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NON-GLYCOSIDIC ANALOGUES OF NUCLEOTIDES: 2′(*R*),3′(*S*),5′-TRIHYDROXYPENTYL DERIVATIVES OF ADENINE AND CYTOSINE

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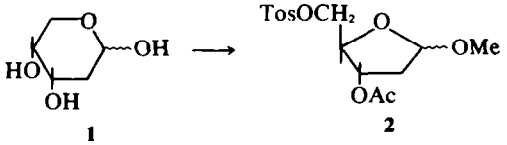
Abstract—Chiral 2′,3′,5′-trihydroxypentyl derivatives of adenine and cytosine in which configurations at C-2′ and C-3′ are opposite to those of the natural nucleosides have been synthesized. The nucleoside analogues were converted into 3′-phosphates and dinucleoside phosphate analogues with 3′–5′ phosphodiester linkages. PMR, UV and CD spectra of the compounds are presented.

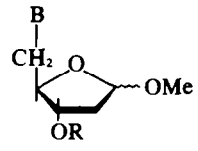
Non-glycosidic analogues of nucleotides have been used extensively for the study of various biological systems.[1-3] Recently we have prepared chiral dihydroxypropyl derivatives of nucleic bases.[4] The present paper deals with the synthesis of 2′(*R*),3′(*S*),5′-trihydroxypentyl derivatives of adenine and cytosine, their phosphates and dinucleoside phosphates, and with examination of CD spectra of these compounds.

2-Deoxy-D-ribose **1** was chosen as a starting compound:



1 **2**

Treatment of **1** with MeOH in the presence of hydrogen chloride in low concentration gave a mixture of α- and β-methyl furanosides;[5] these were converted into α(β)-methyl 3 - O - acetyl - 5 - O - tosyl - 2 - deoxy - D - ribofuranoside **2** by selective tosylation at the primary hydroxyl, and subsequent acetylation. The tosylate **2** thus prepared, was used for alkylation of adenine and N⁴-benzoylcytosine by the method recently developed.[4]



3: B = Ade; R = Ac
4: B = N⁴–Bz–Cyt; R = Ac
5: B = N⁶–Bz–Ade; R = H
6: B = N⁴–Bz–Cyt; R = H
7: B = Ade; R = PO_3H_2
8: B = Cyt; R = PO_3H_2

Various substituents may be attached to the 3′-OH function and the furanose ring is a latent hydroxymethyl group and protects simultaneously a 2′-OH function in these compounds. After selective removal of the acetyl group (in the case of compound **3** the 6-amino group was protected previously by benzoylation), nucleoside analogues **5** and **6** were phosphorylated with β-cyanoethyl phosphate in the presence of mesitylenesulphonyl chloride. Purification of intermediate phosphodiesters by DEAE-cellulose chromatography, removal of N-benzoyl groups with methanolic ammonia and, finally, hydrolysis of cyanoethyl ester by means of 2N LiOH afforded **7** and **8**. Non-glycosidic analogues of 3′-nucleotides **9** and **10** were obtained by mild acidic hydrolysis of the mixture of anomers (Fig. 1), followed by reduction with sodium borohydride, and were isolated in satisfactory yields by chromatography on Dowex 1 (formate form).

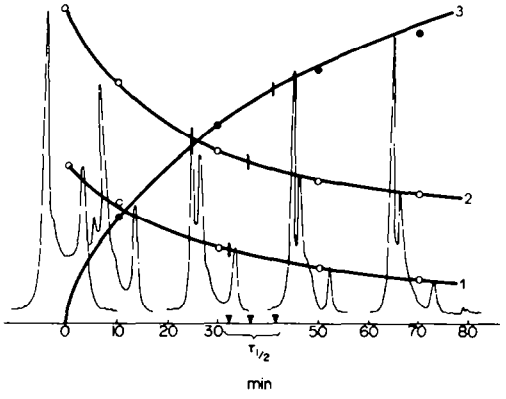
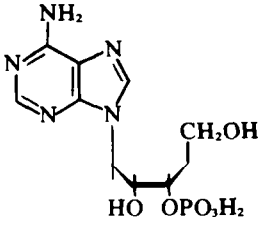


Fig. 1. Signals of α- and β-(1 and 2) anomeric Me groups and MeOH (3) in PMR spectrum of compound **7** as a function of time in 0.05 N ^{2}HCl at 25°.

It should be noted that the reduction succeeded in acidic media only. In neutral and alkaline media insoluble



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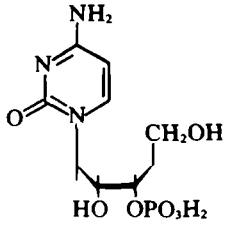

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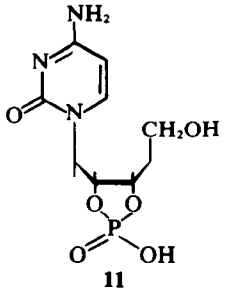
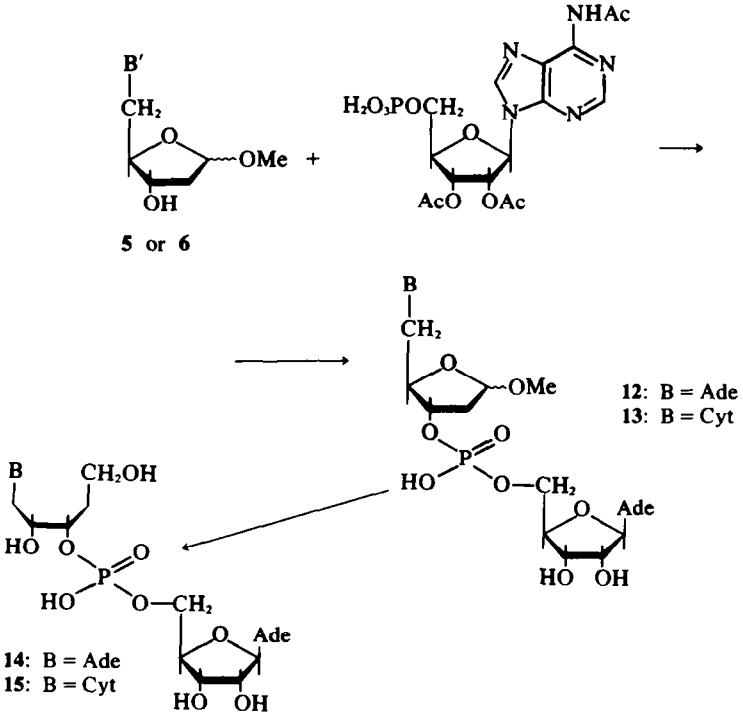


11

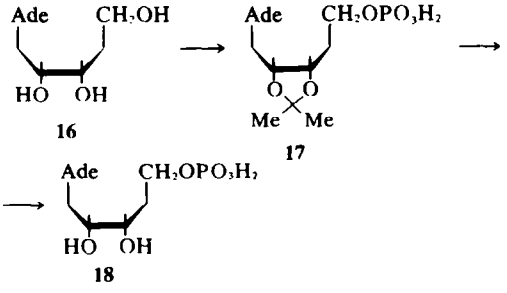
by-products were mainly obtained. The cytosine derivative **10** was converted into the cyclic phosphate **11** by treatment with DCC.

This general scheme was found to be equally applicable to the synthesis of dinucleoside phosphate analogues possessing a 3',5'-phosphodiester linkage, **14** and **15**:



The adenosine analogue **16**, which was prepared from **3** with methanolic ammonia followed by acidic hydrolysis and reduction, upon treatment with phosphorus oxychloride under conditions appropriate for selective phosphorylation of primary hydroxyl groups,[6] yielded a complex mixture. Therefore, the desired 5'-phosphate **18** was prepared by a series of routine reactions.

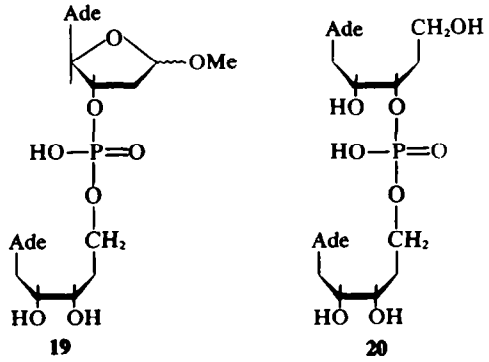
Fully-acetylated **18** was condensed with **5** in the presence of mesitylenesulphonyl chloride. The intermediate was worked up in the manner described above for the nucleotide analogues, to yield dinucleoside phosphate analogues **19** and **20** with both 5'- and 3'-terminal non-glycosidic units **18**.



In order to examine the effect of chirality at C-2' on the CD Spectrum, two dinucleoside phosphate analogues in which natural configuration at C-2' was retained were synthesized.

Isomeric dimers, containing 2', 5' **21** and 3', 5' **22** phosphodiester linkages, were obtained in 80% total yield, and were separated by chromatography on Dowex 1 (formate form).

The compounds prepared were chromatographically and electrophoretically homogeneous. The structures of all compounds were proven by PMR and UV spectra (see Table 1). CD spectra are listed in Table 2.



As seen from Fig. 2, CD spectra of trihydroxypentyl derivatives are surprisingly similar to those of the corresponding common nucleosides. This is quite remarkable, since the compounds under consideration are diastereoisomeric at C-1'. Moreover, the configurations at C-2' and C-3' are opposite to that of D-ribose. It is worthwhile to assume initially that the Cotton effect should depend on the absolute configuration of assymetric site and position of this site relative to the chromophore. The contributions of each bond of the ribofuranose ring to the total rotational strength of B_{2u} transition of pyrimidine nucleosides have been calculated by Miles *et al.*[7] In the case of the *anti*-conformation, this theory predicts a negative contribution of C_2–O and C_3–O bonds in D-ribofuranose in contrast to D-lyxofuranose. It is evident that the trihydroxypentyl substituent is a configurational analogue of D-lyxose in respect to chirality at C-2' and

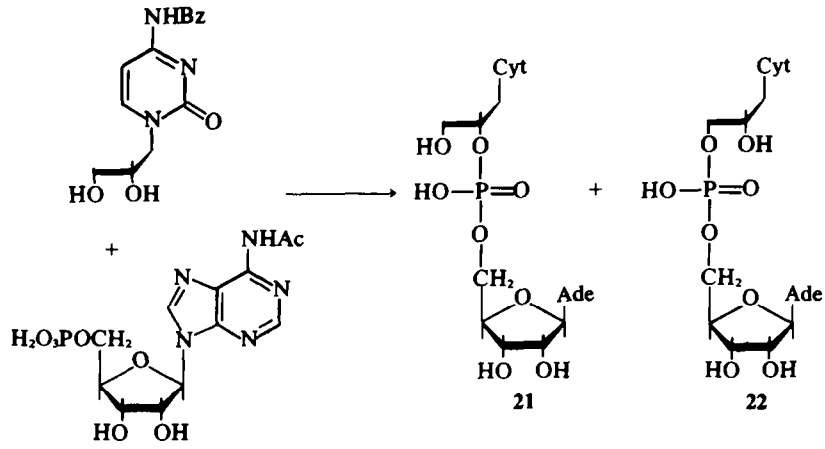


Table 1. PMR and UV spectra of 2′(*R*),3′(*S*),5′-trihydroxypentyl derivatives of adenine and cytosine

Compounds	PMR		UV, λ_{max}, nm (ϵ)		
	Solvent	δ, ppm, (τ, c/s)	pH 1	pH 7	pH 13
2	C^2HCl_3[a]	Tos 7.75d and 7.40d(8)[b]; α- and β-OCH_3 3.20s, 3.30s; $PhCH_3$ 2.44s[c] CH_3CO 2.01s[c]			
3	DMSO-d_6[a]	8-H 8.50s; 2-H 8.45s[d]; 1-H 5.42; α- and β-OCH_3 3.55s and 3.61s; CH_3CO 2.29s[c]; 6-NH_2 7.62s[c]			
5	DMSO-d_6[a]	8-H 8.82s; 2-H 8.51s[d]; Ph 7.70–8.18m; 1-H 5.32; α- and β-OCH_3 3.28s and 3.31s	291(31,200) 252(12,400)	282(24,400)	304(16,400)
6	C^2HCl_3[a]	6-H 7.70d; 5-H 7.42d(7.5); PhCO 7.3–8.0; 1-H 4.94; 2-CH_2 2.12; α- and β-OCH_3 3.26 and 3.29s			
7	^2H_2O[a]	8-H and 2-H 8.08s[c,d]; α- and β-OCH_3 3.09s and 3.24s; 2-CH_2 2.08m	258(11,000)	262(11,600)	262(11,600)
8	^2H_2O[a]	6-H 7.52d; 5-H 5.05(7.5); α- and β-OCH_3 3.30s and 3.35s; 2-CH_2 2.26m	282(7,900)	274(5,400)	274(5,400)
9	^2H_2O[a]	8-H 8.06s; 2-H 8.00s[d]; 5′-CH_2 3.78t (6.5); 4′-CH_2 1.86q(6)	258(12,800)	262(13,600)	262(13,400)
10	^2H_2O[a]	6-H 7.58d; 5-H 5.93d(7.5); 5′-CH_2 3.78t(6.5); 4′-CH_2 1.80q(6)	283(11,600)	275(8,200)	275(8,600)
11			282(11,300)	273(8,000)	273(8,000)
12			257(19,600)	258(18,000)	258(16,200)
13			267(20,300) 289(14,500)[e]	262(19,700)	262(17,900)
14	^2H_2O[a]	8-H 8.17s and 7.90s; 2-H 8.03s and 7.98s[d]; 1′-H 5.94d(5); 5′-CH_2 3.75t(6.5); 4′-CH_2 1.81q(6)	258(22,600)	258(20,200)	258(19,400)
15	^2H_2O[a]	8-H 8.28s; 2-H 7.99s[d]; 6-H 7.21d; 5-H 5.66d(7.5); 1′-H 6.01d(5); 5′-CH_2 3.70t(6); 4′-CH_2 1.85q(6)	267(19,000) 281(15,000)[e]	262(19,200)	262(17,400)
16	CF_3COOH[a]	8-H 8.94s; 2-H 8.25s[d]; 4′-CH_2 1.75q(6)	258(16,000)	262(16,400)	262(16,000)
18			260(17,600)	262(18,000)	262(18,000)
20	^2H_2O[a]	8-H 7.98 and 7.89; 2-H 7.91 and 7.92[d] 5′-CH_2 3.79(6)[b]; 4′-CH_2 1.82q(6)[b]			
21	^2H_2O[a]	8-H 8.28s; 2-H 8.01s; 6-H 7.33d(7.5); 5-H 5.64d(7.5); 1′-H 5.95d(5.2)	267(19,100)	262(19,800)	262(19,200)
22	^2H_2O[a]	8-H 8.31s; 2-H 8.01s; 6-H 7.25d(7.5); 5-H 5.64d(7.5); 1′-H 6.00d(5.2)	267(19,600)	262(20,000)	262(19,500)

[a] At 25°; [b] Two set of signals; [c] Broad singlet; [d] Assignment by exchange of 8-H, for 2 hr at 80° in ^2H_2O; [e] Shoulder

C-3′. Taking account of this, the positive B_{2u} Cotton effect of **10** may be connected with *anti*-like average conformation about the bond from C-1′ to the base in which the N_1–C_2 and $C_{1'}$–$H_{1'}$ (pro-*S*) bonds are coplanar. If this is valid, 1-{2′(*S*),3′-dihydroxypropyl}-cytosine will show negative Cotton effect with decreasing absolute magnitude, on the assumption that reversal of the configuration at C-2′ does not alter conformation significantly. Indeed, as seen from Fig. 2, experimental data are in good agreement with this expectation. It is also of interest to note that the presence of 2′,3′ - O - isopropylidene group causes approximately twofold decrease of the absolute

Table 2. CD spectra of 2'(*R*),3'(*S*),5'-trihydroxypentyl derivatives of adenine and cytosine

Compounds	pH 1			pH 7			pH 13		
	peak λ(Δε)	trough λ(Δε)	crossover λ	peak λ(Δε)	trough λ(Δε)	crossover λ	peak λ(Δε)	trough λ(Δε)	crossover λ
7	268(0.35)	—	—	—	258(−0.44)	—	—	258(−0.47)	—
8	280(6.9)	—	240	271(4.1)	—	—	—	—	—
9	—	244(−0.7)	236	—	255(−1.0)	—	—	256(−1.0)	—
10	280(2.1)	—	242	272(3.2)	—	—	272(3.2)	—	—
12	262(2.5)	247(−1.0)	256	272(7.5)	252(−7.4)	262	271(7.2)	252(−7.7)	262
			235			237			236
13	276(3.8)	—	—	268(5.1)	230(−1.1)	243	268(5.2)	—	240
14	270(3.25)	250(−1.9)	258	269(10.7)	252(−10.8)	261	271(9.8)	252(−10.4)	262
			237			236			236
15	280(4.4)	250(−0.8)	259	275(9.8)	241(−2.5)	258	274(9.2)	240(−3.2)	259
						230			228
20	272(0.45)	251(−1.6)	264	270(2.4)	251(−1.7)	260	270(2.0)	252(−1.3)	260
			231			228			239
21	—	280(−4.4)	235	278(0.9)	258(−3.6)	273	276(1.17)	257(−3.5)	270
						231			229
22	—	283(−2.1)	235	279(0.6)	258(−0.46)	275	278(0.9)	258(−4.8)	272
		255(−1.7)				231			228

magnitude of B_{2u} Cotton effect (see Fig. 2). Since in the case of the compounds investigated this decrease may be impossible to explain by conformational change only, it is reasonable to assume that this phenomenon might be due to an electronic repulsion involving the lone-pair electrons on the eclipsed 2'- and 3'-oxygen atoms.

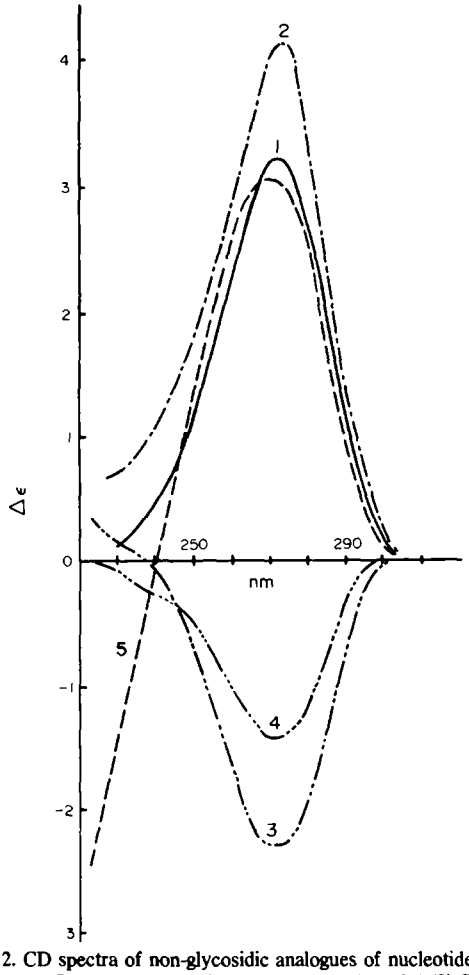


Fig. 2. CD spectra of non-glycosidic analogues of nucleotides in water at pH 7: 1-compound **10**; 2-compound **8**; 3-1-(2'(*S*),3'-dihydroxypropyl) cytosine; 4- its 2',3'-O-isopropylidene derivative in comparison with CD spectrum of Cyd (5).

The dinucleoside phosphate analogues **14** and **15** are found to give Cotton effects quite similar to those of the corresponding common dimers (Fig. 3). A comparison of temperature melting profiles (Fig. 4) indicates that the compounds under consideration have nearly identical thermodynamic parameters of stacking equilibrium. There is little doubt that the conformation of dimers **14** and **15** is *anti–anti* right-handed. On the other hand, dimer **22** containing 1-{2'(*S*),3'-dihydroxypropyl}cytosine as a 3'-linked residue, exhibits a significantly reduced B_{2u} Cotton effect (Fig. 3B). Since this dinucleoside phosphate analogue is conformationally similar to **15**, the low intensity of the Cotton effect is assumed to arise from reversed configuration at C-2' of the 3'-terminal unit. Thus, the experimental data agree with the hypothesis, published recently,[8] in which the "monomeric" dichroicity is regarded as one of the main contributions in CD spectra of oligonucleotides.

EXPERIMENTAL

UV spectra were recorded on a Specord UV-vis spectrophotometer. PMR spectra were determined on an 80 MHz Tesla BS 487C instrument. The chemical shifts are reported in δ values in ppm with t-BuOH (solutions in ^2H_2O) or TMS (in non-polar solvents) as internal standards. CD spectra were obtained on an Jouan-Yvon Dichrographe III. Paper electrophoresis on Whatman 3 MM paper was conducted with the following buffer: 0.01 M $(NH_4)_2CO_3$, pH 9. Silica gel Silufol UV_{254} sheets were used for TLC. The chromatograms were developed with solns of MeOH (1–30%) in $CHCl_3$. Both pyridine and DMFA were dried by heating with CaH_2 under reflux, and were then redistilled before use.

α(β)-Methyl 3 - O - *acetyl* - 5 - O - *tosyl* - 2 - *deoxy* - D - *ribofuranoside* **2**

To a suspension of 2 - deoxy - D - ribose **1** (15.9 g; 0.12 mole) in 396 ml abs MeOH, 4 ml of 10% soln of dry HCl in abs MeOH was added, and this mixture was stirred at room temp. for 1 h. After addition of 50 ml dry pyridine, the resulting soln was evaporated *in vacuo* to a viscous gum which was re-evaporated with dry pyridine (2 × 50 ml). The residue was dissolved in 100 ml dry pyridine and soln of p-toluenesulphonyl chloride (25.2 g; 0.132 mole) in 100 ml dry pyridine was added dropwise for 1 h with vigorous stirring and cooling in an ice-bath. The mixture was kept at

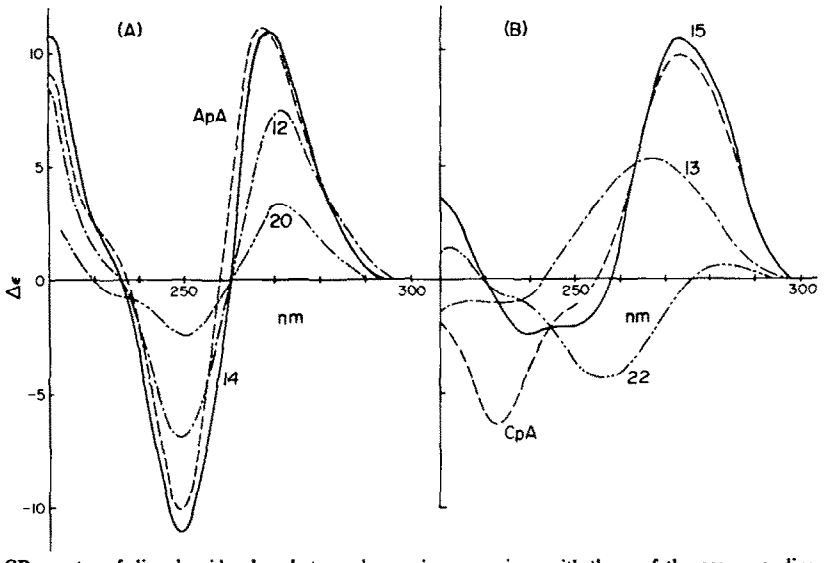


Fig. 3. CD spectra of dinucleoside phosphate analogues in comparison with those of the corresponding common dimers in water at pH 7. The arabic numbers refer to structures mentioned in text.

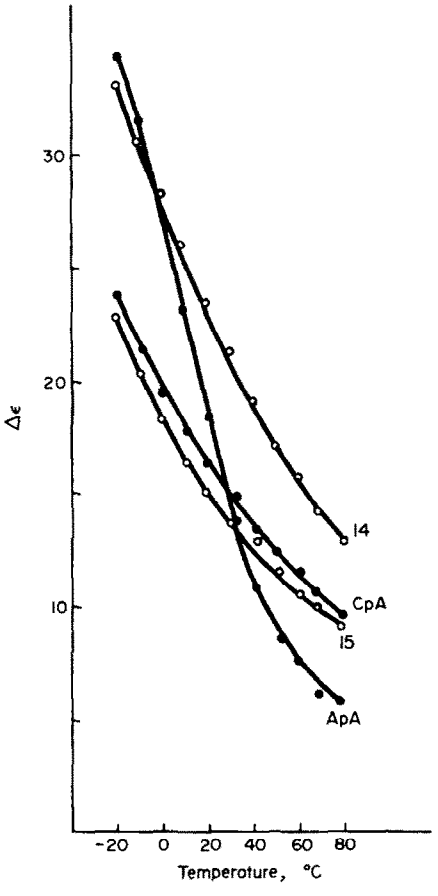


Fig. 4. Temperature melting profiles of dimers **14** and **15** in comparison with ApA and CpA.

0° for 16 h and then at room temp. for 24 h. After addition of 50 ml water, soln was stirred for 2 h at room temp.; pyridine was evaporated to dryness *in vacuo*, re-evaporated with dry pyridine (3 × 50 ml), and the residue was dissolved in 50 ml dry pyridine. To this soln, cooled in ice-bath, 25 ml acetic anhydride was added dropwise with stirring, and the mixture was allowed to stand at room temp. for 16 h. Then, 20 ml water was added, the mixture was stirred at room temp. for 2 h and poured into 1 l. of cool water. The emulsion was extracted with $CHCl_3$, the combined extracts were washed with aq $NaHSO_4$, water, aq $NaHCO_3$, water, and dried. The solvent was removed *in vacuo*, and the residual oil was dried *in vacuo* at 50°. Yield of **2**, 34.2 g (83%).

Alkylation of nucleic bases

A. To a suspension of adenine (8.1 g; 60 mmole) in 240 ml dry DMFA, sodium hydride (1.65 g; 65 mmole) was added in several portions for 30 min, and the suspension was heated at 60° for another 30 min. To this mixture, a soln of (2) (17.1 g) in 60 ml dry DMFA was added. After heating at 60° for 16 h, solvent was removed *in vacuo* at 45° and the residue was triturated with 200 ml water; the mixture was brought to pH 7 and was extracted with $CHCl_3$. The combined extracts were dried, the solvent was removed *in vacuo*, and the residue was re-evaporated with abs EtOH (2 × 20 ml). The ppt was filtered off, washed with abs EtOH and then with ether. After recrystallisation from abs EtOH yield of **3** was 5.5 g (36%). Found: C, 50.77; H, 5.63; N, 22.6; $C_{13}H_{17}N_5O_4$ requires: C, 50.81; H, 5.58; N, 22.79%.

B. A suspension of N^4-benzoylcytosine (12.9 g; 60 mmole), 17.1 g of **2** and anhydrous K_2CO_3 (16.6 g; 120 mmole) in 300 ml dry DMFA was heated at 90° for 20 h. The ppt was removed by filtration with DMFA wash, and combined filtrates were evaporated to dryness *in vacuo* at 45°. The residue was worked up as described in method A. After removal of $CHCl_3$ *in vacuo*, and re-evaporation with abs EtOH, the oil was shaken with 250 ml abs ether and was kept in a refrigerator overnight. The ppt was filtered off, washed with ether and recrystallized from ethyl acetate. Yield of 2.7 g (19.1%). Found: C, 58.87; H, 5.35; N, 10.90. $C_{19}H_{21}N_3O_6$ requires: C, 58.91; H, 5.46; N, 10.85%.

α(β)-Methyl 2,5 - dideoxy - 5(N^6 - benzoyladenyl - 9) - D - ribofuranoside **5**

To a soln of **3** (921 mg; 3 mmole) in 7.5 ml dry pyridine, benzoyl chloride (0.8 ml; 7 mmole) was added, and the mixture was stirred at 20° for 16 h. After evaporation *in vacuo*, the residue was dissolved in $CHCl_3$. This soln was washed in turn with aq $NaHSO_4$, water, aq $NaHCO_3$, water, and dried. The dried extract was evaporated *in vacuo* and re-evaporated twice with EtOH. To the resultant glass, 15 ml EtOH and 15 ml 2 N NaOH was added and the mixture was shaken for 10 min. After addition of 15 ml 2 N HCl, the soln was extracted with $CHCl_3$, the organic layer was washed with aq $NaHCO_3$ and dried. The solvent was removed *in vacuo* and the residue was recrystallized from ethyl acetate. Yield of **5**, 0.7 g (63%). Found: C, 58.65; H, 5.22; N, 19.01. $C_{18}H_{19}N_5O_4$ requires: C, 58.53; H, 5.18; N, 18.96%.

α(β) - Methyl 2,5 - *dideoxy* - 5(N⁴ - *benzoylcytosyl* - 1) - D - *ribofuranoside* **6**

A suspension of **4** (774 mg; 2 mmole) in 10 ml EtOH and 10 ml 2 N NaOH was shaken for 10 min, 10 ml 2 N HCl was added, and the mixture then worked up as described above for adenine derivative. Yield of **6**, 420 mg, (61%). Found: C, 59.07; H, 5.47; N, 12.01. $C_{17}H_{19}N_3O_5$ requires: C, 59.12; H, 5.55; N, 12.17%.

α(β) - Methyl 2,5 - *dideoxy* - 5(*adenyl* - 9) - D - *ribofuranoside*

A suspension of **3** (921 mg; 3 mmole) in 30 ml of methanolic ammonia (half-saturated at 0°) was stirred magnetically at room temp. for 16 h. The resulting soln was evaporated *in vacuo*, and the residue was recrystallized from EtOH. Yield, 600 mg (75%). UV λ_{max} (ε): in 0.1 N HCl 258 (15,600); at pH 7 261 (15,800); in 0.1 N NaOH 261 nm (16,000). PMR δ ppm (in DMSO-d_6): 8-H at 8.25; 2-H at 8.15; α- and β-OCH_3 3.30 and 3.26 (all signals are singlets); 6-NH_2 6.11 (broadening singled).

Phosphorylation of nucleoside analogues

A suspension of 1 mmole of partially protected nucleoside analogue **5** or **6** in 2 ml of 1 M soln of β-cyanoethyl phosphate[9] was evaporated *in vacuo* at temp below 30° and re-evaporated with dry pyridine (3 × 10 ml). The residue was dissolved in 10 ml dry pyridine, and mesitylenesulphonyl chloride (665 mg; 3 mmole) was added. The reaction mixture was sealed to exclude moisture, and was stirred magnetically at 20°. After 3 h, water (10 ml) was added, and the mixture was stirred for a further 2 h. The soln was diluted to 250 ml with water and applied to a DEAE-cellulose anion-exchange column (HCO_3^- form, 500 ml), which was washed with water until the effluent was no longer UV absorbing. The column was then eluted with ammonium bicarbonate (pH 7.5, linear gradient from 0 to 0.05 M; total volume 9 l.). The desired protected nucleotide analogue was normally eluted in the buffer concentration range 0.025–0.035 M. Fractions containing the required β-cyanoethyl ester were combined and evaporated to dryness below 30° under reduced pressure. The residue was re-evaporated with water (5 × 20 ml) and then with EtOH (2 × 10 ml). The residual glass was dissolved in methanolic ammonia (half-saturated at 0°, 10 ml) and the soln was kept at room temp overnight. The solvent was removed *in vacuo* and the residue was dissolved in 5 ml 2 N LiOH. After 10 min, the soln was brought to pH 7.5 with 2 N HCl and was evaporated *in vacuo* below 30°. The crystalline product was filtered off, washed with abs EtOH and dried. Yields of di-lithium salts of **7** and **8**, 290 mg (82%) and 263 mg (79%), respectively.

Dinucleoside phosphate analogues

A. A suspension of pyridinium salt of N⁶,2',3' - O - tri - acetyladenosine 5'-phosphate prepared by acetylation of 1.5 mmole of adenosine 5'-phosphate[10] and 1 mmole of the nucleoside analogues **5** or **6** in 10 ml dry pyridine was evaporated *in vacuo* and re-evaporated several times with dry pyridine. To the residue, dry pyridine (10 ml) and mesitylenesulphonyl chloride (983 mg; 4.5 mmole) was added, and the mixture was stirred at room temp. for 3 h, 10 ml water was then added and the soln was stirred for a further 2 h. The resulting soln was evaporated *in vacuo* to dryness, re-evaporated with abs EtOH, and the residue was dissolved in methanolic ammonia (half-saturated at 0°, 25 ml). After 16 h at room temp., the solvent was removed *in vacuo*, the residue was dissolved in 200 ml water and applied on the DEAE-cellulose column (HCO_3^- form, 500 ml). The chromatography was carried out as mentioned above for nucleotide analogues. The fractions containing the dinucleoside phosphate analogue were combined, evaporated to dryness *in vacuo* below 30° and re-evaporated several times with water. The residue was dissolved in 20 ml of water and lyophilized. The dinucleoside phosphate analogues **12** and **13** were prepared as colourless ammonium salts in 73 and 69% respectively. In this way, dinucleoside phosphate analogue **19** was obtained from 1 mmole of fully-acetylated **18** and 0.6 mmole of **5** in 67% yield.

B. A mixture of chiral 1 - {2'(*S*),3' - dihydroxypropyl} - N⁴ - benzoylcytosine[4] (2 mmole) and pyridinium salt of N⁶,2',3' - O - triacetyladenosine 5' - phosphate (3 mmole) was dried by re-evaporation with dry pyridine as described above. The residue was dissolved in 20 ml dry pyridine, and, after addition of DCC (4.12 g; 20 mmole), this mixture was stirred at room temp. for 6 days. To this, 20 ml water was added, the suspension was stirred for further 12 h. The ppt was removed by filtration, using an aqueous pyridine (8:2 v/v) wash, and the combined filtrates were extracted with cyclohexane (2 × 20 ml). The aqueous layer was evaporated *in vacuo* and re-evaporated with EtOH (2 × 20 ml). The residue was dissolved in 40 ml of methanolic ammonia (half-saturated at 0°) and the soln was allowed to stand at room temp. overnight. After the solvent had been removed *in vacuo* the residue was dissolved in 300 ml water and the soln was applied on a DEAE-cellulose column (HCO_3^- form, 500 ml). Chromatography was carried out as described above. The mixture of ammonium salts of **21** and **22** (overall yield, 82%) was dissolved in 200 ml water and applied to the Dowex 1 column (formate form, 400 ml). The column was washed with water and then eluted with $HCOONH_4$ buffer (pH 4.8; linear gradient from 0.04 to 0.12 M; total volume 9 l.). The fractions containing desired compound were evaporated to dryness *in vacuo*, re-evaporated several times with water and lyophilized. The isomeric dinucleoside phosphate analogues **21** and **22** were obtained as ammonium salts in 39% and 28% yields, respectively.

Opening of furanose ring

A soln of 1 mmole of compound containing the furanose ring in 10 ml 0.05 N HCl was allowed to stand at room temp. for 4 h. To the soln, diluted with 40 ml water, sodium borohydride (1.0 g) was added in several portions over 1 h. The resulting soln (pH *ca.* 8) was kept at room temp. for another 1 h, acidified to pH 5 with acetic acid and applied to a column of Dowex 50 in acid form (20 ml). The column was washed with water until the effluent was no longer UV-absorbing, and then eluted with 2.5% aq NH_3. The fractions containing the analogues were evaporated *in vacuo* to dryness, dissolved in 200 ml water and applied to the Dowex 1 column (formate form, 200 ml). The ion-exchange chromatography was performed as described above for **21** and **22**. The ammonium salts of **9**, **10**, **14**, **15** and **20** were prepared in this manner in 72, 63, 59, 47 and 43% yields, respectively.

The adenosine analogue **16** was obtained likewise by acidic hydrolysis of α(β)-methyl 2,5 - dideoxy - 5(adenyl - 9) - D - ribofuranoside followed by reduction with sodium borohydride. However, in this case, after aqueous ammonia elution the fractions were evaporated to dryness *in vacuo* and the residue was recristallized from water. Yield of **16**, 71%.

9 - (2'(R),3'(S),5' - *trihydroxypentyl)adenine* 5' - *phosphate* **18**

A soln of **16** (400 mg; 1.58 mmole) in 30 ml dry acetone and 5 ml CF_3COOH was kept at room temp. for 3 days and then poured into 20 ml 25% aq NH_3. The resulting soln was evaporated *in vacuo* to a small volume and extracted with $CHCl_3$. The combined $CHCl_3$ extracts were dried, the solvent was evaporated *in vacuo* to dryness and re-evaporated with dry pyridine (2 × 10 ml). The residual oil was dissolved in 4 ml dry pyridine and treated with benzyl chloride (1 ml). After stirring at room temp. for 16 h, to the reaction mixture 1 ml water was added and the soln was stirred for 2 h, evaporated *in vacuo* to dryness, and the residue was dissolved in $CHCl_3$. This soln was washed in turn with aq $NaHSO_4$, water, aq $NaHCO_3$, water and dried. The solvent was removed, the resulting oil was triturated with EtOH, the solvent was removed again and the residue was dissolved with vigorous shake in a mixture of 7 ml EtOH and 7 ml 2 N NaOH. After 20 min the soln was treated with 7 ml 2 N HCl and extracted with $CHCl_3$. The combined extracts were washed with aq $NaHCO_3$, water and dried. The solvent was removed, to the residue 4 ml of 1 M soln of β-cyanoethyl phosphate was added, and the reaction mixture was worked up as described above for **7** and **8**. The di-lithium salt of **17** thus obtained was dissolved in 10 ml 50% aq CH_3COOH, and this soln was heated under reflux for 30 min. After cooling, the soln was evaporated and re-evaporated with water (4 × 10 ml) to remove the excess of acetic acid. The residue was dissolved in 100 ml water and applied a Dowex 1 column (formate form, 20 ml). The column was washed with water and then eluted with 0.2 M

HCOOH. The fractions containing **18** was evaporated *in vacuo* below 30° to a small volume, diluted with EtOH and kept in a refrigerator overnight. Filtration of the crystalline product with abs EtOH wash and drying gave **18** as free acid, in 38% overall yield.

1 - (2'(R),3'(S),5' - *trihydroxypentyl)cytosine* 2',3' - *cyclophosphate* **11**

To a suspension of ammonium salt of **10** (100 mg) in 5 ml abs MeOH, 400 mg of DCC was added, and the reaction mixture was stirred at room temp. for 16 h. After the solvent had been removed, the residue was triturated with 5 ml water and the suspension was filtered using the water wash. The filtrate was extracted twice with ether, and the aqueous layer was filtered again. The new filtrate was concentrated to about half volume and lyophilized. Yield of **11**, 86 mg (92%).

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