SYNTHESIS OF CYTIDYLYL-(3′ → 5′)-CYTIDYLYL-(3′ → 5′)ADENOSINE DERIVATIVES*

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By successive blocking, 5′-O-acetyl-N^4-acetylcytidine 3′-phosphate (*I*) affords 5′-O-dimethoxytrityl-2′-O-tetrahydropyranyl-N^4-dimethylaminomethylenecytidine 3′-phosphate (*V*) and then 5′-O-dimethoxytrityl-2′-O-tetrahydropyranyl-N^4-acetylcytidine 3′-phosphate (*VII*). By successive reactions with compound *VII*, 2,3,5-triisopropylbenzenesulfonyl chloride, and 2-cyanoethanol, and deblocking of the intermediate with 90% aqueous acetic acid, 2′,3′-di-O-benzoyl-N,N-dibenzoyladenosine affords 2′-O-tetrahydropyranyl-N^4-acetylcytidylyl-(3′ → 5′)-2′,3′-di-O-benzoyl-N,N-dibenzoyladenosine P-(2-cyanoethyl) ester (*VIII*). By reaction with compound *VII*, 2,3,5-triisopropylbenzenesulfonyl chloride, and 2-cyanoethanol, compound *VIII* affords 5′-O-dimethoxytrityl-2′-O-tetrahydropyranyl-N^4-dimethylaminomethylenecytidylyl-(3′ → 5′)-2′-O-tetrahydropyranyl-N^4-acetylcytidylyl-(3′ → 5′)-2′,3′-di-O-benzoyl-N,N-dibenzoyladenosine P^1,P^2-bis-(2-cyanoethyl) ester (*IX*). By successive reactions with dimethylformamide dimethylacetal, dimethoxytrityl chloride, benzoyl chloride, and 80% aqueous acetic acid, 3′-O-methyladenosine (*X*) affords 2′-O-benzoyl-3′-O-methyl-N,N-dibenzoyladenosine (*XIII*). By successive condensation with compound *VII* and 5′-O-acetyl-2′-O-tetrahydropyranyl-N^4-acetylcytidine 3′-phosphate (*II*) and treatment of the intermediate with ammonia, compound *XIII* affords 2′-O-tetrahydropyranylcytidylyl-(3′ → 5′)-2′-O-tetrahydropyranylcytidylyl-(3′ → 5′)-3′-O-methyladenosine (*XV*).

The recent biochemical research is interested in compounds that must be prepared by a selective derivatisation of oligonucleotides. In selective substitutions, specifically protected oligonucleotides are used as starting compounds.

In the present paper, we wish to report preparation of two specifically protected derivatives of the t-RNA acceptor sequence. One of these derivatives is represented by a completely protected CCA triester derivative, namely, 5′-O-dimethoxytrityl-2′-O-tetrahydropyranyl-N^4-dimethylaminomethylenecytidylyl-(3′ → 5′)-2′-O-tetrahydropyranyl-N^4-acetylcytidylyl-(3′ → 5′)-2′,3′-di-O-benzoyl-N,N-dibenzoyladenosine P^1,P^2-bis(2-cyanoethyl) ester (*IX*). The nucleotide component for this synthesis was prepared from 5′-O-acetyl-N^4-acetylcytidine 3′-phosphate[1] (*I*) *via* the 2′-O-tetrahydropyranyl derivative *II* reported[1] some time ago (the preparation of compound *II* has been now improved). Removal of acetyl groups by the action of ammonia affords

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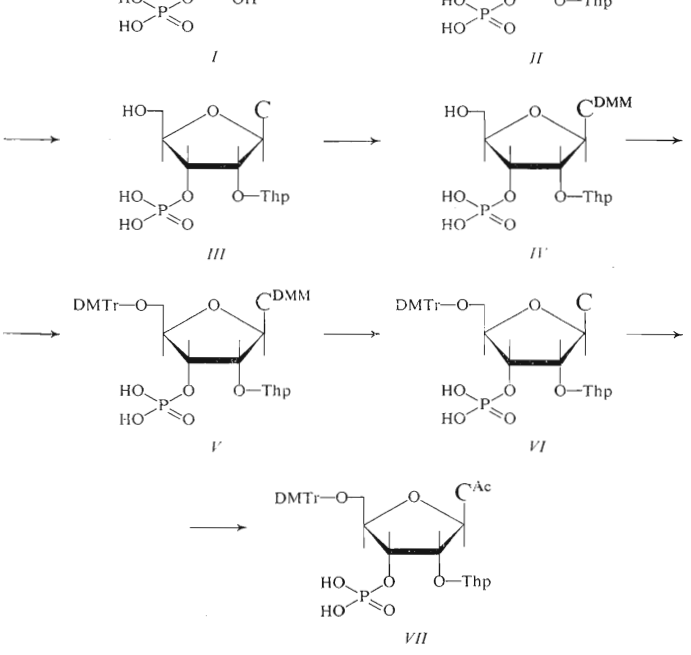
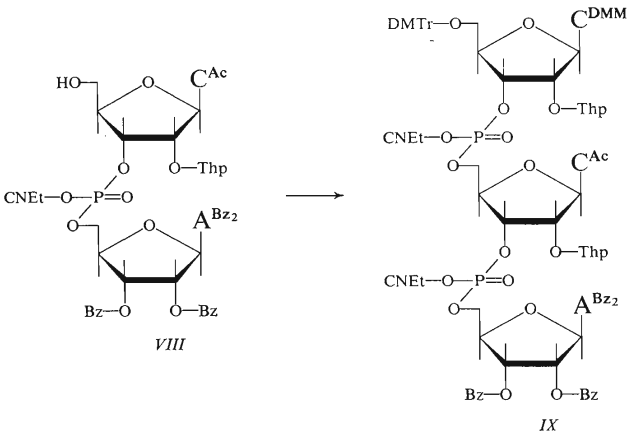
2′-O-tetrahydropyranylcytidine 3′-phosphate (*III*) which (in the form of the pyridinium salt) is substituted on the nitrogen atom on treatment with dimethylformamide dimethylacetal to afford compound *IV*. By reaction with dimethoxytrityl chloride, compound *IV* is converted into 5′-O-dimethoxytrityl-2′-O-tetrahydropyranyl--N^4-dimethylaminomethylenecytidine 3′-phosphate (*V*) which is isolated after extraction with chloroform and pyridine in the form of the pyridinium salt by precipitation with ether. By reaction with ammonia (removal of the dimethylaminomethylene group) and acetylation with acetic anhydride, compound *V* was converted to the



N^4-acetyl derivative *VII* which was isolated in the form of a very stable triethylammonium salt. The phosphate *VII* was condensed with 2′,3′-di-O-benzoyl-N,N-dibenzoyladenosine in the presence of 2,3,5-triisopropylbenzenesulfonyl chloride, the intermediary phosphodiester converted by the action of 2-cyanoethanol into a phosphotriester, and this substance isolated by preparative thin-layer chromatography. Removal of the dimethoxytrityl group gave the protected dinucleotide *VIII* bearing a free $C_{(5')}$-hydroxylic function. The intermediate *VIII* was condensed with the protected phosphate *V* and the final product *IX* isolated by preparative thin-layer chromatography and precipitation of the chloroform solution with ether.

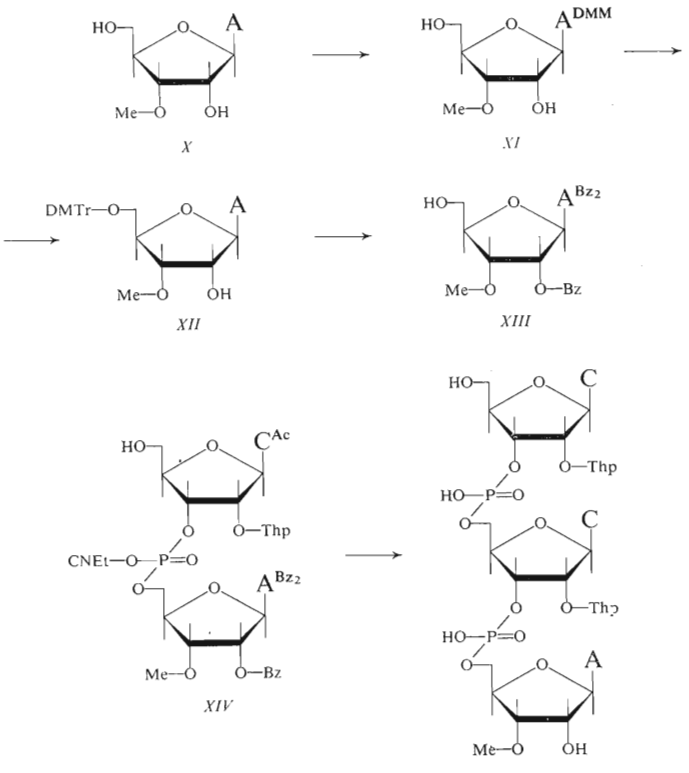


VIII *IX*

The other CCA derivative, namely, 2′-O-tetrahydropyranylcytidylyl-(3′ → 5′)-2′-O-tetrahydropyranylcytidylyl-(3′ → 5′)-3′-O-methyladenosine (*XV*) was prepared from 2′-O-benzoyl-3′-O-methyl-N,N-dibenzoyladenosine (*XIII*) as the starting material. Compound *XIII* was in turn obtained from 3′-O-methyladenosine[2] (*X*) *via* the N-dimethylaminomethylene derivative *XI*, protection of the $C_{(5')}$-hydroxylic function by dimethoxytritylation, deblocking of the nitrogen atom, benzoylation of the resulting compound *XII*, and removal of the dimethoxytrityl group.

The triester derivative of the dinucleotide *XIV* was prepared by condensation of compound *XIII* with the phosphate *VII*. The thus-obtained phosphodiester was converted to the 2-cyanoethyl ester which was isolated and then deblocked on the $C_{(5')}$-hydroxylic function. The further condensation step consisted in the diester

condensation with the phosphate *II*. The reaction mixture was processed with ammonia and the final product *XV* isolated by preparative paper chromatography.



Although the present yields do not exceed the yields of the earlier procedures, the above method is farly more advantageous from the standpoint of time which is required for isolation of products. Our yields of isolated cytidylyl-(3′ → 5′)adenosine derivatives with a free $C_{(5')}$-hydroxylic function are 30% and 42%, resp. Chládek and coworkers[3] report a 40% yield for an analogous derivative of this doublet;

Lohrman and coworkers[4] obtained the $C_{(2')}$-O-acetylnucleotide and the $C_{(2')}$-O-benzoylnucleotide in 38% and 64% yields, resp. Our yields are 36% (the completely protected derivative of the triplet) and 24% (the partly protected derivative; after two reaction steps). By the above authors, the deblocked CCA was obtained in a 44% yield[3] (referred to the starting dinucleoside phosphate) and in a 64% yield[4] (with respect to the recovered unreacted dinucleoside phosphate).

EXPERIMENTAL

Thin-layer chromatography was performed on ready-for-use Silufol UV254 (Kavalier Glassworks, Votice, Czechoslovakia) silica gel sheets in the solvent systems S_2, chloroform–methanol (9 : 1), and S_4, 2-propanol–conc. aqueous ammonia–water (7 : 1 : 2). Preparative thin-layer chromatography was performed on 25 × 20 × 0·6 cm plates of loose macroporous Pitra silica gel (produced by Service Laboratories of this Institute) in the solvent systems S_1, chloroform–pyridine (9 : 1) and S_3, chloroform–pyridine (8 : 2). Bands of the preparative layers were eluted with the solvent system S_E, chloroform–methanol (1 : 1). Unless stated otherwise, all evaporations were performed at 20°C/1 Torr on a rotatory evaporator equipped with a Dry Ice condenser. On preparative chromatographic layers, the dimethoxytrityl group-containing substances were detected by pressing a strip of chromatographic paper to the moist layer, air-drying the paper, and spraying with a 10% solution of perchloric acid in 30% aqueous acetic acid.

5′-O-Acetyl-2′-O-tetrahydropyranyl-N^4-acetylcytidine 3′-Phosphate (*II*), Triethylammonium Salt

A suspension of 5′-O-acetyl-N^4-acetylcytidine 3′-phosphate ammonium salt[5] (4·5 g) in dimethylformamide (40 ml) is treated with stirring with dihydropyran (10 ml) and then 6M hydrogen chloride in dimethylformamide (4·7 ml); on a moistened pH paper, the solution should exhibit the pH value of about 1·5. After 20 h (the R_F value in S_4 changes from 0·26 to 0·45), triethylamine (4·7 ml) is added with stirring and the mixture is cooled down (0°C). The precipitate of triethylamine hydrochloride is filtered off and washed with dimethylformamide (20 ml). The filtrate and washings are combined and poured with stirring into ether (700 ml). The precipitate is collected with suction (sintered glass filter G 3), washed with ether, and dried under diminished pressure. Yield, 5·1 g (78%) of compound *II* triethylammonium salt. Molecular weight determined from a sample processed with ammonia, 650. UV spectra (water): λ_{max} 248 and 300 nm; λ_{min} 237 and 273 nm.

5′-O-Dimethoxytrityl-2′-O-tetrahydropyranyl-N^4-dimethylaminomethylenecytidine 3′-Phosphate (*V*), Pyridinium Salt

A solution of the triethylammonium salt of compound *II* (5 g) in conc. aqueous ammonia (80 ml), pyridine (30 ml), and methanol (50 ml) is kept at room temperature for 4 h and then concentrated (35°C, 15 Torr) until the pH value drops to 7. The neutral concentrate is applied to a column (30 ml) of Dowex 50 (pyridinium form) ion exchange resin and the column is eluted with precooled (0°C) 50% aqueous pyridine (120 ml). The effluent is evaporated (1 Torr) to the volume of about 50 ml, the concentrate diluted with pyridine (50 ml), and evaporated again to the consistence of a sirup. This process is repeated with 6 additional portions of pyridine. The final sirup is dissolved in dimethylformamide (60 ml) and dimethylformamide dimethylacetal (15 ml) is added. The whole is kept at room temperature for 20 h, evaporated, the residual sirup dissolved in pyridine (30 ml),

and dimethoxytrityl chloride (6·74 g) is added. The mixture is kept at room temperature for 20 h, treated with methanol (10 ml), kept for additional 1 h, and shaken with a mixture of chloroform (50 ml) and water (30 ml). The chloroform layer is separated and the aqueous phase is extracted with three 50 ml portions of 1 : 1 chloroform–pyridine. The organic layers are combined, dried over anhydrous magnesium sulfate, and evaporated (first at 15 Torr and later on at 1 Torr). The residue is dissolved in pyridine (40 ml) and the solution is added dropwise with stirring into ether (600 ml). The precipitate is filtered off, washed with ether, and dried under diminished pressure. Yield, 4·3 g (74%) of compound *V*, R_F value 0·59 in S_4. Molecular weight as determined spectrophotometrically from the deblocked sample, 940.

5′-O-Dimethoxytrityl-2′-O-tetrahydropyranyl-N^4-acetylcytidine 3′-Phosphate (*VII*), Triethylammonium Salt

A solution of compound *V* (4·3 g) in pyridine (40 ml) is processed by a mixture of triethylamine (5 ml) and ethanol (10 ml). After 10 min, water is added (20 ml) and the mixture is kept at room temperature for 20 h. The solution is evaporated (1 Torr), the residue coevaporated with three portions of pyridine, and the final residue dissolved in pyridine (50 ml). Acetic anhydride (25 ml) is added, the whole kept at room temperature for 20 h, evaporated, the residue dissolved in 50% aqueous pyridine (50 ml), set aside for 2 h, and extracted with two 50 ml portions of chloroform. The chloroform extracts are combined, dried over anhydrous magnesium sulfate, and evaporated (35°C, 15 Torr). The remaining pyridine solution is added dropwise with stirring into ether (600 ml). The precipitate is collected with suction, washed with ether, and dried under diminished pressure. Yield, 6 g of compound *VII*, R_F value 0·60 in S_4. Molecular weight as determined spectrophotometrically from the deblocked sample, 990.

2′-O-Tetrahydropyranyl-N^4-acetylcytidylyl-(3′ → 5′)-2′,3′-di-O-benzoyl-N,N-dibenzoyladenosine P-(2-Cyanoethyl) Ester (*VIII*)

A mixture of 2′,3′-di-O-benzoyl-N,N-dibenzoyladenosine (2·04 g) and the triethylammonium salt of compound *VII* (6 g) is coevaporated with two portions of pyridine, the residue shaken with pyridine (30 ml) and 2,3,5-triisopropylbenzenesulfonyl chloride (3·65 g) for several minutes, and the mixture evaporated to the consistence of a sirup. After 20 h, pyridine (30 ml) and 2,3,5-triisopropylbenzenesulfonyl chloride (3·65 g) is added, the mixture shaken until homogeneous, and evaporated. The residual sirup is then shaken with 2-cyanoethanol (6·44 ml) for 30 min, kept aside for 4 h, diluted with chloroform (6 ml), and chromatographed on 3 layers of silica gel in the solvent system S_1. The dimethoxytrityl group-containing bands are eluted with the solvent system S_E (400 ml), the eluate is evaporated (40°C, 15 Torr), and the residual sirup coevaporated with toluene (50 ml). The final residue is dissolved in chloroform (5 ml) and the solution is shaken with a mixture of ether (10 ml and cyclohexane (100 ml) until the supernatant is clear. The upper solution is decanted and the remaining resin is dissolved in chloroform (10 ml). To the solution, a mixture of cyclohexane (40 ml) and ether (50 ml) is added. The precipitate is collected with suction, washed with ether, and dried under diminished pressure. Yield, 1·55 g of the 5′-O-dimethoxytrityl derivative of compound *VIII*, R_F value 0·31 in S_2. The substance is dissolved in 80% aqueous acetic acid (50 ml), the solution kept at room temperature for 1 h, evaporated, and the residue coevaporated with 1-butanol (10 ml). The final residue is dissolved in chloroform (10 ml) and the solution is added dropwise into ether (90 ml). The precipitate is collected with suction, washed with ether, and dried under diminished pressure. Yield, 1·05 g (30%) of compound *VIII*, R_F value 0·42 in S_2.

5'-O-Dimethoxytrityl-2'-O-tetrahydropyranyl-N^4-dimethylaminomethylenecytidylyl-(3' → 5')-2'-O-tetrahydropyranyl-N^4-acetylcytidylyl-(3' → 5')-2',3'-di-O-benzoyl-N,N-dibenzoyladenosine P^1,P^2-bis(2-Cyanoethyl) Ester (*IX*)

A mixture of compound *VIII* (1.2 g) and the pyridinium salt of phosphate *V* (2·65 g) is coevaporated with two portions of pyridine, the residue shaken with pyridine (20 ml) and 2,3,5-triisopropylbenzenesulfonyl chloride (1·83 g) for several minutes, and the whole evaporated to the consistence of a sirup. After 20 h, pyridine (20 ml) and 2,3,5-triisopropylbenzenesulfonyl chloride (1·83 g) are added, the mixture shaken till homogeneous, and evaporated. The residue is shaken with 2-cyanoethanol (3·2 ml) for 20 min, the mixture kept at room temperature for 5 h, diluted with chloroform (5 ml), and chromatographed on two layers of silica gel in the solvent system S_3. The dimethoxytrityl group-containing bands (distance from the start line, 10—17 cm) are eluted with S_E (200 ml), the eluate is evaporated (40°C, 15 Torr), the residue coevaporated with toluene (20 ml), and the final residue dissolved in chloroform (5 ml). Ether (200 ml) is added and the mixture shaken till clear. The upper solution is decanted, the material on the bottom dissolved in chloroform (10 ml), and the solution added dropwise into ether (100 ml). The precipitate is collected with suction, washed, and dried under diminished pressure. Yield, 577 mg (36%) of compound *IX*, R_F value 0·49 in S_2. Successive deblocking with aqueous ammonia and 20% aqueous acetic acid (50°C) affords CCA.

5'-O-Dimethoxytrityl-3'-O-methyladenosine (*XII*)

A suspension of 3'-O-methyladenosine[2] (6 g), dimethylformamide (40 ml), and dimethylformamide dimethylacetal (12 ml) is shaken for 4 h, evaporated, and the residue dissolved in pyridine (100 ml). Dimethoxytrityl chloride (8 g) is added, the mixture shaken for 30 min, and kept at room temperature overnight. Conc. aqueous ammonia (50 ml) is added, the mixture set aside for 25h and extracted with chloroform (200 ml). The extract is dried over anhydrous magnesium sulfate, evaporated (40 °C, 15 Torr) and the residue coevaporated with toluene (100 ml). To the final residue, chloroform (50 ml) is added and the resulting suspension is shaken with cyclohexane (90 ml) for 20 h. The solid is collected with suction, washed with 1 : 1 chloroform–cyclohexane and then with light petroleum, and finally dried under diminished pressure. Yield, 10 g (82%) of compound *XII*, m. p. 110—111 °C; R_F value 0.33 in S_2. For $C_{32}H_{33}N_5O_6$ (583.6) calculated: 65.85% C, 5.70% H, 12.00% N; found: 65.55% C, 5.85% H, 11.95% N.

2'-O-Benzoyl-3'-O-methyl-N, N-dibenzoyladenosine (*XIII*)

Benzoyl chloride (7.7 ml) is added to a solution of compound *XII* (7.8 g) in pyridine (70 ml) and the mixture is kept at room temperature for 20 h. Ice is then added (50 g) and after 1 h the mixture is extracted with chloroform (100 ml). The extract is dried over anhydrous magnesium sulfate, evaporated, the residue coevaporated three times with toluene, and the final residue shaken with 80% aqueous acetic acid (100 ml) for 2 h. The solution is evaporated (1 Torr), the residue coevaporated with 1-butanol (30 ml), and finally dissolved in chloroform (200 ml). This solution is shaken with saturated aqueous potassium hydrogen carbonate until the evolution of carbon dioxide ceases. The chloroform solution is separated, dried over anhydrous magnesium sulfate, evaporated, the residue dissolved in chloroform (10 ml), and the solution added dropwise with stirring into cyclohexane (150 ml). The precipitate is collected with suction and the precipitation process is repeated once more. Yield (after drying), 5.95 g (75%) of compound *XIII*, R_F value 0.55 in S_2. The analytical sample, m. p. 112—113 °C (chloroform–cyclohexane). For $C_{32}H_{27}N_5O_7$ (593.6) calculated: 64.75% C, 4.58% H, 11.80% N; found: 65.01% C, 4.90% H, 11.51% N.

2′-O-Tetrahydropyranylcytidylyl-(3′ → 5′)-2′-O-tetrahydropyranylcytidylyl-(3′ → 5′)-3′-O--methyladenosine (*XI*)

A mixture of 2′-O-benzoyl-3′-O-methyl-N, N-dibenzoyladenosine (594 mg) and the triethylammonium salt of compound *VII* (2 g) is coevaporated with two portions of pyridine, the residue dissolved in pyridine (20 ml), the solution shaken for several minutes with 2, 3, 5-triisopropylbenzenesulfonyl chloride (1.2 g), evaporated, and the residual sirup set aside for 20 h. Pyridine (20 ml) and 2, 3, 5-triisopropylbenzenesulfonyl chloride (1.2 g) are then added to the sirup, the whole is shaken until homogeneous, and evaporated. The sirup is shaken with 2-cyanoethanol (2.1 ml) for 30 min and kept at room temperature for 5 h. The reaction mixture is diluted with chloroform and chromatographed on two layers of silica gel in the solvent system S_1. The dimethoxytrityl group--containing bands (distance from the start line, 11—18 cm) are eluted with S_E, the eluate evaporated (40 °C, 15 Torr), the residue coevaporated with toluene (20 ml), and the final residue dissolved in chloroform (5 ml). The solution is shaken with a mixture (1 : 1; 100 ml) of ether and cyclohexane until clear. The upper solution is decanted and the residual material is dissolved in chloroform (5 ml). The solution is added dropwise into ether–cyclohexane (1 : 1; 100 ml) with stirring. The precipitate is collected with suction, washed with ether, and dried under diminished pressure. The substance is kept in 90% aqueous acetic acid for 1 h, the solution evaporated, and the residue coevaporated with 1-butanol (10 ml). The final residue is dissolved in chloroform (10 ml) and the solution added dropwise into ether (100 ml). The precipitate is collected with suction, washed with ether, and dried under diminished pressure. Yield, 360 mg (42%) of compound *XIV*.

A mixture of compound *XIV* (209 mg) and the triethylammonium salt of phosphate *II* (380 mg) is coevaporated with pyridine (20 ml), the residue shaken for several minutes with pyridine (10 ml) and 2,3,5-triisopropylbenzenesulfonyl chloride (360 mg), and the mixture evaporated. After 20 h, conc. aqueous ammonia is added to the sirup and the whole is kept at 50°C for 2 h. The resulting solution is chromatographed on one sheet of paper Whatman No 3 MM in the solvent system S_4. The main UV-absorbing band (R_{Up} 1) is eluted with water and the eluate is freeze-dried to afford 44 mg (23%) of the ammonium salt of compound *XI*. By the action of 20% aqueous acetic acid at 50°C (2 h), this salt gives the free trinucleotide yielding the expected products on degradation with pancreatic ribonuclease.

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