Synthesis and Biological Activity of the Isomers and Analogs of 
\((4E,8E,2S,3R,2'R)\)-N-2'-Hydroxyhexadecanoyl-9-methyl-
4,8-sphingadienine, the Ceramide Portion of the Fruiting-
inducing Cerebroside in a Basidiomycete

_Schizophyllum commune_

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The title compounds were synthesized by employing 2-aminohexadecanoic acid and serine as the chiral sources, and served for an assay of fruiting-inducing activity on _Schizophyllum commune_. The structure-bioactivity relationship among closely related synthetic ceramides is discussed.

In 1982, some cerebroside in mycelia of _Schizophyllum commune_ (Japanese name: Suehiro take) were found by Kawai and Ikeda to stimulate its own fruiting body formation.1) One of the active principles was identified as \((4E,8E,2S,3R,2'R)\)-N-2'-hydroxyhexadecanoyl-1-\(\beta\)-D-glucopyranosyl-9-methyl-4,8-sphingadienine 22) (Fig. 1), which had previously been isolated from a sea anemone (_Metridium senile_) by Karlsson et al.3) Such a minute amount of 2 as 0.1\(\mu\)g induced the fruiting body formation of _S. commune_, and the corresponding ceramide 1a lacking the sugar portion was also as active as 2.2) Very recently, we reported the synthesis of the ceramide 1a and cerebroside 2,4~6) and established the structure and stereochemistry of the natural 2. The remarkable bioactivity of 1a prompted us to study the structure–bioactivity relationship among closely related synthetic ceramides. Herein are described the syntheses of eight stereoisomers as concerned with three asymmetric carbons (C-2,3, and 2'), the (8Z)-
geometrical isomer and the 2'-deoxy analog of the ceramide 1a. The relationships between the structure of these synthetic ceramides and their fruiting-inducing activity against _S. commune_ are also discussed.

**Synthesis of the optically active sphingadienine derivatives 16a~16d, 17a and 17b**

We previously reported the synthesis of 16a and 16b from the (S)-serine (S)-3a via (S)-5.6) In the same manner as described for the (S)-serine series6,7) conversion of the (R)-serine (R)-3a to its methyl ester hydrochloride was
followed by its treatment with benzimino ethyl ether to give the phenyloxazoline ester (R)-4 (Fig. 2). (R)-4 was reduced with DIBAL-H to give an unstable aldehyde (R)-5, which had to be used immediately in the next step due to its instability. The (Z)-alkenylalane 15 was prepared by the same procedure as described for the synthesis of the (£)-alkenylalane 8 from 6. The (£)-alcohol 9a5) was converted to a bromide 10 in the conventional manner via a tosylate 9b. The bromide 10 was converted to a nitrile 11, whose reduction with DIBAL-H furnished an aldehyde 12. Treatment of 12 with triphenylphosphine and carbon tetrabromide according to Corey8) yielded a dibromodiene 13. This gave the alkyne 14 when treated with n-butyllithium. The addition of DIBAL-H to 14 by the established procedure6,7,9,10,11) afforded the desired alkenylalane 15. Alkenylation of (R)-5 with the (£)-alkenylalane 8 gave 16c (21.4% from 7), mp 58.5~59.0°C, [α]D22 +6.6° (CHCl3) and 16d (21.3%), oil, [α]D22 +27.6° (CHCl3)).

**Synthesis of (S)-α-hydroxyhexadecanoic acid**

The (S)-α-hydroxy acid derivatives (S)-19a~19d were synthesized from the (S)-α-amino acid (S)-18 in the same manner as reported previously for the synthesis of the (£)-α-hydroxy acid derivative (£)-19d4~6) (Fig. 3). Deamination of (S)-184'5) was followed by esterification of the resulting crude α-hydroxy acid to give (S)-19a. The optical purity of (S)-19a was estimated to be 93% e.e. by the HPLC analysis of its (£)-α-methoxy-α-trifluoromethylphenylacetate (MTPA ester).12) Alkaline hydrolysis of (S)-19a was followed by recrystallization of the product to give (S)-19b, mp 92~93°C, [α]D22 +3.2° (CHCl3) [lit.13) mp 93.3~93.6°C, [α]D22 +2.7° (CHCl3)]. This was acetylated to give (S)-19c, mp 61~62°C [α]D22 -10.5° (CHCl3)]. Activation of the carboxyl group of (S)-19c was effected by treating (S)-19c with p-nitrophenyl trifluoroacetate in
Synthesis of the Ceramides

The ceramides were synthesized in the following manner (Fig. 3). Treatment of the oxazolines, 16a ~ 16d and 17a, with dil. hydrochloric acid afforded the corresponding 1-O-benzyloxysphingadienine hydrochlorides 21a ~ 21d and 22. These were acylated with (R) or (S)-19d in pyridine to achieve selective N-acylation.6,7 The resulting 1-O-benzyloxysphingadienine N-acylceramides, 23a ~ 23h and 24, were treated with sodium hydroxide to remove both the acetyl and benzoyl groups to give the corresponding ceramides 1a ~ 1h and 25. The 2'-deoxy ceramide 29 was also synthesized to investigate the necessity of the hydroxy group at C-2' for bioactivity. The p-nitrophenyl palmitate 27 was prepared from the palmitic acid 26. The 2'-deoxy ceramide 29 was synthesized from 16a and 27 via 28 in the same manner as already described for the synthesis of 1a ~ 1h. The absolute configurations of 16a and 16b had already been determined by our previous studies.5,6 The enantiomers 16c and 16d were identified with 16a and 16b by 1H-NMR and IR spectra, respectively. The (Z) isomers 17a and 17b were converted to the N-acylsphingadienines 30a and 30b for 13C-
Table I. Synthetic Ceramides and Their Fruiting-inducing Activity against S. commune

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>Geometry at C-8 double bond</th>
<th>Absolute configuration</th>
<th>[α]D (CHCl₃)</th>
<th>Specific activitya (units/mg)</th>
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<td>R</td>
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Natural cerebroside 2

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<th>Compound</th>
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<th>Absolute configuration</th>
<th>[α]D (CHCl₃)</th>
<th>Specific activitya (units/mg)</th>
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NMR measurements. It was known that the ¹³C-NMR data could differentiate the erythro and threo-ceramides; the downfield shift of the C-3 carbon has been observed in 2,3-erythro-ceramide as compared with the 2,3-threo-isomer. For example, erythro and threo-N-oleoy-D-sphingosine have a C-3 carbon at δ⁰⁻MeSi = 74.33 and 71.95, respectively. In our case, the C-3 carbon appeared at δ⁰⁻MeSi = 74.0 (30a, erythro) and 71.8 (30b, threo). Therefore, the stereochemistry of all the synthetic ceramides was determined.

**Fruiting-inducing activity of the synthetic ceramides**

The ceramides prepared in the course of this study were used for an assay of fruiting-inducing activity on S. commune and the results are summarized in Table I. The results provide some important insights into the absolute stereochemistry–activity relationship. The ceramide 1a having a (2S,3R,2'R)-configuration (natural form) was as active as the natural cerebroside 2, and such a minute amount of 1a as 0.1 μg induced the fruiting body formation (12000 units/mg). The other stereoisomers 1b~1h were less active (400~1500 units/mg), the (2S,3R,2'R)-configuration being required for high activity. The 2’-deoxy-ceramide 29 showed a quarter the activity (3000 units/mg) of the 2'R-ceramide 1a, and the 2'S-ceramide 1d was much less active (400 units/mg). This fact indicates that the 2’-hydroxyl group in the ceramide 1a may not be essential for the activity. The introduction of a hydroxyl group at C-2’ with an R-configuration enhances the activity. In contrast, the activity is reduced by a 2'S-hydroxyl group. On the other hand, the activity of the 8Z-isomer 25 equals that of the 8E-ceramide 1a, the activity not being affected by the geometrical configuration at the C-8 double bond. These results suggest that the absolute configurations at C-2,3 and 2’ are important for the activity, and may affect the suitable fit of the ceramide molecule to the critical receptor site on S. commune.

**EXPERIMENTAL**

Melting points (mp) are uncorrected. IR spectra were recorded on a Jasco IRA-102 spectrometer, and ¹H-NMR spectra were recorded on a Hitachi R-24A spectrometer (60 MHz). ¹³C-NMR spectra were recorded on a JEOL JNM FX-100 spectrometer (25 MHz), and optical rotations were measured on a Jasco DIP-140 polarimeter.
(R)-Serine methyl ester hydrochloride (R)-3b. Hydrogen chloride gas was briskly bubbled into a solution of (R)-3a (38.0 g, 0.36 mol) in dry MeOH (400 ml) until the solution became hot (spontaneous refluxing). The solution was left to stand for 14 hr at room temperature, before the MeOH was removed in vacuo. The residue was triturated with ether (60 ml). The solid (R)-3b was collected on a filter, washed with ether and dried in vacuo. Recrystallization from MeOH-ether (1:3) gave 51.2 g (90.9%) of (R)-3b. mp 163~164°C. [α]D -3.44° (c=4.00, MeOH). Its IR spectrum was identical with that of (S)-3b.

(R)-4-Methoxy carbonyl-2-phenyl-1,3-oxazolin-2-ene (R)-4. A solution of PhC(=NH)OEt (64.3 g, 0.43 mol) in CH₂Cl₂ (120 ml) was added to a solution of (R)-3b (37.0 g, 0.24 mol) in water (22 ml), the mixture being vigorously stirred for 24 hr at room temperature. It was then filtered, and the filtrate was diluted with CH₂Cl₂ (100 ml) and water (50 ml). The organic solution was separated, dried (MgSO₄) and concentrated in vacuo. The residue was distilled to give 39.9 g (75.3%) of (R)-4, bp 150-155°C (0.09 mmHg). [α]D -1.19° (c= 1.13, CHCl₃). Its NMR spectra were identical with those of (S)-4.

(R)-4-Formyl-2-phenyl-1,3-oxazolin-2-ene (R)-5. A solution of PhC(=NH)OEt (94.6 g, 0.57 mol) in toluene (30 ml) and rc-hexane (14 ml) was added dropwise to a stirred and cooled solution of (R)-4 (1.5 g, 100%) in toluene (40 ml) and rc-hexane (20 ml). The mixture was stirred for 1 hr at -60°C and for 3 hr at room temperature. The excess reagent was quenched by the addition of HClO₄ (1 ml). After stirring for 30 min, the mixture was poured into saturated NH₄Cl aq. (200 ml). The mixture was partitioned between EtOAc (500 ml) and a saturated aqueous solution of Na₂K tartrate. The organic solution was dried (MgSO₄) and concentrated in vacuo to give 1.2 g (quantitative) of (R)-5 as a crude yellow oil. This was employed in the next step without further purification.

(Z)-5-Methyl-4-tetradecenecarboxylic acid 10. A mixture of 10 (2.1 g, 7.6 mmol) and KCN (880 mg, 13.5 mmol) in DMF (10 ml) and water (3 ml) was stirred at 70°C for 24 hr. It was then poured into an ice-water mixture (100 ml) and extracted with ether (200 ml). The ether solution was washed with water, dried (MgSO₄) and concentrated in vacuo. The residue was chromatographed over SiO₂. Elution with n-hexane gave 2.30 g (85.5%) of 10 as an oil. nD 1.4670. IR νmax cm⁻¹: 1660 (m). NMR δD ppm: 0.87 (3H, deformed t, J=6 Hz), 1.27 (14H, br.s), 1.74 (3H, s), 1.85~2.20 (2H, m), 2.54 (2H, t, J=6 Hz), 3.26 (2H, t, J=7 Hz). 51.2 g (90.9%) of (R)-3b. mp 163~164°C. [α]D -3.44° (c=4.00, MeOH). Its IR spectrum was identical with that of (S)-3b.

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1.80~2.20 (6H, m), 4.85~5.25 (1H, m), 6.40 (1H, t, J = 6 Hz). Anal. Found: C, 50.56; H, 7.30. Calcd. for \( \text{C}_{14} \text{H}_{28} \text{Br}_{2} \): C, 50.54; H, 7.42%. 

(Z)-6-Methyl-5-pentadecene-1-yn e 14. A solution of n-BuLi in n-hexane (1.5 m, 5.6 ml, 8.4 mmol) was added dropwise to a stirred and cooled solution of 13 (1.4 g, 3.6 mmol) in THF (20 ml) at ~70°C under Ar. The mixture was stirred for 1 hr at ~70°C and for 1.5 hr at room temperature, before being poured into an ice-water mixture (50 ml) and extracted with n-hexane. The hexane solution was washed with water, dried (Na\(_2\)SO\(_4\)) and concentrated in vacuo. The residue was chromatographed over SiO\(_2\). Elution with n-hexane gave 645 mg (79.5%) of 14 as an oil. \( n_\text{D}^25 \) 1.4534. IR \( \nu_{\text{max}} \) cm\(^{-1}\): 3400 (s), 3340 (s), 2130 (w). MNR \( \delta_{\text{CDCl}_3} \) ppm: 0.88 (3H, deformed t, \( J = 6 \) Hz), 1.27 (14H, br.s), 1.69 (3H, s), 1.85~2.10 (3H, m), 2.10~2.35 (4H, m), 5.00~5.40 (1H, m). Anal. Found: C, 86.89; H, 12.76. Calcd. for \( \text{C}_{14} \text{H}_{28} \text{Br}_{2} \): C, 87.19; H, 12.81%. 

\( ^{(2'\ E,6'E,4'R,1'S)}-4-(1'-\text{Hydroxy}-7'-\text{methyl}-2',6'-\text{hexadecadienyl})-2\text{-phenyl}-1,3\text{-oxazolin-2-ene} \) 16c and its \( (1'S)\)-isomer 17b. These were prepared by the same procedure as already described. 14 (600 mg, 2.73 mmol) was converted to 15. \( (S)-5 \) (700 mg, 3.40 mmol)\(^{8} \) and 15 yielded a mixture of 17a and 17b. After chromatography on SiO\(_2\) and elution with n-hexane–ether (3:1), the first eluting 17a was isolated in a 24.4% yield (264 mg) from 14.

\( (2'\ E,6'E,4'R,1'S)-4-(1'-\text{Hydroxy}-7'-\text{methyl}-2',6'-\text{hexadecadienyl})-2\text{-phenyl}-1,3\text{-oxazolin-2-ene} \) 16c and its \( (1'S)\)-isomer 17b. These were prepared by the same procedure as already described. 14 (600 mg, 2.73 mmol) was converted to 15. \( (S)-5 \) (700 mg, 3.40 mmol)\(^{8} \) and 15 yielded a mixture of 17a and 17b. After chromatography on SiO\(_2\) and elution with n-hexane–ether (3:1), the first eluting 17a was isolated in a 24.4% yield (264 mg) from 14.

Methyl \( (S)-2\text{-hydroxyhexadecanoate} \) (S)-19a. \( (S)-2\text{-Aminohexadecanoic acid} \) (5.9 g, 21.7 mmol)\(^{5,6} \) was dissolved in 2\text{n}-H\(_2\)SO\(_4\) (40 ml) by heating at 80°C. To this vigorously stirred solution was added a solution of Na\(_2\)SO\(_4\) (3.2 g, 46.4 mmol) in water (34 ml) for 2 hr at 80°C. The mixture was stirred for 2 hr at room temperature, before the mixture was extracted with ether (300 ml). The ether solution was washed with brine and concentrated in vacuo. The residue was added C\(_6\)H\(_6\) (60 ml), MeOH (60 ml) and conc. HCl (0.1 ml). The mixture was stirred and heated under reflux for 3 hr. After cooling, it was poured into an ice-water mixture and extracted with ether. The ether solution was dried (MgSO\(_4\)) and concentrated in vacuo. The residue was dissolved in C\(_6\)H\(_6\) (5 ml) and concentrated in vacuo. The
residual solid was recrystallized twice from acetone–n-hexane (1:5) to give 2.1 g (83.3%) of (S)-19b. mp 92.0 ~ 93.0°C. [α]D20 + 3.2° (c = 0.5, CHCl3) [lit.13] mp 93.3 ~ 93.6°C. [α]D20 + 2.7° (c = 1.98, CHCl3)]. Anal. Found: C, 70.55; H, 11.84%. The IR and NMR spectra of (S)-19b were identical with those of (R)-19b.5,6

(S)-2-Acetoxyhexadecanoic acid (S)-19c. Ac2O (25 ml) was added to a solution of (S)-19b (2.1 g, 7.7 mmol) in pyridine (50 ml). The solution was stirred for 18 hr at room temperature, before being diluted with an ice-water mixture (200 ml) and pyridine (20 ml). After stirring for 10 min, the solution was extracted with ether (500 ml). The ether solution was washed with 2 n-HCl (300 ml) and brine (200 ml × 10) until the aq. layer became neutral. The organic layer was washed with mixed ice–water (100 ml) and concentrated in vacuo. The residue was dissolved in EtOH (5 ml) and concentrated in vacuo. The residual solid was recrystallized from n-hexane to give 2.3 g (95%) of (S)-19c. mp 61.0 ~ 62.0°C. [α]D20 – 10.5° (c = 1.17, CHCl3). Anal. Found: C, 69.09; H, 10.71. Calcd. for C18H34O4: C, 68.75; H, 10.90%. The IR and NMR spectra of (S)-19c were identical with those of (R)-19c.6

p-Nitrophenyl (S)-2-acetoxyhexadecanoyl (S)-19d. A mixture of (S)-19c (1.0 g, 3.2 mmol) and p-nitrophenyl trifluoroacetate (3.2 g, 12.7 mmol) in pyridine (5 ml) was stirred for 18 hr at room temperature, before the pyridine was removed in vacuo. The residue was recrystallized from n-hexane to give ca. 200 mg (quantitative) of 21c, which was then dissolved in pyridine (1 ml). To this solution of 21c was added a solution of (S)-19d (400 mg, 0.92 mmol) in pyridine (1 ml), the mixture being stirred for 24 hr at 45 ~ 50°C. The solvent was removed in vacuo (5 mmHg) and the residue was chromatographed over SiO2. Elution with n-hexane–ether (2:1) gave crystalline 23e, which was recrystallized from n-hexane to give 184 mg (64.2% from 23d) as fine needles. mp 74.0 ~ 75.0°C. [α]D20 – 9.3° (c = 0.7, CHCl3). The IR and NMR spectra were identical with those of 23a.5,6 Anal. Found: C, 74.14; H, 10.07; N, 1.90. Calcd. for C44H73O6N: C, 74.22; H, 10.33; N, 1.97%.

Synthesis of ceramides

i) (4E,8E,2R,3S,2'S)-N-{2'-Acetoxyhexadecanoyl}-1-O-benzoyl-9-methyl-4,8-sphingadienine 23e. This was obtained in a 64.2% yield (pale yellow wax) from 16b and (S)-19d in the same manner as already described. IR v max cm⁻¹: 3500 (s), 3300 (s), 2970 (s), 2920 (s), 2850 (s), 1608 (s), 1510 (s), 1470 (s), 1450 (w), 1370 (w), 1325 (s), 1280 (s), 1170 (w), 1160 (w), 1100 (s), 1050 (m), 1030 (m), 975 (m), 935 (m), 890 (w), 855 (m), 805 (w), 715 (s), 680 (m). NMR δ ppm: 0.75 ~ 1.00 (6H, m), 1.25 (40H, br.s), 1.56 (3H, s), 1.70 ~ 2.30 (6H, m), 2.12 (3H, s), 2.60 ~ 2.80 (1H, m), 4.20 ~ 4.60 (4H, m), 5.00 ~ 5.30 (2H, m), 5.50 ~ 6.10 (2H, m), 6.55 (1H, d, J = 7 Hz), 7.45 ~ 7.70 (3H, m), 8.00 ~ 8.25 (2H, m).

Synthesis of Sphingosine Relatives. Part IV

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ii) \((4E,8E,2S,3S,2'S)-N'2'-Hydroxyhexadecanoyl-9-

methyl-4,8-sphingadienine \(1c\). In the same manner as already described, \(23c\) yielded \(1c\) in a 77.7% yield as a colorless waxy solid. \([\delta]_{D}^{B} -9.7^\circ\) (c=0.5, CHCl3). IR \(v_{\text{max}} \text{ cm}^{-1}: 3350 (s), 2950 (s), 2880 (s), 1620 (s), 1560 (s), 1475 (s), 1385 (w), 1320 (m), 1290 (w), 1130 (m), 1105 (w), 1085 (m), 1050 (w), 995 (m), 905 (w), 890 (w), 845 (w), 760 (w), 720 (w). NMR \(\delta_{\text{CDCl3}} \text{ ppm}: 0.70 - 1.00 (6H, m), 1.25 (40H, br.s), 1.67 (3H, s), 1.70 - 2.30 (6H, m), 3.50 - 4.50 (8H, m), 4.90 - 5.35 (2H, m), 5.45 - 6.05 (2H, m), 7.05 - 7.40 (1H, m). Anal. Found: C, 74.08; H, 11.86; N, 2.55%.}

iii) \((4E,8E,2S,3R,2'S)-N'2'-Hydroxyhexadecanoyl-9-

methyl-4,8-sphingadienine \(1d\). In the same manner as already described, \(23d\) gave \(1d\) in a 72.7% yield as a colorless waxy solid. \([\alpha]_{D}^2 -9.49; N, 3.69.\) Calcd. for C22H35O4N: C, 69.99; H, 9.34; N, 3.71%.}

\((4E,8E,2S,3R,2'R)-N'2'-Acetoxyhexadecanoyl-1-O-

benzoyl-9-methyl-4,8-sphingadienine \(24\). In the same manner as described above for the synthesis of \(23e\), \(21a\) (230 mg) was prepared from \(16a\) (190 mg). A mixture of 27 (243 mg, 0.64 mmol) and \(17a\) (230 mg, 0.48 mmol) in pyridine (2 ml) was stirred for 20 hr at room temperature. The solvent was removed in vacuo and the residue was chromatographed over SiO2. Elution with n-hexane-ether (60:1) gave crystalline 27, which was recrystallized from n-hexane to give 826 mg (91.5%) of 27 as fine needles, mp 61.0 - 61.5°C. IR \(v_{\text{max}} \text{ cm}^{-1}: 1775 (s), 1620 (m), 1540(s).\) NMR \(\delta_{\text{CDCl3}} \text{ ppm: 0.75 - 1.00 (3H, m), 1.23 (40H, br.s), 1.67 (3H, s), 1.75 - 2.60 (2H, m), 2.10 (3H, s), 2.95 - 3.20 (1H, m), 4.20 - 4.80 (4H, m), 4.90 - 5.40 (2H, m), 5.50 - 6.10 (2H, m), 6.65 (1H, d, J=6 Hz), 7.40 - 7.70 (3H, m), 7.90 - 8.20 (2H, m).}

\((4E,8E,2S,3R,2'R)-N'2'-Hydroxyhexadecanoyl-9-

methyl-4,8-sphingadienine \(25\). By the same procedure as described for the synthesis of \(1e\), a colorless waxy solid of 25 (81 mg, 79.0%) was obtained from 24 (129 mg). \([\delta]_{D}^{B} +9.6^\circ\) (c=0.5, CHCl3). IR \(v_{\text{max}} \text{ cm}^{-1}: 3300 (s), 2940 (s), 2870 (s), 1650 (s), 1540 (s), 1470 (s), 1380 (w), 1080 (m), 1050 (w), 1030 (w), 965 (m), 900 (w), 835 (w), 725 (m). NMR \(\delta_{\text{CDCl3}} \text{ ppm: 0.75 - 1.00 (6H, m), 1.27 (40H, br.s), 1.68 (3H, s), 1.80 - 2.20 (6H, m), 3.70 - 4.40 (8H, m), 4.90 - 5.30 (1H, m), 5.40 - 6.10 (2H, m), 7.20 - 7.40 (1H, m). Anal. Found: C, 74.47; H, 11.74; N, 2.50. Calcd. for C35H55O4N: C, 74.28; H, 11.93; N, 2.48%.}

\(p\)-Nitrophenyl palmitate 27. A mixture of palmitic acid (2650 mg, 2.14 mmol) and \(p\)-nitrophenyl trifluoroacetate (1.5 g, 6.3 mmol) in pyridine (2 ml) was stirred for 5 hr at 23°C, before the pyridine was removed in vacuo. The residue was chromatographed over SiO2. Elution with n-hexane-ether (60:1) gave crystalline 27, which was recrystallized from n-hexane to give 826 mg (91.5%) of 27 as fine needles, mp 61.0 - 61.5°C. IR \(v_{\text{max}} \text{ cm}^{-1}: 1775 (s), 1620 (m), 1540(s).\) NMR \(\delta_{\text{CDCl3}} \text{ ppm: 0.75 - 1.00 (3H, m), 1.23 (40H, br.s), 1.67 (3H, s), 1.75 - 2.60 (2H, m), 2.10 (3H, s), 2.95 - 3.20 (1H, m), 4.20 - 4.80 (4H, m), 4.90 - 5.40 (2H, m), 5.50 - 6.10 (2H, m), 6.65 (1H, d, J=6 Hz), 7.40 - 7.70 (3H, m), 7.90 - 8.20 (2H, m).}}
2.55\%.

\((4E,8Z,2S,3R)-N\text{-}C\text{a}cetyl\text{-}9\text{-}methyl\text{-}4,8\text{-}sphingadienine\) 30a. 2 N-HCl (0.7 ml) was added to a solution of 17a (130 mg, 0.33 mmol) in THF (3 ml), and the mixture was stirred for 20 hr at room temperature. It was then diluted with mixed ice-water (10 ml) and extracted with CHCl₃-MeOH (87:13, 25 ml x 3). The organic solution was dried (MgSO₄) and concentrated in vacuo to give ca. 160 mg (quantitative) of 22. This was dissolved in pyridine (2 ml), and \(\alpha\text{-nitrophenyl acetate (74 mg, 0.41 mmol) was added. The mixture was stirred for 24 hr at room temperature. The solvent was removed in vacuo and the residue was chromatographed over SiO₂. Elution with n-hexane–EtOAc (2:1) gave 125 mg (83.9\%) of 1-O-benzoyl derivative of 30a. IR \(\nu_{\text{max}}\) cm\(^{-1}\): 1730 (s), 1660 (s), 1550 (m). This was dissolved in MeOH (9 ml) andaq. NaOH (1N, 1 ml) added. The mixture was stirred for 18 hr at room temperature, before being poured into mixed ice–water (30 ml) and extracted with CHCl₃-MeOH (87:13, 40 ml x 4). The organic solution was washed with brine, dried (MgSO₄) and concentrated in vacuo. The residue was chromatographed over SiO₂. Elution with CHCl₃-MeOH (50:1) gave 81 mg (70.1\%) from 17a as a colorless oil. \(n_f\) 1.4984. \([\alpha]_D^{23}\) -4.7° (c=1.0, CHCl₃). IR \(\nu_{\text{max}}\) cm\(^{-1}\): 3300 (s), 2950 (s), 2870 (s), 1650 (s), 1550 (s), 1440 (m), 1380 (m), 1300 (w), 1180 (w), 970 (m), 890 (w), 835 (w), 720 (w). NMR \(\delta_{\text{CDCl₃}}\) ppm: 0.75–1.00 (3H, m), 1.25 (14H, br.s), 1.64 (3H, s), 1.70–2.30 (6H, m), 2.00 (3H, s), 3.40–4.10 (5H, m), 4.10–4.50 (1H, m), 4.80–5.30 (1H, m), 5.45 (1H, dd, \(J_1=15\text{Hz}, J_2=6\text{Hz}\)), 5.82 (1H, deformed d, \(J=15\text{Hz}\), 6.56 (1H, d, \(J=8\text{Hz}\)). \(^{13}\text{C}-\text{NMR} \delta_{\text{CDCl₃}}\): 14.2, 22.8, 23.2, 23.5, 27.5, 28.1, 29.5, 29.8, 32.0, 33.0, 55.4, 62.9, 71.8, 124.1, 129.5, 133.1, 136.3, 171.9 ppm. Anal. Found: C, 71.08; H, 11.12; N, 3.96\%.

\((4E,8Z,2S,3S)-N\text{-}C\text{a}cetyl\text{-}9\text{-}methyl\text{-}4,8\text{-}sphingadienine\) 30b. This was obtained in a 67.2\% yield from 17b in the same manner as already described. \(n_f\) 1.4971. \([\alpha]_D^{23}\) -8.3° (c=1.2, CHCl₃). IR \(\nu_{\text{max}}\) cm\(^{-1}\): 3300 (s), 2950 (s), 2870 (s), 1650 (s), 1550 (s), 1440 (m), 1380 (w), 1180 (w), 970 (m), 890 (w), 835 (w), 720 (w). NMR \(\delta_{\text{CDCl₃}}\) ppm: 0.75–1.00 (3H, m), 1.25 (14H, s), 1.64 (3H, s), 1.70–2.30 (6H, m), 2.00 (3H, s), 3.50–4.10 (5H, m), 4.20–4.50 (1H, m), 4.90–5.30 (1H, m), 5.43 (1H, dd, \(J=15\text{Hz}\), 5.80 (1H, deformed d, \(J=15\text{Hz}\)), 6.56 (1H, d, \(J=8\text{Hz}\)). \(^{13}\text{C}-\text{NMR} \delta_{\text{CDCl₃}}\): 14.2, 22.8, 23.2, 23.5, 27.5, 28.1, 29.5, 29.8, 32.0, 33.0, 55.4, 62.9, 71.8, 124.1, 129.5, 133.1, 136.3, 171.1 ppm. Anal. Found: C, 71.08; H, 11.20; N, 3.91. Calcd for C₂₁H₃₉O₃N: C, 71.34; H, 11.12; N, 3.96\%.

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