Synthesis of Phosphonate Isosteres of 2′-Deoxy-1′,2′-seco-nucleotides

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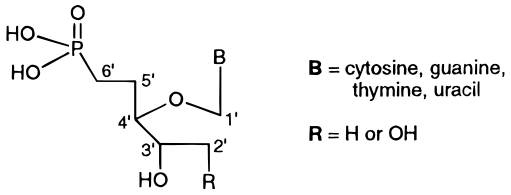
Practical and convergent syntheses of 2′-deoxy-1′,2′-seco-nucleophosphonates and 1′,2′-seco-nucleophosphonates are described. Phosphonate chirons derived from D-isoascorbic acid were used in alkylation of functionalized nucleobases to provide the title phosphonate isosteres in good yields. Subsequent deprotection and deesterification led to the 5′-*C*-methylenephosphonic acids which were conveniently purified using gravity flow C_{18} reverse phase column chromatography.

Introduction

In an effort to find new acyclonucleoside-type antiviral agents which maintain the chiral integrity of the D-pentose moiety, *e.g.*, D-ribose, and with the ability to bypass the initial phosphorylation step, we explored the synthesis of phosphonomethylene analogues of 1′,2′-seco-nucleotides. These isosteres could serve as substrates for host-cell phosphorylating enzymes and eventually be converted to their respective triphosphate counterparts. Such analogues should have the potential to exert activity by inhibiting a viral nucleic acid polymerase.

The concept of using phosphonate isosteres to mimic phosphates is not new and has been employed extensively in nucleoside chemistry.[1] Most of the synthetic pathways in the nucleoside area use either Arbuzov[1,2] or Wittig[1,3] chemistry to attach the 5′-*C*-phosphonomethylene group to a 5′-functionalized nucleoside. More recently an alternative preparative route has been reported in which a suitably protected 5′-*C*-phosphonomethylene sugar[4] /unit[5] is used in the glycosylation reaction with an appropriately functionalized nitrogen heterocycle (nucleobase). We adopted a similar approach in our work



which led to the successful preparation of the title phosphonates. Chirons **1**−**5**[6] (Scheme 1) were chloromethylated and condensed with the desired functionalized nitrogen heterocycle to furnish the blocked 1′,2′-seco-nucleophosphonates of cytosine, guanine, thymine, and uracil.[7] This convergent methodology[8] is far more practical than routes which attach the phosphonomethylene moiety to the acyclic side chain at a later stage[9] in the synthesis. We now wish to describe the synthetic methods leading to the phosphonate esters, their subsequent deprotection, and purification of the resultant phosphonic acids.

Results and Discussion

Our synthetic strategy, which is illustrated in Schemes 1−3, centered on the phosphonate synthons **1**−**5**.[6] They were prepared in good yields by a regiospecific and nucleophilic ring opening of certain chiral epoxides derived from D-isoascorbic acid.[10] Chloromethylation of **1**−**5** was carried out with paraformaldehyde and hydrogen chloride gas in dry dichloromethane (CH_2Cl_2) at 0 °C. If desired, the percent purity of **1a**−**5a** (Scheme 1) can be determined from the characteristic OCH_2Cl resonance typically at δ 5.6 in their respective ^{1}H NMR spectra.[11] In most cases, they were used immediately in the alkylation of the desired nucleobase. For example, when **2a** was condensed with persilylated thymine in the presence of a catalytic amount of tetraethylammonium iodide (TEAI), **6** was obtained in 85% yield. In general, preparation of the pyrimidine 1′,2′-seco-nucleophosphonates **6**−**9**, **12**, **13**, **15**, and **16** were carried out with the persilylated form of the requisite heterocycle, whereas with purines, *i.e.*, 2-amino-6-(benzyloxy)purine and 6-chloropurine,[12] the sodium salt was alkylated. With the exception of **12**−**14**, where the chloromethyl ether of dibenzyl 4(*S*)-(benzyloxy)-3*(R)*-hydroxypentanephosphonate was employed in the alkylation of the functionalized

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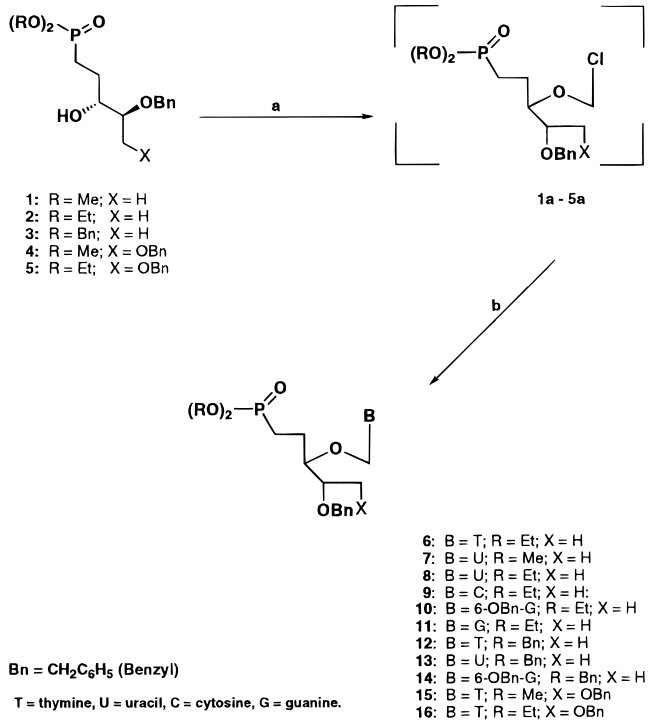
Scheme 1



Reagents: (a) $(CH_2O)_n$, HCl_g, CH_2Cl_2, 0 °C; (b) B-TMS, CH_2Cl_2, TEAI or B-Na, DMF

nucleobase, the yields of the protected 1′,2′-seco-nucleophosphonates ranged from 62−96%.

Deprotection of the 2′- and 3′-*O*-benzyl positions (Scheme 2) was easily accomplished by catalytic transfer hydrogenation[11] over Pearlman's catalyst (20% $Pd(OH)_2/C$). This procedure provided the phosphonate diesters **17**−**22** in high yield.

We next turned our attention to deesterification of the phosphonate moiety. Hydrolysis of nucleoside phosphonate esters has always been problematic,[1a,2,4b] and with this in mind, we explored several established procedures. When the diethyl esters **17** and **19** were reacted with a 1 N ethanolic sodium hydroxide solution, followed by acidification with Dowex-50 resin (H^+) and workup, the monoethyl phosphonate esters **23** and **24**, respectively, were isolated in reasonable yields (Scheme 2). Treating the dimethyl esters **18** and **21** with 4 equiv of bromotrimethylsilane (TMS-Br)[13] in dichloromethane at 0 °C for 1 h in a nitrogen atmosphere furnished the phosphonic acids **25** and **26**, respectively, in near-quantitative yield. We have observed[14] that the same reaction conditions, *i.e.*, 4 equiv of TMS-Br, CH_2Cl_2, N_2, 0 °C, with diethyl phosphonates led only to monoethyl phosphonate esters. A recent report[15] supports this observation and indicates that hydrolysis of ethyl phosphonates requires prolonged reaction times or elevated temperatures. Thus, to realize complete hydrolysis of our compounds, an increase in reaction time (*ca.* 16 h) and temperature (rt) was needed. Although more drastic conditions using bromotrimethylsilane, *i.e.*, a fifteen-fold excess of TMS-Br in acetonitrile and a reaction temperature of 65 °C, have shortened the time required to effect complete deesterification of certain phosphonomethylene nucleotide analogues,[4b] such conditions with our system posed a potential risk of base cleavage[16] in our system. In an effort to minimize such an undesirable side reaction during deesterification of the 1′,2′-seco-nucleophosphonates, the dibenzyl esters **12**−**14** were prepared. Hydrogenolysis of **12**, **13**, and **14** with 10% Pd/C and cyclohexene in ethanol provided a convenient, high yielding, one-step deprotection−deesterification route (Scheme 3) to the phosphonic acids **27**, **25**, and **28**, respectively.

The free phosphonic acids (**25**−**28**) were obtained in high purity by simply passing them through a short, gravity-flow C_{18} reverse phase silica gel column[17] using water or water-methanol as eluant. Although some of our phosphonic acids are hygroscopic and were difficult to analyze, C_{18} reverse phase chromatography followed by lyophilization did allow us to obtain correct elemental analyses without resorting to salt forms. It is worth mentioning that if phosphonic acids are not desired, literature methods are available to convert them to their respective ammonium,[2,4a] pyridinium,[13] or sodium salts.[3,4b]

Experimental Section

Optical rotations were measured on an automatic digital readout polarimeter. ^1H NMR spectra were recorded on either a 90 or 300 MHz spectrometer using Me_4Si (TMS) as an internal standard. All moisture-sensitive reactions were performed using flame-dried glassware. Dichloromethane (CH_2Cl_2) and acetonitrile were dried over CaH_2 and distilled. Evaporations were performed under diminished pressure using a rotary evaporator unless stated otherwise. Thin-layer chromatography was performed on precoated silica gel plates (60-F254, 0.2 mm) manufactured by E. M. Science, Inc., and short-wave ultraviolet light (254 nm) was used to detect the UV absorbing compounds. Silica gel (Merck grade 60, 230−400 mesh, 60 Å) suitable for column chromatography was purchased from Aldrich. C_{18} reverse phase silica gel column chromatography was conducted on custom bonded Davisil (35−75 microns, 60 Å; Alltech Associates). All solvent proportions are by volume unless stated otherwise. Elemental analyses were performed by MHW Laboratories, Phoenix, AZ.

General Procedure for the Chloromethylation of Synthons 1−5.[11] In a three-necked, flame-dried flask fitted with a gas-inlet and a drying tube was added paraformaldehyde and the alcohol (*ca.* 1.5:1) in dry CH_2Cl_2. The mixture was cooled to 0 °C in an ice bath, and dry hydrogen chloride gas was bubbled into the solution for 5 h while maintaining the temperature at 0 °C. At the end of this period, anhyd calcium chloride (*ca.* 5g) was added cautiously and the mixture stirred for 15 min. After filtration, the solution was concentrated under diminished pressure to give the corresponding chloromethyl ether which was used immediately in the alkylation of the desired functionalized heterocycle without further purification.

Physical data for **4(*S*)-(Benzyloxy)-3(*R*)-(chloromethoxy)-1-(diethylphosphonyl)pentane (2a)**: ^1H NMR ($CDCl_3$) δ 1.1−1.6 (m, 9H), 1.65−2.2 (m, 4H), 3.5−4.3 (m, 6H), 4.3−4.7 (t, J = 13.5 Hz, 2H), 5.5−5.8 (q, J = 6 Hz, 2H), 7.3 (s, 5H).

4(*S*)-(Benzyloxy)-3(*R*)-(chloromethoxy)-1-(dibenzylphosphonyl)pentane (3a): ^1H NMR ($CDCl_3$) δ 1.1 (d, J = 6 Hz, 1H), 1.4−2.1 (m, 4H), 3.3−3.9 (m, 2H), 4.5 (m, 2H), 4.7−5.1 (m, 4H), 5.3−5.6 (q, J = 6 Hz, 2H), 7.0−7.4 (m, 15H).

(12) 6-Chloro-9-[[4(*S*)-(benzyloxy)-1-(diethylphosphonyl)-3(*R*)-pentoxy]-methyl]purine was obtained in 54% yield. Physical constants for this compound are: $[\alpha]^{25}_D$ +24.8 (c = 1.174, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 1.08−1.27 (m, 9H), 1.20−1.75 (m, 4H) 3.4-4.1 (m, 6H), 4.38−4.60 (q, J = 15 Hz, 2H), 5.70−5.90 (q, J = 13.5 Hz, 2H), 7.20−7.40 (m, 5H), 8.20 (s, 1H), 8.70 (s, 1H). Anal. ($C_{22}H_{30}ClN_4O_5P$) C, H, N.

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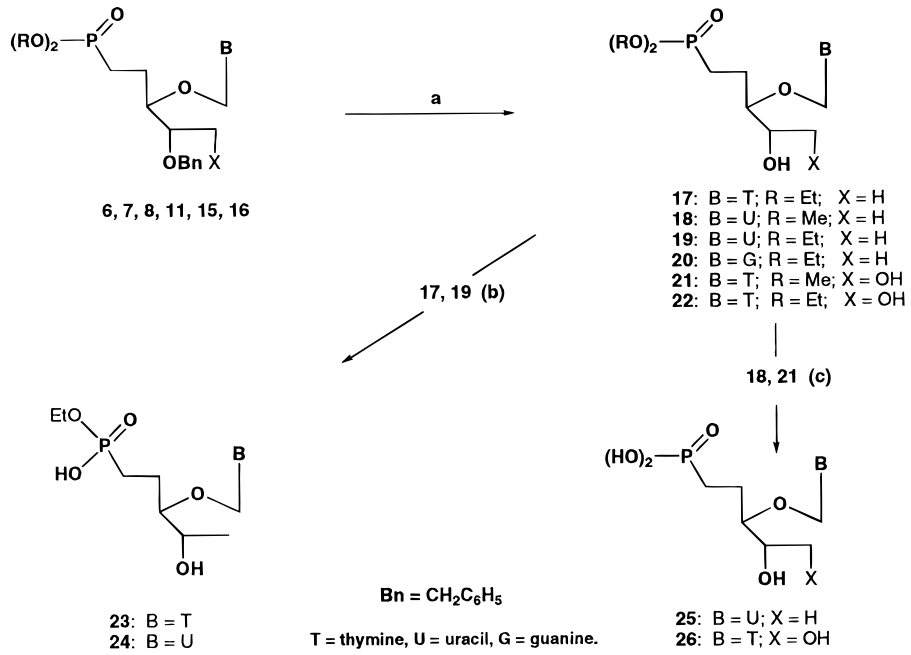
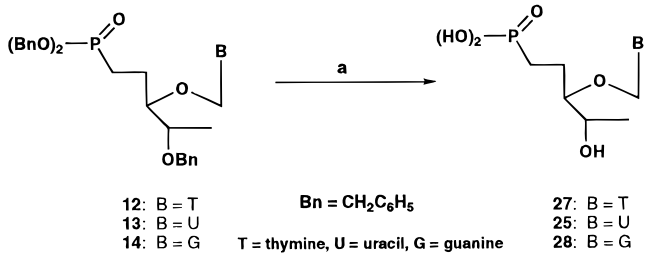
Scheme 2



Reagents: (a) 20% $Pd(OH)_2$/C, EtOH, Cyclohexene, reflux (Δ); (b) NaOH, EtOH-H_2O (1:1), rt; (c) TMS-Br, CH_2Cl_2, N_2, O °C.

Scheme 3



Reagents: (a) 10% Pd/C, EtOH, cyclohexene, reflux (Δ)

1-[[(4(*S*)-(Benzyloxy)-1-(diethylphosphonyl)-3(*R*)-pentoxy]methyl]thymine (6). Thymine (0.54 g, 4.3 mmol) and ammonium sulfate (*ca.* 50 mg) were added to hexamethyldisilazane (HMDS, 10 mL). The reaction mixture was stirred at reflux overnight with the exclusion of moisture. After cooling to room temperature (clear solution), the excess HMDS was removed under reduced pressure and the residue dried under high vacuum. A solution of **2a** (from **2**; 0.948 g, 2.87 mmol) in dry CH_2Cl_2 (20 mL) and tetraethylammonium iodide (TEAI, *ca.* 50 mg) were added to the persilylated thymine, and the mixture was stirred at reflux overnight. The reaction mixture was then diluted with water (2.5 mL) and methanol (15 mL), stirred for 15 min, and evaporated to dryness. The residue was dissolved in CH_2Cl_2 (50 mL) and washed successively with water, 10% $Na_2S_2O_4$ solution, and brine and then dried over anhydrous $MgSO_4$. The viscous material obtained after solvent removal at reduced pressure was chromatographed on a silica gel column, eluting with ethyl acetate–methanol (8:2), to give **6** (1.15 g, 85%) as a gum: $[\alpha]^{25}_D$ +6.6° (*c* = 0.515, EtOH); ^{1}H NMR ($CDCl_3$) δ 1.16 (d, *J* = 6 Hz, 3H), 1.28 (t, *J* = 6 Hz, 6H), 1.44–2.02 (m, 4H), 1.86 (s, 3H), 3.24–4.26 (m, 6H), 4.30–4.72 (m, 2H), 5.18 (AB_q, *J* = 12 Hz, 2H), 7.08 (s, 1H), 7.16 (s, 5H), 9.90 (br s, 1H, D_2O exchangeable). Anal. Calcd for $C_{22}H_{33}N_2O_7P$: C, 56.40; H, 7.10; N, 5.98; P, 6.61. Found: C, 56.58; H, 7.16; N, 5.85; P, 6.76.

1-[[(4(*S*)-(Benzyloxy)-1-(dimethylphosphonyl)-3(*R*)-pentoxy]methyl]uracil (7). Persilylated uracil [obtained from 0.728 g (6.5 mmol) of uracil] was coupled as described for **6**, with the chloromethyl ether **1a**, derived from **1** (1.319 g, 4.4 mmol), in dry CH_2Cl_2 (50 mL) and in the presence of TEAI (30 mg). The reaction mixture was stirred and heated at reflux for 12 h. Workup and chromatography (ethyl acetate) afforded pure **7** (1.78 g, 95%): $[\alpha]^{25}_D$ +14.3° (*c* = 0.595, EtOH); ^{1}H NMR ($CDCl_3$) δ 1.14 (d, *J* = 6 Hz, 3H), 1.45–2.08 (m, 4H), 3.34–3.98 (m, 2H), 3.72 (d, *J* = 11 Hz, 6H), 4.46 (br s, 2H), 5.16 (AB_q, *J* = 12 Hz, 2H), 5.60 (d, *J* = 8 Hz, 1H), 7.28 (br s, 6H), 10.24 (br s, 1H, D_2O exchangeable). Anal. Calcd for $C_{19}H_{27}N_2O_7P$: C, 53.52; H, 6.38; N, 6.57. Found: C, 53.37; H, 6.57; N, 6.35.

1-[[(4(*S*)-(Benzyloxy)-1-diethylphosphonyl)-3(*R*)-pentoxy]methyl]uracil (8). Uracil (2.7g, 24 mmol) was silylated as described for **7** using HMDS (60 mL) and ammonium sulfate (0.1 g). The silylated nucleobase was dissolved in CH_2Cl_2 (100 mL) along with **2a** (16 mmol) and tetrabutylammonium iodide (TBAI, 0.1g). The reaction was carried out as mentioned for **7**. The crude product was chromatographed on a silica gel column using EtOAc-hexane (2:1) as the eluant to furnish pure **8** (5.8 g, 81%): $[\alpha]^{25}_D$ +9.1° (*c* = 1.76, MeOH); ^{1}H NMR ($CDCl_3$) δ 1–1.4 (m, 9H), 1.5–2.1(m, 4H), 3.3–4.2 (m, 6H), 4.3–4.7 (t, *J* = 12.6 Hz, 2H,), 4.9–5.3 (ABq, *J* = 9 Hz, 2H), 5.6 (d, *J* = 7.5 Hz, 1H), 7.2 (br s, 6H) and 10.1 (br s, 1H, D_2O exchangeable). Anal. Calcd for $C_{21}H_{31}N_2O_7P$: C, 55.49; H, 6.87; N, 6.16; P, 6.81. Found: C, 55.29; H, 6.71; N, 5.95; P, 6.97.

1-[[(4(*S*)-(Benzyloxy)-1-(diethylphosphonyl)-3(*R*)-pentoxy]methyl]cytosine (9). Silylation of cytosine (1.67 g, 15 mmol) was carried out using HMDS (35 mL) in presence of ammonium sulfate (0.1 g). The chloromethyl ether **2a** (10 mmol) was then added to the silylated nucleobase in dry CH_2Cl_2 (70 mL) along with TEAI (0.1 g) and the reaction conducted as mentioned for **7**. The crude product was chromatographed on silica gel column using EtOAc as eluant to provide pure **9** (2.97 g, 62%): $[\alpha]^{25}_D$ +9.6° (*c* = 1.35, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 1.0–1.4 (m, 9H), 1.5–2.0 (m, 4H), 3.3–4.3 (m, 6H), 4.35–4.65 (t, *J* = 12.6 Hz, 2H), 4.95–5.45 (q, *J* = 9 Hz, 2H), 5.8 (d, *J* = 7.5 Hz, 1H), 7.0–7.3 (m, 6H) and 7.5 (br s, 1H, D_2O exchangeable). Anal. Calcd for $C_{21}H_{32}N_3O_6P{\cdot}0.5H_2O$: C, 54.54; H, 7.19; N, 9.09. Found: C, 54.32; H, 6.98; N, 8.79.

2-Amino-6-(benzyloxy)-9-[[(4(*S*)-(benzyloxy)-1-(diethylphosphonyl)-3(*R*)-pentoxy]methyl]purine (10). A solution of 2-amino-6-(benzyloxy)purine (0.63, 2.6 mmol) in dry DMF (10 mL) was added dropwise to a stirred suspension of sodium hydride (0.18 g, 60%, 4.5 mmol) in dry DMF (25 mL) over a period of 15 min. The suspension was then stirred for an additional 1 h at rt. Next, chloromethyl ether **2a** (2.5 mmol)

in dry DMF (10 mL) was added dropwise to this mixture and stirred for 18 h under nitrogen. The excess solvent was then removed *in vacuo* and ice−water was added to the resulting residue. The aqueous solution was extracted with EtOAc and the organic layer dried and then concentrated under diminished pressure. The resulting gum was column chromatographed (silica gel) using EtOAc as eluant to afford **10** (1.5 g, 85%): $[\alpha]^{23}_D$ +18.9° (c = 1.42, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 1.0−1.48 (m, 9H), 1.5−2.0 (m, 4H), 3.35−4.2 (m, 6H), 4.3−4.7 (q, J = 12 Hz), 5.1 (br s, 2H), 5.4−5.7 (m, 4H), 7.1−7.55 (m, 10H), 7.65 (s, 1H). Anal. Calcd for $C_{29}H_{38}N_5O_6P$: C, 59.68; H, 6.56; N, 12.00; P, 5.31. Found: C, 59.44; H, 6.61; N, 11.86; P, 5.40.

9-[[(4(*S*)-(Benzyloxy)-1-(diethylphosphonyl)-3(*R*)-pentoxy]methyl]guanine (11). Compound **10** (0.3 g, 0.5 mmol) was dissolved in EtOH (10 mL) containing 2 mL of cyclohexene and to this mixture was added 20% $Pd(OH)_2$/C (0.1 g). The resulting suspension was refluxed for 1.5 h. After this period, the catalyst was removed by filtration (Celite) and the filtrate and wash (EtOH) were combined and concentrated *in vacuo*. The resulting gum on standing in EtOAc solidified to provide **11** (0.15 g, 60%): $[\alpha]^{25}_D$ +18.8° (c = 1.345, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 0.95−1.45 (m, 9H), 1.5−2.0 (m, 4H), 3.3−4.2 (m, 6H), 4.3−4.6 (m, 2H), 5.25−5.60 (m, 2H), 6.75 (br s, 2H), 7.2 (s, 5H), 12.15 (br s, 1H). Anal. Calcd for $C_{22}H_{32}N_5O_6P$: C, 53.54; H, 6.54; N, 14.19; P, 6.28. Found: C, 53.42; H, 6.68; N, 13.96; P, 6.11.

1-[[(4(*S*)-(Benzyloxy)-1-(dibenzylphosphonyl)-3(*R*)-pentoxy]methyl]thymine (12). Thymine (0.9 g, 6.9 mmol) was persilylated with HMDS (30 mL) and ammonium sulfate (0.1 g). The persilylated base was then dissolved in CH_2Cl_2 (50 mL) along with **3a** (5 mmol) and TBAI (0.1 g). The crude product was chromatographed on a silica gel column, and the product was obtained by eluting with EtOAc−CH_2Cl_2 (8:2) to afford pure **12** (0.7 g, 24%): $[\alpha]^{23}_D$ +16.1° (c = 0.745, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 1.1 (d, J = 6 Hz, 3H), 1.4−2.1 (m, 7H), 3.2−3.9 (m, 2H), 4.2−4.6 (q, J = 12 Hz, 2H), 4.7−5.2 (m, 6H), 7.0 (s, 1H), 7.1−7.4 (m, 15H), 8.9 (br s, 1H, D_2O exchangeable). Anal. Calcd for $C_{32}H_{37}N_2O_7P$: C,, 64.85; H, 6.29; N, 4.73; P, 5.23. Found: C, 64.64; H, 6.35; N, 4.57; P, 5.41.

1-[[(4(*S*)-(Benzyloxy)-1-(dibenzylphosphonyl)-3(*R*)-pentoxy]methyl]uracil (13). Uracil (0.67 g, 6 mmol) was silylated with HMDS (30 mL) and ammonium sulfate (0.10 g). The persilylated base was then dissolved in CH_2Cl_2 (30 mL) along with **3a** (5 mmol) and TBAI (0.1 g). The crude product was silica gel column chromatographed using CH_2Cl_2−EtOAc (7.5:2.5) as eluant to provide pure **13** (0.9g, 32%): $[\alpha]^{25}_D$ +14.6° (c = 0.565, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 1.1 (d, J = 6 Hz, 3H), 1.46−2.00 (m, 4H), 3.27−3.82 (m, 2H), 4.3−4.6 (q, J = 12 Hz, 2H), 4.8−5.2 (m, 6H), 5.6 (d, J = 7.5 Hz, 1H), 7.1−7.45 (m, 16H), 9.8 (br s, 1H, D_2O exchangeable). Anal. Calcd for $C_{31}H_{35}N_2O_7P$: C, 64.35; H, 6.10; N, 4.84; P, 5.35. Found: C, 64.23; H, 6.11; N, 4.63; P, 5.49.

2-Amino-6-(benzyloxy)-9-[[(4(*S*)-(benzyloxy)-1-(dibenzylphosphonyl)-3(*R*)-pentoxy]methyl]purine (14). 2-Amino-6-(benzyloxy)purine (1.16 g, 4.8 mmol) in DMF (20 mL) and sodium hydride (0.38 g, 60%, 9.6 mmol) in DMF (25 mL) were combined, and the resulting sodium salt was reacted with **3a** (4.8 mmol) in dry DMF. The reaction was carried out as described for **10**. Pure **14** was obtained after column chromatography (EtOAc−CH_2Cl_2, 1:1) in 32% yield (1.1 g): $[\alpha]^{25}_D$ +9.4° (c = 0.9, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 1.08 (d, J = 6.2 Hz, 3H), 1.6−2.04 (m, 4H), 3.40−3.43 (m, 1H), 3.65−3.69 (m, 1H), 4.40−4.55 (q, J = 11.8 Hz, 2H), 4.85−5.02 (m, 6H), 5.43−5.52 (m, 4H), 7.23−7.37 (m, 18H), 7.47−7.50 (m, 2H), 7.61 (s, 1H). Anal. Calcd for $C_{39}H_{42}N_5O_6P$: C, 66.18; H, 5.98; N, 9.90; P, 4.38. Found: C, 66.33; H, 6.06; N, 9.87; P, 4.44.

1-[[(4(*S*),5-Bis((benzyloxy)-1-(dimethylphosphonyl)-3(*R*)-pentoxy]methyl]thymine (15). Persilylated thymine [obtained from 1.51 g (12 mmol) of thymine] was coupled as described for **6**, with the chloromethyl ether **4a** derived from **4** (2.45 g, 6 mmol), in dry CH_2Cl_2 (50 mL) and in the presence of TEAI (50 mg). The reaction mixture was stirred and heated at reflux for 12 h. Work up and chromatography (EtOAc) afforded pure **15** (3.14 g, 96%): $[\alpha]^{25}_D$ +5.8° (c = 0.89, EtOH); ^{1}H NMR ($CDCl_3$) δ 1.50−2.08 (m, 4H), 1.26 (s, 3H), 3.26−4.18 (m, 4H), 3.64 (d, J = 11 Hz, 6H), 4.46 (s, 2H), 4.62 (s, 2H), 4.86−5.24 (m, 2H), 6.98 (s, 1H,), 7.24 (s, 10H), 10.24 (s, 1H, D_2O exchangeable). Anal. Calcd for $C_{27}H_{35}N_2O_8P$: C, 59.34; H, 6.46; N, 5.13; P, 5.67. Found: C, 59.14; H, 6.52; N, 4.97; P, 5.59.

1-[[(4(*S*),5-Bis(benzyloxy)-1-(diethylphosphonyl)-3(*R*)-pentoxy]methyl]thymine (16). Persilylated thymine [obtained from 2.52 g (20 mmol) of thymine] was coupled as described for **6** with the chloromethyl ether **5a** derived from **5** (4.2 g, 10 mmol) in dry CH_2Cl_2 (50 mL) and in the presence of TEAI (30 mg). The reaction mixture was stirred and heated at reflux for 12 h. Work up and chromatography (EtOAc−MeOH, 9:1) afforded pure **16** (3.6 g, 63%): $[\alpha]^{25}_D$ −6.3° (c = 0.615, EtOH); ^{1}H NMR ($CDCl_3$) δ 1.26 (t, J = 6 Hz, 6H), 1.46−2.12 (m, 4H), 1.82 (s, 3H), 3.59 (br s, 3H), 3.74-4.26 (m, 5H), 4.48 (s, 2H), 4.60 (d, J = 3 Hz, 2H), 5.08 (br s, 2H), 7.06 (s, 1H), 7.26 (s, 10H), 9.74 (s, 1H, D_2O exchangeable). Anal. Calcd for $C_{29}H_{39}N_2O_8P$: C, 60.62; H, 6.84; N, 4.88; P, 5.39. Found: C, 60.40; H, 6.66; N, 4.61; P, 5.51.

1-[[1-(Diethylphosphonyl-4(*S*)-hydroxy)-3(*R*)-pentoxy]-methyl]thymine (17). A solution of compound **6** (0.35 g, 10 mmol) in ethanol (80 mL) and cyclohexene (20 mL) was treated with 20% palladium hydroxide on carbon ($Pd(OH)_2$/C, 1.0 g). The resulting suspension was stirred at reflux for 12 h. After cooling to room temperature, the mixture was filtered and the filtrate was concentrated at reduced pressure. The residue was chromatographed over silica gel (ethyl acetate−methanol, 9:1) to give pure **18** (2.2 g, 78%): $[\alpha]^{25}_D$ −3.1° (c = 0.7, EtOH); ^{1}H NMR ($CDCl_3$) δ 1.12 (d, J = 6 Hz, 3H), 1.28 (t, J = 6 Hz, 6H), 1.42−2.10 (m, 2H), 1.88 (s, 3H), 3.28−4.26 (m, 7H, 1H exchanges with D_2O), 5.18 (br s, 2H), 7.16 (s, 1H), 9.84−10.46 (br m, 1H, D_2O exchangeable). Anal. Calcd for $C_{15}H_{27}N_2O_7P$: C, 47.62; H, 7.19; N, 7.40. Found: C, 47.54; H, 7.27; N, 7.47.

1-[[(1-(Dimethylphosphonyl)-4(*S*)-hydroxy-3(*R*)-pentoxy]methyl]uracil (18). Compound **18** was prepared from **7** (0.690 g, 1.62 mmol) by the method described for **17** using 20% $Pd(OH)_2$/C (0.050 g), cyclohexene (8 mL), and EtOH (9 mL) to give 0.535 g (99%) of **18**: $[\alpha]^{25}_D$ −5.5° (c = 0.435, EtOH); ^{1}H NMR ($CDCl_3$) δ 1.12 (d, J = 6 Hz, 3H), 1.46−2.06 (m, 4H), 3.44−4.02 (m, 2H), 3.72 (d, J = 11 Hz, 6H), 5.22 (s, 2H,), 5.66 (d, J = 8 Hz, 1H), 7.38 (d, J = 8 Hz, 1H). Anal. Calcd for $C_{12}H_{21}N_2O_7P$: C, 42.86; H, 6.29; N, 8.33. Found: C, 42.55; H, 6.42; N, 8.14.

1-[[1-(Diethylphosphonyl)-4(*S*)-hydroxy-3(*R*)-pentoxy]-methyl]uracil (19). Compound **19** was prepared from **8** (4.1 g, 9 mmol) by the method described for **17** using 20% $Pd(OH)_2$/C (1.5 g), cyclohexene (20 mL), and EtOH (100 mL) to provide **19** (2.47 g, 75%): $[\alpha]^{25}_D$ −3.9° (c = 1.275, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.14 (d, J = 6.4 Hz, 3H), 1.28−1.40 (t, J = 7.2 Hz, 6H), 1.73−1.82 (m, 4H), 3.60−3.92 (m, 2H), 4.00−4.18 (m, 4H), 5.20−5.38 (t, J = 7.2 Hz, 2H), 5.75 (d, J = 7.2 Hz, 1H), 6.4 (d, J = 7.2 Hz, 1H), 10.2 (br s, 1H, D_2O exchangeable). Anal. Calcd for $C_{14}H_{25}N_2O_7P$: C, 46.14; H, 6.91; N, 7.69; P, 8.50. Found: C, 46.00; H, 6.79; N, 7.60; P, 8.33.

1[[1-(Diethylphosphonyl)-4(*S*)-hydroxy-3(*R*)-pentoxy]-methyl]guanine (20). Compound **20** was prepared from **11** (1.52 g, 2.6 mmol) by the method described for **17** using 20% $Pd(OH)_2$/C (0.5 g), cyclohexene (10 mL), and EtOH (50 mL). The catalyst was removed by filtration, and the filtrate and wash were concentrated under diminished pressure to a semisolid. On standing in MeOH−EtOAc (1:2) the ester crystallized to afford 0.8 g (80%) of **20**: $[\alpha]^{25}_D$ +4.1° (c =1.335, MeOH); UV λ_{max} (H_2O) 277.5 nm (ϵ = 7388), 251 nm (ϵ = 11781), λ_{max} (pH 1) 281(ϵ = 8946), 256 nm (ϵ = 13179), λ_{max} (pH 11) 263 nm (ϵ = 11651), 257.5 nm (ϵ = 11661) ; ^{1}H NMR (Me_2SO-d_6) δ 0.94 (d, J = 6.3 Hz, 3H), 1.10−1.20 (t, J = 6.9 Hz, 6H), 1.21−1.60 (m, 4H), 3.00−4.10 (m, 6H), 4.77 (d, J = 4.8 Hz, 1H), 5.30−5.47 (q, J = 11.1 Hz, 2H), 6.57 (br s, 2H, D_2O exchangeable), 7.84 (s, 1H), 10.70 (br s, 1H, D_2O exchangeable). Anal. Calcd for $C_{15}H_{26}N_5O_6P$: C, 44.66; H, 6.50; N, 17.36; P, 7.68. Found: C, 44.14; H, 6.68; N, 16.98; P, 7.48.

1-[[4(*S*),5-Dihydroxy-1-(dimethylphosphonyl)-3(*R*)-pentoxy]methyl]thymine (21). Compound **21** was prepared from **15** (1.3 g, 2.38 mmol) by the method described for **17** using 20% $Pd(OH)_2$/C (0.2 g), cyclohexene (9.2 mL), and EtOH (18 mL), to furnish 0.860 g (100%) of **23**: $[\alpha]^{25}_D$ −1.6° (c = 0.94, EtOH); ^{1}H NMR ($CDCl_3$+D_2O) δ 1.46−2.18 (m, 4H), 1.86

(s, 3H), 3.14−4.12 (m, 4H), 3.68 (d, J = 11 Hz, 6H), 5.14 (br s, 2H), 7.18 (s, 1H). Anal. Calcd for $C_{13}H_{23}N_2O_8P$: C, 42.63; H, 6.33; N, 7.65, P, 8.46. Found: C, 42.44; H, 6.42; N, 7.43, P, 8.70.

1-[[1-(Diethylphosphonyl)-4(*S*),5-dihydroxy-3(*R*)-pentoxy]methyl]thymine (22). Compound **22** was prepared from **16** (1.72 g, 3 mmol) by the method described for **17** using 20% $Pd(OH)_2/C$ (0.1 g), cyclohexene (11 mL), and EtOH (21 mL), to give 1.1 g (93%) of **22**: $[\alpha]^{25}_D$ −1.7° (c = 1.5, EtOH); ^{1}H NMR ($CDCl_3$) δ 1.32 (t, J = 6 Hz, 6H), 1.52−2.24 (m, 4H), 1.88 (s, 3H), 3.44−4.66 (m, 10H, 2H are D_2O exchangeable), 5.20 (br s, 2H), 7.26 (s, 1H), 9.34 (br s, 1H, D_2O exchangeable). Anal. Calcd for $C_{15}H_{27}N_2O_8P$: C, 45.69; H, 6.90; N, 7.10. Found: C, 45.47; H, 6.94; N, 6.89.

1-[[1-(Ethylphosphonyl)-4(*S*)-hydroxy-3(*R*)-pentoxy]methyl]thymine (23). Diester **17** (1.0 g, 2.6 mmol) was dissolved in a ethanolic 1 N NaOH solution (18 mL; EtOH−H_2O, 1:1) and stirred at rt for 4 h. The reaction mixture was then diluted with EtOH (10 mL), neutralized with Dowex-50 resin (H^+), and filtered. The filtrate and wash were then concentrated *in vacuo* and lyophilized. The gum which was obtained was chromatographed on a silica gel column, and the column was eluted with MeOH−EtOAc(1:1) to afford pure **23** (0.8 g, 87%) as a hygroscopic gum: $[\alpha]^{25}_D$ −8.9° (c = 1.57, MeOH); ^{1}H NMR (CD_3OD) δ 1.0−1.4 (m, 6H), 1.5−2.0 (m, 7H), 3.6−4.2 (m, 6H), 5.2 (s, 2H), 7.45 (s, 1H). Anal. Calcd for $C_{13}H_{23}N_2O_7P\cdot H_2O$; C, 42.39; H, 6.84. Found: C, 42.59; H, 6.48.

1-[[1-(Ethylphosphonyl)-4(*S*)-hydroxy-3(*R*)-pentoxy]methyl]uracil (24). The preparation of **24** was conducted in the same manner as described for **23**. Diester **19** (1.0 g, 2.7 mmol) furnished **24** (0.7 g) in 73% yield: $[\alpha]^{25}_D$ −10.3° (c =1.925, MeOH); ^{1}H NMR (CD_3OD) δ 1.0−1.4 (m, 6H), 1.5−2.0 (m, 7H), 3.3−4.1 (m, 6H), 5.2 (s, 2H), 5.6 (d, J = 7.5 Hz, 1H), 7.6 (d, J = 7.5 Hz, 1H). Anal. Calcd for $C_{12}H_{21}N_2O_7P$: C, 42.85; H, 6.29; N, 8.33; P, 9.21. Found: C, 42.63; H, 6.10; N, 8.08; P, 9.06.

1-[[(4(*S*)-Hydroxy-1-phosphonyl)-3(*R*)-pentoxy]methyl]uracil (25). Method A. Bromotrimethylsilane (TMS-Br, 0.202 g, 1.32 mmol) was added dropwise, *via* syringe, at 0 °C, to the diester **18** (0.110 g, 0.33 mmol) in CH_2Cl_2 (5 mL) and the reaction mixture was stirred for 1 h at 0 °C. The volatiles were removed under diminished pressure and the residual oil was dissolved in CH_2Cl_2 (5 mL), treated with water (5 mL) and the biphasic solution stirred at rt for 10 min. The aqueous layer was separated and lyophilized to provide **25** (0.097 g, 100%) as a hygroscopic semi-solid: $[\alpha]^{25}_D$ −10.9° (c = 0.61, H_2O); ^{1}H NMR (D_2O) δ 1.14 (d, J = 6 Hz, 3H), 1.46−2.05 (m, 4H), 3.38−4.08 (m, 2H), 5.24 (s, 2H), 5.76 (d, J = 8 Hz, 1H), 7.66 (d, J = 8 Hz, 1H).Anal. Calcd for $C_{10}H_{17}N_2O_7P$: C, 38.96; H, 5.56; N, 9.10; P, 10.05. Found: C, 39.05; H, 5.69; N, 8.99; P, 9.86.

Method B. Compound **13** (0.4 g, 0.7 mmol) was dissolved in a mixture of EtOH (50 mL) and cyclohexene (10 mL). Pd/C (10%, 0.3 g) was added to this mixture, and the resulting suspension was refluxed for 2 h. The catalyst was removed by filtration (Celite), and the filtrate and wash (EtOH) were combined and concentrated to a gummy residue. The residue was dissolved in a minimal amount of water, and the water layer was extracted with CH_2Cl_2. The water layer was then lyophilized, and the resulting residue was purified on a C_{18} reverse phase column using H_2O−MeOH (8:2) as eluant. The material obtained (0.21 g, 96%) was analytically pure and identical in all respects to **25** synthesized from Method A.

1-[[4(*S*),5-Dihydroxy-1-phosphonyl-3(*R*)-pentoxy]methyl]thymine (26). Compound **26** was prepared from **24** (0.220 g, 0.6 mmol) by Method A described for **25** using TMS-Br (0.368 g, 2.4 mmol). The residue, obtained after workup, was purified by C_{18} reverse phase column chromatograpy using water as eluant to provide **26** (0.203 g, 96%): $[\alpha]^{25}_D$ +16.6° (c = 0.565, H_2O); ^{1}H NMR (D_2O) δ 1.36−2.14 (m, 4H), 1.84 (s, 3H), 3.34−3.96 (m, 4H), 5.0−5.4 (m, 2H), 7.58 (s, 1H). Anal. Calcd for $C_{11}H_{19}N_2O_8P$: C, 39.06; H, 5.66; N, 8.28; P, 9.16. Found: C, 38.95; H, 5.76; N, 8.03; P, 9.37.

1[[4(*S*)-Hydroxy-1-phosphonyl-3(*R*)-pentoxy]methyl]thymine (27). Compound **27** was prepared in the same manner described for **25** under Method B. The blocked diester **12** (0.38 g, 1.2 mmol) was dissolved in EtOH (50 mL) and cyclohexene (10 mL). The amount of 10% Pd/C used was 0.3 g. Workup and C_{18} reverse phase column purification afforded analytically pure **27** (0.2 g, 97%): $[\alpha]^{25}_D$ +8.2° (c = 0.025, H_2O); ^{1}H NMR (D_2O) δ 1.2 (d, J = 6 Hz, 3H), 1.5−2.1 (m, 7H), 3.5−4.1 (m, 2H), 5.2−5.4 (m, 2H), 7.55 (s, 1H). Anal. Calcd for $C_{11}H_{19}N_2O_7P$: C, 40.99; H, 5.94; N, 8.69; P, 9.61. Found: C, 41.12; H, 5.95; N, 8.76; P, 9.46.

9-[[4(*S*)-Hydroxy-1-phosphonyl-3(*R*)-pentoxy]methyl]guanine (28). The synthesis of **28** was carried out as described for **25** under Method B. The blocked diester **14** (0.48 g, 0.68 mmol) underwent hydrogenolysis with 0.5 g of 10% Pd/C. Workup and purification furnished **28** (0.212 g, 78%): $[\alpha]^{25}_D$ +5.2° (c = 0.61, H_2O); UV λ_{max} (pH 1) sh 282 nm (ϵ = 9000), 254.5 nm (ϵ = 14000), λ_{max} (H_2O) sh 277 nm (ϵ = 8827), 252 nm (ϵ = 13517), λ_{max} (pH 11) 260 nm (ϵ = 11000); ^{1}H NMR (D_2O) δ 0.93 (d, J = 6.2 Hz, 3H), 1.08−1.56 (m, 4H), 3.41−3.46 (m, 1H) 3.68−3.76 (m, 1H), 5.35−5.43 (ABq, J = 11.4 Hz, 2H), 7.80 (s, 1H). Anal. Calcd for $C_{11}H_{18}N_5O_6P\cdot 3.5H_2O$: C, 32.30; H, 6.14; P, 7.55. Found: C, 32.58; H, 6.40; P, 7.52.

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