Tetrahedron Vol. 41, No. 12, pp. 2369 to 2377, 1985
Printed in Great Britain.

0040-4020/85 $3.00 + .00

SYNTHESIS 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, AND ITS (2R,3S)-ISOMER†

KENJI MORI* and YUJI FUNAKI††

Department of Agricultural Chemistry, The University of Tokyo,
Yayoi 1-1-1, Bunkyo-ku, Tokyo 113, Japan

(*Received in Japan* 21 *February* 1985)

Abstract-- A synthesis of the natural enantiomer as well as its diastereomer of the title compound was accomplished, confirming the structure proposed for the fruiting-inducing cerebroside of Schizophyllum commune.

Fruiting body formation in Basidiomycetes is indeed a spectacular phenomenon especially to those who love to taste mushrooms. Its mechanism, however, is still a mystery in spite of the tremendous efforts to clarify it. Recently Kawai and Ikeda found that the fruiting body formation of Schizophyllm commune (Japanese name: Suéhiro také) can be stimulated by some cerebrosides in its mycelia.[1] They then identified one of the active substances as (4E,8E,2S,3R,2'R)-N-2'-hydroxyhexadecanoyl-1-O-β-D-glucopyranosyl-9-methyl-4,8-sphingadienine **1a** (Fig. 1),[2] which had previously been isolated from a sea anemone (Metridium senile) by Karlsson et al.[3] Such a minute amount of **1a** as 0.1 μg induced the fruiting body formation of S. commune, and the corresponding ceramide **1b** lacking the sugar portion was also found to be bioactive.[2]

The remarkable bioactivity of **1a** prompted us to synthesize it so as to confirm the proposed structure. This paper describes in detail our initial and successful effort to synthesize the ceramide **1b** with correct stereochemistry.[4] The synthetic **1b** was highly active in inducing the

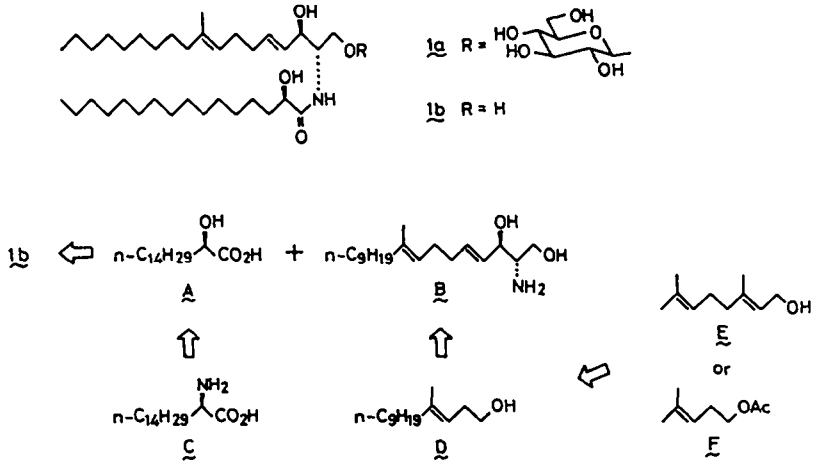

Fig.1. Synthetic plan for the ceramide **1b**.

†Synthesis of Sphingosine Relatives---II. Part I, T. Umemura and K. Mori, Agric. Biol. Chem. 46, 1797 (1982). The experimental part of this work was taken from the forthcoming doctoral dissertation of Y. F.

††Research Fellow on leave from Sumitomo Chemical Co., Ltd. (1983-1985).

fruiting body formation of S. commune.[4]

Our synthetic plan is shown in Fig. 1. We envisioned the assembly of **A** and **B**. (R)-**A** can be obtained from (±)-**C** via resolution followed by deamination. Acylation of (±)-**B** with (R)-**A** will give a diastereomeric mixture from which the desired isomer **1b** can be separated. The precursor of (±)-**B** may be **D** which in turn will be prepared from either **E** or **F**.

Synthesis of the sphingadienine portion B of 1b. The first stage of our work was to synthesize (±)-9-methyl-4,8-sphingadienine **B**. Unlike the usual sphingadienines, this compound possesses a trisubstituted (E)-double bond at C-8, in addition to the disubstituted (E)-double bond at C-4 which is a common feature among sphingolipids. Geraniol **2** (=**E**) was chosen as the starting material, since it contains a trisubstituted (E)-double bond at C-2. In Fig. 2 is shown the conversion of **2** to the intermediate **D**. Treatment of **2** with PBr_3 in ether gave crude geranyl bromide. This was converted to crude homogeranic acid **4a** via **3**.[5] Esterification of **4a** with Me_2SO_4 and K_2CO_3 yielded **4b**. This was shown to be a mixture of methyl homogeranate and homonerate (E/Z=75/25) by GLC analysis. Isomerization of the (E)-double bond at C-2 of **2** must have taken place in the course of its conversion to **3**. After the completion of this work, Gosselin et al. reported a better preparative method of geometrically pure homogeranic acid from **2** avoiding the isomerization.[6] In the present case we proceeded to the next step expecting the possible separation of isomers in a later stage. Thus epoxidation of **4b** with MCPBA yielded **5**. This was cleaved with $HIO_4 \cdot 2H_2O$ to give an aldo ester, whose reduction with $NaBH_4$ gave a hydroxy ester **6a**. The corresponding tosylate **6b** was treated with $(n\text{-}C_6H_{13})_2CuLi$ to give **7**. Finally LAH reduction of **7** yielded a mixture of **8a** (=**D**) and **8a'** in a ratio of 3:1. These two were separable by SiO_2 chromatography, and pure **8a** was obtained in 20 % overall yield from **2** (10 steps).

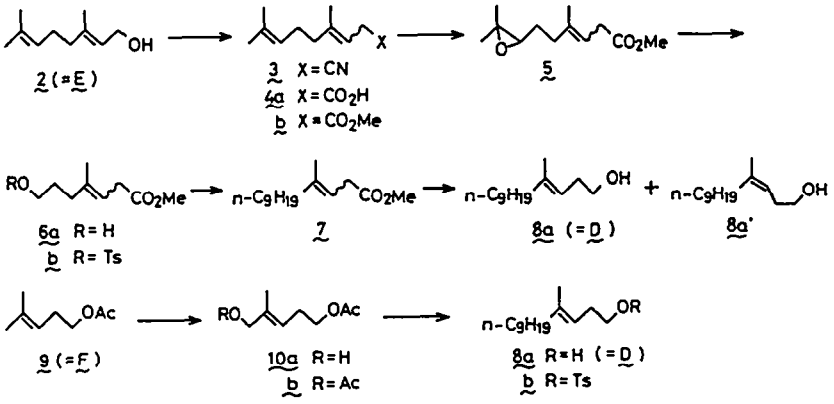

Fig.2. Synthesis of the intermediate D.

A simpler and more efficient synthesis of **8a** was subsequently achieved starting from the known homoprenyl acetate **9** (=**F**).[7] Oxidation of **9** with SeO_2 was followed by $NaBH_4$ reduction to give **10a**, whose acetylation yielded **10b**. The acetate **10b** was treated with $n\text{-}C_8H_{17}MgBr$ in the presence of Li_2CuCl_4 to give **8a** (=**D**) after alkaline hydrolysis in 36 % overall yield from **9**. The identity of the present **8a** with that derived from geraniol was proved by the IR and NMR spectral comparison.

The next stage was the chain-elongation of **8a**. For that purpose **8a** was converted to the corresponding iodide **11** in 96 % yield via **8b** in the conventional manner. The synthesis of the sphingadienine (±)-**17a** from **11** is shown in Fig. 3. Alkylation of the lithio derivative of propargyl alcohol THP ether with **11** was followed by the removal of the THP protective group to give an alcohol **12**. Oxidation of **12** with MnO_2 furnished an aldehyde **13** in 41 % overall yield from **11**. Addition of 2-nitroethanol to **13** in the presence of K_2CO_3 in MeOH[8] afforded a nitrodiol **14** as a diastereomeric mixture. Due to the instability of **14**, its chromatographic separation into the erythro- and threo-isomers was unsuccessful. Conversion of **14** into the corresponding acetonide[9] was not successful either, owing to the retro-aldol reaction. The diastereomeric mixture of **14** was

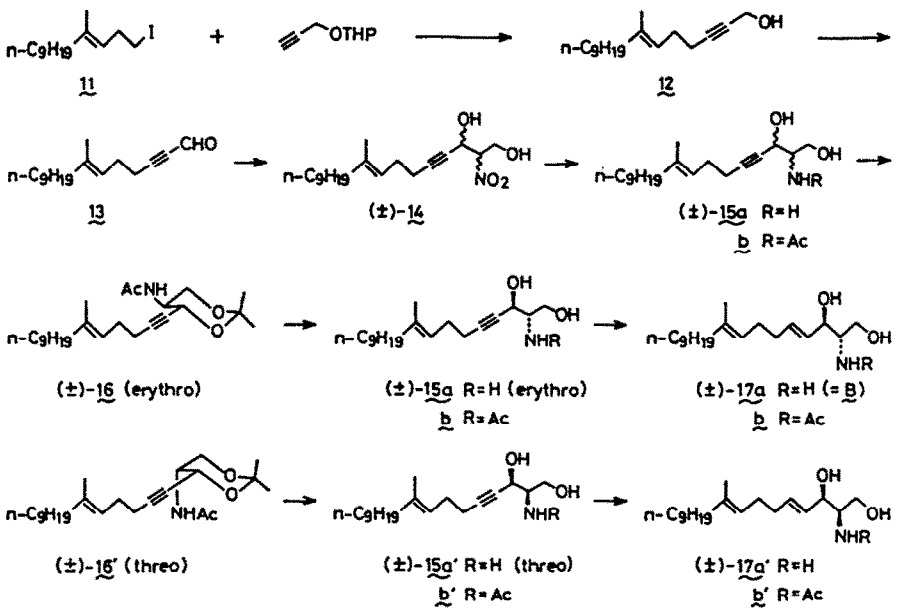

Fig. 3. Synthesis of the sphingadienine B.

therefore directly reduced with Zn and HCl. The resulting **15a** was acetylated with Ac_2O in MeOH to give **15b**, whose EtOAc soln deposited a crystalline mass, m.p. 95.5-96.5°, in 24.6 % yield from **13**. This was later shown to be (±)-threo-**15b'** (vide infra). The mother liquor was treated with $Me_2C(OMe)_2$ and PPTS in Me_2CO to give a mixture of (±)-**16** and **16'**. This was separeted by medium pressure LC over SiO_2 to give (±)-erythro-**16** in 21.5 % yield from **13** together with (±)-threo-**16'** (4.7 % yield from **13**). The assignment of the relative configuration between C-2 and C-3 of **16** and **16'** was made possible by ^{1}H-NMR spectroscopy according to our previous observation.[9,10] The one with an equatorial -NHAc [$\delta(CDCl_3)$ 4.58 (1H, d, J=7 Hz, -CHC≡CCH$_2$-)] was the erythro-isomer (±)-**16**, while the other with an axial -NHAc [$\delta(CDCl_3)$ 4.80 (1H, d, J=2 Hz, -CHC≡CCH$_2$-)] was the threo isomer (±)-**16'**. The aforementioned crystalline (±)-**15b'** yielded (±)-**16'** upon acetonide formation, manifesting its threo-stereochemistry. Removal of the acetonide protective group of (±)-**16** was effected by treatment with p-TsOH in i-PrOH to give (±)-erythro-**15b**. Prior to the reduction of the triple bond, (±)-erythro-**15b** was treated with KOH in aq MeOH to remove the N-acetyl group. The resulting (±)-erythro-**15a** was reduced with LAH to give (±)-erythro-**17a** (=**B**) in 43 % yield from (±)-erythro-**15b**. Similarly (±)-threo-**15b'** yielded (±)-threo-**17a'** in 53 % yield. These two sphingadienine stereoisomers were separately N-acetylated with Ac_2O in MeOH to give (±)-(4E,8E)-2,3-erythro-2-acetamino-9-methyl-4,8-octadecadiene-1,3-diol **17b** and its threo-isomer (±)-**17b'**. The ^{1}H- and ^{13}C-NMR spectra of these two products were compared with those of N-acetyl-9-methyl-4,8-sphingadienine derived from the natural cerebroside **1a** of S. commune.[2] A part of the ^{13}C-NMR data as shown in Table 1 indicated that the sample derived from the natural product possesses the erythro-stereochemistry. The 400 MHz ^{1}H-NMR spectrum of (±)-erythro-**17b** was identical with that of the sample of the natural origin. This concluded the synthesis of (±)-9-methyl-4,8-sphingadienine **17a** (=**B**).

Table 1. ^{13}C-NMR data of the natural and synthetic 2-acetamino-9-methyl-4,8-octadecadiene-1,3-diol

Carbon (No.)	natural ($CDCl_3$, 100 MHz) δ	Synthetic ($CDCl_3$, 22.6 MHz) δ (±)-erythro-**17b**	(±)-threo-**17b'**
-CH_2O- (C-1)	62.19	62.1	63.7
>CHN< (C-2)	54.36	54.5	54.9
>CHO- (C-3)	74.48	74.2	72.5

Synthesis of the ceramide 1b. The remaining task, as shown in Fig. 4, was to prepare (R)-2-

acetoxyhexadecanoic acid **19c** and to N-acylate (±)-**17a** with it. We therefore turned our attention to the preparation of (R)-**19c**. We decided to employ an optically active amino acid (R)-**18a** as the precursor of the desired α-hydroxy acid (R)-**19b** (=**A**). (±)-2-Aminohexadecanoic acid **18a** could readily be prepared by the conventional acetaminomalonic ester synthesis employing diethyl acetaminomalonate and n-tetradecyl bromide as reported by Gerencevic et al.[11] This was converted to the corresponding N-chloroacetyl derivative (±)-**18b**, which was treated with Aspergillus amino acylase[12] to give (S)-**18a** and unhydrolyzed (R)-**18b**. Hydrolysis of (R)-**18b** with dil HCl yielded (R)-**18a**, m.p. 233-236°, $[\alpha]_D^{26}$-21.0°(AcOH). Deamination of (R)-**18a** in dil H_2SO_4 was effected with $NaNO_2$ to give a hydroxy acid, which was immediately esterified with MeOH to give (R)-**19a**, m.p. 45.0-45.5°, $[\alpha]_D^{20}$-1.3°(EtOH), in 52 % yield after chromatographic purification. The optical purity of (R)-**19a** was 85 % e.e. as determined by the HPLC analysis of the corresponding (R)-α-methoxy-α-trifluoromethylphenylacetate (MTPA ester).[13] This rather low e.e. value of (R)-**19a** might be due to the partial racemization in the course of the deamination, since the enantioselectivity of the enzyme reaction was usually almost perfect.[12] The ester (R)-**19a** was hydrolyzed with KOH to give (R)-**19b** (=**A**), m.p. 92-93°, $[\alpha]_D^{20}$-2.9°($CHCl_3$). (R)-2-Hydroxyhexadecanoic acid **19b** was a known compound isolated from wool wax,[14-16] and a synthesis of its (S)-enantiomer by the mixed Kolbe electrolysis was reported by Horn et al.[17] Our recrystallized sample of (R)-**19b** was estimated to be of ca 90 % e.e. by the comparison of its specific rotation (-2.9°) with that of the natural product: $[\alpha]_D$ -3.2°($CHCl_3$).[16] Acetylation of (R)-**19b** with Ac_2O in C_5H_5N yielded (R)-**19c**.

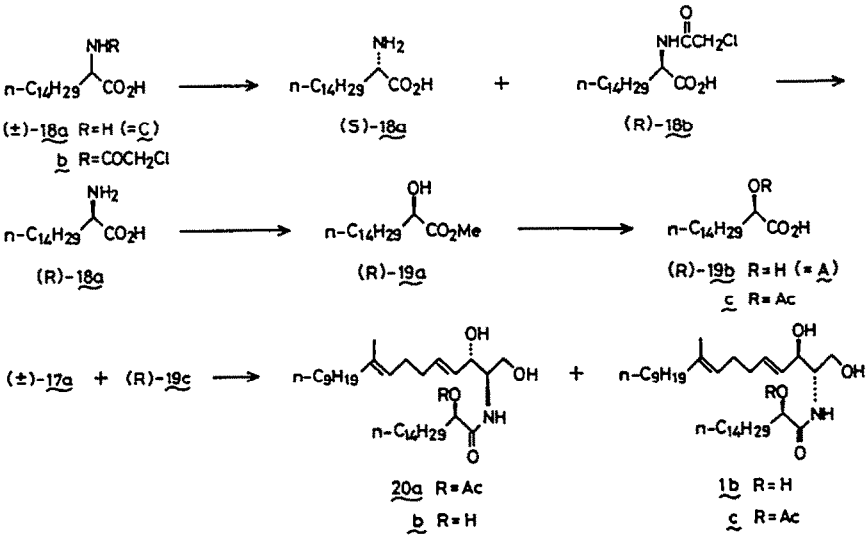

Fig. 4. Synthesis of the ceramide **1b**.

The next and crucial stage of the synthesis was the acylation of the sphingadienine (±)-**17a** with (R)-**19c** and the separation of the resulting diastereomeric mixture. The acylation was successfully executed in 46 % yield from (±)-**17a** by employing 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride as the condensing agent.[18] The resulting mixture of **20a** and **1c** was dissolved in $CHCl_3$ and treated with NaOH in MeOH for 1 h at room temp to give a mixture of **20b** and **1b**. Chromatographic separation of the mixture over a Merck Lobar column gave **20b** (22 % from **17a**), $[\alpha]_D^{21}$+10.6°($CHCl_3$), and the desired ceramide **1b** (21 % from **17a**), m.p. 62-64°, $[\alpha]_D^{21}$+6,4° ($CHCl_3$). An authentic sample of **1b** was prepared from **1a** by the method of Hammarström[19] and showed m.p. 59-61°, $[\alpha]_D^{21}$+7.3°±0.4°($CHCl_3$). Our synthetic **1b** was identified with the natural **1b** by the comparison of IR, ^{1}H-NMR (400 MHz), ^{13}C-NMR (25 MHz) and HPTLC using three different solvent systems. The slightly smaller specific rotation of the synthetic **1b** than that of the authentic **1b** was due to incomplete resolution of (±)-**17a** by the diastereomer separation, because we employed (R)-**19c** of ca 90 % e.e. By simply deviding the $[\alpha]_D$ value of the synthetic **1b** with that of the authentic **1b**, our synthetic **1b** was estimated to be of ca 88 % e.e. The final proof of the

identity of our synthetic **1b** with the ceramide **1b** of natural origin was its very strong fruiting-inducing activity on S. commune. Indeed the specific activity of our **1b** (15,000 units/mg) was higher than that (10,000 units/mg)[1] of the natural cerebroside **1a** itself. The diastereomer **20b** was less active (2,000 units/mg).

In conclusion, the structure **1a** proposed for the fruiting-inducing cerebroside of Schizophyllum commune was confirmed by synthesizing **1b** in 0.2 % overall yield from **9** by a sequence of 19 steps. A rigorous proof of the absolute stereochemistry of **1a** by synthesizing it from L-serine and D-glucose will be the subject of the accompanying paper.[20] We are currently continuing the synthesis of various analogs of **1b** in order to clarify the structure-bioactivity relationship.

EXPERIMENTAL

All b.ps and m.ps were uncorrected. IR spectra were measured as films for oils or as KBr discs for solids on a Jasco IRA-102 spectrometer. NMR spectra were recorded at 60 MHz with TMS as an internal standard on a Hitachi R-24A spectrometer unless otherwise stated. Optical rotations were measured on a Jasco DIP-140 polarimeter.

Geranyl cyanide **3**. PBr_3 (88.6 g, 0.33 mol) was added dropwise to a stirred and cooled soln of **2** (138 g, 0.90 mol) in dry ether (400 ml) containing C_5H_5N (3 drops) at -5°. The stirring was continued for 1 h at -5°. The mixture was then poured into ice-water and extracted with ether. The ether soln was washed with brine, dried ($CaCl_2$) and concentrated in vacuo to give crude geranyl bromide (195 g). This was added dropwise to a stirred suspension of NaCN (45.0 g, 0.92 mol) in dry DMF (500 ml) at 0°. After stirring for 20 h at room temp, the mixture was poured into ice-water and extracted with ether. The ether soln was washed with water, dried ($MgSO_4$) and concentrated in vacuo. The residue was distilled to give 110 g (75.3 %) of **3**, b.p. 81-88°/0.15 mm (lit.[5] b.p. 90-91°/0.2 mm). This was employed in the next step without further purification.

Homogeranic acid **4a**. A soln of KOH (90 g) in water (120 ml) was added to a soln of **3** (80.0 g, 0.49 mol) in MeOH (500 ml), and the mixture was stirred and heated under reflux for 20 h. The solvent was removed in vacuo. The residue was diluted with sat $NaHCO_3$ aq and extracted with ether to remove neutral impurities. The aq layer was acidified with 2 N-HCl and extracted with ether. The ether soln was washed with brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was distilled to give 87.0 g (97.4 %) of **4a**, b.p. 114-124°/0.15 mm (lit.[5] b.p. 108-109°/0.05 mm). This was employed in the next step without further purification.

Methyl homogeranate **4b**. Me_2SO_4 (90.0 g, 0.71 mol) was added dropwise over 1 h to a stirred and ice-cooled mixture of **4a** (90.5 g, 0.50 mol) and K_2CO_3 (100 g, 0.72 mol) in dry Me_2CO (500 ml). The mixture was stirred for 1 h at room temp and heated under reflux for 2 h. Then, after cooling, the mixture was filtered and the filtrate was concentrated in vacuo. The residue was diluted with ice-water and extracted with ether. The ether soln was washed with sat Na_2CO_3 aq and brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was distilled to give 92.0 g (94.3 %) of **4b**, b.p. 75-78°/0.3 mm; ν max 1745 (s), 1660 (w), 1160 (s) cm^{-1}; δ (CCl_4) 1.60 (9H, br.s), 2.01 (4H, br.s), 2.92 (2H, d, J=7 Hz), 3.59 (3H, s), 5.05 (1H, m), 5.28 (1H, t, J=7 Hz); GLC (5 % PEG 20M, 2 m column at 150): E/Z=74.8/25.2; MS (70 eV) m/z 196 (M^+, $C_{12}H_{18}O_2$=196.28).

Methyl 7,8-epoxyhomogeranate **5**. MCPBA (80 % purity, 36.2 g, 0.17 mol) was added portionwise to a stirred and cooled soln of **4b** (30.0 g, 0.15 mol)in CH_2Cl_2 (500 ml) at -10°-5°. The mixture was stirred for 2 h at -10°-5°and filtered. The filtrate was washed with water, sat Na_2CO_3 soln and brine, dried (Na_2SO_4) and concentrated in vacuo. The residue was chromatographed over SiO_2. Elution with n-hexane-EtOAc (15:1) gave 25.5 g (78.7 %) of **5**, n_D^{17}1.4676; ν max 1740 (s), 1160 (s) cm^{-1}; δ (CCl_4) 1.20 (3H, s), 1.22 (3H, s), 1.40-1.80 (2H, m), 1.63 (3H, s), 2.16 (2H, t, J=7 Hz), 2.50 (1H, t, J=6 Hz), 2.93 (2H, d, J=7 Hz), 3.60 (3H, s), 5.31 (1H, t, J=7 Hz). (Found: C, 67.79; H, 9.32. Calc for $C_{12}H_{20}O_3$: C, 67.89; H, 9.50 %).

Methyl 7-hydroxy-4-methyl-3-heptenoate **6a**. $HIO_4 \cdot 2H_2O$ (2.4 g, 9.4 mmol) was added to a stirred and ice-cooled soln of **5** (2.0 g, 9.4 mmol) in ether (80 ml). After stirring for 2 h, the mixture was filtered and the filtrate was concentrated in vacuo. The residue was dissolved in MeOH (20 ml). To this soln was added $NaBH_4$ (0.4 g, 10 mmol) with stirring and ice-cooling. After stirring for 1 h, the mixture was poured into ice-water, acidified with 2 N-HCl and extracted with ether. The ether soln was washed with water, dried ($MgSO_4$) and concentrated in vacuo. The residue was chromatographed over SiO_2. Elution with n-hexane-EtOAc (4:1) gave 1.06 g (65.4 %) of **6a**, $n_D^{25.5}$ 1.4574; ν max 3400 (s), 1740 (s), 1655 (w), 1200 (s), 1160 (s), 1050 (s) cm^{-1}; δ (CCl_4) 1.40-1.85 (5H, m), 1.90-2.30 (2H, m), 2.90 (1H, s, OH), 2.95 (2H, d, J=7 Hz), 3.50 (2H, t, J=6 Hz), 3.61 (3H, s), 5.30 (1H, t, J= 7Hz). (Found: C, 62.60; H, 9.26. Calc for $C_9H_{16}O_3$: C, 62.76; H, 9.36 %).

Methyl 7-tosyloxy-4-methyl-3-heptenoate **6b**. A soln of p-TsCl (2.0 g, 10 mmol) in CH_2Cl_2 (15 ml) was added dropwise to a stirred and ice-cooled soln of **6a** (1.0 g, 5.8 mmol) in C_5H_5N (1.3 g, 16 mmol) and CH_2Cl_2 (15 ml). The soln was stirred for 16 h at room temp. The mixture was poured into ice-water and the CH_2Cl_2 layer was separated. The CH_2Cl_2 soln was washed with sat $CuSO_4$, sat $NaHCO_3$ and brine, dried (Na_2SO_4) and concentrated in vacuo to give 1.76 g (93.0 %) of **6b**, ν max 1745 (s), 1605 (m), 1190 (s), 1180 (s) cm^{-1}; δ (CCl_4) 1.50-1.80 (5H, m, including 2.3H, s, at δ 1.58), 1.96 (2H, t, J=6 Hz), 2.44 (3H, s), 2.89 (2H, d, J= 7Hz), 3.51 (3H, s), 3.96 (2H, t, J= 6Hz), 5.22 (1H, t, J= 7Hz), 7.35 (2H, d, J=8 Hz), 7.80 (2H, d, J= 8Hz). This was employed in the next step without further purification.

Methyl 4-methyl-3-tridecenoate **7**. A soln of n-$C_6H_{13}Li$ (0.8 M, 200 ml, 160 mmol) was added dropwise to a stirred and cooled suspension of CuI (16.9 g, 88.7 mmol) in dry ether (100 ml) at -30°under Ar. To the resulting soln of $(n\text{-}C_6H_{13})_2CuLi$ was added a soln of **6b** (7.3 g, 22.4 mmol) in ether (10 ml) with stirring at -70°. The stirring was continued for 3 h at -70°. Subsequently the mixture was poured into sat NH_4Cl aq. After stirring the mixture for 30 min, the organic layer was

separated, washed with water, dried ($MgSO_4$) and concentrated in vacuo. The residue was chromatographed over SiO_2. Elution with n-hexane-ether (100:1) gave 4.5 g (83.6 %) of **7**, n_D^{24} 1.4445; ν max 1745 (s), 1160 (s) cm^{-1}; δ (CCl_4) 0.88 (3H, deformed t, J=6 Hz), 1.26 (14H, br.s), 1.60 and 1.70 [each s, total 3H (3:1)], 2.00 (2H, br.t, J=7 Hz), 2.92 (2H, d, J=7 Hz), 3.60 (3H, s), 5.28 (1H, t, J=7 Hz); GLC (5 % PEG 20M, 2 m, at 180°) E/Z=3/1. (Found: C, 74.88; H, 11.49. Calc for $C_{15}H_{28}O_2$: C, 74.95; H, 11.74 %).

(E)-4-Methyl-3-tridecen-1-ol **8a**. A soln of **7** (16.0 g, 66.6 mmol) in ether (50 ml) was added dropwise to a stirred and ice-cooled suspension of LAH (3.1 g, 81.6 mmol) in dry ether (350 ml). After stirring for 4 h, the mixture was poured into 0.5 N HCl (400 ml) and ice. The ether layer was separated, washed with water, dried (Na_2SO_4) and concentrated in vacuo to give 13 g of crude **8a** contaminated with **8a'**. This was chromatographed over SiO_2. Elution with n-pentane-ether (10:1) first yielded the (Z)-isomer **8a'** (2.87 g, 20.3 %), n_D^{20} 1.4545; ν max 3350 (s), 1660 (w), 1045 (s) cm^{-1}; ^{1}H-NMR (CCl_4) δ 0.87 (3H, deformed t, J=6 Hz), 1.23 (14H, br.s), 1.67 (3H, br.s), 1.75-2.40 (5H, m), 3.48 (2H, t, J=7 Hz), 5.10 (1H, t, J=7 Hz); ^{13}C-NMR (25 MHz, $CDCl_3$) δ 14.1, 22.7, 23.5, 28.2, 29.4, 29.7, 31.5, 32.0, 62.6, 120.4, 138.9; TLC (SiO_2, developed with n-pentane:ether=1:1) Rf 0.66. (Found: C, 79.33; H, 13.29. Calc for $C_{14}H_{28}O$: C, 79.18; H, 13.29 %). Further elution afforded the (E)-isomer **8a** (10.0 g, 70.5 %), n_D^{20} 1.4555; ν max 3350 (s), 1660 (w), 1045 (s) cm^{-1}; ^{1}H-NMR (CCl_4) δ 0.87 (3H, deformed t, J=6 Hz), 1.23 (14H, br.s), 1.59 (3H, s), 1.95 (2H, m), 2.19 (2H, q, J=7 Hz), 2.92 (1H, br.s, OH), 3.48 (2H, t, J=7 Hz), 5.10 (1H, t, J=7 Hz); ^{13}C-NMR (25 MHz, $CDCl_3$) δ 14.1, 16.1, 22.7, 28.0, 29.4, 29.6, 31.5, 31.9, 39.8, 62.5, 119.5, 139.2; TLC (SiO_2, developed with n-pentane:ether=1:1) Rf 0.60. (Found: C, 79.25; H, 13.38. Calc for $C_{14}H_{28}O$: C, 79.18; H, 13.29 %).

2-Methyl-2-pentene-1,5-diol diacetate **10b**. SeO_2 (18.0 g, 162 mmol) was added to a soln of **9** (22.0 g, 155 mmol) in 95 % EtOH (200 ml) and the mixture was stirred and heated under reflux for 1 h. After cooling, the solvent was removed in vacuo. The residue was diluted with ice-water and extracted with ether. The ether soln was washed with sat $NaHCO_3$ aq and brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was dissolved in THF (100 ml) and water (100 ml). To this was added $NaBH_4$ (3 g, 80 mmol) with stirring and ice-cooling. The soln was stirred for 10 min, neutralized with AcOH (ca 7 ml), diluted with ice-water and extracted with ether. The ether soln was washed with sat $NaHCO_3$ aq and brine, dried ($MgSO_4$) and concentrated in vacuo. The residue **10a** (22 g) was dissolved in C_5H_5N (40 ml) and treated with Ac_2O (40 ml). The soln was stirred for 20 h at room temp. It was then poured into ice-water and extracted with ether. The ether soln was washed with 2 N-HCl, sat $NaHCO_3$ aq and brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was distilled to give 11.8 g (38.0 %) of **10b**, b.p. 95-96 °/5 mm; n_D^{24} 1.4375; ν max 1750 (s), 1240 (s), 1030 (s) cm^{-1}; δ ($CDCl_3$) 1.66 (3H, br.s), 2.03 (3H, s), 2.06 (3H, s), 2.38 (2H, q, J=7 Hz), 4.06 (2H, t, J=7 Hz), 4.44 (2H, br. s), 5.44 (1H, t, J=7 Hz). (Found: C, 59.90; H, 8.01. Calc for $C_{10}H_{16}O_4$: C, 59.98; H, 8.05 %).

(E)-4-Methyl-3-tridecen-1-ol **8a**. A soln of Li_2CuCl_4 in THF (0.1 M, 20 ml, 2 mmol) was added to a stirred and cooled soln of **10b** (9.5 g, 47.5 mmol) in THF at -20° under Ar. To this was added over 30 min a soln of n-$C_8H_{17}MgBr$ in THF (70 ml) prepared from n-$C_8H_{17}Br$ (26.0 g, 135 mmol) and Mg (4.0 g, 165 mg atom). The mixture was stirred for 3 h at room temp, then poured into ice-sat NH_4Cl aq, and extracted with ether. The ether soln was washed with brine, dried ($MgSO_4$) and concentrated in vacuo. A soln of KOH (3 g) in MeOH (100 ml) was added to the residue and the mixture was stirred for 20 h at room temp. It was then poured into ice-water and extracted with EtOAc. The EtOAc soln was washed with brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was chromatographed over SiO_2. Elution with n-hexane-ether (10:1) yielded 9.5 g (94.2 %) of **8a**, whose physical properties were identical with those described previously.

(E)-1-Iodo-4-methyl-3-tridecene **11**. p-TsCl (6.7 g, 35.3 mmol) was added to a soln of **8a** (5.0 g, 23.5 mmol) in C_5H_5N (60 ml) with stirring and ice-cooling. The soln was stirred for 16 h at room temp. The mixture was poured into ice-water and extracted with ether. The ether soln was washed with 2 N-HCl, sat $NaHCO_3$ aq and brine, dried ($MgSO_4$) and concentrated in vacuo. The residual crude **8b** (9.0 g) was dissolved in Me_2CO (50 ml). To this soln was added NaI (7.1 g, 47.4 mmol). The mixture was stirred for 18 h at room temp, poured into ice-water and extracted with ether. The ether soln was washed with $Na_2S_2O_3$ aq and brine, dried ($MgSO_4$) and concentrated in vacuo. The residue in n-hexane was passed through a short column of SiO_2 (5 x 5 cm). Removal of the solvent gave 7.3 g (96.2 %) of **11**, n_D^{21} 1.4924; ν max 1660 (w) cm^{-1}; δ (CCl_4) 0.87 (3H, deformed t, J=6 Hz), 1.25 (14H, br.s), 1.56 (3H, s), 1.75-2.10 (2H, m), 2.45 (2H, q, J=7 Hz), 2.95 (2H, t, J=7 Hz), 5.00 (1H, t, J=7 Hz). This was employed in the next step without further purification.

(E)-7-Methyl-6-hexadecen-2-yn-1-ol **12**. A soln of n-BuLi in hexane (1.5 M, 70 ml, 105 mmol) was added dropwise to a soln of $CH{\equiv}CCH_2OTHP$ (17.0 g, 121 mmol) in THF (80 ml) with stirring and cooling at -15~-10° under Ar. After stirring for 1.5 h at -15~-10°, a soln of **11** (13.0 g, 40.3 mmol) in HMPA (80 ml) was added with stirring and cooling at -15~-10°. The stirring was continued for 1.5 h at -15° and another 1.5 h at 0°. The mixture was then poured into ice-water and extracted with ether. The ether soln was washed with brine, dried ($MgSO_4$) and concentrated in vacuo. MeOH (100 ml) and p-TsOH (1 g) were added to the residue (ca 7 g) and the resulting soln was stirred for 18 h at room temp. It was then poured into ice-water and extracted with EtOAc. The EtOAc soln was washed with sat $NaHCO_3$ aq and brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was chromatographed over SiO_2. Elution with n-hexane-ether (10:1) gave 4.47 g (44.3 %) of **12**, n_D^{24} 1.4690; ν max 3360 (s), 2300 (w), 2240 (w), 1660 (w), 1140 (m), 1080 (s) cm^{-1}; δ (CCl_4) 0.88 (3H, deformed t, J=6 Hz), 1.25 (14H, br.s), 1.58 (3H, s), 1.62-2.30 (7H, m), 4.12 (2H, d, J=6 Hz), 5.11 (1H, t, J=7 Hz). (Found: C, 81.73; H, 12.18. Calc for $C_{17}H_{30}O$: C, 81.53; H, 12.08 %)

(E)-7-Methyl-6-hexadecen-2-ynal **13**. MnO_2 (10.0 g, 115 mmol) was added to a soln of **12** (950 mg, 3.8 mmol) in pet ether (30 ml). The mixture was stirred for 1 h at room temp. It was then filtered and MnO_2 on the filter was washed thoroughly with pet ether. The combined filtrate and washings were concentrated in vacuo to give 870 mg (92.4 %) of **13**, n_D^{22} 1.4676; ν max 2290 (w), 2210 (m), 1675 (s), 1135 (m) cm^{-1}; δ ($CDCl_3$) 0.87 (3H, deformed t, J=6 Hz), 1.23 (14H, br.s), 1.60 (3H, s), 1.82-2.15 (2H, m), 2.17-2.45 (4H, m), 5.12 (1H, t, J=7 Hz), 9.21 (1H, s); TLC (SiO_2, developed with n-hexane-ether=2:1) Rf 0.68. This was employed in the next step without further purification.

A diastereomeric mixture of (E)-2-acetamino-9-methyl-8-octadecen-4-yne-1,3-diol (±)-**15b**. A soln of **13** (650 mg, 2.62 mmol) and 2-nitroethanol (320 mg, 3.51 mmol) in MeOH (2 ml) was added to a stirred and ice-cooled suspension of dry K_2CO_3 (28 mg, 0.2 mmol) in MeOH (1.0 ml). The mixture was stirred for 2 h and then neutralized with conc HCl (42 mg) in MeOH (1 ml). It was then concentrated in vacuo (bath temp <40°). The residue was partitioned between ice-water (20 ml) and ether (50 ml). The ether layer was separated, washed with brine, dried ($MgSO_4$), and concentrated in vacuo to give (±)-**14** (1.0 g) as a pale

yellow oil. Zn powder (1.0 g) and conc HCl (3.8 ml) were added portionwise alternatively over 1 h to a stirred and ice-cooled soln of (±)-**14** (1.0 g) in EtOH (3 ml) at 0°. The mixture was filtered and the solid on the filter was washed with EtOH (3 ml) and water (2 ml). The combined filtrate and washings were made alkaline with 10 N-NaOH aq and extracted with ether. The ether soln was washed with brine, dried ($MgSO_4$) and concentrated in vacuo to give (±)-**15a** (960 mg). This was dissolved in MeOH (20 ml). Ac_2O (5 ml) was added to the soln and the mixture was stirred for 20 h at room temp. It was then poured into ice-water and extracted with ether. The ether soln was washed with sat $NaHCO_3$ aq and brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was dissolved in EtOAc (3 ml) and left to stand in a refrigerator to give (±)-threo-**15b'** as crystals. The solid was collected on a filter and recrystallized from EtOAc to give 226 mg (24.6 % from **13**) of (±)-threo-**15b'**, m.p. 95.5-96.5°. The mother liquor was chromatographed over SiO_2. Elution with EtOAc-C_6H_6 (1:1) gave a mixture of erythro and threo (±)-**15b** (260 mg), which was employed in the next acetonide formation step. (±)-threo-**15b'** showed the following properties: νmax (KBr disc) 3360 (s), 2260 (w), 1670 (s), 1590 (s), 1060 (s), 995 (s) cm^{-1}; δ ($CDCl_3$) 0.85 (3H, deformed t, J=6 Hz), 1.24 (14H, br.s), 1.55 (3H, s), 2.01 (3H, s), 1.75-2.30 (6H, m), 3.60-4.10 (4H, m), 4.20-4.70 (2H, m), 4.90-5.20 (1H, m), 6.56 (1H, d, J=8 Hz). (Found: C, 72.00; H, 10.58; N, 4.02. Calc for $C_{21}H_{37}O_3N$: C, 71.75; H, 10.61; N, 3.99 %).

Two stereoisomers of (E)-2-acetamino-9-methyl-8-octadecen-4-yne-1,3-diol acetonide (±)-erythro-**16** and (±)-threo-**16.'** $Me_2C(OMe)_2$ (2 ml) and PPTS (3 mg) were added to a soln of a distereomeric mixture of (±)-**15b** (260 mg, 0.74 mmol, obtained as the mother liquor after the removal of the crystalline (±)-threo-**15b'**) in Me_2CO (10 ml). The mixture was stirred and heated under reflux for 30 min. It was then poured into ice-water and extracted with ether. The ether soln was washed with sat $NaHCO_3$ aq and brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was chromatographed over SiO_2 (Merck, Art 7734) under medium press. Elution with C_6H_6-EtOAc (20:1) yielded (±)-threo-**16'** (48 mg, 4.7 % from **13**) as the earlier eluted isomer and (±)-erythro-**16** (220 mg, 21.5 % from **13**) as the later eluted isomer. (±)-threo-**16'** was obtained as an oil, n_D^{20} 1.4835; νmax 3370 (s), 2260 (w), 1670 (s), 1530 (s), 1380 (s), 1200 (s), 1085 (s) cm^{-1}; δ ($CDCl_3$) 0.87 (3H, deformed t, J=6 Hz), 1.24 (14H, br.s), 1.46 (6H, s), 1.56 (3H, s), 2.05 (3H, s), 1.70-2.40 (6H, m), 3.62-4.20 (3H, m), 4.80 (1H, d, J=2 Hz), 5.08 (1H, m), 6.34 (1H, d J=8 Hz); TLC (SiO_2, developed with C_6H_6-EtOAc=1:1) Rf 0.34. (Found: C, 73.33; H, 10.61; N, 3.45. Calc for $C_{24}H_{41}O_3N$: C, 73.61; H, 10.55; N, 3.58 %). (±)-erythro-**16** was also obtained as an oil, n_D^{20} 1.4755; νmax 3350 (s), 2260 (w), 1670 (s), 1550 (s), 1380 (s), 1200 (s), 1165 (s), 1080 (s) cm^{-1}; δ ($CDCl_3$) 0.88 (3H, deformed t, J=6 Hz), 1.26 (14H, br.s), 1.43 (3H, s), 1.58 (6H, s), 2.02 (3H, s), 1.82-2.40 (6H, m), 3.65 (1H, dd, J=6 and 10 Hz), 3.72-4.07 (1H, m), 4.25 (1H, dd, J=2 and 10 Hz), 4.58 (1H, d, J=7 Hz), 5.05-5.30 (1H, m), 6.52 (1H, d, J=8 Hz); TLC (SiO_2, developed with C_6H_6-EtOAc=1:1) Rf 0.28. (Found: C, 73.62; H, 10.44; N, 3.58. Calc for $C_{24}H_{41}O_3N$: C, 73.61; H, 10.55; N, 3.58 %). Previously obtained crystalline (±)-threo-**15b'** (10 mg) gave (±)-**16'** (10 mg, 91 %) in the same manner as described above.

(E)-2,3-erythro-2-Acetamino-9-methyl-8-octadecen-4-yne-1,3-diol (±)-erythro-**15b**. p-TsOH (50 mg) was added to a soln of (±)-erythro-**16** (200 mg, 0.51 mmol) in i-PrOH (15 ml). The mixture was stirred and heated at 40°for 40 min. After cooling, the soln was neutralized with 28 % NH_3 aq and concentrated in vacuo. The residue was partitioned between ether and water. The ether soln was separated, dried ($MgSO_4$) and concentrated in vacuo. The residue was chromatographed over SiO_2. Elution with C_6H_6-EtOAc (3:1) gave 141 mg (75.8 %) of (±)-erythro-**15b**, n_D^{19} 1.4861; νmax 3360 (s), 2250 (w), 1650 (s), 1550 (s), 1050 (s) cm^{-1}; δ ($CDCl_3$) 0.88 (3H, deformed t, J=6 Hz), 1.27 (14H, br.s), 1.60 (3H, s), 1.80-2.40 (6H, m), 2.05 (3H, s), 3.55-4.30 (5H, m), 4.50-4.75 (1H, m), 5.05-5.28 (1H, m), 6.17 (1H, d, J=8 Hz). (Found: C, 72.08; H, 10.35; N, 4.23. Calc for $C_{21}H_{37}O_3N$: C, 71.75; H, 10.58; N, 4.02 %).

(E)-2,3-threo-2-Acetamino-9-methyl-8-octadecen-4-yne-1,3-diol (±)-threo-**15b'**. A soln of p-TsOH (1 mg) and (±)-threo-**16'** (16 mg, 0.036 mol) in i-PrOH (2 ml) was stirred and heated at 40°for 30 min. After cooling, the soln was neutralized with dil NH_3 aq, and concentrated in vacuo. The residue was partitioned between ether and ice-water. The ether layer was separated and the aq layer was extracted with ether. The combined ether soln was dried ($MgSO_4$) and concentrated in vacuo to give a solid, which was triturated with n-hexane to give (±)-threo-**15b'** (10 mg, 70.0 %), m.p. 95.5-96.5°. Its IR spectrum was identical with those described previously in this paper.

(4E,8E)-2,3-erythro-2-Amino-9-methyl-4,8-octadiene-1,3-diol (±)-**17a**. N-KOH aq (2 ml) was added to a soln of (±)-erythro-**15b** (122 mg, 0.35 mmol) in MeOH (5 ml) and the mixture was stirred and heated under reflux for 6 h. It was then poured into ice-water and extracted with ether. The ether soln was washed with brine, dried ($MgSO_4$) and concentrated in vacuo to give (±)-erythro-**15a** (113 mg). This was dissolved in THF (3 ml) and added to a stirred suspension of LAH (100 mg, 2.6 mmol) in THF (7 ml). The mixture was stirred and heated under reflux for 6 h. It was then ice-cooled, and the excess LAH was destroyed by the successive addition of water (0.1 ml), 15 % NaOH aq (0.1 ml) and water (0.1 ml). The mixture was filtered, and the filter-cake was washed with THF. The combined filtrate and washings were concentrated in vacuo. The residue was chromatographed over SiO_2. Elution with $CHCl_3$-MeOH (3:1) gave 46 mg (42.6 %) of (±)-**17a**, n_D^{17} 1.4818; ν max 3400 (s), 1675 (m), 1600 (m), 1030 (s), 970 (m) cm^{-1}; δ (100 MHz, $CDCl_3$) 0.85 (3H, deformed t, J=6 Hz), 1.24 (14H, s), 1.55 (3H, s), 1.80-2.20 (6H, m), 2.40-3.00 (5H, m), 3.50-3.70 (2H, m), 3.88-4.08 (1H, m), 4.92-5.10 (1H, m), 5.38 (1H, dd, J=16 and 6 Hz), 5.68 (1H, deformed dt, J=16 and 6 Hz). (Found: C, 73.17; H, 11.72; N, 4.56. Calc for $C_{19}H_{37}O_2N$: C, 73.26; H, 11.97; H, 4.50 %).

(4E,8E)-2,3-threo-2-Amino-9-methyl-4,8-octadiene-1,3-diol (±)-**17a'**. In the same manner as described above for the preparation of (±)-**17a**, (±)-**15b'** (180 mg, 0.51 mmol) gave 84 mg (52.7 %) of (±)-**17a'**, m.p.63.0-64.0°(recrystallized from n-hexane), ν max (KBr disc) 3440 (s), 1680 (w), 1605 (s), 1055 (s), 1040 (s), 975 (s), 950 (s) cm^{-1}; δ (100 MHz, $CDCl_3$) 0.86 (3H, deformed t, J=6 Hz), 1.24 (14H, s), 1.56 (3H, s), 1.83-2.18 (6H, m), 2.52-2.92 (5H, m), 3.49-3.60 (2H, m), 3.97 (1H, t, J=7.5 Hz), 4.92-5.12 (1H, m), 5.40 (1H, dd, J=16 and 6 Hz), 5.70 (1H, deformed dt, J=16 and 6 Hz). (Found: C, 73.46; H, 11.87; N, 4.56. Calc for $C_{19}H_{37}O_2N$: C, 73.26; H, 11.97; N, 4.50 %).

(4E,8E)-2,3-erythro-2-Acetamino-9-methyl-4,8-octadiene-1,3-diol (±)-**17b**. A soln of (±)-**17a** (36mg, 0.12 mmol) in Ac_2O (0.1 ml) and MeOH (2 ml) was stirred for 18 h at room temp. After destroying the excess Ac_2O with 5 % $NaHCO_3$ aq (10 ml), the mixture was extracted with ether. The ether soln was washed with brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was chromatographed over SiO_2. Elution with C_6H_6-EtOAc (1:1) furnished 30 mg (73.4 %) of (±)-**17b** as an oil, n_D^{18} 1.4835; νmax 3300 (s), 1640 (s), 1540 (s), 1045 (s), 965 (s) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.88 (3H, t, J=7.0 Hz), 1.24 (12H, br.s), 1.36 (2H, m), 1.58 (3H, s), 1.94 (2H, t, J=8.0 Hz), 2.03 (3H, s), 2.08 (4H, br.s), 3.50 (1H, br.s), 3.58 (1H, br.s), 3.68 (1H, deformed d, J=11.0 Hz), 3.88 (1H, dt, J_1=7.6 Hz, J_2=3.8 Hz), 3.92 (1H, dd, J_1=11.0 Hz, J_2=3.8 Hz), 4.30

(1H, br.s), 5.09 (1H, t, J=6.0 Hz), 5.53 (1H, dd, J_1=15.6 Hz, J_2=6.4 Hz), 5.79 (1H, ddd, J_1=15.6 Hz, J_2=6.4 Hz, J_3=5.5 Hz), 6.55 (1H, d, J=7.0 Hz); ^{13}C-NMR (22.6 MHz, $CDCl_3$) δ 14.0, 15.8, 22.5, 23.1, 27.5, 27.8, 29.4, 31.8, 32.4, 39.6, 54.5, 62.1, 74.2, 123.0, 128.9, 133.6, 136.2, 171.1. These ^{1}H- and ^{13}C-NMR data were identical with those reported for **17b** derived from **1a**.[2] (Found: C, 71.22; H, 11.03; N, 4.03. Calc for $C_{21}H_{39}O_3N$: C, 71.34; H, 11.12; N, 3.96 %).

(4E,8E)-2,3-threo-2-Acetamino-9-methyl-4,8-octadiene-1,3-diol (±)-**17b'**. A soln of (±)-**17a'** (45 mg, 0.14 mmol) in Ac_2O (0.1 ml) and MeOH (2 ml) was stirred for 18 h at room temp. After destroying the excess Ac_2O with 5 % $NaHCO_3$ aq (10 ml), the mixture was extracted with ether. The ether soln was washed with brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was recrystallized from EtOAc–n-hexane (1:2) to give 45 mg (88.1 %) of (±)-**17b'**, m.p. 86.0–87.0°, ν max (KBr disc) 3300 (s), 1645 (s), 1530 (s), 1040 (s), 960 (s) cm^{-1}; ^{1}H-NMR (90 MHz, $CDCl_3$) δ 0.89 (3H, deformed t, J=6 Hz), 1.27 (14H, s), 1.58 (3H, s), 1.85–2.30 (6H, m), 2.03 (3H, s), 3.30–4.05 (5H, m), 4.20–4.50 (1H, m), 4.95–5.25 (1H, m), 5.47 (1H, dd, J=16 and 6 Hz), 5.81 (1H, deformed dt, J=16 and 6 Hz), 6.37 (1H, d, J=7 Hz); ^{13}C-NMR (22.6 MHz, $CDCl_3$) δ 14.1, 15.9, 22.6, 23.3, 27.6, 28.0, 29.4, 31.9, 32.5, 39.6, 54.9, 63.7, 72.5, 123.2, 129.2, 133.6, 136.2, 171.6. (Found: C, 71.14; H, 11.06; N, 3.95. Calc for $C_{21}H_{39}O_3N$: C, 71.34; H, 11.12; N, 3.96 %).

(±)-2-Aminohexadecanoic acid (±)-**18a**. Diethyl acetaminomalonate (65.3 g, 0.3 mol) in abs EtOH (150 ml) was added to a soln of EtONa [prepared from 6.9 g (0.3 g atom) of Na] in abs EtOH (150 ml). To this was added 1-bromotetradecane (100 g, 0.36 mol) and the mixture was stirred and heated under reflux for 18 h. The solvent was removed in vacuo. The residue was diluted with ice-water and extracted with ether. The ether soln was concentrated in vacuo. The residue was mixed with conc HCl (150 ml) and water (50 ml). The mixture was stirred and heated under reflux for 6 h, diluted with water (400 ml) and further heated under reflux for 18 h. After cooling, 28 % NH_3 aq was added and the precipitated (±)-**18a** was collected on a filter. The solid was washed with water, MeOH and ether, and dried over P_2O_5 to give 66.8 g (81.9 %) of (±)-**18a**, m.p. 221–223 °dec) (lit.[11] m.p. 220–225°), ν max (nujol) ca 3000–2750 (br.s), 2100 (w), 1655 (s), 1620 (s), 1600 (s),1580 (s), 1510 (s), 1415 (s), 1340 (s), 715 (m), 700 (m) cm^{-1}. This was employed in the next step without further purification.

(±)-2-Chloroacetaminohexadecanoic acid (±)-**18b**. $ClCH_2COCl$ (71.2 g, 0.63 mol) and 2 N-NaOH aq (300 ml, 0.6 mol) were added over 1 h dropwise and alternatively to a soln of (±)-**18a** (57.0 g, 0.21 mol) in 2 N-NaOH aq (500 ml, 1.0 mol) and $(EtOCH_2CH_2)_2O$ (400 ml) with stirring and ice-cooling at 10°. After stirring for 1 h, the mixture was acidified with conc HCl. The precipitated solid was collected on a filter, and dissolved in hot EtOAc (1 l). The insoluble (±)-**18a** (11.0 g, 19.3 %) was filtered off. The filtrate was washed with water, dried ($MgSO_4$) and concentrated in vacuo. The residual solid was recrystallized from n-hexane to give 37.0 g (62.6 % based on the consumed (±)-**18a**) of (±)-**18b**, m.p. 97.0–99.0°, νmax(nujol) 3360 (m), 1725 (s), 1600 (s), 1555 (s), 1140 (m) cm^{-1}; δ($CDCl_3$) 0.87 (3H, deformed t, J=6 Hz), 1.24 (24H, br.s), 1.60–2.00 (2H, m), 4.08 (2H, s), 4.40–4.80 (1H, m), 7.06 (1H, d, J=8 Hz), 9.54 (1H, br.s). (Found: C, 62.42; H, 9.87; N, 3.98. Calc for $C_{18}H_{34}O_3NCl$: C, 62.14; H, 9.85; N, 4.03 %).

Enzymatic resolution of (±)-**18b** with amino acylase. Amino acylase (from Aspergillus, 10,000 unit/g, Tokyo Kasei Co., 5 g) and $CoCl_2$ (10 mg) were added to a soln of (±)-**18b** (36.0 g, 0.103 mol) in water (4 l) adjusted to pH 7.3 by the addition of NaOH. The soln was left to stand for 44 h at 37°. The precipitated crystalline (S)-**18a** was collected on a filter, washed with MeOH and ether, and dried over P_2O_5 to give 14.0 g (quantitative) of (S)-**18a**, m.p. 234–236°, $[\alpha]_D^{26}$+21.8°(c=0.1, AcOH); νmax(nujol) 1575 (s), 1510 (s), 1405 (m), 1320 (m), 720 (m) cm^{-1}. (Found: C, 70.51; H, 12.18; N, 5.03. Calc for $C_{16}H_{33}O_2N$: C, 70.80; H, 12.26; N, 5.16 %). The filtrate obtained after removal of (S)-**18a** was acidified with 3 N-HCl. The precipitated solid was collected on a filter, and dissolved in EtOAc (1 l). The insoluble material was filtered off and the filtrate was concentrated in vacuo. The residue was recrystallized from n-hexane to give (R)-**18b** (15.5 g, 86.1 %) m.p. 87.0–88.0°, $[\alpha]_D^{21}$-28.0°(c=0.5, $CHCl_3$). The IR and NMR spectra of (R)-**18b** were identical with those of (±)-**18b**. (Found: C, 62.07; H, 9.75; N, 4.01. Calc for $C_{18}H_{34}O_3NCl$: C, 62.14; H, 9.85; N, 4.03 %).

(R)-2-Aminohexadecanoic acid (R)-**18a**. A mixture of (R)-**18b** and 4 N-HCl (150 ml) was stirred and heated under reflux for 3 h. After cooling, the mixture was neutralized with 28 % NH_3 aq. The precipitated solid was collected on a filter, washed with water, MeOH and ether, and dried over P_2O_5 to give (R)-**18a** (12.0 g, 99.5 %), m.p. 233–236°, $[\alpha]_D^{26}$-21.0°(c=0.1, AcOH). Its IR spectrum was identical with that of (S)-**18a**. (Found: C, 71.09; H, 12.11; N, 5.20. Calc for $C_{16}H_{33}O_2N$: C, 70.80; H, 12.26; N, 5.16 %).

Methyl (R)-2-hydroxyhexadecanoate (R)-**19a**. A soln of $NaNO_2$ (1.52 g, 22.0 mmol) in water (17 ml) was added dropwise to a soln of (R)-**18a** (3.0 g, 11.1 mmol) in 2 N-H_2SO_4 (20 ml) over 2 h. Warming was necessary to maintain the soln homogeneous, which might have caused partial racemization. The mixture was stirred for 2 h at 80°and for 18 h at room temp. It was then extracted with ether. The ether soln was concentrated in vacuo. The residue was dissolved in C_6H_6 and concentrated in vacuo to remove water. The residual crude (R)-**19b** was mixed with MeOH (30 ml), C_6H_6 (40 ml) and conc HCl (3 drops). The soln was stirred and heated under reflux for 3.5 h, cooled, poured into brine and extracted with ether. The ether soln was washed with brine, dried (Na_2SO_4) and concentrated in vacuo. The residue was chromatographed over SiO_2. Elution with n-hexane–ether (40:1) gave crude (R)-**19a**, which was recrystallized from n-hexane to give 1.65 g (52.1 %) of (R)-**19a**, m.p. 45.0–45.5°, $[\alpha]_D^{20}$-1.30°(c=2.8, EtOH) [lit.[15] m.p. 45–46°; $[\alpha]_D^{20}$-1.5°(c=10, EtOH)]; ν max(nujol) 3350 (br.s), 1735 (s), 1275 (s), 1215 (m), 1200 (m), 1130 (m), 1120 (m), 1095 (m), 1080 (m), 720 (m) cm^{-1}; δ($CDCl_3$) 0.90 (3H, deformed t, J=6 Hz), 1.23 (24H, br.s), 1.50–1.80 (2H, m), 2.72 (1H, d, J=6 Hz, OH), 3.78 (3H, s), 4.00–4.30 (1H, m). The optical purity of (R)-**19a** was estimated by the HPLC analysis of the corresponding (R)-MTPA ester (Column, Partisil 5, 25 cm x 4.6 mm; Solvent, n-hexane–THF–MeOH=6000:100:1, flow rate 1.0 ml/min) Rt 12.4 min (92.6 %), 14.9 min (7.4 %). Therefore our (R)-**19a** was of 85.2 % e.e.

(R)-2-Hydroxyhexadecanoic acid (R)-**19b**. KOH (32 mg, 0.53 mmol) was added to a soln of (R)-**19a** (150 mg, 0.52 mmol) in 95 % EtOH (5 ml). The mixture was stirred and heated under reflux for 30 min, and concentrated in vacuo. The residue was acidified with 2 N-HCl and extracted with ether. The ether soln was washed with brine, and concentrated in vacuo. The residue was dissolved in C_6H_6 and concentrated in vacuo. The residual solid was recrystallized from n-hexane (8 ml)–Me_2CO (1 ml) to give 134 mg (94.0 %) of (R)-**19b**, m.p. 92–93°; $[\alpha]_D^{20}$-2.9°(c=1.03, $CHCl_3$) [lit.[16] m.p. 93.3–93.5°; $[\alpha]_D$ -3.2° ($CHCl_3$)]. From the specific rotation, our (R)-**19b** was estimated to be of 90.6 % e.e. νmax(nujol) 3460 (s), 3430 (m), 1750 (s), 1730 (s), 1260 (s), 1135 (s), 1100 (s), 1085 (s), 850 (m), 720 (m) cm^{-1}; δ ($CDCl_3$) 0.86 (3H, deformed t, J=6 Hz), 1.24 (24H, br.s), 1.40–1.90 (2H, m), 4.10–4.40 (1H, m), 6.60–7.00 (2H, br, OH and CO_2H). This was employed in the next step without further purification.

(4E,8E,2S,3R,2'R)-N-2'-Hydroxyhexadecanoyl-2-amino-9-methyl-4,8-octadecadiene-1,3-diol **1b** and its (4E,8E,2R,3S,2'R)-isomer **20b**. Ac_2O (2 ml) was added to a soln of (R)-**19b** (132 mg, 0.48 mmol) in C_5H_5N (4 ml). The mixture was stirred for 16 h at room temp. It was then diluted with ice-water and extracted with ether. The ether soln was washed with 2 N-HCl and brine. EtOH (ca 5 ml) was added to the ether soln, and the soln was concentrated in vacuo. To the residual (R)-**19c** were added (±)-**17a** (76.5 mg, 0.25 mmol), $EtN=C=N(CH_2)_3NMe_2$ HCl (250 mg, 1.3 mmol), CH_2Cl_2 (20 ml), MeCN (20 ml) and MeOH (3 ml). The mixture was left to stand at 40° for 22 h. It was then diluted with ether (100 ml), washed with sat $NaHCO_3$ aq, brine, N-HCl and brine, and concentrated in vacuo. The residue was chromatographed over SiO_2. Elution with C_6H_6-EtOAc (10:1) gave a mixture of **20a** and **1c** (68 mg, 45.6 % from (±)-**17a**), νmax 1740 (s), 1720 (s), 1655 (s), 1555 (s) cm^{-1}; TLC (SiO_2, developed with C_6H_6-EtOAc=3:7) Rf 0.30. This was dissolved in $CHCl_3$ (10 ml) and mixed with NaOH (240 mg) in MeOH (10 ml). The mixture was stirred for 1 h at room temp. It was then diluted with ice-water (30 ml) and extracted with $CHCl_3$. The $CHCl_3$ soln was concentrated in vacuo and the residue was chromatographed over a Merck Lobar®column (Lichroprep®Si60, 40–63 μm). Elution with $CHCl_3$-MeOH (150:1) first yielded **20b** (31 mg, 22.3 % from (±)-**17a**) as a gum, $[\alpha]_D^{21}$ +10.6° (c=0.54, $CHCl_3$); νmax 3350 (br.s), 1655 (s), 1530 (s), 1085 (s), 1045 (s) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.87 (6H, t, J=7.0 Hz), 1.20-1.45 (40H, m), 1.58 (3H, s), 1.95 (2H, t, J=7.5 Hz)), 2.08 (4H, br.s), 3.14 (1H, br.s), 3.27 (1H, br.s), 3.34 (1H, br.s), 3.69 (1H, deformed d, J=11.0 Hz), 3.89 (1H, dt, J_1=8.0 Hz, J_2=4.0 Hz), 3.93 (1H, dd, J_1=11.0 Hz, J_2=4.0 Hz), 4.12 (1H, dd, J_1=7.5 Hz, J_2=3.5 Hz), 4.35 (1H, br.s), 5.09 (1H, t, J=6.0 Hz), 5.53 (1H, dd, J_1=15.5 Hz, J_2=6.5 Hz), 5.81 (1H, dt, J_1=15.5 Hz, J_2=6.0 Hz), 7.21 (1H, d, J=8.0 Hz); TLC (SiO_2, developed with $CHCl_3$-MeOH=93:7) Rf 0.40. Further elution with the same solvent gave **1b** (29 mg, 20.9 % from (±)-**17a**) as a white powder-like solid, m.p. 62–64°(recrystallized from n-hexane); $[\alpha]_D^{21}$ +6.4° (c=0.76, $CHCl_3$); νmax (KBr disc) 3360-3290 (br.s), 2960 (s), 2930 (s), 2860 (s), 1650 (s), 1625 (m), 1540 (s), 1470 (s), 1380 (w), 1330 (w), 1260 (w), 1140 (w), 1100 (w), 1070 (m), 1050 (s), 1025 (m), 960 (m), 885 (w), 720 (m) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.87 (6H, t, J=7.0 Hz), 1.20-1.45 (40H, m), 1.58 (3H, s), 1.95 (2H, t, J=7.5 Hz), 2.08 (4H, br.s), 3.20 (1H, br.s), 3.55 (1H, br.s), 3.70 (1H, br.s), 3.74 (1H, deformed d, J=11.0 Hz), 3.87 (1H, dd, J_1=11.0 Hz, J_2=4.0 Hz), 3.91 (1H, dt, J_1=8.0 Hz, J_2=4.0 Hz), 4.11 (1H, dd, J_1=7.5 Hz, J_2=3.5 Hz), 4.27 (1H, br.s), 5.09 (1H, t, J=6.0 Hz), 5.52 (1H, dd, J_1=15.5 Hz, J_2=6.5 Hz), 5.79 (1H, dt, J_1=15.5 Hz, J_2=6.0 Hz), 7.21 (1H, d, J=8.0 Hz); ^{13}C-NMR (25 MHz, $CDCl_3$) δ14.1, 16.0, 22.7, 25.2, 27.6, 28.1, 29.4, 29.6, 29.7, 31.9, 32.6, 34.7, 39.7, 54.5, 61.9, 72.5, 74.1, 123.1, 128.6, 134.0, 136.2, 175.5; TLC (SiO_2, developed with $CHCl_3$-MeOH=93:7) Rf 0.33; HPTLC (Merck Kieselgel $60F_{254}$) Rf 0.53 (developed with $CHCl_3$-MeOH=9:1), Rf 0.60 (n-hexane-Me_2CO=1:1), Rf 0.16 (C_6H_6-EtOAc=1:4). (Found: C, 73.94; H, 11.92; N, 2.54. Calc for $C_{35}H_{67}O_4N$: C, 74.28; H, 11.93; N, 2.48 %).

Preparation of **1b** from natural **1a**. The glucosyl group of **1a** (from *S. commune*, 11 mg) was removed by the method of Hammarstrom to give 7.0 mg (82 %) of **1b**, m.p. 59–61°, $[\alpha]_D^{21}$ +7.3°(c=0.25, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.87 (6H, t, J=7.0 Hz), 1.20-1.45 (40H, m), 1.58 (3H, s), 1.95 (2H, t, J=7.5 Hz), 2.08 (4H, br.s), 3.00-3.60 (3H, m), 3.74 (1H, deformed d, J=11.0 Hz), 3.89 (1H, dd, J_1=11.0 Hz, J_2=4.0 Hz), 3.92 (1H, dt, J_1=8.0 Hz, J_2=4.0 Hz), 4.12 (1H, dd, J_1=7.5 Hz, J_2=4.0 Hz), 4.28 (1H, deformed t, J=5 Hz), 5.09 (1H, t, J=6.0 Hz), 5.52 (1H, dd, J_1=15.5 Hz, J_2=6.5 Hz), 5.80 (1H, dt, J_1=15.5 Hz, J_2=6.0 Hz), 7.21 (1H, d, J=8.0 Hz); ^{13}C-NMR (25 MHz, $CDCl_3$) δ 14.1, 16.0, 22.7, 25.1, 27.6, 28.1, 29.4, 29.7, 31.9, 32.6, 34.9, 39.8, 54.4, 62.3, 72.5, 74.4, 123.5, 128.8, 134.1, 136.3 (The signal at δ 175.5 could not be observed due to the noise signals). These NMR data as well as the IR, TLC and HPTLC data were identical with those described for our synthetic **1b**. Our synthetic **1b** was estimated to be of 88 % e.e. [(6.4/7.3) x 100] by the comparison of the $[\alpha]_D$ values.

Acknowledgement--We thank Dr. G. Kawai of Noda Institute for Scientific Research, Noda-shi, Chiba-ken, for his kind gift of **1a** and for the bioassay of our synthetic materials. We are grateful to Mr. T. Sugai for his help in preparing the camera-ready manuscript. Financial support of this work by Sumitomo Chemical Co. is gratefully acknowledged.

REFERENCES

1 G. Kawai and Y. Ikeda, Biochim. Biophys. Acta **719**, 612 (1982).
2 G. Kawai and Y. Ikeda, Ibid. **754**, 243 (1983).
3 K.-A. Karlsson, H. Leffler and B. O. Samuelsson, Ibid. **574**, 79 (1979).
4 Preliminary communication: K. Mori and Y. Funaki, Tetrahedron Lett. **25**, 5291 (1984).
5 D. Barnard and L. Bateman, J. Chem. Soc. 926 (1950).
6 P. Gosselin, C. Maignan and F. Rouessac, Synthesis 876 (1984).
7 M. Julia, S. Julia and R. Guégan, Bull. Soc. Chim. France 1072 (1960).
8 C. A. Grob and F. Gadient, Helv. Chim. Acta **40**, 1145 (1957).
9 T. Umemura and K. Mori, Agric. Biol. Chem. **46**, 1797 (1982).
10 K. Mori and T. Umemura, Tetrahedron Lett. **22**, 4429 (1981).
11 N. Gerencevic, A. Castek, M. Sateva, J. Pluscec and M. Prostenik, Monatsh. Chem. **97**, 331 (1966); Chem. Abstr. **65**, 2123b (1966).
12 Y. Masaoka, M. Sakakibara and K. Mori, Agric. Biol. Chem. **46**, 2319 (1982).
13 J. A. Dale and H. S. Mosher, J. Am. Chem. Soc. **95**, 512 (1973).
14 L. Darmstaedter and J. Lifschütz, Ber. Dtsch. Chem. Ges. **29**, 2890 (1896).
15 T. Kuwata, J. Am. Chem. Soc. **60**, 559 (1938).
16 D. H. S. Horn, F. W. Hougen, E. von Rudloff and D. A. Sutton, J. Chem. Soc. 177 (1954).
17 D. H. S. Horn and Y. Y. Pretorius, Ibid. 1460 (1954).
18 S. Hammarström, J. Lipid Res. **12**, 760 (1971).
19 S. Hammarström, Eur. J. Biochem. **15**, 581 (1970).
20 K. Mori and Y. Funaki, Tetrahedron, the accompanying paper.