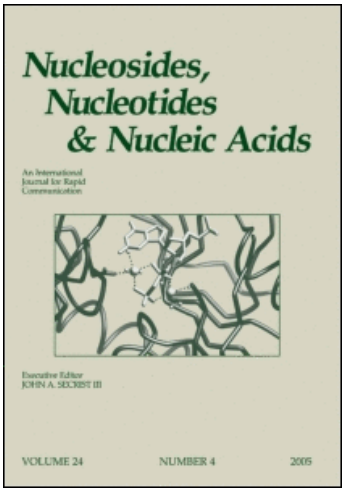

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A New Synthesis of the Potent and Selective Anti-Herpesvirus Agent (*S*)-1-[3-Hydroxy-2-(Phosphonylmethoxy)Propyl]Cytosine

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A NEW SYNTHESIS OF THE POTENT AND SELECTIVE ANTI-HERPESVIRUS AGENT (S)-1-[3-HYDROXY-2-(PHOSPHONYLMETHOXY)PROPYL]CYTOSINE

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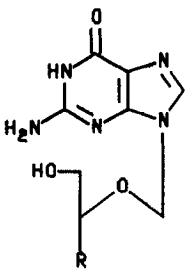
Abstract. A new synthetic approach to (S)-1-[3-hydroxy-2-(phosphonylmethoxy)propyl]cytosine (**3**, (S)-HPMPC) is based on coupling of the heterocyclic moiety with a glycerol-derived side chain, followed by introduction of the phosphonylmethyl ether group.

Acyclic analogues of naturally-occurring nucleosides represent an important class of antiviral agents.[1] For example, the 2'-deoxyguanosine analogue, acyclovir (**1**, ACV), has good activity against herpes simplex viruses (HSV) and varicella-zoster virus (VZV), and has been used extensively for treatment of these viral infections with minimal side effects.[2] The related acyclic derivative ganciclovir (**2**, DHPG) is particularly effective against cytomegalovirus (CMV) and has recently been approved for the treatment of CMV retinitis in AIDS patients. In order to exert an antiviral effect, nucleoside analogues such as ACV and DHPG first require activation to a triphosphate derivative. In the case of acyclovir, a virus-specified thymidine kinase (TK) is responsible for conversion of ACV to ACV-monophosphate. Subsequent phosphorylation is carried out by cellular kinases, and the resulting triphosphate derivative acts by inhibition of viral DNA synthesis through interaction with viral DNA polymerase.[3] ACV has significantly diminished activity against viruses that lack thymidine kinase, such as CMV and TK-deficient strains of HSV.

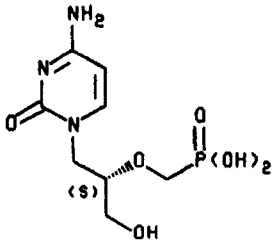
Because the efficiency of the phosphorylation process is an important factor in determining the antiviral activity of these

nucleoside analogues, there has been considerable interest in the synthesis of acyclic analogues of nucleoside phosphates (nucleotides) as well.[4-6] In 1986, De Clercq and Holy introduced a novel class of acyclic nucleotide analogues which possess broad-spectrum antiviral activity.[7,8] Members of this family are characterized by the presence of a stable phosphonylmethyl ether group [RO-CH_2-P(O)$(OH)_2$] in place of the labile phosphate moiety. The oxygen atom β to the phosphorous serves an important role in conferring electronic properties similar to those of the phosphate to the phosphonate group. In addition, these compounds bear an alkyl linkage to the heterocyclic base in place of the usual glycosylic bond. We have been interested in evaluation of the cytosine derivative (S)-1-[3-hydroxy-2-(phosphonylmethoxy)propyl]cytosine (3, HPMPC), a compound which has good in vitro activity against herpes simplex viruses,[9-11] and is more potent than ACV in vivo against both HSV 1 and HSV 2 infections in mice.[9,10] HPMPC is also an effective inhibitor of human cytomegalovirus in vitro[9,12] and has demonstrated greater in vivo efficacy than DHPG in a murine CMV infection model.[10,13] In this paper, we report a new synthetic route to this highly promising antiviral agent which involves simpler isolation procedures and fewer chromatographic purifications than our original route,[9,14] and is amenable to scale-up.[15]



1, R = H (ACV)

2, R = CH_2OH (DHPG)



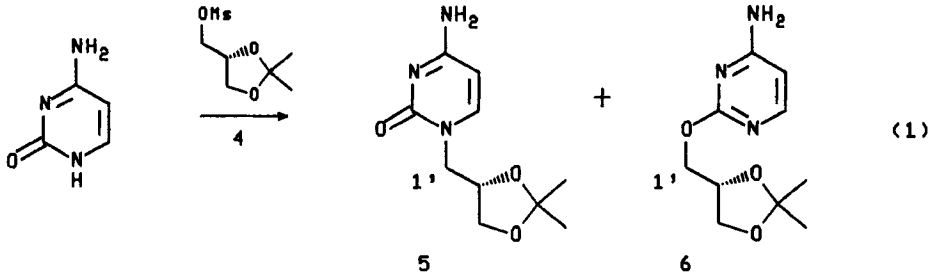
3, HPMPC

Results and Discussion

Our earlier route to HPMPC[9,14] was based on preparation of a protected form of the acyclic HPMP side-chain, followed by coupling with

cytosine, and then deprotection. Since the side-chain can be coupled with a variety of heterocyclic bases, this approach has also proved useful for the synthesis of related HPMP-derived nucleotide analogues. However, a drawback to this route is the absence of crystalline intermediates and the necessity for several chromatographic purifications. In our new approach to HPMPC, the cytosine base is introduced early in the synthetic sequence, and as a result, many of the intermediates are solids which can be isolated by crystallization.

The coupling reaction of cytosine with the mesylate derivative of (S)-2,3-O-isopropylideneglycerol (**4**)[16] was examined first (eq 1). We[9,14] and others[17] have previously reported the formation of both N^1- and O^2-isomeric products in alkylation reactions with cytosine. In a similar fashion, reaction of cytosine with **4** in the presence of cesium carbonate (DMF, 100 °C) provided a 5:1 mixture of **5**[18] and **6**. The use of sodium hydride or potassium tert-butoxide instead of Cs_2CO_3 to promote the coupling reaction did not significantly change the ratio of isomers. Because **5** is soluble in water, an aqueous workup procedure could not be used. Instead, the desired product **5** was isolated in 53-57% yield by concentration of the reaction mixture, followed by simple chromatography to remove the salt by-products formed in the reaction. Alternatively, the O^2-alkylated product **6** could be removed prior to chromatography by treatment of the reaction



(1)

mixture with ethyl acetate. The solid material was collected by filtration to provide a mixture of **5** and insoluble salts, while the less polar isomer **6** remained in solution.

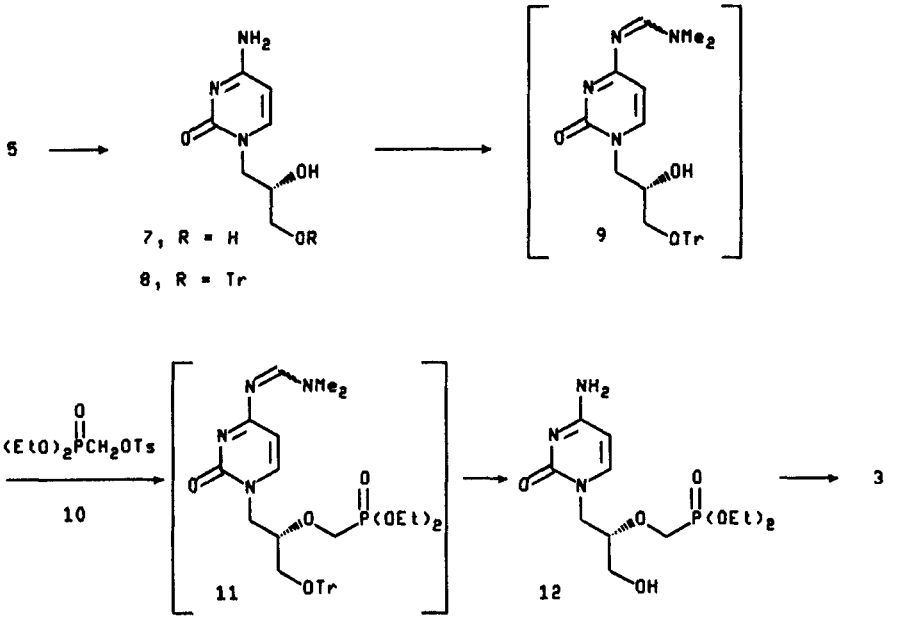
Structural assignments for **5** and **6** are based on their NMR (^1H and ^{13}C) and UV spectral data.[9] The chemical shifts of the cytosine ring carbons and protons are characteristic for each isomer. In addition,

the ^{13}C NMR signal for C-1' appears at 50 ppm for the N-alkyl product **5**, while for the O-alkyl isomer **6**, C-1' is further downfield at 65 ppm. It is interesting to compare these alkylation reactions with glycosylation reactions of cytosine run under Vorbruggen coupling conditions.[19] In the latter case, the isolation of only N-alkylated products may be attributed to equilibration to the thermodynamically-favored isomers. Attempted thermal isomerization of **6** to **5** in a manner analogous to the Chapman rearrangement was unsuccessful.[20]

The acetonide protecting group of **5** was removed under aqueous acidic conditions (80% aq. acetic acid or 0.25 M H_2SO_4) to provide N^1-[(2,3-dihydroxy)propyl]cytosine (**7**)[18,21] in over 90% yield as a highly crystalline solid (Scheme I). In order to selectively introduce the phosphonylmethyl ether group at the 2'-position on the side chain, it was next necessary to protect the primary hydroxyl group, and possibly the amino functionality as well. Reaction of **7** with 1.1 equiv of trityl chloride afforded the 3'-O-monotrityl derivative **8** as the major product, along with smaller amounts of bis- and tris-tritylated by-products. **8** was obtained in 40-50% yield upon crystallization from the crude reaction mixture. Preparation of an N^4,$O^{3'}$-bistrityl derivative by reaction of **7** with 2 equiv of trityl chloride was also examined; however, isolation of the desired product proved difficult since a mixture of several products was formed as a result of competitive tritylation of the secondary hydroxyl and amino groups following initial formation of the primary trityl ether.

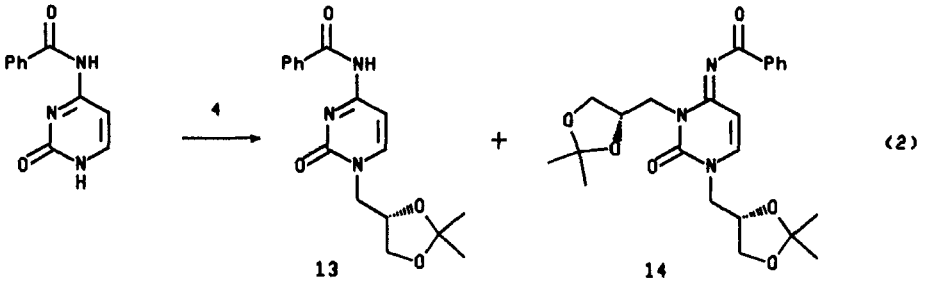
Introduction of the 2'-O-phosphonylmethyl ether group was first attempted by reaction of **8** with sodium hydride (DMF, 22 °C) and diethyl [(tosyloxy)methyl]phosphonate (**10**);[22] however, only a low yield of the desired product was obtained, and examination of the reaction side products indicated that alkylation had occurred on the free amine as well. In order to circumvent this problem, the amine was protected as an amidine derivative.[23] Thus, **8** was treated with DMF dimethyl acetal, the reaction mixture was concentrated, and the product **9** was used without purification in the alkylation reaction with **10** to provide **11**, a fully protected precursor of HPMPC. This product was not purified, but instead treated directly with acetic acid to provide the diethyl ester of HPMPC (**12**)[9,14] in 64% overall yield from **8**. Reaction of **12** with bromotrimethylsilane (TMSBr) in CH_3CN as previously described[9,14] afforded HPMPC (**3**) in 87% yield.

Scheme I



One of the difficulties encountered in the scale-up of this route to HPMPC was the need for chromatography in the isolation of acetonide **5**. A convenient solution to this problem was found in protection of cytosine as its less polar $\underline{N}^4$-benzoyl derivative[24] prior to the coupling reaction with mesylate **4** (eq 2). In this case, salts present in the final reaction mixture could be separated from the alkylated product by extraction with water, thereby simplifying the isolation procedure. For example, following an aqueous workup, **13** was isolated by crystallization in 40% yield from the reaction of $\underline{N}^4$-benzoylcytosine with t-BuOK and **4** (DMSO, 100 °C). The assignment for **13** was based on spectroscopic data, and was confirmed by conversion of **5** to **13** upon treatment with benzoic anhydride in pyridine. An additional product formed in the coupling reaction using either t-BuOK or Cs_2CO_3 was the $\underline{N}^1,\underline{N}^3$-bisalkylated derivative **14**. Chromatographic purification of the reaction mixture from a Cs_2CO_3-promoted coupling provided **14** in 31% yield, along with a 38% yield of **13**, indicating that a significant amount of mesylate **4** was consumed in this side reaction. The structure of **14** was assigned on the basis of its NMR and UV spectra, mass spectrum (parent ion at 443), and elemental analysis.

While similar $\underline{N}^1,\underline{N}^3$-disubstituted derivatives are well-known in alkylation reactions of uracil and thymine,[18,19,21] such side-products are unusual in coupling reactions of cytosine or its acylated derivatives which give rise instead to $\underline{O}$-alkylated side-products.[9,14,17,25]

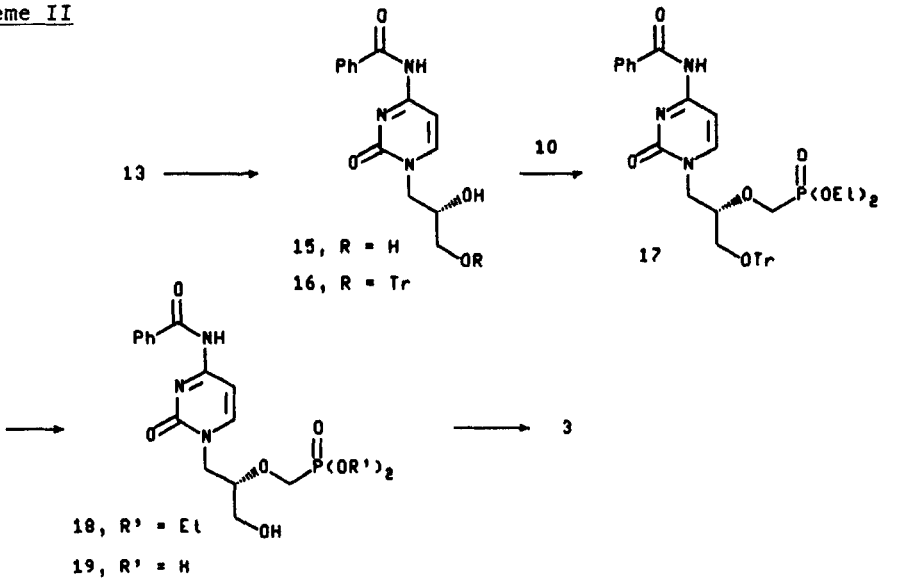


(2)

Even though the isolated yield of **13** was only moderate, the convenient purification of this product offered a clear advantage over the coupling reaction using cytosine. Furthermore, protection of the amine as a benzamide derivative proved useful in subsequent steps in the reaction sequence. Treatment of **13** with HCl in methanol effected removal of the acetonide group to provide diol **15** in 95% yield as a crystalline solid (Scheme II). Reaction of **15** with 1.1 equiv of trityl chloride (DMAP, pyridine) proceeded smoothly and provided the desired monotritylated product **16**. This $\underline{N}^4,\underline{O}^{3'}$-diprotected intermediate could be used without purification in the reaction with diethyl [(tosyloxyl)methyl]phosphonate (**10**)[22] (NaH, DMF). The resulting product **17** was not purified, but treated directly with HCl in CH_2Cl_2 to provide alcohol **18**. The reaction mixture was purified by column chromatography to give **18** as a foam in 62% overall yield for the three-step sequence from **15**. Reaction of **18** with TMSBr in CH_2Cl_2 then afforded crystalline $\underline{N}^4$-benzoyl HPMPC (**19**) in 85% yield. The amino protecting group was removed by treatment with ammonium hydroxide to give HPMPC (**3**) in 87% yield as an ammonium salt. Adjustment of the pH of an aqueous solution of the ammonium salt to 3.5 by addition of HCl resulted in crystallization of HPMPC in its zwitterionic form.

When aqueous acetic acid was used in the conversion of trityl ether **17** to alcohol **18**, hydrolysis of the benzoylcytosine moiety occurred as a side reaction to give the corresponding uracil derivative. The degree of deamination increased with prolonged reaction times and elevated temperatures (>80 °C). To confirm the

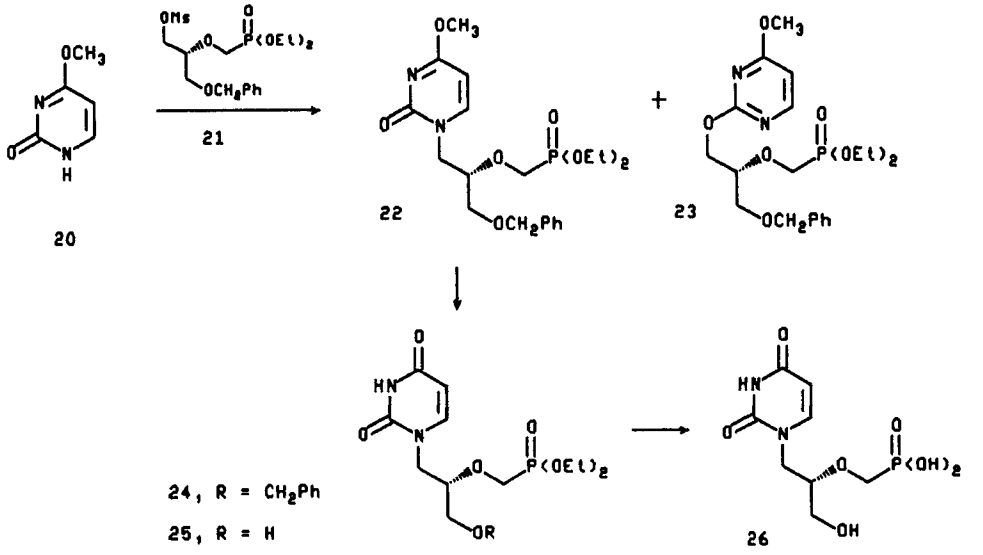
Scheme II



identity of this product, access to the uracil series was achieved by an alternate route (Scheme III). Thus, alkylation of 4-methoxy-2-pyrimidinone (**20**)[26] with phosphonate **21**[9] (Cs_2CO_3, DMF, 100 °C) provided the $\underline{N}^1$-alkylated product **22** in 49% yield after separation from the less polar $\underline{O}^2$-isomer **23** (25% yield) by chromatography. Treatment of **22** with HCl in ethanol afforded the uracil derivative **24** in 90% yield and removal of the benzyl protecting group via transfer hydrogenation (90% yield) then afforded an authentic sample of **25**. The diester was converted to HPMPU (**26**) in quantitative yield by treatment with TMSBr in CH_3CN. The uracil derivatives are clearly distinguishable from their cytosine counterparts by UV and NMR spectroscopy.

Since in vitro biological tests have shown that only the (S)-isomer of HPMPC possesses antiherpesvirus activity, it was important to determine the enantiomeric purity of HPMPC produced in the above synthetic sequences.[27] For this purpose, an HPLC method was developed which employs a reverse phase (C18) column and a mobile phase containing phenylalanine in the presence of cupric sulfate.[28] Resolution of the (R)- and (S)-isomers of HPMPC was readily accomplished under these conditions; furthermore, the order of elution was dependent on whether (D)- or (L)-phenylalanine was used in the mobile phase. Results of the HPLC analysis show that HPMPC produced

Scheme III



by either route contains >99% of the (S)-isomer, indicating that the enantiomeric integrity of the starting glycerol acetonide is maintained throughout both reaction sequences.

In summary, our new approach to (S)-HPMPC has allowed for convenient preparation of this promising antiviral agent. In particular, the route commencing with N^4-benzoylcytosine required only one chromatographic purification, and provided the final product in 17% overall yield. Our studies on cytosine chemistry should prove useful in the synthesis in other cytosine-based derivatives as well.

EXPERIMENTAL

Melting points were determined on an Electrothermal Digital capillary apparatus and are uncorrected. Thin layer chromatography was performed on silica gel 60 F-254 plates purchased from E. Merck and Co.; column chromatography was carried out using Woelm silica gel (32 - 63 micron). IR spectra were obtained using a Perkin-Elmer 1800 FT-IR spectrophotometer. UV spectra were recorded on a Hewlett-Packard 8452A Diode Array spectrophotometer. NMR spectra were recorded on a Bruker AM 300 or 360 NMR spectrometer or a Varian Gemini 300 NMR spectrometer. Chemical shifts are expressed in parts per

million (δ) using tetramethylsilane as an internal standard. Low-resolution mass spectra were measured on a Finnegan 4500 spectrometer. Spectroscopic data and elemental analyses were obtained by the Analytical Research Department, Bristol-Myers Squibb, Wallingford and Syracuse.

(R)-2,3-O-Isopropylideneglycerol methanesulfonate (4)

A solution of (S)-2,3-O-isopropylideneglycerol[29] (50.0 g, 0.378 mol) in CH_2Cl_2 (350 mL) was cooled to 0 °C under argon and treated with Et_3N (57.4 g, 0.567 mol) in one portion. A solution of methanesulfonyl chloride (43.3 g, 0.378 mol) in CH_2Cl_2 (50 mL) was added dropwise over 40 min to give a pale yellow slurry. After 1.5 h at 0 °C, the reaction mixture was allowed to warm to room temperature and was stirred further for 4 h. The slurry was then treated with water (150 mL) and diluted with CH_2Cl_2 (200 mL). The layers were separated, and the organic layer was washed with saturated $NaHCO_3$ solution (150 mL) and saturated NaCl solution (200 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated to provide 78.1 g (98%) of **4** as a clear, pale yellow oil. The material was used in the following reaction without purification. A portion was purified by column chromatography on silica gel (10:1, 1% CH_3OH/CH_2Cl_2) to provide a sample of **4** as a clear, colorless oil: $[\alpha]_D^{20}$ -4.63° (c = 5.5, MeOH) [lit[16] $[\alpha]_D^{27}$ -3.43° (c = 6.42, benzene)]; ^1H NMR ($CDCl_3$) δ 4.29-4.37 (m, 1 H, C<u>H</u>), 4.17 (d, <u>J</u> = 5 Hz, 2 H, C<u>H</u>$_2$OMs), 4.05 (dd, <u>J</u> = 6.5, 9 Hz, 1 H, 1 from C<u>H</u>$_2$O), 3.77 (dd, <u>J</u> = 5, 9 Hz, 1 H, 1 from C<u>H</u>$_2$O), 3.02 (s, 3H, $SO_2C\underline{H}_3$), 1.39 (s, 3 H, C<u>H</u>$_3$), and 1.32 (s, 3 H, C<u>H</u>$_3$); ^{13}C NMR ($CDCl_3$) δ 110.20 (O<u>C</u>O), 73.23 (<u>C</u>H), 69.20 (<u>C</u>H$_2$OMs), 65.81 (<u>C</u>H$_2$O), 37.62 ($SO_2\underline{C}H_3$), 26.64 (<u>C</u>H$_3$), and 25.15 (<u>C</u>H$_3$); mass spectrum (DCI), <u>m/e</u> (rel intensity) 211 (MH^+, 100), 195 (50), 153 (100), 115 (65). Anal. Calcd for $C_7H_{14}O_5S$: C, 39.99; H, 6.71. Found: C, 40.03; H, 7.09.

(S)-2,3-O-Isopropylidene-N^1-[(2,3-dihydroxy)propyl]cytosine (5)

Procedure A. A mixture of mesylate **4** (10.0 g, 0.048 mol), cytosine (6.30 g, 0.057 mol), and Cs_2CO_3 (18.6 g, 0.057 mol) in anhydrous DMF (50 mL) was heated under argon at 100 °C in a 250-mL, three-necked, round-bottomed flask equipped with a mechanical stirrer and reflux condenser. The reaction mixture was stirred at 100 °C for

18 h, then allowed to cool to room temperature, and concentrated in vacuo. The resulting white solid was slurried in hot 15% CH_3OH/CH_2Cl_2. The mixture was allowed to cool to room temperature, and the insoluble material was removed by filtration. The filtrate was concentrated in vacuo to give a white solid which was purified by column chromatography on silica gel (10:1, gradient 1% to 10% CH_3OH/CH_2Cl_2) to afford 6.10 g (57%) of the $\underline{N}^1$-alkylated isomer **5** along with 1.23 g (11%) of the $\underline{O}^4$-isomer **6**. On a larger scale run, reaction of mesylate **4** (65.3 g, 0.31 mol), cytosine (41.4 g, 0.37 mol), and cesium carbonate (121.5 g, 0.37 mol) in 250 mL of DMF provided 37.0 g (53%) of **5**.

Procedure B. A 1-L, three-necked, round-bottomed flask equipped with a mechanical stirrer and reflux condenser was charged with NaH (11.8 g, 80% dispersion in oil, 0.39 mol) and anhydrous DMF (350 mL). Cytosine (43.6 g, 0.39 mol) was added portionwise over 30 min, and the off-white, foamy reaction mixture was heated at 100 °C for 1 h. A solution of mesylate **4** (75.0 g, 0.36 mol) in DMF (50 mL) was added next via cannula over 30 min, and the reaction mixture was stirred at 100 °C for 20 h. The off-white slurry was allowed to cool to room temperature, treated with ethyl acetate (700 mL), and cooled to 0 °C. The solid was collected by filtration and then purified by column chromatography as above to provide 45.4 g (56%) of **5**.

For **5**: mp 272-273 °C (dec); $[\alpha]_D^{20}$ -50.1° (c = 1.26, MeOH); UV_{max} (MeOH) 274 (ε = 7900); ^1H NMR (Me_2SO-$\underline{d}_6$) δ 7.50 (d, $\underline{J}$ = 7 Hz, 1 H, H-6), 7.08 (br s, 2 H, NH_2), 5.65 (d, $\underline{J}$ = 7 Hz, 1 H, H-5), 4.22-4.29 (m, 1 H, H-2'), 3.95 (dd, $\underline{J}$ = 6.5, 8.5 Hz, 1 H, H-3'), 3.82 (dd, $\underline{J}$ = 4, 14 Hz, 1 H H-1'), 3.63-3.71 (m, 2 H, H-1' and H-3'), 1.29 (s, 3 H, $C\underline{H}_3$), and 1.21 (s, 3 H, $C\underline{H}_3$); ^{13}C NMR (Me_2SO-$\underline{d}_6$) δ 165.98 (C-4), 155.90 (C-2), 146.79 (C-6), 108.48 (O$\underline{C}$O), 92.91 (C-5), 73.49 (C-2'), 65.91 (C-3'), 50.68 (C-1'), 26.49 ($\underline{C}H_3$), and 25.17 ($\underline{C}H_3$); mass spectrum (DCI), m/e (rel intensity) 226 (MH^+, 70), 168 (100). Anal. Calcd for $C_{10}H_{15}N_3O_3$: C, 53.32; H, 6.71; N 18.66. Found: C, 53.22; H, 6.79; N 18.60.

For **6**: mp 93-95 °C; UV_{max} (MeOH) 272 (ε = 7000) 228 (ε = 8300); ^1H NMR (Me_2SO-$\underline{d}_6$) δ 7.83 (d, $\underline{J}$ = 6 Hz, 1 H, H-6), 6.85 (br s, 2 H, NH_2), 6.06 (d, $\underline{J}$ = 6 Hz, 1 H, H-5), 4.29-4.37 (m, 1 H, H-2'), 4.15 (d, $\underline{J}$ = 5 Hz, 2 H, H-1'), 4.04 (dd, $\underline{J}$ = 6, 8 Hz, 1 H, H-3'), 3.70 (dd, $\underline{J}$ =

6, 8 Hz, 1 H, H-3'), 1.33 (s, 3 H, $C\underline{H}_3$), and 1.27 (s, 3 H, $C\underline{H}_3$); ^{13}C NMR (Me_2SO-$\underline{d}_6$) δ 165.34 (C-4), 164.49 (C-2), 156.10 (C-6), 108.56 ($O\underline{C}O$), 99.50 (C-5), 73.40 (C-2'), 66.42 (C-1'), 65.82 (C-3'), 26.57 ($\underline{C}H_3$), and 25.32 ($\underline{C}H_3$); mass spectrum (FAB), m/e (rel intensity) 226 (MH^+, 100), 210 (10), 168 (50). Anal. Calcd for $C_{10}H_{15}N_3O_3$: C, 53.32; H, 6.71; N, 18.66. Found: C, 53.17; H, 6.87; N, 18.49.

(S)-N^1-[(2,3-Dihydroxy)propyl]cytosine (7)

Procedure A. A solution of acetonide **5** (20.0 g, 0.089 mol) in 80% aqueous acetic acid (200 mL) was heated on a steam bath for 3 h, allowed to cool to room temperature, and then concentrated in vacuo. The residue was evaporated with three 200-mL portions of water and once with 200 mL of EtOH. The resulting solid was purified by recrystallization from EtOH to afford 14.7 (90%) of **7** as a white solid.

Procedure B. A solution of acetonide **5** (43.0 g, 0.19 mol) in 0.25 M H_2SO_4 solution (625 mL) was stirred at room temperature for 12 h. The reaction mixture was then neutralized to pH 7 by addition of saturated $Ba(OH)_2$ solution. The resulting slurry was heated briefly on a steam bath, and the insoluble material was removed by filtration through Celite, washing the pad with hot water. Concentration of the filtrate provided a white solid which was heated in 250 mL of EtOH on a steam bath and then cooled to 0 °C. The solid was collected by filtration, washed with cold diethyl ether, and dried in vacuo to provide 35.1 g (99%) of **7** as a white solid: mp 190-193 °C; $[\alpha]_D^{20}$ -87.6° (c = 0.94, MeOH); UV_{max} (MeOH) 274 (ε = 7,800); ^1H NMR (Me_2SO-$\underline{d}_6$) δ 7.47 (d, $\underline{J}$ = 7 Hz, 1 H, H-6), 7.07 (br s, 2 H, NH_2), 5.65 (d, $\underline{J}$ = 7 Hz, 1 H, H-5), 4.98 (d, $\underline{J}$ = 5 Hz, 1 H, exch, OH), 4.75 (br s, 1 H, exch, OH), 3.91 (dd, $\underline{J}$ = 4, 13 Hz, 1 H, H-1'), 3.65-3.75 (m, 1 H, H-2'), 3.24-3.45 (m, 3 H, H-1', 2 x H-3'); ^{13}C NMR (Me_2SO-$\underline{d}_6$) δ 165.97 (C-4), 156.44 (C-2), 147.27 (C-6), 92.74 (C-5), 69.40 (C-2'), 63.38 (C-3'), and 51.93 (C-1'); mass spectrum (DCI), m/e (rel intensity) 186 (MH^+, 100), 168 (10). Anal. Calcd for $C_7H_{11}N_3O_3$: C, 45.40; H, 5.99; N, 22.69. Found: C, 45.15; H, 6.08; N, 22.40.

(S)-N^1-[(2-Hydroxy-3-triphenylmethoxy)propyl]cytosine (8)

A solution of **7** (20.0 g, 0.11 mol), triphenylmethyl chloride (36.0 g, 0.13 mol), and 4-(dimethylamino)pyridine (0.66 g, 0.005 mol)

in DMF (200 mL) was treated with Et_3N (21.8 g, 0.22 mol) rapidly via syringe. The resulting yellow slurry was placed in an oil bath preheated to 80 °C and then heated at 80 °C for 3.5 h. The reaction mixture was allowed to cool to room temperature, diluted with ethyl acetate (700 mL), and washed with water (300 mL), saturated $NaHCO_3$ solution (300 mL), and saturated NaCl solution (250 mL). The organic layer was dried briefly over anhydrous Na_2SO_4 and filtered, and the filtrate was allowed to stand at room temperature for 14 h. The resulting precipitate was collected by filtration in two crops to provide a total of 21.7 g (47%) of **8** as a white solid: mp 223-225 °C; $[\alpha]_D^{20}$ -34.5° (c = 0.83, MeOH); UV_{max} (MeOH) 274 (ε = 8,300); ^1H NMR (Me_2SO-d_6) δ 7.25-7.48 (m, 16 H, ArH and H-6), 6.97 (br s, 2 H, NH_2), 5.56 (d, J = 7 Hz, 1 H, H-5), 5.25 (d, J = 6 Hz, 1 H, exch, OH), 3.93-4.05 (m, 2 H, H-1' and H-2'), 3.38 (dd, J = 8, 13 Hz, 1 H, H-1'), and 2.85-2.96 (m, 2 H, H-3'); ^{13}C NMR (Me_2SO-d_6) δ 166.05 (C-4), 156.10 (C-2), 147.26 (C-6), 143.82 (ArC), 128.36 (ArC), 127.95 (ArC), 127.06 (ArC), 92.68 (C-5), 85.89 ($OCPh_3$), 67.61 (C-2'), 65.96 (C-3'), and 48.72 (C-1'); mass spectrum (DCI), m/e (rel intensity) 428 (MH^+, 20), 271 (10), 243 (100), 186 (15). Anal. Calcd for $C_{26}H_{25}N_3O_3$: C, 73.05; H, 5.90; N, 9.83. Found: C, 73.12; H, 6.03; N, 9.76.

Diethyl [(tosyloxy)methyl]phosphonate (10)

A three-necked, 1 L, round-bottomed flask equipped with an argon inlet adapter and reflux condenser was charged with diethyl phosphite (107 g, 0.78 mol), paraformaldehyde (25.6 g, 0.85 mol), and Et_3N (7.80 g, 0.08 mol) and then placed in an oil bath preheated to 110 °C. Within 10 min, the white slurry became a clear, colorless solution. The mixture was stirred at 110 °C for 3 h and then allowed to cool to room temperature.

The resulting clear solution was treated with pyridine (350 mL) and cooled to 0 °C. p-Toluenesulfonyl chloride (163 g, 0.85 mol) was added portionwise over 5 min, and the mixture was allowed to warm to room temperature over 16 h. The yellow slurry was treated with ethyl acetate (300 mL), and insoluble material was removed by filtration. The filtrate was slowly treated with saturated $NaHCO_3$ solution (400 mL), and the organic phase was washed with water (400 mL) and saturated NaCl solution (400 mL), dried over anhydrous $MgSO_4$, and

filtered. The yellow solution was concentrated and the residue was evaporated with toluene (2 x 250 mL) to give 196 g of **10** (79%) as a clear, yellow oil which was used without purification: ^{1}H NMR ($CDCl_3$) δ 7.78 (d, J = 8 Hz, 2 H, TsH), 7.37 (d, J = 8 Hz, 2 H, TsH), 4.05-4.30 (m, 6 H, 2 x $POCH_2$, OCH_2P), 2.42 (s, 3 H, $TsCH_3$), and 1.28 (t, J = 6 Hz, 6 H, $POCH_2CH_3$).

(S)-N^1-[(3-Hydroxy-2-diethylphosphonylmethoxy)propyl]cytosine (12)

DMF dimethylacetal (14.8 g, 0.124 mol) was added in one portion to a solution of **8** (26.5 g, 0.062 mol) in anhydrous DMF (100 mL). The yellow solution was heated at 55 °C for 2.5 h and then concentrated in vacuo. The residue was evaporated from DMF (75 mL) and the resulting thick yellow oil was placed under high vacuum for 16 h. On a separate run, the amidine derivative **9** was purified by column chromatography on silica gel (20:1, gradient 3% to 10% ethanol/CH_2Cl_2): UV_{max} (MeOH) 316 (ε = 21,500); ^{1}H NMR (Me_2SO-d_6) δ 8.56 (s, 1 H, N=CH) 7.57 (d, J = 7 Hz, 1 H, H-6), 7.21-7.42 (m, 15 H, ArH), 5.81 (d, J = 7 Hz, 1 H, H-5), 5.27 (d, J = 6 Hz, 1 H, exch, OH), 4.07 (dd, J = 3.5, 13 Hz, 1 H, H-1'), 3.88-4.02 (m, 1 H, H-2'), 3.47 (dd, J = 9, 13 Hz, 1 H, H-1'), 3.12 (s, 3 H, CH_3), 3.00 (s, 3 H, CH_3) and 2.84-2.96 (m, 2 H, H-3'); ^{13}C NMR (Me_2SO-d_6) δ 171.29 (C-4), 157.46 (N=CH), 155.96 (C-2), 148.37 (C-6), 143.67 (ArC), 128.23 (ArC), 127.79 (ArC), 126.91 (ArC), 100.38 (C-5), 85.78 ($OCPh_3$), 67.26 (C-2'), 65.85 (C-3'), 52.94 (C-1'), 40.65 (CH_3), and 34.65 (CH_3); mass spectrum (FAB), m/e (rel intensity) 483 (MH^+, 100), 243 (100).

Unpurified **9** from above was dissolved in DMF (100 mL) and treated portionwise with sodium hydride (2.80 g, 80% dispersion in oil, 0.093 mol). Some foaming was observed, and the resulting brown cloudy solution was stirred at room temperature for 3 h and then cooled to 0 °C with an ice bath. The cooled slurry was added via cannula (15 gauge) over 40 min to a 0 °C solution of tosylate **10** (29.9 g, 0.093 mol) in DMF (40 mL). The mixture was stirred at 0 °C for 1.5 h and then allowed to warm to room temperature. After a total reaction time of 3 h, EtOH (20 mL) was added to the resulting red slurry. The mixture was diluted with ethyl acetate (1 L) and extracted with water (300 mL). The aqueous layer was extracted with ethyl acetate (300 mL), and the combined organic layers were washed with 50% saturated

$NaHCO_3$ solution (300 mL) and saturated NaCl solution (300 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated.

The residue was dissolved in 80% aqueous acetic acid (300 mL), and the mixture was heated at 90 °C for 3 h. The reaction mixture was allowed to cool to room temperature, water (200 mL) was added, and the slurry was filtered to remove insoluble material (trityl alcohol). Concentration of the filtrate in vacuo, followed by coevaporation of the residue with toluene (2 x 150 mL), and drying in vacuo gave 50 g of a yellow-orange residue. Purification by column chromatography on silica gel (10:1, gradient 3% to 15% CH_3OH/CH_2Cl_2) provided 13.3 g (64%) of **12** as a pale yellow foam: $[\alpha]_D^{20}$ -90.7° (c = 0.53, MeOH); UV_{max} (MeOH) 274 (ε = 7,900), 234 (ε = 6,600); ^1H NMR (Me_2SO-d_6) δ 7.41 (d, J = 7 Hz, 1 H, H-6), 7.25 (br m, 2 H, exch, NH_2), 5.60 (d, J = 7 Hz, 1 H, H-5), 4.81 (br s, 1 H, exch, OH), 3.86-4.03 (m, 6 H, H-1', H-2', 2 x $POCH_2$), 3.76 (dd, J = 5, 14 Hz, 1 H, H-1'), 3.54-3.64 (m, 2 H, OCH_2P), 3.41-3.52 (m, 2 H, H-3'), 1.19 (t, J = 6 Hz, 3 H, $POCH_2CH_3$), and 1.18 (t, J = 6 Hz, 3 H, $POCH_2CH_3$); ^{13}C NMR (Me_2SO-d_6) δ 166.13 (C-4), 156.21 (C-2), 147.08 (C-6), 92.99 (C-5), 80.18 (d, J = 12 Hz, C-2'), 62.83 (d, J = 170 Hz, OCH_2P), 61.78 (d, J = 6 Hz, $POCH_2$), 60.31 (C-3'), 49.48 (C-1'), and 16.33 (d, J = 6 Hz, $POCH_2CH_3$); mass spectrum (DCI), m/e (rel intensity) 336 (MH^+, 100), 318 (15), 290 (25). Anal. Calcd for $C_{12}H_{22}N_3O_6P \cdot 0.25\ H_2O$: C, 42.42; H, 6.68; N, 12.37. Found: C, 42.41; H, 6.67; N, 12.14.

(S)-N^1-[(3-Hydroxy-2-phosphonylmethoxy)propyl]cytosine (3)

Bromotrimethylsilane (34.7 g, 0.227 mol) was added dropwise via syringe to a solution of phosphonate ester **12** (9.50 g, 0.028 mol) in CH_3CN (110 mL). The yellow solution was stirred at room temperature for 20 h, concentrated in vacuo, and then coevaporated with CH_3CN (2 x 100 mL) to afford a yellow foam. The residue was dissolved in 60 mL of water and extracted with CH_2Cl_2, and the aqueous layer was concentrated to a 20 mL volume. Ethanol (200 mL) was added, and the white slurry was stirred at room temperature for 2 h and then cooled to 0 °C. The solid material was collected by filtration and washed with cold ethanol. The solid was dissolved in water and the solution was lyophilized to afford 6.90 g (87%) of **3**[9,14] as a fluffy white powder: mp 260 °C (decomp); $[\alpha]_D^{20}$ -97.3° (c = 0.80, H_2O); ^1H NMR (D_2O)

δ 7.90 (d, J = 8 Hz, 1 H, H-6), 6.17 (d, J = 8 Hz, 1 H, H-5), 4.20 (dd, J = 3, 14 Hz, 1 H, H-1'), 3.79-3.94 (m, 4 H, H-1', H-2', and OCH_2P), and 3.59-3.67 (m, 2 H, H-3'). Anal. Calcd for $C_8H_{14}N_3O_6P$: C, 34.42; H, 5.06; N, 15.06. Found: C, 34.14; H, 5.28; N, 14.76.

(S)-N^1-[2,3-O-Isopropylidene-2,3-(dihydroxy)propyl]-N^4-benzoylcytosine (13)

Procedure A: Via Alkylation of N^4-Benzoylcytosine. Potassium t-butoxide (11.6 g, 0.104 mol) was added portionwise over 5 min to a mechanically-stirred suspension of N^4-benzoylcytosine[24] (20.0 g, 0.093 mol) in DMSO (180 mL) at 100 °C under argon. The yellow solution was stirred at 100 °C for 10 min and then treated dropwise over 5 min with a solution of mesylate **4** (21.8 g, 0.104 mol) in DMSO (20 mL). After 4.5 h at 100 °C, the reaction mixture was allowed to cool to room temperature and was diluted with CH_2Cl_2 (600 mL). The resulting suspension was extracted with water (3 x 150 mL), and the organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated to provide 40 g of a partially crystalline residue. The residue was treated with ethyl acetate (100 mL), and the resulting slurry was cooled to 0 °C and then filtered to provide 9.90 g of a white solid. A second crop was isolated by concentration of the filtrate followed by trituration of the residue with diethyl ether. A total of 11.86 g (38%) of **13** was obtained as a white crystalline solid.

Procedure B: Via Alkylation of N^4-Benzoylcytosine. A solution of mesylate **4** (10.0 g, 0.048 mol) in anhydrous DMF (100 mL) in a 1-L, round-bottomed flask equipped with a mechanical stirrer was treated with N^4-benzoylcytosine[24] (11.3 g, 0.052 mol) and cesium carbonate (17.0 g, 0.052 mol). The thick, white slurry was placed in an oil bath preheated to 100 °C and within 10 min, most of the solid had dissolved. The reaction mixture was stirred vigorously at 100 °C for 3 h and then allowed to cool to room temperature. The insoluble material was removed by filtration and the filtrate was concentrated in vacuo. The residue was treated with CH_2Cl_2 (200 mL), and the mixture refiltered to remove additional solid material. Purification by column chromatography on silica gel (20:1, gradient 1% to 5% CH_3OH/CH_2Cl_2) afforded 6.00 g (38%) of **13** as a white solid along with 3.22 g (31%) of the isomeric dialkylated product **14** as a viscous oil.

Procedure C: Via Benzoylation of 5. A mixture of 5 (4.70 g, 0.021 mol) and benzoic anhydride (4.75 g, 0.021 mol) in pyridine (35 mL) was heated at reflux under argon for 30 min and then allowed to cool to room temperature. The mixture was poured into ice water (250 mL) and the aqueous layer was extracted with CH_2Cl_2 (2 x 75 mL). The combined organic layers were washed with saturated $NaHCO_3$ solution (50 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo to give 8.2 g of a tan solid. The solid was slurried in ethyl acetate (35 mL) at 0 °C for 30 min and the product was collected by filtration to give 5.16 g (75%) of **13** as a white solid.

For **13**: mp 194-195 °C (dec); $[\alpha]_D^{20}$ -54.2 (c = 0.50, MeOH); UV_{max} (MeOH) 306 (ε = 10,200), 260 (ε = 22,700); ^1H NMR (Me_2SO-d_6) δ 11.17 (br s, 1 H, NH), 8.04 (d, J = 7 Hz, 1 H, H-6), 7.99 (d, J = 7 Hz, 2 H, 2 x BzH), 7.47-7.70 (m, 3 H, 3 x BzH), 7.29 (d, J = 7 Hz, 1 H, H-5), 4.35-4.40 (m, 1 H, H-2'), 4.00-4.08 (m, 2 H, H-1' and H-3'), 3.86 (dd, J = 7, 14 Hz, 1 H, H-1'), 3.71 (dd, J = 6, 9 Hz, 1 H, H-3'), 1.32 (s, 3 H, CH_3), and 1.24 (s, 3 H, CH_3) ; ^{13}C NMR (Me_2SO-d_6) δ 167.32 (C=O), 163.26 (C-4), 155.49 (C-2), 151.27 (C-6), 133.27 (BzC), 132.77 (BzC), 128.52 (BzC), 108.93 (OCO), 95.67 (C-5), 72.94 (C-2'), 66.03 (C-3'), 51.82 (C-1'), 26.63 (CH_3), and 25.22 (CH_3); mass spectrum (DCI), m/e (rel intensity) 330 (MH^+, 100), 272 (55). Anal. Calcd for $C_{17}H_{19}N_3O_4$: C, 61.99; H, 5.81; N, 12.76. Found: C, 61.98; H, 5.87, N, 12.76.

For **14**: UV_{max} (MeOH) 318 (ε = 15,000), 242 (ε = 10,000); ^1H NMR (Me_2SO-d_6) δ 8.04 (d, J = 7 Hz, 2 H, 2 x BzH), 7.43-7.61 (m, 4 H, H-6, 3 x BzH), 6.38 (d, J = 7 Hz, 1 H, H-5), 4.26-4.45 (m, 3 H, 3 x H-1'), 4.11-4.17 (m, 1 H, H-1'), 3.93-4.04 (m, 3 H, 2 x H-2', 1 x H-3'), 3.67-3.84 (m, 3 H, 3 x H-3'), 1.33 (s, 3 H, CH_3), 1.32 (s, 3 H, CH_3), and 1.23 (s, 6 H, CH_3); ^{13}C NMR (Me_2SO-d_6) δ 175.11 (C=O), 156.34, 150.14, 143.45, 135.63, 131.84, 128.71, 127.84, 108.40 (OCO), 108.05 (OCO), 72.56 (C-2'), 71.89 (C-2'), 66.62 (C-3'), 65.45 (C-3'), 50.72 (C-1'), 44.52 (C-1'), 26.18 (CH_3), 26.06 (CH_3), 24.91 (CH_3), and 24.67 (CH_3); mass spectrum (DCI), m/e (rel intensity) 444 (MH^+, 100), 386 (50). Anal. Calcd for $C_{23}H_{29}N_3O_6 \cdot 0.25\ H_2O$: C, 61.66; H, 6.64; N, 9.38. Found: C, 61.69; H, 6.52, N, 9.57.

(S)-N^1-[(2,3-Dihydroxy)propyl]-N^4-benzoylcytosine (15)

A mixture of **13** (10.0 g, 0.030 mol) in CH_3OH (100 mL) at room temperature was treated with conc. HCl (10 mL). A white solid

gradually precipitated from the reaction mixture and after 3 h, the resulting thick slurry was cooled to 0 °C. The solid was collected by filtration, washed with cold CH_3OH, and dried in vacuo at 40 °C to provide 8.34 g (95%) of **15** as a white crystalline solid: mp 192-194 °C (dec); $[\alpha]_D^{20}$ -81.95 (c = 1.09, MeOH); UV_{max} (MeOH) 308 (ε = 4800), 258 (ε = 10,800), 236 (ε = 13,600); ^1H NMR (Me_2SO-d_6) δ 8.17 (d, J = 7 Hz, 1 H, H-6), 8.05 (d, J = 7 Hz, 2 H, 2 x BzH), 7.64 (t, J = 7 Hz, 1 H, BzH), 7.53 (t, J = 7 Hz, 2 H, 2 x BzH), 7.32 (d, J = 7 Hz, 1 H, H-5), 5.23 (br m, 3 H, exch, OH and NH), 4.18 (dd, J = 3, 13 Hz, 1 H, H-1'), 3.73-3.80 (m, 1 H, H-2'), 3.55 (dd, J = 9, 13 Hz, 1 H, H-1'), 3.40 (dd, J = 5, 11 Hz, 1 H, H-3'), and 3.30 (dd, J = 5, 11 Hz, 1 H, H-3'); ^{13}C NMR (Me_2SO-d_6) δ 167.53 (C=O), 161.83 (C-4), 154.41 (C-6), 152.81 (C-2), 133.68 (BzC), 132.92 (BzC), 129.06 (BzC), 95.20 (C-5), 68.53 (C-2'), 63.82 (C-3'), and 53.62 (C-1'); mass spectrum (FAB), m/e (rel intensity) 290 (MH^+, 20), 105 (25). Anal. Calcd for $C_{14}H_{15}N_3O_4 \cdot 1.25\ H_2O$: C, 53.94; H, 5.61; N, 13.48. Found: C, 53.96; H, 5.05; N, 13.39.

(S)-N^1-[(3-Triphenylmethoxy-2-hydroxy)propyl]-N^4-benzoylcytosine (16)

A mixture of **15** (30 g, 0.104 mol), triphenylmethyl chloride (31.8 g, 0.114 mol), and 4-(dimethylamino)pyridine (0.63 g, 0.005 mol) in pyridine (300 mL) was heated at reflux for 1 h and then allowed to cool to room temperature. The mixture was concentrated in vacuo, and the resulting syrup was dissolved in CH_2Cl_2 (500 mL) and washed with 10% aqueous HCl solution (3 x 100 mL). The organic solution was washed further with saturated $NaHCO_3$ solution (2 x 100 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated to provide 58.3 g of a yellow foam. The material was used in the following reaction without purification. A pure sample of **16** was obtained by column chromatography on silica gel (10:1, 1% to 2% CH_3OH/CH_2Cl_2): mp 123-126 °C; $[\alpha]_D^{20}$ -42.38 (c = 0.81, MeOH); UV_{max} (MeOH) 306 (ε = 9,000), 260 (ε = 21,500); ^1H NMR (Me_2SO-d_6) δ 11.17 (br s, 1 H, NH), 7.96-8.01 (m, 3 H, H-6, 2 x BzH), 7.22-7.63 (m, 19 H, H-5, 3 x BzH, 15 x TrH), 5.34 (d, J = 6 Hz, exch, OH), 4.20 (dd, J = 3, 13 Hz, 1 H, H-1'), 3.95-4.05 (m, 1 H, H-2'), 3.57 (dd, J = 9, 13 Hz, 1 H, H-1'), 3.00 (dd, J = 5, 9 Hz, 1 H, H-3'), and 2.90 (dd, J = 6, 9 Hz, 1 H, H-3'); ^{13}C NMR (Me_2SO-d_6) δ 166.05 (C=O), 163.17 (C-4), 157.75 (C-2), 151.16 (C-6), 144.12 (TrC),

133.79 (BzC), 129.60, 129.31, 129.26, 129.14, 128.88, 128.83, 128.58, 128.43, 128.38, 128.26, 127.86, 97.02 (C-5), 87.35 ($Ph_3\underline{C}O$), 69.30 (C-2'), 64.89 (C-3') and 54.74 (C-1'); mass spectrum (DCI), m/e (rel intensity) 532 (MH^+, 55), 243 (100), 122 (25), 105 (10). Analysis. Calcd for $C_{33}H_{29}N_3O_4$: C, 74.56; H, 5.50; N, 7.90. Found: C, 74.19; H, 5.50; N, 7.86.

(S)-N^1-[(3-Triphenylmethoxy-2-diethylphosphonylmethoxy)propyl]-N^4-benzoylcytosine (17)

Sodium hydride (9.33 g, 80% dispersion in oil, 0.311 mol) was added in one portion to a solution of **16** (58.3 g, used crude, 0.104 mol) and diethyl [(tosyloxy)methyl]phosphonate **10** (46.7 g, 0.145 mol) in anhydrous DMF (450 mL) at 0 °C under argon. Vigorous hydrogen evolution was noted and over a 30 min period, the internal temperature of the resulting yellow-green suspension rose to 10 °C. After 1 h, HPLC analysis showed that the starting material was consumed. The reaction mixture was then diluted with ethyl acetate (1.5 L) and slowly treated with water (450 mL). The layers were separated, and the organic phase was washed with saturated $NaHCO_3$ solution (450 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated to provide 78.9 g of a viscous yellow oil which was used in the following reaction without purification. An analytical sample of **17** was obtained by column chromatography on silica gel (20:1, gradient 1% to 3% CH_3OH/CH_2Cl_2): $[\alpha]_D^{20}$ -47.67° (c = 0.96, MeOH); UV_{max} (MeOH) 306 (ε = 9,800), 260 (ε = 22,000); ^1H NMR (Me_2SO-$\underline{d}_6$) δ 11.19 (br s, 1 H, exch, NH), 7.98 (apparent d, J = 7 Hz, 3 H, H-6 and BzH), 7.23-7.63 (m, 19 H, 3 x BzH, 15 x TrH, H-5), 3.68-4.09 (m, 9 H, 2 x H-1', H-2', $OC\underline{H}_2P$, 2 x $POC\underline{H}_2$), 3.24-3.31 (m, 1 H, H-3'), 2.94-2.98 (m, 1 H, H-3'), 1.16 (t, J = 7 Hz, 3 H, $POCH_2C\underline{H}_3$), and 1.14 (t, J = 7 Hz, 3 H, $POCH_2C\underline{H}_3$); ^{13}C NMR (Me_2SO-$\underline{d}_6$) δ 167.75 (C=O), 163.85 (C-4), 155.05 (C-2), 151.95 (C-6), 143.54 (TrC), 132.77 (BzC), 128.53, 128.49, 128.33, 128.07, 127.22, 95.75 (C-5), 86.25 ($Ph_3\underline{C}$), 77.96 (d, J = 11 Hz, C-2'), 63.05 (d, J = 165 Hz, $O\underline{C}H_2P$), 62.74 (C-3'), 61.75 (d, J = 6 Hz, $PO\underline{C}H_2$), 51.15 (C-1'), and 16.32 (d, J = 6 Hz, $POCH_2\underline{C}H_3$); mass spectrum (FAB), m/e (rel intensity) 682 (MH^+, 10), 243 (100), 165 (20), 105 (50). Anal. Calcd for $C_{38}H_{40}N_3O_7P \cdot 0.5\ H_2O$: C, 66.07; H, 5.98; N, 6.08. Found: C, 65.96; H, 5.84; N, 6.09.

(S)-N^1-[(3-Hydroxy-2-diethylphosphonylmethoxy)propyl]-N^4-benzoylcytosine (18)

Hydrogen chloride gas was bubbled for 5 min through a solution of trityl ether **17** (65 g of unpurified material, 0.086 mol theoretical) in CH_2Cl_2 (650 mL) at 0 °C. A solution of saturated $NaHCO_3$ (500 mL) was then added slowly and the mixture was stirred vigorously for 10 min. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 x 100 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated to give 64.7 g of a viscous oil. Purification by column chromatography on silica gel (gradient 2.5% to 7% CH_3OH/CH_2Cl_2) afforded 23.2 g (62% overall yield from **15**) of **18** as a pale yellow foam: $[\alpha]_D^{20}$ -75.31 (c = 0.69, MeOH); UV_{max} (MeOH) 306 (ε = 6,300), 260 (ε = 16,300); ^1H NMR (Me_2SO-d_6) δ 11.18 (br s, 1 H, exch, NH), 7.98 (apparent d, J = 7 Hz, 3 H, H-6 and BzH), 7.60 (t, J = 7 Hz, 1 H, BzH), 7.49 (t, J = 7 Hz, 2 H, BzH), 7.27 (br d, J = 7 Hz, 1 H, H-5), 4.88 (t, J = 6 Hz, 1 H, exch, OH), 4.13 (dd, J = 8, 17 Hz, 1 H, H-1'), 3.88-4.01 (m, 5 H, H-2', $POCH_2$), 3.68-3.80 (m, 3 H, H-1', OCH_2P), 3.44-3.57 (m, 2 H, H-3'), 1.18 (t, J = 7 Hz, 3 H, $POCH_2CH_3$), and 1.16 (t, J = 7 Hz, 3 H, $POCH_2CH_3$); ^{13}C NMR (Me_2SO-d_6) δ 167.05 (C=O), 163.16 (C-4), 154.50 (C-2), 151.40 (C-6), 133.45 (BzC), 132.76 (BzC), 128.53 (BzC), 95.45 (C-5), 79.38 (d, J = 11 Hz, C-2'), 62.84 (d, J = 155 Hz, OCH_2P), 61.67 (d, J = 6 Hz, $POCH_2$), 60.35 (C-3'), 50.85 (C-1'), 16.31 (d, J = 6 Hz, $POCH_2CH_3$), and 16.29 (d, J = 6 Hz, $POCH_2CH_3$); mass spectrum (FAB), m/e (rel intensity) 440 (MH^+, 100), 105 (65). Anal. Calcd for $C_{19}H_{26}N_3O_7P \cdot 0.5\ H_2O$: C, 50.89; H, 6.07; N, 9.37. Found: C, 50.99; H, 6.03; N, 9.32.

(S)-N^1-[(3-Hydroxy-2-phosphonylmethoxy)propyl]-N^4-benzoylcytosine (19)

A solution of **18** (21.7 g, 0.049 mol) in CH_2Cl_2 (220 mL) at room temperature under argon was treated with bromotrimethylsilane (22.7 g, 0.148 mol) and the mixture was stirred for 18 h. An additional portion of bromotrimethylsilane (2.90 g, 0.019 mol) was added, and the reaction mixture was stirred for 4 h further and then concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (200 mL) and solution was concentrated to provide 33 g of an orange foam. Addition of water (100 mL) to the residue resulted in gradual formation of a white solid. Ethanol (200 mL) was added next, and the resulting slurry was

stirred at 0 °C for 1 h and then filtered. The collected solid was washed with ethanol (2 x 20 mL) and dried in vacuo to provide 16.1 g (85%) of **19** as a pale pink solid: mp 186-188 °C (dec); $[\alpha]_D^{20}$ -82.0 (c = 0.5, H_2O); UV_{max} (MeOH) 304 (ε = 7,100), 258 (ε = 12,700); ^1H NMR (Me_2SO-d_6) δ 8.04 (d, J = 7 Hz, 1 H, H-6), 7.98 (d, J = 7 Hz, 2 H, 2 x BzH), 7.61 (t, J = 7 Hz, 1 H, BzH), 7.49 (t, J = 7 Hz, 2 H, 2 x BzH), 7.26 (d, J = 7 Hz, 1 H, H-5), 4.11 (dd, J = 4, 13 Hz, 1 H, H-1'), and 3.45-3.81 (m, 6 H, H-1', H-2', 2 x H-3', and OCH_2P); ^{13}C NMR (Me_2SO-d_6) δ 168.01 (C=O), 163.50 (C-4), 155.84 (C-2), 151.90 (C-6), 133.69 (BzC), 133.11 (BzC), 128.86 (BzC), 95.88 (C-5), 80.07 (d, J = 11 Hz, C-2'), 65.68 (d, J = 160 Hz, OCH_2P), 60.70 (C-3'), and 50.38 (C-1'); mass spectrum (FAB), m/e (rel intensity) 384 (MH^+, 20). Anal. Calcd for $C_{15}H_{18}N_3O_7P \cdot 0.5\ H_2O$: C, 45.93; H, 4.84; N, 10.71. Found: C, 46.04; H, 4.67; N, 10.71.

(S)-N^1-[(3-Hydroxy-2-phosphonylmethoxy)propyl]cytosine (3)

Phosphonic acid **19** (16.1 g, 0.042 mol) was treated with concentrated NH_4OH (160 mL) at room temperature. After 4 h, HPLC analysis showed complete consumption of the starting material. CH_2Cl_2 (150 mL) and H_2O (50 mL) were added and the resulting mixture was stirred vigorously for 5 min. The layers were separated and the aqueous phase was extracted further with CH_2Cl_2 (100 mL) and then concentrated in vacuo to give 16.1 g of a white crystalline solid. The material was slurried in ethanol (160 mL) and the solid was collected by filtration and then dried in vacuo at 40 °C to provide 12.8 g (87%) of **3** as its ammonium salt.

The zwitterionic form of HPMPC was obtained by treatment of an aqueous solution of **3** in the ammonium salt form (1 g per 7-10 mL, pH 6.8 solution) with 6 N HCl until the pH of the solution reached 3.5. The product then crystallized from the solution. In this manner, 18.2 g of **3** as the ammonium salt was converted to 17.3 g (94% recovery) of the dihydrate of HPMPC. Anal. Calcd. for $C_8H_{14}N_3O_6P \cdot 2H_2O$: C, 30.49; H, 5.75; N, 13.33. Found: C, 30.89; H, 5.78; N, 13.36.

(S)-N^1-[(3-Benzyloxy-2-diethylphosphonylmethoxy)propyl]-O^4-methyluracil (22)

A mixture of 4-methoxy-2-pyrimidinone (**20**)[26] (1.84 g, 14.6 mmol), mesylate **6**[9,14] (5.00 g, 12.7 mmol) and Cs_2CO_3 (4.75 g, 14.6 mmol) in

DMF (50 mL) was heated at 100 °C in a round-bottomed flask equipped with a mechanical stirrer. After 3 h, the reaction mixture was allowed to cool to room temperature, and the insoluble material was removed by filtration. Concentration of the filtrate gave 7.0 g of a yellow semi-solid residue which was purified by column chromatography on silica gel (20:1, gradient 3% to 9% ethanol/ethyl acetate) to afford 2.63 g (49%) of **22** as a viscous oil along with 1.29 g (25%) of the isomeric O-alkylated product **23**.

For **22**: $[\alpha]_D^{20}$ -64.1° (c = 1.06, MeOH); UV_{max} (MeOH) 276 (ε = 5,900); ^1H NMR ($CDCl_3$) δ 7.49 (d, J = 7 Hz, 1 H, H-6), 7.27-7.35 (m, 5 H, 5 x PhH), 5.79 (d, J = 7 Hz, 1 H, H-5), 4.51 (dd, J = 12, 15 Hz, 2 H, OCH_2Ph), 4.24 (dd, J = 3, 14 Hz, 1 H, H-1'), 3.92-4.10 (m, 9 H, H-1', H-2', 2 x $POCH_2$, OCH_3), 3.54-3.74 (m, 3 H, H-3', OCH_2P), 3.49 (dd, J = 5, 11 Hz, 1 H, H-3'), and 1.26 (t, J = 6 Hz, 6 H, 2 x $POCH_2CH_3$); ^{13}C NMR ($CDCl_3$) δ 172.66 (C-4), 157.35 (C-2), 149.33 (C-6), 138.24 (PhC), 129.06 (PhC), 128.46 (PhC), 128.35 (PhC), 95.40 (C-5), 78.98 (d, J = 12 Hz, C-2'), 73.92 (OCH_2Ph), 69.65 (C-3'), 64.42 (d, J = 170 Hz, OCH_2P), 62.68 (d, J = 6 Hz, $POCH_2$), 62.56 (d, J = 6 Hz, $POCH_2$), 54.63 (OCH_3), 51.57 (C-1'), and 16.62 (d, J = 6 Hz, $POCH_2CH_3$); mass spectrum (DCI), m/e (rel intensity) 441 (M^+ + 1, 100), 127 (25).

For **23**: UV_{max} (MeOH) 260 (ε = 5,900); ^1H NMR ($CDCl_3$) δ 8.13 (d, J = 7 Hz, 1 H, H-6), 7.25-7.30 (m, 5 H, 5 x PhH), 6.34 (d, J = 7 Hz, 1 H, H-5), 4.53 (s, 2 H, OCH_2Ph), 4.45 (d, J = 6 Hz, 2 H, 2 x H-1'), 4.12 (apparent q, J = 7 Hz, 4 H, 2 x $POCH_2$), 3.94-4.03 (m, 3 H, H-2', OCH_2P), 3.91 (s, 3 H, OCH_3), 3.64-3.70 (m, 2 H, H-3'), and 1.27 (t, J = 7 Hz, 6 H, 2 x $POCH_2CH_3$); ^{13}C NMR ($CDCl_3$) δ 172.22 (C-4), 165.88 (C-2), 159.04 (C-6), 138.61 (PhC), 128.95 (PhC), 128.89 (PhC), 128.24 (PhC), 102.73 (C-5), 79.51 (d, J = 12 Hz, C-2'), 73.87 (OCH_2Ph), 70.34 (C-3'), 67.18 (C-1'), 65.01 (d, J = 165 Hz, OCH_2P), 62.82 (d, J = 6 Hz, $POCH_2$), 54.10 (OCH_3), and 16.61 (d, J = 6 Hz, $POCH_2CH_3$); mass spectrum (DCI), m/e (rel intensity) 441 (M^+ + 1, 100), 315 (25).

(S)-N^1-[(3-Benzyloxy-2-diethylphosphonylmethoxy)propyl]uracil (24)

A solution of **22** (2.63 g, 6.00 mmol) in 90% aqueous ethanol (10 mL) was treated with conc. HCl (0.5 mL) and the mixture was stirred at room temperature for 16 h. Concentration gave 2.65 g of a yellow residue which was purified by column chromatography on silica gel

(10:1, gradient 1% to 4% CH_3OH/CH_2Cl_2) to provide 2.29 g (90%) of **24**: $[\alpha]_D^{20}$ -50.5° (c = 0.68, MeOH); UV_{max} (MeOH) 264 (ε = 9,750); ^1H NMR (Me_2SO-d_6) δ 11.25 (br s, 1 H, NH), 7.47 (d, J = 7 Hz, 1 H, H-6), 7.26-7.37 (m, 5 H, 5 x PhH), 5.48 (d, J = 7 Hz, 1 H, H-5), 4.49 (s, 2 H, OCH_2Ph), 3.64-4.01 (m, 9 H, 2 x H-1', H-2', 2 x $POCH_2$, OCH_2P), 3.57 (dd, J = 4, 11 Hz, 1 H, H-3'), 3.48 (dd, J = 5, 11 Hz, 1 H, H-3'), and 1.17 (t, J = 6 Hz, 6 H, 2 x $POCH_2CH_3$); ^{13}C NMR (Me_2SO-d_6) δ 163.85 (C-4), 151.14 (C-2), 146.52 (C-6), 138.19 (PhC), 128.34 (PhC), 127.63 (PhC), 100.44 (C-5), 78.00 (d, J = 12 Hz, C-2'), 72.52 (OCH_2Ph), 69.00 (C-3'), 62.90 (d, J = 165 Hz, OCH_2P), 61.71 (d, J = 6 Hz, $POCH_2$), 48.73 (C-1'), and 16.31 (d, J = 6 Hz, $POCH_2CH_3$); mass spectrum (DCI), m/e (rel intensity) 427 (MH^+ + 1, 100). Anal. Calcd for $C_{19}H_{27}N_2O_7P$: C, 53.52; H, 6.38; N, 6.57. Found: C, 53.39; H, 6.29; N, 6.43.

(S)-N^1-[(3-Hydroxy-2-diethylphosphonylmethoxy)propyl]uracil (25)

A mixture of **24** (1.60 g, 3.75 mmol) and $Pd(OH)_2$ on carbon (1.5 g, 20%) in ethanol/cyclohexene (1:1, 40 mL) was heated at reflux for 3 h. The reaction mixture was filtered while hot through a 1-in. pad of Celite and the pad was washed with hot ethanol (2 x 50 mL). Concentration of the filtrate gave 1.20 g of a colorless opaque oil. Purification on silica gel (20:1, gradient 3% to 7% CH_3OH/CH_2Cl_2) provided 1.14 g (90%) of **25** as a viscous clear oil: $[\alpha]_D^{20}$ -72.5° (c = 0.40, MeOH); UV_{max} (MeOH) 266 (ε = 9,100); ^1H NMR ($CDCl_3$) δ 9.04 (br s, 1 H, NH), 7.30 (d, J = 7 Hz, 1 H, H-6), 5.65 (d, J = 7 Hz, 1 H, H-5), 3.74-4.20 (m, 11 H, 2 x H-1', H-2', 2 x $POCH_2$, OCH_2P, H-3', OH), 3.53-3.60 (m, 1 H, H-3'), 1.32 (t, J = 6 Hz, 3 H, $POCH_2CH_3$), and 1.30 (t, J = 6 Hz, 3 H, $POCH_2CH_3$); ^{13}C NMR ($CDCl_3$) δ 165.04 (C-4), 152.31 (C-2), 146.92 (C-6), 102.17 (C-5), 80.92 (d, J = 12 Hz, C-2'), 64.31 (d, J = 160 Hz, OCH_2P), 63.03 (d, J = 6 Hz, $POCH_2$), 60.58 (C-3'), 49.48 (C-1'), and 16.60 (d, J = 6 Hz, $POCH_2CH_3$); mass spectrum (DCI), m/e (rel intensity) 337 (MH^+ + 1, 100), 319 (20).

(S)-N^1-[(3-Hydroxy-2-phosphonylmethoxy)propyl]uracil (26)

The diethyl ester **25** (1.05 g, 3.10 mmol) was dissolved in CH_3CN (10 mL) and treated with bromotrimethylsilane (3.82 g, 25.0 mmol) at room temperature under argon. The reaction mixture was stirred for 18 h and concentrated in vacuo. The residue was evaporated from CH_3CN (2

x 25 mL) and then placed under high vacuum for 16 h. Water (20 mL) was added and the aqueous solution was extracted with CH_2Cl_2 (25 mL) and lyophilized to afford 870 mg (99%) of **26** as a hygroscopic white solid: $[\alpha]_D^{20}$ -59.26° (c = 0.66, MeOH); UV_{max} (MeOH) 266 (ε = 9,900); ^1H NMR (D_2O) δ 7.62 (d, J = 8 Hz, 1 H, H-6), 5.79 (d, J = 8 Hz, 1 H, H-5), 4.02-4.10 (m, 1 H, H-1'), and 3.60-3.91 (m, 6 H, H-1', H-2', OCH_2P, 2 x H-3'); ^{13}C NMR (D_2O) δ 174.08 (C-4), 159.45 (C-2), 155.32 (C-6), 108.07 (C-5), 86.78 (d, J = 12 Hz, C-2'), 71.59 (d, J = 160 Hz, OCH_2P), 66.83 (C-3'), and 56.06 (C-1'); mass spectrum (FAB), m/e (rel intensity) 281 (MH^+ + 1, 30). Anal. Calcd for $C_8H_{13}N_2O_7P \cdot 1.67\ H_2O$: C, 30.97; H, 5.26; N, 9.03. Found: C, 30.93; H, 4.91; N, 9.04.

HPLC Determination of Enantiomeric Purity

The HPLC system consisted of a Perkin-Elmer Series 4 pump, a Perkin-Elmer ISS-100 injector, a Perkin-Elmer UV95 variable wavelength detector, and a Perkin-Elmer Chromatographics 3 integrator. HPLC analysis was carried out on a Perkin-Elmer 3x3 C-18 cartridge column. The mobile phase consisted of 4 mM cupric sulfate and 8 mM phenylalanine [>98% (D)- or (L)-isomer] in HPLC grade water. The flow rate was set at 0.3 mL/min and UV detection was performed at 270 nm. Using (D)-phenylalanine in the mobile phase, the retention time was 2.4 min for (R)-HPMPC and 2.9 min for (S)-HPMPC. With (L)-phenylalanine in the mobile phase, the retention times were reversed.

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