SYNTHESIS OF OLIGODEOXYRIBONUCLEOTIDES USING *in situ* PREPARED NUCLEOSIDE METHYL PHOSPHOCHLORIDITE INTERMEDIATES*

Jiří Smrt[a], Hana Večerková[b], Jitka Forstová[b], Zdeněk Hostomský[b] and André Rosenthal[c]

[a] *Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6, Czechoslovakia*
[b] *Institute of Molecular Genetics, Czechoslovak Academy of Sciences, 166 10 Prague 6, Czechoslovakia and*
[c] *Zentralinstitut für Molekularbiologie, Akademie der Wissenschaften der DDR, 1115 Berlin-Buch, DDR*

Received February 22nd, 1985

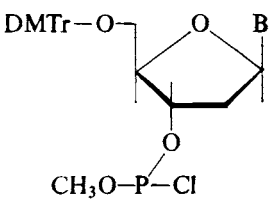
Synthesis of oligodeoxyribonucleotides on silica gel support using 5'-O-dimethoxytrityl-(N-acyl)-deoxyribonucleoside 3'-(O-methyl phosphochloridite) intermediates (*I*) is described. Solutions of these intermediates were prepared before use by mixing of 100 μmol^{-1} solution of methyl phosphodichloridite in 1,2-dichloroethane with the same volume of 120 $\mu mol\,l^{-1}$ solution of 5'-O-dimethoxytrityl-(N-acyl)deoxyribonucleoside in 1,2-dichloroethane containing 1 $mol\,l^{-1}$ of *sym*-collidine. A bench type synthesizer for this methodology is described.

The phosphite method for the synthesis of internucleotidic bond[1] conducted on silica gel support using 5'-dimethoxytrityl-(N-acyl)deoxyribonucleoside 3'-methyl phosphochloridites as intermediates has led to development of rapid procedures for the synthesis of oligodeoxyribonucleotides[2-6]. Chemical instability of phosphochloridites, however, presented a serious limitation to this methodology. Recently, more stable phosphoramidite intermediates have been introduced[7-9]. The phosphochloridite method, however, modified in appropriate manner, could offer a great advantage by using the most simple starting substances, *i.e.* the protected nucleosides and methyl phosphodichloridite.

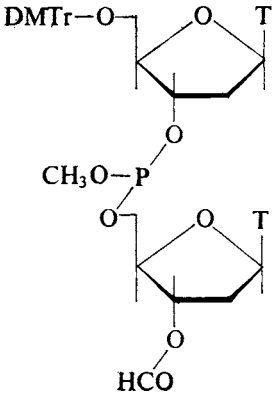
In this paper we wish to report a modification of the phosphochloridite method which has been developed on the basis outlined by Alvarado–Urbina and coworkers[5]. The authors prepared stock solutions (10 $\mu mol\,l^{-1}$ concentration) of nucleoside methyl phosphochloridites in pyridine without removal of collidine hydrochloride. In our hands, however, the condensation yields were far from expected values. It was assumed that the low yields were caused by unavoidable traces of water in the highly

* Part LXXI in the series Oligonucleotidic Compounds; Part LXX: This Journal *48*, 1323 (1983).

hygroscopic solvent. We therefore looked for a nonhygroscopic solvent which would be stable on storage and which would dissolve the collidine hydrochloride to allow the preparation of more concentrated stock solutions of nucleoside methyl phosphochloridites. Pure chloroform would fulfil the last requirement but is unstable to be kept. As suitable solvent 1,2-dichloroethane was selected. This solvent after being distilled from phosphorous pentoxide was stored over molecular sieves for several months without change. It allowed to prepare 0·04 mol l^{-1} solution of 5'-O-dimethoxytritylthymidine 3'-(O-methyl phosphochloridite) by simple mixing of 0·12 mol l^{-1} solution of 5'-O-dimethoxytritylthymidine in 1 mol l^{-1} solution *sym*-collidine with the equal volume of 0·1 mol l^{-1} solution of methyl phosphodichloridite. The solution obtained was kept under argone and its ability to react with 5'-O-dimethoxytritylthymidine to form a phosphite triester was checked by TLC after 5 h and after 20 h. Full reactivity was found after 5 h. After 20 h the reactivity was completely lost. The same findings were made with analogous deoxycytidine and deoxyadenosine derivatives. On the other hand, the deoxyguanosine derivative was unreactive after 5 h storage. To avoid the uncertainty about the true concentration of the reactive component we decided to prepare the solutions of nucleoside methyl phosphochloridites *in situ*[10].



I



II

DMTr = dimethoxytrityl

Another question arose about the possible Michaelis–Arbuzov reaction of the phosphite triester with 1,2-dichloroethane. To test this possibility, a dinucleoside methylphosphite *II* was prepared from 5'-dimethoxytritylthymidine and 3'-O-formylthymidine according[11], omitting the oxidation step. This phosphite triester dissolved in 1 mol l^{-1} *sym*-collidine in 1,2-dichloroethane gave a single peak after 1 h

by ^{31}P NMR spectroscopy. After 24 h another unidentified small peak appeared. Considering the life time of the phosphite triester during the synthetic cycle to be 8min, the amount of phosphonate side product would not exceed 0·1%.

A bench type flow through synthesizer permitting to carry out all repetitive operations was then assembled. In the argone system 0·12 mol l^{-1} solutions of 5'-O-dimethoxytrityl-(N-acyl)deoxyribonucleosides in 1 mol l^{-1} *sym*-collidine in 1,2-dichloroethane were kept in bottles closed with septum for several weeks. 0·1 mol l^{-1} Solution of methyl phosphodichloridite in 1,2-dichloroethane was prepared fresh every day and kept under argone. As solid support HPLC grade silica gel Separon SI (10 μm, 100–200 Å pore size) was selected and derivatized by the described procedures[2,3,5]. Loading amounted to 180 μmol/1 g. Synthetic schedule was adopted according[5], with the exception that chloroform for washing and detritylation was replaced by 1,2-dichloroethane (Table I).

First synthesis of a octanucleotide d(G-G-A-A-T-T-C-C) was carried out starting from 160 mg of the functionalized support (deoxycytidine, 29 μmol) using 5 ml of 0·04 mol l^{-1} (200 μmol, seven-fold excess) of the appropriate nucleoside methyl phosphochloridites *I* prepared *in situ*. The coupling yields up to the last but one step were estimated by determination of absorbance at 500 nm of the detritylation eluate. The ratio of the absorbance obtained from a given cycle to the absorbance obtained in the previous cycle was a measure of the efficiency of condensation. The coupling yields were: 68% (dC), 96% (dT), 86% (dT), 97% (dA), 81% (dA), and 93% (dG). The last dimethoxytrityl group was not removed to facilitate the separation of the longest chain from the shorter untritylated one. The product was deblocked and removed from the support by heating with conc. aqueous ammonia–pyridine mixture[5,11] and isolated by preparative paper chromatography. The eluate from the

TABLE I
Schedule for one cycle in the synthesis

Way No.	Solution	ml min^{-1}	min
4	1,2-dichloroethane	3	2
5	0·25 mol l^{-1} trichloroacetic acid in 1,2-dichloroethane	3	2
6	1,2-dichloroethane	3	2
1	pyridine	3	4
1	ROPCl(OCH_3) in 1,2-dichloroethane	1	5
1	pyridine	3	1
2	0·1 mol l^{-1} I_2 tetrahydrofuran–pyridine–water (5 : 4 : 1)	3	2
3	3% phenyl isocyanate in pyridine	3	2

UV-absorbing and dimethoxytrityl-containing zones was then treated with 80% aqueous acetic acid and the fully deblocked product isolated by preparative paper chromatography. Elution of the main UV-absorbing zone yielded a solution d(G-G-A-A-T-T-C-C), 230 O.D.$_{260}$, assuming $\varepsilon = 80\,000$ for this octamer). Therefore, the overall yield through the seven cycles in the synthesis, deprotection, cleavage from the support, and isolation amounted to 10% based on deoxycytidine initially bound to the support.

Analogously, d(C-G-G-A-T-C-C-G) and d(G-C-T-G-C-A-G-C) were prepared. The results showed that the method outlined in loc. cit.[5] modified by using *in situ* prepared nucleoside methyl phosphochloridites could be reproduced. Lower yields in our synthesis could be caused by using shorter time for condensation (5 min in our work, 7 min in loc. cit.[5]) and lower molar excess of nucleoside methyl phosphochloridite. Following the same procedure a tridecanucleotide d(T-C-C-C-A-G-T-C-A--C-G-A-C) and a pentadecanucleotide d(G-T-T-C-A-T-C-A-T-G-T-T-G-T-C) were prepared. Preparative paper chromatography with longer nucleotide chains did not give good separation and the products were obtained in only 80% purity. At that stage some improvements of the procedure were considered. In the first place, the support originally used was too fine and overloaded, which might cause lower condensation yields when the chain was over ten nucleotides long. Second, the washing solvent (1,2-dichloroethane) was not efficient enough to wash out pyridinium salt after the oxidation step. The deprotection procedure (pyridine–conc. aqueous ammonia) produced 1-methylpyridinium cation which was not well separated from longer chains by the chromatographic procedures.

TABLE II

Schedule for one cycle in the synthesis

Way No.	Solution	ml min^{-1}	min
4	nitromethane	3	2
5	3% trichloroacetic acid in methanol–nitromethane (1 : 99)	3	2–6[a]
6	nitromethane	3	2
1	pyridine	3	3
1	ROPCl(OCH_3) in 1,2-dichloroethane	0·25	8
1	pyridine	3	1
2	0·1 mol l^{-1} I_2 in tetrahydrofuran–collidine–water (3 : 1 : 1)	3	2
3	3% phenyl isocyanate in pyridine	3	2

[a] Detritylation is continued until the eluate is colorless.

A new support with very large pores (Porasil E, 37–75 μm, 1 500 Å pore size) was selected. The loading of this support amounted to 10–12 μmol/1 g. New synthetic schedule was adopted using nitromethane for washing and 3% trichloroacetic acid in nitromethane–methanol (99 : 1) for detritylation according to[6]. Pyridine-containing oxidation solution was replaced by stable 0·1 mol l^{-1} I_2 in tetrahydrofuran–*sym*-collidine–water (3 : 1 : 1). Drying with phenyl isocyanate instead of capping was preserved (Table II). The methyl group was removed from phoshotriester using triethylammonium thiophenoxide in dioxane[12].

According to Table II, synthesis of a pentadecanucleotide d(T-G-A-T-C-A-G-A-T-G-G-C-T-T-T) was carried out starting from 200 mg of deoxythymidine functionalized Porasil E (2 μmol). In every step, 4 ml 0·04 mol l^{-1} (160 μmol) of nucleoside methyl phosphochloridite was used. Monitored yields of the condensation steps (from right to left) were (%): 67, 96, 92, 86, 93, 94, 94, 86, 100, 100, 94, 99, 89, 95. The detritylation times for different deoxynucleotides: 5–6 min (dT), 4–5 min (dC), 3 min (dA), and 1·5–2 min (dG). Low condensation yield (67%) in the first step was regularly observed in every synthesis on Porasil E or Separon SI and could not be enhanced by using double excess of nucleoside methyl phosphochloridite or double reaction time.

After the last condensation step the support was treated with triethylammonium thiophenoxid in dioxane, the dimethoxytrityl-oligonucleotide deprotected, removed from the support by the action of conc. aqueous ammonia at 50°C, and isolated by TLC. After the removal of dimethoxytrityl group, the product was purified by TLC. The overall yield through the synthesis and isolation procedures amounted to 21% based on deoxythymidine initially bound to the support. Using the above described procedure four other pentadecanucleotides were prepared, namely d(C-T-C-C-T-G-G-C-C-A-T-T-C-C-T), d(G-G-G-T-A-C-C-C-A-G-A-A-G-T-C), d(G-C-G-C-T-G-A-G-A-T-C-A-C-C-A), and d(G-C-C-C-G-G-G-G-A-C-G-T-C-T-T). The crude products were further purified on poly(acrylamide) gel electrophoresis in 7 mol l^{-1} urea and characterized by sequencing on anion-exchange cellulose matrix[13,14] (Fig. 1).

EXPERIMENTAL

Materials

For thin-layer chromatography Silufol UV_{254} silica gel foils (20 × 20 cm) were used (Kavalier Glassworks, Votice, Czechoslovakia). For oligonucleotide solutions sterile water was used. Deoxynucleosides were purchased from Papierwerke Waldhof (Mannheim, GFR). 4,4'-Dimethoxytrityl chloride, 5'-O-dimethoxytrityldeoxythymidine and 5'-dimethoxytrityl-N-acyl-deoxynucleosides were prepared according to the published procedures. Porasil E (37–75 μm, 1 500 Å pore size, Waters Associates, Milford, Mass., U.S.A.) and Separon SI (10 μm, 100 to 200 Å pore size, Laboratorní potřeby, Prague) were derivatized by modification of described proce-

dures[2,3,5]. Nitromethane (Fluka, Buchs, Switzerland) was stored over activated (6 h, 190°C, 13 Pa) 4 Å molecular sieves (Merck, GFR). Anhydrous pyridine was obtained by refluxing reagent grade solvent over *p*-toluenesulfonyl chloride for 4 h and distilling. The constant boiling fraction was then refluxed over barium oxide for 4 h and distilled. The constant boiling fraction was stored over activated molecular sieves. 1,2-Dichloroethane was distilled from P_2O_5 and stored over activated molecular sieves. *sym*-Collidine (Fluka) was refluxed over barium oxide, distilled and stored over activated molecular sieves. Phenyl isocyanate (Fluka) was distilled at atmospheric pressure. Methyl phosphodichloridite was prepared using the published procedure and stored as 5 mmol samples under argone in sealed ampoules in icebox.

Equipment

A semi-automated flow through synthetizer was assembled. The 5'-O-dimethoxytrityl-(N-acyl)-deoxyribonucleosides, evaporated previously with pyridine, are stored in septum closed flasks as 0·12 mol l^{-1} solutions in 1 mol l^{-1} solution of *sym*-collidine in 1,2-dichloroethane. These solutions are chemically stable. To every flask a 5 ml all glass syringe with an injection needle reaching to the bottom of the flask is attached through the septum.

Methyl phosphodichloridite is stored in septum closed flask as 0·1 mol l^{-1} solution in 1,2-dichloroethane. This solution is prepared every day. The solution of 5'-dimethoxytrityl-(N-acyl)-nucleoside 3'-(O-methyl phosphochloridite) is prepated by subsequent drawing of equal volumes of the dichloridite and the blocked nucleoside solutions in the appropriate syringe 10 min before the synthetic step. Silica gel loaded with the first nucleoside is packed into a stainless steel column (3 by 20 mm, internal volume). Solvents and reagents are delivered onto the column by a membrane high pressure pump (Development Workshops, Czechoslovak Academy of Sciences) from the centre of an electrically driven 6-way rotary selector valve (Development Workshops). The way No 1 of the valve is connected to the lower end of a glass tube (4 by 100 mm, internal volume). The upper end of the tube is closed by septum through which pyridine is delivered by a manually operated teflon valve into the glass tube during washing steps. Through the same septum, reaction solutions are delivered from the syringes during the synthetic steps. The way No 2 of the valve is connected to a reservoir containing oxidizing solution, the way No 3 to a reservoir containing solution of phenyl isocyanate in pyridine, the ways No 4 and 6 to a reservoir containing washing solvent, and the way No 5 to a reservoir containing detritylating reagent. All flasks containing solutions of nucleoside derivatives, the solution of methyl phosphodichloridite, the tube to the way No 1 and all reservoirs are connected to light overpressure of argone.

Succinylated Aminopropyl-Derivatized Silica Gel (Method *A*)

Silica gel (Separon SI, 10 μm, 100—200 Å pore size, Laboratorní potřeby, Prague; 10 g) was refluxed with 2 mol l^{-1} aqueous hydrochloric acid (200 ml) for 1 h, collected, washed with water, refluxed with water (200 ml), collected, washed with dioxane and dried at diminished pressure (130 Pa, 20°C and then 13 Pa, 150°C). The dry silica was transferred to dry toluene (100 ml) containing 3-aminopropyltriethoxysilane (5 ml) and the suspension was slowly magnetically stirred under reflux for 6 h. The derivatized silica was collected, washed successively (twice each) with toluene, methanol, and ether and dried under diminished pressure. The dried silica was suspended in pyridine (50 ml) and treated with trimethylsilyl chloride (8 ml) at 20°C for 20 h. The silica was collected, washed successively (twice each) with pyridine, methanol, and ether and dried under diminished pressure. The silica was suspended in pyridine (100 ml), succinic anhydride (5 g) and 4-dimethylaminopyridine (1·5 g) added, and the mixture shaken for 20 h at 20°C. The silica was collected, washed successively with pyridine, methanol, and ether and dried under diminished pressure.

5′-O-Dimethoxytrityl-N-benzoyldeoxycytidine Covalently Joined to Silica Gel (Method *A*)

Succinylated aminopropyl-derivatized silica (Separon SI, 1 g) was suspended in pyridine (5 ml), 4-dimethylaminopyridine (60 mg), N,N-dicyclohexylcarbodiimide (2 g), and 5′-O-dimethoxytrityl-N-benzoyldeoxycytidine (215 mg) added and the mixture shaken for 20 h at 20°C. Methanol (0·5 ml) was added and, after 30 min, the silica collected, washed successively with pyridine, N,N-dimethylformamide, methanol, and ether and dried under diminished pressure. Spectroscopic determination of dimethoxytrityl group[13] evaluated the loading of 150 μmol of nucleoside (1 g) silica. Analogous values of loading were achieved with thymidine, deoxyadenosine, and deoxyguanosine derivatives.

Preparation of Solution of N-Hydroxysuccinimide Ester of 5′-O-Dimethoxytrityl-3′-O-succinyldeoxythymidine

To a solution of 5′-O-dimethoxytrityldeoxythymidine (624 mg) in pyridine (10 ml), succinic anhydride (90 mg), and 4-dimethylaminopyridine (122 mg) were added, and after 20 h the solution treated with N-hydroxysuccinimide (138 mg) and N,N-dicyclohexylcarbodiimide (2 g). After 20 h the separated N,N-dicyclohexylurea was centrifuged off and the supernatant used for the functionalization of 3-aminopropyl derivatized silica gel.

5′-O-Dimethoxytrityl-deoxythymidine Covalently Joined to Silica Gel (Method *B*)

Silica gel (Porasil E, 37–75 μm, 1 500 Å pore size, Waters associates; 10 g) was exposed to 15% relative humidity for 24 h by placement in a closed container with a saturated solution of lithium chloride[8]. The silica was then transferred to dry toluene (100 ml) containing 3-aminopropyltriethoxysilane (10 ml) and the suspension was slowly stirred under reflux for 12 h. The silica was collected, washed successively (twice each) with toluene, methanol, and 50% aqueous methanol, suspended in 50% aqueous methanol and shaken for 20 h. The silica was collected, washed with methanol and ether and dried at diminished pressure. The dried silica was suspended in pyridine (100 ml), trimethylsilyl chloride (10 ml) was added and the suspension shaken for 20 h. The silica was filtered off (sinter funnel G 1), washed successively with pyridine, methanol, and ether and dried at diminished pressure. This material (3 g) was treated with a solution of N-hydroxysuccinimide ester of 5′-O-dimethoxytrityl-3′-O-succinyldeoxythymidine for 20 h. Acetic anhydride (2 ml) was added and after 3 h the silica was collected, washed successively with pyridine, N,N-dimethylformamide, methanol, and ether and dried at diminished pressure. Loading 12 μmol/1 g. Analogous loading was achieved with dC, dA, and dG.

Preparation of d(G-G-A-A-T-T-C-C)

Stock solutions (0·12 mol l^{-1}) of 5′-O-dimethoxytrityl-(N-acyl)deoxyribonucleosides were prepared by dissolving of the appropriate amount of the substances (dried previously by evaporating with pyridine) in 1 mol l^{-1} solution of *sym*-collidine in 1,2-dichloroethane (5′-dimethoxytritylthymidine 0·6 mmol, 5 ml; 5′-O-dimethoxytrityl-N-benzoyldeoxycytidine 0·3 mmol, 2·5 ml; 5′-O-dimethoxytrityl-N-benzoyldeoxyadenosine 0·6 mmol, 5 ml; 5′-O-dimethoxytrityl-N-isobutyryldeoxyguanosine 0·6 mmol, 5 ml). The solutions placed in flasks closed by rubber septa were connected to argone system of the synthesizer. Solution of methyl phosphodichloridite (0·1 mol . l^{-1}) was prepared by dissolving of a 5 mmol ampouled sample in 1,2-dichloroethane (50 ml) and kept in septum closed flask connected to argone system of the synthesizer. Reservoirs were filled with appropriate solvents and solutions (Table I). Derivatized silica gel (Method *A*;160 mg, 29 μmol 5′-O-dimethoxytrityl-N-benzoyldeoxycytidine) was packed into the reaction column.

Solution of 5'-O-dimethoxytrityl-N-benzoyldeoxycytidine 3'-(O-methyl phosphochloridite) (5 ml) was prepared by subsequent drawing in the solution of methyl phosphodichloridite (2·5ml) and solution of 5'-O-dimethoxytrityl-N-benzoyldeoxycytidine (2·5 ml) and stirring the mixture by drawing in few bubbles of argone. Through way 4 1,2-dichloroethane was delivered (3 ml per min) into the reaction column, followed by detritylation solution (way No 5). The eluate in this step was collected separately for spectrophotometric determination of condensation yields. The reaction column was then washed with 1,2-dichloroethane (way No 6) followed by pyridine (way No 1). The solution of 5'-O-dimethoxytrityl-N-benzoyldeoxycytidine 3'-methyl-phosphochloridite was slowly expelled from the syringe to the pump (1 ml per min) and into the reaction column. The injection tube and the needle were then washed with pyridine and oxidation solution was pumped in (way No 2). During this step the solution of the next component, a thymidine derivative, was prepared in the appropriate syringe. After the drying step (way No 3), the cycle was repeated according to the schedule (Table I). After the last condensation and oxidation steps, the reaction column was washed with pyridine and the silica bearing the synthetized oligonucleotidic chain was pressed out of the column by reverse pumping with pyridine into a thick-wall tube. Supernatant was decanted and conc. aqueous ammonia (2 ml) was added. The tube was closed with tightened rubber stopper and heated to 50°C for 20 h. The silica was filtered off and washed with two 2 ml portions of 20% aqueous ethanol. The filtrate was concentrated and chromatographed on 2 sheets of Whatman 3 MM in 2-propanol–conc. ammonia–water (55 : 25 : 20). UV-Absorbing and dimethoxytrityl-containing zones were eluted with 1% aqueous ammonia, the eluate evaporated and the residue treated with 80% aqueous acetic acid (2 ml) for 15 min, the solution evaporated (20°C, 130 Pa) and the residue treated with ether (10 ml). The precipitate was centrifuged off, dissolved in 5% aqueous ammonia (1 ml) and the product isolated by preparative paper chromatography (two sheets Whatman 3 MM, 2-propanol–conc. ammonia–water 5 : 3 : 2). The main UV-absorbing zone was eluted with 1% aqueous ammonia and the solution evaporated. Dissolution in water (1 ml) yielded a solution of d(G-G-A-A-T-T-C-C-), 230 $O.D._{260}$ (2·9 μmol), assuming $\varepsilon = 80\,000$ for this octamer. This material on phosphorylation with [γ^{32}P]-ATP and polynucleotide kinase and electrophoresis on poly(acrylamide) gel migrated as 90—95% pure octanucleotide. A sample of the product was further purified by poly(acrylamide) gel electrophoresis and the sequence was shown to be d(G-G-A-A-T-T-C-C) by fingerprinting method (data not shown).

The same procedure was used to synthesize d(C-G-G-A-T-C-C-G), d(G-C-T-G-C-A-G-C), d(T-C-C-C-A-G-T-C-A-C-G-A-C), and d(G-T-T-C-A-T-C-A-T-G-T-T-G-T-C) in comparable yields.

Preparation of d(T-G-A-T-C-A-G-A-T-G-G-C-T-T-T)

The synthesis was carried out following the schedule outlined in Table II starting from derivatized Porasil E (200 mg; 2 μmol dT) and 4 ml 0·04 mol l^{-1} (160 μmol) solution of each nucleoside methyl phosphochloridite. At the end of the synthesis (the drying step) the support was washed with nitromethane and pyridine and pressed out by pumping pyridine into the lower end of the column. Silica gel was collected in 10 ml thick wall test tube, pyridine was removed by suction through a glass tube equipped with sinter glass ending. Silica gel was then washed with dioxane (three 5 ml portions) and treated with a mixture of thiophenol–triethylamine–dioxane (1 : 1 : 2; v/v/v) for 1 h. The solution was removed by suction, silica gel was washed with dioxane (three 5 ml portions, then with one portion of ether and dried by sucking air through it. Conc. aqueous ammonia (2 ml) was added, the test tube closed with rubber stopper and heated to 50°C for 20 h. After cooling, the solution was collected by filtration and the resin washed with four 1 ml portions of 20% aqueous ethanol. To the filtrate 0·5 mol l^{-1} of Tris-base solution (0·2 ml),

was added, the solution was evaporated at 30°C to a volume of cca 0·5 ml and applied to four Silufol sheets which were developed in a mixture of 2-propanol–conc. ammonia–water (55 : : 35 : 10) overnight. The major ultraviolet-absorbing zones containing dimethoxytrityl group were scraped off of the plates, the silica gel suspended in water (3·6 ml) and degassed in water pump vaccuum. The mixture was diluted with ethanol (0·9 ml) and filtered. The resin was washed with four 1 ml portions of 20% aqueous ethanol and the combined filtrates evaporated at 30°C to a volume of about 1 ml, diluted with 1-propanol (3 ml) and evaporated to dryness. To the residue, 80% aqueous acetic acid (0·4 ml) was added, the mixture shaken for 15 min and then applied to two Silufol sheets and immediately developed in a mixture of chloroform–methanol (9 : 1). The plates were dried and then developed in a mixture of 2-propanol–conc. ammonia–water (55 : 35 : 10) overnight. The slowest intensive ultraviolet-absorbing zones were scraped off of the plate, the silica gel was suspended in water (1·6 ml) the mixture degassed in water pump vacuum, diluted with ethanol (0·4 ml) and filtered. The resin was washed with four portions of 20% aqueous ethanol, the filtrates evaporated (30°C). To the residue, 2 ml of water was added, the mixture heated to 50°C for 2 min, cooled and centrifuged. The supernatant (63 $O.D._{260}$, 0·4 μmol, assuming $\gamma = 150000$ for this pentadecamer) was lyophilized affording 3·2 mg of substance. This material on phosphorylation with [γ^{32}P]-ATP and polynucleotide kinase and electrophoresis on poly(acrylamide) gel migrated as pentadecanucleotide of approx. 85% purity. A sample of the product was further purified by poly(acrylamide) gel electrophoresis and the sequence was shown to be d(T-G-A-T-C-A-G-A-T-G-G-T-T-T) by modified Maxam–Gilbert method.

The same procedure was used to synthesize d(C-T-C-C-T-G-G-C-C-A-T-T-C-C-T), d(G-G-G--T-A-C-C-C-A-G-A-A-G-T-C), d(G-C-G-C-T-G-A-G-A-T-C-A-C-C-A), and d(G-C-C-C-G-G-G--G-A-C-G-T-C-T-T) with comparable yields.

REFERENCES

1. Letsinger R. L., Finnan J. I., Heavner G. A., Lunsford W. B.: J. Amer. Chem. Soc. *97*, 3278 (1975).
2. Matteucci M. D., Caruthers M. H.: Tetrahedron Lett. *21*, 719 (1980).
3. Ogilvie K. K., Nemer M. J.: Tetrahedron Lett. *21*, 4159 (1980).
4. Chow F., Kempe T., Palm G.: Nucleic Acids Res. *9*, 2807 (1981).
5. Alvarado-Urbina G., Sathe G. M., Liu W.-Ch., Gillen M. F., Duck P. D., Bender R., Ogilvie K. K.: Science *214*, 270 (1981).
6. Tanaka T., Letsinger R. L.: Nucleic Acids Res. *10*, 3249 (1982).
7. Beaucage S. L., Caruthers M. H.: Tetrahedron Lett. *22*, 1859 (1981).
8. Dorman M. A., Noble S. A., McBride L. J., Caruthers M. H.: Tetrahedron *40*, 95 (1984).
9. Froehler B. C., Matteucci M. D.: Tetrahedron Lett. *24*, 3171 (1983).
10. Smrt J.: Czech. PV 3384—83.
11. Večerková H., Smrt J.: This Journal *48*, 1323 (1983).
12. Daub G. W., van Tamelen E. E.: J. Amer. Chem. Soc. *99*, 3526 (1977).
13. Schaller H., Weimann G., Lerch B., Khorana H. C.: J. Amer. Chem. Soc. *85*, 3821 (1963).
14. Rosenthal A., Hunger H.-D.: Nucleic Acids Res., Symposium Series No *14*, 309 (1984).
15. Maxam A. H., Gilbert W.: *Methods of Enzymology*, Vol. 65, p. 499. Academic Press. New York 1980.
16. Banaszuk A. M., Deugau K. V., Sherwood J., Michalk M., Glick B. R.: Anal. Biochem. *128*, 281 (1983).
17. Rubin C. M., Schmid C. W.: Nucleic Acids Res. *8*, 4613 (1980).

Translated by the author (J. S.).

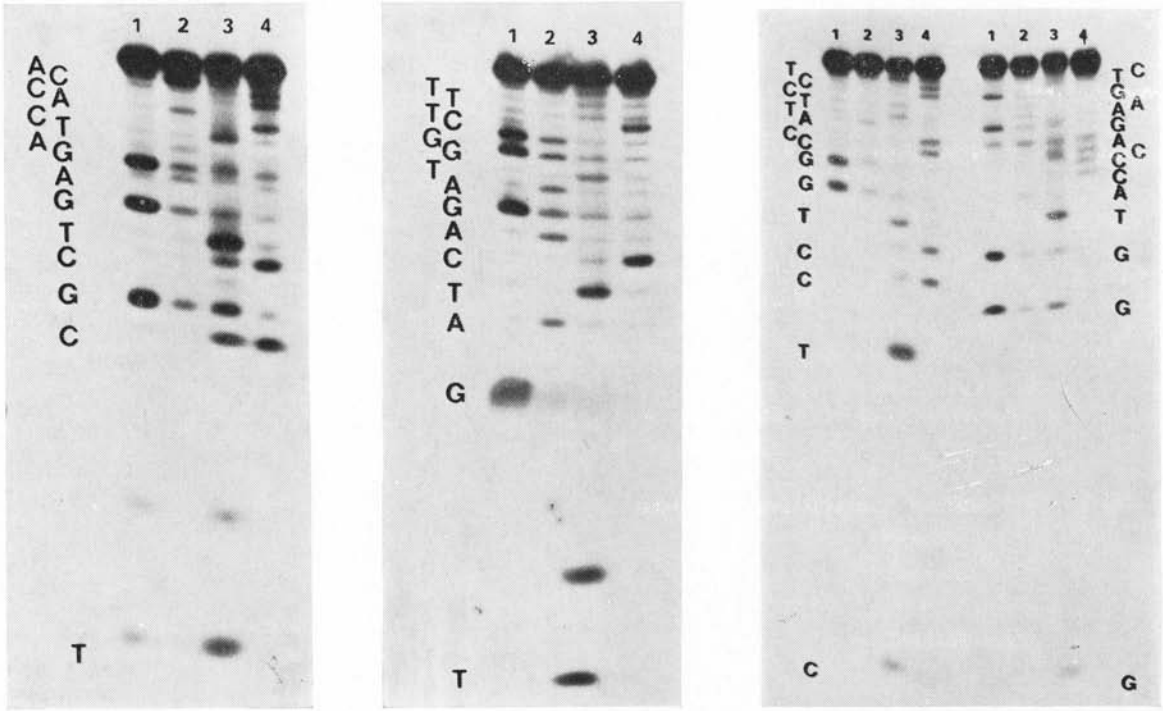


FIG. 1

Sequencing patterns of four pentadecanucleotides. Lane 1-G: (200 µl G-buffer + 2 µl dimethyl sulphoxide) (ref.[15]), 10 min, 20°C; lane 2-A + + G: (80 µl 88% HCOOH) (ref.[16]), 20 min, 20°C; lane 3-T C: (80 µl 10^{-4} mol l^{-1} aqueous $KMnO_4$) (ref.[17]), 20 min, 20°C; lane 4-C: (15 µl water + 40 µl 4 mol l^{-1} $NH_2OH.HCl$, pH 6) (ref.[17]), 20 min, 20°C