3′-O-FORMYL-(N-ACYL)-2′-DEOXYRIBONUCLEOSIDES AS BUILDING UNITS IN THE SYNTHESIS OF OLIGODEOXYRIBONUCLEOTIDES*)**)

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Received January 4th, 1982

5′-O-Dimethoxytrityl-(N-acyl)-2′-deoxyribonucleosides afford 3′-O-formyl-(N-acyl)-2′-deoxyribo nucleosides *Ia—Id* by the action of formic acetic anhydride followed by the action of 80% aqueous acetic acid. The formyl group is removed from *Ia—Id* by treatment with 1 mol l^{-1} triethylamine 3′-O-Formyl-2′-deoxythymidine (*Ia*) gives 3′-O-dimethoxytrityl-2′-deoxythymidine (*V*) by subsequent treatment with acetic anhydride, triethylamine, dimethoxytrityl chloride and methanolic ammonia. The use of compounds *I* for the synthesis of d-GGAGG (*XIX*) and d-T_{16} (*XXXII*) is described. Systems for thin-layer chromatography of 5′-O-dimethyltrityl-oligodeoxyribonucleotides on silica gel are described.

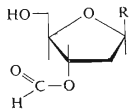
Recent synthetic procedures for oligodeoxyribonucleotides utilized exclusively the bulky dimethoxytrityl group for the protection of building units. As yet, no satisfactorily versatile protecting group for the 3′-OH function removable without impairing the other parts of the fully protected phosphotriester was discovered.

It was recently found that the smallest one of the protecting groups – the formyl group – may meet the requirements. This group was formerly studied[2] and used[3] in this Laboratory in the synthesis of oligoribonucleotides. The conditions for removal of the formyl group[3] were relatively mild (0·1M triethylammonium hydrogen carbonate, pH 8·5, in 90% aqueous methanol). With the aim in mind to use the formyl protected deoxyribonucleosides in solid phase synthesis of oligodeoxyribonucleotides, we considered the water containing reagents not suitable. Therefore methanolysis catalysed by triethylamine was checked. This reaction was tested on 5′-O-dimethoxytrityl-3′-formyl-N-benzoyl-2′-deoxyadenosine, which was prepared by the action of formic acetic anhydride in pyridine on 5′-O-dimethoxytrityl-N-benzoyl-2′-deoxyadenosine. The formyl group was removed by 1 mol l^{-1} triethylamine in methanol in 5–6 min. Thereafter, all four 3′-O-formyl-(N-acyl)-2′-deoxyribonucleosides (*Ia—d*; Scheme 1) were prepared from the corresponding 5′-O-dimethoxytrityl

* Part LXIX in the series Oligonucleotidic Compounds; Part LXVIII: This Journal *47*, 156 (1982).

** The results were preliminarily published[1].

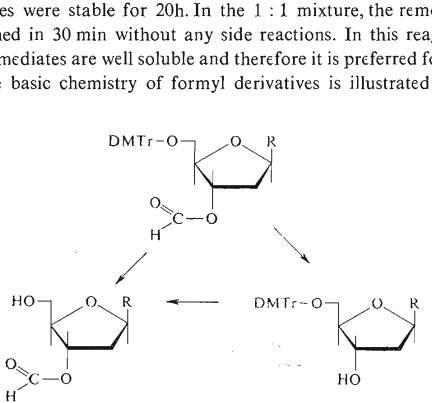
derivatives by the action of formic acetic anhydride followed by detritylation with 80% aqueous acetic acid. Depurination of 2'-deoxyadenosine by means of 80% aqueous acetic acid in case of its 3'-O-formyl derivative was not observed.



Ia, R = thymine (T)
Ib, R = N-benzoylcytosine (C^{Bz})
Ic, R = N-benzoyladenine (A^{Bz})
Id, R = N-isobutyrylguanine (G^{iBu})

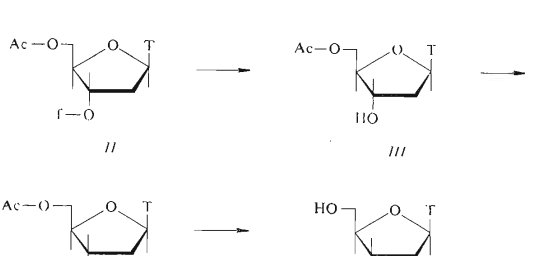
SCHEME 1

Compounds *Ia*–*Id* were transformed to the corresponding (N-acyl)-2'-deoxyribonucleosides by 1 mol l^{-1} triethylamine in methanol during 5–7 min at 20°C. Prolonged treatment showed slow removal of isobutyryl group from deoxyguanosine derivative and benzoyl group from deoxycytidine derivative. Further experiments showed, that 2-chlorophenyl group in phosphotriester and acetyl group on 5'-OH function are stable in deformylation reagent. In order to moderate the rate of methanolysis of the formyl group, 1 mol l^{-1} triethylamine solutions in methanol–tetrahydrofuran mixture were checked. In the mixture containing 90% tetrahydrofuran, the formyl derivatives were stable for 20h. In the 1 : 1 mixture, the removal of formyl group was finished in 30 min without any side reactions. In this reagent the oligonucleotidic intermediates are well soluble and therefore it is preferred for the synthesis in solution. The basic chemistry of formyl derivatives is illustrated by Scheme 2.



SCHEME 2

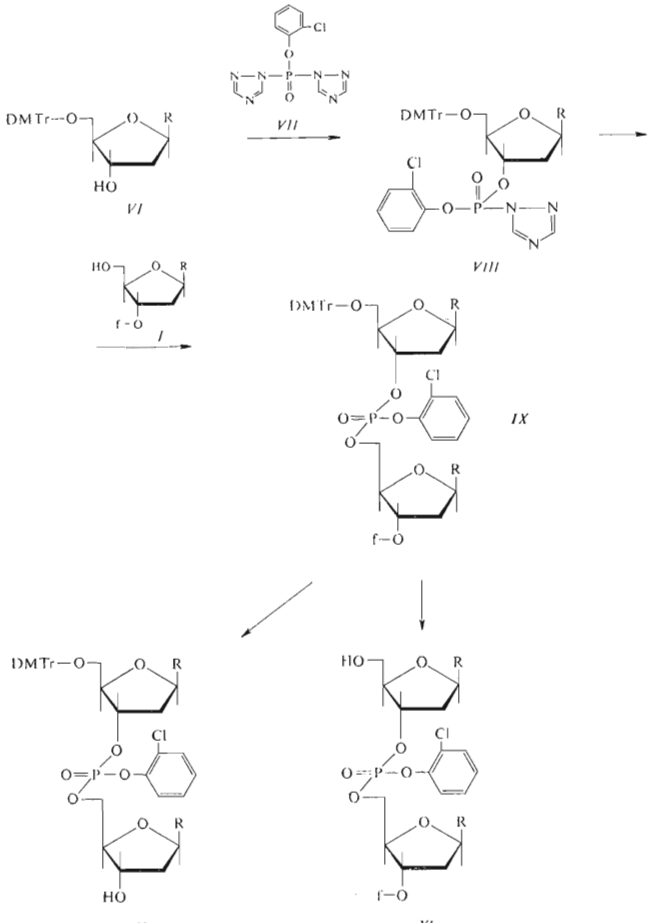
In order to verify versatility of the formyl group, synthesis of 3'-O-dimethoxytrityl--2'-deoxythymidine (*V*) was accomplished, starting from *Ia* (Scheme 3). The compound *V* was isolated in 55% yield after a series of transformations without isolation of intermediates. It differs from the isomeric 5'-O-dimethoxytrityl-2'-deoxythymidine by higher mobility on TLC in chloroform–methanol mixture.



SCHEME 3

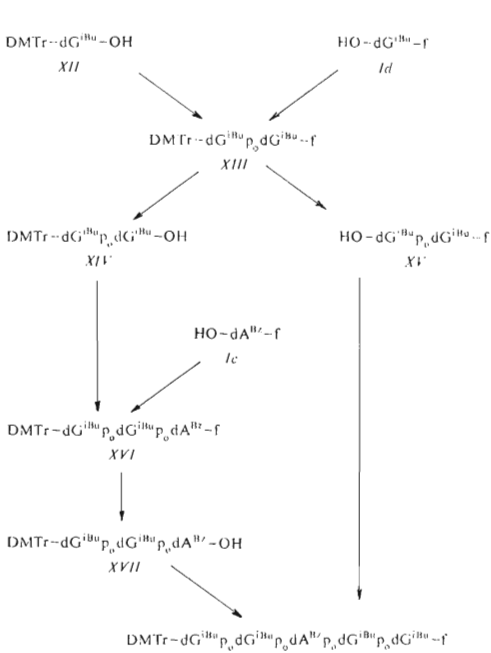
The 3'-O-formyl-2'-deoxyribonucleosides *Ia–Id* may find, due to the minimal steric influence of protection group and to its easy removal, utilization in the synthesis of oligodeoxyribonucleotides as components bearing the free 5'-OH function. As phosphate component 3'-phosphodiester in the presence of condensing agent or 3'-phosphoromonotriazolide in the presence of nucleophilic catalyst[4–7] may serve. In this paper, results of the use of the latter procedure are described (Scheme 4). The product was fully protected phosphotriester *IX*, which could be selectively deprotected either on 3'-OH function, giving the compound *X*, or on the 5'-OH function, giving the compound *XI*. Both of them might serve as components for the synthesis of further internucleotide linkage.

The general Scheme 4 was utilized for the synthesis of purine pentanucleotide d-GGAGG (*XIX*). 5'-O-Dimethoxytrityl-N-isobutyryl-2'-deoxyguanosine (*XII*) served as starting component (Scheme 5). The phosphomonotriazolides were prepared according to[6]. Copulation mixtures were saturated with carbon dioxide before extraction with water in order to prevent the removal of formyl and/or 2-chlorophenyl groups by the action of 4-dimethylaminopyridine in the presence of water. The intermediates were partially purified by precipitation of chloroform solutions with ether. The pentanucleotide derivative *XVIII* was deblocked by subsequent treatment with 4-nitrobenzaldoximtetramethylguanidin[8,9], concentrated ammonia and



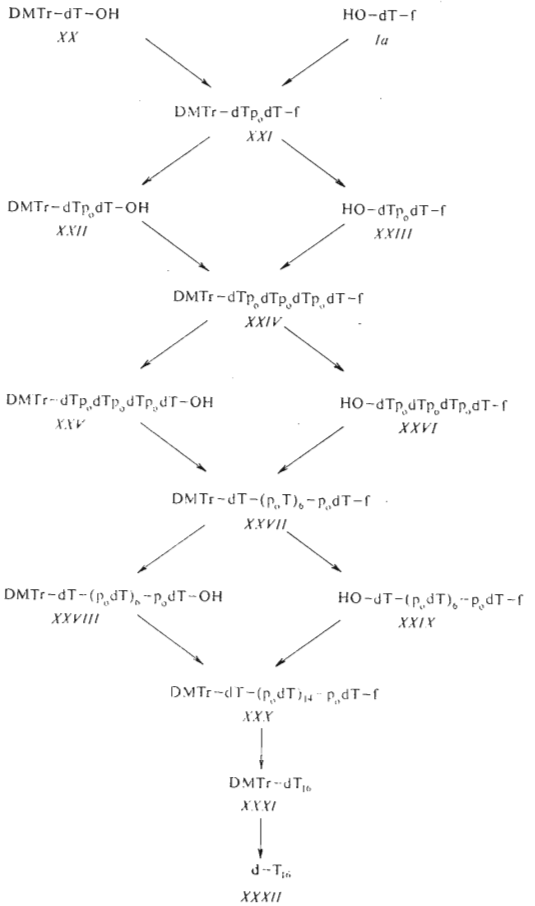
SCHEME 4

80% aqueous acetic acid, and isolated by sodium chloride gradient from DEAE-cellulose in 7 mol l^{-1} urea. The last peak was desalted by absorption of DEAE-cellulose and eluted with 1 mol l^{-1} tetraethylammonium hydrogen carbonate.



SCHEME 5

The second synthesis leading to hexadecathymidylate (*XXXII*) is illustrated by Scheme 6. The approach is characterized by stepwise forming of dinucleotide, tetranucleotide and octanucleotide blocks, each of them being divided in two halves.


DMTr-dT-OH
XX
HO-dT-f
Ia
DMTr-dTp5dT-f
XXI
DMTr-dTp5dT-OH
XXII
HO-dTp5dT-f
XXIII
DMTr-dTp5dTp5dTp5dT-f
XXIV
DMTr-dTp5dTp5dTp5dT-OH
XXV
HO-dTp5dTp5dTp5dT-f
XXVI
DMTr-dT-(p5T)6-p5dT-f
XXVII
DMTr-dT-(p5dT)6-p5dT-OH
XXVIII
HO-dT-(p5dT)6-p5dT-f
XXIX
DMTr-dT-(p5dT)14-p5dT-f
XXX
DMTr-dT16
XXXI
dT16
XXXII

SCHEME 6

One half was deprotected on the 5'-OH function, the second on the 3'-OH function and both connected to doubled chain. It was found in the first procedure, when approximately equimolar amounts of components and reagents were used, that the rate of phosphorylation to monotriazolides slowed down in tetranucleotide and octanucleotide steps and the same the rate of copulation of blocks. In the repeated preparation of *XXXII*, the formation of phosphomonotriazolides from *XXV* and *XXVIII* were performed by use of excess of 2-chlorophenylphosphoditriazolide, which led to fast phosphorylation. The phosphomonotriazolide formed was then separated from excess of the reagent by precipitation with ether and centrifugation. For copulation to *XXVII* and *XXX* higher excess of nucleophilic catalyst was used[10]. The tetranucleotide derivative *XXIV* was purified by removal of low R_F possessing substance by partial ether precipitation. The octanucleotide intermediate was purified by silica gel chromatography in chloroform pyridine system.

As for isolation of the oligonucleotidic products, the first procedure was finished by lithium chloride gradient elution from DEAE-celulose followed by desalting by means of Sephadex G 10. In the second procedure the last fully protected crude derivative *XXX* was processed with oximate. This procedure afforded dimethoxytrityl containing material with high R_F in S_3, which indicated that the intermediate phospho-4-nitrobenzaldoxime derivative might not be hydrolysed, at least in interior of the chain. The spot of low $R_F(S_3)$ appeared only after ammonia treatment. From the reaction mixture, the 5'-O-dimethoxytrityl-d-T_{16} (*XXXI*) was isolated by preparative paper chromatography and its dimethoxytrityl group content was determined. Pure *XXXI* was deblocked by 80% aqueous acetic acid and the compound *XXXII* isolated by preparative paper chromatography.

For monitoring of the chain elongation, TLC on silica gel sheets in modified ammonia systems was applied. The classical system S_8 gives high and not well distinguished R'_F's. In the system 2-propanol–conc. ammonia–water (6 : 3 : 1), the dimethoxytrityl derivatives of d-T_4, d-T_8 and d-T_{16} showed the R'_F's 0·80, 0·60 and 0·35. In the system 2-propanol–conc. ammonia (1 : 1; 25°C) the oligothymidylates d-T_4, d-T_8 and d-T_{16} showed R'_F's 0·48, 0·28 and 0·20.

EXPERIMENTAL

Thin-layer chromatography was performed on ready-for-use Silufol UV_{254} silica gel foils (Kavalier Glassworks, Votice, Czechoslovakia) in the solvent systems S_1, chloroform–methanol (9 : 1), S_2, chloroform–methanol (85 : 15), S_3, 2-propanol–conc. ammonia–water (6 : 3 : 1), S_4, 2-propanol–conc. ammonia (55 : 45), S_5, 2-propanol–conc. ammonia–urea (55 : 44 : 1), S_6, chloroform–methanol (8 : 2), S_7, 2-propanol–conc. ammonia–water (6 : 2 : 2), S_8, 2-propanol–conc. ammonia–water (7 : 1 : 2), S_9, 2-propanol–conc. ammonia water (5 : 2 : 3), S_{10}, 2-propanol–conc. ammonia (1 : 1; 25°C). Unless stated otherwise, R_F's refer to TLC. Column chromatography was performed on macroporous silica gel (Service Laboratory of this Institute). Solutions were taken down on a rotatory evaporator at 40°C, 2 kPa or at 20°C, 130 Pa (1 Torr) on a rotatory evaporator equiped with dry-ice condenser.

The reactions with 2-chlorophenylphosphoditriazolide and the synthesis of internucleotidic phosphotriester bonds were followed by *TLC* in systems S_1 or S_2, dimethoxytrityl containing spots being visualized by spraying with 10% aqueous perchloric acid.

3′-O-Formyl-(N-acyl)-2′-deoxyribonucleosides *Ia—Id*

The solution of 5′-O-dimethoxytrityl-(N-acyl)-2′-deoxyribonucleoside (10 mmol) in pyridine (100 ml) is cooled down in dry ice–ethanol mixture to partial solidification. Formic acetic anhydride (30 ml) is added, the mixture briefly shaken and kept at room temperature till the end of carbon monooxide evolution (*c.* 1 h). Methanol (20 ml) is added and, after 20 min, the mixture is diluted with chloroform (200 ml). The solution is extracted with two 50 ml portions of water, diluted with toluene (100 ml) and evaporated (40°C). The residue is evaporated with several 50 ml portions of toluene (five are usually enough) to afford a solid foam. The foam is shaken with 80% aqueous acetic acid (100 ml), dT and dA derivative 30 min. dC and dG derivative 45 min. The solution is evaporated (20°C), the residue it evaporated with two 30 ml portions of 1-butanol and triturated with ether (200 ml). The mixture is diluted with cyclohexane (80 ml) and, after 20 h at 0°C, the solid is collected, washed with ether–cyclohexane mixture (3 : 1), then with pentane and dried under diminished pressure. 3′-O-Formyl-2′-deoxythymidine (*Ia*), 90% yield. M.p. 150—152°C, R_F–S_1 0·30. 3′-O-Formyl-N-benzoyl-2′-deoxycytidine (*Ib*), 90% yield. M.p. 192—193°C, R_F–S_1 0·35. 3′-O-Formyl-N-benzoyl-2′-deoxyadenosine (*Ic*), 88% yield. M.p. 175—177°C. R_F–S_1 0·33. 3′-O-Formyl-N-isobutyryl-2′-deoxyguanosine (*Id*), 90% yield. M.p. 119—121°C, R_F–S_1 0·18.

3′-O-Dimethoxytrityl-2′-deoxythymidine (*V*)

To the solution of 3′-O-formyl-2′-deoxythymidine (1 mmol) in pyridine (5 ml), acetic anhydride (2·5 ml) is added. After 1 h at room temperature (R_F–S_1 0·25→0·35), the mixture is chilled with ice, methanol (5 ml) is added and, after 20 min, the solution is evaporated (40°C). The residue is distributed between chloroform (30 ml) and water (10 ml), the chloroform layer extracted with water (10 ml), diluted with toluene (20 ml) and evaporated (40°C). The residue is evaporated with three 10 ml portions of toluene and the resulting 5′-O-acetyl-3′-O-formyl-2′-deoxythymidine (*II*) is dissolved in 1 mol l^{-1} solution of triethylamine in methanol–tetrahydrofuran (1 : 1; 5 ml). After 30 min, the solution is evaporated (40°C) and the residue is evaporated with two 5 ml portions of toluene. The resulting 5′-O-acetyl-2′-deoxythymidine (*III*; m.p. 144—145°C, R_F–S_1 0·13) is dissolved in pyridine (5 ml), dimethoxytrityl chloride (1·5 mmol) is added, the mixture briefly shaken a then kept at room temperature for 1 week. Methanol (1 ml) is added and, after 1 h, the solution is diluted with chloroform (20 ml) and extracted with two 7 ml portions of 10% aqueous ethanol. The chloroform solution is diluted with toluene (10 ml), evaporated and the residue evaporated with two 10 ml portions of toluene. The resulting 5′-O-acetyl-3′-O-dimethoxytrityl-2′-deoxythymidine (R_F–S_1 0·56) is dissolved in 6 mol l^{-1} methanolic ammonia (20 ml). After 20 h at room temperature, the solution si evaporated, the residue dissolved in benzene (20 ml), and the solution diluted with cyclohexane (10 ml). After 20 h at 0°C the solid is collected, washed with benzene–cyclohexane (1 : 1), then with pentane and dried under diminished pressure. Yield 55% of *V*. M.p. 128—9°C. For $C_{31}H_{32}N_2O_7$ (544·6) calculated: 68·50% C, 5·93% H, 5·15% N; found: 68·10% C, 6·03% H, 5·09% N. R_F–S_{10} 0·37 (The R_F–S_1 of 5′-O-dimethoxytrityl isomer is 0·25).

DMTr-dG$^{iBu}_{p_0}$dG^{iBu}-f (*XIII*)

The solution of 5'-O-dimethoxytrityl-N-isobutyryl-2'-deoxyguanosine (*XII*; 1·1 mmol) in pyridine (10 ml) is evaporated and the solution of 2-chlorophenylphosphoditriazolide (*VII*; 1 mmol) in tetrahydrofuran (10 ml) is added to the residue. After 2 h (R_F–S_2, the dimethoxytrityl containing material was transformed, 0·45→0·10) the solution is added to the mixture of 3'-O-formyl--N-isobutyryl-2'-deoxyguanosine (*Id*; 1·1 mmol) and 4-dimethylaminopyridine (3·5 mmol) evaporated (20°C) previously with pyridine (10 ml). After 20 h a piece of dry ice is added, the mixture diluted with chloroform (30 ml) and extracted with two 10 ml portions of water. The chloroform solution is diluted with toluene (20 ml) and evaporated (40°C). The residue is evaporated with toluene (10 ml), taken in 3 ml, 2 ml and 1 ml portions of chloroform and the solutions injected into ether (80 ml). The solid is collected, washed with ether and dried under diminished pressure. The substance was dissolved in chloroform (10 ml) and the solution diluted with ether (30 ml). The solid was collected, washed with ether and dried under diminished pressure. Yield 81% of *XIII*, R_F–S_2 0·52. A sample of the product heated with conc. ammonia (80°C, 1 h) gives d-DMTr-GpG (R_{Up}–S_3 6·3) which is transformed by the action of 80% aqueous acetic acid to d-GpG (R_{Up}–S_3 2·0). Snake venom diesterase splits this compound to dG and d-pG (0·95 : 1).

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

The solution of *XIII* (0·2 mmol) in 1 mol l^{-1} solution of triethylamine in methanol–tetrahydrofuran (1 : 1; 5 ml) is after 30 min evaporated (40°C), the residue evaporated (20°C) with two 5 ml portions of pyridine and the resulting compound *XIV* (R_F–S_2 0·33) is treated with the solution of 2-chlorophenylphosphoditriazolide (0·19 mmol) in tetrahydrofuran (2 ml). After 2 h the solution is added to the mixture of 2'-O-formyl-N-benzoyl-2'-deoxyadenosine (*Ic*; 0·2 mmol) and 4-dimethylaminopyridine (0·6 mmol) previously evaporated (20°C) with pyridine (10 ml). To the mixture, a piece of dry ice is added after 20 h, the solution diluted with chloroform (30 ml) and extracted with two 10 ml portions of water. The chloroform solution is diluted with toluene (15 ml), evaporated and the residue evaporated with toluene (5 ml). The residue is taken in 3 ml, 2 ml and 1 ml portions of chloroform and the solutions injected into ether (25 ml). The solid is collected, washed with ether and dried under diminished pressure. Yield 80% of *XVI*, R_F–S_2 0·52, containing *c.* 5% of substance R_F–S_2 0·1.

DMTr-dG^{iBu}p$_0$dG^{iBu}p$_0$dA^{Bz}-OH (*XVII*)

The solution of *XVI* (450 mg) in 1 mol l^{-1} solution of triethylamine in methanol–tetrahydrofuran mixture (1 : 1; 15 ml) is, after 20 min, evaporated (40°C). The residue is dissolved in chloroform (5 ml) and chromatographed on column (3 × 12 cm) of silica gel, suspended in chloroform–triethylamine (99 : 1). Portions (250 ml) of this mixture diluted stepwise with 5, 10 and 15 ml of methanol are used for elution. The main dimethoxytrityl containing fraction is evaporated (40°C), the residue taken subsequently to 3 ml and 2 ml portions of chloroform and the chloroform solutions injected into ether (20 ml). The solid is collected, washed with ether and dried under diminished pressure. Yield 250 mg; 58% of *XVII*, R_F–S_2 0·34. A sample of the product by heating with conc. ammonia (80°C, 2 h) is transformed to d-DMTr-GpGpA (R_{Up}–S_4 2·5). This substance gives by the action of 80% aqueous acetic acid (20 min, 20°C) d-GpGpA (R_{Up}–S_4 2·3) as the main product. The eluate of the main spot is cleaved with snake venom diesterase to dG, d-pG and d-pA (0·9 : 1·05 : 1).

HO-dG^{iBu}p$_0$dG^{iBu}-f (*XV*)

The solution of *XIII* (0·3 mmol) in 80% aqueous acetic acid is kept at 20°C for 45 min and evaporated (20°C). The residue is evaporated with 1-butanol (5 ml), taken in 3 ml and 2 ml portions chloroform and the chloroform solutions injected into ether (20 ml). The solid is collected, washed with ether and dried under diminished pressure. Yield 90% of *XV*, R_F–S_2 0·46.

d-GGAGG (*XIX*)

The solution of *XVII* (0·1 mmol) in pyridine (5 ml) is evaporated (20°C) and the residue treated with the solution of 2-chlorophenylphosphoditriazolide (0·1 mmol) in tetrahydrofuran (0·9 ml). After 5 h the solution is added to the mixture of the substance *XV* (0·15 mmol) and 4-dimethylaminopyridine (0·5 mmol), previously evaporated (20°C) with pyridine (5 ml). After 20 h the reaction mixture is injected into ether (20 ml), the solid is collected, washed with ether and dried under diminished pressure. Crude *XVIII* (270 mg; R_F–S_2 0·45) is dissolved in 0·3 mol l^{-1} solution of *p*-nitrobenzaldoximtetramethylguanidin in 50% aqueous dioxane. The solution is kept for 20 h, diluted with water (5 ml) and neutralised with acetic acid (0·28 ml). The solution is extracted with three 10 ml portions of ether, diluted with pyridine (10 ml) and evaporated (40°C). The residue is dissolved in conc. ammonia (5 ml) and the solution heated in closed flask to 55°C for 5 h. After cooling, the solution is diluted with 1-propanol (10 ml) and evaporated (40°C). The residue is evaporated with 1-propanol (10 ml), dissolved in 80% aqueous acetic acid (10 ml) and after 20 min evaporated (20°C). The residue is dissolved in 7 mol l^{-1} aqueous urea (5 ml), conc. ammonia (0·05 ml) is added and the solution applied onto a column (2·5 × 80 cm) of DEAE-celulose (Cl^-), equilibrated in 7 mol l^{-1} urea. Elution is performed with linear gradient of sodium chloride, (1·5 l 0·05 mol l^{-1} — 1·5 l 0·3 mol l^{-1}). Four UV-absorbing peaks are eluted. The last one, eluted with 0·2 mol l^{-1} sodium chloride (250 ml) is diluted with water to a volume of 1·5 l and the solution passed through a column (2·5 × 15 cm) of DEAE-celulose (HCO_3^-). The column is eluted with 0·06 mol l^{-1} triethylammonium hydrogen carbonate (400 ml) and the substance is eluted with 1 mol l^{-1} solution of the same buffer. UV-Absorbing peak (715 O.D.U. is diluted with the same volume of 1-propanol, evaporated (40°C) and the residue evaporated with three 5 ml portions of ethanol. The residue is dissolved in water (3 m) and lyophilised. Yield 35·5 mg of *XIX*, R_{Up}–S_5 1·1. Snake venom diesterase digest the product to dG, d-pG and d-pA (1·1 : 3·05 : 1).

DMTr-dTp$_0$-dT-f (*XXI*)

The solution of 5′-O-dimethoxytrityl-2′-deoxythymidine (*XX*; 2 mmol) in pyridine (20 ml) is evaporated (20°C) and the residue treated with the solution ol 2-chlorophenylphosphoditriazolide (2 mmol) in tetrahydrofuran (20 ml). After 1 h (R_F–S_1 0·13) the solution is added to the mixture of 3′-O-formyl-2′-deoxythymidine (*Ia*; 2 mmol) and 4-dimethylaminopyridine (6 mmol) previously evaporated (20°C) with pyridine (20 ml). The reaction mixture is kept for 20 h (R_F–S_1 0·35, 90%), a piece of dry-ice is added, the solution diluted with chloroform (30 ml) and extracted with two 10 ml portions of water. The chloroform solution is diluted with toluene (15 ml) and evaporated (40°C). The residue is evaporated with toluene (10 ml), taken to 4 ml, 2 ml and 1 ml portions of chloroform and the solutions injected into the mixture of ether–cyclohexane (4 : 1, 50 ml). After 3 h at 0°C the solid is collected, washed with the mixture of ether–cyclohexane (4 : 1), then with pentane and dried under diminished pressure. Yield 85% of *XXI*, R_F–S_1 0·65 with traces of R_F 0·10. A sample of the product is tranformed to d-DMTr-TpT (R_{Up}–S_3 3·5) by heating with conc. ammonia (80°C, 2 h).

HO-dTp$_0$dT-f (*XXIII*)

The solution of *XXI* (0·5 mmol) in 80% aqueous acetic acid is, after 30 min, evaporated (20°C), the residue evaporated with 1-butanol (5 ml), taken to 3 ml, 2 ml and 1 ml portions of chloroform and the solutions injected into ether–cyclohexane (4 : 1; 50 ml). After 1 h at 0°C the solid is collected, washed with ether–cyclohexane (4 : 1) then with pentane and dried under diminished pressure. Yield 97% of *XXIII*, R_F–S_2 0·25.

DMTr-dTp$_0$dTp$_0$dTp$_0$dT-f (*XXIV*)

The solution of *XXI* (0·5 mmol) in 1 mol l^{-1} solution of triethylamine in methanol–tetrahydrofuran (1 : 1; 10 ml) is, after 30 min at 30°C, evaporated (40°C), the residue evaporated (20°C) with two 10 ml portions of pyridine and the residue treated with the solution of 2-chlorophenylphosphoditriazolide (0·46 mmol) in tetrahydrofuran (5 ml). After 2 h (R_F–S_1 0·15, 95%) the solution is added to the mixture of *XXIII* (0·5 mmol) and 4-dimethylaminopyridine (2·5 mmol) evaporated (20°C) previously with pyridine (10 ml). The reaction mixture is kept for 20 h, ether (2·5 ml) is added and the mixture shaken for 15 min. The solution is decanted from resinuous precipitate and diluted with ether (40 ml). After 20 h at 0°C the solid is collected, washed with ether and dried under diminished pressure. Yield 49% of *XXIV*, R_F–S_2 0·50. A sample of the product is transformed by heating with conc. ammonia (80°C, 2 h) to d-DMTr-TpTpTpT R_{Up}-S_3 3·1.

HO-dTp$_0$dTp$_0$dTp$_0$dT-f (*XXVI*)

The substance is prepared according to the preparation *XXIII*. Yield 97%, R_F–S_1 0·22.

DMTr-dT-(p$_0$dT)$_6$-p$_0$dT-f (*XXVII*)

The solution of *XXVI* (0·25 mmol) in 1 mol l^{-1} triethylamine in methanol–tetrahydrofuran (1 : 1; 15 ml) is, after 30 min, evaporated (40°C), the residue is evaporated (20°C) with two 10 ml portions of pyridine and the residue is treated with the solution of 2-chlorophenylphosphoditriazolide (0·5 mmol) in tetrahydrofuran (4 ml). After 1 h (R_F–S_2 0·25) ether (40 ml) is added and the solid centrifuged under exclusion of atmospheric moisture. A solution of the compound *XXVI* (0·24 mmol) and 4-dimethylaminopyridine (3·8 mmol in pyridine (3 ml)), evaporated (20°C) previously with two 10 ml portions of pyridine, is added to the sediment. After 20 h (R_F–S_1 0·39, 80%), the reaction mixture is injected into ether (200 ml), the solid is collected, washed with ether and dried under diminished pressure. The crude product is dissolved in chloroform–pyridine (9 : 1; 5 ml) and chromatographed on a column (50 ml; 1 : 3) of silica gel suspended in chloroform–pyridine (9 : 1). The column is eluted wit 100 ml portions of chloroform containing 10–40% of pyridine, (20 ml fractions, 2 min). Fractions 10–19, are evaporated to a volume of *c.* 3 ml and diluted with ether (100 ml). After 20 h at 0°C the solid is collected, washed with ether and dried under diminished pressure. Yield 46% of *XXVII*, R_F–S_2 0·45. A sample of the product affords by heating with conc. ammonia (80°C, 2 h) d-DMTr-Tp(Tp)$_6$T, R_{Up}–S_3 1·7.

HO-dT-(p$_0$dT)$_6$-p$_0$dT-f (*XXIX*)

The compound was prepared according to the preparation *XXIII*. R_F–S_2 0·34.

DMTr-dT-(p_0dT)$_{14}$-p_0dT-f (*XXX*)

The solution of *XXVII* (50 μmol) in 1 mol l^{-1} triethylamine in methanol–tetrahydrofuran (1 : 1; 10 ml) is, after 30 min, evaporated (40°C), the residue evaporated (20°C) with two 5 ml portions of pyridine and treated with a solution of 2-chlorophenylphosphoditriazolide (150 μmol) in tetrahydrofuran (1·5 ml). After 1 h (R_F–S_2 0·23) the solution is injected into absolute ether (40 ml). The solid is centrifuged under exclusion of atmospheric moisture and treated with the solution of compound *XXIX* (55 μmol) and 4-dimethylaminopyridine (1·1 mmol) evaporated previously with two 10 ml portions of pyridine, in pyridine (3 ml). The reaction mixture is kept as 37°C for 2 days, injected into ether (50 ml) and the solid is collected and dried under diminished pressure. Yield of crude *XXX* (292 mg) R_F–S_6 0·15—0·35 (main spot) and 0·75 (minor spot). In this system, the octanucleotide derivative *XXVII* has R_F 0·77.

DMTr-d-T_{16} (*XXXI*)

The crude *XXX* (190 mg) is dissolved in 0·3 mol l^{-1} solution of *p*-nitrobenzaldoximtetramethylguanidine in 50% aqueous dioxan (10 ml). After 20 h (R_{Up}–S_3 2·3) the solution is evaporated (40°C) to a volume of 1 ml and conc. ammonia (2 ml) is added. The mixture is heated to 55°C for 6 h in a closed flask, cooled, spotted on two sheets of Whatman 3 MM paper and chromatographed in S_7 for 16 h. UV Absorbing and dimethoxytrityl containing bands (R_{Up} 0·9—1·0) are eluted with 1% aqueous ammonia. The eluate (10 ml) is diluted with 1-propanol (10 ml) and evaporated (40°C). The residue is dissolved in water and lyophilised. Yield 66 mg of *XXXI*, R_{Up}–S_7 1·1. The ratio of dT (measured at 260 nm) and dimethoxytrityl group (measured in perchloric acid–ethanol at 550 nm) is 14·1 : 1.

d-T_{16} (*XXXII*)

A) The synthesis is performed without purification of intermediates starting from *XX* (1 mmol) and *Ia* (1 mmol). The reaction steps are performed according the preparation of *XIII*. The products of individual condensation steps are divided in two halfs, one half deblocked on the 3′-OH function, the second on the 5′-OH function and processed according Scheme 6. The reactions with 2-chlorphenylphosphoditriazolide with the compound *XXV* are performed 8 h, with the compound *XVIII* 20 h. The synthesis of *XXVII* and *XXX* are performed at 37°C (20 h and 45 h resp.). The crude *XXX* (339 mg) is deblocked according the preparation of *XXXI*. The solution is diluted with 1-propanol, evaporated (40°C) the residue evaporated with 1-propanol and dissolved in 80% aqueous acetic acid (5 ml). After 30 min the solution is evaporated (20°C), the residue dissolved in 1 mol l^{-1} ammonia (10 ml) and extracted with three portions of ether. The aqueous solution is chromatographed on a column (2·5 × 90 cm) of DEAE cellulose (Cl^-) using linear gradient of lithium chloride (3 l of 0·05 mol l^{-1}) 3 l of 0·65 mol l^{-1}. Three main peaks are eluted. The fractions of the last one (eluted 0·42 mol l^{-1} lithium chloride) is evaporated to a volume of 30 ml and passed through a column (4·5 × 45 cm) of Sephadex G 10 and eluted with water. UV-absorbing eluate (400 O.D.U.) is concentrated to a small volume and lyophilised. Yield 13 mg of *XXXII*, identical with the product prepared by the procedure *B*.

B) The solution of *XXXI* (60 mg) in 80% aqueous acetic acid is, after 30 min, evaporated (20°C). The residue is dissolved in water, neutralised with conc. ammonia and extracted with two 5 ml portions of ether. The water solution is evaporated to a volume of 2 ml and spotted on two sheets of Whatman 3 MM paper. The paper is developed in S_9 (20 h). UV-Absorbing bands (R_F 0·50) are eluted with 1% aqueous ammonia, the eluate (15 ml) is diluted with 1-propanol

(15 ml) and evaporated (40°C). The residue is dissolved in water (3 ml; 830 O.D.U.) and lyophilised. Yield 39 mg of *XXXII*, R_{Up}–S_9 (paper Whatman No 1) 0·30; R_{Up}–S_{10} 0·70. Snake venom diesterase digests the product to dT and d-pT (ratio 1 : 15·4).

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Translated by the author.