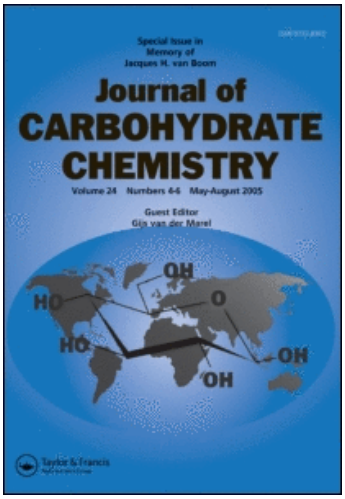

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ABSOLUTE CONFIGURATION OF 4-α-D-GLUCOPYRANOSYL-*MYO*-INOSITOL, ENZYMATIC TRANSGLYCOSYLATION PRODUCT

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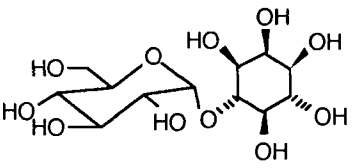
ABSTRACT

The absolute configuration of 4-α-D-glucopyranosyl-*myo*-inositol, one of the enzymatic transglycosylation products from cyclodextrin to *myo*-inositol, was confirmed by chemical synthesis. The glycosylation was shown to proceed in a highly diastereoselective manner by HPLC analysis of the chemically synthesized racemic glycoside.

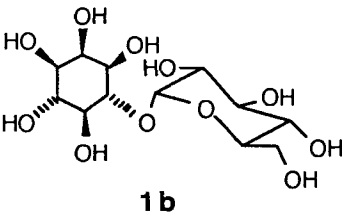
INTRODUCTION

Recently we reported the synthesis of oligoglucosyl-inositols by transglucosylation of the cyclomaltodextrin glucanotransferase (CGTase from *Bacillus ohbensis*) using *myo*-inositol as the acceptor and β-cyclodextrin as the donor.[1] Structures of the products were

determined mainly by ^1H NMR spectroscopy. Among these glucosides, however, the absolute configuration of the inositol part in 4-α-D-glucopyranosyl-*myo*-inositol **1** could not be determined by NMR techniques since *myo*-inositol itself has a symmetry plane in which C-2 and C-5 are involved. We report here that the stereochemistry of the glycoside has been determined to be **1a**, and not **1b**, by an unambiguous chemical synthesis.



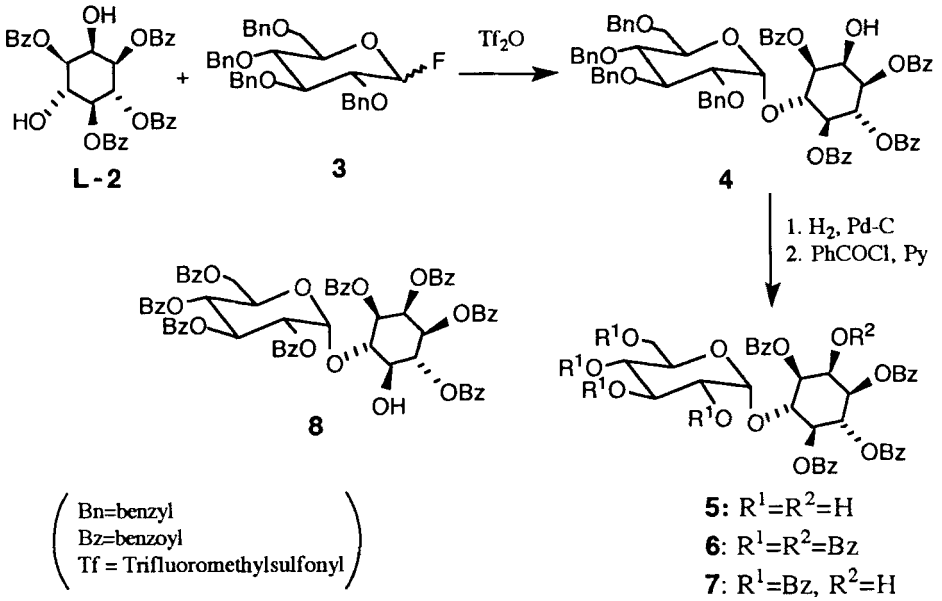
1a



1b

RESULTS AND DISCUSSION

We previously prepared optically active 1L- and 1D-1,3,4,5-tetra-*O*-benzoyl-*myo*-inositol (**L-2** and **D-2**) by kinetic resolution using methyl L- and D-2,3-*O*-cyclohexylidenetartrate respectively.[2] The former **L-2** is a suitable starting material for derivatization to optically active nonabenzoate **6** which can be utilized for determination of the absolute configuration of **1a**. Thus, **L-2** with 74% ee (**L-2**/**D-2** 87 : 13)[3] was selectively glucosylated at C-6 by reaction with 2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl fluoride[4] (α/β 1:1) in the presence of trifluoromethanesulfonic anhydride[5] to give the α-D-glucoside **4** in 76% yield together with β-D-glucoside (*ca.* 11% yield).[6] Removal of the benzyl groups in **4** by hydrogenolysis on Pd-C followed by exhaustive benzoylation gave nonabenzoate **6** (66% yield), accompanied by the 2-hydroxy derivative **7**. On the other hand, the enzymatic glycosylation product[1] **1a** was benzoylated at 60 °C to afford **6** in 96% yield while the reaction at room temperature gave 5-hydroxy derivative **8** in 86% yield as a major product along with 11% yield of **6**. Interestingly, **7** was not formed in this case. The benzoate **6** thus derived from **1a** showed an R_f value on silica gel TLC and spectra (IR and ^1H NMR) identical with those of chemically synthesized major diastereomer **6**. The other diastereomer gave a different ^1H NMR spectrum by comparison with the NMR spectrum of a 1 : 1 diastereomeric mixture of **DL-6** derived from racemic 1,3,4,5-tetrabenzoate **DL-2**. Consequently, the absolute configuration of the glycoside **1** has been unambiguously confirmed to be **1a**.



Scheme 1

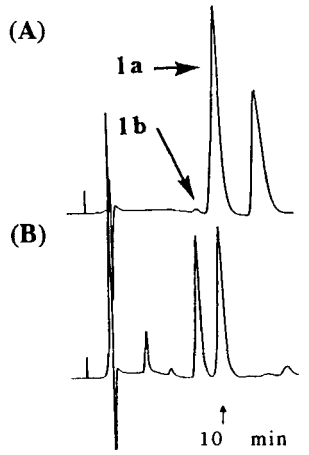
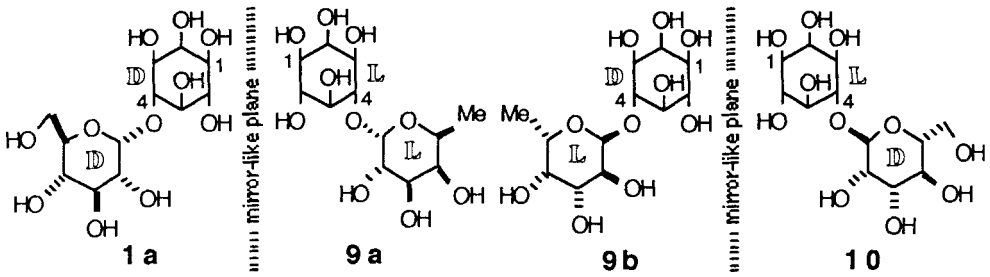


Chart 1. HPLC Profiles of Enzyme-catalyzed (A) and Synthesized (B) Products. The last peak in (A) corresponds to 5-*O*-α-D-glucopyranosyl-*myo*-inositol.

Evaluation of Diastereoselectivity of the Enzyme-Catalyzed Glycosylation. Deprotection of **DL-6** with sodium methoxide afforded **1** quantitatively. The HPLC analysis of **1** showed equal amounts of two peaks. The latter agreed with the larger peak of the enzyme-catalyzed glycosylation product **1a** and the ratio of **1a** and **1b** was 98.8 : 1.2. Consequently, the enzymatic transglucosylation affording **1a** has now been proved to proceed in a highly diastereoselective way.

Comparison of ^{13}C NMR Spectra of 4-Glycosyl-*myo*-inositol Derivatives. The carbon-13 resonances for the inositol ring in **1a** resembled quite closely those from 1L-4-*O*-α-L-fucopyranosyl-*myo*-inositol[7] **9a** which is the product glycosylated at the

enantiomericaly opposite C-4 position of inositol. Chemical shifts of C-3 and C-5 in **9a** were especially different from those in the corresponding diastereomer **9b** as shown in Table 1 and comparison of these shifts seems to be diagnostic for determining absolute configuration of 4-*O*-glycosyl *myo*-inositols. However, it should be noted that accordance of chemical shifts is not related to similarity of the absolute configuration since introduction of a chiral substituent on the hydroxyl group at C-4 in *myo*-inositol results in the formation of diastereomers. It is interesting to point out that there exists an enantiomer-like relation between **1a** and **9a** as illustrated in Scheme 2. This relation might be expressed by the use of the configurational symbols D and L. Thus, **1a** is expressed as D-D when the former and the latter symbols refer to the designations of *myo*-inositol and sugar moieties, respectively. Consequently, **9a** is shown as L-L. A similar enantiomeric relation was observed between 1D-4-*O*-α-L-fucopyranosyl-*myo*-inositol **9b**[7] (= D-L) and 1L-4-*O*-α-D-glucopyranosyl-*myo*-inositol **1b** (= L-D) which was separated from **1a** by preparative HPLC of **1** (Table 1). Furthermore, **9b** (= D-L) showed chemical shifts of inositol carbons similar to those of 1L-4-*O*-α-D-mannopyranosyl-*myo*-inositol **10**[8] (= L-D).



Scheme 2. Enantiomeric relations between **1a** and **9a** and between **9b** and **10**

Table 1. ^{13}C NMR Spectral Data for Inositol Ring Carbons in Compounds **1a**, **1b**, **9a**, **9b**, and **10**

	C-1	C-2	C-3	C-4	C-5	C-6
1a	71.9	73.3	70.7	81.8	75.4	73.1
9a	71.8	73.0	70.6	81.6	75.4	73.3 (Ref. 7)
1b	71.8	73.0	72.4	81.7	73.7	73.4
9b	71.8	73.0	72.5	81.3	73.7	73.3 (Ref. 7)
10	72.0	73.5	72.6	80.8	73.9	73.6 (Ref. 8)

EXPERIMENTAL

General Procedures. NMR spectra (^1H and ^{13}C) were recorded on a JEOL GSX-270 and GSX-500. IR spectra were recorded on a Hitachi EPI-G3. Optical rotations were measured with a Union PM-101. Elemental analyses were performed with a Perkin-Elmer 240C. Thin layer chromatographic analyses were performed on Merck pre-coated plates, Silica Gel 60 F254. Anhydrous reaction atmosphere was achieved by nitrogen gas. Extracts obtained after work-up were dried over $MgSO_4$.

1L-1,3,4,5-Tetra-*O*-benzoyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-*myo*-inositol (4). A solution of 2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl fluoride[4] (**3**, α/β 1:1, 70 mg, 0.13 mmol) and 1L-1,3,4,5-tetra-*O*-benzoyl-*myo*-inositol (**L-2**, 74% ee, 38 mg, 0.063 mmol) in diethyl ether (2 mL) containing powdered 4A molecular sieves was cooled to -20 °C and trifluoromethane-sulfonic anhydride (10.6 μL, 0.063 mmol) was added. The mixture was stirred at room temperature for 18 h, filtered, and diluted with AcOEt. The organic solution was washed with water, aqueous $NaHCO_3$, and brine, dried, and concentrated. The oily residue was chromatographed on silica gel-coated glass plate (ethyl acetate-hexane, 2 : 5) to give **4** (54 mg, 76%) as an amorphous mass, R_f 0.29 (ethyl acetate-hexane, 2 : 5) and β-glycoside, R_f 0.38 (<7 mg, *ca.* 11%) which was contaminated with unidentified products.

4: mp 163-169.5 °C (MeOH and hexane: Diastereomeric ratio did not change before and after recrystallization); $[\alpha]_D^{21}$ 8.4° (*c* 1.4, $CHCl_3$), IR ($CHCl_3$ solution) 3650, 1730 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 3.12 (m, 1H, *J* =10.7Hz), 3.25 (dd, 1H, *J* =9.8 and 3.4 Hz), 3.47 (m, 1H), 3.49 (t, 1H, *J* =9.8 Hz), 3.78 (m, 1H), 3.86 (t, 1H, *J* =9.8 Hz), 3.88-4.78 (complex, 8H), 4.63 (t, 1H, *J* =3.1 Hz), 4.68 (t, 1H, *J* =9.5 Hz), 4.88 (d, 1H, *J* =3.4 Hz, anomeric α-proton), 5.53 (dd, 1H, *J* =9.5 and 3.1 Hz), 5.54 (dd, 1H, *J* =9.5 and 3.1 Hz), 5.92 (t, 1H, *J* =9.5 Hz), 6.36 (t, 1H, *J* =9.5 Hz), 6.95-7.56 (complex, 32H), and 7.83-8.08 (complex, 8H); ^{13}C (67.8 MHz) δ 67.71, 69.00, 69.87, 71.47, 71.99, 72.16, 72.47, 72.94, 73.21, 74.57, 75.38, 77.21, 77.57, 79.35, 81.34, 99.87, 127.25, 127.36, 127.45, 127.49, 127.77, 127.92, 128.00, 128.09, 128.16, 128.21, 128.24, 128.32, 128.43, 128.91, 128.99, 129.63, 129.77, 129.80, 129.93, 132.81, 133.06, 133.24, 133.41, 137.93, 138.44, 138.55, 138.81, 165.30, 165.40 (2C), and 165.82.

Anal. Calcd for $C_{68}H_{62}O_{15}$: C, 72.97; H, 5.68. Found: C, 72.59; H, 5.58.

1L-1,3,4,5-Tetra-*O*-benzoyl-6-*O*-α-D-glucopyranosyl-*myo*-inositol (5). Benzyl ether **4** in methanol and dichloromethane (1 mL each) was stirred with 5% Pd-C

(212 mg) for 17 h at room temperature under a hydrogen atmosphere. Filtration and concentration to dryness gave tetrabenzoate **5** quantitatively (one spot on silica gel TLC); R_f 0.84 (MeOH-CH_2Cl_2 1 : 2); IR (nujol) 3300, 1700 cm^{-1}; ^1H NMR (270 MHz, CD_3OD-$CDCl_3$ 1/5) δ 3.11-3.42 (complex, 6H), 4.62 (t, 1H, J = 2.4 Hz), 4.85 (t, 1H, J = 10.1 Hz), 5.15 (d, 1H, J = 3.7 Hz), 5.50 (dd, 1H, J = 10.1 and 2.4 Hz), 5.60 (dd, 1H, J = 10.1 and 2.4 Hz), 5.93 (t, 1H, J = 10.1 Hz), 6.23 (t, 1H, J = 10.1 Hz), 7.20-7.65 (complex, 12 H), and 7.74-8.22 (complex, 8H).

1L-1,2,3,4,5-Penta-*O*-benzoyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl)-*myo*-inositol (6) and 1L-1,3,4,5-Tetra-*O*-benzoyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl)-*myo*-inositol (7). A mixture of **5** (35.1 mg, 0.046 mmol), benzoyl chloride (161 μL, 1.388 mmol), and 4-dimethylaminopyridine (a catalytic amount) in pyridine (2 mL) was stirred overnight at room temperature. After addition of H_2O, the mixture was stirred for an additional 10 min to decompose the excess of the chloride and diluted with AcOEt. The organic solution was washed with aqueous $KHSO_4$, aqueous $NaHCO_3$, and brine, dried, and concentrated. The residue was purified by preparative TLC (benzene-acetone, 10 : 1) to afford nonabenzoate **6** (39 mg, 66% yield) and octabenzoate **7** (12.5 mg, 23% yield).

6 (diastereomeric ratio, 77 : 23 as analyzed by ^1H NMR): R_f 0.49 (benzene-acetone, 12 : 1); mp 120-28 °C (from MeOH/hexane), IR (nujol) 1720 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) for the major diastereomer δ 3.67 (m, 1H), 4.34 (complex, 2H), 4.99 (t, 1H, J = 10.1 Hz), 5.21 (dd, 1H, J = 9.8 and 3.7 Hz), 5.57 (t, 1H, J = 9.8 Hz), 5.71 (d, 1H, J = 3.7 Hz, 1H), 5.79 (dd, 1H, J = 10.1 and 3.1 Hz), 5.91 (t, 1H, J = 9.8 Hz), 5.96 (dd, 1H, J = 10.1 and 3.1 Hz), 6.00 (t, 1H, J = 10.1 Hz), 6.22 (t, 1H, J = 10.1 Hz), 6.27 (t, 1H, J = 3.1 Hz), and 7.16-8.19 (complex, 45 Hz).

Anal. Calcd for $C_{75}H_{58}O_{20}$: C, 70.42; H, 4.59. Found: C, 70.42; H, 4.61.

7: R_f 0.41 (benzene-acetone, 12 : 1); IR ($CHCl_3$) 3500, 1720 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 3.99 (dd, 1H, J = 12.5 and 4.9 Hz), 4.42 (dd, 1H, J = 12.5 and 2.4 Hz), 4.52 (m, 1H), 4.72 (t, 1H, J = 2.4 Hz), 5.08 (t, 1H, J = 10.1 Hz), 5.15 (dd, 1H, J = 10.4 and 3.7 Hz), 5.46 (t, 1H, J = 10.4 Hz), 5.51 (dd, 1H, J = 10.1 and 2.4 Hz), 5.71 (d, 1H, J = 3.7 Hz), 5.75 (dd, 1H, J = 10.1 and 2.4 Hz), 5.90 (t, 1H, J = 10.4 Hz), 5.91 (t, 1H, J = 10.1 Hz), 6.20 (t, 1H, J = 10.1 Hz), 7.14-7.65 (complex, 24H), and 7.65-8.18 (complex, 16H).

Benzoylation of Enzymatic Glucosylation Product 1a. A pyridine (1 mL) solution of **1a** [21 mg, 0.061 mmol, obtained by recrystallization from H_2O-EtOH for removal of a trace of **1b** and subsequent preparative HPLC using Carbonex™ (*vide infra*)], benzoyl chloride (101.7 μL, 0.88 mmol), and a catalytic amount of 4-

dimethylaminopyridine was stirred for 12 h at room temperature. After further addition of benzoyl chloride (407 μL, 3.51 mmol) the mixture was stirred at 60 °C for 10 h. The work-up procedure and preparative TLC similar to the above mentioned benzoylation procedure yielded nonabenzoate **6** (75.5 mg, 96% yield).

6 (diastereomericaly pure): $[\alpha]_D^{31}$ +65.5° (*c* 1.05, $CHCl_3$); mp 141-2 °C (from benzene). The ^1H NMR spectrum of the compound **6** was identical with that of the predominant diastereomer **6** (the structure illustrated in the Scheme 1) derived chemically from tetrabenzoate **L-2**. The NMR data of the latter **6** are shown above.

When **1a** (50 mg, 0.146 mmol) in pyridine (2 mL) was treated with benzoyl chloride (254.4 μL, 2.192 mmol) and 4-dimethylaminopyridine (a catalytic amount) at room temperature for *ca.* 30 h, 1L-1,2,3,4-tetra-*O*-benzoyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl)-*myo*-inositol (**8**) (148 mg, 86% yield) and **6** (21 mg, 11% yield) were isolated [preparative TCL (benzene-acetone 12 : 1)].

8: R_f 0.41 (benzene-acetone, 12 : 1); mp 180-1 °C (benzene); $[\alpha]_D^{31}$ +68° (*c* 1.02, $CHCl_3$), IR (nujol) 3500, 1720 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 3.75 (dd, 1H, *J* = 12.2 and 2.4 Hz), 4.12 (dd, 1H, *J* = 12.2 and 2.1 Hz), 4.13 (t, 1H, *J* = 10.1 Hz), 4.25 (m, 1H), 4.53 (t, 1H, *J* = 10.1 Hz), 5.35 (dd, 1H, *J* = 10.1 and 3.7 Hz), 5.66 (t, 1H, *J* = 10.1 Hz), 5.67 (dd, 1H, *J* = 10.1 and 2.8 Hz), 5.74 (dd, 1H, *J* = 10.1 and 2.8 Hz), 5.87 (t, 1H, J = 10.1 Hz), 5.92 (d, 1H, *J* = 3.7 Hz), 6.07 (t, 1H, *J* = 10.1 Hz), 6.17 (t, 1H, *J* = 2.8 Hz), 7.20-7.57 (complex, 24H), and 7.64-8.10 (complex, 16H).

Anal. Calcd for $C_{68}H_{54}O_{19}$: C, 69.50; H, 4.63. Found: C, 69.24; H, 4.72.

DL-4-*O*-α-D-Glucopyranosyl-*myo*-inositol (1) from Nonabenzoate DL-6 and Evaluation of Diastereoselectivity of the Glycosylation. To a suspension of benzoate **DL-6** (derived from racemic tetrabenzoate **DL-2**) in MeOH (5 mL) was added 60% NaH in mineral oil (18.6 mg, 0.465 mmol) and the mixture was stirred at room temperature overnight and then NaH (18.6 mg, 0.465 mmol) was further added. The solution was stirred for 2 days and neutralized by addition of cation exchange resin [Diaion SK1B (Mitsubishi Chemical Industries Ltd.), H^+-form]. The resin was filtered and washed with distilled water. The filtrate was passed through anion exchange resin (Diaion SA11A, OH^-- form) and washed with distilled water, and concentrated. The residue was washed with hexane, dichloromethane, and ethyl acetate, and dried under reduced pressure to afford **1** (14.5 mg, quant.); ^1H NMR (270 MHz, $CDCl_3$) δ 3.10-3.94 (complex, 12H), 5.11 (d, 1H, *J* = 3.7 Hz) , and 5.16 (d, 1H, *J* = 3.7 Hz). HPLC analysis of **1** and **1a** was carried out by the use of Hypercarb™ (Shandon Ltd., 100 x 4.7 mm i.d., other conditions; eluent: CH_3CN-H_2O 1 : 99, flow rate: 0.8 mL/min, column temperature: 40 °C). The ratio of **1a** and **1b** in the fraction of mono-glucosylated *myo*-inositols obtained by the enzyme-catalyzed glucosylation was determined to be 98.8 : 1.2.

Separation of DL-4-*O*-α-D-Glucopyranosyl-*myo*-inositol (1) to 1a and 1b by Preparative HPLC. Diastereomeric mixture of **1** thus obtained in the preceding experiment was separated by HPLC on a graphitized carbon column [Column: Carbonex™ (Tonen Ltd., 150 x 10 mm i.d.), eluent: CH_3CN-H_2O 3 : 97, flow rate: 0.7 mL/min, column temperature: ambient] to give **1a** and **1b**.

1b: $[\alpha]_D^{24}$ +55.6° (*c* 0.45, H_2O); ^1H NMR (270 MHz, D_2O, HOD as the reference=4.64 ppm) δ 3.22-3.69 (complex, 10H), 3.89 (dt, 1H, *J* =10.36 and 3.59 Hz), 3.92 (t, 1H, *J* =2.75 Hz), 5.15 (d, 1H, *J* =3.97 Hz); ^{13}C NMR (125.65 MHz) δ 61.26, 70.27, 71.81, 72.35, 72.59, 72.77, 73.00, 73.39, 73.74, 73.89, 81.75, and 100.28.

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