Purpose of review
This review focuses on the chemical and pharmacological rationale behind the development of nucleoside antiviral prodrugs (NAPs).

Recent findings
Highly efficacious NAPs have been developed that extend and improve the quality of lives of individuals infected with HIV and hepatitis B virus (HBV), herpes viruses, and adenovirus infection in immunocompromised individuals. A very high rate of hepatitis C virus (HCV) cure is now possible using NAPs combined with other direct acting antiviral agents (DAAs).

Summary
Prodrug strategies can address the issues of poor oral bioavailability and delivery of active metabolites to the targeted cells. Additionally, NAPs demonstrate potential for improving deficiencies in oral absorption, metabolism, tissue distribution, phosphorylation, and overall potency, in addition to diminishing potential for in-vivo selection of resistant viruses. NAPs continue to be the backbone for the treatment of HIV and HBV, herpesviruses, and adenovirus infections because their active forms are potent, have long intracellular half-lives and are relatively safe with high barrier to resistance.

Keywords
adenovirus, antiviral agents, hepatitis B virus, hepatitis C virus, HIV, herpes simplex virus, nucleoside antiviral agents, prodrugs

INTRODUCTION
Nucleoside antiviral inhibitors (NAIs) have been developed to treat herpes simplex virus (HSV), HIV-1, hepatitis B virus (HBV), and hepatitis C virus (HCV) [1–6]. NAIs are phosphorylated by various cellular kinases to their respective NAI-triphosphate (NAI-TP) forms. NAI-TP compete with the natural nucleotide triphosphates (NTPs) for incorporation by the viral polymerases (V-pol) of HIV-1 (reverse transcriptase), HBV (DNA pol), and HCV (RNA dependent pol, RdRp, NS5B). NAI-TP serve as obligatory chain terminators when they lack a 3′-hydroxyl group on the sugar moiety and as nonobligatory chain terminators when the sugar 3′-hydroxy group is ineffective at chain elongation. In-vivo viral dynamic profiles of NAI depend on their pharmacokinetics, cellular accumulation, and potencies of NAI-TP versus V-pol, and the point of antiviral activity in the viral replication cycle. For example, NAI-TP act primarily on HIV-1 reverse transcriptase prior to integration of HIV-1 into host cell DNA. Once reverse transcription is complete and viral DNA has integrated into host cell DNA, the NAI-TP has no further effect on HIV-1 replication in that cell. The net effect is to block new infections of CD4+ cells, so that the maximal decline rate in plasma HIV-1 reflects the death rate of infected CD4+ lymphocytes [7,8]. In contrast, HBV and HCV do not have preintegration reverse transcriptase steps, and NAI-TP directly inhibit the viral replication in infected cells. Consequently, HBV and HCV plasma decay rates directly reflect the degree of inhibition of the targeted V-pol by the NAI-TP and the subsequent clearance of virus from...
plasma [9,10]. The therapeutic index of NAIIs depends on the cellular accumulation of the NTP, the intracellular half-life, and the relative affinities of the NAI-TP for the V-pol versus host nuclear and/or mitochondrial DNA or RNA-pol [11]. The initial phosphorylation step for NAI may be rate limiting and bypassed by administering a modified NAI containing a masked monophosphate such as a phosphoramidate moiety. An esterase-driven intracellular unmasking to the monophosphate and subsequent phosphorylation by intracellular kinases provides the active NAI-TP form [12]. For phosphonates, phosphonylalkyl esters are widely utilized to mask the polar nature of the NAI to improve the overall pharmacokinetics of phosphonate delivery and subsequent intercellular kinase-driven active NAI-diphosphate formation. This approach was used with the acyclic phosphonate NAI, tenofovir disoproxil fumarate (TDF), as tenofovir-diphosphate has potent activity versus HIV-1 reverse transcriptase and HBV-pol [13]. Tenofovir (TFV) has been used as a ‘backbone’ structure for a variety of prodrugs including TDF (as the disoproxil fumarate), alafenamide fumarate (TAF), and hexadecyloxy-TFV (CMX157) described in this review. Structures and trade names of major compounds in this review are summarized in Figs. 1–4.

**KEY POINTS**

- NAPs overcome deficiencies in oral absorption, tissue distribution, phosphorylation, and cellular accumulation.
- Phosphoramidate containing NAPs overcome deficiencies in rate-limiting monophosphorylation.
- Increased delivery of active nucleoside triphosphate using NAP minimizes the potential for resistant virus selection.
- NAPs extend and improved the quality of life of people infected with HIV and HBV, and are components for potentially curative regimens for HCV.

**Modulating oral absorption and tissue distribution of nucleoside antiviral inhibitors using prodrugs**

Most NAIIs are hydrophylic (polar) and passively diffuse poorly through lipid bilayers, including the gastrointestinal tract. Many NAIIs are actively transported by one or more of the proteins from the solute-membrane-carrier superfamily of the intestine and target cells [14]. However, acyclic NAIIs (e.g., acyclovir, penciclovir, and TFV) are poor substrates for the various gastrointestinal transporters and have limited oral bioavailability, hence the need for prodrug approaches for improving their bioavailability. Various approaches have been explored for improving the intestinal absorption of NAI, including the addition of amino-esters, and less commonly phospholipid moieties onto phosphonate NAI. Other experimental approaches for targeted delivery of NAI include nanoparticle formulations [15].

**FIGURE 1.** Chemical structures of compounds for herpesviruses: valaciclovir ([S]-2-[(2-amino-6-oxo-6,9-dihydro-3H-purin-9-yl) methoxy]ethyl-2-amino-3-methylbutanoate, Trade names: Valtrex/Zelitrex; Glaxo Smith-Kline, and available as a generic; Prodrug: acyclovir. Famciclovir [2-[(acetyloxy)methyl]-4-(2-amino-9H-purin-9-yl)butyl acetate], Trade name: Famvir, Novartis, generics by TEVA Pharmaceuticals and Mylan Pharmaceuticals), prodrug: penciclovir.
Treatment optimisation

to change in log_{10} viral load ($r = -0.74; P = 0.03$). Persons with a viral response survived longer (median 196 versus 54.5 days; $P = 5.04$). No serious adverse events were attributed to CMX001. In a phase 2 study of CMX001 as pre-emptive therapy for ADV infection, in allogeneic hematopoietic cell transplant recipients, a 100 mg twice per week dose demonstrated decreased levels of ADV viremia and showed a potential benefit in reducing both progression to ADV disease and all-cause mortality, compared with participants who received placebo or CMX001 given once per week. Planned intent-to-treat analyses and exploratory analyses in specific patient groups consistently favored this regimen over placebo, although statistical significance was not established in this exploratory study (http://finance.yahoo.com/news/chimerix-announces-topline-data-100000101.html).

Sofosbuvir (Gilead Sciences) and mericitabine (Roche Laboratories, Nutley, New Jersey, USA) are produgs of NAI with origins connecting back to PSI-6130 ($\beta$-$\delta$-deoxy-$\gamma$-fluoro-$\beta$-C-methyl-cytidine; Fig. 3). PSI-6130 is a NAI discovered by Pharmasset Inc. (now Gilead Sciences), which demonstrates potent anti-HCV activity using a replicon assay [59]. Mericitabine is an ester prodrug of PSI-6130 prodrug, licensed to Roche Laboratories, which is undergoing phase 2b testing [60]. Mechanistic studies indicated that PSI-6130 is phosphorylated to the monophosphate, diphosphate, and triphosphate forms when incubated with hepatocytes [35,36,61]. Also, PSI-6130-TP was found to be a potent inhibitor of HCV NS5B via chain termination [61]. However, the uridine derivative of PSI-6130, PSI-6206 was inactive against HCV, as it was not phosphorylated to PSI-6206-MP (PSI-7411) by cellular nucleoside kinases [35,36]. Consequently, a phosphoramide derivative, PSI-6206 was synthesized to bypass the initial phosphorylation step, which yielded PSI-7581, comprising a mixture of two active diastereoisomers, PSI-7976 ($R_{p}$) and PSI-7977 ($S_{p}$). Sofosbuvir, the more potent and more stable $S_{p}$ isomer, is undergoing phase 3 clinical testing for the treatment of HCV in combination with other antiviral agents [62–65]. The cellular metabolism of sofosbuvir is complex, and has been studied in detail [34]. Briefly, the carboxyl ester of PSI-7581 is hydrolyzed stereo-selectively by human CatA and CES1 in hepatocytes. PSI-7977 is a better substrate for CatA and CES1, which ultimately provides a higher concentration of the $5'$-MP (PSI-7411), which is consistent with its more potent activity versus HCV replication. This enzymatic deesterification is followed by a putative nucleophilic attack on the phosphorus by the carboxyl group releasing a molecule of phenol, and the alaninyl phosphate metabolite, PSI-352707, common to both diastereomers. Removal of the amino acid moiety of PSI-352707 is then catalyzed by histidine triad nucleotide-binding protein 1 (Hint1), yielding the $5'$-MP nucleoside PSI-7411, which is phosphorylated to the diphosphate, PSI-7410, and to the active NAI-TP (PSI-7409), by UMP-CMP kinase and nucleoside diphosphate kinase, respectively.

CONCLUSION

Despite demonstrating potency and limited toxicity in vitro, many NAIs are not suitable for clinical development due to pharmacokinetic (absorption, tissue accumulation, metabolism, and elimination) deficiencies. NAP is a well-established approach for altering the physicochemical and hence the pharmacokinetics and phosphorylation of these NAIs. The choice of an appropriate NAP moiety also has an effect on efficacy and safety, by enhancing distribution into virus susceptible tissues (e.g., hepatocytes for HBV/HCV, or lymphatic tissue for HIV), while shielding tissues associated with side-effects (e.g., TAF, but not TDF shields renal tubules from TFV). In addition, some NAIs can be dosed less frequently such as hexadecyloxypropyl tenofovir, which could improve adherence for HIV (or HBV)-infected persons. The chemistry used in the development of NAP continues to become more sophisticated, and has progressed from the use of simple acetates to amino acid esters to phosphoramidates and lipophilic hexadecyloxy. The application of NAP technology has been wide and impactful, and has made it possible to achieve high cure rates for HCV infections, for example, sofosbuvir when combined with other direct acting antiviral agents. Likewise, CMX001 may be a promising therapeutic option for the treatment of severe ADV disease in immunocompromised patients. Although, to date, there is no effective cure for HIV and HBV infections, progression to symptomatic disease can be halted or delayed in most infected persons by using...
a combination of agents targeting the various V-pol involved in viral replication. The field of nucleoside chemistry and biology continues to produce numerous highly effective NAls, with improved oral bioavailability and drug delivery to infected tissues, for the treatment of HIV, HBV, and HCV. These drugs have prolonged the lives of millions of infected persons. The ultimate goal in antiviral research is to find affordable cures for chronic infections that produce significant reductions in global morbidity and mortality.

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Conflicts of interest
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REFERENCES AND RECOMMENDED READING
Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
•• of outstanding interest

12. Nal penetration into HIV-1 reservoirs is discussed in this review.
31. Trost LC, Lanier ER, Lampert B, Painter GR. Preclinical evaluation of CMX157: a lipid-conjugated nucleotide analog for the treatment of HIV. 49th Meeting of the Society for Toxicology (SOT), 2010; Abstract # 1057:
32. Salt Lake City, UT, USA.
34. Tippin TK, Lampert BM, Painter GR, Lanier ER. Lipid conjugates of cidovudine and tenofovir are not substrates of human organic ion transporters hOAT1 and hOAT3. World Congress and American Association of Pharmaceutical Scientists, 2010; #33996; New Orleans, LA, USA.
37. Findings led to the discovery of PSI’6206, which later was converted to sofosbuvir.