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Carbohydrate Research 337 (2002) 1775–1779

CARBOHYDRATE RESEARCH

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Note

Conformational studies of a novel cationic glycolipid, glyceroplasmalopsychosine, from bovine brain by NMR spectroscopy

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Received 5 March 2002; received in revised form 10 July 2002; accepted 13 August 2002

Abstract

A novel glycosphingolipid containing a long chain aldehyde conjugated to galactose and glycerol, Gro1(3)-*O*-CH((CH$_2$)$_n$CH$_3$)-*O*-6Galβ-sphingosine (glyceroplasmalopsychosine) has been studied by NMR spectroscopy (Hikita et al. *J. Biol. Chem.* **2001**, *276*, 23084–23091). We further report here on the conformation showing the galactose and the glycerol at the end of two parallel hydrophobic chains, i.e. the sphingosine and the fatty aldehyde. This is proposed based on the interproton distances derived from ROESY experiments and $^3J_{H,H}$ coupling constants. The absence of any intraresidual NOEs between protons in the glycerol residue suggested that the C-1–C-2 and C-2–C-3 bonds in the glycerol may be rotating freely, supporting the proposed conformation in which the unique terminal glycerol is in an environment with a minimal steric hindrance. The present study proposes a conformation of glyceroplasmalopsychosine greatly different from the two conventional plasmalopsychosines possessing a fatty aldehyde chain oriented in an opposite direction to the sphingosine.

Keywords: glyceroplasmalopsychosine; acetal linkage; conformation; ^{1}H and ^{13}C NMR

Plasmaloglycolipids, a unique class of glycosphingolipids in the brain, contain long chain fatty aldehydes. The fatty aldehydes form either 3,4-*O*- or 4,6-*O*-cyclic acetal linkages with galactose of plasmalogalactosylsphingosine (plasmalopsychosine, PLPS),[1,2] plasmalogalactosylceramide[3] and plasmalogalactosylalkylglycerol,[4] or an inter-residual acetal linkage with two different residues, a galactose and a glycerol of Gro1(3)-*O*-plasmal-*O*-6Galβ-sphingosine (trivial name proposed as glyceroplasmalopsychosine, GPP).[5]

Based on minimum-energy calculations, it was proposed that the fatty aldehyde forming the cyclic acetal linkage in 4,6-*O*-plasmalopsychosine (PLPS B) is oriented in an opposite direction to the lipophilic component, sphingosine.[1] It was further speculated that one of these aliphatic chains, presumably sphingosine, may be anchored in the cell membrane and the other chain may function as a hydrophobic antenna extruding from the cell membrane. The phenomena that plasmalopsychosine (PLPS) showed no cytotoxic effect but only weak protein kinase C (PKC) inhibition in contrast with strong cytotoxicity as well as PKC inhibitory activity of psychosine, presented an interesting question concerning the function of the fatty aldehyde chain of glycolipids on the membrane surface. In the present report, we describe the solution conformation of GPP based on NMR analysis focussing on the orientation of the fatty aldehyde chain.

GPP consists of two stereoisomers, tentatively named GPP^A and GPP^B here, varying with respect to the

Abbreviations: DQF-COSY, double quantum-filtered correlated spectroscopy; ^{1}H–^{13}C COSY, ^{1}H–^{13}C heteronuclear correlated spectroscopy; HMBC, heteronuclear multibond correlation; ROESY, rotating frame Overhauser effect spectroscopy; PKC, protein kinase C; Gro, glycerol; PLPS, plasmalopsychosine; PLPS A, 3,4-*O*-plasmalopsychosine; PLPS B, 4,6-*O*-plasmalopsychosine; GPP, glyceroplasmalopsychosine, Gro1(3)-*O*-CH((CH$_2$)$_n$CH$_3$)-*O*-6Galβ-sphingosine.

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0008-6215/02/$ - see front matter
PII: S0008-6215(02)00290-2

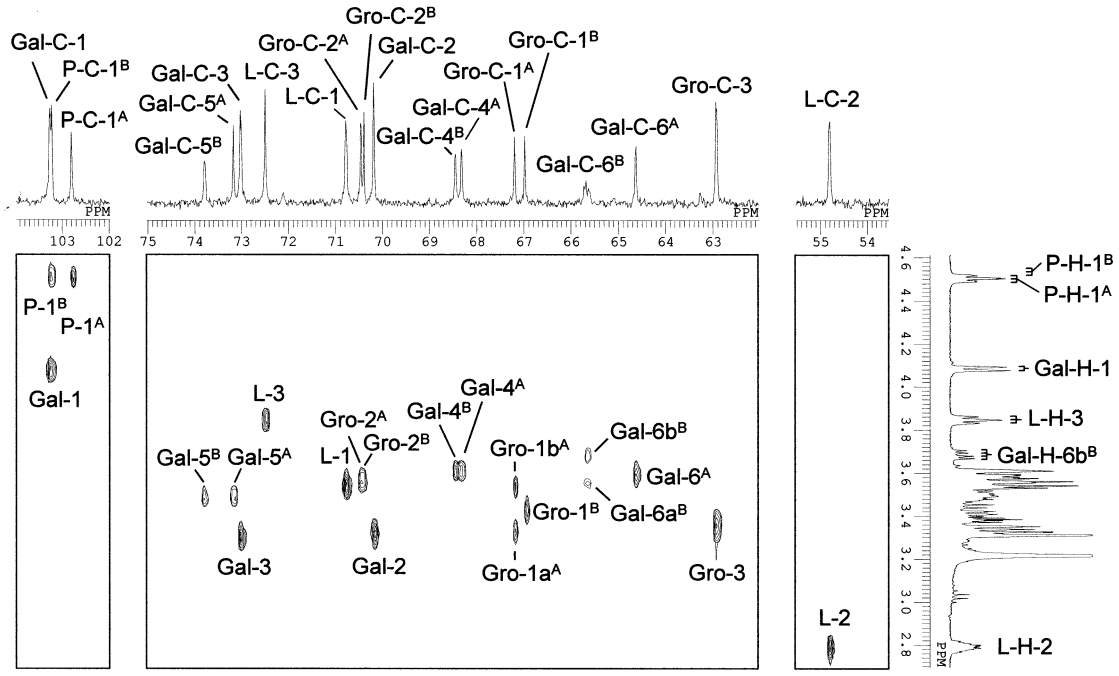


Fig. 1. $^{1}H-^{13}C$ COSY spectrum of GPP. Arabic numerals refer to the position of protons (H) and carbons (C) in Gal, long-chain base (sphingosine, L), fatty aldehyde (P), and glycerol (Gro) residues. The symbols of plasmal isomers GPP^{A} and GPP^{B} are marked with superscript A and B, e.g., $P–H-1^{B}$, $Gal-C-1^{A}$, and $Gro-C-2^{A}$, etc., when the signals are distinct from each other.

Table 1
^{1}H and ^{13}C chemical shifts of GPP in DMSO-d_6/D_2O (98:2) at 60 °C

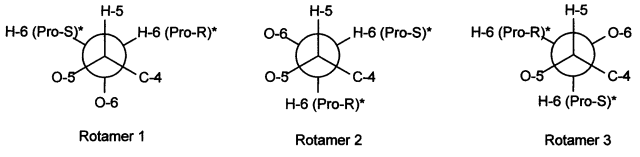
Residue	Proton	^{1}H-chemical shift (ppm)			Carbon	^{13}C-chemical shift (ppm)		
		GPP^{A}	GPP^{B}	Δ (B-A)		GPP^{A}	GPP^{B}	Δ (B-A)
Galactose	H-1	4.07	4.07		C-1	103.26	103.26	
	H-2	3.31	3.31		C-2	70.19	70.19	
	H-3	3.28	3.28		C-3	73.00[a]	73.03[a]	
	H-4	3.59	3.59		C-4	68.33	68.45	0.12
	H-5	3.48	3.47	−0.01	C-5	73.17	73.79	0.62
	H-6a	3.56	3.54	−0.02	C-6	64.62	65.66	1.04
	H-6b	3.60	3.67	0.07				
Sphingosine	H-1a	3.53	3.53		C-1	70.78	70.78	
	H-1b	3.54	3.54		C-2	54.80	54.80	
	H-2	2.78	2.78		C-3	72.49	72.49	
	H-3	3.83	3.83		C-4	130.65	130.65	
	H-4	5.45	3.45		C-5	131.02	131.02	
	H-5	5.58	3.58					
Acetal	H-1	4.48	4.49	0.01	C-1	102.80	103.23	0.43
	H-2	1.50	1.50		C-2	32.89[b]	33.10[b]	
Glycerol	H-1a[a]	3.31	3.39	0.08	C-1[a]	67.20	66.98	−0.22
	H-1b[a]	3.52	3.44	−0.08	C-2	70.47	70.39	−0.08
	H-2	3.56	3.56		C-3[a]	62.94[b]	62.91[b]	
	H-3a[a]	3.32	3.32					
	H-3b[a]	3.37	3.38	0.01				

[a] The position of C-1 in the glycerol residue was tentatively assigned.
[b] Chemical shifts of GPP^{A} and GPP^{B} are exchangeable.

Table 2
^{1}H–^{1}H coupling constants ($^{3}J_{H,H}$) of GPP

Residue		Coupling constants (Hz) GPP^{A}	GPP^{B}
Galactose	$^{3}J_{G1,2}$	6.8*	6.8*
	$^{3}J_{G2,3}$	9.6*	9.6*
	$^{3}J_{G3,4}$	3.2*	3.2*
	$^{3}J_{G4,5}$	<1.0	<1.0
	$^{3}J_{G5,6a}$	5.0	7.0
	$^{3}J_{G5,6b}$	7.0	4.9
	$^{2}J_{G6a,6b}$	−10.8	−11.0
Aldehyde	$^{3}J_{P1,2}$	5.5	5.5
Glycerol	$^{2}J_{Gro1a,1b}$	−9.9	−9.9
	$^{3}J_{Gro1a,2}$	6.1	6.0
	$^{3}J_{Gro1b,2}$	5.0	6.0
	$^{3}J_{Gro2,3a}$	6.0	6.0
	$^{3}J_{Gro2,3b}$	5.0	5.0
	$^{2}J_{Gro3a,3b}$	−11.0	−11.0

* The strong coupling between the Gal-H-2 and Gal-H-3 may change the apparent values.



Rotamer	Population GPP^{A}	GPP^{B}
1	0.09 (0.03)	0.09 (0.03)
2	0.64 (0.45)	0.64 (0.45)
3	0.27 (0.52)	0.27 (0.52)

Fig. 2. Projections and populations of the staggered conformers in relation to the C-5–C-6 bond of galactose. The coupling constants $^{3}J_{5,6(pro\text{-}R)}$ and $^{3}J_{5,6(pro\text{-}S)}$ in three rotamers were estimated using the seven-parameter equation of Hassnoot et al.;[7] 0.5 and 3.0 Hz in rotamer 1, 10.6 and 3.0 Hz in rotamer 2, and 0.5 and 10.6 Hz in rotamer 3, respectively. The populations were calculated using coupling constants listed in Table 2. The proton resonating at the lower field, Gal-H-6b, was tentatively assigned to *pro*-R in GPP^{A} and *pro*-S in GPP^{B}, that is, $^{3}J_{5,6(pro\text{-}R)} = 7.0$ Hz and $^{3}J_{5,6(pro\text{-}S)} = 4$ Hz in both stereoisomers GPP^{A} and GPP^{B}. The numbers given in parentheses are conformer populations calculated using the reverse assignment.

asymmetric C-1 carbon of the aldehyde as previously reported.[5] Fig. 1 shows a part of ^{1}H–^{13}C COSY spectrum of GPP. The ^{13}C chemical shift differences (Δ (B-A) in Table 1) between the isomers, GPP^{A} and GPP^{B}, were largest at C-6 and C-5 of Gal, C-1 of acetal and C-1(3) of Gro. Taken together with ^{1}H chemical shift differences,[5] this supported our previous assignment that the two GPPs are stereoisomers with regard to the acetal C-1.

The $^{3}J_{5,6a}$ and $^{3}J_{5,6b}$ coupling constants of GPP^{A} were 5 and 7 Hz, respectively, while those of GPP^{B} were 7 and 5 Hz (Table 2). According to the seven-parameter equation of Haasnoot et al. based on the Karplus relationship between vicinal coupling constants, $^{3}J_{H,H}$, and torsional angle, H–C–C–H,[6,7] we estimated the $^{3}J_{5,6(pro\text{-}R)}$ and $^{3}J_{5,6(pro\text{-}S)}$ values in three possible ro-

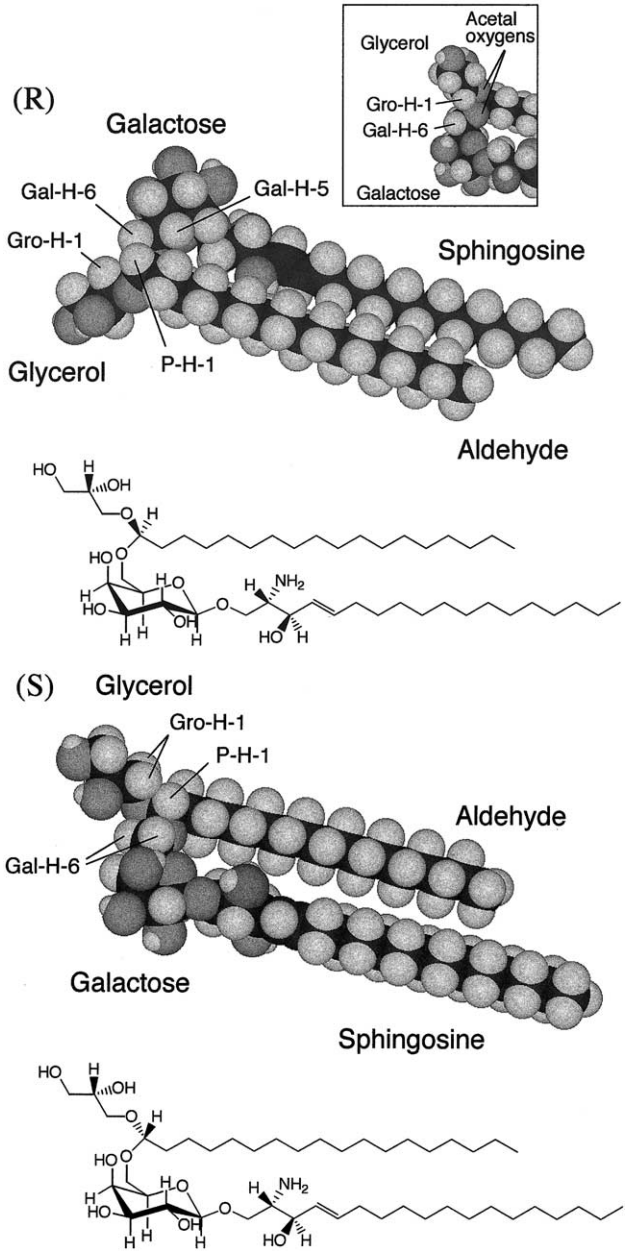


Fig. 3. Space filling models of the two stereoisomers of GPP. Predicted conformational models of GPP with *R*-configuration (R) and *S*-configuration (S) around the asymmetric C-1 carbon of plasmal. The rear side of GPP with *R*-configuration is shown in the inset. The models were derived from one of the molecular species containing an aldehyde (16:0) and a sphingosine (d18:1). Other two molecular species, 18:1/d18:1 and 18:0/d18:1, have been identified as the major ones of GPP.[5]

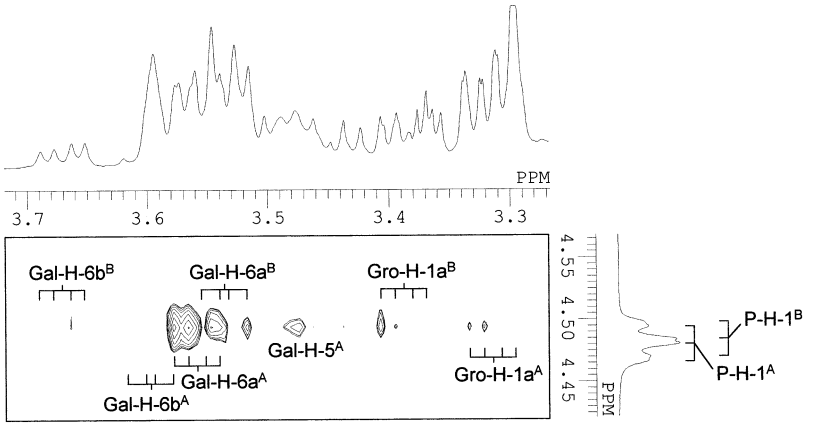


Fig. 4. 2D ROESY spectrum of GPP. The abbreviations for symbols are similar to those in Fig. 1.

tamers around the C-5–C-6 bond (Fig. 2) and calculated the molar fractions of the three staggered conformers for GPP^A and GPP^B.[8] The populations of 0.09, 0.64 and 0.27 for rotamer 1, 2 and 3, respectively, are remarkably different from those of 4,6-*O*-plasmalopsychosine (PLPS B) for which rotamer 1 is the exclusive conformer, while the populations are similar to those of galactosylceramide which has an unsubstituted hydroxyl group at C-6 and the $^3J_{5,6a}$ and $^3J_{5,6b}$ values of around 6 Hz.[9] Furthermore, $^3J_{Gro1a,2}$, $^3J_{Gro1b,2}$, $^3J_{Gro2,3a}$ and $^3J_{Gro2,3b}$ in the glycerol residue of GPP^A and GPP^B were in the range of 5–6 Hz, showing that the configuration around the C-1–C-2 and C-2–C-3 bonds in glycerol has the molar fractions of rotamers similar to the freely rotating C-5–C-6 bond in GalCer. We propose conformations for the two stereoisomers of GPP with the major configuration as shown in Fig. 3. The conformation of the two hydrophobic chains, fatty aldehyde and sphingosine, are oriented in parallel to satisfy the flexibility of the glycerol residue in both of GPPs.

Inter-residual $^1H-^1H$ contacts for the acetal linkage were studied by ROESY experiments (Fig. 4) and NOE distances (Table 3) were evaluated from cross-peak volumes. The H-1 proton of the fatty aldehyde (P-H-1) showed a strong NOE with Gal-H-6a of GPP^A and a weak NOE with Gal-H-6b and Gal-H-5 of GPP^A and Gal-H-6a of GPP^B. The NOE contact of P-H-1/Gal-H-5 was observed solely for the GPP^A isomer, leading us to a conclusion that GPP^A is the isomer with *R*-configuration around the asymmetric P-C-1 carbon (Fig. 3(R)). In regard to the acetal-*O*-Gro linkage, P-H-1 had NOEs, though very weak, with Gro-1a and 1b protons of GPP^A and GPP^B. In addition, any intra-residual NOE contact between protons in the glycerol residue was not observed. Together with the intra-residual $^3J_{Gro1a,2}$, $^3J_{Gro1b,2}$, $^3J_{Gro2,3a}$ and $^3J_{Gro2,3b}$ coupling constants being in the range of 5–6 Hz, this suggests that the conformation around the C-1–C-2 and C-2–C-3 bonds of the glycerol residue is as flexible as that of glycerol in the state with a minimal steric hindrance, supporting the structures shown in Fig. 3.

Recent reports have shown that NMR parameters of the carbohydrate portion of the glycolipids in $(CD_3)_2SO$ are very similar to those of the glycolipids anchored in D_2O/dodecylphosphocholine-d_{38} micelles,[10,11] and even in bilayers as shown by the solid-state ^{13}C NMR technique.[8] These results indicate that glycolipids assume similar conformational properties in both $(CD_3)_2SO/D_2O$ and biological membranes. Thus, the conformation of GPPs proposed here may most probably represent the major conformation under biological conditions, in which two parallel hydrophobic chains are buried in the outer leaflet of the lipid bilayers and the two polar residues, galactose and glycerol, are extending out into the hydrophilic environment on the surface of the cell membranes. The glycerol, extruding from the membrane surface, may have some function either independently or in cooperation with the galactose head.

It has been reported that cationic lipids possessing a free amino group in the sphingosine modulate the activity of growth factor receptor kinase, protein kinase C, or other membrane-bound signal transducer molecules.[12–15] While plasmalopsychosine showed no cytotoxic effect and a weak PKC inhibition, psychosine

Table 3
Inter-residual contacts and NOE distance for GPP^A and GPP^B derived form ROESY experiments

Contact	Distance (Å)	
	GPP^A	GPP^B
P–H-1/Gal-H-6a	2.7	3.0
P–H-1/Gal-H-6b	3.0	>3.5
P–H-1/Gal-H-5	3.1	>3.5
P–H-1/Gro-H-1a	3.5	>3.5
P–H-1/Gro-H-1b	>3.5	>3.5

showed both strong cytotoxicity and strong PKC inhibitory activity, suggesting that the fatty aldehyde chain may reduce the toxic effects of psychosine.[1] The conformational and functional studies of glyceroplasmalopsychosine would help understanding of the biological function of these glycolipids and the role of their fatty aldehyde chains.

1. Experimental

Cationic GSLs were extracted from bovine brain white matter (Pel-Freez Biologicals, Rogers, AR) and purified using ion-exchange chromatography on a CM-Sephadex column and Iatrobeads chromatography as described previously.[5]

^{1}H NMR spectra were obtained using a GX-400 spectrometer (JEOL, Tokyo) at 60 °C. Tetramethylsilane was used as an internal standard for chemical shifts. The purified GSL was deuterium-exchanged with CD_3OD and dried over P_2O_5 in a vacuum, and then dissolved in $(CD_3)_2SO/D_2O$ (98:2) to the final concentration of approximately 5 mM. The ^{1}H assignment and determination of $J_{H,H}$ coupling constants were performed by DQF-COSY with the resolution for $\omega 2$ dimension of 0.98 Hz.[5] ^{13}C-signals were assigned on the basis of ^{1}H–^{13}C COSY and HMBC experiments.[16,17] The ^{1}H–^{13}C heteronuclear COSY spectrum[18] was recorded with 64 increments in $t1$ and 1680 scans were collected for each $t1$ experiment with the spectral widths of 12.5 kHz in $\omega 2$ (for ^{13}C) and 2 kHz in $\omega 1$ (for ^{1}H). After zero-filling, the time domain spectrum was transformed to give a 4096×128 data point matrix with resolutions for $\omega 1$ and $\omega 2$ dimensions of 6.1 and 31 Hz/point, respectively.

NOEs were demonstrated by 2D-ROESY experiments.[19] The spectra were acquired with 112 scans per increment and the spin-lock mixing time was 200 ms. The resolutions for $\omega 1$ and $\omega 2$ dimensions were 9.8 and 2.4 Hz/point, respectively. Distance evaluation was based on the proportionality $V_{ij} \propto r_{ij}^{-6}$, where V_{ij} is the cross-peak volume and r_{ij} the proton–proton distance for proton pair H_i–H_j.[20] The fixed intraresidual distance between H-1 and H-5 of the galactosyl residue (0.24 nm) was used as an internal reference.

To obtain the minimum energy state around the conformation predicted from NMR analyses, molecular mechanics (MM2) force field calculation was performed using standard parameters in the CS Chem 3D Pro program (Cambridge Soft. Com.).[21]

References

1. Nudelman, E. D.; Levery, S. B.; Igarashi, Y.; Hakomori, S. *J. Biol. Chem.* **1992**, *267*, 11007–11016.
2. Levery, S. B.; Nudelman, E. D.; Hakomori, S. *Biochemistry* **1992**, *31*, 5335–5340.
3. Yachida, Y.; Kashiwagi, M.; Mikami, T.; Tsuchihashi, K.; Daino, T.; Akino, T.; Gasa, S. *J. Lipid Res.* **1998**, *39*, 1039–1045.
4. Yachida, Y.; Kashiwagi, M.; Mikami, T.; Tsuchihashi, K.; Daino, T.; Akino, T.; Gasa, S. *J. Lipid Res.* **1999**, *40*, 2271–2278.
5. Hikita, T.; Tadano-Aritomi, K.; Iida-Tanaka, N.; Anand, J. K.; Ishizuka, I.; Hakomori, S. *J. Biol. Chem.* **2001**, *276*, 23084–23091.
6. Karplus, M. *J. Chem. Phys.* **1959**, *30*, 11–15.
7. Haasnoot, C. A. G.; de Leeuw, F. A. A. M.; Altona, C. *Tetrahedron* **1980**, *36*, 2783–2792.
8. Bruzik, K. S.; Nyholm, P. G. *Biochemistry* **1997**, *36*, 566–575.
9. Dabrowski, J.; Egge, H.; Hanfland, P. *Chem. Phys. Lipids* **1980**, *26*, 187–196.
10. Acquotti, D.; Poppe, L.; Dabrowski, J.; von der Lieth, C. W.; Sonnino, S.; Tettamanti, G. *J. Am. Chem. Soc.* **1990**, *112*, 7772–7778.
11. Poppe, L.; von der Lieth, C. W.; Dabrowski, J. *J. Am. Chem. Soc.* **1990**, *112*, 7762–7771.
12. Hakomori, S.; Igarashi, Y. *Adv. Lipid. Res.* **1993**, *25*, 147–162.
13. Hakomori, S. *Adv. Pharmacol.* **1996**, *36*, 155–171.
14. Sakakura, C.; Igarashi, Y.; Anand, J. K.; Sadozai, K. K.; Hakomori, S. *J. Biol. Chem.* **1996**, *271*, 946–952.
15. Megidish, T.; Takio, K.; Titani, K.; Iwabuchi, K.; Hamaguchi, A.; Igarashi, Y.; Hakomori, S. *Biochemistry* **1999**, *38*, 3369–3378.
16. Kochanowski, B.; Fischer, W.; Iida-Tanaka, N.; Ishizuka, I. *Eur. J. Biochem.* **1993**, *214*, 747–755.
17. Matsuda, K.; Ishizuka, I.; Kasama, T.; Handa, S.; Yamamoto, N.; Taki, T. *Biochim. Biophys. Acta* **1997**, *1349*, 1–12.
18. Iida-Tanaka, N.; Ishizuka, I. *Carbohydr. Res.* **2000**, *324*, 218–222.
19. Iida-Tanaka, N.; Fukase, K.; Utsumi, H.; Ishizuka, I. *Eur. J. Biochem.* **2000**, *267*, 6790–6797.
20. Neuhaus, D.; Williamson, M. *The Nuclear Overhauser Effect in Structural and Conformational Analysis*; VCH Publishers: New York, 1989.
21. Kawakami, J.; Kawakami, Y.; Nakamura, K.; Kojima, H.; Ito, S.; Tamai, Y. *Glycoconj. J.* **1998**, *15*, 107–113.