(10) (a) For the dimethyl analogues **7** and **8** see D. Van Haver, D. Tavernier, M. Anteunis, and M. Vandewalle, *Tetrahedron*, **30**, 105 (1974). (b) Compounds **9** and **10** were synthesized in our laboratory: W. Van Brussel, Ph.D. Thesis, Ghent, Belgium, 1975.
(11) P. De Clercq and M. Samson, *Org. Magn. Reson.*, in press; *Bull. Soc. Chim. Belg.*, **85**, 872 (1976).
(12) The "axial–equatorial" terminology used in cyclohexane has been retained, although in cyclopentanes the dihedral angles will not generally equal those in cyclohexane. If ψ is the dihedral angle between cis vicinal bonds, and if there is no angular deformation, then $\psi_{ea} = \psi$; $\psi_{aa} = 120° + \psi_{ea}$; $\psi_{ee} = 120° - \psi_{ea}$. Considering the most puckered part of the molecule we used $\psi = 48.1°$ for the twist conformation and $\psi = 46.1°$ for the envelope conformation.[15d]
(13) These calculations will be discussed in detail elsewhere. The method used was derived from the work of Ouannes[15d] and Altona.[15e] The shape of the potential barrier restricting pseudorotation was calculated from torsional barriers only; nonbonded interactions and dipole–dipole interactions were not calculated but were considered during the study of Dreiding models. The method consists basically in calculating the torsional strain around each carbon–carbon bond of the ring from the rotational barrier of known acyclic compounds and summation of these values for each of the 20 basic conformations met along the itinerary of pseudorotation.
(14) Although the concept of pseudorotation was first introduced[15f] in relation with the conformation of unsubstituted cyclopentane, we will use the same term in the discussion of the conformational behavior of 2,3-dialkyl-1,4-cyclopentanediols.
(15) (a) E. L. Eliel, N. L. Allinger, S. J. Angyal, and G. A. Morisson, "Conformational Analysis", Wiley, New York, N.Y., 1965; (b) C. Altona, H. R. Buys, and E. Havinga, *Recl. Trav. Chim. Pays-Bas*, **85**, 973 (1966); (c) D. Korver, *ibid.*, **88**, 1070 (1969); (d) C. Ouannes and J. Jacques, *Bull. Soc. Chim. Fr.*, 3603 (1965); (e) ref 15b; C. Altona, H. R. Buys, H. J. Hageman, and E. Havinga, *Tetrahedron*, **23**, 2265 (1967); (f) J. E. Kilpatrick, K. S. Pitzer, and R. Spitzer, *J. Am. Chem. Soc.*, **69**, 2483 (1947); (g) C. Altona and M. Sundaralingam, *ibid.*, **94**, 8205 (1972).
(16) See, e.g., P. v. R. Schleyer, D. S. Trifan, and R. Bacskai, *J. Am. Chem. Soc.*, **80**, 6691 (1958); M. Oke and H. Iwamura, *Bull. Chem. Soc. Jpn.*, **32**, 306 (1959).
(17) F. De Pessemier, Ph.D. Thesis, Gent, Belgium, 1976.
(18) G. Germain, J. P. De Clercq, and M. Van Meerssche, *Bull. Soc. Chim. Belg.*, **85**, 557 (1976).
(19) The ^{10}E form is a high-energy conformation, whereas an approximate value for the hydrogen bond is 1.5 kcal/mol. Thus the stabilization of the hairpin conformation (4.9 kcal/mol)[5] would normally be the leading factor in the determination of the composition of the conformational equilibrium for prostaglandin $F_{1\alpha}$ methyl ester in an apolar solvent. Since this is not the case (at least not exclusively) we assume that the nature of the solvent will in great part determine if the aligned conformation is indeed a preferred one.
(20) So far, the difference between prostaglandins with 15(*R*) or 15(*S*) configuration has been made only on the base of their TLC behavior; e.g., J. Ide and K. Sakai, *Tetrahedron Lett.*, 1367 (1976).
(21) The chemical shift values of the methyl groups in 2,3-dimethyl-1,4-diacetoxycyclopentane (**7**) are greatly influenced by the considered configuration: M. Samson, Ph.D. Thesis, Ghent, Belgium, 1976.

Synthesis of Deoxyribooligonucleotides by Means of Cyclic Enediol Pyrophosphates

Fausto Ramirez,*[1] Eleftheria Evangelidou-Tsolis, Aleksander Jankowski, and James F. Marecek

Department of Chemistry, State University of New York at Stony Brook, Stony Brook, New York 11794

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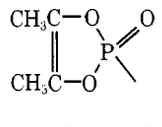
A new method of synthesis of deoxyribooligonucleotides by means of di(1,2-dimethylethenylene) pyrophosphate is described. Reaction of 5′-*O*-*p*-methoxytritylthymidine with the pyrophosphate in dichloromethane (triethylamine as proton acceptor) gives a cyclic enediol phosphate derivative, which is allowed to couple in dimethylformamide (triethylamine as catalyst) with unprotected thymidine to yield the 1-methylacetonyl ester of (5′-*O*-*p*-methoxytrityl)thymidylyl-(3′ → 5′)-thymidine. This protected dinucleotide triester is converted first into the deprotected triester by trifluoroacetic acid in dichloromethane solution, and then into $TpT^-(C_2H_5)_3NH^+$ by triethylamine in aqueous acetonitrile. The protected dinucleotide triester is converted into the protected tri- and tetranucleotide triesters by repetition of the reaction with pyrophosphate and the coupling with thymidine. The tetranucleotide, $TpTpTpT^{3-}[(C_2H_5)_3NH^+]_3$, is obtained after removal of the *p*-methoxytrityl 5′-OH protecting group, and the 1-methylacetonyl phosphate blocking group, from the protected triester. The di- and tetranucleotides are isolated as hydrated triethylammonium salts after DEAE–cellulose chromatography.

One of the strategies employed in the nonenzymatic synthesis of deoxyribooligonucleotides involves the establishment of the 3′ → 5′ internucleotide bond as a phosphotriester, $(R^IO)(R^{II}O)(BLO)PO$, where BL represents the phosphate blocking group, which must eventually be removed to produce the desired phosphodiester, $(R^IO)(R^{II}O)P(O)OH$. This approach, introduced by Todd[2] and explored initially by Letsinger,[3] by Reese,[4] and by Cramer and Eckstein,[5] has been used by many investigators,[6-14] sometimes in conjunction with a search for new reagents to convert the two nucleosides into the triester intermediate. Intensive research effort during the past four years[15-28] discloses a continuing interest in deoxyribooligonucleotide syntheses, in spite of the solution by Khorana and his co-workers[29] of the problem of constructing genes by a combination of nonenzymatic and enzymatic techniques.[30]

The synthesis of ribooligonucleotides is also receiving much attention, in particular by Ikehara, Ohtsuka, and their coworkers, who have developed methods to produce segments suitable for conversion into larger units with amino acid acceptor activity.[31-34] The construction of tRNA's by a combination of nonenzymatic and enzymatic procedures seems possible based on these results.

Work in this Laboratory[35-39] has focused on the development of phosphorylating reagents capable of being applied



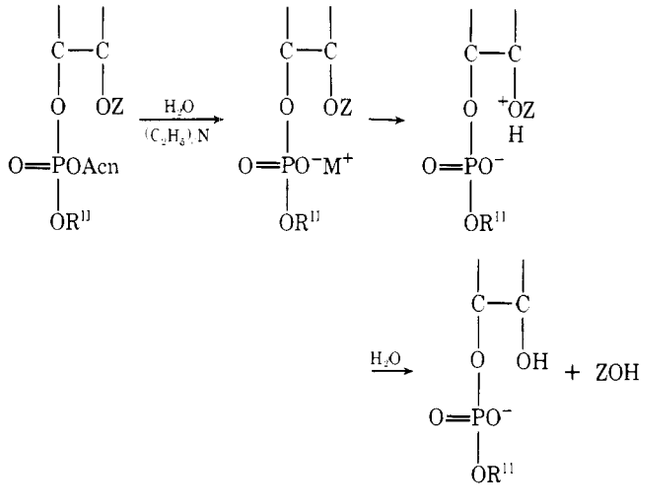
by standard procedures to the synthesis of complex phosphodiesters, such as the phospholipids of biological membranes,[40] and both types of oligonucleotides. Previous papers have described the preparation of several derivatives of the 1,2-dimethylethenylenedioxyphosphoryl group, abbreviated X=P(O)–, which are useful for this purpose.[35-37] The conversion of the alcohols R^IOH and $R^{II}OH$ into the phosphodiester can be achieved as "three-, two-, or one-flask" syntheses, according to the number of intermediates isolated and purified:

$$R^IOH + X{=}P(O)Y + B \rightarrow X{=}P(O)OR^I + Y^-BH^+ \quad (1)$$

$$R^{II}OH + X{=}P(O)OR^{I} \rightarrow (R^{I}O)R^{II}O)P(O)OCH(CH_3)COCH_3 \quad (2)$$

$$(R^{I}O)(R^{II}O)P(O)OCH(CH_3)COCH_3 + H_2O \rightarrow (R^{I}O)(R^{II}O)P(O)OH + HOCH(CH_3)COCH_3 \quad (3)$$

An attractive feature of this approach is the interdependence of the techniques used to remove the 1-methylacetonyl group (Acn) and the various alcohol-protecting groups in complex molecules.[41,42] A *tert*-butyldimethylsilyl group[43-45] (Z) on an alcohol adjacent to the phosphotriester function is relatively stable in mildly acidic and basic conditions. The Acn



group is easily removed in basic medium, and the silyl group in the phosphodiester becomes extremely acid labile, probably due to a high local acid concentration.[42]

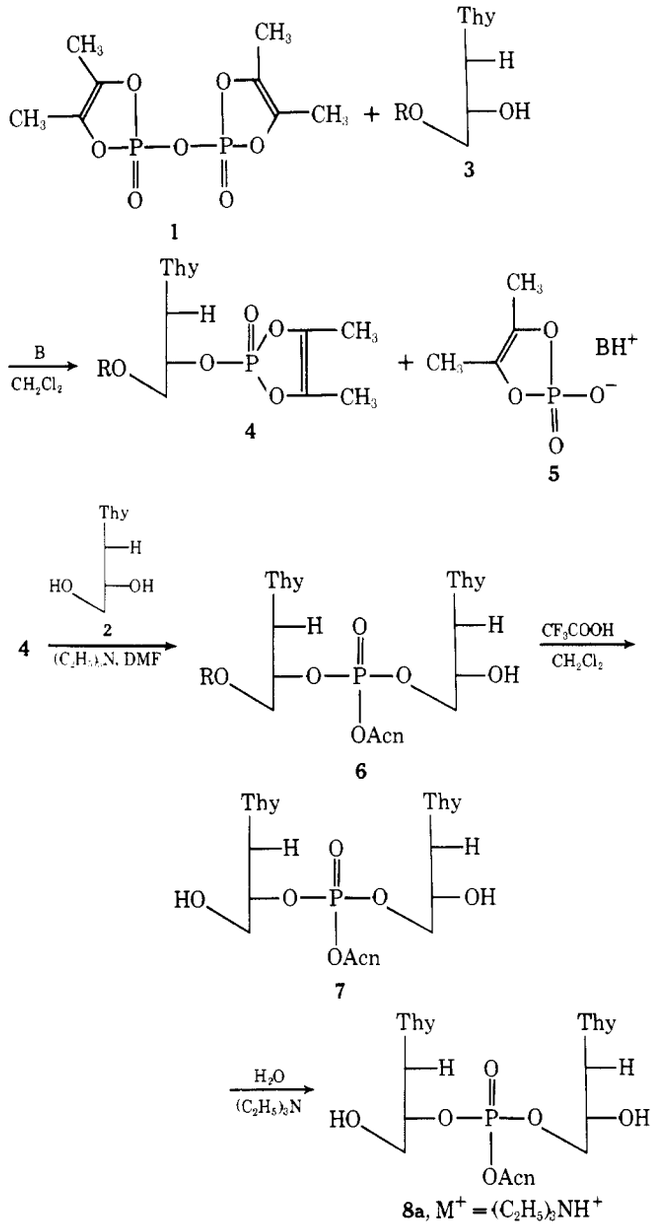
The neighboring group effect which operates in β-silyloxyethyl α-methylacetonyl phosphates can be exploited, in conjunction with other acid-labile alcohol-protecting groups, e.g., *p*-methoxytrityl,[46] in ribooligonucleotide synthesis. The present paper deals with one aspect of this general problem, namely, the application of the pyrophosphate, **1**, to the synthesis of di- and tetranucleotides from thymidine,[47] *using the p-methoxytrityl group to protect the C(5′)-OH function of only the first nucleoside of the chain.* The emphasis of the work is on isolation of the oligonucleotides in the form of their triethylammonium salts. The replacement of this cation by monovalent and divalent metal ions utilizing techniques similar to those that have been developed in the field of cardiolipins[40] would provide specimens needed for basic research on oligonucleotide–cation associations.[48]

Results

Stepwise Deoxyribooligonucleotide Synthesis. Thymidine (**2**) is converted into its dinucleotide, TpT, by means of the pyrophosphate **1**, according to the procedure outlined in Scheme I. The dinucleotide is isolated as the triethylammonium triaquo salt, **8a**·$3H_2O$. The first step of the synthesis is a displacement at the cyclic phosphorus atom by the 5′-protected nucleoside **3** with ring retention, which yields the cyclic phosphate **4** and the salt **5**. The reaction is carried out in dichloromethane solution in the presence of triethylamine as proton acceptor. The solvent is evaporated and replaced by dimethylformamide for the second step, which is a displacement at the cyclic phosphorus atom by the C(5′)–OH of *unprotected* thymidine (**2**), this time with ring opening. Triethylamine is an effective catalyst in this phosphorylation.[37] The protected dinucleotide triester is isolated as its monohydrate, **6**·H_2O, in 82% yield based on **3**, after silica gel chromatography; see Table I.

The third step of the synthesis is the removal of the *p*-

Scheme I[a]



[a] R = $(p\text{-}CH_3OC_6H_4)(C_6H_5)_2C$; Acn = $-CH(CH_3)COCH_3$; B = $(C_2H_5)_3N$.

methoxytrityl protective group by means of trifluoroacetic acid in dichloromethane solution.[49] The dinucleotide triester is isolated as its monohydrate, **7**·H_2O, in 90% yield based on **6**·H_2O, after silica gel chromatography.

The final step is the removal of the 1-methylacetonyl blocking group from the triester, **7**·H_2O, and is carried out for 2.5 h at 0 °C in a mixture of water and acetonitrile in the presence of 2 molar equiv of triethylamine. The crude product from this step contains 85–90% of TpT as its triethylammonium triaquo salt, **8a**·$3H_2O$, according to the analytical criteria described in the Experimental Section.[50] The pure salt, **8a**·$3H_2O$, is isolated in 85% of the theoretical yield based on **7**·H_2O, after chromatography on DEAE–cellulose.[51]

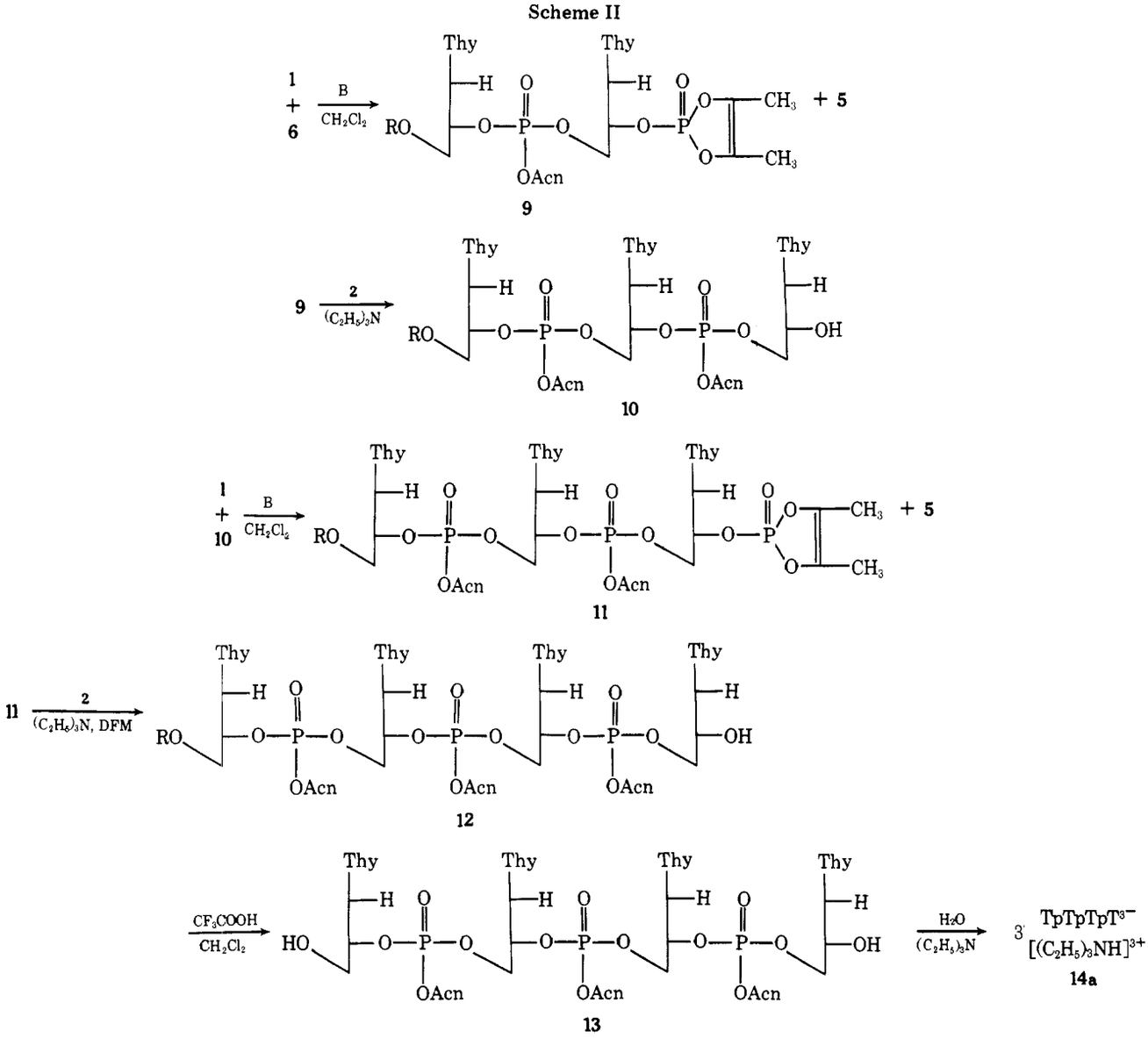
The conversion of thymidine into its tetranucleotide, isolated as the tris(triethylammonium) heptaaquo salt, **14a**·$7H_2O$, is shown in Scheme II. Reaction of the pyrophosphate **1** with the 5′-protected dinucleotide triester **6**, which has been rendered anhydrous by evaporation of a pyridine solution, generates the new cyclic phosphate **9**. The latter is allowed to couple with more thymidine to give the protected trinucleotide triester **10**·$2H_2O$, isolated in 61% yield based on dinucleotide

Table I. Properties of Synthetic Thymidine Oligonucleotides and Their Derivatives [R = (p-$CH_3OC_6H_4$)(C_6H_5)$_2$C; Acn = -$CH(CH_3)COCH_3$]

Registry no.	Compd no.	Compd	Mp, °C	R_f in TLC[a,b]
62930-00-1	4	5′-OR-T-3′-Cyclophosphate	92–100	...
62930-01-2	**6**·H_2O	5′-OR-Tp(Acn)T·H_2O	120–125	0.24 (I,B); 0.80 (I,C); 0.44 (II,D)
62962-23-6	**7**·H_2O	Tp(Acn)T·H_2O	105–114	0.42 (I,A); 0.08 (I,B); 0.63 (I,C); 0.13 (II,D)
62930-02-3	**8a**·3H_2O	TpT$^-$(C_2H_5)$_3$$NH^+$·3$H_2O$	68–76	0.15 (I,C); 0.33 (III,F)[c,d]
62930-03-4	**10**·2H_2O	5′-OR-Tp(Acn)Tp(Acn)T·2H_2O	126–132	0.57 (I,A); 0.06 (I,B); 0.31 (II,D)
62930-04-5	**12**·3H_2O	5-OR-Tp(Acn)Tp(Acn)Tp(Acn)T·3H_2O	130–140	0.29 (I,A); 0.21 (II,D)
62930-05-6	**13**·2H_2O	Tp(Acn)Tp(Acn)Tp(Acn)T·2H_2O	90–100	0.16 (I,A); 0.21 (II,E)
62930-06-7	**14a**·7H_2O	TpTpTpT^{3-}[(C_2H_5)$_3$$NH^+$]$_3$·7$H_2O$	140–149	0.06 (III,F)[e]
62930-07-8	**21a**·H_2O	5′-OR-TpT$^-$(C_2H_5)$_3$$NH^+$·$H_2O$	131–139	0.31 (I,C); 0.13 (II,G)
62930-08-9	**17**·H_2O	(5′-OR-T-3′)$_2$p(Acn)·H_2O	125–130	0.50 (I,B)

[a] I = silica gel plates, Eastman Kodak Co., Cat. No. 13179 (polyvinyl alcohol binder). II = silica gel plates, 60 F-254 (0.25-mm thickness), Merck Cat. No. 5760. III = Whatmann 3 MM paper. Solvents: A = THF; B = *c*-C_6H_{12}/CH_3COCH_3/C_5H_5N, 3/1/1; C = *i*-C_4H_9OH/C_5H_5N/H_2O, 7/2/1; D = CH_2Cl_2/CH_3OH, 9/1; E = CH_2Cl_2/CH_3OH, 5.7/1; F = *i*-C_3H_7OH/H_2O/conc NH_4OH, 7/2/1; G = CH_2Cl_2/CH_3OH, 3/1 v/v UV detection. Samples containing the *p*-methoxytrityl group are also detectable after spraying with 70% aqueous $HClO_4$ (2.5 mL) in acetone (50 mL). All phosphates are detectable after spraying with Hanes reagent [C. W. Stanley, *J. Chromatog.*, **16**, 467 (1964)]. [b] Reference R_f: T, 0.74 (I, C), 0.20 (II, D), 0.59 (III, F); 5′-OR-T (**3**), 0.63 (I, B), 0.58 (II, D); Tp, 0.01 (III, F); TpTpTp, 0.13 (III, F). Tentative Tp(Acn) or p(Acn)T (**21** or **22**), 0.40 (III, F). [c] Authentic TpT: R_f, 0.31 (III, F). [d] Paper electrophoresis: *Rm*, 0.30 (pH 7.2), 0.89 (pH 1.9) for **8a**; 0.30 (pH 7.2), 0.89 (pH 1.9) for authentic TpT. Reference: **Tp**, 1.00. Savant flat head for 1 h, in phosphate buffer (pH 7.2), Gilson Model D electrophorator for 1.5 h, in acetic–formic acid buffer (pH 1.9), both at 2000 V, ref 50. [e] Paper electrophoresis: *Rm*, 0.80 (pH 7.2), 1.35 (pH 1.9) for **14a** vs. **Tp** = 1.00. Other reference *Rm* values: trinucleotide TpTpT, 0.56 (pH 7.6), 1.18 (pH 1.9). Tentative Tp(Acn) or p(Acn)T, 0.45 (pH 7.2), 1.11 (pH 1.9); ref 50.

Scheme II



$6 \cdot H_2O$, after silica gel chromatography. Repetition of the phosphorylation and the coupling steps yields the protected tetranucleotide triester $12 \cdot 3H_2O$ via the cyclic phosphate **11**. The triester $12 \cdot 3H_2O$ is obtained in 49% yield based on trinucleotide $10 \cdot 2H_2O$. The removal of the *p*-methoxytrityl group from $12 \cdot 3H_2O$ is carried out also with trifluoroacetic acid and affords the tetranucleotide triester $13 \cdot 2H_2O$ in 82% yield after silica gel chromatography. The final hydrolysis of the 1-methylacetonyl group is carried out as in the synthesis of the dinucleotide. The crude product obtained in this step contains 80–85% of TpTpTpT as the tris(triethylammonium) heptaaquo salt, **14a**$\cdot 7H_2O$ (See Experimental Section[50]). Pure **14a**$\cdot 7H_2O$ is isolated in 75% of the theoretical yield based on $13 \cdot 2H_2O$, after chromatography over DEAE–cellulose.

The purified dinucleotide **8a** and tetranucleotide **14a** were completely degraded by snake venom and spleen phosphodiesterases, and the theoretical pT/T and Tp/T ratios were observed, within the limits of accuracy of these assays, using paper electrophoresis and chromatography as analytical tools.

The pyrophosphate reagent **1** is employed in stoichiometric amounts in the conversion of protected thymidine **3** into the protected dinucleotide triester **6**; however, in subsequent steps, an excess of pyrophosphate corresponding to about 25 mol % for each additional thymidine unit is required to achieve the reported yields of protected tri- and tetranucleotide triesters **10** and **12**. This requirement may be related to increasing difficulty in removing the last traces of water from the larger oligonucleotides. In the purification of the protected triesters **6**, **10**, and **12** by silica gel chromatography, the most efficient eluting solvent is a mixture of dichloromethane and methanol. This solvent is satisfactory for both column and preparative LC techniques, in the case of dinucleotide **6**. However, only PLC is recommended for the purification of the tri- and tetranucleotides **10** and **12** using this solvent, since the more protracted column chromatography results in a significant decrease in the yields of the pure triesters. Mixtures of ethyl acetate and tetrahydrofuran can also be used as the eluting solvent in silica gel column chromatography in all cases, although the efficiency of the separation is lower than in the corresponding CH_2Cl_2/CH_3OH procedure.

By-Products of the Synthesis. The cyclic pyrophosphate **1** is a powerful phosphorylating reagent, and its reactions with compounds which have one unprotected hydroxyl group, **3**, **6** and **10**, are rapid and quantitative. Not surprisingly, the pyrophosphate **1** is also very sensitive to water.

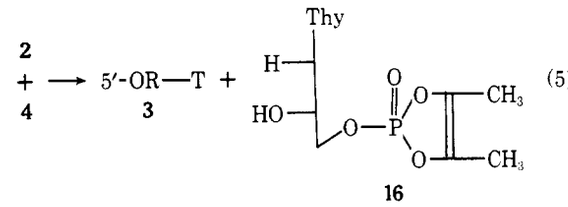
The triethylamine salt of 1,2-dimethylethenylene phosphate does not interfere in the phosphorylation steps required to establish the 3′ → 5′ internucleotide bond. The cyclic *anion*, unlike the corresponding acid and esters (hydrogen or alkyl 1,2-dimethylethenylene phosphates) is not a phosphorylating reagent. The salt is easily removed during the final purification of the triesters.

The cyclic phosphotriesters **4**, **9** and **11** are effective phosphorylating reagents toward alcohols, although they are much less reactive than the pyrophosphate **1**. Consequently, there is no appreciable competition between the cyclic phosphates and the pyrophosphate for the alcohol in the first step of the synthesis. We have shown that the phosphorylation of alcohols by alkyl 1,2-dimethylethenylene phosphates in aprotic solvents is effectively catalyzed by imidazole and by triethylamine.[34] Furthermore, triethylamine is quite effective in increasing the selectivity of the cyclic phosphates for primary alcohol functions in the presence of unprotected secondary alcohol groups in polyols.[34] The attack of the C(3′)–OH group of thymidine (**2**) on the cyclic phosphate **4**, in a displacement at phosphorus with ring opening, would produce the unnatural isomer **15** with the 3′ → 3′ internucleotide bond, according to the equation:

$$2 + 4 \rightarrow \underset{\mathbf{15}}{\text{5′-OR-T-3′p(Acn)-3′-T}} \qquad (4)$$

Isomer **15** is probably responsible for a spot observed in the TLC plates of the crude reaction product, with an R_f value somewhat higher than that of the 3′ → 5′ structure **6**. It is estimated that approximately 2% of the unnatural isomer **15** is formed in the synthesis of the dinucleotide; the by-product **15** is removed in the purification of the triester by silica gel chromatography. We have not detected spots in the TLC plates of the crude reaction products of the tri- and tetranucleotide syntheses[52] that could reasonably be attributed to structures containing the 3′ → 3′ bond resulting from lack of selectivity in the coupling step. It is noteworthy that, without triethylamine as catalyst in the coupling reaction, a significant amount of the unnatural isomers are produced.

A second potential source of by-products in the syntheses is the occurrence of transesterification during the reaction of alcohols with alkyl 1,2-dimethylethenylene phosphates. The transesterification reaction is a displacement at the cyclic phosphorus with ring retention, and we have shown that the triethylamine catalysis of this type of phosphorylation is accompanied by a significant decrease in the extent of transesterification.[34] In the dinucleotide synthesis, transesterification regenerates 5′-OR-T (**3**) and produces a new cyclic phosphate



(5)

16

16, as indicated in eq 5. 5′-OR-T (**3**) is detectable in the TLC plates of the crude reaction product, to an extent estimated as 1–2%. The second by-product **16** could be partly responsible for the spot observed at the origin in the plates. The corresponding transesterification by-products in the synthesis of tri- and tetranucleotides are, respectively, the di- and the trinucleotides **6** and **10**; these by-products are generated in approximately 5% yields in the syntheses. In all cases, these by-products are easily removed by silica gel chromatography.

The transesterification reaction can, in principle, introduce two additional by-products, namely, the symmetrical phosphotriesters, as illustrated in eq 6 and 7, for the dinucleotide case.

$$3 + 4 \longrightarrow \underset{\mathbf{17}}{\text{(5′-OR—T3′)}_2\text{p(Acn)}} \qquad (6)$$

$$2 + 16 \longrightarrow \underset{\mathbf{18}}{\text{(T-5′)}_2\text{p(Acn)}} \qquad (7)$$

Neither of these by-products have been detected.[52] For comparison purposes, compound **17** was independently synthesized from 2 molar equiv of 5′-OR-T (**3**) and the pyrophosphate **1**.

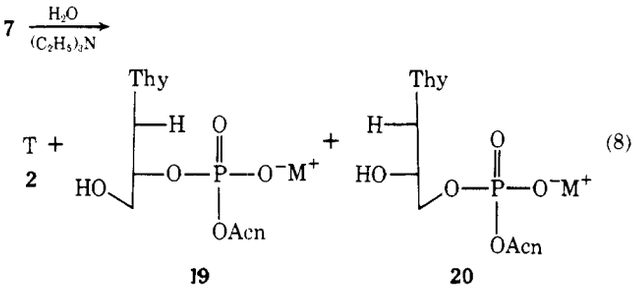
Removal of the *p*-methoxytrityl group from the protected oligonucleotides **6** and **12** is reasonably efficient using trifluoroacetic acid in dichloromethane solution at 0 °C; this method proved to be superior to that involving 75–80% glacial acetic acid as reagent.

The rate of basic hydrolysis of the 1-methylacetonyl group[41] from the oligonucleotide phosphotriesters **7** and **13** is significantly higher than that of simpler dialkyl 1-methylacetonyl phosphates.[37] The hydrolysis is sensitive to temperature and solvent composition. At 0 °C and in water/acetonitrile 1/2

Table II. Main ^{1}H NMR Signals[a] of Synthetic Thymidine Oligonucleotides and Their Derivatives

Assignments[b]	T(2)[i]	5′-OR-T (3)[i]		Cyclophosphate,[c,d] 4		R·Acn·T$_2$ (6·H$_2$O[e])	Acn·T$_2$ (7·H$_2$O[f])	TpT$^-$M$^+$ (8a·3H$_2$O[g])	TpTpTp-T^3-3M$^+$ (14a·7H$_2$O[g])	5′-OR-Tp-T$^-$M$^+$ (21a·H$_2$O[g])	(5′-OR-T-3′)$_2$-p(Acn) (17·H$_2$O)
	C_5D_5N	C_5D_5N	$CDCl_3$	C_5D_5N	$CDCl_3$	C_5D_5N	C_5D_5N	C_5D_5N	C_5D_5N	C_5D_5N	C_5D_5N
C(5)–CH_3	8.10	8.27	8.60	8.43	8.57	8.30 (1) 7.90 (2)	8.20 (1) 7.90 (2)	8.08 (1) 7.70 (2)	8.10 (1) 7.76 (2, 3, 4)	8.30 (1) 7.84 (2)	8.30
C(2′)–H_2	7.32	7.40	7.64	7.17	7.40	7.37 7.07	7.40 7.27	7.30	7.30	7.33	7.15
C(5′)–H_2	5.76	6.40	6.65	6.40	6.48	6.32 (1) 5.31 (2)	5.85 (1) 5.40 (2)	5.70 (1) 5.40 (2)	5.72 (1) 5.40 (2, 3, 4)	6.25 (1) 4.44 (2)	6.41
C(4′)–H	5.56	5.55	6.00	5.45	5.67	5.33	5.40	5.40	5.40	5.44	5.44
C(3′)–H	4.96	5.10	5.50	4.54	4.70	5.18 (2) 4.25 (1)	4.90 (2) 4.30 (1)	4.90 (2) 4.40 (1)	4.90 (4) 4.40 (1, 2, 3)	5.00 (2) 4.44 (1)	4.35
CH_3O		6.37	6.30	6.33	6.20	6.30				6.25	6.37
Acn–CH_3[h]						8.50	8.48				8.54
Acn–CH_3CO						7.76	7.74				7.80

[a] In ppm vs. Me_4Si = 10. Numbers in parentheses refer to nucleoside sequence, 1, 2, . . . n + 1, in a chain of n phosphates and n + 1 nucleosides from left to right. Signals of possible diastereomers of triesters are not resolvable. [b] C(1′)–H is at $\tau \sim 3.0$, C(6)–H at somewhat lower field. Exchangeable and aromatic ^{1}H omitted. [c] $CH_3C = CCH_3$: τ 8.28 (C_5D_5N), 8.10 ($CDCl_3$); ^{31}P δ −11.6 ppm vs. H_3PO_4 = 0 ($CDCl_3$). [d] Spectral changes for **6** → **9** and **10** → **11**, are analogous to those for **3** → **4**. [e] Spectra of **6**, **10**, and **12** are similar. [f] δ ^{31}P −3.7 (C_5D_5N) vs. H_3PO_4 = 0. Spectra of **7** and **13** are similar. [g] $(C_2H_5)_3NH^+$, τ 8.67 (quartet), 6.90 (triplet); J = 6 Hz. [h] Doublet, J = 7 Hz. The methine ^{1}H of Acn gives a multiplet at ~5.2–5.4. [i] Registry no.: **2**, 50-89-5; **3**, 42926-80-7.



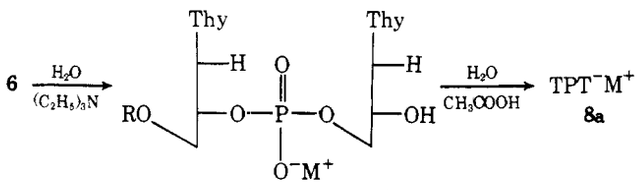
mixtures, the hydrolysis in the presence of triethylamine produces <2–3% phosphodiesters **19** and **20**, resulting from internucleotide bond cleavage (eq 8). This type of by-product is easily removed by chromatography.

Other Chain Building Sequences. Additional synthetic flexibility is provided by a reversal of the sequence of deprotection and deblocking steps, as illustrated in Scheme III. The 1-methylacetonyl group is removed from the protected dinucleotide triester **6** to give the protected diester, isolated as its triethylammonium aquo salt, **21a**·H_2O, after silica gel chromatography. The protected salt **21a** is a useful intermediate for the preparation of metal ion dinucleotide salts, and of other phosphotriesters. The removal of the *p*-methoxytrityl group from **21a** yields the salt of TpT, **8a**·3H_2O. A comparison of the relative deprotection rates of the phosphodiester **21a** and the phosphotriester **6** was carried out in 75% acetic acid; the deprotection of the diester is significantly faster.

State of Hydration and X-Ray Powder Photographs of Solid Deoxyribooligonucleotides. In the solid state, the oligonucleotides are quite hydroscopic. Reproducible results in the elemental analyses of the triesters **6**, **7**, **10**, **12**, **13**, and **17** and of the 5′-O-protected diester salt **21a** are obtained when the material which is isolated after silica gel chromatography is dissolved in tetrahydrofuran and the solution is added to moist diethyl ether. These substances are obtained as free-flowing powders which retain certain stoichiometric amounts of water after drying for 24 h at 25 °C and 0.1 mm. X-ray powder photographs reveal that the hydrated triesters **6**, **7**, **12**, and **13** are noncrystalline solids.

The triethylammonium salts of the oligonucleotides **8a** and **14a** are also obtained as free-flowing noncrystalline hydrates when their aqueous solutions are freeze-dried, and kept several hours at 25 °C and 0.1 mm.

Scheme III



21a, $M^+ = (C_2H_5)_3NH^+$

^{1}H NMR Spectra of Oligonucleotides in Pyridine-d_5. The cyclic phosphates **4**, **9**, and **11** are characterized by the relatively large downfield shift of the C(3′)-H signal of the respective precursors, **3**, **6**, and **10**, as a result of the phosphorylation of the alcohol, C(3′)–OH, and by the sharp signal of the two CH_3 groups on the 1,3,2-dioxaphospholene ring; Table II. The relatively large negative value of the ^{31}P NMR signal, −11 ppm vs. H_3PO_4 = 0, is also typical of the cyclic phosphates.

The spectrum of the protected dinucleotide triester **6** clearly shows the C(5)–CH_3 groups of T-1 and T-2, the latter being at lower magnetic field, possibly as a result of relative deshielding by the phosphoryl group (Table II). The signals from C(3′)–H of T-1 and C(5′)–H_2 of T-2 of **6** move downfield relative to those of protected and unprotected T, respectively, and relative to the signals from C(3′)–H and C(5′)–H_2 of T-2 and T-1 in **6**. These observations confirm the establishment of the 3′ → 5′ internucleotide bond in **6**. The spectra of the protected tri- and tetranucleotide triesters **10** and **12** are very similar to each other and to that of **6**, except that the signal(s) due to the C(5)–CH_3 of the internal nucleosides of the chain are shifted ca. 3 Hz toward high field from that of the terminal T, or T-(n + 1), with n = number of phosphates. The signals of the two internal C(5)–CH_3 of **12** overlap, at 60 MHz.

The isomer 5′-OR-T-3′-p(Acn)-3′-T (**15**) is characterized by signals at τ 8.20 and 5.85, associated with C(5)–CH_3 andC(5′)–H_2, respectively, of T-2 in the molecule of **15**. These two signals are very close to those of the corresponding groups in thymidine, reflecting the similarities in the magnetic environment of the respective protons.

In the salts **8a** and **14a** the C(5)–CH_3 signals of T-1 and T-(n + 1) are widely separated, but those of the internal nucleosides of **14a** are no longer resolvable at 60 MHz, and overlap with the signal of T-(n + 1). In crude samples of the

salts, a very weak signal at ca. τ 7.65 is indicative of 1-methylacetonyl containing by-products, e.g., **19** or **20.**

Discussion

The novel sequence for deoxyribooligonucleotide synthesis described in this paper has several noteworthy features: (1) The protected triesters are produced in essentially a "one-flask" reaction, since the cyclic phosphate intermediates prepared from the pyrophosphate and triethylamine are not isolated. This procedure is simpler than that which uses nicotinamide as proton acceptor.[1b] (2) The first P–O bond [at C(3′)–OH] is formed in a rapid and quantitative reaction. (3) The second P–O bond [at C(5′)–OH] is established on an unprotected nucleoside. The strategy of establishing the internucleotide bond with unprotected nucleosides has already been utilized by several groups.[6a,12a,27,28] (4) The 1-methylacetonyl (or 3-oxo-2-butyl) blocking group is not affected by the conditions required to deprotect the C(5′)–OH function, and conversely, the blocking group can be removed without affecting the 5′ protection. (5) Less than 2% of 3′ → 5′ internucleotide bond cleavage occurs in the phosphorus-deblocking step. (6) No internucleotide bond isomerization is detected in the deblocking step; venom and spleen phosphodiesterases completely degrade the synthetic oligonucleotides, before and after the DEAE–cellulose chromatography.

Experimental Section

Reactions involving derivatives of the 1,2-dimethylethenylenedioxyphosphoryl group must be carried out under strictly anhydrous conditions. The DMF was refluxed with P_2O_5, distilled in vacuum, shaken with Na_2CO_3, and redistilled under vacuum and stored over 4A molecular sieves until used. CH_2Cl_2 was distilled from P_2O_5 then from $CaCO_3$ and stored over molecular sieves. The triethylamine was dried over Na, distilled, and stored over molecular sieves. Thymidine was dehydrated by repeated evaporations from dry pyridine. Except as noted, all evaporations were carried out at ca. 30 °C and 20 mm (rotoevaporator), followed by 0.1 mm.

Elemental analyses of all compounds (except 4) in Table I were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. Values obtained for C, H, N, P, and H_2O (Karl Fischer method) agree with the calculated figures: C, ±0.7; H, ±0.5; N, ±0.3; P, ±0.1; H_2O, ±0.5% [all phosphotriesters were dried for 24 h at 25 °C (0.1 mm) prior to analysis]. The triethylammonium salts were dried as indicated, prior to analysis.

Purifications of oligonucleotide triesters **6, 7, 10, 12,** and **13** in a scale of 1 g or less were carried out on 20 × 20 cm precoated silica gel plates (2 mm-thick, PLC 60 F-254, Merck Cat. No. 5766). The triesters were applied to the plates in CH_2Cl_2 solution (0.3 g/plate), eluted as indicated, and extracted from the adsorbent by means of THF. Large-scale (>1 g) purification of the protected *dinucleotide* triester **6** was performed by column chromatography on silica gel 60 (70–230 mesh; Merck Cat. No. 7734) or silica gel H (short-column chromatography[53]) as indicated. All solvent compositions are v/v.

Di(1,2-dimethylethenylene) Pyrophosphate (Acetoin Enediol Cyclophosphate, 1). The pyrophosphate **1** was prepared as described.[32] The samples utilized exhibited one sharp signal at τ 8.02 (Me_4Si = 10) in their ^1H NMR spectra (60 mg of **1** in 0.5 mL of $CDCl_3$), and were assumed to contain at least 98% pure **1.**

5′-*O*-*p*-Methoxytritylthymidine (3). The procedure in the literature[46] was modified as follows to provide **3** of suitable quality for reaction with the pyrophosphate **1.**

A pyridine solution (100 mL) of T (**2,** 10.08 g) and *p*-methoxytrityl chloride (12.94 g) was stirred at 20 °C for 20 h. The solution was evaporated, the residue dissolved in chloroform (400 mL), and the solution extracted with water (two 400-mL portions, two 200-mL portions), dried (Na_2SO_4), and evaporated. The residue was redissolved in chloroform, and the solution was evaporated (three 25-mL portions). The foamy residue (22 g) was dissolved in CH_2Cl_2 (20 mL) and applied to a silica gel column (445 g, packed in CH_2Cl_2). Elution with 2 L of 49/1 and 1 L of 24/1 CH_2Cl_2/CH_3OH removed impurities. The product appeared in 500 mL of 24/1 CH_2Cl_2/CH_3OH; the solvent was evaporated and the residue was kept 1 h at 0.1 mm. The foamy residue was dissolved in anhydrous benzene (150 mL) and added to cyclohexane (200 mL); crystallization (6 h at 25 °C; 2 h at 0 °C) afforded 18.7 g (87% yield) of 5′-OR-T (**3**), mp 119–120 ° (after drying at 25 °C, 0.5 mm).

Protected Dinucleotide Triester 6. 5′-*O*-*p*-Methoxytritylthymidine (**3**) was dried by evaporation from pyridine (four 25-mL portions) followed by drying at 25 °C (0.1 mm), prior to reaction. A solution of **3** (5.14 g, 10 mmol) in dichloromethane (20 mL) was added, dropwise, to a stirred dichloromethane solution (10 mL) of the pyrophosphate **1** (2.82 g, 10 mmol) containing triethylamine (1.53 mL, 10 mol % in excess of 1 molar equiv) at 0 °C. After 5 h at 0 °C, the mixture was evaporated to yield the crude cyclic phosphate **4,** which was kept 5 min at 25 °C (0.1 mm). A solution of this material in DMF (10 mL) was added, *with protection against moisture,* to a stirred mixture of anhydrous thymidine (**2,** 2.42 g, 10 mmol), triethylamine (1.80 mL, 20 mmol), and DMF (10 mL), at 0 °C. The solution was stirred for 2 h at 0 °C, and was kept 12 h at 0 °C and 2 h at 25 °C. The solution was evaporated at 25 °C, first at 30 mm and then at 0.1 mm to remove all the DMF. The residue was dissolved in dichloromethane and the solution was poured into ice (ca. 200 g). The layers were separated and the aqueous solution was thoroughly extracted with dichloromethane. The combined organic solution was dried (Na_2SO_4) and evaporated at 25 °C (30 mm followed by 0.1 mm, overnight) to yield the crude triester **6**·H_2O (8.63 g). This material was applied (as a CH_2Cl_2 solution) to silica gel PLC plates (0.3 g/plate); two elutions with CH_2Cl_2/CH_3OH 9/1, followed by extraction of the substance from the silica by THF and evaporation of the solvent, afforded the pure triester **6**·H_2O. Purification of larger quantities was also performed by column chromatography, using the same elution solvent, or with ethyl acetate/THF 1/1. The pure triester **6**·H_2O was isolated in 82% of the theoretical yield based on 5′-OR-T (**3**). To obtain a free-flowing powder suitable for elemental analysis, a THF solution of the triester was added to stirred diethyl ether (1/10); the sample was dried several hours at 25 °C (0.1 mm).

Dinucleotide Triester 7. Approximately 20 molar equiv of trifluoroacetic acid[49] was added to a 0.002 M CH_2Cl_2 solution of the protected dinucleotide triester **6**·H_2O at 0 °C. After 20 min at 0 °C, pyridine was added to neutralize the acid, and the solution was evaporated. The crude product was purified by silica gel H (short-column chromatography[53]) using CH_2Cl_2/CH_3OH 9/1. Pure **7**·H_2O was obtained as a free-flowing powder in 90% yield based on **6**·H_2O upon addition of a THF solution to diethyl ether (1/10).

Removal of the *p*-methoxytrityl group from **6**·H_2O was also carried out in 75% acetic acid (0.1 M solution at 20 °C for 5 h). Pure **7**·H_2O was isolated in 65% yield after chromatography (ethyl acetate/THF 1/1 and 1/2 successively).

Thymidylyl-(3′ → 5′)-thymidine (8). Triethylamine (2 molar equiv, as a 0.2 M aqueous solution) was added, over a 15-min period, to a stirred 0.02 M solution of the dinucleotide triester **7**·H_2O in water/acetonitrile (1/5) at 0 °C. After 2.5 h at 0 °C, the solution was diluted with water (1/1) and was evaporated. The crude product was redissolved in water and was again evaporated [three 2-mL portions of H_2O, 25 °C (0.1 mm)]. The residue was dissolved in 2 mL of water and was freeze-dried to yield crude **8a** as a free-flowing powder (after 2 h at 25 °C and 0.1 mm). This crude material was submitted to elemental, NMR spectrometrical, and chromatographic analyses.

The crude dinucleotide was chromatographed over DEAE-Sephadex A-25 (bicarbonate form; equilibrated with 0.1 M NH_4HCO_3, pH 8.5; 1 × 20 cm column). The salt was applied in the minimum volume of buffer; the gradient to 0.25 M NH_4HCO_3 (pH 8.5) was started at fraction 6, ended at fraction 34, and followed by straight 0.25 M buffer. Fractions were ca. 7.5 mL, flow rate 60 mL/h; Isco UA-2 UV analyzer was used. OD 267 were determined on a Beckman D.U. spectrometer with a Gelford attachment. Three components accounted for 90% of the crude dinucleotide: T (2%), Tp(Acn) and/or p(Acn)T (2%), and TpT (96%), eluted successively. The expected nucleotide/nucleoside ratios were obtained in the snake venom and spleen phosphodiesterase digestions[50] of TpT.

Large-Scale Purification of the Triethylammonium Salt of TpT (8a). BioRad Cellex-D, normal capacity DEAE–cellulose was converted into the bicarbonate form by repeated washings with 0.5 M aqueous triethylammonium bicarbonate, followed by deionized water. (A stock 0.5 M triethylammonium bicarbonate solution was made by passing a solution of 0.5 mol of $NaHCO_3$ in 500 mL of deionized water through a Dowex cation-exchange resin in its triethylammonium form and washing the resin with an additional 500 mL of water.)

A solution of the crude triethylammonium salt of the oligonucleotide **8a** in water (ca. 0.2 g in 4 mL) was applied to a column of the DEAE–cellulose (bicarbonate) resin (2.5 × 20 cm). Elution was performed with the gradient which resulted from 1.5 L of deionized water in the mixing vessel and 1.5 L of 0.25 M triethylammonium bicarbonate in the reservoir; 180 17-mL fractions were automatically collected at 9-min intervals. The fractions were monitored by TLC (UV

detection). The combined fractions containing the oligonucleotide were evaporated at 30 °C (20 mm), the residue was dissolved in water (10 mL) and reevaporated, and the residue was dissolved in water (10 mL) and passed through a column of Dowex AG 50W-X8 cation exchange resin (50 g) in its triethylammonium form. The column was washed with water (100 mL) and the combined solution and washing were evaporated. The residue was taken up in 2 mL of H_2O and freeze-dried to give the pure salt **8a**·$3H_2O$ as free-flowing powder. This substance was shown to be homogeneous by TLC and by paper chromatography and electrophoresis. The results of the phosphodiesterase digestions of the oligonucleotide salt were in agreement with its structure.

Protected Trinucleotide Triester 10. The chromatographed protected dinucleotide triester **6**·H_2O (0.575 g, 0.63 mmol) was dehydrated by repeated evaporations from pyridine (five 5-mL portions), followed by drying at 25 °C (0.2 mm). A solution of **6** and triethylamine (80 mg, 0.80 mmol) in dichloromethane (1.0 mL) was immediately added to a stirred dichloromethane solution (0.5 mL) of the pyrophosphate **1** (0.223 g, 0.80 mmol) at 0 °C. An additional 0.2 mL of dichloromethane was used to transfer the material, and the final concentration of the solution was 0.3–0.4 M; this solution was stirred for 5 h at 0 °C and evaporated at 0 °C in vacuum. The residue was suspended in DMF (0.4 mL) at 0 °C and treated with a mixture of anhydrous thymidine (0.153 g, 0.63 mmol) and triethylamine (2 molar equiv) in DMF (1.0 mL). The resulting solution was kept for 12 h at 0 °C and for 2 h at 25 °C. The solvent and the amine were evaporated at 25 °C (0.1 mm; ca. 6 h). The residue was dissolved in dichloromethane and the solution was applied to two 20 × 20 cm PLC plates. The plates were successively eluted with CH_2Cl_2/CH_3OH 9/1 (twice) and 5.6/1 (once). Extraction of the substance from the silica by THF, evaporation of the solution, and drying at 25 °C (0.1 mm; ca. 12 h) afforded the pure triester **10**·$2H_2O$ in 61% of the theoretical yield based on dinucleotide **6**. A free-flowing powder suitable for elemental analysis was obtained by addition of a THF solution of the triester to stirred diethyl ether (1/10), filtration of the precipitate, and drying at 25 °C (0.1 mm).

Protected Tetranucleotide Triester 12. The chromatographed protected trinucleotide triester **10**·$2H_2O$ (0.286 g, 0.22 mmol) was dehydrated and submitted to the same sequence of reactions which transformed the dinucleotide **6**·H_2O into the trinucleotide **10**·$2H_2O$. The following amounts of reagents were employed: triethylamine (0.31 mmol) and dichloromethane (0.5 mL), pyrophosphate **1** (0.093 g, 0.31 mmol) and dichloromethane (0.3 mL) in the first step; thymidine (0.060 g, 0.23 mmol), triethylamine (2 molar equiv), and DMF (0.8 mL) in the second step. The experimental conditions were virtually identical with those described in the synthesis of the lower homologue. The crude tetranucleotide, in CH_2Cl_2 solution, was applied to one 20 × 20 cm PLC plate. The plate was successively eluted with CH_2Cl_2/CH_3OH 9/1 (twice) and 5.6/1 (twice). The pure triester **12**·$3H_2O$ was isolated in 49% of the theoretical yield based on trinucleotide **10**. A free-flowing powder suitable for elemental analysis was prepared as in the case of the lower homologue.

Tetranucleotide Triester 13. The deprotection of **12**·$3H_2O$ was carried out with trifluoroacetic acid as described for the lower homologue. The pure tetranucleotide triester **13**·$2H_2O$ was isolated in 82% yield based on **12**·$3H_2O$, after PLC, with CH_2Cl_2/CH_3OH 3/1 (twice) as the eluting solvent. The analytical sample was prepared by addition of a THF solution of the triester to stirred diethyl ether (1/10).

Removal of the *p*-methoxytrityl group from **12**·$3H_2O$ by 80% acetic acid (0.1 M, 3 h at 20 °C) gave **13**·$2H_2O$ in 71% yield, after chromatography.

TpTpTpT[47] (14). Triethylamine (6 molar equiv, as a 0.2 M aqueous solution) was added, over a 15-min period, to a stirred 0.02 M solution of the tetranucleotide triester **13**·$2H_2O$ in water/acetonitrile (1/5) at 0 °C. After 2.5 h at 0 °C, the solution was worked up as described in the synthesis of TpT (**8a**).

The crude tetranucleotide was chromatographed over DEAE-Sephadex A-25 as described in the case of the lower homologue. Six components accounted for 90% of the crude tetranucleotide: T (1%), Tp(Acn) and/or p(Acn)T (3%), TpT (5%), TpTpT (6%), TpTp(Acn) and/or p(Acn)TpT (6%), and TpTpTpT (79%) eluted successively. Characterization of the pure substances was by paper chromatography and electrophoresis (Table I); the identification of 1-methylacetonyl phosphodiesters is tentative. The expected nucleotide/nucleoside ratios were obtained in the snake venom and spleen phosphodiesterase digestions[50] of the TpTpTpT.

Large-Scale Purification of the Triethylammonium Salt of TpTpTpT (14a). The purification of a 0.2-g sample of **14a** was carried out as in the case of the dinucleotide **8a**. The purified tetranucleotide **14a**·$7H_2O$ was shown to be homogeneous by TLC and by paper chromatography and electrophoresis. The results of the phosphodiester digestions of the salt were in agreement with its structure.

Isolation of Protected Nucleoside Cyclic Phosphate 4 and Protected Dinucleotide Triester Cyclic Phosphate 9. A solution of the respective alcohol, **3** or **6** (1 molar equiv), in CH_2Cl_2 was added dropwise to a stirred solution of the pyrophosphate **1** (1.25 molar equiv) in CH_2Cl_2 containing suspended nicotinamide (1.50 molar equiv) at 0 °C. The final volume of the solution was ca. 0.3 M in alcohol **3** or **6**, and most of the nicotinamide salt of 1,2-dimethylethenylene phosphate formed in the reaction remained in suspension. The mixture was stirred for 4–5 h at 0 °C, and was filtered through a medium-porosity sintered glass funnel under a blanket of N_2 or Ar. The insoluble salt was washed with a few milliliters of CH_2Cl_2 and the solution was evaporated to yield the moisture-sensitive cyclic phosphates **4** and **9** as solid foams. This material contained traces of the nicotinamide salt. The ^1H NMR spectra of the cyclic phosphates are summarized in Table II.

(5′-*O*-*p*-Methoxytrityl)thymidylyl-(3′ → 5′)-thymidine (21). A solution of the purified triester **6**·H_2O (2.65 g, 3 mmol) in a mixture of water (20 mL), acetonitrile, (10 mL), and triethylamine (6 mmol) was stirred at 20 °C for 1 h; TLC indicated completion of the reaction. The solution was evaporated to a 2-mL volume and freeze-dried to a powder (2.54 g). Part of this material (1.0 g) was dissolved in THF (2 mL) and placed on a column of silica gel. Ethyl acetate (50 mL) and THF (50 mL) eluents contained no material. THF/methanol 9/1 (80 mL) and 4/1 (40 mL) eluted a mixture of 5′-OR-TpT$^-$ M^+ (**21a**) and what could be 5′-OR-Tp(Acn)$^-$ M^+, according to their relative TLC mobilities. THF/methanol 4/1 (400 mL) eluted pure **21a** (0.88 g, after evaporation). The salt **21a** was dissolved in water (20 mL) and passed through a column of Dowex AG 50W-X8 cation exchange resin in the $(C_2H_5)_3NH^+$ form. The eluent and the aqueous wash (30 mL) were combined, evaporated to 1 mL, and freeze-dried (0.9 g of **21a**·H_2O, 85% recovery in the purification step). This material was dissolved in THF (7 mL) and the solution was added dropwise with vigorous stirring to 75 mL of diethyl ether. The solid which separated was filtered and dried (50 °C, 0.1 mm, 12 h) to give free-flowing 5′-*O*-*p*-methoxytritylthymidylyl-(3′ → 5′)-thymidine triethylammonium monoaquo salt (**21a**·H_2O).

Removal of the *p*-Methoxytrityl Group from the Protected Dinucleotide Phosphodiester 21. A solution of the salt **21a**·H_2O (0.31 g) in 80% acetic acid (3 mL) was stirred at 20 °C for 3 h. The solution was diluted with water (10 mL) and evaporated. The residue was treated with water (10 mL), the mixture was reevaporated, and the residue was again treated with water (10 mL) and filtered to remove tritanol. The aqueous solution was evaporated to a 2-ml volume and freeze-dried to yield 0.23 g of TpT$^-(C_2H_5)_3NH^+$·$3H_2O$ (**8a**·$3H_2O$) characterized by TLC.

1′-Methylacetonyl Bis[(5′-*O*-*p*-methoxytrityl)thymidylyl-3′] Phosphate (17). (a) A solution containing 5′-OR-T (**3**, 1.02 g, 2 mmol) and the pyrophosphate **1** (0.25 g, 1 mmol) in pyridine (1 mL) was stirred at 20 °C for 48 h. The solution was evaporated and the residue was stirred with water (30 mL) and filtered. The solid was washed with water, dried (25 °C, 0.1 mm, 24 h), and dissolved in THF. The solution was filtered and the filtrate added to diethyl ether. The solid was filtered and dried (30 °C, 0.1 mm, 48 h) to give **17** in ca. 75% yield.

(b) The same material was made by reaction of 5′-OR-T (**3**) with the cyclic phosphate **4**.

Registry No.—**1**, 55894-94-5; **9**, 62930-09-0; **11**, 62930-10-3; **15**, 62930-11-4; *p*-Methoxytrityl chloride, 14470-28-1.

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Boron Compounds. 45.[1] 6-Deoxy-*O*-acyl-α-L-mannofuranoses via *O*-Ethylboranediyl Derivatives

Wilhelm Volker Dahlhoff and Roland Köster*

Max-Planck-Institut für Kohlenforschung, D-433 Mülheim a. d. Ruhr, West Germany

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The O-diethylborylation of 6-deoxy-L-mannopyranose (L-rhamnose) (1) yields 6-deoxy-1,2,3,4-tetrakis-*O*-diethylboryl-α-L-mannopyranose (**2a**), which in the presence of >BH gives a mixture of four isomers **3a–d** and 6-deoxy-1,2:3,5-bis-*O*-ethylboranediyl-β-L-mannofuranose (**4**). Ethylboroxine or bis(ethylpivaloyloxy)diboroxane (BEPDIB) and **1** give over 90% yields of **4** or 6-deoxy-2,3-*O*-ethylboranediyl-α-L-mannofuranose (**5**), depending on the molar ratio used. A reversible trans-O-ethylboranediylation between **4** and **5** occurs on heating **5** or on heating a mixture of **1** and **4** in pyridine. The O-acetylation of **5** gives the 1,5-di-*O*-acetyl derivative **6**, which on deborylation and subsequent O-acylations lead to the boron-free derivatives **7** and **8a** or **8b**, respectively. Ethylboroxine and 6-deoxy-2,3-*O*-isopropylidene-L-mannofuranose (**9**) react to give the 1,5-ethylboranediyl derivative **10** in high yield. The hydride numbers (HZ) of 1 H_2O, **3a**, **4**, **5**, **7**, **8a**, and **9** were determined using propyldiborane(6).

Previous investigations[2–5] on the structures and properties of *O*-ethylboranediyl derivatives of some polyhydroxy compounds have shown that they are sometimes attractive alternatives to conventionally protected compounds for regioselective transformations to the O derivatives. The ease of introduction and removal of the *O*-ethylboranediyl protective group has been illustrated with xylitol,[2] D-mannitol,[3] dulcitol,[4] and several methyl glycosides.[5] In our previous publication with some methyl glycosides as model compounds, no ring isomerization could occur. The present study on the *O*-ethylboranediyl derivatives of 6-deoxy-L-mannopyranose (L-rhamnose) (1) shows that facile pyranose/furanose isomerizations and anomerizations can occur with the *O*-ethylboranediyl derivatives of the free monosaccharides.

Results and Discussion

A. The Indirect O-Ethylboranediylation of 1. The O-diethylborylation of the crystalline 6-deoxy-α-L-mannopyranose monohydrate[6] (1·H_2O) with activated triethylborane[7] at room temperature gives 6-deoxy-1,2,3,4-tetrakis-*O*-