diverse types of prodrugs was carried out including alkoxyalkyl (hexadecyloxypropyl, octadecyloxyethyl, hexadecyloxyethyl), pivaloyloxymethyl (POM), 2,2,2-(trifluoro)ethyl,2-butylsalicylyl esters as well as peptide-conjugated phosphonates. New synthetic procedures including the utilization of hexafluorophosphate coupling agents for esterification of the phosphonate function were developed. All HPMPDAP and cHPMDAP prodrugs were synthesized as phosphonate monoesters. A detailed anti-poxvirus and other antiviral testing as well as comparison of properties of single types of prodrugs was carried out. Alkoxyalkyl esters emerged as the most potent anti-poxvirus prodrugs of HPMPDAP and its cyclic form.

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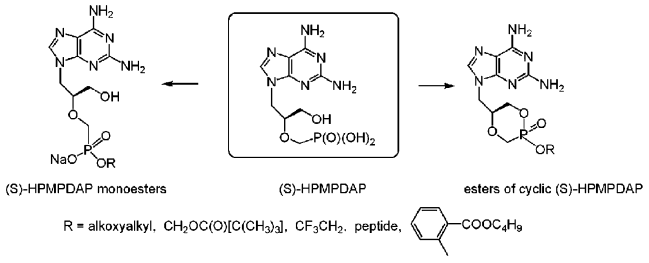

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New Nucleoside and *bis*-Nucleoside-Phosphonate Conjugates: Design, Stability, and Anti-HIV Evaluation

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Design of depot forms of anti-HIV drugs are widely used to reduce toxicity, improve drug pharmacokinetics, and overcome drug resistance. The obvious design rational for depot forms is that the conjugate will be converted by hydrolysis or enzyme action to active agents upon penetration into cells. Herein, we report the design, stability, and anti-HIV properties of phosphonate derivatives containing AZT, 3TC, and *bis*-nucleosides composed of mono- and heteronucleoside analogs with general structure Nu–O–P(O)(R)–O–Nu (Nu = AZT or 3TC). Among phosphonate depot forms of AZT or 3TC, the most perspective were their aminocarbonyl derivatives. They were stable in blood serum, displayed good anti-HIV activity and low toxicity in cell culture, improved pharmacokinetics, lower acute toxicity, and absence of cumulative effect if compared to that of AZT or 3TC, respectively. The stability in blood serum, anti-HIV activity and toxicity of phosphonate derivatives of *bis*-nucleosides were dependent on the structure of phosphonate moiety. Stability of morpholinecarbonyl-*bis*-AZT in blood serum was 30 min, at the same time for heterodimer ($R = ClCH_2$, Nu = AZT, 3TC) and homodimer ($R = CH_3–(CH_2)_5–NHC(O)$, Nu = AZT) was $\gg$6 h. The majority of compounds were less potent than parent nucleosides but their toxicity (CD_{50}) was considerably lower than those of the appropriate nucleoside. Therefore, a higher CD_{50} allowed better selectivity indexes (SI). Pharmacokinetic parameters of some phosphonate derivatives of nucleoside analogs and that of *bis*-nucleosides will be reported.

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RNA Polymerase Fidelity Variants of the Picornaviruses Uncover A Novel, Indirect RNA Mutagenic Activity for Amiloride Compounds

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Our laboratory studies the effects of RNA mutagens, and base analogs, ribavirin, 5-azacytidine and 5-fluorouracil on picornaviruses (poliovirus, Coxsackie B virus). In a screen to isolate RNA mutagen resistant variants of Coxsackie B3 virus, we identified a mutant presenting an A372V change in the viral RNA dependent RNA polymerase that conferred resistance to all 3 base analogs. This resistance was found to result from an increased polymerase fidelity, similar to our recently published work on ribavirin-resistant polioviruses that mapped to a different region of the polymerase (Vignuzzi et al., 2008). Interestingly, the A372V mutant had been previously isolated in a screen for resistance to amiloride compounds (inhibitors of Na^+ ion channels and the Na^+/H^+ exchanger), along with another polymerase mutant, S299T (Harrison et al., 2008). Since the same mutation would not expectedly confer resistance to two different antiviral mechanisms, we hypothesized that amiloride compounds had a previously unknown mutagenic activity. Indeed, we find that amiloride treatment of both Coxsackie virus and poliovirus increases their mutation frequencies. Furthermore, we show that higher fidelity variants of both viruses, presenting lower basal mutation frequencies, are more resistant to the RNA mutagenic effects of these compounds. Our results suggest that in addition to being replication inhibitors (as observed by other groups), amiloride compounds are the first described, non-nucleoside, indirect RNA mutagens. We are currently determining whether this mutagenic activity is the result of a direct interaction with the polymerase, or the result of intracellular alterations stemming from the inhibition of ion channels. Our data raises the question of whether this mutagenic activity is strong enough to act as an antiviral (through lethal mutagenesis) or whether it promotes viral evolution (through moderate mutagenesis). Implications for future drug development will be discussed.

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Overlap in Virus Specificity Leads to the Discovery of Small Molecules Active Against Rabies Virus, Cytomegalovirus, and Monkey Pox Virus

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The Prosetta Platform for cell-free protein synthesis (CFPS) and assembly of viral capsid-like structures has been used successfully to identify novel compounds with antiviral activity (see other