SYNTHESIS OF 2'-END LIPOPHILIZED DERIVATIVES OF 2'-5'-TRIADENYLATES*

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Synthesis of protected derivatives of 2—5 A_3 core starting from 2',3'-O-ethoxymethylene-N^6--benzoyladenosine (*I*) by traizolidate and/or modified triester method are described. Preparation of adenylyl-(2'-5')-adenylyl-(2'-5')-2',3'-O-(1-methoxyhexadecylidene)adenosine (*X*), adenylyl-(2'-5')-adenylyl-(2'-5')-2'(3')-O-palmitoyladenosine (*XIII*) and 5'-phosphoryladenyl-(2'-5')-adenylyl-(2'-5')-2',3'-O-(1-methoxyhexadecylidene)adenosine (*XVI*) are described.

Proposed role of 2—5 A system in the mechanism of interferon's antiviral action suggests its use in chemotherapy of virus diseases or cancer. Such applications of 2—5 A are limited by a relatively short half-life since it is rapidly degraded by a 2',5'--phosphodiesterase[1,2] and second, the ionic character of 2—5 A oligonucleotides prohibits the penetration into the intact cells[3-6]. It was demonstrated that the phosphodiesterase activity responsible for degradation of 2—5 A may require an intact terminal ribose with its *cis*-diol system as recognition site for the enzyme action. An introduction of a substituent on 2',3'-*cis*-diol system of the 2'-end of the molecule may retard the rate of oligonucleotide chain scission[7]. If this substituent would possess lipoid character it may enhance penetration through the cell membrane.

As an extension of our work[8-11] we should like to report the synthesis of derivatives of 2—5 A which was expected to be more resistant to 2',5'-diesterase and which was expected to penetrate the membrane of intact cells. To achieve our goal we decided to synthesize adenylyl-(2'-5')-adenylyl-(2'-5')-2',3'-O-(1-methoxyhexadecylidene) adenosine (*X*), which should serve as starting compound for the preparation of other lipoid derivatives.

The strategy we adopted for the synthesis of such 2—5 A_3 derivatives consisted of the following steps: *1*) preparation of properly protected derivative of adenosine, *2*) synthesis of fully protected 2—5 linked trimer, *3*) specific deprotection of 2',3'-

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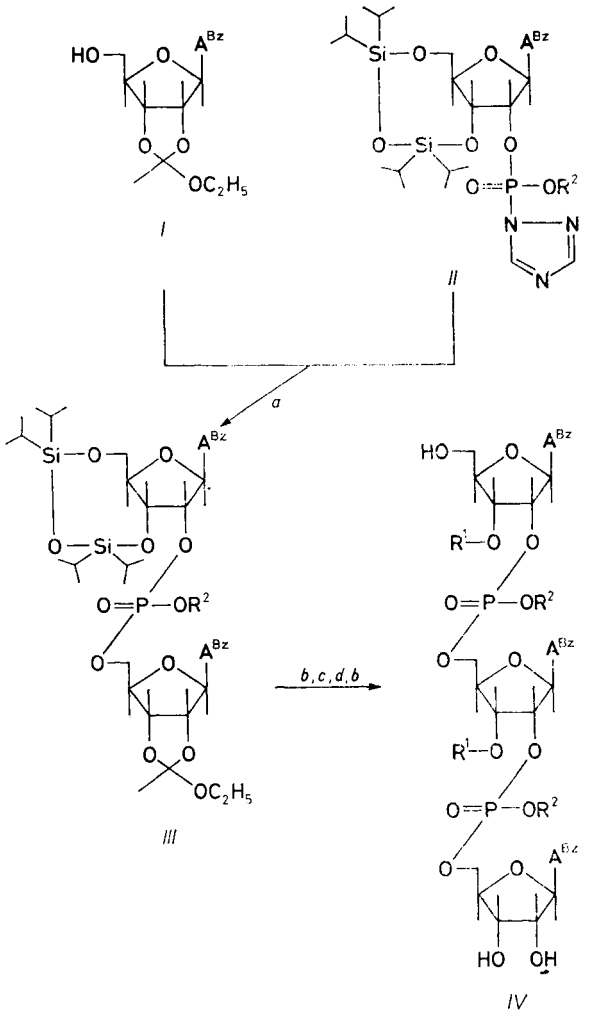
and 5′-hydroxyl groups, *4*) introduction of lipoid substituent to 2′,3′-*cis*-diol system, *5*) phosphorylation of the 5′-hydroxyl, *6*) final deprotection, and finally *7*) transformation of 2′,3′-orthoester to 2′(3′)-O-acyl derivative.

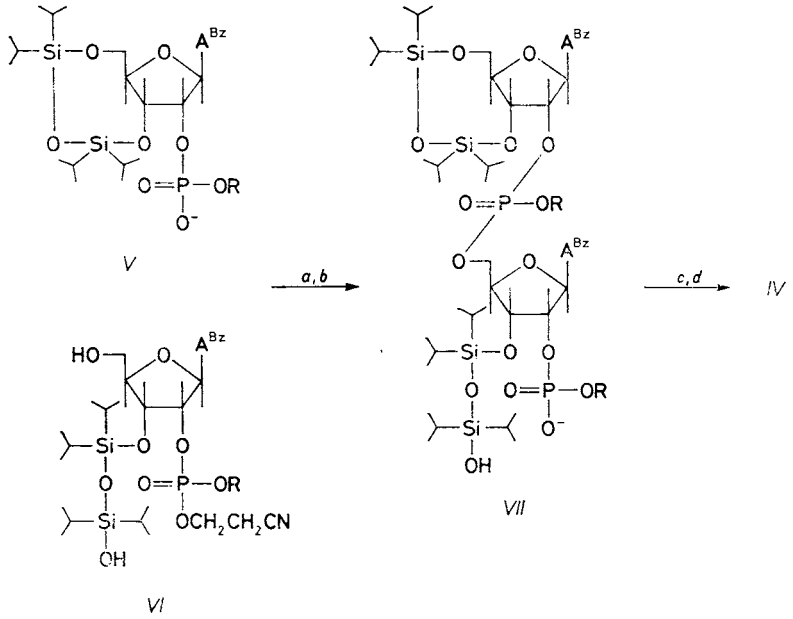
N^6-Benzoyl-2′,3′-O-ethoxymethyleneadenosine (*I*) was easily accessible for the 2′-end of the trimer. Two distinct approaches were used for the assemblage of partially protected trimer *IV*. In the first approach (Scheme 1) 3′,5′-O-(tetraisopropyl-



In formulae *II*–*IV*: R^1 = —Si(i-Pr)$_2$—O—Si(i-Pr)$_2$—OH; R^2 = 2-chlorophenyl.
a, 4-Dimethylaminopyridine *b*, HCl, water, dioxane *c*, triethyl orthoformate, HCl *d*, *II*, 4-dimethylaminopyridine

SCHEME 1

disiloxane-1,3-diyl)-N^6-benzoyladenosine-2′ (2-chlorophenyl) phosphoro-(1,2,4)-triazolidate[10,11] (*II*) was used for elongation. After the condensation *I* and *II* in the presence of 4-dimethylaminopyridine the fully protected dimer derivative *III* was treated with a mixture of aqueous hydrochloric acid and dioxane. By this treatment 5′-hydroxyl function was deprotected by scission of disiloxane ring[12] and simultaneously 2′,3′-*cis*-diol system at the 2′-end was deprotected by acid catalysed orthoester degradation. The 2′,3′-O-ethoxymethylene group was then reintroduced by the action of ethyl orthoformate[13] in the presence of an acid. The free 5′-hydroxyl function was reacted again with *II* and the resulting fully protected trimer partially deprotected to *IV* (29% overall yield).



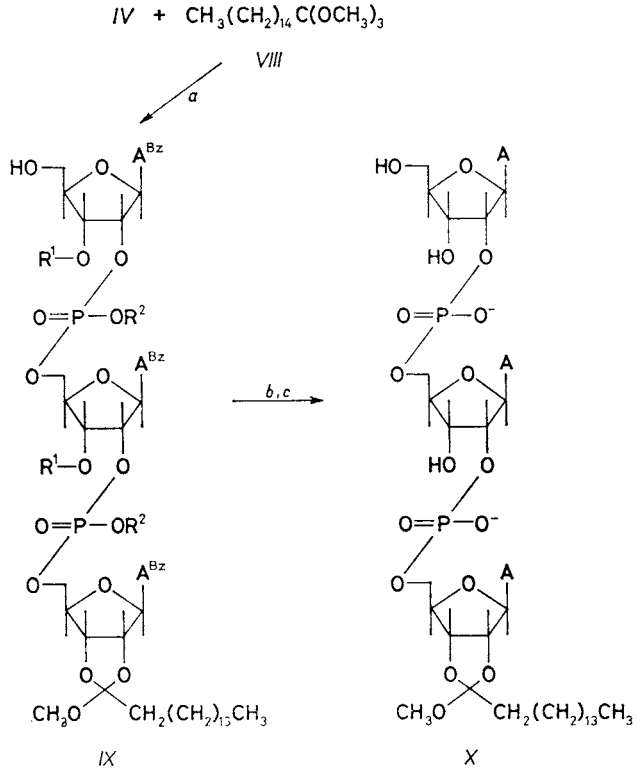
In formulae *V* – *VII*: R = 2-chlorophenyl.

a, 2,4,6-Triisopropylbenzenesulfonyl chloride, 1-methylimidazole *b*, triethylamine, acetonitrile *c*, *I*, 2,4,6-triisopropylbenzenesulfonyl chloride, 1-methylimidazole *d*, HCl, water, dioxane

SCHEME 2

For the second approach (Scheme 2) to the synthesis of *IV*, the triazolidate *II* was hydrolysed to 3′,5′-O-(1,1,3,3-tetraisopropyldisiloxane-diyl)-N^6-benzoyladenosine-2′ (2-chlorophenyl) phosphate (*V*) and then transformed to 3′-O-(1,1,3,3-tetraisopropyldisiloxane-yl)-N^6-benzoyladenosine-2′ (2-chlorophenyl) (2-cyanoethyl) phosphate

(*VI*). The phosphodiester *V* was condensed with 5'-hydroxyl function of the compound *VI* by the action of 2,4,6-triisopropylbenzenesulfonyl chloride and 1-methylimidazole[14] affording a fully protected dimer with masked 2'-phosphoryl group. After removal of the 2-cyanoethyl group the resulting 2'-phosphodiester *VII* was condensed with compound *I* affording fully protected trimer which was then transformed to *IV*. This second approach gave somewhat better yield than the first one (34%). Attempted use of 2',3'-O-(1-methoxyhexadecylidene)adenosine (*XII*) instead of *I* in the step *c* (Scheme 2) was not successful (data not shown). The compounds *IV* from both approaches were deprotected according to the described procedure[8,9] and afforded pure adenylyl-(2'-5')-adenylyl-(2'-5')-adenosine, compared with an authentic sample.

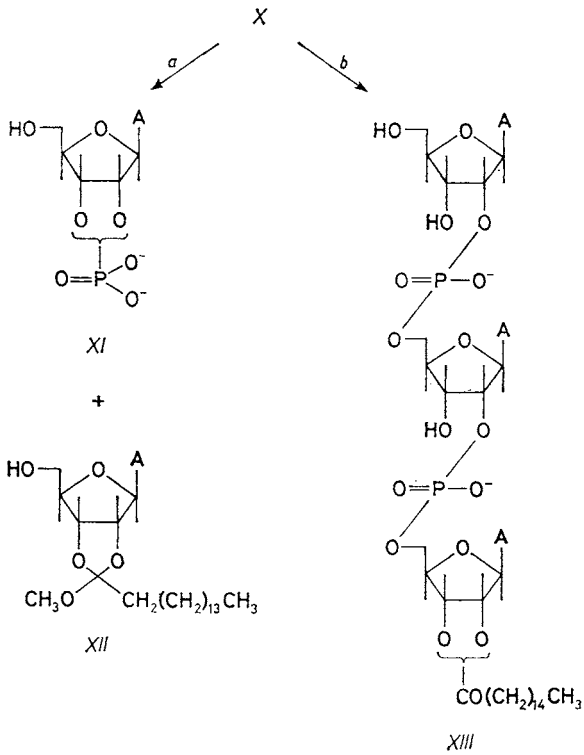


In formulae *IX*, *X*: $R^1 = -Si(i\text{-}Pr)_2-O-Si(i\text{-}Pr)_2-OH$; R^2 = 2-chlorophenyl.

a, Methanesulfonic acid or Dowex-50 (H^+) *b*, ammonia, water, dioxane
c, tetrabutylammonium fluoride, tetrahydrofuran

SCHEME 3

The lipoid moiety was introduced to *IV* by acid-catalysed reaction with orthoester[13], namely trimethyl orthopalmitate (*VIII*) in the presence of methanesulfonic acid or Dowex-50 (H^+) (Scheme 3). The resulting partially protected trimer (*IX*) was deprotected by successive treatment of aqueous ammonia and tetrabutylammonium fluoride to afford adenylyl-(2′-5′)-adenylyl-(2′-5′)-2′,3′-O-(1-methoxyhexadecylidene) adenosine (*X*). The compound was not degraded by snake venom diesterase. Sodium hydroxide degradation (Scheme 4) afforded adenosine-2′(3′)-phosphate (*XI*) and 2′,3′-O-(1-methoxyhexadecylidene)adenosine (*XII*) (ratio 2 : 1). The 2′,3′-orthoester could be quantitatively transformed to 2′(3′)-O-palmitoyl derivative by short action of trifluoroacetic acid. By this method adenylyl-(2′-5′)-adenylyl-(2′-5′)-2′(3′)-O-palmitoyladenosine (*XIII*) was prepared.

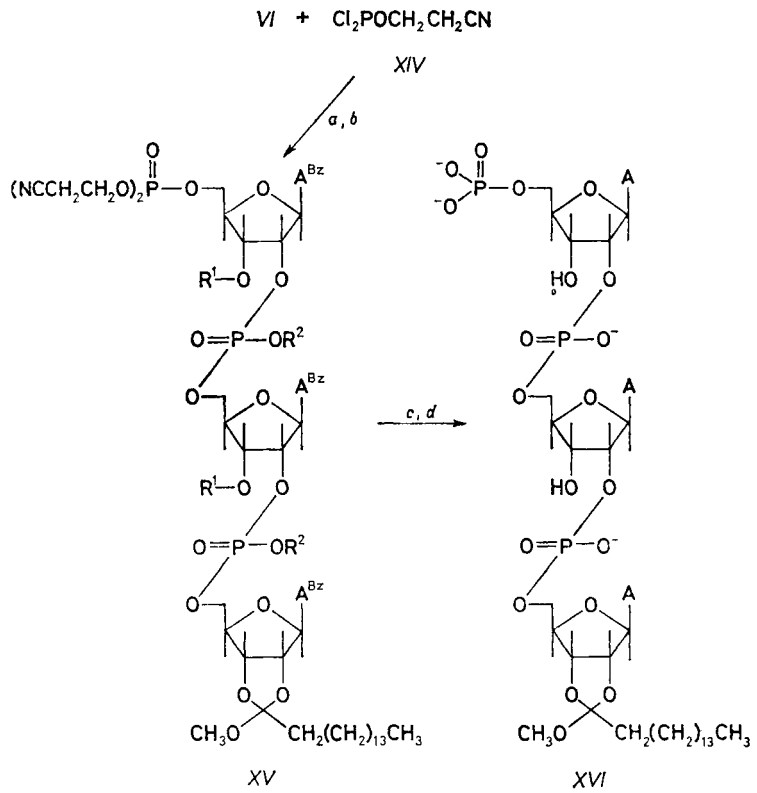


a, 0.25 M aqueous sodium hydroxide *b*, trifluoroacetic acid

SCHEME 4

For the introduction of phosphoryl group into the 5′-position of *IX* (2-cyanoethoxy)dichlorophosphine *XIV* (ref.[15]) was used. The primary product of the phos-

phitylation was transformed to phosphite triester by the action of 3-hydroxypropionitrile, oxidized to phosphate triester and the product *XV* was isolated by preparative TLC. Deprotection afforded 5'-phosphoryladenylyl-(2'-3')-adenylyl-(2'-5')-2',3'-O--(1-methoxyhexydecylidene) adenosine *XVI* (Scheme 5).



In formula *XV*: $R^1 = -Si(i\text{-}Pr)_2-O-Si(i\text{-}Pr)_2-OH$; R^2 = 2-chlorophenyl.
a, 2,4,6-Collidine, 3-hydroxypropionitrile *b*, iodine, tetrahydrofuran, 2,6-lutidine, water *c*, ammonia, water, dioxane *d*, tetrabutylammonium fluoride, tetrahydrofuran

SCHEME 5

The results described in this paper show the approach to 2–5 A derivatives containing a lipophilic group at the 2'-end of the molecule. The availability of compounds of this type opens the way for biological investigation.

EXPERIMENTAL

Methods. Thin-layer chromatography (TLC) was performed on ready-for-use Silufol UV_{254} silica gel foils (Kavalier-Glassworks, Votice, Czechoslovakia) in the solvent systems: S1, chloroform–methanol (9 : 1); S2, chloroform–methanol (95 : 5); S3, 2-propanol–aqueous ammonia–water (7 : 2 : 1); S4, 2-propanol–aqueous ammonia–water (55 : 35 : 10). Preparative TLC was performed on PSC Fertigplatten 5 733 (Merck, F.R.G.).

Column chromatography was performed on a macroporous silica gel (30–60 μm; Service Laboratory of this Institute). Solutions were taken down on a rotatory evaporator at 20–40°C, 130 Pa, or on a rotatory evaporator equipped with dry ice condenser at 20–30°C, 10 Pa.

Enzymic cleavage with alkaline phosphatase (bacterial): The substance (1 mg) dissolved in aqueous Tris-HCl (*c* 50 μmol l^{-1}, pH 8) was incubated with 5 μl of enzyme preparation (Sigma, U.S.A.) at 37°C for 2 h.

Materials. Pyridine was distilled from *p*-toluenesulfonyl chloride (10 g pro 1 l), then from barium oxide and stored over molecular sieves 4 A. N,N-Dimethylformamide was distilled from calcium hydride. Tetrahydrofuran was distilled from lithium aluminum hydride and stabilized with 0·025% 2,6-di-tertbutyl-4-methylphenol. Dichloromethan was passed through a column of basic aluminum oxide. Methanol was dried by being refluxed with magnesium methoxide, distilled and stored over molecular sieves 4 A.

1,3-Dibromo-1,1,3,3-tetraisopropyldisiloxane[12], (2-chlorophenyl)phosphorobis-(1,2,4-triazolidate)[8,15], N^6-benzoyladenosine[16] (modified), 3′,5′-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-N^6-benzoyladenosine[12], (2-cyanoethoxy)dichlorophosphine[17], palmitonitrile[18] were prepared as described.

2′,3′-O-Ethoxymethylene-N^6-benzoyladenosine (*I*)

A mixture of N,N-dimethylformamide (70 ml), triethyl orthoformate (11 ml; 72 mmol), N^6-benzoyladenosine (*II*, 7·42 g; 20 mmol) and 2M solution of hydrogen chloride in N,N-dimethylformamide (70 ml) was stirred 4 h. Pyridine (30 ml) was added, the mixture diluted with water (50 ml) and extracted with chloroform (100 ml). The chloroform solution was evaporated, the residue coevaporated with pyridine (100 ml) and with two 50 ml portions of toluene and dissolved in chloroform (20 ml). The chloroform solution was added into stirred ether (700 ml). The solid was collected, washed with ether (2 × 300 ml) and dried under diminished pressure. Yield 7·37 g (80%), R_F 0·36 (S1). For $C_{20}H_{21}N_5O_6$ (427·4) calculated: 56·20% C, 4·95% H, 16·39% N; found: 55·79% C, 4·92% H, 16·32% N.

3′,5′-O-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-N^6-benzoyladenosine-2′ (2-Chlorophenyl) Phosphoro-(1,2,4-triazolidate) (*II*)

3′,5′-O-(Tetraisopropyldisiloxane-1,3-diyl)-N^6-benzoyladenosine (6·13 g; 10 mmol) was evaporated with two 10 ml portions of pyridine. To the residue the solution of (2-chlorophenyl)phosphorobis-(1,2,4-triazolidae) (10 mmol) in tetrahydrofuran (8 ml) was added. After 18 h standing at room temperature (R_F 0·76 → 0·0 (S1)) the solution containing the product *II* was used for condensation.

3′,5′-O-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-N^6-benzoyladenosine-2′ (2-Chlorophenyl) Phosphate Triethylammonium Salt (*V*)

The solution of the compound *II* in tetrahydrofuran was diluted with 1M aqueous solution of triethylammonium hydrogen carbonate (100 ml) and the mixture extracted with chloroform

(200 ml). The organic layer was evaporated and the residue evaporated with two 100 ml portions of toluene. Yield 8·91 g (99%) of glassy foam, R_F 0·0 (S1). For $C_{41}H_{62}ClN_6O_9PSi_2$ (905·6) calculted: 54·38% C, 6·90% H, 9·28% N; found: 54·33% C, 7·10% H, 8·99% N.

3′-O-(1,1,3,3-Tetraisopropyldisiloxane-1-yl)-N^6-benzoyladenosine-2′ (2-Chlorophenyl) (2-Cyanoethyl) Phosphate (*VI*)

A mixture of compound *V* (2·7 g; 3 mmol) and 3-hydroxypropionitrile (1·2 ml; 15 mmol) was evaporated with three 10 ml portions of pyridine. Pyridine (30 ml) and 2,4,6-triisopropylbenzenesulfonyl chloride (2·7 g; 9 mmol) were added, the mixture stirred for 5 min and 1-methylimidazole (1·2 ml; 15 mmol) added. After 2 h standing the reaction flask was immersed into an ice-water bath. After 10 min acetic acid (1·2 ml) was added and the mixture partitioned between water (50 ml) and chloroform (50 ml). The organic layer was diluted with toluene (50 ml), evaporated and the residue evaporated with toluene (50 ml). The residue was chromatographed on a silica gel column (50 g) using ether–chloroform (1 : 1; 300 ml), chloroform (300 ml), chloroform–methanol (99 : 1; 300 ml), chloroform–methanol (98 : 2; 300 ml) and chloroform–methanol (97 : 3; 300 ml). Appropriate fractions containing diastereoisomers with R_F 0·30 and 0·42 (S2) were evaporated and the residue dissolved in dioxane (18 ml). Aqueous 2M hydrochloric acid (2 ml) was added and, after 2 h, the solution neutralized with pyridine (20 ml). The mixture was partitioned between water (50 ml) and chloroform (50 ml). The chloroform layer was dried (Na_2SO_4) and evaporated. Yield, 1·98 g (77%) of a mixture of diastereoisomers of *VI*, R_F 0·38 and 0·30 (S1).

Preparation of Protected Trinucleotide *IV* Using Triazolide Method

A solution of *I* (1·71 g; 4 mmol) and 4-dimethylaminopyridine (1·95 g; 16 mmol) in pyridine (20 ml) was evaporated and the residue evaporated with two 20 ml portions of pyridine and two 20 ml portions of toluene. Solution of (2-chlorophenyl) phosphorobis-(1,2,4-triazolidate) (*II*) (refs[8,15], 4 mmol) in tetrahydrofuran (3·2 ml) was added and the reaction mixture stirred for 18 h. The chilled mixture (5°C) was diluted with pyridine (20 ml), acetic acid (1·2 ml) was added and the mixture partitioned between chloroform (100 ml) and water (50 ml). The chloroform layer was evaporated and the residue chromatographed on a silica gel column (200 g) using the following elution systems: chloroform–triethylamine (99·5 : 0·5; 300 ml) and chloroform–methanol–triethylamine (98·5 : 1 : 0·5; 600 ml). Collected fractions with R_F 0·52 and 0·43 (S2) were evaporated affording fully protected dinucleotide *III* (3·86 g; 80%).

The product was dissolved in dioxane (36 ml) and aqueous 2M hydrochloric acid (4 ml) was added. The solution was kept 2 h at 20°C, then diluted with pyridine (20 ml) and partitioned between chloroform (50 ml) and water (40 ml). The chloroform layer was dried (Na_2SO_4) and evaporated. The residue was dissolved in N,N-dimethylformamide (5 ml), 2M solution of hydrogene chloride in N,N-dimethylformamide (3·8 ml) and triethyl orthoformate (2 ml) were added and the mixture stirred 4 h at 20°C. Pyridine (40 ml) was added, the mixture diluted with water (100 ml) and extracted with chloroform (200 ml). The chloroform layer was evaporated and the residue evaporated with two 50 ml portions of pyridine and two 50 ml portions of toluene affording 2′,3′-O-ethoxymethylene derivative, R_F 0·60 (S1). A mixture of this product and 4-dimethylaminopyridine (1·32 g; 11 mmol) was evaporated with two 20 ml portions of pyridine and two 20 ml portions of toluene and the solution of phosphorylating derivative *II* (3·2 ml; 4 mmol) was added and the mixture kept 20 h at room temperature. Pyridine (20 ml), acetic acid (1·2 ml) and water (50 ml) were added and the mixture extracted with chloroform (100 ml). The chloroform layer was evaporated and the residue chromatographed on a silica gel column (200 g), using the same elution systems as in the isolation of dinucleotide derivative. Appropriate

fractions with R_F 0·45 and 0·36 (S1, diastereoisomeric mixture) were evaporated affording fully protected trinucleotide. The intermediate was dissolved in dioxane (18 ml), aqueous 2M hydrochloric acid (2 ml) was added and, after 2 h, the mixture diluted with pyridine and partitioned between water (50 ml) and chloroform (50 ml). The chloroform layer was evaporated affording 2·26 g (29% overall yield) of partially protected trinucleotide *IV* as a mixture of diastereoisomers (R_F 0·25 and 0·15 (S1)). Removal of the protecting groups according to the described procedure[8,9] afforded (2'-5')core identical with the described compound.

Preparation of Protected Trinucleotide *IV* Using Modified Triester Method

The mixture of *V* (1·81 g; 2 mmol) and *VI* (1·53 g; 1·7 mmol) was evaporated with three 20 ml portions of pyridine and two 20 ml portions of toluene and dissolved in tetrahydrofuran (20 ml). 2,4,6-Triisopropylbenzenesulfonyl chloride (1·82 g; 6 mmol) and 1-methylimidazole (0·96 ml; 12 mmol) were added and the mixture kept at room temperature for 24 h. The mixture was diluted with pyridine (10 ml), acetic acid (1·2 ml) was added and the mixture partitioned between chloroform (100 ml) and water (50 ml). The chloroform layer was dried (Na_2SO_4) and evaporated. The resdiue was chromatographed on a silica gel column (50 g) using ethyl acetate–pyridine (99·5 : 0·5; 500 ml) elution system. Appropriate fractions with R_F 0·5 and 0·6 (S1) were evaporated affording 2·44 g (86%) of a dinucleotide derivative. The intermediate was dissolved in a mixture of acetonitrile–triethylamine (4 : 1; 20 ml) and, after 2 h, evaporated. The residue was evaporated with three 40 ml portions of etanol; R_F 0·1 (S1) (*VII*). 2',3'-O-Ethoxymethylene-N^6-benzoyladenosine (*I*; 0·7 g; 1·65 mmol) was added and the mixture evaporated with three 20 ml portions of pyridine and three 20 ml portions of toluene and dissolved in tetrahydrofuran. 2,4,6-Triisopropylbenzenesulfonyl chloride (1·3 g; 4·26 mmol) and 1-methylimidazole (0·7 ml; 8·5 mmol) were added, the mixture kept at room temperature for 24 h, diluted with pyridine (20 ml) and acetic acid (1·2 ml) and partitioned between chloroform (80 ml) and water (40 ml). The chloroform layer was dried (Na_2SO_4) and evaporated. The fully protected trinucleotide derivative was isolated by the same procedure as the dinucleotide derivative; yield 1·37 g (46%), R_F 0·46 and 0·36 (S2). The fully protected intermediate was dissolved in dioxane (18 ml), aqueous 2M hydrochloric acid (2 ml) was added. After 2 h, pyridine (4 ml) was added and the mixture partitioned between water (50 ml) and chloroform (50 ml). Chloroform layer was dried (Na_2SO_4) and evaporated affording trinucleotide *IV* (1·14 g; 34% overall).

Trimethyl Orthopalmitate (*VIII*)

A mixture of palmitonitrile[18] (186 g; 0·71 mol), methanol (29 ml; 0·71 mol) and a 4·7M solution of hydrogene chloride in dry ether (215 ml) was refluxed 6 h. The mixture was diluted with ether (200 ml), the solid collected, washed with ether (400 ml) and dried under diminished pressure. The resulting iminoester hydrochloride (118·9 g; 55%) was refluxed in a mixture of methanol (312 ml; 7·8 mol) and ether (320 ml) for 10 h. The filtered solution was diluted with ether and stirred with a 10% aqueous solutions of sodium carbonate (1 l) for 0·5 h. The organic layer was separated, water extracted with ether (200 ml) and the combined ether extracts were kept over potassium carbonate (150 g) for 4 h. The filtrate was concentrated and the residue was fractionally distilled to afford 74·6 g (62%) of *VIII*; b.p. 145—150°C (30 Pa). For $C_{19}H_{40}O_3$ (316·5) calculated: 72·10% C, 12·74% H; found: 71·80% C, 12·55% H.

2',3'-O-(1-Methoxyhexadecylidene)adenosine (*XII*)

A suspension of adenosine (5·34 g; 20 mmol in a mixture of N,N-dimethylformamide (50 ml), trimethyl orthopalmitate (*VIII*, 10·5 ml; 24 mmol) and methanesulfonic acid (4 ml; 62 mmol)

was stirred for 6 h, diluted with pyridine (10 ml) and partitioned between chloroform (100 ml) and water (50 ml). The organic layer was evaporated, the residue coevaporated successively with pyridine (20 ml) and toluene (50 ml), dissolved in chloroform (5 ml) and chromatographed on a silica gel column (150 g) using the following systems: ether–chloroform–triethylamine (30 : 69 : 1; 300 ml), chloroform–triethylamine (99 : 1; 300 ml), chloroform–methanol–triethylamine (98 : 1 : 1; 300 ml), and chloroform–methanol–triethylamine (97 : 2 : 1; 600 ml). Appropriate fractions with R_F 0·23 (S2) were evaporated and the residue coevaporated with ethanol (50 ml) affording glassy foam of *XII* (7·08 g; 68%). For $C_{27}H_{45}N_5O_5$ (519·7) calculated: 62·40% C, 8·73% H, 13·48% N; found: 62·18% C, 8·74% H, 13·26% N.

Adenylyl-(2′-5′)-adenylyl-(2′-5′)-2′,3′-O-(1-methoxyhexadecylidene)adenosine (*X*)

A) Solution of *IV* (1·98 g; 1·0 mmol) in N,N-dimethylformamide (10 ml) was concentrated to a volume of 5 ml. Trimethyl orthopalmitate (*VIII*, 2·2 ml; 5 mmol and methanesulfonic acid (0·6 ml; 10 mmol) were added. After 2 h, the reaction mixture was diluted with pyridine (10 ml) and water (20 ml) and extracted with chloroform (30 ml). The organic layer was evaporated and coevaporated with pyridine (3 × 20 ml) and toluene (3 × 20 ml). The residue was chromatographed on a silica gel column (50 g) in elution systems chloroform–ether–triethylamine (69 : 30 : : 1; 200 ml), chloroform–triethylamine (99 : 1; 200 ml), chloroform–methanol–triethylamine (98 : 1 : 1; 200 ml). Collected fractions with R_F 0·40 were evaporated and coevaporated with ethanol (3 × 10 ml). Yield, 0·98 g (40%) of *IX*. The product was suspended in a mixture of dioxane (1 ml) and aqueous ammonia (1 ml) and the mixture heated in a stoppered tube to 60°C for 8 h, evaporated and dissolved in 1M solution of tetrabutylammonium fluoride in tetrahydrofuran. After 20 h the solution was diluted with 50% aqueous pyridine and passed through a column (15 ml) of Dowex 50 (pyridinium). The column was washed with 50% aqueous pyridine (15 ml). Ammonia (1 ml) was added to the combined eluates and the mixture evaporated. The residue was dissolved in methanol (0·2 ml) and the solution diluted with ether (2 ml). After 20 h at 0°C the solid was collected, washed with ether and dried under diminished pressure; yield 235 mg (50%) of *X*, R_F 0·66 (S3). A sample (1 mg) of the product was dissolved in 0·25M aqueous sodium hydroxide (50 µl) and the mixutre analysed after 18 h in S4. The products of the cleavage were adenosine-2′(3′)phosphate (*XI*) and 2′,3′-O-(1-methoxyhexadecylidene)-adenosine (*XII*) in 2 : 1 ratio.

B) To the solution of *IV* (1·98 g; 1 mmol) in a mixture of N,N-dimethylformamide (5 ml) and trimethyl orthopalmitate (*VIII*, 2·2 ml; 5 mmol) dry Dowex-50 (H^+; 1 g) was added and the suspension stirred for 20 h. Pyridine (2 ml) was added, the resin filtered off, washed with pyridine (2 ml), and the combined filtrates partitioned between chloroform (30 ml) and water (20 ml). The chloroform layer was worked up according to the procedure described under *A*). The overall yield of *X* was 60% (method *A* gave 20%).

Adenylyl-(2′-5′)-adenylyl-(2′-5′)-2′(3′)-O-palmitoyl adenosine (*XIII*)

The compound *X* (5 mg) was dissolved in trifluoroacetic acid (0·2 ml) and the solution, after 10 min, evaporated (130 Pa, 20°C). The residue was evaporated with three 0·1 ml portions of 1-propanol and dried under diminished pressure. The sole product *XIII* (R_F 0·46 (S3)) afforded by the action of conc. aqueous ammonia (1 h) adenylyl-(2′-5′)-adenylyl-(2′-5′)-adenosine (compared with an authetic sample[8]) and palmitic acid (visualized by phosphomolybdic acid in ethanol (5%) spray[19] and heating to 120°C).

5′-Phosphoryladenylyl-(2′-5′)-adenylyl-(2′-5′)-2′,3′-O-
-(1-methoxyhexadecylidene)adenosine (*XVI*)

1-Methoxyhexadecylidene derivative of the protected trinucleotide *VI* (310 mg; 0·14 mmol) was dried for 10 h at 13 Pa, dissolved in a mixture of tetrahydrofuran (5 ml) and 2,4,6-collid ne (2·1 ml). To the cooled solution (0°C) (2-cyanoethoxy)dichlorophosphine (*XIV*, 0·5 ml; 4 mmol) was added. After 2 h standing a solution of 3-hydroxypropionitrile (0·82 ml; 12 mmol) in tetrahydrofuran (5 ml) was added dropwise under stirring. After 2 h, 2M solution of iodine in tetrahydrofuran–2,6-lutidine–water (7 : 2 : 1; 45 ml) was added and the reaction mixture evaporated. The residue was dissolved in a mixture of chloroform (75 ml) and pyridine (25 ml) and the solution extracted with 5% aqueous solution of sodium thiosulfate (25 ml), then with water (25 ml) and evaporated. The residue was evaporated with two 30 ml portions of toluene and dissolved in chloroform and spotted on two preparative silica gel plates and developed in S2. UV-Absorbing bands R_F 0·18 were eluted with S1 (50 ml) and the solution evaporated. The protecting groups were removed by the same procedure as in the preparation *X* from *IX* affording 33 mg (20% overall) of *XVI* R_F 0·47 (S4). Alkaline phosphatase teatment transformed the product to *X*.

Analyses were performed in the Analytical Department (Dr J. Horáček, Head) of this Institute

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