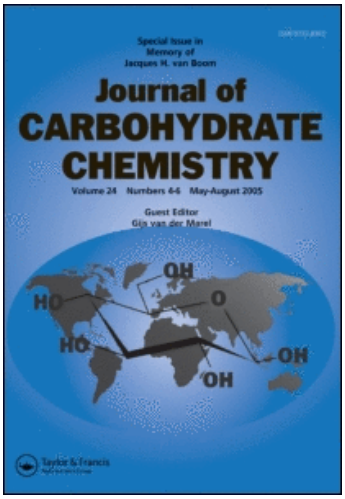

Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

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To cite this Article Bock, Klaus , Skrydstrup, Troels and Refn, Susanne(1991) 'Synthesis Of Isomaltose Analogues', Journal of Carbohydrate Chemistry, 10: 6, 969 — 980
To link to this Article: DOI: 10.1080/07328309108543966
URL: http://dx.doi.org/10.1080/07328309108543966

J. CARBOHYDRATE CHEMISTRY, 10(6), 969-980 (1991)

SYNTHESIS OF ISOMALTOSE ANALOGUES

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January 31, 1991 - Final form August 1, 1991

ABSTRACT

Preparation of the α-glucosides **11**, **12**, **13** and **14** were accomplished through glycosylation of racemic *trans*-1-hydroxy-2-(hydroxymethyl)cyclohexane using 2-thiopyridyl tetra-*O*-benzyl-glucoside as the glycosyl donor in acceptable overall yield for α-selectivity, but with poor regioselectivity. Glycosylation under thermodynamic control using tetrabenzyl glucopyranose acetate and trimethylsilyl triflate as the promotor gave similar results. The unprotected glucosides **12** and **13** were separated and characterized by NMR spectroscopy. Similarly methyl 4-deoxy-α-isomaltoside (**5a**) was prepared through halide catalyzed glycosylation of methyl 2,3-di-*O*-benzoyl-4-deoxy-α-D-glucopyranoside (**15**) in acceptable yield and the unprotected compound characterized by NMR spectroscopy. Compounds **5a**, **12a**, **13a** and the mixture **11a** and **14a** were all tested as substrates for the enzyme glucoamylase from *Aspergillus niger* and proved to be very poor substrates for the enzymic hydrolysis.

INTRODUCTION

The enzyme amyloglucosidase (AMG, EC. 3.2.1.3) from *Aspergillus niger* is one of the four amylases which displays an ability to hydrolyse amylose, not only at the α-1,4-glycosidic linkages, but also at the α-1,6 linkages.[1] Due to its industrial importance, as well as the fact that

no crystal structure of the enzyme has been reported, considerable efforts have been launched to map the active site, as well as determining the specific binding mode of maltodextrins to the enzyme.[2] In model studies using methyl ß-maltoside analogues, it was shown that for the enzymatic hydrolysis of methyl ß-maltoside (1) (the simplest amylose model) by amyloglucosidase from *Aspergillus niger*, the 3, 4' and 6' hydroxy groups are essential, and it was suggested that these three hydroxy groups represent the key polar interaction of maltoside with the catalytic site.[3] These findings immediately raised the question, as to how the same enzyme can hydrolyse the α-1,6 glycosidic linkage, as in methyl ß-isomaltoside (2), albeit at a slower rate compared to 1, when considering the relative positions of the key polar hydroxy groups with respect to the glucosidic linkage. A plausible explanation can be found if one considers the fact that isomaltoside (2) possesses three stable solution conformations around the C5-C6 bond as depicted in Fig. 1. The population of the three conformers in aqueous solution has been determined to be tg:gg:gt = 0:65:35.[4] If, however, the minimum energy conformation (HSEA program) of methyl ß-isomaltoside, whereby the O_6-C_6-C_5-O_5 torsional angle is set to 180° (tg-conformer), is compared to the ground state conformation of methyl ß-maltoside by direct overlap of the non-reducing sugars, one observes overlap of the 4-OH group of isomaltoside (2) with the 3-OH group of maltoside (1). Hence, it was postulated that instead of the 3-OH group acting as a key polar group for the enzymatic hydrolysis of 2, the 4-OH group should now overtake this role, bearing in mind that the enzyme accepts these substrates close to their minimum energy conformations in solution (induced fit).

Recently, Lemieux et al.[5] have added support to the above postulation by studying the enzymatic hydrolysis of isomaltoside analogues, in which one of the C6 protons is replaced by a methyl group thereby shifting the conformational equilibrium of the C5-C6 bond towards the tg-conformation.[5] We chose another approach, whereby the glucoside 3 was considered, being an isomaltoside model locked in the tg-conformation and possessing the required 4-OH group. We have recently described the synthesis of 3 by selective glycosylation of the (*R*)-C-OH group of the corresponding aglycone, as well as compound 6 which represents a 3-deoxy maltose analogue.[6] However, the ability of the glucosidase to accept compound 3 as a substrate could be hindered sterically, due to the additional ring in 3 compared to 2. Hence, in a study of the enzymatic hydrolysis of 3, we also required the glucoside 4, not only for a direct comparison as to the importance of the O5-C5-C6-O6 torsional bond angle, but also as to what influence the three extra carbons in 3 could provoke. In addition, a need arose for the methyl 4-deoxy-α-isomaltoside (5) in order to investigate the importance of the 4-OH group. The results of this study are disclosed below.

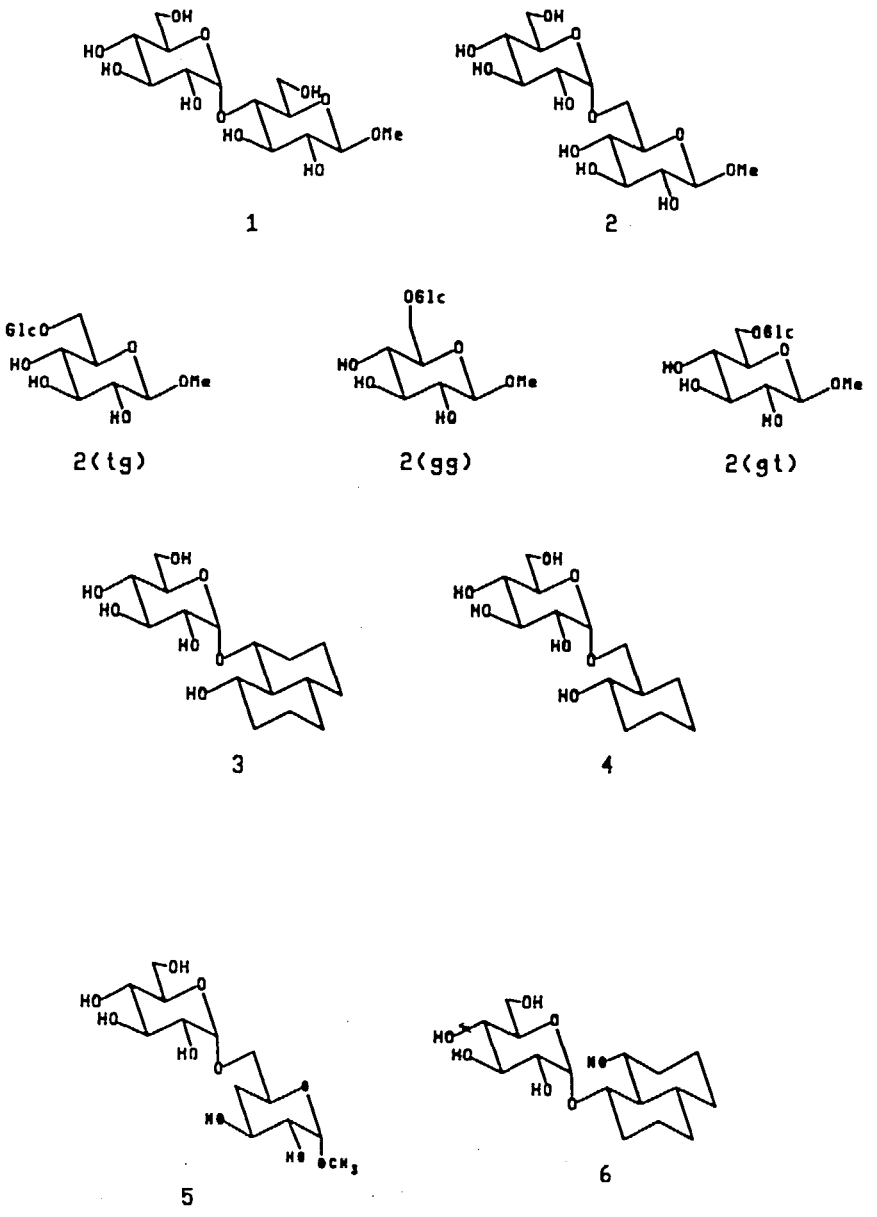


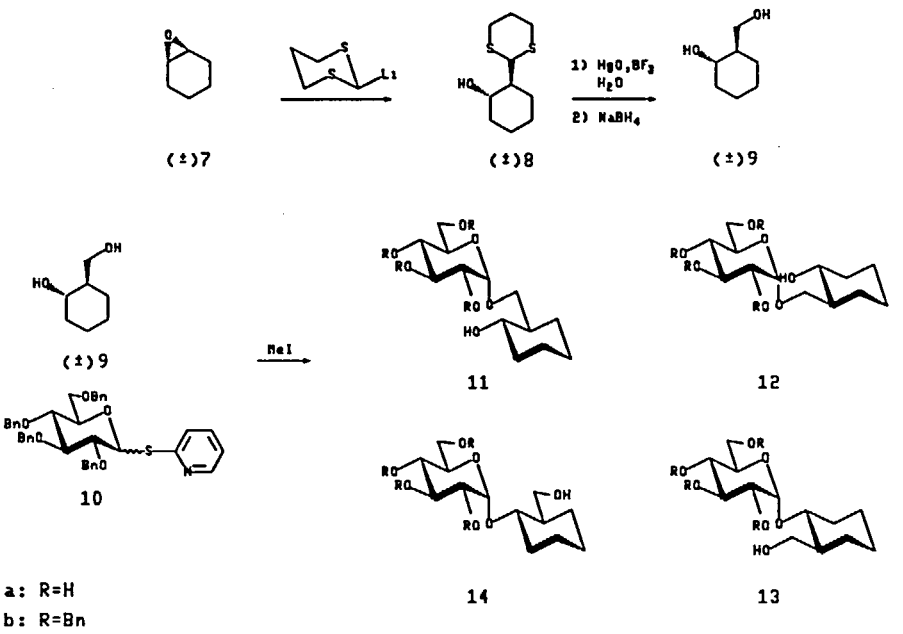
Figure 1

RESULTS AND DISCUSSION

Synthesis of the racemic cyclohexane (±)-**9**, whereby a *trans*-relation of the substituents is obtained, was accomplished by a three step procedure. Opening of cyclohexene oxide (**7**) with lithiodithiane[7] to **8** followed by hydrolysis and subsequent reduction gave (±)-**9** in an overall yield of 69%. Glycosylation was performed using the 2-thiopyridyl glucoside (**10**) in presence of methyl iodide,[8] giving rise to four tetrabenzyl-α-glucosides **11b**, **12b**, **13b** and **14b** in the ratio of approx. 3:3:1:1 and in a combined yield of 47%. Whereas **12b** and **13b** were obtained in homogenous form, **11b** and **14b** proved inseparable by chromatographic means even after acetylation or hydrogenation. An attempt to silylate selectively the primary hydroxy group of **14b** with TBDPSiCl proved unsuccessful as judged from NMR experiments of the product mixture. Hydrogenation proceeded smoothly to provide the unprotected glucosides **11a**, **12a**, **13a** and **14a**. Assignment of the two analogues "6-linked" glucosides ((+/-)-trans-1-hydroxy-2-hydroxymethyl cyclohexanes) to structures **11a** and **12a** were arbitrary due to their similar chemical shifts displayed in both the ^{1}H and ^{13}C NMR spectra. On the other hand, the assignment of **13a** and **14a** proved facile by comparing the chemical shifts of the C1' carbons and H1' protons, respectively, with those of the structurally related compounds **3** and **6** (100.9 (C1'), 5.05 (H1') for **13a** compared to 101.2 (C1'), 5.10 (H1') for **3**, and 94.7 (C1'), 5.12 (Hl') for **14a** compared to 93.8 (Cl'), 5.26 (H1') for **6**).[6]

The relative configuration of compounds **11a** and **12a** however, were determined by acid hydrolysis of the glycosides **12a** and **13a** and benzoylation in pyridine followed by separation of the benzoylated aglycones and measurement of the optical rotations of the two components. These proved to be identical and the configuration of the aglycone in **12a** was therefore assumed to be (4R,5S) based on the assignment of the configuration (4R,5S) of the aglycone in **13a** as determined from ^{13}C-NMR spectroscopy (see above).[6]

Of particular interest was also the distribution of the tetrabenzyl-α-glucosides which could be obtained under thermodynamic control[6]. Hence glycosylation of (±)-**9** with 1-O-acetyl-2,3,4,6-tetra-O-benzyl-*D*-glucopyranose (α/ß mixture: 5/1) using TMSOTf, afforded among other products, **11b**, **12b**, **13b** and **14b** in the ratio of approx. 6:4:4:1, but without substantial improvement in regioselectivity. On the other hand, it is interesting to note that the higher selectivity obtained for the formation of **13b** in comparison to **14b** under thermodynamic control. This is in direct agreement with results earlier obtained in the synthesis of glucoside **3**, when considering the stability of the two products in terms of the *exo*-anomeric effect.

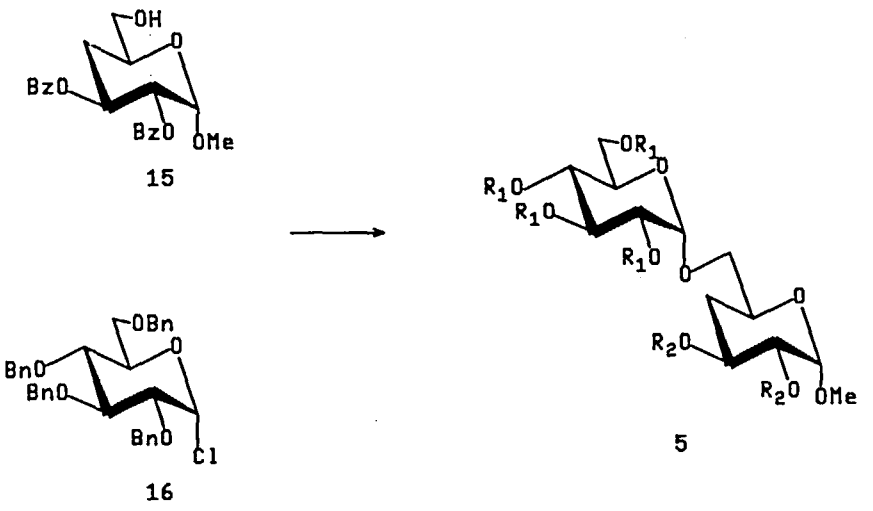


Methyl 4-deoxy-α-isomaltoside was obtained through a glycoside synthesis. Selective tritylation of methyl 4-deoxy-α-D-glucopyranoside,[9] followed by benzoylation and acid hydrolysis provided the methyl 2,3-dibenzoyl-α-glucoside (**15**). Bromide catalyzed glycosylation of **15** with 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl chloride[10] (**16**) gave the disaccharide (**5b**) in 32% yield, which in turn was deprotected to furnish **5a**, characterized as its hexaacetate (**5c**).

Compounds **5a**,**12a**, **13a**, and the mixture of **11a** and **14a** were all subjected to hydrolysis with the enzyme glucoamylase from *Aspergillus niger* under standard conditions[3] where methyl ß-maltoside is completely hydrolysed with a half time for the reaction of about 35 min. None of the compounds tested proved to be hydrolysed to any significant degree under the same experimental conditions during about a week and must therefore all be concluded to be very poor substrates for the enzyme. However, more detailed analysis of the enzyme kinetics of these compounds and of **3** and **6** will be reported in the future.

EXPERIMENTAL

NMR spectra were recorded on a BRUKER AM-500 spectrometer using either $CDCl_3$ or D_2O as solvent at 27 °C. In the latter, acetone (δ 2.225) for ^1H NMR spectra and dioxane



a: $R_1=R_2=H$

b: $R_1=Bn$, $R_2=Bz$

c: $R_1=R_2=Ac$

(67.4 ppm) for ^{13}C NMR spectra were used as internal references. Optical rotations were measured on a Perkin Elmer 241 polarimeter. Column chromatography was performed using Kieselgel 60H for thin-layer chromatography, obtained from the Merck Co. Microanalyses were determined at LEO Pharmaceutical, Ballerup, Denmark.

1-Hydroxy-trans-2-(hydroxymethyl)cyclohexane (±)-9. 1-Hydroxy-*trans*-2-(1,3-dithiolanyl)cyclohexane (**8**) (1.51 g, 6.93 mmol)[7] dissolved in a minimum amount of THF, was added over a period of 10 min to a rigorously stirred mixture of HgO (3.00 g, 13.9 mmol) and BF_3:Et_2O (1.71 mL, 13.9 mmol) in a 15% aqueous solution of THF (20 mL). After stirring for 20 min, ether (30 mL) was added, followed by filtration of the colorless precipitate through a pad of celite. The organic phase was washed with aq. Na_2CO_3 and brine, and then dried ($MgSO_4$) and concentrated to dryness. The residue was redissolved in methanol (60 mL), cooled to 0 °C and $NaBH_4$ (ca. 1 g) was added. Stirring was continued for 2 h at 0 °C. The solution was neutralized with acetic acid and then concentrated to dryness. The residue obtained was extracted with several portions of ethyl acetate. Concentration and chromatography (EtOAc:Hexane, 2:1) gave the diol **9** (695 mg, 77%) as a colorless oil, δ_C [$CDCl_3$] 75.8, 68.0, 45.7, 35.0, 29.5, 27.1, 24.9 and 24.3.

***Glycosylation of diol (±)-9 with 2-(2,3,4,6-tetra-O-benzyl-α,β-D-glucopyranosylthio) pyridine* (10).** A solution of diol (±)-9 (140 mg, 1.08 mmol), 2-(2,3,4,6-tetra-*O*-benzyl-α,β-D-glucopyranosylthio)pyridine (**10**), (α:β, 1:4.2, 725 mg, 1.14 mmol)[8] in CH_2Cl_2 (10 mL) containing 6% MeI, and in the presence of 3Å molecular sieves (500 mg, crushed) was refluxed at 50 °C for 48 h. After filtration through a pad of celite and evaporation, the residue was chromatographed (Hexane:EtOAc, 3:1) to give three fractions. The first fraction consisted of a 4-linked glucosyl analogue contaminated with unreacted 2-thiopyridyl glucoside (**10**). This could be separated by dissolving the mixture in CH_2Cl_2 and treatment with *m*-chloroperbenzoic acid followed by work-up and chromatography (hexane:EtOAc, 3:1) affording the glucoside (+)-trans-1-(2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyloxy)-2-hydroxymethylcyclohexane (**13b**), (40 mg) as a colorless syrup, $[\alpha]^{23}_D$ +12.4° (*c* 1.02, $CHCl_3$), δ_H [$CDCl_3$] 7.43-7.25 (18 H, m), 7.19 (2 H, d, *J* 6,8 Hz), 4.95 (1 H, d, *J* 10.8 Hz, CHPh), 4.87 (1 H, d, *J* 10.8 Hz, CHPh), 4.86 (1 H, d, *J* 10.8 Hz, CHPh), 4.84 (1 H, d, *J* 12.0 Hz, CHPh), 4.83 (1 H, d, *J* 3,8 Hz, H 1'), 4.71 (1 H, d, *J* 12.0 Hz, CHPh), 4.63 (1 H, d, *J* 12.0 Hz, CHPh), 4,51 (1 H, d, *J* 10.8 Hz, CHPh), 4.47 (1 H, d, *J* 12.0 Hz, CHPh), 4.01 (1 H, dd, *J* 9.3 Hz, *J'* 9.3 Hz, H 3'), 3.95 (1 H, dm, *J* 9.8 Hz, H 5'), 3.75 (1 H, dd, *J* 10.5 Hz, *J'* 3.8 Hz, H 6'a), 3.65 (1 H, dd, *J* 9.8 Hz, *J'* 9.3 Hz, H 4'), 3.65-3.58 (3 H, m, H 6'b, H 6a, H 6b), 3.55 (1 H, dd, *J* 9.3 Hz, *J'* 3.8 Hz, H 2'), 3.26 (1 H, ddd, *J* 10.2Hz, *J* 10.2 Hz, *J''*, 3.9 Hz, H 4), 2.25 (1 H, m), 1.75-1.63 (3 H, m), 1.41-1.16 (4 H, m), 1.03-0.95 (1 H, m); δ_C [$CDCl_3$] 99.8, 88.5, 82.2, 79.3, 77.7, 75.7, 74.9, 73.7, 73.4, 70.6, 68.6, 68.5, 45.8, 33.8, 28.3, 25.1 and 24.6 (aromatic signals not included).

Anal. Calcd for $C_{41}H_{48}O_7$: C,75.43 ; H,7.41. Found: C, 75.43; H, 7.48.

The *O*-benzyl groups of glycoside (**13b**) were removed by hydrogenolysis (MeOH, 10 mL; AcOH, 1 mL) by stirring overnight under a hydrogen atmosphere in the presence of Pd/C (50 mg) to give (+)-*trans*-1-(α-D-glucopyranosyloxy)-2-hydroxymethylcyclohexane (**13a**, 16 mg, 90%) as a colorless syrup, δ_H [D_2O] 5.05 (1 H, d, *J* 4.0 Hz, H 1'), 3.84 (1 H, dd, *J* 11.7 Hz, *J'* 1.8 Hz H 6'a), 3.80 (1 H, m, H 5'), 3.76 (1 H, dd, *J* 11.7 Hz, *J'* 5.0 Hz, H 6'b), 3.74 (1 H, dd, *J* 11.0 Hz, *J'* 5.0 Hz, H 6a), 3.68 (1 H, dd, *J* 10.0 Hz, *J'* 9.2 Hz, H 3'), 3.63 (1 H, dd, *J* 11.0 Hz, *J* 5.0 Hz, H 6b), 3.53 (1 H, dd, *J* 10 Hz, *J'* 4.0 Hz, H 2), 3.46 (1 H, ddd, *J* 10.1 Hz, *J'* 10.1 Hz, *J''* 4.3, H 4), 3.40 (1 H , dd, *J* 9.2 Hz, *J'* 9.2 Hz, H 4'), 2.10 (1 H, m), 1.80-1.70 (2 H, m), 1.68-1.59 (2 H, m), 1.46-1.36 (1 H, m), 1.33-1.05 (3 H, m); δ_C [D_2O] 100.9, 83.9, 73.9, 72.7, 72.3, 70.5, 65.2, 61.3, 45.9, 34.2, 28.5, 25.2 and 25.1. Further elution from the column afforded a second fraction consisting of a ca. 3:1 mixture of a "6-linked" (-)-*trans*-1-hydroxy-2-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyloxy)hydroxymethylcyclohexane (**11b**) and "4-linked" glucoside (-)-*trans*-1-(2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyloxy)-2-

hydroxymethylcyclohexane (**14b**) analogue, respectively (162 mg) which were inseparable even after acetylation or after treatment with TBDPSiCl. δ_H [$CDCl_3$] for the major product **11b**, 4.98 (1 H, d, *J* 11.0 Hz, CHPh), 4.88 (1 H, d, *J* 11.0 Hz, CHPh), 4.83 (1 H, d, *J* 11.0 Hz, CHPh), 4.83 (1 H, d, *J* 11.0 Hz, CHPh), 4.68 (1 H, d, *J* 3.8 Hz, H 1'), 4.68 (1 H, d, *J* 12.3 Hz, CHPh), 4.63 (1 H, d, *J* 12.3 Hz, CHPh), 4.52 (2 H, d, *J* 11.7 Hz, CHPh), 4.11 (1 H, bs, OH), 4.00 (1 H, dd, *J* 9.3 Hz, *J'* 9.3 Hz, H 3'), 3.86 (1 H, dd, *J* 9.3 Hz, *J'* 3.8 Hz, H 6), 3.81 (1 H, dm, *J* 9.3 Hz, H 5) 3.73 (1 H, dd, *J* 10.8 Hz, *J'* 4.1 Hz, H 6'), 3.66 (1 H, dd, *J* 10.8 Hz, *J'* 10.5 Hz, H 6'), 3.66 (1 H, dd, *J* 9.3 Hz, *J'* 9.3 Hz, H 4'), 3.58 (1 H, dd, *J* 9.3 Hz, *J'* 3.8 Hz, H 2'), 3.53 (1 H, m, H 4), 3.23 (1 H, dd, *J* 9.3 Hz, *J'* 9.3 Hz, H 6). δ_C [$CDCl_3$] for the major product (**11b**), 97.8, 82.2, 79.6, 77.4, 75.7, 75.3, 74.9, 74.5, 73.4, 73.3, 70.4, 68.4, 43.8, 34.5, 27.2, 25.0 and 24.3 (aromatic signals not included).δ_H [$CDCl_3$] for the minor product (**14b**), 5.08 (1 H, d, *J* 3.9 Hz, H'). δ_C [$CDCl_3$] for the minor product (**14b**), 93.3, 82.2, 79.6, 78.0, 77.4, 75.7 (2C) 75.0, 73.2, 71.2, 68.4, 63.9, 45.3, 30.5, 28.2, 25.3 and 24.6 (aromatic signals not included).

The *O*-benzyl groups of glycosides (**11b**) and (**14b**) were removed by hydrogenolysis (MeOH, 20 mL; AcOH, 3 mL) by stirring overnight under a hydrogen atmosphere in the presence of Pd/C (70 mg) to give the two glucosides **11a** and **14a** (64 mg, 88%) as a colorless syrup. δ_H [D_2O] 4.89 (1 H, d, *J* 3.9 Hz, H 1') for major product **11a**, δ_H [D_2O] 5.12 (1 H, d, *J* 3.8 Hz, H') for minor product **14a**. δ_C [D_2O] for the major product, δ_C [D_2O] 98.8, 74.0, 72.9, 72.6, 72.2, 71.1, 70.4, 61.4, 44.8, 35.2, 28.8, 25.3 and 25.0, and for the minor product δ_C [D_2O] 94.7, 75.8, 73.9, 73.0, 71.9, 70.4, 64.1, 61.3, 45.1, 30.3, 28.6, 25.4 and 24.8.

Further elution from the column afforded a third fraction consisting of a "6-linked" glucoside (+)-*trans*-1-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyloxy)-2-hydroxymethyl cyclohexane (**12b**) as a colorless syrup (126 mg), δ_H [$CDCl_3$] 4.98 (1 H, d, *J* 10.8 Hz, CHPh), 4.87 (1 H, d, *J* 10.8 Hz, CHPh), 4.84 (1 H, d, *J* 10.8 Hz, CHPh), 4.80 (1 H, d, *J* 3.6 Hz, H 1') 4.79 (1 H, d, *J* 12.0 Hz, CHPh), 4.70 (1 H, d, *J* 12.0 Hz, CHPh), 4.63 (1 H, d, *J* 12.0 Hz, CHPh), 4.52 (1 H, d, *J* 10.8 Hz, CHPh), 4.51 (1 H, d, *J* 12.0 Hz, CHPh), 3.97 (1 H, dd, *J* 9.3 Hz, *J'* 9.3 Hz, H 3'), 3.81 (1 H, dm, *J* 9.3 Hz, H 5'), 3.75 (1 H, dd, *J* 10.5 Hz, *J'* 3.8 Hz, H 6'a), 3.68 (1 H, dd, *J* 10.5 Hz, *J'* 5.0 Hz, H 6'b), 3.67 (1 H, dd, *J* 10.5 Hz, *J'* 4.5 Hz, H 6), 3.66 (1 H, dd, *J* 9.3 Hz, *J'* 9.3 Hz, H 4'), 3.60 (1 H, dd, *J* 10.5 Hz, *J'* 4.5 Hz, H 6), 3.59 (1 H, dd, *J* 9.3 Hz, *J'* 3.6 Hz, H 2'), 3.54 (1 H, ddd, *J* 10.2 Hz, *J'* 10.2 Hz, *J''* 4.2 Hz, H 4), 3.44 (1 H, bs, OH), 2.04 (1 H, m) 1.79 (1 H, m), 1.74-1.60 (3 H, m), 1.37-1.19 (3 H, m) and 1.02 (1 H, m). δ_C [$CDCl_3$] 96.9, 82.1, 79.7, 77.5, 75.7, 75.0, 73.9, 73.4, 73.3, 72.3, 70.5, 68.5, 43.6, 34.8, 27.7, 25.2 and 24.4 (aromatic signals not included).

The *O*-benzyl groups of glycoside (**12b**) were removed by hydrogenolysis (MeOH, 15 mL; AcOH, 2 mL) by stirring overnight under a hydrogen atmosphere in the presence of Pd/C (50 mg) to give (-)-*trans*-1-(α-D-glucopyranosyloxy)-2-hydroxymethylcyclohexane (**12a**) (51 mg, 91%) as a colorless syrup. δ_H (D_2O) 4.90 (1 H, d, *J* 3.8 Hz, H 1'), 3.85 (1 H, dd, *J* 12.1 Hz, *J'* 1.8 Hz, H 6'a) 3.79 (1 H, dd, *J* 10.0 Hz, *J'* 3.8 Hz, H 6a), 3.75 (1 H, dd, *J* 12.1 Hz, *J'* 5.3 Hz, H 6'b), 3.71 (1 H, dd, *J* 9.2 Hz, *J'* 9.2 Hz, H 3'), 3.68 (1 H, m, H 5), 3.57 (1 H, dd, *J* 10.0 Hz, *J'* 5.7 Hz, H 6b), 3.55 (1 H, dd, *J* 9.2 Hz, *J'* 3.8 Hz, H 2), 3.53 (1 H, ddd, *J* 10.2 Hz, *J'* 10.2 Hz, *J* 4.7 Hz, H 4), 3.40 (1 H, dd, *J* 9.2 Hz, *J'* 9.2 Hz, H 4'), 1.95 (1 H, m), 1.82 (1 H, m), 1.73 (1 H, m), 1.65 (1 H, m), 1.56 (1 H, m), and 1.32-1.16 (4 H, m); δ_C [D_2O] 99.3, 74.0, 72.7, 72.4, 71.9, 70.7, 70.5, 61.4, 45.4, 35.2, 29.0, 25.6 and 25.2.

Determination of the relative configuration of 11a and 12a. The glucosides **12a** and **13a** were individually subjected to hydrolysis by refluxing in N HCl for 4 h. After concentration the residue was benzoylated using standard conditions. The benzoylated aglycones were isolated by chromatography (hexane:EtOAc 6:1) and their optical rotations measured. The dibenzoate (-)-*trans*-1-*O*-benzoyl-2-benzoyloxymethylcyclohexane obtained from **12a** gave $[\alpha]^{23}_D$ -77.4° (*c* 0.19, $CHCl_3$) and similarly the aglycone from **13a** gave $[\alpha]^{23}_D$ -78.5° (*c* 0.27, $CHCl_3$). NMR data for the benzoylated aglycone: δ_H [$CDCl_3$] 5.06 (1 H, ddd, *J* 10.5, 10.5, 4.5 Hz, H1), 4.49 (1 H, dd, *J* 12.0, 4.5 Hz, H7a), 4.30 (1 H, dd, *J* 12.0, 6.0 Hz, H7b), 2.25 (1 H, m, H2), 1.2-2.4 (8 H, m, H3, H4, H5 and H6), δ_C [$CDCl_3$], 74.3 C1; 42.0, C2; 31.8, 28.4, 24.9 and 24.4, C3, C4, C5 and C6; 66.1 C7.

Glycosylation of diol (±)-9 under thermodynamic control. A solution of diol (±)-9 (32mg, 0.24 mmol) and 1-*O*-acetyl-2,3,4,6-tetra-*O*-benzyl-D-glucopyranose (an α:ß mixture of 5:1), 150 mg, 0.26 mmol) dissolved in CH_2Cl_2 (3 mL) in the presence of 3A molecular sieves (400 mg, crushed) was stirred for 1h at 22 °C. The mixture was cooled to -22 °C and trimethylsilyl triflate (53 µL, 0.29 mmol) was injected followed by slowly warming to 22 °C (1 h) and the stirring for an additional 3 h. After filtration through a pad of celite, the filtrate was washed with aqueous $NaHCO_3$(sat.), dried ($MgS\Theta_4$) and concentrated to dryness. The product distributions were obtained by measuring the integrals of the carbon chemical shifts of the anomeric carbon atoms.

Methyl 2,3-Di-O-benzoyl-4-deoxy-α-D-glucopyranoside (15). A solution of methyl 4-deoxy-α-*D*-glucopyranoside[9] (500 mg, 2.81 mmol) and triphenylmethyl bromide (3 g, 9.29 mmol) in pyridine (20 mL) was stirred at 60-65 °C for 3.5 h. The reaction was followed by TLC with ethyl acetate as eluant. The reaction mixture was cooled in an ice-bath and benzoyl chloride (1 mL 8.67 mmol) in pyridine (9 mL) was added, the mixture was stirred for 3 days, and then

filtered and concentrated. The residue was stirred with ice water and extracted 3 times with dichloromethane (3x30 mL). The organic phase was washed with 2M HCl (10 mL), $NaHCO_3$(aq) and water, dried (Na_2SO_4) and concentrated yielding crude methyl 2,3-di-*O*-benzoyl-4-deoxy-6-*O*-triphenylmethyl-α-D-glucopyranoside together with triphenyl carbinol (3 g). The mixture (1.6 g) was dissolved in acetic acid (50 mL) and water (20 mL) and heated on a steam bath for 2 h and filtered after cooling. The filtrate was washed with hexane (5x25 mL) to remove triphenyl carbinol. The acetic acid-water phase was concentrated and the resulting syrup was purified by preparative TLC by elution with EtOAc-hexane (1:1) and **15** was isolated and only characterized by its NMR data. ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 5.14(1H,d, *J* 3.6 Hz, H-1), 5.24(1H, dd, *J* 3.6, 10.1 Hz, H-2), 5.76(1H, ddd, *J* 10.1, 5.3 and 12.7 Hz, H-3), 2.30(1H, ddd, *J* 5.3, 12.7, 2.6 Hz, H-4a), 1.84(1H, q, *J* 11.0, 12.7, 12.2 Hz, H-4b), 4.08(1H, m, H-5), 3.75(1H, dd, *J* 3.0, 12.0 Hz, H-6a), 3.64(1H, dd, *J* 6.0, 12.0 Hz, H-6b), 3.42, OMe. ^{13}C-NMR (125.7 MHz, $CDCl_3$) δ_C 97.9, C1; 72.8, C2; 68.6, C3; 32.4, C-4; 67.8, C5; 64.9, C6; 55.3, OMe.

Methyl 6-O-(2,3,4,6-Tetra-O-benzyl-α-D-glucopyranosyl)-2,3-di-O-benzoyl-4-deoxy-α-D-glucopyranoside (5b). A solution of **15** (267 mg, 0.69 mmol) and methyl ammonium bromide (313 mg, 1.49 mmol) in dichloromethane (1.5 mL) and DMF (0.4 mL) was stirred with 4 Å molecular sieves (1 g) under nitrogen for 1 h. A solution of freshly prepared 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl chloride[10] (**16**) (900 mg, 1.61 mmol) in dichloromethane (1 mL) was added, and the mixture was stirred for 22 h. The mixture was diluted with dichloromethane (25 mL) and stirred for 1 h and then filtered. The filtrate was diluted with dichloromethane (25 mL) and the solution was washed with hydrogen chloride (0.1 M), a solution of sodium hydrogen carbonate, and water followed by drying ($MgSO_4$) and concentration. The crude product (1.21 g) was purified by preparative TLC by elution with EtOAc-hexane 1:2 and was isolated as a syrup (200 mg, 31.8%).

De-*O*-benzoylation of **5b** (200 mg, 0.22 mmol) in methanol (5 mL) and sodium methoxide in methanol (1 mL 1M) gave a syrup (137 mg, 0.20 mmol, 89%), which was debenzylated as follows. The product (137 mg, 0.20 mmol) in methanol (20 mL) and acetic acid (4 mL) was added 5% palladium on activated carbon (40 mg). The mixture was stirred for 20 h at 100 kPa hydrogen pressure. The mixture was filtered and the catalyst was washed well with methanol. The combined filtrate and washings were concentrated and water (5 mL) was evaporated twice. The crude product (86 mg) was dissolved in pyridine (4 mL) and acetic anhydride and the solution left at room temperature for 16 h followed by concentration. Toluene (5 mL) was coevaporated twice. The product (149 mg) was purified by preparative TLC by elution with EtOAc-hexane 1:1 and methyl 6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl)-2,3-di-*O*-acetyl-

4-deoxy-α-*D*-glucopyranoside (**5c**) was isolated (50 mg, 0.08 mmol) together with some of its 4'-benzyl-derivative (40 mg). Compound **5c** was a syrup with $[\alpha]^{23}_{D}$ 167.6° (*c* 1.918, $CHCl_3$), and further characterized by its NMR data. ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 5.07(1H, d, *J* 3.7 Hz, H-1'), 4.81(1H, dd, *J* 3.7, 10.3 Hz, H-2'), 5.40(1H, t, *J* 10.3, 10.3 Hz, H-3'),4.98(1H, t, *J* 10.3, 10.3 Hz, H-4'), 4.03-4.07(2H, m, H-5', H-6'$_b$), 4.15(1H, dd, *J* 2.5, 12.7 Hz, H-6'$_a$), 4.83(1H, d, *J* 3.7 Hz, H-1), 4.74 (1H, dd, *J* 3.7, 10.3 Hz, H-2), 5.22(1H, ddd, *J* 5,4, 10.3, 12.0 Hz, H-3), 2.08(1H, ddd, *J* 5.4, 12.0, 2.4 Hz., H-4e), 1.54(1H, q, *J* 12.0, 12.0, 12.0 Hz., H-4$_a$), 3.97(1H, m, H-5), 3.62(1H, dd, *J* 5.8, 11.0 Hz., H-6$_a$), 3.47(1H, dd, *J* 3.2, 11.0 Hz., H-6$_b$). ^{13}C-NMR (125.7 MHz, $CDCl_3$): 95.9 ppm, C1'; 70.7, C2'; 70.1, C3'; 68.5, C4'; 67.4, C5'; 61.9, C6'; 97.4, C1; 71.9, C2; 67.7, C3; 32.6, C4; 66.1, C5; 69.4, C6; 55.2, COMe.

Anal. Calcd for $C_{25}H_{36}O_{16}$: C,50.66 ; H,6.08. Found: C, 50.63; H, 6.44.

Metkyl 6-O-(α-D-glucopyranosyl)-4-deoxy-α-D-glucopyranoside (5a). De-*O*-acetylation of **5c** (50 mg, 0.08 mmol) in methanol (10 mL) and sodium methoxide in methanol (0.2 mL 1M) gave crude **5a** which was purified by chromatography on Sephadex G-15 column using methanol:water, 1:1 as eluant. This gave **5a** as a pure syrup (25 mg, 0.07 mmol), which was characterized by its NMR data. ^{1}H NMR (500 MHz, D_2O) δ_H 4.95(1H, d, *J* 3.8 Hz., H-1'), 3.57(1H, dd, *J* 3.8, 10.0 Hz., H-2'), 3.74(1H, t, *J* 10.0, 10.0 Hz., H-3'), 3.43(1H, t, *J* 10.0, 10.0 Hz., H-4'), 3.70(1H, m, H-5'), 3.87(1H, dd, *J* 2.4, 12.5 Hz, H-6'a), 3.78(1H, dd, *J* 4.8, 12.5 Hz., H-6'b), 4.86(1H, d, *J* 3.8 Hz., H-1'), 3.51(1H, dd, *J* 3.8, 9.8 Hz., H-2), 3.93(1H, ddd, *J* 9.8, 11.9, 5.2 Hz., H-3), 1.56(1H, ddd, *J* 11.9, 12.5, 11.3 Hz., H-4a), 2.04(1H, ddd, *J* 5.2, 12.5, 2.1 Hz, H-4e), 4.12(1H, m, H-5), 3.79(1H, dd, *J* 4.8, 11.4 Hz., H-6a), 3.66(1H, dd, *J* 3.3, 11.4 Hz., H-6b). ^{13}C-NMR (125.7 MHz, D_2O) δ_C 98.7ppm, C1'; 72.2, C2'; 73.8, C3'; 70.4, C4'; 72.7, C5'; 61.3, C6'; 100.8, C1; 73.7, C2; 68.1, C3; 35.0, C4; 67.8, C5; 69.7, C6; 55.9, OMe.

ACKNOWLEDGEMENTS

This work was supported by a grant (TS) from The Danish Technical Science Research Council. The 500 MHz NMR facilities were provided by the Danish Natural Science Research Council and The Carlsberg Foundation.

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