Synthetic Analogues of Polynucleotides. Part 15.[1] The Synthesis and Properties of Poly(5'-amino-3'-*O*-carboxymethyl-2',5'-dideoxy-*erythro*-pentonucleosides) containing 3'(*O*) → 5'(*C*) Acetamidate Linkages

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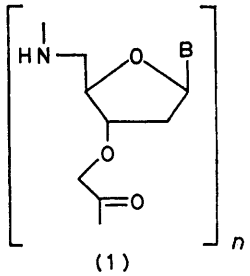
Poly(5'-amino-3'-*O*-carboxymethyl-5'-deoxythymidine) was prepared by the polymerisation of 5'-chloroacetamido-5'-deoxythymidine under basic conditions to give a polymer which contained in addition to 3'(*O*) → 5'(*C*) acetamidate linkages, a substantial proportion of 3'(*O*) → 3-acetamidate linkages. To obtain a polymer which contains 3'(*O*) → 5'(*C*) linkages. 5'-amino-5'-deoxythymidinylacetamido[3'(*O*) → 5'(*C*)]-5'-deoxythymidin-3'-ylacetic acid (7) was synthesised and polymerised. Compound (7) was obtained by the reduction of the corresponding 5'-azido compound. The latter was obtained by the condensation of the pentachlorophenyl ester of 5'-azido-3'-*O*-carboxymethyl-5'-deoxythymidine with 5'-amino-3'-*O*-carboxymethyl-5'-deoxythymidine. Poly(5'-amino-3'-*O*-carboxymethyl-2',5'-dideoxycytidine) was obtained by the polymerisation of 4-*N*-acetyl-5'-chloroacetamido-2',5'-dideoxycytidine (10) under basic conditions followed by removal of the 4-*N*-acetyl groups by mild acidic hydrolysis. This polymer contained 3'(*O*) → 5'(*C*) acetamidate linkages and probably <6% of other acetamidate linkages. Compound (10) was obtained from the corresponding 5'-*p*-tolylsulphonyloxy compound *via* the 5'-azido compound. None of these polymers showed any evidence of base stacking or of interaction with their complementary polyribonucleotides.

In a previous Part [2] attempts to obtain a polynucleotide analogue of structure (1; B = thymidin-1-yl) by condensation polymerisation of 5'-amino-3'-*O*-carboxymethyl-5'-deoxythymidine (2) under a variety of conditions were described. No polymer was formed, however, and the major product was the lactam (3). This result was in contrast to that obtained with 3'-*O*-carboxymethylthymidine which gave a polymer and no lactone analogous to (3).[3] Further attempts have been made to synthesise (1; B = thymidin-1-yl) which did not involve the polymerisation of (2).



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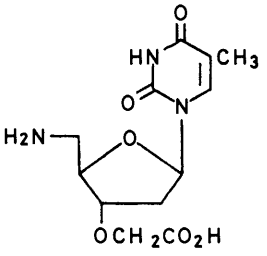

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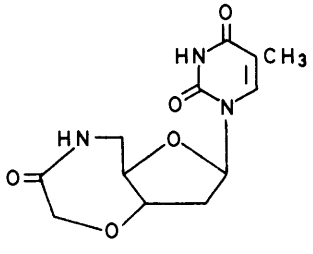
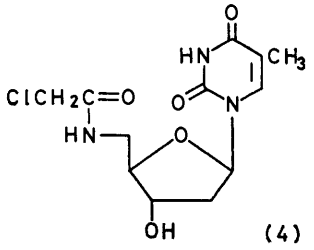


(3)

First, 5'-chloroacetamidyl-5'-deoxythymidine (4) was obtained by the action of chloroacetyl pentachlorophenate on 5'-amino-5'-deoxythymidine. Compound (4) was polymerised by treatment with sodium hydride in dimethyl sulphoxide, 5'-*O*-triphenylmethylthymidine (0.1 mol. equiv.) being added to provide an end group. Removal of the triphenylmethyl end group and exhaustive dialysis of the reaction mixture gave a 7.5% yield of water-soluble polymer. No lactam (3) appeared



(4)

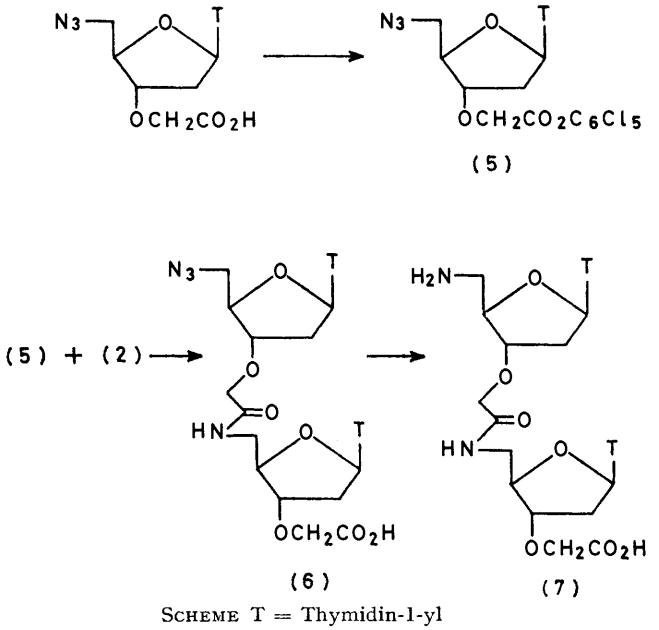
to be produced during the polymerisation. The polymer did not have the required structure (1; B = thymidin-1-yl), however. Strong acidic hydrolysis of the polymer gave in addition to the expected thymine, 3-carboxymethylthymine (35%) thus showing that during the polymerisation, alkylation had occurred on the 3- as well as on the 3'-*O*-position.

Upon the failure of this procedure to produce the required polymer it was decided to synthesise a molecule in which two nucleoside units are linked and then to polymerise the resulting 'dimer'. The required compound, 5'-amino-5'-deoxythymidinylacetamido-[3'(*O*) → 5'(*C*)]-5'-deoxythymidin-3'-ylacetic acid (7) was obtained as in the Scheme.

5'-Azido-3'-*O*-carboxymethyl-5'-deoxythymidine [2] was converted into its pentachlorophenyl ester (5) by treatment with pentachlorophenol and dicyclohexylcarbodiimide. Condensation of (5) with 5'-amino-3'-*O*-carboxymethyl-5'-deoxythymidine (2) gave compound (6). Reduction of (6) to the required amino compound (7) was accomplished by catalytic hydrogenation using a platinum catalyst. This always proved to be difficult, however, and it was necessary to remove starting

material from the product by ion-exchange chromatography.

Compound (7) was polymerised to give the required polymer (1; B = thymidin-1-yl) by the use of triphenylphosphine and 2,2′-dipyridyl disulphide.[4] To serve as

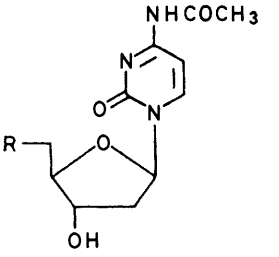


SCHEME T = Thymidin-1-yl

end group in the polymerisation, 5′-t-butyloxycarbonylamino-3′-*O*-carboxymethyl-5′-deoxythymidine, obtained by treatment of compound (2) with t-butyloxycarbonyl azide, was used. The polymer was isolated by dialysing away low molecular weight material, first against dimethylformamide and then against water. The polymer was isolated in an overall yield of 54%. Its solubility in water was low (0.18 mg ml^{-1}) but sufficient to enable investigations on its interaction with polynucleotides to be carried out. The molecular weight was determined by gel permeation chromatography at pH 10 on Sephadex G25 and G50. The polymer was very polydisperse but at the peak of the elution curve the chain length was *ca.* 10—13 ' thymidine ' residues. The polymer showed a typical thymidine spectrum and there was no hyperchromic effect upon alkaline hydrolysis to the monomer (2). It is highly probable that the polymer has the desired structure (1) because of the reluctance of thymidine derivatives to acylate on the nitrogen at position 3 and the lability of compounds so acylated.

Synthesis of the corresponding polymer containing cytosine residues (1; B = cytosin-1-yl) was carried out by the polymerisation of 4-*N*-acetyl-5′-chloroacetamido-2′,5′-dideoxycytidine (10). This route was chosen since that involving a ' dimer ' analogous to compound (7) is excessively long because of the necessity of protecting the cytosine residues. The route taken to obtain compound (10) was as follows. 4-*N*-Acetyl-2′-deoxy-5′-*O*-*p*-tolylsulphonylcytidine was treated with sodium azide to give 4-*N*-acetyl-5′-azido-2′,5′-dideoxycytidine (8). This was converted into the corresponding 5′-amino compound (9) by treatment with hydrogen in the presence of a platinum catalyst. Previous workers had attempted to obtain similar derivatives of 5′-amino-2′,5′-dideoxycytidine by the reaction of the azide with hydrogen in the presence of a catalyst, but without success.[5] We found that the reaction conditions, namely treatment with hydrogen at atmospheric pressure and 20° for 30 min in the presence of Adams' platinum catalyst, were critical. A longer reaction time resulted in appreciable reduction of the cytosine ring. The yield of compound (9) (isolated as the hydrochloride) was poor, because deacetylation occurred during the isolation. The next stage of the synthesis was usually carried out without isolating compound (9). This involved treatment with chloroacetyl pentachlorophenolate to give the required compound (10).

Polymerisation of compound (10) was carried out in dimethyl sulphoxide with at least 5 mol. equiv. of sodium hydride. The resulting polymer was isolated by exhaustive dialysis against dimethylformamide and then against water. The u.v. absorption of the polymer was typical of a 4-*N*-acylcytidine derivative. To give a polymer of the required structure, the 4-*N*-acetyl groups were removed by treatment with M-hydrochloric acid at 20° for 16 h. The polymer, isolated after exhaustive dialysis of this reaction mixture, had a u.v. absorption spectrum typical of a cytidine derivative. The overall



(8) R = N_3
(9) R = H_2N
(10) R = $ClCH_2CONH$

yield of polymer from this process was extremely low, however (1—4%), considerable loss of polymeric material occurring during the removal of the acetyl groups. The use of milder conditions resulted in incomplete deacetylation. The lability of the acetamidate linkages in the polymer to acidic conditions appeared to be greater than was the case with a model compound containing the same linkage.[2]

During a polymerisation of this type it is possible that unwanted linkages could have been formed, through either the cytosine residues or the amide nitrogen at position 5′. Vigorous acidic hydrolysis of the polymer, however, gave cytosine but no detectable 3-carboxymethylcytosine. Because the latter is decomposed very slowly indeed under the conditions used, it can be concluded that the number of linkages through cytosine residues must be <3%. The number of unwanted linkages through the 5′-*N* position was not determined

directly but reaction at this position would be no more probable than reaction at the cytosine residues. The number of unwanted linkages is probably ≤6%. Therefore the polymer has essentially the structure (1; B = cytosin-1-yl). The small amounts of the polymer obtained and its polydisperse nature precluded determination of its molecular weight. Its retention within a dialysis bag after exhaustive dialysis suggests that the polymer must have a chain length of at least six units.

Attempts were made to hybridise the two polynucleotide analogues with their complementary polynucleotides, namely polyadenylic acid and polyinosinic acid. A wide range of conditions was investigated and a number of techniques were used to detect any interaction, but none was found. There was also no evidence for base-stacking in these analogues. In the case of the thymine-containing polymer the measurements were complicated by the fact that it was adsorbed on glass and on plastic surfaces. Thus these acetamidate-linked polymers differed from the acetate-linked polymers previously studied which did interact with their complementary polynucleotides.[1,3,6] The most probable explanation of this difference is that the greater rigidity of the amide linkage compared to that of the ester linkage, prevented the polymeric acetamidate analogues adopting a conformation which favoured interaction with a complementary polynucleotide. This possibility was not predicted from the examination of a model of a molecule containing only one acetamidate linkage, namely, thymidinylacetamido[3′(*O*) ⟶ 5′(*C*)]-5′-deoxythymidine,[2] but became apparent in a model of a compound containing three acetamidate linkages.

EXPERIMENTAL

T.l.c. plates were coated with silica gel (MN-Kieselgel G/UV$_{254}$; Machery, Nagel and Co.). The silica gel used for column chromatography was Kieselgel (0.05—0.2 mm; 70—325 mesh ASTM; type 7734) supplied by E. Merck, A.G. Darmstadt. N.m.r. spectra were run at 100 MHz. Unless stated otherwise the solvent was $(CD_3)_2SO$.

Pentachlorophenyl Chloroacetate.[7]—*N*-Cyclohexyl-*N*′[2-(*N*-methylmorpholino)ethyl]carbodi-imide toluene-*p*-sulphonate (5 g) was dissolved in dichloromethane (62 ml) and pentachlorophenol (3.37 g) added. The mixture was stirred at room temperature for 18 h and then poured into ether (500 ml) and the resulting precipitate of the isourea filtered off and dried (yield 7 g). This was then added to a solution of chloroacetic acid (1 g) in dry dimethylformamide (20 ml) which was stirred at room temperature for 18 h. The solution was then poured into water, the resulting precipitate filtered off, dried, and crystallised from propan-2-ol to give pentachlorophenyl chloroacetate (2.4 g), m.p. 124—126° (Found: C, 28.3; H, 0.7; Cl, 62.2. Calc. for $C_8H_2Cl_6O_2$: C, 28.0; H, 0.6; Cl, 62.1%).

5′-Chloroacetamidyl-5′-deoxythymidine.— 5′-Amino-5′-deoxythymidine [8] (400 mg) was dissolved in dry dimethylformamide (6 ml) and pentachlorophenyl chloroacetate (684 mg) added. The mixture was left at room temperature for 18 h and then evaporated to dryness. Co-evaporation of the residue with ethanol gave a solid which was crystallised from ethanol to give *5′-chloroacetamidyl-5′-deoxythymidine* (385 mg, 73%), m.p. 213—215° (Found: C, 45.1; H, 5.0; Cl, 11.2; N, 13.1. $C_{12}H_{16}ClN_3O_5$ requires C, 45.4; H, 5.0; Cl, 11.2; N, 13.2%); $\lambda_{max.}$ 267 nm (ε 9 300), $\lambda_{min.}$(EtOH) 236 nm; δ 1.78 (3 H, s, CH_3), 2.05 (2 H, m, H-2′), 3.35 (H-5′ and H_2O), 3.7 (1 H, m, H-4′), 4.03 (2 H, s, $ClCH_2CO$), 4.1 (1 H, m, H-3′), 6.1 (1 H, d, H-1′), 7.4 (1 H, s, H-6), 8.3br (1 H, s, NH-5′), and 11.25br (1 H, s, NH-3).

Polymerisation of 5′-Chloroacetamidyl-5′-deoxythymidine.—*5′-O*-Triphenylmethylthymidine monobenzene adduct (15 mg, 0.027 mmol) was added to a mixture of sodium hydride (6 mg, 0.25 mmol) in dimethyl sulphoxide (0.4 ml). After shaking the mixture for 5 min, 5′-chloroacetamidyl-5′-deoxythymidine (10 mg, 0.035 mmol) was added and the mixture shaken at room temperature. At intervals, portions of dimethyl sulphoxide (0.3 ml), sodium hydride (4 mg), and 5′-chloroacetamidyl-5′-deoxythymidine (14 mg) were added in that order (6 additions of each) over a total of 55 h. To the mixture there was then added 98% formic acid (50 ml). After standing at room temperature for 5 min the mixture was evaporated to dryness and to the residue there was added water (20 ml) and chloroform (20 ml). The aqueous phase of the mixture was then separated, repeatedly extracted with chloroform, separated from insoluble material, and exhaustively dialysed against distilled water. The solution inside the dialysis bag was freeze-dried to give solid polymeric material (4 mg). A sample of this material was hydrolysed by treatment with 98% formic acid at 175° for 2 h. Paper chromatography and paper electrophoresis of the hydrolysate showed that thymine and 3-carboxymethylthymine were present in the ratio 13 : 7.

Reaction of Sodium Chloroacetate with 5′-Azido-5′-deoxythymidine.—5′-Azido-5′-deoxythymidine [8] (8.2 g) was dissolved in dry dimethyl sulphoxide (100 ml); sodium hydride (1.84 g) was added, the mixture left at room temperature for 1 h and then sodium chloroacetate (3.6 g) added. The mixture was stirred at 20° for 66 h and then diluted with water (100 ml) and the solution passed down a column of Amberlite IR 120 resin (H^+ form) (41 cm × 2.3 cm diam.). The eluate and washings were evaporated to a small volume and the dimethyl sulphoxide removed by distillation in high vacuum. The resulting oily residue was dissolved in water (20 ml) and chromatographed on a column of DEAE-cellulose (Whatman DE 32; 40 cm × 3.6 cm diam.). Elution with a formic acid gradient (0—0.38M over 1 800 ml and then to 0.5M over a further 2 200 ml) gave three nucleoside-containing peaks, the first being starting material (39%). The fractions containing the second component were combined, evaporated to dryness, and the residue crystallised from water to give 5′-azido-3-*O*-carboxymethyl-5′-deoxythymidine (45%),[2] m.p. 145—146° (Found: C, 44.1; H, 4.6; N, 21.4. Calc. for $C_{12}H_{15}N_5O_6$: C, 44.3; H, 4.7; N, 21.5%); $\lambda_{max.}$ 267 nm (ε 9 760); $\lambda_{min.}$ 235 nm at pH 5; $\lambda_{max.}$ 267 nm (ε 7 530); $\lambda_{min.}$ 247 nm at pH 13. The compound ran at 6.0 cm kV^{-1} h^{-1} to the anode upon paper electrophoresis at pH 6.9.

The fractions containing the third component were combined and freeze-dried to give a hydroscopic solid (15% yield) which was identified as *5′-azido-3-carboxymethyl-3′-O-carboxymethyl-5′-deoxythymidine*, m.p. 126—127° (Found: C, 43.2; H, 4.4; N, 17.8. $C_{14}H_{17}N_5O_8$,0.3H_2O requires C, 43.2; H, 4.6; N, 18.0%); $\lambda_{max.}$ 268 nm (ε 8 380), $\lambda_{min.}$ 237 nm at pH 5; $\lambda_{max.}$ 268 nm (ε 8 350), $\lambda_{min.}$ 238 nm at pH 13; $\nu_{max.}$ 2 100 cm^{-1} (azide); δ 1.89 (3 H, s, CH_3), 2.3 (2 H,

m, H-2'), 3.65 (2 H, d, H-5'), 4.11 (2 H, s, OCH_2), 4.15 (2 H, m, H-3' and H-4'), 4.48 (2 H, s, NCH_2), and 7.63 (1 H, s, H-6). The compound ran at 7.5 cm kV^{-1} h^{-1} to the anode upon paper electrophoresis at pH 6.9.

Pentachlorophenyl Ester of 5'-Azido-3'-O-carboxymethyl-5'-deoxythymidine (5).—5'-Azido-3'-*O*-carboxymethyl-5'-deoxythymidine (170 mg) was dissolved in dry dioxan and pentachlorophenol (140 mg) and dicyclohexylcarbodi-imide (230 mg) added. The solution was kept at room temperature for 18 h, the *NN'*-dicyclohexylurea which had formed was filtered off, and the filtrate evaporated to dryness. The residue was dissolved in acetonitrile and chromatographed on a column of silica gel (28 cm × 2.6 cm diam.). The fractions containing the required compound were combined and evaporated to dryness. The solid residue was crystallised from acetonitrile to give needles of the *required ester* (145 mg, 49%), m.p. 150° (decomp.) (Found: C, 37.9; H, 2.3; Cl, 30.7; N, 12.5. $C_{18}H_{14}Cl_5N_5O_6$ requires C, 37.7; H, 2.5; Cl, 30.9; N, 12.2%).

5'-Amino-3'-O-carboxymethyl-5'-deoxythymidine (2).[2]—5'-Azido-3'-*O*-carboxymethyl-5'-deoxythymidine (0.9 g) was dissolved in AnalaR methanol (100 ml) and Adams' platinum catalyst (0.48 g) added. The mixture was hydrogenated at room temperature for 4 h under 1 atmosphere of hydrogen. The catalyst was filtered off with the help of filter aid and the filtrate evaporated to dryness. The residue was dissolved in water and freeze-dried to give the product (0.72 g) as a solid which softened at 166° and melted at 173° (decomp.) (Found: C, 45.7; H, 6.2; N, 13.0. Calc. for $C_{12}H_{17}N_3O_6,H_2O$: C, 45.5; H, 6.0; N, 13.3%); $\lambda_{max.}$ 267 nm (ε 9 270); $\lambda_{min.}$ 237 nm at pH 5; $\lambda_{max.}$ 268 nm (ε 7 820); $\lambda_{min.}$ 249 nm at pH 13. The compound ran as a single component upon paper electrophoresis at pH 2 with a mobility of 6 cm kV^{-1} h^{-1} towards the cathode. It also ran as a single component upon t.l.c. in acetonitrile–water (9 : 1) (R_F 0.04) and in ethanol–water (9 : 1) (R_F 0.19).

5'-Azido-5'-deoxythymidinylacetamidyl[3'(O) ⟶ 5'(C)]-3'-O-*carboxymethyl-5'-deoxythymidine* (6).—5'-Amino-3'-*O*-carboxymethyl-5'-deoxythymidine (299 mg, 1.00 mmol) was dissolved in dry dimethylformamide (16 ml). The pentachlorophenyl ester of 5'-azido-3'-*O*-carboxymethyl-5'-deoxythymidine (575 mg, 1.00 mmol) was then added and the solution kept at room temperature (*ca.* 20°) for 18 h. The solvent was evaporated off, the residue dissolved in methanol (5 ml), and this solution added dropwise to toluene (500 ml) with constant stirring. The resulting precipitate was centrifuged off, washed well with toluene, and dried to give the required product (410 mg), m.p. 120—122°. It gave an elemental analysis which indicated that it contained a trace of impurity (probably toluene). It was shown to be homogeneous, however, upon paper electrophoresis at pH 6.9 (4.3 cm kV^{-1} h^{-1} towards the anode) and upon t.l.c. in acetonitrile–water (17 : 3) (R_F 0.12), $\lambda_{max.}$ 267 nm (ε 16 200), $\lambda_{min.}$(EtOH) 236 nm; $\lambda_{max.}$ 267 nm (ε 12 900); $\lambda_{min.}$ 251 nm at pH *ca.* 13; δ 1.82 (6 H, s, CH_3), 2.25 (4 H, m, H-2'), 3.4 (2 H, d, $NHCH_2$), 3.6 (2 H, d, N_3CH_2), 3.99—4.06 [8 H, m, H-3' (2 H), H-4' (2 H), and OCH_2 (4 H)], 6.16 (2 H, d, H-1'), 7.51 (2 H, s, H-6), 7.98 (1 H, s, CONH), and 11.25br (2 H, s, NH).

5'-Amino-5'-deoxythymidinylacetamidyl[3'(O) ⟶ 5'(C)]-3'-O-*carboxymethyl-5'-deoxythymidine* (7).—The compound obtained as described above (200 mg) was dissolved in AnalaR methanol (100 ml), Adams' platinum catalyst (390 mg) added and the mixture shaken under hydrogen at 1 atmosphere at room temperature for 5 h. The catalyst was filtered off and the solvent evaporated from the filtrate to give a residue. This was dissolved in methanol (100 ml) and hydrogenated under similar conditions for a further 4 h. The solid which was obtained (203 mg) was applied in aqueous solution to a column (6 cm × 1.4 cm diam.) of Amberlite IR120 ion exchange resin (H^+ form). After washing away the impurities with water the product was eluted with a 1% aqueous solution of ammonia. The eluate was evaporated to dryness and the residue dissolved in water and the solution freeze-dried to give the *required product* (105 mg), m.p. 170—180° (Found: C, 45.1; H, 5.8; N, 13.5. $C_{24}H_{38}N_6O_4,3H_2O$ requires C, 45.4; H, 6.0; N, 13.2%); $\lambda_{max.}$ 267 nm (ε 17 800); $\lambda_{min.}$ 236 nm at pH 5; $\lambda_{max.}$ 268 nm (ε 13 900); $\lambda_{min.}$ 247 nm at pH 13; δ (D_2O) 1.89 (6 H, s, CH_3), 2.45 (4 H, m, H-2'), 3.35 (2 H, d, CH_2NH), 3.60 (2 H, d, CH_2NH_2), 3.98 (2 H, s, OCH_2COOH), 4.16 (2 H, s, OCH_2CONH), 4.3 (4 H, m, H-3' and -4'), 6.16 (2 H, m, H-1'), and 7.47 (2 H, s, H-6).

5'-t-Butyloxycarbonylamino-3'-O-carboxymethyl-5'-deoxythymidine.— 5'-Amino-3'-*O*-carboxymethyl-5'-deoxythymidine (322 mg) was dissolved in dimethyl sulphoxide (5 ml) and redistilled triethylamine (0.28 ml) and t-butyloxycarbonyl azide (0.15 ml) were added. After 20 h at room temperature the mixture was diluted with water (15 ml) and extracted with ether (3 × 15 ml). The aqueous phase was brought to pH 3 by the addition of citric acid and then extracted with ethyl acetate (3 × 25 ml). The organic phases were combined, washed with water (10 ml), and dried ($MgSO_4$). The solvent was then removed by evaporation and the residue dissolved in water (100 ml) and the solution freeze-dried to give the *required product* (221 mg), m.p. 75° (decomp.) (Found: C, 49.1; H, 6.5; N, 9.7. $C_{17}H_{25}N_3O_8,H_2O$ requires C, 48.9; H, 6.2; N, 10.1%); $\lambda_{max.}$ 268 nm (ε 8 980); $\lambda_{min.}$ (EtOH) 234 nm; δ 1.38 (9 H, s, Me_3C), 1.92 (3 H, s, CH_3), 2.2 (2 H, m, H-2'), 3.2 (2 H, d, H-5'), 4.05—4.1 (3 H, m, H-3' and OCH_2), 6.13 (1 H, m, H-1'), 7.0 (1 H, s, amide NH), 7.53 (1 H, s, H-6), and 11.26 (1 H, s, thymine NH). The compound moved as a single component upon paper electrophoresis at pH 7.5 (5.2 cm kV^{-1} h^{-1} towards the anode) and upon t.l.c. in acetonitrile–water (9 : 1) (R_F 0.12) and in ethanol–water (9 : 1) (R_F 0.45).

Polymerisation of 5'-Amino-5'-deoxythymidinylacetamidyl-[3'(O) ⟶ 5'(C)]-3'-O-*carboxymethyl-5'-deoxythymidine.*— The above mentioned compound (4.7 mg), compound (7) (140 mg), and triphenylphosphine (584 mg) were dissolved in dry pyridine (3.9 ml) and 2,2'-dipyridyldisulphide (485 mg) added. The mixture was kept at room temperature for 14 h and then dimethylformamide (2 ml) added and the mixture dialysed exhaustively against dimethylformamide and then against water. The material inside the dialysis bag contained a precipitate. This was centrifuged off and a portion of the supernatant liquid was freeze-dried to give a solid P_1 (10 mg). The precipitate was dissolved in dilute aqueous sodium hydroxide and dialysed exhaustively against water. The material remaining inside the dialysis bag was a suspension; this was freeze-dried to give a solid P_2 (23 mg). P_1 had $\lambda_{max.}$ 269 nm, $\lambda_{min.}$ 237 nm at pH 5; $\lambda_{max.}$ 269 nm, $\lambda_{min.}$ 246 nm at pH 13. P_2 had $\lambda_{max.}$ 271 nm, $\lambda_{min.}$ 240 nm at pH 1; $\lambda_{max.}$ 268 nm, $\lambda_{min.}$ 246 nm at pH 13.

The molecular weights of these products were estimated by gel filtration on Sephadex G25 and G50 at pH 10. This high pH was chosen because there appeared to be an interaction between the Sephadex gels and the thymine residues

of the polymers at lower pH values. The molecular weight calculated from the results depends upon the model selected for comparison. With these uncharged polymers it was considered that the dextrans provided the best model. This assumption was justified when applied to monomeric and dimeric derivatives fractionated on Sephadex G25. The results showed that P_1 and P_2 were very heterogeneous with regard to molecular weight and that there was little difference between the two. The chain length of the molecules was *ca.* 10—13 ' thymidine ' units.

4-N-*Acetyl-5'-azido-2',5'-dideoxycytidine* (8).—4-*N*-Acetyl-2'-deoxy-5'-*p*-tolylsulphonylcytidine (2.8 g) [9] was dissolved in dry dimethylformamide (100 ml), sodium azide (0.87 g) added, and the mixture stirred at 65° for 6 h in an oxygen-free atmosphere. The solvent was removed by evaporation and co-evaporation with ethanol to give a gummy residue. This was extracted with chloroform (3 × 100 ml) and the chloroform extract evaporated to dryness. The resulting solid was fractionated by chromatography on a column of silica gel with chloroform–ethanol (17 : 3) as the eluant. The fractions containing the required product were pooled and evaporated to dryness to give 4-N-*acetyl-5'-azido-2',5'-dideoxycytidine* as a solid (1.56 g, 80%) (Found: C, 43.8; H, 5.0; N, 27.6. $C_{11}H_{14}N_6O_4,0.5H_2O$ requires C, 43.6; H, 5.0; N, 27.7%); $\lambda_{max.}$ 249 (ε 14 670) and 302 nm (ε 7 350), $\lambda_{min.}$(EtOH) 276 nm (ε 3 460); $\nu_{max.}$ 2 100 cm^{-1} (azide); δ 2.11—2.33 (5 H, m, H-2' and CH_3CO), 3.64 (2 H, d, H-5'), 3.97 (1 H, q, H-4'), 4.20 (1 H, m, H-3'), 5.45br (1 H, s, OH-3'), 6.20 (1 H, t, H-1'), 7.26 (1 H, d, H-5), 8.11 (1 H, d, H-6), and 11.14br (1 H, s, NH).

4-N-*Acetyl-5'-amino-2',5'-dideoxycytidine* (9).—To a solution of the above-mentioned compound (248 mg) in dry methanol (80 ml) there was added Adams' platinum oxide catalyst (90 mg) and the mixture shaken for 30 min at 20° under 1 atmosphere of hydrogen. The mixture was then filtered through Hyflo Supercel filter aid and to the clear filtrate there was added 4M-hydrochloric acid (0.22 ml) and the solution evaporated to dryness to give a solid (this consisted of the required product and deacylated material in the ratio 7 : 3). This was dissolved in water (4 ml), acetone (20 ml) was added, and the solution kept at 2° for 48 h. The resulting crystals were filtered off and dried to give 4-N-*acetyl-5'-amino-2',5'-dideoxycytidine hydrochloride* (30 mg), m.p. 204—210° (decomp.) (Found: C, 42.7; H, 5.8; Cl, 11.2; N, 17.9. $C_{11}H_{17}ClN_4O_4,0.5H_2O$ requires C, 42.1; H, 5.8; Cl, 11.3; N, 17.9%); $\lambda_{max.}$ 244 (ε 9 970) and 308 nm (ε 12 000), $\lambda_{min.}$ 272 nm (ε 3 570) at pH 1; δ(D_2O) 2.22 (3 H, s, CH_3CO), 2.52 (2 H, t, H-2'), 3.39 (2 H, t, H-5'), 4.12—4.65 (m, H-3', H-4', and H_2O), 6.18 (1 H, t, H-1'), 7.32 (1 H, d, H-5), and 8.08 (1 H, d, H-6).

4-N-*Acetyl-5'-chloroacetamido-2',5'-dideoxycytidine* (10).—4-*N*-Acetyl-5'-azido-2',5'-dideoxycytidine (1.75 g) was dissolved in dry methanol (157 ml), Adams' platinum catalyst (855 mg) was added, and the mixture shaken for 30 min under 1 atmosphere of hydrogen at 20°. The mixture was then filtered through Hyflo Supercel filter aid, to the filtrate there was added pentachlorophenyl chloroacetate (2.07 g) and the mixture stirred at room temperature for 18 h. It was then evaporated to dryness and the resulting solid fractionated by chromatography on silica gel (350 g). The column was eluted first with chloroform–ethanol (17 : 3, 1 500 ml) and then with chloroform–ethanol (3 : 2). Fractions containing the required product were collected and evaporated to dryness to give the almost pure product as a solid (1.06 g, 52%). A sample was crystallised from propan-2-ol to give 4-N-*acetyl-5'-chloroacetamido-2',5'-dideoxycytidine*, m.p. 165—180° (decomp.) (Found: C, 45.0; H, 4.8; Cl, 10.1; N, 16.2. $C_{13}H_{17}ClN_4O_5$ requires C, 45.3; H, 5.0; Cl, 10.3; N, 16.3%); $\lambda_{max.}$ 249 (ε 16 000) and 302 nm (ε 8 140), $\lambda_{min.}$ (EtOH) 275 nm (ε 4 020); δ 2.10 (3 H, s, CH_3CO), 3.60 (1 H, m, H-4'), 3.90 (1 H, m, H-3'), 4.08 (2 H, s, $ClCH_2CO$), 5.30 (1 H, d, OH-3'), 6.09 (1 H, t, H-1'), 7.22 (1 H, d, H-5), 8.12 (1 H, d, H-6), 8.40 (1 H, m, NH-5'), and 10.83 (1 H, s, NH-4).

Poly(5'-amino-3'-O-carboxymethyl-2',5'-dideoxycytidine).—Sodium hydride (708 mg) was dissolved in dimethyl sulphoxide (5 ml) and 4-*N*-acetyl-5'-chloroacetamido-2',5'-dideoxycytidine (515 mg) was added and the mixture kept at room temperature for 68 h. 98% Formic acid (15 ml) was then added and the mixture kept at room temperature for 5 min and then mixed with water (20 ml). The resulting solution was neutralised with 0.1M-sodium hydroxide, ($\lambda_{max.}$ 249 and 300 nm, $\lambda_{min.}$ 276 nm) and dialysed against distilled water (6 × 4 l) at 4° for 3 days. The solution inside the dialysis bag was freeze-dried to give a solid (47 mg, 9%). A portion of this (38 mg) was dissolved in 0.1M-hydrochloric acid (50 ml) and the solution kept at room temperature for 16 h, $\lambda_{max.}$ 280 nm, $\lambda_{min.}$ 247 nm at pH 1. The solution was neutralised with 0.1M-sodium hydroxide and dialysed against water (3 × 2 l) at 4° for a total of 25 h. The solution inside the dialysis bag contained a precipitate which was centrifuged off. The supernatant liquid was freeze-dried to give the required polymer (2.5 mg); $\lambda_{max.}$ 274 nm, $\lambda_{min.}$ 256 nm at pH 5; $\lambda_{max.}$ 280 nm, $\lambda_{min.}$ 248 nm at pH 1. The precipitate was centrifuged off, suspended in water, and the suspension freeze-dried to give a white solid (1 mg). This proved to be polymeric material similar to that obtained from the solution. The overall yield was therefore *ca.* 1%. Retreatment of the polymer with 0.1M-hydrochloric acid at room temperature for 16 h and then exhaustive dialysis resulted in a loss of 50% of the material.

Acidic Hydrolysis.—The polymer (1 mg) was dissolved in M-hydrochloric acid (5 ml) and the solution heated on a boiling water-bath for 4 h. Examination of the reaction mixture by paper chromatography in propan-2-ol–water–10M-hydrochloric acid (68 : 15.5 : 16.5) showed the presence of cytosine (R_F 0.46) and another component (R_F 0.2). The latter gave a positive reaction for the presence of a deoxypentose residue and was probably 5'-amino-3'-*O*-carboxymethyl-2',5'-dideoxycytidine. This conclusion is supported by the fact that upon hydrolysis for longer times the amount of cytosine increased while that of the component of R_F 0.2 decreased. 3-Carboxymethylcytosine was included as a marker (R_F 0.6). None of this was present in any of the hydrolysates. 3-Carboxymethylcytosine was only slightly hydrolysed to cytosine under these conditions; 2'-deoxycytidine was completely hydrolysed to cytosine.

Study of the Interaction of the Polymers with Polynucleotides.—Possible interactions of poly(5'-amino-3'-*O*-carboxymethyl-5'-deoxythymidine) and poly(5'-amino-3'-*O*-carboxymethyl-2',5'-dideoxycytidine) with polyadenylic acid and polyinosinic acid respectively, were studied by u.v. spectroscopy, o.r.d. measurements, and gel permeation chromatography. None of these methods showed evidence of interaction. The polymeric thymidine derivative was strongly adsorbed onto glass and certain plastic surfaces thus making measurements difficult. The polymers showed no change in u.v. absorption upon increasing the temperature and there was no hyperchromic effect upon alkaline hydrolysis.

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