A Synthesis of Pseudouridine

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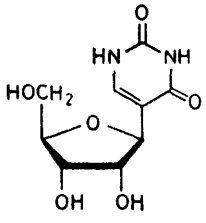
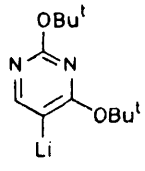
2,3,5-Tri-*O*-benzylribose and 2,4-di-t-butoxy-5-lithiopyrimidine (from the 5-bromo-compound and butyl-lithium) in THF at −78 °C give 5-(2,3,5-tri-*O*-benzyl-D-*altro*-pentahydroxypentyl)-2,4-di-t-butoxypyrimidine and its D-*allo*-epimer (ratio 5 : 2), which cyclise stereospecifically in ethanolic hydrochloric acid to 5-(2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl)uracil and its α-anomer. Debenzylation by boron trichloride gives 5-β-D-ribofuranosyl-uracil, pseudouridine, and its α-anomer. 2,3,5-Tri-*O*-methylribose reacts analogously. 2,3-*O*-Isopropylidene-ribose and its 5-*O*-trityl derivative give no polyol, but instead 2,4-di-t-butoxy-5-(2-hydroxyisopropyl)pyrimidine.

A NUMBER of syntheses of pseudouridine (5-β-D-ribofuranosyluracil) (1) have been described (for a review see ref. 1). In our early synthesis the 5-lithiopyrimidine (2) was coupled with the aldehydo-sugar 2,4-3,5-di-*O*-benzylideneribose to give a protected polyol. The protecting groups were removed and the polyol cyclised by acid in one step to give α- and β-pseudouridine.[2] The route was later made more efficient.[3] Subsequently, other workers have shown that protected hemiacetal sugars may conveniently be brought into reaction with Grignard reagents, leading to the corresponding 1-substituted polyols. Thus Buchanan *et al.*[4] and Moffatt *et al.*[5] have successfully employed 2,3,5-tri-*O*-benzylribose (3) in *C*-nucleoside synthesis. It was of some



(1)



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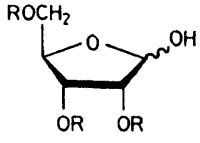


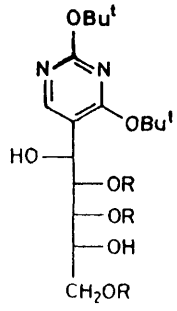
(3) a; R = CH_2Ph
b; R = Me

interest to know if such intermediates, which are more readily available than the aldehydo-sugar derivatives, would prove useful in reactions with more basic organometallic reagents such as (2).

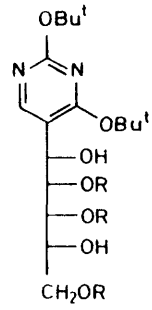
RESULTS AND DISCUSSION

In the present experiments[6] it was found that the reaction between (2) and tribenzylribose (3a) gave, in high yield, a mixture of the two protected polyols (4a) and (5a), epimeric at C-1′. It was not possible to separate these preparatively, but analysis by h.p.l.c. showed that the two isomers were formed in the ratio 5 : 2. It became clear that the major isomer had the D-*altro*-configuration (4a) for the following reasons. In the first place the preferential formation of the D-*altro*- over the D-*allo*-epimer in the reaction between tribenzylribose (3a) and ethynylmagnesium bromide has been noted and rationalised[4,5] by reference to the cyclic model of Cram *et al.* and Karabatsos *et al.*[7] More importantly, we found that the epimeric mixture, when treated with ethanolic hydrochloric acid, gave directly and in high yield a mixture of β- and α-5-(2,3,5-tri-*O*-benzylribosyl)uracil, (6a) and (7a), also in the ratio 5 : 2. The structure of these followed from their conversion to pseudouridine (1) and its α-anomer (7c).

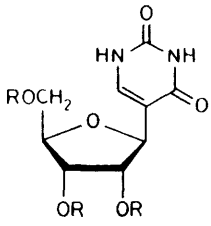
Although debenzylation could not be effected by



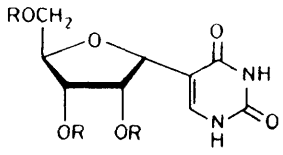
(4) a; R = CH_2Ph
b; R = Me



(5) a; R = CH_2Ph
b; R = Me



(6) a, R = CH_2Ph
b; R = Me



(7) a, R = CH_2Ph
b, R = Me
c, R = H

catalytic hydrogenolysis, since 5-ribityluracil was the only product formed,[8] boron trichloride[9,10] at −78 °C in dichloromethane was very effective, giving no evidence of isomerisation. No pyranose isomers were formed. The anomeric pseudouridines were formed quantitatively, the β-anomer being the major one. Both products were isolated in a pure state, and shown to be identical to those previously described.[2]

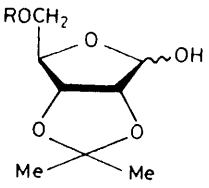
The above evidence shows that the acid-catalysed cyclisation is stereospecific. Since this must therefore

involve an S_N2-type process and the predominant nucleoside has the β-configuration, it follows that the polyol giving rise to it must have the D-*altro*-configuration (4). High stereospecificity has already been observed in the cyclisation of the unprotected polyols corresponding to (4) and (5).[3]

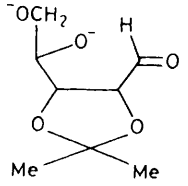
When the mixed protected polyols, (4a) and (5a), were directly treated at low temperature with boron trichloride in dichloromethane, ion-exchange analysis of the products after completion of the reaction showed approximately equal amounts of α- and β-pseudouridine and virtual absence of pyranose isomers. Thus cyclisation and deprotection can be effected in one step but the proportion of the desired β-isomer is reduced.

In view of the ready debenzylation of (4a) and (5a) with boron trichloride the above synthetic scheme was applied to 2,3,5-tri-*O*-methylribose (3b). The reaction with (2) gave the protected polyols (4b) and (5b) in high yield and in the ratio 5 : 1. This favourable ratio was maintained on acid-catalysed cyclisation to (6b) and (7b). Partial fractionation of these could be effected by silicic acid chromatography. Boron trichloride demethylation was very slow. Boron tribromide,[11] though faster, required several days to remove all the methyl groups; it also led to some isomerisation. Boron trichloride with 3 equiv. tetra-n-butylammonium iodide reacted faster, as observed in the 2′-deoxypseudouridine-series,[12] but again led to isomerisation.

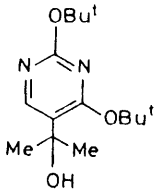
2,3-*O*-Isopropylidene-D-ribose (8a) and its 5-*O*-trityl-



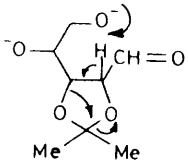
(8) a; R = H
b; R = CPh_3



(9)



(10)



(11)

derivative (8b) have been widely used as precursors for *C*-nucleoside synthesis.[1,13] Reaction with ethynylmagnesium bromide led almost exclusively to compounds with the D-*allo*-configuration in this [14] and in the mannose series, but in the latter the ethyl-Grignard gave mainly the D-*altro* product.[15] Because of this it seemed of interest to submit these derivatives to reaction with the lithiopyrimidine (1). In the reaction with (8a), 3 mol of (1) were used on the assumption that the dianion (9) would first be generated, and that this would then be attacked by the carbanion. In the event no polyol derivative was formed under these, or other conditions. Instead, a product was obtained which was shown by high-resolution mass spectrometry and by its ^1H n.m.r. spectrum to be (10). This must come from acetone generated in the reaction, and indeed (10) is formed when acetone is used as the sole carbonyl component. In explanation it is suggested that base-catalysed elimination of acetone from the dianion occurs as in (11) or by an equivalent mechanism in which the C(3)-oxide is involved. The corresponding reaction with the 5-*O*-trityl derivative (8b) was more sluggish; again only (9) was formed but in reduced yield. In view of these results it is of some interest that 3,4-*O*-isopropylidene-2-deoxyribose reacts normally with the lithiopyrimidine, giving epimeric protected polyols which can be cyclised, to α- and β-2′-deoxypseudouridine.[6]

EXPERIMENTAL

N.m.r. spectra were obtained on Varian HA-100D and XL-100 spectrometers at 100 MHz. Mass spectra were measured using AEI MS30 and MS902 (for high resolution) spectrometers. Chromatography was carried out using silica gel (Merck; 70—230 mesh ASTM unless otherwise stated) and neutral alumina (Woelm). For t.l.c. Kieselgel 60.F.254 (Merck) and Cellulose F (Merck) were used. When t-butyl ethers were chromatographed, triethylamine (0.1%) was added to all solvents.

5-(2,3,5-*Tri*-O-*benzyl*-D-altro-*pentahydroxypentyl*)-2,4-*di*-t-*butoxypyrimidine* (4a) *and its* D-allo-*Epimer* (5a).—A solution of 5-lithio-2,4-di-t-butoxypyrimidine [from the 5-bromopyrimidine (8.06 g, 24.2 mmol) and n-butyl-lithium (24.5 mmol)] in dry oxygen-free tetrahydrofuran (250 ml) at −78 °C was stirred for 25 min, and then added during 10 min to a solution of 2,3,5-tri-*O*-benzylribose [16] (4.64 g, 11.0 mmol) in tetrahydrofuran (150 ml) at −78 °C. After 4 h at −78 °C the solution was allowed to warm to room temperature overnight. Solvent was removed under reduced pressure and the residual syrup partitioned between chloroform and water. Evaporation of the dried chloroform layer gave a syrup (9.53 g) containing no starting sugar (t.l.c.). The syrup (2.4 g) was chromatographed on silica (150 g, Mallinkrodt cc-7). After elution with dichloromethane–chloroform (1 : 1 v/v) (200 ml), chloroform (300 ml) and then chloroform–methanol (99 : 1) eluted the mixed epimers. Removal of solvent gave a syrup (1.13 g, 64%). It showed one spot on t.l.c. and two peaks (ratio 5 : 2) on h.p.l.c. [30-cm Bondapak C-18 column, acetonitrile–water (4 : 6)]; δ (100 MHz, $CDCl_3$) 1.52, 1.58 (s, 9 H, Bu^tO), 3.4—5.2 (complex), 7.28 (m, 15 H, Ph), 8.26 (s, 1 H, C-6-H-*altro*), and 8.38 (s, C-6-H-*allo*); *m/e* 664 (M^+, weak) and 423.

5-(2,3,5-*Tri*-O-*methyl*-D-altro-*pentahydroxypentyl*)-2,4-*di*-t-*butoxypyrimidine* (4b) *and its* D-allo-*Epimer* (5b).—These were prepared in the same way as the tribenzyl ethers, above, from 5-bromo-2,4-di-t-butoxypyrimidine (4.17 g, 13.75 mmol) and 2,3,5-tri-*O*-methylribose [17] (1.2 g, 6.25 mmol). On chromatography on silica (200 g), the *product* was eluted with chloroform–methanol (95 : 5) (400 ml) and formed a syrup (1.73 g, 68%). It showed one spot on t.l.c. and two compounds (ratio 5 : 1) on h.p.l.c. [25-cm CN column, propan-2-ol–heptane (1 : 9)]; δ (100 MHz, $CDCl_3$) 1.60 and 1.65 (s, 9 H, t-BuO), 3.1—4.1 (complex), 5.04 (m,

1 H, C-1′-H), and 8.28 and 8.38 (s, 1 H, C-6-H-*altro* and -*allo*, respectively); *m/e* 416 (M^+) and 383 (M^+ − MeO) (Found: M^+, 416.253 2. $C_{20}H_{36}N_2O_7$ requires M, 416.252 2).

5-(2,3,5-*Tri*-O-*benzyl*-β-D-*ribofuranosyl*)*uracil and its* α-*Anomer*.—The above tribenzyl ether (250 mg, mixed epimers) in methanol (4.5 ml) and concentrated hydrochloric acid (0.5 ml) was kept at room temperature for 24 h. Evaporation of solvent *in vacuo* then repeated evaporation with methanol gave the *product* as a syrup (180 mg, 90%). It had R_F 0.43 [chloroform–methanol (9 : 1)] and consisted of two compounds (α- and β-anomers; ratio 2 : 5) on h.p.l.c. [30-cm Bondapak C-18 column, acetonitrile–water (6 : 4)]; δ (100 MHz, $CDCl_3$) 3.5—5.2 (complex, PhCH_2 and sugar protons) and 7.28 (m, 15 H, Ph); *m/e* 514 (M^+) and 423 (M − C_7H_7) (Found: M^+, 514.209 5. $C_{30}H_{30}N_2O_6$ requires M, 514.210 3).

When the tribenzyl ether was hydrogenolysed in methanol over 5% palladium–charcoal at room temperature, t.l.c. showed a single product, R_F, 0.32 on cellulose in butanol–acetic acid–water (5 : 2 : 3). It was characterised as 5-ribityluracil by ion-exchange analysis, according to the method of Cohn,[18] by its elution position.

5-(2,3,5-*Tri*-O-*methyl*-β-D-*ribofuranosyl*)*uracil* (6b) *and its* α-*Anomer* (7b).—The mixed epimers of the trimethyl ether [(4b) and (5b)] (312 mg) were treated with ethanolic hydrochloric acid as for the benzyl ether [(4a) and (5a)] and gave the *product* as a syrup (179 mg, 83%). H.p.l.c. [50-cm Partisil column, propan-2-ol–heptane (1 : 9)] showed two compounds in the ratio 5 : 1.

Partial separation of the two anomers on silica gel chromatography with chloroform–methanol (95 : 5) gave the β-anomer containing *ca.* 10% of the α-anomer (h.p.l.c.), R_F 0.31; δ (100 MHz, [$^2H_6$]DMSO) 3.0—5.0 (PhCH_2, sugar and Me protons) and 8.25 (s, 1 H, C-6-H); *m/e* 285 (M^+ − H) and 243 (M^+ − HCNO).

5-β-D-*Ribofuranosyluracil* (1) *and its* α-*Anomer* (7c).—(*a*) The above tribenzyl ether [(6a) and (7a)] (120 mg) in dry dichloromethane (5 ml) was cooled to −78 °C, boron trichloride (5 ml) in dichloromethane (25 ml) at −78 °C added, and the solution kept at −78 °C overnight. After warming to −50 °C, dichloromethane–methanol (1 : 1) (50 ml), cooled to the same temperature was added. Evaporation to dryness *in vacuo* and co-evaporation with methanol to remove all the hydrogen chloride gave a product shown by ion exchange analysis to contain β- and α-pseudouridine in a ratio of about 5 : 2.

The anomers were separated by ion-exchange chromatography essentially as described earlier.[2,18] 5-β-D-Ribofuranosyluracil (23 mg, 42%) crystallised from ethanol, m.p. 223—224 °C. It was identical (t.l.c., n.m.r., u.v., m.s., mixed m.p.) with a sample of the natural product (Found: C, 43.7; H, 5.0; N, 11.4. Calc. for $C_9H_{12}O_6N_2$: C, 44.1; H, 4.9; N, 11.5%).

(*b*) The tribenzyl ether of the polyol [(4a) and (5a)] (25 mg) in dichloromethane (1 ml) was treated at −78 °C with boron trichloride (0.5 ml) in dichloromethane (2 ml). After 18 h it was worked up as above. Ion-exchange analysis showed the presence of α- and β-pseudouridine in a ratio of 1 : 1.

(*c*) To a solution of the trimethyl ether [(6b) and (7b)] (100 mg, enhanced β-fraction) in dichloromethane (4.5 ml) was added boron tribromide (3.5 ml) in dichloromethane (13 ml) at −78 °C. After 6 days at this temperature boron trichloride and solvent were removed *in vacuo* and the residue was then treated at −78 °C with dichloromethane–methanol. The product was worked up by ion-exchange chromatography to yield β-pseudouridine (43 mg, 53%). The α-anomer was present but not isolated.

The Reaction of 5-*Lithio*-2,4-*di*-t-*butoxypyrimidine and* 2,3-O-*Isopropylideneribose*.—The reaction between the lithiopyrimidine [from the 5-bromo-compound (5 g, 16.5 mmol)] and isopropylideneribose (0.96 g, 5 mmol) was carried out as in the reactions described above and the product chromatographed on silica. Chloroform–methanol (9 : 1) eluted 2,4-*di*-t-*butoxy*-5-(2-*hydroxyisopropyl*)*pyrimidine* as a syrup (165 mg), R_F 0.40 [chloroform–methanol (95 : 5)]; δ (100 MHz, CD_3OD) 1.54 (s, 6 H, Me), 1.60 and 1.68 (s, 9 H, t-BuO), and 8.34 (s, 1 H, C-6-H); *m/e* 282 (M^+), 267 (weak, M^+ − Me), 265 (weak, M^+ − OH), 240, 155, and 141 (Found: M, 282.194 7; $C_{15}H_{26}O_3N_2$ requires 282.194 2).

The same product was obtained in corresponding reactions with 5-*O*-trityl-2,3-*O*-isopropylideneribose [19] (we thank Mr. D. K. Donald for a sample) and with acetone.

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