PREPARATION OF C-5 SUBSTITUTED CIDOFOVIR DERIVATIVES

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1-[(*S*)-3-Hydroxy-2-(phosphonomethoxy)propyl]cytosine (HPMPC, cidofovir) was modified by substitution on the base moiety in positions C-5 and N^4. Key intermediates of these syntheses, diisopropyl esters of (*S*)-1-[2-(phosphonomethoxy)-3-(triphenylmethoxy)propyl]-5-alkylcytosines (**6** and **7**) prepared from 5-alkyl-4-methoxypyrimidin-2(1*H*)-ones were transformed to the corresponding 5-substituted cytosine or N^4-alkylcytosine derivatives by the action of ammonia or primary amines, respectively. These fully protected phosphonate esters gave by treatment with bromotrimethylsilane followed by hydrolysis free phosphonic acids: 1-[(*S*)-3-hydroxy-2-(phosphonomethoxy)propyl]-5-methylcytosine (5-methyl-HPMPC, **10**), 5-ethyl-1-[(*S*)-3-hydroxy-2-(phosphonomethoxy)propyl]cytosine (5-ethyl-HPMPC, **11**) and a series of 5-ethyl-HPMPC analogues **17**–**21** bearing various substituents in N^4 position (cyclopropyl, cyclopentyl, 2-hydroxyethyl, allyl, 2-(dimethylamino)ethyl). 5-Ethynyl-1-[(*S*)-3-hydroxy-2-(phosphonomethoxy)propyl]cytosine (5-ethynyl-HPMPC, **26**) was prepared from 5-iodocytosine derivative **23** using Sonogashira coupling with (trimethylsilyl)acetylene, CuI and $[PdCl_2(Ph_3P)_2]$. None of the prepared compounds exhibited antiviral activity in vitro.

Keywords: Acyclic nucleotide analogues; Phosphonates; Pyrimidines; Nucleosides; 5-Alkylcytosines; Antivirals; HPMPC; Sonogashira cross-coupling.

Cidofovir (HPMPC, CDV, (*S*)-1-[3-hydroxy-2-(phosphonomethoxy)propyl]-cytosine)[1] is a powerful antiviral agent active against all types of DNA viruses. Several reviews refer to its activity[2–5], mechanism of action[6], pharmacology[7,8] and therapeutic applications[9,10]. As the systemic drug Vistide™ it is approved for intravenous treatment of cytomegalovirus retinitis[11] in AIDS patients, but at the present time, it is clinically important particularly in severe cases of (malignizing) papillomatoses (anogenital, laryngeal)[12], progressive multifocal leukoencephalopathy[13], adenovirus infections[14] and some rather obscure severe infections caused by poxviruses (vaccinia[15], orf, molluscum contagiosum)[16]. The attractivity of cidofovir is dramatically enhanced by its supreme activity against smallpox virus[17]and related monkeypox virus[18].

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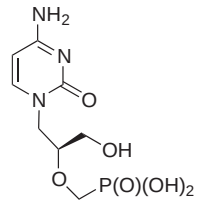


(*S*)-HPMPC (cidofovir)

Cidofovir is not yet available for clinical use in an oral prodrug form, it is currently available only for intravenous use as a solution; the testing of cidofovir in the form of aerosols or eye drops is still under way in animal models. Orally bioavailable cidofovir prodrugs are under preclinical investigation. In this area the main attention was paid to investigation of neutral ester prodrugs[19] and modification of the phosphonate-bearing side chain[20]. The side effect of cidofovir, its nephrotoxicity, is also the limiting factor in its widespread use. These facts substantiate our effort in developing additional cidofovir derivatives with potentially improved pharmacological properties. In contrast to modifications of the phosphonate-bearing side chain, the methodology of cytosine moiety modification in cidofovir is not worked out in detail. Recently we published two papers dealing with preparation of its N^4-substituted derivatives[21,22] and at present time we deal systematically with investigation of the influence of 4-amino group substitution of cytosine in acyclic nucleotide analogues on their antiviral activity.

Important modification of pyrimidine nucleosides and/or nucleobases generally, is their substitution in position C-5. Many compounds of this group are efficient virostatics, especially 2′-deoxyuridines with the following substituents: ethyl (aedurid), bromovinyl (BVDU), trifluoromethyl (trifluridin), vinyl, ethynyl and also their 2′-deoxycytidine counterparts[23]. Some 5-substituted nucleobases are used as anticancer drugs, e.g. 5-fluorouracil (5-FU). 5-Ethynyluracil[24] (eniluracil) is utilized as accompanying substance with 5-fluorouracil. 5-Fluorouracil itself is easily decomposed by enzyme dihydropyrimidine dehydrogenase (DPD). Eniluracil is a potent, suicide substrate for DPD, becomes covalently linked to the enzyme[25] and so enables also oral administration of 5-FU. In the field of acyclic nucleotide analogues (ANPs), antiviral activity was found at some 5-substituted 2,4-diamino-6-[2-(phosphonomethoxy)ethoxy]pyrimidines[26]. Other types of 5-alkylated pyrimidine ANPs were not systematically studied.

These facts substantiate our effort to prepare a series of cidofovir analogues bearing various substituents in C-5 position and also "double substituted" analogues, bering substituents in C-5 and N^4 positions simultaneously and to study the influence of such substitution to biological activity with comparison with the mother compound, HPMPC (cidofovir).

RESULTS AND DISCUSSION

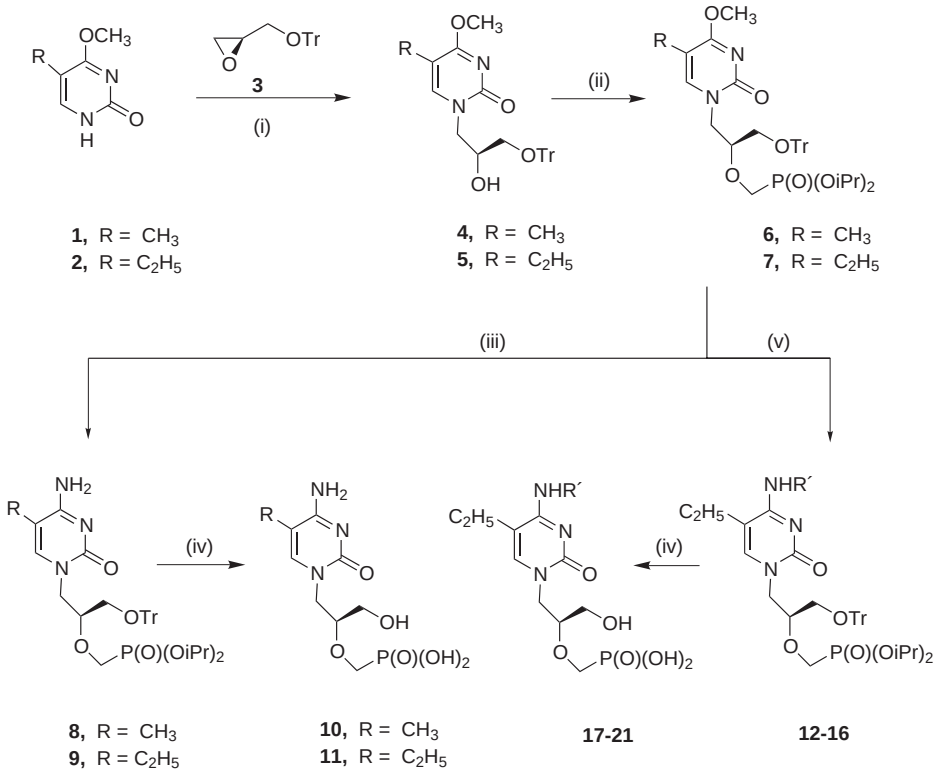
The first approach to substituted cytosine derivatives including C-5 substituted HPMPC exploits 5-alkyl-4-methoxypyrimidin-2(1*H*)-ones (**1** and **2**) which are accessible on large scale by three-step synthesis from the corresponding 5-alkyluracils (thymine, 5-ethyluracil).

This procedure, described originally for uracil and thymine, includes the treatment with $POCl_3$ giving 2,4-dichloropyrimidine, reaction with sodium methoxide leading to 2,4-dimethoxypyrimidine and its final transformation to 4-methoxypyrimidin-2(1*H*)-one by the action of acetyl chloride followed by sodium methoxide[27]. We used this method for transformation of 5-ethyluracil to 5-ethyl-4-methoxypyrimidin-2(1*H*)-one (**2**). The starting 5-ethyluracil was synthesized from ethyl butyrate according to a general procedure for preparation of 5-alkyluracils (formylation of carboxylic esters, followed by cyclization reaction with thiourea and subsequent hydrolysis with trichloroacetic acid)[28].

Reaction of 4-methoxypyrimidin-2(1*H*)-ones **1** and **2** with (2*S*)-2-[(trityloxy)methyl]oxirane (**3**) performed in DMF and catalyzed with cesium carbonate afforded (*S*)-5-alkyl-1-[2-hydroxy-3-(triphenylmethoxy)propyl]-4-methoxypyrimidin-2(1*H*)-ones **4** and **5** which were subsequently treated with diisopropyl [(tosyloxy)methyl]phosphonate in the presence of sodium hydride to give fully protected phosphonate esters **6** and **7** as key intermediates for the following syntheses (Scheme 1). The preparation of 5-methyl derivatives **4** and **6** has been published recently in the paper dealing with preparation of HPMPT and its side-chain fluorinated analogue[29].

The reaction of 4-methoxypyrimidin-2(1*H*)-one phosphonates **6** and **7** with ammonia at 120 °C in an autoclave afforded the corresponding cytosine derivatives **8** and **9**; deprotection of ester groups with bromotrimethylsilane in acetonitrile followed by hydrolysis gave free phosphonic acids: 1-[(*S*)-3-hydroxy-2-(phosphonomethoxy)propyl]-5-methylcytosine (**10**) and 5-ethyl-1-[(*S*)-3-hydroxy-2-(phosphonomethoxy)propyl]cytosine (**11**). The intermediate **7**, diisopropyl ester of 5-ethyl-4-methoxy-1-[(*S*)-2-(phosphonomethoxy)-3-(trityloxy)propyl]pyrimidin-1(2*H*)-one was also utilized for the reaction with various primary amines the selection of which was made on

the basis of activity of other ANP analogues substituted in the amino group[30]. This reaction, performed in refluxing dioxane, afforded N^4-alkyl-5-ethylcytosine phosphonates **12**–**16** which, after simultaneous deprotection of ester and trityl groups with bromotrimethylsilane, afforded a series of free HPMP-5-ethylcytosines bearing N^4-substituents **17**–**21** as final products for biological screening (Scheme 1, Table I).



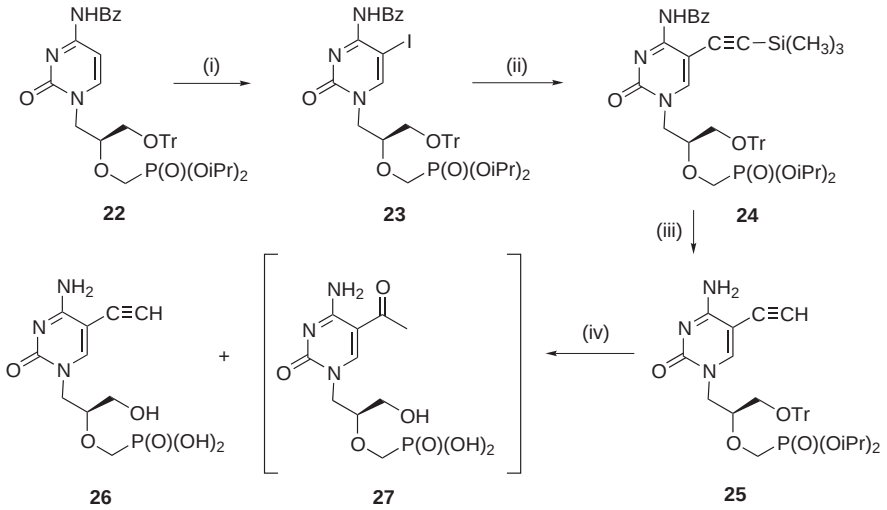
(i) Cs_2CO_3, DMF, 80 °C, 10 h; (ii) $TsOCH_2P(O)(OiPr)_2$, NaH, THF, r.t. ;
(iii) NH_3/CH_3OH, 120 °C; (iv) $(CH_3)_3SiBr$, CH_3CN, r.t.; (v) $R´NH_2$, dioxane, reflux

SCHEME 1

Quite a different approach to C-5 substituted cidofovir derivatives is based on Pd^{II} catalyzed coupling reactions[23b]. We have used Sonogashira coupling for introduction of ethynyl (or other alkynyl groups) to the C-5 position (Scheme 2). The starting compound, fully protected cidofovir ester **22** was first iodinated with *N*-iodosuccinimide. The thus obtained 5-iodo derivative **23** was then treated with (trimethylsilyl)acetylene under

TABLE I
Preparation of some N^4-substituted 5-ethyl-HPMPC derivatives from compound **7**

R′	Phosphonate ester	Yield of ester, %	Phosphonic acid	Yield of phosphonic acid, %
Cyclopropyl	**12**	46	**17**	74
Cyclopentyl	**13**	52	**18**	48
$HOCH_2CH_2$–	**14**	78	**19**	62
CH_2=CH–CH_2–	**15**	52	**20**	69
$(CH_3)_2NCH_2CH_2$–	**16**	not isolated	**21**	41



(i) *N*-Iodosuccinimide, AIBN, toluene, 80 °C; (ii) (trimethylsilyl)acetylene, $[PdCl_2(Ph_3P)_2]$, CuI, $(C_2H_5)_3N$, dioxane, r.t.; (iii) NH_3/CH_3OH, 4 °C; (iv) $(CH_3)_3SiBr$, CH_3CN, 0 °C

SCHEME 2

$[PdCl_2(Ph_3P)_2]$ and CuI catalysis to give 5-[2-(trimethylsilyl)ethynyl] derivative **24**, which was subsequently deprotected to compound **25** with methanolic ammonia.

The Sonogashira coupling ranks among the methods utilized in nucleoside chemistry very frequently[31]. In most cases, it is performed with nucleosides or nucleobases and, due to high reactivity of ethynyl group, it is always used as a last synthetic step. If these conditions are fulfilled, usually

no problems occur and such reactions can be performed even on a large scale (e.g. industrial synthesis of 5-ethynyluracil[32]). In our case, compound **25** was not a final product; its deprotection with bromotrimethylsilane turned out to be a critical step of the synthesis. This reaction when performed under usual conditions (room temperature, high excess of bromotrimethylsilane added in one portion and time-consuming purification on ion exchange resin columns) afforded no desired HPMP-5-ethynylcytosine (**26**) but 5-acetyl derivative **27** only as a product of a nucleophilic addition of water to triple bond. This product was also contaminated with a small amount of the 5-bromo derivative. In order to get over this problem we had to elaborate mild reaction and purification conditions consisting in a dropwise addition of bromotrimethylsilane at low temperature in dry acetonitrile and a very short contact with Dowex 50 at low temperature in the desalting process performed under stirring. Subsequent purification on Dowex 1 in acetate form is not possible. Therefore, final purification of HPMP-5-ethynylcytosine (**26**) was performed by preparative HPLC in neutral conditions to achieve the separation of a small amount of 5-acetyl derivative **27** present as impurity.

In conclusion, we synthesized a series of various base-modified cidofovir analogues as compounds for antiviral screening. Special attention was paid to preparation of 5-ethynyl-HPMPC which was obtained by the Sonogashira reaction; for this purpose, we optimized reaction conditions to prevent transformation of acid-sensitive ethynyl group during removal of ester groups and subsequent purification. All target compounds were tested for antiviral activity in vitro in cell cultures. None of the compounds was active against CMV, VZV, herpes viruses, vaccinia virus, VSV and retroviruses (HIV).

EXPERIMENTAL

Unless stated otherwise, solvents were evaporated at 40 °C/2 kPa and compounds were dried at 13 Pa. Melting points were determined on a Kofler block and are uncorrected. Analytical TLC were performed on silica gel 60 F_{254} plates (Merck, Germany), chromatographic systems are described in the text. Column chromatography was performed on silica gel 60 μm (Fluka) or aluminum oxide (50–150 μ, pH 7.0 ± 0.5; Fluka). Reverse phase HPLC separations were performed on a Waters Delta 600 instrument with a Waters 2487 dual λ absorbance detector using columns XTerra® RP_{18} (3.9 × 150 mm, analytical column) and Luna Phenomenex® C-18 (21 × 250 mm, preparative column). ^{1}H NMR spectra were measured on a Varian Unity 500 instrument (at 500 MHz) in DMSO-d_6 solutions (referenced to the solvent signal at δ 2.50) or in D_2O solutions with internal standard sodium 3-(trimethylsilyl)-propane-1-sulfonic acid (DSS). ^{1}H NMR chemical shifts (δ, ppm) and coupling constants (*J*, Hz) were obtained by first-order analysis of the spectra and decoupling experiments. ^{13}C NMR

spectra were recorded on the same instrument (at 125.7 MHz) using APT pulse sequence in DMSO-d_6 (referenced to the solvent signal δ 39.70). The numbering system for assignment of NMR signals is outlined in Fig. 1. Mass spectra were measured on a ZAB-EQ (VG Analytical) spectrometer using FAB (ionization with xenon, accelerating voltage 8 kV, glycerol matrix). Optical rotations were measured on an Autopol IV polarimeter (Rudolph Research Analytical, U.S.A.) at 20 °C; $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1} and solution concentrations in g/100 ml.

Materials and Solvents

Most chemicals and ion-exchange resins (Dowex 50WX8-200 and Dowex 1X2-400) were purchased from Sigma–Aldrich (Czech Republic). Dimethylformamide, tetrahydrofuran and acetonitrile were dried by distillation from CaH_2 (DMF in vacuo) and stored over molecular sieves (4Å). Diisopropyl [(tosyloxy)methyl]phosphonate was prepared by literature procedure[1]. (2*S*)-2-[(Trityloxy)methyl]oxirane (**3**) was purchased from DAISO, Co. Ltd (Japan).

5-Ethyl-4-methoxypyrimidin-2(1*H*)-one (**2**)

A mixture of 5-ethyl-2,4-dimethoxypyrimidine[33] (20 g, 117 mmol) and acetyl chloride (110 ml, 1.55 mol) was stirred at room temperature for 24 h, then evaporated, the solid residue coevaporated with toluene (2 × 200 ml) and mixed with methanol (25 ml). 1 M Sodium methoxide was added until alkaline reaction persisted and the solution was heated for 40 °C for 1 h. The mixture was neutralized with acetic acid to pH 7 and taken down. The crude product was crystallized from the mixture methanol–ethanol (1:3), crystals were collected by suction, washed with a small amount of acetone, then with water and finally again with acetone and dried in vacuo to give 7 g (39%) of **2**. Mother liquors were evaporated and chromatographed on a column of silica gel (1500 ml) in system chloroform–methanol (20:1) to give additional portion of **2**. Overall yield 14.9 g (83%), white crystals, m.p. 166 °C. For $C_7H_{10}N_2O_2$ (154.2) calculated: 54.54% C, 6.54% H, 18.17% N; found: 54.09% C, 6.49% H, 17.91% N. FAB MS, *m/z* (%): 155 (100) [M + H]. ^{1}H NMR (DMSO-d_6): 11.15 br, 1 H (NH); 7.45 s, 1 H (H-6); 3.82 s, 3 H (OCH_3); 2.26 q, 2 H, $J(CH_2,CH_3)$ = 7.4 (CH_2); 1.02 t, 3 H (CH_3). ^{13}C NMR (DMSO-d_6): 170.65 (C-4); 156.43 (C-2); 141.93 (C-6); 107.98 (C-5); 53.81 (OCH_3); 19.20 (CH_2); 13.54 (CH_3).

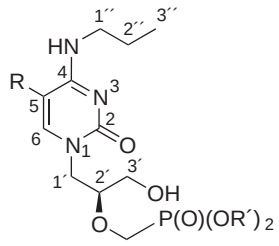


FIG. 1
General numbering scheme for assignment of NMR signals

(*S*)-5-Ethyl-1-[2-hydroxy-3-(triphenylmethoxy)propyl]-4-methoxypyrimidin-2(1*H*)-one (**5**)

(2*S*)-2-[(Trityloxy)methyl]oxirane (**3**; 9.0 g, 28.5 mmol) and cesium carbonate (945 mg, 2.9 mmol) were added to a solution of 5-ethyl-4-methoxypyrimidin-2(1*H*)-one (**2**; 4.39 g, 28.5 mmol) in DMF (200 ml) and the mixture stirred at 75 °C for 10 h. After cooling to room temperature, the mixture was taken down, the residue codistilled with toluene (150 ml) and chromatographed on a column of aluminum oxide (500 ml). Elution was performed with toluene until residues of **3** and TrOH were completely removed, then with ethyl acetate (1 l) and finally, the pure **5** was eluted with a mixture ethyl acetate–ethanol (10:1). Yield 12.78 g (93%) of a white foam, $[\alpha]_D$ –36.2 (*c* 0.443, $CHCl_3$). For $C_{29}H_{30}N_2O_4 \cdot 0.5H_2O$ (479.6) calculated: 72.63% C, 6.51% H, 5.84% N; found: 72.98% C, 6.54% H, 5.89% N. FAB MS, *m/z* (%): 471 (3) [M + H], 243 (100) [trityl]. ^{1}H NMR (DMSO-d_6): 7.60 s, 1 H (H-6); 7.42 d, 6 H, 7.33 t, 3 H and 7.26 t, 3 H (H-arom.); 5.27 d, 1 H, *J*(OH,2′) = 5.9 (OH); 4.05 dd, 1 H, *J*(1′a,2′) = 4.0, *J*(gem) = 12.9 (H-1′a); 3.99 m, 1 H (H-2′); 3.83 s, 3 H (OCH_3); 3.58 dd, 1 H, *J*(1′b,2′) = 8.3 (H-1′b); 2.97 dd, 1 H, *J*(3′a,2′) = 5.0, *J*(gem) = 9.4 (H-3′a); 2.90 dd, 1 H, *J*(3′b,2′) = 5.4 (H-3′b); 2.23 q, 2 H, *J*(CH_2,CH_3) = 7.4 (CH_2); 1.00 t, 3 H (CH_3). ^{13}C NMR (DMSO-d_6): 169.79 (C-4); 155.56 (C-2); 146.82 (C-6); 143.69, 3 C, 128.42, 6 C, 128.02, 6 C and 127.16, 3 C (trityl); 107.60 (C-5); 86.03 (trityl); 67.08 (C-2′); 66.25 (C-3′); 53.95 (OCH_3); 53.16 (C-1′); 19.20 (CH_2); 13.28 (CH_3).

(*S*)-1-{2-[(Diisopropoxyphosphoryl)methoxy]-3-(triphenylmethoxy)propyl}-5-ethyl-4-methoxypyrimidin-2(1*H*)-one (**7**)

Sodium hydride (60% dispersion in oil, 1.52 g, 38 mmol) was added to a solution of **5** (12 g, 25.5 mmol) and diisopropyl [(tosyloxy)methyl]phosphonate (10.7 g, 30.6 mmol) in tetrahydrofuran (350 ml) at –20 °C and stirred for 20 min. The stirring was then continued at 25 °C for 24 h and finally at 40 °C for 2 h. The mixture was filtered through Celite, the filtrate evaporated and the remaining oily residue (18 g) chromatographed on a column of silica gel (1500 ml), starting with chloroform–triethylamine (100:1) until the residue of diisopropyl [(tosyloxy)methyl]phosphonate was removed and then continuing with chloroform–methanol–triethylamine (100:7:1). Yield 13 g (79%) of **7** as colourless oil, $[\alpha]_D$ –29.0 (*c* 0.186, $CHCl_3$). For $C_{36}H_{45}N_2O_7P$ (648.7) calculated: 66.65% C, 6.99% H, 4.32% N, 4.77% P; found: 66.85% C, 6.98% H, 4.36% N, 4.74% P. FAB MS, *m/z* (%): 672 (0.6) [M + Na], 649 (0.3) [M + H], 407 (1.4) [M – trityl + 2 H], 243 (100) [trityl]. ^{1}H NMR (DMSO-d_6): 7.60 s, 1 H (H-6); 7.41 d, 6 H, 7.33 t, 6 H and 7.26 t, 3 H (H-arom.); 4.56 m, 2 H (P-OCH); 3.95 m, 3 H (H-1′, H-2′); 3.82 s, 3 H (OCH_3); 3.78 dd, 1 H, *J*(P,CH_a) = 8.8, *J*(gem) = 13.7 (PCH_a); 3.68 dd, 1 H, *J*(P,CH_b) = 9.7 (PCH_b); 3.24 dd, 1 H, *J*(3′a,2′) = 5.0, *J*(gem) = 9.5 (H-3′a); 2.97 dd, 1 H, *J*(3′b,2′) = 5.0 (H-3′b); 2.23 q, 2 H, *J*(CH_2,CH_3) = 7.4 (CH_2); 1.21 d, 3 H, 1.20 d, 3 H, 1.185 d, 3 H and 1.16 d, 3 H, *J*(CH_3,CH) = 6.1 (CH_3); 1.02 t, 3 H, *J*(CH_2,CH_3) = 7.4 (CH_3). ^{13}C NMR (DMSO-d_6): 169.86 (C-4); 155.39 (C-2); 146.40 (C-6); 143.64, 3 C, 128.39, 6 C, 128.04, 6 C and 127.23, 3 C (trityl); 107.96 (C-5); 86.34 (trityl); 78.18 d, *J*(P,C) = 12.7 (C-2′); 70.27 d, 2 C, *J*(P,C) = 6.4 (P-OCH); 64.08 d, *J*(P,C) = 165.5 (P-C); 62.85 (C-3′); 53.96 (OCH_3); 50.50 (C-1′); 23.88 d, 2 C, *J*(P,C) = 3.4, 23.83 d, *J*(P,C) = 4.9 and 23.72 d, *J*(P,C) = 4.4 (CH_3); 19.29 (CH_2); 13.04 (CH_3).

Diisopropyl Esters of C-5 Substituted (*S*)-1-[2-(Phosphonomethoxy)-3-(triphenylmethoxy)propyl]cytosines. General Procedure

A solution of an appropriate 4-methoxy derivative **6** or **7** (4.7 mmol) in 30% methanolic ammonia (40 ml) was heated in an autoclave at 110 °C for 16 h. After cooling to room temperature, the mixture was taken down and the residue chromatographed on a column of silica gel (200 ml) in system ethyl acetate–acetone–ethanol–water (18:3:2:2).

(S)-1-{2-[(Diisopropoxyphosphoryl)methoxy]-3-(triphenylmethoxy)propyl}-5-methylcytosine (**8**). Yield 1.63 g (54%) of a colourless foam, $[\alpha]_D$ –19.0 (*c* 0.297, $CHCl_3$). For $C_{34}H_{42}N_3O_6P{\cdot}0.5H_2O$ (637.3) calculated: 64.08% C, 6.96% H, 6.59% N, 4.86% P; found: 64.10% C, 6.96% H, 6.72% N, 4.79% P. FAB MS, *m/z* (%): 642 (0.5) [M + Na], 378 (2) [M – trityl + 2 H], 243 (100) [trityl], 126 (8) [5-methylcytosine + H]. ^{1}H NMR (DMSO-d_6): 7.40 d, 6 H, 7.34 t, 6 H and 7.26 t, 3 H (H-arom.); 7.21 br s, 1 H (H-6); 7.15 br, 1 H and 6.65 br, 1 H (NH_2); 4.59 m, 2 H (P-OCH); 3.84 m, 2 H (H-1′a, H-2′); 3.74 dd, 1 H, *J*(P,CH) = 8.9, *J*(gem) = 13.7 (PCH_a); 3.72 dd, 1 H, *J*(1′b,2′) = 8.4, *J*(gem) = 14.6 (H-1′b); 3.69 dd, 1 H, *J*(P,CH_b) = 9.6 (PCH_b); 3.19 dd, 1 H, *J*(3′a,2′) = 3.2, *J*(gem) = 10.6 (H-3′a); 2.89 dd, 1 H, *J*(3′b,2′) = 3.3 (H-3′b); 1.73 d, 3 H, *J*(CH_3,H-6) = 1.0 (CH_3); 1.24 d, 3 H, 1.22 d, 3 H, 1.21 d, 3 H and 1.19 d, 3 H, *J*(CH_3,CH) = 6.1 (CH_3). ^{13}C NMR (DMSO-d_6): 165.82 (C-4); 155.91 (C-2); 144.37 (C-6); 143.69, 3 C, 128.40, 6 C, 128.07, 6 C and 127.22, 3 C (trityl); 100.11 (C-5); 86.23 (trityl); 78.63 d, *J*(P,C) = 12.7 (C-2′); 70.33 d, *J*(P,C) = 6.3 (P-OCH); 70.31 d, *J*(P,C) = 5.9 (P-OCH); 64.17 d, *J*(P,C) = 165.5 (P-C); 62.81 (C-3′); 49.88 (C-1′); 23.94 d, 2 C, *J*(P,C) = 3.9, 23.87 d, *J*(P,C) = 4.4 and 23.78 d, *J*(P,C) = 4.4 (CH_3).

(S)-1-{2-[(Diisopropoxyphosphoryl)methoxy]-3-(triphenylmethoxy)propyl}-5-ethylcytosine (**9**). Yield 1.5 g (50%) of a colourless foam, $[\alpha]_D$ –9.0 (*c* 0.321, $CHCl_3$). For $C_{35}H_{44}N_3O_6P$ (633.7) calculated: 66.34% C, 7.00% H, 6.63% N, 4.89% P; found: 65.73% C, 7.03% H, 6.46% N, 4.90% P. FAB MS, *m/z* (%): 656 (0.5) [M + Na], 243 (100) [trityl]. ^{1}H NMR (DMSO-d_6): 7.38 d, 6 H, 7.32 t, 6 H and 7.26 t, 3 H (H-arom.); 7.18 s, 1 H (H-6); 7.10 br, 1 H and 6.75 br, 1 H (NH_2); 4.55 m, 2 H (P-OCH); 3.85 m, 2 H (H-1′a, H-2′); 3.75 dd, 1 H, *J*(1′b,2′) = 8.7, *J*(gem) = 14.0 (H-1′b); 3.66 dd, 1 H, *J*(P,CH_a) = 9.5, *J*(gem) = 13.4 (PCH_a); 3.60 dd, 1 H, *J*(P,CH_b) = 9.8 (PCH_b); 3.18 dd, 1 H, *J*(3′a, 2′) = 2.8, *J*(gem) = 10.6 (H-3′a); 2.90 dd, 1 H, *J*(3′b,2′) = 3.2 (H-3′b); 2.14 br q, 2 H (CH_2); 1.205 d, 3 H, 1.19 d, 3 H, 1.18 d, 3 H and 1.16 d, 3 H, *J*(CH_3,CH) = 6.1 (CH_3); 0.96 t, 3 H, *J*(CH_3,CH_2) = 7.4 (CH_3). ^{13}C NMR (DMSO-d_6): 165.40 (C-4); 156.06 (C-2); 143.85, 3 C (trityl); 143.70 (C-6); 128.56, 6 C, 128.26, 6 C and 127.44, 3 C (trityl); 106.38 (C-5); 86.43 (trityl); 78.84 d, *J*(P,C) = 13.2 (C-2′); 70.67 d, 2 C, *J*(P,C) = 6.3 (P-OCH); 64.29 d, *J*(P,C) = 165.5 (P-C); 63.07 (C-3′); 50.29 (C-1′); 24.09 d, 2 C, *J*(P,C) = 3.4, 24.02 d, *J*(P,C) = 4.4 and 23.93 d, *J*(P,C) = 4.4 (CH_3); 19.985 (CH_2); 12.76 (CH_3).

Reaction of Compound **7** with Primary Amines. General Procedure

An appropriate primary amine (40 mmol) was added to a solution of compound **7** (1 mmol) in dry dioxane (20 ml) and the mixture stirred under reflux for 5–15 h until a complete conversion was achieved (TLC check). The solution was evaporated, the residue coevaporated with xylene (3 × 20 ml) and chromatographed on a column of silica gel (200 ml) in the system described below. The following compounds were prepared by this procedure:

N^4-Cyclopropyl-1-{(S)-2-[(diisopropoxyphosphoryl)methoxy]-3-(triphenylmethoxy)propyl}-5-ethylcytosine (**12**). Chromatographed in the system chloroform–methanol–triethylamine (100:7:1). Yield 307 mg (46%) of a colourless syrup, $[\alpha]_D$ –13.4 (*c* 0.209, $CHCl_3$). For $C_{38}H_{48}N_3O_6P$ (673.8) calculated: 67.74% C, 7.18% H, 6.24% N, 4.60% P; found: 67.63% C,

7.13% H, 6.49% N, 4.51% P. FAB MS, *m/z* (%): 674 (0.5) [M + H], 432 (2) [M – trityl + 2 H], 243 (100) [trityl]. ^{1}H NMR (DMSO-d_6): 7.40 d, 6 H, 7.33 t, 6 H and 7.26 t, 3 H (H-arom.); 7.14 s, 1 H (H-6); 6.95 d, 1 H, *J*(NH,CH) = 4.0 (NH); 4.55 m, 2 H (P-OCH); 3.87 m, 2 H and 3.75 m, 1 H (H-1′, H-2′); 3.76 dd, 1 H, *J*(P,CH_a) = 8.8, *J*(gem) = 13.6 (PCH_a); 3.67 dd, 1 H, *J*(P,CH_b) = 9.8 (PCH_b); 3.20 dd, 1 H, *J*(3′a,2′) = 2.9, *J*(gem) = 10.7 (H-3′a); 2.90 dd, 1 H, *J*(3′b,2′) = 4.4 (H-3′b); 2.83 m, 1 H (cyclopropyl); 2.13 m, 2 H (CH_2); 1.21 d, 3 H, 1.20 d, 3 H, 1.18 d, 3 H and 1.16 d, 3 H, *J*(CH_3,CH) = 6.2 (CH_3); 0.94 t, 3 H, *J*(CH_2,CH_3) = 7.3 (CH_3); 0.67 m, 2 H and 0.54 m, 2 H (cyclopropyl). ^{13}C NMR (DMSO-d_6): 163.97 (C-4); 155.64 (C-2); 143.70, 3 C (trityl); 142.45 (C-6); 128.41, 6 C, 128.055, 6 C and 127.22, 3 C (trityl); 106.40 (C-5); 86.24 (trityl); 78.74 d, *J*(P,C) = 12.7 (C-2′); 70.30 d, 2 C, *J*(P,C) = 6.3 (P-OCH); 64.13 d, *J*(P,C) = 165.0 (P-C); 62.91 (C-3′); 50.07 (C-1′); 24.235 (NCH); 23.94 d, 2 C, *J*(P,C) = 3.4, 23.86 d, *J*(P,C) = 4.9 and 23.76 d, *J*(P,C) = 3.9 (CH_3); 19.39 (CH_2); 12.65 (CH_3); 6.34 and 6.29 (cyclopropyl CH_2).

N^4-Cyclopentyl-1-{(S)-2-[(diisopropoxyphosphoryl)methoxy]-3-(triphenylmethoxy)propyl}-5-ethylcytosine (**13**). Chromatographed in the system ethyl acetate–ethanol (12:1). Yield 440 mg (52%) of a colourless syrup, $[\alpha]_D$ –11.8 (*c* 0.196, $CHCl_3$). FAB MS, *m/z* (%): 702 (1) [M + H], 243 (48) [trityl]. ^{1}H NMR (DMSO-d_6): 7.39 d, 6 H, 7.31 t, 6 H and 7.27 t, 3 H (H-arom.); 7.12 s, 1 H (H-6); 6.66 d, 1 H, *J*(NH,CH) = 7.5 (NH); 4.56 m, 2 H (P-OCH); 3.88 m, 1 H (H-2′); 3.84 dd, 1 H, *J*(1′a,2′) = 3.4, *J*(gem) = 12.9 (H-1′a); 3.76 dd, 1 H, *J*(P,CH_a) = 9.2, *J*(gem) = 13.6 (PCH_a); 3.74 dd, 1 H, *J*(1′b,2′) = 6.7 (H-1′b); 3.67 dd, 1 H, *J*(P,CH_b) = 9.8 (PCH_b); 3.19 dd, 1 H, *J*(3′a,2′) = 2.6, *J*(gem) = 10.7 (H-3′a); 3.04 m, 1 H (NCH); 2.89 dd, 1 H, *J*(3′b,2′) = 4.2 (H-3′b); 2.19 m, 2 H (CH_2); 1.66 m, 2 H, 1.58 m, 2 H, 1.50 m, 2 H and 1.44 m, 2 H (cyclopentyl CH_2); 1.21 d, 3 H, 1.20 d, 3 H, 1.18 d, 3 H and 1.16 d, 3 H, *J*(CH_3,CH) = 6.2 (CH_3); 0.97 t, 3 H, *J*(CH_3,CH_2) = 7.3 (CH_3). ^{13}C NMR (DMSO-d_6): 162.28 (C-4); 155.66 (C-2); 143.74, 3 C (trityl); 142.44 (C-6); 128.41, 6 C, 128.055, 6 C and 127.22, 3 C (trityl); 106.40 (C-5); 86.24 (trityl); 78.71 d, *J*(P,C) = 12.7 (C-2′); 70.30 d, 2 C, *J*(P,C) = 6.3 (P-OCH); 64.12 d, *J*(P,C) = 165.5 (P-C); 62.89 (C-3′); 51.68 (NCH); 50.02 (C-1′); 31.95 and 31.88 (cyclopentyl CH_2); 24.01 d, *J*(P,C) = 3.9, 23.94 d, *J*(P,C) = 4.9, 23.90 d, *J*(P,C) = 4.9 and 23.84 d, *J*(P,C) = 3.9 (CH_3); 23.73 and 23.63 (cyclopentyl CH_2); 19.44 (CH_2); 12.66 (CH_3).

1-{(S)-2-[(Diisopropoxyphosphoryl)methoxy]-3-(triphenylmethoxy)propyl}-5-ethyl-N^4-(2-hydroxyethyl)cytosine (**14**). Chromatographed in the system ethyl acetate–methanol (4:1). Yield 528 mg (78%) of a colourless syrup, $[\alpha]_D$ –17.6 (*c* 0.203, $CHCl_3$). FAB MS, *m/z* (%): 678 (1.3) [M + H], 243 (100) [trityl]. ^{1}H NMR (DMSO-d_6): 7.39 d, 6 H, 7.32 t, 6 H and 7.26 t, 3 H (H-arom.); 7.15 s, 1 H (H-6); 7.01 t, 1 H, *J*(NH,CH_2) = 5.5 (NH); 4.85 t, 1 H, *J*(OH,CH_2) = 5.5 (OH); 4.55 m, 2 H (P-OCH); 3.87 m, 1 H (H-2′); 3.86 dd, 1 H, *J*(1′a,2′) = 4.8, *J*(gem) = 14.6 (H-1′a); 3.76 dd, 1 H, *J*(1′b,2′) = 8.4 (H-1′b); 3.76 dd, 1 H, *J*(P,CH_a) = 8.9, *J*(gem) = 13.6 (PCH_a); 3.68 dd, 1 H, *J*(P,CH_b) = 9.8 (PCH_b); 3.49 br q, 2 H, *J* = 5.6 (NCH_2); 3.19 dd, 1 H, *J*(3′a,2′) = 2.2, *J*(gem) = 10.5 (H-3′a); 3.09 br q, 2 H, *J* = 5.6 (OCH_2); 2.90 dd, 1 H, *J*(3′b,2′) = 4.2 (H-3′b); 2.16 m, 2 H (CH_2); 1.21 d, 3 H, 1.20 d, 3 H, 1.18 d, 3 H and 1.17 d, 3 H, *J*(CH_3CH) = 6.2 (CH_3); 0.98 t, 3 H, *J*(CH_3,CH_2) = 7.4 (CH_3). ^{13}C NMR (DMSO-d_6): 162.85 (C-4); 155.62 (C-2); 143.71, 3 C (trityl); 142.61 (C-6); 128.42, 6 C, 128.07, 6 C and 127.23, 3 C (trityl); 106.44 (C-5); 86.26 (trityl); 78.72 d, *J*(P,C) = 13.2 (C-2′); 70.34 d, 2 C, *J*(P,C) = 6.4 (P-OCH); 63.86 d, *J*(P,C) = 165.5 (P-C); 60.10 (C-3′); 59.75 (OCH_2); 50.02 (NCH_2); 43.15 (C-1′); 23.95 d, 2 C, *J*(P,C) = 3.4, 23.88 d, *J*(P,C) = 4.4 and 23.78 d, *J*(P,C) = 3.9 (CH_3); 19.49 (CH_2); 12.60 (CH_3).

N^4-Allyl-1-{(S)-2-[(diisopropoxyphosphoryl)methoxy]-3-(triphenylmethoxy)propyl}-5-ethylcytosine (**15**). Chromatographed in the system chloroform–methanol (9:1). Yield 349 mg (52%) of a white foam, $[\alpha]_D$ –30.6 (*c* 0.239, $CHCl_3$). For $C_{38}H_{48}N_3O_6P$ (673.8) calculated: 67.74% C, 7.18% H, 6.24% N, 4.60% P; found: 67.41% C, 7.42% H, 5.98% N, 4.93% P. FAB MS, *m/z* (%): 674 (1) [M + H], 243 (100) [trityl]. ^{1}H NMR (DMSO-d_6): 7.39 d, 6 H, 7.32 t, 6 H and 7.26 t, 3 H (H-arom.); 7.23 t, 1 H, *J*(NH,1″) = 5.7 (NH); 7.17 s, 1 H (H-6); 5.87 ddt, 1 H, *J*(2″,1″) = 5.2, *J*(2″,3″cis) = 10.3, *J*(2″,3″trans) = 17.2 (H-2″); 5.08 dq, 1 H, *J*(3″trans,1″) = *J*(gem) =1.7 (H-3″trans); 5.04 dq, 1 H, *J*(3″cis,1″) = *J*(gem) = 1.7 (H-3″cis); 4.56 m, 2 H (P-OCH); 3.93 br tt, 2 H (H-1″); 3.86 m, 2 H (H-1′a, H-2′); 3.76 dd, 1 H, *J*(P,CH_a) = 9.0, *J*(gem) = 13.6 (PCH_a); 3.75 dd, 1 H, *J*(1′b,2′) = 7.8, *J*(gem) = 14.0 (H-1′b); 3.67 dd, 1 H, *J*(P,CH_b) = 9.6 (PCH_b); 3.19 dd, 1 H, *J*(3′a,2′) = 2.7, *J*(gem) = 10.6 (H-3′a); 2.89 dd, 1 H, *J*(3′b,2′) = 4.4 (H-3′b); 2.18 m, 2 H (CH_2); 1.21 d, 3 H, 1.20 d, 3 H, 1.18 d, 3 H and 1.16 d, 3 H, *J*(CH_3,CH) = 6.1 (CH_3); 0.99 t, 3 H, *J*(CH_3,CH_2) = 7.3 (CH_3). ^{13}C NMR (DMSO-d_6): 162.47 (C-4); 155.64 (C-2); 143.71, 3 C (trityl); 142.77 (C-6); 135.58 (C-2″); 128.42, 6 C, 128.07, 6 C and 127.23, 3 C (trityl); 114.98 (C-3″); 106.38 (C-5); 86.24 (trityl); 78.68 d, *J*(P,C) = 13.2 (C-2′); 70.31 d, 2C, *J*(P,C) = 6.3 (P-OCH); 64.13 d, *J*(P,C) = 165.5 (P-C); 62.91 (C-3′); 50.04 (C-1′); 42.35 (C-1″); 23.95 d, 2 C, *J*(P,C) = 4.4, 23.88 d, *J*(P,C) = 4.9 and 23.78 d, *J*(P,C) = 4.4 (CH_3); 19.53 (CH_2); 12.78 (CH_3).

1-{(S)-2-[(Diisopropoxyphosphoryl)methoxy]-3-(triphenylmethoxy)propyl}-N^4-[2-(dimethylamino)-ethyl]-5-ethylcytosine (**16**). The crude product (1.2 g, ca. 75% purity) was used for the synthesis of **21** without chromatography. FAB MS, *m/z* (%): 705 (1) [M + H], 243 (100) [trityl].

Preparation of 1-[(*S*)-3-Hydroxy-2-(phosphonomethoxy)propyl] Derivatives of Substituted Cytosines **10**, **11** and **17**–**21**. General Procedure

Bromotrimethylsilane (1.5 ml, 11 mmol) was added to a solution of an appropriate phosphonate ester **8**, **9**, **12**–**16** (1 mmol) in acetonitrile (15 ml) and kept in the dark at room temperature for 48 h. The mixture was evaporated and the residue coevaporated with acetonitrile (20 ml). 90% Aqueous methanol (25 ml) was added, the solution neutralized with 1 M triethylammonium hydrogencarbonate (TEAB) to pH 7–8 and evaporated. The residue was codistilled with water (2 × 25 ml) to decompose remaining TEAB, then dissolved in water (150 ml) and extracted with ether (2 × 50 ml). The aqueous layer was concentrated to the 5 ml volume and applied onto a column of Dowex 50 (H^+ form, 40 ml). Elution was performed with water (600 ml), followed by 2.5% aqueous ammonia. The UV-absorbing ammonia fraction was evaporated to dryness, then dissolved in water (5 ml) and applied onto a column of Dowex 1 (acetate form, 40 ml). After elution with water (200 ml), the product was eluted with 0.5 M acetic acid. The product-containing fraction was evaporated, the residue coevaporated with water (4 × 30 ml) and the pure phosphonic acid crystallized from water or lyophilized. The following free phosphonic acids were prepared by this procedure:

1-[(S)-3-Hydroxy-2-(phosphonomethoxy)propyl]-5-methylcytosine (**10**). Yield 232 g (76%) of white crystals, m.p. 162–165 °C, $[\alpha]_D$ –64.5 (*c* 0.372, H_2O). For $C_9H_{16}N_3O_6P{\cdot}2/3H_2O$ (305.2) calculated: 35.42% C, 5.72% H, 13.77% N, 10.14% P; found: 35.60% C, 5.88% H, 13.45% N, 9.73% P. FAB MS, *m/z* (%): 294 (100) [M + H]. ^{1}H NMR (D_2O): 7.73 br q, 1 H, *J*(6,CH_3) = 1.0 (H-6); 4.13 dd, 1 H, *J*(1′a,2′) = 3.3, *J*(gem) = 14.4 (H-1′a); 3.86 dd, 1 H, *J*(1′b,2′) = 8.1 (H-1′b); 3.82 dd, 1 H, *J*(3′a,2′) = 3.9, *J*(gem) = 12.4 (H-3′a); 3.77 m, 1 H (H-2′); 3.75 dd, 1 H, *J*(P,CH_a) = 9.1, *J*(gem) = 12.9 (PCH_a); 3.60 dd, 1 H, *J*(3′b,2′) = 4.2 (H-3′b); 3.57 dd, 1 H, *J*(P,CH_b) = 9.6 (PCH_b); 2.02 d, 3 H, *J*(CH_3,6) = 1.0 (CH_3). ^{13}C NMR (D_2O): 160.37 (C-4); 150.42 (C-2);

147.64 (C-6); 102.99 (C-5); 79.49 d, *J*(P,C) = 11.7 (C-2′); 66.08 d, *J*(P,C) = 157.7 (P-C); 60.07 (C-3′); 49.81 (C-1′); 11.47 (CH_3).

5-Ethyl-1-[(S)-3-hydroxy-2-(phosphonomethoxy)propyl]cytosine (**11**). Yield 225 mg (73%) of white crystals, m.p. 126 °C, $[\alpha]_D$ –106.6 (*c* 0.357, H_2O). For $C_{10}H_{18}N_3O_6P$ (307.3) calculated: 39.09% C, 5.90% H, 13.68% N, 10.08% P; found: 39.12% C, 6.10% H, 13.38% N, 9.79% P. FAB MS, *m/z* (%): 308 (100) [M + H], 140 (17) [5-ethyluracil + H]. ^{1}H NMR (D_2O): 7.72 s, 1 H (H-6); 4.14 dd, 1 H, *J*(1′a,2′) = 2.5, *J*(gem) = 14.2 (H-1′a); 3.89 dd, 1 H, *J*(1′b,2′) = 8.0 (H-1′b); 3.83 dd, 1 H, *J*(3′a,2′) = 3.9, *J*(gem) = 12.4 (H-3′a); 3.78 m, 1 H (H-2′); 3.76 dd, 1 H, *J*(P,CH_a) = 9.4, *J*(gem) = 13.1 (PCH_a); 3.62 dd, 1 H, *J*(3′b,2′) = 3.9 (H-3′b); 3.58 dd, 1 H, *J*(P,CH_b) = 9.5 (PCH_b); 2.42 q, 2 H (CH_2); 1.18 t, 3 H, *J*(CH_3,CH_2) = 7.4 (CH_3). ^{13}C NMR (D_2O): 159.08 (C-4); 149.27 (C-2); 147.18 (C-6); 108.32 (C-5); 79.36 d, *J*(P,C) = 11.7 (C-2′); 66.03 d, *J*(P,C) = 157.2 (P-C); 59.98 (C-3′); 49.94 (C-1′); 18.95 (CH_2); 11.25 (CH_3).

N^4-Cyclopropyl-5-ethyl-1-[(S)-3-hydroxy-2-(phosphonomethoxy)propyl]cytosine (**17**). Yield 257 mg (74%) of a white amorphous solid, $[\alpha]_D$ –69.5 (*c* 0.679, H_2O). HR MS (FAB): for $C_{13}H_{23}N_3O_6P$ [M + H] calculated 348.1325, found 348.1345. FAB MS, *m/z* (%): 348 (100) [M + H]. ^{1}H NMR (D_2O): 7.62 s, 1 H (H-6); 4.14 dd, 1 H, *J*(1′a,2′) = 3.4, *J*(gem) = 14.3 (H-1′a); 3.90 dd, 1 H, *J*(1′b,2′) = 7.9 (H-1′b); 3.83 dd, 1 H, *J*(3′a,2′) = 3.9, *J*(gem) = 12.2 (H-3′a); 3.79 m, 1 H (H-2′); 3.75 dd, 1 H, *J*(P,CH_a) = 9.4, *J*(gem) = 13.2 (PCH_a); 3.69 dd, 1 H, *J*(3′b,2′) = 4.0 (H-3′b); 3.57 dd, 1 H, *J*(P,CH_b) = 9.4 (PCH_b); 2.77 m, 1 H (NCH); 2.36 br q, 2 H, *J*(CH_2,CH_3) = 7.5 (CH_2); 1.13 t, 3 H (CH_3); 1.06 m, 2 H and 0.84 m, 2 H (CH_2). ^{13}C NMR (D_2O): 159.08 (C-4); 149.52 (C-2); 145.28 (C-6); 108.64 (C-5); 79.40 d, *J*(P,C) = 12.2 (C-2′); 66.09 d, *J*(P,C) = 156.7 (P-C); 60.03 (C-3′); 49.93 (C-1′); 23.46 (NCH); 18.88 (CH_2); 11.23 (CH_3); 6.87, 2 C (CH_2).

N^4-Cyclopentyl-5-ethyl-1-[(S)-3-hydroxy-2-(phosphonomethoxy)propyl]cytosine (**18**). Yield 179 mg (48%) of a white amorphous solid, $[\alpha]_D$ –70.0 (*c* 0.165, H_2O). HR MS (FAB): for $C_{15}H_{27}N_3O_6P$ [M + H] calculated 376.1637, found 376.1622. FAB MS, *m/z* (%): 376 (1.5) [M + H]. ^{1}H NMR (D_2O): 7.51 s, 1 H (H-6); 4.22 m, 1 H (NCH); 4.12 dd, 1 H, *J*(1′a,2′) = 3.4, *J*(gem) = 14.4 (H-1′a); 3.88 dd, 1 H, *J*(1′b,2′) = 7.9 (H-1′b); 3.83 dd, 1 H, *J*(3′a,2′) = 3.8, *J*(gem) = 12.3 (H-3′a); 3.79 m, 1 H (H-2′); 3.74 dd, 1 H, *J*(P,CH_a) = 9.5, *J*(gem) = 13.1 (PCH_a); 3.62 dd, 1 H, *J*(3′b,2′) = 4.0 (H-3′b); 3.58 dd, 1 H, *J*(P,CH_b) = 9.5 (PCH_b); 2.41 q, 2 H, *J*(CH_2,CH_3) = 7.4 (CH_2); 2.10 m, 2 H and 1.78 m, 2 H (CH_2); 1.69 m, 4 H (CH_2); 1.16 t, 3 H (CH_3). ^{13}C NMR (D_2O): 156.53 (C-4); 149.85 (C-2); 144.64 (C-6); 108.84 (C-5); 79.48 d, *J*(P,C) = 11.7 (C-2′); 66.14 d, *J*(P,C) = 157.2 (P-C); 60.09 (C-3′); 54.685 (NCH); 49.81 (C-1′); 31.50, 31.48 and 23.40, 2 C (CH_2); 18.96 (CH_2); 11.25 (CH_3).

5-Ethyl-N^4-(2-hydroxyethyl)-1-[(S)-3-hydroxy-2-(phosphonomethoxy)propyl]cytosine (**19**). Yield 217 mg (62%) of a white solid (after lyophilization), $[\alpha]_D$ –57.8 (*c* 0.250, H_2O). For $C_{12}H_{22}N_3O_7P{\cdot}0.5H_2O$ (360.3) calculated: 40.00% C, 6.43% H, 11.66% N, 8.60% P; found: 39.98% C, 6.42% H, 11.38% N, 8.06% P. FAB MS, *m/z* (%): 352 (13) [M + H]. ^{1}H NMR (D_2O): 7.60 s, 1 H (H-6); 4.12 dd, 1 H, *J*(1′a,2′) = 3.5, *J*(gem) = 14.3 (H-1′a); 3.87 dd, 1 H, *J*(1′b,2′) = 7.8 (H-1′b); 3.83 t, 2 H (NCH_2); 3.82 dd, 1 H, *J*(3′a,2′) = 3.8, *J*(gem) = 12.4 (H-3′a); 3.78 m, 1 H (H-2′); 3.74 dd, 1 H, *J*(P,CH_a) = 9.4, *J*(gem) = 13.1 (PCH_a); 3.68 t, 2 H, *J*(CH_2,CH_2) = 5.2 (OCH_2); 3.61 dd, 1 H, *J*(3′b,2′) = 4.2 (H-3′b); 3.58 dd, 1 H, *J*(P,CH_b) = 9.4 (PCH_b); 2.42 q, 2 H, *J*(CH_2,CH_3) = 7.6 (CH_2); 1.18 t, 3 H (CH_3). ^{13}C NMR (D_2O): 158.64 (C-4); 150.73 (C-2); 144.92 (C-6); 109.01 (C-5); 79.54 d, *J*(P,C) = 11.7 (C-2′); 66.17 d, *J*(P,C) = 157.7 (P-C); 60.012 (C-3′); 59.40 (OCH_2); 49.91 (C-1′); 44.39 (NCH_2); 19.06 (CH_2); 11.27 (CH_3).

N^4-Allyl-5-ethyl-1-[(S)-3-hydroxy-2-(phosphonomethoxy)propyl]cytosine (**20**). Yield 240 mg (69%) of a white foam, $[\alpha]_D$ –54.7 (*c* 0.167, H_2O). HR MS (FAB): for $C_{13}H_{23}N_3O_6P$ [M + H] calculated 348.1324, found 348.1330. FAB MS, *m/z* (%): 348 (100) [M + H]. ^{1}H NMR (D_2O):

7.65 s, 1 H (H-6); 5.92 ddt, 1 H, *J*(2″,1″) = 4.6, *J*(2″,3″cis) = 10.6, *J*(2″,3″trans) = 17.2 (H-2″); 5.29 dq, 1 H, *J*(3″cis,1″) = *J*(gem) =1.8 (H-3″cis); 5.25 dq, 1 H, *J*(3″trans,1″) = 1.8 (H-3″trans); 4.17 dt, 2 H (H-1″); 4.13 dd, 1 H, *J*(1′a,2′) = 3.4, *J*(gem) = 14.3 (H-1′a); 3.88 dd, 1 H, *J*(1′b,2′) = 8.0 (H-1′b); 3.83 dd, 1 H, *J*(3′a,2′) = 3.9, *J*(gem) = 12.3 (H-3′a); 3.80 m, 1 H (H-2′); 3.78 dd, 1 H, *J*(P,CH_a) = 9.3, *J*(gem) = 13.2 (PCH_a); 3.62 dd, 1 H, *J*(3′b,2′) = 4.9 (H-3′b); 3.60 dd, 1 H, *J*(P,CH_b) = 9.5 (PCH_b); 2.45 q, 2 H (CH_2); 1.19 t, 3 H, *J*(CH_2,CH_3) = 7.5 (CH_3). ^{13}C NMR (D_2O): 156.98 (C-4); 149.22 (C-2); 145.44 (C-6); 129.87 (C-2″); 117.07 (C-3″); 108.64 (C-5); 79.40 d, *J*(P,C) = 11.7 (C-2′); 65.85 d, *J*(P,C) = 157.7 (P-C); 60.03 (C-3′); 49.94 (C-1′); 44.15 (C-1″); 19.09 (CH_2); 11.30 (CH_3).

N^4-[2-(Dimethylamino)ethyl]-5-ethyl-1-[(S)-3-hydroxy-2-(phosphonomethoxy)propyl]cytosine (**21**). Yield 166 mg (41%) of a white amorphous solid, $[\alpha]_D$ +15.9 (*c* 0.178, H_2O). For $C_{14}H_{27}N_4O_6P{\cdot}1.5H_2O$ (405.4) calculated: 41.48% C, 7.46% H, 13.82% N, 7.64% P; found: 41.80% C, 7.61% H, 13.81% N, 7.72% P. FAB MS, *m/z* (%): 379 (85) [M + H]. ^{1}H NMR (D_2O): 7.44 s, 1 H (H-6); 4.07 dd, 1 H, *J*(1′a,2′) = 3.5, *J*(gem) = 13.8 (H-1′a); 3.85 dd, 1 H, *J*(1′b,2′) = 7.6 (H-1′b); 3.82 t, 2 H, *J*(CH_2,CH_2) = 5.6 (NCH_2); 3.80 dd, 1 H, *J*(3′a,2′) = 4.0, *J*(gem) = 12.2 (H-3′a); 3.78 m, 1 H (H-2′); 3.68 dd, 1 H, *J*(P,CH_a) = 9.4, *J*(gem) = 12.8 (PCH_a); 3.60 dd, 1 H, *J*(3′b,2′) = 4.2 (H-3′b); 3.56 dd, 1 H, *J*(P,CH_b) = 9.4 (PCH_b); 3.42 t, 2 H (NCH_2); 2.98 s, 6 H (NCH_3); 2.34 q, 2 H, *J*(CH_2,CH_3) = 7.4 (CH_2); 1.16 t, 3 H (CH_3). ^{13}C NMR (D_2O): 164.09 (C-4); 158.14 (C-2); 143.57 (C-6); 110.19 (C-5); 80.13 d, *J*(P,C) = 12.2 (C-2′); 66.63 d, *J*(P,C) = 157.2 (P-C); 60.56 (C-3′); 57.57 (NCH_2); 50.23 (C-1′); 43.14, 2 C (NCH_3); 36.24 (NCH_2); 19.21 (CH_2); 11.38 (CH_3).

N^4-Benzoyl-1-{(*S*)-2-[(diisopropoxyphosphoryl)methoxy]-3-(triphenylmethoxy)propyl}-5-iodocytosine (**23**)

A solution of protected HPMPC [1] (**22**; 6.0 g, 8.5 mmol) in dry toluene (40 ml) was heated to 80 °C. *N*-Iodosuccinimide (2.9 g, 13 mmol) was added, followed by AIBN (180 mg, 1.1 mmol), the mixture was heated to 80 °C for 4 h and then set aside at room temperature for 12 h. The resulting dark red solution was concentrated to 1/3 of its original volume, applied onto a column of silica gel (800 ml) equilibrated in the system toluene–triethylamine (100:1) and the column eluted with this system until *N*-iodosuccinimide and all iodinated (red-coloured) by-products were removed. The desired product **23** was eluted with the system toluene–ethyl acetate–triethylamine (200:100:1). Yield 5 g (70%) of a yellowish foam, $[\alpha]_D$ –44.3 (*c* 0.439, $CHCl_3$). For $C_{40}H_{43}IN_3O_7P$ (835.7) calculated: 57.49% C, 5.19% H, 15.19% I, 5.03% N, 3.71% P; found: 57.71% C, 5.31% H, 14.89% I, 4.80% N, 3.97% P. FAB MS, *m/z* (%): 836.2 (0.3) [M + H], 243 (100) [trityl], 105 (30) (benzoyl). ^{1}H NMR (DMSO-d_6): 13.05 br s, 1 H (NH); 8.32 s, 1 H (H-6); 8.25 d, 2 H, 7.61 t, 1 H, 7.53 t, 2 H, 7.41 d, 6 H, 7.34 t, 6 H and 7.25 t, 3 H (H-arom.); 4.56 m, 2 H (P-OCH); 4.02 dd, 1 H, *J*(1′a,2′) = 7.9, *J*(gem) = 13.9 (H-1′a); 3.94 m, 2 H (H-1′b, H-2′); 3.79 dd, 1 H, *J*(P,CH_a) = 8.5, *J*(gem) = 13.9 (PCH_a); 3.73 dd, 1 H, *J*(P,CH_b) = 8.7 (PCH_b); 3.24 dd, 1 H, *J*(3′a,2′) = 3.8, *J*(gem) = 10.6 (H-3′a); 2.99 dd, 1 H, *J*(3′b,2′) = 3.8 (H-3′b); 1.22 d, 3 H, 1.21 d, 3 H, 1.205 d, 3 H and 1.19 d, 3 H, *J*(CH_3,CH) = 6.2 (CH_3). ^{13}C NMR (DMSO-d_6): 178.39 (C=O); 157.87 (C-4); 153.35 (C-6); 148.01 (C-2); 143.63, 3 C (trityl); 136.71, 132.985, 129.68, 2 C and 128.59, 2 C (benzoyl); 128.41, 6 C, 128.15, 6 C, 127.305, 3 C and 86.48 (trityl); 77.76 d, *J*(P,C) = 11.2 (C-2′); 70.45 d, *J*(P,C) = 6.3 (P-OCH); 70.40 d, *J*(P,C) = 5.9 (P-OCH); 68.29 (C-5); 64.00 d, *J*(P,C) = 164.5 (P-C); 62.71 (C-3′); 50.26 (C-1′); 23.97 d, 2 C, *J*(P,C) = 3.9 (CH_3); 23.85 d, 2 C, *J*(P,C) = 4.4 (CH_3).

N^4-Benzoyl-1-{(*S*)-2-[(diisopropoxyphosphoryl)methoxy]-3-(triphenylmethoxy)propyl}-5-[(trimethylsilyl)ethynyl]cytosine (**24**)

Dry dioxane (20 ml) was added under argon to a mixture of iodo derivative **23** (3.70 g, 4.43 mmol), CuI (44 mg, 0.22 mmol) and $[PdCl_2(Ph_3P)_2]$ (62 mg, 0.089 mmol), followed by trimethylsilylacetylene (1.06 ml, 7.53 mmol) and triethylamine (0.66 ml, 4.8 mmol). The mixture was stirred at room temperature for 2 h, then taken down and the residue partitioned between ethyl acetate (250 ml) and a saturated solution of ammonium chloride and EDTA (1:1, 200 ml). The organic layer was dried with anhydrous $MgSO_4$, evaporated in vacuo and the residue chromatographed on silica gel column (750 ml) in the system chloroform–methanol (95:5). Yield 2.5 g (70%) of a yellowish foam, $[\alpha]_D$ –54.1 (*c* 0.376, $CHCl_3$). For $C_{45}H_{52}N_3O_7PSi$ (806.0) calculated: 67.06% C, 6.50% H, 5.21% N, 3.84% P; found: 66.81% C, 6.41% H, 5.31% N, 3.92% P. FAB MS, *m/z* (%): 806.6 (0.3) [M + H], 243 (100) [trityl], 105 (41) (benzoyl). ^{1}H NMR (DMSO-d_6): 12.70 br s, 1 H (NH); 8.00–7.20 m, 21 H (H-6 + H-arom.); 4.57 m, 2 H (P-OCH); 4.00 m, 3 H and 3.76 m, 2 H (H-1′, H-2′, PCH_2); 3.25 dd, 1 H, *J*(3′a,2′) = 3.0, *J*(gem) = 10.6 (H-3′a); 2.95 dd, 1 H, *J*(3′b,2′) = 3.5 (H-3′b); 1.22 d, 6 H and 1.21 d, 6 H, *J*(CH_3,CH) = 6.2 (CH_3); 0.16 s, 9 H ($SiCH_3$). ^{13}C NMR (DMSO-d_6): 177.15 (C=O); 156.70 (C-4); 153.87 (C-2); 151.69 (C-6); 143.63, 3 C (trityl); 136.58, 132.71, 129.54, 2 C and 128.86, 2 C (benzoyl); 128.40, 6 C, 128.12, 6 C and 127.28, 3 C (trityl); 98.76 (C≡); 97.35 (C-5); 96.70 (C≡); 86.41 (trityl); 77.67 d, *J*(P,C) = 10.0 (C-2′); 70.40 d, 2 C, *J*(P,C) = 6.3 (P-OCH); 63.95 d, *J*(P,C) = 164.6 (P-C); 62.59 (C-3′); 50.26 (C-1′); 23.90 d, 2 C, *J*(P,C) = 4.9 (CH_3); 23.80 d, 2 C, *J*(P,C) = 4.4 (CH_3); –0.15 ($SiCH_3$).

1-{(*S*)-2-[(Diisopropoxyphosphoryl)methoxy]-3-(triphenylmethoxy)propyl}-5-ethynylcytosine (**25**)

A solution of **24** (1.5 g, 1.86 mmol) in 30% methanolic ammonia was kept in refrigerator (4 °C) for 24 h, then evaporated and the residue chromatographed on silica gel (400 ml) in the system chloroform–methanol (95:5). Yield 1.13 g (96%) of a white foam, $[\alpha]_D$ –24.0 (*c* 0.240, $CHCl_3$). For $C_{35}H_{40}N_3O_6P$ (629.7) calculated: 66.76% C, 6.40% H, 6.67% N, 4.92% P; found: 66.87% C, 6.32% H, 7.00% N, 5.18% P. FAB MS, *m/z* (%): 604.5 (0.3) [M – acetylen + H], 243 (100) [trityl]. ^{1}H NMR (DMSO-d_6): 7.82 s, 1 H (H-6); 7.60 brs, 1 H (NH_2); 7.40 d, 6 H, 7.35 t, 6 H and 7.27 t, 3 H (H-arom.); 6.72 br s, 1 H (NH_2); 4.55 m, 2 H (P-OCH); 4.32 s, 1 H (≡CH); 3.94 dd, 1 H, *J*(1′a,2′) = 4.0, *J*(gem) = 12.9 (H-1′a); 3.87 m, 1 H (H-2′); 3.81 dd, 1 H, *J*(1′b,2′) = 7.5 (H-1′b); 3.75 dd, 1 H, *J*(P,CH_a) = 8.8, *J*(gem) = 13.8 (PCH_a); 3.64 dd, 1 H, *J*(P,CH_b) = 9.7 (PCH_b); 3.19 dd, 1 H, *J*(3′a,2′) = 3.4, *J*(gem) = 10.6 (H-3′a); 2.89 dd, 1 H, *J*(3′b,2′) = 4.3 (H-3′b); 1.23 d, 3 H, 1.21 d, 3 H, 1.20 d, 3 H and 1.18 d, 3 H, *J*(CH_3,CH) = 6.2 (CH_3). ^{13}C NMR (DMSO-d_6): 164.855 (C-4); 154.23 (C-2); 151.375 (C-6); 143.67, 3 C, 128.42, 6 C, 128.13, 6 C and 127.28, 3 C (trityl); 88.205 (-C≡); 86.29 (trityl); 85.86 (≡CH); 78.19 d, *J*(P,C) = 12.7 (C-2′); 75.92 (C-5); 70.38 d and 70.37 d, *J*(P,C) = 6.4 (P-OCH); 64.03 d, *J*(P,C) = 165.0 (P-C); 62.59 (C-3′); 50.53 (C-1′); 23.96 d and 23.93 d, *J*(P,C) = 4.9 (CH_3); 23.79 d, 2 C, *J*(P,C) = 4.4 (CH_3).

5-Ethynyl-1-[(*S*)-3-hydroxy-2-(phosphonomethoxy)propyl]cytosine (**26**)

Bromotrimethylsilane (0.9 ml, 6.6 mmol) was added dropwise during 30 min to a stirred solution of ester **25** (1 g, 1.6 mmol) in freshly distilled acetonitrile (20 ml) cooled to 0 °C. After addition of bromotrimethylsilane, the mixture was slowly warmed up to room temper-

ature and set aside in the dark for 24 h. 0.5 M Triethylammonium hydrogencarbonate (20 ml) was added, the solution evaporated at 30 °C and coevaporated in water (2 × 20 ml). The residue dissolved in water (5 ml) was applied onto a column of Dowex 50 (H^+, 50 ml), elution performed with cold (0 °C) 50% aqueous acetone (200 ml) followed by water (0 °C, 1 l). Dowex 50 was then removed from the column, stirred with cold water (0 °C) and 10% NH_4OH was added until pH 9. The ion exchanger was filtered off by suction, washed with water (50 ml) and combined filtrates were evaporated in vacuo. The crude product was purified by preparative HPLC using 1% aqueous methanol (isocratic elution, flow rate 12 ml/min). Two products were obtained: HPMP-5-ethynylcytosine **26** (retention time 4.3 min on analytical column, 20 min on preparative column) and HPMP-5-acetylcytosine **27** (retention time 5.0 min on analytical column, 23 min on preparative column). Corresponding fractions were evaporated to dryness and lyophilized from water. Yield 250 mg (52%) of **26** as a white amorphous solid, $[\alpha]_D$ –22.1 (*c* 0.165, H_2O). FAB MS, *m/z* (%): 304 (23) [M + H]. ^{1}H NMR (D_2O): 8.01 s, 1 H (H-6); 4.10 dd, 1 H, *J*(1′a,2′) = 3.7, *J*(gem) = 14.0 (H-1′a); 3.82 dd, 1 H, *J*(1′b,2′) = 7.6 (H-1′b); 3.80 dd, 1 H, *J*(3′a,2′) = 3.3, *J*(gem) = 12.2 (H-3′a); 3.79 s, 1 H (≡CH); 3.70 dd, 1 H, *J*(P,CH_a) = 9.5, *J*(gem) = 12.8 (PCH_a); 3.58 dd, 1 H, *J*(3′b,2′) = 4.4 (H-3′b); 3.57 dd, 1 H, *J*(P,CH_b) = 9.5 (PCH_b). ^{13}C NMR (D_2O): 165.84 (C-4); 157.10 (C-2); 152.50 (C-6); 91.36 (C-5); 85.69 (-C≡); 80.39 d, *J*(C,P) = 12.1 (C-2′); 74.65 (≡CH); 66.96 d, *J*(C,P) = 157.3 (P-C); 60.92 (C-3′); 51.28 (C-1′).

5-Acetyl-1-[(*S*)-3-hydroxy-2-(phosphonomethoxy)propyl]cytosine (**27**)

The compound was obtained as a by-product in preparation of **26**. Yield 40 mg (8%) of a white amorphous solid. HR MS (FAB): for $C_{10}H_{17}N_3O_7P$ [M + H] calculated 322.0804, found 322.0792. FAB MS, *m/z* (%): 322 (10) [M + H]. ^{1}H NMR (D_2O): 8.65 s, 1 H (H-6); 4.25 dd, 1 H, *J*(1′a,2′) = 3.3, *J*(gem) = 14.0 (H-1′a); 3.88 dd, 1 H, *J*(1′b,2′) = 8.4 (H-1′b); 3.85 dd, 1 H, *J*(3′a,2′) = 4.0, *J*(gem) = 12.5 (H-3′a); 3.77 m, 1 H (H-2′); 3.74 dd, 1 H, *J*(P,CH_a) = 9.5, *J*(gem) = 12.8 (PCH_a); 3.63 dd, 1 H, *J*(3′b,2′) = 4.3 (H-3′b); 3.52 dd, 1 H, *J*(P,CH_b) = 9.5 (PCH_b); 2.52 s, 3 H (CH_3). ^{13}C NMR (D_2O): 198.99 (C=O);163.80 (C-4); 156.65 (C-6); 156.48 (C-2); 104.50 (C-5); 79.60 d, *J*(C,P) = 12.7 (C-2′); 66.50 d, *J*(C,P) = 156.7 (P-C); 60.22 (C-3′); 51.40 (C-1′); 26.30 (CH_3).

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