(apparent q, J = 6.4 Hz, 1 H, C1'H), 5.41 (m, 1 H, C1''H), 5.29 (t, J = 4.6 Hz, 1 H, C3'-O*H*), 4.88 (bs, 1 H, C5'-O*H*), 4.36 (m, 1 H, C3'H), 3.84-3.73 (m, 1 H, C4'H), 3.75 (s, 3 H, OCH_3), 3.60-3.46 (m, 2 H), 3.13-3.07 (m, 1 H), 2.75-2.61 (m, 1 H), 2.34-2.19 (m, 2 H), 1.86-1.71 (m, 2 H); IR (DMSO-d_6) 3504, 3454, 2935, 1731, 1691, 1663, 1602, 1514, 1462, 1366, 1244, 1106, 924 cm^{-1}; UV (H_2O) λ_{max} 208, 238, 256 nm; MS (FAB, positive ion, nitrobenzyl alcohol matrix) m/z 494 (MH^+, 37), 273 (37), 242 (37), 226 (100), 219 (62), 165 (75); HRMS calcd for MH^+, $C_{26}H_{28}N_5O_7$ 494.2040, found 494.2023.

10-(Acetyloxy)-1,9-dihydroxy-1,2,3,4-tetrahydroanthracene (10). A solution of quinone methide **3** (from 88.9 mg, 0.347 mmol, of phenol **1**) and $CDCl_3$ (1 mL) was added to a solution of water (2 mL) and CH_3CN (2 mL). This solution was stirred at room temperature until the reaction was complete (30 min). The reaction mixture was extracted with $CHCl_3$ (2 × 15 mL). The combined organic extracts were dried ($NaSO_4$), concentrated, and chromatographed (4:1 hexane/ethyl acetate) to afford 25.3 mg (27%) of the unstable compound **10** as a yellow oil (9:1 mixture of **10** and **3**): ^1H NMR (300 MHz, $CDCl_3$) δ 8.90 (bs, 1 H, ArO*H*), 8.32 (dd, J = 8.3, 1.2 Hz, 1 H, ArH), 7.61 (d, J = 8.0 Hz, 1 H, ArH), 7.50-7.39 (m, 2 H, ArH), 5.08 (apparent q, J = 6.5 Hz, 1 H, C1-H), 2.80-2.59 (bm, 3 H, C4-2H, C1-OH), 2.44 (s, 3 H, OAc), 2.22 (m, 1 H), 1.94-1.69 (m, 3 H); IR ($CDCl_3$) 3581, 3345, 2946, 2868, 1759, 1662, 1637, 1596, 1576, 1451, 1370, 1213, 1179, 1065 cm^{-1}; UV (H_2O) λ_{max} 208, 238, 264 nm; MS (FAB, positive ion, nitrobenzyl alcohol matrix) m/z 272 (M^+, 14), 255 (65), 228 (11), 212 (100), 197 (9), 165 (10); HRMS calcd for $C_{16}H_{16}O_4$ 272.1049, found 272.1034; (M - OH) calcd for $C_{16}H_{15}O_3$ 255.1021, found 255.1014.

1-Hydroxy-1,2,3,4-tetrahydroanthraquinone (12). Chromatography of high R_f material isolated in the purification of **7** and **9** (9:1 hexane/ethyl acetate) afforded quinone **12** as a pale brown solid: mp 98-99 °C; ^1H NMR (300 Hz, $CDCl_3$) δ 8.05 (m, 2 H, ArH), 7.70 (m, 2 H, ArH), 4.93 (m, 1 H, C1-H), 3.37 (s, 1 H, OH), 2.75-2.65 (m, 1 H, C4-H), 2.52-2.41 (m, 1 H, C4-H), 1.97-1.84 (m, 3 H, CH_2), 1.79-1.69 (m, 1 H, CH_2); ^{13}C NMR (75 Hz, $CDCl_3$) δ 186.28, 185.16, 146.28, 143.25, 133.80, 133.68, 132.01, 131.90, 126.28, 126.16, 62.94, 29.31, 23.55, 17.15; IR ($CDCl_3$) 3583, 2954, 2870, 1662, 1624, 1596, 1420, 1331, 1292, 1251, 1170, 1079, 996 cm^{-1}; MS (EI, 70 eV) m/z 228 (M^+, 100), 210 (17), 200 (37), 181 (21), 173 (62), 115 (35); HRMS calcd for $C_{14}H_{12}O_3$ 228.0786, found 228.0794.

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Supplementary Material Available: ^1H NMR and ^{13}C NMR spectra (19 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

Calophycin, a Fungicidal Cyclic Decapeptide from the Terrestrial Blue-Green Alga *Calothrix fusca*

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A broad-spectrum fungicide, calophycin, has been isolated from *Calothrix fusca* EU-10-1, a terrestrial blue-green alga belonging to Nostocaceae, and identified to be a cyclic decapeptide, cyclic (L-Ala-D-Asp-L-Asn-L-Gln-Gly-L-Arg-L-*N*-MeAsn-L-Pro-(2*R*,3*R*,4*S*)-Hamp-L-Val), where Hamp is a (2*R*,3*R*,4*S*)-3-amino-2-hydroxy-4-methylpalmitic acid unit and MeAsn is an *N*-methylasparagine residue. Its total structure, including absolute stereochemistry, was determined by a combination of spectral and chemical studies, including synthesis of the unusual β-amino acid Hamp.

In screening over 1000 strains of laboratory-cultured blue-green algae for fungicidal activity, we have found that extracts of more than 10% of these prokaryotes show activity against one or more of five test organisms, viz., *Aspergillus oryzae*, *Candida albicans*, *Penicillium notatum*, *Saccharomyces cerevisiae*, and *Trichophyton mentagrophytes*.[1,2] Nucleosides[3] and macrolides belonging to the scytophycin class[4] have frequently been identified in extracts that exhibit potent, broad-spectrum activity. We report here the isolation and total structure determination of a strongly antifungal cyclic decapeptide, calophycin (1), from *Calothrix fusca* (Kutzing) Bornet & Flahault, strain EU-10-1.[5]

The alga was isolated from a freshwater stream on the island of Oahu and grown in mass culture. Using a bioassay-directed isolation scheme, the extract (70% ethanol) of the lyophilized alga was subjected to repeated reversed-phase chromatography on C-18 and normal-phase chromatography on silica gel to give **1** as an amorphous white solid in 0.18% yield. The FAB mass spectrum indicated that the molecular weight was 1248 Da and detailed analyses of the ^{13}C and ^1H NMR spectra suggested

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(5) In a disc-diffusion soft agar plate assay calophycin at 1.2 μg/disc showed zones of inhibition of 13, 7, 12, 12, and 15 mm against *A. oryzae*, *C. albicans*, *P. notatum*, *S. cerevisiae*, and *T. mentagrophytes*, respectively (zones of inhibition at other doses and comparison with amphotericin B given in supplementary material). MIC values for calophycin against *C. albicans*, *T. mentagrophytes*, and *Aspergillus fumigatus* were found to be 1.25, 2.5, and 1.25 μg/mL, respectively, using Sabouraud dextrose broth as the test medium; by comparison amphotericin B showed MIC values of 0.625 and 1.25 μg/mL against *C. albicans* and *A. fumigatus*, respectively. Calophycin appeared to be moderately cytotoxic (IC_{50} 0.24 μg/mL against the KB cell line, a human nasopharyngeal carcinoma).

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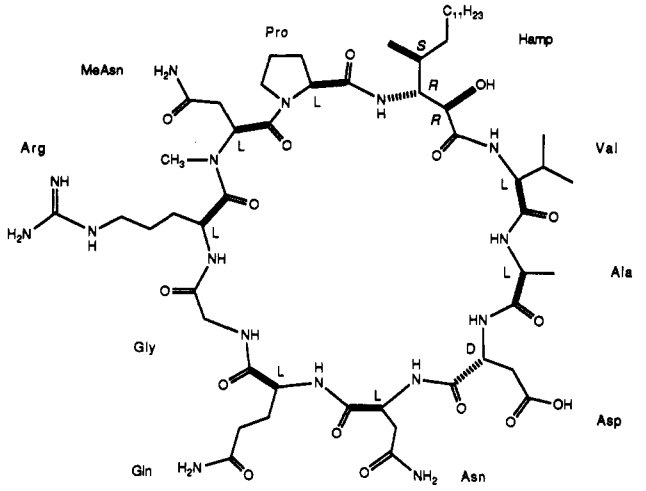
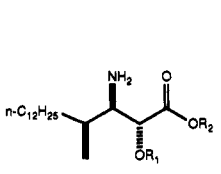


1

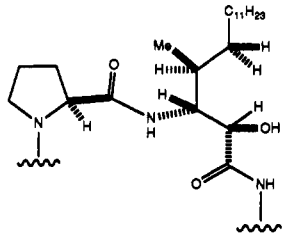
that the molecular formula was $C_{56}H_{96}N_{16}O_{16}$.

Gross Structure. Extensive two-dimensional NMR studies in DMSO-d_6 and MeOH-d_4 established the ten partial structures shown in Chart I. Nine were common α-amino acid units, viz., alanyl, arginyl, aspartyl, asparaginyl, glutaminyl, glycyl, *N*-methylasparaginyl (MeAsn), prolyl, and valyl units. The remaining unit, however, was 3-amino-2-hydroxy-4-methylpalmitoyl, a β-amino acid unit that is also a component of the cyclic decapeptide puwainaphycin E from *Anabaena* sp. BQ-16-1.[6] In support of the presence of these ten units, acid hydrolysis of 1 led to alanine, arginine, aspartic acid (2 equiv), glutamic acid, glycine, *N*-methylaspartic acid, proline, valine, and 3-amino-2-hydroxy-4-methylpalmitic acid (Hamp, 2). Hamp was characterized as the triester derivative 3.



2 $R_1 = R_2 = H$

3 $R_1 = Ac$; $R_2 = Me$



1a

In deducing the gross structures of the three α-amino acid units with primary amide functionalities, viz., the Asn, Gln, and MeAsn units, it was necessary to rigorously determine that the free carboxylic acid group was present in an aspartyl unit and not in a glutamyl or *N*-methylaspartyl unit.[7] An *N*-methylaspartyl unit could be ruled out, as the NOESY spectrum of 1 clearly indicated NOEs between the primary amide protons (5.93 and 7.22 ppm) and one of the protons (2.0 ppm) on C-3 of the MeAsn unit. Unfortunately we were not able to observe NOEs between the protons of the two other primary amide groups and the adjacent methylenes.

NMR analysis of 1 that had been uniformly enriched with ^{13}C to 80% and ^{15}N to 90%, however, permitted us to determine exactly where the carboxylic acid group was located. First of all, the 14 carbonyl carbon signals in the ^{13}C NMR spectrum were rigorously assigned from a COSY-X spectrum. The carbonyl carbon signal (177.2 ppm) that showed no coupling to ^{15}N was ascribed to the carbon of the carboxylic acid group and the COSY-X data established that it was located in an Asp unit. The ^{15}N-coupled signals at 173.7, 176.1, and 178.2 ppm were attributed to the primary amide carbons of the MeAsn, Asn, and Gln units, respectively.

A NOESY spectrum allowed us to connect the ten amino acid units into a total gross structure for 1. NOEs were observed between the following pairs of protons: Ala 2-H and Asp NH, Asp 2-H and Asn NH, Asn NH and Gln NH, Gln 2-H and Gly NH, Gly NH and Arg NH on C-2, Arg 2-H and MeAsn *N*-CH_3, MeAsn 2-H and Pro 5-H_2, Pro 2-H and Hamp NH, Hamp 2-H and Val NH, and Val 2-H and Ala NH. An HMBC experiment confirmed the amino acid sequence implied by the NOESY data with the following three-bond ^{13}C-^1H correlations: Ala C-2 and Asp NH, Asp C-2 and Asn NH, Asp C-1 and Asn 2-H, Gln C-2 and Gly NH, Gln C-1 and Gly 2-H_2, Gly C-2 and Arg NH (on C-2), Arg C-1 and MeAsn 2-H, Pro C-2 and Hamp NH, Pro C-1 and Hamp 3-H, Hamp C-2 and Val NH, Val C-2 and Ala NH, and Val C-1 and Ala 2-H.

Calophycin is structurally related to the puwainaphycins,[7] none of which, however, show antifungal activity. Puwainaphycin E[6] (deschloropuwainaphycin C), for example, differs from 1 in possessing *O*-MeThr, Thr, Thr, and (*E*)-Dhb ((*E*)-didehydrobutyrinyl) units in lieu of Arg, Asn, Asp, and Ala units.

Stereochemistry. Marfey analysis[8] of the amino acids in the acid hydrolyzate of calophycin indicated that the Ala, Arg, Gln, Gly, MeAsn, Pro, and Val units in 1 were L. The aspartic acid, however, was a 1:1 mixture of the L and D isomers, which meant that the Asp and Asn units were either D and L or L and D, respectively.

The Asp unit was found to be D as follows: Calophycin was subjected to a Hofmann reaction with *I,I*-bis(trifluoroacetoxy)iodobenzene,[9] whereby the primary amide groups of the Asn, Gln, and MeAsn units were converted to primary amines. Subsequent acid hydrolysis of the Hofmann product gave a mixture of α-amino acids that lacked L-Asp, L-Glu, and L-*N*-methylaspartic acid as shown by the Marfey analysis. In the HPLC analysis, however, the peak for D-Asp could be clearly seen.

The relative and absolute stereochemistry of Hamp was deduced by a combination of spectral and chemical studies. First of all we knew that the CD spectra of the 2-hydroxy-3-amino-4-methylpalmitic and stearic acids associated with the puwainaphycins,[6] one of which was Hamp in puwainaphycin E, exhibited a negative Cotton effect at 215 nm, strongly suggesting that the configuration of C-2 in all of these α-hydroxy-β-amino acids was *R*.[10] Second, the coupling constants between 2-H and 3-H (J = 10.5 Hz) of the corresponding 2-oxazolidone derivatives of these amino acids indicated that these two protons were cis to each other[11] and therefore the configurations of C-2 and C-3 were both *R*. Hence, C-2 and C-3 in the α-hydroxy-β-amino acids (e.g., Hamp) had to be both *R* (erythro). Finally, analysis of a NOESY spectrum of 1 hinted that C-4 in Hamp might have the *S* configuration, since cross peaks were noted between the following signals, 2-H and 3-H, 2-H and 5-H, 2-H and 5-H′, OH on C-2 and 3-H, NH and 4-H, and 3-H and the methyl protons on C-4.

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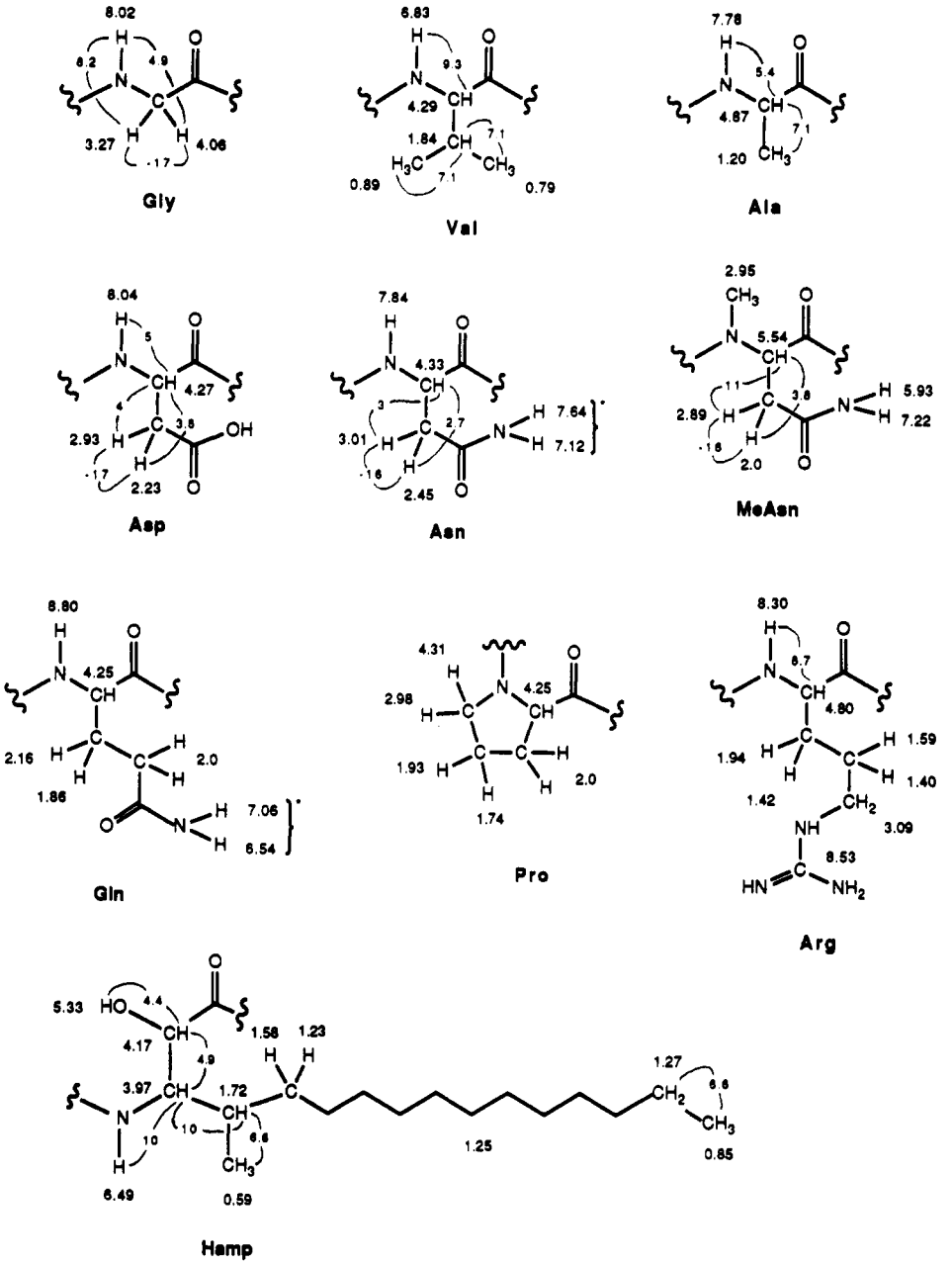
Chart I. Gross Structures of Amino Acid Units in Calophycin Showing ^1H Chemical Shifts and Coupling Constants in DMSO-d_6[a]



[a] Asterisk denotes tentative assignment.

These data suggested that (1) the absolute stereochemistry of Hamp was 2*R*,3*R*,4*S* and (2) the preferred conformation of this region in the calophycin molecule was as depicted in **1a**.

A stereoselective synthesis of (2*R*,3*R*,4*S*)-Hamp (Scheme I) was carried out to confirm the proposed absolute stereochemistry. Decylmagnesium bromide was added to (*R*)-(+)-citronellal and the resulting alcohol **4** was reduced, via mesylate **5**, to the hydrocarbon **6**. Ozonolysis led to aldehyde **7**, which was oxidized to carboxylic acid **8** and esterified to give methyl (4*S*)-4-methylpalmitate (**9**). Using the selenoxide elimination procedure,[12] ester **9** was converted into the E-α,β-unsaturated ester **10** and then reduced to the allylic alcohol **11**. Sharpless asymmetric epoxidation[13] yielded 2*R*,3*S*,4*S* epoxy alcohol **12**, which was oxidized[14] to the epoxy acid **13** and esterified to **14**. Epoxide cleavage with azidotrimethylsilane in the presence of a catalytic amount of zinc chloride[15] gave a 1:1 mixture of azido alcohols that were separated by chromatography. The desired 2*R*,3*R*,4*S* azide **15** was catalytically hydrogenated to the amino ester **16** and finally acetylated to (2*R*,3*R*,4*S*)-Hamp (**3**), which was identical in all respects, including optical properties, with **3** from degradation of calophycin.

Experimental Section

Spectral Analysis. NMR spectra were determined on 11.75- and 7.05-T instruments operating at 500 and 300 MHz for ^1H and

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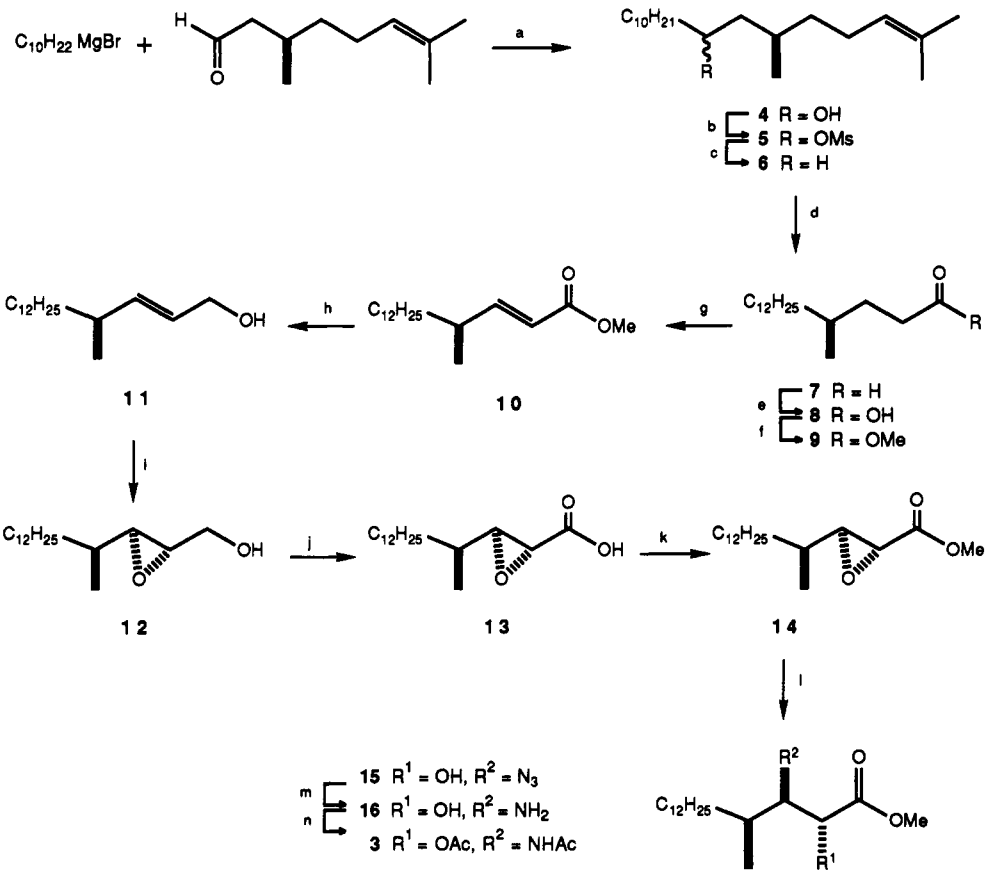
Scheme I. Stereoselective Synthesis of (2*R*,3*R*,4*S*)-Hamp (3)[a]



[a] Reagents, conditions, and yields: (a) −20 °C, 4 h, 99%; (b) MsCl, Et_3N; (c) $LiAlH_4$ (1 M in THF), 86% from 4; (d) O_3, CH_2Cl_2, −78 °C; EtSEt, 95%; (e) $NaClO_2$, *t*-BuOH, 20 h; (f) CH_2N_2, Et_2O, 88% from 7; (g) LDA (1.5 M in cyclohexane), THF, −78 °C, 1 h; PhSeSePh, −78 °C for 30 min and rt for 30 min; $NaIO_4$, THF/MeOH/H_2O, 4 h, 92%; (h) DIBAL-H, hexane, 98%; (i) Ti(O-*i*-Pr)$_4$, *tert*-butyl hydroperoxide, L-(+)-DET, −23 °C, 20 h, 70%; (j) RuO_4, $NaIO_4$, 48 h, 80%; (k) CH_2N_2, Et_2O, 100%; (l) Me_3SiN_3, $ZnCl_2$, 70 °C, 48 h, 45%; (m) Pd–C, H_2, 24 h; (n) Ac_2O, pyridine, 15 h, 95% from 15.

125 and 75 MHz for ^{13}C, respectively. ^1H and ^{13}C NMR chemical shifts are referenced to solvent peaks: δ_H 7.26 (residual $CHCl_3$) and δ_C 77.0 for $CDCl_3$, δ_H 3.30 (residual CHD_2OD) and δ_C 49.0 for methanol-d_4, and δ_H 2.49 (residual DMSO-d_5) and δ_C 39.5 for DMSO-d_6. Homonuclear ^1H connectivities were determined by the COSY experiment and heteronuclear ^1H–^{13}C connectivities were determined by HMQC and HMBC experiments.[16,17] Mass spectra were determined on a VG-70SE mass spectrometer operating in the EI or FAB mode.

Culture Conditions. *Calothrix fusca* (Kützing) Bornet & Flahault, designated strain number EU-10-1, was isolated from an algal sample collected in July, 1987, from a pond in an abandoned irrigation canal, Waimano Stream, Oahu. The cyanophyte was mass cultured in 20-L glass bottles containing a modified inorganic medium, designated A_3M_7, using a previously described procedure.[18,19] Cultures were harvested by filtration after 21 to 34 days to give yields of lyophilized cells ranging from 0.18 to 0.39 g/L.

Isolation of Calophycin. Freeze-dried alga (15 g) was extracted with 7:3 ethanol/H_2O (1 L) for 24 h at room temperature. The deep brown extract was concentrated under reduced pressure and the aqueous concentrate was washed with ethyl ether to remove pigments and then freeze-dried. The resulting dark brown powder (2.0 g) was dissolved in a minimum amount of 1:4 MeOH/H_2O and applied to a 40- × 70-mm column of reverse-phase silica (C-18, YMC) and the column was successively eluted with (a) water (100 mL), (b) 3:7 MeOH/H_2O (100 mL), (c) 1:1 MeOH/H_2O (100 mL), (d) 7:3 MeOH/H_2O (100 mL), (e) 9:1 MeOH/H_2O (100 mL), (f) MeOH (200 mL), and (g) EtOAc (100 mL). Antifungal activity was detected in the MeOH eluate. The material in the MeOH eluate was rechromatographed on a short column of silica gel (45- × 55-mm, 230–425 mesh), eluting with (a) 1:1 THF/MeOH (55 mL), (b) MeOH (50 mL) followed by 1:1 MeOH/H_2O (50 mL), and (c) 1:1 MeOH/H_2O (75 mL). Eluate b, which showed antifungal activity, was subjected to reversed-phase HPLC, first on a Alltech C-18 column with 1:8:1 THF/MeOH/H_2O and then on a YMC C-18 column with 1:8:1 MeCN/MeOH/H_2O to give 27 mg of pure calophycin as a white amorphous powder, $[\alpha]_D$ −85.8° (*c* 2.0, MeOH): FABMS (glycerol) *m/z* 1287 (MK^+), 1271 (MNa^+), 1249 (MH^+); HRFABMS *m/z* 1248.7152 ($C_{56}H_{96}N_{16}O_{16}$, MH^+, Δ3.8 mmu). ^1H NMR (500 MHz, DMSO-d_6): see Chart I. ^1H NMR (500 MHz, MeOH-d_4), amino acid unit δ (carbon position, multiplicity; *J* in Hz): Ala 4.86 (2, q; 7.0), 1.34 (3, d; 7.0); Asp 4.47 (2, t; 3.6), 3.17 (3, dd; −18 and 3.6), 2.58 (3, dd; −18 and 3.6); Asn 4.55 (2, t; 3.6), 3.27 (3, dd; −17 and 3.6), 2.63 (3, dd; −17 and 3.6); Gln 4.48 (2, m), 2.41 (3, m), 1.98 (3, m), 2.19 (4-H_2, m); Gly 3.55 (2, d; −17), 4.21 (2, d; −17); Arg 5.00 (2, dd; 11 and 2.3), 2.05 (3, m), 1.55 (3, m), 1.75 (4, m), 1.50 (4, m), 3.19 (5-H_2, m); MeAsn 5.69 (2, dd; 12 and 2.6), 3.15 (3, dd; −16 and 12), 2.09 (3, dd; −16 and 2.6), 3.06 (*N*-methyl, s); Pro 4.45 (2, m), 2.17 (3-H_2, m), 2.09 (4, m), 1.94 (4, m), 4.53 (5, m), 3.13 (5, m); Hamp 4.42 (2, d; 5.0), 4.10 (3, dd; 11 and 5.0), 1.76 (4, m), 0.72 (methyl on C-4, d; 6.5), 1.74 (5, m), 1.30 (5, m), 1.28 (18 H on C-6,7,8,9,10,11,12,13,14), 1.30 (15-H_2, m), 0.89 (16, t; 6.5); Val 4.31 (2, d; 5.5), 1.98 (3, m), 1.00 (4-H_3; 7.0), 0.88 (4′-H_3; 6.5). ^{13}C NMR (125 MHz, DMSO-d_6), amino acid unit δ (carbon position): Ala 173.12 (1), 48.16 (2), 17.21 (3); Asp 172.38 (1), 50.04 (2), 39.2 (3), 173.83 (4); Asn 170.46 (1), 49.30 (2), 35.36 (3), 173.54

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(4); Gln 171.46 (1), 51.25 (2), 25.83 (3), 31.43 (4), 174.08 (5); Gly 169.07 (1), 42.11 (2); Arg 172.75 (1), 48.84 (2), 26.59 (3), 25.36 (4), 39.9 (5), 156.72 (guanido); MeAsn 167.39 (1), 50.71 (2), 33.64 (3), 171.00 (4), 30.11 (*N*-methyl); Pro 171.10 (1), 59.92 (2), 29.90 (3), 23.71 (4), 46.15 (5); Hamp 169.30 (1), 69.88 (2), 56.01 (3), 31.98 (4), 15.67 (methyl on C-4), 33.24 (5), 24.96, 29.45, 28.87, 28.77, 28.77 (10), 28.77 (11), 28.71, 28.39, 30.99 (14), 21.77 (15), 13.59 (16); Val 170.01 (1), 55.16 (2), 31.87 (3), 17.70 (4), 18.86 (4'). ^{13}C NMR (125 MHz, MeOH-d_4), amino acid unit δ (carbon position): Ala 176.79 (1), 50.54 (2), 17.44 (3); Asp 174.89 (1), 52.47 (2), 39.73 (3), 177.23 (4); Asn 173.40 (1), 51.20 (2), 36.24 (3), 176.09 (4); Gln 174.35 (1), 53.19 (2), 27.52 (3), 32.74 (4), 178.24 (5); Gly 172.30 (1), 43.72 (2); Arg 174.75 (1), 50.83 (2), 28.34 (3), 26.97 (4), 41.61 (5), 158.49 (guanido); MeAsn 169.97 (1), 51.99 (2), 34.57 (3), 173.73 (4), 31.06 (*N*-methyl); Pro 174.68 (1), 61.81 (2), 31.56 (3), 25.13 (4), 48.75 (5); Hamp 173.01 (1), 70.95 (2), 59.08 (3), 34.25 (4), 16.59 (methyl on C-4), 35.06 (5), 27.05, 31.40, 30.88, 30.79, 30.79 (10), 30.79 (11), 30.79, 30.47, 33.07 (14), 23.72 (15), 14.44 (16); Val 172.67 (1), 57.40 (2), 34.35 (3), 18.11 (4), 20.05 (4').

$^{13}C,^{15}N$-Enriched Calophycin. The alga was grown on $Na^{13}CO_3$ and $Na^{15}NO_3$ using a previously described procedure[20] to give 1.42 g of dried alga from an 8-L culture from which 2.4 mg of labeled calophycin, estimated by ^{13}C NMR analysis to be uniformly enriched to 80% ^{13}C and 90% ^{15}N, was isolated. ^{13}C NMR (125 MHz, DMSO-d_6), amino acid unit C=O carbon position δ (multiplicity; coupling constants in Hz): Ala 173.12 (dd); Asp C1 172.38 (dd), Asp C4 173.83 (d); Asn C1 170.46 (dd), Asn C4 173.54 (dd); Gln C1 171.46 (dd), Gln C5 174.08 (dd); Gly 169.07 (dd); Arg 172.75 (dd); MeAsn C1 167.39 (dd), MeAsn C4 171.00 (dd); Pro 171.10 (dd); Hamp 169.30 (dd); Val 170.01 (dd). ^{13}C NMR (125 MHz, MeOH-d_4), amino acid unit C=O carbon position δ (multiplicity; coupling constants in Hz): Ala 176.79 (dd; J_{CC} = 53.7, J_{CN} = 14.8); Asp C1 174.89 (dd), Asp C4 177.23 (d; J_{CC} = 51.0); Asn C1 173.40 (dd), Asn C-4 176.09 (dd; J_{CC} = 47.0, J_{CN} = 16.1); Gln C1 174.35 (dd; J_{CC} = 53.7, J_{CN} = 14.8), Gln C5 178.24 (dd; J_{CC} = 48.3, J_{CN} = 16.1); Gly 172.30 (dd; J_{CC} = 53.6, J_{CN} = 17.4); Arg 174.75 (dd); MeAsn C1 169.97 (dd; J_{CC} = 56.4, J_{CN} = 14.8), MeAsn C4 173.73 (dd; J_{CC} = 48.3, J_{CN} = 16.1); Pro 174.68 (dd); Hamp 173.01 (dd); Val 172.67 (dd).

Acid Hydrolysis. Calophycin (4.1 mg) was dissolved in 3 M HCl and the solution heated to 100 °C for a day. The reaction mixture was freeze-dried and the acid hydrolyzate, in water, was passed through a 2- × 0.9-cm column of C-18 (BondElut), eluting first with 12 mL of water and then with 12 mL of MeOH. A mixture of alanine, arginine, aspartic acid (2 equiv), glutamic acid, glycine, *N*-methylaspartic acid, proline, and valine was found in the water fraction by standard amino acid analysis and 3-amino-2-hydroxy-4-methylpalmitic acid (**2**, Hamp, 0.4 mg) was found in the MeOH fraction.

Hofmann Reaction and Acid Hydrolysis. A solution of calophycin (1 mg) in DMF (100 μL) was mixed with a DMF solution of *I,I*-bis(trifluoroacetoxy)iodobenzene (200 μL, 0.16 M) and the mixture was stirred at room temperature for 5 h.[9] After addition of water and extraction with ether to remove excess reagent, the mixture was freeze-dried and the residue subjected to acid hydrolysis as described above.

Amino Acid Analysis. The amino acids in the acid hydrolyzates of calophycin and the Hofmann degradation product of calophycin were derivatized with Marfey's reagent[8] (1-fluoro-2,4-dinitrophenyl-5-L-alanine amide) and analyzed by reversed-phase HPLC on a C-18 column (Pierce Spheri-5, 100 mm). A linear gradient of 10 to 40% acetonitrile in 0.05 M triethylamine phosphate buffered at pH 2.96 (flow rate 2 mL/min; UV detection at 340 nM) was used to separate the amino acid derivatives. The absolute stereochemistry of each compound was determined by comparing the retention time with those for the authentic L and D amino acid derivatives. The retention times (min) and configurations of the amino acids in the acid hydrolyzate of calophycin were found to be 12.3 (L-Arg), 16.1 (L-Asp), 18.1 (L-Glu), 18.3 (L-*N*-MeAsp), 19.1 (Gly), 20.0 (D-Asp), 20.3 (L-Ala), 22.8 (L-Pro), and 30.1 (L-Val). In the HPLC trace for the Marfey derivatives of the amino acids in the acid hydrolyzate of the Hofmann degradation product, the peak at 16.1 min (L-Asp) was missing, but the peak at 20.0 min (D-Asp) was still present.

Conversion of Hamp to Compound 3. The crude Hamp was dissolved in acetic anhydride (0.5 mL) and pyridine (0.4 mL) and the mixture was stirred overnight. The reaction mixture was diluted with ether and the ether layer was washed successively with water, saturated sodium bicarbonate, and brine, dried over anhydrous magnesium sulfate, and evaporated in vacuo to give the diacetate. The diacetate was dissolved in ether and methylated with diazomethane. The methyl ester of Hamp diacetate (**3**) was purified by HPLC on silica (Alltech Econosil, 10 μm) with 1:1 hexane/THF and had the following physical properties: mp 44 °C; $[\alpha]_D$ +17.4° (*c* 0.75, $CHCl_3$); CD (1,4-dioxane) $[\theta]_{211}$ −3314; EIMS *m/z* 399 (M^+); ^1H NMR (500 MHz, $CDCl_3$) δ 5.51 (d, *J* = 10.5 Hz, NH), 5.02 (d, *J* = 5.7 Hz, 2-H), 4.47 (ddd, *J* = 10.5, 5.7 and 4.8 Hz, 3-H), 3.74 (s, OMe), 2.14 (s, OAc), 2.02 (s, OAc), 1.72 (m, 4-H), 1.49 (m, 2 H), 1.32 (m, 2 H), 1.25 (m, 18 H), 0.90 (d, *J* = 6.6 Hz, Me on C-4), 0.88 (t, *J* = 6.7 Hz, 16-H_3).

(6*S*)-2,6-Dimethyl-2-octadecene (6). To a solution of decylmagnesium bromide (11 mL, 1.0 M) in ether was added dropwise at −20 °C a solution of (*R*)-(+)-citronellal (1.60 g, 96% pure, 10 mmol) in ether. The mixture was stirred for 2 h at 0 °C and then diluted with 1:1 hexane/ether, washed successively with 1% acetic acid in water, water, and brine, dried over anhydrous $MgSO_4$, and evaporated. The residual oil was purified by chromatography (15:1 hexane/ether, silica gel) to give a 1:1 mixture of (6*R*,8*R*)- and (6*R*,8*S*)-8-hydroxy-2,6-dimethyl-2-octadecene (**4**) (2.93 g, 99%) as an oil: HREIMS *m/z* 296.3067 ($C_{20}H_{40}O$, M^+, Δ1.2 mmu); ^1H NMR (300 MHz, $CDCl_3$) δ 5.10 (t, *J* = 7.0 Hz, 1 H), 3.68 (m, 1 H), 1.98 (m, 2 H), 1.68 (s, 3 H), 1.60 (s, 3 H), 1.40 (m, 5 H), 1.26 (m, 18 H), 1.17 (m, 1 H), 0.91/0.89 (d, *J* = 6.8 Hz, 3 H), 0.87 (t, *J* = 6.9 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 131.20/131.16 (C), 124.76 (CH), 69.96/69.60 (CH), 45.14/44.90 (CH_2), 38.37 (CH_2), 37.94/37.74 (CH_2), 36.63 (CH), 31.90 (CH_2), 29.70 (CH_2), 29.62 (CH_2), 29.33/29.26 (CH_2), 28.88 (CH_2), 25.70 (CH_3), 25.54 (CH_2), 25.47/25.34 (CH_2), 22.68 (CH_2), 20.32 (CH_2), 19.16 (CH_3), 17.64 (CH_3), 14.11 (CH_3).

The mixture of (6*R*,8*R*)- and (6*R*,8*S*)-8-hydroxy-2,6-dimethyl-2-octadecene (**4**) (3 g, 10 mmol) was dissolved in 20 mL of methylene chloride and the solution was cooled to −20 °C. Five milliliters of Et_3N was added followed by a dropwise addition of 1.5 mL of methanesulfonyl chloride (98%, 18 mmol). The mixture was stirred for 1 h at −20 °C, diluted with 1:1 hexane/ether, washed successively with water and brine, and dried over anhydrous $MgSO_4$. After evaporation of the solvent, the residual mesylate **5** was dried in vacuo. A solution of **5** in 10 mL of THF was added dropwise to 15 mL of 1 M $LiAlH_4$ in THF at −20 °C. After being stirred for 4 h at room temperature, the reaction mixture was diluted with 1:1 hexane/ether and cooled to −20 °C and the excess $LiAlH_4$ was decomposed by adding water dropwise. The mixture was filtered and evaporated to give alkene **6** (2.4 g, 86%) as an oil:[21] $[\alpha]_D$ −0.69° (*c* 1.6, $CHCl_3$); HREIMS *m/z* 280.3130 ($C_{20}H_{40}$, M^+, Δ0.0 mmu); ^1H NMR (300 MHz, $CDCl_3$) δ 5.12 (t, *J* = 7.0 Hz, 1 H), 1.98 (dm, *J* = 7.0 Hz, 2 H), 1.70 (s, 3 H), 1.62 (s, 3 H), 1.28 (m, 23 H), 1.12 (m, 2 H), 0.90 (t, *J* = 6.2 Hz, 3 H), 0.87 (d, *J* = 6.3 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 130.86 (C), 125.13 (CH), 37.20 (CH_2), 37.05 (CH), 32.47 (CH_2), 32.00 (CH_2), 30.09 (CH_2), 29.78 (5CH_2), 29.44 (CH_2), 27.10 (CH_2), 25.72 (CH_3), 25.63 (CH_2), 22.75 (CH_2), 19.62 (CH_3), 17.61 (CH_3), 14.14 (CH_3); El mass spectrum *m/z* 280 (M^+).

Methyl (4*S*)-4-Methylhexadecanoate (9). Ozone was passed through a solution of alkene **6** (2.8 g, 10 mmol) in methylene chloride (150 mL) at −78 °C until a blue color appeared. The solution was then flushed with oxygen and ethyl sulfide (1.2 mL) was added. The mixture was stirred for 1 h and then the cooling bath was removed. After being stirred for another 2 h, the mixture was washed successively with water and brine, dried over anhydrous $MgSO_4$, and evaporated to afford (4*S*)-4-methylhexadecanal (**7**) (2.5 g, 95%) as an oil:[21] $[\alpha]_D$ +0.045° (*c* 2.85, $CHCl_3$); HREIMS *m/z* 254.2694 ($C_{17}H_{34}O$, M^+, Δ−8.4 mmu); ^1H NMR (300 MHz, $CDCl_3$) δ 9.76 (t, *J* = 1.8 Hz, 1 H), 2.41 (m, 2 H), 1.67 (m, 1 H), 1.42 (m, 3 H), 1.25 (m, 21 H), 1.17 (m, 1 H), 0.873 (t, *J* = 6.2 Hz, 3 H), 0.868 (d, *J* = 6.0 Hz, 3 H); ^{13}C NMR

(20) See footnote 8 in Carmeli, S.; Moore, R. E.; Patterson, G. M. L.; Corbett, T. H.; Valeriote, F. A. *J. Am. Chem. Soc.* **1990**, *112*, 8195–8197.

(21) Shown to be ≥99% a single component by HPLC analysis.

(75 MHz, $CDCl_3$) δ 203.13 (CH), 41.70 (CH_2), 36.66 (CH), 32.37 (CH_2), 31.90 (CH_2), 31.65 (CH_2), 29.90 (CH_2), 29.66 ($4CH_2$), 29.34 (CH_2), 28.88 (CH_2), 26.93 (CH_2), 22.68 (CH_2), 19.33 (CH_3), 14.10 (CH_3).

A mixture of aldehyde **7** (2.54 g, 10 mmol), *tert*-butyl alcohol (75 mL), saturated aqueous sodium phosphate (monobasic, 50 mL), 2-methyl-2-butene (10 mL), and sodium chlorite (1.35 g, 80%, 12 mmol) was stirred overnight at room temperature. The reaction mixture was extracted with 1:1 hexane/ethyl acetate, the extract was dried ($MgSO_4$) and evaporated, and the residual acid **8** was dissolved in ether and treated with excess diazomethane. After removal of solvent the crude ester was purified by chromatography (20:1 hexane/EtOAc, silica) to give **9** (2.5 g, 88% from **7**) as an oil: $[\alpha]_D$ −0.33° (*c* 7.3, $CHCl_3$); HREIMS *m/z* 284.2723 ($C_{18}H_{36}O_2$, M^+, Δ−0.8 mmu); ^1H NMR (300 MHz, $CDCl_3$) δ 3.61 (s, 3 H), 2.26 (m, 2 H), 1.61 (m, 1 H), 1.37 (m, 2 H), 1.22 (m, 21 H), 1.16 (m, 1 H), 0.83 (t, *J* = 6.3 Hz, 3 H), 0.82 (d, *J* = 6.4 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.29 (C), 51.24 (CH_3), 36.61 (CH), 32.33 (CH_2), 31.85 (CH_2), 31.76 (CH_2), 29.87 (CH_2), 29.63 ($6CH_2$), 29.32 (CH_2), 26.87 (CH_2), 22.62 (CH_2), 19.16 (CH_3), 14.00 (CH_3). Short-path distillation at 120 °C (2.8 mm) gave pure **9**. Anal. Calcd for $C_{18}H_{36}O_2$: C, 76.00; H, 12.75. Found: C, 75.92; H, 12.84.

Methyl (4*S*)-4-Methyl-2(*E*)-hexadecenoate (10). Ester **9** (2.84 g, 10 mmol) was dissolved in anhydrous THF (100 mL) and a lithium diisopropylamide solution (8 mL, 1.5 M in cyclohexane) was added at −78 °C. The mixture was stirred for 30 min and then a solution of diphenyl diselenide (3.74 g, 12 mmol) in THF (5 mL) was added via cannula. After stirring for 30 min at −78 °C, the cooling bath was removed and stirring was continued for another hour. The reaction mixture was acidified with saturated NH_4Cl solution and extracted with 1:1 hexane/ether. The extract was washed with water and brine, dried ($MgSO_4$), and evaporated. Chromatography (hexane and then 40:1 hexane/ether, silica) afforded a yellowish oil, which was dissolved in 1:5 THF/MeOH (60 mL) and treated with a solution of sodium periodate (2.56 g, 12 mmol) in water (10 mL). After stirring this mixture vigorously at room temperature for 1 h, sodium bicarbonate (700 mg) was added. After an additional hour, 1:1 hexane/ether was added and the organic layer was washed with saturated bicarbonate and brine, dried ($MgSO_4$), and evaporated to give the α,β-unsaturated ester **10** (2.6 g, 92%) as an oil:[21] $[\alpha]_D$ +8.2° (*c* 3.0, $CHCl_3$); HREIMS *m/z* 282.2549 ($C_{18}H_{34}O_2$, M^+, Δ0.9 mmu); ^1H NMR (300 MHz, $CDCl_3$) δ 6.88 (dd, *J* = 15.7 and 7.8 Hz, 1 H), 5.83 (dd, *J* = 15.7 and 0.9 Hz, 1 H), 3.74 (s, 3 H), 2.30 (m, 1 H), 1.34 (m, 2 H), 1.26 (m, 20 H), 1.05 (d, *J* = 6.7 Hz, 3 H), 0.89 (t, *J* = 6.6 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.34 (C), 155.07 (CH), 119.06 (CH), 51.33 (CH_3), 36.53 (CH), 36.00 (CH_2), 31.89 (CH_2), 29.62 ($5CH_2$), 29.54 (CH_2), 29.33 (CH_2), 27.17 (CH_2), 22.66 (CH_2), 19.37 (CH_3), 14.08 (CH_3).

***trans*-(4*S*)-4-Methylhexadec-2-en-1-ol (11).** A solution of diisobutylaluminum hydride (DIBAL-H, 10 mL, 1 M in hexane) was added to ester **10** (2.50 g, 9.2 mmol) at −20 °C. The mixture was stirred for 2 h at room temperature and then water was added dropwise to decompose excess hydride. The mixture was diluted with ether, filtered, and evaporated to give the allylic alcohol **11** (2.3 g, 98%) as a colorless oil:[21] $[\alpha]_D$ +12.1° (*c* 1.0, $CHCl_3$); HREIMS *m/z* 236.2512 ($C_{17}H_{32}$, $M - H_2O^+$, Δ−0.8 mmu); ^1H NMR (300 MHz, $CDCl_3$) δ 5.58 (m, 1 H), 5.56 (m, 1 H), 4.07 (d, *J* = 4.2 Hz, 2 H), 2.11 (m, 1 H), 1.24 (m, 23 H), 0.96 (d, *J* = 6.7 Hz, 3 H), 0.87 (t, *J* = 6.5 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 139.28 (CH), 126.96 (CH), 63.85 (CH_2), 36.82 (CH), 36.29 (CH_2), 31.91 (CH_2), 29.67 (6 CH_2), 29.35 (CH_2), 27.27 (CH_2), 22.67 (CH_2), 20.35 (CH_3), 14.10 (CH_3).

(2*R*,3*S*,4*S*)-2,3-Epoxy-4-methyl-1-hexadecanol (12). Titanium tetraisopropoxide (1.5 mL, 5 mmol) and L-(+)-diethyl tartarate (0.86 mL, 5 mmol) were added sequentially to CH_2Cl_2 (50 mL) cooled to −25 °C with stirring. After 5 min allylic alcohol **11** (1.27 g, 5 mmol) and a 3.0 M *tert*-butyl hydroperoxide solution in 2,2,4-trimethylpentane (3.3 mL, 10 mmol) were added. The resulting solution was placed in the freezer overnight. A 10% aqueous L-(+)-tartaric acid solution (13 mL) was then added with stirring at −25 °C and after 30 min the cooling bath was removed and stirring was continued for 1 h. The mixture was diluted with 1:2 hexane/ether and the organic layer was washed with brine and evaporated. Chromatography of the residue on silica gel (4:1 hexane/ether) afforded the epoxide **12** (0.95 g, 70%) as an oil:[21] $[\alpha]_D$ −14.0° (*c* −0.55, $CHCl_3$); HREIMS *m/z* 270.2504 ($C_{17}H_{34}O_2$, M^+, Δ5.5 mmu); ^1H NMR (300 MHz, $CDCl_3$) δ 3.89 (dd, *J* = −12.5 and 2.4 Hz, 1 H), 3.57 (dd, *J* = −12.5 and 4.4 Hz, 1 H), 2.91 (ddd, *J* = 4.4, 2.4, and 2.0 Hz, 1 H), 2.75 (dd, *J* = 7.1 and 2.0 Hz, 1 H), 1.49 (m, 1 H), 1.38 (m, 2 H), 1.24 (m, 21 H), 0.92 (d, *J* = 6.7 Hz, 3 H), 0.88 (t, *J* = 6.6 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 61.91 (CH), 60.64 (CH), 57.16 (CH_2), 35.27 (CH), 34.44 (CH_2), 31.90 (CH_2), 31.56 (CH_2), 29.86 (CH_2), 29.64 ($4CH_2$), 29.34 (CH_2), 26.83 (CH_2), 22.62 (CH_2), 15.76 (CH_3), 14.09 (CH_3).

(2*R*,3*S*,4*S*)-Methyl 2,3-Epoxy-4-methylhexadecanoate (14). To a mixture of the epoxy alcohol **12** (270 mg, 1 mmol), sodium bicarbonate (0.5 g, 6.5 mmol), and sodium periodate (0.8 g, 3.9 mmol) in carbon tetrachloride (3 mL), acetonitrile (3 mL), and water (4 mL) was added ruthenium chloride hydrate (10 mg, 0.048 mmol). The mixture was stirred vigorously at room temperature for 48 h and then the organic layer was separated and washed with 1 M sodium bicarbonate. The aqueous portions were combined, carefully acidified with 1 N HCl at 0 °C, and extracted with EtOAc. The extract, which contained acid **13**, was dried ($MgSO_4$), treated for 20 min with excess ethereal diazomethane, filtered, and evaporated to give a residue, which was chromatographed on silica gel with 10% ether in hexane to give methyl ester **14** (240 mg, 80%) as an oil: $[\alpha]_D$ −3.6° (*c* 5.4, $CHCl_3$); HREIMS *m/z* 298.2522 ($C_{18}H_{34}O_3$, M^+, Δ−1.4 mmu); ^1H NMR (300 MHz, $CDCl_3$) δ 3.77 (s, 3 H), 3.22 (d, *J* = 1.9 Hz, 1 H), 2.98 (dd, *J* = 6.9 and 1.9 Hz, 1 H), 1.51 (m, 1 H), 1.42 (m, 2 H), 1.37 (m, 2 H), 1.25 (m, 18 H), 0.94 (d, *J* = 6.7 Hz, 3 H), 0.87 (t, *J* = 6.6 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.92 (C), 63.09 (CH), 52.36 (CH), 51.63 (CH_3), 35.39 (CH), 34.27 (CH_2), 31.89 (CH_2), 29.76 (CH_2), 29.62 (3 CH_2), 29.55 (2 CH_2), 29.33 (CH_2), 26.70 (CH_2), 22.66 (CH_2), 15.64 (CH_3), 14.08 (CH_3). Short-path distillation at 130 °C (2.8 mm) gave pure **14**. Anal. Calcd for $C_{18}H_{34}O_3$: C, 72.44; H, 11.48. Found: C, 72.26; H, 11.69.

(2*R*,3*R*,4*S*)-Methyl 3-Acetamido-2-acetoxy-4-methylpalmitate (3). A mixture of epoxy ester **14** (100 mg, 0.36 mmol), 58 mg (0.5 mmol) of azidotrimethylsilane, and 5 mg (0.037 mmol) of zinc chloride was stirred at 70 °C for 48 h and then, with continued stirring at room temperature, treated with 0.5 mL of tetrahydrofuran, 40 μL of acetic acid, and 20 μL of concentrated hydrochloric acid for 30 min. Extraction with CH_2Cl_2 gave a 1:1 mixture of (2*R*,3*R*,4*S*)-methyl 3-azido-2-hydroxy-4-methylhexadecanoate (**15**) and the 2-azido-3-hydroxy isomer, which was separated by silica gel chromatography with 20% ether in hexane to yield 51 mg (45%) of **15**:[21] $[\alpha]_D$ +6.9° (*c* 0.85, $CHCl_3$); HREIMS *m/z* 313.2625 ($C_{18}H_{35}NO_3$, $M - N_2^+$, Δ−0.8 mmu); ^1H NMR (300 MHz, $CDCl_3$) δ 4.30 (d, *J* = 5.6 Hz, 1 H), 3.85 (s, 3 H), 3.42 (dd, *J* = 6.0 and 5.6 Hz, 1 H), 1.91 (m, 1 H), 1.45 (m, 2 H), 1.26 (m, 21 H), 0.97 (d, *J* = 6.7 Hz, 3 H), 0.88 (t, *J* = 6.6 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.44 (C), 71.32 (CH), 70.04 (CH), 53.11 (CH_3), 33.90 (CH), 33.77 (CH_2), 31.91 (CH_2), 29.65 ($6CH_2$), 29.34 (CH_2), 26.59 (CH_2), 22.68 (CH_2), 15.19 (CH_3), 14.11 (CH_3).

A mixture of 15 mg (0.047 mmol) of azide **15** and 12 mg of 5% palladium on activated carbon in 1 mL of methanol was vigorously stirred under hydrogen. After 48 h the mixture was filtered and the methanol evaporated to give the methyl ester of Hamp (**16**), which was acetylated with 0.5 mL of 1:1 acetic anhydride/pyridine at room temperature with stirring for 15 h. After evaporation the crude diacetate was purified by silica gel chromatography with 40% ethyl acetate in hexane to give 17.8 mg (95% from **15**) of **3** as a solid: mp 44 °C; $[\alpha]_D$ +17.1° (*c* 0.75, $CHCl_3$); CD (1,4-dioxane) $[\theta]_{211}$ −3312; HREIMS *m/z* 399.2980 ($C_{22}H_{41}NO_5$, M^+, Δ0.5 mmu); ^1H NMR (500 MHz, $CDCl_3$) δ 5.51 (d, *J* = 10.5 Hz, NH), 5.02 (d, *J* = 5.7 Hz, 1 H), 4.47 (ddd, *J* = 10.5, 5.7, and 4.8 Hz, 1 H), 3.74 (s, 3 H), 2.14 (s, 3 H), 2.02 (s, 3 H), 1.72 (m, 1 H), 1.49 (m, 2 H), 1.32 (m, 2 H), 1.25 (m, 18 H), 0.90 (d, *J* = 6.6 Hz, 3 H), 0.88 (t, *J* = 6.7 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.15 (C), 170.01 (C), 169.13 (C), 72.92 (CH), 52.52 (CH), 51.89 (CH_3), 33.98 (CH), 33.49 (CH_2), 31.90 (CH_2), 29.63 ($6CH_2$), 29.33 (CH_2), 26.85 (CH_2), 23.30 (CH_3), 22.67 (CH_2), 20.62 (CH_3), 14.60 (CH_3), 14.10 (CH_3). Anal. Calcd for $C_{22}H_{41}NO_5$: C, 66.13: H, 10.34. Found: C, 66.45; H, 10.61.

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Supplementary Material Available: 500-MHz ^1H NMR

spectrum of 1 in DMSO-d_6 and MeOH-d_4, table comparing antifungal activity of calophycin and amphotericin B, 300-MHz ^{1}H and 75-MHz ^{13}C NMR spectra of compounds 4, 6, 7, 9–12, 14, and 15 in $CDCl_3$, and 500-MHz ^{1}H NMR spectrum of synthetic 3 in $CDCl_3$ (22 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

A Route to Several Stereoisomers of Castanospermine

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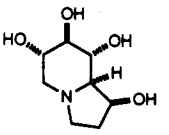
The following three stereoisomers of castanospermine have been prepared: (1*R*,6*R*,7*R*,8*S*,8a*R*)-1,6,7,8-tetrahydroxyindolizidine (1), (1*R*,6*S*,7*S*,8*S*,8a*R*)-1,6,7,8-tetrahydroxyindolizidine (2), and (1*R*,6*R*,7*S*,8*S*,8a*R*)-1,6,7,8-tetrahydroxyindolizidine (3). Each of these compounds was synthesized via asymmetric allylation of pentose derivatives with [(*Z*)-γ-(methoxymethoxy)allyl]diisopinocampheylborane, an approach which is, in principle, suitable for preparations of a total of eight stereoisomers of castanospermine. Compounds 1–3 were tested as possible inhibitors of various glycosidase enzymes and for anti-HIV-1 activity in cell cultures. They are very poor inhibitors of glycosidase enzymes; however, preliminary tests indicate indolizidines 1 and 3 have weak but significant anti-HIV activities.

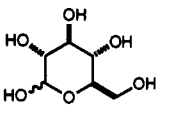
Introduction

Polyhydroxylated indolizidines show varied biological activities based on their ability to act as competitive inhibitors of glycosidase enzymes.[1-11] There is particular interest in inhibition of the glycoprotein-processing enzyme glucosidase I because this interferes with the processing of the surface glycoproteins of HIV-1, suppressing viral replication and virus-induced syncitia formation.[12]

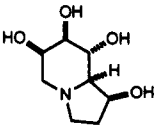
Castanospermine, isolated from *Castanospermum australe* and *Alexa leiopetala*,[13,14] is a potent glucosidase I inhibitor.[15] The stereochemistry of this molecule corresponds to the pyranose form of glucose; consequently, one might expect that, compared with its stereoisomers, castanospermine would be the most potent inhibitor of glucosidase enzymes. However, other observations imply this prediction is not necessarily correct. For instance, 6-epicastanospermine resembles mannose, but it is a poor inhibitor of several mannosidases and an effective inhibitor of amyloglucosidase.[16]



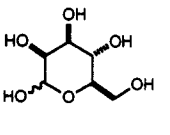
(+)-castanospermine



D-glucose



(+)-6-epicastanospermine



D-mannose

A theoretical study indicates topographic similarity with the mannopyranosyl cation, not with mannose itself, is the key to inhibition of mannosidase enzymes.[17] Calculations comparing castanospermine derivatives with the glucopyranosyl cation, however, have not been reported.[18] In any event their predictive value would be suspect since the mechanistic origins of the enzyme activity have not been elucidated and no structural information is available for the active sites of glucosidase I. At this time the *only* reliable way to formulate a structure/activity relationship for these compounds is to synthesize them and determine their biological activities.

Only eight of the 31 stereoisomers of castanospermine have been synthesized. Some of these were prepared by design[19-22] while others were obtained as byproducts en

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