Synthesis of 6-(*S*) Deuterium-Labelled Derivatives of Maltose and Isomaltose

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Bock, K. and Pedersen, H., 1988. Synthesis of 6-(*S*) Deuterium-Labelled Derivatives of Maltose and Isomaltose. Acta Chem. Scand., Ser. B 42: 190–195.

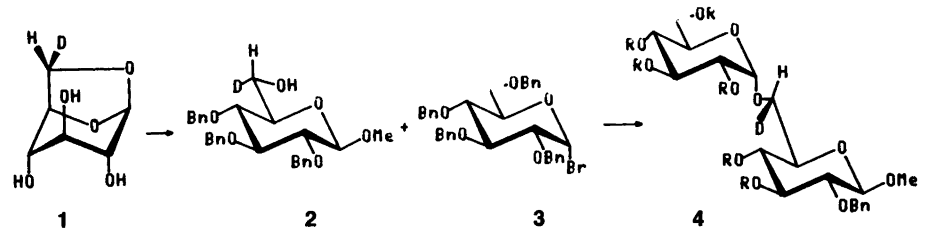
The 6-(*S*) deuterium-labelled compounds methyl β-isomaltoside (**4a**), methyl β-maltoside (**8a**) and methyl 4,6-di-*O*-(α-D-glucopyranosyl)-β-D-glucopyranoside (**12**) have been synthesized from deuterium-substituted 1,6-anhydro compounds. The 6-(*R*) deuterium-labelled compound methyl β-maltoside (**10**) has been prepared by inversion at the 6-position of the corresponding 6-(*S*) derivative. The compounds have all been examined by ^{1}H NMR spectroscopy at 500 MHz and their preferred solution conformation has been inferred from the J_{56R} and J_{56S} coupling constants.

The recently reported synthetic method for the preparation of specifically C-6 deuterium-labelled hexopyranoside[1–3] based on the photobromination of tri-*O*-benzoyl-1,6-anhydro-D-glucose first described by Ferrier and Furneaux[4] has facilitated the analysis of the preferred rotamer population of the C-5 hydroxymethyl group using NMR spectroscopy.[5–7] This is of particular importance for the conformational analysis of 6-linked oligosaccharides such as isomaltose, but the conformational preference of maltose has also been shown[8,9] to be dependent on the conformation of the C-5 hydroxymethyl group. We have therefore synthesized isomaltose and maltose derivatives labelled with deuterium specifically in the 6-(*S*) position of the reducing sugar unit, as well as the 6-(*S*) deuterium-labelled β-methyl glycoside of the branch point trisaccharide of starch, i.e. methyl 4,6-di-*O*-(α-D-glucopyranosyl)-β-D-glucopyranoside, in order to study their preferred conformations in solution on the basis of results obtained from an NMR spectroscopic analysis.

Results and discussion

The 6-(*S*) labelled isomaltose derivative (**4c**) was synthesized (in 36 % yield) by a halide-catalyzed[10] glycosylation reaction involving tetra-*O*-benzyl-α-D-glucopyranosyl bromide (**3**) and methyl 6-(*S*)-2,3,4-tri-*O*-benzyl-6-^{2}H$_1$-β-D-glucopyranoside (**2**). The deuterium-labelled aglycone **2** was prepared from 6-(*S*)-^{2}H$_1$-1,6-anhydro-β-D-glucopyranose (**1**)[3] which was converted into **2** as described previously.[11] Deprotection of **4c** was accomplished by hydrogenation with Pd/C in acetic acid-methanol and the product **4a** was isolated in 92 % yield and characterized through its ^{1}H and ^{13}C NMR parameters.

The 6-(*S*) deuterium-labelled methyl maltoside derivative **8a** was synthesized analogously to the above-described glucoside (**2**), using maltosan hexaacetate as starting material for the photobromination. The 6-(*S*)-6-bromomaltosan derivative (**6**) was isolated crystalline in 64 % yield and reduced with tributyltin deuteride to the 6-(*S*) deuterium-labelled maltosan derivative **7** in 82 % yield. The position of the deuterium label was confirmed by the ^{1}H NMR spectrum of **7** and by comparison with the corresponding spectra of the glucosan derivatives.[3] Opening of the 1,6-anhydro compound **7** to the 6-OH compound **8b** was carried out in 50 % yield as described recently.[12] Finally, the product was de-*O*-acetylated to give the unprotected methyl β-6-(*S*)-^{2}H$_1$-β-maltoside (**8a**) which was characterized by ^{1}H NMR spectroscopy.[13,14]



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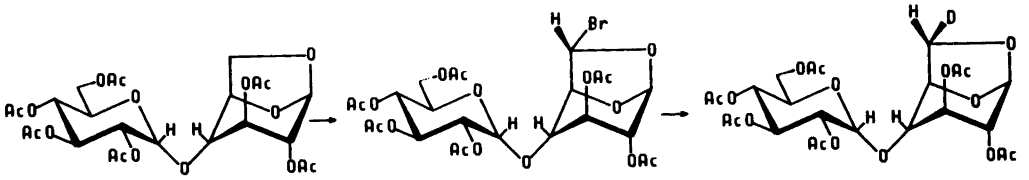

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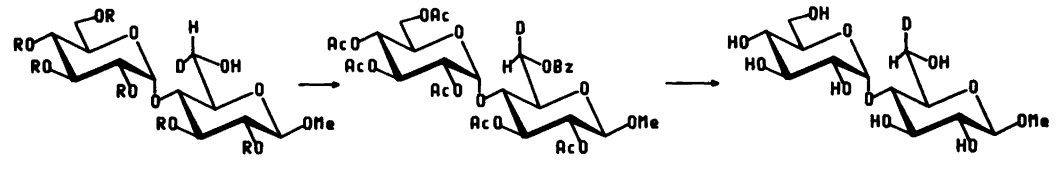
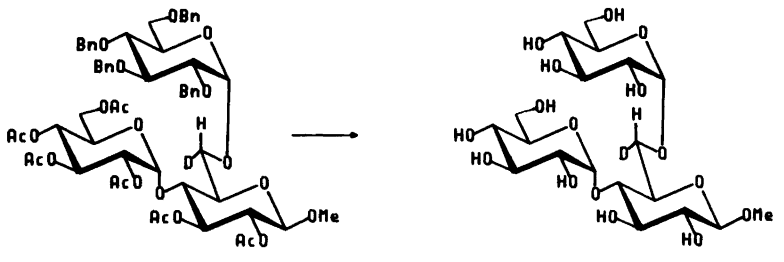
The corresponding 6-(*R*)-labelled compound was prepared by treatment of **8b** with benzoic acid, triphenylphosphine and diethyl azocarboxylate, giving the 6-benzoate (**9**) with inverted stereochemistry at this center in 26 % yield. The product was de-*O*-acylated to **10**, which was isolated in 67 % yield and characterized by its ^{1}H NMR spectral data.

Finally, the branched trisaccharide (**12**) was synthesized with a deuterium label in the 6-(*S*) position using **8a** as the aglycone and **3** as the glycosyl donor in a halide-catalyzed glycosylation



8 9 10



11 12

a: R = H
b: R = Ac
c: R = Bn

as described for the non-labelled compound.[13] Compound **11** was isolated in 24 % yield, and was deprotected as previously described in 50 % yield and finally characterized by its ^{1}H NMR data.

The ^{1}H NMR parameters (protons H-5, H-6R and H-6S) for compounds **4a**, **8a**, **10** and **12** are given in Table 1 together with the data for the non-labelled compounds. These data are in agreement with those recently reported by Ohrui *et al.*,[1] but differ from those previously published.[6,14] The results discussed below are based on the coupling constants J_{56R} and J_{56S} using limiting values from the empirical data published by Altona *et al.*[15] A graphical analysis of the data (Fig. 1) indicates that isomaltose exists predominantly in the "gg" conformation[16] (65 %), that a substantial amount of the "gt" conformer is present (35 %) and that the amount of the "tg" conformer is very low. The observed value of J_{56S} indicates, using the limiting data of Altona *et al.*, a negative population, which most likely means that these values should be changed; however, this will not have any significant influence on the results discussed above. Furthermore, the graph (Fig. 1) shows that the determination of the ratio of "gg" and "gt" is insensitive to the value of J_{56S}.

A similar analysis of the coupling constants J_{56R} = 5.0 Hz and J_{56S} = 1.8 Hz for the reducing unit in methyl maltoside indicates that the population of the preferred rotamer of the C-5 hydroxymethyl group is not significantly different from those for glucose[1-4] or the non-reducing unit of isomaltose. The graphical presentation of the results (Fig. 1) indicates population of "gg" (58 %), "gt" (42 %) and "tg" (0 %). Finally, the data suggest that the "gg" conformation is 52 % populated in compound **12** and that the "tg" conformer is not present to any significant degree.

Table 1. ^{1}H NMR Chemical shifts and coupling constants for protons H-5, H-6(*R*) and H-6 (*S*) in the reducing unit of the deuterium-labelled compounds **4a**, **8a**, **10**, **12** and non-labelled analogues.

Compound	Chemical Shifts/ppm[a] (Coupling Constants/Hz)[b]		
	H-5	H-6(*R*)	H-6(*S*)
Methyl β-isomaltoside	3.65	3.99 (4.3)	3.77 (1.8, 11.0)
4a	3.66	3.99 (4.3)	–[c]
Methyl β-maltoside	3.58	3.74 (5.0)	3.95 (1.8, 12.0)
8a	3.58	3.74 (5.0)	–
10	3.58	–	3.95 (1.8)
Non-labelled **12**[d]	3.75	3.97 (5.8)	3.92 (2.2, 11.6)
12	3.76	3.97 (5.8)	–

[a]Measured at 500 MHz in D_2O at 300 K using acetone (2.22 ppm) as internal reference. [b]Accuracy ± 0.1 Hz. [c]J_{6R6S}, J_{56R} and J_{56S} are expected to be 1/6 for the deuterated compounds, but the small coupling constants are not resolved in the spectra. [d]Ref. 13.

Experimental

Melting points are uncorrected. Optical rotations were measured on a Perkin Elmer 241 polarimeter. NMR spectra were recorded on Bruker WH-90, HX-270 and AM-500 NMR instruments. The spectra of protected compounds were measured in $CDCl_3$. Proton-proton coupling constants are given only if they are different from expected values. Spectra of unprotected compounds were measured in D_2O relative to the internal reference: acetone (δ 2.22) for ^{1}H NMR spectra and dioxane (67.4 ppm) for ^{13}C NMR spectra. Microanalyses were performed by Novo Microanalytical Laboratory, Copenhagen, Denmark. TLC was performed on silica gel-coated plates (Merck F-254). Preparative TLC was performed on 20×40 cm plates coated with 1 mm of silica gel.

Methyl 6-(S)-2,3,4-tri-O-benzyl-6-$^2H_1$-β-D-glucopyranoside (**2**). 6-(*S*)-6-$^2H_1$-1,6-Anhydro-β-D-glucopyranose (**1**)[3] (745 mg, 4.57 mmol) was transformed into **2** by the same procedure as used for the preparation of the corresponding non-labelled material.[11] Yield 700 mg (1.51 mmol, 33 %) with m.p. 78–80 °C, $[\alpha]_D^{23}$ +7.4° (*c* 0.6, $CHCl_3$). Lit.[11] m.p. 90–91 °C, $[\alpha]_D$ 10° ($CHCl_3$). ^{1}H NMR (270 MHz, $CDCl_3$): δ 4.33 (H-1); 3.39 (H-2); 3.55, 3.66 (H-3, H-4); 3.34 (H-5); 3.68 (H-6R); 3.56 (OMe).

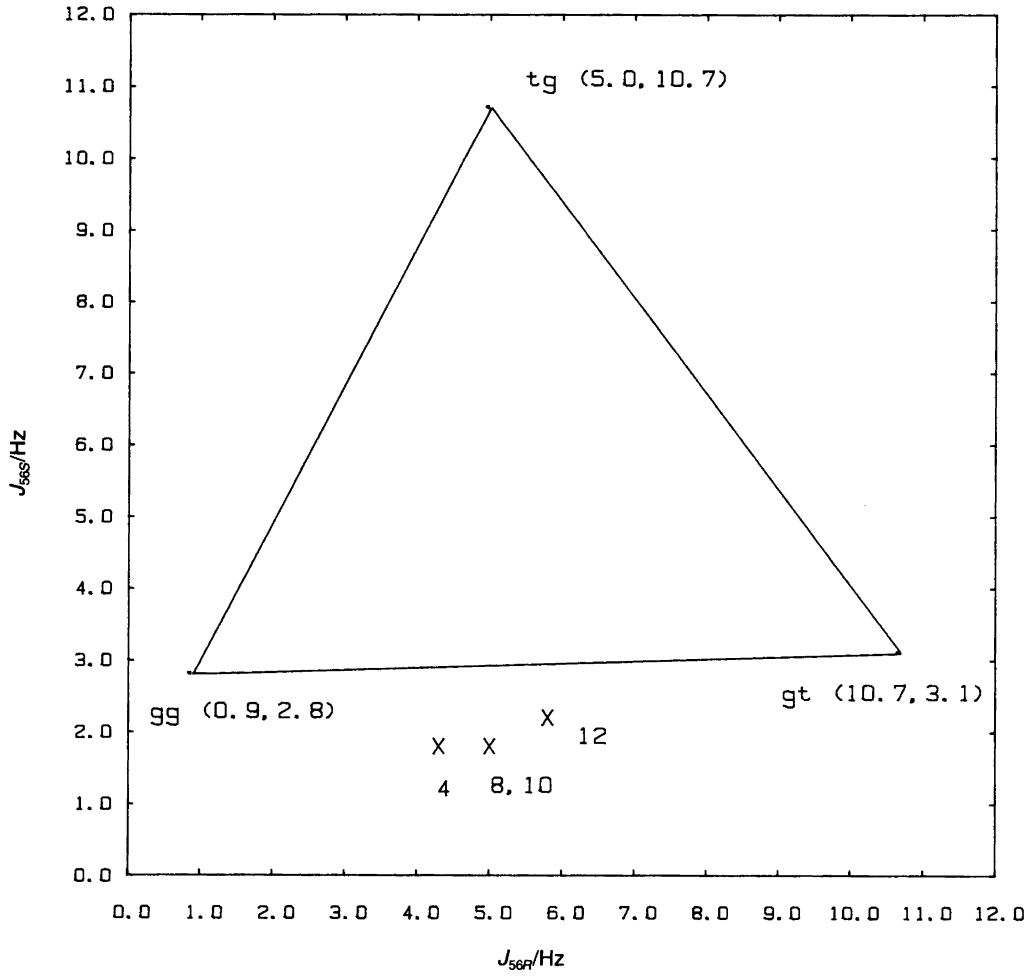


Fig. 1. Plot of coupling constants J_{56R} and J_{56S} using the limiting values of Altona *et al.* to define the staggered conformations "tg", "gt" and "gg". The observed values for J_{56R} and J_{56S} (see Table 1) for compounds **4a**, **8a**, **10** and **12** are shown by ×. The populations can be estimated from these values using a lever rule.

Methyl 6-(S)-2,3,4,2',3',4',6'-hepta-O-benzyl-6-$^2H_1$-β-isomaltopyranoside (**4c**). A solution of **2** (300 mg, 0.645 mmol) and $Et_4N^+Br^-$ (300 mg, 1.45 mmol) in CH_2Cl_2 (1 ml) and DMF (0.5 ml) was stirred with 4 Å molecular sieves (1 g) under N_2 for 1 h. A solution of freshly prepared 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl bromide (**3**)[17] (750 mg, 1.29 mmol) in CH_2Cl_2 (1 ml) was added and the mixture stirred for 16 h. The mixture was diluted with CH_2Cl_2 (25 ml) and stirred for 1 h. After filtering, the filter cake was stirred with acetonitrile for 1 h and the mixture was filtered again. Concentration of the combined filtrates gave a residue which was dissolved in CH_2Cl_2 (50 ml), and the organic phase was washed 3 times with water (25 ml). Drying over $MgSO_4$ and concentration gave a syrup. Purification by preparative TLC [EtOAc – pentane (1:3)] yielded a syrup (247 mg), from which **4c** (228 mg, 0.23 mmol, 36 %) with m.p. 103–105 °C, $[\alpha]_D^{23}$ +39.5 ° (*c* 2.3, $CHCl_3$) could be crystallized using EtOAc – pentane. Physical data for the non-labelled compound were as follows: m.p. 107–108 °C, $[\alpha]_D^{23}$ +39.0 ° (*c* 1.6 $CHCl_3$). Anal. $C_{62}H_{66}O_{11}$: C, H. ^1H NMR data for **4c** (500 MHz, $CDCl_3$): δ 5.05 (H-1', J_{12} = 3.6 Hz); 3.56 (H-2'); 3.98 (H-3');

3.62 (H-4′); 3.87 (H-5′); 3.68 (H-6a′); 3.61 (H-6b′); 4.28 (H-1, J_{12} = 7.8 Hz); 3.30 (H-2); 3.62 (H-3); 3.63 (H-4); 3.50 (H-5); 3.82 (H-6a); 3.50 (OMe). ^{13}C NMR data for **4c** (125.7 MHz, $CDCl_3$): 96.9 ppm (C-1′); 104.4 (C-1); 84.4, 82.3, 81.6, 79.9, 77.7, 77.5, 74.5 (C-2′, C-3′, C-4′, C-5′, C-2, C-3, C-4); 70.0 (C-5′); 68.4 (C-6′); 65.4 (C-6, triplet due to D-substitution); 72.3, 73.2, 74.6, 74.7, 74.8, 75.4, 75.4 (CH_2-Oφ); 56.8 (OMe).

Methyl 6-(S)-6-$^2H_1$-β-isomaltopyranoside (**4a**). Deprotection was accomplished by dissolving **4c** (150 mg, 0.15 mmol) in MeOH (5 ml) and HOAc (3 ml), and adding 5 % palladium-on-carbon (30 mg). The mixture was stirred for 16 h at 1 atm hydrogen pressure and the catalyst was filtered off and washed with EtOH. The filtrate was concentrated by evaporation to give **4a** as a syrup (50 mg, 0.14 mmol, 92 %). The ^{1}H NMR (500 MHz, D_2O) spectrum was identical with spectra published for isomaltose,[13,14] except that the signal for the H-6(*S*) proton at δ 3.77 was missing and the signal at δ 3.99 [H-6(*R*)] had collapsed to a doublet (J = 4.3 Hz): δ 4.95 (H-1′, J_{12} = 3.6 Hz); 3.56 (H-2′); 3.72 (H-3′); 3.42 (H-4′); 3.72 (H-5′); 3.84 (H-6a′); 3.78 (H-6b′); 4.40 (H-1, J_{12} = 7.8); 3.28 (H-2); 3.55 (H-3); 3.50 (H-4); 3.63 (H-5); 3.96 (H-6 (R), J_{56} = 4.3); 3.56 (OMe): ^{13}C NMR data (125.77 MHz, D_2O): 98.6 ppm (C-1′); 72.3 (C-2′); 73.9 (C-3′); 70.4 (C-4′); 72.6 (C-5′); 61.3 (C-6′); 104.2 (C-1); 73.9 (C-2); 76.8 (C-3); 70.3 (C-4); 75.0 (C-5); 65.9 (C.6); 58.0 (OMe).

6-(S)-2,3,2′,3′,4′,6′-Hexa-O-acetyl-1,6-anhydro-6-bromo-β-maltose (**6**). To a solution of maltosan hexaacetate (**5**)[12] (2.0 g, 3.47 mmol) in chlorobenzene (100 ml) was added bromine (0.5 ml, 9.75 mmol). The mixture was heated under reflux for 30 min using a heat lamp (250 W). The cooled solution was washed twice with 10 % $Na_2S_2O_3$ solution (50 ml) and twice with saturated $NaHCO_3$ solution (50 ml). Drying over $MgSO_4$ and concentration of the organic phase yielded a syrup (2.5 g), from which **6** (1.45 g, 2.21 mmol, 64 %) with m.p. 146–166 °C could be crystallized by addition of ether. Recrystallizations from EtOAc – pentane gave **6** with m.p. 178–179 °C, $[\alpha]_D^{23}$ +10.5° (*c* 0.4, $CHCl_3$). Anal. $C_{24}H_{31}BrO_{16}$: C, H. ^{1}H NMR (270 MHz, $CDCl_3$): δ 5.27 (H-1′); 4.82 (H-2′); 5.51 (H-3′); 5.01 (H-4′); 4.38 (H-5′); 4.19 (H-6a′); 4.16 (H-6b′); 5.85 (H-1); 4.58 (H-2); 4.83 (H-3); 3.44 (H-4); 5.02 (H-5); 6.34 (H-6). ^{13}C NMR (67.89 MHz, $CDCl_3$): 97.5 ppm (C-1′); 70.0 (C-2′); 69.4 (C-3′); 68.5 (C-4′, C-5′); 62.3 (C-6′); 102.0 (C-1); 67.2 (C-2); 70.5 (C-3); 74.5 (C-4); 78.8 (C-5); 85.0 (C-6).

6-(S)-2,3,2′,3′,4′,6′-Hexa-O-acetyl-1,6-anhydro-6-$^2H_1$-β-maltose (**7**). To a solution of **6** (0.5 g, 0.76 mmol) in toluene (40 ml) was added tri-*n*-butyltin deuteride (1.0 g, 3.4 mmol) and α, α′-azo-bis-isobutyronitrile (10 mg, 0.06 mmol). The mixture was heated under reflux under N_2 for 1 h and then concentrated to dryness. Pentane (150 ml) was added and the amorphous product was filtered off. The product was crystallized from EtOH and the crystals were washed well with pentane. This gave **7** (360 mg, 0.62 mmol, 82 %) with m.p. 178–180 °C. The deuterium incorporation was 80 % as judged from the ^{1}H NMR spectrum, which was identical with that of **5** except that the signal at 3.75 ppm was absent and the signal at 3.95 ppm had collapsed to a broad singlet. Changes was also seen at 4.71 ppm (H-5).

Methyl 6-(S)-2,3,2′,3′,4′,6′-Hexa-O-acetyl-6-$^2H_1$-β-maltoside (**8b**). Compound **7** (1.0 g, 1.73 mmol) was transformed into **8b** by a sequence analogous to that used for the preparation of the non-labelled compounds.[12] Yield 525 mg (0.86 mmol, 50 %), m.p. 131–135 °C. The ^{1}H NMR spectrum was identical with that of methyl 2,3,2′,3′,4′,6′-hexa-*O*-acetyl-β-maltoside except that the signal at 3.97 ppm was absent. The signal at 3.53 ppm (H-6R) had collapsed to a doublet and the one at 3.86 ppm (H-5) to a double doublet.

Methyl 6-(S)-6-$^2H_1$-β-maltoside (**8a**). De-*O*-acetylation of **8b** (100 mg, 0.16 mmol) with 0.1 % sodium methoxide in MeOH yielded **8a** (55 mg, 0.15 mmol, 96 %) as a syrup which could be crystallized from EtOH. This gave **8a** as the monohydrate with m.p. 112–120 °C. The ^{1}H NMR spectrum of **8a** was identical with that of methyl maltoside[13] except that the signal at 3.95 ppm was missing and that at 3.74 ppm (H-6R) had collapsed to a doublet. Changes were also seen at about 3.58 ppm (H-5).

Methyl 6-(R)-2,3,2′,3′,4′,6′-hexa-O-acetyl-6-O-benzoyl-6-$^2H_1$-β-maltoside (**9**). To a solution of **8b** (100 mg, 0.16 mmol) in ether (2 ml) were added benzoic acid (50 mg, 0.42 mmol), triphenylphos-

phine (100 mg, 0.38 mmol), and diethyl azodicarboxylate (50 mg, 0.32 mmol), and the mixture was stirred for 16 h. The resulting mixture was purified by preparative TLC, eluting with EtOAc-pentane (1:1). This yielded **9** (30 mg, 0.41 mmol, 26 %) as a syrup. The 90 MHz ^{1}H NMR spectrum of **9** showed, among other signals, a complex spin system centered at 7.8 ppm (6-*O*-benzoyl).

*Methyl 6-(*R*)-6-$^2H_1$-β-maltoside* (**10**). Compound **9** (30 mg, 0.042 mmol) was treated with 0.1 % sodium methoxide in MeOH (10 ml) for 2 h. The mixture was neutralized with solid carbon dioxide and evaporated to dryness. The residue was chromatographed on Sephadex G-15, eluting with MeOH–H_2O (1:1). This yielded **10** (10 mg, 0.028 mmol, 67 %), which had ^{1}H NMR data as for methyl maltoside[13] except that one signal at 3.74 ppm was absent and one at 3.95 ppm had collapsed to a doublet.

*Methyl 6-(*S*)-$^2H_1$-4,6-di-*O*-(α-D-glucopyranosyl)-β-D-glucopyranoside* (**12**). Compound **8b** (100 mg, 0.16 mmol) was reacted with **3** (193 mg, 0.32 mmol) as described for the preparation of the non-labelled material by halide ion catalysis.[10] This yielded the protected derivative (**11**) as a syrup (44 mg, 0.038 mmol, 24 %), which was deprotected as described for the preparation of non-labelled material.[13] This yielded **12** as a syrup (10 mg, 0.019 mmol, 50 % from **11**). The ^{1}H NMR spectrum of **12** was identical with that of the non-labelled material[13] except that the signal at 3.92 ppm was absent and the one at 3.97 ppm had collapsed to a doublet.

Acknowledgements. This work has been supported by a grant from the Danish Technical Research Council and Novo A/S to H. P. and from a Nato travel grant (RG 85.0021) to K. B. The 500 MHz spectrometer was provided by the Danish Natural Science Research Council and the Carlsberg Foundation.

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Received October 19, 1987.