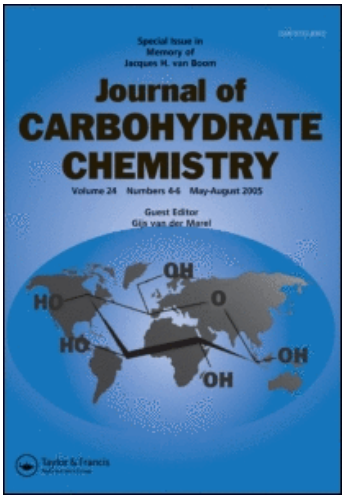

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Syntheses and ^{1}H-NMR Studies on Mucin-Type Sugars Chirally Deuterated at the C-6 Position

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SYNTHESES AND ^{1}H-NMR STUDIES ON MUCIN-TYPE SUGARS CHIRALLY DEUTERATED AT THE C-6 POSITION

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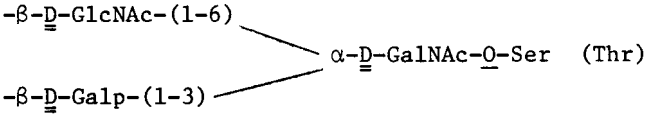
ABSTRACT

1-O-Methyl analogs of mucin oligosaccharide components, D-GalNAc (1a and 1b), β-D-Galp-(1-3)-D-GalNAc (2) and β-D-Galp(1-3)-[β-D-GlcNAc-(1-6)]-D-GalNAc (3) in which the H-6proS proton was selectively replaced by a deuterium, were synthesized to study the solution conformations about the C5-C6 fragments by ^{1}H-NMR spectroscopy. The study revealed the preference of the *gt*-conformer for these sugars.

INTRODUCTION

Mucins contain several types of oligosaccharide chains bound to polypeptides with an O-glycosyl link between D-GalNAc and either a serine (Ser) of threonine (Thr). Many of them have a core structure where the D-GalNAc serves as branching point as indicated below.[1,2]



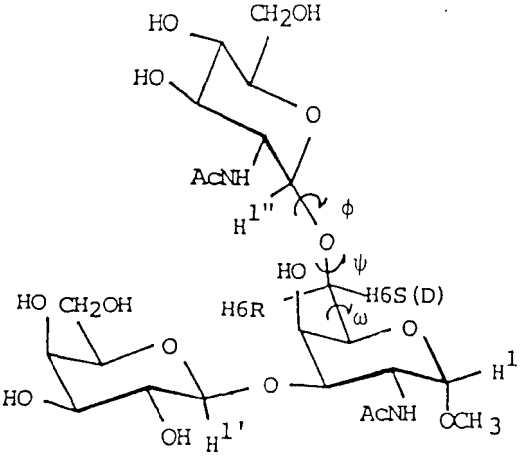


FIG. 1. Structure of tri-saccharide 3 and its (6S)-deuterated analog [(6S)-3]. H-1, H-1' and H-1'' are defined for each anomeric proton of D-GalNAc, D-Galp and D-GlcNAc residues, respectively.

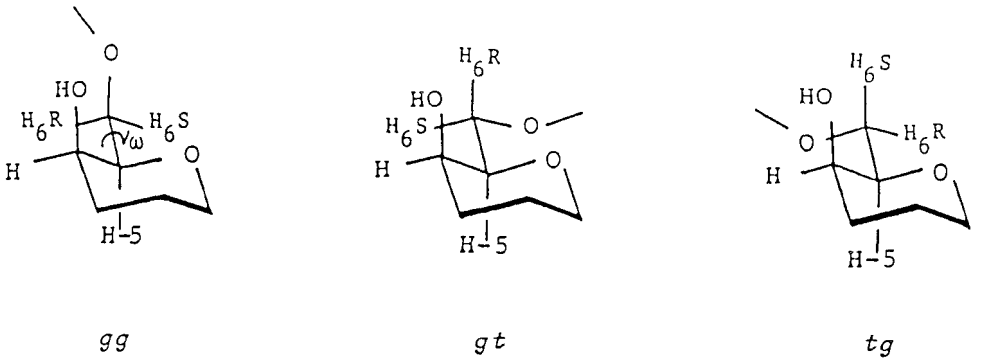


FIG. 2. Three possible conformers about C-5 - C-6 bond.

Determination of the solution conformations, particularly at the (1-6)-linkage moiety of the D-GalNAc, provides key information on the three dimentional structures of mucins. In our preceding studies[3-9] we have applied our method of chiral deuteration at the C-6 position of D-hexoses[3-5] for the ^{1}H NMR studies of conformations of the exocyclic C5-C6 bonds of D-hexoses[6,7] and (1-6)-linked disaccharides.[8,9] In the present study we extend this approarch to the conformational study of miucin components 1-3 as below. We describe herein the syntheses of

Me-α-D-GalNAc 1a

Me-β-D-GalNAc 1b

β-D-Galp-(1-3)-Me-α-D-GalNAc 2

β-D-GlcNAc-(1-6)
>Me-α-D-GalNAc 3
β-D-Galp-(1-3)

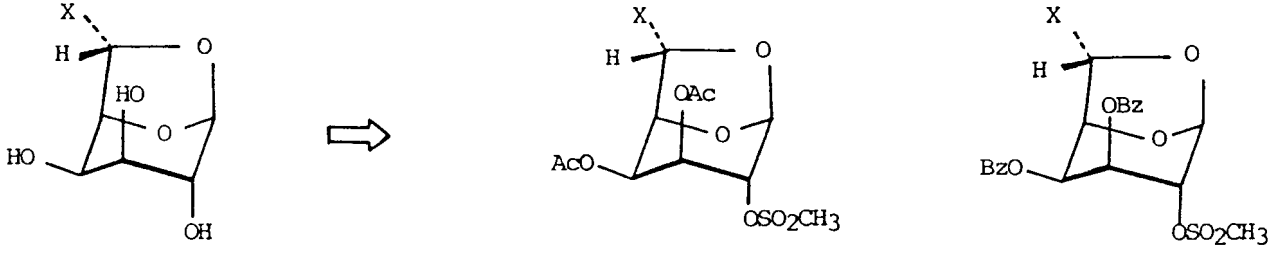
(6S),(6-$^2H_1$)-1~3 where the H-6proS proton is selectively replaced by a deuterium and the use of these compounds for conformational analyses about the C5-C6 bonds[10](FIGs 1 and 2).

RESULTS AND DISCUSSION

A) Chiral Deuteration at the C-6 Position of D-GalNAc Derivatives

In our preceding paper[4] we reported the synthesis of 1,6-anhydro (6S)-deuterated-β-D-galactopyranose [(6S)-4]. From this compounds, it is possible to prepare a desired (6S)-deuterated D-GalNAc according to a reported method.[11,12] Here we used the reported basic method and also attempted a photobromination and a chiral deuteration on 5 and 7 (SCHEME 1) in order to minimize the reaction pathways using the deuterated intermediates. Photobromination of both 5[11] and 7 under the conditions of Ferrier and Furneaux[13] proceeded regio- and stereoselectively at the C-6exo position to give 6 (syrup) and 8 (mp 169-170 °C), respectively. The stereochemical outcome of these reactions could be determined by ^1H NMR analyses because the H-6exo and H-6endo protons of 5 and 7 can be unequivocally assigned by the rule of the coupling constants as $J_{H5,H6exo} > J_{H5,H6endo}$.[5,8] Furthermore, the compound (6S)-5 derived from (6S)-4 with known configuration[5,8] showed the same ^1H NMR spectrum as that of the compound prepared from 5, thus confirming its (6S)-configuration. The deuterated compounds [(6S)-5 and (6S)-7] were converted into 9 (NaOMe/MeOH) and then into 11 without isolating the epoxide 10. Treatment of 9 with excess NaH in dimethylformamide(DMF, 0 °C for 1.5 h) and then with benzyl bromide afforded the benzylated compound 11 (95% from 9). Acid catalyzed acetolysis of 12 (CF_3COOH/Ac_2O) afforded 13 after the epoxide 11 was reacted with sodium azide in DMF at 135 °C for 8 h to give 12. Mehtanolysis of 13 in 15% HCl/MeOH (refluxed for 1 h) gave a 1:1 mixture of 14a and 14b which were separated by silica gel column chromatography using benzene-ethyl acetate. Catalytic hydrogenolysis of the each isomer (Pd-black in 0.5% Ac_2O-MeOH) gave desired (6S)-1a and (6S)-1b, respectively.

SCHEME 1



4 : X= H

(6S)-4: X= D

5 : X= H

6 : X= Br

(6S)- 5 : X= D

7 : X= H

8 : X= Br

(6S)- 7 : X= D

TABLE 1. ^{1}H-NMR Data for Compounds 5 and 7 and their C-6 Bromo and Deuterated Compounds.

Compounds	H-1	H-2	H-3	H-4	H-5	H6endo	H6exo	O-Ac[c]	SO_2Me		
	(Coupling patterns and coupling constants (Hz))										
5	5.54	4.51	5.30	5.20	4.51	4.35	3.76	2.07	3.17		
	(t[d], 1.6)	(s)	(m)	(d, 5.4)	(m)	(d, 7.8)	(dd, 5.3 7.8)	2.14 (S)	(s)		
6	5.91	4.49	5.30	5.20	4.80	6.59	-	2.10	3.18		
	(t, 1.6)	(t, 1.6)	(m)	(dd, 5.4 3.9)	(dd, 1.5 3.9)	(s)	-	2.15 (s)	(s)		
(6S)-5	5.54	4.51	5.30	5.20	4.51	4.33	-	2.07 2.14	3.17		
	(t, 1.6)	(s)	(s)	(d, 5.4)	(m)	(s)		(s)	(s)		
7	~5.67[b]	~4.75	~5.7	~5.60[b]	~4.75[b]	4.61	3.91		3.26	7.82=8.06	7.26-7.63
	(s)	(s)		(dd, 3.9 5.8)		(d, 7.8)	(dd, 5.3 7.8)		(s)	(m)	(m)
8	6.04	4.74	5.70	5.60	5.04	6.82	-		3.27	7.83-8.03	7.30-7.65
	(t, 1.5)	(t, 1.5)	(m)	(dd, 3.9 5.8)	(dd, 1.3 3.9)	(s)			(s)	(m)	(m)
(6S)-7	~5.67[b]	~4.75[b]	~5.7[b]	~5.60[b]	~4.75[b]	4.59	-		3.26	7.83-8.06	7.26-7.63
	(s)	(s)		(dd, 3.9 5.8)		(s)			(s)	(m)	(m)

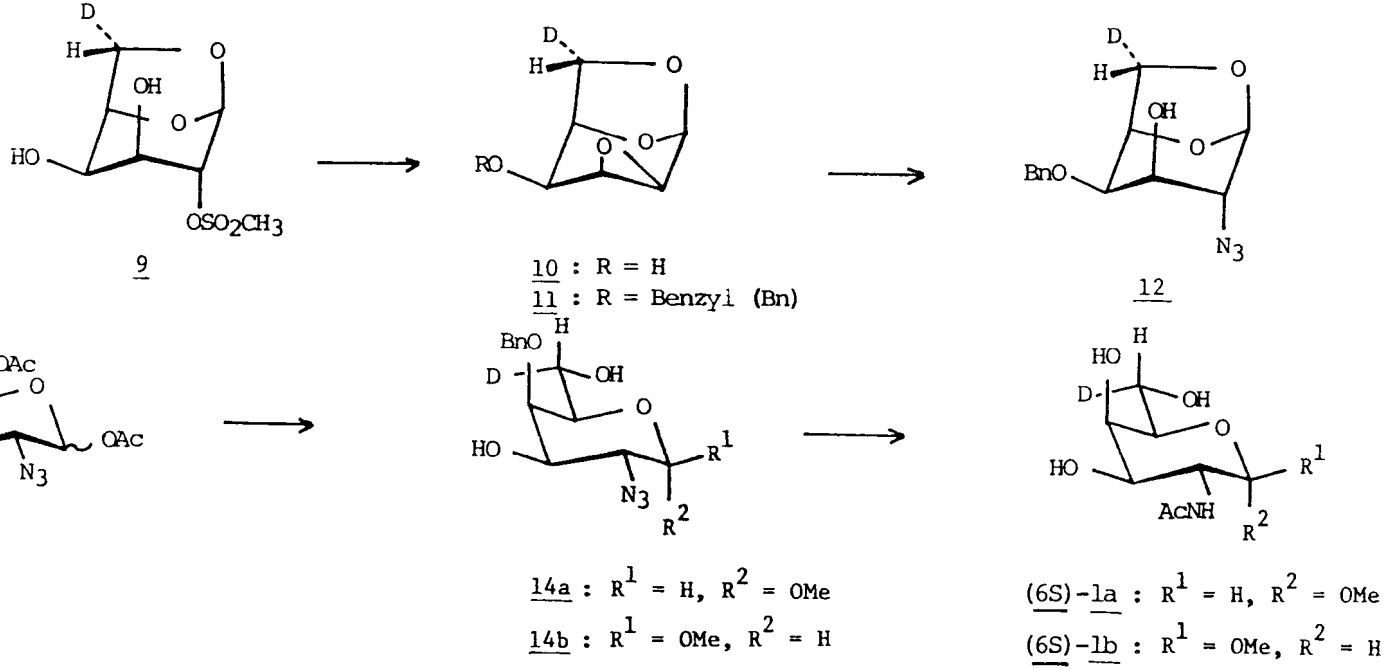
a. Measured at 99.5 MHz using $CDCl_3$ as solvent and TMS as internal standard (0.000 ppm).

b. Signals completely or partially overlapping.

c. Obtained by first-order analysis.

d. The letter t, d, dd and m means triplet, doublet, double-doublet and multiplet including broad ones, respectively.

SCHEME 2



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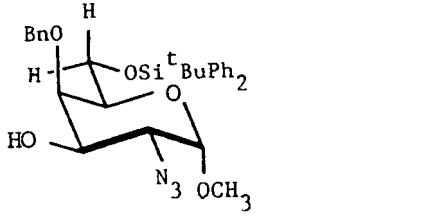

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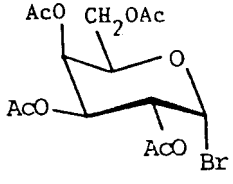

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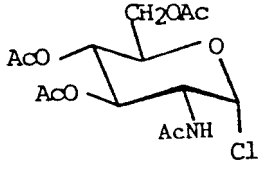

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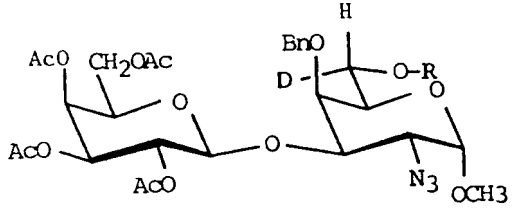
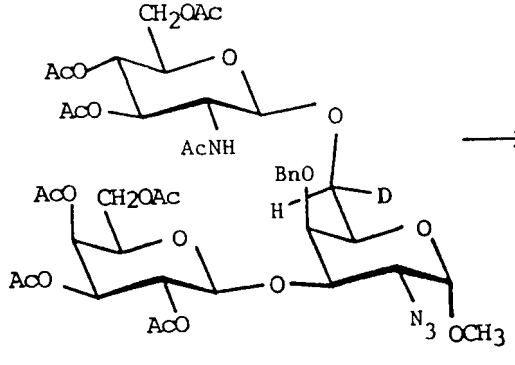


16 : R = Si tBuPh2

17 : R = H

(6S)-2



20

(6S)-3

A disaccharide (6S)-2 was prepared from 14a via selective silylation at the O-6 position to afford 15 and followed by β-glycosidation with acetobromo D-Glcp (18) by the method of Hanessian and Banoub[14] (AgOTf, tetramethylurea in CH_2Cl_2) to give 16. The desilylated compound 17 was also used for the synthesis of a trisaccharide (6S)-3. β-Glycosidation of 17 with a chloride 19[15] gave 20 in ca. 40% yield. Usual deprotection method for 17 and 20 gave desired (6S)-2 and (6S)-3, respectively. The ^{1}H NMR spectrum of (6S)-3 showed the signals for the three anomeric protons at 4.750 ppm (doublet, J = 3.8 Hz, H-1 of α-D-GalNAc), 4.440 ppm (doublet, J = 7.7 Hz, H-1 of β-D-Galp) and 4.528 ppm (doublet, J = 8.5 Hz, H-1 of β-D-GlcNAc) to indicate its β(1-6)-configurations.

B) ^{1}H NMR Assignments of the H-6proR and H-6proS Signals in 1, 2 and 3 and their Conformational Preferences about the C5-C6 Bonds.

The two C-6 protons, namely H-6proR and H-6proS, of 1a and 1b were magnetically unequivalent and gave separate signals (FIG. 3). The vicinal coupling constants of the two protons with H-5 provide key information on the conformations about the C5-C6 bond in the solution. Although it is generally difficult to discriminate the two protons and thereby to determine unequivocally the conformations from the coupling constants, the (6S)-deuterated analogs [(6S)-1a and (6S)-1b] enabled us to solve this problem as indicated in FIG. 3. The $^3J_{H5,H6proR}$ values estimated from the spectra of the deuterated compounds were in accord with the values obtained by ABX-analysis and spin simulations for the H-6proR, H-6proS and H-5 (and H-4 for spin simulations). However, the chemical shifts of H-6proR of the deuterated compounds were at slightly (ca. 0.015-0.020 ppm) higher field compared with the signals of the non-deuterated compounds. This deuterium effect on the chemical shifts was a common feature among the compounds studied here and also previously.[3-9] The complete assignments of all protons of 1a and 1b thus derived from the deuterated analogs are summarized in Table 2, and the rotameric distributions calculated from the vicinal coupling constants are listed in Table 4.

Historically, D-galactoses had been believed to prefer *tg*-conformers. In our previuos studies,[6,8] we revised this concept as follows:

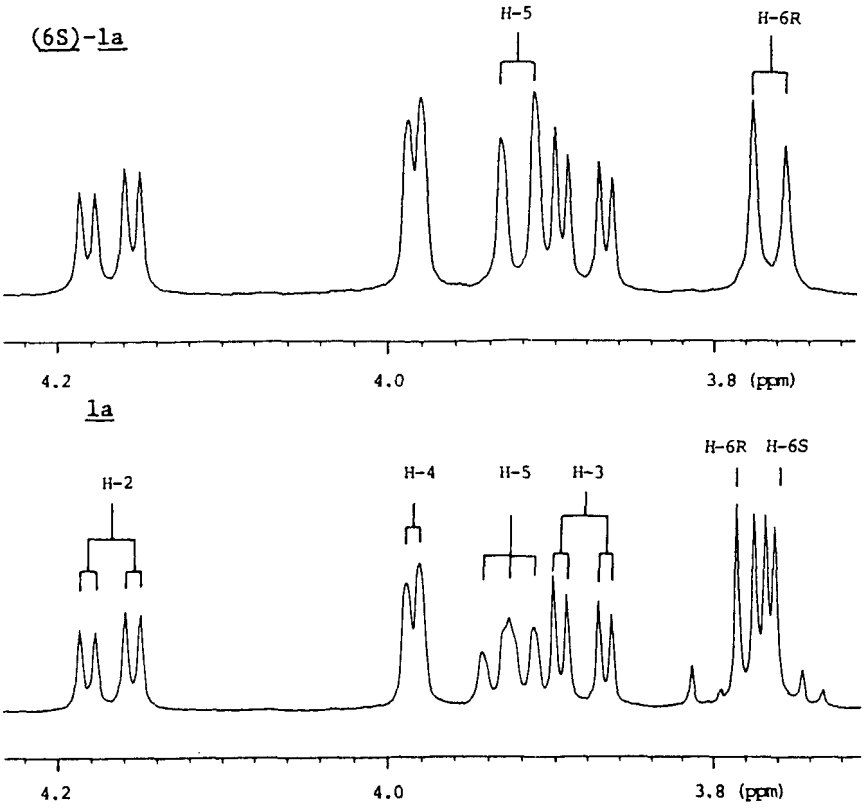


FIG. 3. Partial 400 MHz ^{1}H-NMR Spectra of (6S)-1a (upper) and 1a (below) in D_2O Solution.

D-galactose and methyl D-galactopyranoses (both anomers) predominate in *gt* conformations over *gg* and *tg* conformations in water solution, but in dimethylsulfoxide solution or as their per-O-acylated derivatives (Ac and Bz) in $CDCl_3$ solution prefer the *tg*-conformer to *gg* and gt. The large $^3J_{H5,H\text{-}6proR}$ values (ca. 8.0 Hz) of 1a and 1b also showed the *gt*-preference in a water solution. Calculations of the three rotamers by three different equations A,[16] B[17] and C[18] showed the populations of the *gt*-conformations near 60% - 70%.

Similar analysis was performed on the disaccharide 2. By the first-order analysis of ^{1}H NMR spectrum of (6S)-2 (FIG. 4) the $^3J_{H5,H\text{-}6proR}$ value could be estimated to be 8.4 Hz, and by iterative spin simulation the $^3J_{H5,H\text{-}6proS}$ value was approximated to 4.0 Hz. These values also indicated the *gt*-preference of 2. The analysis on

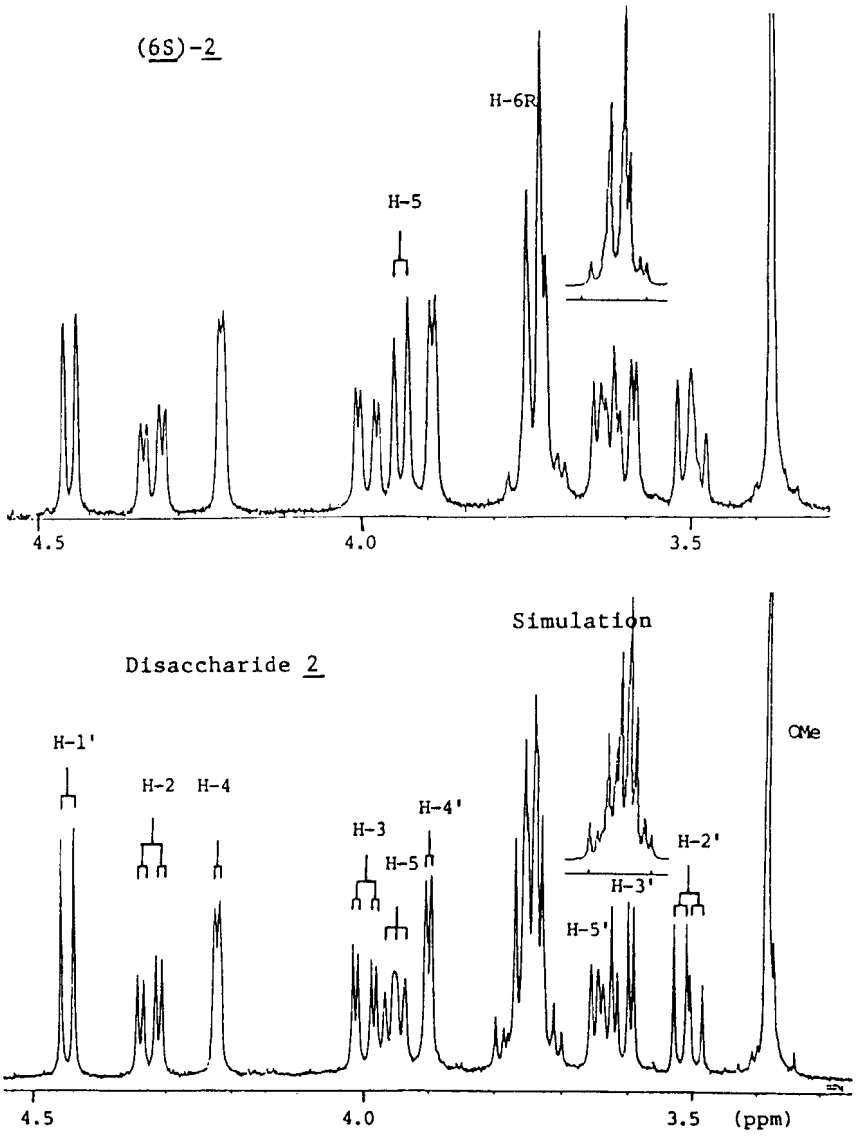


FIG. 4. Partial 400 MHz ^{1}H-NMR Spectra of (6S)-2 (upper) and 2 (below) in D_2O solution and simulation spectrum for H-6proR, H-6proS, H-6proR' and H-6proS' (parameters used for the simulations are listed in Table 3).

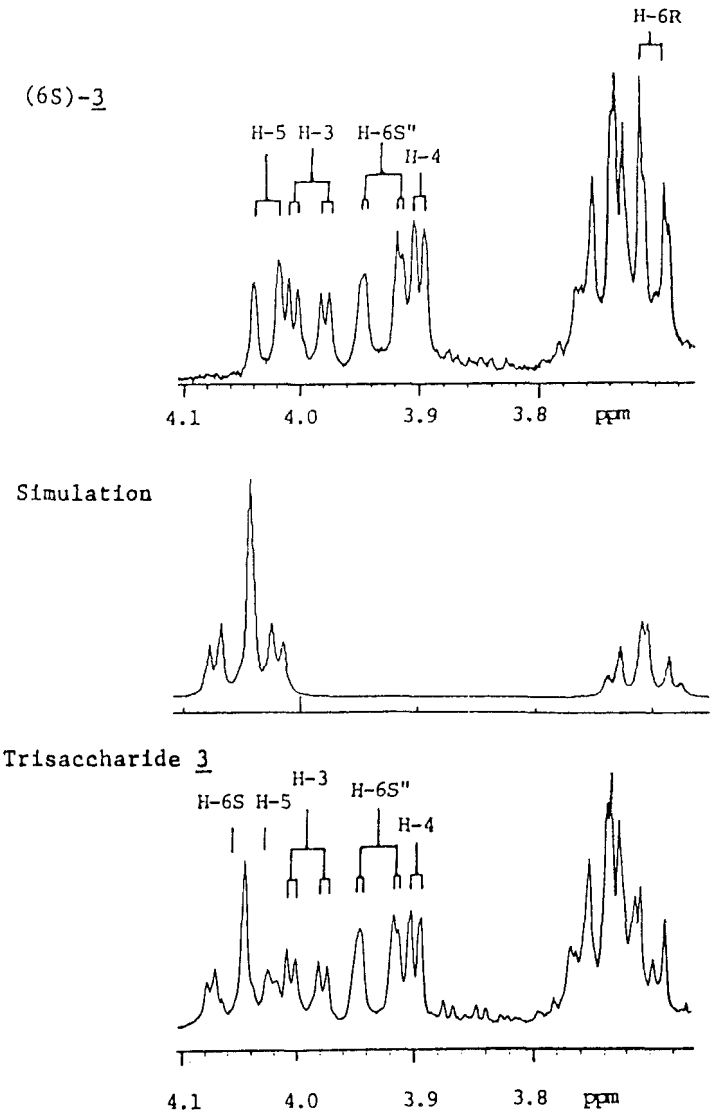


FIG. 5 Partial 400 MHz ^{1}H-NMR Spectra of (6S)-3 (upper) and 3 (below) and simulation spectrum for H-5, H-6proR and H-6proS for 3 (parameters used for this simulation are listed in Table 3).

Table 2. ^{1}H-NMR Data for Compounds 1a and 1b and their (6S)-Deuterated Analogs in D_2O.

Compounds	H-1 [b] ($J_{1,2}$)	H-2 ($J_{2,3}$)	H-3 ($J_{3,4}$)	H-4 ($J_{4,5}$)	H-5[c]	H-6R[c] ($J_{5,6R}$)	H-6S[c] ($J_{5,6S}$, $J_{6R,6S}$)	-OMe	-NDAc
1a	4.779 (3.7Hz)	4.168 (11.0)	3.882 (3.1)	3.984 (1.0)	3.927	3.786 (7.9)	3.760 (4.7, -11.2)	3.390	2.050
(6S)-1a	4.779 (3.7)	4.168 (11.0)	3.883 (3.1)	3.983 (1.0)	3.925	3.765 (8.0)		3.390	2.050
1b	4.393 (8.4)	3.900 (10.5)	3.721 (3.4)	3.942 (1.0)	3.700	3.826 (8.4)	3.781 (3.6, -12.1)	3.520	2.049
(6S)-1b	4.390 (8.4)	3.897 (10.5)	3.721 (3.4)	3.942 (1.0)	3.692	3.806 (8.3)		3.520	2.049

a. Measured at 400 MHz at 296K using 3-(trimethylsilyl)propanesulfonic acid sodium salt as internal standard.

b. Observed first-order couplings.

c. Obtained by ABX analysis.

TABLE 3. ^{1}H-NMR Data for Compounds 2 and 3 in D_2O Solution

Compounds	H-1 ($J_{1,2}$)	H-2 ($J_{2,3}$)	H-3 ($J_{3,4}$)	H-4 ($J_{4,5}$)	H-5	H-6R ($J_{5,6R}$)	H-6S ($J_{5,6S}$, $J_{6R,6S}$)	-OMe	-NDAc
2									
Me-α-GalNAc	~4.78[c]	4.324	3.999	4.221	3.952	3.773	3.739	3.384	2.012
	(3.7)	(11.0)	(2.8)	(0.8)		(8.4)	(4.0, -11.0)		
Residues									
β-D-Galp.	4.448	3.507	3.609	3.900	3.641	3.758[b]	3.725[b]		
	(7.9)	(9.9)	(3.3)	(0.8)		(8.6)	(3.7, -11.0)		
3									
Me-α-GalNAc	4.750	4.33	3.993	4.200	4.032	~3.71[c]	4.060	3.349	2.008
	(3.8)	(11.2)	(2.9)	(0.8)		(8.5)	(4.0, -11.0)		
β-D-Galp.	4.440	3.500	3.606	3.900	3.638	~3.75[b,c]	~3.72[b,c]		
	(7.7)	(10.1)	(3.3)	(1.0)					
β-D-GlcNAc	4.528	3.712	3.534	~3.43[c]	~3.45[c]	~3.73[b,c]	3.933[b]		2.008
	(8.5)	(10.5)	(9.8)	(10.3)		(5.3)	(2, -12.0)		

a. Measured at 400 MHz at 298K using internal acetone standard (2.22 ppm).

b. Assignments of H6proR and H6proS signals may be reversed.

c. Partially or completely overlapped.

TABLE 4. Rotameric Distributions About C5-C6 Bonds of 1, 2 and 3 in D_2O Solution

Compounds	Coupling constants		Rotameric Distributions (%)[a] Method A			B			C		
	$J_{5,6R}$	$J_{5,6S}$	*gg*	*gt*	*tg*	*gg*	*gt*	*tg*	*gg*	*gt*	*tg*
1a	7.9	4.7	21	53	25	16	62	22	62	15	24
1b	8.4	3.6	23	64	14	19	73	7	17	72	11
2	8.4	4.0	21	62	18	16	71	12	14	69	16
3	8.5	4.0	20	63	17	15	72	12	13	70	16

a. Method A : As/Ar = 1.3/1.3 Bs/Br = 2.7/11.5 Cs/Cr = 11.7/5.8 (ref. 16)

B : 2.8/0.9 3.1/10.7 10.7/5.0 (ref. 17)

C 3.6/0.7 2.4/10.8 11.2/4.9 (ref. 18)

for general equations

$$As\,gg + Bs\,gt + Cs\,tg = J_{5,6S} \quad \text{--- (1)}$$

$$Ar\,gg + Br\,gt + Cr\,tg = J_{5,6R} \quad \text{--- (2)}$$

$$gg + gt + tg = 1 \quad \text{--- (3)}$$

the trisaccharides 3 and (6S)-3 (FIG. 5) revealed that the *gt*-preference was maintained at the β(1-6)-linkage moiety. Here it is of significance to note that the H-6proS proton is highly deshielded compared with the H-6proR or H-6proS proton of 1 and 2 by ca. 0.3 ppm. Since the conformation about the C5-C6 bond is little different from that of 1 and 2, the selective deshielding of the H-6proS proton of 3 may be ascribed to the effect of the conformational property along the C-1'' - O-1''(O-6) - C-6 bonds defined by ϕ and ψ angles (FIG. 1). In our previous studies,[8,9] we found a similar deshielding of the H-6proS proton at the β(1-6)-linkage of di-D-glucopyranoses and D-galactopyranoses and also a comparable shift of H-6proR proton at the α(1-6)-linkages. These suggest a conformational equivalence about the β(1-6)-linkages among these disaccharides.[19] The detailed analysis by using Nuclear Enhancement and energy calculations will explain the above described results more precisely.

Consequently, three types of compound 1, 2 and 3, which constitute a core structure of mucin oligosaccharides, were found to take the *gt*-conformation at the D-GalNAc moiety in a water solution. The C-6 position of the D-GalNAc moiety is known to be subject to dehydrogenation by D-galactose oxidase[8,20] and to be glycosylated with D-GlcNAc by a transferase.[21] In these biological processes the *gt*-conformation of D-GalNAc moiety may make significant contribution in influencing the stereo-[7,20] and regioselectivities[2,21] observed with these enzymatic reactions.

EXPERIMENTAL

General Procedures. Melting points were recorded on Yanako model P-type melting point apparatus and uncorrected. IR spectra were recorded on a Jasco A-203 spectrometer between KBr plates for liquid materials and as KBr discs for solid ones. ^{1}H NMR spectra were recorded with JNM FX-100 at 99.5 MHz and GX-400 at 399.5 Hz (JEOL). The NMR solvents used were cited in the text and tetramethylsilane (TMS) was used as an internal standard for $CDCl_3$ solution, with other standards, being cited in the text. Optical rotations were recorded on a Jaco J-20 spectrometer at 589 nm and calibrated with 5% sucrose solution ($[\alpha]_D^{22}$ +66.47° (water)). Kiesel gel 60 F_{254} (Merck) was used for analytical TLC and Kiesel gel 60 (70-230 mesh ASTM, Merck) for silica gel

column chromatography. Solutions were concentrated in vacuo after being dried over $MgSO_4$. Every organic solvent was purified by careful distillation and dried over appropriate salts before use.

1,6-Anhydro-3,4-di-O-acetyl-2-O-methansulfonyl-β-D-galactopyranose (5). 1,6-Anhydro-2-O-methanesulfonyl-β-D-galactopyranose[11] (5 g) was dissolved in dry pyridine (20 mL) - Ac_2O (3 mL) mixture, and the solution was stirred for 12 h at room temperature and concentrated with ethanol-toluene mixture repeatedly to give syrupy 5 (7.2g, 100%). $[\alpha]_D^{22}$ -23.6° (c 0.76, chloroform).

Anal. Calcd for $C_{11}H_{16}O_9S$: C, 40.73; H, 4.98. Found: C, 39.34; H, 4.98.

(6R)-1,6-Anhydro-3,4-di-O-acetyl-6-bromo-2-O-methanesulfonyl-β-D-galactopyranose (6). A mixture of 5 (5 g) and N-bromosuccimide (NBS, 5 g) in carbon tetrachloride (CCl_4, 400 mL) was refluxed over a 300-W heat lamp for 3 h. The reaction mixture was passed through a pad of celite and washed with sat. aq. $NaHCO_3$ and water and processed in a general manner to give a yellow syrup 6, which was purified by silica gel column with 10:1 benzene - ethyl acetate to give analytically pure syrup (5.3 g, 85%), $[\alpha]_D^{22}$ -82.9° (c 0.8, chloroform).

Anal. Calcd for $C_{11}H_{1-}O_9SBr$: C, 32.76; H, 3.76; Br, 19.82. Found: C, 31.44; H, 3.70; Br, 19.69.

(6S),(6-$^2H_1$)-1,6-Anhydro-3,4-di-O-acetyl-2-O-methanesulfonyl-β-D-galactopyranose [(6S)-5]. A mixture of 6 (2 g), n-Bu_3SnD (3 g) and azobisisobutyronitrile (AIBN, 100 mg) in toluene (200 mL) was refluxed for 1 h under nitrogen. The cooled mixture was concentrated, and the residue was chromatographed on silica gel column with 50:1 benzene - ethyl acetate to give syrupy (6S)-5 (1.5g, 90%).

Anal. Calcd for $C_{11}H_{17}(^2H= 2 \times {}^1H)O_9S$: C, 40.61; H, 5.28. Found: C, 40.24; H, 5.31.

1,6-Anhydro-3,4-di-O-benzoyl-2-O-methanesulfonyl-β-D-galactopyranose (7). A mixture of 1,6-anhydro-2-O-methanesulfonyl-β-D-galactopyranose[11] and benzoyl chloride (1 g) in dry pyridine (50 mL) was stirred at 0 °C for 3 h and then at room temperature for 12 h. To the cooled mixture was added 1 mL of water and then 5 mL of sat. aq. $NaHCO_3$ solution, and the mixture was stirred for 3 h, extracted with chloroform and processed in a usual manner to give a syrupy 7 (1.8 g, 92%), $[\alpha]_D^{22}$ -31.5° (c 0.5, chloroform).

Anal. Calcd for $C_{21}H_{20}O_9S$: C, 56.24; H, 4.50. Found: C, 56.30; H, 4.53.

(6R)-1,6-Anhydro-3,4-di-O-benzoyl-6-bromo-2-O-methanesulfonyl-β-D-galactopyranose (8). A mixture of 7 (1 g) and NBS (500 mg) in CCl_4 (100 mL) was processed in the same manner as described for the synthesis of 6 to give 8 in 80% yield, mp 169-170 °C, $[\alpha]_D^{22}$ -132.1° (c 0.8, chloroform).

Anal. Calcd for $C_{21}H_{19}O_9SBr$: C, 47.82; H, 3.64; Br, 15.15. Found: C, 48.02; H, 3.77; Br, 15.18.

Amberite IR 120 and filtered, it was condensed under reduced pressure to give a syrupy residue. The residue was chromatographed on a column of silica gel with 10:1 benzene - ethyl acetate to give 14a as the faster eluting compound (400 mg, 36%) and 14b (410 mg, 37%) as the slower eluting compound. 14a: mp 121-122 °C, $[\alpha]_D^{22}$ +156.1° (c 0.65, chloroform), ^1H NMR ($CDCl_3$); δ3.41 (s, 3H, OMe) and 4.85 (d, 1H, J = 3.4 Hz, H-1).

Anal. Calcd for $C_{14}H_{20}O_5N_3$: C, 54.17; H, 6.51; N, 13.57. Found: C, 54.34; H, 6.56; N, 13.57.

14b: mp 139-140 °C, $[\alpha]_D^{22}$ +6.1° (c 0.16, chloroform); ^1H NMR ($CDCl_3$); δ 3.57 (s, 3H, OMe) and 4.18 (d, 1H, J = 7.8 Hz, H-1).

Anal. Found: C, 54.37; H, 6.62; N, 13.55.

Methyl (6S),(6-$^2H_1$)-2-N-Acetyl-2-deoxy-α-D-galactopyranoside [(6S)-1a] and its β-isomer [(6S)-1b]. The compound 14a (200 mg) was dissolved in methanol (10 mL) and hydrogenolyzed with Pd-black at room temperature for 1 h, and after adding Ac_2O (0.5 mL) to the solution the hydrogenolysis was continued further for 6 h at room temperature. The catalyst was removed by filtration and the filtrate was concentrated to dryness to give a white powder (6S)-1a (125 mg, 82%), mp 220 °C (decomp.), $[\alpha]_D^{22}$ +171.9° (c 0.32, water).

In the same way the compound 14b afforded (6S)-1b in ca. 80% yield, mp 250 °C (decomp.), $[\alpha]_D^{22}$ +13.7° (c 0.3, water).

Methyl (6S),(6-$^2H_1$)-2-Azido-4-O-benzyl-6-O-(t-butyldimethylsilyl)-2-deoxy-α-D-galactopyranoside (15) and its β-anomer. A mixture of 14a (1 g), N,N-dimethylaminopyridine (300 mg), triethylamine (500 mg) and t-butyldimethylsilyl chloride (700 mg) in dichloromethane (CH_2Cl_2, 20 mL) was stirred at room temperature for 6 h. The mixture was diluted with chloroform (30 mL), washed with sat. aq. $NaHCO_3$ solution and with

water and processed as usual to give syrupy 15. By silica gel column with 100:1 benzene - ethyl acetate, the crude syrup was purified (1.3 g, 95%), $[\alpha]_D^{22}$ +127.5° (c 0.39, chloroform).

By the same procedure compound 14b afforded β-isomer 15, mp 113-114 °C, $[\alpha]_D^{22}$ +4° (c 0.75, chloroform).

Anal. Calcd for $C_{20}H_{34}N_3Si$: C, 56.56; H, 8.09; N, 9.90. Found: C, 56.95; H, 7.81; N, 9.68.

(6S),(6-$^2H_1$)-1,6-Anhydro-3,4-di-O-benzoyl-2-O-methanesulfonyl-β-D-galactopyranose [(6S)-7]. Amixture of 8 (2 g), n-Bu_3SnD (1.5 g) and AIBN (100 mg) in toluene (100 mL) was processed in the same way for the synthesis of (6S)-5 to afford syrupy (6S)-7 (1.5 g, 88%).

Anal. Calcd for $C_{21}H_{21}O_9S$: C, 56.11; H, 4.72. Found: C, 56.09; H, 4.70.

(6S),(6-$^2H_1$)-1,6;2,3-Dianhydro-4-O-benzyl-β-D-talopyranose (11). Deacylation of (6S)-5 and (6S)-7 by sodium methoxide in mehtanol gave (6S)-(6-$^2H_1$)-1,6-anhydro-2-O-methanesulfonyl-β-D-galactopyranose 9. The compound 9 (1 g) was dissolved in N,N-dimethylformamide (DMF, 10 mL), and the solution was stirred with sodium hydride (60% oil mixture, 200 mg) at 0 °C for 1 h and then benzyl bromide (1 g) at 20 °C for 3 h. To the mixture methanol was added dropwise to decompose excess sodium hydride, and the mixture was diluted with sat. aq. NaCl solution (50 mL), extracted with ethyl acetate and processed in the usual manner to give syrupy 11 (1.3 g, 100%), $[\alpha]_D^{22}$ -91.9° (c 0.3, chloroform).

(6S),(6-$^2H_1$)-1,6-Anhydro-2-azido-4-O-benzyl-2-deoxy-β-D-galacto-pyranose (12). A mixture of 11 (1 g) and sodium azide (500 mg) in DMF (200 mL) was heated at 135 °C for 8 - 12 h. After the mixture was cooled, the mixture was diluted with sat. aq. NaCl solution (50 mL), and the aqueous solution was extracted with ethyl acetate. The ethyl acetate layer was processed as usual to give a crude syrupy 12. Part of this compound was treated with p-nitrobenzoyl chloride in pyridine to afford a crystalline benzoylated derivative, mp 113-114 °C (lit.[11] 115-117 °C for the non-deuterated compound). The benzoylated derivative was deacylated with sodium methoxide in methanol to give an analytically pure syrup of 12 after silica gel column purification with 50:1 benzene - ethyl acetate; $[\alpha]_D^{22}$ +22.3° (c 0.55, chloroform); IR νmax(cm^{-1}); 3500 (OH), 2100 (N_3).

Methyl (6S),(6-$^2H_1$)-2-Azido-4-O-benzyl-2-deoxy-α-D-galactopyranoside (14a) and its β-isomer (14b). A solution of 12 (1 g) in 15% trifluoroacetic acid - Ac_2O (20 ml) was stirred at room temperature for 12 h and concentrated repeatedly with ethyl alcohol to give syrupy 1,3,6-tri-O-acetyl-2-azido-3-O-benzyl-2-deoxy-D-galactopyranose (13). The compund compound 13 was treated with 0.05 M sodium methoxide in methanol (20 mL) for 30 min.. After the mixture was neutralized with

Methyl (6S),(6-$^2H_1$)-3-O-(β-D-Galactopyranosyl)-2-N-acetyl-2-deoxy-α-D-galactopyranoside [(6S)-2]. Compound 17 (200 mg) was dissolved in methanol and hydrogenolyzed with Pd-black at room temperature for 1 h. After 0.1 mL of Ac_2O was added to the solution, hydrogenolysis was continued for 6 h at room temperature. After the catalyst was filtered off, the filtrate was concentrated repeatedly with ethanol. The residual syrup was dissolved in methanol containing a catalytic amount of barium (as barium methoxide), and the solution was stirred for 3 h, treated with Amberite IR-120 (H^+ form) to remove Ba^{2+} and concentrated to dryness. The residue was treated with ethanol - ethyl ether to give a white powder (6S)-2 (54.7 mg, 77%), mp 182-187 °C (decomp.), $[\alpha]_D^{22}$ +92.7° (c 0.3, water).

Methyl (6S),(6-$^2H_1$)-3-O-(2,3,4-Tetra-O-acetyl-β-D-galactopyranosyl)-O-(2-N-acetyl-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-2-azido-4-O-benzyl-2-deoxy-α-D-galactopyranoside (20). To a stirred mixture of 17 (240 mg), 1,1,3,3-N,N-tetramethylurea (60 mg), AgOTf (126 mg) and molecular sieves 4Å (100 mg) in CH_2Cl_2 (15 mL) was added dropwise a solution of 2-N-acetyl-3,4,6-tri-O-acetyl-2-deoxy-α-D-glucopyranosyl chloride[14] (180 mg) for 5 min at 0 °C. The mixture was stirred at 20 °C for 24 h, diluted with 50 mL of chloroform, filtered to remove salt and washed with sat. aq. $NaHCO_3$ solution and water, and processed as usual. The residual syrup was chromatographed on a silica gel column in 100:1 chloroform - methanol to afford glassy 20 (145 mg, 40% yield based on 17): IR ν max; 3350 (NH), 2100 (N_3), 1740 (OAc), 1670 (NHAc), 1530 (NHAc), 730 (benzyl) and 698 (benzyl).

Methyl (6S),(6-$^2H_1$)-3-O-(β-D-Galactopyranosyl)-6-O-(2-N-acetyl-2-deoxy-β-D-glucopyranosyl)-2-N-acetyl-α-D-galactopyranoside [(6S)-3]. Compound 20 (100 mg) was dissolved in methanol (20 mL) and hydrogenolyzed with Pd-black (100 mg) for 1h. After 0.2 mL Ac_2O was added to the solution, hydrogenolysis was continued for 8 h at room temperature.

The catalyst was filtered off, and the solution was concentrated to dryness to afford a compound where C-2 azido group in 20 was reduced to N-acetyl group and O-4 benzyl group was deprotected: IR ν max; 3500 (OH), 3400 (NH), 3300 (NH), 1740 (OAc), 1650 (NHAc) and 1540 (NHAc).

This compound was treated with barium methoxide in methanol for 3 h and then Amberite IR-120 (H^+), and the solution was concentrated to dryness. The residual syrup was treated with a small amount of ethanol to afford a white powder (6S)-3 (48 mg, 77%), mp 187 °C (decomp.) - 190 °C (completed), $[\alpha]_D^{22}$ +47° (c 0.3, water).

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