Biosynthetic Studies of the Notoamides: Isotopic Synthesis of Stephacidin A and Incorporation into Notoamide B and Sclerotiamide

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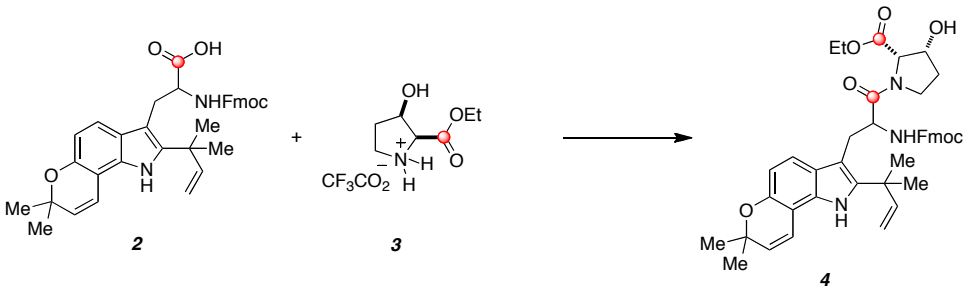
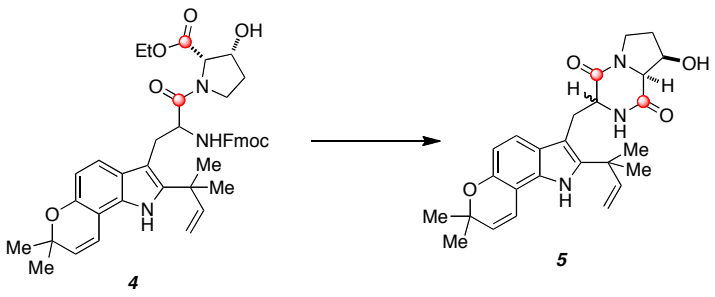
SUPPORTING INFORMATION

General Methods. Unless otherwise noted, all materials were obtained from commercial sources and used without purification. All reactions requiring anhydrous conditions were performed under a positive pressure of argon using flame-dried glassware. Dichloromethane, acetonitrile, toluene, and tetrahydrofuran were degassed with argon and dried through a solvent purification system (J.C. Meyers of Glass Contour). Flash chromatography was performed on standard grade silica gel (230 x 400 mesh) from Sorbent Technologies with the indicated solvent. ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian 300 or 400 MHz or JEOL JNM-ECX 400 spectrometers as indicated. Infrared spectra were recorded on a Nicolet Avatar 320-FT IR spectrometer. Mass spectra were obtained at Colorado State University CIF on a Fisons VG Autospec or at

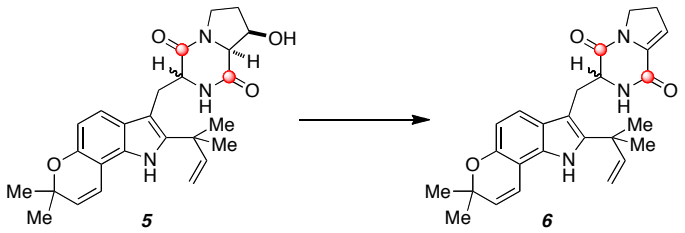
Kumamoto University on a BRUKER esquier3000 mass spectrometer. Melting points were obtained on a Mel-Temp Laboratory Device. Optical rotations were measured on a AUTOPOL III automatic polarimeter. CD spectra was obtained at Kumamoto University on a JASCO J-720 spectropolarimeter.



Peptide (**4**). To a solution of acid **2** (400 mg, 0.692 mmol) in CH_3CN (4 mL) was added *cis*-3-hydroxyproline ethyl ester **3**[1] (199 mg, 0.727 mmol), HATU (395 mg, 1.04 mmol) and $^{i}Pr_2NEt$ (482 mL, 2.77 mmol) successively at room temperature. The mixture was stirred at room temperature for 3 hours. The resulting solution was quenced with 1M aqueous HCl and extracted with CH_2Cl_2. The combined extracts were dried (Na_2SO_4) and concentrated. Purification by silica gel chromatography (ethyl acetate : hexanes, 3 : 1) afforded amide **4** as a mixture of diastereomers and amide rotamers (463 mg, 93%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.20-1.28 (m, 3H), 1.26 (s, 3H), 1.40-1.43 (m, 6H), 1.60 (s, 3H), 2.10-2.35 (m, 2H), 3.05-3.45 (m, 4H), 3.70-3.76 (m, 1H), 4.20-4.75 (m, 5H), 5.16-5.26 (m, 2H), 5.66 (d, J = 9.8 Hz, 1H), 5.91 (d, J = 8.1 Hz, 1H), 6.07-6.14 (m, 1H), 6.50-6.70 (m, 2H), 7.22-7.45 (m, 6H) 7.60-7.85 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.0, 14.4, 21.3, 22.9, 27.5, 27.6, 27.87, 27.92, 27.94, 28.7, 31.8, 39.4, 50.6, 53.6, 55.3, 60.6, 61.6, 65.4, 67.1, 67.6, 75.8, 104.7, 106.3, 110.6, 112.5, 117.1, 118.5, 120.1, 120.3, 124.9, 125.3, 125.4, 127.2, 127.3, 127.8, 129.8, 139.2, 141.4, 144.0, 144.1, 146.3, 148.8, 155.9, 170.6, 170.9, 172.9; HRMS (ESI/APCI) calcd for $C_{41}[^{13}C]_2H_{48}N_3O_7$ (M+H) 720.3554, found 720.3559.

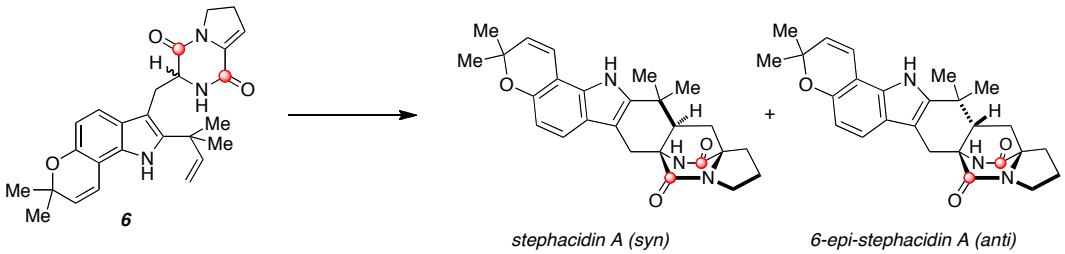


Diketopiperazine (**5**). To a solution of amide **4** (317 mg, 0.44 mmol) in THF (18 mL) at room temperature was added morpholine (4.5 mL). The mixture was stirred at room temperature for 2 hours. The resulting solution was concentrated to afford diketopiperazine **5** as a mixture of diasteromers (190 mg, 96%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.41 and 1.43 (s, 6H), 1.49 and 1.51 (s, 6H), 1.98-2.05 (m, 1H), 2.12-2.17 (m, 1H), 3.07-3.14 (m, 1H), 3.19-3.25 (m, 1H), 3.37-3.43 (m, 1H), 3.47-3.52 (m, 1H), 3.56-3.57 (m, 1H), 3.64-3.69 (m, 2H), 3.76-3.88 (m, 2H), 4.13-4.27 (m, 1H), 4.33-4.36 (m, 1H), 4.52 and 4.65 (br t, J = 3.2 and 3.6 Hz, respectively, 1H), 5.11-5.18 (m, 2H), 5.62-5.66 (m, 1H), 6.09 (dd, J = 10.4, 28.0 Hz, 1H), 6.52-6.64 (m, 2H), 7.16-7.23 (m, 1H), 7.77 and 7.82 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.2, 27.3, 27.4, 27.8, 27.9, 29.6, 29.8, 30.2, 39.0, 39.1, 43.9, 44.0, 54.5, 55.0, 58.0, 58.6, 63.4, 64.0, 64.2, 64.7, 70.8, 70.8, 75.6, 75.7, 104.5, 104.9, 105.3, 110.5, 110.9, 111.8, 112.7, 116.7, 116.7, 117.9, 118.7, 123.6, 129.8, 130.0, 130.7, 130.8, 140.0, 140.1, 145.6, 146.1, 148.7, 148.9, 165.6, 166.1, 167.3, 167.5; HRMS (ESI/APCI) calcd for $C_{24}[^{13}C]_2H_{32}N_3O_4$ (M+H) 452.2454, found 452.2450.



Enamide (**6**). To a solution of alcohols **5** (255 mg, 0.565 mmol) in CH_2Cl_2 (11 mL) at room temperature was added DEAD (772 μL, 1.70 mmol). The mixture was stirred at room temperature for 10 minutes and PBu_3 (423 μL, 1.70 mmol) was added. The solution stirred for 3 hours at room temperature. The reaction mixture was quenched

with 10% aqueous citric acid and extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated. Purification by silica gel chromatography (ethyl acetate : hexanes, 1 : 1 to 3 : 1) yielded enamide **6** as a colorless oil (175 mg, 71%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43 (s, 3H), 1.50 (s, 3H), 2.75 (t, *J* = 7.2 Hz, 2H), 3.10-3.17 (m, 1H), 3.61-3.65 (m, 1H), 4.01-4.10 (m, 2H), 4.42-4.49 (m, 1H), 5.12 (d, *J* = 10.4 Hz, 1H), 5.13 (d, *J* = 18 Hz, 1H), 5.65-5.67 (m, 2H), 6.04-6.11 (m, 2H), 6.54 (d, *J* = 9.6 Hz, 1H), 6.63 (d, *J* = 7.6 Hz, 1H), 7.21-7.22 (m, 1H), 7.83 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.3, 27.8, 27.9, 30.7, 39.0, 45.5, 57.1, 57.6, 75.6, 104.8, 104.9, 110.8, 112.2, 116.8, 118.2, 118.8, 123.4, 129.8, 130.8, 132.7, 133.4, 140.2, 145.8, 148.8, 156.4, 162.5; HRMS (ESI/APCI) calcd for $C_{24}[^{13}C]_2H_{30}N_3O_3$ (M+H) 434.2349, found 434.2346.



(±)-Cycloadducts. To a solution of enamide **6** (175 mg, 0.404 mmol) in MeOH (28 mL) at 0 ºC was added 20% aqueous KOH (7 mL). The reaction mixture was slowly warmed to room temperature over 1 hour and stirred for 18 hours. The resulting solution was quenched with saturated aqueous NH_4Cl and extracted with CH_2Cl_2. The combined extracts were dried over Na_2SO_4 and concentrated. Purification by silica gel chromatography (MeOH : CH_2Cl_2, 3 : 97) afforded (±)-stephacidin A and (±)-6-*epi*-stephacidin A as a 2.4 : 1 mixture of diastereomers, respectively (155 mg, 89%). Recrystallization with MeOH afforded (±)-stephacidin A as a white solid (109 mg, 62%). The filtrate was concentrated to afford (±)-*epi*-stephacidin A (45.6 mg, 26%); (±)-stephacidin A: ^{1}H NMR (400 MHz, d^6-DMSO) δ 1.00 (s, 3H), 1.30 (s, 3H), 1.37 (s, 3H), 1.37 (s, 3H), 1.79-1.88 (m, 2H), 1.95-2.09 (m, 3H), 2.39-1.45 (m, 1H), 2.53-2.56 (m, 1H), 2.64 (dd, *J* = 6.8, 15.6 Hz, 1H), 3.17-3.28 (m, 2H), 3.37 (d, *J* = 4.4 Hz, 1H), 5.71 (d, *J* = 9.6 Hz, 1H), 6.47 (d, *J* = 8.4 Hz, 1H), 6.99 (d, *J* = 9.6 Hz, 1H), 7.08 (d, *J* = 0.21 Hz, 1H), 8.68 (s, 1H), 10.57 (s, 1H); ^{13}C NMR (100 MHz, d^6-DMSO) δ 21.9, 24.2, 24.5,

27.5, 28.4, 29.1, 30.5, 35.0, 44.0, 49.7, 59.8, 60.3, 66.1, 75.4, 104.2, 105.3, 109.0, 117.9, 118.7, 121.9, 129.3, 133.2, 140.1, 147.9, 168.9, 173.5; HRMS (ESI/APCI) calcd for $C_{24}[^{13}C]_2H_{30}N_3O_4$ (M+H) 434.2349, found 434.2359.

(±)-6-*epi*-stephacidin A: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.07 (s, 3H), 1.16 (s, 3H), 1.24 (s, 3H), 1.26 (s, 3H), 1.66-1.74 (m, 1H), 1.81-2.00 (m, 4H), 2.10 (dd, *J* = 2.8, 10 Hz, 1H), 2.52-2.60 (m, 1H), 2.71 (d, *J* = 18 Hz, 1H), 3.31-3.37 (m, 2H), 3.58 (dd, *J* = 2.8, 20.8 Hz, 1H), 5.46 (d, *J* = 10 Hz, 1H) 6.42 (d, *J* = 8.4 Hz, 1H), 6.64 (d, *J* = 9.6 Hz, 1H), 7.03 (d, *J* = 8.4 Hz, 1H) ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.6, 27.0, 27.6, 27.8, 28.2, 30.5, 31.4, 32.8, 36.4, 48.0, 49.9, 65.2, 70.8, 79.4, 106.9, 109.3, 113.4, 121.6, 121.7, 133.1, 137.1, 152.1, 160.9, 166.9, 173.9, 177.6; HRMS (ESI/APCI) calcd for $C_{24}[^{13}C]_2H_{30}N_3O_4$ (M+H) 434.2349, found 434.2344.

General procedure for feeding experiment with *Aspergillus versicolor*. A culture of *Aspergillus versicolor* NRRL 35600 was obtained from the Department of Agriculture in Peoria, IL. This culture was transferred to malt extract agar slants and allowed to incubate for 14 days. Potato Dextrose Broth was prepared by dissolving 48 g of the medium and 6 g tryptose in 2L of doubly distilled H_2O (DDH_2O). The solution was heated to aid in dissolving the medium, which was then transferred to fernbach flasks (4 x 500 mL) and autoclaved. Spores of *A. versicolor* were added to the broth from the agar slants. The fernbach flasks were covered and gently placed in the incubator for 14 days. (±)-$[^{13}C]_2$-Stephacidin A (**1**) (60 mg, 0.138 mmol) was dissolved in 1.0 mL DMSO and added to 75 mL of hot DDH_2O containing 10 mL of 1% TWEEN 80. The solution cooled to room temperature and was added to 350 mL of a sterile trace element solution (35 mM $NaNO_3$, 5.7 mM K_2HPO_4, 4.2 mM $MgSO_4{\cdot}7H_2O$, 1.3 mM KCl, 36 μM $FeSO_4{\cdot}7H_2O$, 25 μM $MnSO_4{\cdot}H_2O$, 7 μM $ZnSO_4{\cdot}7H_2O$, and 1.5 μM $CuCl_2{\cdot}2H_2O$). The fungus broth was decanted and the fungal cells were washed with 100 mL sterile DDH_2O. The precursor/trace element solution (110 mL) was added to each flask using a syringe and needle. The fungal cells were incubated at 25°C for 21 days and each flask was swirled daily to ensure even distribution of the labeled compound.

Isolation and purification. The trace element solution was decanted, and the fungus was pureed in a blender with 1:1 MeOH-$CHCl_3$. The puree was transferred to a 2 L Erlenmeyer flask, diluted to 1.2 L with 1:1 MeOH-$CHCl_3$, and placed in the shaker for 24 hours. Celite (30 g) was added to the flask and allowed to shake for an extra 10 minutes. The suspension was filtered through Whatman #2 paper and the filtrate was stored at 4°C. The mycelia "cake" was diluted with 600 mL 1:1 MeOH-$CHCl_3$ and placed on the shaker for an additional 48 hours. The suspension was filtered through Whatman #2 paper, and the combined filtrates were concentrated under vacuum. The residue was dissolved in 250 mL H_2O and extracted with EtOAc (3 x 300 mL). The organic layer was concentrated and partitioned between MeCN and hexanes. The layers were separated, and the MeCN layer was concentrated under vacuum. The crude material was purified via silica gel flash column chromatography (1% MeOH in DCM – 3% MeOH in DCM) to afford four fractions that were each analyzed by ^{13}C NMR spectroscopy. Further purification was carried out on those fractions containing ^{13}C-labeled material via preparative thin layer chromatography (1000 μm, 3% MeOH in DCM x5) to afford doubly labeled (+)-stephacidin A (29 mg, 0.069 mmol), (+)-notoamide B (1.6 mg, 0.005 mmol) and (+)-sclerotiamide (0.8 mg, 0.0017 mmol).

Calculation of percent incorporation from *Aspergillus versicolor* experiment via mass spectra. The percentage of ^{13}C-enrichment in (+)-notoamide B and (+)-sclerotiamide from isotopically labeled (±)-stephacidin A was calculated according to the method by Lambert et al.[2] These calculations are based on the comparison of the mass spectrum of the labeled material to the mass spectrum of the unlabeled material. For these experiments, electrospray mass spectrum was used, thus the base peak was the M+H.

(+)-Notoamide B percent incorporation:

Native Notoamide B ($C_{26}H_{29}N_3O_4$)
[M+H] (448.22): 73.8%
[M+H+1] (449.22): 21.93%
[M+H+2] (450.21): 3.75%
[M+H+3] (451.21): 0.47%
[M+H+4] (452.20): 0.05%

^{13}C Notoamide B from incorporation experiments:
[M+H] (448.20): 49.2%
[M+H+1] (449.20): 0.1%
[M+H+2] (450.19): 50.5%
[M+H+3] (451.19): 0.0%
[M+H+4] (452.18): 0.0%

Calculation of native Notoamide B: (assume 100 molecules)

[M+H]:	^{12}C: 26 x 73.8 = 1918.8	
[M+H+1]:	^{12}C: 25 x 21.93 = 548.25	^{13}C: 1 x 21.93 = 21.93
[M+H+2]:	^{12}C: 24 x 3.75 = 90.0	^{13}C: 2 x 3.75 = 7.5
[M+H+3]:	^{12}C: 23 x 0.47 = 10.81	^{13}C: 3 x 0.47 = 1.41
[M+H+4]:	^{12}C: 22 x 0.05 = 1.1	^{13}C: 4 x 0.05 = 0.2
total ^{12}C: 2568.96		total ^{13}C: 31.04

Native Notoamide B ^{13}C content: 31.04/(2568.96 +31.04) x 100% = 1.2%

Calculation for ^{13}C Notoamide B from incorporation experiments: (assume 100 molecules)

[M+H]:	^{12}C: 26 x 49.2 = 1279.2	
[M+H+1]:	^{12}C: 25 x 0.1 = 2.5	^{13}C: 1 x 0.1 = 0.1
[M+H+2]:	^{12}C: 24 x 50.5 = 1212	^{13}C: 2 x 50.5 = 101
[M+H+3]:	^{12}C: 23 x 0.0 = 0	^{13}C: 3 x 0.0 = 0
[M+H+4]:	^{12}C: 22 x 0.0 = 0	^{13}C: 4 x 0.0 = 0
total ^{12}C: 2493.7		total ^{13}C: 101.1

Notoamide B from incorporation experiments ^{13}C content: 101.1/(2493.7 + 101.1) x 100% = 3.9%

Percent incorporation of intact doubly ^{13}C-labeled Stephacidin A = 3.9% - 1.2% = 2.7%

(+)-Sclerotiamide percent incorporation:

Native sclerotiamide ($C_{26}H_{29}N_3O_5$)
[M+H] (464.21): 72.8%
[M+H+1] (465.21): 22.1%
[M+H+2] (466.22): 3.4%
[M+H+3] (467.22): 0.42%
[M+H+4] (468.23): 0.08%

^{13}C sclerotiamide from incorporation experiments:
[M+H] (464.21): 18.4%
[M+H+1] (465.21): 1.7%
[M+H+2] (466.22): 79.6%
[M+H+3] (467.24): 0.0%
[M+H+4] (468.24): 0.1%

Calculation of native sclerotiamide: (assume 100 molecules)

[M+H]:	^{12}C: 26 x 72.8 = 1892.8	
[M+H+1]:	^{12}C: 25 x 22.1 = 552.5	^{13}C: 1 x 22.1 = 22.1
[M+H+2]:	^{12}C: 24 x 3.4 = 81.6	^{13}C: 2 x 3.4 = 6.8
[M+H+3]:	^{12}C: 23 x 0.42 = 9.66	^{13}C: 3 x 0.42 = 1.26
[M+H+4]:	^{12}C: 22 x 0.08 = 1.76	^{13}C: 4 x 0.08 = 0.32
total ^{12}C: 2538.3		total ^{13}C: 30.5

Native sclerotiamide ^{13}C content: 30.5/(2538.5 +30.5) x 100% = 1.2%

Calculation for ^{13}C sclerotiamide from incorporation experiments: (assume 100 molecules)

[M+H]:	^{12}C: 26 x 18.4 = 478.4	
[M+H+1]:	^{12}C: 25 x 1.7 = 42.5	^{13}C: 1 x 1.7 = 1.7
[M+H+2]:	^{12}C: 24 x 79.6 = 1910.4	^{13}C: 2 x 79.6 = 159.2
[M+H+3]:	^{12}C: 23 x 0.0 = 0.0	^{13}C: 3 x 0.0 = 0.0
[M+H+4]:	^{12}C: 22 x 0.1 = 2.2	^{13}C: 4 x 0.1 = 0.4
total ^{12}C: 2433.5		total ^{13}C: 161.3

Sclerotiamide from incorporation experiments ^{13}C content: 161.3/(2433.5 + 161.3) x 100% = 6.2%

Percent incorporation of intact doubly ^{13}C-labeled Stephacidin A = 6.2% - 1.2% = 5.0%

General procedure for feeding experiment with *Aspergillus* sp. MF297-2. Culture of the marine-derived *Aspergillus* sp. was maintained on agar plates (20 g malt extract, 5 g peptone and 20 g agar per liter of 50% seawater) in an incubator at 25 ˚C. The spores from the agar plates was cultured in three 1 L flasks containing a medium (3 x 300 mL) composed of 50% seawater with 2.0% malt extract and 0.5% peptone at 25 ˚C for 14 days. $[^{13}C]_2$-stephacidin A (**1**) (40 mg, 0.0928 mmol) was dissolved in 1.0 mL DMSO and added to 75 mL of hot DDH_2O containing 10 mL of 1% TWEEN 80. The solution

cooled to room temperature and was added to 300 mL of a sterile trace element solution (35 mM $NaNO_3$, 5.7 mM K_2HPO_4, 4.2 mM $MgSO_4 \cdot 7H_2O$, 1.3 mM KCl, 36 μM $FeSO_4 \cdot 7H_2O$, 25 μM $MnSO_4 \cdot H_2O$, 7 μM $ZnSO_4 \cdot 7H_2O$, and 1.5 μM $CuCl_2 \cdot 2H_2O$). The fungal broth was decanted and the fungal cells were washed with 100 mL sterile DDH_2O. The precursor/trace element solution (125 mL) was added to each flask. The fungal cells were incubated at 25°C for 14 days and each flask was swirled daily to ensure even distribution of the labeled compound.

Isolation and purification. The trace element solution was decanted, and the fungus was extracted with 1:1 MeOH-$CHCl_3$ three times. The extract (1.2 g) was dissolved in 100 mL H_2O and extracted with EtOAc (3 x 100 mL). The organic layer was concentrated and partitioned between 90% MeOH and hexane. The aqueous MeOH layer was concentrated under vacuum. The crude material (110 mg) was purified by ODS flash column chromatography (30, 96, 100% MeOH in H_2O). The fractions were each analyzed by ^{13}C NMR spectroscopy. The fraction eluted with 96% MeOH-H_2O containing ^{13}C-labeled material was further purified by ODS HPLC with 65% MeOH-H_2O to afford doubly labeled (–)-notoamide B (0.24 mg, 0.53 μmol) and (–)-sclerotiamide (0.34 mg, 0.73 μmol). The fungal broth was extracted with *n*-BuOH (3 x 300 mL). The organic layer was concentrated and partitioned between 90% MeOH and hexane. The aqueous MeOH layer was concentrated under vacuum. The crude material (490 mg) was purified by ODS flash column chromatography (30, 96, 100% MeOH in H_2O). The fractions eluted with 96% MeOH-H_2O and MeOH were combined and purified by ODS HPLC with 60% MeOH-H_2O to afford doubly labeled (–)-stephacidin A (18 mg, 0.041 mmol). The absolute configurations of notoamide B, sclerotiamide, and stephacidin A were determined by their CD spectra.

Calculation of percent incorporation from *Aspergillus* sp. MF297-2 via mass spectra. The percentage of ^{13}C-enrichment in (–)-notoamide B and (–)-sclerotiamide from isotopically labeled (±)-stephacidin A was calculated according to the method by Lambert et al.[2] These calculations are based on the comparison of the mass spectrum of

the labeled material to the mass spectrum of the unlabeled material. For these experiments, electrospray mass spectrum was used, thus the base peak was the M-H.

(–)-Notoamide B percent incorporation:

Native Notoamide B ($C_{26}H_{29}N_3O_4$)
[M-H] (446.10): 63.3%
[M-H+1] (447.00): 35.3%
[M-H+2] (448.00): 0.7%
[M-H+3] (449.00): 0.0%
[M-H+4] (450.00): 0.7%

^{13}C Notoamide B from incorporation experiments:
[M-H] (446.00): 0.0%
[M-H+1] (447.00): 0.0%
[M-H+2] (447.98): 64.6%
[M-H+3] (449.06): 21.7%
[M-H+4] (450.23): 13.7%

Calculation of native Notoamide B: (assume 100 molecules)

[M-H]:	^{12}C: 26 x 63.3 = 1645.8	
[M-H+1]:	^{12}C: 25 x 35.3 = 882.5	^{13}C: 1 x 35.3 = 35.3
[M-H+2]:	^{12}C: 24 x 0.7 = 16.8	^{13}C: 2 x 0.7 = 0.7
[M-H+3]:	^{12}C: 23 x 0.0 = 0.0	^{13}C: 3 x 0.0 = 0.0
[M-H+4]:	^{12}C: 22 x 0.7 = 15.4	^{13}C: 4 x 0.7 = 2.8
total	^{12}C: 2560.5	total ^{13}C: 38.8

Native Notoamide B ^{13}C content: 38.8/(2560.5 +38.8) x 100% = 1.5%

Calculation for ^{13}C Notoamide B from incorporation experiments: (assume 100 molecules)

[M-H]:	^{12}C: 26 x 0.0 = 0.0	
[M-H+1]:	^{12}C: 25 x 0.0 = 0.0	^{13}C: 1 x 0.0 = 0.0
[M-H+2]:	^{12}C: 24 x 64.6 = 1550.4	^{13}C: 2 x 64.6 = 129.2
[M-H+3]:	^{12}C: 23 x 21.7 = 499.1	^{13}C: 3 x 21.7 = 65.1
[M-H+4]:	^{12}C: 22 x 13.7 = 301.4	^{13}C: 4 x 13.7 = 54.8
total	^{12}C: 2350.9	total ^{13}C: 249.1

Notoamide B from incorporation experiments ^{13}C content: 249.1/(2350.9 + 249.1) x 100% = 9.6%

Percent incorporation of intact doubly ^{13}C-labeled Stephacidin A = 9.6% - 1.5% = 8.1%

(–)-Sclerotiamide percent incorporation:

Native sclerotiamide ($C_{26}H_{29}N_3O_5$)
[M-H] (462.10): 71.7%
[M-H+1] (463.20): 24.5%
[M-H+2] (464.00): 3.7%
[M-H+3] (465.00): 0.0%
[M-H+4] (466.00): 0.9%

^{13}C sclerotiamide from incorporation experiments:
[M-H] (462.00): 0.0%
[M-H+1] (462.90): 5.7%
[M-H+2] (464.01): 62.5%
[M-H+3] (464.99): 22.5%
[M-H+4] (465.90): 9.3%

Calculation of native sclerotiamide: (assume 100 molecules)

[M-H]:	^{12}C: 26 x 71.7 = 1864.2	
[M-H+1]:	^{12}C: 25 x 24.5 = 612.5	^{13}C: 1 x 24.5 = 24.5
[M-H+2]:	^{12}C: 24 x 3.7 = 88.8	^{13}C: 2 x 3.7 = 7.4
[M-H+3]:	^{12}C: 23 x 0.0 = 0.0	^{13}C: 3 x 0.0 = 0.0
[M-H+4]:	^{12}C: 22 x 0.9 = 19.8	^{13}C: 4 x 0.9 = 3.6
total	^{12}C: 2585.3	total ^{13}C: 35.5

Native sclerotiamide ^{13}C content: 35.5/(2585.3 +35.5) x 100% = 1.4%

Calculation for ^{13}C sclerotiamide from incorporation experiments: (assume 100 molecules)

[M-H]:	^{12}C: 26 x 0.0 = 0.0	
[M-H+1]:	^{12}C: 25 x 5.7 = 142.5	^{13}C: 1 x 5.7 = 5.7
[M-H+2]:	^{12}C: 24 x 62.5 = 1500.0	^{13}C: 2 x 62.5 = 125.0
[M-H+3]:	^{12}C: 23 x 22.5 = 517.5	^{13}C: 3 x 22.5 = 67.5
[M-H+4]:	^{12}C: 22 x 9.3 = 204.6	^{13}C: 4 x 9.3 = 13.3
total	^{12}C: 2364.6	total ^{13}C: 211.5

Sclerotiamide from incorporation experiments ^{13}C content: 211.5/(2364.6 + 211.5) x 100% = 8.2%

Percent incorporation of intact doubly ^{13}C-labeled Stephacidin A = 8.2% - 1.4% = 6.8%

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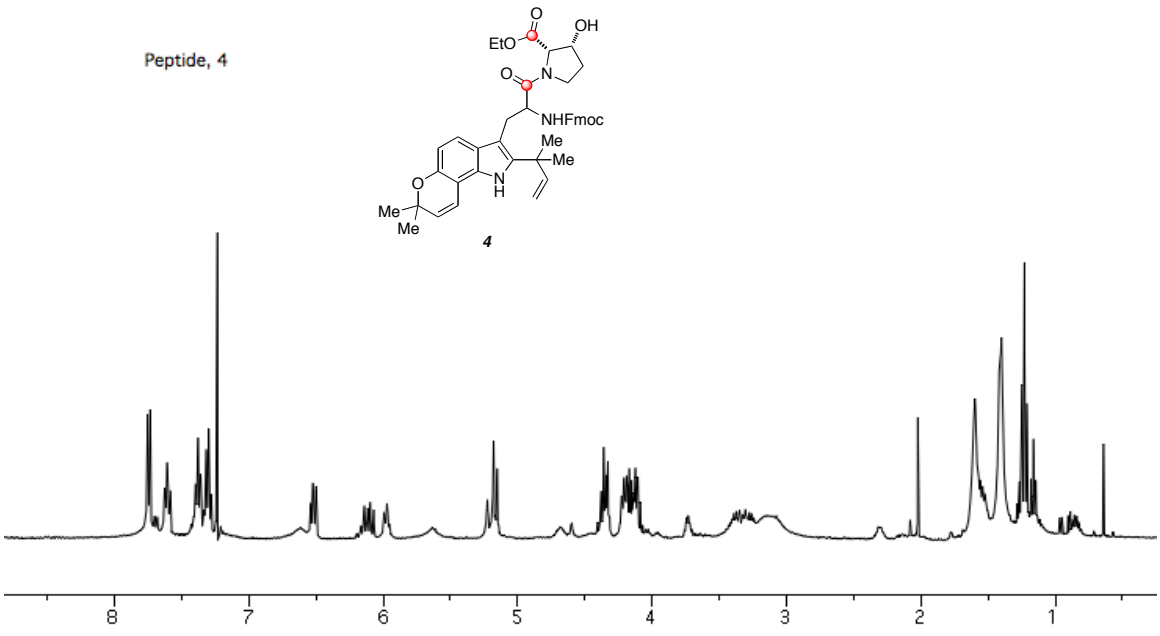
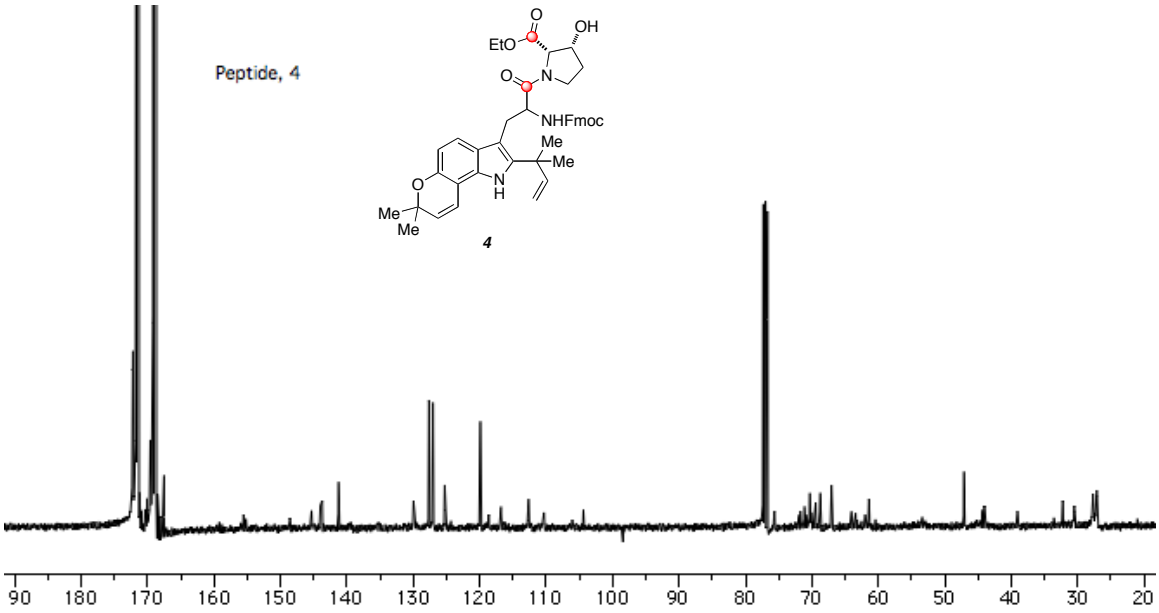
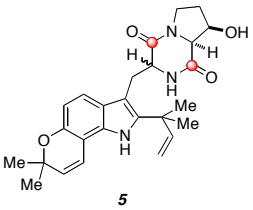
[1] Prepared from ^{13}C-labeled glycine following the same procedure to synthesize unlabeled *cis*-3-hydroxyproline ethyl ester: Greshock, T. J.; Williams, R. M. *Org. Lett.* **2007**, *9*, 4255-4258.

[2] Calculated according to the method outlined in: Lambert, J. B.; Shurvell, H. B.; Lightner, D. A.; Cooks, R. G. *Organic Structural Spectroscopy*; Prentice Hall: Upper Saddle River, NJ, 1998; pp 447-448.





Diketopiperazine, 5



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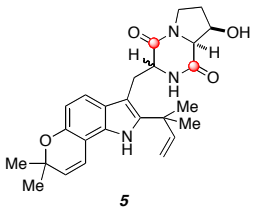
Diketopiperazine, 5



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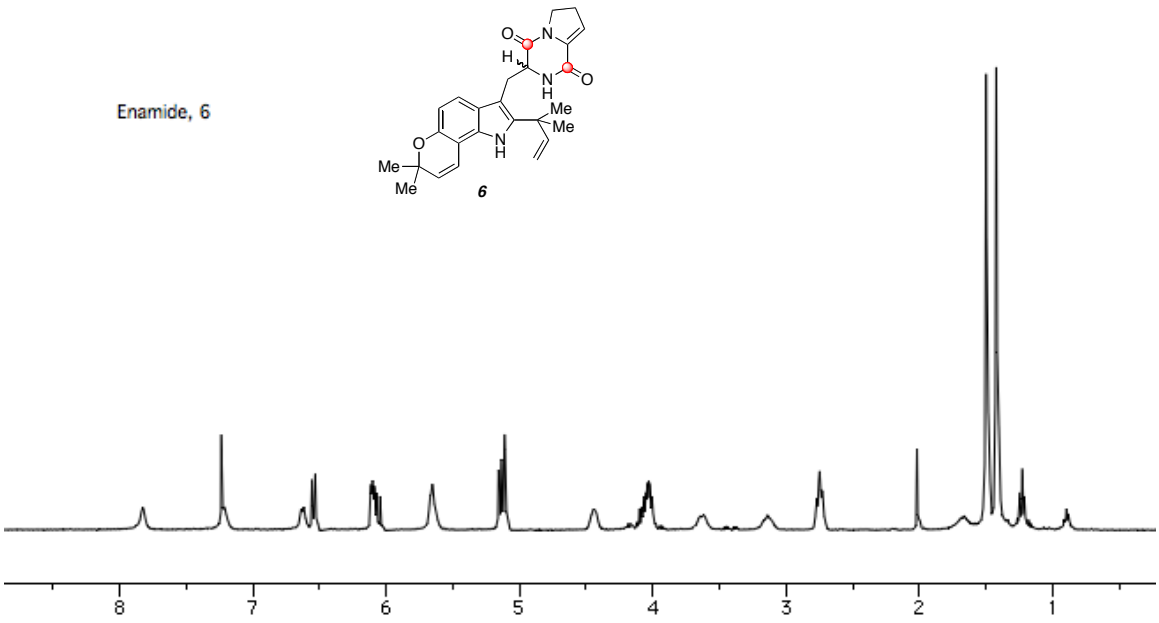
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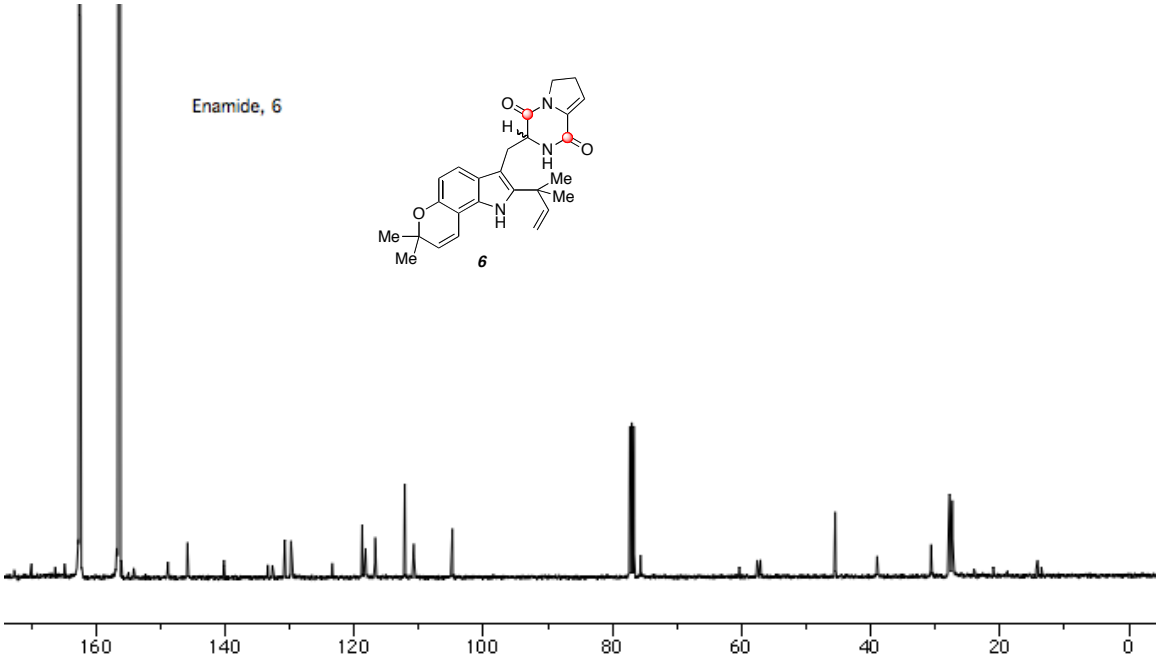
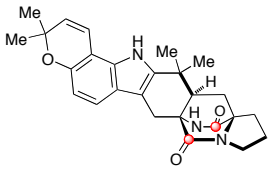
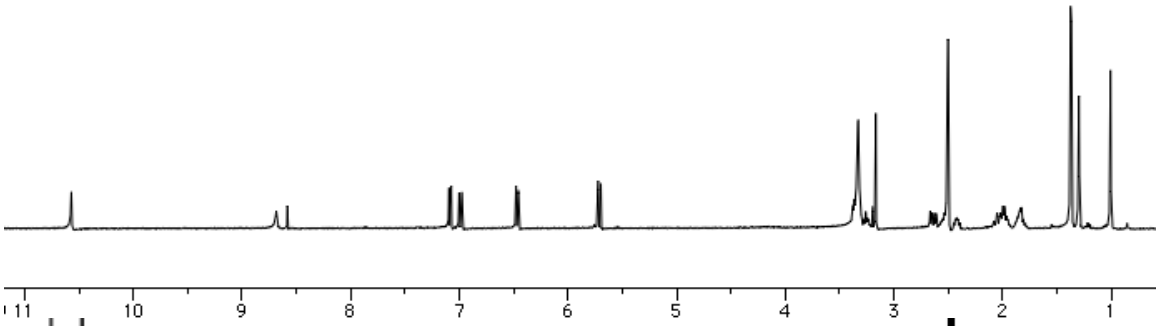
6
8
7
6
5
4
3
2
1

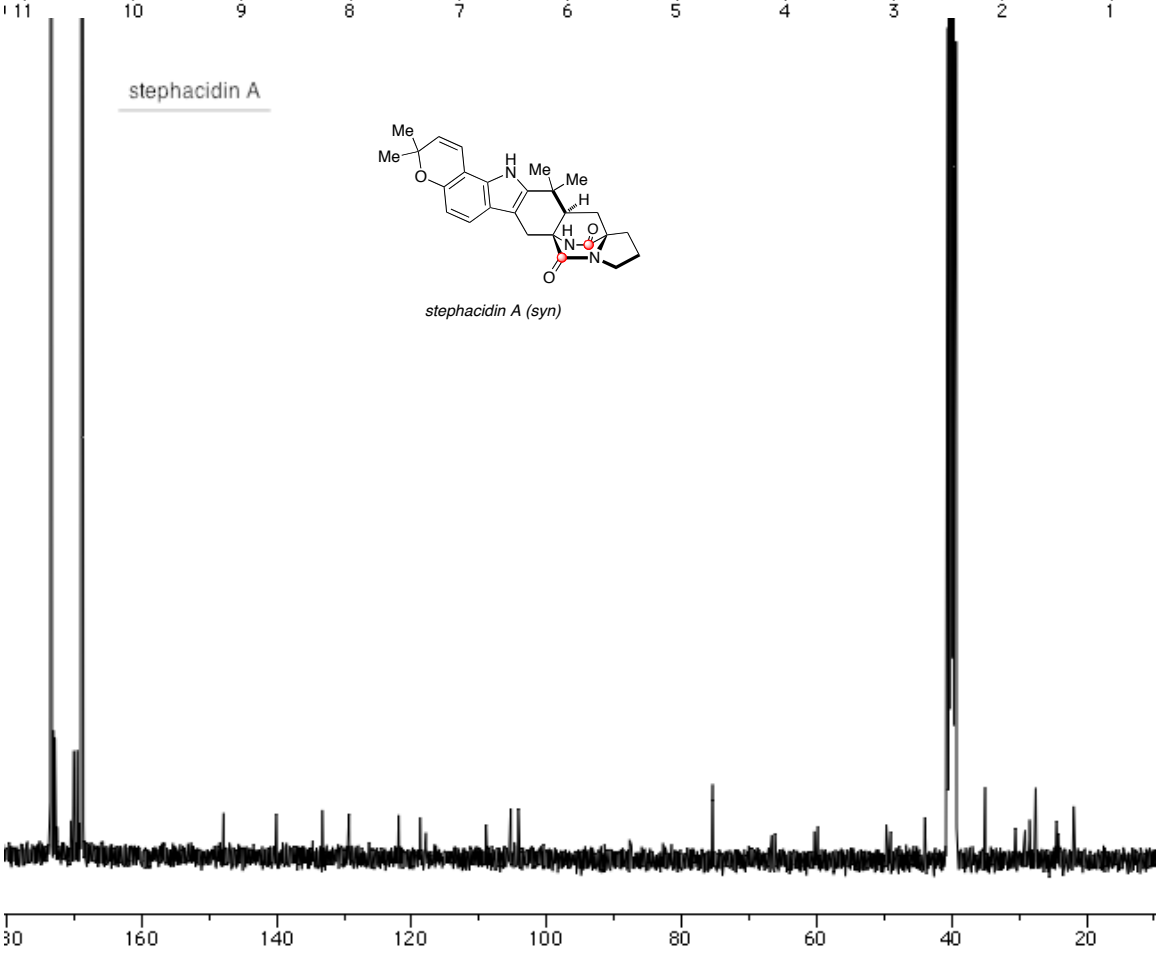

Enamide, 6
6
160
140
120
100
80
60
40
20
0

stephacidin A



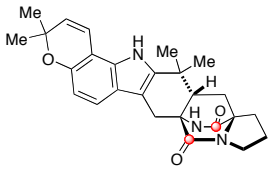
stephacidin A (syn)



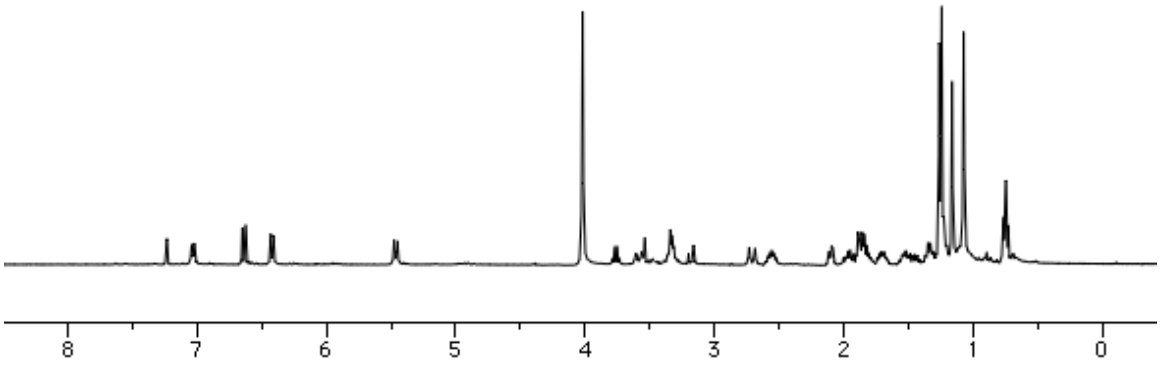


stephacidin A (syn)

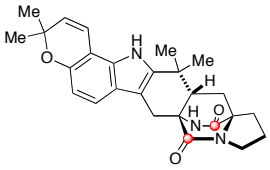
epi-stephacidin A



6-epi-stephacidin A (anti)



epi-stephacidin A



6-epi-stephacidin A (anti)