Synthesis of Nucleoside Phosphate and Phosphonate Prodrugs

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1. INTRODUCTION

For many decades, the design of new nucleoside analogs as potential therapeutic agents focused on both sugar and nucleobase modifications. These nucleoside analogs rely on cellular kinases to undergo stepwise addition of phosphate groups to form the corresponding active nucleoside triphosphate to express their therapeutic effect. However, nucleosides triphosphates cannot be considered as viable drug candidates as they usually have poor chemical stability along with high polarity that hinders them from transporting across cell membranes. Within the nucleoside analog phosphate activation process, the first phosphorylation has often been identified as the limiting step, which led medicinal chemists to prepare stable "protected" monophosphate nucleosides capable of delivering nucleoside monophosphates intracellularly. These nucleoside monophosphate prodrugs are designed to efficiently cross the biological barriers (as opposed to nucleoside monophosphates; Figure 1, eq 1) and reach the targeted cells or tissues. Once inside the cell, the biolabile protecting groups are then degraded enzymatically and/or chemically, releasing the free nucleoside analog in the monophosphate form, which can often efficiently express its therapeutic potency by intracellular conversion to the corresponding nucleoside triphosphate (Figure 1, eq 2).

Interestingly, the use of such phosph(on)ate prodrugs has not only proved to enhance the activity of parent nucleosides, but also generated potent compounds otherwise inactive in their nucleoside form because of a lack of monophosphorylation. Proof of concept for monophosphate prodrugs has now been clinically validated in the human immunodeficiency virus (HIV), hepatitis B (HBV), and hepatitis C virus (HCV) fields, leading to several potent and selective prodrugs such as the phase II pradefovir, the phase III GS-7340 (TAF), and the FDA-approved tenofovir disoproxil fumarate (TDF) and sofosbuvir (GS/PSI-7977) (Figure 2).

Several strategies allowing intracellular delivery of nucleotide analogs were developed over the past 20 years based on the...
the design of many different types of phosphate and phosphonate nucleoside prodrugs (Figure 3). Reviews on nucleoside phosph(on)ate prodrugs generally focus on their enhanced biological activities, potential therapeutic interest, and their physicochemical properties, but almost completely neglect their sometimes challenging synthetic preparation. Herein, we review the most important mono-, di-, and triphosphate and phosphonate prodrug approaches applied to nucleoside analogs (Figure 3) from a chemical point of view, detailing the strengths and limitations of each approach. We will focus on the various synthetic pathways discussing (1) the chemical variation of the biolabile phosph(on)ate masking groups; (2) the reliability of using P(III) and/or P(V) chemistry for both phosphate and phosphonate prodrug synthesis; (3) the influence of the masking group(s) introduction conditions (solvent, temperature, stoichiometry) on the overall outcome for each method; (4) the various protection/deprotection strategies used to impart improved yield and regioselectivity relative to the nature of the nucleobase and the sugar; and (5) the influence of reaction conditions or protective groups on the stereoselectivity (Rp/Sp) observed at the phosphorus center as well as the methods employed to separate both Rp and Sp isomers along with the asymmetric strategies for the synthesis of predominantly single diastereoisomers at the phosphorus center.

2. NOMENCLATURE

Phosphorus is often covalently bonded to common atoms such as C, H, O, N, S, forming various chemical species or functional groups. The different categories of phosphorus functional groups are so extensive that confusion in nomenclature or misused terms is common. As a resource and useful for an in depth understanding of this Review, Table 1 presents an extensive summary of the nomenclature of the principal phosphorus moieties widely used in organic chemistry. Structures and functional group names are classified by the presence of O, C, N, and/or S attached to the phosphorus atom and its valence (III or V).
3. NUCLEOSIDE MONOPHOSPHATE PRODRUGS

3.1. Nucleoside Phosphates and Phosphonates O-PO(OR)₂ and C-PO(OR)₂

3.1.1. Carbonyloxymethyl (Including POM, POC).

To date, the only nucleosides phosph(on)ate prodrugs approved by the FDA are the acyclic nucleoside phosphonates adeovir dipivoxil [bis(pivaloyloxymethyl), POM] and tenofovir disoproxil fumarate [bis(iso propyloxymethyl carbonate, POC)]. Adefovir dipivoxil was initially developed for HIV, but studies were stopped due to severe kidney toxicity at the dosage necessary for good antiviral response. In 2002, further investigation of the compound for the treatment of HBV infection led to FDA approval of adefovir dipivoxil. The structurally related tenofovir disoproxil fumarate had a more favorable toxicity profile and was approved in 2001 for the treatment of HIV infection. Both POM and POC groups have been shown to increase oral bioavailability and overall systemic exposure to the parent phosphonic acid compound. More recently, LB80380, a nucleotide bis(POM)-prodrug, completed a phase II clinical trial for the treatment of lamivudine resistant HBV infection (Figure 4).

The degradation of POC-prodrugs involves the enzymatic cleavage of the carbonate by an esterase leading to an unstable carboxylate intermediate that undergoes two subsequent chemical degradations to form carbon dioxide, formaldehyde, and the nucleotide POC-monoester. Repetition of this sequence with the second POC group or its direct cleavage by phosphodiesterase frees the nucleoside monophosphate (Figure 5).

Figure 5. Activation of carbonate-type prodrugs (including POC, R = i-Pr).

In the case of nucleoside prodrugs bearing POM protecting groups, the ester is cleaved to form an unstable hydroxymethyl alcoholate intermediate that undergoes chemical rearrangement to form formaldehyde and the free monophosphate after the second POM degradation (Figure 6).

The synthetic approaches for carbonyloxymethyl phosphate nucleoside prodrugs are summarized in Figure 7: (A) coupling of a nucleoside monophosphate with a halogeno carbonyloxymethyl derivative (POM-Cl for example), (B) reaction of a bis(carbonyloxymethyl)-phosphorochloridate with a nucleoside under basic conditions, (C) Mitsunobu coupling between a nucleoside and bis(carbonyloxymethyl)-phosphate, and (D) 5'-iodination of a nucleoside followed by bis(POM)-phosphate salt nucleophilic substitution.

The more limited methods to access carbonyloxymethyl phosphonates prodrugs are compiled in Figure 8: (A) reaction of the phosphonic acid nucleoside with an halogeno carbon-
yloxymethyl derivative, and (B) direct conversion of dimethylphosphonate nucleoside using sodium iodide and a halogeno carbonyloxymethyl derivative.

3.1.1.1. Synthesis of Carbonyloxymethyl Phosphates

Diesters. In 1984, Farquhar and co-workers were first to report the synthesis of bis(carbonyloxymethyl)phosphate derivatives along with their stability in different buffers, in the presence of liver esterase and in plasma.\textsuperscript{17,18} They developed two synthetic routes to synthesize the bis(POM)-monophosphate prodrug of 5-FdU,\textsuperscript{17a,18} either by Mitsunobu coupling of (1a, b) with bis(POM)-phosphate 7 or by substitution of a 5-iodo nucleoside 3 with bis(POM)-phosphate silver salt 8 (Scheme 1). The later method was found to be low yielding, and the 3′-acetate could not be removed selectively because of the lack of POM group stability under deprotection conditions. Using this approach, numerous nucleosides of biological interest were transformed in their bis(PO M)-monophosphate prodrugs including 5-FdU,\textsuperscript{17a,18} 2′,3′-dideoxyuridine (ddU),\textsuperscript{19} 3′-azido-3′-deoxythymidine (AZT),\textsuperscript{20} and thymidine.\textsuperscript{21}

Both phosphates 7 and 8 were prepared from common intermediate 6 (Scheme 2), obtained by the reaction between disilver aryl phosphate 5 and iodomethyl pivalate at room temperature. Hydrogenation of 6, precipitation as a cyclo-

hexammonium salt, and ion exchange on H+-resin provided (7). Subsequent transformation of bis(POM)-phosphate 7 into its sodium salt with Na+-resin and final treatment with an aqueous solution of silver nitrate lead to desired silver salt 8 (Scheme 2).

Rose et al.\textsuperscript{22} reported the synthesis of α/β 2′-deoxy-4′-thioadenosine bis(POM)-monophosphate prodrug 10 by Mitsunobu coupling between bis(POM)-phosphate 7 and purine nucleosides 9 (Scheme 3).

Interestingly, applied to the synthesis of 8-bromo-2′-deoxyadenosine bis(POM)-phosphate prodrug, the same method\textsuperscript{22} led to an unexpected side reaction of elimination/dehydration, yielding exocyclic methylene compound 12 (Scheme 4). To circumvent this elimination problem, nucleoside monophosphate 13 was coupled with commercially available chloromethyl pivalate. According to the authors, the low yield of 8-bromo-2′-deoxyadenosine bis(POM)-prodrug 14 (19%) was due to repeated chromatographic purification.

In 1995, considering the cumbersome preparation of bis(POM)-phosphate nucleoside prodrugs, Imbach et al. developed a new approach allowing conversion of a nucleoside...
monophosphate into its corresponding bis(POM)-monophosphate. Thus, AZT monophosphate was reacted with iodomethyl pivalate and diisopropyl ethylamine in acetonitrile for 4 days at room temperature to afford AZT bis(POM)-monophosphate prodrug in 22% yield (Scheme 5).

To increase the reactivity of the nucleoside monophosphate during the coupling with POM-I, Kang et al. choose to preactivate the phosphate moiety as a tributylstannyl salt by using tributyltin methoxide. As illustrated in Scheme 6, 2′-azido-2′-deoxyuridine monophosphate was first reacted with 2 equiv of tributylstannyl methoxide, then coupled with iodomethyl pivalate in the presence of tetrabutylammonium bromide to deliver bis(POM)-prodrug after purification on reverse phase HPLC. Despite a good overall yield, the use of tin derivatives represents a serious limitation because of the possible presence of toxic tin residues incompatible with further biological evaluations.

In 2004, Hwang and Cole developed a new approach using new bis(POM)-phosphorochloridate. This reagent was synthesized efficiently in five steps from trimethylphosphate by treatment with sodium iodide and chloromethyl pivalate, monodeprotection, and subsequent chlorination with oxalyl chloride. The coupling of AZT with bis(POM)-phosphorochloridate in the presence of triethylamine allowed for the formation of desired AZT bis(POM)-monophosphate prodrug in 47% yield (Scheme 7).

3.1.1.2. Synthesis of Carbonyloxymethyl Phosphate Monoesters. The POM-phosphate monoesters have also been synthesized. Although these compounds are sometimes evaluated for their biological activities, they are generally prepared as a reference for metabolic degradation studies. Farquhar et al. reported the synthesis of 5-FdU POM-phosphate monoester as a reference during the degradation study of 5-FdU bis(POM)-prodrug. Starting from the dibenzyl phosphate silver salt, the POM-protecting group was introduced by reaction with chloromethyl pivalate. POM-Phosphate was obtained by catalytic hydrogenation, precipitation of cyclohexylammonium salts, and neutralization over acidic resin. The coupling between the dihydrogen POM-phosphate and 5-FdU with DCC in pyridine afforded POM-5-FdU monophosphate monoester prodrug in 53% yield (Scheme 8).

3.1.1.3. Synthesis of 3′,5′-Cyclic Carbonyloxymethyl Phosphates. Tsien et al. prepared acetyloxymethyl ester produgs of N6,O3′-dibutyl adenosine- and N6,O3′-dibutyl guanosine-3′,5′-cyclic monophosphate, with the intention of increasing intracellular delivery of second messengers cAMP and cGMP. The coupling of either diisopropylethylamine or silver salts of adenosine-3′,5′-cyclic monophosphate with acetyloxymethyl bromide afforded the acetyloxymethyl prodrug as a mixture of two diastereoisomers separated by silica gel chromatography. Interestingly, the diastereomeric ratio was found to be dramatically different depending on the method used as the first one afforded a 65:35 mixture in favor of the fast eluting isomer contrary to the 23:77 mixture obtained with the second method (Scheme 9). On the other hand, cGMP prodrug was prepared as a nonseparable mixture.

Scheme 4. Synthesis of 8-Bromo-2′-deoxyadenosine Bis(POM)-phosphate Prodrug

Scheme 5. Conversion of a Monophosphate into Its Corresponding Bis(POM)-monophosphate Nucleoside

Scheme 6. Preactivation of the Phosphate Moiety as a Tributylstannyl Salt

Scheme 7. Use of Bis(POM)-phosphorochloridate

Scheme 8. Synthesis of 5-FdU-POM-Phosphate Monoester

Scheme 9. Synthesis of an Acetyloxyethyl Phosphate Monoester
of two diastereoisomers (from derivative X = H) using the DIPEA method.

Scheme 9. Synthesis of N₂, O²⁺-Dibutyryl Adenosine-3',5'-cyclic Monophosphate

In 2007, Gunic et al. 27 reported the synthesis of base modified 2'-C-methyl ribonucleosides cyclic monophosphate prodrugs that exhibited potent anti-HCV activities. 5'-Phosphorylation of nucleosides 27 with POCl₃ and P(O)-(OMe)₃ and subsequent cyclization using DCC in pyridine afforded cyclic monophosphate nucleosides 29 in 30% yield (Scheme 10). Finally, coupling with either chloromethyl pivalate or chloromethyl pivalate afforded cyclic POM- and POC-prodrugs 30 in low to moderate yields (Scheme 10).

Scheme 10. Synthesis of 2'-C-Methyl Ribonucleosides Cyclic Monophosphates

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3.1.1.4. Carbonyloxymethyl Phosphonates. The first synthesis of bis(carbonyloxymethyl)-nucleoside phosphate prodrug was reported by Starrett et al. 13,28 who prepared the bis(POM)-, bis(isobutyryloxymethyl)-, and bis-(propionyloxymethyl)-prodrugs of adefovir (PMEA). At first, the coupling between chloromethyl pivalate or iodomethyl pivalate and various inorganic (Ag⁺, Li⁺, K⁺, Na⁺, Cs⁺) or organic salts (Et₂NH⁺, (i-Pr)₂N⁺EtH, n-Bu₂N⁺) of PMEA did not lead to the desired prodrug 31. Finally, bis(POM)-PMEA was obtained in 40% yield from PMEA by using N,N'-dicyclohexylmethane carboxamidine (DCMC) as the coupling agent and chloromethyl pivalate. However, the same procedure was not found suitable for 3-hydroxy-2-phosphonomethoxypropyl nucleosides such as HPMP-5-azaC, because the reaction lead to an inseparable mixture of bis(POM)-ester 32 and cyclic POM-monoester phosphonates 33 (Scheme 11). Optimization of the reaction conditions (using other salts in place of DCMC, temperature, and solvents) was not successful. 29

Scheme 11. Difference of Reactivity between PMEA versus HPMP-5-azaC Derivatives

A similar procedure was used by Choi et al. 15 for the synthesis of 9-[1-phosphonomethoxy cyclopropyl)methyl]-6-deoxyguanine dipivoxil LB80380. The nucleoside prodrug was obtained in two steps by hydrolysis of the diisopropyl phosphonate diester 34 with trimethylsilyl bromide and coupling of the resulting phosphonic acid 35 with POM-Cl in the presence of triethylamine and 1-methyl-2-pyrrolidinone (Scheme 12).

Scheme 12. Synthesis of LB80380

The same procedure was used by Tang et al. 30 to synthesize several PMEA and PMPA bis(alkoxyoxymethyl)-carbonate prodrugs. Chloromethyl carbonates 37 were prepared in 60–75% yield from methyl chloroformate, by chlorination with a large excess of sulfuryl chloride in the presence of catalytic AIBN, followed by addition of the corresponding alcohol in pyridine. The coupling of PMEA or PMPA 38 with 4.5 equiv of chloromethyl carbonates, 37, gave crude 39, which were converted into their more stable fumarate salts 40 in 50–70% yield (Scheme 13).
The same procedure was reported by Mackman et al.\textsuperscript{31} to prepare bis(POC)-5′-phosphonomethoxy prodrugs of potent nucleosides such as d4T, AZT, ddC, or ddT. Phosphonomethoxy-d4T and -ddC derivatives were synthesized by electrophilic addition of dimethyl hydroxymethyl phosphonate to furanoid glycal\textsuperscript{41,32,33}. After oxidative deselenylation, deprotection of the phosphonate moiety and hydrogenation of the double bond, the resulting phosphonic acid salt\textsuperscript{45} was converted to the bis(POC)-prodrug\textsuperscript{46} by coupling with chloromethylisopropyl carbonate in the presence of triethylamine (Scheme 14).

This method was later used for the synthesis of the bis(POC)-5′-phosphonomethoxy 2′-Fd4A prodrug (GS-9148) as shown in Scheme 15.\textsuperscript{34,35}

To increase the solubility of highly polar phosphonic acid nucleoside derivative during coupling reactions and also to reduce the formation of side-products, lipophilic protecting groups are often temporarily introduced. Thus, Benzaria et al.\textsuperscript{36} reported the synthesis of bis(POM)-PMEA\textsuperscript{51} by protection of PMEA derivative\textsuperscript{48} N\textsuperscript{6}-position with a MMTr-group prior to phosphate hydrolysis with TMSBr and subsequent treatment with triethylammonium bicarbonate (Scheme 16). Finally, the reaction of compound 50 with iodomethyl pivalate followed by MMTr-deprotection under acidic conditions allowed for the bis(POM)-PMEA\textsuperscript{51} formation in 18% yield over two steps. MMTr-protection was also employed by Chand and co-workers to protect both amine and hydroxyl groups during the synthesis of various C1′-substituted 9-[2-(phosphonomethoxy)ethyl]adenine\textsuperscript{37} and 9-[3-(phosphonomethoxy)propyl]adenine\textsuperscript{38–40} bis(POM)- and bis(POC)-prodrugs derivatives. MMTr-protection of adenosine intermediate 52 and subsequent selective removal of the pivaloyl group with NaOH in MeOH afforded compound 54. The phosphonate moiety was then introduced by coupling with tosylate 55 in the presence of sodium hydride. The protected dialkyl phosphonate 56 was then hydrolyzed with TMSI in the presence of triethylamine to avoid degradation of the MMTr-protecting groups. Finally, the alkylation of 57 with POM-Cl or POC-Cl proceeded efficiently and gave the bis(POM)- and bis(POC)-prodrugs 58 in 69–99% yields, respectively, after deprotection under mild acidic conditions (Scheme 17).

In 2011, Agrofoglio and co-workers\textsuperscript{41} reported the synthesis of 5-substituted uracil butenyl acyclic bis(POM)-phosphonate nucleoside\textsuperscript{62} by, first, cross-metathesis reaction between crotylated uracil\textsuperscript{60} and dimethyl allylphosphonate\textsuperscript{59}, followed by direct reaction with chloromethylpivalate and sodium iodide (Scheme 18). In parallel, the same team developed a more convergent method for the synthesis of 5-substituted uracil butenyl acyclic bis(POM)- and bis(POC)-phosphonates\textsuperscript{64 and 65} by using a bis(POM)- or bis(POC)-allylphosphonate as cross-metathesis partner.\textsuperscript{57} Bis(POM)- and bis(POC)-allylphosphonates were generated by reaction of dimethyl allylphosphonate with either POM-Cl and POC-Cl in the presence of sodium iodide (Scheme 19). Interestingly, the authors showed that very low conversion rates were observed when diethylallyl phosphonate was used instead of dimethyl allylphosphonate.
The bis(POM)-prodrugs were finally obtained after cross-metathesis with crotylated uracil using ruthenium catalyst A at 40 °C. The known instability of carbonates pushed Agrofoglio’s team to find milder reaction conditions; thus, the preparation of bis(POC)-prodrugs was achieved by using IPr indenylidene catalyst B at room temperature (Scheme 19). A similar procedure was used by Montagu et al. for the preparation of 5-substituted analogs.43

Because of the lack of reactivity of ruthenium catalysts in the presence of purines, an alternative strategy was envisaged for the synthesis of butenyl acyclic purine bis(POM)-phosphonate nucleoside 62.44 Cross-metathesis between (Z)-2-buten-1,4-diol and bis(POM)-allylphosphonate 63a afforded the desired (E)-bis(POM)-4-hydroxy-but-2-en-1-yl phosphonate reagent 66 in 74% yield (Scheme 20). Mitsunobu coupling between 66 and adenine, 6-chloropurine, or 2-amino-6-chloropurine led to the corresponding bis(POM)-phosphonate nucleosides 67–69. Further acidic hydrolysis with formic acid in water gave hypoxanthine 70 and guanine 71 derivatives in 86% and 85% yields, respectively, while treatment with cyclopropylamine gave 6-cyclopropylamino-72 and 2-amino-6-cyclopropylamino-73 derivatives in 82% and 77% yields, respectively.

More recently, 5′-methylene phosphonate furanonucleoside bis(POM)-prodrugs have been prepared through a Horner–Wadsworth–Emmons reaction between correctly protected 5′-ketal nucleoside intermediates and a tetra(POM)-bisphosphonate reagent.45 Uridine, N4(Boc)2-cytosine, N6(Boc)2-adenine, 2-N(Boc)2-6-benzyloxy-purine, and 2-N(Boc)2-6-azido-purine 2′-methyl-2′-F-nucleosides 74 underwent oxidation using IBX. Subsequent treatment with deprotonated tetra(POM)-bisphosphonate reagent 75 afforded vinyl phosphonate nucleosides 76. TBDMS deprotection with aqueous formic acid and hydrogenation over palladium hydroxide afforded the desired prodrugs 77 (Scheme 21).

3.1.1.5. Carbonyl Oxymethyl Phosphonate Monoester.

Starrett et al.13,28 reported the synthesis of PMEA POM-phosphonate monoester 80. Reaction of diphenyl PMEA 78 with sodium benzoate led to the unexpected formation of benzyl monoester PMEA after spontaneous degradation of the dibenzyl PMEA intermediate. The POM-prodrug 80 was then obtained by coupling the PMEA benzyl monoester 79 with...
chloromethyl pivalate in the presence of triethylamine, and subsequent hydrogenation of the benzyl group with palladium hydroxide on carbon (Scheme 22).

Tang et al.\textsuperscript{30} also reported the synthesis of PMEA-carbonyloxymethyl monoester\textsuperscript{82} by direct coupling of the phosphonic acid\textsuperscript{81} with 1.2 equiv of benzyl or allyl chloromethyl carbonate in the presence of triethylamine (Scheme 23).

A similar procedure was used by Krecmerova et al.\textsuperscript{46} for the synthesis of the 2,6-diaminopurine HPMPC (HPMPC-DAP) POM-monoester prodrug\textsuperscript{84} by reaction of 83 with POM-Cl in the presence of DCMC (Scheme 24).

Scheme 24. Synthesis of (HPMPC-DAP) POM-Monoester Prodrug

isolated pure on small scale by HPLC purification. The chair conformation was elucidated by inspection of spin–spin coupling constants from $^1$H NMR spectrum.\textsuperscript{29} In 2010, Krecmerova et al. took advantage of this later procedure to prepare the 2,6-diaminopurine cyclic POM-monoester prodrugs (cHPMP-DAP). The ratio was found to be 6:1 in favor of the less polar $trans$-isomer. As before, the diastereoisomers were distinguished by characteristic values of $^{31}P$ chemical shifts, as well as $H-H$, $H-P$, and $C-P$ coupling constants.

3.1.1.7. Mixed Prodrugs and Miscellaneous. In 1994, Starrett et al.\textsuperscript{13} reported the synthesis of PMEA mixed glyoxamide POM-diester as part of their pioneering work on PMEA prodrugs (Scheme 26). Activation of PMEA with thionyl chloride to form the bis(chloro)-intermediate\textsuperscript{88} and subsequent reaction with $N,N$-diethylacetamide generated bis(glyoxamide)-PMEA\textsuperscript{89}. Selective hydrolysis with sodium hydroxide followed by alkylation with chloromethyl pivalate in the presence of triethylamine gave the desired PMEA mixed glyoxamide POM-diester\textsuperscript{91}.

In 2007, Fu et al.\textsuperscript{48} reported the synthesis of adefovir bis(L-amino acid)-oxymethyl prodrugs. The desired adefovir prodrugs\textsuperscript{92} were obtained by coupling of $N$-Boc protected L-amino acid chloromethyl esters to PMEA in the presence of DCMC followed by deprotection under acidic conditions (Scheme 27). Interestingly, these compounds were found to be 2 times more potent against HBV and 10 times less toxic than adefovir dipivoxil.


3.1.2. S-Acyl-2-thioethyl (SATE) and S-[2-Hydroxyethyl)sulfydyl]-2-thioethyl (DTE). In the early 1990s, a French group first reported that mononucleoside phosphotriesters, incorporating a thioethyl chain where the thiol is masked as a thioester (SATE groups, Figure 9), were able to liberate the parent S'-nucleoside monophosphate inside the cell. It has been demonstrated that the decomposition of bis(SATE)-phosphotriester derivatives involves an esterase-dependent activation process leading to an unstable O-2-mercaptoethylphosphotriester. This intermediate decomposes spontaneously via intramolecular nucleophilic displacement into the corresponding phosphodiester with expulsion of ethylene sulfide (Figure 9). Removal of the remaining SATE group follows a similar mechanism giving the desired S'-nucleoside monophosphate. The same team also reported a related prodrug containing dithioethanol (DTE) masking groups whose activation to the same mercaptoethyl intermediate is achieved by a reductase (Figure 9). The assumed toxicity concern associated with the ethylene sulfide byproduct has largely limited the advancement of SATE/DTE prodrugs into development, but it is commonly used in the in vitro studies to deliver phosph(on)ates intracellularly.

Figure 9. Activation of (SATE)- or (DTE)-nucleoside prodrugs.

Nucleosides phosph(on)ate prodrug containing dithioethanol (DTE, Figure 10) can be prepared by (A) coupling of bis(DTE)-phosphate intermediate to the nucleoside, and (B) coupling of a nucleoside phosphate with 2-substituted (disulfanyl)ethanol derivatives. Bis(SATE)-phosphotriesters nucleosides can be prepared by (A) coupling of a H-phosphonate nucleoside with an hydroxythioester reagent, (B) coupling of a monophosphate nucleoside with an hydroxythioester derivative, and (C) coupling of a N,N-diisopropylphosphoramidite reagent to a nucleoside followed by oxidation of the phosphorus atom (Figure 11).

3.1.2.1. Bis(DTE)- and Bis(SATE)-Monophosphate. Historically, the interest for disulfide monophosphate prodrugs began in the early 1990s with the synthesis and the study of dithioethanol (DTE) phosphotriester of AZT23,49 and ddU.51 Dithioethanol is first monoprotected with a MMTr group, then phosphorylated with POCl₃ to give bis(MMTr-DTE) phosphate intermediate 93 in moderate yields. Condensation of either AZT or ddU with compound 93 led to the

Scheme 26. Synthesis of PMEA Mixed Glyoxamide POM-Diester Prodrug

Scheme 27. Synthesis of Adefovir Bis(1-amino acid) Oxyethyl Phosphonate Prodrugs

Figure 10. Access to bis(DTE)-phosphotriesters and bis(DTE)-phosphonodiesters.

Figure 11. Access to bis(SATE)-phosphotriesters and bis(SATE)-phosphonodiesters.
corresponding bis(DTE)-monophosphate prodrugs 94a and 94b (Scheme 28).

Scheme 28. Synthesis of Bis(DTE)-monophosphate Prodrugs

![Scheme 28](image)

Direct reaction of nucleosides with bis(SATE)-phosphoramidite is the most commonly used approach to prepare (SATE)-monophosphate prodrugs.

Lannuzel et al.\textsuperscript{52} described the synthesis of AZT (t-BuSATE)-pronucleotide 96 by first preparing AZT-MP 95. The phosphonate derivative was then activated by TPSCl and coupled with the S-pivaloyl-2-thioethanol to give the bis(t-BuSATE)-monophosphate prodrug 96 in good yields (Scheme 29).

Scheme 29. Synthesis of Bis(t-Bu-SATE)-Monophosphate Prodrug 96

![Scheme 29](image)

Perigaud et al.\textsuperscript{51} reported the synthesis of bis(SATE)-ddUMP 99 using H-phosphonate chemistry (yields not provided). Dideoxy uridine (ddU) was first converted to the corresponding 5'-hydrogen-phosphonate 97 by reaction with phosphoric acid in the presence of pivaloyl chloride and pyridine (Scheme 30). Compound 97 was then reacted with 2-acetylthioethanol 98 upon pivaloyl chloride activation, to give bis(SATE)-ddUMP 99.

Scheme 30. Synthesis of Bis(MeSATE)-ddUMP Using H-Phosphonate Chemistry

![Scheme 30](image)

The most common strategy to prepare (SATE)-phosphate prodrugs involves the coupling of a phosphoramidite intermediate 100 with a nucleoside in the presence of 1H-tetrazole followed by in situ oxidation with tert-butyl hydroperoxide or m-CPBA. This method has been successfully applied to the synthesis of various derivatives of AZT (101),\textsuperscript{23} adenallene (102),\textsuperscript{53} 9-(2'-β-C-methyl-β-D-ribofuranosyl) substituted purines (103, 104),\textsuperscript{54,55} pyrrolopyrimidine nucleoside (105),\textsuperscript{56} and IsoddA (106) (Scheme 31).\textsuperscript{57}

However, this method has several limitations related to the nature of the starting materials. For instance, the presence of exocyclic amines on the base can lead to competitive substitution and low solubility of the starting material in commonly used organic solvents.\textsuperscript{58} Therefore, bases like G or C have been temporarily protected with groups such as MMTr or DMT (Scheme 32).

The presence of a 3'-hydroxy group can also lead to the formation of undesired 3'- and 5',3'-phosphotriester derivatives. Separation of 3'- and 5'-isomers is not always straightforward and can require several steps of difficult chromatographic purification as reported for the synthesis of compound 116 (Scheme 33).\textsuperscript{59} In other examples, acid labile protective groups such as Boc and TBDMS have been used to circumvent the above-mentioned problem (not shown).\textsuperscript{60}

Ribо nucleosides have also been protected by formation of a 2',3'-isopropylidene group (Scheme 34).\textsuperscript{22} 3.1.2.2. Bis(SATE-) and Bis(DTE)-Phosphonate. 2'-C-Methyl adenosine phosphate prodrug was successfully synthesized by Koh et al.\textsuperscript{61} as potential anti-HCV inhibitors. The bis(SATE)-prodrug 127 was found to be slightly more potent than its phosphate parent 126b but also more toxic. Starting from 2'-C-methyl adenosine 123, compound 124 was obtained via a silylation, benzoylation, and dehydroxylation sequence. Oxidation of 124 produced the corresponding 5'-aldehyde, which was subsequently engaged in a Wittig reaction with diphenylphosphoranylidene methylene phosphonate to yield the corresponding 5',6'-vinyl phosphate (not shown). Catalytic hydrogenation of the double bond followed by transesterification gave the saturated phosphonate ester 125. The 3'-hydroxyl group was protected with a TBDMS group followed by removal of the benzoyl group with ammonia and hydrolysis of the benzyl ester to give 3'-protected phosphonate 126a. Finally, treatment of 126a with S-(2'-hydroxyethyl)-2,2-dimethylpropanethiolate followed by desilylation lead to desired bis(t-Bu-SATE)-phosphono nucleoside 127 in good yield (Scheme 35, eq 1). Interestingly, the authors had to go through this long sequence of selective protection/deprotection of the 3'-hydroxyl because direct reaction of S-(2'-hydroxyethyl)-2,2-dimethylpropanethiolate with phosphonate 126b in the presence of MSNT yielded a 3',5'-cyclic phosphodiester 128 instead of the desired bis(SATE)-derivative 127 (Scheme 35, eq 2).

Benzaria et al.\textsuperscript{36,49} also prepared and studied bis(SATE)- and bis(DTE)-prodrugs of the antiviral agent PMEA 131 (Scheme 36). Hydroxystioesters precursors were condensed with N-MMTr-protected PMEA derivative 129 in pyridine in the presence of 1-mesityl-2-sulfonfyl-3-nitro-1,2,4-triazole (MSNT) to afford the corresponding phosphonodiesters 130 with monooesters as byproducts. Finally, deprotection under acidic conditions provided the target PMEA prodrugs 131.

Li et al.\textsuperscript{62} prepared the 6'-fluoro-6'-methyl-5'-noradenosine phosphonic acid bis(SATE)-prodrug 133 by reaction of

\[ \text{ddU} \xrightarrow{\text{HPO}_3} \text{ddUMP} \]

![Reaction](image)
3.1.2.3. Cyclic Monophosphate Nucleoside Prodrug Bearing SATE Group. Several cyclic monophosphate (cMP) prodrugs of heterobase-modified 2′-C-methyl ribonucleoside were synthesized in 2007 by Gunic et al.63 Coupling of (29) (refer to Scheme 10 for the synthesis) with appropriate hydroxythioester in the presence of MSNT in pyridine gives the corresponding cMP prodrugs 104 and 134 (Scheme 38).

Interestingly, (SATE)-cMP prodrugs of nucleosides 29 displayed remarkable improvement in HCV replicon inhibition (7000−11 000-fold) without significant toxicity. Activities of these (SATE)-cMP prodrugs have been shown to be similar to regular 5′-bis(SATE)-MP prodrugs of nucleosides.55

In 2010, Liu et al.64 successfully prepared 3′,5′-cyclic (SATE)-phosphonodiester nucleoside 136 by reacting adenine phosphonic acid 135 with S-(2-hydroxyethyl)-2,2-dimethylpropanethioate in the presence of MSNT (Scheme 39).

3.1.2.4. Mixed SATE Approach. 3.1.2.4.1. Aryl(SATE)-phosphotriester. The main decomposition pathway of these aryl (SATE)-phosphotriesters involves loss of the SATE moiety by action of an esterase, followed by hydrolysis into the corresponding nucleoside monophosphate through phosphodiesterase enzymatic activity (Figure 12).

Scheme 31. Traditional (SATE)-Prodrugs Strategies

Scheme 32. Protection of Base Competitive Sites

Scheme 33. Mixtures with Sugar Competitive Sites

Scheme 34. 2′,3′-Isopropylidene Group To Mask Competitive Sites

phosphonic acid 132 with S-(2-hydroxyethyl)-2,2-dimethylpropanethioate in the presence of MSNT (Scheme 37).

Villard et al.65 along with Schlienger et al.66 prepared a series of AZT phenyl(SATE)-phosphotriesters derivatives (Scheme
Various thioesters 137 were reacted with phenyl-dichlorophosphate to give the corresponding SATE derivatives 138, which were directly coupled with AZT in the presence of NMI. Removal of the various protecting groups was carried out using either TFA or aqueous acetic acid to provide the desired prodrugs 139 in high yields.

Parigaud’s team reported the synthesis of (SATE)-phosphotriesters bearing modified L-tyrosinyl residues by phosphoramidite P(III) chemistry. Condensation of tyrosinyl precursors with (SATE)-phosphorobis(amidite) reagent 100
led to the corresponding tyrosinyl(SATE)-phosphoramidite intermediates 140. Reaction of (140) with AZT, followed by in situ oxidation with t-BuOOH and treatment of intermediates under acidic conditions (30% HCl in Et2O or 10% TFAA in DCM), afforded the desired prodrugs 141 in good overall yields (Scheme 41).

**Scheme 41. Synthesis of (SATE)-Phosphotriesters Bearing Modified l-Tyrosinyl Residues**

3.1.2.4.2. (SATE)-Phosphoramidate Diester. These (SATE)-phosphoramidate diesters containing either simple aliphatic and aromatic amines or amino acid esters have been shown to deliver 5′-nucleoside monophosphates after simple esterase activation followed by phosphoramidase-catalyzed cleavage of the amino portion (Figure 14).

Despite the fact that IDX184 development for HCV treatment was stopped in phase IIb in August 2012, the (SATE)-phosphoramidate diester prodrug of 2′-C-methylguanosine remains at present the only example of the successful application of this technology to reach human study. IDX184 was prepared using the H-phosphonate chemistry similar to that described above. Thus, the key H-phosphonate monoester precursor was prepared in a few steps from commercially available 2,2-dimethyl-3-hydroxypropanoic acid methyl ester, by protection of the alcohol followed by saponification, leading to compound 146 in 92% yield without purification. Installation of the side chain was performed by peptidic coupling between compound 146 and 2-mercaptopeto-thanol to generate alcohol 147. Finally, treatment of compound 147 with phosphorus acid and pivaloyl chloride, followed by quenching the reaction with triethylammonium bicarbonate (TEAB), generated H-phosphonate monoester precursor 148 in 90% over two steps. 2′-C-Methylguanosine was then reacted with 148 in the presence of pivaloyl chloride to furnish intermediate 149, which was further treated with benzylamine to generate Tr-protected phosphoramidate diester 150 in quantitative yield. Classical deprotection with trifluoroacetic acid led to the isolation of IDX184 in 39% yield (Scheme 43).

**Scheme 43. Synthesis of IDX184**

3.1.2.4.3. (SATE)-Glucosyl Phosphorothiolates. This type of mixed (SATE)-phosphodiesters is based on the combination of the iso(SATE)- and the bis(SGTE)-70 prodrugs, two structural modifications previously studied by Perigaud’s group.

The postulated unmasking pathway of the (SATE)-glucosyl phosphorothiolate derivatives may involve an esterase activation leading to the loss of the SATE group and formation of glucosyl phosphorothiolatediester (Figure 15).
intermediates should then undergo a glucosidase-mediated cleavage of the anomeric bond followed by a rearrangement process similar to the decomposition process proposed for bis(isoSATE)-pronucleotides.\textsuperscript{71}

The synthesis of such (SATE)-glucosyl phosphorothiolate derivatives involves both P(III) and P(V) intermediates and was developed using AZT as a model system. The (SATE)-H-phosphonate monoester precursor \textsuperscript{153} was obtained from \textit{S}-(2-hydroxyethyl)-2,2-dimethylpropanethioate using salicyl chlorophosphite. Condensation of intermediate \textsuperscript{153} with AZT, in the presence of pivaloyl chloride, led to the corresponding H-phosphonate diester, which was in situ oxidized into phosphorothioate \textsuperscript{154} using elemental sulfur (diastereomeric mixture 1:1). For the glucosyl phosphorothiolate portion, \textit{ab o r o n t r i} fluoride etherate-induced glycosylation of a pentaacetyl glucopyranose derivative with 2-bromoethanol gave the corresponding \(\alpha\)-glucopyranoside \textsuperscript{151}. A Finkelstein halogen-exchange reaction with sodium iodide was followed by coupling of the resulting 2-iodoethyl-\(\alpha\)-D-glucosides \textsuperscript{152} with phosphorothioate diester \textsuperscript{154}. Phosphorothiolate derivatives \textsuperscript{155} were obtained, as a 1:1 diastereomeric mixture (Scheme 44).\textsuperscript{71}

### 3.1.2.4.4. (SATE)-Halogeno Phosphodiesters

Egron et al.\textsuperscript{72} tried to improve the anti HIV activity of AZT 5'-fluorophosphate by preparing (t-Bu-SATE)-prodrug \textsuperscript{156}. Starting from H-phosphonate diester \textsuperscript{143} (refer to Scheme 44 for preparation), fluorination was achieved using iodine and triethylamine trishydrofluoride. Pure phosphoro-fluoridate \textsuperscript{156} can be obtained as a 1:1 mixture of diastereoisomers using reverse phase column chromatography purification with an isocratic mixture of acetonitrile in water. It is noteworthy that purification of compounds \textsuperscript{156} on silica gel column chromatography using MeOH as eluent led to the formation of methylphosphate byproduct \textsuperscript{157}. However, this approach was not pursued due to the limited chemical stability of the (SATE)-phosphorofluoridate diester, which also provide \textsuperscript{158} in buffer media as a side product (Scheme 45).

### 3.1.2.4.5. \(\text{S,S}'\)-Bis(\(\text{O-acyl-2-oxyethyl}\)) Phosphorodithiolates: Iso(SATE)-pronucleotides

Schlienger et al.\textsuperscript{70a} studied an isomeric form of (SATE)-pronucleotides, mononucleoside \(\text{S,S}'\)-bis(\(\text{O-acyl-2-oxyethyl}\)) phosphorodithiolates (iso(SATE)-pronucleotides). The proposed decomposition pathway of the iso(SATE)-pronucleotides involves: (1) an esterase activation leading to intermediate A; (2) nucloephilic attack of the resulting free alcohol on the phosphorus atom, giving rise to five-membered ring intermediate B; and (3) conversion of B into 2-mercaptoethylphosphotriester C followed by spontaneous elimination of episulphide. Removal of the second iso(SATE) functional group is achieved by a similar mechanism or by action of phosphodiesterases, allowing the intracellular delivery of the corresponding nucleoside \(\text{S}'\)-monophosphate (Figure 16).

Mononucleoside phosphorodithiolates \textsuperscript{161} were obtained in a one-pot procedure involving (pyrrolidino)phosphoramidites Figure 15. Activation of (SATE)-glucosyl phosphorothiolate prodrugs.

Scheme 44. Synthesis of (SATE)-Glucosyl Phosphorothiolate Derivatives

Scheme 45. Preparation of (t-Bu)SATE Prodrug 156

Figure 15. Activation of (SATE)-glucosyl phosphorothiolate prodrugs.

Figure 16. Decomposition pathway of iso(SATE)-nucleoside prodrugs.
159 and 1H-tetrazole activation, followed by oxidation of 160 with t-BuOOH (Scheme 46).

**Scheme 46. One-Pot Procedure Involving (Pyrrolidino)phosphoramidites**

\[
\text{NuOH} \quad \text{Nu} = \text{ddA, d4T} \rightarrow 1\text{H-tetrazole} \quad \text{MeCN, CH}_2\text{Cl}_2 \rightarrow 159 \rightarrow 160 \rightarrow 161
\]

**3.1.3. Cyclosaligenyl (cycloSal) Phosphate and Phosphonate Prodrug Approach.** cycloSal phosphate and phosphonate prodrugs, originally introduced by Chris Meier and co-workers, are one of the most extensively explored types of masked nucleotides.73 This concept is based on the use of salicylic alcohols to mask the phosphate functional group of a nucleoside monophosphate (Nu-MP) and has been successfully applied to the intracellular delivery of a number of antiviral nucleotides (e.g., AZT, d4T, and acyclovir74). Meier’s research group extensively studied this prodrug and demonstrated that the intracellular cleavage of cycloSal pronucleotides is based on an entirely pH-driven chemical hydrolysis mechanism with no enzymatic activation required. Under basic conditions, the aryl ester P−O bond is cleaved first, followed by spontaneous cleavage of the P−O benzyl ester bond (Scheme 47).

**Scheme 47. Hydrolysis Pathways of the CycloSal-d4TMP Triesters**

As the cycloSal pronucleotides were designed to release the active drug via a chemical cascade mechanism, the stability and hydrolysis pathways of these pronucleotides have been finely tuned by varying the nature of substituent in the boxed structure (Figure 17). Various diols were obtained by reduction of commercially available or prepared salicylic aldehydes, acids, or esters with NaBH₄ or LiAlH₄ (Path A). Other variations were achieved using ortho-formylation of substituted phenols followed by reduction (Path B) or mild basic formylation direct hydroxymethylation reactions (Path C). On the other hand, 7-methylated salicylic alcohols were prepared by alkylation of their corresponding aldehydes with methylolithium (Path D).

The coupling of the cycloSal phosphate moiety to the S'-hydroxyl group of a nucleoside is achieved using either P(III) or P(V) chemistry (Figure 18). However, the strategy using P(III) remains the most common one, due to the usual lack of reactivity of P(V) species. However, the synthesis of cycloSal phosphonates is done exclusively through P(V) chemistry starting directly from nucleoside phosphonates. It is noteworthy that all of these approaches give no diastereoselectivity with respect to the configuration at the phosphorus center. Thus, all compounds are obtained as diastereomERIC mixtures. Nevertheless, Meier and co-workers filed a patent75 reporting the use of chiral auxiliaries for the synthesis of cycloSal phosphate moieties. Those species can be separated before coupling with the nucleoside, leading to diastereomerically pure cycloSal nucleotide prodrugs.

**3.1.3.1. First Generation. 3.1.3.1.1. CycloSal Phosphate.** In a general manner, cycloSal derivative of nucleosides bearing a pyrimidine base such as AZT77 or d4T78 can be easily obtained using two different methods. In the first approach (Scheme 48), diols 162 are reacted with PCl₅, to yield the cyclic intermediate saligenylchlorophosphane 163. Target molecules 164 are then obtained in a “one-pot” procedure by coupling nucleosides analogs with (163), followed by in situ oxidation with t-BuOOH.

An alternative synthetic approach toward such compounds involves the less reactive P(V) chemistry (Scheme 49). The reaction of d4T with phosphorus oxychloride yields phosphodi-
chloridate 165, which is further reacted with salicyl alcohol to give the desired triester 166. However, this last approach leads to yields remarkably lower (37%) than the one obtained with the above P(III) approach.

Finally, a third approach\(^7\) has been used to prepare cycloSal pronucleotides of carbocyclic nucleoside, the phosphorochloridate chemistry (Scheme 50). Alcohol 167 is first reacted with phosphorus oxychloride to give 3-methyl- cyclosaligenylphosphorochloridate 168. Next, reaction of chlorinated intermediate 168 with nucleoside 169 in pyridine gave the phosphate triester 170 in 60% yield. However, this method failed to produce the cycloSal phosphate triester in the case of the 3'-epi isomer of 169, most likely due to steric hindrance or intramolecular cyclization.

In contrast to thymidine nucleosides, cytosine derivatives cause considerable obstacles when reacted with chlorophos-
Spáčílova et al. described the synthesis of 6-heteroaryl-7-deazapurine ribonucleosides cycloSal-phosphate pronucleotides 179 as potential adenosine kinase inhibitors. Interestingly, they demonstrated the relative stability of the cycloSal prodrug 178 toward Pd-catalyzed transformations: despite the partial decomposition of the cycloSal phosphate under basic conditions, Stille and Suzuki cross-coupling reactions can be performed (Scheme 53). Moreover, like for the protection of exocyclic amines, protection of the sugar moiety was achieved by choosing an acid labile group such as an isopropylidene group that can be easily removed using 90% aqueous trifluoroacetic acid.

Meier et al.83 also described the synthesis of cycloSal-BVdUMP triesters 183 from either BVDU 180 or 3'-O-levulinylated BVdU 181 (Scheme 54), using the phosphoramidite/oxidation method previously shown in Scheme 51. Interestingly, after removal of the levulinyl protection under mild condition by treatment with hydrazine hydrate, both methods gave similar overall yields (31−50%).

As mentioned earlier, the chirality of the phosphorus atom leads to the formation of nucleotide prodrugs as mixtures of two diastereoisomers (Rp and Sp) in an almost 1:1 ratio. Moreover, the chromatographic separation of these diastereoisomers, when possible, is often a very difficult task to achieve.

In 2011, Meier and co-workers85−87 reported the first synthetic route to prepare isomerically pure cycloSal-phosphotriesters. Their strategies revolved around the use of chiral auxiliaries that were introduced by reaction with phosphorus oxychloride followed by esterification of the resulting dichlorophosphoramidate with salicylic alcohol. At this stage, their strategy required the facile separation of the diastereoisomers by chromatography or recrystallization. Final nucleophilic displacement of the chiral auxiliary by the protected nucleoside generated diastereomerically pure cycloSal-phosphotriesters, provided that this reaction took place with clean inversion of configuration at the phosphorus atom (SNP reaction). Thus, reaction of (S)-4-isopropylthiazolidine-2-thione with POCl₃ leads to the formation of intermediate 188 that can be further reacted with 2-(hydroxymethyl)phenol in the presence of DBU to afford a mixture of two diastereoisomers 189a and 189b. At this stage, the two compounds can be separated, and the Sp-configuration of (189b) was confirmed by X-ray crystallography. The desired isomer Rp-189a is coupled with nucleoside 3'-OAc-dT using t-BuMgCl to give access to the diastereomerically pure monophosphate prodrug 190 (Scheme 56). The authors

Scheme 53. Pd-Catalyzed Reactions with cycloSal Prodrugs

Scheme 54. Deprotection of a Levulinylate Group on cycloSal-BVdUMP Triesters

Scheme 55. Preparation of Several 5-[125I]Iodo-uridine cycloSal MP Prodrugs
assigned the stereochemistry of the final products, by assuming that the mechanism of this reaction proceeds with inversion of configuration at the phosphorus atom.

Although the reaction conditions worked well for unsubstituted salicylic alcohol, the same sequence was surprisingly not applicable to the synthesis of 3- and 5-substituted cycloSal derivatives because the diastereoisomers were the only ones that could be separated at the phosphoramidate level. Compounds 191a and 191e were coupled with cycloSal-phosphochloridates generating intermediates 193 as a 1:1 mixture of diastereoisomers.

Diastereoisomers $R_p$-193a and $S_p$-193b were separated by chromatography, and the stereochemistry of $S_p$-193b was confirmed by X-ray crystallography. $R_p$-193a and $S_p$-193b, which were more stable than their $S_p$- and $R_p$-counterparts, were coupled with AZT or d4T to form the expected phosphotriesters 194. After nucleophilic displacement by the nucleoside, both enantiomerically pure ($S_p$-) and ($R_p$-) phosphotriesters 194 could be isolated. Optimization of the reaction conditions for the third step was also investigated. Racemization at the phosphorus atom was suppressed when using $[\text{Cu}(\text{BEN})_\text{2}]$-$(\text{OTf})_3$ complex in dichloromethane.

It is noteworthy that the authors considered also using chiral thiophosphoramidates, but this strategy failed in the last step as the $P=S$ was not electrophilic enough to allow for nucleophilic displacement of the chiral auxiliary by the nucleoside (not shown).

Expanding their nucleoside prodrug research program, Meier and co-workers reported the development of bis(cycloSal)-pronucleotides (Figure 19) designed to deliver two molecules of active drug for each biomolecule administrated.

Conversion of tetrols 197 into corresponding phosphitylating agents 200 was realized by treatment with $\text{PCl}_3$ under basic condition. Careful control of the temperature conditions appeared to be critical to selectively obtain compound 199. Thus, reduction of the reaction temperature from $-20$ to $-40$ °C helped decrease the quantity of byproducts resulting from the formation of seven-membered ring 201 (Scheme 58).

The first attempt of coupling between two molecules of d4T and crude chlorophosphite 200 led to the targeted pronucleotides 203 after tedious chromatography and in poor yield (8%, Scheme 59). The synthesis of these bis(cycloSal) compounds via the phosphoramidite chemistry was also investigated but did not lead to any improvement in yields. As presented before,
cycloSal-pronucleotides were always obtained as a mixture of two diastereoisomers \((R_P/S_P\) configuration). In the case of bis(cycloSal)-d4TMP, two stereogenic centers are formed in the course of their preparation. Hence, they should be obtained as a mixture of three isomers \((R_P/R_P, R_P/S_P, \text{and } S_P/S_P\) configuration) in a ratio approaching 1:2:1 depending on the influence of the nucleoside chirality. In the case of compounds 203, all three diastereoisomers were isolated close to the expected 1:2:1 ratio. However, according to \(^1\)H and \(^3\)P NMR spectroscopy, compound 203 was obtained as a mixture of three isomers in a ratio of 1:2:2. According to the authors, that stereodifferentiation may be due to steric interactions between the two cycloSal-d4TMP units in 3,3'-bis(cycloSal)-d4TMP 203.

Another type of bis(cycloSal)-pronucleotides was also developed by Ahmadibeni et al. 89 Thus, 3'-fluoro-3'-deoxythymidine (FLT) and 3'-azido-3'-deoxythymidine (AZT) bis(cycloSal) prodrugs 207 were prepared from tetrol 204 by first formation of bis(chlorophosphite) 205 using PCl\(_3\) and then coupling with either AZT or FLT at low temperature. The subsequent oxidation using \(t\)-butyl hydroperoxide (TBHP) afforded the desired AZT and FLT bis(cycloSal) derivative as inseparable mixtures of diastereoisomers (Scheme 60).

3.1.3.1.2. cycloSal Phosphonates. The cycloSal prodrug approach has also been applied to the synthesis of phosphonate nucleosides such as PMEA 48 using P(V) chemistry. First attempts to prepare cycloSal-PMEAs 209, directly from the diethyl ester of PMEA 48, without protection of the exocyclic amino group, led to a complex mixture of reaction products.90 To overcome this problem, the diethyl ester exocyclic amino group was blocked by a monomethoxytrityl protective group (Scheme 61). Treatment of the intermediate with trimethylsilyl bromide then gave the bis(trimethylsilyl) ester that was immediately converted into the corresponding dichloride 208 using PCl\(_3\). The dichloro intermediate 208 was then reacted with different salicylic alcohols to give the protected cycloSal-PMEA diesters in low to moderate yields. Finally, the MMTr group was cleaved by treatment with TFA, which led to the target cycloSal-PMEAs 209 in S3–82% yield.

Unexpectedly, cycloSal-PMEA derivatives 209 appeared to be unstable especially in acidic conditions (pH = 2) and led to the design of possibly more stable cycloaminobenzyl-PMEA (i.e., cycloAmb-PMEA) phosphoramidates 211.90 In these molecules, the cycloSal phenolic oxygen atom is replaced by a nitrogen hypothesizing that the less electronegative nitrogen would reduce the electrophilicity of the phosphorus atom and consequently increase the stability of the prodrug. The first attempt to prepare the cycloAmb-PMEAs, using the reaction sequence shown in Scheme 61, led to the isolation of the targeted compounds 211 (Scheme 62) in very poor yield (3–7%). Another approach was then envisaged were PMEA was converted into its corresponding dichloride derivative 210 by treatment with oxalyl chloride. Addition of DMF led to the in situ protection of the nucleobase with a formamidine group. 2-Aminobenzyl alcohols were then condensed to intermediate 210 to provide corresponding cycloAmb-PMEA derivatives 211 in 25–42% yield. Interestingly these cycloAmb-PMEA derivatives 211 appeared dramatically more stable than their cycloSal-PMEA counterparts 209 while still displaying anti-HIV activity.
3.1.3.2. “Lock-In” cycloSal-Triesters. Because of the lipophilic character of cycloSal phosphate triesters and their chemically triggered delivery mechanism, a drug concentration equilibrium is generated across the cell membrane. To trap cycloSal triester inside the cells and avoid the formation of this equilibrium, so-called “lock-in” cycloSal pronucleotides were developed.91 These triesters are designed to be enzymatically converted inside the cell into a more polar compound (Figure 20).

Elaborated acyloxy systems, such as the acetoxyethyl (AM), isopropylcarboxyloxyethyl (POC), pivaloyloxymethyl (POM),92 and amino acid,93 were used to release the corresponding carboxylates. Starting from compound 212, obtained using the standard chlorophosphite procedure, deprotection with TFA led to cycloSal-d4TMP acid 213. POC and POM groups can be introduced by reaction of (213) with the corresponding chloromethyl alkyl reagent to give compounds 214. On the other hand, a peptidic coupling between (213) and various amino acids leads to the corresponding amide-containing cycloSal derivatives 215 (Scheme 63).

Meier et al. developed another type of “lock-in” cycloSal-pronucleotide that bears a (carboxy)esterase-cleavable geminal dicarboxylate91,94 or an acetoxyvinyl95 group attached to the aromatic ring of the saligenyl unit. Those new “lock-in” cycloSal-pronucleotides are enzymatically transformed into a more polar aldehyde or ketone inside cells (Figure 21).

The synthesis of these compounds starts with the conversion of 4-formylsalicylic alcohols 218 into cycloSal triesters 220 using a standard P(III)-chemistry route. Next, triesters 220 are reacted with acetic anhydride and zirconium(IV) chloride to give the corresponding final prodrugs 221 in 23–45% yield. Interestingly, for some compounds, a separation of the two diastereoisomers (Rp or Sp) was achieved. The Sp form of the cycloSal triesters demonstrated improved antiviral activity as compared to the Rp form (Scheme 64).

3.1.4. Cyclic 1-Aryl-1,3-propanyl Ester HepDirect. HepDirect prodrugs are aryl substituted cyclic 1,3-propanyl esters developed in the early 2000s by Metabasis Therapeutics, Inc. as a liver-directed prodrug combining high plasma and tissue stabilities. So far, three drugs including MB0781196 and two nucleosides pradefovir2 and MB0713397 have been advanced to human clinical trials (Figure 22). Pradefovir is a 3-chlorophenyl HepDirect prodrug of Adefovir in development.
for hepatitis B infection therapy, while MB07133, a 4-pyridyl HepDirect prodrug of cytarabine, has been developed for hepatocellular carcinoma treatment. MB07811 was considered as a candidate for the treatment of hyperlipidemia.

These cyclic 1,3-propanyl esters were designed to undergo oxidative cleavage catalyzed by the cytochrome P450 (CYP) enzyme 3A, expressed predominantly in the liver. The hemiketal intermediate can undergo ring opening to form a negatively charged phosph(on)ate, which subsequently delivers the free phosph(on)ate nucleoside after spontaneous β-elimination. The aryl vinyl ketone released during the process is then rapidly detoxified by glutathione S-transferase, an enzyme present in high concentration in liver cells.

Interestingly, it was shown that the cleavage of the prodrug portion depends on the stereochemistry at the benzylic position. Indeed, only the phosphates with a cis-relationship between the aryl group and the nucleoside portion (and not the trans) were found to be activated by CYP3A. In addition, modifications at the phenyl moiety revealed the importance of an electron-drawing group for sufficient chemical stability (Figure 23).

HepDirect phosphate prodrugs can be prepared by coupling a nucleoside with a phosphorylating agent derived from a 1-arylpropane-1,3-diol using either P(III) (diisopropylphosphoramidite reagent) or P(V) (nitrophenylphosphate) chemistry. On the other hand, synthesis of phosphonates is achieved by direct coupling of 1-arylpropane-1,3-diol with a phosphonate nucleoside (Figure 24).

Because HepDirect prodrugs have two chiral centers (the benzylic position and the phosphorus atom), nonelective HepDirect prodrug formation results in the formation of four diastereoisomers. However, starting from an enantiomerically pure diol results in the formation of only two diastereoisomers identified as cis and trans that differ only in the configuration of the newly formed phosphorus chiral center (Figure 25).

3.1.4.1. Synthesis of Aryl-Substituted Cyclic 1,3-Propanyl Esters. Enantiomerically pure (R)- and (S)-1-aryl-propane-1,3-diols 223 were obtained through chromatographic separation of diastereomeric (−)-menthone ketals (Scheme 65). Alternatively, they can be synthesized by asymmetric reduction of the aryl ketoacid 224 with (−)- or (+)-B-chlorodiisopinocam-
pheylborane (DIP-Cl) followed by reduction of the resulting β-hydroxy acid with LiAlH₄ or BH₃·Et₂O with ee’s > 96%.²d,98

In certain cases such as 4-pyridyl derivatives, the separation of diastereoisomers as menthone ketals is difficult, and thus other chiral moieties have been employed. Esterification of racemic β-hydroxy ester 225 with N,N-dimethyl phenylalanine led to an easy separation of both diastereoisomers 226 in high optical purities and gave the desired diol S-226b after removal of the phenylalanate portion (Scheme 66).⁹⁷

Scheme 66. Preparation of Enantiomerically Pure (R)- and (S)-1-Aryl-propane-1,3-diols Using N,N-Dimethyl-phenylalanine

Enantiomerically enriched (S)-1-(4-pyridyl)-propane-1,3-diol was also obtained by lipase-mediated resolution in the presence of porcine pancreatic lipase (PPL) and vinyl acetate in 35–40% conversion and >95% ee. Final hydrolysis of the acetate groups led to compound S-226b.⁹⁹

3.1.4.2. HepDirect Phosphate Prodrugs. The first method developed by Erion et al.⁹⁸ used P(III) chemistry and the reaction of a phosphoramidite and a free nucleoside followed by the oxidation of the phosphate intermediate.

Phosphoramidite 228 is synthesized by reaction of diol S-223 and commercially available 1,1-dichloro-N,N-diisopropylphosphinamine 227 (Scheme 67). Compound 228 was stable and was purified by column chromatography on silica gel. The desired HepDirect prodrug of Lamivudine 229 was obtained as a mixture of cis- and trans-phosphate cyclic diesters after coupling of phosphoramidite 228 with 3TC followed by oxidation with t-BuOOH. Reddy et al. used the same phosphite approach to prepare 4-pyridyl HepDirect prodrug of ara-A.⁹⁹ The phosphorylation step was found to be almost instantaneous at 0 °C, giving a mixture of cis and trans isomers after oxidation. However, it was found that the thermal epimerization of the cis–trans mixture (60 °C, 3 h) enables the selective formation of the thermodynamically more stable trans-phosphoramidite. Finally, the stereospecific oxidation of P(III) phosphate 231 into P(V) phosphate derivative resulted in the exclusive formation of trans-HepDirect phosphate prodrug 232 (Scheme 68).

Scheme 67. Synthesis of the HepDirect Prodrug of Lamivudine

Scheme 68. Formation of trans-HepDirect Phosphate Prodrug 232

The stereochemistry of the trans isomer 232 was established using NOE studies, ³¹P NMR, and comparison with similar prodrugs previously reported in the literature.

This coupling reaction was also studied to develop a high throughput synthesis of HepDirect prodrugs.¹⁰⁰ DMSO can be also used as a cosolvent when nucleosides are not totally soluble in DMF (i.e., G nucleosides). The reaction failed to proceed in low polarity solvents because of the poor solubility of unprotected nucleosides. Optimization of the stoichiometry of phosphoramidite relative to coupling agent shows that the best yield (31% ± 14%) can be obtained when 6 equiv of both reagents were used. These conditions were applied to 148 different nucleosides and show an excessive production of doubly phosphorylated products. Moreover, the desired monophosphorylated derivative was only obtained for 52% of the substrates. The use of 2 equiv generally resulted in a decreased yield (11% ± 9%), but led to a better rate of success with 80% of cases giving the desired phosphorylated products. The stoichiometry 1:2:2 (nucleoside:phosphoramidate:coupling agent) is the one generally used for creating nucleoside libraries. For purification, the most efficient method was determined to be preparative reverse-phase HPLC with mass-based fraction collection after filtration of the crude reaction.
mixture. The process was chosen for its automation capabilities and ease of HepDirect prodrug preparation. Normal-phase silica gel cartridge-based purification can also be used but was less efficient because several sample preparation steps were needed prior to chromatography. The HPLC purity of these compounds (obtained with the stoichiometry 1:2:2) was acceptable (90% ± 7%), and the cis–trans ratio was slightly in favor of the cis-compound.

To obtain the desired cis-isomer prodrugs in a completely selective manner, Erion et al.98 developed a chiral p-nitrophenylphosphate reagent that can react through a SN2-type reaction with a nucleoside.

The p-nitrophenylphosphate trans-isomer can be prepared by reaction of p-nitrophenyl phosphorodichloridate and 1,3-propanediols to give 234 as a 40:60 cis:trans mixture of diastereoisomers (Scheme 69). Interestingly, stirring the reaction mixture overnight in the presence of an excess of nitrophenylphosphate reagent that can react through a SN2-type reaction with a nucleoside.

The reaction mixture overnight in the presence of an excess of p-nitrophenol in Et$_3$N shifts the equilibrium toward the more thermodynamically favorable trans-isomer with less than 3% of cis compound remaining. In the same manner, the reaction of disopropylphosphoramidites 233 with p-nitrophenol can be driven to the exclusive formation of the single trans-isomer 235 when stirred at room temperature for 8 h. Subsequent oxidation with t-BuOOH gives access to the desired p-nitrophenylphosphate trans-isomer 236b. The stereochemistry of the final compounds was determined by NOE experiments and comparison of benzylic methane proton chemical shift with literature examples.

Unexpectedly, the coupling of p-nitrophenylphosphate reagents 236a,b with a free nucleoside in the presence of bases such as LiH, KOt-Bu, or KNH$_2$ led to the formation of a cis–trans mixture and/or extensive hydrolysis of the phosphate ester. Surprisingly, the use of sodium hydride selectively generated the trans-isomer nucleoside prodrug (in low yield) through an unknown reaction mechanism. Finally, it was found that the use of t-BuMgCl on 2′,3′-protected nucleosides resulted in the exclusive formation of cis-isomers as illustrated in Scheme 70 with cytarabine (52%)99 and 2′-Me-A (35%).101

Scheme 70. Formation of cis-Isomers

Determination of the stereochemistry of the final product was established by comparison of NMR data with literature examples. Isopropylidine and TBS protective groups were finally removed after phosphorylation under acidic condition or by treatment with a source of fluoride (TEAF, TBAF). It is noteworthy that substrates, bearing leaving groups such as chloro, 4-chlorophenoxy, and 2,4-dichlorophenoxy groups in place of the nitrophenoxy group, were also tested, but were found to epimerize during coupling with the nucleoside.

Bookser et al.102 prepared 3′-amino-3′-deoxyguanosine monophosphate HepDirect prodrug 242 using temporary protection of pyrimidine 2-NH$_2$ and sugar 3′-hydroxyl with N,N-dibenzyloxymethyl and TBS groups, respectively. These protections served two purposes: first, they render the extremely polar guanosine more manageable in terms of solubility and purification, and they also prevent side reactions. Thus, protected compound 240 was reacted with p-nitrophenylphosphate reagent 236a in the presence of t-BuMgCl to generate corresponding HepDirect intermediate 241 in 93% yield. Finally, treatment with triethylammonium formate (TEAF) then TFA allowed for the removal of both the formamidine and the TBS groups.

Boy er et al.97 also showed that in the case of cytosine nucleosides, such as ara-C, N$_9$-protection was necessary to avoid N$_9$-phosphorylation. Starting from dimethylformamidine derivative 243, coupling with p-nitrophenylphosphate reagent 236b in the presence of t-BuMgCl followed by deprotection under acidic conditions allowed for the preparation of 4-pyridyl ara-C HepDirect prodrug 244 in gram quantities (Scheme 72).

Scheme 69. Preparation of Enantiomerically Pure trans p-Nitrophenylphosphates

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Compound</th>
<th>Conditions</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Et$_3$N</td>
<td>234</td>
<td>THF, 78°C to 25°C</td>
<td>48%</td>
</tr>
<tr>
<td>2. t-BuOOH</td>
<td>235</td>
<td>-40°C to rt</td>
<td>78%</td>
</tr>
</tbody>
</table>

The 3′-trans product can be prepared by reaction of cis-trans mixture with 4-nitrophenol and 5-(methythio)-1H-tetrazole to give 236a, which is subsequently reduced under classical Staudinger reaction conditions to give desired 3′-amino-3′-deoxyguanosine monophosphate HepDirect prodrug 242 in quantitative yield (Scheme 71). Determination of the stereochemistry of the final product was established by comparison of NMR data with literature examples.

Bookser et al.102 prepared 3′-amino-3′-deoxyguanosine monophosphate HepDirect prodrug 242 using temporary protection of pyrimidine 2-NH$_2$ and sugar 3′-hydroxyl with N,N-dibenzyloxymethyl and TBS groups, respectively. These protections served two purposes: first, they render the extremely polar guanosine more manageable in terms of solubility and purification, and they also prevent side reactions. Thus, protected compound 240 was reacted with p-nitrophenylphosphate reagent 236a in the presence of t-BuMgCl to generate corresponding HepDirect intermediate 241 in 93% yield. Finally, treatment with triethylammonium formate (TEAF) then TFA allowed for the removal of both the formamidine and the TBS groups. The 2′-N$_9$ group was subsequently reduced under classical Staudinger reaction conditions to give desired 3′-amino-3′-deoxyguanosine monophosphate HepDirect prodrug 242 in quantitative yield (Scheme 71). Determination of the stereochemistry of the final product was established by comparison of NMR data with literature examples.

Bookser et al.102 prepared 3′-amino-3′-deoxyguanosine monophosphate HepDirect prodrug 242 using temporary protection of pyrimidine 2-NH$_2$ and sugar 3′-hydroxyl with N,N-dibenzyloxymethyl and TBS groups, respectively. These protections served two purposes: first, they render the extremely polar guanosine more manageable in terms of solubility and purification, and they also prevent side reactions. Thus, protected compound 240 was reacted with p-nitrophenylphosphate reagent 236a in the presence of t-BuMgCl to generate corresponding HepDirect intermediate 241 in 93% yield. Finally, treatment with triethylammonium formate (TEAF) then TFA allowed for the removal of both the formamidine and the TBS groups. The 2′-N$_9$ group was subsequently reduced under classical Staudinger reaction conditions to give desired 3′-amino-3′-deoxyguanosine monophosphate HepDirect prodrug 242 in quantitative yield (Scheme 71). Determination of the stereochemistry of the final product was established by comparison of NMR data with literature examples.

Boy er et al.97 also showed that in the case of cytosine nucleosides, such as ara-C, N$_9$-protection was necessary to avoid N$_9$-phosphorylation. Starting from dimethylformamidine derivative 243, coupling with p-nitrophenylphosphate reagent 236b in the presence of t-BuMgCl followed by deprotection under acidic conditions allowed for the preparation of 4-pyridyl ara-C HepDirect prodrug 244 in gram quantities (Scheme 72). Unambiguous structural assignment was made by single-crystal X-ray structure determination of the final product and confirmed the relative stereochemistry between the aryl ring and the nucleoside as cis.

3.1.4.3. HepDirect Phosphonate Prodrugs. HepDirect phosphonate prodrugs can be readily prepared from a phosphonic acid nucleotide. In fact, adefovir HepDirect

Scheme 71. Formation of cis-Isomers

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Compound</th>
<th>Conditions</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. t-BuMgCl</td>
<td>236a</td>
<td>DMF, 0°C to rt</td>
<td>92%</td>
</tr>
<tr>
<td>2. TEAF, THF</td>
<td>236b</td>
<td>65% over two steps</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Compound</th>
<th>Conditions</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
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<td>1. t-BuMgCl</td>
<td>238</td>
<td>DMF, -10°C to rt</td>
<td>62%</td>
</tr>
<tr>
<td>2. TEAF, THF</td>
<td>239</td>
<td>65% over two steps</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Compound</th>
<th>Conditions</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. t-BuMgCl</td>
<td>236a</td>
<td>DMF, 0°C to rt</td>
<td>92%</td>
</tr>
<tr>
<td>2. TEAF, THF</td>
<td>239</td>
<td>65% over two steps</td>
<td></td>
</tr>
</tbody>
</table>

9177
prodrug was initially prepared by peptidic coupling conditions of (S)-1-(3-chlorophenyl)-propane-1,3-diol as a mixture of racemic cis- and trans-isomers (ratio from 55:45 to 60:40, favoring the cis isomer) separable by chromatography and fractional crystallization (Scheme 73). Stereochemistry of the cis versus the trans isomers was determined by 1H and 31P NMR experiments as well as comparison with known similar examples from the literature.

To favor the formation of the cis-isomers, alternative coupling procedures and conditions were evaluated. Boyer and collaborators2d,98 found that nucleophilic substitution at low temperature of an activated bis-chlorophosphonate 210 led to the formation of 246 in a 75:25 cis:trans ratio (Scheme 74). Finally, the cis-isomer 247 was obtained after deprotection of the imine group with acetic acid and purification in de > 95%. The stereochemistry was assigned on the basis of 1H and 31P NMR experiments as well as comparison with known similar examples from the literature.

3.1.5. 3′,5′-Cyclic Phosphate Ester Prodrugs. 3′,5′-Cyclic phosphate ester prodrugs (Figure 26) are part of an interesting prodrug concept that led to the discovery of PSI-352938, a compound that demonstrated anti-HCV efficacy in vitro and in human phase 1 trials. The activation of these derivatives to the monophosphate involves, first, an enzymatic P–O-dealkylation by CYP3A4 and then cleavage of the 3′-phosphorus–oxygen bond by phosphodiesterases103

PSI-352938104 and related analogs were prepared by reacting 6-substituted purine nucleoside 248a with tetra-isopropyl phosphorodiamidite in the presence of 4,5-dicyanoimidazole (DCI) and then oxidation to the corresponding cis- and trans-cyclic phosphate 250 using either I2 or t-BuOOH (Scheme 75). Interestingly, the authors found that by heating the mixture of cis and trans phosphate isomers 249 at 50 °C for several hours, the thermodynamically more stable intermediate cis-249 was favored (>95%). It is noteworthy that cis- and trans-phosphate isomers cis-249 and trans-249 can be isolated by simple column chromatography and that the structure and stereochemistry of cis-249 was elucidated using X-ray crystallographic analysis.

An alternative approach using P(V) chemistry was developed to stereoselectively prepare PSI-352938 on multigram scale (Scheme 76). Thus, after optimization of the reaction conditions, the desired cis-cyclic phosphate PSI-352938 was obtained as the major isomer by reacting nucleoside 248b with isopropylidychlorophosphate in the presence of NMI and Et3N.105 The target compound PSI-352938 was obtained with a purity above 99.5% after either column chromatography or recrystallization.
3.2. Alkoxyalkyl Monoester (HDP, ODE)

Alkoxyalkyl monoesters prodrugs, including the hexadecyloxypropyl (HDP) and octadecyloxyethyl (ODE), are ether lipid phospho-conjugates (LPC) developed by Hostetler and co-workers in the mid 1990s. This strategy led to the discovery of CMX-001, a HDP prodrug of cidofovir currently in phase II clinical trials for CMV and adenovirus infections, and to CMX-157, a HDP prodrug of adefovir, currently in clinical development for treatment of HIV infection. Using a similar approach, fozivudine tidoxil, a thioether lipid prodrug of AZT, reached phase II clinical trials for the treatment of HIV infection (Figure 27).

The concept of these prodrugs is based on the mimicking of lysophosphatidylcholine (LPC), a naturally occurring phospholipid. By replacing the choline moiety by a drug, the prodrug is supposed to use the LPC natural uptake pathway in the small intestine to reach targeted tissues and achieve high oral availability. Once delivered into the desired tissue, specific intracellular enzymes such as phospholipase C cleave the lipid carrier to free the nucleoside monophosphate (Figure 28).

To build a more robust prodrug and prevent undesired metabolic reactions, the initial LCP structure was modified over the years. Thus, the acyl linkage at the sn-1 position of the glycerol backbone was changed to an ether linkage to prevent cleavage by lysolecinase, and the hydroxyl group at the sn-2 position was either reduced or substituted to prevent a second acylation by acyl transferases. Overall, alkoxyalkyl monoesters prodrugs, such as HDP, are the result of a series of chain length, substitutions, and saturation optimizations (Figure 29).

The synthesis of nucleosides alkoxyalkyl phosphate prodrugs, summarized in Figure 30, involves (A) Mitsunobu coupling between a nucleoside and an alkoxyalkyl phosphate moiety, (B) phosphorylation of a nucleoside and subsequent introduction of the alkoxyalkyl alcohol, (C) coupling between a nucleoside and an alkoxyalkyl phosphoramidite moiety followed by oxidation of the phosphorus atom, and (D) phosphate condensation and subsequent oxidation.

On the other hand, the synthesis of alkoxyalkyl phosphonate prodrugs, shown in Figure 31, has been achieved by (A) monosaponification of bis(alkoxyalkyl)-nucleoside phosphonate, (B) direct coupling of a nucleoside phosphonic acid with an alkoxyalkyl alcohol under Mitsunobu or DCC coupling.
conditions (strategy employed in most cases), (C) substitution of monochloro activated nucleoside phosphonates, (D) direct alkylation of nucleoside phosphonic acid with alkoxyalkyl halides, and (E) direct introduction of the phosphonate moiety bearing the alkoxyalkyl chain in a single step by substitution with a phosphonoalkoxyalkyl oxymethylmethyl tosylate.

3.2.1. Alkoxyalkyl Phosphate Monoester Prodrugs. The first synthesis of nucleoside alkoxyalkyl phosphate monoester was reported by Piantadosi in 1991 who prepared ether lipid conjugates monophosphate prodrugs of AZT and ddI. Alkoxyalkyl monophosphates were prepared by three possible methods (Scheme 77): (1) by reacting alkoxyalkyl alcohols with diphenyl chlorophosphate followed by catalytic hydrogenation; (2) by reacting alkoxyalkyl alcohols with phosphorus oxychloride followed by hydrolysis of the chlorinated intermediate; or (3) by Arbuzov rearrangement (reaction of an alkoxyalkyl bromide derivative and trimethylphosphite) and subsequent removal of the methoxy groups using trimethylsilyl bromide.

The DCC-mediated coupling of the alkoxyalkyl phosphate derivatives 251a–c with AZT afforded the corresponding alkoxyalkyl phosphate prodrugs 252 in 22–28% yields (Scheme 78).

Mavromoustakos et al. demonstrated that AZT alkoxyalkyl monophosphate prodrugs can be prepared in a more efficient manner by simply using temporarily protected phosphate derivative 258 (Scheme 79). Thus, starting from protected glycerol derivative 255, reaction with hexadecyl bromide followed by acidic removal of the trityl group provided intermediate 256. Compound 256 was then reacted with o-chlorophenyl phosphodi-1,2,4-triazolide 254 and treated with triethylamine and water to afford the desired alkoxyalkyl triethylammonium phosphate salt 257. Finally, the coupling of AZT with compound 257 in the presence of MSNT was followed by deprotection with TBAF, which allowed for the preparation of AZT prodrug 252 in 68% yield.

In 1997, Hostetler et al. reported a new chemical approach for the synthesis of octadecyl glycerol (ODG) and HDP phosphate prodrugs involving the formation of the nucleoside monophosphate before introduction of the alkoxyalkyl chain. Thus, the ODG-acyclovir phosphate prodrug 261 (Scheme 80) was prepared in three steps from the N₂-acetyl protected acyclovir 259. The phosphonate moiety was first introduced on the protected nucleoside 260 by a DCC-mediated coupling with cyanethyl phosphate. Resulting nucleoside cyanethyl phosphate 260 was subsequently coupled with 1-octadecylglycerol in the presence of MSNT and NMI in a low 17% yield.
Cleavage of the cyanoethyl and N\textsuperscript{2}-acetyl protective groups with ammonia afforded the desired prodrug 261 in 92% yield.

In a similar manner, ODG-AZT was obtained by direct DCC-mediated coupling of 1-octadecyl-glycerol with AZT monophosphate in 25% yield (Scheme 81).

Beadle and co-workers\textsuperscript{110} reported the synthesis of HDP-acyclovir phosphate prodrug 265 by coupling of 2-chlorophenyl phosphodi-1,2,4-triazolide 254 with N\textsuperscript{2}-MMTr-protected acyclovir in the presence of HDPOH and NMI (Scheme 82). The subsequent removal of the MMTr group with acetic acid was followed by deprotection of the phenol group under basic conditions to afford the desired prodrug in 78% yield (Scheme 83).

Scheme 80. Synthesis of ODG-Acyclovir Monophosphate Prodrug, MSNT = 1-Mesitylenesulfonyl-3-nitro-1,2,4-triazole

Scheme 81. Synthesis of ODG-AZT Monophosphate Prodrug

Scheme 82. Synthesis of HDP-Acyclovir Phosphate Prodrug

Scheme 83. Preparation of HDP and ODE Dioxolane Prodrugs

Scheme 84. Synthesis of Alkoxyalkyl Phosphate Monoester Prodrug of 5-Fluoro-2′-deoxyuridine

subsequent removal of the MMTr group with acetic acid was followed by deprotection of the phenol group under basic conditions to afford the desired acyclovir HDP phosphate prodrug 265 in 78% yield.

A similar procedure was used by Liang et al. in 2006 for the preparation of HDP- and ODE-(−)-β-D-(2R,4R)-dioxolane-thymine (DOT) monophosphate prodrug in 60% yield.\textsuperscript{111} The 2-chlorophenyl deprotection was conducted with a 0.5 N NaOH solution in THF to afford the desired prodrug in 93% yield (Scheme 83).

Ludwig et al.\textsuperscript{112} prepared an alkoxyalkyl phosphate monoester prodrug of 5-fluoro-2′-deoxuryidine 272 using P(III) chemistry (Scheme 84). The alkoxyalkyl phosphonate reagent was prepared by reacting 1-O-octadecyl-2-O-acetyl-glycerol with salicylchlorophosphite in the presence of pyridine, followed by hydrolysis. Reaction of phosphite reagent 270 with 3′-acetyl-5-fluorodeoxyuridine in the presence of pivaloyl chloride formed the nucleoside phosphonate intermediate 271. Oxidation of P(III) to P(V) with iodine in water and removal of the acetate group using ammonia in methanol afforded the desired alkoxyalkyl phosphate prodrug 272 in 82% yield.

P(III) chemistry has also been used by Sigmund et al.\textsuperscript{113} for the synthesis of AZT and 3′-deoxyadenosine phospholipid conjugates. 2-(4-Nitrophenyl)ethoxy-protected (NPE) alkoxyalkyl phosphoramidites 274 were first obtained by reacting alkoxyalkyl alcohols 273 and diisopropylaminol-(4-nitrophenyl)ethoxy] chloro phosphate. 1H-Tetrazole-mediated coupling of NPE-protected 3′-deoxyadenosine with phosphoramidite 275 followed by phosphorus oxidation with iodine afforded the desired protected prodrugs 276 in excellent yields. The subsequent deprotection by treatment with DBU in

dx.doi.org/10.1021/cr5002035 | Chem. Rev. 2014, 114, 9154–9218

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Publication Date (Web): August 21, 2014 | doi: 10.1021/cr5002035
pyridine afforded the desired prodrug 277 obtained in 71% yield (Scheme 85).

**Scheme 85. Synthesis of 3′-Deoxyadenosine Phospholipid Conjugates, NPE = 2-(4-Nitrophenyl)ethoxycarbonyl**

A similar strategy was later used in an attempt to synthesize S-aza-HPMPC\textsuperscript{117} alkoxyalkyl prodrug 281. S-Aza-cHPMPC cyclic phosphate 86 was synthesized as shown in Scheme 25. This latter compound was reacted with hexadecyloxethyl bromide affording the alkoxyalkyl prodrug in 53% yield as a 3:2 trans/cis ratio at the newly formed phosphorus chiral center. Surprisingly, Mitsunobu coupling conditions between phosphonic acid and alkoxyalkyl alcohol afforded the corresponding prodrug 280 in only 6.5% yield with recovery of the starting material. However, the authors were unable to obtain the desired HPMP-S-aza-C alkoxyalkyl prodrug 281 due to S-aza-cytosine instability\textsuperscript{29} under basic conditions (Scheme 87).

This strategy was also used by Krecmerova et al.\textsuperscript{46} for the synthesis of the alkoxyalkyl prodrugs of 2,6-diaminopurine HPMP analog (HPMPDAP). Pure trans-cHPMPDAP 283 was first obtained by reacting HPMPDAP with DCC and DCMC. Conversion of phosphonic acid 283 into its corresponding tetrabutylammonium salt and subsequent alkylation with hexadecyloxypropyl bromide afforded the corresponding HDP prodrug 284 in 46% yield as a mixture of trans and cis isomers (1.6:1), partially separable by chromatography. The stereochemistry of the cyclic phosphonate 283 and phosphonate esters cis-284 and trans-284 was assigned on the basis of \( ^1H \) and \( ^31P \) NMR and comparison with the literature. Final saponification of the mixture of diasteroisomers 284 afforded ring opening and formation of the HDP-PMPDAP prodrug 285 in 54% yield (Scheme 88). An alternative method for the synthesis of alkoxyalkyl cidofovir prodrug analogs was developed by Wan et al.\textsuperscript{118} and involves a Mitsunobu-type coupling between cHPMPC and oleyloxypropyl alcohol in the presence of triphenylphosphine and diisopropyl azodicarboxylate (DIAD). Ring opening of 287 under basic conditions and subsequent neutralization with acetic acid afforded the desired OLE-HPMPC prodrug 288 in 42% yield (Scheme 89). This method was later used for the synthesis of glycero prodrug derivatives such as 1-\( ^{18} \)-octadecyl-2-\( ^{1} \)-O-benzyl-sn-glycero-3-cidofovir. This prodrug was shown to target the lungs more specifically.\textsuperscript{119}

Valiaeva et al. reported the preparation of HDP-PMEG prodrug 291.\textsuperscript{120} 2-Amino-6-chloropurine phosphonic acid 290 was synthesized\textsuperscript{121} by, first, alkylation of diisopropyl 2-chloroethoxymethylphosphonate with 2-amino-6-chloropurine in the presence of DBU, followed by phosphate deprotection with TMSBr. Interestingly, introduction of the alkoxyalkyl chain was carried out at this stage of the synthesis on the 2-amino-6-chloropurine nucleoside phosphonic acid, presumably to avoid competitive alkylation at the \( ^{O^6} \)-position of guanosine...
and/or increase the solubility of the nucleoside. The coupling reaction of phosphonic acid 290 with HDPOH in the presence of DCC afforded the corresponding 2-amino-6-chloropurine acyclic nucleoside phosphonate prodrug 291 in 47% yield. Subsequent acidic hydrolysis with a 1 M HCl solution and basic neutralization converted nucleotide 292 into the desired guanosine derivative in 75% yield (Scheme 90).

Choo et al. reported the preparation of the alkoxyalkyl cis-5-phosphono-2-en-1-yl nucleoside prodrug 299.123 Uracil diethyl phosphonate derivative 297 was first synthesized by Mitsunobu coupling between N3-benzoylated uracil and (Z)-diethyl (5-hydroxypent-3-en-1-yl)phosphonate followed by debenzoylation with ammonia in methanol. Triisopropylphenylsulfonylation and subsequent aminolysis converted the uracil diethyl phosphonate 297 into its corresponding cytosine derivative 298 in 75% yield. Deprotection of the phosphonate moiety with TMSBr afforded phosphonic acid, which was subsequently coupled to HDPOH in the presence of DCC and DMAP (Scheme 92).

Beadle et al.124 developed an attractive alternative and more convergent strategy for the synthesis of related HPMP adenine prodrug 303 based on the introduction of a phosphonate moiety already bearing the alkoxyalkyl chain. The key alkoxyalkyl tosylate 301 was synthesized from the diethyl derivative 300 by, first, deprotection with TMSBr, chlorination of the resulting phosphonic acid, selective substitution with alkoxyalkyl alcohol, and, finally, hydrolysis with a saturated NaHCO3 solution. The HPMP-adenine analog 303 was readily obtained by alkylation of (S)-9-(3-trityloxy-2-hydroxypropyl)-N6-trityl-adenine 302 with alkoxyalkyl tolenesulfonyloxy methylphosphonate 301 followed by deprotection under acidic condition (Scheme 93).

Alternative preparations of the related HDP-tosylate 309, through a bis(HDP) P(III) derivative, have been reported by Vrbkova et al. (Scheme 94).125 HDPOH was first treated by
Bis(phosphonomethoxy)-acyclic nucleoside 311 was prepared in a 60% yield by reacting 310 with 2 equiv of HDP tosylate 301 in DMF. Monosubstituted compound 312 was obtained as a side product in 25% yield (Scheme 95).

Similarly, the 5-fluorocytosine HPMP derivative 316 was obtained in 69% yield in three steps: (1) alkylation of the free alcohol of 313 with HDP tosylate 301, (2) Bz-deprotection by aminolysis, and (3) trityl removal in acidic conditions. The direct deprotection of 315 with 80% acetic acid afforded the 5-fluourouracil derivative in 54% yield (Scheme 96).120

Interestingly, the 2,6-diaminopurine HPMP derivative could be obtained following the same procedure, but without nucleobase protection (Scheme 97).120

Using the same key intermediate 301, HDP-PMPDAP (2,6-diaminopurine) alkoxyalkyl prodrugs 321a and its 2-amino-6-cyclopropyl analog 321b were synthesized by Valiaeva et al.120 Thus, purines were reacted with 1,3-dioxolan-2-one, and subsequent alkylation with alkoxyalkyl tosylate 301 afforded the desired alkoxyalkyl prodrugs 321 (Scheme 98).

In a similar manner, HDP-PMEDAP, an open ring analog based on the 2,4,6-triaminopyrimidine, was obtained in 19% yield by reaction of tetrahydropyranyloxyethylamine with 2,4-diamino-6-chloropyrimidine 322 followed by acidic hydrolysis. Subsequent alkylation of compound 323 with HDP tosylate 309 afforded HDP-PMEDAP 324 in 15% yield (Scheme 99).120

Valiaeva et al.127 prepared ODE-(S)-MPMP guanosine compound 327, which was found to be active against HCV.

**Scheme 92. Preparation of Alkoxyalkyl \( \text{cis-5-Phosphono-}
\text{pent-2-en-1-yl Nucleoside Prodrug 299} \)**

**Scheme 93. Synthesis of HPMP-Adenine Prodrug 303**

**Scheme 94. Preparations of HDP-Tosylate 309**

**Scheme 95. Preparation of Bis(phosphonomethoxy)-acyclic Nucleoside 311**

**Scheme 96. Synthesis of 5-Fluorocytosine HPMP Derivatives 315 and 316**

**Scheme 97. Synthesis of 2,6-Diaminopurine HPMP Derivative 316**

**Scheme 98. Preparation of Alkoxyalkyl cis-5-Phosphono-pent-2-en-1-yl Nucleoside Prodrug 299**

**Scheme 99. Preparation of Bis(phosphonomethoxy)-acyclic Nucleoside 311**

PCl₃ in pyridine produced bis(HDP)-phosphate 304. Standard hydroxymethylation with paraformaldehyde and triethylamine was followed by tosylation, and afforded bis(HDP)-tosylate 308 in 25% yield. A more efficient synthetic pathway to bis(HDP)-tosylate 308 involves bis-activation of phosphonic acid 307 with oxalyl chloride and subsequent substitutions with HDPOH. Following a known procedure,127 monodeprotection by treatment with excess LiN₃ in DMF at high temperature led to HDP hydroxymethyl tosylate 309 in high yield (Scheme 94).
Compound 327 was easily synthesized in two steps from the O-benzylated guanosine derivative 325 by first coupling with alkoxyalkyl tosylate 301 in the presence of t-BuONa, followed by removal of the benzyl group under acidic conditions (Scheme 100).

Finally, an alternative approach allowing direct conversion of the free PME-C, -G, and -A nucleosides into their corresponding alkoxyalkyl prodrugs was developed by Vrbkova et al.125 Thus, reaction of PMEG with oxalyl chloride in DMF allowed for the one-pot chlorination of the phosphorus atom and formation of a formamidine functional group at the C-2 position. Intermediate 328 was reacted with HDPOH in pyridine to form a bis(HDP)-substituted compound. Subsequent deprotection in 80% acetic acid and removal of one of the alkoxyalkyl chain by treatment with an excess of LiN₃ afforded HDP guanine prodrug 330 in high yield (Scheme 101).

Tichy et al.128 also used a selective hydrolysis of bis(HDP)-monophosphate derivatives to prepare HDP-(S)-HPMP and HDP-2-(2-phosphonoethoxy)ethyl (PEE) prodrugs 333 and 336. Thus, starting from bis(isopropyl)-phosphonoethoxyethyl chloride, treatment with TMSBr, chlorination of the resulting phosphonic acid, and coupling with HDPO gave the desired bis(HDP)-chloro derivative 331. 2-Amino-6-chloropurine was then introduced under basic condition, and the resulting intermediate was hydrolyzed with AcOH to give the bis(HDP)guanine prodrug 332. Finally, treatment with LiN₃ in DMF afforded the desired HDP-PEE prodrug 333 (Scheme 102).

The related HDP-(S)-HPMP prodrug 336 was prepared in six steps from bis(isopropyl)-phosphonoethoxyethyl tosylate 306. Thus, 306 was reacted with TMSBr to give the corresponding phosphonic acid, which was then chlorinated and reacted with HDPOH. The resulting bis(HDP)-phos-
nate 308 was then reacted with 334 in the presence of NaH to
give the bis(HDP)-(S)-HPMPG 335 after deprotection under
acidic conditions. Finally, selective hydrolysis was achieved by
with NaOH in a mixture of dioxane and water to
provide the desired HDP-HPMPG prodrg 336. Interestingly,
the authors also prepared the cyclic monoester 337 by
treatment of 336 with PyBOP in the presence of i-Pr2EtN
(Scheme 103).

Scheme 103. Hydrolysis of Bis(HDP)-MP Derivatives To
Prepare HDP-(S)-HPMPG

3.2.3. Alkoxyalkyl Phosphoramidates. Liang et al.\textsuperscript{111}
reported the synthesis of alkoxyalkyl phosphoramidate DOT
prodrg 339. This mixed prodrg was generated by reaction of
diphenyl phosphite with DOT, followed by addition of
alkoxyalkyl alcohol. The amino acid portion of the prodrg
was finally introduced by reaction of phosphite intermediate
338 with alanine (Scheme 104).

Scheme 104. Synthesis of Alkoxyalkyl Phosphoramidate
DOT Prodrug 339

3.3. Phosphoramidates and Phosphonamidates
\(O-PO(OR)\textsubscript{2}\textsuperscript{129} \) and \(C-PO(OR)\textsubscript{2}\textsuperscript{130}

3.3.1. Aryloxy Amino Acid Amidate ProTide. Aryloxy-
phosphoramidate prodrugs, also called “ProTides”, contain a
phosphorus atom bearing an amino acid alkyl ester and an
arylxy group. Pioneered in the early 1990s by McGui and
co-workers, this prodrg approach was the result of several
years of SAR studies during which several types of masked
phosphate moieties were evaluated including bis(alkyloxy)- and
haloalkyloxyphosphates\textsuperscript{129} bis(aryloxyphosphate)\textsuperscript{130} cyclic\textsuperscript{131}
and noncyclic alklyloxyphosphoramidates\textsuperscript{129,132} and phosphor-
odiamidates\textsuperscript{133}

Because of their ability to increase or even reveal activity of
nucleosides, but also because they are relatively easy to prepare,
this type of prodrg was used in drug discovery settings by
medicinal chemists for the biological evaluation of new
nucleosides/tides candidates in vitro.\textsuperscript{334} The proof-of-principle
in humans demonstrated with sofosbuvir (PSI-7977, originally
discovered by Pharmasset, Inc., and approved for the treatment
of HCV) paved the way for the development of several other
aryloxyphosphoramidate prodrgs that have now advanced to
clinical trials for HIV treatment (GS-7340 to phase III,\textsuperscript{135} GS-
9131 to phase II,\textsuperscript{136} stampidine, to phase I\textsuperscript{137}), cancer
(thymectacin, in phase I/II for the treatment of colon
cancer\textsuperscript{138}), and HCV treatment (INX-08189\textsuperscript{139} to phase II,
PSI-353661\textsuperscript{140} to phase I) (Figure 32).

The mode of action of these aryloxyphosphoramidates,
leading to the intracellular delivery of active nucleoside
monophosphates, has been studied in detail over the years.\textsuperscript{141} After
crossing the cell membrane, the monophosphate
deprotection is initiated by an esterase or cathepsin A
producing carboxylate A\textsuperscript{142} A spontaneous intramolecular
cyclization to a five-member ring occurs, releasing a molecule
of phenol. Cyclic intermediate B undergoes chemical opening
in the presence of water leading to phosphoramidase dieter C.
Finally, cleavage of C by intracellular phosphoramidase or
histidine triad nucleotide-binding protein 1 (HINT-1)\textsuperscript{143} frees
the nucleoside monophosphate (Figure 33).\textsuperscript{144a,144}

Aryloxyphosphoramidate nucleoside prodrgs are generally
prepared by three different methods highlighted in Figure 34:
(A) coupling of the nucleoside with a phosphorochloridate reagent, (B) coupling of a nucleoside with a diarylphosphite and subsequent oxidative amination, and (C) coupling of an amino acid to a nucleoside aryl phosphate.

It is noteworthy that these different synthetic approaches generally lead to approximate 1:1 mixtures of diastereoisomers at the phosphorus center often inseparable by flash chromatography. The discovery that \( S_p \) and \( R_p \) isomers had different in vitro biological properties lead to the development of a diastereoselective approach using enantiomerically pure aryloxy phosphoramidate reagents (Method \( A' \), Figure 34).

On the other hand, the aryloxyphosphonamidate nucleoside prodrugs are obtained from the nucleoside phosphonic acid as shown in Figure 35: (A) bis-chlorination and subsequent oxidative amination, (B) DCC coupling with phenols (1 equiv) followed by chlorination of the nucleoside aryl phosphate and subsequent substitution with an amino acid, and (C) selective saponification leading to a nucleoside monoaryl phosphate and subsequent coupling with an amino acid.

### 3.3.1.1. Aryloxy Amino Acid Phosphoramidate

#### 3.3.1.1.1. Phosphorochloridate Coupling

Aryloxyphosphoramidate nucleoside prodrugs are generally prepared by coupling of nucleosides with phosphorochloridate by either activation of the imidazolium intermediate with NMI or by 5'-deprotonation of the nucleoside with \( t-BuMgCl \) and subsequent substitution with the chlorophosphoramidate (Figure 36).

Over the past 20 years, substitution of the phosphorochloridate reagent has been explored by modifying (1) the nature of the aryloxy portion (substituted phenols or naphthols), (2) the amino acid, and (3) the amino acid ester. Key phosphorochloridate reagents are generally prepared by reaction of phosphorus oxychloride with an aryl alcohol in the presence of triethylamine followed by addition of the appropriate amino acid alkyl ester.

Phosphorochloridates are generally obtained as a 1:1 mixture of \( R_p \) and \( S_p \) diastereoisomers. They are often used crude after a simple extraction or filtration, but cleaner reaction and higher yields are observed when purified by a flash chromatography. From all of the natural amino acids, L-alanine is the most commonly used, while the nature of the aryl group and carboxyl ester portion is dependent on the nucleoside and/or its application. In a general manner, the replacement of the natural amino acid with, for instance, D-amino acids or dialkylglycine led to significant loss of activity. The only counterexample is the dimethyl glycine that showed potency almost similar to that of L-alanine. In the same way, any attempts to replace the amino acid moiety by simple amines or to incorporate methylene linker between the nitrogen and the ester group of the amino acid led to almost total loss of in vitro activity.

Since the first use of the NMI-mediated coupling for the synthesis AZT aryloxyphosphoramidate prodrug by McGuigan et al. in 1992, numerous nucleoside prodrugs have been successfully prepared using this approach including AZT.
Figure 37. NMI method.

Figure 38. t-BuMgCl method.
On the contrary, Yoo et al. had to use the NMI-approach to prepare t-prodrug after 36 h, while carbovir was converted to the corresponding phosphorochloridate, abacavir was phosphorylated in 43% yield with three steps, while the other one will be completely ineffective. Indeed, competitive hydroxyl group(s) on the nucleoside: For instance, the phosphorylation of unprotected nucleosides bearing competitive hydroxyl group(s) can lead to a mixture of 5′-mono and 3′,5′-bisphosphorylated products (often separable by chromatography). However, the same reaction can also produce, in certain cases, a 5′-monophosphorylated regiosomer hardly separable from the 3′-phosphorylated product.

These two approaches are substrate-dependent, and therefore it is very difficult to predict a priori which one to use for the best outcome. In some cases, they both provide similar yields like in the case of 2′-C-methyl-2-amino-6-substituted-purine nucleoside analogs and 5′-FdU (Scheme 105).

In most cases, both approaches provide the expected product prodrug, albeit one in better yields than the other as observed by Kumar et al. during the synthesis of 6-thio-7-deaza-2′-deoxyguanosine phosphoramidate (Scheme 106, eq 1).

### Scheme 105. Comparable Efficiency between the NMI and t-BuMgCl Methodologies

<table>
<thead>
<tr>
<th>Scheme 105</th>
<th>NMI</th>
<th>t-BuMgCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>360</td>
<td>11-28%</td>
<td>22-25%</td>
</tr>
<tr>
<td>R = Cl, OMe, OEt, NMe, SMe</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O(CH2)2CH2OCH2, NH2CH2Bn</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NNNb, OCH(Me2)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Scheme 106. NMI versus t-BuMgCl Methodology

#### (1) Other free hydroxyls groups on the sugar backbone of the nucleoside: For instance, the phosphorylation of unprotected nucleosides bearing competitive hydroxyl group(s) can lead to a mixture of 5′-mono and 3′,5′-bisphosphorylated products (often separable by chromatography). However, the same reaction can also produce, in certain cases, a 5′-monophosphorylated regiosomer hardly separable from the 3′-phosphorylated product.

#### (2) The nature of the nucleobase: Uridine and adenine nucleoside analogs can, in general, be phosphorylated with both methods. That is, no side products resulting from nucleobase phosphorylation are typically observed for the uridine derivatives, while minor N₆-phosphorylation of adenine can be observed, but are easily removed during chromatography. Cytosine nucleoside prodrugs can be obtained by NMI-mediated coupling; however, the high nucleophilicity of the amine can lead to partial N₆-phosphorylation, and therefore the anionic method (t-BuMgCl) is generally preferred.

Similarly, conversion of guanosine, 2,6-diaminopurine, and hypoxanthine nucleoside analogs to their corresponding phosphoramidate prodrugs can often be problematic. Indeed, competitive O₅-phosphorylation can occur, and their low solubility often limits the efficiency of the reaction. However, both NMI and t-BuMgCl methods can be used; nevertheless, the NMI-mediated phosphorylation can fail when solubility of the substrate is very low.

Thus, reaction of allene derivative 369 with 2.15 equiv of phosphoramidate in the presence of 4.15 equiv NMI led to the formation of allenic phosphoramidate 370 along with a side product presumably identified as the O₅-phosphorylated compound. Interestingly, this bis-phosphorylated side product was not isolated, because treatment with silica gel and methanol led to its disappearance. A similar byproduct was observed during the synthesis of butenol nucleoside prodrug 372, in which the reaction mixture was treated with 80% AcOH to hydrolyze the O₅-phosphoramidate prior to chromatography (Scheme 107).
Qiu et al. observed the same phenomena during the synthesis of E- and Z-methyleneциклопропане ацетиль аргил лигназид арлокофосфорамид проидрugs. The phosphorylation reaction was performed by treating nucleoside with 5 equiv of phosphorochloridate and 10 equiv of NMI with added pyridine as a solubility enhancing cosolvent. As a result, the bis-phosphorylated derivate was formed as the major product. This compound could then be converted into the desired prodrug in 80% yield after acidic hydrolysis (Scheme 108).

### 3.3.1.1.1.2. Protection of Competitive Sites on the Nucleobase.

In addition to the use of polar cosolvents such as pyridine or DMF that can sometimes be beneficial, temporary protection of the nucleobase or the sugar moiety can alternatively be used to increase the solubility of the nucleoside and mask other competitive hydroxy and amino groups.

Thus, Ambrose et al. prepared cytosine methyleneциклопропане ацетиль аргил лигназид by first N-protecting of compound with a benzoyl group, followed by reaction with phosphorochloridate via the NMI-mediated coupling. In this case, the benzoyl group prevents N-phosphorylation reaction but also allows for the separation of Z and E nucleoside isomers and 377b. Selective benzoyl group deprotection was carried out by treatment with hydrazine in a 4:1 mixture of pyridine/AcOH to give the desired prodrug in 29% yield (Scheme 109).

DMF-protected acyclovir was efficiently phosphorylated with the NMI method in 51% yield. Deprotection in refluxing propanol afforded the desired acyclovir prodrug in 90% yield. Additional arylsulfoxiphosphoramidate N^2-dimethylformamidine protected analogs were later prepared with the t-BuMgCl method in yields ranging from 31% to 93%. However, the deprotection step usually led to modest yields (2–25%). These low yields were partially due to the additional HPLC reverse phase purification step after the classical flash chromatography.

Formamidine protections can also be used to temporarily protect the cytidine N^4-exocyclic amine. Thus, Nilsson et al. synthesized 4′-азидо-2′-дезо-2′-метилцитидин проидрugs by first reaction of (383) with dimethylformamide dimethylacetel, followed by reaction with a chlorophosphoramidate in the presence of NMI and final deprotection of the amino group under acidolytic conditions (Scheme 111).

### 3.3.1.1.1.3. Protection of Competitive Sites on the Sugar Moiety.

Along with the nucleobase protection, temporary groups can also be introduced on the sugar moiety to increase the solubility of the starting material and avoid competitive phosphorylation.
For instance, while the direct phosphorylation of 4′-azido adenosine afforded the desired prodrug in very low yield (6%), the 2′,3′-diol protection with a cyclopentylidene moiety allowed the coupling of (388) with chlorophosphoramidates in yields ranging from 71% to 92%. Mild acidic deprotection using 80% formic acid in water for 4 h afforded free prodrugs 390 in 47−55% yield (Scheme 112).

McGuigan et al. protected nucleoside 391 with an isopropylidene group using a catalytic amount of perchloric acid in acetone. Phosphorylation of 392 with 2 equiv of t-BuMgCl and various phosphorochloridates afforded the corresponding protected aryloxyphosphoramidate nucleosides in 30−88% yield. The following deprotection was carried out in acidic conditions to afford free prodrugs 393 in moderate to good yields (Scheme 113). Similar alkylidene protection strategies were also employed for the preparation of several nucleoside analogs such as 5-substituted uridine, 4′-azidocytidine and inosine, 2′-C-Me-cytidine and adenosine, or ribavirin using the t-BuMgCl method for the phosphorylation reaction (not shown).

Alternative protecting groups such as benzoxycarbonyl (Cbz) have also been proven to be compatible with the synthesis of phosphoramidate prodrugs. Thus, Cho et al. prepared Cbz-protected A, U, G, and C derivatives using a high-yielding three-step process: first 5′-hydroxy TBDMS-protection, followed by 2′,3′-hydroxyl groups Cbz-protection (along with the N4-position in the case of cytosine derivative), and final TBDMS-removal using Et3N·HF. The NMI-mediated coupling of 395 with phosphorochloridate afforded corresponding derivatives in yields ranging from 94% to 98%. Finally, catalytic hydrogenolysis of (396) delivered the desired A, U, G, and C prodrugs in almost quantitative yields (Scheme 114). It is noteworthy that catalytic hydrogen transfer with cyclohexa-1,4-diene and palladium on charcoal was preferred for uridine derivatives to avoid partial reduction of the C(5)−C(6) double bond. Despite a long sequence, the excellent overall yield represents a real improvement to the direct phosphorylation of unprotected nucleoside (86% to 10% from cytidine, respectively). This method was eventually applied to the synthesis of aryloxyphosphoramidate prodrugs of both 2′,3′-α-fluoro-2′-β-C-methyl uridine and cytidine in 87% and 86% yields.

More recently, Cho et al. showed that N6-carbamoyl adenosines nucleosides can also be efficiently coupled with chlorophosphoramidate using t-BuMgCl (Scheme 115).

A temporary levulinate protecting group has also been reported by Shen et al. for the synthesis of the vidarabine aryloxyphosphoramidate prodrug 404 using t-BuMgCl (Scheme 115).

Vidarabine was sequentially silylated at the 5′-position and acylated at the 2′- and 3′-positions with levulinic anhydride. Selective desilylation using TBAF in acetic acid afforded the...
correctly protected nucleoside 403 in 87% yield. Interestingly, acetic acid was critical in this reaction to prevent the levulinyl group from shifting from the 3′- to the 5′-position. Desired prodrugs were finally obtained by NMI-mediated phosphor-ylation and subsequent deprotection with hydrazine hydrate in a pyridine/acetic acid buffer (Scheme 116).

Shen et al.206 also used a similar sequence involving temporary protection of compound 405 with levulinites to prepare the triciribine prodrug 407 (Scheme 116). Interestingly, Di Francesco et al.207 found the direct formation of 7-substituted phosphoramidate prodrug 410 from the corresponding parent nucleoside to be problematic and decided to use tetrahydropyranyl (THP) groups to both protect the secondary hydroxyl group and the pyrazole moiety. Thus, key intermediate 409 was obtained in four steps from 408 by 5′-silylation followed by protection of the 3′-hydroxyl, selective desilylation using TBAF, and Suzuki coupling with THP-protected pyrazole boronic acid. Finally, reaction of 408 with chlorophosphoramidate 340 in the presence of i-BuMgCl followed by removal of the two THP groups in AcOH afforded the desired 7-substituted 7-deaza-adenine nucleoside prodrug 409 (Scheme 118).

In the presence of competitive 5′- and 3′-hydroxyl groups, it is worth mentioning that reaction conditions can sometimes be optimized to minimize the formation of undesired species without the use of protective groups (Scheme 119). Lehsten et al.208 reported reaction conditions for the large-scale synthesis of NB1011, the phosphoramidate of (E)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU). They found that the temperature and the rate of addition of the electrophilic phosphoramidating species were critical factors to selectively phosphorylate the 5′-over the 3′-hydroxyl groups. The optimized conditions used dichloromethane as the solvent for the entire process. A ratio of 1.4:1 for the amino acid HCl salt to PhOP(=O)Cl2 allowed for the optimum formation of B. Maintaining the temperature between −10 and 0 °C, a solution of NMI in dichloromethane was then added dropwise. The reactive mixture is transferred
slowly into a mixture of BVDU in dichloromethane at $-5\, ^\circ\text{C}$. The nucleoside, poorly soluble in DCM, slowly dissolves in the presence of the excess NMI, and this allows further control of the reaction providing $\sim 1\, \text{kg}$ of NB1011 of high purity (>99% by HPLC) in 53% yield after silica gel chromatography.

3.3.1.1.2. Phosphite Approach. Another alternative approach to the synthesis of arylxoxyphosphoramide nucleoside prodrugs involves the reaction of a nucleoside with a diaryl phosphite and subsequent amination under Atherton–Todd conditions with amino acids.

This method was developed by Li et al. using d4T and AZT as models.\textsuperscript{209} The key diaryl phosphite was prepared in a two-step procedure involving reaction of phenol with phosphorus trichloride and subsequent reaction with phosphorus acid (Scheme 120).\textsuperscript{210} The arylxoxyphosphoramide nucleoside prodrugs are then formed in a one-pot two-step procedure. First, the addition of the nucleoside to a mixture of 1.5 equiv of diarsyl phosphite and catalytic amounts of triethylamine in THF at low temperature quickly yielded the nucleoside aryl phosphate intermediate byproducts, which necessitates the protection of the nucleobase and/or the sugar moiety.

3.3.1.1.2.1. Byproducts. One drawback of this phosphite approach is the potential formation of complex products mixtures including dinucleotides and diamino acids species. To overcome these problems, a different synthetic pathway to the key nucleoside aryl phosphate intermediate was reported by Jiang et al. based on P(III) substitution.\textsuperscript{213} Using d4T and AZT as models, this three-component Arbuzov reaction was initiated by reacting the nucleoside with phosphorodichloridate (1 equiv), $t$-BuOH, and triethylamine to yield the corresponding nucleoside aryl phosphate 416 (Scheme 122). These conditions afforded the nucleoside aryl phosphate cleanly and in high yield (86% for the $p$-methoxy phenol derivative). The addition of 1 equiv of the amino acid, NCS, and 4 equiv of triethylamine to the solution containing the intermediate aryl nucleoside 5′-phosphate afforded almost quantitatively the desired arylxoxyphosphoramide prodrugs 417 in overall yields that ranged from 63% to 79% over two steps.

3.3.1.1.2.2. Protection of Competitive Sites on the Nucleobase and/or the Sugar Moiety. The overall yield of the sequence can usually be improved by masking competitive nucleophilic sites, which also increases the substrate solubility in commonly utilized solvents.

For example, Leisvuori et al.\textsuperscript{215} prepared 2′-OMe cytidine arylxoxyphosphoramide prodrug 422 by first 5′-hydroxyl silylation of 418, trienylation of the N4-position, and protection of the 3′-hydroxyl with levulinic acid and DCC in dioxane. Selective 5′-desilylation carried out with TBAF in a mixture of THF and acetic acid afforded the appropriately protected nucleoside 420. This latter compound was reacted with 1.2 equiv of diphenyl phosphite in pyridine followed by 1.5 equiv of alanine methyl ester in the presence of carbon tetrachloride and triethylamine to afford the protected nucleoside produg 421 in 70% yield. Treatment of 421 with hydrazine, acetic acid, and pyridine cleaved the levulinyl group, while the MMT group was removed by 80% aqueous AcOH at 65 °C, affording the desired prodrug 422 in 90% yield (Scheme 123).

In the same vein, Leisvuori and co-workers\textsuperscript{219} used levulinyl groups to prepare ribavirin phosphoramide prodrug 426. 2′,3′-Bis-levulinoylated ribavirin 423 was reacted with 1.5 equiv of diphenyl phosphite in pyridine to allow formation of the nucleoside phosphite phenyl ester and subsequently to alanine methyl ester in the presence of carbon tetrachloride and triethylamine to form the protected nucleoside produg 425 in

Like for the phosphorochloridate coupling, the phosphite approach presents some limitations mainly with the formation of byproducts, which necessitates the protection of the nucleobase and/or the sugar moiety.
67% yield. Deprotection of the levulinoyl groups afforded the desired ribavirin aryloxyphosphoramidate prodrug \(426\) in 60% yield (Scheme 124).

In the case of a ribonucleoside, protection of the 2',3'-diol with an isopropylidene group can also be envisaged. Thus, Donghi et al. used this approach for the synthesis of 2'-C-Me-cytidine aryloxyphosphoramidate prodrugs bearing \(\beta\)-amino alcohols (Scheme 125).\(^{216}\) 2'-C-Me-cytidine was first protected with an isopropylidene group after treatment with 2,2-dimethoxypropane and \(p\)-TsOH in acetone in 80% yield. The protected nucleoside was reacted with diphenyl phosphite in pyridine followed by amino alcohol to give \(429\) in 40% yield over two steps. The final deprotection with TFA in water afforded the desired prodrugs \(430\) as a mixture of phosphorus diastereoisomers that were later separated by HPLC or supercritical fluid chromatography (SFC).

### 3.3.1.1.3. Miscellaneous Approaches

Another approach involving P(V) chemistry was developed by Nillroth et al. for the synthesis of FLT-prodrugs.\(^{217}\) FLT was first reacted with 2 equiv of 2-chlorophenyl phosphorodichloridate and excess 1,2,4-triazole in the presence of triethylamine to form the nucleoside aryloxy triazolide phosphoramidate intermediate \(431\). The subsequent addition of glycine methyl ester hydrochloride and triethylamine afforded the FLT-aryloxyphosphoramidate prodrug \(432\) in 80% yield (Scheme 126).

In the same study,\(^{217}\) the use of a cyclic phosphorodiamidate reagent for the synthesis of a FLT \(\sigma\)-(methanesulfonamino)-phenyl methoxy glycine prodrug analog was also reported (Scheme 127). Cyclic phosphorochloridate \(433\) was reacted

### Scheme 124. Protection of Competitive Sites — Levulinate Sugar Protection

In the case of a ribonucleoside, protection of the 2',3'-diol with an isopropylidene group can also be envisaged. Thus, Donghi et al. used this approach for the synthesis of 2'-C-Me-cytidine aryloxyphosphoramidate prodrugs bearing \(\beta\)-amino alcohols (Scheme 125).\(^{216}\) 2'-C-Me-cytidine was first protected with an isopropylidene group after treatment with 2,2-dimethoxypropane and \(p\)-TsOH in acetone in 80% yield. The protected nucleoside was reacted with diphenyl phosphate in pyridine followed by amino alcohol to give \(429\) in 40% yield over two steps. The final deprotection with TFA in water afforded the desired prodrugs \(430\) as a mixture of phosphorus diastereoisomers that were later separated by HPLC or supercritical fluid chromatography (SFC).

### Scheme 125. Protection of Competitive Sites — Isopropylidene Group

In the same study,\(^{217}\) the use of a cyclic phosphorodiamidate reagent for the synthesis of a FLT \(\sigma\)-(methanesulfonamino)-phenyl methoxy glycine prodrug analog was also reported (Scheme 127). Cyclic phosphorochloridate \(433\) was reacted

### Scheme 126. Other Approach Using P(V) Chemistry

In the same study,\(^{217}\) the use of a cyclic phosphorodiamidate reagent for the synthesis of a FLT \(\sigma\)-(methanesulfonamino)-phenyl methoxy glycine prodrug analog was also reported (Scheme 127). Cyclic phosphorochloridate \(433\) was reacted

### Scheme 127. Use of Cyclic Phosphorochloridate

In the same study,\(^{217}\) the use of a cyclic phosphorodiamidate reagent for the synthesis of a FLT \(\sigma\)-(methanesulfonamino)-phenyl methoxy glycine prodrug analog was also reported (Scheme 127). Cyclic phosphorochloridate \(433\) was reacted
with glycine methyl ester in the presence of triethylamine to form phosphorodiamidate reagent 434. The crude compound was then directly reacted with FLT, affording the corresponding FLT-prodrug 435 in 86% yield.

Because the direct coupling of dipropylglycine phosphoro-

chloridate with d4T afforded the desired phosphoramidate prodrug 437 in only 2% yield, McGuigan et al. designed an alternative approach for its synthesis.150 However, the coupling of d4T 5′-monophenyl phosphate 436 with 2 equiv of dipropyl glycine methyl ester and 2.5 equiv of MSNT in pyridine afforded the desired prodrug 437 in a low 7% yield (Scheme 128).

### Scheme 128. Synthesis of D4T Phosphoramidate Prodrug 437

![Diagram of Scheme 128](attachment:image.png)

3.3.1.4. Asymmetric Synthesis. It has been proven over time that $S_p$ and $R_p$ diastereoisomers can display different biological profiles, and it is not uncommon to see 10-fold or more difference in terms of in vitro potency between two phosphorus diastereomers.5,219 The separation of phosphorus diastereomeric mixtures can be realized, in some cases, by HPLC, selective crystallization, or flash chromatography on silica gel. However, chemists have more recently developed diastereoselective approaches based on a phosphorus SN2-type mechanism with chiral phosphor(odi)amidate reagents.

Thus, Romań et al.219,220 designed a phosphorodiamidate reagent bearing a ($S$)-4-isopropylthiazolidine-2-thione as chiral auxiliary (Scheme 129). This chiral auxiliary allows the separation of the $S_p$ and $R_p$ diastereoisomers and acts as a leaving group during a SN2 reaction with a nucleoside. ($S$)-4-Isopropylthiazolidine-2-thione was reacted with phosphoryl chloride in the presence of triethylamine to give dichlorophosphate 188. The diastereoselective introduction of the aryl moiety was then carried out with either DBU or Et$_3$N in acetone at $-91 \, ^\circ\text{C}$. The phosphorochloridates 438 were obtained in 59–93% yields and diastereomeric excess (de) between 28% and 87% depending on the nature of the aromatic moiety. It is noteworthy that the use of substoichiometric amounts of phenol was required to avoid formation of diaryl phosphoramide byproducts that are hardly separable from the desired product. The introduction of the amino acid was carried out by reacting the diastereomerically enriched mixture of phosphorochloridates with a single equivalent of L-alanine methyl or benzyl ester hydrochloride and 3 equiv of triethylamine in dichloromethane. Interestingly, the diastereomeric ratios were found generally lower ($\sim$15–85%) than those for the starting phosphorochloridate, pointing out a possible isomerization. However, these phosphorodiamidate reagents can be separated via flash chromatography to deliver pure diastereoisomers (de > 95%). Crystals of the major diastereoisomer were obtained, and its structure and ($R$)-configuration were confirmed by X-ray crystallography. Finally, the coupling of d4T with 1 equiv of diastereomerically pure phosphoramidate 439 and 3 equiv of i-ButMgCl in a mixture of THF and pyridine (1:1) for 5 days at room temperature afforded the desired prodrugs 440 as single diastereoisomers in 11–50% yields (85–95% de). The stereochemistry of the ($S_p$)-diastereomer was assigned by comparison with analytical data from the literature.

Ross et al.221 also prepared several chiral phosphoramidate reagent bearing substituted phenols that would act as leaving groups during the phosphorylation step, $p$-Nitrophenyl phosphoramidate reagent 441 was prepared from the commercially available $p$-nitrophenyl dichlorophosphate by reaction with phenol and amino acid hydrochloride (Scheme 130). At this stage, two successive crystallizations in disopropyl ether afforded the pure $S_p$-reagent in 22% yield (96% de). The stereochemistry of the phosphorus center was assigned by X-ray crystallographic analysis. On the other hand, pure $R_p$-

### Scheme 129. Use of a Chiral Auxiliary

![Diagram of Scheme 129](attachment:image.png)

### Scheme 130. Crystallization of Phosphoramidate Reagent

![Diagram of Scheme 130](attachment:image.png)
was obtained (de > 99.9%) by purification of the enriched mixture by supercritical fluid chromatography using a chiral stationary phase. The synthesis of PSI-7977 was then conducted with the \( S \)\(_p\) isomer 441 and \( t \)-BuMgCl, affording the desired produg 442 in 40% yield (99.7% de after two recrystallizations from dichloromethane).

In the same paper, the authors also investigated the influence of other electronegatively substituted phenol moieties (nitro groups and halogens). 2,4-Dinitrophenol and pentafluorophenol phosphoramidates were found to be the most reactive reagents. The 2,4-dinitrophenol phosphoramidate had low selectivity between 3'- and 5'-hydroxy groups, leading to a higher proportion of 3,5'-bis(phosphorylated)-nucleoside, whereas the pentafluorophenyl reagent was more selective and therefore was selected in further studies.

Compound 443 was prepared by reaction of phenyl dichlorophosphate with \( t \)-alanine isopropyl ester hydrochloride followed by pentafluorophenol addition (Scheme 131). After filtration of the salts, the crude solid was triturated in a mixture of 20% ethyl acetate in hexanes solubilizing only the desired \( S \)\(_p\) isomer (de > 98%). The stereochemistry of \( (S)_p \)-443 was determined by X-ray crystallography. Coupling conditions of (443) with \( 2'\)-Me\(_2\)F-nucleoside were optimized, and it was found that low temperature of reaction (\(-5 \)°C) and slow addition of reagent lowered the formation of both 3'-phosphorylated and 3',5'-bis(phosphorylated) side products while maximizing reaction conversion. Finally, the multigram scale synthesis of PSI-7977 was carried out by treating \( 2'\)-C-Me\(_2\)-2'F-nucleoside with 2.1 equiv of \( t \)-BuMgCl in THF at \(-5 \)°C followed by the addition of 1.2 equiv of pentafluorophenol phosphoramidate 443. After being stirred for 18 h at 5 °C and two successive crystallizations, PSI-7977 was obtained in an excellent 68% yield (de > 99.7%).

### 3.3.1.1.5 Post Modifications of Phosphoramidate Nucleoside Prodrugs

Interestingly, aryloxophosphoramidate nucleoside prodrugs have proven to be stable enough to undergo further modifications. Thus, Velázquez et al. prepared AZT, d4T, and thymidine heterodimers with TSAO-T as potential inhibitors of HIV-1 reverse transcriptase.\(^222\) Thymidine aryloxophosphoramidate prodrug formation was performed using 2 equiv of phosphorochloridate 340 and 6 equiv of NMI (Scheme 132). The corresponding nucleoside phosphoramidate underwent \( N \)\(^2\)-alkylation with 1,3-dibromopropane, and 444 was then coupled to TSAO-T using potassium carbonate. Desired heterodimer 446 was obtained in 81% yield.

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**Scheme 132. Post Modifications – Alkylation Reactions**

As seen previously for the removal of Cbz groups, hydrogenation conditions are compatible with the aryloxophosphoramidate moiety. Thus, reduction of \( 1'\)-Cd4A produg 447 gave the corresponding \( 1'ddA \) phosphoramidate 449 in 49% yield (Scheme 133).\(^172\)

**Scheme 133. Hydrogenation of \( 1'\)-Cd4A to \( 1'ddA \) phosphoramidate**

Similarly, Hatton et al. reported the reduction of the \( 4'\)-C,3'-O-propylene-linked bicyclic pyrimidine nucleoside (Scheme 134).\(^23\) While the cytosine analog 450a was hydrogenated with hydrogen over Pd/C, the uracil derivative 450b was hydrogenated by transfer hydrogenation over Pd/C to minimize the simultaneous uracil base hydrogenation.

**Scheme 134. Post Modifications – Hydrogenation Reactions**

hydrogen over Pd/C, the uracil derivative 450b was hydrogenated by transfer hydrogenation over Pd/C to minimize the simultaneous uracil base hydrogenation.

Postmodification of phosphoramidate nucleoside prodrugs by palladium-catalyzed reactions has also been reported by Perlikova et al.\(^224\) (Scheme 135). 6-Chloro-7-deazapurine ribonucleoside was first protected with an isopropylidene group before reaction with \( t \)-BuMgCl and phosphorochloridates. Phenyl, furyl, thienyl, and dibenzofuryl groups were then introduced at the 6-position using Suzuki–Miyaura or Stille
cross-coupling reactions. Finally, isopropylidene deprotection with 90% TFA at room temperature led to the desired aryloxyphosphoramidate prodrugs in yields ranging from 40% to 87%. It is noteworthy that partial hydrolysis of aryloxyphosphoramidate ester group was observed during the deprotection reaction.

3.3.1.2. Aryloxy Amino Acid Phosphonamidate. Despite its long running success with regular phosphate nucleosides, ProTide technology has not been widely exploited with phosphonate nucleoside until recently. One method developed by Ballatore et al. involves the bis-chlorination of the phosphonic acid nucleoside (PMPA) with thionyl chloride and subsequent substitutions with phenol and L-alanine methyl ester in the presence of triethylamine. Interestingly, the nucleoside was reacted again with thionyl chloride between the two substitutions, presumably to reactivate the potential hydrolyzed product. In these conditions, PMPA-aryloxyphosphonamidate prodrug was only obtained in 5% yield (Scheme 136).

A more efficient method was developed by Chapman et al. for the kilogram scale synthesis of GS-7171, an isopropyl ester aryloxyphosphoramidate prodrug of PMPA (Scheme 137). This method first involves a DCC-mediated coupling between PMPA and phenol in NMP at high temperature. The activation of the remaining hydroxyl with thionyl chloride and the subsequent substitution with the amino acid isopropyl ester at −78 °C afforded GS-7171 in 47% yield. At this stage, R<sub>p</sub> and S<sub>p</sub> isomers were separated using simulated moving bed chromatography to give GS-7340 (de > 98%).

Mackman et al. used a third strategy for the synthesis of the phosphonomethyloxy cyclic nucleoside GS-9131 (Scheme 138). The conversion of nucleoside into phosphonate intermediate 461 was accomplished by oxidation of the 5'-hydroxyl using Jones' reagent, glycal formation under Mitsunobu conditions, and treatment with IBr and diphenyl hydroxymethyl phosphate. Oxidation of the iodine with NaOCl and treatment with aqueous ammonia afforded the nucleoside phenyl phosphonate monoester 462 in 18% yield. Coupling of this compound with L-alanine ethyl ester hydrochloride and PyBOP afforded the desired prodrug GS-9131 in 19% yield.

3.3.2. 3',5'-Cyclic Phosphoramidate. 3',5'-Cyclic phosphoramidates have been recently designed as an alternative to McGuigan Protides to mainly eliminate the potential toxicity associated with the release of phenol moieties. Thus, Gardelli et al. prepared 2'-C-methylcytidine-3',5'-cyclic phosphoramidate by reacting 2'-C-Me-C with a chlorophosphoramidate reagent bearing a 4-chlorophenyl, in the presence of t-BuMgCl. At this stage, both fast eluting (464a F,E) and slow
eluting (464b S.E.) isomers were separated by RP-HPLC and reacted with t-BuOK to form the desired cyclic prodrugs. Interestingly, isomer 464a F.E. was found to give the desired cyclic prodrug 465a (S<sub>P</sub>) in 67% yield, while the other isomer 464b S.E. yields the corresponding cyclic compound 465b F.E. (R<sub>P</sub>) in only 35% along with monophosphate 466 (Scheme 139). The absolute stereochemistry of the phosphorus center on both cyclic compounds (S<sub>P</sub>-465a and (R<sub>P</sub>-465b was assigned by NOE experiments.

Scheme 139. 2′-C-Me-Cytidine-3′-5′-cyclic Phosphoramidate

Jain et al.<sup>228</sup> developed a one-step method for the synthesis of 3′,5′-cyclic phosphoramidate prodrug using novel phosphoramidate reagent 468 that was prepared in two steps from 4-nitrophenol by first reaction with POCl<sub>3</sub> to give chloro intermediate 467, and then reaction with alanine methyl ester in the presence of Et<sub>3</sub>N. Reaction of 5-fluoro-2′-deoxyuridine (FdUrd) with intermediate 468 in the presence of DBU afforded cyclic prodrug 469 as a 5:1 mixture of diasteroisomers as determined by 31P NMR (Scheme 140).

Scheme 140. 2′-C-Me-FdU 3′-5′-Cyclic Phosphoramidate

3.3.3. Amino Acid Amidate Monoester. Amino acid phosphoramidate nucleoside monoester prodrugs were pioneered in the 1990s by Wagner and co-workers.<sup>229</sup> This prodrug was designed as a modification of the aryloxophosphoramide strategy detailed previously. The intention was to explore whether the lipophilic aryl group was indispensable or not while increasing the water solubility of the prodrug and losing the chirality of its phosphorus center. It was designed in such a way that the amino acid phosphoramidate mono ester biodegradation involves the direct cleavage of the amino acid group by the action of a phosphoramidase (Figure 39).<sup>144b,c,230,231</sup>

Interestingly, amino acid phosphoramidate nucleoside monoesters regained some interest in recent years with the discovery, by Herdewijn et al., of their ability to act as a triphosphate mimic and thus to be substrates of reverse transcriptases (including HIV-1). It was demonstrated that amino acid phosphoramidate nucleosides with specific amino acids such as L-aspartic acid and particularly L-histidine, in their acid form, are fulfilling the requirements of structural and electronic properties that allow proper alignment of α-phosphorus atom in the polymerase active site, mimicking a nucleoside triphosphate (Figure 40).<sup>232,233</sup> The success of this recent approach led to its extension to modified or unnatural amino acids derivatives.<sup>234</sup>

The synthesis of amino acid phosphoramidate mono ester nucleosides prodrugs can be achieved by different synthetic pathways shown in Figure 41: (A) formation of a hydroxyl cyanoethyl nucleoside phosphite, oxidation to the monophosphate, and subsequent coupling/deprotection, (B) formation of a methyl cyanoethyl nucleoside phosphite and subsequent oxidative amination/deprotection, (C) formation of a protected H-phosphonate, and subsequent oxidative amination/deprotection, (D) hydrolysis of phosphorothioate, (E) hydrolysis of phosphoramidates, (F) transformation of the nucleoside di- or triphosphate by transient persilylation and substitution of a mono or diphosphate unit, and (G) direct coupling of an amino acid with a nucleoside monophosphate. Their phosphonate counterparts can be prepared by coupling one amino acid to phosphonate nucleoside (Figure 42).
3.3.3.1. Amino Acid Phosphoramidate Monoester. In 1994, the first synthesis of amino acid nucleoside phosphoramidate monoester was reported by Wagner and co-workers\textsuperscript{235} to synthesize new AZT, FLT, and d4T prodrugs. AZT was first reacted with 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite and subsequently treated with 1\textsuperscript{H}-tetrazole and methanol to yield the methyl cyanoethyl nucleoside phosphite intermediate\textsuperscript{470}. Subsequent reaction with phenyl alanine methyl ester in the presence of iodine and final hydrolysis afforded nucleoside phosphoramidate\textsuperscript{471} in 38\% yield. Removal of the cyanoethyl group with ammonia in methanol and purification on acidic resin afforded the desired prodrug\textsuperscript{472} in 88\% yield (Scheme 141).

In 1996, a more commonly used method was reported by Abraham et al.\textsuperscript{230a} for the synthesis of amino acid phosphoramidate mono ester prodrugs of Ara-C and 5-FdU and involves the direct coupling of the amino acid to the nucleoside monophosphate. Thus, Ara-C was selectively phosphorylated at the 5\textsuperscript{′}-position with phosphorus oxychloride in triethyl phosphate. After hydrolysis, a DCC-mediated coupling with phenylalanine methyl ester afforded the desired amino acid Ara-C phosphoramidates\textsuperscript{474a, 474b} (Scheme 142).

In 1997, the same approach was used by Abraham et al.\textsuperscript{236} for the preparation of amino acid phosphoramidate monoester of acyclovir (Scheme 143). After phosphorylation of acyclovir, a DCC-mediated coupling with various amino esters afforded the desired prodrugs\textsuperscript{475} in 28–89\% yield. It is noteworthy that the coupling of ACV with cyanoethyl phosphate afforded a mixture of products and was abandoned.

EDC has also been used as a coupling agent with 2\textsuperscript{′}-deoxyadenosine to perform the reaction at room temperature, and compound\textsuperscript{476} was obtained in an improved 63\% yield (Scheme 144).\textsuperscript{230, 234d}

Interestingly, Abraham et al.\textsuperscript{237} observed a lack of selectivity between 5-FdU’s 5\textsuperscript{′}- and 3\textsuperscript{′}-hydroxy groups during phosphorylation, and thus had to use a series of temporary protecting groups. First, an MMtr group was introduced at the 5\textsuperscript{′}-position of 5-FdU before acetylation of the 3\textsuperscript{′}-hydroxy group. Selective 5\textsuperscript{′}-deprotection and DCC-mediated coupling with cyanoethyl phosphate afforded protected 5-FdU intermediate\textsuperscript{477}.

Deprotection under basic conditions followed by coupling
phenylalanine or tryptophan methyl esters afforded the desired 5-FdU prodrugs \(^{(479)}\) (Scheme 145).

Adel'inskaya et al. prepared different amino acid 2′-deoxy adenosine phosphoramidate mono esters (Scheme 146).\(^{(232)}\)

DCC-mediated coupling between 2′-deoxy adenosine monophosphate \(^{(480)}\) and various protected amino acids afforded the amino acid methyl ester nucleoside phosphoramidates \(^{(481)}\) in 39–94% yield. The subsequent saponification was carried out with 0.4 M NaOH to afford the amino acid nucleoside phosphoramidates \(^{(482)}\) in 22–94% yield. Alternative deprotection with potassium carbonate in 2:1 MeOH:water could also be used.\(^{(234a)}\) A similar procedure was used for the synthesis of 1-deaza-, 3-deaza-, and 7-deaza- adenine analogs (not shown).\(^{(238)}\)

Nucleoside phosphoramidate monoesters bearing amino acid in their acidic form, such as \(^{(484)}\) and \(^{(486)}\), are also intermediates of aryloxyphosphoramidate bioactivation and were synthesized as part of the activation studies (Scheme 147). These compounds were prepared by simple saponification of their corresponding aryloxyphosphoramidates \(^{(483)}\) and \(^{(485)}\), in aqueous triethylamine.\(^{(184b,201)}\)

Alternatively, the amino acid nucleoside phosphoramidate monoesters can be generated using \(H\)-phosphonate intermediates.\(^{(239)}\) AZT triethylammonium \(H\)-phosphonate was generated in 69% yield from AZT by first treatment with diphenyl phosphate in pyridine and subsequent hydrolysis in aqueous triethylamine. Transient silylation of \(^{(487)}\) followed by oxidation with iodine, substitution with amino acids methyl ester, and basic hydrolysis afforded the desired amino acid nucleoside phosphoramidate \(^{(488)}\) in 31–70% yield. Treatment with a methylamine solution in methanol gave the corresponding methyl amide analogs \(^{(489)}\) in good to quantitative yields (Scheme 148).

Other alkyl amine derivatives were directly prepared from the \(H\)-phosphonate nucleoside as previously described for the methyl ester analogs in 28–56% yield (Scheme 149).
The same method was used by Chang et al. for the preparation of amino acid 2',3'-dideoxyadenosine (ddA) phosphoramidate monoester without requiring nucleoside protection. The oxidative amination of the nucleoside H-phosphonate with different amino acids afforded the desired ddA prodrugs in yields ranging from 28% to 51% (Scheme 150).

One major drawback of such strategies is the need to use reverse phase or ion-exchange chromatography for the purification of these very polar nucleoside phosphate monoesters. To overcome this limitation, Zhu et al.241 used a fluorenylmethyl protecting group (Scheme 151). A slight excess of diphenyl phosphite was reacted successively with FmOH and with adenosine in pyridine to afford nucleoside H-phosphonate intermediate 493. The Atherton−Todd amination with amino acids hydrochloride, carbon tetrachloride, and triethylamine afforded the protected nucleoside phosphoramidates 494 in 73−82% yield. Deprotection with piperidine in dichloromethane led to the desired amino acid adenosine phosphoramidate monoesters (yields not provided).

Phosphite intermediates can also be generated through phosphoramidites as reported by Whalen et al. for the preparation of cytidine phosphoramidates monoester.243 Tribenzoyl cytidine phosphoramidite 501 was prepared by a tetrazole-mediated coupling of tribenzoyl cytidine with allyl phosphoramidite 500. Hydrolysis and oxidative amination under Atherton−Todd conditions afforded the protected nucleoside phosphoramidates 502 in 30−78% yield. Removal of the allyl group with Pd(PPh3)4 and subsequent treatment with NaOMe and NaOH afforded the desired phosphoramidates 503 in 80% yield for all examples (Scheme 153).

Fu et al. reported the synthesis of amino acid nucleoside phosphoramidate monoester from nucleosides di- and triphosphates (Scheme 154).244 Treatment of ADP with TMSCl and various amino acid methyl ester in pyridine and subsequent hydrolysis with 2 M ammonium hydroxide afforded...
the desired nucleoside monophosphate prodrugs in roughly 50% yield.

Using a similar approach, Zhu et al. prepared various amino acid nucleoside phosphoramidate monoesters from thymidine, uridine, adenosine, and guanosine triphosphates (Scheme 155).245

Amino acid nucleoside phosphoramidate monoesters can also be obtained by hydrolysis of phosphorothioamidate derivatives (Scheme 156).216,246 2-Chloro-3-methyl-1,3,2-thiazaphospholidin-4-one 2-oxide was first reacted with glycine methyl ester hydrochloride in the presence of triethylamine to provide intermediate 506. Subsequent reaction with FLT and hydrolysis with 10% Et3N in dichloromethane over silica gel at 40 °C afforded the amino acid nucleoside phosphoramidate monoesters 508.

De Napoli et al. used thymidine as a model for the synthesis of nucleoside phosphoramidates monoesters on solid support.247 Tentagel HL resin was first linked to the thymidine phosphoramidite 509 via a 3-chloro-4-hydroxyphenylacetic linkage by a tetrazole-mediated coupling. After oxidation with iodine in pyridine and water, the 3′-trityloxy group was replaced by an acetoxy group, and the cyanethyl group was cleaved in the presence of triethylamine in pyridine. With key intermediate 511 in hand, introduction of various amino acids was carried out successfully after tosylation of the phosphate ester. For optimal results, the coupling was repeated three times before the desired nucleoside phosphoramidate was detached from the solid support with concentrated aqueous ammonia (Scheme 157).

3.3.3.2. Amino Acid Phosphonamidate Monoester. The synthesis of amino acid nucleoside phosphonamidate monoesters, such as 513, is much less represented in the literature when compared to their phosphoramidate counterparts. McKenna et al.248 reported the coupling of cidofovir with valine methyl and ethyl esters in the presence of EDC in water in 35−40% (Scheme 158).

A similar procedure was used by Adelinskaya et al. for the synthesis of an aspartic acid adefovir derivative.232b DCC-mediated coupling of aspartic acid and adefovir and subsequent saponification with sodium hydroxide in methanol and water afforded the desired adefovir prodrug 514 in 58% yield (Scheme 159).
3.3.4. Borch’s Methylaryl Haloalkylamidates. As part of their research program on nucleosides, the Borch team developed a neutral methyl aryl haloalkyl phosphoramidate prodrug capable of passing through the cell membrane. These prodrugs are designed to undergo intracellular activation to generate unstable phosphoramidate anion intermediate B, which in turn undergoes spontaneous cyclization and P−N bond cleavage by water to liberate the nucleoside monophosphate (Figure 43). It is noteworthy that their first prodrug approach involved a haloethyl (instead of halobutyl) nucleoside phosphoramidate. However, further mechanistic studies revealed that after cyclization of the haloethyl phosphoramidate anion, nonselective nucleophilic attack of water at the carbon and phosphorus centers of the pyrolidinium ion intermediate was observed, delivering the NuMP, but also an undesired hydroxyethyl byproduct.

The coupling of the methyl aryl haloalkyl phosphoramidate moiety to the nucleosidic part was achieved either using either P(III) or P(V) chemistry (Figure 44). No chiral synthesis has been developed so far, and final methylaryl haloalkyl phosphoramidate prodrugs are always obtained as a mixture of diastereoisomers at the phosphorus center.

This prodrug approach has been successfully applied to the intracellular delivery of antitumor nucleotide 5-fluoro-2′-deoxyuridine-5′-monophosphate (FdUMP). This compound was synthesized using P(III) chemistry as shown in Scheme 160. Phosphorus trichloride is reacted with the corresponding alcohol in the presence of disopropylethylamine followed by reaction with N-methyl-N-(4-chlorobutyl)amine hydrochloride to yield the desired product.

Scheme 157. Synthesis of Nucleoside Phosphoramidates Monoester Libraries on Solid Phase

Scheme 158. Synthesis of Cidofovir Phosphonamidate Monoester Prodrug

Scheme 159. Phosphonamidate of Adefovir

Scheme 160. FdU Borch’s Phosphoramidate
chloride to generate chlorophosphoramidite 515. This intermediate is directly reacted with SFDU in situ and then oxidized with tert-butyl hydroperoxide to yield S-nitrofurfuryl N-methyl-N-(4-chlorobutyl) phosphoramidate 517 in 34% yield.

Wu et al.\textsuperscript{252} investigated the influence of hydrophilic modification of S-FdU phosphoramidates by replacing the N-methyl group with an N-dihydroxypropyl chain. Selective phosphorylation on the S′-hydroxyl group of S-FdU using phosphoramide 518, generated in situ by reaction of N-allyl-4-chlorobutan-1-amine hydrochloride with POCl\textsubscript{3}, provided compound 520. The -Obt moiety was then displaced by either benzyl alcohol or S-nitrofurfuryl alcohol in the presence of DMAP to furnish the corresponding methylaryl haloalkyl phosphoramidates derivatives. Dihydroxylation with OsO\textsubscript{4}/NMO afforded the final N-2,3-dihydroxypropyl-N-(4-chlorobutyl) phosphoramidates 521 (Scheme 161).

### Scheme 161. N-Dihydroxypropyl Phosphoramidates

Interestingly, attempts to synthesize directly the methylaryl haloalkyl phosphoramidate of cytosine derivatives, such as cytarabine\textsuperscript{253} or gemcitabine,\textsuperscript{254} using either P(III) or P(V) approaches, were unsuccessful presumably because of possible side reactions and very low solubility of the nucleosides. To circumvent this problem, the cytosine amino group had to be protected with an allyloxycarbonyl group that was removed, after the phosphorylation step, by treatment with Pd(PPh\textsubscript{3})\textsubscript{4} and p-toluenesulfinate (Scheme 162).

### Scheme 162. Allyloxycarbonyl Group as Transient Protective Group

3.4. Phosphorodiamidates and Phosphonodiamidates

\(\text{O-PO(NR}_{2}\text{)}\textsubscript{2}\) and \(\text{C-PO(NR}_{2}\text{)}\textsubscript{2}\)

Phosphorodiamidate produgs have rarely been used for the last 20 years, probably due to the success of aryloxyphosphoramidates. It has only been recently that this prodrug approach was reinvestigated because, unlike aryloxyphosphoramidates, it bears an achiral phosphorus center and releases only natural amino acids upon metabolism.

A putative mechanism of unmasking to the monophosphate was proposed by McGuigan et al.\textsuperscript{255} in which carboxypeptidase cleaves the ester function of the amino acid, inducing spontaneous cyclization of the carboxylate of the free amino acid onto the phosph(on)ate moiety. After a spontaneous hydrolysis, the nucleoside phosphoramidate monoester is cleaved into the free nucleoside monophosph(on)ate by action of phosphoramidase (Figure 45).

### Figure 45. Mechanism of action of O- and C-phosphorodiamidate nucleoside produgs.

Bis(aminoc acid) nucleoside phosphorodiamidates can be prepared as shown in Figure 46: (A) phosphorylation of the nucleoside with phosphorus oxychloride and subsequent bis-substitution with amino acids, and (B) chlorination of a nucleoside monophosphate and coupling with the amino acids. On the other hand, the synthesis of the bis(aminoc acid) nucleoside C-phosphorodiamidate involves three different methods highlighted in Figure 47: (A) direct coupling of the phosphonic acid nucleoside with amino acids, (B) TMSBr-deprotection of the phosphate alkyl ester nucleoside and...
subsequent coupling with amino acids, and (C) chlorination of a nucleoside phosphonate and coupling with amino acids.

3.4.1. Bis(amino acid) O-Phosphorodiamidates. The first syntheses of bis(amino acid) O-phosphorodiamidate nucleosides were described in 1991 by McGuigan and coworkers who used AZT256 and FdU257 as substrates. AZT was reacted with phosphorus oxychloride in triethylphosphate to generate AZT monophorodichloridate \(525\). Subsequent substitution with excess amino acids in the presence of triethylamine afforded the corresponding AZT phosphorodiamidate prodrugs \(526\) in 21–44% yield (Scheme 163).

McGuigan et al. also reported the preparation of bis(amino acid)-2'-methyl-6-methoxyguanosine O-phosphorodiamidate prodrug as part of an extensive SAR study (Scheme 164).255 Compounds were prepared either using the conditions described above or by slight modification of the procedure. Nucleoside was first phosphorylated with POCl\(_3\) at \(-78^\circ\)C in THF. Subsequent displacement with amino acids was carried out in the presence of diisopropylethylamine.

Non-symmetrical O-phosphorodiamidates have been obtained in yields ranging from 4% to 17%255 following a similar protocol with successive addition of two different amino acids (Scheme 165).

3.4.2. Bis(amino acid) C-Phosphorodiamidates. The bis(amino acid) nucleoside phosphorodiamidates are generally obtained from phosphonic acid intermediates after activation as phosphorodichloridates. Thus, Serafinowska et al. reported the synthesis of acyclophosphonate prodrug \(538\) in 15% yield by treatment of ethyl phosphonate derivative \(535\) with TMSBr, reaction of the corresponding silyl ether \(536\) with PCl\(_3\), and

\[\text{Scheme 163. AZT O-Phosphorodiamidates Synthesis}\]

\[\text{Scheme 164. 2'-Methyl-6-alkoxyguanosine Phosphorodiamidate Prodrug}\]

\[\text{Scheme 165. Non-symmetrical O-Phosphorodiamidates}\]

\[\text{Scheme 166. O-Phosphorodiamidates Nucleoside Prodrugs from Phosphate Nucleosides}\]

\[\text{AA} = \text{amino acid.}\]
reaction with alanine methyl ester hydrochloride in the presence of triethylamine and NMI (Scheme 167).

Scheme 167. Synthesis of [(Phosphonomethoxy)ethoxy]adenine Prodrug 538

Formation of such bis(aminoc acid) nucleoside phosphorodiamidates can also sometimes require temporary protection of the nucleobase. Thus, Dang et al. (Scheme 168, eq 1) \(^{260}\) treated PMEA with oxalyl chloride in the presence of DMF, allowing simultaneous chlorination of the phosphonic acid and protection of the \(N^6\)-position. Subsequent reaction with 2-methylalanine ethyl ester and triethylamine gave the protected diamidates prodrugs 541. Finally, hydrolysis of the formamide protection with acetic acid in isopropanol afforded the desired prodrugs 542 in 16% yield. Interestingly, reactions carried out with the glycine methyl ester without nucleobase protection failed to produce the corresponding prodrug.

Scheme 168. Synthesis of PMEA Bis(aminoc acid) Nucleoside Phosphorodiamidates with Protective Groups

Bis(aminoc acid) PMEA prodrugs such as 543 \(^{260}\) have also been prepared by direct coupling of PMEA with glycine ethyl ester in pyridine and treatment with a premixed solution of triphenylphosphine and 2,2'-dipyridyl disulfide (Scheme 168, eq 2). Interestingly, the reaction was reported to fail when using 2-methylalanine ethyl ester presumably because of the steric hindrance at the nitrogen.

A similar procedure was used by Mackman et al. \(^{35}\) for the synthesis of GS-9148 bis(aminoc acid) prodrug derivative (Scheme 169). Diethyl nucleoside phosphonate 544 was first treated with ammonium hydroxide generating 6-aminopurine nucleobase while deprotecting one of the phosphonate esters. Subsequent treatment with TMSBr afforded phosphonic acid 545. The coupling of different amino acids in the presence of 2,2'-dithiopyridine, triphenyl phosphate, and triethylamine in pyridine afforded the desired prodrugs 546 in 11–73% yield.

Jansa et al. \(^{261}\) reported the synthesis of bis(aminoc acid) nucleoside phosphonamidate prodrugs directly from the bis(alkyl) nucleoside phosphonate 549 by coupling of transient silyl ester phosphonate intermediate 548 with amino acids (Scheme 170). This procedure prevents tedious isolation of nucleoside phosphonic acids.

Scheme 170. Nucleoside Phosphonamidate Prodrugs Directly from the Bis(alkyl) Nucleoside Phosphonates

4. NUCLEOSIDE DI- AND TRIPHOSPHATE PRODRUGS

For two decades, numerous prodrug strategies have been developed to deliver nucleoside monophosphates into the cells. The monophosphate’s delivery allows bypassing the first phosphorylation, which is often the rate-limiting step to NTP formation. However, di- and triphosphate prodrugs have rarely been studied. This lack of research can be explained by the generally efficient second and third phosphorylations (for most nucleosides) and the inherent instability of the phosphate...
anhydride bond. This bond is only kinetically stable because of the negative charge resonance that avoids the nucleophilic attack at phosphorus moiety.

The di- and triphosphate prodrug strategy has been mainly applied to AZT. AZT is a highly potent anti-HIV drug that was the first FDA-approved nucleoside analog for treatment of HIV infection. AZT is efficiently converted to the monophosphate, but only slowly to the diphosphate, resulting in intracellular accumulation of AZT-MP, which is responsible for some of its side effects. In an ideal situation, the delivery of AZT-DP or AZT-TP would retain the antiviral activity of the parent compound, but avoid the toxicity associated with the AZT-MP. This approach was also widely applied to ara-C to increase its bioavailability and to avoid base deamination.

The main strategy for the synthesis of di- and triphosphate prodrugs developed in the early 1980s involves the introduction of an alkyl or acyl lipophilic chain to the last phosphate unit (β-phosphorus for diphosphate and γ- for triphosphate). The synthesis of these lipophilic di- and triphosphate prodrugs commonly involves the coupling of a lipophilic chain bearing a phosphate or pyrophosphate moiety to a NDP or NTP. The lipophilic chain itself can be also directly coupled to a NDP or NTP. Recently, a new strategy was developed as acyloxybenzyl β-diester diphosphate using P(III) chemistry with the coupling of a phosphoramidite and a NuMP (Figure 48).

4.1. Nucleoside Di- and Triphosphate Glycerides

Several nucleoside di- and triphosphate prodrugs bearing acyl and alkyl glyceride moieties have been reported. The rational design of these prodrugs was based on naturally occurring phospholipid cytidine diphosphate diglyceride, which is a natural intermediate in the biosynthesis of anionic glycerophospholipid in mammalian cells. Theses prodrugs were mainly developed to reach HIV reservoirs such as macrophages and related cells involved in phagocytosis and antigen presentation. However, administration of antiviral nucleosides such as AZT, dDC, or 3′-deoxythymidine as nucleoside diphosphate diglycerides was found to deliver monophosphorylated anti-HIV agents intracellularly, due to the cleavage of the pyrophosphate unit between the α- and β-phosphorus (Figure 49).

Phosphatidic acid was commonly used for the synthesis of nucleosides di- and triphosphate diglycerides. Historically, the direct coupling of nonactivated phosphatidic acid and nucleoside monophosphate was first reported on natural nucleosides but provided only low yields. Therefore, the most common strategy involves the coupling of phosphatidic acid to a nucleoside 5′-monophosphate activated as a morpholidate (Scheme 171). The activation of the phosphatidic acid rather than the nucleoside monophosphate was reported later to give better yields and to facilitate the purification step. NTPs diglycerides were less described and generally synthesized by coupling of phosphatidic acid and an activated NDP in low yield: for instance, AZT-TP distearoylglycerol was prepared by condensation of AZT diphosphate with distearoylphosphatidic acid morpholidate. It was shown to deliver a mixture of AZT and AZT-MP to the cells. Numerous saturated and unsaturated NTPs diglycerides were described and used in preclinical and clinical studies.

Figure 48. BLG = biolabile group.

Figure 49. Mechanism of action for nucleoside diphasphate glycerides.

Scheme 171. AZT DP-Prodrug
unsaturated lipophilic chains linked to the glycerol moiety have been reported such as myristyl, palmityl, stearyl, or oleyl.

The coupling of a morpholidate activated phosphatidic acid was also used for the preparation of a myristoyl glyceride DP derivative of acyclovir.\textsuperscript{268b} This compound (not shown) was found to be active on ACV resistant herpes TK\textsuperscript{−}, indicating an efficient delivery of ACV-MP.\textsuperscript{270}

This prodrug approach was also applied to vidarabine (ara-A) and cytarabine (ara-C), which are known for their antiviral and anticancer activity, respectively (not shown). These compounds bearing free hydroxyl groups in 2\textsuperscript{′} and 3\textsuperscript{′}-position did not require any protection to perform the synthesis of their diphosphate prodrugs. While NDP-prodrugs were found less active that parent ara-C in in vitro antiproliferative studies, they were actually much more potent in mice.\textsuperscript{271} In the form of a diphosphate prodrug, ara-C was found to be protected from the cytosine deamination, which leads to the biologically ineffective ara-U.\textsuperscript{272}

Some studies also reported the synthesis of oxyalkyl and thioalkyl ether glyceride of anti-HIV and anticancer agents. The synthetic strategy remains the same with the coupling of the glycerophospholipid part to a NMP activated as morpholidate (Scheme 172). The oxyalkyl and thioalkyl ether glycerophospholipid were previously synthesized by successive alkylation and acylation. After removal of the protective trityl group, the alcohol is treated with POCl\textsubscript{3} followed by hydrolysis.

4.2. Lipids and Steroids Nucleoside Di- and Triphosphates or Phosphonophosphates

4.2.1. Acyl Phosphates. The literature reports several examples of lipophilic acyl chains linked to the NDP or NTP. After cell penetration, the acylphosphate is expected to be cleaved by a hydrolase to give the corresponding NDP or NTP (Figure 50). Interestingly, the preferential cleavage of the mixed carboxylic phosphoric anhydride part (C–O–P) over the phosphoric anhydride (P–O–P) was observed in buffer and in culture media. Thus, the instability of this prodrug in cell culture media did not allow for an efficient transmembrane diffusion resulting in poor cellular uptake.\textsuperscript{275}

The syntheses of octanoyl, lauroyl, myristoyl, and palmitoyl acyl nucleoside diphosphates of AZT (not shown) and d\textsubscript{4}T (560) were first reported by coupling an acyl pyrophosphate unit to the NDP or NTP. This method appeared to be efficient for the formation of 2',3'-deoxynucleosides prodrugs, but low yields were observed with ribofuranosyl purine nucleotides due to additional potential acylation sites. To circumvent this problem, the authors used an ethyl chloroformate activated form of myristoic acid that selectively reacted with ADP or ATP in good yields (Scheme 174). Kreimeyer et al.\textsuperscript{278} used a similar approach to prepare a cholesterol carbonate prodrug of adenosine triphosphate 563 and showed that this compound was effectively transported across the membrane bilayer of liposomes (Figure 51).

4.2.2. Ether Phosphates. Steroids and lipids ether diphosphates nucleosides were also developed by Hong et al. Because of the ether linkage between the lipid chain and the phosphorus moiety, hydrolysis by hydrolase is impossible, but the intracellular cleavage of the pyrophosphate unit allows for the delivery of nucleoside monophosphate (Figure 52).
Steroids diphosphate derivatives of ara-C were prepared by coupling between ara-CMP morpholidate and various phosphocorticosteroids (Scheme 175). These phosphocorticosteroids were synthesized by either condensation of the steroid with 2-cyanoethylphosphate in the presence of DCC followed by deprotection of the cyanoethyl group or by treatment of 21-iodocorticosteroid with phosphoric acid. This second method was generally preferred because of easy purification of product 566 by simple crystallization. It is noteworthy that these corticosteroid diphosphate prodrugs of ara-C, 567, showed similar activities in vitro as compared to their corresponding monophosphate prodrugs, but were found to be generally less active in vivo. These differences were attributed to the high hydrolysis rate of the phosphoric anhydride bond of the diphosphate prodrugs.

4.2.3. Phosphonophosphates Derivatives. Alkylidiphosphate and alkylphosphonophosphate derivatives of naturally occurring nucleosides such as cytidine, deoxyxycytidine, thymidine, and adenosine have been reported to exhibit antiproliferative activities that were attributed to the phospholipidic chain. Thus, based on this work, alkylphosphonophosphate ara-C derivatives, (compound 568 is shown in Scheme 176 as a representative example) were prepared as a prodrug that would increase the bioavailability of the nucleoside while avoiding the deamination of the cytosine occurring at the nucleoside level and deliver ara-CTP and a phospholipidic chain, two cytotoxic principles.

Ruiz et al. reported the synthesis of PMEA and HPMPC phosphonophosphate HDP and ODE prodrugs (Scheme 177). The phosphate bearing the lipophilic group was obtained by reaction of phosphorus oxychloride and HDP−OH or ODE−OH. Alkoxalkylphosphates were then activated as phosphomorpholidates using DCC, followed by reaction with DMTr-protected HPMPC in the presence of pyridine. Following DMT deprotection with TFA, phosphonophosphates HDP and ODE prodrugs were afforded in 40% and 20% yield, respectively. Unfortunately, these compounds were found to exert less antiviral activity than their HDP and ODE phosphonate prodrugs.

Scheme 174. ADP or ATP Prodrugs

Scheme 175. Preparation of Ara-C Steroids Diphosphate Derivatives

Scheme 176. Preparation of ara-C Alkylphosphono Phosphate Derivatives

Scheme 177. Synthesis of PMEA and HPMPC Phosphonophosphate HDP and ODE Prodrugs

The phosphate bearing the lipophilic group was obtained by reaction of phosphorus oxychloride and HDP—OH or ODE—OH. Alkoxalkylphosphates were then activated as phosphomorpholidates using DCC, followed by reaction with DMTr-protected HPMPC in the presence of pyridine. Following DMT deprotection with TFA, phosphonophosphates HDP and ODE prodrugs were afforded in 40% and 20% yield, respectively. Unfortunately, these compounds were found to exert less antiviral activity than their HDP and ODE phosphonate prodrugs.
4.3. para-Methoxybenzyl Diphosphate Diester

More recently, Meier’s group proposed to use various biolabile protecting groups to synthesize diphosphate prodrugs to efficiently deliver diphosphate nucleosides. The first attempt was realized using a cycloSal (see section 3.1.3) protecting group to mask both hydroxyl groups of the β-phosphate moiety. However, after preparation of several aryl substituted cycloSal diphosphate (synthesis not reported), they observed the predominant release of NMP, by hydrolysis of the phosphorus anhydride bond. To circumvent the hydrolysis of the pyrophosphate unit, the use of a para-acyloxybenzyl (see section 3.1.3) protecting group was investigated (Figure 53).

![Figure 53. Use of a para-acyloxybenzyl group to synthesize diphosphate prodrugs.](image)

Unlike the cycloSal, deprotection of para-acyloxybenzyl is initiated by enzymatic or chemical cleavage of the ester group, and not by nucleophilic attack at the phosphorus moiety.

AZT (not shown) and d4T (574) DP prodrugs were synthesized using P(III) chemistry via a dicynoimidazole-mediated coupling of bis-para-acyloxybenzylphosphoramidite, 573, and bis(tetra-n-butylammonium) nucleoside monophosphates, followed by a subsequent oxidation with tert-butyl hydroperoxide. Phosphoramidites were previously synthesized by reaction of para-acyloxybenzylicalcohol and diisopropyl phosphoramidous dichloride (Scheme 178). Interestingly, these compounds proved to possess a high chemical stability in buffer but also to undergo fast and highly selective enzymatic cleavage in cell extract to deliver NDPs. The retained antiviral activities of d4T diphosphate prodrugs (no marked toxicity) proved their ability to penetrate the cells and release biologically active metabolites intracellularly.

Scheme 178. AZT and d4T Bis(para-acyloxybenzyl)-phosphoramidites DP Prodrugs

5. CONCLUSION

Despite that the concept of phosph(on)ates prodrugs was originally developed in the 1990s and led to the FDA approval of potent antiviral such as TDF in 2001, it is only very recently that the synthesis of phosph(on)ate prodrugs became systematic in the nucleoside field. Indeed, the large number of examples in the literature of phosph(on)ate prodrugs increasing the activity of a nucleoside or even better, revealing the activity of a inactive parent nucleoside, has led the nucleoside community to consider prodrug evaluation as indispensable. As presented in this Review, multiple synthetic methodologies were developed to prepare a large variety of phosph(on)ates prodrugs. However, several challenges remain, including the development of efficient methods for the preparation of chiral phosph(on)ates prodrugs because one diastereomer may possess overall biological properties superior to those of the other. Improvements in the targeting of prodrugs to particular organs and cellular compartment as well as the development of nanoparticles containing nucleoside prodrugs are needed. Finally, the ultimate goal remains the development of efficient triphosphate prodrugs that would completely overcome the phosphorylation issues by delivering the active compound directly to the target polymerase. The application of prodrg technology has had a large impact on the development of nucleoside and nucleotide antiviral therapies, and provides great hope for persons suffering from deadly viruses such as HIV, HBV, and HCV. Finally, it is likely that the lessons learned from these viruses with novel nucleoside prodrugs will be applied to new emerging viruses such as Noro, Hendra, Dengue, and Chikungunya viruses.

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Notes

Dr. Schinazi is the founder and chairman of RFS Pharma, LLC. He was a Founder of Idenix and Pharmasset, Inc., now acquired by Merck and Gilead Sciences, respectively.

Biographies

Ugo Pradere was born in Poitiers (France) in 1983 where he studied chemistry until his master degree. In 2006, he joined the University of Orleans (France) and obtained his Ph.D. in 2009 under the direction of Professor Luigi A. Agrofoglio working on the metallo-catalyzed synthesis of nucleoside analogs and the development of a new convergent synthetic pathway for the preparation of phosphate prodrugs. In 2010, he joined Dr. Raymond F. Schinazi at Emory University (Atlanta, GA) as a Postdoctoral Fellow where he focused on the synthesis of nucleoside phosphate and phosphate prodrugs targeting HCV inhibition with an emphasis on the conversion of furanoselide analogs into their corresponding phosphate prodrug derivatives. In 2011, he joined Professor Jonathan Hall at the ETH of Zurich for a second postdoctoral fellow and is currently working on the synthesis of multiple labeled long modified oligoribonucleotides (RNA) and their use in biological assays. He is now a senior scientist in Hall’s group.
Ethel C. Garnier-Amblard received a M.Sc. degree in Chemistry from the University of Orleans (France) in 2000. She then began graduate studies at the same university in collaboration with the Commissariat à l’Énergie Atomique (CEA, Le Ripault), where she received a Ph.D. in organic chemistry under the direction of Professor G. Guillaumet in 2004. She conducted postdoctoral studies first with Professor Lanny S. Liebeskind (2005), working on organometallic chemistry and catalysis, and then with Professor Dennis C. Liotta (2007) at Emory University (Atlanta, GA), working on medicinal projects involving sphingolipid analogs for oncology applications. Two years later, she was appointed Faculty at the Emory University - Department of Pharmacology, where she worked in the field of heterocyclic and nucleoside chemistry. In 2013, she joined RFS Pharma, LLC (a biopharmaceutical company focused on developing novel, differentiated therapeutics for the treatment of hepatitis viruses) where she is currently a Senior Scientist. Dr. Garnier-Amblard’s research interests concern the discovery of new drugs for the treatment of HIV-1 and hepatitis infections as well as emerging viruses. Her research interests include the development of practical new methodologies, the synthesis of active pharmaceutical ingredients, and asymmetric synthesis in general.

Steve Coats obtained his doctorate in organic chemistry under the direction of Albert Padwa at Emory University in Atlanta, Georgia. He completed a postdoctoral fellowship with Harry Wasserman at Yale University in New Haven, Connecticut. He then spent 3 years at Eli Lilly and Johnson & Johnson Research and Development in Philadelphia, Pennsylvania. In 2006 he moved to RFS Pharma in Atlanta, Georgia, where he is currently Senior Director of Chemistry. His research interests include medicinal chemistry, heterocycles, nucleosides, nucleotides, and prodrugs.

Franck Amblard was born in Châteauroux, France. He studied chemistry at the University of Orléans (France), where he received his Ph.D. in 2004 under the guidance of Professor Luigi A. Agrofoglio working on the synthesis of new nucleosides analogs using metathesis and palladium-catalyzed reactions. In 2005, he moved to the U.S. to join Professor Raymond F. Schinazi’s research group at Emory University (Atlanta, GA) and worked, as a postdoctoral fellow, on new nucleosides and nucleotides prodrugs. He is now Assistant Professor at the Department of Pediatrics, Emory University School of Medicine. His main research interests include the study of nucleosides analogs and small molecules as potential antiviral agents as well as the isolation and characterization of natural compounds from traditional medicines.

Dr. Raymond F. Schinazi is the Frances Winship Walters Professor of Pediatrics and Director of the Laboratory of Biochemical Pharmacology at Emory University. He serves as Senior Research Career Scientist at the Atlanta Department of Veterans Affairs, and Director of the Scientific Working Group on Viral Eradication within the NIH-sponsored Emory University Center for AIDS Research. Dr. Schinazi is a world leader in the area of nucleoside chemistry having published more than 500 papers and been issued 100 U.S. patents. He is the founder of several biotechnology companies focusing on antiviral drug discovery and development, including Pharmasset, Inc., Triangle Pharmaceuticals, Idenix Pharmaceuticals, and RFS Pharma, LLC. He is best known for his innovative and pioneering work on stavudine, lamivudine, emtricitabine, telbivudine, and sofosbuvir, all of which have been approved by the United States Food and Drug Administration. More than 94% of HIV-infected individuals take at least one of the drugs he invented.

ACKNOWLEDGMENTS

This work was supported in part by NIH grants 2P30-AI-050409 and the Department of Veterans Affairs.


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Review

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