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A novel diketo phosphonic acid that exhibits specific, strand-transfer inhibition of HIV integrase and anti-HIV activity

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Abstract—We have synthesized novel phosphonic acid analogues of β-diketo acids. Interestingly, the phosphonic acid isostere, **2**, of our anti-HIV compound, **1**, was an inhibitor of only the strand transfer step, in stark contrast to **1**. Compound **2** had lower anti-HIV activity than **1**, but was more active and less toxic than the phosphonic acid analogue of **L-708906**. These isosteric compounds represent the first examples of β-diketo phosphonic acids of structural, synthetic, and antiviral interest.

The *pol* gene of the human immunodeficiency virus (HIV) encodes viral enzymes that are required for HIV replication.[1–3] Drug design and discovery work directed at inhibitors of two of these enzymes, HIV reverse transcriptase (RT) and HIV protease (PR), have created clinically useful compounds for the treatment of acquired immunodeficiency syndrome (AIDS).[4–8] However, research efforts on drug discovery pertaining to the third enzyme of the *pol* gene, HIV integrase, have not produced a single approved drug whose mechanism of action is inhibition of HIV integrase.[9–11] As HIV integrase has no human counterpart and is important in HIV replication, it remains a key viral target for the design and discovery of new anti-HIV agents.

HIV-1 integrase is encoded at the 3′-end of the HIV *pol* gene and is a relatively small (32 kDa) viral protein. Its catalysis results in the incorporation of HIV DNA into host chromosomal DNA.[12–16] This process begins in the cytoplasm where the viral DNA, produced by reverse transcription, is assembled on HIV integrase. Following this assembly, there is specific endonucleolytic cleavage of two nucleotides from each 3′-end of double-stranded viral DNA. This step, referred to as 3′-processing, produces tailored and recessed viral DNA. The next step, which occurs in the nucleus, is identified as strand transfer. Following staggered nicking of chromosomal DNA and joining of each 3′-end of the recessed viral DNA to the 5′-ends of the host DNA, there is repair, which completes the integration process. The strand transfer step, occurring in the nucleus, is partitioned from the 3′-processing step in the cytoplasm.

While many structurally diverse compounds have been reported to be inhibitors of HIV integrase,[8–10,17–28] only a few compounds of one group, the β-diketo acids, and their related compounds, represent the most convincing, biologically validated inhibitors[23,24] of this viral enzyme. Nair and coworkers have designed a conceptually new β-diketo acid with a nucleobase scaffold, compound **1**, which is a powerful inhibitor of both 3′-processing and strand transfer steps of HIV-1 integrase and which exhibits remarkably potent in vitro anti-HIV activity (Fig. 1).[29,30]

Phosphonic acids have been viewed commonly as mimics of carboxylic acids, particularly with reference to biological activity. For example, amino phosphonic acids, isosteres of amino acids, reveal diverse biological properties.[31] With this concept in mind, we designed the β-diketo phosphonic acids, **2** and **3**, the phosphorus-based isosteres of compounds **1** and **L-708906**. In this report, we describe the methodology for the syntheses of **2** and **3**, and discuss their integrase inhibition and antiviral data.

Keywords: Synthesis; Phosphonates; HIV integrase inhibitors; Mechanism of inhibition; Anti-HIV activity.
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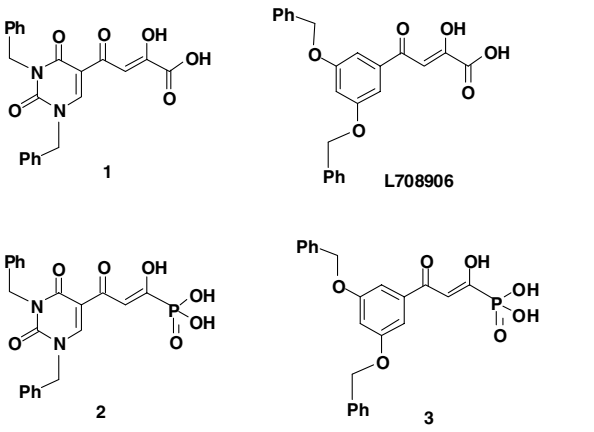
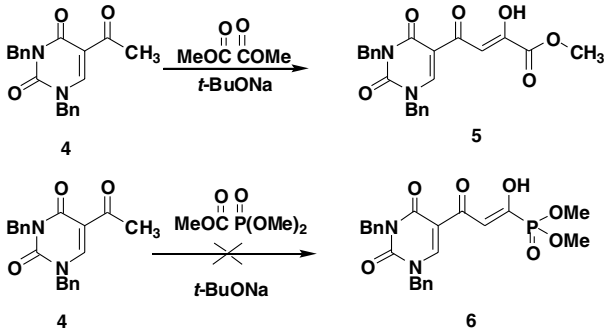


Figure 1. Structures of two biologically active diketo acids (top) and their phosphonic acid isosteres (bottom).

Although the β-diketo acid **1** was synthesized, using as the key step, the condensation of acetyl uracil **4** with dimethyl oxalate under basic conditions,[29] the related reaction of **4** with trimethyl phosphonoformate was unsuccessful (Scheme 1). The reason for this may be the instability of the C(O)–P(O) bond of trimethyl phosphonoformate under basic conditions.[32] Therefore, an alternative synthetic route was devised (Scheme 2).

Thus, 5-formyl uracil **7** was benzylated to give **8** (89%). Compound **8** was condensed with ethyl diazoacetate in the presence of tin (II) chloride[33] to afford **9**, which was transformed into its protected form **10** (28% yield from **8**). Reduction of **10** to the corresponding aldehyde with diisobutylaluminum hydride at −78 °C, followed by a Pudovik reaction with dimethyl phosphite and triethylamine, proceeded to form the phosphonate **11** (50% for two steps). The ketal group of **11** was deprotected to produce the key intermediate **12**, which was purified by HPLC (65% yield) and fully characterized by multinuclear NMR and HRMS data.[34] Dess–Martin oxidation of **12** produced diketone **6**, which exists largely in the enolic form. Compound **6** was deprotected by stirring with NaI in acetone for 3 days.[35] The resulting precipitate was collected and washed with acetone to afford the sodium methyl phosphonate **13** (67% yield). However, compound **13** could not be further deprotected with NaI, even under acetone reflux conditions, possibly because of the presence of the negative charge on the phosphonyl group and the poor solubility of **13** in acetone.[35] The problem was circumvented by conversion of **13** to its protonated form by ion-exchange chromatography with Dowex 50 × 8 in methanol to afford **14**. When compound **14** was heated under reflux with 5 equiv of NaI in acetone for 24 h and the resulting crystalline precipitate was collected and washed with acetone, the target phosphonate **2** (monosodium salt) was produced in a highly purified form (51% yield). Compound **3** was synthesized by a similar procedure to compound **2**. The structures of **2** and **3** were confirmed by ^{1}H, ^{13}C, and ^{31}P NMR, and HRMS data.[34]

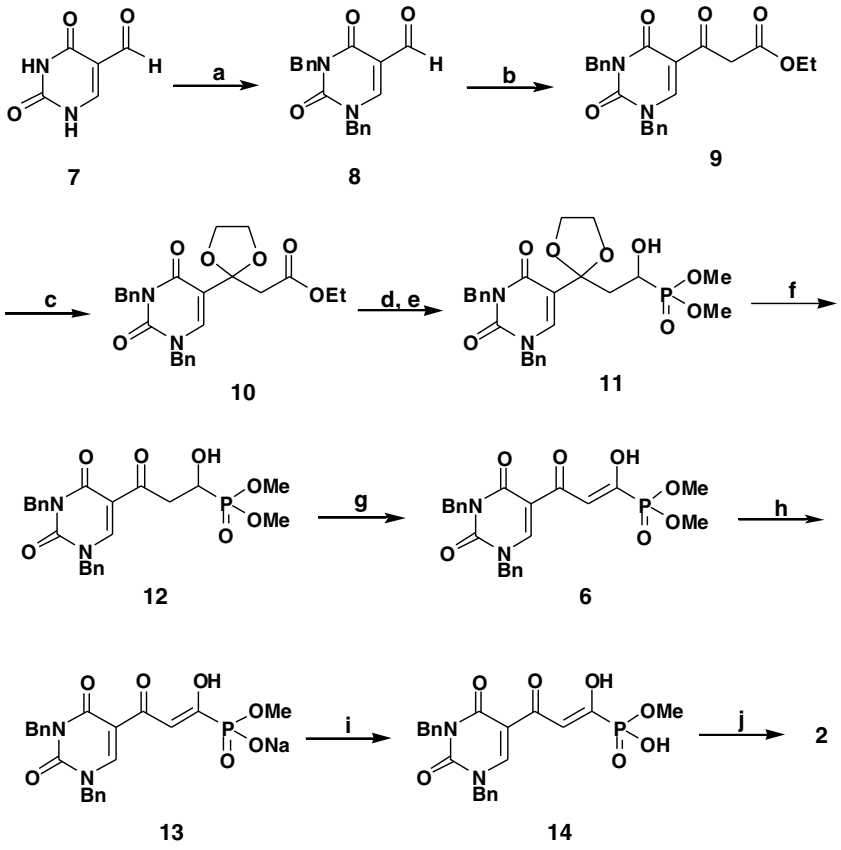


Scheme 1. The difference in the reactivity of **4** with dimethyl oxalate compared to trimethyl phosphonoformate.

Integrase inhibition studies were conducted with recombinant wild-type HIV-1 integrase and a 21-mer oligonucleotide substrate following a previously described procedure.[36,37] Target compound **2** was not an inhibitor of the 3′-processing step of HIV integrase (IC_{50} > 333 μM) but inhibited the strand transfer step (IC_{50} 20.2 ± 7.2 μM).[38] The integrase inhibition activity of **2** is in sharp contrast to that of compound **1**, which is a potent inhibitor of both the 3′-processing and strand transfer steps of integrase (IC_{50} 3.7±1.0 μM and 0.2 ± 0.1 μM, respectively,[29,39] and 14.4±1.6 μM and 4.5 ± 2.5 ± μM, respectively[38]).

Compound **2** was tested in a PBMC-based, microtiter anti-HIV assay against the clinical isolates, HIV-1_{TEKI} (NSI phenotype) and HIV-1_{NL4-3} (SI phenotype).[29] Antiviral determinations were performed in triplicate with serial 1/2 log 10 dilution of the test materials (six to nine concentrations total). The overall performance of both assays was validated by the MOI-sensitive positive control compound, AZT. As summarized in Table 1, in vitro anti-HIV studies against HIV-1 isolates in PBMC showed that compound **2** (highest test concentration = 200 μM) was active with IC_{50} values in the low μM range and with antiviral efficacy data [therapeutic indices, TI = CC_{50}/IC_{50} of >50 (HIV-1_{TEKI}) and >62 (HIV-1_{NL4-3})]. Cell viability data showed only mild cellular cytotoxicity at the highest test concentrations; however, a CC_{50} (>200 μM) was not reached. In contrast, compound **3** was significantly less active and more toxic [TI = 5.8 (HIV-1_{TEKI}) and 4.0 (HIV-1_{NL4-3})]. The control compound, AZT (highest test concentration = 1 μM), gave therapeutic indices of >504 (HIV-1_{TEKI}) and >573 (HIV-1_{NL4-3}).

In summary, we have developed a methodology for the synthesis of a new β-diketo phosphonic acid, **2**, an isostere of our highly active, anti-HIV compound, **1**. In complete contrast to **1**, compound **2** specifically inhibits only the strand transfer step of WT HIV-1 integrase. Anti-HIV data show that the exchange of the carboxylate functionality with a phosphonate results in a reduction in in vitro anti-HIV activity. The anti-HIV data in PBMC may be a reflection of the difference in integrase inhibitory activity of **1** compared to **2** and the result of decreased cellular permeability of phosphonate **2** compared to the less polar carboxylate **1**. Interestingly, compound **2** is more anti-HIV active and less toxic than the phosphonic acid analogue of **L-708906** (compound **3**). Finally, these isosteric compounds represent the first



Scheme 2. Synthesis of integrase inhibitor **2**. Reagents and conditions: (a) BnBr/DMF/K_2CO_3; (b) $N_2CHCO_2Et/SnCl_2/CH_2Cl_2$; (c) ethylene glycol/$CH(OEt)_3$/PTS; (d) dibal-H/toluene/−78 °C; (e) $HP(O)(OMe)_2/Et_3N$/MeOH; (f) PTS/acetone/H_2O; (g) Dess–Martin periodinane/CH_2Cl_2; (h) NaI/acetone; (i) Dowex50Wx8-100/MeOH; (j) NaI/acetone/reflux.

Table 1. Antiviral data for compounds of Figure 1 in PBMC

Compound	HIV-1 isolate cell line	IC_{50}	CC_{50}	TI
1[a]	HIV-1_{TEKI} (PBMC)	50 nM	>200 μM	>4000
	HIV-1_{NL4-3} (PBMC)	<20 nM	>200 μM	>10,000
L708906[b]	HIV-1 (IIIB) (MT-4)	5.5 μM	88.3 μM	16
2	HIV-1_{TEKI} (PBMC)	4.0 μM	>200 μM	>50
	HIV-1_{NL4-3} (PBMC)	3.2 μM	>200 μM	>62
3	HIV-1_{TEKI} (PBMC)	8.1 μM	46.9 μM	5.8
	HIV-1_{NL4-3} (PBMC)	11.8 μM	46.9 μM	4.0

[a] Ref. 29.
[b] Ref. 26.

examples of β-diketo phosphonic acids of structural, synthetic, and antiviral interest.

Acknowledgments

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34. Data for dimethyl 3-(1,3-dibenzyl-2,4-dioxo-1,2,3,4-tetra-hydropyrimidin-5-yl)-1-hydroxy-3-oxopropylphosphonate (**12**). ^{1}H NMR ($CDCl_3$): 8.29 (s, 1H), 7.30–7.50 (m, 10H), 5.18 (s, 2H), 5.05 (d, 1H, J = 15), 5.02 (d, 1H, J = 15), 4.61 (m, 1H), 3.87 (d, 3H, J = 11.0), 3.86 (d, 3H, J = 10.5), 3.57–3.64 (m, 1H), 3.42–3.48 (m, 1H). ^{13}CNMR ($CDCl_3$): 194.9 (d, J = 17.6), 160.8, 150.9, 149.3, (136.2, 134.3, 129.5, 129.2, 129.1, 128.7, 128.5, 128.1, phenyl ring), 111.7 (d, J = 2.31), 64.3 (d, J = 169.4), 53.8, (53.72, 53.68, 53.66, 53.61, P-OCH_3), 45.1, 43.9 (d, J = 3.82). ^{31}PNMR ($CDCl_3$): 26.5. FAB-HRMS: calcd for $C_{23}H_{26}N_2O_7P$ $(M+H)^+$ 473.1478; Found 473.1469. Data for 3-(1,3-dibenzyl-2,4-dioxo-1,2,3,4-tetrahydro-pyrimidin-5-yl)-1-hydroxy-3-oxoprop-1-enylphosphonic acid (**2**) as its monosodium salt: mp 224 °C (dec). ^{1}H NMR (CD_3OD): 8.58 (s, 1H), 7.22–7.39 (m, 11H), 5.12 (s, 2H), 5.10 (s, 2H). ^{13}CNMR (CD_3OD): 188.1 (d, J = 14.5), 185.9 (d, J = 190.4), 161.5, 152.5, 150.6, (138.3, 137.1, 130.2, 129.6, 129.5, 129.3, 128.7, phenyl ring), 111.2 (d, J = 4.90), 104.5 (d, J = 24.4), 54.4, 45.7. ^{31}PNMR (CD_3OD): 0.82. FAB-HRMS: calcd for $C_{21}H_{19}N_2NaO_7P$ $(M+H)^+$ 465.0828; Found 465.0840. Data for 3-(3,5-bis(benzyloxy)phenyl)-1-hydroxy-3-oxoprop-1-enyl- phosphonic acid (**3**) as its monosodium salt: mp 201 °C (dec). ^{1}H NMR (CD_3OD): 7.29–7.46 (m, 10H), 7.25 (d, 2H, J = 2.5), 6.89 (d, 1H, J = 7.0), 6.85 (t, 1H, J = 2.5), 5.12 (s, 4H). ^{13}CNMR (CD_3OD): 193.3 (d, J = 13.4), 185.0 (d, J = 191.3), [161.8, 140.0 (d, J = 4.27), 138.4, 129.7, 129.2, 128.9, 108.3, 107.8, phenyl ring], 101.6 (weak), 71.5. ^{31}PNMR (CD_3OD): 0.91. FAB-HRMS: calcd for $C_{23}H_{21}NaO_7P$ $(M+H)^+$ 463.0923; Found 463.0927.
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