*Anal.* Calcd for $C_{12}H_{18}O_3$: C, 68.55; H, 8.63. Found: C, 68.49; H, 8.53.

**2-[3(*a*)-Hydroxy-2-decalylidine]acetic Acid γ-Lactone (5).**—The hydroxy lactone **25** (300 mg) was dissolved in pyridine and a solution of thionyl chloride in pyridine was added. The mixture became hot during the addition and was allowed to stand for 20 min. It was evaporated *in vacuo*. Water was added and extracted with chloroform. The chloroform extract was dried ($MgSO_4$), filtered, and evaporated *in vacuo*, which gave a red oil. The oil was chromatographed on silica gel (40 g) developed with chloroform and 75-ml fractions were collected. Fractions 3 and 4 contained 175 mg of a fairly pure sample of **5**: ir ($CCl_4$) 1778, 1754 (C=O), 1642 $cm^{-1}$ (C=C); nmr δ 5.72 (m, 1, $W_{1/2}$ = 5 Hz), 5.12 (triplet with further fine splitting, 1, $J$ = 7 Hz, C-3 H). In an attempt to purify the sample for analysis, it was chromatographed twice on preparative thin layer chromatography (Brinkman, silica gel, 20 × 20 cm). The first time it was developed two times with chloroform; the second, three times with 50% benzene–chloroform. This treatment completely epimerized the sample to the equatorial butenolide **4**.

**2-[2(*e*),3(*a*)-Dihydroxy-2(*a*)decalyl]acetic Acid (27).**—The reaction mixture from the Reformatsky reaction with 3(*a*)-acetoxy-*trans*-2-decalone (**23**) (5.69 g) was hydrolyzed by heating overnight on a steam bath with 10% sodium hydroxide (30 ml). A precipitate formed which was soluble on addition of water. The basic solution was extracted with chloroform and the chloroform extract was discarded. The aqueous solution was acidified with 10% HCl and allowed to stand for 3 hr, during which time a precipitate formed. The aqueous mixture was extracted with chloroform. The chloroform was washed with sodium bicarbonate solution, dried ($MgSO_4$), filtered, and evaporated to give 2.42 g of **25**, mp 108–111°. The acidic aqueous solution from above was filtered to give 530 mg of **27**, mp 109–115°. Recrystallization of **27** from methanol–chloroform did not improve the melting point, which was quite variable. It was then recrystallized from acetone and again the melting point was variable. However, if placed in an oil bath at 113° it melted immediately, but if the bath was 111° the range was 111–115°: ir (KBr) 3400–2500 (broad series of peaks), 1705 $cm^{-1}$ (C=O); nmr ($CD_3COCD_3$) 2.6 (2, s, $CH_2CO$), 3.77 (m, 1, $W_{1/2}$ = 6 Hz, C-3 H), 4.33 (m, 3, $W_{1/2}$ = 24 Hz, OH).

*Anal.* Calcd for $C_{12}H_{20}O_4$: C, 63.14; H, 8.83. Found: C, 63.10; H, 9.03.

**Lactonization of 2-[2(*e*),3(*a*)-decalyl]acetic Acid (27).**—The acid **27** (30 mg) was heated on a steam bath overnight in benzene containing a trace of *p*-toluenesulfonic acid. Solvent was removed *in vacuo*, leaving 2-[2(*e*),3(*a*)-dihydroxy-2(*a*)-decalyl]acetic acid γ-lactone (**28**) as an oily brown solid: nmr ($CD_3COCD_3$) δ 2.38 (d, 1, $J_{gem}$ = 16 Hz, $CH_2CO$), 2.72 (d, 1, $J_{gem}$ = 16 Hz, $CH_2CO$), 3.37 (m, 1, OH) 4.47 (t, 1, $J$ = 8 Hz, C-3 H). When the reaction was repeated using 200 mg of **27**, the benzene accidentally evaporated. The residue was epimerized butenolide **4**.

Attempts to purify **28** by recrystallization resulted in hydrolysis of the lactone to **27**. Treatment of **28** (30 mg) with pyridine and thionyl chloride according to the procedure for **20** gave a brown oil (20 mg). The nmr spectrum of this oil showed the presence of axially fused butenolide **5**.

**Registry No.**—**4**, 37107-56-5; **5**, 37107-57-6; **6**, 37107-58-7; **10**, 37107-59-8; **14**, 37107-60-1; **15**, 37107-61-2; **16**, 37107-62-3; **17**, 37107-63-4; **18**, 37107-64-5; **19**, 37107-65-6; **20**, 37107-66-7; **23** dinitrophenylhydrazone, 37107-67-8; **24**, 37107-68-9; **25**, 37107-69-0; **27**, 37107-70-3; **28**, 37107-71-4; 2-(3-keto-*trans*-2-decalyl)acetic acid, 37107-72-5; 3(*a*)-acetoxy-*trans*-2(*a*)-decalol, 29121-93-5.

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# Deoxy Oligonucleotide Synthesis *via* the Triester Method

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The β-cyanoethyl β′,β′,β′-trichloroethyl phosphate group is used in the triester method of deoxy oligonucleotide synthesis. The utility of this protecting function, and the triester method, is indicated by the synthesis of a number of deoxy di-, tri-, and tetranucleotides, including dCpdCpdTp, dTpdCpdTp, dTpdCpdTpdCp, and dApdTpdTpdCp. The tetranucleotides were prepared by block condensation from two dinucleotide units.

There are compelling biochemical reasons for the synthesis of oligonucleotides of known sequence. The two general chemical approaches, the diester and the triester methods, differ in that in the first the phosphate groups carry an acidic hydrogen while in the second they are fully esterified and, hence, neutral. The diester method is, at present, the better developed; Khorana, *et al.*, have synthesized a gene for alanine transfer ribonucleic acid by the combination of this method and biochemical procedures.[2] The triester method offers three advantages over the diester method: the product can be rapidly purified by chromatography on silica gel, making large-scale synthesis possible; the yields do not fall rapidly with chain length; and the phosphate backbone, being fully esterified, is not susceptible to attack by the condensating agent during each condensation step. Triester methods of oligonucleotide synthesis have been explored using β,β,β-trichloroethyl,[3,4] phenyl,[5] *o*-chlorophenyl,[6] and β-cyanoethyl[7] as phosphate protecting groups.

During an attempt to synthesize DNA codons *via*

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(1) (a) J. C. Catlin was Recipient of a Fulbright-Hays Travel Grant. (b) Requests for reprints should be sent to Professor F. Cramer, Max-Planck-Institut für Experimentelle Medizin, Abteilung Chemie, 34 Göttingen/Germany, Hermann-Rein-Str. 3. (c) Abbreviations of oligonucleotides according to IUPAC–IUB recommendations, 1970. See, *e.g.*, *Eur. J. Biochem.*, **15**, 203 (1970): dT = thymidine, dC = deoxy cytidine, dA = deoxyadenosine, dG = deoxyguanosine, dN = any deoxy nucleoside, $dbz^6A$ = $N^6$-benzoyldeoxyadenosine, $dac^2G$ = $N^2$-acetyldeoxyguanosine, $dan^4C$ = $N^4$-anisoyldeoxycytidine, $dbz^4C$ = $N^4$-benzoyldeoxycytidine, [(MeO)Tr] dA = 5′-monomethoxytrityldeoxyadenosine, [$(MeO)_2$Tr] dA = 5′-dimethoxytrityldeoxyadenosine, pdA = deoxyadenosine 5′-phosphate, dAp = deoxyadenosine 3′-phosphate, dAp(CNEt) = deoxyadenosine 3′-phosphate β-cyanoethyl ester, dAp(CNEt,$Cl_3$Et) = deoxyadenosine 3′-phosphate β-cyanoethyl β′,β′,β′-trichloroethyl ester (triester).

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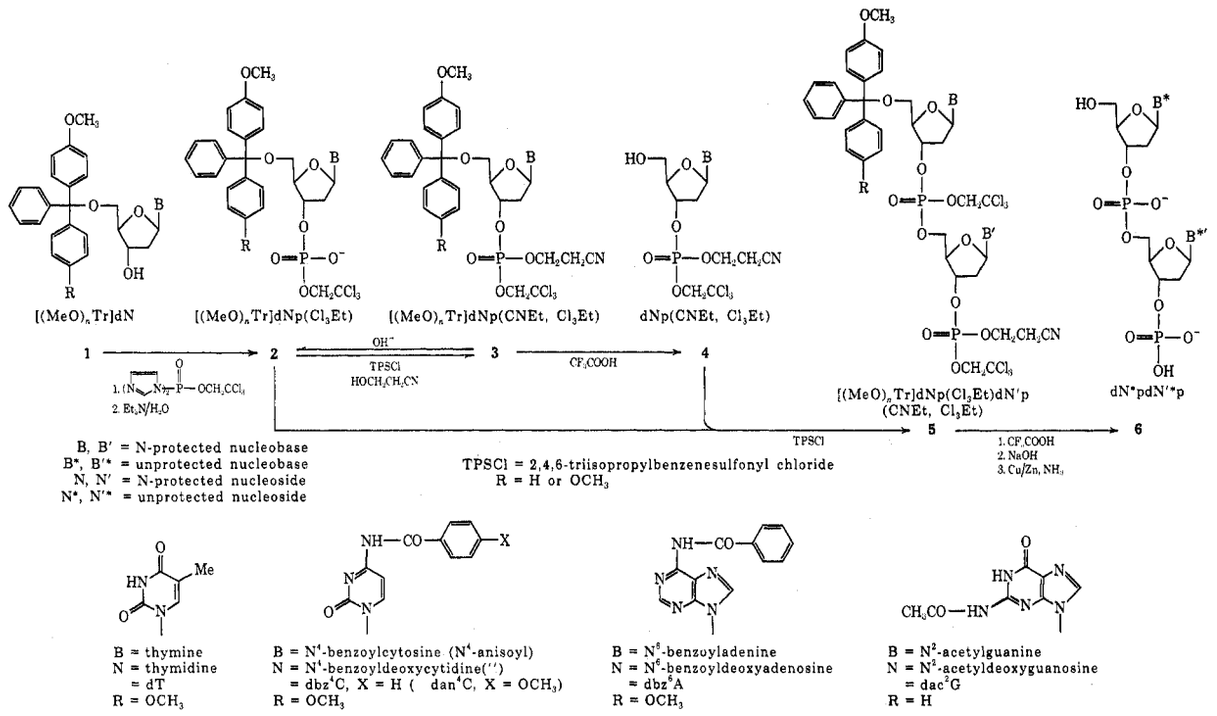


the β,β,β-trichloroethyl triester method, it was found that acetyl and other acyl protecting groups for the 3′-hydroxyl group in certain di- and trinucleotides were unexpectedly stable, perhaps owing to the steric hindrance, and could not be removed without loss of portions of the amino protecting groups.[8] Thus it was necessary to find an alternative protecting group for the 3′-hydroxyl group for use in oligonucleotide synthesis *via* the β,β,β-trichloroethyl triester method. A masked phosphate as protecting group for the 3′-hydroxyl group would have the advantage of permitting the introduction of the phosphate at the mononucleotide stage, rather than before each subsequent condensation.

In our synthetic approach, a nucleoside 3′-phosphate β,β,β-trichloroethyl ester, which carries an acid-labile blocking function on the 5′-hydroxyl, is condensed with the free 5′-hydroxyl group (primary hydroxyl) of a second nucleoside 3′-phosphate β,β,β-trichloroethyl ester in which the phosphate carries an additional base-labile group. The resulting fully protected dinucleotide can then be selectively deblocked at either the 5′ or the 3′ terminal by use of acid or base. The resulting partially protected dinucleotide can be used in a further condensation reaction. In the triester method it is necessary to allow a 3′-phosphate to react with a 5′-hydroxyl group; the reaction of a 3′-hydroxyl (secondary hydroxyl) with a 5′-phosphate ester goes in very low yield owing to the low reactivity of the secondary hydroxyl group.[3]

## Results

An extensive series of di-, tri, and tetranucleotides was synthesized (formulae **1**–**6**, Tables II and III) using the above described concept. The dimethoxytrityl group was used as the acid-labile function to block the 5′-hydroxyl. The 3′ terminal was blocked by conversion of the nucleoside 3′-phosphate β,β,β-trichoroethyl ester to a triester by reaction with β-cyanoethanol. The β-cyanoethyl function is readily cleaved with dilute base. The combination of the dimethoxytrityl and β-cyanoethyl functions gave protected nucleotides and oligonucleotides, which could be deblocked at either the 5′ or the 3′ terminal.

**Preparation of Protected Mononucleotides.**—5′-O- and N-protected nucleosides **1** were prepared according to the procedures of Khorana, *et al.*,[9,10] and converted to the 3′-β,β,β-trichloroethyl phosphate esters (**2**) by reaction with β,β,β-trichloroethyl phosphodiimidazolidate.[8] Condensation of these protected diesters with β-cyanoethanol using triisopropylbenzenesulfonyl chloride gave the fully protected 3′-nucleotide triesters (**3**). Treatment of these protected nucleotides with dilute trifluoroacetic acid gave the desired protected 3′ nucleotides with a free 5′-hydroxyl (**4**), which were to be used at the 3′ terminal in our oligonucleotide synthesis. The 3′ protecting group could also be removed independently (**3** → **2**). The fully protected mononucleotides synthesized and the yields obtained in their preparation and in the selective cleavage of the dimethoxytrityl and β-cyanoethyl function are summarized in Table I.

**Preparation of Oligonucleotides.**—In order to synthesize the dinucleotides (**5, 6**) summarized in Table II a 5′-N-protected 3′-nucleotide β,β,β-trichloroethyl ester (**2**) was condensed with a N-protected 3′-nucleotide β-cyanoethyl β′,β′,β′-trichloroethyl ester (**4**); triisopropylbenzenesulfonyl chloride was used as the condensing agent. In synthesizing a trinucleotide a further condensation step, preceded by a cleavage of the β-cyanoethyl group, was applied. In the case of the tetranucleotides the same procedure as with the dinucleotides was followed, but dinucleotides were used as starting material (summarized in Table III).

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(9) H. Schaller, G. Weimann, B. Lerch, and H. G. Khorana, *J. Amer. Chem. Soc.*, **85**, 3821 (1963).

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TABLE I

MONONUCLEOTIDE DERIVATIVES SYNTHESIZED AND CHARACTERIZED

| 5′ Protected 3′ nucleotides with two phosphate protecting groups | Registry no. | $R_f$ Value[a] (solvent) | Yield, % | 3′ Nucleotides with two phosphate protecting groups | Registry no. | $R_f$ Value[a] (solvent) | Yield, % | 5′ Protected 3′ nucleotides with one phosphate protecting group | Registry no. | $R_f$ Value[a] (solvent) | Yield, % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| [(MeO)$_2$Tr]dTp(CNEt,Cl$_3$Et)[b,c] | 36872-19-2 | 0.7 (D, 5%)<br>0.5 (D, 1:1) | 84 | dTp (CNEt,Cl$_3$Et)[b-d] | 36872-23-8 | 0.2 (D, 1:1)<br>0.5 (D, 1:2) | 79 | [(MeO)$_2$Tr]dTp(Cl$_3$Et)[e,f] | 37042-47-0 | 0.4 (Pr, 20%) | 77 |
| [(MeO)$_2$Tr]dbz$^4$Cp(CNEt,Cl$_3$Et)[b,c] | 36872-20-5 | 0.7 (D, 1:1) | 84 | dbz$^4$Cp (CNEt,Cl$_3$Et)[b-d] | 36872-24-9 | 0.5 (D, 1:1) | 81 | [(MeO)$_2$Tr]dbz$^4$Cp(Cl$_3$Et)[e,f] | 36872-28-3 | 0.4 (Pr, 20%) | 82 |
| [(MeO)$_2$Tr]dan$^4$Cp(CNEt,Cl$_3$Et) | 36872-21-6 | 0.5 (Pr, 1:1) | 5 | dan$^4$Cp (CNEt,Cl$_3$Et)[d] | 36872-25-0 | 0.3 (Pr, 1:1) | 66 | | | | |
| [(MeO)$_2$Tr]dbz$^6$Ap(CNEt,Cl$_3$Et)[b,c,g] | 36872-22-7 | 0.3 (D, 1:1) | 68 | dbz$^6$Ap (CNEt,Cl$_3$Et)[b-d] | 36872-26-1 | 0.5 (D, 60%) | 51 | [(MeO)$_2$Tr]dbz$^6$Ap(Cl$_3$Et)[f] | 36872-29-4 | 0.3 (D, 20%) | 84 |
| [(MeO)Tr]dac$^2$Gp(CNEt,Cl$_3$Et)[c] | 36900-98-8 | 0.3 (Pr, 7%) | 63 | dac$^2$Gp (CNEt,Cl$_3$Et)[d] | 36872-27-2 | 0.3 (Pr, 10%) | 71 | [(MeO)Tr]dac$^2$Gp(Cl$_3$Et)[f] | 36872-30-7 | 0.4 (D, 30%) | 59 |

[a] Chromatography on silica gel plates: D = thin layer plates (Merck, Darmstadt, Germany), Pr = preparative layer plates (Merck, Darmstadt, Germany); 5, 7, 10, and 20% methanol in chloroform, 1:1 and 1:2 benzene:acetone, 60% dioxane in chloroform. [b] Satisfactory analytical data (±0.3% for C, H, N, P, Cl) obtained. [c] Nmr spectrum consistent with the assigned structure. [d] Structure supported by incorporation into a dinucleotide and subsequent degradation into the expected 3′-mononucleotide. [e] Satisfactory analytical data (±0.3% for P, Cl) obtained. [f] Compared *via* thin layer chromatography with an authentic sample. [g] Analytical value for Cl, −0.5%.

In most cases the benzoyl function was found to be a satisfactory N-protecting group for deoxycytidine, as can be seen by the incorporation of $N^4$-benzoyldeoxycytidine into several di-, tri-, and tetranucleotides. The characteristic uv spectrum of $N^4$-benzoyldeoxycytidine was of considerable help in the characterization of the oligonucleotides prepared. Unfortunately, the benzoyl group was partially lost in the preparation of dinucleotides which contained $N^6$-benzoyldeoxyadenosine. Thus it was necessary to use $N^4$-anisoyldeoxycytidine.

The dimethoxytrityl and β-cyanoethyl functions were each removed independently from the fully protected compounds prepared, with either dilute trifluoroacetic acid or dilute sodium hydroxide solution. The conditions of deblocking were not closely studied, but during this work they were continually varied. The preferred cleavage conditions are reported in the Experimental Section.

The purity of the mononucleotides used in the preparation of di- and higher nucleotides seemed to be of minor importance, except in the case of deoxyguanosine, where the purity of each intermediate step in the preparation of 5′-dimethoxytrityl-$N^2$-acetyldeoxyguanosine 3′-phosphate β-cyanoethyl β′,β′,β′-trichloroethyl ester and the 5′-deblocked compound was of utmost importance.

On silica gel chromatography 0.5 g of protected mono- or oligonucleotide could readily be purified on one 100 × 20 cm plate which has a 2-mm layer of silica gel.

The yields obtained in the synthesis and stepwise degradation of the various oligonucleotides are reported in Tables II and III. The tetranucleotides prepared *via* block condensation of two dinucleotides were obtained in yields similar to those obtained for the di- and trinucleotides. The similarity of yields is of significance since equal molar proportions of the nucleotide and nucleoside components were used.

The characterization of the oligonucleotides and their derivatives by chromatography and, in part, by combustion analyses, spectroscopy, and enzymatic degradation is given in the tables. All compounds gave the expected uv spectra.

## Discussion

The combination of the dimethoxytrityl group and the β-cyanoethyl β′,β′,β′-trichloroethyl phosphate diester has been shown to be of value as 5′ and 3′ terminal blocking groups in deoxy oligonucleotide synthesis *via* the triester method. They should be of equal utility in ribooligonucleotide synthesis. In addition, it should be possible to extend the use of the mixed phosphate diester blocking groups to other schemes of oligonucleotide synthesis where the phosphate protecting function is relatively stable to base, as is, *e.g.*, the phenyl group.

The value of the triester method of oligonucleotide synthesis with β,β,β-trichloroethyl as the phosphate blocking group has been extended by the synthesis of many di-, tri-, and tetranucleotides. However, it should be noted that at present the overall yields of unprotected oligonucleotides are quite low. The yields obtained for removal of the trichloroethyl groups are lower than reported in the literature.[3] This

TABLE II

DINUCLEOTIDES AND DERIVATIVES SYNTHESIZED AND CHARACTERIZED

| 5′-Protected dinucleotides with fully protected phosphate groups | Registry no. | $R_f$ Value[a] (solvent) | Yield, % | 5′-Protected dinucleotides with one protecting group per phosphate | Registry no. | $R_f$ Value[a] (solvent) | Yield, % |
|---|---|---|---|---|---|---|---|
| [(MeO)$_2$Tr]dbz$^4$Cp(Cl$_3$Et)-dTp(CNEt,Cl$_3$Et) | 36921-48-9 | (0.6) (Pr, 1:1) | 54 | [(MeO)$_2$Tr]dbz$^4$Cp(Cl$_3$Et)-dTp(Cl$_3$Et) | 36872-40-9 | 0.3 (0.4) (Pr, 20%) | 70 |
| [(MeO)$_2$Tr]dTp(Cl$_3$Et)-dbz$^4$Cp(CNEt,Cl$_3$Et) | 36921-49-0 | (0.3) (HM, 5%) | 61 | [(MeO)$_2$Tr]dTp(Cl$_3$Et)-dbz$^4$Cp(Cl$_3$Et) | 36872-41-0 | 0.3 (0.5) (HM, 20%) | 78 |
| [(MeO)$_2$Tr]dbz$^4$Cp(Cl$_3$Et)-dbz$^4$Cp(CNEt,Cl$_3$Et) | 36872-31-8 | 0.4 (0.5) (HM, 1:1) | 55 | [(MeO)$_2$Tr]dbz$^4$Cp(Cl$_3$Et)-dbz$^4$Cp(Cl$_3$Et) | 36872-42-1 | 0.5 (0.7) (HM, 20%) | 44 |
| [(MeO)$_2$Tr]dbz$^6$Ap(Cl$_3$Et)-dCp(CNEt,Cl$_3$Et)[e,f] | 36872-32-9 | 0.3 (0.5) (HM, 1:1) | 49 | [(MeO)$_2$Tr]dbz$^6$Ap(Cl$_3$Et)-dCp(Cl$_3$Et) | 36872-43-2 | (0.8) (HM, 20%) | 50 |
| [(MeO)$_2$Tr]dbz$^6$Ap(Cl$_3$Et)-dan$^4$Cp(CNEt,Cl$_3$Et) | 36872-33-0 | (0.5) (Pr, 7%) | 70 | [(MeO)$_2$Tr]dbz$^6$Ap(Cl$_3$Et)-dan$^4$Cp(Cl$_3$Et) | 36872-44-3 | 0.4 (0.5) (Pr, 20%) | 93 |
| [(MeO)$_2$Tr]dbz$^6$Ap(Cl$_3$Et)-dTp(CNEt,Cl$_3$Et) | 36872-34-1 | (0.4) (Pr, 5%) | 34 | [(MeO)$_2$Tr]dbz$^6$Ap(Cl$_3$Et)-dTp(Cl$_3$Et) | 36872-45-4 | (0.4) (Pr, 30%) | 66 |
| [(MeO)$_2$Tr]dTp(Cl$_3$Et)-dbz$^6$Ap(CNEt,Cl$_3$Et) | 36872-35-2 | 0.4 (0.6) (Pr, 7%) | 27 | [(MeO)$_2$Tr]dTp(Cl$_3$Et)-dbz$^6$Ap(Cl$_3$Et) | 36872-46-5 | 0.5 (Pr, 30%) | 59 |
| [(MeO)Tr]dac$^2$Gp(Cl$_3$Et)-dTp(CNEt,Cl$_3$Et) | 36872-36-3 | 0.4 (Pr, 1:1) | 40 | [(MeO)Tr]dac$^2$Gp(Cl$_3$Et)-dTp(Cl$_3$Et) | 36872-47-6 | 0.1 (Pr, 20%) | 40 |
| [(MeO)Tr]dac$^2$Gp(Cl$_3$Et)-dac$^2$Gp(CNEt,Cl$_3$Et) | 36872-37-4 | 0.2 (Pr, 1:1) | 7 | [(MeO)Tr]dac$^2$Gp(Cl$_3$Et)-dac$^2$Gp(Cl$_3$Et) | 36872-48-7 | 0.6 (D, 30%) | 59 |
| [(MeO)$_2$Tr]dbz$^4$Cp(Cl$_3$Et)-dbz$^6$Ap(CNEt,Cl$_3$Et)[e,i] | 36872-38-5 | 0.4 (0.7) (Pr, 5%) | 13 | dbz$^4$Cp(Cl$_3$Et)-dbz$^6$Ap(CNEt,Cl$_3$Et)[j] | 36872-52-3 | 0.4 (Pr, 5%) | 63 |
| [(MeO)$_2$Tr]dan$^4$Cp(Cl$_3$Et)-dbz$^6$Ap(CNEt,Cl$_3$Et) | 36872-39-6 | 0.4 (0.7) (Pr, 5%) | 41 | [(MeO)$_2$Tr]dan$^4$Cp(Cl$_3$Et)-dbz$^6$Ap(Cl$_3$Et) | 36921-50-3 | 0.3 (Pr, 20%) | 62 |

[a] Chromatography on silica gel plates: D = thin layer plates (Merck, Darmstadt, Germany), Pr = preparative layer plates (Merck, Darmstadt, Germany), HM = "home-made" preparative layer plates; PC = paper chromatography; 5, 7, 20, and 30% methanol in chloroform, 1:1 benzene:acetone, 7:3 ethanol:1 $N$ ammonium acetate, 55:10:35 isopropyl alcohol:concentrated ammonia:water (occasionally before developing a plate with 7:3 or 55:10:35 it was first developed with 1:1 methanol:chloroform). $R_f$ values are reported for after developing once and twice ( ). [b] $\beta,\beta,\beta$-Trichloroethyl groups cleaved with Zn. [c] Dinucleotide cleaved with spleen

suggests that further refinement of the deblocking reaction is needed. The question of how large an oligonucleotide can be prepared and purified by the triester method must still be explored.

## Experimental Section

Pyridine was purified by distillation from $P_2O_5$ and then from $CaH_2$; it was stored over $CaH_2$ and redistilled immediately before use. Tetrahydrofuran was distilled twice from $CaH_2$, stored over $CaH_2$, and distilled immediately before use. Ethyl ether was distilled from $LiAlH_4$ immediately before use. Uv spectra were measured on a Cary 14 with methanol or water as solvent. Nmr spectra were measured in $CDCl_3$ on a Varian HA-100.

**Preparation of 5′,N-Protected $\beta,\beta,\beta$-Trichloroethyl 3′ Nucleotides.**—The appropriate protected nucleoside (2–30 g) was dried by distillation of pyridine *in vacuo*, followed by distillation of tetrahydrofuran. To an ice-cold solution of imidazole (13 equiv) in tetrahydrofuran was slowly added $\beta,\beta,\beta$-trichloroethyl phosphodichloridate[3] (3 equiv). The resulting mixture was stirred for 1 hr at 0° and filtered (to remove imidazole-HCl) and the filtrate was added to the predried nucleoside. The reaction solution was left overnight at room temperature. It was chilled in ice, water was added, and the pH was adjusted to 7.5–8 by the addition of triethylamine. The reaction mixture was stirred for 2 hr at room temperature, evaporated *in vacuo*, and dried by distillation of benzene. The protected nucleotide could be purified by chromatography on silica gel (plates developed with 20% MeOH/$CHCl_3$ or column eluted with a gradient of $CHCl_3$–15% MeOH/$CHCl_3$ containing 1% triethylamine). Yields of the protected nucleotides after purification were 40–80%.

**Preparation of 5′,N-Protected $\beta$-Cyanoethyl $\beta',\beta',\beta'$-Trichloroethyl 3′ Nucleotides.**—The 5′,N-protected $\beta,\beta,\beta$-trichloroethyl 3′ nucleotide (0.1–15 g) and 3–5 equiv of $\beta$-cyanoethanol were dried by distillation of pyridine *in vacuo*. A pyridine solution of 2,4,6-triisopropylbenzenesulfonyl chloride, an amount equal to, or in slight excess of, the $\beta$-cyanoethanol, was added to the dry mixture. After standing overnight at room temperature the reaction mixture was poured into water and the product was extracted into $CHCl_3$. The $CHCl_3$ solution was washed with water, dried over $Na_2SO_4$, and evaporated *in vacuo*. The crude product was purified by chromatography (Table I).

**Cleavage of the $\beta$-Cyanoethyl Moiety.**—The fully protected 3′ nucleotide or oligonucleotide (4–550 mg), or the derived material without 5′ protection, was dissolved in pyridine (100 mg/10 ml) and chilled in ice; ice-cold 0.1 $N$ NaOH (1 ml/4 ml of pyridine) was added. After 5 min in ice the reaction was neutralized by the addition of a slight excess of 0.1 $N$ HCl in aqueous pyridine or with Dowex 50 (pyridinium form). The reaction mixture was evaporated *in vacuo* and the product was purified on silica gel plates (Tables I, II, III).

**Cleavage of the Mono- and Dimethoxytrityl Group.**—An ice-cold solution of 1% trifluoroacetic acid in methylene chloride was added to the fully protected 3′ nucleotide (60–5000 mg) or oligonucleotide (10–550 mg), or to the analogous material after cleavage of the $\beta$-cyanoethyl moiety (60 ml/g). After 20 min in ice, the reaction was neutralized by addition of pyridine and evaporated *in vacuo*. The product was purified by chromatography on silica gel plates (Tables I, II, III).

**Preparation of Protected Oligonucleotides.**—Equal molar proportions of protected nucleotide component (80–2800 mg) and nucleoside component were dried by distillation of three portions of pyridine at 20–40°. To the dried mixture was added a two- to tenfold excess of triisopropylbenzenesulfonyl chloride in pyridine. After about 40 hr at room temperature the reaction mixture was evaporated *in vacuo*. Alternatively, the reaction mixture was poured into water and the product was extracted into $CHCl_3$, and the $CHCl_3$ solution, after being washed with $H_2O$ and dried over $Na_2SO_4$, was evaporated *in vacuo*. The product was isolated following chromatography on silica gel (Tables II, III). A typical reaction was run on a 0.1-m$M$ scale, although some were done on a much larger scale, in 10 ml of pyridine. Less than 40 hr was required if the reaction mixture was concentrated to an oil.

**Cleavage of the $\beta,\beta,\beta$-Trichloroethyl, $N$-Benzoyl, and $N$-Acetyl Groups.**—A sample of 3′ nucleotide or oligonucleotide (100–300 OD units), after cleavage of the mono- or dimethoxytrityl and $\beta$-cyanoethyl groups, was dissolved in 2 ml of absolute dimethyl formamide (in one case 5% acetic acid in pyridine). To this solution was added about 0.2 g of Zn/Cu couple.[11] The reaction mixture was shaken for 10 min, the solution was decanted, and the residue was washed with 25% aqueous $NH_3$. The combined solutions were evaporated *in vacuo* and the solid obtained was dissolved in 25% aqueous $NH_3$. The zinc ions were precipitated by bubbling $H_2S$ through the solution. The resulting mixture

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| Dinucleotides with one protecting group per phosphate | Registry no. | $R_f$ Value[a] (solvent) | Yield, % | Dinucleotide | Registry no. | $R_f$ Value[a] (solvent) | Yield, % | Relation of nucleotides | $R_f$ Value[a] (solvent) |
|---|---|---|---|---|---|---|---|---|---|
| dbz⁴Cp(Cl₃Et)-dTp(Cl₃Et) | 36900-99-9 | 0.5 (HM, 20%) | 70 | dCpdTp[b] | 3922-65-4 | 0.5 (PC, 55:10:35) | 3 | dCp:dTp[c] 1:1 | (PC, 7:3)[d] |
| dTp(Cl₃Et)-dbz⁴Cp(Cl₃Et) | 36872-49-8 | 0.4 (0.6) (HM, 20%) | 70 | dTpdCp[b] | 4105-16-2 | 0.1 (0.3) (D, 7:3) | 23 | dTp:dCp[c] 0.9:1 | 0.3/0.2 (PC, 7:3) |
| dbz⁴Cp(Cl₃Et)-dbz⁴Cp(Cl₃Et) | 36872-50-1 | 0.4 (0.7) (HM, 20%) | 56 | dCpdCp[b] | 3930-16-3 | 0.1 (D, 7:3) | 18 | | |
| dbz⁶Ap(Cl₃Et)-dCp(Cl₃Et) | 36872-51-2 | (0.7) (HM, 20%) | 50 | dApdCp[b] | 3930-15-2 | 0.5 (D, 7:3) | 17 | dAp:dCp[e] 1:0.9 | 0.6/0.5 (D, 7:3) |
| dbz⁶Ap(Cl₃Et)-dan⁴Cp(Cl₃Et)[g] | 36872-53-4 | 0.5 (0.9) (Pr, 30%) | 13 | dApdCp[h] | | 0.5 (PC, 55:10:35) | 37 | dAp:dCp[e] 1:1 | 0.8/0.3 (PC, 7:3) |
| dbz⁶Ap(Cl₃Et)-dTp(Cl₃Et) | 36872-54-5 | 0.5 (Pr, 30%) | 57 | dApdTp[h] | 6818-27-5 | 0.5 (D, 7:3) | 55 | dAp:dTp[e] 1:1 | 0.5/0.6 (D, 7:3) |
| dTp(Cl₃Et)-dbz⁶Ap(Cl₃Et) | 36872-55-6 | 0.5 (Pr, 30%) | 100 | dTpdAp[b] | 3922-64-3 | 0.4 (D, 7:3) | 25 | dTp:dAp[e] 1:1 | 0.6/0.3 (PC, 7:3) |
| dac²Gp(Cl₃Et)-dTp(Cl₃Et) | 36872-56-7 | (Pr, 30%)[d] | 71 | dGpdTp[h] | 36872-65-8 | 0.2 (PC, 55:10:35) | 65 | dGp:dTp[e] 0.95:1 | 0.1/0.4 (PC, 7:3) |
| dac²Gp(Cl₃Et)-dac²Gp(Cl₃Et) | 36872-57-8 | 0.2 (D, 30%) | 32 | dGpdGp[h] | 4417-99-6 | 0.2 (PC, 55:10:35) | 30 | | |
| dbz⁴Cp(Cl₃Et)-dbz⁶Ap(Cl₃Et)[k] | 36872-58-9 | 0.8 (D, 20%) | 59 | | | | | | |
| dan⁴Cp(Cl₃Et)-dbz⁶Ap(Cl₃Et)[k] | 36872-59-0 | (0.8) (Pr, 30%) | 62 | | | | | | |

phosphodiesterase and components separated chromatographically according to ref 3. [d] $R_f$ value not recorded. [e] Dinucleotides containing both dA and dC usually lost the *N*-benzoyl from dC. [f] [(MeO)₂Tr]dbz⁶Ap(Cl₃Et)dbz⁴Cp(CNEt,Cl₃Et) isolated from this reaction in 5% yield. [g] Run at room temperature and not the usual 0°. [h] β,β,β-Trichloroethyl groups cleaved with Zn/Cu. [i] [(MeO)₂Tr]dCp(Cl₃Et)dbz⁶Ap(CNEt,Cl₃Et) isolated from a similar reaction in 41% yield. [j] Order of cleavage of protecting groups reversed. [k] Not further degraded.

## TABLE III

### TRI- AND TETRANUCLEOTIDES SYNTHESIZED AND CHARACTERIZED

| | Registry no. | $R_f$ Value[a] (solvent) | Yield, % |
|---|---|---|---|
| 5′-Protected tri- and tetranucleotides with fully protected phosphate groups | | | |
| [(MeO)₂Tr]dbz⁴Cp(Cl₃Et)dbz⁴Cp(Cl₃Et)dTp(CNEt,Cl₃Et) | 36901-00-5 | 0.5 (Pr, 7%) | 57 |
| [(MeO)₂Tr]dTp(Cl₃Et)dbz⁴Cp(Cl₃Et)dTp(CNEt,Cl₃Et) | 36901-01-6 | 0.4 (0.5) (D, 7%) | 48 |
| [(MeO)₂Tr]dTp(Cl₃Et)dbz⁴Cp(Cl₃Et)dTp(Cl₃Et)dbz⁴Cp(CNEt,Cl₃Et) | 36872-67-0 | 0.3 (Pr, 1:1) | 54 |
| [(MeO)₂Tr]dbz⁶Ap(Cl₃Et)dTp(Cl₃Et)dTp(Cl₃Et)dbz⁴Cp(CNEt,Cl₃Et) | 36901-02-7 | 0.2 (0.3) (D, 1:1) | 39, 21[g] |
| Tri- and tetranucleotides with fully protected phosphate groups | | | |
| dbz⁴Cp(Cl₃Et)dbz⁴Cp(Cl₃Et)dTp(CNEt,Cl₃Et) | 36872-68-1 | 0.9 (D, 10%) | 40 |
| dTp(Cl₃Et)dbz⁴Cp(Cl₃Et)dTp(CNEt,Cl₃Et) | 36872-69-2 | 0.4 (Pr, 10%) | 73 |
| dTp(Cl₃Et)dbz⁴Cp(Cl₃Et)dTp(Cl₃Et)dbz⁴Cp(CNEt,Cl₃Et) | 36900-88-6 | 0.5 (Pr, 10%) | 24 |
| dbz⁶Ap(Cl₃Et)dTp(Cl₃Et)dTp(Cl₃Et)dbz⁴Cp(CNEt,Cl₃Et) | 36900-89-7 | 0.7 (Pr, 10%) | 61 |
| 5′-Protected tri- and tetranucleotides with one protecting group per phosphate | | | |
| [(MeO)₂Tr]dbz⁴Cp(Cl₃Et)dbz⁴Cp(Cl₃Et)dTp(Cl₃Et) | 36900-90-0 | 0.3 (Pr, 20%) | 46 |
| [(MeO)₂Tr]dTp(Cl₃Et)dbz⁴Cp(Cl₃Et)dTp(Cl₃Et) | 36900-91-1 | 0.2 (D, 20%) | 47 |
| [(MeO)₂Tr]dTp(Cl₃Et)dbz⁴Cp(Cl₃Et)dTp(Cl₃Et)dbz⁴Cp(Cl₃Et) | 36900-92-2 | 0.3 (Pr, 20%) | 24 |
| [(MeO)₂Tr]dbz⁶Ap(Cl₃Et)dTp(Cl₃Et)dTp(Cl₃Et)dbz⁴Cp(Cl₃Et) | 36872-70-5 | 0.3 (Pr, 20%) | 11[h] |
| Tri and tetranucleotides with one protecting group per phosphate | | | |
| dbz⁴Cp(Cl₃Et)dbz⁴Cp(Cl₃Et)dTp(Cl₃Et) | 36872-71-6 | 0.2 (D, 20%) | 100, 39[b] |
| dTp(Cl₃Et)dbz⁴Cp(Cl₃Et)dTp(Cl₃Et) | 36900-93-3 | 0.4 (D, 30%) | 68, 47[b] |
| dTp(Cl₃Et)dbz⁴Cp(Cl₃Et)dTp(Cl₃Et)dbz⁴Cp(Cl₃Et) | 36872-75-0 | 0.6 (D, 30%) | 74 |
| dbz⁶Ap(Cl₃Et)dTp(Cl₃Et)dTp(Cl₃Et)dbz⁴Cp(Cl₃Et) | 36921-51-4 | 0.5 (D, 30%) | 81, 45[b] |

| Tri-, tetra-nucleotide | Registry no. | $R_f$ Value[a] (solvent) | Yield, % | Relation of nucleotides | $R_f$ Value[a] (solvent) |
|---|---|---|---|---|---|
| dCpdCpdTp[c] | 36872-72-7 | 0.3 (D, 55:10:35) | 9 | dCp:dTp (2:1.2)[d] | 0.5/0.6 (D, 7:3) |
| dTpdCpdTp[c] | 36872-73-8 | 0.5 (D, 55:10:35) | 11 | dTp:dCp (2:1.3)[d] | 0.5/0.3 (D, 7:3) |
| dTpdCpdTpdCp[e,f] | 36900-94-4 | 0.3 (PC, 55:10:35) | 31 | dTp:dCp (1:1.2)[d] | 0.3/0.2 (PC, 7:3) |
| dApdTpdTpdCp[e] | 36872-74-9 | 0.3 (PC, 55:10:35) | 52 | dAp:dTp:dCp (1:2.1:1.2)[d] | 0.8/0.5/0.4 (PC, 7:3) |

[a] Chromatography on silica gel plates: D = thin layer plates (Merck, Darmstadt, Germany), Pr = preparative layer plates (Merck, Darmstadt, Germany), HM = "homemade" preparative layer plates; PC = paper chromatography; 7, 10, 20, and 30% methanol in chloroform, 1:1 benzene acetone, 7:3 ethanol:1 *N* ammonium acetate, 55:10:35 isopropyl alcohol:concentrated ammonia:water (occasionally before developing a plate with 7:3 or 55:10:35 it was first developed with 1:1 methanol:chloroform). $R_f$ values are reported for after developing once and twice ( ). [b] First yield for cleavage of [(MeO)₂Tr] from 5′-protected tri- and tetranucleotides with one protecting group per phosphate; second yield for cleavage of (CNEt) from tri- and tetranucleotides with fully protected phosphate groups. [c] β,β,β-Trichloroethyl groups cleaved with Zn. [d] Oligonucleotides cleaved with spleen phosphodiesterase and components separated chromatographically according to ref 3. [e] β,β,β-Trichloroethyl groups cleaved with Zn/Cu. [f] Chromatography on Whatman DE 81 paper, developing with 0.75 *M* $(NH_4)HCO_3$: dTpdCpdTpdCp, $R_f$ 0.45, dTpdCp, $R_f$ 0.62. [g] Two overlapping spots in thin layer chromatography, the lower spot being $HClO_4$ negative. [h] Product used in this reaction only ~50% pure; compare footnote *g*.

was centrifuged and the solution was decanted. The residue was washed with 25% aqueous $NH_3$ and the combined solutions were evaporated *in vacuo*. The residue was then allowed to stand overnight in 3 ml of pyridine:25% aqueous $NH_3$ (1:2, v/v). The solution was then evaporated *in vacuo* and the free nucleotide or oligonucleotide was isolated *via* chromatography (Tables II, III). In all cases where a lower yield than 30% was obtained, zinc in 5% acetic acid in pyridine or in 80% aqueous acetic acid was used instead of the above procedure to cleave the β,β,β-trichloroethyl function.

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# Activated Phosphate Triesters. The Synthesis and Reactivity of *N*-Hydroxysuccinimide and *N*-Mercaptosuccinimide Esters

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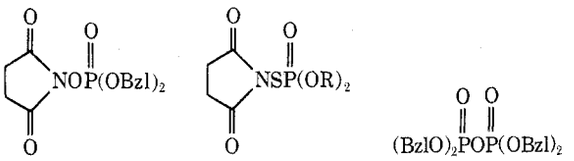
Phosphate esters based upon *N*-hydroxysuccinimide and *N*-mercaptosuccinimide have been prepared. It is shown that the reaction of *N*-hydroxysuccinimide with dibenzyl phosphate through the agency of diisopropylcarbodiimide can give a variety of products. Low temperatures in nonpolar solvents give the desired esters exclusively. Higher temperatures and polar solvents give mainly tetrabenzyl pyrophosphate. *O,O*-Dibenzyl *O*-(*N*-succinimidyl) phosphate phosphorylates benzyl alcohol in high yield: it does not react, however, with 3′-acetylthymidine. The thio esters phosphorylate benzyl alcohol in low yield, giving a large number of side products.

Although there have been remarkable achievements in the field of nucleotide synthesis,[1] it is clear that current methods for the synthesis of the internucleotide phosphate linkage are not satisfactory. The most successful procedures involve the use of condensing agents that remove a molecule of water between a nucleotide and a nucleoside. The most popular of these agents, dicyclohexylcarbodiimide[2] and triisopropylbenzenesulfonyl chloride,[3] are known to produce undesirable side reactions which become more serious when oligonucleotides are condensed; the starting materials are degraded[4-6] and larger and larger excesses of the phosphate-containing unit are required as the oligonucleotides grow in size. Just as the use of active esters[7,8] in peptide synthesis constituted an important advantage, the isolation of an activated phosphate species followed by coupling with a nucleoside hydroxyl group would be expected to result in much cleaner reactions. The possible utility of this scheme in phosphorylation reactions has been demonstrated with various reactive phosphates, *e.g.*, phosphorochloridates,[9] the adduct of phosphorochloridates with dimethylformamide,[10] phosphoromorpholidates,[11] imidazoyl phosphates,[12] oxidized or alkylated thio esters,[13] and activated phosphate esters with 2,4-dinitrophenol,[14] *p*-nitrophenol,[15] 2-hydroxypyridine,[16] and 2-mercaptopyridine.[17]

As a model for possible nucleotide synthesis we have prepared and studied phosphate *N*-hydroxysuccinimide and *N*-mercaptosuccinimide esters. These are *O,O*-dibenzyl *O*-(*N*-succinimidyl) phosphate (**1**), *O,O*-diethyl *S*-(*N*-succinimidyl) phosphorothioate (**2**), and *O,O*-di-*tert*-butyl *S*-(*N*-succinimidyl) phosphorothioate (**3**).




**1** **2**, R = Et; **3**, R = *t*-Bu **4**

It was our purpose to study phospho triesters because of advantages in maintaining phospho triester linkages during oligonucleotide synthesis[18] and their high susceptibility to attack by hydroxide ion.[19] *N*-Hydroxysuccinimide active esters have proven their value in peptide synthesis.[20]

The synthesis of **1** was accomplished by the reaction of dibenzyl phosphate (DBP) and *N*-hydroxysuccinimide (NHS) with diisopropylcarbodiimide in acetonitrile or anisole at low temperature. It was seen that solvent polarity or basicity[21,22] and temperature play an important role in determining the course of the reaction which can proceed to give **1**, tetrabenzyl

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