Stereospecific Deuteration in the Synthesis of Methyl α-(4-^{2}H)-Cellobioside

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Isotopic labeling of compounds is of great help in areas that investigate, for example, reaction mechanisms and biosynthesis and also in structural investigations of biomolecules. Unstable isotopes such as ^{3}H, ^{11}C, and ^{14}C are important in biochemical studies as well as in medicine. The use of stable isotopes combined with mass spectrometry offers great advantages in handling and storage of compounds that are used in these studies. In NMR investigations of large biomolecules such as proteins, isotope enrichment with ^{13}C and ^{15}N is commonly performed to alleviate spectral overlap by resorting to higher dimensional NMR spectroscopy.[1] The use of fractional deuteration in these studies has recently found application for even larger structures.[2] If ^{2}H instead of ^{1}H is present, certain relaxation pathways can be eliminated. This is of interest, in particular, when a problem is difficult to solve, even with existing NMR methodology. We have previously synthesized a site-specific deuterium-substituted methyl β-D-glucan decasaccharide and a methyl β-cellobioside analogue thereof.[3] Recently, we published a conformational study of methyl α-cellobioside using ab initio, molecular mechanics and NMR methods.[4] To continue the detailed analysis of carbohydrate conformation and dynamics, we have performed a stereospecific synthesis of a deuterated methyl α-cellobioside, which we here report.

The synthesis of β-D-Glc*p*-(1→4)-α-D-(4-^{2}H)-Glc*p*-OMe (**1**) started from **2** by the removal of the 4,6-benzylidene group with 90% trifluoroacetic acid to give **3**.[5] A slightly modified oxidation of the diol **3**, based on the method described by David and Thieffry,[6] was achieved by activation with dibutyltin oxide in toluene followed by regioselective oxidation with 1,3-dibromo-5,5-dimethylhydantoin[7] in chloroform, which is faster in **3** than for the derivative having the *gluco*-configuration. This led to methyl 2,3-di-*O*-benzyl-α-D-*xylo*-hexopyranosid-4-ulose (**4**), which after flash chromatography was isolated in 92% yield. Reduction of the latter compound or its O-6-protected analogue with $NaBH_4$ leads to a 9:1 mixture of the *galacto*:*gluco* isomers. To convert the *galacto* derivative to the desired *gluco* isomer, inversion of the configuration at C-4 must be achieved. However, a strategy based on a stereoselective intramolecular reduction with ^{2}H, followed by regioselective protection of O-6, would give the 4-deuterated *gluco* derivative **6**, in a convenient and efficient way. Sodium triacetoxyborohydride [$NaBH(OAc)_3$] is known to be a mild reducing agent which can selectively reduce aldehydes in the presence of ketones.[8,9] In the presence of an alcohol (ROH), $NaBH(OR)(OAc)_2$ is formed which can carry out reduction of ketones.[10−12]

Treatment of the 4-ulose derivative **4** with [$NaB^2H(OAc)_3$], generated in situ from sodium borodeuteride and deuterated acetic acid, led to an intermediate in which the reducing agent is attached at O-6, from where an intramolecular reduction with high stereoselectivity can take place. This resulted in **5** as the *sole* product (isolated in 84% yield from **3**), having the *gluco* configuration as determined by comparison to the nondeuterated analogue of **5**. The extent of deuteration was >98% as calculated by integration of the remaining H-4 signal in the ^{1}H NMR spectrum. Activation of diol **5** with dibutyltin oxide in methanol, followed by addition of benzoyl chloride at −10 °C, gave the regioselectively benzoylated derivative **6** which was isolated in 90% yield. A silver trifluoromethanesulfonate (AgOTf) mediated coupling[13,14] of 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl bromide[15] with **6** produced disaccharide **7** in 91% yield. Subsequent deprotection by hydrogenolysis (H_2, Pd−catalytic) followed by debenzoylation with methanolic sodium methoxide gave after gel permeation chromatography the title compound **1** in 88% yield (Scheme 1). The stereospecific intramolecular reduction using $NaB^2H(OAc)_3$ followed by a glycosylation reaction resulted in a site-specifically deuterated methyl α-cellobioside, which is presently being used for further conformational studies of carbohydrate flexibility and dynamics using NMR spectroscopy.

Experimental Section

General. Concentrations were performed under reduced pressure at temperatures < 40 °C (bath). Optical rotations were determined at the sodium D line and measured at 22 °C for solutions in chloroform or water. $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}. NMR spectra were recorded at 30 °C for solutions in $CDCl_3$ and DMSO-d_6 or at 27 °C in D_2O. High-resolution fast atom bombardment mass spectrometry (HR-FABMS) was performed in the positive mode at a resolution of 10 000 using triethyleneglycol or 3-nitrobenzylalcohol as a matrix.

Methyl 2,3-Di-*O*-benzyl-α-D-(4-^{2}H)-glucopyranoside (5). A suspension of diol **3**[5] (1.1 g, 2.94 mmol) and dibutyltin oxide (739 mg, 2.97 mmol) and 3 Å molecular sieves in anhydrous toluene were refluxed for 3 h. The solvent was evaporated under reduced pressure and the material further dried under vacuum for 1 h. The crude stannylene derivative was dissolved in dry chloroform (10 mL). The mixture was stirred at room temperature under a nitrogen atmosphere for 10 min, whereafter 1,3-dibromo-5,5-dimethylhydantoin (424 mg, 1.48 mmol) was added. After 15 min TLC (toluene−acetone 3:1) showed complete conversion of the starting material and the reaction mixture was diluted with chloroform (10 mL) and washed with sodium

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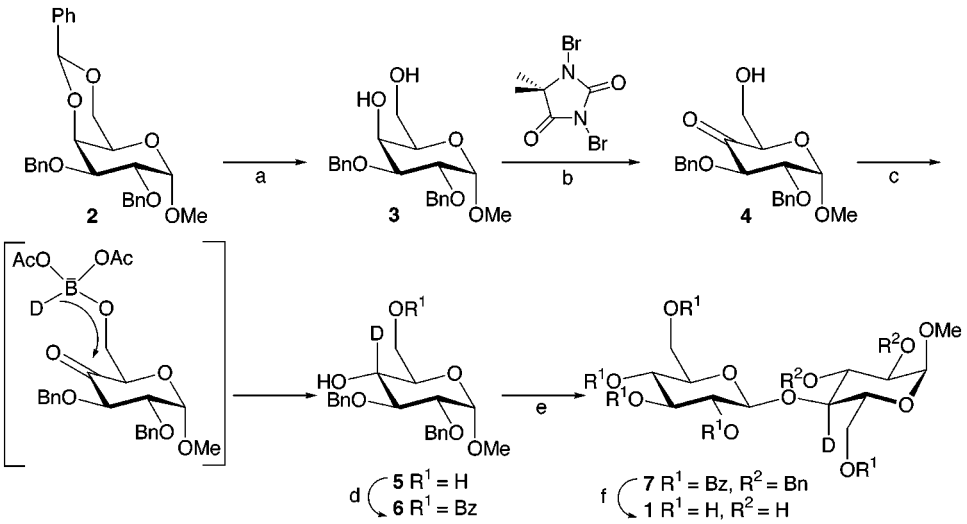
Scheme 1[a]



[a]Reaction conditions: (a) 90% TFA aqueous, $CHCl_3$, 25 °C, see also ref 5; (b) Bu_2SnO, toluene, 3 h, reflux; 1,3-dibromo-5,5-dimethylhydantoin, 15 min, 25 °C; (c) $NaB^2H(OAc)_3$, 1 h, 0 °C; (d) Bu_2SnO, methanol, 3 h, reflux; CH_2Cl_2, benzoyl chloride, −10 °C; (e) Bz_4GlcBr, AgOTf, CH_2Cl_2, -30 °C; (f) H_2-Pd/C, EtOAc, 100 psi, 15 h; NaOMe/MeOH, 30 min, 25 °C.

thiosulfate and water. The organic phase was dried and concentrated in vacuo. Flash column chromatography (silica gel, toluene−EtOAc 6:1 and 2:1) gave methyl 2,3-di-*O*-benzyl-α-D-*xylo*-hexopyranosid-4-ulose (**4**) (1.01 g, 92%): $[\alpha]_D$ +75° (*c* 1.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 3.48 (s), 3.79 (1H, dd, J = 3.5, 10.0 Hz), 3.88 (2H), 4.13 (1H, dd, $J \approx 5$ Hz), 4.45 (1H, d, J = 10.0 Hz), 4.78 (1H, d, J = 3.5 Hz), 4.66, 4.69, 4.86, 4.95 (4H, d, $J \approx$ 12 Hz), 7.25−7.44; ^{13}C NMR ($CDCl_3$) δ 56.1, 60.6, 72.8, 73.9, 74.5, 80.0, 82.5, 98.5, 127.9−128.5, 137.6, 137.7, 203.9.

Sodium borodeuteride (330 mg, 7.88 mmol) was added to acetic acid-*d* (10 mL) cooled to 0 °C. After 30 min methyl 2,3-di-*O*-benzyl-α-D-*xylo*-hexopyranosid-4-ulose (0.98 g, 2.63 mmol) was added under vigorous stirring and the reaction mixture was allowed to attain room temperature. Stirring was continued for 1 h or until TLC (toluene−acetone 1:1) showed complete reaction, and the mixture was concentrated in vacuo. Purification by flash column chromatography (silica gel, toluene−acetone 1:1) provided **5** as the sole product (916 mg, 93%): $[\alpha]_D$ +26° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 3.38 (s), 3.50 (1H, dd, J = 3.6, 9.6 Hz), 3.62 (1H, dd, $J \approx 4$ Hz), 3.74 (1H, dd, J = 4.6, 12 Hz), 3.79 (1H, d, J = 9.6 Hz), 3.81 (1H, dd, J = 3.8, 12 Hz), 4.61 (1H, d, J = 3.6 Hz), 4.66, 4.77 (2H, d, J = 12.1 Hz), 4.70, 5.03 (2H, d, J = 11.6 Hz), 7.26−7.38; ^{13}C NMR ($CDCl_3$) δ 55.2, 62.3, 69.9 (t), 70.7, 73.1, 75.4, 79.8, 81.3, 98.2, 125.3, 127.9−129.0, 138.0, 138.7; HR-FABMS [M + Na]$^+$ *m*/*z* calcd for $C_{21}H_{25}O_6$DNa 398.1690, found 398.1676.

Methyl 2,3-Di-*O*-benzyl-6-*O*-benzoyl-α-D-(4-^{2}H)-glucopyranoside (6). Compound **5** (860 mg, 2.29 mmol) and dibutyltin oxide (628 mg, 2.52 mmol) were mixed together in MeOH (25 mL) and refluxed for 3 h. The solvent was removed under reduced pressure. The crude stannylene derivative was dissolved in CH_2Cl_2 (20 mL) and cooled to −10 °C. Benzoyl chloride (0.27 mL, 2.34 mmol) was added and stirring continued at −10 °C until TLC (toluene−EtOAc 1:1) indicated complete reaction. The mixture was brought up to 0 °C and quenched with water (5 mL). The phases were separated, and the organic phase was washed with saturated aqueous $NaHCO_3$ (2 × 10 mL) and water (10 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Flash column chromatography (silica gel, toluene−EtOAc 3:1) gave **6** (989 mg, 90%): $[\alpha]_D$ +25° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 3.40 (s), 3.53 (1H, dd, J = 3.5, 9.6 Hz), 3.83 (1H, d, J = 9.6 Hz), 3.88 (1H), 4.51 (1H, dd, J = 2.1, 12.1 Hz), 4.62 (1H, dd, J = 4.9, 12.1 Hz), 4.65 (1H, d, J = 3.5 Hz), 4.67, 4.76, 4.78, 5.01 (4H, d, $J \approx$ 12 Hz), 7.28−8.03; ^{13}C NMR ($CDCl_3$) δ 55.2, 63.7, 69.4, 69.7 (t), 73.1, 75.6, 79.6, 81.1, 98.0, 125.3, 127.9−130.2, 133.0, 133.5, 137.9, 138.5, 166.7; HR-FABMS [M + Na]$^+$ *m*/*z* calcd for $C_{28}H_{29}O_7$DNa 502.1952, found 502.1956.

Methyl 2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→4)-2,3-di-*O*-benzyl-6-*O*-benzoyl-α-D-(4-^{2}H)-glucopyranoside (7). Compound **6** (600 mg, 1.25 mmol) and 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl bromide (1.07 g, 1.68 mmol) were mixed together with *sym*-collidine and 4 Å molecular sieves (2 g) in CH_2Cl_2 (30 mL) and cooled to −30 °C. AgOTf (460 mg, 1.79 mmol) was added under stirring, and the temperature was kept at −30 °C until the reaction was complete as indicated by TLC (toluene−EtOAc 5:1). The reaction was quenched with triethylamine (1 mL), filtered through Celite, and concentrated. The crude residue was purified by flash column chromatography (silica gel, toluene−EtOAc 8:1) to give **7** (1.20 g, 91%): $[\alpha]_D$ +49° (*c* 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 3.30 (s), 3.48 (1H, dd, J = 3.7, 9.6 Hz), 3.84 (1H, m), 3.86 (1H, m), 4.01 (1H, d, J = 9.6 Hz), 4.25, 4.35, 4.40, 4.51 (4H, dd), 4.54 (1H, d), 4.58, 4.72, 4.94, 5.08 (4H, d, $J \approx$ 12 Hz), 5.09 (1H, d, J = 8 Hz), 5.57 (1H, dd, J = 8, 10 Hz,), 5.63, 5.79 (2H, dd, $J \approx$ 10 Hz), 7.17−7.93; ^{13}C NMR ($CDCl_3$) δ 55.2, 62.7, 62.8, 68.1, 69.5, 72.2, 72.3, 73.0, 73.4, 75.2, 79.4, 79.7, 97.8, 101.1, 125.3, 127.1−133.6, 138.0, 139.0, 165.0−165.9; HR-FABMS [M + Na]$^+$ *m*/*z* calcd for $C_{62}H_{55}O_{16}$DNa 1080.3529, found 1080.3511.

Methyl β-D-Glucopyranosyl-(1→4)-α-D-(4-^{2}H)-glucopyranoside (1). Compound **7** (900 mg, 0.85 mmol) was dissolved in EtOAc (10 mL), and a catalytic amount of 10% Pd/carbon was added. Hydrogenolysis was performed under an H_2 atmosphere at a pressure of 100 psi. After 15 h the mixture was filtered through Celite, the solvent was evaporated, and the residue was dissolved in CH_2Cl_2 (20 mL). Methanolic sodium methoxide (0.1 M, 5 mL) was added, and the reaction mixture was stirred at room temperature for 30 min. The mixture was filtered through a column of Dowex-50(H^+), the solvent was evaporated, and the product was purified by gel filtration chromatography (Bio-Gel P-2, pyridinium acetate buffer, pH 5.4) to yield **1** (265 mg, 88%): $[\alpha]_D$ +82° (*c* 0.9, H_2O); ^{1}H NMR (D_2O) 3.29 (1H, dd, J = 7.9, 9.2 Hz), 3.39 (1H, dd, $J \approx$ 9.4 Hz), 3.39 (s), 3.46 (1H, ddd), 3.48 (1H, dd, $J \approx$ 9.1 Hz), 3.58 (1H, dd, J = 3.9, 9.8 Hz), 3.70 (1H, dd, J = 5.9, 12 Hz), 3.74 (1H), 3.75 (1H, d, $J \approx$ 9 Hz), 3.82 (1H, dd, J = 4.8, 12 Hz), 3.89, 3.90 (2H, dd, J = 2.3, 12 Hz), 4.48 (1H, d, J = 7.9 Hz), 4.78 (1H, d, J = 3.9 Hz); HR-FABMS [M + Na]$^+$ *m*/*z* calcd for $C_{13}H_{23}O_{11}$DNa 380.1279, found 380.1286.

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