New Methodology for *C*-Nucleoside Synthesis: Preparation of 1,2-dideoxy-1-(3-pyridyl)-D-ribofuranose

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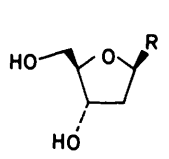
An eleven-step synthesis of 1,2-dideoxy-1-(3-pyridyl)-D-ribofuranose (**3**) from 2-deoxyribopyranose (**4**) is described. This procedure includes methods for selective protection/deprotection and cyclisation which should be applicable to the synthesis of other 1-substituted 1,2-dideoxyriboses. The key steps were reaction of the selectively protected D-ribose (**9**) with 3-lithiopyridine, and subsequent ring closure of the resultant 1-(3-pyridyl)ribitols (**10**) using methanesulphonyl chloride. Dideoxyriboses are of interest as nucleoside analogues for incorporation into synthetic DNA duplexes for the investigation of factors affecting DNA helix stability, especially since they are likely to cause changes in base stacking interactions.

We are attempting to design nucleoside analogues for incorporation into synthetic DNA strands, such that after hybridisation with complementary strands, the stability of the DNA duplexes can be assessed, and factors affecting helix stability may be identified. To this end we have already prepared[1] 1,2-dideoxy-D-ribofuranose (**1**) and 1,2-dideoxy-1-phenyl-β-D-ribofuranose (**2**). Both of these nucleoside analogues produced destabilisation of DNA duplexes, and we reasoned that this could be due in part to the lack of H-bonding potential. In addition, refined quantum mechanical studies[2] have shown that the phenyl analogue (**2**) cannot participate in base stacking. Substitution of a heterocyclic ring for the phenyl ring should overcome the H-bonding deficiency, and since the rings would possess a dipole moment, base stacking should be more favoured. The simple pyridine analogue (**3**) was an obvious initial target for synthesis.

Two main approaches have been used for the construction of *C*-nucleosides:[3] (*a*) nucleophilic displacement of a halogeno group from C-1 of ribose by a complete heteroaryl moiety or by a suitably functionalised precursor; and (*b*) reaction of a *C*-nucleophile (Wittig reagent, Grignard reagent, malonate anion or equivalent) at C-1, with spontaneous or chemically mediated ring closure to reform a furanose ring. Our approach falls into the latter category.

The starting material for the synthesis was 2-deoxyribopyranose (**4**) and this was first converted into the methyl 2-deoxy-D-ribofuranoside (**5**)[4] (MeOH, HCl) prior to protection of the C-3 and C-5 hydroxy groups [*p*-toluoyl chloride, pyridine, 70% overall from (**4**)].[5] This ditoluoylriboside (**6**) was treated with ethanethiol in the presence of zinc bromide to produce the thioacetal (**7**) (76% yield). It was now necessary to differentiate between the C-3/C-4 and C-5 hydroxy groups, and this was achieved *via* removal of the toluoyl groups (NaOMe, MeOH, 95%), and reaction with dimethoxytrityl chloride to yield the thioacetal (**8**) (77% yield overall). The C-3 and C-4 hydroxy groups could now be protected by reaction with 4-methoxybenzyl chloride (NaH, DMF, 90%), and the thioacetal grouping was then removed (I_2, $NaHCO_3$, 80%) to provide the aldehyde (**9**).

This key intermediate has appropriate functionality for the introduction of the 1-substituent, and for subsequent cyclisation. Thus reaction with 3-lithiopyridine (3-bromopyridine, BuLi, −78 °C)[5,6] yielded the alcohols (**10**) in variable yield (28—50%), which upon reaction with methanesulphonyl chloride (pyridine, 0 °C) provided the methanesulphonates. Cyclisation with concomitant deprotection at C-4 produced a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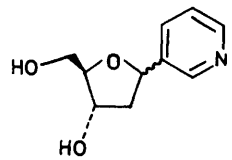
(2) R = Ph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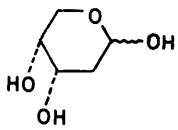
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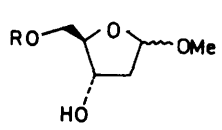
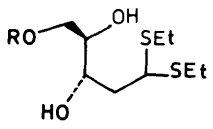
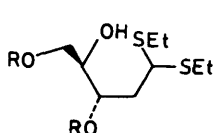
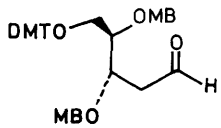

(5) R = H
(6) R = MeC_6H_4CO-


(8) R = dimethoxytrityl


(7) R = MeC_6H_4CO-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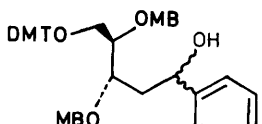
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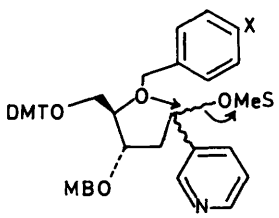
DMT = dimethoxytrityl
MB = 4-methoxybenzyl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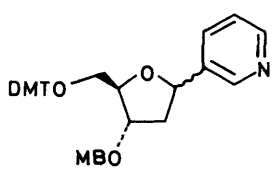
(11)

mixture of 1-(3-pyridyl)-2-deoxyribofuranose derivatives (**11**) (70%). This cyclisation is similar to one reported by Buchanan,[7] who proposed the intermediacy of a species such as (**12**; X = H). A 4-methoxy group would be expected to stabilise such an intermediate (**12**; X = OMe), and a similar mechanism seems likely.

Selective removal of the dimethoxytrityl group was effected with acetic acid (80% yield), and the C-3-protected α- and β-anomers could now be easily separated by flash chromatography. Finally, treatment of each anomer with trityl tetrafluoroborate,[8] then water, provided samples of the α- and β-anomers of 1,2-dideoxy-1-(3-pyridyl)-D-ribofuranoses (**3α**) and (**3β**).

The α-anomer crystallised from ethyl acetate, and an *X*-ray crystal structure will be reported elsewhere. The β-anomer has thus far resisted all efforts at crystallisation, but exhibited in its ^{1}H n.m.r. spectrum a clear double doublet for 1-H at 5.2 p.p.m. (J = 6 and 10 Hz) consistent with the proposed structure.

The two compounds were evaluated for anti-tumour activity using mouse leukaemia L1210 cells, mouse carcinoma FM3A cells, and human lymphoblast Raji cells, but exhibited no activity. T_m (the temperature in °C at which statistically 50% of DNA molecules have been converted from the native form into the denatured form) studies have not yet been completed. The synthetic methodology described should allow access to other heteroaryl analogues.

Experimental

^{1}H N.m.r. spectra were measured at 60 MHz with a Perkin-Elmer R24B spectrometer, at 220 MHz with a Perkin-Elmer R24 spectrometer and at 400 MHz with a Bruker B. WH400 spectrometer (S.E.R.C. Warwick) using TMS as internal reference. T.l.c. was carried out on Merck plates pre-coated with silica gel $60F_{254}$. Mass spectral data were supplied by M-Scan Limited, Ascot. All solvents and reagents were purified in accordance with general laboratory procedures. M.p.s (uncorrected) were determined on a Baird and Tatlock electrothermal m.p. apparatus. Elemental analysis was carried out by Butterworth's Laboratories, Middlesex.

1,2-*Dideoxy*-3,5-*di*-O-p-*toluoyl*-D-*ribose Diethyl Dithioacetal* (**7**).—The ditoluoylribofuranoside[4] (**6**) (15.6 g, 41 mmol) was dissolved in dry dichloromethane (30 ml) at room temperature and ethanethiol (10.2 g, 164 mmol) was added. After 3 min anhydrous zinc bromide (9.2 g, 41 mmol) was added and the reaction mixture stirred for a further period of 30 min, then poured into aqueous sodium hydroxide (5% w/v, 200 ml) and extracted with dichloromethane (4 × 100 ml). The separate organic extracts were washed with brine, combined, dried (Na_2SO_4), and concentrated under reduced pressure. The residual oil was chromatographed on silica gel, and elution with hexane–diethyl ether (7:3)→(6:4) gave the title compound (**7**) as a clear oil (14.6 g, 76%), R_F 0.24 (hexane–diethyl ether 1:1); δ_H(60 MHz; $CDCl_3$) 1.18 (3 H, t, J 7 Hz), 1.20 (3 H, t, J 7 Hz), 2.40 (6 H, s), 2.55 (6 H, m), 3.10 (1 H, m), 4.00 (1 H, dd, J 6 and 10 Hz), 4.40 (3 H, m), 5.60 (1 H, m), 7.22 (4 H, d, J 8 Hz), and 7.92 (4 H, d, J 8 Hz); m/z 476 (M^+, 5%), 119 (100), and 91 (45) (Found: M^+, 476.1689. $C_{25}H_{32}O_5S_2$ requires M, 476.1691).

1,2-*Dideoxy*-5-O-(4,4′-*dimethoxytrityl*)-D-*ribose Diethyl Dithioacetal* (**8**).—The thioacetal (**7**) (12.0 g, 25 mmol) was dissolved in dry methanol (30 ml) and sodium methoxide (1M; 15 ml) was added. After 3 h at room temperature the reaction mixture was neutralised using Dowex-50 (pyridinium form) ion exchange resin. The resin was filtered, washed with methanol, and the combined filtrates were concentrated under reduced pressure. Chromatography on silica gel with dichloromethane–methanol (95:5) gave a clear oil (5.65 g, 93%). This oil (4.82 g, 20 mmol) was dissolved in anhydrous pyridine (300 ml), cooled in an ice-bath and a solution of 4,4′-dimethoxytrityl chloride (7.45 g, 22 mmol) in anhydrous pyridine (100 ml) slowly added. After 1 h the reaction mixture was poured into methanol (100 ml) and concentrated under reduced pressure. The residue was dissolved in dichloromethane (100 ml) and extracted with saturated sodium hydrogen carbonate (200 ml). The aqueous layer was back-extracted with dichloromethane (4 × 50 ml), the combined organic layers dried (Na_2SO_4), and evaporated under reduced pressure. Chromatography on silica gel and elution with hexane–diethyl ether–triethylamine (50:49:1) gave the title compound (**8**) as an oily foam (8.8 g, 81%), R_F 0.8 (dichloromethane–methanol 9:1) and 0.28 (diethyl ether–hexane 8:2); δ_H(220 MHz; $CDCl_3$) 1.26 (6 H, m), 1.74 (1 H, br s), 1.86 (1 H, m), 2.02 (1 H, m), 2.68 (5 H, m), 3.32 (1 H, m), 3.64 (1 H, m), 3.78 (1 H, m), 3.82 (6 H, s), 4.06 (2 H, m), 6.84 (4 H, d, J 8 Hz), 7.18 (2 H, m), 7.28 (6 H, m), and 7.43 (1 H, m); m/z [240 (M + H$^+$) − $C_{21}H_{19}O_2$, 5%], 303 (70), and 121 (100) (Found: M + H$^+$ − $C_{21}H_{19}O_2$ 240.0850. $C_9H_{20}O_3S_2$ requires 240.0854).

2-*Deoxy*-3,4-*bis*-O-(4-*methoxybenzyl*)-5-O-(4,4′-*dimethoxytrityl*)-D-*ribose* (**9**).—The diol (**8**) (10.0 g, 18.5 mmol) dissolved in dry tetrahydrofuran (THF) (15 ml) was added dropwise to a suspension of sodium hydride (60% dispersion, 1.8 g, 44.4 mmol) in dry dimethylformamide (10 ml) at 0 °C. The mixture was stirred under nitrogen for 1 h, after which a solution of 4-methoxybenzyl chloride (7.21 g, 44.4 mmol) in dry THF was added. The reaction mixture was stirred for a further 3 h at room temperature, quenched by slowly pouring into ice-cold saturated sodium hydrogen carbonate (100 ml), and extracted with dichloromethane (4 × 50 ml). The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure. The residual oil was fractionated by short column chromatography on silica gel: the appropriate fractions, eluted with dichloromethane, were combined and evaporated under reduced pressure to give the fully protected dithioacetal (13.0 g, 90%) as a clear oil. The oil (13.0 g, 16.6 mmol) was dissolved in a mixture of acetone (200 ml) and water (33 ml), and sodium hydrogen carbonate (3.1 g, 36.5 mmol) was added. The mixture was cooled in an ice-bath and iodine (9.28 g, 36.5 mmol) added portionwise over 10 min. The reaction mixture was allowed to warm to room temperature and after 1.5 h, a solution of saturated sodium thiosulphate (200 ml) was added and the mixture stirred for a further 5 min. After partial evaporation under reduced pressure, the reaction mixture was poured into saturated sodium hydrogen carbonate (200 ml) and extracted with dichloromethane (4 × 50 ml). The combined organic layers were dried (Na_2SO_4) and evaporated under reduced pressure to give a yellow oil which was fractionated by column chromatography on silica gel with hexane–diethyl ether (1:1) as eluant yielding the title compound (**9**) as a white oily foam (8.25 g, 74%), R_F 0.29 (diethyl ether–hexane 8:2); δ_H(220 MHz; $CDCl_3$) 2.58 (2 H, m), 3.28 (2 H, m), 3.64 (1 H, m), 3.78 (12 H, m), 4.15 (1 H, m), 4.34—4.80 (4 H, m), 6.70—6.90 (8 H, m), 7.00—7.50 (13 H, m), and 9.64 (1 H, t, J 2 Hz); m/z 304 (55%) and 121 (100).

2-*Deoxy*-3,4-*bis*-O-(4-*methoxybenzyl*)-5-O-(4,4′-*dimethoxytrityl*)-1-(3-*pyridyl*)-D-altro- *and* D-allo-*ribitol* (**10**).—Anhydrous diethyl ether (200 ml) was cooled to −78 °C (CO_2–acetone) and stirred under an atmosphere of dry nitrogen. A solution of butyl-lithium [9.5M in hexane (1.86 g, 29.6 mmol)] in dry diethyl ether (10 ml) was slowly added and the pale yellow solution stirred for 10 min at −78 °C. An ethereal solution of 3-bromopyridine (4.7 g, 29.6 mmol) was added dropwise and the pale yellow suspension stirred for a further 10 min at −78 °C. A

solution of the aldehyde (**9**) (5.0 g, 17.4 mmol) in dry diethyl ether (10 ml) was added over 5 min and the reaction mixture allowed to warm to room temperature. After a further 1 h, the brown suspension was poured into aqueous sodium hydrogen carbonate (5% w/v, 100 ml) and extracted with dichloromethane (4 × 50 ml). The organic layers were combined, dried (Na_2SO_4), and concentrated under reduced pressure to give a brown foam. The residue was chromatographed on silica gel and elution with diethyl ether–ethanol (97:3) afforded the title alcohols (**10**) (1.5 g, 27%) as a white foam, R_F 0.16 (diethyl ether–ethanol 8:2); δ_H (400 MHz; $CDCl_3$) 1.87 (2 H, m), 3.30 (2 H, m), 3.78 (14 H, m), 3.95 (1 H, m), 4.30—4.75 (4 H, m), 4.86 (1 H, m), 6.84 (8 H, m), 7.10—7.65 (15 H, m), and 8.48 (2 H, m); *m/z* 452 ($M^+ - C_{21}H_{19}O_2$, 5%), 304 (100), 303 (75), 273 (37), and 121 (100) (Found: $M^+ - C_{21}H_{19}O_2$ 452.2079. $C_{26}H_{30}NO_6$ requires 452.2080).

1,2-*Dideoxy*-5-O-(4,4′-*dimethoxytrityl*)-3-O-(4-*methoxybenzyl*)-1-(3-*pyridyl*)-α-*and*β-D-*ribofuranose* (**11**).—The mixture of alcohols (**10**) (2.01 g, 2.66 mmol) was dissolved in anhydrous pyridine (300 ml) and di-isopropylethylamine (0.77 g, 6 mmol) was added. The solution was cooled in an ice-bath and a solution of methanesulphonyl chloride (0.68 g, 6 mmol) in dry pyridine (10 ml) was slowly added. The reaction mixture was stirred at 0 °C for a further 10 min and then stirred at room temperature for 1 h. The resulting brown solution was poured into aqueous sodium hydrogen carbonate (5% w/v, 50 ml) and extracted with dichloromethane (4 × 50 ml). Evaporation of the solvent under reduced pressure gave a brown foam which was chromatographed on silica gel. Elution with dichloromethane–ethanol–triethylamine (97:2:1) gave an anomeric mixture of the title compound (**11**) (1.23 g, 75%) as a white foam, R_F 0.24 (diethyl ether–ethanol); δ_H(60 MHz; $CDCl_3$) 1.60—2.35 (2 H, m), 3.02 (2 H, m), 3.55 (9 H, s), 4.10—4.50 (4 H, m), 5.10 (1 H, m), 6.80 (6 H, m), 7.00—7.50 (13 H, m), and 8.50 (2 H, m); *m/z* 618 ($M + H^+$, 3%), 316 (56), 303 (80), and 121 (100) (Found: $M + H^+$ 618.2856. $C_{39}H_{40}NO_6$ requires 618.2856).

1,2-*Dideoxy*-1-(3-*pyridyl*)-α- *and* β-D-*ribofuranose* (**3α**) *and* (**3β**).—The anomeric mixture (**11**) (1.23 g, 0.2 mmol) was dissolved in acetic acid–water (8:2, 50 ml) and the orange solution stirred for 2 h at room temperature. The reaction mixture was concentrated under reduced pressure to give a yellow oil which was chromatographed on silica gel. Elution with diethyl ether–ethanol (99:1)→(97:3) gave the 3-*O*-protected β-anomer (270 mg, 43%) and the 3-*O*-protected α-anomer (270 mg, 43%) as pale yellow oils.

Each anomer (250 mg, 0.79 mmol) was dissolved in dry dichloromethane (15 ml) and triphenylmethyl tetrafluoroborate (1.57 g, 4.76 mmol) was added. The reaction mixtures were stirred at room temperature for 1 h, water (20 ml) was added to each mixture, and stirring was continued for a further 45 min. The aqueous layers were separated and the organic layers washed with water (3 × 20 ml). The aqueous fractions were concentrated under reduced pressure to give pale yellow powders for each anomer.

The product corresponding to the α-anomer was purified by chromatography on silica gel. Elution with dichloromethane–methanol–triethylamine (95:4:1) and recrystallization from ethyl acetate gave compound (**3α**) (130 mg, 85%), m.p. 116—118 °C, R_F 0.14 (dichloromethane–methanol 9:1); δ_H(60 MHz; CD_3OD) 1.95 (1 H, m), 2.75 (1 H, m), 3.70 (2 H, m), 4.05 (1 H, m), 4.40 (1 H, m), 4.80 (2 H, br s), 5.15 (1 H, t, *J* 7 Hz), 7.42 (1 H, m), 7.90 (1 H, m), and 8.45 (2 H, m); *m/z* 196 ($M + H^+$, 100%) (Found: C, 61.45; H, 6.7; N, 7.05. $C_{10}H_{13}NO_3$ requires C, 61.54; H, 6.67; N, 7.18%).

The product corresponding to the β-anomer was also purified by chromatography on silica gel. Elution with dichloromethane–methanol–triethylamine (95:4:1) gave compound (**3β**) as a clear oil (145 mg, 94%) R_F 0.14 (dichloromethane–methanol 9:1); δ_H(60 MHz; CD_3OD) 1.70—2.50 (2 H, m), 3.70 (2 H, m), 4.00 (1 H, m), 4.40 (1 H, m), 4.80 (2 H, br s), 5.20 (1 H, dd, *J* 6 and 10 Hz), 7.45 (1 H, m), 7.90 (1 H, m), and 8.50 (2 H, m); *m/z* 196 ($M + H^+$, 100%) (Found: $M + H^+$ 196.0974. $C_{10}H_{14}NO_3$ requires 196.0974).

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References

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