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Synthetic analogues of polynucleotides. Part XIV. The synthesis of poly (3'-O-carboxymethyl-2'-deoxycytidine) and its interaction with polyinosinic acid

R. C. Bleaney, A. S. Jones and R. T. Walker

Chemistry Department, University of Birmingham, Birmingham B15 2TT, UK

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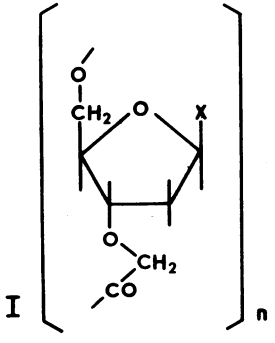
ABSTRACT

Poly (3'-O-carboxymethyl-2'-deoxycytidine) (VII) has been synthesised by the polymerisation of 3'-O-carboxymethyl-4-N-phenoxyacetyl-2'-deoxycytidine (V) and removal of the phenoxyacetyl groups under acidic conditions. V was obtained by the action of 2,4-dinitrophenyl phenylacetate on 3'-O-carboxymethyl-5'-O-triphenylmethyl-2'-deoxycytidine (III) followed by removal of the triphenylmethyl group under carefully controlled acidic conditions. The polymer, VII gave a hypochromic effect of about 20% at 250nm when mixed with poly(I)in 0.2M acetate, pH 5.0. It appeared, therefore, that a complex was formed. Upon heating a solution of this complex there was an initial decrease in optical density followed by a much larger increase to give a T_m of about 60°. Attempts to form the 3'-O-carboxymethyl derivative of 4-N-phenoxyacetyl-5'-O-triphenylmethyl-2'-deoxycytidine to give a shorter synthetic route to VII were not successful. 3'-O-Carboxymethyl-2'-deoxycytidine was obtained by removal of the triphenylmethyl group from III. Attempts to polymerise this compound in concentrated aqueous solution with a water-soluble carbodiimide were not successful.

INTRODUCTION

Analogues of oligonucleotides in which the phosphodiester linkages of the



natural compounds have been replaced by acetate ester linkages (as in I) have been

synthesised.[1-4] Compounds of structure I (X = thymine residue) have been shown to interact with poly(A) and a polymer of structure I (R = adenine residue) has been shown to interact with poly(U). The present paper reports the synthesis of a similar polymer in which X = cytosine residue and its interaction with poly(I).

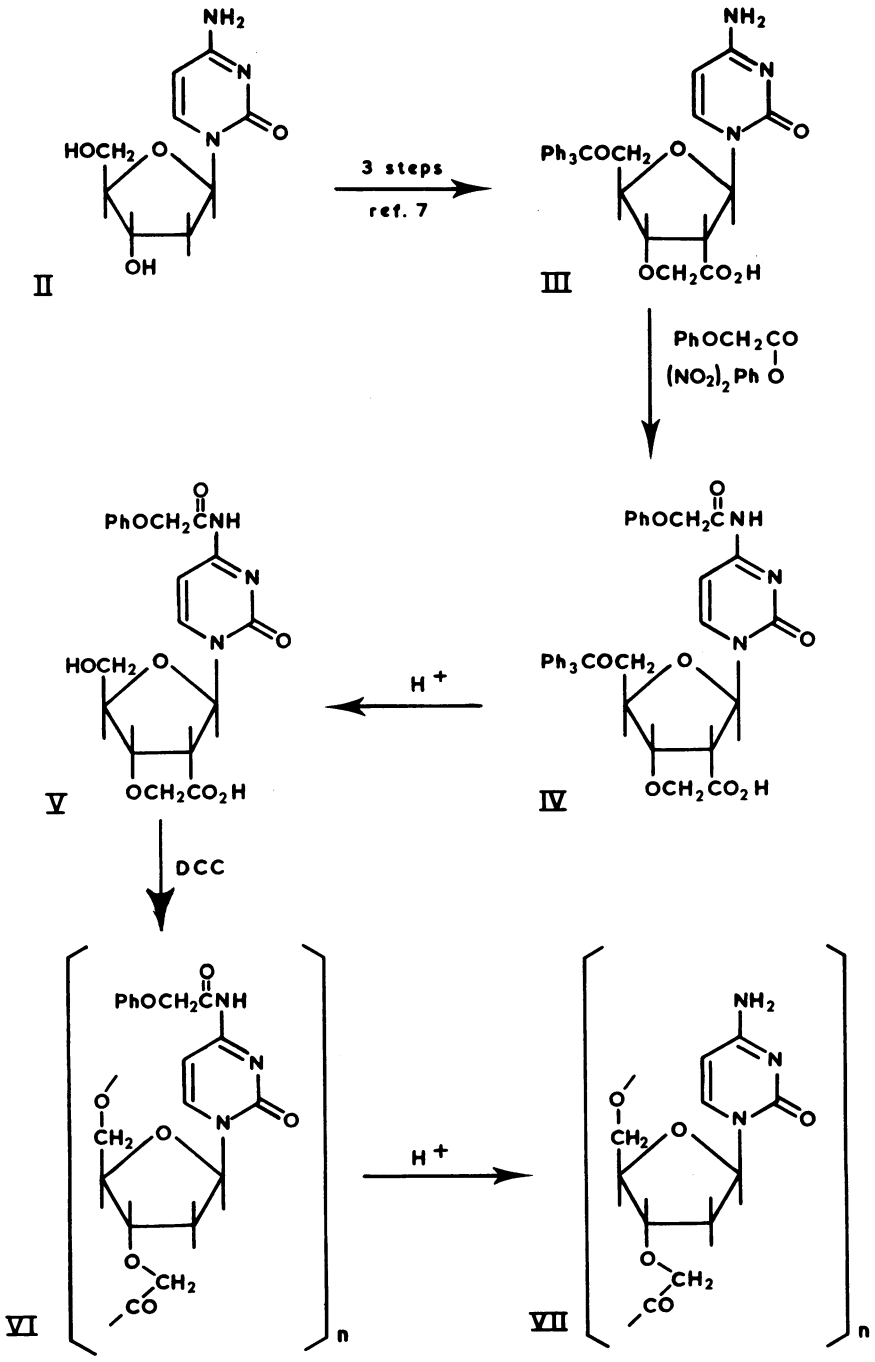
RESULTS AND DISCUSSION

The thymine-containing and the adenine-containing polymers of structure I were obtained by polymerising the appropriate 3'-O-carboxymethylnucleoside without the necessity of protecting the base residues during the condensation polymerisation. It is known, however, that the base residue in deoxycytidine and similar compounds is readily acylated and so the cytosine residue must be blocked before proceeding with the polymerisation. The phenoxyacetyl group was found to be suitable for this purpose.[5] The synthesis was carried out therefore according to the scheme indicated in II—VII.

The conversion of 2'-deoxycytidine (II) into 3'-O-carboxymethyl-5'-O-triphenylmethyl-2'-deoxycytidine (III) has been described before.[6] The phenoxyacetyl derivative (IV) was obtained by the use of 2,4-dinitrophenyl phenoxyacetate. The next stage, which involves the removal of the triphenylmethyl group from IV without removal of the phenoxyacetyl group was critical, but was satisfactorily achieved by the use of formic acid-chloroform (1:1) at room temperature (20°) for 2 min. The resulting compound V was treated with a tenfold molar excess of dicyclohexylcarbodiimide to give the polymer VI which upon treatment with formic acid-water (1:1) and exhaustive dialysis against formic acid-water (1:1) gave the required poly (3'-O-carboxymethyl-2'-deoxycytidine) (VII).

Attempts were made to obtain a shorter synthetic route to VII. Thus 4-N-phenyoxyacetyl-5'-O-triphenylmethyl-2'-deoxycytidine was made in two stages from 2'-deoxycytidine, but attempts to convert this into the required 3'-O-carboxymethyl derivative always resulted in the removal of the phenoxyacetyl group. Because of the relatively unreactive nature of the 4-N position of the cytosine residue in aqueous solutions,[7] 3'-O-carboxymethyl-2'-deoxycytidine was prepared and attempts to polymerise it in very concentrated aqeuous solution in the presence of a water-soluble carbodiimide were made, but without success.

The polymer VII showed evidence of interaction with poly(I), in that a hypochromicity of about 20% at 250nm was obtained in 0.2M sodium acetate, pH 5.0 at a point where about 60% analogue was present (figure 1). The solution having the lowest absorbance at 250nm was heated to give the results shown in figure 2, namely an


NH2
HOCH2
OH
II
3 steps
ref. 7
Ph3COCH2
OCH2CO2H
III
PhOCH2CO
(NO2)2Ph O
PhOCH2CNH
O
IV
H+
V
DCC
CH2
CO
VI
n
VII

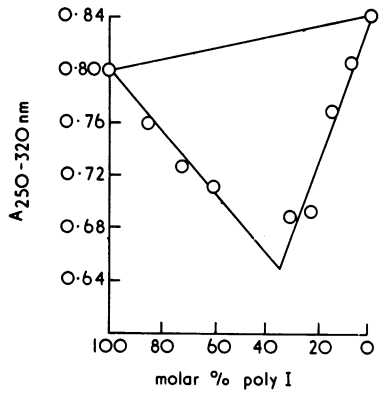


Figure 1. Ultraviolet absorption of solutions containing poly (3'-O-carboxymethyl-2'-deoxycytidine) (VII) and poly(I) in 0.2M acetate, pH 5.0. The solutions were kept at 4° for 18h and then the optical density at 250nm and 320nm determined.

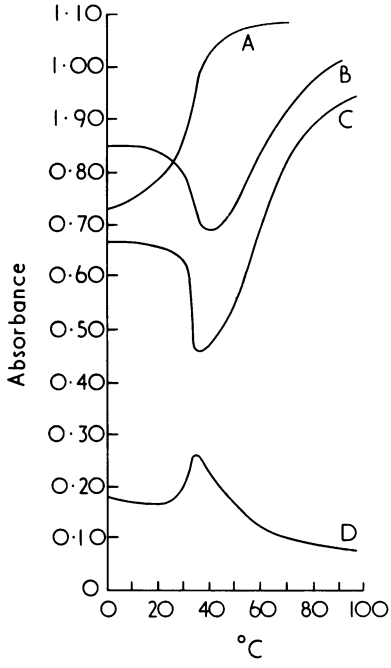


Figure 2. The effect of temperature on the U.V. absorption of a mixture of VII and poly(I). A, poly(I) at 250nm; B, VII + poly(I) at 250nm; C, VII + poly(I) at 250nm - the absorbance at 320nm; D, VII + poly(I) at 320nm.

initial decrease in absorbance at 250nm upon heating to 30° followed by a substantial increase upon further heating to give a T_m of about 60°. There was also a sharp rise in absorbance at 320nm at 30°. A possible explanation of the above phenomena is that the solutions were incompletely hybridised and that heating to 30° caused additional

hybridisation and decrease in absorbance. Further heating then caused the complex to dissociate leading to an increase in absorbance. The maximum shown at 30° at 320nm indicated that the complex was not completely soluble under these conditions and that this absorbance is due to scattering. The solution of VII also showed absorbance at 320nm. Attempts were made to obtain results at higher pH values, but they were unsuccessful because of the low solubility and instability of VII under these conditions.

The results of this and previous investigations[1-4] have shown that polynucleotide analogues containing acetate ester linkages (I) do interact with polynucleotides in solution and that, therefore, these polymers are sterically favourable for such interaction. It has also been shown in the case of the adenine-containing analogue[4,8] that it interferes with the binding of Phe-tRNA^{Phe} to ribosomes in the presence of poly(U) and in the in vitro biosynthesis of polyphenylalanine in the presence of poly (U). In addition it has been possible to synthesise chemically in a stepwise manner oligonucleotide analogues of the general structure I[2,3], the largest so far obtained having five nucleoside units (T_4C). As yet no definite biological activity has been detected in these compounds, but this has been due almost entirely to difficulty in testing them because of their low solubility and relative instability. It is hoped that by the production of analogues containing nucleoside units linked by acetamidate linkages[9] that these difficulties may be overcome.

EXPERIMENTAL

The silica gel used for t.l.c. was G/UV$_{254}$ supplied by Machery and Nagel Co. and that used for column chromatography was Kieselgel 0.05-0.2mm (70-325 mesh ASTM) (type 7734) supplied by E. Merck A. G. Darmstadt.

3'-O-Carboxymethyl-4-N-phenoxyacetyl-5'-O-triphenylmethyl-2'-deoxycytidine. To a suspension of 3'-O-carboxymethyl-5'-O-triphenylmethyl-2'-deoxycytidine (5.4g)[6] in dry dioxan (200ml) there was added 2,4-dinitrophenyl phenoxyacetate (3.7g) and the suspension stirred at 20° for 12h. Examination by t.l.c. showed that little reaction had taken place. This was still the case after the addition of more (3.0g) of 2,4-dinitrophenyl phenoxyacetate and a further 5h stirring. As it appeared that this lack of reaction may have been due to the insolubility of the starting material in dioxan, water (10ml) was added. It then appeared that a rapid reaction had taken place and after stirring for a further 3h the solution was evaporated to dryness in the presence of a small amount of silica gel. The resulting powder was applied to the top of a silica column (38cm x 3.5cm dia.). The column was eluted with acetonitrile

until the yellow dinitrophenol was removed. The required product was obtained by elution with acetonitrile-water (4:1). The fractions containing this product were combined and evaporated to an oil which was dissolved in a small volume of chloroform. Addition of a large volume of light petroleum (b.p. 40-60°) gave a precipitate which was filtered off and dried in vacuo to give the sodium salt of the required product (3.6g, 55% yield) (Found: C, 66.6; H, 5.3; N, 6.3. $C_{37}H_{34}N_3O_8Na$ required C, 66.2; H, 5.1; N, 6.25%) λ_{max} 245nm (ε, 14.6×10^3), 305nm (ε, 10.35×10^3) in chloroform-ethanol (9:1). δ 8.05 (1H,d,H-6), 7.10 (21H,m,H-5, Ph, Ph_3C), 6.10 (2H,s,OCH_2CO_2H), 4.75 (2H,s, $-COCH_2-$), 4.20 (2H,m,H-3', H-4'), 3.90 (2H,m,H-2'), 3.25 (2H,d,H-5').

3'-0-Carboxymethyl-4-N-phenoxyacetyl-2'-deoxycytidine. The foregoing compound (500mg) was dissolved in chloroform (3ml), formic acid (98% Analar, 3ml) was added with rapid mixing and after 2 min at 20° the solvents were rapidly removed in vacuo. Last traces of formic acid were removed by repeated co-evaporation with acetone. The residue was dissolved in aqueous acetone and the solution was dried onto a small quantity of silica which was applied to the top of a silica column. Elution with acetonitrite removed the triphenylmethanol and the product was then eluted with water-acetonitrile (1:4) which was evaporated to dryness to give the sodium salt of the required product as a powder (210mg, 75%) m.p. 120° (Found: C, 47.5; H, 5.2; N, 9.1. $C_{19}H_{20}N_3O_8Na\ 2\ H_2O$ requires C,47.5; H, 5.0; N, 8.8%) λ_{max} 247nm (ε, 15.4×10^3), 302nm (ε, 7.78×10^3) in water. δ 8.40 (1H,d,H-6) 7.10 (6H, H-5 and Ph), 4.50 (1H,m,H-3'), 4.10 (2H,m,H-2').

Poly (3'-0-carboxymethyl-2'-deoxycytidine). The compound obtained as above (124mg) was converted to the pyridinium salt by the use of an ion exchange resin and the compound thoroughly dried in vacuo over phosphoric anhydride. It was then dissolved in dry dimethylformamide (0.1ml) and dry pyridine (0.9ml) and dicyclohexylcarbodiimide (570mg) added. The solution was then kept at room temperature for 7 days. The resulting viscous solution was then co-evaporated with ethanol under reduced pressure until all the pyridine had been removed. Formic acid-water (1:1) (20ml) was then added to the residue and the mixture shaken at room temperature for 2h. The dicyclohexylurea which was thus formed was filtered off and the filtrate dialysed against formic acid-water (1:1) (3 x 500ml) over a period of 24h. Examination by t.l.c. of the material which remained in the dialysis bag showed only one component and this did not move from the base line when examined by t.l.c. in

acetonitrile-water (4:1). The amount of material remaining in the dialysis bag was determined from its U.V. absorption spectrum (which was similar to that of 2'-deoxycytidine) and found to correspond to a yield of 20%. Most of the formic acid was removed from the solution by extraction with ether and the aqueous solution of a sample of the polymer was diluted with 0.2M acetate buffer pH 5 to give an optical density of 0.8 at 250nm. It was then tested for hybridisation against polyinosinic acid in the usual way to give the results shown in figure 1. The effect of changing the temperature on the interaction is shown in figure 2. The poly (3'-O-carboxymethyl-2'-deoxycytidine) upon hydrolysis with alkali gave 3'-O-carboxymethyl-2'-deoxycytidine). There was a hyperchromic effect at 260nm of 5.3%.

4-N-Phenoxyacetyl-2'-deoxycytidine. A solution of 2'-deoxycytidine (1g) and 2,4-dinitrophenyl phenoxyacetate (1.4g) in dioxan (50ml) was boiled for 2h. The solution was cooled and water added until a faint suspension had been formed. Careful, dropwise, addition of this to a large volume of water gave a precipitate which was filtered off, washed with water and crystallised twice from ethanol to give the product (0.53g, 35% yield) in almost pure form. An analytically pure sample of 4-N-phenoxyacetyl-2'-deoxycytidine was obtained by column chromatography on silica gel using ethanol-chloroform (1:1) as eluant. M.p. 166-169° (Found: C, 56.7; H, 5.3; N, 11.3. $C_{17}H_{19}N_3O_6$ requires C, 56.5; H, 5.3; N, 11.6%). λ_{max} 247nm (ε, 16.65 x 10^3), 304nm (ε, 8.30 x 10^3). δ 8.15 (1H,d,H-6), 7.20 (6H,m,H-5), 4.80 (2H,s,-$COCH_2O$-), 4.25 (1H,m,H-3'), 4.10 (1H,t,H-1'), 3.85 (1H,m,H-4'), 3.60 (2H,d,H-5'), 2.05 (2H,m,H-2').

4-N-Phenoxyacetyl-5'-O-triphenylmethyl-2'-deoxycytidine. A solution of the foregoing compound (228mg) and triphenylmethyl chloride (214mg) in dry pyridine (20ml) was kept at room temperature for seven days. Examination of the reaction mixture by t.l.c. in ethanol-chloroform (2:25) showed the presence of a major component and a small amount of a slower-running component which was starting material. To obtain a pure product it was necessary to carry out two fractionations on columns of silica, the first with ethanol-chloroform (2:23) and the second with methanol-chloroform (3:47). The product was finally obtained as a white solid (81mg) by precipitation from chloroform with light petroleum. M.p. 117° (Found: C, 70.4; H, 5.6; N, 6.6. $C_{36}H_{33}N_3O_6$ 0.5 H_2O requires C, 70.5; H, 5.4; N, 6.85%) λ_{max} 246nm (ε, 16.1 x 10^3), 301nm (ε, 5.30 x 10^3). δ 8.00 (1H,d,H-6), 7.75 (20H,m,Ph_3C and Ph), 6.85 (1H,d,H-5), 6.05 (1H,t,H-1'), 4.75 (2H,s,-$COCH_2O$-), 3.95 (1H,m,H-3'),

2.25 (2H,m,H-2').

Attempts to convert this compound into its 3'-O-carboxymethyl derivative resulted in the removal of the phenoxyacetyl group.

3'-O-Carboxymethyl-2'-deoxycytidine. 3'-O-Carboxymethyl-5'-O-triphenylmethyl-2'-deoxycytidine (sodium salt, 500mg) was added to formic acid (98% Analar, 2ml). Within a few minutes triphenylmethanol was precipitated. The suspension was evaporated to dryness, last traces of formic acid being removed by repeated co-evaporation with benzene. To the residue there was added chloroform (50ml) and water (50ml) and the mixture well shaken. The aqueous layer was separated, evaporated to dryness and dissolved in pyridine-water (1:1, 5ml) and the product purified on a column of Zeokarb 225 resin (pyridinium form) (50mg) using aqueous pyridine (1:1, 200ml) as eluant. The solution obtained from the column was evaporated to dryness, the residual oil was co-evaporated several times with ethanol to remove last traces of pyridine and the residue dissolved in water. The solution was then freeze-dried and the resulting solid dried in vacuo over phosphoric anhydride at 78° to give 3'-O-carboxymethyl-2'-deoxycytidine (180mg, 70% yield) (Found: C, 46.4; H, 5.2; N, 14.3. $C_{11}H_{15}N_3O_6$ requires C, 46.3; H, 5.2; N, 14.7%) λ_{max} 278nm (ε, 8.8×10^3) in ethanol. δ 8.25 (1H,d,H-6), 7.85 (1H,d,H-5), 6.85 (2H,b,NH), 6.15 (3H,m,H-1' and $-OCH_2CO_2-$), 4.30 (1H,m,H-3'), 4.10 (2H,m,H-2' and H-4'), 3.50 (2H,d,H-5').

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REFERENCES

1 Halford, M.H. and Jones, A.S. (1968) J.Chem.Soc.(C), 2667-2670
2 Edge, M.D. and Jones, A.S. (1971) J.Chem.Soc.(C), 1933-1939
3 Edge, M.D., Hodgson, A., Jones, A.S. and Walker, R.T. (1972) J.Chem.Soc. Perkin Transactions I, 1991-1996
4 Jones, A.S., MacCoss, M. and Walker, R.T. (1973) Biochim.Biophys Acta, 294, 365-377
5 Bleaney, R.C., Unpublished results
6 Edge, M.D., Hodgson, A., Jones, A.S., MacCoss, M. and Walker, R.T. (1973) J.Chem.Soc. Perkin Transactions I, 290-294
7 Steward, D.L., Herndon, W.C. and Schell, K.R. (1972) Biochim.Biophys Acta, 262, 227-232
8 Cowling, G.J., Jones, A.S. and Walker, R.T. (1971) Biochim.Biophys Acta, 254, 452-456
9 Gait, M.J., Jones, A.S. and Walker, R.T. (1974) J.Chem.Soc. Perkin Transactions I, 1684-1686