was stirred for 48 h, the reaction was determined complete (TLC analysis). The solvent was removed in vacuo and the product added to diethyl ether (10 mL). The organic phase was washed with saturated ammonium chloride (5 mL) and brine (5 mL) and dried over anhydrous magnesium sulfate. Filtration and concentration in vacuo gave 198 mg of a light oil. Chromatography afforded 181 mg (86%) of 1-hydroxy-15-(*tert*-butyldimethylsiloxy)-PGB, 11: ^{1}H NMR (CCl_4) δ 6.6 (1 H, d, J = 15 Hz, olefinic, H_{13}), 5.9 (1 H, dd, J_{13-14} = 15 Hz, J_{14-15} = 5 Hz, olefinic, H_{14}), 4.3–4.05 (1 H, dt, allylic α to $OSiR_3$, H_{15}), 3.7–3.5 (2 H, t, J = 6 Hz, CH_2 α to OH), 2.5–1.8 (6 H, m, 3 CH_2), 1.6–1.1 (18 H, m, $(CH_2)_4$, $(CH_2)_5$, 0.9–0.75 (12 H, s and t, CH_3 and $SiC(CH_3)_3$), 0.0 (6 H, 2 s, $Si(CH_3)_2$); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 209.6, 163.2, 141.4, 140.3, 123.8, 75.6, 72.3; IR (liquid film) 3400, 3050–2860, 1700, 1665, 1600, 1470, 1390, 1360, 1260, 1100, 1040, 970, 840, 775 cm^{-1}.

Preparation of (±)-PGA (12). A solution of enone **10** (97 g, 0.22 mmol) in 20 mL of acetone at −5 °C was treated with 0.16 mL of Jones reagent (2.5 equiv) over a period of 15 min. After being stirred for 30 min between −5 and 0 °C, the mixture was treated with 2-propanol (0.5 mL) and filtered through Celite. The filtrate was added to water (20 mL) and extracted with ethyl acetate (3 × 20 mL), and the combined organics were concentrated in vacuo. A solution of the crude acid in acetonitrile (5 mL) was treated with an aqueous hydrogen fluoride solution and the desilylated product was isolated after 1 h. Chromatography afforded (±)-PGA as an oil, 52 mg, 71% yield: ^{1}H NMR ($CDCl_3$) δ 7.4–7.5 (1 H, dd, J = 6 Hz), 6.1–6.0 (1 H, dd, J = 6 Hz, J = 2 Hz), 5.6–5.4 (2 H, m, olefins), 4.05 (1 H, m, allylic α to OH), 3.2 (1 H, m, doubly allylic); IR (liquid film) 3420, 3080–2740, 1715, 1700, 1590; λ_{max} (EtOH) 217 nm (ϵ 10 000).

Preparation of (±)-PGB (13). The enone **11** (132 mg, 0.3 mmol) was oxidized with 2.8 equiv of Jones reagent in acetone at −5 °C and desilylated exactly as reported above for (±)-PGA **12**. Short-path chromatography gave (±)-PGB **13** as an oil, 78 mg, 78% yield: ^{1}H NMR ($CDCl_3$) δ 6.75 (1 H, d, J = 15 Hz, H_{13}), 6.15 (1 H, dd, J_{13-14} = 15 Hz, $J_{14,15}$ = 5 Hz, H_{14}), 4.4–4.1 (1 H, dt, allylic α to OH, H_{15}), 2.9–2.0 (8 H, m, 4 CH_2), 1.9–1.1 (16 H, 8 CH_2), 1.1–0.75 (3 H, t, CH_3); IR (liquid film) 3440, 1730, 1695, 1650, 1660, 970 cm^{-1}; λ_{max} (EtOH) 279 nm (ϵ 26 500).

Preparation of 14. An ethereal solution of the mixed cyano-*n*-butylcuprate (3.55 mmol in 25 mL, prepared from *n*-butyllithium and cuprous cyanide as reported for **3** above), cooled to −78 °C, was treated with a solution of the enol phosphate **8** in diethyl ether (0.95 mmol in 5 mL). After being stirred at this temperature for 1 h, and a further hour at ca. −45 °C, the reaction mixture was quenched by the addition of a saturated ammonium chloride solution (10 mL). The inorganic salts were removed by filtration through Celite, and the resulting organic phase was washed with brine (15 mL) and dried over anhydrous magnesium sulfate. Filtration and concentration in vacuo gave 620 mg of a light brown oil. TLC and spectral analysis showed no starting material, and the only product was isolated by column chromatography as a light oil, 290 mg, 58% overall yield from **7** (silica gel 60, 70–230 mesh, diethyl ether; R_f 0.53): ^{1}H NMR (360 MHz, $CDCl_3$) δ 5.60–5.49 (2 H, m, olefinic), 5.41–5.40 (1 H, br s, ring olefin), 5.41–5.40 (1 H, br s, ring olefin), 4.52–4.47 (1 H, m, allylic α to OH), 4.21–4.13 (4 H, m, 2 OCH_2), 4.12–4.06 (1 H, m, allylic α to $OSiR_3$), 2.86–2.84 (1 H, m, allylic ring proton), 2.72–2.70 (1 H, m, allylic ring proton), 1.44–1.19 (20 H, t and m, 2 OEt, $(CH_2)_4$, $(CH_2)_3$), 0.92–0.83 (15 H, s and m, 2 CH_3, $SiC(CH_3)_3$), 0.0 (6 H, 2 s, $Si(CH_3)_2$); IR (liquid film) 3400, 3050–2860, 1655, 1470, 1260, 1180, 1040, 980, 880, 840, 780 cm^{-1}.

Preparation of 15. The *tert*-butyl adduct **15** was obtained as a light yellow oil following chromatography (silica gel 60, 230–400 mesh, diethyl ether; R_f 0.49; 58% overall yield from **7**), using the procedure reported above for the preparation of **14** (*tert*-butyllithium substituted for *n*-butyllithium): ^{1}H NMR (CCl_4) δ 5.60–5.35 (3 H, M, olefinic), 4.3–3.8 (6 H, m, allylic α to OH, allylic α to $OSiR_3$, 2 OCH_2CH_3), 2.8–2.55 (2 H, m, ring allylic), 1.60–1.1 (14 H, m, 2 OCH_2CH_3, $(CH_2)_4$), 0.95 (9 H, s, *tert*-butyl), 0.85 (12 H, s and m, *tert*-butyl, CH_3), 0.0 (6 H, 2 s, $Si(CH_3)_2$); IR (liquid film) 3420, 3050–2860, 1650, 1470, 1400, 1370, 1260, 1170, 1040, 960, 840 cm^{-1}.

Acknowledgment. We thank the donors of the Petroleum Research Fund, administered by the American Chemical Society, for partial support of this research and the National Cancer Institute of the PHS (CA 22237) for support of the work. We also acknowledge the National Science Foundation for providing funds for the purchase of a Bruker 360-MHz spectrometer.

Registry No. (±)-1, 51064-01-8; (±)-**2**, 60208-93-7; (±)-3, 78823-07-1; **4**, 78823-17-3; **5**, 78823-18-4; **6**, 78823-19-5; **7**, 78823-20-8; **8**, 78823-21-9; (±)-**9** (isomer 1), 78823-22-0; (±)-**9** (isomer 2), 78855-67-1; (±)-**10**, 78823-23-1; (±)-**11**, 78823-24-2; (±)-**12**, 78855-68-2; (±)-**13**, 17204-83-0; **14**, 78823-25-3; **15**, 78823-26-4; 1-lithio-7-(trimethylsiloxy)heptane, 78823-27-5; lithium cyano-*n*-butylcuprate, 41742-63-6; lithium cyano-*tert*-butylcuprate, 78856-98-1.

Useful Syntheses of *erythro*- and *threo*-*N*-Oleoyl-D-sphingosines (Ceramides) and Galactosylceramides (Cerebrosides) from L-Serine[1]

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Received April 15, 1981

The 4-(carbomethoxy)-2-phenyl-Δ^2-oxazoline formed from L-serine provides the basis for a useful synthesis of ceramides and cerebrosides. The natural erythro configuration and its threo epimer are formed in equal amounts but are readily separated chromatographically, so that both epimers are available for experiments in which the properties of erythro and threo configurations are to be compared. The method produces the final cerebroside product on a scale of 100 mg or more. The optical purity of the product was shown to be close to 100%. In addition to the threo epimer, other analogues of the natural cerebrosides with different chain lengths and stereochemistries should be readily available by using the route developed. Such molecules are of interest to us for ^{13}C NMR and related studies of membrane organization.

Cerebrosides (**1**, Figure 1) are a subclass of glycosphingolipids and are one of the main constituents of brain membranes.[2,3] They are the simplest type of glycosphingolipid and thus serve as a model for the hydrophobic and interfacial regions of more complex glycosphingolipids. Cerebroside and cerebroside sulfate have been shown to bind stereospecifically morphinelike compounds, a nec-

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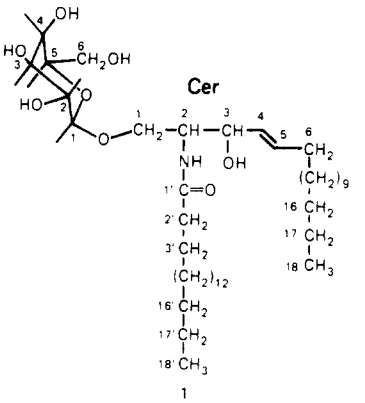
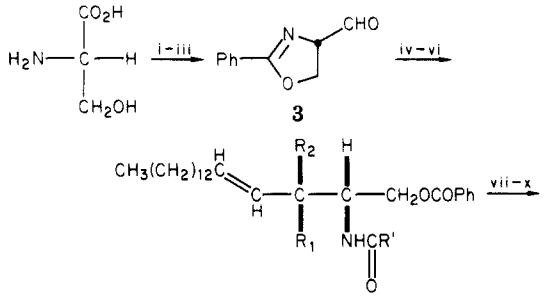


Figure 1. Structural formula of cerebroside containing a saturated fatty acyl chain and showing the numbering system used in ^{13}C NMR assignments. (The oleoylcerebroside has a cis double bond at C-9′,C-10′.)

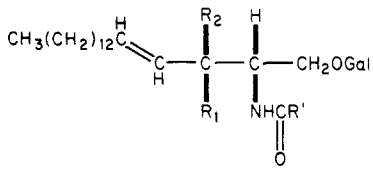
essary requirement for possible receptor functioning.[4,5] Other glycosphingolipids have been implicated as receptors for toxins,[6,7] hormones,[8,9] and interferon.[10] Cerebrosides have been shown to have the structure 1, which includes sphingosine (D-*erythro*-1,3-dihydroxy-2-amino-*trans*-4-octadecene),[11,12] a long-chain fatty acid attached in an amide linkage,[2,3,13,14] and a hexose, in the brain predominantly galactose, attached in a β-glycosidic linkage to sphingosine C-1.[15]

The need to synthesize substantial quantities of cerebrosides and related glycosphingolipids for ^{13}C NMR and other studies of their effects on membrane organization led us to seek an efficient route, which we are reporting herein. Questions concerning the stereospecificity of membrane organization can be answered by comparing the effects of natural and various unnatural stereochemical configurations of cerebrosides on ^{13}C relaxation times in bilayer membranes. A major question is whether the C-3 hydroxyl group is involved in stereospecific hydrogen bonding; therefore, we want to examine the threo isomer of cerebroside. We developed a synthesis based on the sphingosine synthesis of Newman,[16] and we elected to concern ourselves with efficiency rather than stereoselectivity. Our route gives essentially equimolar quantities of D-erythro product and its threo C-3 epimer; however, we were able to separate them readily by chromatography, and for study of structures differing in additional stereochemical features or in chain lengths, we would need to synthesize both erythro and threo isomers in any case. The route developed appears to be applicable to the synthesis of a variety of such structures. Prior synthetic work on sphingosine and cerebrosides has been reviewed by Shapiro[17] (see also ref 18).

Scheme I[a,b]



7a, R_1 = OH; R_2 = H
b, R_1 = H; R_2 = OH



12a, R_1 = OH; R_2 = H
b, R_1 = H; R_2 = OH

[a] (i) MeOH, HCl. (ii) HN=C(Ph)OEt, CH_2Cl_2, H_2O. (iii) DIBAL-H, −70 °C, $PhCH_3$-C_6H_{14}. (iv) *trans*-$CH_3(CH_2)_{12}CH$=$CHAl(i\text{-}Bu)_2$ (4), C_6H_{14}-Et_2O-$PhCH_3$. (v) aqueous HCl, 25 °C, THF. (vi) *cis*-$CH_3(CH_2)_7CH$=$CH(CH_2)_7CO_2Ph$-*p*-NO_2, pyridine. (vii) TbDpSiCl (*tert*-butylchlorodiphenylsilane), imidazole, DMF. (viii) $NaOCH_3$, CH_3OH, 25 °C. (ix) (A) Tetraacetylgalactosyl bromide, $Hg(CN)_2$, C_6H_6-CH_3NO_2, 80 °C; (b) $NaOCH_3$, CH_3OH, 25 °C. (x) $(n\text{-}C_4H_9)_4N^+F^-$, THF. [b] R′ = *cis*-$CH_3(CH_2)_7CH$=$CH(CH_2)_7$.

Results

Ceramides. As shown in Scheme I, L-serine was converted into an oxazoline derivative of sphingosine and its epimer. The L-serine was esterified as previously described,[19] and the amine and hydroxyl groups were then protected by formation of an oxazoline under conditions shown not to cause epimerization,[19] (yield 79% from the ester). The ester group could then be reduced to an aldehyde with diisobutylaluminum hydride (DIBAL-H) at −70 °C. Subsequent reaction with the *trans*-vinylalane (4), readily prepared from 1-pentadecyne, gave the sphingosine oxazoline and its epimer as a 1:1 mixture. These erythro and threo epimers were found to be nicely separable by silica gel flash chromatography[20] with ether–petroleum ether (10:7) as the eluting solvent.

Ceramides were prepared from these oxazolines. Hydrolysis of the oxazoline with aqueous HCl gave the 1-benzoate hydrochloride of sphingosine or the threo epimer which could be acylated selectively at nitrogen to give **7** by using *p*-nitrophenyl oleate in pyridine.[21,22] Treatment

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with sodium methoxide/methanol deprotected the C-1 hydroxyl, giving the corresponding *erythro*- and *threo*-ceramides.

The ^{13}C NMR spectra of these epimers were used for conclusive differentiation. Ceramide was derived from bovine cerebrosides[23] for comparison, and also myristoyl ceramide containing bovine sphingosine was prepared in our laboratory (see supplementary material, Table I). Assignments were based on studies of several cerebrosides, psychosine (galactosylsphingosine), and bovine sphingosine,[1b,c,24] as well as literature data on analogous molecules,[1b,c,24] and are considered unambiguous. All corresponding absorptions are essentially identical for synthetic *erythro*- and *threo*-oleoylceramide (*erythro*-Cer/*threo*-Cer 18:1) and the bovine substances, known to be erythro,[12] except for the sphingosine Cer C-3 and C-4 carbons and the Cer C-2′ of bovine ceramide which is shown to be anomalous by the bovine Cer (14:0) data, possibly because of heterogeneous fatty acid chain types present in the bovine ceramide. The ca. 2.4-ppm difference between erythro and threo epimers at Cer C-3 clearly demonstrates which isomer is which [$\delta_C^{Me_4Si}$ = 74.33 (erythro) and 71.95 (threo)].

Cerebrosides. The ceramide molecules could not be converted to cerebrosides without prior protection of the allylic hydroxyl group at C-3. However, the 1-benzoates **7** could be nicely protected by using *tert*-butyldiphenylsilyl chloride, the resulting substances being stable during subsequent deprotection and glycosylation at C-1, as well as deprotection of the galactose introduced. Glycosylation with α-D-bromotetraacetylgalactose[25] was successful, and removal of the acetyl groups, followed by removal of the *tert*-butyldiphenylsilyl group with tetra-*n*-butylammonium fluoride, afforded pure cerebroside **12a** and its threo epimer **12b**.

As with the ceramides, ^{13}C NMR data differentiate the erythro and threo epimers (see supplementary material, Table II). Bovine Gal-Cer (18:1), synthetic **12a** (erythro), and **12b** (threo) have Cer C-3 at $\delta_C^{Me_4Si}$ = 73.16, 73.17, and 71.44, respectively.

Optical Purity. Possible racemization of the synthetic sphingosine was examined by measurement of the optical rotation of the triacetylsphingosine derivatives. The optical rotation of pure D-*erythro*-triacetylsphingosine is reported as $[\alpha]^{24}_D$ −12.8° (mp 103.5–104 °C),[26] while triacetylsphingosine derived from natural cerebroside gave $[\alpha]^{25}_D$ −11.7° (mp 101–102 °C).[27] Since natural cerebroside contains a few percent of dihydrosphingosine chains, the slightly lower optical rotation seems to be derived essentially entirely from the presence of dihydrotriacetylsphingosine, which has $[\alpha]^{30}_D$ +18.0°,[27] and not from any racemization. This is also consistent with the slightly lower melting point of the natural derivative.

We prepared sphingosine from bovine brain cerebroside[23] and peracetylated it according to the previous method,[27] giving $[\alpha]^{24}_D$ −11.8°. This material was chromatographically pure erythro, as confirmed by ^{13}C NMR. Therefore, any racemization would have had to result from epimerizations at both C-2 and C-3, lending further support to the conclusion that the rotation of natural triacetylsphingosine is accounted for by a small percentage of dihydro form. In turn, these observations support the value −12.8°[26] for optically pure D-*erythro*-triacetylsphingosine.

Triacetyl derivatives were prepared from our synthetic sphingosine and threo epimer. The 1-*O*-benzoate hydrochlorides were N-acetylated by using *p*-nitrophenyl acetate in pyridine and debenzoylated with sodium methoxide/methanol, paralleling the preparation of ceramides shown in Scheme I. Treatment with acetic anhydride in pyridine[27] gave the desired triacetyl derivatives: erythro, $[\alpha]^{24}_D$ −12.9°; threo $[\alpha]^{24}_D$ +8.43°.

These results demonstrate essentially complete optical purity in the synthetic scheme. Absolute confirmation of the optical purity of the cerebroside itself is difficult, and the best method is degradation to form triacetylsphingosine;[23,27] we did not want to commit such large quantities of synthetic cerebrosides to degradation as yet, but intend to do so in due course. However, the operations for conversion of ceramides to cerebrosides are considered highly unlikely to produce any racemization, especially since epimerization at C-2 would be required. Epimerization at the allylic C-3 position cannot affect optical purity, since the diastereomeric cerebroside so formed would be removed chromatographically in purification; none was seen in ^{13}C NMR.

Conclusions. The synthetic scheme developed here produces practical quantities of cerebrosides of high optical purity without the necessity for an optical resolution step during the course of the synthesis. The *erythro*- and *threo*-oxazolines **5** are readily separable at an early stage, i.e., as soon as the second chiral center is established. For purposes of comparison of NMR properties, or for other comparisons including physiological properties, both the erythro and threo series are desirable. The synthesis can be readily expanded to produce a series of cerebrosides that differ in chain length, stereochemistry, or composition of the saccharide unit from the derivatives described above.

Experimental Section[1b]

Materials and Methods. All solvents used were ACS grade. Petroleum ether (bp 31.9–56.1 °C) was used in column chromatographic separations of cerebroside derivatives. Flash column chromatography[20] used EM silica gel 60 (230–400 mesh). These columns could be reused for up to five separations by washing with one bed volume of CH_3OH and then reequilibrating with an equal volume of the eluting solvent.[20] EM silica gel 60 glass-backed plates were used for TLC monitoring of column fractions. The lipid spots were developed by immersion of the plate in an ethanolic solution of phosphomolybdic acid.[28] Methanol and nitromethane were dried by distillation from CaH_2; pyridine and *N*,*N*-dimethylformamide (DMF) were stirred with KOH before distillation from CaH_2. Toluene, hexane, tetrahydrofuran (THF), and ether (diethyl) were dried by being refluxed over metallic sodium with benzophenone indicator and were distilled immediately prior to use. Solvent mixtures are by volume before mixing.

^1H NMR spectra were recorded on a Bruker WM-250 spectrometer using 5-mm tubes. Chemical shifts are given in parts per million relative to internal Me_4Si. IR spectra were recorded on a Perkin-Elmer 735 spectrometer. Optical rotations were measured on a Perkin-Elmer 241 polarimeter using a 1-dm cell.

4-(Carbomethoxy)-2-phenyl-Δ²-oxazoline (2). From L-serine (0.1 mol), the methyl ester hydrochloride was prepared as previously described:[19] yield 92%; mp 133.5–134 °C (lit.[29] mp 134 °C). The hydrochloride (10.9 g, 70 mmol) in 7 mL of H_2O was mixed with a solution of benziminoethyl ether[30,31] (15.6 g, 105

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mmol) in 45 mL of CH_2Cl_2, and the flask was stoppered and shaken for 16 h. Sufficient water was then added to dissolve the precipitated NH_4Cl; the product was extracted with CH_2Cl_2, the pooled extracts were dried over $MgSO_4$, and the solvent was removed by rotary evaporation. The residue was distilled under vacuum: bp 109–110 °C (0.01 torr) [lit.[19] 110–112 °C (0.02 torr)]; yield 11.3 g (55 mmol, 79%); IR (neat) 1740 (ester), 1640 (C=N) cm^{-1}; NMR ($CDCl_3$) δ_H 3.79 (s, 3), 4.57 (dd, 1, J = 10.7, 8.8 Hz), 4.68 (dd, 1, J = 8.4, 8.5 Hz), 4.95 (dd, 1, J = 10.7, 8.2 Hz), 7.36–7.49 (m, 3), 7.96–7.99 (m, 2).

4-Formyl-2-phenyl-Δ²-oxazoline (3). The oxazoline ester **2** (3.1 g, 15 mmol) in 120 mL of dry hexane/toluene (1:3) was cooled to −77 °C (solid CO_2–acetone bath), and diisobutylaluminum hydride (DIBAL-H, Aldrich, 15 mmol) in toluene was added dropwise to the stirring reaction mixture at a rate such that the temperature did not exceed −70 °C. When all the DIBAL-H had been added, the mixture was kept at −70 °C for an additional 3 h, and then 0.5 mL of CH_3OH was added slowly, maintaining the temperature at −70 °C for an additional 30 min. A mixture of 10 mL of ethyl acetate and 40 mL of saturated aqueous sodium potassium tartrate solution was added to the mixture, and the temperature was allowed to rise to ca. 23 °C. The reaction mixture was then transferred to a 2-L separatory funnel and extracted by using additional ethyl acetate and saturated tartrate solution (sufficient to dissolve all of the aluminum salts which precipitated during workup). The ethyl acetate extracts were combined, dried over $MgSO_4$, filtered, and evaporated by rotary evaporation followed by high vacuum to give a yellow oil (2.95 g), shown by TLC in $CHCl_3$–CH_3OH (95:5) to be ca. 90% complete in aldehyde (R_f 0.22) with a trace of unreacted methyl ester (R_f 0.60). This aldehyde was stored in benzene at −20 °C prior to use: IR (neat) 1720 (aldehyde), 1640 (C=N) cm^{-1}. The aldehyde was not sufficiently stable for microanalysis.

***erythro*- and *threo*-4-(1-Hydroxy-2-hexadecenyl)-2-phenyl-Δ²-oxazoline (5).** The *trans*-vinylalane (**4**) resulting from cis addition of DIBAL-H to 1-pentadecyne (30 mmol) was synthesized as previously reported,[16,32,33] the resulting colorless solution being cooled in an ice bath. Then the aldehyde **3** (30 mmol) in 12 mL of ether–toluene (3:1) was added dropwise, maintaining the temperature of the reaction mixture at 5–10 °C. The bath was removed and the mixture stirred for 1 h while warming to ca. 23 °C. The product mixture was then worked up by using the ethyl acetate–tartrate extraction previously described for **3**. The erythro and threo products were separated on a 5-cm-diameter silica gel flash column with ether–petroleum ether (10:7) as the eluting solvent. TLC in the same solvent gave erythro product **5a** (R_f 0.39) and threo product **5b** (R_f 0.23).

Erythro product **5a** was isolated: yielded 3.0 g (26%); mp 89–90 °C; IR (KBr) 1640 (C=N) cm^{-1}; NMR ($CDCl_3$) δ_H 0.88 (t, 3, J = 6.6 Hz), 1.26 (s, 22), 1.62 (m, 2), 2.42 (br s, 1), 4.39 (m, 3), 4.52 (m, 1), 5.44 (dd, 1, J = 15.4, 5.9 Hz), 5.81 (dt, 1, J = 15.4, 7.1 Hz), 7.35–7.50 (m, 3), 7.91 (m, 2).

Anal. Calcd for $C_{25}H_{39}NO_2$: C, 77.92; H, 10.13. Found: C, 77.69; H, 10.08.

Similarly, threo product **5b** was isolated: yield 2.9 g (25%); mp 70–70.5 °C; IR (KBr) 1640 (C=N) cm^{-1}; NMR ($CDCl_3$) δ_H 0.88 (t, 3, J = 6.7 Hz), 1.26 (s, 22), 1.62 (m, 2), 2.21 (br s, 1), 4.37 (m, 3), 4.50 (m, 1), 5.40 (dd, 1, J = 15.3, 6.0 Hz), 5.80 (dt, 1, J = 15.4, 7.1 Hz), 7.36–7.53 (m, 3), 7.91 (m, 2).

Anal. Calcd for $C_{25}H_{39}NO_2$: C, 77.92; H, 10.13. Found: C, 78.20; H, 10.04.

The total yield of erythro plus threo was thus 51%.

***N*-Oleoyl-1-*O*-benzoyl-D-sphingosine (7).** Either oxazoline, **5a** or **5b** (1.15 g, 3 mmol), in 20 mL of THF was stirred during addition of 2.5 mL of 2 N HCl and then for 16 h at 25 °C. The product was isolated by extraction with 100 mL of $CHCl_3$–CH_3OH azeotrope (87:13) and 50 mL of H_2O; the organic layer was dried over $MgSO_4$, filtered, and evaporated to dryness. The hydrochloride residue **6** (1.27 g) gave only one spot on TLC with $CHCl_3$–CH_3OH (95:5) and was dissolved in 4 mL of dry pyridine, to which was added *p*-nitrophenyl oleate (1.17 g, 2.9 mmol) in 6 mL of dry pyridine with stirring. The reaction was allowed to proceed for 16 h at 25 °C, i.e., until the hydrochloride **6** was no longer detectable by TLC, and the solvent was then removed by using a rotary evaporator equipped with a solid CO_2–acetone condenser. The residue was lyophilized from benzene, and the resulting solid was chromatographed on a silica gel flash column (4 cm in diameter) by using ether–petroleum ether (2:1) as the eluting solvent, giving *erythro*-1-*O*-benzoylceramide (**7a**): yield 1.55 g (78%); mp 46–47 °C; IR (KBr) 1720 (ester), 1650 (amide) cm^{-1}; NMR ($CDCl_3$) δ_H 0.88 (t, 6, J = 6.9 Hz), 1.25 (s, 44), 1.55 (m, 2), 1.99 (m, 4), 2.19 (t, 2, J = 7.7 Hz), 3.12 (br s, 1), 4.28–4.60 (m, 4), 5.33 (m, 2), 5.53 (dd, 1, J = 15.1, 6.9 Hz), 5.76 (dt, 1, J = 15.0, 8.0 Hz), 6.11 (d, 1, J = 7.4 Hz), 7.40–8.00 (m, 3), 8.03–8.10 (m, 2).

Anal. Calcd for $C_{43}H_{73}NO_4$: C, 77.36; H, 10.94. Found: C, 77.32; H, 11.15.

Similarly, the threo epimer of 1-*O*-benzoylceramide (**7b**) was isolated: yield 1.56 g (78%); mp 47.5–48.5 °C; IR (KBr) 1720 (ester), 1650 (amide) cm^{-1}; NMR ($CDCl_3$) δ_H 0.88 (t, 6, J = 6.6 Hz), 1.25 (s, 44), 1.59 (m, 2), 2.01 (m, 4), 2.20 (t, 2, J = 7.3 Hz), 2.78 (br s, 1), 4.29–4.54 (m, 4), 5.30–5.36 (m, 2), 5.47 (dd, 1, J = 15.4, 5.9 Hz), 5.76 (dt, 1, J = 15.4, 7.4 Hz), 5.94 (d, 1, J = 8.0 Hz), 7.42–7.61 (m, 3), 8.01–8.05 (m, 2).

Anal. Calcd for $C_{43}H_{73}NO_4$: C, 77.36; H, 10.94. Found: C, 77.17; H, 10.95.

***N*-Oleoyl-D-sphingosine (8).** Either *erythro*-1-*O*-benzoylceramide **7a** or the threo epimer **7b** (0.15 g, 0.22 mmol) was dissolved in 90 volumes of warm CH_3OH; the solution was cooled to 25 °C, 10 volumes of 1 N NaOH was added, and the mixture was stirred for 16 h at 25 °C. When the reaction was complete, TLC ($CHCl_3$–CH_3OH, 95:5) showed only one major spot. The reaction mixture was transferred to a 250-mL separatory funnel with a rinse of 40 mL of $CHCl_3$; 20 mL of H_2O was added, and the extracted organic layer was dried over $MgSO_4$, filtered, and evaporated by rotary evaporation. The residue was lyophilized from benzene and then purified by flash chromatography on silica gel with $CHCl_3$–CH_3OH (97.5:2.5) for elution. Fractions containing pure *erythro*-oleoylsphingosine **8a** were combined, evaporated, and lyophilized from benzene, giving a white powder: yield 0.11 g (92%); mp 89–90 °C; IR (KBr) 1655 (amide) cm^{-1}; ^{13}C NMR presented in Table I of the supplementary material.

Anal. Calcd for $C_{36}H_{69}NO_3$: C, 76.73; H, 12.26. Found: C, 76.51; H, 12.32.

Similarly, the threo epimer of *N*-oleoyl-D-sphingosine (**8b**) was isolated: yield 0.11 g (92%); mp 84–86 °C; IR (KBr) 1660 (amide) cm^{-1}; ^{13}C NMR presented in Table I of the supplementary material.

Anal. Calcd for $C_{36}H_{69}NO_3$: C, 76.73; H, 12.26. Found: C, 76.84; H, 12.28.

***N*-Oleoyl-1-*O*-benzoyl-3-*O*-(*tert*-butyldiphenylsilyl)-D-sphingosine (9).** Either *erythro*-1-*O*-benzoylceramide **7a** or the threo epimer **7b** (667 mg, 1 mmol) and imidazole (102 mg, 1.5 mmol) were dissolved in 2 mL of dry DMF and cooled in an ice bath, and then *tert*-butylchlorodiphenylsilane (401 mg, 1.5 mmol) was added followed by stirring at 0 °C for 10 min, allowing to warm to 25 °C, and heating at 50 °C for 4 h. The product was extracted with ether (ca. 70 mL) and 5% aqueous HCl (ca. 30 mL), and the ether layer was dried over $MgSO_4$ and then evaporated on a rotary evaporator. The resulting crude product was purified by flash column chromatography on a 4-cm-diameter column packed with silica gel with ether–petroleum ether (4:1) for elution. The eluted fractions were monitored by TLC using the same solvent. Fractions containing pure erythro product **9a** were combined and evaporated, giving an oil: yield 860 mg (95%); $[\alpha]^{24}_D$ −5.9° ($CHCl_3$); IR (neat) 1740 (ester), 1650 (amide) cm^{-1}; NMR ($CDCl_3$) δ_H 0.88 (t, 6, J = 6.6 Hz), 1.06 (s, 9), 1.26 (s, 44), 1.81–1.88 (m, 4), 1.94–2.02 (m, 4), 4.32–4.47 (m, 3), 4.56–4.63 (dd, 1, J = 11.4, 7.7 Hz), 5.28–5.46 (m, 4), 5.55 (d, 1, J = 9.5 Hz), 7.34–7.43 (m, 9), 7.61–7.97 (m, 6).

Anal. Calcd for $C_{59}H_{91}NO_4Si$: C, 77.89; H, 10.00. Found: C, 77.61; H, 10.27.

Similarly, the threo epimer **9b** was isolated as an oil: yield 850 mg (94%); $[\alpha]^{24}_D$ −0.12° ($CHCl_3$); IR (neat) 1730 (ester), 1650 (amide) cm^{-1}; NMR ($CDCl_3$) δ_H 0.88 (t, 6, J = 6.6 Hz), 1.07 (s,

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9), 1.26 (s, 44), 1.78–1.90 (m, 4), 1.99 (m, 4), 4.29–4.43 (m, 4), 5.26–5.49 (m, 4), 5.72 (d, 1, J = 9.0 Hz), 7.30–7.44 (m, 9), 7.60–7.93 (m, 6).

Anal. Calcd for $C_{59}H_{91}NO_4Si$: C, 77.89; H, 10.00. Found: C, 77.74; H, 10.25.

***N*-Oleoyl-3-*O*-(*tert*-butyldiphenylsilyl)-D-sphingosine (10).** Either **9a** or **9b** (680 mg, 0.75 mmol) was dissolved in 20 mL of dry CH_3OH, and a solution of sodium metal (10 mg) in 2 mL of dry CH_3OH was slowly added with stirring. The solution was stirred for 16 h at 25 °C, and when the starting ester **9** was no longer detectable by TLC [ether–petroleum ether (2:1)], the product **10** was extracted by addition of $CHCl_3$ (ca. 100 mL) and H_2O (ca. 25 mL). The $CHCl_3$ layer was dried over $MgSO_4$, filtered, and evaporated by rotary evaporation, and the resulting oil was purified on a silica gel flash chromatography column (4 cm in diameter) with ether–petroleum ether (2:1) as the eluting solvent. Fractions containing pure erythro product **10a** were combined and evaporated, giving a clear oil: yield 580 mg (96%); $[\alpha]^{24}_D$ −13.7° ($CHCl_3$); IR (neat) 1660 (amide) cm^{-1}; NMR ($CDCl_3$) δ_H 0.88 (t, 6, J = 6.6 Hz), 1.07 (s, 9), 1.26 (s, 44), 1.98 (m, 8), 3.17 (br s, 1), 3.60 (m, 1), 3.90 (m, 2), 4.34 (m, 1), 5.29–5.41 (m, 4), 5.94 (d, 1, J = 7.3 Hz), 7.32–7.45 (m, 6), 7.61–7.66 (m, 4).

Anal. Calcd for $C_{52}H_{87}NO_3Si$: C, 77.90; H, 10.86. Found: C, 77.83; H, 10.91.

Similarly, the threo epimer **10b** was isolated as an oil: yield 570 mg (95%); $[\alpha]^{24}_D$ +11.8° ($CHCl_3$); IR (neat) 1660 (amide) cm^{-1}; NMR ($CDCl_3$) δ_H 0.88 (t, 6, J = 6.6 Hz), 1.07 (s, 9), 1.26 (s, 44), 1.99 (m, 8), 2.77 (br s, 1), 3.59 (m, 1), 3.92 (m, 2), 4.27 (m, 1), 5.25–5.39 (m, 4), 5.93 (d, 1, J = 7.4 Hz), 7.33–7.45 (m, 6), 7.61–7.68 (m, 4).

Anal. Calcd for $C_{52}H_{87}NO_3Si$: C, 77.90; H, 10.86. Found: C, 77.71; H, 10.80.

***N*-Oleoyl-1-*O*-β-D-galactosyl-3-*O*-(*tert*-butyldiphenylsilyl)-D-sphingosine (11).** The protected ceramide **10**, either erythro or threo, was glycosylated by the method of Helferich and Weis[25] as modified by Shapiro et al.[17] Either **10a** or **10b** (0.4 g, 0.5 mmol) reacted with α-D-bromotetraacetylgalactose in the presence of $Hg(CN)_2$ in dry benzene–nitromethane (1:1) to give a crude product which was deacetylated with sodium methoxide in methanol.[17,25] The corresponding 3-*O*-*tert*-butyldiphenylsilyl cerebroside, **11a** or **11b**, was extracted by addition of $CHCl_3$ (ca. 100 mL) and H_2O (ca. 25 mL); then the $CHCl_3$ layer was dried over $MgSO_4$, filtered, and evaporated. TLC of the residue with $CHCl_3$–CH_3OH (95:5) showed some unreacted **10**, which was separated from **11** by flash chromatography on a 4-cm-diameter silica gel column with $CHCl_3$-CH_3OH (95:5) for elution. Recovered, unreacted starting material (ca. 0.12 g) was recycled in the reaction scheme to improve the yield. Erythro isomer **11a** (0.24 g, 0.25 mmol, 50%) was obtained after the reaction was performed once, and the yield was increased to 0.31 g (0.32 mmol, 64%) after recycling: mp 42–43 °C; IR (KBr) 1660 (amide) cm^{-1}; NMR ($CDCl_3$) δ_H 0.88 (t, 6, J = 6.3 Hz), 1.06 (s, 9), 1.26 (s, 44), 1.83–2.03 (m, 8), 2.45 (br s, 1), 2.78 (br s, 1), 2.89 (br s, 1), 3.47–3.58 (m, 4), 3.79–3.84 (m, 2), 3.93–4.00 (m, 2), 4.08–4.13 (br s, 1), 4.18 (d, 1, J = 7.3 Hz), 4.23–4.27 (m, 1), 4.66 (br s, 1), 5.30–5.43 (m, 4), 5.66 (d, 1, J = 7.5 Hz), 7.33–7.46 (m, 6), 7.60–7.67 (m, 4).

Anal. Calcd for $C_{58}H_{97}NO_8Si{\cdot}H_2O$: C, 70.95; H, 10.09. Found: C, 70.96; H, 10.12.

Similarly, the threo epimer **11b** was isolated: yield 0.29 g (60%, after recycling of unreacted **10b**); mp 41–42 °C; IR (KBr) 1660 (amide) cm^{-1}; NMR ($CDCl_3$) δ_H 0.88 (t, 6, J = 6.4 Hz), 1.07 (s, 9), 1.26 (s, 44), 1.84–2.05 (m, 8), 2.45 (br s, 1), 2.78 (br s, 1), 2.86 (br s, 1), 3.45–3.60 (m, 4), 3.76–3.84 (m, 2), 3.95–4.03 (m, 2), 4.10–4.19 (m, 3), 4.65 (br s, 1), 5.25–5.39 (m, 4), 5.65 (d, 1, J = 7.4 Hz), 7.30–7.45 (m, 6), 7.61–7.68 (m, 4).

Anal. Calcd for $C_{58}H_{97}NO_8Si{\cdot}H_2O$: C, 70.95; H, 10.09. Found: C, 71.08; H, 10.14.

***N*-Oleoyl-1-*O*-β-D-galactosyl-D-sphingosine (*erythro*- and *threo*-12).** Tetra-*n*-butylammonium fluoride (0.5 mL of a 1 M solution in THF) was added to a solution of **11a** or **11b** (0.24 g, 0.25 mmol) in 0.5 mL of THF, and the resulting solution was stirred for 3 h, until **11** was no longer detectable by TLC ($CHCl_3$–CH_3OH, 87:13). To extract the product, 50 mL of $CHCl_3$–CH_3OH azeotrope (87:13) and 20 mL of 5% aqueous HCl were added to the reaction mixture in a separatory funnel, and the cerebroside was extracted into the organic layer. The procedure was repeated twice more with 30-mL aliquots of $CHCl_3$–CH_3OH azeotrope, and the combined organic layers were dried over $MgSO_4$, filtered, and evaporated to dryness. The cerebroside was purified on a 4-cm-diameter silica gel flash column with $CHCl_3$–CH_3OH (84:16) for elution. The fractions containing cerebrosides were combined, evaporated, and then lyophilized from benzene, giving *erythro*-oleoylcerebroside **12a** as a white powder: yield 0.16 g (88%); IR (KBr) 1650 (amide) cm^{-1}; ^{13}C NMR presented in Table II of the supplementary material.

Anal. Calcd for $C_{42}H_{79}NO_8{\cdot}0.5H_2O$: C, 68.67; H, 10.89. Found: C, 68.84; H, 10.60.

Similarly, the threo epimer of oleoylcerebroside **12b** was isolated: yield 0.16 g (88%); IR (KBr) 1650 (amide) cm^{-1}; ^{13}C NMR presented in Table II of the supplementary material.

Anal. Calcd for $C_{42}H_{79}NO_8{\cdot}H_2O$: C, 67.83; H, 10.90. Found: C, 68.10; H, 10.91.

It will be noted that water of hydration differs slightly in these very hygroscopic compounds even when samples are prepared under closely similar conditions.

***N,O,O*-Triacetylsphingosine Derivatives.** Sphingosine derived from bovine cerebrosides[23] was peracetylated according to the procedure of Carter et al.[27] The $CHCl_3$-extracted product was lyophilized from benzene, and the resulting powder was purified on a 3-cm-diameter silica gel flash column with ether–petroleum ether (4:1) for elution, the effluent being monitored by TLC using the same solvent ratio. Fractions containing pure triacetylsphingosine were combined, evaporated, and lyophilized from benzene: yield 77%; mp 101–101.5 °C (lit.[27] mp 101–102 °C); $[\alpha]^{24}_D$ −11.8° ($CHCl_3$) [lit.[27] $[\alpha]^{25}_D$ −11.7° ($CHCl_3$)]; IR (KBr) 1740 (ester), 1660 (amide) cm^{-1}; NMR ($CDCl_3$) δ_H 0.88 (t, 3, J = 6.4 Hz), 1.26 (s, 22), 1.98–2.13 (m, 11; contains 3 s, δ 1.98, 2.05, 2.07, each ca. 3 H), 4.04 (dd, 1, J = 11.6, 4.0 Hz), 4.30 (dd, 1, J = 11.6, 5.8 Hz), 4.39–4.48 (m, 1), 5.28 (dd, 1, J = 7.35, 5.9 Hz), 5.39 (dd, 1, J = 15.2, 7.35 Hz), 5.68 (d, 1, J = 8.8 Hz), 5.79 (dt, 1, J = 15.1, 6.6 Hz). The ^{13}C NMR spectrum was similar to that published.[34]

The synthetic *erythro*- and *threo*-triacetylsphingosines were prepared from the corresponding oxazolines **5** via the 1-benzoate hydrochlorides **6** using *p*-nitrophenyl acetate in pyridine and following the procedure described above for synthesis of oleoylsphingosine **8a** and its epimer **8b**. After the 16-h reaction period, the crude *N*-acetyl-1-*O*-benzoylsphingosine (or its epimer) was extracted by using $CHCl_3$ (ca. 80 mL) and 5% aqueous HCl (ca. 20 mL), and the $CHCl_3$ layer was dried over $MgSO_4$, filtered, and evaporated. The erythro product was purified on a 4-cm-diameter silica gel flash column with ether–petroleum ether (3:1) for elution; product-containing fractions were combined and evaporated, and the residue was lyophilized from benzene: 84% yield from 0.5 g of **5a**; mp 51–52 °C; IR (KBr) 1730 (ester), 1650 (amide) cm^{-1}; NMR ($CDCl_3$) δ_H 0.88 (t, 3, J = 6.6 Hz), 1.25 (s, 22), 1.90–2.07 (m, 5; contains s, δ 2.00, 3 H), 3.01 (br s, 1), 4.30 (m, 1), 4.40–4.44 (m, 2), 4.50–4.58 (m, 1), 5.53 (dd, 1, J = 15.4, 6.6 Hz), 5.78 (dt, 1, J = 15.4, 6.3 Hz), 6.15 (d, 1, J = 8.1 Hz), 7.47–7.61 (m, 3), 8.00–8.03 (m, 2).

Anal. Calcd for $C_{27}H_{43}NO_4$: C, 72.81; H, 9.66. Found: 72.68; H, 9.86.

Similarly, the threo epimer of *N*-acetyl-1-*O*-benzoylsphingosine was prepared from **5b**: yield 85%; mp 47–48 °C; IR (KBr) 1740 (ester), 1660 (amide) cm^{-1}; NMR ($CDCl_3$) δ_H 0.88 (t, 3, J = 6.6 Hz), 1.25 (s, 22), 1.99–2.04 (m, 5; contains s, δ 2.02, 3 H), 2.77 (br s, 1), 4.28–4.41 (m, 3), 4.52–4.56 (m, 1), 5.48 (dd, 1, J = 15.4, 5.9 Hz), 5.77 (dt, 1, J = 15.4, 6.6 Hz), 6.00 (d, 1, J = 7.4 Hz), 7.43–7.62 (m, 3), 8.01–8.05 (m, 2).

Anal. Calcd for $C_{27}H_{43}NO_4$: C, 72.81; H, 9.66. Found: C, 72.64; H, 9.83.

These products were debenzoylated as described in the preparation of **8** above, except that the dried ($MgSO_4$) $CHCl_3$ extract was filtered and evaporated first by rotary evaporation and then under high vacuum for 4 h. This product was then acetylated (without chromatographic purification) by addition of 0.8 mL of acetic anhydride–dry pyridine (1:1) at 25 °C (reaction time 16 h). The *erythro*-triacetylsphingosine was purified in the same way as bovine triacetylsphingosine (above): 85% yield from 0.42

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g of benzoylated starting material; mp 103.5–104.5 °C; $[\alpha]^{24}_D$ −12.9° ($CHCl_3$) [lit.[26] $[\alpha]^{24}_D$ −12.8°]; IR (KBr) 1740 (ester), 1650 (amide) cm^{-1}; NMR ($CDCl_3$) δ_H 0.88 (t, 3, J = 6.3 Hz), 1.26 (s, 22), 1.98–2.14 (m, 11; contains 3 s, δ 1.98, 2.06, 2.07, each ca. 3 H), 4.04 (dd, 1, J = 11.8, 3.7 Hz), 4.30 (dd, 1, J = 11.4, 5.8 Hz), 4.38–4.48 (m, 1), 5.28 (dd, 1, J = 7.35, 6.62 Hz), 5.39 (dd, 1, J = 15.4, 7.35 Hz), 5.68 (d, 1, J = 8.8 Hz), 5.79 (dt, 1, J = 15.1, 6.6 Hz).

Anal. Calcd for $C_{24}H_{43}NO_5$: C, 67.76; H, 10.12. Found: C, 67.66; H, 10.27.

The threo epimer of triacetylsphingosine was prepared in the same way, yield 84%, mp 43–43.5 °C, $[\alpha]^{24}_D$ +8.43° ($CHCl_3$); IR (KBr) 1740 (ester), 1660 (amide) cm^{-1}; NMR ($CDCl_3$) δ_H 0.88 (t, 3, J = 6.6 Hz), 1.25 (s, 22), 2.00–2.13 (m, 11; contains 3 s, δ 2.00, 2.07, 2.08, each ca. 3 H), 4.08 (m, 2), 4.35–4.45 (m, 1), 5.33–5.44 (m, 2), 5.67 (d, 1, J = 9.6 Hz), 5.78 (dt, 1, J = 14.7, 6.6 Hz). The multiplet at 4.08 ppm is the splitting pattern of the diastereotopic sphingosine C-1 protons which are coupled to the C-2 proton with J = 5.9 and 5.1 Hz and differ in chemical shift by 1.8 Hz.

Anal. Calcd for $C_{24}H_{43}NO_5$: C, 67.76; H, 10.12. Found: C, 67.99; H, 10.27.

Acknowledgment. Support of this work by the National Institutes of Health and assistance in the purchase of the NMR spectrometers by National Science Foundation Departmental Instrument grants are gratefully acknowledged. We also thank Dr. W. B. Wise for his technical assistance, Ms. D. Ward and Ms. T. Denial for their experimental assistance, and Ms. H. Brachowski for preparing the manuscript.

Registry No. 2, 78715-83-0; **3**, 78715-84-1; **4**, 41765-25-7; **5a**, 78715-85-2; **5b**, 78739-31-8; *erythro*-**6**, 78739-32-9; *threo*-**6**, 78739-33-0; **7a**, 78715-86-3; **7b**, 78779-90-5; **8a**, 5966-28-9; **8b**, 78779-91-6; **9a**, 78715-87-4; **9b**, 78779-92-7; **10a**, 78715-88-5; **10b**, 78779-93-8; **11a**, 78715-89-6; **11b**, 78779-94-9; **12a**, 73039-25-5; **12b**, 78779-95-0; L-serine, 56-45-1; L-serine methyl ester hydrochloride, 5680-80-8; *p*-nitrophenyl oleate, 17363-90-5; *tert*-butychlorodiphenylsilane, 58479-61-1; α-D-bromotetraacetylgalactose, 3068-32-4; *erythro*-*N*-acetyl-1-*O*-benzoylsphingosine, 78715-90-9; *threo*-*N*-acetyl-1-*O*-benzoylsphingosine, 78715-91-0; *erythro*-triacetylsphingosine, 2482-37-3; *threo*-triacetylsphingosine, 78779-96-1.

Supplementary Material Available: Carbon-13 NMR assignments for *erythro*- and *threo*-ceramides and galactosylceramides (3 pages). Ordering information is given on any current masthead page.

Novel Maytansinoid Tumor Inhibitors from *Trewia nudiflora*: Trewiasine, Dehydrotrewiasine, and Demethyltrewiasine[1]

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Received April 6, 1981

Alcoholic extracts of *Trewia nudiflora* seed have yielded several new maytansinoid tumor inhibitors that are exceptionally active in the PS, B1, and KB systems. These include trewiasine (**1**), dehydrotrewiasine (**2**), and demethyltrewiasine (**3**), which were characterized by NMR, mass spectral, and chemical correlation with other known maytansinoids. Two additional maytansinoids, trenudine (**10**) and treflorine (**11**), have been partially characterized. The *Trewia* ansa macrolides differ from others in this series in that they possess an additional methoxy group at C-15; and, with the exception of **1**, they contain substituents at C-3 which differ from other known maytansinoids. Detailed ^{13}C NMR assignments for maytansine and some related maytansinoids are presented.

In a search for tumor inhibitors of plant origin, we found that ethanolic extracts of *Trewia nudiflora* L. (Euphorbiaceae) seed[3] showed significant activity in vitro against human carcinoma of the nasopharynx (KB) and in vivo against P388 lymphocytic leukemia (PS).[4] *Trewia* extracts also inhibit initiation and growth of crown-gall tumors on potato disks.[5] Previous studies by other workers have demonstrated that *T. nudiflora* seed contains a highly unusual glyceride oil,[6] several novel pyridone alkaloids,[7-9] and an inhibitor of protein synthesis.[10] In this paper, we report the isolation and structural elucidation of three new maytansinoid tumor inhibitors: trewiasine (**1**), dehydrotrewiasine (**2**), and demethyltrewiasine (**3**). In addition, we describe the partial characterization of two more new maytansinoids, trenudine (**10**) and treflorine (**11**). Maytansinoids, including maytansine (**4**), colubrinol (**5**), and maytanbutine (**6**), have previously been reported as constituents of *Maytenus* and *Putterlickia* spp. (Celastraceae),[11] *Colubrina texensis* (Rhamnaceae),[12] and fermentation broths of a *Nocardia* sp.[13] These compounds are

(1) Presented in part at the 180th National Meeting of the American Chemical Society and the Second Chemical Congress of the North American Continent, Las Vegas, NV, Aug 24–29, 1980.

(2) The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

(3) We thank Dr. James Duke, USDA, Beltsville, MD, for supplying seed material in accordance with the program developed by the National Cancer Institute. A 27.2-kg recollection of *Trewia nudiflora* L. seed was purchased in 1978 from Pratap Nursery, Dehra Dun, India.

(4) Cytotoxic and antitumor activities were assayed under the auspices of the National Cancer Institute by the procedures described by: Geran, R. I.; Greenberg, N. H.; MacDonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep., Part 3*, **1972**, *3*, 1. Trewiasine (**1**) was cytotoxic at the 2.0×10^{-4} μg/mL level. In addition, **1** gave T/C values of 126–168 in the dosage range 1.0–31.0 μg/kg against PS and T/C values of 165–207 in the dosage range 4.0–32.0 μg/kg against B1.

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