period of time, and the mixture was then filtered and washed as described under the thymidine assay procedure. The filtrate was evaporated to dryness, and the residue was treated for 1 hr with 1 ml of concentrated ammonium hydroxide. The hydrolysate was concentrated to a small volume and chromatographed on Whatman No. 40 filter paper. The uv-absorbing bands were cut out and eluted with water, and the solution was analyzed spectrophotometrically.

Deacetylation of Polymer-Supported 3-O-Acetate Derivatives.—The hydrolyzing medium was prepared by dilution of 2 *M* potassium hydroxide in methanol with nine volumes of dioxane. In a typical hydrolysis reaction 8 ml of this solution was added to 300 mg of dry TpTOAc polymer, and the mixture was continuously agitated at room tempeature for 30 min. The mixture was filtered and the polymer washed with eight 8-ml portions of methanol–dioxane (1:9, v/v), 15 min for each portion, then with methanol, and vacuum dried as before.

In those instances where ion exchange was desired, the polymer was washed with four or five portions of methanol–dioxane mixture, 5 min each, then with eight portions of 10% triethylammonium acetate in pyridine (or 10% pyridinium acetate in pyridine) for 30 min each, then twice with dimethylformamide to remove a trace of unidentified flocculent white solid, and finally with several changes of reagent grade pyridine, then with methanol, and dried.

Higher Oligomers.—Further condensation to higher oligomers was conducted as described for the initial supported thymidine–pTOAc condensation.

Enzymic Hydrolyses.—Spleen phosphodiesterase solution was prepared by dissolving 10–15 units of lyophilized enzyme[23] in 2 ml of 0.2 *M* aqueous ammonium acetate (pH 5.7). To ten OD_{267} units of oligonucleotide in 10 μl of water was added 40 μl of enzyme solution and the mixture was incubated at 37° for 5–6 hr. The hydrolysis mixture was spotted on Whatman No. 40 paper and chromatographed.

Venom phosphodiesterase solution was prepared by dissolving 5–7 mg of lyophilized enzyme[23] in 1 ml of 0.1 *M* Tris HCl buffer (pH 8.9). Samples of 20 μl solution per ten OD units of oligonucleotide were used in the hydrolysis with subsequent treatment as above. Nucleotide/nucleoside mole ratios found were within ±8% of theory.

Calculation of Corrected Values of Polymer-Bound Products.—If it is assumed that the weight increase of the polymer during the reaction

$$\text{Ⓟ}_c\text{Tr(OCH}_3\text{)T(pT)}_n\text{OAc} \longrightarrow \text{Ⓟ}_c\text{Tr(OCH}_3\text{)T(pT)}_{n+1}\text{OAc}$$

is due only to the added protected nucleotide, then the polymer weight increase is kA, where k is the molecular weight of the protected nucleotide[24] times 10^{-6} and A is the number of μmoles of $T(pT)_{n+1}OAc$ oligomer formed. If B is the number of μmoles of $T(pT)_{n+1}OAc$ oligomer *found* per gram of product polymer, then for 1.000 g of starting polymer

$$B = \frac{A}{1.000 + kA} \quad \text{or} \quad A = \frac{B}{1.000 + kB}$$

When $n = 0$, $k = 447 \times 10^{-6}$; $k = 405 \times 10^{-6}$ for all other values of n. The value of A thus determined was used to calculate the percentage conversion for all reactions described herein.

Registry No.—Pentathymidine tetraphosphate, 17853-36-0.

Acknowledgments.—We wish to thank Dr. R. E. Benson for helpful discussions and Miss Eleanor Applegate for invaluable technical assistance.

(23) Worthington Biochemical Corp., Freehold, N. J.

(24) The weight increase included 1 mol of triethylamine.

Oligonucleotide Syntheses on Insoluble Polymer Supports. III. Fifteen Di(deoxyribonucleoside) Monophosphates and Several Trinucleoside Diphosphates

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The previously described insoluble styrene–divinylbenzene copolymer containing methoxytrityl functional groups has been condensed with the N-acylated deoxyribonucleosides N-benzoyldeoxyadenosine, N-anisoyldeoxycytidine, and N-acetyldeoxyguanosine to give the corresponding supported nucleosides in amounts corresponding to 325–360 μmol/g of polymer. Condensation of these products, and a similar thymidine-containing polymer, with the protected nucleotides N-3′-O-diacetyldeoxyadenosine 5′-phosphate, N-anisoyl-3′-O-acetyldeoxycytidine 5′-phosphate, N-3′-O-diacetyldeoxyguanosine 5′-phosphate, and 3′-O-acetylthymidine 5′-phosphate in the presence of 2,4,6-triisopropylbenzenesulfonyl chloride gave 15 dinucleoside phosphates in isolated conversions of 10–60% based on polymer-bound nucleoside. Several dinucleoside monophosphate-containing polymers were 3′-O-deacetylated and further condensed to trinucleoside diphosphate derivatives from which were isolated deoxyadenylyl-(3′→5′)-thymidylyl-(3′→5′)-thymidine (dApTpT), deoxyguanylyl-(3′→5′)-thymidylyl-(3′→5′)-thymidine (dGTpT), deoxycytidylyl-(3′→5′)-deoxycytidylyl-(3′→5′)-thymidine (dCpCpT), deoxycytidylyl-(3′→5′)-thymidylyl-(3′→5′)-thymidine (dCpTpT), and thymidylyl-(3′→5′)-deoxycytidylyl-(3′→5′)-thymidine (dTpCpT) in conversions of 10–75% based on dinucleoside phosphate. Specific enzymic hydrolysis showed the products to contain exclusively 3′→5′ phospho diester linkages.

A large proportion of previously reported work on polymer-supported oligonucleotide syntheses has dealt with thymidine-containing homooligonucleotides[1–5] while only limited studies on polymer-supported heterooligonucleotides have been described. These include the synthesis of thymidylyl-(3′→5′)-deoxyadenosine (dTpA), thymidylyl-(3′→5′)-deoxycytidine (dTpC), and thymidylyl-(3′→5′)-deoxyguanosine (dTpG)[6] on a soluble support as reported by Khorana and coworkers,[1] the deoxycytidine-containing products deoxycytidylyl-(3′→5′)-thymidine (dCpT), deoxycytid-

(1) (a) H. Hayatsu and H. G. Khorana, *J. Amer. Chem. Soc.*, **88**, 3182 (1966); (b) *ibid.*, **89**, 3880 (1967).

(2) F. Cramer, R. Helbig, H. Hettler, K. H. Scheit, and H. Seliger, *Angew. Chem. Intern. Ed. Engl.*, **5**, 601 (1966).

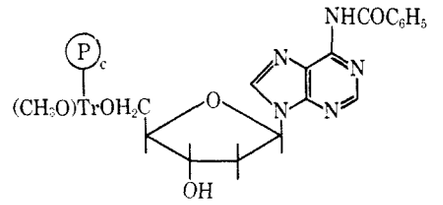
(3) (a) L. R. Melby and D. R. Strobach, *J. Amer. Chem. Soc.*, **89**, 450 (1967); (b) *J. Org. Chem.*, **34**, 421 (1969).

(4) (a) R. L. Letsinger, M. H. Caruthers, and D. M. Jerina, *Biochemistry*, **6**, 1379 (1967); (b) R. L. Letsinger, M. H. Caruthers, P. S. Miller, and K. K. Ogilvie, *J. Amer. Chem. Soc.*, **89**, 7146, (1967).

(5) G. M. Blackburn, M. J. Brown, and M. R. Harris, *J. Chem. Soc., C*, 2438 (1967).

(6) Conventional oligonucleotide symbolism and abbreviations are used throughout this paper; see previously cited references. For simplicity, dinucleoside phosphates will sometimes be referred to as dimers and trinucleoside diphosphates as trimers.

CHART I
NUCLEOSIDE POLYMERS[a]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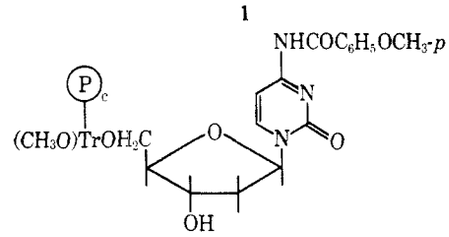
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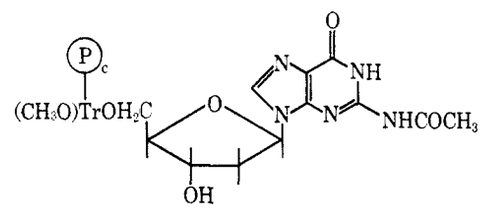

3

[a] —Ⓟc = cross-linked polystyrene backbone and $(CH_3O)Tr$ = pendant 4-methoxytrityl group. These formulations will be further abbreviated as Ⓟo $(CH_3O)TrdA^{Bz}$, etc., indicating 5′ attachment of nucleoside to the polymer.

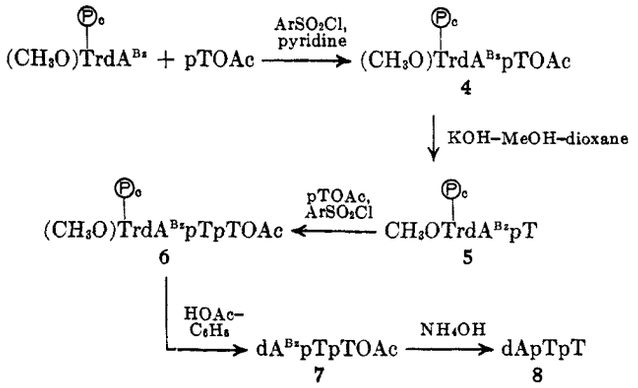
ylyl-(3′→5′)-thymidylyl-(3′→5′)-thymidine (dCpTpT) and deoxycytidylyl-(3′→5′)-thymidylyl-(3′→5′)-thymidylyl-(3′→5′)-thymidine (dCpTpTpT),[7] and, more recently, the guanine-containing dimer deoxyguanylyl-(3′→5′)-deoxyguanosine[8] prepared on an *insoluble* support by Letsinger and coworkers.

This paper will present the results of our initial studies on the synthesis of 15 of the possible 16 di(deoxyribonucleoside) monophosphates derived from the four major natural deoxynucleosides deoxyadenosine (dA), deoxycytidine (dC), deoxyguanosine (dG), and thymidine (T) using the *insoluble* methoxytrityl-containing styrene–divinylbenzene (DVB) copolymer support described in our previous work[3]; the latter dealt exclusively with TpT and its oligomers which will not be considered here. In addition, the transformation of several polymer-supported dinucleoside monophosphates to trinucleoside diphosphates containing dA, dC, and dG will be discussed.

Polymer-Supported Nucleosides and Their Assay.—N-Benzoyldeoxyadenosine[9] (dA^{Bz}), N-anisoyldeoxycytidine[9] (dC^{An}), and N-acetyldeoxyguanosine[10] (dG^{Ac}) were condensed, in anhydrous pyridine, with 1% cross-linked divinylbenzene (DVB)–styrene copolymer bearing methoxytrityl chloride functional groups[3] to give the supported nucleoside derivatives **1**, **2**, and **3** (Chart I) containing, respectively, 360, 325, and 350 μmol of protected nucleoside per gram of polymer.[11] These

(7) (a) R. L. Letsinger and V. Mahadevan, *J. Amer. Chem. Soc.*, **87**, 3526 (1965); (b) *ibid.*, **88**, 5319 (1966).
(8) T. Shimidzu and R. L. Letsinger, *J. Org. Chem.*, **33**, 708 (1968).
(9) H. Schaller, G. Weimann, B. Lerch, and H. G. Khorana, *J. Amer. Chem. Soc.*, **85**, 3821 (1963).
(10) S. A. Narang, T. M. Jacob, and H. G. Khorana, *ibid.*, **87**, 2988 (1965).
(11) In previous publications we have cited loadings to the nearest whole number, but here we cite values to the nearest multiple of five to conform more realistically to the limits of accuracy of the assay procedures.

SCHEME I



values correspond to about 55–60% conversions based on active chloride in the starting polymer. We had achieved much higher conversions in the thymidine series using a mixed benzene–pyridine solvent, but this mixture was unsuitable for the present work because of the relatively low solubility of these acylated nucleosides.

Infrared spectra of the three nucleoside polymers exhibit strong bands in the 5.9–6.1-μ region characteristic of the acylated nucleosides. Quantitative assay was carried out by hydrolysis of the polymers with 1% trifluoroacetic acid in benzene at room temperature for 24 hr. In this way intact N-anisoyldeoxycytidine was released quantitatively and was directly estimated spectrophotometrically. N-Benzoyldeoxyadenosine was quantitatively released and degraded to N-benzoyladenine. This was hydrolyzed with aqueous ammonia to free adenine which was isolated by paper chro-

TABLE I
DI(DEOXYRIBONUCLEOSIDE) MONOPHOSPHATE SYNTHESES

Product	—Chromatographic mobility, solvent A[a] R_f	R_{pT}[d]	—λ_{max}, mμ— H_2O	pH 2	ε max[b] (H_2O)	Polymer, μmol/g	% convn[c]
dApA	0.37	2.8	258	256	30,800	35	10
dApC	0.35	2.7	262	265	22,200	90	26
dApG	0.19	1.5	253	256	27,300	65	17
dApT	0.34	3.5	261	261	20,500	130	39
dCpA	0.36	2.7	262	267	22,200	30	9
dCpC	0.33	3.0	270	279	27,000	95	24
dCpG	0.16	1.2	254	277	19,700	85	24
dCpT	0.32	3.0	268	273	18,800	180	60
dGpA	0.16	1.3	255	257	27,300	35	10
dGpC	0.15	1.4	256		19,700	95	25
dGpG[e]	0.06	0.5	252	255	27,400	135	40
dGpT	0.17	1.5	256	256	20,800	190	59
dTpA	0.36	4.0	260	260	20,500	110	21
dTpC	0.35	3.9	268	273	18,800	205	42
dTpG	0.17	1.9	255	257	20,800	90	17

[a] 2-Propanol–concentrated NH_4OH–water (7:1:2, v/v). [b] Several of these molar extinction coefficients were taken from ref 14b, the remainder were calculated by summing the extinctions of the constituent nucleotides and nucleosides at the appropriate wavelengths and ignoring hypochromicity. The number of μmoles of material were determined using these molar extinctions. [c] Conversions based on nucleoside polymers having, respectively, 360 μmol of dA^{Bz}, 325 μmol of dC^{An}, 350 μmol of dG^{Ac}, and 550 μmol of T/g of polymer, respectively. The per cent values were corrected for polymer weight gain (see ref 3b). [d] Mobility relative to pT. [e] Because of its very low mobility dGpG does not form a well-resolved band in solvent A; thus its per cent conversion figure may be considerably more in error than the ±2% limits for the others.

TABLE II

DEOXYADENYLYL-(3′→5′)-THYMIDYLYL-(3′→5′)-THYMIDINE

Polymer stage	Component	Chromatographic mobility, solvent A R_f	Chromatographic mobility, solvent A R_{pT}	λ_{max}, mμ (H_2O)	ϵ[a] (H_2O)	Polymer, μmol/g	% convn
Nucleoside	dA^{Bz}					360	
Dinucleoside phosphate	dApT	0.34	3.5	261	20,500	70	19
Trinucleoside diphosphate	dApT					100	
	dApTpT	0.14	1.0	263	28,900	55	79[b]

[a] A. M. Duffield and A L. Nussbaum [*J. Amer. Chem. Soc.*, **86**, 111 (1964)] report ε 20,500 for dpTpA. For the trimer we added 8400, the extinction of pT at 260 mμ. [b] Conversion based on dinucleoside phosphate of precursor, not corrected for weight gain.

TABLE III

DEOXYGUANYLYL-(3′→5′)-THYMIDYLYL-(3′→5′)-THYMIDINE

Stage	Component	Chromatographic mobility, solvent A R_f	Chromatographic mobility, solvent A R_{pT}	λ_{max}, mμ (H_2O)	ϵ[a] (H_2O)	Polymer, μmol/g	% convn[b]
Nucleoside	dG^{Ac}					350	
Dinucleoside phosphate	dGpT	0.17	1.5	257	20,800	105	30
Trinucleoside diphosphate	dGpT					115	
	dGpTpT	0.06	0.45	256	29,000	65	62

[a] Reference 14b reports ε 20,800 for dGpT. For the trinucleoside diphosphate we added 8200, the extinction of pT at 256 mμ. [b] Not corrected for weight gain.

TABLE IV

DEOXYCYTIDINE–THYMIDINE OLIGOMERS[a]

Dinucleoside phosphate polymer	Derived trimer polymer products	Chromatographic mobility, solvent A R_f	Chromatographic mobility, solvent A R_{pT}	Spectral data, pH 2 λ_{max}, mμ	Spectral data, pH 2 ε	Polymer, μmol/g	% convn
dCpC						95	
	dCpC	0.32	2.0	279	27,000[b]	50	
	dCpT	0.38	2.4	273	21,200[c]	20	
	dCpCpT	0.12	0.8	276	31,500[d]	15	16
dCpT						100	
	dCpT	0.40	2.4	273	21,200	75	
	dCpTpT	0.15	0.9	270	30,100[e]	10	10
dTpC						120	
	dTpC	0.36		273	21,200[c]	30	
	TpT	0.45		267	18,500	30	
	dTpCpT	0.10		271	30,100[e]	25	21

[a] In contrast to the polymers of Tables I–III which were prepared by nucleotide condensation with triisopropylbenzenesulfonyl chloride and 3′-O-deacetylated with KOH–MeOH–dioxane, the dinucleoside phosphate precursors of Table IV were prepared by dicyclohexylcarbodiimide condensation (see ref 3a) and deacetylated with the crown ether–KOH complex of ref 18. [b] Sum of dpC and dC extinctions. [c] Value taken from that reported for dTpC, P. T. Gilham and H. G. Khorana, *J. Amer. Chem. Soc.*, **80**, 6212 (1958). [d] Value taken from dTpCpC, A. L. Nussbaum, G. Scheuerbrandt, and A. M. Duffield, *ibid.*, **86**, 102 (1964). [e] Sum of dCpT and pT at 270 mμ.

matography and determined spectrophotometrically.[12] Acid treatment of the N-acetyldeoxyguanosine polymer quantitatively liberated the guanine-containing constituents as a mixture of N-acetylguanine and undegraded N-acetyldeoxyguanosine. The mixture was hydrolyzed with ammonia; the two constituents (guanine and deoxyguanosine) were isolated by paper chromatography and separately assayed spectrophotometrically, the molar sum of the products being equivalent to the amount of initially bound N-acetyldeoxyguanosine.

(12) One should be able to estimate the amount of N-benzoyladenine by direct spectrophotometric analysis, but at this point we had to be assured that the hydrolysate did not contain a mixture of N-benzoyladenine and undegraded N-benzoyldeoxyadenosine.

Condensation of Polymer-Supported Derivatives with Protected Nucleotides.—The nucleoside polymers **1**, **2** and **3**, as well as the thymidine analog,[3] were condensed with the acylated 5′ nucleotides N-3′-O-diacetyldeoxyadenosine 5′-phosphate[13] ($dpA^{Ac}OAc$), N-anisoyl-3′-O-acetyldeoxycytidine 5′-phosphate[14] ($dpC^{An}OAc$), N-3′-O-diacetyldeoxyguanosine 5′-phosphate[15] ($dpG^{Ac}OAc$), and 3′-O-acetylthymidine 5′-phosphate[16] (pTOAc), as their bis(triethylammonium) salts in the presence of 2,4,6-triisopropylbenzenesulfonyl chloride[17,3b] to obtain the polymer-supported acylated dinucleoside phosphates (*e.g.*, **4**) (Scheme I). In those instances where the dimer polymer was carried to the trinucleoside diphosphate stage (*e.g.*, **6**), the 3′-O-acetyl group of the acylated dimer was most conveniently removed by treatment with 0.2 *M* potassium hydroxide in methanol–dioxane.[3b,18] Subsequent nucleotide condensation with polymer of type **5** gave the acylated derivative (**6**) from which the trinucleoside diphosphate was isolated by acidic release (see below), ammoniacal deacylation, and paper chromatography. Per cent conversions, chromatographic mobilities, and ultraviolet spectral data for 15 dinucleoside monophosphates (excluding TpT) are listed in Table I while data for the trinucleoside diphosphates are included in Tables II–IV.

Acidic Removal of Products from Oligonucleotide Polymer.—In previous publications[3] we described the preferred use of acetic acid–water–benzene mixture (16:4:5, v/v) for cleavage of products from the sup-

(13) R. K. Ralph and H. G. Khorana, *J. Amer. Chem. Soc.*, **83**, 2926 (1961).

(14) (a) H. G. Khorana, A. F. Turner, and J. P. Vizsolyi, *ibid.*, **83**, 686 (1961); (b) H. Schaller and H. G. Khorana, *ibid.*, **85**, 3828 (1963).

(15) R. K. Ralph, W. J. Connors, H. Schaller, and H. G. Khorana, *ibid.*, **85**, 1983 (1963).

(16) H. G. Khorana and J. P. Vizsolyi, *ibid.*, **83**, 675 (1961).

(17) R. Lohrmann and H. G. Khorana, *ibid.*, **88**, 829 (1967).

(18) In some experiments (Table IV) 3′-O-deacetylation was accomplished with the dicyclohexyl-18-crown-6-ether–potassium hydroxide complex in benzene developed by C. J. Pedersen, *J. Amer. Chem. Soc.*, **89**, 7017 (1967). Our results with this reagent were somewhat variable, and we have since preferred to use the KOH–MeOH–dioxane medium.

TABLE V
ENZYMIC HYDROLYSIS[a]

Substrate	Enzyme	Products: Identity	Ratio: Theory	Ratio: Found
dCpC	S	dCp/C	1	0.9
dCpT	S	dCp/T	1	1.0
dCpCpT	S	dCp/T	2	2.3
dApT	S	dAp/T	1	1.2
dApTpT	V	pT/dA	2	2.2
dGpTpT	V	pT/dG	2	2.2

[a] These represent experiments which were quantitated. Other products were hydrolyzed by spleen phosphodiesterase (S) and purified venom phosphodiesterase (V) and found qualitatively to give the expected results based on comparison of paper chromatographic mobilities of products with known reference compounds.

porting methoxytrityl polymer. It was shown that this reagent nearly quantitatively released products from polymer of low cross-linkage (0.5–0.75% DVB) in about 15 min while more highly cross-linked polymer (1–2% DVB) required longer times for maximal product release. Because of its somewhat superior handling properties we have preferred to use 1% cross-linked polymer which imposes the need for longer acid release times. This presents no problem with oligonucleotides containing only pyrimidine bases for which acid exposure times of 5–18 hr were used without deleterious effect. However, since the acid-labile purine nucleosides, deoxyadenosine and deoxyguanosine, cannot tolerate such long exposures to acid, we compromised by using 15-min release times for their derivatives.

Purity of Products.—Since the nucleotide condensation reactions do not proceed quantitatively, the isolated dinucleoside phosphates are accompanied by unchanged nucleoside, although with 15-min acidic release times the amount of nucleoside is negligibly small. Similarly, trinucleoside diphosphate is accompanied by dinucleoside monophosphate and nucleoside. These, of course, can readily be separated by paper or column chromatography. Isolation of the trinucleoside derivatives clearly focus an important aspect of the nucleotide condensation reaction. Thus in the dApT→dApTpT transformation, the amount of dimer (dApT) carried by the trimer polymer exceeds that in the precursor dimer polymer (Table II); the additional dimer was obviously formed by nucleotide condensation with nucleoside which had escaped reaction in the first condensation step. This conclusion is further reflected in the results listed in Tables III and IV for the trinucleoside diphosphates dGpTpT, dCpCpT, and dTpCpT. In the later two the isolation problem is complicated by the presence of not one, but two dimers; *i.e.*, dCpCpT is accompanied by dCpC and dCpT; dTpCpT is accompanied by dTpC and TpT.

The above considerations refer to purity in terms of the gross product comprising a mixture of oligonucleotides of different chain length and/or base sequence. Such mixtures may present separation problems, but, in any event, the products have the desired 3′→5′ phospho diester constitution of natural nucleic acids. This conclusion stems from the results of the specific enzymic hydrolyses outlined in Table V and is further supported by proton nuclear magnetic resonance studies which have been carried out in this laboratory.[19]

A remaining question of purity relates to the presence of "non-natural" by-products derived from undesirable side reactions. Paper chromatograms of many of the gross products (particularly the pyrimidine-only derivatives) exhibited bands comprising only the desired oligonucleotides together with nucleoside and aromatic carboxamide derived from the ammonolysis of N-aroyl derivatives. However, with many of the purine-containing products several contaminants were evident; these were readily resolved and proved to be present in only minor amounts. For example, the dApTpT preparation showed two well-resolved major bands which proved to be the desired trimer and related dimer dApT, minor nucleoside and aromatic carboxamide bands, and two extraneous bands, one with a very low mobility (<dApTpT) and one with intermediate mobility.[20] Since such contaminants were of minor importance they were not further investigated.

Summary.—We have applied our insoluble polymer-supported synthesis technique to the preparation of all 16 di(deoxyribonucleoside) monophosphates derivable from the four major nucleosides and to several trinucleoside diphosphates. Many of the per cent conversions reported here are quite low, but most of the preparations represent a single attempt based on generalized reaction conditions developed for thymidine homooligonucleotide synthesis.[3] Alteration of various reaction conditions to conform with reactivity differences of different reactants and polymer-bound substrates can reasonably be expected to lead to increased percentage conversions and product purity.

Experimental Section

General Method and Materials.—Paper chromatography and solvent systems, spectrophotometry, and general procedures were as described in previous publications.[3]

The acylated nucleosides dA^{Bz}, dC^{An}, and dG^{Ac} were prepared according to published procedures.[9,10] Protected nucleotides $dpA^{Ac}OAc$, $dpC^{An}OAc$, $dpG^{Ac}OAc$, and pTOAc were also prepared as pyridinium salts according to published procedures[13–16]; they were purified by ether precipitation and were converted, as 0.2 *M* solutions in anhydrous pyridine, to bis(triethylammonium) salts by addition of the appropriate amount of purified triethylamine.

Nucleoside Polymer Preparation and Assay.—To a suspension of 1% cross-linked methoxytrityl chloride polymer[3] in anhydrous pyridine (10–15 ml/g) was added the nucleoside (~0.8 mmol/g of polymer) and the mixture was agitated for 48 hr at room temperature and worked up as previously described.[3a]

To assay the polymers, hydrolysis in 1% trifluoroacetic acid in benzene was carried out as previously described.[3b] N-Anisoyldeoxycytidine was determined by direct spectrophotometric examination [dC^{An}, λ_{max} 302 mμ (ε 24,500) in water[9]]. The acid hydrolysate from the dA^{Bz} polymer was evaporated to dryness, taken up in pyridine–concentrated NH_4OH (1:3 v/v), and allowed to stand at room temperature for 48 hr. Paper chromatography on Whatman No. 40 paper in solvent A afforded adenine which was eluted with water and determined spectrophotometrically [λ_{max} 263 mμ (ε 13,100)]. The dG^{Ac} polymer was assayed similarly to the dA^{Bz} analog except that paper chromatography afforded dG [λ_{max} 255 mμ (ε 12,300), pH 2] and guanine [λ_{max} 248 mμ (ε 11,400), pH 2] which were separately determined and the molar amounts summed.

Nucleotide Condensation and Product Isolation.—Nucleoside

(19) C. C. McDonald, W. D. Phillips, L. R. Melby, and D. R. Strobach, unpublished work.

(20) Paper chromatography in solvent system A; see Experimental Section.

polymer was condensed with protected nucleotide bis(triethylammonium) salt in the presence of 2,4,6-triisopropylbenzenesulfonyl chloride as previously described.[3b] Products containing only pyrimidines were released from the polymer by agitation for 5 hr with HOAc–H_2O–C_6H_6 (16:4:5, v/v) while those containing purines were acid treated for only 15 min and immediately freed of acid by rapid evaporation under vacuum in a rotary evaporator at room temperature. The dried crude products were then hydrolyzed for 48 hr in pyridine–concentrated NH_4OH (1:3, v/v) and paper chromatographed.

For the trinucleoside diphosphate preparations of Tables II and III, 3′-O-deacetylation was effected with 0.2 *M* KOH in methanol–dioxane (1:9, v/v) as described previously.[3b] For the preparations in Table IV an equivalent amount of 0.2 *M* crown ether–KOH complex in benzene[18] was used as the hydrolysis medium and the polymer was washed exhaustively with benzene, pyridine, and methanol, then dried prior to the second nucleotide condensation.

Enzymic Hydrolyses.—The purified oligonucleotides were hydrolyzed with spleen and venom phosphodiesterases as previously described;[3b] the results are summarized in Table V.

Registry No.—Deoxyadenylyl-(3′→5′)-thymidylyl-(3′→5′)-thymidine, 17862-43-0; deoxyguanylyl-(3′→5′)-thymidylyl-(3′→5′)-thymidine, 17853-31-5; deoxycytidine–thymidine oligomers, 17853-32-6.

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Pyrimidine Nucleosides. III. Nucleoside Derivatives of Certain 4-Substituted 6-Pyrimidones[1]

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The synthesis of 4,6-disubstituted pyrimidine nucleosides possessing two hydrogen-bonding groups has been achieved for the first time using a modified Hilbert–Johnson procedure. 4-Amino-1-(β-D-ribofuranosyl)-6-pyrimidone (**8**) and 4-amino-1-(2-deoxy-β-D-ribofuranosyl)-6-pyrimidone (**9**) have been prepared by direct utilization of 4-amino-6-pyrimidone (**5**) *via* silylation and subsequent treatment with the appropriate glycosyl halide in acetonitrile. This procedure applied to 4-methylthio-6-pyrimidone gave 4-methylthio-1-(β-D-ribofuranosyl)-6-pyrimidone (**2**). Reductive desulfurization of **2** gave 1-(β-D-ribofuranosyl)-6-pyrimidone (**3**). Oxidation of a blocked derivative of **2** provided the corresponding methyl sulfone **7** which was successfully converted into **8** and also into 4-methoxy-1-(β-D-ribofuranosyl)-6-pyrimidone (**6**).

The success achieved in the preparation of pyrimidine nucleosides *via* the silylation and alkylation procedures[2,3] suggested the possible extension of this work to 4-substituted 6-pyrimidones. Although the Hilbert–Johnson procedure has recently been successfully employed in the case of 4,6-dimethoxypyrimidine[4] to yield 4-methoxy-1-(β-D-ribofuranosyl)-6-pyrimidone (**6**), the methoxy group could not be successfully changed to other substituents. Attempts to prepare the corresponding 2-deoxy-β-D-ribofuranosyl derivative by the Hilbert–Johnson procedure gave only 0.67% of desired product.[4] A recent paper[5] describes the use of the mercuri procedure for ribosylation of 4-substituted 6-pyrimidones. In most cases the *O*-glycosyl derivatives were found to predominate in a mixture of *O*- and *N*-ribosylated products. The use of acetonitrile, however, gave predominately *N*-ribofuranosyl derivatives.

In the present study 4-amino-6-pyrimidone[6] **5** was treated with hexamethyldisilazane to give the trimethylsilyl derivative which was in turn treated directly in acetonitrile with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide. The product obtained after work-up and purification on silica gel was 4-amino-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-6-pyrimidone (**10**) (Scheme I). Deblocking of crude **10** with methanolic ammonia gave 4-amino-1-(β-D-ribofuranosyl)-6-pyrimidone (**8**) in 61% over-all yield. The physical properties of **8** agree with those recorded by Prystas[5] for this compound prepared by the mercuri procedure. Reaction of **12** with 2-deoxy-3,5-di-*O*-*p*-toluyl-D-ribofuranosyl chloride[7] in acetonitrile yielded after purification by alumina column chromatography a 75% yield of a syrupy mixture of blocked anomers (**11** and **11a**). Deblocking of this material with methanolic ammonia gave a 75% yield of a crystalline mixture of anomers **9** and **9a**. 4-Amino-1-(2-deoxy-α-D-ribofuranosyl)-6-pyrimidone (**9a**) was isolated from this mixture by fractional crystallization. 4-Amino-1-(2-deoxy-β-D-ribofuranosyl)-6-pyrimidone (**9**) was isolated from the mother liquors enriched in **9** by preparative tlc with silica gel adsorbent. The assignment of configuration was readily made by a comparison of the pmr of **9** and **9a** measured in deuterium oxide with an internal standard of sodium 2,2-dimethyl-2-silapentane-5-sulfonate. The anomeric proton of **9** exhibited a pseudotriplet centered at 6.27 ppm (width 13.2 cps, $J_{1',2'}$ = 6.6 cps). The anomeric proton of **9a** consisted of a multiplet of four peaks centered at 6.20 ppm (width 9.3 cps, "$J_{H1'}$" = 2.3 and 7.0 cps). These data clearly allow assignment[8-11] of **9** as the β-D anomer and **9a** as the α-D anomer. The ratio of the anomers **9a**:**9** obtained by this silylation and alkylation procedure was approxi-

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