

Synthesis of the 5'-phosphonate of 4(*S*)-(6-amino-9H-purin-9-yl)tetrahydro-2(*S*)-furanmethanol [*S,S*-IsoddA]

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Abstract

4(*S*)-(6-Amino-9H-purin-9-yl)tetrahydrofuran-2(*S*)-ylmethyl phosphonic acid, a 5'-C-phosphonate analog of the potent anti-HIV compound *S,S*-IsoddA, was synthesized in order to bypass the critical initial intracellular phosphorylation. Key phases in this multistep synthesis were the Arbuzov reaction of the 5-iodofuranose with triethylphosphite and the Mitsunobu/coupling reaction of the phosphonate with adenine. The structure of the final product was confirmed by HRMS and multinuclear NMR data.

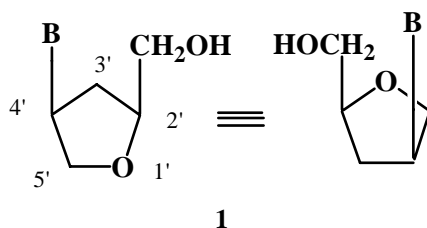
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Introduction

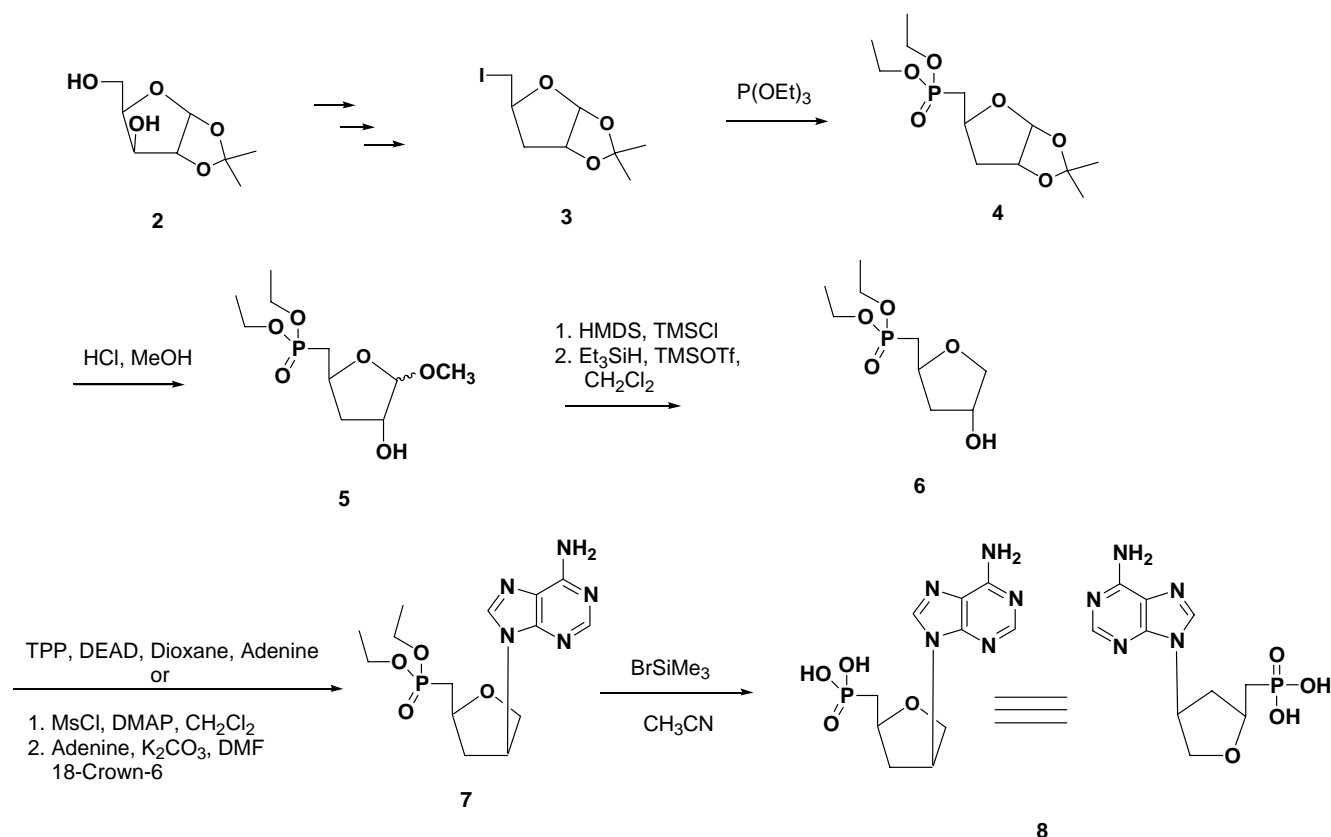
The unique properties of phosphonate analogs of the natural phosphoric acid esters make them suitable for use in a continuously increasing variety of applications. Replacement of an *O*-phosphate group in a biologically-active molecule by a C-phosphonic acid group¹ might be expected to have interesting biological effects. This modification can confer greater stability on these isosteres as the C-P bond that replaces the C-O-P bond cannot be hydrolyzed by the enzymes involved in *O*-phosphate ester cleavage. In 1986, an acyclic nucleoside phosphonate analog, (*S*)-9-(3-hydroxy-2-phosphonomethoxy propyl)adenine, was found to be active against a number of viruses.² Since that time, the use of the apparently membrane permeable phosphonate moiety has been intensively studied in the design of more useful antiviral agents. For example, the anti-HIV activities of dideoxynucleosides are critically dependent on their initial intracellular phosphorylation.^{3,4} One way to overcome the difficulty of the first phosphorylation step is to work with prodrugs that deliver intracellularly the monophosphate forms.^{5,6} Another approach to bypass the first phosphorylation step more completely is through phosphonate analogs, that, after intracellular conversion to their diphosphate forms, can serve as inhibitors/ chain terminators in the HIV RT reaction.⁷⁻¹⁰ A unique feature common to all nucleoside phosphonates is their prolonged antiviral action.

Results and Discussion

Nair and coworkers have reported previously on the synthesis¹¹ and biological studies^{12,13} of the isomeric dideoxynucleoside, 4(*S*)-(6-amino-9*H*-purin-9-yl)tetrahydro-2(*S*)-furanmethanol, [(*S,S*)-IsoddA] (**1**). This compound is almost totally resistant to deamination by adenosine deaminase. The triphosphate of (*S,S*)-IsoddA is a potent inhibitor of HIV reverse transcriptase (K_i 16 nM). However, the *in vitro* anti-HIV activity of IsoddA against HIV-1 and HIV-2, although significant, is in the low μ M range. Consistent with this is the low level of intracellular phosphorylation of the compound.¹² In enzymatic studies, we have shown that (*S,S*)-IsoddA is a poor substrate for deoxycytidine kinase, the most likely enzyme for the initial phosphorylation step.¹⁴ In order to circumvent the critical first phosphorylation step, we have synthesized and studied a phosphatase-stable 5'-C-phosphonate analog of (*S,S*)-IsoddA and the synthesis of this compound is reported here. Only key experimental steps are provided.



3,5-Dideoxy-5-iodo-1,2-*O*-isopropylidene- α -D-xylo-furanose (**3**)¹⁵ was prepared in six steps starting from 1,2-*O*-isopropylidene- α -D-xylofuranose (**2**) (Scheme 1). The Arbuzov reaction of **3** with triethylphosphite gave 3,5-dideoxy-1,2-*O*-isopropylidene-5-diethylphosphonyl- α -D-xylo-furanose (**4**) in 85% yield. Acid-catalyzed methanolysis of **4** afforded the α - and β -methylglycosides **5**. Reductive demethoxylation of **5** utilized a methodology¹¹ which first involved protection of the 2-hydroxyl group by silylation followed *in situ*, by treatment of this product with triethylsilane and TMS-triflate in 1,2-dichloroethane, which produced the tetrahydrofuran **6** in 46% yield. Compound **6** can be converted into 4(*S*)-(6-amino-9*H*-purin-9-yl)-2(*S*)-diethylphosphonylmethyltetrahydrofuran (**7**) either under Mitsunobu conditions or by mesylation (89%) followed by condensation with adenine (49%). COSY and NOESY NMR data of this compound established unambiguously that only the β -isomer was obtained during the condensation step. Dealkylation of the phosphonate ester **7** with bromotrimethylsilane gave the corresponding phosphonic acid, 4(*S*)-(6-amino-9*H*-purin-9-yl)tetrahydrofuran-2(*S*)-ylmethyl phosphonic acid (**8**) which was purified by preparative HPLC (85% yield). It was characterized by ¹H, ¹³C and ³¹P NMR data, and high-resolution negative ion mass spectral data. However, evaluation of the compound for *in vitro* anti-HIV activity in infected CEM-SS cells showed that the activity was much lower than the parent compound, (*S,S*)-IsoddA.



Scheme 1

Experimental Section

General Procedures. Nuclear magnetic resonance spectra were recorded on Bruker Model AC300 and WM 360 systems. Ultraviolet spectra were recorded on a Varian Cary Model 3 spectrophotometer. High-resolution FAB mass spectra were obtained on a VG ZAB-HF mass spectrometer. Flash chromatography used 230-400 mesh silica gel. HPLC analyses were carried out on a Beckman-Coulter instrument with C-18 reversed-phase columns.

4(S)-(6-Amino-9H-purin-9-yl)-2(S)-diethylphosphonylmethyltetrahydrofuran (7). A mixture of the mesylate of **6** (178 mg, 0.563 mmole), adenine (152 mg, 1.127 mmole), anhydrous K_2CO_3 (156 mg, 1.127 mmole), and 18-crown-6 (149 mg, 0.563 mmole) in DMF (5 mL) was heated at 75 °C for 24 h. The solvent was then evaporated under reduced pressure and the residue was stirred with CHCl_3 (250 mL) and filtered. Evaporation of the solvent and column chromatography of the residue under reduced pressure (0 to 5% MeOH/ CHCl_3) gave pure coupled product **7** (98 mg, 49%): ^1H NMR (CDCl_3) δ 8.32 (s, 1H, H-2), 8.00 (s, 1H, H-8), 5.86 (br s, 2H, NH_2), 5.29 (m, 1H, H-2'), 4.28 (m, 1H, H-4') 4.17 (dd, 1H, H-1', $J_{\text{H}1'-\text{H}2'}=2.4$ Hz, $J_{\text{H}1'-\text{H}1''}=10.2$ Hz), 4.11 and 4.09 (each dq, 2H, $\text{OCH}_2\text{CH}_3 \times 2$, $J_{\text{H-H}}=7.2$ Hz, $J_{\text{H-P}}=10.8$ Hz), 4.04

(dd, 1H, H-1'', $J_{H1'-H1''}=10.2$ Hz, $J_{H1''-H2''}=6.6$ Hz), 2.87 (ddd, 1H, H-3'', $J_{H4'-H3''}=7.2$ Hz, $J_{H2'-H3''}=8.4$ Hz, $J_{H3'-H3''}=13.8$ Hz), 2.37 (ddd, 1H, H-5', $J_{H5'-H4'}=5.4$ Hz, $J_{H5'-H5''}=15.0$ Hz, $J_{H5'-P}=18.6$ Hz), 2.13 (ddd, 1H, H-5'', $J_{H4'-H5''}=7.8$ Hz, $J_{H5'-H5''}=15.0$ Hz, $J_{H5'-P}=18.6$ Hz) 1.30 and 1.29 (each t, 3H, $\text{OCH}_2\text{CH}_3 \times 2$, $J=7.2$ Hz); ^{13}C NMR (CDCl_3): δ 155.75 (ethylenic C-4 or C-5), 152.83 (C-2), 149.60 (ethylenic C-4 or C-5), 138.04 (C-8), 119.27 (C-6), 74.22 (C-2'), 72.51 (C-5'), 61.79 (m, $\text{P}(\text{OCH}_2\text{CH}_3)_2$), 54.22 (C-4'), 40.03 (d, C-3', $J_{C-P}=7.1$ Hz), 31.95 (d. P-CH₂, $J_{C-P}=140.5$ Hz), 16.31 and 16.23 ($\text{P}(\text{OCH}_2\text{CH}_3)_2$); ^{31}P NMR (CDCl_3): δ 26.44 (s).

4(S)-(6-Amino-9H-purin-9-yl)tetrahydrofuran-2(S)-ylmethyl phosphonic acid (8). To a solution of **7** (70 mg, 0.197 mmole) in CH_3CN (3 mL) containing a catalytic amount of dry pyridine (< 0.1 mL) was added Me_3SiBr (453 mg, 2.96 mmole) and the reaction mixture was stirred for 96 h at 25 °C. Pyridine (0.4 mL) and water (4 mL) was added and stirring was continued for an additional 2 hours. The reaction mixture was washed with ether (2 x) and the residue was purified by HPLC to give **8** as a crystalline solid (50 mg, 85%): ^1H NMR (D_2O) δ 8.43 and 8.40 (each s, 1H, H-2 and H-8), 5.41 (m, 1H, H-4'), 4.45-4.10 (m, 3H, H-2', H-5' and H-5'') 3.05-2.90 (m, 1H, H-3''), 2.35-1.95 (m, 3H, H-3' and P-CH₂); ^{13}C NMR (D_2O): δ 148.77 (ethylenic C-4 or C-5), 146.18 (C-2), 143.55 (ethylenic C-4 or C-5), 140.16 (C-8), 116.09 (C-6), 73.86 (C-2'), 69.15 (C-5'), 53.59 (C-4'), 36.99 (d, C-3', $J_{C-P}=6.5$ Hz), 31.75 (d. P-CH₂, $J_{C-P}=131.1$ Hz); ^{31}P NMR (D_2O): δ 21.92 (s); HRMS (ESI) calcd for $\text{C}_{10}\text{H}_{14}\text{N}_5\text{O}_4\text{P}$: ($\text{M} - \text{H}$)⁻ 298.0704, found: 298.0706.

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