Antifungal Metabolites from the Marine Sponge *Pachastrissa* sp.: New Bengamide and Bengazole Derivatives

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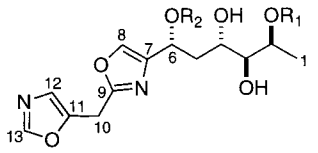
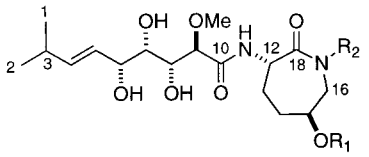
This paper reports the studies of components of an undescribed sponge in the genus *Pachastrissa* sp., collected along the Djibouti coast. The extract showed activity against *Candida albicans*. Six new bengazoles (**1**−**6**) and a new bengamide, named bengamide L (**16**), in addition to the known bengazoles (**7**−**11**), bengamides A (**12**), B (**13**), E (**14**), and F (**15**), and a lactone (**17**) are described in this paper. All structures were determined on the basis of spectroscopic studies.

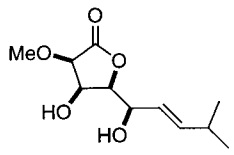
In the course of an investigation of bioactive metabolites from marine sponges collected along the Djibouti coast (Musha Archipelago), we have investigated the CH_2Cl_2 extract of the sponge *Pachastrissa* sp. (family Calthropellidae, order Astrophorida), which showed a potent antifungal activity against *Candida albicans* (MIC = 7 μg/mL). Along with metabolites previously isolated from *Jaspis* sponges (family Coppatiidae, order Astrophorida), bengazoles (**7**−**11**),[1] bengamides A (**12**), B (**13**), E (**14**), F (**15**), and a lactone (**17**),[2,3] we isolated six new bengazole derivatives (**1**−**6**) and the new bengamide L (**16**). Compound **11** was obtained previously from the hydrolysis of certain bengazoles but has not been reported as a natural product.[1,4]



1	R_1 = $CO(CH_2)_{14}CH_3$	R_2 = H
2	R_1 = H	R_2 = $CO(CH_2)_{14}CH_3$
3	R_1 = $CO(CH_2)_{12}CH(CH_3)_2$	R_2 = H
4	R_1 = H	R_2 = $CO(CH_2)_{12}CH(CH_3)_2$
5	R_1 = $CO(CH_2)_{13}CH_3$	R_2 = H
6	R_1 = H	R_2 = $CO(CH_2)_{13}CH_3$
7	R_1 = $CO(CH_2)_{11}CH(CH_3)_2$	R_2 = H
8	R_1 = H	R_2 = $CO(CH_2)_{11}CH(CH_3)_2$
9	R_1 = $CO(CH_2)_{12}CH_3$	R_2 = H
10	R_1 = H	R_2 = $CO(CH_2)_{12}CH_3$
11	R_1 = H	R_2 = H



12 Bengamide A	R_1 = $CO(CH_2)_{12}CH_3$	R_2 = H
13 Bengamide B	R_1 = $CO(CH_2)_{12}CH_3$	R_2 = Me
14 Bengamide E	R_1 = H	R_2 = H
15 Bengamide F	R_1 = H	R_2 = Me
16 Bengamide L	R_1 = $CO(CH_2)_{11}CH(CH_3)_2$	R_2 = H



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Results and Discussion

An EtOH extract of *Pachastrissa* sp. (120 g) was partitioned between *n*-hexane, CH_2Cl_2, and *n*-BuOH. The material obtained from the active CH_2Cl_2 fraction was subjected to bioassay-guided fractionation using Si gel column chromatography to afford four active fractions, F2−F5 (eluted with EtOAc−MeOH, 85:15). Reversed-phase HPLC chromatography of F5 with MeOH−H_2O (90:10) give eight fractions (F5H1−F5H8) and the antifungal fractions F5H2−F5H6 were studied.

HPLC fraction F5H6 was analyzed first. The ^{1}H NMR spectrum revealed the presence of a mixture of compounds. As previously observed, the complex mixture could not be separated by reversed-phase HPLC.[1] Purified compounds were successfully obtained by diol-phase HPLC chromatography with hexane−EtOAc (10:90) to afford bengazoles **1** and **2** and bengamide A (**12**). The molecular formula ($C_{29}H_{48}N_2O_7$) of **1** was established by HRFABMS of the ion at *m*/*z* 537 and indicated the presence of seven unsaturations in the molecule. The structure of **1** was determined by a detailed analysis of 1D and 2D NMR spectra. The ^{13}C NMR spectrum displayed one ester carbonyl (175.4 ppm); six aromatic signals (161.4−124.7 ppm); four methines linked to oxygen (77.8, 71.0, 70.4, and 66.2 ppm), three methylenes at 40.7, 35.3, and 25.6 ppm; and carbons corresponding to a side chain. The ^{1}H NMR spectrum showed three aromatic protons at 8.14, 7.73, and 7.06 ppm; four methines at 5.11, 4.90, 3.51, and 3.33 ppm bonded to oxygen; three methylenes at 4.28, 2.29, and 2.26/1.85 ppm; one methyl as a doublet at 1.22 ppm, and protons at 1.27 ppm corresponding to the side chain. The HMQC, COSY, and HMBC data, and comparison of NMR spectral data with the literature[1,4] supported the proposed structure for **1**. Comparison of the chemical shifts and the coupling constants of **1** with the corresponding literature values revealed that **1** has the same relative configuration at the four chiral centers. However, the LRFABMS showed a

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molecular ion at *m/z* 281 $[M - CH_3(CH_2)_{14}CO_2]^+$ indicating the loss of a palmitic acid unit at C-2. Thus, bengazole **1** is analogous to bengazoles previously isolated. Bengazole **2** showed the same $[M + H]^+$ peak as **1** but the cross peak HMBC correlation observed between H-6 (6.02 ppm) and the carbonyl (173.0 ppm) suggested the presence of an ester function at C-6. Compound **12** displayed an $[M + H]^+$ ion at *m/z* 585 ($C_{31}H_{57}N_2O_8$). Analysis of ^1H and ^{13}C NMR data, interpretation of 2D NMR spectra, and comparison with the bengamides reported by Crews and co-workers[1−3] established that **12** is the known bengamide A.

Two compounds, bengazoles **3** and **4**, were isolated from the HPLC fraction F5H5 (diol column hexane−EtOAc, 30:70). Bengazole **3** {*m/z* 537 $[M + H]^+$ ($C_{29}H_{49}N_2O_7$)} displayed NMR data almost identical to **1** except that the ^1H NMR spectrum of **3** revealed the absence of the terminal methyl and the presence of an isopropyl group (doublet at 0.86 ppm, 6H). Thus, compound **3** differed from **1** by the replacement of the hexadecanoyl side chain with an isohexadecanoyl group. Compound **4** is analogous to **2** with an isohexadecanoyl side chain.

The two other bengazoles (**5** and **6**) isolated from HPLC fraction F5H4 (diol column hexane−EtOAc, 30:70) were found to differ from **1** and **2** by one CH_2 group. This fact was deduced from their HRFABMS, which showed the $[M + H]^+$ ion at *m/z* 523.3383 ($C_{28}H_{47}N_2O_7$), 14 amu less than **1** and **2**, indicating the presence of a pentadecanoate chain. Finally, HPLC fractions F5H3 and F5H2 (diol column hexane−EtOAc, 30:70) provided the known bengazoles **7** and **8** {*m/z* 523 $[M + H]^+$ ($C_{28}H_{47}N_2O_7$)} and bengazoles **9** and **10** {*m/z* 509 $[M + H]^+$ ($C_{27}H_{45}N_2O_7$)}, respectively.

Compounds **13** and **16** were isolated by diol-phase HPLC (hexane−EtOAc, 40:60) of the F5H7 fraction. The NMR spectra of compounds **13** and **16** were very similar to compound **12** except for the presence of a NMe group at 3.10 ppm for **13** and the substitution of the terminal methyl by an isopropyl group (doublet at 0.86 ppm, 6H) for **16**. Both exhibited the same mass spectral data {*m/z* 599 $[M + H]^+$ ($C_{32}H_{59}N_2O_8$)}, with a base peak at *m/z* 369 $[M + 2H - C_{11}H_{19}O_5]^+$ due to the loss of the amide chain. Thus, compound **13** is the known bengamide B and compound **16** is a new bengamide, named bengamide L, differing from bengamide A (**12**) by the replacement of the myristic acid side chain with an isopentadecanoyl group.

Finally, the *n*-BuOH fraction was subjected to XAD-2 resin column, followed by a Sephadex LH-20 column. Fraction 2 was purified by reversed-phase HPLC chromatography to afford bengamide E (**14**), bengamide F (**15**), and lactone **17**. Their structures were determined by comparison with known NMR spectral data. [2,3]

The antifungal properties and the minimum inhibitory concentration (MIC) of the compounds isolated from *Pachastrissa* sp. were evaluated against *Candida albicans* CIP 118079 and *Saccharomyces cerevisiae* ATCC 28383. Only bengazoles **1**−**10** were found to be very active against *Candida albicans*, with MIC values from 0.8 to 1.5 μg/mL, but these compounds did not display any inhibitory activity against *Saccharomyces cerevisiae*. Thus, variation in the length of the side chain and esterification at positions C-2 or C-6 are not related to differences in antifungal activity. The hydrophilic derivative bengazole **11** (no esterification with a fatty acid) and bengamides **12**−**16** were inactive in these assays.[5]

Experimental Section

General Experimental Procedures. The NMR spectra were recorded on a JEOL JNM-LA400 NMR spectrometer at 399.65 MHz for ^1H and 100.40 MHz for ^{13}C, using $CDCl_3$ and CD_3OD as solvents. The chemical shifts are expressed as parts per million (δ) with TMS as internal standard and coupling constants (*J*) in Hertz. LRFABMS and HRFABMS were obtained from a Fisons VG Zab Spec TOF Micromass spectrometer. HPLC was performed with a Thermo Separation Products instrument equipped with an ERC-7515A differential refractometer, using Hibar Lichrospher RP_{18}e Merck (250 × 10 mm) and Nucleosil Diol Macherey−Nagel (250 × 10.5 mm) columns.

Fungal growth was measured in vitro using a liquid-phase turbidimetric system (Bioscreen from Labsystems, Paris, France) and automatically evaluated every 30 min for 24 h using various concentrations of compounds.[6]

Animal Collection. The sponge was collected by scuba at Musha Archipelago, Republic of Djibouti, in a rocky slope at water deeper than 25 m and immediately frozen until extraction. The specimens were attached to the shaded sides of cobbles. This is an orange sponge in life (white in EtOH) with encrusting to subspherical growth shape. The live sponge texture is very smooth, while in EtOH it becomes friable. Skeletal components (spicules) consist of calthrops with varying number of rays (3−4) usually with some small spines (acanthocalthrops), oxea of two size classes (1−1.5 × 39−48 μm and 2−8.5 × 228−325 μm) and euasters (oxyaster type) of two size classes. The latter are distributed mainly in the sponge cortex, but there are also some in the choanosome. The oxea, on the other hand, are dispersed in the choanosome not in any particular order and with none protruding the surface. No triaenes were found. The fact that the species possessed oxea separates it from the genus *Calthropella*, whereas the absence of triaenes differentiates it from the genera *Chelotropaena* and *Chelotropella*. A voucher specimen is deposited in the Israel National Collections of Natural History at Tel Aviv University (reg. no. ZMTAU SP25106).

Extraction and Isolation. The sponges were freeze-dried (600 g wet wt) and then extracted with absolute EtOH. The EtOH extract was decanted and concentrated in vacuo. The viscous concentrate (120 g) was partitioned between *n*-hexane and 10% aqueous MeOH. The aqueous MeOH phase was diluted to 30% with H_2O and extracted with CH_2Cl_2. After removal of MeOH, the residual aqueous solution was finally extracted with *n*-BuOH. The organic layers were evaporated under reduced pressure to yield 7.9 g of an *n*-hexane extract, 13 g of a CH_2Cl_2 extract, and 7.0 g of a *n*-BuOH extract.

The CH_2Cl_2 extract, showing antifungal activity against *Candida albicans* (MIC = 7 μg/mL), was subjected to column chromatography (40 × 5 cm, Si gel 60, 0.063−0.200 mm) by stepped gradient elution from hexane−EtOAc (70:30) to MeOH, affording nine fractions. The active fraction 6 was subjected to flash column chromatography (20 × 3 cm, Si gel 60, 0.040−0.063 mm) by stepped gradient elution from hexane−EtOAc (10:90) to MeOH to afford four active fractions F2 to F5 (eluted with EtOAc−MeOH, 85:15). Reversed-phase HPLC chromatography of active fraction F5 with MeOH−H_2O (90:10) and then diol-phase HPLC chromatography with hexane−EtOAc gave bengazoles **1** (8 mg, 0.006% wet wt), **2** (30 mg, 0.025%), **3** (9 mg, 0.007%), **4** (25 mg, 0.021%), **5** (12 mg, 0.010%), **6** (60 mg, 0.050%), **7** (27 mg, 0.022%), **8** (53 mg, 0.044%), **9** (22 mg, 0.018%), **10** (30 mg, 0.025%), bengamide A (**12**) (196 mg, 0.163%), bengamide B (**13**) (79 mg, 0.066%), bengamide L (**16**) (48 mg, 0.040%), and the lactone **17** (18 mg, 0.015%).

The *n*-BuOH portion was passed through an Amberlite XAD-2 column, and the organic compounds were eluted by MeOH after a first step with H_2O. The MeOH eluates were concentrated under reduced pressure to give 1.6 g of material, which was chromatographed on Sephadex LH-20 with MeOH−H_2O (2:1) as eluent, giving seven main fractions. Fraction 2 was submitted to reversed-phase HPLC chromatography with MeOH−H_2O (50:50) to afford pure bengamide E (**14**) (8 mg, 0.007%) and bengamide F (**15**) (10 mg, 0.008%), and with MeOH−H_2O−TFA (30:70:0.1) to give compound **11** (18 mg, 0.015%).

Bengazole 1: $[\alpha]_D$ −9.5° (*c* 0.3, MeOH); IR (neat) ν_{max} 3600−3100, 1747, 1533 cm^{-1}; UV (MeOH) λ_{max} 209 nm; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.84 (1H, s, H-13), 7.55 (1H, s, H-8), 6.99 (1H, s, H-12), 5.26 (1H, qd, J = 6.7, 2.4 Hz, H-2), 4.94 (1H, dd, J = 9.5, 2.2 Hz, H-6), 4.19 (2H, s, H-10), 3.63 (1H, td, J = 7.8, 2.2 Hz, H-4), 3.38 (1H, dd, J = 7.8, 2.4 Hz, H-3), 2.36 (2H, t, J = 7.5 Hz, H-15), 2.30 (1H, dt, J = 14.6, 2.2 Hz, H-5), 1.89 (1H, dt, J = 14.6, 9.5 Hz, H-5), 1.62 (2H, quint, J = 7.5 Hz, H-16), 1.33 (3H, d, J = 6.7 Hz, H-1), 1.25 (24H, H-17 to H-28), 0.86 (3H, d, J = 6.7, H-29); ^1H NMR (CD_3OD, 400 MHz) δ 8.14 (1H, s, H-13), 7.73 (1H, s, H-8), 7.06 (1H, s, H-12), 5.11 (1H, dq, J = 6.6, 3.0 Hz, H-2), 4.90 (1H, under solvent, H-6), 4.28 (2H, s, H-10), 3.51 (1H, ddd J = 10.0, 7.9, 2.5 Hz, H-4), 3.33 (1H, under solvent, H-3), 2.29 (2H, t, J = 7.5 Hz, H-15), 2.26 (1H, m, H-5), 1.85 (1H, ddd, J = 14.0, 9.5, 6.9 Hz, H-5), 1.59 (2H, quint, J = 7.5 Hz, H-16), 1.27 (24H, H-17 to H-28), 1.22 (3H, d, J = 6.6 Hz, H-1), 0.89 (3H, t, J = 6.9 Hz, H-29); ^{13}C NMR (CD_3OD, 100 MHz) δ 175.4 (s, C-14), 161.4 (s, C-9), 153.3 (d, C-13), 148.2 (s, C-11), 145.1 (s, C-7), 137.2 (d, C-8), 124.7 (d, C-12), 77.8 (d, C-3), 71.0 (d, C-2), 70.4 (d, C-4), 66.2 (d, C-6), 40.7 (t, C-5), 35.3 (t, C-15), 33.1 (t, C-28), 30.7−30.1, 26.0 and 27.7 (t, C-17 to C-27), 25.6 (t, C-10), 16.9 (q, C-1), 14.4 (q, C-29); HRFABMS m/z 537.3540 $[M + H]^+$ (calcd for $C_{29}H_{49}N_2O_7$ 537.3540, Δ −0.0 mmu); LRFABMS using NBA as matrix m/z 537 $[M + H]^+$ (100), 519 (19), 281 (24), 263 (10), 245 (29), 219 (15), 205 (6), 177 (56).

Bengazole 2: $[\alpha]_D$ −11.4° (*c* 1.1, MeOH); IR (neat) ν_{max} 3600−3100, 1754, 1531 cm^{-1}; UV (MeOH) λ_{max} 209 nm; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.85 (1H, s, H-13), 7.64 (1H, s, H-8), 7.00 (1H, s, H-12), 6.02 (1H, dd, J = 9.0, 4.5 Hz, H-6), 4.21 (2H, s, H-10), 4.00 (1H, qd, 6.3, 3.3 Hz, H-2), 3.73 (1H, dddd, 9.0, 4.0, 2.1 Hz, H-4), 3.29 (1H, br s, H-3), 2.29 (2H, dd, 8.0, 6.6 Hz, H-15), 2.23 (1H, dddd, 13.8, 9.0, 2.1 Hz, H-5), 2.08 (1H, dddd, 13.8, 9.0, 4.5 Hz, H-5), 1.58 (2H, m, H-16), 1.25 (24 H, m, H-17 to H-28), 1.17 (3H, d, 6.3 Hz, H-1), 0.80 (3H, t, 6.8 Hz, H-29); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.0 (s, C-14), 159.5 (s, C-9), 151.0 (d, C-13), 145.6 (s, C-11), 138.8 (s, C-7), 137.7 (d, C-8), 124.3 (d, C-12), 76.8 (d, C-3), 69.8 (d, C-4), 66.9 (d, C-2), 65.8 (d, C-6), 35.9 (t, C-5), 34.3 (t, C-15), 31.8, 29.6−29.0 and 22.6 (t, C-17 to C-28), 25.2 (t, C-10), 24.7 (t, C-16), 19.3 (q, C-1), 14.0 (q, C-29); HRFABMS m/z 537.3529 $[M + H]^+$ (calcd for $C_{29}H_{49}N_2O_7$ 537.3540, Δ −1.1 mmu); LRFABMS using NBA as matrix m/z 537 $[M + H]^+$ (8), 281 (87), 263 (5), 245 (15), 235 (4), 219 (23), 205 (8), 177 (100).

Bengazole 3: $[\alpha]_D$ −12.9° (*c* 0.2, MeOH); IR (neat) ν_{max} 3600−3100, 1740, 1536 cm^{-1}; UV (MeOH) λ_{max} 209 nm; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.84 (1H, s, H-13), 7.55 (1H, s, H-8), 6.99 (1H, s, H-12), 5.25 (1H, qd, J = 6.6, 2.4 Hz, H-2), 4.93 (1H, dd, J = 9.6, 2.3 Hz, H-6), 4.18 (2H, s, H-10), 3.64 (1H, m, H-4), 3.38 (1H, m, H-3), 2.35 (2H, t, J = 7.5 Hz, H-15), 2.30 (1H, dt, J = 14.6, 2.3 Hz, H-5), 1.89 (1H, dt, J = 14.6, 9.5 Hz, H-5), 1.62 (2H, quint, J = 7.5 Hz, H-16), 1.50 (1H, m, H-27), 1.32 (3H, d, J = 6.6 Hz, H-1), 1.25 (20H, H-17 to H-26), 0.86 (6H, d, J = 6.6, H-28 and H-29); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.9 (s, C-14), 159.2 (s, C-9), 150.9 (d, C-13), 145.9 (s, C-11), 143.9 (s, C-7), 134.9 (d, C-8), 124.2 (d, C-12), 76.7 (d, C-3), 71.5 (d, C-4), 69.4 (d, C-2), 67.3 (d, C-6), 38.9 (t, C-26), 38.4 (t, C-5), 34.4 (t, C-15), 34.3 (d, C-27), 29.9−29.0 (t, C-17 to C-25), 25.2 (t, C-10), 24.9 (t, C-16), 22.8 (q, C-28 and C-29), 16.8 (q, C-1); HRFABMS m/z 537.3531 $[M + H]^+$ (calcd for $C_{29}H_{49}N_2O_7$ 537.3540, Δ −0.9 mmu); LRFABMS using NBA as matrix m/z 537 $[M + H]^+$ (100), 519 (23), 281 (23), 263 (15), 245 (47), 219 (17), 205 (9), 177 (100).

Bengazole 4: $[\alpha]_D$ −8.5° (*c* 0.9, MeOH); IR (neat) ν_{max} 3600−3100, 1749, 1530 cm^{-1}; UV (MeOH) λ_{max} 209 nm; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.85 (1H, s, H-13), 7.63 (1H, s, H-8), 7.00 (1H, s, H-12), 6.03 (1H, dd, J = 8.7, 4.4 Hz, H-6), 4.21 (2H, s, H-10), 4.02 (1H, qd, J = 6.3, 3.6 Hz, H-2), 3.78 (1H, dddd, J = 9.9, 3.6, 1.8 Hz, H-4), 3.29 (1H, t, J = 3.6, H-3), 2.30 (2H, dd, J = 7.9, 6.9 Hz, H-15), 2.25 (1H, dddd, J = 13.9, 8.7, 1.8 Hz, H-5), 2.08 (1H, dddd, J = 13.9, 9.9, 4.4 Hz, H-5), 1.58 (2H, m, H-16), 1.50 (1H, m, H-27), 1.25 (20 H, m, H-17 to H-26), 1.19 (3H, d, J = 6.3 Hz, H-1), 0.87 (6H, d, J = 6.6 Hz, H-28 and H-29); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.0 (s, C-14), 159.5 (s, C-9), 151.0 (d, C-13), 145.6 (s, C-11), 138.8 (s, C-7), 137.7 (d, C-8), 124.3 (d, C-12), 76.8 (d, C-3), 69.8 (d, C-4), 66.9 (d, C-2), 65.8 (d, C-6), 38.7 (t, C-26), 35.9 (t, C-5), 34.3 (t, C-15), 29.6−29.0 (t, C-17 to C-28), 25.2 (t, C-10), 24.7 (t, C-16), 22.6 (q, C-28 and C-29), 14.0 (q, C-1); HRFABMS m/z 537.3536 $[M + H]^+$ (calcd for $C_{29}H_{49}N_2O_7$ 537.3540, Δ −0.4 mmu); LRFABMS using NBA as matrix m/z 537 $[M + H]^+$ (34), 281 (100), 263 (4), 245 (11), 235 (5), 219 (18), 205 (7), 177 (77).

Bengazole 5: $[\alpha]_D$ −10.5° (*c* 0.6, MeOH); IR (neat) ν_{max} 3600−3100, 1751, 1528 cm^{-1}; UV (MeOH) λ_{max} 209 nm; ^1H and ^{13}C NMR, see bengazole **1**; HRFABMS m/z 523.3379 $[M + H]^+$ (calcd for $C_{28}H_{47}N_2O_7$ 523.3383, Δ −0.4 mmu); LRFABMS using NBA as matrix m/z 523 $[M + H]^+$ (100), 505 (20), 281 (16), 263 (11), 245 (35), 219 (9), 205 (6), 177 (63).

Bengazole 6: $[\alpha]_D$ −9.3° (*c* 1.8, MeOH); IR (neat) ν_{max} 3600−3100, 1743, 1530 cm^{-1}; UV (MeOH) λ_{max} 209 nm; ^1H and ^{13}C NMR, see bengazole **2**; HRFABMS m/z 523.3385 $[M + H]^+$ (calcd for $C_{28}H_{47}N_2O_7$ 523.3383, Δ +0.2 mmu); LRFABMS using NBA as matrix m/z 523 $[M + H]^+$ (50), 281 (100), 263 (4), 245 (10), 219 (15), 205 (6), 177 (67).

Bengamide L (16): $[\alpha]_D$ +18.5° (*c* 1.4, MeOH); IR (neat) ν_{max} 3550−3210, 1673, 1665 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.97 (1H, d, J = 6.5 Hz, NH-11), 6.81 (1H, t, J = 7.1 Hz NH-17), 5.78 (1H, dd, J = 15.4, 6.4 Hz, H-4), 5.44 (1H, dd, J = 15.4, 7.2 Hz, H-5), 4.64 (2H, m, H-15 and H-12), 4.21 (1H, t, J = 7.2 Hz, H-6), 3.82 (2H, br s, H-8, H-9), 3.61 (1H, br s, H-7), 3.51 (3H, s, OMe), 3.35 (2H, m, H-16), 2.29 (2H, t, J = 7.2 Hz, H-20), 2.29 (1H, m, H-3), 2.18 (1H, m, H-14), 2.16 (1H, m, H-13), 1.95 (1H, dq, J = 13.3, 2.8 Hz, H-14), 1.74 (1H, m, H-13), 1.60 (2H, m, H-21), 1.51 (1H, m, H-31), 1.26 (19H, H-22 to H-30), 1.00 (3H, d, J = 6.7 Hz, H-1), 0.99 (3H, d, J = 6.7 Hz, H-2), 0.86 (6H, d, J = 6.6 Hz, H-32 and H-33); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.5 (s, C-19), 172.8 (s, C-18), 171.7 (s, C-10), 141.6 (d, C-4), 125.4 (d, C-5), 81.5 (d, C-9), 74.1 (d, C-6), 72.5 (d, C-8), 72.4 (d, C-7), 70.7 (d, C-15), 59.7 (q, OMe), 51.2 (d, C-12), 44.8 (t, C-16), 38.9 (t, C-30), 34.2 (t, C-20), 32.8 (t, C-14), 31.8 (t, C-13), 30.6 (d, C-3), 28.7 (d, C-31), 29.5−28.9 (t, C-22 to C-29), 24.7 (t, C−21), 23.0 (q, C-32 and C-33), 22.1 (q, C-1), 21.9 (q, C-2); HRFABMS m/z 599.4199 $[M + H]^+$ (calcd for $C_{32}H_{59}N_2O_8$ 599.4271, Δ −7.2 mmu); LRFABMS using NBA as matrix m/z 599 $[M + H]^+$ (12), 581 (10), 563 (7), 545 (19), 481 (24), 469 (55), 440 (21), 395 (23), 369 (67), 324 (17), 163 (28).

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