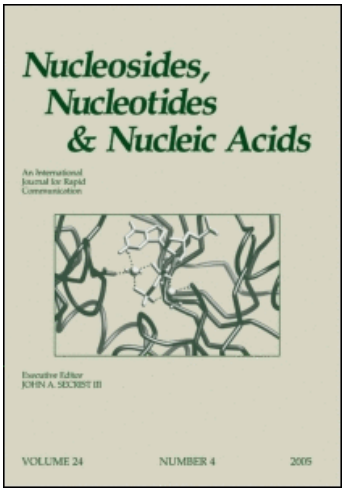

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Synthesis And Anti-Hiv Activity Of Cyclic Pyrimidine Phosphonomethoxy Nucleosides And Their Prodrugs: A Comparison Of Phosphonates And Corresponding Nucleosides

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SYNTHESIS AND ANTI-HIV ACTIVITY OF CYCLIC PYRIMIDINE PHOSPHONOMETHOXY NUCLEOSIDES AND THEIR PRODRUGS: A COMPARISON OF PHOSPHONATES AND CORRESPONDING NUCLEOSIDES

Richard L. Mackman, Lijun Zhang, Vidya Prasad, Constantine G. Boojamra, James Chen, Janet Douglas, Deborah Grant, Genevieve Laflamme, Hon Hui, Choung U. Kim, Jay Parrish, Antitsa D. Stoycheva, Swami Swaminathan, KeYu Wang, and Tomas Cihlar □ *Gilead Sciences, Inc., Foster City, California, USA*

□ *Cyclic phosphonomethoxy pyrimidine nucleosides that are bioisosteres of the monophosphate metabolites of HIV reverse transcriptase (RT) inhibitors AZT, d4T, and ddC have been synthesized. The RT inhibitory activities of the phosphonates were reduced for both dideoxy (dd) and dideoxydidehydro (d4) analogs compared to the nucleosides. Bis-isopropyloxymethylcarbonyl (BisPOC) prodrugs were prepared on selected compounds and provided > 150-fold improvements in antiviral activity.*

Keywords Phosphonate; HIV; antiviral; prodrugs

INTRODUCTION

The backbone of antiretroviral therapy usually includes the use of two nucleoside and nucleotide HIV reverse transcriptase inhibitors (NRTIs and NtRTIs, respectively) selected from a panel of eight drugs that include a tenofovir prodrug (bisPOC PMPA, Viread, (**1**), Figure 1) as a unique nucleotide analog.[1–3] Due to the emergence of resistance and drug related toxicities, new N(t)RTIs with optimal resistance and toxicity profiles, along with the convenience of once daily dosing regimens are highly desirable. In an effort to identify new nucleoside phosphonate inhibitors of RT, we synthesized phosphonomethoxy analogs (**2–7**) (Figure 1) of cyclic pyrimidine nucleosides, for example, d4T, AZT, and ddC, compared and contrasted their respective RT inhibitory activity, and evaluated the antiviral potency and resistance profile of selected bisPOC prodrugs.

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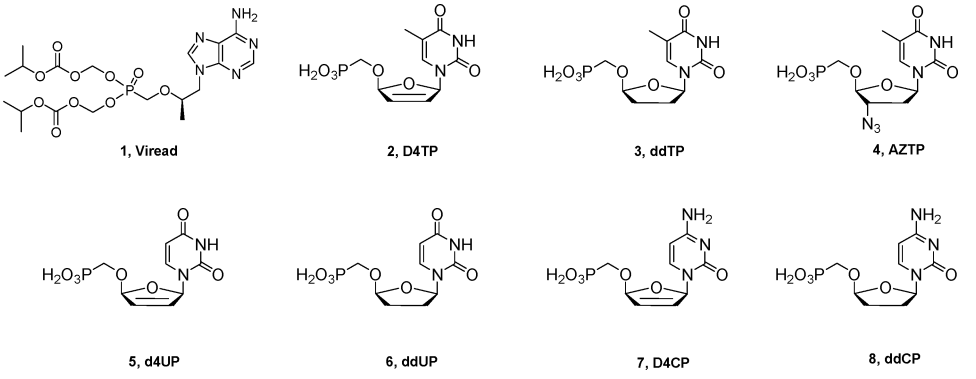
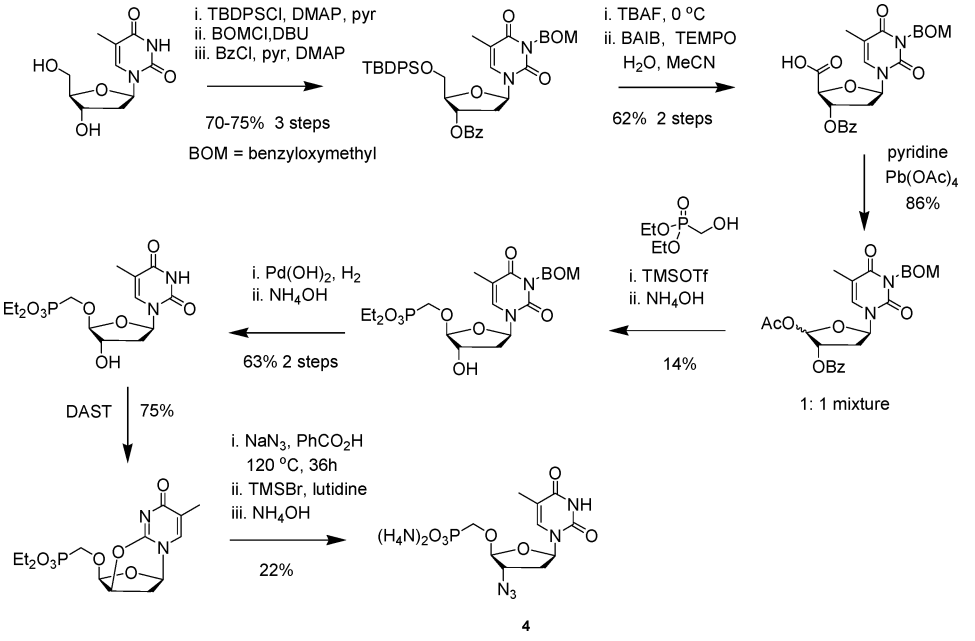


FIGURE 1 Structures of phosphonates (**1–8**).

SYNTHESIS

The phosphonomethoxy analogs of d4t, ddT, d4U, and ddU, analogs (**2–3**), and (**5–6**), respectively (Figure 1), were prepared following procedures similar to those reported earlier.[4] The phosphonomethoxy AZT analog (**4**) was prepared from thymidine according to Scheme 1. The poor yield in the transacetalization step likely is due to the prolonged reaction times, which resulted in a mixture of products including some anomerization at the C1 center. However, none of the α-anomer at the newly created C4 anomeric



SCHEME 1 Synthesis of AZT phosphonate (**4**).

center was isolated from the mixture. The bisPOC prodrugs were prepared from the phosphonate diacids by treatment with chloromethylisopropyl carbonate in the presence of Hünigs base.[5] D4CP analog (**7**) was prepared from the phosphonate diester of (**5**) using typical procedures for conversion of uridine to cytidine[6] followed by removal of the phosphonate ester groups using TMSBr. The dd analog (**8**) was prepared from the phosphonate ester of (**7**) by catalytic reduction followed by TMSBr treatment. Nucleosides were obtained from commercial sources and their respective triphosphates from Trilink Biotechnologies, Inc. (San Diego, CA, USA) or Chemcyte, Inc. (San Diego, CA, USA).

RESULTS AND DISCUSSION

Only d4T phosphonate (**2**) demonstrated antiviral activity below 200 μM (Table 1). Poor antiviral activity can be the result of one or more of the following factors; (1) poor permeation of the phosphonate diacid into the cells; (2) poor metabolism to the diphospho-phosphonates; and (3) poor inhibition of RT. To probe the latter, the diphosphates of (**2**, **3**, **5**, and **6**) were prepared and in each case, weaker RT activity than the corresponding nucleoside triphosphates was observed (Table 1). Thus, the poor antiviral effects are at least in part, due to the weaker RT inhibition properties of the phosphonates. The weaker RT activity of the dd phosphonates (**3**) and (**6**) compared to their respective d4 phosphonates (**2**) and (**5**) mirrors the trend observed for the corresponding dd and d4 nucleoside analogs. This parallel trend implies that the phosphonates likely have similar binding modes to their nucleoside counterparts in the active site of RT.

TABLE 1 Anti-HIV RT activity and antiviral potency for nucleosides, phosphonates, and bisPoc prodrugs

	HIV RT IC_{50} [μM]		WT HIV EC_{50} [μM]		
	Nucleoside triphosphate	Diphospho-phosphonate of (**2–8**)	Nucleoside	Phosphonate (**2–8**)	BisPOC phosphonate of (**2–8**)
d4T	0.06 ± 0.03	0.39 ± 0.17	4.8 ± 0.02	26 ± 8.8	0.91 ± 0.42
ddT	0.12 ± 0.09	5.4 ± 2.1	>200	>200	1.3 ± 0.8
AZT	0.05 ± 0.03	n.d.	0.16 ± 0.12	>200	n.d.
d4U	0.55 ± 0.15	5.7	>200	>200	2.8 ± 0.6
ddU	0.83 ± 0.28	31	>200	>200	>5
d4C	n.d.	n.d.	2.6[a]	>200	n.d.
ddC	0.16 ± 0.08	n.d.	2.5 ± 0.9	>200	n.d.

Cell based antiviral cytopathic assays were conducted in MT-2 cells infected with HIV-1, strain IIIb. Antiviral effect was determined after 5 days of incubation with serial dilutions of tested compounds using XTT-based cell viability method.[8] Enzyme inhibition assays were performed with recombinant p66/p51 heterodimer RT.[9]

[a]Data from.[10]

TABLE 2 Antiviral resistance profile represented as fold EC_{50} change relative to wild-type virus

		Fold EC_{50} change relative to wild-type HIV-1		
Compound	WT EC_{50} [μM]	K65R	6 TAMs[a]	M184V
d4T	4.8 ± 2.3	2.3 ± 1.1	5.5 ± 2.5	1.2 ± 0.2
BisPOC d4TP (**2**)	0.91 ± 0.42	6.7 ± 2.6	6.8 ± 0.7	1.1 ± 0.7[b]

All values are the result of at least 2 experiments. Assays were conducted in MT-2 cells using HIV-1 HXB2 wild-type strain side-by-side with corresponding site directed recombinant mutant strains.

[a]6TAMs virus contains mutations M41L/D67N/K70R/L210W/T215Y/K219Q in HIV RT gene.

[b]M184V resistance determined for parent nucleoside phosphonate.

Given that several of the diphospho-phosphonate analogs were shown to be RT inhibitors (Table 1), albeit weak inhibitors, the potential impact of limited cellular uptake on anti-viral activity was then addressed by preparation of bisPOC prodrugs. The bisPOC prodrug of d4T phosphonate (**2**) improved anti-HIV activity by 29-fold, and for ddT phosphonate (**3**) by >150-fold respectively. Improvements in potency also were found for the corresponding bisPOC prodrug of d4U phosphonate (**5**) thereby indicating that this prodrug strategy is broadly applicable toward improving the antiviral potency of cyclic phosphonates, similar to that observed for Viread (**1**).[3] It is noteworthy that the antiviral activity of bisPOC d4T phosphonate is now several-fold improved over nucleoside d4T despite being a weaker inhibitor of RT. It, therefore, is likely that higher levels of active metabolites are generated by d4T phosphonate (**2**) through superior metabolism properties and a longer intracellular half-life of the active species.[3]

The resistance profile of bisPOC (**2**) toward viruses containing six thymidine analog mutations (6TAMs), K65R or M184V was evaluated and compared to d4T (Table 2). A comparable resistance profile was observed toward the M184V and 6TAMs, whereas a small increase was observed in the K65R resistance. The close overlap of the resistance profiles further supports the conclusion that the phosphonates and their corresponding nucleosides interact with WT and mutant RT through similar binding modes.

CONCLUSION

Evaluation of several cyclic pyrimidine phosphonates reveals that the phosphonates are 'bioisosteres' of their corresponding nucleoside phosphates but weaker RT inhibitors. Antiviral potency can be improved considerably by the application of bisPOC prodrugs. The trends observed for pyrimidine analogs have recently been extended to purine analogs leading to the discovery of the novel clinical candidate, 2′F-d4A phosphonate, GS9148.[7]

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