Preferred Conformation about the C5–C6 Bond of *N*-Acetylneuraminyl(2–6)-D-galacto- and -D-glucopyranosides in Solution

Hiroshi Ohrui,* Yoshihiro Nishida, Hisashi Itoh, and Hiroshi Meguro*

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

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Diastereoselective deuteration and ^{1}H NMR spectroscopy were applied in a conformational study of the C5–C6 bond in four stereoisomeric methyl *N*-acetylneuraminyl(2–6)-β-D-hexopyranosides [NeuNAcα(2–6)Gal, NeuNAcβ(2–6)Gal, NeuNAcα(2–6)Glc, and NeuNAcβ(2–6)Glc]. In aqueous solution, NeuNAcα(2–6)Gal prefers a *gt* conformer (*gg:gt:tg* = ca. 20:60:20), NeuNAc(2–6)Glc isomers prefer a *gg* (*gg:gt:tg* = ca. 60:40:0), and NeuNAcβ(2–6)Gal exists equally in the three conformers.

N-Acetylneuraminic acid (NeuNAc) and its acylated derivatives (the sialic acids) are terminal units in glycoproteins and glycolipids which mediate a variety of biochemical processes.[1] They are linked to the O-3 or O-6 position of D-galactose (Gal) or *N*-acetyl-D-galactosamine (GalNAc) residue with an α(2–3)- or α(2–6)-linkage, and several approaches have been reported to determine the preferred conformation about the NeuNAc linkages.[2-8] Recently[2-4,6] a unique conformation has been proposed for sialooligosaccharides with a common NeuNAcα(2–6)-Galβ(1–4)GlcNAc unit; the terminal NeuNAc residue is folded back toward the GlcNAc residue. This orientation allows a close interaction between the terminal NeuNAc and internal sugars and therefore seems to have a significant role in determining the biochemical or biophysical properties of sialooligosaccharides. This conformation strongly depends on the *gt* preference (Figure 1) of the C5–C6 bond at the NeuNAcα(2–6)Gal linkage,[2c] but little direct evidence has been obtained to verify it probably because of the complexity of the NMR spectra and the difficulty in differentiating H-6*proR* and H-6*proS* at the linkage, although ^{1}H NMR data of these two protons should provide key information.[9,10]

In this paper, we studied the preferred conformation about the C5–C6 bond of four simple sialosaccharides: NeuNAcα(2–6)Gal (**9a**), NeuNAcβ(2–6)Gal (**9b**), NeuNAcα(2–6)Glc (**13a**), and NeuNAcβ(2–6)Glc (**13b**) by using diastereoselective deuteration at the C-6 position to discriminate the two C-6protons in the ^{1}H NMR analysis.[9,10] We wish to show particularly that the *gt* conformation is predominant in the natural isomer **9a**, but the preferred conformation varies among isomers with linkage configurations and stereochemistries at the C-4 position of the 6-O-sialylated hexoses.

Results and Discussion

Syntheses of Diastereoselectively Deuterated NeuNAc(2–6) Sugars. We have already reported the syntheses of (6*S*)-[6-^{2}H]methyl β-D-galactopyranoside **1** and (6*S*)-[6-^{2}H]methyl β-D-glucopyranoside **3** (Figure 2).[9] They were converted into glycosylation acceptors **2** and **4**, respectively, via silylation at O-6 with *tert*-butyldimethylsilyl chloride followed by per-O-benzylation and desilylation. Glycosylation between **2** and NeuNAc-Cl **5**[11,12] with $Hg(CN)_2$ and $HgBr_2$[12,13] gave an α,β-mixture of NeuNAc(2–6)Gal derivatives (**6a** and **6b**) which were separated with silica gel column chromatography and deprotected in the usual way to give **9a** and **9b**, respectively. In a similar manner, **4** was coupled with **5** to afford **10a** and **10b**, which were separated and deprotected to give **13a** and **13b**. Linkage configurations of the products were determined by the reported method using NMR spectroscopy[14,15] (Table II).

^{1}H NMR Assignments of H-6*proR* and H-6*proS* in NeuNAc(2–6) Linked Sugars. Unequivocal discrimination between the H-6*proR* and H-6*proS* protons at the NeuNAc(2–6) linkage is crucial for the ^{1}H NMR study to determine the conformations about the C5–C6 bond because the ^3J(H5,H6*proR*) and ^3J(H5,H6*proS*) values provide the key information. In this study, our diastereoselective deuteration[9] was successfully applied to circumvent this problem. In Figure 3, partially relaxed ^{1}H NMR spectra of **9a** (Figure 3A) and its nondeuterated analogue (Figure 3B) are compared. The inversion recovery method with a pulse sequence of 180°–500 ms–90° was used to simplify the complicated ^{1}H NMR spectrum. Two double doublet peaks at 3.63 and 3.96 ppm of a nondeuterated compound in Figure 3B disappear in the spectrum of the deuterated compound in Figure 3A, while negative doublet signals appear at 3.94 ppm. Since a H-6*proS* proton of **9a** was selectively replaced with a deuterium, the missing signal at 3.63 ppm is assignable to H-6*proS*, and the signal at 3.96 ppm is assigned to H-6*proR*.

In the same way, H-6*proR* and H-6*proS* of the other sialosaccharides are unequivocally assigned, and their

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Table I. ^{1}H NMR Spectral Data for Methyl β-D-Galactopyranoside and Methyl β-D-Glucopyranoside Residues in NeuNAc(2–6)-D-hexopyranosides in D_2O Solution

compd	chem shifts (ppm) and coupling constants (Hz)[a]							
	H-1	H-2	H-3	H-4	H-5	H-6*proR*	H-6*proS*	C1-OMe
8a	4.306	3.496	3.637	3.909	3.781	4.006	3.684	3.568
	(8.1)	(9.9)	(3.3)	(0.9)		(7.6, −10.2)	(4.6)	
9a	4.319	3.496	3.641	3.941	3.780	3.955	3.633	3.581
	(8.1)	(9.8)	(3.3)	(0.9)		(7.6, −10.1)	(4.6)	
9b	4.351	3.512	3.647	3.974	3.816	3.484	3.752	3.585
	(8.1)	(10.0)	(3.1)	(1.0)		(6.1, −10.0)	(5.7)	
Me β-D-galactopyranoside	4.324	3.508	3.653	3.929	3.705	3.802	3.763	3.579
	(8.0)	(9.9)	(3.3)	(0.9)		(8.0, −11.2)	(4.4)	
12a	4.352	3.260	~3.48	~3.48	~3.48	4.051	3.778	3.553
	(8.1)	–[b]	–[c]	–[c]		(3.9, −11.0)	(1.8)	
12b	4.378	3.264	3.462	~3.57	~3.57	3.518	3.963	3.579
	(8.1)	(9.0)	–[b]	–[c]		(5.2, −10.7)	(1.9)	
13a	4.353	3.250	~3.46	~3.46	3.529	3.983	3.767	3.549
	(8.1)	–[c]	–[c]	–[c]		(5.1, −11.0)	(1.8)	
13b	4.365	3.258	3.470	~3.53	~3.53	~3.53	~3.9	3.560
	(8.1)	(8.8)	–[b]	–[c]		–[c]	–[b]	
Me β-D-glucopyranoside	4.383	3.263	3.494	3.380	3.463	3.727	3.932	3.578
	(8.1)	(9.2)	(9.5)	(9.5)		(6.0, −12.1)	(2.2)	

[a] Measured at 400 MHz in D_2O at 23 °C with an internal TPS (3-(trimethylsilyl)propanesulfonic acid sodium salt (0.0000 ppm)). Digital resolution of 0.24 Hz. [b] Not estimated correctly due to vertual couplings or overlapping of signals. [c] Not estimated correctly due to second order couplings or overlapping of signals.

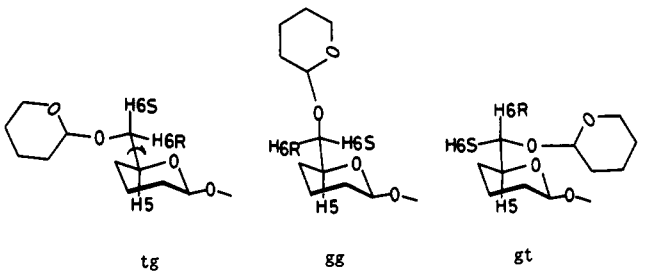

Figure 1. Three rotamers about the C5–C6 bond at an NeuNAc(2–6) linkage.

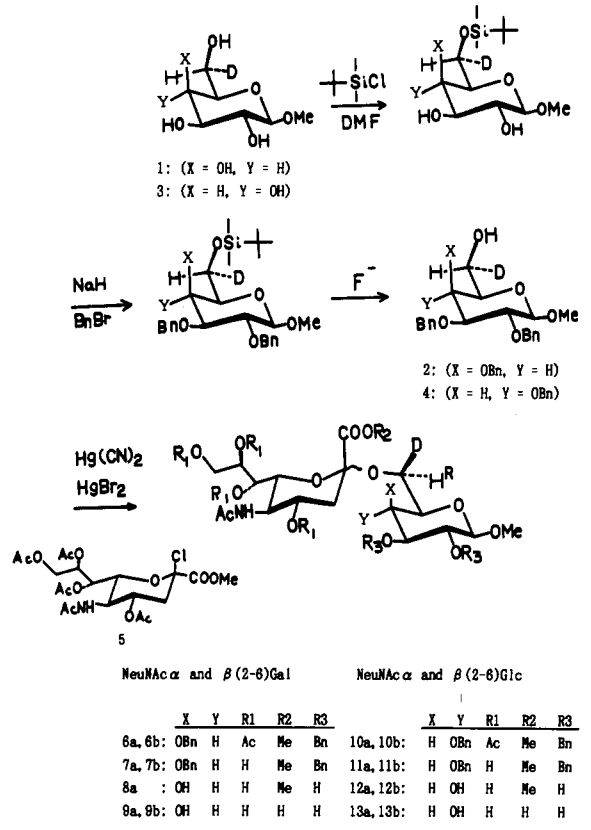

Figure 2. Syntheses of diastereoselectively deuterated NeuNAc(2–6)Gal (**9a** and **9b**) and NeuNAc(2–6)Glc (**13a** and **13b**).

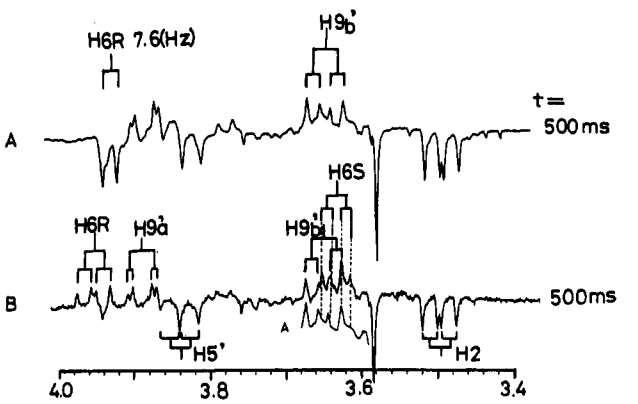

Figure 3. Partially relaxed ^{1}H NMR spectra (400 MHz, D_2O) of (6*S*)-deuterated NeuNAc(2–6)Gal (**9a**, spectrum A) and its nondeuterated analogue (spectrum B).

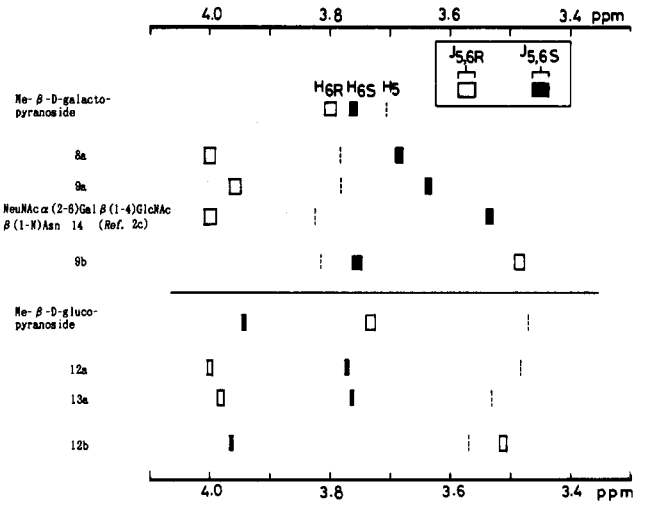

Figure 4. Comparison of chemical shifts and vicinal coupling constants of H-6*proR* and H-6*proS* of NeuNAc(2–6)hexopyranosides in D_2O.

vicinal coupling constants and chemical shifts derived by first-order analysis are summarized in Table I and illustrated in Figure 4. It should be noticed that for **9a** and **13a** with a NeuNAcα(2–6) linkage, H-6*proR* shifts to lower field, while for **9b** and **13b** with a NeuNAcβ(2–6) linkage,

Table II. ^{1}H NMR Spectral Data for the NeuNAc Residues in NeuNAc(2–6)-D-hexopyranosides in D_2O

	chem shift (ppm) and coupling constants (Hz)[a]										
compd	H-3ax′	H-3eq′	H-4′	H-5′	H-6′	H-7′	H-8′	H-9a′	H-9b′	NHAc	COOMe
8a	1.846	2.723	~3.76	~3.88	~3.57	~3.57	~3.86	~3.86	3.656	2.039	3.886
	(12.4)	(4.8, 12.8)	(–	–	–	–	–	–)[b]	(6.7, 12.5)		
9a	1.704	2.741	3.690	3.839	3.721	3.593	~3.9	3.888	3.648	2.044	
	(12.5)	(4.6, 12.5)	(10.1)	(10.8)	(1.6)	(9.3)		(2..5)	(7.0, 12.5)		
9b	1.658	2.393	4.100	3.891	3.834	3.545	~3.88	~3.88	3.667	2.055	
	(12.3)	(5.0, 12.8)	(10.0)	(10.6)	<1.0	(9.3)		–	(6.1, 12.3)		
12a	1.843	2.741	3.762	~3.88	~3.86	3.567	~3.85	~3.85	3.659	2.040	3.888
	(12.3)	(4.4, 12.8)	–	–	–	(8.0)		–	(6.6, 13.0)		
12b	1.797	2.504	4.13	3.947	~3.95	~3.57	~3.86	~3.86	3.656	2.059	3.866
	(11.5)	(4.9, 13.2)	–	–	–	–		–	(6.6, 12.5)		
13a	1.762	2.723	3.729	3.849	3.766	~3.57	~3.86	3.854	3.626	2.028	
	(12.1)	(4.8, 12.5)	(10.4)	(10.3)	(1.5)	(8.0)		(2.4)	(6.8, 12.7)		
13b	1.726	2.438	4.122	~3.92	~3.92	~3.54	~3.88	~3.86	3.638	2.044	
	(11.7)	(4.8, 12.8)	–	–	–	–		(2.5)	(6.1, 12.8)		

[a] Measured at 400 MHz and 23 °C with an internal TPS standard (0.0000 ppm). [b] Not estimated due to the overlapping of signals or second-order couplings.

H-6*proS* shifts to lower field. The same relation is maintained in the protected sugars regardless of the solvents and the conformational changes about the C5–C6 bonds. This relation seems to be useful for the assignment of H-6*proR* and H-6*proS* signals in more complicated sialylated sugars; for example, the ^{1}H NMR data of NeuNAcα(2–6)Galβ(1–4)GlcNAcβ(1–N)Asn (**14**)[2c] were assigned accordingly (Figure 4).

In comparison with the NMR data of asialo methyl β-D-galactopyranoside or methyl β-D-glucopyranoside in D_2O,[10] H-6*proR* in NeuNAcα(2–6) sugars (**8a**, **9a**, **12a**, and **13a**) is deshielded by ca. 0.2–0.3 ppm and H-6*proS* is shielded by ca. 0.1–0.2 ppm, while for NeuNAcβ(2–6) sugars (**9b**, **12b**, and **13b**) H-6*proR* is shielded by ca. 0.2–0.3 ppm, but the chemical shift of H-6*proS* is hardly changed. These shifts can be ascribed to the substitution of the sialic acid but cannot be explained exactly only by the substituting effect because the effect of this kind would appear equally on H-6*proR* and H-6*proS*. The diversity of the chemical shift changes between the two protons would reflect the conformation about the (2–6) linkage surrounding the two protons.

Since the conformation about the C5–C6 bonds is similar in the asialo and sialo saccharides as described later, the chemical shift changes seem to be mainly associated with the orientation of the C2–O6–C6 bonds defined by ϕ and ψ angles, respectively. The deshielding of H-6*proR* and shielding of H-6*proS* in NeuNAcα(2–6) sugars strongly suggest a conformation in which H-6*proR* is close to the ring oxygen and H-6*proS* is in the shielding region of the carbonyl group of NeuNAc, i.e., ϕ(C1–C2–O6–C6) = ca. –60° and ψ(C2–O6–C6–C5) = ca. 180° which follows an exoanomeric effect[16] and our previous results of α(1–6) linked hexopyranosides.[17,18] A similar conformation has been proposed by Breg et al.[2c] for a trisaccharide (**14**) on the basis of HSEA calculations. On the other hand, the large shielding of H-6*proR* in NeuNAcβ(2–6) sugars suggests a conformation in which H-6*proR* is in the strong shielding region of the carbonyl group. The small changes in H-6*proS* chemical shifts can be explained in two ways: (a) although the conformation follows an exoanomeric effect[16] and our previous results with β(1–6) linked hexopyranosides,[17,18] i.e., ϕ = ca. +60° and ψ = ca. 180°, the deshielding effect of the ring oxygen of NeuNAc on H-6*proS* is cancelled by the shielding effect by the carbonyl group or (b) the ϕ or/and ψ angles changed as a result of a change in conformation.

Preferred Conformations about the C5–C6 Bond in Solution. It is generally accepted that the conformation about the C5–C6 bond of hexoses defined by ω angles (O5–C6–C6–O6) exists in an equilibrium of three staggered conformers, namely *gg* (ω = ca. –60°), *gt* (ca. 60°), and *tg* (ca. 180°) (Figure 1). Three-dimensional structures of NeuNAc(2–6) sugars depend largely on the conformation about this bond, and the *gt* preference is considered to be essential for the back-folding of the terminal NeuNAc as described before.

A set of two vicinal coupling constants, ^3J(H5,H6*proR*) and ^3J(H5,H-6*proS*) is useful to determine the preferred conformation about C5–C6 bond. Here, three types of equations, A,[19,20] B,[21,22] and C,[10] were employed for calculating the time-averaged distributions of the three rotamers (Table III). For the NeuNAc(2–6)Gal isomers, eq B was presumed to be the most suitable based on the expected deviation of the dihedral angle about the C5–C6 bond from perfect staggering,[10] while for the NeuNAc(2–6)Glc isomers, eqs A and C were presumed as the most suitable ones.

The large ^3J(H5,H6*proR*) value (7.6 Hz) and a smaller ^3J(H5,H6*proS*) value (4.6 Hz) of the natural isomer **9a** clearly shows that the *gt* conformer is the most populated. The calculation reveals a *gg*:*gt*:*tg* ratio of ca. 20:60:20 (eq B). Compared with asialo methyl β-D-galactopyranoside,[10] the *gt* population of **9a** is slightly decreased. Little difference is observed in the conformation between **9a** with a COO^- anion and **8a** with a COOMe group. A natural trisaccharide (**14**)[2] also shows a high *gt* population of nearly 80% (eq B). This result agrees well with the conclusion of Breg et al. based on the ^{1}H NMR NOE study.[2c] The higher *gt* population of trisaccharide **14** compared with **9a** suggests an attractive interaction between the terminal NeuNAc and the internal sugar residues to stabilize the *gt* conformation.

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Table III. ^{1}H NMR Spectral Data for H-6*proR* and H-6*proS* Signals of NeuNAc(2–6)-D-heoxpyranosides and the Related Monosaccharides and the Rotameric Distributions of the Three Rotamers about the C5–C6 Bond

compd	solv	chem shift, (ppm) H-6*proR*	chem shift, (ppm) H-6*proS*	coupling constant, Hz J(H5,H6-*proR*)	coupling constant, Hz J(H5,H6-*proS*)	rotamer distributions A[a] *gg*	A[a] *gt*	A[a] *tg*	B[a] *gg*	B[a] *gt*	B[a] *tg*	C[a] *gg*	C[a] *gt*	C[a] *tg*
Me β-D-galactopyranoside	D_2O	3.802	3.763	8.0	4.4	22	56	22	21	62	17	14	63	23
8a	D_2O	4.006	3.684	7.6	4.6	24	51	25	23	57	20	16	58	26
9a	D_2O	3.955	3.633	7.6	4.6	24	51	25	23	57	20	16	58	26
NeuNAcα(2–6)Galβ(1–4) GlcNAcβ(1–N)Asn[b]	D_2O	3.996[b]	3.528[b]	9.2[b]	3.6[b]	16	72	12	15	78	7	7	79	14
9b	D_2O	3.484	3.752	6.1	5.7	32	30	38	29	37	34	23	38	39
6a	$CDCl_3$	3.880	3.513	5.7	6.2	32	24	44	30	31	39	23	32	45
6b	$CDCl_3$	3.560	3.650	6.2	6.4	27	28	45	23	35	42	16	37	47
Me β-D-glucopyranoside	D_2O	3.727	3.932	6.0	2.2	52	45	3	57	52	−9	54	48	−2
12a	D_2O	4.051	3.778	3.9	1.8	74	25	1	80	34	−14	80	26	−6
12b	D_2O	3.518	3.963	5.2	1.9	61	38	1	67	46	−13	65	40	−5
13a	D_2O	3.983	3.767	5.1	1.8	63	37	0	68	45	−13	67	39	−6
13b	D_2O	~3.53	~3.9	–	–									
10a	$CDCl_3$	4.169	3.404	3.7	2.6	71	19	10	76	28	−4	75	21	4
10b	$CDCl_3$	3.786	3.870	2.1	2.6	85	2	13	92	12	−4	92	3	5

[a] Equation A: As = 1.3, Bs = 2.7, Cs = 11.7, Ar = 1.3, Br = 11.5, Cr = 5.8 for A*sgg* + B*sgt* + C*stg* = J(H5,H6*proS*) (1). Equation B: As = 2.9, Bs = 3.0, Cs = 11.2, Ar = 1.0, Br = 11.2, Cr = 4.9 for A*rgg* + B*rgt* + C*rtg* = J(H5,H6*proR*) (2). Equation C: As = 2.2, Bs = 2.4, Cs = 11.1, Ar = 1.7, Br 10.8, Cr = 4.1 for *gg* + *gt* + *tg* = 1 (3). [b] Obtained from a report of Breg et al. (ref 2c).

Unnatural isomer **9b** with a β(2–6) linkage and protected derivatives **6a** and **6b** in $CDCl_3$ solution show smaller ^3J(H5,H6*proR*) values and larger ^3J(H5,H6*proS*) values than **9a** reflecting the decrease of the *gt* population and the increase of the *tg* population (Table III).

Larger conformational change was observed for NeuNAc(2–6)Glc isomers **10–13**. These isomers favor a *gg* conformation more than *gt* and *tg*, and the *tg* population is nearly zero (eq A). Compared with asialo methyl β-D-glucopyranoside,[10] the *gg* population of NeuNAc(2–6)Glc increases by ca. 10–20%. Particularly, compounds **10b** and **12a** show the highest *gg* preference that has never been observed for mono-,[17] di-, and tri-D-glucopyranosides[18] in our preceding studies.

The conformational change between NeuNAc(2–6)Gal and NeuNAc(2–6)Glc isomers is similar to the change between methyl β-D-galactopyranoside and methyl β-D-glucopyranoside and therefore seems to be ascribable to the difference in the 1,3-syn interaction between the C4–O4 and C6–O6 bonds.[10] The conformational change between **9a** and **9b** is difficult to explain but may be rationalized by theoretical or semiempirical calculations.

Conclusion

The conformation about the C5–C6 bond in the NeuNAc(2–6)hexopyranosides was found to change significantly with the linkage configurations and stereochemistries at the C-4 position in the sialosaccharides. In the four stereoisomers studied here, NeuNAcα(2–6)Gal (**9a**) with a natural linkage showed the highest *gt* preference which is essential for back-folding of the NeuNAc unit. This result may well rationalize the fact that in nature NeuNAc selectively links with Gal or GalNAc with an α(2–6) bond and indicates a significance of the back-folding structure in the biochemical roles of sialooligosaccharides. The present study also suggests an additional stabilization of the *gt* conformation in trisaccharide **14**.

Experimental Section

General Procedures. Melting points are uncorrected, ^{1}H NMR spectra were recorded at 400 MHz (JEOL). Tetramethylsilane (TMS) was used for $CDCl_3$ and CD_3OD solution and 3-(trimethylsilyl)propanesulfonic acid sodium salt (TPS) was used as an internal standard (δ 0.0000 ppm) for D_2O solution. Optical rotations were calibrated with 5% sucrose solution ($[\alpha]^{22}_D$ +66.5° (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. Unfortunately, NMR analyses showed that glassy products **6a–13b** (not crystallized) were contaminated by small amounts of solvents used for chromatography and deprotection reactions. Consequently, elemental analyses for compounds **6a**, **6b**, **10a**, and **10b** were unsatisfactory. The identification and establishment of purity of the title compounds were based primarily on satisfactory high-resolution ^{1}H NMR spectra. Representative spectra for **6a–13b** are provided in the supplementary material.

(6*S*)-[6-^{2}H]Methyl 2,3,4-Tri-*O*-benzyl-β-D-galactopyranoside (2). A mixture of (6*S*)-[6-^{2}H]methyl-β-D-galactopyranoside (1)[10] (2.0 g, 10.3 mmol), triethylamine (3.12 g, 30.8 mmol), and 4-(dimethylamino)pyridine (0.188 g, 1.54 mmol) in dimethylformamide (DMF, 25 mL) was stirred at room temperature for 15 min. To the solution was added *tert*-butyldimethylsilyl chloride (2.6 g, 15.5 mmol) dropwise for 20 min. After the solution was stirred for 3 h, triethylamine (1 g, 9.9 mmol), sodium hydride (2.47 g, 0.10 mol), and benzyl bromide (11.2 mL, 0.10 mol) were successively added at 0 °C, and the mixture was stirred for 6 h at room temperature. To the mixture were added dropwise methanol (2 mL) and then saturated NaCl aqueous solution (200 mL). The solution was extracted with ethyl acetate (50 mL × 3), and the extracts were collected, washed with saturated NaCl solution, and concentrated. The residual syrup was dissolved in a mixture of 1 M tetrabutylammonium fluoride–tetrahydrofuran solution (10 mL) and ethanol (10 mL), and the solution was refluxed for 3 h, concentrated, and diluted with $CHCl_3$ (50 mL). The solution was washed with water, dried over $MgSO_4$, and concentrated. The residue was chromatographed on silica gel column using *n*-hexane–ethyl acetate (10:1) to elute benzyl bromide or benzyl methoxide followed by *n*-hexane–ethyl acetate–methanol (10:10:1) to elute **2** (2.61 g, 54%) as a waxy solid, mp 100–103 °C ($[\alpha]^{22}_D$ −1.7° (*c* 0.4, $CHCl_3$)). ^{1}H-NMR: δ 4.289 (1 H, d, J = 7.8 Hz, H-1), 3.827 (1 H, dd, J = 7.8 and 9.8 Hz, H-2), 3.532 (1 H, dd, J = 2.8 and 9.8 Hz, H-3), 3.781 (1 H, dd, J = 0.7 and 2.8 Hz, H-4), 3.375 (1 H, dd, J = 0.7 and 6.9 Hz, H-5), 3.752 (1 H, br d, J = 6.9 Hz, H-6), 3.557 (3 H, s, OMe), benzyl methylene protons appeared at 4.661, 4.742, 4.765, 4.815, 4.913, and 4.961 (1 H, d, J = 12.1 Hz). Anal. Calcd for $C_{28}H_{31}O_6D$: C, 72.24; H, 7.14. Found: C, 71.98; H, 6.98.

(6*S*)-[6-^{2}H]Methyl 2,3,4-Tri-*O*-benzyl-β-D-glucopyranoside (4). From (6*S*)-[6-^{2}H]methyl β-D-glucopyranoside (3)[9] a compound 4 was obtained as a waxy solid (53%) in the same manner for the preparation of **2**, mp 76–77 °C, $[\alpha]^{22}_D$ +11.8° (*c* 4.67, $CHCl_3$). ^{1}H NMR: δ 4.335 (1 H, d, J = 7.9 Hz, H-1), 3.400 (1 H, dd, J = 7.9 and 9.0 Hz, H-2), 3.672 (1 H, t, J = 9.0 Hz, H-3), 3.569 (1 H, t, J = 9.5 Hz, H-4), 3.368 (1 H, dd, J = 4.2 and 9.7 Hz, H-5), 3.702

(1 H, br d, J = 4.2 Hz, H-6), 7.2–7.4 (15 H, m, phenyl protons). Benzyl methylene protons appeared as a doublet signal (J = 12.1 Hz) at 4.641, 4.711, 4.811, 4.870, 4.911, and 4.932 ppm. Anal. Calcd for $C_{28}H_{31}O_6D$: C, 72.24; H, 7.14. Found: C, 71.54; H, 7.10.

(6*S*)-[6-^{2}H]Methyl 2,3,4-Tri-*O*-benzyl-[methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onate]-(2–6)-β-D-galactopyranoside (6a) and (6*S*)-[6-^{2}H]Methyl 2,3,4-Tri-*O*-benzyl-[methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosyl)onate]-(2–6)-β-D-galactopyranoside (6b). A method in literature[12,13] using $HgBr_2$ and $Hg(CN)_2$ was applied for preparing a mixture of **6a** and **6b** from a compound **2** and methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosyl chloride)onate (**5**).[11,12] To a stirred mixture of **2** (182 mg, 39.2 mmol), $HgBr_2$ (133 mg, 52.6 mmol), $Hg(CN)_2$ (66 mg, 18.3 mmol), and molecular sieves 4A (200 mg) in dry CH_2Cl_2 (10 mL) was added **5** (200 mg, 39.3 mmol) in CH_2Cl_2 (1 mL). The mixture was stirred at room temperature for 22 h and then diluted with ethyl acetate (50 mL), and the solution was washed with 30% KI aqueous solution (50 mL × 3), dried over $MgSO_4$, and concentrated. The residual syrup was chromatographed on silica gel column (benzene–ethyl acetate, 2:1, and then benzene–ethyl acetate–methanol, 50:100:1) to afford **6a** (47 mg, 13%) in slower moving fractions and **6b** (49 mg, 13%) with recovered **2** (83 mg, 46%). **6a**: glass, $[\alpha]^{22}_D$ +18.8° (c 4.0, MeOH). ^{1}H NMR Gal residue: δ 4.305 (1 H, d, J = 7.6 Hz, H-1), 3.787 (1 H, dd, J = 7.6 and 9.8 Hz, H-2), 3.539 (1 H, d, J = 9.8 and 2.8 Hz, H-3), 3.902 (1 H, br d, J = 2.8 Hz, H-4), 3.56 (H-5, overlapped with OMe signal), 3.841 (1 H, br d, J = 5.7 Hz, H-6), 3.569 (3 H, s, OMe), 7.25–7.4 (15 H, m, phenyl H), benzyl methylene appeared at 4.651, 4.961, 4.715, 4.760, 4.742, and 4.889 ppm as doublet. NeuNAc residue: *O*- and *N*-acetyl proton appeared as a singlet signal at 1.882, 2.017, 2.034, 2.112, and 2.131 ppm; 3.613 (3 H, s, COOMe), 1.951 (1 H, dd, J = 11.6 and 12.0 Hz, H-3ax′), 2.591 (1 H, dd, J = 4.7 and 11.6 Hz, H-3eq′), 5.210 (1 H, m (virtual coupling), H-4′), 4.07 (2 H, m, H-5′ + H-6′), 5.314 (1 H, dd, J = 1.6 and 8.3 Hz, H-7′), 5.355 (1 H, ddd, J = 8.3, 5.5, and 2.9 Hz, H-8′), 4.313 (1 H, dd, J = 2.9 and 12.7 Hz, H-9a′), 4.083 (1 H, dd, J = 5.5 and 12.7 Hz, H-9b′).

6b: glass, $[\alpha]^{22}_D$ +10.2° (c 4.2, MeOH). ^{1}H NMR Gal residue: δ 4.301 (1 H, d, J = 7.5 Hz, H-1), 3.787 (1 H, dd, J = 7.5 and 9.7 Hz, H-2), 3.623 (1 H, dd, J = 3.1 and 9.8 Hz, H-3), 4.083 (1 H, br d, J = 3.0 Hz, H-4), 3.668 (1 H, dd, J = 6.2 and <1 Hz, H-5), 3.57 (H-6, overlapped with an OMe peak), 3.559 (3 H, s, OMe), 7.25–7.4 (15 H, m, phenyl). Benzyl methylene ^{1}H signals appeared at 4.728, 4.879, 4.775, 5.448, 4.832, and 4.832 ppm. NeuNAc residue: *O*- and *N*-acetyl proton appeared as a singlet signal at 1.715, 1.980, 2.065, 2.072, and 2.228 ppm; 3.801 (3 H, s, COOMe), 1.853 (1 H, dd, J = 11.6 and 12.6 Hz, H-3ax′), 2.280 (1 H, dd, J = 4.8 and 11.6 Hz, H-3eq′), 4.8 (1 H, H-4′, overlapped with a benzyl proton signal), 4.0 (2 H, H-5′ + H-6′, overlapped), 5.101 (1 H, dd, J = 2.1 and 6.9 Hz, H-7′), 5.215 (1 H, ddd, J = 2.4, 5.5, and 6.9 Hz, H-8′), 4.562 (1 H, dd, J = 2.4 and 12.5 Hz, H-9a′), 4.029 (1 H, dd, J = 5.5 and 12.5 Hz, H-9b′).

(6*S*)-[6-^{2}H]Methyl 2,3,4-Tri-*O*-benzyl-[methyl (5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onate]-(2–6)-β-D-galactopyranoside (7a). A mixture of **6a** (58 mg, 0.062 mmol) and barium metal (3 mg) was stirred in dry methanol (5 mL) for 30 min, and the solution was neutralized with cation exchange resin (Dowex 50) and concentrated to give **7a** (29 mg, 61%) as a glass, $[\alpha]^{22}_D$ +8.6° (c 3.83, MeOH). ^{1}H NMR (CD_3OD) of Gal residue: δ 4.316 (1 H, d, J = 7.2 Hz, H-1), 3.987 (1 H, br d, J = 2 Hz, H-4), 3.669 (1 H, br d, J = 5.8 Hz, H-5), 3.928 (1 H, br d, J = 5.8 Hz, H-6), 3.513 (3 H, s, OMe), 7.25–7.4 (15 H, m, phenyl H). Benzyl methylene protons appeared at 4.61, 4.71, 4.72, 4.72, 4.82, and ~4.9 (overlapped with HOD signal). NeuNAc residue: δ 1.735 (1 H, t, J = 12.5 Hz, H-3ax′), 2.688 (1 H, dd, J = 4.7 and 12.5 Hz, H-3eq′), 4.218 (1 H, ddd, H-8′), 3.703 (3 H, s, COOMe), 2.0 (NHAc).

(6*S*)-[6-^{2}H]Methyl 2,3,4-Tri-*O*-benzyl-[methyl (5-acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosyl)onate]-(2–6)-β-D-galactopyranoside (7b). A mixture of **6b** (50 mg) and barium metal (3 mg) in methanol was processed in the same way for the preparation of **7a** to afford **7b** (31 mg, 75%) as a glass. $[\alpha]^{22}_D$ −11.5° (c 4.4, MeOH). ^{1}H NMR (CD_3OD) of Gal residue: δ 4.276 (1 H, d, J = 7.1 Hz, H-1), 4.210 (1 H, br d, J = 2.5 Hz, H-4), 3.484 (3 H, s, OMe), 7.25–7.4 (15 H, m, phenyl H). Benzyl methylene protons appeared at 4.675, 4.701, 4.710, 4.720, 4.800, and ~4.9 (overlapped). NeuNAc residue: δ 1.807 (1 H, t, J = 12.5 Hz, H-3ax′), 2.477 (1 H, dd, J = 4.7 and 12.5 Hz, H-3eq′), ~4.22 (1 H, H-8′, overlapped with H-4 (Gal) signal), 3.346 (3 H, s, COOMe), 2.027 (3 H, s, NHAc).

(6*S*)-[6-^{2}H]Methyl [Methyl (5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onate]-(2–6)-β-D-galactopyranoside (8a) and (6*S*)-[6-^{2}H]Methyl [(5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onic]-(2–6)-β-D-galactopyranoside (9a). A mixture of **6a** (30 mg) and Pd black (100 mg) in methanol–acetic acid (50 mL:10 mL) was treated with H_2 at room temperature for 8 h. After the catalyst was filtered off, the solution was concentrated to give **8a** (12 mg, 60%) as a glass. ^{1}H NMR (D_2O) of Gal residue: δ 4.306 (1 H, d, J = 8.1 Hz, H-1), 3.496 (1 H, dd, J = 8.1 and 10.0 Hz, H-2), 3.637 (1 H, dd, J = 3.3 and 10.0 Hz, H-3), 3.909 (1 H, br d, J = 3.3 Hz, H-4), 3.781 (1 H, br d, J = 7.7 Hz, H-6), 3.568 (3 H, s, OMe). NeuNAc residue: δ 1.846 (1 H, t, J = 12.4 Hz, H-3ax′), 2.723 (1 H, dd, J = 12.8 and 4.8 Hz, H-3eq′), 3.656 (1 H, dd, J = 6.7 and 12.5 Hz, H-9a′), 2.039 (3 H, s, NHAc), 3.886 (3 H, s, COOMe). H-6′ and H-7′ signals were overlapped with each other at ca. 3.57 ppm, H-8′ and H-9b′ signals were overlapped with each other, and H-4′ overlapped with H-5 (Gal) signals at ca. 3.76 ppm. A solution of **8a** (10 mg) in 0.2 N NaOH aqueous solution (2 mL) was stirred at room temperature for 30 min and then neutralized with cationic resin (Amberlite A-120). The filtered solution was lyophilized to give **9a** (5.3 mg, 55%) as a glass. ^{1}H NMR data of **9a** are summarized in Tables I and II in the text.

(6*S*)-[6-^{2}H]Methyl [(5-Acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosyl)onic]-(2–6)-β-D-galactopyranoside (9b). Hydrogenation for **6b** (30 mg) in the same way for **6a** gave a glassy **9b** (12 mg, 59% yield). ^{1}H NMR data are given in Tables I and II in the text.

(6*S*)-[6-^{2}H]Methyl 2,3,4-Tri-*O*-benzyl-[methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onate]-(2–6)-β-D-glucopyranoside (10a) and (6*S*)-[6-^{2}H]Methyl 2,3,4-Tri-*O*-benzyl-[methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosyl)onate]-(2–6)-β-D-glucopyranoside (10b). The same procedure as that described for the preparation of **6a** and **6b** was applied for the preparation of **10a** and **10b** with **4** (364 mg, 0.78 mmol), **5** (400 mg, 0.8 mmol), $Hg(CN)_2$ (266 mg), $HgBr_2$ (132 mg), and molecular sieves 4A (100 mg).

10a (58 mg). ^{1}H NMR ($CDCl_3$) of Glc residue: δ 4.256 (1 H, d, J = 7.9 Hz, H-1), 3.39 (1 H, dd, J = 7.9 and 8.9 Hz, H-2), 3.650 (1 H, t, J = 8.9 Hz, H-3), 3.599 (1 H, t, J = 8.9 Hz, H-4), 3.40 (1 H, dd, J = 4.2 and 9.0 Hz, H-5), 4.156 (1 H, d, J = 4.2 Hz, H-6), 3.542 (3 H, s, OMe), benzyl protons (6 H) appeared at 4.699, 4.765, 4.785, 4.881, and 4.894 ppm as doublet, and phenyl ring protons (15 H) appeared between 7.25 and 7.33 ppm as multiplet. NeuNAc residue: δ 1.96 (1 H, t, J = 12.5 Hz, H-3ax′), 2.667 (1 H, dd, J = 4.6 and 12.5 Hz H-3eq′), 5.306 (1 H, dd, J = 1.8 and 9.2 Hz, H-7′), 5.409 (1 H, ddd, J = 2.7, 5.1, and 9.2 Hz, H-8′), 3.968 (1 H, dd, J = 5.1 and 12.6 Hz, H-9a′), 4.195 (1 H, dd, J = 2.6 and 12.6 Hz, H-9b′), 3.755 (3 H, s, $COOCH_3$), 5.120 (1 H, br d, J = 9.7 Hz, NH), 1.869 (3 H, s, $COCH_3$), 1.929 (3 H, s, $COCH_3$), 2.029 (6 H, s, $2COCH_3$) and 2.135 (3 H, s, $COCH_3$). H-4′ peaks overlapped with benzyl methylene ones (ca. 4.8 ppm), and H-5′ and H-6′ signals overlapped with each other at ca. 4.07 ppm.

10b (80 mg). ^{1}H NMR ($CDCl_3$) of Glc residue: δ 4.364 (1 H, d, J = 7.7 Hz, H-1), 3.438 (1 H, dd, J = 7.7 and 8.9 Hz, H-2), 3.638 (1 H, t, J = 9.0 Hz, H-3 or H-4), 3.787 (1 H, t, J = 9.0 Hz, H-4 or H-3), 3.451 (1 H, dd, J = 2.4 and 10.0 Hz, H-5), 3.767 (1 H, d, J = 2.4 Hz, H-6), 3.627 (3 H, s, OMe), benzyl protons (6 H) appeared at 4.709, 4.750, 4.819, 4.867, 4.910, and 4.919 ppm, and phenyl protons (15 H) appeared between 7.25 and 7.35 ppm. NeuNAC residue: δ ~1.9 (H-3ax′, overlapped with OAc signals), 2.438 (1 H, dd, J = 4.8 and 12.8 Hz, H-3eq′), 5.276 (1 H, ddd, J = 4.9, 10.4, and 11.3 Hz, H-4′), 4.162 (1 H, q, J = 10.2, 10.4, and 10.4 Hz, H-5′), 4.288 (1 H, dd, J = 2.6 and 10.4 Hz, H-5′), 4.288 (1 H, dd, J = 2.6 and 10.4 Hz, H-6′), 5.409 (1 H, dd, J = 2.4 and 2.6 Hz, H-7′), 5.275 (1 H, ddd, J = 2.3, 2.5, and 9.2 Hz, H-8′), 4.104

(1 H, dd, J = 8.9 and 12.3 Hz, H-9a'), 5.001 (1 H, dd, J = 2.4 and 12.3 Hz, H-9b'), 3.698 (3 H, s, COOMe), 6.00 (1 H, br d, J = 10.2 Hz, NH). O- or N-Acetyl protons (15 H) appeared at 1.880, 2.015, 2.024, 2.029, and 2.156 ppm.

(6*S*)-[6-^{2}H]Methyl 2,3,4-Tri-*O*-benzyl-[methyl (5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onate]-(2-6)-β-D-glucopyranoside. (11a). A mixture of **10a** (38 mg) and barium metal (3 mg) was processed in the same way for the preparation of **7a** to give a glassy **11a** (23 mg, 74%). ^{1}H NMR (CD_3OD) of Glc residue: δ 4.311 (1 H, d, J = 8.1 Hz, H-1), 3.529 (3 H, s, OMe), benzyl protons (15 H) appeared at 4.512, 4.662, 4.714, 4.744, 4.774, and 4.85 ppm, and phenyl protons appeared between 7.2 and 7.35 ppm. NeuNAc: δ 1.780 (1 H, t, J = 12.2 Hz, H-3ax'), 2.773 (1 H, dd, J = 4.6 and 12.2 Hz, H-3eq'), 2.001 (3 H, s, NAc) 3.811 (3 H, s, COOMe).

(6*S*)-[6-^{2}H]Methyl 2,3,4-Tri-*O*-benzyl-[methyl (5-acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosyl)onate]-(2-6)-β-D-glucopyranoside (11b). A mixture of **10b** (41 mg) and barium (3 mg) in methanol was processed in the same way for the preparation of **7a** to afford a glassy **11b** (16 mg, 48%). ^{1}H NMR (CD_3OD) of a Glc residue: δ 4.334 (1 H, d, J = 8.1 Hz, H-1), 3.568 (3 H, s, OMe), benzyl protons appeared between 4.55 and 4.80 ppm, and phenyl protons appeared between 7.2 and 7.4 ppm. NeuNAc: δ 1.689 (1 H, t, J = 12.5 Hz, H-3ax'), 2.443 (1 H, dd, J = 4.8 and 12.8 Hz, H-3eq'), 2.022 (3 H, s, NAc), 3.621 (3 H, s, COOMe).

(6*S*)-[6-^{2}H]Methyl [Methyl (5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onate]-(2-6)-β-D-glucopyranoside (12a) and (6*S*)-[6-^{2}H]Methyl [(5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onic]-(2-6)-β-D-glucopyranoside (13a). A mixture of **11a** (25 mg) and Pd black (20 mg) in methanol–acetic acid (5:1, 5 mL) was saturated with H_2 at room temperature for 8 h. After the catalyst was filtered off, the solution was concentrated to give a glassy **12a** (11.9 mg, 70%). ^{1}H NMR (400 MHz, D_2O) of a Glc residue: δ 4.352 (1 H, d, J = 8.1 Hz, H-1), 3.260 (1 H, t, virtually coupling), 3.48 (3 H, multiplet, H-3 + H-4 + H-5), 4.033 (1 H, d, J = 3.3 Hz, H-6), 3.553 (3 H, s, OMe). NeuNAc: δ 1.843 (1 H, t, J = 12.2 Hz, H-3ax'), 2.741 (1 H, dd, J = 4.4 and 12.8 Hz, H-3eq'), 3.762 (1 H, ddd, J = 4.5, 9.1, and 12.2 Hz, H-4'), 3.88 (1 H, overlapped with COOMe signals at 3.888 ppm, H-5'), 3.567 (1 H, br d, J = 8.0 Hz, H-7'), 3.659 (1 H, dd, J = 6.6 and 13.0 Hz, H-9a'), 3.85 (3 H, multiplet, H-6' + H-8' + H-9b'), 3.888 (3 H, s, COOMe), 2.040 (3 H, s, NAc).

A solution of **12a** (10 mg) in 0.2 N NaOH aqueous solution (2 mL) was stirred at room temperature for 30 min and then neutralized with Amberlite A-120. The filtered solution was lyophilized to give a glassy **13a** (5.8 mg, 60%). ^{1}H NMR data of **13a** are given in Tables I and II in the text.

(6*S*)-[6-^{2}H]Methyl [Methyl (5-acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosyl)onic]-(2-6)-β-D-glucopyranoside (12b) and (6*S*)-[6-^{2}H]Methyl [(5-Acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosyl)onic]-(2-6)-β-D-glucopyranoside (13b). The same procedure as that described for the preparations of **12a** and **13a** was taken for the preparations of **12b** and **13b** from **11b** (30 mg). **12b** (16 mg, 80%), glassy solid. ^{1}H NMR (400 MHz, D_2O) of a Glc residue: δ 4.378 (1 H, d, J = 8.1 Hz, H-1), 3.264 (1 H, t, J = 9.0 Hz, H-2), 3.462 (1 H, t, virtually coupling, H-3), 3.57 (2 H, m, H-4 + H-5), 3.51 (1 H, d, J = 5.2 Hz, H-6), 3.579 (3 H, s, OMe), NeuNAc residue: δ 1.797 (1 H, dd, J = 11.5 and 13.2 Hz, H-3ax'), 2.504 (1 H, dd, J = 4.9 and 13.2 Hz, H-3eq'), 4.13 (1 H, m, virtually coupling), 3.95 (2 H, m, H-5' + H-6'), 3.86 (2 H, m, H-8' + H-9a'), 3.656 (1 H, dd, J = 6.6 and 12.5 Hz, second-order coupling), 3.866 (3 H, s, COOMe), 2.059 (3 H, s, NAc). **13b** (4 mg from 10 mg of **12b**, 41%), glassy solid. ^{1}H NMR data are given in Tables I and II in the text.

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Supplementary Material Available: NMR spectra for compounds **6a–13b** (26 pages). Ordering information is given on any current masthead page.

Clefts in Simple Acyclic Organic Molecules. Correlated Stereodynamics of *N-tert*-Alkylbenzylamines Studied by Dynamic NMR Spectroscopy,[1] X-ray Diffraction, and Molecular Mechanics Calculations

J. E. Anderson* and D. A. Tocher

Chemistry Department, University College London, Gower Street, London WC1E 6BT, United Kingdom

D. Casarini and L. Lunazzi*

Dipartimento di Chimica Organica, "A. Mangini", Universita, Viale Risorgimento 4, 40136 Bologna, Italy

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In *N-tert*-butyl-*N*-neopentylbenzylamine (1), the slowing of four processes can be observed in the NMR spectrum at low temperature. The rate constant for 120° rotation of the *N-tert*-butyl group is three times that for 180° rotation of the phenyl group at all temperatures studied, suggesting correlated rotation of these groups. Barriers for the two processes specified are 6.8 and 7.1 kcal/mol, respectively, at −120 °C. The barrier to interconversion of enantiomeric configurations by nitrogen inversion plus rotation is 9.2 kcal/mol at −77 °C. The barrier to rotation about the *tert*-butyl–CH_2 bond is 5.95 kcal/mol at −143 °C. The anti arrangement of the *t*-BuNCH_2-*t*-Bu part of the molecule greatly limits the space available for the benzyl substituent, and only one conformation about the N–benzyl bond is populated. Molecular mechanics calculations suggest that the benzyl group occupies a pocket or cleft, defined by the *tert*-butyl groups, undergoing several kcal/mol of repulsion and attraction from opposite directions. The barrier to rotation about the phenyl–CH_2 bond is unprecedentedly high for a simple molecule but is comparable to or smaller than some found in polypeptides of rigid tertiary conformation, where concerted rotation is also a feature. Similar results obtain when either *tert*-butyl group is replaced by an adamantyl group to give *N*-(1-adamantyl)-*N*-neopentylbenzylamine (**2**) and *N-tert*-butyl-*N*-(1-adamantylmethyl)benzylamine (**3**). A crystal structure determination of **2** shows a conformation very close to that predicted by molecular mechanics calculations.

A major difference between simple organic molecules and biologically significant polymers such as proteins is the organized folding of the latter, which leads to groups being close in space, although separated by many bonds

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