SYNTHESIS OF CYTIDYLYL-(3′ → 5′)-2′-O (AND 3′-O)-METHYLADENOSINE 3′-O(AND 2′-O)-N-FORMYL-L-METHIONYL DERIVATIVES*

L.A.ALEXANDROVA** and J.SMRT

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6

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Cytidylyl-(3′ → 5′)-2′-O-methyl-3′-O-(N-formyl-L-methionyl)adenosine (*XIIa*) and cytidylyl-(3′ → 5′)-2′-O-(N-formyl-L-methionyl)-3′-O-methyladenosine (*XIIb*) were synthesised by the action of N-formyl-L-methionylimidazole (*XI*) on 5′-O-dimethoxytrityl-2′-O-tetrahydropyranyl-N^4-dimethylaminomethylenecytidylyl-(3′ → 5′)-2′-O(or 3′-O)-methyl-N^6-dimethylaminomethyleneadenosine (*Xa*, *Xb*) followed by successive removal of acidolabile protecting groups. Contrary to N,N′-dicyclohexylcarbodiimide, *p*-toluenesulfonyltriazole (*II*) in pyridine did not racemise N-formyl-L-methionine. By the action of compound *II*, 5′-O-dimethoxytrityl-2′-O(or 3′-O)-methyl-N^6-dimethylaminomethyleneadenosine (*Ia*, *Ib*) affords the 3′-O(or 2′-O)-(N-formyl-L-methionyl) derivatives *IIIa* and *IIIb*, resp.

In connection with investigations on the functions of ribosomes, the synthesis of two isomeric derivatives of cytidylyl-(3′ → 5′)-adenosine was required. In one (*XIIa*) of these derivatives, the *cis*-diol grouping of the adenosine residue should bear a methyl group at position $C_{(2')}$ and a N-formyl-L-methionyl group at position $C_{(3')}$; in the other derivative *XIIb*, the order of substituents should be reversed. The synthesis of analogous L-phenylalanyl derivatives (Chládek and coworkers[1]) consisted in condensation of N-benzyloxycarbonyl-L-phenylalanine with partially blocked derivatives of O-methyladenosine by the action of N,N′-dicyclohexylcarbodiimide. The thus-obtained aminoacyl derivative of the nucleoside was deblocked at position $C_{(5')}$ and then condensed with blocked cytidine 3′-phosphate.

Preliminary attempts on introduction of the N-formyl-L-methionyl group into the 2′-O(or 3′-O)-methyladenosine derivatives *Ia* and *Ib* by the action of N,N′-dicyclohexylcarbodiimide resulted in a very low conversion. In order to find more efficient condensation agents for our purpose, the recently reported aromatic sulfonyltriazoles[2] have been now tested. Two of them, namely, *p*-toluenesulfonyltriazole (*II*) and mesitylenesulfonyltriazole were checked along with N,N′-dicyclohexylcarbodiimide from the standpoint of the racemisation of N-formyl-L-methionine in pyridine.

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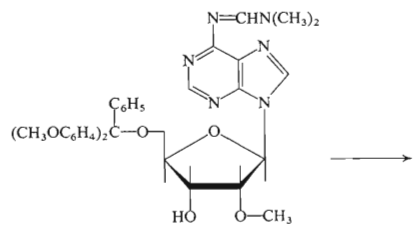
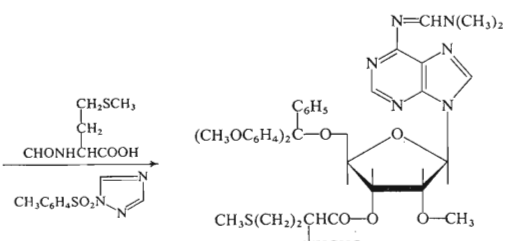
** Present address: Institute of Molecular Biology, Academy of Sciences of USSR, Moscow.

No racemisation of this compound was observed in the case of *p*-toluenesulfonyltriazole (*II*), a weak racemisation was encountered with mesitylenesulfonyltriazole while in the presence of N,N'-dicyclohexylcarbodiimide a complete racemisation occurred. The aminoacylation of compounds *Ia* and *Ib* was consequently performed in the presence of the triazole derivative *II* at 37°C for 3 days. After this period of time, an almost quantitative conversion was indicated by thin-layer chromatography with the formation of a more mobile substance, the N-formylmethionyl group of which was detected on treatment of the chromatogram with iodine. In the attempted removal of the dimethoxytrityl group and the dimethylaminomethylene group from compounds *IIIa* and *IIIb* by the action (*cf.* ref.[1]) of 1-butanol–acetic



Ia
Ib, 3'-O—CH_3

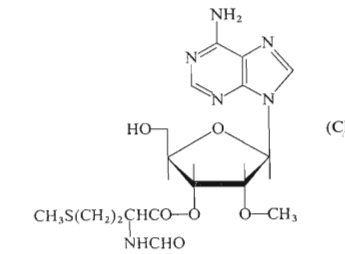


II *IIIa*
IIIb, 3'-O—CH_3, 2'-O—COCH(CH_2)$_2$$SCH_3$, NHCHO

acid–water (solvent system S_3), the expected N-formyl-L-methionyl-O-methyladenosines *IVa* and *IVb* were accompanied by substances that travelled a little faster on the chromatogram. The UV spectrum of these by-products indicated the presence

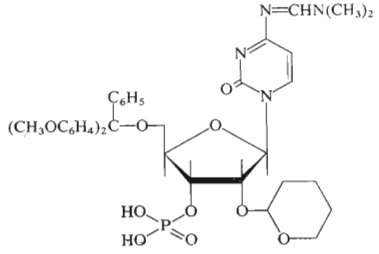
of an acyl group on the amino group of adenosine (λ_{max} 272 nm). The N-formylmethionyl group thus considerably changes conformation of the ribose ring and hence the reactivity of the dimethylaminomethylene group at position N^6 of adenosine since compounds *Ia* and *Ib* lacking the N-formylmethionyl group were unambiguously deblocked to O-methyladenosines by the action of the same solvent system S_3. Similarly, the dimethylaminomethylene derivative of deoxyadenosine affords products with a free amino group under analogous conditions[3].

Since the presence of an acyl on the amino group of adenosine would complicate the isolation process in further steps of the synthesis, conditions were looked after for an improved removal of the dimethylaminomethylene group from compounds *IIIa* and *IIIb* by means of agents of a different acidity. Strongly acidic solutions (99% formic acid, 0·1M trifluoroacetic acid) remove the dimethylaminomethylene group very slowly with the exclusive formation of the N^6-formyl derivative. The 1M trifluoroacetic acid in 80% aqueous dioxane adjusted to pH 2 or 7 by triethylamine affords quantitatively the N^6-formyl derivative within 2 h. On the other hand, solutions of some acids in aqueous 1-butanol (pH value of about 2 on moistened reagent paper) remove the dimethylaminomethylene group with the formation of a pure substance bearing a free amino group (in the case of trifluoroacetic acid) or the substance is accompanied by traces of the N^6-acyl derivative (formic acid) or by 20 to 30% of the N^6-acyl derivative (acetic acid as the agent). To the preparation of compounds *IVa* and *IVb*, the system 1-butanol–trifluoroacetic acid–water (85 : 0·5 : 14·5) was thus applied, the pH of the reaction mixture being maintained at about 2. The yield of the nucleoside derivatives *IVa* and *IVb* was about 40%.



IVa

IVb, 3′-O—CH_3, 2′-O—$COCH(CH_2)_2SCH_3$
NHCHO



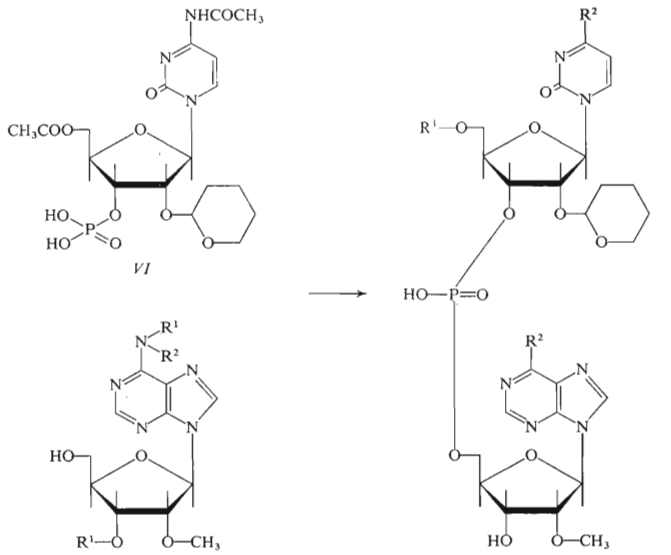
V

In the synthesis of the internucleotidic bond, an excess of compounds *IVa* or *IVb* was condensed with the nucleotide component *V* to circumvent the formation of phosphoamide derivatives on the free amino group (N,N′-dicyclohexylcarbodiimide

was used as the condensing agent). After 7 days, thin-layer chromatography indicated a very low conversion. The mixture was then processed with an acetic acid-containing system (S_3). This system removes the dimethylaminomethylene group from cytidine without any formation of the N-acyl derivative. After the final work-up with 20% aqueous acetic acid (removal of the tetrohydropyranyl group), the mixture was subjected to preparative paper chromatography in the solvent system S_3. The N-formylmethionyldinucleoside phosphates *XIIa* and *XIIb* were identified in bands containing (as indicated by electrophoresis) considerable amounts of cytidine 2',3'-cyclic phosphate and dinucleoside phosphates lacking the N-formylmethionyl group. In view of the low content of the required substances this route was abandoned and the preparative separation of the above mixtures was not performed.

Another route was therefore selected for the preparation of N-formylmethionyl dinucleoside phosphates *in substantia* consisting in a more economic use of the starting O-methyladenosines. The internucleotidic bond was synthesised with the application of an excess of the nucleotide component which was protected by less bulky substituents. The N-formylmethionyl group should be introduced in the stage of the dinucleoside phosphate. As the nucleoside components, derivatives of O-methyladenosines were used; except for the $C_{(5')}$-hydroxylic function, all the remaining reactive groups of these derivatives were blocked. These components make possible to perform the condensation with an excess of the phosphate. From the standpoint of steric hindrance, 5'-O-acetyl-2'-O-tetrahydropyranyl-N^4-acetylcytidine 3'-phosphate[4,5] is less hindered than the phosphate *V*. The N-formylmethionyl group was introduced by means of the imidazole method[6-8].

The nucleoside components of the above stated type were prepared *via* the 5'-O-dimethoxytrityl-O-methyladenosines that were obtained from O-methyladenosines *via* the N^6-dimethylaminomethylene derivatives. Benzoylation of 5'-O-dimethoxytrityl-3'-O-methyladenosine and removal of the dimethoxytrityl group afforded 2'-O-benzoyl-3'-O-methyl-N,N-dibenzoyladenosine[4] (*VIIb*). The corresponding 2'--O-methyl isomer *VIIa* was isolated as a mixture with 2'-O-methyl-3'-O-benzoyl--N-benzoyladenosine; this circumstance is without detriment to the further synthetic step. In the preparation of 3'-O-methyl-2'-O-acetyl-N'-acetyladenosine (*VIII*) by acetylation of the corresponding 5'-O-dimethoxytrityl derivative with acetic anhydride in pyridine and removal of the dimethoxytrityl group, a mixture was obtained containing 40% of 3'-O-methyl-2'-O-acetyladenosine. Compound *VIII* with the acetylated amino group was obtained in 45% yield. The nucleoside components *VIIa*, *VIIb* or *VIII* were condensed with 3 equivalents of the pyridinium salt of the phosphate *VI* in the presence of N,N'-dicyclohexylcarbodiimide and pyridinium Dowex 50 ion exchange resin. After 4 – 5 days, the reaction mixtures were processed with aqueous ammonia and the 2'-O-tetrahydropyranyldinucleoside phosphates *IXa* and *IXb* were isolated by gradient elution on DEAE-cellulose in 65 – 70% yields with respect to the nucleoside component.



VI

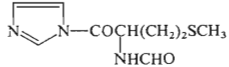
VIIa, $R^1 = R^2 = COC_6H_5$
VIIb, 3′-O—CH_3, 2′-O—COC_6H_5
VIII, $R^1 = COCH_3$, $R^2 = H$

IXa, $R^1 = H$, $R^2 = NH_2$
IXb, 3′-O—CH_3
Xa, $R^1 = C(C_6H_5)(C_6H_4OCH_3)_2$, $R^2 = N{=}CHN(CH_3)_2$
Xb, 3′-O—CH_3

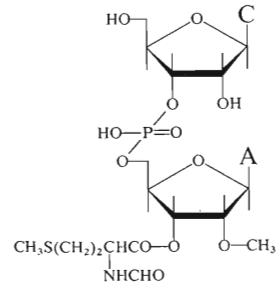
In order to circumvent the formation of by-products in the aminoacylation step, the reactants were blocked on the amino groups of cytidine and adenosine and on the $C_{(5')}$-hydroxylic function of cytidine by a successive treatment with dimethylformamide dimethylacetal and dimethoxytrityl chloride. The products *Xa* abd *Xb* were extracted with 1-butanol.

As shown by preliminary experiments, compound *Xa* is converted by the action of N-formyl-L-methionylimidazole (*XI*) for 20 h into a mixture of more mobile substances (R_F 0·55 and 0·75). Taking into account the relatively high mobility of products (the R_F value of the starting compound *Xa* is 0·1), the primary reaction product is represented by the neutral acylphosphate (R_F 0·55) which undergoes a further aminoacylation with the formation of a substance possessing the R_F 0·75. Virtually, deblocking of the less mobile product with 30% aqueous acetic acid af-

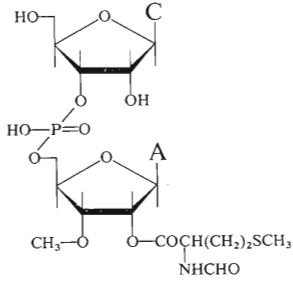
forded cytidylyl-(3′ → 5′)-2′-O-methyladenosine (R_F 0·27 in S_3) whereas the faster product yielded the substance *XIIa* (R_F 0·41 in S_3) containing N-formyl-L-methionine. Since the reaction of the nucleoside derivative *Ia* with the imidazole derivative *XI* (as shown by a separate experiment) was farly slower than that of the dinucleoside phosphate *Xa*, it cannot be excluded that the N-formylaminoacylation takes place with participation of the mixed anhydride of N-formyl-L-methionine and the phosphodiester. Taking into account that the acyl-phosphomonoester should be a better acylating agent, the preparative reactions of compounds *Xa* and *Xb* with the imidazole derivative *XI* were performed in the presence of 0·1 equivalent of N,$O^{2'}$,$O^{3'}$-triacetyladenosine 5′-phosphate. The thus-obtained N-formylmethionyl derivatives *Xa* and *Xb* were subjected to the action of a 1-butanol–formic acid–water mixture (from the preparative point of view, this mixture is more versatile than that containing trifluoroacetic acid because of the higher content of the acid). Precipitation of the methanolic solution with ether yielded the 2′-O-tetrahydropyranyl derivatives of compounds *XIIa* and *XIIb* (these derivatives were stable towards the pancreatic



XI



XIIa



XIIb

ribonuclease). The removal of the tetrahydropyranyl group was accomplished by the action of 20% aqueous acetic acid at 50°C. Under these conditions, a partial removal of the formyl group from the formylamino grouping also takes place. Pure compounds *XIIa* and *XIIb* were finally obtained by column chromatography on cellulose in 19% and 24% yields, resp. These compounds were split by ammonia with the formation of N-formylmethionine and cytidylyl-(3′ → 5′)-O-methyladenosine. The pan-

creatic ribonuclease degradation (pH 7·5) affords N-formylmethionine, cytidine 3′-phosphate, and the corresponding O-methyladenosine. The formation of N-formylmethionine cannot be explained by the presence of proteolytic enzyme in the ribonuclease since the same preparation of pancreatic ribonuclease did not split off N-formylmethionine from 2′-O-tetrahydropyranyl derivatives of compounds *XII*.

Despite the relatively high yields of completely protected compounds in the imidazole condensation, the removal of acidolabile protecting groups is accompanied by losses of the material, particularly in the case of the N-formyl-L-methionyl derivatives. The analogous 2′-O-(benzyloxycarbonyl-L-phenylalanyl) derivative of cytidylyl-(3′ → 5′)-3′-O-methyladenosine was prepared[9] by this method in 42% yield.

EXPERIMENTAL

Thin-layer chromatography was performed on ready-for-use Silufol UV_{254} (Kavalier Glassworks, Votice, Czechoslovakia) silica gel sheets in the solvent systems S_1, chloroform–methanol (8 : 2); S_2, chloroform–methanol (95 : 5); S_3, 1-butanol–acetic acid–water (5 : 2 : 3); S_4, chloroform–methanol (9 : 1); and S_5, 2-propanol–conc. aqueous ammonia–water (7 : 1 : 2). Paper chromatography was performed on papers Whatman in systems S_3 and S_5. Electrophoresis was carried out in 0·05M triethylammonium hydrogen carbonate (pH 7·5) or 6% aqueous acetic acid on paper Whatman No 1 dipped in tetrachloromethane. N-Formylmethionine and the N-formylmethionine-containing compounds were detected on Silufol by exposure to iodine vapours (white spot), removal of excess iodine by a stream of air, and spraying with 10% aqueous perchloric acid (deep purple spot). Unless stated otherwise, evaporations were carried out at 20°C and 1 Torr on rotatory evaporators equipped with Dry Ice condensers.

Racemisation of N-Formyl-L-methionine by the Action of Triazole Derivatives of Aromatic Sulfonic Acids or N,N′-Dicyclohexylcarbodiimide

The 1M solutions of N-formyl-L-methionine in pyridine (1 ml) were treated with *a*) mesitylenesulfonyltriazole (1·4 mmol), *b*) *p*-toluenesulfonyltriazole (2 mmol), and *c*) N,N′-dicyclohexylcarbodiimide (2 mmol). After 20 h, the mixtures were diluted with water (1 ml) and made up with pyridine to the volume of 10 ml. After 2 h, the specific rotation was determined as follows: *a*) −8·01°, *b*) −11·3°, and *c*) +1·7°. The original 1M solution when processed similarly except for the addition of the reagent, exhibited the specific rotation −10°.

5′-O-Dimethoxytrityl-2′-O-methyl-N^6-dimethylaminomethyleneadenosine (*Ia*)

A suspension of 2′-O-methyladenosine (2·44 g; 8·5 mmol), dimethylformamide (15 ml), and dimethylformamide dimethylacetal (4·3 ml) is shaken for 5 h. The resulting homogeneous solution is concentrated to half of the original volume and the concentrate is shaken with pyridine (10 ml) and dimethoxytrityl chloride (3·18 g) until homogeneous and then set aside for 20 h. Methanol (5 ml) is added followed (after 1 h) by chloroform (50 ml) and water (10 ml). The whole is briefly shaken, the chloroform layer separated, dried over anhydrous magnesium sulfate, and evaporated (40°C, 15 Torr). The residue is coevaporated with two portions of toluene and finally dissolved in benzene (25 ml). The solution is added dropwise with stirring into cyclohexane (200 ml). The precipitate is washed with light petroleum and dried under diminished pressure. Yield, 4·6 g

(85%) of compound *Ia*, R_F value 0·63 in S_1. UV spectrum: λ_{max} 307 nm. 5′-O-Dimethoxytrityl--3′-O-methyl-N^6-dimethylaminomethyleneadenosine (*Ib*) was prepared analogously from 3′-O--methyladenosine; the chromatographic behaviour of *Ib* was the same as that of *Ia*.

5′-O-Dimethoxytrityl-2′-O-methyl-3′-O-(N-formyl-L-methionyl)-N^6-dimethylaminomethyleneadenosine (*IIIa*)

Compound *Ia* (2·8 g) is added to a solution of N-formyl-L-methionine (2·22 g) in pyridine (10 ml) and the whole is evaporated. The sirup is coevaporated with two portions of pyridine, the residue dissolved in pyridine (5 ml) and *p*-toluenesulfonyltriazole[2] (*II*; 5·8 g) is added. The mixture is shaken until homogeneous, evaporated, and the residue kept at 37°C for 3 days. Ethyl acetate (100 ml) is added, the organic solution decanted from the insoluble residue, washed with saturated aqueous potassium hydrogen carbonate and three portions of water, dried over anhydrous magnesium sulfate, evaporated (35°C, 1 Torr), the residue coevaporated with two portions of toluene, and dried at 0·1 Torr. Yield, 3·2 g (93%) of compound *IIa*, m.p. 106—116°C; R_F value 0·5 in S_2. The presence of N-formylmethionine in compound *IIa* was detected by exposure of the Silufol to iodine vapours and spraying with 10% aqueous perchloric acid. 5′-O-Dimethoxytrityl-3′-O--methyl-2′-O-(N-formyl-L-methionyl)-N^6-dimethylaminomethyleneadenosine (*IIIb*) is prepared analogously to compound *IIIa* and shows the same chromatographic properties. The dimethylaminomethylene group was removed from samples (about 2 mg each) of compounds *Ia* and *IIIa* by the action of the reagents (50 μl each) given: *a*) *Ia*, solvent system S_3, 20 h; *b*) *IIIa*, S_3, 20 h; *c*) *IIIa*, 99% formic acid, 2 h; *d*) *IIIa*, 0·1M trifluoroacetic acid in 80% aqueous dioxane, 2 h; *e*) *IIIa*, 1M triethylammonium trifluoroacetate (pH 2) in 80% aqueous dioxane, 2 h; *f*) *IIIa*, 1M triethylammonium trifluoroacetate (pH 7) in 80% aqueous dioxane, 2 h; *g*) *IIIa*, 1-butanol--water–trifluoroacetic acid (85 : 14·5 : 0·5), 20 h; and *h*) *IIIa*, 1-butanol–water–formic acid (16 : : 3 : 1), 20 h. When the time stated ellapsed, the mixtures were chromatographed on Silufol in the solvent system S_3, the spots eluted with methanol, and the UV spectrum of the eluate was determined. The experiments yielded the following products: *a*) pure 2′-O-methyladenosine; *b*) 80% of *IVa* and 20% of its N-acetyl derivative; *c*) and *d*) a mixture of N^6-dimethylaminomethylene and N^6-acyl derivatives of compound *IVa*; *e*) and *f*) pure N^6-acyl derivative of compound *IVa*; *g*) pure *IVa*; and *h*) *IVa* and trace of its N^6-acyl derivative.

2′-O-Methyl-3′-O-(N-formyl-L-methionyl)adenosine (*IVa*)

To a solution of compound *IIIa* (795 mg) in a mixture (15 ml) of 1-butanol–water–trifluoroacetic acid (85 : 14·5 : 0·5), trifluoroacetic acid (0·1 ml) is added. After 20 h, the mixture is evaporated and the residue triturated with ether (20 ml). The ethereal solution is discarded and the residue dissolved in chloroform (5 ml). The solution is added dropwise with stirring into ether (50 ml), the precipitate collected with suction, washed with ether, and dried under diminished pressure. Yield, 189 mg (43%) of compound *IIIa*, R_F 0·58 in S_1. UV spectrum (methanol): λ_{max} 260 nm, λ_{min} 233 nm; A_{250}/A_{260} 0·83; A_{280}/A_{260} 0·376. By the action of conc. aqueous ammonia, compound *IVa* is split to N-formylmethionine and 2′-O-methyladenosine. 3′-O-Methyl-2′-O-(N-formyl-L-methionyl)adenosine (*IVb*) was prepared analogously to compound *IVa*. UV spectrum (methanol): λ_{max} 260 nm, λ_{min} 233 nm; A_{250}/A_{260} 0·815; A_{280}/A_{260} 0·35.

Condensation of Compounds *IVa* and *IVb* with 5′-O-dimethoxytrityl-2′-O-tetrahydropyranyl--N^4-dimethylaminomethylenecytidine 3′-Phosphate (*V*)

A mixture of the pyridinium salt of the phosphate *V* (0·8 mmol) and compound *IVa* (1 mmol) is coevaporated with two portions of pyridine and the residue dissolved in pyridine (5 ml). N,N′-

-Dicyclohexylcarbodiimide (8 mmol) is added and the mixture is kept at room temperature for 7 days. Ice (1 g) is then added, followed (after 10 min) by 50% aqueous pyridine (50 ml) and the mixture is washed with three portions of light petroleum. The aqueous layer is filtered, the filtrate evaporated, and the residue coevaporated with three portions of toluene. The final residue is kept in S_3 (100 ml) for 20 h and the mixture is evaporated. The residue is heated (50°C) in 20% aqueous acetic acid (20 ml) for 1 h. One tenth of the volume is chromatographed on one sheet of paper Whatman No 3 MM in S_3. The main UV-absorbing band (R_F of about 0·4) is eluted with 10% aqueous acetic acid at 0°C (3900 A_{260} at pH 2). A sample of the eluate is subjected to electrophoresis to afford three UV-absorbing spots, cytidine 2',3'-cyclic phosphate, cytidylyl-(3' → 5')-2'-O-methylcytidine, and the product *XIIa*. The yield of the procedure was 11% as calculated from the UV-absorption of the spot eluate of the product. The analogous condensation with compound *IVb* yielded 8% of the product *XIIb*.

2'-O-Methyl-3'-O-benzoyl-N,N-dibenzoyladenosine (*VIIa*)

Compound *VIIa* was prepared analogously to the reported[4] 3'-O-methyl isomer *VIIb* in 50% yield in an amorphous form (R_F 0·89 in S_4) and contained 20% of 2'-O-methyl-3'-O-benzoyl-N-benzoyladenosine (R_F 0·72 in S_4).

3'-O-Methyl-2'-O-acetyl-N^6-acetyladenosine (*VIII*) and 3'-O-Methyl-2'-O-acetyladenosine

A solution of 5'-O-dimethoxytrityl-3'-O-methyladenosine (7 mmol) in pyridine (20 ml) is kept with acetic anhydride (10 ml) for 20 h. Methanol (20 ml) is then added with cooling followed (after 1 h) by water (50 ml) and chloroform (100 ml). The mixture is briefly shaken, the chloroform layer separated, dried over anhydrous magnesium sulfate, and evaporated (40°C, 15 Torr). The residue is coevaporated with two portions of toluene, kept at room temperature in 90% aqueous acetic acid (25 ml) for 90 min, evaporated (1 Torr), and the residue coevaporated with 1-butanol (10 ml). The final residue is dissolved in chloroform (15 ml) and the solution is treated dropwise with stirring with cyclohexane (50 ml) to deposit crystals. Another portion (45 ml) of cyclohexane is then added and the mixture is set aside for 20 h. The monoacetyl derivative is collected with suction; 910 mg; m.p. 202—209°C; R_F 0·2 in S_4. For $C_{13}H_{17}N_5O_5$ (323·3) calculated: 48·20% C, 5·20% H, 21·66% N; found: 48·21% C, 5·15% H, 21·41% N. The filtrate is diluted with cyclohexane to the volume of 200 ml. The precipitate is collected with suction, washed with cyclohexane, dried under diminished pressure, and dissolved in chloroform (15 ml). The solution is added dropwise with stirring into cyclohexane (150 ml), the precipitate collected with suction, washed with light petroleum, and dried. Yield, 1·04 g (45%) of compound *VIII*, a solid, R_F 0·26 in S_4. UV spectrum (methanol): λ_{max} 272 nm. For $C_{15}H_{19}N_5O_6$ (365·3) calculated: 49·31% C, 5·24% H, 19·17% N; found 48·85% C, 5·35% H, 18·91% N.

2'-O-Tetrahydropyranylcytidylyl-(3' → 5')-2'-O-methyladenosine (*IXa*)

A solution of the triethylammonium salt of 2'-O-tetrahydropyranyl-5'-O-acetyl-N^4-acetylcytidine 3'-phosphate[4] (6 mmol) in 50% aqueous pyridine (10 ml) is passed through a column (25 ml) of pyridinium Dowex 50 ion exchange resin and the column is eluted with precooled (0°C) 50% aqueous pyridine (60 ml). The eluates are combined and concentrated to one fifth of the original volume. The concentrate is coevaporated with six 50 ml portions of pyridine and finally dissolved in pyridine (30 ml). To the solution, the nucleoside derivative *VIIa* (3 mmol), N,N'-dicyclohexylcarbodiimide (6 g), and dry pyridinium Dowex 50 ion exchange resin (3 g) are added. The mixture is kept at room temperature for 5 days and then water (5 ml) is added followed (after 10 min)

by methanol (30 ml), conc. aqueous ammonia (20 ml), and light petroleum (40 ml). The whole is briefly shaken, heated at 50°C for 30 min, and filtered. The filtrate is concentrated (40°C, 15 Torr) to the volume of about 50 ml, the concentrate decanted from the resinous material, and applied to a column (2 l) of DEAE-cellulose (HCO_3^-). The column is washed with water (1 l) and then eluted with the use of a linear gradient (4 l of water in the mixing chamber and 4 l of 0·15M triethylammonium hydrogen carbonate in the reservoir). The peak eluate (at about 0·07M buffer concentration on the top of the column) is evaporated, the residue coevaporated with three portions of ethanol and finally dissolved in 50% aqueous ethanol. Yield, 67% (42000 A_{260} at pH 1) of the triethylammonium salt of compound *VIIa*; R_F 0·60 in S_5; $E_{UP}^{7,5}$ 0·40; stable towards the pancreatic ribonuclease degradation. The dinucleoside phosphate *IXb* was prepared analogously starting from the nucleoside derivatives *VIIb* or *VIII*.

5′-O-Dimethoxytrityl-2′-O-tetrahydropyranyl-N^4-dimethylaminomethylenecytidylyl-(3′→5′)-2′-O-methyl-N^6-dimethylaminomethyleneadenosine (*Xa*)

A solution of the triethylammonium salt of compound *IXa* in 50% aqueous ethanol (14200 A_{260}; 0·68 mmol) is evaporated, the residue coevaporated with two 10 ml portions of dimethylformamide, the final residue dissolved in dimethylformamide (5 ml), and the solution treated with dimethylformamide dimethylacetal (2·5 ml). The mixture is kept at room temperature for 20 h, evaporated, and the residue dissolved in pyridine (10 ml). The solution is kept with dimethoxytrityl chloride (473 mg) for 20 h. Water (10 ml) is then added, followed (after 10 min) by 1-butanol (80 ml) and water (100 ml). The mixture is briefly shaken, the 1-butanol layer separated, and the aqueous layer extracted with additional 1-butanol (20 ml). The 1-butanol solutions are combined, washed with water (30 ml), dried over anhydrous magnesium sulfate, and concentrated to the volume of 10 ml. The concentrate is added dropwise with stirring into ether (100 ml), the precipitate collected with suction, washed with ether, and dried under diminished pressure. Yield, 602 mg of the pyridinium salt of compound *Xa*; R_F 0·65 in S_5. The action of 80% aqueous acetic acid on compound *Xa* affords the starting substance *IXa* along with the detetrahydropyranylated dinucleoside phosphate. The isomer *Xb* derived from 3′-O-methyladenosine was prepared analogously to *Xa*.

Cytidylyl-(3′→5′)-2′-O-methyl-3′-O-(N-formyl-L-methionyl)adenosine (*XIIa*)

1,1′-Carbonyldiimidazole (430 mg; 2·5 mmol) is added to a solution of N-formyl-L-methionine (1·8 mmol; 320 mg) in dimethylformamide (2 ml). When the evolution of carbon dioxide ceases (2—3 min), this mixture is added to a solution of compound *Xa* (600 mg) and the pyridinium salt of $N,O^{2'},O^{3'}$-triacetyladenosine 5′-phosphate (50 mg) in dimethylformamide (2·5 ml). After 6 days, 50% aqueous pyridine (5 ml) is added and after 6 h, the mixture is evaporated to dryness. The residue is dissolved in 50% aqueous pyridine (10 ml) and the solution is extracted with chloroform (50 ml). The chloroform layer is separated and the aqueous layer is extracted with chloroform (10 ml). The chloroform extracts are combined, dried over anhydrous magnesium sulfate, and evaporated. The residue is coevaporated with two 20 ml portions of toluene and finally dissolved in a mixture of 1-butanol (16 ml), water (3 ml), and formic acid (1 ml). The mixture is kept at room temperature for 20 h and evaporated. The residual sirup is diluted with methanol (5 ml) and ether (100 ml) is added. The precipitate is collected with suction, washed with ether, and dried under diminished pressure. Yield, 474 mg of the 2′-O-tetrahydropyranyl derivative of compound *XIIa*, R_F 0·48 in S_3; stable towards the pancreatic ribonuclease degradation. The substance is dissolved in acetic acid (2 ml) and water is added (8 ml). The non-homogeneous mixture is heated at 50°C for 90 min. The resulting solution is applied to a column (4 × 60 cm) of cellulose (Whatman,

Standard Grade) prewashed with the solvent system 1-butanol–water–acetic acid (78 : 17 : 5). The column is eluted with the same solvent system, 15 ml fractions being taken in 20 min intervals. The first 600 ml of the eluate are discarded. The product is contained in the next 300 ml which are evaporated (1 Torr). The residue is dissolved in methanol (5 ml) and the solution is added dropwise into ether (5 ml). The precipitate is collected with suction, washed with ether, and dried under diminished pressure. Yield, 87 mg (19%) of compound *XIIa*, R_F 0·41 in S_3; $E^{7\to5}_{UP}$ 0·35; $E^{2,4}_{adenosine}$ 0·55. UV spectrum (water): λ_{max} 266 nm, λ_{min} 234 nm; A_{250}/A_{260} 0·745; A_{280}/A_{260} 0·795; A_{290}/A_{260} 0·52. Molecular weight 710 (calculated 745), as determined from the absorbance at 260 nm (pH 1). By the action of conc. aqueous ammonia, compound *XIIa* is split into cytidylyl-(3′ → 5′)-2′-O-methyladenosine and N-formylmethionine. The pancreatic ribonuclease degradation at pH 7·5 affords quantitatively cytidine 3′-phosphate and 2′-O-methyladenosine (ratio 1 : 1·04) and N-formylmethionine. After the elution of compound *XIIa*, the column is eluted with the solvent system S_3 to afford 95 mg of cytidylyl-(3′ → 5′)-2′-O-methyladenosine. The column is then made ready for the next run by washing with the solvent system 1-butanol–water–acetic acid (78 : 17 : 5). Cytidylyl-(3′ → 5′)-2′-O-(N-formyl-L-methionyl)-3′-O-methyladenosine (*XIIb*) was prepared analogously in 24% yield from compound *Xb*. The chromatographical and electrophoretical properties of compounds *XIIa* and *XIIb* are almost identical.

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