‘Upenamide: An Unprecedented Macrocyclic Alkaloid from the Indonesian Sponge *Echinochalina* sp.

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‘Upenamide (**1**) represents a new class of macrocyclic marine alkaloid possessing both spirooxaquinolizidinone and hemiaminal ring systems. It was isolated from the Indonesian sponge *Echinochalina* sp. The gross structure of **1** was elucidated by spectroscopic methods and accurate mass measurements. A suggestion is made as to its biogenetic origin.

Over the past 15 years, an increasing number of macrocyclic diamine alkaloids have been reported from marine sponges. To date, more than 10 classes of polycyclic alkaloids have been described in the literature: saraines (from *Reniera sarai*, family Chalinidae),[1] haliclamines (from *Haliclona* sp., family Chalinidae),[2] xestospongins (from *Xestospongia exigua*, family Petrosiidae),[3] petrosins (from *Petrosia seriata*, family Petrosiidae),[4] papuamines (*Haliclona* sp., family Chalinidae),[5] manzamines (=keramamine, from *Xestospongia* sp., family Petrosiidae, and *Pellina* sp., family Oceanapiidae),[6] cyclostelletamines (from *Stelleta maxima*),[7] mandangamine (from *Xestospongia ingens*, family Petrosiidae),[8] xestocyclamines (from *Xestospongia* sp., family Petrosiidae),[9] ircinols (*Ircina* sp. and *Amphimedon* sp.),[10] halicyclamine A (from *Haliclona* sp., family Chalinidae),[11] ingenamines (from *Xestospongia ingens*, family Petrosiidae),[12] and halitoxins (from *Haliclona rubens*, family Chalinidae, and *Callyspongia fibrosa*, family Callyspongiidae).[13] Despite possessing quite different structural frameworks, they appear to be biogenetically derived from bis-3-alkylpyridine or reduced bis-3-alkylpyridine units.[14]

In our continuing search for new biologically active marine natural products from the Indo-Pacific area, work was begun on the crude extract of a tough and elastic, reddish brown, branching sponge, *Echinochalina* sp. (Protolithospongia) (order Poecilosclerida, family Microcionidae), collected from Derawan Island, Indonesia. The freeze-dried sponge was extracted in methanol and dichloromethane, and the crude extract was subjected to liquid−liquid partition (Kupchan procedure)[15] followed by size-exclusion, normal, and reversed-phase chroma-

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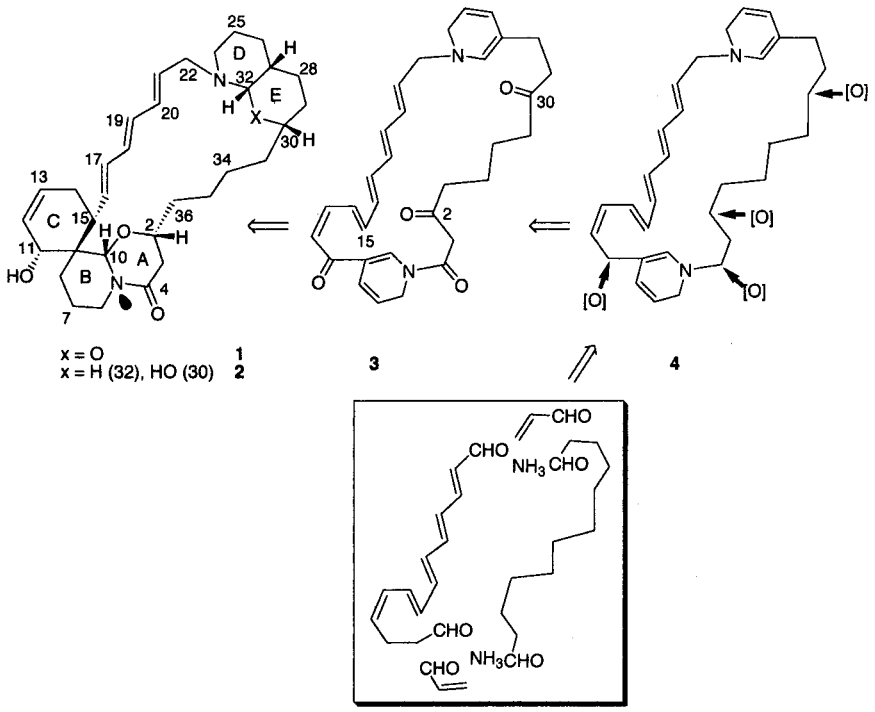


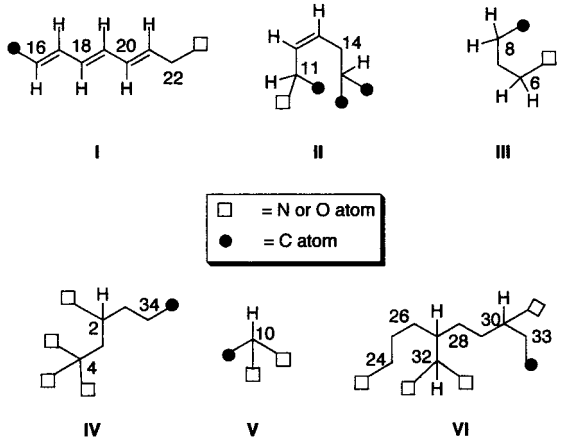
Figure 1. Proposed biogenesis of 'upenamide (**1**).

tography to afford 'upenamide (**1**, Figure 1).[16] 'Upenamide represents a new class of macrocyclic diamine alkaloid possessing both spirooxaquinolizidinone and hemiaminal ring systems.

'Upenamide (**1**) was obtained as an amorphous white solid with a molecular formula of $C_{32}H_{46}N_2O_4$ as established by HRFABMS, *m/z* $[M + H]^+$ 523.3538. The ^{13}C NMR spectrum of **1**, which showed resolved resonances for all 32 carbon atoms (2 C, 15 CH, 15 CH_2) (Table 1), contained nine deshielded resonances that could be assigned to olefinic and amide carbonyl carbons. IR bands at 3411 and 1676 cm^{-1} and ^{13}C NMR resonances at δ 70.0 (CHOH: C-11) and δ 169.4 (CO: C-4) were assigned to a secondary alcohol and an amide carbonyl unit. The anchor points for structural analysis were the well-resolved ^1H NMR double bond resonances between δ 6.72 and δ 5.48. The $^1H-^1H$ COSY and HOHAHA NMR spectra justified the connectivities between C-11/C-14 and between C-16/C-22, respectively, with a cis double bond geometry at C-12/C-13 and all-trans double bond geometry at C-16/C-21 based on proton coupling constants (Chart 1).

Fragments I and II were connected through C-15 (δ 44.8) on the basis of HMBC correlation between H-15 (δ 2.64) and C-16 (δ 135.8) as well as vicinal coupling (COSY spectrum) between H-15 and H-16 (δ 5.72). Furthermore, fragment III could also be connected to fragments I/II through C-9 (δ 44.3) on the basis of HMBC correlations between C-9 and H-11 (δ 4.82)/H-15. The proton resonance at δ 4.18 (H_{eq}-6; nitrogen-bearing methylene) was instrumental in completing the structure of fragment III. Despite overlapping signals, the $^1H-^1H$ COSY spectrum showed cross-peaks between H_{eq}-6 and H_{ax}-6 (δ 2.88), H_2-7 (δ 1.52, 2.08), and H_2-8 (δ1.61, 1.99). The C-6 (δ 41.9)/C-7 (δ 21.7)/C-8 (δ 22.2) connection was also confirmed by HMBC correlations from H_{ax}-6 to C-4/C-8/C-10 (δ 88.7). Connection of fragments I/II/III completed rings B and C and the point of attachment of the alkenyl side chain at C-15.

Chart 1. Structural Fragments Deduced from the $^1H-^1H$ COSY and HMBC Spectrum



The proton resonances at δ 4.78 (H-10; nitrogen- and oxygen-bearing methine) and δ 3.62 (H-2; oxygen-bearing methine) were pivotal in structure analysis/connection of fragments IV to V. The proton signal associated with H-10 showed no COSY cross-peaks to any other proton, but the HMBC spectrum showed correlations between H-10 and C-2 (δ 73.3)/C-4/C-9/C-11/C-15 linking these two fragments. In contrast, the proton signal at δ 3.62 showed COSY cross-peaks to H-3 (δ 2.27, 2.38)/H-36b (δ 1.43), and no HMBC correlations were observed. These observations completed the structure of ring A, the point of attachment of the C-33/C-36 side chain at C-2, and the connection of ring A to the B/C ring system. Thus, the lower portion of 'upenamide consists of a novel spirooxaquinolizidinone unit.

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Table 1. NMR Spectral Data for 'Upenamide (1) with COSY, NOESY, and HMBC[a]

position	^{1}H (mult; J = Hz)	^{13}C	COSY	NOESY	HMBC
1					
2	3.62 (ddt; 3, 3, 11)	73.3	3_{eq}, 3_{ax}, 36	10, 35, 36	
3	eq: 2.27 (dd; 3, 17)	39.6	2, 3_{ax}	2, 3_{ax}, 36	4
	ax: 2.38 (dd; 11, 17)		2, 3_{eq}	3_{eq}, 36	2, 4, 36
4		169.4			
5					
6	eq: 4.18 (ddd; 5, 5, 15)	41.9	6_{ax}, 7	6_{ax}, 7	4, 8, 10
	ax: 2.88 (ddd; 5, 10, 13)		6_{eq}, 7	10	
7	eq: 2.08 (m)	21.7			
	ax: 1.52 (m)				
8	eq: 1.99 (m)	22.2	8_{ax}	10	6, 7, 9, 10, 11
	ax: 1.61 (m)		7, 8_{eq}		6, 7, 9, 11
9		44.3			
10	4.78 (s)	88.7		2, 6_{ax}, 8_{ax}, 16, 17	2, 4, 9, 11, 15
11	4.82 (s)	70.0	12, 13	12, 15	9, 10
12	5.48 (dd; 1.1, 10)	133.1	11, 13, 14	11, 13, 14	9, 11, 13, 14
13	5.63 (ddd; 2, 2, 10)	126.6	11, 12, 14	12, 14	14
14	eq: 2.00 (m)	30.8			9, 12, 13
	ax: 1.90 (m)				9
15	2.64 (ddd; 6, 11, 11)	44.8	14, 16	11	8, 9, 11, 14, 16, 17
16	5.72 (dd; 11, 15)	135.8	15, 17	10, 15, 17	9, 14, 18
17	6.64 (dd; 11, 15)	129.3	16, 18	10, 14	15, 18, 19
18	6.01 (dd; 11, 15)	130.6	17	16	16, 17, 20
19	5.97 (dd; 11, 15)	128.8	20	21	17, 20, 21
20	6.72 (dd, 11, 15)	131.3	19, 20, 22	22, 32	22
21	5.69 (ddd; 4, 11, 15)	130.2	20, 22a, 22b	19, 22, 32	19
22	a: 3.58 (t; 11)	56.4	21, 22b	32	20, 21, 32
	b: 3.16 (m)		21, 22a		21
23					
24	a: 2.77 (ddd; 3, 12, 12)	49.0	24b, 25a, 25b		
	b: 2.57 (br d; 12)		24a, 25a, 25b		
25	a: 1.71 (m)	25.9			
	b: 1.66 (m)				
26	a: 1.76 (m)	23.9			
	b: 1.30 (m)				
27	1.63 (m)	35.7	32	29, 32	
28	a: 1.72 (m)	29.8			
	b: 1.59 (m)				
29	a: 1.36 (m)	28.1			
	b: 1.28 (m)				
30	3.17 (m)	76.3	29, 33	28, 32, 33	
31					
32	4.12 (s)	86.3	27	21, 22, 25, 27, 30	22, 26, 28, 30
33	a: 1.51 (m)	34.8			34, 35
	b: 1.46 (m)				
34	a: 1.68 (m)	24.21			
	b: 1.53 (m)				
35	a: 1.68 (m)	21.18			
	b: 1.53 (m)				
36	a: 1.65 (m)	34.5		34, 35	
	b: 1.43 (m)			2, 34, 35	

[a] Spectra of **1** were recorded in CD_3OD at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR.

Completion of fragment VI was accomplished by HMBC and ^{1}H−^{1}H COSY correlations. The proton signal resonating at δ 4.12 (H-32, nitrogen- and oxygen-bearing methine) indicated only one ^{1}H−^{1}H COSY correlation to H-27 (δ 1.63); HMBC cross-peaks to C-22 (δ 56.4)/C-30 (δ 76.3) established the connections between C-32 (δ 86.3) and C-22 (across N-23) and C-30 (across O-31), thus providing the point of attachment of the alkenyl chain to ring D. The diagnostic proton signals of H_2-24 (δ 2.57, 2.77) showed COSY cross-peaks to H_2-25 (δ 1.66, 1.71), whereas H-30 (δ 3.17) showed COSY correlations to H_2-29 (δ 1.28, 1.36) and H_2-33 (δ 1.46, 1.51), providing sufficient evidence to complete assignment of rings D/E and also the point of attachment of the C-33/C-36 chain. Connection of all fragments yielded 'upenamide (**1**), containing a unique hemiaminal ring system in the upper portion of the molecule.

Compound **1** showed no Bohlman absorptions in its IR spectrum, thus suggesting absence of a *trans*-oxaquinolizidine system.[17] The NOESY spectrum of **1** indicated cross-peaks between H-10 and H-2/H-6_{ax}/H-8_{ax}/H-16/H-17, clearly indicating that rings A/B are cis-oriented and the C-15 carbon on ring C is β-oriented with reference to rings A and B. Further evidence was obtained from the ^{1}H and ^{13}C NMR data of **1** as compared to previously isolated oxaquinolizidine-containing compounds.[3] Particularly interesting is the downfield chemical shift for the H-10 signal (δ 4.78) due to the effects of the lone pair electrons of N-4,[18] and an opposite upfield shift is observed for C-10 (δ 88.7).[19] Also, the NOESY spectrum of **1** revealed a cross-peak between H-11 and H-15, thus

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(19) In xestospongin C, which contains both *cis* and *trans* oxaquinolizidine systems, the proton signal for H-10 appears at $\boldsymbol{\delta}$ 4.40 in the cis case versus H-10′, which appears at δ 3.13 in the trans case. The ^{13}C signal for C-10 appears at δ 88.18 in the cis case versus C-10′, which appears at δ 96.22 in the trans case.[3a]

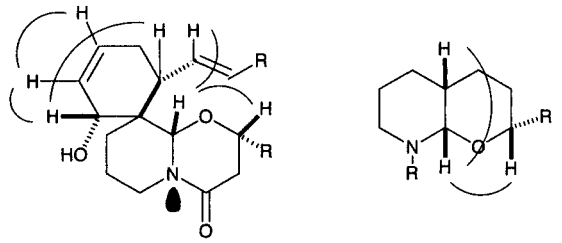

Figure 2. Relevant NOEs obtained from 1D and 2D NOE spectra.

suggesting that both C-11 hydroxyl and C-15 alkenyl chain are α-oriented with respect to ring C (Figure 2).

The absolute configuration for the lower portion of 'upenamide was determined through Mosher analysis.[20,21] Chemical shift differences between the *S*- and *R*-Mosher esters[22] suggested the 11*R* configuration for the carbinol center and the absolute configuration for all stereogenic centers on rings A/B/C as 2*R*,9*S*,10*S*,11*R*,15*R* (Figure 3).

The relative configuration of the hemiaminal portion of 'upenamide was not straightforward, due to the severe crowding of upfield resonances between δ 1.2 and δ 1.8; however, tracing the NOESY and 1D gNOE NMR[23] spectra between H-32 to H-27 and H-32 to H-30 provided the final piece of the puzzle: a cis decalin-like arrangement of the D/E rings (Figure 2). A cis-junction of rings D/E is also confirmed, because H-27 and H-32 appear as broad singlets in the proton spectrum, attesting to a very small coupling constant and lowfield displacement of the anomeric proton H-32 (δ 4.12). Syntheses of rare mixed O−N-bisheterobicycles with cis-junction selectivity have been conducted by Duhamel and co-workers, which clearly supports the relative configuration of the hemiaminal portion of 'upenamide.[24] To determine the absolute configuration of rings D/E, **1** was treated with $NaCNBH_3$ in THF:MeOH (1:1) under reflux to afford the corresponding piperidine diol **2**.[25] Unfortunately, no chemical shift differences were seen upon derivatization of **2** to its corresponding *S*- and *R*-Mosher esters, presumably due to the lack of proper conformation. Attempts to use more exotic derivatizing reagents were precluded because of the lack of sample.

'Upenamide (**1**) has a skeleton without prior precedent and appears to be closely related to a hypothetical haliclamine such as **4**.[2,14,26] The basic biogenetic building blocks of **1** consist of ammonia, a three carbon unit present in propenal, and a variable saturated or unsaturated linear dialdehyde accounting for the formation of the bis-3-alkyldiyhydropyridine macrocycle **4**. Formation of the C ring in **1** could be envisaged as a Michael-type addition of C-9 onto C-15 initiated by the nitrogen lone pair (Figure 1). In a purely formal sense, 'upenamide may be considered to be made up of two NCO fragments connected by two alkyl chains. Isolation of **1** expands our knowledge of the biosysnthetic pathways leading to 3-alkylpyridine and bis-3-alkylpyridine. In bioassays **1** does not show in vitro growth inhibition effects against P388, A549, and HT29 cancer cell lines.

Experimental Section

Spectral Analysis. NMR spectra were determined on a General Electric GN Omega 500 spectrometer operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C. Gradient NOE experiments were carried on a Bruker DRX600. ^{1}H chemical shifts are referred to CD_3OD (offset set to δ 3.30 for C*H*D_2OD impurity); ^{13}C chemical shifts are referred to CD_3OD (49.0 ppm). Homonuclear ^{1}H connectivities were determined by using the 2D double-quantum filtered COSY and 1D decoupling experiments. Homonuclear ^{1}H NOEs were obtained by difference NOE experiments using a 2 s irradiation period. One-bond heteronuclear ^{1}H−^{13}C connectivities were determined by 2D proton-detected HMQC experiments; two- and three-bond ^{1}H−^{13}C connectivities were determined by 2D proton-detected HMBC experiments. High-resolution mass spectra were determined in the FAB modes. Optical rotations were measured on a Jasco-DIP-700 instrument using methanol at 20 °C at the sodium D line (589 nm). IR spectra were recorded on a Perkin-Elmer 1600 FTIR.

Animal Material. The sponge was collected in March 1996 at a depth of 80 feet at Old Pier, Derawan, Indonesia (2° 17" 28" N, 118° 14' 13" E). The sponge is erect, branching to produce-fan-shape blades, and has a smooth, velvety upper surface. The opposing face is covered in small holes arranged in a circle. The texture is very firm and fibrous, with a central diffuse axis. In life the sponge is reddish brown, and light brown in ethanol. The skeleton is composed of robust spongin-bound reticulate tracts, heavily cored by oxea that run parallel to the axis of the sponge; each tract is echinated heavily by smaller oxea. At the surface, oxea form dendritic diverging tracts. The sample is an undescribed species of the genus *Echinochalina* (Protolithospongia, Order Poecilosclerida, Family Microcionidae). A voucher specimen has been deposited at the Natural History Museum, London, United Kingdom (BMNH 1996.11.20.2).

Extraction and Isolation. The wet sponge (588 g) was extracted in methanol (1.5 L) and methylene chloride (0.5 L). The solutions were combined and concentrated to dryness. The crude extract was partitioned between chloroform/water (1:1) and the aqueous layer reextracted with *n*-butanol to afford fractions A (63 mg), B (170 mg), and C (214 mg). Fractions A and B were combined and subjected to liquid−liquid partitioning using the Kupchan procedure.[15] The crude material from

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(21) *S*- and *R*-MTPA esters were prepared by reacting 1 with *R*-MTPA-Cl or *S*-MTPA-Cl in dry pyridine at room temperature. Attempts to purify either Mosher ester led to complete decomposition and loss of material. After careful examination of the ^{1}H NMR spectra of both esters, some important information could be extracted for chemical shift comparison.

(22) *S*-MTPA ester of **1**: ^{1}H NMR (500 MHz, pyridine-d_5) δ 4.598 (H-10), 6.24 (H-11), 5.455 (H-12), 5.841 (H-13), 5.879 (H-16), 6.716 (H-17), 6.00 (H-18), 6.161 (H-19), 6.936 (H-20), 5.643 (H-21). *R*-MTPA ester of **1**: ^{1}H NMR (500 MHz, pyridine-d_5) δ 4.653 (H-10), 6.3125 (H-11), 5.2075 (H-12), 5.756 (H-13), 5.874 (H-16), 6.732 (H-17), 6.03 (H-18), 6.168 (H-19), 6.9505 (H-20), 5.639 (H-21).

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(25) 'Upenamide (**1**) (2.5 mg) was refluxed in THF:MeOH (1.0 mL, 1:1, 2 h) and the crude product purified by reversed-phase HPLC to yield 1.5 mg of the piperidine diol 2: ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.68 (m, H-2), 2.46 (dd, J = 3 and 17 Hz, H-3$_{eq}$), 2.26 (dd, J = 12 and 17 Hz, H-3$_{ax}$), 4.45 (dt, J = 4, 4, and 14 Hz, H-4$_{eq}$), 2.71 (ddd, J = 4, 13, and 13 Hz, H-4$_{ax}$), 2.16 (m, H-7$_{eq}$), 1.59 (m, H-7$_{ax}$), 4.66 (s, H-10), 4.99 (s, H-11), 5.53 (dd, J = 1.5 and 10 Hz, H-12), 5.63 (m, H-13), 2.05−1.89 (m, H_2-14), 2.65 (ddd, J = 6, 11, and 11 Hz, H-15), 5.65 (dd, J = 11 and 15 Hz, H-16), 6.53 (t, J = 11 Hz, H-17), 5.95 (dd, J = 11 and 15 Hz, H-18), 5.93 (dd, J = 11 and 15 Hz, H-19), 6.55 (t, J = 11 Hz, H-20), 5.77 (ddd, J = 4, 11, and 15 Hz, H-21), 3.34 (dd, J = 3 and 112 Hz, H-22a), 2.56 (dd, J = 12 and 12 Hz, H-22b), 2.77 (m, H-24a), 2.04 (m, H-24b), 1.90 (m, H-27), 3.71 (q, J = 4.5 Hz, H-30), 3.08 (m, H-32a), 1.34 (m, H-32b), 1.65 (m, H-36a), 1.61 (m, H-36b). Severe overlapping obscured signals for H-8, H-25, H-26, H-28, H-29, and H-33 to H-35. ^{13}C NMR (125 MHz, $CDCl_3$) δ 72.7 (C-2), 38.4 (C-3), 167.8 (C-4), 40.7 (C-6), 21.1 (C-7), 42.4 (C-9), 70.7 (C-11), 130.7 (C-12), 125.6 (C-13), 30.3 (C-14), 42.6 (C-15), 133.7 (C-16), 127.9 (C-17), 128.5 (C-18), 128.6 (C-19), 128.0 (C-20), 132.2 (C-21), 61.8 (C-22), 56.2 (C-24), 30.1 (C-27), 70.9 (C-30), 58.4 (C-32), 34.8 (C-36).

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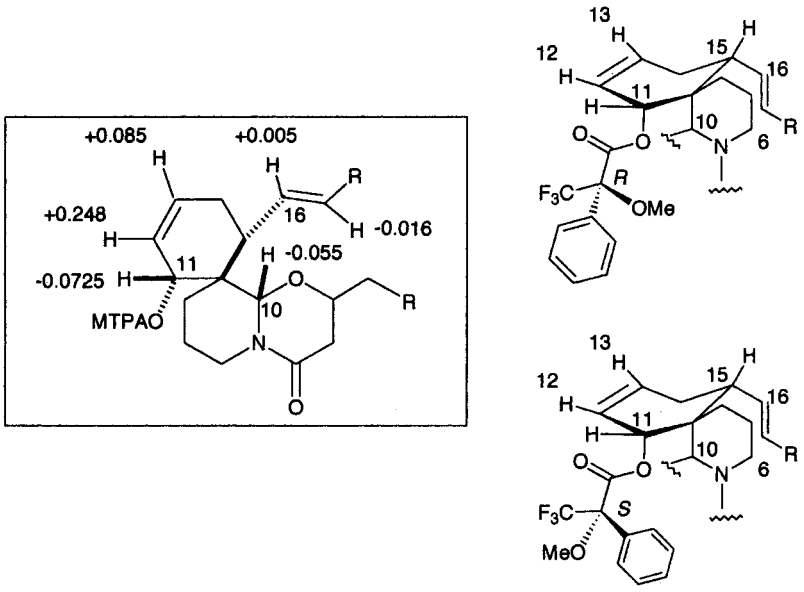

Figure 3. $\Delta\delta$ values obtained for MTPA esters of **1**.

the methylene chloride extraction was loaded onto an ODS flash column eluting with various mixtures of methanol:water to methanol affording seven major fractions (A−G). Fraction E was further separated by Sephadex LH-20 [methanol/chloroform (1:1)], normal phase column chromatography (hexanes/ethyl acetate), and reversed-phase HPLC (Cosmosil 5 C18-AR, 80:20 MeCN/water) to yield 'upenamide (2.2 mg). Using the same isolation protocol, a freeze-dried sponge sample (100.0 g) afforded additional compound **1** (5.0 mg).

'Upenamide (1): colorless solid, 2.2 mg (0.00037% based on wet weight); $[\alpha]_D$ −9.44° (*c* 2.34, MeOH); for ^{1}H and ^{13}C NMR data, see Table 1 (assignments were made by interpretation of COSY, HMQC, and HMBC data); HRFABMS (oxalic acid:thioglycerol:glycerol) *m/z* obsd 523.3538 $[M + H]^+$ ($C_{32}H_{46}N_2O_4$, Δ 0.5 ppm); IR (thin film) υ_{max} 3411, 2928, 1676, 1630, 1453, 1370, 1351, 1201, 1131, 970, 788 cm^{-1}.

Cytotoxicity Testing. Cytotoxicity assays were carried out by Instituto Biomar, S. A., Madrid, Spain.

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Supporting Information Available: ^{1}H, ^{13}C, COSY, NOESY, HMQC, and HMBC NMR spectra for 'upenamide are available.This material is available free of charge via the Internet at http/::pubs.acs.org.

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