SYNTHESIS OF N^4-SUBSTITUTED DERIVATIVES OF 1-[2-(PHOSPHONOMETHOXY)ETHYL]CYTOSINE AND ITS DIISOPROPYL ESTER AS A MODEL REACTION FOR THE SYNTHESIS OF N^4-SUBSTITUTED DERIVATIVES OF CIDOFOVIR

Šárka CHALUPOVÁ[1,*], Antonín HOLÝ[2] and Milena MASOJÍDKOVÁ[3]

Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, 166 10 Prague 6, Czech Republic; e-mail: [1] chalupova@uochb.cas.cz, [2] holy@uochb.cas.cz, [3] masojid@uochb.cas.cz

Received September 12, 2005
Accepted October 21, 2005

We have studied the reaction of 1-[2-(phosphonomethoxy)ethyl]cytosine (**1**) and its diisopropyl ester (**2**) with triethylammonium hydrogensulfite in 60% aqueous methanol. In the presence of some primary or secondary amine salts, at 25–70 °C, this reaction affords transaminated derivatives **4a**–**4e** and **5a**, **5b** as main products accompanied by uracil compounds. However, with certain amines the reaction failed.

Keywords: Cytosine modification; Antivirals; Cidofovir; HPMPC; Acyclic nucleoside phosphonates; Nucleotides; Pyrimidines; Transamination; Amines.

Acyclic nucleoside phosphonates (ANP), isopolar analogues of nucleotides, which contain phosphonomethoxy function linked to an acyclic side chain instead of phosphate group attached to the nucleoside sugar moiety, attract considerable attention. The success of the acyclic nucleoside phosphonate analogs (ANPs) as antiviral agents with potent and selective activity results from selective interactions of their diphosphate metabolites, which act as both competitive inhibitors and alternative substrates, with the viral DNA polymerase. One of the most important ANP representatives is 1-[(*S*)-3-hydroxy-2-(phosphonomethoxy)propyl]cytosine (CAS 113852-37-2, HPMPC, Cidofovir) which shows a general anti-DNA-viral activity. It is also used in the therapy of acyclovir- and foscarnet-resistant HSV-1 lesions and in other viral diseases, such as papillomavirus-induced warts and nasopharyngeal carcinoma. The new drug application was approved in 2001 in the emergency treatment of smallpox. Potential nephrotoxicity and poor oral bioavailability, however, could limit its widespread use in the event of a smallpox outbreak. The efficacy of this class of compounds as antiviral

doi:10.1135/cccc20052066

agents and the continuing need to discover and develop additional compounds which may prove useful particularly against orthopoxvirus infections including variola virus, causing smallpox, substantiate our attempts to synthesize N^4-substituted derivatives of HPMPC. Within the scope of this study we are systematically investigating the influence of the 4-amino group substitution in the PME and HPMP derivatives of cytosine on their antiviral activity.

There are three different approaches to the synthesis of N^4-substituted cytosine derivatives of the HPMPC type: (i) preparation of individual N^4-substituted bases (cytosines) and their transformation to the HPMP derivatives by several synthetic steps or, (ii) reaction of a common synthetic intermediate of the protected HPMP structure, e.g. 1-substituted 4-methoxypyrimidin-2-one derivative, with an appropriate amine, (iii) direct exchange of the amino group in the cytosine base of HPMPC or its derivatives. While the first two alternatives are described in the accompanying paper[1], the present study concerns direct conversions of the cytosine 4-amino group. Contrary to adenine derivatives, where it is in some cases easy to achieve such transformations using the Dimroth rearrangement[2], such a possibility does not exist in the cytosine series.

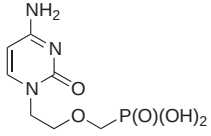
On the other hand, an important feature of the chemistry of uracil and cytosine is the susceptibility of their ring systems to nucleophilic attack at the 5,6-double bond[3–6]. Addition of hydrogensulfite across the 5,6-double bond of cytosine derivatives leads either to deaminated compounds[7,8] or in the presence of certain amines to the transaminated derivatives[7,8]. The reaction proceed under mild conditions with selected aliphatic and aromatic amines which compete successfully with water as a nucleophile. Nevertheless, according to the literature[9,10] the transamination reaction entirely fails with certain amines, where solely uracil derivatives are formed.

In search for a simple and convenient method of preparation of N^4-substituted derivatives of PMEC, PMEC-iPr$_2$ and HPMPC, we have thus reinvestigated also this reaction using alternative reagents, with special attention to the easy removal of excess of salts during the work-up. As a model compound we have taken neutral ester of the phosphonate, PMEC-iPr$_2$ (**2**).

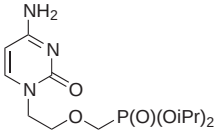
Originally, the reaction was performed with $NaHSO_3$; in order to overcome solubility problems of PMEC-iPr$_2$, we decided to work in aqueous MeOH and replaced $NaHSO_3$ by tetrabutylammonium hydrogensulfite prepared by introduction of SO_2 to a solution of tetrabutylammonium hydroxide. However, under these conditions the reaction was not successful. Neither in aqueous methanol nor in DMF, THF, dioxane or acetonitrile, any reaction took place. In order to simplify work-up of the reaction mixture, we

have replaced $NaHSO_3$ by "triethylammonium hydrogensulfite" of which we expected a volatility in vacuo similar as with triethylammonium hydrogencarbonate. It was prepared by introduction of SO_2 into triethylamine in 60% aqueous methanol. This compound decomposed in vacuo and could be removed by repeated codistillation with water. Further study was performed with this material.

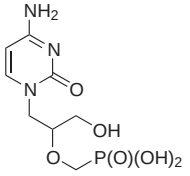
Synthesis



PMEC (**1**)

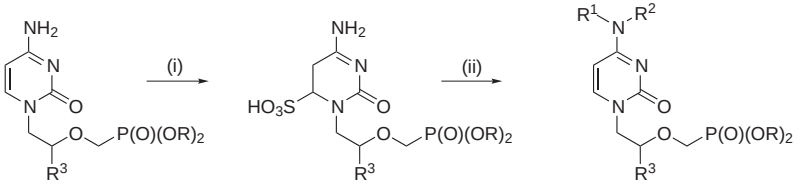


PMEC-iPr$_2$ (**2**)



HPMPC (**3**)

The reaction of 1-[2-(phosphonomethoxy)ethyl]cytosine (**1**) and its neutral diisopropyl ester (**2**) with triethylammonium hydrogensulfite in 60% methanol in the presence of an amine salt affords transaminated derivatives accompanied by varying amounts of their uracil congeners (Scheme 1). The reaction



1, 2, 3 **4a - 4e, 5a - 5b, 6**

(i) $Et_3N^+HSO_3^-$, 60% MeOH; (ii) $R_1R_2NH_2^+$ CH_3COO^- or $R_1R_2NH_2^+$ HCO_3^-

	R	R^1	R^2	R^3
4a	iPr	H	methyl	H
4b	iPr	H	ethyl	H
4c	iPr	H	cyclopropyl	H
4d	iPr	H	allyl	H
4e	iPr	H	butyl	H
5a	H	H	allyl	H
5b	H	H	cyclopropyl	H
6	H	H	cyclopropyl	CH_2OH

SCHEME 1
Synthesis of N^4-substituted PME, PME-iPr$_2$ and HPMP derivatives

occurs already at 25 °C but its rate increases at 70 °C. However, with certain amines the reaction completely failed (Table I); a possible interpretation of this failure could be a steric hindrance. The reaction proceeds with primary amine acetate or secondary amine carbonate compared with their salts with strong acids (HCl), when the reaction completely failed.

Concluding, we have re-examined the transformation of cytosine derivatives to its N^4-amino-substituted congeners based on the hydrogensulfite addition to the cytosine 5,6-double bond followed by reaction with a salt of a primary or secondary amine. The application of this reaction in transformation of the biologically important cytosine ANPs gave the expected

TABLE I
Reaction of primary and secondary amines $R^1R^2NH_2^+X^-$ with PMEC-iPr$_2$ (**2**) to N^4-substituted cytosine derivatives **4** (isolated yield)[a]

R^1	R^2	Salt X^-	Product	Yield, % r.t.	Yield, % 70 °C
H	methyl	OAc^-	**4a**	75.7	
H	cyclopropyl	OAc^-	**4b**	57.8	
H	allyl	OAc^-	**4c**	51.3	
H	ethyl	OAc^-	**4d**	14.4	72.4
H	butyl	OAc^-	**4e**	42	51
H	propyl	OAc^-			
H	cyclohexyl	HCO_3^-			
H	2-methoxyethyl	HCO_3^-			
H	isopropyl	OAc^-			
H	isobutyl	OAc^-			
H	benzyl	HCO_3^-			
H	2-aminoethyl	OAc^-		reaction failed	
H	hydroxyethyl	HCO_3^-			
Methyl	methyl	OAc^-			
Ethyl	ethyl	HCO_3^-			
Butyl	butyl	HCO_3^-			
H	morpholin-4-yl	HCO_3^-			
H	pyrrolidin-N-yl	HCO_3^-			

[a] For composition of the reaction mixture, see Experimental.

N^4-substituted derivatives of both 1-[2-(phosphonomethoxy)ethyl]cytosine (PMEC) and its diisopropyl ester accompanied by uracil derivatives. The optimum conditions include pretreatment of cytosine derivative with triethylammonium hydrogensulfite followed by reaction with acetate of a primary amine or hydrogencarbonate of a secondary amine. Though the method is far from being ideal and general, its simplicity and performance in aqueous medium could be useful in some cases, e.g. for modifications of zwitterionic compounds, like HPMPC. It could be also speculated that, with a suitable method of anchoring HPMPC to polymer support, this transformation could be used for the synthesis of a library of related derivatives. Such an application is under development.

Biological activity tests of the above compounds are still in process.

EXPERIMENTAL

Unless otherwise stated, solvents were evaporated at 40 °C/2 kPa and compounds were dried overnight at 2 kPa over P_2O_5. Melting points were determined on a Büchi Melting Point B-545 apparatus. TLC was performed on Silica gel 60 F_{254} plates (Merck, Germany) in systems chloroform:methanol 9:1 and propan-2-ol:aqueous ammonia:water 7:1:2. ^{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 disilapentanesulfonate (DSS) as internal standards. ^{1}H NMR chemical shifts (δ, ppm) and coupling constants (*J*, Hz) were obtained by first-order analysis of the spectra. The numbering system for assignment of NMR signals is outlined in Fig. 1.

Materials

All amines were purchased from Sigma Aldrich (Prague, Czech Republic). Dowex 50X8 and Dowex 1X2 were obtained from Fluka (Switzerland). Sulfur dioxide was purchased from Sigma–Aldrich (Prague, Czech Republic). HPMPC was a gift from Gilead Sciences, PMEC and its diisopropyl ester were prepared as described in ref.[1]

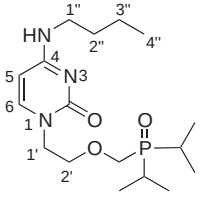


FIG. 1
General numbering scheme for assignment of NMR signals in cytosine derivatives

Tetrabutylammonium hydrogensulfite. Sulfur dioxide was introduced to a solution of tetrabutylammonium hydroxide (100 ml, 0.3 mol) in methanol (300 ml) under stirring and cooling with ice till saturation. The nature of the species formed in the solution was not investigated and the term hydrogen*sulfite* is only tentative.

Triethylammonium hydrogensulfite. To a solution of 60% methanol (500 ml) triethylamine (167.7 ml, 1.2 mol) was added dropwise and at the same time sulfur dioxide was introduced into this solution till saturation. The whole reaction mixture was kept at –10 °C. The nature of the species formed in the solution was not investigated and the term hydrogen*sulfite* is only tentative.

Preparation of Amine Salts. General Procedure

a) Acetate. Acetic acid (5 ml, 0.08 mol) in aqueous ethanol solution (10 ml) was alkalified with an appropriate amine (0.1 mol), evaporated in vacuo and dissolved in MeOH to give 2 M solution.

b) Hydrogencarbonate. Carbon dioxide was introduced into water under cooling with ice under simultaneous dropwise addition of an appropriate amine until saturation and made up to 2 M solution with water. The resulting mixture was kept at –10 °C.

General Procedures

Deionisation of the Reaction Mixture

The reaction mixture containing product was diluted with water, evaporated in vacuo and the residue repeatedly codistilled with water to remove the salts. The thus-obtained residue in water (5–10 ml) was applied onto a column of Dowex 50X8 (H^+ form) (30 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 then continued with 2.5% ammonia (in water and 20% aqueous methanol, respectively) and the UV-absorbing eluate was collected and evaporated in vacuo.

*Isolation of the Free Phosphonates (**5**, **6**) by Dowex 1X2 Column Chromatography*

Unless stated otherwise, 50-ml columns of Dowex 1X2 (100–200 mesh, acetate form) were used. The above deionized sample in water (5–10 ml) was alkalified with aqueous ammonia and 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 and the UV-absorbing eluate was collected, evaporated and repeatedly codistilled with water.

Reaction of Cytosine Derivatives with Hydrogensulfites and Amine Salts

Compound **2** (0.033 g, 0.13 mmol) was dissolved in a 1:1 mixture of 0.15 M potassium phosphate buffer, pH 7.1 (0.33 ml) and 3 M sodium hydrogensulfite (0.33 ml). After standing overnight, this solution was treated with alkylammonium hydrochloride. No reaction took place. Changes in the concentration of the buffer (1 or 2 mol/l) or changes in sodium hydrogensulfite concentration (1 or 2 mol/l) did not bring any improvement.

N^4-Substituted Derivatives of Diisopropyl 1-[2-(Phosphonomethoxy)ethyl]cytosine

Compound **2** (0.2 g, 0.6 mmol) was dissolved in 2 M triethylammonium hydrogensulfite (3 ml, 0.006 mol) and the solution was left standing at ambient temperature overnight. Then alkylamine acetate was added (1.2 mmol) and the mixture was heated at 70 °C for 16 h. The course of the reaction was checked by TLC (chloroform:methanol 9:1). The mixture was then evaporated in vacuo and the residue codistilled with water (3 × 10 ml) until SO_2 was removed. After deionization of the residue on a Dowex 50X8 (H^+ form) column (50 ml), the UV-absorbing eluate was evaporated in vacuo and the product was purified by HPLC techniques. The following compounds were prepared by this procedure.

Diisopropyl 1-[2-(phosphonomethoxy)ethyl]-N^4-methylcytosine (**4a**). Yield 0.157 g (75.7%) of a thick colorless oil. FAB HRMS for $C_{13}H_{24}N_3O_5P$ (347.3) calculated: 348.1688, found: 348.1690. FAB MS, *m/z* (%): 348 (100) [M + H], 126 (30) [4-(methylamino)pyrimidin-2(1*H*)-one + H]. ^{1}H NMR (DMSO-d_6): 7.5 br q, 1 H (NH), *J*(NH,CH_3) = 4.6; 7.39 d and 5.6 d, 1 H (H-6, H-5), *J*(5,6) = 7.2; 3.79 t, 2 H (1′-CH_2) and 3.67 t, 2 H (2′-CH_2), *J*(1′,2′) = 5.1; 3.73 d, 2 H (P-CH_2), *J*(P-CH_2) = 8.4; 4.55 m, 2 H (P-OCH); 1.22 d and 1.20 d (6 H), *J* = 6.1; 2.72 d, 3 H (N-CH_3), *J*(CH_3-NH) = 4.6.

Diisopropyl 1-[2-(phosphonomethoxy)ethyl]-N^4-ethylcytosine (**4b**). Yield 0.156 g (72.4%) of a thick colorless oil. FAB HRMS for $C_{15}H_{28}N_3O_5P$ (361.3) calculated: 362.1844, found: 362.1852. FAB MS, *m/z* (%): 362 (100) [M + H], 139 (30) [4-(ethylamino)pyrimidin-2(1*H*)-one + H]. ^{1}H NMR (DMSO-d_6): 7.6 t, 1 H (NH), *J*(NH,CH_2) = 5.2; 7.4 d and 5.6 d, 1 H (H-6, H-5), *J*(5,6) = 7.2; 3.79 t, 2 H (1′-CH_2) and 3.67 t, 2 H (2′-CH_2), *J*(1′,2′) = 5.1; 3.74 d, 2 H (P-CH_2), *J*(P-CH) = 8.3; 4.55 m, 2 H (P-OCH); 1.22 d and 1.20 d (6 H), *J* = 6.2; 3.24 qd, 2 H (N-Et), *J* = 5.2 and 7.2; 1.08 t, 3 H (N-Et), *J* = 7.2. ^{13}C NMR (DMSO-d_6): 155.71 (C-2); 163.64 (C-4); 145.61 (C-6); 93.63 (C-5); 70.44 d, *J* = 12.7 (C-2′); 70.31 d, *J* = 6.3 (P-OCH); 64.85 d, *J*(P,C) = 164.6; 48.27 (C-1′); 23.97 d and 23.86 d, *J* = 3.9 and 4.4 (CH_3).

Diisopropyl 1-[2-(phosphonomethoxy)ethyl]-N^4-cyclopropylcytosine (**4c**). Yield 0.129 g (57.4%) of a thick colorless oil. FAB HRMS for $C_{16}H_{28}N_3O_5P$ (373.3) calculated: 374.1844, found: 374.1839. FAB MS, *m/z* (%): 374 (100) [M + H], 290 (30) [{[2-(4-amino-2-oxopyrimidin-1(2*H*)-yl)ethoxy]methyl}phosphonic acid + H]. ^{1}H NMR (DMSO-d_6): 7.65 d, 1 H (NH), *J*(NH,CH) = 3.5; 7.4 d and 5.55 d, 1 H (H-6, H-5), *J*(5,6) = 7.1; 3.79 t, 2 H (1′-CH_2) and 3.68 t, 2 H (2′-CH_2), *J*(1′,2′) = 5.0; 3.74 d, 2 H (P-CH_2), *J*(P-CH) = 8.4; 4.56 m, 2 H (P-OCH); 1.22 d and 1.20 d (6 H), *J* = 6.2. ^{13}C NMR (DMSO-d_6): 166.21 (C-4); 155.86 (C-2); 146.77 (C-6); 92.92 (C-5); 70.42 d, *J* = 12.7 (C-2′); 70.30 d, *J* = 6.3 (P-OCH); 64.85 d, *J*(P,C) = 164.1; 48.34 (C-1′); 23.96 d and 23.86 d, *J* = 3.9 and 4.4 (CH_3); 23.49 (N-CH); 6.26, 2 C (CH_2).

Diisopropyl 1-[2-(phosphonomethoxy)ethyl]-N^4-allylcytosine (**4d**). Yield 0.114 g (51.3%) of a thick colorless oil. FAB HRMS for $C_{16}H_{28}N_3O_5P$ (373.3) calculated: 374.1844, found: 374.1849. FAB MS, *m/z* (%): 374 (80) [M + H], 152 (20) [4-(allylamino)pyrimidin-2(1*H*)-one + H]. ^{1}H NMR (DMSO-d_6): 7.8 t, 1 H, *J*(NH,1″) = 5.8 (NH); 7.43 d, 1 H, *J*(6,5) = 7.3 (H-6); 5.86 ddt, 1 H, *J*(2″,1″) = 5.2, *J*(2″,3″) = 10.4 and 17.2 (H-2″); 5.67 d, 1 H, *J*(5,6) = 7.3 (H-5); 5.15 dq, 1 H, *J*(3″,1″) ~ *J*(gem) = 1.8, *J*(3″,2″) = 17.2 (H-3″ trans); 5.08 dq, 1 H, *J*(3″,1″) ~ *J*(gem) = 1.7, *J*(3″,2″) = 10.4 (H-3″ cis); 4.55 d sept, 2 H, *J*(CH,CH_3) = 6.2, *J*(P,CH) = 7.8 (P-OCH); 3.80 t, 2 H, *J*(1′,2′) = 5.1 (H-1′); 3.74 d, 2 H, *J*(P,CH) = 8.3 (P-CH_2); 3.67 t, 2 H, *J*(2′,1′) = 5.1 (H-2′); 1.22 d, 6 H and 1.20 d, 6 H, *J*(CH_3,CH) = 6.2 (CH_3). ^{13}C NMR (DMSO-d_6): 163.89 (C-4); 155.81 (C-2); 145.88 (C-6); 135.20 (C-2″); 115.55 (C-3″); 93.52 (C-5); 70.47 d, *J*(P,C) = 11.2 (C-2′); 70.32 d, 2 C, *J*(P,C) = 5.9 (P-OCH); 64.83 d, *J*(P,C) = 164.6 (P-CH_2); 48.32 (C-1″); 42.01 (C-1′); 23.95 d, 2 C and 23.86 d, 2 C, *J*(P,C) = 4.9 (CH_3).

Diisopropyl 1-[2-(phosphonomethoxy)ethyl]-N^4-butylcytosine (**4e**). Yield 0.105 g (51%) of a thick colorless oil. FAB HRMS for $C_{13}H_{24}N_3O_5P$ (389.4) calculated: 390.2157, found: 390.2152. FAB MS, *m/z* (%): 390 (100) [M + H], 126 (30) [4-(butylamino)pyrimidin-2(1*H*)-one + H]. ^{1}H NMR (DMSO-d_6): 7.51 br t, 1 H (NH), *J*(NH,CH_2) = 5.0; 7.39 d and 5.6 d, 1 H (H-6, H-5), *J*(5,6) = 7.2; 3.98 t, 2 H (1′-CH_2) and 3.67 t, 2 H (2′-CH_2), *J*(1′,2′) = 4.5; 3.73 d, 2 H (P-CH_2), *J*(P-CH) = 8.3; 3.10 m, 2 H (N-CH_2); 1.45 m and 1.31 m (2 H); 0.89 t, 3 H (CH_3), *J*(CH_3-CH_2) = 7.3; 4.55 m, 2 H (P-OCH); 1.22 d, 6 H and 1.19 d, 6 H (CH_3), *J*(CH_3-CH) = 6.2. ^{13}C NMR (DMSO-d_6): 164.00 (C-4); 155.91 (C-2); 145.52 (C-6); 93.66 (C-5); 70.47 d, *J*(P,C) = 11.2 (C-2′); 70.32 d, 2 C, *J*(P,C) = 6.4 (P-OCH); 64.85 d, *J*(P,C) = 164.5 (P-CH_2); 48.29 (C-1″); 41.55 (C-1′); 30.92 (C-2″); 23.98 d, 2 C, *J*(P,C) = 3.9 (CH_3); 23.87 d, 2 C, *J*(P,C) = 4.9 (CH_3); 19.83 (C-3″); 13.88 (C-4″).

N^4-Substituted 1-[2-(phosphonomethoxy)ethyl]cytosines (**5**). General Procedure

Triethylamine (5 ml) was added to a solution of compound **1**; (0.2 g, 0.8 mmol) in water (10 ml) and the solution evaporated in vacuo. The residue in water (5 ml) was added to 2 M solution of triethylammonium hydrogensulfite (3 ml, 0.006 mol). The solution was then left standing at room temperature overnight. Then a solution of an appropriate amine acetate (1.2 mmol) was added and the mixture was heated at 70 °C for 16 h. The course of the reaction was checked by TLC in the system propan-2-ol:aqueous ammonia:water 7:1:2. The mixture was evaporated in vacuo and codistilled with water (3 × 10 ml) until SO_2 was removed. After deionization of the residue on a Dowex 50X8 (H^+ form) column (50 ml), the UV-absorbing eluate was evaporated in vacuo and the product was purified by HPLC. The residue was dissolved in water (3 ml), the solution applied onto a column (50 ml) of Dowex 1X2 (acetate form) and was washed first with water and then with 1 M acetic acid. The UV-absorbing eluate was evaporated in vacuo, codistilled with water and the residue dried over P_2O_5 in vacuo. The following compounds were prepared by this procedure.

1-[2-(Phosphonomethoxy)ethyl]-N^4-allylcytosine (**5a**). Yield 0.068 g (30%) of a white solid. FAB HRMS for $C_{10}H_{16}N_3O_5P$ (289.2) calculated: 290.0905, found: 290.0918. FAB MS, *m/z* (%): 290 (65) [M + H]. ^{1}H NMR (DMSO-d_6): 7.59 d, 1 H, *J*(6,5) = 7.3 (H-6); 5.96 d, 1 H, *J*(5,6) = 7.3 (H-5); 5.96 m, 1 H (H-2″); 5.24 brd, 1 H, *J*(3″,2″) = 17.3 (H-3″); 5.19 brd, 1 H, *J*(3″,2″) = 10.1 (H-3″); 3.97 m, 4 H (H-1″ and H-1″); 3.81 t, 2 H, *J*(2′,1′) = 5.2 (H-2′); 3.50 d, 2 H, *J*(P,CH) = 8.4 (P-CH_2). ^{13}C NMR (DMSO-d_6): 164.30 (C-4); 157.09 (C-2); 145.94 (C-6); 133.69 (C-2″); 115.88 (C-3″); 96.42 (C-5); 70.05 d, *J*(P,C) = 9.8 (C-2′); 69.08 d, *J*(P,C) = 149.9 (P, C); 48.99 (C-1′); 42.46 (C-1″).

1-[2-(Phosphonomethoxy)ethyl]-N^4-cyclopropylcytosine (**5b**). Yield 0.066 g (28%) of a white solid. FAB HRMS for $C_{10}H_{16}N_3O_5P$ (289.2) calculated: 290.0905, found: 290.0914. FAB MS, *m/z* (%): 290 (50) [M + H]. ^{1}H NMR (DMSO-d_6): 7.41 d and 7.68 d, 1 H, *J*(6,5) = 7.3 (H-6); 5.74 d and 6.27 d, 1 H, *J*(5,6) = 7.3 (H-5); 3.86 t, 2 H, *J*(1′,2′) = 5.0 (H-1′); 3.69 t, 2 H, *J*(2′,1′) = 5.0 (H-2′); 3.38 d, 2 H, *J*(P,CH) = 8.4 (P-CH_2); 2.60 m and 2.48 m, 1 H (N-CH); 0.70 m, 2 H and 0.45 m, 2 H (CH_2).

1-[(*S*)-3-Hydroxy-2-(phosphonomethoxy)propyl]-N^4-cyclopropylcytosine (**6**)

Compound **3** (2 g, 7.1 mmol) was dissolved in water (15 ml) and triethylamine (10 ml) was added, the reaction mixture was stirred for 5 min and then evaporated in vacuo. The residue was dissolved in a solution of triethylammonium hydrogensulfite (30 ml, 0.06 mol) and the solution was left standing overnight. Then a solution of an appropriate alkylamine acetate

was added (21.3 mmol) and the mixture was heated at 70 °C for 16 h. The course of the reaction was checked by TLC in the system propan-2-ol:aqueous ammonia:water 7:1:2. The mixture was evaporated in vacuo and codistilled with water (3 × 10 ml) until SO_2 was removed. After the application of the residue onto a Dowex 50X8 (H^+ form) column (150 ml), the column was eluted with water until the acidity of the eluate dropped. The product was then eluted with 2.5% aqueous ammonia, the UV-absorbing eluate was evaporated in vacuo and the residue was purified by HPLC. The purified product in water (3 ml) was applied onto a column (150 ml) of Dowex 1X2 (acetate form) and the column was washed first with water and then with 1 M acetic acid. The UV-absorbing eluate was evaporated in vacuo and codistilled with water and dried over P_2O_5 in vacuo. Yield 1 g (50%) of compound **6** as a white powder, 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)pyrimidin-2(1*H*)-one + H]. ^{1}H NMR (D_2O): 7.3 d (H-6) and 6.02 d (H-5), *J*(5,6) = 7.7; 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 (3′-CH_2), *J*(3′a,2′) = 3.8 (H-3′a), *J*(gem) = 12.4; 3.62 dd, 1 H (3′-CH_2), *J*(3′b,2′) = 4.0 (H-3′b), *J*(gem) = 12.4; 3.75 dd, 1 H (P-CH_2), *J*(P-CH) = 9.0, *J*(gem) = 13.2; 3.57 dd, 1 H (P-CH_2), *J*(P-CH) = 9.8, *J*(gem) = 13.2; 2.77 m, 1 H (N-CH); 0.97 m, 2 H (CH_2) and 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 (C-2′), *J* = 11.7; 66.11 d (P-C), *J* = 158.7; 60.07 (C-3′); 50.06 (C-1′); 23.25 (N-CH); 6.45, 2 C (CH_2). α_D^{20} = –88.5.

This study is a part of research project of the Centre of new antivirals and antineoplastics ID 1M0508 and was supported by the grant No. 1UC1AIO62540-01 of the National Institute of Health. The authors' thanks are due to Gilead Sciences (Foster City (CA) U.S.A.) for financial support and the gift of cidofovir.

REFERENCES

1. Chalupová S., Holý A., Masojídková M.: *Collect. Czech. Chem. Commun.* **2005**, *70*, 2053.
2. Holý A., Günter J., Dvořáková H.,Masojídková M., Andrei G., Snoeck R., Balzarini J., De Clercq E.: *J. Med. Chem.* **1999**, *42*, 2064.
3. Shapiro R., Cohen B. I., Servis R. E.: *Nature* **1970**, 227.
4. Hayatsu H., Wataya Y., Kai K., Iida S.: *Biochemistry* **1970**, *9*, 2858.
5. Hayatsu H., Miura A.: *Biochem. Biophys. Res. Commun.* **1970**, *39*, 983.
6. Kikugawa K., Hayatsu H., Ukita T.: *Biochim. Biophys. Acta* **1967**, *134*, 221.
7. Shapiro R., Servis R. E., Welcher M.: *J. Am. Chem. Soc.* **1970**, *92*, 422.
8. Hayatsu H., Watayu Y., Kai K.: *J. Am. Chem. Soc.* **1970**, *92*, 724.
9. Shapiro R., Klein R. S.: *Biochemistry* **1967**, *6*, 3576.
10. Hayatsu H., Miura A.: *Biochem. Biophys. Res. Commun.* **1970**, *39*, 156.