SYNTHESIS OF MODIFIED TERMINATOR (UGA) AND INITIATOR (AUG) CODONS CONTAINING SOME HYDROXYALKYL ANALOGUES OF NUCLEOSIDES*

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Syntheses of uridylyl-(3′ → 5′)-guanylyl-(3′ → 4′)-9-(4′-hydroxypropyl)adenine (*VII*) and 9-(3′-hydroxypropyl)adenine-3′-phosphorylyl-(3′ → 5′)-uridylyl-(3′ → 5′)-guanosine (*XII*) were reported. In the synthesis of *VII*, N^6-benzoyl-9-(4′-hydroxybutyl)adenine (*II*) was the nucleoside component and 5′-O-dimethoxytrityl-2′-O-tetrahydropyranyl-N^2-acetylguanosine 3′-phosphate (*I*) along with 5′-O-dimethoxytrityl-2′-O-tetrahydropyranyluridine 3′-phosphate (*IV*) were the nucleotide components. In the synthesis of *XII*, 2′,3′-di-O-acetyl-N^2-acetylguanosine (*VIII*) was the nucleoside component and compound *IV* along with N^6-benzoyl-9-(3′-hydroxypropyl)adenine 3′-phosphate (*X*) were the nucleotide components. The internucleotidic bond was synthesised by means of 2,3,5-triisopropylbenzenesulfonyl chloride and protected in each step by the 2-cyanoethyl group. The CD spectra of *VII*, *XII*, and guanylyl-(3′ → 4′)-9-(4′-hydroxybutyl)adenine (*V*) were discussed.

The nonglycosidic nucleotide analogues have been reported for the first time by Ikehara and coworkers[1]. Recently, the syntheses of dinucleoside phosphates containing hydroxyalkyl analogues of nucleosides have been described[2]. As indicated by CD spectra, the conformation of these substances in solution resembles that of the corresponding naturally occurring dinucleoside phosphates[3]. Because of the higher conformational flexibility in contrast to the natural substances, oligonucleotides containing the hydroxyalkyl analogues of nucleosides might produce more stable complexes with enzymes and thus inhibit interactions with naturally occurring substances[4]. Virtually, some inhibitory effects in various biological systems have been observed with substances of this type[1,5,6].

In the present paper, we wish to report the synthesis and CD spectra of modified terminator (UGA) and initiator (AUG) codons containing some hydroxyalkyl analogues of nucleosides. In the proteosynthesis, these codons are of the key importance and protein factors are required for their function. The hydroxyalkyl analogues of these codons could affect the proteosynthesis by inhibition of these factors.

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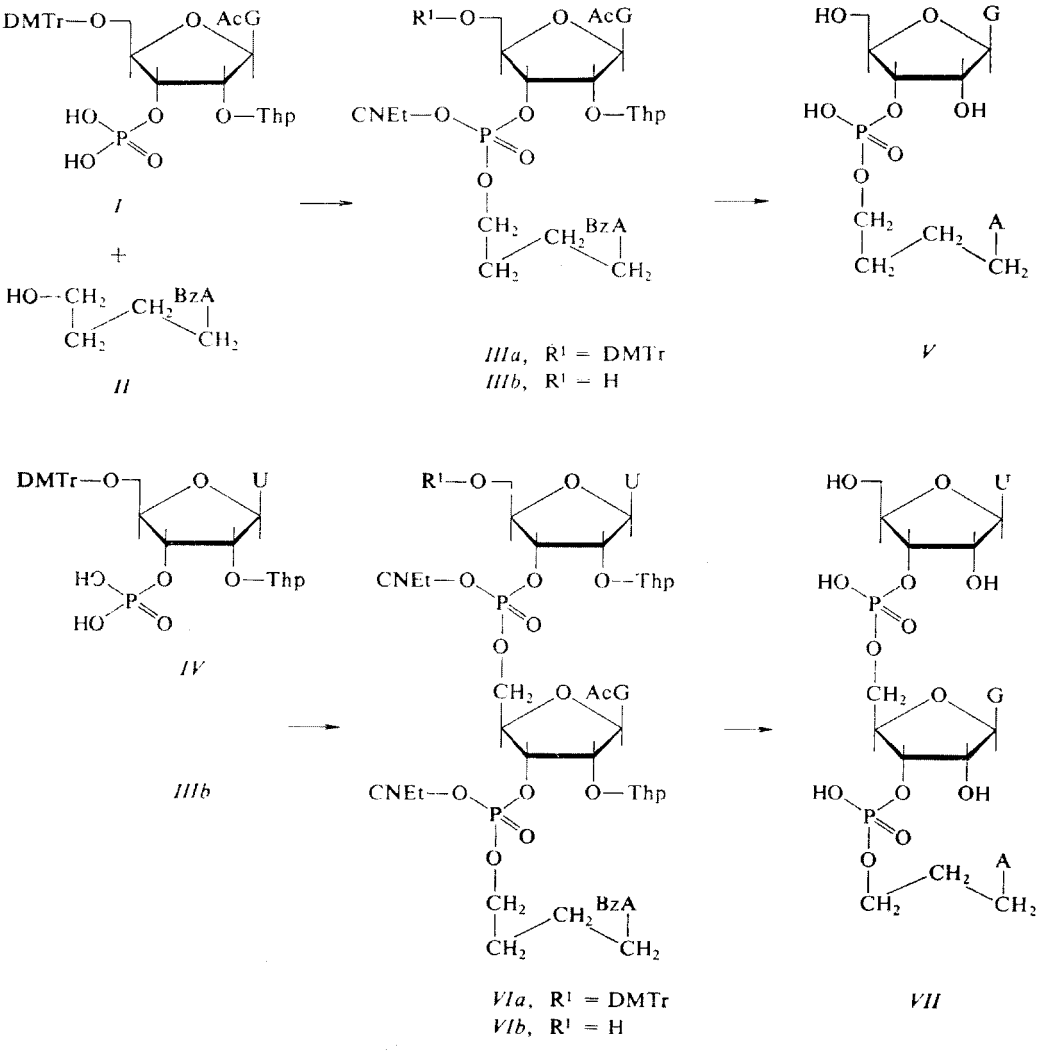
In the analogue *VII* of one of the terminator codons (UGA), the adenosine 5′-phosphate residue is replaced by the 9-(4′-hydroxybutyl)adenine 4′-phosphate residue. The analogue *XII* of the AUG initiator codon contains the 9-(3′-hydroxypropyl)adenine 3′-phosphate residue instead of the adenosine 3′-phosphate residue. The two



SCHEME 1

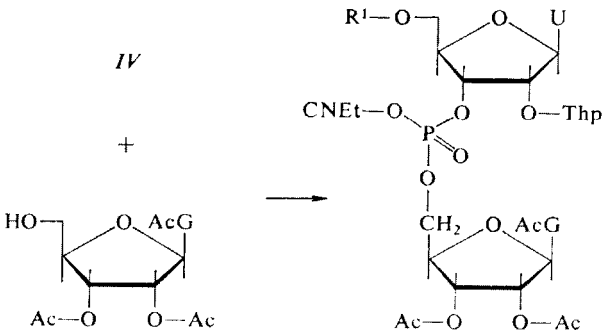
In formulae *I*—*XII* DMTr = dimethoxytrityl, Ac = acetyl, Bz = benzoyl, Thp = tetrahydropyranyl, CNEt = 2-cyanoethyl, G = guanyl, AcG = N^2-acetylguanyl, U = uracilyl, A = adenyl, BzA = N^6-benzoyladenyl.

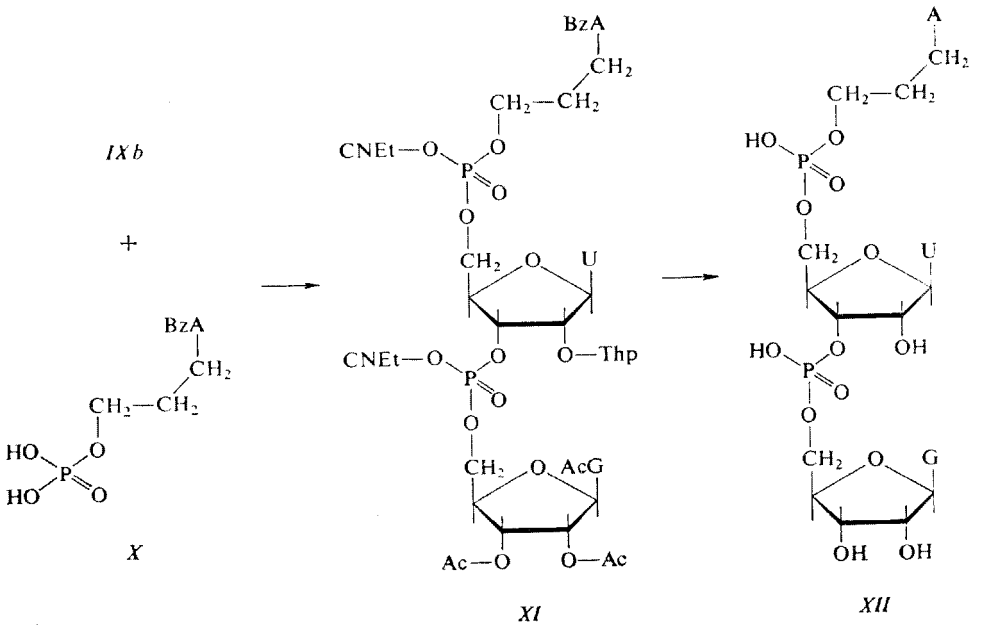
analogues have been prepared by a combined synthesis starting from protected nucleoside derivatives bearing a free $C_{(5')}$-hydroxylic function such as N^6-benzoyl-9-(4'-hydroxybutyl)adenine[7] (*II*) and 2',3'-di-O-acetyl-N^2-acetylguanosine (*VIII*). The original[8] preparation of compound *VIII* has been modified as follows. By reaction with dimethylformamide dimethylacetal, guanosine was converted into N^2-dimethylaminomethyleneguanosine[9] which afforded 5'-O-dimethoxytrityl-N^2-dimethylaminomethyleneguanosine on treatment with dimethoxytrityl chloride. The dimethoxytrityl derivative was isolated by extraction with chloroform containing about 50% of pyridine (the derivative is insoluble in chloroform containg lesser amounts of pyridine). The dimethylaminomethylene group was removed by the action of ammonia and the resulting 5'-O-dimethoxytritylguanosine was acetylated with acetic anhydride in the presence of tetraethylammonium hydroxide. On removal of the dimethoxytrityl group with 90% aqueous acetic acid at 20°C, a mixture of the required product *VIII* with a considerable amount of 2',3'-di-O-acetylguanosine was obtained. Compound *VIII* was isolated from this mixture on the basis of a higher solubility in the chloroform–ether solvent mixture. The conditions according to ref.[8] do not thus appear as suitable for the complete acetylation of the guanosine group. The attempted acetylation of 5'-O-dimethoxytritylguanosine with acetyl chloride in pyridine yielded a mixture of compound *VIII* with a chromatographically faster higher-acetylated by-product[10].

5'-O-Dimethoxytrityl-2'-O-tetrahydropyranyl-N^2-acetylguanosine 3'-phosphate (*I*), 5'-O-dimethoxytrityl-2'-O-tetrahydropyranyluridine 3'-phosphate[11] (*IV*), and N^6-benzoyl-9-(3'-hydroxypropyl)adenine 3'-phosphate (*X*) were used as the nucleotidic components in syntheses of the codon analogues. In the preparation of compound *I*, guanosine 2',3'-cyclic phosphate[12] as the starting compound was treated with acetic anhydride in pyridine to afford 5'-O-acetylguanosine 2',3'-cyclic phosphate[13] which was then converted into 5'-O-acetylguanosine 3'-phosphate[14] by the action of a mixture of ribonucleases T 1 and T 2. Depending on the reaction time, a variable amount of 5'-O-acetyl-N^2-acetylguanosine 2',3'-cyclic phosphate is also formed in the acetylation step; this by-product is not cleaved by the above mixture of enzymes[15]. The required 3'-phosphate is separated from this by-product by precipitation of the neutralised incubation mixture with the theoretical amount of calcium chloride in ethanol. The thus-purified 5'-O-acetylguanosine 3'-phosphate is converted to 5'-O-acetyl-2'-O-tetrahydropyranylguanosine 3'-phosphate by reaction with dihydropyran. As the primary product of this reaction, there is formed a bis(tetrahydropyranyl) derivative[13] from which one tetrahydropyranyl group is spontaneously split off after drying of the calcium salt (precipitated with ether) and washing with ethanol[14]. The pyridinium salt of the product is then acetylated in the presence of tetraethylammonium hydroxide to afford 5'-O-acetyl-2'-O-tetrahydropyranyl-N^2-acetylguanosine 3'-phosphate which is converted to 2'-O-tetrahydropyranyl-N^2-acetylguanosine 3'-phosphate by the action of 1M-NaOH at 0°C (analogously to ref.[16]). This intermediate is treated with dimethoxytrityl chloride to afford compound *I* which is iso-

lated in the form of the triethylammonium salt by extraction with pyridine-containing chloroform.

The modified nucleotide *X* was prepared from N^6-benzoyl-9-(3′-hydroxypropyl)-adenine[7] which was treated with dianilidophosphochloridate[17]. The resulting N^6--benzoyl-9-(3′-hydroxypropyl)adenine 3′-phosphodianilidate was converted on treatment with isopentyl nitrite to the phosphate *X* which was isolated in the form of the barium salt.





SCHEME 2

The synthesis of internucleotidic bonds was performed by the standard technique[18], *i.e.*, by reaction of 2 equivalents of the protected 3'-phosphate with 1 equivalent of the hydroxylic component in the presence of 3 equivalents of 2,3,5-triisopropylbenzenesulfonyl chloride. After the reaction period of one to two days, the reaction mixture was treated with additional 5 equivalents of the sulfonyl chloride and with 2-cyanoethanol (16 equivalents). The whole mixture was kept at room temperature for 20 h and then subjected to preparative thin-layer chromatography in a pyridine-containing solvent system. The dimethoxytrityl-group-positive broad bands (R_F 0·5–1·0) were eluted and the products (*IIIa*, *IXa*) obtained as chromatographically homogeneous solids by precipitation of the chloroform solutions with ether. The dimethoxytrityl group was removed by the action of 90% aqueous acetic acid, the thus-obtained substances (*IIIb*, *IXb*) purified by precipitation with ether, subjected to a further condensation with the 3'-phosphate, and the resulting trinucleoside diphosphate derivatives *VIa* and *XI* isolated analogously to the preceding dinucleoside phosphate derivatives. Compound *XI* lacks the dimethoxytrityl group which is used as marker in thin-layer chromatography in pyridine-containing solvent systems to locate the corresponding bands; nevertheless, compound *XI* was successfully isolated from bands of the same distance from the start line as in the case of compound *VIa*.

The dinucleoside phosphate *IVb* was deblocked (methanolic ammonia; 1M-HCl in 7M urea) and the free guanylyl-(3' → 4')-9-(4'-hydroxybutyl)adenine (*V*) isolated by chromatography on DEAE-cellulose. The derivative *VIb* was deblocked similarly to afford compound *VII*. Compound *XII* was obtained by the final deblocking with 20% aqueous acetic acid at 50°C and isolated by preparative paper chromatography. Since the triester products of the above syntheses were not entirely pure (as shown by deblocking of samples) despite the homogeneity on thin-layer chromatography,

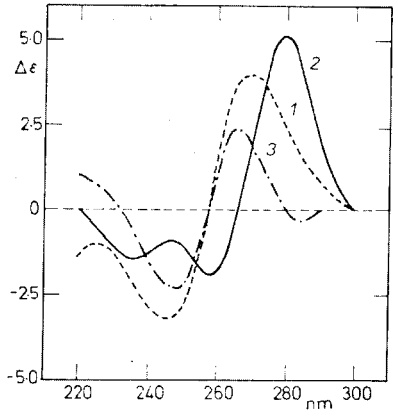


FIG. 1

CD Spectra

1 Uridylyl-(3' → 5')-guanylyl-(3' → 4')-9-(4'-hydroxybutyl)adenine; *2* 9-(3'-hydroxypropyl)adenine-3'-phosphorylyl-(3' → 5')-uridylyl-(3' → 5')-guanosine; *3* guanylyl-(3' → 4')-9-(4'-hydroxybutyl)adenine.

the yields of unprotected compounds *V*, *VII*, and *XII* refer to the starting nucleoside derivatives *II* and *VIII*. The characterisation of products was performed by enzymatic degradations.

The CD spectrum of the trinucleoside diphosphate *VII* and *XII* may be formally compared with that of uridylyl-(3′ → 5′)-guanosine[19]. The analogue *VII* exhibits a nonchiral chromophore Y at position $C_{(3')}$ of UG (UGY) while in the analogue *XII*, the nonchiral chromophore Y is at position $C_{(5')}$ of UG (YUG). The CD spectrum of compound *XII* (YUG) is analogous to that of UG while the CD spectrum of compound *VII* (UGY) strongly differs from that of UG. The difference in CD spectra of compounds *VII* and *XII* might be thus explained by the different location of the nonchiral chromophore on the basic structure of UG (location at position $C_{(3')}$ changes the spectrum). On the other hand, the CD spectrum of the analogue of the dinucleoside phosphate *V* bearing a nonchiral residue at position $C_{(3')}$ strongly resembles the CD spectrum of 9-(3′-hydroxypropyl)adenine-3′-phosphorylyl-(3′ → 5′)--guanosine[3] with the nonchiral chromophore at position $C_{(5')}$.

EXPERIMENTAL

Thin-layer chromatography was performed on ready-for-use Silufol UV_{254} silica gel foils (Kavalier Glassworks, Votice, Czechoslovakia) in the solvent systems S_1, chloroform–methanol (9 : 1); S_2, chloroform–methanol (8 : 2); and S_3, 2-propanol–conc. aqueous ammonia–water (7 : 1 : 2). Preparative thin-layer chromatography was carried out on layers (16 × 32 × 0·6 cm) of loose silica gel containing a fluorescent indicator (produced by Service Laboratories of this Institute, Prague - Suchdol) in the solvent system S_4, chloroform–pyridine–methanol (8 : 1 : 1). The solvent system S_e, chloroform–methanol (1 : 1) was used as eluant. The UV spectra were taken on a Beckman DU apparatus. The CD spectra were measured on a Roussel-Jouan Dichrographe 2 apparatus. Solutions and reaction mixtures were taken down on a rotatory evaporator equipped with a Dry-Ice condenser at 20°C/1 Torr unless stated otherwise.

Enzymatic degradations. a) Pancreatic ribonuclease. To a solution of the appropriate substance (1 mg) in 0·05M Tris-HCl pH 7 (50 µl) there is added a solution of the enzyme (5 mg per 1 ml; 5 µl) and the mixture is incubated at 37°C for 5 h. *b*) T1 Ribonuclease. To a solution of the corresponding substance (1 mg) in 0·05M Tris-HCl pH 7·5 (50 µl) there is added a solution of the enzyme (10000 units per 1 ml; 5 µl) and the whole mixture is incubated at 37°C for 5 h.

2′,3′-Di-O-acetyl-N^2-acetylguanosine (*VIII*)

A suspension of guanosine (6·4 g; dried at 50°C/0·1 Torr for 3 h) in a mixture of dimethylformamide (50 ml) and dimethylformamide dimethylacetal (10 ml) is stirred at room temperature for 20 h. The solution is evaporated, the residue dissolved in pyridine (50 ml), the resulting solution treated with dimethoxytrityl chloride (7·7 g), and the whole mixture shaken until the solid dissolves. After 20 h, methanol is added and after additional 30 min the solution is poured into a mixture of pyridine (100 ml), chloroform (100 ml), and water (30 ml). The whole mixture is shaken (the precipitate, if any, is dissolved by the addition of pyridine), the chloroform layer separated, and evaporated at 35°C/15 Torr. The residual pyridine solution is diluted with an equal volume of

conc. aqueous ammonia, heated at 50°C for 2 h, evaporated at 35°C/15 Torr to remove ammonia, the residual liquid treated with 25% tetraethylammonium hydroxide (47 g), and the whole evaporated to the consistence of a sirup. The sirup is coevaporated at 15 Torr with three 150 ml portions of pyridine, the final residue dissolved in pyridine (150 ml) the solution treated with acetic anhydride (150 ml), and the whole kept at room temperature for 4 days. The reaction mixture is evaporated, the residue dissolved in pyridine (50 ml), and the solution poured onto a mixture of crushed ice (1 kg) and chloroform (300 ml) with stirring. The chloroform layer is separated, evaporated, the residue coevaporated with two portions of toluene, and finally dissolved in 90% aqueous acetic acid. After 80 min at 20°C, the solution is concentrated to the volume of about 50 ml and the concentrate is poured into ether (700 ml). The precipitate is collected with suction, washed with ether, and air-dried. The thus-obtained mixture (5·3 g) of 2',3'-di-O-acetylguanosine and compound *VIII* (R_F values: 0·48 and 0·63 in S_2) is dissolved in chloroform (200 ml), the solution diluted with ether (500 ml) under stirring, and kept at room temperature for 20 h to deposit 2',3'-di-O-acetylguanosine which is collected with suction. The filtrate is evaporated, the residue dissolved in chloroform (20 ml), and the solution poured with stirring into ether (300 ml). The precipitate is collected with suction, washed with ether, and dried under diminished pressure. Yield, 1·7 g of compound *VIII*, R_F 0·63 in S_2. For $C_{16}H_{19}N_5O_8$ (409·32) calculated: 46·94% C, 4·68% H, 17·11% N; found: 46·82% C, 4·52% H, 17·01% N.

5'-O-Acetylguanosine 3'-Phosphate, Calcium Salt

An anhydrous solution of the dicyclohexylguanidinium salt of guanosine 2',3'-cyclic phosphate (prepared[12] from 10 g of guanosine 2'(3')-phosphate sodium salt) in a mixture of dimethylformamide (50 ml) and pyridine (200 ml) is treated with acetic anhydride (100 ml). The whole mixture is shaken at room temperature for 20 h, evaporated, and the residue dissolved in 50% aqueous pyridine (200 ml). After 1 h, the solution is evaporated and the residue is coevaporated with two 100 ml portions of 80% aqueous pyridine. The final residue is dissolved in a mixture of water (80 ml), dimethylformamide (40 ml), and 1M ammonium acetate (10 ml); if necessary, the solution is adjusted to pH 6·5. A dialysate of the mixture of ribonucleases T1 and T2 (prepared[14] from 10 g of Sanzyme R, Calbiochem, Los Angeles) is then added, the whole mixture incubated at 37°C for 24 h, adjusted to pH 7 by the addition of dilute aqueous ammonia, and an aliquot subjected to electrophoresis. The faster spot is eluted and the content of 5'-O-acetylguanosine 3'-phosphate is determined spectrophotometrically in the eluate. The neutralised solution is evaporated to the volume of about 200 ml at 35°C/15 Torr and the concentrate is poured into a solution of calcium chloride (an equivalent with respect to the 3'-phosphate present) in ethanol (1 500 ml). The precipitate is collected by centrifugation, washed twice with 80% aqueous ethanol, once with 99% ethanol, and finally with ether, and dried over phosphorus pentoxide. Yield, 10·3 g of the calcium salt of 5'-O-acetylguanosine 3'-phosphate.

5'-O-Dimethoxytrityl-2'-O-tetrahydropyranyl-N^2-acetylguanosine 3'-Phosphate (*I*), Triethylammonium Salt

A mixture of the calcium salt of 5'-O-acetyl-2'-O-tetrahydropyranylguanosine 3'-phosphate[14] (13·5 g), 50% aqueous pyridine (100 ml), and pyridinium Dowex 50 ion exchange resin (50 ml) is stirred until the salt passes into solution. Another portion (50 ml) of the resin in the pyridinium cycle is then added, the stirring continued for 10 min, the resin filtered off, and washed with 50% aqueous pyridine precooled to 0°C (300 ml). The filtrate and washings are combined and 25% aqueous tetraethylammonium hydroxide (47 ml) is added. The whole mixture is evaporated and

the residue is coevaporated with five 100 ml portions of pyridine. The final residue is dissolved in pyridine (100 ml), the solution treated with acetic anhydride (50 ml), the whole mixture kept at room temperature for 2 days, cooled down to 0°C, and treated slowly with methanol (60 ml). The mixture is kept at 20°C for 1 h, the methyl acetate evaporated at 30°C/15 Torr, the residual pyridine solution diluted with water (50 ml), kept for 1 h, and precipitated with aqueous ethanolic calcium chloride solution (10 g of calcium chloride, 20 ml of water, and 2500 ml of ethanol). After 20 h, the precipitate is collected by centrifugation and washed twice with ethanol and once with ether. A suspension of the ether-containing sediment, 50% aqueous pyridine (100 ml), and pyridinium Dowex 50 ion exchange resin (50 ml) is then stirred at 0°C until the salt passes into solution. A further portion of the resin (50 ml) is added, the whole stirred for 5 min, and filtered with suction. The material on the filter is washed with precooled (0°C) 50% aqueous pyridine (300 ml). The filtrate and washings are combined and evaporated. The residue is dissolved in precooled (0°C) 1M-NaOH (200 ml), the solution stirred at 0°C for 5 min, and adjusted to pH 7·5 by the addition of pyridinium Dowex 50 ion exchange resin. The resin is then filtered off and washed with precooled 50% aqueous pyridine (300 ml) at 0°C. The filtrate and washings are combined, evaporated, and the residue coevaporated with five 100 ml portions of pyridine. The final residue is dissolved in pyridine (100 ml) and the solution is treated with dimethoxytrityl chloride (10 g). The whole mixture is stirred for 1 h, kept at room temperature for 20 h, cooled down to 0°C, and diluted with a mixture of triethylamine (100 ml) and methanol (50 ml). Water (100 ml) is added after 15 min, the mixture washed with ether (300 ml), the aqueous layer diluted with pyridine (100 ml), and extracted first with chloroform (300 ml) and then with two portions of a mixture of chloroform (100 ml) and pyridine (50 ml). The combined chloroform–pyridine extracts are dried over anhydrous magnesium sulfate and evaporated at 30°C/15 Torr. The remaining pyridine solution is concentrated at about 1 Torr to the volume of about 50 ml and the concentrate added dropwise with stirring into a mixture of ether (900 ml) and triethylamine (5 ml). The precipitate is collected with suction, washed with ether, dissolved in pyridine (50 ml), and the solution precipitated with ether again. The product is collected with suction, washed with ether, and dried under diminished pressure. Yield, 9·5 g of the triethylammonium salt of compound *I*, R_F 0·43 in S_3. For $C_{38}H_{42}N_5O_{12}P.C_6H_{15}N$ (892·9) calculated: 3·47% P, 9·41% N; found: 3·12% P, 9·52% N.

N^6-Benzoyl-[9-(3′-hydroxypropyl)adenine] 3′-Phosphate (*X*), Calcium Salt

A solution of N^6-benzoyl-9-(3′-hydroxypropyl)adenine[7] (1·75 g; 5·9 mmol) and dianilidophosphochloridate (1·73 g; 6·5 mmol) in pyridine (20 ml) is kept at room temperature for 20 h, then stirred with 1M potassium acetate (3 ml) for 1 h, and diluted with water (50 ml). The mixture is extracted with two 100 ml portions of chloroform, the extracts dried over anhydrous magnesium sulfate, evaporated, and the residue coevaporated with two portions of toluene. The residual dianilidate of the phosphate *X* (R_F 0·50 in S_1) is dissolved in a mixture (1 : 1) of pyridine–acetic acid (120 ml), the solution is treated with isopentyl nitrite (16 ml), the whole kept at room temperature for 5 h, and evaporated. The residue is coevaporated with five portions of pyridine and finally dissolved in water (20 ml) containing barium acetate (2·16 g). The solution is washed with chloroform (20 ml) and the aqueous solution diluted with ethanol (800 ml). The precipitate is collected with suction, washed successively with 80% aqueous ethanol, 99% ethanol, and ether, and dried over phosphorus pentoxide under diminished pressure. Yield, 2·5 g (77%) of the barium salt of compound *X*. R_F value: 0·49 in S_3. UV spectrum: λ_{max} 290 nm (E = 22000). For $C_{15}H_{14}$ . . $BaN_5O_5.2\,H_2O$ (548·6) calculated: 5·66% P; found: 5·59% P.

N^2-Acetyl-2′-tetrahydropyranylguanylyl-(3′ → 4′)-N^6-benzoyl-9-(4′-hydroxybutyl)adenine [P-(2-Cyanoethyl) Ester] (*IIIb*)

A mixture of the triethylammonium salt of the phosphate *I* (4 mmol) and N^6-benzoyl-9-(4′-hydroxybutyl)adenine[7] (*II*; 2 mmol) is coevaporated with two portions of pyridine and then dissolved in 30 ml of pyridine. The solution is shaken for several minutes with 2,3,5-triisopropylbenzenesulfonyl chloride (1·8 g) and evaporated to the consistence of a sirup. The sirup is kept at room temperature for 20 h, shaken for several minutes with 2-cyanoethanol (2·15 ml), 2,3,5-triisopropylbenzenesulfonyl chloride (3 g), and pyridine (30 ml) and the whole evaporated. The residual sirup is kept at room temperature for 20 h, diluted with chloroform (5 ml) and chromatographed on three layers of loose silicagel in S_4. The dimethoxytrityl-positive bands (R_F 0·5—1·0) are eluted with the solvent system S_e (500 ml) and the eluate is evaporated. The residue is coevaporated with two portions of toluene, dissolved in chloroform (10 ml), and the solution diluted with ether (90 ml). After 20 h at 0°C, the product is collected with suction, washed with ether, and dried under diminished pressure. Yield, 1·87 g of compound *IIIa*, R_F 0·50 in S_1. Compound *IIIa* (1·8 g) is then dissolved in 90% aqueous acetic acie (50 ml), the solution kept at room temperature for 1 h (after this period of time, the detritylation was quantitative as shown by thin-layer chromatography in S_1), and evaporated. The residue is coevaporated with two protions of 1-butanol, dissolved in chloroform (20 ml), and the solution precipitated with ether (80 ml). The precipitate is collected with suction, washed with ether, and dried under diminished pressure to afford 1·8 g of compound *IIIb*, R_F 0·18 (in S_1) and 0·50 (in S_2).

Guanylyl-(3′ → 4′)-9-(4′-hydroxybutyl)adenine (*V*)

A solution of compound *IIIb* (147 mg in 4M methanolic ammonia (10 ml) is kept at room temperature for 40 h, evaporated at 35°C/15 Torr, and the residue dissolved in 1M-HCl in 7M aqueous urea (5 ml). After 2 h, the mixture is diluted with triethylamine (0·7 ml) in water (500 ml) and the whole is applied to a column (600 ml) of DEAE-cellulose (HCO_3^-). The column is washed with water until the UV-absorption of the eluate disappears, and then eluted with a linear gradient of triethylammonium hydrogen carbonate (0·0—0·2M; total volume, 4 l). The UV-absorbing peak (eluted with about 0·1M buffer solution) is evaporated, the residue coevaporated repeatedly with ethanol, and the final residue treated with 2M lithium chloride (2 ml). The solution is evaporated and a mixture (100 ml) of acetone–ethanol (9 : 1) is added to the residue. The insoluble solid is collected by centrifugation, washed with acetone–ethanol (9 : 1) and then ether, and dried under diminished pressure to afford 40 mg (27%, referred to the starting nucleoside *II*) of the lithium salt of compound *V*. R_{Up} (paper) 1·4 in S_3. E_{Up} 0·32. Degradation of compound *V* with ribonuclease T1 affords quantitatively a mixture of guanosine 3′-phosphate and 9-(4′-hydroxybutyl)-adenine in the ratio of 0·99 : 1 (the incubation mixture was chromatographed in S_3 for 15 h).

5′-O-Dimethoxytrityl-2′-O-tetrahydropyranyluridylyl-(3′ → 5′)-2′-O-tetrahydropyranyl-N^2-acetylguanylyl-(3′ → 4′)-N^6-benzoyl-9-(4′-hydroxybutyl)adenine [Bis-P^1,P^2-(2-cyanoethyl) Ester] (*VIa*)

A mixture of the triester *IIIb* (480 mg) and the triethylammonium salt (860 mg) of 5′-O-dimethoxytrityl-2′-O-tetrahydropyranyl-uridine 3′-phosphate[11] (*IV*) is coevaporated with two portions of pyridine and the residue is finally dissolved in 20 ml of pyridine. The solution is shaken for several minutes with 2,3,5-triisopropylbenzenesulfonyl chloride (520 mg), evaporated, and the sirup kept at room temperature for 40 h. 2-Cyanoethanol (1·22 ml), a further portion of 2,3,5-triisopropylbenzenesulfonyl chloride (2·45 g), and pyridine (20 ml) are added, the whole mixture is shaken for

several minutes, and evaporated to the consistence of a sirup. The sirup is kept at room temperature for 20 h, diluted with chloroform (3 ml), and chromatographed on one layer of loose silica gel in the solvent system S_4. The dimethoxytrityl-positive band (R_F 0·5—1·0) is eluted with S_e (200 ml), the eluate evaporated at 15 Torr, and the residue coevaporated with two portions of toluene. The final residue is dissolved in chloroform (10 ml) and the solution is precipitated with ether (90 ml). The precipitate is collected with suction, washed with ether, and dried under diminished pressure to afford 575 mg of the ester *VIa*, R_F 0·52 in S_1.

2′-O-Tetrahydropyranyluridylyl-(3′→5′)-2′-O-tetrahydropyranyl-N^2-acetylguanylyl-(3′→4′)--N^6-benzoyl-9-(4′-hydroxybutyl)adenine [bis-P^1,P^2-(2-Cyanoethyl) Ester] (*VIb*)

A solution of compound *VIa* (570 mg) in 90% aqueous acetic acid (20 ml) is kept at 20°C for 1 h, evaporated, the residue coevaporated with two portions of 1-butanol, and the final residue dissolved in chloroform (20 ml). The solution is precipitated with ether (80 ml), the precipitate collected with suction, washed with ether, and dried under diminished pressure to afford 400 mg of the ester *VIb*; R_F values: 0·90 (in S_1) and 0·36 (in S_2).

Uridylyl-(3′→5′)-guanylyl-(3′→4′)-9-(4′-hydroxybutyl)adenine (*VIII*)

Methanolic ammonia (20 ml) is added to a solution of compound *VIb* (390 mg) in pyridine (20 ml), the whole mixture is kept at 20°C for 40 h, and evaporated at 15 Torr. The residue is coevaporated with two portions of toluene and then stirred in an open vessel with 1M-HCl in 7M aqueous urea (5 ml) and chloroform (5 ml) for 2 h. Water (500 ml) and 2M ammonium hydrogen carbonate (5 ml) are added and the solution is applied to a column (600 ml) of DEAE-cellulose (HCO_3^-). The column is washed with water (2 l) and then eluted with a linear gradient of triethylammonium hydrogen carbonate (0·0—0·2M; total volume 4 l). The UV-absorbing peak (eluted with about 0·18M buffer solution) is evaporated at 15 Torr and the residue is coevaporated repeatedly with ethanol. The final residue is treated with 2M lithium chloride (2 ml), the solution evaporated, and the residue triturated with a mixture (100 ml) of acetone–ethanol (9 : 1). The insoluble substance is collected by centrifugation, washed with a mixture of acetone–ethanol (9 : 1) and then with ether, and dried under diminished pressure to afford 45 mg of the lithium salt of compound *VII* (overall yield 6%, referred to the starting nucleoside *II*). R_{Up} (paper) 0·61 in S_4; E_{Up} 0·55. Pancreatic ribonuclease degradation of compound *VII* affords quantitatively a mixture of uridine 3′-phosphate and compound *V* in the ratio of 1·12 : 1 (separated in S_3, 40 h).

5′-O-Dimethoxytrityl-2′-O-tetrahydropyranyluridylyl-(3′→5′)-2′,3′-di-O-acetyl-N^2-acetylguanosine [P-(2-Cyanoethyl) Ester] (*IXa*) and 2′-O-Tetrahydropyranyluridylyl-(3′→5′)--2′,3′-di-O-acetyl-N^2-acetylguanosine [P-(2-Cyanoethyl) Ester] (*IXb*)

Compounds *IXa* and *IXb* were prepared analogously to compounds *IIIa* and *IIIb* starting from the triethylammonium salt of the phosphate *IV* (4 mmol) and 2′,3′-di-O-acetyl-N^2-acetylguanosine (*VIII*; 2 mmol). Yield, 550 mg of the ester *IXb*; R_F values: 0·14 (S_1) and 0·53 (S_2).

N^6-Benzoyl-[9-(3′-hydroxypropyl)adenine]-3′-phosphorylyl-(3′→5′)-2′-O-tetrahydropyranyluridylyl-(3′→5′)-2′,3′-di-O-acetyl-N^2-acetylguanosine [P^1,P^2-bis(2-Cyanoethyl) Ester] (*XI*)

Compound *XI* was prepared analogously to compound *VIa* starting from the pyridinium salt of N^6-benzoyl-[9-(3′-hydroxypropyl)adenine] 3′-phosphate (*X*; 0·6 mmol) and compound *IXb*

(260 mg). Because of the absence of the dimethoxytrityl marker in thin-layer chromatography, the band of the same mobility as of compound *VIa* was eluted. Yield, 156 mg of compound *XI*; R_F 0·68 (S_2).

9-(3′-Hydroxypropyl)adenine-3′-phosphorylyl-(3′ → 5′)-uridylyl-(3′ → 5′)-guanosine (*XII*)

A solution of compound *XI* (140 mg) in 4M methanolic ammonia (10 ml) is kept at room temperature for 40 h, evaporated, and the residue dissolved in 20% aqueous acetic acid (5 ml). The solution is heated at 50°C for 1 h, cooled down, and chromatographed for 3 days on 2 sheets of paper Whatman 3 MM in the solvent system S_3. The UV-absorbing bands (R_{Up} 0·80) are eluted with 1% aqueous ammonia and the eluate freeze-dried to afford 36 mg of the ammonium salt of compound *XII* (overall yield 10%, refererd to the starting nucleoside *VIII*). Pancreatic ribonuclease degradation of compound *XII* proceeds by 98% and affords a mixture of guanosine and 9-(3′-hydroxypropyl)adenine-3′-phosphorylyl-(3′ → 5′)-uridine 3′-phosphate in the ratio of 1·08 : 1 (the incubation mixture was separated in the solvent system S_3 for 40 h).

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