Supporting Information

Meeting key synthetic challenges in amanitin synthesis with a new cytotoxic analog: 5'-hydroxy-6'-deoxy-tryptathionine-(*R*)-sulfoxide.

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Materials and Methods

General Information. Reactions were performed in flame-dried borosilicate round-bottom flasks fitted with a rubber septum under a positive pressure of Ar, unless otherwise noted. Air/moisture sensitive liquids and solutions were transferred via syringe under positive Ar pressure. Controlled temperature reactions were performed using a mineral oil bath and a temperature controlled hot plate (Corning, PC 420D). Analytical thin layer chromatography (TLC) was performed using pre-coated Merck aluminum backed silica gel plates (Silica gel 60 F254). Visualization was achieved using ultraviolet light (254 nm) and chemical staining with silica gel impregnated with iodine, potassium permanganate, bromocresol green, and ninhydrin as appropriate. Flash column chromatography purification was performed using silica gel 60 (230-400 mesh, Silicycle, Quebec, Canada and 230-400 mesh, high purity 9385, Sigma Aldrich, Germany). Prior to use, silica gel was washed with four volumes of ammonium hydroxide/dichloromethane/ethanol (0.5:4:4.5) solution, filtered, and baked to neutralize small amounts of acid on silica gel. Solvents were dried according to standard methods.[1] Reagents and solvents were purchased from Sigma-Aldrich, Novabiochem, Alfa Aesar, Acros Organics, AK Scientific Inc., Oakwood Chemical, TCI America and used without further purification unless noted otherwise. Sep-Pak C18 cartridges were purchased from Waters (Sep-Pak C18 35 cc Vac Cartridge, 10 g, 55-105 µm). Tryptathionine containing compounds were quantified using tryptathionine as a chromophore with the extinction coefficient of 10,000 $M^{-1}cm^{-1}$ (for compounds with sulfur atom in thioether oxidation state) and 12,600 $M^{-1}cm^{-1}$ (for compounds with sulfur atom in sulfoxide or sulfone oxidation state) at λ_{max} (in MeOH or H_2O). Typically, quantities >5 nmol are accurately quantified by UV-Vis spectroscopy using a 0.5-mL cuvette.

Instrumentation. ^{1}H, ^{19}F, ^{13}C, and 2D NMR spectra were recorded on Bruker Avance 300 (300 MHz), Bruker Avance 400dir (400 MHz), Bruker Avance 400inv (400 MHz), and Bruker Avance 600-CRP (600 MHz) spectrometers. Chemical shifts (δ) are reported in ppm and referenced to the appropriate residual solvent peaks (acetone-D_6, CD_3CN, CD_2Cl_2, or $CDCl_3$, DMSO-D_6). Low-resolution mass spectrometry (LRMS) in Electrospray Ionization (ESI) mode was obtained using Waters ZQ mass spectrometer equipped with ESCI ion source and Waters 2695 HPLC. High-resolution mass spectrometry (HRMS) in ESI mode was obtained using Water/Micromass LCT-TOF mass spectrometer equipped with ESI ion source. Circular dichroism (CD) spectra were obtained using JASCO J-815 CD Spectrometer. UV-Vis spectroscopy was performed using Cary5000 Spectrophotometer. All HPLC chromatograms were generated on an Agilent 1100 system equipped with an auto injector and a diode array detector. Analytical injections were performed using an Agilent Eclipse XDB C-18, 5 µm (4.6 x 250 mm) column with a flow rate of 2 mL/min. In cases of closely-eluting peaks, integration was performed with a standard data analysis software package by drawing a line between two peaks (without peak correction).

List of HPLC gradients

HPLC **gradient A**: 2 mL/min; solv. A = 0.1% formic acid (FA) H_2O and solv. B = 0.1% FA ACN.

Time (min)	% B (0.1% FA ACN)
0	5
18	50
21	100
26	100
31	5

HPLC **gradient B**: 2 mL/min; solv. A = 0.1% FA H_2O and solv. B = 0.1% FA ACN.

Time (min)	% B (0.1% FA ACN)
0	5
27	18
30	100
34	100
36	5

HPLC **gradient C**: 2 mL/min; solv. A = 0.1% FA H_2O and solv. B = 0.1% FA ACN.

Time (min)	% B (0.1% FA ACN)
0	5
21	18
34	30
39	100
43	100

HPLC **gradient D:** 2 mL/min; solv. A = 0.1% FA H_2O and solv. B = 0.1% FA ACN.

Time (min)	% B (0.1% FA ACN)
0	10
25	13
28	13
30	20
36	35
40	100
42	100

HPLC **gradient E**: 2 mL/min; solv. A = 0.1% FA H_2O and solv. B = 0.1% FA ACN.

Time (min)	% B (0.1% FA ACN)
0	10
25	15
28	15
30	20
33	100
37	100
40	10

HPLC **gradient F**: 2 mL/min; solv. A = 0.1% TFA H_2O and solv. B = 0.1% FA ACN.

Time (min)	% B (0.1% FA ACN)
0	5
26	20
27	21
30	100
33	100

HPLC **gradient G**: 2 mL/min; solv. A = 0.1% FA H_2O and solv. B = 0.1% FA ACN.

Time (min)	% B (0.1% FA ACN)
0	5
24	19
27	21
30	100
33	100

Molecular modeling: docking of (*R*)-5'-OH-6'-deoxy-amanitin, **2**

The crystal structure of target-bound α-amanitin was extracted from the RNA pol II co-crystal (Research Collaboratory for Structural Bioinformatics PDB ID: 6EXV) structure using PyMol.[2-4] Avogadro was used for displaying chemical structures and energy minimized conformers.[5-6] The structures were corrected for crystallographic errors using Avogadro software. The three dimensional geometry minimized structure of (*R*)-5'-OH-6'-deoxy-amanitin (**2**) was generated using Avogadro software;[5-6] further energy and geometry minimization was performed with AutoDock Vina molecular docking program.[7-8] AutoDock Tools was used to assign hydrogens, Gasteiger charges and rotatable bonds to the ligand. The ligand ((*R*)-5'-OH-6'-deoxy-amanitin, **2**) and the receptor (6EXV) pdbqt docking files were prepared using AutoDockTools software. Ligand docking into the binding site of 6EVX was performed with the AutoDock Vina software. A docking grid with dimensions 20 Å × 20 Å × 20 Å, centered at the 154.579, 142.371, 116.633 coordinates in *x*, *y* and *z* directions was used. The receptor (6EXV) was kept rigid during docking, maintaining flexibility of rotatable bonds in the ligand ((*R*)-5'-OH-6'-deoxy-amanitin, **2**). The docking predictions were generated by AutoDock Vina and the most energetically favorable pose of (*R*)-5'-OH-6'-deoxy-amanitin (**2**) was compared to the crystal structure pose of α-amanitin. Further assessment and visualization of the results was performed using PyMol.[2-4]

Geometry optimization of compounds **12** and **4**

The three dimensional energy minimized structures of compounds **12** and **4** were generated using Avogadro software.[5-6] Further energy and geometry optimization was performed using Gaussian software[9] with the B3LYP hybrid energy functional.[10] The initial optimization was performed using 6g basis set. The structures were then further geometry optimized using 6-31g(d) basis set. Final assessment and visualization of the results was performed using PyMol.[2-4]

Cell culture

Materials and methods: MEM_{α} cell culture medium, fetal bovine serum (FBS), 0.25% trypsin (containing 1.3 mM EDTA), 0.85% Trypan blue, and the antibiotic mixture Pen/Strep (10K U/mL penicillin, 10K mg/mL streptomycin) were purchased from Gibco. Cell culture plastic ware was obtained from Corning or Falcon. Cells were cultured at 37 °C in a humidified chamber with 5% CO_2. CellTiter 96® AQ_{ueous} One Solution cell proliferation assay (MTS) reagent was purchased from Promega. Absorbance measurements of the 96-well plates were obtained using a Beckman-Coulter DTX 880 multimode detector, equipped with an excitation filter of 490 nm. All experiments are carried out in a laminar flow culture cabinet, unless otherwise noted. Chinese hamster ovary (CHO) cells (ATCC CCL-61) were cultured in MEM_{α} medium supplemented with 10% (v/v) fetal bovine serum and 100 U/mL penicillin and 10 mg/mL streptomycin in a T-25 flask at 37 °C and 5% CO_2. Sub-cultivation ratio of 1:5 was used to split cells at the point of 70-80% confluency.

In vitro cell proliferation assay

To assay cell viability, CHO cells were trypsinized and seeded in 96-well plates (3,000 cells/well). The cells were allowed to form a monolayer over a period of 24 hours. The compounds analyzed (α-amanitin, thioether-5'-OH-6'-deoxy-ama , (*R*)-sulfoxide-5'-OH-6'-deoxy-ama , (*S*)-sulfoxide-5'-OH-6'-deoxy-ama , and sulfone-5'-OH-6'-deoxy-ama) were dissolved in MEM_{α} medium added to each well to achieve desired concentration. Media and blank controls were run in parallel and all readings were normalized against the controls. The cells were incubated at 37 °C for 72 hours in a humidified, 5% CO_2 atmosphere. Following a 72-h period, 20 µL of MTS reagent were added to each well and the cells were incubated for another 3 hours at 37 °C in a humidified, 5% CO_2 atmosphere. The absorbance reading was recorded at 490 nm using a 96-well plate reader. IC_{50} (drug concentration required to inhibit cell viability by 50%) values were calculated using GraphPad Prism 7 software with non-linear 3-parameter regression fit to the following equation: Y=Bottom + (Top-Bottom)/(1+10^((X-LogIC50))) with Hill slope of -1.0. Experiments were performed in triplicate, and the error bars were calculated as the standard error of the mean.

In vitro RNA pol II transcription assay

Chemicals and Reagents
Primers were purchased from IDT. dNTP and rNTP were purchased from Thermo Fisher. *GoTaq* polymerase and HeLaScribe® Nuclear Extract in vitro Transcription System was purchased from Promega. *Taq* polymerase was purchased from NEB. [^{32}P] α-dGTP (3000Ci/mmol 10mCi/ml EasyTide) and [^{32}P] α-GTP (3000Ci/mmol 10mCi/ml EasyTide) were purchased from Perkin Elmer. QIAquick PCR purification kit was purchased from Qiagen. AcGFP1-N1 was a gift from Michael Davidson (Addgene plasmid # 54705; http://n2t.net/addgene:54705; RRID:Addgene_54705).

DNA Primer sequences

P1	AcGFP1.FOR.V4	CAGTCGACGGTACCGC
P2	AcGFP1.REV.V4	GCCCTCGAACTTCACCTC
P3	AcGFP1.FOR.V2	CGCGGGCCCGGGATCCAC
P4	AcGFP1.REV.V2	ACCTCGGCGCGCGACTTGT

Synthesis of the DNA template for runoff transcription
The DNA template for runoff transcription was synthesized by PCR with template pAcGFP-N1, and primers P1 and P2. To a final volume of 20 μL, 1 x GoTaq Buffer, 250 μM of each dNTP, 500 nM of each primer (P1 and P2), 10 pg/μL pAcGFP-N1 and 0.05 U/μL GoTaq were employed to thermocycle for 30 cycles (30 s at 95 °C, 30 s at 50 °C and 60 s at 72 °C, Bio-Rad). A 1 μL aliquot of the amplified solution was resolved with 6 x DNA loading dye in a 1% agarose gel containing 1% ethidium bromide and then visualized using GelDoc XR imager (Bio-Rad). Purification was completed via QIAquick PCR purification kit (Qiagen).

Synthesis of the radioactive chromatographic standard
The radioactive chromatographic standard was synthesized by PCR with template pAcGFP-N1, and primers P3 and P4. To a final volume of 50 μL, 1 x Thermopol Buffer, 200 μM of each dNTP, 500 nM of each primer (P3 and P4), 10 pg/μL pAcGFP-N1 and 0.02 U/μL Vent polymerase, 1 μL [^{32}P] α-dGTP were employed to thermocycle for 30 cycles (30 s at 95 °C, 30 s at 62 °C and 30 s at 72 °C, Bio-Rad). A 1 μL aliquot of the amplified solution was resolved with 6 x DNA loading dye in 8% denaturing PAGE and then visualized by autoradiography via the Typhoon 9200 imager (Molecular namics-Amersham-GE). Purification was completed via QIAquick PCR purification kit (Qiagen).

Runoff transcription assay and statistical analysis
The runoff transcription assay was modified from HeLaScribe® Nuclear Extract in vitro Transcription System (Promega). A 1.35X master mixture was formulated with 1.35X HeLa Nuclear Extract Transcription Buffer, 4.05 mM $MgCl_2$, 540 μM rATP, 540 μM rCTP, 540 μM rUTP and 21.6 μM rGTP, 5.4 ng/μL DNA template and 2-4 μL [^{32}P] α-GTP, depending on the specific activity on the day of experimentation. To a final volume of 8 μL, 1 X master mixture, 0 - 300 nM aqueous solutions containing α-amanitin or amanitin analogs (**1**, **2**, **11**-**13**), 0.32 U/μL HeLaScribe® Nuclear Extract were combined to transcribe for 60 min. The reaction was quenched by 56 μL HeLa Extract Stop Solution, followed by phenol-chloroform extraction and EtOH precipitation. The pellet was resolved by 8% denaturing PAGE and then visualized by

autoradiography via the Typhoon 9200 imager (Molecular dynamics-Amersham-GE). Dosimetry was calculated by ImageJ,[11] and the 3 parameter logistic fit was completed by Origin 2019 (OriginLab). For inhibitor I that demonstrates transcription activity E with half inhibitory concentration IC_{50},

$$E = \frac{IC_{50}}{IC_{50} + [I]} \quad (1)$$

This assumes symmetry around IC_{50} (asymmetry factor $S = 1$) and no cooperativity (Hill's slope $H = -1$). All data were presented in mean ± standard deviation ($n = 3$).

Summarized transcription activities of amanitin analogs with IC_{50} curves

Table S1. Transcription activities of HeLa nuclear extract in varying amanitin analog concentrations.

Concentration (nM)	Transcription activity E (%)				
	(*R*)-5'-OH-6'-deoxy-ama (**2**)	S-deoxy-5'-OH-6'-deoxy-ama (**11**)	(*S*)-5'-OH-6'-deoxy-ama (**12**)	SO_2-5'-OH-6'-deoxy-ama (**13**)	α-ama (**1**)
0.2	100 ± 18	-	-	-	-
0.8	89 ± 17	-	-	-	-
1.0	-	-	-	-	85 ± 2
3.0	-	-	-	-	69 ± 9
3.4	-	-	-	105 ± 17	-
3.7	-	96 ± 17	109 ± 29	-	-
4.0	76 ± 7	-	-	-	-
10.00	-	-	-	92 ± 4	48 ± 10
11.00	-	93 ± 7	112 ± 13	-	-
20.00	48 ± 4	-	-	-	-
30.00	-	-	-	77 ± 14	23 ± 3
33.00	-	65 ± 9	97 ± 16	-	-
91.00	-	-	-	52 ± 3	-
100.000	22 ± 14	41 ± 9	93 ± 6	-	9 ± 4
272.000	-	-	-	27 ± 2	-
300.000	-	25 ± 9	66 ± 7	-	-
IC_{50} (nM)	18 ± 1.5	79 ± 10	710 ± 150	100 ± 2.2	7.9 ± 0.9
R^2	0.963	0.973	0.842	0.999	0.991

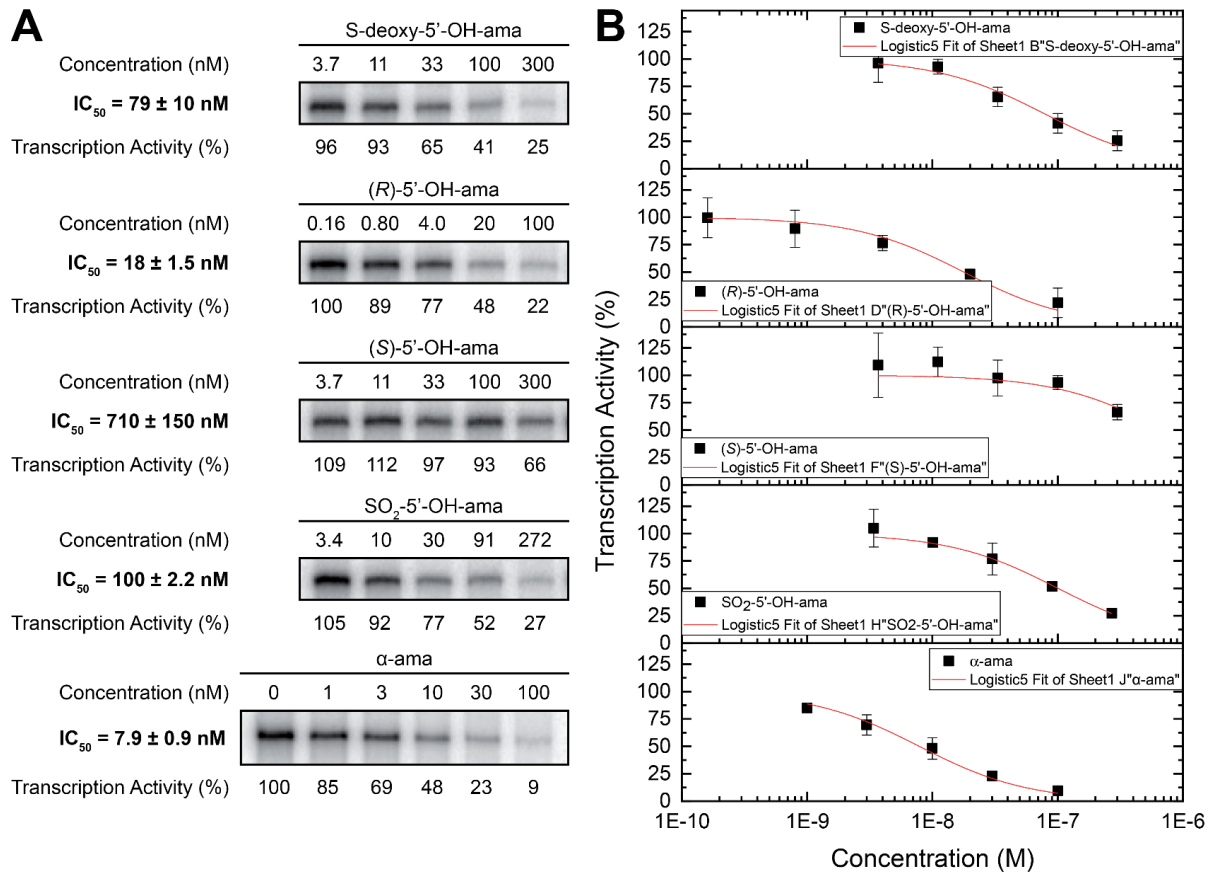
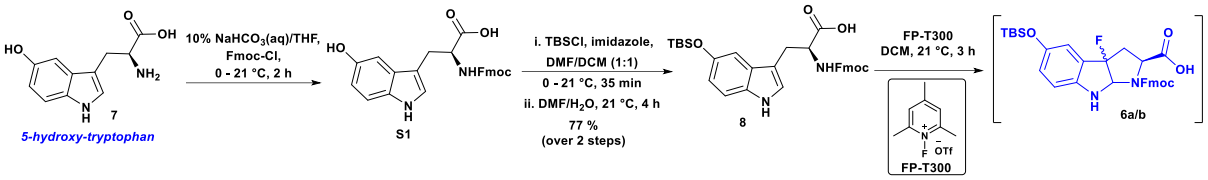
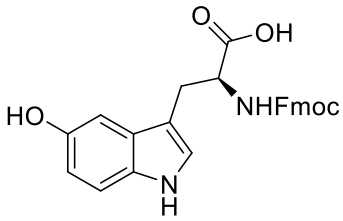


Figure S1. Characteristic inhibition of transcription by α-amanitin and its analogs under varied concentrations (n = 3). **(A)** [^{32}P]-autoradiogram of runoff transcription assay resolved by 8% denaturing PAGE. **(B)** IC_{50} curves of α-amanitin and its analogs. 5'OH-ama refers to the cpds investigated here that lack a 6'OH.

Synthesis and characterization of *N*$^{\alpha}$Fmoc-5'-OH-Trp-OH compound **S1**



Scheme S1. Synthesis of *N*$^{\alpha}$Fmoc-FPI(5'-OTBS)-OH from commercial 5-hydroxy-tryptophan.



Chemical Formula: C26H22N2O5
Exact Mass: 442.15
Molecular Weight: 442.47

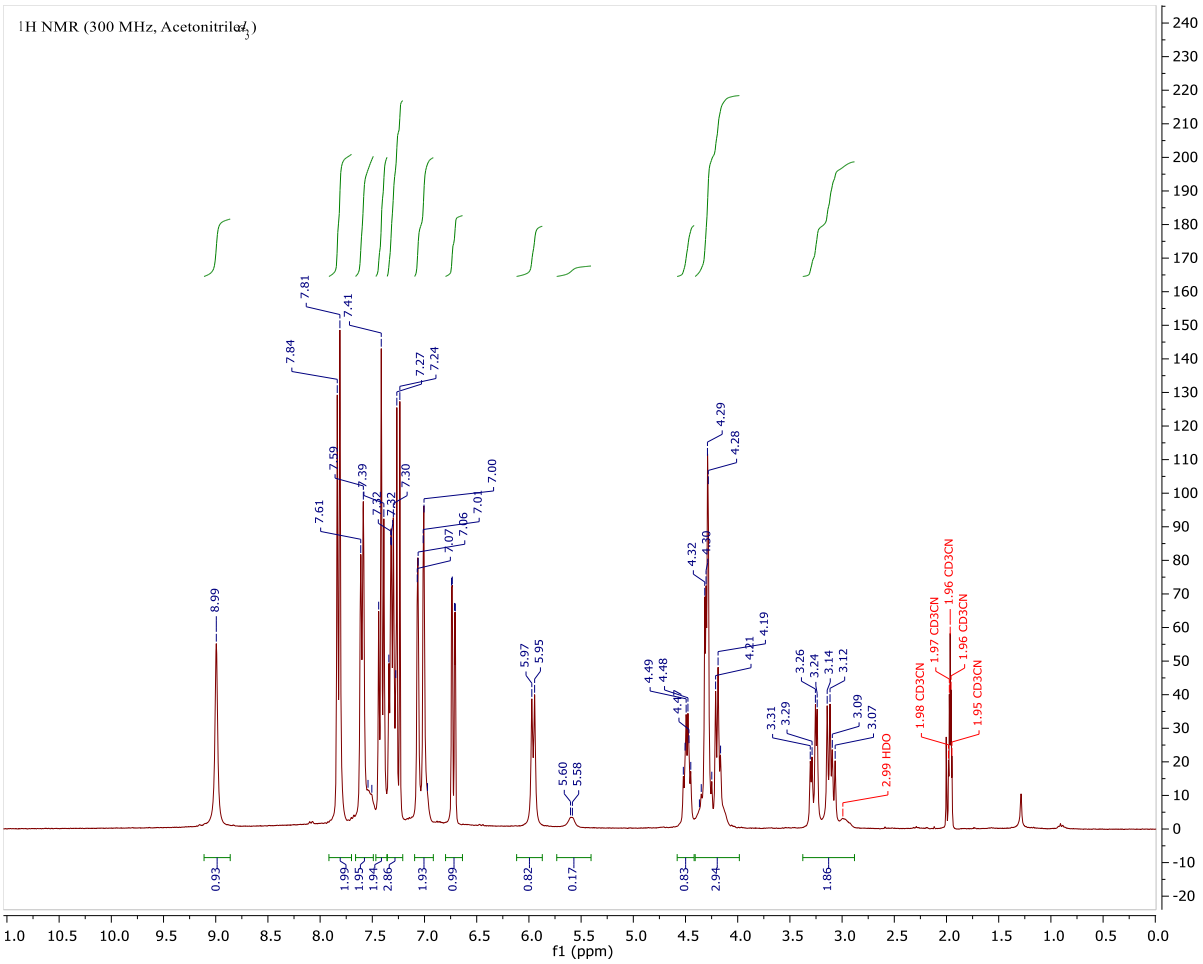
(S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(5-hydroxy-1H-indol-3-yl)propanoic acid (S1). A round bottom flask was charged with 5-hydroxy-tryptophan **7** (1.06 eq., 4.00 g, 18.2 mmol) in 10% $NaHCO_3$(aq)/THF (120 mL/40 mL). The light brown solution was cooled to 0 °C in an ice bath and fluorenylmethyloxycarbonyl chloride (1 eq., 4.42 g, 17.1 mmol) dissolved in 40 mL of THF was added dropwise over 15 mins. The reaction mixture was stirred at 0 °C for 30 mins and then warmed to 21 °C and stirred for additional 1.5 hours. The reaction mixture was concentrated under reduced pressure to remove THF. The brown, clear reaction mixture was acidified to pH 2 with 1 M HCl (aq) and extracted with EtOAc (4x100 mL). The organic layer was dried with $MgSO_4$, filtered and concentrated under reduced pressure to produce brown solid. The crude solid was immediately purified using silica gel flash column chromatography (gradient elution, 95.9:4:0.1 to 92.9:7:0.1 DCM/MeOH/acetic acid) to obtain *N*$^{\alpha}$Fmoc-5'-OH-Trp-OH **S1** (6.81 g, 15.4 mmol) as a beige solid in 90% yield.

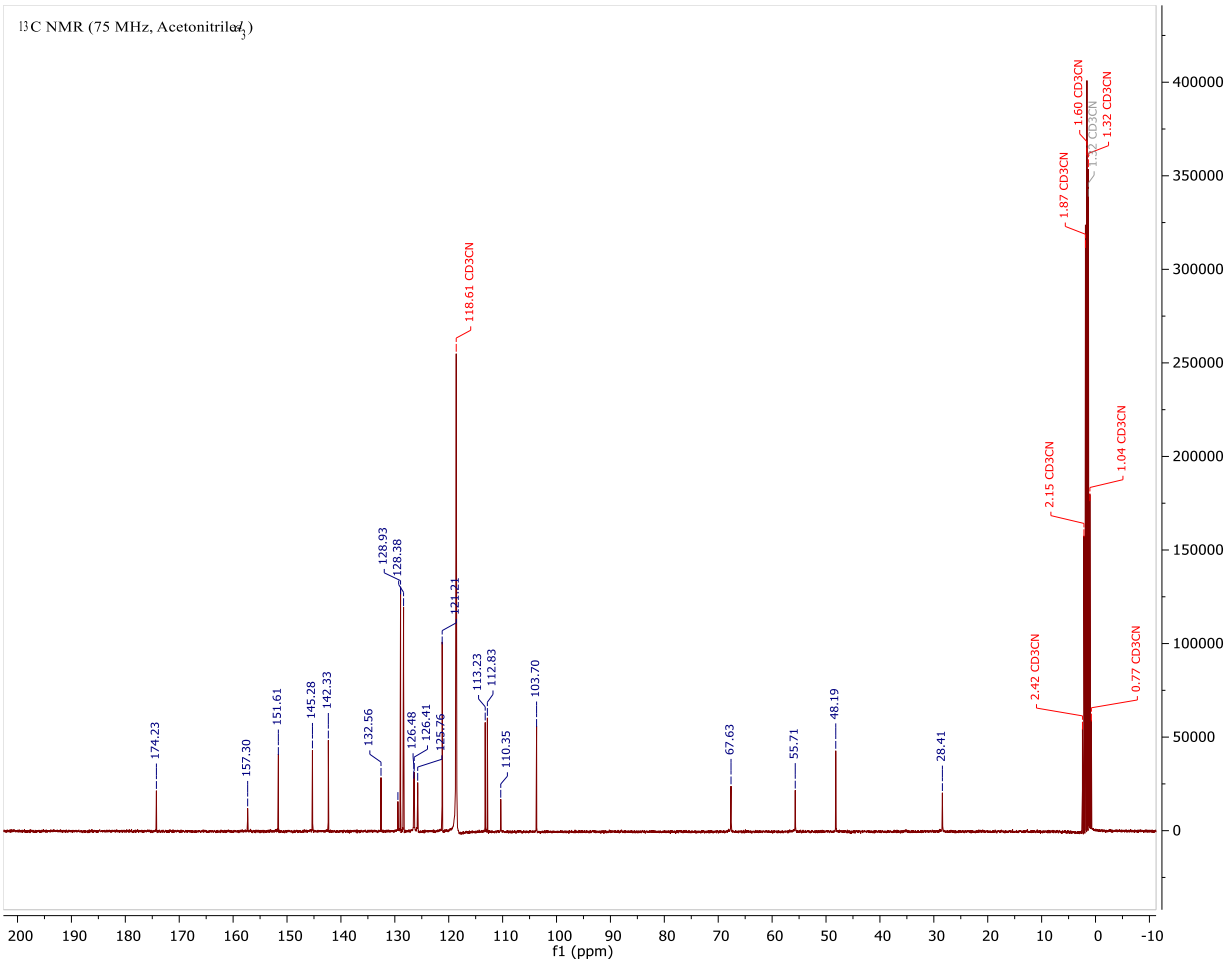
^{1}H NMR (300 MHz, Acetonitrile-d_3) δ 8.99 (s, 1H), 7.82 (d, J = 7.6 Hz, 2H), 7.60 (d, J = 7.5 Hz, 2H), 7.41 (t, J = 7.5 Hz, 2H), 7.36 – 7.21 (m, 3H), 7.04 (dd, J = 17.0, 2.4 Hz, 2H), 6.72 (dd, J = 8.7, 2.3 Hz, 1H), 5.96 (d, J = 8.1 Hz, 1H), 4.49 (td, J = 8.1, 5.1 Hz, 1H), 4.41 – 3.98 (m, 3H), 3.38 – 2.88 (m, 2H).

^{13}C NMR (75 MHz, Acetonitrile-d_3) δ 28.41, 48.19, 55.71, 67.63, 103.70, 110.35, 112.83, 113.23, 121.21, 125.76, 126.41, 126.48, 128.38, 128.93, 129.43, 132.56, 142.33, 145.28, 151.61, 157.30, 174.23.

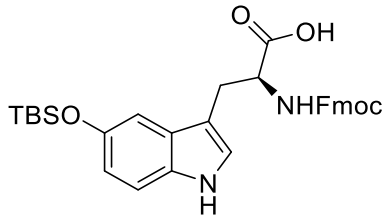
HRMS (ESI-TOF, m/z): $[M+Na]^+$ found 465.1424; calc. 465.1426 for $C_{26}H_{22}N_2O_5Na$.

TLC (DCM:MeOH:acetic acid 92.9:7:0.1 v/v/v): R_f = 0.1 (UV, I_2, bromocresol green).


1H NMR (300 MHz, Acetonitrile-d3)
8.99
7.84
7.81
7.61
7.59
7.41
7.39
7.32
7.32
7.30
7.27
7.24
7.07
7.06
7.01
7.00
5.97
5.95
5.60
5.58
4.49
4.48
4.32
4.30
4.29
4.28
4.21
4.19
3.31
3.29
3.26
3.24
3.14
3.12
3.09
3.07
2.99 HDO
1.98 CD3CN
1.97 CD3CN
1.96 CD3CN
1.96 CD3CN
1.95 CD3CN
0.93
1.99
1.95
1.94
2.86
1.93
0.99
0.82
0.17
0.83
2.94
1.86
f1 (ppm)


13C NMR (75 MHz, Acetonitrile-d3)
174.23
157.30
151.61
145.28
142.33
132.56
128.93
128.38
126.48
126.41
125.76
121.21
118.61 CD3CN
113.23
112.83
110.35
103.70
67.63
55.71
48.19
28.41
2.42 CD3CN
2.15 CD3CN
1.87 CD3CN
1.60 CD3CN
1.32 CD3CN
1.04 CD3CN
0.77 CD3CN
f1 (ppm)

Synthesis and characterization of compound **8**



Chemical Formula: $C_{32}H_{36}N_2O_5Si$
Exact Mass: 556.2393
Molecular Weight: 556.7340

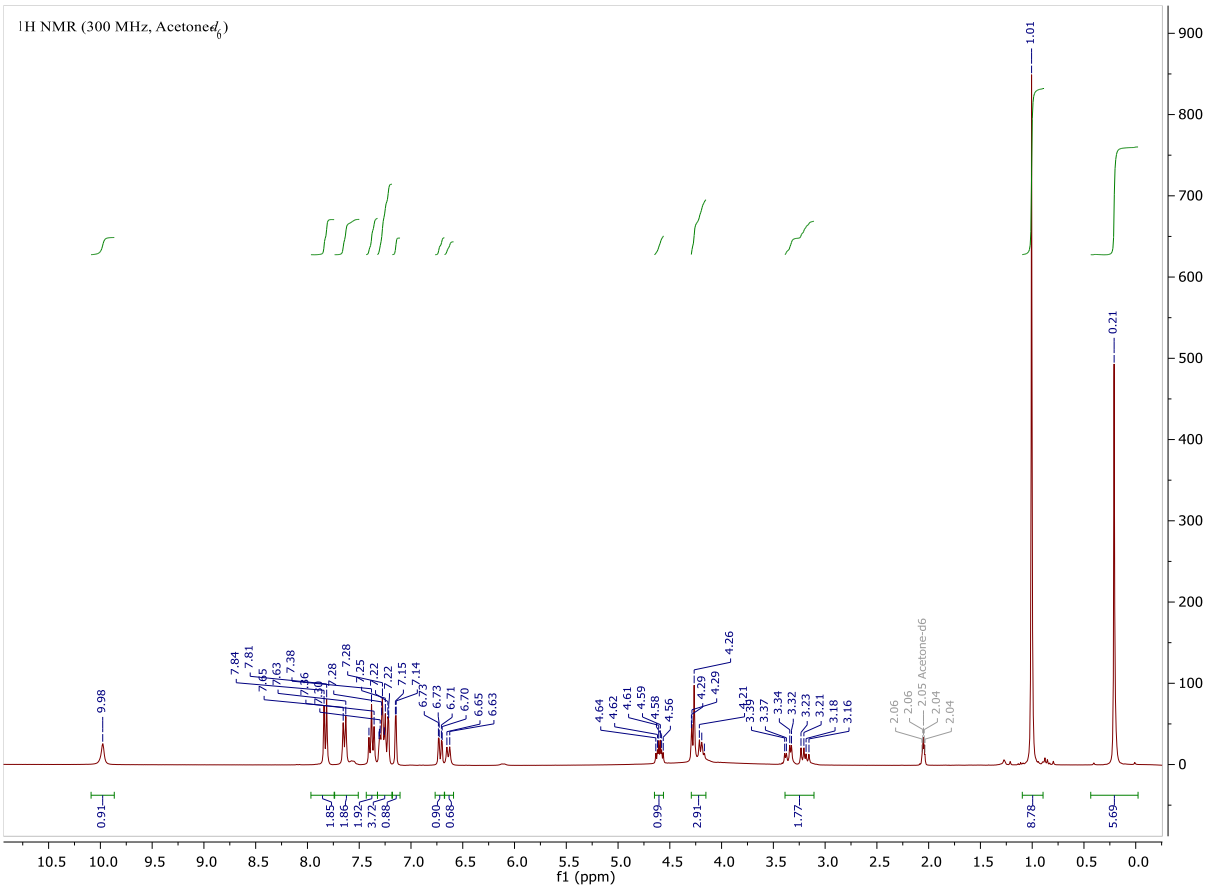
(S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(5-((tert-butyldimethylsilyl)oxy)-1H-indol-3-yl)propanoic acid (8). A flame-dried round bottom flask under positive Ar atmosphere was charged with *N*$^{\alpha}$Fmoc-5'-OH-Trp-OH **S1** (1 eq., 2.00 g, 4.52 mmol) followed by anhydrous dichloromethane (DCM) (35 mL), anhydrous dimethylformamide (DMF, 35 mL), and imidazole (3.9 eq., 1.20 g, 17.6 mmol). The reaction contents were cooled to 0 °C and *tert*-butyldimethylsilyl chloride (4.0 eq., 2.73 g, 18.1 mmol) was added and the reaction was warmed to 21 °C over 35 mins. The reaction mixture (light beige and clear) was then diluted to 200 mL with DCM and washed with pH 3.5 HCl (aq) (4x60 mL), followed by brine (1x60 mL). The organic layer was dried with $MgSO_4$ and concentrated in vacuo to ~2 mL (DMF). The crude residue was further diluted with DMF to 11 mL and treated with 500 µL of H_2O. The reaction mixture was stirred at 21 °C for 4 hours and the product was then precipitated with hexanes. The product was filtered and washed with hexanes to obtain *N*$^{\alpha}$Fmoc-5'-OTBS-Trp-OH **8** (2.16 g, 3.89 mmol, 86% yield) as a white solid.

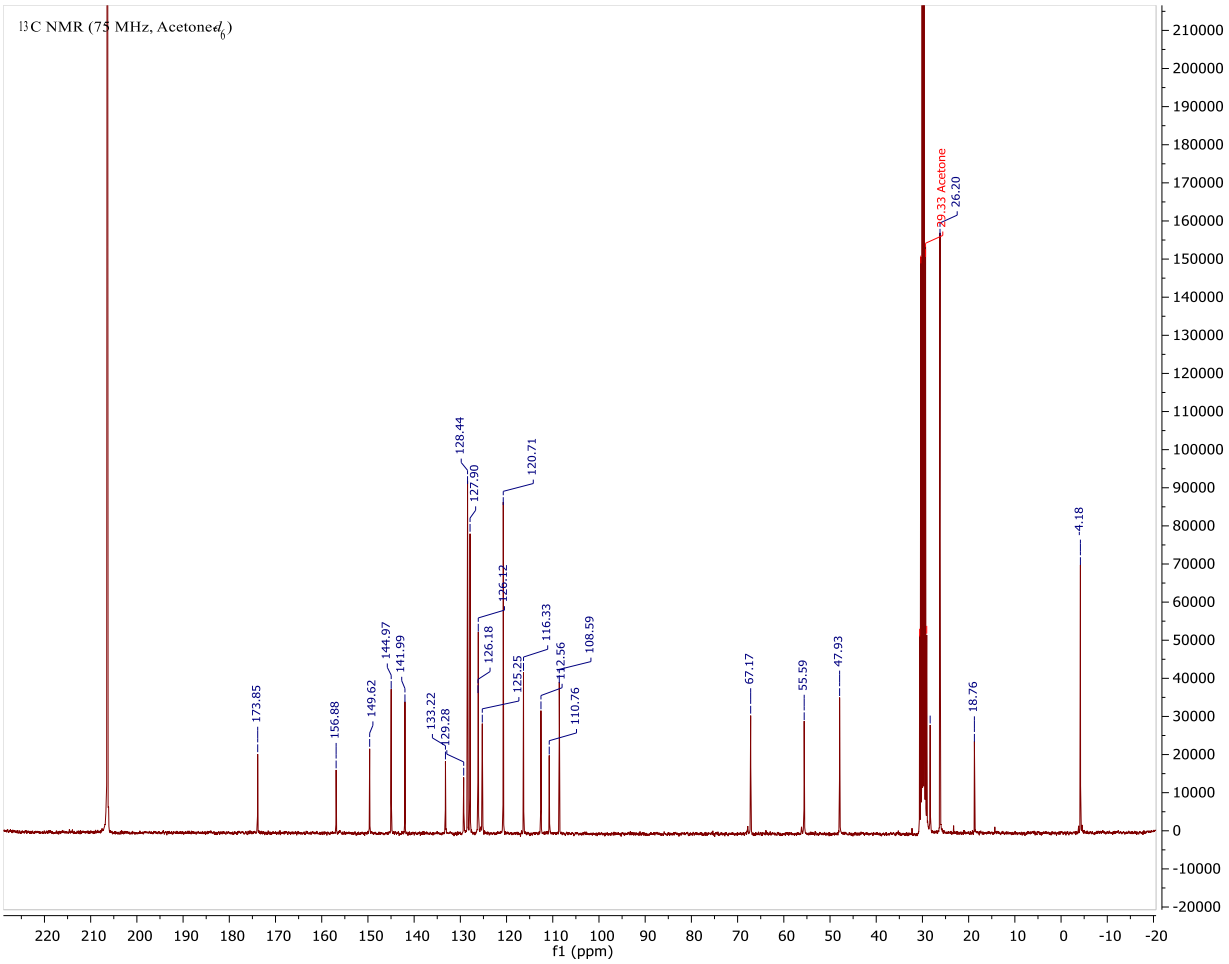
^{1}H NMR (300 MHz, Acetone-d_6) δ 9.98 (s, 1H), 7.83 (d, *J* = 7.5 Hz, 2H), 7.64 (d, *J* = 7.5 Hz, 2H), 7.38 (t, *J* = 7.4 Hz, 2H), 7.26 (ddd, *J* = 18.1, 8.7, 2.8 Hz, 4H), 7.15 (d, *J* = 2.3 Hz, 1H), 6.72 (dd, *J* = 8.6, 2.3 Hz, 1H), 6.64 (d, *J* = 8.3 Hz, 1H), 4.60 (td, *J* = 8.4, 4.8 Hz, 1H), 4.29 – 4.15 (m, 3H), 3.39 – 3.11 (m, 2H), 1.01 (s, 9H), 0.21 (s, 6H).

^{13}C NMR (75 MHz, Acetone-d_6) δ -4.18, 18.76, 26.20, 28.33, 47.93, 55.59, 67.17, 108.59, 110.76, 112.56, 116.33, 120.71, 125.25, 126.12, 126.18, 127.90, 128.44, 129.28, 133.22, 141.99, 144.97, 149.62, 156.88, 173.85.

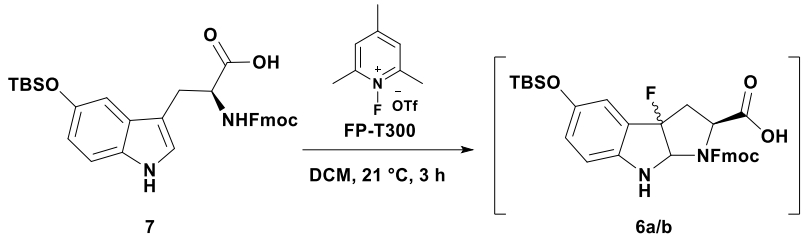
HRMS (ESI-TOF, m/z): $[M+Na]^+$ found 579.2285; calc. 579.2291 for $C_{32}H_{36}N_2O_5NaSi$.

TLC (DCM:MeOH 9:1 v/v): R_f = 0.4 (UV, I_2, bromocresol green).


1H NMR (300 MHz, Acetone-d6)
9.98
7.84
7.81
7.63
7.38
7.28
7.25
7.22
7.15
7.14
6.73
6.71
6.70
6.65
6.63
4.64
4.62
4.61
4.59
4.58
4.56
4.29
4.26
4.21
3.39
3.37
3.34
3.32
3.23
3.21
3.18
3.16
2.06
2.05 Acetone-d6
2.04
1.01
0.21
0.91
1.85
1.86
1.92
3.72
0.88
0.90
0.68
0.99
2.91
1.77
8.78
5.69
f1 (ppm)


13C NMR (75 MHz, Acetone-d_6)
173.85
156.88
149.62
144.97
141.99
133.22
129.28
128.44
127.90
126.18
126.12
125.25
120.71
116.33
112.56
110.76
108.59
67.17
55.59
47.93
30.33 Acetone
26.20
18.76
-4.18
f1 (ppm)

Synthesis and characterization of compound **6a/b**

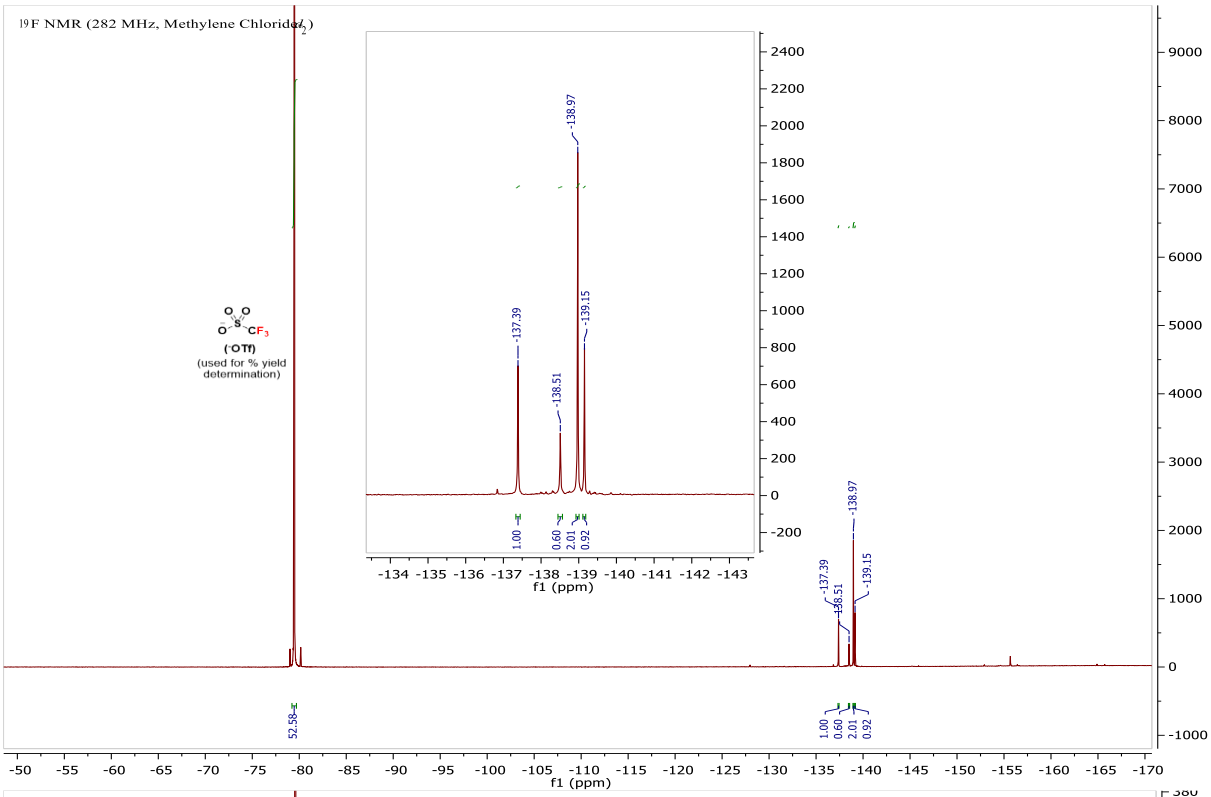


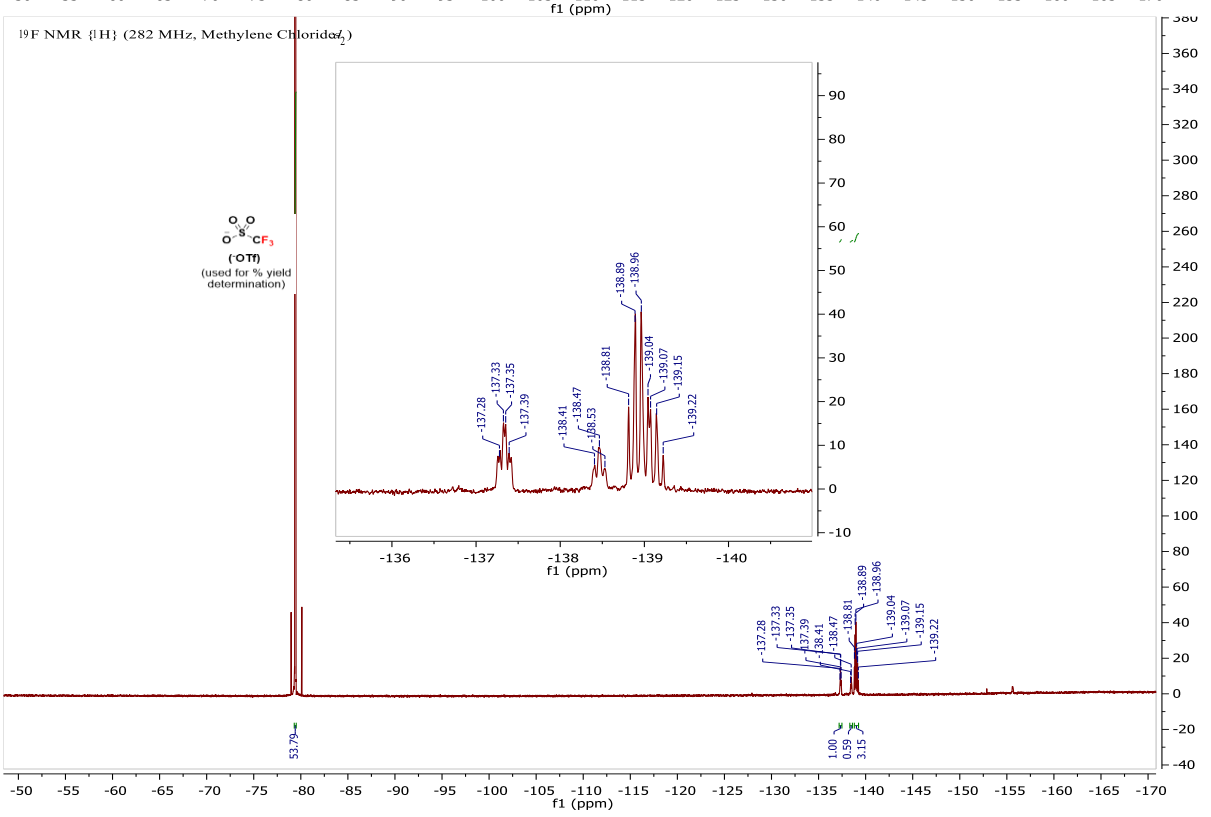
Synthesis of *syn-cis* and *anti-cis* N^{α}Fmoc-FPI(5'-OTBS)-OH (6a/b). A flame-dried round bottom flask under positive Ar atmosphere was charged with N^{α}Fmoc-5'-OTBS-Trp-OH **8** (1.0 equiv, 500 mg, 0.90 mmol) dissolved in 92 mL of anhydrous DCM. Compound **8** was then treated with *N*-fluoro-2,4,6-trimethylpyridinium triflate (FP-T300) (3.3 equiv, 857 mg, 2.97 mmol). The reaction contents were stirred at 21 °C for 3 hours and the reaction progress was monitored by TLC (9:1 DCM/MeOH). Upon completion, the reaction mixture (light brown, clear) was washed with pH 3.5 HCl (aq) (4x25 mL) to remove the excess fluorinating agent and the generated collidinium salt, dried with $MgSO_4$, filtered, and concentrated under reduced pressure to give **6a/b** as a light brown solid. The concentrate was used directly in the next step without further purification. The approximate reaction yield and diastereomeric distribution (d.r.) were determined by concentrating a small amount of the reaction mixture and subjecting it to ^{19}F-NMR. Four distinct peaks were observed in ^{19}F-NMR corresponding to two diastereomers (*syn-cis* and *anti-cis*), each existing as a set of two rotamer. The -C***F***$_3$ group of the triflate counterion (-79.5 ppm, derived from FP-T300) was used as an internal standard for yield determination, **6a/b** was obtained in ~87% yield. The d.r. of 1.8:1 (*syn-cis*:*anti:cis*) was obtained, determined based on the characteristic ^{19}F-peak shape previously reported for *syn-cis* and *anti-cis* diastereomers.[12]

^{19}F NMR (282 MHz, Methylene Chloride-d_2) δ -139.15 (*syn-cis*, minor rotamer), -138.97 (*syn-cis*, major rotamer), -138.51 (*anti-cis*, minor rotamer), -137.39(*anti-cis*, major rotamer).

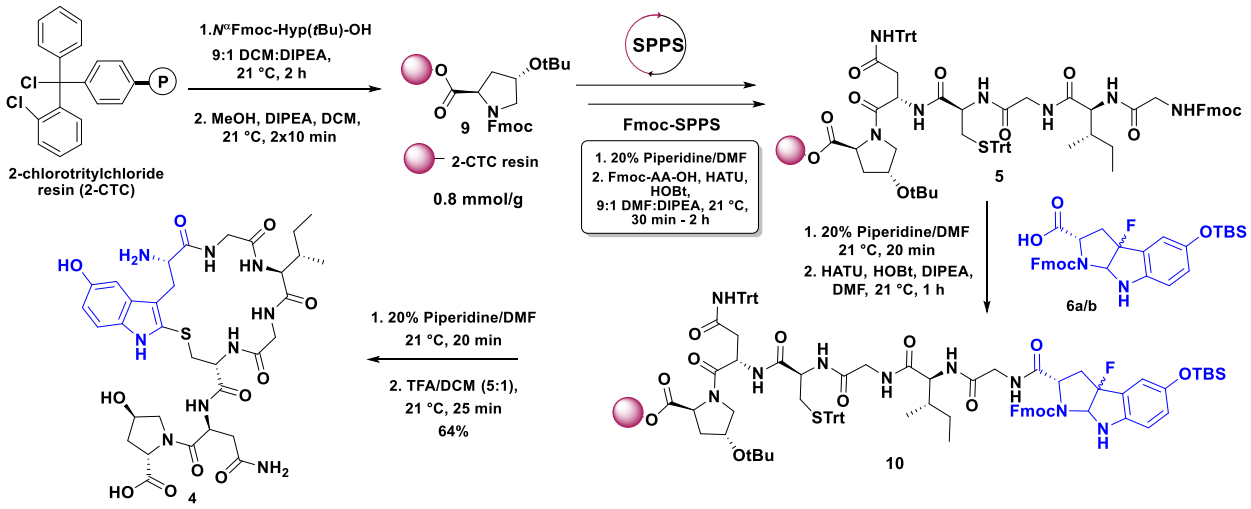
LRMS (ESI-TOF, m/z): $[M+Na]^+$ found 597.5; calc. 579.2 for $C_{32}H_{35}FN_2O_5NaSi$.

TLC (DCM:MeOH 9:1 v/v): R_f = 0.5 (UV, I_2, bromocresol green).


19F NMR (282 MHz, Methylene Chloride-d2)
(-OTf)
(used for % yield determination)
-137.39
-138.51
-138.97
-139.15
1.00
0.60
2.01
0.92
52.58
f1 (ppm)

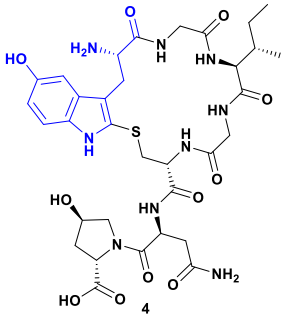

19F NMR {1H} (282 MHz, Methylene Chloride-d2)
(-OTf)
(used for % yield determination)
-137.28
-137.33
-137.35
-137.39
-138.41
-138.47
-138.53
-138.81
-138.89
-138.96
-139.04
-139.07
-139.15
-139.22
53.79
1.00
0.99
3.15
f1 (ppm)

Synthesis and characterization of S-deoxy-5'-OH-heptapeptide monocycle **4**



Scheme S2. Solid-phase synthesis of S-deoxy-5'-OH-heptapeptide monocycle **4**.

Solid phase synthesis of 2-CTC-Hyp(O*t*Bu)Asp(NTrt)Cys(STrt)GlyIleGly*N*HFmoc (**5**). 2-Chlorotrityl chloride resin was dried over P_2O_5 under reduced pressure for 17 hours before use. A peptide synthesis glass vessel (Chemglass, 100-mL) adapted with an Ar(g) flow inlet and a medium size frit filter was charged with the dried 2-chlorotrityl chloride resin (2 g, 1.2 mmol/g loading) and 25 mL of anhydrous DCM. The resin was agitated for 25 mins with a flow of Ar(g), the solvent was filtered and replaced with another 25 mL portion of anhydrous DCM and the resin was washed for 5 mins. The first amino acid loading was performed in the following manner: 3.2 mmol of *N*$^{\alpha}$Fmoc-Hyp(O*t*Bu)-OH were dissolved in 17 mL of 9:1 DMC:DIPEA. The reaction was run for 2 hours and the resin was washed with DCM (5 x 5mins x 25 mL). The resin was capped with 20 mL of 17:2:1 DCM:MeOH:DIPEA solution for 20 mins and then washed with DCM (5 x 5 mins x 25 mL) and DMF (5 x 5 mins x 25 mL). The Fmoc deprotection was performed using 20 mL of 20% piperidine/DMF solution for 20 mins, followed by another 5-minute treatment with 20 mL of 20% piperidine/DMF solution. The resin was washed with DMF and the dibenzofulvene-piperidine adduct was quantified by ultraviolet-visible (UV-Vis) spectroscopy. The absorption measurement was performed at 301 nm using extinction coefficient (ε) of 7800 $M^{-1}cm^{-1}$; loading of 0.8 mmol/g was obtained. The resin was then washed with DMF (5 x 5 mins x25mL) and the next amino acid was coupled. The coupling was performed with 4.1 eq. of *N*$^{\alpha}$Fmoc-Asp(NTrt)-OH, 4 eq. of HATU, and 4 eq. of HOBt in 18 mL of 9:1 DMF:DIPEA. The coupling cocktail was pre-activated for 5 mins prior to addition to the resin. The coupling reaction was run for 30 mins – 2 hours and the completion of the reaction was monitored using Kaiser test. Capping of unreacted amines was performed after every coupling step with 18 mL of 2:3 pyridine:acetic anhydride solution for 20 mins. The resin was then washed with DMF (5 x 5 mins x25mL) and Fmoc-deprotection and amino acid coupling steps were performed as previously described to obtain **2-CTC-Hyp(O*t*Bu)Asp(*N*Trt)Cys(STrt)GlyIleGly*N*HFmoc** (**5**).



4

Chemical Formula: $C_{33}H_{45}N_9O_{11}S$

Exact Mass: 775.30

Molecular Weight: 775.84

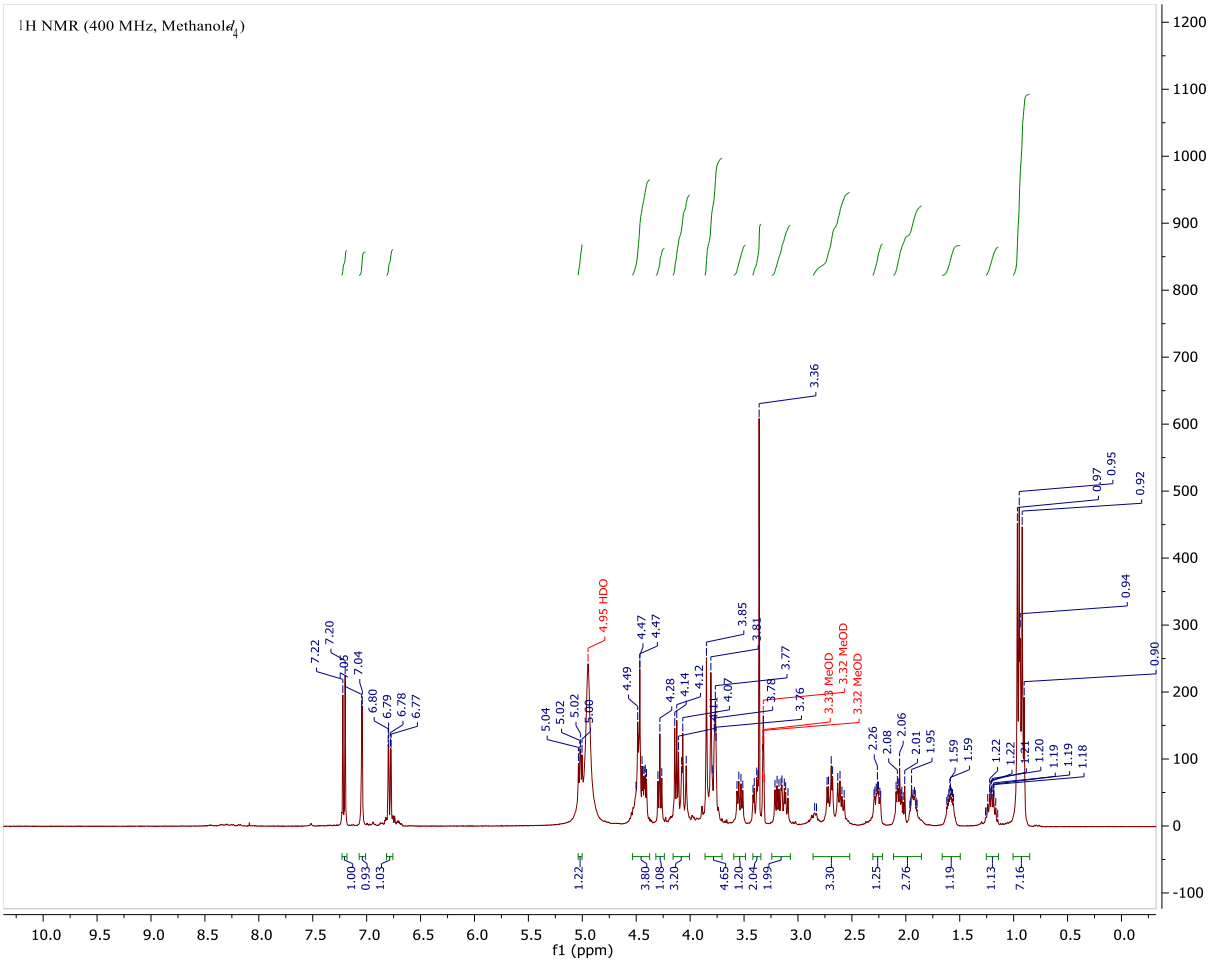
(2S,4R)-1-(((3R,9S,15S)-15-amino-9-((S)-sec-butyl)-18-hydroxy-5,8,11,14-tetraoxo-2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,21-hexadecahydro-[1]thia[4,7,10,13]tetraazacyclooctadecino-[18,17-b]indole-3-carbonyl)-L-asparaginyl)-4-hydroxypyrrolidine-2-carboxylic acid (**4**). *N*$^{\alpha}$Fmoc-FPI(5'-OTBS)-OH **6a/b** (3.3 eq., 274 mg, 0.477 mmol) was dissolved in 10 mL of DMF and combined with HOBt (10 eq., 1.59 mmol, 214 mg), DIPEA (6.5 eq., 1.03 mmol, 180 µL), and HATU (3.3 eq., 181 mg, 0.477 mmol). The coupling cocktail was pre-activated for 5 mins and added to a solid phase glass vessel containing the resin-loaded hexapeptide **2-CTC-Hyp(OtBu)Asp-(NTrt)Cys(STrt)GlyIleGlyNH$_2$** (1 eq., 0.159 mmol, based on the last Fmoc-deprotection step). The reaction was agitated using Ar flow for 1 hour at 21 °C. The reaction completion was confirmed by TLC analysis performed on a small amount of the product cleaved from the resin with 1:4 hexafluoroisopropanol:DCM. The resin was then washed with DMF (5 x 5 mins x10 mL). The Fmoc deprotection was performed using 10 mL of 20% piperidine/DMF solution for 20 mins and the resin was then washed with DMF (5 x 5 mins x10 mL) and DCM (5 x 3 mins x10 mL). The Savige-Fontana reaction was carried out in a 250-mL round bottom flask equipped with a stir-bar by adding 16 mL of DCM followed by 16 mL of trifluoroacetic acid to the resin (safety precaution: TFA is a strong acid, that can cause severe burns to the skin and is very volatile, take caution when handling). The resin was gently stirred with a stir-bar for 25 mins at 21 °C. Upon completion the amber-coloured clear reaction mixture was concentrated under reduced pressure. The reaction concentrate was re-dissolved in 16 mL of TFA:phenol:H_2O:TIPS (88:5:5:2, v:m:v:v) and gently stirred for 40 mins at 21 °C. The clear and amber-coloured reaction mixture was then concentrated under reduced pressure, the resin was filtered and washed with DCM (5x4minsx10mL) and TFA (1 x 3 mins x 6 mL). The combined washes were concentrated under reduced pressure to ~6 mL and the product was triturated with 250 mL of cold diethyl ether. The dried precipitate was purified using Sep-Pak C18 cartridge (gradient elution, 99.9:0.1 H_2O:formic acid to 89.9:10:0.1 H_2O/ACN/formic acid to 85.9:14:0.1 H_2O/ACN/formic acid) and the product containing fractions were lyophilized to obtain **4** (79 mg, 0.102 mmol, 64 % yield, calculated based on the last Fmoc-deprotection step) as a white solid.

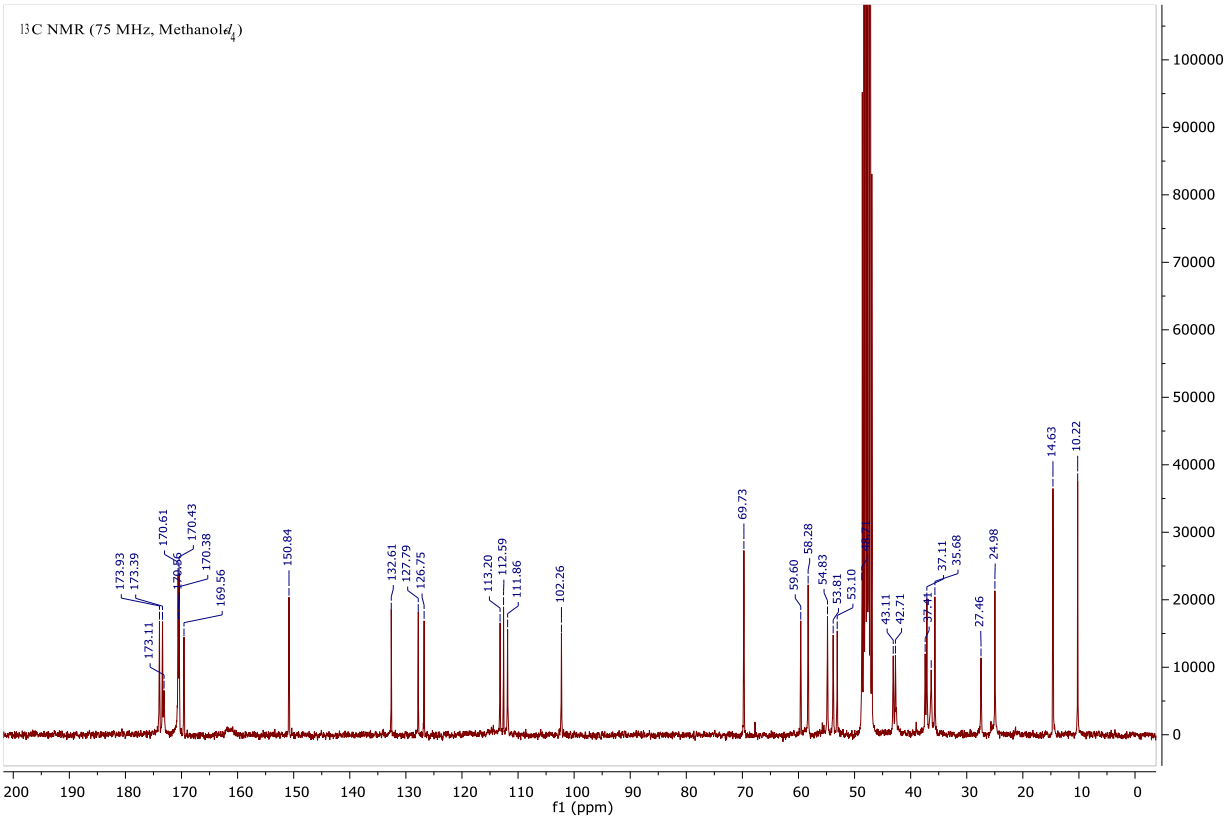
^{1}H NMR (400 MHz, Methanol-d_4) δ 7.21 (d, J = 8.8 Hz, 1H), 7.04 (d, J = 2.3 Hz, 1H), 6.79 (dd, J = 8.7, 2.2 Hz, 1H), 5.02 (dd, J = 8.3, 5.2 Hz, 1H), 4.53 – 4.38 (m, 4H), 4.28 (t, J = 7.2 Hz, 1H), 4.16 – 4.01 (m, 3H), 3.86 – 3.70 (m, 5H), 3.54 (dd, J = 14.8, 6.8 Hz, 1H), 3.42 – 3.34 (m, 2H), 3.15 (ddd, J = 25.7, 14.2, 8.1 Hz, 2H), 2.86 – 2.52 (m, 3H), 2.26 (ddd, J = 12.2, 8.1, 3.3 Hz, 1H), 1.99 (dddd, J = 45.9, 14.8, 7.5, 4.0 Hz, 3H), 1.66 – 1.50 (m, 1H), 1.25 – 1.14 (m, 1H), 1.01 – 0.85 (m, 7H).

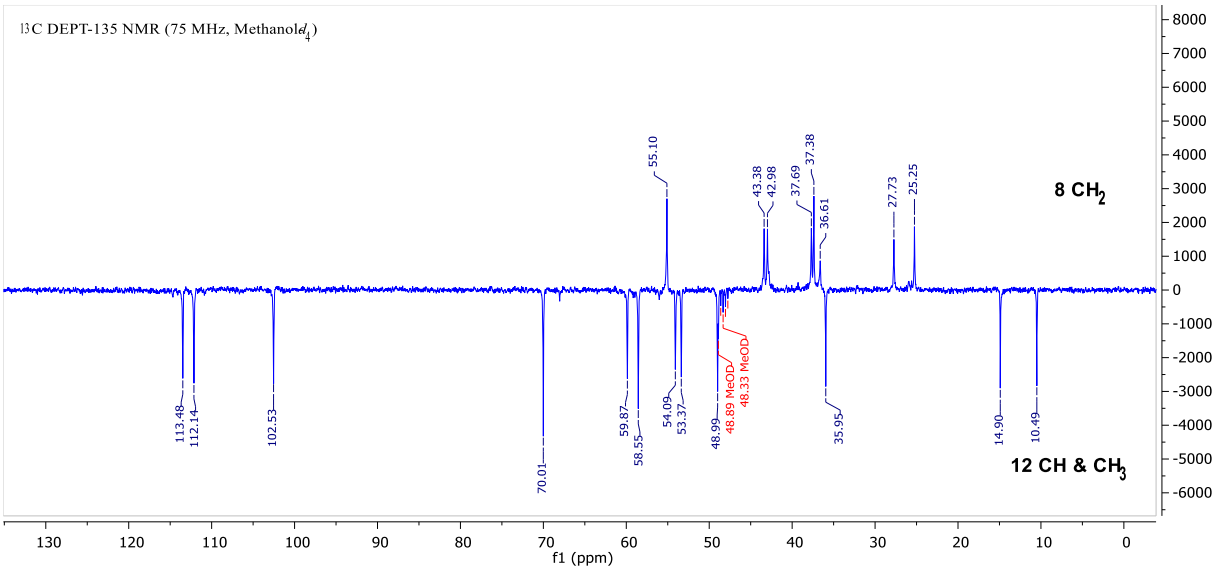
^{13}C NMR (75 MHz, Methanol-d_4) δ 10.22, 14.63, 24.98, 27.46, 35.68, 36.34, 37.11, 37.41, 42.71, 43.11, 48.71, 53.10, 53.81, 54.83, 58.28, 59.60, 69.73, 102.26, 111.86, 112.59, 113.20, 126.75, 127.79, 132.61, 150.84, 169.56, 170.38, 170.43, 170.56, 170.61, 173.11, 173.39, 173.93.

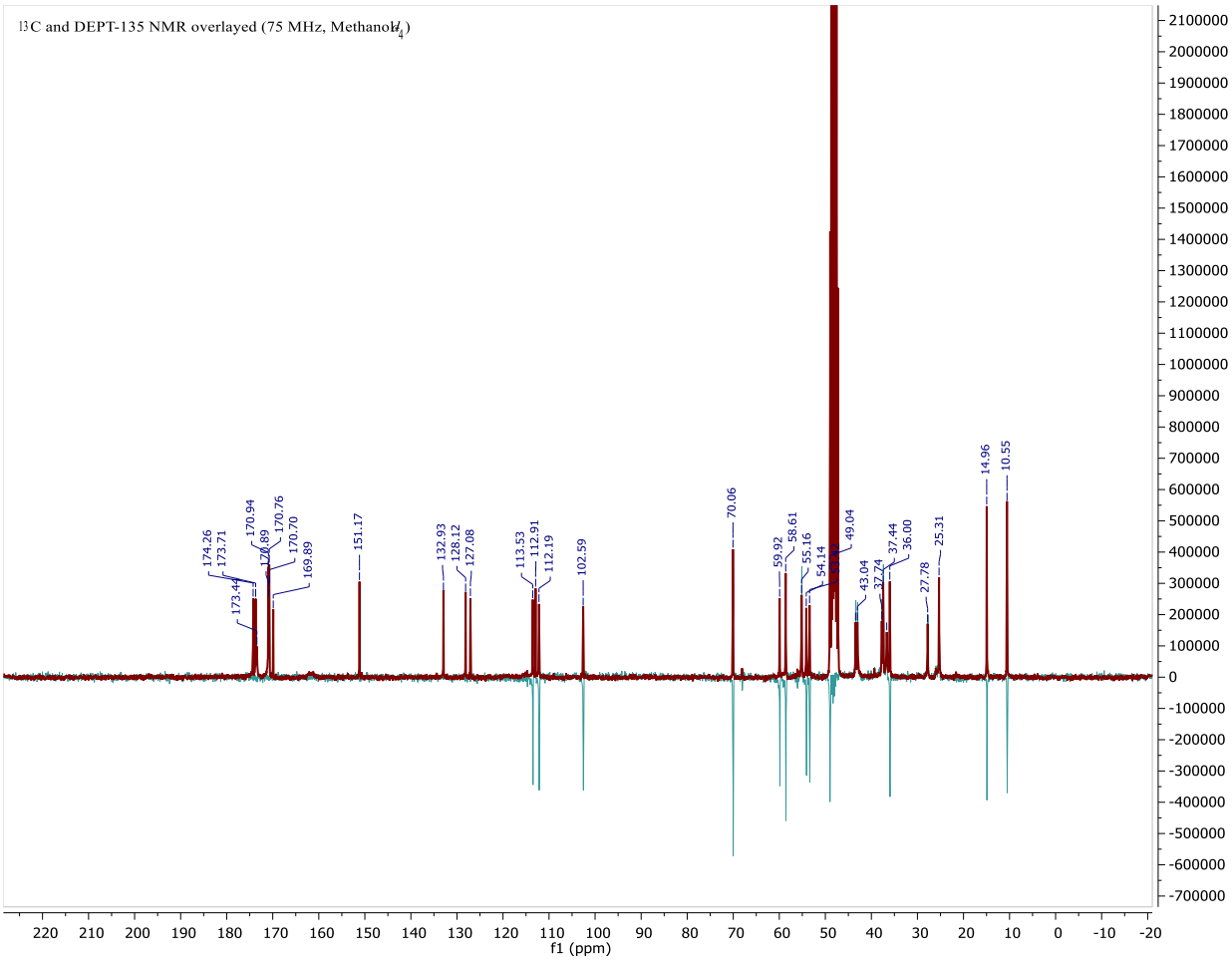
HRMS (ESI-TOF, m/z): $[M+Na]^+$ found 776.3032; calc. 776.3032 for $C_{33}H_{46}N_9O_{11}S$.

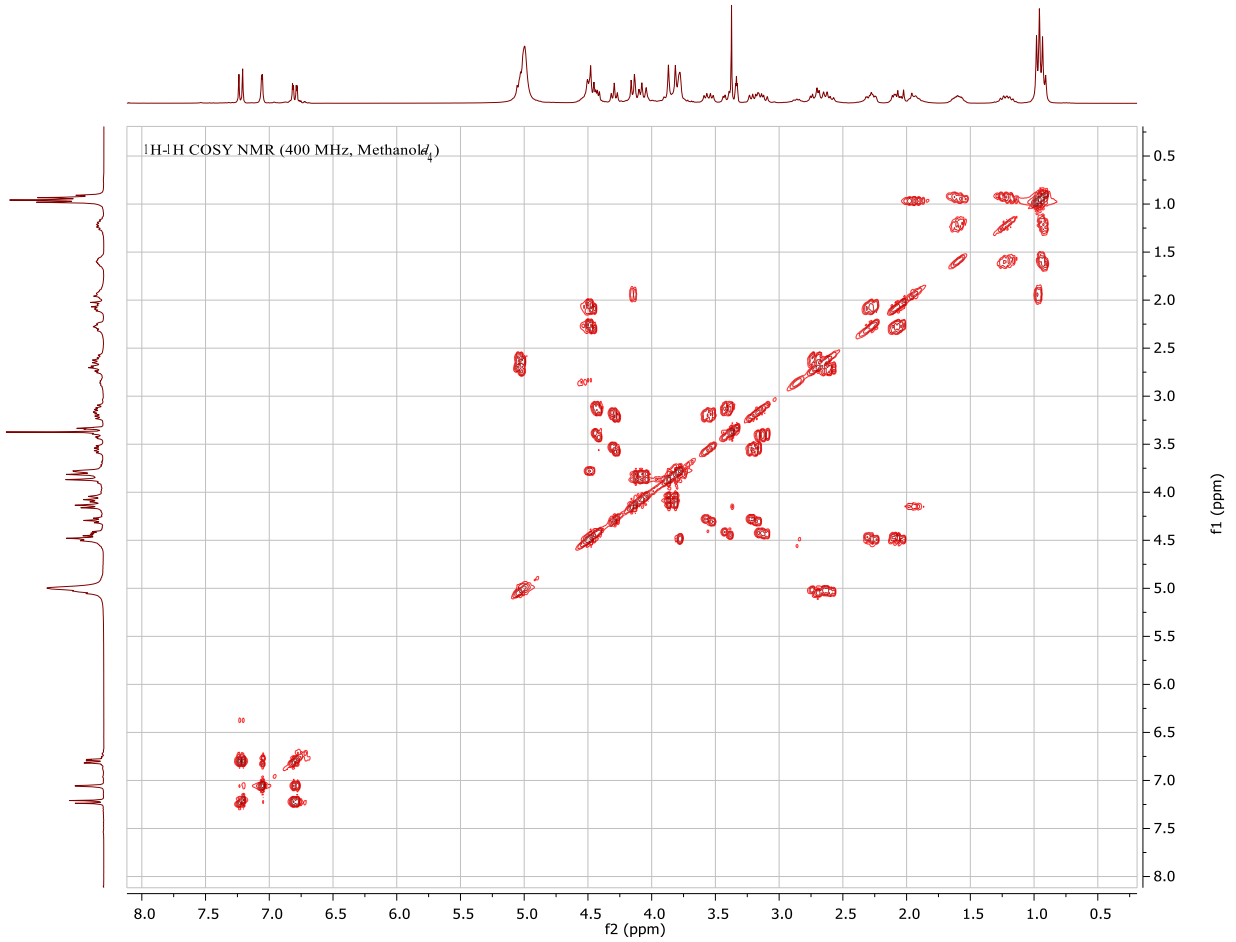
HPLC (gradient A) $\mathbf{t_R}$ = 10.6 min; λ_{max} = 286 nm.

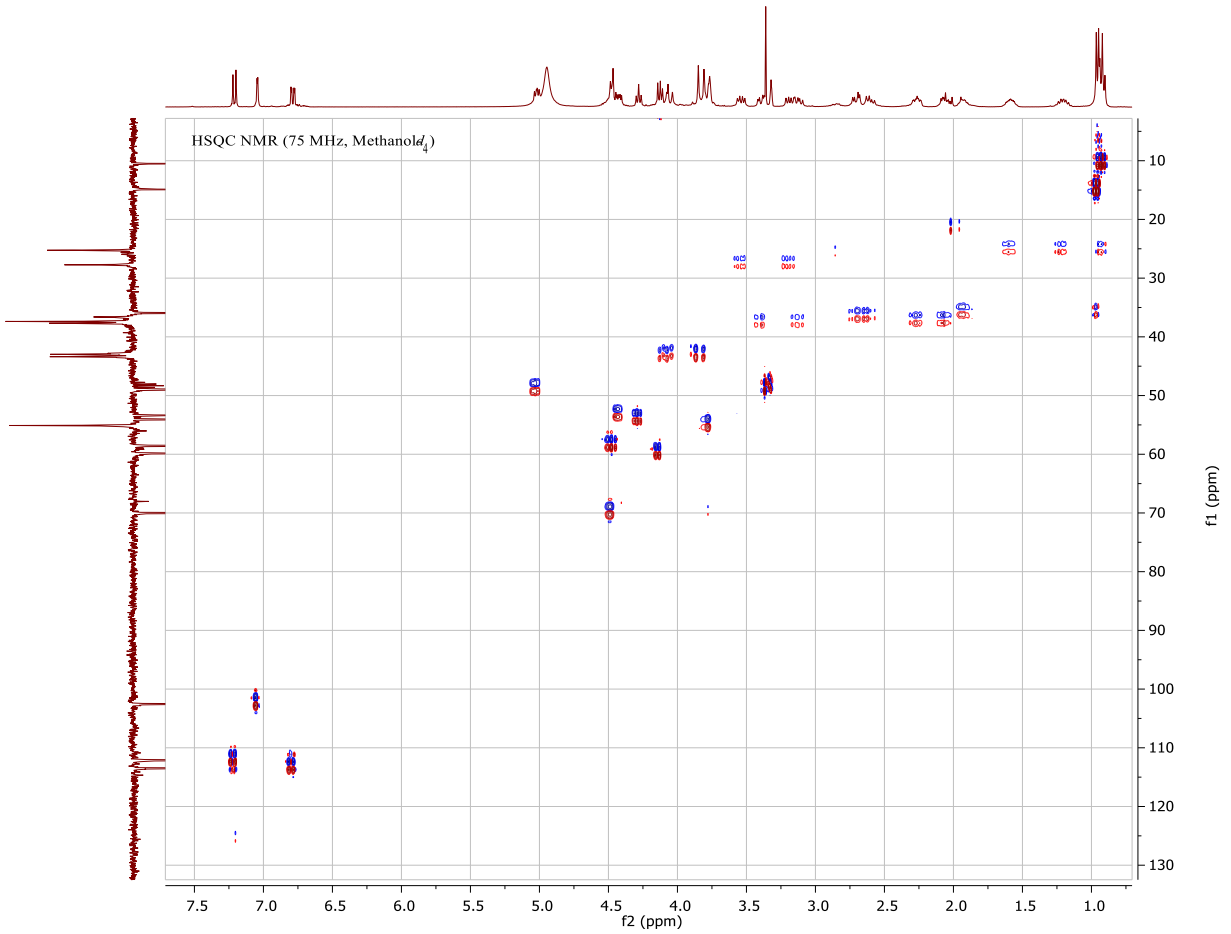


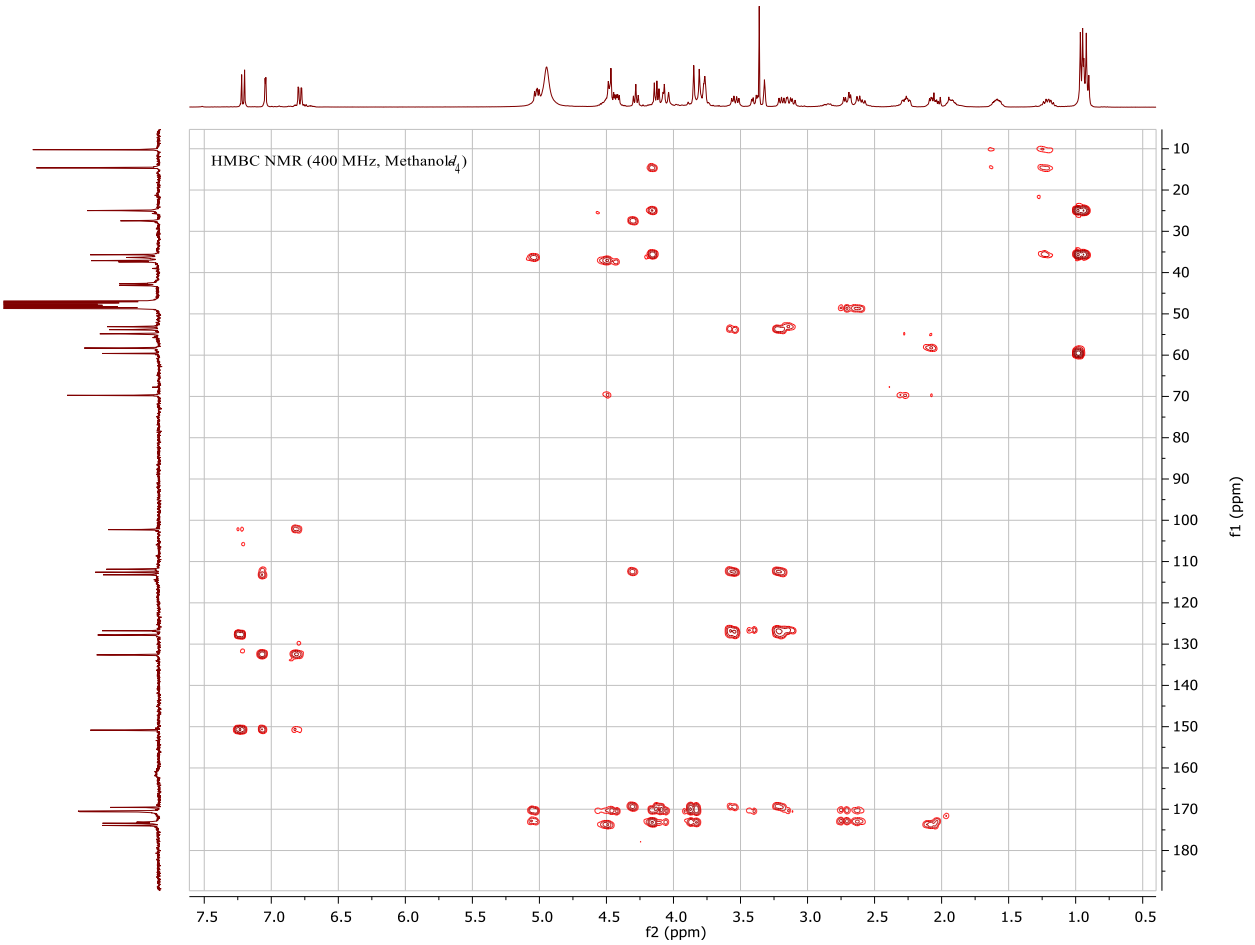

13C NMR (75 MHz, Methanol-d4)
173.93
173.39
173.11
170.61
170.43
170.38
169.56
150.84
132.61
127.79
126.75
113.20
112.59
111.86
102.26
69.73
59.60
58.28
54.83
53.81
53.10
43.11
42.71
37.41
37.11
35.68
27.46
24.98
14.63
10.22
f1 (ppm)


13C DEPT-135 NMR (75 MHz, Methanol-d4)
8 CH2
12 CH & CH3
113.48
112.14
102.53
70.01
59.87
58.55
55.10
54.09
53.37
48.99
48.89 MeOD
48.33 MeOD
43.38
42.98
37.69
37.38
36.61
35.95
27.73
25.25
14.90
10.49
f1 (ppm)

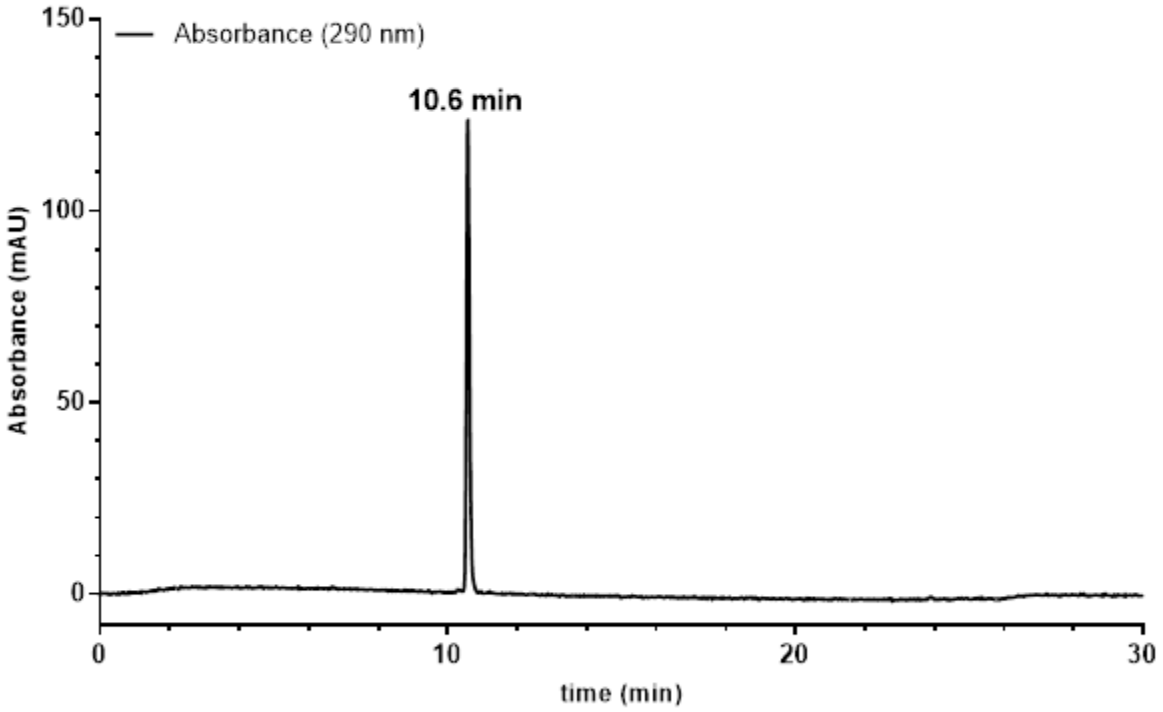

13C and DEPT-135 NMR overlayed (75 MHz, Methanol-d4)
174.26
173.71
173.44
170.94
170.89
170.76
170.70
169.89
151.17
132.93
128.12
127.08
113.53
112.91
112.19
102.59
70.06
59.92
58.61
55.16
54.14
49.04
43.04
37.74
37.44
36.00
27.78
25.31
14.96
10.55
f1 (ppm)


¹H-¹H COSY NMR (400 MHz, Methanol-d_4)
f2 (ppm)
f1 (ppm)

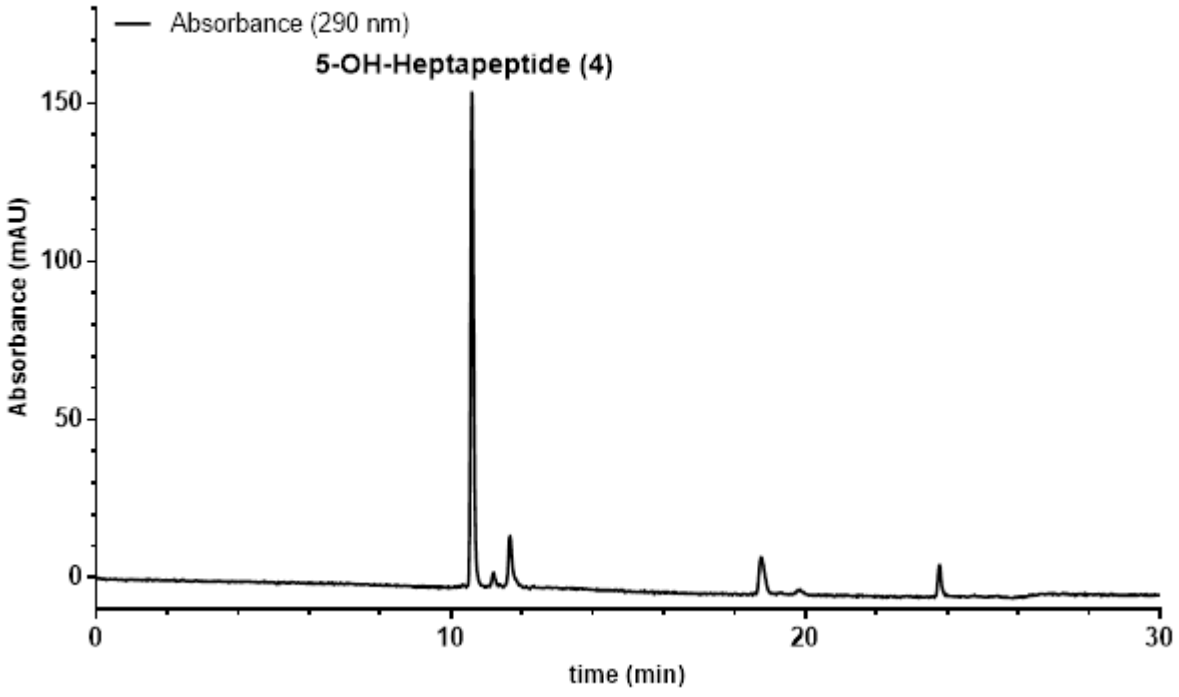

HSQC NMR (75 MHz, Methanol-d_4)
f2 (ppm)
f1 (ppm)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
10
20
30
40
50
60
70
80
90
100
110
120
130


HMBC NMR (400 MHz, Methanol-d_4)
f2 (ppm)
f1 (ppm)

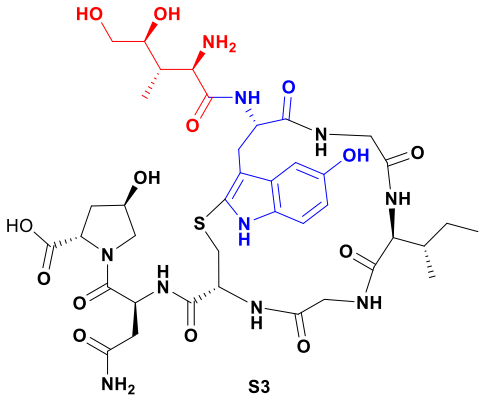
HPLC Chromatogram of 5-OH-Heptapeptide-Monocycle (4)



HPLC Chromatogram of crude reaction mixture following Savige-Fontana reaction showing formation of predominantly 5'-OH-heptapeptide monocycle (4)



Synthesis and characterization of S-deoxy-5'-OH-octapeptide monocycle **S3**



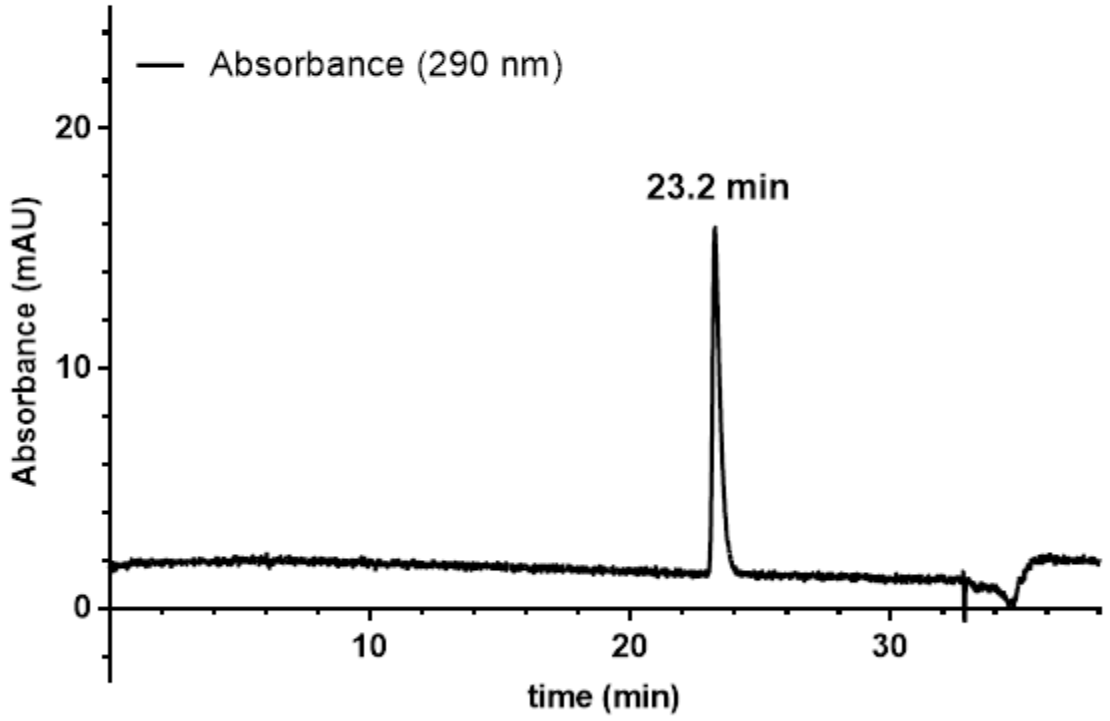
Chemical Formula: $C_{39}H_{56}N_{10}O_{14}S$
Exact Mass: 920.37
Molecular Weight: 920.99

S-deoxy-5'-OH-octapeptide monocycle (**S3**). To a 2-mL Eppendorf tube containing (2*S*,3*R*,4*R*)-O^{γ},O^{δ}-bis-TBS-N^{α}-Fmoc-dihydroxyisoleucine-NHS (1 eq., 5 mg, 7.0 µmol) added 5'-OH-heptapeptide monocycle **4** (3.1 eq., 17 mg, 22 µmol) dissolved in 140 µL of anhydrous DMF. The clear and amber mixture was vortexed and then treated with 8 µL of DIPEA to achieve pH of 8.5. The reaction was stirred for 36 hours at 21 °C, while monitoring the progress with mass spectrometry and HPLC. After 36 hours, 8 µL of Et_2NH (11 eq., 5.7 mg, 77 µmol) were added to the reaction mixture and reaction contents were stirred for 20 hours at 21 °C. The reaction mixture was then treated with 1 µL of acetic acid and 23 µL of 1 M TBAF in THF (3.3 eq., 23 µmol). The reaction mixture was stirred for 4 hours at 21 °C. DMF was then removed under reduced pressure and the concentrate was purified using Sep-Pak C18 (10-g) cartridge (gradient elution, 99.9:0.1 H_2O/formic acid to 89.9:10:0.1 H_2O/ACN/formic acid to 75.9:24:0.1 H_2O/ACN/formic acid). The product containing fractions were lyophilized to obtain monocyclic octapeptide **S3** (3 mg, 3.3 µmol, 47% isolated yield over 3 steps) as a white solid. The product was quantified using tryptathionine as a chromophore with the extinction coefficient of 10,000 $M^{-1}cm^{-1}$ at $\lambda_{max,}$~286 nm (MeOH).

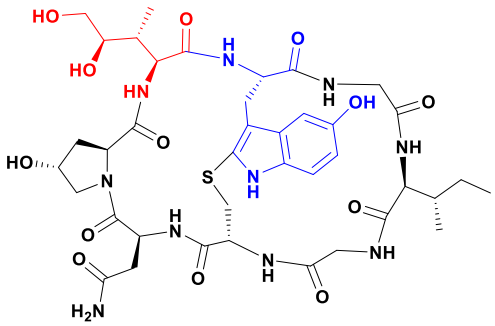
HRMS (ESI-TOF, m/z): $[M+Na]^+$ found 943.3583; calc. 943.3590 for $C_{39}H_{56}N_{10}O_{14}SNa$.

HPLC (gradient B) $\mathbf{t_R}$ = 23.2 min; λ_{max} = 286 nm.

HPLC chromatogram of
S-deoxy-5'-OH-octapeptide monocycle (S3)



Synthesis and characterization of S-deoxy-5'-OH-6'-deoxy-amanitin **11**



Chemical Formula: $C_{39}H_{54}N_{10}O_{13}S$
Exact Mass: 902.36
Molecular Weight: 902.98

S-deoxy-5'-OH-6'-deoxy-amanitin (11). Monocyclic octapeptide **S3** (1 eq., 1.3 mg, 1.46 µmol) was dissolved in 402 µL of dimethyacetamide. This solution was treated with HATU (10 eq., 5.3 mg, 14.6 µmol) and DIPEA (10 eq., 14.6 µmol, 2.5 µL), and stirred for 1 hour at 21 °C. Upon completion, the crude material was purified using Sep-Pak C18 (2 g) cartridge (gradient elution, 99.9:0.1 H_2O/formic acid to 89.9:10:0.1 H_2O/ACN/formic acid to 86.9:15:0.1 H_2O/ACN/formic acid). Sep-Pak fractions were analyzed by HPLC using gradient C and the product containing fractions were lyophilized to obtain the final compound **11** (~0.3 mg, 0.31 µmol, 45% yield) as a white solid. The product was quantified using tryptathionine as a chromophore with the extinction coefficient of 10,000 $M^{-1}cm^{-1}$ at $\lambda_{max,}$~286 nm (in MeOH).

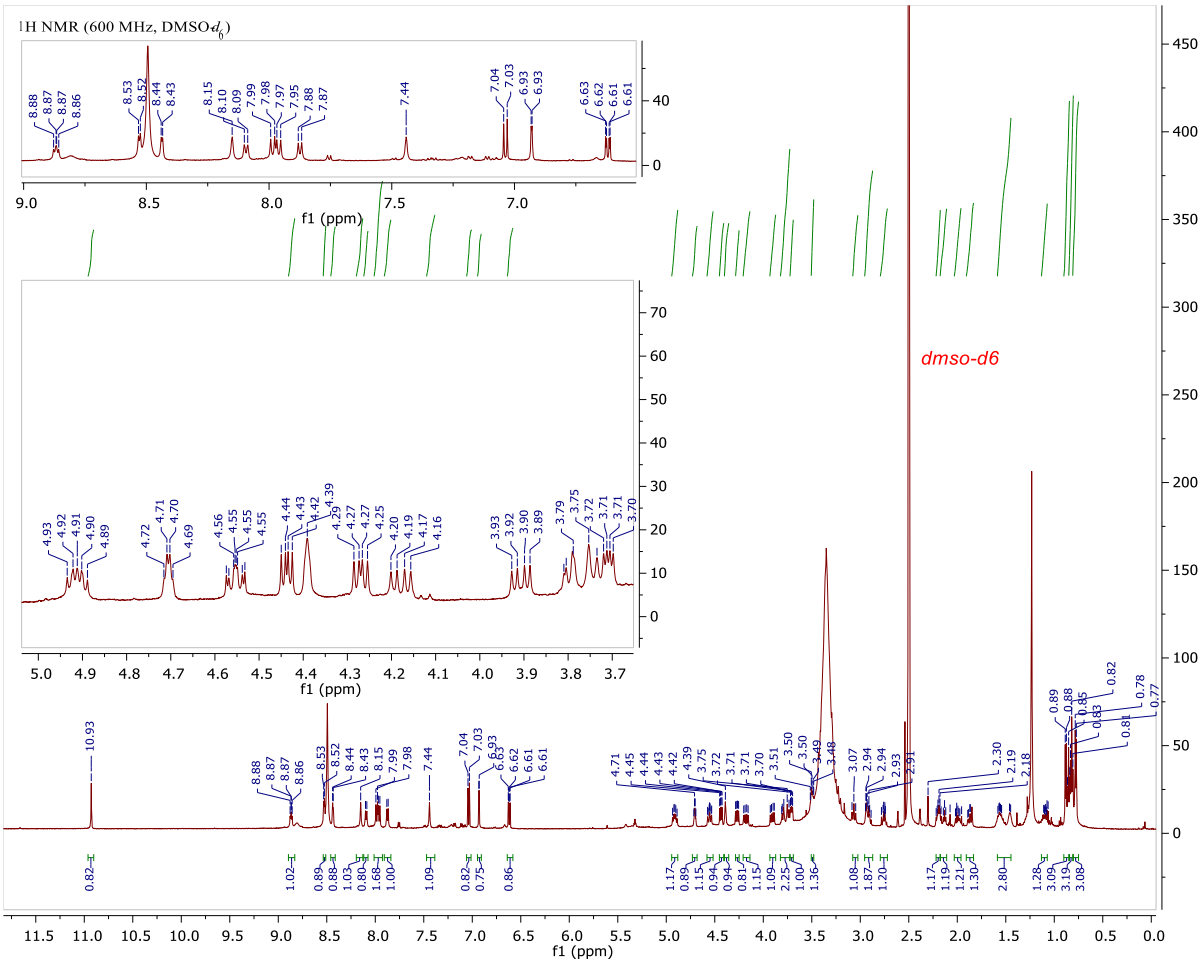
HRMS (ESI-TOF, m/z): $[M+H]^+$ found 903.3658; calc. 903.3659 for $C_{39}H_{55}N_{10}O_{13}S$.

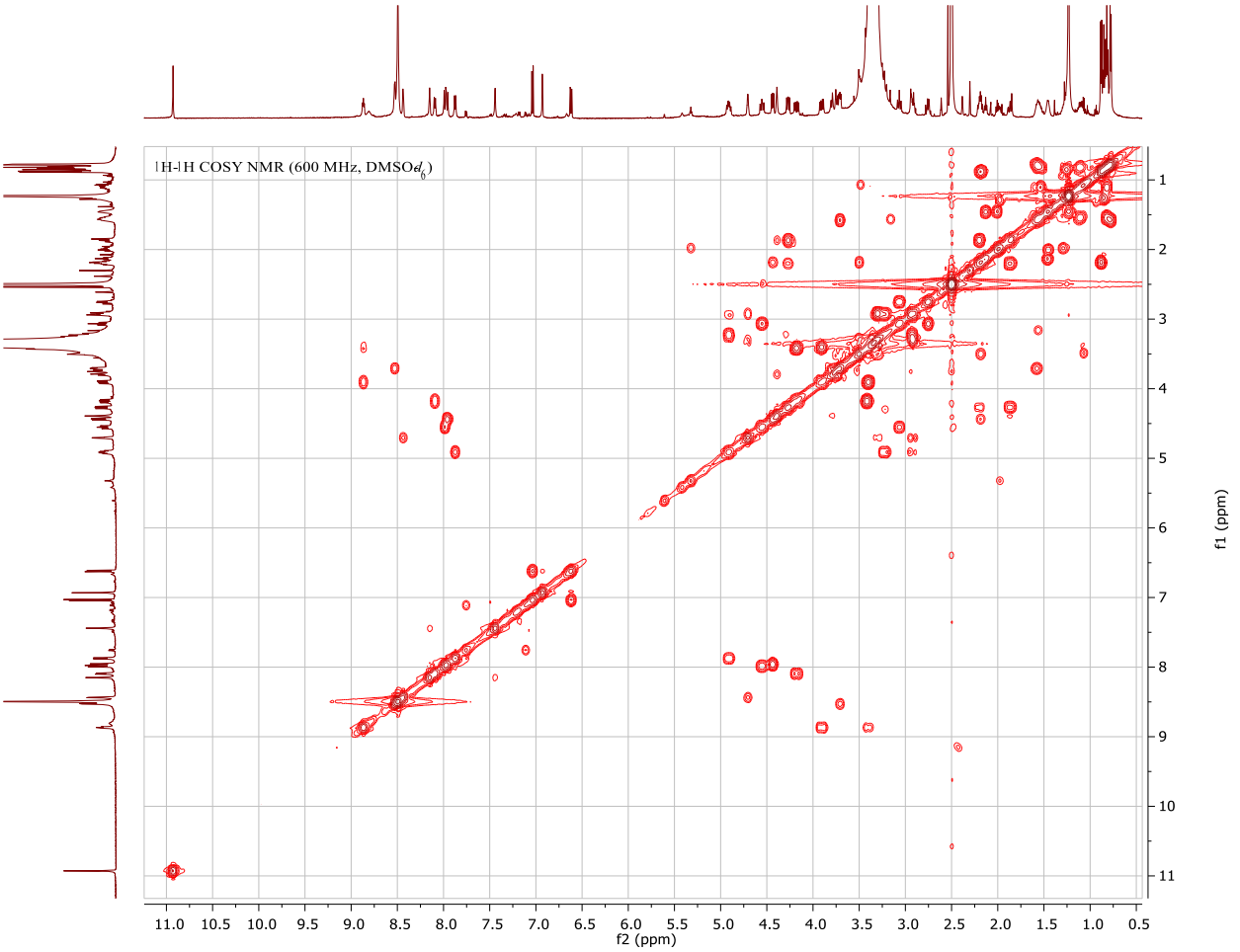
HPLC (gradient C) $\mathbf{t_R}$ = 23.2 min; λ_{max} = 286 nm.

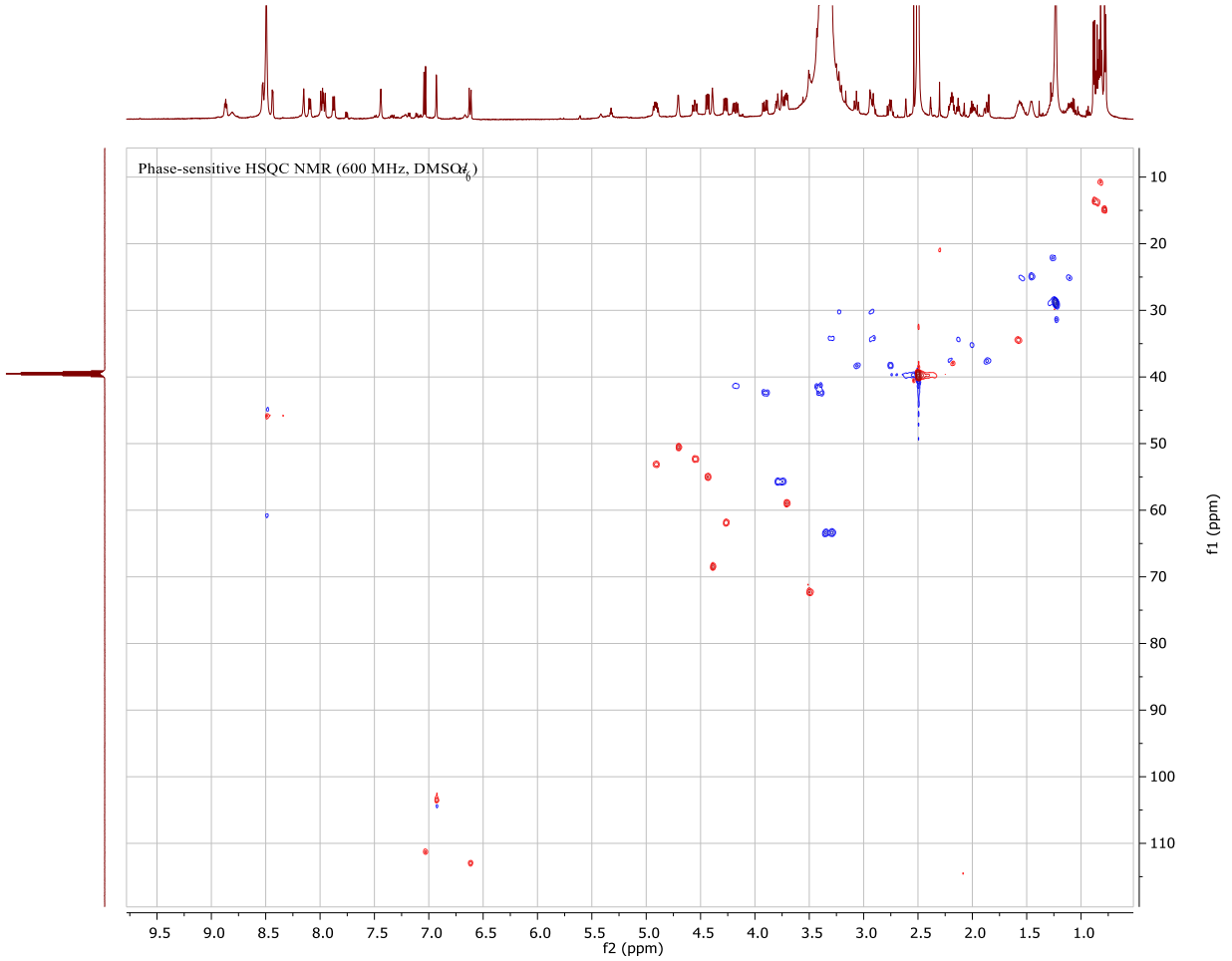
Table S2. ^{1}H-NMR assignments for compound **11** (600 MHz, DMSO-d_6)

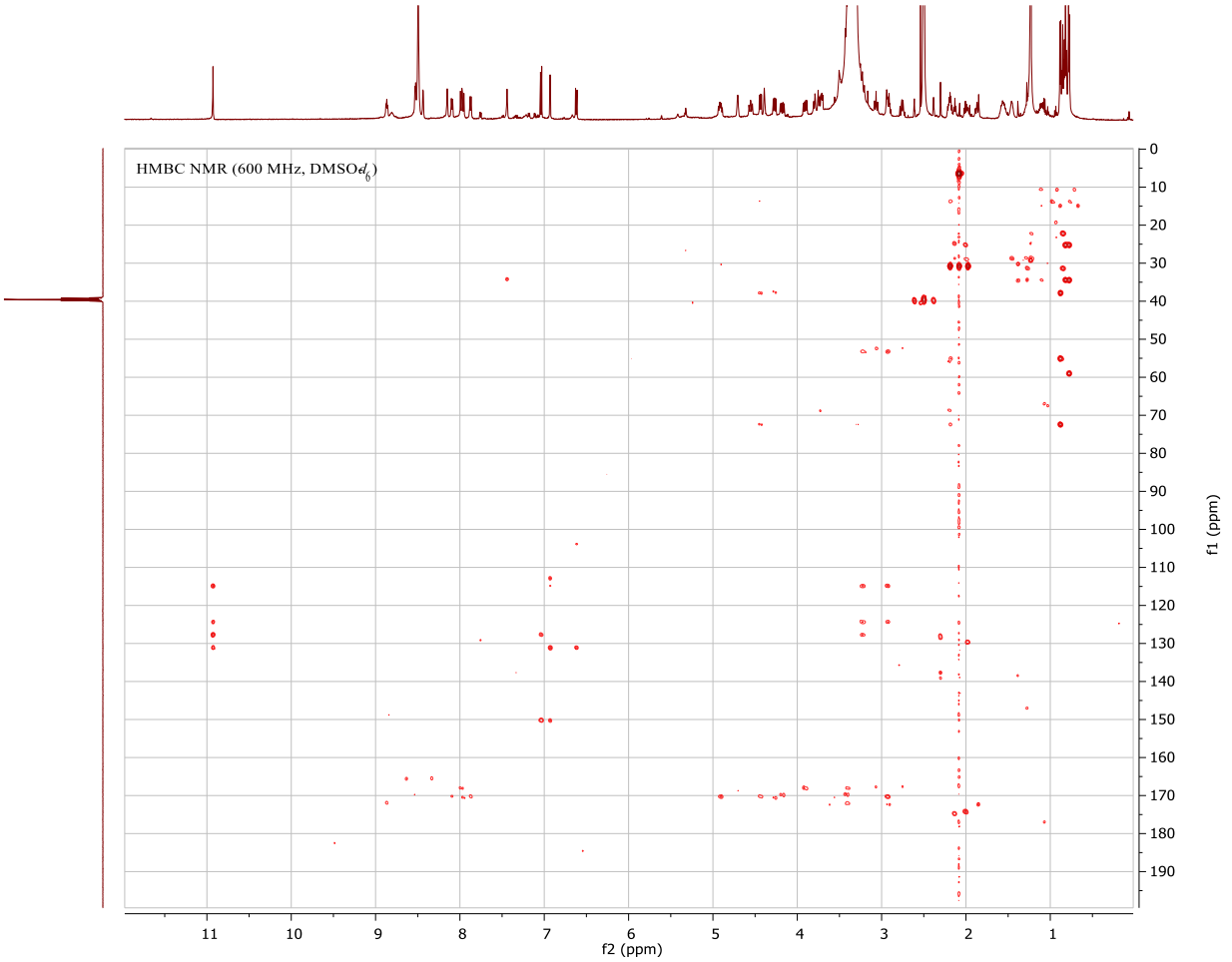
Residue	Proton	Shift (ppm)	Coupling constant (*J*, Hz)
Asn-1	NH	8.44	d, *J* = 3.6
	HCα	4.71	q, *J* = 4.1
	HCβ	2.92	m
	HCβ	3.28	n/d
	H_2N	7.44, 8.15	br s
Hyp-2	HCα	4.27	dd, *J* = 11.5, 7.0
	H'Cβ	1.88	dd, *J* = 12.3, 3.3
	H"Cβ	2.2	dd, *J* = 10.8, 6.3
	HCγ	4.39	m
	H'Cδ	3.74	d, *J* = 11.3
	H"Cδ	3.8	m
	HO	-	-
DIle-3	HN	7.96	d, *J* = 9.5

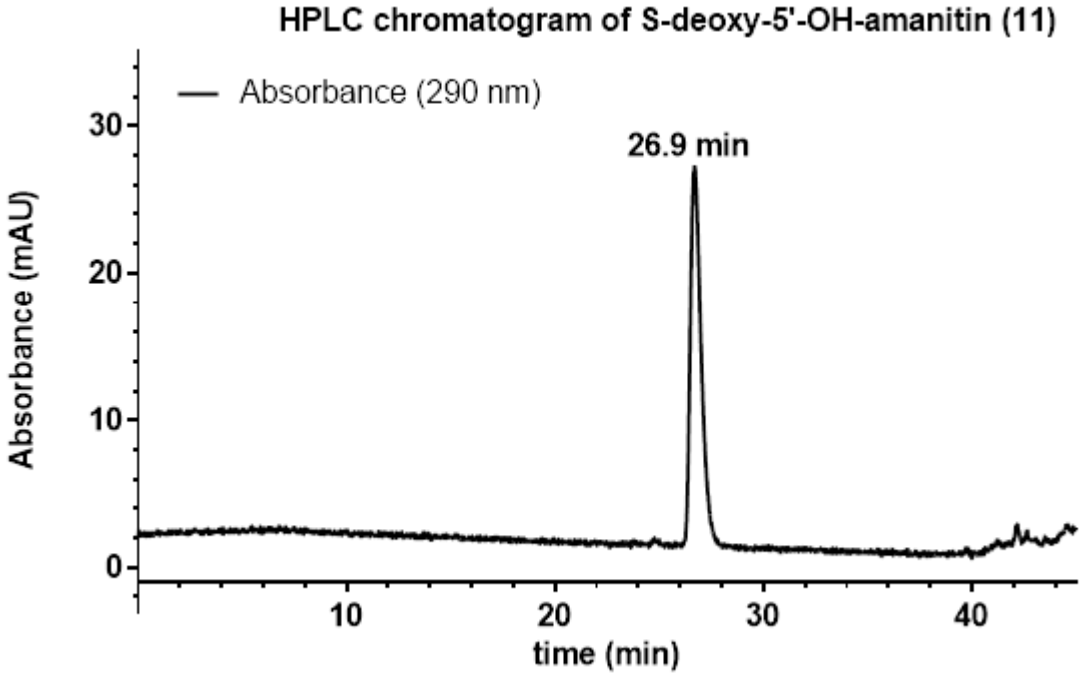
	HCα	4.44	dd, *J* = 9.4, 5.7
	HCβ	2.17	m
	HCγ	3.5	m
	H'Cδ	3.29	n/d
	H"Cδ	3.35	n/d
	CH_3	0.88	d, *J* = 7.1
	HOγ	-	-
	HOδ	-	-
Trp-4	HN	7.87	d, *J* = 8.3
	HCα	4.91	dt, *J* = 13.8, 7.8
	H'Cβ	2.91	m
	H"Cβ	3.24	m
	H-4'	6.93	d, *J* = 2.3
	H-6'	6.62	dd, *J* = 8.7, 2.3
	H-7'	7.04	d, *J* = 8.6
	HN-Indole	10.93	s
	HO-5	-	-
Gly-5	HN	8.09	d, *J* = 7.7
	H'Cα	3.41	m
	H"Cα	4.18	dd, *J* = 18.6, 8.4
Ile-6	HN	8.53	d, *J* = 4.5
	HCα	3.71	dd, *J* = 8.7, 4.5
	HCβ	1.53	m
	H'Cγ	1.56	m
	H"Cγ	1.1	m
	CH_3β	0.82	t, *J* = 7.3
	CH_3γ	0.78	d, *J* = 6.7
Gly-7	HN	8.87	m
	H'Cα	3.4	m
	H"Cα	3.91	dd, *J* = 17.3, 7.5
Cys-8	HN	7.99	d, *J* = 9.9
	HCα	4.55	ddd, *J* = 11.9, 9.9, 3.5
	H'Cβ	2.75	m
	H"Cβ	3.07	m

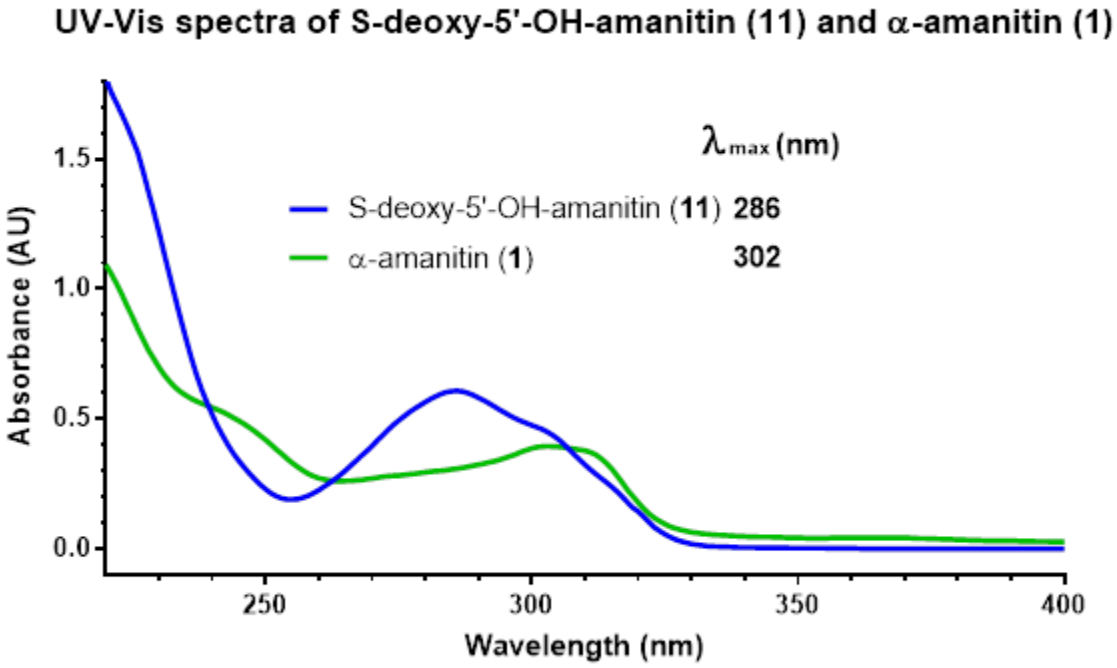

1H NMR (600 MHz, DMSO-d6)
dmso-d6
f1 (ppm)


^{1}H-^{1}H COSY NMR (600 MHz, DMSOd_6)
f2 (ppm)
f1 (ppm)

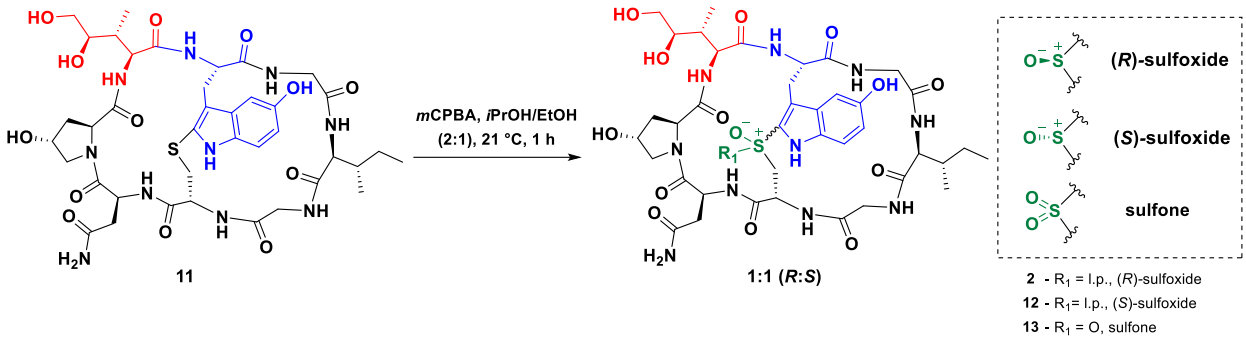

Phase-sensitive HSQC NMR (600 MHz, DMSO-d_6)
f1 (ppm)
f2 (ppm)


HMBC NMR (600 MHz, DMSO-d_6)
f1 (ppm)
f2 (ppm)


HPLC chromatogram of S-deoxy-5'-OH-amanitin (11)
Absorbance (290 nm)
26.9 min
Absorbance (mAU)
30
20
10
0
10
20
30
40
time (min)


UV-Vis spectra of S-deoxy-5'-OH-amanitin (11) and α-amanitin (1)
λmax (nm)
S-deoxy-5'-OH-amanitin (11) 286
α-amanitin (1) 302
Absorbance (AU)
1.5
1.0
0.5
0.0
250
300
350
400
Wavelength (nm)

Synthesis of (*R*)-5'-OH-6'-deoxy-amanitin (**2**), (*S*)-5'-OH-6'-deoxy-amanitin (**12**), and SO_2-5'-OH-6'-deoxy-amanitin (**13**) via *m*CPBA oxidation of S-deoxy-5'-OH-6'-deoxy-amanitin (**11**)



Compound **11** (1 eq., 150 nmol, 135 µg) was dissolved in 50 µL of 2:1 *i*PrOH/EtOH. This solution was treated with *m*CPBA (1.3 eq., 195 nmol) in 3 µL of *i*PrOH/EtOH (2:1). The reaction was run for 1 hour at 21 °C and then diluted to 200 µL with the HPLC buffer (0.1% formic acid in H_2O) and immediately subjected to HPLC purification. HPLC analysis (gradient D) revealed formation of three products in 4.4:4.6:1 ratio corresponding to (*R*)-sulfoxide:(*S*)-sulfoxide:sulfone (**2**:**12**:**13**). The HPLC purification was performed using gradient D, product containing eluent was lyophilized to give (*R*)-sulfoxide **2** (58 nmol) and (*S*)-sulfoxide **12** (47 nmol) with overall 70% yield in sulfoxide. The product was quantified using tryptathionine as a chromophore with the extinction coefficient of 12,600 $M^{-1}cm^{-1}$ at $\lambda_{max,}$~288 nm (in MeOH or H_2O).

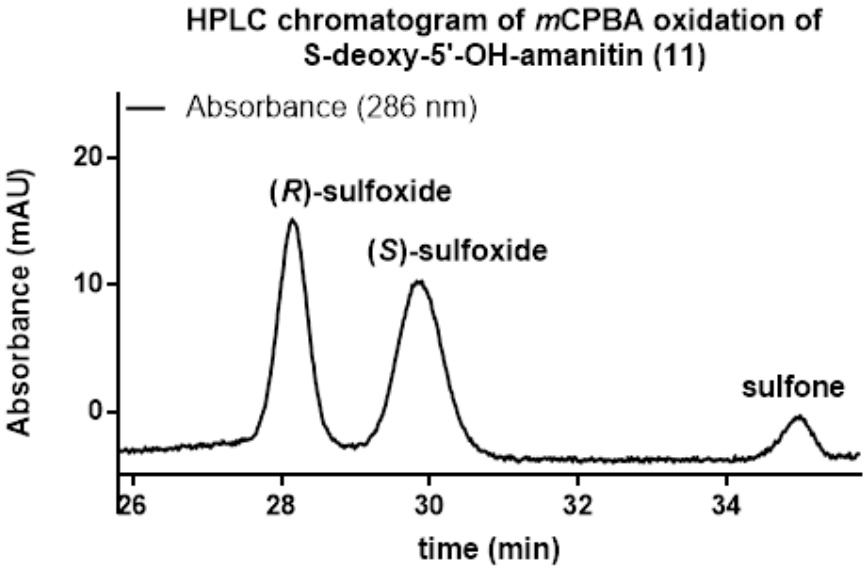
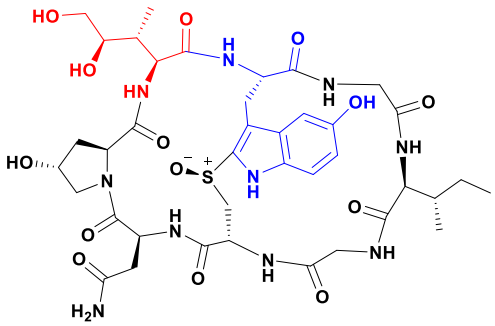


Figure S2. HPLC chromatogram of *m*CPBA sulfoxidation carried out on S-deoxy-5'-OH-6'-deoxy-amanitin **11** showing formation of (*R*)-, (*S*)-sulfoxides and sulfone.

Synthesis and characterization of (*R*)-5'-OH-6'-deoxy-amanitin **2**

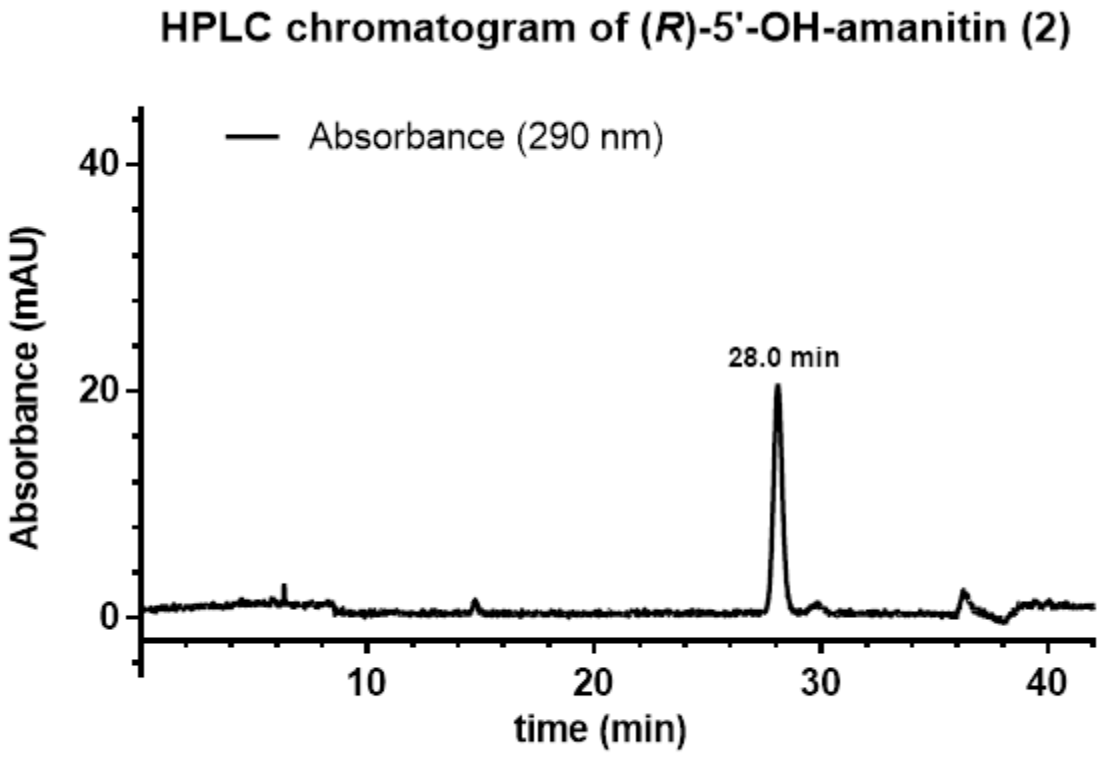


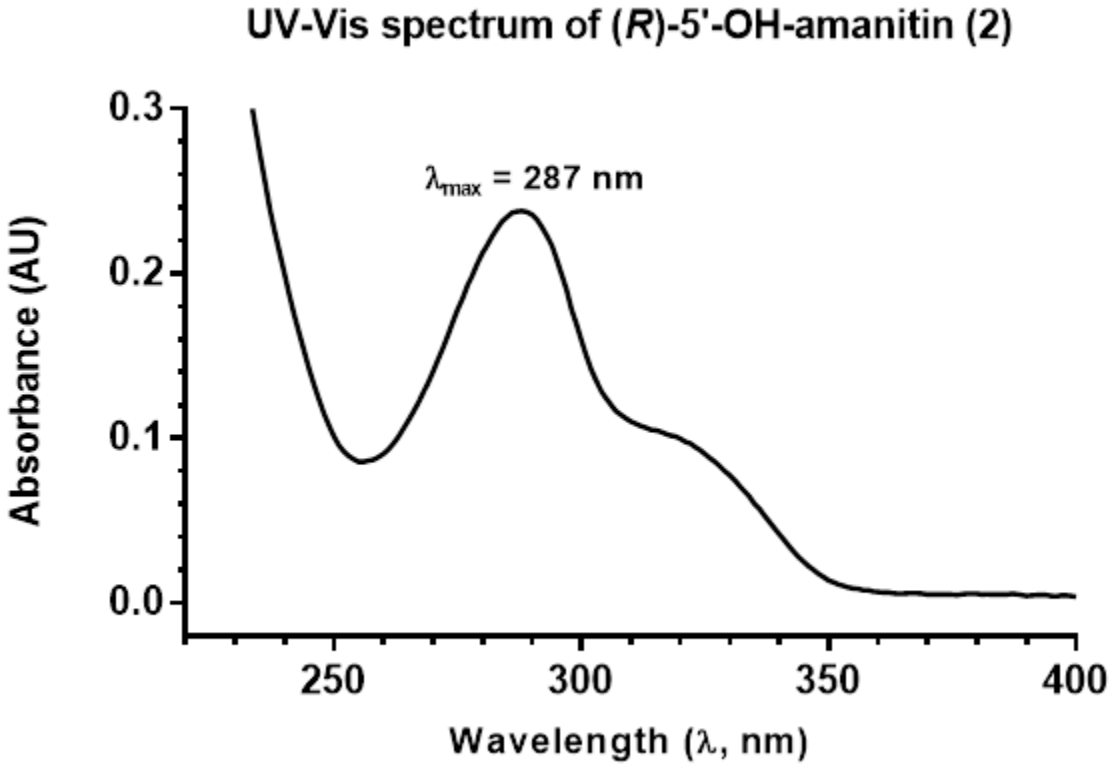
Chemical Formula: $C_{39}H_{54}N_{10}O_{14}S$
Exact Mass: 918.35
Molecular Weight: 918.98

(*R*)-5'-OH-6'-deoxy-amanitin (2)

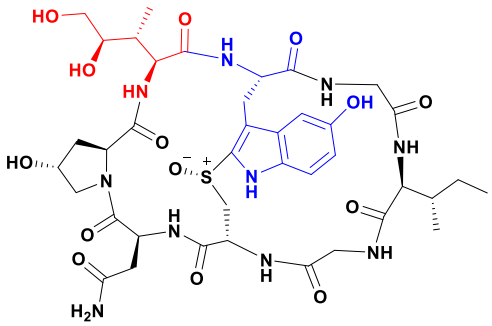
HRMS (ESI-TOF, m/z): $[M+H]^+$ found 919.3623; calc. 919.3614 for $C_{39}H_{55}N_{10}O_{14}S$.

HPLC (gradient D) $\mathbf{t_R}$ = 28.0 min; λ_{max} = 287 nm.




UV-Vis spectrum of (R)-5'-OH-amanitin (2)
Absorbance (AU)
0.3
0.2
0.1
0.0
λmax = 287 nm
250
300
350
400
Wavelength (λ, nm)

Synthesis and characterization of (*S*)-5'-OH-6'-deoxy-amanitin **12**

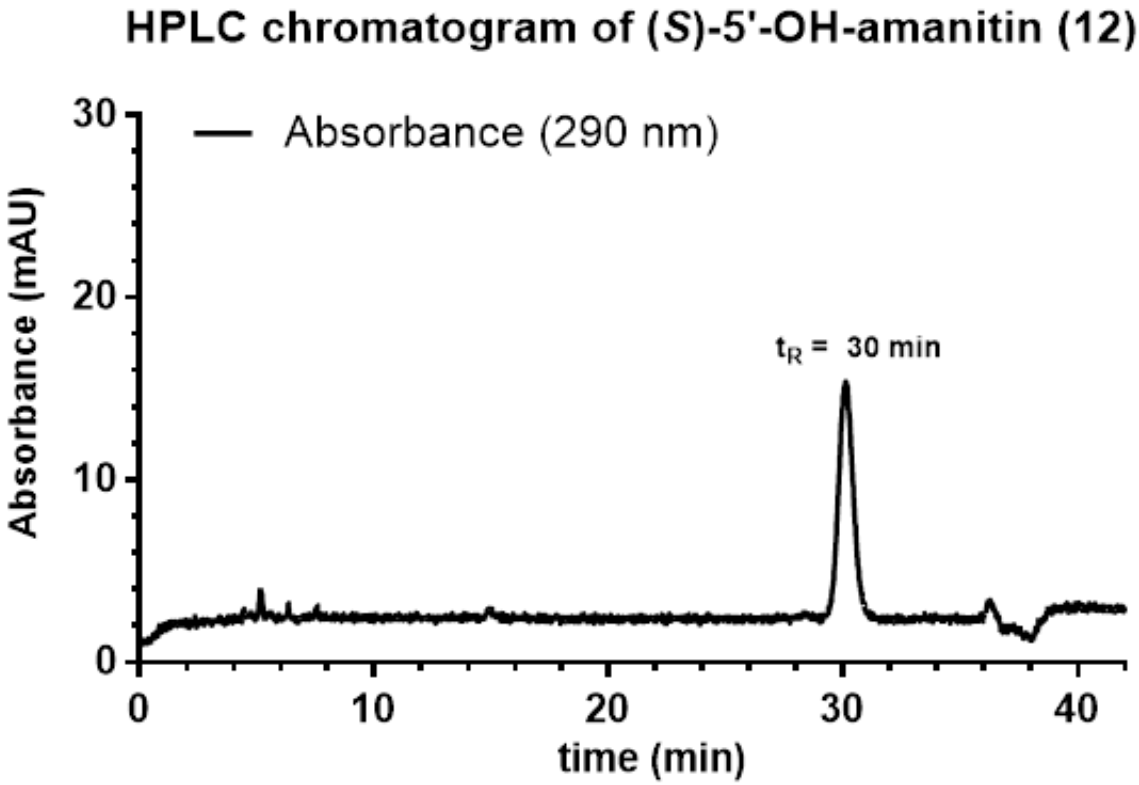


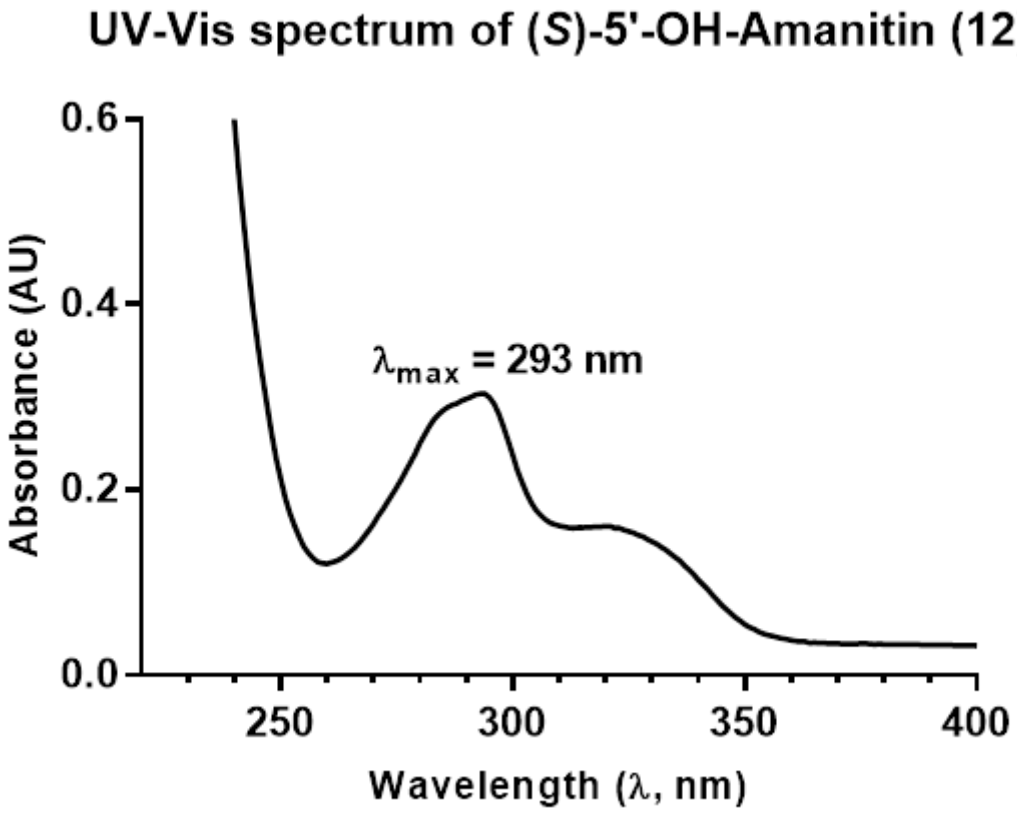
Chemical Formula: $C_{39}H_{54}N_{10}O_{14}S$
Exact Mass: 918.35
Molecular Weight: 918.98

(*S*)-5'-OH-6'-deoxy-amanitin (12)

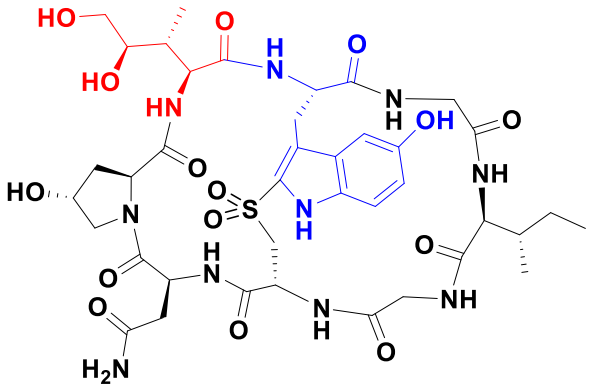
HRMS (ESI-TOF, m/z): $[M+H]^+$ found 919.3607; calc. 919.3614 for $C_{39}H_{55}N_{10}O_{14}S$.

HPLC (gradient D) $\mathbf{t_R}$ = 30 min; λ_{max} = 293 nm.




UV-Vis spectrum of (S)-5'-OH-Amanitin (12)
Absorbance (AU)
0.6
0.4
0.2
0.0
λmax = 293 nm
250
300
350
400
Wavelength (λ, nm)

Synthesis and characterization of SO_2-5'-OH-6'-deoxy-amanitin **13**

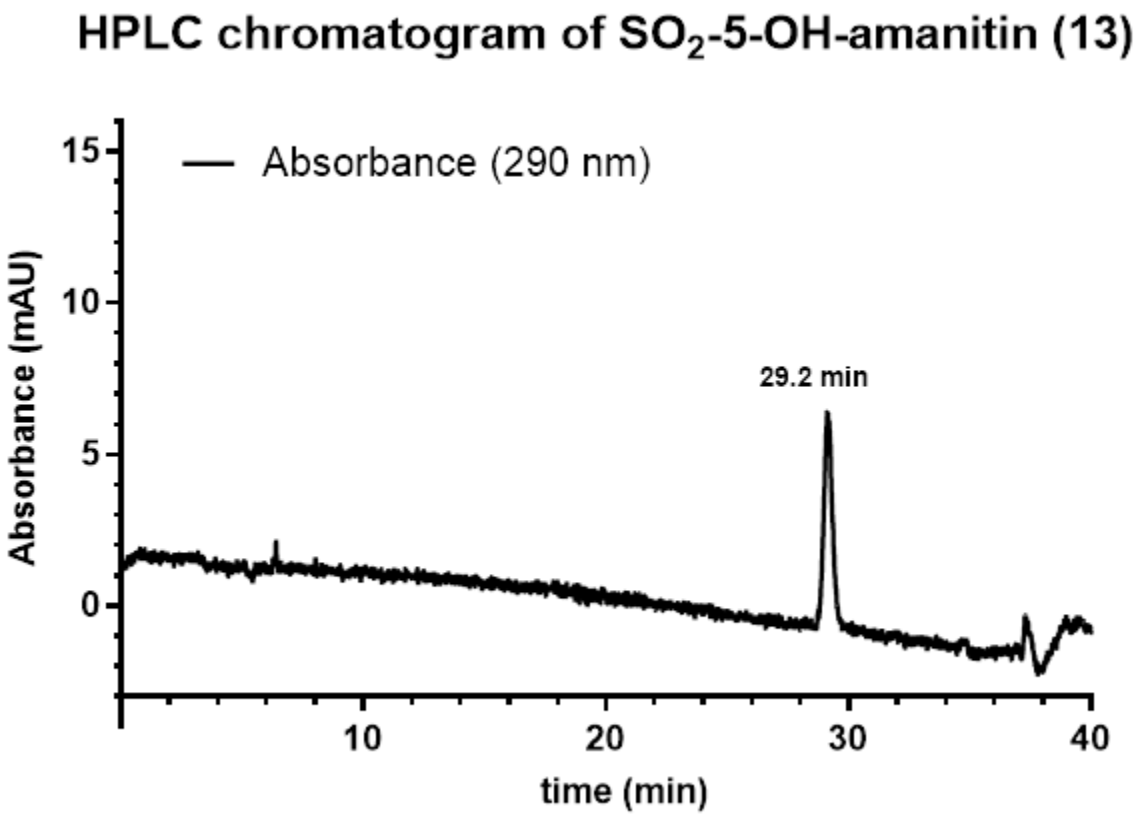


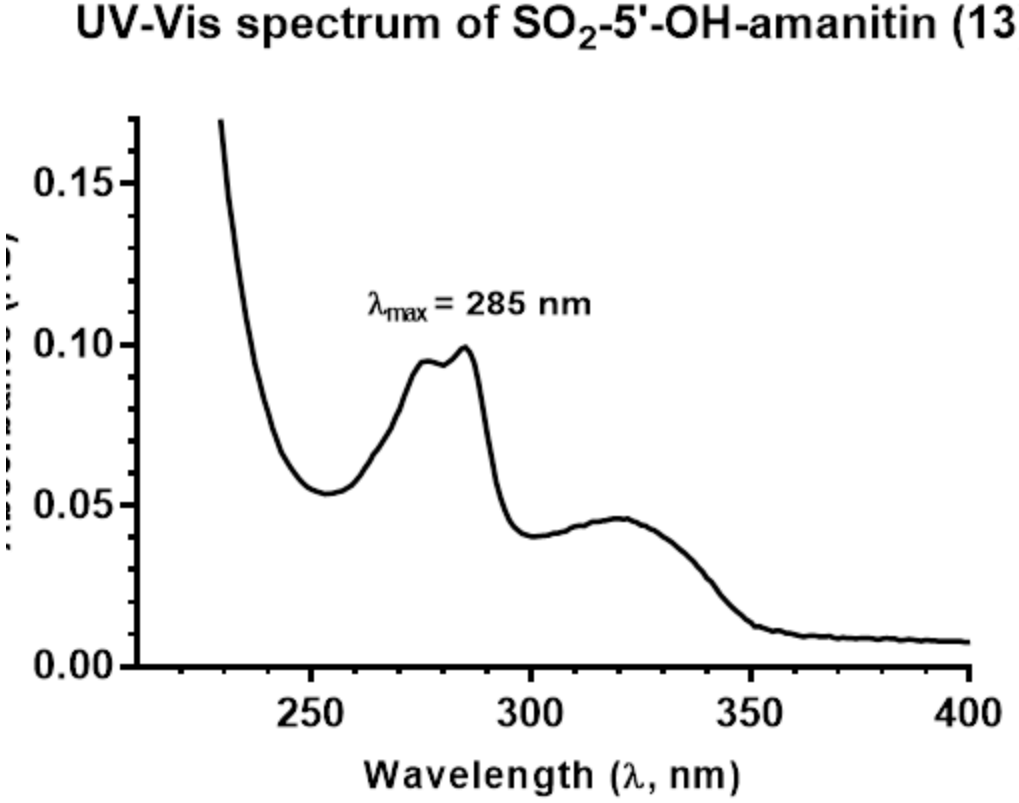
Chemical Formula: $C_{39}H_{54}N_{10}O_{15}S$
Exact Mass: 934.35
Molecular Weight: 934.98

SO_2-5'-OH-6'-deoxy-amanitin (13)

HRMS (ESI-TOF, m/z): $[M+Na]^+$ found 957.3383; calc. 957.3383 for $C_{39}H_{54}N_{10}O_{15}SNa$.

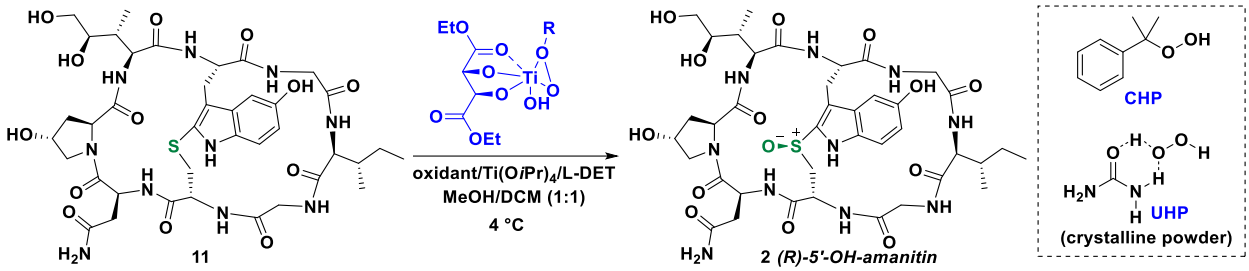
HPLC (gradient E) $\mathbf{t_R}$ = 29.2 min; λ_{max} = 285 nm.




UV-Vis spectrum of SO2-5'-OH-amanitin (13)
0.15
0.10
0.05
0.00
λmax = 285 nm
250
300
350
400
Wavelength (λ, nm)

Screening of Ti(O*i*Pr)$_4$/L-DET sulfoxidation conditions to synthesize (*R*)-5'-OH-6'-deoxy-amanitin **2**

Table S3. Screening of Ti(O*i*Pr)$_4$/L-DET sulfoxidation conditions to synthesize (*R*)-5'-OH-6'-deoxy-amanitin **2** carried out on S-deoxy-5'-OH-6'-deoxy-amanitin **11**.



Entry	Conditions	(*R*:*S*)	Yield (%)*
1	CHP/Ti(O*i*Pr)$_4$/L-DET (1:1:2), 17 h	**1:1.1**	**61**
2	UHP/Ti(O*i*Pr)$_4$/L-DET (1:1:2), 17 h	**2.6:1**	**66**
3	UHP, 17 h	**1:2.8**	**80**

*HPLC sulfoxide yield

General procedure A, entries 1-3.
A small Eppendorf tube containing bicyclic S-deoxy-5'-OH-6'-deoxy-amanitin **11** (1 eq., 12.5 nmol) dissolved in 6 µL of MeOH was cooled to 4 °C. Compound **11** was then treated with 2.5 µL of DCM solution containing Ti(O*i*Pr)$_4$ (0.8 eq., 10 nmol) and L-DET (1.6 eq., 20 nmol). The reaction mixture was kept at 4 °C for 30 mins and then 3.5 µL of DCM at 4 °C were added to the reaction followed by 0.5 µL of oxidant (UHP or CHP; 1 eq., 12.5 nmol) solution in MeOH. The reaction was run for 17 hours at 4 °C. The reaction mixture was concentrated, re-dissolved in 100 µL of 1:4 MeOH/H_2O(0.1% formic acid) and subjected to HPLC analysis using gradient E. The ratio between (*R*) and (*S*) sulfoxides was determined by integrating HPLC signals. Note: no Ti(O*i*Pr)$_4$/L-DET was added to reaction described in entry 3, instead 2.5 µL of DCM were added and the reaction was carried out as described.
Note: solvents were found to have an effect on the reaction outcome. DCM was freshly distilled over CaH and used within a day, while MeOH was used as is (Sigma, HPLC grade).

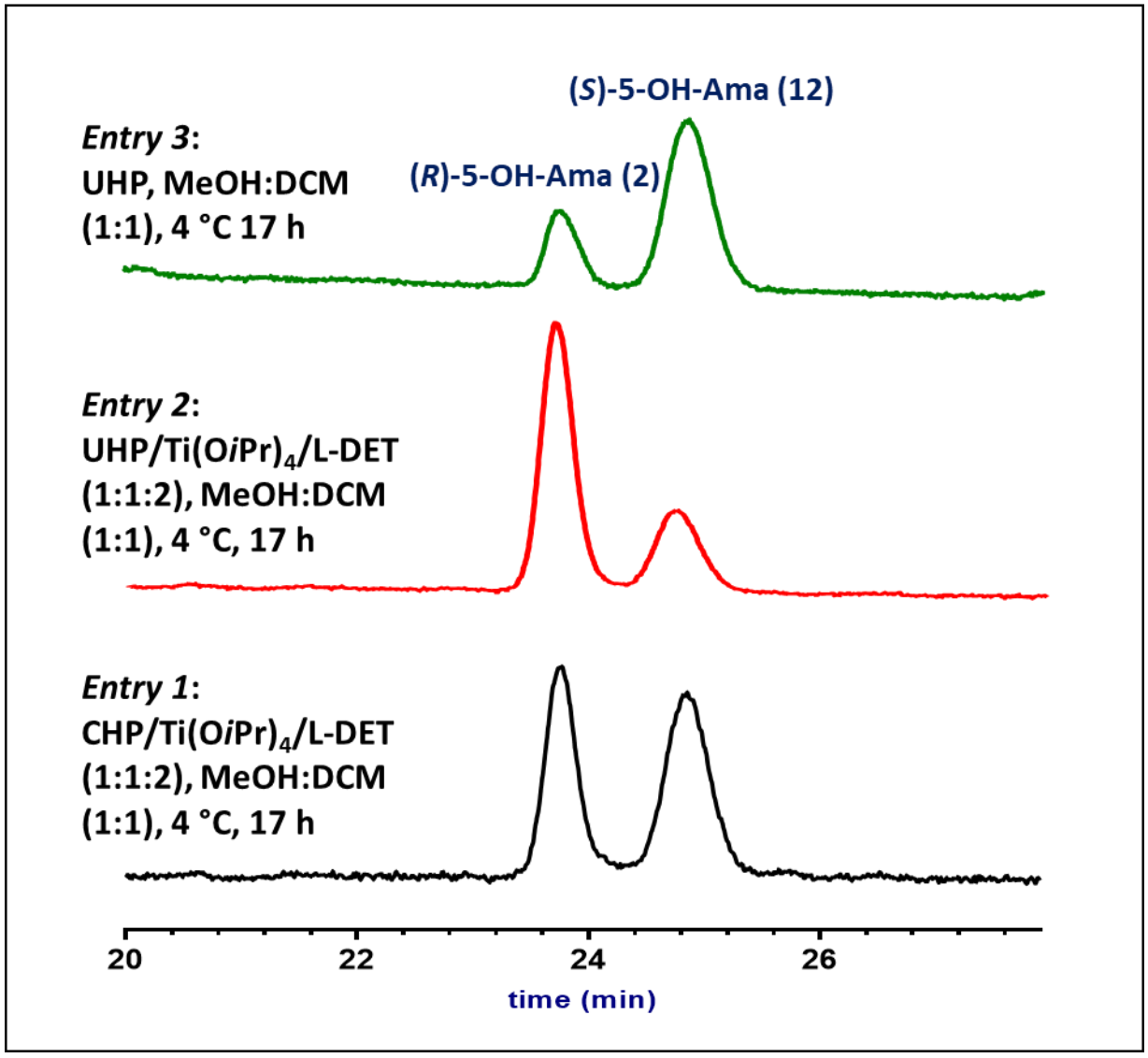
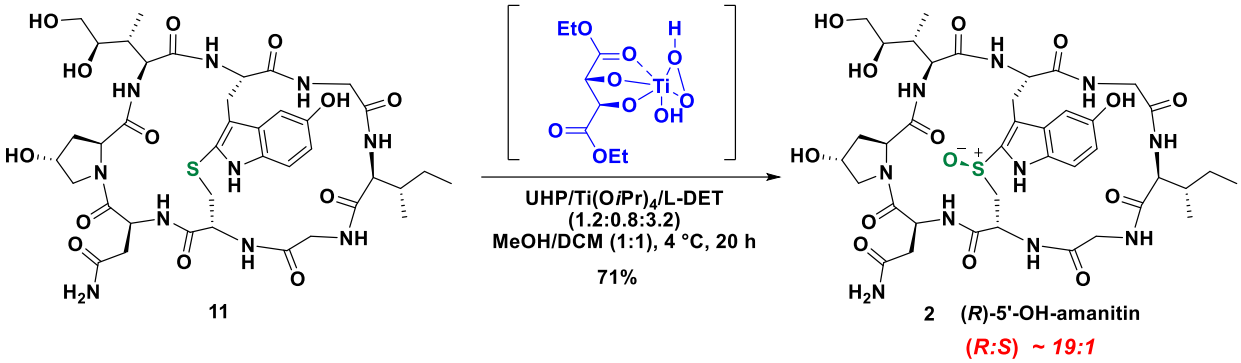


Figure S3. Entries 1 and 2 - HPLC chromatograms (286 nm) showing (*R*:*S*)-sulfoxide distribution obtained via Ti^{IV}-catalyzed diastereoselective sulfoxidation in the presence of L-DET as a ligand and CHP and UHP as oxidants; Entry 3 - HPLC chromatogram (286 nm) showing (*R*:*S*)-sulfoxide distribution obtained via UPH-assisted oxidation in the absence of Ti^{IV}-source and L-DET ligand; cumene hydroperoxide (CHP) and urea hydroperoxide (UHP).

Diastereoselective sulfoxidation of S-deoxy-5'-OH-6'-deoxy-amanitin **11** in the presence of UHP/Ti(O*i*Pr)$_4$/L-DET to synthesize (*R*)-5'-OH-6'-deoxy-amanitin **2**



A small Eppendorf tube containing bicyclic S-deoxy-5'-OH-6'-deoxy-amanitin **11** (1 eq., 100 nmol) dissolved in 50 µL of MeOH was cooled to 4 °C. Compound **11** was then treated with 31.9 µL of DCM solution containing Ti(O*i*Pr)$_4$ (0.8 eq., 80 nmol) and L-DET (3.2 eq., 320 nmol). The reaction mixture was kept at 4 °C for 30 mins and then 18 µL of DCM at 4 °C were added to the reaction followed by 7.5 µL of UHP solution in MeOH (1.15 eq., 115 nmol). The reaction was run for 20 hours at 4 °C. The reaction mixture was then concentrated, re-dissolved in 200 µL of 1:4 MeOH/H$_2$O(0.1% formic acid) and subjected to HPLC purification using gradient D. Following the HPLC analysis and purification, starting material was recycled and subjected to a second cycle of sulfoxidation. A diastereomeric distribution of 19:1 (*R*:*S*) was achieved and (*R*)-5'-OH-6'-deoxy-amanitin (**2**) was obtained in 71% yield (71 nmol). The ratio between (*R*) and (*S*) sulfoxides was determined by integrating HPLC signals (~25% of the starting material remained unreacted after 20 hours). Extinction coefficient of 12,600 $M^{-1}cm^{-1}$ (290 nm) was used for quantification.

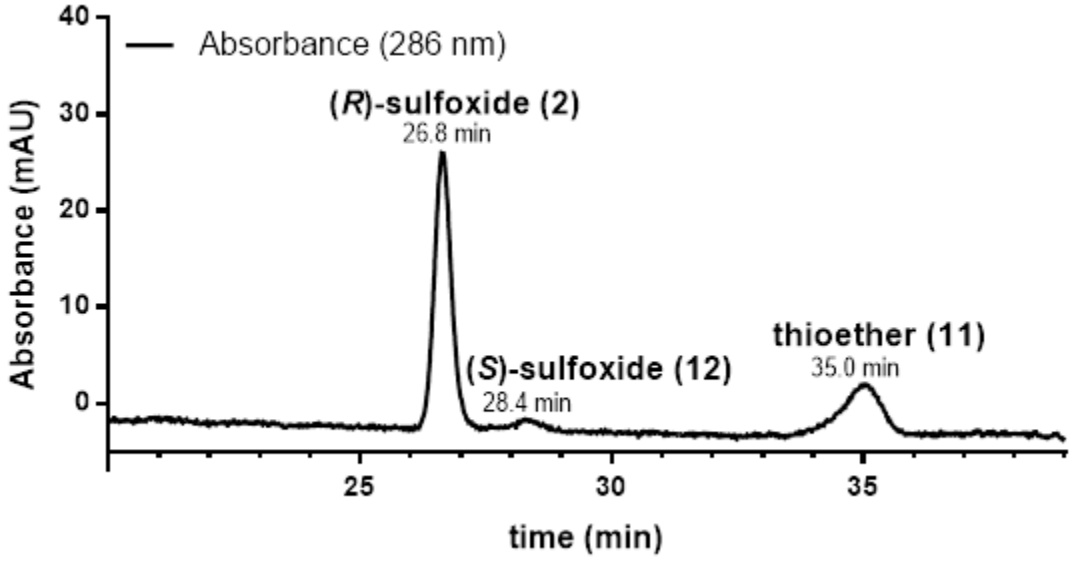
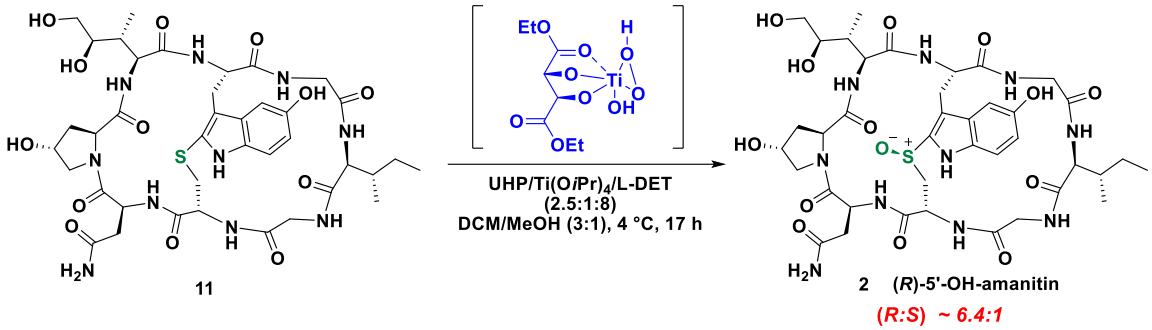


Figure S4. HPLC chromatogram of asymmetric sulfoxidation of bicyclic octapeptide **11** with UHP in the presence of Ti(IV) and L-DET.

Diastereoselective sulfoxidation of S-deoxy-5'-OH-6'-deoxy-amanitin **11** in the presence of UHP/Ti(O*i*Pr)$_4$/L-DET in 2.5:1:8 ratio



A small Eppendorf tube containing bicyclic S-deoxy-5'-OH-6'-deoxy-amanitin **11** (1 eq., 6 nmol) dissolved in 1.7 µL of MeOH was cooled to 4 °C. Compound **11** was then treated with 7.2 µL of DCM solution containing Ti(O*i*Pr)$_4$ (1 eq., 6 nmol) and L-DET (8 eq., 48 nmol). The reaction mixture was kept at 4 °C for 30 mins and then treated with 1.0 µL of UHP solution in MeOH (2.5 eq., 15 nmol). The reaction was run for 17 hours at 4 °C. The reaction mixture was then concentrated, re-dissolved in 100 µL of 1:9 MeOH/H_2O (0.1% formic acid) and subjected to HPLC analysis using gradient D. The ratio between (*R*) and (*S*) sulfoxides was determined by integrating HPLC signals (~4% of the starting material remained unreacted after 17 hours). A diastereomeric distribution of 6.4:1 (*R*:*S*) was achieved and two sulfoxides were obtained in 96% yield (HPLC calculated yield, ~5.8 nmol).

Note: solvent conditioning greatly affected the reaction outcome. DCM was freshly distilled over CaH and used within a day, while MeOH was used as is (Sigma, HPLC grade).

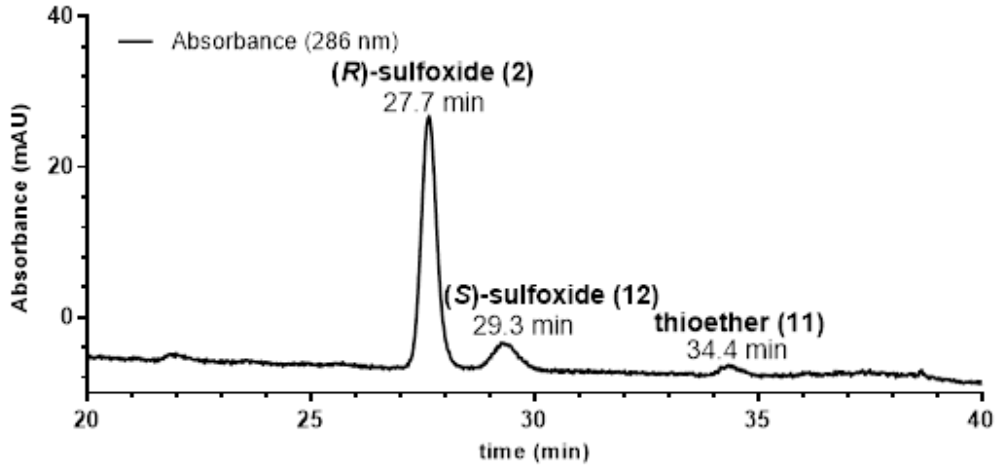
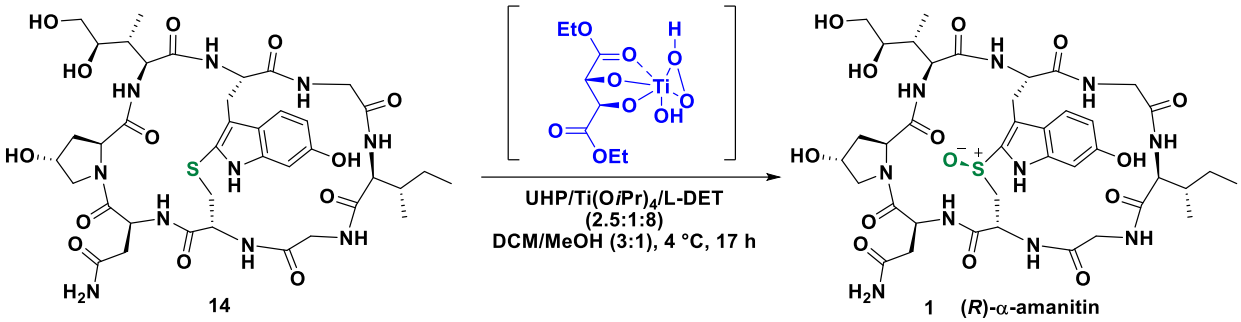


Figure S5. HPLC chromatogram of asymmetric sulfoxidation of bicyclic octapeptide **11** in the presence of UHP/Ti(O*i*Pr)$_4$/L-DET in 2.5:1:8 ratio

Diastereoselective sulfoxidation of S-deoxy-6'-OH-amanitin 14 in the presence of UHP/Ti(OiPr)4/L-DET to synthesize (*R*)-α-amanitin 1



A small Eppendorf tube containing bicyclic S-deoxy-6'-OH-amanitin **14** (1 eq., 6 nmol; synthesized according to previously reported procedure[13]) dissolved in 1.7 µL of MeOH was cooled to 4 °C. Compound **14** was then treated with 7.2 µL of DCM solution containing Ti(O*i*Pr)$_4$ (1 eq., 6 nmol) and L-DET (8 eq., 48 nmol). The reaction mixture was kept at 4 °C for 30 mins and then treated with 1.0 µL of UHP solution in MeOH (2.5 eq., 15 nmol). The reaction was run for 17 hours at 4 °C. The reaction mixture was then concentrated, re-dissolved in 100 µL of 1:9 MeOH/H_2O(0.1% formic acid) and subjected to HPLC analysis using gradient D. Upon HPLC analysis only (*R*)-6'-OH-amanitin i.e. α-amanitin (**1**) was detected as a product in 84% yield (HPLC calculated yield, ~5 nmol). Approximately 16% of the starting material remained unreacted after 17 hours.

Note: solvent conditioning greatly affected the reaction outcome. DCM was freshly distilled over CaH and used within a day, while MeOH was used as is (Sigma, HPLC grade).

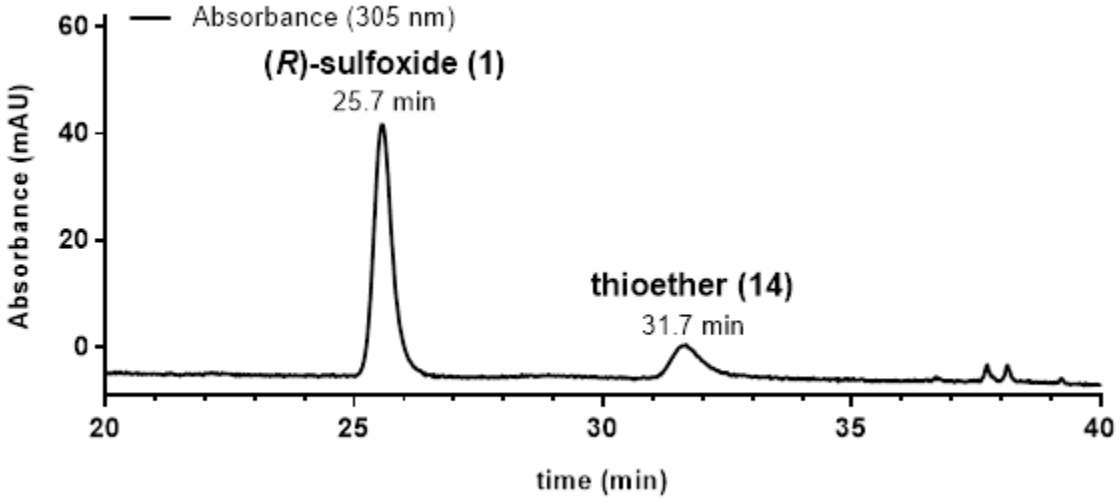


Figure S6. HPLC chromatogram of asymmetric sulfoxidation of bicyclic octapeptide **14** with UHP in the presence of Ti(IV) and L-DET to give α-amanitin.

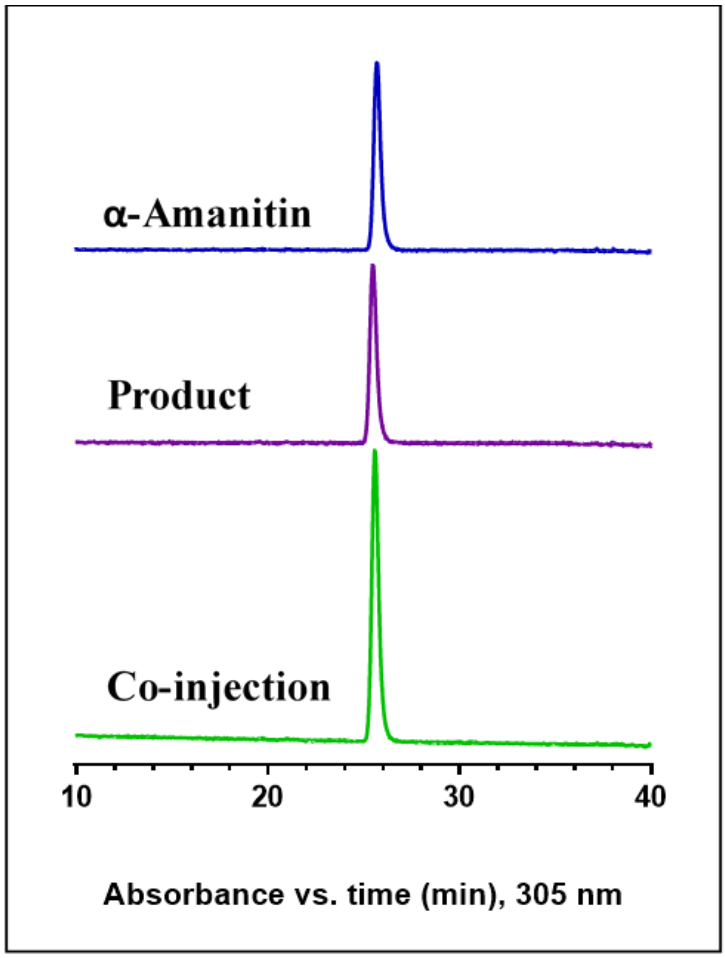
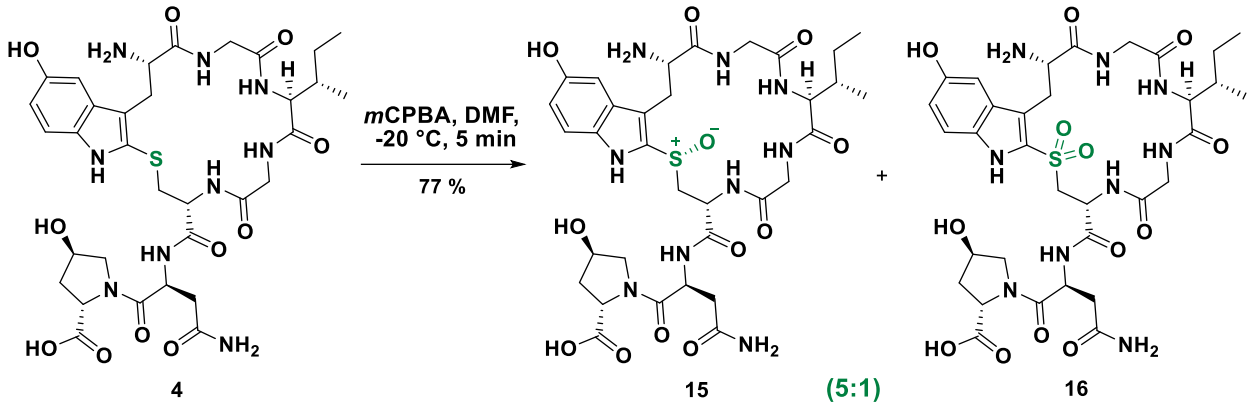


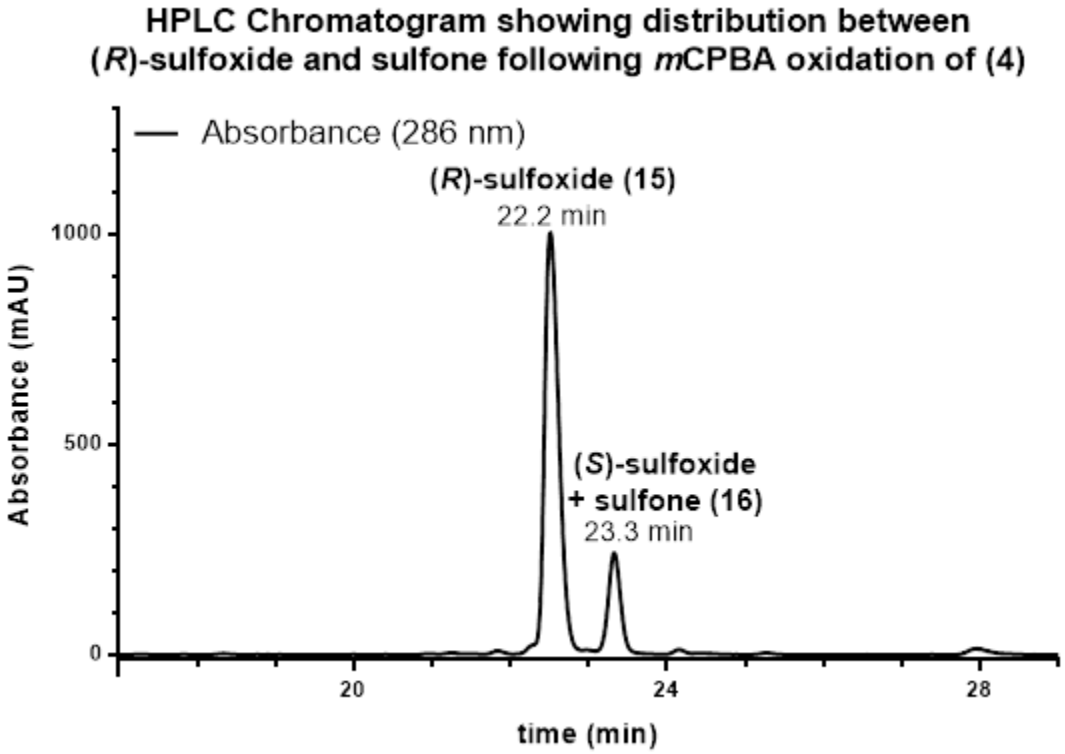
Figure S7. HPLC co-injection of α-amanitin (Sigma) and product obtained via asymmetric sulfoxidation of bicyclic octapeptide **14** with UHP in the presence of Ti(IV) and L-DET confirming (*R*)-α-amanitin formation; HPLC analysis was performed with gradient D.

Sulfoxidation of 5'-OH-heptapeptide monocycle **4**

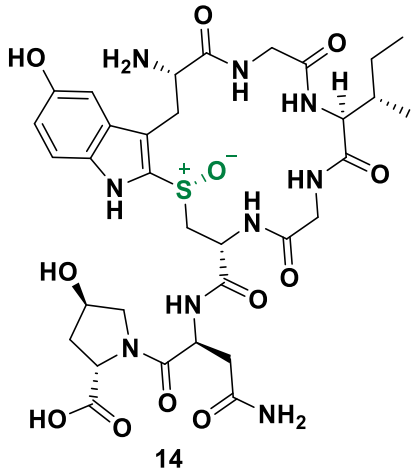


An Eppendorf tube containing S-deoxy-5'-OH-heptapeptide monocycle **4** (1 eq., 0.78 mg, 1.0 µmol) dissolved in 278 µL of DMF was cooled to -20 °C. The reaction mixture was then treated with 33 µL of a cooled *m*CPBA solution (DMF, 1.5 eq., 0.26 mg, 1.5 µmol). The reaction mixture was kept at -20 °C for 5 mins, then diluted with 2 mL of H_2O (0.1% formic acid) and subjected to HPLC purification (gradient F) to give (*R*)-5'-OH-heptapeptide **15** as a white solid in 77% yield (0.6 mg, 0.77 µmol). The ratio between (*R*)-sulfoxide **15** (peak 1) and inseparable (*S*)-sulfoxide/sulfone **16** (peak 2) was determined by integrating HPLC signals (5:1). The oxidation state of the products was determined by mass spectrometry. Extinction coefficient of 12,600 $M^{-1}cm^{-1}$ (290 nm) was used for quantification.

Note: *m*CPBA reagent purity varies batch to batch, therefore, a small-scale reaction should be carried out first to determine an appropriate amount of reagent required to avoid overoxidation.



Synthesis and characterization of (*R*)-5'-OH-heptapeptide monocycle **15**



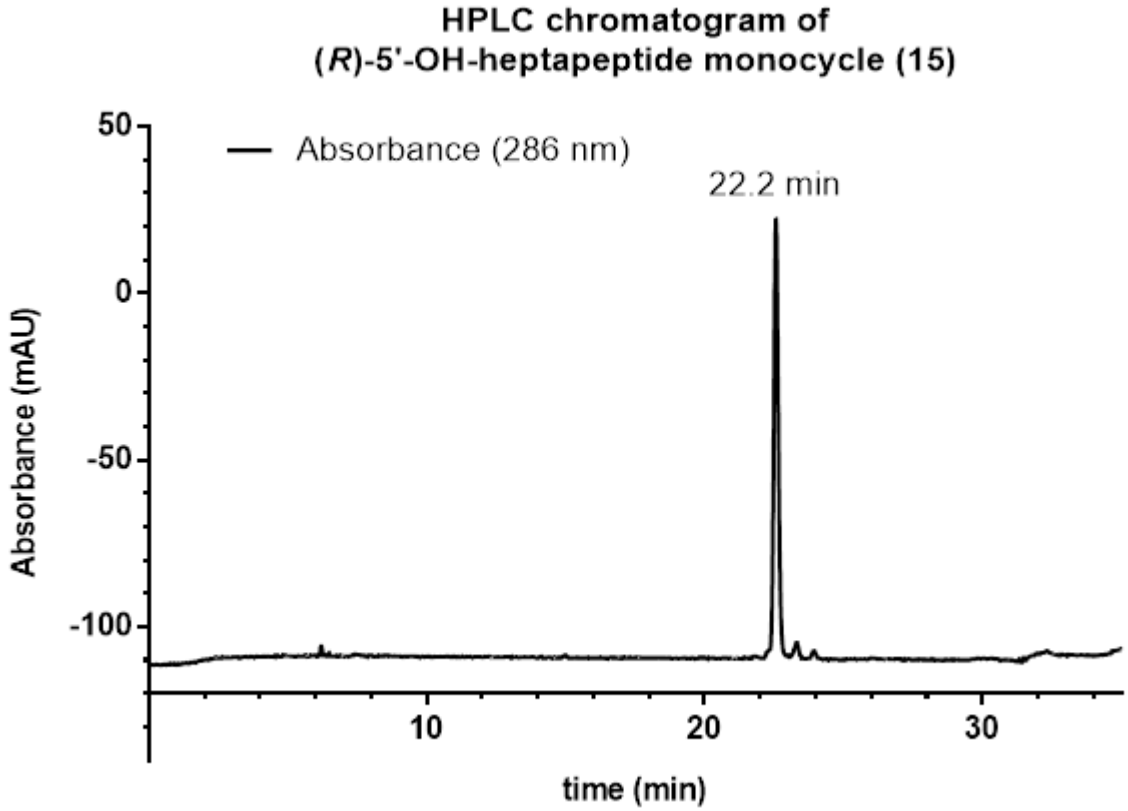
14

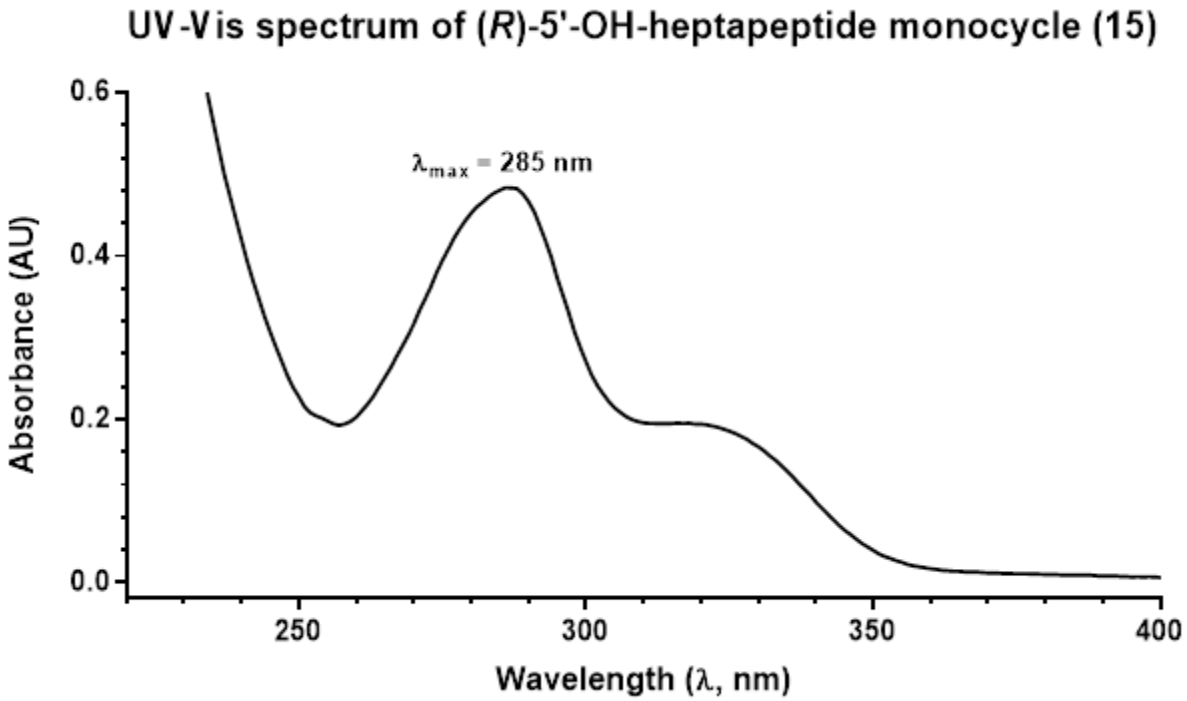
Chemical Formula: $C_{33}H_{45}N_9O_{12}S$
Exact Mass: 791.29084
Molecular Weight: 791.83400

(*R*)-5'-OH-heptapeptide monocycle (15)

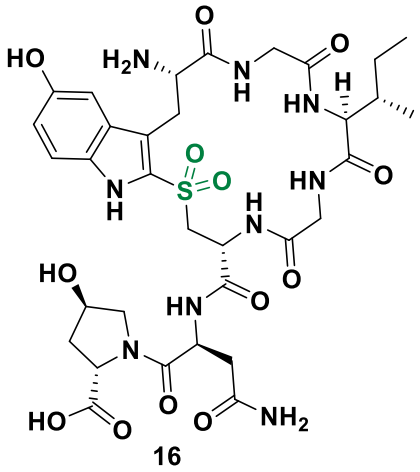
HRMS (ESI-TOF, m/z): $[M+H]^+$ found 792.2985; calc. 792.2987 for $C_{33}H_{46}N_9O_{12}S$.

HPLC (gradient F) $\mathbf{t_R}$ = 22.2 min; λ_{max} = 285 nm.




UV-Vis spectrum of (R)-5'-OH-heptapeptide monocycle (15)
λmax = 285 nm
Absorbance (AU)
0.0
0.2
0.4
0.6
250
300
350
400
Wavelength (λ, nm)

Synthesis and characterization of SO_2-5'-OH-heptapeptide monocycle **16**



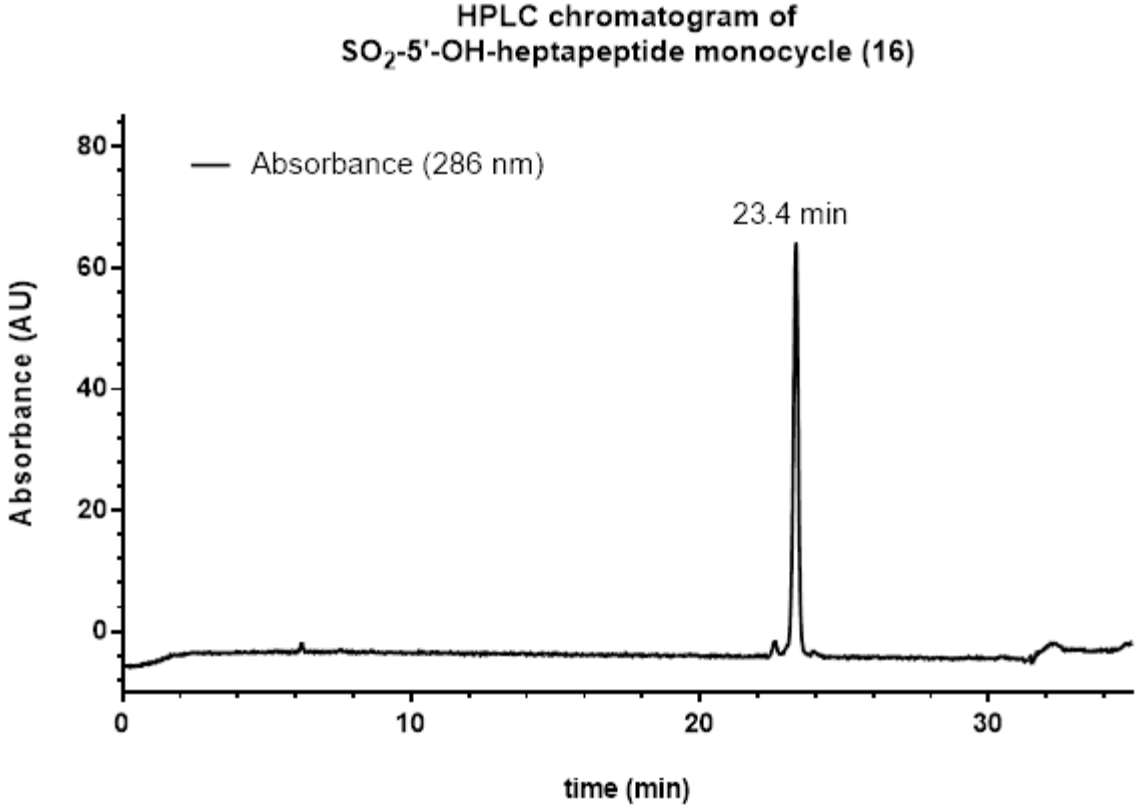
16

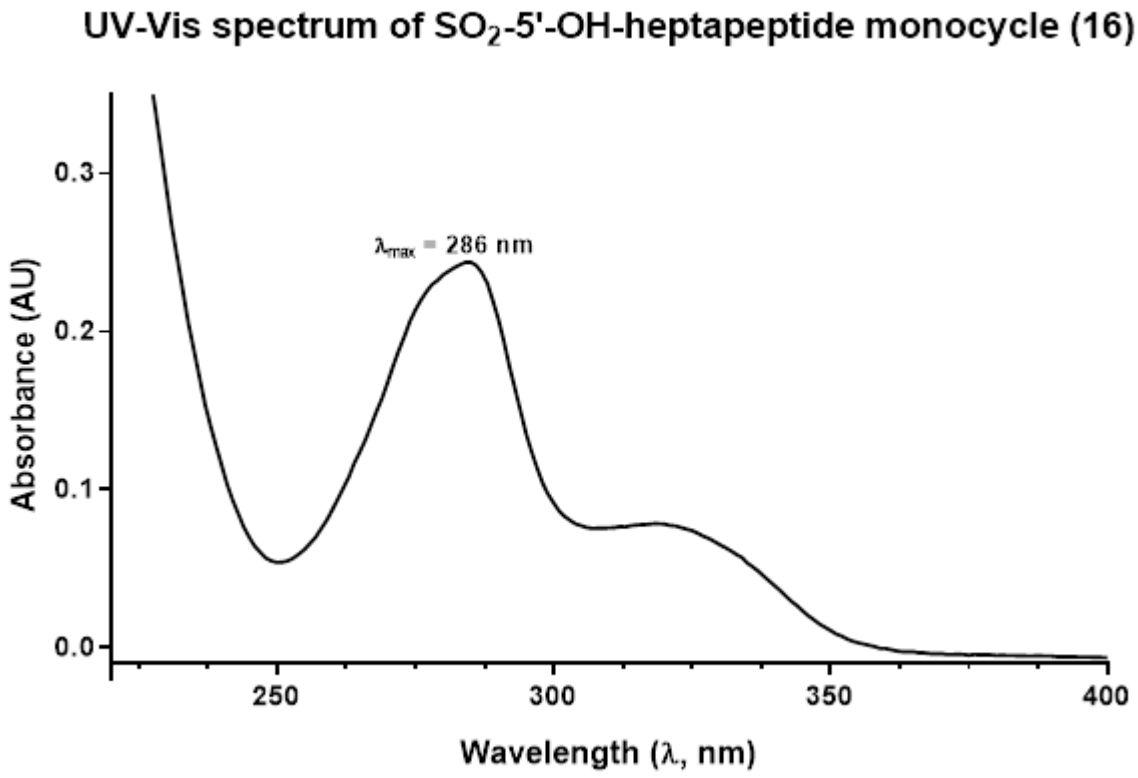
Chemical Formula: $C_{33}H_{45}N_9O_{13}S$
Exact Mass: 807.28575
Molecular Weight: 807.83300

SO_2-5'-OH-heptapeptide monocycle (16)

HRMS (ESI-TOF, m/z): $[M+H]^+$ found 808.2922; calc. 808.2930 for $C_{33}H_{46}N_9O_{13}S$.

HPLC (gradient F) $\mathbf{t_R}$ = 23.4 min; λ_{max} = 286 nm.

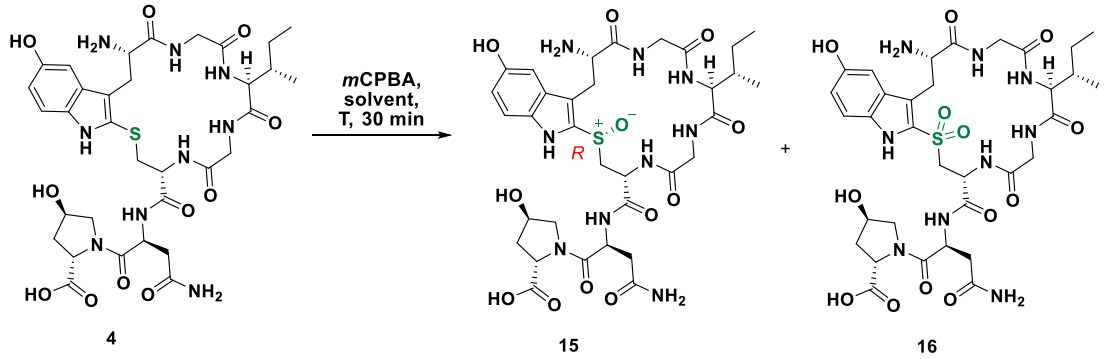



UV-Vis spectrum of SO2-5'-OH-heptapeptide monocycle (16)
λmax = 286 nm
Absorbance (AU)
0.0
0.1
0.2
0.3
250
300
350
400
Wavelength (λ, nm)

Optimization of 5'-OH-heptapeptide **4** sulfoxidation with *m*CPBA

General procedure:
An Eppendorf tube containing S-deoxy-5'-OH-heptapeptide monocycle **4** (1 eq.) was dissolved in a solvent of choice and cooled to an appropriate temperature when necessary. The reaction mixture was then treated with *m*CPBA (1.5 or 0.3 eq.) dissolved in the reaction solvent. The reaction was run for 30 mins, then diluted with H_2O (0.1% formic acid) and subjected to HPLC purification using gradient F to give (*R*)-5'-OH-heptapeptide monocycle (**15**) and an inseparable mixture of (S)-sulfoxide/SO_2-5'-OH-heptapeptide monocycle (**16**). The ratio between (*R*)-sulfoxide and sulfone was determined by integrating HPLC signals. The oxidation state of the products was determined by mass spectrometry. In all cases, no other side-products were observed. The results are summarized in **Table S4** (see below).
Note: *m*CPBA reagent purity varies batch to batch, therefore, a small-scale reaction should be carried out first to determine an appropriate amount of reagent required to avoid overoxidation.

Table S4. Optimization of heptapeptide sulfoxidation with *m*CPBA.

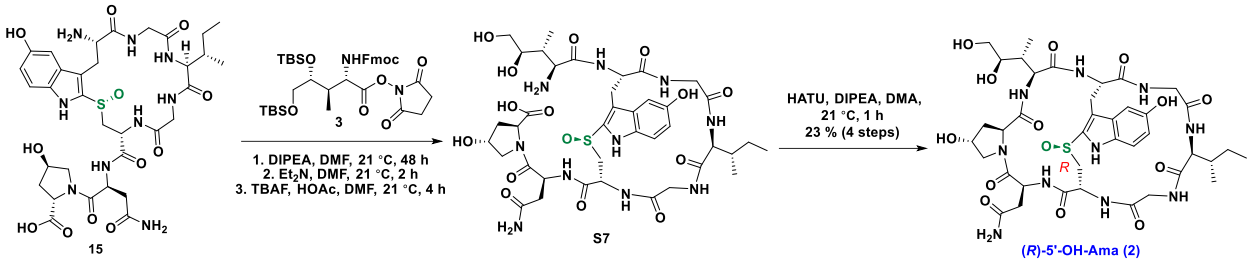


4 **15** **16**

Entry	Solvent	Temperature (°C)	Equiv. *m*CPBA	Scale (nmol of **4**)	(*R*)-sulfoxide, (%)	(*S*)-sulfoxide+ sulfone (%)	SM **4** (%)
1	ACN/H_2O (6.5:1)	21	1.5	30	27	73	-
2	*i*PrOH/MeOH (2:1)	21	1.5	30	31	69	-
3	*i*PrOH	21	1.5	30	31	69	-
4	EtOH	21	1.5	30	40	60	-
5	Acetic acid	21	1.5	30	40	60	-
6	TFE/H_2O (3:1)	21	1.5	30	39	61	-
7	MeOH	21	1.5	30	39	61	-
8	DMA	21	1.5	30	50	50	-
9	H_2O/DMF (14.9:0.1)	21	1.5	30	54	46	-
10	NMP	21	1.5	30	60	40	-
11	DMF	21	1.5	30	62	38	-
12	DCM/DMF (2:1)	21	1.5	30	32	68	-
13	DMF/toluene (2.7:1)	21	1.5	30	41	59	-
14	DMF	0	1.5	30	78	22	-
15	DMF	-20	1.5	30	79	21	-
16	DMF	-10	1.5	1000	83	17	-
17	DMF	-10	0.4	30	27	10	63

*SM = starting material

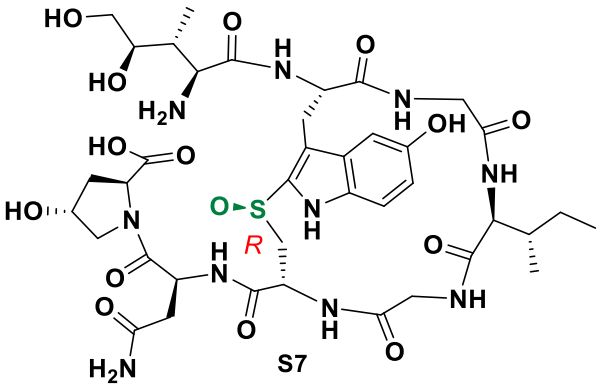
Synthesis of (*R*)-5'-OH-6'-deoxy-amanitin **2** from (*R*)-5'-OH-heptapeptide monocycle **15**



Scheme S3. Synthesis of (*R*)-5'-OH-6'-deoxy-amanitin **2** from (*R*)-5'-OH-heptapeptide monocycle **15**: coupling of DHIle, Fmoc- and TBS-deprotection, followed by macrolactamization to give (*R*)-5'-OH-6'-deoxy-amanitin **2**

(*R*)-5'-OH-octapeptide monocycle (**S7**). To a 2-mL Eppendorf tube containing (2*S*,3*R*,4*R*)-O^{γ},O^{δ}-bis-TBS-N^{α}-Fmoc-dihydroxyisoleucine-NHS **3** (4.2 eq., 3.3 mg, 4.2 µmol) added (*R*)-5'-OH-heptapeptide monocycle **15** (1 eq., 0.8 mg, 1 µmol) dissolved in 40 µL of anhydrous DMF. The solution was vortexed and then treated with 2.5 µL of DIPEA (14.7 eq., 1.9 mg, 14.7 µmol) to achieve pH 8.5. The reaction was stirred for 48 hours at 21 °C, while monitoring the progress with mass spectrometry and HPLC. After 48 hours, 4.8 µL of Et_2NH (46 eq., 3.4 mg, 46 µmol) were added to the reaction mixture and reaction contents were stirred for 2 hours. The reaction progress was monitored by mass spectrometry and HPLC. Following the completion of Fmoc-deprotection, the reaction mixture was treated with 0.7 µL of acetic acid and 12 µL of 1 M TBAF in THF (12 eq., 12 µmol). The reaction mixture was stirred for 4 hours at 21 °C. DMF was then removed under reduced pressure and the concentrate was purified using Sep-Pak C18 (10-g) cartridge (gradient elution, 99.9:0.1 H_2O/formic acid to 89.9:10:0.1 H_2O/ACN/formic acid to 75.9:24:0.1 H_2O/ACN/formic acid). The fractions were analyzed by HPLC using gradient A. The product containing fractions were lyophilized to obtain monocyclic octapeptide **S7** (~0.4 mg, 0.43 µmol, 43% yield over 3 steps). The product was quantified using tryptathionine as a chromophore with the extinction coefficient of 12,600 $M^{-1}cm^{-1}$ at $\lambda_{max,}$~286 nm (MeOH).

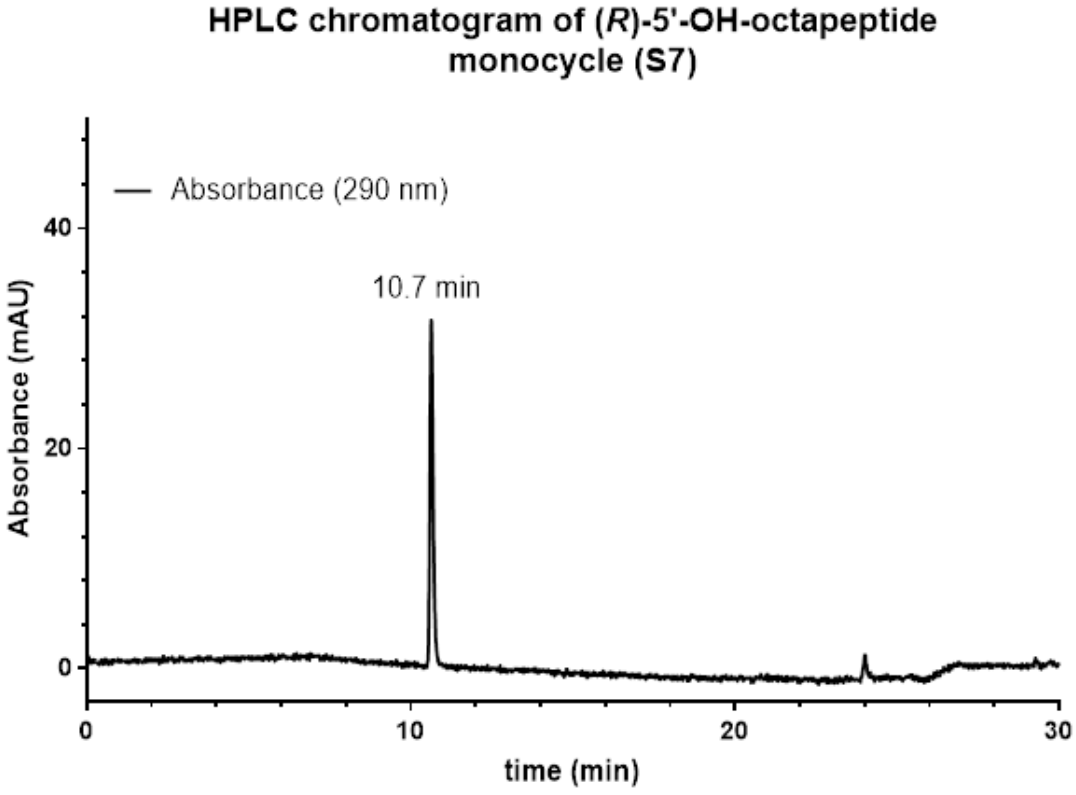
Synthesis of (*R*)-5'-OH-octapeptide monocycle **S7**



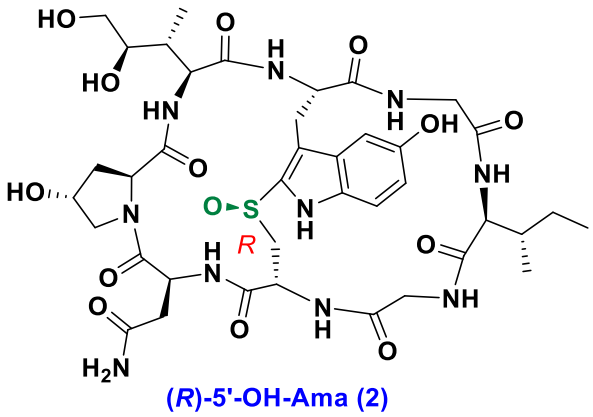
Chemical Formula: $C_{39}H_{56}N_{10}O_{15}S$
Exact Mass: 936.36473
Molecular Weight: 936.99200

HRMS (ESI-TOF, m/z): $[M+H]^+$ found 937.3722; calc. 937.3720 for $C_{39}H_{57}N_{10}O_{15}S$.

HPLC (gradient A) $\mathbf{t_R}$ = 10.7 min; λ_{max} = 286 nm.



Synthesis and characterization of (*R*)-5'-OH-6'-deoxy-amanitin **2** obtained from (*R*)-5'-OH-heptapeptide monocycle **15**



(*R*)-5'-OH-6'-deoxy-amanitin (2). (*R*)-5'-OH-octapeptide monocycle **S7** (1 eq., 0.4 mg, 0.43 µmol) was dissolved in 114 µL of dimethyacetamide. HATU (10 eq., 4 µmol, 5.3 mg) and DIPEA (10 eq., 14.6 µmol, 2.5 µL) were added to the reaction mixture and the reaction was run for 1 hour at 21 °C. Upon completion, the crude material was purified by HPLC using gradient D to obtain compound **2** (0.23 µmol, 23% yield over 4 steps from **15**). The product was quantified using tryptathionine as a chromophore with the extinction coefficient of 12,600 $M^{-1}cm^{-1}$ at λ_{max},~286 nm (in MeOH). The product was subjected to an HPLC co-injection with compound **2** obtained via a previously reported *m*CPBA sulfoxidation[13] of the bicyclic precursor **11** (see **Figure S8**).

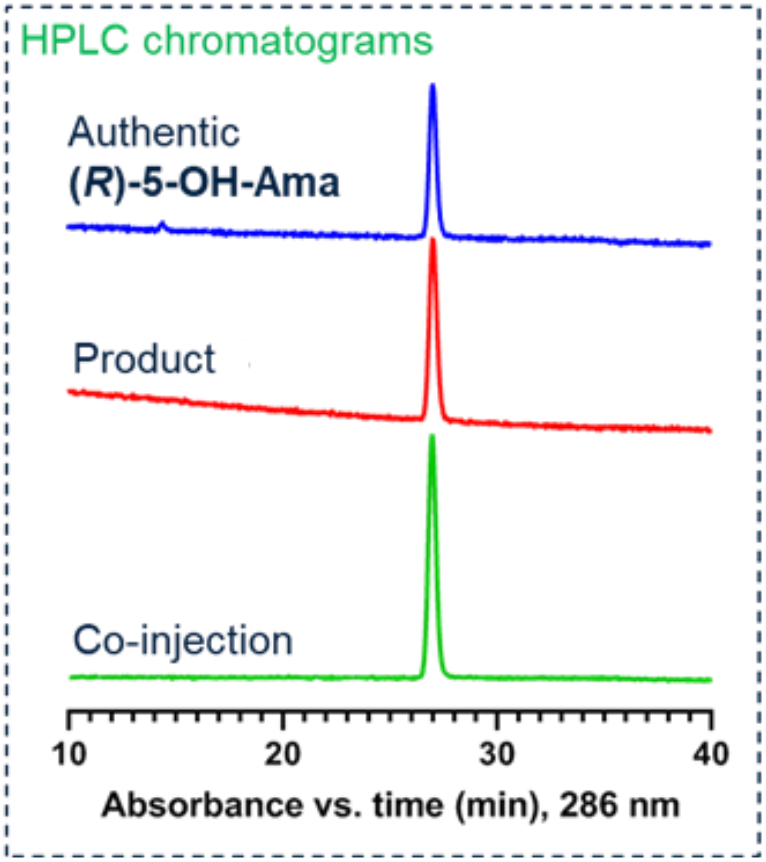
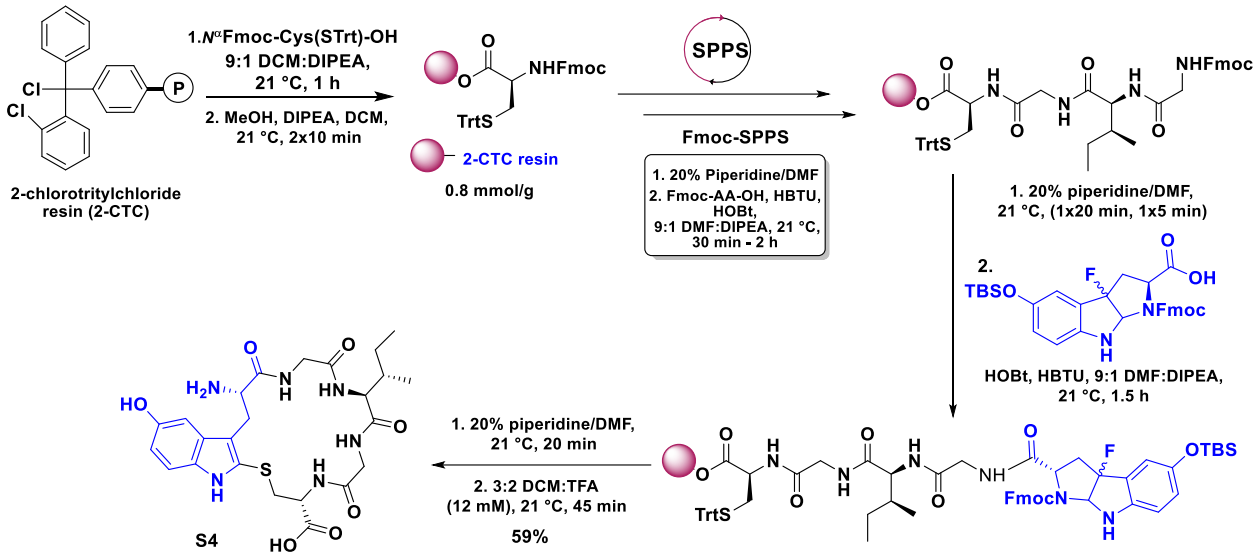


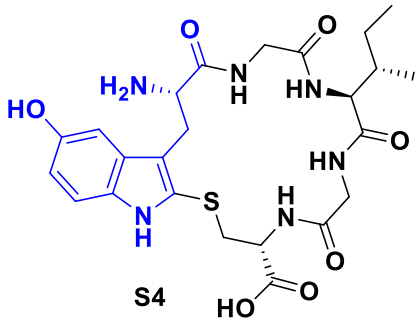
Figure S8. HPLC chromatogram of the sulfoxide containing 5'-OH-6'-deoxy-amanitin product obtained through compound **15** and co-injection with (*R*)-5'-OH-6'-deoxy-amanitin **2** produced via a reported *m*CPBA oxidation route[13] confirming (*R*)-sulfoxide formation during heptapeptide sulfoxidation; HPLC analysis was performed with gradient D.

Synthesis and characterization of S-deoxy-5'-OH-pentapeptide monocycle **S4**



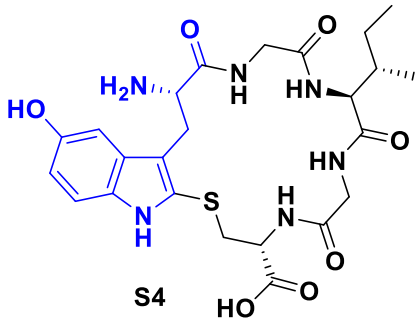
Scheme S4. Solid-phase synthesis of S-deoxy-5'-OH-pentapeptide monocycle **S4**.

Solid phase synthesis of 2-CTC-Cys(STrt)GlyIleGly*N*HFmoc. 2-Chlorotrityl chloride resin (10 g) was dried over P_2O_5 under reduced pressure for 17 hours prior to use. A peptide synthesis glass vessel (Chemglass, 250-mL) adapted with an Ar(g) flow inlet and a medium size frit filter was charged with the dried 2-chlorotrityl chloride resin (10 g, 1.2 mmol/g loading) and 100 mL of anhydrous DCM. The resin was agitated for 25 mins with a flow of Ar(g). The solvent was then filtered, replaced with another 100 mL portion of anhydrous DCM and the resin was washed for 3 mins. The first amino acid loading was performed in the following manner: 14.4 mmol of Fmoc-Cys(STrt)-OH (1.2 eq.) were dissolved in 85 mL of 9:1 DMC:DIPEA. The reaction was run for 1 hour and the resin was washed with DCM (3x3 minx100 mL). The resin was capped with 100 mL of 17:2:1 DCM:MeOH:DIPEA solution for 20 mins and then washed with DCM (5x3 minx100 mL) followed by DMF (5x3 minx100 mL). The Fmoc-deprotection was performed using 100 mL of 20% piperidine/DMF solution for 20 mins, followed by another 5-minute treatment with 100 mL of 20% piperidine/DMF solution. The resin was washed with DMF (3x5 minx100 mL) and the dibenzofulvene-peperidine adduct was quantified by ultraviolet-visible (UV-Vis) spectroscopy. The absorption measurement was performed at 301 nm using extinction coefficient (ε) of 7800 $M^{-1}cm^{-1}$. Resin loading of ~ 0.8 mmol/g was obtained. The resin was then washed with DMF (5x5 minx100 mL) and the next amino acid was coupled. The coupling was performed with 4.1 eq. of *N*$^{\alpha}$Fmoc-Gly-OH, 4 eq. of HBTU and 4 eq. of HOBt in 65 mL of 9:1 DMF:DIPEA. The coupling cocktail was pre-activated for 5 mins prior to addition to the resin. The coupling reaction was run for 30 min – 2 hours and the completion of the reaction was monitored using Kaiser test. The resin was then washed with DMF (5x5 minx100 mL) and Fmoc-deprotection and amino acid coupling steps were performed as described above to obtain **2-CTC-Cys(STrt)GlyIleGly*N*HFmoc**.



Chemical Formula: $C_{24}H_{32}N_6O_7S$
Exact Mass: 548.21
Molecular Weight: 548.62

Monocyclic S-deoxy-5'OH-pentapeptide S4. Fmoc-FPI(5OTBS)-OH **6a/b** (2.05 eq., 5.05 g, 8.7 mmol) was dissolved in 44 mL of 9:1 DMF:DIPEA followed by HOBt (2 eq., 1.15 g, 8.5 mmol), and HATU (2.0 eq., 3.23 g, 8.5 mmol). The coupling cocktail was pre-activated for 5 mins. The resin-loaded tetrapeptide, **2-CTC-Cys(STrt)GlyIleGly*N*HFmoc**, was Fmoc-deprotected using 50 mL of 20% piperidine/DMF solution for 20 mins, followed by another 5-minute treatment with 50 mL of 20% piperidine/DMF solution and DMF resin wash (3x5 minx50 mL). **2-CTC-Cys(STrt)GlyIleGly-NH_2** (1 eq., 4.24 mmol, based on the last Fmoc-deprotection step) was treated with the coupling cocktail. The reaction was agitated using Ar(g) flow for 1.5 hour at 21 °C. The reaction completion was confirmed by TLC (89:10:1 DCM:MeOH:acetic acid) analysis performed on a small amount of the product cleaved from the resin with 4:1 DCM:hexafluoroisopropanol. Upon reaction completion, the resin was washed with DMF (5 x 5 min x50 mL). The Fmoc-deprotection was performed using 50 mL of 20% piperidine/DMF solution for 20 mins followed by DMF (5 x 5 min x 50 mL) and DCM (5 x 5 min x 50 mL) washes.
The resin was split into two batches and transferred into two 500-mL round bottom flasks each equipped with a stir-bar. The Savige-Fontana reaction was carried out in two 500-mL round bottom flasks by adding 280 mL of 3:2 DCM:TFA to the resin (**safety precaution**: TFA is a strong acid, that can cause severe burns to the skin and is very volatile, take caution when handling). The resin was gently stirred with for 45 mins at 21 °C. Upon completion the amber-coloured clear reaction mixture was concentrated under reduced pressure. The reaction concentrate was re-dissolved in 60 mL of TFA:H_2O:TIPS (93:5:2, v:v:v) and stirred gently for 40 mins at 21 °C. The clear and amber-coloured reaction mixture was then concentrated under reduced pressure, the resin was filtered and washed with DCM (5x4 minx40 mL) and TFA (1x3 minx30 mL). The combined washes were concentrated under reduced pressure to ~20 mL and the product was triturated with cold diethyl ether. The precipitate was purified using Sep-Pak C18 packed column (~42 g C18 column, gradient elution, 99.9:0.1 H_2O/formic acid to 89.9:10:0.1 H_2O/ACN/formic acid to 87.9:12:0.1 H_2O/ACN/formic acid) and the product containing fractions were lyophilized to obtain **S4** (1.38 g, 2.55 mmol, 59% yield calculated based on initial resin loading) as a beige solid.



Chemical Formula: $C_{24}H_{32}N_6O_7S$
Exact Mass: 548.21
Molecular Weight: 548.62

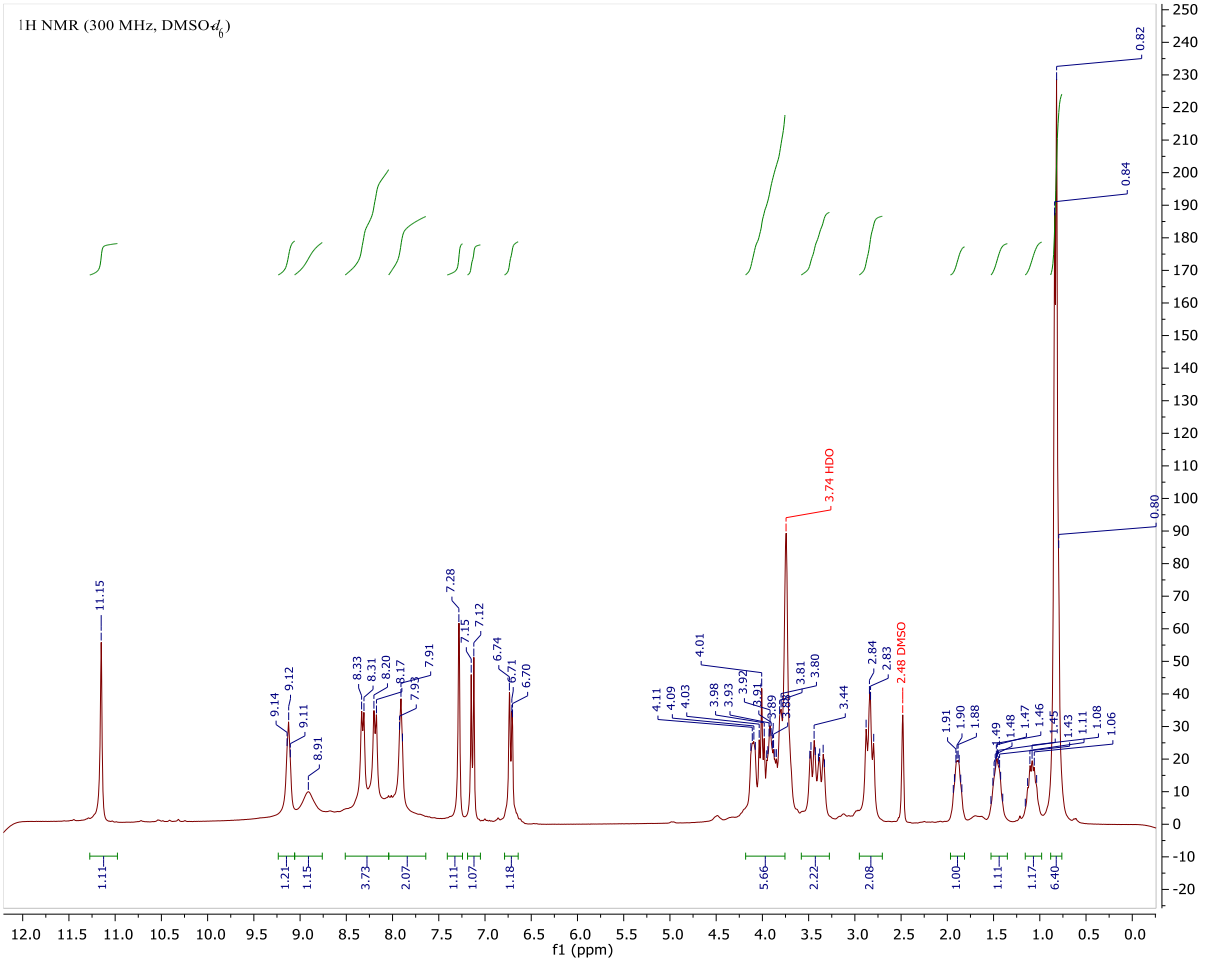
S-deoxy-5'-OH-pentapeptide monocycle (S4)

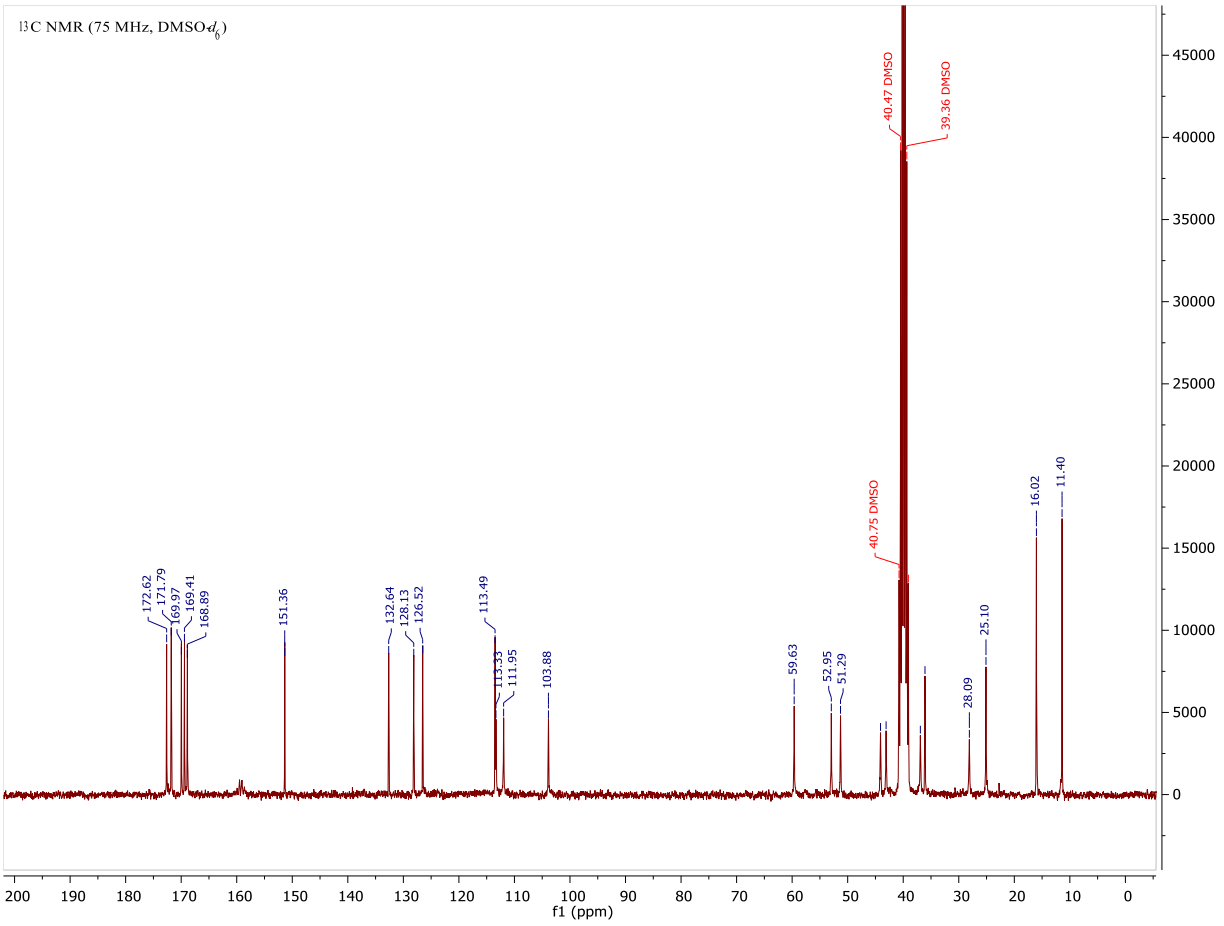
^{1}H NMR (300 MHz, DMSO-d_6) δ 11.15 (s, 1H), 9.12 (t, *J* = 5.3 Hz, 1H), 8.91 (s, 1H), 8.25 (m, 4H), 7.91 (t, *J* = 4.9 Hz, 2H), 7.28 (s, 1H), 7.14 (d, *J* = 8.5 Hz, 1H), 6.79 – 6.64 (m, 1H), 4.18 – 3.76 (m, 6H), 3.41 (ddd, *J* = 29.7, 14.6, 3.9 Hz, 2H), 2.95 – 2.70 (m, 2H), 1.90 (dh, *J* = 12.2, 4.9, 3.7 Hz, 1H), 1.53 – 1.35 (m, 1H), 1.08 (dq, *J* = 15.2, 7.5 Hz, 1H), 0.83 (m, 6H).

^{13}C NMR (75 MHz, DMSO-d_6) δ 11.40, 16.02, 25.10, 28.09, 36.08, 36.92, 43.08, 44.09, 51.29, 52.95, 59.63, 103.88, 111.95, 113.33, 113.49, 126.52, 128.13, 132.64, 151.36, 168.89, 169.41, 169.97, 171.79, 172.62.

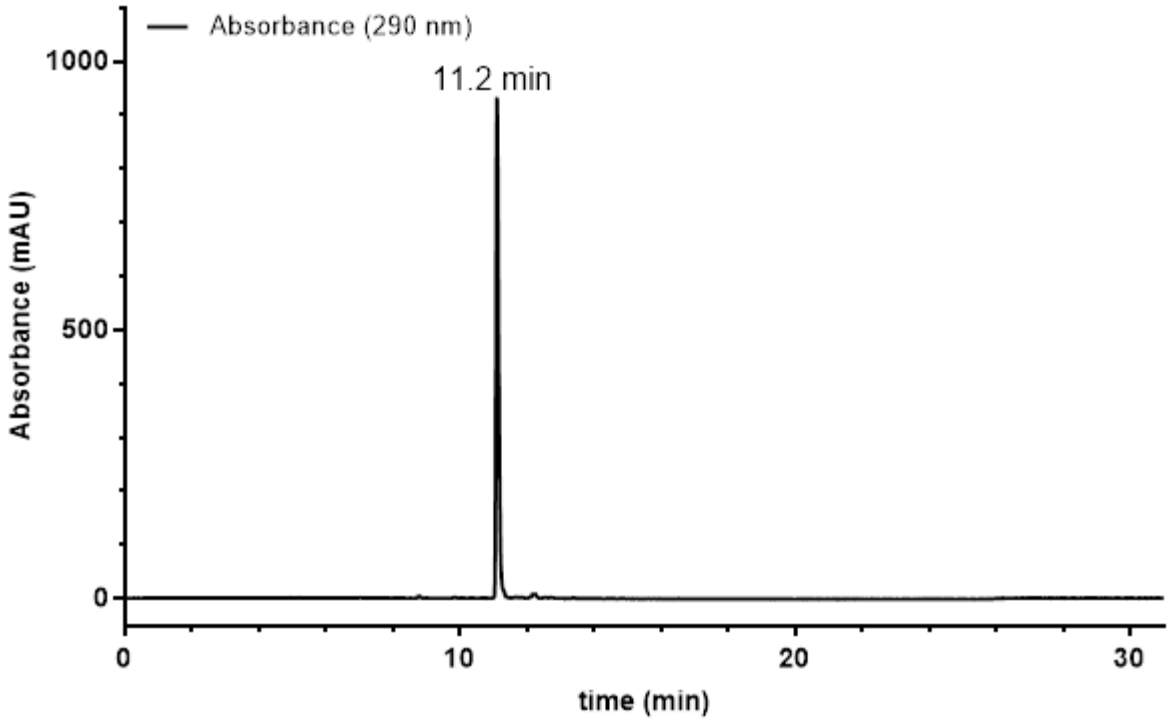
HRMS (ESI-TOF, m/z): $[M+H]^+$ found 548.2053; calc. 548.2048 for $C_{24}H_{32}N_6O_7S$ (ion species detected $C_{24}H_{33}N_6O_7S$, $[M+H]^+$).

HPLC (gradient A) $\mathbf{t_R}$ = 11.2 min; λ_{max} = 288 nm.

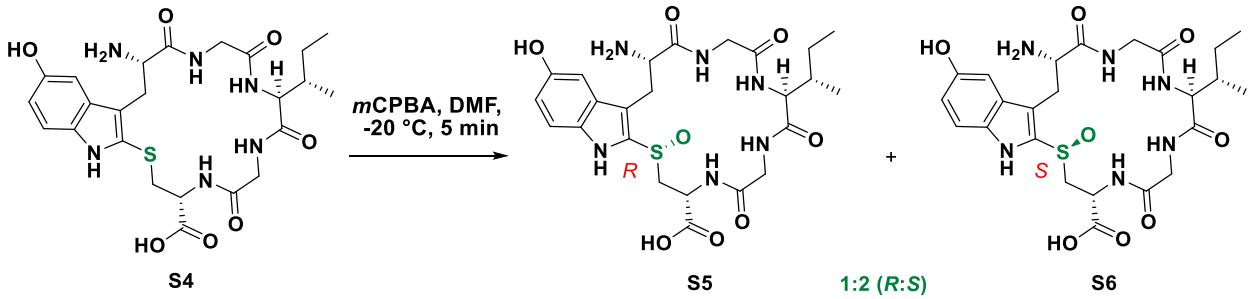

1H NMR (300 MHz, DMSO-d_6)
11.15
9.14
9.12
9.11
8.91
8.33
8.31
8.20
8.17
7.93
7.91
7.28
7.15
7.12
6.74
6.71
6.70
4.11
4.09
4.03
4.01
3.98
3.93
3.92
3.91
3.89
3.88
3.81
3.80
3.74 HDO
3.44
2.84
2.83
2.48 DMSO
1.91
1.90
1.88
1.49
1.48
1.47
1.46
1.45
1.43
1.11
1.08
1.06
0.82
0.84
0.80
1.11
1.21
1.15
3.73
2.07
1.11
1.07
1.18
5.66
2.22
2.08
1.00
1.11
1.17
6.40
f1 (ppm)


13C NMR (75 MHz, DMSO-d6)
172.62
171.79
169.97
169.41
168.89
151.36
132.64
128.13
126.52
113.49
113.33
111.95
103.88
59.63
52.95
51.29
40.75 DMSO
40.47 DMSO
39.36 DMSO
28.09
25.10
16.02
11.40
f1 (ppm)

HPLC chromatogram of
5-OH-pentapeptide monocycle (S4)



Sulfoxidation of monocyclic 5'-OH-pentapeptide **S4**



An Eppendorf tube containing S-deoxy-5'-OH-pentapeptide monocycle **S4** (1 eq., 55 µg, 100 nmol) dissolved in 25 µL of DMF was cooled to -20 °C. The reaction mixture was then treated with 18.5 µL of a cooled solution of *m*CPBA (DMF, 0.9 eq., 90 nmol). The reaction was run at -20 °C for 5 mins, then diluted with H_2O (0.1% formic acid) and subjected to HPLC analysis using gradient F. The ratio between (*R*)- and (*S*)-sulfoxides was determined by integrating HPLC signals (1:2). The oxidation state of the products was determined by mass spectrometry and the stereochemistry was assigned based on the HPLC elution pattern.
Note: *m*CPBA reagent purity varies batch to batch, therefore, a small-scale reaction should be carried out first to determine an appropriate amount of reagent required to avoid overoxidation.

(*R*)-5'-OH-Pentapeptide monocycle (S5)

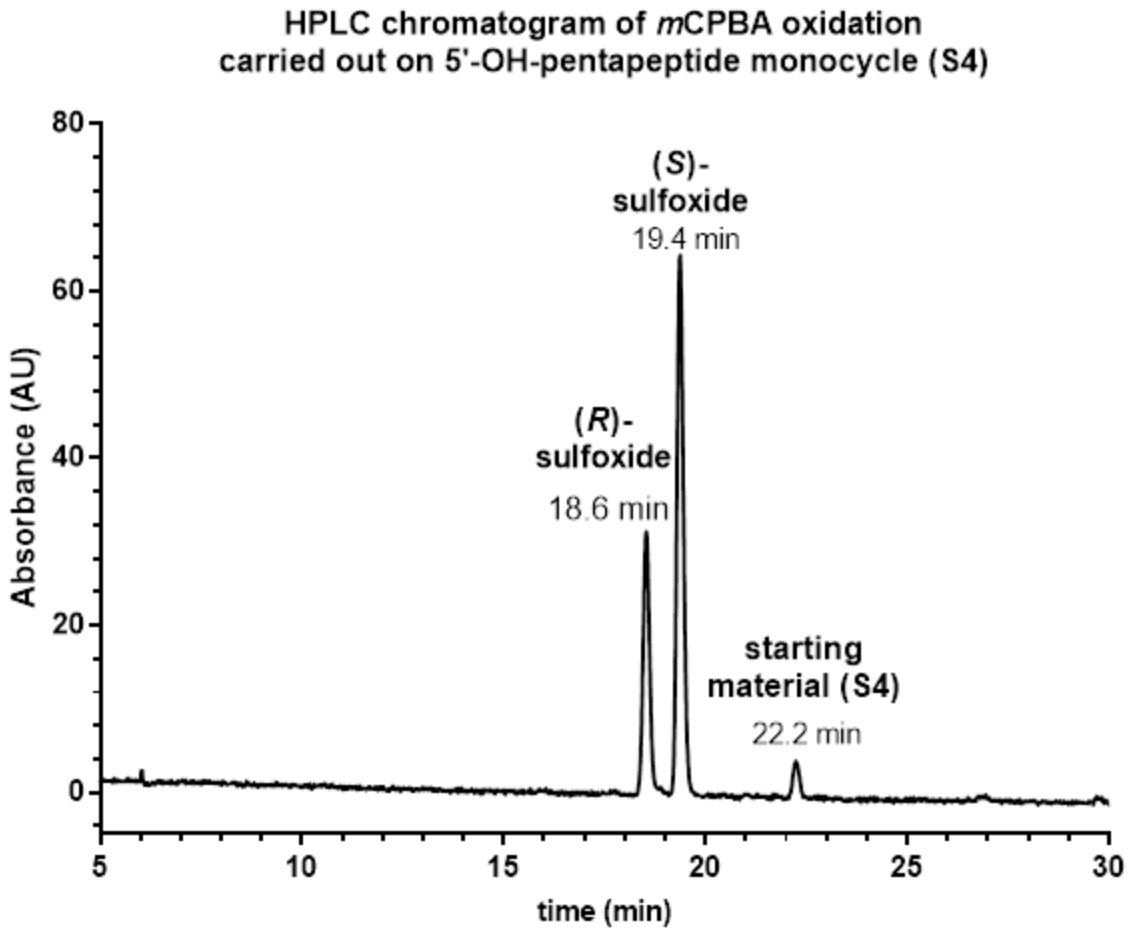
LRMS (ESI, m/z): $[M+H]^+$ found 565.2; calc. 565.2 for $C_{24}H_{33}N_6O_8S$.

HPLC (gradient F) $\mathbf{t_R}$ = 18.6 min.

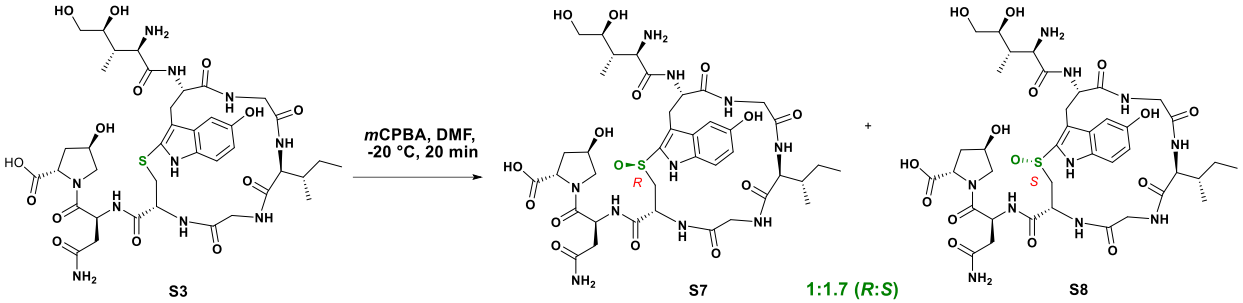
(*S*)-5'-OH-Pentapeptide monocycle (S6)

LRMS (ESI, m/z): $[M+H]^+$ found 565.3; calc. 565.2 for $C_{24}H_{33}N_6O_8S$.

HPLC (gradient F) $\mathbf{t_R}$ = 19.4 min.


HPLC chromatogram of mCPBA oxidation
carried out on 5'-OH-pentapeptide monocycle (S4)
Absorbance (AU)
time (min)
(S)-
sulfoxide
19.4 min
(R)-
sulfoxide
18.6 min
starting
material (S4)
22.2 min
0
20
40
60
80
5
10
15
20
25
30

Sulfoxidation of monocyclic 5'-OH-octapeptide **S3**



An Eppendorf tube containing S-deoxy-5'-OH-octapeptide monocycle **S3** (1 eq., 46 µg, 50 nmol) dissolved in 48 µL of DMF was cooled to -20 °C. The reaction contents were then treated with *m*CPBA (1.0 eq., 50 nmol) dissolved in 2.2 µL of cooled DMF. The reaction was run at -20 °C for 20 mins, then diluted with H_2O (0.1% formic acid) and subjected to HPLC analysis using gradient G. The ratio between (*R*)-sulfoxide and (*S*)-sulfoxide was determined by integrating HPLC signals (1:1.7). The oxidation state of the products was determined by mass spectrometry and the stereochemistry was assigned based on the HPLC elution pattern.
Note: *m*CPBA reagent purity varies batch to batch, therefore, a small-scale reaction should be carried out first to determine an appropriate amount of reagent required to avoid overoxidation.

(*R*)-5'-OH-Octapeptide monocycle (S8)

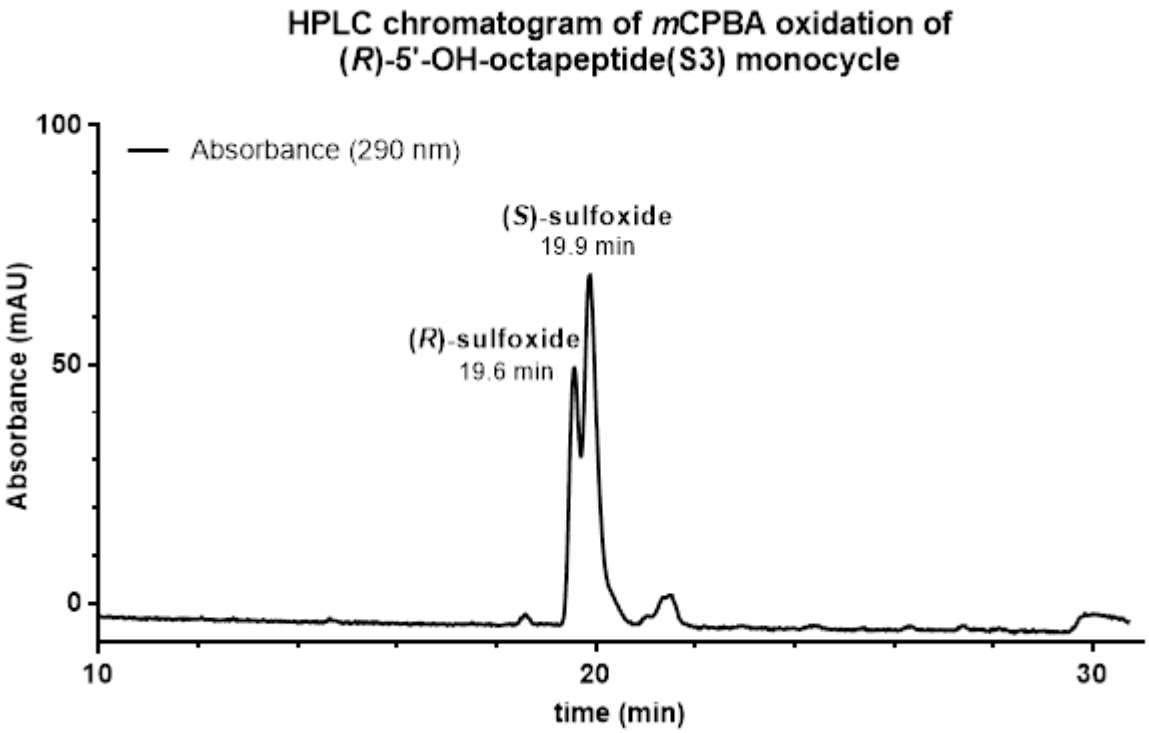
LRMS (ESI, m/z): $[M+H]^+$ found 937.3; calc. 937.4 for $C_{39}H_{57}N_{10}O_{15}S$.

HPLC (gradient G) $\mathbf{t_R}$ = 19.6 min.

(*S*)-5'-OH-Octapeptide monocycle (S9)

LRMS (ESI, m/z): $[M+H]^+$ found 937.3; calc. 937.4 for $C_{39}H_{57}N_{10}O_{15}S$.

HPLC (gradient G) $\mathbf{t_R}$ = 19.9 min.


HPLC chromatogram of mCPBA oxidation of
(R)-5'-OH-octapeptide(S3) monocycle
Absorbance (290 nm)
(S)-sulfoxide
19.9 min
(R)-sulfoxide
19.6 min
Absorbance (mAU)
100
50
0
10
20
30
time (min)

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