Synthetic Analogues of Polynucleotides. Part VIII.[1] Analogues of Oligonucleotides containing Carboxymethylthymidine

By **M. D. Edge, A. Hodgson, A. S. Jones,*** and **R. T. Walker,** Chemistry Department, The University of Birmingham, Birmingham B15 2TT

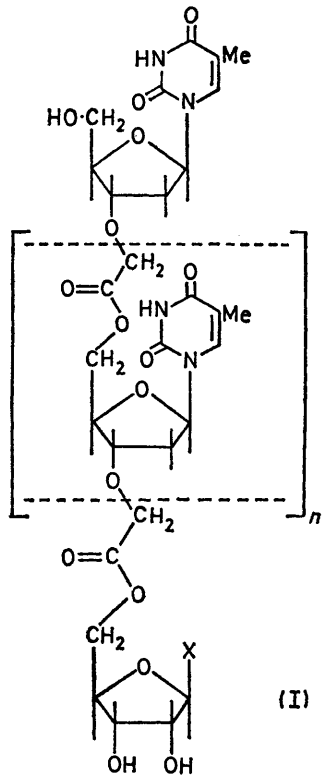
Analogues of oligonucleotides in which the nucleoside units are linked by acetate ester linkages (·O·CH_2·CO_2·) instead of the phosphodiester linkages of the natural compounds, have been synthesised. The compounds obtained were thymidinylacetyl-(3'→5')-thymidinylacetyl-(3'→5')-thymidinylacetyl-(3'→5')-uridine, thymidinylacetyl-(3'→5')-thymidinylacetyl-(3'→5')-thymidinylacetyl-(3'→5')-thymidinylacetyl-(3'→5')-uridine, and thymidinylacetyl-(3'→5')-thymidinylacetyl-(3'→5')-thymidinylacetyl-(3'→5')-thymidinylacetyl-(3'→5')cytidine. The last was also obtained labelled with tritium in the cytidine residue. An oligomer of similar structure to these compounds having a molecular weight between 4×10^3 and $4{\cdot}7 \times 10^4$ was obtained by copolymerising 3'-*O*-carboxymethylthymidine with 0·1 mol. equiv. of 2',3'-*O*-isopropylideneuridine and removing the blocking group. This oligomer and the other two uridine-containing oligomers gave a small but significant hypochromic effect when mixed with polyadenylic acid in solution.

We have previously described,[2] the synthesis of analogues of trinucleoside diphosphates in which the phosphodiester linkages were replaced by acetate ester linkages (O·CH_2·CO_2·). The compounds had the structure (I; $n = 1$, X = uracil, cytosine, adenine, guanine, or hypoxanthine residue). In order to study further the interaction of compounds of this type with polynucleotides and their effects in biological systems, oligomers of structure (I; $n > 1$, X = uracil or cytosine residue) have been synthesised. The techniques used were essentially the same as those previously reported;[2] the triphenylmethyl group was used to block the 5'-hydroxy-group, the isopropylidene group for protecting vicinal 2'- and 3'-hydroxy-groups, the dimethylaminomethylene group for the 4-amino-group of the cytosine residue, and the 2-cyanoethyl group for the carboxy-group. The 2-cyanoethyl group was removed by treating the ester with 4 mol. equiv. of potassium t-butoxide in dimethylformamide at 100° for 10 min instead of 2 mol. equiv. under the same conditions for 2 h.[2] Complete removal of the 2-cyanoethyl group was achieved with only 5% hydrolysis of the internucleoside ester linkages instead of the 12% hydrolysis obtained by the previous procedure. For the removal of the dimethylaminomethyleme group from the cytosine residues, instead of the usual treatment with boiling ethanol,[2] the protected compound, after chromatography in chloroform–ethanol was left adsorbed on the silica of a t.l.c. plate for 36 h at 20° and then eluted. This was a convenient procedure for use on a small scale. It was not ascertained whether the effect was due to the silica, to the adsorbed ethanol, or to both. Both the triphenylmethyl and the isopropylidene groups were removed by acidic hydrolysis. It was possible to remove the triphenylmethyl group

[1] Part VII, M. J. Cooper, R. S. Goody, A. S. Jones, J. R. Tittensor, and R. T. Walker, *J. Chem. Soc.* (*C*), 1971, 3183.
[2] M. D. Edge and A. S. Jones, *J. Chem. Soc.* (*C*), 1971, 1933.

selectively in the presence of the isopropylidene group, as exemplified by the synthesis of thymidinylacetyl-(3′→5′)-(2′,3′-*O*-isopropylidene)uridine [dThd-a-Urd(iP); see Scheme for abbreviations].

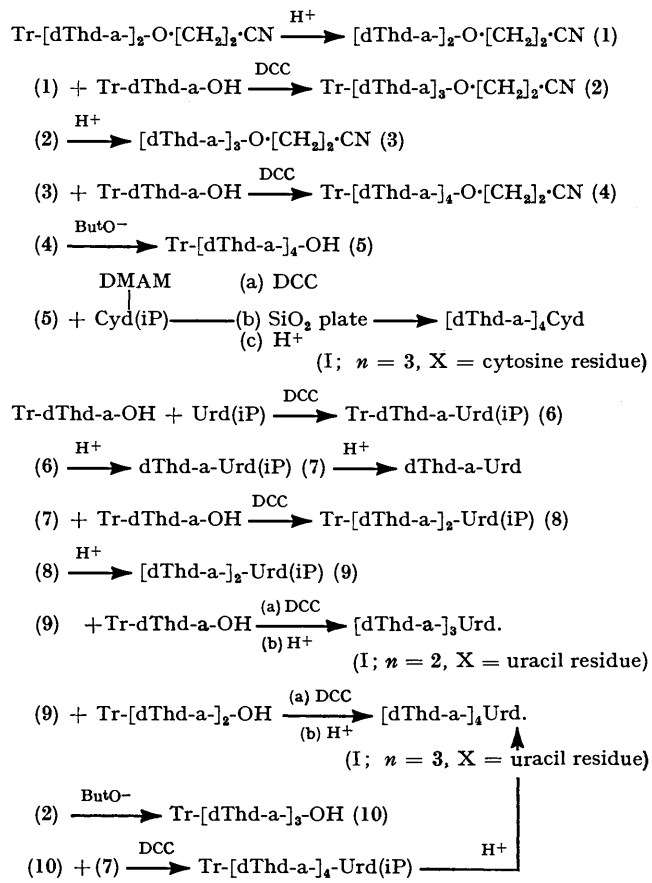
The final products of the syntheses were thymidinylacetyl(3′→5′)uridine (dThd-a-Urd), which was used in addition to the nucleosides and other intermediates



(I)

as a model for the interpretation of n.m.r. spectra, thymidinylacetyl-(3′→5′)-thymidinylacetyl-(3′→5′)-thymidinylacetyl-(3′→5′)-uridine (I; $n = 2$, X = uracil residue) ([dThd-a-]$_3$Urd), thymidinylacetyl(3′→5′)-thymidinylacetyl-(3′→5′)-thymidinylacetyl-(3′→5′)-thymidinylacetyl-(3′→5′)-uridine (I; $n = 3$, X = uracil residue) ([dThd-a-]$_4$Urd), and the corresponding cytosine compound (I; $n = 3$, X = cytosine residue) ([dThd-a-]$_4$Cyd). The 3′→5′ internucleoside acetate ester linkages were in each case produced by condensation of the appropriate 3′-*O*-carboxymethyl derivative with the appropriate derivative with a free 5′-hydroxy-group by means of dicyclohexylcarbodi-imide in pyridine according to the Scheme shown.

The compounds were characterised by their u.v. absorption spectra, by alkaline hydrolysis to their constituent nucleoside units, by elemental analysis, and by n.m.r. spectra. The presence of the 3′→5′ acetate ester linkage was confirmed by the following two observations. (*a*) The formation of the 3′→5′-acetate linkage caused the 5′-H to resonate at lower field than in the parent nucleoside. Although these signals could not be readily distinguished from other signals, their absence from their usual position indicated that the 5′-*O*-position was substituted. On the other hand the terminal 5′-H resonated at the same field as the parent nucleoside and so its signal could be distinguished



Abbreviations: Tr = 5′-*O*-triphenylmethyl, dThd = thymidine residue, -a- = 3′→5′ acetate ester linkage (O·CH$_2$·CO), Urd = uridine residue, Cyd = cytidine residue, DMAM = dimethylaminomethylene, iP = 2′,3′-*O*-isopropylidene, DCC = dicyclohexylcarbodi-imide.

SCHEME Synthesis of oligonucleotide analogues

and the ratio of terminal nucleoside to total bases determined. With 5′-*O*-triphenylmethyl derivatives this was not possible because the 5′-*O*-triphenylmethyl group also caused a similar shift in the signal of the 5′-H. (*b*) In a number of the oligomers the presence of one NH signal per thymine or uracil residue indicated that none of the nucleosides was linked by N-3 of the base. Details of the n.m.r. spectra are given in the Table.

The cytosine-containing compound (I; $n = 3$, X = cytosine residue) was only obtained in a small amount so it was not characterised by n.m.r. Its structure was established by the fact that it gave 4 mol of 3′-*O*-carboxymethylthymidine for every 1 mol of cytidine upon alkaline hydrolysis and that the u.v. absorption spectrum showed that the cytosine residue was not acylated. A sample of this compound labelled with tritium in the cytidine residue was prepared in order

to study its hybridisation with mouse satellite deoxyribonucleic acid, results of which will be published elsewhere.[3]

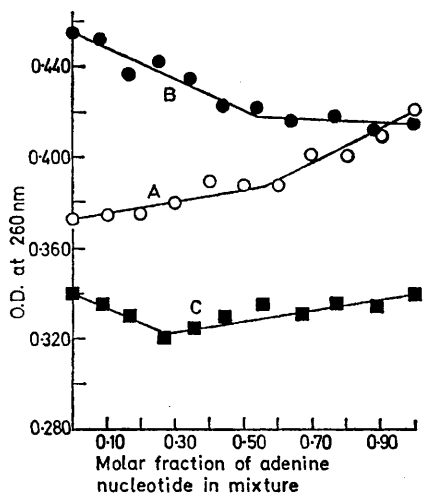
An oligomer of structure (I; X = uracil residue) of indefinite chain length ([dThd-a-]$_n$Urd) was synthesised by polymerising 3′-*O*-carboxymethylthymidine with 0·1 mol. equiv. of 2′,3′-*O*-isopropylideneuridine in the presence of dicyclohexylcarbodi-imide and removing the isopropylidene group by acidic hydrolysis. The resulting oligomer was isolated by dialysing away the low molecular weight material. Determination of the average chain length by measurement of the ratio of 3′-*O*-carboxymethylthymidine to uridine in an alkaline hydrolysate gave a value of 168 nucleoside units (*i.e.* mol. wt. 4·7 × 10^4). This must be considered to be a maximum value, however, because some chains probably did not terminate in uridine residues. The molecular weight of the smallest molecules was probably not less than about 4—8 × 10^3 because molecules of about this molecular weight are able to diffuse through the dialysis membranes used.

The oligomers [dThd-a-]$_3$Urd, [dThd-a-]$_4$Urd, and [dThd-a-]$_n$Urd were examined for their interaction with polyadenylic acid by determining the hypochromicity of the u.v. absorption obtained by mixing solutions of the complementary polymers. The results (see Figure) showed that in all three cases there was a small but definite hypochromic effect at 260 nm. With [dThd-a-]$_n$-Urd the effect was about 5% and with the other two oligomers about 3%. Effects of similar magnitude were also observed at 267 nm, and with [dThd-a-]$_n$Urd there was no observable shift in the $\lambda_{max.}$ of the solutions from that expected of a mixture of the two components.

N.m.r. data [δ (p.p.m.), number of protons]

Compound *	H-6		H-5	H-1′		H-5′	CH_2·CN	Me of thymidine	Me_2C	NH	Remarks
	Uridine	Thymidine		Thymidine	Uridine						
Uridine	7·85(d), 1		5·60(d), 1		5·77(d), 1	3·60(s), 2					
Thymidine		7·80(s), 1		6·25(t), 1		3·65(s), 2		1·81(s), 3		11·3(s), 1	
dThd-a-Urd(iP)	7·72(d)	7·72(s)	5·67(d), 1	6·17(t) 1	5·82(d), 1	3·60br, 2		1·79(s), 3	1·50(s), 3 1·30(s), 3		H-6 for uridine + thymidine = 2H
dThd-a-Urd	7·86(d), 1	7·70(s), 1	5·61(d), 1	6·17(t), 1	5·78(d), 1	3·65br		1·77(s), 3			HCO_3H, δ 8·3(s), 1H
[dThd-a-]$_2$Urd(iP)	7·74(d), 1	7·75(s), 1 7·50(s), 1	5·70(d), 1	6·20(t), 2	5·84(d), 1	Indistinct		1·80(s), 6	1·50(s), 3 1·30(s), 3		
[dThd-a-]$_3$Urd	7·60(d), 1	7·42(s), 3	5·65(d)	6·15(m), 3	5·80(d)	3·60(m), 2		1·80(s), 9		11·5(s), 4	Uridine H-5 + H-1′ = 2H
[dThd-a-]$_4$Urd	7·58(d), 1	7·42(s), 4	5·65(d)	6·13(t), 4	5·73(d)	3·50br, 2		1·79(s), 12		11·3(s), 5	Ditto
Tr-dThd-a-OH		7·50(s), 1		6·20(t), 1				1·45(s), 3			
Tr-[dThd-a-]$_2$-O·[CH_2]$_3$·CN		7·5—7·4 †		6·20(t), 2			2·90(t), 2	1·76(s), 3 1·47(s), 3		11·5(s), 2	
dThd-a-O·[CH_2]$_2$·CN		7·68(s), 1		6·23(t), 1		3·60br, 2	2·90(t), 2	1·78(s), 3			
[dThd-a-]$_2$-O·[CH_2]$_2$·CN		7·70(s), 1 7·48(s), 1		6·20(t), 2		3·60br, 2	2·92(t), 2	1·79(s), 6			
[dThd-a-]$_3$-O·[CH_2]$_2$·CN		7·71(s), 3 7·49(s), 3		6·28(t), 3		3·60br, 2	2·92(t), 2	1·80(s), 9			
Tr-[dThd-a-]$_4$-O·[CH_2]$_3$·CN ‡		†		6·18(t), 4			2·88(t), 2	1·76(m), 6 1·41(m), 3		11·4(s), 4	

* For abbreviations see Scheme. † Merges with Ph_3C protons. ‡ At 60 MHz.



Optical density at 260 nm of mixtures of polyadenylic acid and carboxymethylthymidine-containing analogues of oligonucleotides. Conditions as in text. A, [dThd-a-]$_3$Urd; B, [dThd-a-]$_4$Urd; C, [dThd-a-]$_n$Urd

The [dThd-a-]$_n$Urd was only partly soluble in aqueous solutions, so for this experiment it was dissolved in dimethylformamide and the solution was diluted with aqueous salt solution to give a dimethylformamide concentration of 10%. This procedure gave a more satisfactory solution but the dimethylformamide may have reduced the hypochromicity. This result should be compared with those obtained previously with an oligomer of structure [dThd-a-]$_n$ in which a hypochromicity of 8% was obtained in the absence of dimethylformamide but at lower ionic strength.[2] These results confirm, therefore, that this type of oligonucleotide analogue can interact with polynucleotides in solution. In the case of [dThd-a-]$_3$Urd and [dThd-a-]$_4$-Urd the maximum hypochromicity appears to occur when the molar ratio of thymidine to adenosine residues is approximately 1 : 1, whereas with [dThd-a-]$_n$Urd the maximum hypochromicity occurs at a much higher proportion of thymidine residues (2—3 : 1). This suggests that the shorter oligomers may be forming 1 : 1 complexes whereas the longer oligomer is forming a 2 : 1 complex, although this conclusion requires confirmation. Such a conclusion is in accord with the behaviour of the complementary oligonucleotides; it has been shown that polyadenylic acid and a thymidine hexanucleotide form a 1 : 1 complex under conditions where polyadenylic acid and thymidine decanucleotide form a 1 : 2 complex.[4]

[3] P. M. B. Walker, to be published.
[4] R. Naylor and P. T. Gilham, *Biochemistry*, 1967, **5**, 2722.

The oligomer [dThd-a-]$_n$Urd showed some evidence of instability in the freeze-dried state (containing 20% water) when stored at −40° for 3—4 weeks. From measurements on the stability of a similar compound in solution,[5] details of which will be published later, it appears that the half-life of these oligomers at 37° at pH 7 is about 26 h.

EXPERIMENTAL

For t.l.c., silica gel powder, MN Silica Gel UV254 (Machery, Nagel & Co., Germany), and for column chromatography, Kieselgel, 0·05—0·2 mm (70—325 Mesh ASTM), type 7734 (Merck, Germany) were used.

General Techniques.—(a) *Condensations.* These were carried out between the pyridinium salt of the appropriate carboxylic acid (prepared from the sodium salt by use of the pyridinium form of Zeo Karb 225) and the appropriate 5′-hydroxy-compound in dry pyridine at a concentration of 0·5—1·0 mmol ml^{-1} in the presence of dicyclohexylcarbodi-imide (4—5 mol. equiv.) at about 20° for for 18—24 h. Most of the excess of dicyclohexylcarbodiimide was removed by extraction with cyclohexane or sometimes converted into dicyclohexylurea by addition of water. Dicyclohexylurea formed in this way or during the reaction was mainly removed by taking advantage of its low solubility in cold acetone. In most of the cases the required products were isolated by silica gel column chromatography with a ratio of silica gel to compound of about 100 : 1 by weight. The required products were usually obtained as solids by precipitation from acetone, or other suitable solvent, with light petroleum.

(b) *Removal of triphenylmethyl groups.* This was carried out by hydrolysis with acetic acid–water (4 : 1) at 100° for 15 min, or with formic acid–water (2 : 1) at 20° for 4 h, or with formic acid (98%) at 20° for 5—10 min. The last procedure caused selective removal of the triphenylmethyl group in the presence of an isopropylidene group.

(c) *Removal of 2-cyanoethyl groups.* This was carried out either as previously described [2] or by the use of 4 mol. equiv. of potassium t-butoxide in dimethylformamide at 100° for 10 min.

Determination of the triphenylmethyl group. This was carried out by a procedure similar to but not identical with that described by Duffield and Nussbaum.[6] The compound was dissolved in a mixture of glacial acetic acid and conc. sulphuric acid (2 : 1 v/v) and the optical density was read at 436 nm. Triphenylmethyl chloride was used as standard. A 2·64 × 10^{-2} mM-solution gave an optical density of 1·00 in a 1 cm cell.

2-Cyanoethyl Thymidinylacetyl-(3′→5′)-thymidin-3′-ylacetate.—2-Cyanoethyl 5′-*O*-triphenylmethylthymidinylacetyl-(3′→5′)-thymidin-3′-ylacetate (1·0 g) [2] was treated with acetic acid to remove the triphenylmethyl group. The product was isolated by silica gel column chromatography, with ethyl acetate to elute triphenylmethanol and acetone–ethyl acetate (4 : 1) to elute the *product* (530 mg), which was obtained as a chromatographically pure white powder (Found: C, 51·0; H, 5·1; N, 11·2. $C_{27}H_{33}N_5O_{13}$ requires C, 51·1; H, 5·2; N, 11·0%), $\lambda_{max.}$ (H_2O) 267 nm (ε 18·6 × 10^3).

2-Cyanoethyl 5′-O-Triphenylmethylthymidinylacetyl-(3′→5′)-thymidinylacetyl-(3′→5′)-thymidin-3′-ylacetate.— 3′-*O*-Carboxymethyl-5′-*O*-triphenylmethylthymidine (0·5 mmol) and the foregoing compound (0·5 mmol) were condensed together. Column chromatography of the products on silica gel was carried out by applying the compound dissolved in chloroform to the column and then eluting with ethyl acetate followed by acetone–ethyl acetate (1 : 4). The *required compound* (350 mg, 52%) was obtained as a chromatographically homogeneous white powder (Found: C, 59·7; H, 5·6; N, 8·2. $C_{58}H_{61}N_7O_{19}$ requires C, 60·0; H, 5·8; N, 8·5%), $\lambda_{max.}$ (EtOH) 266 nm (ε 25·5 × 10^3). Alkaline hydrolysis gave 3′-*O*-carboxymethylthymidine and 3′-*O*-carboxymethyl-5′-*O*-triphenylmethylthymidine in the molar ratio of 2·1 : 1. The compound contained 1 trityl group to 3·0 thymidine residues (the latter being measured by u.v. absorption.

2-Cyanoethyl Thymidinylacetyl-(3′→5′)-thymidinylacetyl-(3′→5′)-thymidin-3′-ylacetate.—The foregoing compound (200 mg) was treated with 98% formic acid to remove the triphenylmethyl group. The products (applied in pyridine solution) were fractionated on a silica gel column, which was eluted with ethyl acetate and then with acetone. The *product* (150 mg) was obtained as a chromatographically homogeneous, white powder (150 mg) (Found: C, 51·2; H, 4·7; N, 10·4. $C_{39}H_{47}N_7O_{19}$ requires C, 51·0; H, 5·1; N, 10·7%), $\lambda_{max.}$ (EtOH) 267 nm (ε 25·6 × 10^3).

Thymidinylacetyl-(3′→5′)-(2′,3′-O-isopropylidene)uridine.—3′-*O*-Carboxymethyl-5′-*O*-triphenylmethylthymidine (7 g) [7] and 2′,3′-*O*-isopropylideneuridine (3·4 g) were condensed together and the product was treated with 98% formic acid to remove the triphenylmethyl group. The mixture, which consisted of a major component (which gave 3′-*O*-carboxymethylthymidine and 2′,3′-*O*-isopropylideneuridine upon alkaline hydrolysis) and three minor components, was fractionated on a silica gel column [elution with ethanol–chloroform (1 : 19 then 2 : 23)]. The *product* (2·6 g) was isolated in pure form as a white solid (Found: C, 51·2; H, 5·1; N, 10·1. $C_{24}H_{30}N_4O_{12}$ requires C, 50·9; H, 5·3; N, 9·9%), $\lambda_{max.}$ (H_2O) 264, $\lambda_{min.}$ 232; $\lambda_{max.}$ (alkali) 264, $\lambda_{min.}$ 246 nm.

Thymidinylacetyl-(3′→5′)-uridine.—The foregoing compound (200 mg) was treated with formic acid–water (2 : 1) to remove the isopropylidene groups. Removal of the solvents left pure thymidinylacetyl-(3′→5′)-uridine (140 mg) containing one mol. equiv. of formic acid (Found: C, 46·3; H, 4·6; N, 10·0. $C_{21}H_{26}N_4O_{12}$,HCO_2H requires C, 46·2; H, 4·6; N, 9·8%) (n.m.r. indicated the presence of formic acid). Alkaline hydrolysis gave 3′-*O*-carboxymethylthymidine and uridine.

Thymidinylacetyl-(3′→5′)-thymidinylacetyl-(3′→5′)-(2′,3′-O-isopropylidine)uridine.—The foregoing compound (1·13 g) was condensed with 3′-*O*-carboxymethyl-5′-*O*-triphenylmethylthymidine (1·16 g). The product was isolated by silica gel column chromatography with ethanol–chloroform (2 : 23) as eluant. The triphenylmethyl group was then removed with 98% formic acid and the mixture was fractionated on a silica gel column [applied in pyridine solution and eluted with chloroform followed by ethanol–chloroform (3 : 22)] to give the *product* (400 mg) in pure form (Found: C, 50·6; H, 5·1; N, 9·9. $C_{36}H_{44}N_6O_{18}$ requires C, 50·9; H, 5·2; N, 9·9%).

2-Cyanoethyl 5′-O-Triphenylmethylthymidinylacetyl-(3′→5′)-thymidinylacetyl-(3′→5′)-thymidinylacetyl-(3′→5′)-thymidin-3′ylacetate.—2-Cyanoethyl thymidinylacetyl-

[5] A. S. Jones and M. MacCoss, unpublished results.

[6] A. M. Duffield and A. L. Nussbaum, *Analyt. Biochem.*, 1964, **7**, 254.

[7] M. H. Halford and A. S. Jones, *J. Chem. Soc.* (*C*), 1968, 2667.

(3′→5′)-thymidinylacetyl-(3′→5′)-thymidin-3′-ylacetate (48 mg) was condensed with 3′-*O*-carboxymethyl-5′-*O*-triphenylmethylthymidine (30 mg). The mixture was fractionated by silica gel column chromatography [ethyl acetate then acetone–ethyl acetate (1 : 1) as eluant]. The product was isolated as a white solid (40 mg) which contained 1 triphenylmethyl group to every 3·95 thymine residues. An analytically pure *sample* (13% yield) was obtained by t.l.c. [ethanol–chloroform (2 : 23)] followed by silica column chromatography [Found (after drying *in vacuo* at 20°): C, 56·0; H, 5·6; N, 8·4. $C_{70}H_{75}N_9O_{25},3H_2O$ requires C, 56·0; H, 5·6; N, 8·4%], $\lambda_{max.}$ ($CHCl_3$) 267 nm (ε 34,650), 1 triphenylmethyl group to 3·95 thymine residues. Alkaline hydrolysis gave 3′-*O*-carboxymethylthymidine and 3′-*O*-carboxymethyl-5′-*O*-triphenylmethylthymidine in a molar ratio of 2·9 : 1.

Thymidinylacetyl-(3′→5′)-thymidinylacetyl-(3′→5′)-thymidinylacetyl-(3′→5′)-uridine.— 3′-*O*-Carboxymethyl-5′-*O*-triphenylmethylthymidine (54 mg) was condensed with thymidinylacetyl-(3′→5′)-thymidinylacetyl(3′→5′)-(2′,3′-*O*-isopropylidene)uridine (85 mg). The product was isolated by silica gel column chromatography [elution with ethanol–chloroform (2 : 23)] and the protecting groups were removed with formic acid–water (2 : 1). Triphenylmethanol was extracted with chloroform and the *product* (20 mg) was precipitated with ethyl acetate from solution in dimethylformamide. Alkaline hydrolysis of this material gave 3′-*O*-carboxymethylthymidine and uridine in a molar ratio of 2·9 : 1. It was characterised by n.m.r. spectroscopy (see Table).

5′-O-*Triphenylmethylthymidinylacetyl-(3′→5′)-thymidinylacetyl-(3′→5′)-thymidinylacetyl-(3′→5′)-thymidinylacetyl-(3′→5′)-(2′,3′-O-isopropylidene)uridine.*— 2-Cyanoethyl 5′-*O*-triphenylmethylthymidinylacetyl-(3′→5′)-thymidinylacetyl-(3′→5′)-thymidin-3′-ylacetate (80 mg, 0·069 mmol) was treated with potassium butoxide (31·2 mg, 0·276 mmol) in dry dimethylformamide (20 ml) at 100° for 10 min to remove the 2-cyanoethyl group. The resulting acid was condensed with thymidinylacetyl-(3′→5′)-(2′,3′-*O*-isopropylidene)uridine (40 mg) in the usual way. The mixture was fractionated by column chromatography on silica gel [elution with chloroform, then ethanol–chloroform (1 : 49 then 1 : 19)]. The *product* (47 mg) was isolated as a white powder [Found (after drying *in vacuo* at room temperature): C, 54·1; H, 5·3; N, 7·8. $C_{79}H_{86}O_{29}N_{10},6H_2O$ requires C, 54·3; H, 5·6; N, 8·0%]. Alkaline hydrolysis gave 3′-*O*-carboxymethyl-5′-*O*-triphenylmethylthymidine, 3′-*O*-carboxymethylthymidine, and 2′,3′-*O*-isopropylideneuridine in the molar ratio of 1·1 : 3·1 : 1·0.

Thymidinylacetyl-(3′→5′)-thymidinylacetyl-(3′→5′)-thymidinylacetyl-(3′→5′)-thymidinylacetyl-(3′→5′)-uridine.— The foregoing compound (40 mg) was treated with formic acid–water (2 : 1) to remove the triphenylmethyl and isopropylidene groups. The mixture was extracted with chloroform to remove triphenylmethanol and with ether to remove formic acid, and the *product* (20 mg) was obtained chromatographically pure as a white solid. Alkaline hydrolysis gave 3′-*O*-carboxymethylthymidine and uridine in a molar ratio of 3·8 : 1. This compound was characterised by n.m.r. spectroscopy.

An identical product was obtained by the condensation of 5′-*O*-triphenylmethylthymidinylacetyl-(3′→5′)-thymidin-3′-ylacetic acid [2] with thymidinylacetyl-(3′→5′)-thymidinyl-(3′→5′)-(2′,3′-*O*-isopropylidene)uridine followed by treatment with formic acid–water (2 : 1).

4-N-*Dimethylaminomethylene-2′,3′-O-isopropylidenecytidine.*—This was obtained by a procedure described by Zemlicka and Holy [8] for the synthesis of similar compounds. 2′,3′-*O*-Isopropylidenecytidine (0·20 g) was dissolved in dry dimethylformamide (3·5 ml) and dimethylformamide dimethyl acetal (0·36 ml) was added. The solution was set aside at room temperature for 18 h, then evaporated under reduced pressure. The residue was crystallised by addition of ether to give the *product* (0·21 g), which was recrystallised from chloroform and light petroleum; m.p. 179—180°, $\lambda_{max.}$ (EtOH) 316 (ε 3·6 × 10⁴), $\lambda_{min.}$ 245 nm (ε 1750) (Found : C, 53·6; H , 6·6; N, 16·2. $C_{15}H_{22}N_4O_5$ requires C, 53·3; H, 6·5; N, 16·6%), homogeneous on t.l.c. in ethanol–chloroform (2 : 23).

Thymidinylacetyl-(3′→5′)-thymidinylacetyl-(3′→5′)-thymidinylacetyl-(3′→5′)-thymidinylacetyl-(3′→5′)-cytidine. 2-Cyanoethyl 5′-*O*-triphenylmethylthymidinylacetyl-(3′→5′)-thymidinylacetyl-(3′→5′)-thymidinylacetyl-(3′→5′)-thymidin-3′-ylacetate (8 mg) was treated with potassium t-butoxide in the usual way to remove the 2-cyanoethyl group and then condensed with 4-*N*-dimethylaminomethylene-2′,3′-*O*-isopropylidenecytidine (1·5 mg) in the usual way. After removal of excess of dicyclohexylcarbodi-imide and dicyclohexylurea the mixture was subjected to t.l.c. in ethanol–chloroform (2 : 23). The compound was left adsorbed to the silica for 36 h at 20° then eluted with ethanol–chloroform (1 : 1). The eluate was evaporated to dryness to give a white solid which had no absorption at 310 nm. (This showed that the dimethylaminomethylene group had been removed by the prolonged contact with the silica.) The other protecting groups were removed by treatment with formic acid–water (2 : 1) at room temperature for 16 h and the product, dissolved in acetic acid–water (1 : 4), subjected to paper electrophoresis for 9 kVh in 0·05M-acetate (pH 3·6). Two major components and two minor components were detected. The former were eluted and again subjected to paper electrophoresis. The faster-running component remained homogeneous. This was eluted with acetic acid–water and salts were removed by dissolving in dry dimethylformamide, filtering, and precipitating the *product* from solution with ethanol and ether; $\lambda_{max.}$ [AcOH–H_2O (1 : 4)] 268—269, $\lambda_{min.}$ 243 nm. Alkaline hydrolysis gave 3′-*O*-carboxymethylthymidine and cytidine in the molar ratio of 4·1 : 1.

A sample of this compound labelled with tritium in the cytidine residue was obtained by carrying out the synthesis with labelled 4-*N*-dimethylaminomethylene-2′,3′-*O*-isopropylidenecytidine. The labelled compound was identical with the unlabelled compound with regard to chromatographic and electrophoretic mobility and u.v. absorption spectrum; *ca.* 1 mg of material (specific activity of 236 mCi $mmol^{-1}$) was obtained.

Polymerisation of 3′-O-Carboxymethylthymidine in the Presence of 2′,3′-O-Isopropylideneuridine.—3′-*O*-Carboxymethylthymidine [pyridinium salt (0·28 mmol)] and 2′,3′-*O*-isopropylideneuridine (0·028 mmol) were exhaustively dried and dissolved in dry pyridine (1·5 ml); dicyclohexycarbodi-imide (500 mg) was added and the mixture was kept at 20° for 16 h. The pyridine was evaporated off *in vacuo* to leave an oil which was dissolved in 98% formic acid (6 ml); water (3 ml) was added and the mixture was kept at 20° for 4 h, filtered free from

[8] J. Zemlicka and A. Holy, *Coll. Czech. Chem. Comm.*, 1967, **32**, 3159.

dicyclohexylurea, and evaporated to dryness. The residue was dissolved in dimethylformamide (20 ml) and dialysed against dimethylformamide (2 × 200 ml for 4 h; 1 × 1·5 l for 16 h) at 20° and then against water (2 × 500 ml for 4 h; 1 × 1 l for 4 h) at 20°. The suspension inside the dialysis bag was then freeze-dried to give the oligomer (14 mg, 12%). Hydrolysis in alkali gave 3′-*O*-carboxymethylthymidine and uridine in the molar ratio of 167 : 1. The product did not migrate on paper electrophoresis at pH 6·8.

Interaction with Polyadenylic Acid.—This was studied by measurement of the u.v. absorption of solutions containing the oligomers and polyadenylic acid (mol. wt. $>10^5$; PL Biochemicals Inc.) in various proportions by the standard procedures already used.[7,9] The solvent was 0·3M-sodium chloride, 0·01M-glycylglycine (pH 6·3). In the case of [dThd-a-]$_n$Urd the solution contained 10% (v/v) of dimethylformamide and 0·03M-sodium citrate (instead of glycylglycine). The solutions were kept at 4° for 18 h, then allowed to warm up to 20°, and the optical density at 260 and at 267 nm was measured. The results (at 260 nm) are shown in the Figure.

N.m.r. Spectra.—The spectra (100 MHz) (Table) were recorded for solutions in [$^2H_6$]dimethyl sulphoxide with tetramethylsilane as internal reference.

We thank the Medical Research Council and the Cancer Research Campaign for research grants and Messrs. Arthur Guinness, Son and Co. Ltd., for a research studentship (to M. D. E.).

[2/365 *Received*, 18*th February*, 1972]

[9] M. G. Boulton, A. S. Jones, and R. T. Walker, *Biochim. Biophys. Acta*, 1971, **246**, 197.