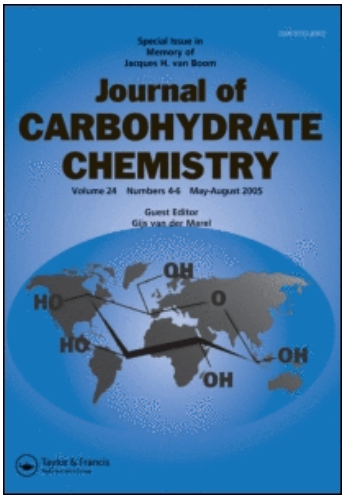

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CONFORMATIONAL ANALYSIS OF HYDROXYMETHYL GROUP OF D-MANNOSE DERIVATIVES USING (6S)- AND (6R)-(6-$^2H_1$)-D-MANNOSE

Hiroshi Hori, Yoshihiro Nishida, Hiroshi Ohrui,* and Hiroshi Meguro*

Department of Food Chemistry, Faculty of Agriculture, Tohoku University
Tsutsumidori-Amamiyamachi 1-1, Aoba-ku, Sendai 981, Japan

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ABSTRACT

D-Mannose derivatives stereospecifically deuterated at C-6 were synthesized and the unequivocal ^1H NMR assignments of H-6*proS* and H-6*pro R* were made. The preferred conformation of the hydroxymethyl groups of these compounds are discussed.

INTRODUCTION

Recently much interest has been focused on the chemistry and physiology of glycoproteins.[1] The conformational properties of their carbohydrate portions have been extensively studied[2] using NMR spectroscopy and theoretical calculations. Knowledge of the three-dimentional structures of the carbohydrate moieties is essential to an understanding of the biological activities of the glycoproteins. Conformational analysis around the C5-C6 bond or the (1-6)-glycosidic linkages of hexoses in solution is very difficult, compared with that of other glycosidic linkages involving secondary hydroxy groups, due to the difficulty in making the ^1H NMR assignments of the two protons H-6*proS* and H-6*pro R*. The ambiguous assignments of these two protons might lead to misinterpretation of the rotamer populations.

We have already reported highly stereoselective chiral deuteration at C-6 and C-5 of hexopyranoses[3-5] and pentofuranoses,[6] respectively.

Study on these deuterated sugars gives an unambiguous solution to this problem.[7] Previous assignments of the two protons at C-6 and the preferred rotamers about C5-C6 bonds of D-galactose derivatives[8] and *N*-acetyl -D-glucosamine[9] have been shown to be erroneous by our ^{1}H NMR studies on the corresponding chirally deuterated sugars.[10, 11]

Because D-mannose is an important hexose that exists in the branching positions and other portions of asparagine-linked oligosaccharides, we have undertaken a ^{1}H NMR study of some specifically deuterated mannose derivatives. Here we report in detail: 1) syntheses of (6S) and (6R)-(6-^{2}H$_1$)-D-mannose derivatives, 2) the conclusive ^{1}H NMR assignment of H-6*proS* and H-6*pro R* of some D-mannose derivatives and 3) the rotamer populations around their C5-C6 bonds based on the above assignments.

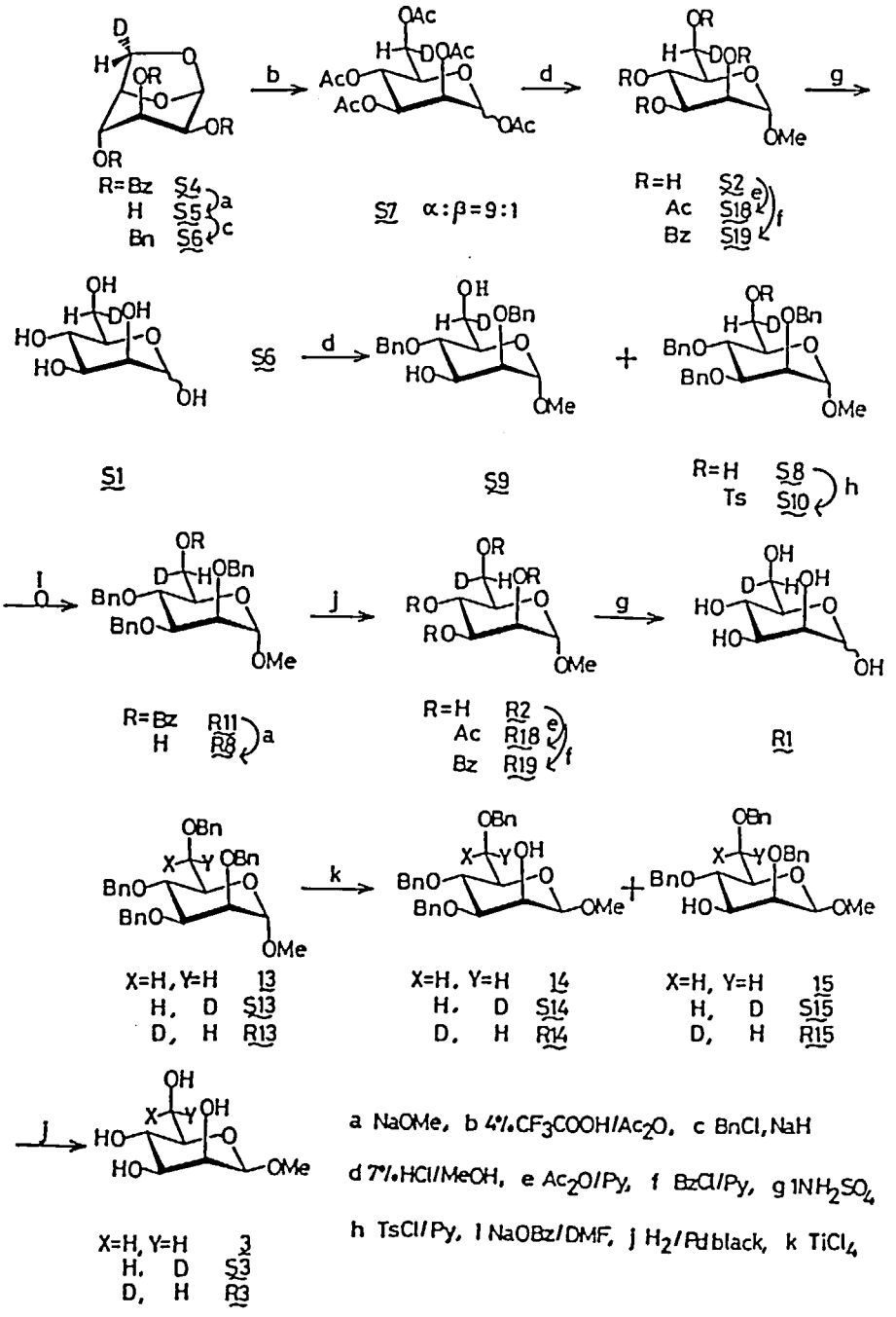
RESULTS AND DISCUSSION

Syntheses of Chirally Deuterated Mannose Derivatives.

Chiral deuteration at C-6 of mannose was accomplished by our method;[5] photobromination[12] of 1,6-anhydro-2,3,4-tri-O-benzoyl-β-D-mannopyranose followed by reduction with Bu_3SnD gave stereospecifically the (6S)-(6-^{2}H$_1$)-mannose derivative S4. Deprotection of S4 followed by acetolysis gave S7, which was treated with HCl/MeOH to give deuterated methyl α-glycoside S2. Compound S1 was prepared by hydrolysis of S2 or S5.

(6R)-(6-^{2}H$_1$) derivatives were prepared by S_N2 inversion at C-6 of the (6S)-(6-^{2}H$_1$) compound as follows. The protecting groups of S4 were replaced by benzyl groups to give S6. S6 was solvolyzed with HCl/MeOH to give S8 (77%) together with unexpected S9 (14%), which is expected to be useful as a glycosyl acceptor for 3,6-branched manno-oligosaccharides. Tosylation of S8 gave S10, which was heated with NaOBz in DMF to give R11, which was converted to methyl α-glycoside R2 by two deprotection steps. Compound R1 was prepared by acid catalyzed hydrolysis of R2.

α-Mannoside 13 was converted to β-mannoside 14 (41%) and 15 (11%) by reaction with $TiCl_4$.[13] Anomerization accompanied by de-O-benzylation was found in the course of the studies concerned with selective de-O-benzylation with Lewis acids.[14] Compound 14 and 15 were hydrogenolyzed to give 3. Compounds S3 and R3 were prepared from S13 and R13, respectively, by the same method. Location of the OH group at C-2 of 14 and



C-3 of 15 was determined from the ^{1}H NMR spectra of their benzoates 16 and 17, respectively. The down-field shifts of H-2 and H-3 were respectively observed in those spectra. The mechanism of the reaction will be discussed elswhere.

^{1}H NMR Studies and Conformational Analyses.

Non-protected compounds. ^{1}H NMR spectra of non-protected compounds measured in D_2O at 400 or 270 MHz are shown in Fig.s 1-3. The prochiral protons H-6*proS* and H-6*proR* were unequivocally assigned from these spectra of deuterated compounds. Simultaneously, signals from H-5 and the proton attached to the deuterated C-6 changed from a ddd and dd to a dd and d, respectively. Isotope shifts (0.02 ppm) by a deuteron were observed for H-6 but not the other protons. Line broadening of H-6 signals by coupling with a deuteron was observed but split peaks were not observed. The chemical shifts of H-6*proS* and H-6*proR*, and coupling constants $J_{5,6proS}$ and $J_{5,6proR}$ are listed in the Table. ^{1}H NMR data from other protons are given in experimental section.

H-6*proS* resonated at a lower-field than H-6*proR* in all compounds. H-6*proS* of the α-anomers resonated at higher-field than those of the corresponding β-anomers, On the other hand, H-6*proR* of the α-anomers resonated at lower-field than those of the β-anomers. These results were very similar to those of the corresponding gluco-derivatives.[7,15] Our assignments of H-6*proS* and *proR*. signals confirmed the assumption of Brisson *et al.*[16] The coupling constants $J_{5,6proS}$ were smaller than $J_{5,6proR}$ in all compounds. Line broadening of H-6 and additional splitting of H-3 were observed in 2, S2 and R2 because of strong coupling between H-4 and H-5. Accurate coupling constants were obtained by spin simulation (LAOCON III). Both coupling constants $J_{5,6proS}$ and $J_{5,6proR}$ of α-anomers are smaller than those of the corresponding β-anomers.

The populations of three stable staggered rotamers (Fig. 4) about the C5-C6 single bond were calculated on the basis of $J_{5,6proS}$ and $J_{5,6proR}$ using the equations employed by Wu *et al.*[18] (Table). In our preceding study it was concluded that these equations were suitable for estimating the population about C5-C6 bond[15] of D-glucoses and D-mannoses. Major rotamers were gg and gt and the populations of tg rotamers were below 3% in all non-protected compounds. These results

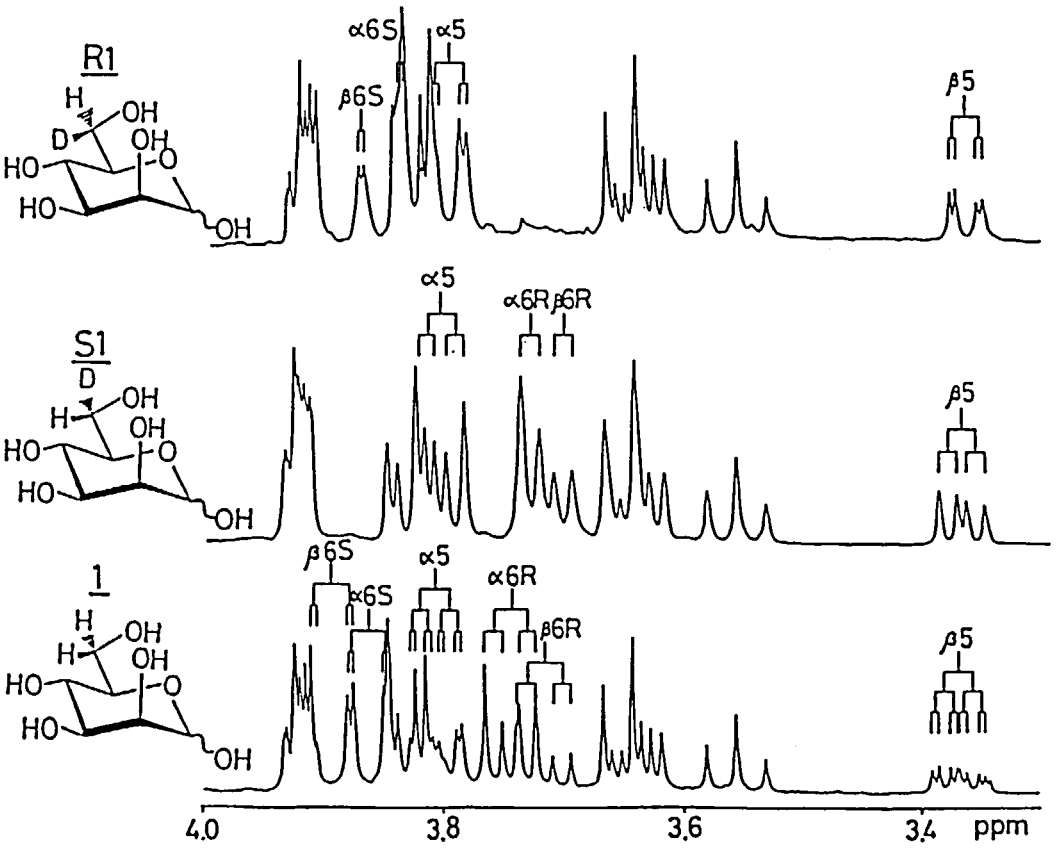


Fig. 1 ^{1}H NMR spectra of the H-5 and H-6 protons of 1, S1 and R1 in D_2O at 400 MHz.

can be explained in terms of both the gauche effect,[19] which stabilizes the gg and gt rotamers, and the 1,3-syn interaction[17,20] between O-4 and O-6, which destabilizes the tg rotamer.

The populations of rotamer gg for the β-anomers β1 and 3 were smaller (7-8%) than those for the α-anomers α1 and 2. Although a similar trend was observed for glucose and methyl glucopyranosides,[15] the populations of gg for β1, 2 and 3 were slightly smaller (4-5%) and those of gt + tg were larger compared with those of the corresponding gluco-derivatives. However, there were little differences in the rotamer populations between α-glucose and α-mannose. It was

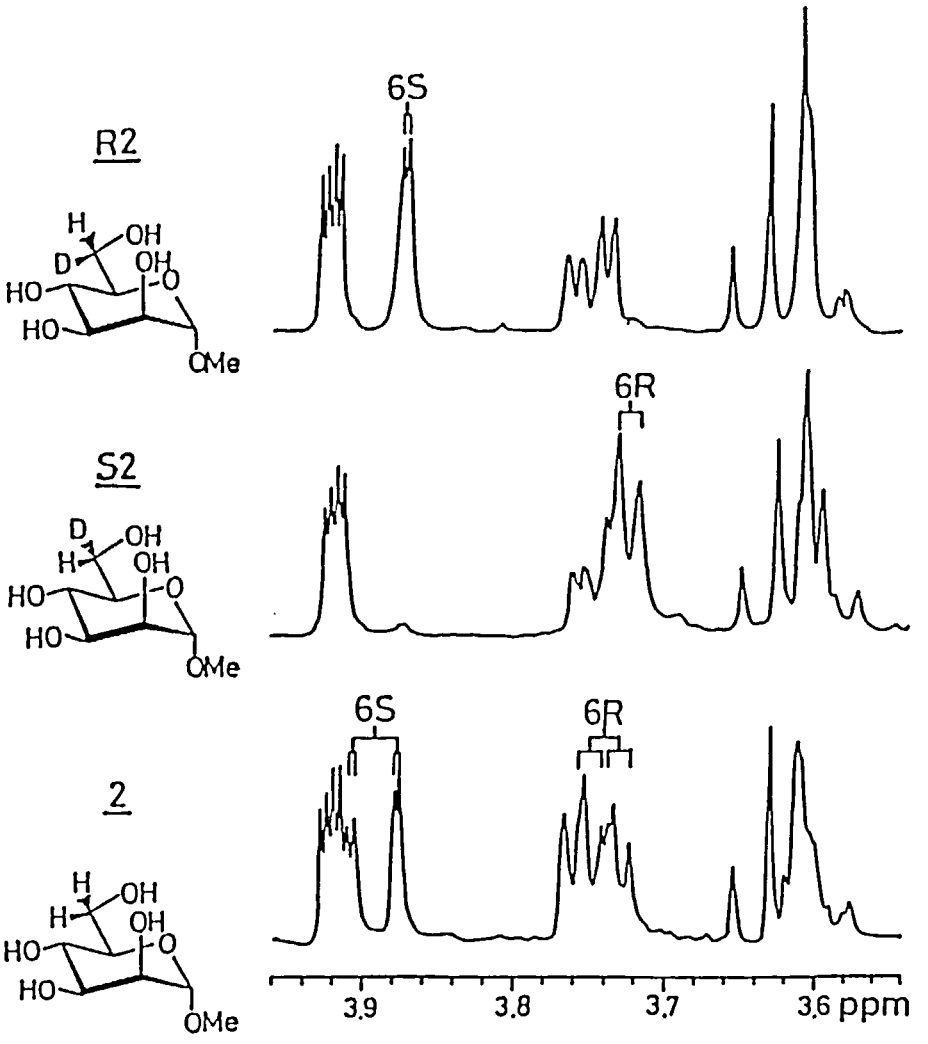


Fig. 2 ^{1}H NMR spectra of the H-6 protons of 2, S2 and R2 in D_2O at 400 MHz.

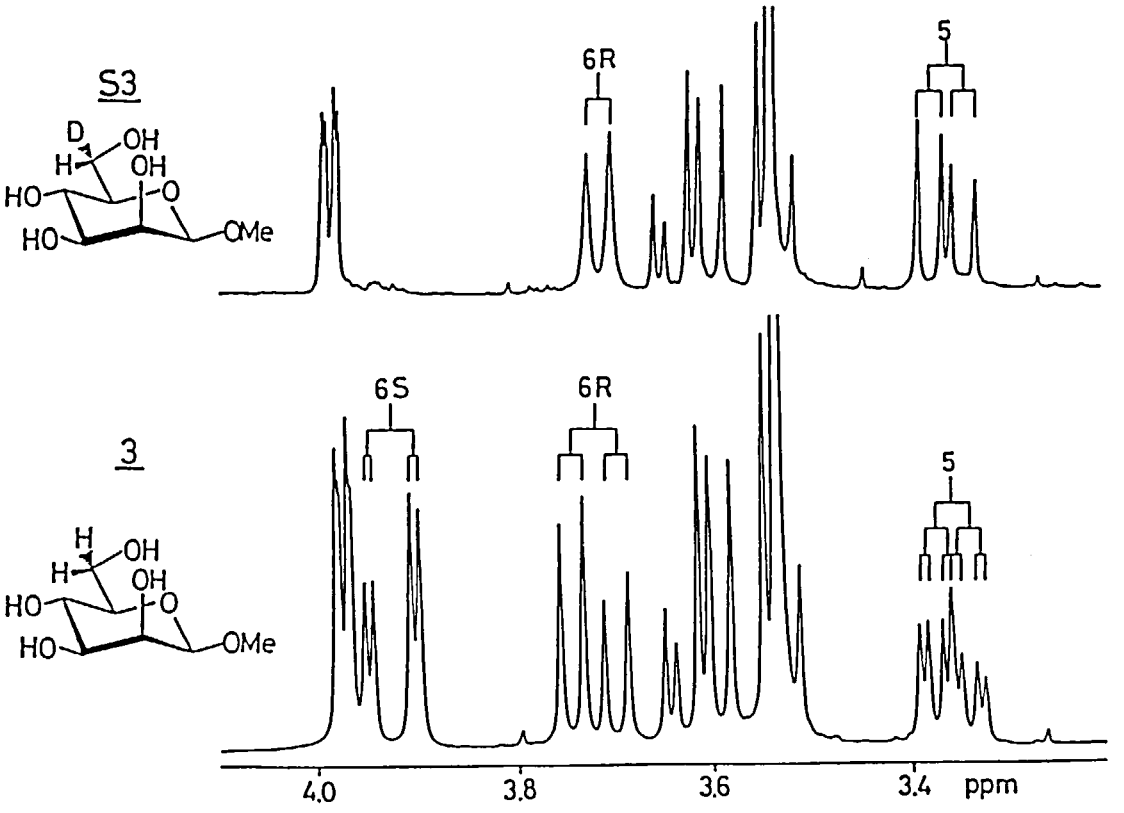


Fig. 3 ^{1}H NMR spectra of the H-5 and H-6 protons of 3 and S3 in D_2O at 270 MHz.

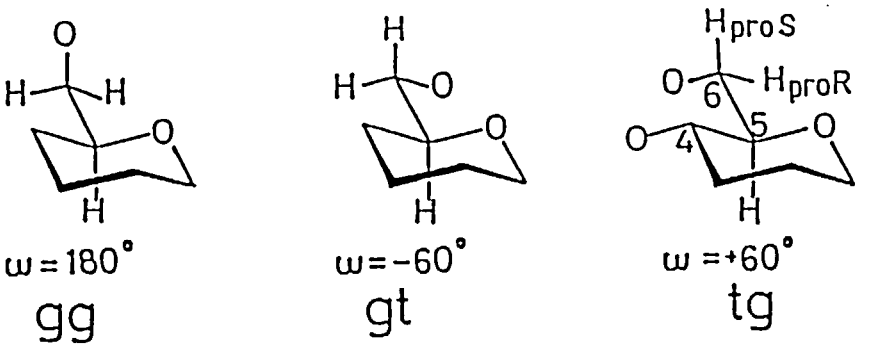
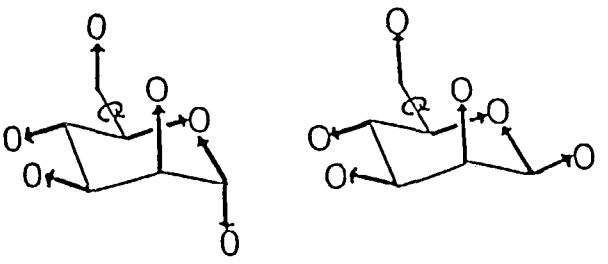


Fig. 4 Three staggered rotamers around the C5-C6 bond.



α-D-Mannopyranose β-D-Mannopyranose

Fig. 5 Dipoles of the α and β-D-mannopyranose gg rotamer.

characteristic for mannose derivatives that the gg populations were almost equal or smaller than those of gt for β-anomers β1 and 3. The decrease of the gg population for the β-anomers would be favorable in reducing the dipole moment. Rotamer gg of the β-anomers would have a large dipole moment because the four oxygen atoms at C-1,2,3 and 6 are oriented to the β face of the pyranose rings. It would be reduced by the decrease of the gg population and also by the increase of those of gt or tg. The gt population increases in practice since conformer tg causes unfavorable 1,3-syn interaction described above.

Protected compounds. ^{1}H NMR spectra of deuterated or non-deuterated protected compounds 14 and 18-22 were measured in $CDCl_3$ at 100 MHz. The chemical shifts and the coupling constants listed in the Table showed that H-6*proS* resonated at lower-field than H-6*proR* in all cases as observed for non-protected compounds with acetate 18 being an exception. The inversion of the chemical shifts of H-6*proS* and *proR* has already been observed in acetylated glucose.[17] The coupling constant $J_{5,6proS}$ of 21 was exceptionally large compared with other ones and was near to $J_{5,6proR}$.

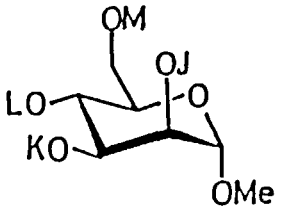
We used Wu's equations again without modification for acylated or silylated compounds because small differences of electronegativities between hydroxy and acyloxy[19] or silyloxy groups would cause little change of coupling constants.

General trend of the rotamer populations was gg/gt=6/4 in the protected compounds, irrespective of anomeric configuration, except for

Table ^{1}H NMR Data and Calculated Roatmer Populations

Compound	δ (ppm)		J (Hz)		Population (%)		
	H-6*proS*	H-6*proR*	$J_{5,6proS}$	$J_{5,6proR}$	gg	gt	tg
α1	3.863	3.731	2.0	5.8	55	44	1
β1	3.894	3.716	2.3	6.4	48	49	3
2	3.893	3.743	2.0	6.0	54	46	0
3	3.926	3.721	2.3	6.6	46	51	3
14	3.75	3.70	2.0	5.4	59	40	1
18	4.11	4.27	2.2	5.4	58	39	3
19	4.70	4.48	2.7	4.6	62	28	10
20	4.59	4.56	2.0	4.4	68	29	3
21	~3.90	3.85	4.6	5.4	44	28	28
22	3.85	3.84	1.0	5.4	65	44	-9

Compounds α1-3 and 14-22 were measured in D_2O at 400 MHz and in $CDCl_3$ at 100 MHz, respectively. Preparation of 20-22 will be described elsewhere.



J	K	L	M	
Bn	Bz	Bn	Bz	20
Bn	Bn	H	Si+	21
Bn	H	Bn	Si+	22

21. Benzoate 19 showed slightly larger populations of tg (10%) and gg (68%) compared with other cases. A similar population was observed for methyl 2,3,4,6-tetra-O-benzoyl-α-D-glucopyranoside.[15] Considerable population of tg for 21 would be possibly ascribed to intramolecular hydrogen bonding between O-6 and 4-OH in a hydrophobic environment. A negative tg population was calculated for 2 because of a small coupling constant ($J_{5,6proS}$ = 1.0 Hz). Slightly larger coupling constants for $J_{5,6proS} \geq 1.8$ Hz were expected for the silylated compounds when low electronegativity of silicon was taken into account.

In the case of 22, it could be assumed that the stable torsion angles for rotamers gg and/or gt are respectively shifted to small angles, to some extent, from the ideal angles of 180° and -60°.[13] The shifts would be ascribed to the eletrostatic repulsion between O-5 and O-6, because a silicon atom would increase the electron density of O-6.

In conclusion, mannose derivatives stereospecifically deuterated at C-6 were synthesized for the conformational analyses of the exocyclic bond by ^{1}H NMR. The general characteristics of the chemical shifts of the prochiral protons, H-6*proS*, H-6*pro R* (reversed for 18) and the coupling constants, $J_{5,6proS}$, $J_{5,6pro\,R}$ were assigned by the use of the deuterated compounds. The major rotamers about the C5-C6 single bond were found to be gg and gt, similar to those of the glucose derivatives. However, the axial hydroxy group at C-2 of unprotected mannose derivatives, especially of β-anomers, caused an increase of gt and tg populations and a decrease of gg compared with the corresponding rotamers of glucose derivatives which have an equatorial oxygen at C-2.

EXPERIMENTAL

General Procedures. Melting points were taken with Yanako Model P hotplate and were uncorrected. ^{1}H NMR spectra were recorded on JEOL JNM FX-100, GX-270, GX-400 spectrometers at 100, 270 and 400 MHz, respectively, at 21-23 °C. TMS and TPS were used as internal standards in $CDCl_3$ and D_2O, respectively. All digital resolutions were 0.24 Hz. All ^{1}H NMR data in the EXPERIMENTAL were recorded at 100 MHz except for 1, S1, R1, 2, S2, R2, 3, and S3. ^{13}C NMR spectra were recorded on a JEOL JNM FX-100 spectrometer at 25 MHz (complete proton-decoupled mode). IR spectra were recorded on a Jasco A-202 Infrared spectrometer. Specific rotations were measured on a Jasco J-20 polarimeter at 589 nm. Merck silica gel (Art. 7734) was used for column chromatography and Merck silica gel (Art. 5548) was used for analytical thin layer chromatography (TLC).

(6S)-(6-^{2}H$_1$)-1,6-Anhydro-β-D-mannopyranose (*S5*). A solution of (6S)-(6-^{2}H$_1$)-1,6-anhydro-2,3,4-tri-O-benzoyl-β-D-mannopyranose S4[5] (8.5 g) and sodium methoxide (70 mg of Na in 200 mL of dry MeOH) was refluxed for 3 h. The cooled solution was neutralized with Dowex 50(H^+). The resin was removed by filtration and washed with MeOH. The filtrate and the washing were combined and concentrated to give a syrup which

was dissolved in water and washed with C_6H_6. Evaporation of the solvent and coevaporation with toluene gave a white powder S5 (2.6 g, 90%). $[\alpha]_D^{21}$ -128° (c, 0.1, H_2O), IR (KBr) 3370, 2180 cm^{-1}, ^{13}C NMR (D_2O) δ 66.9 (t, $J_{C,D}$ = 22 Hz, C-6), 68.5 (C-2), 72.8 (C-3), 74.1 (C-4), 78.3 (C-5), and 103.7 ppm (C-1); ^1H NMR (D_2O) δ 5.39 (1H, bs, H-1), 4.58 (1H, bs, H-5), 4.18 (1H, d, $J_{5,6endo}$ = 1.0 Hz, H-6endo), and 3.7-4.0 ppm (3H, m, H-2,3,4).

Anal. Calcd for $C_6H_9DO_5$: C, 44.17; H, 6.79. Found: C, 44.05; H, 6.85.

(6S)-(6-$^2H_1$)-1,6-Anhydro-2,3,4-tri-O-benzyl-β-D-mannopyranose (*S6*). To a solution of S5 (400 mg) in DMF (10 mL) was added NaH (220 mg). After 30 min, benzyl chloride (1.3 g) was added to the solution in small portions at 0 °C and the mixture was stirred overnight at room temperature. The reaction was stopped by addition of MeOH (2 mL). The reaction mixture was concentrated, and the product was extracted with $CHCl_3$. The extract was washed with saturated NH_4Cl and dried over $MgSO_4$. Evaporation of the solvent gave a syrup, which was purified by chromatography ($CHCl_3$). The syrupy S6 (1.0 g, 94%) was crystallized from EtOH: $[\alpha]_D^{21}$ -30° (c, 0.1, $CHCl_3$), mp 86-87 °C; IR (KBr) 2170, 735, 127.9, 127.7 (C_6H_5- x 3), 100.1 (C-1), 76.6 (C-4), 74.6 (C-2,3), 74.0 (C-5), 73.4, 71.4, 71.3 (OCH_2Ph x 3), and 64.7 ppm (t, $J_{C,D}$ = 22 Hz, C-6); ^1H NMR ($CDCl_3$) δ 7.3 (15H, m, C_6H_5- x 3), 5.45 (1H, bs, H-1), 4.67, 4.48 (2H, ABq, OCH_2Ph), 4.54 (2H, s, OCH_2Ph), 4.44 (2H, s, OCH_2Ph), 4.46 (1H, m, H-5), 4.06 (1H, d, $J_{5,6endo}$ = 1.0 Hz, H-6endo), 3.81 (1H, sep, $J_{2,3}$ = 5.37, $J_{1,3}$ = $J_{3,4}$ = $J_{3,5}$ = 1.7 Hz, H-3), 3.57 (1H, dd, $J_{1,2}$ = 1.7, $J_{2,3}$ = 5.37 Hz, H-2), and 3.46 ppm (1H, t, $J_{3,4}$ = $J_{4,5}$ = 1.7 Hz, H-4).

Anal. Calcd for $C_{27}H_{27}DO_5$: C, 74.80; H, 6.74. Found: C, 74.63; H, 6.81.

Methyl (6S)-(6-$^2H_1$)-α-D-Mannopyranoside (*S2*). A solution of S5 (400 mg) and sulfuric acid (0.25 mL) in acetic anhydride (15 mL) was stirred at 0 °C for 3 h. The solution was poured into 6% aq NaOAc and was extracted with $CHCl_3$. The extract was successively washed with sat. NaCl, sat. $NaHCO_3$ and water, and dried over $MgSO_4$. Evaporation of the solvent gave a syrupy mixture of (6S)-(6-$^2H_1$)-1,2,3,4,6-penta-O-acetyl-α- and β-D-mannopyranose (α : β = 9 : 1) S7: IR (Nujol) 1750 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.09 (0.9H, d, $J_{1,2}$ = 1.7 Hz, αH-1), and 5.74 ppm (0,1H, d, $J_{1,2}$ = 1.2 Hz, βH-1).

A solution of S7 (530 mg) in 7% HCl/MeOH was refluxed for 1 h. Concentration of the solution gave a syrup, which was dissolved in water and neutralized with Dowex 1(OH^-). Evaporation of water gave a syrup, which was crystallized from n-PrOH to give S2 (205 mg, 78%): mp 190 °C; $[\alpha]_D^{21}$ +78° (c, 0.1, H_2O): ^1H NMR (D_2O, 400 MHz) δ 4.745 (1H, d, $J_{1,2}$ = 1.7 Hz, H-1), 3.923 (1H, dd, $J_{1,2}$ = 1.7, $J_{2,3}$ = 3.4 Hz, H-2), 3.748 (1H, dd containing second order split, $J_{2,3}$ = 3.4, $J_{3,4}$ = 9.9 Hz, H-3), 3.728 (1H, bd, $J_{5,6R}$ = 6.0 Hz, H-6R), 3.628 (1H, t, $J_{3,4}$ = $J_{4,5}$ = 9.9 Hz, H-4), 3.593 (1H, dd, $J_{4,5}$ = 10.0, $J_{5,6R}$ = 6.0 Hz, H-5), and 3.400 (3H, s, OCH_3).

Anal. Calcd for $C_7H_{13}DO_6$: C, 43.07; H, 7.75. Found: C, 43.12; H, 7.58.

(6S)-(6-$^2H_1$)-D-Mannose (*S1*). Compound S5 (240 mg) in 1N H_2SO_4 (5 mL) was refluxed for 8 h. The cooled solution was diluted with water (5 mL) and treated with charcoal and then neutralized with $BaCO_3$. The suspension was centrifugated (600 r.p.m. x 15 min) and the supernatant was concentrated to give a syrup which was crystallized from a mixture of MeOH and i-PrOH to afford crystalline S1 (224 mg, 84%): mp 132 °C; ^1H NMR (D_2O, 400 MHz) δ 5.165 (0.7 H, d, $J_{1,2}$ = 1.8 Hz, αH-1), 4.885 (0.3H, d, $J_{1,2}$ = 1.0 Hz, βH-1), 3.931 (0.3H, dd, $J_{1,2}$ = 1.0, $J_{2,3}$ = 3.3 Hz, βH-2), 3.921 (0.7 H, dd, $J_{1,2}$ = 1.8, $J_{2,3}$ = 3.3 Hz, αH-2), 3.832 (0.7 H, dd, $J_{2,3}$ = 3.3, $J_{3,4}$ = 9.7 Hz, αH-3), 3.804 (0.7 H, dd, $J_{4,5}$ = 9.7, $J_{5,6S}$ = 6.0 Hz, αH-5), 3.726 (0.7 H, d, $J_{5,6R}$ = 6.0 Hz, αH-6R), 3.700 (0.3 H, d, $J_{5,6R}$ = 6.4 Hz, βH-6R), 3.644 (0.3 H, dd, $J_{2,3}$ = 3.3, $J_{3,4}$ = 9.7 Hz, βH-3), 3.641 (0.7H, t, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, αH-4), 3.557 (0.3 H, t, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, βH-4), and 3.66 ppm (0.3 H, dd, $J_{5,6R}$ = 6.1, $J_{4,5}$ = 9.7 Hz, βH-4).

Anal. Calcd for $C_6H_{11}DO_6$: C, 39.73; H, 7.23. Found: C, 39.91; H, 7.35.

Methyl (6S)-(6-$^2H_1$)-2,3,4-Tri-O-benzyl-6-O-p-toluenesulfonyl-α-D-mannopyranoside (*S10*). To a solution of S8 (2 g) in pyridine (25 mL) was added p-toluenesulfonyl chrolide (2 g). After the mixture was stirred for 1 day, it was poured into ice-water (300 mL) and stirred for 2 h. The mixture was extracted with $CHCl_3$ and the extract was successively washed with water, sat. $NaHCO_3$ and then water. The organic layer was dried over $MgSO_4$ and concentrated to a syrup, which was purified by chromatography ($CHCl_3$) and crystallized from EtOH to give

S10 (2.4 g, 92%); mp 108-109 °C, $[\alpha]_D^{21}$ +20° (c, 1.0, $CHCl_3$); IR (KBr) 2200, 1365, 1175, 740, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.77 (2H, bd, J = 8.6 Hz, H-2,2' of tosyl group), 7.1-7.4 (17H, m, H-3,3' of tosyl group and C_6H_5- x 3), 4.88 and 4.46 (2H, ABq, J_{HCH} = 11.0 Hz, OCH_2Ph), 4.68 (2H, s, OCH_2Ph), 4.57 (2H, s, OCH_2Ph), 4.64 (1H, d, $J_{1,2}$ = 1.5 Hz, H-1), 4.20 (1H, m, H-6R), 3.6-3.4 (4H, m, H-2,3,4,5), 3.25 (3H, s, OCH_3), and 2.39 ppm (3H, s, CH_3 of tosyl group).

Anal. Calcd for $C_{35}H_{37}DSO_8$; C, 67.83; H, 6.34; S, 5.17, Found: C, 67.77; H, 6.23; S, 5.10.

Methyl (6R)-(6-$^2H_1$)-6-O-Benzoyl-2,3,4-tri-O-benzyl-α-D-manno-pyranoside (*R11*). To a solution of S10 (2.4 g) in DMF (70 mL) was added BzONa (1.7 g). The mixture was refluxed for 3 h and then evaporated to give a syrup. The syrup was chromatographed ($CHCl_3$) to give a syrupy R11 (1.9 g, 86%): $[\alpha]_D^{21}$ +16° (c, 0.5, $CHCl_3$); IR (Nujol) 2200, 1720, 740, 715, and 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.03 (2H, m, o-H x 2 of C_6H_5CO), 7.2-7.6 (18H, m, m-H x 2 and p-H of C_6H_5CO and C_6H_5- x 3), 4.95 and 4.60 (2H, ABq, J_{HCH} = 10.7 Hz, OCH_2Ph), 4.80 and 4.67 (2H, ABq, J_{HCH} = 12 Hz, OCH_2Ph), 4.66 (2H, s, OCH_2Ph), 4.78 (1H, d, $J_{1,2}$ = 1.7 Hz, H-1), 4.57 (1H, d, $J_{5,6S}$ = 2 Hz, H-6S), 3.8-4.25 (4H, m, H-2,3,4,5), and 3.34 (3H, s, OCH_3).

Methyl (6R)-(6-$^2H_1$)-α-D-Mannopyranoside (*R2*). A mixture of R11 (130 mg) and Pd-black (5 mg) in MeOH (20 mL) was shaken under H_2 (1 atm) for 2 h. The catalyst was filtered off and the filtrate was concentrated to give methyl (6R)-(6-$^2H_1$)-6-O-benzoyl-α-D-mannopyranoside R12 (67 mg, 98%): IR (Nujol) 3400, 2200, and 1720 cm^{-1}. A solution of R12 (54 mg) in dry MeOH (10 mL) containing catalytic amount of Na was refluxed for 3 h. The cooled solution was neutralized with Dowex 50 (H^+) and the resin was filtered off. The filtrate was concentrated to give a syrup, which was dissolved in water and washed with C_6H_6. Evaporation of the solvent gave a syrup, which was crystallized from a mixture of water and n-PrOH to give R11 (14 mg, 80%): $[\alpha]_D^{21}$ +78° (c, 0.1, H_2O), mp 190-193 °C; ^1H NMR (D_2O, 400 MHz) δ 4.745 (1H, d, $J_{1,2}$ = 1.7 Hz, H-1), 3.922 (1H, dd, $J_{1,2}$ = 1.7, $J_{2,3}$ = 3.4 Hz, H-2), 3.873 (1H, d, $J_{5,6S}$ = 2.2 Hz, H-6S), 3.749 (1H, dd contains second order split, $J_{2,3}$ = 3.4, $J_{3,4}$ = 9.8 Hz, H-3), 3.633 (1H, t, $J_{3,4}$ = $J_{4,5}$ = 9.9 Hz, H-4), 3.593 (1H, bd, $J_{4,5}$ = 9 Hz, $J_{5,6S}$ = 2 Hz, H-5), and 3.400 (3H, s, OCH_3).

Anal. Calcd for $C_7H_{13}DO_6$: C, 43.07; H, 7.75. Found: C, 43.23; H, 7.57.

(6R)-(6-$^2H_1$)-D-Mannose (R1). Compound R1 was prepared from R12 by the similar procedure described for S1 in 83%. R1: ^1H NMR (D_2O, 400 MHz) δ 5.17 (0.7H, d, $J_{1,2}$ = 1.8 Hz, αH-1), 4.89 (0.3H, d, $J_{1,2}$ = 1.0 Hz, βH-1), 3.931 (0.3H, d, $J_{1,2}$ = 1.0, $J_{2,3}$ = 3.3 Hz, βH-2), 3.92 (0.7H, d, $J_{1,2}$ = 1.8, $J_{2,3}$ = 3.3 Hz, αH-2), 3.875 (0.3H, d, $J_{5,6S}$ = 2.3 Hz, βH-6S), 3.842 (0.7H, $J_{5,6S}$ = 2.0 Hz, αH-6S), 3.833 (0.7H, dd, $J_{2,3}$ = 3.3, $J_{3,4}$ = 9.7 Hz, αH-3), 3.802 (0.7H, dd, $J_{4,5}$ = 9.7, $J_{5,6S}$ = 2.0 Hz, αH-5), 3.646 (0.3H, dd, $J_{2,3}$ = 3.3, $J_{3,4}$ = 9.7 Hz, βH-3), 3.646 (0.7H, t, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, αH-4), 3.562 (0.3H, t, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, βH-4), and 3.367 ppm (0.3H, dd, $J_{4,5}$ = 9.7, $J_{5,6S}$ = 2.3 Hz, βH-5).

Methyl (6R)-(6-$^2H_1$)-2,3,4-Tri-O-benzyl-α-D-mannopyranpside (*R8*). A solution of R11 (390 mg) in dry MeOH containing a catalytic amount of Na was refluxed for 3 h. The cooled solution was neutralized with Dowex 50 (H^+). After the resin was filtered off and washed with MeOH, the filtrate and washing were concentrated to give a syrup, which was purified by chromatography ($CHCl_3$-EtOAc, 4:1) to afford R8 (280 mg, 83%); $[\alpha]_D^{21}$ +35° (c, 0.05, $CHCl_3$), IR (Nujol) 3450, 2150, 749 and 695 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.3 (15H, m, C_6H_5- x 3), 4.80 and 4.65 (2H, ABq, J_{HCH} = 12.5 Hz, OCH_2Ph), 4.71 (1H, d, $J_{1,2}$ = 1.7 Hz, H-1), 4.94 and 4.64 (2H, Abq, J_{HCH} = 11.0 Hz, OCH_2Ph), 4.62 (2H, s, OCH_2Ph), 3.5-4.1 (5H, m, H-2, 3,4,5,6S), 3.28 (3H, s, OCH_3), and 2,1 (1H, bs, 6-OH).

Methyl (6S)-(6-$^2H_1$)-2,3,4,6-Tetra-O-benzyl-α-D-mannopyranoside (*S13*). and Methyl (6R)-(6-$^2H_1$)-2,3,4,6-Tetra-O-benzyl-α-D-mannopyranoside (*R13*). Compound S8 (170 mg) and R8 (1.13 g) were benzylated (BnCl, NaH/DMF). Syrupy S13 (200 mg, 99%) and R13 (1.13 g, 81%) were obtained by chromatographic purification ($CHCl_3$: benzene = 1 : 1). S13: M^+/z = 556; $[\alpha]_D^{21}$ + 27. 0° (c, 0.5, $CHCl_3$), IR (Nujol) 740 and 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.1-7.4 (20H, m, C_6H_5- x 4), 4.89 and 4.45 (2H, ABq, J_{HCH} = 10.7 Hz, OCH_2Ph), 4.73 (2H, s, OCH_2Ph), 4.60 (2H, s, OCH_2Ph), 4.68 and 4.50 (2H, Abq, J_{HCH} = 12.2 Hz, OCH_2Ph), 4.77 (1H, d, $J_{1,2}$ = 1.7 Hz, H-1), 3.6-4.1 (5H, m, H-2,3,4,5,6R), and 3.31 (3H, s, OCH_3). R13: M^+/z = 556; $[\alpha]_D^{21}$ +27.5° (c, 0.6, $CHCl_3$), IR (Nujol) 740 and 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.1-7.4 (20H, m, C_6H_5- x 4), 4.89 and 4.45 (2H, ABq, J_{HCH} = 10.7 Hz, OCH_2Ph), 4.73 (2H, s, OCH_2Ph), 4.60 (2H, s, OCH_2Ph), 4.69 and 4.50 (2H,

ABq, J_{HCH} = 12.2 Hz, OCH_2Ph), 4.77 (1H, d, $J_{1,2}$ = 1.7 Hz, H-1), 3.6-4.1 (5H, m, H-2,3,4,5,6S), and 3.32 (3H, s, OCH_3).

Methyl 3,4,6-Tri-O-benzyl-β-D-mannopyranoside (*14*) and Methyl 2,4,6-Tri-O-benzyl-β-D-mannopyranoside (*15*). A solution of 13[20] (4.30 g) and $TiCl_4$ (1.40 g) in CH_2Cl_2 (100 mL containing trace amount of DMF) was stirred for 10 h at room temperature. The reaction mixture was poured into sat. Na_2CO_3 and the organic layer was separated. The aqueous layer was extracted with CH_2Cl_2. The combined extracts were washed with sat. NaCl, dried over $MgSO_4$ and concentrated to a syrup. The syrup was chromatographed (C_6H_6 : EtOAc, 3 : 1) to give two fractions. Concentration of the first and the second fractions gave crystalline 15 (1.49 g, 41%) and oily 14 (400 mg), respectively. 14: $[\alpha]_D^{21}$ -22.3° (c, 0.5, $CHCl_3$); ^1H NMR δ 7.1-7.4 (15H, m, C_6H_5- x 3), 4.88 and 4.51 (2H, ABq, J_{HCH} = 10.7 Hz, OCH_2Ph), 4.77 and 4.63 (2H, ABq, J_{HCH} = 11.5 Hz, OCH_2Ph), 4.63 and 4.51 (2H, ABq, J_{HCH} = 12.5 Hz, OCH_2Ph), 4.30 (1H, d, $J_{1,2}$ < 1.0 Hz, H-1), 4.08 (1H, dd, $J_{1,2}$ < 1.0, $J_{2,3}$ = 2.9 Hz, H-2), 3.87 (1H, t, $J_{3,4}$ = $J_{4,5}$ = 9.3 Hz, H-4), 3.74 (2H, m, H-6S,R), 3.56 (1H, dd, $J_{2,3}$ = 2.9 Hz, H-3), 3.53 (3H, s, OCH_3), 3.42 (1H, m, H-5), and 2.5 ppm (1H, bs, 2-OH). 15: mp 66 °C; $[\alpha]_D^{21}$ -68° (c, 0.2, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.2-7.4 (15H, m, C_6H_5- x 3), 5.06 and 4.60 (2H, ABq, J_{HCH} = 11.7 Hz, OCH_2Ph), 4.85 and 4.51 (2H, ABq, J_{HCH} = 11.0 Hz, OCH_2Ph), 4.68 and 4.54 (2H, ABq, J_{HCH} = 12.2 Hz, OCH_2Ph), 4.40 (1H, d, $J_{1,2}$ = 0.7 Hz, H-1), 3.55 (3H, s, OCH_3), 3.4-4.0 (6H, m, H-2,3,4,5,6S,R), and 2.45 ppm (1H, bs, 3-OH).

Methyl (6S)-(6-$^2H_1$)-3,4,6-Tri-O-benzyl-β-D-mannopyranoside (*S14*), Methyl (6R)-(6-$^2H_1$)-3,4,6-Tri-O-benzyl-β-D-mannopyranoside (*R14*), Methyl (6S)-(6-$^2H_1$)-2,4,6-Tri-O-benzyl-β-D-mannopyranoside (*S15*), and Methyl (6R)-(6-$^2H_1$)-2,4,6-Tri-O-benzyl-β-D-mannopyranoside (*R15*). Compounds S14 and S15, and R14 and R15 were prepared from S13 and R13, respectively, by the same procedure described for 14 and 15.

Methyl (6S)-(6-$^2H_1$)-2,3,4,6-Tetra-O-acetyl-α-D-mannopyranoside (*S18*) and Methyl (6R)-(6-$^2H_1$)-2,3,4,6-Tetra-O-acetyl-α-D-mannopyranoside (*R18*). Compounds S2 and R2 were acetylated (Ac_2O/pyridine) to give S18 and R18, respectively. S18: ^1H NMR ($CDCl_3$) δ 5.1-5.5 (3H, m, H-2,3,4), 4.72 (1H, d, $J_{1,2}$ = 1.7 Hz, H-1), 4.27 (1H, bd, $J_{5,6R}$ = 5.4 Hz, H-6R), 4.00 (1H, m, H-5), and 2,16, 2.11, 2.04 and 2.00 ppm (12H, 4s, CH_3CO x 4).

R18: ^{1}H NMR ($CDCl_3$) δ 5.1-5.5 (3H, m, H-2,3,4), 4.72 (1H, d, $J_{1,2}$ = 1.7 Hz, H-1), 4.11 (1H, bd, $J_{5,6S}$ = 2.2 Hz, H-6S), 4.00 (1H, m, H-5), and 2.16, 2.11, 2.04 and 2.00 ppm (12H, 4s, CH_3CO x 4).

Methyl 2,3,4,6-Tetra-O-benzoyl-α-D-mannopyranoside (*19*), Methyl (6S)-(6-$^2H_1$)-2,3,4,6-Tetra-O-benzoyl-α-D-mannopyranoside (*S19*) and Methyl (6R)-(6-$^2H_1$)-2,3,4,6-Tetra-O-benzoyl-α-D-mannopyranoside (*R19*). Compounds 2, S2 and R2 were benzoylated (BzCl/pyridine) to give 19 (M^+/z = 611), S19 (M^+/z = 612) and R19 (M^+/z = 612). 19: ^{1}H NMR ($CDCl_3$) δ 7.2-8.2 (20H, m, C_6H_5- x 4), 6.13 (1H, t, $J_{3,4}$ = 10.0 , $J_{4,5}$ = 9.5 Hz, H-4), 5.91 (1H, dd, $J_{2,3}$ = 3.0, $J_{3,4}$ = 10.0 Hz, H-3), 5.71 (1H, dd, $J_{1,2}$ = 1.7, $J_{2,3}$ = 3.2 Hz, H-2), 5.01 (1H, d, $J_{1,2}$ = 1.7 Hz), 4.3-4.9 (3H, m, H-5,6S,R), and 3.55 ppm (3H, s, OCH_3).

Methyl 2-O-Benzoyl-3,4,6-tri-O-benzyl-β-D-mannopyranoside (*16*). Compound 14 was benzylated (BzCl/pyridine) to give a syrupy 16: ^{1}H NMR ($CDCl_3$) δ 7.9-8.1 (2H, m, 2o-H of benzoyl group), 7.0-7.5 (18H, m, p-H, and 2m-H of benzoyl group and C_6H_5- x 3), 5.84 (1H, dd, $J_{1,2}$ = 1.0, $J_{2,3}$ = 1.9 Hz, H-2), 4.89 and 4.55 (2H, ABq, J_{HCH} = 10.8 Hz, OCH_2Ph), 4.84 and 4.55 (2H, ABq, J_{HCH} = 11.5 Hz, OCH_2Ph), 4.79 and 4.59 (2H, ABq, J_{HCH} = 12.2 Hz, OCH_2Ph), 4.49 (1H, d, $J_{1,2}$ = 1.0 Hz, H-1), 4.01 (1H, t, $J_{3,4}$ = $J_{4,5}$ = 9.0 Hz, H-4), 3.87 (1H, m, H-6S,R), 3.76 (1H, dd, $J_{2,3}$ = 2.9, $J_{3,4}$ = 9.0 Hz, H-3), 3.54 (1H, m, H-5), and 3.51 (1H, s, OCH_3).

Methyl 3-O-Benzoyl-2,4,6-tri-O-benzyl-β-D-mannopyranoside (*17*). Compound 15 was benzoylated (BzCl/pyridine) to give a syrupy 17: $[\alpha]_D^{21}$ -90° (c, 0.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.0 (2H, m, o-H x 2 of benzoyl group), 7.0-7.7 (18H, m, p-H and m-H x 2 of benzoyl group, and C_6H_5- x 3), 5.12 (1H, dd, $J_{2,3}$ = 3.2, $J_{3,4}$ = 9.8 Hz, H-3), 4.87 and 4.60 (2H, ABq, J_{HCH} = 12.2 Hz, OCH_2Ph), 4.72 and 4.56 (2H, ABq, J_{HCH} = 12 Hz, OCH_2Ph), 4.69 and 4.51 (2H, ABq, J_{HCH} = 10.7 Hz, OCH_2Ph), 4.52 (1H, d, $J_{1,2}$ = 0.7 Hz, H-1), 4.15 (1H, t, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 4.9 (1H, dd, $J_{1,2}$ = 1.0, $J_{2,3}$ = 3.2 Hz, H-2), 3.82 (2H, m, H06S,R), 3.58 (1H, m, H-5), and 3.58 (3H, s, OCH_3).

Methyl (6S)-(6-$^2H_1$)-β-D-Mannopyranoside (*S3*). A mixture of S14 (74 mg) and Pd-black (5 mg) in MeOH (10 mL) was shaken under H_2 (1 atm) for 5 h. The catalyst was filtered off and the filtrate was concentrated to give a syrup. The syrup was dissolved in H_2O (10 mL) and the solution was washed with C_6H_6. The aqueous layer was

lyophylized to give S3 (M^+/z = 195) as powder in a quantitative yield. S3: ^{1}H NMR (D_2O) δ 4.565 (1H, d, $J_{1,2}$ = 0.8 Hz, H-1), 3.972 (1H, dd, $J_{1,2}$ = 0.8, $J_{2,3}$ = 3.1 Hz, H-2), 3.705 (1H, d, $J_{5,6R}$ = 6.6 Hz, H-6R), 3.622 (1H, dd, $J_{2,3}$ = 3.1, $J_{3,4}$ = 9.4 Hz, H-3), 3.543 (1H, t, $J_{3,4}$ = 9.4, $J_{5,6R}$ = 6.6 Hz, H-5).

Methyl (6R)-(6-^{2}H$_1$)-β-D-Mannopyranoside (R3). R3 was prepared from R14 by the same procedure as described for S3. R3: ^{1}H NMR (D_2O) δ 4.565 (1H, d, $J_{1,2}$ = 0.8 Hz, H-1), 3.972 (1H, dd, $J_{1,2}$ = 0.8, $J_{2,3}$ = 3.1 Hz, H-2), 3.946 (1H, dd, $J_{5,6S}$ = 2.2 Hz, H-6S), 3.548 (1H, t, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4), 3.533 (3H, s, OCH_3), and 3.354 (1H, dd, $J_{4,5}$ = 9.4, $J_{5,6S}$ = 2.2 Hz, H-5).

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