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Note

Synthesis of plasmalopsychosines A and B, two novel lysosphingolipids found in human brain †

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INTRODUCTION

Glycosphingolipids (GSLs) are present in most mammalian tissues and play important roles in cellular recognition and transmembrane signaling[1–5]. In this connection, sphingosine (Sph), *N*,*N*-dimethylsphingosine, *N*,*N*,*N*-trimethylsphingosine, and lysosphingolipids such as lyso-GM_3 and psychosine have been identified as potent inhibitors of protein kinase C (PKC)[1,2,6–9]. These findings led to a search for this class of compounds in neural tissues, and a recent report from this laboratory[10] described the isolation of two novel lysosphingolipids from the white matter of human brain. These compounds, termed plasmalopsychosines A and B (PP-A and -B, (formulas **1** and **2**) were structurally identified as conjugates of "plasmals" (long chain aliphatic aldehydes)[11] with the galactose residue of psychosine (**3**) through 3,4- and 4,6-cyclic acetal linkages, respectively. Preliminary biological studies of PP-A and -B have shown a clear (though weak) inhibition of PKC activity; however, the functional role, if any, of these compounds in transmembrane signaling remains unclear. The purpose of the present work was to provide synthetic samples of PP-A and -B to facilitate PKC-related studies. We describe the synthesis of PP-A (**1**), PP-B (**2**), and a structural analogue of PP-B (**8**).

RESULTS AND DISCUSSION

Both the target structures **1** and **2** are cyclic acetals of a C_{16} aliphatic aldehyde and psychosine (**3**). The aldehyde, hexadecanol (**5**), was readily obtained by

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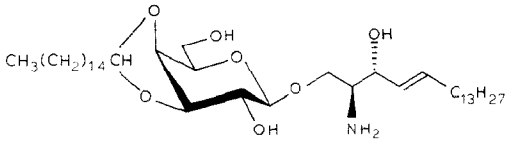

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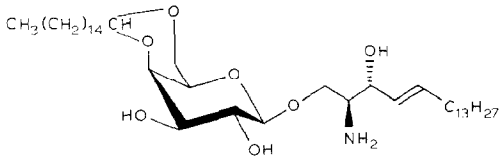

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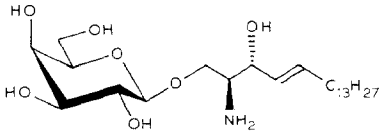

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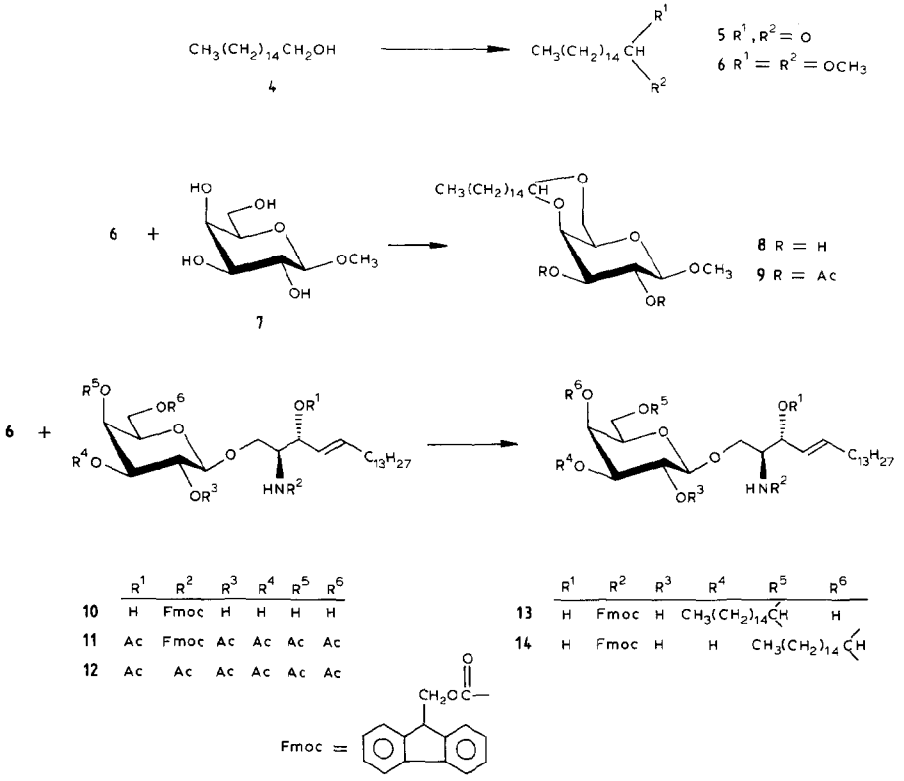
oxidation of *n*-hexadecanol (**4**) (Scheme 1) with pyridinium chlorochromate[12]. However, when it was treated with methyl β-D-galactopyranoside[13] (**7**) in the presence of an acid catalyst, the reaction was slow and did not reach completion even after 7 days at 50°C. Therefore, **5** was converted into a more reactive derivative, 1,1-dimethoxyhexadecane[14] (**6**) with the aid of trimethyl orthoformate[15] and Amberlite® IR-120. The efficacy of **6** in forming cyclic acetals with sugars was then tested in a simple case. Condensation of the galactoside **7** with **6** in *N*,*N*-dimethylformamide (DMF) containing a trace of *p*-toluenesulfonic acid (*p*-TsOH) gave a 7:1 mixture of cyclic acetal **8** and its 3,4-isomer. Separation of this mixture by repeated chromatography gave pure **8** in 71% yield. In the ^{1}H NMR spectrum of **8**, the signal for the methine proton of the acetal function appeared at 4.563 ppm, whereas the same signal in the spectrum of the 3,4-isomer was observed at 5.009 ppm. The 4,6 position of the acetal linkage in **8** was confirmed by acetylation and determination of the ^{1}H NMR spectrum of the product (**9**), which showed characteristic deshielded signals at 5.313 and 4.865 ppm for H-2 and H-3, respectively, indicating lack of substitution at positions 2 and 3 in **8**.

Having evaluated the efficacy of **6**, we focused our attention on the synthesis of target molecules **1** and **2**. Psychosine (**3**) was either prepared synthetically[16] or obtained through alkaline hydrolysis[17] of galactocerebroside isolated from bovine brain[18]. Treatment of a solution of psychosine in 3:1 chloroform–water, with 9-fluorenylmethyl chloroformate (FmocCl) in the presence of K_2CO_3 gave N-protected Fmoc-psychosine (**10**). The ^{1}H NMR of **10** showed signals for aromatic protons and for H-5 and H-4 of Sph at 7.76–7.73, 5.70, and 5.48 ppm, respectively,



but the region between 4.5 and 3.0 ppm was not clearly assignable. To obtain a well-resolved spectrum, crude product mixture (containing untransformed psychosine) was acetylated, yielding fully substituted Fmoc-psychosine (**11**) and psychosine (**12**). The ^{1}H NMR spectra of both these compound were completely assigned, and a prominent deshielded signal for H-3 of Sph at 5.304 ppm was observed in both spectra. Fmoc-psychosine (**10**) was then treated with **6** in essentially the same way as described for **7**, yielding a mixture of 3,4- and 4,6-cyclic acetals (**13** and **14**), which could not be separated by chromatography. Removal of the Fmoc protecting group[19] by treatment with piperidine followed by HPLC purification on an Iatrobeads® column gave target compounds **1** and **2** (PP-A and -B), chromatographically indistinguishable from their natural counterparts.

Structures of the synthesized **1** and **2** were confirmed by ^{1}H NMR, positive-ion fast atom bombardment mass spectrometry (FABMS), and methylation analysis. The ^{1}H NMR of **1** showed a signal for the methine proton of the acetal function at 4.99 ppm, whereas the signal for the same proton in **2** appeared at 4.58 ppm. These chemical shifts are indicative of a five-membered cyclic structure in **1** and a six-membered cyclic structure in **2**. FABMS of **1** and **2** showed identical fragmentation patterns, including ion peaks at m/z 684 $(M + H)^+$ and m/z 282 $(Sph - 17)^+$,

confirming that both are indeed the condensation products of psychosine and a C_{16} aliphatic aldehyde. Permethylation of **1** and **2** followed by acid hydrolysis, reduction, acetylation, and analysis of the resulting partially methylated alditol acetates by GLC–MS gave 1,3,4,5-tetra-*O*-acetyl-2,6-di-*O*-methylgalactitol and 1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methylgalactitol, respectively, confirming the presence of a 3,4-cyclic acetal structure in **1** and a 4,6-cyclic acetal in **2**.

In conclusion, PP-A (**1**) and PP-B **2**) were synthesized and characterized as being identical to compounds isolated from human brain.

EXPERIMENTAL

General methods.—Optical rotations were determined with a Perkin–Elmer Model 241 MC polarimeter. ^{1}H NMR spectra were recorded on a Bruker WM-500 spectrometer. Chemical shifts (δ) are referenced to internal Me_4Si. TLC and HPTLC were conducted on Silica Gel 60 F-254 plates (Merck, Darmstadt, Germany), and visualized by spraying with 0.5% orcinol in 10% aq H_2SO_4 or 0.2% ninhydrin in EtOH, followed by heating. Flash chromatography[20] was performed on silica gel (230–400 mesh) columns (EM Science, Gibbstown, NJ). Compounds were judged pure on the basis of their ^{1}H NMR spectra, copies of which were furnished to the editor for verification. FABMS and high-resolution MS were performed on a JEOL JMS-HX 110/DA-5000 mass spectrometer/data system. 3-Nitrobenzyl alcohol (NBA) was used as a matrix. Sodium acetate was added to the matrix to obtain $(M + Na)^+$ ions. Methylation[21], followed by hydrolysis, reduction, and per-*O*-acetylation, were performed as described previously[22], and data were analyzed on an ELQ 400 GLC–MS system (DB-5 column; splitless injection; temperature program 140–250°C at 4°C/min; EIMS mode), with identification of the partially methylated alditol acetates by retention time and characteristic electron-impact mass spectra[23,24]. Identifications were confirmed by comparison with mixtures of known standards.

Hexadecanal (**5**).—To a solution of *n*-hexadecanol (**4**) (10 g, 41.2 mmol) in CH_2Cl_2 (200 mL), pyridinium chlorochromate (17.7 g, 82.5 mmol) was added. The mixture was stirred for 2 h at room temperature, then hexane (300 mL) was added, and the supernatant was decanted from the black gum. The insoluble residue was thoroughly washed with hexane, and the combined organic solution was concentrated to one half its original volume and filtered through a short column of Florisil® and silica. Evaporation of the filtrate gave **5** as a white solid in quantitative yield; mp 45°C; ^{1}H NMR ($CDCl_3$): δ 9.758 (s, 1 H, HCO), 2.37 (m, 2 H, CH_2CO), 1.625 (m, 2 H, CH_2), 1.257 (m, 24 H, CH_2), and 0.879 (t, 3 H, CH_3).

1,1-Dimethoxyhexadecane (**6**).—A solution of **5** (1 g, 4.15 mmol) and trimethyl orthoformate (540 mg, 5.08 mmol) in dry MeOH (7 mL) containing Amberlite® IR-120 (H^+) (2.5 g, washed with dry MeOH) was refluxed for 2 h. The resin was filtered off and the filtrate was evaporated in vacuo. Chromatography of the residue on a silica column with toluene as eluent gave **6** (950 mg, 80%) as a

colorless oil; ^{1}H NMR ($CDCl_3$): δ 4.343 (t, 1 H, *J* 5.8 Hz, CH), 3.305 (s, 6 H, OCH_3), 2.157, 1.571, 1.253 (3 m, 28 H, CH_2), and 0.876 (t, 3 H, *J* 6.9 Hz, CH_3).

*Methyl 4,6-*O*-hexadecylidene-β-*D*-galactopyranoside* (**8**).—To a solution of **7** (218 mg, 1.12 mmol) in DMF (3 mL), **6** (385 mg, 1.34 mmol) and *p*-TsOH (125 mg, 0.65 mmol) were added at 20°C. After 24 h in the mixture was neutralized with Et_3N and evaporated in vacuo. The residue was chromatographed on SiO_2 in 4:1 toluene–MeOH, which gave a 7:1 mixture of compound **8** and a minor product. Separation of the mixture by HPLC using a Nova-Pak® (HR Silica 60 Å) column and a $CHCl_3$–MeOH gradient gave pure **8** (328 mg, 71%); $[\alpha]_D$ − 16.2° (*c* 1.5, 1:1 $CHCl_3$–MeOH); ^{1}H NMR ($CDCl_3$): δ 4.563 (t, 1 H, *J* 5.2 Hz, CH), 4.186 (dd, $J_{5,6}$ 0.9, $J_{6,6'}$ 12.5 Hz, H-6′), 4.151 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 3.968 (d, 1 H, $J_{3,4}$ 3.6 Hz, H-4), 3.851 (dd, 1 H, H-6), 3.680 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 9.6 Hz, H-2), 3.605 (dd, 1 H, H-3), 3.554 (s, 3 H, OCH_3), 3.372 (s, 1 H, H-5), 1.692 (m, 2 H, CH_2CH), 1.252 (br m, 26 H, CH_2), and 0.876 (t, 3 H, CH_3); high resolution FABMS: calcd for $C_{30}H_{51}NO_9$ [M + NBA]$^-$, 569.3564; found, 569.3569.

*Methyl 2,3-di-*O*-acetyl-4,6-*O*-hexadecylidene-β-*D*-galactopyranoside* (**9**).—To a solution of **8** (6 mg, 0.104 mmol) in pyridine (0.5 mL), acetic anhydride (0.1 mL) was added and the mixture was stirred for 20 h at room temperature. To destroy excess Ac_2O, EtOH (0.1 mL) was added and solvents were evaporated in vacuo. The residue was suspended in 5 mL water and passed through a Bond Elut® C-18 column. The column was rinsed with water to remove water-soluble impurities, and finally the desired compound was eluted with MeOH. Evaporation of the eluate gave pure **8** in quantitative yield, ^{1}H NMR data ($CDCl_3$): δ 5.313 (dd, 1 H, $J_{1,2}$ 8, $J_{2,3}$ 10.3 Hz, H-2), 4.865 (dd, 1 H, $J_{3,4}$ 3.6 Hz, H-3), 4.492 (t, 1 H, CH), 4.380 (dd, $J_{5,6}$ 2, $J_{6,6'}$ 12.3 Hz, H-6′), 4.376 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.129 (d, 1 H, H-4), 3.823 (dd, 1 H, H-6), 3.492 (s, 3 H, OCH_3), 3.394 (s, 1 H, H-5), 2.067 (m, 2 H, CH_2CH), 1.254 (br m, 26 H, CH_2), and 0.877 (t, 3 H, CH_3); high resolution FABMS: calcd for $C_{26}H_{45}O_7$ (M − CH_3O)$^+$, 469.3166; found, 469.3158.

O-β-D-Galactopyranosyl-(1 → 1)-(2S,3R,4E)-2-(9-fluorenylmethoxycarbonylamino)-4-octadecene-1,3-diol (**10**).—To a solution of **3** (250 mg, 0.54 mmol) in 4:1 $CHCl_3$–water (20 mL), 9-fluorenylmethyl chloroformate (154 mg, 0.59 mmol) and K_2CO_3 (149 mg, 1.07 mmol) were added. The mixture was stirred for 14 h at room temperature and then $CHCl_3$ was removed by evaporation. The residue was suspended in 5 mL water and transferred to a preconditioned Bond Elut® C-18 column. The column was rinsed with water to wash out water-soluble impurities, and finally eluted with MeOH to recover the lipophilic product. Evaporation of the solvent gave an amorphous solid composed of **10** and some unreacted started material (**3**). Chromatography on a silica gel column with 3:1 toluene–MeOH as eluent yielded **10** (320 mg, 86.5%). ^{1}H NMR (4:1 $CDCl_3$–CD_3OD): δ 7.76–7.73 (m, 8 H, aromatic), 5.70 (m, 1 H, H-5 of Sph), 5.48 (m, 1 H, H-4 of Sph), 3.604 (d, 1 H, $J_{3,4}$ 2.5 Hz, H-4), 1.99 (m, 2 H, H-6,6′ of Sph), 1.1–1.4 (br m, 22 H, CH_2), and 0.87 (t, 3 H, CH_3); high resolution FABMS: calcd for $C_{39}H_{57}NNaO_9$ [M + Na]$^+$, 706.3930; found, 706.3927.

O-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 1)-(2S,3R,4E)-3-O-acetyl-2-(9-fluorenylmethoxycarbonylamino)-4-octadecene-1,3-diol (**11**) *and O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-(1 → 1)-(2S,3R,4E)-2-acetamido-3-O-acetyl-4-octadecene-1,3-diol* (**12**).—To a mixture of **3** and **10** (5 mg) obtained from the previous reaction, pyridine (0.5 mL) and Ac_2O (0.2 mL) were added at 0°C. The reaction mixture was stirred for 24 h and solvents were evaporated in vacuo. Chromatography of the residue over a silica gel column using 1 : 1 hexane–EtOAc as eluent gave **11** and **12**, R_f 0.71 and 0.16, respectively, in 1 : 1 toluene–EtOAc.

^{1}H NMR data for **11** ($CDCl_3$): δ 7.9–7.2 (m, 8 H, aromatic), 5.806 (m, 1 H, H-5 of Sph), 5.385 (d, 1 H, $J_{3,4}$ 3.6 Hz, H-4), 5.381 (m, 1 H, H-4 of Sph), 5.304 (t, 1 H, H-3 of Sph), 5.183 (dd, 1 H, $J_{1,2}$ 8, $J_{2,3}$ 10.4 Hz, H-2), 5.014 (dd, 1 H, H-3), 4.462 (m, 1 H, H-1 of Sph), 4.415 (d, 1 H, H-1), 4.380 (m, 1 H, H-1′ of Sph), 4.220 (t, 1 H, H-2 of Sph), 4.135 (m, 2 H, H-6,6′), 4.0 (br m, 2 H, CH_2 of Fmoc), 3.863 (t, 1 H, H-5), 2.153 (s, 3 H, Ac), 2.07 (m, 2 H, H-6,6′ of Sph), 2.044, 2.032, 1.99, (3 s, 9 H, Ac), 1.38–1.18 (m, 22 H, CH_2), and 0.883 (t, 3 H, CH_3); high resolution FABMS: calcd for $C_{49}H_{67}N_9NaO_{14}$ $[M + Na]^+$, 916.4459; found, 916.4411.

^{1}H NMR data for **12** ($CDCl_3$): δ 5.774 (dt, 1 H, H-5), 5.729 (d, 1 H, NH), 5.38 (d, 1 H, H-4), 5.37 (dd, 1 H, H-4 of Sph), 5.266 (t, 1 H, H-3 of Sph), 5.148 (dd, 1 H, $J_{2,3}$ 10.5 Hz, H-2), 5.015 (dd, 1 H, $J_{3,4}$ 3.5 Hz, H-3), 4.439 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.344–4.274 (m, 1 H, H-2 of Sph), 4.122–4.174 (m, 2 H, H-6,6′), 3.966–3.874 (m, 2 H, H-1,1′ of Sph), 3.607 (dd, 1 H, H-5), 2.160, 2.061, 2.046, 1.986, 1.957 (5 s, 18 H, Ac), 1.4–1.2 (m, 22 H, CH_2), and 0.881 (t, 3 H, CH_3); high resolution FABMS: calcd for $C_{34}H_{56}NO_{11}$ $(M - CH_3CO_2)^+$, 654.3853; found, 654.3844.

O-(3,4-O-Hexadecylidene-β-D-galactopyranosyl)-(1 → 1)-(2S,3R,4E)-2-amino-4-octadecene-1,3-diol (**1**) *and O-(4,6-O-hexadecylidene-β-D-galactopyranosyl)-(1 → 1)-(2S,3R,4E)-2-amino-4-octadecene-1,3-diol* (**2**).—To a stirred solution of **10** (47.0 mg, 0.065 mmol) in DMF (1 mL), **6** (22.6 mg, 0.078 mmol) and *p*-TsOH (16 mg) were added. The mixture was stirred for 44 h at 20°C, with the further addition of *p*-TsOH (16 mg) and **6** (30 mg, 0.103 mmol) after 22 h. After completion of the reaction, Et_3N was added to neutralize the acid and the solvents were evaporated in vacuo. Column chromatography of the residue on silica gel in 5 : 1 toluene–MeOH gave a mixture of **13** and **14** in 78% yield. To this mixture piperidine (3 mL) was added, and the solution was stirred for 3 h at 20°C. Evaporation of the liquids in vacuo gave a mixture of **1** and **2**, which was separated by HPLC using an Iatrobead 10-μm column. Gradient elution was started with 98 : 1.8 : 0.2 $CHCl_3$–MeOH–NH_4OH, with increase in the polar components to 80 : 18 : 2 over a period of 500 min.

^{1}H NMR data for **1** (2 : 1 $CDCl_3$-CD_3OD): δ 5.763 (dt, 1 H, $J_{5,6} = J_{5,6'} = 6.4$ Hz, H-5 of Sph), 5.465 (dd, 1 H, $J_{3,4}$ 7.2, $J_{4,5}$ 15.5 Hz, H-4 of Sph), 4.992 (t, 1 H, CH), 4.029 (t, 1 H, H-3 of Sph), 2.076 (m, 2 H, H-6,6′ of Sph), 1.41–1.19 (m, 48 H, CH_2), 0.901, and 0.887 (2 t, 6 H, CH_3); FABMS (positive mode): *m/z* 684 $[M + H]^+$, 282 $(Sph - 17)^+$; high resolution FABMS: calcd for $C_{40}H_{78}NO_7$ $[M + H]^+$, 684.5778; found, 684.5787.

^{1}H NMR data for **2** (2:1 $CDCl_3$–CD_3OD): δ 5.775 (dt, 1 H, $J_{5,6} = J_{5,6'} = 6.8$, $J_{4,5}$ 15 Hz, H-5 of Sph), 5.455 (dd, 1 H, $J_{3,4}$ 7 Hz, H-4 of Sph), 4.582 (t, 1 H, J 5.3 Hz, CH), 2.065 (m, 2 H, H-6,6′ of Sph), 1.7–1.2 (m, 48 H, CH_2), and 0.887 (t, 6 H, CH_3); FABMS (positive mode): m/z 684 $[M + H]^+$, 282 $(Sph - 17)^+$; high resolution FABMS: calcd for $C_{40}H_{78}NO_7$ $[M + H]^+$, 684.5778; found, 684.5787.

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