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First Total Synthesis of Leucamide A

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Abstract: The first total synthesis of marine bioactive cyclic heptapeptide Leucamide A has been accomplished, including a simple method for construction of the 4,2-bisheterocycle tandem pair substructure that employs a DAST-mediated cyclization of β-hydroxy amide and final HBTU-promoted ring closing.

Leucamide A (**1**) was first isolated from the dichloromethane extract of the Australian marine sponge *Leucetta microraphis* recently.[1] This bioactive cyclic heptapeptide contains a unique mixed 4,2-bisheterocycle tandem pair consisting of a methyloxazole and thiazole subunit and showed moderate cytotoxicity toward several tumor cell lines. The 4,2-bisheterocycle-containing peptide has been reported to have potent antibiotic activity, correlating with the location and the identity of the tandem pairs.[2] Therefore, such moieties may be useful pharmacophores in combinatorial libraries for new lead discovery.

In this paper, we report the first total synthesis of Leucamide A, including a simple method for the construction of the 4,2-bisheterocycle-tandem pair system employing a diethylaminosulfur trifluoride (DAST)-mediated cyclization of β-hydroxy amide.

Retrosynthetic analysis to Leucamide A is outlined in Figure 1. Disconnection at the amide linkage NH-(3)/C-25 and NH-(1)/C-7 resulted in a tricyclic fragment **2** and dipeptide **3**. Fragment **2** was rediscounected to give the 4,2-bisheterocycle-tandem pair system **4** and oxazole **5**. The former can easily be prepared from thiazole **6** and threonine employing a DAST-mediated cyclization of β-hydroxy amide. Proline amide bonds are known to have cis/trans geometry;[3] therefore, amidation at NH-(3)/C-25 was chosen as the final cyclization step to form the preferred trans geometry required by the natural Leucamide A. The *tert*-butyl group was selected to protect the C-25 carboxylic acid, which can be removed simultaneously with the NH-(3) *tert*-butoxycarbonyl (Boc) group under mild conditions before cyclization.

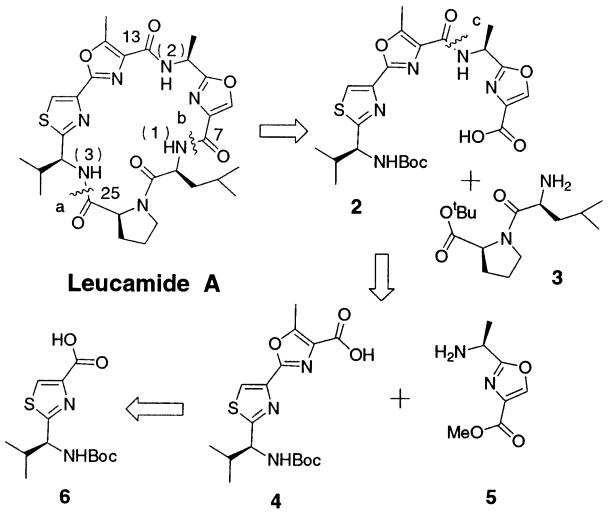

FIGURE 1. Retrosynthetic analysis of Leucamide A.

The oxazole **11** could be prepared from alanine-serine-derived oxazoline by a procedure recently reported by Williams.[4] Amine deprotection was achieved with acetyl chloride in absolute methanol, as described by North,[5] to provide primary amine **5** in 96% yield, as outlined in Scheme 1. The dipeptide fragment **3** was obtained from a coupling of Bu^{t}O-proline and Fmoc-leucine by using *i*-BuOCOCl and NMM and followed by deprotection with piperidine in DMF.

For the synthesis of the 4,2-bisheterocycle tandem moiety **4**, Holzapfel's modified Hantzsch procedure was employed to produce the thiazole fragment,[6,7] followed by saponification with lithium hydroxide in aqueous ethanol to generate the requisite thiazole carboxylic acid **6**, as reported previously by Meyers.[8] The acid **6** was transformed into β-hydroxyamide **7** by coupling with L-threonine methyl ester. The resulting **7** was converted to the desired oxazole **8** by using DAST-mediated cyclization and following the oxidation in the presence of bromotrichloromethane and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU).[9] The expected 4,2-bisheterocycle tandem moiety **8** was obtained in 40% overall yield from acid **6**. Saponification with lithium hydroxide in aqueous methanol gave the carboxylic acid **4** in 98% yield.

To couple the three fragments, it was decided to first join 4,2-bisheterocycle tandem pair moiety **4** with oxazole amide **5**. This coupling reaction was accomplished by the use of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide

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(1) Kehraus, S.; Konig, G. M.; Wright, A. D. *J. Org. Chem.* **2002**, *67*, 4989.

(2) Roy, R. S.; Kelleher, N. L.; Milne, J. C.; Walsh, C. T. *Chem. Biol.* **1999**, *6*, 305.

(3) Tan, T. L.; Williamson, R. T.; Gerwick, W. H.; Watts, K. S.; McGough, K.; Jacobs, R. *J. Org. Chem.* **2000**, *65*, 419. Yokokawa, F.; Sameshima, H.; In, Y.; Minoura, K.; Ishida, T.; Shioiri, T. *Tetrahedron* **2002**, *58*, 8127.

(4) (a) Williams, D. R.; Lowder, P. D.; Gu, Y.; Brooks, D. A. *Tetrahedron Lett.* **1997**, *38*, 331. (b) Downing, S. V.; Aguilar, E.; Meyers, A. I. *J. Org. Chem.* **1999**, *64*, 826.

(5) North, M.; Pattenden, G. *Tetrahedron* **1990**, *46*, 8267.

(6) Bredenkamp, M. W.; Holzapfel, C. W. *Synth. Commun.* **1990**, *20*, 2235.

(7) Wipf, P.; Miller, C. P. *J. Am. Chem. Soc.* **1992**, *114*, 10975.

(8) Ailar, I.; Meyers, A. I. *Tetrahedron Lett.* **1994**, *35*, 2477.

(9) (a) Phillips, A. J.; Uto, Y.; Wipf, P.; Reno, M. J.; Williams, D. R. *Org. Lett.* **2000**, *2*, 1165. (b) Burrell, G.; Evans, J. M.; Jones, G. E.; Stemp, G. *Tetrahedron Lett.* **1990**, *31*, 3649. (c) Lafargue, P.; Lellouche, J. P. *Heterocycles* **1995**, *41*, 947.

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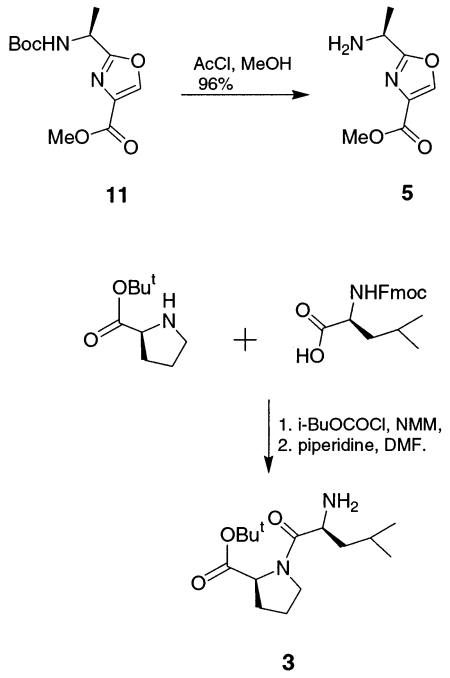
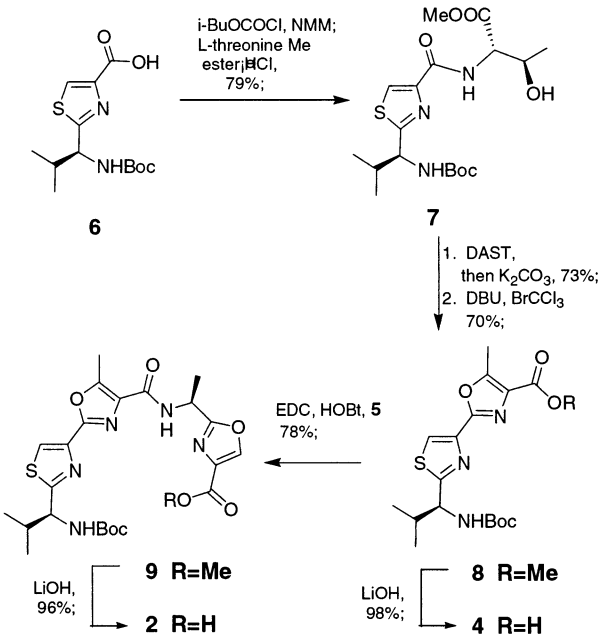
SCHEME 1. Synthesis of Compound 5 and Dipeptide 3



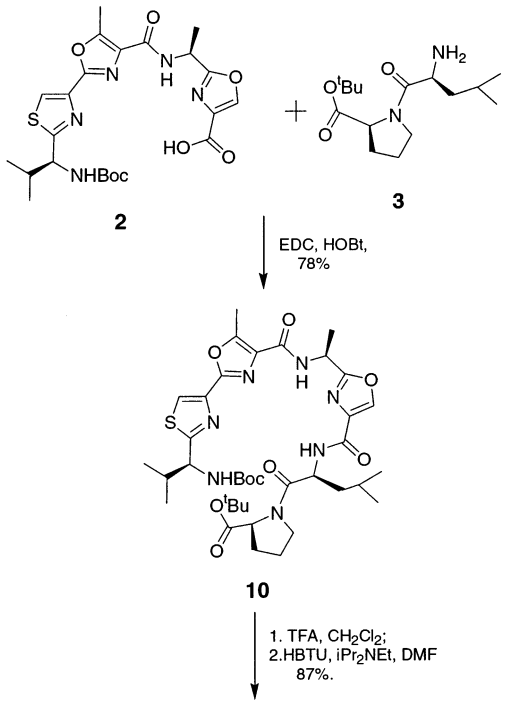
SCHEME 2. Synthesis of Compounds 4 and 2



hydrochloride (EDC) in the presence of 1-hydroxy-benzotriazole (HOBt). This step finally offered the adduct **9** in 78% yield. The corresponding carboxylic acid **2** could be obtained from **9** in almost quantitative yield by employment of well-known deprotection protocols as shown in Scheme 2.

The ring-closure precursor **10** was obtained in 78% yield from a coupling of the pentapeptidic acid **2** with dipeptide **3** by using EDC in the presence of HOBt in DMF, as outlined in Scheme 3. The final ring-closure step was successfully implemented after deprotecting the *tert*-butyl group and Boc group simultaneously in the presence of TFA in CH_2Cl_2 by treatment of the crude amino

SCHEME 3. Synthesis of Leucamide A



acid with HBTU and diisopropylethylamine in dry DMF. Isolation of the product afforded Leucamide A in 87% yield.

The ^1H and ^{13}C NMR data and HREIMS of the synthetic material were found to be identical to those reported for the isolated natural product by comparison of the spectral data. The optical rotation of the pure synthetic sample **1** $[\alpha]_D$ −98 (*c* 0.78 $CHCl_3$), on the other hand, was higher than that for the reported rotation for the isolated natural product $[\alpha]_D$ −69 (*c* 0.6, $CHCl_3$).[1] Even with this deviation, we think that our data strongly support the reported structure.

In summary, the first total synthesis of Leucamide A was reported, and the method described here for the construction of the 4,2-bisheterocycle tandem pair substructure is readily adaptable to the synthesis of related analogues and derivatives. Further efforts aimed at preparation of new 4,2-bisheterocycle-containing compounds and studies of their biological property are in progress.

Experimental Section

The reaction mixture was generally poured into water, and the separated aqueous phase was then thoroughly extracted with the specified solvent. After being washed with 10% aqueous HCl and/or $NaHCO_3$ (if required), water, and saturated aqueous NaCl, the combined organic phases were dried over anhydrous Na_2SO_4 or $MgSO_4$ and then filtered and concentrated under reduced pressure to yield the crude reaction product. DME and THF was distilled from sodium, and DMF was distilled in a vacuum. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$, and spectra were referenced to the residual solvent signals with resonances at δ H/C 7.26/77.0 ppm.

Compound 5. Acetyl chloride (321 μL, 4.5 mmol) was added dropwise to absolute methanol (3 mL) at 0 °C. The oxazole **11**[4] (120 mg, 0.45 mmol) was added as a solid in one portion and

the reaction allowed to stir overnight at room temperature. Concentration of the reaction mixture gave **5** in hydrochloride salt, which was partitioned between CH_2Cl_2 (10 mL) and saturated aqueous $NaHCO_3$ (10 mL). The phases were separated, and the aqueous phase was extracted with CH_2Cl_2. The combined organic phases were then processed in the usual way to provide the free amine **5** (72 mg, 96%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.50 (d, J = 6.9 Hz, 3H), 3.89 (s, 3H), 4.19 (m, 1H), 8.16 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 21.19, 45.24, 51.70, 132.55, 143.58, 161.27, 168.76.

Compound 3. To a stirred solution of Fmoc-leucine (1.062 g, 3 mmol) in dry THF (20 mL) under N_2 at −30 °C were added *N*-methylmorpholine (NMM) (366 μL, 3.3 mmol) and isobutyl chloroformate (414 μL, 3.15 mmol). After 10 min, Bu^{t}O-proline (539 mg, 3.15 mmol) was added in one portion, and then the solution was stirred for 1 h with gradual warming to ambient temperature. The mixture was partitioned between H_2O (50 mL) and EtOAc (50 mL). The phases were separated, and the aqueous phase was extracted with EtOAc. The combined organic phases were then processed in the usual way to yield the dipeptide (1.2 g).

Piperidine (344 μL, 3.48 mmol) was added dropwise to a cold (0 °C) solution of the dipeptide (440 mg, 0.88 mmol) in DMF (3 mL) under N_2 and stirred for 20 min with gradual warming to room temperature. TLC monitoring shows complete consumption of the starting material. The mixture was diluted with 150 mL of EtOAc and washed with water. The organic phases were then processed in the usual way and chromatographed (10:1 $CHCl_3$/EtOAc) to afford **3** (152 mg, 61%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.90 (d, J = 1.2 Hz, 3H), 0.92 (d, J = 1.5 Hz, 3H), 1.40 (s, 9H), 1.80−2.04 (m, 8H), 3.47−3.58 (m, 4H), 4.35 (dd, J = 4.2 Hz, 4.5 Hz, 1H).

Compound 4. To a stirred solution of **6**[6−8] (134 mg, 0.446 mmol) in dry THF (2 mL) under N_2 at −30 °C were added *N*-methylmorpholine (NMM) (109 μL, 0.98 mmol) and isobutyl chloroformate (62 μL, 0.47 mmol). After 10 min, L-threonine methyl ester (80 mg, 0.47 mmol) was added in one portion, and then the solution was stirred for 1 h with gradual warming to ambient temperature. The mixture was partitioned between H_2O (20 mL) and EtOAc (20 mL). The phases were separated, and the aqueous phase was extracted with EtOAc. The combined organic phases were then processed in the usual way to yield crude β-hydroxyamide 146 mg.

DAST (47 μL, 0.36 mmol) was added dropwise to a cold (−78 °C) solution of the crude β-hydroxyamide (125 mg, 0.30 mmol) in CH_2Cl_2 under N_2. After the mixture was stirred for 1 h at −78 °C, anhydrous K_2CO_3 (62.0 mg, 0.45 mmol) was added in one portion and the mixture was allowed to warm to room temperature. The reaction mixture was poured into saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic phases were then processed in the usual way to give the oxazoline intermediate (86 mg, 73%).

Oxazoline (79 mg, 0.20 mmol) dissolved in CH_2Cl_2 (4 mL) was cooled to −10 °C. DBU (34 μL, 0.23 mmol) was added followed by $BrCCl_3$ (27 μL, 0.27 mmol) dropwise. The mixture was stirred overnight while warming to room temperature. The mixture was washed with saturated aqueous NH_4Cl (5 mL × 2), and the aqueous phase was extracted with EtOAc. The combined organic phases were then processed in the usual way to yield the oxazole **8** (58 mg, 70%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.87 (d, J = 6.9 Hz, 3H), 0.94 (d, J = 6.6 Hz, 3H), 1.40 (s, 9H), 2.41 (m, 1H), 2.67 (s, 3H), 3.88 (s, 3H), 4.89 (m, 1H), 5.30 (br, 1H), 7.95 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.07, 17.11, 19.30, 28.17, 33.26, 51.94, 57.89, 79.99, 120.00, 128.24, 142.81, 155.12, 155.31, 156.34, 162.53, 173.97; $[\alpha]_D$ −36.0 (c 0.60, $CHCl_3$).

Lithium hydroxide monohydrate (14 mg, 0.32 mmol) was added to a stirred solution of **8** (46 mg, 0.12 mmol) in 1 mL of 3:1 MeOH/H_2O at 0 °C and stirred for 1 h with gradual warming to room temperature. TLC monitoring shows complete consumption of starting material. The solvents were removed, and the residue was partitioned between EtOAc (10 mL) and H_2O (5 mL). The organic phase was separated, and the aqueous phase was acidified to pH = 2 with 1 M aqueous HCl and then extracted with EtOAc. The combined organic phases were then processed in the usual way to give **4** (44 mg, 96%) as a white solid, which was used in the next step without further purification: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.93 (d, J = 6.3 Hz, 3H), 0.99 (d, J = 6.3 Hz, 3H), 1.44 (s, 9H), 2.43 (m, 1H), 2.71 (s, 3H), 4.90 (m, 1H), 5.37 (m, 1H), 6.10 (br, 1H), 8.02 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.22, 17.18, 19.28, 28.17, 33.24, 57.89, 80.07, 120.35, 128.15, 142.61, 155.18, 155.40, 157.10, 165.60, 174.04.

Compound 2. To oxazole amine **5** (10.8 mg, 0.063 mmol) and 4 Å molecular sieves in DMF (1 mL) at −10 °C were added HOBT (27 mg, 0.2 mmol) and oxazole acid **4** (26 mg, 0.069 mmol), and the resulting mixture was stirred at −10 °C for 20 min. Then EDCI (14 mg, 0.076 mmol) was added, and the mixture was stirred for 2 h with gradual warming to room temperature. The reaction mixture was diluted with 50 mL of EtOAc and 20 mL of H_2O. The aqueous phase was extracted with EtOAc. The combined organic phases were then processed in the usual way and chromatographed (1:1 petroleum ether/EtOAc) to yield **9** (28 mg, 78%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.90 (d, J = 6.6 Hz, 3H), 0.99 (d, J = 6.9 Hz, 3H), 1.43 (s, 9H), 1.68 (d, J = 7.2 Hz, 3H), 2.50 (m, 1H), 2.70 (s, 3H), 3.88 (s, 3H), 4.94 (m, 1H), 5.25 (br, 1H), 5.48 (m, 1H), 7.52 (d, J = 8.7 Hz, 1H), 7.85 (s, 1H), 8.16 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 11.81, 17.03, 19.40, 28.23, 33.14, 42.51, 52.13, 58.05, 80.16, 119.74, 129.54, 133.24, 143.10, 144.05, 153.79, 154.25, 155.35, 161.08, 161.45, 165.17, 174.94; $[\alpha]_D$ −7.3 (c 0.60, $CHCl_3$).

Lithium hydroxide monohydrate (10 mg, 0.24 mmol) was added to a stirred solution of **9** (28 mg, 0.056 mmol) in 1 mL of 4:1 MeOH/H_2O at 0 °C and stirred for 1 h with gradual warming to room temperature. TLC monitoring shows complete consumption of starting material. The solvents were removed, and the residue was partitioned between EtOAc (20 mL) and H_2O (10 mL). The organic phase was separated, and the aqueous phase was acidified to pH = 2 with 1 M aqueous HCl and then extracted with EtOAc. The combined organic phases were then processed in the usual way to give **2** (27 mg, 100%) as a white solid, which was used in the next step without further purification: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.93 (d, J = 6.6 Hz, 3H), 1.00 (d, J = 6.9 Hz, 3H), 1.45 (s, 9H), 1.72 (d, J = 6.9 Hz, 3H), 2.48 (m, 1H), 2.73 (s, 3H), 4.95 (m, 1H), 5.32 (br s, 1H), 5.54(m, 1H), 7.65 (d, J = 7.8 Hz, 1H), 7.87 (s, 1H), 8.24 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 11.79, 17.20, 19.18, 19.34, 28.19, 33.17, 42.58, 57.94, 80.17, 119.84, 129.45, 133.10, 142.73, 144.66, 153.96, 154.25, 155.42, 161.16, 163.51, 165.12, 174.70.

Compound 10. To amine **3** (53 mg, 0.19 mg) and 4 Å molecular sieves in DMF (1 mL) at −10 °C were added HOBT (23 mg, 0.17 mmol) and **2** (27 mg, 0.052 mmol), and the resulting mixture was stirred at −10 °C for 20 min. Then, EDCI (12 mg, 0.063 mmol) was added, and the mixture was stirred for 2 h with gradual warming to room temperature. The reaction mixture was diluted with 50 mL of EtOAc and 20 mL of H_2O. The aqueous phase was extracted with EtOAc. The combined organic phases were then processed in the usual way and chromatographed (1:1.5 petroleum ether/EtOAc) to yield **10** (32 mg, 78%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.90 (d, J = 6.9 Hz, 3H), 0.95 (d, J = 6.3 Hz, 3H), 0.99 (d, J = 6.6 Hz, 3H), 1.01 (d, J = 6.0 Hz, 3H), 1.43 (s, 18H), 1.64 (d, J = 6.9 Hz, 3H), 1.70−1.98 (m, 6H), 2.17 (m, 1H), 2.50 (m, 1H), 2.72 (s, 3H), 3.63 (m, 1H), 3.81 (m, 1H), 4.38 (m, 1H), 4.96 (m, 2H), 5.29 (m, 1H), 5.43 (m, 1H), 7.49 (d, J = 8.4 Hz, 1H), 7.54 (d, J = 9.3 Hz, 1H), 7.89 (s, 1H), 8.08 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 11.79, 17.04, 19.14, 19.37, 21.66, 23.26, 24.53, 24.68, 27.84, 28.18, 28.95, 33.06, 41.66, 42.40, 46.77, 48.31, 58.02, 59.50, 80.04, 81.15, 119.77, 129.55, 135.82, 141.24, 143.09, 153.73, 154.24, 154.31, 160.19, 161.03, 163.91, 170.55, 170.92, 174.82; $[\alpha]_D$ −16.4 (c 0.38, $CHCl_3$).

Leucaminde A (1). To a stirred solution of **10** (22 mg, 0.028 mmol) in CH_2Cl_2 (0.5 mL) at −10 °C was added TFA (0.5 mL) dropwise over a period of 20 min. The reaction mixture was stirred for 4 h with gradual warming to room temperature. TLC monitoring shows complete consumption of starting material. The solvents were removed and then evaporated to dryness by a zero tropic distillation with toluene.

A suspension of the crude solid residue and 4 Å molecular sieves in 2.5 mL of dry DMF was cooled to −10 °C, and diisopropylethylamine (40 μL, 0.2 mmol) and HBTU (52 mg, 0.14

mmol) were added. The resulting mixture was stirred at −10 °C for 2 h and then at room temperature for 12 h, and the mixture was diluted with 100 mL of EtOAc and 20 mL of H_2O. The organic phases were then processed in the usual way and chromatographed (1:2 petroleum ether/EtOAc) to afford 15 mg (87%) of Leucaminde A (**1**) as a white solid: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.87 (d, J = 6.6 Hz, 3H), 0.95 (d, J = 6.6 Hz, 3H), 0.97 (d, J = 6.6 Hz, 3H), 1.15 (d, J = 6.6 Hz, 3H), 1.53 (m, 1H), 1.69 (d, J = 6.6 Hz, 3H), 1.72−1.87 (m, 3H), 2.04 (m, 2H), 2.21 (m, 1H), 2.67 (m, 1H), 2.74 (s, 3H), 3.58 (m, 2H), 4.77 (dd, J = 3.0, 8.1 Hz, 1H), 4.91 (m, 1H), 5.07 (dd, J = 8.4, 7.2 Hz, 1H), 5.21 (m, 1H), 7.81 (s, 1H), 8.14 (s, 1H), 8.61 (d, J = 5.1 Hz), 8.62 (d, J = 8.7 Hz, 1H), 9.48 (d, J = 7.8 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 11.8, 18.7, 19.0, 20.7, 21.2, 23.6, 24.9, 25.0, 25.4, 34.9, 43.2, 44.6, 47.3, 49.2, 56.9, 60.2, 119.5, 130.2, 135.7, 140.9, 143.2, 153.2, 154.4, 159.5, 161.4, 164.3, 169.3, 169.5, 173.3; $[\alpha]_D$ − 98 (*c* 0.78, $CHCl_3$) HREIMS *m/z* 611.2525 (calcd for $C_{29}H_{37}SO_6N_7$ 611.2525).

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Supporting Information Available: Copies of ^{1}H and ^{13}C NMR spectra of compounds **8**−**10** and Leucamide A. This material is available free of charge via the Internet at http://pubs.acs.org.

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