6. Oceanapins A–F, Unique Branched Ceramides Isolated from the Haplosclerid Sponge *Oceanapia* cf. *tenuis* of the Coral Sea

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Dedicated to the memory of *Margherita Ratti*

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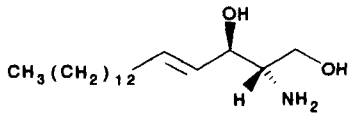
A series of ceramides, called oceanapins A–F (**2–7**), which are unique for branching at both the sphingosine and fatty-acid chains, have been isolated as pure compounds from the haplosclerid sponge *Oceanapia* cf. *tenuis* of the Coral Sea. Following acid hydrolysis, both the fatty-acid and the sphingosine portions were obtained separately, which allowed their unequivocal structural definition. The absolute configuration was secured *via* protection of C(1′)–OH and *Mosher*'s esterification at C(3′)–OH of the oceanapins.

1. Introduction. – Sphingosine (**1**) [1] is long known from hydrolysates of brain tissues [2] which, like all animal sphingolipids [3], also contain sphingosine amides of long-chain fatty acids (ceramides) and their glycosyl derivatives (cerebrosides).



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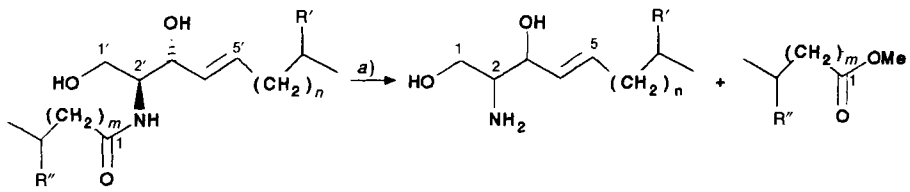
Sphingosine derivatives were later also isolated from seaweeds, both green [4] [5] and red [6], symbiontic dinoflagellates [7], and sea anemones [8]. All these sphingosine derivatives are built on linear chains. Isopropyl [9] [10] or ante-iso termini [11] have only been found in the sphingosine portion of ceramides [9] [10] and cerebrosides [11] of a few other marine invertebrates.

With few exceptions [7] [8], sphingosine derivatives were found to occur as mixtures of homologous or isomeric compounds, the separation of which has never been achieved [4–6] [9–12], though eagerly solicited [13]. We report here on the isolation from the tropical sponge *Oceanapia* cf. *tenuis* (Haplosclerida) of pure homologous or isomeric ceramides that are unique for branching on both the sphingosine and the fatty-acid chains. This allowed us also to assign their absolute configuration.

2. Results and Discussion. – 2.1. *Chromatographic Separation.* A combination of FC, *CN*- and *RP*-HPLC proved to be an excellent method for separating oceanapins **2–7**

(*Scheme 1*). As expected, the observed retention times correlate with the length of the lipophilic chain. Accordingly, oceanapins with short and/or branched side chains are eluted first (*Table*).

Scheme 1



2 $n = 9$, $m = 19$, R′ = H, R″ = Me
3 $n = 9$, $m = 19$, R′ = R″ = Me
4 $n = 9$, $m = 20$, R′ = Me, R″ = H
5 $n = 10$, $m = 19$, R′ = R″ = Me
6 $n = 10$, $m = 19$, R′ = Et, R″ = H
7 $n = 10$, $m = 19$, R′ = Et, R″ = Me

8a $n = 9$, R′ = H
b $n = 9$, R′ = Me
c $n = 10$, R′ = Me
d $n = 10$, R′ = Et

9a $m = 19$, R″ = Me
b $m = 20$, R″ = H
c $m = 19$, R″ = H

a) 1.2M H_2SO_4 in 85% MeOH, reflux, 6 h.

Table. *HPLC Retention Times* vs. *Chain Length and Relative Abundance for Oceanapins* **2**–**7**

Oceanapins	No. of C-atoms			Relative abundance[a])	t_R[b])
	total	fatty-acid chain	sphingosine chain		
2	39	23	16	3	13.2
3	40	23	17	1	14.1
4	40	23	17	2.5	15.2
5	41	23	18	7	16.2
6	41	22	19	3	17.4
7	42	23	19	10.7	18.5

[a]) From area integration of reversed-phase HPLC peaks with MeOH, 1 ml/min, λ 215 nm.
[b]) HPLC retention times under conditions defined in [a]).

2.2. *Connectivity and Relative Configuration.* For the abundant oceanapin F (**7**) C-atoms were assigned *via* ^1H, ^{13}C correlations, suggesting the presence of two OH substituents from signals for a deshielded CH_2 (δ(H) 3.70, 3.96) and CH group (δ(H) 4.29), correlated to the δ(C) 62.52 (*t*) and the δ(C) 74.72 (*d*) signals, respectively. An amide functionality (CONH) was also suggested by the signals at δ(C) 173.85 (*s*) and δ(H) 6.30 (br. *d*) signals, and by correlation of the signal at δ(H) 3.89 (*dt*) with δ(C) 54.45 (*d*, *CH*NH), in agreement with IR absorption bands at 3360 and 1640 cm^{-1}. An (*E*)-olefinic bond was indicated by signals at δ(H) 5.51 and 5.76 ($J = 15.3$ Hz) correlated to 2 *d*'s at δ(C) 128.80 and 134.30, respectively. At this point, differential decoupling irradiations and ^1H, ^1H and long-range ^1H,^{13}C experiments allowed us to establish the C(1′)–C(5′) portion (*Exper. Part*).

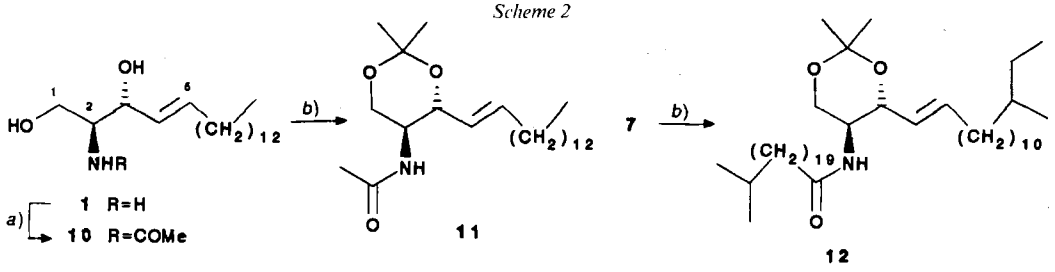
Long aliphatic chains were suggested by a δ(H) 1.24 (br. *s*) and a series of *t* between δ(H) 29.1 and 30.0 that also occur with the other oceanapins. The chains proved to be either unbranched (δ(H) 0.87 (*t*), correlated to δ(C) 14.09 (*q*), for **2**, **4**, and **6**), or branched with either i-Pr terminus (δ(H) 0.85 (*d*), correlated to δ(C) 22.64 (*q*) and 27.95 (*d*), for **2**–**5** and **7**) or ante-iso terminus (δ(H) 0.84 (*t*) and 0.85 (*d*), correlated to δ(C) 11.39 (*q*), and 19.21 (*q*), and 34.38 (*d*), for **6** and **7**). Branching of the ante-iso type for oceanapins **6** and **7** was also supported by calculations through the empirical *Lindeman-Adams* rule [14].

Mass spectra afforded further structural insight by showing fragment ions for loss of H_2O from the molecular ion, besides diagnostic fragment ions at either *m/z* 378 for **2**–**5**, and **7** or *m/z* 364 for **6**[1]) of compositions $C_{24}H_{46}NO$ and $C_{25}H_{48}NO$ (HR-EI-MS) for **6** and **7**, respectively, which must derive from the cleavage of the C(2)–C(3) bond. EI-MS data (*Exper. Part*) indicated a C_{23} amidic chain for oceanapins B (**3**) and D (**5**), which have the same chain terminus.

The whole C–C connectivity was assigned from the sphingosine and fatty-acid moieties obtained as pure compounds from acid hydrolysis of the oceanapins (sphingosines **8a–d** and methyl esters **9a–c**; *Scheme 1*).

The *erythro*-configuration for acetonide **12**, prepared from oceanapin F (**7**; *Scheme 2*), was deduced from the identity of the NMR spectra ($\delta(H-C(2'))$ 3.83 ppm, $J(2',1') = 5.2$ and 9.1, $J(2',3') = 9.6$) with acetonide **11**, prepared from the *N*-acyl derivative **10** of commercial D-*erythro*-sphingosine (**1**). This established *erythro*-configuration also for oceanapin F (**7**). This conclusion was also supported by molecular-mechanics calculations for acetonide **11** in both *threo*- and *erythro*-configuration. Thus, calculations for the preferred conformation of **11** (*erythro*) gave coupling constants in agreement with the experiment: $J(2',1') = 5.2$ and 11.0 (for torsional angle 2 H–C(1')–C(2')–H 55° and 172°), and $J(2',3') = 9.9$ (for torsional angle H–C(2')–C(3')–H 177°). In contrast, the corresponding *J* for the preferred conformation of **11** (*threo*) were found to be different from **12**: $J(2',1') = 2.4$ and 1.7, corresponding to torsional angle 2 H–C(1')–C(2')–H of 51° and 67°.

Scheme 2



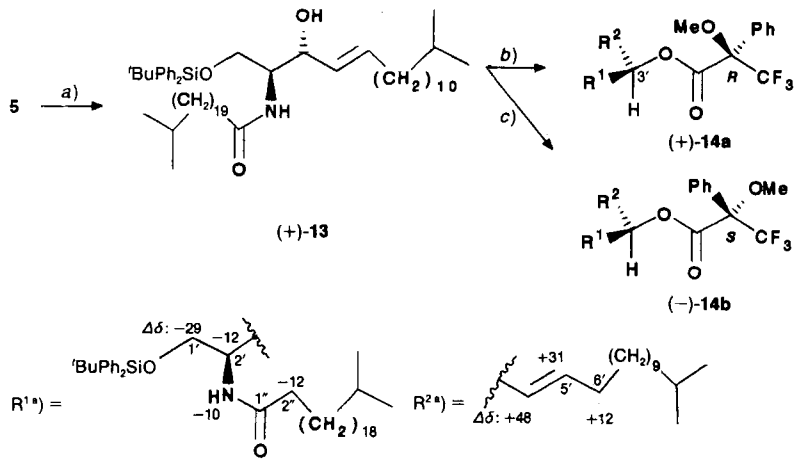
a) Ac_2O, EtOH, 0°, 1 h. *b*) Acetone, $CuSO_4$, reflux, 6 h.

2.3. *Absolute Configuration.* Previous assignments of the absolute configuration of acylsphingosines were either based on CD measurements on mixtures, such as for caulerpicin (2'*S*,3'*R*) [4c] and the ceramide portion of cerebrosides [15], or on total synthesis, such as for symbioramide [7] (= (2*S*,3*R*,2'*R*,3'*E*)-*N*-(2'-hydroxyoctadec-3'-enoyl)-dihydrosphingosine) [16] and the sphingosine derivative of *Anemonia sulcata* [10], (2*R*,3*S*) [17].

For the oceanapins we have decided to prepare both *Mosher*'s esters [18] (+)-**14a** and (–)-**14b** of the silyl ether (+)-**13** [19] derived from **5**. The NMR data, expressed as $\Delta\delta$ ($\delta_S - \delta_R$) in *Scheme 3*, show that the ^{1}H-NMR signals for 2 H–C(1'), H–C(2'), 2 H–C(2") and N–H of ester (+)-**14a** are observed upfield from the corresponding protons of ester (–)-**14b**. The opposite trend is observed for H–C(4'), H–C(5'), and 2 H–C(6'). This result can be consistently explained by the diamagnetic effect of the Ph ring, thus indicating the (*R*)-configuration at C(3'). Since *erythro*-configuration has been estab-

[1]) Reversed-phase HPLC (*Table*) gave two other homologous oceanapins, albeit in too small amounts (*ca.* 0.2 mg each) for complete structural study. The one eluted at t_R 11.6 min gave two significant EI-MS fragment ions, *m/z* 561 ($[M - H_2O]^+$), indicating a total of 37 C-atoms, and *m/z* 336, suggesting a C_{20} amidic chain. For the oceanapin eluted at t_R 12.4 min, fragment ions *m/z* 364 and 589 ($[M - H_2O]^+$) indicated a C_{22} amidic chain or a total of 39 C-atoms, respectively. For the latter, comparison of t_R with oceanapin A (**2**; *Table*) suggested branching at both chains.

Scheme 3



a) 'BuPh$_2$SiCl, 1*H*-imidazole, DMF, 60°, 12 h.
b) (*S*)-MTPACl, pyridine, CCl_4, r.t., 24 h.
c) (*R*)-MTPACl, pyridine, CCl_4, r.t., 24 h.

[a]) The $\Delta\delta = \delta_S - \delta_R$ represent the difference, in Hz, g the corresponding ^{1}H-NMR resonances between the (*S*)-MTPA ester (−)-**14b** and the (*R*)-MTPA ester (+)-**14a**.

lished between C(2′) and C(3′), the absolute configuration of oceanapin D (**5**) is thus (2′*S*,3′*R*).

We thank Dr. *Jane Fromont*, Sir Fisher Marine Biology Institute, University James Cook, Townsville Qld., for the sponge identification, Mrs. *M. Rossi* and *A. Sterni* for skilled technical aid, and *MURST* (Progetti 40%) and *CNR* (Roma) for financial support. This work has been carried out within the collaborative program *ORSTOM-CNRS* on 'Marine Substances of Biological Interest'.

Experimental Part

1. *General.* All evaporations were carried out at reduced pressure. Yields are given on reacted compounds. M.p.: *Kofler* hot-stage microscope. Pyridine was freshly distilled from BaO and DMF (stored on flamed 4 Å molecular sieves) were used. Flash-chromatography (FC): *Merck Si-60*, 15–25 μm. TLC: *Merck* 'Kieselgel' *60 PF_{254}* plates. HPLC: *Merck LiChrosorb CN*; reversed-phase (RP) HPLC: *Merck LiChrosorb RP18*; 7 μm, 25 × 1-cm columns; solvent flux 5 ml/min; UV monitoring λ 215 nm, if not otherwise stated. Polarimetric data: *JASCO-DIP-181* polarimeter. IR: *Perkin-Elmer-337* spectrometer; ν_{max} in cm^{-1}. NMR ($CDCl_3$): δ in ppm rel. to internal $SiMe_4$ (= 0 ppm), *J* in Hz; *Varian-XL-300* spectrometer; ^{1}H at 299.94 MHz (the coupling pattern of many protons has been clarified by differential decoupling irradiations [20] and ^{1}H,^{1}H COSY [21]); ^{13}C at 75.43 MHz, multiplicities from DEPT experiments [22]; H–C assignments from one-bond [23a] and long-range ^{1}H,^{13}C COSY experiments [23b]. For natural oceanapins A–E (**2–6**), only the ^{13}C- and ^{1}H-NMR signals that differ from those of oceanapin F (**7**) are reported; all other signals for oceanapins **2–6** proved to be superimposable. In analogy, for sphingosines **8b–d**, only NMR signals that differ from those of **8a** are reported. EI-MS (*m/z*, (%)): *Kratos-MS80* mass spectrometer with home-built data system and equipped with a *Vacumetrics-DIP* gun for FAB spectra. Molecular mechanics calculations: MMX, PCMOD.4, *Serena Software*, Bloomington, Indiana.

2. *Collection and Isolation.* The sponge was collected by scuba diving in Woodin Channel, New Caledonia, in August 1985 and was identified by Dr. *Jane Fromont*. The sponge was immediately frozen, freeze-dried (600 g), and CH_2Cl_2 extracted. The solvent was evaporated and the residue (5.04 g) subjected to FC (hexane/AcOEt gradient

elution) collecting 20 fractions of 100 ml each. *Fr. 13* was further subjected to FC (Et_2O) and then to HPLC (*CN*, hexane/*i*-PrOH 96:4), giving a mixture of oceanapins **2–7** (62 mg, 0.01%; t_R 8.7 min) which could be cleanly separated by reversed-phase HPLC (MeOH): **2** (6.0 mg), **3** (2.0 mg), **4** (4.9 mg), **5** (14.3 mg), **6** (6.2 mg), and **7** (21.5 mg); t_R values in the *Table*.

3. *Oceanapin A* (= N-*[(2S,3R,4E)-1,3-Dihydroxyhexadec-4-en-2-yl]-21-methyldocosanamide*; **2**). White powder. M.p. (hexane) 65–75°. ^{1}H-NMR: 1.24 (br. *s*, CH_2(8′) to CH_2(15′), CH_2(4) to CH_2(20)); 0.87 (*t*, *J*(16′, 15′) = 6.6, 3 H–C(16′)). ^{13}C-NMR: 29.1–30.0 (series of *t*, C(7′)–C(13′), C(4)–C(18)); 31.90 (*t*, C(14′)); 22.67 (*t*, C(15′)); 14.09 (*q*, C(16′)). EI-MS: 589 (1, $[M - H_2O]^+$), 572 (1), 558 (1), 379 (11), 378 (9), 337 (4), 111 (6), 43 (100).

4. *Oceanapin B* (= N-*[(2S,3R,4E)-1,3-Dihydroxy-15-methylhexadec-4-en-2-yl]-21-methyldocosanamide*; **3**). White powder. M.p. (hexane) 63–68°. ^{1}H-NMR: 1.24 (br. *s*, CH_2(8′) to CH_2(14′), CH_2(4) to CH_2(20)); 1.49 (*m*, H–C(15′), H–C(21)); 0.85 (*d*, *J* = 6.6, 2 Me–C(15′), 2 Me–C(21)). ^{13}C-NMR: 29.1–30.0 (series of *t*, C(7′)–C(12′), C(4)–C(18)); 27.41 (*t*, C(13′), C(19)); 39.05 (*d*, C(14′), C(20)); 27.96 (*d*, C(15′), C(21)); 22.65 (*q*, C(16′), *Me*–C(15′), *Me*–C(22), *Me*–C(21)). EI-MS: 603 (1, $[M - H_2O]^+$), 589 (1), 586 (0.7), 573 (1), 379 (17), 378 (14), 111 (7), 83 (26), 57 (63), 43 (100).

5. *Oceanapin C* (= N-*[(2S,3R,4E)-1,3-Dihydroxy-15-methylhexadec-4-en-2-yl]tricosanamide*; **4**). White powder. M.p. (hexane) 65–70°. ^{1}H-NMR: 1.24 (br. *s*, CH_2(8′) to CH_2(14′), CH_2(4) to CH_2(22)); 1.49 (*m*, H–C(15′)); 0.85 (*d*, *J* = 6.6, 2 Me–C(15′)); 0.87 (*t*, *J*(23,22) = 6.6, 3 H–C(23)). ^{13}C-NMR: 29.1–30.0 (series of *t*, C(7′)–C(12′), C(4)–C(20)); 27.41 (*t*, C(13′)); 39.05 (*t*, C(14′)); 27.96 (*d*, C(15′)); 22.65 (*q*, C(16′), *Me*–C(15′)); 31.90 (*t*, C(21)); 22.67 (*t*, C(22)); 14.09 (*q*, C(23)). EI-MS: 603 (0.5, $[M - H_2O]^+$), 572 (1), 379 (3), 378 (3), 111 (6), 83 (24), 57 (60), 43 (100).

6. *Oceanapin D* (= N-*[(2S,3R,4E)-1,3-Dihydroxy-16-methylheptadec-4-en-2-yl]-21-methyldocosanamide*; **5**). White powder. M.p. (hexane) 70–72°. ^{1}H-NMR: 1.24 (br. *s*, CH_2(8′) to CH_2(15′), CH_2(4) to CH_2(20)); 1.49 (*m*, H–C(16′), H–C(21)); 0.85 (*d*, *J* = 6.6, 2 Me–C(16′), 2 Me–C(21)). ^{13}C-NMR: 29.1–30.0 (series of *t*, C(7′)–C(13′), C(4)–C(18)); 27.40 (*t*, C(14′), C(19)); 39.05 (*t*, C(15′), C(20)); 27.95, (*d*, C(16′), C(21)); 22.65 (*q*, C(17′), *Me*–C(16′), C(22), *Me*–C(21)). EI-MS: 617 (0.8, $[M - H_2O]^+$), 586 (1), 379 (21), 378 (18), 337 (2), 83 (27), 82 (20), 57 (62), 43 (100). FAB-MS (3-nitrobenzyl alcohol matrix): 658 (0.5, $[M + Na]^+$), 618 (1.1, $[M + H - H_2O]^+$).

7. *Oceanapin E* (= N-*[(2S,3R,4E)-1,3-Dihydroxy-16-methyloctadec-4-en-2-yl]docosanamide*; **6**). White powder. M.p. (hexane) 65–68°. ^{1}H-NMR: 1.24 (br. *s*, CH_2(8′) to CH_2(15′), CH_2(17′), CH_2(4) to CH_2(21)); 0.87 (*t*, *J*(22,21) = 6.6, 3 H–C(22)). ^{13}C-NMR: 29.1–30.0 (series of *t*, C(7′)–C(13′), C(17′), C(4)–C(19)); 31.90 (*t*, C(20)); 22.68 (*t*, C(21), 14.09 (*q*, C(22)). EI-MS: 617 (2, $[M - H_2O]^+$), 586 (3), 366 (22), 364 (31), 252 (4), 111 (13), 83 (48), 82 (45), 57 (94), 43 (100). HR-EI-MS: 364.3576 ± 0.0050 ($C_{24}H_{46}NO$, calc. 364.3579).

8. *Oceanapin F* (= N-*[(2S,3R,4E)-1,3-Dihydroxy-16-methyloctadec-4-en-2-yl]-21-methyldocosanamide*; **7**). White powder. M.p. (hexane) 70–75°. $[\alpha]_D^{20} = -2.1$, $[\alpha]_{365}^{20} = -8.0$ (*c* = 0.35, $CHCl_3$)[2]. IR (nujol): 3360 (br.), 2920*vs*, 2850*vs*, 1640*vs*, 1470*s*, 1360*m*, 1178*m*, 1068*s*, 988*m*. ^{1}H-NMR: 3.70 (br. *dd*, J_{gem} = 11.0, 3.7), 3.96 (*dt*, J_{gem} = 11.0, 3.7, 2 H–C(1′)); 3.89 (*ddt*, *J* = 6.6, 5.6, 3.7, H–C(2′)); 4.29 (br. *ddd*, *J*(3′,2′) = 5.6, *J*(3′,4′) = 6.6, *J*(OH,3′) = 4.7, H–C(3′)); 2.62 (*d*, *J*(OH,3′) = 4.7, OH–C(3′)); 5.51 (*ddt*, *J*(4′,5′) = 15.3, *J*(4′,3′) = 6.6, *J*(4′,6′) = 1.2, H–C(4′)); 5.76 (*dtd*, *J*(5′,4′) = 15.3, *J*(5′,6′) = 6.9, *J*(5′,3′) = 1.2, H–C(5′)); 2.02 (‘*q*’, *J*(6′,5′) ≈ *J*(6′,7′) = 6.9, CH_2(6′)); 1.30 (*m*, CH_2(7′), H–C(16′)); 1.24 (br. *s*, CH_2(8′) to CH_2(15′), CH_2(17′), CH_2(4) to CH_2(20)); 0.84 (*t*, *J*(18′,17′) = 6.6, 3 H–C(18′)); 6.30 (br. *d*, *J*(NH,2′) = 6.6, NH–C(1)); 2.22 (*t*, *J*(2,3) = 7.5, CH_2(2)); 1.60 (*m*, CH_2(3)); 1.49 (*m*, H–C(21)); 0.85 (*d*, *J* = 6.6, 2 Me–C(21), Me–C(16′)). ^{13}C-NMR: 62.52 (*t*, C(1′)); 54.45 (*d*, C(2′)); 74.72 (*d*, C(3′)); 128.80 (*d*, C(4′)); 134.30 (*d*, C(5′)); 32.25 (*t*, C(6′)); 29.1–30.0 (series of *t*, C(7′)–C(13′), C(17′), C(4)–C(18)); 27.10 (*t*, C(14′)); 36.63 (*t*, C(15′)); 34.38 (*d*, C(16′)); 11.39 (*q*, C(18′)); 19.21 (*q*, *Me*–C(16′)); 173.85 (*s*, C(1)); 36.83 (*t*, C(2)); 25.74 (*t*, C(3)); 27.40 (*t*, C(19)); 39.05 (*t*, C(20)); 27.95 (*d*, C(21)); 22.64 (*q*, C(22), *Me*–C(21)). EI-MS: 631 (1, $[M - H_2O]^+$), 614 (1), 602 (2), 379 (27), 378 (16), 350 (4), 337 (3), 252 (2), 111 (8), 83 (30), 82 (18), 57 (69), 43 (100). HR-EI-MS: 378.3735 ± 0.0040 ($C_{25}H_{48}NO$, calc. 378.3736).

9. *Acid Hydrolysis of Oceanapins A–F* (**2–7**). Each single, pure oceanapin (2–10 mg) was dissolved in 3 ml of 1.2M H_2SO_4 in 85% MeOH and heated at reflux for 6 h. The mixture was then cooled, treated with H_2O (1 ml), and then extracted with hexane (3 × 3 ml). The org. phase was percolated through a *Whatman* phase-separation filter and evaporated: methyl ester **9**. The aq. layer was treated with 2M NaOH, then extracted with AcOEt (3 × 3 ml), percolated through a *Whatman* phase-separation filter, and finally evaporated: sphingosine **8**.

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[2]) Also for the other oceanapins, we obtained low [α], values, which, coupled to the scarce availability of these compounds, resulted in large uncertainties in [α].

(4E)-2-Aminohexadec-4-ene-1,3-diol (**8a**): ^{1}H-NMR: 3.67, 3.61 (2*dd*, J_{gem} = 11.0, J(1,2) = 5.7, CH_2(1)); 2.83 ('*q*', J(2,1) ≈ J(2,3) = 5.4, H–C(2)); 4.03 (*ddd*, J(3,4) = 6.6, J(3,2) = 5.4, J(3,5) = 1.2, H–C(3)); 5.45 (*ddt*, J(4,5) = 15.3, J(4,3) = 6.6, J(4,6) = 1.2, H–C(4)); 5.73 (*dtd*, J(5,4) = 15.3, J(5,6) = 6.6, J(5,3) = 1.2, H–C(5)); 2.03 (*q*, J(6,5) = J(6,7) = 6.6, CH_2(6)); 1.35 (*quint.*, J(7,6) ≈ J(7,8) = 6.6, CH_2(7)); 1.24 (*m*, CH_2(8) to CH_2(15)); 0.86 (*t*, J(16,15) = 6.6, 3 H–C(16)); 2.45 (br. *s*, OH). ^{13}C-NMR: 64.04 (*t*, C(1)); 56.15 (*d*, C(2)); 75.39 (*d*, C(3)); 129.30 (*d*, C(4)); 134.68 (*d*, C(5)); 32.33 (*t*, C(6)); 29.7–29.2 (series of *t*, C(7)–C(13)); 31.90 (*t*, C(14)); 22.67 (*t*, C(15)); 14.09 (*q*, C(16)).

(4E)-2-Amino-15-methylhexadec-4-ene-1,3-diol (**8b**): ^{1}H-NMR: 1.24 (*m*, CH_2(8) to CH_2(14)); 1.50 (*m*, H–C(15)); 0.85 (*d*, J = 6.6, 2 Me–C(15)). FAB-MS (3-nitrobenzyl alcohol matrix): 286 (5, $[M + H]^+$), 268 (9, $[M + H - H_2O]^+$).

(4E)-2-Amino-16-methylheptadec-4-ene-1,3-diol (**8c**): ^{1}H-NMR: 1.50 (*m*, H–C(16)); 0.85 (*d*, J = 6.6, 2 Me–C(16)). ^{13}C-NMR: 27.41 (*t*, C(14)); 39.05 (*t*, C(15)); 27.96 (*d*, C(16)); 22.66 (*q*, C(17), *Me*–C(16)). FAB-MS: (3-nitrobenzyl alcohol matrix): 300 (8, $[M + H]^+$), 282 (12, $[M + H - H_2O]^+$).

(4E)-2-Amino-16-methyloctadec-4-ene-1,3-diol (**8d**): ^{1}H-NMR: 1.24 (br. *s*, CH_2(8) to CH_2(15), CH_2(17)); 1.40 (*m*, H–C(16)); 0.84 (*t*, J(18,17) = 6.6, 3 H–C(18)); 0.85 (*d*, J(Me–C(16),16) = 6.6, Me–C(16)). ^{13}C-NMR: 29.1–29.9 (series of *t*, C(7)–C(13), C(17)); 27.41 (*t*, C(14)); 36.33 (*t*, C(15)); 34.39 (*d*, C(16)); 11.40 (*q*, C(18)); 19.21 (*q*, *Me*–C(16)). FAB-MS (3-nitrobenzyl alcohol matrix): 314 (4, $[M + H]^+$), 296 (6, $[M + H - H_2O]^+$).

Methyl 21-Methyldocosanoate (**9a**): ^{1}H-NMR: 2.29 (*t*, J(2,3) = 7.2, CH_2(2)); 1.24 (br. *s*, CH_2(3) to CH_2(20)); 1.50 (*m*, H–C(21)); 0.85 (*d*, J = 6.6, 2 Me–C(21)); 3.65 (*s*, MeO). ^{13}C-NMR: 174.37 (*s*, C(1)); 34.12 (*t*, C(2)); 24.96 (*t*, C(3)); 31.92 (*t*, C(4)); 29.2–29.7 (series of *t*, C(5)–C(18)); 27.42 (*t*, C(19)); 39.05 (*t*, C(20)); 27.96 (*d*, C(21)); 22.66 (*q*, 2 *Me*–C(21)); 51.44 (*q*, MeO). EI-MS: 368 (39, $M^{+\cdot}$), 337 (2), 325 (9), 87 (70), 74 (100).

Methyl Tricosanoate (**9b**): ^{1}H-NMR: 2.29 (*t*, J(2,3) = 7.2, CH_2(2)); 1.24 (*m*, CH_2(3) to CH_2(22)); 0.87 (*t*, J = 6.9, 3 H–C(23)). ^{13}C-NMR: 174.4 (*s*, C(1)); 34.12 (*t*, C(2)); 24.9 (*t*, C(3)); 31.91 (*t*, C(4)); 29.2–29.7 (series of *t*, C(5)–C(20)); 31.92 (*t*, C(21)); 22.69 (*t*, C(22)); 14.10 (*q*, C(23)); 51.44 (*q*, MeO). EI-MS: 368 (10, $M^{+\cdot}$), 337 (1), 325 (9), 87 (70), 74 (100).

Methyl Docosanoate (**9c**): ^{1}H-NMR: 2.29 (*t*, J(2,3) = 7.2, CH_2(2)); 1.24 (br. *s*, CH_2(3) to CH_2(21)); 0.87 (*t*, J = 6.9, 3 H–C(22)). ^{13}C-NMR: 174.37 (*s*, C(1)); 34.12 (*t*, C(2)); 24.96 (*t*, C(3)); 31.91 (*t*, C(4)); 29.1–29.7 (series of *t*, C(5)–C(19)); 31.92 (*t*, C(20)); 22.69 (*t*, C(21)); 14.11 (*q*, C(22)); 51.44 (*q*, MeO). EI-MS: 354 (8, $M^{+\cdot}$), 87 (70), 74 (100).

10. *N-[(2S,3R,4E)-1,3-(Isopropylidenedioxy)octadec-4-enyl]acetamide* (**11**). To D-*erythro*-sphingosine (= (2*S*,3*R*,4*E*)-2-aminooctadec-4-ene-1,3-diol; **1**; *Sigma*; 3.0 mg, 0.010 mmol) in EtOH (0.5 ml) was added an excess of Ac_2O and stirred for 1 h at r.t. The mixture was evaporated to give monoacetate **10** (3.3 mg, 97%) which was then dissolved in dry acetone (1 ml). Excess dry $CuSO_4$ was added and the mixture heated at reflux with stirring for 6 h, then cooled, filtered, and evaporated: **11** (3.2 mg, 87%).

N-Acetoxysphingosine (= *N-[(2S,3R,4E)-1,3-Dihydroxyoctadec-4-en-2-yl]acetamide*; **10**): ^{1}H-NMR: 3.95, 3.70 (2 br. *d*, J_{gem} = 10.8, CH_2(1)); 3.89 (*m*, H–C(2)); 4.31 (*m*, H–C(3)); 5.52 (*dd*, J(4,5) = 15.3, J(4,3) = 6.3, H–C(4)); 5.78 (*dt*, J(5,4) = 15.3, J(5,6) = 6.9, H–C(5)); 2.04 (*m*, CH_2(6)); 1.35 (*m*, CH_2(7)); 1.24 (br. *s*, CH_2(8) to CH_2(17)); 0.87 (*t*, J(18,17) = 6.9, 3 H–C(18)); 2.03 (*s*, NHCO*Me*). ^{13}C-NMR: 62.39 (*t*, C(1)); 54.37, (*d*, C(2)); 74.35 (*d*, C(3)); 128.70 (*d*, C(4)); 134.29 (*d*, C(5)); 32.26 (*t*, C(6)); 29.1–29.7 (series of *t*, C(7)–C(15)); 31.91 (*t*, C(16)); 22.68 (*t*, C(17)); 14.10 (*q*, C(18)); 170.74 (*s*, NH*C*OMe); 23.40 (*q*, *Me*CO). EI-MS: 324 (1), 323 (0.5, $[M - H_2O]^{+\cdot}$), 310 (1), 292 (1), 102 (69), 85 (98), 57 (24), 43 (100).

Data of **11**: ^{1}H-NMR: 4.00, 3.61 (2*dd*, J_{gem} = 11.3, J(1,2) = 5.2, 9.1, CH_2(1)); 3.83 (*dddd*, J(2,1) = 5.2, 9.1, J(2,3) = 9.6, J(2,NH) = 8.3, H–C(2)); 4.04 (*dd*, J(3,2) = 9.6, J(3,4) = 7.5, H–C(3)); 5.41 (*ddt*, J(4,5) = 15.4, J(4,3) = 7.5, J(4,6) = 1.4, H–C(4)); 5.74 (*dt*, J(5,4) = 15.3, J(5,6) = 6.8, H–C(5)); 2.02 (*q*, J = 6.8, CH_2(6)); 1.35 (*m*, CH_2(7)); 1.24 (br. *s*, CH_2(8) to CH_2(17)); 0.87 (*t*, J(18,17) = 6.9, 3 H–C(18)); 2.04 (*s*, NHCO*Me*); 1.47, 1.41 (2*s*, Me_2C); 5.19 (br. *d*, J = 8.4, NH). ^{13}C-NMR: 62.70 (*t*, C(1)); 48.28 (*d*, C(2)); 74.12 (*d*, C(3)); 127.25 (*d*, C(4)); 136.53 (*d*, C(5)); 32.30 (*t*, C(6)); 29.1–29.7 (series of *t*, C(7)–C(15)); 31.90 (*t*, C(16)); 22.67 (*t*, C(17)); 14.10 (*q*, C(18)); 169.75 (*s*, NH*C*OMe); 23.38 (*q*, *Me*CO); 98.87 (*s*, Me_2C); 28.40, 28.96 (2*q*, Me_2C). EI-MS: 366 (1.6, $[M - 15]^+$), 324 (2), 323 (1), 102 (10), 85 (65), 82 (23), 57 (49), 43 (100).

11. *N-[(2S,3R,4E)-1,3-(Isopropylidenedioxy)-16-methyloctadec-4-en-2-yl]-21-methyldocosanamide* (**12**). As described for **11**, **7** (3.9 mg, 0.06 mmol) was transformed into **12** (4.0 mg, 97%). ^{1}H-NMR: 3.99, 3.64 (2*dd*, J_{gem} = 11.3, J(1′,2′) = 5.1 or 9.3, CH_2(1′)); 3.82 (*dddd*, J(2′,1′) = 9.3, 5.3, J(2′,3′) = 9.6, J(2′,NH) = 8.1, H–C(2′)); 4.07 (*dd*, J(3′,2′) = 9.6, J(3′,4′) = 7.8, H–C(3′)); 3.82 (*dddd*, J(2′,1′a) = 5.1, J(2′,1′b) = 9.3, J(2′,3′) = 9.6, J(2′,NH) = 8.1, H–C(2′)); 5.39 (*ddt*, J(4′,5′) = 15.3, J(4′,3′) = 7.8, J(4′,6′) = 1.2, H–C(4′)); 5.13 (*d*, J(NH,2′) = 8.1, NH); 1.48, 1.41 (2*s*, Me_2C). ^{13}C-NMR: 62.72 (*t*, C(1′)); 48.11 (*d*, C(2′)); 74.10 (*d*, C(3′)); 127.28 (*d*,

C(4′)); 136.51 (*d*, C(5′)); 32.33 (*t*, C(6′)); 29.2–29.7 (series of *t*, C(7′)–C(13′), C(17′), C(4)–C(18)); 27.11 (*t*, C(14′)); 36.64 (*t*, C(15′)); 34.39 (*d*, C(16′)); 19.21 (*q*, *Me*–C(16′)); 11.40 (*q*, C(18′)); 172.88 (*s*, C(1)); 36.89 (*t*, C(2)); 25.69 (*t*, C(3)); 27.41 (*t*, C(19)); 39.05 (*t*, C(20)); 27.95 (*d*, C(21)); 22.65 (*q*, 2 *Me*–C(21)); 98.83 (*s*, Me_2C); 28.99, 28.47 (2*s*, Me_2C). EI-MS: 674 (2, $[M - 15]^+$), 632 (1.5), 379 (63), 378 (12), 278 (18), 85 (25), 82 (23), 57 (60), 43 (100).

12. Mosher's *Esters*. A soln. of **5** (9.0 mg, 0.014 mmol), 1 H-imidazole (2.5 mg, 0.036 mmol), and (*tert*-butyl)diphenylsilyl chloride (*Aldrich*; 5.0 mg, 0.018 mol) in dry DMF (1 ml) was stirred at 60° under N_2 for 12 h. The mixture was cooled, treated with H_2O (2 ml), and extracted with AcOEt (3 × 3 ml). The org. phase was washed with sat. aq. NaCl soln., dried (Na_2SO_4), and evaporated to give a residue that was subjected to prep. TLC (hexane/AcOEt 7:3): pure (+)-**13** (10.8 mg, 89%), R_f 0.7. To a soln. of (+)-**13** (5.3 mg, 0.006 mmol) in dry pyridine (0.2 ml) and CCl_4 (0.2 ml) were added 3 mol-equiv. of (+)-(*S*)-MTPACl (*Aldrich*) and stirred at r.t. for 24 h. Sat. aq. $CuSO_4$ soln. (3 ml) was added and the mixture percolated through a *Whatman* phase-separation filter. The filtrate was evaporated and the residue subjected to HPLC (*CN*, hexane/i-PrOH 98:2, λ = 225 nm): pure (+)-**14a** (t_R 5.3 min; 4.8 mg, 90%) besides unreacted (+)-**13** (t_R 9.3 min, 1 mg).

Following the same procedure with (−)-(*R*)-MTPACl and (+)-**13** (4.0 mg), pure (−)-**14b** (t_R 5.5 min; 4.4 mg, 88%) was obtained.

N-*[(2S,3R,4E)-1-[(*tert*-Butyl)diphenylsilyloxy]-3-hydroxy-16-methylheptadec-4-en-2-yl-21-methyldocosanamide* ((+)-**13**): $[\alpha]_D^{20} = +5$, $[\alpha]_{365}^{20} = +37$ (*c* = 0.4, $CHCl_3$). ^{1}H-NMR: 3.95, 3.75 (2*m*, CH_2(1′), H–C(2′)); 4.20 (*m*, H–C(3′)); 5.46 (*ddt*, *J*(4′,5′) = 15.3, *J*(4′,3′) = 6.0, *J*(4′,6′) = 1.2, H–C(4′)); 5.76 (*dtd*, *J*(5′,4′) = 15.3, *J*(5′,6′) = 6.9, *J*(5′,3′) = 1.2, H–C(5′)); 2.02 ('*q*', *J*(6′,7′) ≈ *J*(6′,5′) = 6.9, CH_2(6′)); 1.30–1.20 (*m*, CH_2(7′) to CH_2(15′), CH_2(4) to CH_2(20)); 1.50 (*m*, H–C(16′), H–C(21)); 0.85 (*d*, *J* = 6.6, 2 Me–C(16′), 2 Me–C(21)); 6.10 (*d*, *J*(NH,2′) = 8.1, NH); 2.18 (*t*, *J*(2,3) = 7.5, CH_2(2)); 1.60 (*m*, CH_2(3)); 3.56 (br. *d*, *J* = 7.2, OH); 1.06 (*s*, Me_3C); 7.61, 7.39 (2*m*, Ph). ^{13}C-NMR: 63.98 (*t*, C(1′)); 53.92 (*d*, C(2′)); 74.28 (*d*, C(3′)); 128.93 (*d*, C(4′)); 133.38 (*d*, C(5′)); 32.32 (*t*, C(6′)); 29.2–29.9 (series of *t*, C(7′)–C(13′), C(4)–C(18)); 27.41 (*t*, C(14′), C(19)); 36.82 (*t*, C(15′)); 27.95 (*t*, C(16′), C(21)); 22.65 (*q*, C(17′), *Me*–C(16′), C(22), *Me*–C(21)); 173.33 (*s*, C(1)); 36.82 (*t*, C(2)); 25.76 (*t*, C(3)); 26.84 (*q*, Me_3C); 19.14 (*s*, Me_3C); 132.37 (*s*, 2 C, Ph); 130.07 (*d*, 2 C, Ph); 127.89 (*d*, 4 C, Ph); 135.49 (*d*, 4 C, Ph). EI-MS: 855 (0.5, $[M - H_2O]^+$), 799 (45), 798 (68), 264 (9), 199 (73), 57 (50), 43 (47).

*(2S,3R,4E)-1-[(*tert*-Butyl)diphenylsilyloxy]-16-methyl-2-(21-methyldocosanamido)hexadec-4-en-3-yl (R)-3,3,3-Trifluoro-2-methoxy-2-phenylpropanoate* ((+)-**14a**): $[\alpha]_D^{20} = +8.0$ (*c* = 0.2, $CHCl_3$). ^{1}H-NMR: 3.78, 3.66 (*AB* of *ABX*, *J*(*AB*) = 10.8, *J*(*AX*) = 3.9, *J*(*BX*) = 4.2, CH_2(1′)); 4.29 (*m*, H–C(2′)); 5.63 (*t*, *J*(2′,3′) ≈ *J*(3′,4′) = 7.5, H–C(3′)); 5.27 (*ddt*, *J*(4′,5′) = 15.3, *J*(4′,3′) = 7.2, *J*(4′,6′) = 0.9, H–C(4′)); 5.80 (*dt*, *J*(5′,4′) = 15.3, *J*(5′,6′) = 6.9, H–C(5′)); 1.95 (*m*, CH_2(6′)); 1.20–1.30 (*m*, CH_2(7′) to CH_2(15′)); 1.50 (*m*, H–C(16′), H–C(21″)); 0.85 (*d*, *J* = 6.6, 2 Me–C(16′), 2 Me–C(21″)); 1.97 (*m*, CH_2(2″)); 1.60 (*m*, CH_2(3″)); 5.44 (br. *d*, *J*(NH,2′) = 9.0, NH); 1.06 (*s*, Me_3C); 7.59, 7.40 (2*m*, Ph); 3.37 (*q*, ^{5}*J*(H,F) = 0.9, MeO). ^{13}C-NMR: 63.55 (*t*, C(1′)); 51.71 (*d*, C(2′)); 75.77 (*t*, C(3′)); 32.26 (*t*, C(6′)); 29.2–30.0 (series of *t*, C(7′)–C(13′), C(4″)–C(18″)); 27.42 (*t*, C(14′), C(19″)); 36.82 (*t*, C(15′)); 27.96 (*d*, C(16′), C(21″)); 22.65 (*q*, 2 *Me*–C(16′), 2 *Me*–C(21″)); 172.40 (*s*, C(1″)); 36.82 (*t*, C(2″)); 25.63 (*t*, C(3″)); 26.88 (*q*, Me_3C); 19.27 (*s*, Me_3C); 55.40 (*q*, MeO); 138.99 (*d*); 135.54 (*d*); 130.02 (*d*); 129.51 (*d*); 128.27 (*d*); 127.90 (*d*); 127.42 (*d*); 123.60 (*d*). EI-MS: 856 (1, $[M - MTPAO]^+$), 799 (100), 462 (27), 199 (23), 57 (86), 43 (62).

*(2S,3R,4E)-1-[(*tert*-Butyl)diphenylsilyloxy]-16-methyl-2-(21-methyldocosanamido)hexadec-4-en-3-yl (S)-3,3,3-Trifluoro-2-methoxy-2-phenylpropanoate* ((−)-**14b**): $[\alpha]_D^{20} = -10.0$ (*c* = 0.2, $CHCl_3$). ^{1}H-NMR (only signals that differ from (+)-**14a** are reported): 3.65, 3.60 (*AB* of *ABX*, *J*(*AB*) ≈ 10.5, *J*(*AX*) ≈ *J*(*BX*) = 3.6, CH_2(1′)); 4.25 (*m*, H–C(2′)); 5.65 (*t*, *J*(2′,3′) ≈ *J*(3′,4′) = 7.5, H–C(3′)); 5.43 (*ddt*, *J*(4′,5′) = 15.3, *J*(4′,3′) = 7.3, *J*(4′,6′) = 1.3, H–C(4′)); 5.91 (*dt*, *J*(5′,4′) = 15.3, *J*(5′,6′) = 6.9, H–C(5′)); 1.99 (*m*, CH_2(6′)); 1.93 (*m*, CH_2(2″)); 5.41 (br. *d*, *J*(NH,2′) = 9.0, NH); 3.48 (*q*, ^{5}*J*(H,F) = 0.9, MeO). ^{13}C-NMR (only signals are reported that differ from those of (+)-**14a**): 76.03 (*t*, C(3′)); 172.50 (*s*, C(1″)); 36.75 (*t*, C(2″)); 55.45 (*q*, MeO); 139.78 (*d*); 135.53 (*d*); 129.99 (*d*); 128.69 (*d*); 128.34 (*d*); 127.51 (*d*); 127.26 (*d*); 124.00 (*d*). EI-MS: practically superimposable to that of (+)-**14a**.

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