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Leucamide A: A New Cytotoxic Heptapeptide from the Australian Sponge *Leucetta microraphis*

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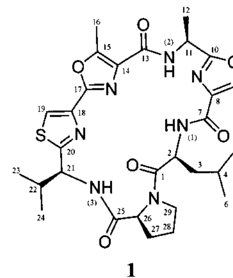
Abstract: Leucamide A (**1**), a bioactive cyclic heptapeptide containing a unique mixed 4,2-bisheterocycle tandem pair consisting of a methyloxazole and thiazole subunit was isolated together with the known compound BRS1 (**2**), from the dichloromethane extract of the Australian marine sponge *Leucetta microraphis*. The planar structure of leucamide A (**1**) was elucidated by employing spectroscopic techniques (NMR, MS, UV, and IR). Its absolute stereochemistry was established by chemical degradation, derivatization, and chiral GC–MS analysis. A conformational analysis of **1** was made using MMFF. Leucamide A (**1**) was found to be moderately cytotoxic toward several tumor cell lines.

Cyclic peptides have been isolated from diverse marine organisms,[1–6] but not from sponges of the genus *Leucetta*. These molecules are characterized by their often remarkably potent biological activity.[7–11] *Leucetta* sp. have previously been shown to contain bioactive imidazole alkaloids.[12–22] As part of an ongoing chemotaxonomic investigation of several sponges belonging to the genera *Leucetta* and *Pericharax*, the CH_2Cl_2 extract of a sample of *Leucetta microraphis* Haeckel, 1872, was examined. In the current report, details of the isolation and structural elucidation of the new cyclic heptapeptide (**1**, leucamide A) are described. Leucamide A is characterized by the presence of seven amino acids and amino acid derived residues: L-leucine, oxazole, L-alanine, methyloxazole, thiazole, L-valine, and L-proline. Mixed 4,2-bisheterocycle tandem pairs in peptides are extremely rare, and the direct connection between the two residues methyloxazole and thiazole is described here for the first time. Similar subunits consisting of oxazoline and thiazole or oxazole and thiazole, respectively, are to be found in the cyclic peptides amythiamicin A and GE2270 A, both isolated from actinomycetes,[23,24] and in the *Escherichia coli* peptide microcin B17.[25] Peptides known to contain 4,2-tandem heterocycle pairs are reported to have potent antibiotic activity,[26,27] and such moieties may be useful pharmacophores in combinatorial libraries since their activity appears to correlate with the location and the identity of the tandem pairs.[25]

The sample of *L. microraphis* investigated in this study was collected at the Pompey Group of reefs, the Great Barrier Reef, Australia. Freeze-dried animal tissues were exhaustively extracted with CH_2Cl_2, and the lipophilic extract was fractionated by repeated normal- and reversed-phase chromatography to yield the new cyclic heptapeptide (**1**, leucamide A) and the known compound BRS1 (**2**).[28]



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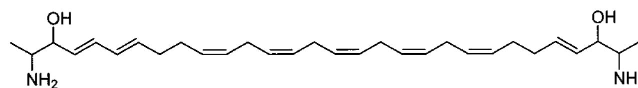

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Leucamide A (**1**) was obtained as a white amorphous solid whose elemental composition was found to be $C_{29}H_{37}N_7O_6S$, by mass spectral analysis. Its ^{13}C NMR

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(1) Admi, V.; Uzi, A.; Carmeli, S. *J. Nat. Prod.* **1996**, *59*, 396–399.
(2) Banker, R.; Carmeli, S. *J. Nat. Prod.* **1998**, *61*, 1248–1251.
(3) Fernández, R.; Omar, S.; Feliz, M.; Quinoá, E.; Riguera, R. *Tetrahedron Lett.* **1992**, *33*, 6017–6020.
(4) Toske, S. G.; Fenical, W. *Tetrahedron Lett.* **1995**, *36*, 8355–8358.
(5) Foster, M. P.; Concepcion, G. P.; Caraan, G. B.; Ireland, C. M. *J. Org. Chem.* **1992**, *57*, 6671–6675.
(6) Kobayashi, J.; Nakamura, T.; Tsuda, M. *Tetrahedron* **1996**, *52*, 6355–6360.
(7) Bonnington, L. S.; Tanaka, J.; Higa, T.; Kimura, J.; Yoshimura, Y.; Nakao, Y.; Yoshida, W. Y.; Scheuer, P. J. *J. Org. Chem.* **1997**, *62*, 7765–7767.
(8) Ojika, M.; Nemoto, T.; Nakamura, M.; Yamada, K. *Tetrahedron Lett.* **1995**, *36*, 5057–5058.
(9) Sone, H.; Kigoshi, H.; Yamada, K. *Tetrahedron* **1997**, *53*, 8149–8154.
(10) Tong Tan, L.; Williamson, R. T.; Gerwick, W. H.; Watts, K. S.; McGough, K.; Jacobs, R. *J. Org. Chem.* **2000**, *65*, 419–425.
(11) Rudi, A.; Aknin, M.; Gaydou, E. M.; Kashman, Y. *Tetrahedron* **1998**, *54*, 13203–13210.
(12) Carmely, S.; Kashman, Y. *Tetrahedron Lett.* **1987**, *28*, 3003–3006.
(13) He, H.; Faulkner, D. J. *J. Org. Chem.* **1992**, *57*, 2176–2178.
(14) Mancini, I.; Guella, G.; Debitus, C.; Pietra, F. *Helv. Chim. Acta* **1995**, *78*, 1178–1184.
(15) Xiong, F.; Barnes, J. R.; Schmitz, F. J. *J. Nat. Prod.* **1997**, *60*, 497–498.
(16) Carmely, S.; Ilan, M.; Kashman, Y. *Tetrahedron* **1989**, *45*, 2193–2200.
(17) Carroll, A. R.; Bowden, B. F.; Coll, J. C. *Aust. J. Chem.* **1993**, *46*, 1229–1234.
(18) Alvi, K. A.; Peters, B. M.; Hunter, L. M.; Crews, P. *Tetrahedron* **1993**, *49*, 329–336.
(19) Akee, R. K.; Carroll, A. R.; Yoshida, W. Y.; Scheuer, P. J. *J. Org. Chem.* **1990**, *55*, 1944–1946.
(20) Chan, G. W.; Mong, S.; Hemling, M. E.; Freyer, A. J.; Offen, P. H.; DeBrosse, C. W.; Sarau, H. M.; Westley, J. W. *J. Nat. Prod.* **1993**, *56*, 116–121.
(21) Plubrukarn, A.; Smith, D. W.; Cramer, R. E.; Davidson, B. S. *J. Nat. Prod.* **1997**, *60*, 712–715.
(22) Xiong, F.; Schmitz, F. J.; Tanner, R. S.; Kelly-Borges, M. *J. Nat. Prod.* **1998**, *61*, 384–386.

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TABLE 1. ^{1}H NMR and ^{13}C NMR Spectral Data for Compound 1[a]

amino acid	position	^{13}C[b] (δ, ppm)	^{1}H[b] (δ (ppm), mult, J (Hz))	HMBC[d]	NOESY/ROESY
L-leucine	1	173.3 (s)[c]		(1), 26	
	2	49.2 (d)	4.91 (1H, ddd, J = 1.8, 8.8, 10.6)		(1), 3, 6, 29
	3	43.2 (t)	1.55 (1H, m); 1.72 (1H, m)	(1), 5, 6	(1), 2, 29
	4	24.9 (d)	1.85 (1H, m)		(1)
	5	23.6 (q)	0.95 (3H, d, J = 6.2)		
	6	21.2 (q)	1.14 (3H, d, J = 6.2)		2, 29
	(1)		8.77 (1H, d, J = 8.8)		2, 3, 4
oxazole	7	159.5 (s)		(1)	
	8	135.7 (s)		9	
	9	140.9 (d)	8.15 (1H, s)		11
	10	164.3 (s)		(2), 9, 11, 12	
L-alanine	11	44.6 (d)	5.19 (1H, dd, J = 5.9, 6.6)	9, 12	(2), 9, 12
	12	20.7 (q)	1.68 (3H, d, J = 6.6)	11	(2), 11
	(2)		8.62 (1H, d, J = 5.9)		11, 12
Me-oxazole	13	161.3 (s)		(2), 16	
	14	130.2 (s)		16	
	15	153.2 (s)		16	
	16	11.8 (q)	2.74 (3H, s)		19
	17	154.4 (s)		16, 19	
thiazole	18	143.2 (s)		19	
	19	119.5 (d)	7.81 (1H, s)		16
	20	169.3 (s)		(3), 19, 21, 22	
L-valine	21	56.9 (d)	5.06 (1H, dd, J = 7.0, 8.4)	(3), 19, 22, 23, 24	(3), 22, 23, 24
	22	34.9 (d)	2.21 (1H, m)	21, 23, 24	(3), 21, 23, 24
	23	19.0 (q)	0.97 (3H, d, J = 6.6)	21	(3), 21, 22
	24	18.7 (q)	0.87 (3H, d, J = 6.6)	21	(3), 21, 22
	(3)		9.48 (1H, d, J = 8.4)		21, 22, 23, 24, 26
L-proline	25	169.5 (s)		(3), 26, 27	
	26	60.2 (d)	4.77 (1H, dd, J = 2.9, 8.1)	28, 29	(3), 27
	27	25.0 (t)	1.80 (1H, m); 2.67 (1H, m)		26, 28
	28	25.4 (t)	2.04 (2H, m)		27, 29
	29	47.3 (t)	3.58 (2H, m)	26	2, 3, 6, 28

[a] $CDCl_3$, 300 MHz. [b] Assignments are based on extensive 1D and 2D NMR measurements (HMBC, HSQC, COSY). [c] Implied multiplicities determined by DEPT (C = s; CH = d; CH_2 = t; CH_3 = q). [d] Numbers refer to proton resonances.

data contained a total of 29 resonances for 6 methyl, 4 methylene, and 8 methine groups and 11 quarternary carbons. These data also revealed the presence of 10 double bonds (3 × CC; 3 × CN; 4 × CO) as the only multiple bonds within the molecule; **1** was thus pentacyclic. The peptidic nature of **1** was evident from its ^{1}H NMR spectrum, in particular, the three exchangeable NH resonances at δ 8.62, 8.77, and 9.48, and the resonances of the α-amino groups seen between δ 4.90 and 5.20. Moreover, characteristic IR bands at 3382, 3261, and 1668 cm^{-1} showed the presence of peptide bonds.[29] The planar structure of **1** was determined by analysis of its 1D and 2D NMR spectral data. Thus, after assignment of all protons to their directly bonded carbon atoms via a one-bond ^{1}H−^{13}C 2D NMR shift correlated measurement (HSQC), it was possible to deduce major fragments of the molecule from the results of a ^{1}H−^{1}H COSY measurement. Analysis of the COSY spectrum of **1** gave evidence for fragments a−d, Figure 1. Fragment a was established from couplings observed between H_3-5 and H_3-6 to H-4, between H-4 and H_2-3, which further coupled to H-2, and between H-2 and NH-(1). Coupling between H_3-12 and H-11 and between H-11 and NH-(2) established fragment b. A third major molecular fragment, c, was established on the basis of couplings observed between both H_3-23 and H_3-24 to H-22, between H-22 and H-21, and between H-21 and NH-(3). Fragment d was deduced from couplings observed between H-26 and H_2-27, between H_2-27 and H_2-28, and between H_2-28 and H_2-29. Characteristic low-field-shifted ^{1}H and ^{13}C resonances for both CH-26 and CH_2-29 (δ 4.77 and 60.2; 3.58 and 47.3) indicated both C-26 and C-29 to be α to nitrogen making fragment d a likely proline residue. Long-range ^{1}H−^{13}C 2D NMR shift correlated measurements (HMBC; see Table 1) using two different values for $J_{CHlong\text{-}range}$ (5 and 8 Hz) permitted the sequence and connection of

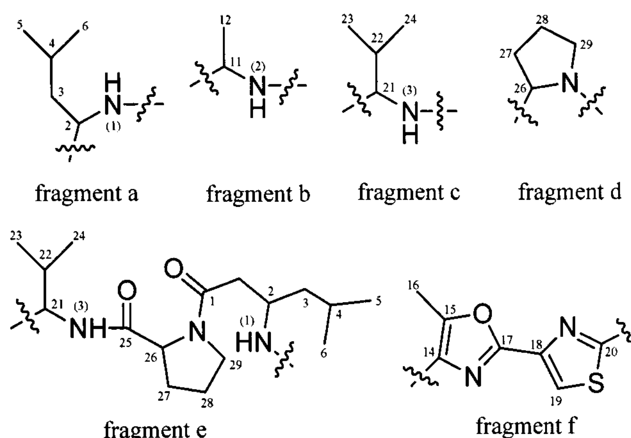

FIGURE 1. Partial structures of compound **1**.

(23) Shimanaka, K.; Takahashi, Y.; Iinuma, H.; Naganawa, H.; Takeuchi, T. *J. Antibiot.* **1994**, *47*, 1153−1159.
(24) Tavecchia, P.; Gentili, P.; Kurz, M.; Sottani, C.; Bonfichi, R.; Lociuro, S.; Selva, E. *J. Antibiot.* **1994**, *47*, 1564−1567.
(25) Roy, R. S.; Kelleher, N. L.; Milne, J. C.; Walsh, C. T. *Chem. Biol.* **1999**, *6*, 305−318.
(26) Shimanaka, K.; Kinoshita, N.; Iinuma, H.; Hamada, M.; Takeuchi, T. *J. Antibiot.* **1994**, *47*, 668−674.
(27) Clough, B.; Rangachari, K.; Strath, M.; Preiser, P. R.; Wilson, R. J. M. *Protist* **1999**, *150*, 189−195.
(28) Willis, R. H.; De Vries, D. J. *Toxicon* **1997**, *35*, 1125−1129.
(29) Silverstein, R. M.; Bassler, G. C.; Morill, T. C. *Spectrometric Identification of Organic Compounds*; John Wiley & Sons: New York, 1981; pp 124−126.

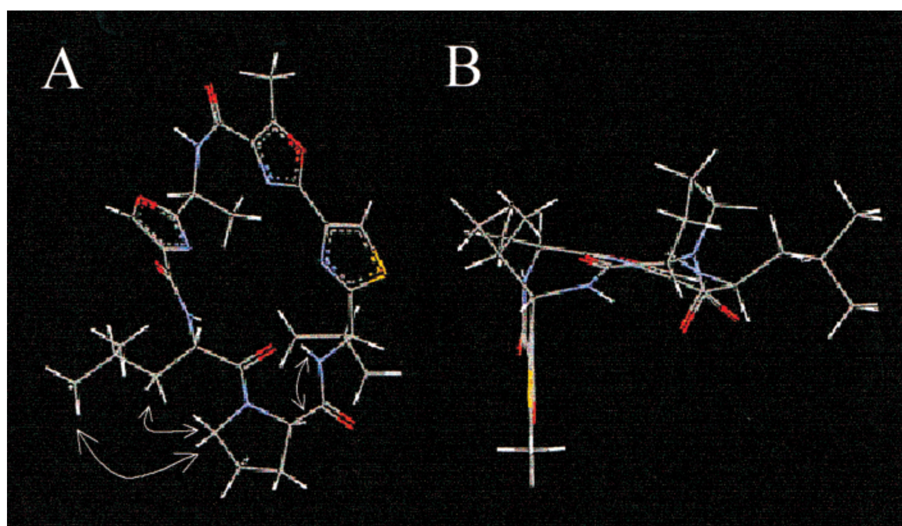

FIGURE 2. Molecular modeling of leucamide A (**1**): (A) view of **1** from above with selected NOE correlations; (B) view showing the two planes within **1**.

amino acid residues, and other units (thiazole, oxazole, methyloxazole) of **1**, to be further elaborated. Thus, long-range CH couplings observed between amide carbonyl C-25 (δ 169.5) and both NH-(3) and H-26, between C-26 and H_2-29, and between amide carbonyl C-1 (δ 173.3), and both H-26 and NH-(1) clearly delineated the sequence of amino acids as valine, proline, and leucine (fragment e, Figure 1). The ^{13}C NMR chemical shifts for C-8, C-9, and C-10, together with the singlet proton resonance at δ 8.15 (H-9), as well as the long-range CH couplings observed between both C-8 and C-10 and H-9 were all indicative of the presence of an oxazole ring in **1** and correlated well with corresponding values reported for other marine derived oxazole containing cyclic peptides.[1] The high field ^{13}C NMR chemical shift of C-16 (δ 11.8) together with chemical shifts for C-14, C-15, and C-17 were in good agreement with values reported for the methyloxazole unit present in the tenuecyclamides.[2] Long-range CH couplings between H_3-16 and C-14, C-15, and C-17 supported this deduction. Based on the ^{13}C NMR chemical shifts of C-18 and C-20, the singlet proton resonance at δ 7.81, assigned to H-19, together with the resonance of its associated carbon atom (δ 119.5), the fourth heterocyclic ring was concluded to be a thiazole moiety.[2] A long-range CH coupling between C-17 and H-19 allowed the connectivity between C-17 to C-18, and thus that between the thiazole and the methyloxazole rings, to be established, fragment f (Figure 1). The bond between C-20 and C-21, and thus between fragments e and f, was also established from CH long-range couplings, this time observed between C-20 and both H-21 and H-22. At this stage of the structural analysis the amide carbonyl groups C-7 and C-13 still required assignment. C-7 had to be connected to NH-(1) because of its CH long-range coupling to NH-(1), and C-13 to both NH-(2) and C-14 based on the CH long-range couplings between C-13 and H_3-16 and NH-(2). C-10 had to be linked to C-11 because of an HMBC coupling observed between C-10 and H_3-12. The final connection between C-7 and C-8, and hence the planar structure of **1**, followed by deduction.

Ozonolysis and acid hydrolysis followed by amino acid analysis using chiral GC−MS enabled the absolute stereochemistry of **1** to be deduced. The L-configuration for the amino acid residues valine, leucine, proline, and alanine was established by comparative GC analysis of their pentafluoropropyl isopropyl ester derivatives with correspondingly derivatized standard D- and L-amino acids (see the Experimental Section). Proline amide bonds are known to have cis/trans geometry,[10] which correlates with the differential value between the ^{13}C NMR resonances of proline β and γ carbons ($\Delta\delta_{\beta\gamma}$).[30] The low value of $\Delta\delta_{\beta\gamma}$ (0.4) seen for **1** is indicative of a trans geometry, a finding that was supported by ROESY interactions observed between H_2-29 and both H-2 and H_2-3.

Molecular modeling studies provided information about the preferred conformation of leucamide A (**1**). The 3D structure obtained for **1** from a conformational analysis (Figure 2) was in good agreement with experimental NOESY and ROESY spectral data. It is evident from this analysis (Figure 2B) that the methyloxazole and thiazole are coplanar and lie approximately orthogonal to the plane formed by the remaining amino acid residues. The valine side chain and the proline take a pseudoaxial orientation above the plane formed by the majority of the amino acid residues, while the leucine and alanine are pseudoequatorial and in plane with this part of the molecule.

Leucamide A (**1**) was found to inhibit the growth of the three tumor cell lines HM02, HepG2, and Huh7 with GI_{50} values of 5.2 μg/mL (HM02), 5.9 μg/mL (HepG2), and 5.1 μg/mL (Huh7). These results are not surprising since a number of other cyclic peptides have been reported to be cytotoxic toward several similar cell lines.[31−35] The antimicrobial, antialgal, and HIV-1 reverse transcriptase inhibitory activities of leucamide A were also investigated and showed the compound to be inactive in all of the applied assays at the 50 μg level.

(30) Siemion, I. Z.; Wieland, T.; Pook, K.-H. *Angew. Chem.* **1975**, *19*, 712−714.
(31) Fusetani, N.; Matsunaga, S. *Chem. Rev.* **1993**, *93*, 1793−1806.

Cyclic peptides similar to leucamide A have been isolated from a variety of marine invertebrates and microorganisms. Scanning electromicrographs of *L. microraphis* show the presence of microbial symbionts, including cyanobacteria, in the sponge tissue. Leucamide A may therefore be produced by these microorganisms and not by the sponge itself. Similar proposals have been made for other marine invertebrate metabolites.[36] The complexity of associations in marine organisms, especially in sponges, however, makes it extremely difficult to definitively state the source of many natural products. Simply comparing the isolated compounds with similar structural features of natural products produced by microorganisms could thus lead to false conclusions concerning a compound's origin. To avoid such situations, it is clearly necessary to determine at least the cellular localization of the sponge metabolites.[37] If indeed metabolites such as leucamide A are of microbial origin, the ecological significance of their generally cytotoxic secondary metabolites is clearly an area that should be attracting much more attention.

Experimental Section

General Experimental Procedures. HPLC was carried out using a Merck-Hitachi system consisting of a L-6200A pump, a L-4500 A photodiode array, and a Knauer K-2300 differential refractometer as detectors together with a D-6000 A interface. ^{1}H and ^{13}C, COSY, NOESY, ROESY, HSQC, and HMBC NMR spectra were recorded in $CDCl_3$. Spectra were referenced to residual solvent signals with resonances at $\delta_{H/C}$ 7.26/77.0 ($CDCl_3$). All other experimental details were as previously reported.[38]

Animal Material. *L. microraphis* was collected in March of 1995 at reef 21-097, Pompey Group, Great Barrier Reef, Australia, and stored at −20 °C until required. A voucher specimen is deposited at the Queensland Museum, voucher no. QMG316040.

Extraction and Isolation. Freeze-dried sponge tissue (67.7 g) was extracted with CH_2Cl_2 (3 × 0.5 L) to yield 0.9 g (1.33%) of green extract. This extract was fractionated by vacuum liquid chromatography (VLC) over Si gel (Merck; 5−40 μm) using gradient elution from petroleum ether containing increasing proportions of CH_2Cl_2, followed by MeOH, to yield 5 fractions. ^{1}H NMR investigations of these fractions indicated fractions 4 and 5 to be of further interest, based on the presence of a large number of resonances attributable to aromatic protons. Fraction 4 was further fractionated by VLC over Polygoprep 60−50 C_{18} material using gradient elution from H_2O (100%) to MeOH (100%), to yield two fractions. Reversed-phase (RP) HPLC separation of the second of these two fractions (column: Knauer C_8 Eurospher-100, 250 × 8 mm, 5 μm; MeOH/H_2O (8:2), 2.0 mL/min) yielded semipure compound **1**. Final purification was achieved by RP HPLC (column: Phenomenex Max C_{12}, 250 × 4.6 mm, 5 μm; MeOH/H_2O (8:2), 0.8 mL/min) to afford 7.2 mg of **1** as a white amorphous solid. Fraction 5 was rechromatographed by solid-phase extraction (Bakerbond SPE C_{18}) using gradient elution from H_2O/MeOH (7:3) to MeOH (100%) to yield 19.7 mg (0.029%) of **2** (elution with MeOH/H_2O (9:1)) as a colorless oil.

Ozonolysis of 1. A stream of O_3 was bubbled through a CH_2Cl_2 solution of **1** (200 μg in 1 mL) for 15 min. Solvent was removed under a stream of N_2, and the resulting residue was subjected to amino acid analysis as outlined below.

Amino Acid Analysis by Chiral GC. A 0.2 mg sample of **1** and the residue of the ozonolysis were each dissolved in 1 mL of 6 N HCl and hydrolyzed in a sealed vial at 104 °C for 20 h. After removal of HCl by repeated evaporation in vacuo, the hydrolysate was heated with acetyl chloride (150 μL) and ^{i}PrOH (500 μL) at 100 °C for 45 min. The mixtures were evaporated to dryness using a stream of N_2 and the residues treated with pentafluoropropionic anhydride (0.3 mL) in CH_2Cl_2 (0.6 mL) at 100 °C for 15 min. Excess reagents were removed by evaporation under a stream of N_2 and the residues resolved in CH_2Cl_2 (1 mL). The derivatized amino acids were then analyzed by GC−MS using a Chirasil Val column (25 m × 0.25 mm; 0.16 μm; program rate: column temperature held at 50 °C for 3 min; 50 °C − 180 °C at 4 °C/min; flow: 0.6 mL/min; Inj.: 250 °C). The retention times of the *N*-pentafluoropropionyl isopropyl ester derivatives of the amino acids established the presence of L-Ala (12.24 min), L-Val (14.22 min), L-Leu (17.29 min), and L-Pro (19.93 min).

Leucamide A (1): white amorphous solid (7.2 mg, 0.011%); $[\alpha]^{23}_D$ −69.3° (*c* 0.60, $CHCl_3$); UV ($CHCl_3$) λ 300−240 nm (br); λ_{max} 246 nm (ϵ 9267); IR (ATR) ν_{max} 3382, 3261, 2959, 1668, 1628, 1518, 1445, 877, 732 cm^{-1}; ^{1}H and ^{13}C NMR data (see Table 1); EIMS *m/z* 611 (44), 568 (40), 555 (36), 499 (56), 70 (100); HREIMS *m/z* 611.2522 (calcd for $C_{29}H_{37}N_7O_6S$, 611.2525).

Biological Assays Performed with Leucamide A (1). Agar diffusion assays were undertaken using the bacteria *Bacillus megaterium*, *E. coli*, the fungi *Microbotryum violaceum*, *Eurotium repens*, *Mycotypha microsporum*, and the green microalga *Chlorella fusca*.[39] An ELISA-based enzyme inhibition assay against HIV-1 reverse transcriptase was performed as previously described.[40] Cytotoxicity tests against the cell lines HM02 (stomach carcinoma), HepG2 (liver carcinoma), and Huh7 (liver carcinoma with mutated p53) followed the standards of the NCI.[41]

Molecular Modeling. Leucamide A (**1**) was mimicked by conformation search (grid scan) using a MMFF force field as implemented in the Cerius^2 4.0 (MSI) molecular modeling software package. The models were further refined with 500 iterations of steepest gradient minimization, followed by conjugate gradient minimization. Calculations were performed using a Silicon Graphics O2 workstation (Irix 6.5.6).

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Supporting Information Available: EIMS, ^{1}H NMR, ^{13}C NMR, and 2D NMR spectra of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(32) Bewley, C. A.; Faulkner, D. J. *Angew. Chem.* **1998**, *110*, 2280−2297.

(33) Ishida, K.; Nakagawa, H.; Murakami, M. *J. Nat. Prod.* **2000**, *63*, 1315−1317.

(34) Chill, L.; Kashman, Y.; Schleyer, M. *Tetrahedron* **1997**, *53*, 16147−16152.

(35) Clarc, W. D.; Corbett, T.; Valeriote, F.; Crews, P. *J. Am. Chem. Soc.* **1997**, *119*, 9285−9286.

(36) Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J.; Corbett, T. H. *J. Am. Chem. Soc.* **2001**, *123*, 5418−5423 and references therein.

(37) Bewley, C. A.; Holland, N. D.; Faulkner, D. J. *Experientia* **1996**, *52*, 716−722.

(38) Wright, A. D.; König, G. M.; Angerhofer, C. K.; Greenidge, P.; Linden, A.; Desqueyroux-Faundez, R. *J. Nat. Prod.* **1996**, *59*, 710−716.

(39) Schulz, B.; Sucker, J.; Aust, H. J.; Krohn, K.; Ludewig, K.; Jones, P. G.; Döring, D. *Mycol. Res.* **1995**, *99*, 1007−1015.

(40) Höller, U.; Wright, A. D.; Matthée, G. F.; König, G. M.; Dräger, S.; Aust, H. J.; Schulz, B. *Mycol. Res.* **2000**, *11*, 1354−1365.

(41) Grever, M. R.; Schepartz, S. A.; Chabner, B. A. *Semin. Oncol.* **1992**, *19*, 622−638.