

The ProTides Boom

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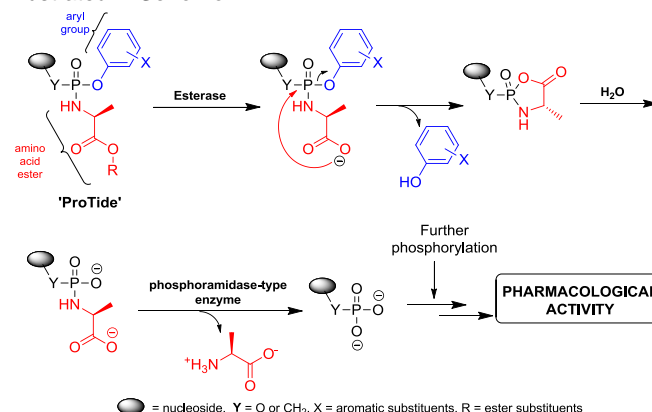
Dedicated to Prof. Chris McGuigan (1958-2016)

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Phosphate prodrug strategies have been successfully used in the discovery of various nucleotide therapeutics that are nowadays used daily in the clinics to treat viral infections in particular hepatitis B and C (HBV and HCV, respectively) and HIV.^[1] Over the last few years, the 'ProTide' technology - pioneered by Chris McGuigan (Cardiff University, UK)- has emerged as a powerful strategy in the discovery of nucleotide therapeutics.^[2] Indeed, this technology was used in the successful discovery and development of two FDA-approved 'ProTides', Sovaldi™ and tenofovir alafenamide. The pipelines of several pharma and biotech companies seem to be stocked with a selection of 'ProTides', some of which are currently in Phase III clinical trials, while there is an increasing number of reports on 'ProTides' undergoing preclinical studies. Collectively, these reflect a 'ProTides' boom that could in the future be translated into the approval of more 'ProTides' to treat patients from viral infections and cancer.

The in vivo activation of many antiviral and anticancer nucleosides involves phosphorylation into their active di- or triphosphate counterparts.^[3] Among the three kinase-dependent phosphorylation steps required for the bioactivation of this class of therapeutics, the first phosphorylation step that converts nucleosides into their 5'-O-monophosphate derivatives is often found to be the rate-limiting step.^[4] To overcome this inefficient activating metabolic step, numerous strategies that deliver nucleoside 5'-O-monophosphates into cells have been developed.^[1] Nowadays, one of the most applied phosphate prodrug approaches is the 'ProTide' technology. The development^[2] of this technology started by masking the 5'-O-monophosphate groups of therapeutic nucleosides with simple dialkyl and then haloalkyl groups. However, these attempts did not lead to improved biological activity due most likely to the inability of these masking groups to be hydrolyzed in vivo to release the nucleoside monophosphate, which can subsequently be further phosphorylated to the active species. Next, McGuigan and co-workers synthesised alkyloxy and haloalkyl phosphoramidate prodrugs and these showed better activities than their parent nucleosides.^[5] This was the first breakthrough in the development of the 'ProTides' and which provided evidence that masking of phosphate groups with biocleavable motifs may yield an effective prodrug system for the delivery of therapeutic nucleoside monophosphates. These initial studies identified L-alanine as a superior amino acid in the alkyloxy phosphoramidates, an observation that has informed recent drug discovery programs that yielded FDA-approved drugs.^[5b] Encouraged by this, the phosphate group was masked with two amino acid esters. Back then, this was found not to be beneficial as the prodrugs were largely inactive. Diaryl phosphates were studied next and these showed very good activity. Hence, the

McGuigan lab combined the amino acid ester from the alkyloxy and haloalkyl phosphoramidates and the aryl masking group to generate aryloxy triester phosphoramidates.^[6] These were found to be superior in the delivery of therapeutic nucleoside 5'-O-monophosphates. Since then, the masking of the phosphate group with an amino acid ester and an aryl motif, nowadays known as the 'ProTide' technology, has become an approved prodrug strategy in the discovery of nucleotide therapeutics. The mechanism by which the 'ProTides' are metabolized in vivo to release the nucleoside monophosphate is believed to proceed through the action of two enzymes; an esterase, e.g. cathepsin A,^[7] and phosphoramidase-type enzyme, e.g. hint-1^[8], as illustrated in **Scheme 1**.^[2]



Scheme 1. Postulated mechanism of the in vivo metabolism of the 'ProTides' to release nucleoside monophosphates.

'ProTides' as clinical candidates and drugs

To date, at least ten 'ProTides' have reached clinical trials and been investigated as treatments for viral infections and cancer [**Figure 1**]. The McGuigan lab discovered and developed the anti-HCV agent INX-189, a 'ProTide' of 6-O-methyl-2'-C-methyl guanosine (**1**).^[9] This compound was at that time the most potent inhibitor of HCV replication in cell-based replication assays (EC₅₀ = 0.01 μ M; EC₉₀ = 0.04 μ M, CC₅₀ = 7 μ M). Critically, it generated significantly higher levels of the 6-O-methyl-2'-C-methyl guanosine triphosphate than the parent nucleoside and had a half-life of over 24 hrs. Given the excellent pharmacokinetics (PK) and pharmacodynamics (PD) profiles of INX-189, it was chosen as a clinical candidate that was then developed by Inhibitex Inc.^[10] Following successful early clinical results, INX-189 was acquired by Bristol-Myers Squibb (BMS) and studied in Phase III clinical trials in combination with daclatasvir, another anti-HCV experimental drug of BMS. However, cardiotoxicity was observed and further development was suspended.^[11]

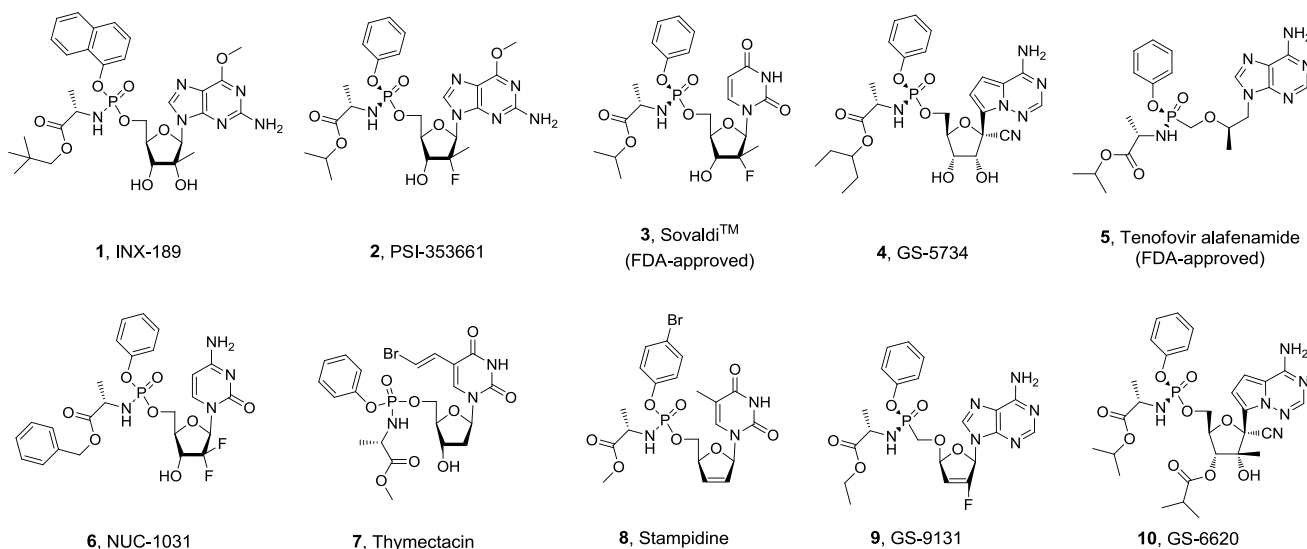


Figure 1. Chemical structures of a selection of 'ProTides' that have reached clinical trials and those that have been FDA-approved.

Other 'ProTides' for the treatment of hepatitis C, particularly PSI-353661 (**2**)^[12] and PSI-7977 (**3**)^[13], proceeded to clinical evaluation. PSI-353661 is a 'ProTide' of 6-O-methyl-2'-deoxy-2'-fluoro-2'-C-methylguanosine while PSI-7977 is a 'ProTide' of 2'-deoxy-2'-fluoro-2'-C-methyluridine. Both compounds showed potent anti-HCV activity through efficient delivery of the parent nucleosides 5'-O-monophosphates. As a result of the impressive early data from the clinical trials of these two agents, they were acquired by Gilead Sciences, Inc. Out of the two compounds, PSI-7977, which subsequently became GS-7977, completed successfully clinical evaluations and became known as Sofosbuvir (Sovaldi™), the first 'ProTide' approved for clinical use against hepatitis C.

GS-5734 (**4**)^[14] is a C-nucleoside 'ProTide', which is currently undergoing Phase I clinical trials for the treatment of Ebola.^[14] Preclinical data showed that GS-5734 exerted potent antiviral activity against variants of the Ebola virus. The 'ProTide' showed potent inhibition of EBOV replication (EC_{50} = 0.06 to 0.14 μ M) while the parent C-nucleoside was not as effective (EC_{50} = 0.77 to >20 μ M). Impressively, in rhesus monkeys infected with Ebola virus, once-daily intravenous administration of GS-5734 led to significant suppression of the Ebola virus replication and 100% protection of infected animals against lethal disease. GS-5734 also showed promising inhibition of the replication of other pathogenic RNA viruses, e.g. arenaviruses, filoviruses, and coronaviruses, indicating the wide-spectrum of activity of this 'ProTide'.

GS-7340 (**5**)^[15], a 'ProTide' of the acyclic nucleoside phosphonate Tenofovir, of which the oral prodrug, Tenofovir disoproxil, is FDA-approved for HIV therapy, exhibited improved anti-HIV activity and better in vivo stability than Tenofovir. Impressively, the 'ProTide' GS-7340 generated 10- to 30-fold higher levels of Tenofovir and its phosphorylated metabolites following incubation of peripheral blood mononuclear cells compared with Tenofovir disoproxil and Tenofovir, respectively.^[16] Although GS-7340 is similar to the FDA-approved tenofovir disoproxil, it is more potent and thus in Phase III clinical studies therapeutic effects were achieved at lower doses and less incidence of side effects. In late 2015, GS-7340, now known as tenofovir alafenamide, was approved in

combination with other anti-HIV agents for the treatment of HIV-1 infection.

NUC-1031 (**6**)^[17] is an anticancer 'ProTide' that was discovered by the McGuigan lab and is currently being developed by NuCana Biomed Ltd. It is a prodrug of the FDA-approved anticancer drug Gemcitabine (Gemzar™). This compound overcame three resistance mechanisms that limit the efficacy of the parent nucleoside Gemcitabine. Results from phase I/II clinical trials showed that NUC-1031 was effective against a wide range of cancers and was well-tolerated by patients. Impressively, five out of 68 patients achieved tumor shrinkage of $\geq 30\%$ while an additional thirty-three patients had achieved stable disease. NuCana is also pushing forward the clinical development of NUC-3373^[18], a 'ProTide' of 5-fluoro-2'-deoxyuridine (FUDR) [structure not shown].

At least a further four 'ProTides' were reported to have undergone clinical evaluation, i.e. Thymectacin (**7**) for cancer, Stampidine (**8**) and GS-9131 (**9**) for HIV; and GS-6620 (**10**) for hepatitis C.

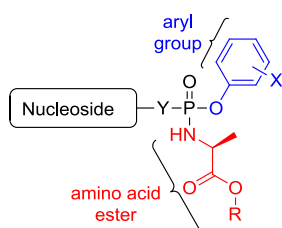
The plethora of 'ProTides' that have been, still undergoing or successfully completed clinical trials clearly highlights the effectiveness of this technology to deliver nucleotide therapeutics. Coupling this to the large number of 'ProTides' that are currently undergoing preclinical evaluation, it is safe to say that more 'ProTides' will progress into clinical studies increasing the chances of more 'ProTides' being approved in the future to treat viral infections and cancer. This has been made possible by the pioneering work of Prof. Chris McGuigan that started in the early 1990s and years later yielded the 'ProTide' technology as we know it today. Since its development, the 'ProTide' technology has been widely adopted and adapted by academic research groups, small and large pharmaceutical companies to discover new medicines that have already improved treatment outcomes and consequently the quality of life of many patients.

Keywords: ProTide • Prodrug • Nucleoside • Nucleotide • Phosphorylation

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Table of Contents



X = (un)substituted aryl Y = O or CH₂ R = various esters

Chemical structure of the 'ProTides'

The phosphate and phosphonate 'ProTide' technology, which was invented by the McGuigan lab in the early 1990s, has proven to be effective in the discovery of nucleotide therapeutics. Impressively, it has already inspired the discovery of two FDA-approved drugs with many more in (pre)clinical development.
