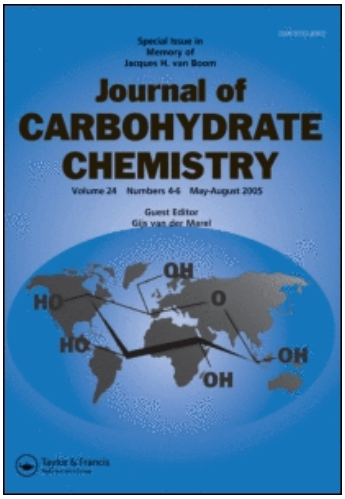


Journal of Carbohydrate Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

Model Compounds for Plasmaloglycolipids: Preparation of Long Chain Cyclic Acetals of Methyl β-d-Galactopyranoside and Determination of Their Regio- and Stereochemistry by Proton Nmr

Khalid Khan Sadozai[a]; Steven B. Levery[b]; Jasbir K. Anand[c]; Sen-itiroh Hakomori[c]
[a] Alpha-Beta Technology, Worcester, Massachusetts [b] Perkin Elmer-Applied Biosystems Division, Foster City, California [c] The Biomembrane institute, Seattle, Washington

To cite this Article Sadozai, Khalid Khan , Levery, Steven B. , Anand, Jasbir K. and Hakomori, Sen-itiroh(1996) 'Model Compounds for Plasmaloglycolipids: Preparation of Long Chain Cyclic Acetals of Methyl β-d-Galactopyranoside and Determination of Their Regio- and Stereochemistry by Proton Nmr', Journal of Carbohydrate Chemistry, 15: 6, 715 — 725

To link to this Article: DOI: 10.1080/07328309608005687

URL: http://dx.doi.org/10.1080/07328309608005687

J. CARBOHYDRATE CHEMISTRY, 15(6), 715-725 (1996)

MODEL COMPOUNDS FOR PLASMALOGLYCOLIPIDS: PREPARATION OF LONG CHAIN CYCLIC ACETALS OF METHYL β-D-GALACTOPYRANOSIDE AND DETERMINATION OF THEIR REGIO- AND STEREOCHEMISTRY BY PROTON NMR

Khalid Khan Sadozai,[+] Steven B. Levery,[◊] Jasbir K. Anand,[⊗] and Sen-itiroh Hakomori[⊗]

[+]Alpha-Beta Technology, One Innovation Drive, Worcester, Massachusetts 01605
[◊]Perkin Elmer-Applied Biosystems Division, 850 Lincoln Centre Drive, Foster City, California 94404
[⊗]The Biomembrane Institute, 201 Elliott Avenue West, Seattle, Washington 98119

Received November 21, 1995 - Final Form May 3, 1996

ABSTRACT

Plasmalopsychosines and plasmalocerebrosides comprise a novel class of human brain glycosphingolipids consisting of long chain fatty aldehydes conjugated to the galactose moiety of psychosine or cerebroside via cyclic acetal linkages. In order to clarify questions concerning the detailed stereochemistry of the acetal linkages in the natural lipids, model compounds having a simplified aglycone were synthesized. Methyl β-D-galactopyranoside was condensed with 1,1-dimethoxyhexadecane in the presence of *p*-TsOH to give a mixture of stereoisomeric cyclic acetals. After acetylation, the mixture of acetals was separated by HPLC and the structure of each isomer was established by 1-D NOE experiments. By comparison of NMR data from the model compounds with spectra of synthetic plasmalopsychosines it was possible to determine the stereochemistry of the acetal chains in the latter, and, by extension, their stereochemistry in the natural lipids.

INTRODUCTION

Considerable evidence has accumulated for regulatory or modulatory functions associated with sphingolipid metabolites and catabolites.[1-7] For example, sphingosine, *N,N*-dimethylsphingosine, and lysosphingolipids such as lyso-GM_3 and psychosine (galactosylsphingosine), have all been identified as potent inhibitors of protein kinase C (PKC).[1-9] Cerebroside (galactosylceramide), in contrast, had no such activity in a PKC inhibition assay.[9] A systematic search for other compounds in neural tissues having potential roles in transmembrane signalling led to the discovery of a new class of glycosphingolipid having long chain fatty aldehydes (predominantly 16:0 and 18:0) conjugated to the galactose moiety of psychosine or cerebroside via cyclic acetal linkages (see Figure 1), either 3,4- (A) or 4,6- (B).[10,11] Plasmalopsychosines A and B, two novel lysosphingolipids, were isolated and characterized from white matter of human brain,[10] while plasmalocerebrosides A and B, neutral compounds found in much lower concentration in brain tissue, were found and characterized as a mixture consisting primarily of the A analog.[11] In a PKC inhibition assay, plasmalopsychosines A and B were found to have a weak activity intermediate between that of psychosine (positive control) and cerebroside (negative control). We have reported[12] the total synthesis of one isomer of plasmalopsychosine A (3,4-*O*-hexadecylidene), and one isomer of plasmalopsychosine B (4,6-*O*-hexadecylidene), and have shown their identities with the compounds isolated from the natural source. However, the precise stereochemistry at C-1 of the acetal moiety (CH") has not yet been determined for any of the compounds, natural or synthetic. In order to clarify this problem, we synthesized C_{16} cyclic acetals of methyl β-D-galactopyranoside. Following peracetylation, it was possible to separate all four relevant positional and diastereomeric isomers. Thus it was possible to compare the general structural features of isomeric long chain cyclic acetals of galactose, and to relate these to the corresponding features of the synthetic and natural plasmalopsychosine analogs. Determination of the structural differences among these four isomers by proton NMR spectroscopy is described below.

RESULTS AND DISCUSSION

Condensation of methyl β-D-galactopyranoside with 1,1-dimethoxyhexadecane in the presence of *p*-TsOH gave a mixture of cyclic acetals. Previously we reported[12] that by

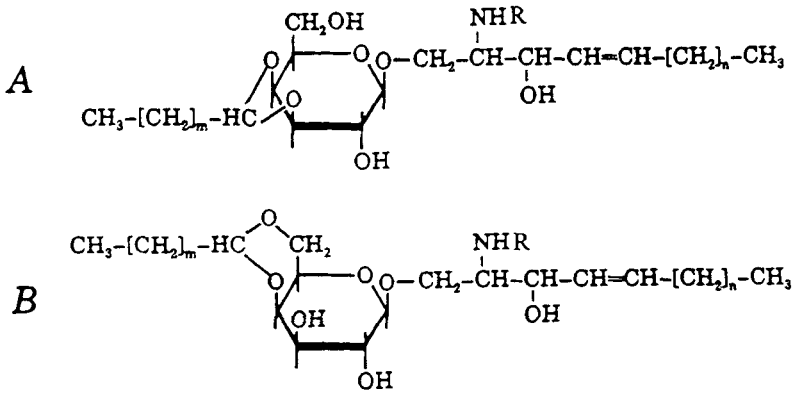


Figure 1. Structures of plasmalopsychosines (R = H) and plasmalocerebrosides R = CO-$[CH_2]_p$-CH_3 isolated and characterized from human brain tissue. For predominant species, m = 14 or 16; n = 12; p = 14, 16, or 22.

repeated chromatography we were able to separate two isomers of these acetals (a 4,6- and a 3,4-). However, when a ^1H NMR spectrum of the unchromatographed product mixture was obtained (not shown), *four* distinct signals for the methine proton were observed at 4.563, 5.009, 5.056, and 5.213 ppm. The appearance of four distinct signals for the methine proton suggested the formation of four isomeric acetal products. Attempted HPLC of the mixture on a Nova-Pak silica column, using a chloroform-methanol gradient, separated the 4,6- isomers from the 3,4- isomers; however, further separation of the isomers was not possible.

The failure of the chromatographic separation tempted us to acetylate the crude mixture of acetals. Upon acetylation, the four isomers became chromatographically distinguishable. Separation of the isomers by HPLC, using a Nova-Pak silica column and a hexane-ethyl acetate gradient gave compounds **1b** (R_f = 0.31); **2b** (R_f = 0.29); **1a** (R_f = 0.25); and **2a** (R_f = 0.24).

Identification of the positional isomers was accomplished by GC-MS of partially methylated galactitol acetates following permethylation, hydrolysis, reduction, and acetylation according to standard procedures.[13] Identification of 2,6-di-*O*-methyl-1,3,4,5-tetra-*O*-acetyl-galactitol as virtually the only product from compounds **1a** and **1b** confirmed their identities as 3,4- acetals, while identification of 2,3-di-*O*-methyl-1,4,5,6-

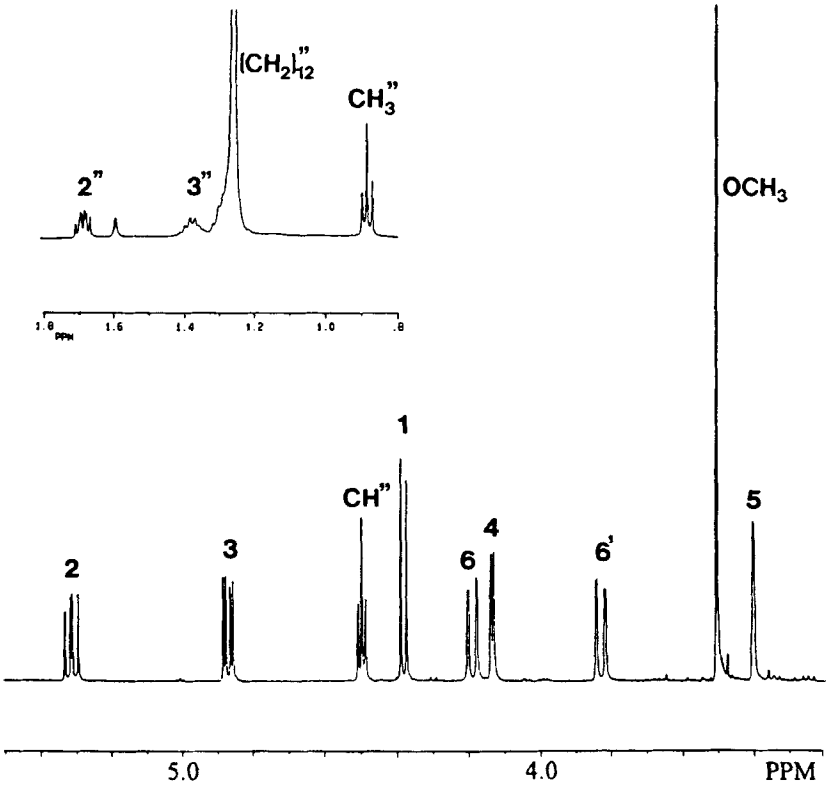


Figure 2. Proton NMR of compound **2a** in $CHCl_3$ at 309 °K. Inset: Upfield region containing resonances from hexadecylidene chain.

tetra-*O*-acetyl-galactitol as the product from compound **2a** confirmed its identity as a 4,6-acetal. Compound **2b** produced a mixture of 2,3-di-*O*-methyl-1,4,5,6-tetra-*O*-acetyl- and 2,6-di-*O*-methyl-1,3,4,5-tetra-*O*-acetyl galactitols in an approximate ratio of 3:1, confirming that the major component was also a 4,6-acetal, with some contamination from one of the 2,3-acetals.

1-D ^1H NMR spectra were obtained for each of the per-*O*-acetyl-*O*-hexadecylidene derivatives of methyl β-galactopyranoside under conditions similar to those used previously for characterizing the synthetic plasmalopsychosines.[12] A typical spectrum (for compound **2a**) is reproduced in Figure 2. Ring protons were assigned in all spectra by sequential decoupling experiments starting from the easily identifiable β- H-1

Table. Proton chemical shifts (ppm)[a] and ^3J coupling constants (Hz) for per-*O*-acetyl-*O*-hexadecylidene derivatives (**1a,b**; **2a,b**) of methyl β-D-galactopyranoside in $CHCl_3$ at 309 °K, compared with those of per-*O*-acetyl-galactosylceramide (reference).[b]

	OCH_3	H-1 ($J_{1,2}$)	H-2 ($J_{2,3}$)	H-3 ($J_{3,4}$)	H-4 ($J_{4,5}$)	H-5 ($J_{5,6}$)	H-6 ($J_{5,6'}$)	H-6' ($J_{6,6'}$)	CH" ($J_{1'',2''}$)
1a	3.467	4.301 (8.2)	4.865 (6.4)	4.152 (6.1)	4.030 (2.3)	3.993 (5.2)	3.398 (7.8)	4.401 (NF)[c]	5.010 (5.0)
1b	3.476	4.280 (8.2)	4.927 (7.3)	4.305 (5.6)	4.027 (1.9)	3.887 (5.9)	4.372 (5.9)	4.372 (eq)[d]	5.282 (5.0)
2a	3.499	4.381 (7.8)	5.313 (10.2)	4.871 (3.9)	4.135 (1.2)	3.396 (1.5)	4.191 (1.9)	3.830 (-12.2)	4.500 (5.1)
2b	3.499	4.349 (7.8)	5.304 (10.2)	4.885 (3.9)	4.272 (1.4)	3.698 (4.9)	4.140 (1.9)	3.887 (-12.5)	5.066 (5.8)
3		4.379 (7.6)	5.079 (10.4)	4.932 (3.8)	5.311 (5.8)	3.833 (4.9)	4.065 (6.7)	4.065 (eq)	

a. Other resonances in common: H-2", 1.69 ppm; H-3", 1.38 ppm; $(CH_2)_{12}$", 1.26 ppm; CH_3", 0.881 ppm. b. Data for per-*O*-acetylgalactosylceramide taken from ref. 14 (reported at 298 °K). c. NF=Non-first order splitting. d. eq=equivalent.

doublet in each spectrum. In each case a downfield 1-proton triplet, not coupled to any of the ring protons, could be assigned to the acetal C-H of the hexadecylidene substituent. Connectivities of this proton to the remainder of the alkyl chain resonances could then be established similarly by decoupling experiments. In two cases (compounds **1b** and **2b**) resonances from impurities were detected. In the spectrum of **1b**, these resonances (present in a ratio between 1:5 and 1:6) were from an unidentifiable impurity; while in the spectrum of **2b**, the resonances clearly represented a contamination from compound **1a** making up about 25% of the mixture. In neither case did the impurity peaks interfere with assignment of the major resonances or with the subsequent NOE difference

experiments (see below). The proton assignments for all four derivatives are summarized in the Table.

Even in the absence of "linkage" analysis by permethylation, the chemical shift and coupling constant data lend support to the primary structure assignments of the four fractions. Compared with data reported for per-*O*-acetylated galactosylceramide under comparable conditions[14] (see Table), the chemical shifts of H-2 for all derivatives show typical downfield values for an acetylated HO-2, while the chemical shifts of H-4 for all four hexadecylidene derivatives show substantial upfield values compared with that for **3**, as expected for an occupation of HO-4 by an acetal rather than an acetyl substituent. In the case of the putative 3,4-*O*-hexadecylidene derivatives, H-3 for both (4.152 and 4.305 ppm for **1a** and **1b**, respectively) show substantial upfield shifts compared with the values observed for the 4,6-*O*-hexadecylidene derivatives (4.871 and 4.885 ppm for **2a** and **2b**, respectively), which in turn show values similar to that for **3** (4.932 ppm), as expected if acetylated at HO-3. The values observed for all H-6 and H-6' can't be interpreted quite as systematically, but two trends clearly support the primary structure assigned to derivatives **2a,b**: (a) the breakdown of chemical shift equivalence, or near-equivalence, of H-6 and H-6', as expected due to immobilization of the exocyclic hydroxymethyl group in a 4,6-*O*-alkylidene ring; (b) the upfield shift of one hydroxymethyl proton for each derivative (3.830 and 3.887 ppm for **2a** and **2b**, respectively), which is assigned in each case to H-6', since the shielding compared with the geminal H-6 (4.191 and 4.140 ppm, respectively) would be expected as a consequence of the enforced antiperiplanar arrangement of H-6' with a lone-pair on O-6. These assignments were borne out by subsequent analysis of dipolar coupling data (see below).

The differences observed for the sets of ^3J coupling constants among the four β-galactopyranoside ring systems reflect the steric constraints imposed on the sugar ring by the five-membered ring of the 3,4-*O*-hexadecylidene substituent. Construction of Dreiding models shows that for compounds **1a** and **1b**, larger $^3J_{3,4}$ and $^3J_{4,5}$ can be expected than for **2a** and **2b**, because the 3,4-*O*-hexadecylidene substituent constrains the H-3/H-4 and H-4/H-5 dihedral angles to somewhat smaller values (away from the Karplus minimum of ca. 90°). A concomitant decrease in the H-2/H-3 dihedral angles (away from the Karplus maximum of ca. 180°) explains the decreased values of $^3J_{3,4}$ in **1a** and **1b**.

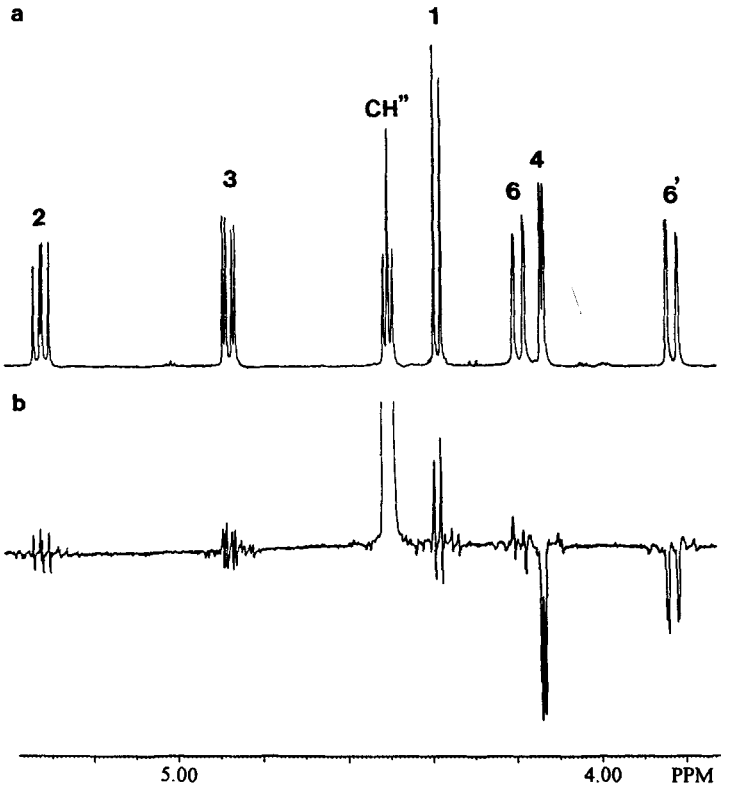
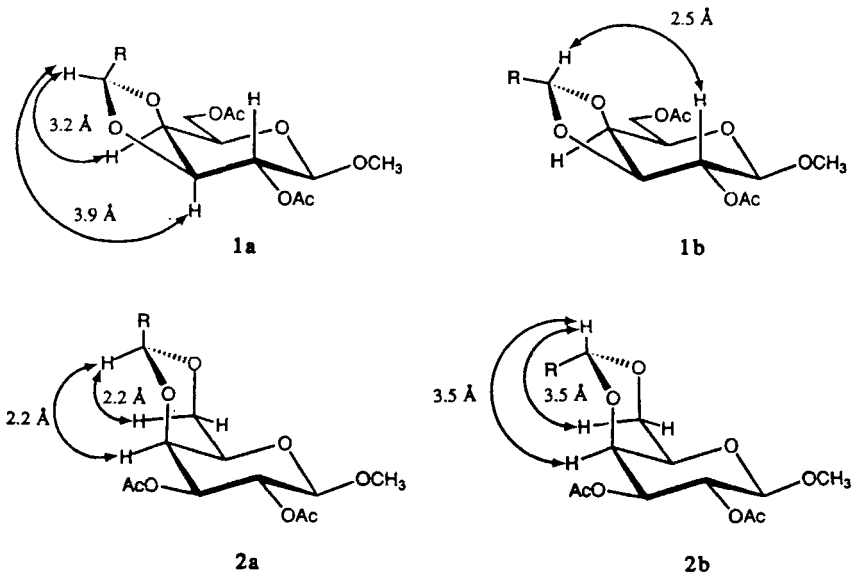


Figure 3. Downfield region of proton NMR of compound **2a** (Panel A) and 1-D SIR-ΔNOE experiment with irradiation of acetal C-H at 4.500 ppm (Panel B).

For the two 3,4-*O*-hexadecylidene derivatives (**1a** and **1b**), resonances from the acetal protons were observed at chemical shifts of 5.010 and 5.282 ppm, respectively. Of these, the former is virtually identical to the value previously obtained for the corresponding synthetic plasmalopsychosine (4.992 ppm). It can therefore be expected, with a high degree of certainty, that the stereochemical configuration at the acetal carbon is the same in **1a** as in the plasmalopsychosine analog. The same reasoning could be applied to the two 4,6-*O*-hexadecylidene derivatives (**2a** and **2b**). In this case, the acetal protons were observed at 4.500 and 5.066 ppm, respectively. The chemical shift observed for the acetal proton in the corresponding plasmalopsychosine was 4.582 ppm, a value much closer to that in **2a**.



$R = CH_2(CH_2)_{14}CH_3$

Figure 4. Stick drawings of per-*O*-acetyl-*O*-hexadecylidene derivatives of methyl β-D-galactopyranoside showing linkage and approximate stereochemical disposition of *O*-hexadecylidene groups (determined by ΔNOE experiments) and nearest neighbor interproton distances from acetal C-H (estimated from Dreiding stereomodels). **1a**, Methyl 2,6-Di-*O*-acetyl-3,4-*O*-*endo*-hexadecylidene-β-D-galactopyranoside; **1b**, Methyl 2,6-Di-*O*-acetyl-3,4-*O*-*exo*-hexadecylidene-β-D-galactopyranoside; **2a**, Methyl 2,3-Di-*O*-acetyl-4,6-*O*-*endo*-hexadecylidene-β-D-galactopyranoside; **2b**, Methyl 2,3-Di-*O*-acetyl-4,6-*O*-*exo*-hexadecylidene-β-D-galactopyranoside.

Four sets of 1-D SIR-ΔNOE spectra were acquired by selective irradiation of the acetal C-H for each derivative, varying the mixing times from 0.04 to 1.2 sec. An example for derivative **2a** is shown Figure 3. In the case of derivative **1a**, NOE difference spectra clearly showed dipolar cross-relaxation between the acetal C-H and the galactopyranose ring H-4 (4.040 ppm; medium intensity) and H-3 (4.152 ppm; weak), while for derivative **1b**, cross-relaxation was observed only with H-2 (4.927 ppm; strong). Dreiding models showed that the close proximities required to observe these interactions are manifested only if the hexadecylidene chain is configured *endo* in **1a** and *exo* in **1b** (Figure 4a,b). Approximate interproton distance measurements from the acetal C-H were 3.2 and 3.9 A

to H-4 and H-3, respectively, in **1a**, and 2.5 A to H-2 in **1b**. Since chemical shift arguments indicate that the acetal carbon of the previously synthesized plasmalopsychosine A analog has a configuration like that in derivative **1a**, that hexadecylidene chain must also be in the *endo* configuration.

NOE experiments for derivative **2a** indicated strong dipolar interactions of C-H with H-4 and H-6' (4.135 and 3.830 ppm, repectively; Figure 3b) of the galactopyranose moiety, while in the case of **2b** only a weak interaction was observed between C-H and H-6' (3.887 ppm) and possibly H-4 (4.272 ppm). Observation of these interactions is expected only if the hexadecylidene chain is *endo* in **2a** and *exo* in **2b** (Figure 4c,d). Interproton distances from the acetal C-H estimated from Dreiding models were 2.2 A to both H-4 and H-6' in **2a** and 3.5 A to these protons in **2b**. By the arguments used previously, the *endo* configuration must also be assigned to the acetal carbon in the corresponding plasmalopsychosine B.

EXPERIMENTAL

General Methods. Optical rotations were determined with a Perkin Elmer Model 241 MC polarimeter. TLC and HPTLC were conducted on silica gel F-254 plates (E. Merck, Darmstadt, Germany) and visualized by spraying with 0.5% orcinol in 10% aq H_2SO_4, followed by heating. Flash chromatography was performed on silica gel (230-400 mesh, EM Science, Gibbstown, NJ) and gel permeation chromatography on Sephadex LH-20 and G-10 (Pharmacia, Piscataway, NJ). Compounds were judged pure on the basis of their ^{1}H NMR spectra. Methylation linkage analysis was carried out as previously described.[13]

^{1}H NMR spectrometry. NMR spectra were recorded on a Bruker (Karlsruhe, Germany) AM-500 Fourier transform spectrometer/Aspect 3000 data system, using quadrature detection. Samples were dissolved in $CDCl_3$ (referenced to Me_4Si @ 0.0 ppm) at 309±2 °K. For 1-D reference spectra of the per-*O*-acetyl-*O*-hexadecylidene derivatives of methyl β-D-galactopyranoside, the sweep width was 4000 Hz, collected over 16 K data points, and the preparatory delay was 2.0 s. 1-D transient NOE spectra[15] of these compounds were obtained by selective inversion-recovery in the difference mode (SIR-ΔNOE).[16] Twelve mixing times (from 10 ms to 1.2 s) were used. The sweep width was 4000 Hz, collected over 16 K data points, and the PD was 3.0 s.

Methyl 2,6-Di-*O*-acetyl-3,4-*O*-*endo*-hexadecylidene-β-D-galactopyranoside (1a); Methyl 2,6-Di-*O*-acetyl-3,4-*O*-*exo*-hexadecylidene-β-D-galactopyranoside (1b); Methyl 2,3-Di-*O*-acetyl-4,6-*O*-*endo*-hexadecylidene-β-D-galactopyranoside (2a); Methyl 2,3-Di-*O*-acetyl-4,6-*O*-*exo*-hexadecylidene-β-D-galactopyranoside (2b). To a solution of methyl β-D-galactopyranoside (218 mg, 1.12 mmol) in DMF (3 mL) were added 1,1-dimethoxyhexadecane (385 mg, 1.34 mmol) and *p*-TsOH (125 mg, 0.65 mmol). After stirring for 24 h at room temperature, the reaction mixture was neutralized by addition of triethylamine and concentrated *in vacuo*. Chromatography of the residue on silica gel (toluene-methanol, 4:1) gave a mixture of four isomeric acetals of methyl β-D-galactopyranoside: ^{1}H NMR ($CDCl_3$) δ 5.21, 5.06, 5.01, and 4.56 (4t, 2:1:4:28, 4 isomeric O-[O-]**CH**"), 3.55 (s, 3H, **OCH$_3$**), 1.8-1.0 (m, 28H, **CH$_2$**), 0.87 (t, 3H, **CH$_3$**).

The two major components of the mixture were separated by HPLC using a Nova-Pak™ HR-Silica 60A column and a chloroform-methanol gradient. The data for one pure component were identical to those reported earlier[12] for (an isomer of) methyl 4,6-*O*-hexadecylidene-β-D-galactopyranoside. The other component was judged to be an isomer of methyl 3,4-*O*-hexadecylidene-β-D-galactopyranoside: $[\alpha]_D$ +4.6°(*c* 0.2, $CHCl_3$-MeOH 1:1); ^{1}H NMR ($CDCl_3$) δ 5.01(t, 1H, J = 5.0 Hz, O-[O-]**CH**"), 5.14 (d, 1H, J = 8.5 Hz, **H-1**), 4.04 (m, 2H, **H-3**, **H-4**), 3.91 (m, 1H, **H-2**), 3.85 (m, 2H, **H-6**, **H-6'**), 3.55 (s, 3H, **OCH$_3$**), 1.69 (m, 2H, **CH$_2$**"CH"), 1.5-1.2 (m, 26H, **CH$_2$**), 0.88 (t, 3H, **CH$_3$**).

To a solution of a crude mixture of acetals (100 mg) in pyridine (2.0 mL), acetic anhydride (0.5 mL) was added and the mixture stirred for 16 h at room temperature. To destroy excess acetic anhydride, ethanol (0.5 mL) was added and stirring continued another 10 min. After evaporation of the solvents *in vacuo*, the residue was suspended in 5 mL water and passed through a Bond-Elut™ C-18 column. The column was washed with water to remove water soluble impurities and finally eluted with methanol to recover the compound retained on the column. Evaporation of the solvent gave a mixture of the desired compounds, which were fractionated by HPLC using a Nova-Pak™ HR-Silica 60 A column and a hexane-ethyl acetate gradient to give four compounds with R_f = 0.31, 0.29, 0.25, and 0.24 (hexane-ethyl acetate 3:1), having the isomeric structures **1b**, **2b**, **1a**, and **2a**, determined by methylation analysis and ^{1}H NMR as described under RESULTS.

ACKNOWLEDGMENT

This study was supported by funds from The Biomembrane Institute.

REFERENCES

1. Y. A. Hannun and R. M. Bell, *Science,* **243**, 500 (1987).
2. S. Hakomori, Y. Igarashi, H. Nojiri, E. Bremer, N. Hanai, and G. Nores, in *Trophic Factors and the Nervous System*, L. A. Horrocks, et al., Eds.; Raven Press, Ltd: New York, 1990, p 135.
3. S. Hakomori, *Cancer Cells*, **3**, 461 (1991).
4. S. Hakomori, *J. Biol. Chem.*, **265**, 18713 (1990).
5. A. H. Merrill, Jr. and V. L. Stevens, *Biochim. Biophys. Acta*, **1010**, 131 (1989).
6. A. H. Merrill, Jr. and D. D. Jones, *Biochim. Biophys. Acta*, **1044**, 1 (1990).
7. Y. Igarashi, H. Nojiri, N. Hanai, and S. Hakomori, *Methods Enzymol.*, **170**, 521 (1989).
8. Y. Igarashi, S. Hakomori, T. Toyokuni, B. Dean, S. Fujita, M. Sugimoto, T. Ogawa, K. El-Ghendy, and E. Racker, *Biochemistry*, **28**, 6796 (1989).
9. Y. A. Hannun and R. M. Bell, *Science*, **235**, 670 (1987).
10. E. D. Nudelman, S. B. Levery, Y. Igarashi, and S. Hakomori, *J. Biol. Chem.*, **267**, 11007 (1992).
11. S. B. Levery, E. D. Nudelman, and S. Hakomori, *Biochemistry*, **31**, 5335 (1992).
12. K. K. Sadozai, J. K. Anand, E. D. Nudelman, and S. Hakomori, *Carbohydr. Res.*, **24**, 301 (1993).
13. S. B. Levery and S. Hakomori, *Methods Enzymol.*, **138**, 13 (1987).
14. S. Gasa, M. Nakamura, A. Makita, M. Ikura, and K. Hikichi, *Eur. J. Biochem.*, **155**, 603 (1986).
15. S. L. Gordon and K. Wuthrich, *J. Am. Chem. Soc.*, **100**, 7094 (1978).
16. N. H. Andersen, K. T. Nguyen, C. Hartzell, and H. Eaton, *J. Magn. Reson.*, **74**, 195 (1987).