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Aryloxy Phosphoramidate Triesters: a Technology for Delivering Monophosphorylated Nucleosides and Sugars into Cells

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Prodrug technologies aimed at delivering nucleoside monophosphates into cells (protides) have proved to be effective in improving the therapeutic potential of antiviral and anticancer nucleosides. In these cases, the nucleoside monophosphates are delivered into the cell, where they may then be further converted (phosphorylated) to their active species. Herein, we describe one of these technologies developed in our laboratories, known as the phosphoramidate protide method. In this approach, the charges of the phosphate group are fully masked to provide efficient passive cell-membrane penetration. Upon entering the cell, the masking groups are enzymatically cleaved to release the phosphorylated biomolecule. The application of this technology to various therapeutic nucleosides has resulted in improved antiviral and anticancer activities, and in some cases it has transformed inactive nucleosides to active ones. Additionally, the phosphoramidate technology has also been applied to numerous antiviral nucleoside phosphonates, and has resulted in at least three phosphoramidate-based nucleotides progressing to clinical investigations. Furthermore, the phosphoramidate technology has been recently applied to sugars (mainly glucosamine) in order to improve their therapeutic potential. The development of the phosphoramidate technology, mechanism of action and the application of the technology to various monophosphorylated nucleosides and sugars will be reviewed.

Introduction

Synthetic nucleoside mimetics have become an integral part of the therapeutic regimens of patients suffering from viral infections and/or cancers.[1,2] In most cases, the active species of nucleosides are their 5′-*O*-triphosphate derivatives, which produce their therapeutic effects as a result of DNA chain termination/polymerase inhibition.[3,4] The formation of these 5′-*O*-triphosphates generally proceeds in three different steps starting from the nucleoside. The inefficiency of these three steps represents a major hurdle for a range of nucleosides. In particular, the first step responsible for the formation of the nucleoside analogue monophosphate is often regarded as the most difficult, and has been shown to be responsible for holding back the full therapeutic potential of certain nucleoside analogues, for example, 2′,3′-didehydro-2′,3′-dideoxythymidine (d4T).[5] Therefore, bypassing this rate-limiting step should improve the therapeutic activities of such compounds. However, because of the charged nature of nucleoside monophosphates under physiological conditions, they show poor cell membrane permeability and are highly susceptible to dephosphorylation; as a results, little therapeutic benefit would be produced following their administration (Figure 1).

Designing a strategy that masks the charges of the nucleoside analogue monophosphates so that they penetrate the membrane and then selectively release the nucleoside analogue monophosphate inside the cell could prove to be useful (Figure 1). The need for such a prodrug approach was previ-

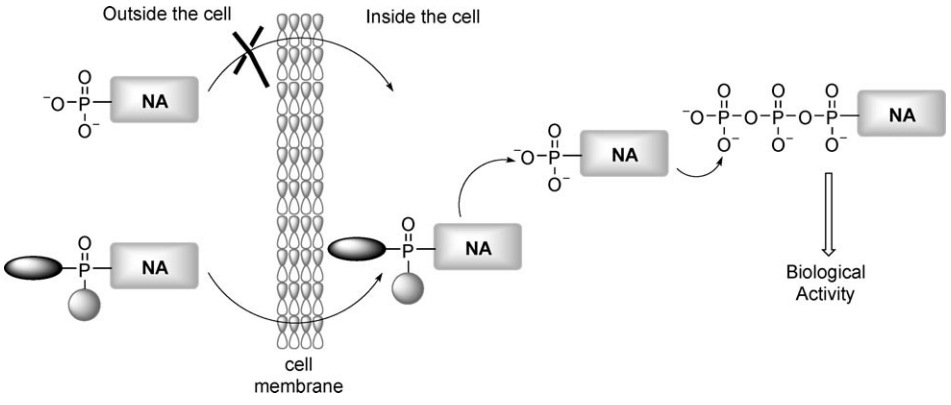

Figure 1. Schematic representation of the pronucleotide (protide) concept (NA: nucleoside analogue).

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ously emphasised in the early 1990s, when a series of nucleotides (phosphonates) were shown to possess interesting antiviral activities.[6]

Over the last decade or so, numerous pronucleotide strategies using different mechanisms of action have been developed,[7–9] and have recently been reviewed.[10] These strategies are based upon either enzymatic and/or chemical intracellular cleavage of the masking groups to release the charged nucleoside monophosphate inside the cell.[11,12] We have designed and developed a pronucleotide approach, known as the phosphoramidate approach, which is based upon intracellular enzymatic activation of the prodrug to release the nucleoside monophosphate (Figure 2).[13] The development of the protide technology has moved from using simple alkyl groups to block the phosphate charges to more sophisticated structures that

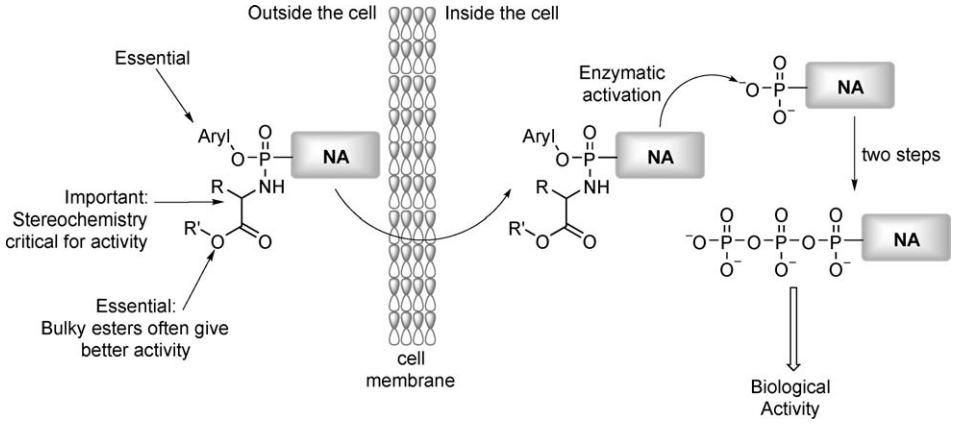


Figure 2. A general representation of how phosphoramidates deliver monophosphates into cells.

may efficiently deliver phosphorylated species into cells. The application of this technology has been shown to improve the activities of various antiviral and anticancer nucleosides as a result of the delivery of their monophosphate derivatives. In fact, several phosphoramidate-based nucleoside analogues have entered clinical trials. In addition, we have recently published the application of the phosphoramidate approach to the antiosteoarthritic carbohydrate-based nutraceutical glucosamine in order to improve its therapeutic potential.[14]

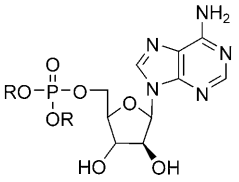
Extensive work has been carried out to establish the mechanism of action of phosphoramidates and lately there has been a great deal of attention paid towards the identification of the enzymes responsible for the metabolism of phosphoramidates that leads to the release of the phosphorylated species.

In this work, we will summarise a) the development of the phosphoramidate approach, b) the mechanism of action of this technology, c) the results of the application of such a technology to various antiviral and anticancer nucleoside analogues, d) the application of the phosphoramidate prodrug approach to glucosamine, and finally, we will briefly report on some phosphoramidate-based drug candidates undergoing clinical trials, as well as a look at the future of this pronucleotide technology.

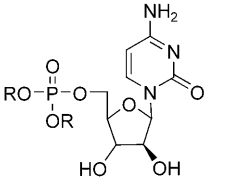
The journey towards aryloxy phosphoramidates triesters

Alkyl and haloalkyl phosphate triesters

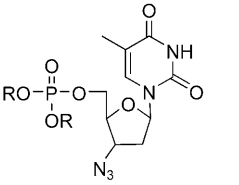
The journey towards the current form of phosphoramidates started with simple dialkyl triesters of the antiviral agent araA (**1**), which showed only moderate biological activity, but did display significantly enhanced stability towards adenosine deaminase.[15] The application of the same strategy on the antineoplastic agent araC (**2**) also provided reasonable biological activity,[16] while dialkyl triester derivatives of the anti-HIV (human immunodeficiency virus) agent AZT (**3**) failed to exert any significant activity in vitro.[17] This latter result was thought to be due to the stability of the dialkyl esters, as they were considered to be relatively stable, and therefore impeded metabolic conversion into the active 5′-*O*-triphosphate form of AZT, which is a prerequisite for biological activity.[17] Various improved haloalkyl masking groups (**4**) were then used to block the charges of AZT monophosphate. Although some of these haloalkyl derivatives showed some anti-HIV activity, they were still less active than the parent nucleoside, AZT.[18] In addition, haloalkyl phosphate triesters of 2′,3′-dideoxycytidine (ddC, **5**) were found to be devoid of any anti-HIV activity.[18] Attempts to boost the anti-HIV activity of these haloalkyl phosphate triesters by



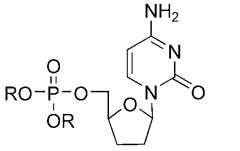
1, R = Me, Et, Pr
6, R = CCl_3CH_2, CF_3CH_2



2, R = Me, Et, Pr
7, R = CCl_3CH_2, CF_3CH_2



3, R = Me, Et, Pr
4, R = CCl_3CH_2, CF_3CH_2



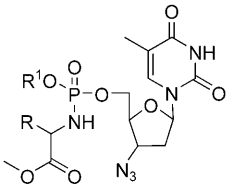
5, R = CCl_3CH_2, CF_3CH_2

changing the degree or nature of halogenation were generally unsuccessful.[19] However, haloalkyl triester derivatives of araA (**6**) and araC (**7**) did display enhanced biological activities.[20] Interestingly, the biological activities of araA and araC derivatives correlated to the lipophilicity of the phosphate triester pro-

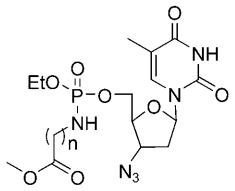
drugs. Thus, it was concluded that the favourable biological activities of the araA and araC (halo)alkyl derivatives were, in part at least, a result of better membrane permeability rather than intracellular phosphate delivery.[15,16,20]

Alkyloxy phosphoramidates

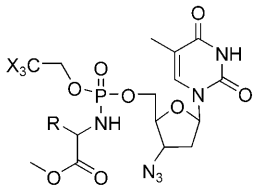
We originally embarked on designing phosphoramidate-based protides following the concept that HIV protease might cleave a suitable oligopeptide from the phosphate moiety of a blocked nucleotide phosphoramidate.[21] Initially, simple monoamino acyl analogues were prepared and evaluated, and showed sufficiently interesting results to pursue in their own right. Thus, a series of simple alkyloxy phosphoramidates of AZT were prepared with a small family of methyl-esterified amino acids (**8**).[22] Interestingly, alkyloxy phosphoramidates of



8, R = H, Me, *i*Pr, CH_2*i*Pr, CHMeEt, Bn
R^1 = Me, Et, Pr, Bu, Hex



9, *n* = 1–6



10, X = H, F, Cl
R = H, Me, *i*Pr

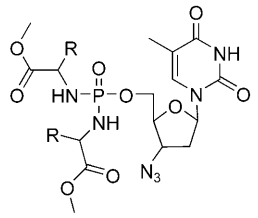
AZT (**8**) showed better anti-HIV activity compared to the AZT dialkyl phosphates (**3** and **4**), which was a first example in the development of phosphoramidates. More importantly, the antiviral potential of alkyloxy phosphoramidates was found to correlate to the amino acid side chain; with alanine being most efficacious, and with leucine and, in particular, isoleucine being less active.[22,23] In contrast, the length of the alkyl phosphate chain had no significant effect on the biological activity.[23]

Further studies focussing on the nature of the amino acid were conducted. In one study, α-amino acids were compared to their β and γ derivatives (**9**), and it was found that the parent α system (glycine) provided maximal activity.[24] Increasing the alkyl spacer length resulted in a significant loss of activity, where changing the length from $n=2$ to $n=1$ leads to >10-fold decrease in activity.[24]

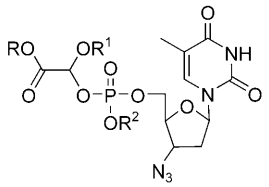
Bearing in mind the improvements seen with haloalkyl phosphates compared with their alkyl derivatives, we wondered whether haloalkyl phosphoramidates would also be more potent. Therefore, a small series of haloalkyl phosphoramidates of AZT (**10**) were prepared and examined.[25] For each of the studied amino acids, glycine, alanine and valine, the alkyl chain was either ethyl, trifluoroethyl or trichloroethyl. Surprisingly, in contrast to earlier observations, no enhancement in antiviral potency over the haloalkyl compounds was observed, with one striking exception. The trichloroethyl alanine compound (**10**) was active against HIV at 0.08 μM, 50 times more potent than either the ethyl or the trifluoroethyl analogues. Interestingly, this significant improvement in antiviral activity was only seen with alanine, while no such activity was seen with the glycine and valine systems.[25] Out of these earlier investigations, alanine emerged as a preferred amino acid, although the mechanistic origins of this preference were at that time unknown. Interestingly, alanine has been the amino acid of choice in many recent examples from a variety of laboratories. Notably, all protides tested to date in the clinic have been alanine derived.

Phosphorodiamidates

The promising activity of alkyl phosphoramidates, particularly those related to alanine, made us wonder whether diamidates would be more efficacious. Thus, several methyl-esterified amino acyl phosphorodimidates (**11**), as well as non-amino



11, R = H, Me*i*Pr, CH_2Ph, CH_2CO_2Me



12, R = Me, Et
R^1 = H, Me
R^2 = Me, *n*Pr, $nC_{12}H_{25}$

acyl phosphorodimidates derived from simple primary and secondary amines, were prepared and tested.[26] Structure–activity relationships (SAR) indicated a strong preference for amino acids such as phenylalanine.[26] Hence, a different amino acid SAR emerged for these diamidates as compared to the earlier alkyl phosphoramidates. Interestingly, this preference for aromatic side chains was also seen by other groups for the rather unrelated phosphoramidate diesters.[12] However, in general diamidates appeared to offer no biological advantage over the amidates, and the chemical yields of the diamidates were significantly lower, and hence they were not further pursued.

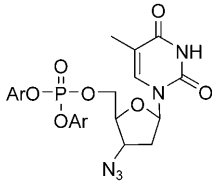
Lactyl-derived systems

In order to establish the importance of the bridging amino acid nitrogen atom to the biological activity of phosphoramidates, we also prepared and evaluated a small family of isosteric O-linked analogues derived from lactic and glycolic acid (**12**).[27] Overall, this study revealed that lengthening the alkyl phosphate chain (R^2) leads to a reduction in potency. Notably, the glycolyl systems ($R^1=H$) were more active than lactyl

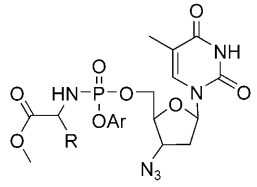
(R^1=Me) by a factor of ~20, which is in contrast to the earlier work where alanine was preferred over glycine.[22,23] A brief hydrolytic stability study conducted on compounds **12** revealed the release of polar compounds and traces of AZT in biological media, but not in DMSO/water. Thus, enzyme-mediated activation is a possibility. However, because of the poor anti-HIV activity of such systems, these compounds were not pursued further.

Diaryl phosphates

One of the main breakthroughs in the development of the current form of phosphoramidate triesters came in 1992, when we found aryloxy phosphates and phosphoramidates to be of very high efficacy and in some cases more active than the parent nucleoside.[28] Using simple phosphorochloridate chemistry, diaryl phosphates of AZT (**13**) were prepared. Interestingly, these diaryl derivatives showed good anti-HIV activity and, for the first time in the development of the phosphoramidate approach, some of the phosphate prodrugs, that is, diaryl derivatives in this case, exerted better activity than the parent nucleoside.[28] More interestingly, some diaryl derivatives were active in cell lines where the parent nucleoside AZT was inactive.[28] Initially, the cell line resistant to AZT that we used (T lymphocyte cell line, JM) was considered to be AZT insensitive as a result of poor phosphorylation.[29] However, it was later found that the poor sensitivity of this cell line towards AZT was due to an AZT efflux pump.[30] Nevertheless, the conclusion remains valid that the diaryl phosphate was better able to retain activity in the JM cell line, and this may imply a degree of intracellular phosphate delivery. The nitro group was believed to be vital to activity as the parent diphenyl phosphate was ~100-fold less active (C8166 cells). The electron-withdrawing power of the *para*-nitro groups and putative enhancements in the aryl leaving group ability were suggested as the major driving force of this SAR.[28] Thus, a series of analogues with various electron-withdrawing groups in the *para* position were prepared (**13**).[31] It soon became very clear that there was a correlation between the electron-withdrawing power of the substituent in the *para* position and the antiviral potency. The nitro- and cyano-substituted compounds were found to be the most potent, the parent phenyl compound showed intermediate activity while the methoxy analogue was the least active, being 500-fold less active than the nitro compound. In order to investigate the effect of the position of the electron-withdrawing nitro group on the aryl moieties, symmetrical bis-*ortho*-nitro and bis-*meta*-nitro analogues were prepared.[32] Evaluating the activity of these compounds against HIV-1 and HIV-2 revealed that the location of the nitro group had little effect on the activity. However, in this study we were able to assess the activity of the phosphate prodrugs in a true thymidine kinase (TK)-deficient human T cell leukaemia cell line (CEM/TK$^-$). The results were quite disappointing as all the diaryl phosphate prodrugs, as well as AZT, failed to show any anti-HIV activity in this cell line. This indicated poor intracellular phosphate delivery, and that the diaryl phosphates were acting largely, if not entirely, as AZT prodrugs and not as AZTMP prodrugs, as intended.[32] However, the earlier work using JM cells on phosphoramidates[28] had indicated that aryloxy phosphoramidates may offer a chance for true nucleoside phosphate delivery, and this became the main focus of our work.



13, Ar = Ph, *p*-X-Ph
X = various



14, R = H, Me, CH_2*i*Pr, CH_2Ph
Ar = *p*-X-Ph
X = H, F, NO_2, Me, Et, *n*Pr, *n*Pnt, MeO

Aryloxy phosphoramidates

As a result of the favourable potency boosts seen with *para*-substituted aryls in the diaryl systems, we thus prepared a series of aryloxy phosphoramidates of AZT with various *para* substituents and amino acids (**14**).[33] These compounds were studied in JM cell lines only to probe potentially implied AZTMP release. From this study, alanine phosphoramidate emerged as strikingly effective compared to other amino acids, for example, leucine, glycine and phenylalanine.[33] In particular, the phenyl methoxy alaninyl phosphoramidate of AZT was the most potent against HIV (EC_{50} = 0.8 μM), whilst the parent nucleoside, AZT, showed an EC_{50} value of ~100 μM in the same assay. We also observed that there was a marked preference for alanine over leucine (10-fold) and glycine (>100-fold). Moreover, whilst electron-withdrawing aryl substitution was very effective in the diaryl system,[31] it was detrimental here. Substitution with a *para*-fluoro group had a slight extraneous effect, but not significantly so, whilst *para*-nitro substitution led to a 100-fold loss of anti-HIV activity.[34]

In a subsequent report, we studied a selection of AZT phosphoramidates with a wide range of aryl substituents. Interestingly, these compounds were tested in true TK$^+$ and TK$^-$ cell lines.[34] However, none of the phosphoramidates retained the high potency (2–4 nM) of AZT in TK competent (TK$^+$) human T cell lines (CEM and MT4) against either HIV-1 or HIV-2. However, they retained substantial activity in the TK-deficient cell line CEM/TK$^-$, whereas AZT lost all of its activity in this same cell line. This was taken as direct proof of intracellular nucleoside phosphate delivery. Again, alanine emerged as an important component, while the glycine analogue was inactive in all HIV-infected CEM/TK$^-$ cultures. In this assay, leucine and phenylalanine were as effective as alanine, although they were less so in CEM/TK$^+$ assays. Thus, the parent phenyl methoxy alanyl phosphoramidate emerged as an important lead which demonstrated efficacy to bypass the nucleoside kinase.[34]

Application to various antiviral and anticancer nucleosides

We, and others, have applied our aryloxy phosphoramidate approach to many nucleosides with antiviral and anticancer activ-

ities. The results generally varied from one nucleoside to another, and sometimes varied significantly from one phosphoramidate to another for the same nucleoside. Additionally, in some cases, the application of the phosphoramidate approach turns an inactive nucleoside to an active one. Here, we will summarise some of the data obtained from applying the phosphoramidate technology to numerous nucleoside analogues.

4′-Azidouridine

4′-Azidouridine (**15**) is a ribonucleoside that is inactive as an inhibitor of HCV replication,[35] while the analogous cytosine derivative (4′-azidocytidine) is a well-known potent inhibitor of the virus.[36] The active species of 4′-azidocytidine was found to be its triphosphate derivative. We hypothesised that 4′-azidouridine was inactive as a result of poor phosphorylation, and consequently applied our phosphoramidate approach to 4′-azidouridine.[37]

Numerous 4′-azidouridine phosphoramidates (**15a–c**; Table 1) with varying amino acid, aryl and ester groups were

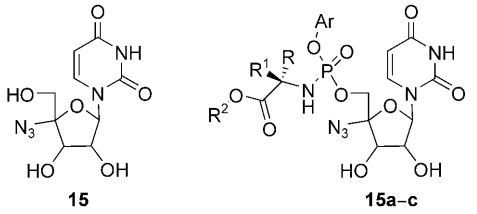


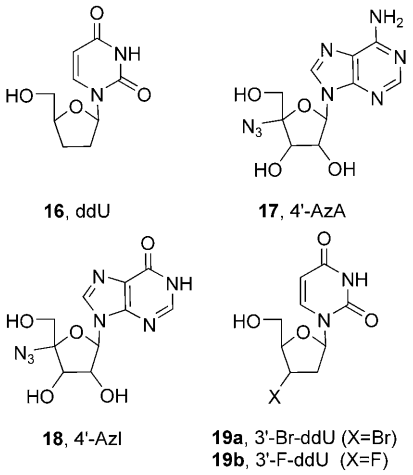
Table 1. Anti-HCV activity of 4′AzU (**15**) and a series of its phosphoramidate derivatives (**15a–c**).

Compd	Ar	Amino acid (R,R^1)	Ester (R^2)	EC_{50} [μM]	CC_{50} [μM]
15a	Phenyl	L-Ala	2-Bu	0.63	>100
15b	Phenyl	L-Ala	Bn	0.61	>100
15c	Naphthyl	L-Ala	Bn	0.22	>100
15	–	–	–	>100	>100

EC_{50}: 50% effective concentration or compound concentration required to inhibit HCV replication by 50%; CC_{50}: 50% cytotoxic concentration as determined with the MTS dye viability staining method. The HCV replicon assay was performed in the stable replicon cell line 2209-23 derived from Huh-7 cells stably transfected with a bicistronic HCV replicon (genotype 1b) expressing the renilla luciferase reporter gene. Further details of the anti-HCV assay can be found in reference [37].

prepared. Most of the compounds showed improved anti-HCV activity and had no toxicity in the replicon assay (CC_{50} >100 μM). Some of the compounds inhibited the replication of HCV at submicromolar concentrations (<1 μM), while the parent nucleoside was devoid of any activity (EC_{50} >100 μM). In terms of SAR, L-alanine phosphoramidates showed better activity than other amino acids such as valine, β-alanine and *N*-methylglycine. In addition, naphthyl phosphoramidates exhibited improved anti-HCV activity over their phenyl counterparts, most likely due to the better lipophilicity of the naphthyl derivatives, which enhanced their ability to cross the cell membrane. In one case, the distereoisomers of a phosphoramidate (naphthyl L-alanine benzyl ester) were separated, their absolute stereochemistry determined by molecular modelling and NMR, and subsequently tested. In this case, no significant differences in HCV inhibition were observed for the two distereoisomers (*Rp*, EC_{50} = 0.39 μM; *Sp*, EC_{50} = 0.43 μM). The triphosphate derivative of 4′azidouridine was also synthesised and tested against the HCV polymerase. The data revealed that 4′-azidouridine triphosphate was as potent an inhibitor of HCV polymerase as 4′-azidocytidine triphosphate. This finding, coupled with the success of the phosphoramidate approach to improve the anti-HCV activity of 4′-azidouridine, indicates that these improvements in activity are a direct result of bypassing the first phosphorylation step. This suggested that the inability of 4′-azidouridine to exert any anti-HCV activity was due to poor intracellular phosphorylation to the monophosphate.

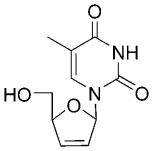
As in the case of 4′-azidouridine, the application of our phosphoramidate technology was successful in transforming various inactive nucleosides to active ones, such examples include 2′,3′-dideoxyuridine (ddU, **16**),[38,39] 4′-azidoadenosine (4′-AzA, **17**),[40] 4′-azidoinosine (4′-AzI, **18**),[41] and 3′-fluoro- and 3′-bromo derivatives of 2′,3′-dideoxyuridine (**19a**, 3′-BrddU; 3′-F-ddU, **19b**)[42].



2′,3′-Didehydro-2′,3′-dideoxythymidine (d4T)

An FDA-approved anti-HIV agent, d4T, exhibits a selective anti-HIV activity in cell culture, and also a reduced toxicity in certain cell types (e.g. bone marrow progenitor cells) compared with other antiretroviral agents such as AZT.[43–45] The impressive profile of d4T (**20**), as well as the fact that the phosphorylation of d4T to the monophosphate is known to be of low efficiency,[46] highlighted d4T as an ideal candidate for our phosphoramidate technology. We prepared a family of d4T phosphoramidates and investigated their anti-HIV activity.[47,48]

Various substituted aryl- and ester-modified d4T phosphoramidates (**20a–e**; Table 2) were prepared and tested against HIV-1 and HIV-2. Generally, d4T phosphoramidates were signifi-



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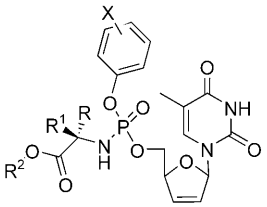


20a–e

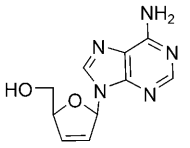
Table 2. Anti-HIV activity of d4T (**20**) and a series of its phosphoramidate derivatives (**20a–e**).

Compd	X	Amino acid (R,R^1)	Ester (R^2)	EC_{50} [μM] HIV-1 CEM(0)	HIV-2 CEM(0)	HIV-2 CEM-TK$^-$	CC_{50} [μM]
20a	*p*-Cl	L-Ala	Me	0.005	0.007	0.006	11
20b	*p*-Br	L-Ala	Me	0.009	0.016	0.018	17
20c	*m*-I	L-Ala	Me	0.008	0.007	0.016	25
20d	–	L-Ala	*n*Pr	0.42	1.1	0.4	≥250
20e	–	L-Ala	*n*Bu	0.50	0.69	0.22	168
20	–	–	–	0.651	0.770	33	267

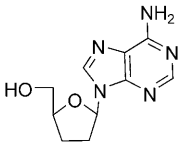
EC_{50} is the 50% effective compound concentration required to protect CEM cells against the cytopathicity of HIV by 50%. Data are the mean of 2–3 independent experiments. CC_{50} is the 50% cytostatic compound concentration required to inhibit CEM cell proliferation by 50%. Experimental details can be found in Reference [48].

cantly more active than the parent nucleoside, d4T. In addition, several compounds showed a >10-fold superior selectivity index (SI) compared with d4T.[48] Although halogen- substituted aryl phosphoramidates of d4T had better lipophilicity profiles compared with other aryl-substituted compounds, no correlation between lipophilicity and anti-HIV activity was observed in this study.[48] Regarding amino acid variations, it was initially found that L-alanine phosphoramidates of d4T possessed better anti-HIV activity compared with other d4T phosphoramidates with other natural non-L-alanine amino acids.[47] However, we later observed that L-alanine could be substituted with some unnatural amino acids with no significant loss of anti-HIV activity.[49] The impressive anti-HIV data obtained with the d4T phosphoramidates led us to investigate the possible ways of improving the aqueous solubility of these agents.[50] We found that substitution of polyether side chains on the aryl ring resulted in a significant improvement in aqueous solubility. The replacement of the phenol moiety with, for example, a tyrosine substantially increases aqueous solubility, but at the cost of antiviral activity, confirming the importance of maintaining prodrug lipophilicity.

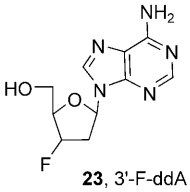
Unlike 4′-AzU, 4′-AzA and ddU, in this case the application of the phosphoramidate approach was successful in significantly improving the antiviral activities of numerous nucleosides that already exert good antiviral activities. Such success was also observed with 2′,3′-didehydro-2′,3′-dideoxyadenosine (d4A, **21**),[51] 2′,3′-dideoxyadenosine (ddA, **22**)[51] and 2′,3′-dideoxy-3′-fluoroadenosine (3′-F-ddA, **23**)[52].



21, d4A



22, ddA

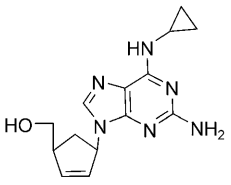


23, 3′-F-ddA

Abacavir

Carbocyclic nucleosides, lacking the labile glycosidic linkage between the heterocycle and sugar moieties, have long been proposed to offer an attractive in vivo stability advantage over the 2′,3′-dideoxynucleosides. The first member of this structurally unique family to be approved for clinical use was abacavir (**24**), which is used in the treatment of HIV.[53–55] This agent is activated via two distinct stages; initially abacavir is metabolised to its monophosphate derivative after which the compound is deaminated by a distinct cytosolic deaminase.[56,57] The deaminated product, carbovir monophosphate, is then converted to the triphosphate form by two successive phosphorylation processes.[56] Although abacavir is a relatively potent anti-HIV agent, it is still less potent than numerous other approved anti-HIV drugs such as AZT, 3TC and ddC. Consequently, we wondered whether applying our protide approach would improve the anti-HIV activity of this agent, and whether the micromolar in vitro anti-HBV activity of abacavir could be improved after conversion to its protide form.

We synthesised a wide range of abacavir phosphoramidates (**24a–d**; Table 3) with varying amino acids and esters. These



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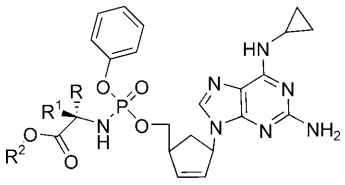


24a–d

compounds were studied for their anti-HIV as well as anti-HBV activities.[58,59] The parent nucleoside (abacavir) had an EC_{50} value of ~2.0 μM against both HIV-1 and HIV-2. All of the studied phosphoramidates of abacavir showed significant improvements in antiviral activities. The most potent phosphoramidate was the phenyl L-alanine methyl ester derivative (HIV-1, EC_{50} = 0.05 μM; HIV-2, EC_{50} = 0.07 μM). However, this derivative was more toxic in vitro relative to the parent nucleoside (CC_{50} = 13 μM and 160 μM, respectively).[59] In terms of anti-HBV activi-

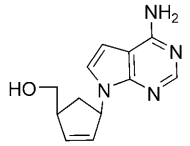
Table 3. Anti-HIV and anti-HBV activities of abacavir (**20**) and a series of its phosphoramidate derivatives (**24a–d**).

Compd	Amino acid (R,R^1)	Ester (R^2)	EC$_{50}$ [μM] HIV-1 CEM	EC$_{50}$ [μM] HIV-2 CEM	CC$_{50}$ [μM] CEM	EC$_{50}$ [μM] HBV HepG 2 2.2.15	CC$_{50}$ [μM] HepG2
24a	L-Ala	Me	0.05	0.05	13	0.55	17
24b	L-Ala	Et	0.07	0.061	12	0.35	18
24c	L-Ala	*i*Pr	0.48	0.85	17	0.33	10
24d	DiMeGly	Me	0.067	0.064	>130	0.019	5.5
24	–	–	1.9	3.0	78	5.6	110

EC$_{50}$: effective concentration required to inhibit HIV or HBV-induced cytopathicity by 50%. CC$_{50}$: cytostatic concentration required to inhibit HepG2 cell proliferation by 50%. Details of the HIV and HBV assays can be found in Reference [59]. Data are the mean of at least two independent experiments.

ty, the most potent protide is that derived from the unusual achiral amino acid, dimethylglycine, which was ~300-times more potent than abacavir, but again with an increase in cytotoxicity.

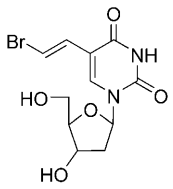
We also applied our phosphoramidate approach to carbocyclic 2′,3′-dideoxy-2′,3′-didehydro-7-deazaadenosine (**25**, 7-deaza-d4A), which is a nucleoside with poor anti-HIV and anti-HBV activities (IC$_{50}$ = 280 μM and 2 μM, respectively).[60] The protides of this nucleoside showed significant improvements in both anti-HIV and anti-HBV activities. In regard to anti-HIV activity, the most potent phosphoramidate was the phenyl L-alanine methyl ester derivative (IC$_{50}$ = 0.091 μM), offering a 3000-fold boost in potency. This compound also showed potent anti-HBV activity (IC$_{50}$ = 0.09 μM), though the phenyl dimethylglycine and phenylalanine methyl ester derivatives were found to be more potent against HBV, with IC$_{50}$ values of 0.05 μM and 0.055 μM, respectively.[60]



25, carbocylic 7-deaza-d4A

BVDU

(*E*)-5-(2-Bromovinyl)-2′-deoxyuridine (BVDU, **26**) was among the other antiviral nucleosides to which our phosphoramidate technology was applied. This nucleoside analogue exhibits potent anti-HSV-1 and anti-VZV activity in cell culture.[61,62] BVDU is activated intracellularly to the triphosphate derivative by viral thymidine/thymidylate kinase to form the mono- and diphosphate forms, respectively, after which the triphosphate species of BVDU is formed by cellular kinases. Thus, we were interested in using our protide approach on BVDU to see if we could improve the antiviral activity. A series of phosphoramidates of BVDU (**26a–d**) were synthesised and tested against VZV, HSV-1 HSV-2 and also vaccinia virus (Table 4).[63] The results showed the phosphoramidates to be less potent than the parent nucleoside against VZV, HSV-1 and HSV-2, although the phenyl benzyl L-alanine derivative of BVDU had an activity comparable to BVDU against HSV-1, and a significantly improved inhibitory activity against HIV-1 TK$^-$ than the parent BVDU. In addition, some of the protides were relatively more potent against vaccinia virus compared with BVDU.



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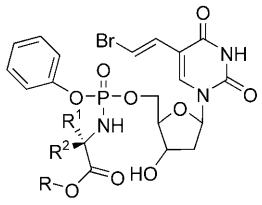


26a–d

To our surprise, NewBiotics reported that some BVDU phosphoramidates possess potent anticancer activities.[64] Thus, we were interested in studying the cytostatic (antiproliferative) activity of a large series of BVDU phosphoramidates. Our interest was enhanced by the fact that the phenyl L-alanine methoxy phosphoramidate of BVDU (thymectacin) entered clinical trials against colon cancer.[65] A number of BVDU phosphoramidates were synthesised and tested against various types of cancer cells, such as breast, prostate and bladder cancers.[66–68]

Table 4. Anti-VZV, Anti-HSV and antivaccinia activities of BVDU (**26**) and a series of its phosphoramidate derivatives (**26a–d**).

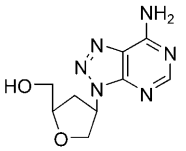
Compd	Amino acid (R,R^1)	Ester (R^2)	Anti-VZV activity EC$_{50}$ [μM] VZV (OKA)	VZV (YS)	TK$^-$ VZV (YS)	CC$_{50}$ [μM]	Anti-HSV and Anti-vaccinia activities EC$_{50}$ [μM] HSV-1 (KOS)	HSV-2 (G)	TK$^-$ HSV-1 (KOS)	Vaccinia virus	MCC [μM]
26a	L-Ala	Me	0.05	0.26	>50	>200	0.384	>48	48	9.6	>400
26b	L-Ala	Bn	0.026	0.025	>50	81	0.002	>16	0.348	0.256	80
26c	L-Val	Me	11	11	>200	>200	1.92	>240	>240	32	>400
26d	L-Ala	Me	0.07	0.13	>20	74	0.015	>16	16	0.128	400
26 (BVDU)	–	–	0.003	0.01	96	>200	0.001	>80	>48	0.384	>400

EC$_{50}$: effective concentration required to inhibit virus-induced cytopathicity by 50%. CC$_{50}$: cytotoxic concentration required to inhibit HEL cell proliferation by 50%. MCC: minimal cytotoxic concentration or compound concentration required to result in a microscopically visible alteration of cell morphology. Further details of the VZV and HSV assays could be found in Reference [63].

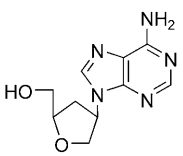
Most of the studied BVDU phosphoramidates exhibited anticancer activities varying from moderate to high (submicromolar) in some cases. One of our compounds, which is the phenyl L-alanine benzyl ester derivative of BVDU, revealed potent cytostatic activity ($IC_{50} = 1.4$ μM) against colon cancer (HT115), and offered a 175-fold improvement in activity over thymectacin against colon cancer cells. The introduction of substitutions at the phenyl ring led to a series of compounds with potent anticancer activities. In particular, the *p*-chlorophenyl L-alanine benzyl ester derivative of BVDU showed activity against breast, colon and prostate cancer cells, with IC_{50} values of 6.2, 3.4 and 2.4 μM, respectively. In terms of amino acid variations, the results were favourable. Most of the non-L-alanine compounds retained good activity, and a significant number of them possessed improved anticancer activities over those with L-alanine. In fact, the naphthyl dimethylglycine methyl derivative was found to be most potent against breast cancer MDAMB231 ($IC_{50} = 0.32$ μM), however, its activity was not as potent against a human prostate cancer cell line (PC-3). In addition, the naphthyl L-proline methyl derivative was potent against bladder cancer cells T24 ($IC_{50} = 0.4$ μM), while again such a potent activity was not retained against other types of cancers, including breast and prostate. Thus, it soon became clear that certain phosphoramidate derivatives could be very potent against one cancer cell type, while not necessarily potent against others. The reasons for such differences between cancer types are still unknown, but it may be a result of the levels or types of enzymes present in these cells, which are involved in metabolising phosphoramidates, or it may be related to cell access or efflux.

Application to other nucleosides

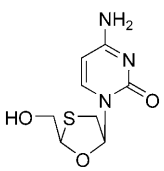
The first application of the aryl phosphoramidate technology to an isonucleoside was reported in 1994 by Franchetti and co-workers.[69] In fact, they reported the application of the technology to 8-aza-isoddA (**27**) and isoddA (**28**). The result was a significant boost in the antiviral potency, where the aryl phosphoramidates of 8-aza-isoddA (**27**) showed >25-fold increase in antiviral activity, while those of isoddA (**28**) displayed a 350–800-fold increase in activity. This was an important study, which demonstrated the power of the aryloxy phosphoramidate approach in greatly improving the biological profiles of weakly active nucleosides. In this study, for example, isoddA (**28**) was transformed from 32 μM activity against HIV-2, to 40 nM activity upon phosphoramidate formation. Further studies on these compounds revealed that such improvements in antiviral activity were due to an efficient intracellular phosphate delivery by aryloxy phosphoramidates.[70]



27, 8-aza-isoddA



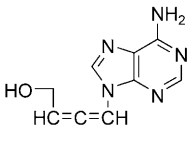
28, isoddA



29, 3TC

As a result of the impressive success of phosphoramidates in significantly improving the antiviral activity of isonucleosides, we became interested in applying phosphoramidates to sugar-modified nucleosides. Thus, we applied the approach to 2′,3′-dideoxy-3′-thiacytidine (3TC, **29**).[71] However, the antiviral activity of 3TC phosphoramidates was not as impressive as that seen with the protides of isonucleosides. In fact, the phenyl L-alanine methoxy phosphoramidate derivative of 3TC was less active than the parent nucleoside in deoxycytidine (dCyd) kinase (dCK) expressing HIV-1- and HIV-2-infected cells.[71] However, conducting the assay in dCK-deficient cells indicated far less of an impact on the potency for the 3TC phosphoramidate than in the parent CEM cells (~20-fold vs. 2000-fold). Interestingly, both compounds were equally effective against hepatitis B virus in hepatoma G2 cells indicating efficient protide activation in these cells but not in CEM cells used for the HIV assay.[71] This was the first indication that the efficacy of phosphoramidates might be cell-line dependent.

Zemlicka and co-workers used the phosphoramidate approach on various nucleosides, and interestingly they applied such a strategy on nonclassical nucleosides.[72] Initially, phenyl



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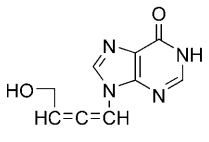

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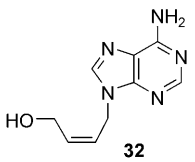

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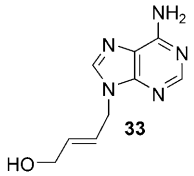

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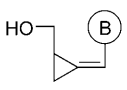

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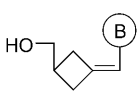
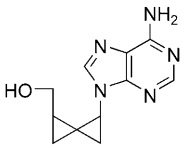


35

B = various bases



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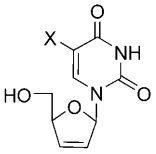


37, X = F, Cl, I

methoxy alaninyl phosphoramidates of the anti-HIV agent, adenallene (**30**), and the inactive compound, hypoxallene (**31**) were prepared.[73] The phosphoramidate derivative of adenallene was shown to exert a 10–20-fold boost in anti-HIV activity. Another study looking at alkenyl adenine nucleosides of structures **32** and **33** revealed some interesting results. In these cases, both (*Z*)-**30** and (*E*)-**31** nucleosides were inactive, whilst the phosphoramidate of **30** was active in the 1–10 μM range

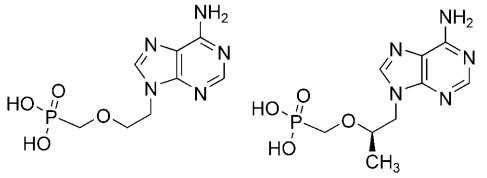
and nontoxic; the isomeric phosphoramidate of **33** remained inactive. The hypoxanthine analogue of **32** was also poorly active.

In a subsequent study, Zemlicka and co-workers investigated the activity of methylenecyclopropane nucleoside (**34**) phosphoramidates in several antiviral assays.[74–77] In addition to the active *Z*-isomer, the inactive *E*-isomers were also studied. This study led to interesting conclusions in terms of structure–activity relationships. The results revealed that the adenine and 2,6-diaminopurine phosphoramidates were most potent against HIV. Although they were relatively toxic, the SI values of these agents were superior to those of the parent compounds. Notably, these protides were 10-fold more potent against HIV-2 compared with HIV-1.[77] Strikingly, the phosphoramidates of the inactive (*E*)-isomer were also found to be active. In addition, the phosphoramidates of the (*Z*)-guanine analogues were active against a wide selection of viruses (HCMV, HBV, EBV, and VZV). In these cases, no significant toxicities were noted. The phosphoramidate approach was then applied to cyclobutane **35** and spiropentane **36** nucleosides with varying degrees of success.[78,79]

To further pursue the kinase bypass approach, we prepared some novel d4T inactive antiviral (i.e. HIV) analogues with 5-halo substituents in place of the 5-methyl group (**37**) and converted them to their phosphoramidates.[80] Although the parent nucleosides were only weakly active or inactive at >10–50 μM, all the phosphoramidates were active. In particular, the phenyl methoxy alaninyl phosphoramidate of the 5-chloro compound was active at submicromolar concentrations and nontoxic.[80]

Application to acyclic nucleoside phosphonates

As well as the application to nucleoside analogues, in 2001 we reported the first application of the aryl phosphoramidate technology to nucleoside analogue phosphonates, in particular PMEA (**38**) and (R)PMPA (**39**).[81] These two nucleoside phosphonate analogues exist in their monophosphonate forms (containing a stable P–C bond). Thus, applying a protide approach to these agents is an attractive possibility. A series of phosphoramidates with substituted phenyl moieties and varying amino acids and esters were prepared and tested against two different HIV-infected cell lines (MT4 and CEM). All the phosphoramidates showed potent activity against HIV-1 and HIV-2 with boosts in activity between 30–100 fold. In our assay, (R)PMPA (tenofovir) was active against HIV-1 at 2.3 μM and HIV-2 at 1.4 μM in MT4 cells, while it was active against HIV-1 and HIV-2 at 3.67 μM in CEM cells. As for the phosphoramidates, the majority of them exhibited submicromolar inhibitory activities. In particular, the phenyl L-alanine methyl ester derivative of tenofovir was most potent in MT4 cells (HIV-1, $EC_{50}=0.029$ μM; HIV-2, $EC_{50}=0.026$ μM). In CEM cells, the same phosphoramidate derivative was also very potent (HIV-1, $EC_{50}=0.053$ μM; HIV-2, $EC_{50}=0.090$ μM). PMEA phosphoramidates were also relatively potent especially the phenyl L-alanine methyl ester. This agent also exhibited inhibitory activity at submicromolar concentrations though it also displayed some toxicity. Most of the other phosphoramidates of both PMEA and (R)PMPA did not possess significant toxicity.

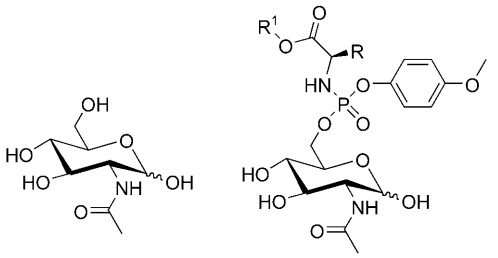


38, PMEA **39**, PMPA

Following our work on PMEA and (R)PMPA in 2001,[81] there have been some reports in the literature, mainly from Gilead Sciences,[82–84] on the application of the phosphoramidate technology to acyclic nucleoside phosphonates. Indeed such application has resulted in two very potent anti-HIV drugs, GS-7340 and GS-9131, entering clinical trials, which will be discussed later.

Application to glucosamine

Glucosamine has been used for a long time in the prophylaxis and treatment of osteoarthritis.[85] Despite the different hypotheses regarding the mechanism of action of glucosamine, the action of the 6-phosphate metabolite on cartilage degradation appears to be a favoured putative mechanism.[86] Thus, a series of phosphoramidates of *N*-acetyl glucosamine (**40a**–**e**; Table 5) were synthesised and tested for antiosteoarthritic activity.[14]



40 **40a–e**

Although all the phosphoramidates synthesised had the same aryl motif (4-*O*-methoxy phenol), they differed in their amino acid and ester moieties. The assay used for measuring the activity of these compounds was based on the induced GAG (glycosaminoglycan) release. In this assay, glucosamine had some protective activity at 10 mM concentration, though some toxicity was observed. Notably, no protective activity was detected at 1 mM concentration. However, the phosphoramidates of *N*-acetyl glucosamine showed better activity and some of them showed activity at concentrations as low as 0.1 mM with no observed toxicity. Hence, they are approximately $\geq$100-fold more active than glucosamine in this assay.

Notably, unusual and interesting SARs relative to nucleoside phosphoramidates were observed for the glucosamine protides. In particular, the often favoured L-alanine phosphoramidates showed poor activity, while their D-counterparts were more potent. The reasons for such differences are currently under investigation in our laboratories.

Table 5. Antiosteoarthritic activity of *N*-acetylglucosamine (**40**) and a series of its phosphoramidate derivatives (**40 a–e**).[a]

Compd	Amino acid (R)	Ester (R′)	Control GAG fold	GAG fold	Reduction GAG fold[b] [%]	MTT[b]
40 a	Gly	Bn	3.9	4.2	−12.2 (±15.42)	99 (±1.92)
40 b	L-Val	Et	3.8	4.4	−21.1 (±25.80)	94 (±3.19)
40 c	L-Pro	*n*Bu	3.8	4.1	−2.2 (±4.44)	104 (±1.2)
40 d	L-Ala	Bn	4.5	4.6	−1.9 (±10.19)	89 (±1.29)
40 e	D-Ala	Bn	4.2	3.4	24.3 (±16.28)	96 (±2.91)
40	–	–	4.5	4.3	5.4 (±0.41)	107 (±2.03)

[a] Activity measured at 0.1 mM concentration. The average fold increase in GAG release into the culture media in IL-1-treated cultures is calculated using the appropriate control (minus IL-1) for explants cultured in the absence (control GAG fold) and presence (GAG fold) of glucosamine compounds at concentrations ranging from 10 to 0.1 mM; values indicated are the average of these experiments. The percent reduction in GAG-fold was calculated for each experiment as the percent difference observed for each experiment using the following calculation: {[(control GAG fold)−(sample GAG fold)]/(control GAG fold)}×100. The effects of different concentrations of glucosamine compounds on chondrocyte viability were assessed using the MTT assay. The number of experiments carried out varied from one sample to another. Further details on the assay could be found in Reference [14]. [b] Values given in parentheses are the standard error.

Mechanism of action

The activation of aryloxy phosphoramidates to release the monophosphate species is suggested to proceed in four steps: two enzyme-mediated and two spontaneous (Figure 3). The activation process begins with the cleavage of the ester moiety by an esterase-type activity. In an effort to model this in a predictive sense, we exposed various esters to pig liver esterase and followed the progress of the reaction by ^{31}P NMR.[86] Upon esterase treatment, the two signals of the starting material, corresponding to the two diastereoisomers of phosphoramidates, disappeared to give a downfield singlet ($\delta_p \sim 8.2$), which was characterised as the amino acyl phosphate monoester. Whilst the rate of cleavage of various esters did not correlate with antiviral activity, we did note that esterase lability was a necessary condition (but not sufficient alone) for high biological potency. Notably, the *tert*-butyl ester was not hydrolysed in this assay, and the *tert*-butyl ester-derived phosphoramidate was the least potent of all tested compounds.[86] Recently, scientists at Gilead identified cathepsin A as the major enzyme responsible for the cleavage of the ester motif.[87] This study also looked at the rate of ester hydrolysis, which often correlates with antiviral activity, and the findings of such an investigation were striking. Phosphoramidates containing alanine were hydrolysed quicker than those with branched amino acids. In fact, cathepsin A was unable to hydrolyse phosphoramidates with heavily branched amino acids such as leucine, valine and isoleucine. The reason behind this hydrolysing selectivity remains unclear. Interestingly, another relatively minor enzyme able to hydrolyse phosphoramidates was also detected in this study, but has yet to be identified.

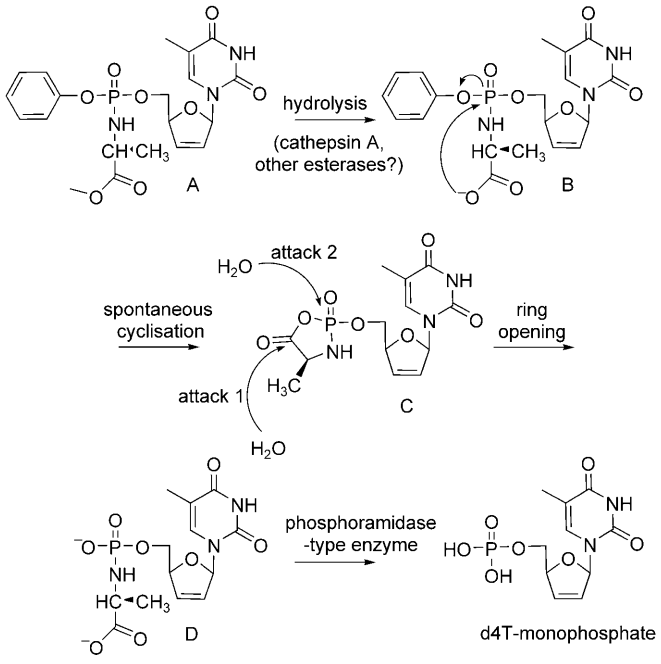


Figure 3. Postulated mechanism of phosphoramidate activation.

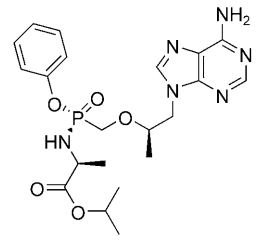
Upon ester hydrolysis, an intracellular displacement of the phenoxy group by the carboxylate anion (B) takes place (Figure 3). This results in the formation of a five-membered ring mixed anhydride (C), with the speed of this process being dependent on the structure of the analogue. Thus, cyclisation of intermediate B of AZT was more difficult than that of d4T.[88] More interestingly, phosphoramidates with β-alanine only went as far as the ester hydrolysis, as no subsequent metabolites were formed.[88]

The cyclic anhydride intermediates then undergo hydrolysis, which is not enzyme assisted, to give phosphoalaninate (D). The cleavage of the P–N bond is ruled out at this stage,[89] hydrolysis can only proceed by either attack on the phosphorous atom or the carbonyl functionality. Often the hydrolysis or methanolysis of mixed carboxylic and phosphoric anhydrides proceeds by attack on the carboxylate moiety (attack 1).[89] However, in the case of five-membered ring anhydrides, attack on the phosphate group (attack 2) can prevail.[90,91] Notably, phosphoalaninates of d4T phosphoramidates exhibited an anti-HIV effect comparable to the parent compound, d4T.[92]

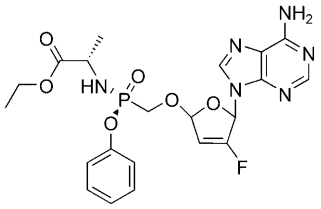
The final activation step of phosphoramidates is the cleavage of the P–N bond of the phosphoalanine intermediate to generate the monophosphate nucleoside analogue. Although this step is believed to be a result of the activity of an intracellular phosphoramidase,[92,93] direct evidence is still lacking.

Aryloxy phosphoramidates under clinical investigations

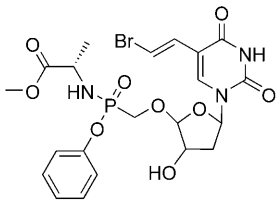
At least four different aryloxy triester phosphoramidates have reached clinical evaluation; (**41**, GS-9140; **42**, GS-9131; **43**, thymectacin (NB1011); **44**, stampidine). Apart from thymectacin, which is being developed as an anticancer agent, the other three drugs have been pursued as anti-HIV agents.



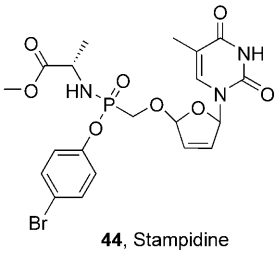
41, GS-7340



42, GS-9131



43, Thymectacin



44, Stampidine

GS-7340 and GS-9131

GS-7340 (**41**) and GS-9131 (**42**) are two phosphoramidate triester-based nucleoside analogues that are being developed by Gilead Sciences for the treatment of HIV/AIDS.[94,95] As well as the potent anti-HIV activities exerted by these agents, they also possessed favourable antiviral drug resistance profiles and low cytotoxicities.[87,94,95]

GS-7340 (**41**) is a phosphoramidate prodrug of the FDA-approved anti-HIV phosphonate tenofovir (TFV, (R)PMPA). TFV is an acyclic nucleotide analogue of dAMP, and is a potent in vitro and in vivo inhibitor of HIV-1 replication.[96] TFV is converted to its active species, which acts as a potent inhibitor of HIV-1 reverse transcriptase, following two intracellular phosphorylations by kinases.[97,100] TFV exhibits low cellular permeability and poor oral bioavailability because a charged phosphate group is inherent in the molecule. Numerous prodrugs have been designed to address these problems; the bis-isopropoxycarbonyloxymethyl ester prodrug of TFV (TDF) has been approved for HIV treatment. Although TDF is well tolerated, infrequent development of resistance and minor long-term toxicities are associated with its use.[96,101,102] In addition, despite the initial high systemic levels of TFV following TDF administration, the rapid systemic degradation of TDF to TFV, which is charged under physiological conditions, limits its uptake into the target cells.

In order to develop plasma-stable prodrugs of TFV, the Gilead group applied the phosphoramidate approach. Upon optimisation, they identified the phenyl L-alanine isopropyl ester of TFV (GS-7340, **41**) as a good clinical candidate. As well as the improved potency in anti-HIV activity compared to TFV, GS-7340 (**41**) was found to be stable in blood.[103] Further investigations revealed that 10-fold and 30-fold higher levels of TFV and its phosphorylated metabolites were recorded following incubation of peripheral blood mononuclear cells (PBMC) with GS-7340 (**41**), compared with the incubation with bis-POC TFV and TFV, respectively.[103] Next, the Gilead group developed a practical large-scale synthesis of GS-7340 (**41**), which also allowed separation of the distereoisomers.[104] Interestingly, the group chose the *S*-isomer for development as an HIV inhibitor because it was found to be ~10-fold more potent than the *R*-isomer.[104] However, Gilead recently discontinued the development of GS-7340 (**41**) as they did not believe that GS-7340 (**41**) has a profile that differentiates it to an extent that supports its continued development.

GS-9131 (**42**) is a phosphoramidate prodrug of the novel nucleoside analogue 2′-α-fluoro-2′,3′-didehydro-2′,3′-dideoxyadenosine. In fact, it is the phenyl L-alanine ethyl ester derivative of this nucleoside that was chosen for development and clinical studies. The encouraging factors for the Gilead group, which are also responsible for the development of GS-9131 (**42**), were: a) the good anti-HIV potency of this agent against HIV-1-resistant virus strains, b) the favourable in vivo pharmacological properties of GS-9131 and c) the ability of GS-9131 (**42**) to effectively deliver the active diphosphate metabolite into PBMC.[94,105] GS-9131 (**42**) is currently under clinical evaluation.

Thymectacin

As mentioned above, the phenyl L-alanine methoxy derivative of BVDU (**43**) surprisingly showed potent antitumour activities. Developed by NewBiotics, this BVDU phosphoramidate, known as thymectacin, is currently undergoing combined phase I/II clinical trials for the treatment of colon cancer. It is a novel agent that selectively targets tumour cells with a high expression of thymidylate synthase (TS).[106–109] It is non-cross-resistant with TS-targeted agents and was found to be at least 10-fold more cytotoxic to 5-FU-resistant, TS over-expressing colorectal tumour cells than to normal cells.[108] Monotherapy trials are complete, and thymectacin (**43**) was found to be active and feasible in fluoropyrimidine-resistant metastatic CRC.[109]

Stampidine

Stampidine (**44**) is a *para*-bromophenyl methoxy-L-alaninyl phosphoramidate derivative of d4T. As well as showing good anti-HIV activity against wild-type HIV-1 strains, stampidine (**44**) also displayed good anti-HIV-1 activity against virus strains carrying NNRTI mutations.[110,111] This compound also showed good activity, tolerability and toxicity profiles in vivo.[112–114] Stampidine (**44**) has been proposed as a treatment for patients infected with highly drug-resistant strains of HIV-1. Despite the impressive in vitro and in vivo activities of stampidine (**44**), there have been no recent reports regarding its further development, and no clinical trials are underway at present.

Conclusions and Outlook

The phosphoramidate triester protide approach has been well investigated and established as a viable method for the intracellular delivery of monophosphate nucleoside analogues. This approach has been proven to considerably improve the antiviral and anticancer profiles of many nucleoside analogues. Unlike other pronucleotide approaches, phosphoramidate

triesters are activated intracellularly by enzymes to release the phosphorylated biomolecule. Although some of the enzymes involved in this activation process have been identified, more work is needed in this area to confirm the full activation mechanism.

Nevertheless, the phosphoramidate triester pronucleotide technology has proved effective as a tool for drug discovery, and so far at least four phosphoramidate triester-based drugs are undergoing clinical trials (one anticancer and three antiviral agents). In the future, we can expect to see this technology applied to non-nucleoside phosphorylated compounds, either as therapeutic molecules or as biochemical tools to study biological phenomena. In addition, phosphoramidate-based compounds may be explored as treatments of a wide range of diseases that are not just limited to viral infections and cancers.

Keywords: drug delivery · nucleosides · nucleotides · phosphoramidates · prodrugs

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