SYNTHESIS OF OLIGODEOXYRIBONUCLEOTIDES FROM 3'-O-FORMYL-(N-ACYL)-2'-DEOXYRIBONUCLEOSIDES USING *o*-CHLOROPHENYLPHOSPHODITRIAZOLIDE AND/OR METHYL PHOSPHODICHLORIDITE. REMOVAL OF METHYL GROUP FROM PHOSPHOTRIESTER BY THE ACTION OF AQUEOUS PYRIDINE*

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d-GGAATTCC (*XI*) was prepared by phosphomonotriazolide method in solution. Synthesis of oligodeoxyribonucleotides by phosphite method in solution using methyl phosphodichloridite was studied. Preparation of P-methyl esters of protected d-CA (*XVII*), d-CAT (*XIX*), d-TT (*XXI*) and d-ATT (*XXIII*) is described. The removal of methyl group from methyl containing phosphotriester by the action of aqueous pyridine is described.

In the last paper of this series[1] preparation of 3'-O-formyl-(N-acyl)-2'-deoxyribonucleosides and their use in the synthesis of oligodeoxyribonucleotides by phosphomonotriazolide[2] method was described. An analogous method was applied in the present work for the synthesis of d-GGAATTCC (*XI*) (Scheme 1). The protected dinucleotide d-CC (*VI*) bearing the free $C_{(5')}$-hydroxyl function represented the starting component. As the elongation components protected derivatives of d-TT (*I*), d-AA (*II*) and d-GG (*III*) containing free $C_{(3')}$-hydroxyl functions were used. In the condensation steps increasing excess of phosphomonotriazolides was applied. The ratio of 4-dimethylaminopyridine (*V*) to phosphomonotriazolide was 10–20 : 1. Because of the observation that *V* is capable to split off the formyl and the *o*-phenyl groups during the working up of reaction mixtures that base was neutralized by car-

DMTr-dTp_odTp_o-OH (*I*)

DMTr-$dA^{Bz}p_odA^{Bz}$-OH (*II*)

DMTr-$dG^{iBu}p_odG^{iBu}$-OH (*III*)

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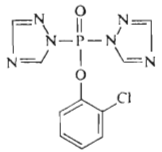
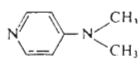
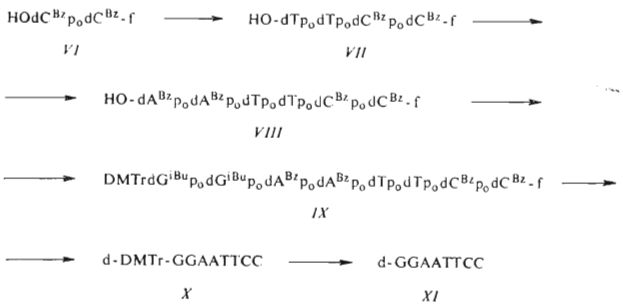


IV



V

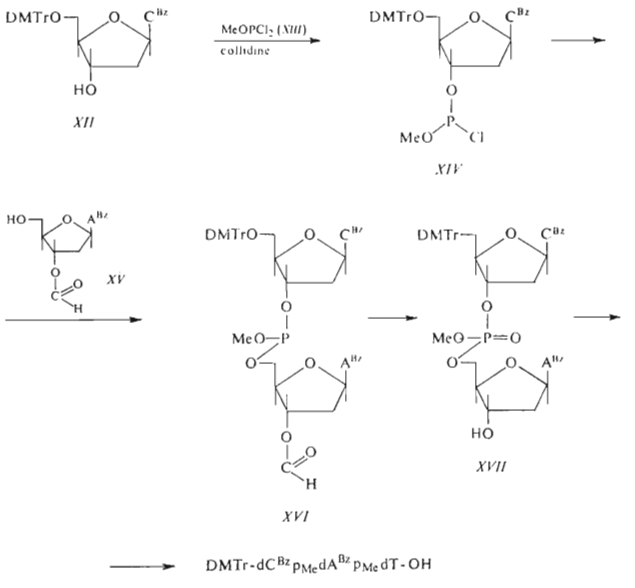
bon dioxide or removed in supernatant after precipitation of the reaction mixture with ether as described in the preceding paper[1]. In the present work a standard procedure was introduced to remove *V* by dilution of the reaction mixture with chloroform containing pyridinium acetate and extraction of salts with water. Further methodical improvement leading to quick purification of the products was brought by the use of step gradient elution with pyridine in chloroform during short column chromatography. The elution was accelerated by suction or by slight air pressure. Marked correlation between the length of the oligonucleotidic chain and the elution concentration of pyridine was observed. The protected dinucleotides were eluted



SCHEME 1

with chloroform containing 10%, the tetranucleotide 20%, the hexanucleotide 30 to 35% and the octanucleotide 55–60% of pyridine. Analogous correlation is apparent between the length of chain of 5'-O-dimethoxytrityl-oligonucleotides after ammonia treatment in S_2. R_{Up} of tetranucleotide is 5·0, that of hexanucleotide 3·0 and of octanucleotide 2·0, respectively. The classical preparative paper chromatography was used in two-step purification of the product. Nevertheless, the last impurities had to be removed by column chromatography in 7 mol l^{-1} urea.

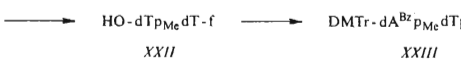
The acceleration of the stepwise synthesis might be achieved by application of Letsinger's phosphite method[4]. We have been studying this method and used methyl phosphodichloridite as bifunctional reagent. This reagent was originally reported by Daub and van Tamelen[5] for the phosphite synthesis of a oligoribonucleotide. These authors introduced also thiophenolate anion for selective removal of methyl-



SCHEME 2

group from the phosphotriester. Recently, methyl phosphodichloridite has been exploited in the synthesis of oligodeoxyribonucleotides on silica gel support[6–10]. In original phosphite procedure[4], the bifunctional reagent was used in less than equimolar quantity. In our study, we applied an excess of the reagent in order to suppress the formation of 3',3'-diester. The excess of the reagent was reacted with additional $C_{(5')}$-hydroxyl function bearing component. The 5',5'-diester formed could be easily removed after deformylation which procedure transformed it in substance

bearing two free hydroxyl groups. Because of poor solubility of 3'-O-formyl-N-acyl--2'-deoxynucleosides in tetrahydrofuran these substances were applied in pyridine solutions. By this modified procedure we prepared two protected dinucleotides, d-CA (*XVII*, 58%) and d-TT (*XXI*; 67%), and a protected trinucleotide, d-CAT (*XIX*; 48%) (Scheme 2). In the synthesis of protected d-ATT (*XXIII* (Scheme 3) the

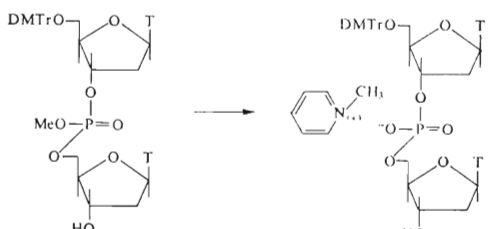


SCHEME 3

$C_{(5')}$-hydroxyl function bearing component was the protected d-TT (*XXII*). In this case the 3'-hydroxyl component was phosphitylated by excess of methylphosphodichloridite which was removed in the next step by precipitation of the resulting phosphomonochloridite with light petroleum and centrifugation. The monochloridite in excess was then reacted with the 5'-hydroxyl component. Yield of isolated *XXIII* reached 77%. All products were isolated by step gradient chromatography accelerated by slight air pressure on spherical silica gel Silpearl. This chromatographic material did not cause any detritylation and therefore the chromatography was performed without addition of base. The combination of phosphite method and pressure forced step gradient chromatography allowed to accomplish one synthetic step in one day.

The phosphite method using methyl phosphodichloridite introduced methyl group as protecting group for the internucleotidic linkage. The idea of using methyl group for the protection of internucleotidic linkage, based on known lability of trimethyl phosphate in alkaline medium, occurred in this Laboratory some time ago. The first experiments to prepare 5'-O-dimethoxytrityl-2'-deoxythymidin 3'-(methylphosphate) by the reaction of the deoxynucleoside derivative with methyl phosphodichloridate in pyridine led to 5'-O-dimethoxytrityl-2'-deoxythymidin 3'-phosphate under the removal of methyl group[11]. In further experiments the P-methyl ester was prepared by other method and the removal of methyl group by aqueous ammonia was tested on HO-dTpMedT-OH (ref.[12]) which procedure unambiguously had to lead, as more recent findings showed, to random splitting of the phosphotriester[13]. The majority of recent users of methyl group took for its removal the unpleasant thiophenol for granted. A non traditional treatment used Ogilvie and coworkers[8] who just heated the methyl-protected oligodeoxyribonucleotide bonded to silica gel support with a mixture of pyridine and aqueous ammonia. We have been testing this approach on DMTr-dTpMedT-f and found no random splitting. TLC of the reaction mixtures

in ammonia system (S_2) showed always an intensive UV-absorbing spot at the origin. We identified this spot as methylpyridinium and found later that the methyl containing phosphotriester, although having been stable in dry pyridine, lost the methyl group in aqueous pyridine solution (Scheme 4). This finding explained lower isolated



XXIV *XXV*

SCHEME 4

yields of our products. The methyl group is also labile in the presence of triethylamine as we found (to our regret) after preservation of a considerable quantity of DMTr-dTp_{Me}dT-OH after chromatographic purification on silica gel in the presence of triethylamine in form of glassy foam at room temperature. After two weeks the substance by the action of triethylamine present was transformed in 90% yield to phosphodiester.

EXPERIMENTAL

Thin-layer chromatography was performed on ready-for-use Silufol UV_{254} (Kavalier Glassworks, Votice, Czechoslovakia) in the solvent systems S_1, chloroform–methanol (9 : 1), S_2, 2-propanol–conc. ammonia–water (7 : 2 : 1), S_3, chloroform–methanol (85 : 15). Column chromatography was performed on macroporous silica gel (Service laboratory of the Institute of Organic Chemistry and Biochemistry) or on spherical silica gel Silpearl (Kavalier Glassworks). Condensation components were dried by dissolving in pyridine and distilling off the pyridine two times. For other methods see loc. cit.[1].

DMTr-dTp_0dT-OH (*I*)

To 5′-O-dimethoxytrityl-2′-O-deoxythymidine (2·2 mmol) the solution of 2-chlorophenyl phosphoditriazolide (*IV*; 2 mmol) in tetrahydrofuran (10 ml) was added. After 1 h the solution was added to the mixture of 3′-O-formyl-2′-deoxythymidine (1·77 mmol) and 4-dimethylaminopyridine (*V*; 17 mmol), evaporated previously twice with pyridine (10 ml). After 2 h the mixture was diluted with a mixture of chloroform (50 ml), pyridine (10 ml) and acetic acid (1·15 ml),

shaken and extracted with two 25 ml portions of water. The chloroform solution was filtered through silica gel (3·5 × 6 cm), washed with chloroform (20 ml), the combined filtrates diluted with toluene (30 ml) and evaporated (40°C). The residue was evaporated with toluene (5 ml), taken in 5 ml and 2 ml portions of chloroform and the solution injected into the mixture of ether (40 ml) and cyclohexane (20 ml). The solid was collected after 20 h at 0°C, washed with the mixture ether–cyclohexane (2 : 1; 20 ml), then with pentane and dried under diminished pressure. The fully protected derivative (R_F-S_1 0·69) was dissolved in 1 mol l^{-1} solution of triethylamine in methanol–tetrahydrofuran (1 : 1; 40 ml) and evaporated (40°C) after 45 min. Yield 1·6 mmol (90%) of *I*, R_F-S_1 0·26.

HO-d$C^{Bz}p_0$dC^{Bz}-f (*VI*)

5'-O-Dimethoxytrityl-N-benzoyl-2'-deoxycytidine (1·9 mmol), was treated with the solution of *o*-chlorophenyl phosphoditriazolide (1·7 mmol) in tetrahydrofuran (10 ml). After 1 h the solution was added to the mixture of 3'-O-formyl-N-benzoyl-2'-deoxycytidine (1·5 mmol) and 4-dimethylaminopyridine (20 mmol) evaporated previously with pyridine, the mixture shaken for 30 min and then allowed to stand 20 h. A mixture of chloroform (50 ml), pyridine (10 ml) and acetic acid (1·3 ml) was added and the solution extracted with two 25 ml portions of water. The chloroform solution was filtered through a column (3·5 × 5 cm) of silica gel, the column was washed with chloroform (50 ml), the combined filtrates diluted with toluene and evaporated (40°C). The residue was evaporated with toluene (30 ml), taken in 5 ml and 3 ml of chloroform and the solutions injected into a mixture of ether and cyclohexane (3 : 1; 80 ml). After 20 h at 0°C the solid was collected, washed with ether–cyclohexane (3 : 1; 80 ml), then with pentane and dried under diminished pressure. The fully protected derivative was dissolved in 80% aqueous acetic acid (50 ml) and, after 45 min, evaporated (20°C). The residue was evaporated with 1-butanol (20 ml), taken to 6 ml, 4 ml and 2 ml of chloroform and the solutions injected into ether (100 ml). After 2 h at 0°C the solid was collected, washed with ether and dried under diminished pressure. Yield, 1·14 mmol (76%), R_F-S_1 0·38.

HO-dTp_0dTp_0d$C^{Bz}p_0$dC^{Bz}-f (*VII*)

The substance *I* (0·8 mmol) evaporated previously with pyridine was treated with the solution of *o*-chlorophenyl phosphoditriazolide (0·7 mmol) in tetrahydrofuran (5 ml) for 3 h and the solution added to the dried mixture of the substance *VI* (0·55 mmol) and 4-dimethylaminopyridine (10 mmol). After 20 h the solution was diluted with a mixture of chloroform (30 ml), pyridine (10 ml) and acetic acid (0·8 ml) and extracted with two portions of water. The solution was diluted with toluene (30 ml) and evaporated (40°C). The residue was dissolved in a mixture of chloroform and pyridine (9 : 1; 50 ml) and the solution filtered through a column (3·5 × 4 cm) of silica gel and the filtrate discarded. The column was then eluted with a mixture of chloroform and pyridine (4 : 1, 100 ml), the filtrate evaporated, and the residue evaporated with toluene (10 ml). The residue was taken to 3 ml, 2 ml and 1 ml of chloroform and the solutions injected into ether (20 ml). After 2 h at 0°C the solid was collected, washed with ether and dried under diminished pressure. The fully protected derivative of d-TTCC, R_F-S_1 0·27, was obtained. A sample of this substance was transformed by the action of conc. ammonia (80°C; 2 h) to d--DMTr-TTCC (R_{Up}-S_2 5·0). The product was dissolved in 80% aqueous acetic acid (10 ml) and, after 45 min, evaporated (20°C). The residue was evaporated with 1-butanol (2 ml), taken to 3 ml, 2 ml and 1 ml of chloroform and the solutions injected into ether (20 ml). After 2 h at 0°C the solid was collected, washed with ether and dried under diminished pressure. Yield, 0·26 mmol (47%), R_F-S_1 0·20.

DMTr-dA^{Bz}p$_0$dA^{Bz}-OH (*II*)

The substance was prepared analogously to *VI* from 5'-O-dimethoxytrityl-N-benzoyl-2'-deoxyadenosine (2·8 mmol) and 3'-O-formyl-N-benzoyl-2'-deoxyadenosine (2·6 mmol). Yield, 1·5 mmol (57%), R_F-S$_1$ 0·27.

HO-dA^{Bz}p$_0$dA^{Bz}p$_0$dTp$_0$dTp$_0$dC^{Bz}p$_0$dC^{Bz}-f (*VIII*)

The condensation was carried out according to *VII*, starting from *II* (0·5 mmol), *II'* (0·45 mmol), *VII* (0·26 mmol) and *V* (4·5 mmol). The reaction mixture was diluted with a mixture of chloroform (30 ml), pyridine (15 ml) and acetic acid (0·3 ml), extracted with two 20 ml portions of water, diluted with the same volume of toluene and evaporated (40 °C). The residue was dissolved in 5% solution of pyridine in chloroform and the solution applied onto a column (3·5 × 4 cm) of silica gel. The column was eluted stepwise with 15 ml portions of chloroform containing 10, 15, 20, 25, 30 and twice 35% of pyridine with slight pressure applied on the top of the column. TLC in S$_1$ showed the presence of the substance in fractions containing 35% of pyridine. The eluate was evaporated, the residue evaporated with two 10 ml portions of toluene. A sample of the fully protected product afforded by the action of conc. ammonia d-DMTr-AATTCC, R_{Up}-S$_2$ 3·00. The product was dissolved in 80% aqueous acetic acid (10 ml) and the solution, after 40 min, evaporated (20°C). The residue was evaporated with 1-butanol (3 ml), taken in 3 ml and 2 ml portions of chloroform and the solution injected into ether (20 ml). After 2 h at 0°C the solid was collected, washed with ether and dried under diminished pressure. Yield, 0·22 mmol (50%), R_F-S$_3$ 0·40.

DMTr-dG^{iBu}p$_0$dG^{iBu}p$_0$dA^{Bz}p$_0$dA^{Bz}p$_0$dTp$_0$dTp$_0$dC^{Bz}p$_0$dC^{Bz}-f (*IX*)

The condensation was carried out according to *VII*, starting from *III* (ref.[1]) (0·45 mmol), *VIII* (0·11 mmol), *IV* (0·4 mmol) and *V* (4 mmol). The reaction mixture was diluted with a mixture of chloroform (30 ml), pyridine (15 ml) and acetic acid (0·3 ml) and extracted with two 20 ml portions of water. The chloroform solution was diluted with toluene (20 ml) and evaporated (40°C). The residue was dissolved in a mixture of chloroform and pyridine (9 : 1; 50 ml) and filtered through a column (3·5 × 4 cm) of silica gel. By application of a slight pressure the column was successively eluted with 50 ml portions of chloroform containing 20, 30, 40, 50, 60 and 70% of pyridine. TLC in S$_3$ showed the presence of the product in eluates containing 50 and 60% of pyridine. These eluates were evaporated, the residue evaporated with toluene and dried in *vacuo*. Yield, 59 μmol (54%), R_F-S$_3$ 0·40.

d-DMTr-GGAATTCC (*X*)

The suspension of *IX* (45 μmol) in 0·3 mol l^{-1} solution of tetramethylguanidinium *p*-benzaldoximate in 50% aqueous dioxane (10 ml) was stirred 20 h and evaporated (40°C). To the residue conc. ammonia (10 ml) and pyridine (0·5 ml) was added and the mixture heated in a closed flask to 55°C for 7 h. The resulting solution was applied onto 2 sheets of paper Whatman 3MM and chromatographed in 2-propanol–conc. ammonia–water (55 : 15 : 30). UV-Absorbing and dimethoxytrityl containing bands (R_F 0·4) were eluted with 1% aqueous ammonia, the eluate diluted with the same volume of 1-propanol (to prevent foaming) and evaporated (40°C) to a volume of 2 ml, diluted again with 1-propanol (10 ml) and evaporated. Yield, 58 mg, R_{Up}-S$_2$ 2·0.

d-GGAATTCC (*XI*)

The substance *X* (56 mg) was dissolved in 80% aqueous acetic acid (5 ml) and the solution, after

30 min, evaporated (20°C). The residue was dissolved in 5% aqueous ammonia (4 ml) and chromatographed on 2 sheets of paper Whatman 3MM in 2-propanol–conc. ammonia–water (45 : 25 : 30). The main UV absorbing bands (R_F 0·55) were eluted with 1% aqueous ammonia, the eluate diluted with the same volume of 1-propanol and evaporated (40°C). The residue was dissolved in water (5 ml) and lyophilized. The product (48 mg), R_{Up}-S_2 1·0 contained traces of faster moving substances. A part of this product (30 mg) was dissolved in 7 mol l^{-1} aqueous urea and chromatographed on a column (2·5 × 80 cm) of DEAE-cellulose (Cl^-) equilibrated with 0·05 mol l^{-1} sodium chloride in 7 mol l^{-1} aqueous urea. The elution was carried out using a linear gradient of sodium chloride in 7 mol l^{-1} urea (1·5 l, 0·05 mol l^{-1} ÷ 1·5, 10·4 mol l^{-1}). After two minor peaks the main peak was eluted with 0·24 mol l^{-1} of sodium chloride. The combined fractions (250 ml) were passed through a column of Sephadex G-10 and the column eluted with water. UV-Absorbing fraction (200 ml) was evaporated, the residue dissolved in water (50 ml) and the Sephadex operation repeated. UV-Absorbing fraction (60 ml; 274 O.D.U.) was evaporated to a volume of 5 ml and stored in frozen state. A sample of the product (2 O.D.U.) was incubated with crude snake venom and the products chromatographed on paper Whatman No 1 in 1-butanol–ethanol–5 mol l^{-1} hydrochloric acid (3 : 2 : 2). Spectrophotometric evaluation of the spots revealed the ratio guanine : adenine : dC : dT (1·1 : 1·05 : 0·85 : 1).

DMT-dC^{Bz}p$_{Me}$dA^{Bz}-OH (*XVII*)

The solution of 5′-O-dimethoxytrityl-N-benzoyl-2′-deoxycytidine (*XII*; 3·5 mmol) in tetrahydrofuran (9 ml) was added to the stirred solution of 2,4,6-collidine (2·75 ml) and methyl phosphodichloridite (*XIII*; 3·85 mmol) in tetrahydrofuran (5 ml) cooled to −78°C. After 15 min, the solution of 3′-O-formyl-N-benzoyl-2′-deoxyadenosine (*XV*; 4·55 mmol) in pyridine (15 ml; prepared by heating the suspension and by quick cooling in ethanol–dry ice mixture) was added. The mixture was stirred another 15 min at −78°C, then 1 h at room temperature, and cooled down to −10°C. The 0·1 mol l^{-1} solution of iodine in 2 : 1 tetrahydrofuran–water was added to remaining slight yellow coloration. Few drops of conc. aqueous solution of sodium thiosulfate were added to remove the coloration, the mixture was diluted with water (100 ml) and extracted with two 100 ml portions of chloroform. The chloroform extracts were diluted with toluene (50 ml) and evaporated (40°C). The residue was dissolved in 1 mol l^{-1} solution of triethylamine in 1 : 1 tetrahydrofuran–methanol (50 ml) and, after 35 min, evaporated (40°C). The residue was dissolved in tetrahydrofuran, the solution diluted with toluene and evaporated again. The residue was dissolved in chloroform and applied onto a column (4 × 8 cm) of Silpearl suspended in chloroform. By application of a slight air pressure the column was eluted with chloroform (500 ml), then with chloroform–methanol 99 : 1 and 98 : 2 (250 ml portions). Fractions of 80 ml were collected in 7 min intervals. TLC in S_1 revealed the presence of the product in eluate with 1% of methanol. The appropriate fractions were evaporated (40°C) and the residue dried at 13 Pa. Yield, 2·02 mmol (58%), R_F-S_1 0·29. A sample of the product by the action of conc. ammonia (80°C; 1 h) was transformed quantitatively to d-DMTr-CA, R_{Up}-S_2 8·0.

DMTr-dC^{Bz}p$_{Me}$dA^{Bz}p$_{Me}$dT-OH (*XIX*)

The solution of *XVII* (3·9 mmol) in tetrahydrofuran (14 ml) was added to the stirred solution of 2,4,6-collidine (4·1 ml) and methyl phosphodichloridite (4·7 mmol) in tetrahydrofuran (5 ml) at −78°C. The progress of the reaction was followed by TLC in S_1. After 35 min, the solution of 3′-O-formyl-2′-deoxythymidine (*XVIII*; 5·85 mmol) in pyridine (10 ml) was added. The mixture was stirred for 10 min at −78°C and 1 h at room temperature. The mixture was now cooled to −10°C and 0·1 mol l^{-1} solution of iodine in 2 : 1 tetrahydrofuran–water was added to re-

maining slight yellow coloration. Few drops of conc. aqueous solution of sodium thiosulfate was added to discolor the solution, water (150 ml) was added and the mixture was extracted with two 150 ml portions of chloroform. The chloroform extract was diluted with toluene (100 ml) and evaporated (40°C). The residue was dissolved in 1 mol l^{-1} solution of triethylamine in 1 : 1 methanol–tetrahydrofuran (50 ml), after 45 min evaporated (40°C), the residue dissolved in tetrahydrofuran (30 ml), the solution diluted with toluene (30 ml) and evaporated again. The residue was dissolved in chloroform (60 ml) and applied onto a column (4 × 11 cm) of Silpearl suspended in chloroform. By application of slight air pressure the column was eluted with chloroform and then with seven 500 ml portions of chloroform containing 1–7% of methanol. Fractions of 80 ml were collected in 7 min intervals and checked in S_1. Fractions containing the product afforded 1·85 mmol (48%) of *XIX*, R_f-S_1 0·24. A sample of the product was heated with conc. ammonia (80°C; 1 h) and afforded d-DMTr-CAT, R_{Up}-S_2 6·6. This substance was detritylated (evaporation, 80% aqueous acetic acid, 40 min, evaporation, trituration with ether) and the resulting d-CAT was cleaved with snake venom diesterase, affording dC, d-pA and d-pT in equimolar quantities.

HO-dTp_{Me}dT-f (*XXII*)

The synthesis of the fully protected derivative *XXI* was performed according to the preparation of *XVII* starting from 5′-O-dimethoxytrityl-2′-deoxythymidine (6·62 mmol). The chloroform extract after iodine oxidation was evaporated, the residue dissolved in 10 ml, 5 ml and 3 ml portions of chloroform and the solutions injected into 1 : 1 ether–cyclohexane (200 ml). The solid was collected, washed with 1 : 1 ether–cyclohexane, then with pentane and dried under diminished pressure. The substance was dissolved in 80% aqueous acetic acid (150 ml) and, after 45 min, evaporated (20°C). The residue was evaporated with 1-butanol (20 ml), dissolved in chloroform (60 ml) and chromatographed on a column (4 × 16 cm) of Silpearl suspended in chloroform. The elution was performed according to preparation of *XIX*, using eleven 250 ml portions of chloroform, containing successively 0–10% of methanol. The product was eluted with 8–9% solution of methanol. Yield, 4·43 mmol (67%), R_f-S_1 0·20. A sample of the product was transformed by the action of conc. ammonia (80°C; 1 h) to d-TT, R_{Up}-S_2 4·8.

DMTr-d$A^{Bz}p_{Me}$dTp_{Me}dT-f (*XXIII*)

The solution of 5′-O-dimethoxytrityl-N-benzoyl-2′-deoxyadenosine (1·5 mmol) in tetrahydrofuran (6·5 ml) was added during 3 min to the stirred and cooled (−78°C) mixture of 2,4,6-collidine (0·39 ml) and methyl phosphodichloridite (3·85 mmol) in tetrahydrofuran (2 ml) placed in 250 ml centrifugation tube. After 15 min the mixture was diluted with light petroleum (150 ml) and centrifuged. The supernatant was decanted, the tube containing the sediment cooled to −78°C and the solution of *XXII* (1 mmol) in pyridine (20 ml) was added. The tube was briefly shaken and then the mixture stirred at −78°C for 1 h, allowed to warm up to −10°C and treated with 0·1 mol l^{-1} solution of iodine in 2 : 1 tetrahydrofuran–water (13 ml). After 5 min the solution was discolored by adding of conc. aqueous sodium thiosulfate, diluted with water (40 ml) and extracted with two 40 ml portions of chloroform. Toluene (30 ml) was added and the solution evaporated (40°C). The residue was dissolved in chloroform (35 ml) and chromatographed (see prep. *XIX*) on 3·5 × 6 cm column of Silpearl, using eight 100 ml portions of chloroform, containing 0–7% of methanol. The substance was eluted by 4–5% methanol solution. Yield, 0·77 mmol (77%), R_f-S_1 0·26. A sample of the product was transformed by the action of conc. ammonia (80°C, 1 h) to d-DMTr-ATT, R_{Up}-S_2 6·6. Detritylation and snake venom diesterase digestion (see prep. *XIX*) afforded dA and d-pT (1 : 2·05).

Reaction of DMTr-dTp$_{Me}$dT-OH (*XXIV*) with Pyridine

Samples of *XXIV* (3 mg) were dissolved in 0·1 ml portions of *a*) pyridine, *b*) 99%, *c*) 95% and *d*) 50% aqueous pyridine and heated to 60°C for 5 h. TLC in S_1 showed the dimethoxytrityl containing spot on the origin (phosphodiester) in experiments: *a*) traces, *b*) *c*. 20%, *c*) *c*. 80%, *d*) 100%. TLC in S_2 showed UV-absorbing spot of N-methylpyridinium on the origin of increasing intensity from *a*) to *d*).

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