Bengamides Revisited: New Structures and Antitumor Studies

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The structural chemistry and biological activity of the bengamide class of compounds have been further characterized. Extracts prepared from recollected *Jaspis* cf. *coriacea* from five sites in Fiji were pooled. Six new bengamides, M (**7b**), N (**8a**), O (**8b**), P (**9a**), Q (**9b**), and R (**10**), were identified, accompanied by the known bengamides A (**1a**), B (**1b**), E (**3a**), F (**3b**), Y (**5**), Z (**6**), L (**7a**), G (**11a**), H (**11b**), and I (**12**). The structures of the new compounds were determined from spectroscopic data, and some were additionally confirmed by semisynthesis. Cytotoxicity screening data were obtained from the NCI-DTP 60 cell screen for bengamides A, B, and P. Bengamides A and B were more potent than bengamide P, with average IC_{50} values of 0.046, 0.011, and 2.70 FM, respectively. The *in vitro* antitumor activity against MDA-MB-435 human mammary carcinoma was also determined for natural bengamides A, B, E, F, P, M, O, and Z and for synthetic samples of B and O. The best activity was observed for the natural bengamides A (IC_{50} = 1 nM) and O (IC_{50} = 0.3 nM).

Introduction

The bengamide class of sponge-derived natural products has been studied for over a decade. Bengamides contain unusual structural elements consisting of fused ketide and amino acid biosynthetic moieties that together impart interesting antiparasitic, antimicrobial, and cytotoxic activity.

In 1986, the UC Santa Cruz (UCSC) group disclosed the first examples of this class, (+) bengamide A (**1a**) and (+) B (**1b**), as an easily separable mixture from a small sponge collection, subsequently identified as *Jaspis* cf. *coriacea* (family Coppatiidae, order Choristida = Astrophorida).[1] The absolute stereochemistry of each of the six chiral sites in bengamide A was eventually and unequivocally established from our extensive spectroscopic studies[2] and reaffirmed by total syntheses reported by groups headed by Boeckman,[3a] Mukai,[3b] Ogawa,[3c,d] Marshall,[3e] Ohrui,[3f] Broka,[3g] and Gurjar.[3h] In 1992, the UCSC samples of bengamides A, B, and P were evaluated *in vitro* in the NCI 60 cell line screen and found to have a unique profile compared to that of standard antitumor compounds in the NCI database. Significant *in vivo* antitumor activity was also observed with bengamide B on subcutaneously implanted MDA-MB-435 human breast carcinoma xenografts grown in nude mice.[4] The novel bengamide structures, unique *in vitro* cytotoxicity profile, and preliminary *in vivo* data prompted a long-term collaborative UCSC−Novartis Institute of Biomedical Research (NIBR) reinvestigation of bengamide-containing sponges. The goal was to carefully probe bengamide antiproliferative properties as a function of structure and to examine the most potent analogues in antitumor models. The latter required larger scale isolations to afford additional amounts of the known bengamides, but also provided opportunities to identify additional new analogues present as minor components. A medicinal chemistry program was also initiated to provide additional novel bengamides and samples of the natural products derived from total or semi synthesis.

Results and Discussion

The four known classes of bengamides, shown in Figure 1, consist of bengamides A (**1a**)/B (**1b**), C (**2a**)/D (**2b**), E (**3a**)/F (**3b**), and *iso*-E (**4**).[2] During the re-isolation of the bengamides, it was important to be able to rapidly dereplicate[5] among the 15 known bengamide analogues (see Figures 1 and 2)[2,6] and numerous bengazole[7] derivatives described to date from this and related sponges. The selected ^{1}H NMR signals shown in Figure 3 are diagnos-

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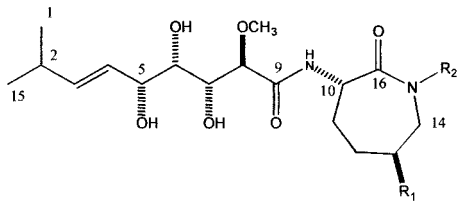


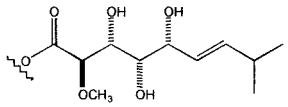
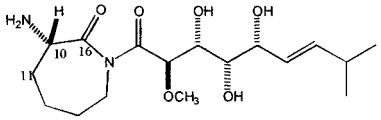
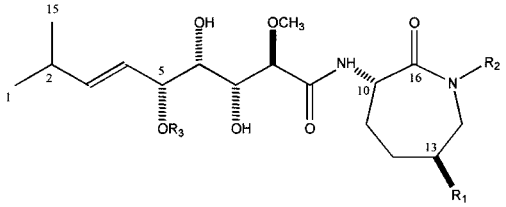
Type	Bengamide		R_1	R_2
I	A	(**1a**)	$-OCO(CH_2)_{12}CH_3$	H
I	B	(**1b**)	$-OCO(CH_2)_{12}CH_3$	CH_3
II	C/D	(**2a/2b**)		H/ CH_3
III	E	(**3a**)	H	H
III	F	(**3b**)	H	CH_3
IV	iso-E	(**4**)		

Figure 1. Four known classes of bengamides.



Bengamide		R_1	R_2	R_3
L	(**7a**)	$OCO(CH_2)_{11}CH(CH_3)_2$	H	H
M	(**7b**)	$OCO(CH_2)_{11}CH(CH_3)_2$	CH_3	H
N	(**8a**)	$OCO(CH_2)_{10}CH(CH_3)_2$	H	H
O	(**8b**)	$OCO(CH_2)_{10}CH(CH_3)_2$	CH_3	H
P	(**9a**)	H	H	$CO(CH_2)_{12}CH_3$
Q	(**9b**)	H	CH_3	$CO(CH_2)_{12}CH_3$
R	(**10**)	H	H	$CO(CH_2)_{14}CH_3$
Y	(**5**)	OH	H	H
Z	(**6**)	OH	CH_3	H
G	(**11a**)	$OCO(CH_2)_{11}CH_3$	H	H
H	(**11b**)	$OCO(CH_2)_{11}CH_3$	CH_3	H
I	(**12**)	$OCO(CH_2)_{13}CH_3$	H	H

Figure 2. Summary of additional Type I bengamides.

tic of one or more substructures that can be present in a bengamide, consisting of a 2(*R*)-methoxy-3(*R*),4(*S*),5(*R*)-trihydroxy-8-methylnon-6(*E*)-enoyl moiety; *S*-cyclolysine; a 3(*S*)-hydroxy- *S*-cyclolysine; and an unbranched linear R_1 fatty acid moiety. Searching the ^1H NMR spectra of semipure fractions for the eight signal clusters shown in Figure 3 allows rapid identification of the presence or absence of each of these substructures.

The rationale behind our choice of which collections to pursue deserves brief comment. We have repeatedly observed that isomorphic sponge populations from different geographical areas sometimes exist as chemotypes and can afford different sets of analogues.[8] During the past 12 years, we sought and located specimens of bengamide-containing *Jaspis* cf. *coriacea*, from numerous locations throughout the Indo-Pacific including Indonesia, Fiji, Papua New Guinea, and the Solomon Islands. This development quickly put aside our early belief[2a] that bengamide-containing sponges were restricted to Fiji and nearby locales. Surprising considering its apparent abundance,[9] none of the general taxonomy guides mention this sponge.

The bengamides and the accompanying bengazoles appear restricted to a small group of related (if not the same) species that are widespread in their biogeographical distribution (Table 1). Bengamide and bengazole derivatives have been reported from four species of sponge collected from a broad geographic range. These have been described as *Jaspis* cf. *coriacea* Carter 1886, collected in many Indo-Pacific locations (Fiji, Solomons, Papua New Guinea and Indonesia);[1,2] *Jaspis carteri* Ridley 1884, from New Caledonia;[6a] *Jaspis digonoxea* de Laubenfels 1950, discovered in South Africa,[6c] and *Pachastrissa* sp. Lendenfeld 1903 from Djibouti.[6b] A comparative study is underway at UCSC in order to establish taxonomic relationships between the four chemically related sponges. Each of the four specimens appears to be morphologically similar orange encrusting sponges, and vouchers of three of the four specimens have been examined. In most cases, bengamide-containing sponges are described as having the morphology we commonly observe shown in the underwater photo in Table 1, but with varying spicule types and taxonomic names.

The isolation work reported below began after a 1997 collection of *Jaspis* cf. *coriacea* from several Fiji locations was pooled to provide 4 L of malodorous viscous oil. The isolation conducted, shown in Chart 1, provided both known and new bengamides. Some of the fractions obtained from flash chromatography over silica gel were further purified and yielded the known bengamides A (**1a**),[1,2] B (**1b**),[1,2] E (**3a**),[2] F (**3b**),[2] Y (**5**),[2,6c] Z (**6**),[2,6c] L (**7a**),[6b] G (**11a**),[6] H (**11b**),[6] and I (**12**).[6]

Fractions obtained from silicagel chromatography were examined by NMR to identify those containing unknown bengamides and subsequently pooled. These were further fractionated using reversed-phase HPLC, but the isolations were complicated by the presence of a large number of compounds in trace amounts. Some metabolites possess intense UV absorbance while the bengamide analogues lack chromophores and are not easily detected even by diode array UV. Novel bengamides were finally isolated by examining each semi-preparative fraction by analytical HPLC with UV monitoring at 210 nm. However, this was not straightforward because even a major bengamide component could only be seen as a minute peak in the chromatogram. Several purifications using reversed-phase C_{18} HPLC afforded additional amounts

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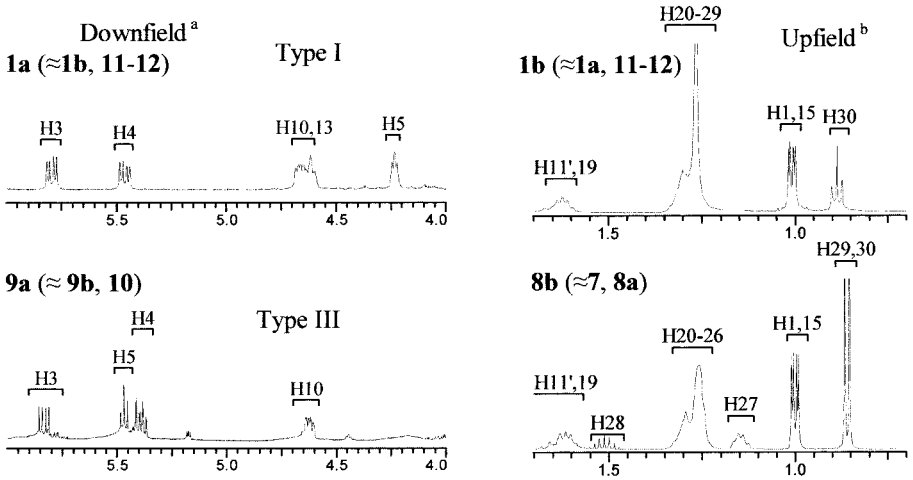
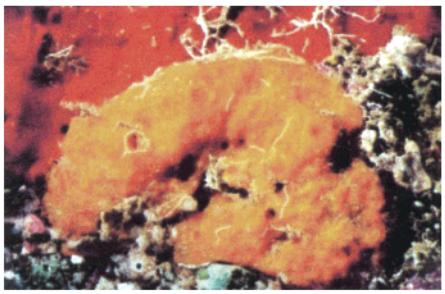
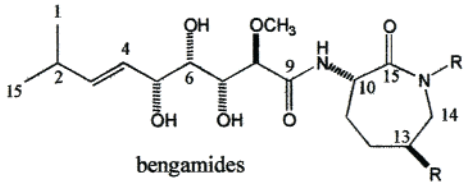


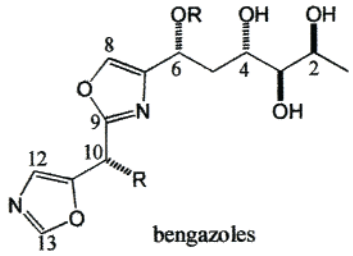
Figure 3. Diagnostic ^{1}H NMR (500 MHz) resonances for bengamides. Key: (a) characteristic downfield region (δ4−6) of a Type I or Type III bengamide in which the ester side chain occurs at C13 or C5; (b) characteristic upfield region (δ 0.7−1.7) of a bengamide with an ester side chain that is unbranched vs branched.

Table 1. Summary of Bengamide/Bengazole Sponge Sources



Jaspis sponge

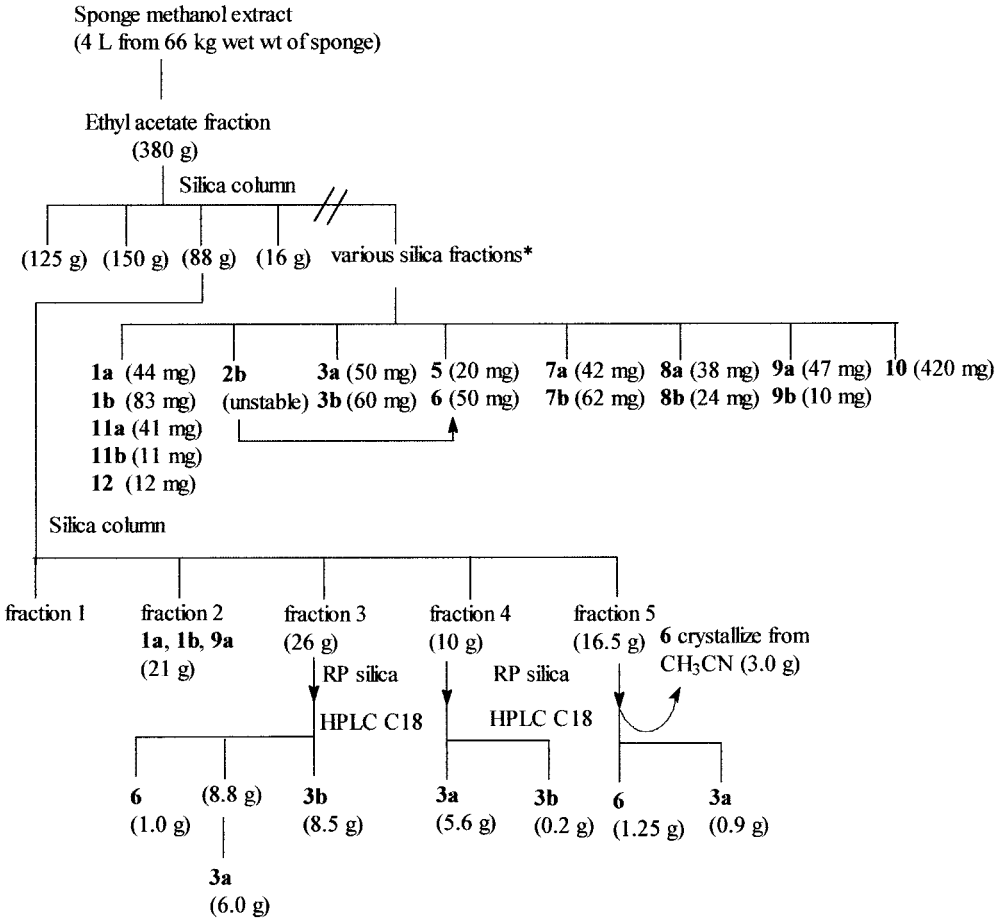




taxonomic identification	spicule type	collection site	compd types	ref
Jaspis cf. *coriacea* (Diaz & van Soest)	oxea/strongyloxea oxyaster	Fiji, Solomon Is., Papua New Guinea, Indonesia	Beng A/B Beng C/D Beng E/F Bengazoles	2a,b
Jaspis carteri (Hooper)	oxea/strongyloxea oxyaster	New Caledonia	Beng A/B	5a
Pachastrissa sp. (Ilam)	oxea/strongyloxea oxyaster I oxyaster II	Djibouti (Red Sea, Gulf of Aden)	Beng A/B Beng E/F bengazoles	5b
Jaspis digonoxea (van Soest)	oxea microxea oxyaster	South Africa	Beng A/B Bengazoles	6c
Jaspis sp. (van Soest)		Australia	Beng A/B	5c
Jaspis sp. (van Soest)		Australia	bengazoles	6d

of known compounds (see the Experimental Section) and six new compounds: 38 mg of bengamide M (**7b**), 38 mg of bengamide N (**8a**), 24 mg of bengamide O (**8b**), 3.6 mg of bengamide P (**9a**), 10.7 mg of bengamide Q (**9b**), and 21.9 mg of impure bengamide R (**10**) (structures are shown in Figure 2).

Chart 1. Scheme To Emphasize Isolation of Bengamides



* Combined fractions from numerous silica runs

The process to establish structures of the six new bengamides began once each molecular formula was established. The next step was to search for the five diagnostic low-field resonances at δ 4.0−6.0, shown in Figure 3 for H3, H4, H5, H10, or H13. A similar evaluation was conducted to identify methyl groups expected in the upfield region at δ 0.8−1.5. These resonances were used to count the number of terminal isopropyl groups containing isochronous or anisochronous methyl resonances and to identify methyl groups at the terminus of a long aliphatic chain. In addition, the ^{13}C NMR data of bengamide A (**1a**) and of bengamide E (**3a**), shown in Table 2, served as a benchmark to rapidly categorize structural variations within the new analogues. Finally, the NMR data were employed to divide the six new compounds into two sets. The first set possessed one isopropyl moiety with diastereotopic methyls (consistent with Me1 and Me15 of bengamide A) plus one isopropyl array with isochronous methyls (an isopropyl terminus of a long aliphatic ester residue attached at C13). The compounds consistent with this profile include bengamide M (**7b**), N (**8a**), and O (**8b**). The second set had a methyl terminated long chain aliphatic residue at C5 and consisted of bengamide P (**9a**), Q (**9b**), and R (**10**).

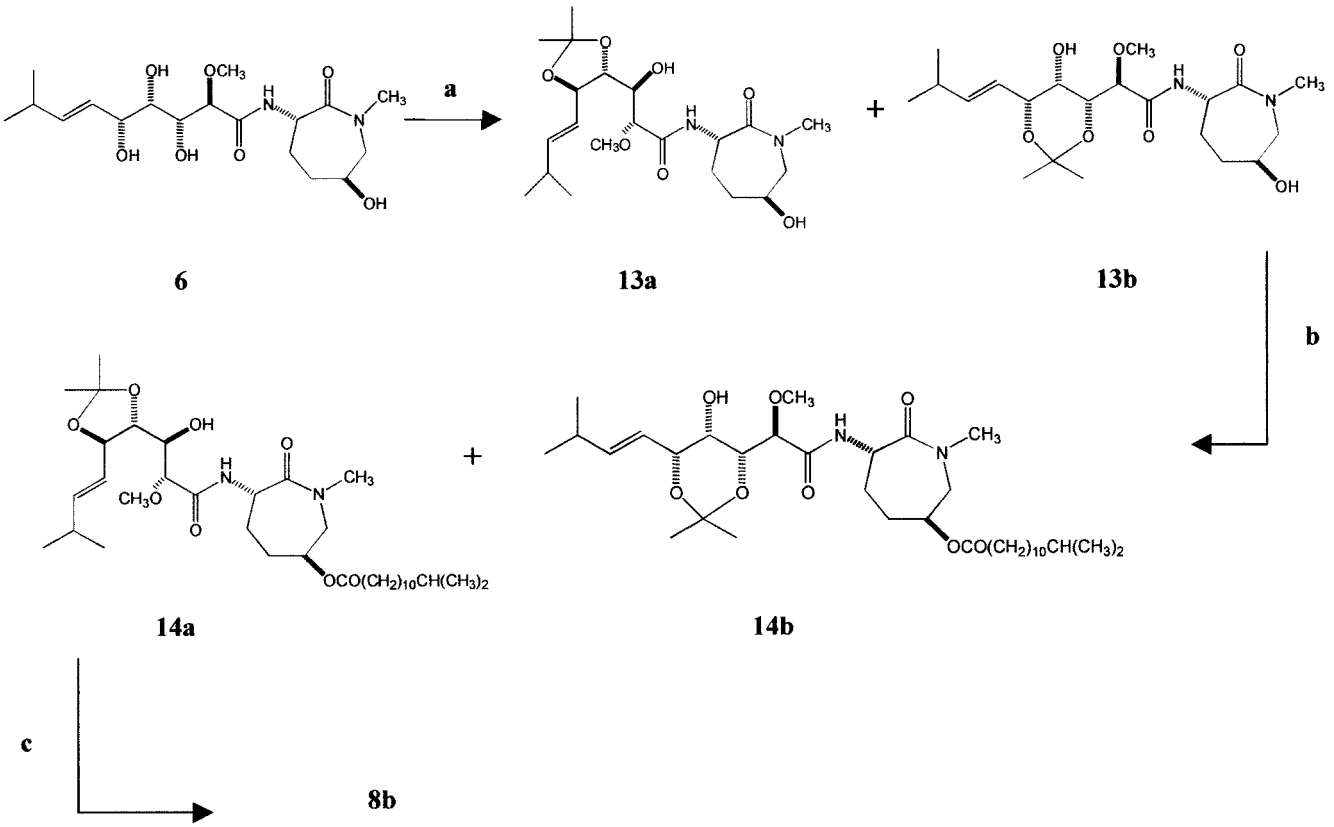
Table 2. Comparative ^{13}C NMR Data for Classes of Bengamides in $CDCL_3$ (125.7 MHz)

carbon no.	**1a**[2]	**3a**[2]	**8a**	**9a**[a]
17	174.2 (s)		174.9 (s)	175.1 (s)
16	173.0 (s)	175.2 (s)	173.1 (s)	173.6 (s)
9	172.3 (s)	171.7 (s)	172.1 (s)	172.2 (s)
3	141.9 (d)	141.6 (d)	142.0 (d)	144.1 (d)
4	125.5 (d)	125.5 (d)	125.6 (d)	122.0 (d)
8	81.3 (d)	81.7 (d)	81.7 (d)	80.8 (d)
5	74.3 (d)	74.1 (d)	74.4 (d)	75.9 (d)
7	72.8 (d)	72.4 (d)	72.7 (d)	72.0 (d)*
6	72.5 (d)	72.7 (d)	72.7 (d)	71.4 (d)*
13	70.9 (d)	28.7 (t)	70.9 (d)	29.1 (t)
OMe	59.7 (q)	59.9 (q)	59.9 (q)	60.2 (q)
10	51.5 (d)	52.0 (d)	51.6 (d)	52.2 (d)
14	45.2 (t)	42.0 (t)	45.2 (t)	42.3 (t)
18	34.4 (t)		34.5 (t)	34.9 (t)
12	33.0 (t)	28.0 (t)	34.2 (t)	28.2 (t)
28	32.0 (t)		33.1 (d)	32.1 (t)
2	30.9 (d)	30.8 (d)	32.1 (d)	30.0 (d)
20−26	29.7−29.2 (t)		31.0−27.6 (t)	30.0−29.3 (t)
27	29.7−29.2 (t)		39.2 (t)	30.0−29.3 (t)
11	28.9 (t)	31.0 (t)	31.0−27.6 (t)	31.4 (t)
19	25.0 (t)		25.1 (t)	25.2 (t)
29	22.8 (t)		22.8 (q)	22.9 (t)
1,15	22.3, 22.2 (q)	22.2, 22.1 q	22.4, 22.3 (q)	22.2, 22.1 (q)
30	14.2 (q)		22.8 (q)	14.3 (q)

[a] The signals marked with an asterisk can be interchanged.

Identification of the structures of bengamides M, N, and O relied on comparing their HRFABMS-derived molecular formulas and NMR data with comparisons of other closely related compounds. For example, the molecular formula of bengamide M (**7b**), $C_{33}H_{60}O_8N_2$, was the same as the known compound, bengamide J.[6a] Comparison of the NMR data between bengamide M (**7b**) and the known compound bengamide L (**7a**)[6b] substantiated that this pair differed only by an *N*-Me group. Similarly, the molecular formula of bengamide N (**8a**),

Scheme 1. Semisynthesis of Bengamide O (8a) from Natural Bengamide Z (6)[a]



[a] Key: (a) anhyd $ZnCl_2$, anhyd H_3PO_4, acetone, rt, 1 h; (b) DMAP, EDCI, 12-methyltridecanoic acid; (c) TFA, H_2O, 0.5 h, 0 °C.

$C_{31}H_{56}O_8N_2$, has one CH_2 less than that of bengamide L (**7a**). Their comparative NMR data allowed the missing methylene group to be placed in the aliphatic ester chain. Likewise, the molecular formula of bengamide O (**8b**), $C_{32}H_{58}O_8N_2$, contained an additional CH_2 versus that of bengamide N (**8a**). Once again, the comparative NMR data allowed the difference in this pair to be ascribed to replacement of *N*-H by *N*-Me in the caprolactam group.

The bengamides P, Q, and R were seen to be related to bengamides E/F because resonances for H13 (see Figure 3) were absent from the δ 4.6 region. However, both ^{13}C and ^1H NMR data revealed that P, Q, and R each contained a fatty acid moiety, not present in E/F. The molecular formula of bengamide P (**9a**), $C_{31}H_{56}O_7N_2$, was one oxygen less than that of bengamide A (**1a**), which meant that a myristate group must have replaced one of the hydroxyl groups in the C-10 side chain of the bengamide E framework. The $^1H-^1H$ COSY data of P (**9a**) revealed that the shift of H5 was at δ 5.48 versus δ 4.25 (Figure 3). The logical conclusion that the myristate group was attached to C5 was further confirmed by HMBC NMR data.

Bengamides Q and R proved to be close in structure to that of P. The molecular formula of bengamide Q (**9b**), $C_{32}H_{58}O_7N_2$, along with the NMR data indicated it was the caprolactam *N*-Me analogue of bengamide P (**9a**). Similarly, the molecular formula of bengamide R (**10**) $C_{33}H_{60}O_7N_2$ indicated that it was the C_2H_4 homologue of P (**9a**) while its NMR properties showed the caprolactam *N*-Me was absent and the other resonances were virtually identical to that of P (**9a**). This suggested that a palmitate rather than a myristate fatty acid moiety is attached to C5 of the nonenoyl side chain.

The bengamide analogues C (**2a**) and D (**2b**) once again eluded efforts to obtain them in pure form. Several years[2b] ago, we reported that bengamide C proved to be very acid sensitive and rapidly hydrolyzed to give 1:1 mixtures of a bengamide informally called hydroxy bengamide E (recently renamed bengamide Y)[6c] and a nonenoyl lactone. The same is true for bengamide D which also rapidly hydrolyzes to give 1:1 mixtures of a bengamide informally called hydroxy bengamide F (recently renamed bengamide Z[6c]) and nonenoyl lactone. A small amount of impure and unstable **2b** was obtained (Chart 1). Gram quantities of bengamide Z (**6**) were isolated and utilized as an advanced intermediate for semisynthesis of bengamide analogues. During this work we assumed that the absolute stereochemical elements of the additional bengamides isolated, G−I, L−R, as well as those of Y and Z were identical to those of the bengamides whose stereochemistry had been rigorously established.[2,3] This seemed to be justified in that the optical properties and ^1H NMR chemical shifts of and coupling constants to each methine resonance of all bengamides are nearly identical.

Synthetic schemes were developed and implemented to obtain additional samples of bengamides for biological evaluation. The convergent total syntheses that start with commercially available α-D-glucoheptonic-γ-lactone and 5(*R*)-5-hydroxy-L-lysine provide gram quantities of bengamide B (**2b**), and E (**3a**), as will be reported elsewhere.[10] Bengamide O (**8b**) was prepared semisynthetically from bengamide Z (**6**) (Scheme 1). Bengamide Z (**6**) was converted to a 4:1 mixture of acetonides **13** in 95% yield. Selective esterification at the 13-OH position with 12-methyl-tridecanoic acid afforded the

Table 3. Selected NCI-DTP[a] Antitumor (in Vitro) Activity Results of Bengamides (IC_{50}, μM)[b] against 7 Cell Lines

tumor type	A (**1a**) (NSC 613012)	B (**1b**) (NSC 646846)	P (**9a**) (NSC 646847)
NSCL			
A549	0.019	0.001.9	0.69
HOP92	0.200	0.0068	5.6
NCI-H522	0.060	0.0063	3.1
colon			
HCT 116	0.018	0.0024	0.73
HCT 15	0.260	0.130	2.80
COLO 205	0.018	0.025	0.30
CNS			
SNB 75	0.190	0.063	3.3
SNB 19	0.024	0.0086	5.4
melanoma			
UACC 62	0.015	0.0052	2.5
LOX IMVI	0.023	0.0023	1.1
MALME-3M	0.180	0.022	6.0
ovarian			
OVCAR 3	0.010	0.010	4.0
OVCAR 8	0.007	0.0051	1.9
renal			
UO 31	0.370	0.025	0.990
786-0	0.024	0.0035	0.940
leukemia			
CCRF-CEM	0.027	0.0073	3.10
average IC_{50}	0.046 ± 0.005	0.011 ± 0.001	2.70 ± 0.23

[a] Data provided by the National Cancer Institute−Development Therapeutics Program (NCI-DTP). For the comprehensive data set against 60 cell lines use the NSCs above at http://dtp.nci.nih.gov/. [b] $IC_{50} = LC_{50}$ single point data reported on the website given above.

corresponding mixture of **14a** and **14b**. Finally, TFA-promoted deprotection gave bengamide O (**8b**), whose properties were identical to the natural product.

The original biological screening of the bengamides revealed their significant anthelminthic activity[11] and cytotoxicity.[12] In 1993, we reported that bengamides A (**1a**), E (**3a**), F (**3b**), P (**9a**), and Q (**9b**) were active at 0.1 μM concentrations in an *in vitro* anthelminthic screen while F (**3b**) showed *in vivo* activity.[11] Bengamides A (NSC 613012), B (NSC 646846), and P (NSC 646847) were submitted to the National Cancer Institute−Development Therapeutics Program (NCI-DTP) 60 cell line screen for[13] further evaluation of their cytotoxicity. A subset of the data obtained is reported for the first time in Table 3 and the full set is openly available by inputting the individual NSCs at the Web address shown. The entries chosen for Table 3 illustrate that there can be differential sensitivities to the bengamides for the individual cell lines within each of seven different tumor types. The overall level of activity (given as average IC_{50}) was 0.046 μM for bengamide A, 0.011 μM for bengamide B, and 2.7 μM for bengamide P. As has been reported previously for bengamide Z (NSC not available),[6c] differential levels of growth inhibition were observed among cell lines within a particular tumor class. Use of the COMPARE algorithm to analyze the *in vitro* data suggests that bengamide cytotoxicity is not correlated with any of the reported molecular targets. This further suggests that bengamide activity may be due to inhibition of a novel target.

Detailed studies on the anti-proliferative properties of eight bengamide derivatives were performed by the Oncology Research group at NIBR. The comparative IC_{50} data on several bengamides shown in Table 4 against MDA-MB-435 illustrates several important SAR features. The presence and location of the fatty acyl chain provides substantial differences in *in vitro* potency. Bengamides substituted at the 13-position with long chain esters (A, B, M, and O) are 100−1000-fold more potent than bengamide Z and the lysine-derived bengamides (E, F, and P). Bengamide Z is derived from hydroxylysine but lacks the fatty acyl chain common to some other members of this class. Bengamide P, which is a myristylated lysine-derived bengamide, is only as potent as its nonmyristylated counterpart (bengamide E). Finally *N*-methylation of the caprolactam causes only minor perturbation in activity.

The bengamides occur as complex mixtures, making absolute purification difficult if not impossible. This made it essential to use a side-by-side evaluation of natural products and synthetic materials as further proof of *in vitro* SAR trends. Replicate experiments were run on bengamides B and O and synthetic or semisynthetic derived compounds. In each case, a difference of no more than a factor of 10 was observed in the IC_{50} data. Thus, the data collected in Table 4 further substantiate the anti-proliferative profile of the bengamide series.

Conclusions

We have speculated but to date have not been able to prove that bengamides are formed via a mixed ketide-amino acid biosynthesis.[2] The terminal methyl groups can arise from a branched amino acid such as leucine while the other end of the molecule, a cyclized L-lysine, flanks a diketide subunit. This kind of mixed ketide-amino acid biosynthesis is uncommon but has an interesting parallel, as shown in Chart 2, to barbamide isolated from the marine cyanobacterium *Lyngbya majuscula*, and experimentally shown to be comprised of three amino acids plus an acetate unit.[14]

Our consistent isolation of the bengamides from extracts of *Jaspis* cf. *coriacea* suggested that these compounds might be chemical markers for this sponge genus. However, the literature surveyed in Table 1 indicates that this may not be the case. Yet these compounds seem restricted to the *Jaspis*/*Pachastrissa* genera. In general, the bengamides are obtained in relatively high yield from the organisms shown in Table 1; thus, there is no reason to suspect that the biogenesis of bengamides depends on involvement of microorganisms.[15] Alternatively, there are reports of caprolactams isolated from the fungi *Periconia* circinata[16a] and *Streptomyces griseus*.[16b] We have successfully explored several specimens of *J*. cf. *coriacea* for

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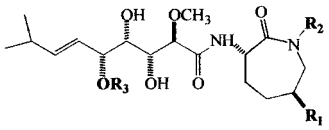
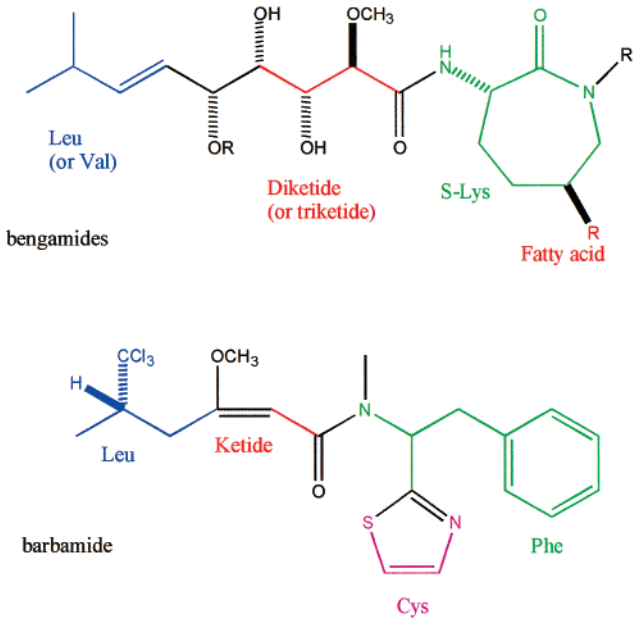
Table 4. ***In Vitro*** **Anti-Proliferative Activity of Selected Bengamides (IC_{50}, μM) on MDA-MB-435 Human Mammary Carcinoma Cells**



bengamide	R_1	R_2	R_3	MDA-MB-435 (IC_{50}, μM)
A (**1a**)	$OCO(CH_2)_{12}CH_3$	H	H	0.001 ± 0.0006
B (**1b**)	$OCO(CH_2)_{12}CH_3$	CH_3	H	0.012 ± 0.003
M (**7b**)	$OCO(CH_2)_{11}CH(CH_3)_2$	CH_3	H	0.0101 ± 0.0021
O (**8b**)	$OCO(CH_2)_{10}CH(CH_3)_2$	CH_3	H	0.00029 ± 0.0005
Z (**6**)	OH	CH_3	H	2.9 ± 1.5
E (**3a**)	H	H	H	3.3 ± 1.2
F (**3b**)	H	CH_3	H	2.9 ± 2.9
P (**9a**)	H	H	$CO(CH_2)_{12}CH_3$	1.2 ± 7.9
B (**1b**)-s	$OCO(CH_2)_{12}CH_3$	CH_3	H	0.0024 ± 0.0008
O (**8b**)-ss	$OCO(CH_2)_{10}CH(CH_3)_2$	CH_3	H	0.0008 ± 0.0005

s: prepared by total synthesis; ss: prepared by semi-synthesis starting with **6** (Scheme 1).

Chart 2. Biosynthetic Origins of the Bengamides vs Barbamide[20]



fungi that are chemically prolific when grown in saltwater culture. These include *Paecilomyces* cf. *javanica* as a source of deoxynortrichoharzin and diketopiperazines,[17] as well as an *Aspergillus* fungus which produces the chlorocarolides,[17b] but no bengamide-like compounds have been detected in the crude extracts of these fungi grown in saltwater culture. Caprolactams have also been isolated from unidentified Gram-positive marine bacteria cultured from deep ocean sediments.[18] It is noteworthy that simple natural product caprolactams have also shown cytotoxic, antibiotic, and antiviral activity.[19]

The best anti-proliferative *in vitro* activity is found for bengamides having a fatty acid attached to the caprolactam ring (bengamides A and B) and not on the polyhydroxylated side chain (bengamides P−R). Bengamides A, B, M, and O are exceptionally potent *in vitro* against the MDA-MB-435 human breast carcinoma cell line, and bengamide B has also demonstrated significant anti-proliferative effects against MDA-MB-435 xenografts in nude mice.[4] The *in vitro* potency of the bengamides, as well as the novel pattern of inhibitory activity in the NCI-DTP 60 cell line screen, firmly demonstrate the interesting cytotoxic properties for this class. The results reported above suggest that further evaluation of the bengamides as novel antitumor compounds is warranted.

Experimental Section

General Experimental Procedures. The NMR spectra ($CDCl_3$) were recorded at 500 MHz (^1H) and 125.7 MHz (^{13}C). Final NMR assignments were based on previously published data[2,6b] and 2D NMR data derived from HMQC, HMBC, and $^1H-^1H$ COSY. HPLC was performed with a 10 μm ODS column.

Biological Material, Collection, and Identification. Specimens of *Jaspis* cf. *coriacea* were collected from five different study sites in the Fiji Islands, using scuba, at depths of 20−100 feet: Benga Lagoon (97000), (S 18° 22.100′, E 177° 58.490′); Savu Savu (97015), (S 16° 47.70′, E 179° 53.25′); Somo Somo Strait (97016), (S 16°45.286′, W 179° 59.629′); Taveuni/Tasman Strait (97017), (S 16° 43.746′, W 179° 49.552′); and Ngau Island (97021) (S 17° 57.69′, E 179° 15.330′). The sponge is commonly found in an encrusting form (0.5−4 cm thick), under ledges. It is dull orange in color, with a rubbery texture, membranous oscules, easily torn and with crustaceans on the under side. Megascleres consist of oxea-strongyloxea, 550 μm × 6 μm (300 μm × 5 μm − 720 μm × 10 μm), microscleres are oxyasters, with 8−12 microspined rays, 24 μm (10−35 μm). An underwater photo is shown in Table 1.

It is important to note that *Jaspis* cf. *coriacea* and *J. carteri* are very similar, both in external morphology and skeletal character. However, the slight difference in size and arrangement of skeletal elements justifies separation into two distinct species.[20] After careful examination of the specimen described as *Pachastrissa* sp., there is strong evidence for considering it as congeneric with other *Jaspis* sponges. Under current parameters for assigning species to the genus *Jaspis* Gray 1967,[21] the spicules from the specimen described as *Pachas-*

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trissa sp. are considered as oxyasters, rather than true calthrops. Although in this case the definition between the two spicule types is difficult to determine, there is a high degree of variation in ray number and length in both the *Pachastrissa* voucher and other *Jaspis* species. Therefore, in this group of sponges, the spicules are considered as oxyasters. This determination would conclude that the specimen does not belong in the family Calthropellidae. This observation, in combination with the similarity of chemical profiles, suggests that the *Pachastrissa* specimen be placed in the genus *Jaspis*. It does, however, differ substantially from both *Jaspis* cf. *coriacea* and *J. carteri*, having at least two different classes of oxyaster, and should therefore be described as a separate species. A future examination of both the *Jaspis digonoxea* (South Africa) and *Jaspis sp*. (Australia) specimens will determine their taxonomic relationship to the other three sponges.

Extraction and Isolation of Bengamides. The sponges were initially preserved according to our standard procedure as described previously.[22] From previous experience[2] with bengamides, we were able to easily direct the extraction toward fractions with a high bengamide content. The sponge was soaked six times in CH_3OH and the resulting oil (4 L) extracted with 3 × 1.5 L of EtOAc. The EtOAc fraction (380 g) was applied to a silica column (4.5 kg silica, sample in CH_2Cl_2) and eluted with 16 L of CH_2Cl_2 (resulting in 125 g oil), 16 L of EtOAc (resulting in 150 g oil), 24 L of 20% $MeOH/CH_2Cl_2$ (bengamide fraction, 88 g) followed by 16 L of CH_3OH wash (resulting in 16 g of tar). The bengamide-containing fraction (88 g) was then further fractionated on a silica gel column (1.76 kg of silica, 21 cm bed height flushed with 4.4 L of EtOAc/hexanes 1:1, sample applied in 220 mL of EtOAc/hexanes 1:1) and eluted with 6.2 L of EtOAc (fraction 1), 8.8 L of 5% CH_3OH/EtOAc (fraction 2), 13 L of 10% CH_3OH/CH_2Cl_2 (fraction 3 and 4), and 4.4 L of CH_3OH (fraction 5). Bengamides were found in fractions 2−5. RP silica was used to remove colored nonpolar components. The resulting fractions were then further purified by preparative HPLC to afford 12.5 g of bengamide E (**3a**), 8.7 g of bengamide F (**3b**), and 5.25 g of bengamide Z (**6**). Final purification was carried out using gradient reversed-phase HPLC (CH_3CN/H_2O 15:85 or 20:80 and CH_3OH/H_2O 40:60 or 35:65 up to 100:0) on various fractions to yield **1a**,**b**, **3a**,**b**, **5**, **6**, **7a**,**b**, **8a**,**b**, **9a**,**b**, **10**, **11a**,**b**, and **12** as shown in Chart 1.

In Vitro Cell Proliferation Assay. Data in Table 4 were generated using an adaptation of published procedures.[23] MDA-MB-435 cells were plated in 96-well plates at an initial density of 3000 cells/well. Cell number was measured by MTS detection before and after a 72 h exposure to bengamide analogues and compared to the corresponding cell densities of vehicle treated cells. Plots of net cell growth vs compound concentrations were used to determine concentrations resulting in 50% growth inhibition (IC_{50}). IC_{50} values shown in Table 4 are the average values for two to six replicate experiments. IC_{50} values shown in Table 3 are the average GI_{50} values for two to four replicate experiments, obtained from the NCI DTP web site (http://dtp.nci.nih.gov/).

Bengamide M (7b): clear colorless oil; 62 mg; $[\alpha]_D$ +2.1° (*c* 61.9, MeOH); HRFABMS *m/z* 635.4227 $[M + Na]^+$ (100) (calcd for $C_{33}H_{60}O_8N_2Na$ 635.4247, Δ −2.0 mmu); ^1H NMR δ 8.11 (1H, d, J = 6.0 Hz, N-H), 5.79 (1H, dd, J = 6.5, 15.5 Hz, H-3), 5.46 (1H, dd, J = 6.5, 15.5 Hz, H-4), 4.67 (1H, m, H-10), 4.61 (1H, m, H-13), 4.28 (bs, OH), 4.23 (1H, t, J = 6.5, Hz, H-5), 3.81 (2H, m, H-7, H-8), 3.67 (1H, dd, J = 10.5, 14.5 Hz, H-14), 3.61 (1H, bd, J = 6.0 Hz, H-6), 3.54 (3H, s, OMe), 3.23 (1H, bd, J = 15.0 Hz, H-14′), 3.11 (3H, s, NMe), 2.31 (3H, m, H-2, H-18), 2.15 (2H, m, H-11, H-12), 1.96 (1H, m, H-12′), 1.61 (3H, m, H-11′, H-19), 1.52 (1H, m, H-29), 1.26 (16H, H-20 to H-27), 1.15 (2H, m, H-28), 1.00 (6H, dd J = 2.5, 7.0 Hz, H-1, H-15), 0.86 (6H, d J = 6.5 Hz, H-30, H-31); ^{13}C NMR δ 173.2 (s, C-17), 172.4 (s, C-16), 172.0 (s, C-9), 142.1 (d, C-3), 125.6 (d, C-4), 81.0 (d, C-8), 74.5 (d, C-5), 73.1 (d, C-7), 72.5 (d, C-6), 69.4 (d, C-13), 60.3 (q, OMe), 53.6 (t, C-14), 51.5 (d, C-10), 39.3 (t, C-28), 36.6 (q, NMe), 34.6 (t, C-18), 32.9 (t, C-12), 31.0 (d, C-2), 30.1 (d, C-29), 29.9−27.6 (t, C-11 and C-20 to C-27), 25.1 (t, C-19), 22.9 (q, C-30 and C-31), 22.4−22.3 (q, C-1, C-15).

Bengamide N (8a): clear colorless oil; 38 mg; $[\alpha]_D$ +20.7° (*c* 38.0, MeOH); HRFABMS *m/z* 607.3901 $[M + Na]^+$ (100), (calcd for $C_{31}H_{56}O_8N_2Na$ 607.3934, Δ −3.3 mmu); ^1H NMR δ 7.99 (1H, d, J = 5.5 Hz, N-H_a), 6.00 (1H, bs, N-H_b), 5.80 (1H, dd, J = 6.5, 15.5 Hz, H-3), 5.47 (1H, dd, J = 6.5, 15.5 Hz, H-4), 4.66 (1H, m, H-10), 4.60 (1H, m, H-13), 4.24 (1H, t, J = 6.5, Hz, H-5), 3.84 (1H, bd, J = 6.5, H-7*), 3.80 (1H, d, J = 6.5, H-8*), 3.61 (1H, bd, J = 6.5 Hz, H-6), 3.56 (3H, s, OMe), 3.38 (1H, m, H-14), 3.32 (1H, m, H-14′), 2.31 (2H, m, H-2, H-18), 2.19 (2H, m, H-12, H-11), 1.97 (1H, m, H-12′), 1.75 (1H, m, H-11′), 1.61 (2H, m, H-19), 1.52 (1H, m, H-28), 1.27 (14H, H-20 to H-26), 1.15 (2H, m, H-27), 1.01 (6H, dd J = 2.5, 6.5 Hz, H-1, H-15), 0.87 (6H, d J = 7.5 Hz, H-29, H-30); ^{13}C NMR δ 174.9 (s, C-17), 173.1 (s, C-16), 172.1 (s, C-9), 142.0 (d, C-3), 125.6 (d, C-4), 81.7 (d, C-8), 74.4 (d, C-5), 72.7 (d, C-6, C-7), 70.9 (d, C-13), 59.9 (q, OMe), 51.6 (d, C-10), 45.2 (t, C-14), 39.2 (t, C-27), 34.5 (t, C-18), 34.2 (t, C-12), 33.1 (d, C-28), 32.1 (d, C-2), 30.1 (t, C-11), 30.0−27.6 (t, C-20 to C-26), 25.1 (t, C-19), 22.8 (q, C-29 and C-30), 22.4−22.3 (q, C-1, C-15).

Bengamide O (8b): clear colorless oil; 24 mg; $[\alpha]_D$ +35.8° (*c* 23.9, MeOH); HRFABMS *m/z* 621.4074 $[M + Na]^+$ (100) (calcd for $C_{32}H_{58}O_8N_2Na$ 621.4091, Δ −1.7 mmu); NMR data (^{13}C and ^1H) identical to that of bengamide M (**7b**).

Bengamide P (9a): clear colorless oil; 47 mg; $[\alpha]_D$ +47.2° (*c* 3.6, MeOH); HRFABMS *m/z* 607.3732 $[M + K]^+$ (100) (calcd for $C_{31}H_{56}O_7N_2K$ 607.3725, Δ 0.7 mmu); ^1H NMR: δ 8.00 (1H, d, J = 6.5 Hz, N-H_a), 6.17 (1H, bs, N-H_b), 5.83 (1H, dd, J = 6.5, 16.0 Hz, H-3), 5.47 (1H, t, J = 7.5 Hz, H-5), 5.39 (1H, dd, J = 7.5, 16.0 Hz, H-4), 4.54 (1H, m, H-10), 3.74 (3H, bs, H-6, H-7, H-8), 3.53 (3H, s, OMe), 3.29 (2H, m, H-14), 2.34 (1H, m, H-2), 2.34 (2H, t, J = 7.5, H-18), 2.07 (2H, m, H-11, H-12), 1.86 (2H, m, H-12′, H-13), 1.60 (3H, m, H-19, H-11′), 1.43 (1H, m, H-13′), 1.26 (20H, H-20 to H-29), 0.98 (6H, d J = 7.0 Hz, H-1, H-15), 0.89 (3H, t J = 7.0 Hz, H-30); ^{13}C NMR δ 175.1 (s, C-17), 173.6 (s, C-16), 172.2 (s, C-9), 144.1 (d, C-3), 122.0 (d, C-4), 80.8 (d, C-8), 75.9 (d, C-5), 72.0 (d, C-7/C-6)*, 71.4 (d, C-7/C-6)*, 60.2 (q, OMe), 52.2 (d, C-10), 42.3 (t, C-14), 34.9 (t, C-18), 32.1 (t, C-28), 31.4 (t, C-11), 31.0 (d, C-2), 30.0−29.3 (t, C-20 to C-27), 29.1 (t, C-13), 28.2 (t, C-12), 25.2 (t, C-19), 22.9 (t, C-29), 22.2−22.1 (q, C-1, C-15), 14.3 (q, C-30).

Bengamide Q (9b): clear colorless oil; 10 mg; $[\alpha]_D$ +14.1° (*c* 10.7, MeOH); HRFABMS *m/z* 605.4170 $[M + Na]^+$ (100) (calcd for $C_{32}H_{58}O_7N_2Na$ 605.4142, Δ 2.8 mmu); ^1H NMR δ 8.14 (1H, d, J = 6.5 Hz, N-H_a), 5.83 (1H, dd, J = 6.5, 16.0 Hz, H-3), 5.46 (1H, t, J = 7.5 Hz, H-5), 5.39 (1H, dd, J = 7.5, 16.0 Hz, H-4), 4.62 (1H, m, H-10), 3.74 (3H, bs, H-6, H-7, H-8), 3.62 (1H, dd, J = 15.5, 11.5, H-14), 3.54 (3H, s, OMe), 3.22 (1H, dd, J = 5.5, 15.5, H-14′), 3.05 (3H, s, NMe), 2.34 (1H, m, H-2), 2.34 (2H, t, J = 7.5, H-18), 2.02 (2H, m, H-11, H-12), 1.84 (2H, m, H-12′, H-13), 1.63 (3H, m, H-19, H-11′), 1.47 (1H, m, H-13′), 1.26 (20H, H-20 to H-29), 0.98 (6H, d J = 6.5 Hz, H-1, H-15), 0.89 (3H, t J = 6.5 Hz, H-30); ^{13}C NMR δ 173.6 (s, C-17), 172.4 (s, C-16), 172.1 (s, C-9), 144.0 (d, C-3), 122.0 (d, C-4), 80.6 (d, C-8), 75.9 (d, C-5), 71.9 (d, C-7/C-6)*, 71.4 (d, C-7/C-6)*, 60.3 (q, OMe), 52.1 (t, C-14), 50.6 (d, C-10), 36.3 (q, NMe), 34.9 (t, C-18), 32.1 (t, C-28), 31.5 (t, C-11), 31.0 (d, C-2), 29.9−29.3 (t, C-20 to C-27), 27.9 (t, C-13), 26.8 (t, C-12), 25.2 (t, C-19), 22.9 (t, C-29), 22.2−22.1 (q, C-1, C-15), 14.3 (q, C-30).

Bengamide R (10): impure sample; oil; 420 mg; no $[\alpha]_D$ measured; HRFABMS *m/z* 619.4301 $[M+Na]^+$ (100), (calcd for $C_{33}H_{60}O_7N_2Na$ 619.4298, Δ 0.3 mmu); NMR data (^{13}C and ^1H) identical to that of bengamide P (**9a**).

Preparation of Bengamide O (8b). For the ketal protection, a solution of anhydrous $ZnCl_2$ (275 mg) and anhydrous H_3PO_4 (20 mg) in 10 mL of acetone was added to **6** (250 mg). TLC (10% $MeOH/CH_2Cl_2$) showed spot to spot conversion after 1 h at room temperature. The reaction was quenched with 2.4 M K_2CO_3 (1 mL), extracted with CH_2Cl_2 (30 mL), dried with Na_2SO_4, and evaporated to give 270 mg of crude oil. The

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product was purified on a short silica column with 10% MeOH/CH_2Cl_2 to give 260 mg of a 4/1 mixture of **13a** and **13b** (95% yield). This mixture was added to a solution of (dimethylamino)pyridine (54 mg), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimde hydrochloride (168 mg) and 12-methyl-tridecanoic acid (100 mg) in 5 mL of CH_2Cl_2. TLC (100% EtOAc) showed complete reaction after 1.5 h. Purification of the reaction solution was carried out on a silica column (50% EtOAc/CH_2Cl_2) to give a mixture of **14a** and **14b** (180 mg, 60% yield). This mixture was dissolved in a solution of TFA (3 mL), THF (3 mL) and H_2O (2 mL) at 0 °C. After 0.5 h, the reaction was evaporated under high vacuum and the residue purified on silica column (EtOAc) to give **8b** (96 mg, 57% yield).

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Supporting Information Available: ^{13}C and ^{1}H NMR spectra of bengamides M, N, P, and Q. This material is available free of charge via the Internet at http://pubs.acs.org.

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