainly been explored for the synthesis of RNA and DNA and their incorporation into oligomeric structures for improvement of potency and efficacy of antisense compounds.[34] Because the 5'-hydroxyl of the nucleoside is prone to metabolism in-*vivo*,[35] the development of 5'-modified nucleosides has become of interest. Adding a steric bulk adjacent to the 5'-primary hydroxyl should improve the pharmacokinetic profile and reduce the metabolic lability.[35]

Particularly, the 2'-deoxy-5'-methyl DNA[7] (**157**, Figure 5.10) bearing a methyl at the 5'-position next to the phosphodiester linkage, is known to reduce recognition by nuclease.[36, 37] Moreover the 5'-methyl facilitates cellular uptake by slightly increasing the lipophilicity.[7]



157

Figure 5.10. Structure of 5'-methyl DNA.[7]

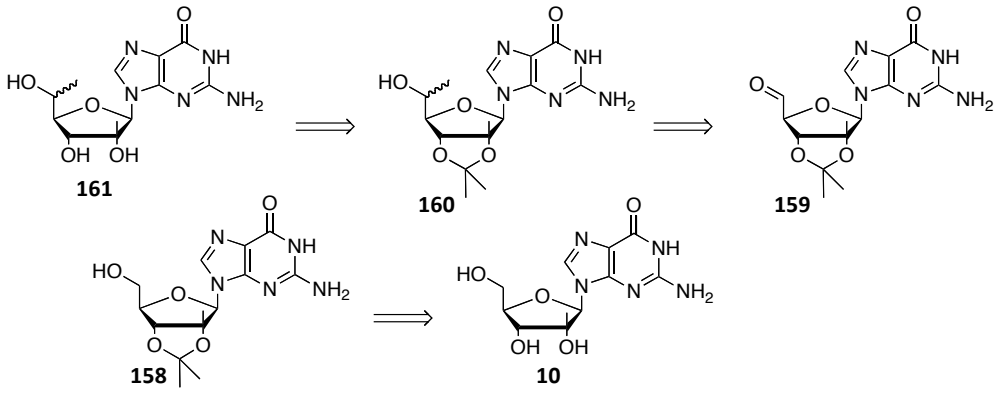
Numerous pathways have been reported for the synthesis of 5'-methyl modified nucleosides and most of them described the synthesis of 5'-methyl *via* oxidation of the 5'-hydroxyl at the ribose level before condensation with the nucleobase.[38-40] In particular, Alios developed 5'-*C*-methyl-2'-*C*-methyl guanosine and other nucleoside analogues *via* oxidation at the riboside level.[41]

Preliminary docking studies into HCV NS5B polymerase predicted that a nucleoside triphosphate bearing a methyl group at C-5' may be a good substrate of the enzyme. Moreover, in an attempt to investigate the effect of 5'-methyl group towards the reduction of dephosphorylation by phosphatase[42] occurring before entrance into the cells, and eventually increasing the activity against HCV RNA replication, it was decided to develop 5'-*C*-methyl modification starting from the 2'-*C*-methyl guanosine nucleosides and further transpose the synthetic route to 6-*O*-methyl-2'-*C*-methyl guanosine derivatives.

3.2. Synthetic route to 5'-*C*-methyl-2'-*C*-methyl guanosine

The common method for methylation at the 5'-position of a nucleoside is reported *via* oxidation of the 5'-hydroxyl to aldehyde. This intermediate is often used for introduction of substituents at C-4' position.[43] Nevertheless, only few methods report the formation of 5'-methyl modification at a nucleoside level, and they concern mostly pyrimidine nucleosides.[35, 44] Despite the chemoselectivity of certain oxidising reagents, acetonide protection of 2'- and 3'-hydroxyls is most commonly used to avoid formation of by-products as well as to increase solubility of guanosine derivatives.[45] Substitution of the methyl at *C*-5' is often accomplished *via* Grignard reaction onto the 5'-aldehyde nucleoside.[46]

The retrosynthetic pathway investigated to form 5'-*C*-methyl-2'-*C*-methyl guanosine **161** takes into consideration the different synthetic aspects described above and is reported in Scheme 5.9.



Scheme 5.9. Retrosynthetic pathway to 5'-*C*-methyl-2'-*C*-methyl guanosine **161**.

Formation of 2',3'-isopropylidene protected 2'-*C*-methyl guanosine **158** was performed following standard procedures, using 60% aqueous solution of perchloric acid ($HClO_4$) in anhydrous acetone,[47] and yielded 32% of **158** after purification by flash chromatography.

The reaction conditions for oxidation of the 5'-position were then investigated. Oxidation reactions are extensively used in organic chemistry, and a wide variety of reagents have been developed. Nowadays, milder and more selective oxidants are used, such as activated dimethysulfoxide reagents,[48] enzymes,[49] or catalytic oxidants[50] as well as hypervalent iodine reagent, such as Dess-Martin periodinane.[51] Such reagents enable

to overcome chemoselectivity issues and are compatible with a variety of functional groups.[52]

3.2.1. Direct oxidations

3.2.1.1. Oxidizing reagents and mechanisms

The formation of the 5'-aldehyde **159** (Scheme 5.9) was first performed using direct oxidation reaction using reagent such as the Dess-Martin periodinane (**162**),[51, 53-57] pyridinium chlorochromate[58, 59] (**163**), dimethylsufoxide with oxalyl chloride (**164**)[38] or radical reagents such as TEMPO (tetramethylpiperidine-1-oxyl)[60] / TCC (trichlorocyanuric acid)[61] (**165**) (Table 5.7). Each reagent has specific characteristics reported in Table 5.7.

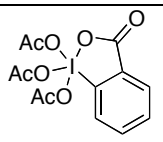
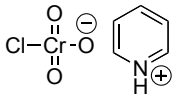
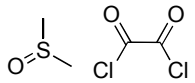
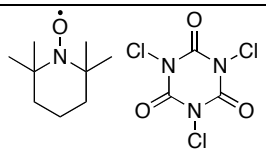
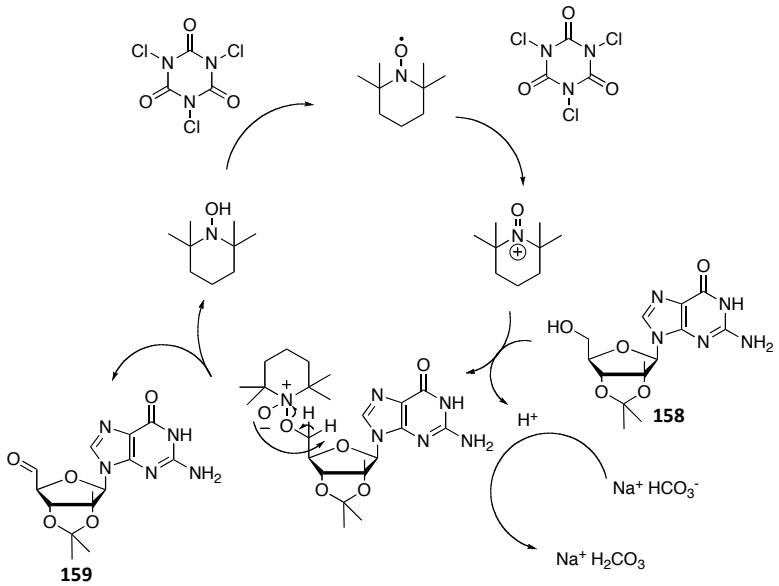
	 **162**	 **163**	 **164**	 **165**
Oxidation	$R^{I}OH$ to RCHO $R^{II}OH$ to RCO	$R^{I}OH$ to RCHO to RCOOH $R^{II}OH$ to RCO	$R^{I}OH$ to RCHO $R^{II}OH$ to RCO	$R^{I}OH$ to RCHO
By-products	Acetic acid		Carbon dioxide Carbon monoxide Dimethyl sulfide Triethylamonium chloride	
Advantages	Mild High yielding Easy work-up High selectivity	Mild High efficiency High selectivity No overoxidation	Mild Wide tolerance to functional groups	Mild Basic conditions High yielding
Disadvantages	Does not work well for adenosine	Highly toxic	by-products are volatiles but toxic and odorous	overoxidation to carboxylic acid in aqueous solvents

Table 5.7. Characteristics of oxidizing reagents commonly used for direct oxidation.

The mechanism of the radical reaction using TEMPO/TCC (**165**) is described in Scheme 5.10. While TCC act as a second oxidant to regenerate TEMPO, sodium

hydrogenocarbonate ($NaHCO_3$) is used as a base to trap the proton release from the reaction.

The mechanisms and reaction conditions of Dess-Martin (**162**), Swern oxidations (**164**) or oxidation with PCC (**163**) are similar. The attack of the primary 5'-hydroxyl of **158** onto the iodine of **162**, chromium of **163** or the sulfur atom of **164**, previously activated by triethylamine, is followed by deprotonation of 5'-methylene group to afford the nucleoside 5'-carboxaldehyde **159** (Scheme 5.9).



Scheme 5.10. Proposed catalytic cycle for TEMPO/TCC catalysed oxidation under basic conditions.[60]

3.2.1.2. Attempts for the formation of 5'-aldehyde by direct oxidation

Because of eventual hydration, the formation of the nucleoside 5'-aldehyde is extremely difficult, and only few examples concerning purine nucleosides are reported in the literature.[56, 62] Numerous attempts varying the reaction conditions and the reagents described previously were unsuccessful (Table 5.8).

Attempt	Reagent	Conditions	Work-up	Results and observations
1	Dess-Martin (1.4 eq)	anh. DCM, rt, 3 hrs	aq. $Na_2S_2O_3$/ aq. $NaHCO_3$	TLC base line products only
2	Dess-Martin (1.5 eq)	anh. DCM, 0 °C, 3 hrs	aq. $Na_2S_2O_3$/ aq. $NaHCO_3$	TLC base line products only
3	Dess-Martin (1.5 eq)	anh. DCM / anh. ACN, rt, 4 hrs	none	TLC base line products only
4	PCC (1.3 eq)	anh. DCM, rt, 3 hrs	none	TLC base line products only
5	Oxalyl chloride (1.4 eq), anh. NEt_3 (4.8 eq)	anh. DMSO, anh. DCM, -70 °C to rt, 2 hrs	H_2O / DCM	TLC base line products only
6	TEMPO (0.025 eq) / TCC (0.76 eq)	anh. DMF, $NaHCO_3$ (30.0 eq), 0 °C, 4 hrs	none	Purification by flash chromatography

Table 5.8. Reaction conditions of direct oxidations attempted.

Being compatible with both *tert*-butyl-dimethyl silyl and isopropylidene protecting groups,[61] the Dess-Martin oxidation was first attempted on **158**. However, changing the temperature or the solvent system (attempt 2 and 3, Table 5.8) did not afford the 5'-aldehyde **159** or the starting material **158**, but only degradation products which were not characterised. During the third attempt, no aqueous work-up was performed in order to avoid eventual hydration,[62] epimerisation of C-4' or elimination of the acetonide function[63] of **159**. Unfortunately the outcome was similar to the first two attempts. Further investigations of the oxidative step with PCC (attempt 4, Table 5.8) or *via* Swern conditions (attempt 5, Table 5.8) were equally vain.

As a last attempt, the catalytic combination of TEMPO/TCC (attempt 6, Table 5.8) was tested. Never reported on nucleosides but on unprotected glycosides,[60] these conditions were applied both to 2'-*C*-methyl guanosine **10** and its acetonide protected analogue **158**. NMRs of the purified residue were not conclusive since neither the aldehydic proton, nor the riboside or acetonide protons were visible. In both cases, the starting material was not recovered.

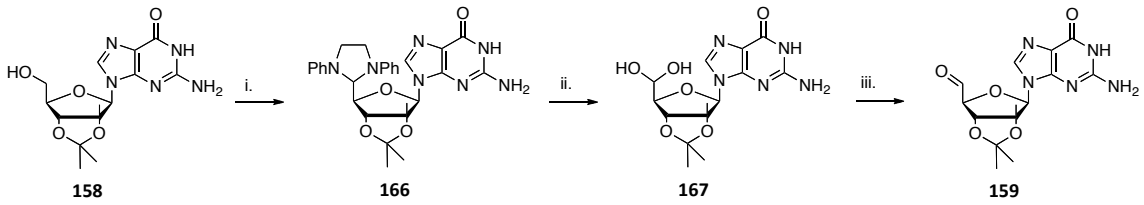
These reactions were performed one more time, and without attempting to isolate the nucleoside 5'-aldehyde **159**, the methylation step was performed with the Grignard reagent methyl magnesium bromide (MeMgBr).[38] In all cases, NMR and mass spectroscopy supported the conclusion that methylation did not happen, hence suggesting that the oxidation step failed.

3.2.2. Indirect oxidations

The lack of success of direct oxidations performed at a nucleoside level led us to investigate oxidation of the 5'-position *via* indirect routes.

3.2.2.1. Pfitzner-Moffatt route

Discovered in 1963,[45] the Pfitzner-Moffatt route[62] is used to oxidise selectively primary and secondary alcohols to aldehydes,[63] without reaching the carboxylic acid. The four-step synthesis described on 2',3'-isopropylidene-*N*-6-protected adenosine[65] was adapted to 2'-*C*-methyl guanosine starting from **158** (Scheme 5.11).



158 **166** **167** **159**

(i. anh. DMSO, DCC (3.0 eq), $Cl_2CHCOOH$ (0.5 eq), 0 °C to rt, 1hr 30min, anh. MeOH, oxalic acid dihydrate (2.0 eq), rt, 30min, N,N'-diphenylethylene diamine (1.1 eq), rt, 1hr, 62%; ii. Dowex 50WX4-50, H_2O/THF (1:1), rt, 1hr, 18%; iii. Dean-Stark, toluene, 1hr).

Scheme 5.11. Pfitzner-Moffatt oxidation route.

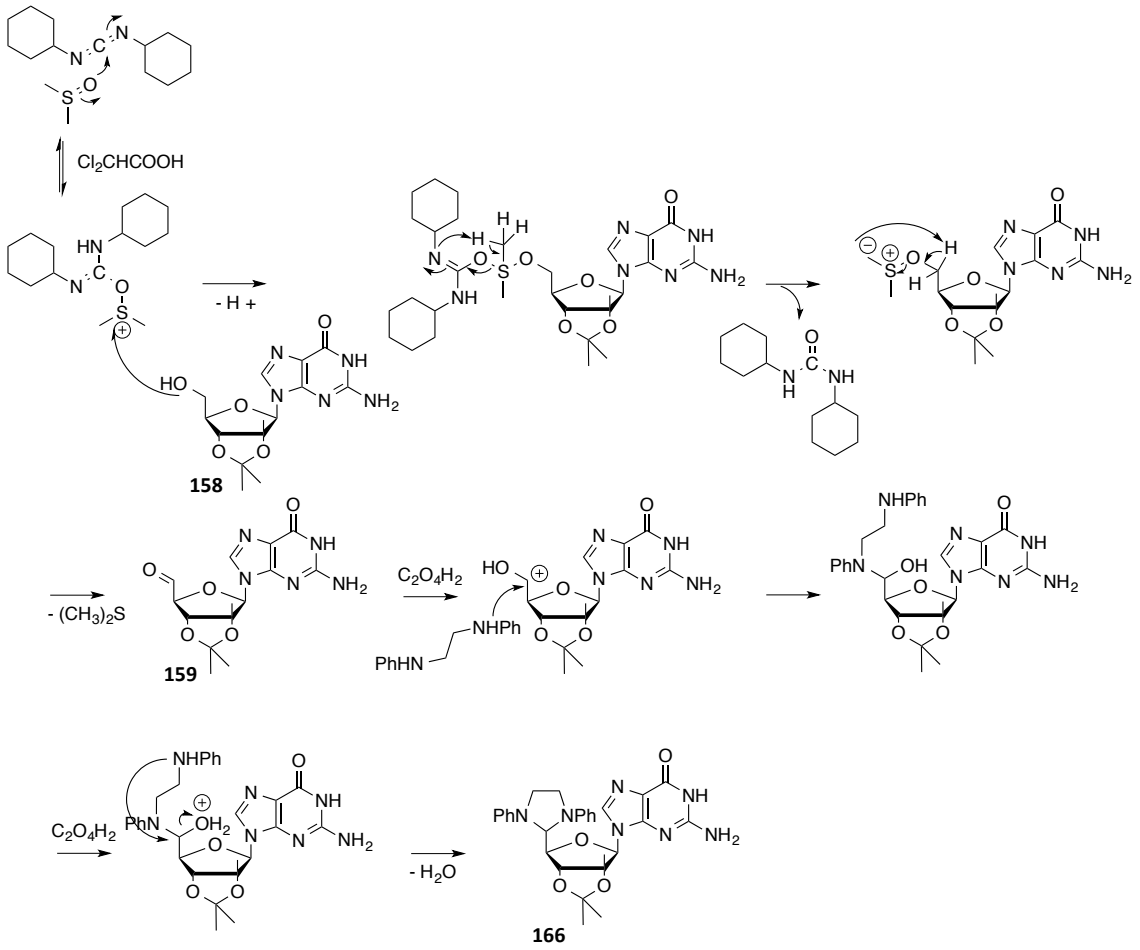
Despite requiring several steps, this mild oxidation enables the isolation of the 5'-aldehyde (**159**) as a stable 5'-dihydrate (**167**, Scheme 5.9), after treatment of the 5', 5'-(*N*,*N*'-diphenylethylenediamine) intermediate **166** with acidic Dowex resin.

166 was obtained by attack of the 5'-hydroxyl of **158** onto the ylide intermediate formed by dichloroacetic acid, followed by release of urea and ylide sulfonium to give **159**. The latter then reacts with *N*,*N*'-diphenylethylenediamine (Scheme 5.12) to afford **166** in 62%. After work-up, the structure was confirmed by the presence of characteristic ^{1}H-NMR peaks, such as aromatic protons in the range of 6.82-8.32 ppm and a multiplet at 3.72 ppm corresponding to the methylene groups. **166** was then treated with strongly acidic Dowex resin to afford the nucleoside 5'-dihydrate **167** (Scheme 5.11). The spectrum of ^{13}C-NMR confirms the presence of a tertiary carbon at 88.46 ppm in deuterated methanol, corresponding to the C-5'.

Isolation of the desired nucleoside 5'-aldehyde **159** failed and only **167** was recovered by precipitation in toluene. The nucleoside 5'-dihydrate **167** appeared extremely stable and rather poorly soluble in toluene.

Aiming at obtaining the nucleoside 5'-methylated intermediate **160** (Scheme 5.9), the nucleoside 5'-dihydrate **167** was treated with an excess of Grignard reagent (MeMgBr, ~10 eq).[63] However **167** being poorly soluble in anhydrous dichloromethane, the reaction failed and only the starting material was recovered.

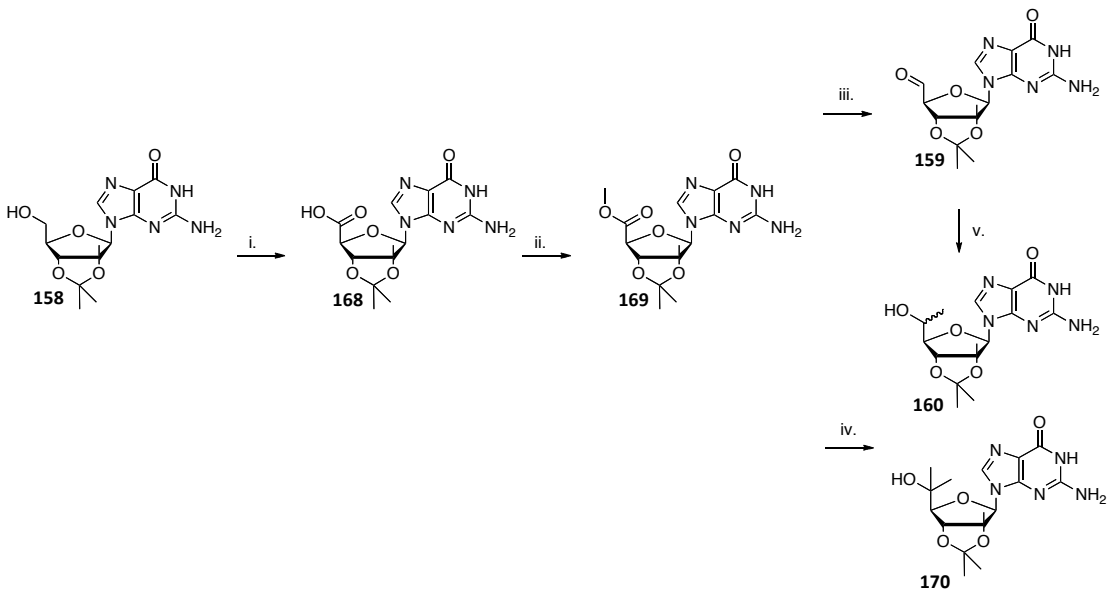
Despite the Pfitzner-Moffatt oxidation being widely used in nucleoside chemistry, this method failed to provide the desired nucleoside 5'-aldehyde intermediate **159** or its 5'-methylated derivative **160**. This is mainly due to **167** being highly hydrophilic (ClogP ~ -2.25) and highly stable to the conditions used. Protection of the exocyclic amine of the heterobase with benzyl would probably enhance the solubility of **167**.



Scheme 5.12. Mechanism of Pfitzner-Moffatt oxidation.

3.2.2.2. Route *via* TEMPO / BAIB and reduction

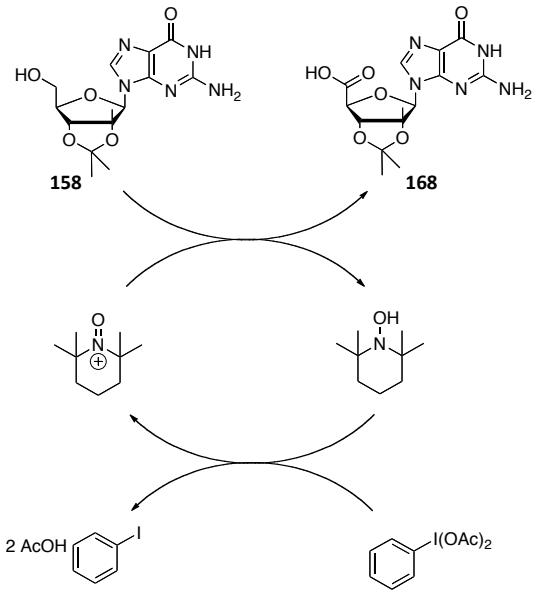
The TEMPO (2,2,6,6-tetramethyl-1-piperidinyl oxide) / BAIB ([bis(aceoxy)-iodo]benzene) route has been reported in the literature on both ribosides and nucleosides, and in particular for access to the guanosine 5'-methylcarboxylate *via* the 5'-carboxylic acid intermediate.[66] While Moffatt and co-workers described similar reaction using diazomethane, the combination of TEMPO and BAIB provide a much safer route, as described Scheme 5.13. These mild and selective conditions[40] are compatible with numerous protecting groups as well as variety of functional groups.[44]



(i. anh ACN / H_2O (1:1), TEMPO (0.25 eq), BAIB (2.2 eq), rt, overnight, 95%; ii. anh. MeOH, 30min, $SOCl_2$ (5.0 eq), 0 °C to rt, overnight, 18%, iii. anh. Et_2O, DIBAL-H (1M n hexanes, 2.0 eq), - 78 °C, 2 hrs; iv and v. anh. THF, MeMgCl (1.0 to 10 eq), rt or -78 °C, 1 hr).

Scheme 5.13. TEMPO/BAIB route to 2',3'-protected 5'-methyl-2'-*C*-methyl guanosine **160**.

The oxidation step of **158** to its corresponding 5'-carboxylic acid **168** proceeds *via* the formation of an *N*-oxoammonium salt by acetic acid catalysis.[40, 44] The former enables the oxidation of the alcohol while it is reduced to hydroxylamine. The organic oxidant BAIB regenerates TEMPO by oxidation of the hydroxylamine (Scheme 5.14).



Scheme 5.14. Mechanism of TEMPO / BAIB oxidation.

In contrast with the guanosine derivative, which required the addition of 2.0 eq of sodium bicarbonate to obtain its 5'-carboxlic acid in good yield,[44] **168** was recovered in 95% yield without purification, and characterised by NMR. The carbonyl group appeared at 171.95 ppm in ^{13}C-NMR, and a broad singlet at 10.67 ppm in ^{1}H-NMR confirmed the presence of the carboxylic acid group at C-5'.

Esterification of **168** was performed using thionyl chloride ($SOCl_2$) in methanol *via* the formation of the acyl chloride intermediate. Acting as a good leaving group, the chlorine was substituted by methanol and afforded **169** in 18% yield, after flash chromatography.

Reduction of **169** to the nucleoside 5'-aldehyde **159** was attempted using diisobutylaluminium hydride (DIBAL-H), which acts a reducing reagent after formation of a Lewis acid-base complex. DIBAL-H was introduced at low temperature to form the tetrahedral intermediate, which should be converted to the aldehyde by addition of hydrochloric acid. Unfortunately, the reaction failed and only compound **169** was recovered.

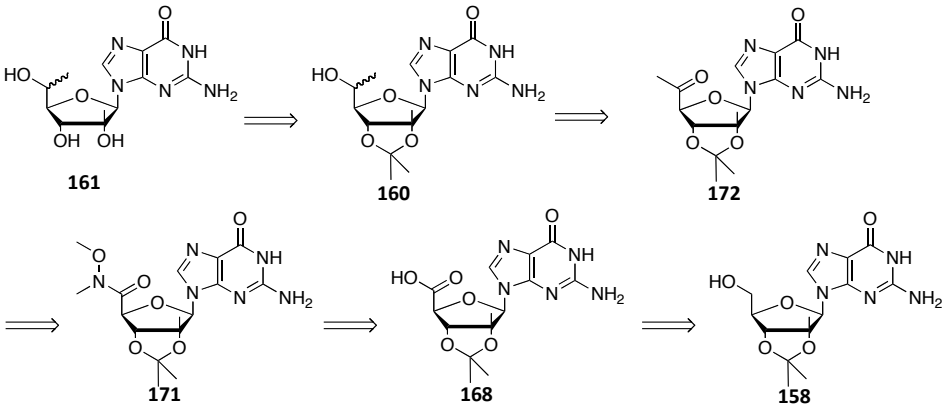
Another attempt of reduction was carried out using either 1.0 or 10 equivalents of the Grignard reagent (methyl magnesium chloride) to test the reactivity of the 5'-methyl ester derivative **169**. The use of Grignard does not favour the formation of secondary alcohol because the methyl keto intermediate is much more reactive than the ester functionality. Thus, it is likely that the use of Grignard reagent will afford the 5',5'-

dimethyl substituted nucleoside **170**. However, neither the amount of Grignard introduced, nor the control of temperature (-78 °C), enabled the formation of desired protected 5'-methyl-2'-*C*-methyl guanosine **160** or its 5',5'-dimethylated analogue **170**.

A recently published one-pot procedure subjects the 5'-methyl ester carbocycle intermediate, formed using TEMPO/BAIB procedure, to methyl magnesium bromide to obtain both the 5'-methyl and 5'-dimethyl analogues.[34] Moreover, Verma and co-workers also propose the use of methyl lithium (MeLi) at low temperature to obtain 5'-dimethyl carbasugar.[35] If time had allowed, these conditions could have been tested on 2',3'-isopropylidene 2'-*C*-methyl guanosine **158**.

3.2.2.3. Route *via* TEMPO / BAIB and Weinreb amide

Instead of targeting the 5'-aldehyde **159** as an intermediate towards the synthesis of 5'-methyl-2'-*C*-methyl-guanosine **161**, an alternative method focused on synthesising the 5'-methylketo intermediate **172** from the 5'-Weinreb amide nucleoside **171** (Scheme 5.15). The latter is an effective acylating agent due to its highly activated carbonyl group. It is reported to be a good intermediate for accessing highly functionalised ketones.[67]



Scheme 5.15. Retrosynthesis route to 5'-methyl-2'-*C*-methyl guanosine **161** *via* 5'-methylketo **172** and 5'-Weinreb amide **171** intermediates.

Oxidation to **168** was performed using the TEMPO/BAIB procedure as described previously. Considering that the 5'-carboxylic acid is sterically hindered, the formation of 5'-carboxylic acid amides, such as **171,** requires the use of coupling reagents such as 1-hydroxybentriazole (HOBt), commonly used for amide synthesis, in combination with

an acid activating agent such as bromo-*tris*-pyrrolidinophosphonium hexafluorophosphate (PyBrOP), in the presence of base such as *N*,*N*-diisopropylethylamine (DIPEA).[42] *N*,*O*-dimethylhydroxylamine hydrochloride was then added and **171** was obtained in moderate yield (48%). Anhydrous conditions are essential for the success of this reaction, hence the importance of obtaining the starting material **168** as a precipitate and drying it over two days. **171** was characterised by ^{1}H-NMR and ^{13}C-NMR, and characteristic peaks of the 5'-carboxylic acid amide appeared at 36.36 ppm (ON($\underline{CH_3}$)OCH_3), 61.51 ppm (ON(CH_3)O$\underline{CH_3}$) and the peak of the 5'-carbonyl group shifted upfield (169.5 ppm) compared to its starting material **168**.

The 5'-carboxylic amide function is reported to react quickly with Grignard and organolithium reagents to form 5'-alkylketo products, without obatining a tertiary alcohols even with an excess of reagent.[67] The mechanism of formation of nucleoside 5'-methylketo **172** is described in Scheme 5.16, and proceeds *via* the formation of a chelated intermediate which prevents the addition of a second nucleophile[68] and collapses by hydrolysis.

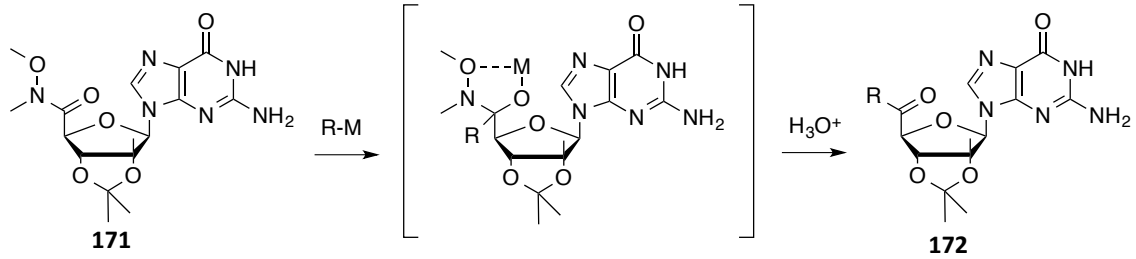


Figure 5.16. Reaction mechanism of **171** with organometallic reagents.

In order to investigate the formation of 5'-methylketo **172** from **171**, several reaction conditions were performed (Table 5.9). Unfortunately, the methyl magnesium bromide (MeMgBr, attempts 1.a-e) seemed not to be a suitable reagent for the nucleophilic attack onto the Weireb amide intermediate **171**. Even under high temperature or high concentration of reagent, only the starting material was recovered. As a last attempt, butyl lithium (BuLi, attempts 2.a-b) was used to test the reactivity if **171**. Introduction of the organolithium reagent at low temperature was unhelpful in the recovery of 5'-butylketo nucleoside, and once again **171** was recovered with some degradation products. Mass of the crude confirmed that the reaction did not take place.

Attempt	Reagent	Conditions	Observations
1.a	CH_3MgBr (3.0 M in Et_2O, 2.0 eq)	anh. THF, 0 °C, 30 min	**171**
1.b		+ rt, 30 min	**171**
1.c	+ CH_3MgBr (3.0 M in Et_2O, 2.0 eq)	+ rt, 1 hr	**171**
1.d		+ rt, overnight	**171**
1.e		+ 65 °C, 5 hrs	**171**
2.a	BuLi (2.0 M in pentane, 2.0 eq)	anh. THF, -30 °C, 1 hr	**171**
2.b		+ rt, 3 hrs	**171** and degradation products

Table 5.9. Conditions attempted for nucleophilic attack onto **171**.

Being unexpected and disappointing, these results suggested the high stability of the Weinreb amide intermediate **171**, and the use of a stronger nucleophilic reagent would be required to obtain of the desired 5'-metylketo **172**. Once obtained, the subsequent reduction to its secondary alcohol analogue **160**, followed by deprotection of 2',3'-hydroxyls would provide the desired 5'-methyl-2'-*C*-methyl guanosine **161**, likely as a mixture of stereoisomers at *C*-5'.

3.3. Conclusion

Despite numerous routes investigated, attempts to substitute a methyl group at *C*-5' of 2'-*C*-methyl guanosine **10** remained vain. Direct oxidations of the 5'-hydroxyl performed with common oxidative reagents resulted only in degradation products, while indirect oxidations were likely to be more successful, however increased considerably the number of synthetic steps.

The TEMPO/BAIB oxidation gave good yields and pure 5'-carboxylic acid **168** without purification. However, the 5'-dihydroxy hydrate intermediate **167**, resulting from the Pfitzner-Moffatt oxidation, the 5'-methylester **169** and the 5'-weinreb amide intermediate **171** appeared extremely stable under the reaction conditions used. Thus, preventing any nucleophilic substitution at *C*-5' or reduction to the 5'-aldehyde intermediate **159**.

It would be interesting to enhance the solubility of the 5'-dihydroxy hydrate **167** using a protection of the exocyclic amine *N*-2 with monomethoxytrityl, which is reported to increase considerably the solubility of guanosine derivatives.[45] Moreover, one-pot oxidation-methylation[35] should be attempted and the use of organolithium reagents should be further investigated.

Adaptation of any route performed on 2',3'-isopropylidene 2'-*C*-methyl guanosine **158** to the 2'-*C*-methyl-6-*O*-methyl guanosine analogue **10** would require the use of another protecting group at 2'- and 3'-hydroxyls since the formation of 2',3'-isopropylidene 2'-*C*-methyl-6-*O*-methyl guanosine is very low yielding.

Such 5'-methylated or 5'-dimethylated nucleosides have an important interest for the treatment of several diseases, and such modifications have been reported to enhance the pharmacokinetic profile compared to their parent nucleosides.[35] Their metabolic stability make them important compounds, thus, further investigation is necessary to develop a quick and efficient synthetic route at a nucleoside level, in comparison to the ones already developed at a furanose level.

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Chapter Six: Acyclic nucleosides and prodrugs

1. Introduction and objectives

In the search of expanding the SAR study of 2'-*C*-methyl-6-*O*-methyl guanosine **11**, disconnection of the chain of the furanose ring was investigated and novel acyclic nucleosides were designed as potential inhibitors of the HCV NS5B polymerase.

1.1. Acyclic nucleosides

Since 1970 and the discovery of acyclovir (ACV, **173** Figure 6.1) as a potent antiviral approved for the treatment of herpes simplex virus (HSV), varicella zoster virus (VZV) and human cytomegalovirus (HCMV),[1] numerous syntheses of acyclic nucleosides have been reported and have led to the development of other antiviral agents displaying broad-spectrum activity against several viral infections,[2] microbial infections[3] and cancer.[3] Among many purine acyclic nucleosides, penciclovir (**174**, Figure 6.1) used for the treatment of HSV,[4] and other acyclic nucleoside phosphonates, such as (*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine ((*S*)-HPMPA, **175**, Figure 6.1), exhibited activity against DNA viruses.[5] The latter served as a template for currently clinically used phosphonates, which exhibit prolonged antiviral response and impressive resistance profile,[6] such as previously mentioned anti-HBV adefovir dipivoxyl **4.a** (Figure 1.9, chapter 1).[5, 6]

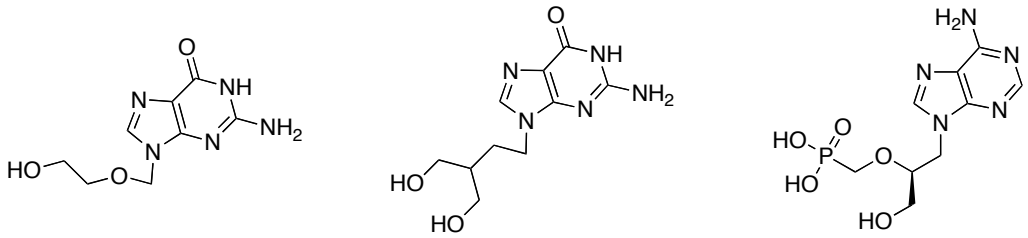


Figure 6.1. Structures of acyclovir **173**, penciclovir **174** and *(S)*-HPMPA **175**.

Mimicking natural cyclic sugar nucleosides, acyclic nucleosides act as inhibitors[7] of viral reverse transcriptase, DNA polymerase[8] or nucleoside phosphorylase.[9] Moreover,

the conformation of the acyclic chain is essential for interaction and affinity with metabolic enzymes.[10]

Aiming at developing highly potent and selective molecules, alterations of the nucleobase[11] and/or acyclic chain have been extensively investigated.[12, 13]

1.2. Acyclic nucleosides and analogues for HCV treatment

The lack of efficient therapy against HCV has led several groups to investigate acyclic nucleosides and phosphonates as inhibitors of HCV RNA replication, however this field is still to be further explored.

Several triazole-based acyclic nucleosides have been designed as anti-HCV agents,[14, 15] and micromolar activity against HCV RNA replication was reported when the triazole bore a rigid triple bond linked to a π-conjugated aromatic system, such as **176** (Figure 6.2).[14] Despite exhibiting lower potency (EC_{50} = 22 ± 3 μg/mL in Huh-5-2 cells) than ribavirin **3** (Figure 1.6, chapter 1) (EC_{50} = 7 ± 2 μg/mL, CC_{50} > 21 ± 11 μg/mL in Huh-5-2 cells), **176** was much less cytotoxic (CC_{50} > 50 μg/mL in Huh-5-2 cells).[14]

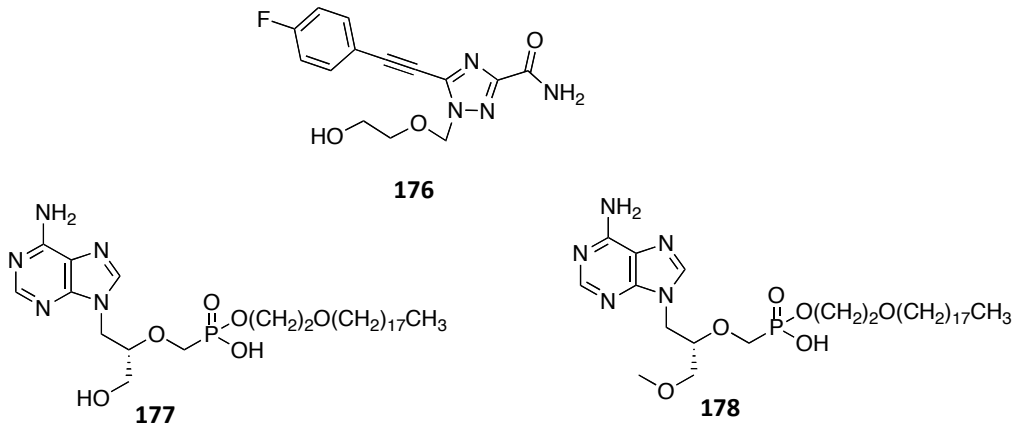


Figure 6.2. Acyclic nucleoside **176** and acyclic nucleoside phosphonates **177** and **178** as inhibitors of HCV RNA replication.

Moreover, efforts to target the HCV polymerase led to the development of acyclic nucleoside phosphonates, known to be efficient inhibitors of DNA viruses and retroviruses.[16] Recently, Wyles and co-workers reported the synthesis and biological activity of **177** (ODE-(*S*)-HPMPA, Figure 6.2).[17] The latter is not only the first acylic nucleoside phosphonate reported to be potent against a RNA virus,[17, 18] but is also the first of this class to exhibit anti-HCV activity versus genotypes 1b (EC_{50} = 1.55 ± 0.5 μM) and 2a (EC_{50} = 1.65 ± 0.33 μg/mL).[19] To address the issue of cytoxicity (CC_{50} =

35.6 ± 6.8 μM) displayed by **177**, other related analogues were synthetised.[17, 19] Replacement of the hydroxyl chain by a methoxy group led to **178** (ODE-(*S*)-MPMPA, Figure 6.2) which reduced considerably the cytotoxicity while retaining micromolar potency against HCV replication in cells (genotype 1b: EC_{50} = 1.43 ± 0.38 μM, genotype 2a: EC_{50} = 2.38 ± 1.09 μM, CC_{50} > 150 μM).[19]

1.3. Objectives

Acyclic pyrimidine[12] and purine[20] nucleosides (Figure 6.3), based on the features of penciclovir (**174**, Figure 6.1) and the antitumor and antibiotic neplanocin A (**179**, Figure 6.3),[21] have been reported and tested against herpes simplex viruses (HSV). Only guanine derivatives (**180** and **181**, Figure 6.3) exhibited moderate activity respectively against HSV-2 (EC_{50} = 11.7 μg/mL) and HSV-1 (EC_{50} = 32.7 μg/mL), without displaying cellular toxicity (CC_{50} > 100 μg/mL).[20] The lack of antiviral activity is reported to be likely due to poor phosphorylation during nucleoside activation.[12]

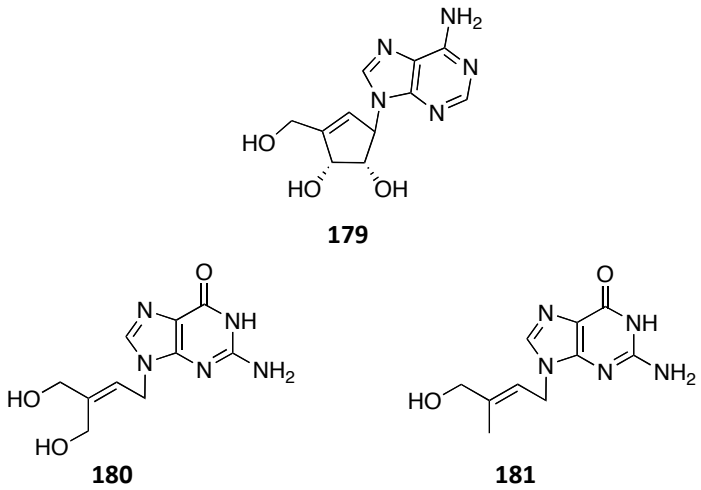


Figure 6.3. Antibiotic neplanocin A (**179**) and acyclic guanine nucleosides (**180** and **181**).

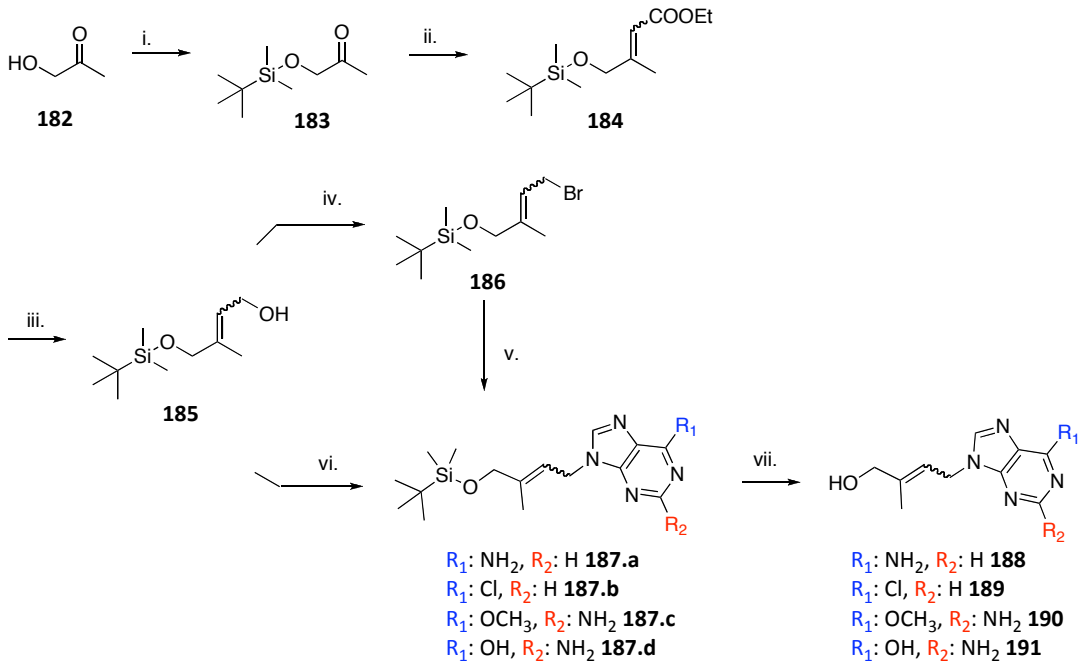
Taking these facts into considerations and aiming at developing acyclic nucleosides as potential inhibitors of HCV RNA replication, acyclic purine nucleosides, sharing similar structure with **181**, and their phosphoramidate analogues were synthesised. Fixing the acyclic chain, only the heterobase moiety was varied, using 6-chloropurine, adenine, guanine and 6-*O*-methyl guanine.

2. Synthesis of acyclic purine nucleosides and prodrugs

2.1. Acyclic purine nucleosides

Coupling of the heterobase with a carbon substrate[22] or formation of the heterobase from aminoalkanes[23] are common methods for the preparation of acyclic nucleosides, which are not efficient in the case of electron-rich purines. Reported methods for carbon-base bond formation consist of allylic ester palladium-catalysed displacement, nucleophilic displacement of halides or activated alcohols, and Mitsunobu coupling.[24] While the first two methods give moderate yields because of competition at *N*-7 and *N*-9 of the purine base,[25, 26] the mild conditions and stereospecificity of the Mitsunobu coupling make it an attractive method.[27]

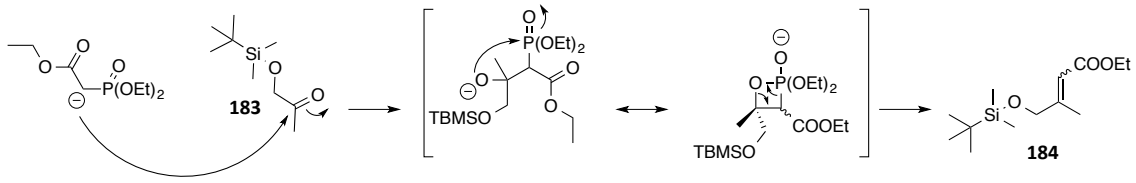
The synthetic pathway towards acyclic purine nucleoside (Scheme 6.1) analogues of **181** (Figure 6.3), was reported by Hong and co-workers, *via* displacement of allylic bromide.[12, 20] This synthetic route (Scheme 6.1) was first investigated and compared with the results obtained from the Mitsunobu coupling.



(i. anh. DMF, imidazole (2.5 eq), TBDMSCl (2.2 eq), rt, 36 hrs, 81%; ii. anh. THF, NaH (60% dispersion in oil, 1.0 eq), $(EtO)_2POCH_2COOEt$ (1.2 eq), rt, 1 hr, 51%; iii. anh. DCM, DIBAL-H (1.0 M in hexane, 2.0 eq), 0 °C, 2 hrs, rt, 2 hrs, 24%; iv. anh. DCM, PPh_3 (1.1 eq), NBS (1.1 eq), 0 °C to rt, 4 hrs, 38%; v. anh. DMF, adenine (3.2 eq), NaH (60% dispersion in oil, 3.2 eq), rt, overnight, 21%; vi. anh. THF, purine (1.0 eq), PPh_3 (1.1 eq), DIAD (1.0 eq), rt, overnight; vii. anh. THF, TBAF (2.2 eq), 30 min, rt.)

Scheme 6.1. Synthetic route to acyclic purine nucleosides.

Commercially available hydroxypropan-2-one **182** was protected with *tert*-butyl-dimethyl silyl chloride (TBDMSCl) before being subjected to Horner-Emmons olefination,[28] whose mechanism proceeding *via* nucleophilic attack is described in Scheme 6.2. After deprotonation by sodium hydride, the activated α-carbon of triethylphosphonoacetate ($(EtO)_2POCH_2COOEt$) attacks onto the carbonyl group of **182** (Scheme 6.2) to form an intermediate, which then undergoes elimination to afford the α,β-unsaturated ester **184**. The latter was recovered as a mixture of separable *E*- and *Z*-isomers (*E*/*Z* ratio: 22/78) in 51% yield. The mixture was reduced by diisobutyl aluminium hydride (DIBAL-H) to its corresponding mixture of unsaturated allylic alcohol **185**.[29, 30] A portion of the major isomer could be easily obtained pure after flash chromatography, whereas the other was always obtained as a mixture. However since it was not necessary, the isomers were not separated at this stage.



Scheme 6.2. Mechanism of Horner-Emmons reaction.

The first pathway investigated is reported in the literature,[12, 20] and the allylic bromide **186** was formed with *N*-bromosuccinimide (NBS). Subsequent displacement of allylic bromide **186** by adenine under basic conditions afforded *N*-9 alkylated **187.a** in 21% yield, despite the 64% reported.[20] The yield over two steps (30%) was only comparable to the one reported in the literature (35-72%).[20, 29, 30] The low yield is mainly due to the poor solubility of the heterobase in dimethylformamide. We can speculate that the use of cesium carbonate would have probably enhance the solubility.[20, 31]

As an attempt to increase the yield, one-pot reaction was performed under Mitsunobu conditions. This approach gives good *N*-9 regioselectivity under mild conditions.[32] Generation of the phosphonium intermediate by action of diisopropylazodicarboxylate (DIAD) and triphenylphosphine (PPh_3) and activation of the heterobase, are key steps for condensation of the latter onto activated allylic phosphonium intermediate (Scheme 6.3). Despite moderate yields reported in the literature,[33, 34] **187.a** was recovered in 26% yield, comparable to the 30% obtained from the two-step synthesis. The poor solubility of the heterobase in tetrahydrofuran, best solvent for Mitsunobu reaction, and the competing nucleophilicity of the exocyclic amino group at *N*-6, are presumably the main reasons for the poor yield obtained.

While the first route enabled the recovery of a separable mixture of regioisomers (94/6), the Mitsunobu coupling of **185** with adenine afforded only one isomer. The latter and the major isomer formed during the first reaction shared identical NMR spectra. They were they were tentatively assigned with *E* geometry at the double bond. This hypothesis was confirmed by comparing ^{13}C-NMR analysis of both isomers. In fact, the characteristic ^{13}C-NMR peaks of unsaturated acyclic nucleoside *E*-isomers often appears at a downfield shifts compared to their *Z*-isomers.[35, 36] The major isomer corresponded to the *trans*-configuration (*E*-isomer) (141.46 ppm, 116.36 ppm, 67.15 ppm, 40.79 ppm, 13.66 ppm),[20] while the signals of the minor *Z*-isomer appeared with upfield shifts (141.07 ppm, 116.09 ppm, 66.16 ppm, 40.58 ppm, 13.57 ppm).

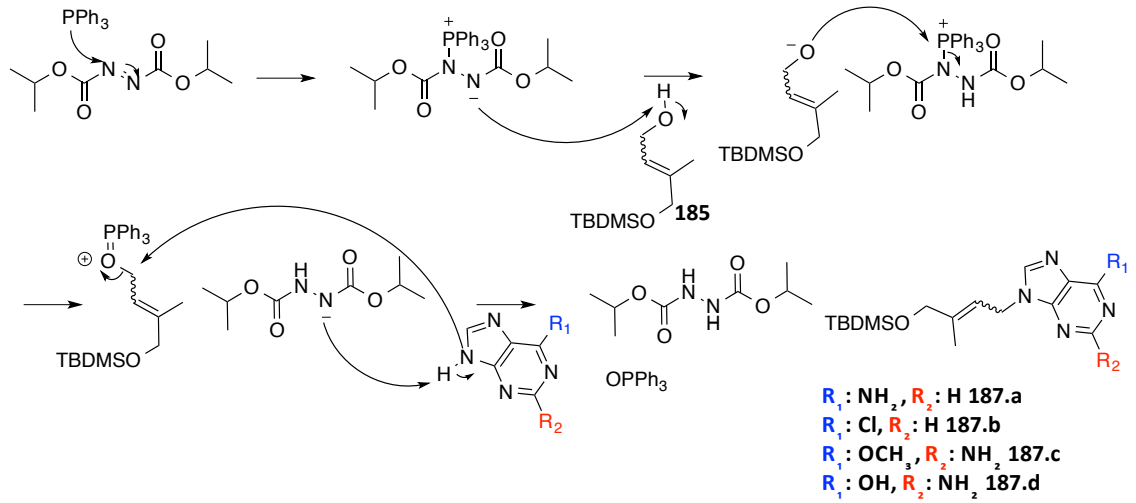


Figure 6.3. Mechanism of Mitsunobu coupling.

From these results and because the yield of the one-step Mitsunobu coupling was similar to the two-step synthesis *via* displacement of allylic bromide **186**, the latter route was abandoned. **185** was then coupled with 6-chloro purine, 6-*O*-methyl guanine and guanine heterobases under Mitsunobu conditions, and results are described in Table 6.1. The acyclic 6-chloro purine nucleoside **187.b** was recovered in high yield (Table 6.1), which is twice the yield reported *via* the allylic bromide intermediate **186**.[20] Comparison of NMR data with the literature[20] confirmed the *cis*-configuration of the double bond (*Z*-isomer).

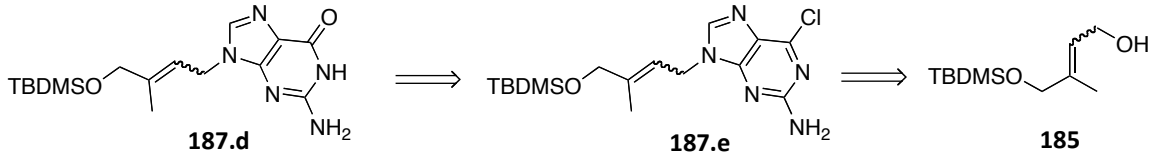
Coupling with 6-*O*-methyl guanine afforded **187.c**, which was recovered as a mixture of separable isomers and surprisingly the formation of the sterically hindered *Z*-isomer was favoured.

	187.a	**187.b**	**187.c**
Yield	26%	70%	16%
***E*-isomer**	100	0	18
***Z*-isomer**	0	100	87

Table 6.1. Yields of Mitsunobu coupling and ratio of different isomers of acyclic chain of nucleosides.

In contrast, the insolubility of guanine in tetrahydrofuran did not allow the reaction to take place under Mitsunobu conditions, and the same happened when using the

commercially available *N*-2-acetylated guanine. A new approach *via* the formation of the 6-chloro guanine intermediate **187.e** was then investigated (Scheme 6.4).



Scheme 6.4. Retrosynthetic route to guanine acyclic nucleoside **187.d** *via* 6-*Cl*-guanine acyclic intermediate **187.e**.

Mistunobu coupling between 6-chloro guanine and **185** allowed the formation of **187.e** (Scheme 6.4) in 79% yield as a mixture of isomers. Subsequent hydrolytic dechlorination treatement using an aqueous solution of formic acid (95-97%) at -78 °C for two hours,[27] did not suggest formation of the desired **187.d**, and total degradation of the starting material occured. It was then decided to obtain the guanine derivative after deprotection of **187.c** (acyclic 6-*O*-methyl guanine derivative), followed by treatment with sodium iodide (NaI) and trimethylsilyl chloride (TMSCl).[37]

The mixture of isomers **187.a** obtained *via* allylic bromide route and intermediates **187.b-c** were deprotected using *tetra*-butylammonium fluoride (TBAF, step vii, Scheme 6.1) as a source of fluoride, and acyclic nucleosides **188**,[20] **189** and **190** were obtained in respectively 58%, 31% and 32% yield. *E*- and *Z*-isomers of both **188** and **190** were separated by flash chromatography and their configurations were assigned by ^{1}H-NMR and ^{13}C-NMR (Table 6.2). In both cases, the *E*-isomer was more apolar than the *Z*-isomer. The peak of the vinylic proton appeared as a triplet in the case of the *E*-isomer, whereas a triplet of quadruplet was observed for the *Z*-isomer. This multiplet corresponds to a coupling with the methylene group linked to the heterobase (CH<u>C</u>H$_2$-base) and an allylic *cis* coupling with the methyl group in proximity only in the cis-configuration of the double bond (*Z*-isomer).

		***E*-isomer (a)**	***Z*-isomer (b)**
188	**E/Z ratio**	31	69
	^{1}H (MeOD)	5.54 (t, *J* = 7.30 Hz, C<u>H</u>)	5.74 (tq, *J* = 7.10 Hz, 1.40 Hz, C<u>H</u>)
	^{13}C (MeOD)	142.44 (<u>C</u>(CH_3)CH_2OH), 121.71 (<u>C</u>H), 61.64 (<u>C</u>H_2OH), 42.05 (CH<u>C</u>H_2-base), 13.95 (<u>C</u>H_3)	142.28 (<u>C</u>(CH_3)CH_2OH), 118.75 (<u>C</u>H), 67.52 (<u>C</u>H_2OH), 42.04 (CH<u>C</u>H_2-base), 13.86 (<u>C</u>H_3)
190	**E/Z ratio**	40	60
	^{1}H ($CDCl_3$)	5.63 (t, *J* = 7.50 Hz, C<u>H</u>)	5.71 (tq, *J* = 7.00 Hz, 1.50 Hz, C<u>H</u>)
	^{13}C ($CDCl_3$)	142.22 (<u>C</u>(CH_3)CH_2OH), 120.16 (<u>C</u>H), 61.52 (<u>C</u>H_2OH), 41.34 (CH<u>C</u>H_2-base), 13.70 (<u>C</u>H_3)	141.23 (<u>C</u>(CH_3)CH_2OH), 117.43 (<u>C</u>H), 66.97 (<u>C</u>H_2OH), 40.70 (CH<u>C</u>H_2-base), 13.63 (<u>C</u>H_3)

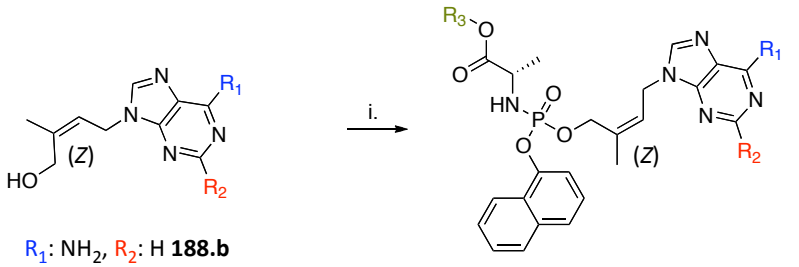
Table 6.2. Ratio of different isomers and ^{13}C chemical shifts (ppm) of acyclic chain of nucleosides **188** and **190**.

The *E*-isomer of molecule **190** (**190.a**) was treated with sodium iodide (NaI) and trimethylsilyl chloride (TMSCl)[37] to afford its acyclic guanine analogue **191** in 8% yield. The triplet peak corresponding to the vinylic proton confirmed the *trans*-configuration of the alkene.

After confirmation of purity, acyclic purine nucleosides **188.a (***E*-isomer), **188.b (***Z*-isomer), **189 (***Z*-isomer), **190.a (***E*-isomer), **190.b (***Z*-isomer) and **191 (***E*-isomer) were sent to Inhibitex *Inc.* for evaluation of their cellular toxicity and potency against HCV RNA replication.

2.2. Acyclic purine ProTides

α-Naphthyl *L*-alanine ester phosphoramidates of acyclic purine nucleosides **188.b**, **189** and **190.b** were synthesised according to the standard Uchiyama procedure, using *tert*-butyl magnesium chloride as a base (Scheme 6.5). Starting from the (*Z*)-isomer of each nucleoside, only ProTide endowed with a *cis*-configuration of the double bond were synthesised. The ester was the only moiety varied at the ProTide level, using neopentyl (**a**), cyclohexyl (**b**) or benzyl (**c**) esters. Acyclic 6-chloropurine nucleoside **189** was not obtained in sufficient quantity to form the *α*-naphthyl *L*-alanine benzyl ester ProTide.



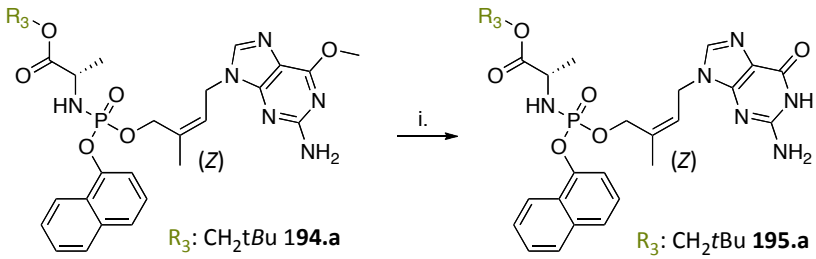
R_1: NH_2, R_2: H **188.b**
R_1: Cl, R_2: H **189**
R_1: OCH_3, R_2: NH_2 **190.b**

* R_1: NH_2, R_2: H, R_3: CH_2tBu **192.a**
R_3: cHex **192.b**
R_3: Bn **192.c**
* R_1: Cl, R_2: H, R_3: CH_2tBu **193.a**
R_3: cHex **193.b**
* R_1: OCH_3, R_2: NH_2, R_3: CH_2tBu **194.a**
R_3: cHex **194.b**
R_3: Bn **194.c**

(i. anh. THF, tBuMgCl (2.0 eq), α-naphthyl L-Alanine ester phosphochloridate (2.0 eq), rt, overnight.)

Scheme 6.5. Synthesis of *α*-naphthyl *L*-Alanine ester Protides of acyclic purine nucleosides.

Acyclic guanine Protides were obtained from their corresponding 6-*O*-methyl derivatives by treatment with sodium iodide (NaI) and trimethylsilyl chloride (TMSCl) (Scheme 6.6).[37]



R_3: CH_2tBu **194.a**
R_3: cHex **194.b**
R_3: Bn **194.c**

R_3: CH_2tBu **195.a**
R_3: cHex **195.b**
R_3: Bn **195.c**

(i. anh. ACN, NaI (1.5 eq), TMSCl (1.5 eq), rt, 1-4 hrs).

Scheme 6.6. Synthesis of naphthyl *L*-Alanine ester acyclic guanine Protides **195**.

All acyclic ProTides were recovered as mixtures of diastereoisomers at the phosphorus center. Yields, phosphorus chemical shifts (^{31}P δ) in deuterated chloroform ($CDCl_3$) and ratio of diastereoisomers are reported Table 6.3.

		192	193	194	195
a.	**Yield**	18%	2%	47%	2%
	^{31}P δ (ppm) / ratio	3.16, 3.81 (44/56)	2.82, 2.63 (36/64)	4.23, 3.91 (39/61)	4.12, 3.93 (41/59)
b.	**Yield**	47%	20%	44%	2%
	^{31}P δ (ppm) / ratio	2.89, 2.76 (50/50)	2.83, 2.69 (36/64)	3.09, 2.90 (43/57)	4.19, 3.96 (46/54)
c.	**Yield**	65%	-	44%	10%
	^{31}P δ (ppm) / ratio	4.11, 3.72 (50/50)	-	4.22, 3.82 (33/67)	4.10, 3.71 (44/56)

Table 6.3. Yields, ^{31}P -NMR chemical shifts and ratio of diastereoisomers of acyclic purine ProTides.

The yields were quite variable, and even starting from the same parent acyclic nucleoside, poor to moderate yields were obtained (**192.a**/**192.c** and **193.a**/**193.b**). Formation of acyclic guanine ProTide *via* demethylation of the 6-position was poor yielding (**195.a-c**), and the reaction time varied depending on the ester moiety.

The R_p and S_P isomers of acyclic adenine ProTides (**192.a-c**) were formed in a 50/50 ratio. Only the neopentyl ester (**192.a**) favours the formation of one diastereoisomer (^{31}P δ = 3.81 ppm).

In the case of acyclic 6-chloro purine (**193.a-b**), 6-*O*-methyl guanine (**194.a-c**) and guanine (**195.a-b**) ProTides, the major diastereoisomer formed always appeared to be the one corresponding with an upfield phosphorus peak. Because the diastereoisomers have not been separated, it was not possible to assign the configuration of the favoured isomer.

α-Naphthyl *L*-Alanine ester ProTides **192.a-c**, **193.a-b**, **194.a-c**, **195a-c** were tested as mixture of diastereoisomers against HCV replication.

3. Biological evaluation

Six acyclic purine nucleosides **188.a (***E*-isomer), **188.b (***Z*-isomer), **189 (***Z*-isomer), **190.a (***E*-isomer), **190.b (***Z*-isomer) and **191 (***E*-isomer) and eleven ProTides (**192.a-c**, **193.a-b**, **194.a-c**, **195a-c**) were tested in replicon-based assays by Inhibitex *Inc.* to determine their inhibitory potency (EC_{50}) against HCV replication and their eventual cellular toxicity (CC_{50}). The results of these assays are reported Table 6.4.

isomer	R_3	adenine			6-chloro-purine		
		Cpd	EC_{50} (µM)	CC_{50} (µM)	Cpd	EC_{50} (µM)	CC_{50} (µM)
(E)	-	**188.a**	>100	63	-	-	-
(Z)	-	**188.b**	>100	>100	**189**	62.8	45
(Z)	CH_2tBu	**192.a**	> 40	45	**193.a**	38	51
(Z)	cHex	**192.b**	> 40	46	**193.b**	> 40	>100
(Z)	Bn	**192.c**	> 40	>100	-	-	-

isomer	R_3	6-OMe guanine			guanine		
		Cpd	EC_{50} (µM)	CC_{50} (µM)	Cpd	EC_{50} (µM)	CC_{50} (µM)
(E)	-	**190.a**	>100	>100	**191**	>10	>100
(Z)	-	**190.b**	>100	>100	-	-	-
(Z)	CH_2tBu	**194.a**	> 40	>100	**195.a**	> 40	48
(Z)	cHex	**194.b**	> 40	45	**195.b**	23	36
(Z)	Bn	**194.c**	> 40	68	**195.c**	7.1	>100

Table 6.4. Inhibitory activity (EC_{50}) and cellular toxicity (CC_{50}) of acyclic purine nucleosides and ProTides.

According to these data, neither acyclic purine nucleosides, nor their ProTides, are efficient in inhibiting HCV replication in replicon-based assays. The *cis*- or *trans*-configuration of the double bond present in the acyclic chain does not have a significant impact on the activity, since both isomers are inactive at a nucleoside level (**188.a-b** and **190.a-b**). While poor efficiency was expected at the nucleoside level due to potential poor phosphorylation,[20] the ProTide technology may have overcome this issue.

With the exception of **195.c**, exhibiting micromolar potency against HCV RNA replication (EC_{50} = 7.1 µM) and being not cytotoxic (EC_{50} > 100 µM), other ProTides are either inactives, or are moderately active, but only at cytotoxic concentrations.

The acyclic guanine family (**191**, **195.a-c**) appears somehow more potent than any other. The guanine base is probably better accepted by metabolic enzymes or fits better in the active site of the NS5B polymerase. It is stricking that the introduction of a

methoxy substituent at *C*-6 of the heterobase (**190.a-b**, **194.a-c**) leads to total loss of activity compared to its guanine analogue. Thus, in this case it might be that the acyclic 6-*O*-methyl guanine analogues are not substrates of the enzymes responsible for 6-*O*-ether hydrolysis.

In conclusion, the lack of anti-HCV activity of these acyclic derivatives is likely to be associated with an unfavourable conformation of the double bond, which may prevent nucleosides and ProTides to be good substrates for metabolic enzymes responsible for phosphorylation and/or hydrolysis of the heterobase. On the other hand, it is also possible that the 5'-triphosphate of such acyclic nucleosides is not an inhibitor of HCV NS5B polymerase. Work to prepare and assay the 5'-triphosphate directly against the viral polymerase would clarify this, but was beyond the scope of our work, given the poor antiviral activity of the agents prepared.

4. Conclusion and future work

Starting from the potent anti-HCV 2'-*C*-methyl-6-*O*-methyl guanosine **11**, the sugar moiety was modified to an acyclic chain containing an unsaturated bond. A different method than the one reported in the literature[20] was investigated for the formation of such acyclic purine nucleosides. Mitsunobu coupling enabled the synthesis of four acyclic purine nucleosides, from which two were obtained as a separable mixture of *cis*- and *trans*-isomers. The former isomer (*Z*-isomer) appeared to be the preferred conformation despite steric hindrance. In order to overcome eventual poor metabolism of the acyclic nucleoside, ProTides of the (*Z*-isomer) of each acyclic nucleoside were synthesised, with variation at the ester moiety.

Due to poor solubility of the guanine nucleobase in tetrahydrofuran, the preferred solvent for the Mitsunobu reaction, the acyclic guanine nucleosides and phosphoramidates were obtained by demethoxylation of their corresponding 6-O-methyl derivatives.[32] From this method acyclic guanine analogues were more easily accessible in comparison with the two-step synthesis reported in the literature.[20]

Acyclic purine nucleosides were inactive in HCV replicon assay, and unfortunately the ProTide technology did not reinstate the potency without increasing the cytotoxicity. These disappointing results are likely to be related to inefficient metabolism resulting

from the unfavourable conformation of the alkene, or possibly to poor activity of the 5'-triphosphate derivative against HCV RNA replication.

The acyclic guanine family appeared as the most potent in cell-based assays. Moreover, taking into consideration the remarkable loss of potency related to the introduction of the 6-*O*-methyl group at the heterobase, it is presumable that such acyclic nucleoside are not substrate for metabolic enzymes responsible for base hydrolysis.

The *α*-naphthyl *L*-alanine benzyl ester phosphoramidate (**195.c**) of acyclic guanine nucleoside was the most promising derivative among all acyclic analogues synthesised. Further work would concentrate on tuning the acyclic moiety by removing the unsaturation of the nucleoside and develop an extensive structure-activity relationship, to better understand the mode of action of acyclic derivatives towards inhibition of NS5B polymerase.

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Chapter Seven: Conclusion

This thesis reports the design, synthesis and biological evaluation of prodrugs of *β*-2'-*C*-methyl-6-*O*-methyl guanosine (**11**), and of several modified analogues as potential back-up molecules for INX-08189 (BMS-986094) an efficient inhibitor of HCV previously discovered in our laboratory. The rationale behind each of these modifications has been discussed in this report.

HCV replicon based assays revealed that modifications at the nucleobase or sugar moieties tend to be unfavourable. However, the ProTide technology somehow restored the inhibitory potency against HCV replication, at the exception of acyclic nucleosides. While the latter were not promising, future investigations should take into consideration tuning the prodrug motif and/or applying the phosphorodiamidate prodrug technology on the 7-deaza family in order to deliver more efficiently the 5'-monophosphate species intracellularly.

Enzymatic hydrolysis of phosphorodiamidates, 2'-O-modified and base modified nucleoside prodrugs using carboxypeptidase Y and cell lysates supported the putative mechanism of activation and the results obtained in biological assays. Additionally, molecular modelling studies were performed using cathepsin A, human hint phosphoramidase, inosine monophosphate dehydrogenase and adenosine deaminase-like protein-1, to investigate the intracellular mechanism of bioactivation.

Despite the lack of significant inhibitory activity of modified nucleosides against HCV, the development of novel synthetic pathways towards varied modifications performed at a nucleoside level represents a significant achievement. We believe that the work presented herein will be the foundation of future research in the development of novel antivirals or anticancer molecules.

Chapter Eight: Experimental Section

1. General experimental methods

Solvent and Reagents:

All solvents were anhydrous and purchased from Aldrich: chloroform ($CHCl_3$), dichloromethane (DCM), diethyl ether (Et_2O), acetonitrile (ACN), methanol (MeOH), N,N-dimethylformamide (DMF), tetrahydrofuran (THF), pyridine, acetone. All reagents bought from Aldrich, Fischer, TCI, Carbosynth, Hande Pharma or CiVentiChem were used without purification. All glassware was dried in the oven at 130 °C before use.

Thin Layer Chromatography (TLC):

Thin layer chromatography (TLC) was performed on commercially available aluminium backed plates from Merck, (60 F_{264}, thickness 0.2 mm), and separated fractions were visualised using ultra-violet light (245-366 nm).

Preparative Chromatography:

Preparative TLC was performed on 20x20 cm, 500 to 2000 μm silica gel plates from Merck.

Column Chromatography (CC):

Column chromatography was peformed using silica gel (60 Å, 35-70 μm) from Fischer as stationary phase. Glass column was packed with the appropriate solvent and the residues were loaded and pre-absorbed on the silica. Identification of the fractions was performed on TLC plates and the solvent was evaporated under reduced pressure.

Nuclear Magnetic Resonance (NMR) Spectroscopy:

^{1}H, ^{13}C, ^{19}F and ^{31}P spectra were recorded on a Bruker Advance 500 spectrometer at 25°C with respective frequencies of 500, 125, 470 and 202 MHz. Spectra were calibrated to the residual signal of the deuterated solvent used for the experiment. The external standard for ^{31}P NMR is 85% of phosphoric acid. Chemical shifts (δ) are given in parts per million (ppm) and coupling constants (*J*) in Hertz (Hz). The NMR signals

are assigned for each molecule using the following abbreviations: s (singlet), bs (broad singulet), d (doublet), dd (doublet of doublet), t (triplet), m (multiplet).

Mass Spectroscopy (MS):

Mass spectroscopy experiments were performed by the School of Chemistry, Cardiff University. Low resolution mass spectra were run on a Waters GCT Premier spectrometer in either positive or negative mode. High resolution mass spectra were run using electrospray ionisation source (EI). *m/z* values are reported in Da.

High Performance Liquid Chromatography (HPLC):

Analytical High Performance Liquid Chromatography (HPLC) were run either on a Varian Prostar LC workstation with a Varian Prostar 335 LC detector, and pro-star 210 delivery system using an analytical column Varian Polaris C18-A (10 μm) with Galaxie Chromatography Data System as software, or on a Thermo-Fischer workstation with a Spectra System UV2000 detector, and Spectra System P4000 coupled with a Spectra System SCM1000 delivery system using an analytical Thermo-Fischer C18-A (10 μm) column (reverse phase) with Chrom Quest 5.0 as software.

Semi-preparative HPLC was only performed on the first instrument mentioned above. Only compounds which purity was ≥ 95% were sent for biological evaluation.

UV Spectroscopy (UV):

The UV experiments were run on Varian 50 Bio UV-visible spectrophotometer, and recordered by the WinUV version 3.1 software.

2. Standard procedures

Standard procedure A: Preparation of protected amino acid ester.[1]

To the Boc-protected amino acid (1.0 eq) dissolved in anh. DCM (20 mL/g of amino acid) was added the alcohol (1.4 eq), DCC (1.0 eq) and DMAP (0.1 eq) and the solution was stirred overnight at room temperature. After removal of solvent under reduced pressure, flash chromatography using a gradient of hexane/ethyl acetate was performed to afford a white solid.

Standard procedure B1: Preparation of protected amino ester tosylate salt.

The protected amino acid ester (1.0 eq) and pTSA (1.0 eq) was dissolved in EtOAC and the solution was refluxed overnight, then cooled down to room temperature to enable precipitation of the desired sulfonate salt.

Standard procedure B2: Preparation of protected amino ester tosylate salt.

To the amino acid (1.0 eq) dissolved in toluene (20 mL/g of amino acid) was added the alcohol (5 to 15 eq) and pTSA (1.1 eq). The solution was heated under reflux overnight using Dean-Stark apparatus. After removal of solvent under reduced pressure, the amino acid was precipitated from Et_2O to afford a white solid.

Standard procedure C: Preparation of amino acid ester α-naphthyl phosphochloridates.[2]

The amino ester sulfonate salt (1.0 eq) dissolved in anh. DCM (5 mL) was added to the *α*-naphthyl phosphorodichloridate (1.0 eq) and the solution cooled down to -78 °C, at which NEt_3 (2.0 eq) was added dropwise. After one hour at - 78 °C, the reaction was left to warm at room temperature and stirred for further 30 min. The volatiles were evaporated under reduced pressure and the residue was purified by flash chromatography using Hexane/EtOAc (1:1).

Standard procedure D: Preparation of ProTides via tBuMgCl method.[2]

*t*BuMgCl (1M in THF, 2.0 eq) was added dropwise to 2'-C-Me-6-OMe guanosine (1.0 eq) in anh. THF (1.5 mL) and the solution was stirred for 20 min, after which the amino acid ester α-naphthyl amino acid ester phosphochloridate (2.0 eq) in anh. THF (1.5 mL) was added. The solution was stirred overnight at room temperature before the removal

of solvent under reduced pressure. The residue was purified on by flash chromatography using $CHCl_3$/MeOH as eluents.

Standard procedure E: Preparation of phosphorodiamidates.

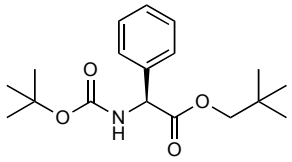
To a suspension of the nucleoside (1.0 eq) in anh. THF was added NEt_3 (1.2 eq). After stirring for 30 min at room temperature, $POCl_3$ (1.2 eq) is added dropwise at -78 °C and the reaction mixture is stirred 30 min at -78 °C, then allowed to warm to room temperature over 30 min. Anh. DCM was added, followed by the amino acid ester salt/amine (5.0 eq) and NEt_3 (10.0 eq.) at -78 °C. After stirring at room temperature overnight, water was added and the layers were separated. The aqueous phase was extracted with DCM and the organic phase washed with brine. The combined organic layers are dried over anh. Na_2SO_4, filtered and evaporated to dryness. The resulting residue is purified by flash chromatography column chromatography using a gradient of DCM/MeOH.

3. Experimental procedures related to chapter 3

3.1. Preparation of *β*-2'-*C*-methyl-6-*O*-methyl guanosine phosphoramidates

3.1.1. Preparation of protected amino acid esters

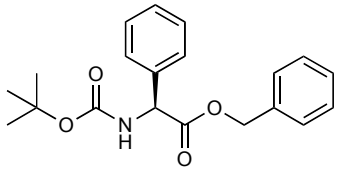
Preparation of Boc protected L-Phenylglycine-neopentyl ester



Prepared according to standard procedure A, using *N*-Boc-*L*-α-phenylglycine (1.69 g, 6.72 mmol, 1.0 eq) in anh. DCM (31 mL) was added the neopentyl alcohol (0.83 g, 9.42 mmol, 1.4 eq), DCC (1.39 g, 6.76 mmol, 1.0 eq) and DMAP (0.08 g, 0.68 mmol, 0.1 eq). After overnight stirring, the solvent was removed under reduced pressure and the residue was purified by flash chromatography using Hexane/EtOAc (9:1) to afford a white solid (1.88 g, 5.84 mmol, 87%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 7.42-7.34 (m, 5H, Ph), 5.25 (s, 1H, CH), 3.92 & 3.76 (AB system, 2H, J = 10.5 Hz, O$\underline{CH_2}$C$(CH_3)_3$), 1.47 (s, 9H, NHCOOC($\underline{CH_3}$)$_3$), 0.84 (s, 9H, $OCH_2C(\underline{CH_3})_3$).

Preparation of Boc protected L-Phenylglycine-benzyl ester

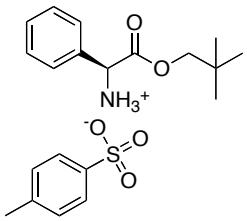


Prepared according to standard procedure A, using *N*-Boc-*L*-α-phenylglycine (1.52 g, 6.06 mmol, 1.0 eq) in anh. DCM (33 mL) was added the benzyl alcohol (0.85 mL, 8.20 mmol, 1.4 eq), DCC (1.26 g, 6.26 mmol, 1.0 eq) and DMAP (0.07 g, 0.60 mmol, 0.1 eq). After overnight stirring, the solvent was removed under reduced pressure and the residue was purified by flash chromatography using Hexane/EtOAc (9:1) to afford a white solid (1.35 g, 3.95 mmol, 65%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 7.36 to 7.34 (m, 5H, Ph), 7.31 (m, 3H, $OCH_2\underline{Ph}$), 7.22 (t, 2H, J = 3.5 Hz, $OCH_2\underline{Ph}$), 5.56 (m, 1H, NH), 5.40 (d, 1H, J = 7 Hz, CH), 5.18-5.15 (m, 2H, O$\underline{CH_2}$Ph), 1.46 (s, 9H, NHCOOC($\underline{CH_3}$)$_3$).

3.1.2. Preparation of amino acid ester salts or free amino acids

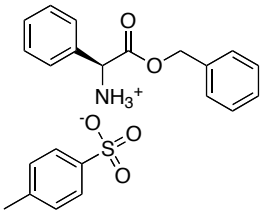
Preparation of L-Phenylglycine-neopentyl ester tosylate salt (12.a)



Prepared according to standard procedure B1, using Boc protected *L*-phenylglycine neopentyl ester (1.05 g, 3.27 mmol, 1.0 eq), pTSA (0.63 g, 3.31 mmol, 1.0 eq) dissolved in EtOAc (37 mL) and refluxed overnight. The solution was then cooled down to RT and the product precipitated. After filtration and diethyl ether washes a white solid was recovered white product (0.88 g, 2.25 mmol, 68%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.68 (bs, 3H, NH_3^+), 7.57 (d, J = 8.0 Hz, 2H, 2 x CH ortho Ts), 7.49 -7.33 (m, J = 7.5 Hz, 5H, 2 x CH meta Ts, 2 x CH meta Ph, CH para Ph), 7.08 (d, J = 7.5 Hz, 2H, 2 x CH ortho Ph), 5.10 (s, 1H, CH), 3.81 & 3.67 (AB system, J = 10.3 Hz, 2H, O$\underline{CH_2}$C$(CH_3)_3$), 2.37 (s, 3H, CH_3 Ts), 0.72 (s, 9H, $OCH_2C(\underline{CH_3})_3$).

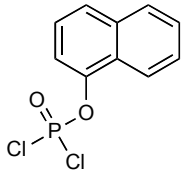
Preparation of L-Phenylglycine-benzyl ester tosylate salt (12.b)



Prepared according to standard procedure B1, using Boc protected *L*-phenylglycine benzyl ester (1.35 g, 3.95 mmol, 1.0 eq), pTSA (0.75 g, 3.91 mmol, 1.0 eq) dissolved in EtOAC (44 mL) and refluxed overnight. The solution was then cooled down to RT and the product precipitated. After filtration and diethyl ether washes a white solid was recovered (1.53 g, 3.71 mmol, 94%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.70 (bs, 3H, NH_3^+), 7.54 (d, 2H, J = 8.0 Hz, 2 x CH ortho Ts), 7.31-7.14 (m, 7H, CH Bn, 2 x CH meta Ts), 7.06 (d, 2H, J = 7.0 Hz, 2 x CH ortho Ph), 7.01 (d, 3H, J = 8.0 Hz, 2 x CH meta, CH para Ph), 5.14 (s, 1H, CH), 5.03 & 4.95 (AB system, 2H, J =12.5 Hz, O$\underline{CH_2}$Ph), 2.33 (s, 3H, CH_3 Ts).

3.1.3. Preparation of *α*-naphthyl phosphorodichloridate (***13***)[2]



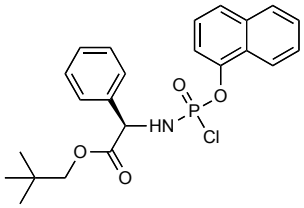
Phosphorus oxychloride (1.95 mL, 20.9 mmol, 1.0 eq) and *α*-naphthol (3.01 g, 20.9 mmol, 1.0 eq) were stirred in anh. Et_2O (20 mL) under argon. Anh. NEt_3 (2.91 mL, 20.9 mmol, 1.0 eq) was added at -78 °C and stirred for 30 min, then the solution was allowed to warm to room temperature over 45 min. The triethylamine chloride salt was filtered under reduced pressure to give bright yellow oil (2.7936 g, 10.7 mmol, 51%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.13 (d, 1H, *J* = 8.5 Hz, H-8 Ar), 7.93 (d, 1H, *J* = 8.0 Hz, H-6 Ar), 7.84 (d, 1H, *J* = 8.5 Hz, H-4 Ar), 7.61 (m, 3H, H-9, H-7, H-3 Ar), 7.49 (t, 1H, *J* = 7.75 Hz, H-2 Ar).

^{31}P (202 MHz, $CDCl_3$) δ_P (ppm): 3.74 (s).

3.1.4. Preparation of *α*-naphthyl phosphorochloridates

Preparation of α-naphthyl L-Phenylglycine-neopentyl phosphochloridate (14.a)

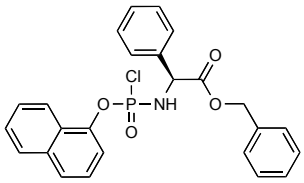


Prepared according to procedure C, using *L*-phenylglycine-neopentyl ester tosylate salt (**12.a**) (0.71 g, 1.80 mmol, 1.0 eq) in anh. DCM (10.5 mL), *α*-naphthyl phosphorodichloridate (**13**) (0.51 g, 1.95 mmol, 1.0 eq) and NEt_3 (0.52 mL, 3.73 mmol, 2.0 eq). The solvent was removed under reduced pressure and the residue purified by flash chromatography using Hexane/EtOAc (1:1) to obtain the desired molecule (0.61 g, 1.38 mmol, 76%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.11-8.01 (m, 1H, H-8 Ar), 7.82 (d, 1H, *J* = 8.0 Hz, H-6 Ar), 7.68 (d, 1H, *J* = 8.0 Hz, H-4 Ar), 7.55-7.46 (m, 3H, H-3, H-9, H-7 Ar), 7.40-7.08 (m, 6H, 5 x CH Ph, H-2 Ar), 5.31-4.91 (m, 4H, NHCHPhCO, NHCHPhCO, $OCH_2C(CH_3)_3$), 0.81 (s, 9H, $OCH_2C(CH_3)_3$).

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm): 7.72 (s), 7.44 (s).

Preparation of α-naphthyl L-Phenylglycine-benzyl ester phosphochloridate (14.b)

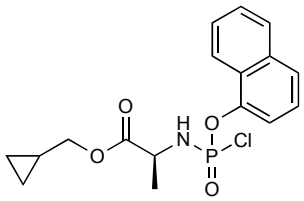


Prepared according to procedure C, using *L*-phenylglycine-benzyl ester tosylate salt (**12.b**) (1.11 g, 2.69 mmol, 1.0 eq) in anh. DCM (5 mL), *α*-naphthyl phosphorodichloridate (**13**) (0.72 g, 2.74 mmol, 1.0 eq) and NEt_3 (0.76 mL, 5.45 mmol, 2.0 eq). The solvent was removed under reduced pressure and the residue purified by flash chromatography using Hexane/EtOAc (1:1), to obtain the desired molecule (0.92 g, 1.98 mmol, 73%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.11-8.01 (m, 1H, H-8 Ar), 7.82 (d, 1H, *J* = 8.0 Hz, H-6 Ar), 7.68 (d, 1H, *J* = 8.0 Hz, H-4 Ar), 7.55-7.46 (m, 3H, H-3, H-9, H-7 Ar), 7.40-7.08 (m, 11H, 5 x CH Ph, 5 x CH OCH_2Ph, H-2 Ar), 5.31-4.91 (m, 4H, NHCHPhCO, NHCHPhCO, OCH_2Ph).

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm): 7.50 (s), 7.24 (s).

Preparation of α-naphthyl L-Phenylglycine-cyclopropylmethyl ester phosphochloridate (14.c)



Prepared according to procedure C, using *L*-alanine-cyclopropylmethyl ester tosylate salt (0.61 g, 1.92 mmol, 1.0 eq) in anh. DCM (11 mL), α-naphthyl phosphorodichloridate (**13**) (0.54 g, 2.06 mmol, 2.0 eq) and anh. NEt_3 (0.54 mL, 3.84 mmol, 2.0 eq). The solvent was removed under reduced pressure and the residue purified by flash chromatography using Hexane/EtOAc (1:1) to yield the desired molecule (0.65 g, 1.86 mmol, 97%).

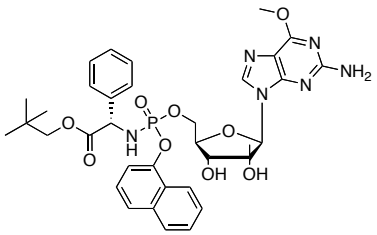
^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.10-8.08 (d, 1H, *J* = 9.0 Hz, H-8 Ar), 7.84 (d, 1H, *J* = 6.0 Hz, H-6 Ar), 7.70 (d, 1H, *J* = 8.0 Hz, H-4 Ar), 7.61 (t, 1H, *J* = 5.0 Hz, H-3 Ar), 7.52 (m, 2H, H-7, H-9 Ar), 7.51 (m, 1H, H-2 Ar), 4.93 (m, 1H, NHCH(CH_3)CO), 4.33 (m, 1H, NHCH(CH_3)CO), 4.02 (m, 2H, OCH_2cProp), 1.15 (d, 3H, *J* =2.5 Hz,

NHCH(CH$_3$)CO), 0.85 (m, 1H, CH cProp), 0.51 (m, 2H, 2 x CH cProp), 0.45 (m, 2H, 2 x CH cProp).

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm): 8.38 (s), 8.22 (s).

3.1.5. Preparation of *β*-2'-*C*-methyl-6-*O*-methyl guanosine ProTides

Synthesis of α-naphthyl L-Phenylglycine-neopentyl ester β-2'-C-methyl-6-O-methyl guanosine phosphoramidate (15.a)



This ProTide was prepared according to the Standard Procedure D, using *t*BuMgCl (1M in THF, 1.38 mL, 1.38 mmol, 2.0 eq), *β*-2'-*C*-Me-6-*O*Me guanosine (**11**) (0.22 g, 0.70 mmol, 1.0 eq) in anh. THF (1.3 mL) and *α*-naphthyl *L*-Phenylglycine neopentyl ester phosphochloridate (**14.a**) (0.61 g, 1.38 mmol, 2 eq) in anh. THF (1.3 mL). After overnight stirring at RT, the solvent was removed under reduced pressure and the residue was purified by flash chromatography using $CHCl_3$/MeOH as eluents to yield a white solid (0.23 g, 0.32 mmol, 46%).

HPLC (MeOH/H_2O): Rt = 13.37 min, 14.40 min.

HPLC (ACN/ H_2O): Rt = 21.33 min.

MS (TOF ES$^+$): 721.28 (M + H$^+$), 759.23 (M + K$^+$).

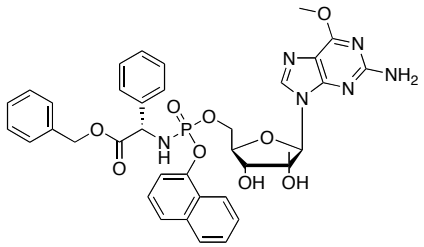
^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.01 (m, 2H, H-8 Ar diastereoisomers), 7.83 (m, 4H, H-6 Ar, H-8 base diastereoisomers), 7.66 (m, 2H, H-4 Ar diastereoisomers), 7.58-7.25 (m, 18H, H-2, H-3, H-7, H-9 Ar, 5 x CH Ph diastereoisomers), 6.01 & 5.89 (2s, 2H, H-1' diastereoisomers), 5.20-5.12 (m, 6H, OCH$_2$C(CH$_3$)$_3$, NHCHPhCO diastereoisomers), 4.70-4.58 (m, 2H, H-5' diastereoisomers), 4.51-4.45 (m, 2H, H-5' diastereoisomers), 4.45-4.32 (m, 2H, H-4'diastereoisomers), 4.19-4.16 (m, 2H, H-3' diastereoisomers), 4.10 & 4.08 (2s, 6H, 6-*O*-CH$_3$ base diastereoisomers), 0.91 & 0.90 (2s, 6H, CH$_3$-2' diastereoisomers), 0.74 & 0.73 (2s, 18H, OCH$_2$C(CH$_3$)$_3$ diastereoisomers).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 172.89 & 172.21 (2d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 7.45 Hz, NHCHPhCO diastereoisomers), 161.65 (s, C-6 base), 159.41 (s, C-2 base), 152.80 (s, C-4 base), 145.02 (s, C-1 Ar), 137.75 & 137.68 (2s, C-8 base diastereoisomers), 137.55

(s, C-1 Ph), 134.73 (s, C-5 Ar), 128.79 & 128.65 & 128.51 & 128.37 (4s, C-2, C-3, C-5, C-6 Ph), 127.78 & 127.67 (2s, C-6 Ar diastereoisomers), 126.90 & 126.71 & 126.61 & 126.58 & 126.35 & 125.41 & 125.36 & 125.10 (8s, C-3, C-7, C-8, C-10 Ar diastereoismers), 124.98 (s, C-9 Ar), 121.41 (s, C-4 Ar), 115.09 (s, C-2 Ar), 115.07 (s, C-5 base), 91.65 & 91.57 (2s, C-1' diastereoisomers), 82.05 & 81.86 (2d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 6.3 Hz, C-4' diastereoisomers), 80.77 & 80.45 (2s, O<u>C</u>H$_2$C(CH$_3$)$_3$ diastereoisomers), , 79.27 & 79.17 (2s, C-2' diastereoisomers), 74.64 & 74.27 (2s, C-3' diastereoisomers), 66.24 & 65.76 (2d, $J^2_{P\text{-}O\text{-}C}$ = 5.04 Hz, C-5' diastereoisomers), 58.45 & 58.27 (2s, NH<u>C</u>HPhCO diastereoisomers), 53.89 (s, 6-*O*-<u>C</u>H$_3$ base), 31.41 (s, OCH$_2$<u>C</u>(CH$_3$)$_3$), 26.03 & 26.01 (2s, OCH$_2$C(<u>C</u>H$_3$)$_3$ diastereoisomers), 20.37 & 20.32 (2s, CH$_3$-2' diastereoisomers).

^{31}P NMR (202 MHz, CDCl$_3$) δ_P (ppm): 3.97 (s) (62%), 3.60 (s) (38%).

Synthesis of α-naphthyl L-Phenylglycine-benzyl ester β-2'-C-methyl-6-O-methyl guanosine phosphoramidate (15.b)



This ProTide was prepared according to the Standard Procedure D, using *t*BuMgCl (1M in THF, 1.49 mL, 1.49 mmol, 2.0 eq), *β*-2'-*C*-methyl-6-*O*-methyl guanosine (**11**) (0.23 g, 0.75 mmol, 1.0 eq) in anh. THF (1.5 mL), and *α*-naphthyl *L*-Phenylglycine benzyl ester phosphochloridate (**14.b**) (0.69 g, 1.49 mmol, 2.0 eq) in anh. THF (1.5 mL). After overnight stirring at RT, the solvent was removed under reduced pressure and the residue was purified by flash chromatography using CHCl$_3$/MeOH as eluents to yield a white solid (0.16 g, 0.22 mmol, 32%).

HPLC (MeOH/H$_2$O): Rt = 19.35 min, 19.89 min.

HPLC (ACN/ H$_2$O): Rt = 23.54 min.

MS (TOF ES$^+$): 741.25 (M + H$^+$), 763.23 (M + Na$^+$), 779.21 (M + K$^+$).

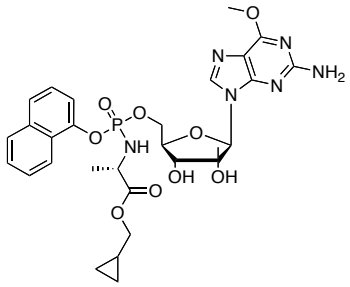
^{1}H NMR (500 MHz, CDCl$_3$) δ_H (ppm): 8.02-8.00 (m, 2H, H-8 Ar diastereoisomers), 7.85 (m, 4H, H-6 Ar, H-8 base diastereoisomers), 7.66 & 7.64 (2d, 2H, *J* = 8.5 Hz, H-4 Ar diastereoisomers), 7.58-7.13 (m, 28H, H-2, H-3, H-7, H-9 Ar, 5 x CH Ph, 5 x CH OCH$_2$<u>Ph</u> diastereoisomers), 5.84 & 5.81 (2s, 1H, H-1' diastereoisomers), 5.18-5.07 (m, 6H, O<u>CH$_2$</u>Ph, NH<u>CH</u>PhCO diastereoisomers), 4.53-4.49 (m, 2H, H-5'

diastereoisomers), 4.36-4.34 (m, 1H, H-5' diastereoisomers), 4.30-4.25 (m, 2H, H-4' diastereoisomers), 4.18-4.16 (m, 2H, H-3' diastereoisomers), 4.03 & 4.02 (2s, 3H, 6-*O*-$\underline{CH_3}$ base diastereoisomers), 0.90 & 0.89 (2s, 3H, CH_3-2' diastereoisomers).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 172.54 & 172.18 (2d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 7.50 Hz, NHCHPh$\underline{C}$O diastereoisomers), 161.67 (s, C-6 base), 159.38 (s, C-2 base), 152.80 (s, C-4 base), 145.43 (s, C-1 Ar), 137.69 & 137.25 (2s, C-8 base diastereoisomers), 134.68 (s, C-1 Ph), 132.79 (s, C-5 Ar), 128.90 & 128.76 (2s, C-2, C-6 Ph), 128.46 & 128.33 (2s, C-3, C-5 $OCH_2\underline{Ph}$), 127.83 & 127.77 & 127.69 (4s, C-3, C-5 Phe, C-6 Ar), 126.90 & 126.63 & 126.39 (3s, C-2, C-4, C-6 $OCH_2\underline{Ph}$, C-4 Ph), 125.67 & 125.36 & 125.12 & 124.98 (2s, C-3, C-7, C-8, C-10 Ar), 124.70 (s, C-9 Ar), 121.40 (s, C-4 Ar), 115.98 (s, C-2 Ar), 114.78 (s, C-5 base), 91.84 & 91.62 (2s, C-1' diastereoisomers), 81.16 & 81.08 (2s, C-4' diastereoisomers), 79.80 & 79.29 (2s, C-2' diastereoisomers), 74.86 & 74.54 (2s, C-3' diastereoisomers), 67.68 & 67.42 (2s, $O\underline{CH_2}Ph$ diastereoisomers), 66.24 & 65.76 (2s, C-5' diastereoisomers), 58.55 & 58.28 (2s, NH$\underline{C}$HPhCO diastereoisomers), 53.90 & 53.82 (2s, 6-*O*-$\underline{CH_3}$ base diastereoisomers), 20.36 & 20.31 (2s, CH_3-2' diastereoisomers).

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm): 3.82 (s) (20%), 3.41 (s) (80%).

Synthesis of α-naphthyl L-Alanine-cyclopropylmethyl ester β-2'-C-methyl-6-O-methyl guanosine phosphoramidate (15.c)



This ProTide was prepared according to the Standard Procedure D, using *t*BuMgCl (1M in THF, 1.90 mL, 1.90 mmol, 2eq), *β*-2'-*C*-Me-6-*O*Me guanosine (**11**) (0.29 mg, 0.93 mmol, 1.0 eq) in anh. THF (2.0 mL), and *α*-naphthyl *L*-Alanine-cyclopropylmethyl ester phosphochloridate (**14.c**) (0.65 g, 1.86 mmol, 2.0 eq) in anh. THF (2.0 mL). After overnight stirring at RT, the solvent was removed under reduced pressure and the residue was purified by flash chromatography using $CHCl_3$/MeOH as eluents to yield a white solid (0.19 g, 0.30 mmol, 32%).

HPLC (MeOH/H_2O): Rt = 13.04 min, 13.65 min.

HPLC (ACN/H_2O): Rt = 22.54 min.

MS (TOF ES^+): 643.25 ($M + H^+$).

^1H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.13-8.05 (m, 2H, H-8 Ar diastereoisomers), 7.81-7.75 (m, 4H, H-6 Ar, H-8 base diastereoisomers), 7.63-7.58 (m, 2H, H-4 Ar diastereoisomers), 7.53-7.45 (m, 6H, H-2, H-3, H-7 Ar diastereoisomers), 7.37-7.02 (m, 2H, H-9 Ar diastereoisomers), 5.98 & 5.96 (2s, 2H, H-1' diastereoisomers), 4.73-4.70 (m, 2H, H-5' diastereoisomers), 4.66-4.61 (m, 2H, H-5' diastereoisomers), 4.52-4.46 (m, 2H, H-3' diastereoisomers), 4.23-4.19 (m, 1H, H-4' diastereoisomers), 4.11-4.07 (m, 2H, NHCH(CH_3)CO diastereoisomers), 4.03 & 4.01 (2s, 6H, 6-OCH_3 base diastereoisomers), 3.87-3.73 (m, 4H, OCH_2-cProp diastereoisomers), 1.36-1.33 (m, 6H, NHCH(CH_3)CO diastereoisomers), 0.99-0.95 (m, 2H, C-1 cProp diastereoisomers), 0.92 & 0.89 (2s, 6H, CH_3-2' diastereoisomers), 0.47-0.45 (m, 4H, 4 x CH cProp diastereoisomers), 0.18-0.15 (m, 4H, 4 x CH cProp diastereoisomers).

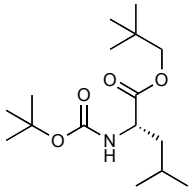
^{13}C NMR (125 MHz, $CDCl_3$) δ_c (ppm): 173.85 & 173.53 (2d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 7.56 Hz, NHC(CH_3)CO diastereoisomers), 161.59 (s, C-6 base), 159.58 (s, C-2 base), 152.89 (s, C-4 base), 146.43 (s, C-1 Ar), 137.65 (s, C-8 base), 134.72 & 134.69 (2s, C-5 Ar diastereoisomers), 127.78 & 127.65 (2s, C-6 Ar diastereoisomers), 126.69 & 126.65 & 126.55 & 126.45 (4s, C-7, C-8 Ar diastereoisomers), 126.37 & 126.32 (2s, C-10 Ar diastereoisomers), 125.48 & 125.42 (2s, C-3 Ar diastereoisomers), 124.97 & 124.91 (2s, C-9 Ar diastereoisomers), 121.48 & 121,42 (2s, C-4 Ar diastereoisomers), 115.03 (s, C-2 Ar), 114.91 (s, C-5 base), 91.61 & 91.52 (2s, C-1' diastereoisomers), 80.96 & 80.79 (2d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 7.5 Hz, C-4' diastereoisomers), 79.16 & 79.12 (2s, C-2' diastereoisomers), 73.95 (s, C-3'), 70.40 & 70.38 (2s, OCH_2-cProp diastereoisomers), 66.14 & 65.78 (2s, C-5' diastereoisomers), 53.88 (s, 6-*O*CH_3 base), 50.46 & 50.40 (2d, $J^2_{P\text{-}N\text{-}C}$ = 7.5 Hz, NHCH(CH_3)CO diastereoisomers), 20.92 & 20.74 (2d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 5.0 Hz, NHCH(CH_3)CO diastereoisomers), 20.51 & 20.21 (2s, CH_3-2' diastereoisomers), 9.74 & 9.54 (2s , C-1 cProp diastereoisomers), 3.19 & 3.14 (2s, CH_2 cProp diastereoisomers).

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm): 4.07 (s) (43%), 3.90 (s) (57%).

3.2. Preparation of *β*-2'-*C*-methyl-6-*O*-methyl guanosine phosphorodiamidates

3.2.1. Preparation of protected amino acid esters

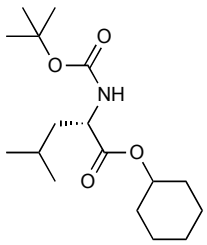
Preparation of Boc protected L-Leucine-neopentyl ester



Prepared according to standard procedure A, using *N*-Boc-*L*-Leucine (4.01 g, 17.3 mmol, 1.0 eq) in anh. DCM (95 mL) was added the neopentyl alcohol (2.00 g, 22 mmol, 1.3 eq), DCC (3.57 g, 17.3 mmol, 1.0 eq) and DMAP (1.729 g, 0.21 mmol, 0.1 eq). After overnight stirring, the solvent was removed under reduced pressure and the residue was purified by flash chromatography using Hexane/EtOAc (9:1) to afford a white solid (5.16 g, 17.12 mmol, 99%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 4.96 (d, 1H, J = 6.9 Hz, NH), 4.33 (d, 1H, J = 5.5 Hz, NHCH($CH_2CH(CH_3)_2$)CO), 3.82 & 3.77 (AB system, 2H, J = 10.55 Hz, O$CH_2C(CH_3)_3$), 1.73 (m, 1H, NHCH($CH_2CH(CH_3)_2$)CO), 1.59 (m, 1H, NHCH($CH_2CH(CH_3)_2$)CO), 1.48 (m, 1H, NHCH($CH_2CH(CH_3)_2$)CO), 1.42 (s, 9H, NHCOOC($CH_3)_3$), 0.94 (s, 9H, $OCH_2C(CH_3)_3$), 0.93 (s, 6H, NHCH($CH_2CH(CH_3)_2$)CO).

Preparation of Boc protected L-Leucine-cyclohexyl ester

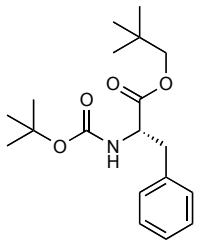


Prepared according to standard procedure A, using *N*-Boc-*L*-Leucine (4.73 g, 20.5 mmol, 1.0 eq) in anh. DCM (95 mL) was added the cylohexanol (4.30 mL, 40.9 mmol, 2.0 eq), DCC (4.23 g, 20.5 mmol, 1.0 eq) and DMAP (0.25 g, 2.05 mmol, 0.1 eq). After overnight stirring, the solvent was removed under reduced pressure and the residue was purified by flash chromatography using Hexane/EtOAc (9:1) to afford colourless oil (4.24 g, 13.53 mmol, 66%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 4.95 (bs, 1H, NH), 4.79 (m, 1H, CH cHex), 4.24 (m, 1H, NHCH($CH_2CH(CH_3)_2$)CO), 1.81 (m, 4H, 2 x CH_2 ortho cHex), 1.70 (m, 6H, 2

x CH_2 meta cHex & CH_2 para cHex), 1.56-1.49 (m, 3H, NHCH($\underline{CH_2}\underline{CH}(CH_3)_2$)CO), 1.43 (s, 9H, NHCOOC($\underline{CH_3}_3$), 0.93 (s, 6H, NHCH($CH_2CH(\underline{CH_3}_2$)CO).

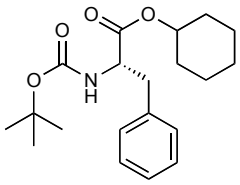
Preparation of Boc protected L-Phenylalanine-neopentyl ester



Prepared according to standard procedure A, using *N*-Boc-*L*-Phenylalanine (4.00 g, 15.1 mmol, 1.0 eq) in anh. DCM (83 mL), neopentyl alcohol (1.73 g, 19.6 mmol, 1.3 eq), DCC (3.12 g, 15.1 mmol, 1.0 eq) and DMAP (0.19 g, 1.51 mmol, 0.1 eq). After overnight stirring, the solvent was removed under reduced pressure and the residue was purified by flash chromatography using Hexane/EtOAc (9:1) to afford a white solid (5.26 g, 14.68 mmol, 97%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 7.26 (m, 2H, 2 x CHmeta Ph), 7.20 (m, 1H, CH para Ph), 7.13 (m, 2H, 2 x CH ortho Ph), 5.06 (d, 1H, J = 6.95 Hz, NH), 4.59 (d, 1H, J = 6.8 Hz, CH), 3.82 & 3.68 (AB system, 2H, J = 10.5 Hz, O$\underline{CH_2}C(CH_3)_3$), 3.06 (m, 2H, $\underline{CH_2}$Ph), 1.39 (s, 9H, NHCOOC($\underline{CH_3}_3$), 0.88 (s, 9H, $OCH_2C(\underline{CH_3}_3$).

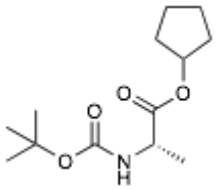
Preparation of Boc protected L-Phenylalanine-cyclohexyl ester



Prepared according to standard procedure A, using *N*-Boc-*L*-Phenylalanine (4.22 g, 15.9 mmol, 1.0 eq) in anh. DCM (85 mL), cyclohexanol (3.40 mL, 31.8 mmol, 2.0 eq), DCC (3.30 g, 15.9 mmol, 1.0 eq) and DMAP (0.21 g, 1.59 mmol, 0.1 eq). After overnight stirring, the solvent was removed under reduced pressure and the residue was purified by flash chromatography using Hexane/EtOAc (9:1) to afford a colourless oil (5.19 g, 4.94 mmol, 94%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 7.28 (m, 2H, 2 x CH ortho Ph), 7.24 (m, 1H, H para Ph), 7.16 (m, 2H, 2 x CH meta Ph), 5.03 (d, 1H, J = 5.0 Hz, NH), 4.79 (m, 1H, CH cHex), 1.80 (m, 4H, 2 x CH_2 ortho cHex), 1.69 (m, 4H, 2 x CH_2 meta cHex), 1.69 (m, 2H, CH_2 para cHex), 1.51 (s, 9H, NHCOOC($\underline{CH_3}_3$).

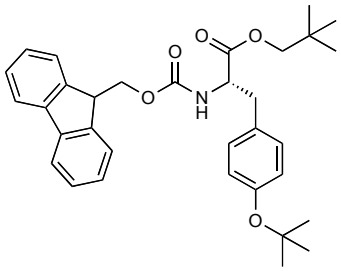
Preparation of Boc protected L-Alanine-cyclopentyl ester



Prepared according to standard procedure A, using *N*-Boc-*L*-Alanine (7.00 g, 37.0 mmol, 1.0 eq) in anh. DCM (140 mL) was added the cyclopentyl alcohol (4.40 mL, 48.1 mmol, 1.3 eq), DCC (7.66 g, 37.0 mmol, 1.0 eq) and DMAP (0.463 g, 3.70 mmol, 0.1 eq). After overnight stirring, the solvent was removed under reduced pressure and the residue was purified by flash chromatography using Hexane/EtOAc (9:1) to afford a white solid (7.82 g, 30.3 mmol, 82%).

^{1}H NMR (500 MHz, MeOD) δ_H (ppm): 5.20 (m, 1H, CH cPent), 4.11-4.05 (m, 1H, NHCH(CH$_3$)CO), 1.88 (m, 2H, CH$_2$ cPent), 1.76-1.72 (m, 6H, 3 x CH$_2$ cPent), 1.45 (s, 9H, NHCOOC(CH$_3$)$_3$), 1.33 (d, 3H, *J* = 7.30 Hz, NHCH(CH$_3$)CO).

Preparation of Fmoc-Tyrosine-(tert-butyl)-neopentyl ester

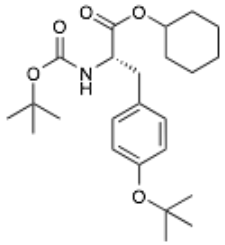


Prepared according to standard procedure A, using using Fmoc-*L-tert*-butyl)-OH (3.04 g, 6.61 mmol, 1.0 eq) in anh. DCM (61 mL) was added the neopentyl alcohol (1.17 g, 13.2 mmol, 2.0 eq), DCC (1.36 g, 6.61 mmol, 1.0 eq) and DMAP (0.081 g, 0.66 mmol, 0.1 eq). After overnight stirring, the solvent was removed under reduced pressure and the residue was purified by flash chromatography using Hexane/EtOAc (9:1) to afford a white solid (3.28 g, 6.19 mmol, 94%).

^{1}H NMR (500 MHz, CDCl$_3$) δ_H (ppm): 7.76 (d, 2H, *J* = 7.55 Hz, 2 x CH Fmoc), 7.60 (t, 2H, *J* = 7.30 Hz, 2 x CH Fmoc), 7.40 (t, 2H, *J* = 7.45 Hz, 2 x CH Fmoc), 7.32 (m, 2H, 2 x CH Fmoc), 7.08 (d, 2H, *J* = 8.15 Hz, 2 x CH ortho Ar), 6.94 (d, 2H, *J* = 8.20 Hz, 2 x CH meta Ar), 5.60 (d, 1H, *J* = 8.35 Hz, NH), 4.74 (q, 1H, *J* = 7.85 Hz, NHCH(CH$_2$ArOC(CH$_3$)$_3$)CO), 4.45 (m, 1H, CH$_2$ Fmoc), 4.43 (m, 1H, CH$_2$ Fmoc), 4.35 (t, 1H, *J* = 7.10 Hz, CH Fmoc), 3.88 & 3.80 (AB system, 2H, *J* = 10.5 Hz,

O$\underline{CH_2}$C(CH_3)$_3$), 3.18-3.07 (m, 2H, NHCH($\underline{CH_2}$ArOC(CH_3)$_3$)CO), 1.35 (s, 9H, NHCH(CH_2ArOC($\underline{CH_3}$)$_3$)CO), 0.88 (s, 9H, $OCH_2C(\underline{CH_3})_3$).

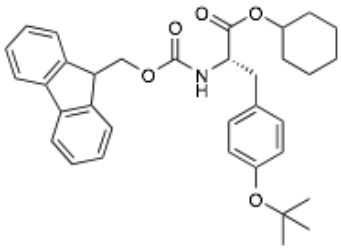
Preparation of Boc protected L-Tyrosine-(tert-butyl)-cyclohexyl ester



Prepared according to standard procedure A, using *N*-Boc-*L*-Tyrosine-O-*tert*-butyl (5.00 g, 14.8 mmol, 1.0 eq) in anh. DCM (100 mL) was added the cyclohexanol (3.09 mL, 29.6 mmol, 1.3 eq), DCC (3.10 g, 14.8 mmol, 1.0 eq) and DMAP (0.19 g, 1.48 mmol, 0.1 eq). After overnight stirring, the solvent was removed under reduced pressure and the residue was purified by flash chromatography using Hexane/EtOAc (9:1) to afford a white solid (6.20 g, 14.78 mmol, 99%).

^{1}H NMR (500 MHz, MeOD) δ_H (ppm): 7.14 (d, 2H, J = 8.35 Hz, 2 x CH ortho Ar), 6.92 (d, 2H, J = 8.25 Hz, 2 x CH meta Ar), 4.76-4.73 (m, 1H, CH cHex), 4.33 (m, 1H, NH$\underline{CH}$(CH_2ArOC(CH_3)$_3$)CO), 3.06-3.02 (m,1H, NHCH($\underline{CH_2}$ArOC(CH_3)$_3$)CO),), 2.93-2.89 (m, 1H, NHCH($\underline{CH_2}$ArOC(CH_3)$_3$)CO),), 1.92-1.53 (m, 10H, 2 x CH_2 meta cHex & 2 x CH_2 ortho cHex & CH_2 para cHex), 1.42 (s, 9H, NHCH(CH_2ArOC($\underline{CH_3}$)$_3$)CO),), 1.36 (s, 9H, NHCOOC($\underline{CH_3}$)$_3$).

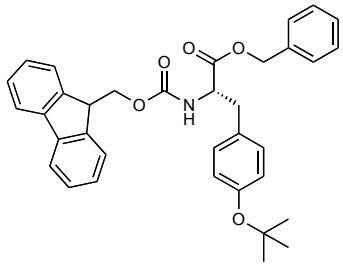
Preparation of Fmoc-Tyrosine-(tert-butyl)-cyclohexyl ester



Prepared according to standard procedure A, using Fmoc-*L*-*tert*-butyl)-OH (3.12 g, 6.80 mmol, 1.0 eq) in anh. DCM (63 mL) was added the cyclohexanol (1.42 mL, 13.6 mmol, 2.0 eq), DCC (1.40 g, 6.80 mmol, 1.0 eq) and DMAP (0.083 g, 0.68 mmol, 0.1 eq). After overnight stirring, the solvent was removed under reduced pressure and the residue was purified by flash chromatography using Hexane/EtOAc (9:1) to afford a white solid (3.67 g, 6.78 mmol, 99%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 7.68 (d, 2H, *J* = 7.55 Hz, 2 x CH Fmoc), 7.56-7.52 (m, 2H, 2 x CH Fmoc), 7.33-7.30 (m, 2H, 2 x CH Fmoc), 7.25-7.21 (m, 2H, 2 x CH Fmoc), 7.04 (d, 2H, *J* = 8.15 Hz, 2 x CH ortho Ar), 6.87 (d, 2H, *J* = 8.25 Hz, 2 x CH meta Ar), 5.83 (d, 1H, *J* = 8.25 Hz, NH), 4.76-4.75 (m, 1H, CH cHex), 4.63-4.59 (m, 1H, NHCH(CH_2ArOC(CH_3)$_3$)CO), 4.41-4.34 (m, 2H, CH_2 Fmoc), 4.29-4.26 (m, 1H, CH Fmoc), 3.09-2.99 (m, 2H, NHCH(CH_2ArOC(CH_3)$_3$)CO), 1.84-1.61 (m, 8H, 2 x CH_2 meta cHex & 2 x CH_2 ortho cHex), 1.53-1.36 (m, 2H, CH_2 para cHex), 1.27 (s, 9H, NHCH(CH_2ArOC(CH_3)$_3$)CO).

Preparation of Fmoc-Tyrosine-(tert-butyl)-benzyl ester

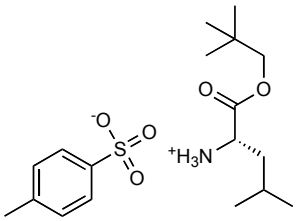


Prepared according to standard procedure A, using N-Fmoc-*L*-Tyrosine(*tert*-butyl)-OH (2.02 g, 4.40 mmol, 1.0 eq) in anh. DCM (41 mL) was added the benzyl alcohol (0.17 mL, 8.80 mmol, 2.0 eq), DCC (0.91 g, 4.40 mmol, 1.0 eq) and DMAP (0.054 g, 0.44 mmol, 0.1 eq). After overnight stirring, the solvent was removed under reduced pressure and the residue was purified by flash chromatography using Hexane/EtOAc (9:1) to afford a white solid (2.28 g, 4.15 mmol, 94%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 7.79 (d, 2H, *J* = 7.45 Hz, 2 x CH Fmoc), 7.59 (d, 2H, *J* = 7.15 Hz, 2 x CH Fmoc), 7.44-7.32 (m, 9H, 4 x CH Fmoc & 5 x OCH_2Ph), 6.92 (d, 2H, *J* = 8.10 Hz, 2 x CH ortho Ar), 6.86 (d, 2H, *J* = 8.30 Hz, 2 x CH meta Ar), 5.28 (d, 1H, *J* = 8.00 Hz, NH), 5.20-5.14 (m, 2H, CH_2 Fmoc), 4.70-4.68 (m, 1H, NHCH(CH_2ArOC(CH_3)$_3$)CO), 4.59-4.35 (m, 2H, OCH_2Ph), 4.32-4.28 (m, 1H, CH Fmoc), 3.11-3.0 (m, 2H, NHCH(CH_2ArOC(CH_3)$_3$)CO), 1.34 (s, 9H, NHCH(CH_2ArOC(CH_3)$_3$)CO).

3.2.2. Preparation of amino acid ester salts or free amino acids

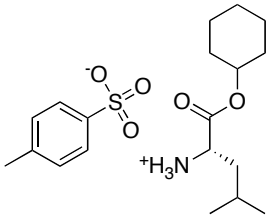
Preparation of L-Leucine-neopentyl ester tosylate salt (12.c)



Prepared according to standard procedure B1, using Boc protected *L*-Leucine neopentyl ester (5.16 g, 17.12 mmol, 1.0 eq), pTSA (3.30 g, 17.3 mmol, 1.0 eq) dissolved in EtOAc (190 mL) and refluxed overnight. The solution was then cooled down to RT and the product crashed down on an ice bath. After filtration and diethyl ether washes a white solid was recovered (2.83 g, 7.58 mmol, 44%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.23 (bs, 3H, NH_3^+), 7.79 (d, 2H, J = 8.1 Hz, 2 x CH ortho Ts), 7.15 (d, 2H, J = 8.0 Hz, 2 x CH meta Ts), 3.95 (t, 1H, J = 7.2 Hz, NHCH($CH_2CH(CH_3)_2$)CO), 3.82 & 3.74 (AB system, 1H, J = 10.5 Hz, OCH_2C(CH_3)$_3$), 2.36 (s, 3H, CH_3 Ts), 1.71-1.62 (2m, 3H, NHCH($CH_2CH(CH_3)_2$)CO), 0.90 (s, 9H, $OCH_2C(CH_3)_3$), 0.80 (d, 6H, NHCH($CH_2CH(CH_3)_2$)CO).

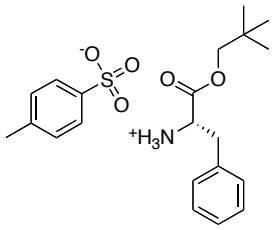
Preparation of L-Leucine-cyclohexyl ester tosylate salt (12.d)



Prepared according to standard procedure B1, using Boc protected *L*-Leucine-cyclohexyl ester (4.24 g, 13.5 mmol, 1.0 eq) and pTSA (2.58 g, 13.5 mmol, 1.0 eq) dissolved in EtOAc (149 mL) and refluxed overnight (65°C). The solution was then cooled down to RT and the product crashed down on an ice bath. After filtration and diethyl ether washes a white solid was recovered (4.90 g, 12.7 mmol, 94%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.17 (s broad, 3H, NH_3^+), 7.79 (d, 2H, J = 7.95 Hz, 2 x CH ortho Ts), 7.15 (d, 2H, J = 7.80 Hz, 2 x CH meta Ts), 4.78 (m, 1H, CH cHex), 3.91 (m, 1H, NHCH($CH_2CH(CH_3)_2$)CO), 2.38 (s, 3H, CH_3 Ts), 1.77-1.64 (m, 6H, CH_2 ortho cHex, CH_2 meta cHex, NHCH($CH_2CH(CH_3)_2$)CO), 1.51-1.49 (m, 3H, CH_2 para cHex, NHCH($CH_2CH(CH_3)_2$)CO), 1.41-1.20 (m, 4H, CH_2 ortho cHex, CH_2 meta cHex), 0.83 (t, 6H, J = 8.5 Hz, NHCH($CH_2CH(CH_3)_2$)CO).

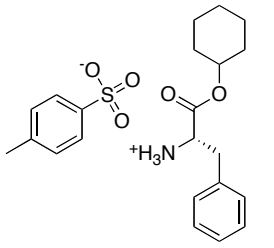
Preparation of L-Phenylalanine-neopentyl ester tosylate salt (12.e)



Prepared according to standard procedure B1, using Boc protected *L*-Phenylalanine neopentyl ester (5.26 g, 14.7 mmol, 1.0 eq), pTSA (3.02 g, 14.7 mmol, 1.01 eq) dissolved in EtOAc (174 mL) and refluxed overnight. The solution was then cooled down to RT and the product crashed down on an ice bath. After filtration and diethyl ether washes a white solid was recovered (1.24 g, 3.04 mmol, 19%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.29 (s broad, 3H, NH_3^+), 7.77 (d, 2H, J = 8.1 Hz, 2 x CH ortho Ts), 7.19-7.11 (d, 8H, 2 x CH meta Ts, 5 x CH Ph), 4.34 (q, 1H, J = 5.2 Hz, NH<u>CH</u>(CH_2Ph)CO), 3.64 & 3.58 (AB system, 1H, J = 10.5 Hz, O<u>CH_2</u>C(CH_3)$_3$), 3.29 (dd, 1H, J = 5.2 & 14.2 Hz, NHCH(<u>CH_2</u>Ph)CO), 3.10 (dd, 1H, J = 8.2 & 14.2 Hz, NHCH(<u>CH_2</u>Ph)CO), 2.35 (s, 3H, CH_3 Ts), 0.72 (s, 9H, OCH_2C(<u>CH_3</u>)$_3$).

Preparation of L-Phenylalanine-cyclohexyl ester tosylate salt (12.f)

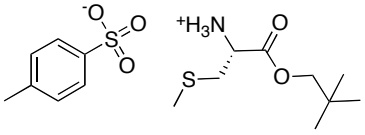


Prepared according to standard procedure B1, using Boc protected *L*-Phenylalanine-cyclohecxyl ester (5.16 g, 14.9 mmol, 1.0 eq), pTSA (2.85 g, 15.0 mmol, 1.0 eq) dissolved in EtOAc (182 mL) refluxed overnight. The solution was then cooled down to RT and the product crashed down on an ice bath. After filtration and diethyl ether washes a white solid was recovered (3.77 g, 8.98 mmol, 60%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.20 (s broad, 3H, NH_3^+), 7.77 (d, 2H, J = 8.10 Hz, 2 x CH ortho Ts), 7.19-7.14 (m, 7H, 2 x CH meta Ts, 5 x CH Ph), 4.66 (m, 1H, CH cHex), 4.28 (m, 1H, NH<u>CH</u>(CH_2Ph)CO), 3.31-3.27 (m, 1H, NHCH(<u>CH_2</u>Ph)CO), 3.14-3.09 (m, 1H, NHCH(<u>CH_2</u>Ph)CO), 2.36 (s, 3H, CH_3 Ts), 1.62-1.53 (m, 4H, CH_2 ortho

cHex, CH_2 meta cHex), 1.44 (m, 2H, CH_2 para cHex), 1.21-1.19 (m, 4H, CH_2 ortho cHex & CH_2 meta cHex).

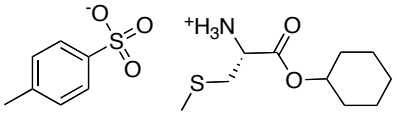
Preparation of S-methyl-L-Cysteine-neopentyl ester tosylate salt (12.g)



Prepared according to standard procedure B2, using commercially available *S*-methyl-*L*-Cysteine (2.56 g, 18.9 mmol, 1.0 eq), pTSA (3.96 g, 20.8 mmol, 1.1 eq) and neopentyl alcohol (25.0 g, 284 mmol, 15 eq) dissolved in toluene (28 mL) and refluxed overnight. The solution was then cooled down to RT and the product crashed down on an ice bath. After filtration and diethyl ether washes a white solid was recovered (6.53 g, 17.3 mmol, 91%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.36 (bs, 3H, NH_3^+), 7.79 (d, 2H, J = 6.80 Hz, 2 x CH ortho Ts), 7.15 (d, 2H, J = 6.80 Hz, 2 x CH meta Ts), 4.35 (m, 1H, $\underline{CH}(CH_2SCH_3)CO$), 3.85 & 3.76 (AB system, 2H, J =10.2 Hz, $O\underline{CH_2}C(CH_3)_3$), 3.09 (bs, 2H, $CH(\underline{CH_2}SCH_3)CO$), 2.37 (s, 3H, CH_3 Ts), 2.04 (s, 3H, $CH(CH_2S\underline{CH_3})CO$), 0.91 (s, 9H, $OCH_2C(\underline{CH_3})_3$).

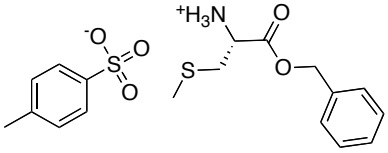
Preparation of S-methyl-L-Cysteine-cyclohexyl ester tosylate salt (12.h)



Prepared according to standard procedure B2, using commercially available *S*-methyl-*L*-Cysteine (2.51 g, 18.6 mmol, 1.0 eq), cyclohexanol (29 mL, 279 mmol, 15 eq) and pTSA (3.90 g, 20.4 mmol, 1.1 eq) dissolved in toluene (27 mL) and refluxed overnight. The solution was then cooled down to RT and the product crashed down on an ice bath. After filtration and diethyl ether washes a white solid was recovered (5.87 g, 15.07 mmol, 81%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.32 (bs, 3H, NH_3^+), 7.79 (d, 2H, J = 8.10 Hz, 2 x CH ortho Ts), 7.15 (d, 2H, J = 7.90 Hz, 2 x CH meta Ts), 4.82-4.77 (m, 1H, CH cHex), 4.29-4.27 (m, 1H, $\underline{CH}(CH_2SCH_3)CO$), 3.17-3.04 (m, 2H, $CH(\underline{CH_2}SCH_3)CO$), 2.36 (s, 3H, CH_3 Ts), 2.04 (s, 3H, $CH(CH_2S\underline{CH_3})CO$), 1.79-1.67 (4H, CH_2 meta cHex, CH_2 ortho cHex), 1.51-1.38 (m, 6H, CH_2 meta cHex, CH_2 ortho cHex, CH_2 para cHex).

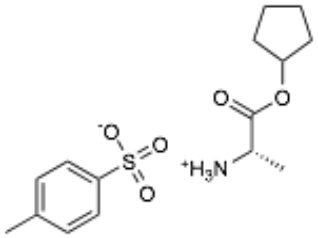
Preparation of S-methyl-L-Cysteine-benzyl ester tosylate salt (12.i)



Prepared according to standard procedure B2, using commercially available *S*-methyl-*L*-Cysteine (2.50 g, 18.5 mmol, 1.0 eq), benzyl alcohol (25 mL, 240 mmol, 13 eq) and pTSA (3.87 g, 20.3 mmol, 1.0 eq) dissolved in toluene (27 mL) and refluxed overnight. The solution was then cooled down to RT and the product crashed down on an ice bath. After filtration and diethyl ether washes a white solid was recovered (2.09 g, 5.25 mmol, 28%).

^{1}H NMR (500 MHz, MeOD) δ_H (ppm): 7.74 (d, 2H, *J* = 8.15 Hz, 2 x CH ortho Ts), 7.45-7.37 (m, 5H, 5 x CH Ph), 7.26 (d, 2H, *J* = 7.95 Hz, 2 x CH meta Ts), 5.31 (d, 2H, *J* = 2.50 Hz, O$\underline{CH_2}$Ph), 4.36-4.34 (m, 1H, $\underline{CH}(CH_2SCH_3)CO$), 3.14 (dd, *J* = 4.70 Hz & 14.85 Hz, 1H, $CH(\underline{CH_2}SCH_3)CO$), 2.99 (dd, 1H, *J* = 7.45 Hz & 14.85 Hz, $CH(\underline{CH_2}SCH_3)CO$), 2.39 (s, 3H, CH_3 Ts), 2.11 (s, 3H, $CH(CH_2S\underline{CH_3})CO$).

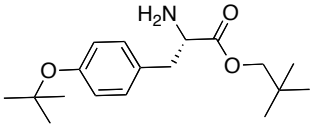
Preparation of L-Alanine-cyclopentyl ester tosylate salt (12.j)



Prepared according to standard procedure B1, using *L*-Alanine-cyclopentyl ester (7.82 g, 30.4 mmol, 1.0 eq), pTSA (5.78 g, 30.4 mmol, 1.0 eq) dissolved in EtOAc (275 mL) and refluxed overnight. The solution was then cooled down to RT and the product crashed down on an ice bath. After filtration and diethyl ether washes a white solid was recovered (7.00 g, 21.3 mmol, 70%).

^{1}H NMR (500 MHz, MeOD) δ_H (ppm): 7.73 (d, 2H, *J* = 8.15 Hz, 2 x CH meta Ts), 7.25 (d, 2H, *J* = 7.95 Hz, 2 x CH ortho Ts), 5.31-5.28 (m, 1H, CH cPent), 4.06-4.02 (m, 1H, $NH\underline{CH}(CH_3)CO$), 2.39 (s, 3H, CH_3 Ts), 1.96-1.92 (m, 2H, CH_2 (2) cPent), 1.79-1.74 (m, 4H, CH_2 (5) cPent, CH_2 (3) cPent), 1.69-1.67 (m, 2H, CH_2 (4) cPent), 1.52 (d, 3H, *J* = 7.25 Hz, $NHCH(\underline{CH_3})CO$).

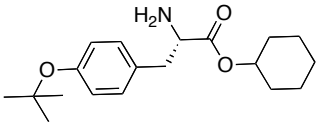
Preparation of L-Tyrosine-(tert-butyl)-neopentyl ester (12.k)



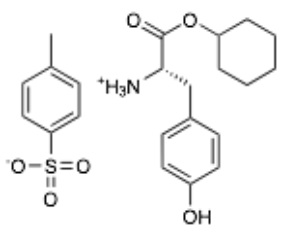
Fmoc protected *L*-Tyrosine-(*tert*-butyl)-neopentyl ester (3.28 g, 6.19 mmol, 1.0 eq) was dissolved in anh. DMF (15 mL) and morpholine (15 mL) was added. The solution was left stirring for 30 min at RT. The reaction was quenched by addition of 2-propanol. The solvents were evaporated under reduced pressure and the residue was purified by flash chromatography using petroleum ether/EtOAc (25%) to afford an oil corresponding to the desired material (1.87 g, 6.08 mmol, 98%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 6.87 (d, 2H, *J* = 6.50 Hz, 2 x CH ortho Ar), 6.71 (m, 2H, 2 x CH meta Ar), 3.49-3.41 (m, 1H, , NHCH(CH_2ArOC(CH_3)$_3$)CO), 3.32 & 3.20 (AB system, 2H, *J* = 9.50 Hz, OCH_2C(CH_3)$_3$), 3.10-3.04 (m, 2H, NHCH(CH_2ArOC(CH_3)$_3$)CO), 1.25 (s, 9H, NHCH(CH_2ArOC(CH_3)$_3$)CO), 0.68 (s, 9H, OCH_2C(CH_3)$_3$).

Preparation of L-Tyrosine-(tert-butyl)-cyclohexyl ester (12.l)



Route 1: Prepared according to standard procedure B1, using Boc protected *L*-Tyrosine-(*tert*-butyl)-cyclohexyl ester (6.20 g, 14.8 mmol, 1.0 eq), pTSA (2.87 g, 14.8 mmol, 1.0 eq) dissolved in EtOAc (217 mL) and refluxed overnight. The solution was then cooled down to RT and the product crashed down on an ice bath. The residue was filtered and washed with diethyl ether and dried to recover a white product (5.02 g, 11.5 mmol, 78%) corresponding to the *tert*-butyl deprotected *L*-Tyrosine- cyclohexyl ester pTSA salt, described below.

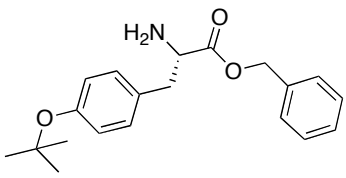


^{1}H NMR (500 MHz, MeOD) δ_H (ppm): 7.73 (d, 2H, *J* = 8.20 Hz, 2 x CH ortho Ts), 7.25 (d, 2H, *J* = 7.95 Hz, 2 x CH meta Ts), 7.09 (d, 2H, *J* = 8.50 Hz, 2 x CH ortho Ar), 6.79 (d, 2H, *J* = 8.55 Hz, 2 x CH meta Ar), 4.78 (m, 1H, CH cHex), 4.19 (t, 1H, *J* = 6.90 Hz, NHCH(CH_2ArOH)CO), 3.13-3.10 (m, 2H, NHCH(CH_2ArOH)CO), 2.39 (s, 3H, CH_3 Ts), 1.87-1.68 (m, 4H, CH_2 meta cHex, CH_2 ortho cHex), 1.58-1.52 (m, 6H, CH_2 meta cHex & CH_2 ortho cHex & CH_2 para cHex).

Route 2: Fmoc protected *L*-Tyrosine-(*tert*-butyl)-cyclohexyl ester (3.67 g, 6.78 mmol, 1.0 eq) was dissolved in anh. DMF (11 mL) and morpholine (11 mL) was added. The solution was left stirring for 30 min at RT. After quenching by addition of 2-propanol. The solvents were evaporated under reduced pressure and the residue was purified by flash chromatography using petroleum ether/EtOAc (25%) to afford an oil corresponding to the desired material (1.96 g, 6.14 mmol, 91%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 6.83 (t, 2H, J = 6.30 Hz, 2 x CH ortho Ar), 6.63 (m, 2H, 2 x CH meta Ar), 4.49 (m, 1H, CH cHex), 3.38-3.36 (m, 1H, NHCH(CH_2ArOC(CH_3)$_3$)CO), 2.71-2.68 (m, 1H, NHCH(CH_2ArOC(CH_3)$_3$)CO), 2.60-2.56 (m, 1H, NHCH(CH_2ArOC(CH_3)$_3$)CO), 1.54-1.24 (m, 6H, CH_2 ortho cHex, CH_2 meta cHex, CH_2 para cHex), 1.06 (s, 9H, NHCH(CH_2ArOC(CH_3)$_3$)CO), 0.98-0.96 (m, 4H, CH_2 ortho cHex, CH_2 meta cHex).

Preparation of L-Tyrosine-(tert-butyl)-benzyl ester (12.m)

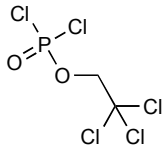


Fmoc protected *L*-Tyrosine-(*tert*-butyl)-benzyl ester (2.78 g, 5.04 mmol, 1.0 eq) was dissolved in anh. DMF (15 mL) and morpholine (15 mL) was added. The solution was left stirring for 30 min at RT. The reaction was quenched by addition of 2-propanol. The solvents were evaporated under reduced pressure and the residue was purified by flash chromatography using petroleum ether/EtOAc (25%) to afford an oil corresponding to the desired material (1.50 g, 4.59 mmol, 91%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 7.38-7.32 (m, 5H, 5 x CH Ph), 7.06 (d, 2H, J = 8.45 Hz, 2 x CH ortho Ar), 6.91 (d, 2H, J = 8.45 Hz, 2 x CH meta Ar), 5.15 (s, 2H, OCH_2Ph), 3.78-3.76 (m, 1H, NHCH(CH_2ArOC(CH_3)$_3$)CO), 3.07-3.03 (m, 1H, NHCH(CH_2ArOC(CH_3)$_3$)CO), 2.88-2.85 (m, 1H, NHCH(CH_2ArOC(CH_3)$_3$)CO), 1.34 (s, 9H, NHCH(CH_2ArOC(CH_3)$_3$)CO).

3.2.3. Intermediate from TCE pathway

Preparation of 2,2,2-trichloroethoxy phosphorodichloridate (16) [3]



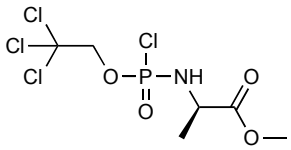
To a solution of $POCl_3$ (5.08 mL, 54.5 mmol, 1.0 eq) in anh. Et_2O (125 mL) was added dropwise, at -80 °C under vigorous stirring, a solution of 2,2,2-trichloroethanol (5.25 mL,5.45 mmol, 1.0 eq) and NEt_3 (7.50 mL, 53.8 mmol, 0.99 eq) in anh. Et_2O (100 mL). After the completion of addition, the solution was left 2hrs at -78 °C then the mixture was allowed to warm to RT while stirring overnight. The mixture was filtered with anh. Et_2O and the solvent was removed under reduced pressure to yield the phosphorodichloridate as an orange oil. The product was purified by distillation (0.80 g, 3.02 mmol, 6%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 4.81 (d, 1H, J = 6.50 Hz, O$\underline{CH_2}CCl_3$), 4.51 (d, 1H, 5.31 (d, 1H, J = 6.45 Hz, PO$\underline{CH_2}CCl_3$).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 93.14 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 14.1 Hz, $OCH_2\underline{C}Cl_3$), 78.35 (d, $J^2_{P\text{-}O\text{-}C}$ = 7.5 Hz, O$\underline{C}H_2CCl_3$).

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm): 7.70 (s).

Preparation of L-Alanine-methyl ester 2,2,2-trichloroethoxy phosphochloridate (17)[4]



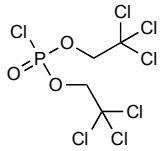
A solution of 2,2,2-trichloroethoxy phosphorodichloridate (**16**) (0.80 g, 3.02 mmol, 1.0 eq) in anh. DCM (24 mL) was added to a solution of *L*-alanine methylester hydrochloride (0.47 g, 3.32 mmol, 1.1 eq) in anh. DCM (94 mL), and the solution was cooled down to -80 °C. Anh. NEt_3 (0.84 mL, 6.04 mmol, 2.0 eq) in anh. DCM (15 mL) was added dropwise at -80 °C. After the completion of the addition, the solution was allowed to warming at RT, and stirred for a further 1hr 45min. The solvent was then removed under reduced pressure to give a yellowish oil (1.11 g, 3.33 mmol) used crude for the next step.

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 5.31 & 4.62 (2d, 2H, J = 6.50 Hz, PO$\underline{CH_2}CCl_3$), 4.17 (s, 3H, NHCH(CH_3)COO$\underline{CH_3}$), 3.92 (m, 1H, NH$\underline{CH}$(CH_3)COOCH_3), 1.26 (d, 3H, J = 7.00 Hz, NHCH($\underline{CH_3}$)COOCH_3).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 169.60 (s, NHCH(CH_3)$\underline{C}$OOCH$_3$), 96.38 (s, OCH$_2\underline{C}$Cl$_3$), 76.32 (s, O$\underline{CH_2}$CCl$_3$), 52.81 (s, NHCH(CH_3)COO$\underline{CH_3}$), 48.96 (s, NH$\underline{CH}$(CH_3)COOCH$_3$), 20.91 (s, NHCH($\underline{CH_3}$)COOCH$_3$).

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm): 11.73 (s), 11.17 (s).

Preparation of bis(2,2,2-trichloroethoxy) phosphorochloridate (20) [5]



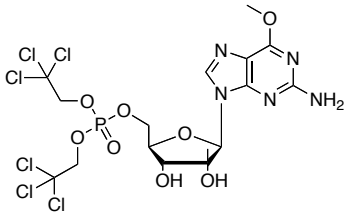
To a solution of $POCl_3$ (5.2 mL, 55.8 mmol, 1.0 eq) in anh. Et_2O (50 mL) was added dropwise, at -80 °C under with vigorous stirring, a solution of 2,2,2-trichloroethanol (11 mL, 114 mmol, 2.04 eq), NEt_3 (16.0 mL, 115 mmol, 2.06 eq) in anh. Et_2O (100 mL). Then the mixture was allowed to warm to RT and left stirring overnight. The reaction mixture was filtered with anh. Et_2O and the solvent was removed under reduced pressure to yied a yellow solid that was then distillated under reduced pressure. The product came out at 120°C as a colourless oil (0.34 g, 0.90 mmol, 2%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 4.77 (m, 4H, O$\underline{CH_2}$CCl$_3$).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 94.30 & 93.62 (2d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 11.25 & 12.50 Hz, , OCH$_2\underline{C}$Cl$_3$), 76.90 & 76.18 (2s, O$\underline{C}$H$_2$CCl$_3$)**.**

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm): 3.10 (s)**.**

Preparation of bis(2,2,2-trichloroethoxy) phosphate (21)



The bis(2,2,2-trichloroethoxy) phosphorochloridate (**20**) (0.34 g, 0.90 mmol, 2.0 eq) was added to a solution of *β*-2'-*C*-me-6-*O*-Me guanosine (**11**) (0.14 g, 0.46 mmol, 1.0 eq) in pyridine (10 mL) and left stirring for 63 hrs at RT. The reaction was monitored by phosphorus NMR. The solvent was then removed under reduced pressure and the residue was purified by flash chromatography using MeOH/$CHCl_3$ as eluents to yield a white solid (0.13 g, 1.20 mmol, 43%).

HPLC (MeOH/H_2O): Rt = 16.64 min

HPLC (ACN/H_2O): Rt = 25.63 min.

MS (TOF ES^+): 655.92 ($M + H^+$).

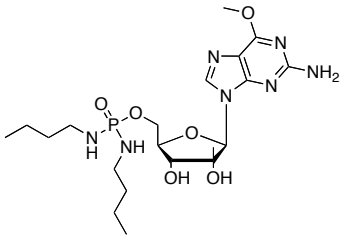
^1H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 7.79 (s, 1H, H-8 base), 6.01 (s, 1H, H-1'), 5.32 (bs, 2H, NH_2 base), 4.76-4.75 (m, 1H, H-5'), 4.68 (d, 2H, J = 6.5 Hz, O$\underline{CH_2}CCl_3$), 4.64 (d, 2H, J = 6.5 Hz, O$\underline{CH_2}CCl_3$), 4.60-4.58 (m, 1H, H-5'), 4.52 (d, 1H, J = 7.0 Hz, H-3'), 4.32 (m, 1H, H-4'), 4.08 (s, 3H, 6-OCH_3 base), 1.04 (s, 3H, CH_3-2').

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 161.65 (s, C-6 base), 159.58 (s, C-2 base), 152.77 (s, C-4 base), 137.65 (s, C-8 base), 115.52 (s, C-5 base), 91.77 (s, C-1'), 80.74 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 7.0 Hz, C-4'), 79.07 (s, C-2'), 77.05 & 76.79 (2s, 2 PO$\underline{CH_2}CCl_3$), 74.18 (s, C-3'), 67.89 (d, $J^2_{P\text{-}O\text{-}C}$ = 7.0 Hz, C-5'), 54.07 (s, 6-OCH_3 base), 20.30 (s, CH_3-2').

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm): - 4.23 (s).

3.2.4. Preparation of phosphorodiamidates of *β*-2'-*C*-methyl-6-*O*-methyl guanosine

Synthesis of N-butylamino β-2'-C-methyl-6-O-methyl guanosine phosphorodiamidate (19.b)



Prepared according to procedure E, using *β*-2'-C-Me-6-OMe guanosine (**11**) (1.04 g, 3.35 mmol) in anh. THF (17 mL), anh. NEt_3 (0.56 mL, 4.02 mmol, 1.2 eq), $POCl_3$ (0.38 mL, 4.08 mmol, 1.2 eq), commercially available *N*-butylamine (1.70 mL, 17.20 mmol, 5.0 eq) in anh. DCM (17 mL), anh. NEt_3 (4.70 mL, 33.7 mmol, 10 eq). After overnight stirring at RT and flash chromatography using MeOH (3%)/$CHCl_3$, the desired material was recovered (0.42 g, 0.83 mmol, 25%).

HPLC (MeOH/H_2O): Rt = 12.31 min

HPLC (ACN/H_2O) : Rt = 12.79 min

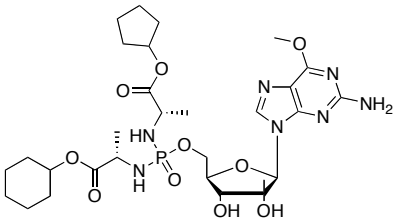
MS (TOF ES^+): 502.26 ($M + H^+$), 524.24 ($M + Na^+$), 565.26 ($M + MeCN + Na^+$).

^1H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 7.86 (s, 1H, H-8 base), 6.05 (s, 1H, H-1'), 5.68 (bs, 2H, 2 x $\underline{NH}CH_2CH_2CH_2CH_3$), 4.44-4.23 (m, 4H, H-5', H-3', H4'), 4.04 (s, 3H, 6-*O*CH_3 base), 2.88-2.85 (m, 4H, 2 x NH$\underline{CH_2}CH_2CH_2CH_3$), 1.41-1.38 (m, 4H, 2 x $NHCH_2\underline{CH_2}CH_2CH_3$), 1.27-1.23 (m, 4H, 2 x $NHCH_2CH_2\underline{CH_2}CH_3$), 0.99 (s, 3H, CH_3-2'), 0.82-0.79 (m, 6H, 2 x $NHCH_2CH_2CH_2\underline{CH_3}$).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 161.40 (s, C-6 base), 159.69 (s, C-2 base), 153.07 (s, C-4 base), 137.64 (s, C-8 base), 115.24 (s, C-5 base), 91.36 (s, C-1'), 81.11 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 6.25 Hz, C4'), 79.36 (s, C-2'), 73.41 (s, C-3'), 64.02 (s, C-5'), 53.75 (s, 6-$O$$CH_3$ base), 41.02 & 40.91 (2s, 2 x $NH\underline{C}H_2CH_2CH_2CH_3$), 33.91 (d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 6.3 Hz, $NHCH_2\underline{C}H_2CH_2CH_3$), 33.87 (d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 6.4 Hz, $NHCH_2\underline{C}H_2CH_2CH_3$), 20.22 (s, CH_3-2'), 19.82 & 19.80 (2s, 2 x $NHCH_2CH_2\underline{C}H_2CH_3$), 14.07 &13.66 (2s, 2 x $NHCH_2CH_2CH_2\underline{C}H_3$).

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm): 17.40 (s).

Synthesis of LAlanine-cyclohexyl ester / L-Alanine-cyclopentyl ester β-2'-C-methyl-6-O-methyl guanosine phosphorodiamidate (23)



To a solution of *β*-2'-C-Me-6-OMe guanosine (**11**) (0.55 g, 1.76 mmol) in anh. THF (8 mL), anh. NEt_3 (0.30 mL, 2.11 mmol, 1.2 eq) was added and then mixture was left stirring at RT for 30 min, after which the solution was cooled to -78 °C and $POCl_3$ (0.20 mL, 2.11 mmol, 1.2 eq) was added dropwise. The solution was stirred 1 hr at -78 °C and then 30 min at RT. The formation of the intermediate was monitored by phosphorus NMR. Then *L*-Alanine-cyclohexyl tosylate salt (provided by Inhibitex *Inc.*) (0.37 g, 1.76 mmol, 1.0 eq) in anh. DCM (2 mL) was added, and the reaction was cooled down at -78 °C at which anh. NEt_3 (0.50 mL, 3.52 mmol, 2.0 eq) was added dropwise. The temperature was maintained at -78 °C for 1h, and the solution was then allowed to return to RT for 4 hrs. *L*-Alanine-cyclopentyl tosylate salt (provided by Inhibitex *Inc.*) (2.90 g, 8.80 mmol, 5.0 eq) in anh. DCM (5 mL) was added. The reaction was cooled down at -78 °C at which anh. NEt_3 (2.50 mL, 17.60 mmol, 10 eq) was added dropwise. The solution was left stirring overnight, and after flash chromatography using MeOH (3%)/$CHCl_3$, the desired molecule was recovered (0.28 g, 0.41 mmol, 23%).

HPLC (MeOH/H_2O) : Rt = 14.97 min

HPLC (ACN/H_2O) : Rt = 17.37 min

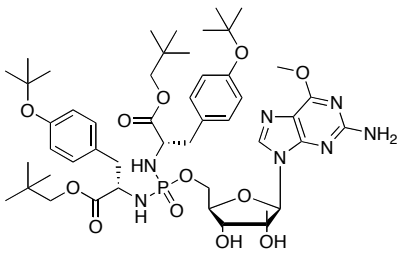
MS (TOF ES^+): 684.32 ($M + H^+$), 706.27 ($M + Na^+$), 722.27 ($M + K^+$).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 7.74 (s, 1H, H-8 base), 5.96 (s, 1H, H-1'), 5.51 (bs, 2H, NHCH(CH_3)COO-cHex, NHCH(CH_3)COO-cPent), 5.18-5.13 (m, 1H, H-3'), 4.76-4.68 (m, 1H, CH cPent), 4.61-4.59 (m, 1H, H-5'), 4.51-4.48 (m, 1H, CH cHex), 4.32 (m, 1H, H-5'), 4.23-4.20 (m, 1H, H-4'), 4.06 (s, 6-*O*CH_3 guanine), 3.92-3.88 (m, 2H, NHCH(CH_3)COO-cPent, NHCH(CH_3)COO-cHex), 1.86-1.57 (m, 18H, 4 x CH_2 cPent, 5 x CH_2 cHex), 1.36 (m, 6H, NHCH(CH_3)COO-cPent, NHCH(CH_3)COO-cHex), 1.00 (s, 3H, CH_3-2').

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 174.29 (d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 6.3 Hz, NHCH(CH_3)COO-cHex), 173.65 (d, , $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 6.3 Hz, NHCH(CH_3)COO-cPent), 161.61 (s, C-6 base), 159.61 (s, C-2 base), 152.93 (s, C-4 base), 137.89 (s, C-8 base), 115.80 (s, C-5 base), 91.84 (s, C-1'), 81.14 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 5.0 Hz, C-4'), 79.44 (s, C-2'), 78.51 (s, C-3'), 74.60 (s, C-1 cPent), 73.91 (s, C ipso cHex), 65.01 (d, $J^2_{P\text{-}O\text{-}C}$ = 3.75 Hz, C-5'), 53.82 (s, 6-*O*CH_3 guanine), 50.97 (d, $J^2_{P\text{-}N\text{-}C}$ = 6.3 Hz, NHCH(CH_3)COO-cHex), 49.95 (d, $J^2_{P\text{-}N\text{-}C}$ = 6.3 Hz, NHCH(CH_3)COO-cPent), 32.68 & 32.46 (2s, 2 x C cPent (2 & 5)), 31.40 & 31.32 (2s, 2 x C ortho cHex), 25.23 (s, C para cHex), 23.66 & 23.54 (2s, 2 x C meta cHex, 2 x C cPent (3 & 4)), 21.04 & 20.99 (2s, NHCH(CH_3)COO-cHex, NHCH(CH_3)COO-cPent), 20.36 (s, CH_3-2').

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm): 13.68 (s) (59%), 13.48 (s) (41%).

Synthesis of L-Tyrosine-(tert-butyl)-neopentyl ester β-2'-C-methyl-6-O-methyl guanosine phosphorodiamidate (24.a)



Prepared according to procedure E, using *β*-2'-C-Me-6-OMe guanosine (**11**) (0.36 g, 1.17 mmol) in anh. THF (6.25 mL), anh. NEt_3 (0.20 mL, 1.40 mmol, 1.2 eq), $POCl_3$ (0.12 mL, 1.40 mmol, 1.2 eq), *L*-Tyrosine-(*tert*-butyl)-neopentyl ester (**12.k**) (1.80 g, 5.85 mmol, 5.0 eq) in anh. DCM (7.0 mL), and anh. NEt_3 (1.60 mL, 11.7 mmol, 10 eq). After overnight stirring at RT and flash chromatography using MeOH (3%)/$CHCl_3$ and preparative chromatography, the desired material was recovered (0.35 g, 0.36 mmol, 31%).

HPLC (MeOH/H_2O) : Rt = 32.05 min

HPLC (ACN/H_2O) : Rt = 28.53 min

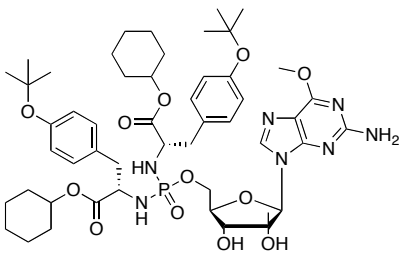
MS (TOF ES^+): 992.49 ($M + Na^+$).

^1H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 7.64 (s, 1H, H-8 base), 7.10 (d, 2H, *J* = 8.4 Hz, 2 x CH ortho Ar), 6.99 (d, 2H, *J* = 8.4 Hz, 2 x CH ortho Ar), 6.89-6.85 (m, 4H, 4 x CH meta Ar), 5.89 (s, 1H, H-1'), 5.38 (bs, 2H, 2 x NHCH($CH_2ArOC(CH_3)_3$)CO), 4.43-4.38 (m, 2H, H-5', H-3'), 4.21-4.13 (m, 2H, 2 x NHCH($CH_2ArOC(CH_3)_3$)CO), 4.07 (s, 3H, 6-*O*CH_3 base), 4.05-4.02 (m, 1H, H-4'), 3.93-3.88 (m, 1H, H-5'), 3.79-3.72 (m, 4H, 2 x O$CH_2C(CH_3)_3$), 3.05-2.83 (m, 4H, 2 x NHCH(CH_2ArOC$(CH_3)_3$)CO), 1.32 & 1.30 (2s, 18H, 2 x NHCH($CH_2ArOC(CH_3)_3$)CO), 0.97 (s, 3H, CH_3-2'), 0.92 & 0.88 (m, 18H, 2 x $COOCH_2C(CH_3)_3$).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 173.60 (s, NHCH($CH_2ArOC(CH_3)_3$)CO), 173.05 (s, NHCH($CH_2ArOC(CH_3)_3$)CO), 161.63 (s, C-6 base), 159.55 (s, C-2 base), 154.42 & 154.07 (2s, 2 x C para Ar), 152.94 (s, C-4 base), 137.96 (s, C-8 base), 131.46 & 130.65 (2s, 2 x C-1 Ar), 130.11 &130.07 & 139.89 & 129.86 (4s, 4 x C ortho Ar), 124.32 & 124.24 & 124.16 & 124.09 (4s, 4 x C meta Ar), 115.83 (s, C-5 base), 91.50 (s, C-1'), 80.73 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 6.3 Hz, C-4'), 79.55 (s, C-2'), 78.79 & 78.41 (2s, 2 x NHCH($CH_2ArOC(CH_3)_3$)CO), 74.90 & 74.79 (2s, 2 x $COOCH_2C(CH_3)_3$), 74.62 (s, C-3'), 64.64 (s, C-5'), 55.44 & 55.15 (2s, 2 x NHCH($CH_2ArOC(CH_3)_3$)CO), 53.82 (s, 6-*O*CH_3 base), 39.77 (s, 2 x NHCH($CH_2ArOC(CH_3)_3$)CO), 31.26 & 31.21 (2s, 2 x $OCH_2C(CH_3)_3$), 28.81& 28.75 (2s, 6 x NHCH($CH_2ArOC(CH_3)_3$)CO), 26.41 & 26.38 (2s, 6 x $OCH_2C(CH_3)_3$), 20.32 (s, CH_3-2').

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm): 13.72 (s).

Synthesis of L-Tyrosine-(tert-butyl)-cyclohexyl ester β-2'-C-methyl-6-O-methyl guanosine phosphorodiamidate (24.b)



Prepared according to procedure E, using *β*-2'-C-Me-6-OMe guanosine (**11**) (0.37 g, 1.19 mmol) in anh. THF (6.40 mL), anh. NEt_3 (0.20 mL, 1.43 mmol, 1.2 eq), $POCl_3$ (0.12 mL, 1.43 mmol, 1.2 eq), *L*-Tyrosine-(*tert*-butyl)-cyclohexyl ester (**12.l**) (1.90 g, 5.95 mmol, 5.0 eq) in anh. DCM (7.0 mL), and anh. NEt_3 (1.63 mL, 11.90 mmol, 10

eq). After overnight stirring at RT and flash chromatography using MeOH (2.5%)/$CHCl_3$ and preparative chromatography, the desired material was recovered (0.22 g, 0.22 mmol, 18 %).

HPLC (MeOH/H_2O) : Rt = 32.02 min

HPLC (ACN/H_2O) : Rt = 29.10 min

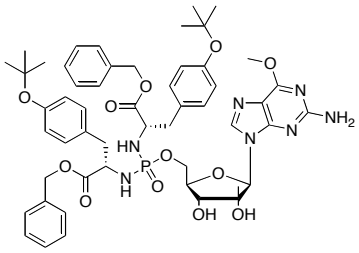
MS (TOF ES^+): 1016.49 ($M + Na^+$).

^1H NMR (500 MHz, MeOD) δ_H (ppm): 7.95 (s, 1H, H-8 base), 7.09-7.04 (m, 4H, 4 c CH ortho Ar), 6.88-6.84 (m, 4H, 4 x CH meta Ar), 5.98 (s, 1H, H-1'), 4.66-4.56 (m, 2H, CH cHex), 4.25-4.19 (m, 3H, H-5', H-3'), 4.14-4.11 (m, 1H, H-4'), 4.04 (s, 3H, 6-*O*CH_3 base), 4.02-3.95 (m, 2H, 2 x NH<u>C</u>H(CH_2ArOC(CH_3)$_3$)CO), 2.94-2.75 (m, 4H, 2 x NHCH(<u>CH_2</u>ArOC(CH_3)$_3$)CO), 1.78-1.62 (m, 10H, 4 x CH_2 ortho cHex, CH_2 para cHex), 1.32 & 1.30 (2s, 18H, 2 x NHCH(CH_2ArOC(<u>CH_3</u>)<u>$_3$</u>)CO), 1.31-1.18 (m, 10H, 4 x CH_2 meta cHex, 2 x CH_2 para cHex), 0.99 (s, 3H, CH_3-2').

^{13}C NMR (125 MHz, MeOD) δ_C (ppm)**:** 173.93 (d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 3.8 Hz, NHCH(CH_2ArOC(CH_3)$_3$)<u>C</u>O), 173.86 (d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 5.0 Hz, NHCH(CH_2ArOC(CH_3)$_3$)<u>C</u>O), 161.79 (s, C-6 base), 161.94 (s, C-2 base), 155.46 & 155.35 (2s, 2 x C para Ar), 154.63 (s, C-4 base), 139.39 (s, C-8 base), 133.18 & 133.10 (2s, 2 x C-1Ar), 131.20 & 130.98 (2s, 4 x C ortho Ar), 125.49 & 125.30 & 125.17 & 124.98 (4s, 4 x C meta Ar), 115.69 (s, C-5 base), 93.18 (s, C-1'), 82.31 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 7.6 Hz, C-4'), 80.03 (s, C-2'), 79.53 & 79.49 (2s, 2 x NHCH(CH_2ArO<u>C</u>(CH_3)$_3$)CO), 75.01 (s, C-3'), 74.95 (m, C-1 cHex), 66.56 (d, $J^2_{P\text{-}O\text{-}C}$ = 5.0 Hz, C-5'), 57.00 & 56.91 (2s, 2 x NH<u>C</u>H(CH_2ArOC(CH_3)$_3$)CO), 54.30 (s, 6-*O*CH_3 base), 41.00 (d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 5.0 Hz, NHCH(<u>C</u>H_2ArOC(CH_3)$_3$)CO), 40.86 (d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 7.6 Hz, NHCH(<u>C</u>H_2ArOC(CH_3)$_3$)CO), 32.58 & 32.53 (2s, 4 x C ortho cHex), 29.31 & 29.28 (2s, 2 x NHCH(CH_2ArOC(<u>C</u>H_3)$_3$)CO), 26.55 & 26.40 (2s, 2 x C para cHex), 24.73 (s, 4 x C meta cHex), 20.41 (s, CH_3-2').

^{31}P NMR (202 MHz, MeOD) δ_P (ppm)**:** 13.50 (s).

Synthesis of L-Tyrosine-(tert-butyl)-benzyl ester β-2'-C-methyl-6-O-methyl guanosine phosphorodiamidate (24.c)



Prepared according to procedure E, using *β*-2'-C-Me-6-OMe guanosine (**11**) (0.31 g, 0.99 mmol) in anh. THF (5.30 mL), anh. NEt_3 (0.17 mL, 1.19 mmol, 1.2 eq), $POCl_3$ (0.10 mL, 1.19 mmol, 1.2 eq), *L*-Tyrosine-(*tert*-butyl)-benzyl ester (**12.m**) (1.62 g, 4.95 mmol, 5.0 eq) in anh. DCM (5.0 mL), and anh. NEt_3 (1.36 mL, 9.90 mmol, 10 eq). After overnight stirring at RT and flash chromatography using MeOH (3%)/$CHCl_3$ and preparative chromatography, the desired material was recovered (0.16 g, 0.16 mmol, 16%).

HPLC (MeOH/H_2O) : Rt = 31.10 min

HPLC (ACN/H_2O) : Rt = 27.08 min

MS (TOF ES^+): 1032.42 ($M + Na^+$).

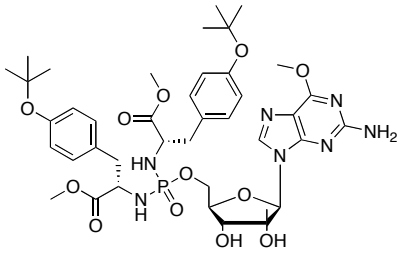
^{1}H NMR (500 MHz, MeOD) δ_H (ppm): 7.90 (s, 1H, H-8 base), 7.32-7.24 (m, 10H, 2 x $OCH_2\underline{Ph}$), 6.96 (d, 4H, J = 8.4 Hz, 4 x CH ortho Ar), 6.79-6.77 (dd, 4H, J = 2.8 Hz, 8.5 Hz, 4 x CH meta Ar), 5.95 (s, 1H, H-1'), 5.13-5.02 (m, 4H, 2 x $O\underline{CH_2}Ph$), 4.22-4.05 (m, 6H, H-3', H-5', H-4', 2 x $NH\underline{C}H(CH_2ArOC(CH_3)_3)CO$), 4.05 (s, 3H, 6-*O*$CH_3$ base), 2.89-2.77 (m, 4H, 2 x $NHCH(\underline{CH_2}ArOC(CH_3)_3)CO$), 1.29 & 1.27 (2s, 18H, 2 x $NHCH(CH_2ArOC(\underline{CH_3})_3)CO$), 0.98 (s, 3H, CH_3-2').

^{13}C NMR (125 MHz, MeOD) δ_C (ppm)**:** 174.21 (s, $NHCH(CH_2ArOC(CH_3)_3)\underline{C}O$), 174.12 (d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 5.0 Hz, $NHCH(CH_2ArOC(CH_3)_3)\underline{C}O$), 162.76 (s, C-6 base), 161.92 (s, C-2 base), 155.47 & 155.37 (2s, 2 x C para Ar), 154.60 (s, C-4 base), 139.35 (s, C-8 base), 137.02 & 137.00 (2s, 2 x C-1 $OCH_2\underline{Ph}$), 132.98 & 132.88 (2s, 2 x C-1 Ar), 131.12 & 131.08 (2s, 4 x C ortho Ar), 129.57 & 129.52 & 129.31 (3s, 4 x C ortho, 4 x C meta. 2 x C para $OCH_2\underline{Ph}$), 125.14 & 125.13 (2s, 4 x C meta Ar), 115.63 (s, C-5 base), 93.14 (s, C-1'), 82.20 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 7.6 Hz, C-4'), 79.96 (s, C-2'), 79.52 (2s, 2 x $NHCH(CH_2ArO\underline{C}(CH_3)_3)CO$), 74.87 (s, C-3'), 67.99 & 67.94 (2s, 2 x $O\underline{C}H_2Ph$), 66.36 (s, C-5'), 57.01 & 56.91 (2s, 2 x $NH\underline{C}H(CH_2ArOC(CH_3)_3)CO$), 54.21 (s, 6-*O*$CH_3$ base), 40.56 (d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 6.3 Hz, $NHCH(\underline{C}H_2ArOC(CH_3)_3)CO$), 40.39 (s,

NHCH(CH$_2$ArOC(CH$_3$)$_3$)CO), 29.25 & 29.23 (2s, 6 x NHCH(CH$_2$ArOC(CH$_3$)$_3$)CO), 20.31 (s, CH$_3$-2').

^{31}P NMR (202 MHz, MeOD) δ_P (ppm)**:** 13.45 (s).

Synthesis of L-Tyrosine-(tert-butyl)-methyl ester β-2'-C-methyl-6-O-methyl guanosine phosphorodiamidate (24.d)



Prepared according to procedure E, using *β*-2'-C-Me-6-OMe guanosine (**11**) (0.26 g, 0.85 mmol) in anh. THF (4.55 mL), anh. NEt$_3$ (0.14 mL, 1.02 mmol, 1.2 eq), POCl$_3$ (0.09 mL, 1.02 mmol, 1.2 eq), commercially available *L*-Tyrosine-(*tert*-butyl)-methyl ester chloridate salt (1.07 g, 4.25 mmol, 5.0 eq) in anh. DCM (3.4 mL), and anh. NEt$_3$ (1.19 mL, 8.5 mmol, 10 eq). After overnight stirring at RT and flash chromatography using MeOH (3%)/CHCl$_3$ and preparative chromatography, the desired material was recovered (0.12 g, 0.14 mmol, 16%).

HPLC (MeOH/H$_2$O) : Rt = 29.01 min

HPLC (ACN/H$_2$O) : Rt = 20.40 min

MS (TOF ES$^+$): 858.38 (M + H$^+$), 880.37 (M + Na$^+$).

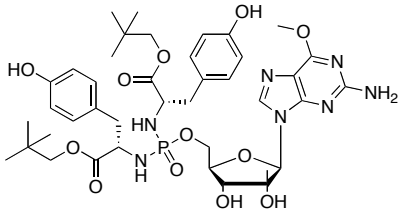
^{1}H NMR (500 MHz, CDCl$_3$) δ_H (ppm): 7.69 (s, 1H, H-8 base), 7.03 (d, 2H, *J* = 8.4 Hz, 2 x CH ortho Ar), 6.98 (d, 2H, *J* = 8.4 Hz, 2 x CH ortho Ar), 6.87 (dd, 4H, *J* = 3.0 Hz, 8.40 Hz, 4 x CH meta Ar), 5.95 (s, 1H, H-1'), 5.45 (bs, 2H, 2 x NHCH(CH$_2$ArOC(CH$_3$)$_3$)CO), 4.35-4.30 (m, 2H, H-3', H-5'), 4.09-4.06 (m, 3H, 2 x NHCH(CH$_2$ArOC(CH$_3$)$_3$)CO, H-4'), 4.05 (s, 3H, 6-*O*CH$_3$ base), 4.01-3.96 (m, 1H, H-5'), 3.63 & 3.62 (2s, 6H, 2 x COOCH$_3$), 3.31-3.28 (m, 4H, 2 x NHCH(CH$_2$ArOC(CH$_3$)$_3$)CO), 1.31 & 1.30 (2s, 18H, 2 x NHCH(CH$_2$ArOC(CH$_3$)$_3$)CO), 1.98 (s, 3H, CH$_3$-2').

^{13}C NMR (125 MHz, CDCl$_3$) δ_C (ppm)**:** 173.68 (s, NHCH(CH$_2$ArOC(CH$_3$)$_3$)CO), 173.36 (d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 5.0 Hz, NHCH(CH$_2$ArOC(CH$_3$)$_3$)CO), 161.60 (s, C-6 base), 159.61 (s, C-2 base), 154.40 & 154.15 (2s, 2 x C para Ar), 153.03 (s, C-4 base), 137.85 (s, C-8 base), 131.30 & 130.80 (2s, 2 x C-1 Ar), 130.29 & 129.96 & 129.96 & 129.87 (4s, 4x C ortho Ar), 124.20 & 124.12 (2s, 4 x C meta Ar), 115.70 (s, C-5 base), 91.47

(s, C-1'), 80.80 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 6.3 Hz, C-4'), 79.36 (s, C-2'), 78.68 & 78.42 (2s, 2 x NHCH(CH_2ArO<u>C</u>(CH_3)$_3$)CO), 74.39 (s, C-3'), 64.77 (s, C-5'), 55.37 & 55.22 (2s, 2 x NH<u>C</u>H(CH_2ArOC(CH_3)$_3$), 53.81 (s, 6-*O*CH_3 base), 52.24 & 52.18 (2s, 2 x COO<u>C</u>H_3), 39.63 & 30.57 (2s, 2 x NHCH(<u>C</u>H_2ArOC(CH_3)$_3$)CO), 28.82-22.77 (2s, 6 x NHCH(CH_2ArOC(<u>C</u>H_3)$_3$)CO), 20.29 (s, CH_3-2').

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm)**:** 13.31 (s).

Synthesis of L-Tyrosine-neopentyl ester β-2'-C-methyl-6-O-methyl guanosine phosphorodiamidate (25.a)



L-Tyrosine-(*tert*-butyl)-neopentyl ester phosphorodiamidate (**24.a**, 0.15 g, 0.16 mmol) was dissolved in anh. DCM (1.60 mL). Trifluoroacetic acid (0.20 mL) was added and the solution was stirred at RT for 30 min. The solvent was removed under reduced pressure and the residue was purified by flash chromatography using $CHCl_3$/MeOH (6.5%), to recover a white off solid (0.067 g, 0.08 mmol, 49%).

HPLC (MeOH/H_2O) : Rt = 27.20 min

HPLC (ACN/H_2O) : Rt = 17.52 min

MS (TOF ES$^+$): 858.38 (M + H^+), 880.36 (M + Na^+).

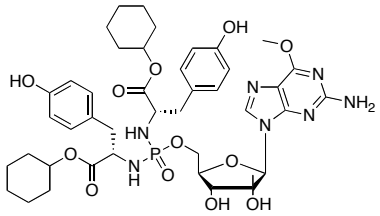
^{1}H NMR (500 MHz, MeOD) δ_H (ppm): 8.03 (s, 1H, H-8 base), 7.01-6.95 (dd, 4H, *J* = 8.5 Hz, 22 Hz, 4 x CH ortho Ar), 6.72-6.66 (dd, 4H, *J* = 8.5 Hz, 18.2 Hz, 4 x CH meta Ar), 5.97 (s, 1H, H-1'), 4.17-4.06 (m, 4H, H-3', H-4', H-5'), 4.04 (s, 3H, 6-*O*CH_3 base), 4.02-3.95 (m, 2H, 2 x NH<u>CH</u>(CH_2ArOH)CO), 3.73-3.62 (m, 4H, 2 COO<u>C</u>H_2C(CH_3)$_3$), 2.90-2.77 (m, 4H, 2 x NHCH(<u>C</u>H_2ArOH)CO), 0.99 (s, 3H, CH_3-2'), 0.87 & 0.84 (2s, 18H, 2 x COOCH_2C(<u>C</u>H_3)$_3$).

^{13}C NMR (125 MHz, MeOD) δ_C (ppm)**:** 174.77 (s, NHCH(CH_2ArOH)<u>C</u>O), 174.70 (d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 5.0 Hz, NHCH(CH_2ArOH)<u>C</u>O), 162.65 (s, C-6 base), 161.88 (s, C-2 base), 157.48 & 157.39 (2s, 2 x C para Ar), 154.56 (s, C-4 base), 139.26 (s, C-8 base), 131.59 & 131.52 (2s, 4 x C ortho Ar), 128.72 & 128.71 (2s, 2 x C-1 Ar), 116.38 & 116.35 (2s, 4 x C meta Ar), 115.66 (s, C-5 base), 93.32 (s, C-1'), 82.52 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 7.6 Hz, C-4'), 79.48 (s, C-2'), 75.56 & 75.52 (2s, 2 x COO<u>C</u>H_2C(CH_3)$_3$), 74.93 (s, C-3'), 66.54 (d, $J^2_{P\text{-}}$

$_{O\text{-}C}$ = 3.78 Hz, C-5'), 57.18 & 57.07 (2s, 2 x NH<u>C</u>H(CH_2ArOH)CO), 54.42 (s, 6-*O*CH_3 base), 40.73 (d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 6.30 Hz, NHCH(<u>C</u>H_2ArOH)CO), 40.61 (d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 6.30 Hz, NHCH(<u>C</u>H_2ArOH)CO), 32.07 & 32.01 (2s, 2 x COOCH_2<u>C</u>$(CH_3)_3$), 26.82 & 26.79 (2s, 6 x COOCH_2C(<u>C</u>$H_3)_3$), 20.36 (s, CH_3-2').

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm)**:** 13.39 (s).

Synthesis of L-Tyrosine-cyclohexyl ester β-2'-C-methyl-6-O-methyl guanosine phosphorodiamidate (25.b)



L-Tyrosine-(*tert*-butyl)-cyclohexyl ester phosphorodiamidate (**24.b**, 0.12 g, 0.12 mmol) was dissolved in anh. DCM (1.20 mL). Trifluoroacetic acid (0.13 mL) was added and the solution was stirred at RT for 30 min. The solvent was removed under reduced pressure and the residue was purified by flash chromatography using $CHCl_3$/MeOH (6%), to recover a white off solid (0.042 g, 0.05 mmol, 40%).

HPLC (MeOH/H_2O) : Rt = 27.80 min

HPLC (ACN/H_2O: Rt = 17.60 min

MS (TOF ES^+): 882.38 ($M + H^+$), 904.36 ($M + Na^+$).

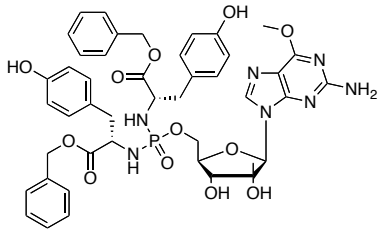
^{1}H NMR (500 MHz, MeOD) δ_H (ppm): 7.91 (s, 1H, H-8 base), 7.00-6.96 (m, 4H, 4 x CH ortho Ar), 6.71-6.68 (m, 4H, 4 x CH meta Ar), 5.98 (s, 1H, H-1'), 4.66-4.57 (m, 2H, 2 x CH cHex), 4.21-4.11 (m, 4H, H-3', H-4', H-5'), 4.04 (s, 3H, 6-*O*CH_3 base), 3.97-3.91 (m, 2H, 2 x NH<u>CH</u>(CH_2ArOH)CO), 2.85-2.68 (m, 4H, 2 x NHCH(<u>C</u>H_2ArOH)CO), 1.75-1.64 (m, 8H, 2 x CH_2 meta cHex, 2 x CH_2 ortho cHex), 1.49 (m, CH_2 para cHex), 1.41-1.18 (m, 10H, 2 x CH_2 meta cHex, 2 x CH_2 ortho cHex, CH_2 para cHex), 0.99 (s, 3H, CH_3-2').

^{13}C NMR (125 MHz, MeOD) δ_C (ppm)**:** 174.14 (d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 3.8 Hz, NHCH(CH_2ArOH)<u>C</u>O), 174.03 (d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 5.0 Hz, NHCH(CH_2ArOH)<u>C</u>O), 162.79 (s, C-6 base), 161.90 (s, C-2 base), 157.40 & 157.34 (2s, 2 x C para Ar), 154.56 (s, C-4 base), 139.40 (s, C-8 base), 131.70 & 131.68 & 131.65 & 131.51 (4s, 4 x C ortho Ar), 128.78 & 128.73 (2s, 2 x C-1 Ar), 116.61 & 116.58 & 116.30 & 116.28 (3s, 4 x C meta Ar), 115.66 (s, C-5 base), 93.19 (s, C-1'), 82.36 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 7.6 Hz, C-4'), 79.99 (s,

C-2'), 75.08 (s, C-3'), 75.01 & 74.94 (2s, 2 x C-1 cHex), 66.63 (d, $J^{2}_{P\text{-}O\text{-}C}$ = 5.0 Hz, C-5'), 57.13 (2s, 2 x NHCH(CH$_2$ArOH)CO), 54.31 (s, 6-*O*CH$_3$ base), 40.71 (d, $J^{3}_{P\text{-}N\text{-}C\text{-}C}$ = 6.3 Hz, NHCH(CH$_2$ArOH)CO), 40.62 (d, $J^{3}_{P\text{-}N\text{-}C\text{-}C}$ = 6.3 Hz, NHCH(CH$_2$ArOH)CO), 32.54 & 32.52 & 32.48 & 32.44 (m, 4 x C ortho cHex), 26.41 & 26.38 (2s, 2 x C para cHex), 24.66 (s, 4 x C meta cHex), 20.36 (s, CH$_3$-2').

^{31}P NMR (202 MHz, MeOD) δ_P (ppm)**:** 13.48 (s).

Synthesis of L-Tyrosine-benzyl ester β-2'-C-methyl-6-O-methyl guanosine phosphorodiamidate (25.c)



L-Tyrosine-(*tert*-butyl)-benzyl ester phosphorodiamidate (**24.c**, 0.099 g, 0.098 mmol) was dissolved in anh. DCM (1.00 mL). Trifluoroacetic acid (0.11 mL) was added and the solution was stirred at RT for 30 min. The solvent was removed under reduced pressure and the residue was purified by flash chromatography using CHCl$_3$/MeOH (6%), to recover a white off solid (0.02 g, 0.02 mmol, 24%).

HPLC (MeOH/H$_2$O) : Rt = 25.75 min

HPLC (ACN/H$_2$O) : Rt = 16.45 min

MS (TOF ES$^+$): 898.32 (M + H$^+$), 920.30 (M + Na$^+$), 936.27 (M + K$^+$).

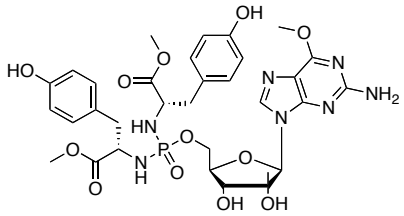
^{1}H NMR (500 MHz, MeOD) δ_H (ppm): 7.87 (s, 1H, H-8 base), 7.31-7.17 (m, 10H, 2 x COOCH$_2$Ph), 6.88-6.85 (m, 4H, 4 x CH ortho Ar), 6.65-6.62 (m, 4H, 4 x CH meta Ar), 5.96 (s, 1H, H-1'), 5.08-4.94 (m, 4H, 2 x COOCH$_2$Ph), 4.21-4.19 (d, 1H, *J* = 8.7 Hz, H-3'), 4.13-4.10 (m, 3H, H-4', H-5'), 4.02 (s, 3H, 6-*O*CH$_3$ guanine), 3.97-3.94 (m, 2H, 2 x NHCH(CH$_2$ArOH)CO), 2.82-2.73 (m, 4H, 2 x NHCH(CH$_2$ArOH)CO), 0.98 (s, 3H, CH$_3$-2').

^{13}C NMR (125 MHz, CDCl$_3$) δ_C (ppm)**:** 174.39 (d, $J^{3}_{P\text{-}N\text{-}C\text{-}C}$ = 3.8 Hz, NHCH(CH$_2$ArOH)CO), 174.28 (d, $J^{3}_{P\text{-}N\text{-}C\text{-}C}$ = 5.0 Hz, NHCH(CH$_2$ArOH)CO), 162.76 (s, C-6 base), 161.89 (s, C-2 base), 157.41 & 157.34 (2s, 2 x C para Ar), 154.55 (s, C-4 base), 139.38 (s, C-8 base), 137.03 & 137.00 (2s, 2 x C ipso Ph), 131.64 & 131.61 (2s, 4 x C ortho Ar), 129.53 & 129.52 & 129.50 & 129.47 & 129.32 & 129.26 (6s, 2 x COOCH$_2$Ph), 128.61 & 128.57 (2s, 2 C ipso Ar), 116.34 & 116.32 (2s, 4 x C meta Ar),

115.66 (s, C-5 base), 93.18 (s, C-1'), 82.31 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 7.6 Hz, C-4'), 79.96 (s, C-2'), 75.00 (s, C-3'), 67.94 & 67.86 (2s, 2 x COO<u>C</u>H_2Ph), 66.49 (d, $J^2_{P\text{-}O\text{-}C}$ = 3.8 Hz, C-5'), 55.22 & 55.12 (2s, 2 x NH<u>C</u>H(CH_2ArOH)CO), 54.24 (s, 6-*O*CH_3 base), 40.49 & 40.39 (2s, 2 x NHCH(<u>CH_2</u>ArOH)CO), 20.34 (s, CH_3-2').

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm)**:** 13.38 (s).

Synthesis of L-Tyrosine-methyl ester β-2'-C-methyl-6-O-methyl guanosine phosphorodiamidate (25.d)



L-Tyrosine-(*tert*-butyl)-methyl ester phosphorodiamidate (**24.d**, 0.49 g, 0.57 mmol) was dissolved in anh. DCM (1.90 mL). Trifluoroacetic acid (0.63 mL) was added and the solution was stirred at RT for 30 min. The solvent was removed under reduced pressure and the residue was purified by flash chromatography using $CHCl_3$/MeOH (6%) and preparative TLC to recover a white off solid (0.077 g, 0.11 mmol, 18%).

HPLC (MeOH/ H_2O) : Rt = 19.76 min

HPLC (ACN/H_2O) : Rt = 10.45 min

MS (TOF ES$^+$): 746.26 (M + H$^+$), 768.24 (M + Na$^+$), 784.20 (M + K$^+$).

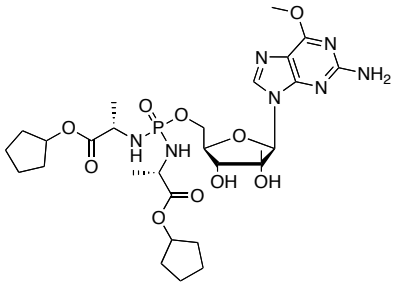
^{1}H NMR (500 Hz, MeOD) δ_H (ppm): 7.91 (s, 1H, H-8 base), 6.99 (d, 2H, *J* = 8.5 Hz, 2 x CH ortho Ar), 6.94 (d, 2H, *J* = 8.4 Hz, 2 x CH ortho Ar), 6.71-6.66 (dd, 4H, *J* = 8.1 Hz, 16.45 Hz, 4 x CH meta Ar), 5.97 (s, 1H, H-1'), 4.20 (d, 1H, *J* = 8.3 Hz, H-3'), 4.10-4.05 (m, 3H, H-5', H4'), 4.00 (s, 3H, 6-*O*CH_3 base), 3.99-3.96 (q, 1H, *J* = 7.8 Hz, NH<u>CH</u>(CH_2ArOH)CO), 3.92-3.88 (q, *J* = 7.0 Hz, 1H, NH<u>CH</u>(CH_2ArOH)CO), 3.63 & 3.61 (2s, 6H, 2 x COO<u>CH_3</u>), 2.89-2.86 (m, 2H, NHCH(<u>CH_2</u>ArOH)CO), 2.78-2.74 (m, 2H, NHCH(<u>CH_2</u>ArOH)CO), 0.99 (s, 3H, CH_3-2').

^{13}C NMR (125 MHz, MeOD) δ_C (ppm)**:** 175.09 (s, NHCH(CH_2ArOH)<u>C</u>O), 174.97 (d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 5.0 Hz, NHCH(CH_2ArOH)<u>C</u>O), 162.78 (s, C-6 base), 161.93 (s, C-2 base), 157.43 & 157.33 (2s, 2 x C para Ar), 154.58 (s, C-4 base), 139.33 (s, C-8 base), 131.62 & 131.52 (2s, 4 x C ortho Ar), 128.77 & 128.73 (2s, 2 x C ipso Ar), 116.30 & 116.27 (2s, 4 x C meta Ar), 115.61 (s, C-5 base), 93.14 (s, C-1'), 82.23 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 7.6 Hz, C-4'), 79.95 (s, C-2'), 74.47 (s, C-3'), 66.29 (s, $J^2_{P\text{-}O\text{-}C}$ = 5.0 Hz, C-5'), 57.06 & 56.98

(2s, 2 x NHCH(CH_2ArOH)CO), 54.24 (s, 6-*O*CH_3 base), 52.58 & 52.52 (2s, 2 x COOCH_3), 40.44 (d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 6.3 Hz, NHCH(CH_2ArOH)CO), 40.35 (d, $J^3_{P\text{-}N\text{-}C\text{-}C$ = 7.6 Hz, NHCH(CH_2ArOH)CO), 20.34 (s, CH_3-2').

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm)**:** 13.42 (s).

Synthesis of L-Alanine-cyclopentyl ester β-2'-C-methyl-6-O-methyl guanosine phosphorodiamidate (27.e)



Prepared according to procedure E, using *β*-2'-C-Me-6-OMe guanosine (**11**) (0.32 g, 1.02 mmol) in anh. THF (5 mL), anh. NEt_3 (0.17 mL, 1.22 mmol, 1.2 eq), $POCl_3$ (0.12 mL, 1.22 mmol, 1.2 eq), *L*-Alanine-cyclopentyl ester tosylate salt (provided by Inhibitex *Inc.*) (1.68 g, 5.10 mmol, 5.0 eq) in anh. DCM (8 mL) and anh. NEt_3 (1.42 mL, 10.2 mmol, 10 eq). After overnight stirring at RT and flash chromatography using MeOH (4%)/$CHCl_3$, the desired material was recovered (0.063 g, 0.09 mmol, 9%).

HPLC (MeOH/ H_2O): Rt = 15.35 min

HPLC (ACN/ H_2O): Rt = 10.27 min

MS (TOF ES^+): 670.30 ($M + H^+$).

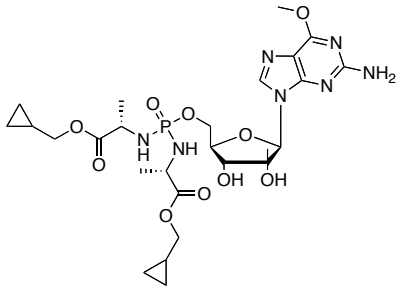
^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 7.70 (s, 1H, H-8 base), 5.89 (s, 1H, H-1'), 5.48 (bs, 2H, 2 x NHCH(CH_3)COO-cPent), 5.06 (m, 2H, CH cPent), 4.46-4.42 (m, 1H, H-5'), 4.37 (d, 1H, *J* = 8.5 Hz, H-3'), 4.26-4.22 (m, 1H, H-5'), 4.11-4.08 (m, 1H, H-4'), 3.94 (s, 3H, 6-*O*CH_3 base), 3.83-3.70 (m, 2H, 2 x NHCH(CH_3)COO-cPent), 1.73-1.45 (m, 16H, 8 x CH_2 cPent), 1.31 (d, 3H, *J* = 8.0 Hz, NHCH(CH_3)COO-cPent), 1.29 (d, 3H, *J* = 8.0 Hz, NHCH(CH_3)COO-cPent), 1.02 (s, 3H, CH_3-2').

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm)**:** 174.00 (d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 5.0 Hz, NHCH(CH_3)COO-cPent), 173.90 (d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 6.3 Hz, NHCH(CH_3)COO-cPent), 161.37 (s, C-6 base), 159.56 (s, C-2 base), 152.99 (s, C-4 base), 137.97 (s, C-8 base), 115.50 (s, C-5 base), 91.76 (s, C-1'), 81.14 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 6.4 Hz, C-4'), 79.22 (s, C-2'), 78.18 & 78.10 (2s, 2 x CH cPent), 73.93 (s, C-3'), 64.95 (s, C-5'), 53.70 (s, 6-*O*CH_3 base), 49.81 & 49.67 (2s, 2 x NHCH(CH_3)COO-cPent), 32.56 & 32.34 (2s, 4 x CH_2

cPent (C-2 & C-5)), 23.55 (s, 4 x CH_2 cPent (C-3 & C-4)), 20.75 & 20.71 (2s, 2 x NHCH(<u>CH_3</u>)COO-cPent), 20.27 (s, CH_3-2').

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm)**:** 12.99 (s).

Synthesis of L-Alanine-cyclopropylmethyl ester β-2'-C-methyl-6-O-methyl guanosine phosphorodiamidate (27.f)



Prepared according to procedure E, using *β*-2'-C-Me-6-OMe guanosine (**11**) (0.29 g, 0.93 mmol) in anh. THF (5 mL), anh. NEt_3 (0.16 mL, 1.12 mmol, 1.2 eq), $POCl_3$ (0.11 mL, 1.12 mmol, 1.2 eq), *L*-Alanine-cyclopropylmethyl ester tosylate salt (provided by Inhibitex *Inc.*) (1.47 g, 4.65 mmol, 5.0 eq) in anh. DCM (8 mL) and anh. NEt_3 (1.30 mL, 9.3 mmol, 10 eq). After overnight stirring at RT and flash chromatography using MeOH (6%)/$CHCl_3$ the desired material was recovered (0.034 g, 0.06 mmol, 7%).

HPLC (MeOH/ H_2O): Rt = 13.03 min

HPLC (ACN/ H_2O): Rt = 11.12 min

MS (TOF ES$^+$): 642.26 (M + H$^+$), 665.25 (M + Na$^+$), 680.22 (M + K$^+$).

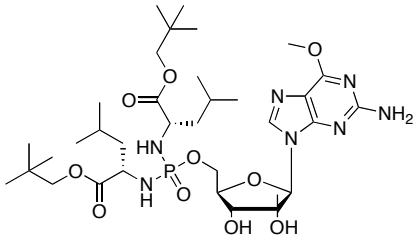
^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 7.77 (s, 1H, H-8 base), 5.99 (s, 1H, H-1'), 5.58 (bs, 2H, <u>NH</u>CH(CH_3)COOCH_2), 4.54 (m, 1H, H-5'), 4.43 (m, 1H, H-3'), 4.34-4.31 (m, 1H, H- 5'), 4.22-4.21 (m, 1H, H-4'), 4.03 (s, 3H, 6-*O*CH_3 base), 3.99-4.86 (m, 6H, NHCH(CH_3)COO<u>CH_2</u>-cProp, NH<u>CH</u>(CH_3)COOCH_2-cProp), 1.38 (d, 3H, *J* = 5.0 Hz, NHCH(<u>CH_3</u>)COOCH_2-cProp), 1.35 (d, 3H, *J* = 7.0 Hz, NHCH(<u>CH_3</u>)COOCH_2-cProp), 1.08 (m, 2H, 2 x CH cProp), 0.99 (s, 3H, CH_3-2'), 0.52 & 0.23 (2s, 8H, 4 x CH_2 cProp).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 174.43 (s, NHCH(CH_3)<u>C</u>OOCH_2), 174.30 (d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 5.0 Hz, NHCH(CH_3)<u>C</u>OOCH_2), 161.54 (s, C-6 base), 159.66 (s, C-2 base), 152.99 (s, C-4 base), 137.87 (s, C-8 base), 115.56 (s, C-5 base), 91.69 (s, C-1'), 81.11 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 6.3 Hz, C-4'), 79.34 (s, C-2'), 74.21 (s, C-3'), 70.23 & 70.16 (2s, 2 x NHCH(CH_3)COO<u>C</u>H_2-cProp), 64.98 (s, C-5'), 53.80 (s, 6-*O*CH3 base), 49.91 & 49.78 (2s, 2 x NH<u>C</u>H(CH_3)COOCH_2-cProp), 20.94 (d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 6.4 Hz,

NHCH(CH$_3$)COOCH$_2$), 20.84 (s, NHCH(CH$_3$)COOCH$_2$-cProp), 20.27 (s, CH$_3$-2'), 9.63 & 8.61 (2s, 2 x CH cProp), 3.23 & 3.17 (2s, 4 x CH$_2$ cProp).

^{31}P NMR (202 MHz, CDCl$_3$) δ_P (ppm): 13.17 (s).

Synthesis of L-Leucine-neopentyl ester β-2'-C-methyl-6-O-methyl guanosine phosphorodiamidate (29.a)



Prepared according to procedure E, using *β*-2'-C-Me-6-OMe guanosine (**11**) (0.25 g, 0.80 mmol) in anh. THF (4.3 mL), anh. NEt$_3$ (0.13 mL, 0.96 mmol, 1.2 eq), POCl$_3$ (0.09 mL, 0.96 mmol, 1.2 eq), *L*-Leucine-neopentyl ester tosylate salt (**12..c**) (1.51 g, 4.05 mmol, 5.0 eq) in anh. DCM (2.3 mL), and anh. NEt$_3$ (1.12 mL, 8.04 mmol, 10 eq). After overnight stirring at RT and flash chromatography using MeOH (2.5%))/CHCl$_3$ the desired material was recovered (0.005 g, 0.008 mmol, 1%).

HPLC (MeOH/ H$_2$O) : Rt = 32.41 min

HPLC (ACN/ H$_2$O) : Rt = 25.39 min

MS (TOF ES$^+$): 758.42 (M + H$^+$), 780.40 (M + Na$^+$).

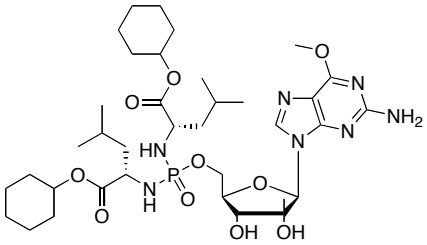
^{1}H NMR (500 MHz, CDCl$_3$) δ_H (ppm): 7.67 (s, 1H, H-8 base), 5.90 (s, 1H, H-1'), 5.51 (bs, 2H, 2 x NHCH(CH$_2$CH(CH$_3$)$_2$)CO), 4.74 (m, 1H, H-5'), 4.66 (m, 1H, H-3'), 4.25 (m, 1H, H-5'), 4.23 (m, 1H, H-4'), 4.06 (s, 3H, 6-*O*CH$_3$ base), 3.97-3.91 (2m, 2H, 2 x NHCH(CH$_2$CH(CH$_3$)$_2$)CO), 3.82-3.75 (m, 4H, 2 x COOCH$_2$C(CH$_3$)$_3$), 1.73-1.68 (m, 2H, 2 x NHCH(CH$_2$CH(CH$_3$)$_2$)CO), 1.58-1.45 (m, 4H, 2 x NHCH(CH$_2$CH(CH$_3$)$_2$)CO), 1.00 (s, 3H, CH$_3$-2'), 0.94 & 0.93 (2s, 30H, 2 x NHCH(CH$_2$CH(CH$_3$)$_2$)CO, 2 x COOCH$_2$C(CH$_3$)$_3$).

^{13}C NMR (125 MHz, CDCl$_3$) δ_C (ppm)**:** 175.20 (s, NHCH(CH$_2$CH(CH$_3$)$_2$)CO), 174.58 (s, NHCH(CH$_2$CH(CH$_3$)$_2$)CO), 161.61 (s, C-6 base), 159.63 (s, C-2 base), 152.86 (s, C-4 base), 137.05 (s, C-8 base), 115.96 (s, C-5 base), 91.25 (s, C-1'), 81.33 (s, C-4'), 79.65 (s, C-2'), 75.19 (s, C-3'), 74.77 & 74.65 (2s, 2 x COOCH$_2$C(CH$_3$)$_3$), 65.59 (s, C-5'), 53.65 (s, 6-*O*CH$_3$ base), 52.96 & 52.82 (2s, 2 NHCH(CH$_2$CH(CH$_3$)$_2$)CO), 43.77 (s, 2 x NHCH(CH$_2$CH(CH$_3$)$_2$)CO), 31.34 & 31.24 (2s, 2 x COOCH$_2$C(CH$_3$)$_3$), 26.52 & 26.49 & 26.43 & 26.40 & 26.38 & 26.32 (6s, 6 COOCH$_2$C(CH$_3$)$_3$), 24.62 & 24.56 (2s, 2

x NHCH(CH_2<u>C</u>H(CH_3)$_2$)CO), 22.68 & 22.45 & 22.31 & 22.01 (2s, 4 x NHCH(CH_2CH(<u>C</u>H_3)$_2$)CO), 20.43 (s, CH_3-2').

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm)**:** 13.76 (s).

Synthesis of L-Leucine-cyclohexyl ester β-2'-C-methyl-6-O-methyl guanosine phosphorodiamidate (29.b)



Prepared according to procedure E, using *β*-2'-C-Me-6-OMe guanosine (**11**) (0.59 g, 1.89 mmol) in anh. THF (8 mL), anh. NEt_3 (0.32 mL, 2.27 mmol, 1.2 eq), $POCl_3$ (0.21 mL, 2.27 mmol, 1.2 eq), *L*-Leucine-cyclohexyl ester tosylate salt (**12.d**) (3.64 g, 9.45 mmol, 5.0 eq) in anh. DCM (5.4 mL), and anh. NEt_3 (2.67 mL, 18.9 mmol, 10 eq). After overnight stirring at RT and flash chromatography using MeOH (3%)/$CHCl_3$, the desired material was recovered (0.1 g, 0.13 mmol, 7%).

HPLC (MeOH/ H_2O) : Rt =22.00 min

HPLC (ACN/ H_2O) : Rt =20.11 min

MS (TOF ES$^+$): 782.42 (M + H$^+$), 804.41 (M + Na$^+$).

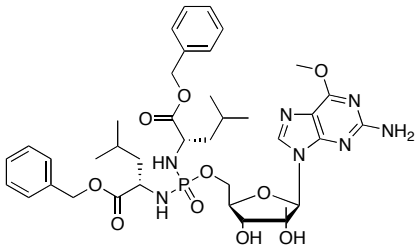
^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 7.71 (s, 1H, H-8 base), 5.95 (s, 1H, H-1'), 5.18 (bs, 2H, 2 x <u>NH</u>CH(CH_2CH(CH_3)$_2$)CO), 4.76-4.70 (m, 2H, 2 x CH cHex), 4.60 (m, 1H. H-5'), 4.48-4.47 (m, 1H, H-3'), 4.25-4.23 (m, 1H, H-5'), 4.20-4.17 (m, 1H, H-4'), 4.02 (s, 3H, 6-*O*CH_3 base), 3.89-3.77 (m, 2H, 2 x NH<u>CH</u>(CH_2CH(CH_3)$_2$)CO), 1.79 (m, 8H, 4 x CH_2 ortho cHex), 1.67-1.65 (m, 4H, 2 x CH_2 para cHex), 1.50-1.24 (m, 14H, 4 x CH_2 meta cHex, 2 x NHCH(<u>CH_2</u>CH(CH_3)$_2$CO, 2 x NHCH(CH_2<u>CH</u>(CH_3)$_2$)CO), 1.24 (s, 3H, CH_3-2'), 0.98 & 0.84 (m, 12H, 4 x NHCH(CH_2CH(<u>CH_3</u>)$_2$)CO).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 174.25 (s, NHCH(CH_2CH(CH_3)$_2$)<u>C</u>O), 173.84 (d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 3.4 Hz, NHCH(CH_2CH(CH_3)$_2$)<u>C</u>O), 161.55 (s, C-6 base), 159.71 (s, C-2 base), 152.93 (s, C-4 base), 137.87 (s, C-8 base), 115.68 (s, C-5 base), 91.93 (s, C-1'), 81.12 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 5.0 Hz, C-4'), 79.41 (s, C-2'), 74.71 (s, C-3'), 73.87 & 73.72 (2s, 2 x CH cHex), 65.23 (s, C-5'), 53.43 (s, 6-*O*CH_3 base), 52.94 & 52.80 (2s, 2 x NH<u>CH</u>(CH_2CH(CH_3)$_2$)CO), 43.60 (d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 2.5 Hz, NHCH(<u>CH_2</u>CH(CH_3)$_2$CO), 43.54 (s, NHCH(<u>CH_2</u>CH(CH_3)$_2$CO), 31.46 & 31.37 (2s, 4 x CH_2 ortho cHex), 25.28 &

25.23 (2s, 2 x CH_2 para cHex), 24.56 & 24.51 (2s, 2 x NHCH(CH_2<u>CH</u>(CH_3)$_2$)CO), 22.81 & 22.73 & 22.61 & 22.53 (4s, 4 x CH_2 meta cHex), 22.05 & 22.03 & 21.99 & 21.97 (2s, 2 x NHCH(CH_2CH(<u>CH_3</u>)$_2$)CO), 20.31 (s, CH_3-2').

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm): 13.78 (s).

Synthesis of L-Leucine-benzyl ester β-2'-C-methyl-6-O-methyl guanosine phosphorodiamidate (29.c)



Prepared according to procedure E, using *β*-2'-C-Me-6-OMe guanosine (**11**) (0.32 g, 1.03 mmol) in anh. THF (5.5 mL), anh. NEt_3 (0.18 mL, 1.28 mmol, 1.2 eq), $POCl_3$ (0.11 mL, 1.24 mmol, 1.2 eq), commercially available *L*-Leucine-benzyl ester tosylate salt (2.04 g, 5.15 mmol, 5.0 eq) in anh. DCM (8 mL), and anh. NEt_3 (1.55 mL, 10.3 mmol, 10 eq). After overnight stirring at RT and flash chromatography using MeOH (4%)/$CHCl_3$, the desired material was recovered (0.22 g, 0.28 mmol, 27%).

HPLC (MeOH/ H_2O) : Rt = 22.12 min

HPLC (ACN/ H_2O) : Rt = 18.92 min

MS (TOF ES$^+$): 798.33 (M + H$^+$), 820.31 (M + Na$^+$).

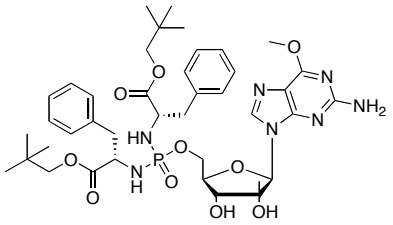
^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 7.72 (s, 1H, H-8 base), 7.33 (m, 10H, COOCH$_2$<u>Ph</u>), 5.92 (s, 1H, H-1'), 5.49 (bs, 2H, 2 x <u>NH</u>CH(CH_2CH(CH_3)$_2$)CO), 5.15 (m, 4H, 2 x COO<u>CH$_2$</u>Ph), 4.55 (m, 1H, H-5'), 4.49 (m, 1H, H-3'), 4.22 (m, 1H, H-5'), 4.12 (m, 1H, H-4'), 4.05 (s, 3H, 6-*O*CH_3 base), 3.57 (t, *J* = 10.5 Hz, 2H, 2 x NH<u>CH</u>(CH_2CH(CH_3)$_2$)CO), 1.66 (m, 2H, 2 x NHCH(CH_2<u>CH</u>(CH_3)$_2$)CO), 1.53 (m, 2H, NHCH(<u>CH_2</u>CH(CH_3)$_2$)CO), 1.44 (m, 2H, NHCH(<u>CH_2</u>CH(CH_3)$_2$)CO), 0.97 (s, 3H, CH_3-2'), 0.87 (m, 12H, 2 x NHCH(CH_2CH(<u>CH_3</u>)$_2$)).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 174.61 (s, NHCH(CH_2CH(CH_3)$_2$)<u>C</u>O), 174.22 (s, NHCH(CH_2CH(CH_3)$_2$)<u>C</u>O), 161.61 (s, C-6 base), 159.62 (s, C-2 base), 152.86 (s, C-4 base), 137.94 (s, C-8 base), 135.45 (2s, 2 x C-1 <u>Ph</u>), 128.63 & 128.59 & 128.56 & 128.45 & 128.42 & 128.38 & 128.31 & 128.25 & 128.19 & 128.10 (m, 10C, 2 x C para, 4 x C meta, 4 x C ortho <u>Ph</u>), 115.72 (s, C-5 base), 91.84 (s, C-1'), 81.07 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 6.3 Hz, C-4'), 79.41 (s, C-2'), 74.40 (s, C-3'), 67.17 & 67.05 (2s, 2 x

COO<u>C</u>H$_2$Ph), 65.07 (d, $J^2_{P\text{-}O\text{-}C}$ = 5.0 Hz, C-5'), 53.84 (s, 6-*O*CH$_3$ guanine), 50.47 & 50.41 (2s, 2 x NH<u>C</u>H(CH$_2$CH(CH$_3$)$_2$)CO), 43.36 & 43.31 (2s, 2 NHCH(<u>C</u>H$_2$CH(CH$_3$)$_2$)CO), 24.51 & 24.49 (2s, 2 x NHCH(CH$_2$<u>C</u>H(CH$_3$)$_2$)CO), 22.71 & 22.62 & 22.53 & 22.42 (4s, 4 x NHCH(CH$_2$CH(<u>C</u>H$_3$)$_2$)CO), 20.32 (s, CH$_3$-2').

^{31}P NMR (202 MHz, CDCl$_3$) δ_P (ppm): 13.87 (s).

Synthesis of L-Phenylalanine-neopentyl ester β-2'-C-methyl-6-O-methyl guanosine phosphorodiamidate (32.a)



Prepared according to procedure E, using *β*-2'-C-Me-6-OMe guanosine (**11**) (0.18 g, 0.57 mmol) in anh. THF (3.1 mL), anh. NEt$_3$ (0.10 mL, 0.69 mmol, 1.2 eq), POCl$_3$ (0.07 mL, 0.69 mmol, 1.2 eq), *L*-Phenylalanine-neopentyl ester tosylate salt (**12.e**) (1.12 g, 2.87 mmol, 5.0 eq) in anh. DCM (4.4 mL), and anh. NEt$_3$ (0.80 mL, 5.73 mmol, 10 eq). After overnight stirring at RT and flash chromatography using MeOH (3%)/CHCl$_3$, the desired material was recovered (0.05 g, 0.06 mmol, 11%).

HPLC (MeOH/ H$_2$O) : Rt = 32.69 min

HPLC (ACN/ H$_2$O) : Rt = 24.91 min

MS (TOF ES$^+$): 826.39 (M + H$^+$), 848.50 (M + Na$^+$).

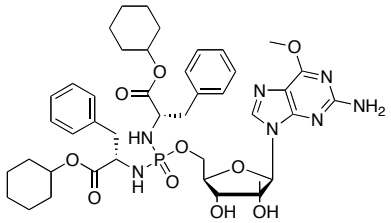
^{1}H NMR (500 MHz, CDCl$_3$) δ_H (ppm): 7.68 (s, 1H, H-8 base), 7.25-7.08 (m, 10H, 2 x NHCH(CH$_2$<u>Ph</u>)CO), 5.92 (s, 1H, H-1'), 5.49 (bs, 2H, <u>NH</u>CH(CH$_2$Ph)CO), 4.42 (m, 1H, H-3'), 4.35 (m, 1H, H-5'), 4.19 (m, 2H, H-4', NH<u>CH</u>(CH$_2$Ph)CO), 4.17-4.08 (m, 2H, H-5', NH<u>CH</u>(CH$_2$Ph)CO), 4.04 (s, 3H, 6-*O*CH$_3$ base), 3.79-3.70 (m, 4H, 2 x COO<u>CH$_2$</u>C(CH$_3$)$_3$), 2.98-2.84 (2m, 4H, 2 x NHCH(<u>CH$_2$</u>Ph)CO), 0.97 (s, 3H, CH$_3$-2'), 0.88 & 0.87 (2s, 18H2 x COOCH$_2$C(<u>CH$_3$</u>)$_3$).

^{13}C NMR (125 MHz, CDCl$_3$) δ_C (ppm): 173.50 (s, NHCH(CH$_2$Ph)<u>C</u>O), 173.03 (s, NHCH(CH$_2$Ph)<u>C</u>O), 161.60 (s, C-6 base), 159.58 (s, C-2 base), 152.96 (s, C-4 base), 137.90 (s, C-8 base), 136.16 & 136.03 (2s, 2 C-1 Ph), 129.48 & 129.42 & 129.39 & 129.35 (4s, 4 x C meta Ph), 128.55 & 128.53 & 128.33 &128.27 (4s, 4 x C ortho Ph), 127.05 & 127.01 (2s, 2 x C para Ph), 115.77 (s, C-5 base), 91.82 (s, C-1'), 81.09 (s, C-4'), 79.47 (s, C-2'), 76.83 (s, C-3'), 74.89 & 74.77 (2s, 2 x COO<u>C</u>H$_2$C(CH$_3$)$_3$), 65.20

(s, C-5'), 55.22 & 55.19 (2s, 2 x NH<u>C</u>H(CH_2Ph)CO), 53.80 (s, 6-*O*CH_3 base), 45.78 (d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 6.3 Hz, NHCH(<u>CH_2</u>Ph)CO), 40.40 (2, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 5.0 Hz, NHCH(<u>CH_2</u>Ph)CO), 31.21 & 31.15 (2s, 2 x $COOCH_2$<u>C</u>(CH_3)$_3$), 26.73 & 26.37 (2s, 2 x $COOCH_2C$(<u>C</u>H_3)$_3$), 20.36 (s, CH_3-2').

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm): 13.10 (s).

Synthesis of L-Phenylalanine-cyclohexyl ester β-2'-C-methyl-6-O-methyl guanosine phosphorodiamidate (32.b)



Prepared according to procedure E, using *β*-2'-C-Me-6-OMe guanosine (**11**) (0.31 g, 1.01 mmol) in anh. THF (5.43 mL), anh. NEt_3 (0.17 mL, 1.21 mmol, 1.2 eq), $POCl_3$ (0.11 mL, 1.21 mmol, 1.2 eq), *L*-Phenylalanine-cyclohexyl ester tosylate salt (**12.f**) (2.13 g, 5.05 mmol, 5.0 eq) was added and anh. DCM (4.0 mL), and anh. NEt_3 (1.41 mL, 10.1 mmol, 10 eq). After overnight stirring at RT and flash chromatography using MeOH (2%)/$CHCl_3$, the desired material was recovered (0.04 g, 0.05 mmol, 5%).

HPLC (MeOH/H_2O) : Rt =22.09 min

HPLC (ACN/H_2O) : Rt = 20.32 min

MS (TOF ES$^+$): 850.39 (M + H$^+$), 872.37 (M + Na$^+$).

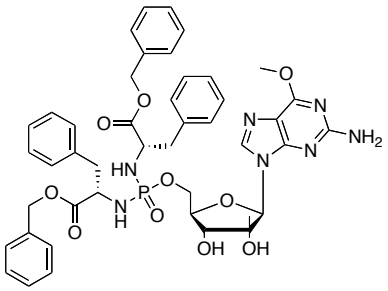
^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 7.65 (s, 1H, H-8 base), 7.28-7.11 (m, 10H, 2 x NHCH(CH_2<u>Ph</u>)CO), 5.91 (s, 1H, H-1'), 5.16 (bs, 2H, <u>NH</u>CH(CH_2Ph)CO), 4.48 (m, 1H, H-3'), 4.38 (m, 1H, H-5'), 4.13-4.12 (m, 2H, H-4', H-5'), 4.06 (s, 3H, 6-*O*CH_3 base), 4.02 (m, 2H, 2 x NH<u>CH</u>(CH_2Ph)CO), 3.59-345 (m, 2H, 2 x CH cHex), 2.98-2.85 (m, 4H, 2 x NHCH(<u>CH_2</u>Ph)CO), 1.80-1.67 (m, 8H, 2 x CH_2 ortho cHex, 2 x CH_2 meta cHex), 1.51 (m, 2H, 2 x CH_2 para cHex), 1.32-1.23 (m, 8H, 2 CH_2 x ortho cHex, 2 x CH_2 meta cHex), 0.98 (s, 3H, CH_3-2').

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm)**:** 172.76 (s, NHCH(CH_2Ph)<u>C</u>O), 172.28 (s, NHCH(CH_2Ph)<u>C</u>O), 161.58 (s, C-6 base), 159.65 (s, C-2 base), 152.96 (s, C-4 base), 137.81 (s, C-8 base), 136.23 & 136.14 (2s, 2 x C-1 Ph), 129.62 & 129.58 & 129.52 & 129.48 (2s, 4 C x meta Ph), 128.81 & 128.44 & 128.41 & 128.12 (4s, 4 x C ortho Ph), 126.97 & 126.92 (2s, 2 x C para Ph), 115.72 (s, C-5 base), 91.76 (s, C-1'), 81.06 (s, C-

4'), 79.46 (s, C-2'), 74.81 (s, C-3'), 74.21 & 74.06 (2s, 2 x C-1 cHex), 65.17 (s, C-5'), 55.30 & 55.26 (2s, 2 x NHCH(CH_2Ph)CO), 53.81 (s, 6-*O*CH_3 base), 40.40 & 40.34 (2s, 2 x NHCH(CH_2Ph)CO), 31.49 & 31.40 (2s, 4 x C ortho cHex), 25.22 (s, 2 x C para cHex), 23.67 (s, 4 x C meta cHex), 20.34 (s, CH_3-2').

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm)**:** 13.16 (s).

Synthesis of L-Phenylalanine-benzyl ester β-2'-C-methyl-6-O-methyl guanosine phosphorodiamidate (32.c)



Prepared according to procedure E, using *β*-2'-C-Me-6-OMe guanosine (**11**) (0.15 g, 0.47 mmol) in anh. THF (2.5 mL), anh. NEt_3 (0.08 mL, 0.56 mmol, 1.2 eq), $POCl_3$ (0.05 mL, 0.56 mmol, 1.2 eq), commercially available *L*-Phenylalanine-benzyl ester tosylate salt (1.00 g, 2.34 mmol, 5.0 eq) in anh. DCM (3.6 mL), anh. NEt_3 (0.65 mL, 4.68 mmol, 10 eq). After overnight stirring at RT and flash chromatography using MeOH (3%)/$CHCl_3$, the desired material was recovered (0.04 g, 0.04 mmol, 9%).

HPLC (MeOH/H_2O) : Rt = 25.97 min

HPLC (ACN/H_2O) : Rt = 21.16 min

MS (TOF ES$^+$): 866.33 (M + H$^+$), 888.35 (M + Na$^+$).

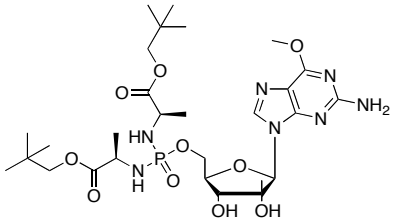
^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 7.66 (s, 1H, H-8 base), 7.33-7.26 (m, 10H, 6 x CH OCH_2Ph, 4 x CH NHCH(CH_2Ph)CO), 7.19-1.17 (m, 6H, 6 x CH NHCH(CH_2Ph)CO), 7.02-7.00 (m, 4H, 4 x CH OCH_2Ph), 5.91 (s, 1H, H-1'), 5,1 (bs, 2H, NHCH(CH_2Ph)CO), 5.14-5.02 (m, 4H, 2 x OCH_2Ph), 4.45 (m, 1H, H-3'), 4.34 (m, 1H, H-5'), 4.18-4.09 (m, 3H, 2 x NHCH(CH_2Ph)CO, H-4'), 4.05 (s, 3H, 6-*O*CH_3 base), 2.92-2.84 (m, 4H, 2 x NHCH(CH_2Ph)CO), 0.97 (s, 3H, CH_3-2').

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 173.18 (s, NHCH(CH_2Ph)CO), 172.69 (s, NHCH(CH_2Ph)CO), 161.62 (s, C-6 base), 159.57 (s, C-2 base), 152.90 (s, C-4 base), 137.94 (s, C-8 base), 135.94 & 135.87 (2s, 2 x C-1 NHCH(CH_2Ph)CO), 135.13 & 135.08 (2s, 2 x C-1 OCH_2Ph), 120.53 & 129.50 & 129.46 & 129.42 (4s, 4 x C meta NHCH(CH_2Ph)CO, 4 x C meta OCH_2Ph), 128.59 & 128.52 & 128.49 & 128.45 (4s, 4

x C ortho, 2 x C para $OCH_2\underline{Ph}$, 4 x C ortho $NHCH(CH_2\underline{Ph})CO$), 127.04 & 127.02 (2s, 2 x C para $NHCH(CH_2\underline{Ph})CO$), 115.86 (s, C-5 base), 91.82 (s, C-1'), 81.04 (s, C-4'), 79.51 (s, C-2'), 74.94 (s, C-3'), 67.33 & 67.19 (2s, 2 x $O\underline{C}H_2Ph$), 65.16 (s, C-5'), 55.30 & 55.24 (2s, 2 x $NH\underline{C}H(CH_2Ph)CO$), 53.44 (s, 6-*O*$CH_3$ base), 40.15 (s, 2 x $NHCH(\underline{C}H_2Ph)CO$), 20.37 (s, CH_3-2').

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm): 13.20 (s).

Synthesis of D-Alanine-neopentyl ester β-2'-C-methyl-6-O-methyl guanosine phosphorodiamidate (34.a)



Prepared according to procedure E, using *β*-2'-C-Me-6-OMe guanosine (**11**) (0.66 g, 2.13 mmol) in anh. THF (11 mL), anh. NEt_3 (0.35 mL, 2.56 mmol, 1.2 eq), $POCl_3$ (0.24 mL, 2.56 mmol, 1.2 eq), *D*-Alanine-neopentyl tosylate salt (provided by M. Aljarah) (3.53 g, 10.65 mmol, 5.0 eq) in anh. DCM (8.0 mL), and anh. NEt_3 (2.95 mL, 21.3 mmol, 10 eq). After overnight stirring at RT, flash chromatography using MeOH (3%)/$CHCl_3$, and work-up with 0.5N HCl in H_2O, the desired material was recovered (0.25 g, 0.38 mmol, 18%).

HPLC (MeOH/H_2O) : Rt = 20.16 min

HPLC (ACN/H_2O) : Rt = 18.33 min

MS (TOF ES^+): 674.33 ($M + H^+$), 696.31 ($M + Na^+$).

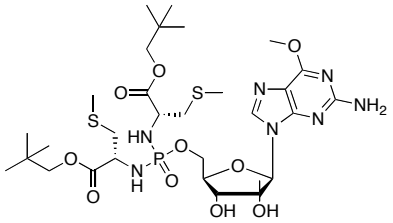
^1H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 7.90 (s, 1H, H-8 base), 6.01 (s, 1H, H-1'), 5.41 (bs, 2H, $\underline{NH}CH(CH_3)CO$), 4.55-4.50 (m, 1H, H-3'), 4.32-4.29 (m, 2H, H-4', H-5'), 4.18 (m, 1H, H-5'), 4.07 (s, 3H, 6-*O*CH_3 guanine), 4.03-3.88 (m, 4H, 2 x $O\underline{CH_2}C(CH_3)_3$), 3.63-3.50 (m, 2H, 2 x $NH\underline{CH}(CH_3)CO$), 1.42 (d, 6H, *J* = 7.9 Hz, 2 x $NHCH(\underline{CH_3})CO$), 0.99 (s, 3H, CH_3-2'), 0.90 & 0.85 (2s, 18H, 2 x $OCH_2C(\underline{CH_3})_3$).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm)**:** 172.76 (s, $NHCH(CH_3)\underline{C}O$), 172.28 (s, $NHCH(CH_3)\underline{C}O$), 161.56 (s, C-6 base), 159.65 (s, C-2 base), 152.96 (s, C-4 base), 137.89 (s, C-8 base), 115.72 (s, C-5 base), 91.18 (s, C-1'), 81.92 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 6.3 Hz, C-4'), 79.45 (s, C-2'), 74.90 & 74.69 (2s, 2 x $O\underline{C}H_2C(CH_3)_3$), 73.41 (s, C-3'), 65.84 (s, C-5'), 53.85 (s, 6-*O*CH_3 base), 49.88 & 49.58 (2s, 2 x $NH\underline{C}H(CH_3)CO$), 31.37 (s, 2 x

NHCH<u>C</u>(CH)$_3$CO), 26.25 & 26.19 (2s, 2 x OCH$_2$C(<u>C</u>H$_3$)$_3$), 21.29 (d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 6.3 Hz, NHCH(<u>C</u>H$_3$)CO), 21.07 (d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 6.3 Hz, NHCH(<u>C</u>H$_3$)CO), 20.42 (s, CH$_3$-2').

^{31}P NMR (202 MHz, CDCl$_3$) δ_P (ppm)**:** 13.61 (s).

Synthesis of S-methyl-L-Cysteine-neopentyl ester β-2'-C-methyl-6-O-methyl guanosine phosphorodiamidate (35.a)



Prepared according to procedure E, using *β*-2'-C-Me-6-OMe guanosine (**11**) (0.33 g, 1.05 mmol) in anh. THF (5.61 mL), anh. NEt$_3$ (0.18 mL, 1.26 mmol, 1.2 eq), POCl$_3$ (0.11 mL, 1.26 mmol, 1.2 eq), *S*-methyl-*L*-Cysteine-neopentyl tosylate salt (**12.g**) (1.98 g, 5.25 mmol, 5.0 eq) in anh. DCM (6.0 mL), and anh. NEt$_3$ (1.44 mL, 10.5 mmol, 10 eq). After overnight stirring at RT, flash chromatography using MeOH (3%)/CHCl$_3$, and preparative chromatography, the desired material was recovered (0.097 g, 0.13 mmol, 12%).

HPLC (MeOH/H$_2$O) : Rt = 28.80 min

HPLC (ACN/H$_2$O) : Rt = 19.89 min

MS (TOF ES$^+$): 788.28 (M + Na$^+$).

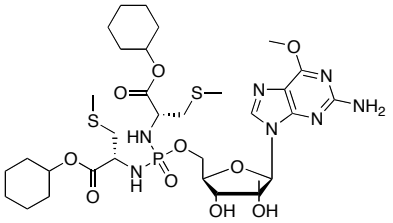
^{1}H NMR (500 MHz, MeOD) δ_H (ppm): 7.98 (s, 1H, H-8 base), 5.98 (s, 1H, H-1'), 4.47-4.38 (m, 2H, H-5'), 4.38 (d, 1H, *J* = 9.0 Hz, H-3'), 4.21-4.14 (m, 2H, 2 x NH<u>CH</u>(CH$_2$SCH$_3$)CO), 4.07 (s, 3H, 6-*O*CH$_3$ base), 3.86-3.73 (m, 5H, H-4', 2 x COO<u>CH$_2$</u>C(CH$_3$)$_3$), 2.89-2.83 (m, 4H, 2 x NHCH(<u>CH$_2$</u>SCH$_3$)CO), 2.13 & 2.09 (2s, 6H, 2 x NHCH(CH$_2$S<u>CH$_3$</u>)CO), 0.99 (s, 3H, CH$_3$-2'), 0.96 & 0.94 (2s, 18H, 2 x COOCH$_2$C(<u>CH$_3$</u>)$_3$).

^{13}C NMR (125 MHz, MeOD) δ_C (ppm)**:** 173.72 (s, NHCH(CH$_2$SCH$_3$)<u>C</u>O), 173.68 (d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 6.3 Hz, NHCH(CH$_2$SCH$_3$)<u>C</u>O), 162.76 (s, C-6 base), 161.92 (s, C-2 base), 154.59 (s, C-4 base), 139.48 (s, C-8 base), 115.60 (s, C-5 base), 93.25 (s, C-1'), 82.43 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 7.6 Hz, C-4'), 79.99 (s, C-2'), 75.76 & 74.96 (2s, 2 x COO<u>C</u>H$_2$C(CH$_3$)$_3$), 74.91 (s, C-3'), 66.88 (s, C-5'), 55.32 & 55.00 (2s, 2 x NH<u>C</u>H(CH$_2$SCH$_3$)CO), 54.21 (s, 6-*O*CH$_3$ base), 39.43 & 39.35 (2s, 2 x CH$_2$ NHCH(<u>C</u>H$_2$SCH$_3$)CO), 32.25 & 32.20 (2s, 2

x $COOCH_2\underline{C}(CH_3)_3$), 26.84 & 26.57 (2s, 6 x $COOCH_2C(\underline{C}H_3)_3$), 20.26 (s, CH_3-2'), 16.15 & 16.11 (2s, 2 x $NHCH(CH_2S\underline{C}H_3)CO$).

^{31}P NMR (202 MHz, MeOD) δ_P (ppm)**:** 13.36 (s).

Synthesis of S-methyl-L-Cysteine-cyclohexyl ester β-2'-C-methyl-6-O-methyl guanosine phosphorodiamidate (35.b)



Prepared according to procedure E, using *β*-2'-C-Me-6-OMe guanosine (**11**) (0.55 g, 1.77 mmol) in anh. THF (9.50 mL), anh. NEt_3 (0.30 mL, 2.12 mmol, 1.2 eq), $POCl_3$ (0.20 mL, 2.12 mmol, 1.2 eq), *S*-methyl-*L*-Cysteine-cyclohexyl ester tosylate salt (**12.h**) (3.44 g, 8.85 mmol, 5.0 eq) in anh. DCM (10 mL), and anh. NEt_3 (2.43 mL, 17.7 mmol, 10 eq). After overnight stirring at RT, flash chromatography using MeOH (3%)/$CHCl_3$, and preparative chromatography, the desired material was recovered (0.11 g, 0.14 mmol, 8%).

HPLC ($MeOH/H_2O$) : Rt = 29.15 min

HPLC (ACN/H_2O) : Rt = 20.10 min

MS (TOF ES^+): 790.30 ($M + H^+$), 812.29 ($M + Na^+$), 828.27 ($M + K^+$).

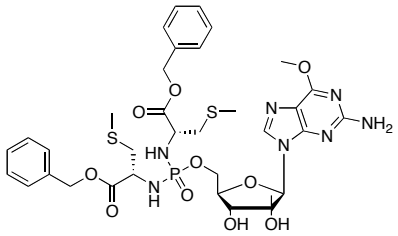
^1H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 7.78 (s, 1H, H-8 base), 5.96 (s, 1H, H-1'), 5.52 (bs, 2H, 2 x $\underline{NH}CH(CH_2SCH_3)CO$), 4.78-4.73 (m, 2H, 2 x CH cHex), 4.57-4.54 (m, 2H, H-5'), 4.43 (m, H-3'), 4.33-4.11 (m, 3H, 2 x $NH\underline{CH}(CH_2SCH_3)CO$, H-4'), 4.03 (s, 3H, 6-*O*CH_3 base), 2.86-2.06 (m, 4H, 2 x $NHCH(\underline{CH_2}SCH_3)CO$), 2.10 & 2.06 (2s, 6H, 2 x $NHCH(CH_2S\underline{CH_3})CO$), 1.79 (m, 4H, 2 x CH_2 ortho cHex), 1.67 (m, 4H, 2 x CH_2 meta cHex), 1.49 (m, 2H, CH_2 para cHex), 1.40-1.19 (m, 10H, 2 x CH_2 ortho cHex , 2 x CH_2 meta cHex, CH_2 para cHex), 0.97 (s, 3H, CH_3-2').

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm)**:** 171.77 (d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 5.0 Hz, $NHCH(CH_2SCH_3)\underline{C}O$), 171.60 (d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 5.0 Hz, $NHCH(CH_2SCH_3)\underline{C}O$), 161.56 (s, C-6 base), 159.62 (s, C-2 base), 152.90 (s, C-4 base), 137.90 (s, C-8 base), 115.51 (s, C-5 base), 91.68 (s, C-1'), 80.98 (s, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 6.3 Hz, C-4'), 79.33 (s, C-2'), 74.54 (s, C-3'), 74.48 & 74.23 (2s, 2 x C ipso cHex), 65.19 (d, $J^2_{P\text{-}O\text{-}C}$ = 5.04 Hz, C-5'), 55.86 & 53.80 (2s, 2 x $NH\underline{C}H(CH_2SCH_3)CO$), 53.73 (s, 6-*O*$CH_3$ base), 38.86 (d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 5.0

Hz, NHCH(CH2SCH$_3$)CO), 38.78 (s, NHCH(CH2SCH$_3$)CO), 31.41 & 31.37 (2s, 4 x CH_2 ortho cHex), 25.19 (s, 2 x CH_2 para cHex), 23.59 & 23.53 (2s, 4 x CH_2 meta cHex), 20.25 (s, CH_3-2'), 16.17 & 15.98 (2s, 2 x NHCH(CH_2SCH_3)CO).

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm)**:** 13.31 (s).

Synthesis of S-methyl-L-Cysteine-benzyl ester β-2'-C-methyl-6-O-methyl guanosine phosphorodiamidate (35.c)



Prepared according to procedure E, using *β*-2'-C-Me-6-OMe guanosine (**11**) (0.31 g, 1.00 mmol, 1.0 eq) in anh. THF (5.35 mL), anh. NEt_3 (0.17 mL, 1.20 mmol, 1.2 eq), $POCl_3$ (0.11 mL, 0.11 mmol, 1.2 eq), *S*-methyl-*L*-Cysteine-benzyl ester tosylate salt (**12.i**) (1.99 g, 5.00 mmol, 5.0 eq) in anh. DCM (8.0 mL), and anh. NEt_3 (1.39 mL, 10.0 mmol, 10 eq). After overnight stirring at RT, flash chromatography using MeOH (3%)/$CHCl_3$, and preparative chromatography, the desired material was recovered (0.053g, 0.07 mmol, 6.6 %).

HPLC (MeOH/H_2O) : Rt = 27.18 min

HPLC (ACN/H_2O) : Rt = 17.86 min

MS (TOF ES$^+$): 806.24 (M + H$^+$), 828.22 (M + Na$^+$), 844.20 (M + K$^+$).

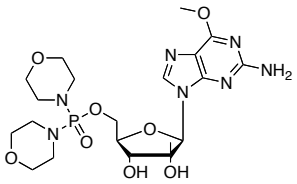
^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 7.74 (s, 1H, H-8 base), 7.34-7.30 (m, 10H, 2 x COOCH$_2$Ph), 5.91 (s, 1H, H-1'), 5.37 (bs, 2H, 2 x NHCH(CH_2SCH_3)CO), 5.20-5.10 (m, 4H, 2 x COOCH$_2$Ph), 4.59-4.52 (m, 2H, H5', H3'), 4.25-4.21 (m, 2H, 2 x NHCH(CH_2SCH_3)CO), 4.16-4.11 (m, 2H, H5', H4'), 4.05 (s, 3H, 6-*O*CH_3 base), 2.87-2.83 (m, 4H, 2 x NHCH(CH$_2$SCH$_3$)CO), 2.03 (s, 3H, NHCH(CH_2SCH$_3$)CO), 2.01 (s, 3H, NHCH(CH_2SCH$_3$)CO), 0.98 (s, 3H, CH_3-2').

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm)**:** 172.27 (s, NHCH(CH_2SCH_3)CO), 172.07 (s, NHCH(CH_2SCH_3)CO), 161.62 (s, C-6 base), 159.51 (s, C-2 base), 152.83 (s, C-4 base), 138.01 (s, C-8 base), 135.02 (s, 2 x C ipso Ph), 128.62 & 128.56 & 128.46 & 128.44 (4s, 4 x C meta Ph, 4 x CH ortho Ph, 2 x C para Ph), 115.57 (s, C-5 base), 91.77 (s, C-1'), 80.96 (s, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 6.3 Hz, C-4'), 79.43 (s, C-2'), 74.42 (s, C-3'), 67.60 & 67.55 (2s, 2 x OCH$_2$Ph), 65.06 (s, C-5'), 55.87 & 53.79 & 53.62 (3s, 2 x

NHCH(CH_2SCH_3)CO, 6-*O*CH_3 base), 38.68 & 38.63 (2s, 2 x NHCH(CH_2SCH_3)CO), 20.35 (s, CH_3-2'), 16.03 & 16.00 (s, 2 x NHCH(CH_2SCH_3)CO).

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm)**:** 13.34 (s).

Synthesis of morpholino β-2'-C-methyl-6-O-methyl guanosine phosphorodiamidate (36)



Prepared according to procedure E, using *β*-2'-C-Me-6-OMe guanosine (**11**) (0.91 g, 2.92 mmol) in anh. THF (14 mL), anh. NEt_3 (0.49 mL, 3.50 mmol, 1.2 eq), $POCl_3$ (0.33 mL, 3.50 mmol, 1.2 eq), commercially available morpholine (1.26 mL, 14.6 mmol, 5.0 eq) in anh. DCM (10 mL), and anh. NEt_3 (4.10 mL, 29.2 mmol, 10 eq). After overnight stirring at RT, flash chromatography using MeOH (6%)/$CHCl_3$, and preparative chromatography, the desired material was recovered (0.034 g, 0.063 mmol, 3%).

HPLC (MeOH/H_2O): Rt = 13.48 min

HPLC (ACN/H_2O): Rt = 12.47 min

MS (TOF ES^+): 530.21 ($M + H^+$).

^{1}H NMR (500 MHz, $CDCl_3$): 7.75 (s, 1H, H-8 base), 6.00 (s, 1H, H-1'), 5.51-4.48 (m, 1H, H-5'), 4.41-4.35 (m, 2H, H-5', H-3'), 4.28-4.26 (m, 1H, H-4'), 4.07 (s, 3H, 6-*O*CH_3 base), 3.66-3.62 (m, 8H, 4 x O(CH_2CH_2)$_2$N), 3.19-3.12 (m, 8H, 4 x O(CH_2CH_2)$_2$N), 1.03 (s, 3H, CH_3-2').

^{13}C NMR (125 MHz, $CDCl_3$): 160.84 (s, C-6 base), 159.56 (s, C-2 base), 152.59 (s, C-4 base), 137.35 (s, C-8 base), 114.40 (s, C-5 base), 91.33 (s, C-1'), 80.62 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 8.8 Hz, C-4'), 78.51 (s, C-2'), 77.98 (s, C-3'), 67.74 & 66.45 & 66.41 & 66.37 (4s, 4 x O(CH_2CH_2)$_2$N), 64.63 (d, $J^2_{P\text{-}O\text{-}C}$ = 3.75 Hz, C-5'), 53.96 (s, 6-*O*CH_3 base), 44.87 & 44.72 & 44.69 & 44.56 (4s, 4 x O(CH_2CH_2)$_2$N), 19.50 (s, CH_3-2').

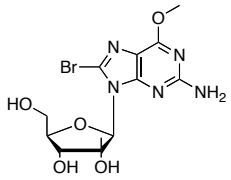
^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm): 14.85 (s).

4. Experimental procedures related to chapter 4

4.1. 8-modified nucleosides and phosphoramidates

4.1.1. 8-modified nucleosides

Synthesis of 8-bromo β-2'-C-methyl-6-O-methyl guanosine (46)

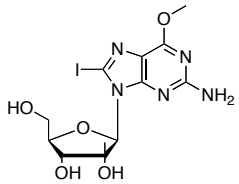


To *β*-2'-C-Me-6-OMe guanosine (**11**) (2.52 g, 8.11 mmol, 1.0 eq) dissolved in anh. MeOH (125 m) was added *N*-bromo succinimide (2.43 g, 12.17 mmol, 1.5 eq), the solution was left stirring for 1 hr at RT. After purification by flash chromatography using MeOH (4%)/CH_2Cl_2, the desired material was recovered (3.05 g, 7.82 mmol, 96%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 6.16 (s, 1H, H-1'), 5.10 (bs, 2H, NH_2 base), 4.66 (dd, 1H, *J* = 6.2 Hz, 8.6 Hz, H-4'), 4.37 (d, 1H, *J* = 6.2 Hz, H-3'), 4.24 (d, 1H, *J* =1.5 Hz, H-5'), 4.22 (d, 1H, *J* =1.5 Hz, H-5'), 4.06 (s, 3H, 6-*O*CH_3 base), 1.04 (s, 3H, CH_3-2').

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 161.00 (s, C-6 base), 158.78 (s, C-2 base), 152.71 (s, C-4 base), 124.16 (s, C-8 base), 116.40 (s, C-5 base), 95.15 (s, C-1'), 82.85 (s, C-4'), 79.64 (s, C-2'), 71.91 (s, C-3'), 60.38 (s. C-5'), 54.23 (s, 6-*O*CH_3 base), 21.53 (s, CH_3-2').

Synthesis of 8-iodo β-2'-C-methyl-6-O-methyl guanosine (47)

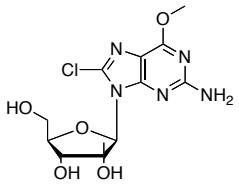


To *β*-2'-C-Me-6-OMe guanosine (**11**) (1.51 g, 4.82 mmol, 1.0 eq) dissolved in anh. THF (57 mL) was added *N*-iodo succinamide (2.90 g, 12.62 mmol, 2.6 eq), and the solution was left stirring for 72 hrs at 35 °C in the dark. After purification by flash chromatography using MeOH (2%)/CH_2Cl_2, the desired material was recovered (0.53 g, 1.21 mmol, 25%).

^{1}H NMR (500 MHz, MeOD) δ_H (ppm): 6.01 (s, 1H, H-1'), 4.62 (m, 1H, H-4'), 4.16 (d, 1H, *J* = 6.0 Hz, H-3'), 4.09 (s, 3H, 6-*O*CH_3 base), 4.08-3.96 (m, 2H, H-5'), 1.05 (s, 3H, CH_3-2').

^{13}C NMR (125 MHz, MeOD) δ_C (ppm): 162.70 (s, C-6 base), 161.20 (s, C-2 base), 154.15 (s, C-4 base), 119.29 (s, C-5 base), 99.22 (s, C-8 base), 98.71 (s, C-1'), 84.32 (s, C-3'), 79.52 (s, C-2'), 74.52 (s, C-4'), 62.30 (s, C5'), 54.93 (s, 6-*O*CH_3 base), 21.49 (s, CH_3-2').

Synthesis of 8-chloro β-2'-C-methyl-6-O-methyl guanosine (48)

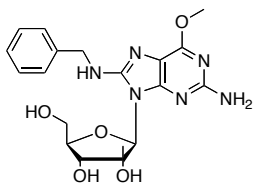


To *β*-2'-C-Me-6-OMe guanosine (**11**) (1.51 g, 4.82 mmol, 1.0 eq) dissolved in anh. THF (56 mL) was added *N*-chloro succinamide (0.78 g, 5.83 mmol, 1.21 eq), and the solution was left stirring for 64 hrs at RT in the dark. After purification by flash chromatography using MeOH (4%)/CH_2Cl_2, the desired material was recovered (1.10 g, 3.19 mmol, 66%).

^{1}H NMR (500 MHz, MeOD) δ_H (ppm): 6.01 (s, 1H, H-1'), 4.58 (d, 1H, *J* = 6.2 Hz, H3'), 4.11-4.06 (m, 2H, H4', H5'), 4.07 (s, 3H, 6-*O*CH_3 base), 3.97-3.92 (m, 1H, H-5'), 1.03 (s, 3H, CH_3-2').

^{13}C NMR (125 MHz, MeOD) δ_C (ppm): 162.03 (s, C-6 base), 161.22 (s, C-2 base), 154.28 (s, C-4 base), 136.59 (s, C-8 base), 114.59 (s, C-5 base), 95.60 (s, C-1'), 84.41 (s, C-4'), 80.37 (s, C-2'), 74.33 (s, C-3'), 62.27 (s. C-5'), 54.68 (s, 6-*O*CH_3 base), 21.13 (s, CH_3-2').

Synthesis of 8-benzylamino β-2'-C-methyl-6-O-methyl guanosine (49)



To a solution of 8-bromo-6-O-methyl-2'-C-methyl guanosine (**46**) (0.51 g, 1.30 mmol, 1.0 eq) dissolved in anh. MeOH (3 mL), was added benzylamine (0.18 mL, 1.69 mmol, 1.3 eq). The mixture was stirred overnight at 115 °C in a sealed vessel. The solvent was

evaporated under reduced pressure and the residue was purified by flash chromatography using MeOH (6%)/$CHCl_3$ and preparative chromatography to recover the desired material (0.04 mg, 0.09 mmol, 6.6%).

HPLC (MeOH/H_2O): Rt = 18.52 min

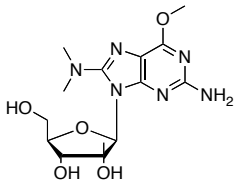
HPLC (ACN/H_2O): Rt = 10.04 min

MS (TOF ES^+): 417.19 ($M + H^+$), 739.17 ($M + Na^+$), 480.19 ($M + MeCNNa^+$).

^1H NMR (500 MHz, MeOD) δ_H (ppm): 7,36-7.22 (m, 5H, Ph), 6.09 (s, 1H, H-1'), 4.63 & 4.44 (AB system, 2H, *J* = 15.1 Hz, $\underline{CH_2}$Ph), 4.21 (m, 1H, H-3'), 4.01 (s, 3H, 6-*O*CH_3 base), 3.99 (m, 1H, 1H H-5'), 3.93 (m, 1H, H-4'), 3.84 (m, 1H, H-5'), 0.94 (s, 3H, CH_3-2').

^{13}C NMR (125 MHz, MeOD) δ_C (ppm): 159.48 (s, C-6 base), 159.34 (s, C-2 base), 152.71 (s, C-8 base), 148.65 (s, C-4 base), 140.84 (s, C ipso Ph), 128.78 & 128.64 & 128.24 & 128.17 & 128.01 & 127.60 (6s, 2 x C Ph, C-5 base), 93.69 (s, C-1'), 82.88 (s, C-4'), 79.85 (s, C-2'), 73.94 (s, C-3'), 53.89 (s, 6-*O*CH_3 base), 46.89 (s, $\underline{C}H_2$Ph), 20.99 (s, CH_3-2').

Synthesis of 8-dimethylamino β-2'-C-methyl-6-O-methyl guanosine (50)



8-bromo-6-O-methyl-2'-C-methyl guanosine (**46**) (0.63 g, 1.62 mmol) was dissolved in anh. DMF (3.30 mL), and dimethylamine (0.97 mL, 1.94 mmol, 1.2 eq) was added. Then anh. NEt_3 (0.59 mL, 4.21 mmol, 2.6 eq) was added and the solution was stirred over 72 hrs in a sealed tube at 115 °C. The solvent was evaporated. The residue was purified by flash chromatography using $CHCl_3$/MeOH (5 %) as eluents, and preparative chromatography (EtOAc) allowed the recovery of the desired material (0.29 g, 0.83 mmol, 51%).

HPLC (MeOH/H_2O) : Rt = 12.20 min

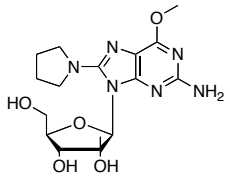
HPLC (ACN/H_2O) : Rt = 3.25 min

MS (TOF ES^+): 355.17 ($M + H^+$).

^1H NMR (500 MHz, MeOD) δ_H (ppm): 5.91 (s, 1H, H-1'), 4.74 (d, 1H, *J* = 8.7 Hz, H-3'), 4.04 (s, 3H, 6-*O*CH_3 base), 4.02-3.99 (m, 2H, H-5' & H-4'), 3.94-3.91 (m, 1H, H-5'), 2.89 (2s, 6H, N($\underline{CH_3}$)$_{\underline{2}}$), 1.12 (s, 3H, CH_3-2').

^{13}C NMR (125 MHz, MeOD) δ_C (ppm)**:** 161.18 (s, C-6 base), 160.25 (s, C-2 base), 157.04 (s, C-4 base), 154.51 (s, C-8 base), 112.94 (s, C-5 base), 96.65 (s, C-1'), 83.75 (s, C-4'), 80.23 (s, C-2'), 74.87 (s, C-3'), 62.36 (s, C-5'), 54.26 (s, 6-*O*CH_3 base), 43.61 (s, N($\underline{CH_3}$)$_2$), 21.58 (s, CH_3-2').

Synthesis of 8-pyrrolidino β-2'-C-methyl-6-O-methyl guanosine (51)



8-bromo-6-O-methyl-2'-C-methyl guanosine (**46**) (0.68 g, 1.75 mmol) was dissolved in anh. DMF (15 mL), and pyrrolidine (0.17 mL, 2.10 mmol, 1.2 eq) was added. Then anh. NEt_3 (0.64 mL, 4.55 mmol, 2.6 eq) was added, and the solution was stirred over the weekend in a sealed tube at 155 °C. The solvent was evaporated. The residue was purified by flash chromatography using $CHCl_3$/MeOH (5 %) as eluent, and a preparative chromatography (EtOAc) allowed the recovery of the desired material (0.06 g, 0.16 mmol, 9%).

HPLC (MeOH/H_2O) : Rt = 4.60 min

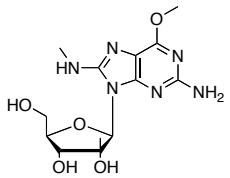
HPLC (ACN/H_2O) : Rt = 2.61 min

MS (TOF ES$^+$): 381.19 (M + H$^+$).

^{1}H NMR (500 MHz, MeOD) δ_H (ppm): 5.98 (s, 1H, H-1'), 4.71 (d, 1H, *J* = 8.8 Hz, H-3'), 4.02 (s, 3H, 6-*O*CH_3 base), 3.98-3.96 (m, 2H, H-5', H4'), 3.91-3.90 (m, 1H, H-5'), 3.58-3.55 (m, 2H, N($\underline{CH_2}CH_2$)$_2$), 3.43-3.39 (m, 2H, N($\underline{CH_2}CH_2$)$_2$), 2.08-1.98 (m, 4H, N($CH_2\underline{CH_2}$)$_2$), 1.15 (s, 3H, CH_3-2').

^{13}C NMR (125 MHz, MeOD) δ_C (ppm)**:** 161.17 (s, C-6 base), 159.70 (s, C-2 base), 157.01 (s, C-4 base), 154.65 (s, C-8 base), 113.28 (s, C-5 base), 94.47 (s, C-1'), 83.81 (s, C-4'), 80.22 (s, C-2'), 74.79 (s, C-3'), 62.72 (s, C-5'), 54.11 (s, 6-*O*CH_3 base), 53.15 & 53.09 (2s, N($\underline{CH_2}CH_2$)$_2$), 26.56 & 26.23 (2s, N($CH_2\underline{CH_2}$)$_2$), 21.71 (s, CH_3-2').

Synthesis of 8-methylamino β-2'-C-methyl-6-O-methyl guanosine (57)



To a solution of 8-chloro-6-O-methyl-2'-C-methyl guanosine (**48**) (0.44 g, 1.27 mmol, 1.0 eq) is anh. MeOH (12 mL), was added anh. methylamine (2.0M in sol. MeOH, 1.28 mL, 2.54 mmol, 2.0 eq). The reaction was left stirring in a sealed vessel for 72 hrs at 115 °C. The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography using MeOH (6%)/$CHCl_3$ to recovered the desired molecule (0.160 g, 0.47 mmol, 37%).

HPLC (MeOH/H_2O): Rt = 5.69 min

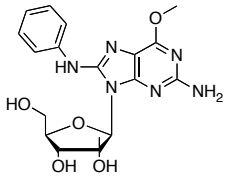
HPLC (ACN/H_2O): Rt = 4.23 min

MS (TOF ES^+): 341.16 ($M + H^+$).

^{1}H NMR (500 MHz, MeOD) δ_H (ppm): 6.06 (s, 1H, H-1'), 4.18 (d, 1H, *J* = 8.9 Hz, H-3'), 4.03 (m, 1H, H-5'), 4.02 (s, 3H, 6-*O*CH_3 base), 3.92-3.86 (m, 2H, H-4', H-5'), 2.89 (s, 3H, NH$\underline{CH_3}$), 0.96 (s, 3H, CH_3-2').

^{13}C NMR (125 MHz, MeOD) δ_C (ppm): 159.48 (s, C-6 base), 159.31 (s, C-2 base), 153.31 (s, C-4 base), 153.72 (s, C-8 base), 112.29 (s, C-5 base), 93.58 (s, C-1'), 82.85 (s, C-4'), 79.85.37 (s, C-2'), 73.88 (s, C-3'), 59.37 (s, C-5'), 53.88 (s, 6-*O*CH_3 base), 29.29 (s, NH$\underline{C}H_3$), 20.95 (s, CH_3-2').

Synthesis of 8-phenylamino β-2'-C-methyl-6-O-methyl guanosine (58)



8-chloro-6-O-methyl-2'-C-methyl guanosine (**48**) (0.52 g, 1.50 mmol) was dissolved in anh. MeOH (15 mL), and aniline (0.27 mL, 3.00 mmol, 2.0 eq) was added. The solution was stirred over the weekend in a sealed tube at 115 °C. The solvent was evaporated and the residue was purified by flash chromatography using $CHCl_3$/MeOH (4.5 %) as eluents, and preparative chromatography allowed the recovery of the desired material (0.19 g, 0.47 mmol, 32%).

HPLC (MeOH/H_2O) : Rt = 18.04 min

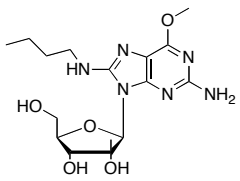
HPLC (ACN/H_2O) : Rt = 13.93 min

MS (TOF ES$^+$): 403.17 (M + H$^+$).

^{1}H NMR (500 MHz, MeOD) δ_H (ppm): 7.50 (m, 2H, 2 x CH ortho Ph), 7.28 (m, 2H, 2 x CH meta Ph), 6.97 (m, 1H, CH para Ph), 6.22 (s, 1H, H-1'), 4.21 (m, 1H, H-3'), 4.11-4.08 (dd, 1H, *J* = 2.0 Hz, 11.9 Hz, H-5'), 4.02 (s, 3H, 6-*O*CH$_3$ base), 3.97-3.91 (m, 2H, H-5', H-4'), 1.03 (s, 3H, CH$_3$-2').

^{13}C NMR (125 MHz, MeOD) δ_C (ppm)**:** 160.33 (s, C-6 base), 160.27 (s, C-2 base), 154.83 (s, C-4 base), 153.12 (s, C-8 base), 141.37 (s, C ipso Ph), 130.13 & 129.92 (2s, 2 x C meta Ph), 123.54 (s, C para Ph), 120.67 &120.37 (2s, 2 x C ortho Ph), 111.99 (s, C-5 base), 93.49 (s, C-1'), 82.69 (s, C-4'), 79.68 (s, C-2'), 74.04 (s, C-3'), 59.49 (s, C-5'), 54.23 (s, 6-*O*CH$_3$ base), 19.57 (s, CH$_3$-2').

Synthesis of 8-butylamino β-2'-C-methyl-6-O-methyl guanosine (59)



8-chloro-6-O-methyl-2'-C-methyl guanosine (**48**) (0.17 g, 0.49 mmol) was dissolved in anh. MeOH (5.0 mL) and *N*-butylamine (0.97 mL, 0.98 mmol, 2.0 eq) was added. The solution was stirred over the weekend in a sealed tube at 115 °C. The solvent was evaporated and the residue was purified by flash chromatography using CHCl$_3$/MeOH (3-4%) and preparative chromatography was performed to recover the desired material (0.050 g, 0.13 mmol, 27%).

HPLC (MeOH/H$_2$O) : Rt = 8.53 min

HPLC (ACN/H$_2$O) : Rt = 12.45 min

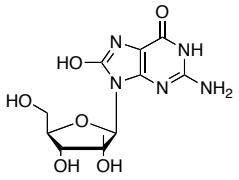
MS (TOF ES$^+$): 383.41 (M + H$^+$).

^{1}H NMR (500 MHz, CDCl$_3$) δ_H (ppm): 6.08 (s, 1H, H-1'), 5.80 (bs, 1H, NHCH$_2$CH$_2$CH$_2$CH$_3$), 4.21 (d, 1H, *J* = 8.5 Hz, H-3'), 4.02 (s, 3H, 6-*O*CH$_3$ base), 3.94-3.88 (m, 1H, H-4'), 3.75-3.54 (m, 2H, H-5'), 3.33-3.32 (m, 2H, NHCH$_2$CH$_2$CH$_2$CH$_3$), 1.64-1.60 (m, 2H, NHCH$_2$CH$_2$CH$_2$CH$_3$), 1.45-1.40 (m, 2H, NHCH$_2$CH$_2$CH$_2$CH$_3$), 1.26 (s, 3H, CH$_3$-2'), 0.98 (t, 3H, *J* = 4.4 Hz, NHCH$_2$CH$_2$CH$_2$CH$_3$).

^{13}C NMR (125 MHz, CDCl$_3$) δ_C (ppm)**:** 159.41 (s, C-6 base), 159.23 (s, C-2 base), 154.55 (s, C-4 base), 154.09 (s, C-8 base), 110.78 (s, C-5 base), 93.57 (s, C-1'), 84.57 (s, C-4'), 79.77 (s, C-2'), 71.98 (s, C-3'), 60.35 (s, C-5'), 53.42 (s, 6-*O*CH$_3$ base), 40.10

(s, $NH\underline{CH_2}CH_2CH_2CH_3$), 31.55 (s, $NHCH_2\underline{CH_2}CH_2CH_3$), 21.13 (s, $NHCH_2CH_2\underline{CH_2}CH_3$), 19.98 (s, CH_3-2'), 13.73 (s, $NHCH_2CH_2CH_2\underline{CH_3}$).

Synthesis of 8-oxo β-2'-C-methyl guanosine (63)



8-bromo-6-O-methyl-2'-C-methyl guanosine (**46**) (0.45 g, 1.15 mmol, 1.0 eq) was suspended in anh. pyridine (14 mL) and sodium acetate (0.95 g, 11.6 mmol, 10 eq), and mixed with silver acetate (0.20 g, 1.17 mmol, 1.0 eq). A solution of 1-methylimidazole (0.035 mL, 0.44 mmol, 0.38 eq) in acetic anhydride (1.15 mL, 12.19 mmol, 10.6 eq) was added dropwise at 0 °C and refluxed overnight. The excess of acetic anhydride was evaporated under high reduced pressure, followed by hydrolysis with ammonium bicarbonate ($(NH_4)HCO_3$) at 0 °C. The solution was then evaporated to dryness and the residue was extracted with DCM. The organic layer was washed several times and after evaporation of the solvent, the residue was loaded on Sephadex G-10 for purification (ACN/H_2O). The residue was purified by preparative TLC to recover the desired material (0.016 g, 0.052 mmol, 5%)

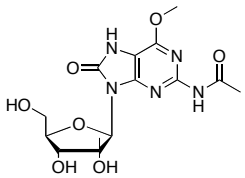
HPLC ($MeOH/H_2O$) : Rt = 2.31 min

MS (TOF ES^+): 336.27 ($M + Na^+$).

^1H NMR (500 MHz, DMSO) δ_H (ppm): 10.66 (bs, 1H, NH base), 6.47 (bs, 2H, NH_2 base), 5.69 (s, 1H, H-1'), 4.99 (m, 2H, 3'-OH. 8-OH base), 4.79 (s, 1H, 2'-OH), 4.55 (m, 5'-OH), 4.29 (t, 1H, J = 7.4 Hz, H-3'), 3.76 (m, 2H, H-5'), 3.73 (m, 1H, H-4'), 1.02 (s, 3H, CH_3-2').

^{13}C NMR (125 MHz, DMSO) δ_C (ppm)**:** 155.3 (s, C-6 base), 153.6 (s, C-2 base), 149.6 (s, C-8 base), 148.1 (s, C-4 base), 115.2 (s, C-5 base), 91.32 (s, C-1'), 84.33 (s, C-4'), 79.93 (s, C-2'), 75.09 (s, C-3'), 63.23 (s, C-5'), 20.67 (s, CH_3-2').

Synthesis of 8-oxo β-2'-C-methyl-6-O-methyl-N-2-acetate guanosine (64)



Prepared as **63**. The residue was purified by preparative TLC to recover the desired material (0.009 g, 0.025 mmol, 2%)

HPLC ($MeOH/H_2O$) : Rt = 2.43 min

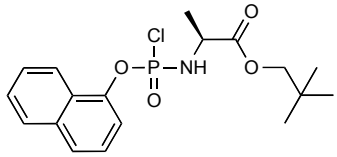
MS (TOF ES^+): 392.12 ($M + Na^+$).

^1H NMR (500 MHz, DMSO) δ_H (ppm): 11.43 (bs, 1H, NH-7 base), 10.28 (s, 1H, NHCOCH$_3$ base), 5.80 (s, 1H, H-1'), 4.92 (bs, 1H, 3'-OH), 4.87 (s, 1H, 2'-OH), 4.50 (bs, 1H, 5'-OH), 4.43 (m, 1H, H-3'), 3.99 (s, 3H, 6-*O*CH$_3$ base), 3.80-3.77 (m, 1H, H-4'), 3.71-3.61 (m, 2H, H-5'), 2.18 (s, 3H, NHCOCH$_3$ base), 1.01 (s, 3H, CH$_3$-2').

^{13}C NMR (125 MHz, DMSO) δ_C (ppm)**:** 166.6 (s, NHCOCH$_3$ base), 158.4 (s, C-6 base), 152.0 (s, C-2 base), 149.9 (s, C-8 base), 135.2 (s, C-4 base), 102.2 (s, C-5 base), 89.01 (s, C-1'), 83.38 (s, C-4'), 77.77 (s, C-2'), 73.54 (s, C-3'), 62.10 (s, C-5'), 55.87 (s, 6-*O*CH$_3$ base), 24.35 (s, NHCOCH$_3$ base), 20.16 (s, CH$_3$-2').

4.1.2. Preparation of phosphorochloridates

Preparation of α-naphthyl L-Alanine-neopentyl ester phosphochloridate (14.d)



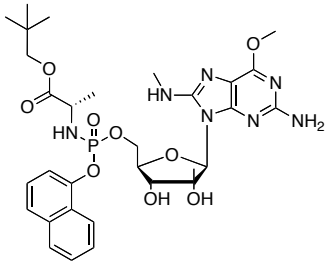
Prepared according to procedure C, using *L*-Alanine-neopentyl tosylate salt (provided by M. Aljarah) (0.66 g, 2.00 mmol, 1.0 eq) in anh. DCM (11 mL), α-naphthyl phosphorodichloridate (***12***) (0.57 g, 2.18 mmol, 1.1 eq), NEt_3 (0.57 mL, 4.00 mmol, 2.0 eq). The solvent was removed under reduced pressure and the residue purified by flash chromatography using Hexane/EtOAc (1:1) to yield the desired molecule (0.60 g, 1.55 mmol, 78%).

^1H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.08 (m, 1H, H-9 Ar), 7.82 (m, 1H, H-6 Ar), 7.70 (m, 1H, H-4 Ar), 7.61 (m, 1H, H-3 Ar), 7.50 (m, 2H, H-7, H-8 Ar), 7.39 (m, 1H, H-2 Ar), 4.11 (m, 1H, NHCH(CH$_3$)CO), 3.91 (m, 2H, COOCH$_2$C(CH$_3$)$_3$), 1.56 (d, 3H, *J* = 2.5 Hz, NHCH(CH$_3$)CO), 0.95 (2s, 9H, COOCH$_2$C(CH$_3$)$_3$).

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm): 8.41 (s), 8.25 (s).

4.1.3. Preparation of 8-modified ProTides

Synthesis of α-naphthyl L-Alanine-neopentyl ester 8-methylamino β-2'-C-methyl-6-O-methyl guanosine (68)



This ProTide was prepared according to the Standard Procedure D, using *t*BuMgCl (1.16 mL, 2.0 eq), 8-methylamino-2'-*C*-Me-6-OMe-guanosine (**57**) (0.198 g, 0.58 mmol) in anh. THF (1.70 mL), and *α*-naphthyl *L*-Alanine-neopentyl ester phosphochloridate (***14.d***) (0.472 g, 1.17 mmol, 2.0 eq) in anh. THF (1.1 mL). After overnight stirring at RT, the solvent was removed under reduced pressure, and the residue was purified by flash chromatography, using $CHCl_3$/MeOH (4%) as eluents, and preparative chromatography to yield the starting material (**57**) and the desired ProTide (0.011 g, 0.015 mmol, 3%).

HPLC (MeOH/H_2O): Rt = 28.29 min, 28.39 min

HPLC (ACN/ H_2O): Rt = 18.20 min

MS (TOF ES^+): 688.28 ($M + H^+$), 710.26 ($M + Na^+$).

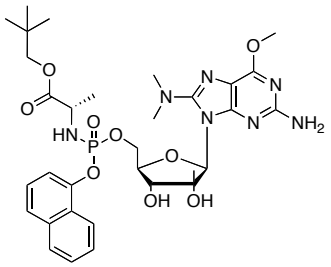
^1H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.13 (d, 2H, *J* = 7.5 Hz, H-9 Ar diastereoisomers), 7.86 (d, 2H, *J* = 7.0 Hz, H-6 Ar diastereoisomers), 7.68 (d, 2H, *J* = 6.7 Hz, H-4 Ar diastereoisomers), 7.55 (m, 6H, H-7, H-8, H-3 Ar diastereoisomers), 7.43-7.39 (m, 2H, H-2 Ar diastereoisomers), 5.88 & 5.85 (2s, 1H, H-1' diastereoisomers), 4.74-4.69 (m, 4H, $COO\underline{CH_2}C(CH_3)_3$ diastereoisomers), 4.64-4.62 (m, 2H, H-3' diastereoisomers), 4.54-4.42 (m, 2H, H-5' diastereoisomers), 4.19-4.15 (m, 4H, H-4', H-5' diastereoisomers), 4.04 (s, 6H, 6-*O*Me base diastereoisomers), 3.89-3.858 (m, 4H, $NH\underline{CH}(CH_3)CO$, $\underline{NH}CH_3$ base diastereoisomers), 2.97 (d, 3H, *J* = 2.3 Hz, $NH\underline{CH_3}$ base), 2.93 (d, 3H, *J* = 2.0 Hz, $NH\underline{CH_3}$ base), 1.61 (bs, 2H, $\underline{NH}CH(CH_3)CO$ diastereoisomers), 1.44 (d, *J* = 7.0 Hz, $NHCH(\underline{CH_3})CO$), 1.34 (d, *J* = 7.0 Hz, $NHCH(\underline{CH_3})CO$), 1.05 & 0.98 (2s, 6H, CH_3-2' diastereoisomers), 0.93 & 0.90 (2s, 18H, $COOCH_2C(\underline{CH_3})_3$ diastereoisomers).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 173.38 & 173. 21 (2s, $NHCH(CH_3)\underline{C}O$ diastereoisomers), 158.12 (s, C-6 base), 157.19 (s, C-2 base), 156.64 (s, C-8 base),

154.10 & 152.30 (2s, C-4 base, C-1 Ar), 134.83 (s, C-5 Ar), 127.95 (s, C-6 Ar), 126.78 & 126.61 & 126.58 & 126.35 (4s, C-8, C-7 Ar diastereoisomers), 125.48 & 125.25 & 125.19 (3s, C-10, C-2, C-3 Ar), 121.20 (s, C-9 Ar), 115.24 & 115.12 (2s, C-4 Ar diastereoisomers), 112.9 (s, C-5 base), 92.43 & 92.03 (2s, C-1' diastereoisomers), 82.47 & 82.36 (2s, C-4' diastereoisomers), 81.93 & 81.89 (s, C-2' diastereoisomers), 81.58 & 81.50 (2s, C-3' diastereoisomers), 74.98 & 74.17 (2s, $COO\underline{C}H_2C(CH_3)_3$ diastereoisomers), 65.24 & 65.19 (2s, C-5' diastereoisomers), 53.53 & 53.49 (2s, 6-*O*Me base diastereoisomers), 50.59 & 50.47 (2s, $NH\underline{C}H(CH_3)CO$ diastereoisomers), 31.41 & 31.35 (2s, $COOCH_2\underline{C}(CH_3)_3$ diastereoisomers), 29.78 & 29.62 (s, $NH\underline{C}H_3$ base diastereoisomers), 26.28 (s, $COOCH_2C(\underline{C}H_3)_3$), 21.19 & 21.11 (s, CH_3-2' diastereoisomers), 20.97 & 20.89 (s, $NHCH(\underline{C}H_3)CO$ diastereoisomers).

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm): 3.99 (s, 46%), 3.30 (s, 54%).

Synthesis of α-naphthyl L-Alanine-neopentyl ester 8-dimethylamino β-2'-C-methyl-6-O-methyl guanosine (69)



This ProTide was prepared according to the Standard Procedure D, using *t*BuMgCl (1M in THF, 0.70 mL, 2.0 eq), 8-dimethylamino-2'-*C*-Me-6-OMe-guanosine (**50**) (0.12 g, 0.35 mmol, 1.0 eq) in anh. THF (1.02 mL). *α*-naphthyl *L*-Alanine-neopentyl ester phosphochloridate (***14.d***) (0.33 g, 0.85 mmol, 2.4 eq) in anh. THF (1.00 mL). After overnight stirring at RT, the solvent was removed under reduced pressure, and the residue was purified by flash chromatography, using $CHCl_3$/MeOH (2%) as eluents, and preparative chromatography to yield the starting material (0.037 g, 0.05 mmol, 15%).

HPLC ($MeOH/H_2O$): 28.49 min, 29.13 min

HPLC (ACN/H_2O): 18.25 min, 18.81 min

MS (TOF ES^+): 702.21 ($M + H^+$).

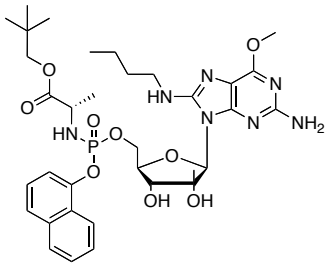
^1H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.15-8.13 (2m, 2H, H-9 Ar diastereoisomers), 7.84-7.82 (m, 2H, H-6 Ar diastereoisomers), 7.66-7.63 (m, 2H, H-4 Ar diastereoisomers), 7.54-7.34 (m, 8H, H-2, H-3, H-7, H8 Ar diastereoisomers), 5.95 &

5.92 (2s, 2H, H-1' diastereoisomers), 5.19 (bs, 2H, NHCH(CH_3)CO diastereoisomers), 4.24-4.19 (m, 2H, H-3' diastereoisomers), 4.17-4.07 (m, 4H, NHCH(CH_3)CO, H-4' diastereoisomers), 4.0 & 3.98 (2s, 6H, 6-*O*Me base diastereoisomers), 3.81-3.64 (m, 8H, H-5', $COOCH_2C(CH_3)_3$ diastereoisomers), 2.88 & 2.87 (m, 12H, $N(CH_3)_2$ diastereoisomers), 1.33 & 1.32 (2d, 6H, J = 3.5 Hz, NHCH(CH_3)CO diastereoisomers), 1.12 & 1.08 (2s, 6H, CH_3-2' diastereoisomers), 0.87 & 0.86 (2s, 18H, $COOCH_2C(CH_3)_3$ diastereoisomers).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 174.53 (d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 6.3 Hz, NHCH(CH_3)CO), 173.41 (d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 7.6 Hz, NHCH(CH_3)CO), 159.58 & 159.57 (2s, C-6 base diastereoisomers), 158.28 & 158.15 (2s, C-2 base diastereoisomers), 155.63 & 155.53 (2s, C-4 base diastereoisomers), 153.32 & 153.29 (2s, C-8 base diastereoisomers), 146.60 & 146.48 (2s, C-1 Ar diastereoisomers), 134.73 (s, C-5 Ar), 127.79 (s, C-6 Ar), 126.68 & 126.65 & 126.46 & 126.39 (4s, C-7, C-8, C-3, C-10 Ar), 124.56 & 124.88 (2s, C-4 n Ar diastereoisomers), 121.56 & 121.47 (2s, C-9 Ar diastereoisomers), 115.11 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 2.5 Hz, C-2 Ar), 114.94 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 2.5 Hz, C-2 Ar), 112.81 & 112.62 (2s, C-5 base diastereoisomers), 91.63 & 91.58 (2, C-1' diastereoisomers), 80.55 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 6.3 Hz, C-4'), 80.23 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 6.3 Hz, C-4'), 79.94 & 79.76 (2s, C-2' diastereoisomers), 76.19 & 75.94 (2s, C-3' diastereoisomers), 74.94 & 74.71 (2s, $COOCH_2C(CH_3)_3$ diastereoisomers), 67.22 & 67.18 (2s, C-5' diastereoisomers), 53.44 & 53.40 (2s, 6-*O*Me base diastereoisomers), 50.61 & 50.26 (2s, NHCH(CH_3)CO diastereoisomers), 43.24 & 43.23 (2s, $N(CH_3)_2$ diastereoisomers), 31.43 & 31.39 (s, $COOCH_2C(CH_3)_3$ diastereoisomers), 26.38 & 26.25 (2s, $COOCH_2C(CH_3)_3$ diastereoisomers), 21.10 & 21.01 (2s, CH_3-2' diastereoisomers), 20.68 & 20.64 (2s, NHCH(CH_3)CO diastereoisomers).

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm): 4.32 (s, 55%), 4.07 (s, 45%).

Synthesis of α-naphthyl L-Alanine-neopentyl ester 8-butylamino β-2'-C-methyl-6-O-methyl guanosine (70)



This ProTide was prepared according to the Standard Procedure D, using *t*BuMgCl (0.68 mL, 2.0 eq), 8-butylamino-2'-*C*-Me-6-OMe-guanosine (**59**) (0.130 g, 0.34 mmol) in anh. THF (1.00 mL), and *α*-naphthyl *L*-Alanine-neopentyl ester phosphochloridate (***14.d***) (0.261 g, 0.68 mmol, 2.0 eq) in anh. THF (0.8 mL). After overnight stirring at RT, the solvent was removed under reduced pressure, and the residue was purified by flash chromatography, using $CHCl_3$/MeOH (4%) as eluents, and preparative chromatography to yield the starting material (0.008 g, 0.011 mmol, 4%).

HPLC (MeOH/H_2O): 28.90 min, 29.15 min

HPLC (ACN/H_2O): 20.72 min, 20.75 min.

MS (TOF ES^+): 730.34 ($M + H^+$).

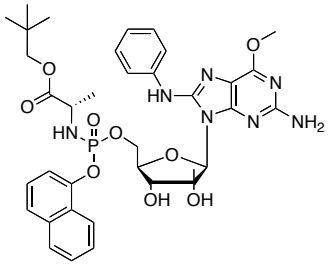
^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.13-8.10 (m, 2H, H-9 Ar diastereoisomers), 7.86-7.84 (m, 2H, H-6 Ar diastereoisomers), 7.68 (m, 2H, H-7 Ar diastereoisomers), 7.54-7.51 (m, 6H, H-4, H-2, H-3 Ar diastereoisomers), 7.42-7.38 (m, 2H, H-8 Ar diastereoisomers), 5.89 & 5.86 (2s, 2H, H-1' diastereoisomers), 4.86 (bs, 1H, $\underline{NH}CH_2CH_2CH_2CH_3$), 4.64 (bs, 1H, $\underline{NH}CH_2CH_2CH_2CH_3$), 4.22-4.12 (m, 6H, H-3', H-4', $NH\underline{CH}(CH_3)CO$ diastereoisomers), 4.03 & 4.02 (2s, 6H, 6-*O*Me base diastereoisomers), 3.86-3.67 (m, 8H, $COO\underline{CH_2}C(CH_3)_3$, H-5' diastereoisomers), 3.40-3.38 (m, 4H, $NH\underline{CH_2}CH_2CH_2CH_3$ diastereoisomers), 1.60-1.54 (m, 6H, $NHCH_2\underline{CH_2}CH_2CH_3$, $\underline{NH}CH(CH_3)CO$ diastereoisomers), 1.41 (d, 3H, J = 7.0 Hz, $NHCH(\underline{CH_3})CO$), 1.31 (d, 3H, J = 7.1 Hz, $NHCH(\underline{CH_3})CO$), 1.28-1.26 (m, 4H, $NHCH_2CH_2\underline{CH_2}CH_3$ diastereoisomers), 1.07 & 1.01 (s, 6H, CH_3-2' diastereoisomers), 0.89 & 0.85 (2s, 18H, $COOCH_2C(\underline{CH_3})_3$ diastereoisomers), 0.82 & 0.80 (2t, 3H, J = 7.3 Hz, $NHCH_2CH_2CH_2\underline{CH_3}$ diastereoisomers).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 173.88 (s, $NHCH(CH_3)\underline{C}O$), 173.45 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 7.6 Hz, $NHCH(CH_3)\underline{C}O$), 158.14 & 158.06 (s, C-6 base diastereoisomers), 156.80 & 156.64 (2s, C-2 base diastereoisomers), 153.52 & 153.23 (2s, C-8 base

diastereoisomers), 151.32 & 151.10 (2s, C-4 base diastereoisomers), 146.39 & 146.25 (2d, $J^2_{P\text{-}O\text{-}C}$ = 7.6 Hz, 6.30 Hz, C-1 Ar diastereoisomers), 134.80 & 134.78 (2s, C-5 Ar diastereoisomers), 127.91 & 127.8 (2s, C-6 Ar diastereoisomers), 126.74 & 126.54 & 126.40 & 126.35 & 126.30 & 126.24 (6s, C-8, C-7, C-10 Ar diastereoisomers), 125.47 & 125. 23 & 125.14 & 125.08 (4s, C-4, C-3 Ar diastereoisomers), 121.27 & 121.23 (2s, C-9 Ar diastereoisomers), 115.15 & 115.01 (2s, C-2 Ar, Ar diastereoisomers), 112.55 & 112.23 (2s, C-5 base diastereoisomers), 92.13 & 91.93 (2s, C-1' diastereoisomers), 81.71 & 81.03 (2s, C-4' diastereoisomers), 80.25 & 70.98 (2s, C-2' diastereoisomers), 79.06 & 78.95 (2s, C-3' diastereoisomers), 74.95 & 74.90 (2s, $COO\underline{C}H_2C(CH_3)_3$ diastereoisomers), 67.90 (d, $J^2_{P\text{-}O\text{-}C}$ = 5.1 Hz, C-5'), 67.84 (d, $J^2_{P\text{-}O\text{-}C}$ = 5.2 Hz, C-5'), 53.56 & 53.52 (2s, 6-*O*Me base diastereoisomers), 50.57 & 50.39 (2s, $NH\underline{C}H(CH_3)CO$ diastereoisomers), 42.81 & 42.78 (s, $NH\underline{CH_2}CH_2CH_2CH_3$ diastereoisomers), 31.73 & 31.37 (2s, $COOCH_2\underline{C}(CH_3)_3$ diastereoisomers), 31.36 & 30.90 (2s, $NHCH_2\underline{C}H_2CH_2CH_3$ diastereoisomers), 26.25 & 26.24 (2s, $COOCH_2C(\underline{C}H_3)_3$ diastereoisomers), 21.18 & 21.03 (2s, CH_3-2' diastereoisomers), 21.00 & 20.97 (2s, $NHCH(\underline{C}H_3)CO$ diastereoisomers), 20.15 & 20.09 (2s, $NHCH_2CH_2\underline{C}H_2CH_3$ diastereoisomers), 13.80 & 13.74 (2s, $NHCH_2CH_2CH_2\underline{C}H_3$ diastereoisomers).

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm): 4.18 (s, 67%), 3.75 (s, 33 %).

Synthesis of α-naphthyl L-Alanine-neopentyl ester 8-phenylamino β-2'-C-methyl-6-O-methyl guanosine (71)



This ProTide was prepared according to the Standard Procedure D, using *t*BuMgCl (0.84 mL, 2.0 eq), 8-aniline-2'-*C*-Me-6-OMe-guanosine (**58**) (0.170 g, 0.42 mmol, 1.0 eq) in anh. THF (1.23 mL), and *α*-naphthyl *L*-Alanine-neopentyl ester phosphochloridate (***14.d***) (0.32 g, 0.84 mmol, 2.0 eq) in anh. THF (0.80 mL). After overnight stirring at RT, the solvent was removed under reduced pressure, and the residue was purified by flash chromatography, using $CHCl_3$/MeOH (2.5%) as eluents,

and preparative chromatography to yield the starting material (0.005 g, 0.007 mmol, 2%).

HPLC (MeOH/H_2O): Rt = 30.93 min, 31.93 min

HPLC (ACN/H_2O): Rt = 21.40 min, 21.71 min

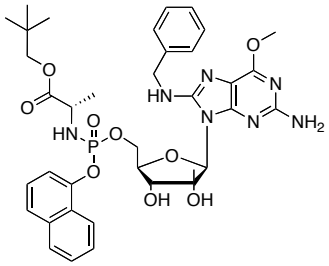
MS (TOF ES^+): 750.30 ($M + H^+$), 772.28 ($M + Na^+$).

^1H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.13 (m, 2H, H-9 Ar diastereoisomers), 8.04 (d, 2H, *J* = 8.4 Hz, H-6 Ar diastereoisomers), 7.84-7.82 (m, 2H, H-4 Ar diastereoisomers), 7.65-7.60 (m, 6H, CH para Ph, H-7 Ar, H-8 Ar diastereoisomers), 7.54-7.45 (m, 6H, H-2 Ar, H-3 Ar, CH ortho Ph diastereoisomers), 7.36-7.32(m, 2H, CH ortho Ph diastereoisomers), 7.26-7.7.18 (m, 2H, CH meta Ph diastereoisomers), 6.97-6.90 (m, 2H, CH meta Ph diastereoisomers), 6.03 & 5.99 (2s, 1H, H-1' diastereoisomers), 4.94 (bs, 2H, NHPh diastereoisomers), 4.27-4.25 (m, 2H, H-3' diastereoisomers), 4.17-4.11 (m, 4H, NHCH(CH_3)CO, H-4' diastereoisomers), 4.09 & 4.07 (s, 6H, 6-*O*CH_3 base diastereoisomers), 3.81-3.64 (m, 8H, H-5', COOCH_2C(CH_3)$_3$ diastereoisomers), 1.82 (bs, 2H, NHCH(CH_3)CO diastereoisomers), 1.30-1.27 (m, 6H, NHCH(CH_3)CO diastereoisomers), 1.04 & 0.99 (2s, 6H, CH_3-2' diastereoisomers), 0.89 & 0.87 (2s, 9H, COOCH$_2$C(CH_3)$_3$ diastereoisomers).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 173.36 & 173.30 (2s, NHCH(CH_3)CO diastereoisomers), 159.03 (s, C-6 base), 157.70 (s, C-2 base), 152.92 (s, C-4 base), 146.87 (s, C-8 base), 146.30 (s, C-1 Ar), 134.76 & 134.68 (2s, C ipso Ph diastereoisomers), 129.85 & 129.07 (2s, C meta Ph diastereoisomers), 127.85 & 127.77 (2s, C-6 Ar diastereoisomers), 126.64 & 126.51 & 126.33 & 126.28 (4s, C-7 Ar, C-8 Ar diastereoisomers), 125.47 & 125.40 & 125.04 & 124.99 (4s, C-3, C-10 Ar diastereoisomers), 122.41 & 122.25 (2s, C para Ph diastereoisomers), 118.96 & 118.74 (2s, C ortho Ph diastereoisomers), 115.14 & 114.96 (2s, C-2 Ar diastereoisomers), 112.09 (s, C-5 base), 92.41 & 91.29 (2s, C-1' diastereoisomers), 81.37 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 6.3 Hz, C-4'), 81.12 (s, C-4'), 80.45 & 80.20 (2s, C-2' diastereoisomers), 79.45 & 79.17 (2s, C-3' diastereoisomers), 74.81 & 74.73 (2s, C5'), 74.32 & 73.87 (2s, COOCH_2C(CH_3)$_3$ diastereoisomers), 54.12 & 53.87 (2s, 6-*O*Me base), 50.53 (d, $J^2_{P\text{-}N\text{-}C}$ = 7.6 Hz, NHCH(CH_3)CO), 50.30 (d, $J^2_{P\text{-}N\text{-}C}$ = 7.7 Hz, NHCH(CH_3)CO), 31.46 & 31.29 (2s, COOCH$_2$C(CH_3)$_3$ diastereoisomers), 26.36 & 26.22 (2s, COOCH$_2$C(CH_3)$_3$ diastereoisomers), 20.92 & 20.88 (2s, CH_3-2' diastereoisomers), 20.28 & 20.09 (2s, NHCH(CH_3)CO).

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm): 4.37 (s, 36%), 4.30 (s, 64%).

Synthesis of α-naphthyl L-Alanine-neopentyl ester 8-benzylamino β-2'-C-methyl-6-O-methyl guanosine (72)



This ProTide was prepared according to the Standard Procedure D, using *t*BuMgCl (0.80 mL, 2.0 eq), 8-benzylamino-2'-*C*-Me-6-OMe-guanosine (**49**) (0.165 g, 0.40 mmol) in anh. THF (1.17 mL), and *α*-naphthyl *L*-Alanine-neopentyl ester phosphochloridate (***14.d***) (0.304 g, 0.80 mmol, 2.0 eq) in anh. THF (1.1 mL). After overnight stirring at RT, the solvent was removed under reduced pressure, and the residue was purified by flash chromatography, using $CHCl_3$/MeOH (4%) as eluents, and preparative chromatography to yield the starting material (11.7 g, 0.015 mmol, 4%).

HPLC (MeOH/H_2O): 30.57 min, 30.85 min

HPLC (ACN/H_2O): 22.55 min

MS (TOF ES^+): 764.32 ($M + H^+$), 786.30 ($M + Na^+$).

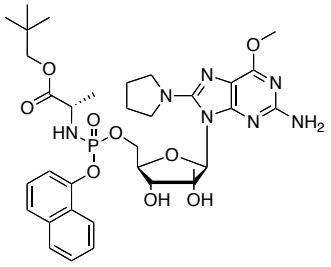
^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.07-8.01 (m, 2H, H-9 Ar diastereoisomers), 7.82-7.78 (m, 2H, H-6 Ar diastereoisomers), 7.65-7.63 (m, 4H, H-4 Ar, CH para Ph diastereoisomers), 7.51-7.41 (m, 10H, CH ortho Ph, H-7, H-8, H-3, H-2 Ar diastereoisomers), 7.36-7.15 (m, 6H, CH ortho Ph, CH meta Ph), 5.94 & 5.92 (2s, 2H, H-1' diastereoisomers), 4.94 (bs, 2H, NHCH_2Ph diastereoisomers), 4.65-4.62 (m, 4H, NHCH_2Ph diastereoisomers), 4.43-4.35 (m, 4H, COOCH_2C$(CH_3)_3$ diastereoisomers), 4.16-4.13 (m, 2H, H-3' diastereoisomers), 4.02 & 4.01 (2s, 6H, 6-*O*Me diastereoisomers), 3.89-3.58 (m, 8H, H-4', H-5', NHCH(CH_3)CO diastereoisomers), 2.19 (bs, 2H, NHCH(CH_3)CO diastereoisomers), 1.41 (d, 3H, *J* = 3.5 Hz, NHCH(CH_3)CO), 1.32 (d, 3H, *J* = 3.3 Hz, NHCH(CH_3)CO), 1.07 & 1.02 (2s, 6H, CH_3-2' diastereoisomers), 0.96 & 0.88 (2s, 9H, $COOCH_2C(CH_3)_3$ diastereoisomers)

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 173.31 & 173.15 (2s, NHCH(CH_3)CO diastereoisomers), 158.35 (s, C-6 base), 157.02 & 156.94 (2s, C-2 base diastereoisomers), 153.64 (s, C-8 base), 151.01 (s, C-4 base), 147.2 (s, C-1 Ar), 139.12

(s, C-5 Ar), 134.76 & 134.74 (2s, C ipso Ph diastereoisomers), 128.49-125.26 (18s, C-10, C-8, C-3, C-7, C-6 Ar, Ph diastereoisomers), 125.04 & 125.00 (2s, C-9 Ar diastereoisomers), 121.35 & 121.30 (2s, C-4 Ar diastereoisomers), 115.07 & 114.96 (2s, C-2 Ar diastereoisomers), 112.45 (s, C-5 base), 92.09 & 91.87 (2s, C-1' diastereoisomers), 81.52 & 81.47 (2s, C-4' diastereoisomers), 81.25 & 81.17 (2s, C-2' diastereoisomers), 81.06 & 81.01 (2s, C-3' diastereoisomers), 74.82 & 74.78 (2s, $COO\underline{C}H_2C(CH_3)_3$ diastereoisomers), 74.19 & 74.02 (2s, C-5' diastereoisomers), 53.62 & 53.59 (2s, 6-$O\underline{C}H_3$ base diastereoisomers), 50.60 & 50.45 (2s, $NH\underline{C}H(CH_3)CO$ diastereoisomers), 46.75 & 46.42 (2s, $NH\underline{CH_2}Ph$ diastereoisomers), 31.34 & 30.89 (2s, $COOCH_2\underline{C}(CH_3)_3$ diastereoisomers), 26.38 & 26.25 (2s, $COOCH_2C(\underline{C}H_3)_3$ diastereoisomers), 21.07 & 21.03 (2s, CH_3-2' diastereoisomers), 20.98 (d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 5.0 Hz, $NHCH(\underline{C}H_3)CO$), 20.83 (d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 5.1 Hz, $NHCH(\underline{C}H_3)CO$).

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm): 4.09 (s, 60%), 3.86 (s, 40%).

Synthesis of α-naphthyl L-Alanine-neopentyl ester 8-pyrrolidino β-2'-C-methyl-6-O-methyl guanosine (73)



This ProTide was prepared according to the Standard Procedure D, using *t*BuMgCl (1M in THF, 1.28 mL, 2.0 eq), 8-pyrrolidine-2'-*C*-Me-6-OMe-guanosine (**51**) (0.24 g, 0.64 mmol, 1.0 eq) in anh. THF (1.87 mL), and *α*-naphthyl *L*-Alanine-neopentyl ester phosphochloridate (***14.d***) (0.55 g, 1.43 mmol, 2.2 eq) in anh. THF (1.10 mL). After overnight stirring at RT, the solvent was removed under reduced pressure, and the residue was purified by flash chromatography, using $CHCl_3$/MeOH (2%) as eluents, and preparative chromatography to yield the starting material (0.058 g, 0.08 mmol, 12%).

HPLC (MeOH/H_2O): Rt = 22.57 min

HPLC (ACN/H_2O): Rt = 19.41 min, 20.01 min

MS (TOF ES^+): 728.32 ($M + H^+$).

^1H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.16-8.12 (m, 2H, H-9 Ar diastereoisomers), 7.89-7.86 (m, 2H, H-6 Ar diastereoisomers), 7.71-7.68 (t, 2H, *J* = 8.4 Hz, H-4 Ar

diastereoisomers), 7.58-7.40 (m, 8H, H-2, H-3, H-7, H-8 Ar diastereoisomers), 6.00 & 5.95 (2s, 1H, H-1' diastereoisomers), 5.21-5.14 (m, 2H, NHCH(CH_3)CO diastereoisomers), 4.90-4.86 (m, 4H, COOCH_2C(CH_3)$_3$ diastereoisomers), 4.34-4.29 (m, 4H, H-5' diastereoisomers), 4.24-4.21 (m, 1H, NHCH(CH_3)CO), 4.13-4.10 (m, 1H, NHCH(CH_3)CO), 4.02 & 3.99 (2s, 6H, 6-*O*Me base diastereoisomers), 3.87-3.73 (m, 4H, H-3', H-4' diastereoisomers), 3.61-3.58 (m, 4H, NH(CH_2CH_2)$_2$ diastereoisomers), 3.48-3.41 (m, 4H, NH(CH_2CH_2)$_2$ diastereoisomers), 1.98-1.95 (m, 8H, NH(CH_2CH_2)$_2$ diastereoisomers), 1.42 & 1.34 (2d, 3H, J = 7.1 Hz, 7.1 Hz, NHCH(CH_3)CO diastereoisomers), 1.20 & 1.14 (2s, 6H, CH_3-2' diastereoisomers), 0.92 & 0.91 (s, 18H, COOCH$_2$C(CH_3)$_3$ diastereoisomers).

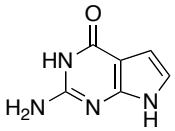
^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 174.89 (s, NHCH(CH_3)CO), 173.40 (d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 7.6 Hz, NHCH(CH_3)CO), 159.06 (s, C-6 base), 157.83 & 157.65 (2s, C-2 base diastereoisomers), 154.08 & 153.40 (2s, C-4 base diastereoisomers), 146.87 & 146.52 (2s, C-8 base diastereoisomers), 146.30 (s, C-1 Ar), 134.80 (s, C-5 Ar), 127.90 & 127.87 (2s, C-6 Ar diastereoisomers), 126.79 & 126.72 & 127.42 & 126.60 & 126.49 & 126.45 (6s, C-7, C-8, C-10 Ar diastereoisomers), 125.51 & 125.48 & 125.18 & 125.02 (4s, C-3, C-4 Ar diastereoisomers), 121.48 & 121.44 (2s, C-9 Ar diastereoisomers), 115.19 & 115.02 (2d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 2.5 Hz, 2.5 Hz, C-2 Ar diastereoisomers), 113.16 & 112.93 (2s, C-5 base diastereoisomers), 91.63 & 91.61 (2s, C-1' diastereoisomers), 80.24 & 79.99 (2d, $J^3_{\text{P-O-C-C}}$ = 5.0 Hz, 6.3 Hz, C-4' diastereoisomers), 79.99 & 79.94 (2s, C-2' diastereoisomers), 76.75 & 76.53 (2s, C-3' diastereoisomers), 75.17 & 74.88 (2s, COOCH$_2$C(CH_3)$_3$ diastereoisomers), 67.19 & 67.99 (2d, $J^2_{P\text{-}O\text{-}C}$ = 5.0 Hz, 5.0 Hz, C-5' diastereoisomers), 53.39 & 53.34 (2s, 6-*O*Me base diastereoisomers), 51.73 & 51.71 (2s, NH(CH$_2$CH$_2$)$_2$ diastereoisomers), 50.75 & 50.38 (2s, NHCH(CH_3)CO diastereoisomers), 31.45 & 31.40 (2s, COOCH$_2$C(CH_3)$_3$ diastereoisomers), 26.28 & 26.12 (2s, COOCH$_2$C(CH$_3$)$_3$ diastereoisomers), 25.34 & 25.12 (2s, NH(CH_2CH_2)$_2$ diastereoisomers), 21.38 & 21.22 (2d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 3.8 Hz, 3.6 Hz, NHCH(CH$_3$)CO diastereoisomers), 20.65 & 20.60 (2s, CH_3-2' diastereoisomers).

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm): 4.55 (s, 54%), 4.33 (s, 46%).

4.2. 7-deaza modified nucleosides and phosphoramidates

4.2.1. Preparation of the 7-deaza-6-*O*-methyl guanine

Synthesis of 7-deaza guanine (79) [6]

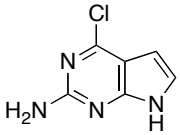


2,4-diamino-6-hydroxypyrimidine (**78**) (3.02 g, 23.6 mmol) was dissolved in H_2O (60 mL) containing NaOAc (1.97 g, 24.0 mmol). The solution was heated to 80 °C until a clear solution was obtained. Then 50% aq. α-chloroacetaldehyde (2.52 mL, 39.0 mmol) was introduced and the solution was left stirring at 80 °C overnight. A brown precipitate was recovered, dried and purified by flash chromatography using $CHCl_3$/MeOH (0.80 g, 5.33 mmol, 23%).

^{1}H NMR (500 MHz, DMSO) δ_H (ppm): 10.94 (s, 1H, NH-9), 10.19 (s, 1H, NH-1), 6.61 (d, 1H, J = 2.2 Hz, H-8), 6.19 (d, 1H, J = 2.1 Hz, H-7), 6.01 (s, 2H, NH_2-2).

^{13}C NMR (125 MHz, DMSO) δ_C (ppm)**:** 158.83 (s, C-6), 152.17 (s, C-2), 151.12 (s, C-4), 116.55 (s, C-8), 101.55 (s, C-7), 99.84 (s, C-5).

Synthesis of 7-deaza-6-chloro guanine (80) [7]

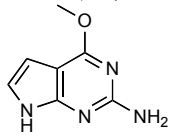


$POCl_3$ (32 mL) and 7-deazaguanine (**79**) (0.80 g, 5.33 mmol) were heated under reflux for 1 hr 30 min. After cooling the mixture was concentrated under reduced pressure. Later some ice was cautiously added and then some water. The mixture was then neutralized with $NaHCO_3$. The precipitated solid was filtered off and dried to yield the desired material (0.57 g, 3.4 mmol, 64%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.30 (bs, 1H, NH-9), 7.00 (s, 1H, H-8), 6.45 (s, 1H, H-7), 4.95 (broad s, 2H, NH_2-2).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 153.19 (s, C-2), 151.78 (s, C-4), 150.68 (s, C-6), 122.09 (s, C-8), 111.98 (s, C-5), 100.78 (s, C-7).

Synthesis of 7-deaza-6-O-methyl guanine (81)



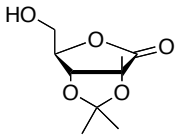
7-deaza-6-chloro-guanine (**80**) (0.350 g, 2.08 mmol) in anh. MeOH (59 mL) with NaOMe (0.337 g, 6.24 mmol, 1.0 eq) was heated under reflux for 5 days. The residue was purified by flash chromatography using MeOH/$CHCl_3$ (15%) to afford the desired material (0.0084, 0.05 mmol, 3%).

^{1}H NMR (500 MHz, MeOD) δ_H (ppm): 6.82 (d, 1H, *J* = 3.5 Hz, H-8), 6.31 (d, 1H, *J* = 3.5 Hz, H-7), 4.02 (s, 3H, 6-*O*CH_3).

^{13}C NMR (125 MHz, MeOD) δ_C (ppm): 163.32 (s, C-6), 153.09 (s, C-2), 151.56 (s, C-4), 120.60 (s, C-8), 112.21 (s, C-5), 99.82 (s, C-7), 54.80 (s, 6-*O*CH_3).

4.2.2. Glycosylation *via* fully protected 2'-C-methyl ribofuranose

Preparation of 2',3'-isopropylidene-2'-C-methyl-D-ribono-1,4-lactone (83)

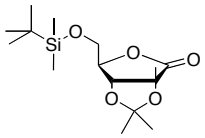


Commercially available 2'-*C*-methyl-ribono lactone (**82**) (3.67 g, 22.7 mmol) was suspended in anh. acetone (90 mL) and perchloric acid (60%, 2.20 mL) was added dropwise at RT. After 1 hr 30 min, the reaction was neutralized with NaOH and the solvent was removed under reduced pressure. The residue was purified by flash chromatography using hexane/ethyl acetate (9:1) as eluents to yield the 2',3'-isopropylidene protected lactone (3.46 g, 17.1 mmol, 76%).

^{1}H NMR (500 MHz, CDCl3) δ_H (ppm): 4.52-4.49 (m, 1H, H-3'), 3.96-3.95 (m, 1H, H-5'), 3.81-3.78 (m, 1H, H-5'), 3.19-3.16 (m, 1H, H-4'), 1.64 (s, 3H, CH_3-2'), 1.43 & 1.41 (2s, 6H, 2 x CH_3).

^{13}C NMR (125 Hz, $CDCl_3$) δ_C (ppm)**:** 177.2 (s, C-1'), 112.9 (s, $\underline{C}(CH_3)_2$), 84.04 (s, C-3'), 83.72 (s, C-2'), 82.55 (s, C-4'), 61.95 (s, C-5'), 26.94 & 26.38 (2s, C($\underline{C}H_3)_2$), 19.75 (s, CH_3-2').

Preparation of 2',3'-isopropylidene-5'-TBDMS-2'-C-methyl-D-ribono-1,4-lactone (84)

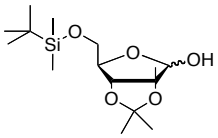


The 2',3'-isopropylidene-2'-*C*-Me-ribono-lactone (**83**) (0.77 g, 3.78 mmol) was dissolved in anh. DMF (10 mL), then imidazole (0.54 g, 7.96 mmol, 2.1 eq) and TBDMSCl (1.25 g, 8.28 mmol, 2.2 eq) were added, and the reaction was left stirring overnight. The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography using $CHCl_3$/MeOH (9:1) to yield a white solid (0.39 g, 1.04 mmol, 28%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 4.41-4.39 (m, 2H, H-3', H-4'), 3.81-3.76 (m, 2H, H-5'), 1.53 (s, 3H, CH_3-2'), 1.34 & 1.32 (2s, 6H, C($\underline{CH_3}$)$_2$), 0.81 & 0.80 (2s, 9H, OSi(CH_3)$_2$C($\underline{CH_3}$)$_3$), 0.009 (s, 6H, OSi($\underline{CH_3}$)$_2$C(CH_3)$_3$).

^{13}C NMR (d-$CDCl_3$) δ_C (ppm): 175.95 (s, C-1'), 112.63 (s, $\underline{C}$(CH_3)$_2$), 83.29 (s, C-3'), 82.96 (s, C-4'), 63.59 (s, C-5'), 27.07 & 26.74 (2s, C($\underline{C}H_3$)$_2$), 26.15 & 25.83 & 25.69 (3s, OSi(CH_3)$_2$C($\underline{C}H_3$)$_3$), 19.87 (s, CH_3-2'), 18.49 (s, OSi(CH_3)$_2\underline{C}$(CH_3)$_3$), -5.29 & -5.52 (2s, OSi($\underline{C}H_3$)$_2$C(CH_3)$_3$).

Preparation of 2',3'-isopropylidene-5'-TBDMS-2'-C-methyl-furanose (85)



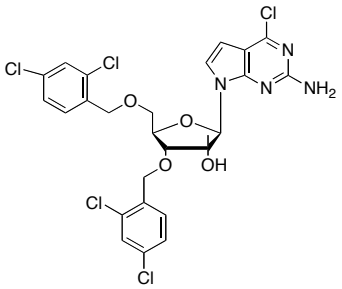
2',3'-isoproylidene-5'-TBDMS protected ribonolactone (**84**) (0.33 g, 1.04 mmol) was dissolved in anh. THF (8.0 mL) and DIBAL-H (1M in hexanes, 3.10 mL, 3.12 mmol, 3.0 eq) was added dropwise at -78 °C and the reaction was stirred for 1 hr and then 3-4 hrs at -15 °C. The reaction was quenched with 8% HCl, diluted with EtOAc and brine. After extraction with EtOAc, the residue was purified by flash chromatography using hexane/EtOAc (9:1) to yield a colorless oil (0.09 g, 0.29 mmol, 28%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 5.11 (d, 1H, J = 10.5 Hz, H-1', β-anomer), 5.02 (bs, 1H, H-1', α-anomer), 4.49 (d, 1H, J = 11.0 Hz, OH, β-anomer), 4.42 (d, 1H, J = 1.0 Hz, H-3', β-anomer), 4.32 (bs, 1H, H-3', α-anomer), 4.18 (m, 1H, H-4' β-anomer), 4.05 (m, 1H, H-4', α-anomer), 3.74-3.66 (m, 5H, OH α-anomer & H-5'), 1.49 (s, 3H, CH_3-

2'), 1.45 (s, 3H, CH_3-2'), 1.42 & 1.41 & 1.40 & 1.38 (4s, 12H, 2 x C($\underline{CH_3}_2$), 0.90 & 0.86 (2s, 18H, 2 x OSi$(CH_3)_2$C($\underline{CH_3}_3$), 0.10 & 0.09 (s, 12H, 2 x OSi($\underline{CH_3}_2$C$(CH_3)_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 113.2 & 112.43 (2s, $\underline{C}(CH_3)_2$ anomers), 104.57 & 102.20 (2s, C-1' anomers), 92.54 & 87.95 (2s, C-2' anomers), 87.50 & 87.05 (2s, C-3' anomers), 86.71 & 82.33 (2s, C-4' anomers), 65.10 & 64.85 (2s, C-5' anomers), 29.62 & 29.28 & 29.08 & 28.45 (4s, C($\underline{C}H_3)_2$ anomers), 26.54 & 26.25 & 26.17 & 25.93 & 25.84 & 25.72 (6s, OSi$(CH_3)_2$C($\underline{C}H_3)_3$ anomers), 21.45 & 20.06 (s, CH_3-2' anomers), 18.42 & 18.40 (2s, OSi$(CH_3)_2\underline{C}(CH_3)_3$ anomers), -5.41 & -5.58 & -5.73 & -5.76 (4s, OSi($\underline{C}H_3)_2$C$(CH_3)_3$ anomers).

4.2.3. Glycosylation with 3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2'-*C*-methyl-1-*O*-methyl-*α*-*D*-ribofuranose

Preparation of 3',5'-O-[2,4-dichlorophenyl)methyl]-2'-C-methyl-6-chloro-7-deaza guanosine (90) [8]

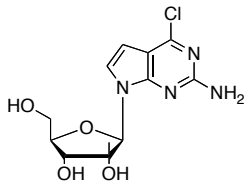


To a precooled solution (0 °C) of commercially available 3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2'-*C*-methyl-1-*O*-methyl-*α*-*D*-ribofuranose (**89**) (1.53 g, 3.08 mmol, 1.0 eq) in anh. DCM (36 mL), was added dropwise HBr (33% in acetic acid, 3.72 mL, 20.5 mmol, 6.7 eq). The reaction was left stirring for 2 hrs at RT and the solvent was evaporated under reduced pressure by coevaporation with toluene. The resulting residue was dissolved in anh. ACN (18 mL) and added dropwise into a well-stirred solution of 7-deaza-6-chloro guanine (**80**) (0.52 g, 3.02 mmol, 1.0 eq), KOH (85% powdered, 0.62 g, 9.24 mmol, 3.0 eq) and tris[2-(2-methoxyethoxy)-ethyl]amine (TDA-1, 0.20 mL, 0.62 mmol, 0.2 eq) in anh. ACN (36 mL). The solution was stirred for 1bhr at RT, filtered and evaporated under reduced pressure. The residue was purified by flash chromatography using Petroleum ether/EtOAc (5:1, 3:1, 2:1 et 1.5:1) to recover the desired material (0.60 g, 0.96 mmol, 31%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 7.44-7.39 (m, 4H, 2 x H-6 & 2 x H-5 DCB), 7.36 (d, 1H, *J* = 3.8 Hz, H-8 base), 7.29-7.25 (m, 2H, 2 x H-3 DCB), 6.34 (d, 1H, *J* = 3.8 Hz, H-7 base), 6.23 (s, 1H, H-1'), 4.82-4.63 (m, 4H, 2 x CH_2 DCB), 4.27-4.24 (m, 1H, H-4'), 4.20 (d, 1H, *J* = 8.2 Hz, H-3'), 3.99-3.97 (m, 1H, H-5'), 3.78-3.76 (m, 1H, H-5'), 1.00 (s, 3H, CH_3-2').

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm)**:** 162.05 (s, C-2 base), 156.72 (s, C-4 base), 155.89 (s, C-6 base), 138.03 & 137.73 & 137.40 & 137.29 & 137.26 & 137.09 (6s, 2 x C-1, 2 x C-4, 2 x C-2 DCB), 134.11 & 133.96 (2s, 2 x C-6 DCB), 132.74 & 134.68 (2s, 2 x C-3 DCB), 131.05 & 130.61 (2s, 2 x C-5 DCB), 126.50 (s, C-8 base), 114.03 (s, C-5 base), 104.04 (s, C-7 base), 93.91 (s, C-1'), 83.09 (s, C-4'), 83.26 (s, C-3'), 82.49 (s, C-2'), 73.69 & 73.44 (2s, 2 x CH_2 DCB), 72.11 (s, C-5'), 21.97 (s, CH_3-2').

Preparation of 7-deaza-6-chloro-2'-C-methyl guanosine (91) [8]

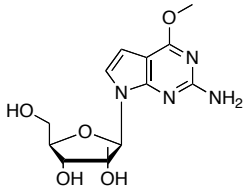


To a solution of 3',5'-*O*-[(2,4-dichlorophenyl)methyl]-2'-*C*-methyl-6-chloro-7-deaza-guanosine (**90**) (0.27 g, 0.42 mmol, 1.0 eq) in anh. DCM (8.40 mL) at -78 °C, was added dropwise BCl_3 (1M in DCM, 4.2 mL, 4.2 mmol, 10 eq). The reaction was left stirring for 2 hrs at -78 °C and then for 2 hrs 30 min at -20 °C. The reaction was quenched with DCM/MeOH (1:1) (4.2/4.2 mL), and left stirring at -20 °C for 30 min, then neutralized at 0 °C with aq. NH_3. The solid formed was filtered and the filtrate was evaporated under reduced pressure. The residue was purified by flash chromatography using $CHCl_3$/MeOH (50:1 and 20:1) to give the desired material (0.088 g, 0.28 mmol, 66%).

^{1}H NMR (500 MHz, MeOD) δ_H (ppm): 7.45 (d, 1H, *J* = 3.6 Hz, H-8 base), 6.41 (d, 1H, *J* = 3.5 Hz, H-7 base), 6.18 (s, 1H, H-1'), 4.11 (d, 1H, *J* = 9.0 Hz, H-3'), 4.02-3.99 (m, 2H, H-4', H-5'), 3.85-3.83 (m, 1H, H-5'), 0.89 (s, 3H, CH_3-2').

^{13}C NMR (125 MHz, MeOD) δ_C (ppm)**:** 161.05 (s, C-2 base), 154.90 (s, C-4 base), 153.41 (s, C-6 base), 124.99 (s, C-8 base), 113.43 (s, C-5 base), 101.06 (s, C-7 base), 92.53 (s, C-1'), 83.70 (s, C-4'), 80.31 (s, C-2'), 74.04 (s, C-3'), 61.47 (s, C-5'), 20.05 (s, CH_3-2').

Synthesis of 7-deaza-6-O-methyl-2'-C-methyl guanosine (88)



To a solution of 7-deaza-6-chloro-2'-C-methyl guanosine (**91**) (0.88 g, 0.28 mmol, 1.0 eq) in anh. MeOH (9 mL) was added NaOMe (0.05 mmol, 0.84 mmol, 3.0 eq) at 0 °C. The reaction was left stirring overnight under reflux at 65 °C. After neutralization of the reaction with amberlite, the solvent was evaporated under reduced pressure and the residue was purified by flash chromatography using $CHCl_3$/MeOH (50:1 and 20:1) to give the desired material (0.043 g, 0.14 mmol, 49%).

HPLC (MeOH/H_2O): Rt = 15.3 min

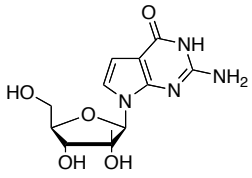
HPLC (ACN/H_2O): Rt = 6.58 min

MS (TOF ES^+): 311.13 ($M + H^+$).

^{1}H NMR (500 MHz, MeOD) δ_H (ppm): 7.10 (d, 1H, *J* = 3.6 Hz, H-8 base), 6.34 (d, 1H, *J* = 3.6 Hz, H-7 base), 6.08 (s, 1H, H-1'), 4.15 (d, 1H, *J* = 9.2 Hz, H-3'), 4.03 (m, 2H, H-5', H-4'), 4.02 (s, 3H, 6-*O*CH_3 base), 3.86 (dd, 1H, *J* =3.3 Hz, 12.3 Hz, H-5'), 0.86 (s, 3H, CH_3-2').

^{13}C NMR (125 MHz, MeOD) δ_C (ppm)**:** 165.34 (s, C-6 base), 160.92 (s, C-2 base), 154.81 (s, C-4 base), 121.54 (s, C-8 base), 100.53 (s, C-7 base), 100.08 (s, C-5 base), 93.47 (s, C-1'), 83.54 (s, C-4'), 80.50 (s, C-2'), 74.22 (s, C-3'), 61.73 (s, C-5'), 54.00 (s, 6-*O*CH_3 guanine), 20.31 (s, CH_3-2').

Synthesis of 7-deaza-2'-C-methyl guanosine (95) [8, 9]



To a solution of 7-deaza-6-O-methyl-2'-C-methyl guanosine (**88**) (8.0 mg, 0.026 mmol, 1.0 eq) in anh. ACN (1.0 mL) was added NaI (0.006g, 0.04 mmol, 1.5 eq) and TMSCl (5 µL, 0.04mmol, 1.5 eq). The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography using CH_2Cl_2/MeOH as eluants, to recover the desired molecule (0.0016 g, 0.0054 mmol, 21%).

HPLC (MeOH/H_2O): Rt = 7.44 min

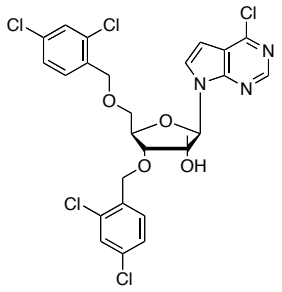
HPLC (ACN/H_2O): Rt = 2.22 min

MS (TOF ES^-): 295.08 (M - H^+), 331.08 (M + Cl^-).

^{1}H NMR (500 MHz, MeOD) δ_H (ppm): 7.09 (d, 1H, *J* = 3.6 Hz, H-8 base), 6.42 (d, 1H, *J* = 3.6 Hz, H-7 base), 6.09 (s, 1H, H-1'), 4.07 (d, 1H, *J* = 9.2, H-3'), 4.01-3.96 (m, 2H, H-4', H-5'), 3.84-3.81 (dd, 1H, *J* = 3.6, 12.70 Hz, H-5'), 0.90 (s, 3H, CH_3-2').

^{13}C NMR (125 MHz, MeOD) δ_C (ppm)**:** 162.05 (s, C-2 base), 154.87 (s, C-4 base), 153.19 (s, C-6 base), 118.98 (s, C-8 base), 102.43 (s, C-7 base), 91.82 (s, C-1'), 82.76 (s, C-4'), 82.51 (s, C-2'), 73.35 (s, C-3'), 60.91 (s, C-5'), 19.30 (s, CH_3-2').

Synthesis of 3',5'-O-[2,4-dichlorophenyl)methyl]-7-deaza-6-chloro-2'-C-methyl purine (105) [8]

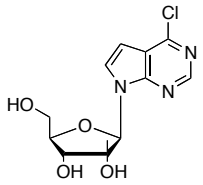


Prepared following the procedure for (**90**), using commercially available 5-bis-*O*-(2,4-dichlorophenylmethyl)-2'-*C*-methyl-1-*O*-methyl-*α*-*D*-ribofuranose (**89**) (1.08 g, 2.17 mmol, 1.0 eq) in anh. DCM (35 mL), HBr (33% in acetic acid, 2.36 mL, 13.02 mmol, 6.0 eq). The resulting residue was dissolved in anh. ACN (6 mL) and added dropwise into a solution of commercially available 6-chloro-7-deaza purine (**104**) (1.00 g, 6.51 mmol, 3.0 eq) and NaH (60% dispersion in oil, 0.26 g, 6.51 mmol, 3.0 eq) in anh. ACN (12 mL). After suspension in water (29 mL) and extraction with EtOAc (57 mL), the organic layer was washed with brine (34 mL) and evaporated under reduced pressure. Purification by flash chromatography using Petroleum ether/EtOAc (3:1, 2:1) afforded the desired material (0.60 g, 0.96 mmol, 44%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.49 (s, 1H, H-2 base), 7.66 (d, 1H, *J* = 3.5 Hz, H-8 base), 7.30-7.22 (m, 4H, 2 x H-3, 2 x H-6 DCB), 7.16-7.14 (dd, 1H, *J* = 2.0 Hz, 8.3 Hz, H-5 DCB), 7.09-7.07 (dd, 1H, *J* = 2.0 Hz, 8.3 Hz, H-5 DCB), 6.42 (d, 1H, *J* = 3.5 Hz, H-7 base), 6.34 (s, 1H, H-1'), 4.70-4.52 (m, 4H, 2 x CH_2 DCB), 4.25-4.22 (dt, 1H, *J* = 2.5 Hz, 8.5 Hz, H-4'), 4.16 (d, 1H, *J* = 8.5 Hz, H-3'), 3.92-3.89 (dd, 1H, *J* = 2.5 Hz, 11.0 Hz, H-5'), 3.72-3.69 (dd, 1H, *J* = 2.5 Hz, 11.0 Hz, H5'), 0.85 (s, 3H, CH_3-2').

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm)**:** 151.96 (s, C-6 base), 150.76 (s, C-4 base), 150.57 (s, C2 base), 134.64 & 134.48 & 134.24 & 133.90 & 133.37 & 133.54 (6s, 2 x C-1, 2 x C-2, 2 x C-4 DCB), 130.84 & 130.23 (2s, 2 x C-6 DCB), 129.57 & 129.12 (2s, 2 x C-3 DCB), 127.40 & 127.07 (2s, 2 x C-5 DCB), 126.83 (s, C-8 base), 117.76 (s, C-5 base), 104.04 (s, C-7 base), 90.88 (s, C-1'), 80.65 (s, C-4'), 80.05 (s, C-3'), 79.29 (s, C-2'), 70.56 & 70.24 (2s, 2 x $\underline{C}H_2$ DCB), 68.95 (s, C-5'), 20.77 (s, CH_3-2').

Preparation of 7-deaza-6-chloro-2'-C-methyl purine (106) [8, 9]

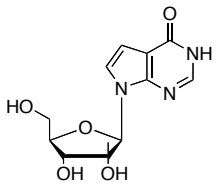


To a solution of 3',5'-O-[2,4-dichlorophenyl)methyl]-7-deaza-6-chloro-2'-C-methyl purine (**105**) in anh. DCM (23 mL) at -78 °C was added BCl_3 (1M in DCM, 11.2 mL, 11.2 mmol, 10 eq). The mixture was stirred at -78 °C for 2 hrs 30 min, then at -30 °C to -20 °C fror 3 hrs. The reaction was quenched with MeOH/DCM (13 mL : 13 mL) and stirred at -15 °C for 30 min, then neutralized with aq. NH_3 at 0 °C and stirred at RT for 15 min. The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography using DCM/MeOH (99:1, 98:2, 95:5 and 90:10) to recover the desire molecule (0.12 g, 0.41 mmol, 37 %).

^{1}H NMR (500 MHz, MeOD) δ_H (ppm): 8.61 (s, 1H, H-2 base), 8.06 (d, 1H, J = 3.8 Hz, H-8 base), 6.72 (d, 1H, J = 3.8 Hz, H-7 base), 6.43 (s, 1H, H-1'), 4.16 (d, 1H, J = 9.2 Hz, H-3'), 4.08-4.03 (m, 2H, H-4', H5'), 3.90-3.87 (dd, 1H, J = 3.3 Hz, 12.6 Hz, H-5'), 0.83 (s, 3H, CH_3-2').

^{13}C NMR (125 MHz, MeOD) δ_C (ppm)**:** 151.68 (s, C-6 base), 151.50 (s, C-4 base), 150.48 (s, C-2 base), 129.41 (s, C-8 base), 117.54 (s, C-5 base), 100.94 (s, C-7 base), 92.93 (s, C-1'), 84.06 (s, C-4'), 81.52 (s, C-2'), 73.71 (s, C-3'), 61.21 (s, C-5'), 19.89 (s, CH_3-2').

Preparation of 7-deaza-2'-C-methyl inosine (107)



To 7-deaza-6-chloro-2'-C-methyl purine (**106**) (1.07 g, 3.56 mmol, 1.0 eq) was added sodium hydroxide (20 g in 500 mL H_2O, 1M, 20 mL). The mixture was heated under reflux for 1 hr, cooled and neutralized with aqueous HCl (2M (2.7 mL) in 50 mL H_2O). The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography using MeOH/$CHCl_3$ (1:4) as eluant to afford the desired material (0.080 g, 0.28 mmol, 8%).

HPLC (MeOH/H_2O): Rt = 7.56 min

HPLC (ACN/H_2O): Rt = 2.34 min

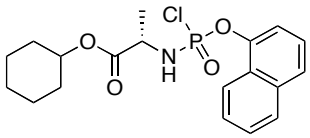
MS (TOF ES^-): 280.08 (M - H^+).

^{1}H NMR (500 MHz, MeOD) δ_H (ppm): 7.94 (s, 1H, H-2 base), 7.53 (d, 1H, *J* = 3.6 Hz, H-8 base), 6.68 (d, 1H, *J* = 3.6 Hz, H-7 base), 6.28 (s, 1H, H-1'), 4.11 (d, 1H, *J* = 8.8 Hz, H-3'), 4.05-4.01 (m, 2H, H-4', H-5'), 3.88-3.84 (dd, 1H, *J* = 3.8 Hz, 12.9 Hz, H-5'), 0.84 (s, 3H, CH_3-2').

^{13}C NMR (125 MHz, MeOD) δ_C (ppm)**:** 161.60 (s, C-6 base), 149.12 (s, C-4 base), 144.12 (s, C-2 base), 123.01 (s, C-8 base), 109.67 (s, C-5 base), 100.59 (s, C-7 base), 92.99 (s, C-1'), 83.82 (s, C-4'), 80.57 (s, C-2'), 73.82 (s, C-3'), 61.45 (s, C-5'), 19.98 (s, CH_3-2').

4.2.4. Preparation of phosphorochloridates

Preparation of α-naphthyl L-Alanine-cyclohexyl ester phosphochloridate (14.e)

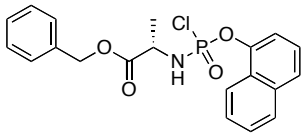


Prepared according to procedure C, using *L*-Alanine-cyclohexyl tosylate salt (provided by Inhibitex *Inc.*) (3.69 g, 17.77 mmol, 1.0 eq) in anh. DCM (93 mL), *α*-naphthyl phosphorodichloridate (***12***) (4.6387 g, 17.77 mmol, 1.1 eq), and NEt_3 (4.95 mL, 35.54 mmol, 2.0 eq). The solvent was removed under reduced pressure and the residue purified by flash chromatography using Hexane/EtOAc (1:1) to yield the desired molecule (4.90 g, 12.28 mmol, 69%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.25-8.21 (m, 2H, H-9 Ar diastereoisomers), 8.09-7.92 (m, 2H, H-6 Ar diastereoisomers), 7.72-7.70 (m, 2H, H-4 Ar diastereoisomers), 7.61-7.56 (m, 2H, H3 Ar diastereoisomers), 7.50-7.51 (m, 4H, H-7, H-8 Ar diastereoisomers), 7.39-7.01 (m, 2H, H-2 Ar diastereoisomers), 4.15-4.10 (m, 2H, CH cHex diastereoisomers), 4.06-3.99 (m, 2H, NHCH(CH_3)CO diastereoisomers), 1.99-1.87 (m, 4H, 2 x CH_2 ortho diastereoisomers), 1.76-1.61 (m, 16 H, 4 x CH_2 meta, 2 x CH_2 para, 2 x CH_2 ortho diastereoisomers), 1.51 & 1.48 (2d, 3H, J = 2.5 Hz, 2.5 Hz, CH_3 L-Ala),.

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm): 8.28 (s), 8.13 (s).

Preparation of α-naphthyl L-Alanine-benzyl ester phosphochloridate (14.f) [10]



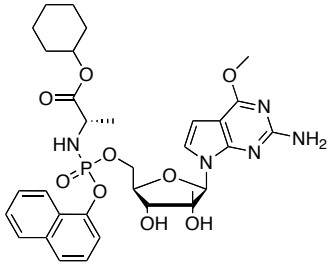
Prepared according to procedure C, using *L*-Alanine-benzyl tosylate salt (provided by Inhibitex *Inc.*) (3.15 g, 8.98 mmol, 1.0 eq) in anh. DCM (52 mL), *α*-naphthyl phosphorodichloridate (***12***) (2.34 g, 8.98 mmol, 1.1 eq), and NEt_3 (17.96 mL, 2.50 mmol, 2.0 eq). The solvent was removed under reduced pressure and the residue purified by flash chromatography using Hexane/EtOAc (1:1) to yield the desired molecule (0.58 g, 1.44 mmol, 16%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.19-8.18 (m, 2H, H-4 Ar diastereoisomers), 7.83-7.82 (m, 2H, H-7 Ar diastereoisomers), 7.69-7.67 (m, 4H, H-2, H-9 Ar diastereoisomers), 7.52-7.47 (m, 4H, H-3, H-8 Ar diastereoisomers), 7.39-7.28 (m, 12H, H-6 Ar, 5 x CH Ph diastereoisomers), 5.22-5.25 (m, 4H, O$\underline{CH_2}$Ph diastereoisomers), 4.47-4.37 (m, 2H, NH$\underline{CH}$(CH_3)CO diastereoisomers), 1.58 & 1.56 (2d, 6H, J = 6.5 Hz, 6.5 Hz, NHCH($\underline{CH_3}$)CO).

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm): 8.72 (s), 8.63 (s).

4.2.5. Preparation of α-naphthyl *L*-Alanine-ester ProTides

Preparation of α-naphthyl L-Alanine-cyclohexyl-ester 2'-C-methyl-6-O-methyl-7-deaza-guanosine ProTide (92)



This ProTide was prepared according to the Standard Procedure D, using *t*BuMgCl (1M in THF, 0.25 mL, 0.25 mmol, 2eq), 7-deaza-6-*O*Me-2'-*C*-Me guanosine (**88**) (0.039 g, 0.13 mmol, 1.0 eq) in anh. THF (0.37 mL). and α-naphthyl *L*-Alanine-cyclohexyl ester phosphochloridate (***14.e***) (0.100 g, 0.25 mmol, 2.0 eq) in anh. THF (0.37 mL). After overnight stirring at RT, the solvent was removed under reduced pressure and the residue was purified by flash chromatography, using $CHCl_3$/MeOH (4%) as eluents, and preparative chromatography to yield the desired molecule (0.010 g, 0.015 mmol, 12%).

HPLC (MeOH/H_2O): Rt = 24.31 min, 24.52 min

HPLC (ACN/H_2O): Rt = 17.64, 18.05 min

MS (TOF ES^+): 670.259 ($M + H^+$).

92.a (fast eluting isomer):

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.16 (d, 1H, *J* = 7.0 Hz, H-9 Ar), 7.88 (1H, d, *J* = 7.5 Hz, H-6 Ar), 7.70 (d, 1H, *J* = 7.5 Hz, H-4 Ar), 7.59-7.54 (m, 3H, H-2, H-7, H-8 Ar), 7.44 (t, 1H, *J* = 8.0 Hz, H-3 Ar), 6.80 (d, 1H, *J* = 3.6 Hz, H-8 base), 6.30 (d, 1H, *J* = 3.6 Hz, H-7 base), 6.00 (s, 1H, H-1'), 4.89 (s, 2H, NH_2 base), 4.77 (m, 1H, NHCH(CH_3)CO), 4.63 (m, 1H, H-3'), 4.22 (m, 1H, H-4'), 4.13-4.08 (m, 1H, CH cHex), 4.02 (s, 3H, 6-*O*CH_3 base), 3.83-3.79 (m, 2H, H-5'), 3.63 (m, 1H, NHCH(CH_3)CO), 1.79-1.76 (m, 2H, CH_2 ortho cHex), 1.72-1.70 (m, 2H, CH_2 ortho cHex), 1.69-1.50 (m, 4H, CH_2 meta, CH_2 para cHex), 1.40 (d, 3H, *J* = 7.0 Hz, NHCH(CH_3)CO), 1.38-1.37 (m, 2H, CH_2 meta), 0.91 (s, 3H, CH_3-2').

^{13}C NMR (125 MHz, $CDCl_3$) δ_c (ppm): 174.24 (s, NHCH(CH_3)CO), 166.35 (s, C-6 base), 160.65 (s, C-2 base), 155.78 (s, C-4 base), 148.53 (s, C-1 Ar), 136.49 (s, C-5 Ar), 128.67 (s, C-6 Ar), 127.96 (s, C-7 Ar), 127.37 (s, C-8 Ar), 126.56 (s, C-10 Ar), 125.80 (s, C-3 Ar), 122.24 (s, C-9 Ar), 121.56 (s, C-8 base), 120.89 (s, C-4 Ar), 116.32 (s, C-2 Ar), 100.98 (s, C-5 base), 100.17 (s, C-7 base), 93.84 (s, C-1'), 84.09 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 7.5 Hz, C-4'), 80.46 (s, C-2'), 74.98 (s, C-3'), 74.36 (s, C ipso cHex), 66.34 (d, $J^2_{P\text{-}O\text{-}C}$ = 5.1

Hz, C-5'), 53.78 (s, 6-*O*Me base), 51.64 (s, NHCH(CH$_3$)CO), 32.67 & 30.97 (2s, 2 x C ortho cHex), 26.23 (s, C para cHex), 20.67 & 20.34 (s, 2 x C meta cHex), 20.76 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 6.8 Hz, NHCH(CH$_3$)CO), 19.73 (s, CH$_3$-2').

^{31}P NMR (202 MHz, CDCl$_3$) δ_P (ppm)**:** 3.86 (s).

92.b (slow eluting isomer containing 13% of fast isomer).

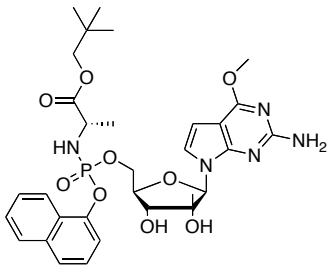
^{1}H NMR (500 MHz, CDCl$_3$) δ_H (ppm): 8.09 (m, 2H, H-9 Ar diastereoisomers), 7.80 (2H, m, H-6 Ar diastereoisomers), 7.70 (d, 2H, *J* = 8.0 Hz, H-4 Ar diastereoisomers), 7.51 & 7.50 & 7.49 & 7.48 & 7.46 & 7.44 (6s, 6H, H-2, H-7, H-8 Ar diastereoisomers), 7.34 (t, 2H, *J* = 8.0 Hz, H-3 Ar diastereoisomers), 6.70 (d, 1H, *J* = 3.5 Hz, H-8 base), 6.39 (d, 1H, *J* =3.5 Hz, H-8 base), 6.21 (d, 1H, *J* = 3.5 Hz, H-7 base), 6.07 (d, 1H, *J* = 3.5 Hz, H-7 base), 5.93 (bs, 2H, H-1' diastereoisomers), 4.85 & 4.81 (s, 2H, NH$_2$ base diastereoisomers), 4.66-4.58 (m, 2H, H-3' diastereoisomers), 4.40-4.36 (m, 2H, H-4' diastereoisomers), 4.11 (m, 2H, NHCH(CH$_3$)CO diastereoisomers), 4.07-4.00 (m, 2H, CH cHex diastereoisomers), 3.93 (s, 3H, 6-*O*CH$_3$ base), 3.91 (s, 3H, 6-*O*CH$_3$ base), 3.87-3.86 (m, 2H, H-5'), 3.83-3.80 (m, 2H, H-5'), 3.70-3.66 (m, 2H, NHCH(CH$_3$)CO diastereoisomers), 1.70-1.65 (m, 4H, 2 x CH$_2$ ortho cHex diastereoisomers), 1.59-1.57 (m, 4H, 2 x CH$_2$ ortho cHex diastereoisomers), 1.53 (m, 8H, 2 x CH$_2$ meta, 2 X CH$_2$ para cHex diastereoisomers), 1.42 (m, 2H, CH$_2$ meta cHex), 1.29 (d, 3H, *J* = 7.0 Hz, NHCH(CH$_3$)CO), 1.25 (d, 3H, *J* = 7.0 Hz, NHCH(CH$_3$)CO), 1.23-1.15 (m, 2H, CH$_2$ meta cHex), 0.81 (s, 3H, CH$_3$-2'), 0.74 (s, 3H, CH$_3$-2').

^{13}C NMR (125 MHz, CDCl$_3$) δ_c (ppm): 174.24 & 174.10 (2s, NHCH(CH$_3$)CO diastereoisomers), 166.83 & 166.35 (2s, C-6 base diastereoisomers), 160.98 & 160.65 (2s, C-2 base diastereoisomers), 156.78 & 156.19 (2s, C-4 base diastereoisomers), 147.65 & 147.53 (2s, C-1 Ar diastereoisomers), 136.63 & 136.49 (2s, C-5 Ar diastereoisomers), 128.93 & 128.67 (2s, C-6 Ar), 127.96 & 127.86 (2s, C-7 Ar diastereoisomers), 127.37 & 127.12 (2s, C-8 Ar diastereoisomers), 126.89 & 126.56 (2s, C-10 Ar diastereoisomers), 126.54 & 125.80 (2s, C-3 Ar diastereoisomers), 122.89 & 122.24 (2s, C-9 Ar diastereoisomers), 121.75 & 121.56 (2s, C-8 base diastereoisomers), 120.89 & 119.67 (2s, C-4 Ar diastereoisomers), 116.87 & 116.32 (2s, C-2 Ar diastereoisomers), 101.21 & 100.98 (2s, C-5 base diastereoisomers), 100.17 & 99.04 (2s, C-7 base diastereoisomers), 94.34 & 93.84 (2s, C-1' diastereoisomers), 84.56 & 84.09 (2d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 7.4 Hz, 8.1 Hz, C-4' diastereoisomers), 80.78 & 80.46 (2s, C-2' diastereoisomers), 74.98 & 74.56 (2s, C-3' diastereoisomers), 74.36 & 74.18 (2s, C ipso

cHex diastereoisomers), 66.34 & 66.18 (2d, $J^{2}_{P\text{-}O\text{-}C}$ =5.1 Hz, 5.0 Hz, C-5' diastereoisomers), 53.78 & 53.53 (2s, 6-*O*Me base diastereoisomers), 51.87 & 51.64 (2s, NHCH(CH_3)CO diastereoisomers), 33.13 & 32.67 & 31.75 & 30.97 (4s, C ortho cHex diastereoisomers), 26.52 & 26.23 (2s, C para cHex diastereoisomers), 20.80 & 20.78 & 20.67 & 20.34 (4s, C meta cHex diastereoisomers), 20.81 & 20.76 (2d, $J^{3}_{P\text{-}O\text{-}C\text{-}C}$ = 6.8 Hz, 6.8 Hz, NHCH(CH_3)CO diastereoisomers), 19.75 & 19.73 (2s, CH_3-2' diastereoisomers).

^{31}P NMR (202 MHz, *d*-$CDCl_3$) δ_P (ppm)**:** 3.86 (s, 13%), 4.62 (s, 87%).

Preparation of α-naphthyl L-Alanine-neopentyl-ester 2'-C-methyl-6-O-methyl-7-deaza-guanosine ProTide (93)



This ProTide was prepared according to the Standard Procedure D, using *t*BuMgCl (1M in THF, 0.28 mL, 0.28 mmol, 2eq), 7-deaza-6-*O*Me-2'-*C*-Me guanosine (**88**) (0.04 mg, 0.14 mmol, 1.0 eq) in anh. THF (0.41 mL), and *α*-naphthyl *L*-Alanine-neopentyl ester phosphochloridate (***14.d***) (0.11 g, 0.28 mmol, 2.0 eq) in anh. THF(0.41 mL). After overnight stirring at RT, the solvent was removed under reduced pressure and the residue was purified by flash chromatography, using $CHCl_3$/MeOH (4%) as eluents, and preparative chromatography to yield the desired molecule (0.0115 g, 0.017 mmol, 12%).

HPLC (MeOH/H_2O): Rt = 28.36 min

HPLC (ACN/H_2O): Rt = 20.29 min, 20.64 min

MS (TOF ES$^+$): 658.26 (M + H^+), 680.25 (M + Na^+).

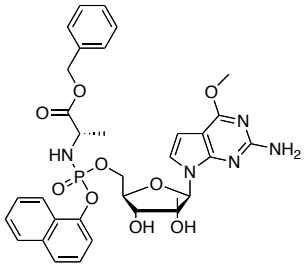
^{1}H NMR (500 MHz, MeOD) δ_H (ppm): 8.22-8.20 (m, 2H, H-9 Ar diastereoisomers), 7.93-7.90 (m, 2H, H-6 Ar diastereoisomers), 7.74-7.71 (m, 2H, H-4 Ar diastereoisomers), 7.59-7.54 (m, 6H, H-2, H-7, H-8 Ar diastereoisomers), 7.45-7.41 (m, 2H, H-3 Ar diastereoisomers), 6.79 (d, 1H, *J* = 4.0 Hz, H-8 base), 6.74 (d, 1H, *J* = 3.5 Hz, H-8 base), 6.18 (s, 1H, H-1'), 6.17 (d, 1H, *J* = 3.5 Hz, H-7 base), 6.15 (s, 1H, H-1'), 5.97 (d, 1H, *J* = 3.5 Hz, H-7 base), 4.50-4.47 (m, 2H, H-3' diastereoisomers), 4.18-4.16

(m, 2H, H-5' diastereoisomers), 4.09-4.04 (m, 4H, H-5', H-4' diastereoisomers), 4.00 & 3.99 (2s, 6H, 6-*O*CH_3 base diastereoisomers), 3.80-3.75 (m, 2H, O$\underline{CH_2}$C$(CH_3)_3$), 3.77-3.75 (m, 2H, NH$\underline{CH}(CH_3)$CO diastereoisomers), 3.65-3.62 (m, 2H, O$\underline{CH_2}$C$(CH_3)_3$), 1.35 (d, 3H, J = 7.5 Hz, NHCH($\underline{CH_3}$)CO), 1.31 (d, 3H, J = 7.0 Hz, NHCH($\underline{CH_3}$)CO), 0.93 & 0.91 (2s, 6H, CH_3-2' diastereoisomers), 0.87 & 0.85 (2s, 18H, OCH$_2$C($\underline{CH_3}$)$_3$ diastereoisomers).

^{13}C NMR (1265 MHz, MeOD) δ_c (ppm): 174.93 & 174.89 (2s, NHCH(CH_3)$\underline{C}$O diastereoisomers), 165.26 & 165.23 (2s, C-6 base diastereoisomers), 161.16 & 160.07 (2s, C-2 base diastereoisomers), 155.32 & 155.22 (2s, C-4 base diastereoisomers), 148.06 & 147.99 (2s, C-1 Ar diastereoisomers), 136.39 & 136.36 (2s, C-5 Ar diastereoisomers), 128.97 & 128.91 (2s, C-6 Ar diastereoisomers), 127.90 & 127.81 (2s, C-7 Ar diastereoisomers), 127.51 & 127.45 (2s, C-8 Ar diastereoisomers), 126.58 & 126.49 (2s, C-10 Ar diastereoisomers), 125.96 & 125.89 (2s, C-3 Ar diastereoisomers), 122.75 & 122.58 (2s, C-9 Ar diastereoisomers), 120.78 & 120.74 (2s, C-8 base diastereoisomers), 120.33 & 120.27 (2s, C-4 Ar diastereoisomers), 116.15 & 115.74 (2d, $J^3_{P\text{-}O\text{-}C\text{-}C}$= 3.5 Hz, 2.9 Hz, C-2 Ar diastereoisomers), 100.99 & 100.88 (2s, C-7 base diastereoisomers), 99.64 & 99.41 (2s, C-5 base diastereoisomers), 92.66 & 92.24 (2s, C-1' diastereoisomers), 81.36 & 81.22 (2d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 11.3 Hz, 8.9 Hz, C-4' diastereoisomers), 80.17 & 80.06 (2s, C-2' diastereoisomers), 74.63 & 74.59 (2s, O$\underline{CH_2}$C$(CH_3)_3$ diastereoisomers), 74.03 & 74.01 (2s, C-3' diastereoisomers), 67.46 & 66.21 (2d, $J^2_{P\text{-}O\text{-}C}$ = 6.2 Hz, 5.7 Hz, C-5' diastereoisomers), 53.85 & 53.77 (2s, 6-*O*CH_3 base diastereoisomers), 51.69 & 51.64 (2s, NH$\underline{C}$H(CH_3)CO diastereoisomers), 32.25 & 32.21 (2s, OCH$_2\underline{C}(CH_3)_3$ diastereoisomers), 26.72 & 26.69 (2s, OCH$_2$C($\underline{CH_3}$)$_3$ diastereoisomers), 20.83 & 20.72 (2d, $J^3_{P\text{-}N\text{-}C\text{-}C}$= 6.5 Hz, 6.9 Hz, NHCH($\underline{C}H_3$)CO diastereoisomers), 19.99 & 19.94 (2s, CH_3-2' diastereoisomers).

^{31}P NMR (202 MHz, MeOD) δ_P (ppm)**:** 4.13 (s, 24%), 4.09 (s, 76%).

Preparation of α-naphthyl L-Alanine-benzyl-ester 2'-C-methyl-6-O-methyl-7-deaza-guanosine ProTide (94)



This ProTide was prepared according to the Standard Procedure D, using *t*BuMgCl (1M in THF, 0.35 mL, 0.35 mmol, 2eq), 7-deaza-6-*O*Me-2'-*C*-Me guanosine (**88**) (0.054 g, 0.17 mmol, 1.0 eq) in anh. THF (1.00 mL), *α*-naphthyl *L*-Alanine-benzyl ester phosphochloridate (***14.f***) (0.141 g, 0.35 mmol, 2.0 eq) in anh. THF (0.51 mL). After overnight stirring at RT, the solvent was removed under reduced pressure and the residue was purified by flash chromatography, using $CHCl_3$/MeOH (3.5%) as eluents, and preparative chromatography to yield the desired molecule (0.0149 g, 0.022 mmol, 13%).

HPLC (MeOH/H_2O): Rt = 23.41 min.

HPLC (ACN/H_2O): Rt = 16.67 & 16.93 min.

MS (TOF ES^+): 678.23 ($M + H^+$).

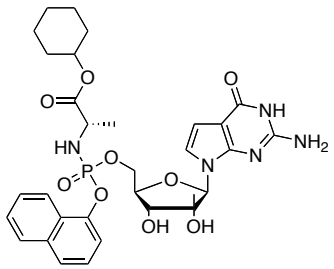
^{1}H NMR (500 MHz, MeOD) δ_H (ppm): 8.21 (2d, 2H, *J* = 8.5 Hz, H-9 Ar diastereoisomers), 7.91-7.89 (m, 4H, OCH_2Ph diastereoisomers), 7.72-7.70 (m, 2H, H-6 Ar diastereoisomers), 7.68 (d, 2H, *J* = 8.2 Hz, H-4 Ar diastereoisomers), 7.56-7.49 (m, 6H, H-2, H-7, H-8 Ar diastereoisomers), 7.43-7.37 (m, 2H, H-3 Ar diastereoisomers), 7.27-7.25 (m, 6H, OCH_2Ph diastereoisomers), 6.80 (d, 1H, *J* = 3.5 Hz, H-8 base), 6.73 (d, 1H, *J* = 4.0 Hz, H-8 base), 6.17 (s, 1H, H-1'), 6.16 (d, 1H, *J* = 3.5 Hz, H7 base), 6.15 (s, 1H, H-1'), 5.94 (d, 1H, *J* = 4.0 Hz, H-7 base), 5.07-4.98 (m, 2H, COOCH_2Ph), 4.58-4.55 (m, 2H, COOCH_2Ph), 4.48-4.45 (m, 2H, H-3' diastereoisomers), 4.15-4.04 (m, 4H, H-4', H-5' diastereoisomers), 4.04 & 3.98 (2s, 6H, 6-*O*CH_3 base diastereoisomers), 3.33-3.32 (m, 4H, H-5', NHCH(CH_3)CO diastereoisomers), 1.32-1.29 (m, 6H, NHCH(CH_3)CO diastereoisomers), 0.85 & 0.79 (2s, 6H, CH_3-2' diastereoisomers).

^{13}C NMR (125 MHz, MeOD) δ_c (ppm): 174.31 & 174.20 (2s, NHCH(CH_3)CO diastereoisomers), 166.75 & 166.12 (2s, C-6 base diastereoisomers), 161.35 & 161.02 (2s, C-2 base diastereoisomers), 156.63 & 156.47 (2s, C-4 base diastereoisomers), 147.42 & 147.18 (2s, C-1 Ar diastereoisomers), 138.65 & 138.18 (2s, C-5 Ar

diastereoisomers), 136.53 & 136.09 (2s, C ipso Ph diastereoisomers), 129.51 & 129.47 & 129.40 & 129.31 & 129.27 & 129.18 & 128.89 & 128.67 (8s, C meta Ph, C para Ph, C-6 Ar diastereoisomers), 127.85 & 127.81 & 127.79 & 127.61 & 127.54 & 127.50 (6s, C ortho Ph, C-7 Ar diastereoisomers), 126.58 & 126.34 (2s, C-8 Ar diastereoisomers), 126.02 & 125.93 (2s, C-10 Ar diastereoisomers), 125.43 & 125.09 (2s, C-3 Ar diastereoisomers), 122.75 & 122.23 (2s, C-9 Ar diastereoisomers), 121.97 & 121.76 (2s, C-8 base diastereoisomers), 120.48 & 120.12 (2s, C-4 Ar diastereoisomers), 115.19 & 115.01 (2s, C-2 Ar diastereoisomers), 101.93 & 100.84 (2s, C-7 base diastereoisomers), 99.45 & 99.16 (2s, C-5 base diastereoisomers), 92.68 & 92.15 (2s, C-1' diastereoisomers), 84.56 & 84.34 (2d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ =7.4 Hz, 8.2 Hz, C-4' diastereoisomers), 81.26 & 80.87 (2s, C-2' diastereoisomers), 74.63 & 74.29 (2s, C-3' diastereoisomers), 73.98 & 73.17 (2s, O<u>CH$_2$</u>Phe diastereoisomers), 67.94 & 67.87 (2d, $J^2_{P\text{-}O\text{-}C}$ = 5.9 Hz, 5.5 Hz, C-5' diastereoisomers), 54.02 & 53.78 (2s, 6-*O*CH$_3$ base diastereoisomers), 51.71 & 51.31 (2s, NH<u>C</u>H(CH$_3$)CO diastereoisomers), 20.78 & 20.45 (2s, CH$_3$-2' diastereoisomers), 19.97 & 19.23 (2d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 6.5 Hz, 6.8 Hz, NHCH(<u>CH$_3$</u>)CO diastereoisomers).

^{31}P NMR (202 MHz, MeOD-d_4) δ_P (ppm)**:** 4.28 (s, 47%), 4.18 (s, 53%).

Preparation of α-naphthyl L-Alanine-cyclohexyl-ester 2'-C-methyl-7-deaza-guanosine ProTide (98)



To a solution of *α*-naphthyl *L*-Alanine-cyclohexyl-ester 2'-C-methyl-6-O-methyl-7-deaza-guanosine ProTide (**92**) (4.5 mg, 0.007 mmol, 1.0 eq) in anh. ACN (1.0 mL) was added NaI (0.0016 g, 0.011 mmol, 1.5 eq) and TMSCl (1.5 µL, 0.011 mmol, 1.5 eq). The reaction was stirred at RT for 1 hr, then the solvent was evaporated under reduced pressure and the residue was purified by preparative chromatography using CH_2Cl_2/MeOH as eluants, to recover the desired molecule (0.0016 g, 0.002 mmol, 34%).

HPLC (MeOH/H_2O): Rt = 25.82 min, 26.55 min

HPLC (ACN/H_2O): Rt = 15.32 min, 15.84 min

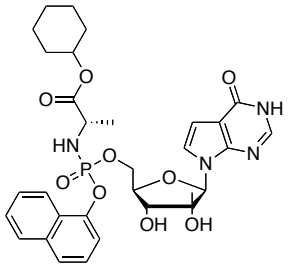
MS (TOF ES^+): 656.23 ($M + H^+$).

^1H NMR (500 MHz, MeOD) δ_H (ppm): 8.23-8.21 (m, 2H, H-9 Ar diastereoisomers), 7.93-7.90 (m, 2H, H-6 Ar diastereoisomers), 7.74-7.71 (m, 2H, H-4 Ar diastereoisomers), 7.59-7.54 (m, 6H, H-2, H-7, H-8 Ar diastereoisomers), 7.45 (t, 2H, *J* = 8.0 Hz, H-3 Ar diastereoisomers), 6.75 (d, 1H, *J* = 3.7 Hz, H-8 base), 6.71 ((d, 1H, *J* = 3.7 Hz, H-8 base), 6.31 (d, 1H, *J* = 3.7 Hz, H-7 base), 6.19 (d, 1H, *J* = 3.7 Hz, H-7 base), 6.11 (s, 1H, H-1'), 6.10 (s, 1H, H-1'), 4.64-4.51 (m, 8H, H-3', H-5', H-4' diastereoisomers), 4.28-4.24 (m, 2H, CH cHex diastereoisomers), 3.99-3.95 (m, 2H, NHCH(CH_3)CO diastereoisomers), 1.74-1.66 (m, 8H, 4 x CH_2 ortho cHex diastereoisomers), 1.51-1.37 (m, 8H, 2 x CH_2 para, 2 x CH_2 meta cHex diastereoisomers), 1.35-1.29 (m, 6H, NHCH(CH_3)CO diastereoisomers), 1.14-1.11 (m, 4H, CH_2 meta diastereoisomers), 0.89 (s, 3H, CH_3-2'), 0.84 (s, 3H, CH_3-2').

^{13}C NMR (125 MHz, MeOD) δ_c (ppm): 170.05 & 169.86 (s, NHCH(CH_3)CO diastereoisomers), 159.87 & 159,35 (s, C-6 base diastereoisomers), 153.67 & 153.40 (s, C-2 base diastereoisomers), 150.10 & 150.02 (s, C-4 base diastereoisomers), 139.86 & 139.72 (s, C-1 Ar diastereoisomers), 136.61 & 136.43 (2s, C-5 Ar diastereoisomers), 128.94 & 128.91 (2s, C-6 Ar diastereoisomers), 127.90 & 127.80 (2s, C-7 Ar diastereoisomers), 127.67 & 127.58 (2s, C-8 Ar diastereoisomers),127.53 & 127.35 (s, C-10 Ar diastereoisomers), 126.53 & 125.81 (2s, C-3 Ar diastereoisomers), 122.98 & 122.54 (2s, C-9 Ar diastereoisomers), 120.76 & 119.55 (2s, C-4 Ar diastereoisomers), 118.99 & 118.54 (2s, C-8 base diastereoisomers), 115.65 & 115.32 (2s, C-2 Ar diastereoisomers), 103.79 & 103.66 (2s, C-7 base diastereoisomers), 99.36 & 99.21 (s, C-5 base diastereoisomers), 92.31 & 92.18 (2s, C-1' diastereoisomers), 81.20 & 81.02 (2s, C-4' diastereoisomers), 78.56 & 78.43 (2s, C-2' diastereoisomers), 73.92 & 73.83 (2s, C-3' diastereoisomers), 73.91 & 73.56 (2s, C ipso cHex diastereoisomers), 66.13 & 66.01 (2s, C-5' diastereoisomers), 51.76 & 51.63 (2s, NHCH(CH_3)CO diastereoisomers), 32.30 & 32.18 (2s, C ortho cHex diastereoisomers), 26.35 & 26.11 (1s, C para cHex diastereoisomers), 25.56 & 25.44 (2s, C meta cHex diastereoisomers), 20.62 & 20.55 (2s, CH_3-2' diastereoisomers), 20.01 & 19.98 (2s, NHCH(CH_3)CO diastereoisomers).

^{31}P NMR (202 MHz, MeOD) δ_P (ppm)**:** 4.18 (s, 68%), 4.14 (s, 32%).

Preparation of α-naphthyl L-Alanine-cyclohexyl-ester 2'-C-methyl-7-deaza-inosine ProTide (108)



This ProTide was prepared according to the Standard Procedure D, using *t*BuMgCl (1M in THF, 0.19 mL, 0.19 mmol, 2eq), 7-deaza-2'-*C*-methyl inosine (**107**) (0.027 g, 0.10 mmol, 1.0 eq) in anh. THF (1.00 mL), and *α*-naphthyl *L*-Alanine-cyclohexyl ester phosphochloridate (***14.e***) (0.075 g, 0.19 mmol, 2.0 eq) in anh. THF (0.51 mL). After overnight stirring at RT, the solvent was removed under reduced pressure and the residue was purified by flash chromatography, using $CHCl_3$/MeOH (3.0%) as eluents, and preparative chromatography to yield the desired molecule (0.001 g, 0.0016 mmol, 2%).

HPLC (MeOH/H_2O): Rt = 26.30 min, 27.04 min

HPLC (ACN/H_2O): Rt = 15.87 min, 16.39 min

MS (TOF ES^+): 641.24 ($M + H^+$).

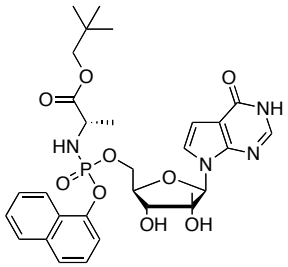
^{1}H NMR (500 MHz, MeOD) δ_H (ppm): 8.23-8.22 (m, 2H, H-2 base diastereoisomers), 7.93-7.91 (m, 4H, H-4, H-9 Ar diastereoisomers), 7.75-7.71 (m, 2H, H-6 Ar diastereoisomers), 7.67-7.64 (m, 2H, H-4 Ar diastereoisomers), 7.59-7.54 (m, 6H, H-3, H-7, H-8 Ar diastereoisomers), 7.45-7.32 (2t, 2H, *J* = 8.0 Hz, 7.5 Hz, H-2 Ar diastereoisomers), 7.11 (d, 1H, *J* = 3.5 Hz, H-8 base), 7.063 (d, 1H, *J* = 3.5 Hz, H-8 base), 6.51 (d, 1H, *J* = 3.5 Hz, H-7 base), 6.35 (d, 1H, *J* = 3.0 Hz, H-7 base), 6.29 & 6.28 (2s, H-1' diastereoisomers), 4.66-4.63 (m, 4H, H-5', H-3' diastereoisomers), 4.55-4.50 (m, 4H, H-5', H-4' diastereoisomers), 4.23-4.20 (m, 2H, NHCH(CH_3)CO diastereoisomers), 4.08 (dd, 2H, *J* = 1.5 Hz, 8.5 Hz, CH cHex diastereoisomers), 1.77-1.65 (m, 12H, 2 x CH_2 para, 4 x CH_2 meta cHex diastereoisomers), 1.52-1.49 (m, 2H, 2 x CH_2 ortho cHex), 1.37-1.29 (m, 12H, 2 x CH_2 ortho cHex, CH_3-2' diastereoisomers), 0.83 (d, 3H, *J* = 3.5 Hz, NHCH($\underline{CH_3}$)CO), 0.78 (d, 3H, *J* = 3.5 Hz, NHCH($\underline{CH_3}$)CO).

^{13}C NMR (125 MHz, MeOD) δ_c (ppm): 171.53 & 171.23 (2s, NHCH(CH_3)$\underline{C}$O diastereoisomers), 161.42 & 161.37 (2s, C-6 base diastereoisomers), 149.35 & 149.28 (2s, C-4 base diastereoisomers), 148.12 & 147.96 (s, C-1 Ar diastereoisomers), 144.24

& 144.21 (2s, C-2 base diastereoisomers), 136.39 & 136.37 (2s, C-5 Ar diastereoisomers), 128.98 & 128.95 (2s, C-6 Ar diastereoisomers), 127.93 & 127.83 & 127.54 & 127.52 (4s, C-7, C-8 Ar diastereoisomers), 126.58 & 126.55 (2s, C-10 Ar diastereoisomers), 125.98 & 125.88 (2s, C-3 Ar diastereoisomers), 122.70 & 122.58 (2s, C-9 Ar diastereoisomers), 122.20 & 122.13 (s, C-4 Ar diastereoisomers), 116.12 & 116.10 (2s, C-8 base diastereoisomers), 115.69 & 115.66 (2s, C-2 Ar diastereoisomers), 109.65 & 109.53 (2s, C-5 base diastereoisomers), 104.05 & 103.97 (2s, C-7 base diastereoisomers), 93.15 & 92.84 (2s, C-1' diastereoisomers), 81.60 & 81.47 (2s, $J^{3}_{P\text{-}O\text{-}C\text{-}C}$ = 7.9 Hz, C-4' diastereoisomers), 80.23 & 80.15 (2s, C-2' diastereoisomers), 74.98 & 74.94 (2s, C-3' diastereoisomers), 74.25 & 73.74 (2s, C ipso cHex diastereoisomers), 67.16 & 66.12 (2d, $J^{2}_{P\text{-}O\text{-}C}$ = 5.1 Hz, C-5' diastereoisomers), 51.80 & 51.20 (2s, NHCH(CH_3)CO diastereoisomers), 32.38 & 32.32 & 30.76 & 30.70 (4s, C ortho cHex diastereoisomers), 26.36 & 24.28 (2s, C para cHex diastereoisomers), 20.80 & 20.78 & 20.75 & 20.70 (4s, C meta cHex diastereoisomers), 20.61 & 20.55 (2s, NHCH(CH_3)CO diastereoisomers), 19.84 & 19.82 (2s, CH_3-2' diastereoisomers).

^{31}P NMR (202 MHz, MeOD) δ_P (ppm)**:** 4.17 (s, 55%), 4.15 (s, 45%).

Preparation of α-naphthyl L-Alanine-neopentyl-ester 2'-C-methyl-7-deaza-inosine ProTide (109)



This ProTide was prepared according to the Standard Procedure D, using *t*BuMgCl (1M in THF, 0.38 mL, 0.38 mmol, 2eq), 7-deaza-2'-*C*-methyl inosine (**107**) (0.053 g, 0.19 mmol, 1.0 eq) in anh. THF (1.00 mL). and *α*-naphthyl *L*-Alanine-neopentyl ester phosphochloridate (**14.d**) (0.15 g, 0.38 mmol, 2.0 eq) in anh. THF (0.50 mL). After overnight stirring at RT, the solvent was removed under reduced pressure and the residue was purified by flash chromatography, using $CHCl_3$/MeOH (5%) as eluents, and preparative chromatography to yield the desired molecule (0.0007 g, 0.0011 mmol, 1%).

HPLC (MeOH/H_2O): Rt = 23.07 min, 23.29 min

HPLC (ACN/H_2O): Rt = 15.75 min, 16.20 min

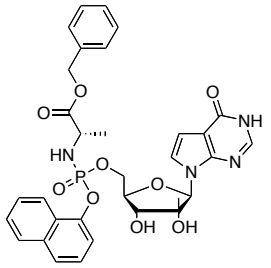
MS (TOF ES^+): 629.22 ($M + H^+$), 652.46 ($M + Na^+$).

^1H NMR (500 MHz, MeOD) δ_H (ppm): 8.23-8.22 (m, 2H, H-2 base diastereoisomers), 7.93-7.91 (m, 4H, H-4, H-9 Ar diastereoisomers), 7.75-7.71 (m, 2H, H-3 Ar diastereoisomers), 7.60-7.54 (m, 6H, H-6, H-7, H-8 Ar diastereoisomers), 7.45-7.43 (m, 2H, H-2 Ar diastereoisomers), 7.09 (d, 1H, *J* =3.5 Hz, H-8 base), 7.05 (d, 1H, *J* =3.5 Hz, H-8 base), 6.50 (d, 1H, *J* =3.0 Hz, H-7 base), 6.35 (d, 1H, *J* =3.5 Hz, H-7 base), 6.29 & 6.27 (2s, H-1' diastereoisomers), 4.60-4.50 (m, 2H, H-5' diastereoisomers), 4.23-4.51 (m, 3H, H-3' diastereoisomers, H-5'), 4.08-4.03 (m, 4H, H-4', NHCH(CH_3)CO diastereoisomers), 3.90-3.86 (m, 1H, H-5'), 3.82-3.77 (AB system, 2H, J_{AB} = 10.5 Hz, COOCH_2C(CH_3)$_3$), 3.69-3.65 (AB system, 2H, J_{AB} = 10.5 Hz, OCH_2C(CH_3)$_3$), 1.38 & 1.34 (2s, 6H, CH_3-2' diastereoisomers), 0/90 & 0.88 (2s, 18H, OCH$_2$C(CH_3)$_3$), 0.83 (d, 3H, *J* = 1.5 Hz, NHCH(CH_3)CO), 0.78 (d, 3H, *J* = 1.5 Hz, NHCH(CH_3)CO).

^{13}C NMR (125 MHz, MeOD) δ_c (ppm): 170.32 & 170.03 (2s, NHCH(CH_3)CO diastereoisomers), 160.70 & 160.67 (2s, C-6 base diastereoisomers), 151.49 & 151.41 (2s, C-4 base diastereoisomers), 148.66 & 148.63 (2s, C-1 Ar diastereoisomers), 144.22 & 144.16 (2s, C-2 base diastereoisomers), 134.46 & 134.43 (2s, C-5 Ar diastereoisomers), 129.39 & 129.35 (2s, C-6 Ar diastereoisomers), 128.05 & 127.96 & 127.94 & 127.85 (2s, C-7, C-8 Ar diastereoisomers), 126.58 & 126.54 (2s, C-10 Ar diastereoisomers), 125.92 & 125.90 (2s, C-3 Ar diastereoisomers), 122.69 & 122.65 (2s, C-9 Ar diastereoisomers), 122.22 & 122.19 (2s, C-4 Ar diastereoisomers), 116.10 & 116.05 (2s, C-8 base diastereoisomers), 115.74 & 115.69 (2s, C-2 Ar diastereoisomers), 110.12 & 110.09 (2s, C-5 base diastereoisomers), 104.05 & 103.99 (2s, C-7 base diastereoisomers), 92.93 & 92.86 (2s, C-1' diastereoisomers), 84.07 & 84.05 (2s, C-4' diastereoisomers), 75.45 & 75.38 (2s, C-2' diastereoisomers), 74.60 & 74.58 (2s, COOCH_2C(CH_3)$_3$ diastereoisomers), 73.76 & 73.72 (2s, C-3' diastereoisomers), 61.21 & 61.20 (2s, C-5' diastereoisomers), 50.23 & 50.20 (2s, NHCH(CH_3)CO diastereoisomers), 30.75 & 30.71 (2s, COOCH$_2$C(CH_3)$_3$), 26.72 & 26.59 (2s, COOCH$_2$C(CH_3)$_3$ diastereoisomers), 19.87 & 19.85 (2s, NHCH(CH_3)CO diastereoisomers), 18.70 & 18.40 (CH_3-2' diastereoisomers).

^{31}P NMR (202 MHz, MeOD) δ_P (ppm)**:** 4.18 (s, 65%), 4.12 (s, 35%).

Preparation of α-naphthyl L-Alanine-benzyl-ester 2'-C-methyl-7-deaza-inosine ProTide (110)



This ProTide was prepared according to the Standard Procedure D, using *t*BuMgCl (1M in THF, 0.36 mL, 0.36 mmol, 2eq), 7-deaza-2'-*C*-methyl inosine (**107**) (0.050 g, 0.18 mmol, 1.0 eq) in anh. THF (1.00 mL), and *α*-naphthyl *L*-Alanine-benzyl ester phosphochloridate (**14.f**) in anh. THF (1.50 mL). After overnight stirring at RT, the solvent was removed under reduced pressure and the residue was purified by flash chromatography, using $CHCl_3$/MeOH (5%) as eluents, and preparative chromatography to yield the desired molecule (0.0022 g, 0.0034 mmol, 2%).

HPLC (MeOH/H_2O): Rt = 25.05 min, 25.80 min

HPLC (ACN/H_2O): Rt = 14.88 min, 15.35 min

MS (TOF ES^-): 683.11 ($M + Cl^-$).

^{1}H NMR (500 MHz, MeOD) δ_H (ppm): 8.21-8.20 (m, 2H, H-2 base diastereoisomers), 7.92-7.90(m, 4H, H-4, H-9 Ar diastereoisomers), 7.73-7.71 (m, 2H, H-3 Ar diastereoisomers), 7.57-7.53 (m, 6H, H-6, H-7, H-8 Ar diastereoisomers), 7.44-7.39 (m, 2H, H-2 Ar diastereoisomers), 7.30-7.26 (m, 10H, $OCH_2\underline{Ph}$ diastereoisomers), 7.08 (d, 1H, *J* = 3.5 Hz, H-8 base), 7.065(d, 1H, *J* = 4.0 Hz, H-8 base), 6.48 (d, 1H, *J* = 3.5 Hz, H-7 base), 6.34 (d, 1H, *J* = 4.0 Hz, H-7 base), 6.28 & 6.27 (2s, H-1' diastereoisomers), 5.10-5.50 (m, 4H, $CO\underline{CH_2}Ph$ diastereoisomers), 4.51-4.48 (m, 2H, H-5' diastereoisomers), 4.21-4.04 (m, 8H, H-5', H-3', H-4', $NH\underline{CH}(CH_3)CO$ diastereoisomers), 1.31 & 1.30 (2s, 6H, CH_3-2' diastereoisomers), 0.83 (d, 3H, *J* = 3.5 Hz, $NHCH(\underline{CH_3})CO$), 0.78 (d, 3H, *J* = 3.5 Hz, $NHCH(\underline{CH_3})CO$).

^{13}C NMR (125 MHz, MeOD) δ_c (ppm): 171.19 & 171.12 (2s, $NHCH(CH_3)\underline{C}O$ diastereoisomers), 161.38 & 161.37 (2s, C-6 base diastereoisomers), 149.33 & 149.27 (2s, C-4 base diastereoisomers), 148.12 & 148.09 (s, C-1 Ar diastereoisomers), 144.24 & 144.11 (2s, C-2 base diastereoisomers), 137.13 & 137.10 (2s, C ipso Ph diastereoisomers), 136.37 & 136.35 (2s, C-5 Ar diastereoisomers), 129.55 & 129.54 & 129.53 & 129.52 (4s, C meta Ph diastereoisomers), 129.32 & 129.28 (2s, C para Ph

diastereoisomers), 129.24 & 129.22 & 129.17 & 129.15 (4s, C ortho Ph diastereoisomers), 128.96 & 128.94 (2s, C-6 Ar diastereoisomers), 127.93 & 127.84 (2s, C-7 Ar diastereoisomers), 127.59 & 127.55 (2s, C-8 Ar diastereoisomers), 126.59 & 126.53 (2s, C-10 Ar diastereoisomers), 126.01 & 125.93 (2s, C-3 Ar diastereoisomers), 122.69 & 122.56 (2s, C-9 Ar diastereoisomers), 122.22 & 122.20 (2s, C-4 Ar diastereoisomers), 116.17 & 116.14 (2s, C-8 base diastereoisomers), 115.80 & 115.77 (2s, C-2 Ar diastereoisomers), 109.53 & 109.50 (2s, C-5 base diastereoisomers), 104.01 & 103.97 (2s, C-7 base diastereoisomers), 93.12 & 92.82 (2s, C-1' diastereoisomers), 81.51 & 81.44 (2d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 6.4 Hz, 6.45 Hz, C-4' diastereoisomers), 80.22 & 80.17 (2s, C-2' diastereoisomers), 74.69 & 73.69 (2s, C-3' diastereoisomers), 67.96 & 67.93 (2s, CO$\underline{CH_2}$Phe), 67.15 & 66.09 (2d, $J^2_{P\text{-}O\text{-}C}$ = 8.4 Hz, 4.7 Hz, C-5' diastereoisomers), 51.78 & 51.73 (2s, NH$\underline{CH}$(CH_3)CO diastereoisomers), 20.54 & 20.37 (2d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 6.4 Hz, 7.7 Hz, NHCH($\underline{CH_3}$)CO diastereoisomers), 19.87 & 19.83 (2s, CH_3-2' diastereoisomers).

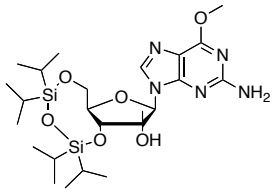
^{31}P NMR (202 MHz, MeOD) δ_P (ppm)**:** 4.21 (s, 61%), 4.08 (s, 39%).

5. Experimental procedures related to chapter 5

5.1. 2'-*O*-modifications of 2'-*C*-methyl-6-*O*-methyl guanosine

5.1.1. Intermediates prepared with the use of Markiewicz's reagent

Preparation of 3',5'-protected 2'-C-methyl-6-O-methyl guanosine (118)

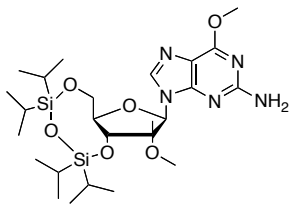


To 2'-*C*-methyl-6-*O*-methyl guanosine (**11**, provided by Inhibitex *Inc.*) (0.81 g, 2.60 mmol) in anh. pyridine (20.0 mL) was added $TPDSCl_2$ (1.2 eq, 3.12 mmol, 1.0 mL). The solution was left stirring 30 min at 0 °C, then overnight at RT. The solvents were evaporated under reduced pressure and the residue was purified by flash chromatography, using $CHCl_3$/MeOH as eluents, to recover a white solid (0.63 g, 1.17 mmol, 45%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 7.91 (s, 1H, H-8 base), 6.05 (s, 1H, H-1'), 4.28 (d, 1H, J = 9.0 Hz, H-3'), 4.23 (d, 1H, J = 1.5 Hz, H-5'), 4.20 (d, 1H, J = 1.5 Hz, H-5'), 4.12 (d, 1H, J = 9.0 Hz, H-4'), 4.01 (s, 3H, 6-*O*CH_3 base), 1.12 (m, 4H, 4 x CH), 1.03 (m, 27H, 8 x CH_3, CH_3-2').

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 161.59 (s, C-6 base), 159.85 (s, C-2 base), 153.14 (s, C-4 base), 136.18 (s, C-8 base), 115.46 (s, C-5 base), 89.99 (s, C-1'), 81.43 (s, C-4'), 79.39 (s, C-2'), 73.44 (s, C-3'), 60.45 (s. C-5'), 53.70 (s, 6-*O*CH_3 base), 20.72 (s, CH_3-2'), 17.45-16.30 (12s, 4 x CH, 8 x CH_3).

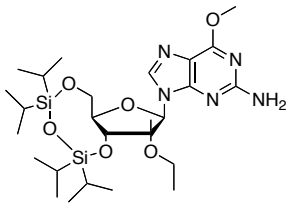
Preparation of 3',5'-protected 2'-C-methyl-2'-O-methyl-6-O-methyl guanosine (119.a)



3',5'-protected 2'-C-methyl-6-O-methyl guanosine (**118**) (1.15 g, 2.14 mmol) was dissolved in anh. DMF (15 mL) and molecular sieves 3Å were added. At 0 °C, NaH (60% in dispersion oil, 0.10 g, 2.35 mmol, 1.1 eq) and methyliodide (MeI) (0.42 mL, 6.42 mmol, 3.0 eq) were added and the solution was stirred for 1 hr. Then NaH (60% in

dispersion oil, 0.10 g, 2.35 mmol, 1.1 eq) was added, and the solution was stirred for 30 min at 0 °C. Absolute EtOH (61 mL) and ice cold EtOAc were added, and the solution was washed with NH_4Cl, dried over Na_2SO_4, the solvents were evaporated under reduced pressure and the residue was purified by flash chromatography, using EtOAc (25-30%)/petroleum ether, to yield the desired unpure material (0.057 g, 0.10 mmol, 5%).

Preparation of 3',5'-protected 2'-C-methyl-2'-O-ethyl-6-O-methyl guanosine (119.b)

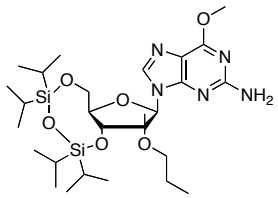


Similar to procedure for **119.a**, using 3',5'-protected 2'-C-methyl-6-O-methyl guanosine (**118**) (0.2092 g, 0.39 mmol) in anh. DMF (4 mL), molecular sieves 3Å, NaH (60% in dispersion oil, 0.0198 g, 0.50 mmol, 1.3 eq), 2-iodoethanol (0.07 mL, 1.18 mmol, 3 eq) and NaH (60% in dispersion oil, 0.0226 g, 0.57 mmol). The solution was stirred for 1 hr at 0 °C. Asbsolute EtOH (11 mL) and ice cold EtOAc (56 mL) were added, and the solution was washed with NH_4Cl, dried over Na_2SO_4, and the solvents were evaporated under reduced pressure. The purification by flash chromatography with EtOAc/Hexane allowed the recovery of the desired material (0.100 g, 0.17 mmol, 44%).

^{1}H NMR (500 MHZ, $CDCl_3$) δ_H (ppm): 8.46 (s, 1H, H-8 base), 6.19 (s, 1H, H-1'), 4.8 (bs, 2H, NH_2 base), 4.27 (m, 2H, H-4', H-3'), 4.15 (dd, 1H, *J* = 1.5 Hz, 12 Hz, H-5'), 4.09 (s, 3H, 6-*O*CH_3 base), 4.03 (dd, 1H, *J* = 2.0 Hz, 12.0 Hz, H5'), 3.93 (m, 1H, O$\underline{CH_2}CH_3$), 3.76 (m, 1H, O$\underline{CH_2}CH_3$), 1.33 (t, 3H, *J* = 7.0 Hz, $OCH_2\underline{CH_3}$), 1.07 (d, 24 H, *J* = 2.0 Hz, 8 x CH_3), 1.05 (m, 4H, 4 x CH), 1.00 (s, 3H, CH_3-2').

^{13}C NMR (125 MHz, $CDCl_3$) δ_c (ppm): 161.55 (s, C-6 base), 159.27 (s, C-2 base), 153.29 (s, C-4 base), 138.02 (s, C-8 base), 115.38 (s, C-5 base), 86.32 (s, C-1'), 83.66 (s, C-2'), 83.16 (s, C-4'), 72.77 (s, C-3'), 60.16 (s, C-5'), 59.19 (s, O$\underline{CH_2}CH_3$), 53.91 (s, 6-*O*CH_3 base), 17.40-17.13 (13s, 8 x CH_3, 4 x CH, CH_3-2'), 15.44 (s, CH_3 $OCH_2\underline{CH_3}$).

Preparation of 3',5'-protected 2'-C-methyl-2'-O-propyl-6-O-methyl guanosine (119.c)



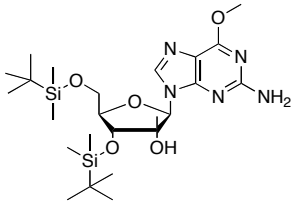
Similar to procedure for **119.a**, using 3',5'-protected 2'-*C*-methyl-6-*O*-methyl guanosine (**118**) (0.05 g, 0.09 mmol) in anh. DMF (1 mL), molecular sieves 3Å, 1-iodopropane (0.04 mL, 0.40 mmol, 4.3 eq), NaH (60% dispersion in mineral oil, 0.03 g, 1.11 mmol, 1.2 eq). The solution was stirred for 1 hr at 0 °C. Asbsolute EtOH (3.0 mL) and ice cold EtOAc (30 mL) were added, and the solution was washed with NH_4Cl, dried over Na_2SO_4, and the solvents were evaporated under reduced pressure. The purification by flash chromatography with EtOAc/Hexane allowed the recovery of the desired material (0.03 g, 0.06 mmol, 62%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.47 (s, 1H, H-8 base), 6.19 (s, 1H, H-1'), 4.82 (bs, 2H, NH_2 base), 4.28 (d, 1H, J = 1.5, H-3'), 4.16 (m, 2H, O$\underline{CH_2}CH_2CH_3$), 4.09 (s, 3H, 6-*O*CH_3 base), 4.02 (d, 1H, H-4'), 3.83 (m, 1H, H-5'), 3.78 (m, 1H, H-5'), 1.29 (m, 2H, $OCH_2\underline{CH_2}CH_3$), 1.09 (s, 24H, 8 x CH_3), 1.07-1.03 (m, 7H, 4 x CH, $OCH_2CH_2\underline{CH_3}$), 0.99 (s, 3H, CH_3-2').

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 161.55 (s, C-6 base), 159.29 (s, C-2 base), 153.28 (s, C-4 base), 138.06 (s, C-8 base), 115.36 (s, C-5 base), 86.24 (s, C-1'), 83.22 (s, C-4'), 83.51 (s, C-2'), 72.87 (s, C-3'), 65.07 (s, C-5'), 60.18 (s, O$\underline{CH_2}CH_2CH_3$), 53.93 (s, 6-*O*CH_3 base), 23.36 (s, $OCH_2\underline{CH_2}CH_3$), 17.3-17.13 (10s, 8 x $\underline{C}H_3$, 4 x $\underline{C}$H), 15.41 (s, CH_3-2'), 10.71 (s, $OCH_2CH_2\underline{CH_3}$).

5.1.2. Intermediates prepared with the use of *tert*-butyl-dimethyl-silyl protecting group

Preparation of 3',5'-protected 2'-C-methyl-6-O-methyl guanosine (124.a)



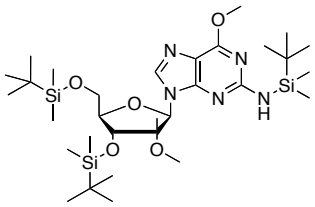
2'-*C*-methyl-6-*O*-methyl guanosine (**11**, provided by Inhibitex *Inc.*) (3.19 g, 10.3 mmol, 1.0 eq) was dissolved in anh. DMF (16 mL). TBDMSCl (6.64 g, 44.1 mmol, 4.3 eq) and imidazole (5.65 g, 83.0 mmol, 8.1 eq) were added and the solution was left stirring

overnight at RT. A purification using EtOAc/petroleum ether was performed to recovered the desired material (2.49 g, 4.61 mmol, 45%).

^{1}H NMR (500 MHz, MeOD) δ_H (ppm): 8.16 (s, 1H, H-8 base), 6.03 (s, 1H, H-1'), 4.31 (d, 1H, *J* = 8.5 Hz, H-3'), 4.12-4.07 (m, 2H, H-5', H4'), 4.05 (s, 6-*O*CH_3 base), 3.91-3.88 (dd, 1H, *J* = 2.0 Hz, 11.95 Hz, H-5'), 1.01 (s, 3H, CH_3-2') 0.98 & 0.94 (2s, 18H, 2 x $Si(CH_3)_2C(\underline{CH_3})_3$), 0.19 (m, 12H, 2 x $Si(\underline{CH_3})_2C(CH_3)_3$).

^{13}C NMR (125 MHz, MeOD) δ_C (ppm)**:** 162.78 (s, C-6 base), 161.92 (s, C-2 base), 154.74 (s, C-4 base), 138.45 (s, C-8 base), 115.38 (s, C-5 base), 91.72 (s, C-1'), 83.88 (s, C-4'), 80.49 (s, C-2'), 74.64 (s, C-3'), 62.25 (s, C-5'), 54.34 (s, 6-*O*CH_3 base), 28.89 & 28.04 (2s, 4 x $Si(CH_3)_2\underline{C}(CH_3)_3$), 27.17-26.64 (m, 6 x $Si(CH_3)_2C(\underline{C}H_3)_3$), 20.53 (s, CH_3-2'), -5.43 & -5.67 (2s, 4 x $Si(\underline{C}H_3)_2C(CH_3)_3$).

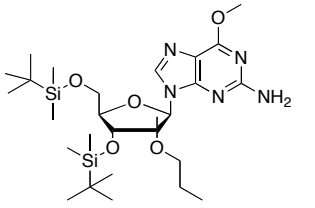
Preparation of 3',5'-protected-N-2-protected 2'-C-methyl-6-O-methyl guanosine (124.b)



Recovered preparing **124.a** after purification by flash chromatography (3.68 g, 5.63 mmol, 55%).

^{1}H NMR (400 MHz, $CDCl_3$) δ_H (ppm): 7.94 (s, 1H, H-8 base), 5.89 (s, 1H, H-1'), 4.67 (bs, 1H, NH base), 4.12 (d, 1H, *J* = 8.5 Hz, H-3'), 4.12-4.07 (m, 2H, H-5', H4'), 3.90 (s, 6-*O*CH_3 base), 3.86-3.82 (dd, 1H, *J* = 2.5 Hz, 11.9 Hz, H-5'), 1.01 (s, 3H, CH_3-2') 0.98 & 0.94 (2s, 18H, 2 x $Si(CH_3)_2C(\underline{CH_3})_3$), 0.19 (m, 12H, 2 x $Si(\underline{CH_3})_2C(CH_3)_3$), 0.16 (2s, $NHSi(\underline{CH_3})_2C(CH_3)_3$).

Preparation of 3',5'-protected 2'-C-methyl-2'-O-propyl-6-O-methyl guanosine (125.b)

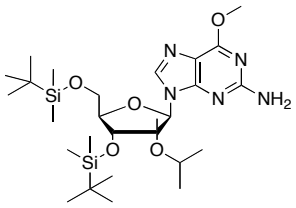


Similar to procedure for **119.a**, using 3',5'-protected 2'-*C*-methyl-*6*-O-methyl guanosine (**124.a**) (0.80 g, 1.48 mmol, 1.0 eq) in anh. DMF (11.0 mL), molecular sieves

3Å and NaH (60% in dispersion oil, 0.066 g, 1.63 mmol, 1.1 eq), propyl iodide (0.43 mL, 4.44 mmol, 3.0 eq). NaH (60% in dispersion oil, 0.066 g, 1.63 mmol, 1.1 eq). The solution was stirred for 30 min at 0 °C. Absolute EtOH (12 mL) and ice cold EtOAc (36 mL) were added and the solution was washed with NH_4Cl, dried over Na_2SO_4, the solvents were evaporated under reduced pressure. The purification was undertaken on flash chromatography with EtOAc (16%)/Hexane to recover only the desired material (0.300 g, 0.57 mmol, 38%).

^{1}H NMR (500 MHz, MeOD) δ_H (ppm): 8.04 (s, 1H, H-8 base), 5.88 (s, 1H, H-1'), 4.12 (d, 1H, *J* = 9.5 Hz, H-3'), 3.93-3.91 (m, 3H, H-4', H-5'), 3.87 (s, 3H, 6-*O*CH_3 base), 3.14 (t, 2H, *J* =1.5 Hz, O$\underline{CH_2}CH_2CH_3$), 1.47 (m, 2H, $OCH_2\underline{CH_2}CH_3$), 1.17 (s, 3H, CH_3-2'), 0.84 (s, 9H, $Si(CH_3)_2C(\underline{CH_3})_3$), 0.78 (s, 9H, $Si(CH_3)_2C(\underline{CH_3})_3$), 0.76 (m, 3H, $OCH_2CH_2\underline{CH_3}$), 0.03 (m, 12H, 2 x $Si(\underline{CH_3})_2C(CH_3)_3$).

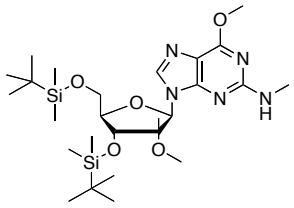
Preparation of 3',5'-protected 2'-C-methyl-2'-O-isopropyl-6-O-methyl guanosine (125.c)



Similar to procedure for **119.a**, using 3',5'-protected 2'-*C*-methyl-*6*-O-methyl guanosine (**124.a**) (1.00 g, 1.85 mmol, 1.0 eq) in anh. DMF (13.5 mL), molecular sieves 3Å, NaH (60% in dispersion oil, 0.081 g, 2.04 mmol, 1.1 eq), isopropyl iodide (0.55 mL, 5.55 mmol, 3.0 eq), and NaH (60% in dispersion oil, 0.081 g, 2.04 mmol, 1.1 eq). The solution was stirred for 30 min at 0 °C. Absolute EtOH (15 mL) and ice cold EtOAc (45 mL) were added and the solution was washed with NH_4Cl, dried over Na_2SO_4. The solvents were evaporated under reduced pressure and the residue was purified by flash chromatography using EtOAc (12 to 25%)/petroleum ether to recover the desired material (0.043 g, 0.07 mmol, 4%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.14 (s, 1H, H-8 base), 5.98 (s, 1H, H-1'), 4.61 (s, 2H, NH_2 base), 4.00-3.98 (m, 1H, H-4'), 3.96 (d, 1H, *J* = 2.0 Hz, H-3'), 3.90 (s, 3H, 6-*O*CH_3 base), 3.88 (m, 1H, O$\underline{CH}(CH_3)_2$), 3.71 (dd, *J* = 1.5 Hz, 12.0 Hz, H-5'), 1.10 (s, 3H, CH_3-2'), 0.82 (s, 9H, $Si(CH_3)_2C(\underline{CH_3})_3$), 0.79 (d, 6H, *J* = 3.0 Hz, $OCH(\underline{CH_3})_2$), 0.75 (s, 9H, $Si(CH_3)_2C(\underline{CH_3})_3$), 0.02 (m, 12H, 2 x $Si(\underline{CH_3})_2C(CH_3)_3$).

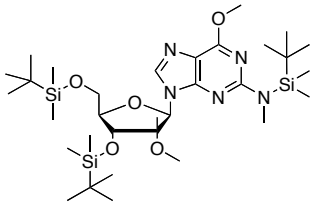
Preparation of 3',5'-protected 2'-C-methyl-2'-O-methyl-N-2-methyl-6-O-methyl guanosine (125.d)



Similar to procedure for **119.a**, using 3',5'-protected 2'-*C*-methyl-*6*-O-methyl guanosine (**124.a**) (1.00 g, 1.85 mmol, 1.0 eq) in anh. DMF (13.5 mL), molecular sieves 3Å and NaH (60% in dispersion oil, 0.081 g, 2.04 mmol, 1.1 eq), methyl iodide (0.36 mL, 5.55 mmol, 3.0 eq), and NaH (60% in dispersion oil, 0.081 g, 2.04 mmol, 1.1 eq). The solution was stirred for 30 min at 0 °C. Absolute EtOH (15 mL) and ice cold EtOAc (75 mL) were added and the solution was washed with NH_4Cl, dried over Na_2SO_4. The solvents were evaporated under reduced pressure and the residue was purified by flash chromatography using EtOAc/Hexane to recover the bis-methylated material (0.380 g, 0.69 mmol, 37%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 7.99 (s, 1H, H-8 base), 5.98 (s, 1H, H-1'), 4.86 (s, 1H, NH base), 4.10 (d, 1H, *J* = 9.5 Hz, H-3'), 3.98-3.91 (m, 2H, H-4', H5'), 3.88 (s, 3H, 6-*O*CH_3 base), 3.69-3.67 (m, 1H, H-5'), 3.38 (s, 3H, O$\underline{CH_3}$), 2.82 (d, 3H, *J* = 5.0 Hz, NH$\underline{CH_3}$), 1.10 (s, 3H, CH_3-2'), 0.81 (s, 9H, Si(CH_3)C($\underline{CH_3}$)$_3$), 0.75 (s, 9H, Si(CH_3)$_2$C($\underline{CH_3}$)$_3$), 0.02 (m, 12H, 2x Si($\underline{CH_3}$)$_2$C(CH_3)$_3$).

Preparation of 3',5'-protected-N-2-protected 2'-C-methyl-2'-O-methyl-N-2-methyl-6-O-methyl guanosine (125.e)

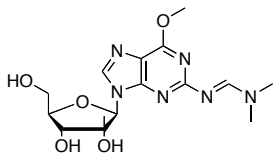


Similar to procedure for **119.a**, using 3',5'-protected-*N*-2-protected 2'-*C*-methyl-*6*-O-methyl guanosine (**124.b**) (3.68 g, 5.63 mmol, 1.0 eq) in anh. DMF (40 mL), molecular sieves 3Å, NaH (60% in dispersion oil, 0.248 g, 6.20 mmol, 1.1 eq), methyl iodide (1.05 mL, 16.89 mmol, 3.0 eq), and NaH (60% in dispersion oil, 0.248 g, 6.20 mmol, 1.1 eq). The solution was stirred for 30 min at 0 °C. Absolute EtOH (161 mL) and ice cold EtOAc (800 mL) were added and the solution was washed with NH_4Cl, dried over

Na_2SO_4. The solvents were evaporated under reduced pressure and the residue was purified by flash chromatography using EtOAc/Hexane to recover the bis-methylated material (0.180 g, 0.26 mmol, 5%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.10 (s, 1H, H-8 base), 5.82 (s, 1H, H-1'), 3.97 (dd, 1H, *J* = 1.5 Hz, 12 Hz, H-4'), 3.92 (m, 1H, H-3'), 3.90 (s, 3H, 6-*O*CH_3 base), 3.73-3.70 (m, 2H, H-5'), 3.33 (s, 3H, O$\underline{CH_3}$), 2.85 (s, 3H, N$\underline{CH_3}$Si(CH_3)C(CH_3)$_3$), 0.93 (s, 3H, CH_3-2'), 0.81, 0.79, 0.76 (3s, 27H, 2 x OSi(CH_3)C($\underline{CH_3}$)$_3$, NCH$_3$Si(CH_3)C($\underline{CH_3}$)$_3$), 0.24 (m, 6H, NCH$_3$($\underline{CH_3}$)$_2$C(CH_3)$_3$), 0.05 (m, 12H, OSi($\underline{CH_3}$)$_2$C(CH_3)$_3$).

Preparation of 2'-C-methyl-2'-O-methyl-N-2-dimethylformamide-6-O-methyl guanosine (127)

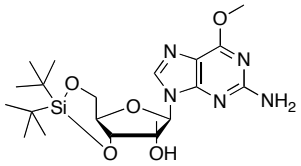


To 2'-*C*-methyl-6-*O*-methyl guanosine (**11**) (1.04 g, 3.33 mmol, 1.0 eq) in anh. DMF (15 mL), was added *N*,*N*-dimethylformamide dimethylacetal (2.22 mL, 16.65 mmol, 5.0 eq) and the reaction was left stirring overnight at RT. Purification by flash chromatography afforded the desired material (0.17 g, 0.46 mmol, 14%).

^{1}H NMR (500 MHz, MeOD) δ_H (ppm): 8.50 (s, 1H, H-8 base), 8.26 (s, 1H, N$\underline{CH}$N(CH_3)$_2$), 5.99 (s, 1H, H-1'), 4.24 (d, 1H, *J* = 9.0 Hz, H-3'), 4.12 (m, 1H, H-5'), 4.06 (s, 3H, 6-*O*CH_3 base)), 4.05-4.02 (m, 1H, H-4'), 3.89-3.86 (dd, 1H, *J* = 2.5 Hz, 12.0 Hz, H-5'), 3.22 (s, 3H, NCHN($\underline{CH_3}$)$_2$), 3.15 (s, 3H, NCHN($\underline{CH_3}$)$_2$), 0.96 (s, 3H, CH_3-2').

5.1.3. Intermediates prepared with the use of di-*tert*-butyl-silyl and dimethylformamide protecting group

Preparation of 3',5'-protected 2'-C-methyl-6-O-methyl guanosine (130)



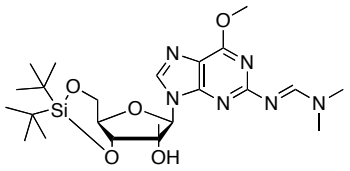
To a suspension of 2'-*C*-methyl-6-*O*-methyl guanosine (**11**) (0.555 g, 1.78 mmol, 1.0 eq) in anh. DMF (9 mL) was added di-tert-bytyl-silyl-triflate (0.64 mL, 1.96 mmol, 1.1 eq) at 0 °C. The solution was stirred 2 hrs 30 min at 0 °C, and the reaction was

quenched with cold EtOH and neutralised with NEt_3. The solvent was removed under reduced pressure and the remaining oil was dissolved in MeOH. A white precipitate crashed out by addition of H_2O. After filtration and drying under Buchner, the desired molecule was recovered (0.203 g, 0.45 mmol, 25%).

^{1}H NMR (500 MHz, MeOD) δ_H (ppm): 7.88 (s, 1H, H-8 base), 5.96 (s, 1H, H-1'), 4.47-4.43 (m, 1H, H-5'), 4.26-4.17 (m, 3H, H-5', H-3', H-4'), 4.07 (s, 3H, 6-OCH_3 base), 1.13 & 1.09 (2s, 18H, 2 x SiC($\underline{CH_3}$)$_3$), 1.04 (s, 3H, CH_3-2').

^{13}C NMR (125 MHz, MeOD) δ_C (ppm): 162.76 (s, C-6 base), 162.11 (s, C-2 base), 154.61 (s, C-4 base), 139.40 (s, C-8 base), 115.29 (s, C-5 base), 94.19 (s, C-1'), 81.58 (s, C-4'), 79.97 (s, C-2'), 75.16 (s, C-3'), 68.52 (s, C-5'), 54.26 (s, 6-OCH_3 base), 32.64 & 34.54 (2s, 2 x Si$\underline{C}$(CH_3)$_3$), 28.06 & 27.71 (2s, 2 x SiC($\underline{C}H_3$)$_3$), 20.19 (s, CH_3-2').

Preparation of 3',5'-protected-N-2-protected 2'-C-methyl-6-O-methyl guanosine (131)

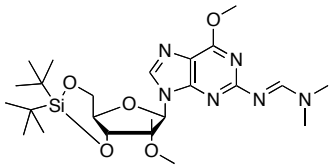


To a solution of 3',5'-protected 2'-C-methyl-6-O-methyl guanosine (**130**) (0.20 g, 0.45 mmol, 1.0 eq) in anh. DMF (3.42 mL) was added *N*,*N*-dimethylformamide dimethylacetate (0.26 mL, 1.80 mmol, 4.0 eq). The reaction mixture was stirred overnight at RT, and the solvent was evaporated. The residue was dissolved in EtOAc and washed with $NaHCO_3$, H_2O and brine. Evaporation of the organic layer afforded the desired material (0.22 g, 0.43 mmol, 95%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.62 (s, 1H, N$\underline{CH}$N(CH_3)$_2$), 7.69 (s, 1H, H-8 base), 6.18 (s, 1H, H-1'), 4.49 (d, 1H, *J* = 3.5 Hz, H-5'), 4.14 (s, 3H, 6-*O*CH_3 base), 4.11-4.08 (m, 3H, H-5', H-3', H-4'), 3.14 & 3.13 (2s, 6H, NCHN($\underline{CH_3}$)$_2$), 1.05 & 1.02 (2s, 18H, SiC($\underline{CH_3}$)$_3$), 1.00 (s, 3H, CH_3-2').

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 162.74 (s, C-6 base), 161.10 (s, C-2 base), 158.45 (s, N$\underline{CH}$N(CH_3)$_2$), 153.15 (s, C-4 base), 138.23 (s, C-8 base), 118.04 (s, C-5 base), 94.93 (s, C-1'), 80.06 (s, C-4'), 78.12 (s, C-2'), 74.82 (s, C-3'), 67.73 (s, C-5'), 53.84 (s, 6-*O*CH_3 base), 41.15 (s, NCHN($\underline{CH_3}$)$_2$), 35.17 (s, NCHN($\underline{CH_3}$)$_2$), 27.73 & 27.40 (2s, 2 x SiC($\underline{CH_3}$)$_3$), 22.81 & 22.71 (2s, 2 x Si$\underline{C}$(CH_3)$_3$), 20.29 (s, CH_3-2').

Preparation of 3',5'-protected-N-2-protected 2'-C-methyl-2'-O-methyl-6-O-methyl guanosine (132)

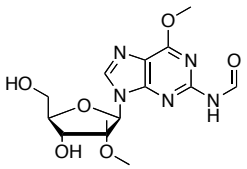


To a solution of 3',5'-protected-*N*-2-protected 2'-*C*-methyl-6-*O*-methyl guanosine (**131**) (0.22 g, 0.43 mmol, 1.0 eq) in anh. DMF (6.5 mL) was added molecular sieves 3Å, methylidodide (0.16 mL, 2.58 mmol, 6.0 eq) at 0 °C. The reaction was left stirring at 0 °C for 15 min, and then NaH (60% dispersion in oil, 0.02 g, 0.52 mmol, 1.2 eq) was added. The mixture was left stirring for 1 hr at 0 °C, and quenched with sodium dihydrogen phosphate (0.5 M) and dissolved in EtOAc. The organic layer was washed with sodium dihydrogen phosphate, H_2O and brine, and the solvent was evaporated under reduced pressure to yield the desired material without further purification needed (0.16 g, 0.30 mmol, 70%).

^{1}H NMR (500 MHZ, $CDCl_3$) δ_H (ppm): 8.60 (s, 1H, NC<u>H</u>N$(CH_3)_2$), 7.64 (s, 1H, H-8 base), 6.10 (s, 1H, H-1'), 4.48-4.44 (m, 1H, H-5'), 4.24-4.19 (m, 1H H-4'), 4.10 (s, 3H, 6-*O*CH_3 base), 4.07-4.03 (m, 2H, H-5', H-3'), 3.52 (s, 3H, O<u>C</u>H$_3$), 3.12 & 3.09 (2s, 6H, NCHN(<u>C</u>H$_3$)$_2$), 1.02 (s, 3H, CH_3-2'), 1.01 & 0.99 (2s, 18H, 2 x SiC(<u>C</u>H$_3$)$_3$).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 162.72 (s, C-6 base), 161.07 (s, C-2 base), 158.47 (s, N<u>C</u>HN$(CH_3)_2$), 153.23 (s, C-4 base), 137.91 (s, C-8 base), 117.95 (s, C-5 base), 82.12 (s, C-1'), 80.01 (s, C-2'), 74.79 (s, C-4'), 74.04 (s, C-3'), 67.82 (s, C-5'), 53.76 (s, 6-*O*CH_3 base), 52.67 (s, O<u>C</u>H$_3$), 41.12 (s, NCHN(<u>C</u>H$_3$)$_2$), 35.15 (s, NCHN(<u>C</u>H$_3$)$_2$), 28.08 & 27.92 (2s, 2 x SiC(<u>C</u>H$_3$)$_3$), 22.73 & 22.66 (2s, 2 x Si<u>C</u>$(CH_3)_3$), 20.92 (s, CH_3-2').

Preparation of N-2-formamide 2'-C-methyl-2'-O-methyl-6-O-methyl guanosine (133.a)



To 3',5'-protected-*N*-2-protected 2'-*C*-methyl-2'-*O*-methyl-6-*O*-methyl guanosine (**132**) (0.33 g, 0.63 mmol, 1.0 eq) in anh. THF (16 mL) was added anh. NEt_3 (0.15 mL, 1.13 mmol, 1.8 eq) and $NEt_3.H_3F$ (0.35 mL, 2.21 mmol, 3.5 eq). The solution was

stirred at RT for 1 hr. The solvent was evaporated under reduced pressure and the residue purified by flash chromatography followed by preparative chromatography to recover the partially hydrolysed formamidine (0.12 g, 0.37 mmol, 59%).

HPLC (MeOH/H_2O): Rt = 15.28 min

HPLC (ACN/H_2O): Rt = 5.87 min

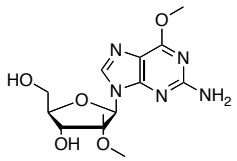
MS (TOF ES^+): 376.12 ($M + Na^+$).

^1H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 9.41 (d, 1H, *J* = 11.0 Hz, NHCHO), 8.76 (d, 1H, *J* = 10.5 Hz, NHCHO), 8.43 (s, 1H, H-8 base), 6.25 (s, 1H, H-1'), 4.28 (d, 1H, *J* = 9.5 Hz, H-3'), 4.20 (dd, 1H, *J* = 2.0 Hz, 13.0 Hz, H-5'), 4.10 (s, 3H, 6-*O*CH_3 base), 4.06 (dd, 1H, *J* = 2.0 Hz, 9.5 Hz, H-4'), 3.97 (m, 1H, H-5'), 3.53 (s, 3H, OCH_3), 0.97 (s, 3H, CH_3-2').

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 163.40 (s, NHCHO), 161.32 (s, C-6 base), 153.43 (s, C-2 base), 151.87 (s, C-4 base), 139.94 (s, C-8 base), 118.25 (s, C-5 base), 86.93 (s, C-1'), 83.65 (s, C-2'), 83.11 (s, C-4'), 73.43 (s, C-3'), 59.67 (s, C-5'), 54.58 (s, 6-*O*CH_3 base), 51.32 (s, OCH_3), 14.79 (s, CH_3-2').

5.1.4. Preparation of 2'-*O*-modified 2'-*C*-methyl-*6*-O-methyl guanosine nucleosides

Preparation of 2'-C-methyl-2'-O-methyl-6-O-methyl guanosine (120)



Route 1: To 3',5'-protected 2'-*C*-methyl-2'-*O*-methyl-6-*O*-methyl guanosine (**119.a**) (0.057 g, 0.10 mmol) dissolved in anh. THF (5.96 mL). TBAF (0.069 mL, 0.22 mmol, 2.2 eq) was added and the mixture was stirred for 3 hrs 30 min at RT. The purification was performed by flash chromatography using CH_2Cl_2/MeOH to recover the desired unpure material (0.007 g, 0.02 mmol, 20%).

Route 2: *N*-2-formamide 2'-*C*-methyl-2'-*O*-methyl-6-*O*-methyl guanosine (**133.a**)

(0.12 g, 0.37 mmol) was dissolved in isopropanol (5 mL) and stirred under reflux overnight. The solvent was evaporated under reduced pressure and the material was recovered after preparative chromatography (0.005 g, 0.015 mmol, 4%).

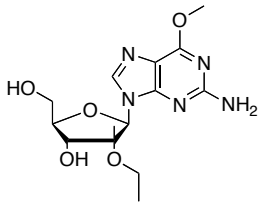
HPLC (MeOH/H_2O): Rt = 12.23 min

HPLC (ACN/H_2O): Rt = 12.09 min

MS (TOF ES^+): 326.14 ($M + H^+$).

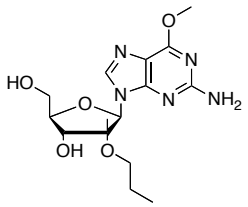
^{1}H NMR (500 MHz, MeOD) δ_H (ppm): 8.29 (s, 1H, H-8 base), 6.17 (s, 1H, H-1'), 4.22 (d, 1H, *J* = 9.5 Hz, H-3'), 4.07 (s, 3H, 6-*O*CH_3 base), 4.04-4.01 (m, 2H, H-4', H-5'), 3.87-3.84 (dd, 1H, *J* = 3.0 Hz, 12.5 Hz, H-5'), 3.56 (s, 3H, O$\underline{CH_3}$), 1.01 (s, 3H, CH_3-2').
^{13}C NMR (125 MHz, MeOD) δ_C (ppm): 163.63 (s, C-6 base), 162.70 (s. C-2 base), 154.58 (s, C-4 base), 139.10 (s, C-8 base), 112.83 (s, C-5 base), 88.62 (s, C-1'), 84.95 (s, C-2'), 83.76 (s, C-4'), 74.77 (s, C-3'), 61.95 (s, C-5'), 54.25 (s, 6-*O*CH_3 base), 51.83 (s, O$\underline{C}H_3$), 15.20 (s, CH_3-2').

Preparation of 2'-C-methyl-2'-O-ethyl-6-O-methyl guanosine (121)



Route 1: Prepared following the procedure to **120**, using 3',5'-protected 2'-*C*-methyl-2'-*O*-ethyl-6-*O*-methyl guanosine (**119.b**) (0.10 g, 0.17 mmol) in anh. THF (10 mL), and TBAF (0.11 mL, 0.38 mmol). The reaction was left stirring for 2 hrs at RT. The solvent was evaporated under reduced pressure, and purified by flash chromatography using MeOH/$CHCl_3$ to yield the desired material (0.15 g, 0.05 mmol, 87%).
HPLC (MeOH/H_2O): Rt = 1.29 min
HPLC (ACN/H_2O): Rt = 2.68 min
MS (TOF ES^+): 340.16 ($M + H^+$).
^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 7.64 (s, 1H, H-8 base), 5.84 (s, 1H, H-1'), 4.97 (bs, 2H, NH_2 base), 4.47 (d, 1H, *J* = 8.5 Hz, H-3'), 4.22 (dd, 1H, *J* = 1.5 Hz, 11.0 Hz, H-5'), 4.15 (dd, 1H, *J* = 2.0 Hz, 10.0 Hz, H-5'), 4.09 (s, 3H, 6-*O*CH_3 base), 3.88-3.82 (m, 3H, O$\underline{CH_2}CH_3$, H4'), 1.31 (t, 3H, *J* = 7.0 Hz, $OCH_2\underline{CH_3}$), 1.02 (s, 3H, CH_3-2').
^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 162.75 (s, C-6 base), 161.84 (s, C-2 base), 154.49 (s, C-4 base), 139.35 (s, C-8 base), 115.36 (s, C-5 base), 92.99 (s, C-1'), 84.15 (s, C-4'), 80.36 (s, C-2'), 73.58 (s, C-3'), 61.18 (s, C-5'), 54.61 (s, 6-*O*CH_3 base), 34.18 (s, O$\underline{CH_2}CH_3$), 20.38 (s, CH_3-2'), 13.98 (s, $OCH_2\underline{CH_3}$).

Preparation of 2'-C-methyl-2'-O-propyl-6-O-methyl guanosine (122)



Route 1: Prepared following the procedure to **120**, using 3',5'-protected 2'-*C*-methyl-2'-*O*-propyl-6-*O*-methyl guanosine (**119.c**) (0.03 g, 0.06 mmol) in anh. THF (2 mL), TBAF (0.03 g, 0.12 mmol, 2.1 eq). The reaction was left stirring for 2 hrs 30 min at RT. The solvent was evaporated under reduced pressure, and was purified by flash chromatography to yield (0.02 g, 0.04 mmol, 82%).

Route 2: To a solution of 3',5'-protected 2'-*C*-methyl-2'-*O*-propyl-6-*O*-methyl guanosine (**125.b**) (0.300 g, 0.57 mmol, 1.0 eq) in anh. THF (7.0 mL) was added TBAF (1M in THF, 1.71 mL, 1.71 mmol, 3.0 eq) at 0 °C. The reaction was left stirring at RT for 2 hrs, and solvents were evaporated under reduced pressure. The crude residue was purified by flash chromatography using petroleum ether/methanol (2 to 4%) to recover the desire molecule (0.121 g, 0.34 mmol, 60%).

HPLC ($MeOH/H_2O$): Rt = 18.46 min

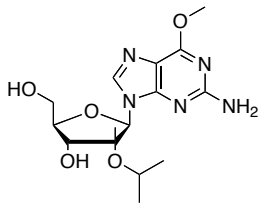
HPLC (ACN/H_2O): Rt = 2.43 min

MS (TOF ES^+): 353.20 (M), 376.15 ($M + Na^+$), 392.13 ($M + K^+$).

^1H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 7.80 (s, 1H, H-8 base), 5.98 (s, 1H, H-1'), 5.03 (bs, 2H, NH_2 base), 4.51 (d, 1H, *J* = 9.0 Hz, H-3'), 4.18 (dd, 1H, *J* = 2.5 Hz, 12.5 Hz, H-5'), 4.09 (s, 3H, 6-*O*CH_3 base), 4.06 (m, 1H, H-4'), 3.99 (dd, 1H, *J* = 2.0 Hz, 12.0 Hz, H-5'), 3.62 (t, 2H, *J* = 6.5 Hz, O$\underline{CH_2}CH_2CH_3$), 1.74 (m, 2H, $OCH_2\underline{CH_2}CH_3$), 1.05 (s, 3H, CH_3-2'), 1.04 (t, 3H, *J* = 7.5 Hz, $OCH_2CH_2\underline{CH_3}$).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 161.96 (s, C-6 base), 159.26 (s, C-2 base), 154.51 (s, C-4 base), 137.86 (s, C-8 base), 115.46 (s, C-5 base), 89.43 (s, C-1') 83.54 (s, C-4'), 83.36 (s, C-2'), 73.81 (s, C-3'), 64.95 (s, C-5'), 60.41 (s, O$\underline{CH_2}CH_2CH_3$), 54.07 (s, 6-*O*CH_3 base), 23.31 (s, $OCH_2\underline{CH_2}CH_3$), 15.81 (s, CH_3-2'), 10.70 (s, $OCH_2CH_2\underline{CH_3}$).

Preparation of 2'-C-methyl-2'-O-isopropyl-6-O-methyl guanosine (123)



Route 2: To a solution of 3',5'-protected 2'-*C*-methyl-2'-*O*-isopropyl-6-*O*-methyl guanosine (**125.c**) (0.111 g, 0.19 mmol, 1.0 eq) in anh. THF (3.0 mL) was added TBAF (1M in THF, 0.57 mL, 0.57 mmol, 3.0 eq) at 0 °C. The reaction was left stirring at RT for 2 hrs, and solvents were evaporated under reduced pressure. The crude residue was purified by flash chromatography using petroleum ether/MeOH (2 to 4%) to recover the desired molecule (0.025 g, 0.071 mmol, 37%).

HPLC (MeOH/H_2O): Rt = 19.95 min

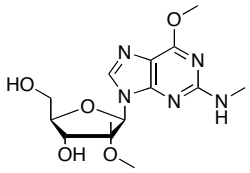
HPLC (ACN/H_2O): Rt = 9.32 min

MS (TOF ES^+): 354.18 ($M + H^+$); MS (TOF ES^-): 388.14 ($M + Cl^-$).

^{1}H NMR (500 MHz, MeOD) δ_H (ppm): 8.24 (s, 1H, H-8 base), 6.00 (s, 1H, H-1'), 4.26 (d, 1H, *J* = 8.5 Hz, H-3'), 4.23-4.17 (m, 2H, H-5', O<u>C</u>H$(CH_3)_2$), 4.06 (s, 3H, 6-*O*CH_3 base), 4.03-4.00 (m, 1H, H-4'), 3.88 (dd, 1H, *J* = 3.5 Hz, 12.5 Hz, H-5'), 1.27 & 1.26 (2s, 6H, OCH(<u>CH_3</u>)<u>$_2$</u>), 0.99 (s, 3H, CH_3-2').

^{13}C NMR (125 MHz, MeOD) δ_C (ppm): 162.53 (s, C-6 base), 161.75 (s. C-2 base), 154.33 (s, C-4 base), 138.23 (s, C-8 base), 114.71 (s, C-5 base), 91.85 (s, C-1'), 85.86 (s, C-2'), 84.83 (s, C-4'), 74.68 (s, C-3'), 72.57 (s, O<u>C</u>H$(CH_3)_2$), 59.73 (s, C-5'), 54.21 (s, 6-*O*CH_3 base), 21.87 (s, OCH(<u>CH_3</u>)<u>$_2$</u>), 19.22 (s, CH_3-2').

Preparation of 2'-C-methyl-2'-O-methyl-N-2-methyl-6-O-methyl guanosine (126)

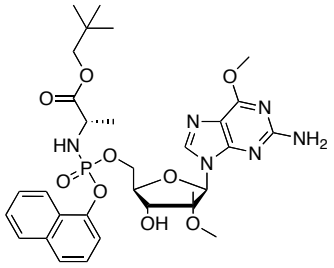


To a solution of 3',5'-protected 2'-*C*-methyl-2'-*O*-methyl-*N*-2-methyl-6-*O*-methyl guanosine (**125.d**) and 3',5'-protected-*N*-2-protected 2'-*C*-methyl-2'-*O*-methyl-*N*-2-methyl-6-*O*-methyl guanosine (**125.e**) (0.380 g, 0.69 mmol, 1.0 eq) in anh. THF (8.5 mL) was added TBAF (1M in THF, 2.07 mL, 2.07 mmol, 3.0 eq) at 0 °C. The reaction was left stirring at RT for 2 hrs, and solvents were evaporated under reduced pressure.

The crude residue was purified by flash chromatography using petroleum ether/methanol (2 to 4%) to recover the desire molecule (0.090 g, 0.265 mmol, 38%).
HPLC (MeOH/H_2O): Rt = 17.08 min
HPLC (ACN/H_2O): Rt = 8.35 min
MS (TOF ES^+): 340.16 ($M + H^-$), 362.13 ($M + Na^+$), 701.28 ($2M + Na^+$).
^1H NMR (500 MHz, MeOD) δ_H (ppm): 8.25 (s, 1H, H-8 base), 6.23 (s, 1H, H-1'), 4.21 (d, 1H, *J* = 9.0 Hz, H-3'), 4.13 (m, 1H, H-4'), 4.06 (s, 3H, 6-*O*CH_3 base), 4.04-4.03 (m, 1H, H-5'), 3.88-3.84 (m, 1H, *J* = 3.5 Hz, 12.5 Hz, H-5'), 3.58 (s, 3H, O$\underline{CH_3}$), 2.96 (s, 3H, NH$\underline{CH_3}$), 1.02 (s, 3H, CH_3-2').
^{13}C NMR (125 MHz, MeOD) δ_C (ppm): 162.33 (s, C-6 base), 161.71 (s. C-2 base), 154.68 (s, C-4 base), 138.51 (s, C-8 base), 114.81 (s, C-5 base), 88.33 (s, C-1'), 85.02 (s, C-2'), 84.63 (s, C-4'), 74.87 (s, C-3'), 61.09 (s, C-5'), 54.00 (s, 6-*O*CH_3 base), 51.63 (s, O$\underline{C}H_3$), 28.79 (s, NH$\underline{C}H_3$), 15.23 (s, CH_3-2').

5.1.5. Preparation of 2'-*O*-modified 2'-*C*-methyl-*6*-O-methyl guanosine ProTides

Preparation of α-naphthyl L-Alanine-neopentyl-ester 2'-C-methyl-2'-O-methyl-6-O-methyl guanosine ProTide (146)



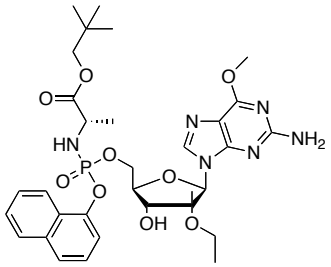
This ProTide was prepared according to the Standard Procedure D, using *t*BuMgCl (1M in THF, 0.50 mL, 0.50 mmol, 2.0 eq), 2'-*C*-methyl-2'-*O*-methyl-6-*O*-methyl guanosine (**120**) (0.080 g, 0.25 mmol, 1.0 eq) in anh. THF (1.00 mL), *α*-naphthyl *L*-Alanine-neopentyl ester phosphochloridate (**14.d**) (0.192 g, 0.50 mmol, 2.0 eq) in anh. THF (0.5 mL). After overnight stirring at RT, the solvent was removed under reduced pressure and the residue was purified by flash chromatography, using $CHCl_3$/MeOH as eluents, and preparative chromatography to yield the desired molecule (0.005 g, 0.007 mmol, 3%).
HPLC (MeOH/H_2O): Rt = 27.43 min, 27.85 min.
HPLC (ACN/H_2O): Rt = 19.01 min, 19.31 min
MS (TOF ES^+): 673.27 ($M + H^+$), 695.25 ($M + Na^+$).

^{1}H NMR (500 MHz, MeOD) δ_H (ppm): 8.37 & 8.24 (2s, 2H, H-8 base diastereoisomers), 8.18-8.13 (m, 2H, H-9 Ar diastereoisomers), 7.89-7.84 (m, 2H, H-6 Ar diastereoisomers), 7.69-7.66 (m, 2H, H-4 Ar diastereoisomers), 7.53-7.45 (m, 6H, H-3, H-7, H-8 Ar diastereoisomers), 7.40-4.35 (m, 2H, H-2 Ar diastereoisomers), 6.27 & 6.26 (2s, 2H, H-1' diastereoisomers), 4.67-4.65 (dd, 2H, *J* = 2.0 Hz, 5.5 Hz, H-5'), 4.60-4.53 (m, 2H, H-5'), 4.29-4.22 (m, 4H, H-3', H-4' diastereoisomers), 4.14 & 4.13 (2s, 6H, 6-*O*CH_3 base diastereoisomers), 4.10-4.03 (m, 2H, NH<u>CH</u>(CH_3)CO diastereoisomers), 3.77 & 3.74 (AB system, 2H, J_{AB} = 10.5 Hz, O<u>CH_2</u>$C(CH_3)_3$), 3.67 & 3.61 (AB system, 2H, J_{AB} = 10.5 Hz, O<u>CH_2</u>$C(CH_3)_3$), 3.56 & 3.55 (2s, 6H, O<u>CH_3</u> diastereoisomers), 1.37 (d, 3H, *J* = 7.0 Hz, NHCH(<u>CH_3</u>)CO), 1.34 (d, 3H, *J* = 7.0 Hz, NHCH(<u>CH_3</u>)CO), 1.03 & 1.00 (2s, 6H, CH_3-2' diastereoisomers), 0.88 & 0.87 (2s, 18H, $OCH_2C($<u>CH_3</u>$)_3$ diastereoisomers).

^{13}C NMR (125 MHz, MeOD) δ_C (ppm): 174.87 & 174.10 (2s, NHCH(CH_3)<u>C</u>O diastereoisomers), 161.72 & 172.57 (2s, C-6 base diastereoisomers), 159.43 & 159.23 (2s, C-2 base diastereoisomers), 155.28 & 155.11 (2s, C-4 base diastereoisomers), 153.43 & 153.09 (2s, C-1 Ar diastereoisomers), 140.27 & 140.12 (2s, C-8 base diastereoisomers), 138.56 & 138.43 (2s, C-5 Ar diastereoisomers), 128.87 & 128.78 (2s, C-6 Ar diastereoisomers), 127.91 & 127.81 (2s, C-10 Ar diastereoisomers), 127.76 & 127.68 (2s, C-7 Ar diastereoisomers), 127.43 & 127.36 (2s, C-4 Ar diastereoisomers), 126.50 & 126.47 (2s, C-8 Ar diastereoisomers), 125.91 & 125.84 (2s, C-9 Ar diastereoisomers), 122.74 & 122.68 (2s, C-3 Ar diastereoisomers), 117.38 & 117.11 (2s, C-5 base diastereoisomers), 116.18 & 116.14 (2s, C-2 Ar diastereoisomers), 89.34 & 89.32 (2s, C-1' diastereoisomers), 84.54 & 84.32 (2s, C-2' diastereoisomers), 81.88 & 81.80 (2d, $J^3_{\text{P-O-C-C}}$ = 6.4 Hz, 6.4 Hz, C-4' diastereoisomers), 75.70 & 75.65 (2s, C-3' diastereoisomers), 75.38 & 75.33 (2s, O<u>C</u>$H_2C(CH_3)_3$), 67.34 & 67.30 (2s, C-5' diastereoisomers), 55.05 & 55.03 (2s, 6-*O*<u>C</u>H_3 base diastereoisomers), 51.84 & 50.81 (2s, O<u>C</u>H_3 diastereoisomers), 50.01 & 49.54 (2s, NH<u>C</u>H(CH_3)CO diastereoisomers), 29.89 & 29.79 (s, OCH_2<u>C</u>$(CH_3)_3$ diastereoisomers), 26.69 & 26.68 (2s, $OCH_2C($<u>CH_3</u>$)_3$ diastereoisomers), 20.58 & 20.52 (2s, NHCH(<u>C</u>H_3)CO diastereoisomers), 15.16 & 15.12 (s, CH_3-2' diastereoisomers).

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm): 4.19 (s, 48%), 4.18 (s, 52%).

Preparation of α-naphthyl L-Alanine-neopentyl-ester 2'-C-methyl-2'-O-ethyl-6-O-methyl guanosine ProTide (147)



This ProTide was prepared according to the Standard Procedure D, using *t*BuMgCl (0.94 mL, 4.7 eq), 2'-*C*-methyl-2'-*O*-ethyl-6-*O*-methyl guanosine (**121**) (0.069 g, 0.20 mmol) in anh. THF (2.0 mL), and *α*-naphthyl *L*-Alanine-neopentyl ester phosphochloridate (**14.d**) (0.360 g, 0.94 mmol, 4.7 eq) anh. THF (1.5 mL). After overnight stirring at RT, the solvent was removed under reduced pressure and the residue was purified by flash chromatography, using $CHCl_3$/MeOH (3.5%) as eluents, and preparative chromatography to yield the desired molecule (0.002 g, 0.003 mmol, 1.4%).

HPLC (MeOH/H_2O): Rt = 25.24 min, 25.67 min

HPLC (ACN/H_2O): Rt = 20.81 min, 20.96 min

MS (TOF ES^+): 687.70 ($M + H^+$).

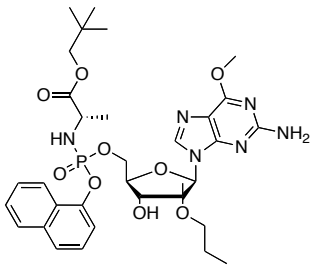
^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 7.87-7.81 (m, 2H, H-9 Ar diastereoisomers), 7.69-7.64 (m, 4H, H-6 Ar, H-8 base diastereoisomers), 7.58-7.36 (m, 10H, H-2, H-3, H-4, H-7, H-8 Ar diastereoisomers diastereoisomers), 5.90 & 5.83 (s, 2H, H-1' diastereoisomers), 4.78 (m, 2H, H-4' diastereoisomers), 4.46 (m, 4H, H-5' diastereoisomers), 4.01 (m, 2H, H-3' diastereoisomers), 4.09 & 4.07 (2s, 6H, 6-*O*CH_3 diastereoisomers), 3.85 (m, 8H, O$\underline{CH_2}CH_3$, COO$\underline{CH_2}C(CH_3)_3$ diastereoisomers), 3.69 (m, 2H, NH$\underline{CH}(CH_3)$CO diastereoisomers), 1.39 & 1.32 (2t, 6H, J = 7.3 Hz, 7.3 Hz, $OCH_2\underline{CH_3}$ diastereoisomers), 1.19 & 1.16 (2d, 6H, J = 5.6 Hz, 5.7 Hz, NHCH($\underline{CH_3}$)CO diastereoisomers), 0.92 & 0.90 (s, 18H, 2 x $COOCH_2C(\underline{CH_3})_3$ diastereoisomers), 0.87 & 0.85 (s, 6H, CH_3-2' diastereoisomers).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 172.83 & 172.65 (2s, NHCH(CH_3)$\underline{C}$O diastereoisomers), 164.26 & 164.18 (2s, C-6 base diastereoisomers), 160.96 & 160.07 (2s, C-2 base diastereoisomers), 155.52 & 155.22 (2s, C-4 base diastereoisomers), 148.09 & 147.96 (2s, C-1 Ar diastereoisomers), 141.03 & 142.87 (2s, C-8 base diastereoisomers), 138.60 & 138.56 (2s, C-5 Ar diastereoisomers), 128.79 & 128.56 (2s,

C-6 Ar diastereoisomers), 127.78 & 127.21 (2s, C-7 Ar diastereoisomers), 126.67 & 126.43 & 126. 12 (3s, C-8, C-7, C-10 Ar diastereoisomers), 125.01 & 124.95 (4s, C-4, C-3 Ar diastereoisomers), 121.27 & 121.23 (2s, C-9 Ar diastereoisomers), 115.01 & 114.92 (2s, C-2 Ar, Ar diastereoisomers), 112.85 & 112.30 (2s, C-5 base diastereoisomers), 94.41 & 94.23 (2s, C-1' diastereoisomers), 82.49 & 82.23 (2s, C-4' diastereoisomers), 80.27 & 80.02 (2s, C-2' diastereoisomers), 79.63 & 79.14 (2s, C-3' diastereoisomers), 74.77 & 74.56 (2s, $COO\underline{C}H_2C(CH_3)_3$ diastereoisomers), 67.87 & 67.56 (2s, C-5' diastereoisomers), 61.07 & 60.87 (2s, $O\underline{C}H_2CH_3$ diastereoisomers), 54.04 & 53.86 (2s, 6-*O*Me diastereoisomers), 50.80 & 50.65 (2s, $NH\underline{C}H(CH_3)CO$ diastereoisomers), 26.27 & 26.08 (s, 2 x $COOCH_2C(\underline{C}H_3)_3$ diastereoisomers), 25.41 & 25.18 (2s, $OCH_2\underline{C}(CH_3)_3$ diastereoisomers), 21.98 & 21.67 (2s, CH_3-2' diastereoisomers), 21.46 & 21.33 (2s, $NHCH(\underline{C}H_3)CO$ diastereoisomers), 15.71 & 15.68 (2s, $OCH_2\underline{C}H_3$ diastereoisomers).

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm): 3.60 (s, 60%), 3.41 (s, 40%).

Preparation of α-naphthyl L-Alanine-neopentyl-ester 2'-C-methyl-2'-O-propyl-6-O-methyl guanosine ProTide (148)



This ProTide was prepared according to the Standard Procedure D, using *t*BuMgCl (0.28 mL, 0.29 mmol, 2.0 eq), 2'-*C*-methyl-2'-*O*-propyl-6-*O*-methyl guanosine (**122**) (0.050 g, 0.14 mmol, 1.0 eq) in anh. THF (0.81 mL), and *α*-naphthyl *L*-Alanine-neopentyl ester phosphochloridate (**14.d**) (0.108 g, 0.28 mmol, 2.0 eq) in anh. THF (0.5 mL). After overnight stirring at RT, the solvent was removed under reduced pressure and the residue was purified by flash chromatography, using $CHCl_3$/MeOH (3.5%) as eluents, and preparative chromatography followed by preparative HPLC to yield the desired molecule (0.0006 g, 0.0009 mmol, 6 %).

HPLC ($MeOH/H_2O$): Rt = 28.48 min, 28.97 min

HPLC (ACN/H_2O): Rt = 21.80 min, 22.14 min

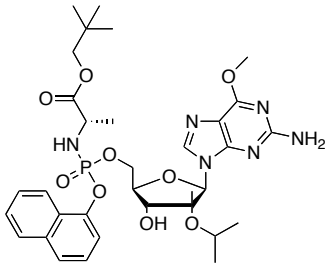
MS (TOF ES^+): 723.28 ($M + Na^+$).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.18-8.01 (m, 2H, H-9 Ar diastereoisomers), 7.86-7.85 (m, 2H, H-6 Ar diastereoisomers), 7.73-7.65 (m, 2H, H-8 base diastereoisomers), 7.72-7.66 (m, 2H, H-4 Ar diastereoisomers), 7.59-7.57 (m, 2H, H-8 Ar diastereoisomers), 7.54-7.51 (m, 4H, H-7, H-3 Ar diastereoisomers), 7.43-7.39 (m, 2H, H-2 Ar diastereoisomers), 6.06 & 6.04 (2s, 2H, H-1' diastereoisomers), 4.96 (bs, 4H, NH_2 base diastereoisomers), 4.70-4.66 (m, 2H, H-5' diastereoisomers), 4.53-4.48 (m, 2H, H-5' diastereoisomers), 4.27-4.23 (m, 2H, H-3' diastereoisomers), 4.19-4.14 (m, 4H, H-4', NHCH(CH_3)CO diastereoisomers), 4.09 & 4.08 (2s, 6H, 6-*O*CH_3 base diastereoisomers), 3.84-3.75 (m, 4H, O$CH_2CH_2CH_3$ diastereoisomers), 3.72-3.59 (2m, 4H, COO$CH_2C(CH_3)_3$ diastereoisomers), 1.89-1.86 (m, 2H, $OCH_2CH_2CH_3$), 1.72-1.65 (m, 2H, $OCH_2CH_2CH_3$), 1.39 & 1.36 (2d, 6H, J = 7.0 Hz, 7.0 Hz, NHCH(CH_3)CO diastereoisomers), 1.04-1.03 (m, 6H, $OCH_2CH_2CH_3$ diastereoisomers), 0.95-0.92 (2s, 18H, $OCH_2C(CH_3)_3$ diastereoisomers), 0.89 & 0.86 (2s, 6H, CH_3-2').

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 173.15 & 17.10 (2s, NHCH(CH_3)$_3$CO diastereoisomers), 161.36 & 161.19 (2s, C-6 base diastereoisomers), 159.84 & 159.45 (2s, C-2 base), 156.18 & 156.02 (2s, C-4 base diastereoisomers), 153.28 & 153.01 (2s, C-1 Ar diastereoisomers), 134.73 & 134.23 (2s, C-5 Ar diastereoisomers), 127.77 & 127.43 (2s, C-6 Ar diastereoisomers), 126.55 & 126.37 (2s, C-7 Ar diastereoisomers), 126.37 & 126.18 (2s, C-8 base diastereoisomers), 125.87 & 125.42 & 125.57 (3s, C-10, C-8, C-3 Ar diastereoisomers), 124.80 & 124.66 (2s, C-4 Ar diastereoisomers), 121.60 & 121.55 (2s, C-9 Ar diastereoisomers), 115.20 & 115.12 (2s, C-5 base), 115.02 & 114.96 (2s, C-2 Ar diastereoisomers), 87.76 & 87.34 (2s, C-1' diastereoisomers), 83.11 & 83.01 (2s, C-2' diastereoisomers), 81.03 & 79.86 (2s, C-4' diastereoisomers), 75.28 & 75.12 (2s, C-3' diastereoisomers), 74.71 & 74.53 (2s, O$CH_2C(CH_3)_3$ diastereoisomers), 65.09 & 64.95 (2s, O$CH_2CH_2CH_3$ diastereoisomers), 53.87 & 53.41 (2s, 6-*O*CH_3 base diastereoisomers), 50.89 50.43 (2s, NHCH(CH_3)CO diastereoisomers), 26.26 & 26.12 (2s, $OCH_2C(CH_3)_3$ diastereoisomers), 23.33 & 23.04 (2s, $OCH_2C(CH_3)_3$ diastereoisomers), 21.40 & 21.32 (2s, $OCH_2CH_2CH_3$ diastereoisomers), 21.40 & 41.23 (2s, NHCH(CH_3)CO diastereoisomers), 15.56 & 15.12 (2s, CH_3-2' diastereoisomers), 10.73 & 10.21 (2s, $OCH_2CH_2CH_3$ diastereoisomers).

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm): 3.45 (s, 17%), 3.40 (s, 83%).

Preparation of α-naphthyl L-Alanine-neopentyl-ester 2'-C-methyl-2'-O-isopropyl-6-O-methyl guanosine ProTide (149)



This ProTide was prepared according to the Standard Procedure D, using *t*BuMgCl (1M in THF, 0.14 mL, 0.14 mmol, 2.0 eq), 2'-*C*-methyl-2'-*O*-isopropyl-6-*O*-methyl guanosine (**123**) (0.025 g, 0.071 mmol, 1.0 eq) in anh. THF (1.00 mL), and *α*-naphthyl *L*-Alanine-neopentyl ester phosphochloridate (**14.d**) (0.055 g, 0.14 mmol, 2.0 eq) in anh. THF (0.5 mL). After overnight stirring at RT, the solvent was removed under reduced pressure and the residue was purified by flash chromatography, using $CHCl_3$/MeOH as eluents, and preparative chromatography followed by preparative HPLC to yield the desired molecule (0.001 g, 0.0014 mmol, 2 %).

HPLC (MeOH/H_2O): Rt = 28.95 min, 29.31 min

HPLC (ACN/H_2O): Rt = 21.89 min, 22.22 min

MS (TOF ES^+): 701.30 ($M + H^+$), 723.28 ($M + Na^+$).

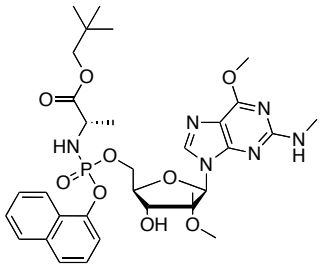
^{1}H NMR (500 MHz, MeOD) δ_H (ppm): 8.23 & 8.21 (2s, 2H, H-8 base diastereoisomers), 8.18-8.16 (m, 2H, H-9 Ar diastereoisomers), 8.12 (m, 1H, H-8 Ar diastereoisomers), 7.93-7.84 (m, 2H, H-6 Ar diastereoisomers), 7.71-7.66 (m, 2H, H-2 Ar diastereoisomers), 7.54-7.35 (m, 6H, H-4, H-3, H-7 Ar diastereoisomers), 5.99 & 5.97 (2s, 2H, H-1' diastereoisomers), 4.40-4.36 (m, 2H, H-3' diastereoisomers), 4.28-4.11 (2m, 4H, O<u>CH</u>$(CH_3)_2$, H-5' diastereoisomers), 4.04 & 4.03 (2s, 6H, 6-*O*CH_3 base diastereoisomers), 3.99-3.93 (m, 2H, H-4' diastereoisomers), 3.93-3.86 (m, 2H, NH<u>CH</u>(CH_3)CO diastereoisomers), 3.76-3.63 (m, 4H, O<u>CH_2</u>C$(CH_3)_3$ diastereoisomers), 1.50 (d, 3H, *J* = 7.1 Hz, NHCH(<u>CH_3</u>)CO), 1.32 (d, 3H, *J* = 6.8 Hz, NHCH(<u>CH_3</u>)CO), 1.27-1.25 (m, 3H, OCH(<u>CH_3</u>)$_2$), 1.21-1.19 (m, 3H, OCH(<u>CH_3</u>)$_2$), 1.01 & 0.99 (2s, 6H, CH_3-2' diastereoisomers), 0.86 & 0.85 (2s, 18H, OCH_2C(<u>CH_3</u>)$_3$ diastereoisomers).

^{13}C NMR (125 MHz, MeOD) δ_C (ppm): 173.67 & 174.10 (2s, NHCH$(CH_3)_3$<u>C</u>O diastereoisomers), 168.10 & 167.89 (2s, C-6 base diastereoisomers), 159.32 & 159.11 (2s, C-2 base diastereoisomers), 155.48 & 155.13 (s, C-4 base diastereoisomers), 153.43 & 153.09 (2s, C-1 Ar diastereoisomers), 140.67 & 140.11 (2s, C-8 base

diastereoisomers), 138.67 & 138.32 (2s, C-5 Ar diastereoisomers), 128.97 & 128.54 (2s, C-6 Ar diastereoisomers), 127.43 & 127.19 (2s, C-10 Ar diastereoisomers), 127.98 & 127.76 (2s, C-7 Ar diastereoisomers), 127.44 & 127.36 (2s, C-4 Ar diastereoisomers), 126.62 & 126.44 (2s, C-3 Ar diastereoisomers), 125.90 & 125.87 (2s, C-9 Ar diastereoisomers), 122.76 & 122.64 (2s, C-8 Ar diastereoisomers), 117.42 & 117.28 (2s, C-5 base diastereoisomers), 116.58 & 116.23 (2s, C-2 Ar diastereoisomers), 89.14 & 89.02 (2s, C-1' diastereoisomers), 84.54 & 84.32 (2s, C-2' diastereoisomers), 80.16 & 79.46 (2d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 6.3 Hz, 6.5 Hz, C-4' diastereoisomers), 75.31 & 75.20 (2s, C-3' diastereoisomers), 75.35 & 75.31 (2s, $O\underline{C}H_2C(CH_3)_3$ diastereoisomers), 71.37& 71.29 (2s, $O\underline{C}H(CH_3)_2$ diastereoisomers), 67.73 & 67.60 (2s, C-5' diastereoisomers), 53.97 & 53.30 (s, 6-$O\underline{C}H_3$ base diastereoisomers), 50.20 & 50.19 (2s, $NH\underline{C}H(CH_3)CO$ diastereoisomers), 30.76 & 30.69 (s, $OCH_2\underline{C}(CH_3)_3$ diastereoisomers), 26.65 & 26.21 (2s, $OCH_2C(\underline{C}H_3)_3$ diastereoisomers), 23.58 & 23.35 (2s, $OCH(\underline{C}H_3)_2$ diastereoisomers), 21.53 & 21.37 (2s, $NHCH(\underline{C}H_3)CO$ diastereoisomers), 18.98 & 18.56 (s, CH_3-2' diastereoisomers).

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm): 4.14 (s, 60%), 4.00 (s, 40%).

Preparation of α-naphthyl L-Alanine-neopentyl-ester N-2-methyl-2'-C-methyl-2'-O-methyl-6-O-methyl guanosine ProTide (150)



This ProTide was prepared according to the Standard Procedure D, using *t*BuMgCl (0.20 mL, 0.20 mmol, 2.0 eq), 2'-*C*-methyl-2'-*O*-methyl-*N*-2-methyl-6-*O*-methyl guanosine (**126**) (0.033 g, 0.098 mmol, 1.0 eq) in anh. THF (1.00 mL), and *α*-naphthyl *L*-Alanine-neopentyl ester phosphochloridate (**14.d**) (0.075 g, 0.20 mmol, 2.0 eq) in anh. THF (0.5 mL). After overnight stirring at RT, the solvent was removed under reduced pressure and the residue was purified by flash chromatography, using $CHCl_3$/MeOH (1.5%) as eluents, and preparative chromatography followed by preparative HPLC to yield the desired molecule (0.0014 g, 0.002 mmol, 20%).

HPLC (MeOH/H_2O): Rt = 28.36 min, 28.85 min

HPLC (ACN/H_2O): Rt = 21.17 min, 21.58 min

MS (TOF ES^+): 709.27 ($M + Na^+$).

^1H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.18-8.16 (m, 2H, H-9 Ar diastereoisomers), 7.86-7.84 (m, 2H, H-6 Ar diastereoisomers), 7.71-7.65 (m, 4H, H-4, H-8 Ar diastereoisomers), 7.57-7.49 (m, 6H, H-8 base, H-2, H-7 Ar diastereoisomers), 7.43-4.39 (m, 2H, H-3 Ar diastereoisomers), 6.14 (s, 2H, H-1' diastereoisomers), 5.02 (bs, 2H, NHCH$_3$ base diastereoisomers), 4.70-4.60 (m, 2H, H-5' diastereoisomers), 4.53-4.49 (m, 2H, H-5' diastereoisomers), 4.19-4.14 (m, 4H, H-3', H-4' diastereoisomers), 4.08 & 4.07 (2s, 6H, 6-*O*CH$_3$ base diastereoisomers), 3.82 & 3.80 (AB system, 2H, J_{AB} = 10.5 Hz, OCH$_2$C(CH$_3$)$_3$), 3.69 & 3.64 (AB system, 2H, J = 10.5 Hz, OCH$_2$C(CH$_3$)$_3$), 3.57 & 3.56 (2s, 6H, OCH$_3$ diastereoisomers), 3.00 (d, 3H, J = 5.0 Hz, NHCH$_3$), 2.96 (d, 3H, J = 5.0 Hz, NHCH$_3$), 2.92 & 2.90 (2d, 2H, J = 5.0 Hz, NHCH$_3$ diastereoisomers), 1.39 & 1.36 (2d, 6H, J = 7.0 Hz, 7.2 Hz, NHCH(CH$_3$)CO diastereoisomers), 1.02 & 1.01 (2s, 6H, CH$_3$-2' diastereoisomers), 0.89 & 0.88 (2s, 18H, OCH$_2$C(CH$_3$)$_3$ diastereoisomers).

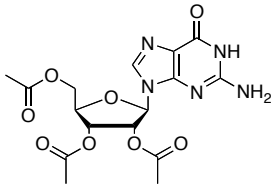
^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 173.15 & 17.10 (2s, NHCH(CH$_3$)$_3$CO diastereoisomers), 161.36 (s, C-6 base), 159.84 (s, C-2 base), 156.18 (s, C-4 base), 153.28 (C-1 Ar), 136.44 & 136.34 (2s, C-5 Ar diastereoisomers), 127.75 (s, C-6 Ar), 126.62-125.59 (6s, C-3, C-7, C-10 Ar, C-8 base diastereoisomers), 125.57 & 125.51 (2s, C-8 Ar diastereoisomers), 124.87 & 124.81 (2s, C-4 Ar diastereoisomers), 121.59 & 121.55 (2s, C-9 Ar diastereoisomers), 115.20 & 115.17 (2s, C-5 base diastereoisomers), 115.06 & 115.04 (2s, C-2 Ar diastereoisomers), 87.17 & 87.10 (2s, C-1' diastereoisomers), 83.21 (s, C-2'), 80.73 & 80.54 (2d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 6.4 Hz, 6.4 Hz, C-4' diastereoisomers), 75.10 & 74.87 (2s, C-3' diastereoisomers), 74.73 (s, OCH$_2$C(CH$_3$)$_3$), 65.98 & 65.42 (2s, C-5' diastereoisomers), 53.64 & 53.32 (s, 6-*O*CH$_3$ base), 51.16 & 50.87 (2s, OCH$_3$ diastereoisomers), 50.43 & 50.38 (2s, NHCH(CH$_3$)CO diastereoisomers), 29.70 (s, OCH$_2$C(CH$_3$)$_3$), 28.78 & 28.72 (2s, NHCH$_3$ diastereoisomers), 26.27 (s, OCH$_2$C(CH$_3$)$_3$), 21.30 & 21.20 (2d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 3.8 Hz, 5.12 Hz, NHCH(CH$_3$)CO diastereoisomers), 14.88 & 14.80 (2s, CH$_3$-2' diastereoisomers).

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm): 3.68 (s, 47%), 3.43 (s, 53%).

5.2. 2'-*O*-modifications of 6-*O*-methyl guanosine

5.2.1. Preparation of 2'-*O*-modified-6-*O*-methyl guanosine nucleosides

Preparation acetylated guanosine (135)

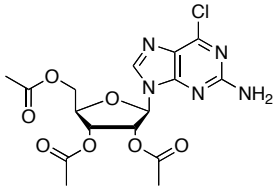


To a supension of guanosine (2.52 g, 8.90 mmol) and DMAP (0.11 g, 0.89 mmol, 0.17 eq) in anh. ACN (95 mL) was added anh. NEt_3 (4.90 mL, 35.2 mmol, 4.0 eq). Acetic anhydride was added dropwise (3.03 mL, 32.1 mmol, 3.6 eq) and the reaction was left stirring at RT overnight. MeOH (30 mL) was added to quench the reaction, a and a white product precipitated (2.25 g, 5.51 mol, 62%). by addition of Et_2O (300 mL).

^{1}H NMR (500 MHz, MeOD) δ_H (ppm): 7.87 (s, 1H, H-8 base), 6.08 (d, 1H, *J* = 4.9 Hz, H-1'), 5.95 (t, 1H, *J* = 5.4 Hz, H-3'), 5.70 (t, 1H, *J* = 5.3 Hz, H-4'), 4.47 (m, 3H, H-5', H-2'), 2.15 (s, 3H, OCO$\underline{CH_3}$), 2.09 & 2.08 (2s, 6H, 2 x OCO$\underline{CH_3}$).

^{13}C NMR (125 MHz, MeOD) δ_C (ppm): 172.33 (s, O$\underline{C}$OCH$_3$), 171.50 (s, O$\underline{C}$OCH$_3$), 171.22 (s, O$\underline{C}$OCH$_3$), 159.29 (s, C-6 base), 155.42 (s, C-2 base), 138.25 (s, C-8 base), 118.27 (s. C-5 base), 87.84 (s, C-1'), 81.38 (s, C-2'), 74.31 (s. C-3'), 72.05 (s, C-4'), 64.24 (s, C-5'), 20.62 (s, OCO$\underline{C}$H$_3$), 20.45 (s, OCO$\underline{C}$H$_3$), 20.29 (s, OCO$\underline{C}$H$_3$).

Preparation acetylated 6-chloroguanosine (136)

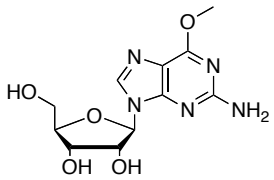


Acetyl guanosine (**135**) (0.59 g, 1.44 mmol), BTEA-Cl (0.66 g, 2.88 mmol, 2eq), *N*,*N*-dimethylaniline (0.20 mL, 1.58 mmol, 1.1 eq) and $POCl_3$ (0.67 mL, 7.20 mmol, 5 eq) in anh. ACN (20 mL) were heated under reflux at 85 °C for 2 hrs. Volatiles were evaporated immediately and the yellow oil recovered was dissolved in $CHCl_3$ and stirred for 30 min at RT with crushed ice. The 2 layers were separated and the aqueous layer was extracted 3 times with $CHCl_3$. Then crushed ice was added to the combined organic layers and washed 3 times with cold H_2O and once with $NaHCO_3$ (5%) in water to reach neutral pH. The organic layer was dried over $MgSO_4$. The solvent was evaporated to give a green yellow oil (0.57 g, 1.33 mmol, 92%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm); 7.89 (s, 1H, H-8 base), 6.01 (d, 1H, *J* = 4.9 Hz, H-1'), 5.94 (t, 1H, *J* = 5.1 Hz, H-3'), 5.72 (t, 1H, *J* = 5.0 Hz, H-4'), 5.51 (bs, 2H, NH_2 base), 4.42 (m, 3H, H-5', H-2'), 2.12 (s, 3H, OCO$\underline{CH_3}$), 2.07 (s, OCO$\underline{CH_3}$), 2.05 (s, 6H, OCO$\underline{CH_3}$).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 170.45 (s, O$\underline{C}$OCH$_3$), 169.58 (s, O$\underline{C}$OCH$_3$), 169.38 (s, O$\underline{C}$OCH$_3$), 159.28 (s, C-6 base), 153.15 (s, C-2 base), 151.66 (s, C-4 base), 140.75 (s, C-8 base), 125.59 (s. C-5 base), 87.29 (s, C-1'), 79.96 (s, C-2'), 72.74 (s. C-3'), 71.45 (s, C-4'), 62.73 (s, C-5'), 20.97 (s, OCO$\underline{C}$H$_3$), 20.65 (s, OCO$\underline{C}$H$_3$), 20.49 (s, OCO$\underline{C}$H$_3$).

Preparation 6-O-methyl guanosine (137) [12]

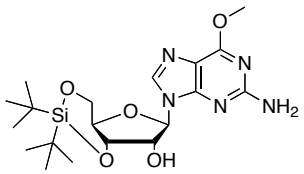


Acetylated 6-chloro-guanosine (**136**) (0.58 g, 1.33 mmol) was dissolved in anh. MeOH (50 mL) and NaOMe (0.48 g, 8.78 mmol, 6.6 eq) was added at 0 °C. The solution was stirred overnight at RT. The solvent was evaporated under reduced pressure after neutralisation with amberlite and filtration. The residue was purified by flash chromatography to yield the desired material (0.2125 g, 0.72 mmol, 54%).

^{1}H NMR (500 MHz, DMSO) δ_H (ppm): 8.10 (s, 1H, H-8 base), 6.43 (s, 2H, NH_2 base), 5.79 (d, 1H, *J* = 6.0 Hz, H-1'), 5.38 (d, 1H, *J* = 5.5 Hz, H-2'), 5.09 (m, 2H, H-4', H-3'), 4.47 (d, 1H, *J* = 5.5 Hz, OH-5'), 4.11 (d, 1H, *J* = 3.5 Hz, OH-4'), 3.97 (s, 3H, 6-*O*CH$_3$ base), 3.91 (d, 1H, *J* = 3.5, OH-3'), 3.65 (m, 1H, H-5'), 3.55 (m, 1H, H-5').

^{13}C NMR (125 MHz, DMSO) δ_C (ppm): 160.67 (s, C-6 base), 159.76 (s, C-2 base), 154.09 (s, C-4 base), 137.95 (s, C-8 base), 113.56 (s, C-5 base), 86.54 (s, C-1'), 85.23 (s, C-4'), 73.44 (s, C-2'), 70.37 (s, C-3'), 61.41 (s, C-5'), 53.16 (s, 6-*O*CH$_3$ base).

Preparation 3',5'-DTBS-protected 6-O-methyl guanosine (138) [13]

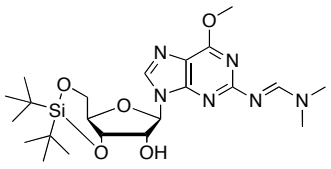


To a suspension of 6-O-methyl guanosine (**137**) (0.23 g, 0.77 mmol, 1.0 eq) in anh. DMF (4.5 mL) was added di-*tert*-butyl-silyl-triflate (0.28 mL, 0.85 mmol, 1.1 eq) at 0 °C. The solution was stirred 30 min at 0 °C, and the reaction was quenched with cold EtOH and neutralised with NEt_3. The solvent was removed under reduced pressure and the remaining oil was dissolved in MeOH. A white solid precipitated by addition of H_2O. After filtration and drying under Buchner, the desired molecule was recovered (0.27 g, 0.61 mmol, 80%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 7.65 (s, 1H, H-8 base), 5.99 (s, 1H, H-1'), 4.64-4.61 (m, 1H, H-4'), 4.57 (m, 1H, H-2'), 4.53-4.48 (m, 1H, H-5'), 4.22-4.17 (m, 1H, H-3'), 4.11-4.09 (m, 1H, H-5'), 4.07 (s, 3H, 6-*O*CH_3 base), 1.13 & 1.07 (2s, 18H, Si(C($\underline{CH_3}$)$_3$)$_2$).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 161.77 (s, C-6 base), 159.38 (s, C-2 base), 152.51 (s, C-4 base), 137.47 (s, C-8 base), 116.20 (s, C-5 base), 90.58 (s, C-1'), 75.53 (s, C-4'), 74.82 (s, C-2'), 73.76 (s, C-3'), 67.60 (s, C-5'), 53.99 (s, 6-*O*CH_3 base), 32.24 & 32.18 (2s, Si($\underline{C}$(CH$_3$)$_3$)$_2$), 27.80 & 27.78 & 27.50 & 27.41 & 27.37 & 27.26 (6s, Si(C($\underline{CH_3}$)$_3$)$_2$).

Preparation 3',5'-DTBS-protected-N-2-formamidine 6-O-methyl guanosine (139)



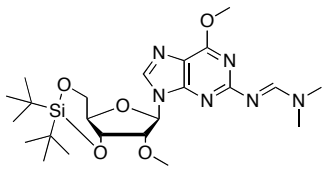
To a solution of 3',5'-protected 6-*O*-methyl guanosine (**138**) (0.19 g, 0.43 mmol, 1.0 eq) in anh. DMF (3.10 mL) was added *N*,*N*-dimethylformamide dimethylacetate (0.24 mL, 1.72 mmol, 4.0 eq). The reaction mixture was stirred overnight at RT, and the solvent was evaporated. The residue was dissolved in EtOAc and washed with $NaHCO_3$, H_2O and brine. Evaporation of the organic layer afforded the desired material (0.21 g, 0.42 mmol, 97%).

^{1}H NMR (500 MHz, MeOD) δ_H (ppm): 8.44 (s, 1H, N$\underline{CH}$N(CH$_3$)$_2$), 7.65 (s, 1H, H-8 base), 5.93 (s, 1H, H-1'), 4.59 (d, 1H, *J* = 5.0 Hz, H-3'), 4.35-3.30 (m, 2H, H-4', H-5'),

4.07-4.01 (s, 1H, H-2'), 3.97 (s, 3H, 6-*O*Me base), 3.96 & 3.86 (m, 1H, H-5'), 2.97 & 2.96 (2s, 6H, NCHN($\underline{CH_3}$)$_2$), 0.93 & 0.89 (2s, 18H, Si(C($\underline{CH_3}$)$_3$)$_2$).

^{13}C NMR (125 MHz, MeOD) δ_C (ppm): 162.32 (s, C-6 base), 160.79 (s, C-2 base), 158.26 (s, N$\underline{C}$HN(CH$_3$)$_2$), 152.51 (s, C-4 base), 138.53 (s, C-8 base), 117.86 (s, C-5 base), 90.66 (s, C-1'), 77.56 (s, C-4'), 76.23 (s, C-2'), 74.43 (s, C-3'), 67.52 (s, C-5'), 53.67 (s, 6-*O*Me base), 41.09 (s, NCHN($\underline{CH_3}$)$_2$), 35.09 (s, NCHN($\underline{CH_3}$)$_2$), 27.67 & 27.56 & 27.42 & 27.37& 27.28 & 27.05 (6s, Si(C($\underline{C}$H$_3$)$_3$)$_2$), 22.61 & 22.56 (2s, Si($\underline{C}$(CH$_3$)$_3$)$_2$).

Preparation 3',5'-DTBS-protected-N-2-formamidine 2'-O-methyl-6-O-methyl guanosine (140.a)

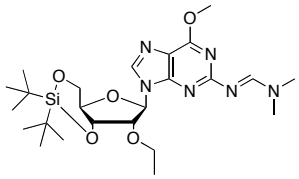


To a solution of 3',5'-DTBS-protected-*N*-2-formamidine 6-*O*-methyl guanosine (**139**) (0.21 g, 0.42 mmol, 1.0 eq) in anh. DMF (5.9 mL) was added molecular sieves 3Å, methylidodide (0.16 mL, 2.52 mmol, 6.0 eq) at 0 °C. The reaction was left stirring at 0 °C for 15 min, and then NaH (60% dispersion in oil, 0.02 g, 0.50 mmol, 1.2 eq) was added. The mixture was left stirring for 1 hr at 0 °C, and quenched with sodium dihydrogen phosphate (0.5 M) and dissolved in ethyl acetate. The organic layer was washed with sodium dihydrogen phosphate, H_2O and brine, and the solvents were evaporated under reduced pressure to yield the desired material without further purification needed (0.2064 g, 0.40 mmol, 97 %).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.64 (s, 1H, N$\underline{CH}$N(CH$_3$)$_2$), 7.72 (s, 1H, H-8 base), 6.02 (s, 1H, H-1'), 4.53-4.27 (m, 2H, H-5', H-4'), 4.22-4.19 (m, 1H H-2'), 4.15 (s, 3H, 6-*O*CH$_3$ base), 4.01-3.97 (m, 2H, H-5', H-3'), 3.64 (s, 3H, O$\underline{CH_3}$), 3.18 & 3.16 (2s, 6H, NCHN($\underline{CH_3}$)$_2$), 1.07 & 1.05 (2s, 18H, Si(C($\underline{CH_3}$)$_3$)$_2$).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 162.74 (s, C-6 base), 161.18 (s, C-2 base), 158.39 (s, N$\underline{C}$HN(CH$_3$)$_2$), 152.81 (s, C-4 base), 140.94 (s, C-8 base), 118.16 (s, C-5 base), 88.82 (s, C-1'), 82.35 (s, C-2'), 77.80 (s, C-4'), 74.34 (s, C-3'), 67.58 (s, C-5'), 53.87 (s, 6-*O*Me base), 52.04 (s, O$\underline{C}$H$_3$), 40.94 (2s, NCHN($\underline{CH_3}$)$_2$), 35.12 (s, NCHN($\underline{CH_3}$)$_2$), 28.70 & 27.85 (2s, Si(C($\underline{C}$H$_3$)$_3$)$_2$), 22.74 & 22.66 (2s, Si($\underline{C}$(CH$_3$)$_3$)$_2$).

Preparation 3',5'-DTBS-protected-N-2-formamidine 2'-O-methyl-6-O-methyl guanosine (140.b)

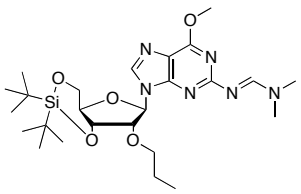


Prepared similarly to **140.a**, using 3',5'-DTBS-protected-*N*-2-formamidine 6-*O*-methyl guanosine (**139**) (0.25 g, 0.51 mmol, 1.0 eq) in anh. DMF (7.5 mL), molecular sieves 3Å, ethyl idodide (0.19 mL, 3.06 mmol, 6.0 eq), and NaH (60% dispersion in oil, 0.025 g, 0.61 mmol, 1.2 eq). The mixture was left stirring for 2 hrs at 0 °C, and quenched with sodium dihydrogen phosphate (0.5 M) and dissolved in EtOAc. The organic layer was washed with sodium dihydrogen phosphate, H_2O and brine, and the solvents were evaporated under reduced pressure to yield the desired material without further purification needed (0.18 g, 0.34 mmol, 67%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.64 (s, 1H, NC<u>H</u>N$(CH_3)_2$), 7.72 (s, 1H, H-8 base), 6.03 (d, 1H, *J* = 7.5 Hz, H-1'), 4.46-4.39 (m, 1H, , H-5'), 4.32-4.30 (m, 1H, H-2'), 4.20-4.16 (m, 1H, H-4'), 4.15 (s, 3H, 6-*O*CH_3 base), 4.13-4.08 (m, 1H, H-3'), 4.01-3.97 (m, 1H, H-5'), 3.74-3.58 (m, 2H, O<u>CH_2</u>CH_3), 3.16 & 3.13 (2s, 6H, NCHN(<u>CH_3</u>)<u>$_2$</u>), 1.25-1.23 (m, 3H, 2OCH_2<u>CH_3</u>), 1.06 & 1.03 (2s, 18H, 2 x Si(C(<u>CH_3</u>)<u>$_3$</u>)<u>$_2$</u>)..

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 162.73 (s, C-6 base), 161.17 (s, C-2 base), 158.38 (s, NC<u>H</u>N$(CH_3)_2$), 152.83 (s, C-4 base), 138.43 (s, C-8 base), 118.20 (s, C-5 base), 89.18 (s, C-1'), 80.71 (s, C-4'), 77.47 (s, C-2'), 74.30 (s, C-3'), 67.51 (s, O<u>CH_2</u>CH_3), 62.79 (s, C-5'), 53.75 (s, 6-*O*CH_3 base), 41.09 & 35.18 (2s, NCHN(<u>CH_3</u>)<u>$_2$</u>), 29.65 & 29.31 (2s, 2 x Si(<u>C</u>$(CH_3)_3)_2$), 27.90 & 27.85 & 27.79 & 27.69 & 27.49 & 27.37 (6s, 2 x Si(C(<u>CH_3</u>)<u>$_3$</u>), 15.37 (s, OCH_2<u>CH_3</u>).

Preparation 3',5'-DTBS-protected-N-2-formamidine 2'-O-propyl-6-O-methyl guanosine (140.c)



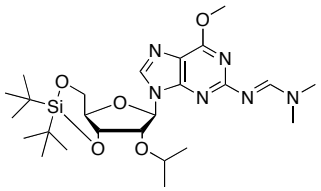
Prepared similarly to **140.a**, using 3',5'-DTBS-protected-*N*-2-formamidine 6-*O*-methyl guanosine (**139**) (0.60 g, 1.22 mmol, 1.0 eq) in anh. DMF (17 mL), molecular sieves

3Å, propyl idodide (0.71 mL, 7.32 mmol, 6.0 eq), and NaH (60% dispersion in oil, 0.059 g, 1.47 mmol, 1.2 eq). The mixture was left stirring for 5 hrs at 0 °C, and quenched with sodium dihydrogen phosphate (0.5 M) and dissolved in EtOAc. The organic layer was washed with sodium dihydrogen phosphate, H_2O and brine, and the solvents were evaporated under reduced pressure to yield the desired material without further purification needed (0.52 g, 0.97 mmol, 80%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.50 (s, 1H, NC<u>H</u>N$(CH_3)_2$), 7.65 (s, 1H, H-8 base), 5.90 (d, 1H, *J* = 6.0 Hz, H-1'), 4.32-4.28 (m, 2H, H-2', H3'), 4.18 (m, 1H, H-4'), 3.99 (s, 3H, 6-*O*CH_3 base), 3.91-3.84 (m, 1H, H-5'), 3.77-3.72 (m, 1H, H-5'), 3.53-3.51 (m, 2H, O<u>C</u>H_2<u>C</u>H_2<u>C</u>H_3), 3.02 & 3.00 (2s, 6H, NCHN(<u>C</u>H_3)$_2$), 1.54-1.47 (m, 2H, OCH$_2$<u>C</u>H_2<u>C</u>H_3), 0.85 & 0.81 (2s, 18H, Si(C(<u>C</u>H_3)$_3$)$_2$), 0.79-0.73 (m, 3H, OCH$_2$CH$_2$<u>C</u>H_3).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 162.52 (s, C-6 base), 160.93 (s, C-2 base), 158.19 (s, N<u>C</u>HN$(CH_3)_2$), 152.64 (s, C-4 base), 138.45 (s, C-8 base), 117.93 (s, C-5 base), 89.01 (s, C-1'), 86.42 (s, C-4'), 80.83 (s, C-2'), 72.86 (s, <u>C</u>H_2<u>C</u>H_2<u>C</u>H_3), 69.32 (s, C-3'), 63.04 (s, C-5'), 54.09 (s, 6-*O*CH_3 base), 36.18 & 34.72 (2s, NCHN(<u>C</u>H_3)$_2$), 29.44 & 29.15 (2s, Si(<u>C</u>$(CH_3)_3)_2$), 26.64 & 26.58 & 26.55 & 26.50 & 24.44 & 26.41 (6s, Si(C(<u>C</u>H_3)$_3$)$_2$), 22.89 (s, OCH$_2$<u>C</u>H_2<u>C</u>H_3), 11.18 (s, OCH$_2$CH$_2$<u>C</u>H_3).

Preparation 3',5'-DTBS-protected-N-2-formamidine 2'-O-isopropyl-6-O-methyl guanosine (140.d)

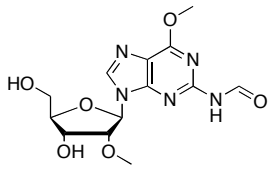


Prepared similarly to **140.a**, using 3',5'-DTBS-protected-*N*-2-formamidine 6-*O*-methyl guanosine (**139**) (0.60 g, 1.22 mmol, 1.0 eq) in anh. DMF (17 mL), molecular sieves 3Å, isopropyl idodide (0.73 mL, 7.32 mmol, 6.0 eq), and NaH (60% dispersion in oil, 0.059 g, 1.46 mmol, 1.2 eq). The mixture was left stirring for 5 hrs at 0 °C, and quenched with sodium dihydrogen phosphate (0.5 M) and dissolved in EtOAc. The organic layer was washed with sodium dihydrogen phosphate, H_2O and brine, and the solvents were evaporated under reduced pressure to yield the desired material without further purification needed (0.40 g, 0.75 mmol, 61%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.57 (s, 1H, NC<u>H</u>N$(CH_3)_2$), 7.93 (s, 1H, H-8 base), 5.87 (d, 1H, *J* = 6.0 Hz, H-1'), 4.32-4.27 (m, 1H, H-2'), 4.10-4.08 (m, 2H, H-3'), 4.08 (s, 3H, 6-*O*CH_3 base), 4.05-3.98 (m, 1H, H-4'), 3.99-3.94 (m, 1H, H-5'), 3.59-3.55 (m, 1H, H-5'), 3.11-3.04 (m, 1H, <u>CH</u>$(CH_3)_2$), 2.89 & 2.81 (2s, 6H, NCHN(<u>CH_3</u>)<u>$_2$</u>), 1.49-1.44 (m, 6H, OCH(<u>CH_3</u>)<u>$_2$</u>), 0.99 & 0.96 (2s, 18H, Si(C(<u>CH_3</u>)<u>$_3$</u>)<u>$_2$</u>).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 162.61 (s, C-6 base), 160.04 (s, C-2 base), 158.83 (s, N<u>C</u>HN$(CH_3)_2$), 152.64 (s, C-4 base), 140.32 (s, C-8 base), 118.46 (s, C-5 base), 89.47 (s, C-1'), 85.46 (s, C-4'), 78.40 (s, C-2'), 73.82 (s, O<u>C</u>H$(CH_3)_2$), 71.49 (s, C-3'), 63.14 (s, C-5'), 53.84 (s, 6-*O*CH_3 base), 35.55 & 35.18 (2s, NCHN(<u>C</u>$H_3)_2$), 29.64 & 29.28 (2s, Si(<u>C</u>$(CH_3)_3)_2$), 27.64 & 27.58 & 27.55 & 27.50 & 27.43 & 27.40 (6s, Si(C(<u>C</u>$H_3)_3)_2$), 20.93 & 19.86 (s, OCH(<u>C</u>$H_3)_2$).

Preparation N-2-formamide 2'-O-methyl-6-O-methyl guanosine (141.a)



To a solution of 3',5'-DTBS-protected-*N*-2-formamidine 2'-*O*-methyl-6-*O*-methyl guanosine (**140.a**) (0.26 g, 0.51 mmol, 1.0 eq) in anh. THF (11 mL) was added anh. NEt_3 (0.13 mL, 0.92 mmol, 1.8 eq) and $NEt_3.H_3F$ (0.29 mL, 1.79 mmol, 3.5 eq). The solution was stirred at rrom temperature for 1 hr. The solvent was evaporated under reduced pressure and the residue purified by flash chromatography using MeOH/$CHCl_3$ to recover the partially DMF cleaved molecules A (0.0066 g, 0.19 mmol, 38 %).

HPLC (MeOH/H_2O): Rt = 1.52 min

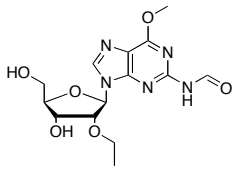
HPLC (ACN/H_2O): Rt = 5.59 min

MS (TOF ES^+): 339.34 ($M + H^+$).

^{1}H NMR (500 MHz, MeOD) δ_H (ppm): 9.49 (bs, 1H, NHC<u>H</u>O), 8.45 (s, H-8 base), 6.14 (d, 1H, *J* = 4.5 Hz, H-1'), 4.50 (m, 1H, H-3'), 4.33 (t, 1H, *J* = 4.7 Hz, H-2'), 4.16 (s, 3H, 6-*O*CH_3 base), 4.11-4.09 (m, 1H, H-4'), 3.92 (dd, 1H, *J* = 2.9 Hz, 12.3 Hz, H-5'), 3.81 (dd, 1H, *J* = 3.5 Hz, 12.2 Hz, H-5'), 3.52 (s, 3H, O<u>CH_3</u>).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 165.58 (s, NH<u>C</u>HO), 162.59 (s, C-6 base), 154.23 (s, C-2 base), 153.51 (s, C-4 base), 142.09 (s, C-8 base), 119.31 (s, C-5 base), 88.12 (s, C-1'), 86.96 (s, C-4'), 85.06 (s, C-2'), 70.34 (s, C-3'), 62.32 (s, C-5'), 58.96 (s, O<u>C</u>H_3), 55.05 (s, 6-*O*CH_3 base).

Preparation N-2-formamide 2'-O-ethyl-6-O-methyl guanosine (141.b)

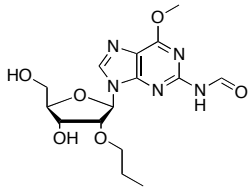


To as solution of 3',5'-DTBS-protected-*N*-2-formamidine 2'-*O*-ethyl-6-*O*-methyl guanosine (**140.b**) (0.18 g, 0.34 mmol, 1.0 eq) in anh. THF (9 mL), anh. NEt_3 (0.086 mL, 0.61 mmol, 1.8 eq) and $NEt_3.H_3F$ (0.19 mL, 1.19 mmol, 3.5 eq) were added. The solution was stirred at RT for 1 hr. The solvent was evaporated under reduced pressure and the residue purified by flash chromatography using $CHCl_3$/MeOH (3-4%) to afford the desired molecule (0.044 g, 0.12 mmol, 35 %).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 9.51 (d, 1H, *J* = 9.5 Hz, NHCHO), 8.51 (d, 1H, *J* = 9.5 Hz, NHCHO), 7.99 (s, 1H, H-8 base), 5.94 (d, 1H, *J* = 6.5 Hz, H-1'), 4.62 (dd, 1H, *J* = 5.0 Hz, 6.5 Hz, H-2'), 4.55 (m, 1H, H-3'), 4.32 (m, 1H, H-4'), 4.16 (s, 3H, 6-*O*CH_3 base), 3.99-3.96 (m, 1H, H-5'), 3.84-3.79 (m, 1H, H-5'), 3.64-3.57 (m, 1H, O$CH_2$$CH_3$), 3.55-3.50 (m, 1H, O$CH_2$$CH_3$), 1.814 (t, 3H, *J* = 7.0 Hz, OCH_2CH_3).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 163.02 (s, NHCHO), 161.75 (s, C-6 base), 151.91 (s, C-2 base), 151.51 (s, C-4 base), 141.37 (s, C-8 base), 119.98 (s, C-5 base), 88.86 (s, C-1'), 86.96 (s, C-4'), 81.02 (s, C-2'), 70.53 (s, C-3'), 67.00 (s, CH_2CH_3), 62.68 (s, C-5'), 54.70 (s, 6-*O*CH_3 base), 15.23 (s, OCH_2CH_3).

Preparation N-2-formamide 2'-O-propyl-6-O-methyl guanosine (141.c)



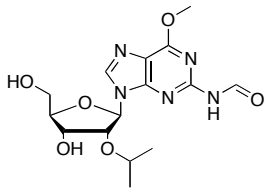
To a solution of 3',5'-DTBS-protected-*N*-2-formamidine 2'-*O*-propyl-6-*O*-methyl guanosine (**140.c**) (0.52 g, 0.97 mmol, 1.0 eq) in THF (25 mL), anh. NEt_3 (0.24 mL, 1.75 mmol, 1.8 eq) and $NEt_3.H_3F$ (0.55 mL, 3.40 mmol, 3.5 eq) were added. The solution was stirred at RT for 1 hr. The solvent was evaporated under reduced pressure and the residue purified by flash chromatography using $CHCl_3$/MeOH (2%) to afford the desired molecule (0.13 g, 0.36 mmol, 37 %).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 9.49 (d, 1H, *J* = 10.0 Hz, NHCHO), 8.67 (d, 1H, *J* = 10.0 Hz, NHCHO), 8.03 (s, 1H, H-8 base), 5.96 (d, 1H, *J* = 5.5 Hz, H-1'), 4.58-4.56

(m, 2H, H-2', H-3'), 4.30 (m, 1H, H-4'), 4.14 (s, 3H, 6-*O*CH_3 base), 3.98-3.96 (m, 1H, H-5'), 3.84-3.79 (m, 1H, H-5'), 3.52-3.38 (m, 2H, O$\underline{CH_2}CH_2CH_3$), 1.53-1.46 (m, 2H, $OCH_2\underline{CH_2}CH_3$), 0.82 (t, 3H, J = 7.0 Hz, $OCH_2CH_2\underline{CH_3}$).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 163.17 (s, NH$\underline{C}$HO), 161.64 (s, C-6 base), 151.94 (s, C-2 base), 151.56 (s, C-4 base), 141.27 (s, C-8 base), 119.19 (s, C-5 base), 88.61 (s, C-1'), 86.84 (s, C-4'), 81.31 (s, C-2'), 73.08 (s, O$\underline{C}H_2CH_2CH_3$), 70.41 (s, C-3'), 62.49 (s, C-5'), 54.65 (s, 6-*O*CH_3 base), 22.82 (s, $OCH_2\underline{C}H_2CH_3$), 10.19 (s, $OCH_2CH_2\underline{C}H_3$).

Preparation N-2-formamide 2'-O-isopropyl-6-O-methyl guanosine (141.d)

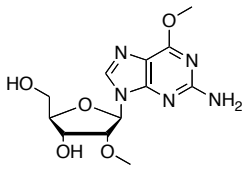


To a solution of 3',5'-DTBS-protected-*N*-2-formamidine 2'-*O*-isopropyl-6-*O*-methyl guanosine (**140.d**) (0.40 g, 0.75 mmol, 1.0 eq) in anh. THF (19 mL). (0.043 g, 0.76 mmol, 4.0 eq), anh. NEt_3 (0.19 mL, 1.35 mmol, 1.8 eq) and $NEt_3.H_3F$ (0.43 mL, 2.63 mmol, 3.5 eq) were added. The solution was stirred at RT for 1 hr. The solvent was evaporated under reduced pressure and the residue purified by flash chromatography using $CHCl_3$/MeOH (2%) to afford the desired molecule (0.070 g, 0.19 mmol, 25 %).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 9.51 (d, 1H, J = 9.8 Hz, NH$\underline{C}$H$\underline{O}$), 8.39 (d, 1H, J = 9.8 Hz, $\underline{NH}$CHO), 7.94 (s, 1H, H-8 base), 5.85 (d, 1H, J = 6.9 Hz, H-1'), 4.70 (dd, 1H, J = 5.0 Hz, 6.8 Hz, H-2'), 4.42 (m, 1H, H-3'), 4.33 (m, 1H, H-4'), 4.18 (s, 3H, 6-*O*CH_3 base), 3.98 (dd, 1H, J = 1.5 Hz, 12.4 Hz, H-5'), 3.82 (d, 1H, J = 12.4 Hz, H-5'), 3.67-3.62 (m, 1H, O$\underline{CH}(CH_3)_2$), 1.16 (d, 3H, J = 6.2 Hz, OCH($\underline{CH_3}$)$_2$), 0.94 (d, 3H, J = 6.1 Hz, OCH($\underline{CH_3}$)$_2$).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 162.88 (s, NH$\underline{C}$HO), 161.82 (s, C-6 base), 151.89 (s, C-2 base), 151.40 (s, C-4 base), 141.50 (s, C-8 base), 120.09 (s, C-5 base), 89.42 (s, C-1'), 87.10 (s, C-4'), 78.97 (s, C-2'), 73.47 (s, O$\underline{C}H(CH_3)_2$), 71.07 (s, C-3'), 62.91 (s, C-5'), 50.69 (s, 6-*O*CH_3 base), 22.59 & 22.25 (s, OCH($\underline{CH_3}$)$_2$).

Preparation 2'-O-methyl-6-O-methyl guanosine (142)



N-2-formamide 2'-*O*-methyl-6-*O*-methyl guanosine (**141.a**) (0.050 g, 0.13 mmol, 1.0 eq) was dissolved in THF/H_2O (10/5 mL) and KOH (0.030 g, 0.53 mmol, 4.0 eq) was added. The reaction was left stirring for 6 hrs at RT. The solvent was evaporated under reduced pressure to give a yellow residue that was purified by preparative chromatography using $CHCl_3$/MeOH (10%) to yield the desired material (0.040 g, 0.13 mmol, 96%).

HPLC (MeOH/H_2O): Rt = 12.62 min

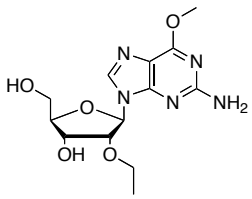
HPLC (ACN/H_2O): Rt = 5.11 min

MS (TOF ES^+): 312.13 ($M + H^+$); MS (TOF ES^-): 346.09 ($M + Cl^-$).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 7.64 (s, 1H, H-8 base), 5.78 (d, 1H, *J* = 7.5 Hz, H-1'), 4.89(bs, 2H, NH_2 base), 4.69 (dd, 1H, *J* = 5.0 Hz, 7.5 Hz, H-2'), 4.57 (dd, 1H, *J* = 5.0 Hz, 5.8 Hz, H-3'), 4.35 (m, 1H, H-4'), 4.10 (s, 3H, 6-*O*CH_3 base), 4.00 (m, 1H, H-5'), 3.79 (m, 1H, H-5'), 3.36 (m, 1H, O$\underline{CH_3}$).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 162.23 (s, C-6 base), 158.75 (s, C-2 base), 151.95 (s, C-4 base), 139.47 (s, C-8 base), 115.76 (s, C-5 base), 89.40 (s, C-1'), 87.86 (s, C-4'), 80.01 (s, C-2'), 70.78 (s, C-3'), 63.41 (s, C 5'), 58.84 (s, OCH_3), 54.08 (s, 6-*O*CH_3 base).

Preparation 2'-O-ethyl-6-O-methyl guanosine (143)



N-2-formamide 2'-*O*-methyl-6-*O*-methyl guanosine (**141.b**) (0.050 g, 0.13 mmol, 1.0 eq) was dissolved in THF/H_2O (10/5 mL) and KOH (0.029 g, 0.52 mmol, 4.0 eq) was added. The reaction was left stirring for 6 hrs at RT. The solvent was evaporated under reduced pressure to give a yellow residue that was purified by preparative chromatography using $CHCl_3$/MeOH (10%) to yield the desired material (0.039 g, 0.12 mmol, 92%).

HPLC (MeOH/H_2O): Rt = 14.13 min

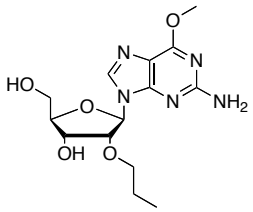
HPLC (ACN/H_2O): Rt = 3.04 min

MS (TOF ES^+): 325.20 (M); MS (TOF ES^-): 360.11 (M + Cl^-).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 7.66 (s, 1H, H-8 base), 5.77 (d, 1H, *J* = 7.5 Hz, H-1'), 4.74 (dd, 1H, *J* = 7.5 Hz, 4.3 Hz, H2'), 5.51 (dd, 1H, *J* = 4.5 Hz, 6.5 Hz, H-3'), 4.35 (m, 1H, H-4'), 4.09 (s, 3H, 6-*O*CH_3 base), 3.99 (dd, 1H, *J* = 1.0 Hz, 12.5 Hz, H-5'), 3.77 (d, 1H, *J* = 12.0 Hz, H-5'), 3.59 (m, 1H, O$\underline{CH_2}CH_3$), 3.47 (m, 1H, O$\underline{CH_2}CH_3$), 1.11 (t, 3H, *J* = 7.0 Hz, $OCH_2\underline{CH_3}$).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 162.23 (s, C-6 base), 158.69 (s, C-2 base), 149.15 (s, C-4 base), 115.23 (s, C-5 base), 139.54 (s, C-8 base), 89.57 (s, C-1'), 87.76 (s, C-2'), 80.44 (s, C-4'), 71.17 (s, C-3'), 67.00 (s, O$\underline{C}H_2CH_3$), 63.39 (s, C-5'), 54.13 (s, 6-*O*CH_3 base), 15.25 (s, 3H, $OCH_2\underline{C}H_3$).

Preparation 2'-O-propyl-6-O-methyl guanosine (144)



N-2-formamide 2'-*O*-propyl-6-*O*-methyl guanosine (**141.c**) (0.060 g, 0.16 mmol, 1.0 eq) was dissolved in THF/H_2O (3/1.5 mL) and KOH (0.041 g, 0.64 mmol, 4.0 eq) was added. The reaction was left stirring for 4 hrs at RT. The solvent was evaporated under reduced pressure to give a yellow residue that was purified by preparative chromatography using $CHCl_3$/MeOH (10%) to yield the desired material (0.056 g, 0.15 mmol, 95%).

HPLC (MeOH/H_2O): Rt = 16.51 min

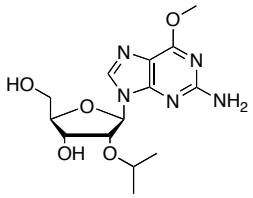
HPLC (ACN/H_2O): Rt = 7.10 min

MS (TOF ES^+): 340.17 (M + H^+); MS (TOF ES^-): 374.12 (M + Cl^-).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 7.65 (s, 1H, H-8 base), 5.76 (d, 1H, *J* = 7.5 Hz, H-1'), 5.20 (bs, 2H, NH_2 base), 4.70 (dd, 1H, *J* = 4.5 Hz, 3.0 Hz, H-2'), 4.49 (dd, 1H, *J* = 4.5 Hz, 5.5 Hz, H-3'), 4.32 (m, 1H, H-4'), 4.06 (s, 3H, 6-*O*CH_3 base), 3.97 (dd, 1H, *J* = 2.0 Hz, 13.0 Hz, H-5'), 3.75 (d, 1H, *J* = 13.0 Hz, H-5'), 3.44-3.28 (2m, 2H, O$\underline{CH_2}CH_2CH_3$), 1.49-1.42 (m, 2H, $OCH_2\underline{CH_2}CH_3$), 0.88 (t, 3H, *J* = 7.5 Hz, $OCH_2CH_2\underline{CH_3}$).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 162.11 (s, C-6 base), 158.92 (s, C-2 base), 151.93 (s, C-4 base), 139.49 (s, C-8 base), 115.99 (s, C-5 base), 89.49 (s, C-1'), 87.67 (s, C-4'), 80.52 (s, C-2'), 73.10 (s, O<u>C</u>$H_2CH_2CH_3$), 71.06 (s, C-3'), 63.34 (s, C-5'), 54.06 (s, 6-*O*CH_3 base), 22.83 (s, OCH_2<u>C</u>H_2CH_3), 10.12 (s, OCH_2CH_2<u>C</u>H_3).

Preparation 2'-O-isopropyl-6-O-methyl guanosine (145)



N-2-formamide 2'-*O*-isopropyl-6-*O*-methyl guanosine (**141.d**) (0.070 g, 0.19 mmol, 1.0 eq) was dissolved in THF/H_2O (3/1.5 mL) and KOH (0.043 g, 0.76 mmol, 4.0 eq) was added. The reaction was left stirring for 5 hrs at RT. The solvent was evaporated under reduced pressure to give a yellow residue that was purified by preparative chromatography using $CHCl_3$/MeOH (10%) to yield the desired material (0.039 g, 0.11 mmol, 60%).

HPLC (MeOH/H_2O): Rt = 15.67 min

HPLC (ACN/H_2O): Rt = 7.22 min

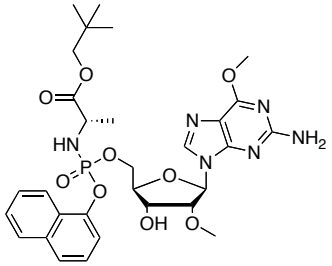
MS (TOF ES$^+$): 340.12 (M + H$^+$), 362.10 (M + Na$^+$).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 7.64 (s, 1H, H-8 base), 5.71 (d, 1H, *J* = 7.5 Hz, H-1'), 5.03 (bs, 2H, NH_2 base), 4.81 (dd, 1H, *J* = 5.0 Hz, 2.5 Hz, H-2'), 4.42 (m, 1H, H-3'), 4.35 (m, 1H, H-4'), 4.10 (s, 3H, 6-*O*CH_3 base), 3.99 (dd, 1H, *J* = 1.5 Hz, 12.5 Hz, H-5'), 3.76 (d, 1H, *J* = 12.5 Hz, H-5'), 3.63-3.57 (m, 1H, O<u>CH</u>$(CH_3)_2$), 1.15 (d, 3H, *J* = 6.0 Hz, OCH(<u>C</u>H_3)$_2$), 0.88 (d, 3H, *J* = 6.5 Hz, OCH(<u>C</u>H_3)$_2$).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 162.19 (s, C-6 base), 158.81 (s, C-2 base), 151.90 (s, C-4 base), 139.60 (s, C-8 base), 115.38 (s, C-5 base), 89.81 (s, C-1'), 87.69 (s, C-4'), 78.51 (s, C-2'), 73.42 (s, O<u>C</u>H$(CH_3)_2$), 71.67 (s, C-3'), 63.50 (s, C-5'), 54.07 (s, 6-*O*CH_3 base), 22.69 & 22.16 (s, OCH(<u>C</u>H_3)$_2$).

5.2.2. Preparation of 2'-*O*-modified-6-*O*-methyl guanosine ProTides

Preparation of α-naphthyl L-Alanine-neopentyl-ester 2'-O-methyl-6-O-methyl guanosine ProTide (151)



This ProTide was prepared according to the Standard Procedure D, using *t*BuMgCl (1M in THF, 0.27 mL, 0.27 mmol, 2 eq), 2'-*O*-methyl-6-*O*-methyl guanosine (**142**) (0.042 g, 0.134 mmol, 1 eq) in anh. THF (1.50 mL), and *α*-naphthyl *L*-Alanine-neopentyl ester phosphochloridate (**14.d**) (0.11 g, 0.27 mmol, 2 eq) in anh. THF (1.0 mL). After overnight stirring at RT, the solvent was removed under reduced pressure and the residue was purified by flash chromatography, using $CHCl_3$/MeOH as eluents, and preparative chromatography to yield the desired molecule (0.2 mg, 0.0003 mmol, 0.2%).

HPLC (MeOH/H_2O): Rt = 26.66 min, 26.88 min

HPLC (ACN/H_2O): Rt = 18.02 min, 18.28 min

MS (TOF ES^+): 659.32 ($M + H^+$), 681.34 ($M + Na^+$), 697.21 ($M + K^+$).

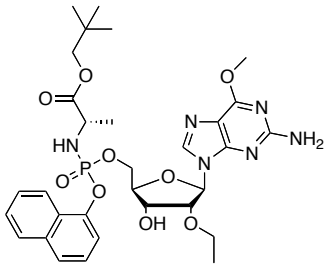
^{1}H NMR (500 MHz, MeOD) δ_H (ppm): 8.17-8.16 (m, 2H, H-9 Ar diastereoisomers), 7.93 & 7.91 (2s, 2H, H-8 base diastereoisomers), 7.73-7.69 (m, 4H, H-7, H-4 Ar diastereoisomers), 7.55-7.49 (m, 6H, H-8, H-6, H-2 Ar diastereoisomers), 7.43-7.40 (m, 2H, H-3 Ar diastereoisomers), 5.99 & 5.98 (2d, 2H, *J* = 5.5 Hz, 5.6 Hz, H-1' diastereoisomers), 4.59-4.56 (m, 2H, H-2' diastereoisomers), 4.51-4.49 (m, 6H, O$\underline{CH_2}$C$(CH_3)_3$ diastereoisomers, H-3'), 4.42-4.37 (m, 1H, H-3'), 4.31-4.26 (2m, 2H, H-4' diastereoisomers), 4.10-4.08 (m, 1H, NH$\underline{CH}(CH_3)$CO), 4.05 & 4.04 (2s, 6H, 6-*O*CH_3 base diastereoisomers), 4.03-4.00 (m, 1H, NH$\underline{CH}(CH_3)$CO), 3.81 (2dd, 2H, *J* = 2.5 Hz, 12.5 Hz, H-5' diastereoisomers), 3.71 (2d, 2H, *J* = 1.5 Hz, 1.6 Hz H-5' diastereoisomers), 2.18 & 2.17 (2s, 6H, O$\underline{CH_3}$ diastereoisomers), 1.34 & 1.31 (2d, 6H, *J* = 3.0 Hz, 3.0 Hz, NHCH($\underline{CH_3}$)CO diastereoisomers), 0.92 & 0.90 (2s, 18H, $OCH_2C(\underline{CH_3})_3$ diastereoisomers).

^{13}C NMR (125 MHz, MeOD) δ_C (ppm): 174.63 & 174.12 (2s, NHCH(CH_3)$\underline{C}$O diastereoisomers), 161.42 & 172.17 (2s, C-6 base diastereoisomers), 159.23 & 159.10

(2s, C-2 base diastereoisomers), 155.38 & 155.10 (2s, C-4 base diastereoisomers), 153.41 & 153.10 (2s, C-1 Ar diastereoisomers), 140.24 & 140.08 (2s, C-8 base diastereoisomers), 138.54 & 138.40 (2s, C-5 Ar diastereoisomers), 128.83 & 128.65 (2s, C-6 Ar diastereoisomers), 127.95 & 127.84 (2s, C-10 Ar diastereoisomers), 127.86 & 127.75 (2s, C-7 Ar diastereoisomers), 127.42 & 127.21 (2s, C-4 Ar diastereoisomers), 126.70 & 126.57 (2s, C-8 Ar diastereoisomers), 125.82 & 125.75 (2s, C-9 Ar diastereoisomers), 122.72 & 122.61 (2s, C-3 Ar diastereoisomers), 117.45 & 117.16 (2s, C-5 base diastereoisomers), 116.24 & 116.11 (2s, C-2 Ar diastereoisomers), 89.34 & 89.23 (2s, C-1' diastereoisomers), 84.45 & 84.22 (2s, C-2' diastereoisomers), 81.97 & 81.84 (2d, $J^{3}_{\text{P-O-C-C}}$ = 6.5 Hz, 6.5 Hz, C-4' diastereoisomers), 75.76 & 75.13 (2s, C-3' diastereoisomers), 75.38 & 75.12 (2s, O<u>C</u>H_2C$(CH_3)_3$), 67.56 & 67.22 (2s, C-5' diastereoisomers), 55.10 & 55.01 (2s, 6-*O*<u>C</u>H_3 base diastereoisomers), 51.96 & 50.78 (2s, O<u>C</u>H_3 diastereoisomers), 50.04 & 49.82 (2s, NH<u>C</u>H(CH_3)CO diastereoisomers), 29.97 & 29.74 (s, OCH$_2$<u>C</u>$(CH_3)_3$ diastereoisomers), 26.70 & 26.55 (2s, OCH$_2$C(<u>C</u>H_3)$_3$ diastereoisomers), 20.56 & 20.48 (2s, NHCH(<u>C</u>H_3)CO diastereoisomers).

^{31}P NMR (202 MHz, MeOD) δ_P (ppm): 4.24 (s, 78%), 4.22 (s, 22%).

Preparation of α-naphthyl L-Alanine-neopentyl-ester 2'-O-ethyl-6-O-methyl guanosine ProTide (152)



This ProTide was prepared according to the Standard Procedure D, using *t*BuMgCl (1M in THF, 0.13 mL, 0.13 mmol, 2 eq), 2'-*O*-ethyl-6-*O*-methyl guanosine (**143**) (0.022 g, 0.066 mmol, 1 eq) in anh. THF (0.75 mL). and *α*-naphthyl *L*-Alanine-neopentyl ester phosphochloridate (**14.d**) (0.053 g, 0.13 mmol, 2 eq) in anh. THF (1.0 mL). After overnight stirring at RT, the solvent was removed under reduced pressure and the residue was purified by flash chromatography, using $CHCl_3$/MeOH as eluents, and preparative chromatography to yield the desired molecule (1.9 mg, 0.0028 mmol, 4%).

HPLC (MeOH/H_2O): Rt = 27.26 min, 26.48 min

HPLC (ACN/H_2O): Rt = 18.95 min, 19.25 min

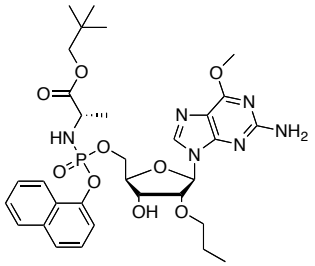
MS (TOF ES$^+$): 673.12 (M + H$^+$), 695.31 (M + Na$^+$).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.15-8.13 (m, 2H, H-9 Ar diastereoisomers), 7.87-7.82 (m, 4H, H-7 Ar, H-8 base diastereoisomers), 7.69-7.63 (m, H-4 Ar diastereoisomers), 7.57-7.49 (m, 6H, H-8, H-6, H-2 Ar diastereoisomers), 7.43-7.33 (m, 2H, H-3 Ar diastereoisomers), 5.88 (bs, 2H, H-1' diastereoisomers), 4.67-4.65 (m, 2H, H-2' diastereoisomers), 4.61-4.58 (m, 2H, O$\underline{CH_2}$C$(CH_3)_3$), 4.49-4.46 (m, 2H, H-3' diastereoisomers), 4.44-4.40 (m, 2H, O$\underline{CH_2}$C$(CH_3)_3$), 4.30-4.28 (m, 2H, H-4' diastereoisomers), 4.16-4.14 (m, 2H, NH$\underline{CH}(CH_3)$CO diastereoisomers), 4.08 & 4.06 (2s, 6H, 6-*O*CH_3 base diastereoisomers), 3.85-3.83 (m, 2H, H- 5' diastereoisomers), 3.72-3.70 (m, 2H, H-5' diastereoisomers), 3.58-3.54 (m, 2H, O$\underline{CH_2}CH_3$), 3.48-3.46 (m, 2H, O$\underline{CH_2}CH_3$), 1.37 & 1.35 (2d, 6H, J = 3.2 Hz, 3.3 Hz, NHCH($\underline{CH_3}$)CO diastereoisomers), 1.19-1.52 (m, 6H, $OCH_2\underline{CH_3}$ diastereoisomers), 0.91 & 0.90 (2s, 18H, $OCH_2C(\underline{CH_3})_3$ diastereoisomers).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 168.54 & 168.45 (2d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 2.9 Hz, 5.4 Hz, NHCH(CH_3)$\underline{C}$O diastereoisomers), 161.76 & 161.73 (2s, C-6 base diastereoisomers), 161.03 & 161.01 (2s, C-2 base diastereoisomers), 149.52 & 149.51 (2s, C-4 base diastereoisomers), 147.33 & 147.29 (2s, C-1 Ar diastereoisomers), 141.06 & 141.03 (2s, C-8 base diastereoisomers), 134.82 & 134.79 (2s, C-5 Ar diastereoisomers), 128.85 & 128.83 (2s, C-6 Ar diastereoisomers), 127.71 & 127.69 (2s, C-7 Ar diastereoisomers), 126.65 & 127.63 (2s, C-8 Ar diastereoisomers), 126.47 & 126.42 (2s, C-10 Ar diastereoisomers), 125.57 & 125.45 (2s, C-3 Ar diastereoisomers), 124.88 & 124.85 (2s, C-9 Ar diastereoisomers), 121.35 & 121.33 (2s, C-4 Ar diastereoisomers), 116.42 & 116.10 (2s, C-2 Ar diastereoisomers), 115.35 & 115.11 (2s, C-5 base diastereoisomers), 87.69 & 87.67 (2s, C-1' diastereoisomers), 87.27 & 87.21 (2s, C-2' diastereoisomers), 82.93 & 82.89 (2d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 2.0 Hz, 2.9 Hz, C-4' diastereoisomers), 74.75 & 74.73 (2s, CO$\underline{C}H_2C(CH_3)_3$ diastereoisomers), 69.85 & 69.782 (2s, C-3' diastereoisomers), 66.95 & 66.94 (2s, 2'-O$\underline{CH_2}CH_3$ diastereoisomers), 66.15 & 66.05 (2d, $J^2_{P\text{-}O\text{-}C}$ = 2.0 Hz, 2.7 Hz, C-5' diastereoisomers), 53.83 & 53.81 (2s, 6-*O*CH_3 base diastereoisomers), 50.45 & 50.39 (2d, $J^2_{P\text{-}N\text{-}C}$ = 1.8 Hz, 2.2 Hz, NH$\underline{C}H(CH_3)$CO diastereoisomers), 29.71 & 29.69 (2s, $COCH_2\underline{C}(CH_3)_3$ diastereoisomers), 26.30 & 26.28 (2s, $COCH_2C(\underline{C}H_3)_3$ diastereoisomers), 21.18 & 21.13 (2s, NHCH($\underline{C}H_3$)CO diastereoisomers) 15.24 & 15.21 (2s, $OCH_2\underline{C}H_3$ diastereoisomers).

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm): 3.70 (s, 45%), 3.35 (s, 55%).

Preparation of α-naphthyl L-Alanine-neopentyl-ester 2'-O-propyl-6-O-methyl guanosine ProTide (153)



This ProTide was prepared according to the Standard Procedure D, using *t*BuMgCl (1M in THF, 0.31 mL, 0.31 mmol, 2 eq), 2'-*O*-propyl-6-*O*-methyl guanosine (**144**) (0.053 g, 0.15 mmol, 1 eq) in anh. THF (1.60 mL), and *α*-naphthyl *L*-Alanine-neopentyl ester phosphochloridate (**14.d**) (0.13 g, 0.31 mmol, 2 eq) in anh. THF (1.0 mL). After overnight stirring at RT, the solvent was removed under reduced pressure and the residue was purified by flash chromatography, using $CHCl_3$/MeOH as eluents, and preparative chromatography to yield the desired molecule (3.8 mg, 0.0055 mmol, 4%).

HPLC (MeOH/H_2O): Rt = 28.01 min, 28.05 min

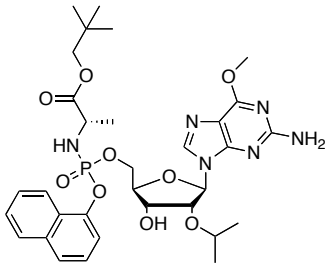
HPLC (ACN/H_2O): Rt = 20.14 min, 20.45 min

MS (TOF ES^+): 687.25 ($M + H^+$), 709.24 ($M + Na^+$); MS (TOF ES^-): 721.26 ($M + Cl^-$).

^1H NMR (500 MHz, MeOD) δ_H (ppm): 8.17-8.13 (m, 2H, H-9 Ar diastereoisomers), 7.94-7.91 (m, 4H, H-7 Ar, H-8 base diastereoisomers), 7.72-7.67 (m, H-4 Ar diastereoisomers), 7.54-7.48 (m, 6H, H-8, H-6, H-2 Ar diastereoisomers), 7.42-7.36 (m, 2H, H-3 Ar diastereoisomers), 5.96 & 5.95 (2d, 2H, *J* = 8.5 Hz, 6.5 Hz, H-1' diastereoisomers), 4.54-4.52 (m, 2H, H-2' diastereoisomers), 4.50-4.48 (m, 2H, $O\underline{CH_2}C(CH_3)_3$), 4.45-4.44 (m, 2H, H-3' diastereoisomers), 4.41-4.37 (m, 2H, $O\underline{CH_2}C(CH_3)_3$), 4.24-4.22 (m, 1H, $NH\underline{CH}(CH_3)CO$ diastereoisomers), 4.17-4.16 (m, 2H, H-4' diastereoisomers), 4.12-4.09 (m, 1H, $NH\underline{CH}(CH_3)CO$ diastereoisomers), 4.08 & 4.07 (2s, 6H, 6-$O\underline{CH_3}$ base diastereoisomers), 4.04 (d, 2H, *J* = 2.0 Hz, $\underline{NH}CH(CH_3)CO$ diastereoisomers), 3.91-3.39 (m, 2H, H-5'), 3.78-3.76 (m, 2H, H-5'), 3.62-3.60 (m, 2H, $O\underline{CH_2}CH_2CH_3$), 3.43-3.39 (m, 2H, $O\underline{CH_2}CH_2CH_3$), 1.71-1.65 (m, 2H, $OCH_2\underline{CH_2}CH_3$), 1.56-1.49 (m, 2H, $OCH_2\underline{CH_2}CH_3$), 1.35 & 1.33 (2d, *J* = 3.5 Hz, 3.0 Hz, $NHCH(\underline{CH_3})CO$ diastereoisomers), 0.90 & 0.89 (2s, 18H, $OCH_2C(\underline{CH_3})_3$ diastereoisomers), 0.84 & 0.83 (2t, 6H, *J* = 6.5 Hz, 6.5 Hz, $OCH_2CH_2\underline{CH_3}$ diastereoisomers).

^{13}C NMR (125 MHz, MeOD) δ_C (ppm): 168.30 & 168.17 (2s, NHCH(CH_3)$\underline{C}$O diastereoisomers), 162.93 & 162.90 (2s, C-6 base diastereoisomers), 161.60 & 161.58 (2s, C-2 base diastereoisomers), 154.14 & 154.10 (2s, C-4 base diastereoisomers), 147.23 & 147.10 (2s, C-1 Ar diastereoisomers), 140.36 & 140.33 (2s, C-8 base diastereoisomers), 134.96 & 134.90 (2s, C-5 Ar diastereoisomers), 128.93 & 128.86 (2s, C-6 Ar diastereoisomers), 127.83 & 127.81 (2s, C-7 Ar diastereoisomers), 127.55 & 127.52 (2s, C-8 Ar diastereoisomers), 127.13 & 127.06 (2s, C-10 Ar diastereoisomers), 126.59 & 126.50 (2s, C-3 Ar diastereoisomers), 126.04 & 125.99 (2s, C-9 Ar diastereoisomers), 122.71 & 122.66 (2s, C-4 Ar diastereoisomers), 116.72 & 116.54 (2s, C-2 Ar diastereoisomers), 116.19 & 116.15 (2s, C-5 base diastereoisomers), 89.26 & 88.36 (2s, C-1' diastereoisomers), 82.57 & 82.55 (2s, C4' diastereoisomers), 79.49 & 79.46 (2s, C-2' diastereoisomers), 75.46 & 75.44 (2s, CO$\underline{CH_2}$C(CH_3)$_3$ diastereoisomers), 73.64 & 73.62 (2s, O$\underline{CH_2}$$CH_2CH_3$ diastereoisomers), 71.29 & 71.27 (2s, C-3' diastereoisomers), 63.53 & 63.46 (2d, $J^2_{P\text{-}O\text{-}C}$ = 1.8 Hz, 2.2 Hz, C-5' diastereoisomers), 54.35 & 54.33 (2s, 6-*O*CH_3 base diastereoisomers), 49.91 & 49.89 (2s, NH$\underline{C}$H(CH_3)CO diastereoisomers), 30.76 & 30.74 (2s, COCH$_2\underline{C}$(CH_3)$_3$ diastereoisomers), 26.75 & 26.73 (2s, COCH$_2$C($\underline{C}H_3$)$_3$ diastereoisomers), 23.87 & 23.84 (2s, OCH$_2\underline{C}$H$_2$CH$_3$ diastereoisomers), 20.73 & 20.68 (2s, NHCH($\underline{C}H_3$)CO diastereoisomers), 10.67 & 10.60 (2s, OCH$_2$CH$_2\underline{C}$H$_3$ diastereoisomers).

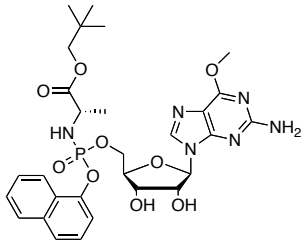
Preparation of α-naphthyl L-Alanine-neopentyl-ester 2'-O-isopropyl-6-O-methyl guanosine ProTide (154)



This ProTide was prepared according to the Standard Procedure D, using *t*BuMgCl (1M in THF, 0.22 mL, 0.22 mmol, 2 eq), 2'-*O*-isopropyl-6-*O*-methyl guanosine (**145**) (0.039 g, 0.11 mmol, 1 eq) in anh. THF (1.20 mL), and *α*-naphthyl *L*-Alanine-neopentyl ester phosphochloridate (**14.d**) (0.22 g, 0.22 mmol, 2 eq) in anh. THF (1.2 mL). After overnight stirring at RT, the solvent was removed under reduced pressure and the

residue was purified by flash chromatography, using $CHCl_3$/MeOH as eluents, preparative chromatography to yield the desired unpure material.
MS (TOF ES^+): 687.21 ($M + H^+$), 709.32 ($M + Na^+$).

Preparation of α-naphthyl L-Alanine-neopentyl-ester 6-O-methyl guanosine ProTide (155)

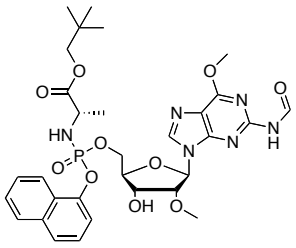


This ProTide was prepared according to the Standard Procedure D, using *t*BuMgCl (1M in THF, 0.42 mL, 0.42 mmol, 2 eq), 6-*O*-methyl guanosine (**137**) (0.061 g, 0.21 mmol, 1 eq) in anh. THF (2.30 mL), and *α*-naphthyl *L*-Alanine-neopentyl ester phosphochloridate (**14.d**) (0.17 g, 0.42 mmol, 2 eq) in anh. THF (2.0 mL) anh anh. pyridine (0.30 mL). After overnight stirring at RT, the solvent was removed under reduced pressure and the residue was purified by flash chromatography, using $CHCl_3$/MeOH as eluents, preparative chromatography to yield the desired material (1.4 mg, 0.002 mmol, 1%).
HPLC (MeOH/H_2O): Rt = 26.23 min, 26.51 min
HPLC (ACN/H_2O): Rt = 16.79 min, 17.05 min
MS (TOF ES^+): 645.22 ($M + H^+$), 667.34 ($M + Na^+$).
^1H NMR (500 MHz, MeOD) δ_H (ppm): 8.16-8.10 (m, 2H, H-9 Ar diastereoisomers), 7.93 (bs, 2H, H-8 base diastereoisomers), 7.89-7.85 (m, 2H, H-7 Ar diastereoisomers), 7.71-7.67 (m, H-4 Ar diastereoisomers), 7.54-7.45 (m, 6H, H-8, H-6, H-2 Ar diastereoisomers), 7,40-7,34 (m, 2H, H-3 Ar diastereoisomers), 5.90-5.89 (2d, 2H, J = 5.5 Hz, H-1' diastereoisomers), 4.65-4.36 (m, 2H, H-2' diastereoisomers), 4.59-4.54 (m, 4H, O$\underline{CH_2}$C$(CH_3)_3$ diastereoisomers), 4.51-4.46 (m, 1H, H-3'), 4.42-4.37 (m, 1H, H-3'), 4.29-4.24 (2m, 2H, H-4' diastereoisomers), 4.07-4.06 (m, 1H, NH$\underline{CH}(CH_3)$CO), 4.05 & 4.04 (2s, 6H, 6-*O*CH_3 base diastereoisomers), 4.02-4.00 (m, 1H, NH$\underline{CH}(CH_3)$CO), 3.80-3.78 (m, 2H, H-5'), 3.70-3.68 (m, 2H, H-5'), 1.33 & 1.31 (2d, J = 3.0 Hz, 3.0 Hz, NHCH($\underline{CH_3}$)CO diastereoisomers), 0.90 & 0.89 (2s, 18H, $OCH_2C(\underline{CH_3})_3$ diastereoisomers).

^{13}C NMR (125 MHz, MeOD) δ_C (ppm): 169.74 & 169.68 (2d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 1.9 Hz, 3.7 Hz, NHCH(CH_3)<u>C</u>O diastereoisomers), 161.43 & 161.17 (2s, C-6 base diastereoisomers), 160.59 & 160.57 (2s, C-2 base diastereoisomers), 149.45 & 149.38 (2s, C-4 base diastereoisomers), 145.31 & 145.22 (2s, C-1 Ar diastereoisomers), 143.95 & 143.93 (2s, C-8 base diastereoisomers), 134.44 & 134.38 (2s, C-5 Ar diastereoisomers), 128.87 & 128.81 (2s, C-6 Ar diastereoisomers), 127.76 & 127.75 (2s, C-7 Ar diastereoisomers), 127.48 & 127. 46 (2s, C-8 Ar diastereoisomers), 126.71 & 126.65 (2s, C-10 Ar diastereoisomers), 126.50 & 126.45 (2s, C-3 Ar diastereoisomers), 125.98 & 125.79 (2s, C-9 Ar diastereoisomers), 122.70 & 122.66 (2s, C-4 Ar diastereoisomers), 116.82 & 116..63 (2s, C-2 Ar diastereoisomers), 116.26 & 116.22 (2s, C-5 base diastereoisomers), 89.96 & 89.87 (2s, C-1' diastereoisomers), 84.41 & 84.33 (2d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 1.7 Hz, 2.6 Hz, C-4' diastereoisomers), 75.42 & 75.39 (2s, CO<u>C</u>H_2C(CH_3)$_3$ diastereoisomers), 74.75 & 74.71 (2s, C-2' diastereoisomers), 71.85 & 71.76 (2s, C-3' diastereoisomers), 67.91 & 67.77 (2d, $J^2_{P\text{-}O\text{-}C}$ = 5.9 Hz, 5.9 Hz, C-5' diastereoisomers), 54.17 & 54.15 (2s, 6-*O*CH_3 base diastereoisomers), 49.88 & 49.84 (2s, NH<u>C</u>H(CH_3)CO diastereoisomers), 30.69 & 30.67 (2s, COCH$_2$<u>C</u>(CH_3)$_3$ diastereoisomers), 26.70 (s, COCH$_2$C(<u>C</u>H_3)$_3$ diastereoisomers), 20.69 & 20.55 (2s, NHCH(<u>C</u>H_3)CO diastereoisomers).

^{31}P NMR (202 MHz, MeOD) δ_P (ppm): 4.32 (s, 49%), 4.21 (s, 51%).

Preparation of α-naphthyl L-Alanine-neopentyl-ester 2'-O-methyl-6-O-methyl-N-2-formamide guanosine ProTide (156)



This ProTide was prepared according to the Standard Procedure D, using *t*BuMgCl (1M in THF, 0.32 mL, 0.32 mmol, 2 eq), 2'-*O*-methyl-6-*O*-methyl-*N*-2-formamide guanosine (**141.a**) (0.059 g, 0.16 mmol, 1 eq) in anh. THF (1.80 mL), and *α*-naphthyl *L*-Alanine-neopentyl ester phosphochloridate (**14.d**) (0.13 g, 0.32 mmol, 2 eq) in anh. THF (1.0 mL). After overnight stirring at RT, the solvent was removed under reduced pressure and the residue was purified by flash chromatography, using $CHCl_3$/MeOH

(8%) as eluents, preparative chromatography to yield the desired material (2.9 mg, 0.0042 mmol, 3%).

HPLC (MeOH/H_2O): Rt = 26.69 min, 27.02 min

HPLC (ACN/H_2O): Rt = 17.72 min, 18.18 min

MS (TOF ES^+): 687.11 ($M + H^+$), 709.32 ($M + Na^+$), 725.24 ($M + K^+$).

^1H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 9.51 & 9.33 (2d, *J* = 10.5 Hz, 10.0 Hz, NHC<u>H</u>O), 8.70 & 8.60 (2d, *J* = 10.5 Hz, 10.0 Hz, <u>NH</u>CHO), 8.13-8.11 (m, 1H, H-9 Ar), 8.05-8.03 (m, 1H, H-9 Ar), 7.94 & 7.89 (2m, 2H, H-8 base diastereoisomers), 7.87-7.85 (m, 1H, H-7 Ar), 7.80-7.79 (m, 1H, H-7 Ar), 7.68-7.66 (m, H-4 Ar), 7.60-7.59-7.57 (m, H-4 Ar), 7.56-7.46 (m, 6H, H-8, H-6, H-2 Ar diastereoisomers), 7.41-7.37 (m, 1H, H-3 Ar), 7.32-7.31 (m, 1H, H-3 Ar), 5.96 & 5.95 (2d, 2H, *J* = 4.0 Hz, H-1' diastereoisomers), 4.68-4.63 (m, 2H, H-3' diastereoisomers), 4.54-4.51 (m, 4H, <u>NH</u>CH(CH_3)CO diastereoisomers, H-5'), 4.39-4.34 (m, 4H, H-2', H-4' diastereoisomers), 4.28-4.26 (m, 2H, H-5'), 4.37-4.15 (m, 2H, NH<u>CH</u>(CH_3)CO diastereoisomers), 4.14 & 4.08 (2s, 6H, 6-*O*CH_3 base diastereoisomers), 3.87 & 3.85 (m, 2H, O<u>CH_2</u>C(CH_3)$_3$), 3.75-3.73 (m, 2H, O<u>CH_2</u>C(CH_3)$_3$), 3.43 & 3.35 (2s, 6H, O<u>CH_3</u> diastereoisomers), 1.40 & 1.38 (2d, *J* = 3.0 Hz, 3.1 Hz, NHCH(<u>CH_3</u>)CO diastereoisomers), 0.92 & 0.91 (2s, 18H, $OCH_2C($<u>CH_3</u>$)_3$ diastereoisomers).

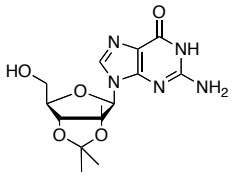
^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 173.38 & 173.13 (2s, NHCH(CH_3)<u>C</u>O diastereoisomers), 163.03 & 162.80 (2s, NH<u>C</u>HO diastereoisomers), 162.39 & 162.32 (2s, C-6 base diastereoisomers), 161.49 & 161.44 (2s, C-2 base diastereoisomers), 152.14 & 151.96 (2s, C-4 base diastereoisomers), 150.75 & 150.65 (2s, C-1 Ar diastereoisomers), 140.33 & 140.28 (2s, C-8 base diastereoisomers), 134.75 & 134.71 (2s, C-5 Ar diastereoisomers), 127.87 & 127.84 (2s, C-6 Ar diastereoisomers), 127.66 & 127.62 (2s, C-7 Ar diastereoisomers), 126.67 & 126.64 (2s, C-8 Ar diastereoisomers), 126.47 & 126.41 (2s, C-10 Ar diastereoisomers), 125.54 & 125.37 (2s, C-3 Ar diastereoisomers), 124.95 & 124.88 (2s, C-9 Ar diastereoisomers), 121.37 & 121.33 (2s, C-4 Ar diastereoisomers), 115.97 & 155.74 (2s, C-5 base diastereoisomers), 115.38 & 115.10 (2s, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 3.2 Hz, 2.8 Hz, C-2 Ar diastereoisomers), 87.28 & 87.15 (2s, C-1' diastereoisomers), 82.78 & 82.72 (2d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 2.8 Hz, 2.43 Hz, C-4' diastereoisomers), 87.35 & 87.29 (2s, C-2' diastereoisomers), 74.85 & 74.82 (2s, CO<u>CH_2</u>C(CH_3)$_3$ diastereoisomers), 69.58 & 69.35 (2s, C-3' diastereoisomers), 65.87 & 65.61 (2d, $J^2_{P\text{-}O\text{-}C}$ = 5.5 Hz, 5.4 Hz, C-5'

diastereoisomers), 58.84 & 58.77 (2s, O<u>C</u>H_3 diastereoisomers), 54.51 & 54.41 (2s, 6-*O*CH_3 base diastereoisomers), 50.47 & 50.39 (2d, $J^2_{P\text{-}N\text{-}C}$ = 3.8 Hz, 5.4 Hz, NH<u>C</u>H(CH_3)CO diastereoisomers), 29.70 & 29.66 (2s, $COCH_2$<u>C</u>(CH_3)$_3$ diastereoisomers), 26.28 & 26.23 (s, $COCH_2C$(<u>C</u>H_3)$_3$ diastereoisomers), 21.18 & 21.12 (2s, NHCH(<u>C</u>H_3)CO diastereoisomers).

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm): 3.47 (s, 38%), 3.31 (s, 62%).

5.3. 5'-*C*-modifications of 2'-*C*-methyl guanosine

Preparation of 2', 3'-isopropylidene protected 2'-C-methyl-guanosine (158) [14]

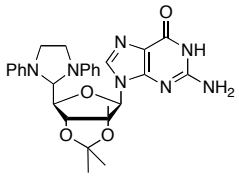


To a solution of 2'-*C*-methyl guanosine (**10**, provided by Inhibitex *Inc.*) (1.03 g, 3.45 mmol, 1 eq) in anh. acetone (50 mL), perchloric acid (60 % in aq. solution, 0.58 mL) was added dropwise at RT and stirred overnight. Ammonium hydroxide was then added dropwise until reaching a neutral pH. The solvent was removed under reduced pressure and the residue was purified by flash chromatography using $CHCl_3$/MeOH (9:1) as eluents to yield a white product (0.37 g, 1.09 mmol, 32%).

^{1}H NMR (500 MHz, DMSO) δ_H (ppm): 10.68 (bs, 1H, OH-5'), 7.95 (s, 1H, H-8 base), 6.50 (bs, 2H, NH_2 base), 6.01 (s, 1H, H-1'), 5.27 (d, 1H, J = 4.6 Hz, H-3'), 4.58 (d, 1H, J = 2.5 Hz, H-5'), 4.17 (dd, 1H, J = 4.0 Hz, 2.5 Hz, H5'), 3.68 (m, 1H, H-4'), 1.53 (s, 3H, CH_3-2'), 1.36 (s, 3H, C($\underline{C}H_3$)$_2$), 1.15 (s, 3H, C($\underline{C}H_3$)$_2$).

^{13} C NMR (125 MHz, DMSO) δ_C (ppm): 156.68 (s, C-6 base), 153.63 (s, C-2 base), 150.81 (s, C-4 base), 135.52 (s, C-8 base), 116.29 (s, $\underline{C}(CH_3)_2$), 112.81 (s, C-5 base), 91.63 (s, C-1'), 89.56 (s, C-2'), 85.67 (s, C-3'), 84.08 (s, C-4'), 61.35 (s, C-5'), 28.09 (s, C($\underline{C}H_3$)$_2$), 26.61 (C($\underline{C}H_3$)$_2$), 19.39 (CH_3-2').

Preparation of 2',3'-isopropylidene-5'-C-diphenylimidazolidin-2'-C-methyl-guanosine (166)



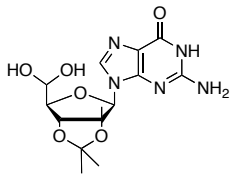
A solution of 2',3'-isopropylidene-2'-*C*-methyl guanosine (**158**) (0.30 g, 0.89 mmol) and DCC (0.55 g, 2.67 mmol, 3.0 eq), in anh. DMSO (2.00 mL) was stirred with ice cooling while dichloroacetic acd (0.04 mL, 0.5 eq) was added dropwise. The mixture was then stirred at RT for 1 hr 30 min and then a solution of oxalic acid dihydrate (0.23 g, 1.78 mmol, 2.0 eq) in anh. MeOH (0.89 mL) was slowly. The mixture was left 30 min at RT, then filtered and wash with cold MeOH. *N*,*N*'-diphenylethylenediamine (0.21 g, 1.02 mmol, 1.1 eq) was added to the combined filtrate and the resulting solution

was stirred for 1 hr at RT. H_2O was added to slight turbidity and filtration was performed. The organic layer was extracted with $CHCl_3$, and the solvent was evaporated under reduced pressure to give a brown solid (0.29 g, 0.55 mmol, 62%).

^{1}H NMR (500 MHz, DMSO) δ_H (ppm): 8.32 (s, 1H, H-8 base), 7.21 (t, 4H, *J* = 7.8 Hz, 4 x CH meta Ph), 6.86 (d, 4H, *J* = 8.0 Hz, 4 x CH ortho Ph), 6.82 (t, 2H, *J* =7.5 Hz, 2 x CH para Ph), 6.01 (s, 1H, H-1'), 4.59 (m, 2H, H-4', H-3'), 3.72-3.68 (m, 4H, PhN$\underline{CH_2}\underline{CH_2}$NPh), 3.35 (d, 1H, *J* = 6.5 Hz, H-5'), 1.53 (s, 3H, CH_3-2'), 1.36 (s, 3H, C($\underline{CH_3}$)$_2$), 1.16 (s, 3H, C($\underline{CH_3}$)$_2$).

^{13}C NMR (125 MHz, DMSO) δ_C (ppm): 165.69 & 164.08 (2s, 2 x C ipso Ph), 155.72 (s, C-6 base), 154.23 (s, C-2 base), 150.32 (s, C-4 base), 135.43 (s, C-8 base), 129.65 & 129.24 (2s, 4 x C meta Ph), 119.24 & 199.10 (2s, 4 x C ortho Ph), 115.32 & 115.28 (s, 2 x C para Ph), 113.32 (s, $\underline{C}(CH_3)_2$), 112.92 (C-5 base), 92.43 (s, C-1'), 89.77 (s, C-2'), 85.83 (s, C-3'), 84.69 (s, C-4'), 65.70 (s, C-5'), 43.39 (s, PhN$\underline{C}H_2\underline{C}H_2$NPh), 25.16 & 25.11 (2s, 2 x C($\underline{C}H_3$)$_2$), 19.22 (s, CH_3-2').

Preparation of 2',3'-isopropylidene-5'-C-dihydroxy-hydrate-2'-C-methyl-guanosine (167)

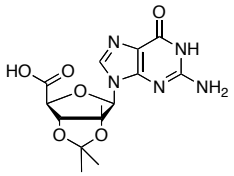


The 2',3'-isopropylidene-5'-*C*-diphenylimidazolidin-2'-*C*-methyl-guanosine (**166**) (0.08 g, 0.15 mmol) was mixed with Dowex 50WX4-50 (0.15 g) in H_2O/THF (1:1) and stirred for 1 hr 30 min at RT. Then the solvent was evaporated under reduced pressure to recover a brown solution. The purification was undertaken by flash chromatography using $CHCl_2$/MeOH to recover of the desired material (9.8 mg, 0.03 mmol, 18%).

^{1}H NMR (500 MHz, DMSO) δ_H (ppm): 10.66 (bs, 1H NH base), 7.94 (s, 1H, H-8 base), 6.51 (s, 2H, NH_2 base), 6.03 (s, 1H, H-1'), 4.77 (m, 2H, OH-5'), 4.60 (d, 1H, *J* = 3.0 Hz, H-3'), 4.33 (m, 1H, H-5'), 3.78 (m, 1H, H-4'), 1.54 (s, 3H, C($\underline{CH_3}$)$_2$), 1.38 (s, 3H, C($\underline{CH_3}$)$_2$), 1.17 (s, 3H, CH_3-2').

^{13}C NMR (125 MHz, DMSO) δ_C (ppm): 156.67 (s, C-6 base), 153.76 (s, C-2 base), 150.82 (s, C-4 base), 135.45 (s, C-8 base), 116.36 (s, $\underline{C}(CH_3)_2$), 112.82 (s, C-5 base), 91.68 (s, C-1'), 89.64 (s, C-2'), 85.76 (s, C-3'), 84.17 (s. C-4'), 48.56 (s, C-5'), 28.13 (s, C($\underline{C}H_3$)$_2$), 27.00 (s, C($\underline{C}H_3$)$_2$), 19.43 (s, CH_3-2').

Preparation of 2',3'-isopropylidene-5'-C-carboxylic acid 2'-C-methyl-guanosine (168)

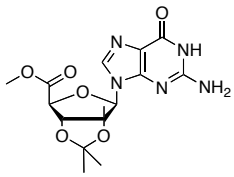


2',3'-isopropylidene-2'-*C*-methyl guanosine (**158**) (0.84 g, 2.49 mmol), TEMPO (0.63 mmol, 0.0978 g, 0.25 eq) and BAIB (5.58 mmol, 1.7978 g, 2.2 eq) was dissolved in MeCN/H_2O (16 mL/16 mL) and the solution was stirred overnight at RT. Acetone (16 mL) and Et_2O (80 mL) were added and the solution was stirred for another 2 hrs 30 min at RT, and the material precipitated (0.83 g, 2.36 mmol, 95%).

^{1}H NMR (500 MHz, DMSO) δ_H (ppm): 10.67 (bs, 1H, OH-5'), 7.92 (s, 1H, H-8 base), 6.51 (s, 2H, NH_2 base), 6.02 (s, 1H, H-1'), 4.37 (m, 1H, H-3'), 3.71 (m, 1H, H-4'), 1.44 (s, 3H, CH_3-2'), 1.16 (s, 3H, C($\underline{CH_3}$)$_2$), 1.08 (s, 3H, C($\underline{CH_3}$)$_2$).

^{13}C NMR (125 MHz, DMSO) δ_C (ppm): 171.96 (s, C-5'), 156.49 (s, C-6 base), 153.22 (s, C-2 base), 150.65 (s, C-4 base), 135.48 (s, C-8 base), 116.67 (s, $\underline{C}$(CH_3)$_2$), 112.14 (s, C-5 base), 91.54 (s, C-1'), 90.11 (s, C-2'), 85.75 (s, C-3'), 84.15 (s, C-4'), 25.33 (s, 2 x C($\underline{C}H_3$)$_2$), 21.02 (s, CH_3-2').

Preparation of 2',3'-isopropylidene-5'-C-methylester-2'-C-methyl-guanosine (169)



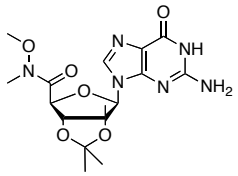
2',3'-isopropylidene-5'-*C*-carboxylic-acid-2'-*C*-methyl-guanosine (**168**) (0.83 g, 2.36 mmol) was dissolved in anh. MeOH (80 mL) and the solution was stirred at 0 °C for 30 min. $SOCl_2$ (8.95 mmol, 0.65 mL, 3.8 eq) was added dropwise at 0 °C, and the solution was left stirring overnight while allowing return to RT. $NaHCO_3$ sat. (4 mL) were cautiously added, then $NaHCO_3$ was added until suspended particles were visible in the solution. Silica was added to the mixture and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography using CH_2Cl_2/MeOH (7%) to yield the desired material (0.16 g, 0.43 mmol, 18%).

^{1}H NMR (500 MHz, DMSO) δ_H (ppm): 10.66 (bs, 1H, NH base), 7.85 (s 1H, H-8 base), 6.32 (bs, 2H, NH_2 base), 6.15 (s, 1H, H-1'), 5.35 (d, 1H, J = 2.5 Hz, H-3'), 4.77 (d, 1H,

J = 2.5 Hz, H-4'), 3.63 (s, 3H, COO$\underline{CH_3}$), 1.33 (s, 6H, 2 x C($\underline{CH_3}$)$_2$), 1.21 (s, 3H, CH_3-2').

^{13}C NMR (125 MHz, DMSO) δ_C (ppm): 170.25 (s, C-5'), 156.60 (s, C-6 base), 153.32 (s, C-2 base), 150.22 (s, C-4 base), 137.00 (s, C-8 base), 116.95 (s, $\underline{C}(CH_3)_2$), 112.61 (s, C-5 base), 91.85 (s, C-1'), 90.31 (s, C-2'), 87.73 (s, C-3'), 83.84 (s, C-4'), 51.94 (s, COO$\underline{CH_3}$), 26.84 (2, 2 x C($\underline{C}H_3)_2$), 19.48 (s, CH_3-2').

Preparation of 2',3'-isopropylidene-5'-C-weinreb amide 2'-C-methyl-guanosine (171)



2',3'-isopropylidene-5'-*C*-carboxylic-acid-2'-*C*-methyl-guanosine (**168**) (0.74 g, 2.11 mmol, 1.0 eq) was dissolved in anh. DMF (4.22 mL), and DIPEA (0.92 mL, 5.28 mmol, 2.5 eq) was added. PyBroP (1.08 g, 2.32 mmol, 1.1 eq) and HOBt.H_2O (0.31 g, 2.32 mmol, 1.1 eq) were added, and after 1 min, subsequent addition of CH_3ONHCH_3.HCl (0.41 g, 4.22 mmol, 2.0 eq). After overnight stirring at RT, the solvent was evaporated under reduced pressure, and the residue was washed with HCl (5%) and $NaHCO_3$. Purification by flash chromatography allowed the recovery of the desired material (0.55 g, 1.39 mmol, 66%).

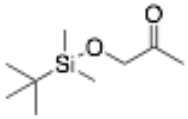
^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 9.32 (bs, 1H, NH base), 8.15 (s, 1H, H-8 base), 7.89 (s, 2H, NH_2 base), 6.03 (s, 1H, H-1'), 4.37 (dd, 1H, *J* = 5.5 Hz, H-3'), 3.75 (d, 1H, *J* = 5.5 Hz, H-4'), 3.81 (s, CON(CH_3)O$\underline{CH_3}$), 3.45 (s, 3H, CON($\underline{CH_3}$)OCH_3), 1.54 (s, 3H, CH_3-2'), 1.31 (s, 3H, C($\underline{CH_3}$)$_2$), 1.22 (s, 3H, C($\underline{CH_3}$)$_2$).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 169.75 (s, C-5'), 152.28 (s, C-6 base), 152.11 (s, C-2 base), 151.79 (s, C-4 base), 139.38 (s, C-8 base), 117.23 (s, $\underline{C}(CH_3)_2$), 113.69 (s, C-5 base), 93.61 (s, C-1'), 91.19 (s, C-2'), 87.35 (s, C-3'), 81.88 (s, C-4'), 61.51 (s, CON(CH_3)O$\underline{C}H_3$), 36.36 (s, CON($\underline{C}H_3$)OCH_3), 27.86 (s, 2 x C($\underline{C}H_3)_2$), 22.12 (s, CH_3-2').

6. Experimental procedures related to chapter 6

6.1. Preparation of acyclic purine nucleosides

Preparation of 1-(tertbutyldimethylsilyloxy)propan-2-one (183) [15]

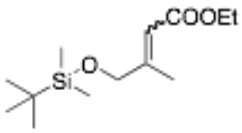


To a solution of hydroxyacetone (**182**) (90%, 10.0 mL, 146.1 mmol, 1 eq) and imidazole (9.91 g, 146.1 mmol, 1.0 eq) in anh. DMF (191 mL) was added TBDMSCl (21.88 g, 321.3 mmol, 2.2 eq). The reaction was left stirring for 36 hrs at RT. Water was added to the mixture and the organic phase was extracted with hexane. The solvent was evaporated under reduced pressure, and the residue was purified by flash chromatography using Petroleum Ether/EtOAc (15:1) to give the desired material (22.29 g, 118.35 mol, 81%) as colourless oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 4.14 (s, 2H, $O\underline{CH_2}COCH_3$), 2.16 (s, 3H, $OCH_2CO\underline{CH_3}$), 0.93 (s, 9H, $Si(CH_3)_2C(\underline{CH_3})_3$), 0.09 (s, 6H, $Si(\underline{CH_3})_2C(CH_3)_3$).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 209.02 (s, $OCH_2\underline{C}OCH_3$), 69.64 (s, $O\underline{C}H_2COCH_3$), 31.85 (s, $Si(CH_3)_2\underline{C}(CH_3)_3$), 26.89 & 26.05 & 25.87 (3s, $Si(CH_3)_2C(\underline{C}H_3)_3$), 18.76 (s, $Si(CH_3)_2\underline{C}(CH_3)_3$), -5.56 & -5.58 (2s, $Si(\underline{C}H_3)_2C(CH_3)_3$).

Preparation of ethyl-4-(tert-butyldimethylsilyloxy)-3-methylbut-2-enoate (184) [15, 16]



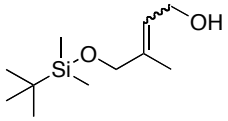
A supension of NaH (4.20 g, 106.0 mmol, 1.0 eq) in anh. THF (170 mL) was added dropwise triethylphosphonoacetate (17.0 mL, 127.18 mmol, 1.2 eq) at 0 °C, The solution was left stirring for 1 hr at RT. 1-(tertbutyldimethylsilyloxy)propan-2-one (**183**) (19.96 g, 105.98 mmol, 1.0 eq) was added and stirring was allowed for 1 hr. The solution was neutralized by AcOH and stirred with EtOAc. The organic phase was washed with brine, and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography using petroleum ether/EtOAc (2-3%) to recover both isomers *E* and *Z* (22%/78%) (13.85 g, 53.59 mmol, 51%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 6.00 (d, 1H, *J* = 1.4 Hz, $\underline{CH}COOCH_2CH_3$), 5.68 (d, 1H, *J* = 1.6 Hz, $\underline{CH}COOCH_2CH_3$), 4.81 (q, 2H, *J* = 1.7 Hz, $COO\underline{CH_2}CH_3$), 4.20-4.17 (q, 2H, *J* = 1.6 Hz, $COO\underline{CH_2}CH_3$,), 4.14 (s, 2H, $\underline{CH_2}OSi(CH_3)_2C(CH_3)_3$), 4.12 (s,

2H, $\underline{CH_2}OSi(CH_3)_2C(CH_3)_3$), 2.07 (t, 3H, *J* = 0.6 Hz, $C(\underline{CH_3})CH_2OSiCH_3)_2C(CH_3)_3$), 1.98 (t, 3H, *J* = 0.6 Hz, $C(\underline{CH_3})CH_2OSiCH_3)_2C(CH_3)_3$), 1.31-1.27 (m, 6H, 2 x $COOCH_2\underline{CH_3}$), 0.94 (s, 9H, $Si(CH_3)_2C(\underline{CH_3})_3$), 0.93 (s, 9H, $Si(CH_3)_2C(\underline{CH_3})_3$), 0.10 (s, 6H, $Si(\underline{CH_3})_2C(CH_3)_3$), 0.09 (s, 6H, $Si(\underline{CH_3})_2C(CH_3)_3$).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 167.04 & 165.98 (2s, 2 x $\underline{C}OOCH_2CH_3$), 160.73 & 157.05 (2s, 2 x $\underline{C}(CH_3)CH_2OSiCH_3)_2C(CH_3)_3$), 114.98 & 113.41 (2s $\underline{C}HCOOCH_2CH_3$), 67.11 & 62.70 (2s, 2 x $\underline{C}H_2OSi(CH_3)_2C(CH_3)_3$), 59.64 & 59.53 (2s, 2 x $COO\underline{C}H_2CH_3$), 34.12 & 3157 (2s, 2 x $Si(CH_3)_2\underline{C}(CH_3)_3$), 26.91 & 26.54 & 26.17 & 25.86 & 25.77 & 25.69 (6s, 6 x $Si(CH_3)_2C(\underline{C}H_3)_3$), 21.37 & 20.14 (2s, 2 x $C(\underline{C}H_3)CH_2OSiCH_3)_2C(CH_3)_3$), 18.26 & 18.01 (2s, 2 x $Si(CH_3)_2\underline{C}(CH_3)_3$), 15.39 & 14.33 (2s, 2 x $OCH_2\underline{C}H_3$), -5.21 & -5.47 (2s, 2 x $Si(\underline{C}H_3)_2C(CH_3)_3$).

Preparation of 4-(tert-butyldimethylsilyloxy)-3-methylbut-2-en-1-ol (185) [16]



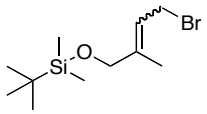
To a solution of ethyl 4-(tert-butyldimethylsilyloxy)-3-methylbut-2-enoate (**184**) (12.20 g, 56.40 mmol, 1.0 eq) in anh. DCM (225 mL) was added dropwise DIBAL-H (1M in hexane, 119.0 mL, 119.0 mmol, 2.1 eq) at -20 °C. The mixture was left stirring at 0 °C for 2 hrs, after which MeOH (1.5 mL) and aq. sodium potassium tartrate tetrahydrate (140 mL) was added. The solution was stirred overnight at RT, and the organic layer was evaporated. The residue was purified by flash chromatography using petroleum ether/EtOAc (10 to 20%) to yield a mixture of isomer (82%/18%) (2.90 g, 13.40 mmol, 24%).

^1H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 6.53-5.60 (m, 1H, *J* = 6.5 Hz, 0.60 Hz, $\underline{CH}CH_2OH$), 5.56-5.53 (m, 1H, *J* = 4.9 Hz, 0.8 Hz, $\underline{CH}CH_2OH$), 4.15 (d, 2H, *J* = 6.7 Hz, $CH\underline{CH_2}OH$), 4.10 (d, 2H, *J* = 6.6 Hz, $CH\underline{CH_2}OH$), 3.98 & 3.76 (2s, 4H, 2 x $\underline{CH_2}OSi(CH_3)_2C(CH_3)_3$), 1.68 & 1.59 (2s, 6H, 2 x $C(\underline{CH_3})CH_2OSiCH_3)_2C(CH_3)_3$), 0.86 & 0.84 (2s, 18H, 2 x $Si(CH_3)_2C(\underline{CH_3})_3$), 0.03 & 0.02 (2s, 6H, 2 x $Si(\underline{CH_3})_2C(CH_3)_3$).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 138.90 & 137.65 (2s, 2 x $\underline{C}(CH_3)CH_2OSiCH_3)_2C(CH_3)_3$), 125.75 & 122.93 (2s, 2 x $\underline{C}HCH_2OH$), 67.69 & 65.43 (2s, 2 x $\underline{C}H_2OSi(CH_3)_2C(CH_3)_3$), 58.84 & 58.06 (2s, 2 x $CH\underline{C}H_2OH$), 26.90 & 26.13 & 25.93 & 25.89 & 25.81 & 25.26 (6s, 6 x $Si(CH_3)_2C(\underline{C}H_3)_3$), 20.84 & 20.13 (2s, 2 x

$Si(CH_3)_2\underline{C}(CH_3)_3$), 13.96 & 13.31 (2s, 2 x $C(\underline{C}H_3)CH_2OSiCH_3)_2C(CH_3)_3$), -5.49 & -5.21 (2s, 2 x $Si(\underline{C}H_3)_2C(CH_3)_3$).

Preparation of (4-bromo-2-methylbut-2-enyloxy)(tert-butyl)dimethylsilane (186) [17, 18]

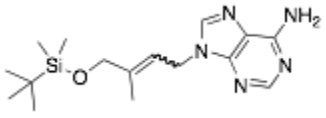


A solution of 4-(tert-butyldimethylsilyloxy)-3-methylbut-2-en-1-ol (**185**) (0.49 g, 2.26 mmol, 1.0 eq) and triphenylphosphine (0.66 g, 2.49 mmol, 1.1 eq) in anh. DCM (6.80 mL), NBS (0.44 g, 2.49 mmol, 1.1 eq) was slowly added at 0 °C and stirred for 4 hrs at RT. DCM was added and the organic layer was washed with brine and H_2O, and was filtrated through Celite. The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography using petroleum ether/EtOAc (10%) to afford the desired molecule in only one major isomer (0.240 g, 0.86 mmol, 38%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 5.76 (m, 1H, *J* = 8.5 Hz, 1.4 Hz, $\underline{CH}CH_2Br$), 4.01 (s, 2H, $\underline{CH_2}OSiCH_3)_2C(CH_3)_3$), 3.98 (d, 2H, *J* = 8.5 Hz, $CH\underline{CH_2}Br$), 1.63 (s, 3H, $C(\underline{CH_3})CH_2OSiCH_3)_2C(CH_3)_3$), 0.86 (s, 9H, $Si(CH_3)_2C(\underline{CH_3})_3$), 0.02 (s, 6H, $Si(\underline{CH_3})_2C(CH_3)_3$).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 140.84 (s, $\underline{C}(CH_3)CH_2OSiCH_3)_2C(CH_3)_3$), 119.36 (s, $\underline{C}HCH_2Br$), 67.21 (s, $\underline{C}H_2OSiCH_3)_2C(CH_3)_3$), 40.08 (s, $Si(CH_3)_2\underline{C}(CH_3)_3$), 28.46 (s, $CH\underline{C}H_2Br$), 25.84 & 25.74 & 25.64 (3s, $Si(CH_3)_2C(\underline{C}H_3)_3$), 18.365 (s, $Si(CH_3)_2\underline{C}(CH_3)_3$), 14.08 (s, $C(\underline{CH_3})CH_2OSiCH_3)_2C(CH_3)_3$), -5.31 & -5.22 (2s, $Si(\underline{C}H_3)_2C(CH_3)_3$).

Preparation of acyclic TBDMS protected adenine (187.a) [17]



Route 1: A solution of (4-bromo-2-methylbut-2-enyloxy)(tert-butyl)dimethylsilane (**186**) (0.240 g, 0.86 mmol, 1.0 eq), adenine (0.38 g, 2.78 mmol, 3.2 eq), NaH (60% in dispersion oil, 0.17 g, 2.78 mmol, 3.2 eq) in anh. DCM (7 mL) was stirred overnight at RT. The reaction was quenched with water and diluted with EtOAc. The organic layer was washed with H_2O and brine, and the solvent evaporated under reduced pressure.

The residue was purified by flash chromatography using DCM/MeOH (5%) to yield the desired material as a mixture of two isomers (6%/94%, 0.06 g, 0.18 mmol, 21%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.38 & 8.18 (2s, 2H, H-2 base), 7.81 & 7.74 (2s, 2H, H-8 base), 6.47 & 5.83 (bs, 4H, 2 x NH_2 base), 5.78-5.75 (m, 1H, *J* = 6.2 Hz & 1.3 Hz, CHCH$_2$-base), 5.65-5.62 (m, 1H, *J* = 7.2 Hz, 2.9 Hz, 1.45 Hz, CHCH$_2$-base), 4.87 (d, *J* = 6.2 Hz, CHCH$_2$-base), 4.75 (d, 2H, *J* = 7.2 Hz, CHCH$_2$-base), 4.09 & 3.98 (2s, 4H, 2 x CH$_2$OSi(CH$_3$)$_2$C(CH$_3$)$_3$), 1.80 & 1.69 (2s, 6H, 2 x C(CH$_3$)CH$_2$OSi(CH$_3$)$_2$C(CH$_3$)$_3$), 0.91 & 0.79 (2s, 18H, 2 x Si(CH$_3$)$_2$C(CH$_3$)$_3$), 0.08 & 0.04 (2s, 212H, 2 x Si(CH$_3$)$_2$C(CH$_3$)$_3$).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 155.53 & 155.11 (2s, 2 x C-6 base), 152.85 & 152.50 (2s, 2 x C-2 base), 149.31 (2s, 2 x C-4 base), 141.57 & 141.18 (2s, 2 x C(CH$_3$)CH$_2$OSiCH$_3$)$_2$C(CH$_3$)$_3$)), 140.18 & 139.89 (2s, 2 x C-8 base), 118.89 & 118.02 (2s, 2 x C-5 base), 116.32 & 116.09 (2s, 2 x CHCH$_2$-base), 67.07 & 66.16 (2s, 2 x CH$_2$OSi(CH$_3$)$_2$C(CH$_3$)$_3$), 40.84 & 40.58 (2s, 2 x CHCH$_2$-base), 25.87 & 25.67 (2s, 2 x Si(CH$_3$)$_2$C(CH$_3$)$_3$), 18.76 & 18.24 (2s, 2 x Si(CH$_3$)$_2$C(CH$_3$)$_3$), 13.71 & 13.47 (2s, 2 x C(CH$_3$)CH$_2$OSi(CH$_3$)$_2$C(CH$_3$)$_3$), -5.61 & -5.32 (2s, Si(CH$_3$)$_2$C(CH$_3$)$_3$).

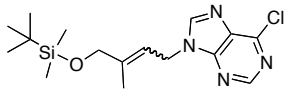
Route 2: To a stirred solution of adenine (0.11 g, 0.84 mmol, 1.1 eq) in anh. THF (11 mL) at RT was added 4-(tert-butyldimethylsilyloxy)-3-methylbut-2-en-1-ol (**185**) (0.20 g, 0.92 mmol, 1.1 eq) followed by triphenylphosphine (0.24 g, 0.92 mmol, 1.1 eq). After few minutes, DIAD (0.17 mL, 0.84 mmol, 1.0 eq) was added dropwise and the reaction was left stirring over 72 hrs. The solvent was evaporated under reduced pressure and the residue purified by flash chromatography using EtOAc and then DCM/MeOH (5%) to recover only one isomer of the desired material (0.08 g, 0,24 mmol, 26%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.35 (s, 1H, H-2 base), 7.79 (s, 1H, H-8 base), 6.09 (bs, 2H, NH_2 base), 5.76-5.73 (t, 1H, *J* = 6.8 Hz, CHCH$_2$-base), 4.84 (d, *J* = 6.8 Hz, CHCH$_2$-base), 4.07 (s, 2H, CH$_2$OSi(CH$_3$)$_2$C(CH$_3$)$_3$), 1.78 (s, 3H, C(CH$_3$)CH$_2$OSi(CH$_3$)$_2$C(CH$_3$)$_3$), 0.89 (s, 9H, Si(CH$_3$)$_2$C(CH$_3$)$_3$), 0.05 (s, 6H, Si(CH$_3$)$_2$C(CH$_3$)$_3$).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 155.55 (s, C-6 base), 152.91 (s, C-2 base), 149.93 (s, C-4 base), 141.46 (s, C(CH$_3$)CH$_2$OSi(CH$_3$)$_2$C(CH$_3$)$_3$), 139.86 (s, C-8 base), 119.48 (s, C-5 base), 116.36 (s, CHCH$_2$-base), 67.15 (s, CH$_2$OSi(CH$_3$)$_2$C(CH$_3$)$_3$), 40.79

(2s, CH<u>C</u>H$_2$-base), 25.83 (s, Si(CH$_3$)$_2$C(<u>C</u>H$_3$)$_3$), 18.32 (s, Si(CH$_3$)$_2$<u>C</u>(CH$_3$)$_3$), 13.66 (s, C(<u>C</u>H$_3$)CH$_2$OSiCH$_3$)$_2$C(CH$_3$)$_3$), -5.43 (s, Si(<u>C</u>H$_3$)$_2$C(CH$_3$)$_3$).

Preparation of acyclic TBDMS protected 6-chloro-purine (187.b) [17]

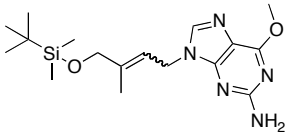


To a stirred solution of 6-chloro purine (0.325 g, 2.10 mmol, 1.0 eq) in anh. THF (28 mL), was added 4-(tert-butyldimethylsilyloxy)-3-methylbut-2-en-1-ol (**185**) (0.500 g, 2.31 mmol, 1.1 eq) followed by triphenylphosphine polymer bound (3 mmol/g, 0.77 g, 2.31 mmol, 1.1 eq). After few minutes, DIAD (0.43 mL, 2.10 mmol, 1.0 eq) was added dropwise and the reaction was left stirring overnight at RT. The solvent was evaporated under reduced pressure and the residue purified by flash chromatography using EtOAc and then DCM/MeOH (3%) to recover only one isomer of the desired material (0.389 g, 1.63 mmol, 70%).

^{1}H NMR (500 MHz, CDCl$_3$) δ_H (ppm): 8.69 (s, 1H, H-2 base), 8.12 (s, 1H, H-8 base), 5.75 (t, 1H, *J* = 5.2 Hz, <u>CH</u>CH$_2$-base), 4.92 (d, *J* = 6.0 Hz, CH<u>CH$_2$</u>-base), 4.12 (s, 2H, <u>CH$_2$</u>OSi(CH$_3$)$_2$C(CH$_3$)$_3$), 1.76 (s, 3H, C(<u>CH$_3$</u>)CH$_2$OSi(CH$_3$)$_2$C(CH$_3$)$_3$), 0.91 (s, 9H, Si(CH$_3$)$_2$C(<u>CH$_3$</u>)$_3$), 0.08 (s, 6H, Si(<u>CH$_3$</u>)$_2$<u>C</u>(CH$_3$)$_3$).

^{13}C NMR (125 MHz, CDCl$_3$) δ_C (ppm): 151.69 (s, C-6 base), 151.60 (s, C-2 base), 150.63 (s, C-4 base), 144.90 (s, C-8 base), 137.30 (s, <u>C</u>(CH$_3$)CH$_2$OSi(CH$_3$)$_2$C(CH$_3$)$_3$), 131.40 (s, C-5 base), 118.61 (s, <u>C</u>HCH$_2$-base), 65.71 (s, <u>C</u>H$_2$OSi(CH$_3$)$_2$C(CH$_3$)$_3$), 41.33 (s, CH<u>C</u>H$_2$-base), 25.73 & 25.62 & 25.39 (3s, Si(CH$_3$)$_2$C(<u>CH$_3$</u>)$_3$), 18.17 (s, Si(CH$_3$)$_2$<u>C</u>(CH$_3$)$_3$), 13.57 (s, C(<u>C</u>H$_3$)CH$_2$OSi(CH$_3$)$_2$C(CH$_3$)$_3$), -5.56 (s, Si(<u>CH$_3$</u>)$_2$C(CH$_3$)$_3$).

Preparation of acyclic TBDMS protected 6-O-methyl-guanine (187.c)



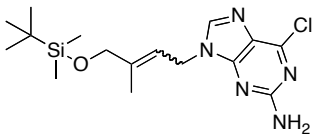
To a stirred solution of 6-*O*-methyl guanine (0.6936 g, 4.20 mmol, 1.0 eq) in anh. THF (60 mL), was added 4-(tert-butyldimethylsilyloxy)-3-methylbut-2-en-1-ol (**185**) (1.00 g, 4.62 mmol, 1.1 eq) at RT, followed by triphenylphosphine polymer bound (3 mmol/g, 1.54 g, 4.62 mmol, 1.1 eq). After few minutes, DIAD (0.83 mL, 4.20 mmol, 1.0 eq) was

added dropwise and the reaction was left stirring overnight. The crude product was purified by flash chromatography to recover the desired molecule as a mixture of isomers (13%/87%) (0.240 g, 0.66 mmol, 16%).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 7.61 (s, 1H, H-8 base), 7.58 (s, 1H, H-8 base), 5.72-5.68 (tq, 1H, *J* = 1.5 Hz, 7.10 Hz, CHCH$_2$-base), 5.45-5.42 (m, 1H, CHCH$_2$-base), 5.38 (bs, 2H, NH_2 base), 4.97 (bs, 2H, NH_2 base), 4.74 (d, 2H, *J* = 7.2 Hz, CHCH$_2$-base), 4.69 (d, 2H, *J* = 7.0 Hz, CHCH$_2$-base), 4.11 (s, 3H, 6-*O*CH$_3$ base), 4.10 (s, 3H, 6-*O*CH$_3$ base), 4.08 (s, 2H, CH$_2$OSi(CH$_3$)$_2$C(CH$_3$)$_3$), 4.06 (s, 2H, CH$_2$OSi(CH$_3$)$_2$C(CH$_3$)$_3$), 1.80 (s, 3H, C(CH$_3$)CH$_2$OSi(CH$_3$)$_2$C(CH$_3$)$_3$), 1.76 (s, 3H, C(CH$_3$)CH$_2$OSi(CH$_3$)$_2$C(CH$_3$)$_3$), 0.91 (s, 9H, Si(CH$_3$)$_2$C(CH$_3$)$_3$), 0.89 (s, 9H, Si(CH$_3$)$_2$C(CH$_3$)$_3$), 0.12 (s, 6H, Si(CH$_3$)$_2$C(CH$_3$)$_3$), 0.10 (s, 6H, Si(CH$_3$)$_2$C(CH$_3$)$_3$).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 161.91 & 159.34 (2s, 2 x C-6 base), 153.76 & 153.39 (2s, 2 x C-2 base), 147.23 & 147.15 (2s, 2 x C-4 base), 140.97 & 140.86 (2s, 2 x C(CH$_3$)CH$_2$OSi(CH$_3$)$_2$C(CH$_3$)$_3$), 138.83 & 137.23 (2s, 2 x C-8 base), 119.76 & 116.80 (2s, 2 x CHCH$_2$-base), 115.87 & 115.61 (2s, 2 x C-5 base), 67.93 & 67.24 (2s, 2 x CH$_2$OSi(CH$_3$)$_2$C(CH$_3$)$_3$), 53.99 & 53.82 (2s, 2 x 6-*O*CH$_3$ base), 40.52 & 40.24 (2s, 2 x CHCH$_2$-base), 31.56 & 29.67 (2s, 2 x Si(CH$_3$)$_2$C(CH$_3$)$_3$), 25.85 & 25.59 (2s, 2 x Si(CH$_3$)$_2$C(CH$_3$)$_3$), 18.34 & 18.13 (2s, 2 x Si(CH$_3$)$_2$C(CH$_3$)$_3$), 14.08 & 13.65 (2s, 2 x C(CH$_3$)CH$_2$OSi(CH$_3$)$_2$C(CH$_3$)$_3$), -5.39 & -5.42 (2s, 2 x Si(CH$_3$)$_2$C(CH$_3$)$_3$ isomers).

Preparation of acyclic TBDMS protected 6-chloro-guanine (187.e)



To a solution of 6-chloro guanine (0.356 g, 2.10 mmol, 1.0 eq) in anh. THF (24 mL), was added 4-(tert-butyldimethylsilyloxy)-3-methylbut-2-en-1-ol (**185**) (0.500 g, 2.31 mmol, 1.1 eq), followed by triphenylphosphine polymer bound (3 mmol/g, 0.77 g, 2.31 mmol, 1.1 eq), at RT. After few minutes, DIAD (0.42 mL, 2.10 mmol, 1.0 eq) was added dropwise and the reaction was left stirring overnight. The crude product was purified by flash chromatography to recover the desired molecule as a mixture of isomers (0.610 g, 1.66 mmol, 79%).

^{1}H NMR (50 MHz, $CDCl_3$) δ_H (ppm): 7.98 & 7.39 (2s, 2H, 2 x H-8 base), 5.62 & 5.23 (2t, 2H, *J* = 7.0 Hz, 7.6 Hz, 2 x CHCH$_2$-base), 5.42 & 5.39 (2bs, 4H, 2 x NH_2 base),

4.78 & 4.23 (2d, 4H, *J* = 7.0 Hz, 7.6 Hz, 2 x CHCH_2-base), 4.32 & 4.03 (2s, 4H, 2 x CH_2OSi(CH_3)$_2$C(CH_3)$_3$), 1.78 & 1.51 (2s, 6H, 2 x C(CH_3)CH_2OSi(CH_3)$_2$C(CH_3)$_3$), 0.96 & 0.81 (2s, 18H, 2 x Si(CH_3)$_2$C(CH_3)$_3$), 0.29 & .0.08 (2s, 12H, 2 x Si(CH_3)$_2$C(CH_3)$_3$).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 159.28 & 156.66 (2s, 2 x C-6 base), 153.56 & 151.01 (2s, 2 x C-4 base), 142.17 & 141.90 (2s, 2 x C-8 base), 138.14 & 137.47 (2s, 2 x C(CH_3)CH_2OSi(CH_3)$_2$C(CH_3)$_3$), 124.81 & 124.54 (2s, 2 x C-5 base), 118.03 & 115.99 (2s, 2 x CHCH_2-base), 62.04 & 61.57 (2s, 2 x CH_2OSi(CH_3)$_2$C(CH_3)$_3$), 40.76 & 40.48 (2s, 2 x CHCH_2-base), 25.80 & 25.78 & 25.61 & 21.95 & 21.68 & 21.27 (6s, 2 x Si(CH_3)$_2$C(CH_3)$_3$), 18.29 & 18.22 (2s, 2 x Si(CH_3)$_2$C(CH_3)$_3$ isomers), 14.00 & 13.55 (2s, 2 x C(CH_3)CH_2OSi(CH_3)$_2$C(CH_3)$_3$), -5.26 & -5.22 & -5.44 & -5.48 (4s, 2 x Si(CH_3)$_2$C(CH_3)$_3$).

Preparation of acyclic adenine nucleoside (188) [17]

Acyclic TBDMS protected adenine (**187.a**) (0.08 g, 0.24 mmol, 1.0 eq) was dissolved in anh. THF (2.5 mL), and TBAF (1M in THF, 1.44 mL, 1.44 mmol, 6.0 eq) was added. The solution was left stirring for 1 hr. Purification by flash chromatography using $CHCl_3$/MeOH (6%) was performed to recover the desired material and the isomers were separated ((*E*) (**188.a**), in mixture 0.0096 g, 0.044 mmol, 18%; (*Z)* (**188.b**), 0.021 g, 0.096 mmol, 40%) (*E*/*Z* ratio: 31%/69%).

HPLC (MeOH/H_2O): Rt = 9.99 min (*Z*), 11.75 min (*E*),

HPLC (ACN/H_2O): Rt = 4.61 min (*Z*), 5.65 min (*E*)

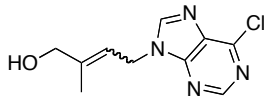
MS (TOF ES$^+$): 242.28 (M + Na^+).

^{1}H NMR (500 MHz, MeOD) δ_H (ppm): 8.23 (s, 1H, H-2 base), 8.21 (s, 1H, H-2 base), 8.18 & 8.11 (2s, 2H, 2 x H-8 base), 5.74-5.71 (tq, 1H, *J* = 7.1 Hz, 1.4 Hz, CHCH_2-base), 5.54-5.51 (t, *J* = 7.3 Hz, CHCH_2-base), 4.95 (d, *J* = 7.4 Hz, CHCH_2-base), 4.92 (d, *J* = 7.2 Hz, CHCH_2-base), 4.29 (s, 2H, C(CH_3)OCH_2OH), 4.00 (s, 2H, C(CH_3)OCH_2OH), 1.87 (s, 3H, C(CH_3)CH_2OH), 1.85 (s, 3H, C(CH_3)CH_2OH).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 155.57 (s, C-6 base), 156.21 (s, C-6 base), 153.71 (s, C-2 base), 153.59 (s, C-2 base), 149.76 (s, C-4 base), 149.12 (s, C-4 base), 142.44 (s, C-8 base), 142.28 (s, C-8 base), 118.97 (s, C-5 base), & 118.25 (s, C-5 base), 121.71 (s, CHCH_2-base), 118.75 (s, CHCH_2-base), 67.52 (s, C(CH_3)OCH_2OH), 61.64

(s, C(CH_3)O<u>C</u>H_2OH), 42.05 (s, CH<u>C</u>H_2-base), <u>40.04</u> (s, CH<u>C</u>H_2-base), 21.80 (s, C(<u>C</u>H_3)CH_2OH), <u>13.86</u> (s, C(<u>C</u>H_3)CH_2OH).

Preparation of acyclic 6-chloro-purine nucleoside (189) [17]



Acyclic TBDMS protected adenine (**187.b**) (0.389 g, 1.63 mmol, 1.0 eq) was dissolved in anh. THF (17 mL) and TBAF (1M in THF, 9.78 mL, 9.78 mmol, 6.0 eq) was added. The solution was left stirring for 4 hrs. Purification by flash chromatography using $CHCl_3$/MeOH (2.5%) was performed to recover the desired material and only one isomer (*Z*) was recovered (0.120 g, 0.50 mmol, 31%).

HPLC (MeOH/H_2O): Rt = 11.20 min

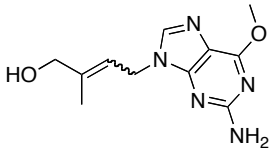
HPLC (ACN/H_2O):): Rt = 7.20 min

MS (TOF ES^-): 273.03 (M + Cl^-), 511.11 (2M + Cl^-).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.71 (s, 1H, H-2 base), 8.14 (s, 1H, H-8 base), 5.76-5.74 (tq, 1H, *J* = 5.7 Hz, 1.4 Hz, <u>CH</u>CH_2-base), 4.94 (d, 2H, *J* = 6.9 Hz, CH<u>C</u>H_2-base), 4.09 (s, 2H, C(CH_3)<u>C</u>H_2OH), 1.85 (s, 3H, C(<u>C</u>H_3)CH_2OH).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 151.88 (s, C-2 base), 151.62 (s, C-6 base), 150.81 (s, C-4 base), 144.92 (s, C-8 base), 142.62 (s, <u>C</u>(CH_3)CH_2OH), 131.41 (s, C-5 base), 116.08 (s, <u>C</u>HCH_2-base), 66.76 (s, C(CH_3)<u>C</u>H_2OH), 41.51 (s, CH<u>C</u>H_2-base), 13.98 (s, C(<u>C</u>H_3)CH_2OH).

Preparation of acyclic 6-O-methyl-guanine nucleoside (190)



Acyclic TBDMS protected 6-*O*-methyl-guanine (**187.c**) (0.463 g, 1.27 mmol, 1.0 eq) was dissolved in anh. THF (14 mL), and TBAF (1M in THF, 7.62 mL, 7.62 mmol, 6.0 eq) was added. The solution was left stirring for 4 hrs. Purification by flash chromatography using $CHCl_3$/MeOH (3%) was performed to recover the desired material as a mixture of separable isomers (0.100 g, 0.40 mmol, 32%) (<u>*E*</u> (**190.a**)/*Z* (**190.b**) ratio: 40%/60%).

HPLC (MeOH/H_2O): Rt (*Z*) = 7.45 min (*Z*), 11.89 min (<u>*E*</u>)

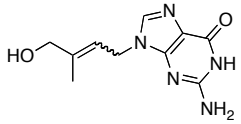
HPLC (ACN/H_2O): Rt = 2.48 min (*Z*), 5.81 min (*E*)

MS (TOF ES^+): 250.13 ($M + H^+$).

^1H NMR (500 MHz, MeOD) δ_H (ppm): 7.81 (s, 1H, H-8 base), 7.34 (s, 1H, H-8 base), 5.71 (tq, 1H, *J* = 1.5 Hz, 7.0 Hz, CHCH$_2$-base), 5.63 (t, 1H, *J* = 7.5 Hz, CHCH$_2$-base), 4.74 (d, 2H, *J* = 7.0 Hz, CHCH$_2$-base), 4.74 (d, 2H, *J* = 7.5 Hz, CHCH$_2$-base), 4.06 & 4.02 (2s, 6H, 2 x 6-*O*CH$_3$ base), 3.99 & 3.94 (2s, 4H, 2 x C(CH$_3$)CH$_2$OH), 1.85 & 1.84 (s, 6H, 2 x C(CH$_3$)CH$_2$OH).

^{13}C NMR (125 MHz, MeOD) δ_C (ppm): 161.98 & 161.81 (2s, 2 x C-6 base), 160.97 & 160.45 (2s, 2 x C-2 base), 154.09 & 153.96 (2s, 2 x C-4 base), 142.22 & 141.23 (2s, 2 x C(CH$_3$)CH$_2$OH), 139.81 & 139.5 (s, C-8 base), 120.16 & 117.43 (2s, CHCH$_2$-base), 114.33 (s, C-5 base), 66.97 & 61.52 (2s, 2 x C(CH$_3$)CH$_2$OH), 53.36 (s, 6-*O*CH$_3$ base), 41.34 & 40.70 (2s, 2 x CH$_2$-base), 13.70 & 13.63 (2s, 2 x C(CH$_3$)CH$_2$OH).

Preparation of acyclic guanine nucleoside (191) [17]



To as solution (*E*)-acyclic 6-*O*-methyl-guanine nucleoside (**190.a**) (0.033 g, 0.13 mmol, 1.0 eq) in anh. ACN (5 mL), was added NaI (0.029 g, 0.20 mmol, 1.5 eq) and TMSCl (0.025 mL, 0.20 mmol, 1.5 eq). The reaction was left stirring for 1 hr at RT and preparative chromatography afforded the desired (*E*)-isomer (0.0024 g, 0.010 mmol, 8%).

HPLC (MeOH/H_2O): Rt = 11.52 min

HPLC (ACN/H_2O): Rt = 2.73 min

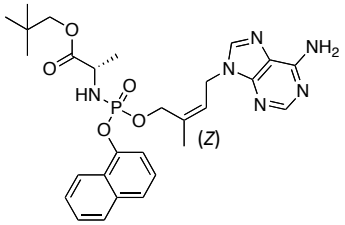
MS (TOF ES^+): 236.11 ($M + H^+$), 258.10 ($M + Na^+$), 274.07 ($M + K^+$), 299.12 ($M + MeCNNa^+$), 471.22 ($2M + H^+$), 493.20 ($2M + Na^+$).

^1H NMR (500 MHz, MeOD) δ_H (ppm): 7.72 (s, 1H, H-8 base), 5.33 (t, 1H, *J* = 7.9 Hz, CHCH$_2$-base), 4.76 (d, 2H, *J* = 7.0 Hz, CHCH$_2$-base), 4.28 (s, 2H, C(CH$_3$)CH$_2$OH), 1.85 (s, 3H, C(CH$_3$)CH$_2$OH).

^{13}C NMR (d-MeOD) δ_C (ppm): 158.83 (s, C-6 base), 154.17 (s, C-2 base), 153.96 (s, C-4 base), 141.44 (s, C(CH$_3$)CH$_2$OH), 137.53 (s, C-8 base), 119.87 (s, CHCH$_2$-base), 114.33 (s, C-5 base), 63.27 (s, C(CH$_3$)CH$_2$OH), 40.85 (s, CHCH$_2$-base), 13.82 (s, C(CH$_3$)CH$_2$OH).

6.2. Preparation of acyclic purine ProTides

Preparation of α-naphthyl L-Alanine-neopentyl-ester (Z)-acyclic adenine ProTide (192.a)



This ProTide was prepared according to the Standard Procedure D, using *t*BuMgCl (1M in THF, 0.46 mL, 0.46 mmol, 2eq), (*Z*)-acyclic adenine (**188.b**) (0.051 mg, 0.23 mmol, 1.0 eq) in anh. THF (0.68 mL), and *α*-naphthyl *L*-Alanine-neopentyl ester phosphochloridate (**14.d**) (0.177 g, 0.46 mmol, 2.0 eq) in anh. THF (0.68 mL). After overnight stirring at RT, the solvent was removed under reduced pressure and the residue was purified by flash chromatography, using $CHCl_3$/MeOH (5%) as eluents, and preparative chromatography to yield the desired molecule (0.024 g, 0.042 mmol, 18%).

HPLC (MeOH/H_2O): Rt = 27.22 min, 27.41 min

HPLC (ACN/H_2O): Rt = 17.45 min

MS (TOF ES^+): 567.25 ($M + H^+$).

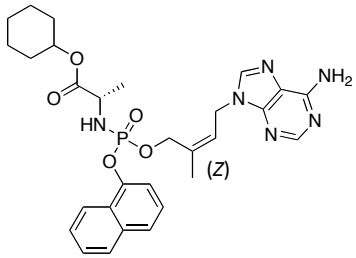
^1H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.31 & 8.28 (2s, 2H, H-2 base diastereoisomers), 8.07-8.06 (m, 2H, H-4 Ar diastereoisomers), 7.82-7.77 (m, 2H, H-6 Ar diastereoisomers), 7.70 (bs, 2H, H-8 base diastereoisomers), 7.65 (d, 2H, J = 8.0 Hz, H-9 Ar diastereoisomers), 7.51-7.43 (m, 6H, H-2, H-7, H-8 Ar diastereoisomers), 7.32 (m, 2H, H-3 Ar diastereoisomers), 5.80 (bs, 4H, NH_2 base diastereoisomers), 5.61 (t, 2H, J = 6.5 Hz, CHCH$_2$-base), 5.24 (t, 2H, J = 6.0 Hz, CHCH$_2$-base), 4.76-4.70 (m, 4H, CHCH$_2$-base diastereoisomers), 4.60-4.45 (m, 4H, C(CH_3)CH$_2$OP diastereoisomers), 4.11-4.04 (m, 2H, NHCH(CH_3)CO diastereoisomers), 3.80-3.71 (2m, 4H, COOCH$_2$C(CH_3)$_3$ diastereoisomers), 1.75 & 1.73 (2s, 6H, C(CH$_3$)CH$_2$OP diastereoisomers), 1.36-1.22 (m, 6H, NHCH(CH$_3$)CO diastereoisomers), 0.89 & 0.87 (2s, 18H, COOCH$_2$C(CH$_3$)$_3$ diastereoisomers).

^{13}C NMR (125 MHz, $CDCl_3$) δ_c (ppm): 173.64 (d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 6.4 Hz, NHCH(CH_3)CO), 173.54 (d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 6.4 Hz, NHCH(CH_3)CO), 155.53 & 155.19 (2s, C-6 base diastereoisomers), 152.96 & 152.90 (2s, C-2 base diastereoisomers), 149.74 & 149.68 (2s, C-4 base diastereoisomers), 146.61 & 146.55 (2s, C-1 Ar diastereoisomers), 139.82 & 139.76 (2s, C-8 base diastereoisomers), 136.53 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 3.8 Hz,

C(CH$_3$)CH$_2$OP), 136.53 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 2.6 Hz, C(CH$_3$)CH$_2$OP), 134.69 & 134.52 (2s, C-5 Ar diastereoisomers), 127.79 & 127.77 (2s, C-6 Ar diastereoismers), 126.53 & 126.44 (2s, C-10 Ar diastereoismers), 126.65 & 126.40 (2s, C-7 Ar diastereoisomers), 126.26 & 126.08 (2s, C-8 Ar diastereoisomers), 125.48 & 125.31 (2s, C-3 Ar diastereoisomers), 124.80 & 124.65 (2s, C-9 Ar diastereoisomers), 121.45 & 121. 41 (2s, CHCH$_2$-base diastereoisomers), 120.64 & 120.32 (2s, C-4 Ar diastereoisomers), 119.28 & 119.01 (2s, C-5 base diastereoisomers), 115.33 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 2.6 Hz, C-2 Ar), 115.21 (s, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 2.6 Hz, C-2 Ar), 74.72 & 74.54 (2s, OCH$_2$C(CH$_3$)$_3$ diastereoisomers), 70.58 (d, $J^2_{P\text{-}O\text{-}C}$ = 3.8 Hz, C(CH$_3$)CH$_2$OP), 70.51 (d, $J^2_{P\text{-}O\text{-}C}$ = 2.6 Hz, C(CH$_3$)CH$_2$OP), 50.51 (d, $J^2_{P\text{-}N\text{-}C}$ = 2.6 Hz, NCH(CH$_3$)CO), 50.43 (d, $J^2_{P\text{-}N\text{-}C}$ = 3.8 Hz, NCH(CH$_3$)CO), 40.70 & 40.67 (2s, CHCH$_2$-base diastereoisomers), 31.40 & 31.34 (2s, OCH$_2$C(CH$_3$)$_3$ diastereoisomers), 26.27 & 26.25 (2s, OCH$_2$C(CH$_3$)$_3$ diastereoisomers), 21.26 & 21.23 (2s, NCH(CH$_3$)CO diastereoisomers), 13.80 & 13.78 (2s, C(CH$_3$)CH$_2$OP diastereoisomers).

^{31}P NMR (202 MHz, CDCl$_3$) δ_P (ppm)**:** 3.16 (s, 44%), 3.81 (s, 56%).

Preparation of α-naphthyl L-Alanine-cyclohexyl-ester (Z)-acyclic adenine ProTide (192.b)



This ProTide was prepared according to the Standard Procedure D, using *t*BuMgCl (1M in THF, 0.34 mL, 0.34 mmol, 2eq), (*Z*)-acyclic adenine (**188.b**) (0.037 mg, 0.17 mmol, 1.0 eq) in anh. THF (0.50 mL), and *α*-naphthyl *L*-Alanine-cyclohexyl ester phosphochloridate (**14.e**) (0.135 g, 0.34 mmol, 2.0 eq) in anh. THF (0.50 mL). After overnight stirring at RT, the solvent was removed under reduced pressure and the residue was purified by flash chromatography, using CHCl$_3$/MeOH (5%) as eluents, and preparative chromatography to yield the desired molecule (0.018 g, 0.008 mmol, 47%).

HPLC (MeOH/H$_2$O): Rt = 27.49 min, 27.71 min

HPLC (ACN/H$_2$O): Rt = 18.36 min

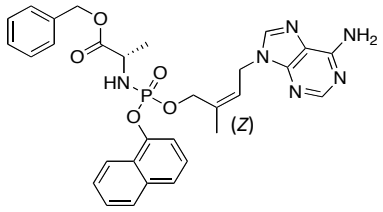
MS (TOF ES$^+$): 579.25 (M + H$^+$).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.37 & 8.36 (2s, 2H, H-2 base diastereoisomers), 8.12-8.08 (m, 2H, H-4 Ar diastereoisomers), 7.83-7.08 (m, 2H, H-6 Ar diastereoisomers), 7.73 (bs, 2H, H-8 base diastereoisomers), 7.64 (d, 2H, J = 8.2 Hz, H-9 Ar diastereoisomers), 7.54-7.47 (m, 6H, H-2, H-7, H-8 Ar diastereoisomers), 7.38 (t, 2H, J = 7.9 Hz, H-3 Ar diastereoisomers), 5.79 (bs, 4H, NH_2 base diastereoisomers), 5.63 (t, 2H, J = 6.6 Hz, $\underline{CH}CH_2$-base), 5.63 (t, 2H, J = 6.5 Hz, $\underline{CH}CH_2$-base), 4.78-4.71 (m, 4H, $CH\underline{CH_2}$-base diastereoisomers), 4.61-4.49 (m, 4H, $C(CH_3)\underline{CH_2}OP$ diastereoisomers), 4.13-4.06 (m, 2H, $NH\underline{CH}(CH_3)CO$ diastereoisomers), 3.94-3.88 (m, 2H, CH cHex diastereoisomers), 1.73 & 1.72 (2s, 6H, $C(\underline{CH_3})CH_2OP$ diastereoisomers), 1.41-1.39 (m, 4H, 2 x CH_2 meta cHex), 1.37-1.35 (m, 4H, 2 x CH_2 ortho cHex), 1.33-1.23 (m, 18H, CH_2 para cHex, $NHCH(\underline{CH_3})CO$ diastereoisomers, 2 x CH_2 meta cHex, 2 x CH_2 ortho cHex).

^{13}C NMR (125 MHz, $CDCl_3$) δ_c (ppm): 173.45 (d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 6.4 Hz, $NHCH(CH_3)\underline{C}O$), 173.36 (d, , $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 6.4 Hz, $NHCH(CH_3)\underline{C}O$), 155.34 & 155.21 (2s, C-6 base diastereoisomers), 152.98 & 152.57 (2s, C-2 base diastereoisomers), 149.47 & 149.13 (2s, C-4 base diastereoisomers), 146.70 & 146.65 (2s, C-1 Ar diastereoisomers), 140.03 & 139.92 (2s, C-8 base diastereoisomers), 136.53 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 3.8 Hz, $\underline{C}(CH_3)CH_2OP$), 136.53 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 2.6 Hz, $\underline{C}(CH_3)CH_2OP$), 134.71 & 134.51 (2s, C-5 Ar diastereoisomers), 127.77 & 127.43 (2s, C-6 Ar diastereoisomers), 126.72 & 126.53 (2s, C-10 Ar diastereoisomers), 126.26 & 126.02 (2s, C-7 Ar diastereoisomers), 126.22 & 126.08 (2s, C-8 Ar diastereoisomers), 125.51 & 125.11 (2s, C-3 Ar diastereoisomers), 124.76 & 124.31 (2s, C-9 Ar diastereoisomers), 121.52 & 121.46 (2s, $\underline{C}HCH_2$-base diastereoisomers), 120.63 & 120.54 (2s, C-4 Ar diastereoisomers), 120.36 & 120.24 (2s, C-5 base diastereoisomers), 115.29 & 115.23 (2s, C-2 Ar diastereoisomers), 74.06 & 73.97 (2s, C ipso cHex diastereoisomers), 70.47 & 70.21 (2s, $C(CH_3)\underline{C}H_2OP$ diastereoisomers), 50.51 & 50.48 (2s, $N\underline{C}H(CH_3)CO$ diastereoisomers), 40.72 & 40.67 (2s, $CH\underline{C}H_2$-base diastereoisomers), 31.39 & 31.31 (2s, CH_2 ortho cHex diastereoisomers), 25.21 & 25.13 (2s, CH_2 para cHex diastereoisomers), 21.56 & 21.43 (2s, $NCH(\underline{C}H_3)CO$ diastereoisomers), 21.21 & 21.13 (2s, CH_2 meta cHex diastereoisomers), 13.83 & 13.78 (2s, $C(\underline{C}H_3)CH_2OP$ diastereoisomers).

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm)**:** 2.89 (s, 50%), 2.76 (s, 50%).

Preparation of α-naphthyl L-Alanine-benzyl-ester (Z)-acyclic adenine ProTide (192.c)



This ProTide was prepared according to the Standard Procedure D, using *t*BuMgCl (1M in THF, 0.46 mL, 0.46 mmol, 2eq), (*Z*)-acyclic adenine (**188.b**) (0.05 mg, 0.23 mmol, 1.0 eq) in anh. THF (0.70 mL), and *α*-naphthyl *L*-Alanine-benzyl ester phosphochloridate (**14.f**) (0.19 g, 0.46 mmol, 2.0 eq) in anh. THF (0.70 mL). After overnight stirring at RT, the solvent was removed under reduced pressure and the residue was purified by flash chromatography, using $CHCl_3$/MeOH (6%) as eluents, and preparative chromatography to yield the desired molecule (0.088 g, 0.15 mmol, 65%).

HPLC (MeOH/H_2O): Rt = 26.29 min, 26.59 min

HPLC (ACN/H_2O): Rt = 17.15 min

MS (TOF ES^+): 587.22 ($M + H^+$), 609.20 ($M + Na^+$).

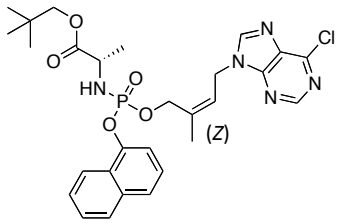
^1H NMR (500 MHz, MeOD) δ_H (ppm): 8.33 & 8.29 (2s, 2H, H-2 base diastereoisomers), 8.08-8.05 (m, 2H, H-4 Ar diastereoisomers), 7.84-7.79 (m, 6H, H-6 naphthyl, 2 x CH meta Ph diastereoisomers), 7.71 (bs, 2H, H-8 base diastereoisomers), 7.66 (d, 2H, J = 8.0 Hz, H-9 Ar diastereoisomers), 7.55-7.42 (m, 12H, 2 x CH ortho Ph, 2 x CH para Ph, H-2, H-7, H-8 Ar diastereoisomers), 7.31 (m, 2H, H-3 Ar diastereoisomers), 5.63 & 6.58 (2t, 2H, J = 6.5 Hz, 6.0 Hz, CHCH$_2$-base diastereoisomers), 5.01 & 4.99 (AB system, 2H, J_{AB} = 8.5 Hz, OCH$_2$Ph), 4.97 & 4.86 (AB system, 2H, J_{AB} = 8.0 Hz, OCH$_2$Ph), 4.75-4.69 (m, 4H, CHCH$_2$-base diastereoisomers), 4.59-4.40 (m, 4H, C(CH$_3$)CH$_2$OP diastereoisomers), 4.10-4.02 (m, 2H, NHCH(CH$_3$)CO diastereoisomers), 1.73 & 1.69 (2s, 6H, C(CH$_3$)CH$_2$OP diastereoisomers), 1.37-1.21 (m, 6H, NHCH(CH$_3$)CO diastereoisomers).

^{13}C NMR (125 MHz, MeOD) δ_c (ppm): 173.61 (d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 6.4 Hz, NHCH(CH$_3$)CO), 173.53 (d, $J^3_{P\text{-}N\text{-}C\text{-}C}$ = 6.4 Hz, NHCH(CH$_3$)CO), 155.52 & 155.45 (2s, C-6 base diastereoisomers), 152.96 & 152.44 (2s, C-2 base diastereoisomers, 149.73 & 149.54 (2s, C-4 base diastereoisomers), 146.60 & 146.56 (2s, C-1 Ar diastereoisomers), 139.80 & 139.11 (2s, C-8 base diastereoisomers), 136.56 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 3.8 Hz, C(CH$_3$)CH$_2$OP), 136.51 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 2.5 Hz, C(CH$_3$)CH$_2$OP), 135.20 & 135.15 (2s, C

ipso Ph diastereoisomers), 134.71 & 134.26 (2s, C-5 Ar diastereoisomers), 128.61 & 128.56 & 128.49 & 128.42 (4s, C meta, C ortho Ph diastereoisomers), 128.19 & 128.14 (2s, C para Ph diastereoisomers), 127.76 & 127.73 (2s, C-6 Ar diastereoismers), 126.49 & 126.41 (2s, C-10 Ar diastereoismers), 126.38 & 128.09 (2s, C-7 Ar diastereoismers), 126.23 & 126.03 (2s, C-8 Ar diastereoismers), 125.49 & 125.21 (2s, C-3 Ar diastereoismers), 124.81 & 124.42 (2s, C-9 Ar diastereoismers), 121.43 & 121.40 (2s, $\underline{C}HCH_2$-base diastereoisomers), 120.62 & 120.46 (2s, C4 Ar diastereoismers), 119.67 & 119.42 (2s, C-5 base diastereoismers), 115.32 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 2.5 Hz, C-2 Ar), 115.28 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 2.5 Hz, C-2 Ar), 70.59 (d, $J^2_{P\text{-}O\text{-}C}$ = 3.8 Hz, $C(CH_3)\underline{C}H_2OP$), 70.54 (d, $J^2_{P\text{-}O\text{-}C}$ = 2.5 Hz, $C(CH_3)\underline{C}H_2OP$), 67.76 & 67.25 (2s, $COO\underline{C}H_2Ph$ diastereoisomers), 50.54 (d, $J^2_{P\text{-}N\text{-}C}$ = 2.5 Hz, $N\underline{C}H(CH_3)CO$), 50.42 (d, $J^2_{P\text{-}N\text{-}C}$ = 3.5 Hz, $N\underline{C}H(CH_3)CO$), 41.09 & 41.89 (2s, $CH\underline{C}H_2$-base diastereoisomers), 21.23 & 21.19 (2s, $NCH(\underline{C}H_3)CO$ diastereoisomers), 13.81 & 13.77 (2s, $C(\underline{C}H_3)CH_2OP$ diastereoisomers).

^{31}P NMR (202 MHz, MeOD) δ_P (ppm)**:** 4.11 (s, 50%), 3.72 (s, 50%).

Preparation of α-naphthyl L-Alanine-neopentyl-ester (Z)-acyclic 6-chloro purine ProTide (193.a)



This ProTide was prepared according to the Standard Procedure D, using *t*BuMgCl (1M in THF, 0.42 mL, 0.42 mmol, 2eq), (*Z*)-acyclic 6-chloro purine (**189**) (0.050 mg, 0.21 mmol, 1.0 eq) in anh. THF (0.65 mL), and *α*-naphthyl *L*-Alanine-neopentyl ester phosphochloridate (**14.d**) (0.161 g, 0.42 mmol, 2.0 eq) in anh. THF (0.65 mL). After overnight stirring at RT, the solvent was removed under reduced pressure and the residue was purified by flash chromatography, using $CHCl_3$/MeOH (1.8%) as eluents, and preparative chromatography to yield the desired molecule (0.002 g, 0.002 mmol, 2%).

HPLC (MeOH/H_2O): Rt = 24.12 min, 24.39 min

HPLC (ACN/H_2O): Rt = 17.72 min

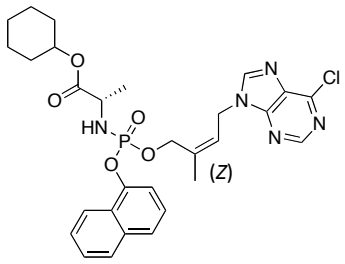
MS (TOF ES^+): 609.18 ($M + Na^+$).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.69 & 8.68 (2s, 2H, H-2 base diastereoisomers), 8.45-8.43 (m, 2H, H-9 Ar diastereoisomers), 8.10-8.07 (m, 2H, H-6 Ar diastereoisomers), 7.90 & 7.89 (2s, 2H, H-8 base diastereoisomers), 7.82-7.70 (m, 2H, H-7 Ar diastereoisomers), 7.67-7.63 (m, 2H, H-8 Ar diastereoisomers), 7.60-7.57 (m, 2H, H-2 Ar diastereoisomers), 7.48-7.43 (m, 2H, H-4 Ar diastereoisomers), 7.36-7.33 (m, 2H, H-3 Ar diastereoisomers), 5.56-5.54 (m, 2H, $\underline{CH}CH_2$-base diastereoisomers), 4.80-4.77 (m, 4H, $CH\underline{CH_2}$-base diastereoisomers), 4.65-4.56 (m, 4H, $C(CH_3)\underline{CH_2}OP$ diastereoisomers), 4.18-4.03 (2m, 2H, $NH\underline{CH}(CH_3)CO$ diastereoisomers), 3.80-3.76 & 3.74-3.72 (2m, 4H, $O\underline{CH_2}C(CH_3)_3$ diastereoisomers), 1.91-1.90 & 1.88-1.84 (m, 6H, $C(\underline{CH_3})CH_2OP$ diastereoisomers), 1.37-1.31 (m, 6H, $NHCH(\underline{CH_3})CO$ diastereoisomers), 0.90 & 0.89 (2s, 18H, $OCH_2C(\underline{CH_3})_3$ diastereoisomers).

^{13}C NMR (125 MHz, $CDCl_3$) δ_c (ppm): 172.54 & 172.43 (2s, $NHCH(CH_3)\underline{C}O$ diastereoisomers), 156.24 & 156.18 (s, C-6 base diastereoisomers), 152.58 & 152.25 (2s, C-2 base diastereoisomers), 148.72 & 148.62 (2s, C-4 base diastereoisomers), 146.86 & 146.72 (2s, C-1 Ar diastereoisomers), 145.28 & 145.01 (2s, C-8 base diastereoisomers), 138.45 & 137.89 (2s, $\underline{C}(CH_3)CH_2OP$), 136.34 & 136.18 (2s, C-5 Ar diastereoisomers), 128.25 & 128.07 (2s, C-6 Ar diastereoisomers), 127.65 & 127.32 (2s, C-10 Ar diastereoisomers), 125.45 & 125.19 (2s, C-7 Ar diastereoisomers), 125.41 & 125.22 (2s, C-8 Ar diastereoisomers), 124.36 & 124.14 (2s, C-3 Ar diastereoisomers), 124.98 & 124.13 (2s, C-9 Ar diastereoisomers), 122.52 & 121.98 (2s, $\underline{C}HCH_2$-base diastereoisomers), 120.88 & 120.72 (2s, C-4 Ar diastereoisomers), 118.42 & 118.13 (2s, C-5 base diastereoisomers), 115.19 & 115.11 (2s, C-2 Ar diastereoisomers), 74.67 & 74.45 (2s, $O\underline{C}H_2C(CH_3)_3$ diastereoisomers), 72.13 & 72.01 (2s, $C(CH_3)\underline{C}H_2OP$ diastereoisomers), 51.20 & 51.09 (2s, $NH\underline{C}H(CH_3)CO$ diastereoisomers), 40.95 & 40.23 (2s, $CH\underline{C}H_2$-base diastereoisomers), 31.56 & 31.34 (2s, $COOCH_2\underline{C}(CH_3)_3$ diastereoisomers), 26.78 & 26.45 (2s, $COOCH_2C(\underline{C}H_3)_3$ diastereoisomers), 21.02 & 20.88 (2s, $NHCH(\underline{C}H_3)CO$ diastereoisomers), 14.02 & 13.86 (2s, $C(\underline{C}H_3)CH_2OP$ diastereoisomers).

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm)**:** 2.82 (s, 36%), 2.63 (s, 64%).

Preparation of α-naphthyl L-Alanine-cyclohexyl-ester (Z)-acyclic 6-chloro purine ProTide (193.b)



This ProTide was prepared according to the Standard Procedure D, using *t*BuMgCl (1M in THF, 0.42 mL, 0.42 mmol, 2eq), (*Z*)-acyclic 6-chloro purine (**189**) (0.050 mg, 0.21 mmol, 1.0 eq) in anh. THF (0.65 mL), and *α*-naphthyl *L*-Alanine-cyclohexyl ester phosphochloridate (**14.e**) (0.166 g, 0.42 mmol, 2.0 eq) in anh. THF (0.65 mL). After overnight stirring at RT, the solvent was removed under reduced pressure and the residue was purified by flash chromatography, using $CHCl_3$/MeOH (1.8%) as eluents, and preparative chromatography to yield the desired molecule (0.025 g, 0.041 mmol, 20%).

HPLC ($MeOH/H_2O$): Rt = 24.28 min, 24.51 min.

HPLC (ACN/H_2O): Rt = 19.28 min.

MS (TOF ES^+): 621.22 ($M + Na^+$).

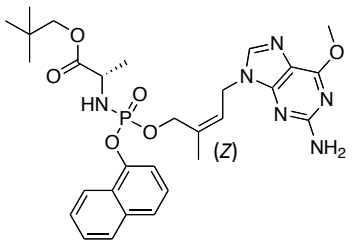
^1H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.75 & 8.74 (2s, 2H, H-2 base diastereoisomers), 8.10-8.07 (m, 2H, H-4 Ar diastereoisomers), 8.01 (s, 2H, H-8 base diastereoisomers), 7.82-7.80 (m, 2H, H-6 Ar diastereoisomers), 7.63 (d, 2H, *J* = 8.2 Hz, H-9 Ar diastereoisomers), 7.52-7.47 (m, 6H, H-2, H-7, H-8 Ar diastereoisomers), 7.37 (m, 2H, H-3 Ar diastereoisomers), 5.58-5.55 (m, 2H, $\underline{CH}CH_2$-base diastereoisomers), 4.84-4.83 (m, 4H, $CH\underline{CH_2}$-base diastereoisomers), 4.78-4.71 (m, 2H, NH diastereoisomers), 4.62-4.48 (m, 4H, $C(CH_3)\underline{CH_2}OP$ diastereoisomers), 4.13-4.04 (m, 2H, CH cHex diastereoisomers), 3.94-3.88 (m, 2H, $NH\underline{CH}(CH_3)CO$ diastereoisomers), 1.83 & 1.82 (2s, 6H, $C(\underline{CH_3})CH_2OP$ diastereoisomers), 1.79-1.54 (m, 16H, 2 x CH_2 meta cHex, CH_2 para cHex diastereoisomers, 2 x CH_2 ortho cHex), 1.40 (d, 6H, *J* = 7.1 Hz, $NHCH(\underline{CH_3})CO$), 1.36 (d, 6H, *J* = 7.1 Hz, $NHCH(\underline{CH_3})CO$), 1.34-1.28 (m, 4H, 2 x CH_2 ortho cHex).

^{13}C NMR (125 MHz, $CDCl_3$) δ_c (ppm): 173.45 & 173.36 (2s, $NHCH(CH_3)\underline{C}O$ diastereoisomers), 155.34 & 155.16 (2s, C-6 base diastereoisomers), 151.95 & 151.23 (2s, C-2 base diastereoisomers), 147.47 & 147.12 (2s, C-4 base diastereoisomers), 146.55 & 146.09 (2s, C-1 Ar diastereoisomers), 144.62 & 144.31 (2s, C-8 base

diastereoisomers), 137.87 & 137.36 (2s, C(CH$_3$)CH$_2$OP diastereoisomers), 135.78 & 135.42 (2s, C-5 Ar diastereoisomers), 127.82 & 127.65 (2s, C-6 Ar diastereoisomers), 126.55 & 126.36 (2s, C-10 Ar diastereoisomers), 126.27 & 126.19 (2s, C-7 Ar diastereoisomers), 126.14 & 126.04 (2s, C-8 Ar diastereoisomers), 125.50 & 125.19 (2s, C-3 Ar diastereoisomers), 124.83 & 124.32 (2s, C-9 Ar diastereoisomers), 121.46 & 121.40 (2s, CHCH$_2$-base diastereoisomers), 120.48 & 120.14 (2s, C-4 Ar diastereoisomers), 119.24 & 119.07 (2s, C-5 base diastereoisomers), 115.20 & 115.16 (2s, C-2 Ar diastereoisomers), 74.82 & 74.12 (2s, C ipso cHex diastereoisomers), 70.10 & 69.94 (2s, C(CH$_3$)CH$_2$OP diastereoisomers), 50.20 & 50.03 (2s, NCH(CH$_3$)CO diastereoisomers), 41.19 & 41.02 (2s, CHCH$_2$-base diastereoisomers), 31.31 & 31.27 (2s, 2 x CH$_2$ ortho cHex diastereoisomers), 25.20 & 25.11 (2s, CH$_2$ para cHex diastereoisomers), 21.25 & 21.19 (2s, 2 x CH$_2$ meta cHex diastereoisomers), 13.86 & 13.67 (2s, C(CH$_3$)CH$_2$OP).

^{31}P NMR (202 MHz, CDCl$_3$) δ_P (ppm)**:** 2.83 (s, 36%), 2.69 (s, 64%).

Preparation of α-naphthyl L-Alanine-neopentyl-ester (Z)-acyclic 6-O-methyl guanine ProTide (194.a)



This ProTide was prepared according to the Standard Procedure D, using *t*BuMgCl (1M in THF, 0.28 mL, 0.28 mmol, 2eq), (*Z*)-acyclic 6-*O*-methyl guanine (**190.b**) (0.035 mg, 0.14 mmol, 1.0 eq) in anh. THF (0.41 mL), and *α*-naphthyl *L*-Alanine-neopentyl ester phosphochloridate (**14.d**) (0.108 g, 0.28 mmol, 2.0 eq) anh. THF (0.41 mL). After overnight stirring at RT, the solvent was removed under reduced pressure and the residue was purified by flash chromatography, using CHCl$_3$/MeOH (2%) as eluents, and preparative chromatography to yield the desired molecule (0.039 g, 0.065 mmol, 47%).

HPLC (MeOH/H$_2$O): Rt = 22.92 min, 23.13 min

HPLC (ACN/H$_2$O): Rt = 17.44 min

MS (TOF ES$^+$): 597.29 (M + H$^+$), 619.27 (M + Na$^+$).

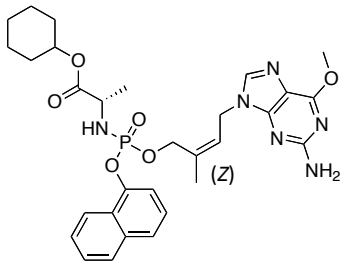
^{1}H NMR (500 MHz, CDCl$_3$) δ_H (ppm): 8.26-8.24 (m, 2H, H-8 base diastereoisomers), 8.20-8.18 (m, 2H, H-4 Ar diastereoisomers), 7.85-7.82 (m, 2H, H-8 Ar

diastereoisomers), 7.77-7.76 (m, 1H, H-9 Ar), 7.59-7.56 (m, 1H, H-9 Ar), 7.50-7.48 (m, 2H, H-7 Ar diastereoisomers), 7.45-7.39 (m, 4H, H-6, H-3 Ar diastereoisomers), 6.83 & 6.83 (2d, 2H, J = 7.0 Hz, 7.5 Hz, H-2 Ar diastereoisomers), 5.69-5.64 (m, 1H, CHCH$_2$-base), 5.56-5.51 (m, 1H, CHCH$_2$-base), 4.79 (d, 2H, J = 7.0 Hz, CHCH$_2$-base), 4.72 (d, 2H, J = 6.5 Hz, CHCH$_2$-base), 4.58 (s, 2H, C(CH$_3$)CH$_2$OP), 4.45 (s, 2H, C(CH$_3$)CH$_2$OP), 4.02 & 4.00 (2s, 6-*O*CH$_3$ base diastereoisomers), 3.76-3.67 (m, 4H, OCH$_2$C(CH$_3$)$_3$ diastereoisomers), 3.57-3.54 (m, 2H, NHCH(CH$_3$)CO diastereoisomers), 1.95 & 1.94 (2s, 6H, C(CH$_3$)CH$_2$OP diastereoisomers), 1.22 (d, 3H, J = 7.0 Hz, NHCH(CH$_3$)CO), 1.20 (d, 3H, J = 7.0 Hz, NHCH(CH$_3$)CO), 0.92 & 0.90 (2s, OCH$_2$C(CH$_3$)$_3$ diastereoisomers).

^{13}C NMR (125 MHz, $CDCl_3$) δ_c (ppm): 171.96 & 171.65 (2s, NHCH(CH$_3$)CO diastereoisomers), 161.20 & 161.14 (2s, C-6 base diastereoisomers), 159.14 & 159.02 (2s, C-2 base diastereoisomers), 153.98 & 153.92 (s, C-4 base diastereoisomers), 148.34 & 148.12 (2s, C-1 Ar diastereoisomers), 138.72 & 138.62 (2s, C-8 base diastereoisomers), 135.43 & 135.15 (2s, C(CH$_3$)CH$_2$OP diastereoisomers), 134.28 & 134.12 (2s, C-5 Ar diastereoisomers), 126.63 & 126.21 (2s, C-6 Ar diastereoisomers), 126.12 & 126.01 (2s, C-10 Ar diastereoisomers), 125.98 & 125.53 (2s, C-7 Ar diastereoisomers), 125.16 & 125.06 (2s, C-8 Ar diastereoisomers), 124.83 & 124.32 (2s, C-3 Ar diastereoisomers), 124.42 & 124.24 (2s, C-9 Ar diastereoisomers), 121.54 & 121.18 (2s, CHCH$_2$-base diastereoisomers), 120.97 & 120.86 (2s, C-4 Ar diastereoisomers), 115.22 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 3.5 Hz, C-2 Ar), 115.14 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 3.3 Hz, C-2 Ar), 104.83 & 104.67 (2s, C-5 base diastereoisomers), 82.10 & 81.89 (2s, OCH$_2$C(CH$_3$)$_3$ diastereoisomers), 70.32 (d, $J^2_{P\text{-}O\text{-}C}$ = 5.5 Hz, C(CH$_3$)CH$_2$OP), 70.15 (d, , $J^2_{P\text{-}O\text{-}C}$ = 5.0 Hz, C(CH$_3$)CH$_2$OP), 53.86 & 53.79 (2s, 6-*O*CH$_3$ base diastereoisomers), 50.43 (d, $J^2_{P\text{-}N\text{-}C}$ = 8.3 Hz, NHCH(CH$_3$)CO), 50.37 (d, $J^2_{P\text{-}N\text{-}C}$ = 8.0 Hz, NHCH(CH$_3$)CO), 40.84 & 40.64 (2s, CHCH$_2$-base diastereoisomers), 30.98 & 30.76 (2s, OCH$_2$C(CH$_3$)$_3$ diastereoisomers), 26.57 & 26.19 (2s, OCH$_2$C(CH$_3$)$_3$ diastereoisomers), 20.96 & 20.93 (2s, NHCH(CH$_3$)CO diastereoisomers), 13.64 & 13.60 (2s, C(CH$_3$)CH$_2$OP diastereoisomers).

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm)**:** 4.23(s, 39%), 3.91 (s, 61%).

Preparation of α-naphthyl L-Alanine-cyclohexyl-ester (Z)-acyclic 6-O-methyl guanine ProTide (194.b)



This ProTide was prepared according to the Standard Procedure D, using *t*BuMgCl (1M in THF, 0.28 mL, 0.28 mmol, 2eq), (*Z*)-acyclic 6-*O*-methyl guanine (**190.b**) (0.035 mg, 0.14 mmol, 1.0 eq) in anh. THF (0.41 mL), and *α*-naphthyl *L*-Alanine-cyclohexyl ester phosphochloridate (**14.e**) (0.112 g, 0.28 mmol, 2.0 eq) anh. THF (0.41 mL). After overnight stirring at RT, the solvent was removed under reduced pressure and the residue was purified by flash chromatography, using $CHCl_3$/MeOH (2.5%) as eluents, and preparative chromatography to yield the desired molecule (0.037 g, 0.061 mmol, 44%).

HPLC (MeOH/H_2O): Rt = 23.25 min, 23.48 min

HPLC (ACN/H_2O): Rt = 17.32 min

MS (TOF ES^+): 609.29 ($M + H^+$), 631.28 ($M + Na^+$).

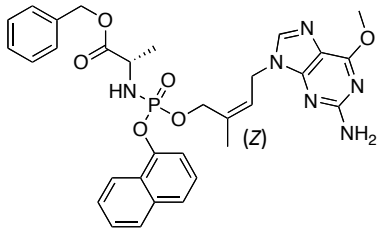
^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.26-8.24 (m, 2H, H-8 base diastereoisomers), 8.20-8.18 (m, 2H, H-4 Ar diastereoisomers), 7.85-7.83 (m, 2H, H-8 Ar diastereoisomers), 7.77-7.76 (m, 1H, H-9 Ar), 7.60-7.55 (m, 1H, H-9 Ar), 7.50-7.48 (m, 1H, H-7 Ar), 7.45-7.39 (m, 3H, H-6 Ar diastereoisomers, H-7 Ar), 7.34-7.25 (m, 2H, H-3 Ar diastereoisomers), 6.83 (d, 1H, *J* = 7.5 Hz, H-2 Ar), 6.81 (d, 1H, *J* = 7.5 Hz, H-2 Ar), 5.78-5.76 & 5.74-5.71 (2m, 2H, CHCH$_2$-base diastereoisomers), 4.81-4.79 (m, 2H, CHCH$_2$-base diastereoisomers), 4.45-4.40 (m, 4H, C(CH_3)CH_2OP diastereoisomers), 4.05 & 4.03 (2s, 6-*O*CH_3 base diastereoisomers), 3.99-3.96 (m, 2H, CH cHex diastereoisomers), 3.87-3.82 (m, 2H, NHCH(CH_3)CO diastereoisomers), 1.95 & 1.94 (2s, 6H, C(CH_3)CH_2OP diastereoisomers), 1.91-1.89 (m, 8H, 2 x CH_2 ortho cHex diastereoisomers), 1.37-1.31 (m, 12H, 2 x CH_2 meta, CH_2 para cHex diastereoisomers), 1.22 (d, 3H, *J* = 7.0 Hz, NHCH(CH_3)CO), 1.20 (2 d, 3H, *J* = 7.0 Hz, NHCH(CH_3)CO).

^{13}C NMR (125 MHz, $CDCl_3$) δ_c (ppm): 171.90 & 171.74 (2s, NHCH(CH_3)CO diastereoisomers), 161.60 & 161.43 (2s, C-6 base diastereoisomers), 159.41 & 159.32 (2s, C-2 base diastereoisomers), 153.54 & 153.19 (2s, C-4 base diastereoisomers),

148.24 & 148.08 (2s, C-1 Ar diastereoisomers), 138.77 & 138.41 (2s, C-8 base diastereoisomers), 135.87 & 135.57 (2s, C(CH$_3$)CH$_2$OP diastereoisomers), 134.68 & 134.39 (2s, C-5 Ar diastereoisomers), 128.43 & 128.18 (2s, C-6 Ar diastereoisomers), 126.31 & 126.30 (2s, C-10 Ar diastereoisomers), 126.19 & 125.98 (2s, C-7 Ar diastereoisomers), 125.46 & 125.21 (2s, C-8 Ar diastereoisomers), 124.62 & 124.24 (2s, C-9 Ar diastereoisomers), 123.15 & 123.04 (2s, C-3 Ar diastereoisomers), 121.40 & 121.09 (2s, CHCH$_2$-base diastereoisomers), 120.00 & 119.92 (2s, C-4 Ar diastereoisomers), 115.32 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 3.2 Hz, C-2 Ar), 115.20 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 3.2 Hz, C-2 Ar), 108.87 & 108.52 (2s, C-5 base diastereoisomers), 70.73 (d, $J^2_{P\text{-}O\text{-}C}$ = 5.0 Hz, C(CH$_3$)CH$_2$OP), 70.65 (d, $J^2_{P\text{-}O\text{-}C}$ = 5.1 Hz, C(CH$_3$)CH$_2$OP), 74.12 & 74.01 (2s, C ipso cHex diastereoisomers), 54.18 & 54.12 (2s, 6-*O*CH$_3$ diastereoisomers), 49.98 (d, $J^2_{P\text{-}N\text{-}C}$ = 8.0 Hz, NHCH(CH$_3$)CO), 49.68 (d, $J^2_{P\text{-}N\text{-}C}$ = 8.2 Hz, NHCH(CH$_3$)CO), 41.60 & 41.25 (2s, CHCH$_2$-base), 30.75 & 30.52 (2s, 2 x CH$_2$ ortho cHex diastereoisomers), 30.43 & 30.12 (2s, CH$_2$ para cHex diastereoisomers), 23.73 & 23.41 (2s, 2 x CH$_2$ meta cHex diastereoisomers), 20.50 & 20.34 (2s, NHCH(CH$_3$)CO diastereoisomers), 13.86 & 13.21 (2s, C(CH$_3$)CH$_2$OP diastereoisomers).

^{31}P NMR (202 MHz, CDCl$_3$) δ_P (ppm)**:** 3.09(s, 43%), 2.90 (s, 57%).

Preparation of α-naphthyl L-Alanine-benzyl-ester (Z)-acyclic 6-O-methyl guanine ProTide (194.c)



This ProTide was prepared according to the Standard Procedure D, using *t*BuMgCl (1M in THF, 0.24 mL, 0.24 mmol, 2eq), (*Z*)-acyclic 6-*O*-methyl guanine (**190.b**) (0.030 mg, 0.12 mmol, 1.0 eq) in anh. THF (0.29 mL). and *α*-naphthyl *L*-Alanine-benzyl ester phosphochloridate (**14.f**) (0.097 g, 0.24 mmol, 2.0 eq) anh. THF (0.30 mL). After overnight stirring at RT, the solvent was removed under reduced pressure and the residue was purified by flash chromatography, using CHCl$_3$/MeOH (3%) as eluents, and preparative chromatography to yield the desired molecule (0.033 g, 0.053 mmol, 44%).

HPLC (MeOH/H$_2$O): Rt = 22.00 min, 22.29 min

HPLC (ACN/H$_2$O): Rt = 16.33 min

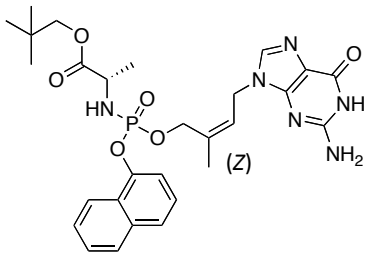
MS (TOF ES$^+$): 617.26 (M + H$^+$), 639.25 (M + Na$^+$).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 8.09-8.06 (m, 4H, H-8 base, H-4 Ar diastereoisomers), 7.82-7.80 (s, 2H, 2 x CH meta Ph), 7.65-7.20 (m, 4H, H-8, H-9 Ar diastereoisomers), 7.49-7.47 (m, 6H, H-2, H-6 Ar diastereoisomers, 2 x CH meta Ph), 7.37-7.24 (m, 10H, H-7, H-3 Ar, 2 x CH ortho, CH para Ph diastereoisomers), 5.60 (td, 1H, *J* = 1.5 Hz, 7.0 Hz, CHCH$_2$-base), 5.55 (td, *J* = 1.5 Hz, 7.4 Hz, CHCH$_2$-base), 5.09 (s, 2H, OCH$_2$Ph), 4.96 (s, 2H, OCH$_2$Phe), 4.61-4.57 (m, 4H, CHCH$_2$-base diastereoisomers), 4.53-4.41 (m, 4H, C(CH$_3$)CH$_2$OP diastereoisomers), 4.18-4.11 (m, 2H, NHCH(CH$_3$)CO diastereoisomers), 4.08 & 4.05 (2s, 6H, 6-*O*CH$_3$ diastereoisomers), 1.76 & 1.72 (2s, 6H, C(CH$_3$)CH$_2$OP diastereoisomers), 1.38 (d, 3H, *J* = 7.0 Hz, NHCH(CH$_3$)CO), 1.35 (d, 3H, *J* = 7.5 Hz, NHCH(CH$_3$)CO).

^{13}C NMR (125 MHz, $CDCl_3$) δ_c (ppm): 171.90 & 171.74 (2s, NHCH(CH$_3$)CO), 161.60 & 161.13 (2s, C-6 base diastereoisomers), 159.41 & 159.26 (2s, C-2 base diastereoisomers), 153.54 & 153.36 (2s, C-4 base diastereoisomers), 148.24 & 148.02 (2s, C-1 Ar diastereoisomers), 138.77 & 138.72 (2s, C-8 base diastereoisomers), 135.87 & 135.52 (2s, C(CH$_3$)CH$_2$OP diastereoisomers), 135.19 & 135.01 (2s, C ipso Ph diastereoisomers), 134.68 & 134.41 (2s, C-5 Ar diastereoisomers), 128.61 & 128.56 & 128.49 & 128.42 (4s, C meta, C ortho Ph diastereoisomers), 128.19 & 128.14 (2s, C para Ph diastereoisomers), 126.56 & 126.11 (2s, C-10 Ar diastereoisomers), 126.31 & 126.30 (2s, C-6 Ar diastereoisomers), 126.11 & 125.98 (2s, C-7 Ar diastereoisomers), 125.49 & 125.12 (2s, C-8 Ar diastereoisomers), 124.83 & 124.68 (2s, C-9 Ar diastereoisomers), 124.61 & 124.42 (2s, C-3 Ar diastereoisomers), 121.83 & 121.52 (2s, C-4 Ar diastereoisomers), 121.40 & 121.09 (2s, CHCH$_2$-base diastereoisomers), 115.32 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 3.3 Hz, C-2 Ar), 115.20 (d, $J^3_{P\text{-}O\text{-}C\text{-}C}$ = 3.3 Hz, C-2 Ar), 104.38 & 104.17 (2s, C-5 base diastereoisomers), 70.73 (d, $J^2_{P\text{-}O\text{-}C}$ = 5.2 Hz, C(CH$_3$)CH$_2$OP), 70.65 (d, $J^2_{P\text{-}O\text{-}C}$ = 5.1 Hz, C(CH$_3$)CH$_2$OP), 67.23 & 67.79 (2s, OCH$_2$Ph diastereoisomers), 53.88 & 53.80 (2s, 6-*O*CH$_3$ base diastereoisomers), 50.45 (d, $J^2_{P\text{-}N\text{-}C}$ = 8.3 Hz, NHCH(CH$_3$)CO), 50.41 (d, $J^2_{P\text{-}N\text{-}C}$ = 8.2 Hz, NHCH(CH$_3$)CO), 40.48 & 40.46 (2s, CHCH$_2$-base diastereoisomers), 20.95 & 20.91 (2s, NHCH(CH$_3$)CO diastereoisomers), 13.74 & 13.70 (2s, C(CH$_3$)CH$_2$OP diastereoisomers).

^{31}P NMR (202 MHz, $CDCl_3$) δ_P (ppm)**:** 4.22 (s, 33%), 3.82 (s, 67%).

Preparation of α-naphthyl L-Alanine-neopentyl-ester (Z)-acyclic guanine ProTide (195.a)



To a solution of *α*-naphthyl *L*-Alanine-neopentyl-ester (Z)-acyclic 6-*O*-methyl guanine ProTide (**194.a**) (0.015 g, 0.025 mmol) in anh. ACN (0.84 mL), NaI (0.006 mg, 0.038 mmol, 1.5 eq) was added followed by TMSCl (0.004 mL, 0.038 mmol, 1.5 eq). The reaction was left stirring for 2 hrs at RT and the solvent was evaporated under reduced pressure. The residue was purified by preparative HPLC to recover the desired material (0.002 g, 0.0003 mmol, 2%).

HPLC (MeOH/H_2O): Rt = 21.75 min, 22.00 min

HPLC (ACN/H_2O): Rt = 14.57 min

MS (TOF ES^-): 581.23 (M - H^+), 617.21 (M + Cl^-).

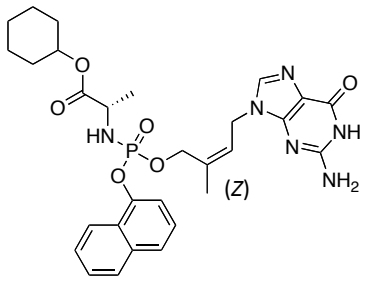
^{1}H NMR (500 MHz, MeOD) δ_H (ppm): 8.15-8.13 (m, 2H, H-9 Ar diastereoisomers), 7.87-7.85 (m, 2H, H-6 Ar diastereoisomers), 7.69-7.67 (m, 2H, H-4 Ar diastereoisomers), 7.61 & 7.59 (2s, 2H, H-8 base diastereoisomers), 7.52-7.49 (m, 4H, H-3, H-8 Ar diastereoisomers), 7.47-7.36 (m, 4H, H-2, H-7 Ar diastereoisomers), 5.55-5.50 (m, 2H, $\underline{CH}CH_2$-base diastereoisomers), 4.66-4.59 (2m, 4H, $CH\underline{CH_2}$-base diastereoisomers), 4.57-4.52 (2m, 4H, $C(CH_3)\underline{CH_2}OP$ diastereoisomers), 4.08-4.05 (m, 2H, $NH\underline{CH}(CH_3)CO$ diastereoisomers), 3.82-3.79 & 3.76-3.74 (2m, 4H, $O\underline{CH_2}C(CH_3)_3$ diastereoisomers), 1.81-1.79 (m, 6H, $C(\underline{CH_3})CH_2OP$ diastereoisomers), 1.38-1.35 (m, 6H, $NHCH(\underline{CH_3})CO$ diastereoisomers), 0.92 & 0.91 (2s, 9H, $OCH_2C(\underline{CH_3})_3$ diastereoisomers).

^{13}C NMR (125 MHz, MeOD) δ_c (ppm): 171.21 & 171.19 (2s, $NHCH(CH_3)\underline{C}O$ diastereoisomers), 156.54 & 156.32 (2s, C-6 base diastereoisomers), 152.75 & 152.12 (2s, C-2 base diastereoisomers), 146.27 & 146.09 (2s, C-4 base diastereoisomers), 145.92 & 145.90 (2s, C-1 Ar diastereoisomers), 142.50 & 142.32 (2s, C-8 base diastereoisomers), 136.64 & 136.44 (2s, $\underline{C}(CH_3)CH_2OP$ diastereoisomers), 135.29 & 135.12 (2s, C-5 Ar diastereoisomers), 127.81 & 127.43 (2s, C-6 Ar diastereoisomers), 126.83 & 126.51 (2s, C-10 Ar diastereoisomers), 126.25 & 126.07 (2s, C-7 Ar diastereoisomers), 125.98 & 125.11 (2s, C-8 Ar diastereoisomers), 125.46 & 125.03 (2s,

C-3 Ar diastereoisomers), 124.85 & 124.12 (2s, C-9 Ar diastereoisomers), 121.47 & 121.42 (2s, CHCH$_2$-base diastereoisomers), 120.44 & 120.21 (2s, C-4 Ar diastereoisomers), 119.02 & 118.87 (2s, C-5 base diastereoisomers), 115.20 & 115.01 (2s, C-2 Ar diastereoisomers), 81.23 & 80.98 (2s, OCH$_2$C(CH$_3$)$_3$ Ar diastereoisomers), 70.01 & 69.92 (2s, C(CH$_3$)CH$_2$OP diastereoisomers), 49.68 & 49.51 (2s, NCH(CH$_3$)CO diastereoisomers), 40.81 & 40.23 (2s, CHCH$_2$-base), 31.29 & 31.01 (2s, OCH$_2$C(CH$_3$)$_3$ diastereoisomers), 26.45 & 26.32 (2s, OCH$_2$C(CH$_3$)$_3$ diastereoisomers), 20.97 & 20.80 (2s, NCH(CH$_3$)CO diastereoisomers), 14.02 & 14.00 (2s, C(CH$_3$)CH$_2$OP diastereoisomers).

^{31}P NMR (202 MHz, MeOD) δ_P (ppm)**:** 4.12 (s, 41%), 3.93 (s, 59%).

Preparation of α-naphthyl L-Alanine-cyclohexyl-ester (Z)-acyclic guanine ProTide (195.b)



To a solution of *α*-naphthyl *L*-Alanine-neopentyl-ester (Z)-acyclic 6-*O*-methyl guanine ProTide (**194.b**) (0.015 g, 0.025 mmol) in anh. ACN (0.84 mL), NaI (0.004 mL, 0.026 mmol, 1.03 eq) was added followed by TMSCl (0.004 mL, 0.026 mmol, 1.03 eq). The reaction was left stirring for 4 hrs at RT and the solvent was evaporated under reduced pressure. The residue was purified by preparative HPLC to recover the desired material (0.002 g, 0.0003 mmol, 2%).

HPLC (MeOH/H$_2$O): Rt = 22.11 min, 22.37 min

HPLC (ACN/H$_2$O): Rt = 15.24 min

MS (TOF ES$^-$): 593.24 (M - H$^+$), 529.21 (M + Cl$^-$).

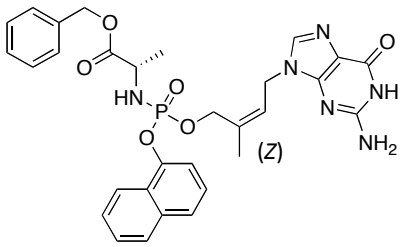
^{1}H NMR (500 MHz, MeOD) δ_H (ppm): 8.13-8.11 (m, 2H, H-9 Ar diastereoisomers), 7.90-7.85 (m, 4H, H-6, H-4 Ar diastereoisomers), 7.69-7.67 (m, 2H, H-8 base diastereoisomers), 7.60-7.59 (m, 2H, H-2 Ar diastereoisomers), 7.52-7.50 (m, 2H, H-7 Ar diastereoisomers), 7.46-7.38 (m, 4H, H-3, H-8 Ar diastereoisomers), 5.54-5.50 (m, 2H, CHCH$_2$-base diastereoisomers), 4.66-4.64 (m, 4H, CHCH$_2$-base diastereoisomers), 4.57-4.52 (m, 4H, C(CH$_3$)CH$_2$OP diastereoisomers), 4.16-4.13 (m, 1H, CH cHex), 4.01-

3.98 (m, 1H, CH cHex), 2.85-2.83 (m, 2H, NHCH(CH$_3$)CO diastereoisomers), 1.81 & 1.79 (2s, 6H, C(CH$_3$)CH$_2$OP diastereoisomers), 1.75-1.50 (m, 16H, 2 x CH$_2$ meta cHex, CH$_2$ para cHex diastereoisomers, 2 x CH$_2$ ortho cHex), 1.35 (d, 3H, *J* = 7.1 Hz, NHCH(CH$_3$)CO), 1.31 (d, 3H, *J* = 7.1 Hz, NHCH(CH$_3$)CO), 0.92-0.90 (m, 4H, CH$_2$ ortho cHex).

^{13}C NMR (125 MHz, MeOD) δ_c (ppm): 171.20 & 171.18 (2s, NHCH(CH$_3$)CO diastereoisomers), 156.64 & 156.23 (2s, C-6 base diastereoisomers), 152.95 & 152.72 (2s, C-2 base diastereoisomers), 146.17 & 146.02 (2s, C-4 base diastereoisomers), 145.95 & 145.93 (2s, C-1 Ar diastereoisomers), 142.52 & 142.16 (s, C-8 base diastereoisomers), 136.67 & 136.46 (2s, C(CH$_3$)CH$_2$OP diastereoisomers), 135.28 & 135.12 (2s, C-5 Ar diastereoisomers), 126.82 & 126.42 (2s, C-6 Ar diastereoisomers), 126.25 & 126.02 (2s, C-10 Ar diastereoisomers), 126.17 & 126.02 (2s, C-7 Ar diastereoisomers), 125.97 & 125.84 (2s, C-8 Ar diastereoisomers), 125.45 & 125.19 (2s, C-3 Ar diastereoisomers), 124.83 & 124.72 (2s, C-9 Ar diastereoisomers), 121.64 & 121.31 (2s, CHCH$_2$-base diastereoisomers), 120.48 & 120.13 (2s, C-4 Ar diastereoisomers), 119.04 & 118.96 (2s, C-5 base diastereoisomers), 115.19 & 115.03 (2s, C-2 Ar diastereoisomers), 74.22 & 74.11 (2s, C ipso cHex diastereoisomers), 69.89 & 69.43 (2s, C(CH$_3$)CH$_2$OP diastereoisomers), 49.97 & 49.65 (2s, NCH(CH$_3$)CO diastereoisomers), 41.20 & 41.15 (2s, CHCH$_2$-base), 31.01 & 30.98 (2s, 2 x CH$_2$ ortho cHex diastereoisomers), 25.19 & 25.12 (2s, CH$_2$ para cHex diastereoisomers), 21.45 & 21.19 (2s, 2 x CH$_2$ meta cHex diastereoisomers), 21.08 & 20.97 (2s, NCH(CH$_3$)CO diastereoisomers), 14.11 & 14.07 (2s, C(CH$_3$)CH$_2$OP diastereoisomers).

^{31}P NMR (202 MHz, MeOD) δ_P (ppm)**:** 4.19 (s, 46%), 3.96 (s, 54%).

Preparation of α-naphthyl L-Alanine-benzyl-ester (Z)-acyclic guanine ProTide (195.c)



To a solution of *L*-Alanine-benzyl-ester (*Z*)-acyclic 6-*O*-methyl guanine ProTide (**194.c**) (0.033 g, 0.054 mmol) in anh. ACN (2.00 mL), NaI (0.012 mg, 0.081 mmol, 1.5 eq) was added, followed by TMSCl (0.017 mL, 0.081 mmol, 1.5 eq). The reaction was

left stirring for 1 hr at RT and the solvent was evaporated under reduced pressure. The residue was purified by preparative HPLC, followed by preparative HPLC to recover the desired molecule (0.0031 g, 0.005 mmol, 10%).

HPLC (MeOH/H_2O): Rt = 25.35 min, 25.62 min

HPLC (ACN/H_2O): Rt = 15.60 min

MS (TOF ES^+): 625.19 ($M + Na^+$).

^1H NMR (500 MHz, MeOD) δ_H (ppm): 8.13 (d, 2H, *J* = 11.2 Hz, H-9 Ar diastereoisomers), 7.87 (d, 2H, *J* = 7.9 Hz, H-6 Ar diastereoisomers), 7.68 (2H, d, *J* = 8.1 Hz, H-4 Ar diastereoisomers), 7.59 & 7.57 (2s, H-8 base diastereoisomers), 7.52-7.35 (m, 8H, 2 x CH meta Ph, H-3, H-8 Ar diastereoisomers), 7.31-7.26 (m, 10H, 2 x CH ortho, CH para Ph, H-2, H-7 Ar diastereoisomers), 5.49-5.47 (m, 2H, $\underline{CH}CH_2$-base diastereoisomers), 5.09 & 5.08 (2s, 4H, $O\underline{CH_2}Ph$ diastereoisomers), 4.63-4.61 (m, 4H, $CH\underline{CH_2}$-base diastereoisomers), 4.49 & 4.48 (2s, 4H, $C(CH_3)\underline{CH_2}OP$ diastereoisomers), 4.06-4.05 (m, 2H, $NH\underline{CH}(CH_3)CO$ diastereoisomers), 2.04 & 2.03 (2s, 6H, $C(\underline{CH_3})CH_2OP$ diastereoisomers), 1.36 & 1.34 (2s, 6H, $NHCH(\underline{CH_3})CO$ diastereoisomers).

^{13}C NMR (125 MHz, MeOD) δ_c (ppm): 171.20 & 171.18 (2s, $NHCH(CH_3)\underline{C}O$ diastereoisomers), 156.64 & 156.42 (2s, C-6 base diastereoisomers), 152.65 & 152.32 (2s, C-2 base diastereoisomers), 146.17 & 146.03 (2s, C-4 base diastereoisomers), 145.95 & 145.93 (2s, C-1 Ar diastereoisomers), 142.52 (2s, C-8 base diastereoisomers), 136.67 & 136.46 (s, $\underline{C}(CH_3)CH_2OP$), 135.28 & 135.12 (2s, C-5 Ar diastereoisomers), 128.76 & 128.51 (2s, C meta Ph diastereoisomers), 127.80 & 127.69 (2s, C para Ph diastereoisomers), 126.82 & 126.62 (2s, C-6 Ar diastereoisomers), 126.57 & 126.43 (2s, C ortho Ph), 126.25 & 126.12 (2s, C-10 Ar diastereoisomers), 126.17 & 126.04 (2s, C-7 Ar diastereoisomers), 125.97 & 125.38 (2s, C-8 Ar diastereoisomers), 125.45 & 125.11 (2s, C-3 Ar diastereoisomers), 124.83 & 124.65 (2s, C-9 Ar diastereoisomers), 121.76 & 121.38 (2s, $\underline{C}HCH_2$-base diastereoisomers), 120.48 & 120.32 (2s, C-4 Ar diastereoisomers), 119.04 & 118.86 (2s, C-5 base diastereoisomers), 115.19 & 115.01 (2s, C-2 Ar diastereoisomers), 69.98 & 69.23 (2s, $C(CH_3)\underline{C}H_2OP$ diastereoisomers), 67.18 & 67.05 (2s, $COO\underline{C}H_2Ph$ diastereoisomers), 49.65 & 49.13 (2s, $N\underline{C}H(CH_3)CO$ diastereoisomers), 40.75 & 40.43 (2s, $CH\underline{CH_2}$-base diastereoisomers), 20.85 & 20.79 (2s, $NCH(\underline{C}H_3)CO$ diastereoisomers) 14.00 & 13.94 (2s, $C(\underline{C}H_3)CH_2OP$ diastereoisomers).

^{31}P NMR (202 MHz, MeOD) δ_P (ppm)**:** 4.10 (s, 44%), 3.71 (s, 56%).

7. Enzymatic assays: standard procedures

Carboxypeptidase Y assays

1 to 5 mg of phosphoramidate/phosphoradiamidate was dissolved in 50 to 200 µL of deuterated solvent (D_2O or d_6-acetone) and 100 to 400 µL of Trizma buffer (pH ~ 7.6). ^{31}P-NMR was recorded as reference (t = 0 min). 0.1 mg of carboxypeptidase Y (purchased from Sigma, EC 3.4.16.1) dissolved in 50 to 200 µL of Trizma buffer (pH ~ 7.6) was added to the previous mixture. ^{31}P-NMR spectra were recoreded overnight (over 14 hours), every 2 to 7 minutes, at RT.

Cell lysate assays

The cell lysate experiments were performed using Huh-7 human hepatoma cells lysate (10^7 cells), which were incubated with the phosphoramidate dissolved in D_2O. ^{31}P-NMR spectra were recoreded overnight (over 14 hours), every hour, at 37 °C.

Adenosine deaminase assays

Stock solution of nucleoside analogue was dissolved in phosphate buffer (pH ~ 7.5, 0.05 M) to reach a final concentration of 44 µM. To a solution of adenosine deaminase from calf intestinal mucosa (solution in 50% glycerol, 0.01 M potassium phosphate, pH ~ 6.0, Sigma-Aldrich), phosphate buffer (pH ~ 7.5, 0.05 M) was added to reach a final volume of 2.0 mL.

For each assay, 1 mL of the solution of 44 µM of nucleoside analogue was place in a 1 cm UV cuvet, and one UV spectrum was recorded over the range of 400 to 220 nm, at 25 °C. 30 to 500 µL of the enzyme solution was added to the cuvet and UV spectra were recorded in 1 min interval for 30 min, and then at every 30 min or every hour, over 4 to hours.

8. Molecular modelling

Molecular modelling was performed using MOE software (version 2008 and 2010) with the crystal structure of cathepsin A enzyme (PNB 1YSC), of human Hint-1 enzyme with AMP co-crystallised structure (PNB 1KPF), and the homology model of adenosine deaminase-like protein-1 (ADAL-1) created by Dr. A. Brancale.

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APPENDIX 1

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Phosphorodiamidates as a Promising New Phosphate Prodrug Motif for Antiviral Drug Discovery: Application to Anti-HCV Agents

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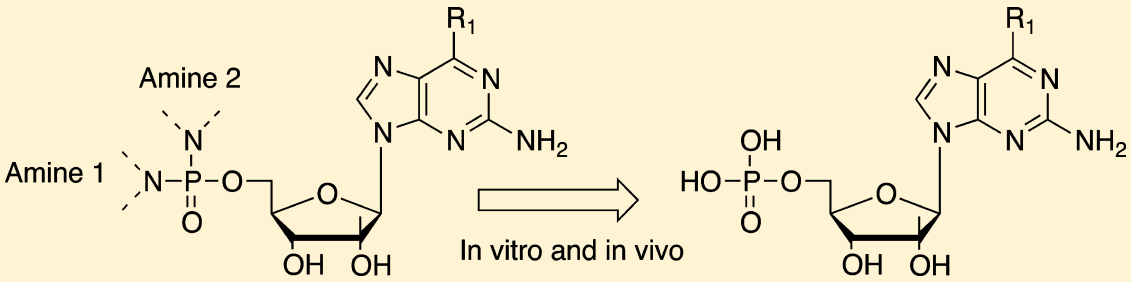
Supporting Information



ABSTRACT: We herein report phosphorodiamidates as a significant new phosphate prodrug motif. Sixty-seven phosphorodiamidates are reported of two 6-*O*-alkyl 2′-*C*-methyl guanosines, with significant variation in the diamidate structure. Both symmetrical and asymmetric phosphorodiamidates are reported, derived from various esterified amino acids, both D and L, and also from various simple amines. All of the compounds were evaluated versus hepatitis C virus in replicon assay, and nanomolar activity levels were observed. Many compounds were noncytotoxic at 100 μM, leading to high antiviral selectivities. The agents are stable in acidic, neutral, and moderately basic media and in selected biological media but show efficient processing by carboxypeptidases and efficiently yield the free nucleoside monophosphate in cells. On the basis of in vitro data, eight leads were selected for additional in vivo evaluation, with the intent of selecting one candidate for progression toward clinical studies. This phosphorodiamidate prodrug method may have broad application outside of HCV and antivirals as it offers many of the advantages of phosphoramidate ProTides but without the chirality issues present in most cases.

INTRODUCTION

There is an urgent ongoing need for improved therapeutic agents for hepatitis C Virus (HCV) infection, with an increasing emphasis on direct acting antivirals (DAAs), and in particular, inhibitors of the viral NS5B RNA polymerase.[1] Nucleoside-based inhibitors of the polymerase are considered particularly valuable on the basis of the high genetic barrier to resistance.[2] Thus, a number of nucleoside modifications (**1**−**4**, Figure 1) have emerged with anti-HCV activity in vitro, and several have progressed to clinical evaluation.

One issue common to all nucleoside analogues, either antiviral or anticancer, is an absolute need for nucleoside kinase-mediated activation to their 5′-monophosphate forms. In some cases, as in the present anti-HCV field, further phosphorylation to the 5′-triphosphate is also required, and in general the first phosphorylation step is considered rate-limiting.[3] With this in mind, and given that the free phosphate derivatives are not considered useful drug entities due to poor cell uptake, a number of phosphate (nucleotide) prodrug motifs have emerged.[4] These have included our aryl phosphoramidate ("ProTide") approach,[5] the amidate diester method of Wagner[6] and McKenna,[7] the lipid diester approach of Hosteller,[8] thioester approaches of Gosselin,[9] cytochrome based methods,[10] and the chemical driven cycloSAL method of Meier.[11] Each of these methods has its strengths and weaknesses. In general, fully blocked prodrugs (ProTides, CycloSAL, etc.) may give better delivery but do often generate a chiral phosphate center leading to isomer issues. Phosphate diester methods avoid the chirality issue but may have delivery challenges.[4] Despite this, several phosphate prodrugs of antiviral nucleosides have progressed into clinical trials. This includes Inhibitex's INX-189[12] (**5**, Figure 2) and Pharmasset's PSI-7977[13] (**6**), both based directly on phosphoramidate ProTides, and Idenix's

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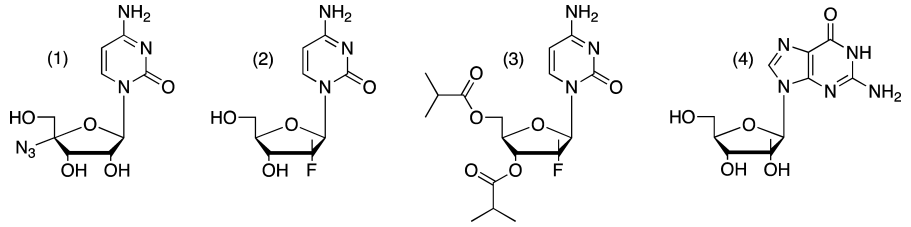


Figure 1. Some anti-HCV nucleoside analogues.

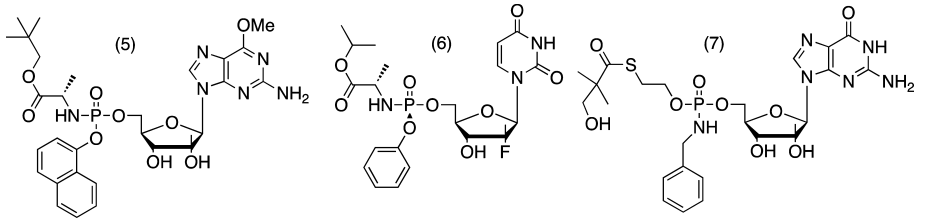
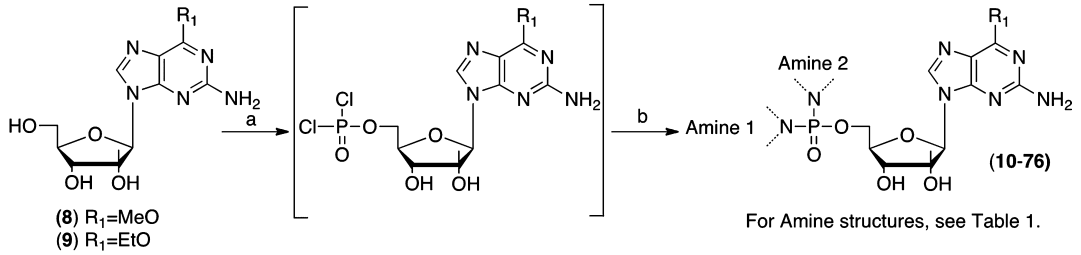


Figure 2. Some anti-HCV phosphate prodrug compounds in clinical trials.

Scheme 1. Synthetic Route to Phosphorodiamidates[a]



[a]Reagents and conditions: (a) Et_3N (1 equiv), $POCl_3$ (1 equiv), dry THF; (b) for symmetrical diamidates, amine (5 equiv), Et_3N (10 equiv), dry DCM; for asymmetric diamidates, amine 1 (1 equiv), Et_3N (2 equiv) then amine 2 (5 equiv), Et_3N (10 equiv), dry DCM.

IDX184[14] (**7**), which is a hybrid phosphoramidate/SATE prodrug. Each of these is now in human trials for efficacy versus HCV.

Most chiral nucleotide prodrugs that have entered into the clinic to date have been progressed as diastereoisomeric mixtures at the phosphate center. NewBiotics' anticancer agent NB1011 is a further example in this category.[15] In general, when tested, both stereoisomers often display similar biological activity in vitro and always release the same pharmacophore after initial metabolism.[16] One exception to this in the HCV field to date is Pharmasset, who has developed a large scale separation technique for their mixed compound PSI-7851, now pursued as the single stereoisomer **6** as above.[13]

With this in mind, we sought to revisit the notion of an achiral phosphate prodrug motif with a phosphoramidate core. In addition, we particularly wanted to formulate a prodrug whose promoieties were nontoxic and preferably natural. Thus our attention turned to phosphorodiamidates. Indeed, over 20 years ago, our group was among the first to report phosphorodiamidate-based nucleotide prodrugs. Thus, seeking to deliver the 5′-monophosphate of the anti-HIV agent AZT into cells, we prepared a series of L-amino acid phosphorodiamidates with a variety of esters.[17] There have since been reports of this motif on phosphonates[18] and non-nucleosides[19] but little further work on nucleosides.[20−22]

In this paper, we report the very promising profile of a family of such phosphorodiamidates of 2′-*C*-methylguanosine and its derivatives, leading toward selection of a clinical candidate.

CHEMISTRY

Following the success of **5** in early clinical trials for HCV[23] and our recent disclosure that alternative 6-alkoxy (and other) groups may be equally effective,[24] we used the 6-alkoxy derivatives of 2′-*C*-methylguanosine as our starting point. These were prepared by methods we have described.[12] Following our much earlier reports on phosphoramidates of AZT,[17] we used a single synthetic route based on treating the unprotected nucleoside with phosphoryl chloride to generate the intermediate dichloridate which was not isolated (Scheme 1). In the first instance, we used the 6-methoxy analogue **8**, the base nucleoside behind **5**. Reaction with $POCl_3$ in THF in the presence of Et_3N for 45 min (when ^{31}P NMR showed no presence of $POCl_3$) followed by addition of an excess of various amino acid esters (or other amines), lead to compounds **10**−**76** as shown in Table 1.

As L-alanine is often a preferred motif in phosphoramidate ProTides and the final putative activating step for these diamidates would be the same loss of amino acid, we thought that L-alanine may be beneficial here, so our first SAR family was symmetrical substituted L-alanines with linear esters methyl-(**10**), ethyl-(**11**), *n*-propyl-(**12**), *n*-butyl-(**13**), and *n*-pentyl-(**14**); branched esters isopropyl-(**15**) and (*R*,*S*)-2-butyl-(**16**), and 3,3-dimethylbutyl-(**17**); followed by cyclic esters cyclobutyl-(**18**), cyclopentyl-(**19**) cyclohexyl-(**20**), and 2-tetrahydropyranyl-(**24**). We have often reported that benzyl esters can be rather effective in ProTides, so we prepared the benzyl-(**21**), *S*-phenethyl-(**22**), 2,4-difluorobenzyl-(**23**), and 2-indanyl-(**25**) L-alanine esters. Given the extremely high potency

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Table 1. Replicon Data and Cytotoxicity of Nucleoside Analogues and Phosphorodiamidates

				EC_{50} (μM)		EC_{90} (μM)		
compd	AA ester/amine	AA ester/amine	nucleoside	av	SD	av	SD	CC_{50} (μM)
4			2′CMeG	2.2	1.4	8.3	7.5	>100
8			6OMe2′CMeG	4.4	2.2	19.4	10.2	>100
9			6OEt2′CMeG	9.5	1.84	24.5	1.78	>100
5	L-Ala OCH_2tBu	NaphO	6OMe2′CMeG	0.01	0.01	0.04	0.02	7.01
10	L-Ala OMe	L-Ala OMe	6OMe2′CMeG	5.90	0.76	>10		>100
11	L-Ala OEt	L-Ala OEt	6OMe2′CMeG	1.20	0.08	4.10	0.09	>100
12	L-Ala OnPr	L-Ala OnPr	6OMe2′CMeG	0.28	0.05	1.20	0.53	>100
13	L-Ala OButyl	L-Ala OButyl	6OMe2′CMeG	0.07	0.01	0.23	0.02	>100
14	L-Ala OPentyl	L-Ala OPentyl	6OMe2′CMeG	0.03	0.01	0.11	0.02	65
15	L-Ala OiPr	L-Ala OiPr	6OMe2′CMeG	0.49	0.18	2.10	0.09	>100
16	L-Ala *O*-(*R*,*S*)-2Bu	L-Ala *O*-(*R*,*S*)-2Bu	6OMe2′CMeG	0.15	0.00	0.53	0.10	>100
17	L-Ala *O*-3,3-dimethylbutyl	L-Ala *O*-3,3-dimethylbutyl	6OMe2′CMeG	0.02	0.01	0.09	0.07	71
18	L-Ala OcBu	L-Ala OcBu	6OMe2′CMeG	0.32	0.05	0.80		>100
19	L-Ala OcPentyl	L-Ala OcPentyl	6OMe2′CMeG	0.06	0.01	0.22	0.03	>100
20	L-Ala OcHx	L-Ala OcHx	6OMe2′CMeG	0.05	0.02	0.19	0.08	>100
21	L-Ala OBn	L-Ala OBn	6OMe2′CMeG	0.49		1.60		>100
22	L-Ala OSPhEt	L-Ala OSPhEt	6OMe2′CMeG	0.49		1.70		88
23	L-Ala *O*-2,4-diFBn	L-Ala *O*-2,4-diFBn	6OMe2′CMeG	0.26	0.21	1.07	0.92	54
24	L-Ala OTHP	L-Ala OTHP	6OMe2′CMeG	13.33	13.02	29.30	15.14	>100
25	L-Ala OIndanol	L-Ala OIndanol	6OMe2′CMeG	0.58	0.39	1.30	0.10	70
26	L-Ala OCH_2tBu	L-Ala OCH_2tBu	6OMe2′CMeG	0.06	0.04	0.20	0.12	>100
27	L-Ala OCH_2iPr	L-Ala OCH_2iPr	6OMe2′CMeG	0.07	0.01	0.26	0.01	>100
28	L-Ala OCH_2cPropyl	L-Ala OCH_2cPropyl	6OMe2′CMeG	0.21	0.00	0.66	0.001	>100
29	D-Ala OCH_2tBu	D-Ala OCH_2tBu	6OMe2′CMeG	0.11	0.03	0.50	0.19	>100
30	L-Asp OMe	L-Asp OMe	6OMe2′CMeG	10.18	3.17	>40		>100
31	L-Asp OBn	L-Asp OBn	6OMe2′CMeG	0.61	0.08	1.90	0.14	>100
32	L-Gly OBn	L-Gly OBn	6OMe2′CMeG	0.60	0.13	1.80	0.09	>100
33	L-Gly OCH_2tBu	L-Gly OCH_2tBu	6OMe2′CMeG	0.13	0.02	0.60	0.20	>100
34	L-Leu OcHx	L-Leu OcHx	6OMe2′CMeG	0.45	0.52	2.00	2.15	20
35	L-Leu OBn	L-Leu OBn	6OMe2′CMeG	0.38	0.02	1.31	0.21	39
36	L-Leu OCH_2tBu	L-Leu OCH_2tBu	6OMe2′CMeG	0.47	0.15	2.40	0.71	27
37	L-Ile OMe	L-Ile OMe	6OMe2′CMeG	5.17	1.94	29.5	2.66	>100
38	L-Ile OcHx	L-Ile OcHx	6OMe2′CMeG	4.00	0.47	7.10	1.16	14
39	L-Ile OBn	L-Ile OBn	6OMe2′CMeG	0.40	0.11	1.56	0.10	24
40	L-Ile OCH_2tBu	L-Ile OCH_2tBu	6OMe2′CMeG	2.90	0.33	6.30	1.36	15
41	L-Met OcHx	L-Met OcHx	6OMe2′CMeG	0.60	0.17	2.60	0.11	51
42	L-Met OBn	L-Met OBn	6OMe2′CMeG	0.25	0.04	0.77	0.24	>100
43	L-Met OCH_2tBu	L-Met OCH_2tBu	6OMe2′CMeG	2.22		5.00		>100
44	L-Phe OcHx	L-Phe OcHx	6OMe2′CMeG	0.50	0.59	2.00	2.20	25
45	L-Phe OBn	L-Phe OBn	6OMe2′CMeG	0.32	0.14	1.35	1.07	67
46	L-Phe OCH_2tBu	L-Phe OCH_2tBu	6OMe2′CMeG	0.05	0.02	0.21	0.12	24
47	L-Pro OBn	L-Pro OBn	6OMe2′CMeG	0.52	0.21	1.80	0.23	>100
48	L-Pro OCH_2tBu	L-Pro OCH_2tBu	6OMe2′CMeG	0.81	0.05	2.00	0.21	56
49	L-Val OcHx	L-Val OcHx	6OMe2′CMeG	2.50	0.35	10.00	0.72	20
50	L-Val OBn	L-Val OBn	6OMe2′CMeG	0.12	0.04	0.66	0.35	49
51	L-Val OCH_2tBu	L-Val OCH_2tBu	6OMe2′CMeG	0.72		2.50		32
52	L-Tyr (tBu) OMe	L-Tyr (tBu) OMe	6OMe2′CMeG	0.11	0.04	0.50	0.10	89
53	L-PhG OcHx	L-PhG OcHx	6OMe2′CMeG	0.32	0.00	1.80	0.04	18
54	L-PhG OCH_2tBu	L-PhG OCH_2tBu	6OMe2′CMeG	0.27	0.03	1.50	0.09	24
55	L-Val-L-Ala OCH_2tBu	L-Val-L-Ala OCH_2tBu	6OMe2′CMeG	0.54	0.26	2.40	0.46	>100
56	*β*-Ala OBn	*β*-Ala OBn	6OMe2′CMeG	3.80	2.03	>10		>100
57	butylamine	butylamine	6OMe2′CMeG	37.80	3.80	>40		>100
58	morpholine	morpholine	6OMe2′CMeG	>100		>100		>100
59	L-Ala OcPentyl	L-Ala OcHx	6OMe2′CMeG	0.04	0.01	0.16	0.03	>100
60	L-Ala OCH_2tBu	L-Ala OBn	6OMe2′CMeG	0.15	0.04	0.40	0.07	>100
61	L-Ala OCH_2tBu	L-Ala OtBu	6OMe2′CMeG	0.15	0.01	0.57	0.08	>100
62	L-Ala OCH_2tBu	L-Ala OcHx	6OMe2′CMeG	0.04	0.01	0.15	0.02	>100
63	L-Ala OCH_2tBu	L-Pro OMe	6OMe2′CMeG	0.49		2.20		>100
64	L-Ala OCH_2tBu	L-Val OMe	6OMe2′CMeG	0.17	0.06	0.69	0.15	>100

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Table 1. continued

compd	AA ester/amine	AA ester/amine	nucleoside	EC_{50} (μM) av	EC_{50} (μM) SD	EC_{90} (μM) av	EC_{90} (μM) SD	CC_{50} (μM)
65	L-Ala OBn	butylamine	6OMe2′CMeG	0.44	0.28	1.68	0.94	>100
66	L-Ala OCH_2tBu	butylamine	6OMe2′CMeG	0.41	0.28	1.50	0.75	>100
67	L-Ala OCH_2tBu	pentylamine	6OMe2′CMeG	0.11	0.02	0.52	0.05	>100
68	L-Ala OCH_2tBu	cyclopropylamine	6OMe2′CMeG	0.87	0.11	4.70	0.45	>100
69	L-Ala OCH_2tBu	BnNH	6OMe2′CMeG	0.43	0.02	1.90	0.11	>100
70	L-Ala OCH_2tBu	PhNH	6OMe2′CMeG	0.27	0.01	0.82	0.00	>100
71	L-Ala OCH_2tBu	NaphNH	6OMe2′CMeG	0.13	0.05	0.56	0.18	>100
72	L-Ala OCH_2tBu	diethylamine	6OMe2′CMeG	3.70		>10		>100
73	L-Ala OCH_2tBu	pyrrolidine	6OMe2′CMeG	0.87	0.02	4.00	0.16	>100
74	L-Ala OcHx	L-Ala OcHx	6OEt2′CMeG	0.05	0.00	0.15	0.02	69
75	L-Ala OBn	L-Ala OBn	6OEt2′CMeG	0.29	0.04	1.00	0.29	62
76	L-Ala OCH_2tBu	L-Ala OCH_2tBu	6OEt2′CMeG	0.04	0.01	0.11	0.04	72

of the neopentyl alanine compound as a naphthyl ProTide (**5**),[12] we were keen to explore the methylene bridged family starting with the neopentyl-(**26**) and 2-methylpropyl-(**27**) compounds and also the methylene cyclopropyl-(**28**).

On the basis of our recent report that the 6-ethoxy group can well substitute for the purine 6-methoxy as a core nucleoside for anti-HCV ProTides,[21] we applied the diamidate method to the *O*6-ethyl-2′-*C*-methylguanosine nucleoside, preparing the symmetrical cyclohexyl-(**74**), benzyl-(**75**), and neopentyl-(**76**) L-alanine analogues.

In aryl phosphoramidate ProTides, we have reported on several occasions the sometimes strong preference, for L-amino acids over D-analogues,[25] and all of the first examples reported here are L-amino acid derived. However, we did synthesize the neopentyl-D-alanine analogue **29** of **8**.

Although L-alanine is generally strongly preferred, other natural and unnatural amino acids are also effective as ProTides,[26] and so we did vary the amino acid motif itself. An attempt was made to consistently select from the same three ester groups, neopentyl, cyclohexyl, and benzyl, for each new amino acid to facilitate comparison. Thus, symmetrical diamidates were prepared from dimethyl-(**30**) and dibenzyl-(**31**) L-aspartic acid, benzyl-(**32**) and neopentyl-(**33**) glycine, cyclohexyl-(**34**), benzyl-(**35**), and neopentyl-(**36**) L-leucine, methyl-(**37**), cyclohexyl-(**38**), benzyl-(**39**), and neopentyl-(**40**) L-isoleucine, cyclohexyl-(**41**), benzyl-(**42**), and neopentyl-(**43**) L-methionine, cyclohexyl-(**44**), benzyl-(**45**), and neopentyl-(**46**) L-phenylalanine, benzyl-(**47**) and neopentyl-(**48**) L-proline, and cyclohexyl-(**49**), benzyl-(**50**), and neopentyl-(**51**) L-valine. The tyrosine methyl ester diamidate was also prepared as its *para*-*O*-*tert*-butyl ether-(**52**). Diamidates of the unnatural amino acid L-phenylglycine were prepared as its cyclohexyl-(**53**) and neopentyl-(**54**) esters. In a prior program on ProTides of d4T for HIV, we reported a complete loss of activity on extending from alanine to β-alanine and beyond,[27] but it was unclear whether similar restrictions would apply here, hence we prepared the symmetrical benzyl-β-alanine diamidate-(**56**).

Finally, in this series, we wondered if the chemistry methodology would extend to a dipeptide and whether such an adduct might be active and so we successfully incorporated an L-valyl-L-alanine neopentyl ester (**55**).

Simple amines have not been found to be useful as the amino component of aryl phosphoramidates, although Idenix has successfully incorporated benzylamine into their clinical analogue IDX184.[14] However, for diamidates, the SAR was unknown, and so we prepared the symmetrical butylamine-(**57**) and morpholinyl-(**58**) diamidates. Notably, from a synthetic perspective, none of the above amino acid and amine variations presented particular challenges although the yields from these reactions were not high and remain unoptimized.

Besides seeking to probe the SAR and potential advantages of symmetrical diamidates, as above, we also wondered if the synthetic method would allow access to asymmetrically mixed diamidates. To do this, we slightly adapted the synthetic route (Scheme 1) to allow the stepwise introduction of two separate amino acids or one amino acid and one amine. To limit the large number of possible combinations, and to facilitate interpretation of the data, one of the amines was generally kept constant as neopentyl L-alanine and variations were made in the second amine. Initially, different L-alanine esters, benzyl-(**60**), *tert*-butyl-(**61**), and cyclohexyl-(**62**), were combined with neopentyl L-alanine, followed by different amino acids methyl L-proline-(**63**) and methyl L-valine-(**64**), and then a number of different simple amines (**65**−**73**). Synthetically, it did not matter much which amine was introduced first. Each of these asymmetric diamidates was isolated as a roughly 1:1 mixture of phosphate diastereoisomers as revealed by ^{31}P NMR and HPLC. No attempt was made to separate the diastereoisomers, and they were tested as mixtures.

In this way, a substantial set of symmetrical and asymmetric phosphorodiamidates of 6-*O*-methyl-2′-*C*-methylguanosine and three derivatives of the 6-*O*-ethyl analogue were prepared and fully characterized. As expected, all of the symmetrical diamidates reported above were observed as one peak by ^{31}P NMR and one signal on analytical HPLC. The ^{31}P NMR shifts of the symmetrical amino aryl diamidates were ca. 13 ppm, being rather downfield of our usual aryloxy phosphoramidate ProTides.[28] The asymmetric diamidates gave two peaks by ^{31}P NMR and for HPLC, in roughly 1:1 ratios, as typically observed for phosphoramidate ProTides. Other spectroscopic and analytical data fully confirmed the structure and purity of the diamidates herein reported.

BIOLOGICAL ACTIVITY IN REPLICON

Each of the diamidates described above were tested for HCV inhibition in a replicon assay, with the clinical anti-HCV ProTide (**5**) as positive control (Table 1). Both EC_{50} and EC_{90} values are reported, along with standard deviations, in a 72 h HCV replicon assay. In general EC_{90} values were 2−5-fold higher than EC_{50} values, and the discussion below will focus on

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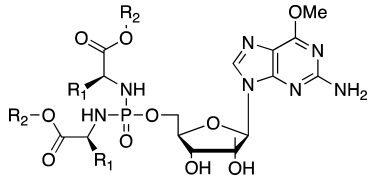
Table 2. Replicon Activity for Symmetrical Phosphorodiamidates



		replicon EC_{50} (μM) (R_2)			
amino acid	compd	cyclohexyl	benzyl	neopentyl	CC_{50} in Huh7 cells (μM)
L-alanine	**20**	0.05			>100
	21		0.49		>100
	19			0.06	>100
D-alanine	**29**			0.11	>100
glycine	**32**		0.60		>100
	33			0.13	>100
L-leucine	**34**	0.45[a]			20
	35		0.38		39
	36			0.47	27
L-isoleucine	**38**	4.0			14
	39		0.40		24
	40			2.9	15
L-valine	**49**	2.5			20
	50		0.12		49
	51			0.72[b]	32
L-proline	**47**		0.52		>100
	48			0.81	56
L-methionine	**41**	0.60			51
	42		0.25		>100
	43			2.22[b]	>100
L-phenylglycine	**53**	0.32			18
	54			0.27	24
L-phenylalanine	**44**	0.50[a]			25
	45		0.32		67
	46			0.05	24
L-aspartic (OBn)	**31**		0.61		>100

[a]Large standard deviation. [b]Single assay result.

the EC_{50} numbers. In addition, the cell cytotoxicities (CC_{50}) in the replicon cell line (Huh7) are reported.

As noted in Table 1, the three parent nucleosides, 2′-C-methyl guanosine **4** and the 6-methoxy **8** and 6-ethoxyl **9** analogues, display only modest anti-HCV activity, with EC_{50} values in the 2−10 μM range. By comparison, **5** is active at nanomolar levels, with an EC_{50} of 10 nM and an EC_{90} of 40 nM, representing a ca. 400-fold potency boost over the respective nucleoside.[12] Compound **5** does show some cytotoxicity to Huh7 cells in this assay at 7 μM, but its high potency still leads to a significant SI of ca. 700.

Examining the potency of the first family of symmetrical L-alanine phosphorodiamidates, we note a consistent and clear increase in potency for the *n*-alkyl esters as they extended from methyl (**10** EC_{50} = 6 μM) to *n*-pentyl (**14** EC_{50} = 0.03 μM). The *n*-pentyl L-alanine **14** is thus about 3 times less active than **5**, but notably it is also about 10 times less cytotoxic. It is interesting to note that the calculated lipophilicity (ClogP) values for this series range from 0.5 (methyl) to 4.7 (*n*-pentyl). It maybe that lipophilicity and potency correlate, however, there are exceptions. Clearly, the *n*-pentyl **14** is an interesting compound with a very attractive SI.

Branching the amino acid ester at the α (Table 1, **15**, **16**), β (**26**, **27**), or γ (**17**) position does not significantly change activity or toxicity relative to the straight chain compounds. Thus, the isopropyl ester **15** and the *n*-propyl ester **12** have

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similar activities, and the β branched neopentyl ester **26** is similar to the *n*-pentyl ester **14**. Indeed, several of these branched esters such as compounds **17** and **27** are very potent, with excellent SI values. We were particularly interested in the neopentyl L-alanine analogue **26** given its similarity to our clinical agent **5**. This compound reveals an EC_{50} of 0.06 μM and EC_{90} of 0.2 μM and is only 5-fold less active in this assay than **5**. However, in common with many of the phosphorodiamidates, this compound is significantly less cytotoxic, with CC_{50} >100 μM. Thus, the SI for **26** is >1600, which exceeds that of **5**.

Cyclic esters of L-alanine such as cyclopentyl-(**19**) and cyclohexyl-(**20**) are also very potent in the replicon assay and show no Huh7 cell toxicity at 100 μM. However, the cyclobutyl derivative **18** is about 5-fold less active with an EC_{50} of 0.32 μM, and the tetrahydropyranyl ester **24** is more than 200-fold less active compared to **20**.

Interestingly, the benzyl ester derivatives of L-alanine (Table 1, **21**−**23**) are somewhat less active than many of the alkyl ester analogues, being 5−10-times less active than the cyclohexyl compound for example. This is in marked contrast to the phosphoramidate ProTides SAR and much of our prior experience.[28]

An observation from our phosphoramidate SAR is that the purine C-6-substituent can be varied considerably and that the 6-ethoxy may be particularly effective.[24] As noted in Table 1, the same applies to this series, with the cyclohexyl-(**74**), benzyl-(**75**), and neopentyl-(**76**) purine C-6-ethoxy derivatives all being equipotent with their C-6-methoxy analogues. Indeed, with an EC_{90} of 110 nM, the neopentyl L-alanine analogue of the 6-ethoxy nucleoside **76** emerged as one of the most potent compounds in the present study.

The SAR next turned to symmetrical phosphorodiamidates with amino acids other than L-alanine. The subtlest change was to make the D-alanine analogue as its neopentyl ester **29**. In contrast to our earlier work on D-amino acids in aryl phosphoramidate ProTides,[25] here we see only a slight (ca. 2-fold) loss of activity. This again points to quite a new and separate SAR for these diamidates as compared to aryloxy phosphoramidates.

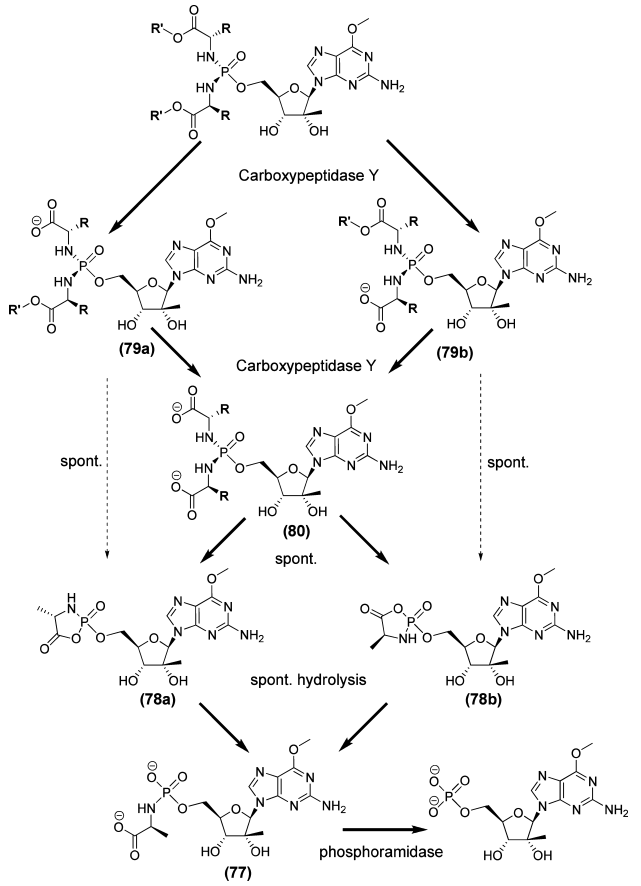
In generating the amino acid SAR in this series of symmetrically substituted phosphorodiamidates, effort was made to use three similar esters for each different amino acid derivative to facilitate comparison. The three ester groups selected were the cyclohexyl, benzyl, and neopentyl. Table 2 shows the compiled data.

A number of observations can be made from the data in Table 2. First, the benzyl esters do not greatly distinguish the different amino acids. The replicon activities for nine amino acids with benzyl ester groups range from 0.25 to 0.61 μM, only 2- to 3-fold, which is similar to the variability in the replicon assay.

The neopentyl and cyclohexyl ester groups distinguish the different amino acids and give a similar rank order. Thus, the best amino acid for both the cyclohexyl and the neopentyl esters is the L-alanine, whereas the worst is L-isoleucine.

Overall evaluation of the amino acid SAR leads to conclusion that small amino acid substituents like glycine or alanine are preferred and that increasing the size of the amino acid R_1 group as for L-leucine and L-methionine is detrimental to replicon activity, as is branching of the amino acid as for L-valine, L-isoleucine, and L-phenylglycine.

Scheme 2. Putative Initial Activation Route of Phosphorodiamidates



The exception to this rule is that the neopentyl L-phenylalanine derivative **46** is very potent (EC_{50} = 0.05 μM) in the replicon assay. The cyclohexyl L-phenylalanine derivative **44** was tested twice, and one value was 10-fold higher than the other (EC_{50} = 0.083 and 0.92). It is possible that further testing would refine these data. Further supporting this surprising L-phenylalanine SAR, the L-tyrosine derivative **52**, as the *para*-*O*-*tert*-butyl ether, also had good activity and limited toxicity in the replicon assay (EC_{50} = 0.11 μM, CC_{50} = 89 μM).

A further observation in this series of symmetrical phosphorodiamidates is that some cytotoxicity is observed in Huh7 cells for the amino acids other than alanine and glycine. In fact, the cyclohexyl L-isoleucine **38** demonstrates that not all phosphorodiamidates are equivalent. Compound **38** has an EC_{50} of 4 μM and a CC_{50} of 14 μM, for an SI of only ca. 4, and being the most cytotoxic diamidate studied.

As an extension of the growing symmetrical phosphorodiamidate SAR, a more complicated, dipeptide, L-Val-L-Ala neopentyl ester **55**, in which the L-valine nitrogen forms the phosphoramidate linkage, was prepared. Testing in the replicon assay shows that **55** has similar replicon activity (Table 1, EC_{50} = 0.54 μM) as the neopentyl L-valine derivative **51**. It is unclear how this dipeptide would fit in with the putative metabolic process (see Scheme 2) and may suggest the existence of another metabolic pathway leading to the triphosphate formation for this compound.

Working the SAR in the opposite direction, simple amine substituted phosphorodiamidates were tested in the replicon assay. The *n*-butyl amine analogue **57**, which may be

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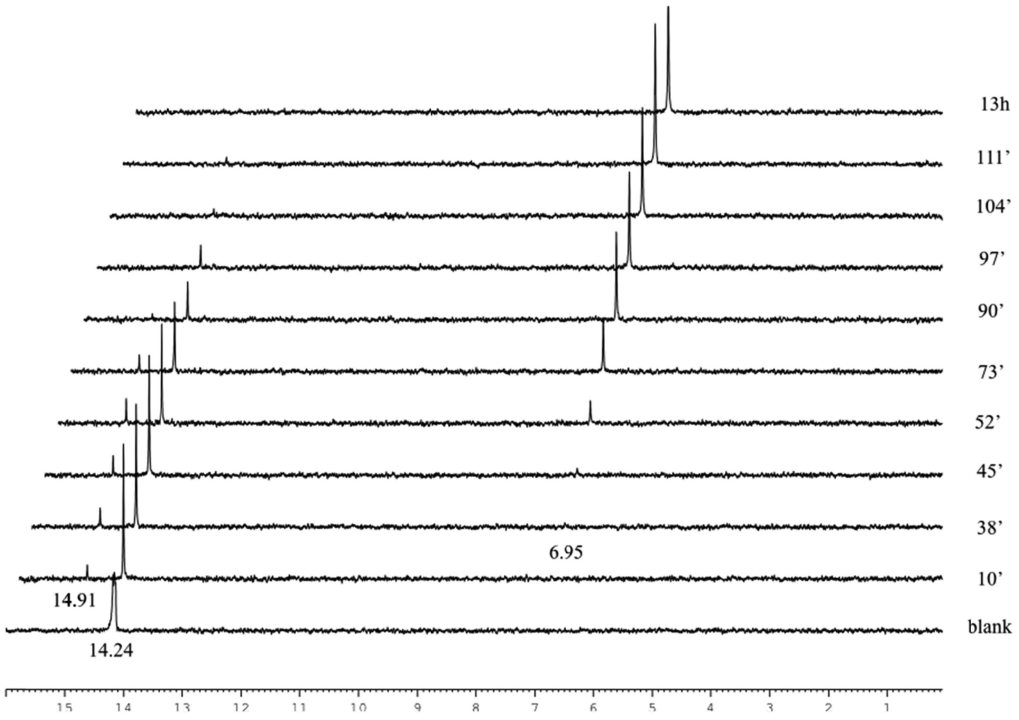

Figure 3. ^{31}P NMR kinetic study of **26** in the presence of carboxypeptidase Y. Conditions: 5.0 mg of **26** in 200 μL of acetone-d_6 + 400 μL of Trizma buffer (pH 7.4), 0.3−0.5 mg of carboxypeptidase Y in 200 μL of Trizma buffer (pH 7.4).

considered similar to the L-leucine derivatives, but without the α carboxylate, has a replicon EC_{50} of 38 μM, which is about 100-fold less active than the L-leucine derivatives. This is consistent with the proposed metabolic route (Scheme 2) where the carboxylate anion plays a key role. The positioning of the carboxylate β to the amine, as for the β-alanine analogue **56**, results in moderate activity in the replicon assay (Table 1, EC_{50} = 3.8 μM). The morpholino phosphorodiamidate **58**, an example of a secondary amine, is inactive even at 100 μM in the replicon assay. Both of these simple amine phosphorodiamidates **57** and **58** are much less active than the parent nucleoside **8** in the replicon assay, supporting our understanding that there is a limited direct cleavage of the P−O bond, leading to free nucleoside, in the replicon assay.

Although one of the key motivations for this new ProTide motif was to generate an achiral phosphate center, we were interested in understanding some aspects of the asymmetric diamidate SAR. Our synthetic route (Scheme 1) did allow the stepwise addition of two different amines. Each of these asymmetric diamidates were isolated as a roughly 1:1 mixture of phosphorus diastereoisomers, and no attempt was made to separate them and they were tested as mixtures. To facilitate comparison, generally, one of the two amines was maintained as the L-alanine neopentyl ester (Table 1).

From this basis, our SAR study was focused in three areas. First, we explored compounds **60**−**62** in which the second amine was also L-alanine but with different ester groups. The replicon results indicated that the presence of the L-alanine neopentyl ester provides good antiviral potency even in the presence of difficult to cleave[29] *tert*-butyl L-alanine ester **61**. The presence of two easily cleavable esters such as for derivatives **59** and **62** slightly increased the replicon activity comparing to **61** but one "cleavable" group was sufficient.

Next, the SAR of asymmetrical phosphorodiamidates, where the neopentyl L-alanine is combined with different amino acids was briefly explored. The methyl L-proline **63** and the methyl L-valine **64** amino acids had moderate replicon activity (Table 1), more similar to the symmetrical L-prolines and L-valines than the very potent symmetrical neopentyl L-alanine **26**. It might be that compounds **63** and **64** have different metabolic intermediates (**78**, Scheme 2) than does **26**.

Third, the phosphorodiamidate SAR of simple amines combined with neopentyl L-alanine was extensively studied (**66**−**73**, Table 1). In general, these compounds had moderate to good activity, with EC_{50} values ranging from 0.1 to 0.9 μM for monosubstituted amines, more active than the symmetrical simple amines, but less active than the symmetrical neopentyl L-alanine derivative (**26**). Presumably, conversion of compounds **66**−**73** involves cleavage of the L-alanine neopentyl ester followed by the elimination of the simple amine to form the key metabolic intermediate (**78**, Scheme 2). Thus, it might be expected that the amines that make the best leaving groups such as phenylamine **70** and naphthylamine **71**, would be the most active (EC_{50} = 0.27 and 0.13 μM) and that a poor leaving group such as diethyl amine, in derivative **72**, would be the least active (EC_{50} = 3.7 μM, Table 1).

In conclusion, all but one of the 67 phosphorodiamidates tested in the replicon assay were active at or below micromolar concentrations, with 55 of them active below 1 μM and 12 active below 100 nM. Many are noncytotoxic at high μM levels, some at 100 μM, giving attractive SI values for many compounds. Clearly several of these compounds were worthy of advancing to in vivo studies.

■ STABILITY ASSAYS

Given the new structural motif we are reporting and the very promising replicon data (as described above), we sought to establish some outline stability data under a variety of conditions. To begin with, the lead symmetrical neopentylalanine compound **26** was dissolved in pH 7 phosphate buffer at

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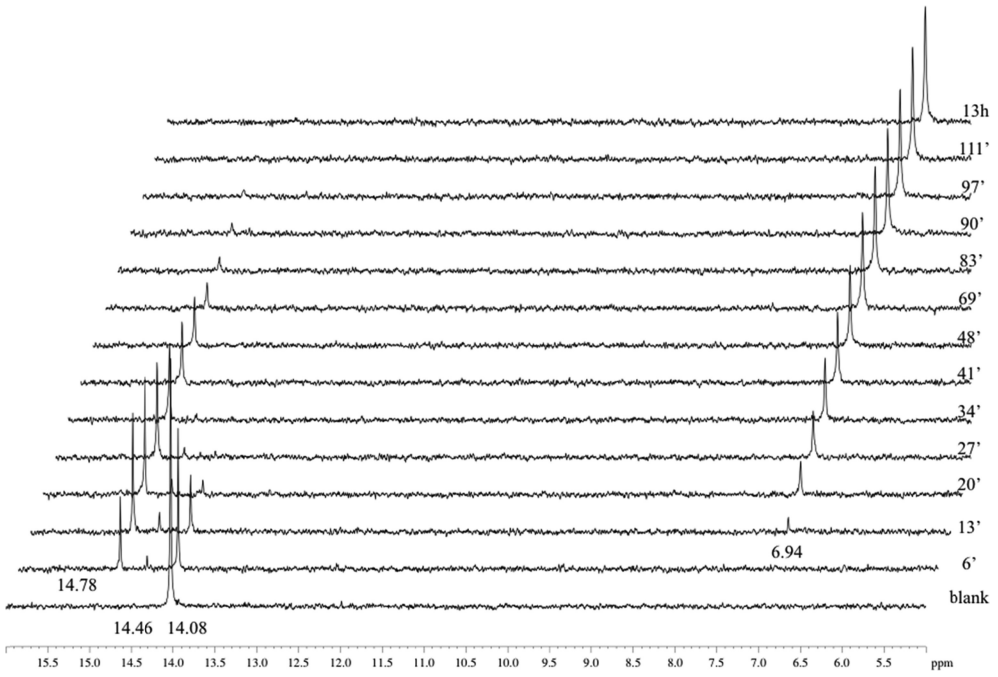

Figure 4. ^{31}P NMR kinetic study of **21** in the presence of carboxypetidase Y. Conditions: 5.1 mg of **21** in 200 μL of acetone-d_6 + 400 μL of Trizma buffer (pH 7.4), 0.3−0.5 mg of carboxypeptidase Y in 200 μL of Trizma buffer (pH 7.4).

25 °C and monitored by ^{31}P NMR over 14 h. Compound **26** was observed at 13.2 ppm, and no additional signal(s) appeared over the course of the experiment (see Supporting Information for stability spectra). HPLC also indicated no detectable decomposition. We have previously reported acid stability data[27] for several acyclovir aryloxy phosphoramidate ProTides. As a representative example, the phosphorodiamidate (**26**) was dissolved in citric acid−HCl buffer at pH 2 and maintained at either 37 or 47 °C while being monitored by ^{31}P NMR over 14 h. Despite the acidic pH and elevated temperatures, the samples remained entirely stable over the course of the assay. A base stability study was conducted on compound **26** at pH 8.5/37 °C and pH 11/37 °C, and these studies revealed slow decomposition. After 17 h at pH 11, the majority species was still unchanged (**26**), with only minor peaks at 6.9 ppm and −4.3 ppm. The peak at 6.9 ppm corresponds to an amino acid phosphoramidate derivative similar to compound **77** (see Scheme 2 below), suggesting one phosphorodiamidate P−N bond on compound **26** had been hydrolyzed under the aqueous basic conditions. However, the decomposition is rather slow, with a half-life exceeding 100 h (if first order). From this initial stability data, it seems possible that the phosphorodiamidates have a pH stability profile that is consistent with oral dosing.

Next, we studied the stability of compound **26** with human serum at 37 °C. As before, the solution was monitored at 1 h intervals over by ^{31}P NMR over 12 h. The phosphorodiamidate **26** gave a peak at 14.5 ppm under these conditions, and no sign of degradation was observed by ^{31}P NMR. Thus, as noted for aryloxy phosphoramidates,[30] the present phosphorodiamidates seem essentially stable in human serum, certainly for periods of hours appropriate for human dosing.

As we have reported previously,[12,30] the initial step of the conversion of aryloxy amino acid ester phosphoramidates (ProTides) to phosphates is thought to involve an enzyme-mediated cleavage of the amino acid ester group. We have also reported on the use of ^{31}P NMR and a buffered solution of the enzyme carboxypeptidase Y as an in vitro model for studying the initial steps of ProTide[30] activation. We sought to apply this method to our new family of anti-HCV phosphorodiamidates. Thus, compounds of interest were dissolved in acetone-d_6 and TRIZMA buffer at pH 7.4, and the ^{31}P NMR spectrum was recorded as the baseline. Then carboxypeptidase Y (cathepsin) was added and spectra recorded at intervals up to 13 h (Figure 3). The data from these experiments were used to map a possible phosphorodiamidate metabolic pathway (Scheme 2).

The first compound studied was the symmetrical neopentyl ester of L-alanine **26**, which has a ^{31}P NMR shift of 14.2 ppm at baseline (Figure 3). After 10 min, a small downfield metabolite peak is observed at 14.9 ppm, which is consistent with cleavage of the neopentyl ester. The peak at 14.9 ppm builds up for approximately one hour, then diminishes, with a new peak emerging at 6.95 ppm. This is first observed at ca. 40 min and continues to grow through the course of the experiment. The peak at 6.95 corresponds to the key amino acid phosphate derivative **77** (R = CH_3) (Scheme 2). This was determined by synthesizing compound **77** (R = CH_3) and using it as an analytical reference standard (data not shown). Intermediate **77** could form by intramolecular attack of the amino acid carboxylate anion onto the phosphorus, with elimination of the second amino acid, followed by spontaneous hydrolysis of the cyclic, mixed anhydride intermediate **78a**,**b**. No direct ^{31}P NMR evidence (Figure 3) was obtained for this cyclic compound, consistent with it being a short-lived metabolic intermediate. As discussed in our publications on aryloxy amino acid phosphoramidates, final conversion to the nucleoside monophosphate is generally thought to be effected by enzymes of the histidine triad nucleotide binding (Hint) family (Scheme 2).[30,33]

This initial evidence indicates that nucleoside phosphorodiamidates are converted to nucleoside monophosphates in a similar fashion as aryl phosphoramidates, that is, via metabolite **77**. To further support this hypothesis, a second phosphor-

dx.doi.org/10.1021/jm2011673 | J. Med. Chem. 2011, 54, 8632–8645

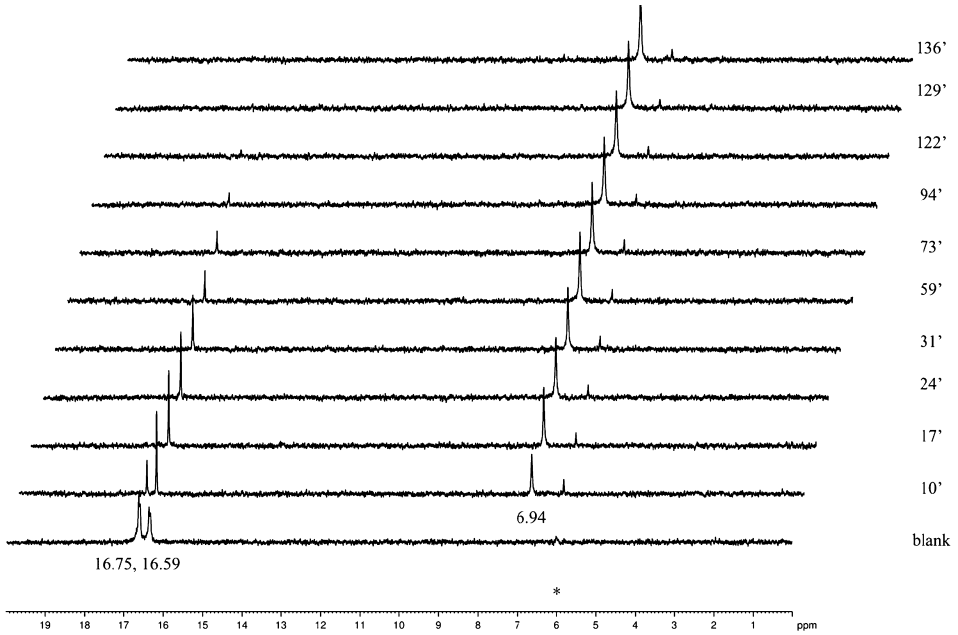

Figure 5. ^{31}P NMR kinetic study of **65** in the presence of carboxypetidase Y. Conditions: 4.9 mg of **65** in 200 μL of acetone-d_6 + 400 μL of Trizma buffer (pH 7.4), 0.3−0.5 mg of carboxypeptidase Y in 200 μL of Trizma buffer (pH 7.4). * Phosphate impurity in Trizma buffer; in blank and remains unchanged throughout.

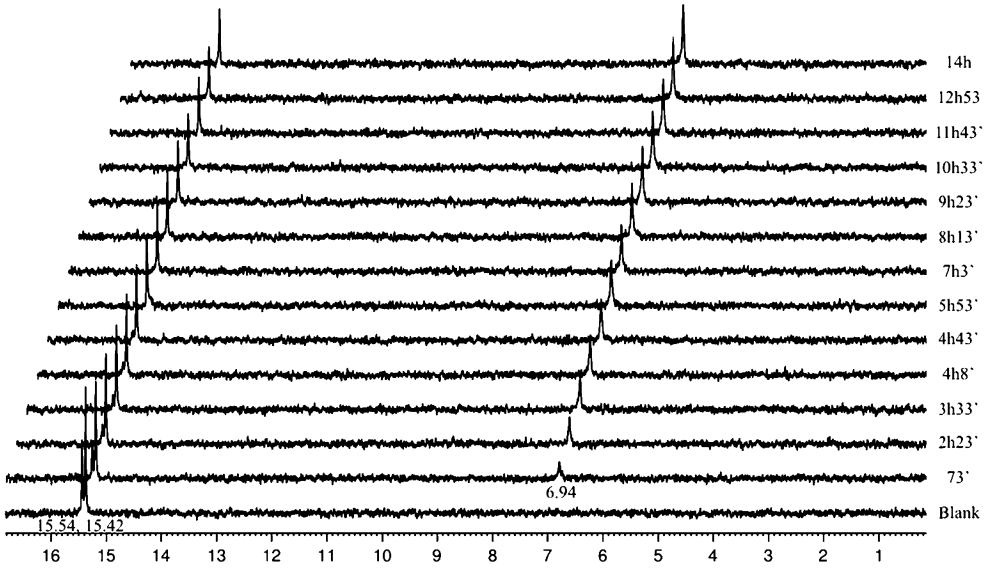

Figure 6. ^{31}P NMR kinetic study of **73** in the presence of carboxypetidase Y. Conditions: 5.3 mg of **73** in 200 μL of acetone-d_6 + 400 μL of Trizma buffer (pH 7.4), 0.3−0.5 mg of carboxypeptidase Y in 200 μL of Trizma buffer (pH 7.4).

odiamidate, the symmetrical benzyl L-alanine derivative **21**, was studied in the same system (Figure 4). In this case, a similar ^{31}P NMR pattern, with the formation of the same key metabolite at 6.94 ppm (**77**, R = CH_3) is observed, but there are subtle differences. Parent **21** shows one phosphorus ^{31}P NMR peak at 14.08 ppm at baseline, which disappears within the first 30 min upon incubation with enzyme. However, in this case, two downfield singlet peaks are observed, one small and transient at 14.46 ppm and the other larger and longer lived at 14.78 ppm. Three possibilities exist to explain these two new downfield peaks: (a) one specific benzyl ester is cleaved by carboxypeptidase Y to give either **79a** or **79b**, but not both, followed by a second ester cleavage to give **80**; (b) both pro-pR and pro-pS benzyl esters are cleaved by carboxypeptidase Y and both **79a** and **79b** are observed, but cleavage of both benzyl esters does not occur; (c) the peak at 14.46 ppm represents mono ester cleavage, where the ^{31}P NMR signals of **79a** and **79b** overlap, and the peak at 14.78 represents diester cleavage to give **80**.

Regardless of how the amino acid ester groups are cleaved by this particular enzyme, the important information from the ^{31}P NMR experiments is that compound **21** goes through the same common intermediate **77** (R = CH_3) as does compound **26**.

In Supporting Information, we present further detail of the kinetics of appearance of each species in the carboxypeptidase Y mediated cleavage of **21**.

It should be noted that **26** is nearly 10-fold more active than **21** in the HCV replicon assay (Table 1), however comparison

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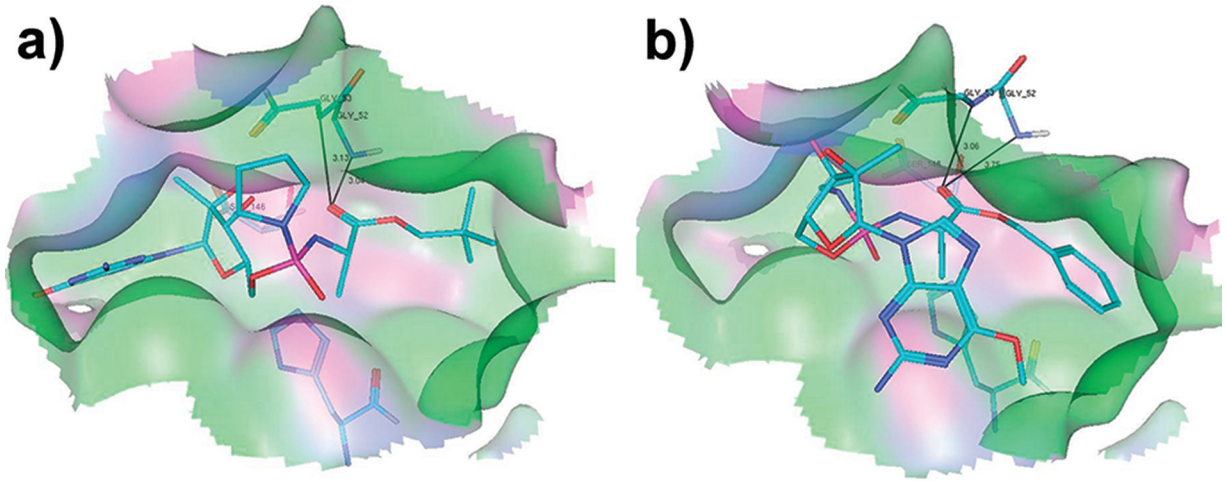

Figure 7. (a) Docking of compound **65−Rp** isomer within the catalytic site of carboxypeptidase Y. (b) Docking of compound **65−Sp** isomer within the catalytic site of carboxypeptidase Y.

Table 3. Rat Liver Triphosphate Levels from PK Analysis of Phosphorodiamidates

compd	AA ester	replicon EC_{50} (μM)	C_{max}[a] (ng/g)	C_{last}[b] (ng/g)	T_{max}[c] (h)	AUC_{0-t}[d] (ng·h/g)
5[e]	L-Ala OCH_2tBu	0.01	1446.7	983.3	8.0	24557
26	L-Ala OCH_2tBu	0.06	1580.0	800.0	8.0	25147
13	L-Ala *O*-*n*-butyl	0.07	1553.3	719.7	8.0	27671
14	L-Ala *O*-*n*-pentyl	0.03	1042.3	506.0	8.0	18600
16	L-Ala O-(*R*,*S*)-2-butyl	0.15	907.7	538.7	8.0	16252
19	L-Ala *O*-cyclopentyl	0.06	1523.3	610.0	8.0	24546
25	L-Ala *O*-2-indanol	0.58	632.0	632.0	24.0	10642
29	D-Ala OCH_2tBu	0.11	598.3	441.7	8.0	11608
50	L-Val OBn	0.12	359.5	73.6	4.0	4478

[a] C_{max}: Maximum observed concentration. [b] C_{last}: Concentration at the last measurable time-point. [c] T_{max}: Time at which maximum concentration was observed. [d] AUC_{0-t}: area under the concentration−time curve from time 0 to the last measurable concentration. [e] Compound **5** is a the phosphoramidate INX-189 (see Figure 2).

of Figures 3 and 4 shows that the speed of carboxypeptidase Y processing is very similar for the two prodrugs, and thus the replicon and enzyme assays do not correlated directly, but we do regard enzyme mediated ester cleavage as a useful tool to study our prodrugs in vitro.

If a carboxylate anion intermediate such as **79a**, **79b**, or **80** can eliminate an amino acid moeity to give intermediate **77**, we wondered if simple primary or secondary amines could likewise be eliminated from asymmetric phosphorodiamidates such as **65** and **73**. Figure 5 shows the ^{31}P NMR traces for an experiment with the benzyl L-alanine, *n*-butyl amine derivative **65**, and carboxypeptidase Y.

Because **65** is asymmetrical, two peaks are seen in the baseline ^{31}P NMR (16.59 and 16.75 ppm). Both peaks disappear within 120 min, but the diastereomer at 16.75 is cleaved faster. Once again. a peak at 6.94, identified as compound (**77**, R = CH_3), is observed growing in magnitude during the course of the experiment. A very similar pattern is observed in Figure 6, when the neopentyl L-alanine, pyrrolidine asymmetrical phosphoramidate **73** was incubated with carboxypeptidase Y. Again compound **77** (R = CH_3) is observed at 6.94 ppm and grows in over 14 h. Interestingly, in this case, only one of the two diastereomers of compound **73** (^{31}P NMR δ = 15.54 ppm) is cleaved by carboxypeptidase Y over the course of the experiment.

Summarizing this portion or our work, we have built on our previous understanding that aryloxy groups are eliminated from asymmetrical phosphoramidates containing an amino acid carboxylate by showing that a second amino acid or a primary amine or a secondary amine can also be eliminated to give the key intermediate **77**. The data on **21**, **26**, **65**, and **73** supports the notion that only one ester cleavage is necessary and that the second amine loss is rapid following the first ester cleavage.

Docking Studies. To further support the above, we conducted some docking studies on several asymmetric diamidates, using published[31] crystal structure of carboxypeptidase Y. Thus, as shown in Figure 7a,b the Sp diastereomer of **65** binds significantly better than the Rp diastereomer.

In the case of the Sp isomer, the stabilization by H-binding of two glycine residues (Gly52, 53) is notable and the nucleophilic active site of Ser146 is also well positioned. These docking data would then suggest that one diastereoisomer of **65** might be processed better than the other. On the basis of the clear kinetic difference noted above (Figure 5), perhaps the more downfield species in ^{31}P NMR of **65** is the Sp isomer. It is interesting to wonder if the differing kinetics of metabolism here may lead to a difference in biological potency as we have noted in some cases for aryl phosphoramidates.[5] However, we were unable to separate the compound **65** diastereomers to test this hypothesis.

Pharmacokinetics. The HCV replicon, stability, and carboxypeptidase Y data suggests that our new phosphorodiamidate prodrug strategy may be a promising means of delivering 2′-*C*-methylguanosine triphosphate into cells. The

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next hurdle was to determine if this prodrug strategy works in vivo in a rodent. Because of our growing experience with phosphoramidates such as **5** in rats, we decided to continue with the rat as our initial PK model for the phosphorodiamidates. Eight symmetrical derivatives were selected for rat PK studies (**13**, **14**, **16**, **19**, **25**, **26**, **29**, and **50**, Table 3) based on activity in the replicon assay and structural considerations. We focused on symmetrical diamidates because they are represented by single diastereoisomers, which would simplify further development. All but two were L-alanine derivatives because we wanted to fully explore the ability of these relatively simple derivatives to provide sufficient liver triphosphate levels.

Compounds were formulated in 95% Capmul MCM/5% Tween 80, and doses of 10 mg/kg were administered by oral gavage to male Sprague–Dawley rats. Liver samples were collected up to 24 h postadministration and were snap-frozen in liquid nitrogen. Liver concentrations of 2′-*C*-methylguanosine triphosphate were determined by LC-MS/MS. Results for these eight phosphorodiamidates are compared to **5** in Table 3.

The main observation is that all compounds tested produced significant levels of triphosphate in rat livers from a 10 mg/kg dose, further validating phosphorodiamidates as effective phosphate prodrugs. In addition, several of the phosphorodiamidates provide similar liver triphosphate exposures as the clinical compound **5**, which has shown efficacy against HCV in phase Ib clinical trials.[23] The amount of 2′-*C*-methylguanosine triphosphate necessary to achieve an EC_{90} in the replicon assay can be determined by measuring triphosphate levels in the replicon cells upon incubation with a 2′-*C*-methylguanosine based inhibitor such as **5**. This EC_{90} triphosphate level in cells can be extrapolated to EC_{90} triphosphate levels in the liver, as measured in ng of triphosphate/gram of liver tissue. The value we have calculated is 243 pmol of triphosphate per gram of liver (equal to 131 ng/g).[32] Thus, for all phosphorodiamidates tested, except for **50**, the level of triphosphate 24 h post dose, is several fold above 131 ng/g level necessary to achieve 90% inhibition of HCV replication (Table 3).

An important part of our evaluation of any new prodrug approach for delivering 2′-*C*-methylguanosine triphosphate, including these phosphorodiamidates, is measurement of systemic nucleoside (2′-*C*-methylguanosine, **4**) levels after oral dosing of the prodrug. Our desire is to limit the systemic exposure of this nucleoside. The plasma 2′-*C*-methylguanosine levels for these eight phosphorodiamidates were measured, and all but compound **13** had lower nucleoside C_{max} values than **5** (<100 nM), and it is only slightly higher (data not shown).

The neopentyl L-alanine diamidate **26** has both the highest C_{max} and C_{last} of any diamidate tested. Figure 8 shows rat liver triphosphate levels after a 10 mg/kg dose for the clinical compound **5** and for **26**. Triphosphate levels were measured at eight time points over 24 h. It is clear that both prodrug strategies produce similarly high levels of triphosphate. The *n*-butyl L-alanine ester **13** has the highest overall triphosphate AUC (Table 3), and the cyclopentyl ester **19** also has excellent AUC and C_{last} values. These compounds along with the *n*-pentyl ester **14** were advanced to monkey PK studies. A combination of rat PK, monkey PK, and preliminary rodent toxicology studies will be used to help select a clinical candidate. These additional studies will be reported elsewhere upon completion.

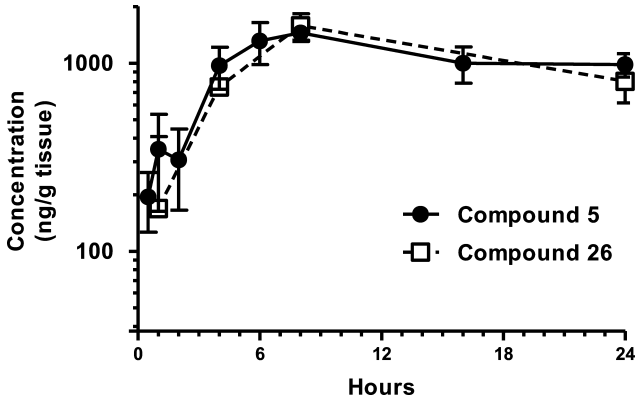

Figure 8. Rat liver 2′-*C*′-methyl guanosine triphosphate levels from compounds **5** and **26**.

CONCLUSIONS

In conclusion, we report on a new family of phosphate prodrugs based on phosphorodiamidates. This type of 5′-monophosphate prodrug has the advantage that it can be designed to be achiral at the phosphate center if desired. We report on a range of novel prodrugs derived from amino acids and simple amines and build a substantial HCV replicon SAR for both symmetrical and asymmetrical phosphorodiamidates of 2′-*C*-methyl-6-*O*-methylguanosine. The replicon data suggests that one aminoacyl ester is essential for potent activity versus HCV. The phosphorodiamidates are stable in acid and mild base and also in human serum. Carboxypeptidase Y is able to activate these compounds to a nucleoside aminoacid phosphate key intermediate, which is also the essential metabolic intermediate for our earlier aryloxy ProTides. Many of the novel compounds in this study show low nanomolar activity versus HCV in replicon coupled with low cytotoxicity in the Huh7 replicon cell line. Eight potent HCV inhibitors were advanced to PK studies in Sprague–Dawley rats, and it was demonstrated that they all provided substantial 2′-*C*-methylguanosine triphosphate levels, in rat livers, that were maintained over a period of 24 h. This body of work has validated phosphorodiamidates as prodrugs for 2′-*C*-methylguanosine both at the in vitro and the in vivo levels. Further in vivo studies are underway that are intended to lead toward selection of a phosphorodiamidate prodrug for HCV clinical studies.

EXPERIMENTAL SECTION

General. Anhydrous solvents were purchased from Aldrich and used without further purification. All reactions were carried out under an argon atmosphere. Reactions were monitored with analytical TLC on silica gel 60-F254 precoated aluminum plates and visualized under UV (254 nm) and/or with ^{31}P NMR spectra. Column chromatography was performed on silica gel (35–70 μM). Proton (^{1}H), carbon (^{13}C), and phosphorus (^{31}P) NMR spectra were recorded on a Bruker Avance 500 spectrometer at 25 °C. Spectra were autocalibrated to the deuterated solvent peak, and all ^{13}C NMR and ^{31}P NMR were proton-decoupled. Analytical and semipreparative HPLC were conducted by Varian Prostar (LC Workstation-Varian prostar 335 LC detector) using Varian Polaris C18-A (10 μM) as an analytic column and Varian Polaris C18-A (10 μM) as a semipreparative column; elution was performed using a mobile phase consisting of water/acetonitrile in gradient (system1, 90/10 to 0/100 v/v in 30 min) or water/methanol (system 2, 90/10 to 0/100 v/v in 30 min). High-resolution mass spectra (HRMS) was performed as a service by Cardiff University, using electrospray (ES). Compound purity was assured by a

combination of high field multinuclear NMR (H, C, P) and HPLC. Purity by the latter was always >95% with no detectable parent nucleoside for all final products.

Standard Procedure A: Synthesis Of Symmetrical Diamidates. To a suspension of the nucleoside (1.0 mol equiv) in anhydrous tetrahydrofuran, triethylamine (1.0−1.2 mol equiv) was added. After stirring for 30 min at room temperature, phosphoryl chloride (1.0−1.2 mol equiv) was added dropwise at −78 °C. The reaction mixture was stirred for 30 min at −78 °C and then allowed to warm to room temperature. Anhydrous dichloromethane was added, followed by amino acid ester (5.0 mol equiv) and triethylamine (10.0 mol equiv) at −78 °C. After stirring at room temperature for 20 h, water was added and the layers are separated. The aqueous phase was extracted with dichloromethane and the organic phase washed with brine. The combined organic layers were dried over anhydrous sodium sulfate, filtered, and evaporated to dryness. The resulting residue was purified by silica gel column chromatography using as an eluent a gradient of methanol in dichloromethane or chloroform. In some cases, a subsequent repurification was necessary either by preparative HPLC (gradient of methanol in water) or preparative TLC.

Standard Procedure B: Synthesis of Symmetrical Diamidates. To a solution of the nucleoside (1.0 mol equiv) in anhydrous triethylphosphate was added phosphoryl chloride (2.0 mol equiv) at 0 °C. The reaction mixture was stirred for 24 h at 5 °C. Anhydrous dichloromethane was added to the reaction mixture followed by amino acid ester (5.0 mol equiv) and diisopropylethylamine (10.0 mol equiv) at 0 °C. After stirring at 5 °C for 5 days, water was added and the layers were separated. The aqueous phase was extracted with dichloromethane and the organic phase washed with brine. The combined organic layers were dried over anhydrous sodium sulfate, filtered, and evaporated to dryness. The resulting residue was purified by silica gel column chromatography using as an eluent a gradient of methanol in dichloromethane. A subsequent repurification, if necessary, was accomplished either by preparative HPLC (gradient of methanol in water) or preparative TLC.

Standard Procedure C: Synthesis of Asymmetrical Diamidates. To a suspension of the nucleoside (1.0 mol equiv) in anhydrous tetrahydrofuran, triethylamine (1.0 mol equiv) was added. After stirring for 30 min at room temperature, phosphoryl chloride (1.0 mol equiv) was added dropwise at −78 °C. The reaction mixture was stirred for 30 min at −78 °C and then allowed to warm to room temperature. Anhydrous dichloromethane was added, followed by the addition of amino acid ester or amine (1 mol equiv) and anhydrous triethylamine (2 or 1 mol equiv, respectively) at −78 °C. Reaction was warmed to room temperature and monitored by ^{31}P NMR. When NMR indicated completion of the reaction (no starting material, presence of monosubstituted product), a second amino acid ester or amine (5 mol equiv) was added followed by the addition of triethylamine (10 or 5 mol equiv, respectively) at −78 °C. After stirring at room temperature for 16−20 h, water was added and the layers were separated. The aqueous phase was extracted with dichloromethane. The combined organic layers were dried over anhydrous sodium sulfate, filtered, and evaporated to dryness. The resulting residue is purified by silica gel column chromatography using as an eluent a gradient of methanol in chloroform.

Example: Synthesis of 2-Amino-6-methoxy-9-(2′-*C*-methyl-β-D-ribofuranosyl) Purine 5′-*O*-Bis(methoxy-L-alaninyl) Phosphate (10). The phosphorodiamidate **10** was prepared according to the standard procedure B.

In a first step, a suspension of 6-*O*-methyl-2′-*C*-methylguanosine (250 mg, 0.803 mmol) in anhydrous tetrahydrofuran (4 mL) was reacted with triethylamine (135 μL, 0.964 mmol) and phosphorus oxychloride (89 μL, 0.964 mmol). In a second step, anhydrous dichloromethane (4 mL), L-alanine methyl ester hydrochloride salt (561.1 mg, 4.02 mmol), and triethylamine (1.12 mL, 8.03 mmol) were added.

After workup, silica gel column chromatography and preparative HPLC, 19.8 mg of (**10**) was obtained in 4.4% yield as an off-white solid. ^{1}H NMR (500 MHz, MeOD-d_4) δ 7.99 (s, 1H, H-8), 5.99 (s, 1H, H1′), 4.44−4.34 (m, 2H, H5′), 4.31 (d, 1H, *J* = 8.9 Hz, H3′), 4.21−4.16 (m, 1H, H4′), 4.07 (s, 3H, OCH_3), 3.98−3.89 (m, 2H, 2× CHα Ala), 3.70, 3.69 (2s, 6H, 2× OCH_3 ester), 1.33 and 1.32 (2d, 6H, *J* = 7.1 Hz, CH_3 Ala), 1.00 (s, 3H, CH_3). ^{13}C NMR (126 MHz, MeOD-d_4) δ 176.10 (2d, $^3J_{C-C-N-P}$ = 4.8 Hz, 2× *C*═O Ala), 162.75 (C6), 161.95 (C2), 154.21 (C4), 139.33 (C8), 115.54 (C5), 93.19 (C1′), 82.33 (d, $^3J_{C-C-O-P}$ = 7.6 Hz, C4′), 80.02 (C2′), 74.73 (C3′), 66.13 (d, $^2J_{C-O-P}$ = 4.8 Hz, C-5′), 54.21 (OCH_3), 52.72 (2× CH_3 ester), 51.01, 50.93 (2d, $^2J_{C-N-P}$ = 2.3 Hz, 2× *C*H Ala), 20.86, 20.67 (2d, $^3J_{C-C-N-P}$ = 6.2 Hz, 2× CH_3 Ala), 20.27 (2′-CH_3). ^{31}P NMR (202 MHz, MeOD-d_4) δ 14.00. HPLC t_R = 8.86 min (system 1).

Example: Synthesis of 2-Amino-6-methoxy-9-(2′-*C*-methyl-β-D-ribofuranosyl) Purine 5′-*O*-Bis(benzoxy-L-alaninyl) Phosphate (21). The phosphorodiamidate **21** was prepared according to the standard procedure B.

In the first step, a solution of 6-*O*-methyl-2′-*C*-methylguanosine (250 mg, 0.803 mmol) in anhydrous triethylphosphate (1 mL) was reacted with phosphorus oxychloride (148 μL, 1.61 mmol). In the second step, anhydrous dichloromethane (4 mL), the tosylate salt of benzoxy-L-alanine (1.41 g, 4.02 mmol), and diisopropylethylamine (1.40 mL, 8.03 mmol) were added to the previous mixture. After workup, silica gel column chromatography and preparative HPLC, 50.1 mg of **21** was obtained in 8.7% yield as an off white solid. ^{1}H NMR (500 MHz, MeOD-d_4) δ 7.96 (s, 1H, H-8), 7.34−7.25 (m, 10H, 2× Ph), 5.99 (s, 1H, H1′), 5.16−5.02 (m, 4H, 2× CH_2 ester), 4.41−4.31 (m, 2H, H-5′), 4.29 (d, 1H, *J* = 9.0 Hz, H3′), 4.21−4.15 (m, 1H, H4′), 4.04 (s, 3H, OCH_3), 4.02−3.94 (m, 2H, 2× CH Ala), 1.33 (d, 6H, *J* = 7.1 Hz, 2× CH_3 Ala), 0.99 (s, 3H, CH_3). ^{13}C NMR (126 MHz, MeOD-d_4) δ 175.42, 175.36 (2d, 2× C═O, $^3J_{C-C-N-P}$ = 6.3 Hz, ester), 162.72 (C6), 161.91 (C2), 154.56 (C4), 139.31 (C8), 137.32, 137.29 (d, 2× C ipso OCH_2Ph), 129.55, 129.5, 129.24, 129.21 (OCH_2Ph), 115.55 (C5), 93.17 (C1′), 82.37 (C4′), 80.01 (C2′), 74.81 (C3′), 67.89,67.87 (2× OCH_2Ph), 66.26 (C5′), 54.19 (OCH_3), 51.13, 51.08 (2d, 2× Cα Ala), 20.79−20.58 (2d, $^3J_{C-C-N-P}$ = 6.3 Hz, 2× CH_3 Ala), 20.26 (2′CCH_3). ^{31}P NMR (202 MHz, MeOD-d_4) δ 13.93. HPLC t_R = 13.16 min (system 1).

Example: Synthesis of 2-Amino-6-methoxy-9-(2′-*C*-methyl-β-D-ribofuranosyl) Purine 5′-*O*-[(Benzoxy-L-alaninyl)-(2,2-dimethylpropoxy-L-alaninyl)] Phosphate (60). The phosphorodiamidate **60** was prepared according to the standard procedure C.

In the first step, a solution of 6-*O*-methyl-2′-*C*-methylguanosine (250 mg, 0.803 mmol) in anhydrous tetrahydrofuran (5 mL) was allowed to react with triethylamine (110 μL, 0.803 mmol) and phosphorus oxychloride (70 μL, 0.803 mmol). The tosylate salt of benzoxy-L-alanine (282 mg, 0.803 mmol) and triethylamine (110 μL, 0.803 mmol) were added. Anhydrous dichloromethane (4 mL) and the tosylate salt of neopentyloxy-L-alanine (1.33 g, 4.02 mmol) and triethylamine (1.12 mL, 8.03 mmol) were added as described in method C. After workup and silica gel column chromatography, 25 mg of the prodrug was obtained in 4% yield as an off-white solid. ^{1}H NMR (500 MHz, MeOH-d_4) 7.97, 7.96 (2s, 1H, H8), 7.36−7.30 (m, 5H, OCH_2Ph), 5.98, 5.97 (2s, 1H, H1′), 5.18- 5.09 (m, 2H, OCH_2Ph), 4.39−4.33 (m, 2H, H5′), 4.28 (2d, *J*= 8.00 Hz, 1H, H3′), 4.20−4.16 (m, 1H, H4′), 4.06, 4.05 (2s, 3H, 6OCH_3), 4.02−3.94 (m, 2H, 2× CHα Ala), 3.84, 3.82, 3.72, 3.67 (2AB, J_{AB}= 10.50 Hz, 2H, $CH_2C(CH_3)_3$), 1.39−1.32 (m, 6H, 2× CH_3 Ala), 0.97 (s, 3H, 2′CCH_3), 0.93, 0.91 (2s, 9H, $CH_2C(CH_3)_3$). ^{13}C NMR (126 MHz, MeOH-d_4) 175.54, 175.43, 175.39 (C═O ester), 162.73, 162.71 (C6), 161.93, 161.89 (C2), 154.57, 154.55 (C4), 139.32, 139.08 (C8), 137.39 (ipso OCH_2Ph), 129.55, 129.35, 129.25, 129.23, 129.20, 129.16, 128.27, 128.00 (OCH_2Ph), 116.19, 115.54 (C5), 93.34, 93.18 (C1′), 82.39, 82.33 (C4′), 80.01, 79.99 (C2′), 75.34, 75.04 ($CH_2C(CH_3)_3$), 74.84, 74.82 (C3′), 67.88, 67.85 (OCH_2Ph), 67.86 (d, $^2J_{C-O-P}$ = 3.75 Hz, C5′), 66.36 (d, $^2J_{C-O-P}$ = 5.50 Hz, C5′), 54.18, 54.01 (6OCH_3), 49.69, 49.64, 49.52, 49.46 (2× Cα Ala), 32.28, 32.25 ($CH_2C(CH_3)_3$), 26.74, 26.71 ($CH_2C(CH_3)_3$), 21.07, 20.90, 20.79, 20.66 (4*d*, $^3J_{C-C-N-P}$ = 6.25 Hz, 2× CH_3 Ala), 20.39, 20.25 (2′CCH_3). ^{31}P NMR (202 MHz, MeOH-d_4) 13.98, 13.94. HPLC t_R = 16.11, 16.80 min (system 1). MS (TOF ES+) *m*/*z*: 716.28 (M + Na^+, 100%). HRMS $C_{30}H_{44}N_7O_{10}P_1$ calculated, 694.2966; found, 694.2956

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Biological Methods. *Replicon Assays.* The HCV inhibitory activity of compounds was evaluated in an Huh7 cell line expressing a stable, bicistronic subgenomic HCV genotype 1b (Con1) replicon encoding the Renilla luciferase reporter gene (Apath, LLC, Brooklyn, NY) as previously described.[32] Cellular cytotoxicity was evaluated using the CellTiter-Glo Luciferase assay (Promega, Madison WI). A day before testing, 2×10^4 Huh7 cells were seeded in 96-well flat bottom white plates (Nunc, Roskilde, Denmark). Four-fold serial drug dilutions were made in growth medium and added to the cells. No drug controls were included in each plate. The plates were incubated in the presence of test compound for 3 days at 37 °C with 5% CO_2. Luciferase reagent was added to cells and plates were incubated for 20 min before measuring relative luminescent units (RLU) in a luminometer (Veritas, Turner Biosystems, Sunnyvale, CA).

Pharmacokinetic Studies in Rats. Rat studies were conducted at Inhibitex, Inc., in accordance with NIH Guidelines and following protocols approved by the Institutional Animal Care and Use Committee (IACUC) of Inhibitex, Inc. Studies were carried out as previously described.[32] Test compounds were formulated in 95% Capmul MCM (ABITEC Corp., Janesville, WI)/5% Tween 80 (Sigma, St. Louis, MO), and doses of 10 mg/kg were administered by oral gavage to male Sprague–Dawley rats (Taconic Farms, Germantown, NY). Liver samples were collected as a terminal procedure up to 24 h postadministration and were snap-frozen immediately upon collection in liquid nitrogen. Liver samples were stored frozen at ≤−80 °C prior to analysis.

Bioanalysis of Pharmacokinetic Samples. The concentration of 2′-*C*-MeGTP in liver samples from rats was measured by LC-MS/MS as described previously.[28] The assay was linear ($r^2 \geq 0.99$) in the concentration range of 100–4000 ng per gram of tissue with ≥85% accuracy and ≤2% CV. Noncompartmental pharmacokinetic analyses were performed on the liver concentration data using WinNonlin v5.2 software (Pharsight, St. Louis, MO) as described previously.[32]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Preparative methods, spectroscopic and analytical data on target compounds plus ^{31}P NMR stability assays and metabolic activation data. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

DAAs, direct acting antivirals; HCV, hepatitis C virus; AZT, 3′azidothymidine; SAR, structure–activity relationships; TLC, thin layer chromatography; HPLC, high performance/liquid chromatography; ClogP, calculated logarithm of the octanol/water partition coefficient; PK, pharmacokinetics

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Synthesis and evaluation against hepatitis C virus of 7-deaza analogues of 2′-*C*-methyl-6-*O*-methyl guanosine nucleoside and L-Alanine ester phosphoramidates

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ABSTRACT

7-Deazapurines are known to possess broad antiviral activity, however the 2′-*C*-methylguanosine analogue displays poor cell permeation and limited phosphorylation, thus is not an efficient inhibitor of hepatitis C virus (HCV) replication. We previously reported the 6-*O*-methyl entity as a prodrug moiety to increase liphophilicity of guanine nucleosides and the ProTide approach applied to 2′-*C*-methyl-6-*O*-methylguanosine has lead to potent HCV inhibitors now in clinical trials. In this Letter, we report the synthesis and biological evaluation of 2′-*C*-methyl-6-*O*-methyl-7-deaza guanosine and ProTide derivatives. In contrast to prior studies, removal of the N-7 of the nucleobase entirely negates anti-HCV activity compared to the 2′-*C*-methyl-6-*O*-methylguanosine analogues. To understand better this significant loss of activity, enzymatic assays and molecular modeling were carried out and suggested 2′-*C*-methyl-6-*O*-methyl-7-deaza guanosine and related ProTides do not act as efficient prodrugs of the free nucleotide, in marked contrast to the case of the parent guanine analogue.

Identified in 1989,[1] Hepatitis C is an infectious disease affecting approximately 180 million people worldwide.[2] The standard treatment of care currently administered to patients consists of the general antiviral agent ribavirin combined with pegylated interferon and one of the recently discovered non-nucleoside inhibitors telaprevir or boceprevir. This treatment is not only long but has limited efficacy[3] and causes numerous side effects,[4] hence the need of more efficient therapy. Several modified purine nucleosides, such as β-2′-*C*-methyl purines and nucleobase modified purines, have exhibited significant anti-HCV activity.[5] After phosphorylation to their triphosphates β-2′-*C*-methyl modified nucleosides are direct inhibitors of HCV replication,[6] and they are known as Direct Acting Antivirals (DAAs). This includes several 7-deaza purines, particularly β-2′-*C*-methyl-7-deaza adenosine (**1**) (Fig. 1) which is reported to be a potent inhibitor of HCV replication (EC_{50} = 0.25 μM, CC_{50} >100 μM),[5,6] whereas its guanosine analogue is inactive (EC_{50} >100 μM, CC_{50} >100 μM). The loss of inhibitory activity is likely to be due to its poor cell uptake and inefficient intracellular metabolism to its 5′-triphosphate form.[6,7] This membrane permeation issue was previously addressed and modifications at the 6-position of nucleobase lead us to develop β-2′-*C*-methyl-6-*O*-methyl guanosine (**2**) (Fig. 1), being however fivefold less potent in HCV replicon than its β-2′-*C*-methyl guanosine analogue.[8] Nevertheless the ProTide technology applied to this nucleoside not only boosted the lipophilicity but also enabled by-pass of the limiting first phosphorylation step, delivering efficiently inside the host cell the corresponding 5′-monophosphate form.[9] The L-Alanine neopentyl phosphoramidate of β-2′-*C*-methyl-6-*O*-methyl guanosine (**3**) (Fig. 1) ,[8] also known as INX-08189 or BMS-986094, is one of the examples of a ProTide developed for HCV treatment.

It has previously been reported that both 2′-*C*-methyl guanosine 7-deaza and 7-aza guanosine triphosphates exhibited sub-micromolar inhibitory potencies against the polymerase (respectively IC_{50} = 0.12 μM and IC_{50} = 0.13 μM).[6] Hence, the polymerase does not discriminate between 7-aza and 7-deaza derivatives. We thus

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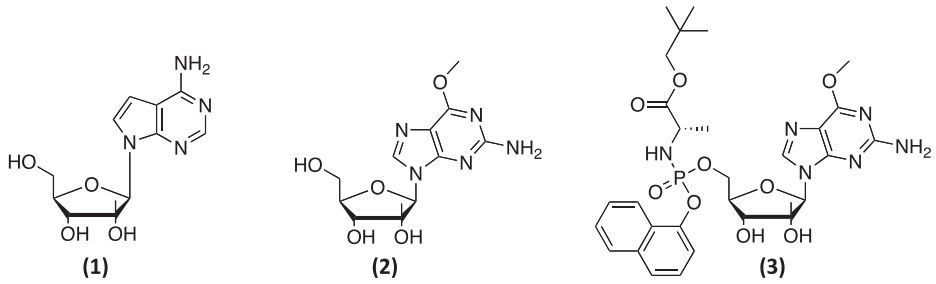

Figure 1. Structures of potent anti-HCV β-2′-*C*-methyl nucleosides (**1**–**2**) and ProTide (**3**).

decided to synthesise 7-deaza analogues of 2′-*C*-methyl-6-*O*-methyl guanosine and related ProTides in order to increase inhibitory potency against HCV.

β-2′-*C*-Methyl-6-*O*-methyl-7-deaza guanosine nucleoside (**8**) was synthesized in two stages: first the formation of the nucleobase (**4**) (Fig. 2), followed by coupling with the commercial 3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2′-*C*-methyl-1-*O*-methyl-α-D-ribofuranose (**5**) (Fig. 2).

7-Deaza-6-chloroguanine (**4**) was formed in moderate yield over two steps as described in the literature.[10,11] Displacement of the 6-chlorine of **4** by sodium methoxide was undertaken under reflux for 5 days but lead mainly to degradation and generation of 6-methoxy-7-deaza guanine in very poor yield (3%). It was then decided to perform this step directly at the nucleoside level. Condensation with the ribofuranose moiety (**5**) was performed, as described in the literature,[6] by in situ conversion to the 1-bromo analogue followed by subsequent reaction with 7-deaza-6-chloroguanine potassium salt (formed in situ with potassium hydroxide powder) to afford only the β-anomer of **6** (Fig. 2). Treatment of **6** with boron trichloride in dichloromethane resulted in the deprotection of dichlorophenylmethyl group to yield 2′-*C*-methyl-6-chloro-7-deazaguanosine (**7**). The latter was then converted to the 6-*O*-methyl analogue by substitution of the chlorine with sodium methoxide in methanol under reflux,[8] affording 2′-*C*-methyl-6-*O*-methoxy-7-deaza guanosine (**8**) after column chromatography, in 50–60% yield. Without prior protection of the nucleoside **8**, three 5′-ProTides (**9**–**11**) bearing the L-Alanine as amino acid moiety were synthesized following our standard procedure[12] using *tert*-butyl magnesium chloride as a base[13] (Fig. 2). Purification by column chromatography and preparative TLC were required to obtain pure compounds. NMR and analytical HPLC confirmed the structure with purity of at least 95% for all phosphoramidates. They were each obtained in 12–13% yield as a mixture of two diastereoisomers, which were only separated in the case of the L-alanine-*O*-cyclohexyl ester ProTide (**9a** and **9b**).

The three phosphoramidates (**9**–**11**) and their parent nucleoside (**8**) were evaluated in a HCV replicon assay (EC_{50}) and for cytotoxicity in Huh-7 cells (CC_{50}) (Table 1). Table 1 compares the biological results of synthesized compounds (**9**–**11**) and their 2′-*C*-methyl-6-*O*-methyl guanosine analogues (**2**, **3**, **12**–**13**).

In vitro results (Table 1) suggest that 2′-*C*-methyl-6-*O*-methyl-7-deaza guanosine (**8**) is not an inhibitor of HCV replication whereas its corresponding ProTides (**9**–**11**) boost the antiviral activity from 3- to 10-fold. So, to some extent the ProTide approach applied to 2′-*C*-methyl-6-*O*-methyl-7-deaza guanosine is effective and the results confirm the delivery of the 5′-triphosphate inside the cells, whereas the N-7 modification of the parent nucleoside (**8**) does not show the improvement of potency desired and either lacks of cell permeation or is a poor substrate for nucleoside kinases responsible for phosphorylation. The separated diastereoisomers **9a** and **9b** exhibit similar potency in replicon assays showing little if any influence of phosphorus stereochemistry on bioactiva-

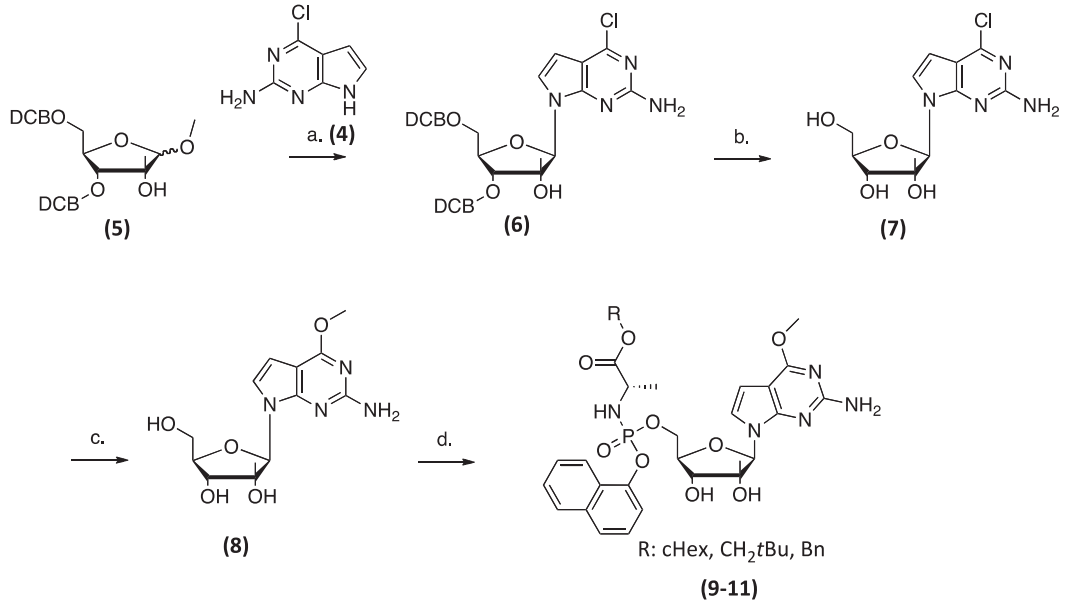

Figure 2. Synthesis of 2′-*C*-methyl-6-*O*-methyl-7-deaza guanosine (**8**) and ProTides (**9**–**11**). Reagents and conditions: (a) anhyd CH_2Cl_2, HBr (33% in acetic acid, 6.7 equiv), 0 °C to rt, 2 h; anhyd ACN, KOH (3.0 equiv), TDA-1 (0.2 equiv), rt, 1 h; (b) anhyd CH_2Cl_2, BCl_3 (10 equiv), −78 °C, 2 h then −20 °C, 2 h 30 min; (c) anhyd MeOH, NaOMe (3.0 equiv), reflux, overnight; (d) anhyd THF, *t*BuMgCl (1.2 equiv), naphthyl L-Alanine ester phosphochloridate (1.2 equiv), rt, overnight.

Table 1
Biological activity of 2′-*C*-methyl-6-*O*-methyl-7-deaza guanosine (**8**) and 2′-*C*-methyl-6-*O*-methyl guanosine (**2**) and their corresponding phosphoramidates

R	2′-*C*-Methyl-6-*O*-methyl-7-deaza guanosine			2′-*C*-Methyl-6-*O*-methyl guanosine		
	Compd	EC_{50} (μM)	CC_{50} (μM)	Compd	EC_{50} (μM)	CC_{50} (μM)
	8	>100	>100	**2**	3	>100
cHex	**9a**	13.3	18	**12**	0.03	6
cHex	**9b**	8.9	13	—	—	—
CH_2tBu	**10**	12.9	27	**3**	0.01	6
Bn	**11**	27.3	27	**13**	0.02	11

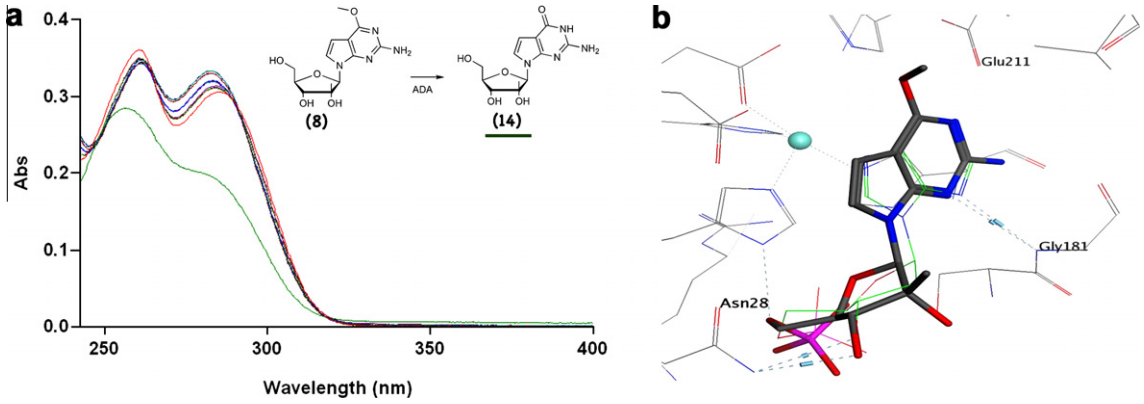

Figure 3. (a) UV spectra of conversion of 2′-*C*-methyl-6-*O*-methyl-7-deaza guanosine (**8**) into 2′-*C*-methyl-7-deaza guanosine (**14**) by adenosine deaminase assay; (b) docking of β-2′-*C*-methyl-6-*O*-methyl-7-deaza guanosine 5′-monophosphate (grey sticks) in ADAL-1 (green line: natural substrate adenosine 5′-monophosphate).

tion and potency. However, comparing the 7-deaza to the parent guanine series, it is remarkable that the removal of the N-7 of the nucleobase leads to a loss of potency over 1000-fold in the case of the neopentyl and benzyl derivatives (3 vs 10 and 13 vs 11). Their *C*log*P* values being similar (3.14 to 4.00), cell uptake is probably not the main issue in this case.

However, phosphoramidates **9**–**11** are somewhat cytotoxic towards Huh-7 cells resulting in a poor selectivity index in comparison to their of 2′-*C*-methyl-6-*O*-methyl guanosine analogues. Because the 2′-*C*-methyl modification of 7-deaza adenosine resulted in significant loss of cellular toxicity[7] and neither 2′-*C*-methyl guanosine nor its 7-deaza analogue exhibited cytotoxicity in Huh-7 cells,[6] and because 7-deaza guanosine lacks antiviral properties in cell cultures but is reported to be orally active in vivo against numerous RNA viruses without high toxicity,[14] the cytotoxicity may result from the 6-*O*-methyl modification. A similar trend has already been reported by our group[8] and this modification would ideally be metabolised to its guanine derivative affording the delivery of the potent 2′-*C*-methyl-7-deaza guanosine 5′-triphosphate (IC_{50} = 0.12 μM).[6] However, our key observation is that the removal of the 7-aza moiety greatly reduces anti-HCV efficacy in this series of nucleosides and activity is not greatly restored by the ProTide.

To further understand the impact of the methine group replacing the N-7 of the nucleobase in the biological activation pathway, some enzymatic assays and molecular docking were carried out with the 7-deaza analogue (**10**) of **3**.

Preliminary UV experiments were carried out incubating **8** with adenosine deaminase (ADA). As expected, the conversion of β-2′-*C*-methyl-6-*O*-methyl-7-deaza guanosine into β-2′-*C*-methyl-7-deaza guanosine does not occur at a nucleoside level (Fig. 3a). However molecular docking of β-2′-*C*-methyl-6-*O*-methyl-7-deaza guanosine 5′-monophosphate in an homology model of adenosine deaminase-like protein1 (ADAL-1),[15] recently reported as the enzyme involved in metabolism of 6-*O*/*N*-substituted purine monophosphates,[16] predicted that the later is a relatively good substrate (Fig. 3b). Thus, the β-2′-*C*-methyl-7-deaza guanosine 5′-monophosphate should be delivered intracellularly.

To further investigate if 2′-*C*-methyl-6-*O*-methyl-7-deaza guanosine phosphoramidates deliver the corresponding 5′-monophosphate intracellularly and thus may lead to the bioactive 2′-*C*-methyl-7-deaza guanosine 5′-triphosphate, enzymatic experiments were carried out to mimic the different stages of the putative mechanism of ProTide activation (Fig. 4). After incubation with carboxypeptidase Y (CPY) in Trizma buffer, enzymatic cleavage of the amino acid ester of phosphoramidate **10** was monitored by ^{31}P NMR. Spectra were recorded regularly and selected spectra are shown in Figure 5. The two peaks at δ_P ~4 ppm correspond to the two diastereoisomers of phosphoramidate **10**. The two phosphorus signals decrease over time confirming conversion of **10** to the amino acyl phosphate **15** lacking both the naphthyl and neopentyl ester moieties represented by the peak at 7.10 ppm (Fig. 5). While 50% of the diastereoisomer corresponding to δ_P ~4.04 ppm is converted after 5 h, more than 9 h is necessary for 50% of the disatereoisomer which peak appears at δ_P ~3.55 ppm, to be converted to its amino acyl phosphate derivative **15**. This difference in metabolism rate is probably a result of different fitting in the enzymatic catalytic site. These observations correspond to a metabolic conversion rate of 10 over 20-times slower than its analogue **3**.[8] This may partly explain the considerable loss of activity exhibited in replicon assays. It is to some extent rather surprising that the remote 7-deaza modification has such an impact on the deesterification step on the ProTide. Compound **15** is a key metabolite for further enzymatic conversion to the corresponding 5′-monophosphate **16** by phosphoramidase type enzymes.

To explain this difference of reactivity towards carboxypetidase-type enzymes, some molecular docking was performed using cathepsin A (PNB 1YSC) whose structure and substrate specificity is similar to CPY.[17] Preliminary docking of phosphoramidate **10** predicted that the S_p isomer fits best in the cathepsin pocket, thus

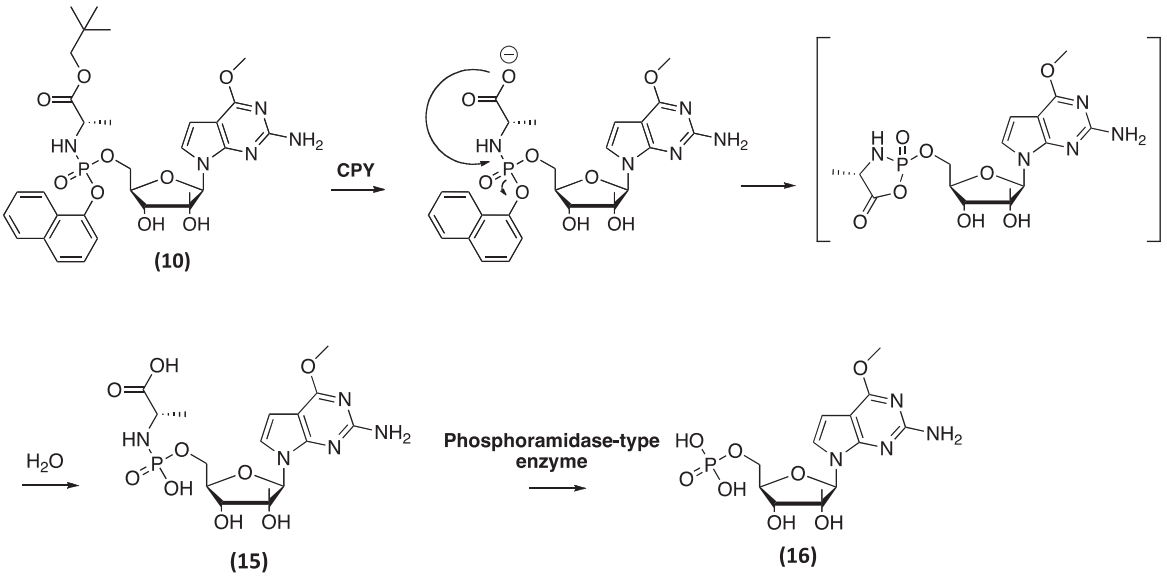

Figure 4. Putative mechanism of activation of 2′-*C*-methyl-6-*O*-methyl-7-deaza guanosine phosphoramidates.

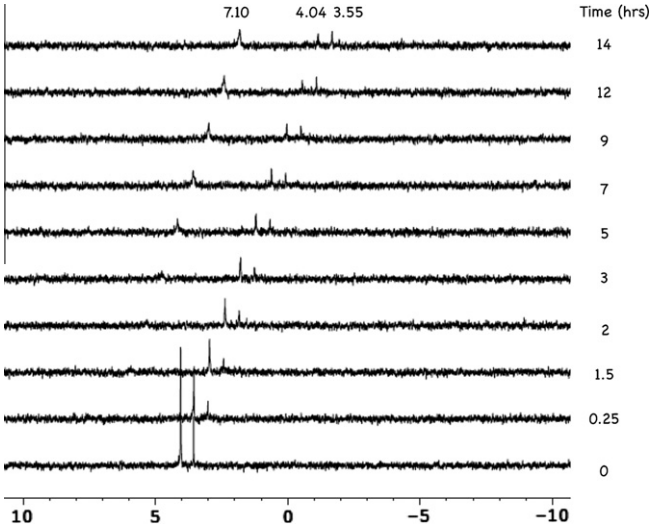

Figure 5. Carboxypetidase Y assay on phosphoramidate **10**.

S_p isomers of both **10** and analogue **3** were docked into the cathepsin active site (Fig. 6a). The removal of N-7 provokes an undesirable effect on the position of the amino acid ester carbonyl group of **10**, which lies towards the front of the pocket and is quite far from the essential catalytic residues Gly52, Gly53 and Ser146, whereas the carbonyl group of **3** faces inside the catalytic pocket resulting in faster ester cleavage. This would suggest that a hydrogen bond with the N-7 in the nucleobase pocket is probably essential to lock the nucleobase in a configuration that enables a good fit of the phosphoramidate carbonyl into the catalytic site of carboxyesterase-type enzymes. Thus, modeling data support the slow release of amino acid monophosphate **15** seen during CPY assays.

The second step of activation was investigated in order to confirm the release of the 5′-monophosphate **16**. ProTide **10** was incubated with Huh-7 cell lysates. Mass spectra and ^{31}P NMR spectra (data not shown) were recorded and suggested the formation of the desired 5′-monophosphate **16** ($M+Na^+ = 413$). Nevertheless conversion of **10** is very slow and even after 48 h, **10** remains the main specie present in the medium ($M+H^+ = 658$, $M+Na^+ = 680$, $M+K^+ = 696$). This result is in correlation with the predictive docking in Human Hint-1 (PNB 1KPF) (Fig. 6b), where the phosphorus lies far from the optimal position near the catalytic residues based on adenosine monophosphate, the natural substrate.

To conclude, we report the synthesis of 7-deaza derivatives of β-2′-*C*-methyl-6-*O*-methyl guanosine. Despite the efficiency of the ProTide technology, replacement of N-7 by a methine group leads to a significant loss of potency against HCV and loss of antiviral selectivity which cannot be salvaged by the ProTide motif. A much

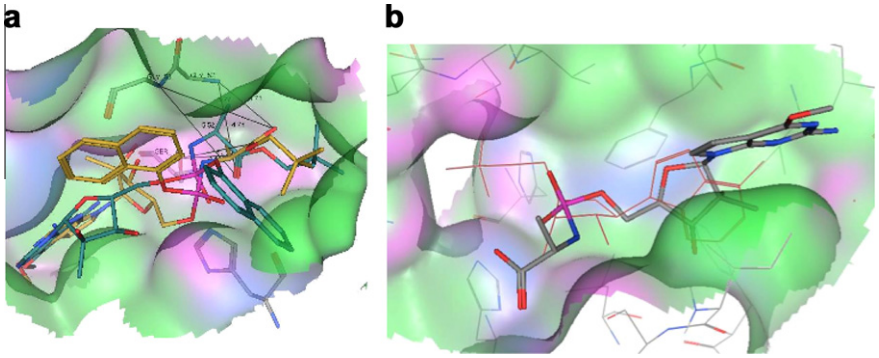

Figure 6. (a) Docking of S_P isomers of **3** (blue) and **10** (yellow) in the catalytic site of cathepsin A (red: oxygen, pink: phosphorus). (b) Docking of aminoacyl intermediate of **10** (grey stick) and adenosine 5′-monophosphate (AMP, red line) in the catalytic site of human Hint-1.

reduced rate of processing of the ProTide to the key amino acyl intermediate and the slow release of the 5′-monophosphate specie may be the reasons for this outcome, since the conversion to β-2′-*C*-methyl-7-deaza guanosine 5′-monophosphate by ADAL-1 should be efficient. The remarkable loss of potency at the ProTide level on formation of the 7-deaza species is a surprising outcome of this study and it shows the need to tune the ProTide motif, and understand its interplay with the nucleoside, on each occasion. Moreover, the lack of activity of these 7-deaza guanosine derivatives is not expected to be due to differences of affinity at the polymerase level compared to guanosine derivatives.[6]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.12.004.

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