PREPARATION OF PURINE 2'-DEOXY-5'-O-PHOSPHONOMETHYL-NUCLEOSIDES AND 2'-DEOXY-3'-O-PHOSPHONOMETHYLNUCLEOSIDES

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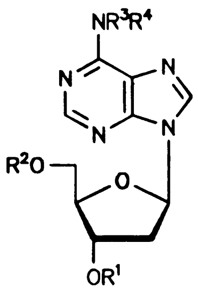
Sodium salt of 2'-deoxy-N^6-dimethylaminomethylene-3'-O-(tetrahydro-2*H*-pyran-2-yl)adenosine (*VIII*) reacted with dibenzyl *p*-toluenesulfonyloxymethanephosphonate (*Ia*) to give dibenzyl ester of 2'-deoxy-N^6-dimethylaminomethylene-5'-O-phosphonomethyl-3'-O-(tetrahydro-2*H*-pyran-2-yl)adenosine (*XI*) which after deprotection afforded the final 2'-deoxy-5'-O-phosphonomethyladenosine (*XII*). 2'-Deoxy-5'-O-hydroxymethanephosphonyladenosine (*XIV*) and 5'-O-benzyloxymethanephosphonyl-2'-deoxyadenosine (*XIII*) were isolated as side products. The preparation of 2'-deoxy-5'-O-phosphonomethylguanosine (*XVI*) and protection of the starting nucleoside were analogous to those for compound *XII*. In the 2'-deoxy-3'-O-phosphonomethylnucleosides series, 2'-deoxy-3'-O-phosphonomethylcytidine (*XXI*) and 2'-deoxy-3'-O-phosphonomethyladenosine (*XXVII*) were prepared, using N^4-benzoyl-5'-O-tert-butyldiphenylsilyl-2'-deoxycytidine (*XVIII*) and 5'-O-tert-butyldiphenylsilyl-2'-deoxy-N^6-dimethylaminomethyleneadenosine (*XXIV*), respectively, as starting compounds.

This communication represents a continuation of our previous studies devoted to syntheses and biochemical investigation of phosphonomethyl derivatives of nucleosides and their analogs (refs[1 – 5] and citations therein). The interesting properties of ribonucleoside phosphonates, particularly their resistance to phosphomonoester hydrolases[1,6] and ability to incorporate into oligonucleotide chains[2] under the catalysis of RNA polymerases, led us to the idea to prepare also phosphonomethyl derivatives of 2'-deoxyribonucleosides and subject them, as well as their triphosphate analogs, to an analogous biochemical scrutiny.

We have hitherto described the synthesis of 2'-deoxy-5'-O-phosphonomethyl derivatives only for the pyrimidine series. In contrast to the procedures developed for the synthesis of analogous derivatives of the ribo-series, it appeared that both the sugar and heterocyclic components in 2'-deoxyribonucleoside series require very specific protection. The reaction conditions for the introduction of the phosphonomethyl residue have to be considerably modified because of an increased lability of the nucleoside bond[7]. Therefore, in our previous paper on the synthesis of 5'-O-phosphonates of pyrimidine 2'-deoxynucleosides, for the reaction of sodium alkoxide of the protected nucleoside we had to use dibenzyl *p*-toluenesulfonyloxymethanephosphonate (*Ia*) instead of the routinely used diethyl or di(2-propyl) esters. The phosphonates, prepared as their dibenzyl esters, were deblocked and converted into the free acids by catalytic hydrogenation.

$TsOCH_2P(O)(OR)_2$ *Ia*, R = Bn
Ib, R = Et

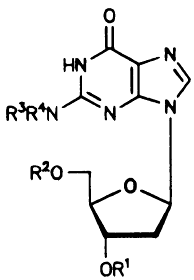
The key problem in the synthesis of purine 2′-deoxynucleoside 5′-O-phosphonomethyl derivatives was again the choice of protecting groups. Originally, for the synthesis of the 2′-deoxyadenosine 5′-phosphonate *XII* we chose N^6,3′-O-dibenzoyl-2′-deoxyadenosine (*II*) as the starting compound. However, reaction of its sodium salt with dibenzyl *p*-toluenesulfonyloxymethanephosphonate in dimethylformamide afforded a complex mixture of products from which on reaction with sodium methoxide, separation from inorganic salts and catalytic hydrogenation the desired 2′-deoxy-5′-O-phosphonomethyladenosine (*XII*) was obtained only in a very low yield, together with considerable amounts of 2′-deoxyadenosine, adenine, sugar derivatives and other unidentified UV-absorbing products. Similar attempts to prepare 2′-deoxy-5′-O-phosphonomethylguanosine (*XVI*) have also shown that protection with benzoyl groups (particularly in the sugar part) is not suitable for such purpose.



	R^1	R^2	R^3	R^4
II	Bz	H	Bz	H
III	H	TBDPS	H	H
IV	THP	TBDPS	H	H
V	THP	TBDPS	THP	H
VI	THP	H	H	H
VII	THP	H	THP	H
VIII	THP	H	$=CHN(CH_3)_2$	
XXIV	H	TBDPS	$=CHN(CH_3)_2$	

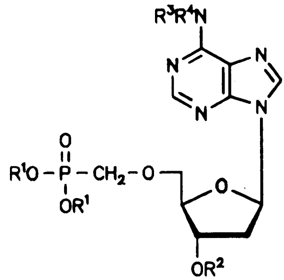
More appropriate for the protection of 2′-deoxyadenosine and 2′-deoxyguanosine appeared to be the tetrahydropyranyl group for blocking the 3′-hydroxyl and the dimethylaminomethylene group for protection of the amino group of the base.

In both cases the starting 2′-deoxynucleosides were first silylated in position 5′ with tert-butylchlorodiphenylsilane using the standard procedure[8] in dimethylformamide with imidazole as the base. Tetrahydropyranylation of the 3′-hydroxyl was carried out in acetonitrile with excess 3,4-dihydro-2*H*-pyran and was catalyzed with trifluoroacetic acid. Under these conditions, the 2′-deoxyadenosine derivative *III* to a limited extent underwent reaction at the base. Thus, beside the desired 3′-O-tetrahydropyranyl derivative *IV*, we also obtained about 20% of N^6,3′-O-bis(tetrahydropyranyl) derivative *V*. After removal of the tert-butyldiphenylsilyl group in position 5′ we obtained 2′-deoxy-3′-O-(tetrahydro-2*H*-pyran-2-yl)adenosine (*VI*) and 2′-deoxy-N^6,3′-O-bis(tetrahydro-2*H*-pyran-2-yl)adenosine (*VII*). Reaction of compound *VI* with dimethylformamide dimethyl acetal afforded 2′-deoxy-N^6-dimethylaminomethylene-3′-O-(tetrahydro-2*H*-pyran-2-yl)adenosine (*VIII*) as the starting compound for the preparation of phosphonomethyl derivative of 2′-deoxyadenosine *XII*. 2′-Deoxy-N^2-dimethylaminomethylene-3′-O-(tetrahydro-2*H*-pyran-2-yl)guanosine (*X*) was obtained in an analogous manner. In this case no tetrahydropyranylation of the base was observed.



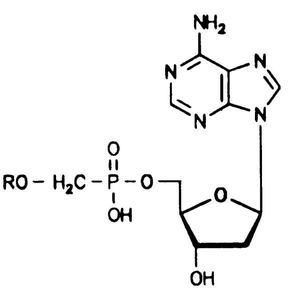
IX, $R^1 = R^3 = R^4 = H$; R^2 = TBDPS

X, R^1 = THP; R^2 = H; R^3, R^4 = =CHN$(CH_3)_2$



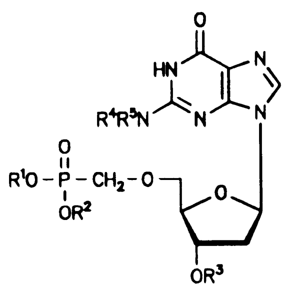
XI, R^1 = Bn; R^2 = THP; R^3, R^4 = =CHN$(CH_3)_2$

XII, $R^1 = R^2 = R^3 = R^4 = H$



XIII, R = Bn

XIV, R = H



XV, R^1 = H; R^2 = Bn; R^3 = THP; R^4, R^5 = =CHN$(CH_3)_2$

XVI, $R^1 = R^2 = R^3 = R^4 = R^5 = H$

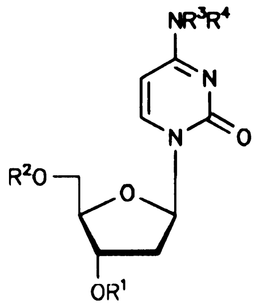
Reaction of sodium salt of 2′-deoxy-N^6-dimethylaminomethylene-3′-O-(tetrahydro-2*H*-pyran-2-yl)adenosine (*VIII*) with dibenzyl ester *Ia* gave dibenzyl-2′-deoxy-N^6-dimethylaminomethylene-5′-O-phosphonomethyl-3′-O-(tetrahydro-2*H*-pyran-2-yl)adenosine (*XI*). After removal of the dimethylaminomethylene group from the base by treatment with ammonia, the tetrahydropyranyl group could be removed with acetic acid under appropriate reaction conditions and with only a minimum cleavage of the nucleoside bond. The dibenzyl ester was subsequently converted into free phosphonate *XII* by catalytic hydrogenation on palladium on charcoal.

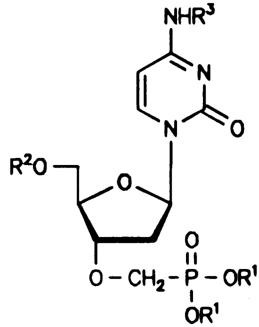
In addition to the phosphonomethyl derivative *XII* we also isolated 2′-deoxy-5′-O-hydroxymethanephosphonyladenosine (*XIV*) and its benzyl derivative *XIII*. The structure of both side-products has been confirmed by spectral methods and also by their electrophoretic mobility which corresponds to the presence of only one negative charge in the molecule. It thus appears that the reaction of nucleoside anion with dibenzyl *p*-toluenesulfonyloxymethanephosphonate may also take place at the phosphorus atom, giving rise to compounds of the type *XIII* and *XIV*, i.e. hydroxymethanephosphonates, in addition to O-phosphonomethyl derivatives.

5′-O-Phosphonomethyl derivative of 2′-deoxyguanosine *XVI* was prepared by an analogous procedure from compound *X*. In contrast to the corresponding 2′-deoxyadenosine derivative, the reaction with dibenzyl *p*-toluenesulfonyloxymethanephosphonate resulted in formation of the monobenzyl ester *XV*, i.e. benzyl 2′-deoxy-N^2-dimethylaminomethylene-5′-O-phosphonomethyl-3′-O-(tetrahydro-2*H*-pyran-2-yl)guanosine, instead of the expected dibenzyl ester. The compound *XV*, which formed after processing the reaction mixture, was successively treated with methanolic ammonia (removal of the dimethylaminomethylene group), acetic acid (removal of the tetrahydropyranyl group) and catalytic hydrogenation to afford the desired 2′-deoxy-5′-O-phosphonomethylguanosine (*XVI*).

In the series of 3′-O-phosphonomethyl derivatives we focused our attention on compounds derived from 2′-deoxycytidine and 2′-deoxyadenosine which deserve further biological investigation. N^4-Benzoyl-5′-O-tert-butyldiphenylsilyl-2′-deoxycytidine (*XVIII*) was used for the condensation with esters of *p*-toluenesulfonyloxymethanephosphonic acid. The protection of the base in 2′-deoxycytidine with benzoyl group is suitable because in the strongly basic medium used in condensation with *p*-toluenesulfonyloxymethanesulfonic acid esters the N-benzoyl group is stable; it neither migrates nor is removed. On the contrary, the dimethylaminomethylene group, which for the same purpose served well with 2′-deoxyadenosine derivatives, is more labile in 2′-deoxycytidine: in our attempted preparation of 2′-deoxy-3′-O-phosphonomethylcytidine from dimethylaminomethylene derivative *XVII* it was split off and 2′-deoxy-N^4,3′-O-bis(diethylphosphonomethyl)cytidine (*XXII*) was obtained as the main product in contrast to phosphonates of purine 2′-deoxyribonucleosides.

2′-Deoxy-3′-O-phosphonomethylcytidine (*XXI*) was prepared by reaction of sodium salt of the starting nucleoside *XVIII* with diethyl *p*-toluenesulfonyloxymethanephosphonate (*Ib*, ref.[1]) which afforded the diethyl ester *XIX*. After removal of the



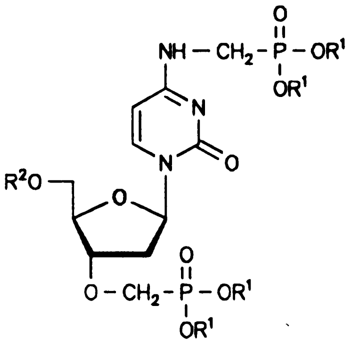


XVII,	R^1 = H; R^2 = TBDPS; R^3, R^4 = =CHN(CH_3)$_2$	*XIX*,	R^1 = Et; R^2 = TBDPS; R^3 = Bz
XVIII,	R^1 = R^3 = H; R^2 = TBDPS; R^4 = Bz	*XX*,	R^1 = Et; R^2 = R^3 = H;
		XXI,	R^1 = R^2 = R^3 = H

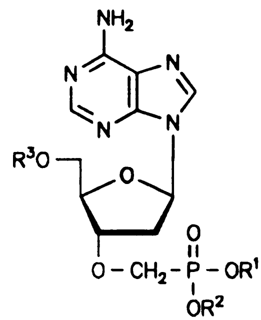
nucleoside protecting groups, the ethyl ester groups were removed by reaction with bromotrimethylsilane followed by hydrolysis. The preparation of phosphonates with the use of diethyl *p*-toluenesulfonyloxymethanephosphonate usually affords higher yields and less side-products than the dibenzyl ester method[7]. Although the removal of ethyl ester groups with bromotrimethylsilane in 2′-deoxynucleoside derivatives often effects either splitting of the nucleoside bond or its anomerization, such side-reactions do not occur in the case of 2′-deoxycytidine whose nucleoside bond is quite resistant to acid-catalyzed cleavage[7,9]. (Analogous results have been described recently for the preparation of 2′,3′-dideoxynucleoside phosphonates where the ester cleavage was performed with iodotrimethylsilane[5].)

Using our previous experience with various protecting groups in the phosphonate synthesis, we chose 5′-O-tert-butyldiphenylsilyl-2′-deoxy-N^6-dimethylaminomethyleneadenosine (*XXIV*) for the preparation of 2′-deoxy-3′-O-phosphonomethyladenosine (*XXVII*); in this case, the synthesis of 5′-phosphonate was performed via the dibenzyl ester *XXV*. After removal of the dimethylaminomethylene group by action of methanolic ammonia and splitting off the tert-butyldiphenylsilyl group with tetrabutylammonium fluoride, the free 2′-deoxy-3′-O-phosphonomethyladenosine (*XXVII*) was obtained by catalytic hydrogenation.

The new compounds were characterized by ^{1}H NMR, IR and mass spectra and elemental analyses. For free 5′- and 3′-O-phosphonomethyl derivatives we also measured the UV spectra and determined the extinction coefficient at the absorption maxima (λ_{max}). The purity of the compounds was checked by TLC and HPLC profiles; free phosphonomethyl derivatives were also subjected to paper electrophoresis to prove the number of negative charges in the molecule.



XXII, R^1 = Et; R^2 = H
XXIII, R^1 = Et; R^2 = Ac



XXV, R^1 = R^2 = Bn; R^3 = TBDPS
XXVI, R^1 = Bn; R^2 = H; R^3 = TBDPS
XXVII, R^1 = R^2 = R^3 = H

Ac, acetyl; Bn, benzyl; Bz, benzoyl; TBDPS, tert-butyldiphenylsilyl; THP, tetrahydro-2*H*-pyran-2-yl; Ts, *p*-toluenesulfonyl

In those cases where the structure was not unequivocally proved by the ^{1}H NMR spectra, we measured the ^{13}C NMR spectra by the APT technique (attached proton test)[10] and the ^{31}P NMR spectra. This concerns particularly compounds *XII* – *XIV* (see Tables I and II). 2′-Deoxy-5′-O-phosphonomethyladenosine (*XII*) is characterized by chemical shifts and coupling constants which agree with the values given already earlier for compounds of this type[7,11]. On the other hand, in the spectra of compounds *XIII* and *XIV*, found as side products, we found non-zero coupling constants of protons H-4′, H-5′ and phosphorus atom which indicate that the phosphonate group is bound to the sugar residue by the P–O–C bond, similarly as in 2′-deoxyadenosine 5′-phosphate[12,13]. Corresponding changes were also found in the ^{13}C NMR spectrum where there is a marked upfield shift of the C-5′ and P–CH_2, a lower value of the coupling constant of C-5′ with phosphorus (^2J(P,C-5′) ≈ 5.5) and a non-zero coupling constant of C-4′ with phosphorus (^3J(P,C-4′) = 7.3). The ^{31}P NMR spectrum of compound *XII* is characterized by a signal at δ 14.54; in the spectra of compounds *XIII* and *XIV* the phosphorus

TABLE I
^{1}H NMR parameters of the compounds *XII* – *XIV* in D_2O

Compound	δ, ppm								
	H-1′	H-2′	H-2″	H-3′	H-4′	H-5′	H-5″	$P-CH_a$	$P-CH_b$
XII	6.49	2.87	2.59	4.71	4.26	3.82	3.78	3.67	3.62
XIII	6.36	2.71	2.56	4.71	4.28	4.11	4.11	3.64	3.60
XIV	6.47	2.84	2.63	4.75	4.30	4.14	4.11	3.71	3.71

Compound	*J*, Hz											
	1′, 2′	1′, 2″	2′, 2″	2′, 3′	2″, 3′	3′, 4′	4′, 5′	4′, 5″	5′, 5″	P, H-4′	P, H-5′	P, H-5″
XII	7.1	6.3	13.9	6.3	3.9	3.4	3.7	5.1	11.0	0	0	0
XIII	7.1	6.6	13.9	6.3	4.1	3.4	3.7	3.7	12.0	1.5	5.6	5.6
XIV	7.1	6.4	14.1	6.3	3.7	3.5	3.7	3.7	11.7	1.0	5.6	5.1

TABLE II
^{13}C NMR parameters of the compounds *XII* – *XIV* in D_2O

Compound	δ, ppm						*J*, Hz		
	C-1′	C-2′	C-3′	C-4′	C-5′	$P-CH_2$	P, C-4′	P, C-5′	P, CH_2''
XII	84.5	39.9	72.3	86.5	73.4	69.4	0	11.0	153.8
XIII	84.9	40.2	72.0	87.0	64.9	65.6	7.3	5.1	159.0
XIV	84.7	40.2	72.2	86.9	65.1	57.7	7.3	5.9	158.2

signal is shifted downfield (δ 18.09 and 20.26) due to the different type of the bond. The found values correspond to those given in the literature[14,15].

EXPERIMENTAL

Unless stated otherwise, solvents were evaporated at 40 °C/2 kPa and the products were dried over phosphorus pentoxide at 13 Pa. Melting points were determined on a Kofler block and are uncorrected.

Thin-layer chromatography was performed on Silufol UV 254 foils (Kavalier, Czech Republic). Spots of compounds were detected by UV light at 254 nm and by spraying with 0.5% solution of 4-(4-nitrobenzyl)pyridine followed by heating and exposure to ammonia vapours[16,17]. Preparative column chromatography was carried out on silica gel (30 – 60 μm) prepared in the Service Laboratories of the Institute. Reversed-phase chromatography was performed on octadecyl silica gel (20 μm, Laboratorní přístroje, Praha) in water, detection at 254 nm on a Uvicord 4701 A instrument (LKB, Sweden). Preparative HPLC was carried out on an Alltech 300 × 51 mm column packed with Separon SGX-RPS 10 μm; the same type of reversed phase was used for analytical chromatography. The eluent systems used are given in the text.

Electrophoreses were done on a Whatman paper No. 3 MM in 0.1 M triethylammonium hydrogen carbonate for 1 h at 20 V/cm. The electrophoretic mobilities are referenced to uridine 5′-phosphate.

UV absorption spectra were measured on a Pye Unicam 8800 instrument, mass spectra on a ZAB-EQ (VG Analytical) spectrometer using the FAB (ionization with Xe, 8 kV) and SIMS (ionization with Cs^+, 35 kV) techniques. As matrices we used glycerol (G), thioglycerol (TG) or mixtures of glycerol, 5% heptafluorobutyric acid and trifluoroacetic acid (GFM).

^1H NMR spectra were measured on a Varian UNITY 200 (200.01 MHz for ^1H) and Varian UNITY 500 (499.8 MHz for ^1H) instruments in hexadeuteriodimethyl sulfoxide with tetramethylsilane as internal standard. Free phosphonates were measured in D_2O with sodium disilapentasulfonate (DSS) as internal standard. ^{13}C and ^{31}P NMR spectra were taken on a Varian UNITY 200 instrument (50.3 MHz for ^{13}C and 80.98 MHz for ^{31}P) in D_2O. The spectra were referenced to dioxane as external standard, $\delta(^{13}C)$(dioxane) = 66.86, and phosphoric acid, $\delta(^{31}P)(H_3PO_4) = 0$. The values of δ are given in ppm, those of J in Hz. Infrared spectra were recorded on an IR Fourier transform spectrometer Bruker IFS-88, wavenumbers of the bands are given in cm^{-1}.

Dimethylformamide was distilled in vacuo from phosphorus pentoxide and then from calcium hydride. Acetonitrile was distilled from calcium hydride immediately before use. Dibenzyl *p*-toluenesulfonyloxymethanephosphonate was prepared according to ref.[7].

Attempted Preparation of 2′-Deoxy-5′-O-phosphonomethyladenosine (*XII*) from N[6],3′-O-Dibenzoyl-2′-deoxyadenosine (*II*)

Dibenzyl ester *Ia* (6.25 g, 14 mmol) was added to a solution of N^6,3′-O-dibenzoyl-2′-deoxyadenosine[18] (*II*, 5.00 g, 10.9 mmol) in dimethylformamide (60 ml). After cooling to −20 °C, a 60% suspension of NaH in mineral oil (1.30 g, 32.5 mmol) was added, the mixture was stirred at this temperature for 30 min and then 5 h at room temperature. The mixture was neutralized with acetic acid (1.30 g, 21.6 mmol), coevaporated with xylene (2 × 100 ml) and the residue was partitioned between water (300 ml) and chloroform (500 ml). Both the aqueous and organic phases (the latter after drying over magnesium sulfate) were evaporated. The residues contained complex mixture of products, most of the UV-absorbing compounds being present in the aqueous phase. Both the residues were debenzoylated by treatment with 0.2 M methanolic sodium methoxide (250 ml) for 48 h. After neutralization with solid carbon dioxide, the reaction mixtures were combined, concentrated to 5 ml and desalted on Dowex 50 (pyridinium form, 150 ml). The mixture of products that had been washed out with water was concentrated, the residue was dissolved in methanol (300 ml) and hydrogenated over 10% palladium on charcoal (0.5 g) for 5 days. The catalyst was filtered off, the filtrate taken down and the residue separated by HPLC on a reversed phase in water. After elution of 2'-deoxy-5'-O-phosphonomethyladenosine (*XII*) the column was eluted with a gradient of methanol (0.25% methanol per min to 20% methanol, then 1.5% methanol per min to 80% methanol); yield 200 mg (5%) of 2'-deoxy-5'-O-phosphonomethyladenosine (E_{Up} 0.79) as a white amorphous residue. The other fractions contained predominantly adenine, several unidentified UV-absorbing minor products and compounds of sugar character.

2'-Deoxy-3'-O-(tetrahydro-2*H*-pyran-2-yl)adenosine (*VI*)

3,4-Dihydro-2*H*-pyran (50 ml), followed by trifluoroacetic acid (1.6 ml), was added to a stirred suspension of 5'-O-tert-butyldiphenylsilyl-2'-deoxyadenosine (10.1 g, 20.6 mmol) in acetonitrile (300 ml). After stirring at room temperature for 4 h, the mixture was neutralized by stirring with sodium hydrogen carbonate (1 g) and evaporated. The residue was partitioned between water (500 ml) and chloroform (500 ml), the chloroform layer was dried over magnesium sulfate and the solvent was evaporated. The obtained crude product *IV* was chromatographed in two portions on silica gel (1 500 ml) in toluene–acetone 2 : 1. After separation of disubstituted derivative *V* (2.71 g, 20%; R_F 0.47), the pure product *IV* (7.68 g, 13.38 mmol) was dissolved in tetrahydrofuran (100 ml), the solution was mixed with 1 M solution of tetrabutylammonium fluoride in tetrahydrofuran (14 ml) and the mixture was set aside for 1 h. After evaporation, the residue was dissolved in chloroform (200 ml), the chloroform solution was washed with water (2 × 100 ml), dried over magnesium sulfate and the solvent was evaporated. The residue was chromatographed on silica gel (800 ml) in ethyl acetate–acetone–ethanol–water 18 : 3 : 2 : 2. The product *VI* was isolated as a white foam (4.0 g, 58%), R_F 0.42. For $C_{15}H_{21}N_5O_4$ (335.4) calculated: 53.72% C, 6.31% H, 20.88% N; found: 52.26% C, 6.07% H, 19.51% N. Mass spectrum (SIMS): 336 (M + H). IR spectrum ($CHCl_3$, dilute solution): 3 527, 3 414 (NH_2 free), 3 238 (OH bonded), ($CHCl_3$, 3%): 3 528, 3 413 (NH_2 free), 3 481, 3 309 (NH_2 bonded), 3 180 (OH bonded), 1 633, 1 593 (ring). ^1H NMR spectrum corresponding to a 1 : 1 mixture of diastereoisomers (hexadeuteriodimethyl sulfoxide): 1.48 m, 4 H (2 × CH_2); 1.75 m, 2 H (CH_2); 2.74 and 2.85 2 × dq, 2 H (H-2', J(2',1') = 8.2; J(2',3') = 5.80; J(2',2'') = 13.67 and J(2',1') = 8.43; J(2',3') = 6.11); 3.49 m, 1 H and 3.77 m, 1 H (OCH_2); 3.60 m, 2 H (2 × H-5'); 4.03 and 4.08 2 × td, 1 H (H-4', J(4,3') = 2.08; J(4',5') = J(4',5'') = 4.52 and J(4',3') = 2.20; J(4',5') = J(4',5'') = 4.15); 4.51 2 × dt, 1 H (H-3'); 4.75 m, 1 H (OCHO); 5.35 and 5.36 2 × t, 1 H (OH, J = 4.3 and J = 5.7); 6.30 and 6.33 2 × dd, 1 H (H-1', J(1',2') = 8.42; J(1',2'') = 6.22 and J(1',2') = 8.18; J(1',2'') = 6.10); 7.34 bs, 2 H (NH_2); 8.14 s, 1 H and 8.34, 8.35 2 × s, 1 H (H-2, H-8); exchange with CD_3COOD: 3.52, 3.54, 3.63 and 3.66 4 × dd, 2 H (2 × H-5').

2'-Deoxy-N^6,3'-O-bis(tetrahydro-2*H*-pyran-2-yl)adenosine (*VII*)

A 1 M solution of tetrabutylammonium fluoride in tetrahydrofuran (5 ml) was added to a solution of compound *V* (2.71 g, 4.12 mmol; obtained as side-product in the preparation of *VI*) in tetrahydrofuran (100 ml). After 1 h the solvent was evaporated, the residue dissolved in chloroform (150 ml), the chloroform solution washed with water (2 × 100 ml), dried over magnesium sulfate and the solvent evaporated. The residue was chromatographed on silica gel (200 ml) in toluene–acetone 1 : 1 to give 1.23 g (71%) of *VII* as a white foam, R_F 0.34. For $C_{20}H_{29}N_5O_5$ (419.5) calculated: 57.26% C, 6.97% H, 16.69% N; found: 58.80% C, 6.97% H, 16.29% N. Mass spectrum (SIMS): 420 (M + H). IR spectrum ($CHCl_3$, dilute): 3 419 (NH free), 3 238 (OH bonded); ($CHCl_3$, 3%): 3 418 (NH), 3 223 (OH bonded), 1 618, 1 588 (ring). ^1H NMR spectrum of 1 : 1 mixture of diastereoisomers (hexadeuteriodimethyl sulfoxide): 1.30 – 1.90 m, 12 H (6 × CH_2 pyranyl); 2.50 overlapped by DMSO (H-2'', J(2'',1') = 6.10; J(2'',3') = 2.32); 2.75 and 2.85 2 × dq, 1 H (H-2', J(2',1') = 8.17; J(2',3') = 5.74; J(2',2'') = 13.67 and J(2',1') = 8.0; J(2',3') = 6.10; J(2',2'') = 13.67); 3.50 m, 2 H (OCH_2); 3.60 m, 2 H (2 × H-5'); 3.80 m, 2 H (OCH_2); 4.03 and 4.08 2 × td, 1 H (H-4', J(4',3') = 2.3; J(4',5') = J(4',5'') = 4.5 and J(4',3') = 2.19; J(4',5') = J(4',5'') = 4.15); 4.51 2 × dt, 1 H

(H-3′); 4.75 m, 1 H (OCHO); 5.26 and 5.27 2 × t, 1 H (OH, J = 5.74 and J = 5.68); 5.50 br, 1 H (OCHO); 6.33 and 6.36 2 × dd, 1 H (H-1′, J(1′,2′) = 8.18; J(1′,2″) = 6.10 and J(1′,2′) = 8.06; J(1′,2″) = 6.23); 8.23 bs, 1 H (NH); 8.27 s, 1 H and 8.43, 8.426 2 × s, 1 H (H-2, H-8); exchange with CD_3COOD: 3.58 4 × dd, 2 H (2 × H-5′).

2′-Deoxy-N^6-dimethylaminomethylene-3′-O-(tetrahydro-2*H*-pyran-2-yl)adenosine (*VIII*)

Dimethylformamide dimethylacetal (20 ml) was added to a solution of compound *VI* (3.9 g, 11.6 mmol) in dimethylformamide (40 ml). After standing for 20 h at ambient temperature, the reaction mixture was concentrated, the residue was mixed with ground dry ice and then with 50% aqueous pyridine (50 ml). After stirring for 1 h the solution was evaporated and the residue codistilled with pyridine (40 ml) and xylene (2 × 40 ml) to give 4.7 g (100%) of chromatographically pure amorphous compound *VIII* (R_F 0.36 in ethyl acetate–acetone–ethanol–water 18 : 3 : 2 : 2). Mass spectrum (SIMS, G): 391 (M + H).

2′-Deoxy-N^2-dimethylaminomethylene-3′-O-(tetrahydro-2*H*-pyran-2-yl)guanosine (*X*)

The title compound was prepared from 5′-O-tert-butyldiphenylsilyl-2′-deoxyguanosine (*IX*; 0.46 g, 0.91 mmol) in the same manner as the above-mentioned 2′-deoxyadenosine derivative *VIII*. The product was isolated as a white foam (130 mg, 35%), R_F 0.30 (ethyl acetate–acetone–ethanol–water 18 : 3 : 2 : 2). Mass spectrum (SIMS, GFM): 407 (M + H).

Dibenzyl 2′-Deoxy-N^6-dimethylaminomethylene-5′-O-phosphonomethyl-3′-O-(tetrahydro-2*H*-pyran-2-yl)adenosine (*XI*)

To a solution of compound *VIII* (4.5 g, 11.5 mmol) in dimethylformamide (100 ml) was added 60% NaH suspension in oil (1.43 g, 35.8 mmol). After stirring for 30 min, dibenzyl ester *Ia* (7.95 g, 17.8 mmol) in dimethylformamide (25 ml) was added and the mixture was stirred at ambient temperature for 3 h. The mixture was neutralized with solid carbon dioxide with addition of two drops of water and the solvent was evaporated. The residue was coevaporated with xylene (2 × 50 ml) and then dissolved in chloroform (500 ml). The chloroform solution was washed with water (2 × 250 ml), dried over magnesium sulfate and taken down. Chromatography of the residue on silica gel (700 ml) in ethyl acetate–acetone–ethanol–water 18 : 3 : 2 : 2 afforded 3.43 g (44.5 %) of pure amorphous product *XI*, R_F 0.36. For $C_{33}H_{41}N_6PO_7$ (664.7) calculated: 4.66% P; found: 4.81% P. Mass spectrum (FAB, TG + G): 666 (M + H).

2′-Deoxy-5′-O-phosphonomethyladenosine (*XII*)

Pure phosphonomethyl derivative *XI* (3.2 g, 4.8 mmol) from the preceding operation was stirred with 10% methanolic ammonia (100 ml) for 24 h at room temperature. The solution was taken down and the residue heated at 50 °C with 70% acetic acid (150 ml). After 1 h the reaction mixture was evaporated, the residue codistilled with water (3 × 100 ml), dissolved in methanol (100 ml) and hydrogenated over 10% palladium on charcoal (1.2 g) at atmospheric pressure and ambient temperature for 48 h and then at 50 °C for 2 h. The catalyst was removed by filtration through Celite, the filtrate was concentrated and the residue (1.2 g) was purified in two portions on a column of DEAE-Sephadex A25 (400 ml). Elution with a gradient 0 – 0.4 M triethylammonium hydrogen carbonate (4 l) gave successively adenine (150 mg; 23%), compounds *XIII* (200 mg; 10%) and *XIV* (150 mg; 9%) and finally the desired product *XII*. Fractions containing *XII* were combined and the residue after evaporation was several times coevaporated with water to decompose all triethylammonium hydrogen carbonate. The product was purified by reversed-phase chromatography with water as eluent. The UV-absorbing eluate was concentrated to 4 ml and poured through a column of Dowex 50 × 8 (Li^+ form, 200 ml). Evaporation afforded 400 mg (24%) of 2′-deoxy-5′-O-phosphonomethyladenosine (*XII*) as its lithium salt, a solid not melting up to 260 °C. E_{Up} 0.79, R_F 0.1 (ethyl acetate–acetone–ethanol–water 13 : 3 : 4 : 5). For $C_{11}H_{14}N_5PO_6Li_2$ (357.1) calculated: 37.00% C, 3.95% H, 19.61% N, 8.67% P; found: 36.81% C, 4.12% H, 19.53% N, 8.58% P. UV spectrum (pH 7): λ_{max} 259 nm, ε_{max} 14 900. Mass spectrum (FAB, G): 346 (M + H), 358 (M + H of the dilithium salt). ^{1}H NMR spectrum (D_2O): 2.60 ddd, 1 H (H-2″, $J(2'',3')$ = 3.9; $J(2'',2')$ = 13.7), 2.81 p, 1 H (H-2′, $J(2',3')$ = 6.3); 3.40 t, 1 H (H-1′, $J(1',2')$ = 6.8; $J(1',2'')$ = 5.9); 3.70 d, 2 H (PCH_2, $J(P,CH)$ = 8.3); 3.83 m, 2 H (2 × H-5′, $J(5',4')$ = 4.5); 4.27 m, 1 H (H-4′, $J(4',3')$ = 3.0); 4.70 m, 1 H (H-3′); 8.10 and 8.39 2 × s, 2 H (H-2, H-8).

Characterization of both side-products, 2′-deoxy-5′-O-hydroxymethanephosphonyladenosine (*XIV*) and 5′-O-benzyloxymethanephosphonyladenosine (*XIII*), by NMR spectra is given in Tables I and II. The electrophoretic mobility of both compounds is E_{Up} 0.47. TLC in ethyl acetate–acetone–ethanol–water (13 : 3 : 4 : 5): R_F 0.42 and 0.18 for compounds *XIII* and *XIV*, respectively.

2′-Deoxy-5′-O-phosphonomethylguanosine (*XVI*)

A suspension of sodium hydride (60%) in oil (30 mg; 0.75 mmol) was added to a solution of derivative *X* (100 mg, 0.25 mmol) in dimethylformamide (4 ml). After stirring for 30 min, dibenzyl ester *Ia* (170 mg, 0.38 mmol) was added and the mixture was stirred for 4 h at room temperature. Further work-up was the same as described for compound *XI*, except that the desired phosphonate, as the monobenzyl ester *XV*, was contained only in the aqueous phase (E_{Up} 0.30). The residue after evaporation of the aqueous phase was treated with methanolic ammonia, acetic acid and hydrogenated as described for the derivative *XI*. The crude product, containing also some inorganic salts, was chromatographed on a DEAE-Sephadex A25 column (HCO_3^- form, 50 ml). The column was washed first with water (200 ml) and then with a gradient 0 – 0.4 M triethylammonium hydrogen carbonate (400 ml). The UV-absorbing eluate was evaporated, the residue codistilled with water (4 × 15 ml) and the product was converted into the lithium salt by passing through a column of Dowex 50 (Li^+ form, 20 ml). The overall yield was 18 mg (20%, calculated for the starting compound *X*). The product was obtained as a white solid, E_{Up} 0.78, R_F 0.10 (in ethyl acetate–acetone–ethanol–water 13 : 3 : 4 : 5). Mass spectrum (SIMS, GFM): 368 (M + H of the lithium salt, 362 (M + H of free phosphonate).

2′-Deoxy-N^4,3′-O-bis(diethylphosphonomethyl)cytidine (*XXII*)

A suspension of sodium hydride in mineral oil (60%, 115 mg, 2.9 mmol) was added to a solution of compound *XVII* (0.5 g, 0.96 mmol) in dimethylformamide (20 ml). After 30 min, compound *Ib* (464 mg, 1.4 mmol) was added. After 6 h, the reaction mixture was worked up similarly as described for compound *XI*. The obtained crude product, 2′-deoxy-N^4,3′-O-bis(diethylphosphonomethyl)-5′-O-tert-butyldiphenylsilylcytidine (1.2 g), was dissolved in tetrahydrofuran (10 ml) and stirred with 1 M tetrabutylammonium fluoride in tetrahydrofuran (1 ml) for 12 h. The solvent was evaporated and the residue dissolved in chloroform (100 ml). The solution was washed with water (50 ml) and dried over magnesium sulfate. After evaporation, the residue was chromatographed on silica gel (30 ml) in ethyl acetate–acetone–ethanol–water (15 : 3 : 4 : 3): yield 150 mg (30%) of compound *XXII*, R_F 0.30. The product was characterized as the acetate *XXIII*.

5′-O-Acetyl-2′-deoxy-N^4,3′-O-bis(diethylphosphonomethyl)cytidine (*XXIII*)

Dimethylaminopyridine (5 mg) and acetyl chloride (0.02 ml) were added to a solution of compound *XXII* (50 mg, 0.09 mmol) in acetonitrile (2 ml). After 2 h, the solvent was evaporated and the residue partitioned between chloroform (5 ml) and water (5 ml). The chloroform solution was dried, the solvent evaporated and the residue chromatographed on silica gel (20 ml) in ethyl acetate–acetone–ethanol–water (18 : 3 : 2 : 2); R_F of the product *XXIII* was 0.33. Yield 45 mg (88%) of amorphous residue of *XXIII*. ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 1.22 t, 6 H (2 × CH_3); 1.24 t, 6 H (2 × CH_3); 2.09 s, 3 H (acetyl); 2.20 m, 2 H (2 × H-2'); 3.69 dd, 1 H (H-5", $J(5",4')$ = 3.5; $J(5",5')$ = 10.6); 3.79 dd, 1 H (H-5', $J(5',4')$ = 3.05); 3.89 d, 2 H (PCH_2, $J(P,CH)$ = 9.2); 3.90 d, 2 H (PCH_2, $J(P,CH)$ = 8.5); 4.06 dq, 8 H (4 × CH_2, $J(CH_2,CH_3)$ = 7.1; $J(P,OCH)$ = 8.4); 4.20 m, 2 H (H-3', H-4'); 6.12 dd, 1 H (H-1', $J(1',2')$ = 5.74; $J(1',2")$ = 7.7); 7.26 d, 1 H (H-5, $J(5,6)$ = 7.4); 7.50 bs, 1 H (NH); 8.22 d, 1 H (H-6).

Diethyl N^4-Benzoyl-5'-O-tert-butyldiphenylsilyl-2'-deoxy-3'-O-phosphonomethylcytidine (*XIX*)

The title compound was prepared from derivative *XVIII* (4.5 g, 7.9 mmol; ref.[7]), 60% sodium hydride suspension (954 mg, 23.8 mmol) and diethyl *p*-toluenesulfonyloxymethanephosphonate (*Ib*; 3.83 g, 11.9 mmol) analogously as described for compound *XI*, the reaction time being 2 h. The crude product was purified by chromatography on silica gel (600 ml) in ethyl acetate (R_F 0.48). The product was obtained as a solid residue (2.8 g, 49%). For $C_{37}H_{46}N_3PO_8S$ (719.8) calculated: 61.74% C, 6.44% H, 5.84% N, 4.30% P; found: 61.53% C, 6.48% H, 5.96% N, 4.47% P. Mass spectrum (SIMS, G and methanol): 720 (M + H). ^1H NMR spectrum (hexadeuteriodimethyl sulfoxide): 1.03 s, 9 H (tert-butyl); 1.233 and 1.238 2 × t, 6 H (2 × CH_3, J = 7.3); 2.20 p, 1 H (H-2", $J(2",1')$ = 6.9; $J(2",3')$ = 6.3; $J(2",2')$ = 14.2); 2.59 ddd, 1 H (H-2', $J(2',1')$ = 6.3; $J(2',3')$ = 2.9); 3.86 2 × dd, 2 H (2 × H-5', $J(5',4')$ = 4.5; $J(5',5")$ = 12.0); 3.89 d, 2 H (PCH_2, $J(P,CH)$ = 9.5); 4.05 2 × dq, 4 H (2 × $POCH_2$, $J(CH_2,CH_3)$ = 7.3; $J(P,OCH)$ = 8.3); 4.18 m, 1 H (H-4'); 4.29 m, 1 H (H-3', $J(3',4')$ = 2.5); 6.13 t, 1 H (H-1', $\sum J$ = 13.2); 7.20 – 8.20 m, 15 H (H-arom.); 7.23 d, 1 H (H-5, $J(5,6)$ = 7.8); 8.19 d, 1 H (H-6); 11.30 bs, 1 H (NH).

Diethyl 2'-Deoxy-3'-O-phosphonomethylcytidine (*XX*)

A solution of compound *XIX* (2.8 g, 3.89 mmol) in an NH_4OH–water–ethanol (1 : 1 : 2, 180 ml) mixture was allowed to stand at ambient temperature for 20 h. After evaporation, the residue was dissolved in tetrahydrofuran (100 ml) and the solution was stirred with 1 M tetrabutylammonium fluoride in tetrahydrofuran (4 ml) at room temperature for 2 h. The solvent was evaporated and the residue chromatographed on silica gel (600 ml) in ethyl acetate–acetone–ethanol–water (15 : 3 : 4 : 3), giving 1.40 g (95%) of amorphous pure product *XX*, R_F 0.31. For $C_{14}H_{24}N_3PO_7$ (377.3) calculated: 8.21% P; found 8.07% P. Mass spectrum (SIMS, TG + G): 378 (M + H). ^1H NMR spectrum (hexadeuteriodimethyl sulfoxide): 1.24 t, 6 H (2 × CH_3, J = 7.1); 1.98 ddd, 1 H (H-2", $J(2",3')$ = 5.9; $J(2",2')$ = 14.4); 2.30 ddd, 1 H (H-2', $J(2',3')$ = 2.0); 3.56 d, 2 H (2 × H-5', $J(5',4')$ = 3.9); 3.85 d, 2 H (PCH_2, $J(P,CH)$ = 9.3); 3.96 td, 1 H (H-4', $J(4',3')$ = 2.0); 4.05 dq, 4 H (2 × $POCH_2$, $J(CH_2,CH_3)$ = 7.1; $J(P,OCH)$ = 8.1); 4.17 m, 1 H (H-3'); 5.10 b, 1 H (OH); 5.74 d, 1 H (H-5, $J(5,6)$ = 7.3); 6.12 dd, 1 H (H-1', $J(1',2')$ = 5.9; $J(1',2")$ = 8.8); 7.20 bs, 2 H (NH_2); 7.78 d, 1 H (H-6).

2'-Deoxy-3'-O-phosphonomethylcytidine (*XXI*)

Bromotrimethylsilane (2.1 ml, 16 mmol) was added to a suspension of compound *XX* (1.20 g, 3.18 mmol) in acetonitrile (80 ml). The suspension turned immediately into a solution which was then set aside in the dark at ambient temperature for 20 h. The reaction course was monitored by paper electrophoresis and reversed-phase HPLC in water. Toluene (50 ml) was added, the mixture concentrated at 30 °C and the residue was codistilled with another portion of toluene (50 ml) and two drops of triethylamine. The residue was dissolved in water (5 ml), the solution made alkaline with 3 drops of triethylamine and applied onto a column of Dowex 1 × 8 (acetate form, 500 ml). The ion exchanger was washed with water (1 500 ml) and then with 0.5 M acetic acid until all UV-absorbing material was eluted. The product-containing fractions were evaporated and the residue was several times codistilled with water to complete removal of acetic acid. The crude product was purified by HPLC on a reversed phase with water as eluent to give 870 mg (85%) of pure product *XXI*, m.p. 155 – 156 °C (water); E_{Up} 0.85. For $C_{10}H_{16}N_3PO_7$ (321.2) calculated:

13.08% N, 9.64% P; found: 12.74% N, 9.50% P. UV spectrum (pH 7): λ_{max} 270 nm, ε_{max} 7 800. Mass spectrum (FAB, G): 332 (M + H). IR spectrum (KBr): 3 448, 3 345 (NH_2); 2 939 (CH_2); 2 769 (POH); 2 451, 2 415 (OH); 1 718 (C=O); 1 683 (NH_2); 1 644 (C=C); 1 539 (C=N); 1 141 (P=O); 1 099, 1 068 (CO); 930 (POH). ^{1}H NMR spectrum (D_2O): 2.29 p, 1 H (H-2″, $J(2″,1′)$ = 7.0; $J(2″,3′)$ = 5.9; $J(2″,2′)$ = 13.7); 2.62 ddd, 1 H (H-2′, $J(2′,1′)$ = 6.3; $J(2′,3′)$ = 2.9); 3.55 and 3.57 2 × d, 2 H (PCH_2, $J(P,CH)$ = 9.3); 3.86 m, 2 H (2 × H-5′); 4.28 m, 2 H (H-3′, H-4′); 6.10 d, 1 H (H-5); 6.31 t, 1 H (H-1′, $\sum J$ = 13.2); 7.89 d, 1 H (H-6).

5′-O-tert-Butyldiphenylsilyl-2′-deoxy-N^6-dimethylaminomethyleneadenosine (*XXIV*)

Dimethylformamide dimethyl acetal (12 ml) was added to a solution of 2′-deoxy-5′-O-tert-butyldiphenylsilyladenosine (4.0 g, 8.2 mmol) in dimethylformamide (20 ml) and the mixture was stirred at ambient temperature for 18 h. After evaporation, ground dry ice was added to the residue, followed by 50% aqueous pyridine (40 ml). After 1 h, the solution was concentrated and the residue codistilled with toluene (3 × 50 ml), yielding 4.5 g of chromatographically pure product *XXIV*; R_F 0.35 (ethyl acetate–acetone–ethanol–water 18 : 3 : 2 : 2). The compound was used in the synthesis of compound *XXV* without further purification.

Dibenzyl 5′-O-tert-Butyldiphenylsilyl-2′-deoxy-3′-O-phosphonomethyladenosine (*XXV*)

A suspension of sodium hydride in mineral oil (60%, 0.96 g, 24 mmol) was added to a solution of compound *XXIV* (4.2 g, 7.7 mmol) in dimethylformamide (60 ml). After 30 min, compound *Ia* (5.26 g, 11.5 mmol) was added, the mixture was stirred at room temperature for 5 h, neutralized with dry ice, and the solvent was evaporated. The residue was codistilled with xylene (2 × 80 ml), partitioned between chloroform (600 ml) and water (300 ml), the organic phase was washed with water (300 ml), dried over magnesium sulfate and the solvent was evaporated. The residue was stirred with 18% methanolic ammonia (200 ml) for 24 h. After evaporation, the remaining crude product was chromatographed on silica gel (750 ml), first in ethyl acetate (800 ml) and then in ethyl acetate–acetone–ethanol–water (36 : 6 : 1 : 1); R_F of product *XXV* was 0.43. The compound was obtained as an amorphous residue; yield 1.03 g (18%). For $C_{41}H_{46}N_5PO_6Si$ (763.9) calculated: 64.47% C, 6.07% H, 9.17% N, 4.05% P; found: 64.24% C, 6.04% H, 9.30% N, 4.03% P. Mass spectrum (FAB, TG + DMSO 3 : 1): 764 (M + H), 136 (BH). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 0.96 s, 9 H (tert-butyl); 2.50 ddd, 1 H (H-2″, $J(2″,3′)$ = 1.9; $\sum J$ = 22.0); 2.92 ddd, 1 H (H-2′, $J(2′,3′)$ = 5.9; $J(2′,1′)$ = 8.0; $J(2′,2″)$ = 13.9); 3.70 dd, 1 H (H-5″, $J(5″,4′)$ = 5.4; $J(5″,5′)$ = 11.0); 3.86 dd, 1 H (H-5′, $J(5′,4′)$ = 5.4); 4.02 d, 2 H (PCH_2, $J(P,CH)$ = 9.0); 4.11 td, 1 H (H-4′, $J(4′,3′)$ = 1.7; $\sum J$ = 12.45); 4.41 m, 1 H (H-3′, $\sum J$ = 9.5); 5.08 and 5.12 2 × s, 4 H (2 × $POCH_2$); 6.28 dd, 1 H (H-1′, $J(1′,2′)$ = 8.05; $J(1′,2′)$ = 6.1); 7.20 – 7.65 m, 22 H (NH_2 + H-arom.); 8.06 s, 1 H and 8.23 s, 1 H (H-2, H-8).

After washing out the product *XXV*, the elution was performed with pure methanol, affording 2.2 g (42%) of 5′-O-tert-butyldiphenylsilyl-2′-deoxy-3′-O-phosphonomethyladenosine monobenzyl ester (*XXVI*), E_{Up} 0.45.

2′-Deoxy-3′-O-phosphonomethyladenosine (*XXVII*)

To a solution of compound *XXV* (1.0 g, 1.3 mmol) in tetrahydrofuran (100 ml) was added 1 M solution of tetrabutylammonium fluoride in tetrahydrofuran (1.5 ml). After 2 h, the solvent was evaporated, the residue dissolved in methanol (150 ml) and the solution hydrogenated over 10% palladium on charcoal (0.6 g) at ambient temperature and pressure for 72 h. The catalyst was removed by filtration through Celite, the filtrate was evaporated and the residue dissolved in water (5 ml). The solution was made alkaline with triethylamine and applied onto a column of DEAE-Sephadex A25 (HCO_3^- form, 400 ml). The elution was carried out with a gradient of triethylammonium hydrogen carbonate (0 – 0.4 mol l^{-1}, 2 000 ml) and then with 0.4 M triethylammonium hydrogen carbonate (500 ml). The UV-absorbing eluate, which contained the product *XXVII*, was evaporated and the residue was coevaporated with water until all the triethylammonium hydrogen carbonate was decomposed. The product was converted to the lithium salt by passing its aqueous solution (5 ml) through a column of Dowex 50 × 8 (Li^+ form, 200 ml). Yield 265 mg (59%) of lithium salt of *XXVII*, R_F 0.10 (ethyl acetate–acetone–ethanol–water 13 : 3 : 4 : 5). E_{Up} 0.85. UV spectrum (pH 7): λ_{max} 259 nm, ε_{max} 14 900. Mass spectrum (FAB, T + G + methanol): 346 (M + H). ^{1}H NMR spectrum (D_2O): 2.76 m, 2 H (2 × H-2', $\sum J$ = 10.0); 3.62 d, 2 H (PCH_2, J(P,CH) = 8.8); 3.86 bs, 2 H (2 × H-5'); 4.39 bs, 1 H (H-4'); 4.45 bs, 1 H (H-3'); 6.43 bt, 1 H (H-1', $\sum J$ = 13.67); 8.14 s, 1 H and 8.28 s, 1 H (H-2, H-8).

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