Supplementary Information

Chemoselective Cyclization of Unprotected Linear Peptides by α-Ketoacid–Hydroxylamine Amide Ligation

Takeo Fukuzumi[a], Lei Ju[a] and Jeffrey W. Bode[b*]

[a]Roy and Diana Vagelos Laboratories, Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania, USA 19104.

[b]Laboratorium für Organische Chemie, ETH-Zürich, HCl F-315 Wolfgang Pauli Strasse 10, 8093, Zürich, Switzerland.

bode@org.chem.ethz.ch

1. General Methods

Reagents: All reactions utilizing air- or moisture-sensitive reagents were performed in dried glassware under an atmosphere of dry Nitrogen. CH_2Cl_2 was distilled over CaH_2. THF was distilled from Na/benzophenone. CH_3OH and DMF were dried by passage over molecular sieves under Ar atmosphere. *N*,*N*-Diisopropylethylamine (DIPEA) was distilled from CaH_2. Other reagents were used without further purification. Oxone was purchased from Alfa Aesar. Thin layer chromatography (TLC) was performed on Merck precoated plates (silica gel 60 F254, Art 5715, 0.25 mm) and was visualized by fluorescence quenching under UV light or by staining with phosphomolybdic acid. Silica-gel preparative thin-layer chromatography (PTLC) was performed using plates prepared from Merck Kieselgel 60 PF254 (Art 7747). Column chromatography was performed on E. Merck Silica Gel 60 (230–400 Mesh) using a forced flow of 0.5–1.0 bar.

Instruments: ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) were measured on a Bruker Avance II 500 spectrometer. Chemical shifts are expressed in parts per million (PPM) downfield from residual solvent peaks and coupling constants are reported as Hertz (Hz). Splitting patterns are indicated as follows: br, broad; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Infrared (IR) spectra were recorded on a JASCO FT/IR-430 spectrophotometer and are reported as wavenumber (cm^{-1}). Optical rotations were measured on a Jasco P–2000 polarimeter operating at the sodium D line with a 100 mm path length cell, and are reported as follows: $[\alpha]_D^T$ (concentration (g/100 mL), solvent). UV-vis spectra were recorded with a JASCO V-570 spectrometer. Analytical HPLC was performed using a C18 column (Shiseido CAPCELL PAK C18 UG120, S-5 μm, 4.6 mm I.D. × 250 mm) or (YMC R-ODS-10A, S-10 μm, 4.6 mm I.D. × 250 mm). All separations involved a gradient of CH_3CN and Millipure water containing 0.1 % trifluoroacetic acid. Standard separation condition: gradient 10–90% CH_3CN/H_2O over 30 min at a flow rate of 1.0 mL/min. Preparative HPLC was performed using a C18 column (YMC-Pack R&D ODS/D, S-10 μm, 20 mm I.D. × 250 mm). Standard separation condition: gradient 10–90% CH_3CN/H_2O over 30 min at a flow rate of 20.0 mL/min.

2. Solid-Phase Peptide Synthesis

2.1 General Procedures

The preparation of sulfur ylide linker[1] and nitrones[2] has been previously described. Peptides were prepared according to the standard Fmoc manual solid-phase synthesis protocols using sulfur ylide linker on Rink amide MBHA resin (substitution: 0.56 mmol/g). The following Fmoc amino acids from Novabiochem were employed: Fmoc-Ala-OH, Fmoc-Asp(O^{t}Bu)-OH, Fmoc-Glu(O^{t}Bu)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Phe-OH, Fmoc-Ser(^{t}Bu)-OH, Fmoc-Tyr(^{t}Bu)-OH, Fmoc-Asn(OTrt)-OH, Fmoc-Gln(OTrt)-OH, Fmoc-Orn(Boc)-OH, Fmoc-Val-OH, Fmoc-^{D}Phe-OH. Coupling on the resin was carried out with Fmoc amino acids (5 equiv), HBTU (5 equiv), HOBt (5 equiv) and DIPEA (10 equiv) in DMF for 2 h. Fmoc deprotections were conducted by treatment with 20% piperidine in DMF for 30 minutes.

2.2 Estimation of Level of Residue Attachment

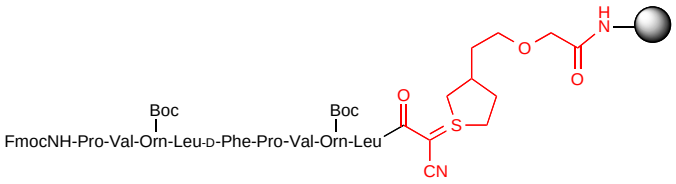
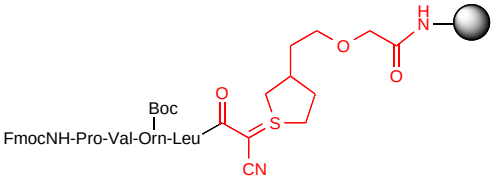
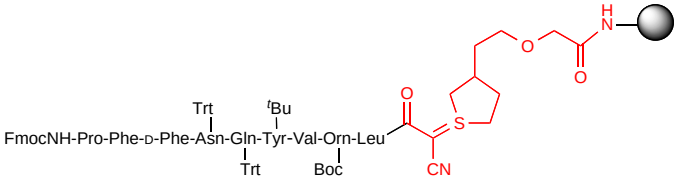
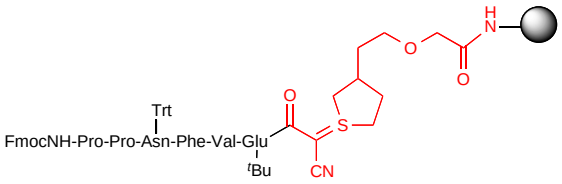
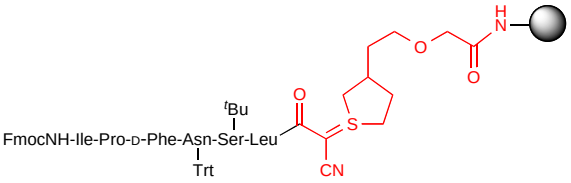
The Fmoc loading of the resin was determined with the following method (**Supplementary Table 1**).[3]

1. Two quartz UV cells were used.
2. A dry Fmoc amino acid resin (5 μmol) was weight carefully into a vial. To the vial was added 2% DBU in DMF (2 mL) and stirred gently for 30 min (solution A). The solution A was filtered into a 10 mL volumetric flask and diluted to the mark with CH_3CN (solution B). The solution B (2 mL) was taken into 25 mL volumetric flask and diluted to the mark with CH_3CN (solution C).
3. A reference solution was prepared as in step 2, without the addition of the resin (solution D).
4. The sample solution (solution C) and the reference solution (solution D) were added to cells.
5. Two cells were placed in a spectrophotometer and recorded optical densities at 304 nm.
6. An estimate of the residue attachment was obtained from equation below.

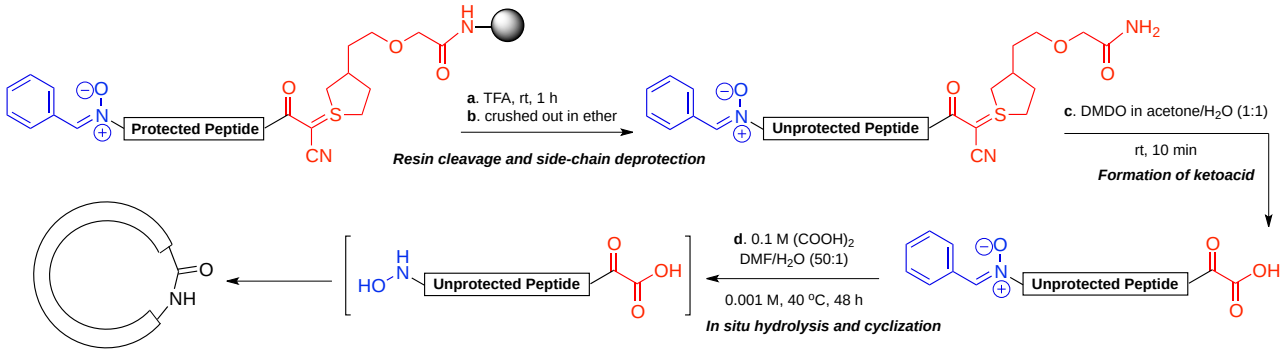
   Fmoc loading: mmol/g = $(Abs_{sample} - Abs_{reference}) \times 16.4$ / (mg of the resin)

(1) Ju, L., Bode, J.W. Amide Formation by Decarboxylative Condensation of Hydroxyamines and α-Ketoacids: *N*-[(1S)-1-Phenylethyl]-benzeneacetamide. *Org. Synth*. **87**, 218–225 (2010).
(2) Medina, S.I., Wu, J. & Bode, J.W. Nitrone protecting groups for enantiopure N-hydroxyamino acids: synthesis of N-terminal peptide hydroxylamines for chemoselective ligations. *Org. Biomol. Chem.* **8**, 3405–3417 (2010).
(3) Novabiochem catalog.

Table 1. Fmoc loading of Resins

Peptide on Rink Amide MBHA Resin	Fmoc Loading
	0.23 mmol/g
	0.32 mmol/g
	0.20 mmol/g
	0.20 mmol/g
	0.23 mmol/g

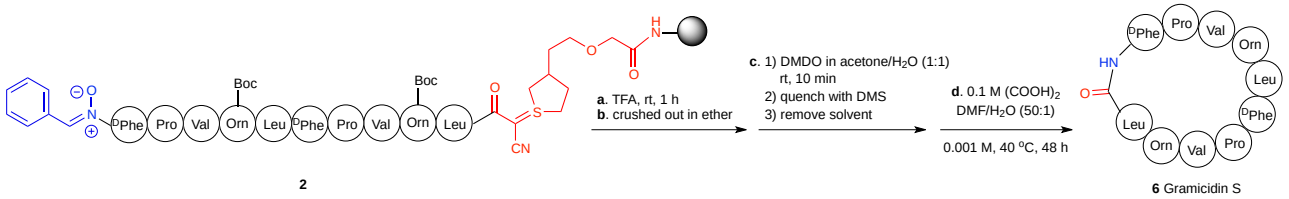
3. General Procedure for peptide cyclization



The resin bound peptide (approximately 0.025 mmol) was cleaved from the resin with TFA (3 mL) at room temperature for 1 h. The mixture was filtered through a small plug of cotton wool into a centrifuge tube and rinsed with a small volume of TFA. The filtrate was reduced to minimum volume by a nitrogen stream. Diethyl ether was added and the white precipitate was collected by centrifugation and dried under reduced pressure to give the crude nitrone-sulfur ylide peptide. To a solution of this crude peptide in acetone/H_2O (1/1 v/v, 0.01 M) was added DMDO (0.067 M in acetone, 4 equiv) at rt, and the mixture was stirred for 10 min. The reaction was quenched with dimethylsulfide (DMS). The solvent was removed under reduced pressure to give the unpurified nitrone-ketoacid peptide.

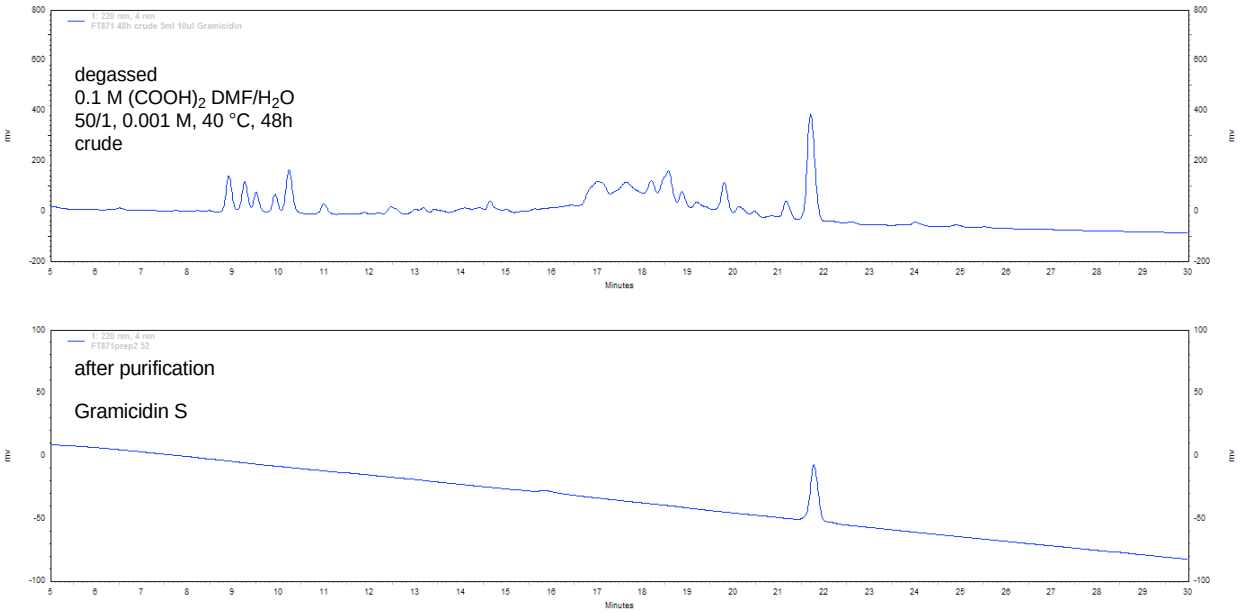
The nitrone-ketoacid peptide was diluted by degassed 0.1 M $(COOH)_2$ in DMF/H_2O (50/1 v/v, 0.001 M) and the temperature was raised to 40 °C for 48 h. The solvent was removed under reduced pressure. The crude was diluted by methanol 5 mL, and 10 μL of this solution was analyzed by HPLC. The HPLC yield was determined by the UV absorption using the standard curves generate from pure material (see Section 4, page 2). The crude cyclic peptide was purified by preparative HPLC using a C18 column (YMC-Pack R&D ODS/D, S-10 μm, 20 mm I.D. × 250 mm), separation condition: gradient 10–60% CH_3CN/H_2O over 30 min at a flow rate of 10.0 mL/min. The solvent was removed by lyophilization to give the cyclized product.

Synthesis of Gramicidin S



2 **6** Gramicidin S

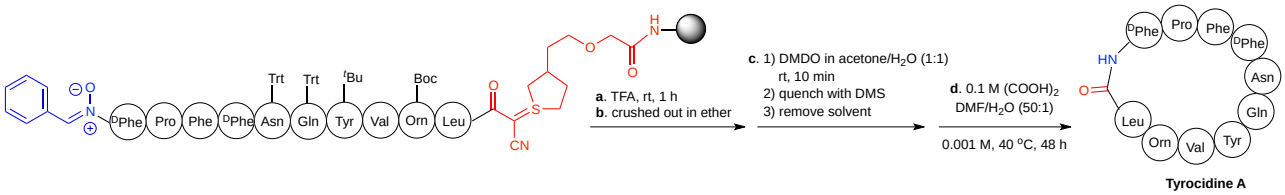
The resin **2** (0.24 mmol/g, 100.5 mg, 0.02412 mmol) was used according to the general procedure. The crude yield was confirmed based on standard curve (13%). The crude peptide was purified by preparative HPLC. The solvent was removed by lyophilization to give Gramicidin S (3.6 mg, 13%).



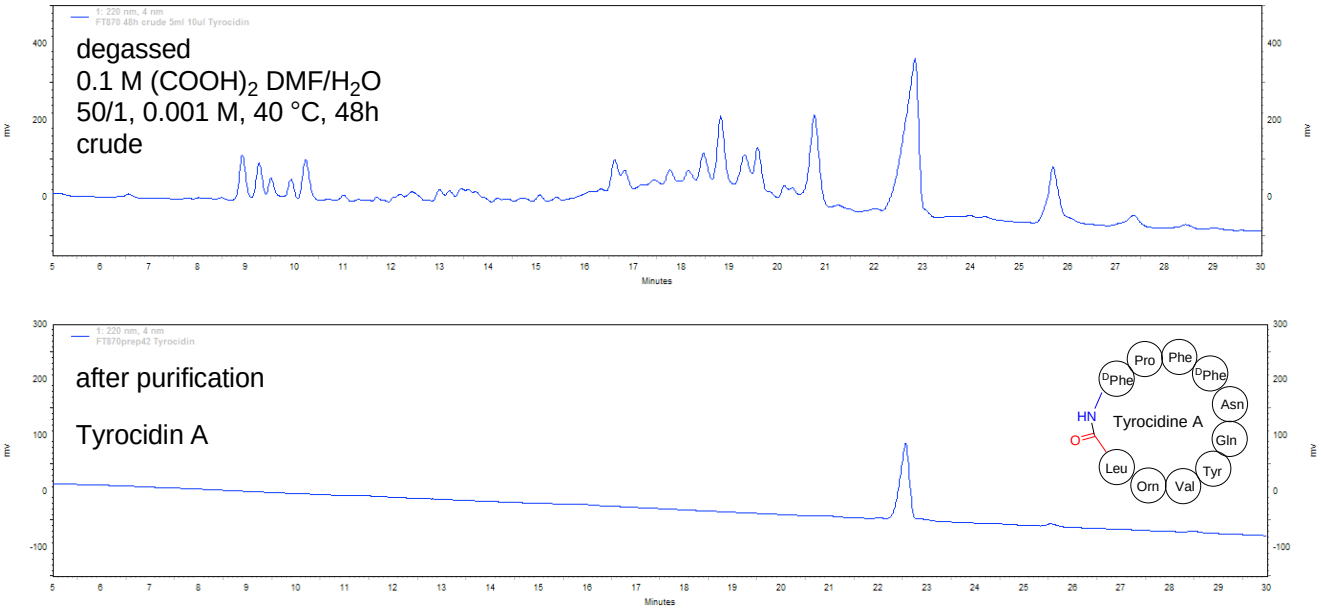
Analytical HPLC: gradient 10–90% CH_3CN/H_2O over 30 min at a flow rate of 1.0 mL/min, 220 nm, C18 column (Shiseido UG120, 4.6 mm × 250 mm, 5 μm). Retention time: 21 min (Gramicidin S). $[\alpha]_D^{20}$ –267 (0.14 M in MeOH); ^{1}H NMR (500 MHz, d_6-DMSO): δ = 9.06 (s, 2H), 8.70 (d, J = 9.2 Hz, 2H), 8.33 (d, J = 9.2 Hz, 2H), 7.66 (br, 6H), 7.40–7.20 (m, 12H), 4.79–4.73 (m, 2H), 4.61–4.55 (m, 2H), 4.42–4.34 (m, 4H), 4.32–4.28 (m, 2H), 3.65–3.55 (m, 2H), 3.00–2.95 (m, 2H), 2.85–2.80 (m, 2H), 2.75–2.70 (m, 2H), 2.10–2.05 (m, 2H), 1.95–1.90 (m, 2H), 1.80–1.70 (m, 2H), 1.80–1.70 (m, 18H), 1.65–1.25 (m, 24H), 0.83–0.76 (m, 24H); LRMS (*m/z*): calcd for $C_{80}H_{93}N_{12}O_{10}$, 1141; found, 1141 $[M+H]^+$, 1163 $[M+Na]^+$. The optical rotation, ^{1}H NMR and mass spectrum were consistent with natural occuring Gramicidin S.[4]

(4) Lit.[ref] $[\alpha]_D^{25}$ –260 (c = 0.20, CH_3OH); a) Tamaki, M. *et al*. Biomimetic formation of gramicidin S by dimerization–cyclization of pentapeptide precursor on solid support. *Tetrahedron Lett*. **47**, 8475–8478 (2006). b) Stern, A., Gibbons, W.A. & Craig. L.C. A conformational analysis of gramicidin S-A by nuclear magnetic resonancea *Proc. Natl. Acad. Sci. U. S. A*, **61**, 734–741

Synthesis of Tyrocidine A



The resin bound peptide (0.20 mmol/g, 101.3 mg, 0.02026 mmol) was used according to the general procedure and the crude yield determined as 32%. The crude peptide was purified by preparative HPLC. The solvent was removed by lyophilization to give Tyrocodine A (4.4 mg, 17%).



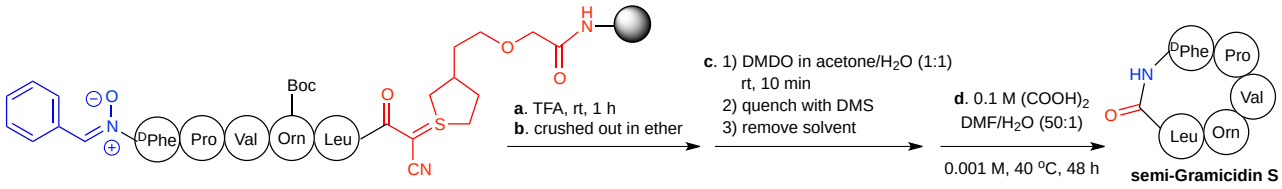
Analytical HPLC: gradient 10–90% CH_3CN/H_2O over 30 min at a flow rate of 1.0 mL/min, 220 nm, C18 column (Shiseido UG120, 4.6 mm × 250 mm, 5 µm). Retention time: 23.0 min (Tyrocidine A).

$[\alpha]_D^{20}$ –124 (c = 0.12, EtOH); ^{1}H NMR: (500 MHz, CD_3OD): δ = 9.29 (br, 1H), 9.18 (br, 1H), 8.87 (d, J = 9.5 Hz, 1H), 8,70 (d, J = 9.0 Hz, 1H), 8.08 (s, 1H), 7.87 (d, J = 8.5 Hz, 1H), 7.55 (s, 1H), 7.51 (d, J = 9.0 Hz, 1H), 7.32–7.10 (m, 15 H), 6.92 (br, 1H), 6.58 (br, 1H), 5.84–5.75 (m, 1H), 5.48–5.42 (m, 1H), 4.70–4.41 (m, 6H), 4.11 (d, J = 8.0 Hz, 1H), 4.06–4.01 (m, 1H), 3.42–2.76 (m, 9H), 2.45–2.35 (m, 1H), 2.22–2.12 (m, 4H), 2.05–1.95 (m, 2H), 1.80–1.70 (m, 3H), 1.68–1.60 (m, 3H), 1.48–1.40 (m, 2H), 1.38–1.28 (m, 1), 0.43 (br, 1 H); LRMS (*m/z*): calcd for $C_{88}H_{88}N_{13}O_{13}$, 1270; found, 1270 $[M+H]^+$, 1292 $[M+Na]^+$. Optical rotation, ^{1}H NMR and mass spectrum consist wild-type Tyrocidine A.[5]

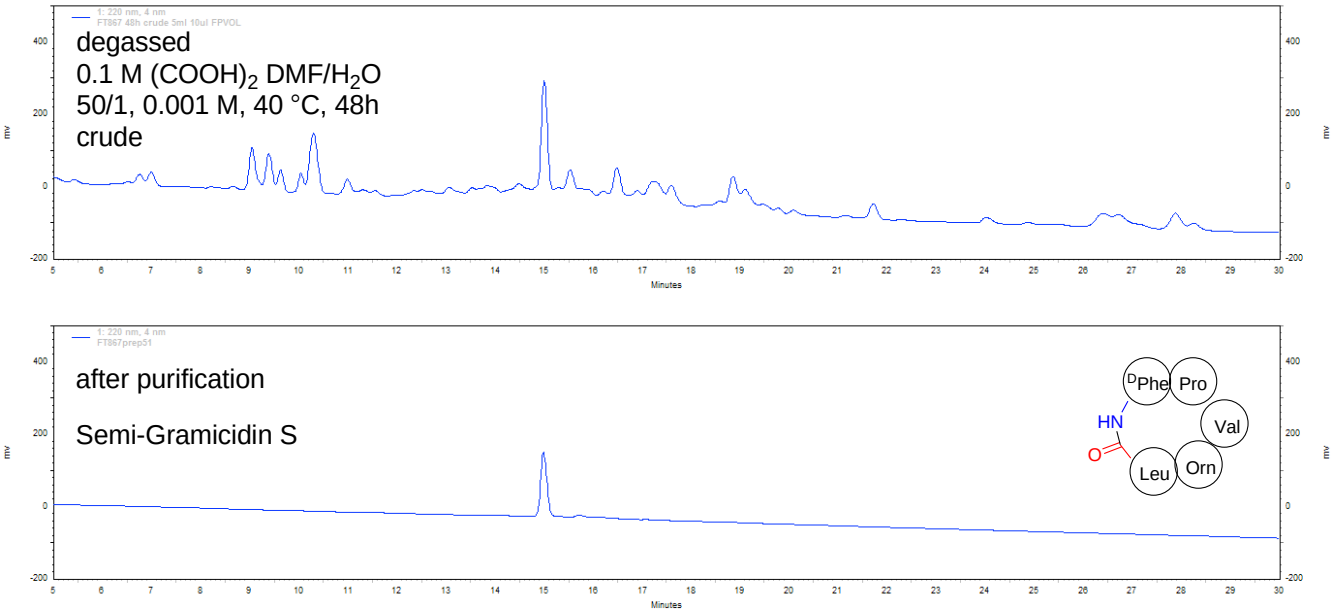
(1968).

(5) Lit.[ref] $[\alpha]_D^{25}$ –108 (*c* = 0.14, 50% ethanol); a) Ohno, M., Izumiya, N. Synthesis of Tyrocidine A. *J. Am. Chem. Soc.*, **88**, 376–378 (1966). b) Hu, H. *et al.* Synthesis and antibacterial activities of *N*-glycosylated derivatives of Tyrocidine A, a macrocyclic peptide antibiotic. *J. Med. Chem.* **52**,

Synthesis of Semi–Gramicidin S



The resin bound peptide (0.32 mmol/g, 102.9 mg, 0.0329 mmol) was used according to the general procedure. The crude yield was confirmed based on standard curve (17 %). The crude peptide was purified by preparative HPLC. The solvent was removed by lyophilization to give the ligation product semi-Gramicidin S (2.8 mg, 15%).

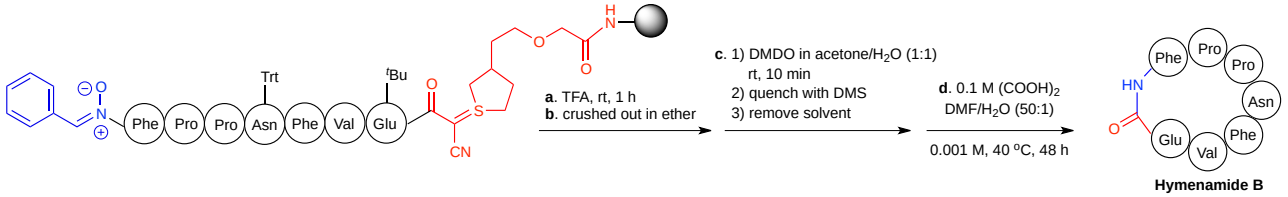


Analytical HPLC: gradient 10–90% CH_3CN/H_2O over 30 min at a flow rate of 1.0 mL/min, 220 nm, C18 column (Shiseido UG120, 4.6 mm × 250 mm, 5 μm). Retention time: 15.0 min.

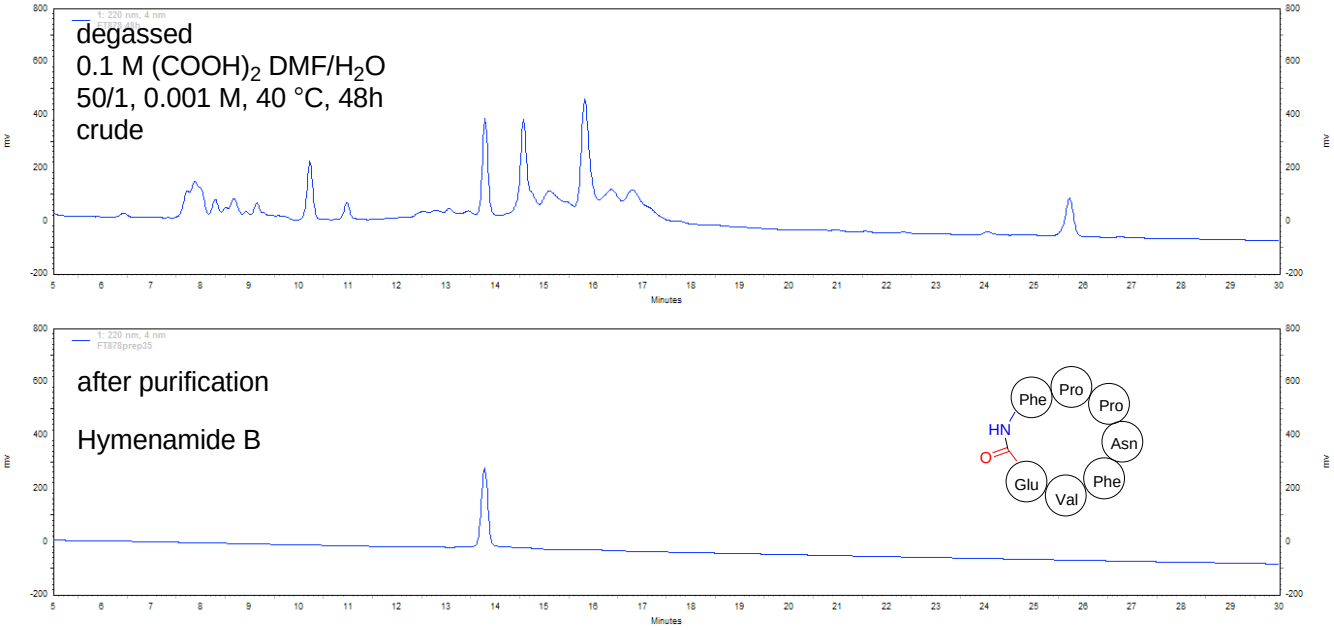
$[\alpha]_D^{22}$ –115 (*c* = 0.11, MeOH). ^{1}H NMR: (500 MHz, d_6-DMSO): δ = 8.53 (d, *J* = 7.0 Hz, 1H), 8.24 (d, *J* = 8.0 Hz, 1H), 8.08 (d, *J* = 8.0 Hz, 1H), 7.62 (br, 3H), 7.29–7.26 (m, 2H), 7.25–7.19 (m, 3H), 6.76 (d, *J* = 9.5 Hz, 1H), 4.53 (dd, *J* = 7.5, 14.5 Hz, 1H), 4.22 (dd, *J* = 8.0, 16.0 Hz, 1H), 4.07 (dd, *J* = 3.5, 9.0 Hz, 1H), 3.96 (t, *J* = 9.5, 1H), 3.81 (dd, *J* = 7.5, 15.5 Hz, 1H), 3.77–3.72 (m, 1H), 2.89–2.85 (m, 3H), 2.78 (dd, *J* = 6.5, 13.0 Hz, 1H), 1.96–1.88 (m, 3H), 1.82–1.69 (m, 4H), 1.67–1.61 (m, 1H), 1.53–1.34 (m, 5H), 0.85 (d, J = 7.0 Hz, 3H), 0.83 (d, *J* = 7.0 Hz, 3H), 0.81 (d, *J* = 6.5 Hz, 3H), 0.77 (d, *J* = 6.5 Hz, 3H). LRMS (ESI): *m/z*: calcd for $C_{30}H_{47}N_6O_5$, 571; found, 571 $[M+H]^+$, 593 $[M+Na]^+$.

2052–2059 (2009).

Synthesis of Hymenamide B



The resin bound peptide (0.27 mmol/g, 106.3 mg, 0.0287 mmol) was used according to the general procedure. The crude yield was confirmed based on standard curve (16%). The crude peptide was purified by preparative HPLC. The solvent was removed by lyophilization to give the product (1.8 mg, 8%).

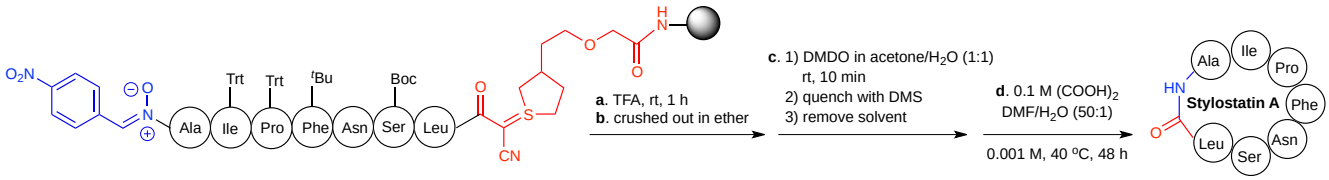


Analytical HPLC: gradient 10–90% CH_3CN/H_2O, 30 min, flow rate = 1.0 mL/min, 220 nm, C18 column (Shiseido UG120, 4.6 mm × 250 mm, 5 μm). Retention time: 13.8 min (hymenamide B).

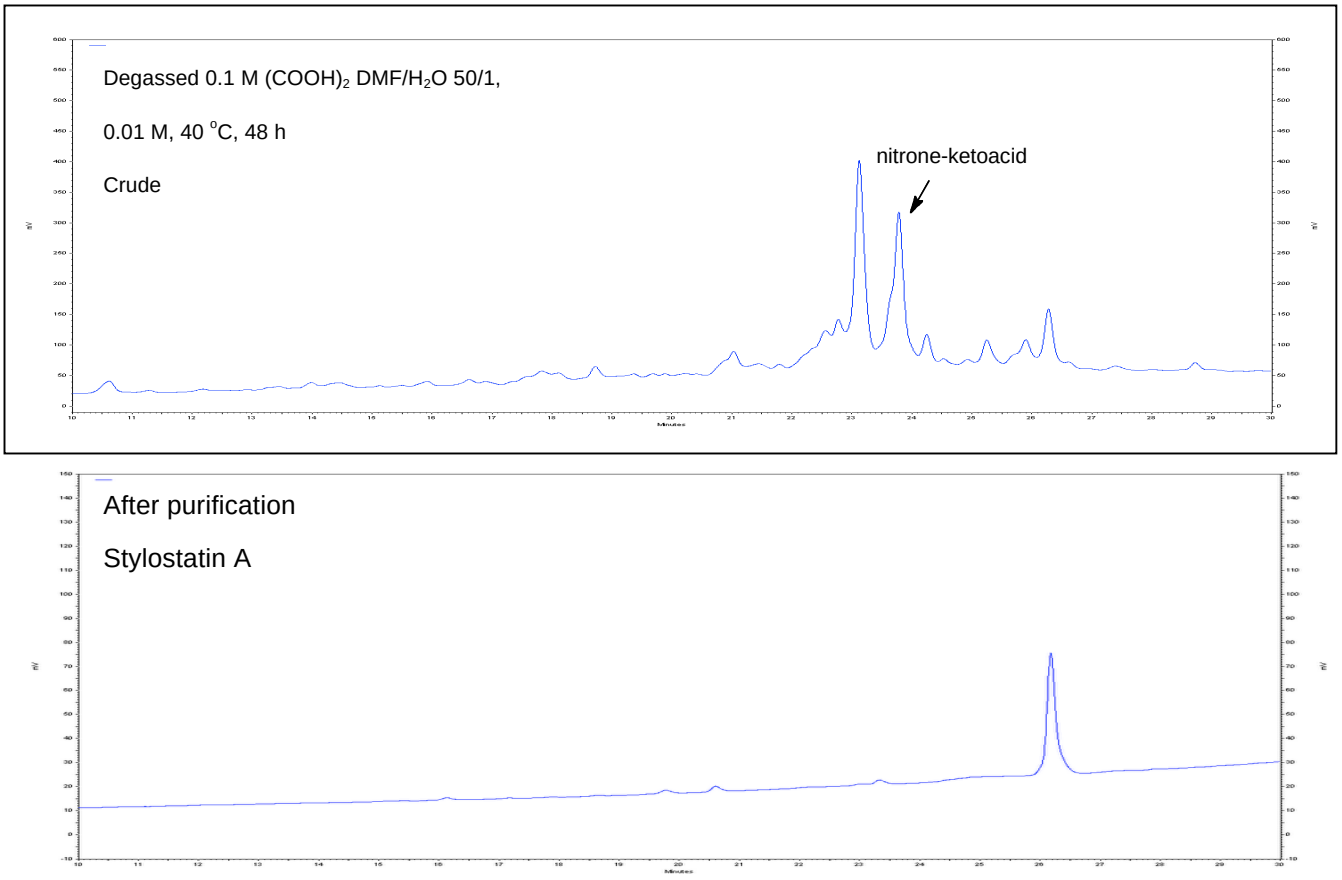
$[\alpha]_D^{20}$ –58 (c = 0.13, MeOH); ^{1}H NMR: (500 MHz, d_6-DMSO): δ = 8.66 (s, 1H), 8.00 (s, 1H), 7.70 (d, J = 9.8 Hz, 1H), 7.46 (d, J = 9.8 Hz, 1H), 7.46 (d, J = 9.8 Hz, 1H), 7.39 (d, J = 6.8 Hz, 2H), 7.29–7.14 (m, 8H), 6.93 (s, 1H), 5.03 (ddd, J = 2.4, 9.8, 10.5 Hz, 1H), 4.71 (ddd, J = 6.8, 7.3, 9.8 Hz, 1H), 4.38 (dd, J = 5.4, 8.3 Hz, 1H), 4.29 (d, J = 8.3 Hz, 1H), 4.17 (ddd, J = 6.4, 9.8, 12.0 Hz, 1H), 4.07 (ddd, J = 4.4, 7.3, 11.2 Hz, 1H), 3.56–3.46 (m, 3H), 3.30–3.40 (m, 2H), 3.19 (dd, J = 7.3, 14.2 Hz, 1H), 2.77 (dd, J = 6.8, 14.2 Hz, 1H), 2.69 (t, J = 10.5 Hz, 1H), 2.10–2.20 (m, 4H), 1.95–1.80 (m, 5H), 1.60–1.80 (m, 4H), 1.07 (d, J = 6.8 Hz), 0.93 (d, J = 7.3 Hz); LRMS (m/z): calcd for $C_{42}H_{55}N_8O_{10}$, 831; found, 831 $[M+H]^+$, 853 $[M+Na]^+$. The rotation, ^{1}H NMR and mass spectrum consist wild-type Hymenamide B.[6]

(6) Lit.[ref] $[\alpha]_D^{25}$ –30 (c = 1.2, CH_3OH); Kobayashi, J. *et al*. Hymenamides a and b, new proline-rich

Synthesis of Stylostatin A



The resin bound peptide (0.24 mmol/g, 100.2 mg, 0.02405 mmol) was used according to the general procedure. Analytical HPLC of the reaction showed that the desired product (13%) was formed along with uncyclizaed starting material (see HPLC trace). The solvent was removed lyophilization to give stylostatin A (1.1 mg, 7%).

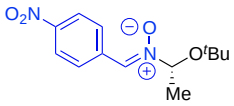


Analytical HPLC: gradient 5-60% CH_3CN/H_2O over 30 min at a flow rate of 1.0 mL/min, 220 nm, C18 column (Shiseido UG120, 4.6 mm × 250 mm, 5 μm). Retention time: 26.2 min (Stylostatin A).

$[\alpha]_D^{20}$ –135 (c = 0.09, CH_3OH); ^1H NMR: (500 MHz, DMSO-d_6): δ8.65 (d, 1H, *J* = 5.0 Hz), 8.59 (d,

cyclic heptapeptides from the okinawan marine sponge *hymeniacidon* sp. *Tetrahedron* **49**, 2391–2402 (1993).

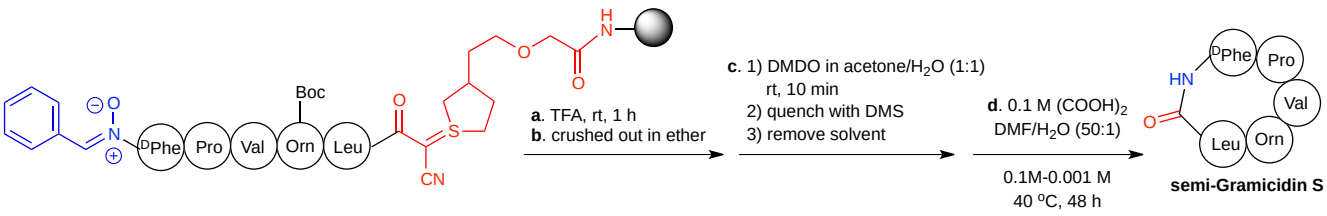
1H, *J* = 7.5 Hz), 8.48 (d, 1H, *J* = 5.0 Hz), 8.03 (d, 1H, *J* = 9.5 Hz), 7.78, (s, 1H), 7.74 (d, 1H, *J* = 5.5 Hz), 7.32 (d, 1H, *J* = 7.0 Hz), 7.29–7.26 (m, 3 H), 7.20 (t, 1H, *J* = 6.5 Hz), 7.12 (d, 2H, *J* = 7.5 Hz), 4.90 (t, 2H, *J* = 7.0 Hz), 4.46–4.41 (m, 2 H), 4.30–4.27 (m, 2 H), 4.16–4.11 (m, 2H), 4.00 (dd, 1H, *J* = 8.6 and 5.0 Hz), 3.84 (dd, 2H, *J* = 9.1 and 4.0 Hz), 3.69–3.66 (m, 2H), 3.24–2.20 (m, 2H), 3.14 (dd, 1H, *J* = 14 and 4.0 Hz), 3.08–3.04 (m, 2H), 3.04–3.02 (m, 1H), 2.62 (t, 1H, *J* = 10.5 Hz), 2.17–2.13 (dd, 1H, *J* = 12.3 and 7.0 Hz), 1.61–1.48 (m, 4 H), 1.27–1.24 (m, 1H), 1.13 (d, 3H, *J* = 6.5 Hz), 0.83 (d, 3H, *J* = 7.0 Hz), 0.81–0.77 (m, 12 H), 0.77–0.71 (m, 1H); LRMS (*m/z*): calcd for $C_{36}H_{54}N_8O_9$, 742.4; found, 765.4 $[M+Na]^+$.[7]



17

$[\alpha]_D^{20}$ –8.58 (c = 0.0.84, $CHCl_3$); mp = 124-127 °C; ^{1}H NMR: (500 MHz, $CDCl_3$): δ = 8.41 (d, 2H, *J* = 9.0 Hz), 8.25 (d, 2H, *J* = 9.0 Hz), 7.59 (s, 1H), 4.74 (qt, 1H, *J* = 7.0 Hz), 1.74 (t, 3H, *J* = 7.0 Hz), 1.47 (s, 9H); ^{13}C NMR: (500 MHz, $CDCl_3$): δ 166.9, 148.2, 16.2, 133.0, 129.3, 124.1, 83..8, 75.0, 28.1, 15.9; IR (thin film) 2980, 2939, 2360, 1740, 1518, 1343, 1157, 862 cm^{-1}; HRMS (*m/z*): calcd for $C_{14}H_{18}N_2O_5$, 294.1216; found, 293.1130 $[M-H]^-$.

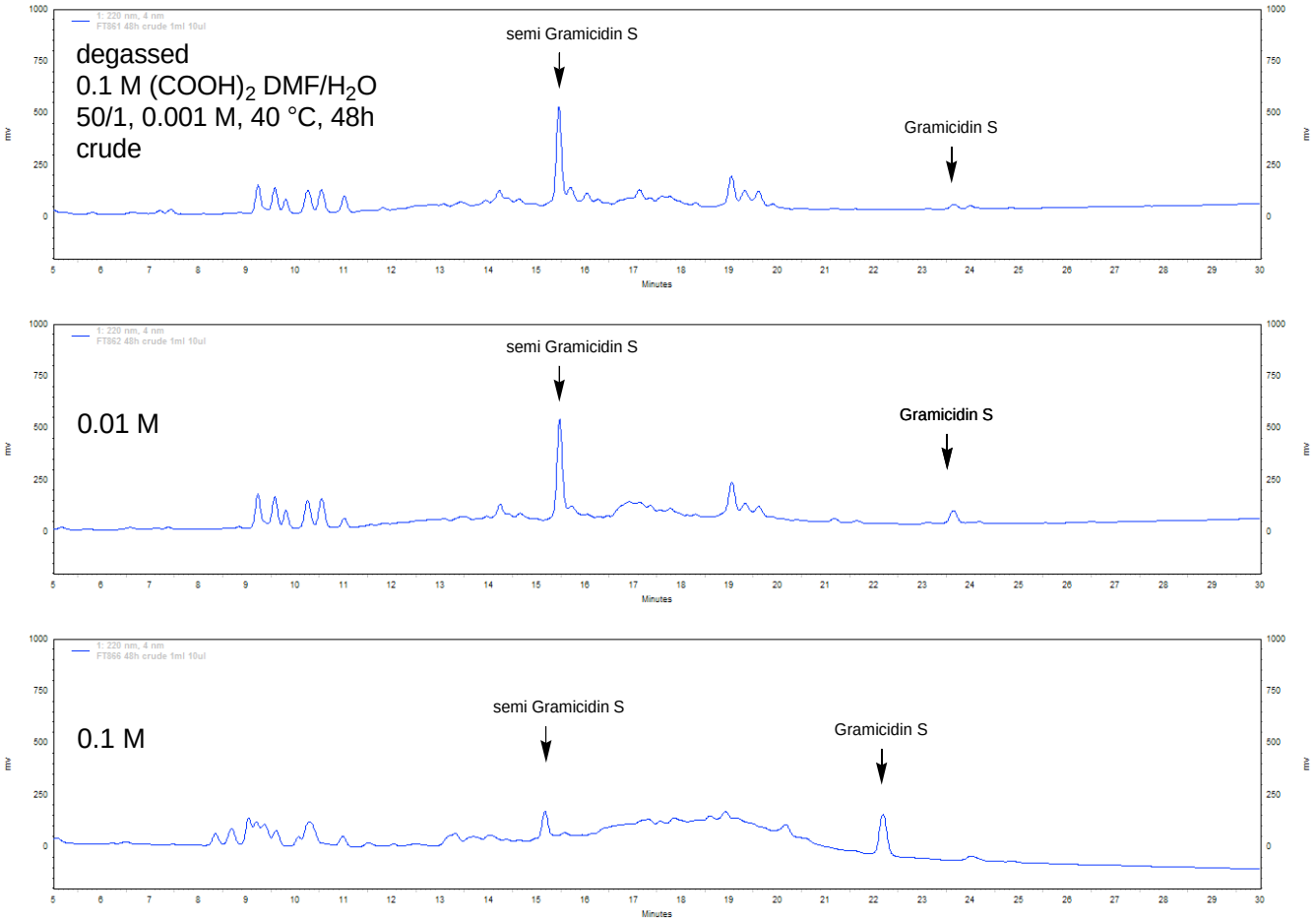
Effect of concentration of peptide cyclization (Semi-Gramicidin S)



The resin (0.32 mmol/g) was used according to the general procedure using different concentration for the cyclization step. The crude yield was determined by UV using a standard curve.

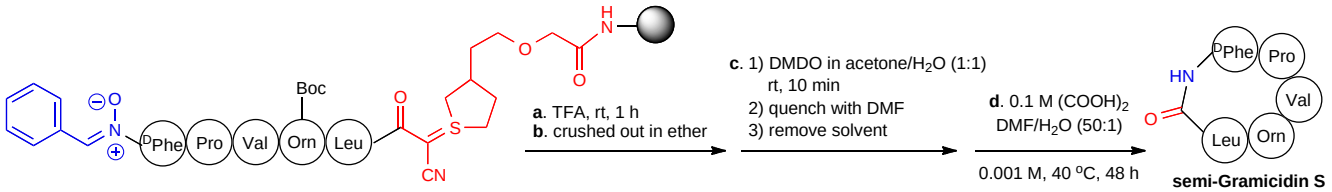
(7) Lit.[ref] $[\alpha]_D^{25}$ –116 (*c* = 0.29, CH_3OH); Pettit, G.R. *et al*. Isolation and structure of Stylostatin 1 from the Papua new guinea marine sponge *stylotella aurantiu*. *J. Org. Chem*. **57**, 7217–7220 (1992).

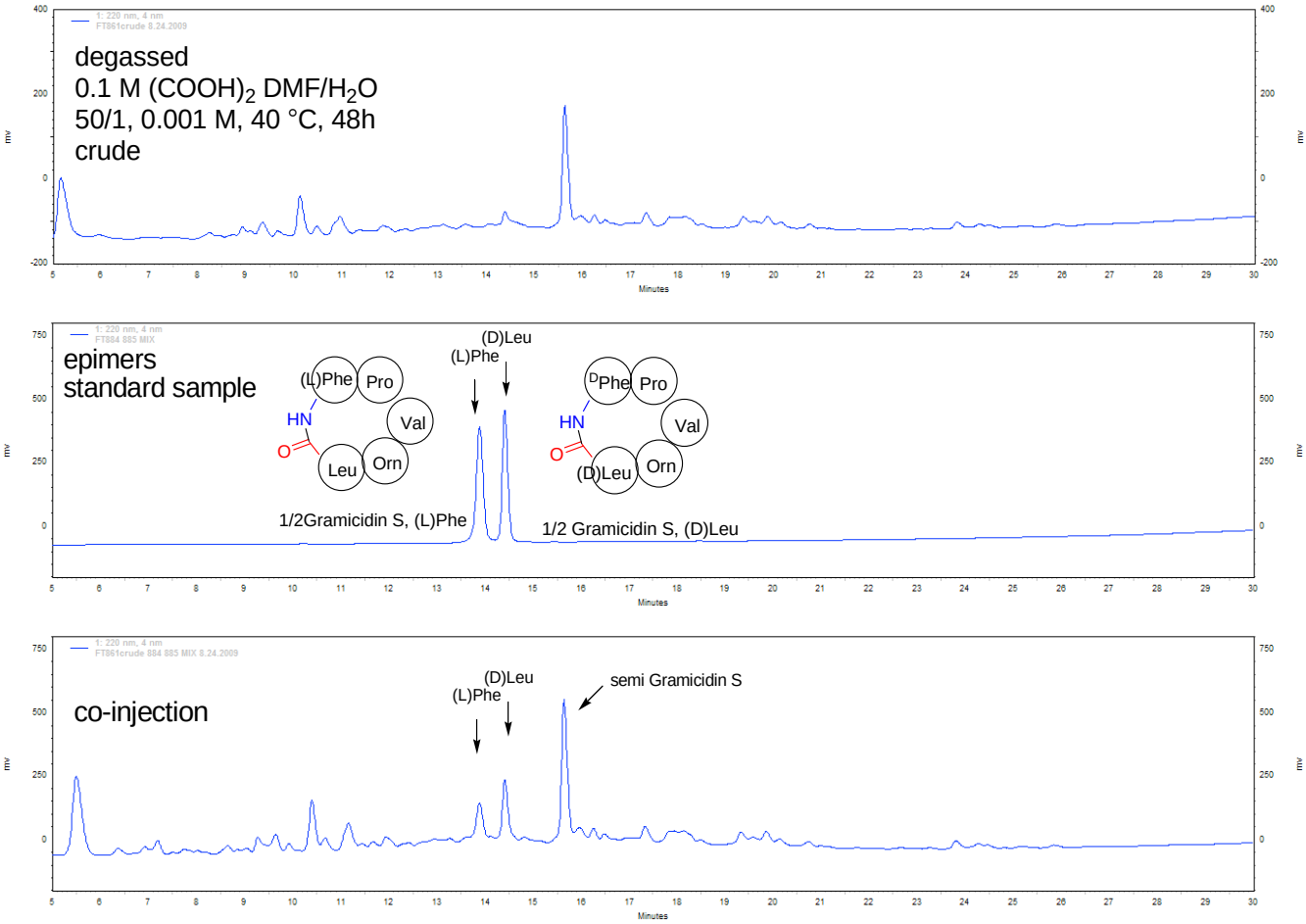
HPLC analysis: gradient 10–90% CH_3CN/H_2O over 30 min at a flow rate of 1.0 mL/min, 220 nm, C18 column (Shiseido UG120, 4.6 mm × 250 mm, 5 μm). Retention time: 15 min (semi Gramicidin S), 24 min (Gramicidin S).



Analytical HPLC: gradient 10–90% CH_3CN/H_2O over 30 min at a flow rate of 1.0 mL/min, 220 nm, C18 column (Shiseido UG120, 4.6 mm × 250 mm, 5 μm). Retention time: 15 min (semi Gramicidin S), 22 min (Gramicidin S).

Epimerization study (semi-Gramicidin S)



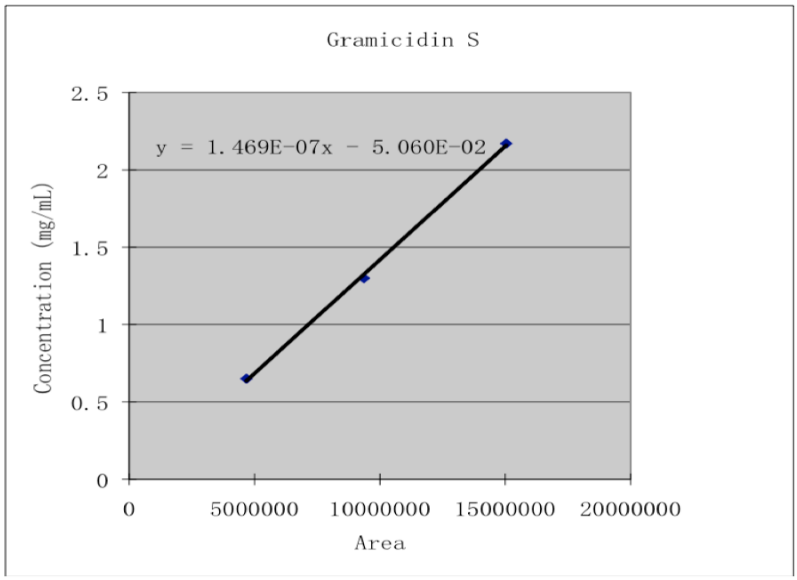


Analytical HPLC: gradient 10–90% CH_3CN/H_2O over 30 min at a flow rate of 1.0 mL/min, 220 nm, C18 column (Shiseido UG120, 4.6 mm × 250 mm, 5 μm). Retention time: 15 min (semi Gramicidin S).

4. Standard Curves

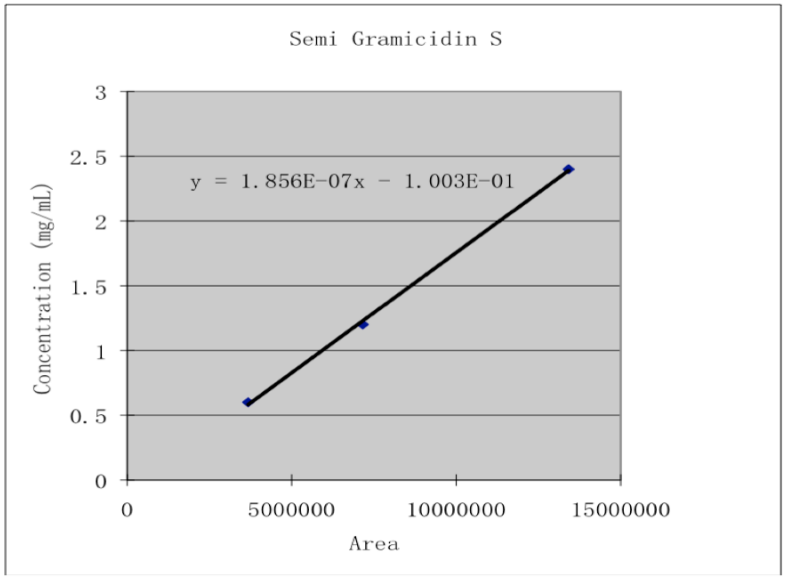
Gramicidin S Standard Curve

	1.3mg/2.00mL	1.3mg/1.00mL	1.3mg/0.60mL
conc [mg/mL]	0.65	1.3	2.17
inject 10 μL [area]	4676497	9363009	15039716



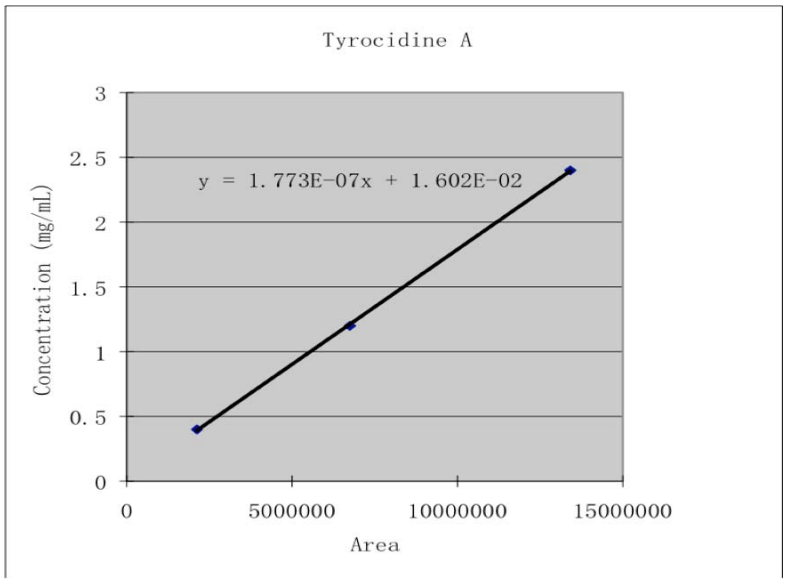
Semi Gramicidin S Standard Curve

	1.2mg/2.00mL	1.2mg/1.00mL	1.2mg/0.50mL
conc [mg/mL]	0.6	1.2	2.4
inject 10 μL [area]	3674333	7159351	13416202



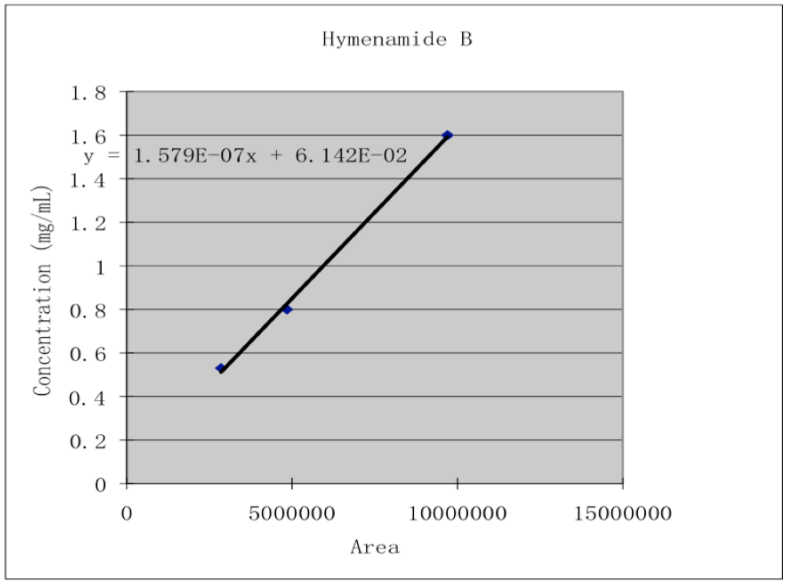
Tyrocidine A Standard Curve

	1.2mg/3.00mL	1.2mg/1.00mL	1.2mg/0.50mL
conc [mg/mL]	0.4	1.2	2.4
inject 10 μL [area]	2123620	6746556	13413946



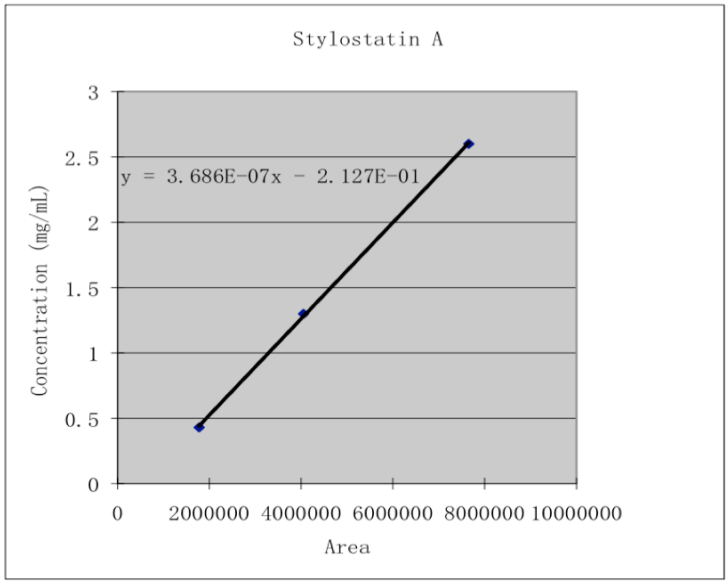
Hymenamide B Standard Curve

	1.6mg/3.00mL	1.6mg/2.00mL	1.6mg/1.00mL
conc [mg/mL]	0.53	0.8	1.6
inject 10 μL [area]	2851202	4843943	9698486

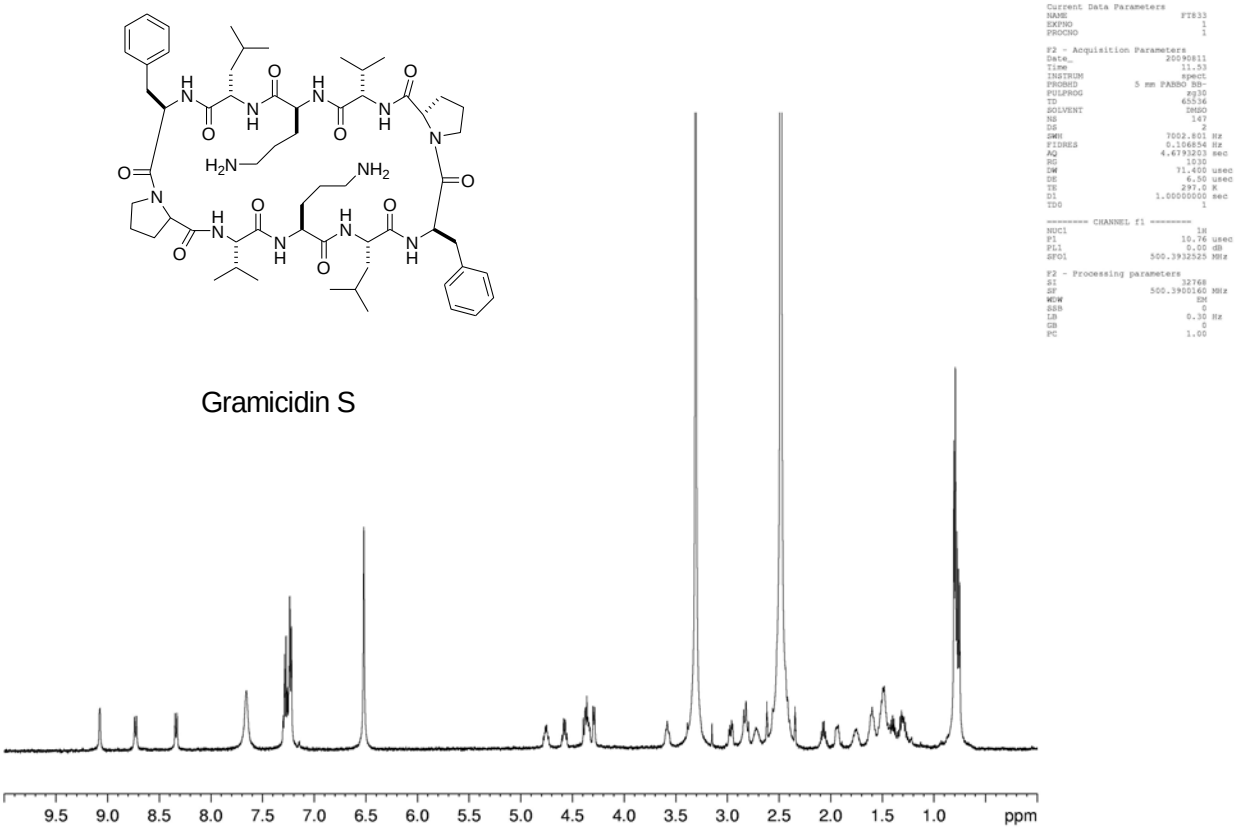


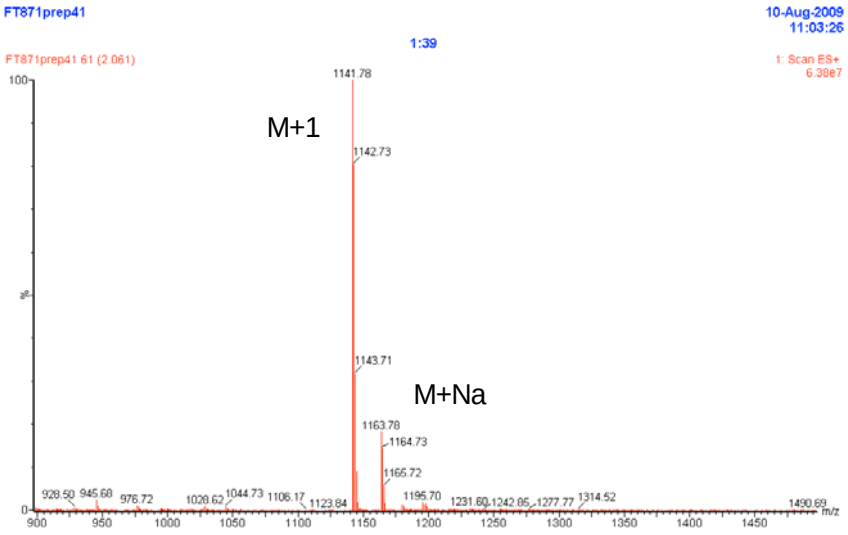
Stylostatin A Standard Curve

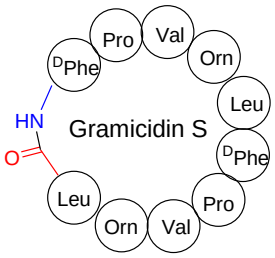
	1.3mg/2.00mL	1.3mg/1.00mL	1.3mg/0.5mL
conc [mg/mL]	0.43	1.3	2.6
inject 10 μL [area]	1776619	4050720	7652189



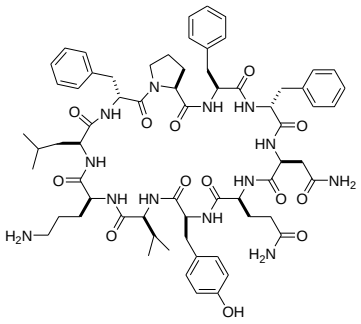
5. NMR and ESI Mass



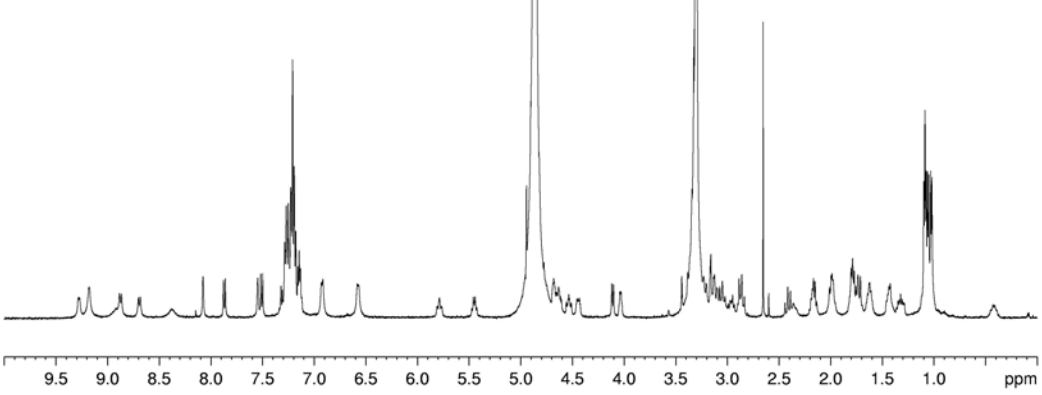


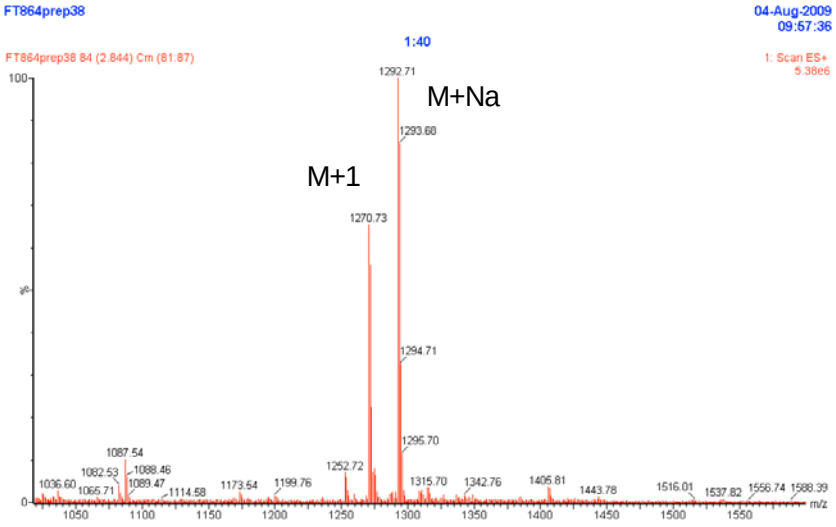


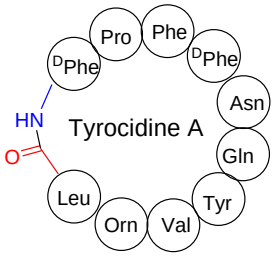
ESIMS spectrum of cyclic peptide (Gramicidin S)



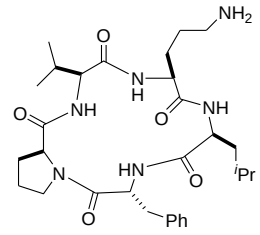
Tyrocidine A



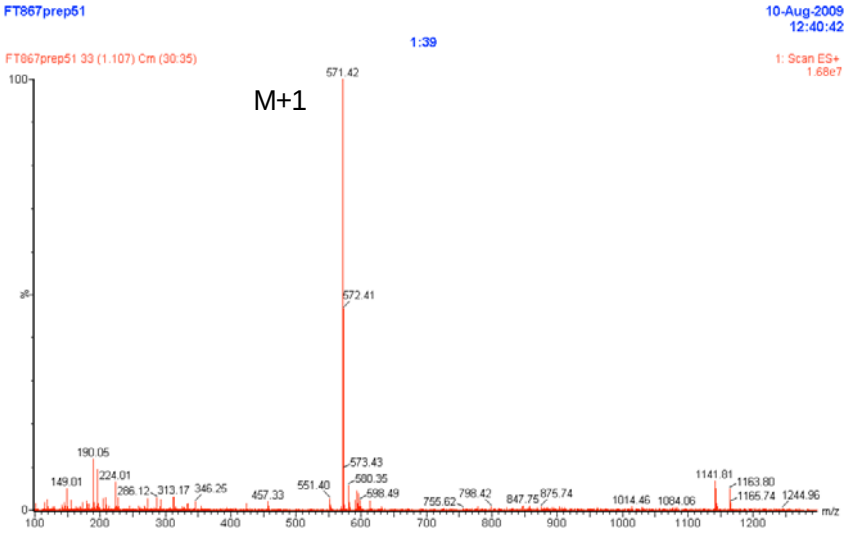


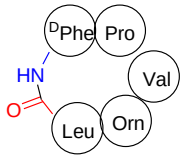


ESIMS spectrum of cyclic peptide (Tyrocidine A)



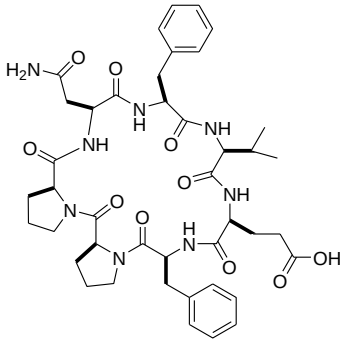
Semi-Gramicidin S



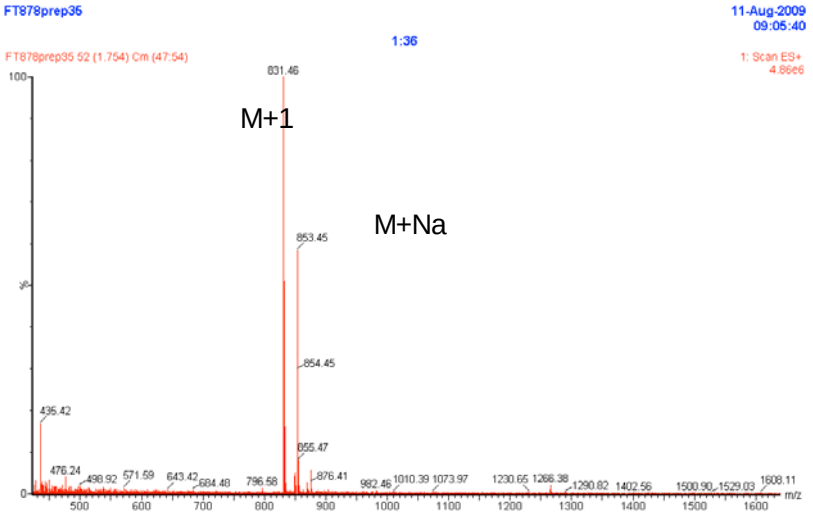


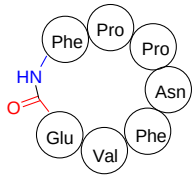
Semi-Gramicidin S

ESIMS spectrum of cyclic peptide (semi Gramicidin S)



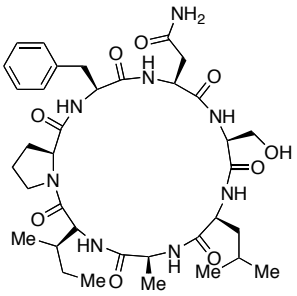
Hymenamide B

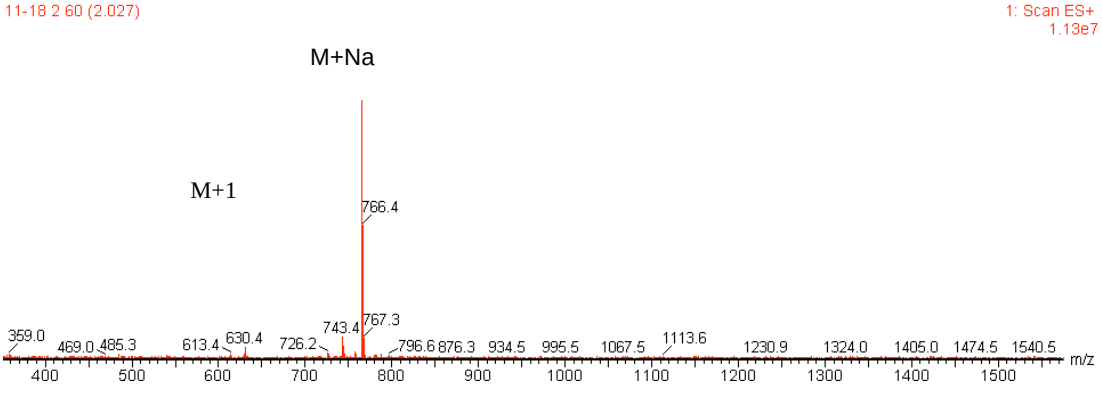




Hymenamide B

ESIMS spectrum of cyclic peptide (Hymenamide B)





ESIMS spectrum of cyclic peptide (Stylostatin A).