Review

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Future Medicinal Chemistry

Phosphonate prodrugs: an overview and recent advances

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Phosphonates, often used as isosteric replacements for phosphates, can provide important interactions with an enzyme. Due to their high charge at physiological pH, however, permeation into cells can be a challenge. Protecting phosphonates as prodrugs has shown promise in drug delivery. Thus, a variety of structures and cleavage/activation mechanisms exist, enabling release of the active compound. This review describes the structural diversity of these pro-moieties, relevant cleavage mechanisms and recent advances in the design of phosphonate prodrugs.

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Prodrugs have become increasingly common as replacements for drug candidates that have encountered obstacles during the development process. Since 2008, over 10% of new chemical entities approved by the US FDA have been prodrugs; more impressively, between 2014 and 2017, 17% of new chemical entities with FDA-approval have been prodrugs [1]. The strategy, despite being used for more than a century, was coined by Adrien Albert in 1958, and is defined as an inactive molecule undergoing some biological transformation in order to release the active metabolite [2]. In essence, use of a prodrug strategy enables a problematic molecule to overcome a biological obstacle, such as poor bioavailability, low absorption, instability, poor specificity, formulation difficulties or other adverse effects or toxicity concerns [3–5]. As drug development programs are fast approaching US$3 billion per FDA-approved drug, the consideration of a prodrug strategy early in development may be more efficient than attempting to save a problematic candidate later in the pipeline [6].

A key point of the prodrug strategy is that traditionally problematic functional groups are masked. One such example is the phosphonate: phosphonates often provide an opportunity for unique interactions with a target, but are characterized by a high negative charge and subsequent poor bioavailability [7]. The phosphonate group is often isosteric to a phosphate by replacing one ester oxygen of the phosphate with a carbon atom. This modification can lead to improved metabolic stability, as enzymes typically associated with cleaving an oxygen-phosphorus bond may not be as efficient at cleaving a carbon–phosphorus bond [8,9]. However, due to the charge of phosphonates at physiological pH, diffusion across biological membranes remains difficult (Figure 1) [10]. To remedy this, a variety of protecting groups exist for phosphonates [11]. Each strategy, however, must strike a balance between enabling sufficient absorption and cleavage of the moiety without generation of toxic byproducts.

Phosphonate prodrugs can be classified according to substituents they include, most commonly esters and amides, and the substitution pattern they carry. Prodrugs of phosphonates may be mono- or di-substituted, and, if di-substituted, they can be symmetrical or asymmetrical (Table 1). When asymmetrically di-substituted, a new chiral center is introduced in the molecule at the phosphorus atom, possibly giving rise to slower cleavage of the protecting groups surrounding the phosphonate. In order to determine the optimal pattern of substitution, the reason for using the prodrug must be considered, as does the mechanism of how the protecting group(s) will be cleaved (Figure 2).

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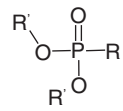
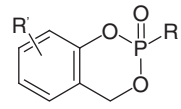
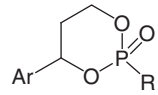
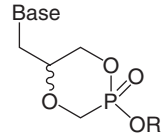
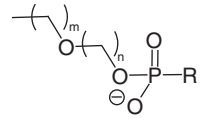
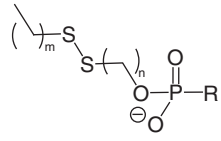
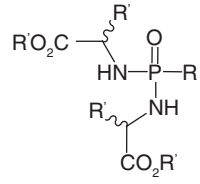
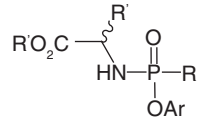
Table 1. Phosphonate prodrugs and nomenclature.

Symmetrical diesters		
	R′ =	
Alkyl	$-(CH_2)_nCH_3$	
Aryl	$-C_6H_5$	
Benzyl	$-CH_2C_6H_5$	
Acyloxyalkyl (POM)	$-CH_2OC(O)C(CH_3)_3$	
Alkoxycarbonyloxyalkyl (POC)	$-CH_2OC(O)OCH(CH_3)_2$	
S-Acylthioalkyl (SATE)	$-CH_2CH_2SC(O)R$	
Asymmetrical diesters		
CycloSal		
HepDirect		
Monoesters		
Internal monoester		
Lipid conjugates		
Disulfide lipid conjugates		
Symmetrical bisamidates		
Amino acid esters		
Mixed amidate/ester		
ProTides		

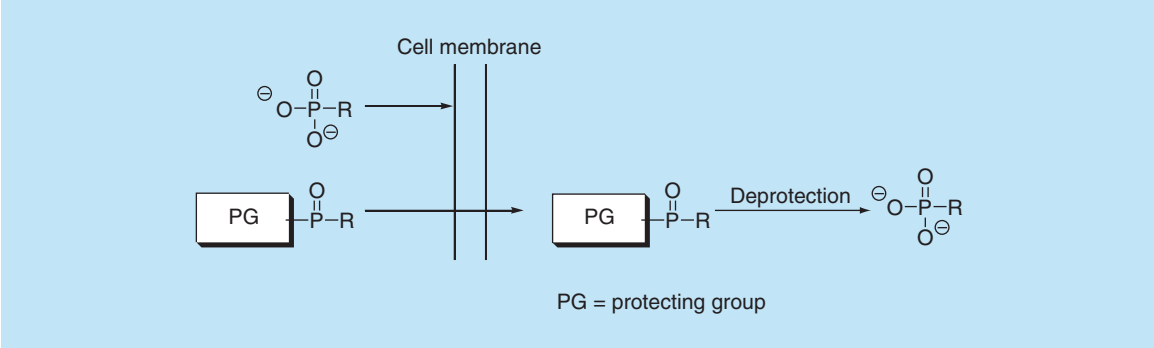


Figure 1. Permeation of a phosphonate prodrug and subsequent deprotection to release the active compound.

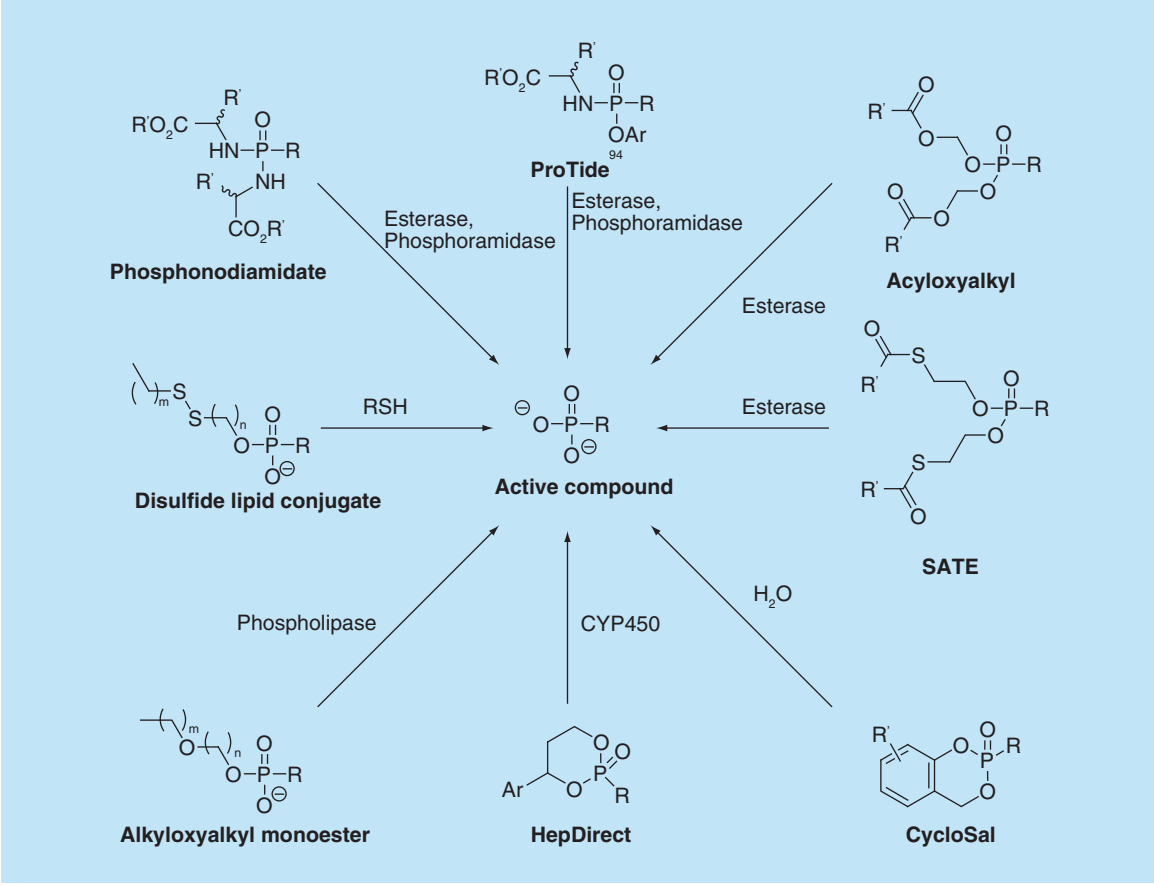


Figure 2. Routes of activation of phosphonate prodrugs. The expanding role of prodrugs in contemporary drug design and development.
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Phosphonate ester prodrugs

Phosphonate esters are a common prodrug strategy employed, with many examples having been prepared and evaluated for biological activity. A recent comprehensive review by Wiemer and Wiemer [9] thoroughly describes both modern and historical examples of varying prodrug approaches. While their review includes phosphates, this review will specifically focus on phosphonates, including recent examples.

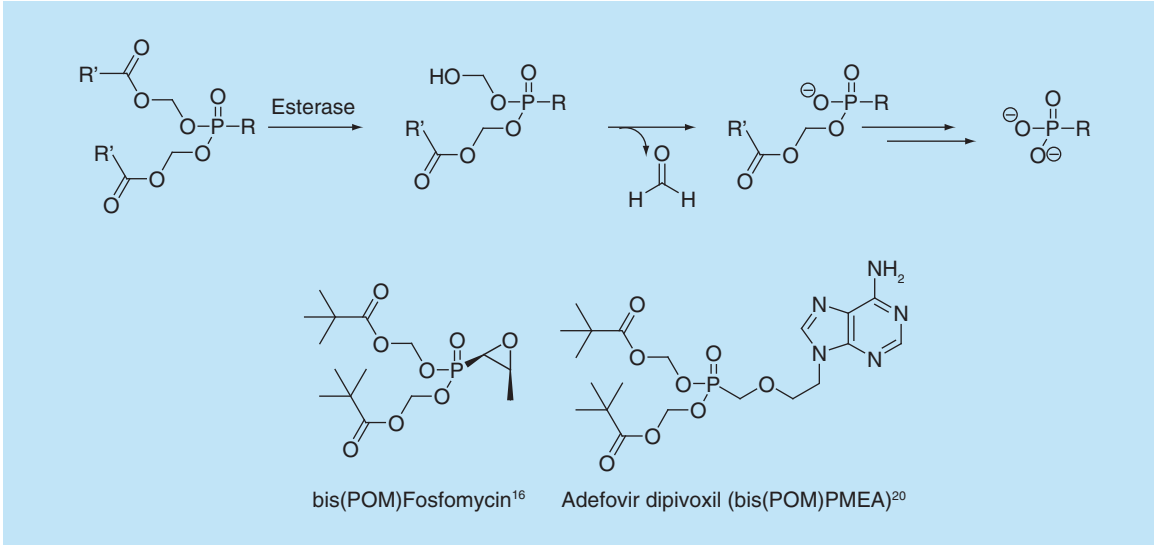

Figure 3. Activation and examples of acyloxyalkyl prodrugs.

Symmetrical diesters

In order to attain a neutral charge at biological pH, diester prodrugs may be the most obvious choice. Symmetrical esters induce no stereocenter. Thus, both esters are cleaved and biologically activated by the same enzyme or group of enzymes. While simple dialkyl esters of phosphonates have been synthesized, they have been reported as overly stable in mammalian systems [12–14]. In contrast, dibenzyl esters are more readily converted to the active drug, but with rates highly dependent upon the substituent pattern of the aryl ring, as unsubstituted benzyl esters cleave at an undesirably slow rate [13,15]. Because aryl esters are more easily hydrolyzed, they function more readily as prodrugs, with the ability to modify rates of hydrolysis by varying the substituent pattern [14].

More complex examples of phosphonate diesters have been evaluated and may be more advantageous prodrugs. Acyloxyalkyl esters are a general first prodrug for many new phosphonate containing compounds, since their first use to protect fosfomycin in 1969 (Figure 3) [7,16]. The success of this strategy is due in part to the improved stability of acyloxyalkyl phosphonates over their phosphate counterparts [7,17–19]. One such acyloxyalkyl prodrug is the pivaloyloxymethyl (POM) moiety. POM prodrugs are cleaved enzymatically to generate a hydroxymethyl intermediate, followed by spontaneous loss of formaldehyde to release the monoester or active compound (Figure 3) [17]. Nucleotide Adefovir dipivoxil, the di-POM prodrug of 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA), was FDA-approved in 2002 and has been used to treat Hepatitis B through inhibition of reverse transcriptase [20]. The di-POM moiety was compared with a range of other prodrug esters, but was ultimately selected as it displayed the highest oral bioavailability in rats, more than twofold higher than free acid PMEA [21]. More recently, POM prodrugs were shown to increase cell permeability of anti-infective and antitumor agents, demonstrating the continued use of the POM moiety in phosphonate drug design [22–28]. Activities of POM prodrugs of fosmidomycin analogs increased activity in whole cell tuberculosis assays over 50-fold [22], while they also increased activity in whole cell *Plasmodium falciparum* assays over tenfold [23,24]. Further, concentrations of POM-protected bisphosphonates required to stimulate Vγ2Vδ2 T-cells were up to 1100-fold lower than that of their parent acids [25–28]. Toxic byproducts from the POM moiety, however, raise concern. Pivalic acid lowers carnitine levels and is toxic to small mammals at high concentrations [29,30], while formaldehyde is a known mutagen [31]. However, formaldehyde released through *in vivo* activation of a prodrug represents a small fraction of daily exposure to formaldehyde when compared with dietary sources and metabolic processes [32]. For these reasons, alternate protecting groups have recently received more attention.

Structurally related to the acyloxyalkyl prodrugs are carbonate esters, most commonly isopropyloxycarbonyloxymethyl (POC) protected phosphonates (Figure 4). Because the structures are related, the metabolic cleavage of POC groups is similar to that of other acyloxyalkyl prodrugs. After enzymatic cleavage of isopropanol, an unstable carboxylate intermediate spontaneously decomposes to lose CO_2 and formaldehyde, releasing the mono-ester phosphonate and subsequently, the free phosphonate after a second enzymatic event (Figure 4) [33]. Tenofovir

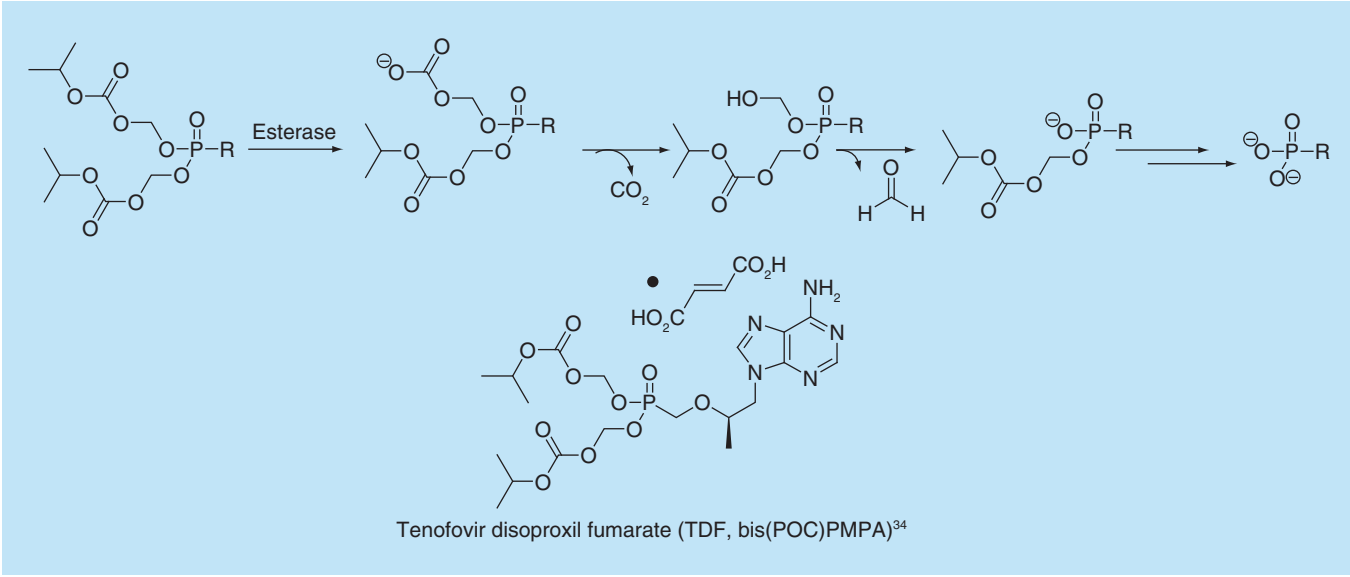

Figure 4. Activation and an example of alkoxycarbonyloxyalkyl prodrug.

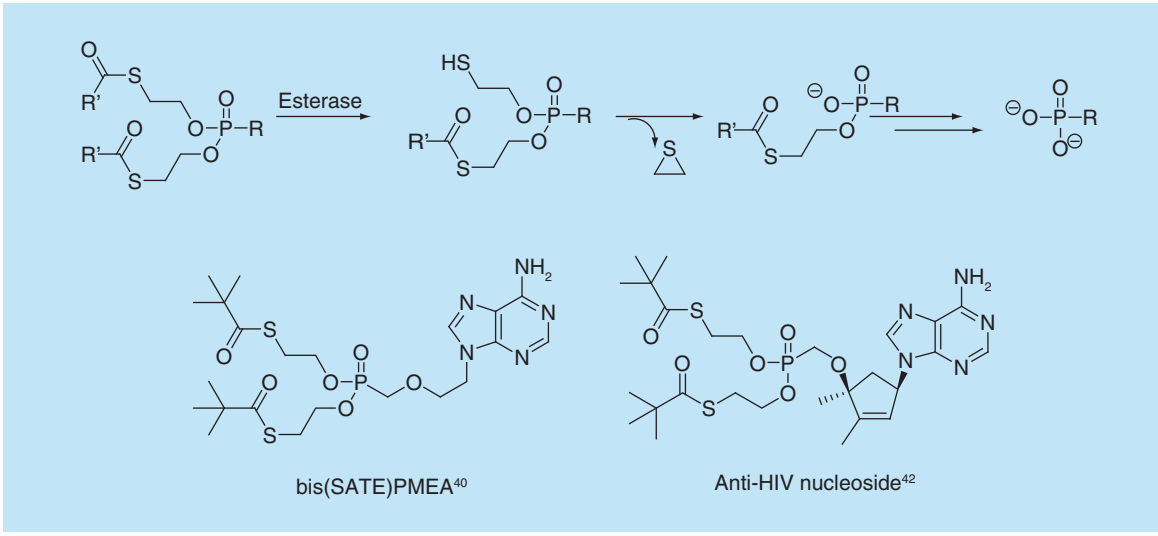

Figure 5. Activation and examples of S-acylthioethyl prodrugs.

disoproxil fumarate (TDF), the di-POC prodrug of 9-[2-(phosphonomethoxy)propyl]adenine (PMPA), is a second nucleotide reverse transcriptase inhibitor. Alone, it is used to treat chronic Hepatitis B. In combination with other antiretrovirals, TDF is used to treat HIV/AIDS [34]. Use of the disoproxil fumarate prodrug over the free phosphonate results in increased oral absorption and tissue permeability of the drug [35]. TDF may also be used in combination as pre-exposure prophylaxis to prevent acquiring HIV and has undergone multiple clinical trials to be used in vaginal rings for the same purpose [36,37]. Similarly, addition of the POC moiety to an inhibitor of glutamate carboxypeptidase II resulted in a product that is orally bioavailable, with a >20-fold increase in total exposure when compared with an equimolar dose of its parent compound [38]. The enzyme is believed to play a key role in some neurological disorders [38].

Compared with acyloxyalkyl prodrugs, such as POM and POC, *S*-acylthioethyl (SATE) ester prodrugs are relatively new, as they were first reported in 1993 (Figure 5) [39]. They have also been used as prodrugs of phosphonate compounds. They take advantage of a similar cleavage mechanism to that of the POM and POC moieties, engaging esterases found in the blood and other tissues. After cleaving the terminal thioester to the

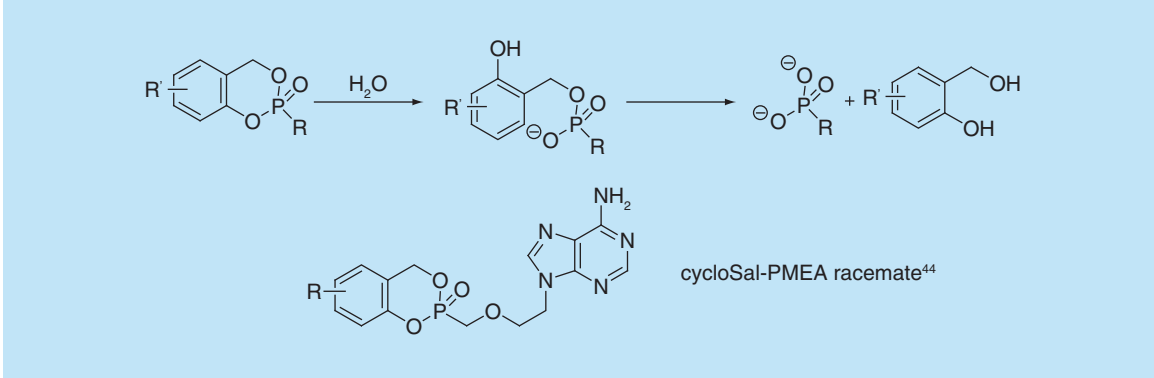

Figure 6. Activation and an example of a cycloSal prodrug.

thiol, the intermediate then decomposes to lose ethylene sulfide and liberate the mono-ester phosphonate [39]. The process repeats to release the active drug (Figure 5). An early example of the SATE moiety was its application to PMEA [40]. The authors found that the bis(tert-butyl-SATE)-PMEA analog retained similar activity to the bis(POM) compound in cells, but saw a half-life increase of over 50-fold in human serum, suggesting the bis(SATE) prodrug may have better *in vivo* efficacy [40]. Since that time, the SATE moiety has seen use in attempts to improve cell permeation of phosphonoformate and nucleosides [41,42]. Depending on the type of ester used in SATE prodrugs, toxicity concerns may arise. One such concern is the release of a thiol intermediate. However, it may be inferred through negative Ames and Comet assays of SATE phosphates that the released byproducts are not toxic [43]. As the bis(tert-butyl-SATE)-PMEA releases pivalic acid, the aforementioned concerns of carnitine depletion should be noted. Other esters, however, may generate less toxic byproducts and avoid the formaldehyde release seen in both POM and POC moieties.

Asymmetrical diesters

If the two esters of the phosphonate contain different substituents, an asymmetrical diester is formed. These phosphonate ester prodrugs have been employed with favorable results. In these compounds, the phosphorus atom becomes a center of chirality, potentially complicating synthetic routes. One strategy for making an asymmetrical ester is by initially masking the phosphonate using a salicyl alcohol (Figure 6). This strategy was first employed to improve cellular penetration of acylic nucleosides of phosphates, but later was used as a protection of PMEA [44]. Interestingly, the cycloSal analogs rely on aqueous hydrolysis as opposed to enzymatic cleavage [44], potentially enabling protected PMEA to permeate out of a cell before it is cleaved and reaches its target. The hydrolysis is pH dependent, and some analogs cleave more rapidly than others. The substitution pattern of the salicyl alcohol can influence the hydrolysis rate, allowing for fine-tuning of the prodrug's properties (Figure 6). Despite having less toxic byproducts, the cycloSal prodrugs of PMEA displayed lower activities than those of bis(POM)-PMEA, but a twofold increase over the PMEA phosphonic acid. While the phosphorus atom is a center of chirality, the cycloSal-PMEA enantiomers were not tested separately for activity, but as a racemic mixture, leaving room for future work to synthesize and test the enantiomers. It has been shown in other systems that different phosphorus enantiomers with cycloSal moieties demonstrate varying levels of activity, with reported three to 80-fold differences between enantiomers in cell-based assays [45].

A variation of the cycloSal prodrug concept utilizes DNA bases rather than a salicyl alcohol [46]. This modification results in prodrugs known as cyclic nucleoside phosphonates (CNPs, Figure 7). Cidofovir (((*S*)-1-(3-hydroxy-2-phosphonomethoxypropyl) cytosine, (*S*)-HPMPC), is a potent inhibitor of various dsDNA viruses and has been US FDA approved for the treatment of cytomegalovirus in patients with AIDS, though nephrotoxic [46]. Cyclic cidofovir (cHPMPC) is less cytotoxic when administered to rats, rabbits and monkeys orally [47], but, like unprotected cidofovir, lacks sufficient oral bioavailability. Where the CNPs struggle, however, is cell penetration due to the single free hydroxyl remaining negatively charged at physiological pH. Prodrugs of cHPMPC and other CNPs, such as a cyclic (*S*)-9-[3-hydroxy-(2-phosphonomethoxy)propyl]adenosine (cHPMPA), mask the hydroxyl group using a variety of protection strategies (Figure 7). These compounds demonstrate remarkable increases in activity and bioavailability over their parent phosphonates against a range of viral infections [48–52]. Peptidomimetics have

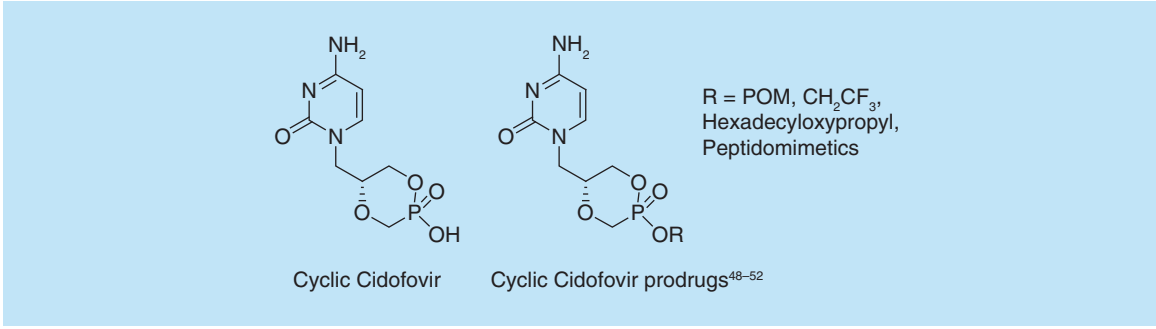

Figure 7. Cyclic cidofovir and prodrugs thereof.

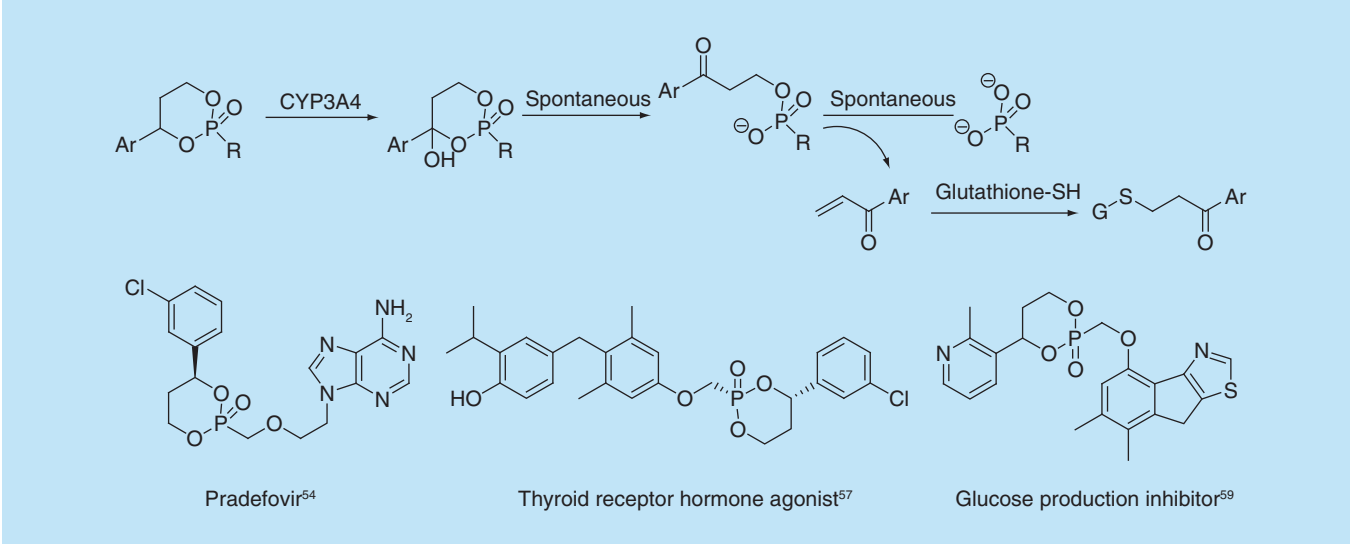

Figure 8. Activation and examples of HepDirect prodrugs.

shown promise, as they are more readily bioavailable over a single amino acid protecting group, up to eight-times higher than their parent phosphonate when administered to rats orally [48–50]. Other protection strategies have included POM groups, alkoxyalkyl lipid-like chains and alkyl esters, with activity depending on the length of chains for these features [51,52].

Another approach to developing asymmetric phosphonate ester prodrugs is the HepDirect strategy(Figure 8) [53]. By protecting the phosphonate with a chiral diol, the phosphorus atom is now also a center of chirality. However, unlike cycloSal prodrugs requiring aqueous cleavage or the aforementioned diesters that may be cleaved prior to cell penetration, HepDirect prodrugs are designed to be activated within hepatocytes in order to reach their intended target [53]. To achieve this goal, Erion *et al.* set out to synthesize molecules that would be activated by enzymes expressed in liver tissue, survive aqueous solution, blood and nonhepatic tissues, and release no toxic byproducts [53]. After oxidation of the benzylic position via a cytochrome P_{450} isozyme (CYP3A4), the resulting hemiketal spontaneously opens and liberates the active phosphonate and an enone. While a Michael acceptor may prove problematic, Erion *et al.* suggest that glutathione present in cells is conjugated to the enone, rather than releasing a potential toxic byproduct [53]. As with other diesters, the HepDirect strategy has been applied to PMEA and other phosphonates. Pradefovir, the HepDirect version of PMEA, was synthesized and subsequently found to demonstrate a 12-fold improvement in levels of PMEA in the liver/kidney over POM prodrug adefovir dipivoxil [54]. This is important to note, because adefovir dipivoxil is only approved for treatment at a suboptimal dose due to its renal toxicity [20]. Pradefovir, though, was put on hold for further clinical testing after increased tumor incidence was observed in animal studies [55], but has recently moved forward with safety studies in healthy human subjects [56]. The HepDirect prodrug strategy has also been employed for a thyroid receptor agonist in order

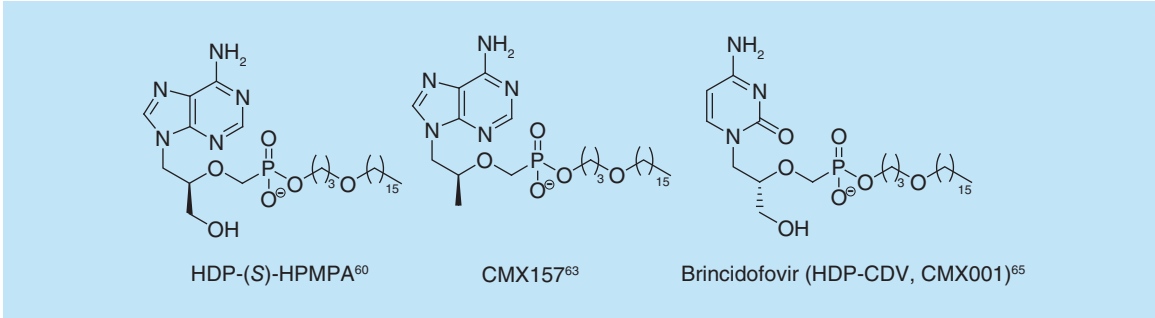

Figure 9. Examples of lipid conjugate prodrugs.

to lower cholesterol by increasing oral bioavailability in rats 40-fold [57,58] and as a means to overcome inhibition of CYP3A4 in a molecule targeting glucose production seen with the use of other pro-moieties [59].

Monoesters

As shown by several examples above, smaller lipophilic diesters mask both negative charges in a phosphonate, and are effective prodrugs. More recently, monoesters, masking only one anion of the phosphonate, have been reported [60]. These compounds, bearing long hydrocarbon chain, lipid-like substituents, have also shown to work well as prodrugs (Figure 9). Although first designed for use in nucleotide monophosphates, the strategy has also been applied to acyclic nucleoside phosphonates (ANPs). Hostetler *et al.* have synthesized several lipid-like nucleotide and nucleoside phosphonates in an attempt to increase efficacy against HIV [60]. Specifically, some of these lipid esters were significantly more active against their respective targets relative to the free phosphonate. In some cases, the activity improved by multiple log units. A hexadecyloxypropyl (HDP) prodrug of (*S*)-9-[3-hydroxy-(2-phosphonomethoxy)propyl]adenosine (HDP-(*S*)-HPMPA) displayed >10,000-fold increase in activity against HIV-1 [60], The same compound showed >5000-fold improvement in activity against the orf virus [61]. The HDP prodrug of cidofovir (HDP-CDV) showed >2000-fold improvement in activity against Epstein-Barr virus (Figure 9) [62]. These gains in activity are attributed to the increased intracellular concentrations of the drugs, thanks to the HDP lipid moiety.

HDP-tenofovir (CMX157) has also been synthesized, originally with the intent of treating HIV and Hepatitis B [63]. It has been tested in combination with each US FDA-approved antiviral used for the treatment of HIV and showed synergistic or additive behavior [64]. Since it was first reported, CMX157 has advanced through a Phase II clinical trial in which it was tested for tolerated dosing in Hepatitis B patients. Because CMX157 is cleaved intracellularly by phospholipase C and/or sphingomyelinase, the nephrotoxicity seen by other tenofovir prodrugs cleaved in plasma may be decreased, and with the aforementioned additive or synergistic behavior, CMX157 may be an intelligent choice to pair with currently approved antivirals to move forward through Phase III studies.

Brincidofovir (CMX001) [65] is an HDP prodrug of cidofovir. CMX001 has excellent activity against a range of viruses including: poxvirus [66–69], cytomegalovirus [70,71] and herpesvirus [62,72], and has been reviewed for its treatment of varying dsDNA viruses [73,74]. Brincidofovir has and is currently undergoing clinical trials in the treatment of adenovirus in pediatric stem cell transplant recipients [75–77]. Krečmerová *et al.* have synthesized varying alkyloxyalkyl monoester prodrugs that have shown activity against dsDNA viruses, as well, demonstrating potency up to 600-fold higher than that of the parent phosphonate [52]. In attempt to synthesize reduction sensitive lipid prodrugs of tenofovir, monoester disulfide conjugates have been synthesized [78,79]. Multiple linking strategies connecting the phosphonate and disulfide moieties were studied, with a butyl linker improving both the measured activity and therapeutic index of the disulfide prodrug tenfold over TDF when tested against HIV-1, though further studies may be needed to elucidate why the disulfide bond may be advantageous over the alkoxyalkyl lipids.

Phosphonate amidate prodrugs

Phosphonates masked with amidate moieties are a newer concept in the field of phosphonate prodrugs, as the first reported phosphoramidate was reported in 1990 [80]. However, amidates have advantages compared with their ester prodrug cousins, as the prodrug moieties for these compounds are commonly amino acids, generating less problematic byproducts than some of the aforementioned esters. Since 1990, significant advances have been made

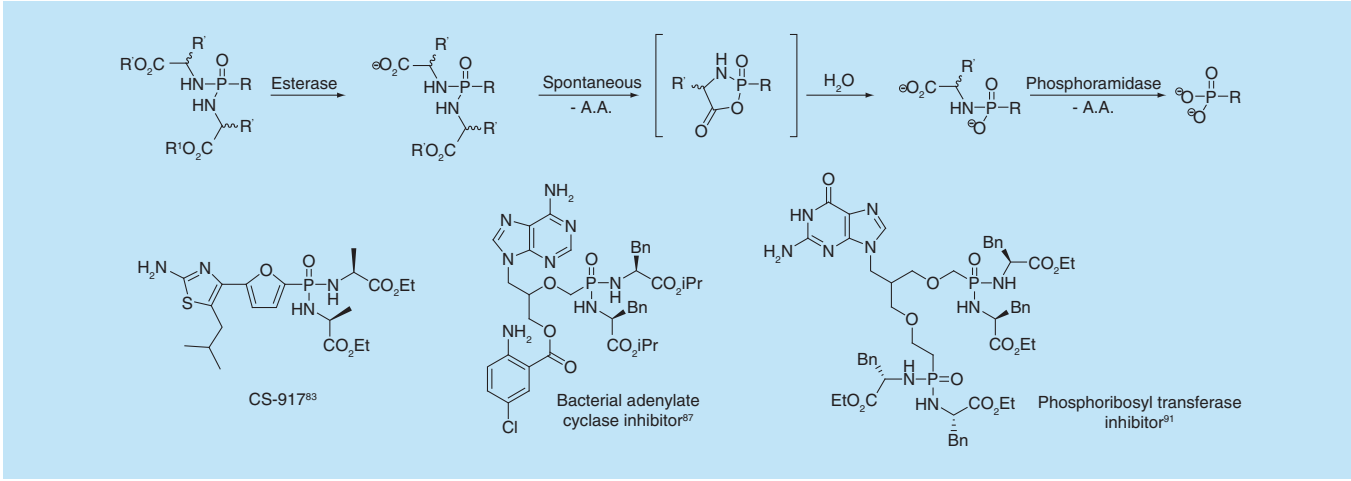

Figure 10. Activation and examples of bisamidate prodrugs.
AA: Amino acid.

in phosphor- and phosphon-amidate analogs, and it is important to note the only US FDA-approved phosphonate prodrug since 2007, tenofovir alafenamide, includes an amidate moiety [1,81].

Symmetrical bisamidates

Although more examples of bisamidates have been reported to protect phosphates, this substituent family has found use with respect to phosphonates. Like phosphonate diesters, the simplest amidate prodrugs would be symmetrical in order to avoid introduction of a stereogenic phosphorus. However, because protection by amino acids still allows for a double negative charge, the amino acids are typically esterified to remain neutral. Due to this esterification, activation of the prodrug is a multistep process. An esterase first cleaves a carboxyester. The free carboxylate cyclizes with the phosphonate, liberating the remaining amino acid ester (Figure 10). Water opens the intermediate ring, allowing a phosphoramidase to cleave the final amino acid from the phosphonate [7]. While the cyclic intermediates are unstable, they have recently been characterized for the first time [82].

One bisamidate to advance to clinical trials is CS-917, a bis-alanine ethyl ester-protected phosphonate, which is an inhibitor of fructose 1,6-bisphosphatase [83]. In treating Type 2 diabetes, CS-917 was shown to have a tenfold higher bioavailability than the free phosphonic acid [84]. The compound was ultimately withdrawn when it failed to significantly reduce long-term glucose exposure [85].

In recent years, phosphonodiamidates have received more attention with respect to acyclic nucleosides. They have been used to target *Bordetella pertussis* adenylate cyclase toxin (ACT) [86]. In targeting ACT, multiple PMEA bisamidates or PMEA analog bisamidates were prepared [86–88]. Despite showing weaker activity than bis(POM)PMEA in cell-based assays, the bisamidates were less cytotoxic and more stable, allowing room for further optimization and developing a better overall profile than bis(POM)PMEA. Bisamidates have also been used to target phosphoribosyltransferases in *Mycobacterium tuberculosis* and *P. falciparum* [89,90]. Through synthesizing various bisamidate purine base ANPs, it was found the prodrugs have low cytotoxicity and improved permeability over the free phosphonic acid analogs, furthering their potential as antimalarials [91,92]. Like bisamidates, monoamidates induce no stereocenter at the phosphorus atom through the delocalization of the negative charge. However, monoamidates have received little attention as prodrugs of phosphates and even less with respect to phosphonates, leaving room for exploration in this area.

Mixed amidate/esters

While bisamidates as prodrugs for phosphonates have received considerable attention, mixed amidate/ester prodrugs of phosphonates have received much more (Figure 11). Like asymmetrical diester prodrugs, the mixed substitution pattern introduces a stereogenic center at the phosphorus atom. Also like other phosphonate prodrugs, this strategy was first applied to phosphates to overcome poor cellular uptake, poor conversion to the active component or to release less toxic byproducts [80]. In 2001, the first mixed amidate/ester prodrugs of PMEA and PMPA were reported [93]. It was found the L-alanine methyl ester paired with a phenoxy moiety worked best in combination,

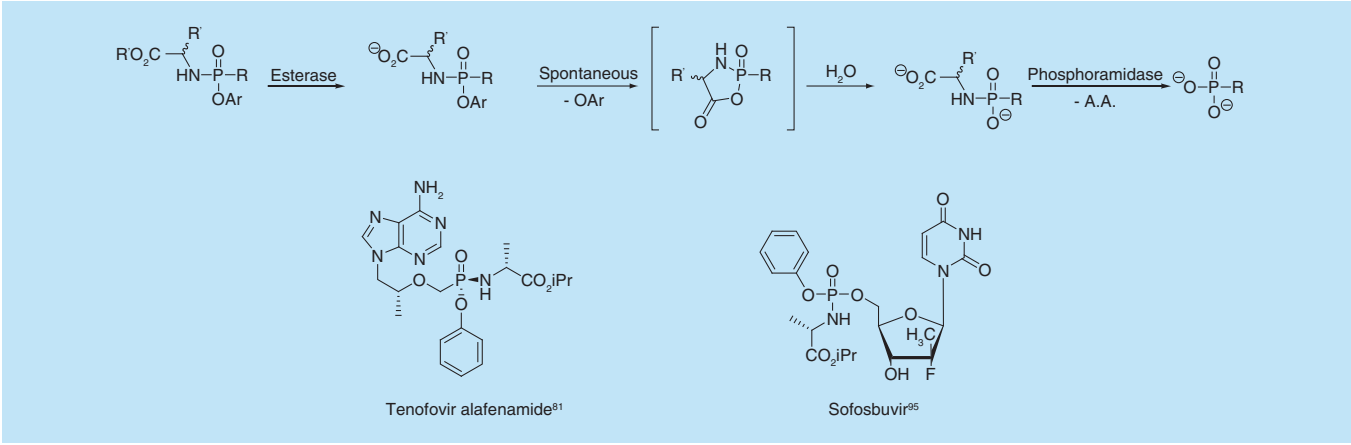

Figure 11. Activation and examples of ProTides.

while the D-alanine methyl ester resulted in extremely reduced activity. Since this discovery, aryloxy phosphonamidate prodrugs have come to be known as the 'ProTide' (pro-nucleotide) [94] technology and have resulted in two US FDA-approved drugs: tenofovir alafenamide for the treatment of HIV (ProTide of PMPA) [81] and sofosbuvir for the treatment of Hepatitis C (Figure 11) [95]. The ProTide technology has been further applied to other areas of interest with favorable results in phosphates, likely serving as precursors to phosphonate analogs [96–105]. The technology has also been extensively reviewed in recent years and is fast becoming a favored approach for nucleotide prodrugs [1,9,106–112].

Current & future applications

Whereas phosphate prodrugs have been explored for decades, phosphonates have received increased attention only more recently. Phosphonate prodrug analogs have been prepared to potentially overcome a pharmacokinetic barrier their parent phosphoric or phosphonic acids could not. Innovation of the ProTide technology has expanded the field of phosphonate prodrugs with relation to target and lowered toxicity found with other moieties. What follows is an update on two phosphonate prodrug strategies: as inhibitors of isoprenoid biosynthesis and newer compounds as antiviral agents.

Isoprenoid biosynthesis

Isoprenoids are a class of molecules derived from two five-carbon building blocks, isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) [113]. Thousands of isoprenoids have been identified to date, of which some notable examples are chlorophyll, carotenoids and ubiquinones [114]. Isoprenoids are vital for biological function of an organism; however, humans and certain types of bacteria and protozoa biosynthetically obtain IPP and DMAPP differently. Humans obtain isoprenoids via the mevalonate pathway (MVA) while Gram-negative bacteria and some protozoa utilize the 2C-methyl-D-erythritol 4-phosphate (MEP) pathway.

Because some bacteria obtain isoprenoids in a different manner than humans, the enzymes involved in the MEP pathway serve as attractive targets for inhibition. Two natural product phosphonates, fosmidomycin and its acetyl analog FR900098, have demonstrated inhibition of the MEP pathway. Because both fosmidomycin and FR900098 are free phosphonic acids, prodrugs of each have been synthesized in attempt to increase the oral bioavailability of the active moiety. We and others have performed SAR optimization and synthesized prodrugs of analogs to target *M. tuberculosis* and *P. falciparum* [23,115–123]. An α,β-unsaturated bis(POM) analog of fosmidomycin displays potent *in vivo* activity against *P. falciparum* (Figure 12) [124]. Other prodrugs have been explored in relation to *M. tuberculosis* and Gram-negative bacteria with varying results, suggesting the use of prodrugs may be organism dependent [125].

While the MEP is an attractive target for infective pathogens, the MVA may be targeted with regards to human diseases. Geranylgeranyl diphosphate synthase and farnesyl diphosphate synthase (FDPS) are attractive targets, as they are essential for growth and metastasis of multiple cancers [126]. Nitrogenous bisphosphonates, structural analogs of natural diphosphates, are inhibitors of FDPS. However, in order to effectively permeate

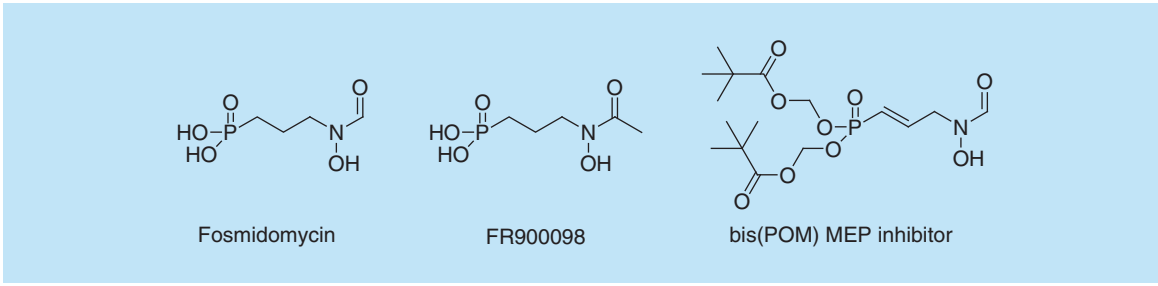

Figure 12. Inhibitors of the 2C-methyl-D-erythritol 4-phosphate pathway.

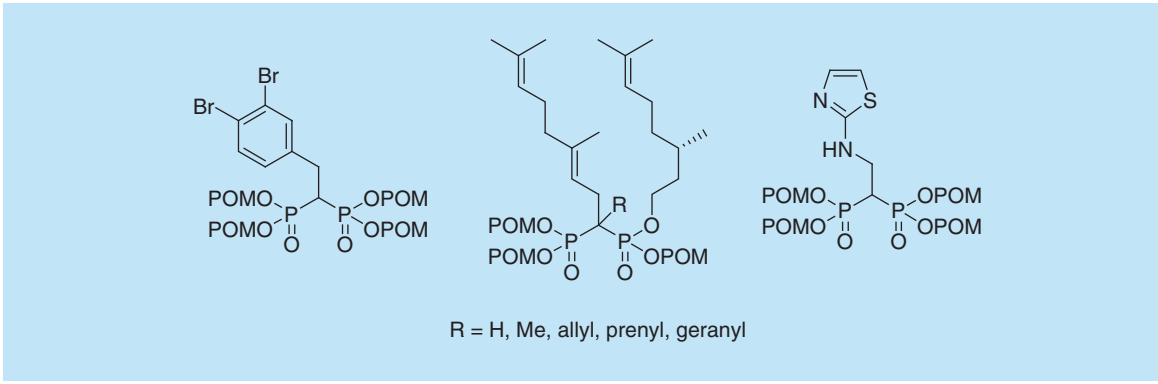

Figure 13. Anticancer inhibitors of the mevalonate pathway.

cells, the negative charge of both phosphonate moieties needs to be masked to some degree. Simple alkyl and aryl esters having been tested [127], but the more labile POM prodrugs have yielded better activity over the free nitrogenous or non-nitrogenous bisphosphonate (Figure 13) [128–130]. While the increase in activity varied between compounds and their respective prodrugs, in general, the trend displayed an increase when the charge was masked, displaying the anticancer potential these compounds exhibit. Efforts have continued in optimizing the substituents of the α-carbon, as well. In myeloma cells, bisphosphonates with isoprenoid substituents at the α-carbon and as one of the phosphonate esters have been synthesized as tris(POM) prodrugs, demonstrating submicromolar activity [131]. As the prodrugs had been tested as diastereomeric mixtures, further work in exploring which isomer(s) may be responsible for activity are ongoing. Bisphosphonates have also received attention in the stimulation of Vγ2Vδ2 T cells and inhibiting tumor cell growth [28]. Through inhibition of FDPS, IPP levels will increase and subsequently stimulate Vγ2Vδ2 T cells through the Vγ2Vδ2 T cell receptors. Tetrakis-pivaloyloxymethyl 2-(thiazole-2-ylamino)ethylidene-1,1-bisphosphonate demonstrated remarkable abilities to stimulate the Vγ2Vδ2 T cells from 81- to 1900-fold more than its free acid analog across 21 tumor cell lines, signifying the potential use of bisphosphonate prodrugs as aids in cancer immunotherapy. Further, this compound stimulates Vγ2Vδ2 T cells more effectively compared with zoledronic acid, the most commonly used stimulant in clinical trials [132].

Nucleotides & nucleosides

As mentioned previously, concerns for toxicity of metabolic byproducts of a POM or POC prodrug beg for investment into other moieties. Adefovir dipivoxil and TDF are both still in use today, along with multiple groups exploring options to both increase activity and reduce toxicity of their metabolic byproducts by exploring the use of other prodrug moieties. In fact, tenofovir alafenamide, which was approved by the US FDA in 2015 for the treatment of HIV-1, is a testament to the advances of ProTide technology [81,133]. Further clinical trials have been performed with tenofovir alafenamide in attempt to replace TDF, mostly due to the toxicity concerns of the byproducts of long-term use of TDF, specifically in bone and renal tissue [134–137]. In fact, a Phase IIIb study found no inferiority in regards to tenofovir alafenamide replacing TDF in a regimen with rilpivirine and emtricitabine in virally suppressed adults [138]. Tenofovir alafenamide also has shown lower mitochondrial toxicity in T cells when

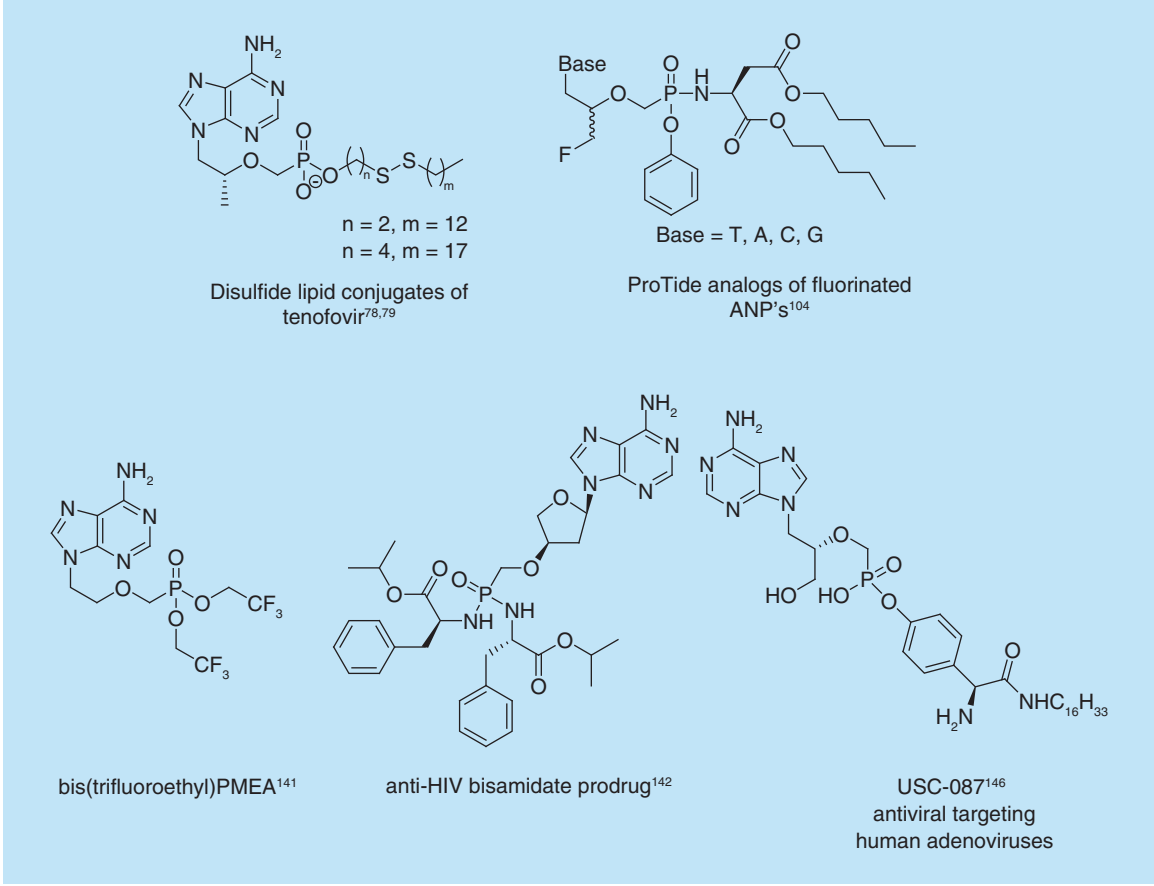

Figure 14. Recent examples of nucleoside prodrugs.

compared with TDF [139]. Because of the toxicity associated with long-term use of TDF, less toxic substitutes are continuously being explored. Mono-protected disulfide lipid conjugates of tenofovir have also shown promise in treating HIV-1 (Figure 14) [78,79]. Other ProTide analogs of TDF and adefovir dipivoxil have also demonstrated better inhibition of HIV and Hepatitis B [99,104]. Despite the demonstration of other prodrugs for tenofovir demonstrating lower toxicity, TDF has still seen further interest in clinical trials and has been tested in vaginal rings as a form of pre-exposure prophylaxis, displaying concentrations higher than needed to inhibit HIV [37]. So, while long-term use of TDF may prove toxic through ingestion, pre-exposure prophylaxis through other means may provide for less systemic side effects.

Though approved by the US FDA for the treatment of Hepatitis B, adefovir dipivoxil suffers from a similar detriment as TDF – the potentially toxic metabolic byproducts. Not only this, but the majority of orally administered adefovir is hydrolyzed to free PMEA in the gastrointestinal tract and is found in the kidneys rather than in circulation, resulting in nephrotoxicity [140]. Various prodrug moieties have been used to mask PMEA in an effort to increase transport to the liver as opposed to hydrolysis while traversing the gastrointestinal tract. A bis(2,2,2-trifluoroethyl)PMEA analog was demonstrated to be at least fourfold more stable in rat plasma in comparison with adefovir dipivoxil (Figure 14), suggesting protecting groups aimed to survive the gastrointestinal tract and deliver to the liver warrant more attention [141].

Recent antiviral advances indicate that phosphonate nucleoside prodrugs show promise outside the realm of HIV/Hepatitis B. Bisamidate L-α-2′-deoxythreosyl prodrugs display a 200-fold increase in potency over their phosphonic acid parent molecule against both HIV-1 and HIV-2 (Figure 14) [142]. Prodrugs of ANPs with purine bases have demonstrated potent inhibition of phosphoribosyltransferases in malaria and tuberculosis [89,90,92,143], with recent advances demonstrating excellent selectivity for the parasitic enzyme over the human homolog [144]. Advances have also been seen with regards to phosphonate prodrugs used in treating DNA viruses, such as Epstein-Barr virus, Herpes virus and cytomegalovirus [52,145]. An *N*-alkyl tyrosinamide phosphonate ester prodrug has

even been shown *in vivo* to protect against human adenoviruses, which otherwise have no FDA-approved drug specific for this activity (Figure 14) [146]. Further research into replacing phosphate prodrugs with phosphonates is warranted [1].

Future perspective

Over the last decade, prodrugs have accounted for more than 10% of newly approved chemical entities from the US FDA. This demonstrates the need to consider the use of a prodrug prior to clinical evaluation, especially in the case of the traditionally problematic phosphonic acid, in order to overcome the double negative charge at physiological pH. As time has progressed, it has now become almost commonplace to test prodrugs of phosphonates prior to clinical evaluation, due to most prodrugs seeing better activity and availability over their parent acid. This does not come without limits, however. The choice of the pro-moiety is important, both because of where the drug is intended to release and what metabolic byproducts are freed from the protecting group. Despite toxicity concerns from some types of prodrugs, they still see use today, both clinically and at the bench. With the innovative ProTide technology and discovery of other nontoxic or less toxic pro-moieties, it would be wise to delve into testing safer metabolite based pro-moieties on previously potent or clinically approved compounds, as has been seen for TDF. Further, it is important to note the mixed amidate/ester approach has received little attention in phosphonates outside nucleotides and may warrant more focus in this arena, especially due to the success seen with tenofovir alafenamide. With the growing interest in phosphonate analogs of phosphates and concerns for developing safer metabolic byproducts, coupled with the increasing amount of prodrugs in clinical trials and receiving US FDA approval, the potential uses for phosphonate prodrugs will surely remain of interest for years to come.

Executive summary

- Phosphonates, at biological pH, exist as a dianion and have trouble penetrating into cells.
- There are many protection strategies and subsequent activation mechanisms that have been developed to deliver phosphonate compounds to their target.
- Not all protection strategies work in every situation, so a variety of acyloxyalkyl, amidate and mixed ester/amidate strategies have been developed.
- The ProTide technology is a more recent development and has resulted in the only US FDA-approved phosphonate prodrug in the past 10 years.

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