Short Communication

Synthesis of 6'-(*S*) Deuterium-labelled Lactose

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The determination of the preferred conformation in solution of the C-5 hydroxymethyl group of hexopyranosides using NMR spectroscopy[1–7] has been facilitated recently by the convenient synthesis of specifically C-6 deuteriated hexopyranoses.[6–9] Using this approach we report the synthesis of two compounds specifically 6-(*S*)-$^2H_1$-labelled in a galactopyranoside unit. These compounds have been used as aglycones in the synthesis of oligosaccharides [10–11] in order to facilitate the assignment of the preferred solution conformation of the hydroxymethyl groups, which is of importance for the overall conformation of the oligosaccharides involved.

Penta-*O*-acetyl-6-(*S*)-$^2H_1$-α-D-galactopyranose (**1**) was prepared as reported[8] and converted into the glycosyl bromide **2** in quantitative yield. Glycosylation of methyl 2,3,6-tri-*O*-benzyl-β-Dglucopyranoside (**3**)[12] with **2** using silver triflate as the promoter gave the disacchardie **4** in 77 % yield. Deprotection by catalytic hydrogenolysis followed by transesterification gave the desired lactoside (**5**) in 92 % yield. The compound was characterized by its ^1H NMR parameters, which were in accord with the pulished values.[13] Hayes *et al.*[13] did not assign the preferred rotamer population of the 6'-hydroxymethyl group, but the results obtained in the present work (with $J_{5,6R} = 8.2$ Hz) suggest, in agreement with the data in Ref. 3 for methyl β-galactoside, that the "*gauche-trans*" conformers (Fig. 1) are predominantly populated in methyl β-lactoside.

The glycosyl bromide **2** was also converted into its β-methyl glycoside (**7**) by treatment with methanol and silver carbonate followered by de-*O*-acetylation. The unprotected compound was isolated crystalline in 61 % yield. This compound was further converted into the 2,3,6-tri--

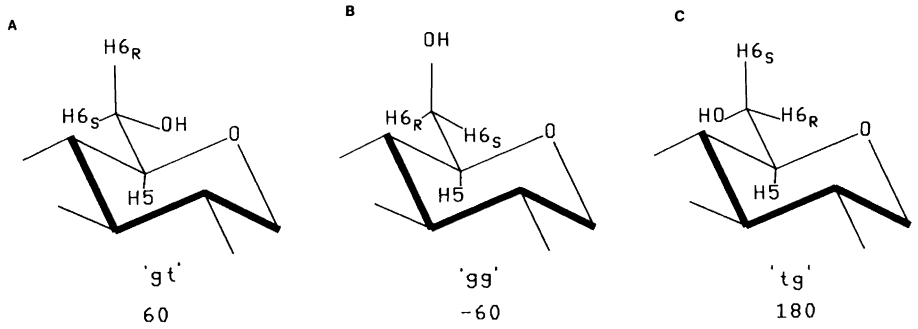
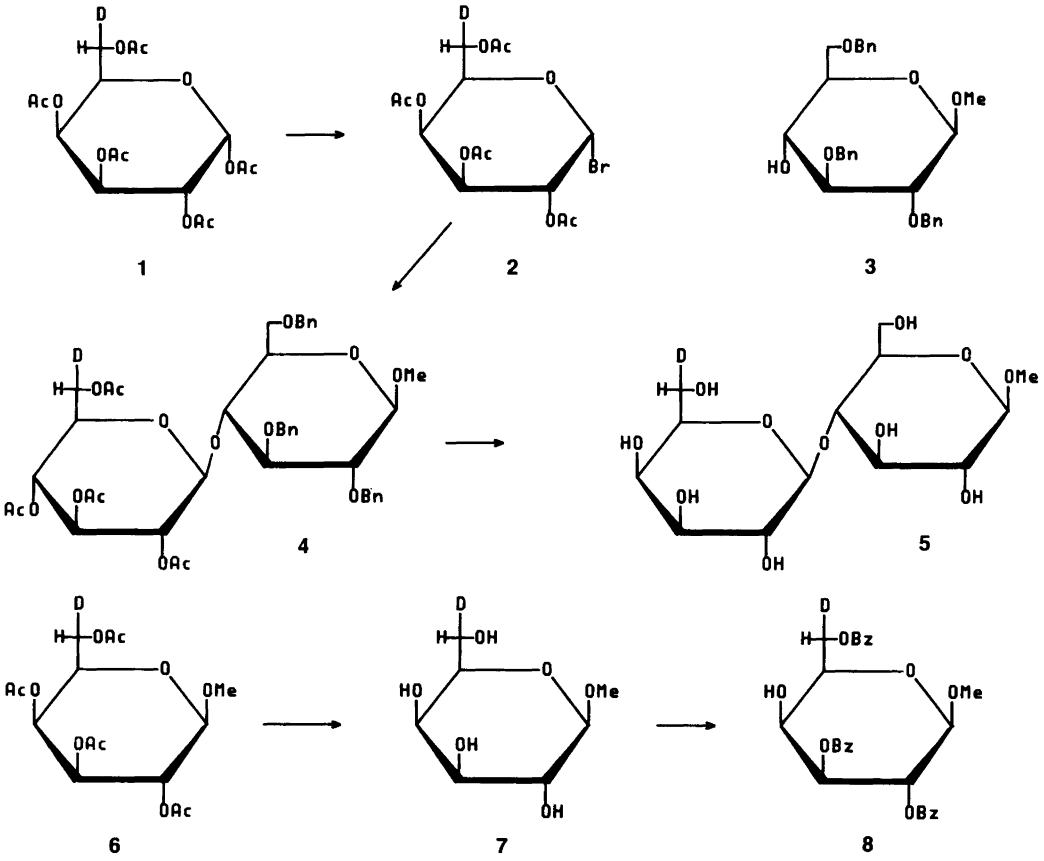


Fig. 1. Description of the preferred conformation of hydroxymethyl groups in hexopyranosides (a) "gt", *gauche-trans*, ω = 60°, (b) "gg", *Gauche-gauche*, ω = −60° and (c) "tg", *trans-gauche*, ω = 180°.



O-benzoyl derivative (**8**)[14] by selective benzoylation. The structure of this compound was confirmed by its ^{1}H NMR spectral data.

Compounds **5** and **7** have been used as receptors in the enzymatic synthesis of α-2,6-linked *N*-acetylneuraminic acid containing di- and trisaccharides,[10] and compound **8** has been used as aglycone in the chemical synthesis of methyl 6-(*S*)-^{2}H$_1$-4-(α-D-galactopyranosyl)-β-D-galactopyranoside.[11] The conformational properties of these oligosaccharides will be discussed elsewhere.[10,11]

Experimental

Melting points are uncorrected. Optical rotations were measured on a Perkin Elmer 241 polarimeter. NMR spectra were obtained on Bruker WH-90 and AM-500 NMR instruments. The spectra of protected compounds were measured in $CDCl_3$. The spectra of unprotected compounds were measured in D_2O relative to the internal references: acetone (δ 2.22) for ^{1}H NMR spectra and dioxane (67.4 ppm) for ^{13}C NMR spectra. TLC was performed on silica gel-coated plates (Merck F-254).

2,3,4,6-Tetra-O-acetyl-6-(S)-$^2H_1$-α-D-galactopyranosyl bromide (**2**). To a solution of of 1,2,3,4,6-penta-*O*-acetyl-6-(*S*)-^{2}H$_1$-α-D-galactopyranose (**1**)[8] (305 mg, 0.78 mmol) in dichloromethane (3 ml) was added acetic acid saturated with hydrogen bromide (3 ml) and the reaction mixture was stirred for 2 h. Ice was added followed by dichloromethane (25 ml), and the organic phase was then washed five times with water (25 ml). Drying over magnesium sulfate and evaporation gave **2** (320 mg, 0.77 mmol, ~ 99 %) as a syrup which was used without further purification.

Methyl 2,3,6-tri-O-benzyl-4-O-[2,3,4,6-tetra-O-acetyl-6-(S)-$^2H_1$-β-D-galacopyranosyl]-β-D-glucopyranoside (**4**). A solution of methyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**3**)[12] (300 mg, 0.65 mmol), silver trifluoromethanesulfonate (200 mg, 0.78 mmol) and *N,N,N',N'*-tetramethylurea (0.093 ml, 0.78 mmol) in dichloromethane (5 ml) was stirred for 1 h with 4 Å molecular sieves under nitrogen. The mixture was cooled to −20 °C and a solution of **2** (320 mg, 0.78 mmol) in dichloromethane (5 ml) was added, followed by stirring for 2 h under nitrogen. The mixture was diluted with dichloromethane (25 ml) and filtered into saturated sodium hydrogen carbonate solution containing ice. The filter was washed with dichloromethane (25 ml) and the combined organic phases were washed once with water (20 ml). Drying (magnesium sulfate) and evaporation to dryness gave a syrup (688 mg), which was purified by preparative TLC using two elutions with ethyl acetate-hexane (1:2). Isolation of the slower-moving fraction yielded **4** (400 mg, 0.50 mmol, 77.4 %) as a syrup, which was characterized by its data. ^{1}H NMR (500 MHz, $CDCl_3$): Glc: α 4.29 (H-1,d), 3.41 (H-2,dd), 3.58 (H-3,t), 3.96 (H-4,dd), 3.37 (H-5,ddd), 3.75 (H-6a, H-6b, d); Gal: 4.64 (H-1d), 5.12 (H-2,dd), 4.84 (H-3,dd), 5.26 (H-4,dd), 3.53 (H-5,dd), 3.84 (H-6,d). Glc: J_{12} 8.0 Hz, J_{23} 9.0, J_{34} 9.0, J_{45} 10.0, J_{56} 2.7; Gal: J_{12} 8.0, J_{23} 10.5, J_{34} 3.5, J_{45} ca 1, J_{56} 6.0.

Methyl 4-O-[6-(S)-$^2H_1$-β-D-galactopyranosyl]-β-D-glucopyranoside (**5**). To a solution of **4** (400 mg, 0.50 mmol) in methanol (10 ml) and acetic acid (4 ml) was added 5 % palladium-on-carbon (150 mg) and the mixture was stirred for 16 h under 1 atm. hydrogen pressure. The catalyst was filtered off and washed with methanol. Filtrate and washings were combined and evaporated to dryness, leaving a syrup (250 mg). The syrup was de-*O*-acetylated in a mixture of methanol (10 ml) and 1M sodium methoxide in methanol (1.5 ml) overnight. Neutralisation and removal of the sodium ions with Amberlite IRC-50 (H^+) ion exchange resin followed by filtration and evaporation yielded **5** as a crystalline compound (165 mg, 0.46 mmol, 92 %) with m.p. 170–175 °C. Recrystallization from 5 % ethanol gave a product with m.p. 207–208 °C; $[\alpha]_D^{20}$ +2.10° (*c* 2.33, H_2O) [lit.[15] m.p. 213–215 °C; $[\alpha]_D^{20}$ +5.1° (H_2O)]. ^{1}H NMR (500 MHz, D_2O): Glc: δ 4.39 (H-1,d), 3.29 (H-2), 3.63 (H-3, H-4), 3.58 (H-5), 3.97 (H-6a,dd), 3.79 (H-6b,dd); Gal: 4.43 (H-1,d), 3.52 (H-2,dd), 3.64 (H-3), 3.91 (H-4,dd), 3.70 (H-5,dd), 3.76 (H-6). Glc: J_{12} 8.3 Hz, J_{56a} 2.2, J_{56b} 5.4, J_{6a6b} 12.6; Gal: J_{12} 8.1, J_{23} 10.2, J_{34} 3.5, J_{45} 1.1, J_{56} 8.4.

Methyl 6-(S)-$^2H_1$-β-D-galactopyranoside (**7**). 2,3,4,6-Tetra-*O*-acetyl-6-(*S*)-$^2H_1$-β-D-galactopyranosyl bromide (**2**) (242 mg, 0.59 mmol) was dissolved in methanol (5 ml) containing silver carbonate (250 mg, 0.9 mmol). The mixture was stirred overnight. The reaction was carried out in the dark with exclusion of moisture. Filtration and evaporation to dryness yielded **6** as a crystalline solid. The crude product was de-*O*-acetylated in a mixture of methanol (5 ml) and 1M sodium methoxide (1 ml). Neutralization and removal of the sodium ions was performed by stirring with Amberlite IRC-50 (H^+). Filtration and evaporation gave crude crystalline product. Recrystallization from dry methanol gave **7** as colourless crystals (70 mg, 0.36 mmol, 61 %) with m.p. 176.5–177 °C (lit.[16] m.p. 178–180 °C). ^{1}H NMR (500 MHz, D_2O): δ 4.30 (H-1,d), 3.49 (H-2,dd), 3.62 (H-3,dd), 3.91 (H-4,dd), 3.67 (H-5,dd), 3.76 (H-6,d); J_{12} 8.0 Hz, J_{23} 9.9, J_{34} 3.3, J_{45} ca. 1, J_{56} 8.0. ^{13}C NMR (125.7 MHz, D_2O): 104.78 ppm (C-1), 71.69 (C-2), 73.77 (C-3), 69.63 (C-4), 76.00 (C-5), 58.07 (oMe).

Methyl 2,3,6-tri-O-benzoyl-6-(S)-$^2H_1$-β-D-galactopyranoside (**8**). A solution of **7** (48 mg, 0.25 mmol) in pyridine (1 ml) under N_2 was stirred and cooled to −20 °C, benzoyl chloride (120 µl, 4.2 equiv.) was added and the temperature kept at −10 °C for 2 h. The mixture was left overnight at room temperature with exclusion of moisture. Most of the pyridine was removed by evaporation and the residue was extracted with dichloromethane (3 × 10 ml). The organic solution was washed twice with hydrochloric acid (2M, 3 ml) and twice with sodium hydrogen carbonate solution, and finally with water and dried (magnesium sulfate). Evaporation gave a syrup (137 mg) which was shown by TLC to contain one major compound. The product was purified by preparative TLC, eluting with ethyl acetate-hexane (1:3). The main fraction gave **8** (64 mg, 0.13 mmol, 51 %) with m.p. 136–140 °C (lit.[14] m.p. 142–143 °C). ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.66 (H-1,d), 5.70 (H-2,dd), 5.37 (H-3,dd), 4.37

(H-4,d), 4.08 (H-5,d), 4.61 (H-6,d); J_{12} 7.7 Hz, J_{23} 10.5, J_{34} 3.1, J_{45} ca. 0.7, J_{56} 6.5.

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