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Fluorinated nucleosides as an important class of anticancer and antiviral agents

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Abstract

Fluorine-containing nucleoside analogues represent a significant class of FDA approved chemotherapeutics widely used in the clinic. The incorporation of fluorine into drug-like agents modulates lipophilic, electronic and steric parameters thus influencing pharmacodynamic and pharmacokinetic properties of drugs. Fluorine can block oxidative metabolism of drugs and the formation of undesired metabolites by changing H-bonding interactions. In this review, we focus our attention on chemical fluorination reagents and methods used in the nucleoside analogues field, including PET radiochemistry. We briefly discuss both the cellular biology and clinical properties of FDA-approved and fluorine-containing nucleoside/nucleotide analogues in development as well as common resistance mechanisms associated with their use. Finally, we emphasize pro-nucleotide strategies used to improve therapeutic outcome of nucleoside analogues in the clinic.

Keywords

Nucleoside analogues, Human equilibrative nucleoside transporter proteins (hENT), Reverse transcriptase (RT), NRTIs, ProTides (phosphoramidates), PET imaging

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Introduction

Over the last three decades medicinal fluorine chemistry has significantly evolved into an area of interest for many scientists. Incorporation of fluorine atoms(s) into a drug-like molecule can impart a range of chemical, physical and biological properties. Combining the benefits of fluorine

substitution with the established nucleoside class therapeutics has led to a number of clinically proven drug molecules used in both antiviral and anticancer therapy. Indeed, among fifteen FDA-approved pyrimidine and purine-based cytotoxic antimetabolites, the fluorine-containing nucleoside analogues make up a significant class of chemotherapeutics widely used in the clinic (Figure 1). In this review we focus on nucleophilic and electrophilic methods of fluorination and highlight novel and interesting synthetic strategies towards fluorinated nucleoside analogues (NAs). In addition, we outline the role ^{18}F plays as a radioisotope in PET imaging. We discuss the cellular biology and clinical properties of FDA approved fluorine-containing nucleoside/nucleotide analogues as well as those in clinical development. Furthermore, we emphasize strategies designed to circumvent the key resistance mechanisms associated with NAs thus improving their therapeutic efficiency in the clinic.

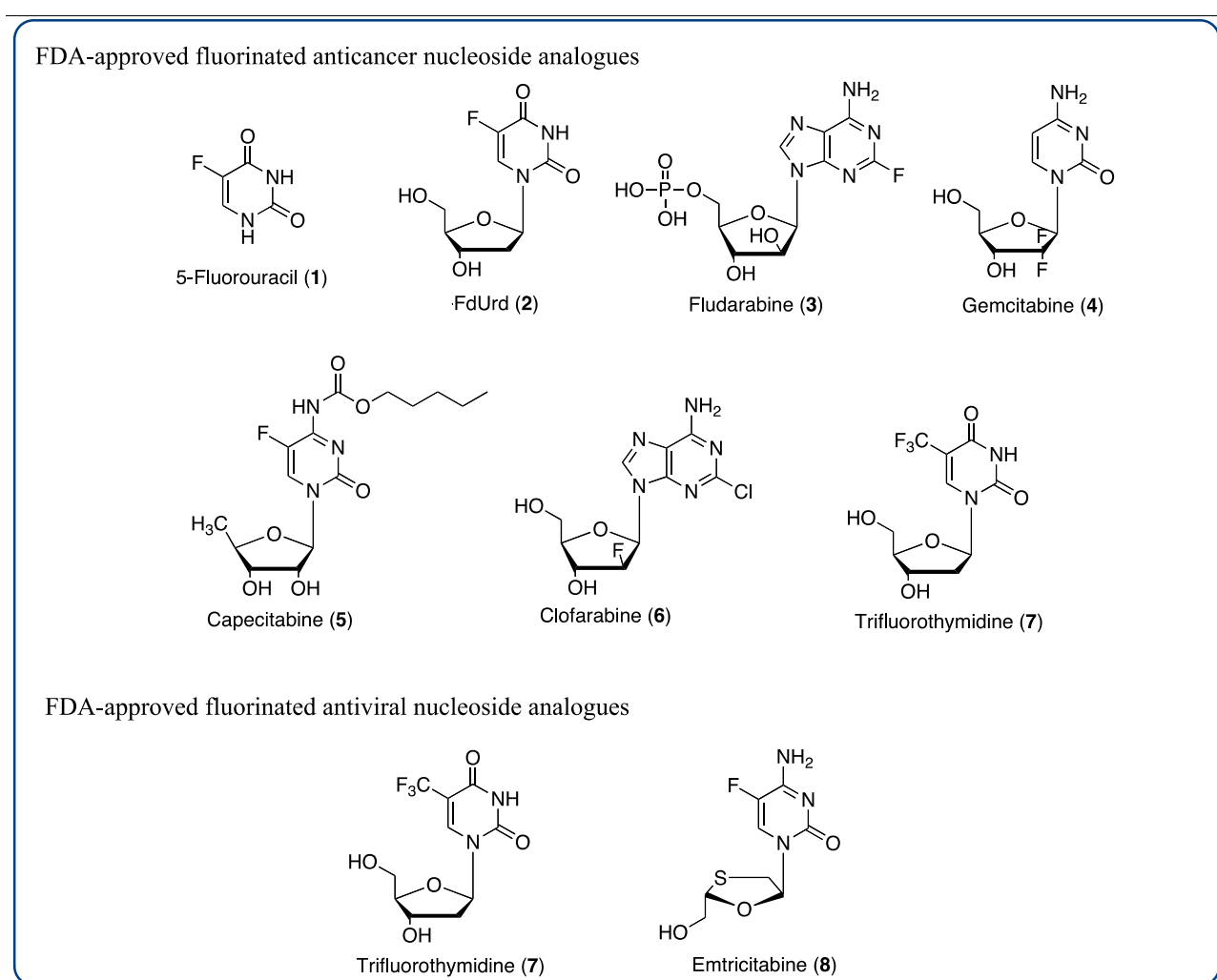


Figure 1. FDA-approved fluorinated anticancer and antiviral nucleoside analogues.

1. Overview of fluorine chemistry applied to nucleosides

More than 20% of medicines and novel drug candidates are estimated to contain fluorine within their chemical structure [1][2], and the special properties of fluorine within the context of drug discovery are summarised in Figure 2 and detailed below. Fluorine has been extensively exploited in the drug discovery and development process, but its incorporation has historically been limited by synthetic chemistry challenges.

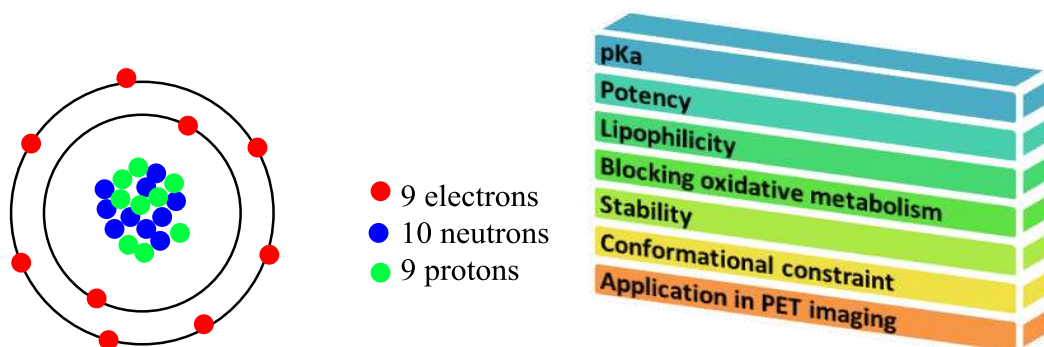


Figure 2. Structure and properties of the fluorine atom

Fluorine has the capability to increase the lipophilicity (logP), which together with the molecular size influences the membrane permeability of a molecule, playing a critical role in the optimization of drug candidates' oral bioavailability. The status of fluorine as the most electronegative atom in the periodic table (3.98 on the Pauling scale) also affects the acidic and basic properties of nearby functional groups by modifying the pKa of the molecule. This will influence its toxicity, selectivity, potency, and both pharmacodynamic and pharmacokinetic properties. Furthermore, the highly polarized C-F bond will often provide better stability to the molecule itself. As fluorine is a very small atom similar in size to H (van der Waals radius of 1.47 Å compared to 1.20 Å for H), fluorine is commonly used to substitute the H or the OH group in bioactive molecules causing minimal steric perturbation. However, by changing H-binding interactions, it can block oxidative metabolism and the formation of undesired metabolites [3][4].

The presence of a fluorine atom in the ribose ring of nucleoside analogues may have a significant impact on the conformation of nucleosides. It affects the dipole-dipole and gauche interactions, the anomeric effect, and the F-base interactions. Predictions on the activity of each single nucleoside are difficult to make, as the outcome of its conformation is dependent on each substitution. Generally, fluorine in the 2'-β-position of the ribose favours a south conformation of a molecule, which is often linked to enhanced anti-HIV activity for example, whereas the fluorine in

the 2'- α -position favours a north conformation (Figure 3). Nevertheless this rule doesn't apply to all fluorinated nucleosides. The pro-nucleotide CycloSal triesters of ara- and ribo-configured 2'-fluorinated-2',3'-dideoxyadenosines (F-ddA) were used to study the effects of the two different sugar pucker conformations induced by two opposite α and β configurations of the fluorine at C2' of the dideoxyribose moiety. F-ara-ddA is an active anti-HIV agent, whereas the ribo-analogue is inactive. Interestingly, the ribo-configured phosphotriesters prepared from the inactive F-ribo-ddA showed a level of anti-HIV activity that was even higher than that of F-ara-ddA [5]. Therefore stability and potential therapeutic activity of both conformations vary according to different substitutions on the nucleosides [6].

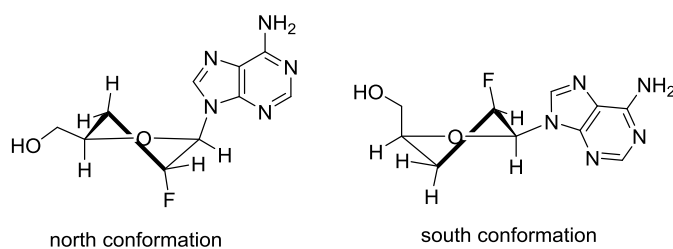


Figure 3. North and south conformation of two anomers of F-ddA

Moreover, fluorine also has a remarkable role in Positron Emission Tomography (PET) due to the convenient half-life of its radioisotope ^{18}F (109.8 min). A relatively short half-life allows synthesis of the tracer and the imaging process on the same day. In addition, ^{18}F is also a low positron emission energy emitter giving a good spatial resolution for imaging. All these factors make it the optimal radioisotope for PET imaging and much effort has been put into the discovery of novel and efficient routes towards synthesis of novel ^{18}F -PET tracers [7][8][9]. The glucose analogue 2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG) is the most ubiquitous PET tracer with multiple applications in oncology, cardiology and neurology [9][10]. However, also many ^{18}F -nucleoside analogues are currently under clinical investigation as proliferation biomarkers, reporter imaging probes for personalised medicine and prediction tools of the response to nucleoside analogue chemotherapy [11]. The challenge of their synthesis is to incorporate the ^{18}F -fluorine into the nucleoside analogues at a late stage in the synthesis so that the synthetic procedure and the imaging can occur within several half-lives to provide good quality scanning data.

1.1 Fluorination chemistry: nucleophilic and electrophilic methods

Numerous treatments against viral infections and cancer involve the use of nucleosides, nucleotides or their prodrugs, with many of them bearing a fluorine atom on the sugar or on the nucleobase moiety. Different synthetic strategies towards nucleosides have been extensively reviewed in recent

years [12][13][14]. Herein we will describe some standard and novel interesting synthetic pathways towards fluorinated nucleoside analogues.

The main approaches used for the synthesis of fluorinated nucleosides consist either in the fluorination of preformed nucleosides (divergent approach), which is particularly used in ^{18}F -PET chemistry, or in the N-glycosylation of the nucleobase with a fluorinated substrate (convergent approach).

1.1.1 Nucleophilic methods

In terms of ease of synthesis, aliphatic fluorinations are the most straightforward, with several fluoride salts being used to perform nucleophilic reactions. Olah's reagents (**9** and **10**, Figure 4) are useful when the conversion of secondary and tertiary alcohols is required, being selective for tertiary over the secondary alcohol.

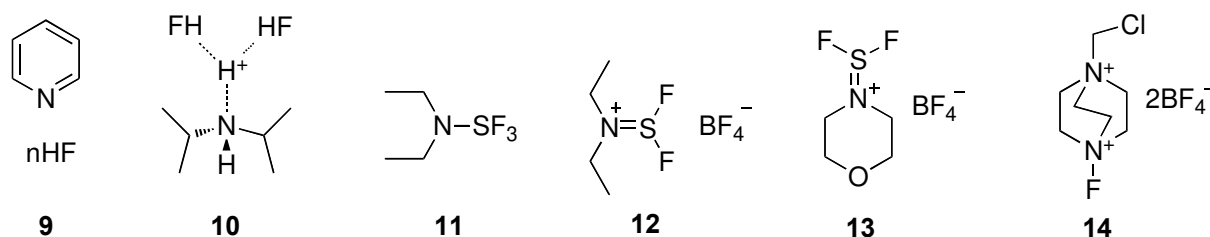
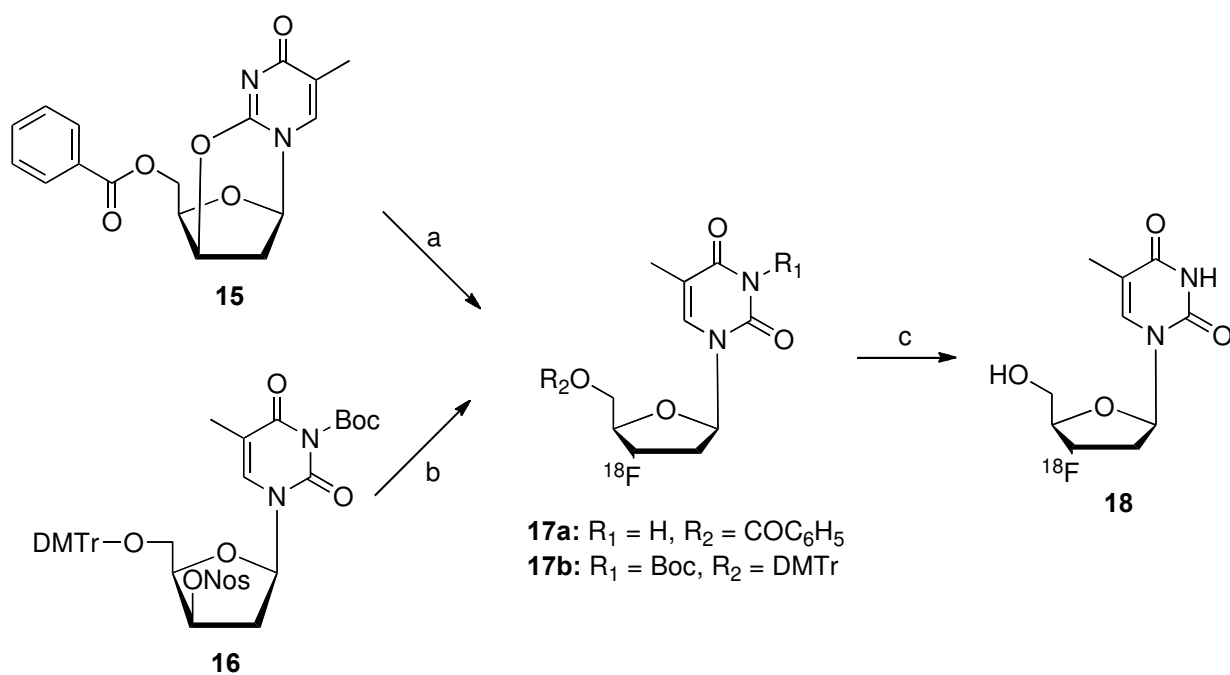


Figure 4. Nucleophilic and electrophilic fluorinating agents

Aliphatic nucleophilic fluorinations such as the one outlined in Figure 5 occur with $\text{S}_{\text{N}}2$ stereochemistry; good leaving groups and aprotic solvents are preferably required due to the low reactivity of fluoride. In PET chemistry crown ethers (such as Kryptofix 222TM) are used to enhance the weak nucleophilicity of ^{18}F -fluoride so that the incorporation can happen in a fast and efficient way. ^{18}FLT , the first human approved ^{18}F -nucleoside as a tumour proliferation biomarker is synthesised through this approach. A rapid and facile synthesis of ^{18}F -FLT consists in the use of two different precursor molecules (**15** and **16**), which need to be deprotected to give [^{18}F]-FLT (**18**) after the fluorination step [15].

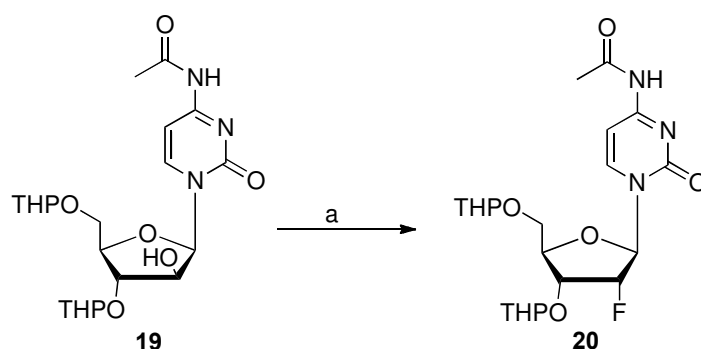
Figure 5. Synthesis of ^{18}FLT via two approaches



Reagents and conditions: (a) $\text{K}/(\text{K}222)^{18}\text{F}$, anhydr. DMSO, 160°C , 10min; (b) $\text{K}/(\text{K}222)^{18}\text{F}$, anhydr. CH_3CN , 135°C , 5min; (c) 1N NaOH, 55°C , 10 min or 1N HCl, 105°C , 5 min.

(Diethylamino)sulphur trifluoride (DAST) (**11**, Figure 4) is another versatile reagent for fluorination particularly useful in nucleoside chemistry. A fluorination reaction with DAST proceeds via an $\text{S}_{\text{N}}2$ mechanism consisting of displacement of the OH group with inversion of stereochemistry (Figure 6) [16].

Figure 6. Synthesis of 2'-F-nucleoside (20) using DAST as fluorinating agent



Reagents and conditions: (a) DAST, pyridine, DCM, 40°C

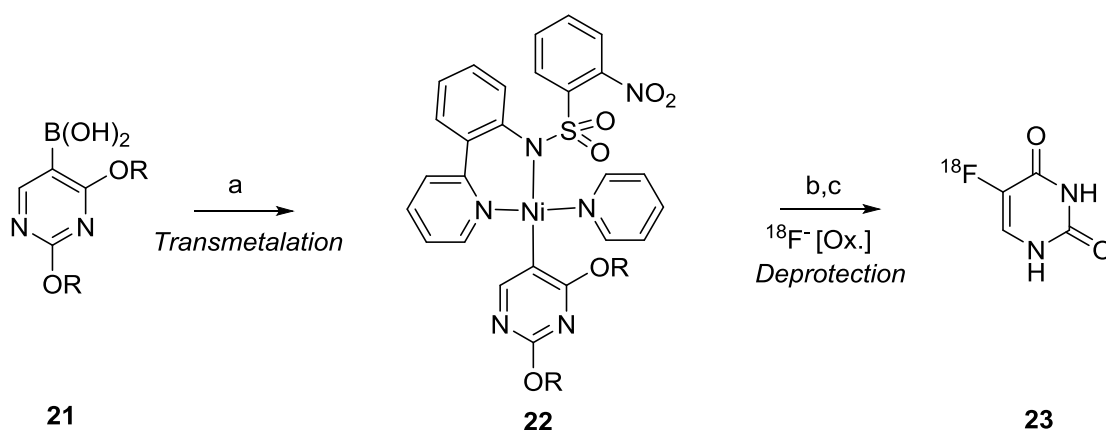
This agent is used to convert primary, secondary, tertiary and allylic alcohols to fluorides, but also for the conversion of aldehydes and ketones to difluorides. Diethylaminodifluorosulfinium tetrafluoroborate (XtalFluor-E, **12**) and morpholinodifluorosulfinium tetrafluoroborate (XtalFluor-M, **13**) shown in Figure 4 are newer fluorinating agents that are more thermally stable than DAST.

They are used to convert alcohols to alkyl fluorides and carbonyl groups into gem-difluorides, being more selective than DAST and leading to fewer elimination side products [16]. Recently, several studies have been carried out to look for improved methods and substrates for nucleophilic fluorination reactions. Further developments have been applied also to radiochemistry, for example in the use of metal-catalysts for allylic fluorination reactions [17].

Nucleophilic aromatic fluorination is operationally more challenging than aliphatic fluorination, and in PET radiochemistry is often used to make radiolabelled arenes. High temperature and activated substrates with electron withdrawing groups on the aromatic rings in the *ortho* or *para* positions are commonly required to counter the normally disfavoured S_NAr mechanism. When the aromatic group contains electron rich groups, a strategy has been developed to circumvent this issue, whereby an electron-donating group is masked with an electron withdrawing group using appropriate protecting group chemistry.

In PET radiochemistry ¹⁸F⁻ fluoride is commonly introduced into the aromatic moiety by the use of arylodonium salts [8]. New studies focus in finding methods to improve reaction yields and new synthetic strategies to access the aryl fluoride. A number of transition metal catalysed nucleophilic fluorinations have been studied. For example, a novel approach uses nickel σ-aryl complexes as pre-catalysts to access ¹⁸F-fluorouracil (**23**), a PET tracer for cancer imaging (Figure 7). This is the first application of a transition-metal-mediated fluorination for clinical application [18].

Figure 7. Synthesis of ¹⁸F-Fluorouracil (23**) via transition-metal-mediated fluorination**

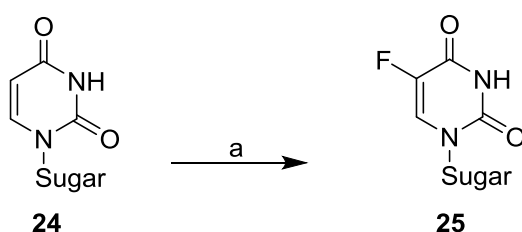


Reagents and conditions: (a) LnNi^{II}X₂, pyridine, 70°C, 1h; (b) PhI(4-OMe-pyridine)₂(OTf)₂, (18-c-6)K¹⁸F, MeCN (0.5% H₂O), 23°C, 1 min; (c) HCl, EtOH, 23°C, 2 min.

1.1.2 Electrophilic fluorination

Fluorination of aromatic rings and/or electron rich-double bonds can be achieved with the use of an electrophilic-fluorinating agent. Since most applications preclude the highly toxic and non-selective fluorine gas, fluorine must bind an electronegative atom such as nitrogen, activated by a strongly withdrawing group such as the sulfonyl group. This synthetic strategy is quite common in nucleoside chemistry when fluorination of a heterocyclic ring is required as depicted in Figure 8. A convenient electrophilic fluorinated agent is Selectfluor (**14**, Figure 4) which, compared to F₂, is safer, solid and soluble in polar solvents. It can also be used in particular cases for fluorination of sugar moieties bearing an electron-rich double bond.

Figure 8. Electrophilic fluorination with Selectfluor (14)

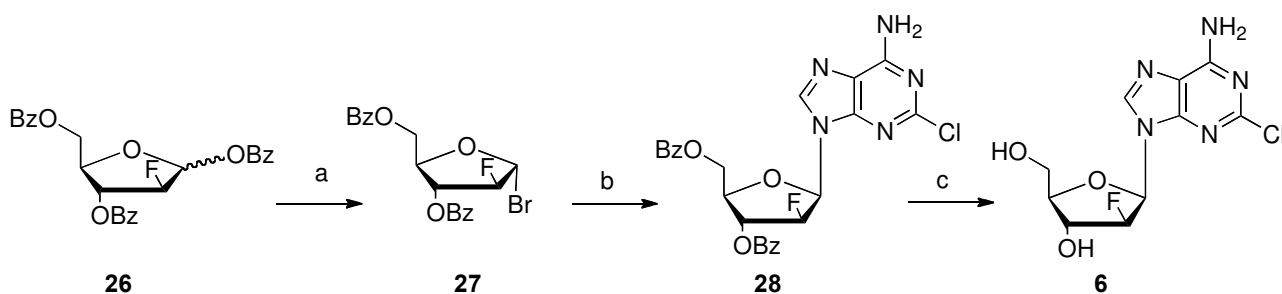


Reagents and conditions: (a) Selectfluor, HOAc-H₂O.

1.2 N-glycosylation of fluorine-containing starting material

The N-glycosylation reaction between (fluorinated) nucleobase and sugar moieties can be performed when later-stage fluorination is either not possible or convenient. This convergent approach is used particularly in the synthesis of 2'-β-fluoro nucleosides. First the fully-protected sugar (**26**) is brominated to form the 1-α-glycosyl bromide (**27**), formation of which leads preferentially to the formation of β-nucleoside (**28**) as reported in the synthesis of the antineoplastic agent clofarabine (**6**) (Figure 9) [19].

Figure 9. Synthesis of clofarabine (6)

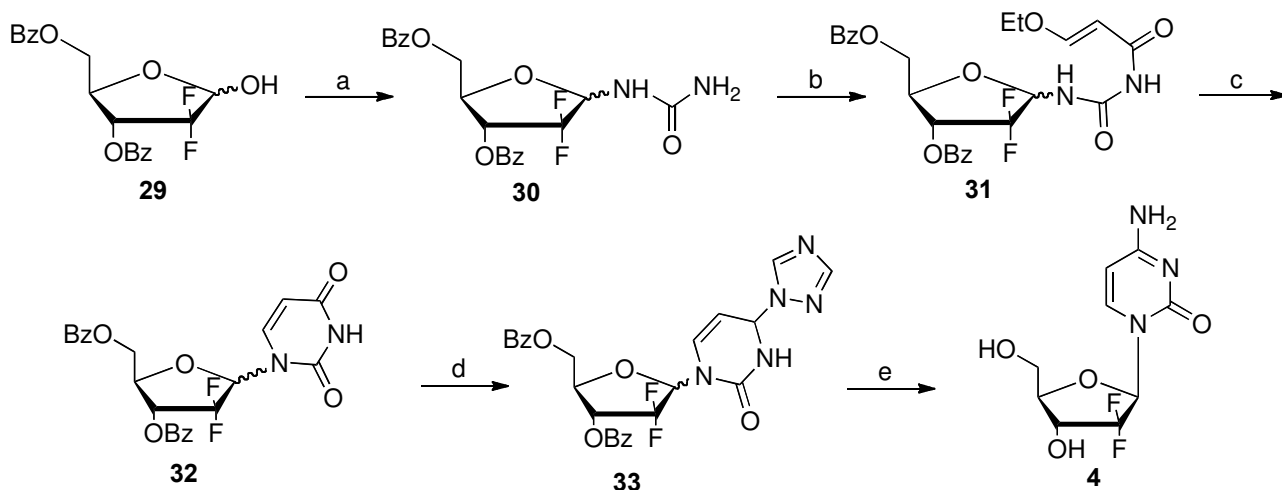


Reagents and conditions: (a) HBr, AcOH, rt; (b) 2-chloroadenine, KO^t-Bu, MeCN, t-AmOH, DCE, 55°C; (c) cat. NaOMe, MeOH, rt.

This synthetic approach is commonly used also in PET chemistry for the synthesis of several ^{18}F - β nucleosides as reported in the novel synthesis of ^{18}F FIAU, a reporter gene for the expression of the herpes simplex virus type-1 thymidine kinase (HSV1-tk) [20][21]. General synthetic approaches for the convergent synthesis of fluorinated nucleosides have recently been extensively reviewed by Schinazi *et al* [22].

Several synthetic approaches have been investigated for the synthesis of gemcitabine (**4**), an established anticancer drug against pancreatic, breast and ovarian cancer. The conventional scheme consists of a convergent synthesis where the 2-deoxy-2,2-difluororibofuranose derivative (**29**) is protected and activated, to further react with an activated cytosine. New linear synthetic approaches towards gemcitabine (**4**) have also been developed achieving moderate anomeric selectivity (Figure 10) [23].

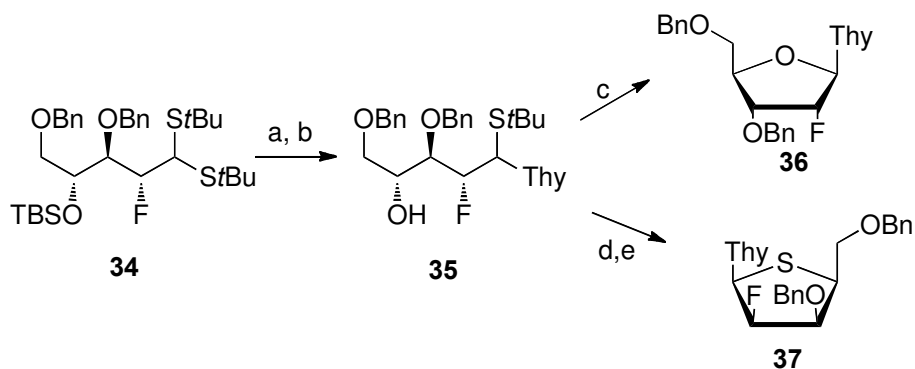
Figure 10. Linear synthesis of gemcitabine (4)



Reagents and conditions: (a) urea, dioxane, rt, 66 h; (b) (*E*)-3-ethoxyacryloyl chloride, MeCN, reflux, 18 h; (c) HCl, AcOH, rt, 24 h; (d) 1,2,4-triazole, 2-chlorophenyl phosphorodichloridate, pyridine, rt, 5 d; (e) 7N NH₃, MeOH, rt, 36 h.

Another recent study also shows advances in diastereoselective synthesis of 2'-fluoro-nucleoside analogues using an acyclic approach. Two nucleoside scaffolds, the 1',2'-*trans*-furanosides (**36**) and 1',2'-*cis*-thiofuranosides (**37**) with a fluorine in the C2'-position have been synthesised using the following strategy with the potential to be applied to many other nucleosides (Figure 11) [24].

Figure 11. Diastereoselective synthesis of 2'-F- nucleoside analogues

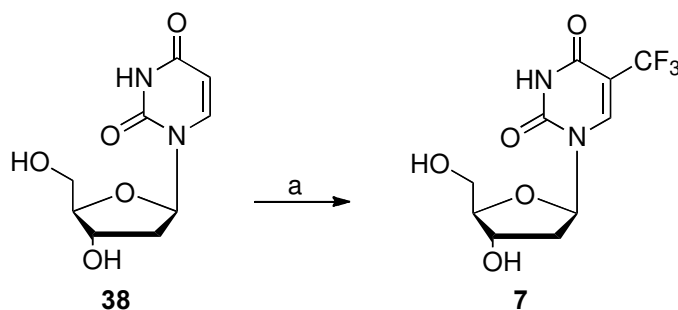


Reagents and conditions: (a) I_2 , Silylated thymidine, THF, 25°C, 48 h; (b) HF-pyridine, THF, 25°C, 16 h (c) $Me_2S(SMe)BF_4$, THF, 25°C, 5 h; (d) $MsCl$, Et_3N , anhydr. DCM, 25°C, 5 h; (e) 2,6-lutidine, 160°C, 48 h.

1.3 Trifluoromethylation

Direct trifluoromethylation of nucleoside analogues can be used as a synthetic approach towards trifluoromethylated nucleosides such as trifluorothymidine (TFT, **7**) an agent with both anticancer and antiviral properties (Figure 12) [25].

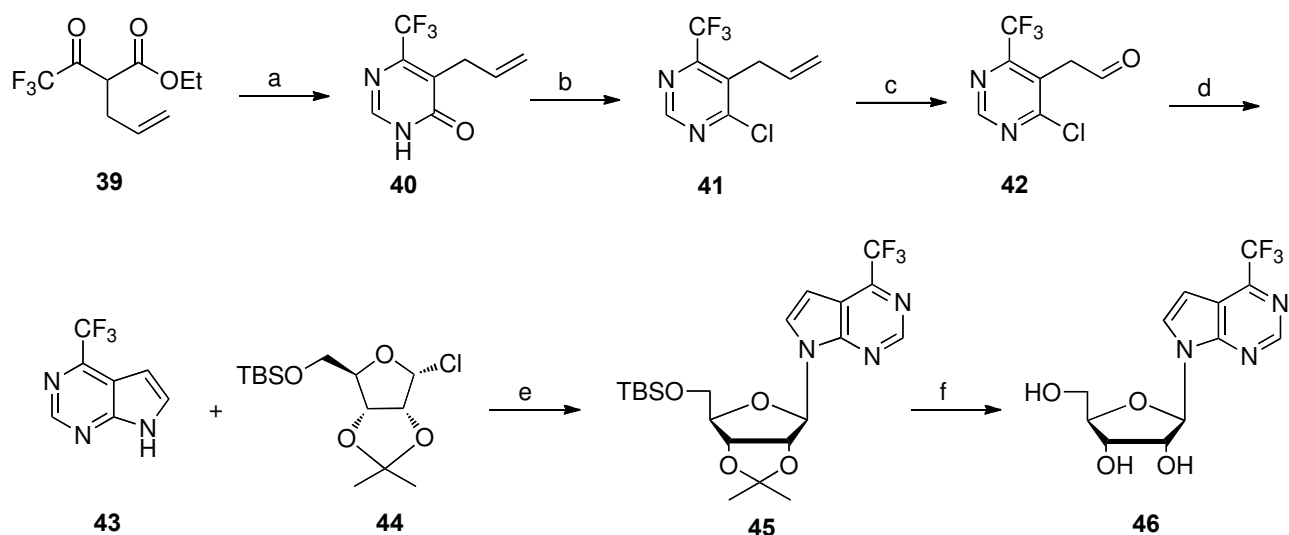
Figure 12. Synthesis of the anticancer and antiviral agent TFT (7)



Reagents and conditions: (a) Trifluoroiodomethane, DMSO, H_2SO_4 , H_2O , $FeSO_4$, 40°C.

Recent studies concerning trifluoromethylation led to the development of a new synthetic pathway based on N-glycosylation of a trifluoromethylated-substrate obtained from an easily accessible building block as shown in Figure 13. Interestingly, the nucleosides were obtained with good β -selectivities [26].

Figure 13. Synthesis of trifluoromethylated nucleoside analogue (46)



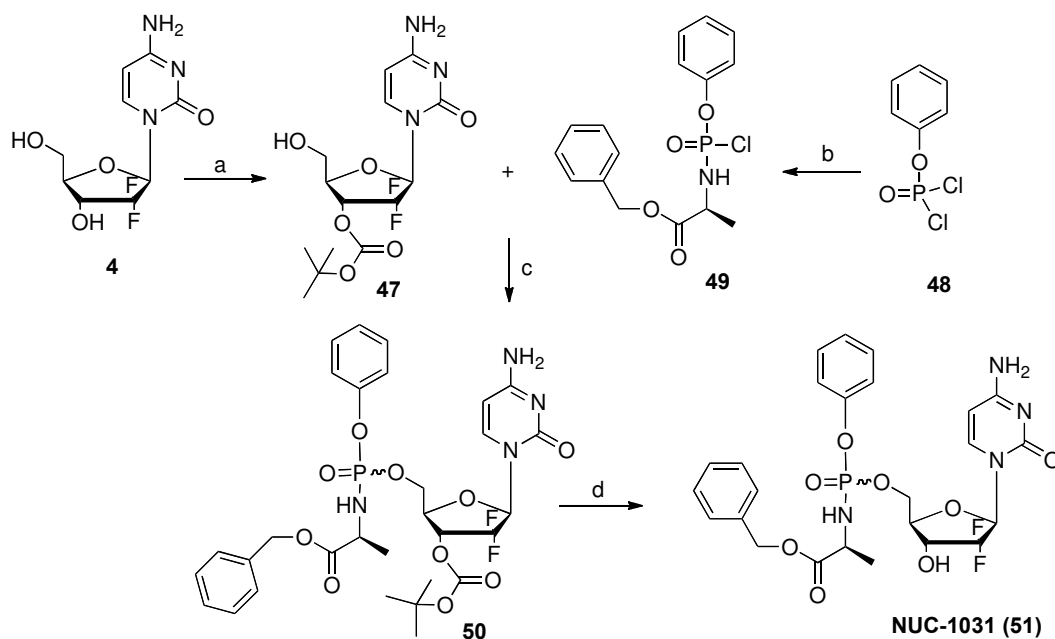
Reagents and conditions: (a) Sodium ethoxide, formamidine hydrochloride, EtOH, reflux, 16 h; (b) POCl₃, reflux, 17 h; (c) O₃, DCM, MeOH, -78°C, 15 min. then DMS, rt, 18 h; (d) NH₃, EtOH, rt, 24 h, then *p*-TsOH, MeOH, rt, 19 h; (e) NaH, MeCN, 0°C, 5 min., rt, 30 min. then **44**, THF, rt, 16 h; (f) CF₃COOH (90% in H₂O, v/v), rt, 5 min.

2. Synthesis of fluorinated nucleotide analogues - the phosphoramidate (ProTide) approach

Despite the widespread utility of nucleoside analogues as both antiviral and anticancer agents, drug resistance represents a major problem for their clinical application. In order to express therapeutic activity, nucleoside analogues need to be phosphorylated by cellular kinases to the corresponding 5'-mono-, di- and triphosphate forms. Although several synthetic strategies have been developed to access the active 5'-triphosphate form [27], they are not viable drug candidates due to their chemical instability and high polarity which disallow effective crossing of these species through cell membranes. In the activation pathway of nucleoside analogues the first phosphorylation is considered to be rate-limiting step therefore many approaches to develop prodrugs of nucleoside monophosphate forms have been developed [28].

The phosphoramidate technology also known as ProTide approach, developed by McGuigan and co-workers in 1995 is the most successful of the pro-nucleotide strategies. Phosphoramidates consist of a 5'-masked monophosphate moiety (built using: an amino acid ester and aryloxy group) introduced into the nucleoside template at the 5'-position, which, after cellular nucleoside-transporter-independent uptake and subsequent intracellular metabolism, deliver the 5'-monophosphate form [29]. Some anticancer and antiviral ProTides are characterized by the presence of fluorine in their chemical structure. The fluorinated nucleoside is used as substrate for their chemical synthesis as exemplified in Figure 14 for gemcitabine phosphoramidate NUC-1031 (**51**) [30].

Figure 14. Synthesis of ProTide NUC-1031 (51)



Reagents and conditions: (a) DBOC, Na₂CO₃, 1,4-dioxane/H₂O (4:1 v/v), rt, 48 h; (b) L-Ala benzyl ester tosylate salt, Et₃N, dry DCM, -78°C, 3 h; (c) *tert*-BuMgCl, THF, rt, 16 h; (d) TFA/DCM (1:1 v/v), 0°C, 4 h.

3. Fluorinated anticancer nucleosides

Nucleoside analogues (NAs) are considered as cornerstones in the treatment of patients with cancer and viral infections since they were first introduced into clinical use over five decades ago. Nucleoside analogues are antimetabolites that mimic natural nucleosides in the way they are metabolised and exert biological activity. After cellular uptake using nucleoside-transporter systems NAs become substrates for specific nucleo(s)tide kinases which convert them to the 5'-mono, -di and triphosphate forms. Such active molecules can interfere with the *de novo* synthesis of DNA/RNA precursors, which leads to the inhibition of DNA and RNA synthesis and eventually to suppression of cell growth and division.

3.1 Pyrimidine-based anticancer fluorinated nucleoside analogues

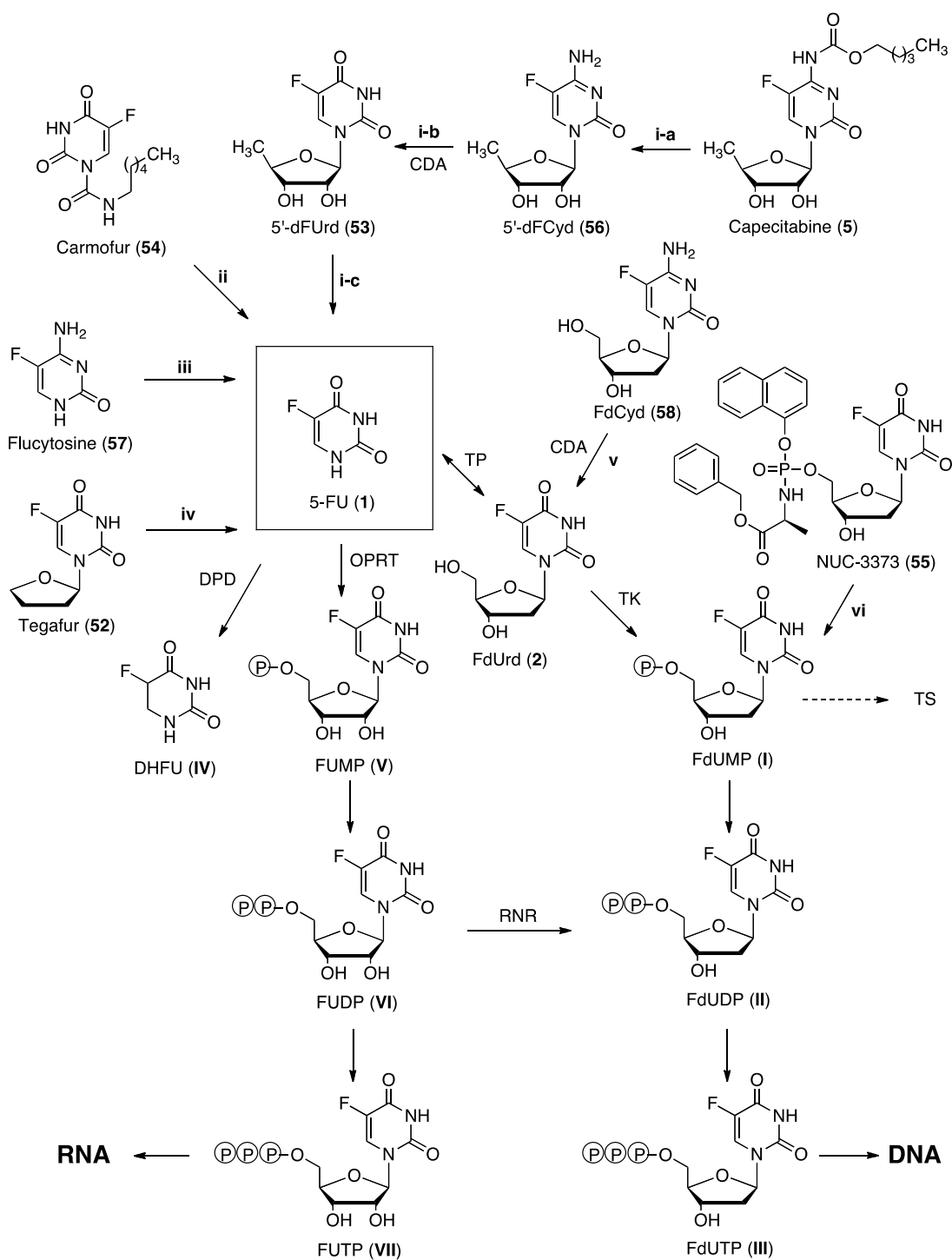
3.1.1 5-Fluorouracil and prodrugs

One of the first FDA approved anticancer agents was 5-fluorouracil (**1**) (included in the WHO list of essential medicines), and along with its nucleoside analogue 5-fluoro-2'-deoxyuridine (FdUrd, **2**), is still in clinical use to treat patients with solid tumours including colon, gastric, breast and pancreatic carcinoma (Table 1). In order to exert activity, both compounds after intracellular uptake need to be metabolized to the 5'-monophosphate form FdUMP (**I**), which forms a stable ternary complex with thymidylate synthase (TS) and the reduced co-factor 5,10-

methylenetetrahydrofolate (5,10-CH₂-THF) acting as a source of the methyl group. The presence of fluorine being more tightly bound to carbon (C-5) than hydrogen in FdUMP, prevents β -elimination reaction to occur with release of TS enzyme. As a consequence, the TS remains trapped in the TS/FdUMP/mTHF ternary complex causing irreversible inhibition of its enzymatic function [31][32]. Therefore, a formation of TMP, a building block for DNA synthesis and repair, is diminished. However, the effectiveness of 5-FU (**1**) in the clinic is limited since less than a third of patients achieve objective responses [33]. Moreover, inherent and acquired resistance mechanisms that are usually associated with the nucleoside analogue 5-FU (such as decreased cellular uptake, decreased activation to the 5'-monophosphate form by thymidine kinase or overexpression of TS) have been also reported. Many prodrugs of 5-FU (**1**) including capecitabine (**5**), tegafur (**52**), doxifluridine (furtulon, **53**), and carmofur (**54**) have been developed to address the key resistance mechanisms and are currently in use in the clinic. An orally administered capecitabine (**5**) is recognized as the most important prodrug of **1** and as a single agent is used for the treatment of metastatic breast cancer (that is resistant to both paclitaxel and anthracycline), metastatic colorectal cancer and adjuvant colon cancer [34].

One of the most effective strategies that address the key resistance mechanisms associated with nucleoside analogues in the clinic is the phosphoramidate (ProTide) technology. Delivery of the free 5'-monophosphate form of a nucleoside analogue inside a cell is particularly important as the first phosphorylation is considered as the rate-limiting step in the activation of most nucleoside analogues. The application of the 5'-phosphoramidate approach to FdUrd (**2**) led to the discovery and further clinical development of L-alanine-based 5'-ProTide NUC-3373 (**55**) [35]. Preclinical *in vitro* data has shown that NUC-3373 exerts its cytostatic activity independently of thymidine kinase in TK-deficient cell lines. In addition, NUC-3373 is resistant to the degradative action of catabolic enzymes such as thymidine phosphorylase (TP), an enzyme often upregulated in tumour cells or expressed in mycoplasma-infected tumour tissue [36], and dihydropyrimidine dehydrogenase (DPD), an enzyme abundantly expressed in the liver. The cytostatic potency of ProTide **55** is maintained in the tumour cell lines that lack hENT1 transporter (CEM/hEnt-0) whilst that of FdUrd (**2**) is reduced by 63-fold [37]. Moreover, *in vitro* NUC-3373 generates up to 363-fold higher intracellular levels of FdUMP (**I**) in comparison with 5-FU in human colorectal cancer cell line HT29. *In vivo* NUC-3373 achieves significantly greater tumour volume reduction than 5-FU in the human colorectal cancer HT29 mouse xenograft model [38]. In 2016, NUC-3373 entered into a Phase I clinical study in patients with advanced solid tumours (Table 1). The metabolism of **1** and bioactivation pathways of its derivative agents is summarised in Figure 15.

Figure 15. Metabolism of 5-FU, and its nucleoside and nucleotide derivatives



Abbreviations of structures: 5'-dFCyd: 5'-deoxy-5-fluorocytidine (**56**); 5'-dFUrd: 5'-deoxy-5-fluorouridine (**53**); DHFU: 5-fluorodihydrouracil (**IV**); FdCyd: 5-fluoro-2'-deoxycytidine (**58**); 5-FU: 5-fluorouracil (**1**); FdUrd: 5-fluoro-2'-deoxyuridine (**2**); FdUMP: 5-fluoro-2'-deoxyuridine-5'-monophosphate (**I**); FdUDP: 5-fluoro-2'-deoxyuridine-5'-diphosphate (**II**); FdUTP: 5-fluoro-2'-deoxyuridine-5'-triphosphate (**III**); FUMP: 5-fluorouridine-5'-monophosphate (**V**); FUDP: 5-fluorouridine-5'-diphosphate (**VI**); FUTP: 5-fluorouridine-5'-triphosphate (**VII**); Enzymes: CDA: cytidine deaminase; DPD: dihydropyrimidine dehydrogenase; OPRT: orotate phosphoribosyl transferase; RNR: ribonucleotide reductase; TK: thymidine kinase; TS: thymidylate synthase; TP: thymidine phosphorylase; Inhibition is represented by dashed lines.

Metabolic pathways of 5-FU and its nucleoside/nucleotide derivatives: **i(a-c):** (a) cleavage of N⁴-pentyl-carbamate in capecitabine (**5**) by carboxylesterase (in liver) to form 5'-dFCyd (**56**); (b) CDA-mediated deamination of 5'-dFCyd (**56**) to 5'-dFUrd (**53**) (in liver, or tumour); (c) TP-mediated conversion of 5'-dFUrd (**53**) to 5-FU (**1**); **ii:** Successive oxidation at the hexylcarbamoyl side-chain followed by excision of the side-chain from the pyrimidine ring; **iii:** Deamination of flucytosine (**57**) by cytosine deaminase; **iv:** Hydroxylation of Tegafur (**52**) to form 5'-hydroxytegafur followed by spontaneous decomposition to 5-FU (**1**); **v:** CDA-mediated deamination of FdCyd (**58**) to FdUrd (**2**); **vi(a-d):** (a) Hydrolysis of the ester moiety in the 5'-ProTide NUC-3373 (**55**) mediated by carboxylesterase-type enzyme; (b) spontaneous cyclization with displacement of the aryl moiety; (c) spontaneous hydrolysis of cyclic mixed anhydride intermediate; (d) P-N bond cleavage mediated by phosphoramidase-type enzyme to deliver FdUMP (**1**).

3.1.2 Gemcitabine and prodrugs

The second prominent and clinically widely used fluorinated anticancer agent is gemcitabine (2'-deoxy-2',2'-difluorocytidine, dFdC, **4**) approved for the treatment of pancreatic, non-small cell lung, ovarian and breast cancers [39][40]. Similarly to 5-FU, the clinical effectiveness of gemcitabine is limited due to resistance mechanisms caused by poor cellular uptake, poor conversion of gemcitabine into active metabolites by deoxycytidine kinases and/or rapid degradation (deamination) into the inactive potentially toxic by-product dFdU catalysed by cytidine deaminase [41][42][43]. The important active form of dFdC, the triphosphate metabolite (dFdC-TP), incorporates into the DNA chain during S-phase of cell cycle causing masked chain termination of DNA synthesis and eventually cell death. Another key active metabolite of dFdC, the diphosphate form (dFdC-DP), contributes significantly to the anticancer activity of dFdC by inhibiting ribonucleotide reductase (RNR). This results in depletion of the natural deoxynucleotide pool (essential substrates for DNA synthesis) and as a consequence enhances the incorporation of dFdC-TP instead of natural deoxynucleotides as a substrate for DNA polymerase [44].

In order to improve the clinical efficacy of gemcitabine, in particular to increase the intracellular generation of the active metabolite, dFdC-TP, as well as increasing the plasma half-life, the level of protection from catabolic action of cytidine deaminase and bioavailability, many modifications at the 4-(*N*) and 5'-sites of gemcitabine have been applied [45]. This has led to development of novel gemcitabine-based prodrugs such as LY2334737 (**59**), CO-101 (**60**) and the phosphoramidate NUC-1031 (**51**) (Figure 16). LY2334737, primarily designed to block the site of deamination of **4**, links valproic acid with gemcitabine via an amide bond at the 4-(*N*)-position of the cytosine base [46]. The first-in-human Phase I clinical trial of LY2334737 (**59**) as a single agent in patients with advanced and metastatic solid tumours has been completed [47]. An additional Phase Ib study of LY2334737 in a combination with capecitabine in patients with advanced solid tumours was designed to determine the recommended dose of **59** for future Phase II studies [48].

However, due to hepatic toxicities observed in patients in this study, further development of LY2334737 was discontinued.

Another gemcitabine-based agent that has entered clinical trials is gemcitabine elaidate CO-101 (CP-4126, **60**). This lipophilic prodrug with an unsaturated fatty acid at the 5'-site of gemcitabine was designed with the aim to increase plasma membrane permeability independently from nucleoside-transporter systems. Inside the cell, CO-101 is first converted to free gemcitabine after carboxyesterase-mediated cleavage of the fatty acid chain and subsequently to its 5'-monophosphate form (dFdC-MP) by deoxycytidine kinase [49][50]. However, in the Phase I study high levels of dFdU were generated suggesting that CO-101 was rapidly deaminated by CDA [51]. Several clinical trials for **60** (in monotherapy and in combination for advanced solid tumours) have been conducted. However, further clinical development of CO-101 was suspended as the agent was not superior to gemcitabine and no difference in overall survival between the two groups was achieved (in either the primary analysis of the hENT1-low patient population, or in the overall intent-to treat population) in metastatic pancreatic cancer trials [52][53].

NUC-1031 (**51**) is the gemcitabine L-alanine-based 5'-phosphoramidate designed to overcome all three of the resistance mechanisms (cellular uptake, kinase mediated phosphorylation and deamination) that have been associated with a poor survival prognosis to gemcitabine therapy [54][55][56]. After cellular uptake, independent of nucleoside transporters, **51** releases the 5'-monophosphate form via consecutive steps mediated by the action of carboxyesterase-type enzyme (ester hydrolysis) and phosphoramidase-type enzyme (P-N bond cleavage) [57]. The first-in-human Phase I study in patients with advanced solid tumours was opened in 2012. Encouraging pharmacokinetics and a favourable efficacy and safety profile have been reported for NUC-1031 in this study. The plasma half-life of NUC-1031 was more favourable than gemcitabine (7.3 hours versus 1.5 hours), and NUC-1031 achieved 217 x higher intracellular dFd-CTP levels than those reported for gemcitabine [58]. Final results of the first-in-human Phase I study of NUC-1031 in patients with solid tumours indicated the achievement of durable disease control in a high proportion of patients including patients refractory to, or who relapsed on prior gemcitabine therapy [59]. Currently, NUC-1031 is being assessed in several clinical studies for the treatment of patients with ovarian, biliary and pancreatic cancers as shown in Table 1.

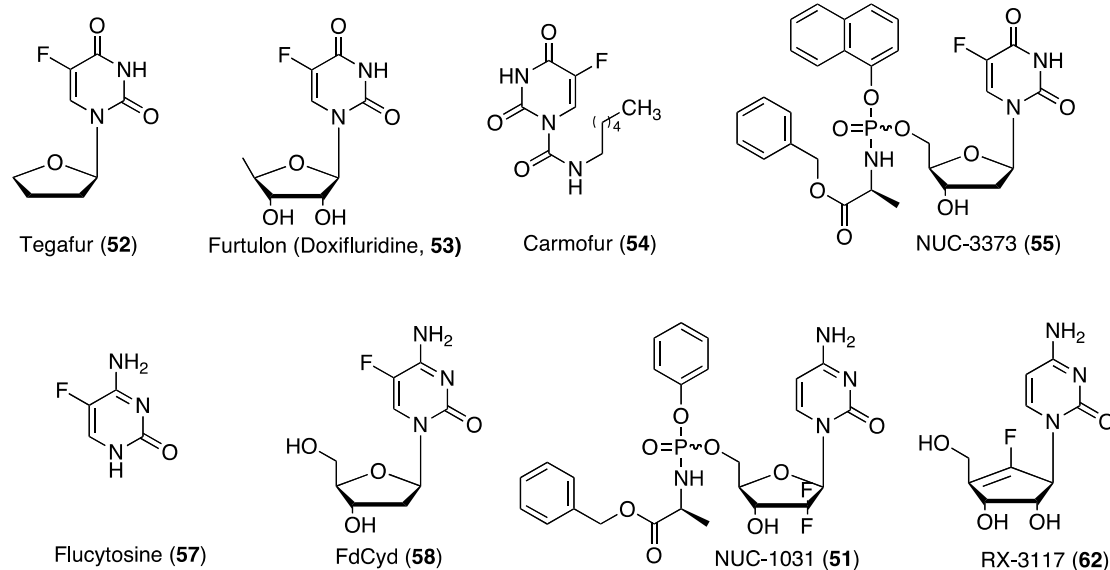
3.1.3 Other pyrimidine-based anticancer fluorinated nucleoside analogues

Trifluorothymidine (TFT, **7**) is a 5-CF₃-bearing nucleoside analogue closely related to thymidine. Within the cell, TFT is phosphorylated by thymidine kinase to the corresponding 5'-monophosphate

form as one of its active metabolites which reversibly inhibits thymidylate synthase (TS) by binding to the active site of the enzyme [60]. The 5'-monophosphate form of TFT is a substrate for further phosphorylation steps to give 5'-di- and 5'-triphosphate forms. The triphosphate form of TFT (TFT-TP) is also an active metabolite that can be incorporated into DNA strands with subsequent induction of fragmentation [61][62]. The key resistance mechanism associated with the use of TFT is its rapid degradation to 5-carboxy-2'-deoxyuridine (5-COOH-dU), 5-trifluorouracil (5-CF₃-U), and 5-carboxyuracil (5-COOH-U) by the action of thymidine phosphorylase [63][64]. Although encouraging initial results from clinical studies on the administration of TFT to patients with breast and colon cancer were observed, further progression of **7** in the clinic as a single agent was halted due to rapid recurrence of disease in patients [65]. Trifluridine was approved in 1980 as an anti-herpes simplex viral drug for treating cold sores and in 2015, as TAS-102 in a combination with tipiracil hydrochloride (a thymidine phosphorylase inhibitor in chemotherapy) for patients with refractory colorectal cancer.

Tezacitabine (FMdC, **61**, Figure 16), the 2'-fluoromethylene deoxycytidine nucleoside analogue of gemcitabine (**4**) exerts anticancer activity via irreversible inhibition of ribonucleotide reductase (RNR) by its diphosphate form (FMdC-DP). It has been postulated that the presence of fluorine in the 2'-vinyl moiety in FMdC-DP facilitates the 3'-hydrogen atom abstraction from the FMdC-DP, by resonance stabilization of the formed 3'-radical [66]. Another active metabolite of tezacitabine, the 5'-triphosphate form (FMdC-TP) incorporates into DNA during replication, or repair leading to chain termination. Tezacitabine, unlike other cytidine nucleoside analogues, is relatively resistant to deamination by cytidine deaminase. Moreover, tezacitabine has shown to enhance the DNA-directed effect of capecitabine in human colon cancer cells through increasing activity of TP, one of the enzymes required for activation of capecitabine in tumours [67]. Phase I and II clinical trials for tezacitabine have been completed for haematologic malignancies and gastric cancer, respectively. In addition, tezacitabine was clinically assessed in Phase I and II either as a single agent or in a combination with 5-FU for treatment in patients with advanced oesophageal cancer or gastric cancer. In 2004, further development of tezacitabine was terminated as it failed to meet Phase II trial endpoints.

Fluorinated anticancer nucleobase, nucleoside and nucleotide analogues in clinical studies



Fluorinated anticancer nucleoside analogues withdrawn from clinical studies

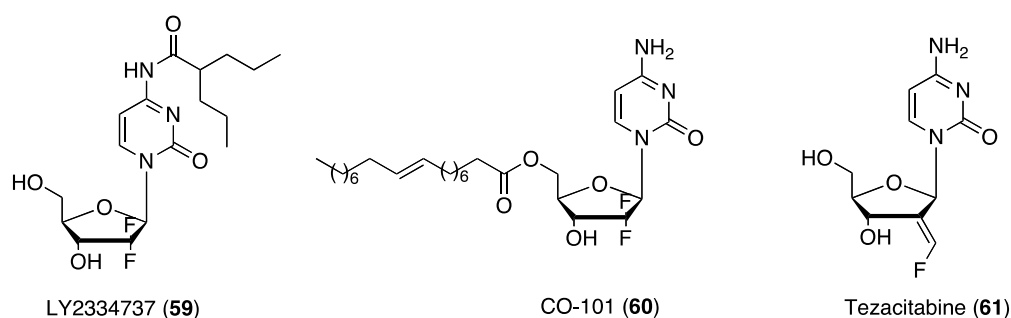


Figure 16. Fluorinated anticancer nucleobase, nucleoside and nucleotide analogues in clinical studies

Fluorocyclopentenylcytosine (RX-3117, **62**) is an orally available, cytosine analogue of naturally occurring neplanocin A with a fluorinated cyclopentenyl moiety (Figure 16) [68]. RX-3117 has anticancer activity in a number of human tumour xenografts. Its metabolic activation begins, after nucleoside transporter 1 (hENT-1) mediated uptake, with a phosphorylation step to 5'-monophosphate (RX-MP) performed by UCK2, one of two uridine-cytidine kinase (UCK) [69][70], and subsequent two phosphorylations to the 5'-di- and triphosphate forms. The latter form (RX-TP) can be incorporated to RNA, whereas the 5'-diphosphate (RX-DP) can be reduced by RNR to its deoxy-analogue (dRX-DP) which can be further converted to the triphosphate form (dRX-TP) and incorporated into DNA. Moreover, RX-3117 can act as an effective demethylating agent by inhibiting DNA methyltransferase (DNMT). Interestingly, unlike gemcitabine, RX-3117 is a poor substrate for cytidine deaminase (CDA) as shown by Peters *et al.* in their *in vitro* studies in the

U397 cell line simultaneously treated with RX-3117 and tetrahydrouridine (THU), an inhibitor of CDA enzyme [69]. A first-in-human trial of RX-3117 in patients with solid tumours has been completed and demonstrated encouraging evidence of the single agent safety and tolerability [71]. Currently, patients are being recruited for two Phase I/II clinical trials focusing on adverse reactions, therapeutic use and proof of concept for RX-3117 use in patients with pancreatic and bladder cancer [71].

3.2 Purine-based anticancer fluorinated nucleoside analogues

Fludarabine (FAMP, **3**) represents an effective and extensively investigated purine nucleoside analogue in indolent B-cell malignancies, and is used as a first- and second-line agent in the treatment of B-cell chronic lymphocytic leukaemia (B-CLL) [72]. Fludarabine is also used in combination treatment, particularly in chemoimmunotherapy in combination with the DNA-alkylating agent cyclophosphamide, and the monoclonal antibody rituximab to treat CLL patients [73]. Structurally, fludarabine is the 5'-monophosphate form of 2'-fluoro-arabinofuranosyladenine and as such, being negatively charged at physiological pH, is unable to enter cells. Thus, fludarabine is first dephosphorylated to its antimetabolite, F-ara-A which after cellular uptake by nucleoside transport systems is re-phosphorylated by deoxycytidine kinase to the 5'-monophosphate form and subsequently to the 5'-di and 5'-triphosphate forms by adenylate kinase and nucleoside diphosphate kinase, respectively. The major active metabolite of fludarabine is the 5'-triphosphate (F-ara-ATP), which as an alternative substrate to the natural deoxynucleotide (dATP), inhibits DNA polymerases. Moreover, F-ara-ATP can inhibit other enzymes involved in DNA synthesis such as DNA primase, DNA ligase and ribonucleotide reductase [74][75][76]. Although the presence of fluorine in F-ara-A increases its relative resistant to deamination by adenosine deaminase [77], F-ara-A can be a subject to phosphorolytic cleavage with the release of 2-fluoroadenine (F-Ade), a metabolite known to accumulate as the toxic triphosphate F-ATP [78]. In fact, many clinical trials have shown that administration of fludarabine may cause (in a dose dependent manner) severe central nervous toxicity (dementia, coma), elevation of liver enzyme levels, and transient somnolence [79][80][81].

Several resistance mechanisms associated with fludarabine have been reported and they are mainly related to biochemical alterations in membrane nucleoside transporters, deoxycytidine kinase and cytoplasmic 5-nucleotidase cN-II activities, and to changes in the expression of miR-34a (small non-coding RNA molecule mediating post-transcriptional gene silencing) [82]. In addition, over-expression of miR-181, miR-221, MYC, SULF2 and down-regulation of miR-29a has been reported in CLL patients resistant to fludarabine [83].

Clofarabine (Cl-F-ara-A, **6**) is a second-generation chemotherapeutic agent that has been designed with the aim to combine structural features of cladribine and fludarabine [84]. Introduction of the fluorine atom into the 2'-arabino position in the scaffold of cladribine gave rise to the structure of clofarabine with increased stability at pH 1 [84]. In addition, clofarabine shows resistance to phosphorolytic cleavage and the deamination process. Clofarabine as a clinical agent is approved for the treatment of relapsed and refractory paediatric acute lymphoblastic leukaemias, and is also being investigated in combination therapy to treat patients with chronic lymphocytic leukaemia, acute myelogenous leukaemia and myelodysplastic syndrome [85]. Clofarabine can enter cells using either facilitated or active nucleoside transport mechanisms and, when at higher concentration and upon longer exposure, by passive diffusion across the lipid membranes [86]. Once inside the cell, clofarabine, like other nucleoside analogues, is phosphorylated to its 5'-monophosphate form by cytosolic deoxycytidine kinase (dCK) as well as mitochondrial deoxyguanosine kinase (dGK) [87]. Subsequently the diphosphate is converted to its active species, the 5'-triphosphate metabolite (Cl-F-ara-ATP) [87]. There are three major mechanisms by which clofarabine exerts its anticancer activity, and these include inhibition of ribonucleotide reductase, inhibition of DNA synthesis, and direct induction of apoptosis [88]. The clofarabine triphosphate form, as an adenosine analogue, can be incorporated by DNA polymerase and lead to DNA chain termination. The fluorine atom, with its electron-withdrawing properties, has been postulated to affect the reactivity of the 3'-OH group and/or the three-dimensional structure of DNA in such a way that incorporation of other nucleotide analogues and hence extension of DNA chain terminated with 2'-fluoro-containing nucleosides is inhibited [89]. In addition, clofarabine may also contribute to RNA-directed events such as inhibition of the polyadenylation process being one of the key steps in the post-transcriptional RNA processing essential for the synthesis and maintenance of mRNA transcripts [90]. Resistance mechanisms commonly associated with nucleoside analogues have been reported also for clofarabine. It has been also hypothesized that the activity of dCK for which clofarabine is a good substrate, may modulate ABCG2-mediated resistance as clofarabine cytotoxicity can be strongly reduced by enhanced ABCG2-mediated efflux [91]. Recently, clofarabine has also been reported as a dual-action inhibitor of HIV-1 replication by both limiting dNTP substrates pool for viral DNA synthesis and by inhibiting the DNA polymerase activity of HIV-1 reverse transcriptase [92].

4. Antiviral fluorinated nucleosides

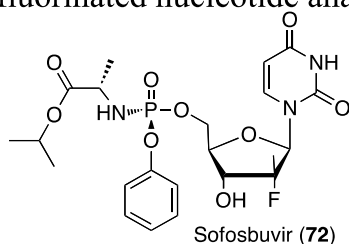
In the last two decades, major advances for the treatment of several viral infections have been achieved. On the other hand, an outburst of novel viral diseases has featured in recent years, for

which effective therapies do not exist. Ebola, Zika and also new strains of hepatitis and herpes viruses have spread with potential for a pandemic outbreak. There are three main classes of viral infections. The first class includes life threatening chronic viruses like the human immunodeficiency virus (HIV), Hepatitis B (HBV) and Hepatitis C (HCV) viruses. The second class comprises acute viral infections such as influenza that are generally non-lethal and self-resolving. The third class includes viral infections (common cold caused by rhinoviruses) that are non-lethal but have a significant economic impact [93].

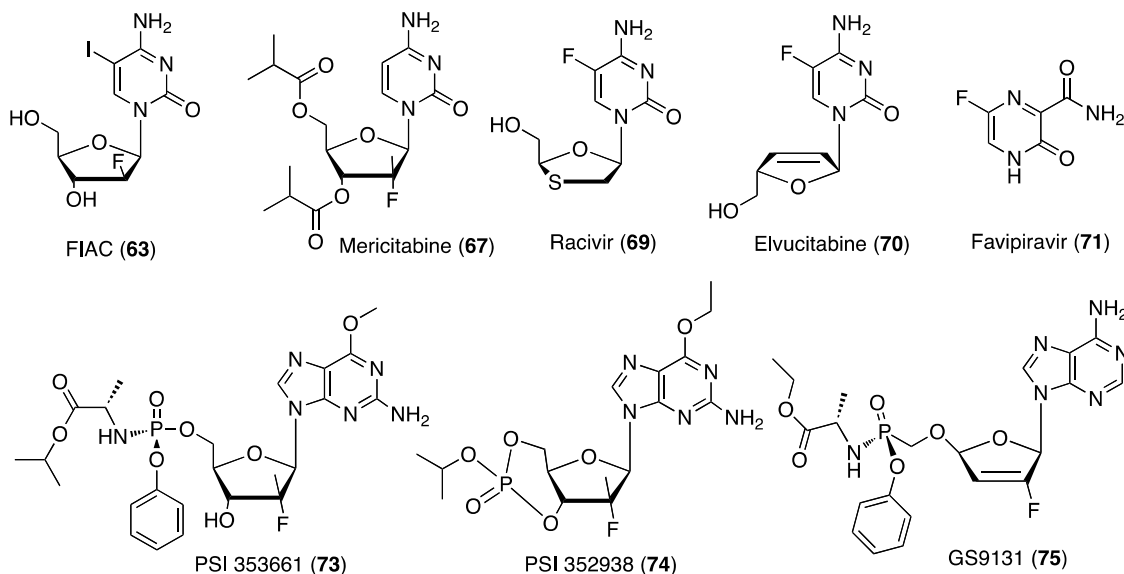
Nucleoside analogues represent a very successful class of antiviral agents. Compared to the anticancer nucleosides, they are structurally more diverse as they include nucleosides, acyclic nucleosides and nucleotides. Additionally, they show a better tolerance profile compared to the anticancer nucleosides due to a low level of activity on mammalian enzymes. In order to exert their antiviral activity, they are converted to their 5'-triphosphate form. The target of nucleoside reverse transcriptase inhibitors (NRTIs) is usually the viral polymerase. The class of NRTI include the compounds mimicking the endogenous natural nucleosides and those that need to be phosphorylated to their 5'-triphosphate form in order to exert biological activity. Viral polymerase catalytic residues interact with the template, the primer and with the incoming nucleoside 5'-triphosphates [93].

Nowadays, nucleoside analogues are used in the clinic or in clinical development for the treatment of several diseases such as HBV, HCV, HIV, human respiratory syncytial virus (HRSV), human cytomegalovirus (HCMV), and Varicella zoster virus (VZV). Despite their widespread use, many limitations are associated with antiviral chemotherapy and resistant viral strains are common. For this reason, research is focusing on the development of new nucleoside analogues which can overcome the resistance mechanisms [93]. Many of these compounds are characterized by the presence of fluorine that has improved biological profiles and provided improved metabolic stability. The properties and expectations that an antiviral drug needs to meet and the acceptable level of toxicity vary according to the type and gravity of the disease. Examples of fluorinated antiviral nucleoside analogues and derivatives (**63-75**) in the clinic and clinical trials are reported in Figure 17.

FDA-approved antiviral fluorinated nucleotide analogue



Fluorinated antiviral nucleoside and nucleotide analogues in clinical studies



Fluorinated antiviral nucleoside analogues withdrawn from clinical studies

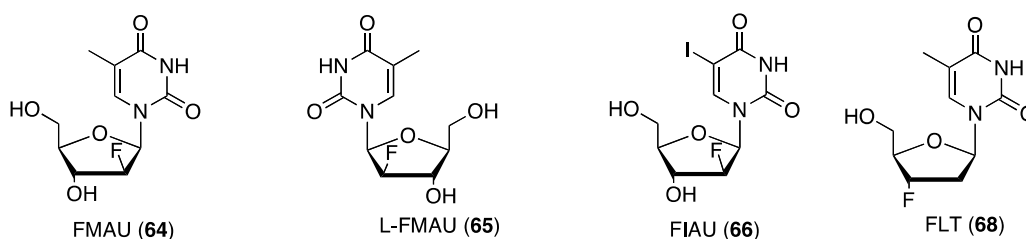


Figure 17. Fluorinated antiviral nucleoside analogues and derivatives in clinical trials.

4.1 Pyrimidine-based antiviral fluorinated nucleosides and prodrugs

The first antiviral nucleoside, Iduviran, was synthesised by Prusoff *et al.* in 1959 [94] and soon after, a 2'-fluorinated derivative was synthesised in order to stabilise the glycosidic bond that was unstable in acidic conditions [95]. Several uridine and cytidine derivatives were subsequently synthesised; in particular 1-(2'-deoxy-2'-fluoro-beta-D-arabinofuranosyl)-5-iodocytosine (FIAC, **63**), and 1-(2'-deoxy-2'-fluoro-beta-D-arabinofuranosyl)-5-methyluridine (FMAU, **64**) showed a

very good activity against HSV, HBV and other viruses such as CMV and Epstein–Barr virus (EBV) [95][96]. L-FMAU (**65**), also known as clevudine, is a very potent antiviral agent for the treatment of HBV. It was approved in South Korea in 2006 [97][98] but recently a Phase III clinical trial was halted due to cases of myopathy in some patients. It was then revoked also from the South Korean market. [99]

Many other 2'-fluorinated nucleosides have been synthesised more recently and studies on the SAR showed that fluorine in the 2'-arabino-position (up) or in the 3'-ribo-position (down) leads to an increased anti-HIV activity [95]. FIAU (**66**) has been selected as a Phase I clinical trial candidate, but it showed mitochondrial toxicity resulting in lactic acidosis and hepatic failure [100]. Martin *et al.* have synthesised a variety of 2'-deoxy-2'-fluoro-pyrimidine based nucleosides and, among them, many fluorinated analogues of 2',3'-dideoxycytidine (ddC), which have showed significant anti-HIV activity [101].

Mericitabine (RG-7128, **67**) is a 3',5'-diisobutyrate prodrug of 2'-deoxy-2'-fluorocytidine (FdC) with potent anti-HCV activity [50]. It has successfully completed Phase I and II clinical trial either alone or in combination with pegylated-interferon and ribavirin or in combination with a first generation of protease inhibitor [102][103]. A clinical trial with mericitabine and other agents in an interferon-free regime was also very successful and clinical trials are likely to continue [52]. 3'-fluoro-3'-deoxythymidine (FLT, **68**), also known as Alovudine, was one of the first antiviral compounds synthesised in 1971 [104] but its anti-HIV activity was not discovered until 1988 [105], showing a major potency compared to azidothymidine (AZT), an established agent for the treatment of HIV [106]. Alovudine inhibits *in vitro* replication of highly resistant nucleoside reverse transcriptase inhibitor (NRTI) HIV strains. However, due to dose-dependent safety concerns in initial development, further clinical investigations of Alovudine were halted [107]. Phosphoramidate prodrugs of FLT have been synthesised and *in vitro* studies showed potent inhibition of HIV-1 and HIV-2 replication, although they were less potent in comparison to the parent nucleoside [108]. Emtricitabine (FTC, **8**) is a 5-fluorodeoxycytidine derivative, with an uncommon sugar, an oxathiolane ring. It is an inhibitor of reverse transcriptase (NRTI) and is used for the treatment of HIV infection [98]. Emtricitabine is included in the list of the essential medication compiled by the World Health Organization [109]. It is chemically similar to the antiviral agent lamivudine and its triphosphate form incorporates faster into growing viral DNA in comparison with lamivudine. Although very active, there are some concerns about the fact that it could be inactive against lamivudine-resistant HBV strains and that it could be associated with similar HBV-resistant mutant development. Nevertheless, the presence of the fluorine boosts its bioavailability and half-life compared to lamivudine [110]. Racivir (**69**) the enantiomer of **8**,

showed also potent anti-HIV activity and studies *in vitro* have revealed that this agent is also active against HBV [111].

Elvucitabine (**70**) is another 5-fluoro NRT inhibitor active as anti-HIV and HBV agent that has recently completed Phase II clinical trial for the treatment of HIV [112]. Favipiravir (T-705, **71**) is a fluorinated pyrazine carboxamide derivative effective in the treatment of influenza viruses. Introduction of fluorine at the 6-position of the pyrazine ring improved the biological activity and pharmacokinetic properties of T-705 when compared to other derivatives [113]. It is currently used in Japan for the treatment of influenza virus and clinical trials are ongoing in the United States. T-705 is converted into the ribofuranosyl triphosphate form by endocellular enzymes and it inhibits the influenza viral polymerase [114]. After the 2014 outbreak of Ebola virus disease (EVD), a lethal condition with no specific treatment yet approved, a multicenter non-randomized trial with favipiravir and standardized care was conducted. Although the trial did not conclude on the efficacy of the drug, it suggests that further studies should be conducted with a favipiravir monotherapy in patients with medium to high viremia [115][116].

Sofosbuvir (Sovaldi®, GS7977, **72**), the nucleotide prodrug of β -D-2'-deoxy-2'- α -fluoro-2'- β -C-methyluridine, is an anti-HCV inhibitor of the NS5B RNA polymerase with a pan-genotypic antiviral activity. Non-structural protein 5B (NS5B) is an RNA-dependent RNA polymerase responsible for the synthesis of both positive and negative-strand genomic RNA. This enzyme is essential for sofosbuvir as **72** acts by mimicking the natural substrate of NS5B polymerase. Once incorporated into the growing RNA, it induces chain termination. It was first synthesized as a diastereoisomeric mixture (GS-9851), comprising sofosbuvir (GS-7977, S_p isomer) and GS-491241 (R_p isomer), with potent inhibitory activity towards the NS5B enzyme [117][118]. Studies *in vitro* showed that GS-9851 is converted into an inactive, achiral intermediate by the enzymes cathepsin A (CatA) and carboxylesterase 1 (CES1). It is then hydrolysed by an histidine triad nucleotide-binding protein 1 (Hint1) into an inactive 5'-monophosphate form which is further phosphorylated to the corresponding 5'-diphosphate form by uridine-monophosphate-cytidine-monophosphate kinase (UMP-CMP) and finally into the active 5'-triphosphate form by nucleoside diphosphate kinase, respectively [82][119]. Sofosbuvir shares the same metabolic pathways as the diastereoisomeric mixture and, because of this specific metabolism, it has a low potential for cytochrome P450-mediated drug-drug interactions. It has showed an impressive sustained virological response rates (SVR) (over 90% of patients) compared to other anti HCV agents and high genetic barrier to resistance. This makes sofosbuvir essential in the care of both treatment-naïve and treatment-experienced patients. Sofosbuvir was FDA-approved in 2013 in the USA for the treatment of chronic HCV infection in patients with genotypes 1, 2, 3 or 4, including those with hepatocellular

carcinoma meeting the Milan criteria (patients awaiting for liver transplantation) and those with HCV/HIV-1 co-infection. In combination therapy with ribavirin, the prodrug **72** was revealed to be effective also in subjects co-infected with HIV. In addition, sofosbuvir prevented a recurrence of HCV infection in most of the patients awaiting liver transplant. Several trials with **72** and other oral direct-acting antivirals (DAAs) are ongoing and will provide oral interferon-free treatment regimens for genotype-1 infections [120]. Recently, after the pandemic spread of Zika virus, sofosbuvir has been also evaluated for the treatment of this disease. *In vitro* studies showed promising results and further studies need to be undertaken [121].

4.2 Purine-based fluorinated nucleosides and prodrugs

PSI-353661 (**73**) and PSI-352938 (**74**) are both purine-based prodrugs of β -D-2'-deoxy-2'- α -fluoro-2'- β -C-methylguanosine-5'-monophosphate. Both compounds have shown a promising *in vitro* anti-HCV activity. They are characterised by a unique resistance profile that makes them desirable candidates for a combination therapy with other HCV inhibitors including other nucleosides and analogous nucleotides. They are currently under clinical evaluation [122]. GS9131 (**75**) is another 2'-F- purine-based phosphoramidate which has been shown to inhibit HIV-1 reverse transcriptase (RT) and proved to have a unique resistance profile toward N(t)RTI resistance mutations. GS9131 is currently under clinical evaluation [123].

Table 1 brings together the various anticancer and antiviral fluorinated nucleosides that have entered clinical development, along with information on the drug developer, disease indication and molecular target.

Table 1. Fluorinated NAs and their prodrugs in clinical use and clinical development for cancer

Drug Name	Originator/Developer	Phase	Disease	Target
Fluorouracil (5-FU, 1)	Roche	Approved (1962)	Colorectal, breast, pancreatic, stomach cancer	TS
Floxuridine (FdUrd, 2)	Roche	Approved (1970)	Liver metastasis	TS
Capecitabine (5)	Roche	Approved (1998)	Metastatic breast, colorectal cancer	TS
Tegafur (52) + Uracil	Taiho Pharmaceutical	Used in Japan, Taiwan II II/III	Advanced GI cancers Colon, combination therapy for hepatocellular carcinoma Gastric cancer	TS, DNA synthesis inhibition
Tegafur (52) + Gimeracil + Oteracil	Taiho Pharmaceutical	III	Advanced gastric cancer in combination with cisplatin	TS
Doxifluridine (53)	Aida Pharmaceuticals	III	Combination therapy for GI cancer	TS
FdCyd(58) + THU	National Cancer Institute	I and II	Neoplasms	DNA methyltransferase
Flucytosine (57)	Tocagen	I/II I	Combination therapy for solid tumours Brain tumours	TS
NUC-3373 (55)	NuCana	I	Colorectal and breast cancer	TS
Gemcitabine (4)	Eli Lilly	Approved (1996)	Non-small cell lung, breast, pancreatic, ovarian, soft tissue sarcoma	DNA polymerase RNR, dCMP deaminase
LY2334737 (59)	Eli Lilly	Discontinued	Malignant and metastatic solid tumours	DNA synthesis inhibition
CO-101 (60)	Clavis Pharma	Discontinued	Advanced solid tumours Metastatic pancreatic dual adenocarcinoma	DNA synthesis inhibition
NUC-1031 (51)	NuCana	III II I	Pancreatic cancer Ovarian cancer Combination therapy for ovarian and biliary tract cancers	DNA synthesis inhibition
RX-3117 (62)	Rexahn Pharmaceuticals/TEVA Pharmaceuticals	I/II I	Solid tumours Combination therapy for ovarian cancer	DNA methyltransferase
Tezacitabine (61)	Aventis/Chiron Corporation	Discontinued	Hematological malignancies	RNR
Trifluorothymidine (7) + Tipiracil HCl (TAS-102)	Taiho Pharmaceutical	Approved (2015)	Metastatic colorectal cancer	TS
Fludarabine (3)	Southern Research Institute/ Bayer HealthCare Pharmaceuticals	Approved (1991)	Hairy cell leukemia, B-cell CLL	DNA polymerase, RNR, DNA primase
Clofarabine (6)	Bioenvision	Approved (2004)	Pediatric refractory ALL	DNA polymerase, RNR

Data in Table 1 were taken from the references 22, 33 and 45 and references therein. Additional references are included in the main text body. Information about status of clinical trials available from www.clinicaltrials.gov

Table 1 (cont.) Fluorinated NAs and their prodrugs in clinical use or clinical development for viral infections

Drug Name	Originator/Developer	Phase	Disease	Viral Target
Trifluorothymidine (7) Viroptic	GlaxoSmithKline	Approved (1998)	Herpes Simplex Virus (HSV)	DNA polymerase
Emtricitabine (8) Emtriva	Emory University/Gilead	Approved (2003)	Human Immunodeficiency Virus (HIV)	Nucleoside reverse transcriptase
Sofosbuvir (72) Sovaldi	Gilead	Approved (2013)	Hepatitis C (HCV)	NS5B RNA-dependent RNA polymerase
Mericitabine (67)	Pharmasset/Hoffmann-LaRoche	II	HCV	NS5B RNA-dependent RNA polymerase
Favipiravir (71)	Toyama Chemical/MediVector	Approved in Japan (2014) III JIKI trial	As stockpiling against Influenza Pandemics Influenza Ebola	RNA-dependent RNA polymerase
Fiacitabine (63, FIAC)	Memorial Sloan-Kettering Cancer Center/Oclassen Pharmaceuticals	II	Cytomegalovirus and HIV infections	DNA polymerase RNR
Elvucitabine (70)	Yale University/Achillion Pharmaceuticals	II	Chronic HIV infections	Nucleoside reverse transcriptase
Racivir (69)	Emory University/Pharmasset	II	HIV	Nucleoside reverse transcriptase
Fialuridine (66, FIAU)	Oclassen Pharmaceuticals/Eli Lilly	Discontinued	HSV, HIV, and Hepatitis B (HBV)	DNA polymerase
Clevudine (65, L-FMAU)	Bukwand/Pharmasset	Discontinued	HBV	DNA polymerase
Alovudine (68, FLT)	Medivir/Beijing Mefuvir Medicinal Technology	Discontinued	HIV	DNA polymerase
PSI 353661 (73)	Pharmasset	Preclinical development	HCV	NS5B RNA-dependent RNA polymerase
PSI 352938 (74)	Pharmasset	I	HCV	NS5B RNA-dependent RNA polymerase
GS9131 (75)	Gilead	I	HCV	Nucleoside reverse transcriptase

Data in Table 1 (cont.) were taken from the references: 1, 95, 98 and 112 and references therein. Additional references are included in the main text body. Information about status of clinical trials available from www.clinicaltrials.gov

Conclusion

In this review we summarise electrophilic and nucleophilic fluorination methods, including ^{18}F -radiolabeling used in the synthesis of fluorine-containing nucleoside analogues. The modulatory and beneficial effect on pharmacological properties of molecules that fluorine incorporation at an appropriate position imparts is well known and reported. Along with cellular biology of FDA-approved fluorinated nucleoside analogues and derivatives, we emphasise some profound effects fluorine adds to nucleos(t)ide analogues such as clofarabine and tezacitabine or FdUrd-MP. These effects include increased stability towards deamination and phosphorytic cleavage or target/ligand binding properties, respectively. We also outline current developments and strategies aiming to

improve a clinical outcome of fluorine-containing nucleoside analogues. Given the unique features and desirable characteristics that fluorine can impart to drugs, its bioisosteric replacement of hydrogen will continue to be used in medicinal chemistry as a method for improving and influencing pharmacodynamic and pharmacokinetic properties.

Executive summary:

- In this review, we briefly discuss the most important fluorinated nucleosides from a biological perspective, highlighting the contribution of fluorine to therapeutic activity.
- As a uniquely small and electronegative atom, fluorine plays an important role within many drug molecules, imparting a variety of physicochemical properties influencing molecular stability, solubility and target binding properties.
- Combining the benefits of fluorine incorporation with the established nucleoside class of therapeutics has resulted in a number of clinically proven drug molecules or clinical drug candidates, mainly in the fields of antiviral and anticancer therapy.
- Recent developments in fluorine incorporation chemistry using nucleophilic fluoride have been instrumental in the evolving field of ^{18}F PET radiochemistry.
- There are significant drawbacks associated with nucleoside therapeutics, such as the necessity for transport-mediated uptake plus the requirement for enzyme-mediated 5'-triphosphorylation.
- To circumvent the drawbacks of nucleosides as therapeutic agents, we outline the application of phosphoramidate-based pro-nucleotide (ProTide) approaches to improve therapeutic outcome, illustrated by the registered drug sofosbuvir (hepatitis C) and the clinical candidate NUC-1031 (solid tumours).

Future Perspective:

- Fluorine incorporation strategies will continue to be strategically employed in drug discovery and development to improve pharmacokinetic and pharmacodynamic properties of candidate molecules.
- The concept of activated nucleotide prodrugs (ProTides), which has recently achieved clinical success (e.g. sofosbuvir for Hepatitis C treatment), will continue to improve the efficacy profile of established nucleoside drugs whose efficacy is limited by poor cellular uptake and the requirement for phosphorylation.

- Technologies for incorporation of the positron-emitter ^{18}F (half-life 109 minutes) will continue to develop, driving the development of ^{18}F -labelled nucleosides for *in vivo* biomarker or drug distribution studies using PET imaging.

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