SYNTHESIS OF SOME N^4-SUBSTITUTED DERIVATIVES OF 1-[(*S*)-3-HYDROXY-2-(PHOSPHONOMETHOXY)PROPYL]CYTOSINE (HPMPC, CIDOFOVIR)

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N^4-Substituted derivatives of HPMPC were synthesized in four-step synthesis which included treatment of 4-methoxypyrimidin-2(1*H*)-one (**1**) with (*S*)-[(trityloxy)methyl]oxirane in DMF. Condensation of intermediary 1-[2-hydroxy-3-(trityloxy)propyl]-4-methoxypyrimidin-2(1*H*)-one (**2**) with (diisopropoxyphosphoryl)methyl tosylate in the presence of sodium hydride resulted in fully protected 4-methoxypyrimidin-2(1*H*)-one derivative **3** which gave on reaction with an appropriate primary amine in dioxane N^4-substituted products **4a**–**4i**. The reaction with bromotrimethylsilane simultaneously cleaved the trityl group and deprotected the phosphonate residue and gave the title HPMP analogues substituted at the cytosine amino group in position N^4 **5a**–**5i**. Compound **4j** was prepared from 4-methoxypyrimidin-2(1*H*)-one (**1**) by reaction with cyclopropylamine in dioxane. The intermediary 4-(cyclopropylamino)pyrimidin-2(1*H*)-one (**6**) then reacts with (*S*)-[(trityloxy)methyl]oxirane in DMF. Fully protected phosphonate **4j** and its deprotected counterpart **5j** was obtained by the same sequence of reactions as in the case of compounds **5a**–**5i**.

Keywords: HPMPC; Cidofovir; Acyclic nucleoside phophonates; Nucleotides; Aminolysis; Cytosine; Pyrimidines.

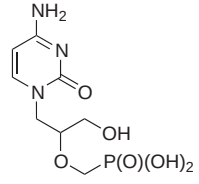
Acyclic nucleoside phosphonates are sometimes considered to be a new dimension in the antiviral chemotherapy. They not only exhibit a pronounced and protracted biological activity that persists for days or even longer after a single administration, but even more importantly, they have a uniquely broad spectrum of indications for clinical use, encompassing both DNA virus and retrovirus infections and also various forms of neoplasia of both viral and non-viral origin[1].

The acyclic nucleoside phosphonate HPMPC, 1-[(*S*)-3-hydroxy-2-(phosphonomethoxy)propyl]cytosine, cidofovir, has a unique profile among the antiviral agents: it is active against a much broader spectrum of DNA viruses than any other antiviral agent known and extends a long-lasting antiviral

activity thus enabling infrequent dosing (in some cases, 1–2 week intervals)[2]. Cidofovir was approved for parenteral use in the treatment of cytomegalovirus retinitis in AIDS patients. However, it suppresses essentially all genera of DNA viruses – herpes-, adeno-, polyoma,- papilloma- and poxviruses and many case reports of successful treatment of patients with diseases caused by these viruses are published in the literature thus proving its general usefulness[3,4].

Recently, serious concerns were expressed about the possible occurrence of variola virus stocks as a potential tool of bioterrorism (variola virus is the causative agent of very dangerous and highly contagious smallpox disease). A major part of the present population has not been vaccinated against smallpox since it has been globally eradicated several decades ago; pregnant women, cardiacs, transplant patients would have to be excluded from the vaccination. Most importantly, the vaccine is not yet available in the required quantity. Therefore, an oral drug formulation that would allow individual application to prevent an epidemic in the case of smallpox (or closely related monkeypox) outbreak would be highly desirable[5–7].

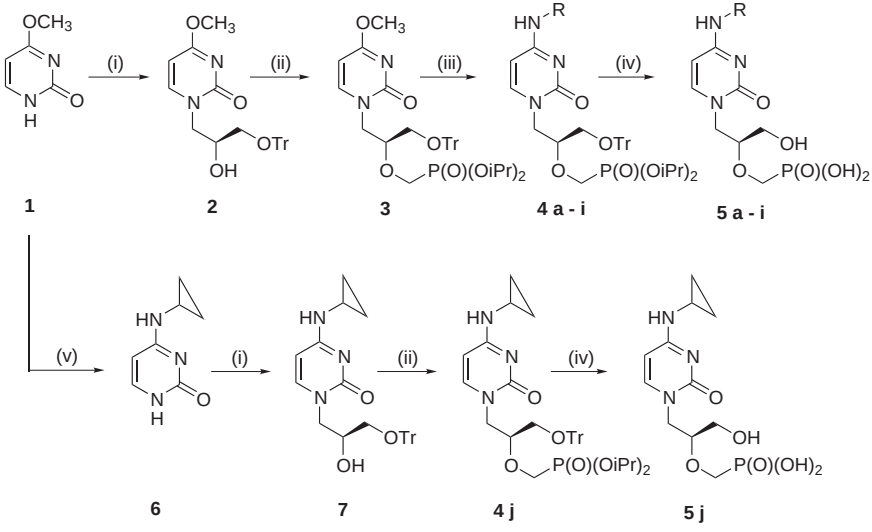
Cidofovir (HPMPC) is the most active compound against poxviruses ever tested[1,8]. Big effort is aimed at the development of cidofovir prodrugs with better penetrability through the cellular membrane[9,10]. No attention was paid to the effect of the cytosine base modification in cidofovir. In this paper, we describe two synthetic approaches to the preparation of N^4-substituted cidofovir derivatives, both resulting in the (*S*)-enantiomers.



Synthesis

There are two general schemes for the synthesis of N^4-substituted derivatives of HPMPC. The first approach (Scheme 1) involves four steps: 4-methoxypyrimidin-2(1*H*)-one (**1**) reacts with (*S*)-[(trityloxy)methyl]-oxirane in DMF using Cs_2CO_3 catalysis so that only *N*-isomer is formed[11]. The thus obtained 1-[2-hydroxy-3-(trityloxy)propyl]-4-methoxypyrimidin-2(1*H*)-one (**2**) reacts with (diisopropoxyphosphoryl)methyl tosylate in THF in the presence of NaH at increased temperature[11]. The third step consists in the reaction of the resulting fully protected phosphonate **3** with an ap-

propriate primary amine in dioxane. With low-boiling amines, the reaction mixture was heated to reflux, in the case of high-boiling amines, at 80 °C. Tritylated diisopropyl esters **4a**–**4i** were obtained by column chromatography on silica gel and after codistillation with acetone they were dried over P_2O_5 in vacuo. Both the trityl and phosphonate protecting isopropyl groups were simultaneously removed by cleavage with bromotrimethylsilane giving products (**5a**–**5i**). The other approach starts from the same compound **1**; in this case it is converted to the N^4-substituted cytosine derivative which then undergoes the same procedure to give the final protected intermediate **4**. It is documented by the synthesis of N^4-cyclopropyl derivative **5j**. Compound **4j** was at first prepared from 4-methoxypyrimidin-2(1*H*)-one (**1**) by reaction with cyclopropylamine in dioxane. The resulting 4-(cyclopropylamino)pyrimidin-2(1*H*)-one (**6**) then reacted with (*S*)-[(trityloxy)methyl]oxirane in DMF giving compound **7**. Fully protected phosphonate **4j** and its deprotected counterpart **5j** were obtained by the same reaction sequence as described for the first approach (compounds **5a**–**5i**, Scheme 1).



a, R = ethyl
b, R = propyl
c, R = allyl
d, R = butyl
e, R = cyclopentyl
f, R = but-2-en-1-yl
g, R =2-methoxyethyl
h, R = 2-hydroxyethyl
i, R = 2-(dimethylamino)ethyl
j, R = cyclopropyl

(i) (*S*)-[(trityloxy)methyl]oxirane, DMF, Cs_2CO_3, 100 °C; (ii) $TsOCH_2P(O)(OiPr)_2$, NaH, THF, 50 °C; (iii) RNH_2, dioxane, reflux; (iv) Me_3SiBr, acetonitrile; (v) cyclopropylamine, dioxane, reflux

SCHEME 1

To increase the efficiency of antiviral screening we also performed the reaction of compound **3** in dioxane with a mixture of five primary amines with similar boiling points. The mixture contained propylamine (b.p. 48 °C), cyclopropylamine (b.p. 50 °C), allylamine (b.p. 53 °C), cycloheptylamine (b.p. 54 °C) and isobutylamine (b.p. 67 °C). The progress of the reaction was followed by TLC and the reaction mixture was refluxed until the starting material was consumed. Other five amines were: *N*,*N*-dimethylethane-1,2-diamine (b.p. 107 °C), ethanolamine (b.p. 170 °C), 2-methoxyethylamine (b.p. 95 °C), cyclohexylamine (b.p. 134 °C) and benzylamine (b.p. 185 °C). Both these mixtures were worked up in the same way as in the case of compounds **5a**–**5i**. HPLC analysis of the resulting material detected four of the five compounds as separate peaks. The area of one of the peaks suggests that it is composed of two compounds.

Concluding, we synthesized a serie of N^4-substituted derivatives of HPMPC. This method gave better results than the direct hydrogensulfite-mediated exchange of the amino group in the cytosine base of HPMPC or its derivatives[12].

Biological Activity

All prepared compounds were tested in Vero cell cultures for their antiviral activity against parainfluenza-3 virus, reovirus-1, sindbis virus, Coxsackie virus B4 and Punta Toro virus. In E_6SM cell cultures these compounds were tested for their activity against herpes simplex virus-1 (KOS), herpes simplex virus-2 (G), vaccinia virus and herpes simplex virus-1 TK^- KOS ACV. In HeLa cell cultures these compounds were tested for their activity against vesicular stomatitis virus, Coxsackie virus B4 and respiratory syncytial virus. None of the prepared compounds was active on these cell cultures. All prepared compounds were also tested for their immunomodulatory activity. None of the test compounds, applied at the screening concentrations of 100 μmol/l, increased production of NO by murine macrophages when given alone or together with LPS.

EXPERIMENTAL

Unless stated otherwise, solvents were evaporated at 40 °C/2 kPa and compounds were dried at 2 kPa over P_2O_5 overnight. Melting points were determined on a Büchi Melting Point B-545 apparatus. TLC was performed on Silica gel 60 F_{254} plates (Merck, Germany). ^{1}H NMR spectra were taken on Varian Unity-200 (at 200 MHz) or Varian Unity-500 (at 500 MHz) instruments in DMSO-d_6, D_2O or D_2O–NaOD solutions with tetramethylsilane (TMS) or sodium dimethylsilapentanesulfonate (DSS) as the respective internal standards. ^{1}H NMR

chemical shifts (δ, ppm) and coupling constants (*J*, Hz) were obtained by the first-order analysis of the spectra. Flash chromatography was made on an ISCO Champion apparatus with silica gel columns. The numbering system for assignment of NMR signals is outlined in Fig. 1.

Materials

All amines, sodium hydride and cesium carbonate were purchased from Sigma Aldrich (Prague, Czech Republic). Dowex 50X8 and Dowex 1X2 were obtained from Fluka (Switzerland). Dimethylformamide was distilled from P_2O_5 in vacuo. (*S*)-[(Trityloxy)methyl]oxirane was obtained from Raylo (Canada). Diisopropyl [(tosyloxy)methyl]phosphonate was prepared according to ref.[9]

General Procedures

Deionisation of the Reaction Mixture

A solution of reaction products in water (4–5 ml) was applied onto a column of Dowex 50X8 (H^+ form) (50 ml if not stated otherwise) and the column was washed with water (20% aqueous methanol for deionisation of phosphonate diesters) until the UV-absorption (254 nm) and acid reaction of the eluate dropped (standard elution rate, 3 ml/min). The elution continued with 2.5% ammonia (in water or 20% aqueous methanol) and the UV-absorbing eluate was collected and evaporated in vacuo.

Purification of Phosphonates by Dowex 1X2 Column Chromatography

Unless stated otherwise, 50-ml columns of Dowex 1X2 (50–100 mesh, acetate form) were used. The deionized sample in water (4–5 ml) was applied onto the column. Elution with water (3 ml/min) was continued until the initial UV-absorption (254 nm) of the eluate dropped. The column was then eluted (3 ml/min, 30-ml fractions) with 1 M acetic acid.

4-(Cyclopropylamino)pyrimidin-2(1*H*)-one (**6**)

A solution of 4-methoxypyrimidin-2(1*H*)-one (**1**; 5 g, 39.6 mmol) in dioxane (250 ml) was heated with cyclopropylamine (50 ml) and the reaction mixture was refluxed until TLC (chloroform:methanol, 9:1) showed the absence of starting material. The mixture was then evaporated in vacuo and dissolved in MeOH. Silica gel (10 g) was added, the mixture was evaporated in vacuo and the residue was chromatographed by flash chromatography on a silica gel column (330 g). The product was eluted with chloroform:methanol (4:1). The product-containing fractions were evaporated in vacuo. The product was crystallized from

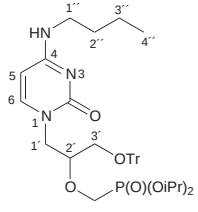


FIG. 1
General numbering scheme for assignment of NMR signals

ethanol, washed with ether (30 ml) and dried overnight at 2 kPa over P_2O_5. Yield 5.5 g (91.8%) of a white powder, m.p. 225 °C. FAB HR MS: for $C_7H_9N_3O$ calculated: 151.0745, found: 151.0751. FAB MS, *m/z* (%): 152 (100) [M + H]. ^{1}H NMR (DMSO-d_6): 10.45 vb, 1 H (NH); 7.66 bs, 1 H (NH); 7.25 bs, 1 H (H-2); 5.53 bs, 1 H (H-3); 2.78 bs, 1 H (H-1′); 0.67 um, 1 H (H-2′); 0.42 um, 1 H (H-2′). ^{13}C NMR (DMSO-d_6): 165.9 (C-4); 157.07 (C-6); 141.52 (C-2); (C-5); 93.37 (C-5); 23.43 (C-1′); 6.26 (C-2′).

4-(Cyclopropylamino)-1-[(*S*)-2-hydroxy-3-(trityloxy)propyl]pyrimidin-2(1*H*)-one (**7**)

The solution of 4-(cyclopropylamino)pyrimidin-2(1*H*)-one (**6**; 4.5 g, 29.7 mmol) and (*S*)-[(trityloxy)methyl]oxirane (9.4 g, 29.7 mmol) in dimethylformamide (148 ml) was preheated to 100 °C for 15 min and cesium carbonate (1.4 g, 4.29 mmol) was added. The resulting mixture was stirred at 100 °C for 2 h. The reaction course was followed by TLC (chloroform:methanol, 25:1). The insoluble material was filtered off and the filtrate was evaporated to dryness in vacuo. The residue was codistilled with toluene (3 × 30 ml) and the mixture was chromatographed on a silica gel column (330 g). The product was eluted with chloroform:methanol (49:1) to which triethylamine (1%) was added. The fractions containing product were evaporated to dryness in vacuo and the product was codistilled with acetone and dried at 2 kPa over P_2O_5 overnight. Yield 8.9 g (64.2%) of a white foam. For $C_{29}H_{29}N_3O_3 \cdot 0.5H_2O$ (467.5) calculated: 73.09% C, 6.35% H, 8.82% N; found: 73.33% C, 6.33% H, 8.62% N. FAB MS, *m/z* (%): 468 (30) [M + H]. ^{1}H NMR (DMSO-d_6): 7.61 bd, 1 H, *J* = 3.5 (NH, H-1′); 7.42 m, 6 H (*o*-H); 7.34 m, 6 H (*m*-H); 7.26 m, 6 H (*p*-H); 7.33 d, 1 H, *J* = 7.0 (H-2, H-3); 5.50 d, 1 H, *J* = 7.0 (H-3); 5.24 bd, 1 H, *J* = 5.5 (OH, H-2″); 3.99 brdd, 1 H, *J* = 13.0 and 3.0 (H-1″a, H-2″); 3.93 m, 1 H (H-2″); 3.40 brdd, 1 H, *J* = 13.0 and 8.5 (H-1″b, H-2″); 2.93 dd, 1 H, *J* = 9.5 and 5.0 (H-3″a, OTr and H-2″, H-3″); 2.88 dd, 1 H, *J* = 9.5 and 5.2 (H-3″b, OTr and H-2″, OTr); 0.68 um, 1 H (H-3′); 0.42 um (H-3′). ^{13}C NMR (DMSO-d_6): 165.17 (C-4); 156.24 (C-6); 146.13 (C-2); 143.91 (OTr); 128.47 (OTr); 128.06 (OTr); 127.17 (OTr); 93.29 (C-5); 85.96 (C-4″); 67.68 (C-2″); 66.00 (C-3″); 52.80 (C-1″); 23.53 (C-1′); 6.27 (C-2′).

1-[(*S*)-3-Hydroxy-2-(phosphonomethoxy)propyl]-N^4-cyclopropylcytosine (**5i**)

A solution of 4-(cyclopropylamino)-1-[2-hydroxy-3-(trityloxy)propyl]pyrimidin-2(1*H*)-one (**7**; 8.9 g, 19 mmol) and (diisopropoxyphosphoryl)methyl tosylate (21.3 g, 60.8 mmol) in dimethylformamide (50 ml) was evaporated to dryness in vacuo. The residue in dimethylformamide (178 ml) was cooled down to –20 °C and sodium hydride (1.3 g, 57 mmol) was added. The reaction mixture got gradually warmer and was at last heated at 50 °C for 2 days. The reaction course was checked by TLC chloroform:methanol (9:1). Detection of the phosphonate function with 2-(4-nitrobenzyl)pyridine resulted in blue spots in the ammonia vapours. The reaction mixture was evaporated to dryness in vacuo and codistilled with toluene (3 × 30 ml). The residue was dissolved in 80% acetic acid (500 ml) and the reaction mixture was refluxed for 30 min. The reaction mixture was evaporated to dryness in vacuo. The residue dissolved in water (100 ml) was extracted with ether (3 × 50 ml). The ether layer was re-extracted with water (3 × 50 ml), the aqueous layer was taken down in vacuo and the residue was dried at 2 kPa over P_2O_5 overnight. The dry residue in acetonitrile (70 ml) was treated with bromotrimethylsilane (14 ml). The mixture was left standing overnight, evaporated in vacuo and the residue was dissolved in water (300 ml), made alkaline by addition of ammonia and evaporated in vacuo. The residue dissolved in water (5 ml)

was applied onto a Dowex 50X8 (H^+ form) column (200 ml), the column was eluted with water until the acidity of the eluate dropped. The column was then eluted with 2.5% aqueous ammonia and the UV-absorbing eluate was taken down in vacuo. The product in water (5 ml) was applied onto a column (200 ml) of Dowex 1X2 (acetate form) and washed with water and then with 1 M acetic acid. The UV-absorbing eluate was evaporated in vacuo and codistilled with water. The product was purified by HPLC, codistilled with ethanol and dried over P_2O_5 in vacuo. Yield 1.5 g (25%) of a white solid, m.p. 245 °C. For $C_{11}H_{18}N_3O_6P \cdot 0.5EtOH$ (319.2) calculated: 42.11% C, 6.18% H, 12.28% N, 9.05% P; found: 42.00% C, 6.13% H, 12.27% N, 9.28% P. FAB MS, *m/z* (%): 320 (100) [M + H], 152 (10) [4-(cyclopropylamino)pyrimidine-2(1*H*)-one + H]. ^{1}H NMR (D_2O): 7.3 d, 1 H, *J*(5,6) = 7.7 (H-6); 6.02 d, 1 H, *J*(5,6) = 7.7 (H-5); 4.14 dd, 1 H, *J*(1′a,2′) = 3.4, *J*(gem) = 14.2 (H-1′a); 3.85 dd, 1 H, *J*(1′b,2′) = 8.3, *J*(gem) = 14.2 (H-1′b); 3.77 m, 1 H (2′-CH); 3.83 dd, 1 H, *J*(3′a,2′) = 3.8, *J*(gem) = 12.4 (3′-CH_2); 3.62 dd, 1 H, *J*(3′b,2′) = 4.0, *J*(gem) = 12.4 (3′-CH_2); 3.75 dd, 1 H, *J*(P-CH) = 9.0, *J*(gem) = 13.2 (P-CH_2); 3.57 dd, 1 H, *J*(P-CH) = 9.8, *J*(gem) = 13.2 (P-CH_2); 2.77 m, 1 H (N-CH); 0.97 m, 2 H (CH_2); 0.75 m, 2 H (CH_2). ^{13}C NMR (D_2O): 161.0 (C-4); 151.75 (C-2); 147.9 (C-6); 94.97 (C-5); 79.54 d, 2 C, *J* = 11.7 (C-2′); 66.11 d, 2 C, *J*(P,C) = 158.7 (P-C); 60.07 (C-3′); 50.06 (C-1′); 23.25 (N-CH); 6.45, 2 C (CH_2). α_D^{20} –88.5.

1-[(*S*)-3-Hydroxy-2-(phosphonomethoxy)propyl]-N^4-alkylcytosines. General Procedure

To a solution of compound **3** (1 g, 1.6 mmol) in dioxane (10 ml) an appropriate amine (3 ml) was added. The reaction mixture was refluxed until the starting material was consumed. The course of the reaction was checked by TLC in chloroform:methanol (25:1). The reaction mixture was evaporated to dryness in vacuo, codistilled with dioxane and chromatographed on a silica gel column (300 ml) in 1% triethylamine in chloroform. The product containing fractions were collected, evaporated to dryness in vacuo, codistilled with acetone (3 × 5 ml) and dried over P_2O_5 in vacuo overnight. The following compounds were prepared by this procedure.

Diisopropyl 1-[(S)-3-hydroxy-2-(phosphonomethoxy)propyl]-N^4-ethylcytosine (**4a**). Yield 0.683 g (66.9%) of white foam. For $C_{35}H_{44}N_3O_6P \cdot H_2O$ (633.7) calculated: 64.5% C, 7.11% H, 6.45% N, 4.75% P; found: 64.80% C, 7.33% H, 6.32% N, 4.73% P. FAB MS, *m/z* (%): 634 (35) [M + H]. ^{1}H NMR (DMSO-d_6): 7.54 t, 1 H, *J*(NH,1″) = 5.4 (NH); 7.40 d, 6 H (*o*-H); 7.34 t, 6 H (*m*-H); 7.26 t, 3 H (*p*-H); 7.27 d, 1 H (H-6); 5.53 d, 1 H, *J*(5,6) = 7.3 (H-5); 4.55 m, 2 H (P-OCH); 3.87 dd, 1 H, *J*(1′a,2′) = 4.6, *J*(gem) = 12.8 (1′-CH_2); 3.84 m, 1 H (2′-CH); 3.76 dd, 1 H, *J*(P,CH) = 8.9, *J*(gem) = 13.7 (P-CH); 3.69 dd, 1 H, *J*(1′b,2′) = 6.7, *J*(gem) = 12.8 (1′-CH_2); 3.67 dd, 1 H, *J*(P,CH) = 9.5, *J*(gem) = 13.7 (P-CH_2); 3.23 m, 2 H (1″-CH_2); 3.19 dd, 1 H, *J*(3′a,2′) = 3.2, *J*(gem) = 10.5 (3′-CH_2); 2.91 dd, 1 H, *J*(3′b,2′) = 4.3, *J*(gem) = 10.5 (3′-CH_2); 1.215, 3 H, *J*(CH_3,CH) = 6.1 (CH_3); 1.21, 3 H, *J*(CH_3,CH) = 6.1 (CH_3); 1.19, 3 H, *J*(CH_3,CH) = 6.1 (CH_3); 1.17, 3 H, *J*(CH_3,CH) = 6.1 (CH_3); 1.07, 3 H, *J*(CH_3,CH_2) = 7.2 (H″). ^{13}C NMR (DMSO-d_6): 163.76 (C-4); 155.94 (C-2); 145.55 (C-6); 143.67, 3 C (OTr); 128.44, 6 C (OTr); 128.10, 6 C (OTr); 127.26, 3 C (OTr); 93.79 (C-5); 86.24 (OTr); 78.70 (C-2′); 70.34 d, 2 C, *J*(P,C) = 6.3 (P-OC); 64.16 d, *J*(P,C) = 166.0 (P-C); 62.75 (C-3′); 49.97 (C-1′); 34.65 (C-1″); 23.89 d, 2 C (iPrCH_3); 23.83 d, 2 C (iPrCH_3); 14.46 (C-2″). α_D^{20} –21.3.

Diisopropyl 1-[(S)-3-hydroxy-2-(phosphonomethoxy)propyl]-N^4-propylcytosine (**4b**). Yield 0.722 g (69.2%) of white foam. For $C_{36}H_{46}N_3O_6P \cdot 0.25H_2O$ (647.7) calculated: 66.29% C, 7.19% H, 6.44% N, 4.75% P; found: 66.07% C, 7.31% H, 6.29% N, 5.09% P. FAB MS, *m/z* (%): 648 (20) [M + H]. ^{1}H NMR (DMSO-d_6): 7.54 t, 1 H, *J* = 5.55 (NH); 7.40 m, 6 H (*o*-H); 7.34 m, 6 H

(*m*-H); 7.27 d, 1 H, *J*(5,6) = 7.3 (H-6); 7.26 m, 3 H (*p*-H); 5.55 d, 1 H, *J*(5,6) = 7.3 (H-5); 4.55 m, 2 H (P-OCH); 3.86 dd, 1 H, *J*(1′a,1′b) = 12.6 (H-1′a); 3.83 m, 1 H (H-2′); 3.75 dd, 1 H, *J*(P,CH) = 8.9, *J*(gem) = 13.7; 3.68 dd, 1 H, *J*(1′a,1′b) = 12.6 (H-1′b); 3.66 dd, 1 H, *J*(P,CH) = 9.6, *J*(gem) = 13.7 (P-CH); 3.19 dd, 1 H, *J*(3′a,2′) = 3.4, *J*(gem) = 10.5 (3′-CH_2); 3.16 m, 2 H (H-1″); 2.91 dd, 1 H, *J*(3′b,2′) = 4.3, *J*(gem) = 10.5 (3′-CH_2); 1.48 m, 1 H, *J*(CH_2,CH_3) = 7.4 (H-2″); 1.215 d, 3 H, *J*(CH_3,CH) = 6.2 (CH_3); 1.21, 3 H, *J*(CH_3,CH) = 6.2 (CH_3); 1.19, 3 H, *J*(CH_3,CH) = 6.2 (CH_3); 1.17, 3 H, *J*(CH_3,CH) = 6.2 (CH_3); 0.88 t, 3 H, *J*(3″,2″) = 7.4 (H-3″). ^{13}C NMR (DMSO-d_6): 163.97 (C-4); 155.93 (C-2); 145.55 (C-6); 143.67, 3 C (OTr); 128.44, 6 C (OTr); 128.10, 6 C (OTr); 127.26, 3 C (OTr); 93.80 (C-5); 86.24 (OTr); 78.74 (C-2′); 70.34 d, 2 C, *J*(P,C) = 6.3 (P-OC); 64.14 d, *J*(P,C) = 164.8 (P-C); 62.74 (C-3′); 49.99 (C-1′); 41.63 (C-1″); 23.89 d, 2 C (iPrCH_3); 23.83 d, 2 C (iPrCH_3); 22.04 (C-2″); 11.63 (C-3″). α_D^{20} –17.9.

Diisopropyl 1-[(S)-3-hydroxy-2-(phosphonomethoxy)propyl]-N^4-allylcytosine (**4c**). Yield 0.593 g (57%) of white foam. For $C_{36}H_{44}N_3O_6P{\cdot}0.5H_2O$ (645.7) calculated: 66.04% C, 6.93% H, 6.42% N, 4.73% P; found: 66.14% C, 6.90% H, 6.30% N, 5.01% P. FAB MS, *m/z* (%): 646 (20) [M + H]. ^{1}H NMR (DMSO-d_6): 7.70 t, 1 H, *J*(NH,H-1″a) = 5.6 (NH); 7.40 m, 6 H (*o*-H); 7.34, 6 H (*m*-H); 7.32 d, 1 H, *J*(5,6) = 5.6 (H-6); 7.26 m, 3 H (*p*-H); 5.86 m, 1 H, *J*(2″,3″a) = 17.2 (H-2″), *J*(2″,3″b) = 10.3 (H-2″), *J*(2″,1″a) = 5.2 (H-2″) and *J*(2″,3″b) = 17.2 (H-2″); 5.60 d, 1 H, *J*(5,6) = 7.2 (H-5); 4.55 m, 2 H (P-OCH); 5.15 dq, 1 H, *J*(3″a,3″b) = 1.7 (H-3″a); 5.09 dq, 1 H, *J*(3″a,3″b) = 1.7 (H-3″b); 3.88 m, 2 H, *J*(1″a,3″a) = 1.7 (H-1″a), *J*(1″b,3″b) = 1.6 (H-1″b); 3.84 dd, 1 H, *J*(1′a,1′b) = 13.5 (H-1′a); 3.84 m, 1 H, *J*(2′,1′b) = 6.9 (H-2′), *J*(2′,1′a) = 4.5 (H-2′); 3.75 dd, 1 H, *J*(P,CH) = 8.9, *J*(gem) = 13.6; 3.66 dd, 1 H, *J*(P,CH) = 9.6, *J*(gem) = 13.6 (P-CH); 3.19 dd, 1 H, *J*(3′a,2′) = 4.4, *J*(gem) = 10.5 (3′-CH_2); 2.91 dd, 1 H, *J*(3′b,2′) = 4.4, *J*(gem) = 10.5 (3′-CH_2); 1.215, 3 H, *J*(CH_3,CH) = 6.2 (CH_3); 1.21, 3 H, *J*(CH_3,CH) = 6.2 (CH_3); 1.19, 3 H, *J*(CH_3,CH) = 6.2 (CH_3); 1.17, 3 H, *J*(CH_3,CH) = 6.2 (CH_3). ^{13}C NMR (DMSO-d_6): 163.85 (C-4); 155.85 (C-2); 145.91 (C-6); 143.67, 3 C (OTr); 135.17 (C-2″); 128.44, 6 C (OTr); 128.10, 6 C (OTr); 127.26, 3 C (OTr); 115.62 (C-3″); 93.70 (C-5); 86.24 (OTr); 78.65 d, *J*(C,P) = 12.6; 70.34 d, 2 C, *J*(C,P) = 6.4 (P-OC); 64.15 d, 2 C, *J*(C,P) = 166.0 (P-C); 62.73 (C-3′); 50.03 (C-1′); 42.05 (C-1″); 23.89 d, 2 C (iPrCH_3); 23.83 d, 2 C (iPrCH_3). α_D^{20} –19.2.

Diisopropyl 1-[(S)-3-hydroxy-2-(phosphonomethoxy)propyl]-N^4-butylcytosine (**4d**). Yield 0.794 g (74.5%) of white foam. For $C_{37}H_{48}N_3O_6P{\cdot}0.5H_2O$ (661.7) calculated: 66.25% C, 7.36% H, 6.26% N, 4.62% P; found: 66.52% C, 7.31% H, 6.08% N, 4.45% P. FAB MS, *m/z* (%): 662 (20) [M + H]. ^{1}H NMR (DMSO-d_6): 7.51 t, 1 H (NH); 7.27 d, 1 H, *J*(5,6) = 7.2 (H-6); 7.40 m, 6 H (*o*-H); 7.34 m, 6 H (*m*-H); 7.26 m, 3 H (*p*-H); 5.54 d, 1 H, *J*(5,6) = 7.2 (H-5); 4.55 m, 2 H (P-OCH); 3.86, 1 H, *J*(1′a,1′b) = 12.8 (H-1′a); 3.84 m, 1 H, *J*(2′,1′b) = 6.3 (H-2′), *J*(2′,1′a) = 4.6 (H-2′); 3.75 dd, 1 H, *J*(P,CH) = 8.9, *J*(gem) = 13.7 (P-CH); 3.68 dd, 1 H, *J*(1′b,2′) = 6.3, *J*(gem) = 12.8 (H-1′b); 3.66 dd, 1 H, *J*(P,CH) = 9.7, *J*(gem) = 13.7 (P-CH); 3.20 m, 2 H (H-1″); 3.20 dd, 1 H, *J*(3′a,2′) = 3.4, *J*(gem) = 10.5 (H-3′); 2.91 dd, *J*(3′b,2′) = 4.3, *J*(gem) = 10.5 (H-3′); 1.45 m, 2 H (H-2″); 1.31 m, 2 H (H-3″); 1.215 d, 3 H, *J*(CH_3,CH) = 6.3 (CH_3); 1.21 d, 3 H, *J*(CH_3,CH) = 6.3 (CH_3); 1.19 d, 3 H, *J*(CH_3,CH) = 6.2 (CH_3); 1.17 d, 3 H, *J*(CH_3,CH) = 6.2 (CH_3); 0.89 t, 3 H, *J*(3″,2″) = 7.3 (CH_3). ^{13}C NMR (DMSO-d_6): 163.94 (C-4); 155.93 (C-2); 145.53 (C-6); 143.67, 3 C (OTr); 128.44, 6 C (OTr); 128.10, 6 C (OTr); 127.26, 3 C (OTr); 86.24 (OTr); 78.69 (C-2′); 70.34 d, 2 C, *J*(C,P) = 6.2 (P-OC); 64.14 d, 2 C, *J*(C,P) = 165.5 (P-C); 62.73 (C-3′); 49.99 (C-1′); 39.70 (C-1″); 30.90 (C-2″); 23.89 d, 2 C (iPrCH_3); 23.83 d, 2 C (iPrCH_3); 19.83 (C-3″); 13.88 (C-4″). α_D^{20} –24.5.

Diisopropyl 1-[(S)-3-hydroxy-2-(phosphonomethoxy)propyl]-N^4-cyclopentylcytosine (**4e**). Yield 0.598 g (55.2%) of white foam. For $C_{38}H_{48}N_3O_6P{\cdot}0.33H_2O$ (673.7) calculated: 67.14% C,

7.22% H, 6.18% N, 4.56% P; found: 67.23% C, 7.17% H, 6.19% N, 4.37% P. FAB MS, *m/z* (%): 674 (40) [M + H]. ^{1}H NMR (DMSO-d_6): 7.52 d, 1 H, *J*(5,6) = 7.3 (H-6); 7.40 d, 6 H (*o*-H); 7.34 t, 6 H (*m*-H); 7.26 t, 3 H (*p*-H); 7.24 d, 1 H, *J*(NH,CH) = 7.0 (NH); 5.53 d, 1 H, *J*(5,6) = 7.3 (H-5); 4.55 m, 2 H (P-OCH); 4.17 br sext, 1 H, *J*(CH,NH) ~ *J*(CH,CH_2) = 6.9; 3.87 dd, 1 H, *J*(1′a,2′) = 4.5, *J*(gem) = 12.6 (1′-CH_2); 3.84 m, 1 H (2′-CH); 3.76 dd, 1 H, *J*(P,CH) = 8.8, *J*(gem) = 13.7 (P-CH_2); 3.68 dd, 1 H, *J*(1′b,2′) = 6.2, *J*(gem) = 12.6 (H-1′b); 3.67 dd, 1 H, *J*(P,CH) = 9.7, *J*(gem) = 13.7 (P-CH_2); 3.20 dd, 1 H, *J*(3′a,2′) = 3.3, *J*(gem) = 10.6 (H-3′); 2.90 dd, *J*(3′b,2′) = 4.4, *J*(gem) = 10.6 (H-3′); 1.85 m, 2 H (CH_2); 1.64 m, 2 H (CH_2); 1.53 m, 2 H (CH_2); 1.38 m, 2 H (CH_2); 1.215 d, 3 H, *J*(CH_3,CH) = 6.1 (CH_3); 1.21 d, 3 H, *J*(CH_3,CH) = 6.1 (CH_3); 1.19 d, 3 H, *J*(CH_3,CH) = 6.1 (CH_3); 1.17 d, 3 H, *J*(CH_3,CH) = 6.1 (CH_3). ^{13}C NMR (DMSO-d_6): 163.46 (C-4); 155.92 (C-2); 145.42 (C-6); 143.67, 3 C (OTr); 128.44, 6 C (OTr); 128.10, 6 C (OTr); 127.26, 3 C (OTr); 93.90 (C-5); 86.24 (OTr); 78.69 d, 2 C, *J*(P,C) = 13.2 (C-2′); 70.34 d, 2 C, *J*(C,P) = 6.3 (P-OC); 64.13 d, 2 C, *J*(C,P) = 165.5 (P-C); 62.73 (C-3′); 51.30 (N-CH); 49.97 (C-1′); 32.38 (CH_2); 32.31 (CH_2); 23.89 d, 2 C (iPrCH_3); 23.83 d, 2 C (iPrCH_3); 23.52 (CH_2). α_D^{20} + 6.6.

Diisopropyl 1-[(S)-3-hydroxy-2-(phosphonomethoxy)propyl]-N^4-(but-2-en-1-yl)cytosine (**4f**). Yield 0.825 g (77.7%) of white foam. For $C_{37}H_{46}N_3O_6P{\cdot}0.5H_2O$ (659.7) calculated: 66.45% C, 7.08% H, 6.28% N, 4.63% P; found: 66.74% C, 7.25% H, 6.24% N, 4.67% P. FAB MS, *m/z* (%): 660 (25) [M + H]. ^{1}H NMR (DMSO-d_6): 7.62 t, 1 H, *J*(NH,1″) = 5.5 (NH); 7.40 m, 6 H (*o*-H); 7.34 m, 6 H (*m*-H); 7.32 d, 1 H, *J*(5,6) = 7.2 (H-6); 7.26 m, 3 H (*p*-H); 5.57 d, 1 H, *J*(5,6) = 7.2 (H-5); 5.57 m, 1 H (H-2″-CH); 5.57 m, 1 H (H-3″-CH); 4.55 m, 2 H (P-OCH); 3.87dd, 1 H, *J*(1′a,2′) = 4.6, *J*(gem) = 12.0 (1′-CH_2); 3.84 m, 1 H (2′-CH); 3.79 m, 2 H (1″-CH_2); 3.75 dd, 1 H, *J*(P,CH) = 8.8, *J*(gem) = 13.6 (P-CH); 3.66 dd, 1 H, *J*(P,CH) = 9.5, *J*(gem) = 13.6; 3.66 dd, 1 H, *J*(1′b,2′) = 6.3, *J*(gem) = 12.0 (H-1′b); 3.20 dd, 1 H, *J*(3′a,2′) = 3.3, *J*(gem) = 10.5 (H-3′); 2.91 dd, *J*(3′b,2′) = 4.3, *J*(gem) = 10.5 (H-3′); 1.65 br dq, 3 H, *J*(CH_3,2″) ~ *J*(CH_3,1″) = 1.2, *J*(CH_3,3″) = 6.3 (CH_3); 1.215 d, 3 H, *J*(CH_3,CH) = 6.1 (CH_3); 1.21 d, 3 H, *J*(CH_3,CH) = 6.1 (CH_3); 1.19 d, 3 H, *J*(CH_3,CH) = 6.1 (CH_3); 1.17 d, 3 H, *J*(CH_3,CH) = 6.1 (CH_3). ^{13}C NMR (DMSO-d_6): 163.68 (C-4); 155.89 (C-2); 145.75 (C-6); 143.67, 3 C (OTr); 128.44, 6 C (OTr); 128.10, 6 C (OTr); 127.79 (C″-CH); 127.26, 3 C (OTr); 126.84 (C-3″); 93.75 (C-5); 86.24 (OTr); 78.73 (C-2′); 70.34 d, 2 C, *J*(C,P) = 6.4 (P-OC); 64.19 d, 2 C, *J*(C,P) = 165.5 (P-C); 62.75 (C-3′); 50.02 (C-1′); 41.55 (C-1″); 23.89 d, 2 C (iPrCH_3); 23.83 d, 2 C (iPrCH_3); 17.64 (C-4″). α_D^{20} –11.3.

Diisopropyl 1-[(S)-3-hydroxy-2-(phosphonomethoxy)propyl]-N^4-(2-methoxyethyl)cytosine (**4g**). Yield 0.855 g (80%) of white foam. For $C_{36}H_{46}N_3O_7P{\cdot}0.5H_2O$ (663.7) calculated: 63.42% C, 7.10% H, 6.16% N, 4.54% P; found: 63.43% C, 7.09% H, 6.08% N, 4.33% P. FAB MS, *m/z* (%): 664 (30) [M + H]. ^{1}H NMR (DMSO-d_6): 7.64 t, 1 H, *J*(NH,1″) = 5.2 (NH); 7.40 d, 6 H (*o*-H); 7.34 d, 6 H (*m*-H); 7.29 d, 1 H, *J*(5,6) = 7.3 (H-6); 7.26 d, 3 H (*p*-H); 5.60 d, 1 H, *J*(5,6) = 7.3 (H-5); 4.55 m, 2 H (P-OCH); 3.87 dd, 1 H, *J*(1′a,2′) = 4.5, *J*(gem) = 12.8 (1′-CH_2); 3.84 m, 1 H (2′-CH); 3.75 dd, 1 H, *J*(P,CH) = 8.9, *J*(gem) = 13.7 (P-CH_2); 3.69 dd, 1 H, *J*(1′b,2′) = 6.7, *J*(gem) = 12.8 (H-1′b); 3.66 dd, 1 H, *J*(P,CH) = 9.5, *J*(gem) = 13.7 (P-CH_2); 3.40 m, 2 H (1″-CH_2); 3.40 m, 2 H (2″-CH_2); 3.38 s, 3 H (OCH_3); 3.19 dd, 1 H, *J*(3′a,2′) = 3.3, *J*(gem) = 10.6 (H-3′); 2.91 dd, *J*(3′b,2′) = 4.3, *J*(gem) = 10.6 (H-3′); 1.215 d, 3 H, *J*(CH_3,CH) = 6.1 (CH_3); 1.21 d, 3 H, *J*(CH_3,CH) = 6.1 (CH_3); 1.19 d, 3 H, *J*(CH_3,CH) = 6.1 (CH_3); 1.17 d, 3 H, *J*(CH_3,CH) = 6.1 (CH_3). ^{13}C NMR (DMSO-d_6): 164.06 (C-4); 155.85 (C-2); 145.72 (C-6); 143.67, 3 C (OTr); 128.44, 6 C (OTr); 128.10, 6 C (OTr); 127.26, 3 C (OTr); 93.83 (C-5); 86.24 (OTr); 78.67 d, 2 C, *J*(P,C) = 13.2 (C-2′); 70.44 (C-2″-OCH_2); 70.34 d, 2 C, *J*(C,P) = 6.4

(P-OC); 64.13 d, 2 C, *J*(C,P) = 165.0 (P-C); 62.74 (C-3′); 58.09 (OCH_3); 49.98 (C-1′); 39.61 (C-1″-NCH_2); 23.89 d, 2 C (iPrCH_3); 23.83 d, 2 C (iPrCH_3). α_D^{20} –13.3.

Diisopropyl 1-[(S)-3-hydroxy-2-(phosphonomethoxy)propyl]-N^4-(2-hydroxyethyl)cytosine (**4h**). Yield 0.809 g (77.3%) of white foam. For $C_{35}H_{44}N_3O_7P \cdot 1.5H_2O$ (649.7) calculated: 62.12% C, 7.00% H, 6.21% N, 4.58% P; found: 61.85% C, 7.17% H, 6.29% N, 4.37% P. FAB MS, *m/z* (%): 650 (20) [M + H]. ^{1}H NMR (DMSO-d_6): 7.62 t, 1 H, *J*(NH,1″) = 5.6 (NH); 7.40 d, 6 H (*o*-H); 7.34 d, 6 H (*m*-H); 7.27 d, 1 H, *J*(5,6) = 5.0 (H-6); 7.26 d, 3 H (*p*-H); 5.61 d, 1 H, *J*(5,6) = 5.0 (H-5); 4.82 t, 1 H, *J*(OH,2″) = 5.0 (OH); 4.55 m, 2 H (P-OCH); 3.87 dd, 1 H, *J*(1′a,2′) = 4.5, *J*(gem) = 13.1 (1′-CH_2); 3.83 m, 1 H (2′-CH); 3.76 dd, 1 H, *J*(P,CH) = 8.9, *J*(gem) = 13.7 (P-CH_2); 3.69 dd, 1 H, *J*(1′b,2′) = 6.8, *J*(gem) = 13.1 (H-1′b); 3.67 dd, 1 H, *J*(P,CH) = 9.5, *J*(gem) = 13.7 (P-CH_2); 3.47 brq, 2 H (2″-CH_2); 3.29 q, 2 H (1″-CH_2); 3.19 dd, 1 H, *J*(3′a,2′) = 3.3, *J*(gem) = 10.5 (H-3′); 2.91 dd, *J*(3′b,2′) = 4.4, *J*(gem) = 10.5 (H-3′); 1.215 d, 3 H, *J*(CH_3,CH) = 6.1 (CH_3); 1.21 d, 3 H, *J*(CH_3,CH) = 6.1 (CH_3); 1.19 d, 3 H, *J*(CH_3,CH) = 6.1 (CH_3); 1.17 d, 3 H, *J*(CH_3,CH) = 6.1 (CH_3). ^{13}C NMR (DMSO-d_6): 164.26 (C-4); 156.04 (C-2); 145.73 (C-6); 143.67, 3 C (OTr); 128.44, 6 C (OTr); 128.10, 6 C (OTr); 127.26, 3 C (OTr); 94.10 (C-5); 86.24 (OTr); 78.80 d, 2 C, *J*(P,C) = 13.2 (C-2′); 70.34 d, 2 C, *J*(C,P) = 6.4 (P-OC); 64.23 d, 2 C, *J*(C,P) = 166.5 (P-C); 62.83 (C-2″-OCH_2); 59.87 (C-3′); 50.03 (C-1′); 42.85 (C-1″-NCH_2); 23.89 d, 2 C (iPrCH_3); 23.83 d, 2 C (iPrCH_3). α_D^{20} –11.8.

Diisopropyl 1-[(S)-3-hydroxy-2-(phosphonomethoxy)propyl]-N^4-(2-dimethylaminoethyl)cytosine (**4i**). Yield 0.545 g (50%) of white foam. For $C_{37}H_{49}N_4O_6P \cdot 1.33H_2O$ (676.7) calculated: 63.41% C, 7.43% H, 7.99% N, 4.42% P; found: 63.32% C, 7.34% H, 7.91% N, 4.54% P. FAB MS, *m/z* (%): 677 (70) [M + H]. ^{1}H NMR (DMSO-d_6): 7.59 t, 1 H, *J*(NH,1″) = 5.5 (NH); 7.40 d, 6 H (*o*-ArH); 7.34 t, 6 H (*m*-H); 7.30 d, 1 H, *J*(5,6) = 7.3 (H-6); 7.26 t, 3 H (*p*-H); 5.62 d, 1 H, *J*(5,6) = 7.3 (H-5); 4.55 m, 2 H (P-OCH); 3.88 dd, 1 H, *J*(1′a,2′) = 4.6, *J*(gem) = 13.2 (1′-CH_2); 3.84 m, 1 H (2′-CH); 3.76 dd, 1 H, *J*(P,CH) = 9.0, *J*(gem) = 13.7 (P-CH_2); 3.70 dd, 1 H, *J*(1′b,2′) = 6.8, *J*(gem) = 13.2 (H-1′b); 3.67 dd, 1 H, *J*(P,CH) = 9.7, *J*(gem) = 13.7 (P-CH_2); 3.47 brq, 2 H (1″-CH_2); 3.20 dd, 1 H, *J*(3′a,2′) = 3.4, *J*(gem) = 10.6 (H-3′); 2.91 dd, *J*(3′b,2′) = 3.8, *J*(gem) = 10.6 (H-3′); 2.51 t, 2 H, *J*(2″,1″) = 6.4 (2″-CH_2); 2.28 s, 6 H (NCH_3); 1.215 d, 3 H, *J*(CH_3,CH) = 6.1 (CH_3); 1.21 d, 3 H, *J*(CH_3,CH) = 6.1 (CH_3); 1.19 d, 3 H, *J*(CH_3,CH) = 6.1 (CH_3); 1.17 d, 3 H, *J*(CH_3,CH) = 6.1 (CH_3). ^{13}C NMR (DMSO-d_6): 164.02 (C-4); 155.84 (C-2); 145.74 (C-6); 143.67, 3 C (OTr); 128.44, 6 C (OTr); 128.10, 6 C (OTr); 127.26, 3 C (OTr); 93.88 (C-5); 86.24 (OTr); 78.68 d, 2 C, *J*(P,C) = 12.7 (C-2′); 70.34 d, 2 C, *J*(C,P) = 6.3 (P-OC); 64.15 d, 2 C, *J*(C,P) = 166.5 (P-C); 62.75 (C-3′); 57.63 (C-2″); 49.96 (C-1′); 44.82, 2 C (NCH_3); 37.45 (C-1″); 23.89 d, 2 C (iPrCH_3); 23.83 d, 2 C (iPrCH_3). α_D^{20} –15.0.

Synthesis of Compounds **5**. General Procedure

To the appropriate N^4-substituted derivative **4** in acetonitrile (15 ml), bromotrimethylsilane (3 ml) was added. The reaction mixture was left standing overnight and then evaporated in vacuo. The residue was stirred in 80% aqueous methanol (50 ml) for 5 min and the reaction mixture was neutralized with several drops of aqueous ammonia. Then it was evaporated to dryness in vacuo and the residue dissolved in water (50 ml) was extracted with ether (3 × 50 ml). Ether extracts were re-extracted with water (20 ml), combined aqueous exctracts were taken down in vacuo and the residue was deionized on Dowex 50X8 (H^+ form) column (50 ml). The UV-absorbing ammonia eluate was evaporated in vacuo, the residue was purified on a column (50 ml) of Dowex 1X2 (acetate form). The UV-absorbing eluate was evapo-

rated in vacuo and codistilled with water (3 × 10 ml). HPLC were used for final purification. The following compounds were prepared by this procedure:

1-[(S)-3-Hydroxy-2-(phosphonomethoxy)propyl]-N^4-ethylcytosine (**5a**). Yield 0.280 g (56.6%) of white solid, m.p. 227 °C. For $C_{10}H_{18}N_3O_6P·0.33H_2O$ (307.2) calculated: 38.34% C, 6.01% H, 13.41% N, 9.89% P; found: 38.48% C, 5.87% H, 13.18% N, 9.58% P. FAB MS, *m/z* (%): 308 (10) [M + H]. ^{1}H NMR (D_2O): 7.71 d, 1 H, *J*(5,6) = 7.8 (H-6); 6.08 d, 1 H, *J*(5,6) = 7.8 (H-5); 4.12 dd, 1 H, *J*(1′a,2′) = 3.2, *J*(gem) = 14.3 (1′-CH_2); 3.84 dd, 1 H, *J*(1′b,2′) = 8.2, *J*(gem) = 14.3 (1′-CH_2); 3.81 dd, 1 H, *J*(3′a,2′) = 3.9, *J*(gem) = 12.5 (3′-CH_2); 3.77 dd, 1 H, *J*(P,CH) = 9.2, *J*(gem) = 13.2 (P-CH_2); 3.76 m, 1 H (2′-CH); 3.61 dd, *J*(3′b,2′) = 3.9, *J*(gem) = 12.5 (3′-CH_2); 3.58 dd, 1 H, *J*(P,CH) = 9.6, *J*(gem) = 13.2 (P-CH_2); 3.43 q, 2 H, *J*(H1″-CH_2,H2″-CH_3) = 7.3 (CH_2); 1.27 t, 3 H, *J*(H1″-CH_2,H2″-CH_3) = 7.3 (CH_3). ^{13}C NMR (D_2O): 156.97 (C-4); 149.56 (C-2); 147.93 (C-6); 94.84 (C-5); 79.35 (C-2′); 65.83 (P-C); 59.94 (C-3′); 49.88 (C-1′); 37.69 (C-1″NCH_2); 12.19 (CH_3). α_D^{20} –96.9.

1-[(S)-3-Hydroxy-2-(phosphonomethoxy)propyl]-N^4-propylcytosine (**5b**). Yield 0.336 g (65%) of white solid, m.p. 231 °C. For $C_{11}H_{20}N_3O_6P·0.2H_2O$ (321.2) calculated: 40.67% C, 6.33% H, 12.93% N, 9.53% P; found: 40.89% C, 6.35% H, 12.99% N, 9.41% P. FAB MS, *m/z* (%): 322 (100) [M + H]. ^{1}H NMR (D_2O): 7.74 t, 1 H, *J*(NH,1″) = 6.0 (NH); 7.46 d, 1 H, *J*(5,6) = 7.3 (H-6); 5.67 d, 1 H, *J*(5,6) = 7.3 (H-5); 3.88 dd, 1 H, *J*(1′a,2′) = 4.0, *J*(gem) = 13.7 (1′-CH_2); 3.63 dd, 1 H, *J*(1′b,2′) = 6.3, *J*(gem) = 13.7 (1′-CH_2); 3.60 d, 1 H, *J*(P,CH) = 9.5, *J*(gem) = 13.7 (P-CH_2); 3.58 m, 1 H (2′-CH); 3.56 dd, 1 H, *J*(P,CH) = 9.5, *J*(gem) = 13.7 (P-CH_2); 3.38 d, 1 H, *J*(3′,2′) = 5.0 (3′-CH_2); 3.38 d, 1 H, *J*(3′,2′) = 5.0 (3′-CH_2); 3.20 m, 2 H (1″-CH_2); 1.49 br sext, 2 H, *J*(1″,2″) = 7.3 (2″-CH_2); 0.88 t, 3 H, *J*(CH_3,CH_2) = 7.4 (CH_3). ^{13}C NMR (D_2O): 163.23 (C-4); 155.70 (C-2); 146.30 (C-6); 94.04 (C-5); 80.45 d, 2 C, *J*(P,C) = 9.8 (C-2′); 66.02 d, 2 C, *J*(P,C) = 161.0 (P-C); 60.61 (C-3′); 49.09 (C-1′); 30.82 (C-1″NCH_2); 19.85 (C-2″NCH_2); 13.89 (CH_3). α_D^{20} –111.0.

1-[(S)-3-hydroxy-2-(phosphonomethoxy)propyl]-N^4-allylcytosine (**5c**). Yield 0.288 g (56%) of white solid, m.p. 211 °C. For $C_{11}H_{18}N_3O_6P$ (319.2) calculated: 41.38% C, 5.68% H, 13.16% N, 9.70% P; found: 41.11% C, 5.81% H, 13.02% N, 9.44% P. FAB MS, *m/z* (%): 320 (70) [M + H]. ^{1}H NMR (D_2O): 7.87 t, 1 H, *J*(NH,1″) = 5.5 (NH); 7.49 d, 1 H, *J*(5,6) = 7.3 (H-6); 5.87 ddt, 1 H, *J*(2″,1″) = 5.4, *J*(2″,3″cis) = 10.2, *J*(2″,3″trans) = 17.2 (2″-CH); 5.71 d, 1 H, *J*(5,6) = 7.3 (H-5); 5.17 dq, 1 H, *J*(3″,1″) ~ *J*(gem) = 1.7, *J*(3″trans,2″) = 17.2 (3″-CH_2); 5.09 dq, 1 H, *J*(3″,1″) ~ *J*(gem) = 1.6, *J*(3″cis,2″) = 10.2 (3″-CH_2); 3.91 m, 2 H (1″-CH_2); 3.90 dd, 1 H, *J*(1′a,2′) = 3.9, *J*(gem) = 13.1 (1′-CH_2); 3.63 dd, 1 H, *J*(1′b,2′) = 6.5, *J*(gem) = 13.1 (1′-CH_2); 3.61 dd, 1 H, *J*(P,CH) = 9.4, *J*(gem) = 13.7 (P-CH_2); 3.58 m, 1 H (2′-CH); 3.56 dd, 1 H, *J*(P,CH) = 9.4, *J*(gem) = 13.7 (P-CH_2); 3.38 d, 1 H, *J*(3′,2′) = 5.0 (3′-CH_2); 3.38 d, 1 H, *J*(3′,2′) = 5.0 (3′-CH_2). ^{13}C NMR (D_2O): 163.60 (C-4); 156.10 (C-2); 146.50 (C-6); 135.03 (C-2″); 115.86 (C-3″); 93.90 (C-5); 80.62 d, 2 C, *J*(P,C) = 9.8 (C-2′); 65.79 d, 2 C, *J*(P,C) = 161.0 (P-C); 60.63 (C-3′); 49.12 (C-1′); 42.23 (C-1″). α_D^{20} –94.0.

1-[(S)-3-Hydroxy-2-(phosphonomethoxy)propyl]-N^4-butylcytosine (**5d**). Yield 0.402 g (74.6%) of white solid, m.p. 185 °C. For $C_{12}H_{22}N_3O_6P·0.5H_2O$ (335.2) calculated: 41.86% C, 6.73% H, 12.20% N, 9.00% P; found: 42.07% C, 6.87% H, 12.17% N, 8.98% P. FAB MS, *m/z* (%): 336 (80) [M + H]. ^{1}H NMR (D_2O): 7.85 t, 1 H, *J*(NH,1″) = 5.5 (NH); 7.48 d, 1 H, *J*(5,6) = 7.3 (H-6); 5.68 d, 1 H, *J*(5,6) = 7.3 (H-5); 3.89 dd, 1 H, *J*(1′a,2′) = 3.9, *J*(gem) = 13.4 (1′-CH_2); 3.63 dd, 1 H, *J*(1′b,2′) = 6.6, *J*(gem) = 13.4 (1′-CH_2); 3.60 d, 1 H, *J*(P,CH) = 9.1, *J*(gem) = 13.6 (P-CH_2); 3.58 m, 1 H (2′-CH); 3.56 dd, 1 H, *J*(P,CH) = 9.1, *J*(gem) = 13.6 (P-CH_2); 3.38 d, 1 H, *J*(3′,2′) = 5.0 (3′-CH_2); 3.38 d, 1 H, *J*(3′,2′) = 5.0 (3′-CH_2); 3.04 m, 2 H (1″-CH_2); 1.47 m, 1 H (2″-CH_2); 1.31 m, 1 H (3″-CH_2); 0.89 t, 3 H, *J*(CH_3,CH_2) = 7.3 (CH_3). ^{13}C NMR (D_2O): 163.74 (C-4);

156.25 (C-2); 146.14 (C-6); 94.04 (C-5); 80.65 d, 2 C, *J*(P,C) = 9.8 (C-2′); 66.02 d, 2 C, *J*(P,C) = 159.7 (P-C); 49.02 (C-1′); 41.79 (C-1″); 30.90 (C-2″); 22.05 (C-3″); 11.66 (CH_3). α_D^{20} –86.3.

1-[(S)-3-hydroxy-2-(phosphonomethoxy)propyl]-N^4-cyclopentylcytosine (**5e**). Yield 0.270 g (48.3%) of white solid, m.p. 188 °C. For $C_{13}H_{22}N_3O_6P \cdot 0.4EtOH \cdot 0.4H_2O$ (347.3) calculated: 44.44% C, 6.81% H, 11.27% N, 8.31% P; found: 44.41% C, 6.97% H, 11.27% N, 8.64% P. FAB MS, *m/z* (%): 348 (100) [M + H]. ^{1}H NMR (D_2O): 7.69 d, 1 H, *J*(5,6) = 7.8 (H-6); 6.04 d, 1 H, *J*(5,6) = 7.8 (H-5); 4.14 m, 1 H (N-CH); 4.13 dd, 1 H, *J*(1′a,2′) = 3.2, *J*(gem) = 14.4 (1′-CH_2); 3.84 dd, 1 H, *J*(1′b,2′) = 8.2, *J*(gem) = 14.4 (1′-CH_2); 3.82 dd, 1 H, *J*(3′a,2′) = 3.4, *J*(gem) = 12.5 (3′- CH_2); 3.76 dd, 1 H, *J*(P,CH) = 9.4, *J*(gem) = 13.1 (P-CH_2); 3.62 dd, 1 H, *J*(3′b,2′) = 4.0, *J*(gem) = 12.5 (3′-CH_2); 3.58 dd, 1 H, *J*(P,CH) = 9.7, *J*(gem) = 13.1 (P-CH_2); 2.04 m, 2 H (CH_2); 1.73 m, 2 H (CH_2); 1.66 m, 4 H (CH_2). ^{13}C NMR (D_2O): 157.05 (C-4); 150.41 (C-2); 147.56 (C-6); 95.08 (C-5); 79.44 d, 2 C (C-2′); 66.02 d, 2 C (P-C); 59.98 (C-3′); 54.12 (N-CH); 49.85 (C-1′); 31.73, 2 C (CH_2); 31.70, 2 C (CH_2); 23.17, 2 C (CH_2). α_D^{20} –71.2.

1-[(S)-3-Hydroxy-2-(phosphonomethoxy)propyl]-N^4-(but-2-en-1-yl)cytosine (**5f**). Yield 0.409 g (76.3%) of white solid, m.p. 121 °C. For $C_{12}H_{20}N_3O_6P \cdot 0.33H_2O \cdot 0.33EtOH$ (333.2) calculated: 42.90% C, 6.44% H, 11.85% N, 8.73% P; found: 42.67% C, 6.55% H, 11.71% N, 9.09% P. FAB MS, *m/z* (%): 334 (45) [M + H]. ^{1}H NMR (D_2O): 7.75 d, 1 H (H-6); 6.11 d, 1 H (H-5); 5.83 m, 1 H (2″-CH); 5.54 m, 1 H (3″-CH); 4.14 dd, 1 H, *J*(1′a,2′) = 3.2, *J*(gem) = 14.4 (1′-CH_2); 3.99 dpent, 2 H (1″-CH_2); 3.86 dd, 1 H, *J*(1′b,2′) = 8.2, *J*(gem) = 14.4 (1′-CH_2); 3.82 m, 1 H (2′-CH); 3.77 dd, 1 H, *J*(P,CH) = 9.2, *J*(gem) = 13.1 (P-CH_2); 3.76 dd, 1 H, *J*(3′a,2′) = 3.4, *J*(gem) = 12.4 (3′-CH_2); 3.62 dd, 1 H, *J*(3′b,2′) = 3.9, *J*(gem) = 12.4 (3′-CH_2); 3.57 dd, 1 H, *J*(P,CH) = 9.5, *J*(gem) = 13.1 (P-CH_2); 1.70 dq, 3 H (CH_3). ^{13}C NMR (D_2O): 157.26 (C-4); 149.68 (C-2); 148.23 (C-6); 130.31 (C2″-CH); 122.01 (C-3″); 94.83 (C-5); 79.33 d, 2 C (C-2′); 65.93 d, 2 C (P-C); 59.95 (C-3′); 49.94 (C-1′); 43.89 (C-1″); 17.02 (CH_3). α_D^{20} –75.2.

1-[(S)-3-Hydroxy-2-(phosphonomethoxy)propyl]-N^4-(2-methoxyethyl)cytosine (**5g**). Yield 0.356 g (65.6%) of white solid, m.p. 146 °C. For $C_{11}H_{20}N_3O_7P \cdot 0.5H_2O$ (337.2) calculated: 38.15% C, 6.11% H, 12.13% N, 8.94% P; found: 38.07% C, 6.16% H, 11.85% N, 8.93% P. FAB MS, *m/z* (%): 338 (70) [M + H]. ^{1}H NMR (D_2O): 7.75 d, 1 H, *J*(5,6) = 7.8 (H-6); 6.13 d, 1 H, *J*(5,6) = 7.8 (H-5); 4.14 dd, 1 H, *J*(1′a,2′) = 3.2, *J*(gem) = 14.4 (1′-CH_2); 3.86 dd, 1 H, *J*(1′b,2′) = 8.2, *J*(gem) = 14.4 (1′-CH_2); 3.83 dd, 1 H, *J*(3′a,2′) = 4.0; *J*(gem) = 12.4 (3′-CH_2); 3.77 m, 2 H (NCH_2); 3.72 dd, 1 H, *J*(P,CH) = 9.3, *J*(gem) = 13.1 (P-CH_2); 3.69 m, 1 H (2′-CH); 3.64 m, 3 H (H-3′b); 3.64 m, 3 H (OCH_2); 3.57 dd, 1 H, *J*(P,CH) = 9.6, *J*(gem) = 13.1 (P-CH_2); 3.40 s, 3 H (OCH_3). ^{13}C NMR (D_2O): 158.62 (C-4); 150.24 (C-2); 148.34 (C-6); 94.88 (C-5); 79.38 d, 2 C (C-2′); 69.07 (C-2″); 65.99 d, 2 C (P-C); 59.98 (C-3′); 58.24 (OCH_3); 49.99 (C-1′); 42.08 (C-1″). α_D^{20} –90.9.

1-[(S)-3-Hydroxy-2-(phosphonomethoxy)propyl]-N^4-(2-hydroxyethyl)cytosine (**5h**). Yield 0.372 g (71.6%) of white solid, m.p. 131 °C. For $C_{10}H_{18}N_3O_7P \cdot 1.25H_2O$ (323.2) calculated: 34.74% C, 5.98% H, 12.15% N, 8.96% P; found: 34.70% C, 5.84% H, 12.04% N, 9.20% P. FAB MS, *m/z* (%): 324 (35) [M + H]. ^{1}H NMR (D_2O): 7.75 d, 1 H, *J*(5,6) = 7.8 (H-6); 6.14 d, 1 H, *J*(5,6) = 7.8 (H-5); 4.14 dd, 1 H, *J*(1′a,2′) = 3.3, *J*(gem) = 14.3 (1′-CH_2); 3.84 dd, 1 H, *J*(1′b,2′) = 8.2, *J*(gem) = 14.4 (1′-CH_2); 3.82 m, 1 H (2′-CH); 3.82 m, 3 H (3′-CH_2); 3.82 m, 3 H (NCH_2); 3.76 dd, 1 H, *J*(P,CH) = 9.3, *J*(gem) = 13.1 (P-CH_2); 3.62 dd, 1 H, *J*(3′b,2′) = 4.0, *J*(gem) = 12.4 (3′-CH_2); 3.60 m, 2 H (OCH_2); 3.57 dd, 1 H, *J*(P,CH) = 9.8, *J*(gem) = 13.1 (P-CH_2). ^{13}C NMR (D_2O): 158.74 (C-4); 150.25 (C-2); 148.30 (C-6); 94.98 (C-5); 79.37 d, 2 C

(C-2′); 65.99 d, 2 C (P-C); 59.97 (C-3′); 58.97 (C-2″OCH_2); 50.00 (C-1′); 44.38 (C-1″NCH_2). α_D^{20} –89.2.

1-[(S)-3-Hydroxy-2-(phosphonomethoxy)propyl]-N^4-(2-dimethylaminoethyl)cytosine (**5i**). Yield 0.229 g (40.7%) of white solid, m.p. 155 °C. For $C_{12}H_{23}N_4O_6P{\cdot}1.5H_2O{\cdot}0.5EtOH$ (350.3) calculated: 39.00% C, 7.30% H, 13.99% N, 7.74% P; found: 38.77% C, 7.25% H, 13.86% N, 8.16% P. FAB MS, *m/z* (%): 351 (35) [M + H]. ^{1}H NMR (D_2O): 7.61 t, 1 H, *J*(5,6) = 7.8 (H-6); 6.00 d, 1 H, *J*(5,6) = 7.8 (H-5); 4.10 dd, 1 H, *J*(1′a,2′) = 3.5, *J*(gem) = 13.2 (1′-CH_2); 3.80 m, 3 H (H-1′b); 3.80 m, 3 H (H-2′); 3.80 m, 3 H (H-3′a); 3.77 brt, 2 H, *J*(1″,2″) = 6.5 (1″-CH_2); 3.71 dd, 1 H, *J*(P,CH) = 9.0, *J*(gem) = 13.2 (P-CH_2); 3.62 dd, 1 H, *J*(3′b,2′) = 3.9, *J*(gem) = 12.4 (3′-CH_2); 3.41 brt, 2 H (2″-CH_2); 2.96 s, 6 H (NCH_3). ^{13}C NMR (D_2O): 165.23 (C-4); 158.56 (C-2); 147.17 (C-6); 96.31 (C-5); 80.02 d, 2 C, *J*(P,C) = 12.2 (C-2′); 66.54 d, 2 C, *J*(P,C) = 156.7 (P-C); 60.44 (C-3′); 57.17 (C-2″); 50.40 (C-1′); 43.05, 2 C (NCH_3); 35.81 (C-1″). α_D^{20} –49.2.

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