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THE SELECTIVE ACYLATION OF THE FUNCTIONAL GROUPS OF CYTIDINE AND 2'-DEOXYCYTIDINE

R. C. BLEANEY, A. S. JONES* and R. T. WALKER
Chemistry Department, The University of Birmingham, Birmingham B15 2TT

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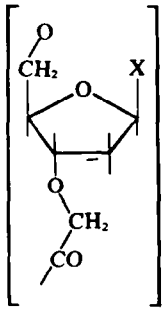
Abstract—A study has been made of the selective acylation of cytidine and 2'-deoxycytidine derivatives. It has been shown that selective 5'-O-acetylation of 2',3'-O-isopropylidenecytidine occurs in aqueous solutions. An improved procedure for the 4-N-acetylation of 2'-deoxycytidine is described. The benzyloxycarbonyl, methoxyacetyl and phenoxyacetyl groups have been used to protect the 4-N-position of cytidine derivatives. The first of these could be removed by hydrogenolysis over Pd—C but the use of Pt as a catalyst resulted in reduction of the cytosine ring. The other two groups could be removed by mild acidic hydrolysis. The 4-N-position of 2',3'-O-isopropylidinecytidine and of 2'-deoxycytidine could be selectively phenoxyacetylated by the use of 2,4-dinitrophenyl phenoxyacetate.

In the synthesis of oligonucleotides by chemical methods it is well known that cytosine residues must be protected in order to avoid the formation of N–P bonds. For this purpose Khorana and his group have used the *p*-anisoyl group which could easily be removed when required, under mildly basic conditions which did not affect the phosphodiester linkages.[1] We have synthesised polynucleotide analogues of structure 1 which contain acetate ester linkages instead of phosphodiester linkages and in which the base residue (X) was either thymine or adenine.[2,3]



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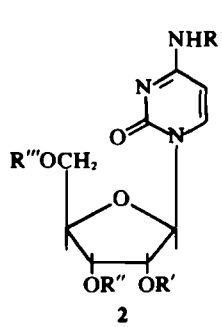
In these syntheses it was found to be unnecessary to protect either of these groups during the polymerisation. We have been synthesising a polymer of structure 1 where X = cytosine residue and this needed a blocking group for the cytosine residues, which could be removed under conditions which did not affect the acetate ester linkages. Clearly this protecting group must be more easily removed than the anisoyl group used in oligonucleotide synthesis because of the lability of the acetate ester linkage in 1. In addition to this, the synthesis of 1 has involved the use of various protecting groups for the OH groups of 2'-deoxycytidine, so a study has been made of methods of selectively acylating cytidine and 2'-deoxycytidine and some of their derivatives.

The first reaction that we considered was the selective acetylation of the exocyclic amino group of 2'-deoxycytidine. This is usually carried out by the method described by Fox *et al.* for the N-acetylation of cytidine and 2'-deoxycytidine.[4,5] The procedure involves treatment of the nucleoside in boiling ethanol solution with a large excess of acetic anhydride added in portions over 5–6 hr. Yields approaching 90% are claimed. We have used this procedure with variable results; low yields of product were often obtained and and TLC examination of the reaction mixture indicated that diacetylation was probably taking place. We found that a 90% yield of 4-N-acetyl-2'-deoxycytidine could be obtained consistently by reacting 2'-deoxycytidine with a large excess of acetic anhydride in boiling ethanol or methanol for only 1 hr. The product was used as a starting material for the synthesis of 3'-O-carboxymethyl-2'-deoxycytidine.[6]

Because of the relative ease of hydrolysis of the acetate ester linkage in 1 it seemed advisable to use as a blocking group for the 4-N position one which could be removed under very mild, non-hydrolytic conditions. An obvious choice was the benzyloxycarbonyl group which can be removed by hydrogenolysis. This group was readily introduced onto the 4-N-position of 2',3'-O-isopropylidenecytidine (*vide infra*) by the action of an excess of benzyl chloroformate. The benzyloxycarbonyl group was readily removed by hydrogenolysis over Pd-C to give the starting material as the major product. When Adam's platinum catalyst was used, however, reduction of the cytosine ring also took place. In view of this and of the known difficulty of hydrogenating polymers and the subsequent successful use of the phenoxyacetyl group to protect the 4-N-position, this work was not continued further.

An investigation was also made of the possibility of using a protecting group which could be removed from the 4-N-position without significant hydrolysis of the acetate ester linkages of 1. To test this, the 4-N-methoxyacetyl and 4-N-phenoxyacetyl derivatives of 5'-O-acetyl-2'3'-O-isopropylidenecytidine; (2, R = H; R',R'' = isopropylidene; R''' = COMe) were synthesised. This latter compound was obtained by the selective O-acetylation of 2',3'-O-isopropylidenecytidine; selective



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O-acetylation of cytidine derivatives appears to take place in aqueous solutions at pH7 and room temperature.[7] The acidic hydrolysis of 4-N-methoxyacetyl-5'-O-acetyl-2',3'-O-isopropylidenecytidine (2, R = $MeOCH_2CO$; R',R'' = isopropylidene; R''' = MeCO) and the corresponding 4-N-phenoxyacetyl derivative was followed spectrophotometrically and the results indicated that the 4-N-phenoxyacetyl derivative (2, R = $PhOCH_2CO$; R'R'' = isopropylidene R''' = MeCO) had a half life of about 35 min in formic acid-water (1:1) at 20°. This meant that the phenoxyacetyl group was suitable for our purpose.

Attempts to phenoxyacetylate the 4-N-position of 2'-deoxycytidine using phenoxyacetic anhydride under the same conditions as were used for the acetylation, were unsuccessful. The selective phenoxyacetylation of the 4-N-position of 2'-deoxycytidine by the use of 2,4-dinitrophenyl phenoxyacetate is described by us elsewhere.[6] In addition this active ester selectively phenoxyacetylated the 4N-position of 2',3'-O-isopropylidenecytidine to give 2 (R = $PhOCH_2CO$; R', R''isopropylidene; R''' = H).

EXPERIMENTAL

The silica gel used for TLC was G/uv$_{254}$ supplied by Machery & Nagel Co. and that used for column chromatography was Kieselgel 0·05–0·2 mm (70–325 mesh ASTM) (type 7734) supplied by E. Merck, A. G., Darmstadt.

4-N-Acetyl-2'-deoxycytidine. 2'-Deoxycytidine (7·4 g) was dissolved in dry, boiling EtOH (500 ml) and Ac_2O (50 ml) added over a period of 30 min. After boiling for a further 30 min the soln was cooled and concentrated to 250 ml by evaporation under reduced pressure. (Examination by TLC showed that 90% of the starting material had been converted into the required N-acetyl derivative). To the soln there was added cyclohexane (200 ml) and the evaporation continued until a white ppt separated. Further evaporations with MeOH and cyclohexane were carried out until all of the AcOH and Ac_2O had been removed. The solid recovered from the residue was characterised as pure 4-N-acetyl-2'-deoxycytidine by elemental analysis, UV spectroscopy and chromatography in comparison to an authentic sample.[5] It was obtained in yields of about 90%.

5'-O-Acetyl-2',3'-O-isopropylidenecytidine. 2',3'-O-Isopropyledinecytidine hydrochloride (1·9 g) was converted into its free base by the use of an ion exchanger and then dissolved in water containing 10% dimethylformamide (100 ml) and Ac_2O (2 ml) was added to the soln which was maintained at pH7 by the addition of NaOH from an autotitrator. Further aliquots (2 ml) of Ac_2O were added over a period of 6 hr, the soln being maintained at pH7. The soln was then extracted with butan-1-ol (3 × 80 ml). The combined butanol solns were dried over $MgSO_4$ and evaporated to dryness in the presence of a small amount of silica gel. The resulting powder was applied to a column of silica gel (30 g) and the column eluted with EtOH–$CHCl_3$ (1:4). The first fractions from the column contained a small amount of material which had a UV absorption spectrum similar to that of a 4-N-acetylated compound. The later fractions had UV absorption spectra similar to that of the starting material. Those fractions which had these spectra and which were also homogeneous by TLC were combined and evaporated to an oil. This was dissolved in a small quantity of $CHCl_3$ and the product was precipitated by the addition of an excess of light petroleum. The ppt was filtered off and dried to give 5'-O-*acetyl*-2',3'-O-*isopropylidenecytidine* (0·83 g), m.p. 65–70° (Found, C, 51·7; H, 6·1; N, 12·6. $C_{14}H_{19}N_3O_6$ requires: C, 51·7; H, 5·8; N, 12·9%), λ_{max} 243 nm (ϵ, 8·25 × 10^3), 270 nm (ϵ, 7·28 × 10^3) in EtOH. δ, $(CD_3)_2SO$, 7·65 (1H, d, H-6), 7·25 (2H, s, NH_2), 5·75 (2H, m, H-1', H-5), 5·00 – 4·80 (2H, m, H-2', H-3'), 4·20 (3H, m, H-4', H-5'), 2·05 (3H, s, MeCO), 1·50 (3H, s, isopropylidene) 1·30 (3H, s, isopropylidene).

4-N-Methoxyacetyl-5'-O-acetyl-2',3'-O-isopropylidenecytidine. Methoxyacetyl chloride (2 ml) was added to 5'-O-acetyl-2'3'-O-isopropylidenecytidine (200 mg) and the mixture gently warmed to effect soln. This soln was kept at 20° for 18 hr and then the excess of methoxyacetyl chloride was distilled off under reduced pressure at < 60°. The resulting oil was dissolved in $CHCl_3$ (20 ml) and the $CHCl_3$ soln shaken for 2 hr with a suspension of $CaCO_3$ (1 g) in water (20 ml). The $CHCl_3$ soln (which showed the presence of only one component on TLC) was dried and evaporated to a volume of about 2 ml. An excess of diethyl ether was then added and the resulting ppt collected and dried. It was a slightly impure sample (78 mg) of the required product. An analytically pure sample of the *compound* was obtained by chromotography on silica gel of a portion of this using EtOH–$CHCl_3$ (1:99) as the eluant. It had m.p. 115–118° (Found C, 51·3; H, 5·8; N, 10·4. $C_{17}H_{23}N_3O_8$ requires: C, 51·4; H, 5·8; N, 10·6%) λ_{max}249 nm (ϵ, 16·3 × 10^3), 303 nm (ϵ, 6·50 × 10^3) in EtOH. $\delta(CD_3)_2SO$, 8·10 (1H, d, H-6), 7·15 (1H, d, H-5), 5·80 (1H, d, H-1'), 5·05 – 4·85 (2H, m, H-2', H-3'), 4·15 (3H, m, H-4', H-5'), 3·90 (2H, s, $COCH_2O$), 3·35 (3H, s, MeO), 1·95 (3H, s, MeCO), 1·50 (3H, s, isopropylidene), 1·30 (3H, s, isopropylidene).

4-N-Phenoxyacetyl-5'-O-acetyl-2',3'-O-isopropylidenecytidine. This was obtained from 5'-O-acetyl-2',3'-O-isopropylidenecytidine (200 mg) and phenoxyacetylchloride (3 ml) as described above. After removal of the phenoxyacetyl chloride, the $CHCl_3$ soln was found by TLC to contain only one component. This was obtained in pure form by precipitation with diethyl ether to give the *required compound* as a white powder (29 mg), m.p. 94–98° (Found: C, 57·6; H, 5·7; N, 9·2. $C_{22}H_{25}N_3O_8$ requires: C. 57·5; H, 5·45; N, 9·15%), λ_{max}249 nm (ϵ, 18·3 × 10^3), 303 nm (ϵ, 7·1 × 10^3) in EtOH. $\delta(CD_3)_2SO$, 8·15 (1-H, d, H-6), 7·20 (6H, m, H-5 and Ph), 5·85 (1H, d, H-1'), 5·05 – 4·80 (4H, m, H-2', H-3', $COCH_2O$), 4·25 (3H, m, H-4', H-5'), 1·95 (3H, s, MeCO), 1·50 (3H, s, isopropylidene), 1·30 (3H, s, isopropylidene).

4-N-Benzyloxycarbonyl-5'-O-acetyl-2',3'-O-isopropylidenecytidine. Benzyl chloroformate (2 ml) was added to 5'-O-acetyl-2',3'-O-isopropylidenecytidine (200 mg) and the mixture kept at 20° for 18 hr. The excess benzyl chloroformate was removed by distillation under reduced pressure and the residue fractionated on a column of silica gel (20 g) using EtOH–$CHCl_3$ (1:49) as eluant. The fractions containing the required product in pure form were combined and evaporated to dryness. The residual oil was dissolved in a minimum of chloroform and an excess of light petroleum added. The resulting ppt was filtered off and dried to give the *required product* (100 mg), m.p. 50–60°. (Found: C, 57·6; H, 5·8; N, 8·9. $C_{22}H_{23}N_3O_8$ requires: C, 57·2; H, 5·5; N, 9·15%) λ_{max}246 nm (ϵ, 16·4 × 10^3), 296 nm (ϵ, 7·00 × 10^3) $\delta(CD_3)_2SO$, 8·00 (1H, d, H-6), 7·40 (5H, m, Ph), 7·05 (1H, d, H-5), 5·80 (1H, d, H-1'), 5·20 (2H, s, CH_2O), 5·05 – 4·30 (2H, m, H-2', H-3'), 4·25 (3H, m, H-4', H-5'), 1·95 (3H, s, MeCO), 1·50 (3H, s, isopropylidene), 1·30 (3H, s, isopropylidene).

Controlled hydrogenation of the above compound in MeOH with Pd/C gave 5'-O-acetyl-2',3'-O-isopropylidene as the only product; no reduction of the cytosine ring occurred. When Adam's platinum catalyst was used, however, no UV absorbing material remained at the end of the reaction thus showing that the cytosine ring had been reduced.

Rates of hydrolysis of 4-N-methoxyacetyl- and 4-N-phenoxyacetyl derivatives of 5'-O-acetyl-2',3'-O-isopropylidenecytidine. These were determined in various concentrations of aqueous formed acid at room temp., the progress of the reactions being determined spectrophotometrically at 310 and 312 nm respectively. The results were as listed below.

% Formic acid in water	Half life (min) of	
	4-N-$MeOCH_2CO$ der.	4-N-$PhOCH_2CO$ der.
10	95	45
20	73	25
30	70	20
40	77	20
50	90	35
60	122	—
70	236	100

4-N-*Phenoxyacetyl*-2',3'-O-*isopropylidenecytidine*. 2',3'-O-Isopropylidenecytidine (549 mg) was added to a soln of 2,4-dinitrophenyl phenoxyacetate (396 mg) in dioxan (40 ml). The mixture was stirred at 20° for 18 hr and then concentrated to dryness under reduced pressure to give a red oil. This was crystallised and recrystallised from EtOH to give white crystals (125 mg) of the *required product*, m.p. 176–177°. (Found: C, 57·7; H, 5·5; N, 10·1. $C_{20}H_{23}N_3O_7$ requires: C, 57·5; H, 5·5; N, 10·05%) λ_{max}248 nm (ϵ, $16{\cdot}3 \times 10^3$), 303 nm (ϵ, $7{\cdot}32 \times 10^3$) in EtOH. $\delta(CD_3)_2SO$, 8·25 (1H, d, H-6), 7·10 (6H, m, H-5 and Ph), 5·85 (1H, D, H-1'), 5·00–4·80 (4H, m, H-2', H-3', $COCH_2O$), 4·20 (1H, m, H-4'), 3·25 (2H, d, H-5'), 1·50 (3H, s, isopropylidene), 1·30 (3H, s, isopropylidene). A further quantity (237 mg) of a slightly yellow sample of the above product was obtained from the crystallisation liquors to give a total yield of 45%.

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