Supplementary Material

For

Atropospecific Total Synthesis of Tryptorubin A

By

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Experimental procedures (synthesis)

General remarks

All reactions were carried out under an inert argon atmosphere with dry solvents under anhydrous conditions unless otherwise stated. Dry acetonitrile (MeCN), dichloromethane (DCM), diethyl ether (Et_2O), tetrahydrofuran (THF), toluene (PhMe), dimethylformamide (DMF), benzene, and triethylamine (TEA) were obtained by passing the previously degassed solvents through activated alumina columns. Reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. Yields refer to chromatographically and spectroscopically (^{1}H NMR or LCMS) homogeneous material, unless otherwise stated. Reactions were monitored by thin layer chromatography (TLC) carried out on 0.25 mm E. Merck silica plates (60F-254), using UV light as the visualizing agent and/or phosphomolybdic acid and heat as a developing agent. Flash silica gel chromatography was performed using E. Merck silica gel (60, particle size 0.043 – 0.063 mm). NMR spectra were recorded on Bruker DRX-600 and AMX-400 instruments and were calibrated using residual undeuterated solvent as an internal reference (chloroform-*d*: ^{1}H NMR δ = 7.26 ppm, ^{13}C NMR δ = 77.16 ppm). The following abbreviations were used to explain NMR peak multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. High-resolution mass spectra (HRMS) were recorded on an Agilent LC/MSD TOF mass spectrometer by electrospray ionization time-of-flight (ESI-TOF) reflectron experiments.

Figure S1, Detailed route to compound 1b

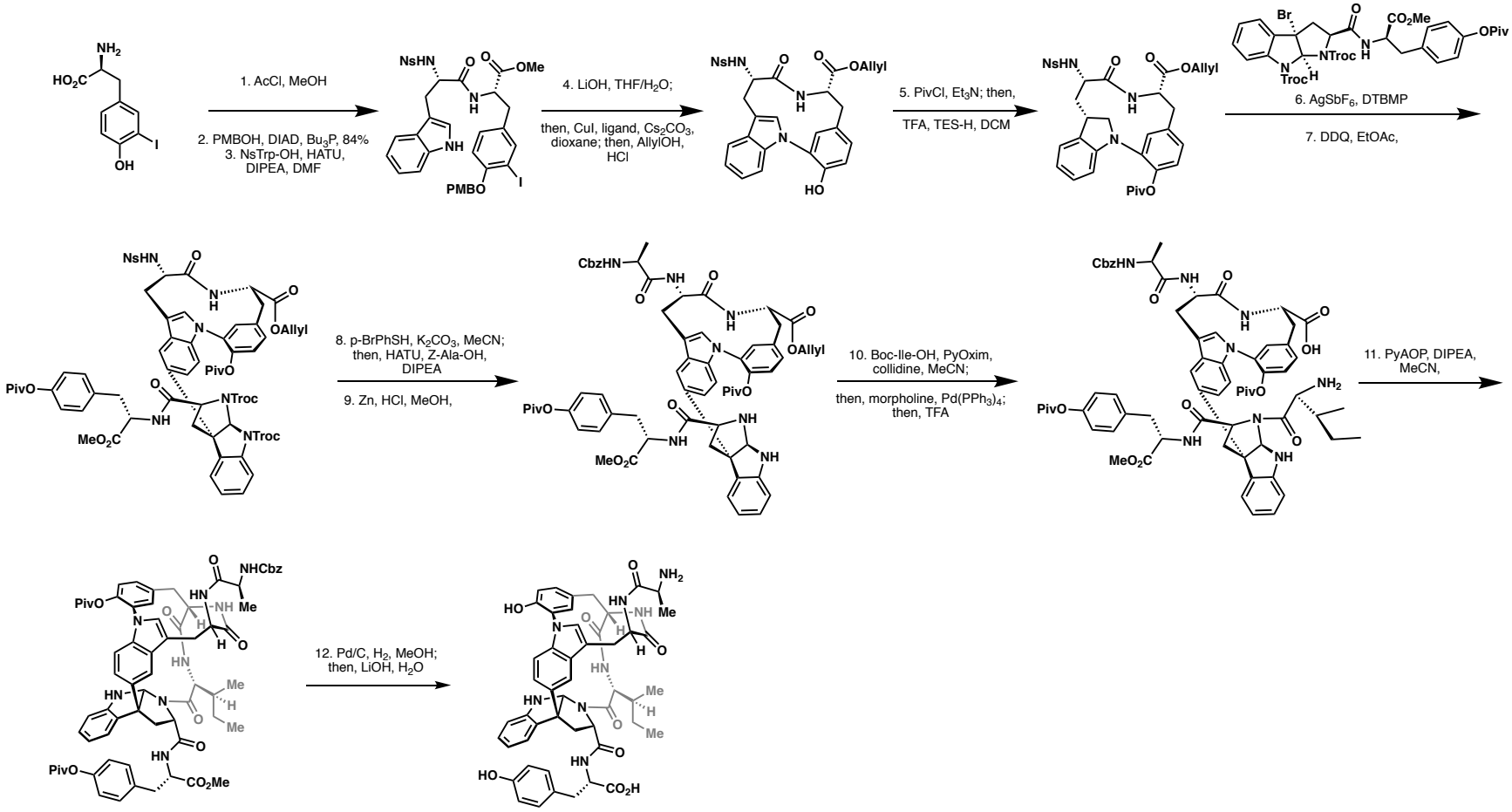


Figure S2, Detailed route to compound 4

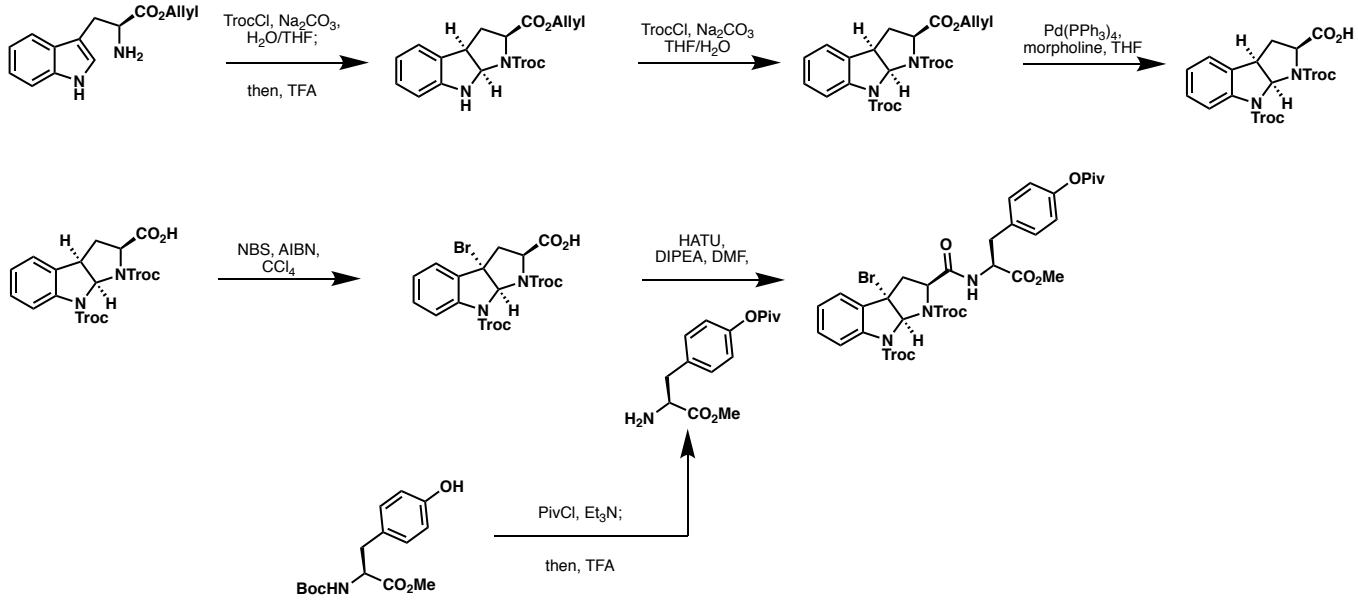


Figure S3, Detailed route to compound 1a

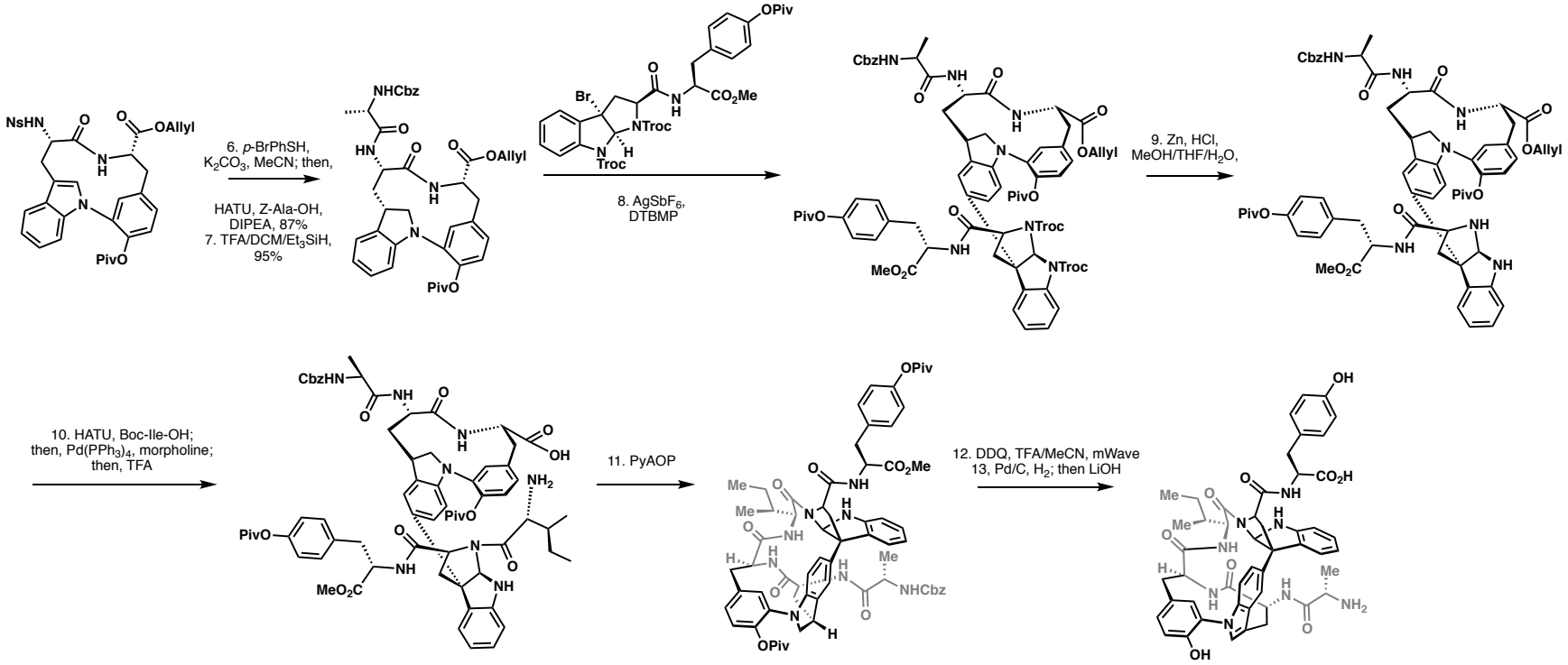


Figure S4, Abridged summary of failed approaches

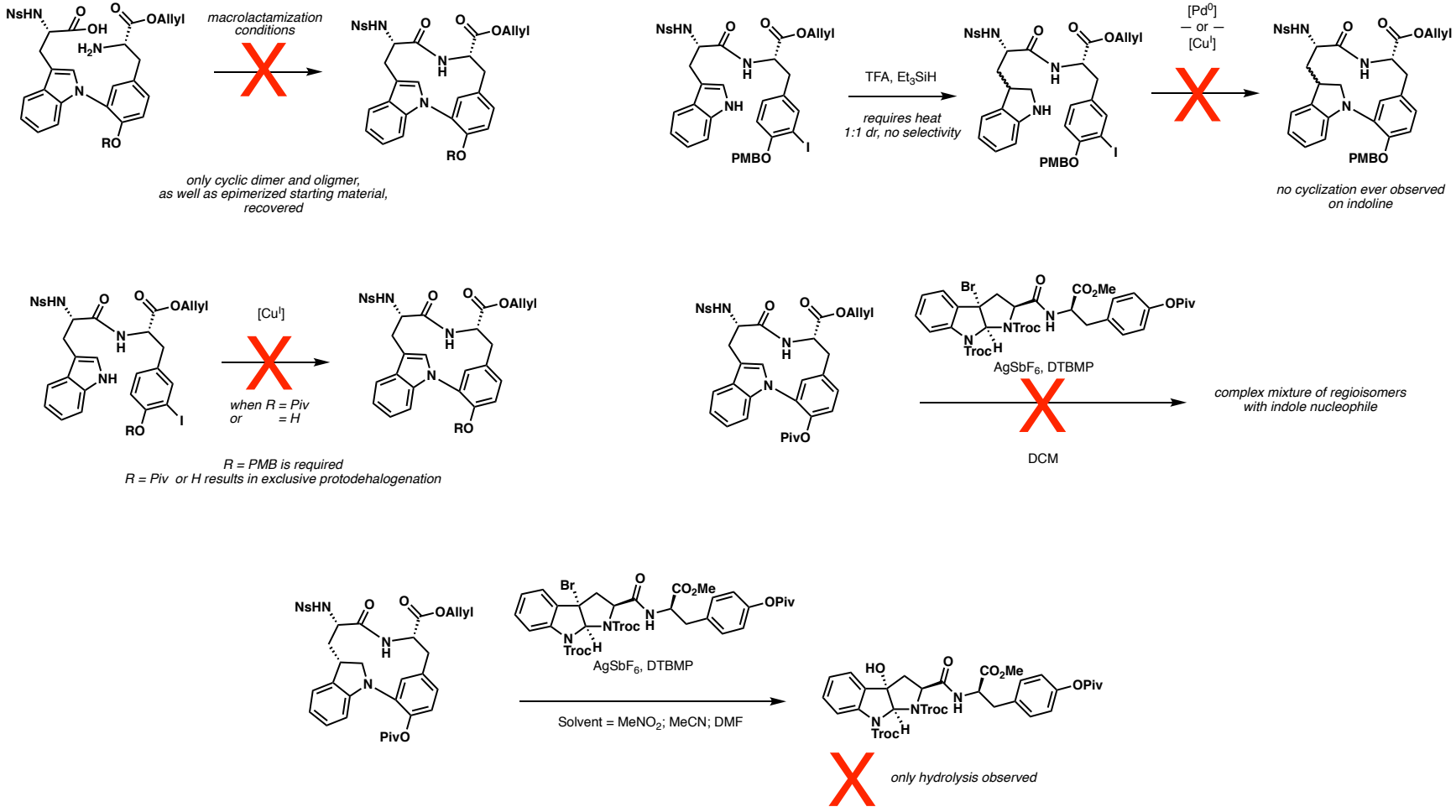


Figure S4, Abridged summary of failed approaches (cont.)

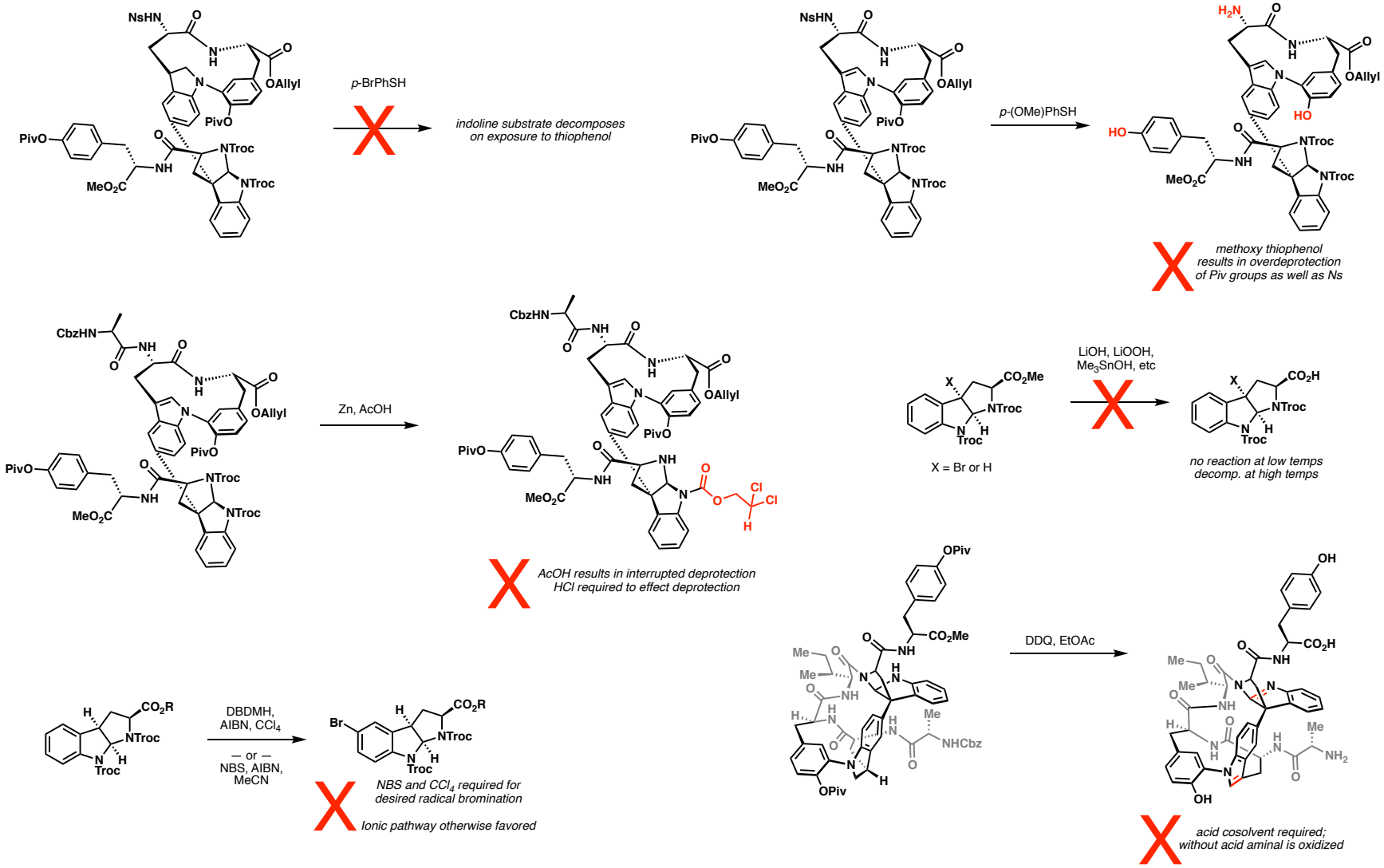


Figure S4, Abridged summary of failed approaches (cont.)

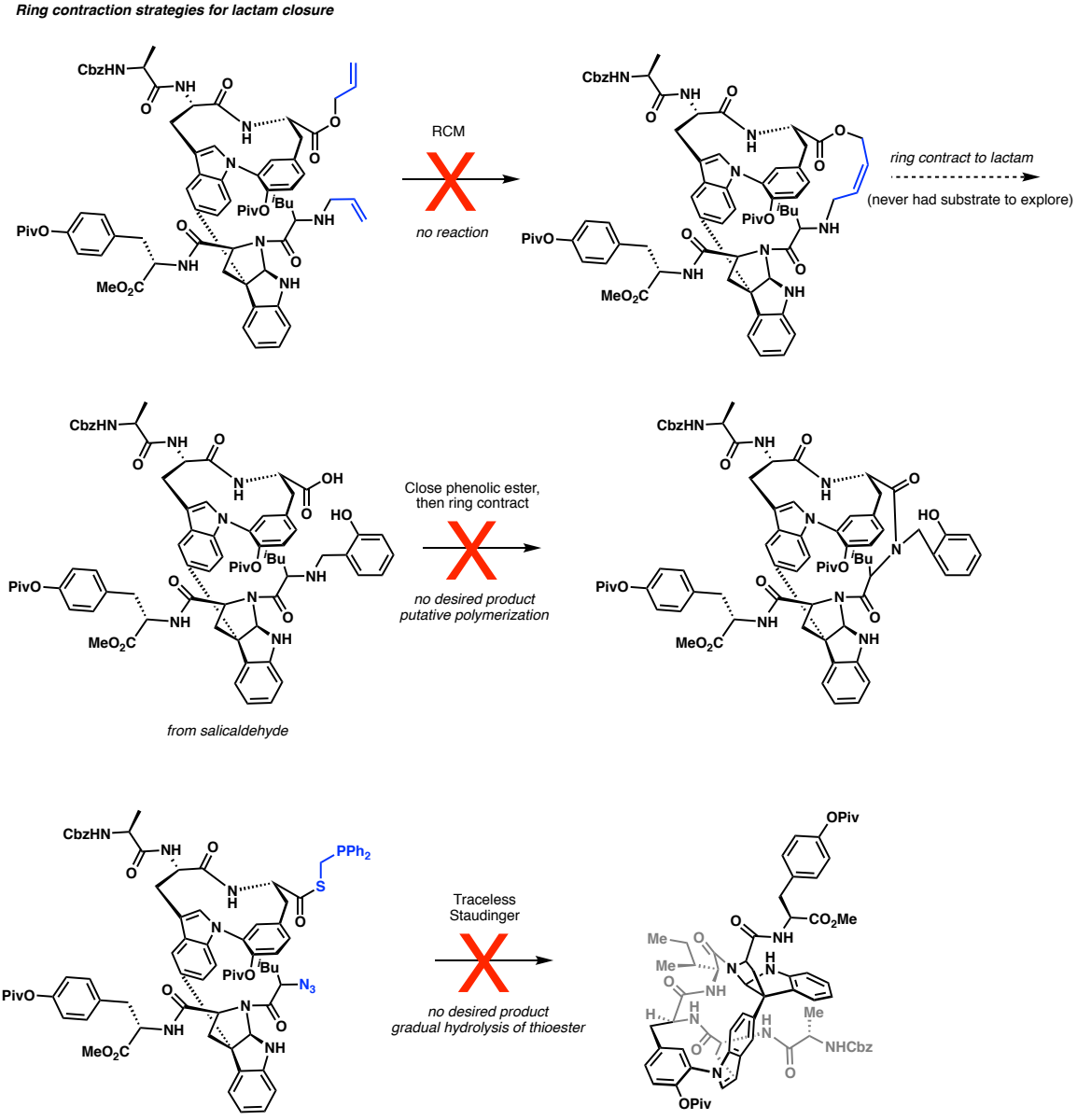
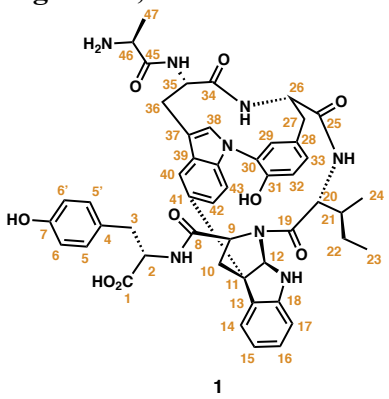
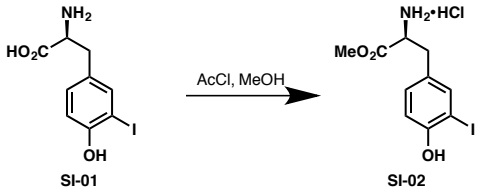


Figure S5, Skeletal numbering system for 1



1

Compound experimentals

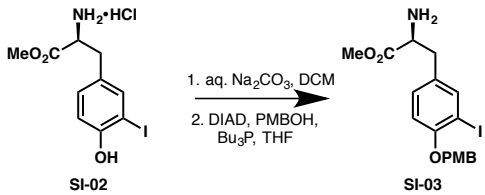


SI-02

To MeOH (200 mL) at 0 °C was added AcCl (14 mL, 200 mmol, 2.0 equiv) dropwise. *L*-3-iodotyrosine **SI-01** (30.7 g, 100 mmol, 1 equiv) was then added as a solid in a single portion. The mixture was heated to reflux and stirred for 2 h, at which time LC/MS analysis showed complete consumption of starting material. The mixture was concentrated *via* rotary evaporation to yield the title product's HCl salt (35.5 g, quant.) as a white solid.

Spectral data matched previous reports;[30] the ^{1}H NMR data is provided here for convenience.

^{1}H NMR: (400 MHz, CD_3OD) δ 7.62 (d, *J* = 2.1 Hz, 1H), 7.09 (dd, *J* = 8.1, 2.0 Hz, 1H), 6.84 (d, *J* = 8.2 Hz, 1H), 4.27 (t, *J* = 6.7 Hz, 1H), 3.84 (s, 3H), 3.16 (dd, *J* = 14.6, 6.0 Hz, 1H), 3.05 (dd, *J* = 14.6, 7.6 Hz, 1H).



[30] He, C., Stratton, T.P.; Baran, P.S. *J. Am. Chem. Soc.,* **2019,** *141,* 29.

SI-03

Immediately before use in the Mistunobu reaction, the amine freebase was prepared according to the following procedure: *L*-3-iodotyrosine methyl ester hydrochloride **SI-02** (35.5 g, 100 mmol, 1 equiv) was partitioned between aqueous Na_2CO_3 (saturated, 300 mL), and DCM (100 mL) and the layers were separated. The aqueous layer was extracted with additional DCM (2 X 100 mL). The combined organics were dried ($MgSO_4$) and concentrated *via* rotary evaporation to give the amine freebase as a yellow foam (32.1 g).

A solution of the resultant *L*-3-iodotyrosine methyl ester freebase (32.1 g, 100 mmol, 1 equiv), paramethoxybenzyl alcohol (16 mL, 130 mmol, 1.3 equiv), and tri-*n*-butylphosphine (32 mL, 130 mmol, 1.3 equiv) in THF (500 mL) was cooled to 0 ^oC. Diisopropylazodiacarboxylate (25 mL, 130 mmol, 1.3 equiv) was added dropwise. The mixture was allowed to warm to rt, then stirred for 30 min, at which time LC/MS analysis showed complete consumption of starting material. The mixture was concentrated *via* rotary evaporation, and the resulting orange residue was taken up in Et_2O (200 mL), then extracted with HCl (1 M, 3 X 100 mL). The combined aqueous layer was basified to pH 10 *via* portionwise addition of solid Na_2CO_3, then extracted with EtOAc (3 X 150 mL). The combined organics were washed with brine (50 mL), dried ($MgSO_4$), and concentrated *via* rotary evaporation to give **SI-03** as a yellow foam (37.0 g, 81%) of sufficient purity to carry forward. Analytically pure samples could be isolated via preparative TLC (SiO_2, 5% MeOH/DCM with 1% Et_3N):

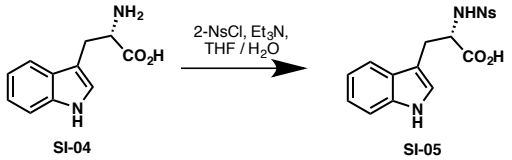
$\mathbf{[\alpha]_D^{20}}$ = -11.4 (*c* = 0.7, $CHCl_3$);

^{1}H NMR (600 MHz, CD_3OD): δ 7.63 (d, *J* = 2.2 Hz, 1H), 7.43 – 7.40 (m, 2H), 7.15 (dd, *J* = 8.4, 2.2 Hz, 1H), 6.96 – 6.92 (m, 3H), 5.07 (s, 2H), 4.60 (s, 1H), 3.81 (s, 3H), 3.68 (s, 3H), 2.92 (dd, *J* = 13.7, 6.3 Hz, 1H), 2.86 (dd, *J* = 13.7, 6.8 Hz, 1H).

^{13}C NMR (151 MHz, CD_3OD): δ 176.0, 161.0, 157.9, 141.2, 132.7, 131.5, 130.2, 130.0, 114.9, 114.1, 87.4, 71.9, 56.6, 55.7, 52.4, 40.2.

HRMS: Calc'd for $C_{18}H_{21}INO_4$, $[M+H]^+$, 442.0510; found, 442.0515.

$\boldsymbol{R_f}$ = 0.32 (Hex:EtOAc = 1:5; UV, $Ce_2(SO_4)_3$ in phosphomolybdic acid)



SI-05

To a solution of *L*-tryptophan **SI-04** (20.4 g, 100 mmol, 1 equiv) and Et_3N (34.8 mL, 250 mmol, 2.5 equiv) in THF:H_2O (1:9, 200 mL) at 0 ^oC was added a solution of 2-nitrobenzenesulfonyl chloride (24.4 g, 110 mmol, 1.10 equiv) in THF (100 mL). The

mixture was warmed to rt and stirred 30 min, at which time TLC analysis showed complete consumption of starting material. The THF was removed *via* rotary evaporation, and the resulting aqueous solution was washed with Et_2O (75 mL). To the aqueous layer, aqueous HCl (2 M) was added until the solution registered pH 1. The mixture was extracted with EtOAc (3 X 100 mL), and the combined organics were washed with brine (75 mL), dried ($MgSO_4$), and concentrated *via* rotary evaporation to give **SI-05** (34.2 g, 88%) as a yellow-brown foam which was carried forward without further purification:

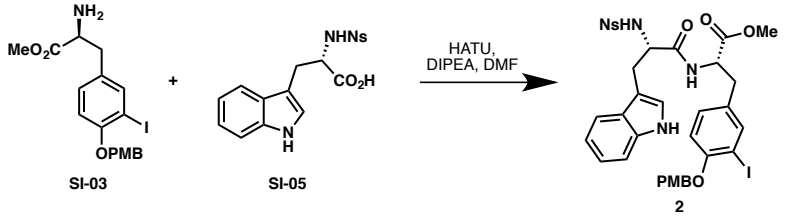
$[\alpha]_D^{24.2}$ = 10.3 (*c* = 1.0, MeOH);

^{1}H NMR (600 MHz, CD_3OD): δ 7.57 (dd, *J* = 16.9, 7.9 Hz, 2H), 7.50 – 7.46 (m, 1H), 7.41 (d, *J* = 7.9 Hz, 1H), 7.29 (t, *J* = 7.7 Hz, 1H), 7.17 (d, *J* = 8.1 Hz, 1H), 7.05 – 7.01 (m, 2H), 6.91 (t, *J* = 7.5 Hz, 1H), 4.31 (dd, *J* = 9.6, 4.2 Hz, 1H), 3.35 – 3.32 (m, 1H), 3.08 (dd, *J* = 14.6, 9.5 Hz, 1H).

^{13}C NMR (151 MHz, CD_3OD): δ 175.5, 148.2, 137.9, 134.5, 134.3, 133.2, 130.7, 128.2, 125.7, 125.3, 122.2, 119.8, 119.2, 112.4, 110.1, 58.9, 29.8.

HRMS: Calc'd for $C_{17}H_{16}N_3O_6S$, $[M+H]^+$, 390.0754; found, 390.0751.

R_f = 0.50 (Hex:Acetone:AcOH = 50:50:1; UV, $Ce_2(SO_4)_3$ in phosphomolybdic acid)



Compound 2

To a solution of **SI-05** (34.2 g, 88.0 mmol, 1.1 equiv) and **SI-03** (37.0 g, 81.1 mmol, 1 equiv) in DMF (200 mL) at 0 °C was added HATU (33.4 g, 88.0 mmol, 1.1 equiv), followed by DIPEA (46.3 mL, 264 mmol, 3.3 equiv). The mixture was stirred for 10 min at 0 °C, then allowed to warm to rt and stirred an additional 30 min. The mixture was diluted with EtOAc (1.0 L) and washed sequentially with aqueous HCl (1 M, 4 X 200 mL), aqueous $NaHCO_3$ (saturated, 100 mL), and brine (100 mL). The organic layer was dried ($MgSO_4$) and concentrated *via* rotary evaporation to yield **2** (59.8 g, 91%) as a yellow foam which was carried forward without further purification.

$[\alpha]_D^{20}$ = 17.2 (*c* = 1.0, $CHCl_3$);

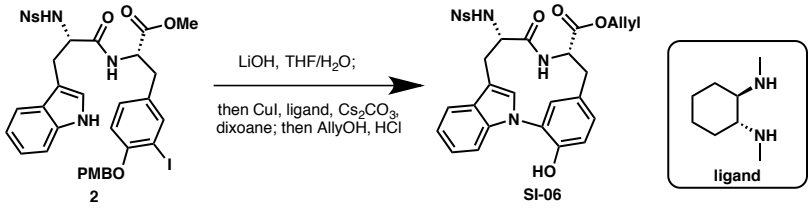
^{1}H NMR (600 MHz, $CDCl_3$): δ 7.99 (s, 1H), 7.85 – 7.82 (m, 1H), 7.47 (d, *J* = 2.1 Hz, 1H), 7.47 – 7.44 (m, 1H), 7.42 – 7.38 (m, 4H), 7.19 – 7.14 (m, 2H), 7.05 – 7.02 (m, 3H), 6.94 – 6.89 (m, 3H), 6.81 (ddd, *J* = 7.9, 7.0, 1.0 Hz, 1H), 6.74 (d, *J* = 8.4 Hz, 1H), 5.94 (d, *J* = 4.4 Hz, 1H), 5.04 (s, 2H), 4.84 – 4.80 (m, 1H), 4.10 (dt, *J* = 9.5, 4.6 Hz, 1H), 3.79 (s,

3H), 3.74 (s, 3H), 3.35 (ddd, J = 14.9, 4.8, 1.0 Hz, 1H), 3.05 (dd, J = 14.1, 5.6 Hz, 1H), 2.97 – 2.87 (m, 2H).

^{13}C NMR (151 MHz, $CDCl_3$): δ 171.2, 170.4, 159.5, 156.6, 146.4, 140.4, 136.5, 133.7, 132.8, 131.8, 131.0, 130.4, 128.9, 128.6, 126.3, 125.7, 124.6, 122.4, 120.0, 118.5, 114.1, 113.0, 111.4, 109.0, 86.9, 70.9, 58.0, 55.4, 53.4, 52.7, 36.5, 29.1.

HRMS: Calc'd for $C_{35}H_{34}IN_4O_9S$, $[M+H]^+$, 813.1086; found, 813.1078.

***R*$_f$** = 0.57 (Hex:EtOAc = 1:2; UV, $Ce_2(SO_4)_3$ in phosphomolybdic acid)



SI-06

To a solution of **2** (32.5 g, 40.0 mmol, 1 equiv) in THF/H_2O (1:1, 200 mL) was added LiOH·H_2O (3.36 g, 80.0 mmol, 2.00 equiv). The mixture was stirred at rt for 6 h, and then the THF was removed *via* rotary evaporation. The resulting aqueous solution was washed with Et_2O (75 mL), then acidified to pH 1 *via* addition of aqueous HCl (2 M). The mixture was extracted with EtOAc (3 X 100 mL) dried (MgSO4), and concentrated *via* rotary evaporation to give a yellow residue, which was directly used for next step without further purification.

The yellow residue and K_2CO_3 (27.6 g, 200 mmol, 5.00 equiv) were dissolved in 1,4-dioxane (1.0 L) and the resulting slurry was degassed with argon for 60 minutes. The septum was quickly removed, CuI[31] (2.28 g, 12.0 mmol, 30.0 mol%) was added, and the septum was quickly replaced. The flask was evacuated and backfilled with argon four times. Ligand *rac*-*trans*-*N,N'*-dimethylcyclohexane-1,2-diamine (2.8 mL, 18 mmol, 45 mol%) was added in one portion. The resultant blue-green mixture was heated to 100 ^oC for 24 h, at which time LC/MS analysis showed complete consumption of starting material. The mixture was cooled to rt, diluted with Et_2O (1.0 L), and extracted with aqueous NH_4OH (1 M, 4 X 300 mL). To the combined aqueous extractions was slowly added aqueous HCl (3 M) until the mixture registered pH 1. The solution was extracted with EtOAc (4 X 300 mL) and the combined organics were washed with brine (100 mL), dried ($MgSO_4$), and concentrated *via* rotary evaporation to give a brown foam which was carried forward without further purification.

To allyl alcohol (100 mL) at 0 ^oC was added AcCl (7.1 mL, 100 mmol, 2.5 equiv) dropwise. To the resultant solution was added the above paragraph's crude brown foam. The mixture was heated to 65 ^oC for 3 h, at which time LC/MS showed complete

[31] Commerical CuI was purified *via* Soxhlet washing with refluxing THF for 24 h, then stored in an Argon-filled glovebox.

conversion. The mixture was concentrated *via* rotary evaporation, and the resultant residue purified by column chromatography (SiO_2, 0 to 5% acetone/DCM) to yield **SI-06** (14.4 g, 61% overall) as a yellow foam:

$[\alpha]_D^{20}$ = 171.2 (*c* = 1.0, $CHCl_3$);

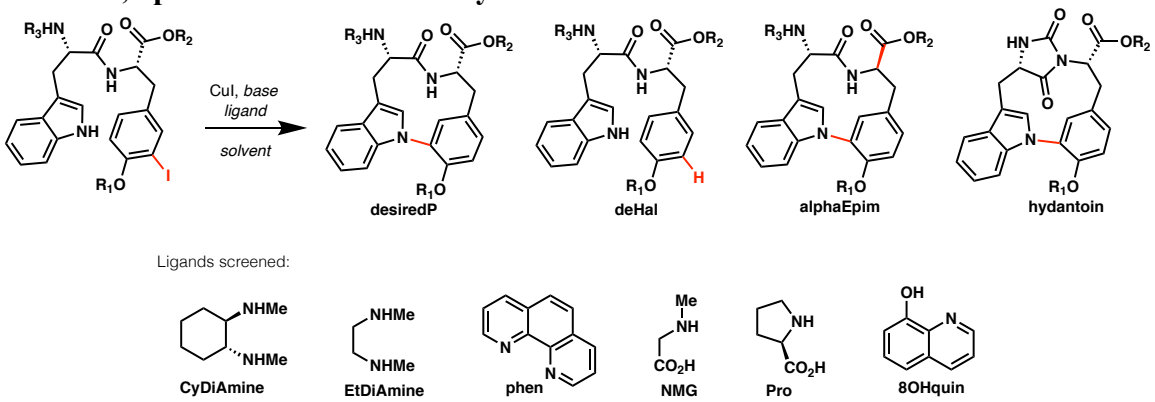
^{1}H NMR (600 MHz, $CDCl_3$): δ 8.16 (dd, *J* = 7.7, 1.7 Hz, 1H), 7.93 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.84 – 7.75 (m, 3H), 7.43 – 7.38 (m, 2H), 7.33 (t, *J* = 7.6 Hz, 1H), 7.12 (d, *J* = 10.3 Hz, 1H), 6.93 (s, 2H), 6.52 (s, 1H), 6.18 (s, 1H), 5.83 (dt, *J* = 16.9, 5.2 Hz, 2H), 5.66 (d, *J* = 7.8 Hz, 1H), 5.29 – 5.21 (m, 2H), 4.48 (t, *J* = 5.6 Hz, 2H), 4.44 – 4.35 (m, 2H), 3.57 (dd, *J* = 14.6, 4.1 Hz, 1H), 3.02 (dd, *J* = 14.6, 3.4 Hz, 1H), 2.75 (dd, *J* = 14.5, 2.0 Hz, 1H), 2.43 (dd, *J* = 14.5, 11.7 Hz, 1H).

^{13}C NMR (151 MHz, $CDCl_3$): δ 170.4, 168.7, 150.4, 149.9, 148.2, 147.4, 134.8, 134.6, 133.2, 132.5, 131.7, 131.5, 129.6, 129.1, 128.4, 127.6, 126.1, 125.5, 124.6, 119.3, 119.2, 117.4, 116.7, 116.6, 66.2, 60.8, 54.5, 35.8, 27.5.

HRMS: Calc'd for $C_{29}H_{27}N4O_8S$, $[M+H]^+$, 591.1544; found, 591.1536.

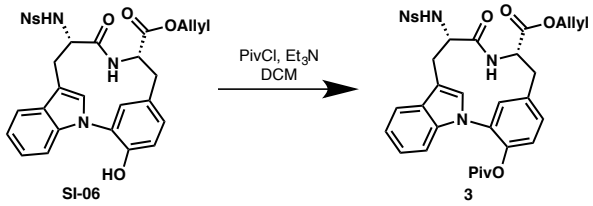
R_f = 0.37 (Hex:EtOAc = 1:1; UV, $Ce_2(SO_4)_3$ in phosphomolybdic acid)

Table S1, optimization of Ullman cyclization



Standard conditions: ligand = **CyDiAmine**; base = K_2CO_3; solvent = dioxane; R_1 = PMB; R_2 = H; R_3 = Ns; concentration = 40 mM

Entry	Difference from standard conditions	Yield desiredP (%)	Major side product
standard conditions	-	66	-
1	R_1 = Piv	-	deHal
2	R_1 = H	-	deHal
3	R_2 = Me	30	alphaEpim
4	R_3 = Cbz	-	hydantoin
5	R_3 = Boc	-	hydantoin
6	ligand = EtDiAmine	54	-
7	ligand = phen	21	deHal
8	ligand = NMG	29	deHal
9	ligand = Pro	28	deHal
10	ligand = 8OHquin	-	deHal
11	base = Cs_2CO_3	66	-
12	base = K_3PO_4	49	deHal
13	base = Barton's base (guanadine)	-	recovered SM
14	base = Et_3N	-	recovered SM
15	solvent = toluene	-	recovered SM
16	solvent = DMF	44	deHal
17	solvent = DMSO	31	deHal
18	concentration = 10 mM	69	-
19	concentration = 1 mM	68	-
20	concentration = 100 mM	44	oligimers
21	additive = NaI, 10 equiv	-	recovered SM
22	additive = TBAI, 10 equiv	-	recovered SM



Compound 3

To a solution of **SI-06** (16.8 g, 28.5 mmol, 1 equiv) and Et_3N (11.9 mL, 85.5 mmol, 3.00 equiv) in DCM (100 mL) at rt was added pivaloyl chloride (4.2 mL, 34 mmol, 1.2 equiv) dropwise. After complete addition, the mixture was stirred for 1 h at rt. The resultant solution was poured over aqueous HCl (1 M, 200 mL) and the aqueous layer extracted with additional DCM (3 X 100 mL). The combined organics were dried ($MgSO_4$) and concentrated *via* rotary evaporation to yield the title product as a yellow foam (19.0 g, 99%), which was carried forward without further purification:

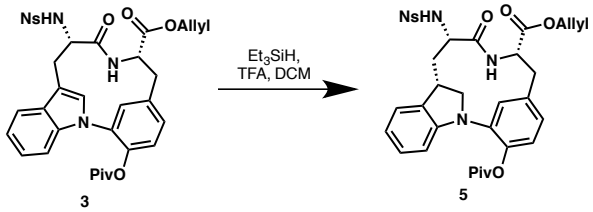
$[\alpha]_D^{24.1}$ = 107.8 (*c* = 0.8, $CHCl_3$);

^{1}H NMR (600 MHz, $CDCl_3$): δ 8.17 (dd, *J* = 7.6, 1.7 Hz, 1H), 7.93 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.82 (td, *J* = 7.7, 1.7 Hz, 1H), 7.79 (td, *J* = 7.6, 1.5 Hz, 1H), 7.74 (d, *J* = 7.7 Hz, 1H), 7.54 (d, *J* = 8.0 Hz, 1H), 7.38 (td, *J* = 7.5, 1.1 Hz, 1H), 7.31 (ddd, *J* = 8.3, 7.2, 1.2 Hz, 1H), 7.11 (d, *J* = 10.3 Hz, 1H), 7.06 (dd, *J* = 8.3, 2.1 Hz, 1H), 7.03 (d, *J* = 8.3 Hz, 1H), 6.54 (s, 1H), 5.95 (d, *J* = 2.1 Hz, 1H), 5.84 (ddt, *J* = 17.2, 10.4, 5.9 Hz, 1H), 5.71 (d, *J* = 8.4 Hz, 1H), 5.30 – 5.20 (m, 2H), 4.54 – 4.45 (m, 2H), 4.45 – 4.35 (m, 2H), 3.56 (dd, *J* = 14.6, 4.2 Hz, 1H), 3.01 (dd, *J* = 14.5, 3.3 Hz, 1H), 2.84 (dd, *J* = 14.4, 2.0 Hz, 1H), 2.51 (dd, *J* = 14.4, 11.8 Hz, 1H), 1.33 (s, 9H).

^{13}C NMR (151 MHz, $CDCl_3$): δ 177.3, 170.3, 168.9, 149.9, 148.2, 146.7, 144.8, 135.8, 135.2, 134.6, 134.2, 133.2, 132.5, 131.8, 131.5, 129.4, 128.6, 126.1, 125.2, 124.2, 123.1, 119.3, 118.8, 117.0, 116.2, 66.3, 61.0, 54.2, 39.3, 35.9, 27.5, 27.3.

HRMS: Calc'd for $C_{34}H_{35}N_4O_9S$, $[M+H]^+$, 675.2119; found, 675.2118.

***R*$_f$** = 0.18 (Hex:EtOAc = 2:1; UV, $Ce_2(SO_4)_3$ in phosphomolybdic acid)



Compound 5

To a solution of **3** (19.0 g, 28.2 mmol) in DCM (100 mL) at rt was added TFA (25 mL) followed by Et_3SiH (25 mL). The mixture was stirred at rt for 5 min, then concentrated

via rotary evaporation, and the residue purified *via* column chromatography (SiO_2, 0-50% EtOAc/hexane) to yield **5** (16.2 g, 85%) as a yellow foam:

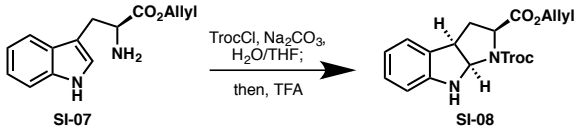
$[\alpha]_D^{20}$ = -60.2 (*c* = 1.0, $CHCl_3$);

^{1}H NMR (600 MHz, $CDCl_3$): δ 8.02 (dd, *J* = 7.6, 1.5 Hz, 1H), 7.82 (d, *J* = 7.8 Hz, 1H), 7.75 – 7.66 (m, 2H), 7.37 (s, 1H), 7.23 (s, 1H), 7.03 (d, *J* = 8.1 Hz, 2H), 6.96 (dd, *J* = 8.1, 1.9 Hz, 1H), 6.92 (d, *J* = 8.1 Hz, 2H), 5.81 (ddt, *J* = 16.5, 10.4, 5.8 Hz, 1H), 5.29 – 5.23 (m, 2H), 4.44 (qdt, *J* = 13.1, 5.9, 1.4 Hz, 2H), 4.09 (ddd, *J* = 11.8, 9.5, 2.3 Hz, 1H), 3.57 (s, 1H), 3.33 (dd, *J* = 11.9, 2.8 Hz, 1H), 2.99 (dd, *J* = 13.2, 2.4 Hz, 1H), 2.54 (s, 1H), 2.46 – 2.35 (m, 2H), 1.32 (s, 9H).

^{13}C NMR (151 MHz, $CDCl_3$): δ 176.8, 170.3, 168.5, 150.2, 148.1, 144.2, 134.9, 134.1, 132.8, 132.5, 131.5, 131.3, 129.3, 125.8, 123.9, 122.1, 119.3, 116.6, 66.1, 57.5, 54.4, 39.5, 39.1, 27.3.

HRMS: Calc'd for $C_{34}H_{37}N_4O_9S$, $[M+H]^+$, 677.2276; found, 677.2278.

R_f = 0.33 (Hex:EtOAc = 2:1; UV, $Ce_2(SO_4)_3$ in phosphomolybdic acid)



SI-08

To a solution of *L*-tryptophan allyl ester (**SI-07**) (19.5 g, 80 mmol, 1 equiv) in THF/H_2O (1:1, 400 mL) was added Na_2CO_3 (12.7 g, 120 mmol, 1.50 equiv). The mixture was stirred at rt for 10 min, then 2,2,2-trichloroethylchloroformate (12.1 mL, 88 mmol, 1.10 equiv) was added dropwise. The mixture was stirred at rt for 30 min, then concentrated *via* rotary evaporation to remove THF. The resultant slurry was extracted with DCM (3 X 200 mL), and the combined organics dried ($MgSO_4$) and concentrated *via* rotary evaporation to yield the carbamate as a yellow foam.

The crude carbamate was dissolved in TFA (200 mL) and stirred at rt for 12 h. At this time, a separate two-necked round-bottom flask fitted with a mechanical stirrer was charged with Na_2CO_3 (310 g), H_2O (600 mL), and DCM (200 mL). While stirring vigorously, the homogeneous TFA solution was slowly (over *ca.* 20 min) poured into to this slurry. The resultant mixture was filtered through a fritted glass funnel, and the filter cake was washed with DCM (3 X 100 mL). The biphasic filtrate's layers were separated, and the aqueous layer extracted with additional DCM (3 X 200 mL). The combined organics were washed with brine (200 mL), dried ($MgSO_4$), and concentrated *via* rotary evaporation, and the residue purified by column chromatography (SiO_2, 0-20% acetone/hexane) to yield **SI-08** as a yellow foam (22.5 g, 67%).

Note: Due to propensity of this compound to tautomerize to its open-chain form, it was used immediately in the next step.

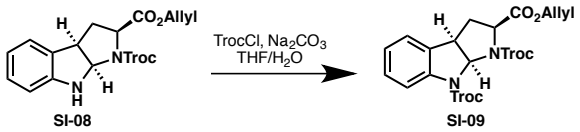
$[\alpha]_D^{24.4}$ = 139.1 (*c* = 1.0, $CHCl_3$);

The NMR spectra presented as a mixture of rotamers:
^{1}H NMR (600 MHz, $CDCl_3$): δ 7.07 – 7.02 (m, 4H), 6.70 (qd, *J* = 7.5, 1.0 Hz, 2H), 6.59 (dd, *J* = 7.6, 0.9 Hz, 2H), 5.68 (d, *J* = 6.6 Hz, 1H), 5.64 (d, *J* = 6.7 Hz, 1H), 5.52 (dddt, *J* = 16.8, 10.7, 8.4, 5.9 Hz, 2H), 5.16 (s, 1H), 5.11 (ddq, *J* = 17.4, 10.5, 1.3 Hz, 4H), 5.02 – 4.98 (m, 2H), 4.75 (dd, *J* = 21.8, 11.9 Hz, 2H), 4.66 – 4.63 (m, 3H), 4.17 (dddt, *J* = 19.0, 13.1, 6.0, 1.3 Hz, 2H), 4.02 – 3.88 (m, 4H), 2.74 – 2.62 (m, 4H).

^{13}C NMR (151 MHz, $CDCl_3$): δ 170.7 (170.4), 153.0 (152.4), 149.9 (149.6), 131.8, 128.94 (128.88), 127.5 (127.3), 124.4 (124.2), 119.2 (119.1), 118.58 (118.55), 109.4 (109.2), 95.6 (95.1), 77.8 (77.3), 75.1 (75.0), 66.2 (66.1), 59.8 (59.4), 46.3 (45.3), 34.7 (33.9).

HRMS: Calc'd for $C_{17}H_{18}Cl_3N_2O_4$, $[M+H]^+$, 419.0327; found, 419.0324.

R_f = 0.80 (Hex/EtOAc = 2:1; UV, $Ce_2(SO_4)_3$ in phosphomolybdic acid)



SI-09
To a solution of **SI-08** (22.5 g, 53.6 mmol, 1 equiv) in THF (150 mL) was added Na_2CO_3 (8.52 g, 80.4 mmol, 1.50 equiv), then H_2O (150 mL). To the resultant mixture was added 2,2,2-trichloroethylchloroformate (8.1 mL, 59.0 mmol, 1.1 equiv) dropwise, and the solution was stirred for 4 h at rt. The mixture was concentrated *via* rotary evaporation to remove THF, and resultant aqueous suspension was extracted with EtOAc (3 X 150 mL). The combined organics were dried ($MgSO_4$) and concentrated *via* rotary evaporation, and the residue purified by column chromatography (SiO_2, 0 – 20% acetone/hexane) to yield **SI-09** as a yellow foam (30.0 g, 94%):

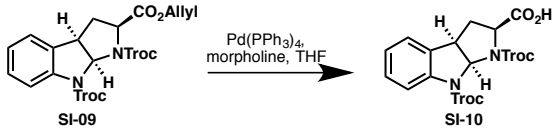
$[\alpha]_D^{20}$ = -1.0 (*c* = 1.0, $CHCl_3$);

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.69 (s, 1H), 7.27 – 7.24 (m, 1H), 7.18 – 7.15 (m, 1H), 7.09 – 7.04 (m, 1H), 6.59 (d, *J* = 6.5 Hz, 1H), 5.53 (ddt, *J* = 16.5, 10.5, 5.8 Hz, 1H), 5.15 – 5.09 (m, 2H), 4.82 (s, 2H), 4.74 (dd, *J* = 9.0, 1.1 Hz, 1H), 4.62 (t, *J* = 18.4 Hz, 1H), 4.18 – 4.11 (m, 2H), 3.88 (ddt, *J* = 13.1, 5.7, 1.5 Hz, 1H), 2.74 (d, *J* = 13.2 Hz, 1H), 2.72 – 2.60 (m, 1H).

^{13}C NMR (151 MHz, $CDCl_3$): δ 170.4, 152.1, 151.8, 141.8, 131.4, 131.3, 129.1, 124.4, 124.3, 118.6, 95.2, 77.7, 75.7, 75.3, 66.2, 59.6, 44.7, 34.1.

HRMS: Calc'd for $C_{20}H_{19}Cl_6N_2O_6$, $[M+H]^+$, 592.9369; found, 592.9362.

$\boldsymbol{R_f}$ = 0.80 (Hex:EtOAc = 2:1; UV, $Ce_2(SO_4)_3$ in phosphomolybdic acid)



SI-10

A solution of **SI-09** (30.0 g, 50.4 mmol, 1 equiv) and morpholine (26.0 mL, 302 mmol, 6 equiv) in THF (250 mL) was sparged with argon for 30 min; then, was added $Pd(PPh_3)_4$ (582 mg, 0.504 mmol, 1.00 mol%) in a single portion. The solution was stirred for 15 min at rt, at which time TLC analysis showed complete consumption of starting material. The mixture was poured over aqueous HCl (1 M, 500 mL) and extracted with EtOAc (3 X 250 mL). The combined organics were dried ($MgSO_4$) and concentrated *via* rotary evaporation, and the residue purified by column chromatography (SiO_2, 0-67% EtOAc/hexane with 0.67% AcOH) to yield **SI-10** as a yellow foam (28.0 g, quant.):

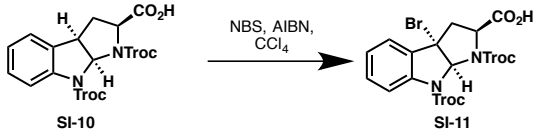
$\boldsymbol{[\alpha]_D^{20}}$ = 17.1 (*c* = 1.0, $CHCl_3$);

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.65 (s, 1H), 7.25 – 7.20 (m, 1H), 7.13 (dd, *J* = 7.6, 1.4 Hz, 1H), 6.99 (td, *J* = 7.5, 1.0 Hz, 1H), 6.55 (d, *J* = 6.6 Hz, 1H), 5.02 (s, 1H), 4.83 (d, *J* = 11.7 Hz, 2H), 4.67 (dd, *J* = 9.2, 1.4 Hz, 1H), 4.57 (s, 1H), 4.10 (t, *J* = 6.9 Hz, 1H), 2.73 – 2.66 (m, 1H), 2.63 (dt, *J* = 13.4, 1.6 Hz, 1H).

^{13}C NMR (151 MHz, $CDCl_3$): δ 175.6, 151.8, 141.7, 131.1, 129.2, 124.4, 124.1, 117.5, 95.2, 77.8, 75.7, 75.4, 59.3, 44.7, 33.9.

HRMS: Calc'd for $C_{17}H_{15}Cl_6N_2O_6$, $[M+H]^+$, 552.9056; found, 552.9049.

$\boldsymbol{R_f}$ = 0.45 (Hex:EtOAc:AcOH = 50:50:1; UV, $Ce_2(SO_4)_3$ in phosphomolybdic acid)



SI-11

A solution of **SI-10** (2.22 g, 4.0 mmol, 1 equiv) in CCl_4 (80 mL) was degassed *via* argon sparging for 45 min. The septum was removed, NBS (1.42 g, 8.0 mmol, 2.00 equiv) and AIBN (657 mg, 4.0 mmol, 1.00 equiv) were added, and the septum quickly replaced. The resultant mixture was heated to reflux for 1 h, at which time LC/MS analysis showed

complete consumption of the starting material. The mixture was concentrated *via* rotary evaporation, and the residue purified by column chromatography (SiO_2, 0-50% EtOAc/hexane) to yield **SI-11** as a yellow foam (1.70 g, 67%):

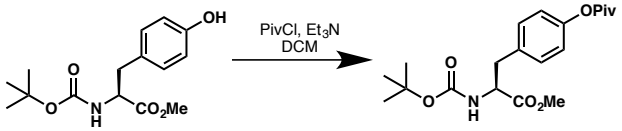
$[\alpha]_D^{25.2}$ = 55.0 (*c* = 1.0, $CHCl_3$);

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.65 (s, 1H), 7.36 – 7.31 (m, 2H), 7.10 (td, *J* = 7.6, 1.0 Hz, 1H), 6.58 (s, 1H), 4.83 (s, 2H), 4.67 – 4.62 (m, 1H), 4.58 (s, 1H), 3.32 (d, *J* = 13.4 Hz, 1H), 3.22 (s, 1H).

^{13}C NMR (151 MHz, $CDCl_3$): δ 174.0, 151.7, 141.0, 132.2, 131.5, 125.3, 124.0, 118.4, 94.9, 85.1, 75.8, 75.5, 59.7, 58.9, 43.7.

HRMS: Calc'd for $C_{17}H_{14}BrCl_6N_2O_6$, $[M+H]^+$, 630.8161; found, 630.8159.

***R*$_f$** = 0.45 (Hex:EtOAc:AcOH = 50:50:1; UV, $Ce_2(SO_4)_3$ in phosphomolybdic acid)



SI-13

To a solution of Boc-Tyr-OMe (**SI-12**) (3.00 g, 10.0 mmol, 1 equiv) and triethylamine (4.2 mL, 30 mmol, 3.0 equiv) in DCM (50 mL) was added pivaloyl chloride (1.85 mL, 15.0 mmol, 1.5 equiv) dropwise at rt. After complete addition, the mixture was stirred for 1 h at rt. The resultant solution was poured over aqueous HCl (1 M, 100 mL) and the aqueous layer extracted with additional DCM (3 X 50 mL). The combined organics were dried ($MgSO_4$) and concentrated *via* rotary evaporation, and the residue was purified by column chromatography (SiO_2, 0-30% EtOAc/hexane) to yield **SI-13** as a colorless oil (3.70 g, 98%).

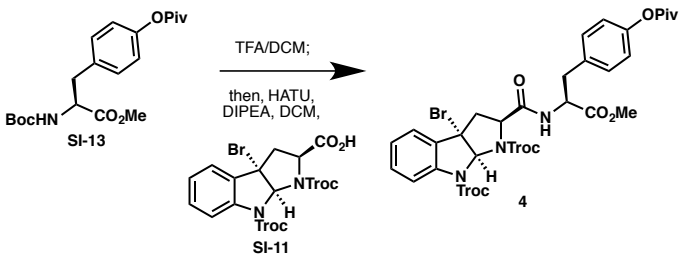
$[\alpha]_D^{22.6}$ = 28.9 (*c* = 1.0, $CHCl_3$);

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.12 (d, *J* = 8.1 Hz, 2H), 7.00 – 6.97 (m, 2H), 4.99 (d, *J* = 8.3 Hz, 1H), 4.56 (q, *J* = 6.8 Hz, 1H), 3.70 (s, 3H), 3.07 (qd, *J* = 14.0, 6.0 Hz, 2H), 1.42 (s, 9H), 1.34 (s, 9H).

^{13}C NMR (151 MHz, $CDCl_3$): δ 177.1, 172.3, 155.2, 150.3, 133.5, 130.3, 121.7, 80.1, 54.5, 52.4, 39.2, 37.8, 28.4, 27.2.

HRMS: Calc'd for $C_{20}H_{29}NNaO_6$, $[M+Na]^+$, 402.1887; found, 402.1888.

***R*$_f$** = 0.83 (Hex/EtOAc = 2:1; UV, $Ce_2(SO_4)_3$ in phosphomolybdic acid)



Compound 4

To solution of **SI-13** (1.26 g, 3.32 mmol, 1.10 equiv) in DCM (10 mL) was added TFA (2.5 mL). The resultant mixture was stirred at rt for 1 h, then diluted with toluene (10 mL) and concentrated *via* rotary evaporation. The residue was co-evaporated with additional toluene (2 X 10 mL) to remove residual TFA. The resultant oil was dissolved in DCM (15 mL), and then **SI-11** (1.40 g, 2.21 mmol, 1 equiv) and DIPEA (1.9 mL, 11.1 mmol, 5 equiv) were sequentially added. Then, HATU (1.01 g, 2.65 mmol, 1.20 equiv) was added, and the mixture stirred at rt for 1 h. The reaction mixture was quenched with saturated aqueous NH_4Cl solution (50 mL) and extracted with DCM (3 X 50 mL). The organic layer was dried ($MgSO_4$) and concentrated, and the residue purified by column chromatography (SiO_2, 0-50% EtOAc/hexane) to yield **4** as a yellow foam (1.40 g, 71%):

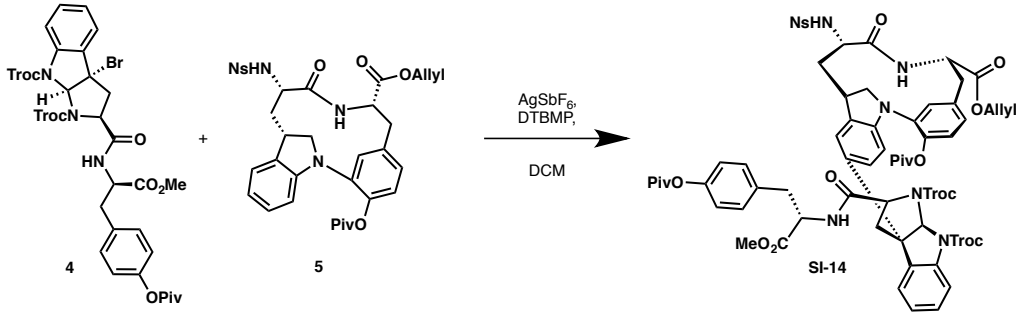
$\mathbf{[\alpha]_D^{20}}$ = 37.5 (*c* = 1.0, $CHCl_3$);

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.74 (d, *J* = 8.2 Hz, 1H), 7.48 (d, *J* = 7.7 Hz, 1H), 7.36 (td, *J* = 7.8, 1.3 Hz, 1H), 7.19 (td, *J* = 7.6, 1.0 Hz, 1H), 6.93 – 6.87 (m, 4H), 6.70 (s, 1H), 5.14 (d, *J* = 11.9 Hz, 1H), 4.88 (d, *J* = 11.9 Hz, 2H), 4.66 (dd, *J* = 9.5, 1.4 Hz, 1H), 4.28 (s, 1H), 3.67 – 3.59 (m, 1H), 3.52 (s, 3H), 3.11 (dd, *J* = 13.5, 9.5 Hz, 1H), 2.60 (dd, *J* = 13.7, 4.7 Hz, 1H), 2.21 (dd, *J* = 13.7, 7.7 Hz, 1H), 1.34 (s, 9H).

^{13}C NMR (151 MHz, $CDCl_3$): δ 177.0, 171.1, 168.1, 151.7, 150.3, 140.0, 132.9, 132.6, 131.2, 123.0, 125.8, 125.5, 121.7, 118.0, 94.8, 94.7, 86.2, 75.9, 75.6, 61.9, 60.0, 53.1, 52.3, 42.5, 39.1, 37.6, 27.2.

HRMS: Calc'd for $C_{32}H_{33}BrCl_6N_3O_9$, $[M+H]^+$, 891.9526; found, 891.9572.

R_f = 0.50 (Hex:EtOAc = 2:1; UV, $Ce_2(SO_4)_3$ in phosphomolybdic acid)



SI-14

Macrocycle **5** (2.00 g, 2.95 mmol, 1 equiv), alkyl bromide **4** (3.96 g, 4.43 mmol, 1.50 equiv), and 2,6-di-^{t}butyl-4-methylpyridine (3.02 g, 14.8 mmol, 5.00 equiv) were combined and azeotroped from benzene three times (3 X 50 mL). After the final azeotrope, the mixture was held under vacuum (<1 torr) for 13 h. The flask was backfilled with argon, and DCM (60 mL) was added. The septum was quickly removed, $AgSbF_6$ (5.05 g, 14.8 mmol, 5.00 equiv) was added as a solid in a single portion, and the septum quickly replaced. The resultant brown slurry was stirred for 1 h at rt, then filtered over a pad of Celite, washing with large amounts of DCM (*ca.* 5 X 100 mL). The filtrate was concentrated *via* rotary evaporation, and the residue purified *via* column chromatography (SiO_2, 0-7% acetone/DCM) to yield **SI-14** (2.42 g, 55%) as a pale-yellow foam:

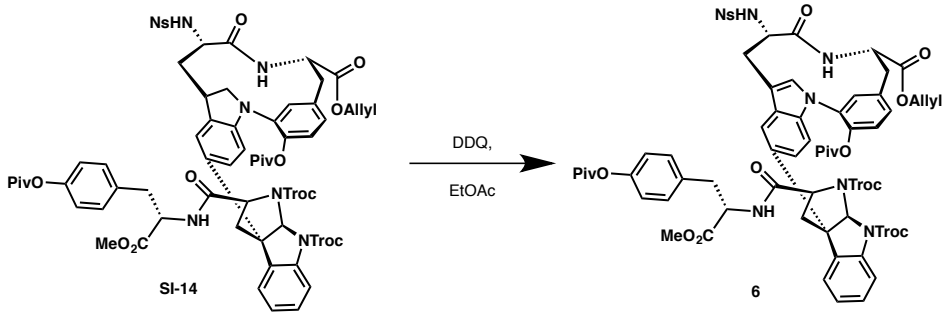
$[\alpha]_D^{20}$ = -37.7 (*c* = 1.0, $CHCl_3$);

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.98 (dd, *J* = 7.8, 1.4 Hz, 1H), 7.83 (dd, *J* = 7.9, 1.3 Hz, 1H), 7.79 (d, *J* = 7.7 Hz, 1H), 7.74 (t, *J* = 7.7 Hz, 1H), 7.67 (td, *J* = 7.7, 1.3 Hz, 1H), 7.49 (d, *J* = 39.6 Hz, 2H), 7.29 (t, *J* = 7.8 Hz, 1H), 7.24 – 7.12 (m, 1H), 7.05 – 6.84 (m, 6H), 5.27 – 5.22 (m, 2H), 4.90 – 4.78 (m, 3H), 4.39 (s, 2H), 4.23 (m, 2H), 4.05 (ddd, *J* = 12.0, 9.8, 2.4 Hz, 1H), 3.52 (s, 3H), 3.41 (d, *J* = 13.5 Hz, 1H), 3.32 (dd, *J* = 11.8, 3.0 Hz, 1H), 3.10 (s, 1H), 2.97 (d, *J* = 12.8 Hz, 1H), 2.66 (dd, *J* = 13.7, 4.6 Hz, 1H), 2.56 (d, *J* = 38.4 Hz, 2H), 2.40 – 2.27 (m, 2H), 1.34 (s, 9H), 1.30 (s, 9H).

^{13}C NMR (151 MHz, $CDCl_3$): δ 177.1, 176.5, 171.4, 170.2, 169.8, 167.9, 151.7, 150.3, 148.9, 148.2, 143.5, 140.2, 135.6, 135.1, 134.6, 134.4, 133.2, 132.9, 132.6, 131.3, 131.1, 130.1, 129.2, 128.1, 125.9, 125.5, 125.4, 124.2, 122.5, 121.7, 119.4, 117.5, 117.1, 95.3, 95.1, 83.9, 75.4, 66.1, 62.5 57.3, 54.5, 53.3, 52.2, 39.5, 39.2 39.1, 37.8, 27.4, 27.3.

HRMS: Calc'd for $C_{66}H_{68}Cl_6N_7O_{18}S$, $[M+H]^+$, 1488.2467; found, 1488.2482.

R_f = 0.68 (Hex/EtOAc = 2:3; UV, $Ce_2(SO_4)_3$ in phosphomolybdic acid)



Compound 6

A stoppered pressure vessel was charged with **SI-14** (2.42 g, 1.62 mmol, 1 equiv), DDQ (1.10 g, 4.86 mmol, 3.00 equiv), and EtOAc (15 mL). The vessel was sealed and heated to 100 ^{o}C for 36 h, at which time LC/MS analysis showed complete consumption of starting material. The mixture was cooled to rt, poured over aqueous $NaHCO_3$ (saturated, 50 mL), and extracted with additional EtOAc (4 X 50 mL). The combined organics were

dried ($MgSO_4$) and concentrated *via* rotary evaporation, and the residue was purified *via* column chromatography (SiO_2, 0-10% actetone/DCM) to yield **6** (2.12 g, 88%) as an orange foam:

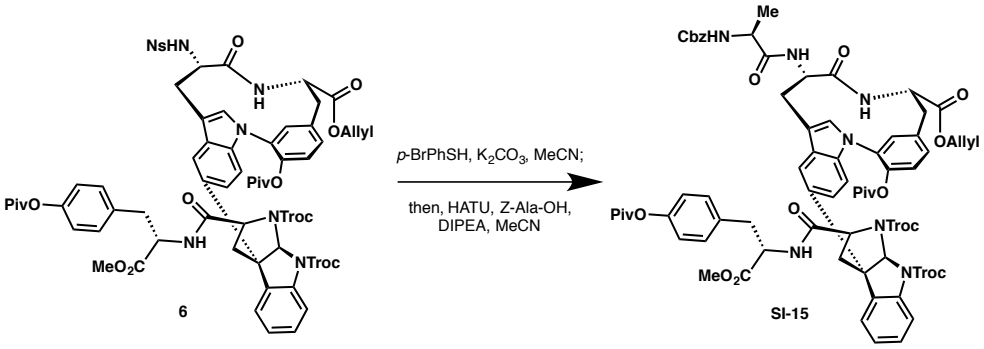
$[\alpha]_D^{20}$ = 50.2 (*c* = 1.0, $CHCl_3$);

^{1}H NMR (600 MHz, $CDCl_3$): δ 8.16 (dd, *J* = 7.7, 1.6 Hz, 1H), 7.96 – 7.90 (m, 2H), 7.86 – 7.78 (m, 3H), 7.51 (d, *J* = 7.6 Hz, 1H), 7.40 (d, *J* = 8.5 Hz, 1H), 7.35 – 7.31 (m, 1H), 7.18 (td, *J* = 7.5, 1.1 Hz, 1H), 7.05 (ddd, *J* = 9.3, 6.1, 3.2 Hz, 2H), 7.03 – 6.99 (m, 3H), 6.95 – 6.92 (m, 2H), 6.85 (s, 1H), 6.56 (s, 1H), 5.96 (d, *J* = 2.2 Hz, 1H), 5.84 (ddt, *J* = 17.2, 10.4, 5.9 Hz, 1H), 5.65 (d, *J* = 7.9 Hz, 1H), 5.31 – 5.21 (m, 2H), 4.93 (dd, *J* = 9.4, 1.9 Hz, 1H), 4.85 (d, *J* = 11.9 Hz, 2H), 4.50 (dq, *J* = 6.0, 1.4 Hz, 2H), 4.43 (ddd, *J* = 12.1, 10.3, 1.9 Hz, 1H), 4.36 (dt, *J* = 7.7, 3.7 Hz, 1H), 3.60 – 3.55 (m, 1H), 3.53 (s, 3H), 3.47 (d, *J* = 13.4 Hz, 1H), 3.29 – 3.18 (m, 1H), 3.03 (dd, *J* = 14.6, 3.4 Hz, 1H), 2.84 (dd, *J* = 14.4, 1.9 Hz, 1H), 2.71 (dd, *J* = 13.6, 4.7 Hz, 1H), 2.52 (dd, *J* = 14.3, 11.8 Hz, 1H), 2.36 (dd, *J* = 13.4, 7.9 Hz, 1H), 1.35 (s, 9H), 1.30 (s, 9H).

^{13}C NMR (151 MHz, $CDCl_3$): δ 177.3, 177.1, 171.5, 170.3, 169.6, 168.8, 151.9, 150.3, 148.9, 148.1, 147.5, 144.7, 140.3, 136.8, 135.5, 135.2, 135.1, 134.7, 134.6, 133.3, 133.3, 132.4, 131.9, 131.5, 130.1, 129.6, 129.3, 128.9, 127.9, 126.3, 126.1, 125.3, 123.9, 123.2, 121.8, 119.3, 117.8, 117.3, 116.1, 115.4, 114.1, 95.1, 95.0, 85.3, 75.6, 75.5, 66.4, 62.7, 60.9, 54.2, 53.4, 52.2, 39.3, 39.2, 37.9, 35.8, 27.5, 27.3, 27.3.

HRMS: Calc'd for $C_{66}H_{66}Cl_6N_7O_{18}S$, $[M+H]^+$, 1486.2311; found, 1486.2327.

R_f = 0.52 (Hex/EtOAc = 2:3; UV, $Ce_2(SO_4)_3$ in phosphomolybdic acid)



SI-15

A solution of **6** (1.61 g, 1.08 mmol, 1 equiv) and 4-bromothiophenol (1.02 g, 5.40 mmol, 5.00 equiv) in MeCN (10 mL) was sparged with argon for 30 min. The septum was quickly removed, and K_2CO_3 (745 mg, 5.40 mmol, 5.00 equiv) was added as a solid in a single portion. The septum was quickly replaced and the mixture stirred at rt for 1 h, at which time LC/MS analysis showed complete consumption of starting material.

At this time, in a separate flask, *N*-Cbz-*L*-Alanine (2.41 g, 10.8 mmol, 10.0 equiv) and HATU (4.11 g, 10.8 mmol, 10.0 equiv) were dissolved in MeCN (10 mL). DIPEA (5.7

mL, 33 mmol, 30 equiv) was then added, and the mixture stirred for 30 sec before being transferred quickly *via* syringe to the substrate/thiophenol-containing flask.
The resultant reaction was stirred for 30 min, then concentrated *via* rotary evaporation to a volume of *ca.* 5 mL, then diluted with EtOAc (60 mL). The mixture was washed with aqueous HCl (1 M, 3 X 50 mL) (added carefully with concomitant gas evolution), then aqueous $NaHCO_3$ (saturated, 2 X 50 mL), then brine (50 mL). The organic layer was dried ($MgSO_4$) and concentrated *via* rotary evaporation, and the residue purified by column chromatography (SiO_2, 0-16% acetone/DCM) to yield **SI-15** (1.08 g, 66%) as a white foam:

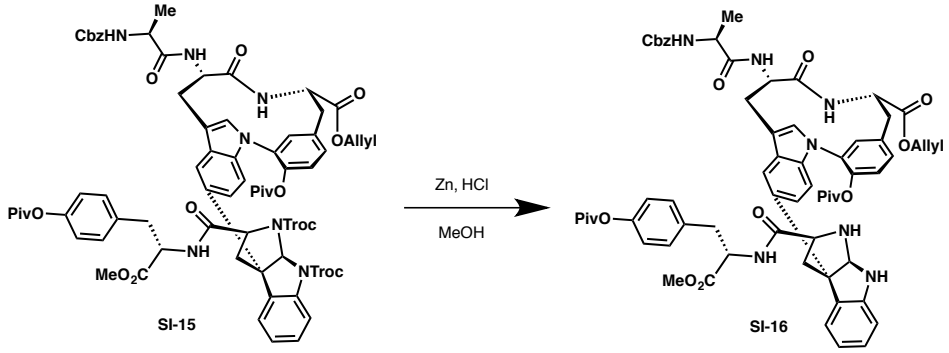
$[\alpha]_D^{20} = 40.1$ (c = 1.0, $CHCl_3$);

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.73 (d, J = 8.2 Hz, 1H), 7.66 (d, J = 8.4 Hz, 1H), 7.55 (d, J = 10.0 Hz, 1H), 7.33 (dd, J = 5.0, 2.0 Hz, 3H), 7.24 – 7.20 (m, 2H), 7.16 – 7.08 (m, 5H), 7.04 (d, J = 8.2 Hz, 1H), 7.00 – 6.92 (m, 3H), 6.85 (s, 2H), 6.61 – 6.52 (m, 3H), 5.99 (d, J = 2.1 Hz, 1H), 5.92 (ddt, J = 17.4, 10.6, 5.7 Hz, 1H), 5.35 – 5.29 (m, 1H), 5.22 (dq, J = 10.6, 1.3 Hz, 1H), 5.16 (d, J = 12.3 Hz, 1H), 4.96 – 4.88 (m, 2H), 4.82 (t, J = 11.8 Hz, 2H), 4.72 (d, J = 11.8 Hz, 1H), 4.62 – 4.56 (m, 3H), 4.37 (ddd, J = 12.4, 10.1, 2.6 Hz, 1H), 4.29 (s, 1H), 4.07 (tt, J = 7.6, 4.3 Hz, 1H), 3.76 (d, J = 12.1 Hz, 1H), 3.53 (s, 3H), 3.53 – 3.47 (m, 1H), 2.94 – 2.83 (m, 2H), 2.68 (dd, J = 14.7, 3.1 Hz, 1H), 2.53 (dd, J = 13.7, 5.3 Hz, 1H), 2.40 (s, 1H), 2.22 (s, 1H), 1.69 – 1.63 (m, 3H), 1.33 (d, J = 6.0 Hz, 17H).

^{13}C NMR (151 MHz, $CDCl_3$): δ 177.5, 177.2, 171.6, 171.5, 170.7, 169.7, 168.7, 157.6, 154.4, 152.4, 150.3, 149.2, 147.6, 144.4, 139.0, 136.4, 136.0, 135.4, 135.3, 135.2, 133.2, 132.0, 130.1, 129.5, 129.3, 129.0, 128.9, 128.8, 128.1, 125.5, 122.9, 121.7, 118.5, 117.3, 117.2, 116.9, 114.6, 94.9, 94.6, 85.9, 76.0, 75.9, 67.6, 66.1, 63.1, 59.0, 57.0, 54.4, 53.4, 52.8, 52.3, 39.5, 39.3, 39.2, 38.3, 34.9, 27.3, 27.2, 24.9, 16.7.

HRMS: Calc'd for $C_{71}H_{74}Cl_6N_7O_{17}$, $[M+H]^+$, 1506.3267; found, 1506.3261.

***R**$_f$* = 0.25 (Hex:EtOAc = 1:1; UV, $Ce_2(SO_4)_3$ in phosphomolybdic acid)



Note: Vigorous stirring is extremely important for this reaction. Reduced stirring vigor will reduce yields. Also, in our hands, attempts to increase this reaction's scale beyond what is reported here resulted in lower yields.

SI-16

To a vigorously (1500 RPM) stirred solution of **SI-15** (100 mg, 0.0663 mmol, 1 equiv) in MeOH (4 mL) was added aqueous HCl (3 M, 1 mL). The resultant slurry was cooled to 0 ^{o}C, then was added Zn dust (1 g) and the mixture was allowed to warm to rt. After 2 h, LC/MS analysis showed complete deprotection. At this time, the mixture was filtered over a pad of Celite, washing with additional MeOH (*ca.* 6 X 5 mL). The filtrate was concentrated *via* rotary evaporation, and the residue purified *via* column chromatography (SiO_2, 0-7% MeOH/DCM with 0.5% Et_3N) to yield **SI-16** (49 mg, 64%) as a white foam:

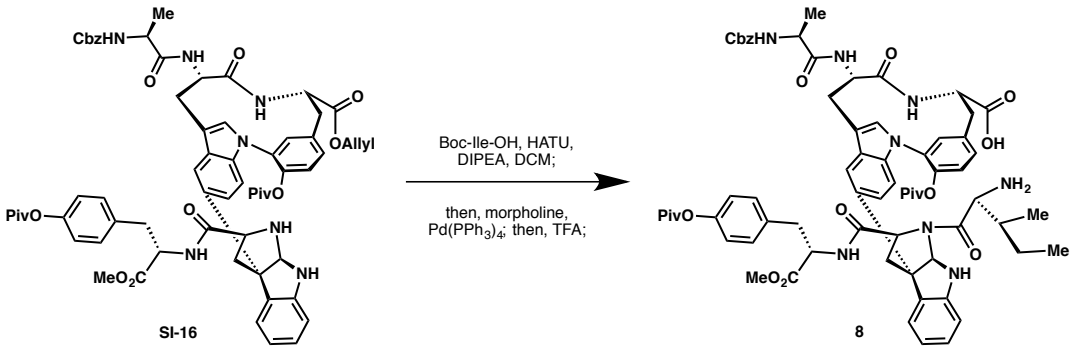
$\mathbf{[\alpha]_D^{20}}$ = 19.7 (*c* = 1.0, $CHCl_3$);

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.59 (d, *J* = 8.5 Hz, 1H), 7.44 (d, *J* = 5.9 Hz, 1H), 7.36 (dd, *J* = 5.1, 1.9 Hz, 3H), 7.25 (d, *J* = 6.2 Hz, 1H), 7.21 (dd, *J* = 7.4, 1.2 Hz, 1H), 7.11 (ddd, *J* = 8.6, 5.4, 2.5 Hz, 8H), 7.04 (d, *J* = 8.3 Hz, 1H), 7.02 – 7.00 (m, 2H), 6.95 (td, *J* = 7.4, 0.9 Hz, 1H), 6.89 (d, *J* = 1.8 Hz, 1H), 6.51 (s, 1H), 6.41 (d, *J* = 7.8 Hz, 1H), 6.37 (d, *J* = 9.1 Hz, 1H), 5.93 – 5.83 (m, 2H), 5.32 – 5.27 (m, 1H), 5.21 – 5.17 (m, 1H), 5.00 (s, 1H), 4.76 (d, *J* = 11.9 Hz, 1H), 4.71 (dt, *J* = 9.1, 3.6 Hz, 1H), 4.57 (dq, *J* = 5.4, 1.8 Hz, 2H), 4.32 (ddd, *J* = 11.5, 9.9, 2.4 Hz, 1H), 4.04 – 3.98 (m, 2H), 3.87 (d, *J* = 11.8 Hz, 1H), 3.81 (dt, *J* = 9.0, 4.5 Hz, 1H), 3.60 (s, 3H), 3.46 – 3.42 (m, 1H), 3.04 (dd, *J* = 13.6, 2.5 Hz, 1H), 2.95 – 2.86 (m, 3H), 2.66 (dt, *J* = 13.9, 3.8 Hz, 3H), 1.34 (d, *J* = 1.0 Hz, 18H), 1.20 (d, *J* = 7.5 Hz, 3H).

^{13}C NMR (151 MHz, $CDCl_3$): δ 177.6, 177.5, 173.0, 172.4, 171.8, 170.6, 169.6, 157.1, 150.4, 148.3, 148.2, 146.3, 144.5, 139.1, 136.4, 135.6, 135.4, 133.7, 133.6, 131.9, 131.7, 130.3, 129.4, 129.1, 128.8, 128.7, 128.6, 128.5, 127.2, 123.7, 123.0, 122.0, 121.7, 118.2, 117.3, 117.1, 116.7, 112.0, 88.2, 67.3, 66.0, 62.6, 62.6, 56.4, 54.4, 53.5, 52.3, 52.0, 46.6, 39.3, 39.2, 37.8, 34.3, 27.3, 27.2, 25.0, 17.7.

HRMS: Calc'd for $C_{65}H_{72}N_7O_{13}$, $[M+H]^+$, 1158.5183; found, 1158.5195.

***R**$_f$* = 0.25 (Hex/EtOAc = 1:4; UV, $Ce_2(SO_4)_3$ in phosphomolybdic acid)



Compound 8

To a stirred solution of **SI-16** (49 mg, 0.042 mmol, 1 equiv) in DCM (0.5 mL) was added *N*-Boc-isoleucine (98 mg, 0.42 mmol, 10 equiv), then HATU (161 mg, 0.423 mmol, 10 equiv), then DIPEA (0.22 mL, 1.3 mmol, 30 equiv). The mixture was stirred at rt for 24 h, at which time LCMS analysis showed complete consumption of starting material.

The reaction was cooled to 0 ^{o}C, then morpholine (0.05 mL, 0.6 mmol, 15 equiv) was added, followed by $Pd(PPh_3)_4$ (0.5 mg, 0.0004 mmol, 1 mol%). The mixture was warmed to rt and stirred for 30 min, at which time LC/MS analysis showed complete conversion to the deallylated product (i.e., complete disappearance of a peak with *m/z* = 1371.6, with concomitant formation of a peak with *m/z* = 1331.6).

At this time, the mixture was cooled to 0 ^{o}C, and TFA (3 mL) was added dropwise. The mixture was again warmed to rt and stirred for 1 h, at which time LC/MS analysis showed complete deprotection of the Boc moiety (i.e., complete disappearance of the peak with *m/z* = 1331.6, with concomitant formation of a peak with *m/z* = 1231.6).

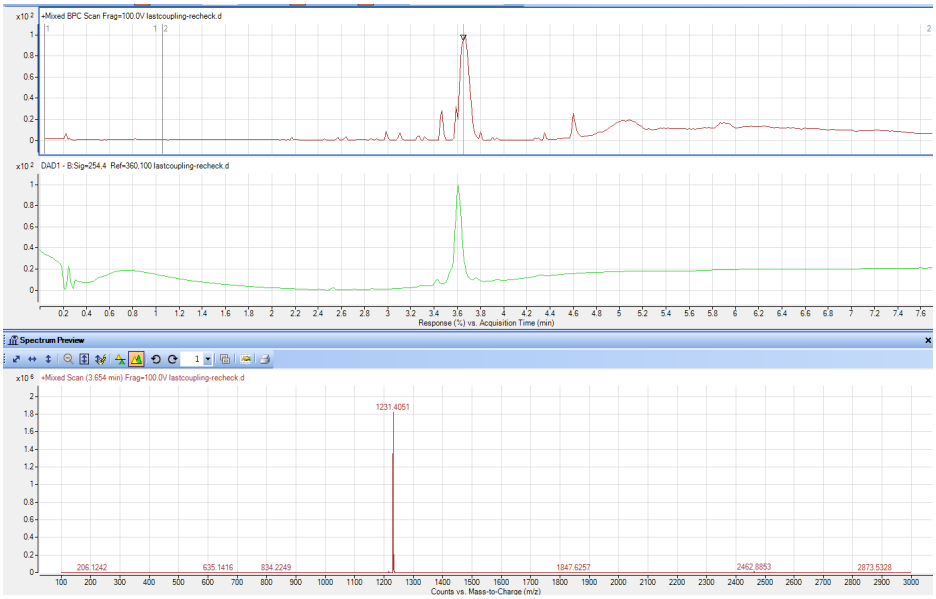
At this time, the reaction mixture was concentrated *via* rotary evaporation, and the viscous oily residue was diluted with DMSO (0.6 mL). The resultant solution was purified *via* preparative HPLC (C18, 45-65% MeCN/H_2O with 0.1% HCO_2H over 12 min; product elutes at 6.4 min). The fractions containing desired material were combined and lyophilized to yield a white foam. This white foam was dissolved in a mixture of MeCN (2 mL) and aqueous HCl (30 mM, 2 mL), and lyophilized a second time, to yield the title product's HCl salt (16 mg, 30%) as a white foam:

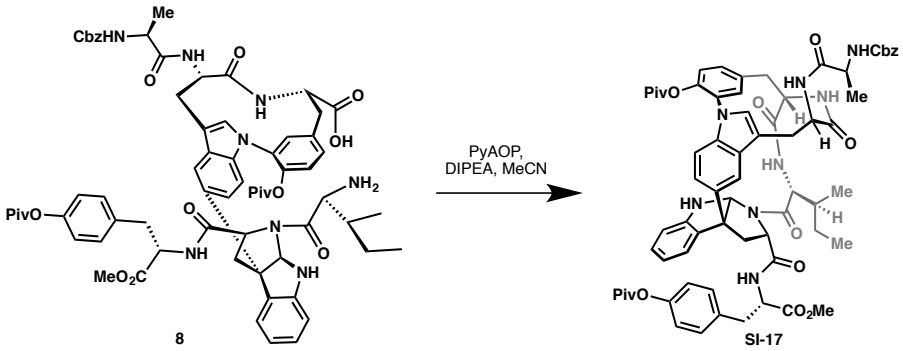
$\mathbf{[\alpha]_D^{24.4}}$ = 39.2 (*c* = 1.0, $CHCl_3$);

NMR: Due to the rotamerism of this compound, its NMR was not assigned (see spectral data).

HRMS: Calc'd for $C_{68}H_{79}N_8O_{14}$, $[M+H]^+$, 1231.5710; found, 1231.5721.

LC/low-resolution MS trace (254 nm absorption in top lane; SIR in bottom lane):





8 SI-17

SI-17

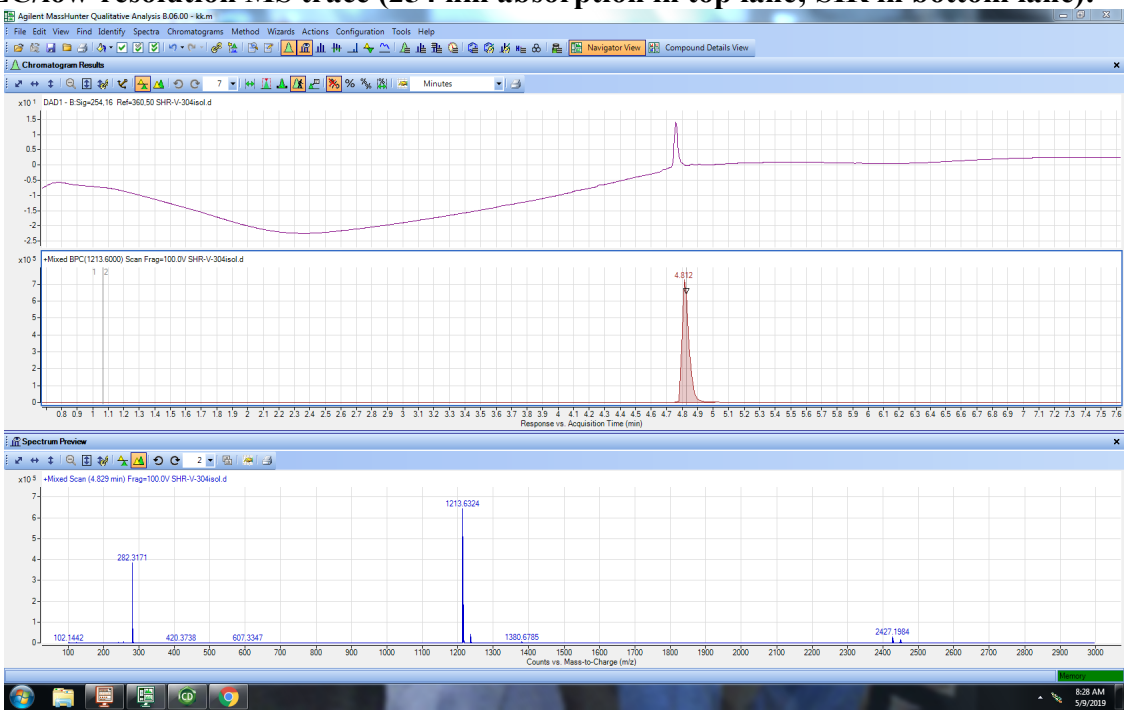
A 24-mL syringe was charged with zwitterion **8** (16 mg, 0.013 mmol, 1 equiv) in MeCN (15 mL). A separate 24-mL syringe was charged with PyAOP (14 mg, 0.026 mmol, 2 equiv) in MeCN (15 mL). A 100-mL round-bottom flask fitted with a septum was charged with DIPEA (3 drops from a 22-guage needle; *ca.* 0.015 mL, *ca.* 0.086 mmol, *ca.* 6 equiv) in MeCN (3 mL). The flask was warmed to 55 ^{o}C; then, the two aforementioned syringes were mounted in a 2-barrel syringe pump and concurrently added to the DIPEA solution over 12 h. After complete addition, LCMS analysis showed complete consumption of starting material. At this time, the reaction was cooled to rt, DMSO (1 mL) was added, and the excess solvent removed *via* rotary evaporation. The resultant DMSO solution was purified *via* preparative HPLC (C18, 65-95% MeCN/H_2O with 0.1% HCO_2H over 12 min; product elutes at 7.7 min). Fractions containing the desired product were combined and lyophilized to yield **SI-17** (0.6 mg, 4%) as a white foam:

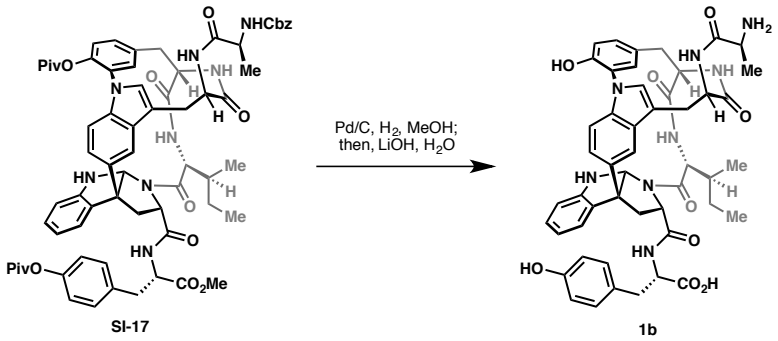
$[\alpha]_D^{23.1}$ = -2.2 (*c* = 1.0, MeOH);

NMR: Due to the rotamerism of this compound, its NMR was not assigned (see spectral data).

HRMS: Calc'd for $C_{68}H_{77}N_8O_{13}$ ($[M+H]^+$), 1213.5610; found, 1213.5608.

LC/low-resolution MS trace (254 nm absorption in top lane; SIR in bottom lane):





atrop-tryptorubin A (1b)

SI-17 (0.6 mg, 0.0005 mmol) was dissolved in MeOH (0.5 mL). The solution was sparged with argon for 30 min, then Pd/C (*ca.* 0.1 mg) was added. The solution was put under an atmosphere of H_2 (tripled party balloon; *ca.* 1.1 atm) and subjected to 3 vaccuum/backfill cycles, then stirred at rt for 24 h, at which time LC/MS analysis showed complete removal of the Cbz protecting group (i.e., complete disappearance of a peak with *m/z* = 1213.6, with concomitant formation of a peak with *m/z* = 1079.5). At this time, the atmosphere was changed to argon, and the mixture was subjected to an additional 3 vacuum/backfill cycles. Then, aqueous LiOH (1N, 0.1 mL) was added, and the mixture was stirred at rt for 2 h at which time LC/MS analysis showed complete conversion (i.e., complete disappearance of the peak with *m/z* = 1079.5, with concomitant formation of a peak with *m/z* = 897.4). The mixture was quenched with HCl (1 M, 0.1

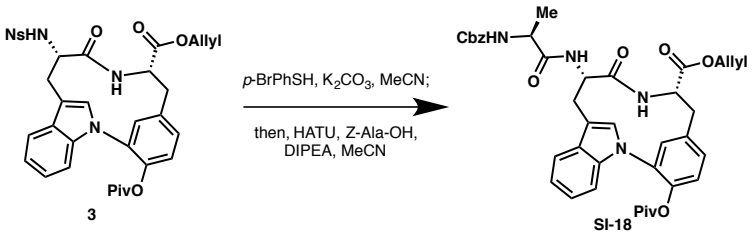
mL), then filtered through a pad of Celite, washing with additional MeOH (*ca.* 3 X 1 mL). The resultant filtrate was diluted with DMSO (0.3 mL) and excess solvent removed *via* rotary evaporation. The concentrated solution was purified *via* preparative HPLC (C18, 15-35% MeCN/H_2O with 0.1% HCO_2H over 12 min; product elutes at 9.5 min), and the fractions containing desired product were combined and lyophilized to yield the title product's formate salt (0.4 mg, 90%) as a white solid.

$[\alpha]_D^{25.8}$ = -19.6 (*c* = 0.2, MeOH);

NMR: See tabulated data, page 52

HRMS: Calc'd for $C_{49}H_{53}N_8O_9$ ($[M+H]^+$), 897.3936; found, 897.3913.

LC/low-resolution MS trace: See *LCMS co-injection of natural and synthetic tryptorubin A*, page 68



SI-18

A solution of **3** (10.1 g, 15.0 mmol, 1 equiv) and *p*-bromothiophenol (14.2 g, 75.0 mmol, 5.00 equiv) were dissolved in MeCN (150 mL) and sparged with argon for 30 min. In one portion, K_2CO_3 (10.4 g, 75.0 mmol, 5.00 equiv) was added, and the mixture stirred at rt for 1 h. At this time, the solution was cooled to 0 ^oC. In a separate flask, *N*-Cbz-*L*-alanine (22.3 g, 100 mmol, 6.67 equiv) and HATU (38.0 g, 100 mmol, 6.67 equiv) were combined in MeCN (100 mL). In one portion, DIPEA (52 mL, 300 mmol, 20 equiv) was added, and the mixture stirred at rt for 30 seconds before being added in a single portion to the cooled macrocycle solution. The mixture was allowed to warm to rt and stir for 30 min. The mixture was then poured into HCl (1 M, 500 mL) and extracted with EtOAc (3 X 300 mL). The combined organics were washed with brine, dried ($MgSO_4$), and concentrated, and the residue was purified *via* column chromatography (SiO_2, 0-80% EtOAc/hex) to yield the title product (9.06 g, 87%) as a yellow foam:

$[\alpha]_D^{25.1}$ = 64.4 (*c* = 1.0, $CHCl_3$);

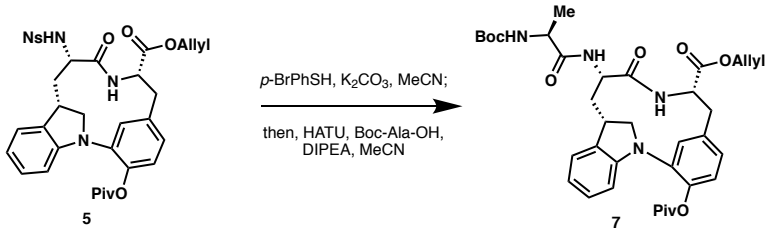
^{1}H NMR (600 MHz, $CDCl_3$): δ 7.56 (d, *J* = 7.7 Hz, 1H), 7.54 (d, *J* = 8.0 Hz, 1H), 7.35 (d, *J* = 9.9 Hz, 1H), 7.33 – 7.27 (m, 4H), 7.17 (t, *J* = 7.6 Hz, 1H), 7.10 (dd, *J* = 8.3, 2.2 Hz, 1H), 7.07 – 7.01 (m, 3H), 6.72 (d, *J* = 9.4 Hz, 1H), 6.58 (s, 1H), 5.94 (d, *J* = 2.2 Hz, 1H), 5.89 (ddt, *J* = 16.4, 10.8, 5.5 Hz, 1H), 5.31 (d, *J* = 17.2 Hz, 1H), 5.21 (dd, *J* = 10.5, 1.5 Hz, 1H), 4.96 (dt, *J* = 9.4, 3.7 Hz, 1H), 4.83 (d, *J* = 3.1 Hz, 1H), 4.73 (d, *J* = 11.7 Hz, 1H), 4.64 – 4.55 (m, 2H), 4.43 (dt, *J* = 9.8, 6.7 Hz, 1H), 4.03 (d, *J* = 11.8 Hz, 1H), 3.98

(qd, *J* = 7.3, 3.0 Hz, 1H), 3.60 (dd, *J* = 14.5, 3.5 Hz, 1H), 2.96 – 2.87 (m, 3H), 1.48 (d, *J* = 7.3 Hz, 3H), 1.34 (s, 9H).

^{13}C NMR (151 MHz, $CDCl_3$): δ 177.4, 171.1, 170.6, 169.7, 156.8, 145.0, 146.1, 144.7, 136.2, 135.6, 134.8, 134.4, 131.7, 129.3, 129.0, 128.6, 128.6, 128.4, 125.0, 123.2, 123.0, 118.4, 118.3, 117.1, 116.9, 67.6, 66.0, 56.4, 54.3, 52.5, 39.3, 34.4, 27.3, 25.5, 17.7.

HRMS: Calc'd for $C_{39}H_{43}N_4O_8$, $[M+H]^+$, 695.3075; found, 695.3090.

***R**$_f$* = 0.20 (Hex:EtOAc = 1:1; UV, $Ce_2(SO_4)_3$ in phosphomolybdic acid)



Compound 7

A solution of **5** (68 mg, 0.10 mmol, 1 equiv) and *p*-bromothiophenol (95 mg, 0.50 mmol, 5.00 equiv) were dissolved in MeCN (1 mL) and sparged with argon for 30 min. In one portion, K_2CO_3 (69 mg, 0.50 mmol, 5.00 equiv) was added, and the mixture stirred at rt for 1 h. At this time, the solution was cooled to 0 ^oC. In a separate flask, Boc-alanine (126 mg, 0.67 mmol, 6.67 equiv) and HATU (253 mg, 0.67 mmol, 6.67 equiv) were combined in MeCN (1 mL). In one portion, DIPEA (0.35 mL, 2 mmol, 20 equiv) was added, and the mixture stirred at rt for 30 seconds before being added in a single portion to the cooled macrocycle solution. The mixture was allowed to warm to rt and stir for 30 min. The mixture was then poured into HCl (1 M, 5 mL) and extracted with EtOAc (3 X 5 mL). The combined organics were washed with brine, dried ($MgSO_4$), and concentrated, and the residue was purified *via* column chromatography (SiO_2, 0-80% EtOAc/hex) to yield the **7** (50 mg, 75%) as a yellow foam. Crystals suitable for X-ray diffraction were formed by dissolving the compound in MeCN (*ca.* 100 mg/mL) and allowing the mixture to slowly evaporate until crystals were observed.

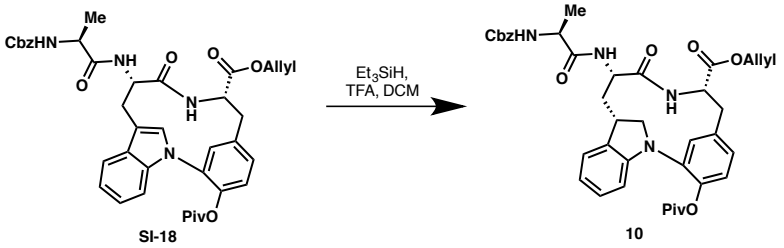
X-ray crystallography: Solved structure; see details on page 134.

$[\alpha]_D^{23.1}$ = -10.2 (*c* = 0.4, MeOH);

^{1}H NMR (600 MHz, DMSO-d_6) (Major rotamer) δ 7.22 – 7.14 (m, 1H), 7.13 – 7.07 (m, 1H), 7.04 (t, *J* = 7.7 Hz, 1H), 7.01 (d, *J* = 8.1 Hz, 1H), 6.92 – 6.77 (m, 3H), 6.59 (d, *J* = 7.8 Hz, 1H), 5.86 (ddt, *J* = 17.5, 10.7, 5.4 Hz, 1H), 5.27 (dd, *J* = 17.3, 1.7 Hz, 1H), 5.18 (dd, *J* = 10.5, 1.5 Hz, 1H), 4.55 (d, *J* = 5.4 Hz, 2H), 4.16 (t, *J* = 10.6 Hz, 1H), 4.08 – 3.96 (m, 1H), 3.96 – 3.81 (m, 1H), 3.69 – 3.53 (m, 1H), 3.37 – 3.31 (m, 2H), 2.98 (d, *J* = 11.2 Hz, 1H), 2.69 – 2.60 (m, 1H), 2.21 – 2.10 (m, 1H), 2.09 – 1.97 (m, 1H), 1.35 (s, 9H), 1.21 (s, 9H), 1.10 (d, *J* = 7.1 Hz, 3H).

^{13}C NMR (151 MHz, DMSO) (Major rotamer) δ 176.5, 171.8, 171.4, 170.2, 155.2, 151.2, 136.8, 136.5, 132.2, 132.2, 132.1, 128.2, 126.9, 124.1, 122.7, 121.1, 118.1, 117.6, 114.6, 78.1, 65.2, 65.1, 54.9, 54.1, 53.3, 49.4, 40.4, 38.5, 36.8, 28.2, 26.8, 17.8.

HRMS: Calc'd for $C_{36}H_{47}N_4O_8$, $[M+H]^+$, 663.3388; found, 663.3389.



Compound 10

To a solution of **SI-18** (9.06 g, 13.1 mmol) in DCM (100 mL) was added sequentially Et_3SiH (100 mL), then TFA (100 mL). The mixture was stirred at rt for 4 h, then the volatiles removed *via* rotary evaporation, and the residue purified *via* column chromatography (SiO_2, 0-16% acetone/DCM) to yield **10** (8.58 g, 94%) as a yellow foam:

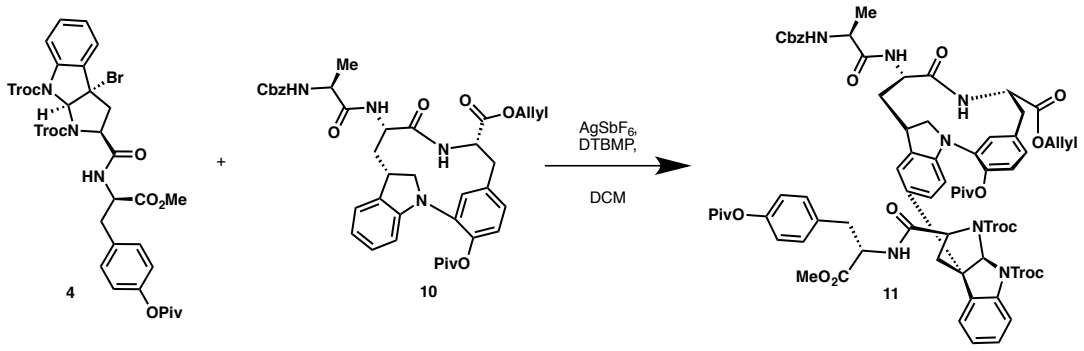
$[\alpha]_D^{24.4}$ = 69.2 (*c* = 1.0, $CHCl_3$);

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.36 – 7.29 (m, 3H), 7.25 – 7.20 (m, 3H), 7.16 – 7.07 (m, 2H), 7.07 – 7.01 (m, 2H), 6.96 – 6.87 (m, 2H), 6.78 (d, *J* = 7.9 Hz, 1H), 6.07 (s, 1H), 5.91 (ddt, *J* = 16.4, 10.9, 5.6 Hz, 1H), 5.34 (dd, *J* = 17.2, 1.5 Hz, 1H), 5.24 (dd, *J* = 10.5, 1.3 Hz, 1H), 5.11 (d, *J* = 11.8 Hz, 1H), 5.02 (s, 1H), 4.73 – 4.59 (m, 3H), 4.54 (s, 1H), 4.31 (t, *J* = 10.3 Hz, 1H), 4.14 (t, *J* = 11.4 Hz, 1H), 3.98 (s, 1H), 3.61 (s, 1H), 3.43 (dd, *J* = 11.6, 3.7 Hz, 1H), 3.08 (dd, *J* = 13.2, 2.3 Hz, 1H), 2.88 (t, *J* = 12.5 Hz, 1H), 2.69 (d, *J* = 15.5 Hz, 1H), 2.26 – 2.06 (m, 1H), 1.36 (d, *J* = 7.3 Hz, 3H), 1.33 (s, 9H).

^{13}C NMR (151 MHz, $CDCl_3$): δ 176.8, 171.6, 170.5, 168.7, 156.3, 150.9, 145.4, 143.8, 136.2, 135.1, 134.2, 131.7, 129.6, 128.8, 128.8, 128.6, 126.1, 125.6, 125.3, 124.0, 120.4, 118.7, 115.7, 67.7, 66.1, 62.1, 54.8, 52.2, 51.7, 39.4, 39.2, 38.2, 32.1, 27.4, 17.7.

HRMS: Calc'd for $C_{39}H_{45}N_4O_8$, $[M+H]^+$, 697.3232; found, 697.3240.

R_f = 0.50 (DCM:acetone = 8:1; UV, $Ce_2(SO_4)_3$ in phosphomolybdic acid)



Compound 11

Macrocycle **10** (8.58 g, 12.3 mmol, 1 equiv), alkyl bromide **4** (16.5 g, 18.5 mmol, 1.50 equiv), and 2,6-di-^{t}butyl-4-methylpyridine (7.6 g, 37 mmol, 3.0 equiv) were combined and azeotroped from benzene three times (3 X 50 mL). After the final azeotrope, the mixture was held under vacuum (<1 torr) for 13 h. The flask was backfilled with argon, and DCM (240 mL) was added. The septum was quickly removed, $AgSbF_6$ (12.7 g, 37 mmol, 3.00 equiv) was added as a solid in a single portion, and the septum quickly replaced. The resultant brown slurry was stirred for 1 h at rt, then filtered over a pad of Celite, washing with large amounts of DCM (*ca.* 5 X 200 mL). The filtrate was concentrated *via* rotary evaporation, and the residue purified *via* column chromatography (SiO_2, 0-20% acetone/DCM) to yield **11** (11.5 g, 62%) as a pale-yellow foam:

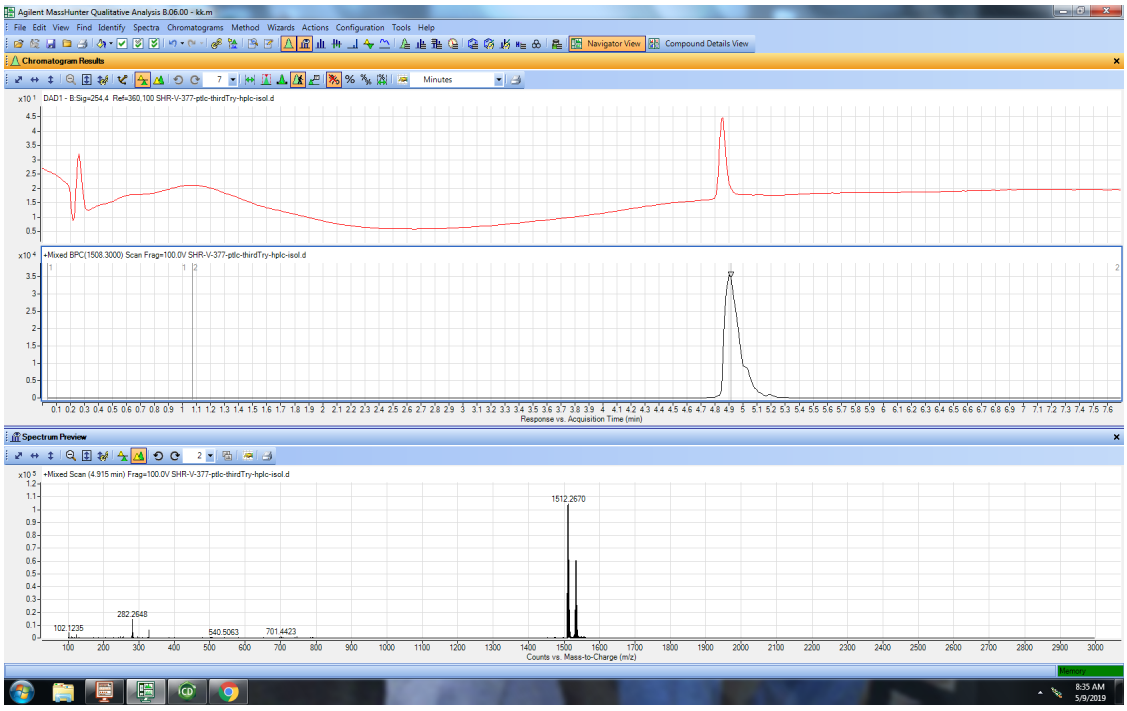
$[\alpha]_D^{23.6}$ = -14.8 (*c* = 1.0, $CHCl_3$);

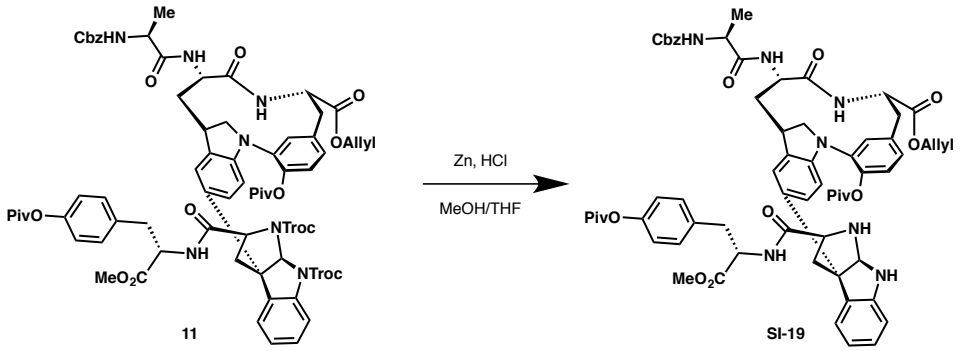
NMR: Due to rotamerism (see spectra), the NMR of this compound was not assigned.

HRMS: Calc'd for $C_{71}H_{76}Cl_6N_4O_{17}$, $[M+H]^+$, 1508.3423; found, 1508.3408.

R_f = 0.45 (DCM:acetone = 8:1; UV, $Ce_2(SO_4)_3$ in phosphomolybdic acid)

LC/low-resolution MS trace (254 nm absorption in top lane; SIR in bottom lane):





SI-19

A solution of **11** (11.5 g, 7.61 mmol) in MeOH/THF (2:1; 1.2 L total volume) was cooled to 0 °C. Then, sequentially, aqueous HCl (3 N, 60 mL, 180 mmol) and Zn^0 (11.7 g, 180 mmol) were added. The mixture was stirred at 0 °C, then filtered over a pad of Celite, washing with additional MeOH. The filtrate was concentrated *via* rotary evaporation, and the residue purified *via* column chromatography (SiO_2, 0-8% MeOH/DCM with 0.5% Et_3N) to yield **SI-19** (7.36 g, 84%) as a white foam:

$[\alpha]_D^{23.1}$ = -22.8 (*c* = 1.0, $CHCl_3$);

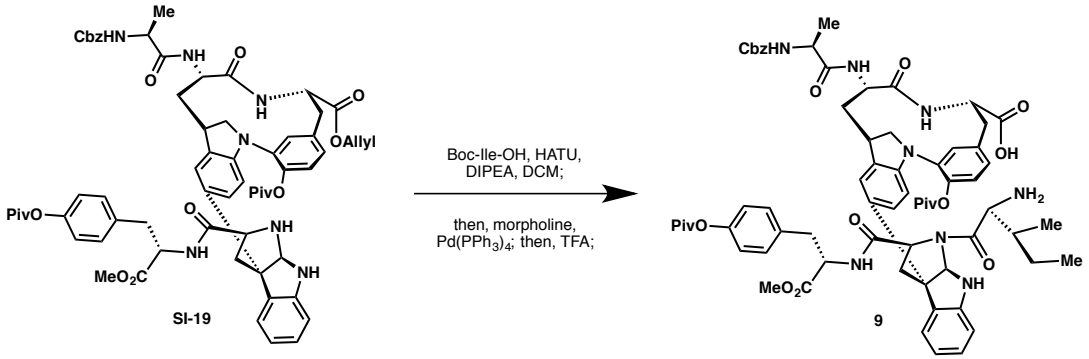
^{1}H NMR (600 MHz, $CDCl_3$): δ 7.49 – 7.30 (m, 6H), 7.24 – 7.16 (m, 2H), 7.11 (d, *J* = 8.4 Hz, 2H), 7.09 – 7.01 (m, 5H), 6.94 – 6.86 (m, 4H), 6.76 (s, 1H), 6.36 (d, *J* = 7.8 Hz, 1H), 6.13 (d, *J* = 9.8 Hz, 1H), 5.89 (ddt, *J* = 16.5, 11.0, 5.7 Hz, 1H), 5.31 (dd, *J* = 17.2, 1.6 Hz, 1H), 5.25 (s, 1H), 5.21 (d, *J* = 10.5 Hz, 1H), 5.14 (d, *J* = 11.9 Hz, 1H), 4.83 (s, 1H), 4.60 (qd, *J* = 13.3, 5.6 Hz, 2H), 4.51 (d, *J* = 11.9 Hz, 1H), 4.25 – 4.17 (m, 1H), 4.16 – 3.96 (m, 2H), 3.93 (d, *J* = 9.2 Hz, 1H), 3.90 – 3.81 (m, 2H), 3.57 (s, 3H), 3.49 (s, 1H),

3.33 (dd, *J* = 11.7, 4.4 Hz, 1H), 3.10 – 3.03 (m, 2H), 2.85 (t, *J* = 12.3 Hz, 1H), 2.65 – 2.53 (m, 3H), 2.47 (dd, *J* = 13.4, 9.3 Hz, 1H), 1.98 (dt, *J* = 15.7, 5.4 Hz, 1H), 1.36 (s, 9H), 1.33 (s, 9H), 1.05 (d, *J* = 7.4 Hz, 3H).

^{13}C NMR (151 MHz, $CDCl_3$): δ 177.6, 176.8, 173.0, 172.4, 172.1, 170.5, 168.3, 157.0, 150.4, 150.2, 148.0, 144.9, 144.5, 136.4, 136.2, 135.9, 133.7, 132.7, 131.9, 130.5, 130.3, 128.8, 128.7, 128.6, 128.4, 127.4, 125.6, 125.5, 123.9, 123.3, 122.1, 121.3, 118.6, 116.5, 112.4, 87.4, 67.4, 66.0, 62.7, 62.7, 62.3, 60.5, 54.8, 54.7, 53.6, 53.5, 52.3, 51.9, 51.8, 51.6, 47.6, 39.2, 39.2, 37.8, 27.4, 27.3, 17.7.

HRMS: Calc'd for $C_{65}H_{74}N_7O_{13}$, $[M+H]^+$, 1160.5339; found, 1160.5352.

R_f: 0.30 (Hex:EtOAc = 1:4; UV, $Ce_2(SO_4)_3$ in phosphomolybdic acid)



Compound 9

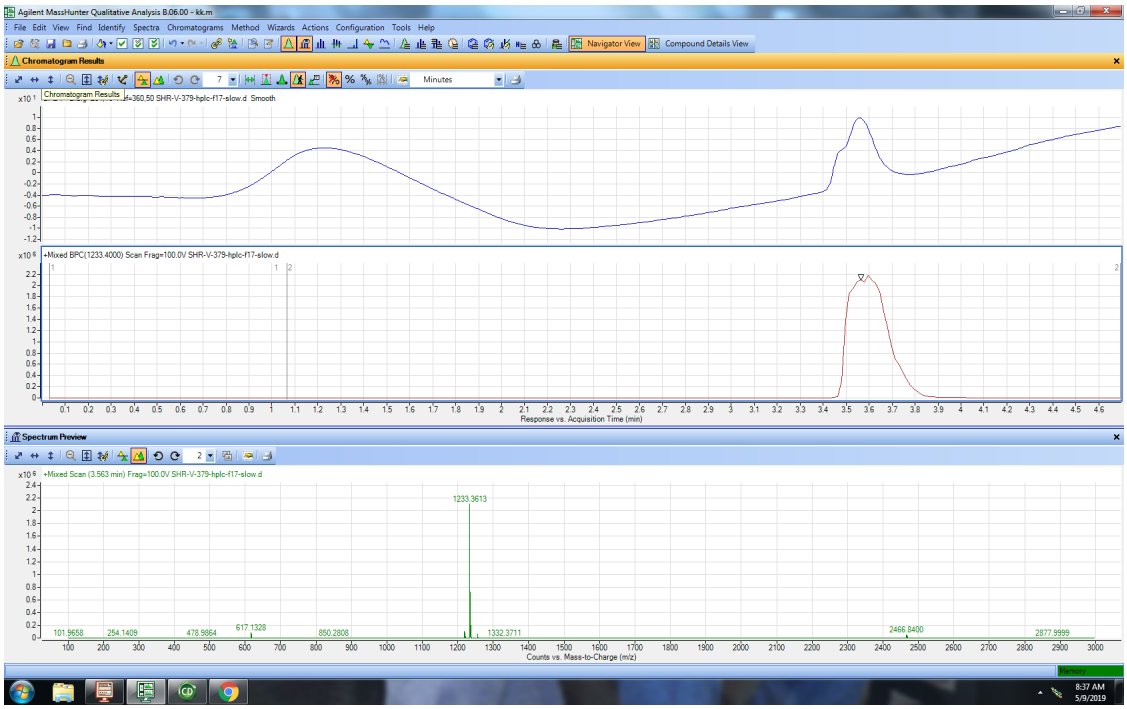
To a solution of **SI-19 (**7.36 g, 6.34 mmol, 1 equiv), *N*-Boc-L-Isoleucine (2.20 g, 9.51 mmol, 1.50 equiv), and HATU (3.61 g, 9.51 mmol, 1.50 equiv) in DCM (120 mL) was added DIPEA (5.0 mL, 29 mmol, 4.6 equiv). The mixture was stirred at rt for 12 h, at which time LCMS showed complete consumption of starting material. The mixture was cooled to 0 ^oC, and morpholine (5.5 mL, 63 mmol, 10 equiv) was added, followed by $Pd(PPh_3)_4$ (73 mg, 0.063 mmol, 1 mol%). The mixture was warmed to rt and stirred 1 h. Then, the volatiles were removed *via* rotary evaporation. The residue was taken up in DCM (40 mL), then TFA (40 mL) was added. After stirring at rt for 20 min, the volatiles were again removed. The residue was taken up in DMSO (2 mL) and purified *via* preparative HPLC (C18, 35-55% MeCN/H_2O with 0.1% HCO_2H over 12 min; product elutes at 10.5 min). Fractions containing the desired product were lyophilized, and the residue was taken up in MeCN/aqueous HCl (10 mL of MeCN and 30 mL of 20 mM HCl) and lyophilized a second time, to yield **9** (2.24 g, 29%) as a yellow foam:

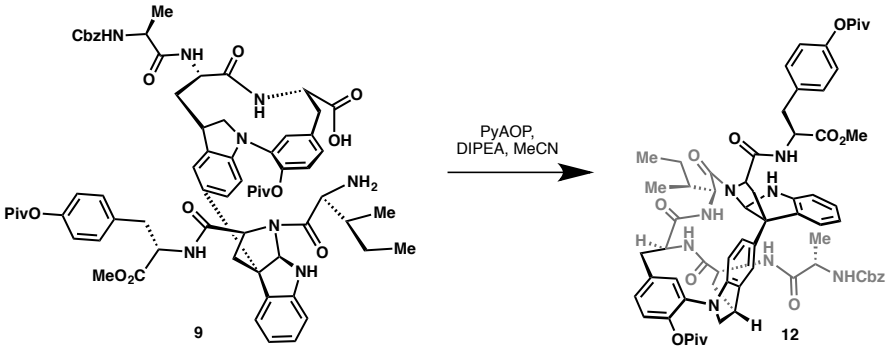
$[\alpha]_D^{25}$ = -72.9 (*c* = 1.0, $CHCl_3$);

NMR: Due to rotamerism (see spectra), the NMR of this compound was not assigned.

HRMS: Calc'd for $C_{68}H_{81}N_8O_{14}$, $[M+H]^+$, 1233.5867; found, 1233.5852.

LC/low-resolution MS trace (254 nm absorption top lane; SIR bottom lane):





9 12

Compound 12

A 24-mL syringe was charged with zwitterion **9** (123 mg, 0.100 mmol, 1 equiv) in MeCN (20 mL). A separate 24-mL syringe was charged with PyAOP (104 mg, 0.200 mmol, 2.00 equiv) in MeCN (20 mL). A 500-mL round-bottom flask fitted with a septum was charged with DIPEA (0.17 mL, 1.0 mmol, 10 equiv) in MeCN (150 mL). The flask was warmed to 60 ^{o}C; then, the two aforementioned syringes were mounted in a 2-barrel syringe pump and concurrently added to the DIPEA solution over 16 h. After complete addition, LCMS analysis showed complete consumption of starting material. At this time, the reaction was cooled to rt, DMSO (1 mL) was added, and the excess solvent removed *via* rotary evaporation. The resultant DMSO solution was purified *via* preparative HPLC (C18, 55-75% MeCN/H_2O with 0.1% HCO_2H over 12 min; product elutes at 8.9 min). Fractions containing the desired product were combined and lyophilized to yield **12** (13 mg, 11%) as a white foam:

$[\alpha]_D^{24.7}$ = -212.2 (*c* = 1.0, $CHCl_3$);

NMR: Due to rotamerism (see spectra), the NMR of this compound was not assigned.

HRMS: Calc'd for $C_{68}H_{79}N_8O_{13}$, $[M+H]^+$, 1215.5761; found, 1215.5741.

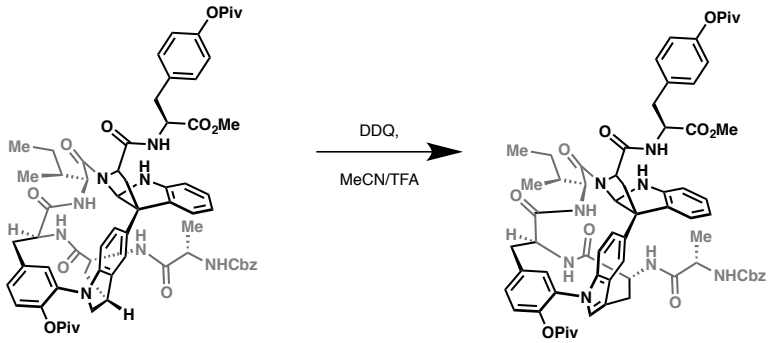
LC/low-resolution MS trace (254 nm absorption in top lane; SIR in bottom lane):

Table S2, optimization of macrolactamization

Standard conditions: base = DIPEA; solvent = MeCN; coupling reagent = PyAOP; final concentration = 0.5 mM; addition method = dual slow addition of PyAOP and substrate

Entry	Difference from standard conditions	Yield 12 (%)	Major side product
standard conditions	-	11	-
1	base = collidine	8	-
2	base = NMM	8	-
3	solvent = DMF	-	dimethylamide
4	solvent = DMA	-	dimethylamide
5	solvent = DMSO	-	-
6	coupling reagent = HATU	3	guanadinylation
7	coupling reagent = PyOxim	9	-
8	coupling reagent = EDCI/DMAP	<1	-
9	coupling reagent = T3P	<1	-
10	final concentration = 0.05 mM	9	-
11	final concentration = 5 mM	2	-
12	mix reagents all at start	-	-

13	Slow add'n of substrate to PyAOP	-	-
14	8 hour dual add'n	7	-



SI-20

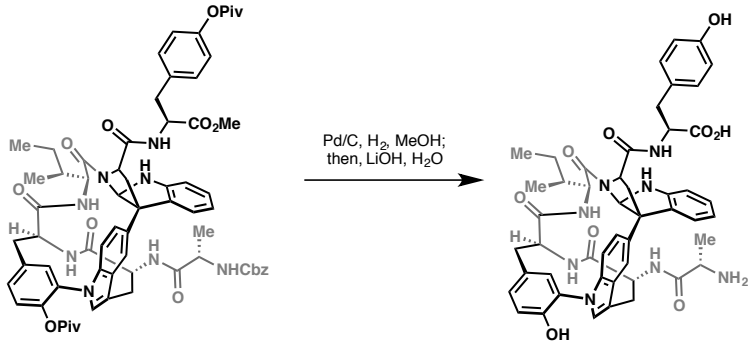
A 0.5 mL microwave vial was charged with **12 (**2.4 mg, 0.0020 mmol, 1 equiv), MeCN (0.15 mL) and TFA (0.15 mL). DDQ (4.5 mg, .020 mmol, 10 equiv) was added, and the microwave vial was sealed and heated to 55 ^{o}C in the microwave for 5 min. The mixture was concentrated *via* rotary evaporation, and the residue taken up in DMSO (0.7 mL) and purified *via* preparative HPLC (C18, 55-75% MeCN/H_2O with 0.1% HCO_2H over 12 min; product elutes at 3.1 min) to yield **SI-20** (1.1 mg, 42%) as a yellow foam:

$[\alpha]_D^{24}$ = -37.2 (*c* = 0.1, $CHCl_3$);

NMR: Due to rotamerism (see spectra), the NMR of this compound was not assigned.

HRMS: Calc'd for $C_{68}H_{77}N_8O_{13}$, $[M+H]^+$, 1213.5605; found, 1213.5602.

LC/low-resolution MS trace (254 nm absorption in top lane; SIR in bottom lane):



SI-20 1a

Synthetic tryptorubin A (1a):

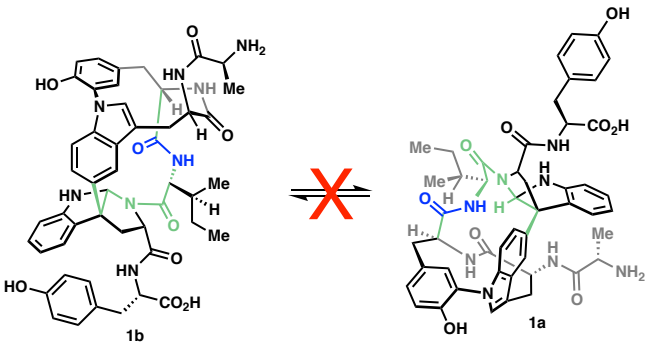
A solution of SI-20 (1.1 mg, 0.91 µmol) in MeOH (0.5 mL) was sparged with Ar for 15 min. Then, Pd/C (10% w/w, *ca.* 1 mg) was added. The reaction atmosphere was replaced with H_2 (1 atm) and the mixture vigorously stirred for 12 h at rt. The atmosphere was replaced back to Ar; then, aqueous LiOH (1 M, 0.2 mL, 0.2 mmol) was added, and the mixture stirred at rt for an additional 2 h. The mixture was filtered and the filtrate purified *via* preparative HPLC (C18, 15-35% MeCN/H_2O with 0.1% HCO_2H over 12 min; product elutes at 3.9 min). Fractions containing desired product were combined and lyophilized, and the resultant white foam was taken up in H_2O/MeCN/AcOH (100:100:1) and lyophilized again to yield the acetate salt of **1a** (0.7 mg, 86%) as a white foam:

$[\alpha]_D^{25.9} = 4.7$ ($c = 0.6$, MeOH);

NMR: See tabulated NMR data, page 54.

HRMS: Calc'd for $C_{49}H_{53}N_8O_9$ ($[M+H]^+$), 897.3936; found, 897.3933.

LC/low-resolution MS trace: See *LCMS co-injection of natural and synthetic tryptorubin A*, page 67.



Attempted thermal interconversion of 1a **and** 1b**:**
Substrate **1a** or **1b** (*ca.* 0.05 mg) was dissolved in DMSO-d6 (0.15 mL) and heated to 120 ^oC for 24 h. The solution was cooled to rt and analyzed by ^1H NMR. In both cases, significant decomposition to unknown species occurred, but no evidence of interconversion to the other atropisomer (i.e., **1a** to **1b**, or **1b** to **1a**, respectively) was observed.

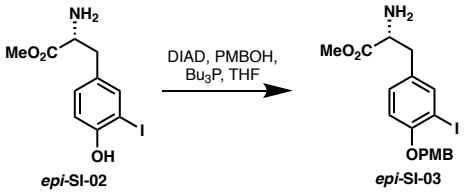
***Epi*-SI-02**

NOTE: There exist many literature precedents for preparation of this compound via *reaction of Tyrosine with I_2 in concentrated aqueous NH_4OH. However, in our hands, this preparation resulted in generation of explosive solid NI_3, and due to safety risks we thus adopted the following procedure.*

To a solution of *D*-Tyrosine (18.1 g, 100 mmol, 1 equiv) and toluene sulfonic acid hydrate (38.0 g, 200 mmol, 2 equiv) in MeCN (1.0 L) was added NIS (23.6 g, 105 mmol, 1.05 equiv) in six portions over two hours. The mixture was stirred at rt for 18 h, and then concentrated *via* rotary evaporation to a yellow oil. A separate flask was charged with MeOH (200 mL), and to this MeOH was added AcCl (14.3 mL, 200 mmol, 2 equiv) dropwise. The resultant methanolic HCl was added *via* cannula to the crude iodo-tryptophan, and the solution stirred at reflux for 2 h. The mixture was concentrated *via* rotary evaporation, and the residue partitioned between aqueous sodium carbonate (saturated, 300 mL) and EtOAc (300 mL). The aqueous layer was extracted with additional EtOAc (5 X 300 mL), and the combined organics were dried ($MgSO_4$) and concentrated. The residue was purified *via* column chromatography (SiO_2, 0-6%

MeOH/DCM with 0.5% Et_3N) to yield the title product as a yellow foam (25.7 g, 84%). Spectral data matched previous literature[32]; ^{1}H NMR data are reported here for convenience:

^{1}H NMR: (400 MHz, CD_3OD) δ 7.62 (d, *J* = 2.1 Hz, 1H), 7.09 (dd, *J* = 8.1, 2.0 Hz, 1H), 6.84 (d, *J* = 8.2 Hz, 1H), 4.27 (t, *J* = 6.7 Hz, 1H), 3.84 (s, 3H), 3.16 (dd, *J* = 14.6, 6.0 Hz, 1H), 3.05 (dd, *J* = 14.6, 7.6 Hz, 1H).



***Epi*-SI-03**

This compound was prepared in a manner identical to **SI-03,** starting from ***Epi*-SI-02** (25.7 g, 84 mmol), and yielding the title product (30.8 g, 83%) as a yellow foam:

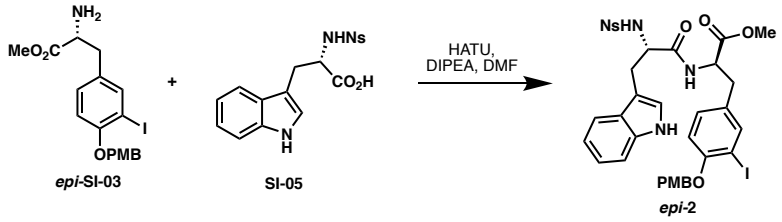
$[\alpha]_D^{20.0}$ = 6.8 (*c* = 1.0, $CHCl_3$);

^{1}H NMR (600 MHz, CD_3OD): δ 7.63 (d, *J* = 2.2 Hz, 1H), 7.43 – 7.40 (m, 2H), 7.15 (dd, *J* = 8.4, 2.2 Hz, 1H), 6.96 – 6.92 (m, 3H), 5.07 (s, 2H), 4.60 (s, 1H), 3.81 (s, 3H), 3.68 (s, 3H), 2.92 (dd, *J* = 13.7, 6.3 Hz, 1H), 2.86 (dd, *J* = 13.7, 6.8 Hz, 1H).

^{13}C NMR (151 MHz, CD_3OD): δ 176.0, 161.0, 157.9, 141.2, 132.7, 131.5, 130.2, 130.0, 114.9, 114.1, 87.4, 71.9, 56.6, 55.7, 52.4, 40.2.

HRMS: Calc'd for $C_{18}H_{21}INO_4$, $[M+H]^+$, 442.0510; found, 442.0516.

***R*$_f$** = 0.32 (Hex:EtOAc = 1:5; UV, $Ce_2(SO_4)_3$ in phosphomolybdic acid)



***Epi*-2**

This compound was prepared in a manner identical to compound **2,** starting from ***Epi*-SI-03** (32.8 g, 69.7 mmol), and yielding the title product (49.9 g, 88%) as a yellow foam:

[32] Chiarello, J. and Joullie M.M. *Syn. Comm.,* **1988,** *18,* 2211.

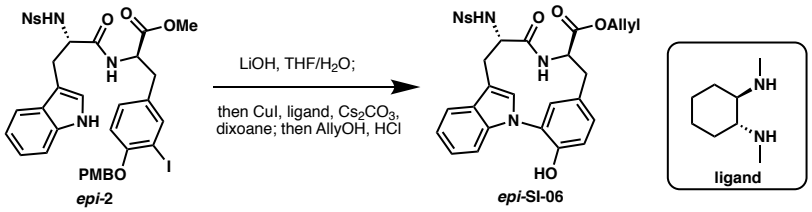
$[\alpha]_D^{20.0}$ = -17.0 (*c* = 1.0, $CHCl_3$);

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.06 (s, 1H), 7.80 (d, *J* = 7.4 Hz, 1H), 7.57 (s, 1H), 7.43 (d, *J* = 7.7 Hz, 1H), 7.39 – 7.31 (m, 4H), 7.20 – 7.14 (m, 2H), 7.12 (d, *J* = 8.1 Hz, 1H), 7.07 (d, *J* = 8.5 Hz, 1H), 7.02 (t, *J* = 7.6 Hz, 1H), 6.96 (s, 1H), 6.89 (d, *J* = 8.4 Hz, 2H), 6.82 – 6.76 (m, 2H), 6.04 (d, *J* = 4.0 Hz, 1H), 4.98 (s, 2H), 4.90 (dt, *J* = 8.5, 5.5 Hz, 1H), 4.11 (dt, *J* = 10.9, 4.5 Hz, 1H), 3.80 (s, 3H), 3.72 (s, 3H), 3.44 (dd, *J* = 14.9, 4.7 Hz, 1H), 3.09 (dd, *J* = 14.1, 5.5 Hz, 1H), 2.99 (dd, *J* = 14.1, 5.5 Hz, 1H), 2.91 (dd, *J* = 15.0, 10.6 Hz, 1H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 171.4, 170.5, 159.4, 156.7, 146.2, 140.1, 136.4, 133.7, 132.7, 131.5, 131.0, 130.9, 130.0, 128.9, 128.7, 126.2, 125.6, 124.6, 122.2, 119.9, 118.49, 114.0, 113.0, 111.4, 108.9, 87.2, 70.9, 58.2, 55.4, 53.3, 52.6, 36.9, 29.0.

HRMS: Calc'd for $C_{35}H_{34}IN_4O_9S$, $[M+H]^+$, 813.1086; found, 813.1081.

R_f: 0.55 (Hex:EtOAc = 1:1; UV, $Ce_2(SO_4)_3$ in phosphomolybdic acid)



***Epi*-SI-06**

This compound was prepared in a manner identical to compound **SI-06,** starting from ***Epi*-2** (32.5 g, 40.0 mmol), and yielding the title product (15.1 g, 64%) as a yellow foam:

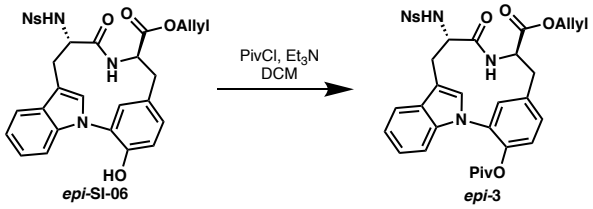
$[\alpha]_D^{22.5}$ = -223.0 (*c* = 1.0, $CHCl_3$);

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.20 (dd, *J* = 7.6, 1.6 Hz, 1H), 7.91 (dd, *J* = 7.6, 1.5 Hz, 1H), 7.83 – 7.75 (m, 2H), 7.40 – 7.36 (m, 1H), 7.33 – 7.30 (m, 1H), 7.25 – 7.20 (m, 2H), 6.90 (s, 2H), 6.39 (d, *J* = 10.1 Hz, 1H), 6.33 (s, 1H), 6.28 (s, 1H), 6.09 (d, *J* = 9.3 Hz, 1H), 5.83 (ddt, *J* = 16.5, 10.4, 5.8 Hz, 1H), 5.75 (s, 1H), 5.27 (dd, *J* = 17.2, 1.5 Hz, 1H), 5.21 (dd, *J* = 10.4, 1.3 Hz, 1H), 4.49 (qd, *J* = 13.1, 5.8 Hz, 2H), 4.35 (ddd, *J* = 12.0, 10.0, 2.1 Hz, 1H), 4.15 (ddd, *J* = 12.9, 9.3, 3.7 Hz, 1H), 3.18 (t, *J* = 12.5 Hz, 1H), 2.86 (dd, *J* = 13.2, 3.6 Hz, 1H), 2.72 (dd, *J* = 14.5, 2.1 Hz, 1H), 2.33 (dd, *J* = 14.5, 11.8 Hz, 1H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 170.7, 169.1, 150.9, 149.7, 147.8, 147.0, 134.8, 134.3, 133.9, 133.3, 131.4, 130.6, 129.4, 129.0, 128.9, 127.1, 125.7, 125.1, 123.7, 119.3, 119.1, 117.4, 116.6, 66.3, 58.7, 54.3, 35.7, 27.9.

HRMS: Calc'd for $C_{29}H_{27}N_4O_8S$, $[M+H]^+$, 591.1544; found, 591.1565.

R_f: 0.40 (Hex:EtOAc = 1:1; UV, $Ce_2(SO_4)_3$ in phosphomolybdic acid)



epi-SI-06 *epi*-3

***Epi*-3**

This compound was prepared in a manner identical to compound **3,** starting from ***Epi*-SI-06** (15.1 g, 25.5 mmol), and yielding the title product (15.7 g, 91%) as a yellow foam:

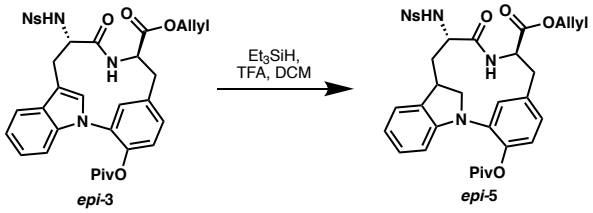
$[\alpha]_D^{23.2}$ = -170.4 (*c* = 1.0, $CHCl_3$);

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.21 (dd, *J* = 7.8, 1.4 Hz, 1H), 7.90 (dd, *J* = 7.9, 1.4 Hz, 1H), 7.82 (td, *J* = 7.7, 1.4 Hz, 1H), 7.77 (td, *J* = 7.7, 1.5 Hz, 1H), 7.50 – 7.43 (m, 1H), 7.36 – 7.30 (m, 1H), 7.22 – 7.12 (m, 2H), 7.06 – 6.99 (m, 2H), 6.51 (d, *J* = 10.0 Hz, 1H), 6.36 (s, 1H), 6.14 (d, *J* = 9.3 Hz, 1H), 5.90 (d, *J* = 2.0 Hz, 1H), 5.82 (ddt, *J* = 17.2, 10.4, 5.8 Hz, 1H), 5.26 (dd, *J* = 17.2, 1.4 Hz, 1H), 5.21 (dd, *J* = 10.5, 1.2 Hz, 1H), 4.48 (dtdd, *J* = 13.1, 11.7, 5.8, 1.4 Hz, 2H), 4.36 (ddd, *J* = 12.0, 10.1, 2.0 Hz, 1H), 4.19 – 4.11 (m, 1H), 3.18 (dd, *J* = 13.2, 11.9 Hz, 1H), 2.86 (dd, *J* = 13.2, 3.6 Hz, 1H), 2.78 (dd, *J* = 14.5, 2.1 Hz, 1H), 2.40 (dd, *J* = 14.4, 11.8 Hz, 1H), 1.33 (s, 9H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 177.3, 170.7, 169.3, 150.5, 147.8, 146.3, 144.6, 136.3, 135.1, 134.8, 133.8, 133.8, 133.3, 131.4, 130.6, 128.9, 128.4, 125.6, 124.8, 123.2, 122.9, 119.2, 118.7, 116.9, 116.5, 66.4, 58.9, 54.0, 39.2, 35.6, 27.9, 27.3.

HRMS: Calc'd for $C_{34}H_{35}N_4O_9S$, $[M+H]^+$, 675.2119; found, 675.2135.

R_f: 0.58 (Hex:EtOAc = 1:1; UV, $Ce_2(SO_4)_3$ in phosphomolybdic acid)



epi-3 *epi*-5

***Epi*-5**

This compound was prepared in a manner identical to compound **5,** starting from ***Epi*-3** (15.7 g, 23.3 mmol), and yielding the title product (12.8 g, 81%) as a yellow foam. The material yielded was homogeneous by TLC and LC-MS analysis, and was therefore assigned as a single diastereomer. The stereochemistry at the indoline C3 position could

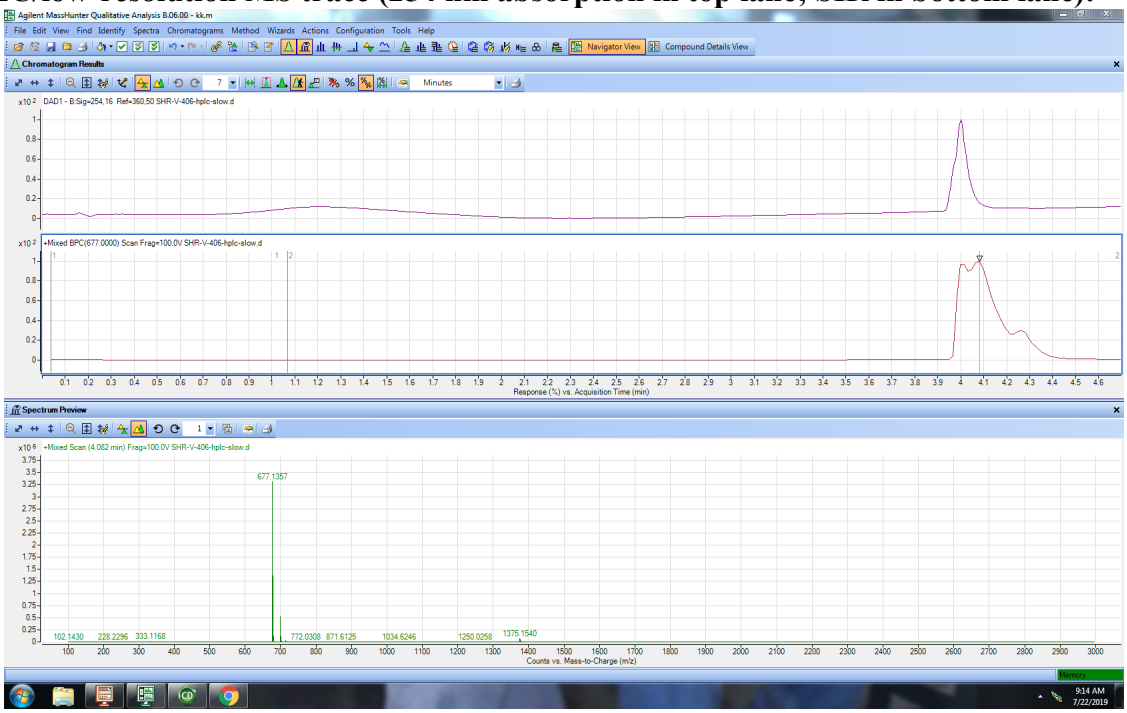
not be assigned by NMR, but is assumed to (*R*) by analogy to derivative ***epi*-SI-21**, whose stereochemistry was determined *via* crystallography.

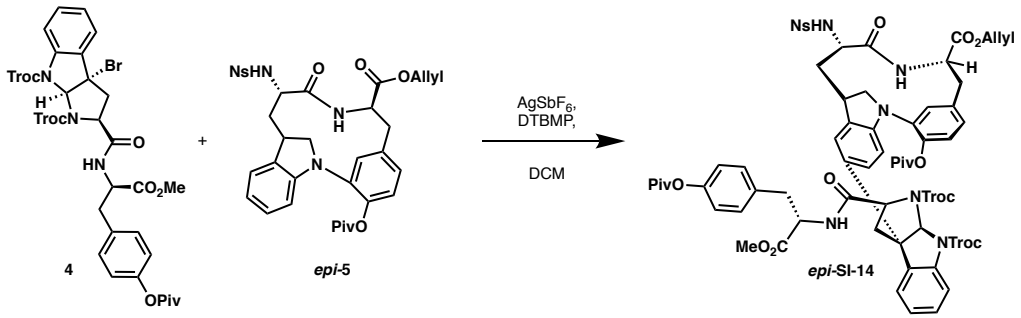
$[\alpha]_D^{24.7}$ = -13.9 (*c* = 1.0, $CHCl_3$);

NMR: Due to rotamerism (see spectra), the NMR of this compound was not assigned.

HRMS: Calc'd for $C_{34}H_{37}N_4O_9S$, $[M+H]^+$, 677.2276; found, 677.2279.

LC/low-resolution MS trace (254 nm absorption in top lane; SIR in bottom lane):





***Epi*-SI-14**

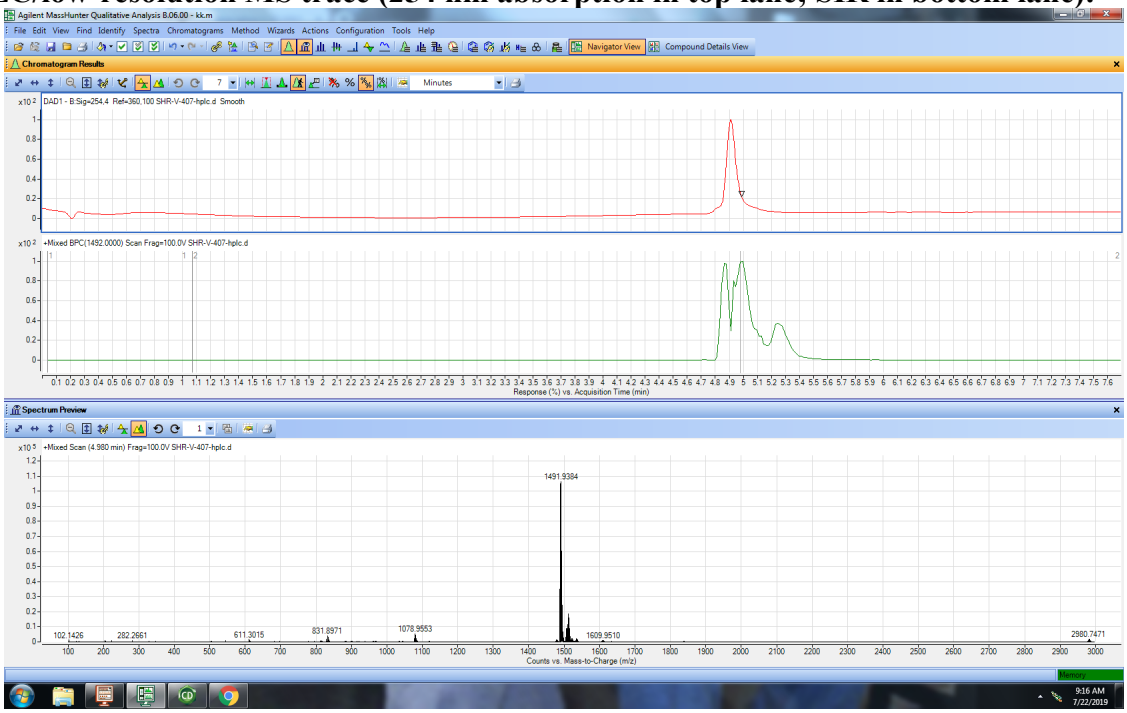
This compound was prepared in a manner identical to compound **SI-14,** starting from ***Epi*-5** (5.36 g, 7.92 mmol), and yielding the title product (6.02 g, 51%) as a yellow foam:

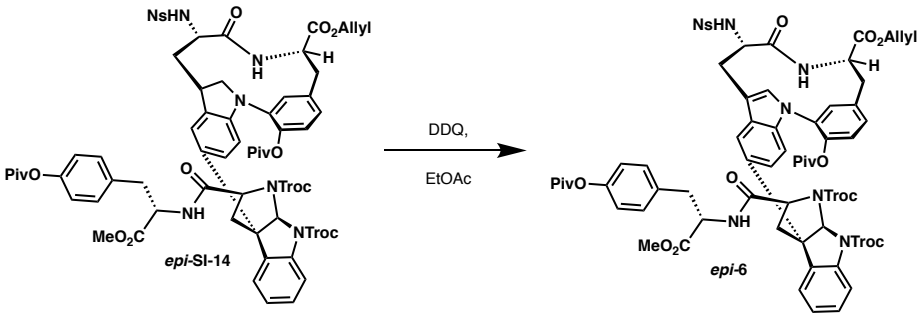
$[\alpha]_D^{24.1}$ = -2.1 (*c* = 1.0, $CHCl_3$);

NMR: Due to rotamerism (see spectra), the NMR of this compound was not assigned.

HRMS: Calc'd for $C_{66}H_{68}Cl_6N_7O_{18}S$, $[M+H]^+$, 1488.2467; found, 1488.2429.

LC/low-resolution MS trace (254 nm absorption in top lane; SIR in bottom lane):





Epi-6

This compound was prepared in a manner identical to compound **6,** starting from ***Epi*-SI-14** (6.02 g, 4.04 mmol), and yielding the title product (4.81 g, 80%) as a red foam:

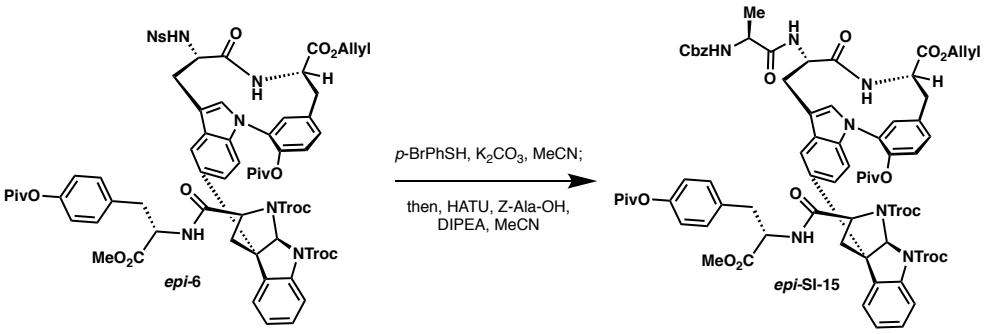
$[\alpha]_D^{20.9}$ = -43.7 (*c* = 1.0, $CHCl_3$);

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.19 – 8.14 (m, 1H), 7.91 – 7.87 (m, 1H), 7.83 (d, *J* = 8.1 Hz, 1H), 7.78 (tt, *J* = 7.5, 5.7 Hz, 2H), 7.60 (d, *J* = 1.9 Hz, 1H), 7.38 – 7.32 (m, 3H), 7.17 (t, *J* = 7.5 Hz, 1H), 7.04 (dd, *J* = 8.3, 2.2 Hz, 1H), 7.00 (d, *J* = 8.1 Hz, 3H), 6.98 – 6.93 (m, 3H), 6.74 (s, 1H), 6.41 (s, 1H), 6.18 (d, *J* = 10.2 Hz, 1H), 5.90 – 5.81 (m, 2H), 5.80

(d, J = 9.6 Hz, 1H), 5.28 (dd, J = 17.2, 1.4 Hz, 1H), 5.23 (dd, J = 10.4, 1.2 Hz, 1H), 5.18 – 5.06 (m, 1H), 4.94 (dd, J = 9.2, 1.7 Hz, 1H), 4.89 (d, J = 12.0 Hz, 1H), 4.60 – 4.50 (m, 2H), 4.44 (ddd, J = 12.2, 10.2, 2.1 Hz, 1H), 4.31 (s, 1H), 4.17 (ddd, J = 11.8, 9.7, 3.7 Hz, 1H), 3.55 (s, 3H), 3.42 (d, J = 13.4 Hz, 1H), 3.31 – 3.23 (m, 1H), 3.18 (t, J = 12.4 Hz, 1H), 2.99 (dd, J = 13.0, 3.7 Hz, 1H), 2.87 (dd, J = 14.4, 2.1 Hz, 1H), 2.70 (dd, J = 13.7, 4.7 Hz, 1H), 2.56 (dd, J = 14.4, 11.9 Hz, 1H), 2.38 (dd, J = 13.7, 7.9 Hz, 1H), 1.35 (s, 9H), 1.30 (s, 9H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 177.2, 177.1, 171.4, 170.3, 169.5, 169.3, 152.0, 150.4, 149.6, 147.7, 147.4, 144.5, 140.5, 135.9, 135.5, 135.3, 135.0, 134.2, 134.0, 133.4, 133.2, 131.4, 130.5, 130.1, 129.4, 128.9, 128.7, 126.0, 125.5, 125.5, 123.3, 123.1, 121.8, 119.4, 117.8, 117.4, 116.5, 116.0, 95.2, 95.0, 85.6, 75.6, 66.5, 62.5, 59.0, 54.1, 53.4, 52.3, 39.3, 39.2, 37.9, 35.8, 28.0, 27.3.

HRMS: Calc'd for $C_{66}H_{66}Cl_6N_7O_{18}S$, $[M+H]^+$, 1486.2311; found, 1486.2341.



***Epi*-SI-15**

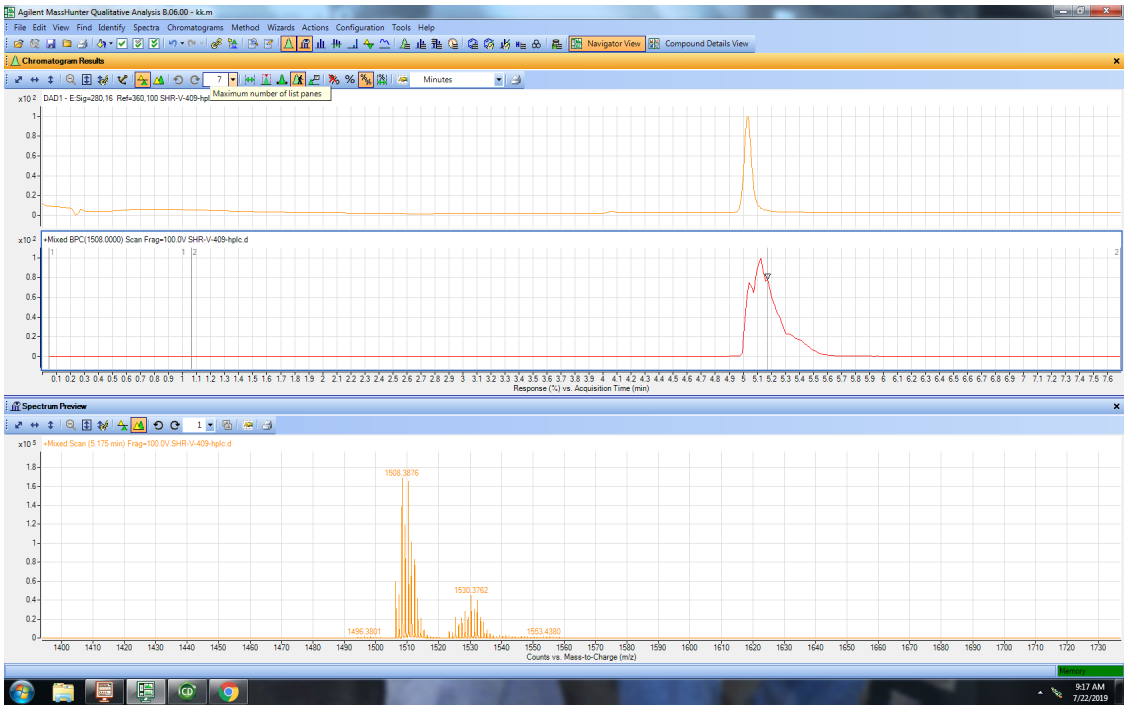
This compound was prepared in a manner identical to compound **SI-15,** starting from ***Epi*-6** (4.81 g, 3.23 mmol), and yielding the title product (2.14 g, 44%) as a white foam:

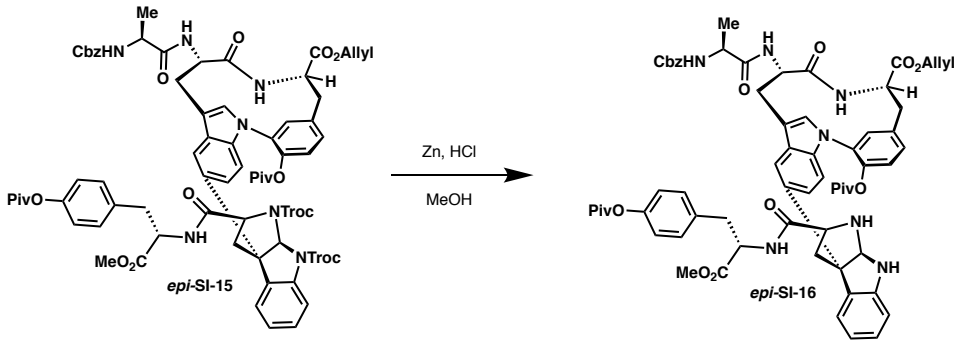
$[\alpha]_D^{24.3}$ = -61.6 (c = 1.0, $CHCl_3$);

NMR: Due to rotamerism (see spectra), the NMR of this compound was not assigned.

HRMS: Calc'd for $C_{71}H_{74}Cl_6N_7O_{17}$, $[M+H]^+$, 1506.3267; found, 1506.3256.

LC/low-resolution MS trace (254 nm absorption in top lane; SIR in bottom lane):





Epi-SI-16

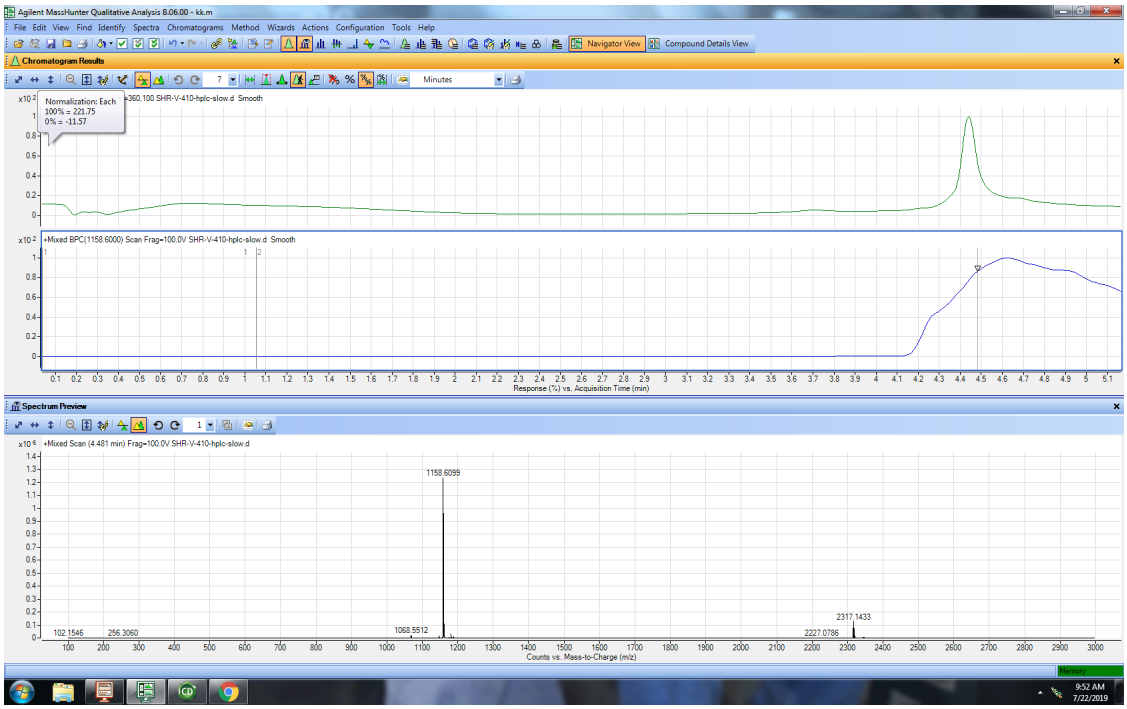
This compound was prepared in a manner identical to compound **SI-16,** starting from ***Epi*-SI-15** (2.14 g, 1.42 mmol), and yielding the title product (840 mg, 51%) as a white foam:

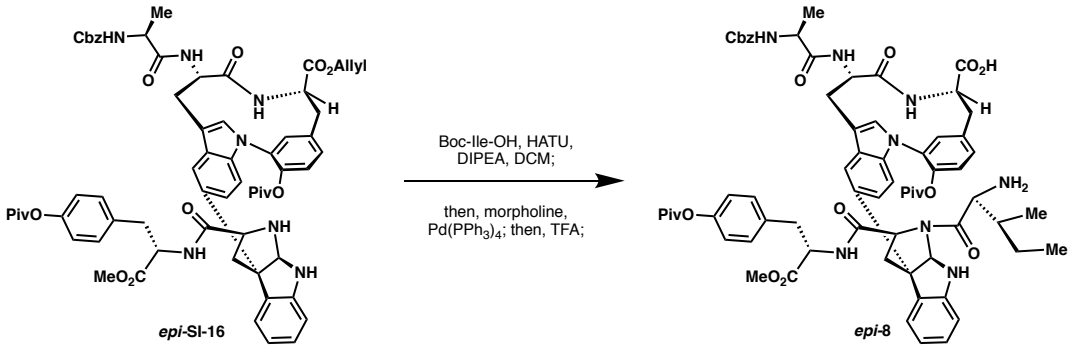
$[\alpha]_D^{24.1}$ = -88.2 (*c* = 0.6, $CHCl_3$);

NMR: Due to rotamerism (see spectra), the NMR of this compound was not assigned.

HRMS: Calc'd for $C_{65}H_{72}N_7O_{13}$, $[M+H]^+$, 1158.5183; found, 1158.5188.

LC/low-resolution MS trace (254 nm absorption in top lane; SIR in bottom lane):





Epi-8

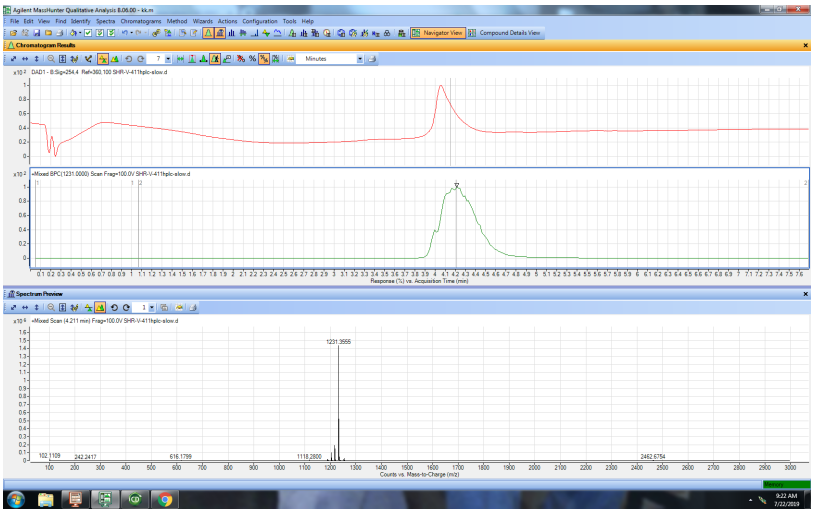
This compound was prepared in a manner identical to compound **8,** starting from ***Epi*-SI-16** (840 mg, 0.73 mmol), and yielding the title product (368 mg, 41%) as a white foam:

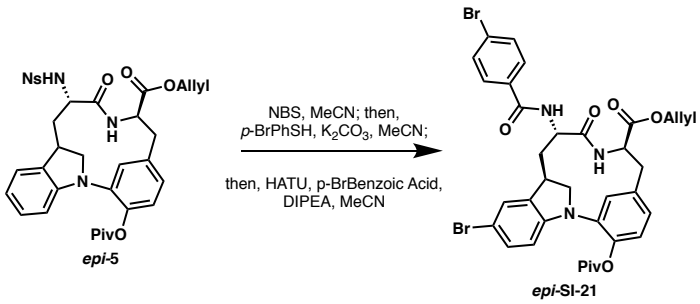
$[\alpha]_D^{20}$ = -62.7 (*c* = 0.1, $CHCl_3$);

NMR: Due to rotamerism (see spectra), the NMR of this compound was not assigned.

HRMS: Calc'd for $C_{68}H_{79}N_8O_{14}$, $[M+H]^+$, 1231.5710; found, 1213.5714.

LC/low-resolution MS trace (254 nm absorption in top lane; SIR in bottom lane):





***Epi*-SI-21**

(*Prepared for purposes of X-ray crystallographic characterization)*

To a solution of ***epi*-5** (68 mg, 0.10 mmol, 1 equiv) in MeCN (1 mL) was added *N*-bromosuccinimide (18 mg, 0.10 mmol, 1.0 equiv). The mixture was stirred at rt for 15 min, at which time LC/MS analysis showed complete consumption of starting material.
To this solution was sequentially added 4-bromothiophenol (95 mg, 0.50 mmol, 5.0 equiv) and K_2CO_3 (69 mg, 0.50 mmol, 5.0 equiv), and the mixture was stirred at rt for 30 min, at which time LC/MS analysis showed removal of the nosyl protecting group.
At this time, in a separate flask, 4-bromobenzoic acid (151 mg, 0.750 mmol, 7.50 equiv) and HATU (285 mg, 0.750 mmol, 7.50 equiv) were dissolved in MeCN (1 mL). DIPEA (0.52 mL, 3.0 mmol, 30 equiv) was then added, and the mixture stirred for 30 sec before being transferred quickly *via* syringe to the substrate/thiophenol-containing flask.
The resultant reaction was stirred for 5 min, then filtered through a pad of cotton to remove solids. The filtrate was directly purified *via* preparative HPLC (C18, 75-95% MeCN/H_2O with 0.1% HCO_2H over 12 min; product elutes at 6.5 min), to yield ***epi*-SI-21** (15 mg, 20%) as a white foam. Crystals suitable for X-ray diffraction were prepared by dissolving the product in DCM (0.2 mL), diluting the solution with *n*-heptane (3 mL), and allowing the mixture to slowly evaporate until crystals were observed.

X-ray crystallography: Solved structure; see details on page 150.

$[\alpha]_D^{20}$ = -118.3 (*c* = 1.0, DMSO);

^{1}H NMR (600 MHz, 1:1 CD_3OD/DMSO-d6): δ 7.84 (d, *J* = 8.5 Hz, 2H), 7.71 (d, *J* = 8.2 Hz, 2H), 7.64 – 7.50 (m, 1H), 7.39 (d, *J* = 8.1 Hz, 1H), 7.17 (s, 1H), 7.10 (s, 2H), 6.77 (d, *J* = 8.4 Hz, 1H), 5.89 (s, 1H), 5.31 (d, *J* = 17.6 Hz, 1H), 5.14 (s, 1H), 4.63 (s, 2H), 4.27 – 4.06 (m, 2H), 3.65 (s, 1H), 3.22 (d, *J* = 11.1 Hz, 1H), 3.11 (d, *J* = 12.6 Hz, 1H), 2.74 (t, *J* = 12.5 Hz, 1H), 2.46 (s, 1H), 2.05 (s, 1H), 1.41 (s, 9H).

^{13}C NMR (151 MHz, 1:1 CD_3OD/DMSO-d6): 177.8, 171.6, 171.0, 167.2, 150.8, 146.0, 145.5, 138.5, 138.2, 137.7, 134.6, 133.7, 133.63, 133.4, 132.6, 132.3, 130.9, 129.9, 126.4, 124.7, 118.8, 118.3, 114.7, 66.7, 64.1, 55.8, 52.0, 31.0, 38.6, 35.9, 27.9.

HRMS: Calc'd for $C_{35}H_{36}Br_2N_3O_6$, $[M+H]^+$, 752.0965; found, 752.0963.

LC/low-resolution MS trace (254 nm absorption in top lane; SIR in bottom lane):

Comparison of natural tryptorubin A, synthetic tryptorubin A (1a), and atrop-tryptorubin A (1b)

Table S3, Reassigned chemical shifts for natural isolate (1a)

Because of small referencing errors (relative to solvent residuals), as well as erroneous peak picking, some chemical shifts in tryptorubin's original isolation report were determined to be incorrect. Table S3 describes correctly re-referenced and re-picked chemical shifts for natural **1a**. All ^{1}H NMR multiplicities and integrations remain unchanged in assignment. The reassigned (right) chemical shifts were used in all subsequent NMR comparisons to synthetic materials.

Natural product original assignment in MeOD			**Natural product reassignment in MeOD**				
Position	ΔC	ΔH	Position	ΔC	ΔH	HMBC to	nOe to
1	173.9	-	1	177.8	-	-	-
2	57.3	4.39	2	57.3	4.39	8	3
3	38.1	3.05; 2.86	3	38.8	3.05; 2.86	1, 2, 5	3, 2, 5
4	129.8		4	129.8		-	-
5	130	6.92	5	131.7	6.92	3, 4	3, 6, 9
6	115.9	6.4	6	115.9	6.40	5, 6, 7	5
7	155.8	-	7	156.8	-	-	-
8	170.6	-	8	173.4	-	-	-
9	63.1	4.70	9	65.3	4.70	8, 10	3, 10, 2NH
10	38.9	3.2; 2.16	10	40.3	3.2; 2.16	8, 9, 11, 12, 13, 14	10, 42
11	59.9	-	11	62.9	-	-	-
12	94.1	6.97	12	94.1	6.97	9, 11, 16	20, 40
13	137	-	13	137	-	-	-
14	123.4	6.86	14	124.2	6.86	11, 16, 18	15, 40
15	118.5	6.77	15	120.6	6.77	16, 19	-
16	128.2	7.18	16	129.4	7.18	15, 16, 18	-

17	112	6.89		17	112	6.89	15	-
18	150.3	-		18	150.3	-	-	-
19	173.9	-		19	176.1	-	-	-
20	57.7	3.87		20	59.7	3.87	21	12, 21, 24
21	36.5	1.71		21	38	1.71	-	24
22	24.3	1.30		22	30.7	1.30	-	21
23	11.5	0.95		23	11.5	0.95	-	21
24	16.3	1.00		24	16.3	1.00	20	21,22
25	170.2	-		25	171.5	-	-	-
26	51.6	4.13		26	53.5	4.13	25, 27	27, 20NH
27	33.4	3; 2.77		27	33.4	3; 2.77	25, 26, 28	26
28	125.9	-		28	127.7	-	-	-
29	131.8	5.94		29	131.8	5.94	27, 30, 31, 33	26NH
30	132.6	-		30	133.8	-	-	-
31	150.2	-		31	150.2	-	-	-
32	117.2	6.76		32	118.2	6.76	-	-
33	128.3	6.74		33	129.9	6.74	-	27
34	169.0	-		34	173.2	-	-	-
35	53.7	4.38		35	53.7	4.38	34	-
36	27.5	2.97; 2.85		36	27.5	2.97; 2.85	37, 38	-
37	120.8	-		37	122.9	-	-	-
38	147.7	6.83		38	147.7	6.83	39, 40, 44	35
39	139.1	-		39	140.2	-	-	-
40	123.6	7.44		40	123.6	7.44	42	36, 12
41	136.1	-		41	136.1	-	-	-
42	123.3	7.44		42	123.3	7.44	44	10, 9
43	116.0	7.17		43	117.2	7.17	39, 41	42
44	154.7	-		44	154.7	-	-	-

45	176.4	-	45	177.1	-	-	-
46	51.0	3.49	46	51	3.45	-	-
47	20.6	1.35	47	21.1	1.25	45, 46	46

Table S4, Comparison of ^{1}H and ^{13}C shifts of natural isolate vs. *atrop*-tryptorubin A (1b)

Natural Isolate			**Synthetic 1b**					
Position	ΔC	ΔH	Position	ΔC	ΔH	multiplicity	ΔΔH	ΔΔC
1	177.8	-	1	174.9	-	-	-	2.9
2	57.3	4.39	2	57	4.43	m	0.04	0.3
3	38.8	3.05; 2.86	3	40.4	3.04; 2.62	m	0.01; 0.14	1.6
4	129.8	-	4	127.5	-	-	-	2.3
5	131.7	6.92	5	131.7	6.65	d, J = 8.1	0.27	0
6	115.9	6.40	6	115.9	6.29	d, J = 8.0	0.11	0
7	156.8	-	7	161.3	-	-	-	4.5
8	173.4	-	8	174	-	-	-	0.6
9	65.3	4.70	9	66.8	4.97	m	0.27	1.5
10	40.3	3.20; 2.16	10	43.4	3.12; 2.24	m	0.08; 0.08	3.1
11	62.9	-	11	63.8	-	-	-	0.9
12	94.1	6.97	12	91.6	6.24	m	0.73	2.5
13	137.0	-	13	139.1	-	-	-	2.1
14	124.2	6.86	14	124.8	7.05	m	0.19	0.6
15	120.6	6.77	15	121.3	6.93	m	0.16	0.7
16	129.4	7.18	16	129.8	7.26	app t, J = 7.8	0.08	0.4
17	112.0	6.89	17	112.7	6.96	d, J = 8.4	0.07	0.7
18	150.3	-	18	149.8	-	-	-	0.5
19	176.1	-	19	176.2	-	-	-	0.1
20	59.7	3.87	20	56.9	4.01	m	0.14	2.8
21	38.0	1.71	21	37.5	1.81	m	0.10	0.5
22	30.7	1.30	22	26.3	1.13; 1.57	m	0.27; 0.17	4.4

23	11.5	0.95	23	10.8	0.88	t, *J* = 7.3	0.07	0.7
24	16.3	1.00	24	16.4	1.01	d, *J* = 6.8	0.01	0.1
25	171.5	-	25	172.9	-	-	-	1.4
26	53.5	4.13	26	53.8	3.95	d, *J* = 7.9	0.18	0.3
27	33.4	3.00; 2.77	27	34.4	2.83; 2.55	m	0.17; 0.22	1
28	127.7	-	28	120.5	-	-	-	7.2
29	131.8	5.94	29	129.9	6.25	s	0.31	1.9
30	133.8	-	30	136.8	-	-	-	3
31	150.2	-	31	156.6	-	-	-	6.4
32	118.2	6.76	32	122.3	6.60	d, *J* = 8.2	0.16	4.1
33	129.9	6.74	33	129.6	6.52	d, *J* = 8.4	0.22	0.3
34	173.2	-	34	170.2	-	-	-	3
35	53.7	4.38	35	57.1	4.19	s	0.19	3.4
36	27.5	2.97; 2.85	36	23.7	3.41; 2.83	m	0.44; 0.02	3.8
37	122.9	-	37	131.4	-	-	-	8.5
38	147.7	6.83	38	145.5	7.07	m	0.24	2.2
39	140.2	-	39	152.5	-	-	-	12.3
40	123.6	7.44	40	117.7	7.15	s	0.29	5.9
41	136.1	-	41	139.2	-	-	-	3.1
42	123.3	7.44	42	118.4	7.33	d, *J* = 8.3	0.11	4.9
43	117.2	7.17	43	127.7	7.04	m	0.13	10.5
44	154.7	-	44	150	-	-	-	4.7
45	177.1	-	45	177.7	-	-	-	0.6
46	51	3.45	46	50.6	3.44	m	0.01	0.4
47	21.1	1.25	47	19.9	0.95	d, *J* = 6.9	0.30	1.2

Table S5, Comparison of ^{1}H and ^{13}C shifts of natural isolate vs. synthetic tryptorubin A (1a)

Natural isolate			**Synthetic 1a**				
Position	ΔC	ΔH	Position	ΔC	ΔH	multiplicity	ΔΔH	ΔΔC
1	177.8	-	1	177.7	-	-	-	0.1
2	57.3	4.39	2	57.1	4.37	m	0.02	0.2
3	38.8	3.05; 2.86	3	38.8	3.05; 2.82	m	0; 0.04	0
4	129.8	-	4	129.7	-	-	-	0.1
5	131.7	6.92	5	131.6	6.93	d, J = 7.8	0.01	0.1
6	115.9	6.40	6	116.0	6.40	d, J = 7.9	0	0.1
7	156.8	-	7	157	-	-	-	0.2
8	173.4	-	8	173.6	-	-	-	0.2
9	65.3	4.70	9	65.3	4.70	m	0	0
10	40.3	3.20; 2.16	10	40.4	3.20; 2.15	m	0; 0.01	0.1
11	62.9	-	11	62.9	-	-	-	0
12	94.1	6.97	12	93.8	6.99	m	0.02	0.3
13	137.0	-	13	137.3	-	-	-	0.3
14	129.4	7.18	14	129.3	7.18	m	0	0.1
15	124.2	6.86	15	124.0	6.86	m	0	0.2
16	120.6	6.77	16	120.5	6.78	m	0.01	0.1
17	112.0	6.89	17	111.9	6.87	m	0.02	0.1
18	150.3	-	18	150.2	-	-	-	0.1
19	176.1	-	19	176.2	-	-	-	0.1
20	59.7	3.87	20	59.7	3.88	d, J = 9.0	0.01	0
21	38.0	1.71	21	38.0	1.70	m	0.01	0
22	30.7	1.30	22	26.8	1.24	m	0.06	3.9
23	11.5	0.95	23	11.3	0.95	app m	0	0.2
24	16.3	1.00	24	16.2	1.00	d, J = 6.5	0	0.1

25	171.5	-	25	171.6	-	-	-	0.1
26	53.5	4.13	26	53.6	4.14	app d, J = 8.0	0.01	0.1
27	33.4	3.00; 2.77	27	33.3	2.98; 2.74	m	0.02; 0.03	0.1
28	127.7	-	28	124.3	-	-	-	3.4
29	131.8	5.94	29	131.9	5.72	s	0.22	0.1
30	133.8	-	30	134.6	-	-	-	0.8
31	150.2	-	31	154.7	-	-	-	4.5
32	118.2	6.76	32	120.6	6.70	m	0.06	2.4
33	129.9	6.74	33	129.6	6.64	m	0.10	0.3
34	173.2	-	34	173.8	-	-	-	0.6
35	53.7	4.38	35	53.5	4.37	m	0.01	0.2
36	27.5	2.97; 2.85	36	27.5	2.95; 2.85	m	0.02; 0	0
37	122.9	-	37	122.0	-	-	-	0.9
38	147.7	6.83	38	147.9	6.85	m	0.02	0.2
39	140.2	-	39	140.2	-	-	-	0
40	123.6	7.44	40	124.8	7.40	s	0.04	1.2
41	136.1	-	41	136.0	-	-	0	0.1
42	123.3	7.44	42	124.0	7.38	d, J = 7.3	0.06	0.7
43	117.2	7.17	43	117.1	7.20	m	0.03	0.1
44	154.7	-	44	154.8	-	-	-	0.1
45	177.1	-	45	177.8	-	-	-	0.7
46	51.0	3.45	46	51.2	3.46	m	0.01	0.2
47	21.1	1.25	47	21.4	1.22	d, J = 7.0	0.03	0.3

Table S6, Comparison of HMBC of natural isolate, synthetic tryptorubin A (1a), and *atrop*-tryptorubin A (1b)[33]

	Natural product	Synthetic **1a**	Synthetic **1b**
Position	HMBC to	HMBC to	HMBC to
H2	C8	C3, C4, C8	
H3	C1, C2, C5	C1, C2, C5	C1, C2, C5
H5	C3, C4	C3, C5, C7	C3, C5, C7
H6	C5, C6, C7	C4, C6, C7	C4, C6
H9	C8, C10	C8, C10	
H10	C8, C9, C11, C12, C13, C14	C8, C11, C12, C41	C8, C9, C13
H12	C9, C11, C16	C9, C10, C11, C41	C9, C10, C13
H14	C11, C16, C18	C14, C18	
H15	C16, C19	C11, C13, C16	C10, C17
H16	C15, C16, C18	C13, C17	C14, C18
H17	C15	C13, C15	C15
H20	C21	C21	C21, C25
H21	C20, C23, C24	C20, C23, C24	C22
H22			C20, C21
H23	C21	C21, C22	C21, C22
H24	C20	C20, C21, C22	C19, C20, C21, C22
H26	C25, C27	C25, C27, C28	C25, C28
H27	C25, C26, C28	C25, C26, C28	C25, C26, C28, C29
H29	C27, C30, C31, C33	C31, C33	
H32	C28, C30	C28, C30, C31	C28
H33		C29, C31	C29, C31
H35	C34	C34	C34
H36	C37, C38		C40
H38	C39, C40, C44	C37, C39, C44	C39, C40, C41
H40	C42	C11, C42, C44	C43, C44
H42	C44	C40, C44	C41
H43	C39, C41	C39, C41	C40
H46		C45, C47	C45, C47
H47	C45, C46	C45, C46	C45, C46

[33] It should be noted for data in this table, and the following ROESY spectral data table, that the natural material's NMR was acquired at Harvard, while NMR of synthetic material was acquired at Scripps. Slight differences in the respective experimental pulse sequences may explain slight differences in observed correlations.

Table S7, Comparison of ROESY of natural isolate, synthetic tryptorubin A (1a), and *atrop*-tryptorubin A (1b)

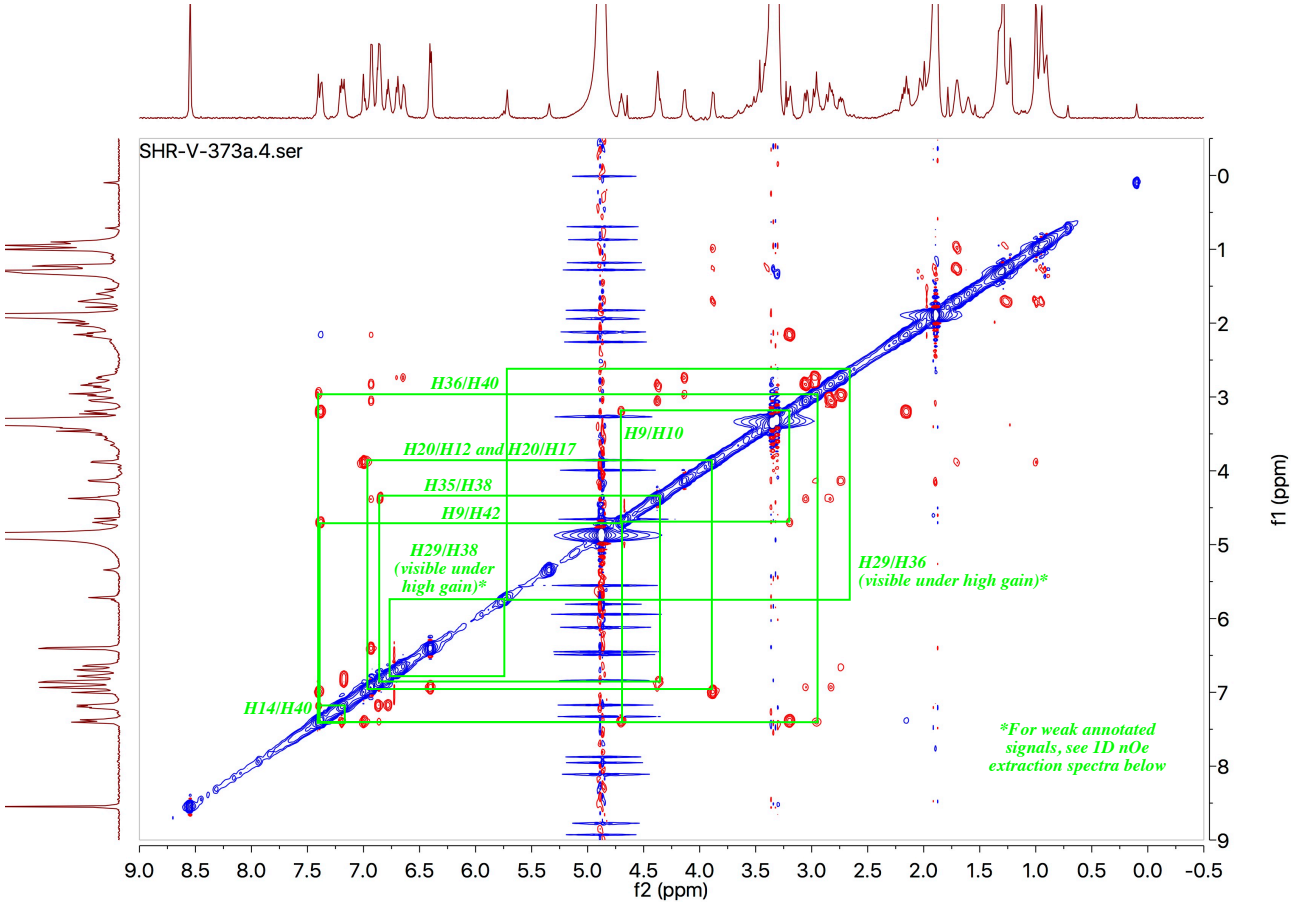
	Natural product	Synthetic **1a**	Synthetic **1b**
Position	nOe to	nOe to	nOe to
H1			
H2	H3	H3	H3, H5
H3	H2, H3, H5	H2, H3, H5	H2, H3, H5
H4			
H5	H3, H6, H9	H2, H3, H6, H10	H2, H3, H10, H15
H6	H5	H5	H5, H10, H15
H7			
H8			
H9	H3, H10, 2NH	H10, H42	H10, H40
H10	H10, H42	H9, H10, H42	H5, H9, H10, H40
H11			
H12	H20, H40	H20, H40	H20, H42
H13			
H14	H15, H40	H6, H15	H10
H15		H10, H14, H40	
H16		H17	H15
H17		H20	H10, H15, H16
H18			
H19			
H20	H12, H21, H24	H12, H21, H22, H24	H12, H21, H22, H24
H21	H24	H20, H22, H23	H20, H22, H23, H24
H22	H21	H20, H21, H23	H20, H21, H22, H23, H24
H23	H21	H21, H22	H21, H22, H24
H24	H21, H22	H20, H21	H20, H21
H25			
H26	H27, 20NH	H27	H27
H27	H26	H26, H27	H26, H27, H29, H33
H28			
H29	26NH	H36, H38, H43	H27, H43
H30			
H31			
H32		H27	H27
H33	H27	H27	H27
H34			
H35	H38	H38	H36
H36	H40	H38, H40	H35, H36, H38, H40
H37			
H38	H35	H29, H35, H36	H29, H36, H47
H39			

H40	H12, H36	H12, H36, H42	H9, H10, H12, H36
H41			
H42	H9, H10	H9, H10	H12, H43
H43	H42	H9, H10, H29, H42	H29, H42
H44			
H45			
H46		H47	H47
H47	H46	H46	H46

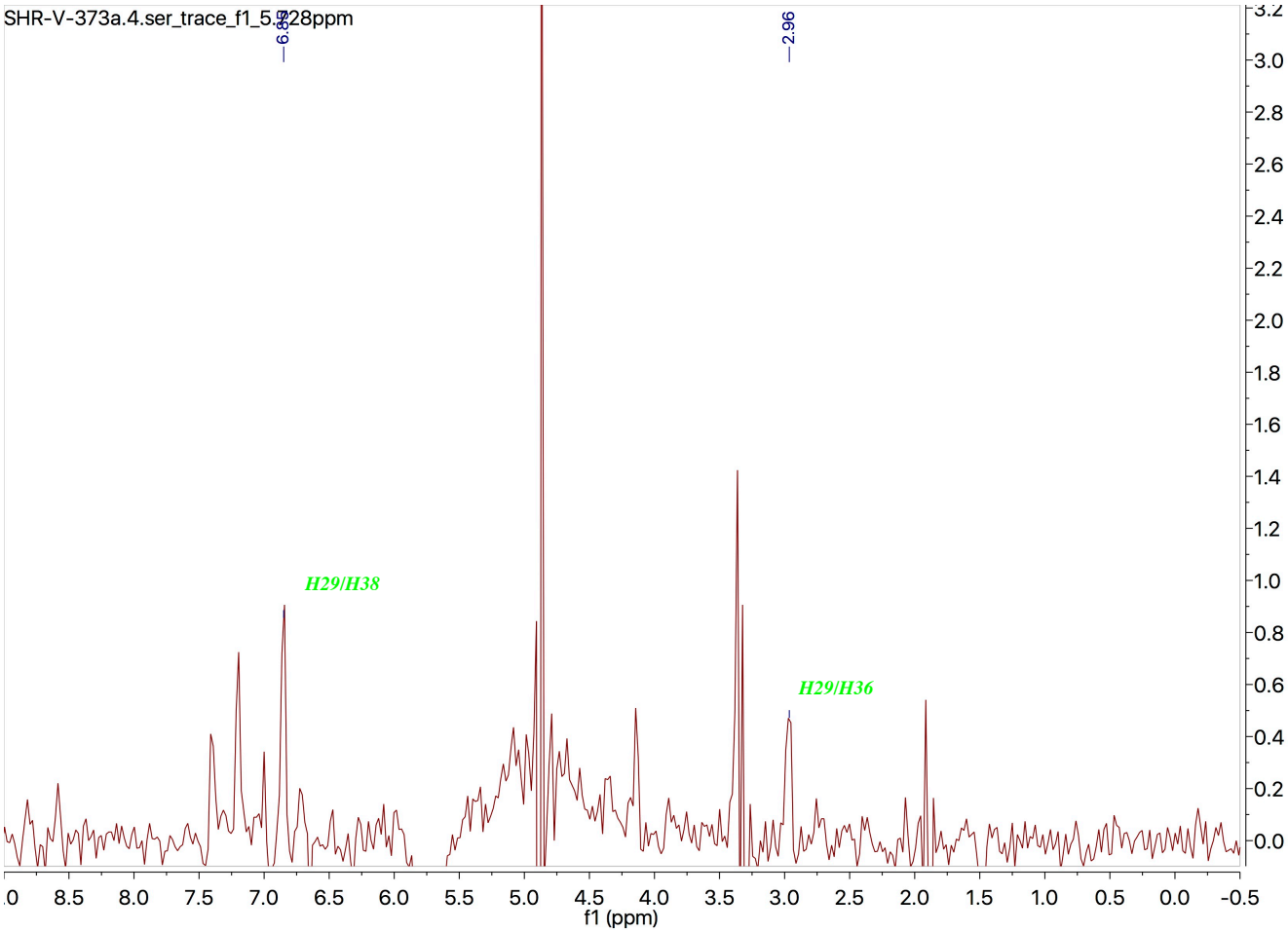
NOTE: An astute observer will note that in the graphical ^{13}C NMR data (p. 79), there are several unassigned peaks that are present in synthetic but not natural tryptorubin A, and also several unassigned peaks that are present in natural but not synthetic tryptorubin A. While the origin of these peaks is unclear, it is note worthy that all ASSIGNED ^{13}C resonances correlate well between the natural and synthetic material. Additionally, the ^{1}H NMR coinjection experiment (p. 78) verifies that these compounds are indeed the same species.

2D NMR spectra for compound 1a (synthetic and natural)

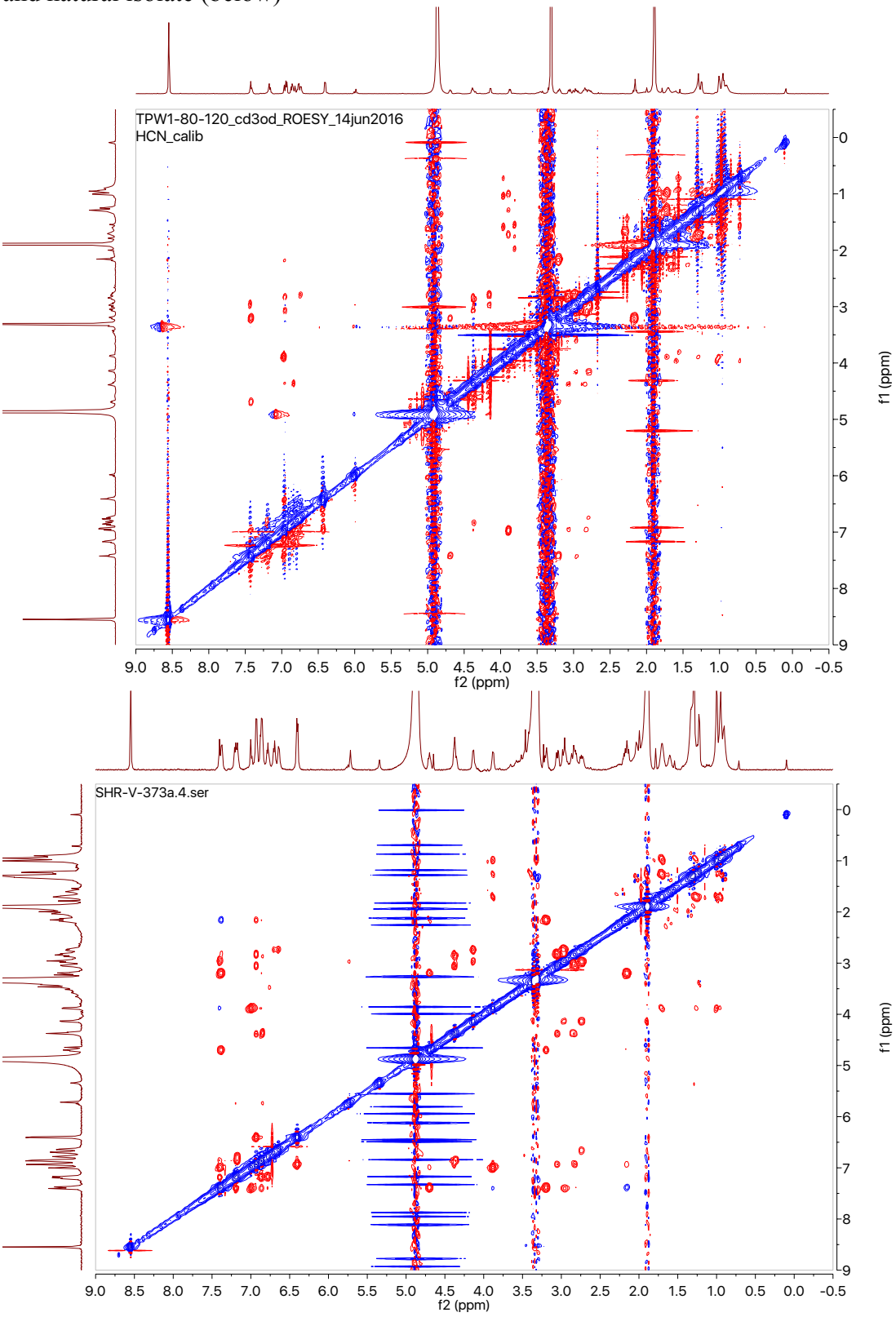
^{1}H-^{1}H ROESY spectrum for **1a** (Note: explicitly annotated correlations are those discussed in following section, "Explanation of NMR analysis of two atropisomers)



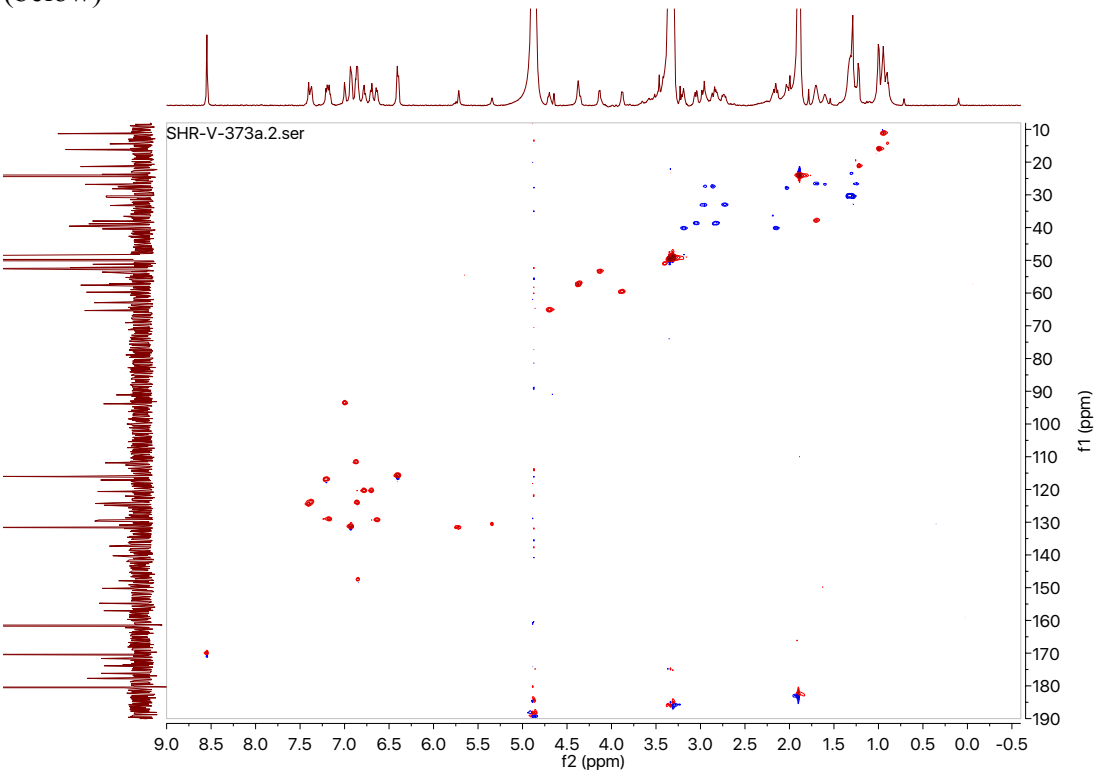
^{1}H-^{1}H 1D nOe extractions for **1a**, F1 = 5.72 ppm (H29)

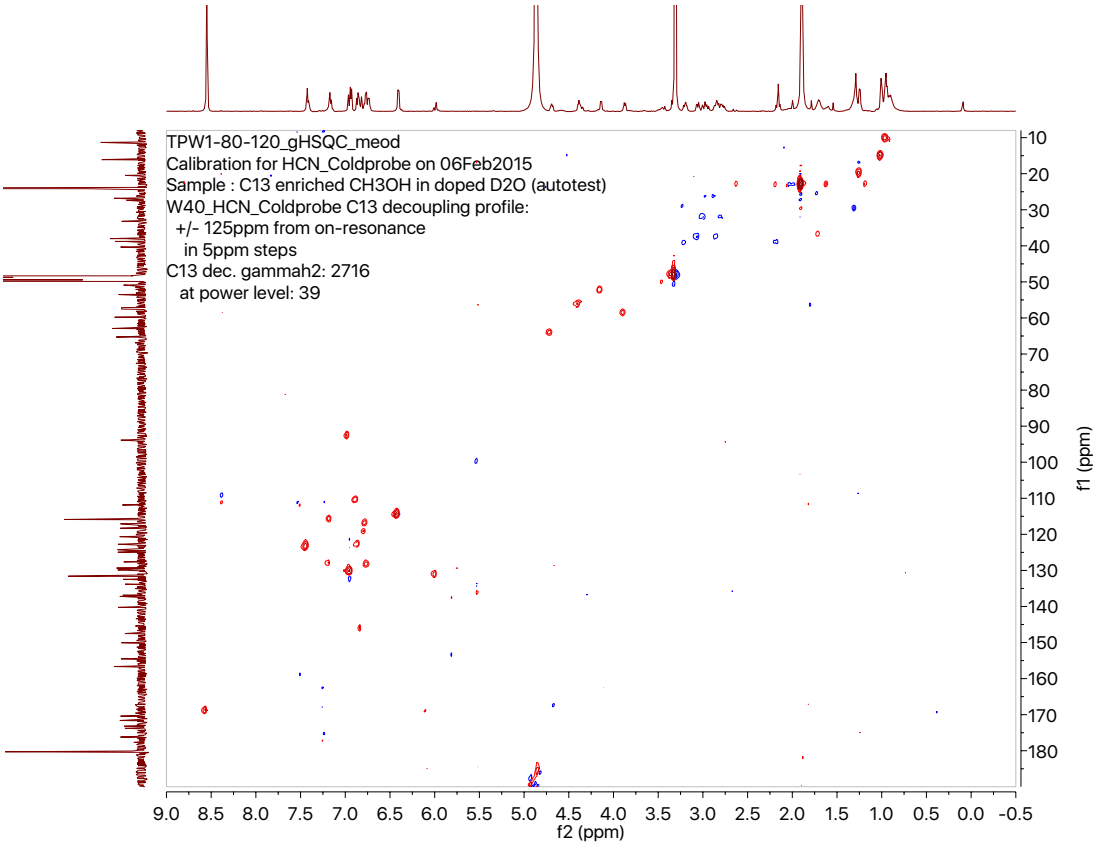


^{1}H-^{1}H ROESY spectrum for **1a**, unannotated comparison: synthetic material (above) and natural isolate (below)

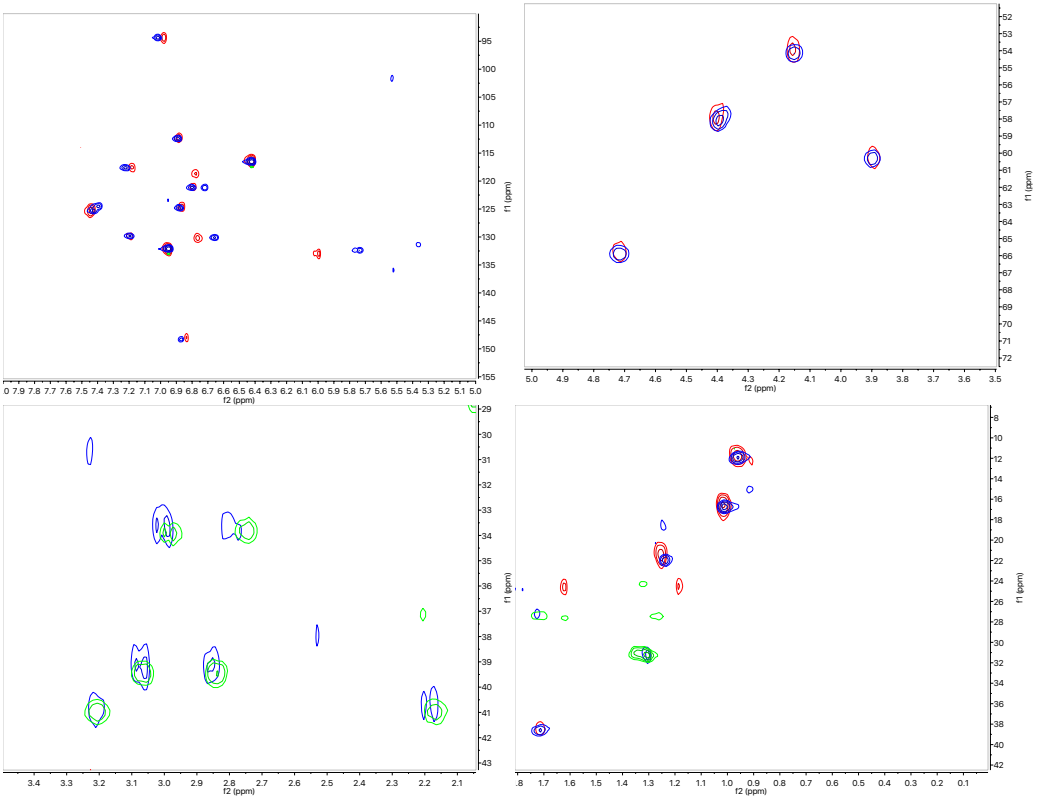


^{1}H-^{13}C HSQC spectrum for **1a:** synthetic material (above) and natural isolate (below)

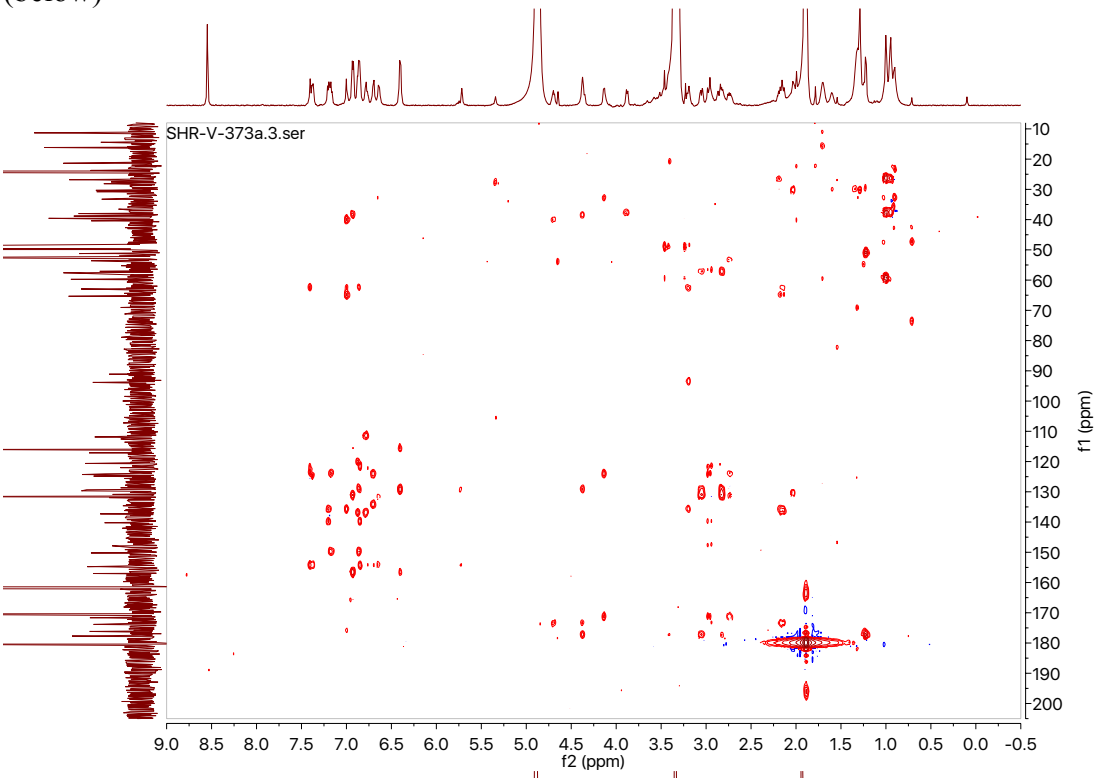


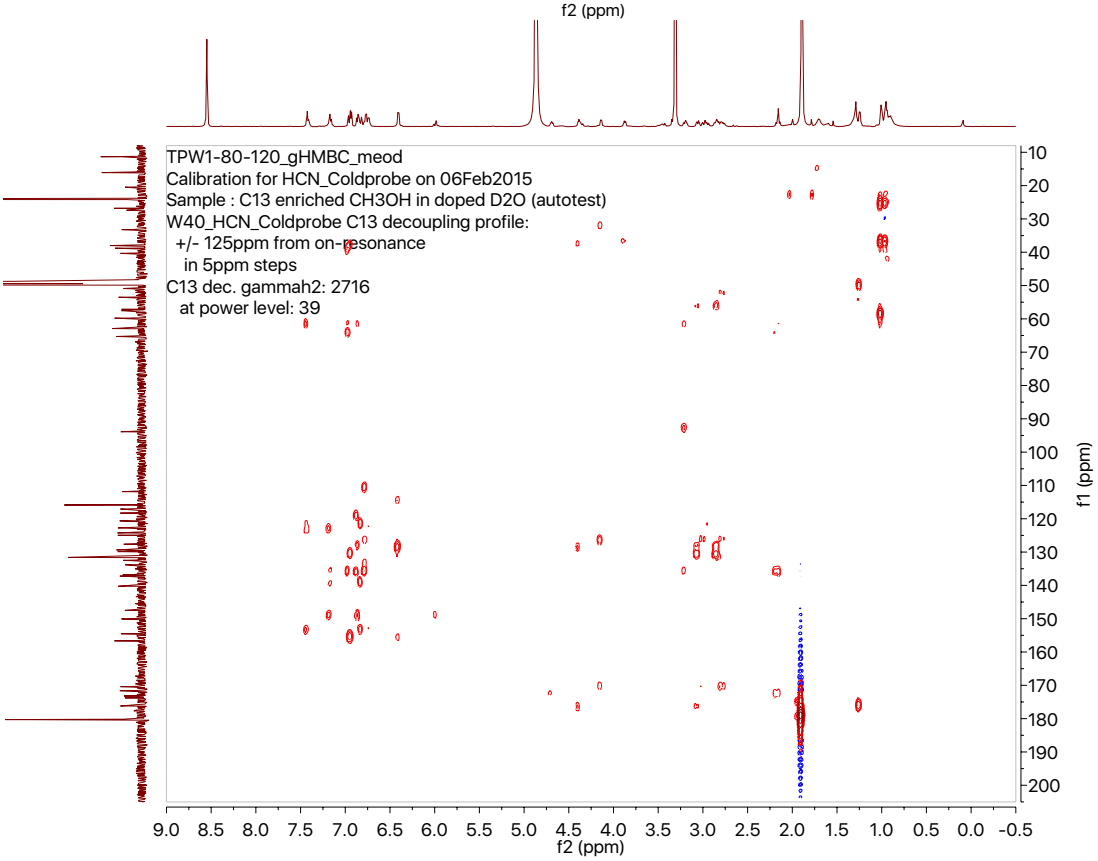


^{1}H-^{13}C HSQC superimposed spectra for **1a,** synthetic material (green-blue) and natural isolate (blue-red). Above left, 8 to 5 ppm f2 region; above right, 5 to 3.5 ppm f2 region; below left, 3.5 to 2 ppm f2 region; below right, 2 to 0 ppm f2 region.



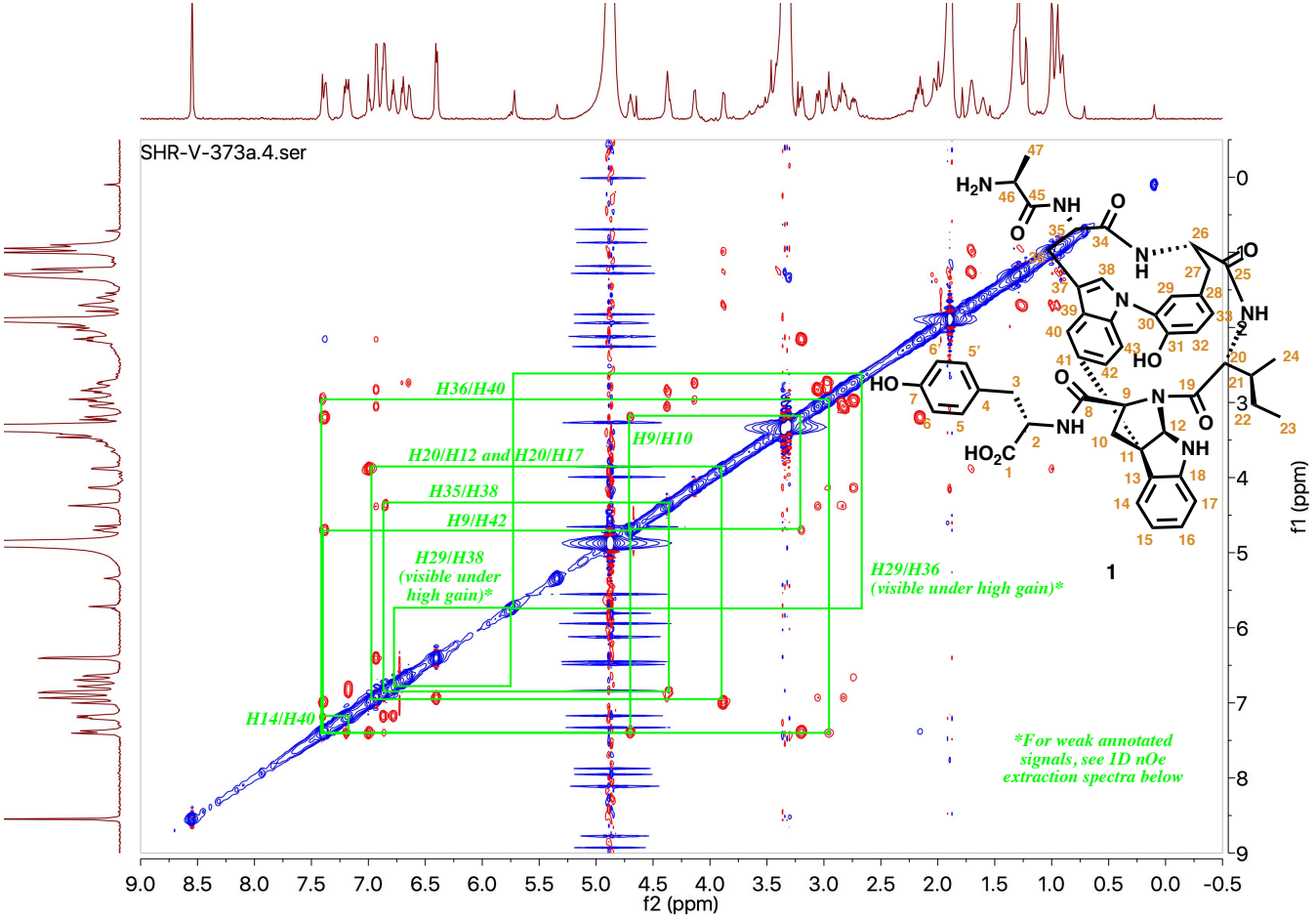
^{1}H-^{13}C HMBC spectrum for **1a:** synthetic material (above) and natural isolate (below)



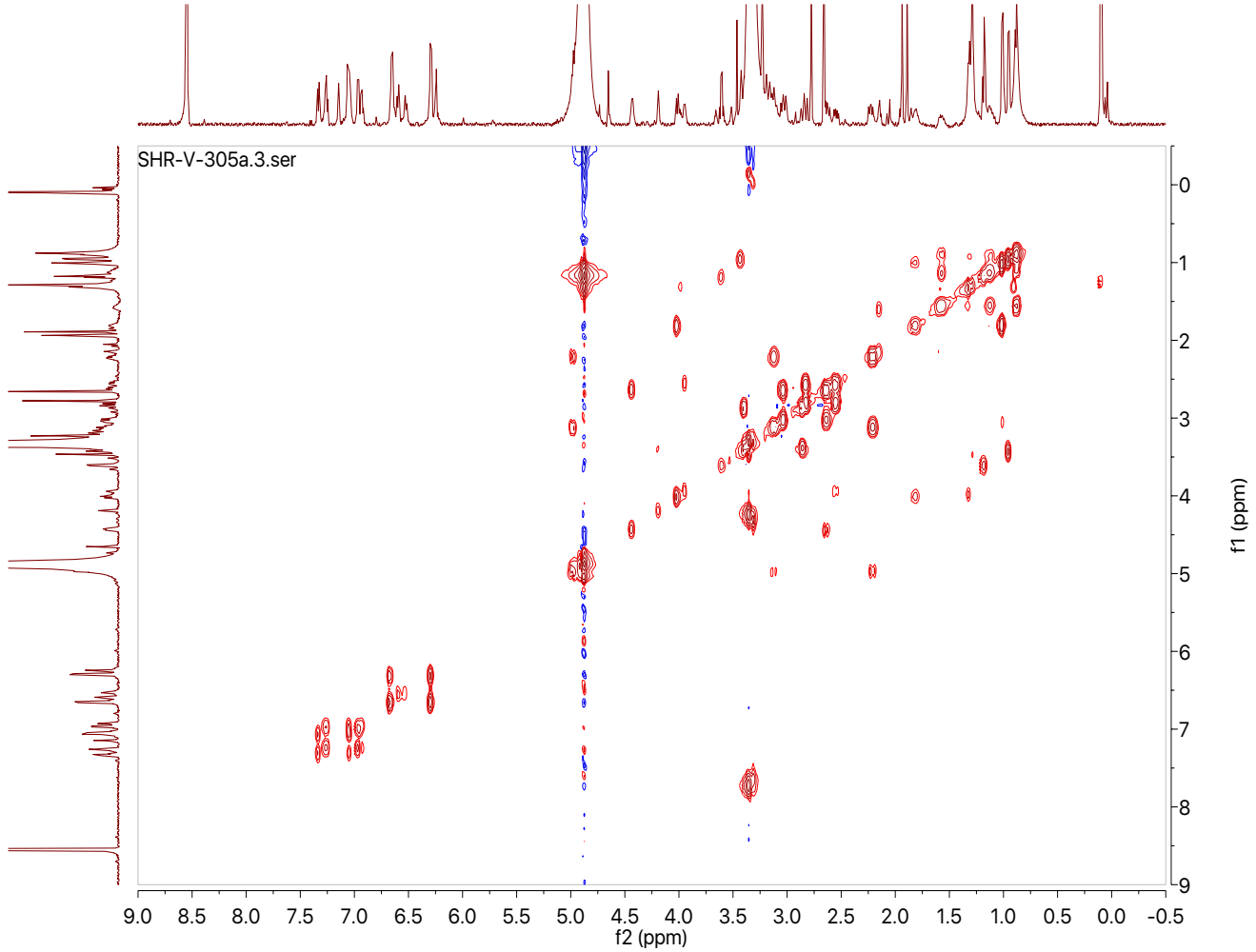


2D NMR spectra for compound 1b

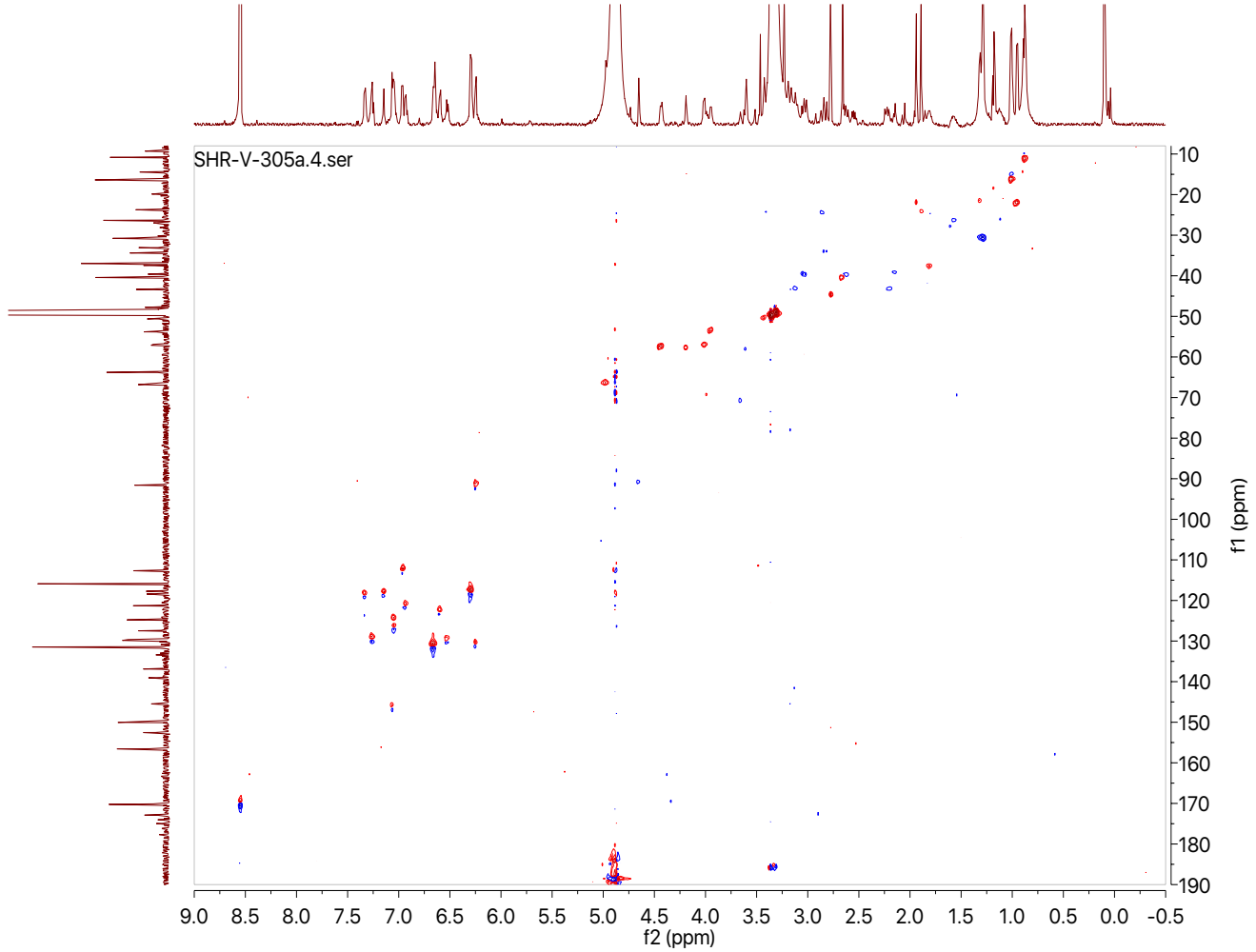
^{1}H-^{1}H ROESY spectrum for **1a** (Note: explicitly annotated correlations are those discussed in following section, "Explanation of NMR analysis of two atropisomers)



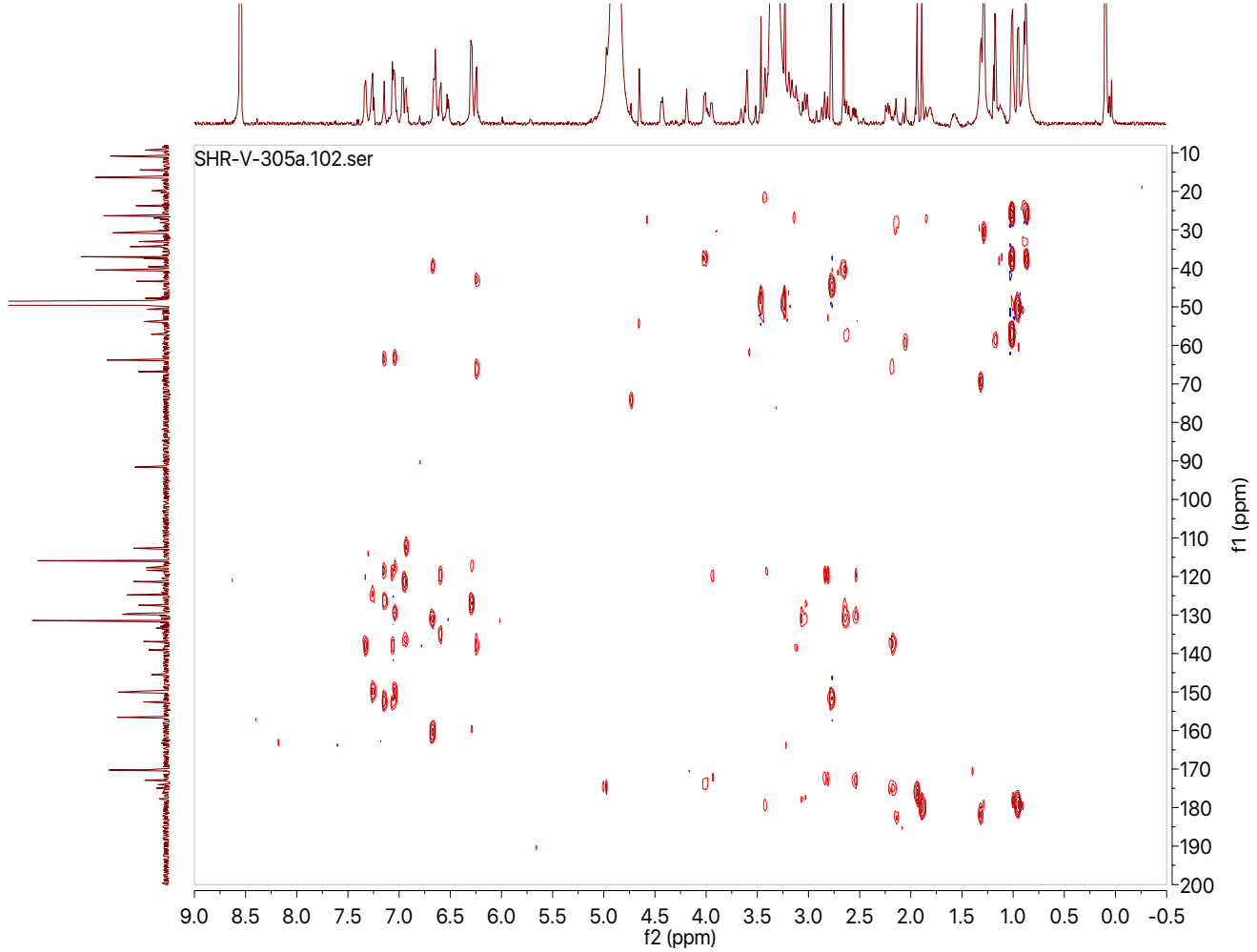
^{1}H-^{1}H COSY spectrum for **1b**



^{1}H-^{13}C HSQC spectrum for **1b**



^{1}H-^{13}C HMBC spectrum for **1b**



Explanation of NMR analysis of the two atropisomers

Prior to our hypothesis that **1a** and **1b** are atropisomers of one another, we considered a variety of other possibilities that could explain the differences in NMR resonances between these two compounds. We first collated the structural features we were confident in:

1. Given the HRMS data, the peptidic nature of the natural product, and the extensive labeling studies reported in the initial isolation disclosure, we felt safe in the assumption that the compound was constructed from six canonical amino acids-- Two tryptophans, two tyrosines, and one each of alanine, and isoleucine.
2. In the initial isolation disclosure, ^{13}C-labeled material allowed for a C-C COSY. This COSY allowed unambiguous correlations across the peptide chain, making us feel secure in the determination that the peptidic backbone sequence was Ala-Trp-Tyr-Ile-Trp-Tyr. A strong HRMS daughter peak at *m/*z = 825.3, corresponding to $[M - Ala]^+$, also further supported this hypothesis. HMBCs across at least one peptide bond (H2 to C8) also confirmed the backbone assignment.
3. Finally, the presence of a pyrroloindoline was confidently postulated; based on the monomeric building blocks at hand, we viewed a pyrroloindoline as the only possible motif corresponding to the ^{13}C NMR shift of C12 (*ca*. 94 ppm).
4. Based on the H-N HMBC studies disclosed in the isolation report (specifically, an H29 to N38 correlation), we felt assured in the assignment of the indole nitrogen-to-phenol (N38 to C30) bond.

Taking these facts together, we reasoned—before considering atropisomerism—that possibilities for a structural difference between **1a** and **1b** were as follows:

1. Either **1a** or **1b** could contain one or more *D*-amino acid α-centers. That is, either the natural product was constructed from one or more *D*-amino acids, or in the synthetic pursuit of **1b**, synthetic manipulations had epimerized one or more α-centers.
2. It was possible that the non-proteogenic pyrroloindoline stereocenters (i.e., C11 and C12) had been incorrectly assigned in either **1b** or **1a**; that is, in either **1a** or **1b**, the pyrroloindoline consisted of *exo* relative stereochemistry.
3. It was possible that the regiochemistry of the C11-to-C41 bond had been misassigned; that is, in either **1a** or in **1b**, the bond actually extended from C11 to C42 (indole C6 alkylation), rather than C41.

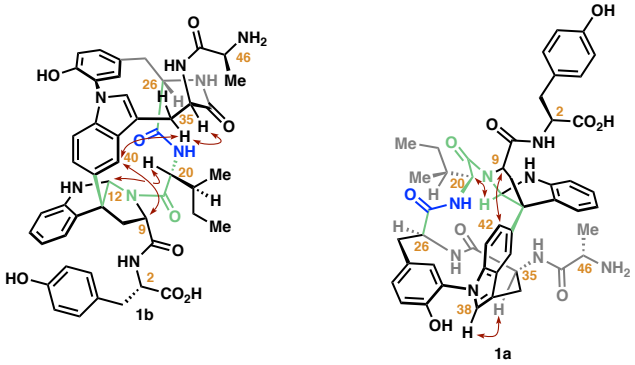
Next, we describe how, for both **1a** and **1b**, we ruled out each of the above possibilities regarding a theoretical misassignment:

1. ***Ruling out a D-amino acid. (See Fig. S6 and S7)***
    a. For ***1a:*** Marfey's analysis, as reported in the initial isolation disclosure, yielded free *L*-alanine and *L*-tyrosine from hydrolysis of the N- and C-

terminal residues, respectively, confirming the configuration of C46 and C2, respectively. An nOe correlation from H9 to H42, and also from H9 to only the convex H10 of diastereomeric methylene C10, unambiguously assigned the configuration of C9 as *L*. An nOe correlation from H20 to H12 unambiguously assigned C20 as *L*. For the molecule to be geometrically feasible, C26 was required to be *L*; the bridged macrocycle could not geometrically form if that stereocenter were *D*. An nOe correlation from H35 to H38 allowed us to unambiguously assign C35 as *L*. We concluded all α-centers were *L* in configuration.

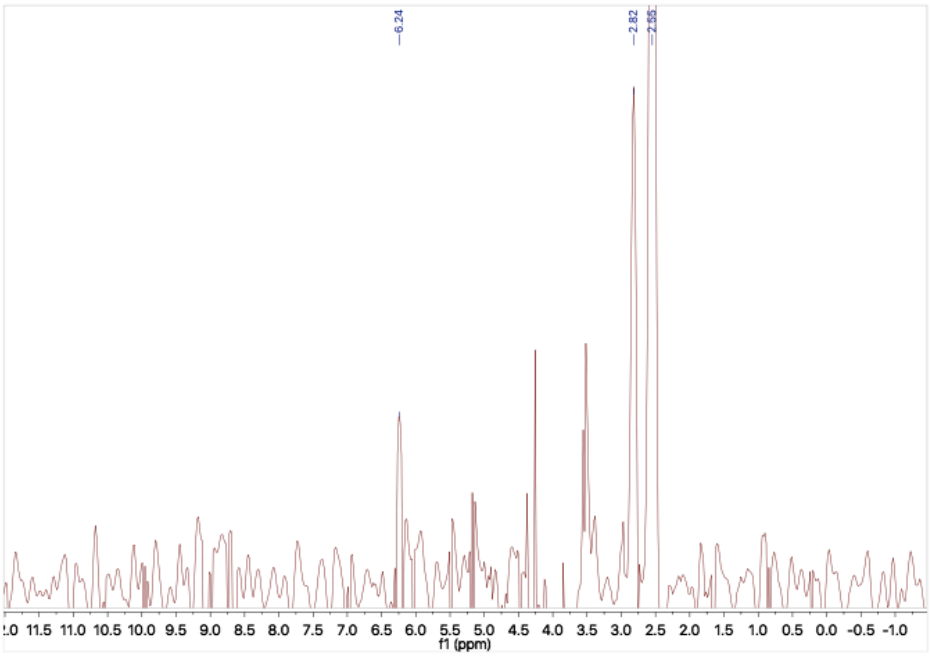
b. For **1b**: In the synthesized material, the fact that we started from *L*-amino acid building blocks and didn't observe diastereomeric erosion was, in itself, support that all amino acids had *L* configuration. Additionally, we had the following evidence via NMR analysis: An nOe correlation from H9 to H40 unambiguously confirmed C9's configuration as *L*. An nOe correlation from H20 to H12 unambiguously confirmed C20's configuration as *L*. An nOe correlation from H35 went to only one hydrogen of the diastereopic methylene H36, and this same hydrogen correlated by nOe to H40. Taken together, these correlations unambiguously confirm the configuration of C35 as *L*. No direct evidence for the configuration of C2 and C46 was observed, but as stated above, it is unlikely that these centers epimerized exclusively to the undesired stereochemistry.

Figure S6, nOe data that supports stereochemical assignment



No 2D NMR correlations could unambiguously confirm the configuration of C26 in **1b**. However, a 1D ROE extraction showed a very weak – but distinct – correlation from H26 to H12, confirming H26's *L* configuration (see Fig. S7).

Figure S7, Weak ROE from H26 to H12



Additionally, X-ray crystallography of a reasonably late-stage intermediate (**7**) showed the correct *L* configuration. However, we viewed these evidences of C26's configuration as insufficient, and thus embarked on a total synthesis campaign to prepare the C26 epimer of **1b** (*vide supra* for synthesis of ***epi*-8**). Having prepared ***epi*-8**, we attempted to close the final macrolactam, and were surprised to observe that this epimer failed to condense to the desired bicycle, despite screening a variety of conditions (Table S8) that were known to cyclize the epimeric congener (i.e., **8** --> **SI-17**). Given these data, we feel that cyclization of *epi*-**8** to the hypothetical *epi*-**SI-17** (Fig. S8) is unfeasible.[34] Similarly, we view it as tremendously unlikely that bicycle **SI-17** could epimerize post-cyclization to *epi*-**SI-17**, given that such an epimerization would require abstraction of an endocyclic proton (Fig. S8). In addition to these data, it is worth noting that intermediates on the route towards *epi*-**SI-17** all had a characteristic rotamerism in their NMR spectra that was absent from the *S*-configured congeners (compare, for example, the ^{1}H NMR spectra of **SI-16** vs. *epi*-**SI-16)**. Final atropisomer **1b** did not show this rotamerism,

[34] Comparison of crystal structures of intermediates with the C26-*epi* configuration, vs. the natural C26 configuration (i.e., **7** vs. ***epi*-SI-21**; Fig. S9), helped us form a mechanistic hypothesis for the *epi* scaffold's reticence to cyclization. Specifically, for the *epi* scaffold to lactamize, the newly-formed ring would have to be supra-facial to the pendant alanine (represented by in ***epi*-SI-21** as a benzamide by analogy), and this steric clash strongly disfavors cyclization. In contrast, the natural stereochemistry allows the macrolactam to be antara-facial to the alanine, which is more sterically feasible.

further supporting its assignment as (*S*). Taken together, we conclude that **SI-17** indeed has the desired C26 (*S*) stereochemistry, and in turn, that **1b** consists of only *L* amino acid residues.

Figure S8, Epimer cyclization experiments to confirm SI-17 stereochemical assignment

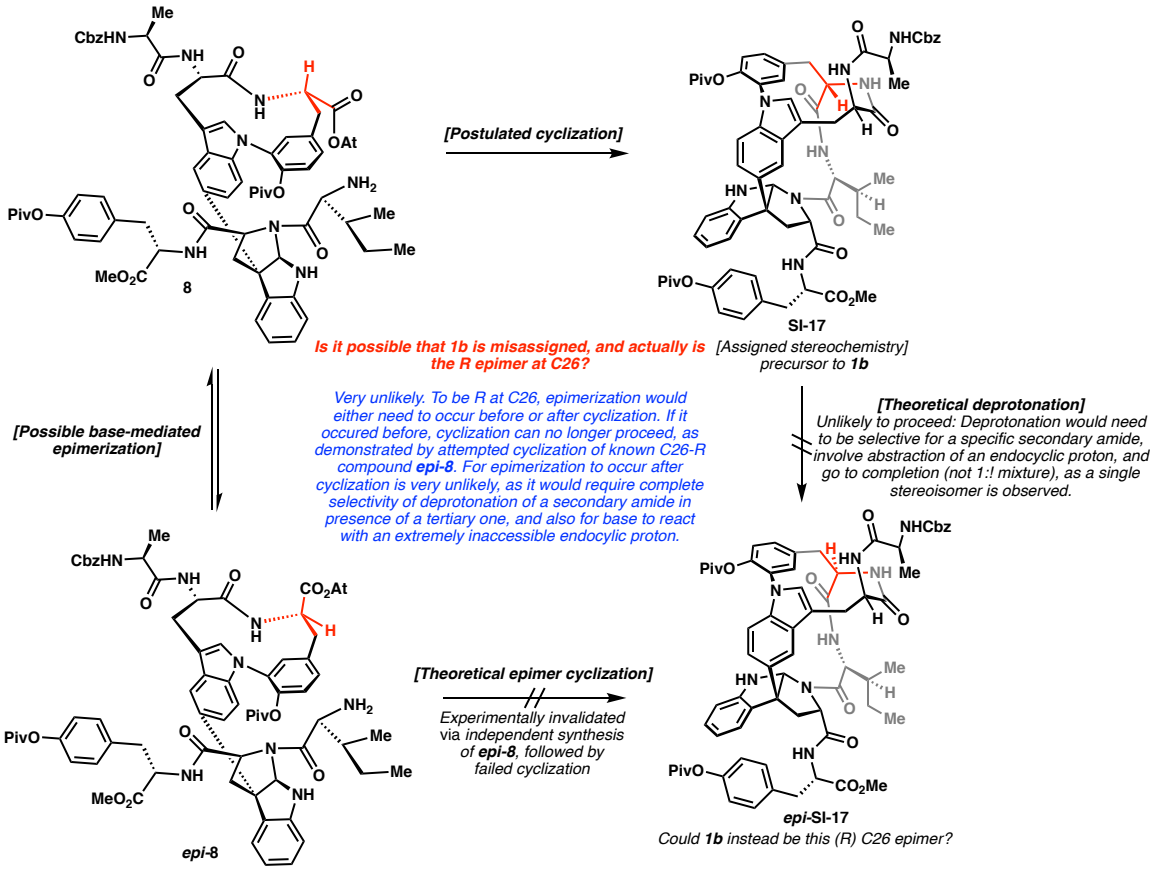


Figure S9, crystallographic analysis that rationalizes the reticence of the *epi* scaffold to cyclize

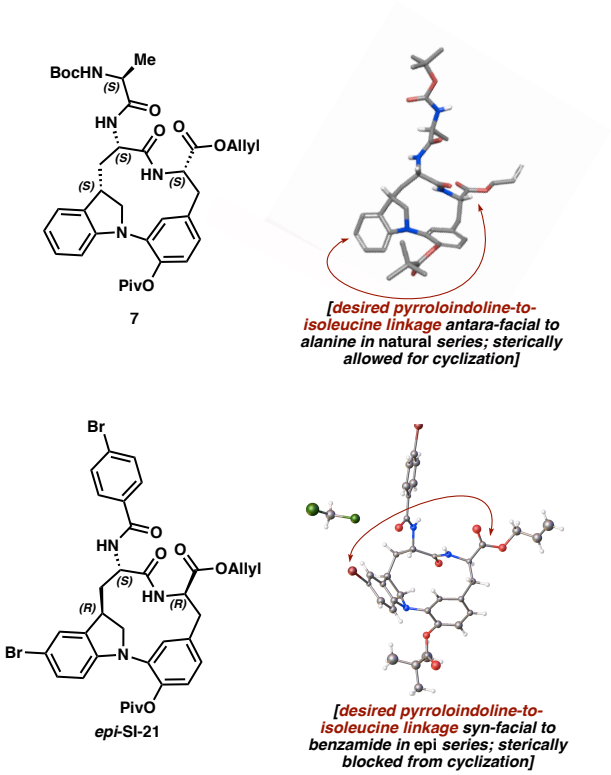


Table S8, Conditions that cyclize 8 but not *epi*-8

Standard conditions: base = DIPEA; solvent = MeCN; coupling reagent = PyAOP; final concentration = 0.5 mM; addition method = dual slow addition of PyAOP and substrate. "Non-zero" product was determined to be present *via* any mass hit of desired product upon LCMS analysis.

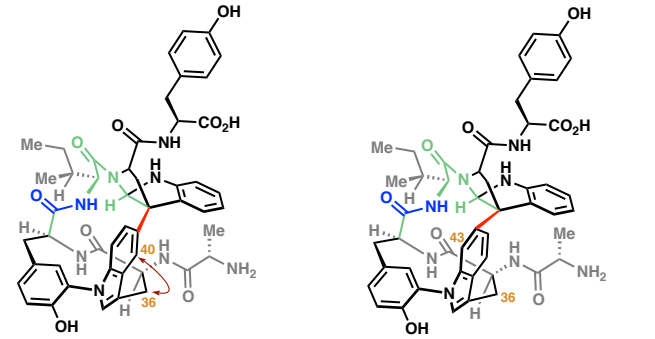
Entry	Difference from standard conditions	Non-zero SI-17 observed from 8	Non-zero *epi*-SI-17 observed from *epi*-8
0	none	✓	✗
1	base = collidine	✓	✗
2	base = NMM	✓	✗
3	solvent = DMF	✓	✗
4	solvent = DMA	✓	✗
5	solvent = DMSO	✗	✗
6	coupling reagent = HATU	✓	✗
7	coupling reagent = PyOxim	✓	✗
8	coupling reagent = EDCI/DMAP	✓	✗
9	coupling reagent = T3P	✗	✗
10	final concentration = 0.05 mM	✓	✗
11	final concentration = 5 mM	✗	✗
12	mix reagents all at start	✗	✗
13	Slow add'n of substrate to PyAOP	✗	✗

14	8 hour dual add'n	✓	×

2. ***Ruling out misassigned stereochemistry at pyrroloindoline (C11/C12) (See Fig. S6):*** For both **1a** and **1b**, nOe correlations from H9 to Trp2 aryl protons (H42 and H40 for **1a** and **1b,** respectively) unambiguously assign the pyrroloindoline relative stereochemistry as *endo*. A misassignment in this relative stereochemistry was thus ruled out.

3. ***Ruling out a regioisomeric bond at C11 (See Fig. S10)*:** Consider, as a thought experiment, if the C11 to C41 bond were regiochemically misassigned; that is, that either **1b** or **1a** contained a bond from C11 to C42 (Note that C42 is "C6" of the indole, a much more common site of alkylation in indole alkaloids). If this had been the case, the sole singlet on Trp2 would have belonged to H43 rather than H40. However, the aryl singlet in both **1a** and **1b** showed an nOe correlation with H36, unambiguously assigning the singlet as H40, and thus the regio-site of alkylation as C41. The key nOe correlation is shown in Fig. S10 for **1a**, and the argument is directly analogous for **1b**.

Figure S10, nOe data that supports regiochemical assignment



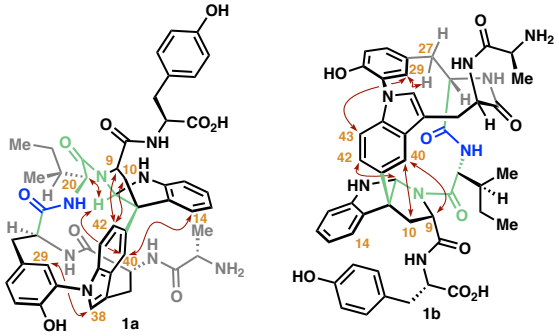
With these other possibilities for structural isomerism ruled out, we felt confident that both the atomic connectivity and stereochemistry of **1a** and **1b** were identical. The striking similarity in the HMBC, and most of the ROESY, for **1a** and **1b** is notable, and supports this assignment.

This left us, by systematic process of elimination, with the hypothesis that **1a** and **1b** were atropisomers. As discussed in the main manuscript, the single geometric constraint of an nOe correlation from H9 to H40 in **1b**, and from H9 to H42 in **1a**, was enough to unambiguously assign the two compounds' respective atropochemistry. The following nOe correlations additionally strengthened this hypothesis (Fig. S11 shows these correlations graphically):

1. H9 to H42 in **1a**; *vs.* H9 to H40 in **1b**

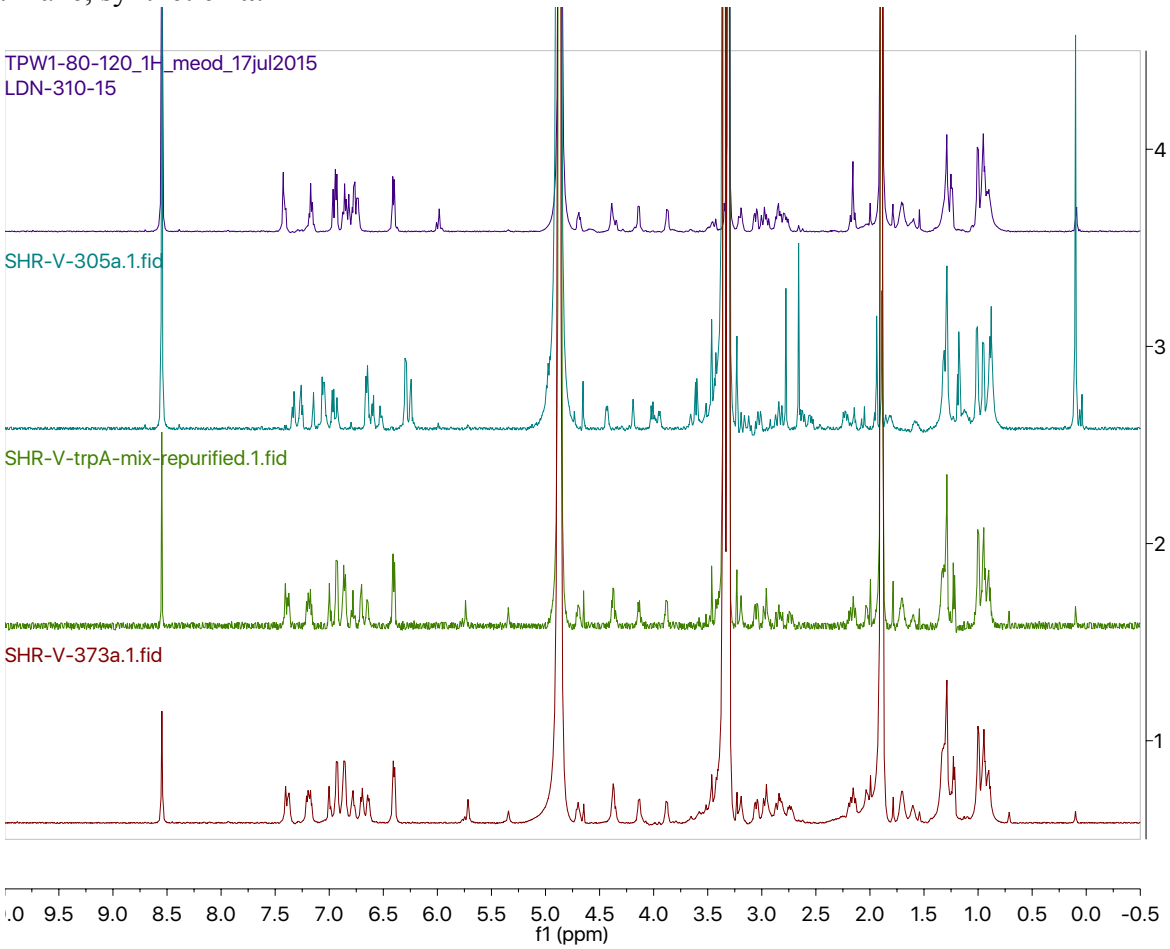
2. H12 to H42 in **1b**; conspicuously absent in **1a.**
3. H14 to H40 in **1a**; conspicuously absent in **1b**.
4. H17 to H20 in **1a**; conspicuously absent in **1b**.
5. H29 to both H38 and H36 in **1a**; conspicuously absent in **1b.**
6. H29 to H27 in **1b**; conspicuously absent in **1a**.

Figure S11, nOe data that supports topisomerism.

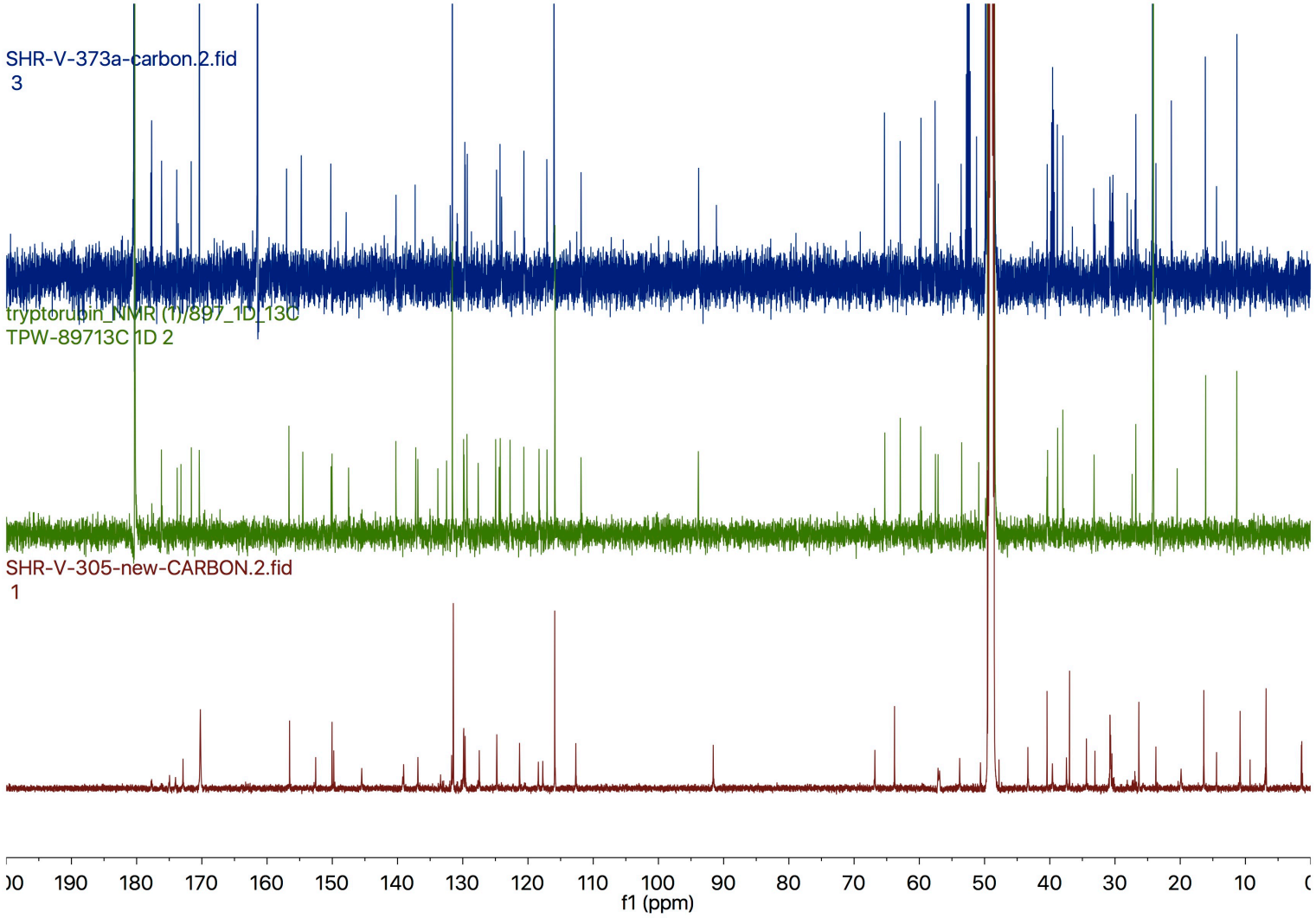


Stacked NMR spectra of natural and synthetic tryptorubin A

^{1}H NMR spectra. Top lane, natural tryptorubin A; second lane, synthetic **1b**; third lane, *ca.* 1:1 mixture of synthetic **1a** and natural tryptorubin A; fourth lane, synthetic **1a**.

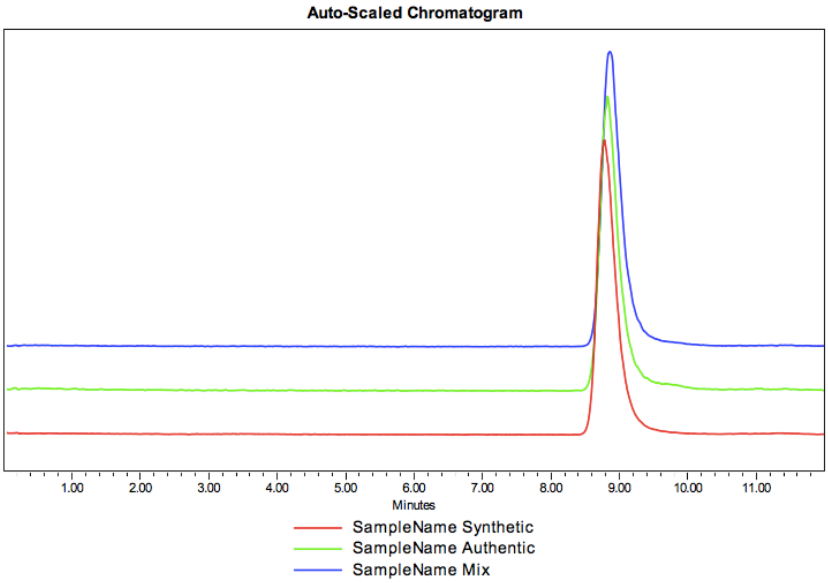


^{13}C NMR spectra. Top lane, synthetic **1a;** middle lane, natural tryptorubin A; bottom lane, synthetic **1b**.



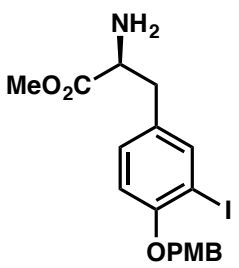
LCMS co-injection of natural tryptorubin A, synthetic 1a, and synthetic 1b

Solutions of 0.05 mg/mL of natural isolate, synthetic **1a**, and a 1:1 mixture of the two were analyzed on a Waters I-Class LC connected to a Waters QDa single quadrupole mass spec. The samples were eluted off of a Waters Cortecs C18 column (1.6 mm, 2.1x55 mm, 90 Å) under isocratic conditions [0.6 mL/min, 84:16 (0.1% formic acid / H2O) : acetonitrile] at 35 °C. The compound of interest and any potential constitutional isomers were detected by Single Ion Recording in ESI+ (m/z = 897.4). The chromatogram below shows identical retention times for natural (green) and synthetic (red) material, and a single peak (blue) with no shouldering when the two compounds are co-injected:



Solutions of 0.05 mg/mL of natural isolate, and synthetic **1b** were analyzed on an Agilent 1260 Infinity LC connected to an Agilent 6230 TOF mass spec. The samples were eluted off of an Agilent Poroshell 120 C18 column (1.6 mm, 2.1x55 mm, 90 Å), eluting with gradient conditions [0.7 mL/min, 10 to 99% MeCN/H_2O with 0.1% formic acid, over 8.8 min, followed by an isocratic hold at 99% MeCN/H_2O with 0.1% formic acid for an additional 3 min] at 35 °C. For the natural compound, the compound of interest and any potential constitutional isomers were detected by Single Ion Recording in ESI+ (m/z = 897.4) (bottom lane). For synthetic **1b**, detection was effected both *via* Single Ion Recording (middle lane) and also *via* monitoring absorbance at 254 nm (top lane). The chromatogram shows that the two compounds are clearly distinct, with **1b** being significantly more retentive under these elution conditions:

NMR spectra: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MeO2C
I
OPMB

SI-03

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6.0
5.5
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3.0
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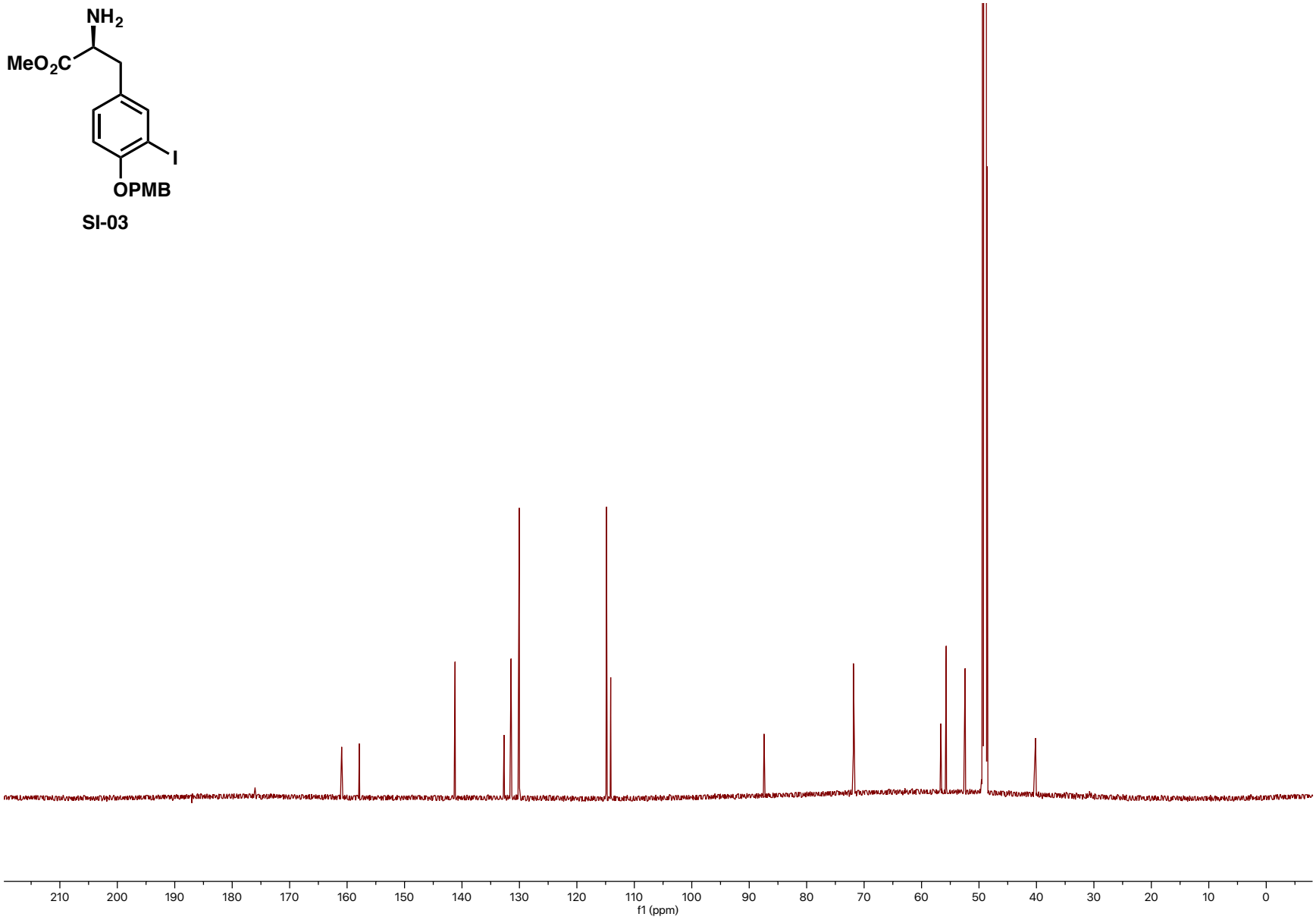
0.5
0.0
-0.5
f1 (ppm)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MeO2C
I
OPMB
SI-03
210
200
190
180
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50
40
30
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10
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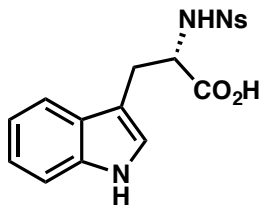
f1 (ppm)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CO2H
N
H

SI-05

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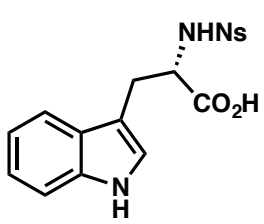
f1 (ppm)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CO2H
N
H

SI-05

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220
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10
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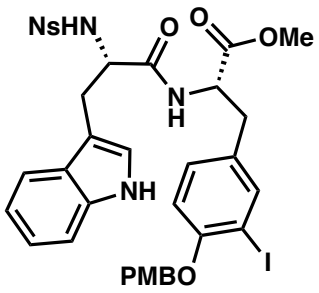
f1 (ppm)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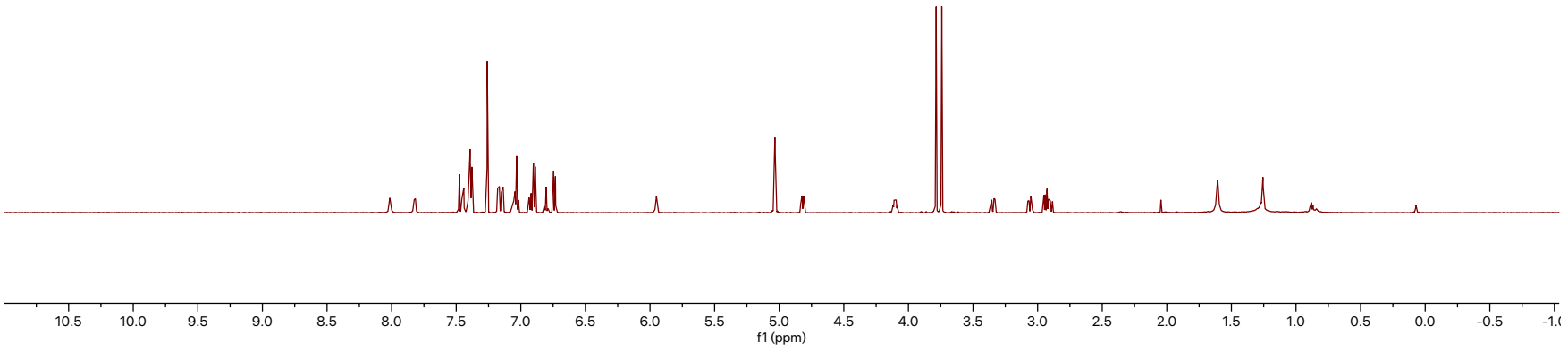
O
OMe
N
H
NH
PMBO
I

2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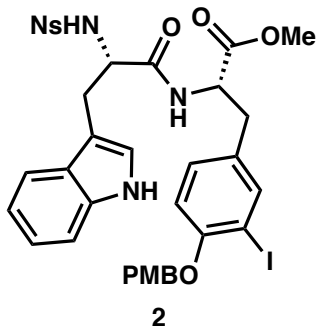
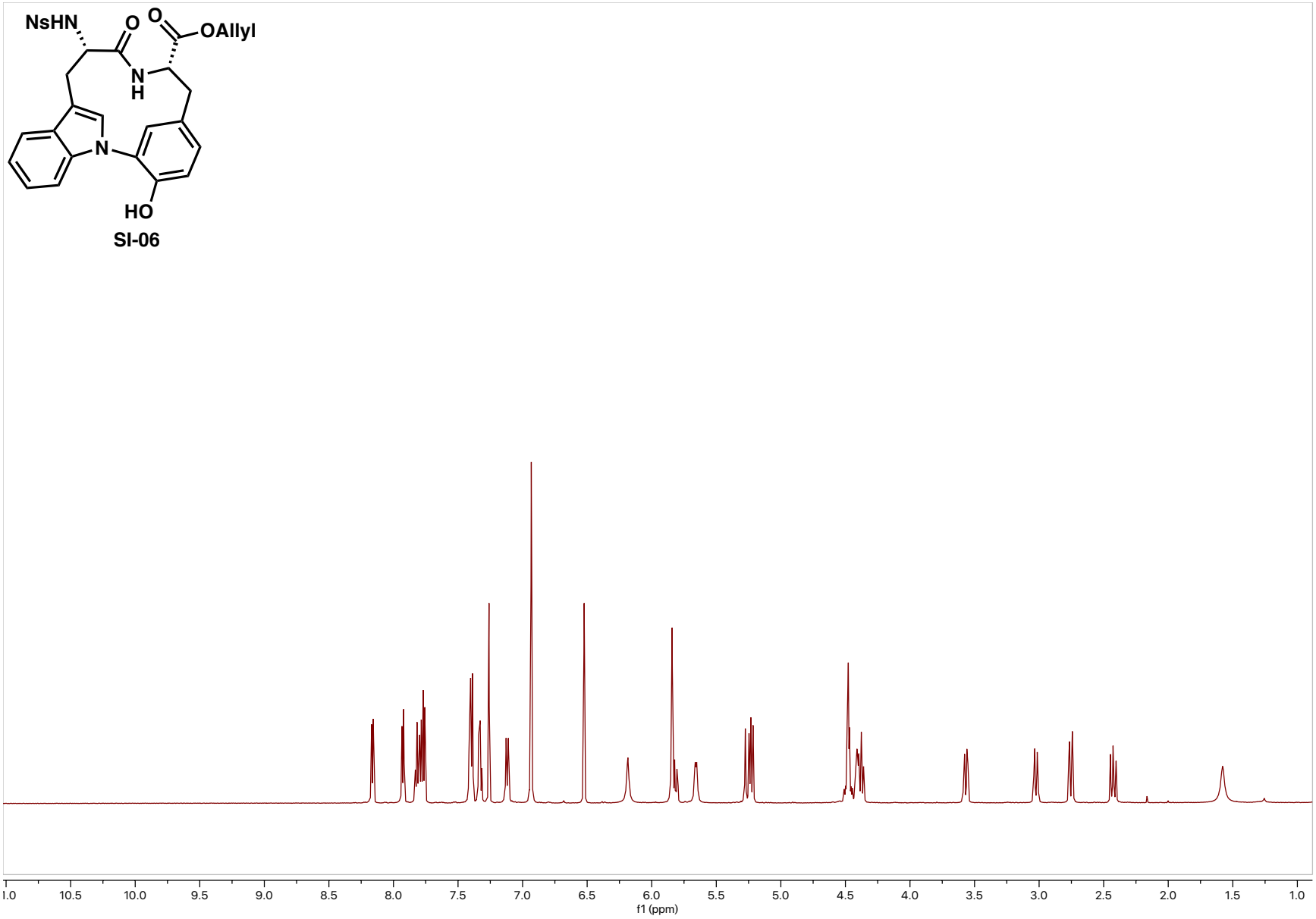
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)



2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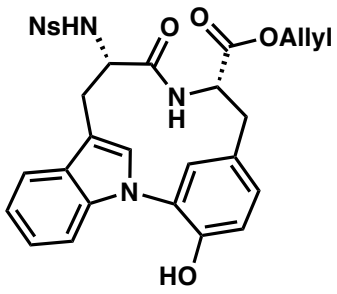
O
O
OAllyl
N
H
N
HO
SI-06
f1 (ppm)


NsHN
O
O
OAllyl
N
H
N
HO

SI-06

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210
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180
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150
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130
120
110
100
90
80
70
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50
40
30
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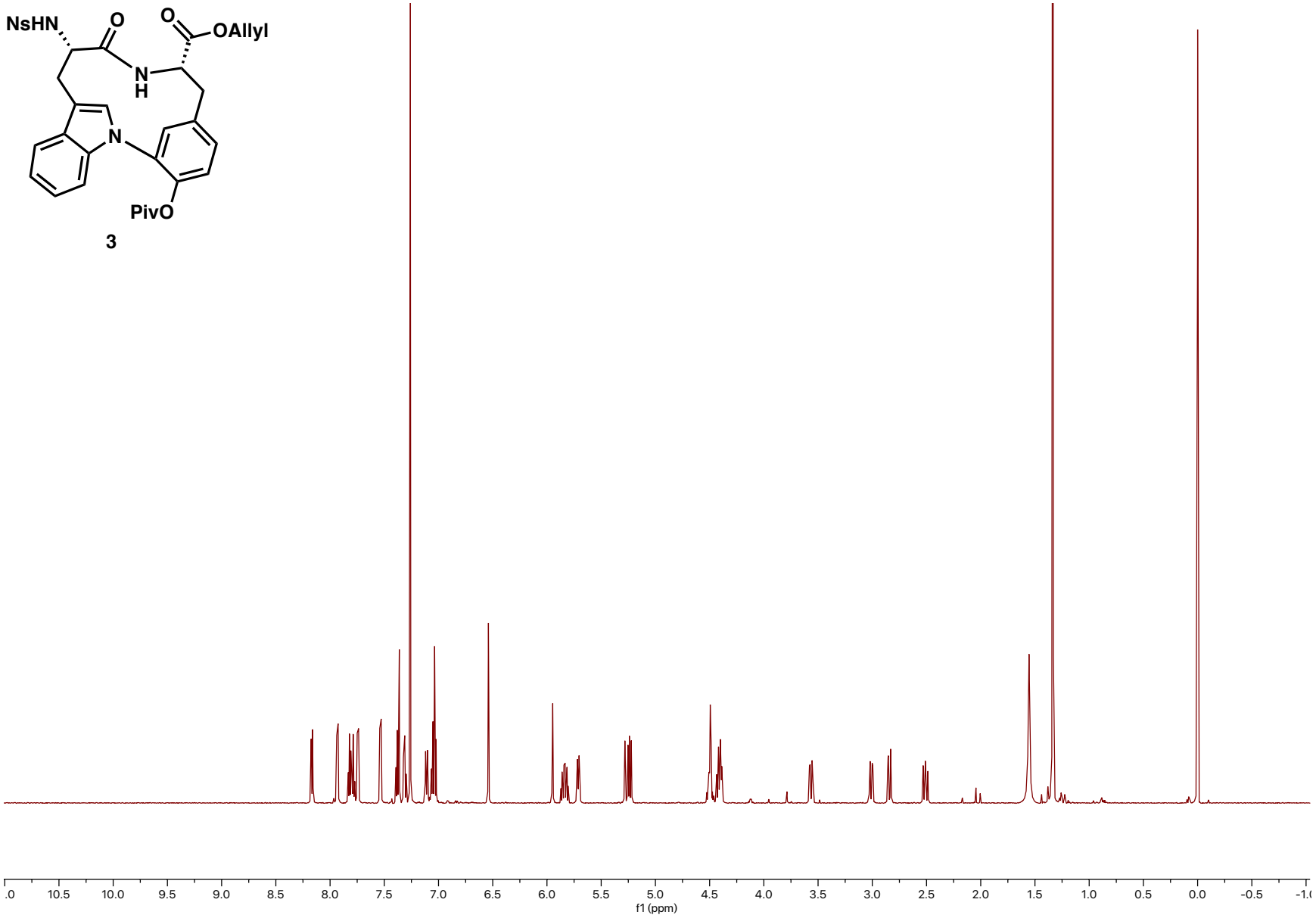
10
0
f1 (ppm)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O
OAllyl
N
H
N
PivO
3
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10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
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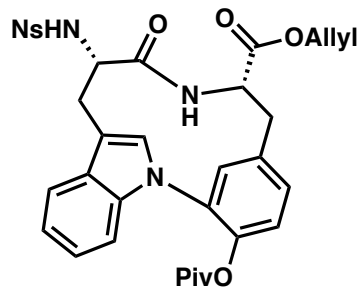
-0.5
f1 (ppm)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O
OAllyl
N
H
N
PivO

3

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40
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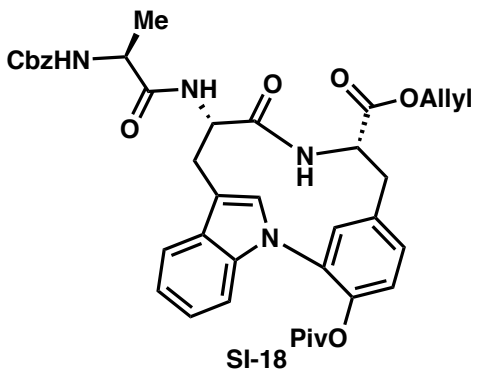
10
0
f1 (ppm)


Me
CbzHN
NH
O
O
O
OAllyl
N
H
N
PivO

Sl-18

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8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
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4.0
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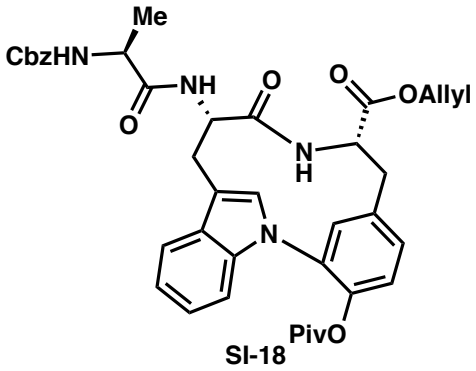
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)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CbzHN
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O
O
O
OAllyl
N
H
N
PivO

SI-18

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90
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70
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50
40
30
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10
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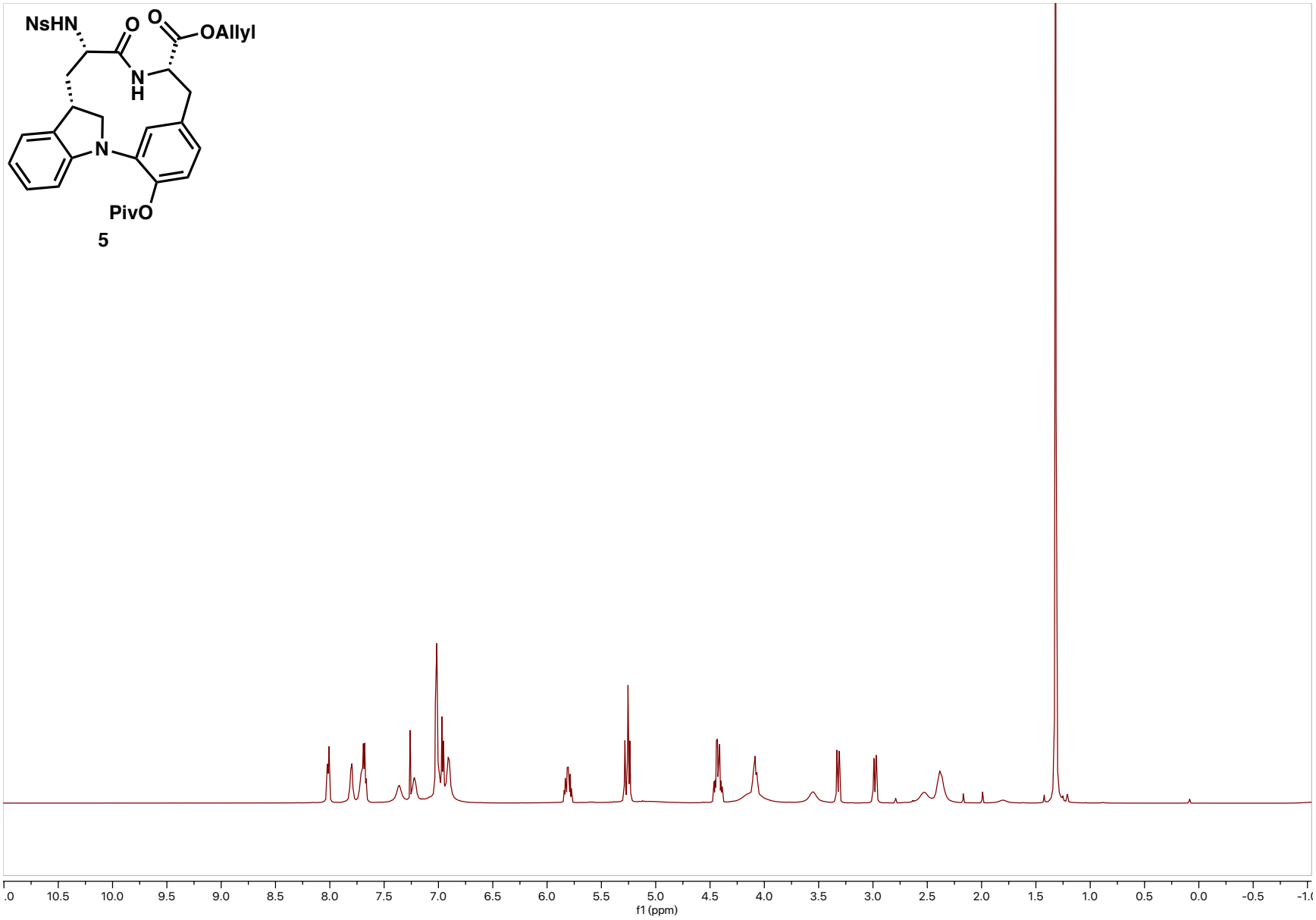
f1 (ppm)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OAllyl
N
H
N
PivO
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8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
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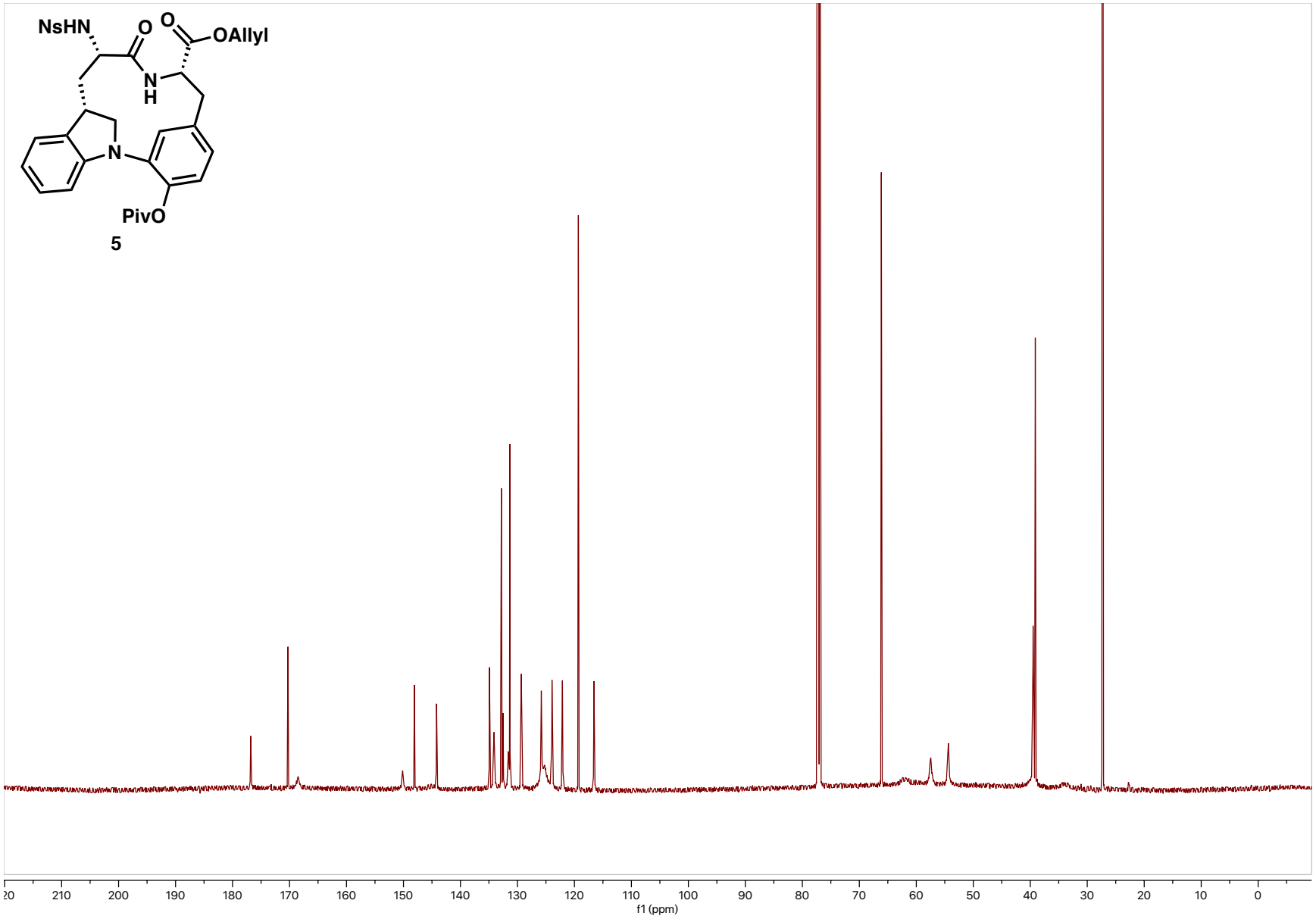
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)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O
OAllyl
N
H
N
PivO
5
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180
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120
110
100
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80
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50
40
30
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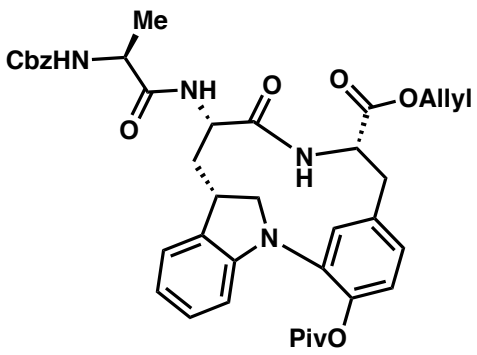
10
0
f1 (ppm)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O
OAllyl
N
H
N
PivO

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8.0
7.5
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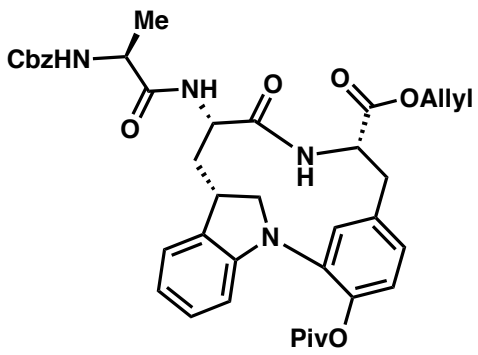
-0.5
f1 (ppm)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O
O
OAllyl
N
H
N
PivO

10

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180
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120
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100
90
80
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50
40
30
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10
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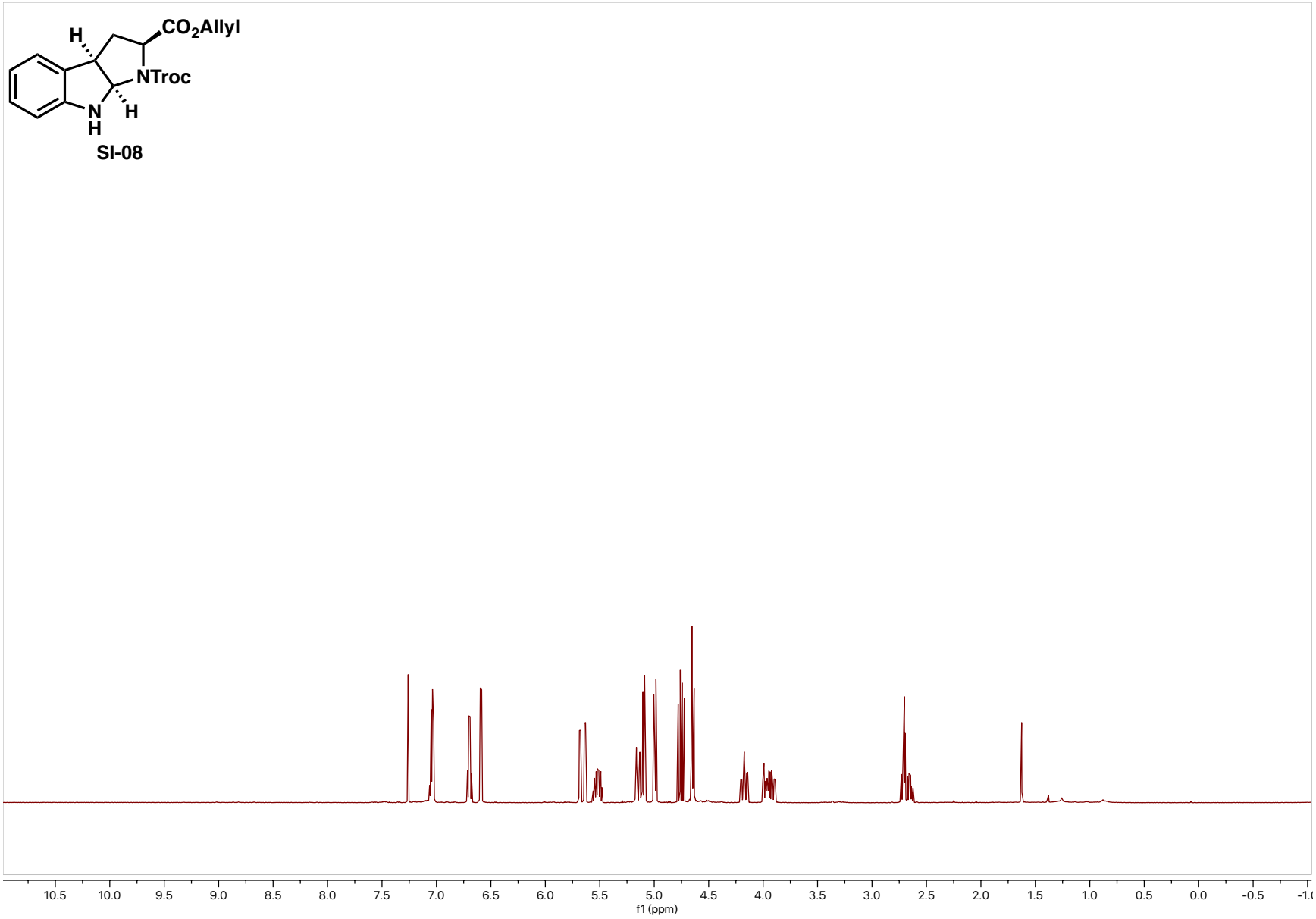
f1 (ppm)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CO2Allyl
NTroc
N
H
H
SI-08
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6.0
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5.0
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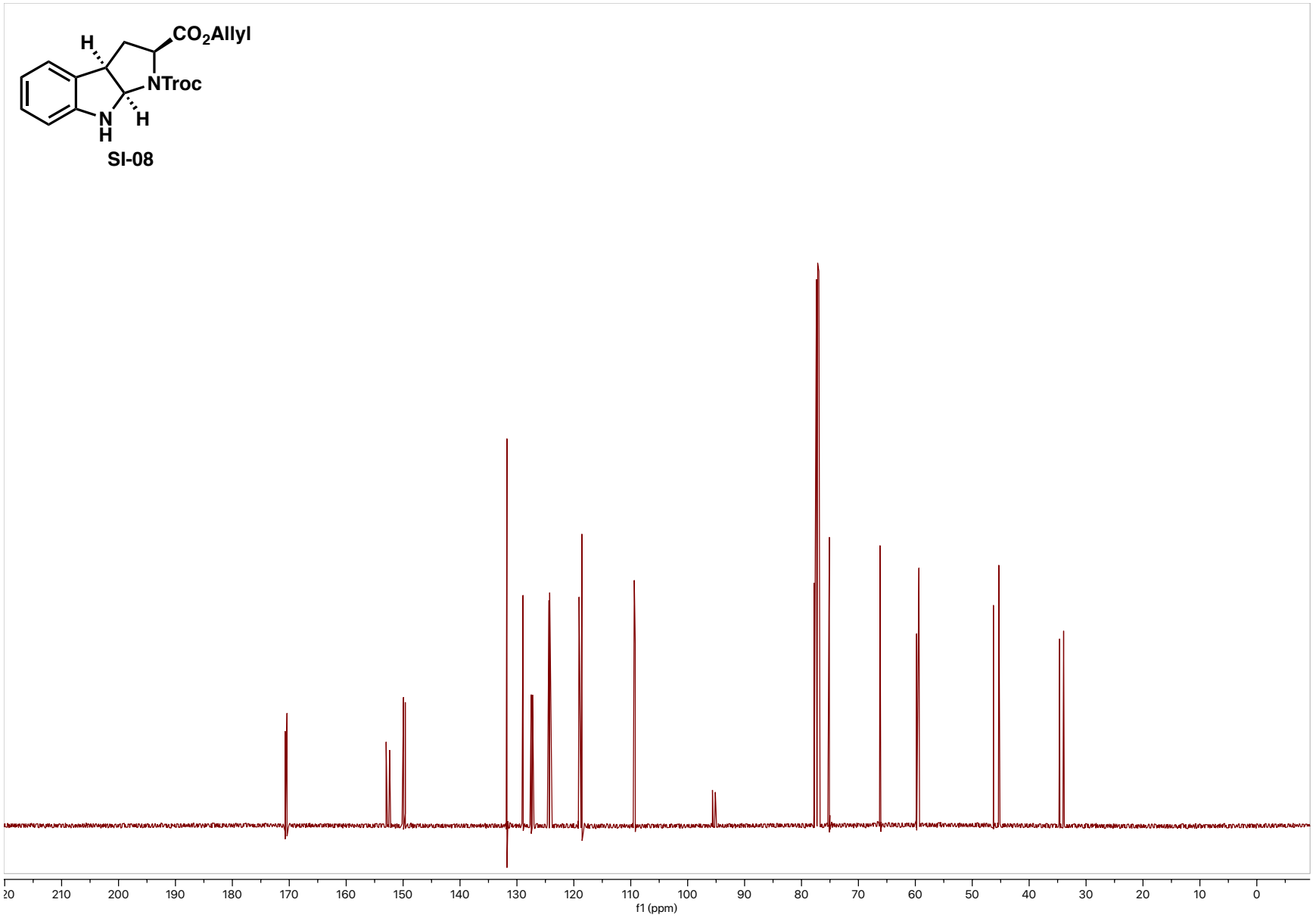
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)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CO2Allyl
NTroc
N
H
H
SI-08
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210
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110
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90
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50
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30
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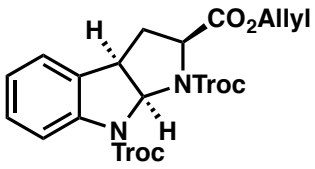
0
f1 (ppm)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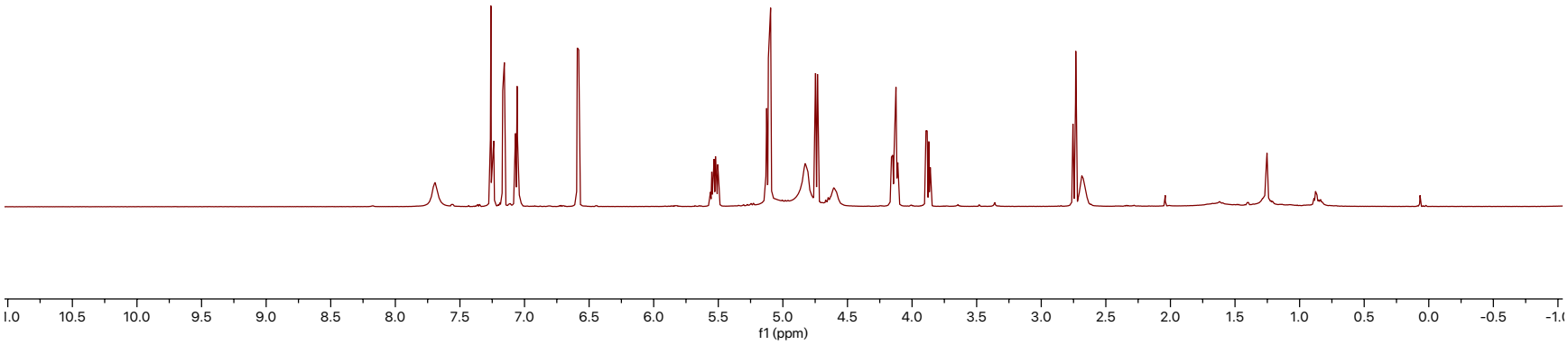
CO2Allyl
NTroc
N
H
Troc

SI-09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2.0
1.5
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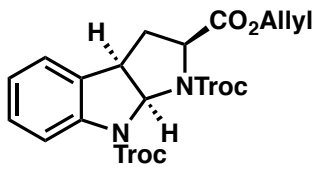
0.5
0.0
-0.5
f1 (ppm)


H
CO2Allyl
NTroc
N
H
Troc

SI-09

210
200
190
180
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160
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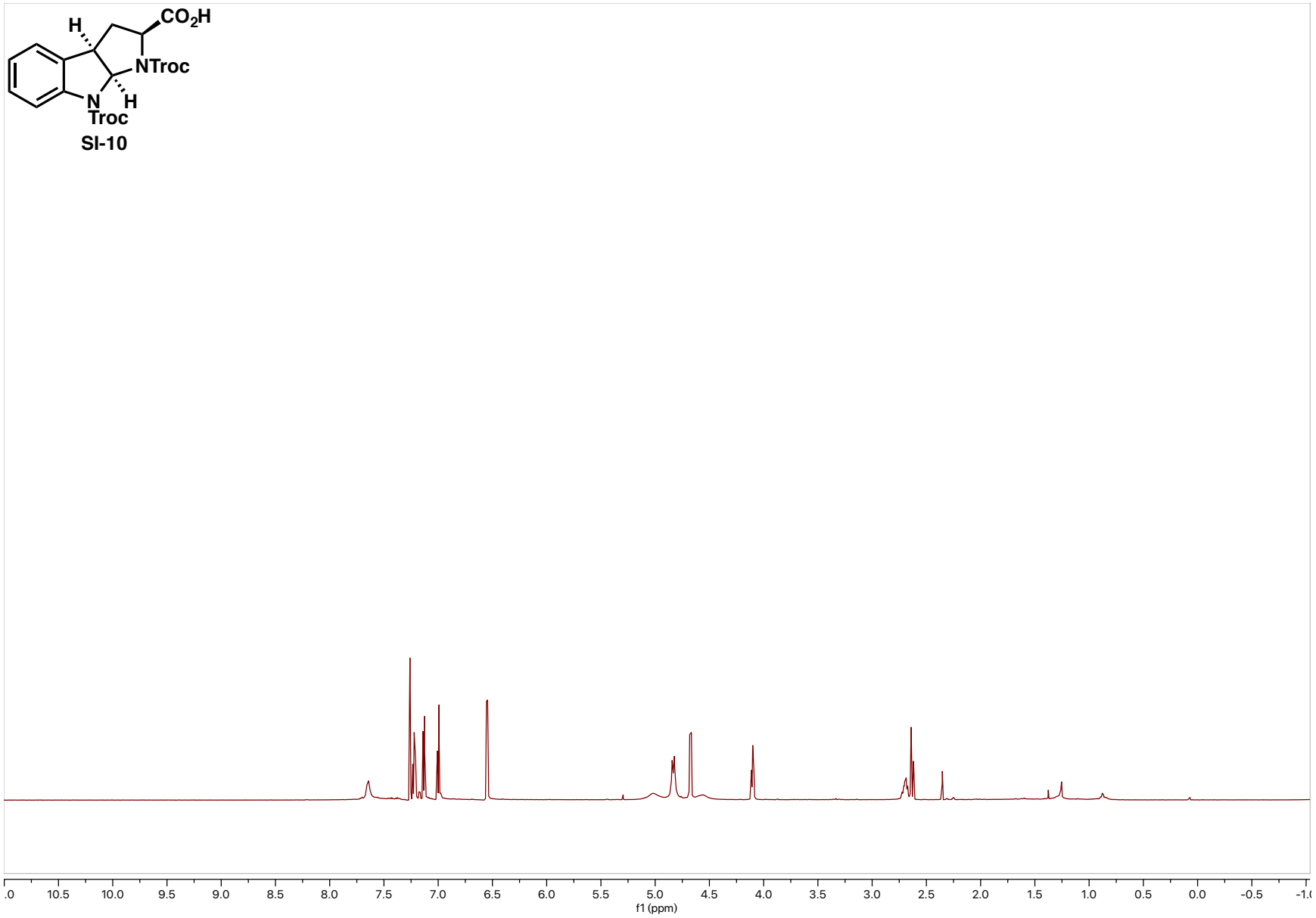
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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NTroc
N
H
Troc
SI-10
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7.0
6.5
6.0
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5.0
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0.5
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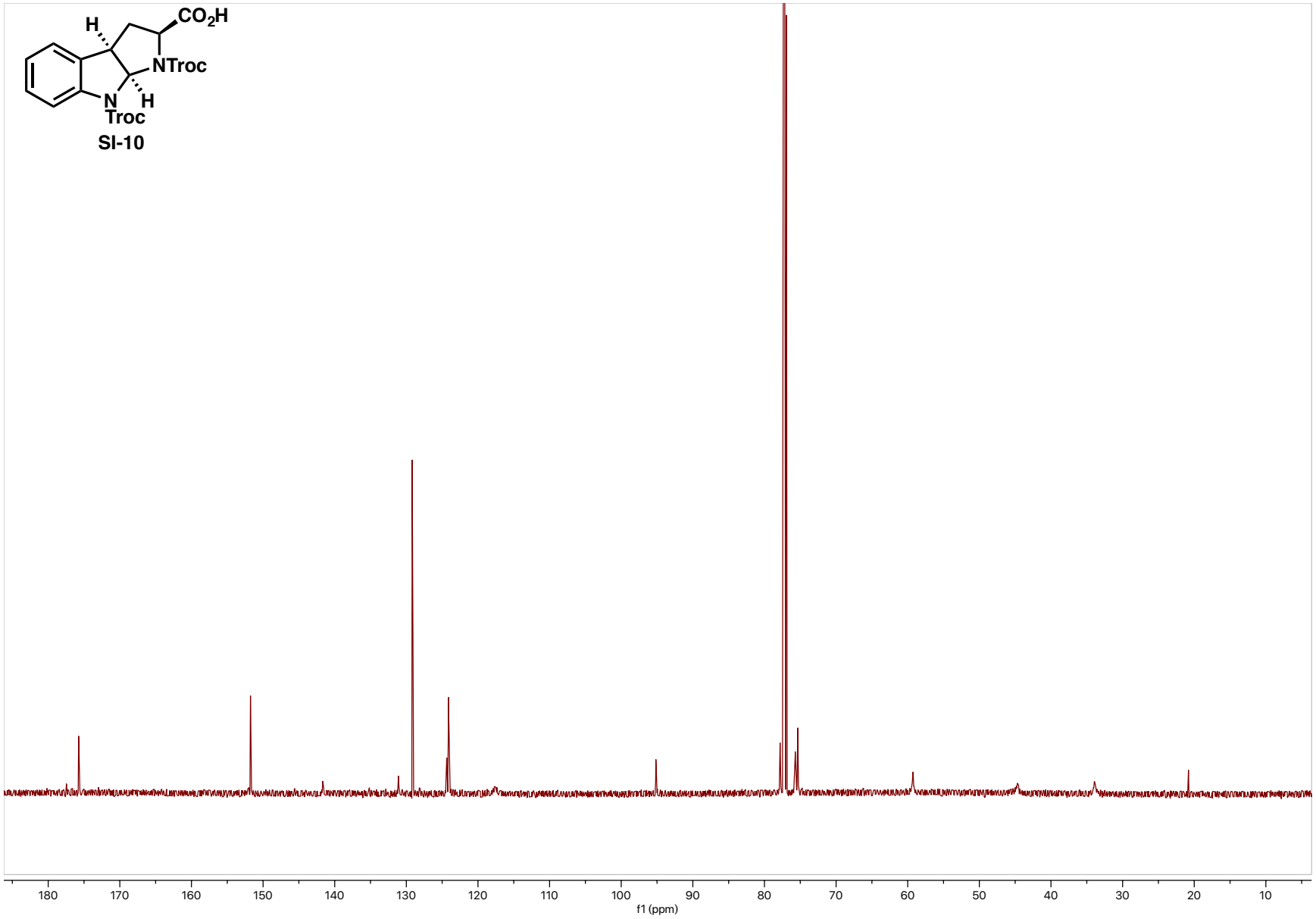
-0.5
f1 (ppm)


H
CO2H
NTroc
N
H
Troc
SI-10
180
170
160
150
140
130
120
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100
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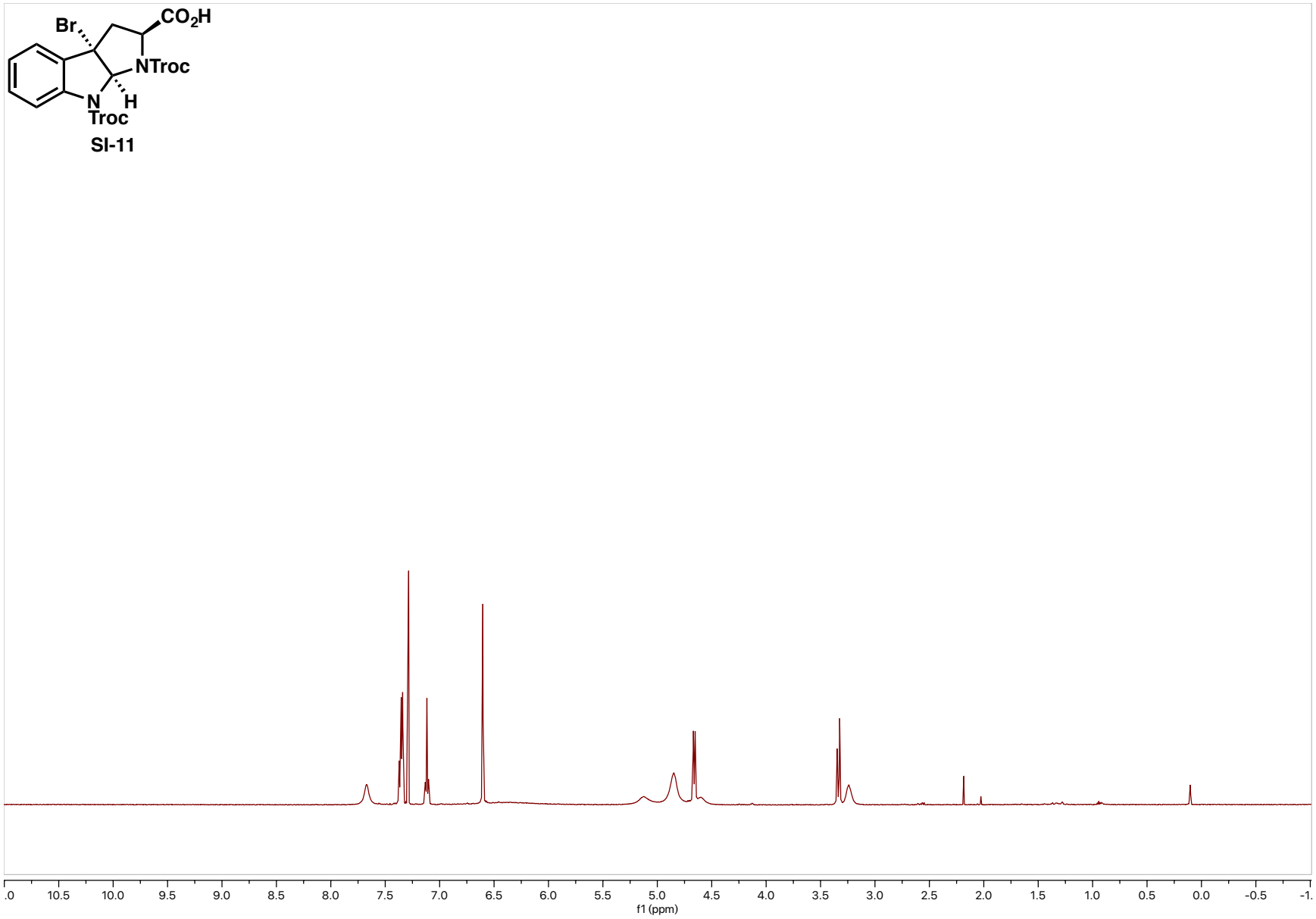
80
70
60
50
40
30
20
10
f1 (ppm)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CO2H
NTroc
N
H
Troc
SI-11
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9.0
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8.0
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7.0
6.5
6.0
5.5
5.0
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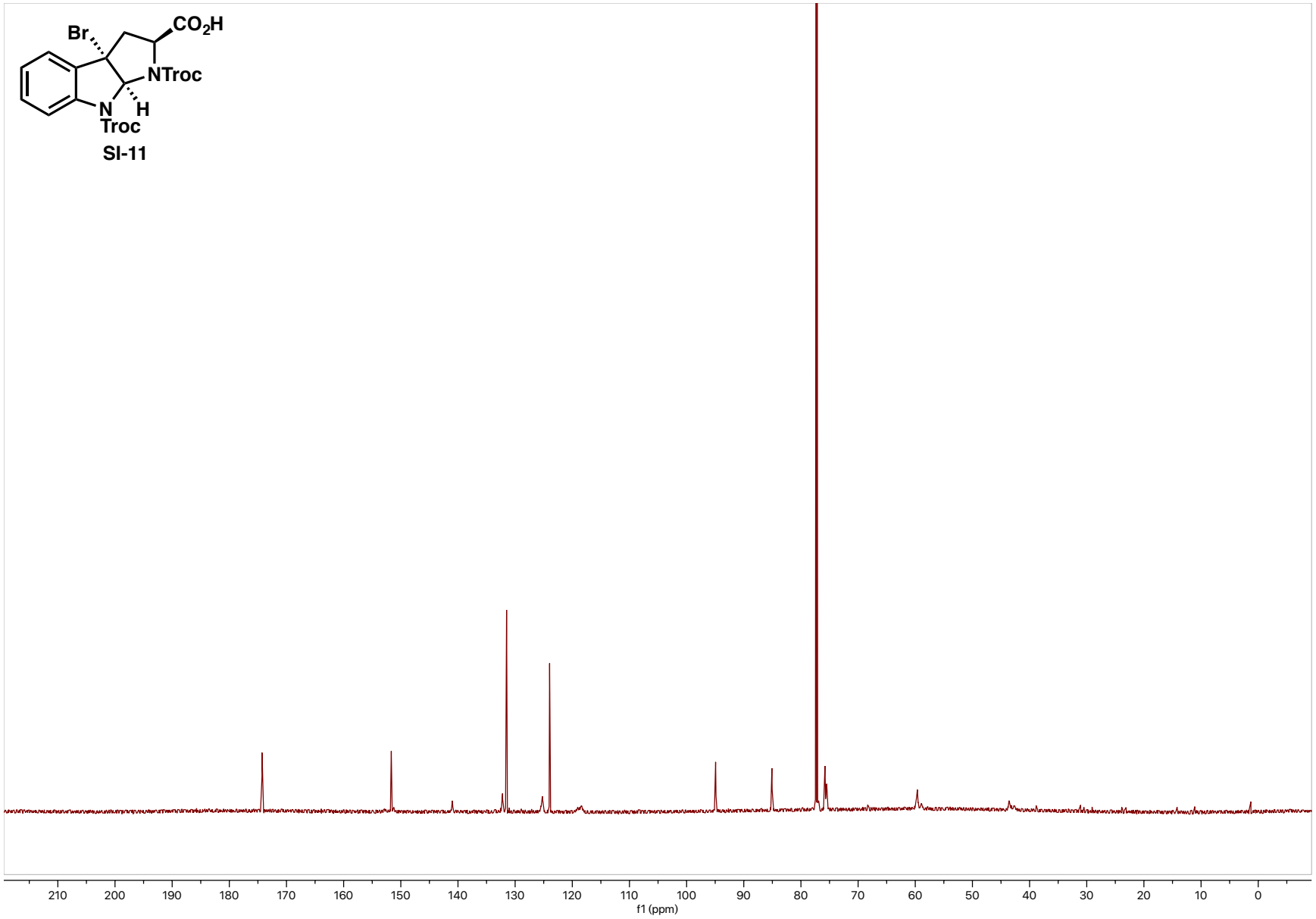
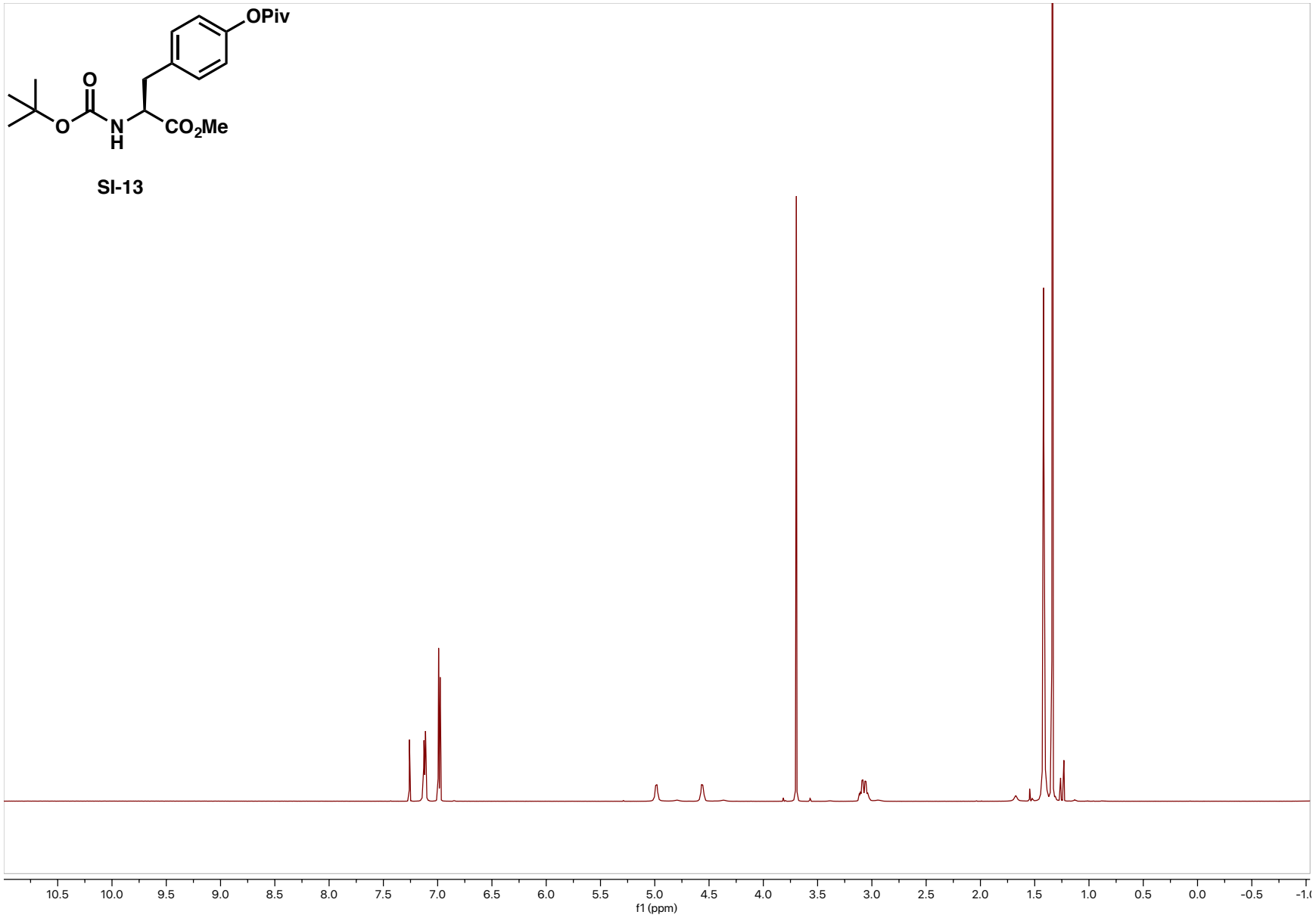
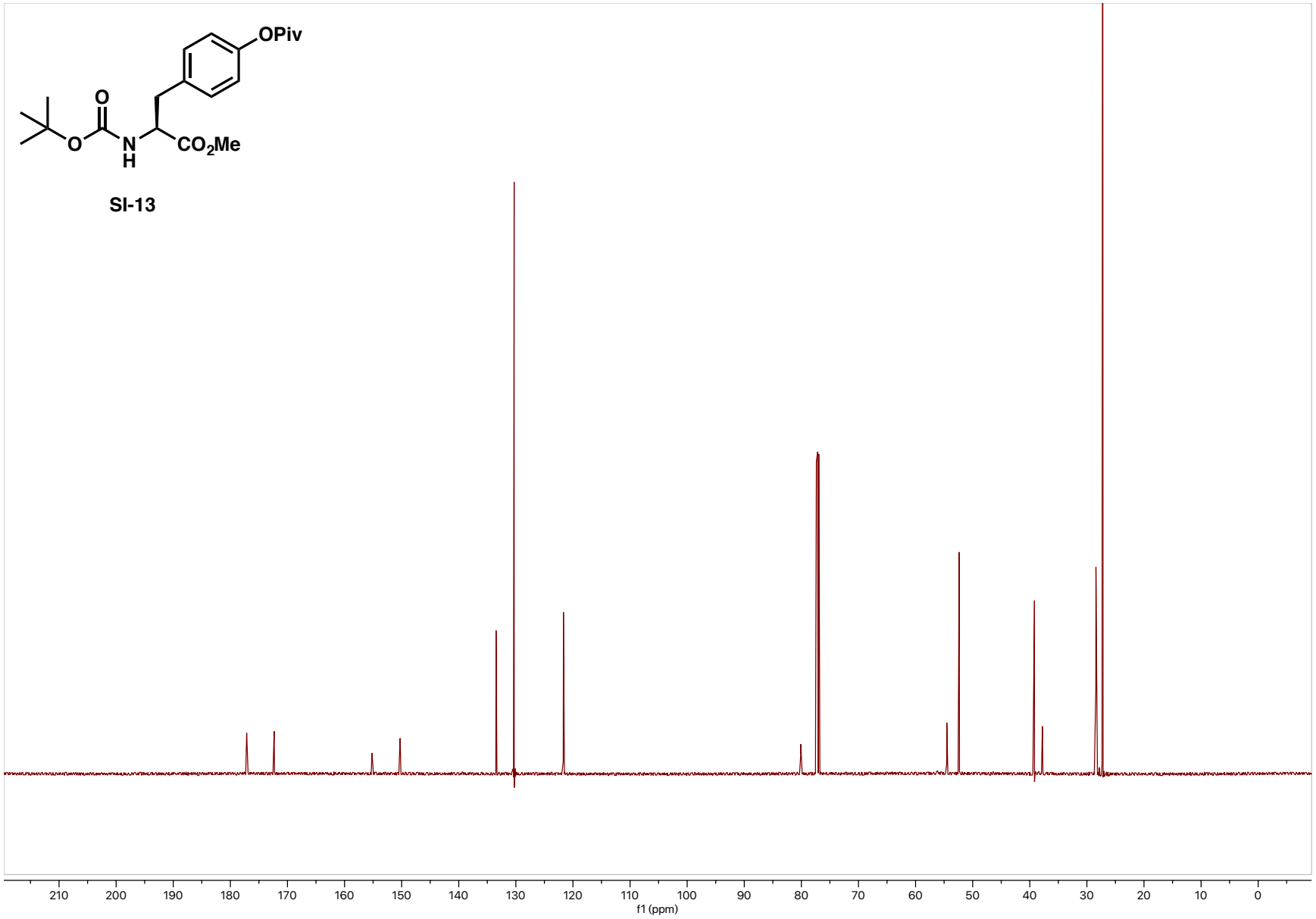
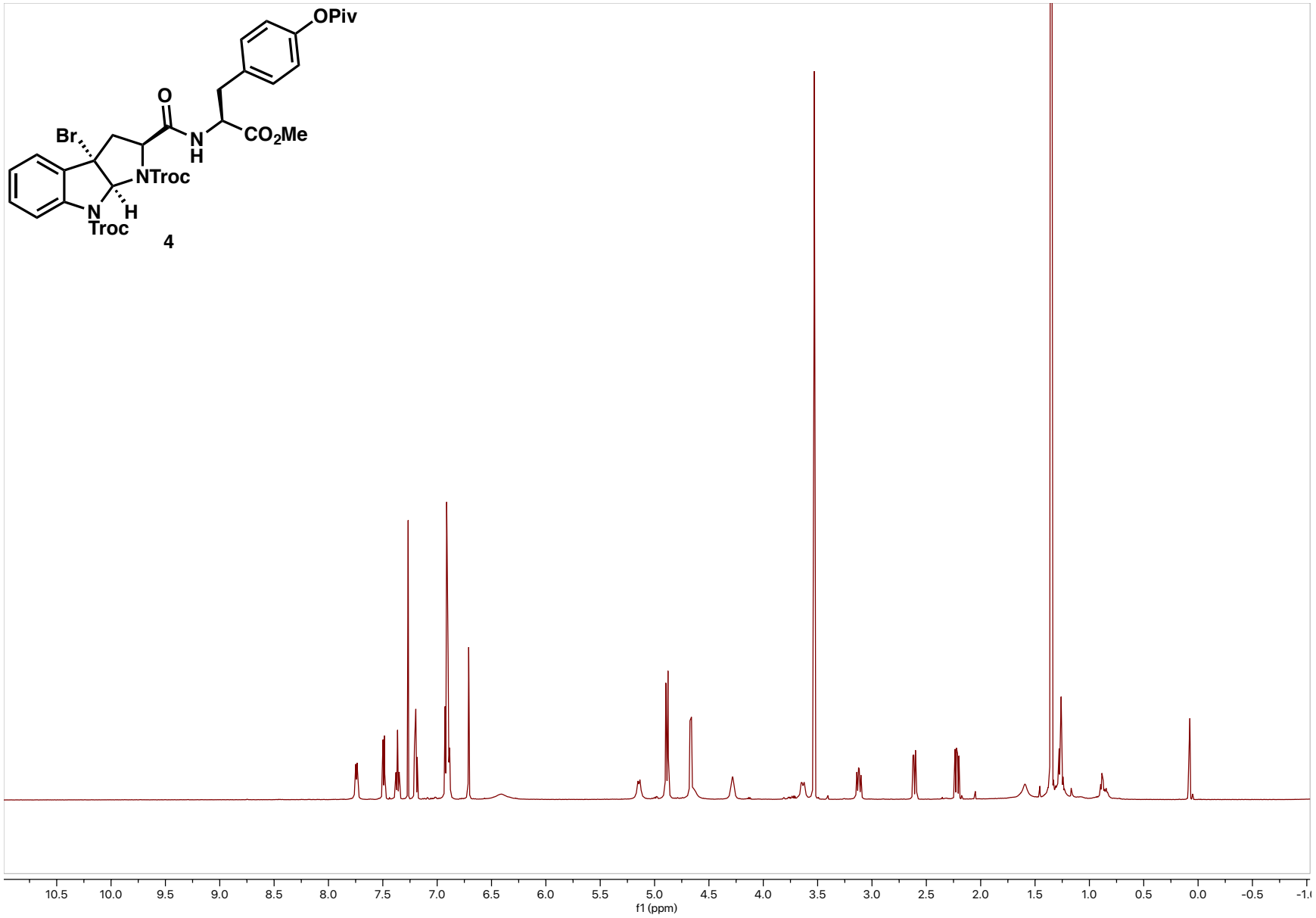
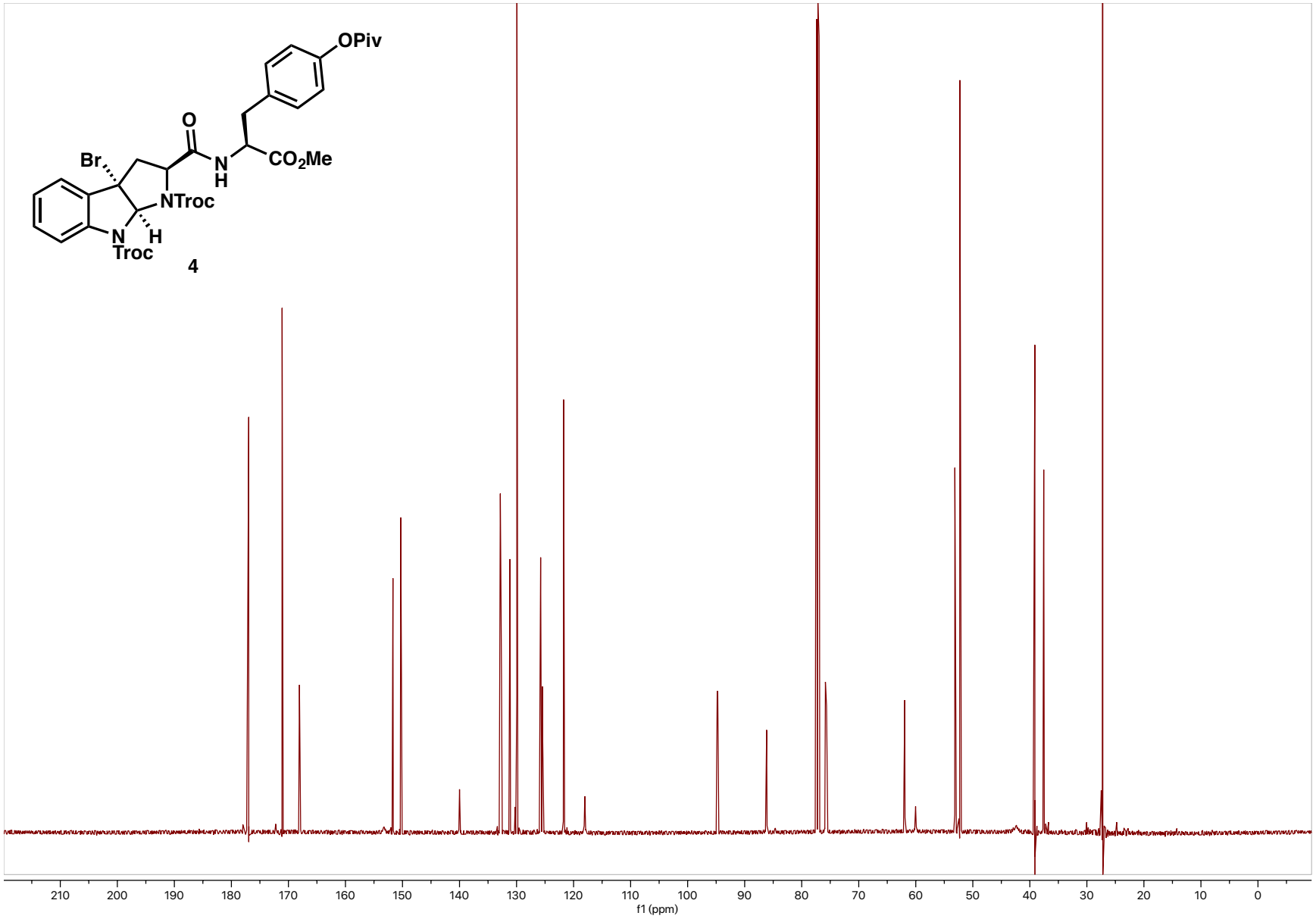
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

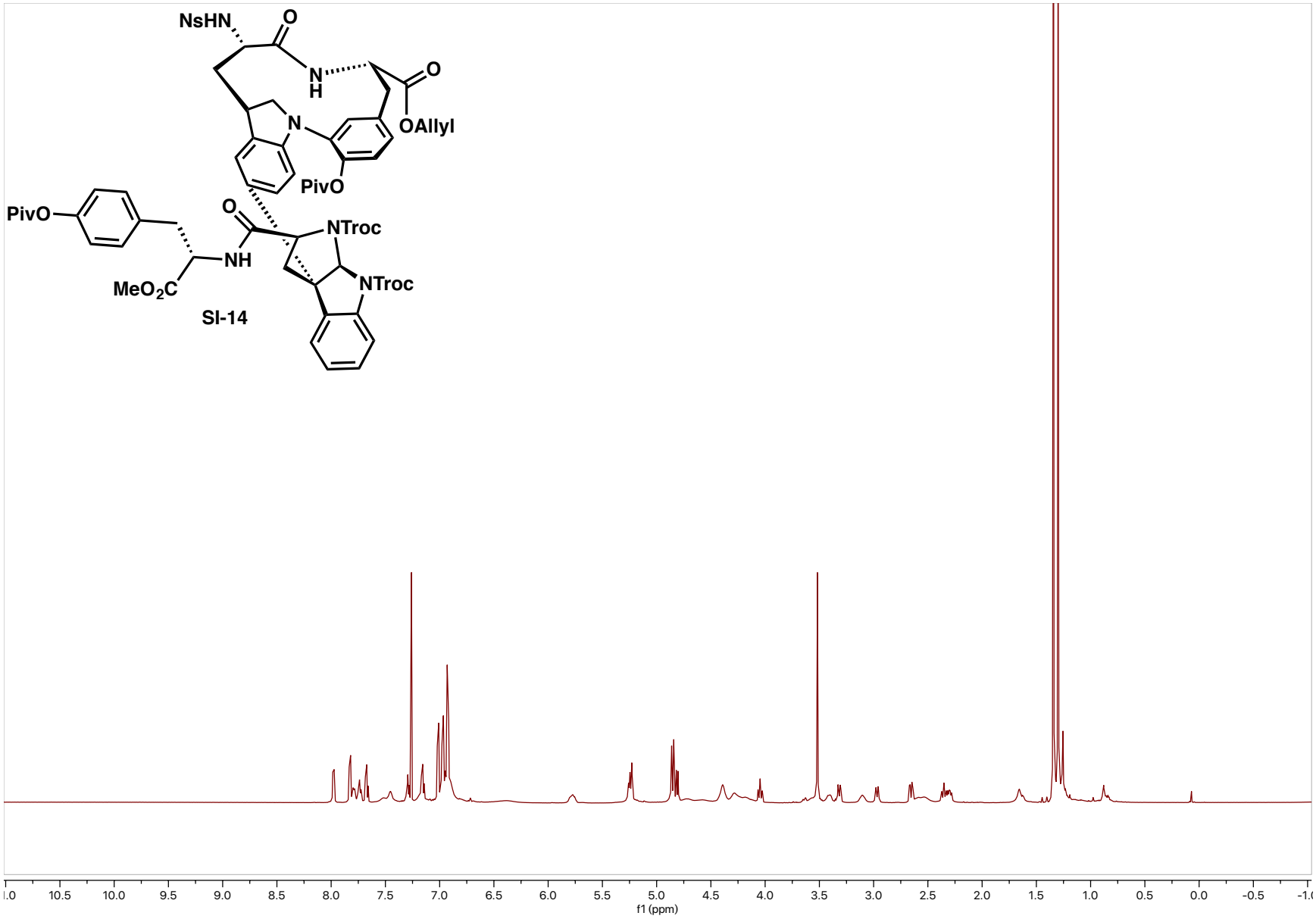

Br
CO2H
NTroc
N
H
Troc
SI-11
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

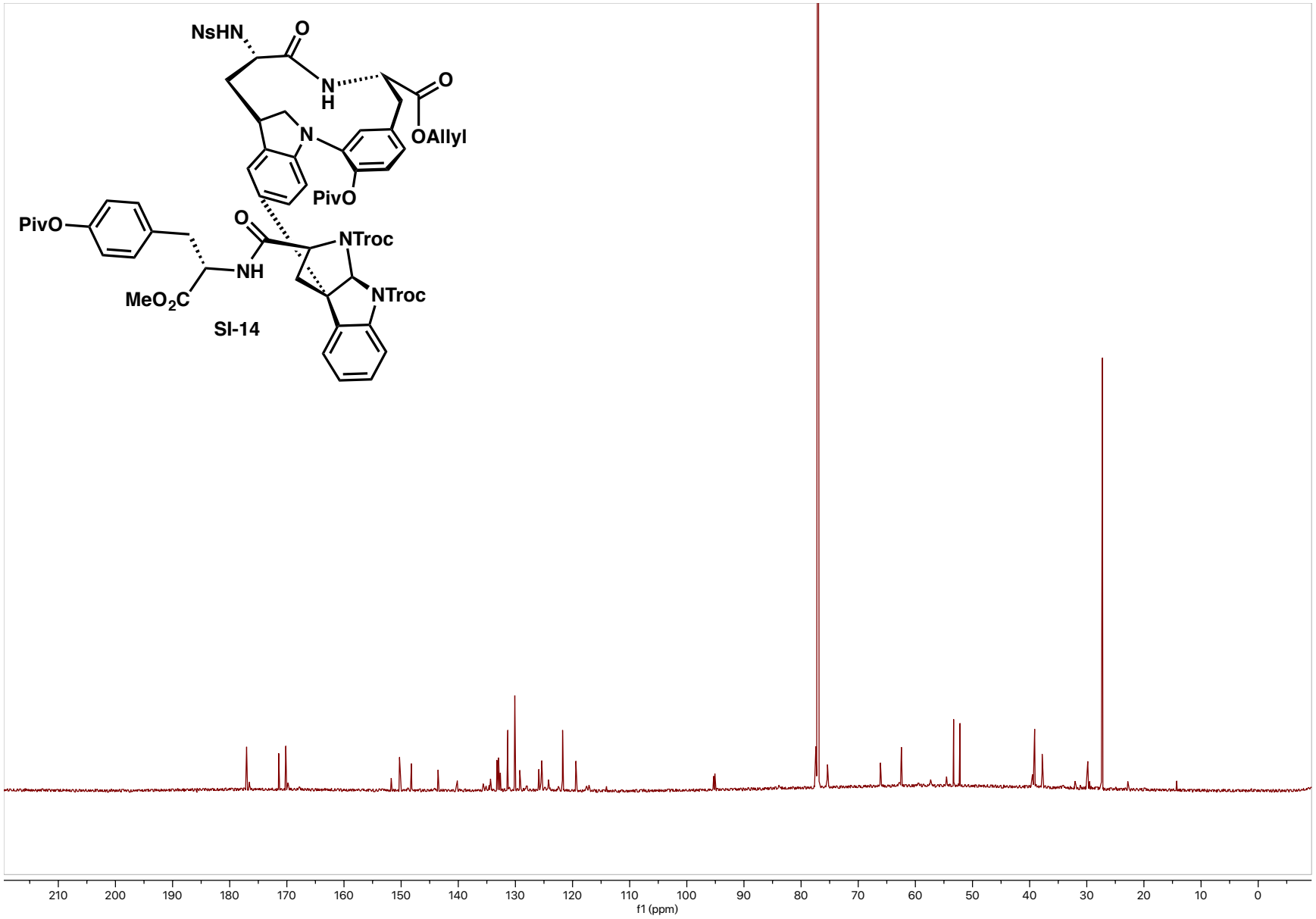

OPiv
O
O
N
H
CO2Me
SI-13
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

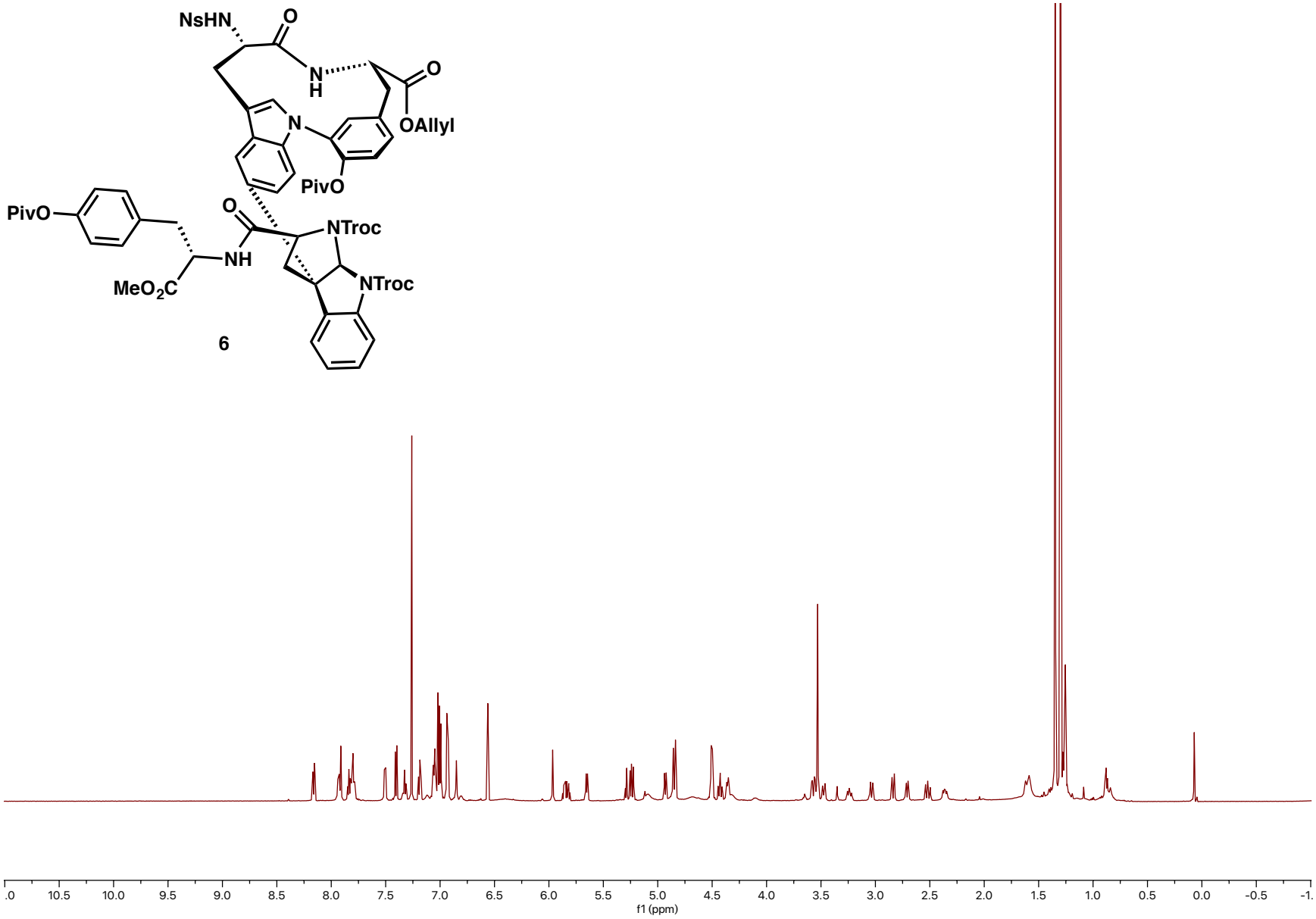

OPiv
O
O
N
H
CO2Me
SI-13
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

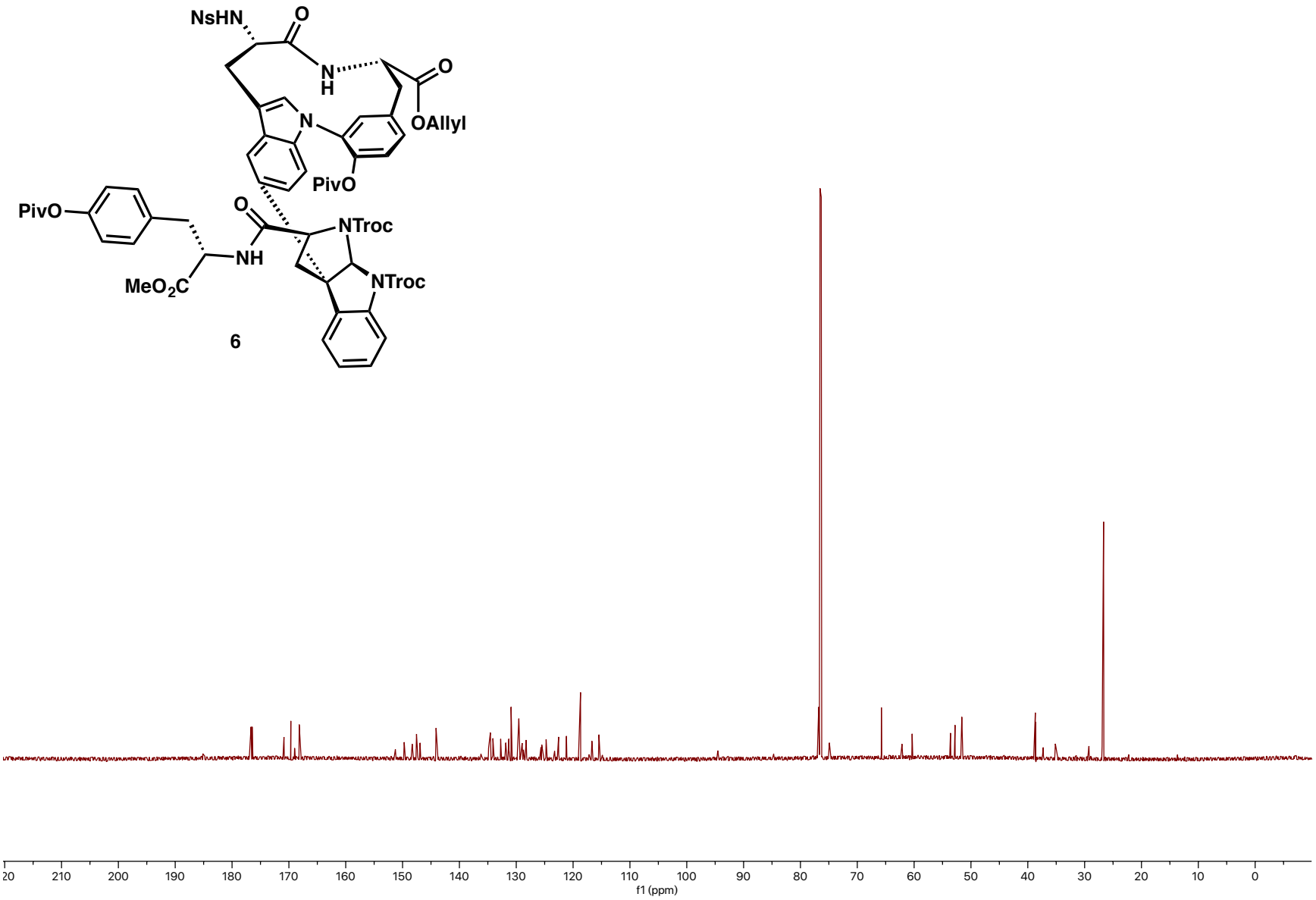

OPiv
O
Br
N
H
CO2Me
NTroc
N
H
Troc
4
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

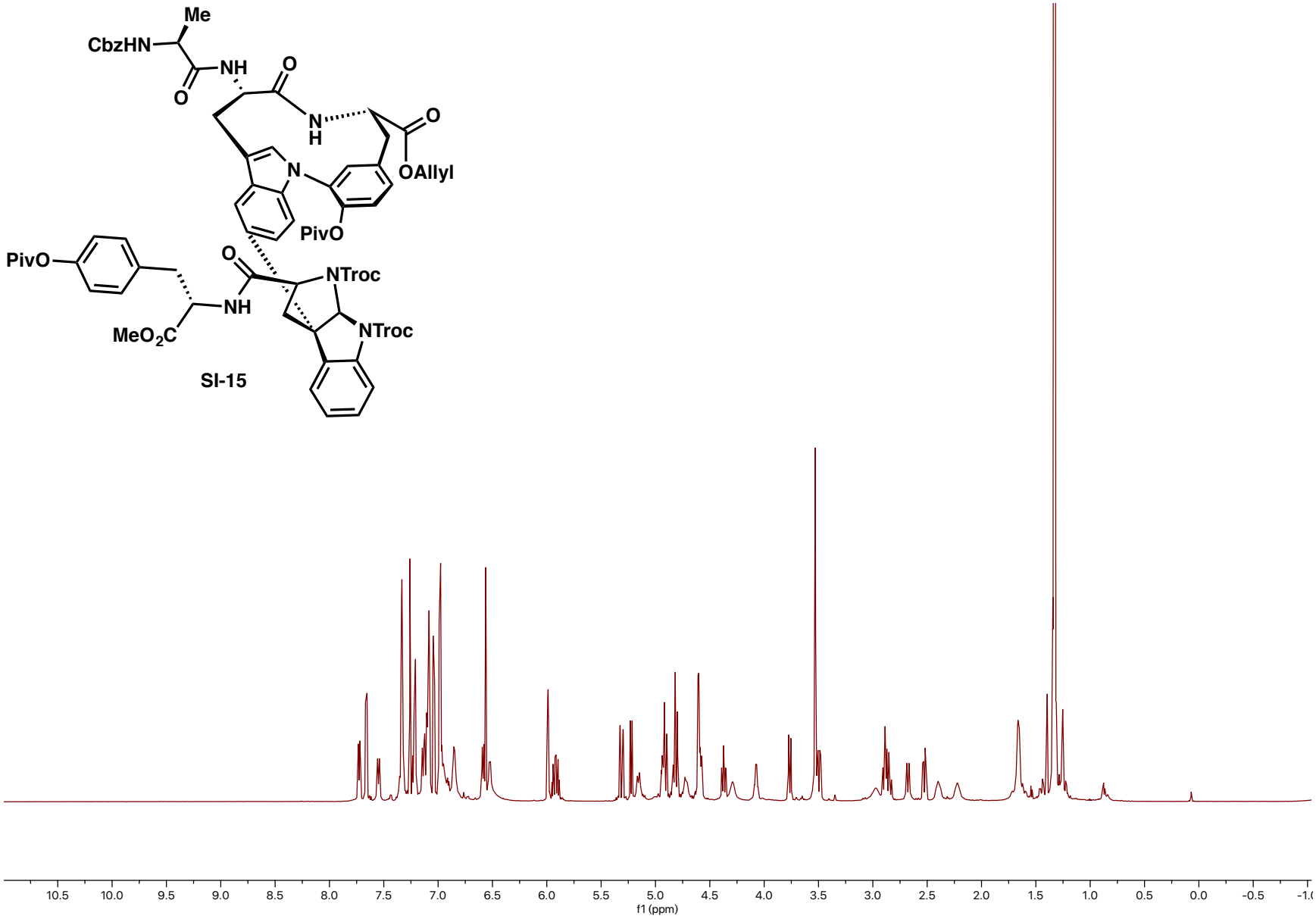

OPiv
O
Br
N
H
CO2Me
NTroc
N
H
Troc
4
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


NsHN
O
N
H
O
OAllyl
N
PivO
PivO
O
NTroc
NH
NTroc
MeO2C
SI-14
10.5
10.0
9.5
9.0
8.5
8.0
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
0.5
0.0
-0.5
f1 (ppm)

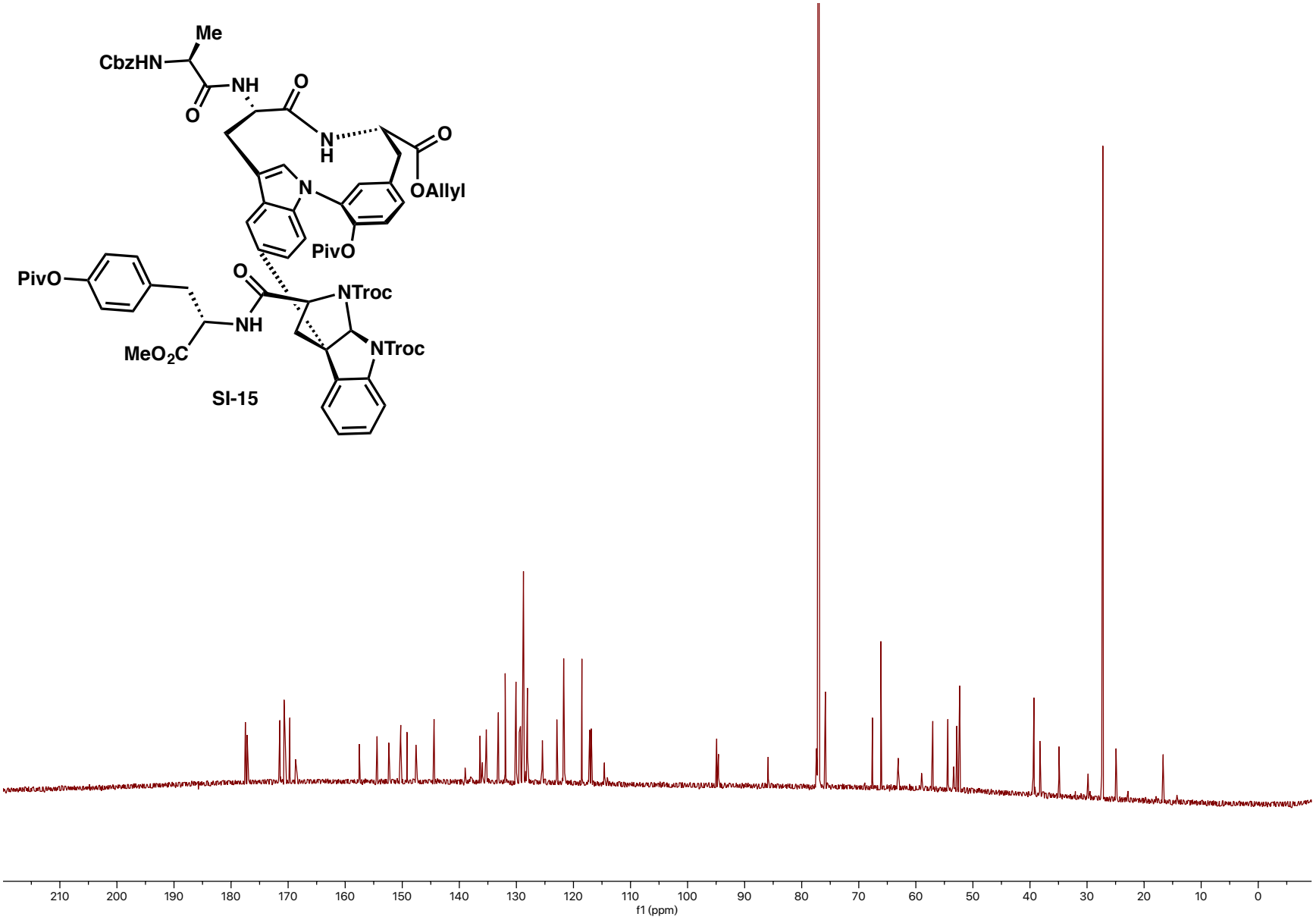

NsHN
O
N
H
O
OAllyl
N
PivO
PivO
O
NTroc
NH
NTroc
MeO2C
SI-14
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

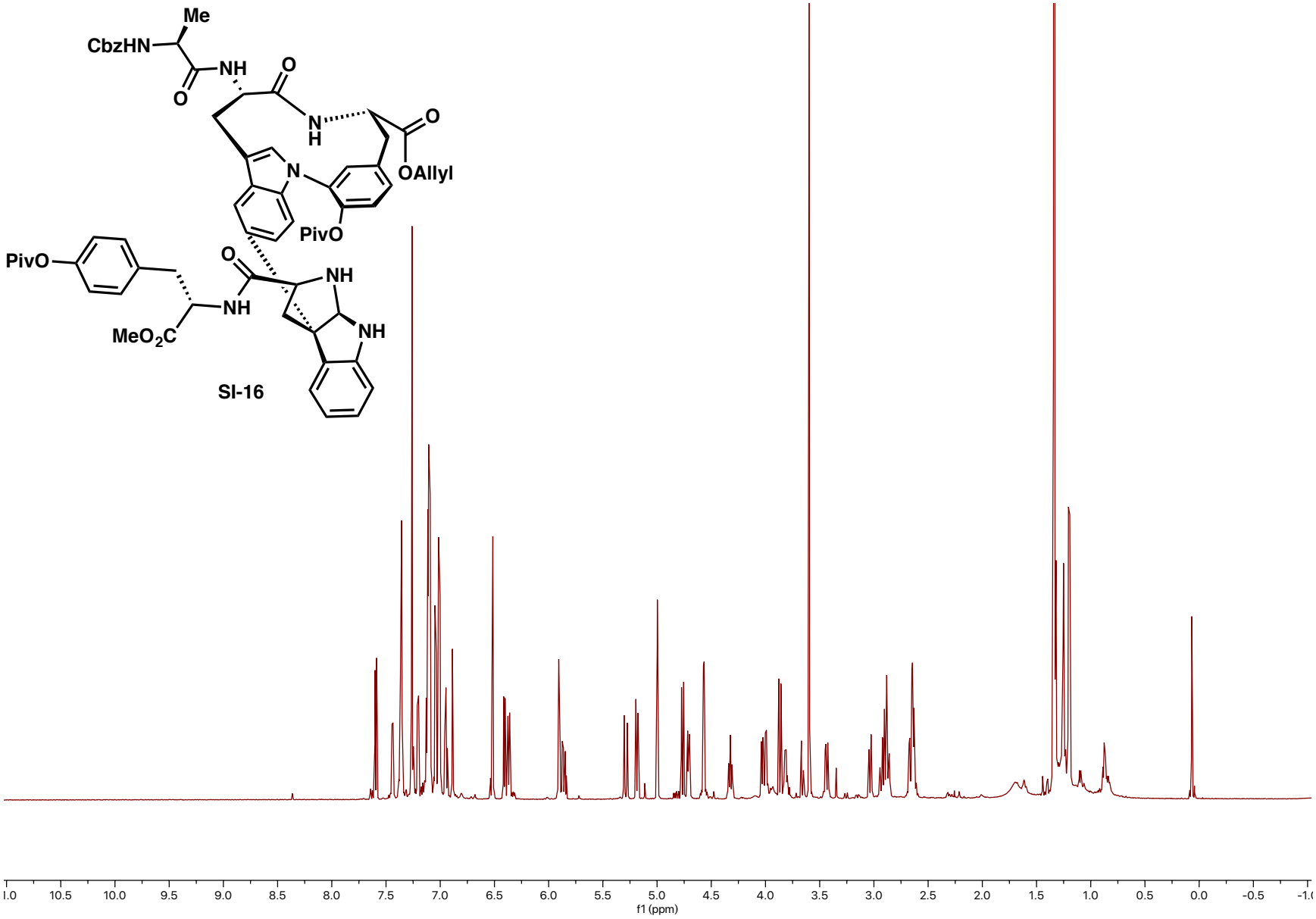

NsHN
O
N
H
O
OAllyl
N
PivO
PivO
O
NTroc
NH
NTroc
MeO2C
6
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

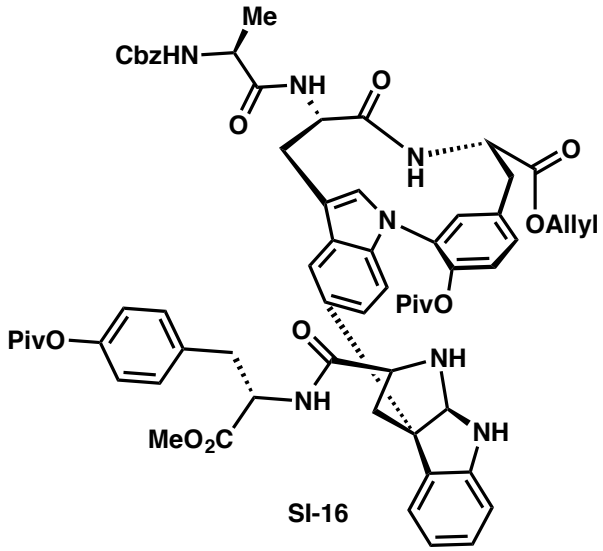

NsHN
O
N
H
O
OAllyl
N
PivO
PivO
O
NTroc
NH
NTroc
MeO2C
6
20
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


Me
CbzHN
NH
O
O
N
H
O
OAllyl
N
PivO
PivO
O
NTroc
NH
NTroc
MeO2C
10.5
10.0
9.5
9.0
8.5
8.0
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
0.5
0.0
-0.5
f1 (ppm)

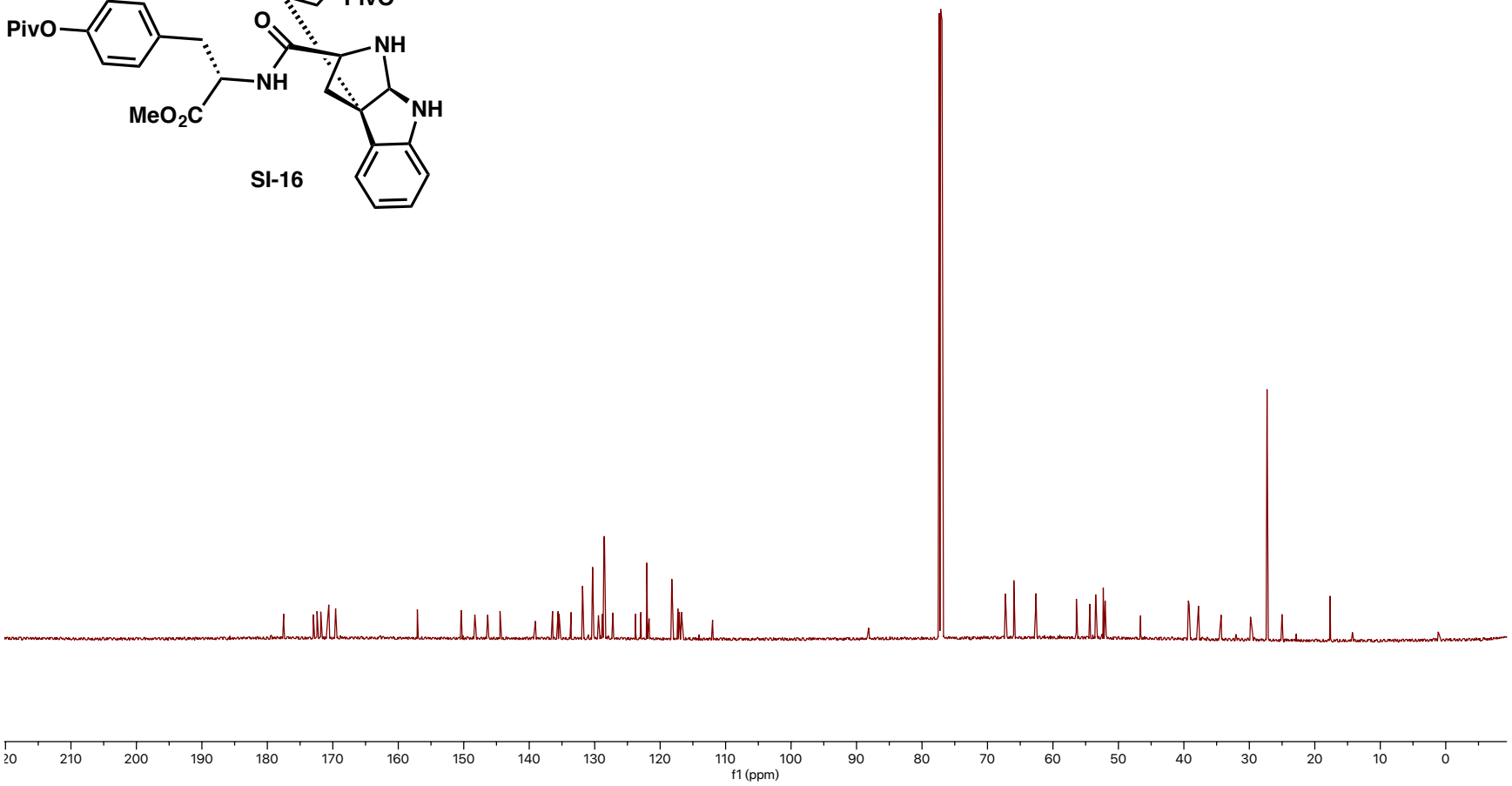
SI-15

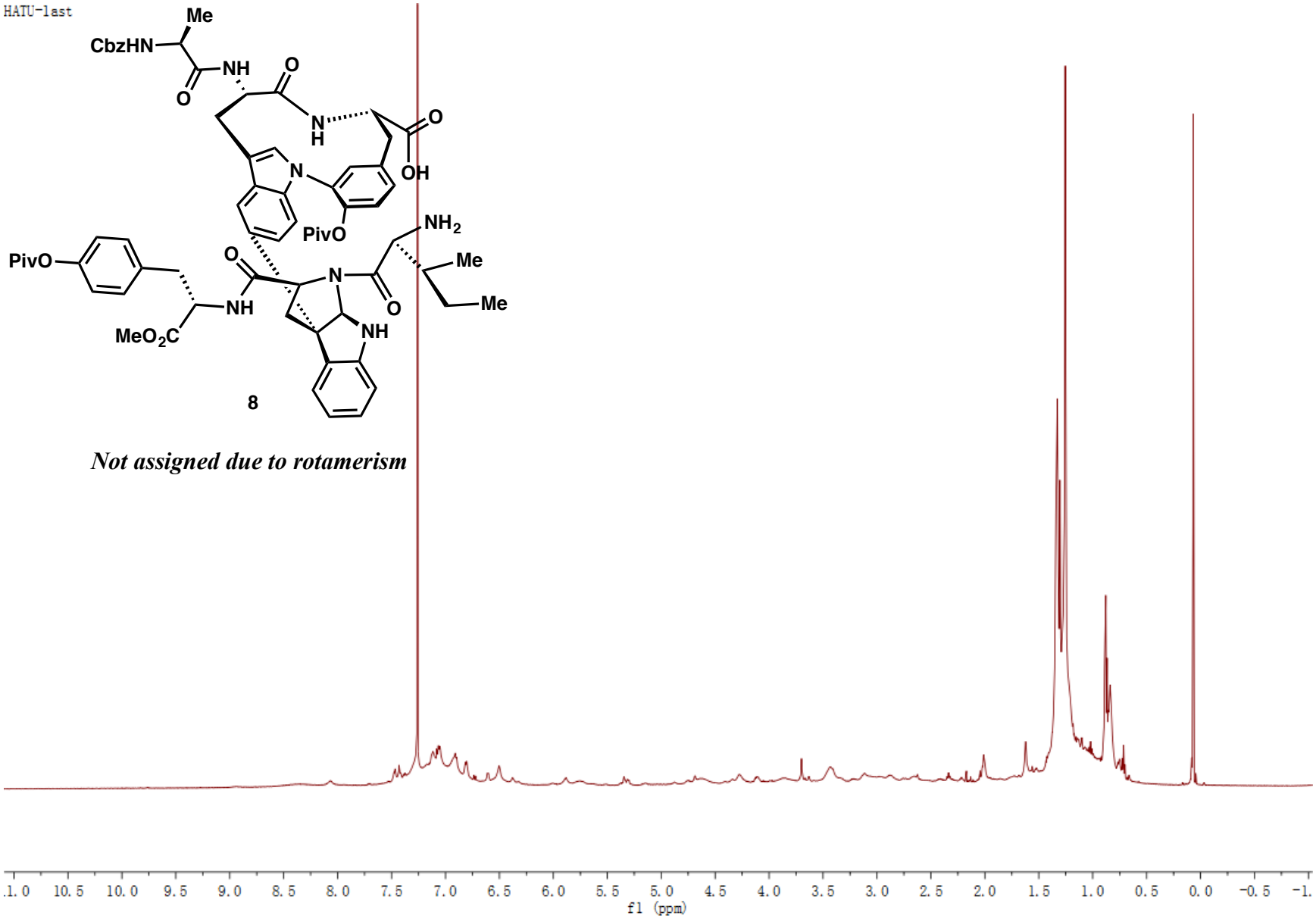

Me
CbzHN
NH
O
O
N
H
O
OAllyl
N
PivO
PivO
O
NTroc
NH
NTroc
MeO2C
SI-15
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


Me
CbzHN
NH
O
O
N
H
O
OAllyl
N
PivO
PivO
O
NH
NH
NH
MeO2C
SI-16
1.0
10.5
10.0
9.5
9.0
8.5
8.0
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
0.5
0.0
-0.5
f1 (ppm)

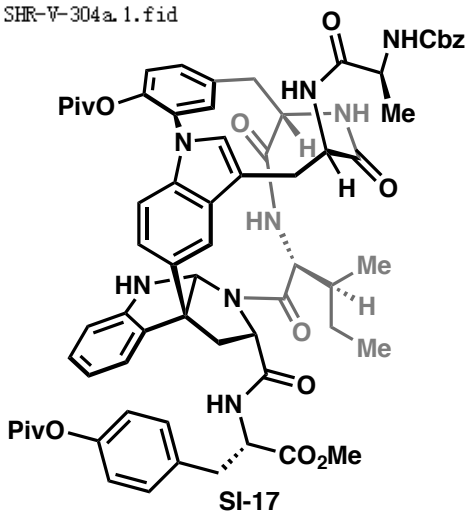

Me
CbzHN
NH
O
O
N
H
O
OAllyl
N
PivO
PivO
O
NH
NH
NH
MeO2C

SI-16


220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

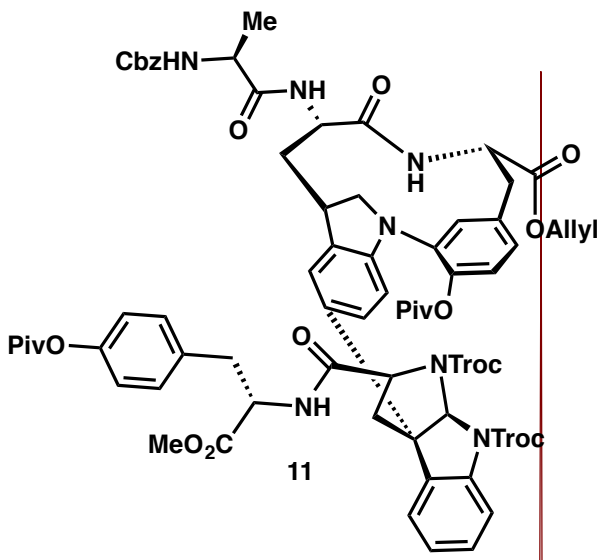

HATU-last
Me
CbzHN
NH
O
O
N
H
O
OH
N
PivO
NH2
PivO
O
N
Me
NH
O
Me
MeO2C
NH
8
Not assigned due to rotamerism
f1 (ppm)

SHR-V-304a.1.fid



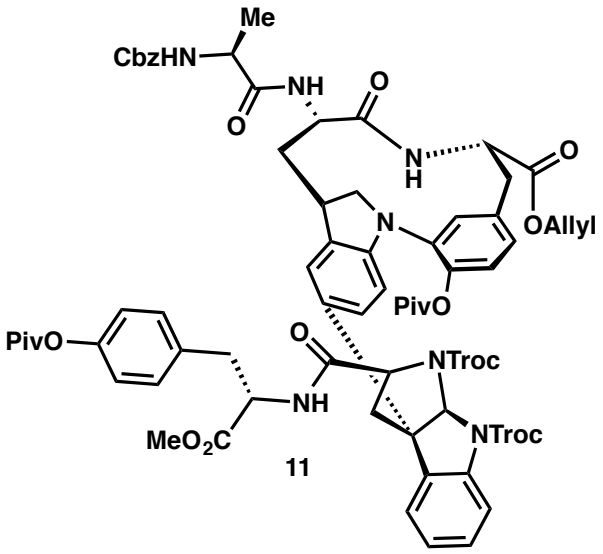
SI-17

Not assigned due to rotamerism



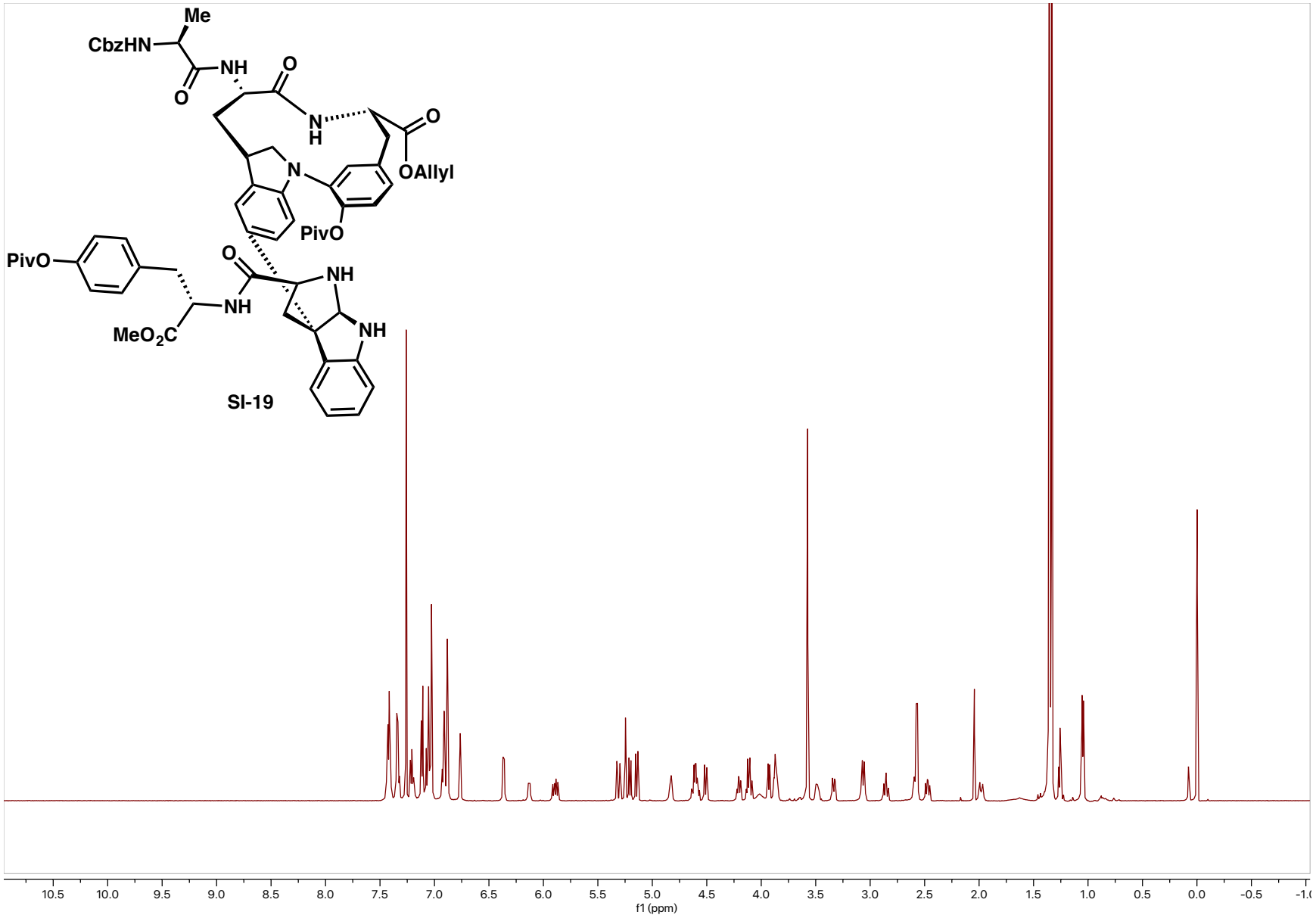
11

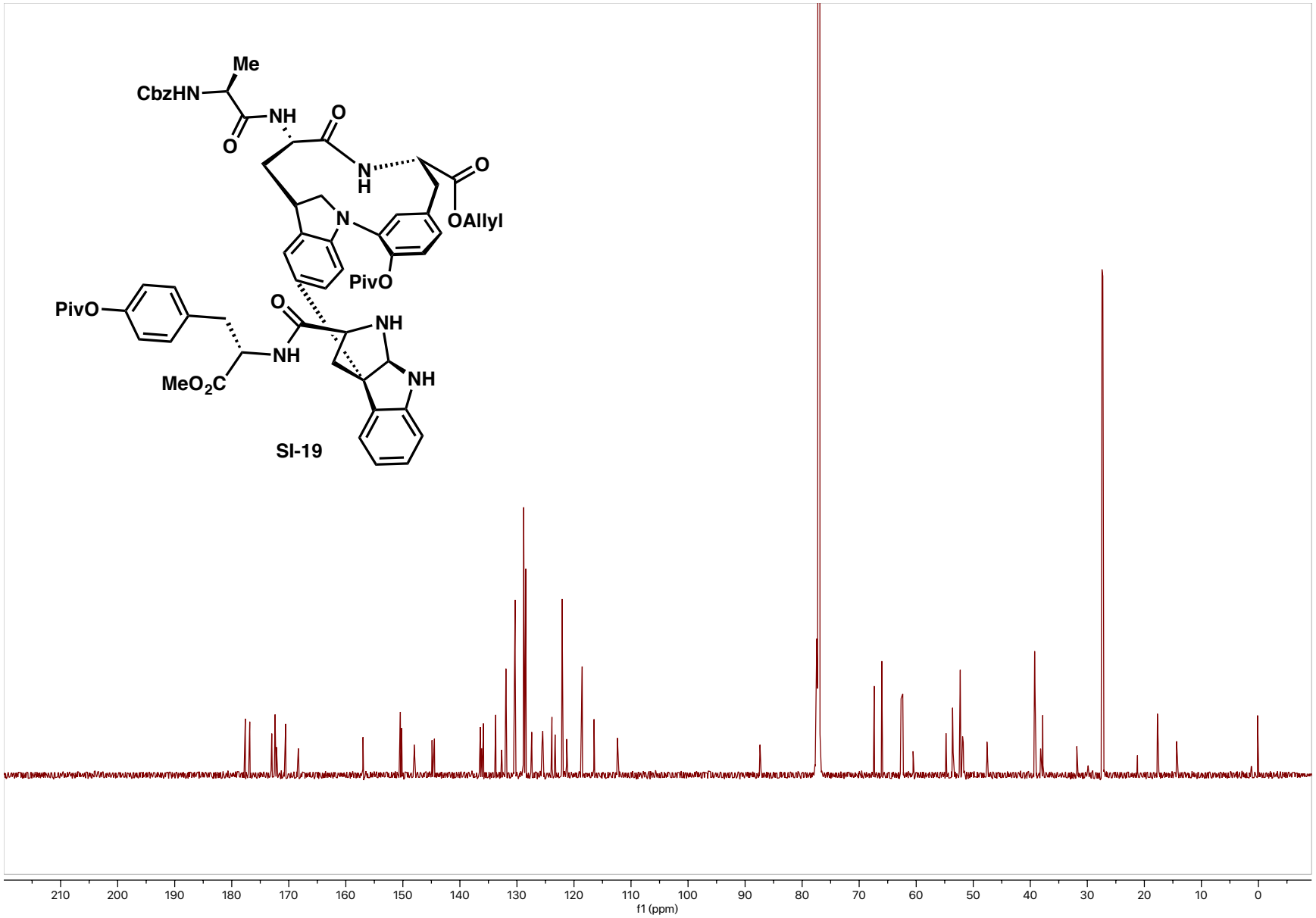
Not assigned due to rotamerism

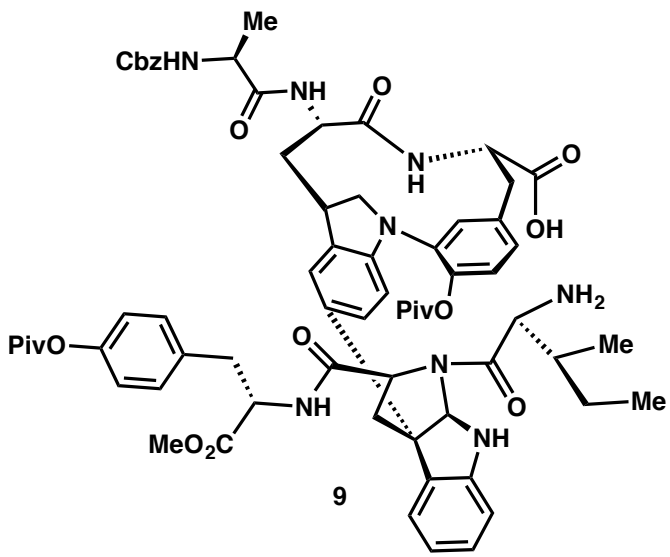


11

Not assigned due to rotamerism

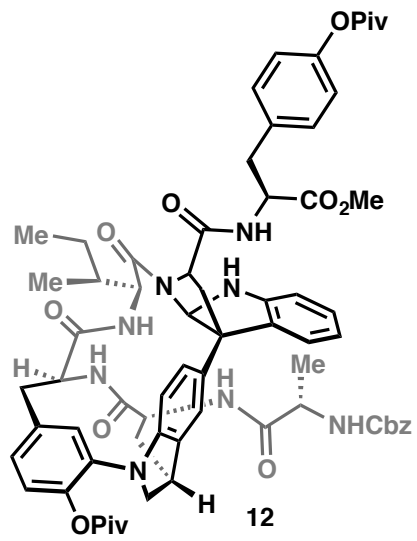

Me
CbzHN
NH
O
O
N
H
O
OAllyl
N
PivO
PivO
O
NH
NH
NH
MeO2C
SI-19
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)


Me
CbzHN
NH
O
O
N
H
O
OAllyl
N
PivO
PivO
O
NH
NH
NH
MeO2C
Sl-19
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)



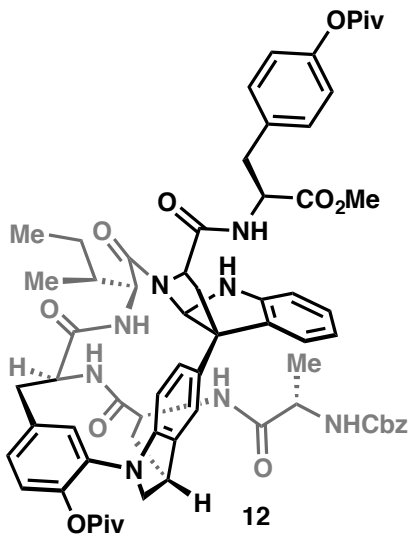
9

Not assigned due to rotamerism



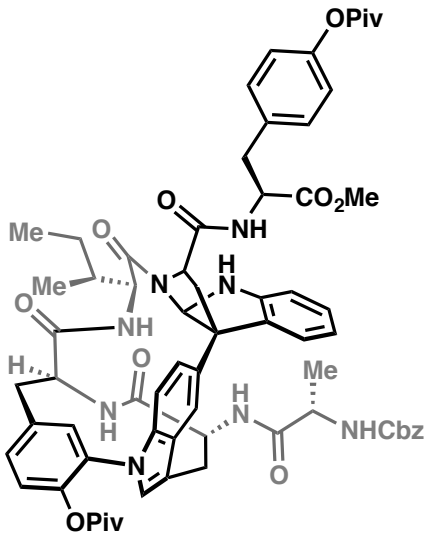
12

Not assigned due to rotamerism



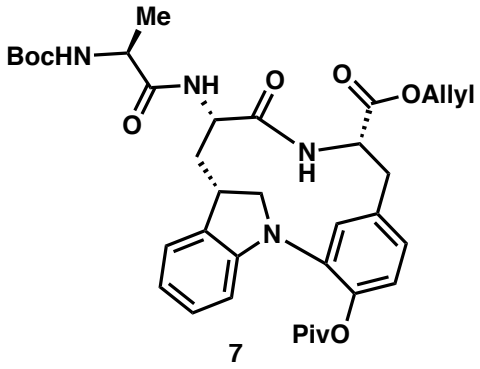
12

Not assigned due to rotamerism



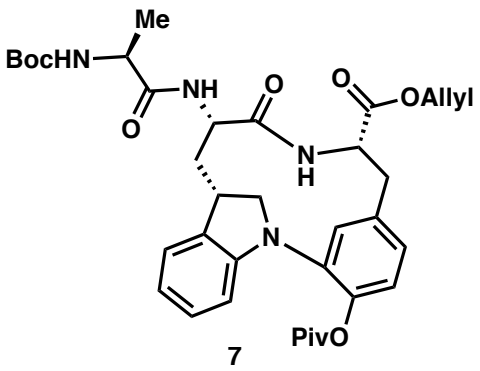
SI-20

Not assigned due to rotamerism


Me
BocHN
NH
O
O
O
OAllyl
N
H
N
PivO

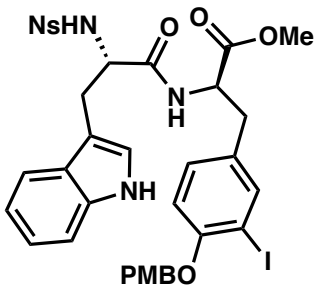
7

10.5
10.0
9.5
9.0
8.5
8.0
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
0.5
0.0
-0.5
f1 (ppm)


Me
BocHN
NH
O
O
O
OAllyl
N
H
N
PivO

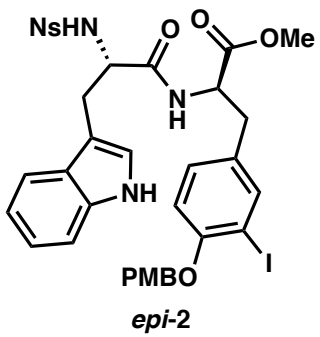
7

220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


NsHN
O
O
OMe
N
H
NH
PMBO
I

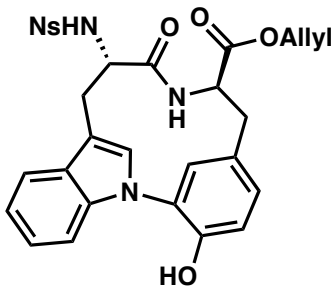
epi-2

10.5
10.0
9.5
9.0
8.5
8.0
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
0.5
0.0
-0.5
f1 (ppm)

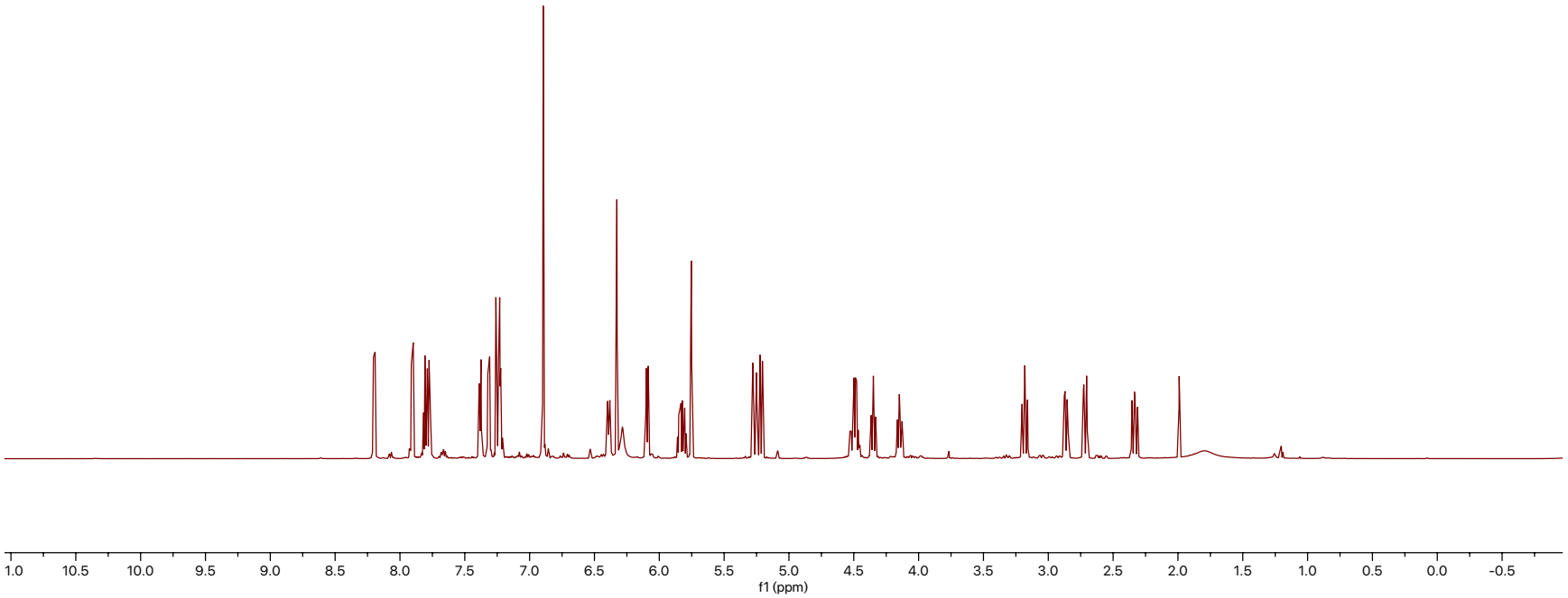

NsHN
O
O
OMe
N
H
NH
PMBO
I

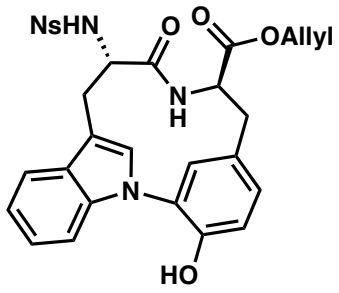
epi-2

220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


NsHN
O
O
OAllyl
N
H
N
HO

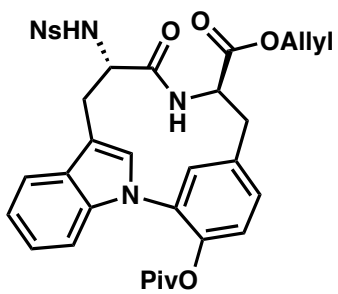
epi-SI-06


1.0
10.5
10.0
9.5
9.0
8.5
8.0
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
0.5
0.0
-0.5
f1 (ppm)


NsHN
O
O
OAllyl
N
H
N
HO

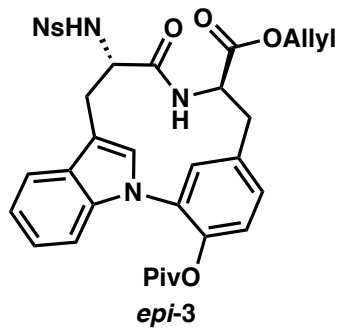
epi-SI-06

220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


NsHN
O
O
OAllyl
N
H
N
PivO

epi-3

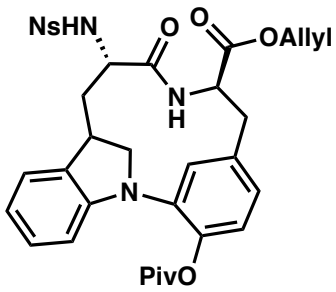
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)


NsHN
O
O
OAllyl
N
H
N
PivO

epi-3

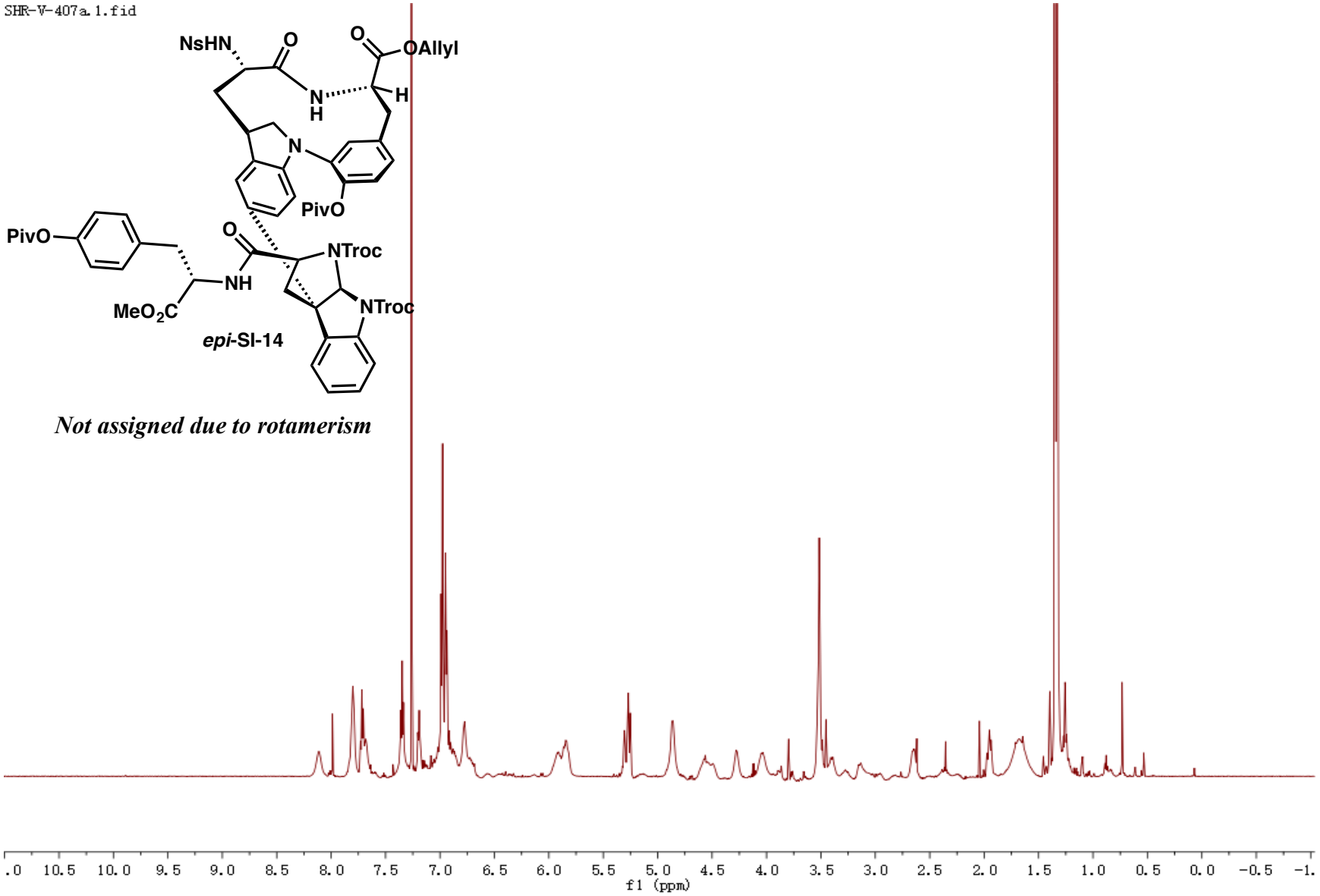
20
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

SHR-V-406a.1.fid

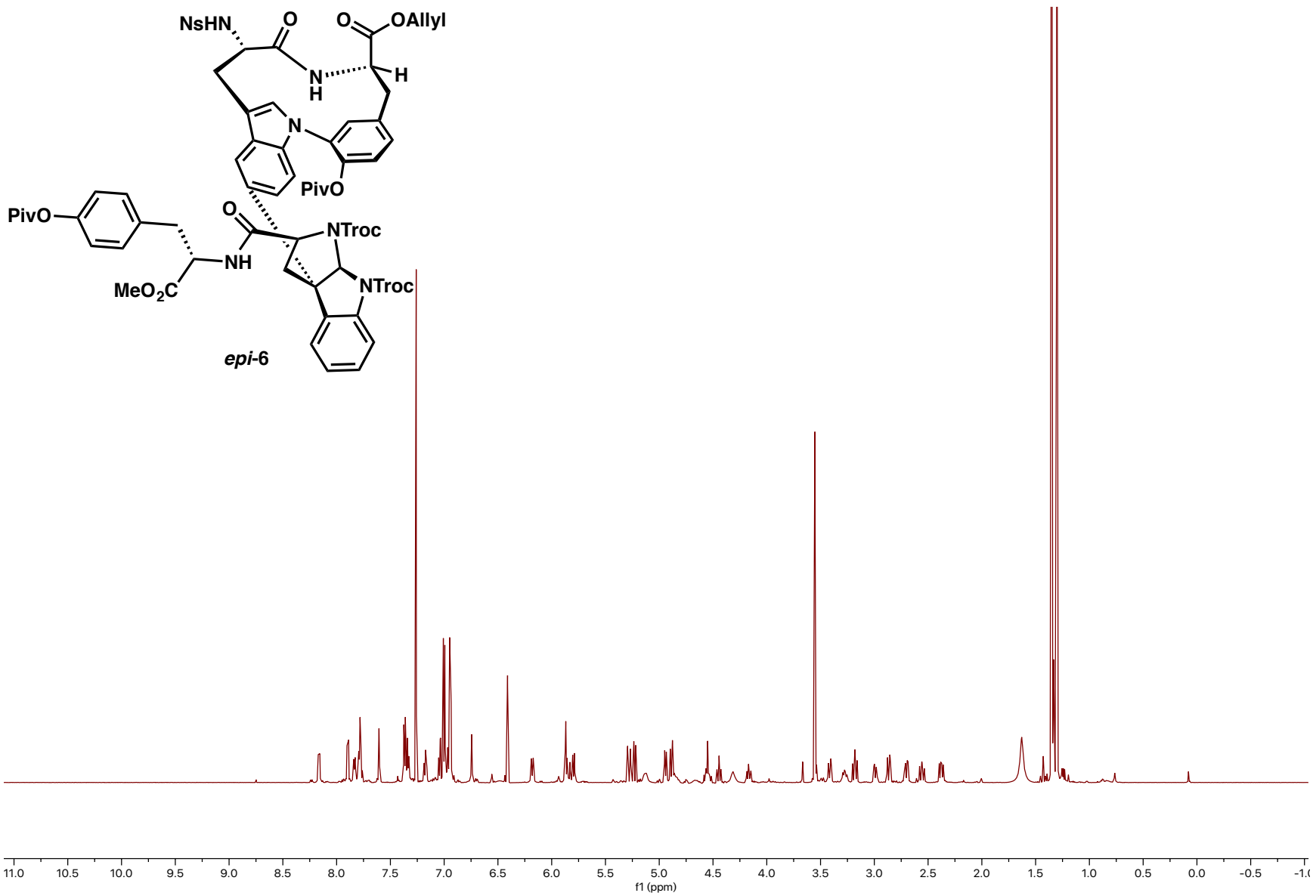


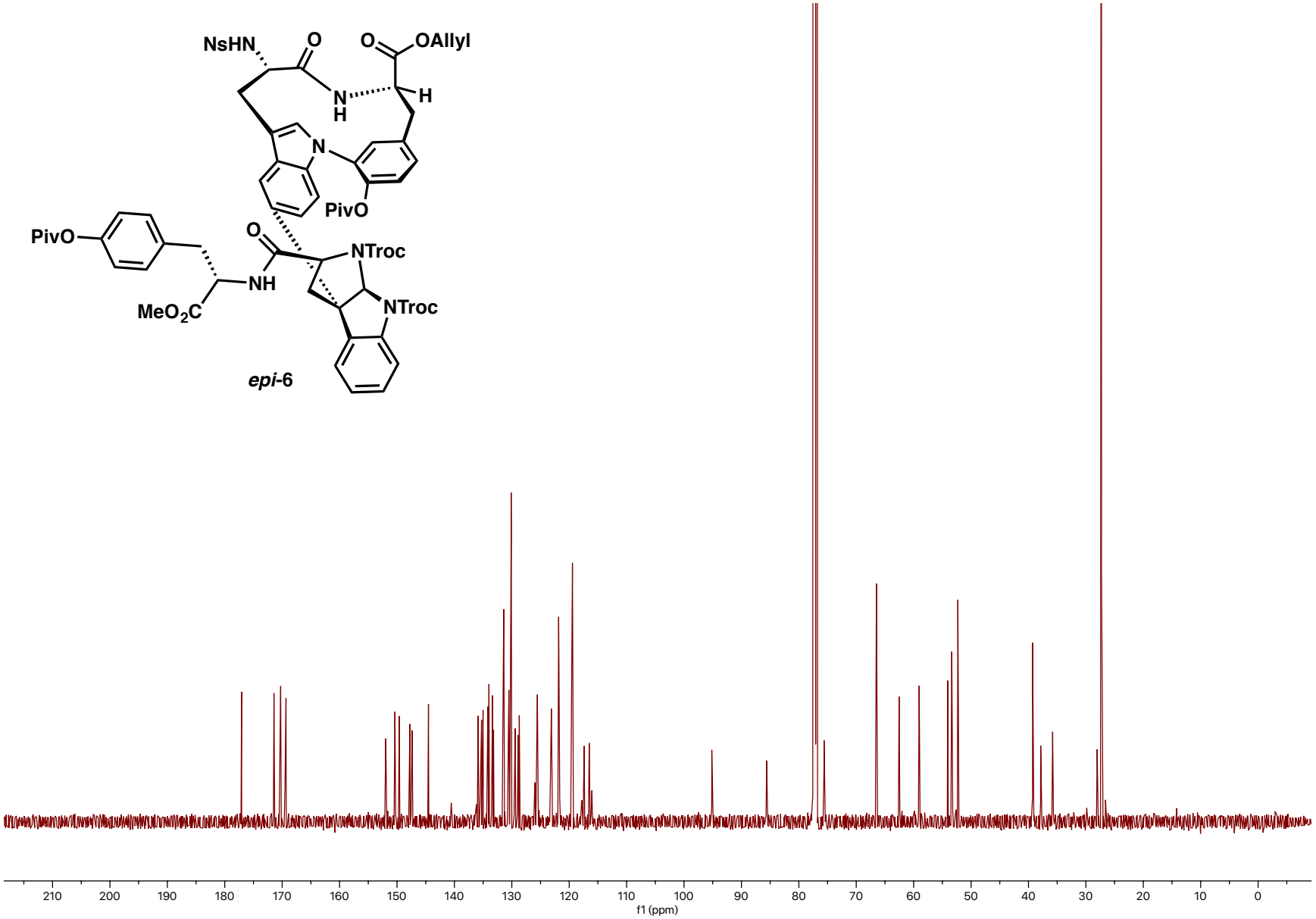
epi-5

Not assigned due to rotamerism



Not assigned due to rotamerism


NsHN
O
O
OAllyl
N
H
H
N
PivO
PivO
O
NTroc
NH
NTroc
MeO2C
epi-6
11.0
10.5
10.0
9.5
9.0
8.5
8.0
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
0.5
0.0
-0.5
f1 (ppm)


NsHN
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O
OAllyl
N
H
H
N
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PivO
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NH
NTroc
MeO2C
epi-6
210
200
190
180
170
160
150
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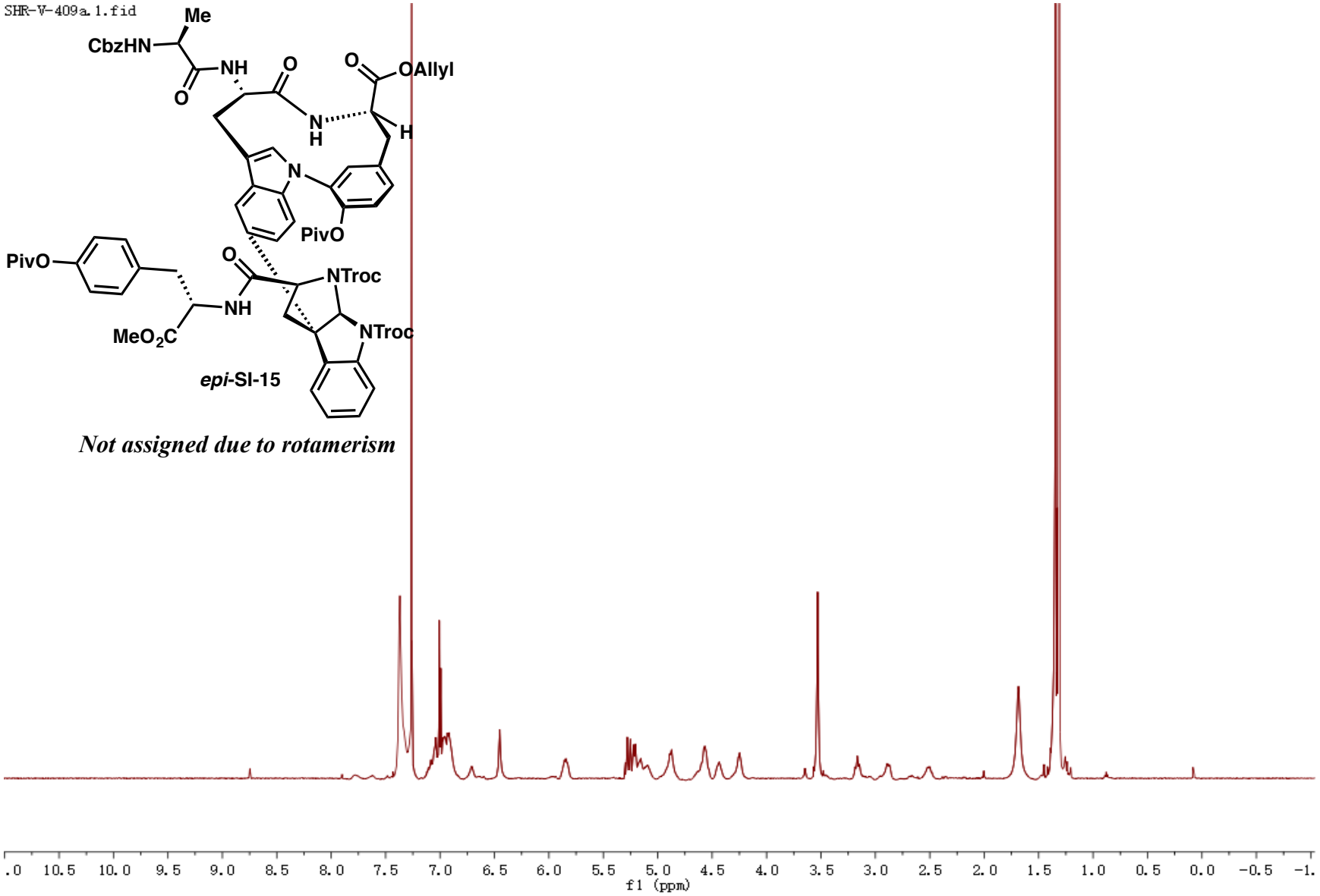
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)



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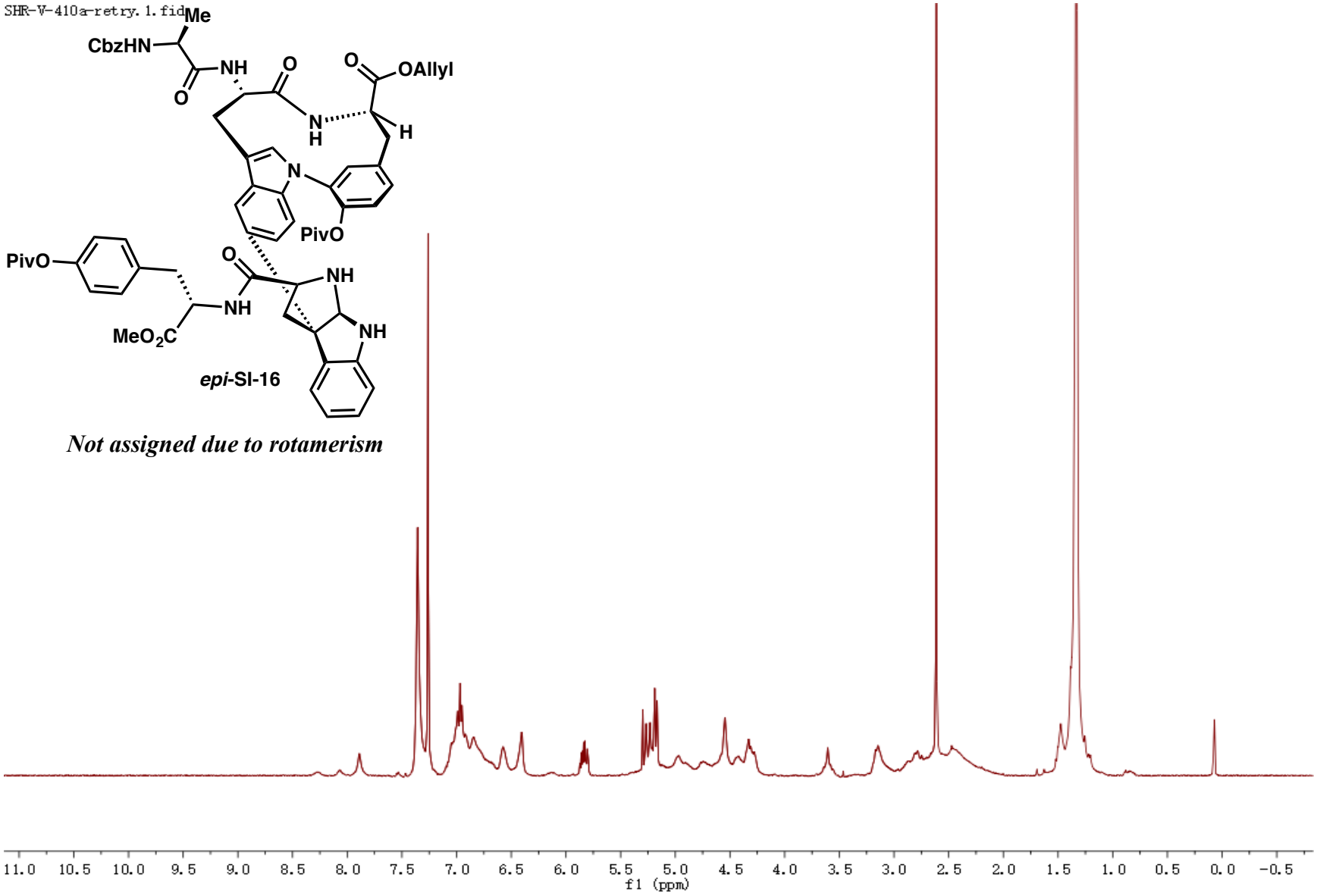

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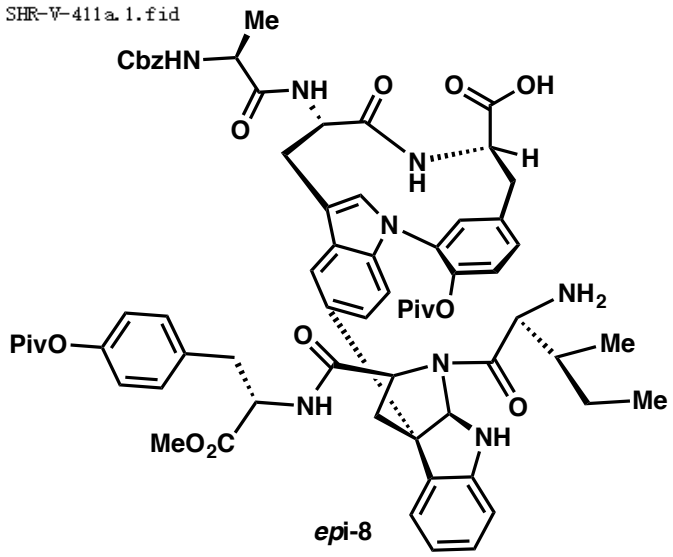
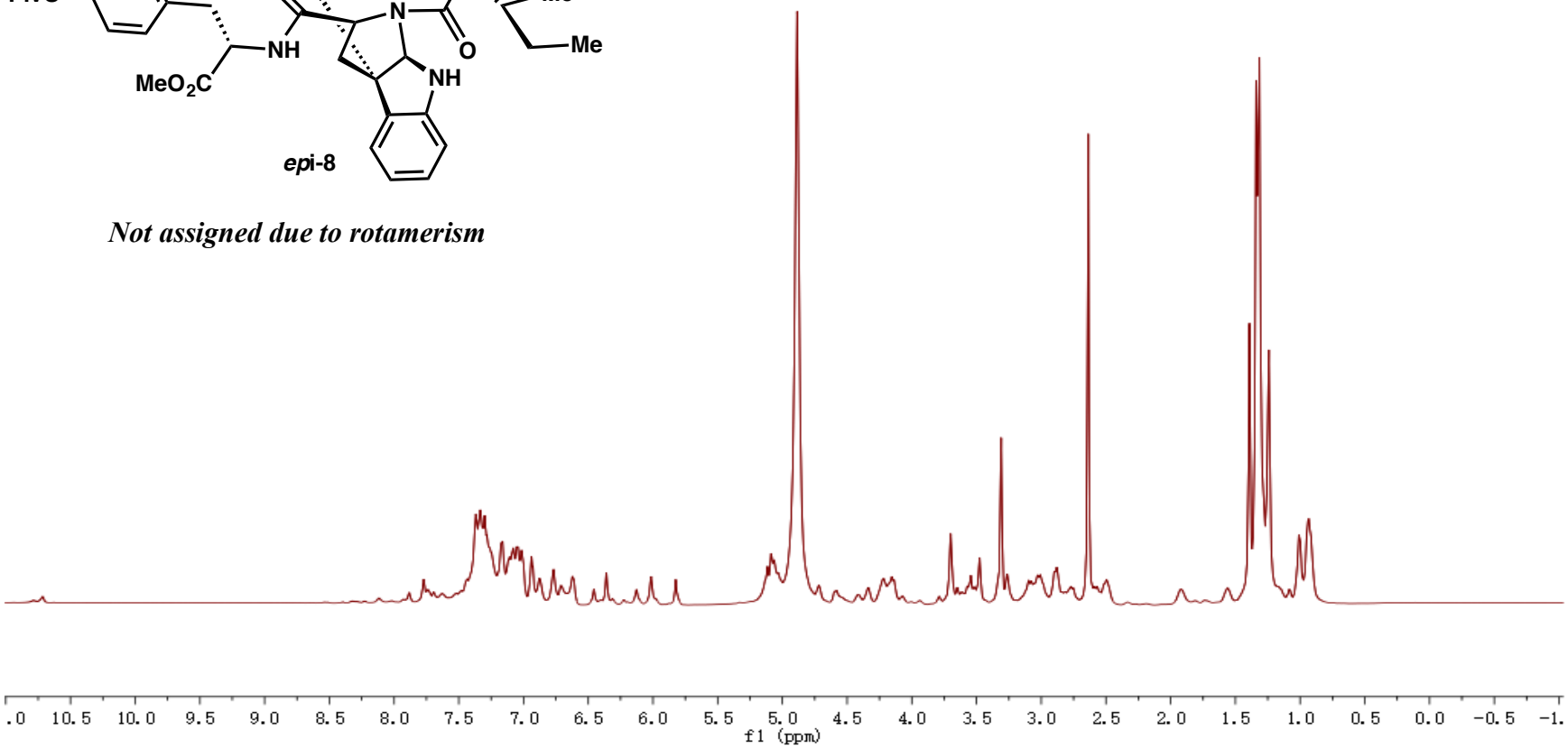
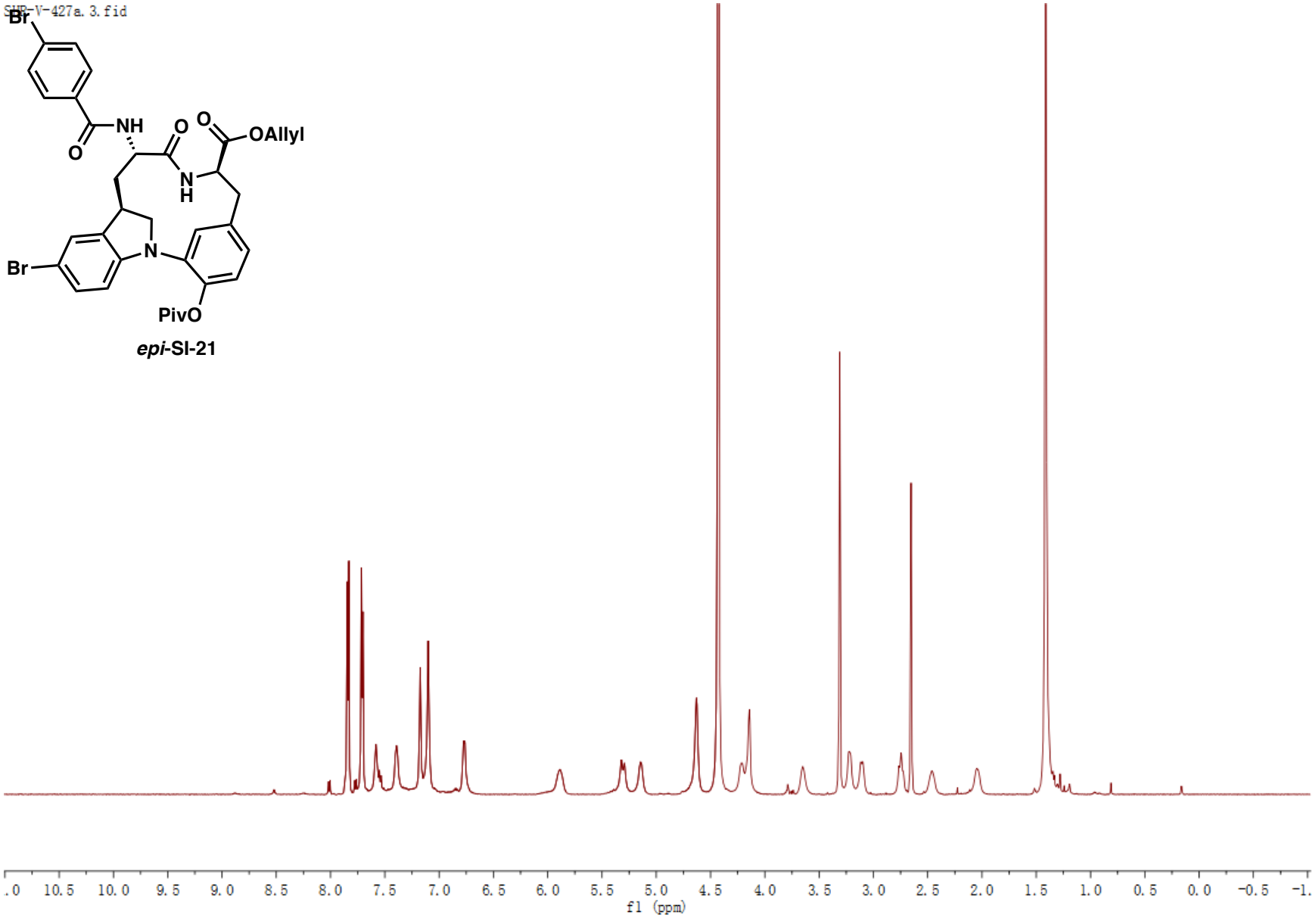
SHR-V-411a.1.fid



epi-8

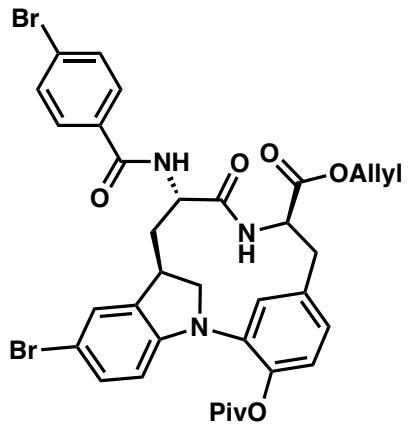
Not assigned due to rotamerism





epi-SI-21

SHR-V-427a.4.fid

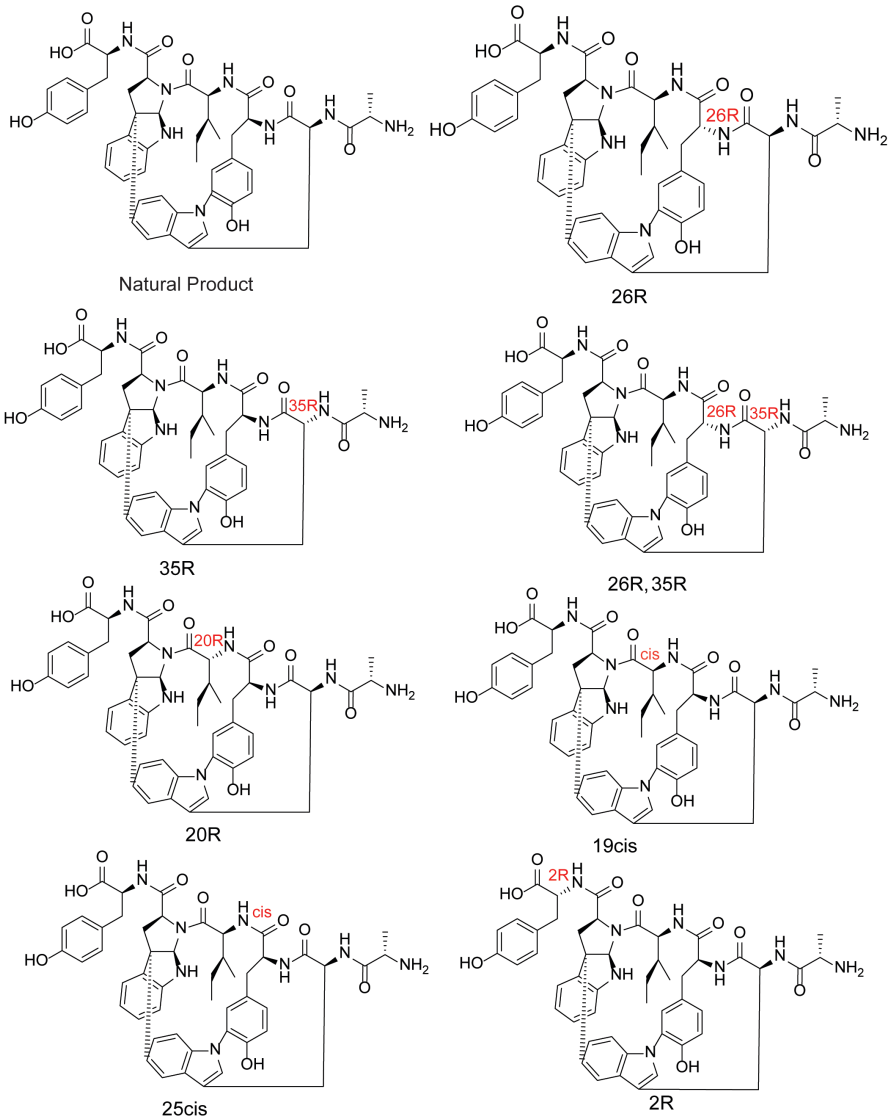


***epi*-SI-21**

DFT calculations and conclusions

In order to computationally explore proposed structures for the natural (**1a**) and synthetic *atrop* (**1b**) structures, we performed DFT calculations to predict ^{13}C NMR spectra on variations of the tryptorubin A structure (Figure S12). We considered various stereoisomers of both the bridge-above and bridge-below shapes, as well as variations of the bridge below shape with *cis*-peptide bonds. To prepare the structures for DFT calculations, we first minimized the energy of the structures in Schrödinger's Maestro software suite and then performed a conformational search on the minimized structure, both using the MMFF force field.

Figure S12: Structural possibilities considered *via* DFT calculations.



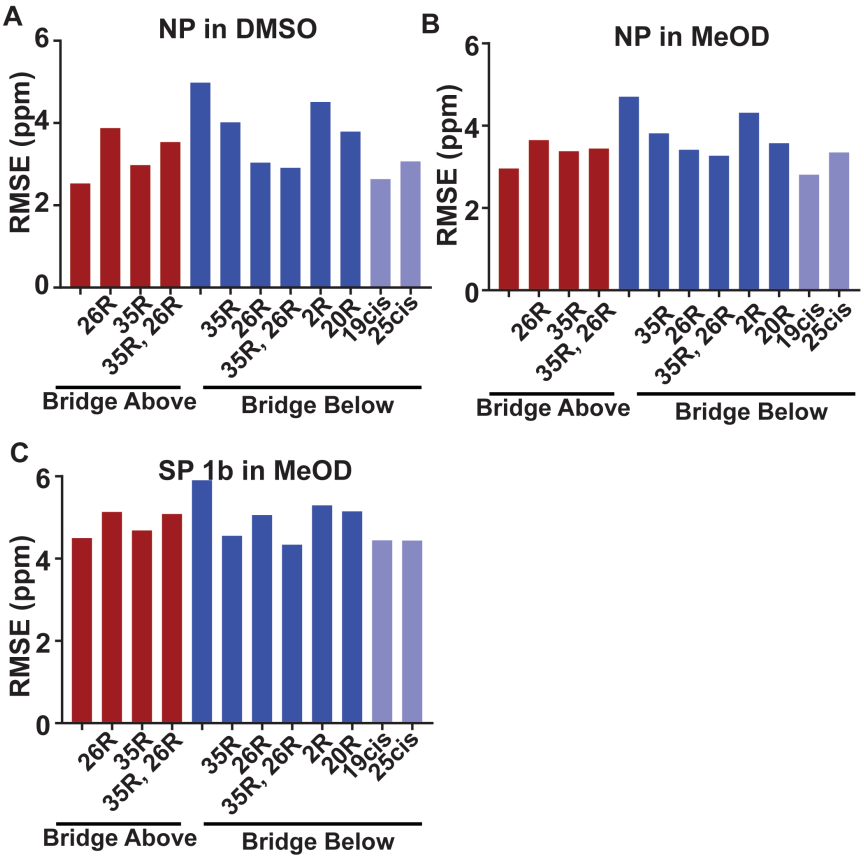
We selected the predicted minimum energy conformation structure for DFT calculations. All DFT calculations were performed with Gaussian 16[35] using the B3LYP function with a 6-31 (d,p) basis set and in gas phase. We first optimized the structure and checked for convergence with a frequency calculation. If the minimization had not converged, we repeated the optimization and frequency calculations until convergence. We then performed an NMR GIAO calculation on the minimized structure. We used the regression coefficients described in reference[36] to calculate the predicted chemical shifts for all carbons.

We then calculated the root mean squared error (RMSE) for the predicted and experimentally-observed chemical shifts for all structures (Figure S13). We selected the several closest matches between the calculated and experimental structures for follow up with alternate low-energy conformers. The two closest matches to the natural product in both DMSO and MeOD were the proposed natural product structure (bridge-above shape, **1a**) and the bridge-below structure with a cis peptide bond at N19-C19 (see Figure S11, **19cis**), so we selected these structures for additional analysis. For the synthetic product **1b**, the closest two matches were the bridge-below structure with stereochemical inversions at C26 and C35 and the bridge below structure with a cis peptide bond at N25-C25.

Figure S13: RMSE's for predicted spectrum for one conformer without reassignment for A) natural product **1a** in DMSO, B) natural product **1a** in MeOD, and C) synthetic product **1b** in MeOD.

[35] Gaussian 16, Revision A.03, Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Petersson, G. A.; Nakatsuji, H.; Li, X.; Caricato, M.; Marenich, A. V.; Bloino, J.; Janesko, B. G.; Gomperts, R.; Mennucci, B.; Hratchian, H. P.; Ortiz, J. V.; Izmaylov, A. F.; Sonnenberg, J. L.; Williams-Young, D.; Ding, F.; Lipparini, F.; Egidi, F.; Goings, J.; Peng, B.; Petrone, A.; Henderson, T.; Ranasinghe, D.; Zakrzewski, V. G.; Gao, J.; Rega, N.; Zheng, G.; Liang, W.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Throssell, K.; Montgomery, J. A., Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M. J.; Heyd, J. J.; Brothers, E. N.; Kudin, K. N.; Staroverov, V. N.; Keith, T. A.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A. P.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Millam, J. M.; Klene, M.; Adamo, C.; Cammi, R.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Farkas, O.; Foresman, J. B.; Fox, D. J. Gaussian, Inc., Wallingford CT, 2016.

[36] I. A. Konstantinov, L. J. Broadbelt, Regression formulas for density functional theory calculated 1H and 13C NMR chemical shifts in toluene-d8. *J Phys Chem A* **115**, 12364-12372 (2011).



In this manner we identified these three structures — the proposed bridge-below structure **1b**, the bridge-below isomer epimerized at C26 (**26R**), and the bridge-below toplogy with a *cis* peptide bond at N19-C19 (**19cis**) for further analysis. For each structure chosen for additional analysis, we performed the DFT calculations as described above for an additional 4 low energy conformers. We averaged the 5 predicted spectra for each structure using a Boltzmann-weighted average and then calculated RMSE (Figure S14). The proposed natural product structure was the closest match to the natural product spectrum taken in DMSO (RMSE = 2.4 ppm) while the bridge-below, *cis* N19-C19 was the closest match to that natural product spectrum taken in MeOD. We then compared the ^{1}H-^{1}H distances for ROESY NMR data[12] (Table S9, Figure S15) and found that only the proposed natural product structure is consistent with the ROESY data with all ^{1}H-^{1}H distances < 5 Å. We therefore concluded that the natural product structure was correctly assigned as the bridge-above shape assignment (**1a**).

For the synthetic product **1b**, the bridge-above structure (**1a**), the bridge-below atropisomer epimerized at C26 (**26R**), the bridge-below atropisomer with 26R and 35R (**26R,35R**), and the bridge-below atropisomer with a *cis* peptide bond between N19-C19 (**cis19**) all had similar RMSE's (4.3 – 4.4 ppm). The bridge-above isomer and the bridge-below with 26R or 26R and 35R structures are not consistent with the observed ROESY correlations (Table S10, Figure S16) because they each have at least one ^{1}H-^{1}H pair that is predicted to be greater than 5 Å apart, yet a correlation for that pair is observed in the ROESY spectrum. We therefore concluded that the synthetic product **1b** is most likely the proposed bridge-below structure with a *cis* peptide bond

at N19-C19 because it is the structure with the closest match to the experimental NMR spectrum that is still consistent with the ROESY spectrum. A full list of predicted ^{13}C chemical shifts and error compared to experimental spectra are listed in Table S11 for the natural isolate and in Table S12 for synthetic product 1b. Previous NMR DFT calculation studies report average errors under 2 ppm for correctly assigned structures while an incorrectly assigned structure had a higher average error, 6.8 ppm.[16] Our errors were also around 2 ppm, with the bridge-above isomer structure having an error of 1.9 and 2.2 ppm for the natural product in DMSO and MeOD, respectively, and the **cis19** structure having an average error of 3.3 ppm when compared to the spectra for the synthetic product **1b** in MeOD.

We also compared the hydrophobic and hydrophilic accessible surface areas of structures with the bridge-above and bridge-below shapes using Maestro's QikProp tool (Table S13). We found that while both structures have similar hydrophobic surface areas, the bridge above structure has a much larger hydrophilic surface area (311.1 vs 274.0 Å^2). This is consistent with the **1b** being significantly more retentive during liquid chromatography than the natural isolate, as discussed above.

Figure S14: RMSE's for predicted spectrum for five conformers for structures selected for further analysis without reassignment for A) natural product in DMSO, B) natural product in MeOD, and C) synthetic product 1b in MeOD.

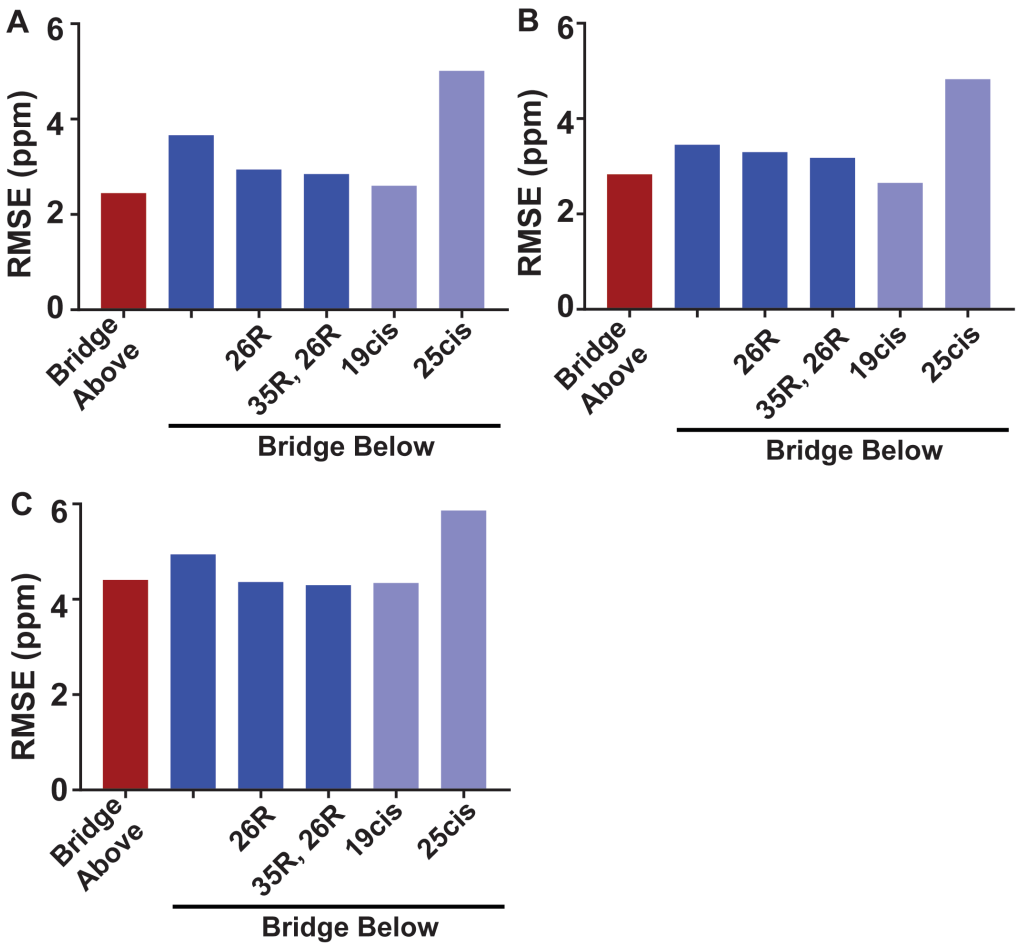


Table S9. ^{1}H-^{1}H distances for observed correlations in the ROESY spectrum of the natural product.

Experimental nOe	Bridge above (Å)	Bridge below cis 19 (Å)
H9-H42	2.6	5.2
H10-H42	2.0	4.7
H12-H20	2.3	4.3
H12-H40	2.3	4.5
H14-H40	3.6	3.7
H27-H33	2.5	2.7
H35-H38	2.7	4.4
H36-H40	2.4	2.4

Figure S15. Images showing 1H-1H distances in Table S1 for the minimum energy A) bridge above and B) bridge below cis19 structures

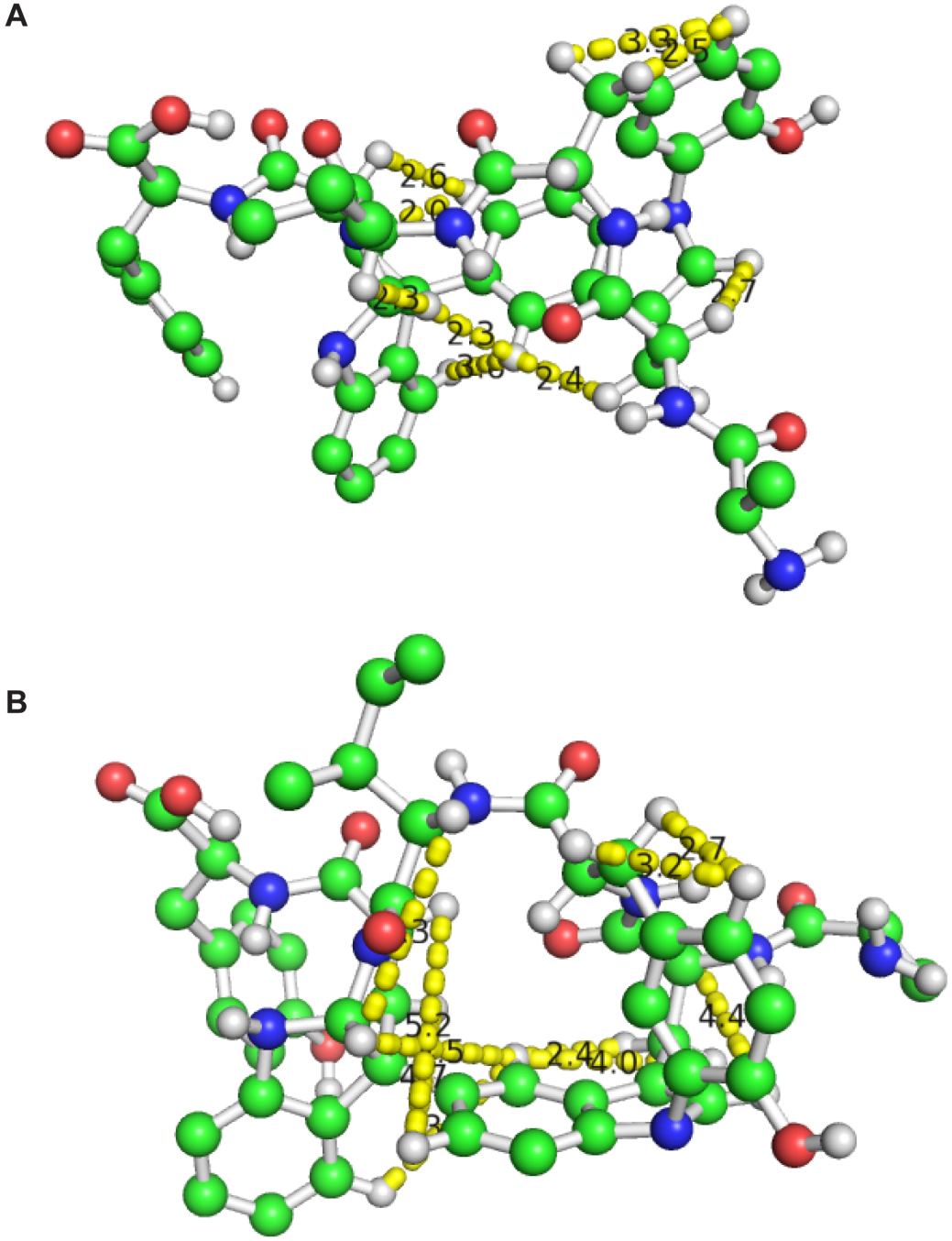


Table S10. 1H-1H distances for observed correlations in the ROESY spectrum of the synthetic product 1b. A * indicates that an alternative low energy conformation had a distance < 4 Å; a ** indicates that rotation about a rotatable bond would allow for a distance < 4 Å.

Experimental nOe	Bridge above (Å)	Bridge below (Å)	Bridge below 26R (Å)	Bridge below 26R 35R (Å)	Bridge below cis 19 (Å)a
H9-H40	5.1	3.7	2.3	2.2	3.3
H10-H14	3.5	5.7*	3.6	3.5	3.7
H10-H17	4.6	2.7	4.8	4.8	4.7
H10-H40	2.0	1.9	2.3	2.4	1.9
H12-H20	2.3	2.7	2.0	2.0	4.3
H12-H40	2.3	3.2	3.6	3.5	4.5
H12-H26	5.0	2.3	5.3	5.4	4.0
H12-H42	4.3	3.6	3.2	3.3	2.1
H27-H29	3.0	3.4	2.7	2.7	3.2
H27-H33	2.5	2.9	2.5	2.5	2.7
H29-H43	3.2	3.8	3.4	3.3	3.5
H36-H38	3.1	2.9	3.2	3.1	3.0
H36-H40	2.4	2.5	2.4	2.4	2.4
H38-H47	5.5	3.8	6.0**	6.6	3.7

Figure S16. Images showing ^{1}H-^{1}H distances in Table S1 for the minimum energy A) bridge above, B) bridge=below (**1b**), C) bridge below 26R, D) bridge below 26R 35R, and E) bridge below cis19 structures.

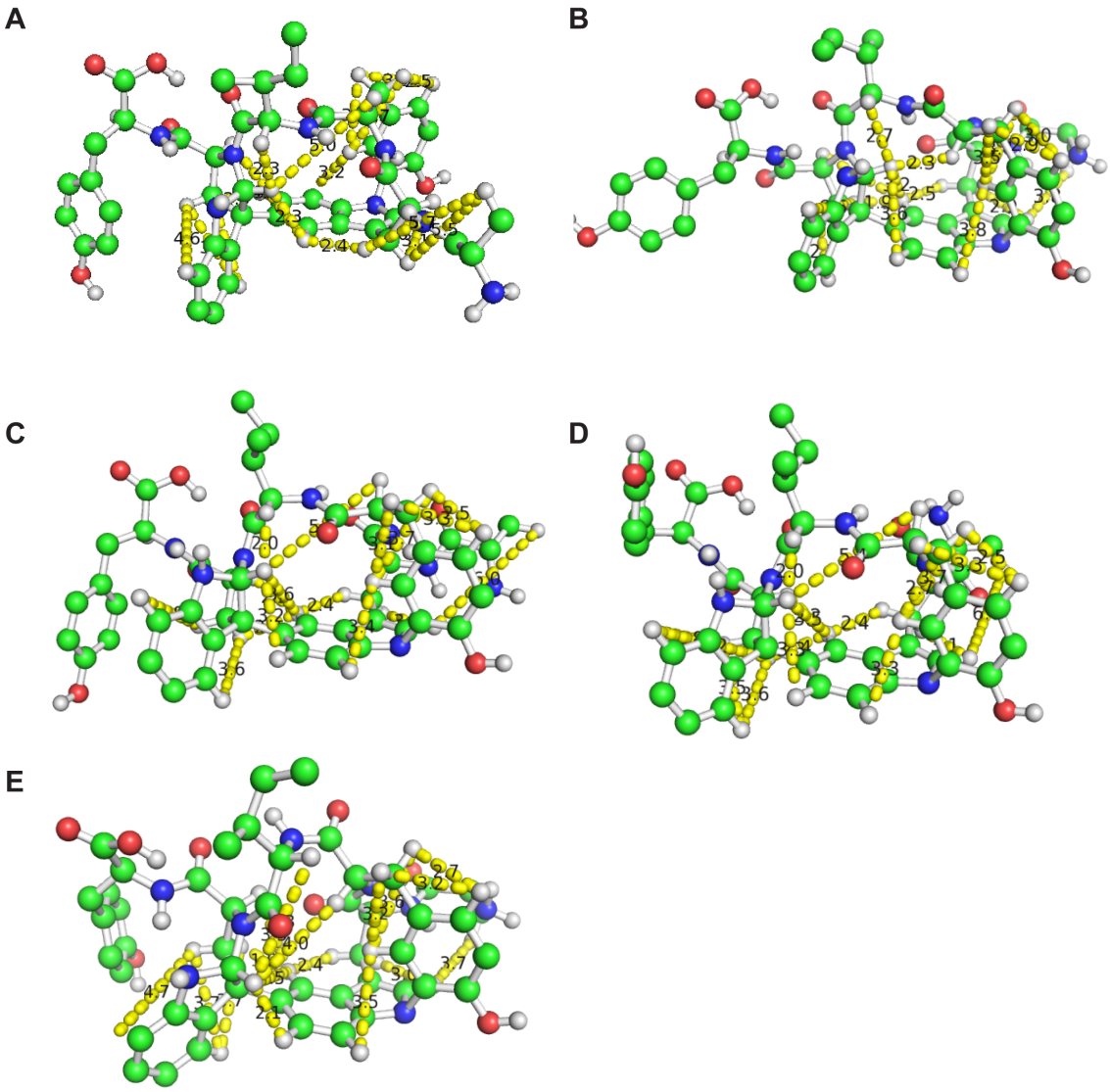


Table S11. Comparison between predicted bridge above ^{13}C shifts and the natural isolate

Position	ΔC predicted	|ΔΔC| (DMSO)	|ΔΔC| (MeOD)
1	169.9	4.1	7.9
2	55.8	0.8	1.5
3	38.6	0.7	0.2
4	129.9	0.1	0.1
5	130.3	0.3	1.4
6	112.6	2.3	3.3
7	155.1	0.7	1.7

8	167.6	2.5	5.8
9	67.6	4.5	2.3
10	38.9	0.2	1.4
11	66.2	5.3	3.3
12	96.2	3.9	2.1
13	136.8	0.5	0.2
14	124.1	0.7	0.1
15	119.9	1.5	0.7
16	128.0	0.2	1.4
17	108.3	1.7	3.7
18	148.4	1.2	1.9
19	171.2	3.6	4.9
20	61.7	3.5	2.0
21	37.8	1.3	0.2
22	25.9	0.6	4.8
23	8.6	2.9	2.9
24	15.2	0.4	1.1
25	168.4	1.8	3.1
26	56.3	4.3	2.8
27	33.3	0.9	0.1
28	128.9	3.0	1.2
29	128.8	3.0	3.0
30	135.0	2.5	1.2
31	148.8	0.8	1.4
32	115.8	1.7	2.4
33	128.7	0.4	1.2
34	171.5	2.5	1.7
35	60.2	6.2	6.5
36	27.9	0.4	0.4
37	122.3	1.4	0.6
38	145.8	0.6	1.9
39	139.6	0.5	0.6
40	120.5	3.1	3.1
41	138.9	2.8	2.8
42	123.2	0.1	0.1
43	116.6	0.9	0.6
44	150.3	2.8	4.4
45	172.0	3.2	5.1
46	53.5	2.8	2.5
47	19.7	2.3	1.4
Average error	N/A	1.9	2.2

Table S12. Comparison between predicted bridge below 19-cis ^{13}C shifts and the synthetic product 1b

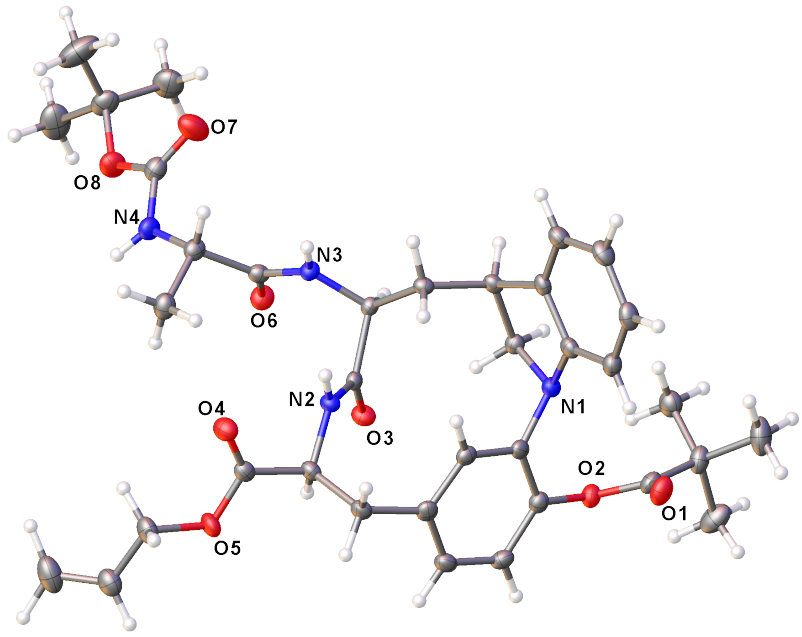
Position	ΔC predicted	|ΔΔC|
1	169.7	5.2
2	57.2	0.2
3	39.1	1.3
4	128.7	1.2
5	130.0	1.7
6	113.1	2.8
7	155.3	6.0
8	176.8	2.8
9	63.9	2.9
10	42.6	0.8
11	64.0	0.2
12	95.7	4.1
13	138.2	0.9
14	124.5	0.3
15	120.4	0.9
16	127.9	1.9
17	109.7	3.0
18	147.1	2.7
19	171.4	4.8
20	65.2	8.3
21	39.8	2.3
22	26.1	0.2
23	7.5	3.3
24	15.2	1.2
25	172.1	0.8
26	54.8	1.0
27	31.1	3.3
28	127.6	7.1
29	129.9	0.0
30	135.5	1.3
31	149.3	7.3
32	114.1	8.2
33	126.8	2.8
34	169.8	0.4
35	56.0	1.1
36	28.5	4.8
37	126.0	5.4
38	146.7	1.2
39	141.2	11.3
40	124.1	6.4

41	135.6	3.6
42	127.1	8.7
43	117.0	10.7
44	154.0	4.0
45	171.4	6.3
46	52.6	2.0
47	19.7	0.2
Average error	N/A	3.3

Table S13. Predicted hydrophobic and hydrophilic accessible surface areas. Surface areas were calculated using the QikProp tool in Maestro.

Structure	Solvent Accessible Surface Area (Å^2)	Hydrophobic Accessible Surface Area (Å^2)	Hydrophilic Accessible Surface Area (Å^2)
Bridge-above (**1a**)	1071.1	380.7	311.1
Bridge-below (**1b**)	1016.7	380.0	274.0

X-ray crystal structures



Compound 7

Table 1. Crystal data and structure refinement for baran698_0m_a.

Identification code	SHR-V-171
Empirical formula	C36 H46 N4 O8
Formula weight	662.77
Temperature	100.0 K
Wavelength	1.54178 Å

Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Unit cell dimensions	a = 9.70320(10) Å α= 90°.
b = 18.7029(3) Å	β= 90°.
c = 19.2126(3) Å	γ = 90°.
Volume	3486.66(9) $Å^3$
Z	4
Density (calculated)	1.263 Mg/m^3
Absorption coefficient	0.733 mm^{-1}
F(000)	1416
Crystal size	0.29 x 0.13 x 0.08 mm^3
Theta range for data collection	4.729 to 68.271°.
Index ranges	-11<=h<=11, -22<=k<=22, -23<=l<=21
Reflections collected	17992
Independent reflections	6357 [R(int) = 0.0325]
Completeness to theta = 67.679°	99.6 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.7531 and 0.6737
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	6357 / 0 / 448
Goodness-of-fit on F^2	1.015
Final R indices [I>2sigma(I)]	R1 = 0.0343, wR2 = 0.0893
R indices (all data)	R1 = 0.0367, wR2 = 0.0906
Absolute structure parameter	0.03(8)
Extinction coefficient	n/a
Largest diff. peak and hole	0.224 and -0.183 e.$Å^{-3}$

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for baran698_0m_a. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

x	y	z	U(eq)	
O(1)	4338(2)	-1292(1)	6362(1)	30(1)
O(2)	6071(2)	-567(1)	6027(1)	22(1)
O(3)	5609(2)	1829(1)	4965(1)	23(1)
O(4)	2062(2)	2403(1)	3388(1)	27(1)
O(5)	3735(2)	1960(1)	2709(1)	26(1)
O(6)	5538(2)	3557(1)	5181(1)	25(1)
O(7)	3931(2)	5266(1)	5867(1)	40(1)
O(8)	5546(2)	5802(1)	5179(1)	32(1)
N(1)	3944(2)	359(1)	6354(1)	20(1)
N(2)	3442(2)	1938(1)	4542(1)	18(1)
N(3)	3425(2)	3143(1)	5480(1)	18(1)
N(4)	4407(2)	4871(1)	4777(1)	26(1)
C(1)	5414(3)	-1524(2)	7677(2)	43(1)
C(2)	6701(3)	-398(2)	7448(1)	32(1)
C(3)	7561(3)	-1555(2)	6939(2)	39(1)
C(4)	6280(3)	-1120(1)	7142(1)	27(1)
C(5)	5421(3)	-1013(1)	6488(1)	23(1)
C(6)	5257(2)	-296(1)	5487(1)	22(1)
C(7)	5655(3)	-410(1)	4803(1)	24(1)
C(8)	4949(3)	-68(1)	4272(1)	24(1)
C(9)	3809(2)	358(1)	4414(1)	21(1)
C(10)	3381(2)	430(1)	5102(1)	20(1)
C(11)	4148(2)	142(1)	5643(1)	19(1)
C(12)	2611(2)	369(1)	6645(1)	20(1)
C(13)	1716(3)	-208(1)	6704(1)	24(1)
C(14)	488(3)	-102(1)	7066(1)	27(1)
C(15)	165(3)	561(2)	7354(1)	28(1)
C(16)	1056(3)	1139(1)	7276(1)	25(1)
C(17)	2280(2)	1041(1)	6919(1)	20(1)
C(18)	3382(2)	1572(1)	6709(1)	19(1)
C(19)	4578(2)	1068(1)	6525(1)	19(1)

C(20)	2827(2)	2047(1)	6112(1)	18(1)
C(21)	3943(2)	2444(1)	5690(1)	17(1)
C(22)	4411(2)	2035(1)	5031(1)	17(1)
C(23)	3716(2)	1586(1)	3884(1)	20(1)
C(24)	3131(3)	810(1)	3858(1)	22(1)
C(25)	3062(2)	2035(1)	3310(1)	21(1)
C(26)	3126(3)	2350(2)	2122(1)	29(1)
C(27)	4109(3)	2351(2)	1544(1)	32(1)
C(28)	4509(4)	2936(2)	1224(2)	46(1)
C(29)	4276(2)	3627(1)	5190(1)	20(1)
C(30)	3549(2)	4243(1)	4824(1)	23(1)
C(31)	3162(3)	3991(1)	4094(1)	28(1)
C(32)	4575(3)	5311(1)	5333(1)	26(1)
C(33)	5848(3)	6386(2)	5666(2)	34(1)
C(34)	6363(4)	6097(2)	6355(2)	44(1)
C(35)	4587(4)	6854(2)	5736(2)	60(1)
C(36)	7008(4)	6773(2)	5298(2)	50(1)

Table 3. Bond lengths [Å] and angles [°] for baran698_0m_a.

O(1)-C(5)	1.197(3)
O(2)-C(5)	1.372(3)
O(2)-C(6)	1.399(3)
O(3)-C(22)	1.230(3)
O(4)-C(25)	1.199(3)
O(5)-C(25)	1.334(3)
O(5)-C(26)	1.468(3)
O(6)-C(29)	1.232(3)
O(7)-C(32)	1.204(3)
O(8)-C(32)	1.349(3)
O(8)-C(33)	1.467(3)
N(1)-C(11)	1.438(3)
N(1)-C(12)	1.409(3)
N(1)-C(19)	1.499(3)
N(2)-H(2)	0.8800
N(2)-C(22)	1.340(3)
N(2)-C(23)	1.450(3)
N(3)-H(3)	0.8800
N(3)-C(21)	1.458(3)
N(3)-C(29)	1.345(3)
N(4)-H(4)	0.8800
N(4)-C(30)	1.442(3)
N(4)-C(32)	1.358(3)
C(1)-H(1A)	0.9800
C(1)-H(1B)	0.9800
C(1)-H(1C)	0.9800
C(1)-C(4)	1.527(4)
C(2)-H(2A)	0.9800
C(2)-H(2B)	0.9800
C(2)-H(2C)	0.9800
C(2)-C(4)	1.529(4)
C(3)-H(3A)	0.9800
C(3)-H(3B)	0.9800
C(3)-H(3C)	0.9800

C(3)-C(4)	1.536(4)
C(4)-C(5)	1.521(3)
C(6)-C(7)	1.387(3)
C(6)-C(11)	1.385(3)
C(7)-H(7)	0.9500
C(7)-C(8)	1.387(4)
C(8)-H(8)	0.9500
C(8)-C(9)	1.390(4)
C(9)-C(10)	1.392(3)
C(9)-C(24)	1.514(3)
C(10)-H(10)	0.9500
C(10)-C(11)	1.387(3)
C(12)-C(13)	1.389(3)
C(12)-C(17)	1.399(3)
C(13)-H(13)	0.9500
C(13)-C(14)	1.394(4)
C(14)-H(14)	0.9500
C(14)-C(15)	1.393(4)
C(15)-H(15)	0.9500
C(15)-C(16)	1.393(4)
C(16)-H(16)	0.9500
C(16)-C(17)	1.384(3)
C(17)-C(18)	1.514(3)
C(18)-H(18)	1.0000
C(18)-C(19)	1.536(3)
C(18)-C(20)	1.548(3)
C(19)-H(19A)	0.9900
C(19)-H(19B)	0.9900
C(20)-H(20A)	0.9900
C(20)-H(20B)	0.9900
C(20)-C(21)	1.544(3)
C(21)-H(21)	1.0000
C(21)-C(22)	1.549(3)
C(23)-H(23)	1.0000
C(23)-C(24)	1.560(3)
C(23)-C(25)	1.524(3)

C(24)-H(24A)	0.9900
C(24)-H(24B)	0.9900
C(26)-H(26A)	0.9900
C(26)-H(26B)	0.9900
C(26)-C(27)	1.463(4)
C(27)-H(27)	0.9500
C(27)-C(28)	1.314(4)
C(28)-H(28A)	1.04(4)
C(28)-H(28B)	1.06(4)
C(29)-C(30)	1.522(3)
C(30)-H(30)	1.0000
C(30)-C(31)	1.527(4)
C(31)-H(31A)	0.9800
C(31)-H(31B)	0.9800
C(31)-H(31C)	0.9800
C(33)-C(34)	1.514(4)
C(33)-C(35)	1.511(4)
C(33)-C(36)	1.513(4)
C(34)-H(34A)	0.9800
C(34)-H(34B)	0.9800
C(34)-H(34C)	0.9800
C(35)-H(35A)	0.9800
C(35)-H(35B)	0.9800
C(35)-H(35C)	0.9800
C(36)-H(36A)	0.9800
C(36)-H(36B)	0.9800
C(36)-H(36C)	0.9800

C(5)-O(2)-C(6)	116.08(18)
C(25)-O(5)-C(26)	114.54(19)
C(32)-O(8)-C(33)	120.5(2)
C(11)-N(1)-C(19)	113.64(18)
C(12)-N(1)-C(11)	120.47(18)
C(12)-N(1)-C(19)	106.18(18)
C(22)-N(2)-H(2)	118.6
C(22)-N(2)-C(23)	122.86(19)

C(23)-N(2)-H(2)	118.6
C(21)-N(3)-H(3)	119.8
C(29)-N(3)-H(3)	119.8
C(29)-N(3)-C(21)	120.41(19)
C(30)-N(4)-H(4)	119.6
C(32)-N(4)-H(4)	119.6
C(32)-N(4)-C(30)	120.8(2)
H(1A)-C(1)-H(1B)	109.5
H(1A)-C(1)-H(1C)	109.5
H(1B)-C(1)-H(1C)	109.5
C(4)-C(1)-H(1A)	109.5
C(4)-C(1)-H(1B)	109.5
C(4)-C(1)-H(1C)	109.5
H(2A)-C(2)-H(2B)	109.5
H(2A)-C(2)-H(2C)	109.5
H(2B)-C(2)-H(2C)	109.5
C(4)-C(2)-H(2A)	109.5
C(4)-C(2)-H(2B)	109.5
C(4)-C(2)-H(2C)	109.5
H(3A)-C(3)-H(3B)	109.5
H(3A)-C(3)-H(3C)	109.5
H(3B)-C(3)-H(3C)	109.5
C(4)-C(3)-H(3A)	109.5
C(4)-C(3)-H(3B)	109.5
C(4)-C(3)-H(3C)	109.5
C(1)-C(4)-C(2)	109.0(2)
C(1)-C(4)-C(3)	110.8(2)
C(2)-C(4)-C(3)	110.5(2)
C(5)-C(4)-C(1)	108.6(2)
C(5)-C(4)-C(2)	110.3(2)
C(5)-C(4)-C(3)	107.7(2)
O(1)-C(5)-O(2)	122.6(2)
O(1)-C(5)-C(4)	126.2(2)
O(2)-C(5)-C(4)	111.2(2)
C(7)-C(6)-O(2)	119.3(2)
C(11)-C(6)-O(2)	119.5(2)

C(11)-C(6)-C(7)	120.8(2)
C(6)-C(7)-H(7)	120.4
C(6)-C(7)-C(8)	119.3(2)
C(8)-C(7)-H(7)	120.4
C(7)-C(8)-H(8)	119.6
C(7)-C(8)-C(9)	120.9(2)
C(9)-C(8)-H(8)	119.6
C(8)-C(9)-C(10)	118.7(2)
C(8)-C(9)-C(24)	121.8(2)
C(10)-C(9)-C(24)	119.1(2)
C(9)-C(10)-H(10)	119.6
C(11)-C(10)-C(9)	120.9(2)
C(11)-C(10)-H(10)	119.6
C(6)-C(11)-N(1)	118.6(2)
C(6)-C(11)-C(10)	119.0(2)
C(10)-C(11)-N(1)	121.9(2)
C(13)-C(12)-N(1)	126.6(2)
C(13)-C(12)-C(17)	121.5(2)
C(17)-C(12)-N(1)	111.8(2)
C(12)-C(13)-H(13)	121.1
C(12)-C(13)-C(14)	117.7(2)
C(14)-C(13)-H(13)	121.1
C(13)-C(14)-H(14)	119.4
C(15)-C(14)-C(13)	121.1(2)
C(15)-C(14)-H(14)	119.4
C(14)-C(15)-H(15)	119.7
C(14)-C(15)-C(16)	120.6(2)
C(16)-C(15)-H(15)	119.7
C(15)-C(16)-H(16)	120.6
C(17)-C(16)-C(15)	118.8(2)
C(17)-C(16)-H(16)	120.6
C(12)-C(17)-C(18)	109.1(2)
C(16)-C(17)-C(12)	120.2(2)
C(16)-C(17)-C(18)	130.5(2)
C(17)-C(18)-H(18)	109.9
C(17)-C(18)-C(19)	101.10(19)

C(17)-C(18)-C(20)	109.18(19)
C(19)-C(18)-H(18)	109.9
C(19)-C(18)-C(20)	116.39(19)
C(20)-C(18)-H(18)	109.9
N(1)-C(19)-C(18)	106.40(18)
N(1)-C(19)-H(19A)	110.4
N(1)-C(19)-H(19B)	110.4
C(18)-C(19)-H(19A)	110.4
C(18)-C(19)-H(19B)	110.4
H(19A)-C(19)-H(19B)	108.6
C(18)-C(20)-H(20A)	108.5
C(18)-C(20)-H(20B)	108.5
H(20A)-C(20)-H(20B)	107.5
C(21)-C(20)-C(18)	114.91(18)
C(21)-C(20)-H(20A)	108.5
C(21)-C(20)-H(20B)	108.5
N(3)-C(21)-C(20)	109.55(18)
N(3)-C(21)-H(21)	108.4
N(3)-C(21)-C(22)	108.51(18)
C(20)-C(21)-H(21)	108.4
C(20)-C(21)-C(22)	113.44(18)
C(22)-C(21)-H(21)	108.4
O(3)-C(22)-N(2)	123.3(2)
O(3)-C(22)-C(21)	121.0(2)
N(2)-C(22)-C(21)	115.67(18)
N(2)-C(23)-H(23)	108.9
N(2)-C(23)-C(24)	112.55(18)
N(2)-C(23)-C(25)	107.77(19)
C(24)-C(23)-H(23)	108.9
C(25)-C(23)-H(23)	108.9
C(25)-C(23)-C(24)	109.76(19)
C(9)-C(24)-C(23)	109.83(19)
C(9)-C(24)-H(24A)	109.7
C(9)-C(24)-H(24B)	109.7
C(23)-C(24)-H(24A)	109.7
C(23)-C(24)-H(24B)	109.7

H(24A)-C(24)-H(24B)	108.2
O(4)-C(25)-O(5)	124.4(2)
O(4)-C(25)-C(23)	124.2(2)
O(5)-C(25)-C(23)	111.38(19)
O(5)-C(26)-H(26A)	110.0
O(5)-C(26)-H(26B)	110.0
H(26A)-C(26)-H(26B)	108.3
C(27)-C(26)-O(5)	108.7(2)
C(27)-C(26)-H(26A)	110.0
C(27)-C(26)-H(26B)	110.0
C(26)-C(27)-H(27)	118.4
C(28)-C(27)-C(26)	123.2(3)
C(28)-C(27)-H(27)	118.4
C(27)-C(28)-H(28A)	114(2)
C(27)-C(28)-H(28B)	121(2)
H(28A)-C(28)-H(28B)	124(3)
O(6)-C(29)-N(3)	123.0(2)
O(6)-C(29)-C(30)	122.3(2)
N(3)-C(29)-C(30)	114.5(2)
N(4)-C(30)-C(29)	112.2(2)
N(4)-C(30)-H(30)	109.1
N(4)-C(30)-C(31)	109.6(2)
C(29)-C(30)-H(30)	109.1
C(29)-C(30)-C(31)	107.7(2)
C(31)-C(30)-H(30)	109.1
C(30)-C(31)-H(31A)	109.5
C(30)-C(31)-H(31B)	109.5
C(30)-C(31)-H(31C)	109.5
H(31A)-C(31)-H(31B)	109.5
H(31A)-C(31)-H(31C)	109.5
H(31B)-C(31)-H(31C)	109.5
O(7)-C(32)-O(8)	126.6(2)
O(7)-C(32)-N(4)	124.5(2)
O(8)-C(32)-N(4)	108.9(2)
O(8)-C(33)-C(34)	111.0(2)
O(8)-C(33)-C(35)	109.0(2)

O(8)-C(33)-C(36)	101.9(2)
C(34)-C(33)-C(36)	109.5(3)
C(35)-C(33)-C(34)	113.4(3)
C(35)-C(33)-C(36)	111.5(3)
C(33)-C(34)-H(34A)	109.5
C(33)-C(34)-H(34B)	109.5
C(33)-C(34)-H(34C)	109.5
H(34A)-C(34)-H(34B)	109.5
H(34A)-C(34)-H(34C)	109.5
H(34B)-C(34)-H(34C)	109.5
C(33)-C(35)-H(35A)	109.5
C(33)-C(35)-H(35B)	109.5
C(33)-C(35)-H(35C)	109.5
H(35A)-C(35)-H(35B)	109.5
H(35A)-C(35)-H(35C)	109.5
H(35B)-C(35)-H(35C)	109.5
C(33)-C(36)-H(36A)	109.5
C(33)-C(36)-H(36B)	109.5
C(33)-C(36)-H(36C)	109.5
H(36A)-C(36)-H(36B)	109.5
H(36A)-C(36)-H(36C)	109.5
H(36B)-C(36)-H(36C)	109.5

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for baran698_0m_a. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + ...\ + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

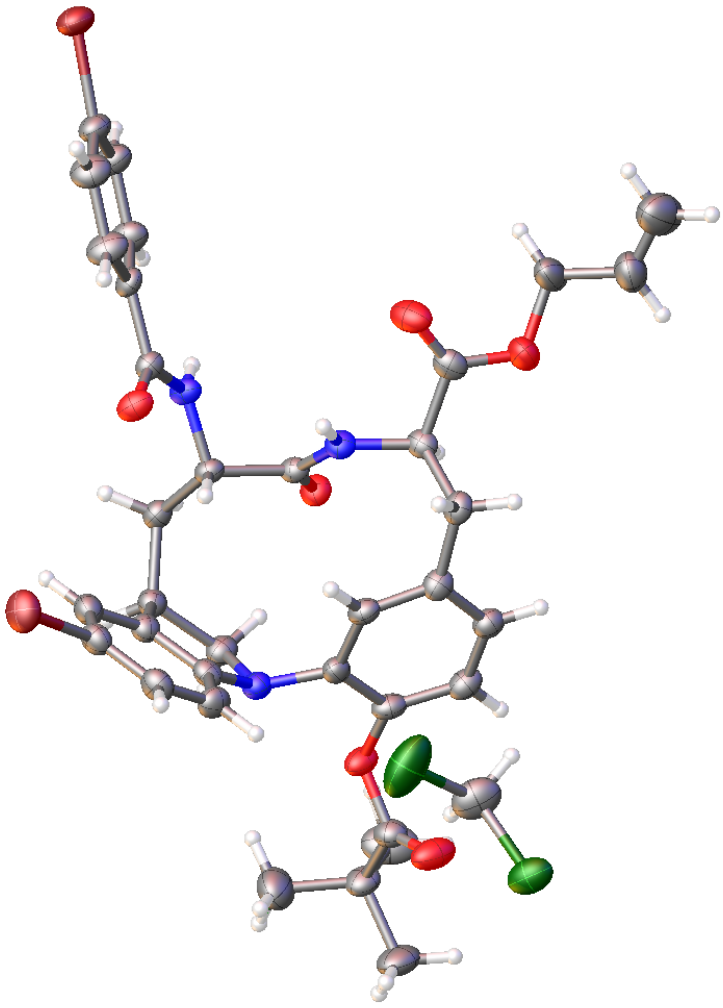
U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}	
O(1)	29(1)	21(1)	39(1)	5(1)	-7(1)	-5(1)
O(2)	22(1)	22(1)	24(1)	4(1)	-2(1)	0(1)
O(3)	16(1)	30(1)	23(1)	-2(1)	1(1)	2(1)
O(4)	24(1)	34(1)	24(1)	0(1)	-2(1)	4(1)
O(5)	28(1)	33(1)	16(1)	5(1)	0(1)	2(1)
O(6)	17(1)	27(1)	30(1)	2(1)	0(1)	-3(1)
O(7)	44(1)	45(1)	31(1)	-8(1)	6(1)	-13(1)
O(8)	37(1)	28(1)	32(1)	-3(1)	-2(1)	-10(1)
N(1)	21(1)	19(1)	18(1)	1(1)	1(1)	-2(1)
N(2)	15(1)	22(1)	16(1)	-1(1)	1(1)	1(1)
N(3)	13(1)	19(1)	21(1)	1(1)	0(1)	1(1)
N(4)	29(1)	21(1)	27(1)	0(1)	2(1)	-4(1)
C(1)	38(2)	48(2)	44(2)	23(2)	-8(1)	-12(1)
C(2)	36(2)	32(1)	27(1)	3(1)	-4(1)	-1(1)
C(3)	33(2)	32(1)	52(2)	-2(1)	-15(1)	7(1)
C(4)	25(1)	25(1)	32(1)	8(1)	-3(1)	-3(1)
C(5)	25(1)	15(1)	29(1)	1(1)	0(1)	2(1)
C(6)	23(1)	18(1)	24(1)	1(1)	-2(1)	-2(1)
C(7)	25(1)	20(1)	27(1)	-4(1)	0(1)	2(1)
C(8)	29(1)	23(1)	20(1)	-5(1)	3(1)	-1(1)
C(9)	25(1)	18(1)	21(1)	-4(1)	-3(1)	-3(1)
C(10)	19(1)	17(1)	23(1)	-1(1)	2(1)	-2(1)
C(11)	20(1)	16(1)	21(1)	1(1)	2(1)	-4(1)
C(12)	22(1)	25(1)	14(1)	3(1)	-2(1)	0(1)
C(13)	28(1)	23(1)	22(1)	4(1)	-2(1)	-2(1)
C(14)	25(1)	32(1)	25(1)	8(1)	-2(1)	-8(1)
C(15)	21(1)	39(1)	22(1)	5(1)	4(1)	-3(1)
C(16)	26(1)	29(1)	19(1)	1(1)	3(1)	-1(1)
C(17)	22(1)	24(1)	14(1)	3(1)	-3(1)	-3(1)
C(18)	20(1)	23(1)	14(1)	-3(1)	-1(1)	-2(1)
C(19)	19(1)	20(1)	19(1)	1(1)	-3(1)	-1(1)

C(20)	15(1)	20(1)	17(1)	-2(1)	2(1)	0(1)
C(21)	15(1)	18(1)	17(1)	1(1)	-2(1)	-1(1)
C(22)	16(1)	17(1)	18(1)	3(1)	2(1)	-2(1)
C(23)	20(1)	24(1)	15(1)	0(1)	1(1)	-1(1)
C(24)	25(1)	24(1)	16(1)	-2(1)	-1(1)	-1(1)
C(25)	20(1)	23(1)	18(1)	-2(1)	0(1)	-5(1)
C(26)	28(1)	37(1)	21(1)	8(1)	-2(1)	0(1)
C(27)	33(2)	40(2)	23(1)	3(1)	-3(1)	-2(1)
C(28)	48(2)	57(2)	32(2)	12(2)	-6(1)	-16(2)
C(29)	18(1)	22(1)	20(1)	-4(1)	0(1)	-2(1)
C(30)	21(1)	23(1)	27(1)	1(1)	3(1)	-2(1)
C(31)	30(1)	27(1)	29(1)	4(1)	-5(1)	-5(1)
C(32)	27(1)	24(1)	28(1)	2(1)	-2(1)	0(1)
C(33)	40(2)	27(1)	35(2)	-7(1)	-10(1)	-3(1)
C(34)	51(2)	44(2)	36(2)	-8(1)	-11(1)	-5(2)
C(35)	52(2)	35(2)	94(3)	-20(2)	-16(2)	7(2)
C(36)	61(2)	44(2)	45(2)	0(2)	-13(2)	-24(2)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$x 10^3) for baran698_0m_a.

x	y	z	U(eq)	
H(2)	2603	2095	4625	21
H(3)	2550	3249	5542	21
H(4)	4827	4970	4383	31
H(1A)	4569	-1254	7773	65
H(1B)	5942	-1580	8108	65
H(1C)	5174	-1996	7493	65
H(2A)	7279	-141	7113	48
H(2B)	7220	-474	7879	48
H(2C)	5874	-115	7548	48
H(3A)	7278	-2030	6778	59
H(3B)	8167	-1605	7344	59
H(3C)	8056	-1308	6565	59
H(7)	6404	-720	4700	28
H(8)	5247	-126	3804	29
H(10)	2552	680	5202	24
H(13)	1932	-658	6503	29
H(14)	-139	-488	7117	33
H(15)	-670	619	7606	33
H(16)	828	1594	7464	30
H(18)	3638	1876	7117	23
H(19A)	5098	1255	6121	23
H(19B)	5218	1022	6924	23
H(20A)	2288	1744	5790	21
H(20B)	2189	2405	6313	21
H(21)	4764	2517	5997	20
H(23)	4735	1569	3808	24
H(24A)	2122	820	3933	26
H(24B)	3309	599	3394	26
H(26A)	2256	2117	1976	34
H(26B)	2915	2848	2262	34

H(27)	4477	1906	1394	38
H(28A)	4060(40)	3400(20)	1415(19)	48(10)
H(28B)	5120(40)	2910(20)	770(20)	68(13)
H(30)	2689	4365	5086	28
H(31A)	2658	4371	3853	43
H(31B)	2579	3564	4127	43
H(31C)	4001	3875	3833	43
H(34A)	5618	5836	6588	66
H(34B)	6663	6494	6651	66
H(34C)	7140	5773	6273	66
H(35A)	4250	6983	5272	91
H(35B)	4826	7289	5993	91
H(35C)	3866	6593	5987	91
H(36A)	7784	6445	5231	75
H(36B)	7306	7181	5580	75
H(36C)	6684	6943	4844	75



Compound ***epi*-SI-21**

Table 1. Crystal data and structure refinement for baran736.

Report date	2019-07-31	
Identification code	baran736_0m_a	
Empirical formula	C36 H36 Br2 Cl2 N3 O6	
Molecular formula	C35 H34 Br2 N3 O6, C H2 Cl2	
Formula weight	837.40	
Temperature	100.15 K	
Wavelength	1.54178 Å	
Crystal system	Hexagonal	
Space group	$P6_1$	
Unit cell dimensions	a = 15.1618(10) Å	α= 90°.
	b = 15.1618(10) Å	β= 90°.
	c = 27.351(2) Å	γ = 120°.
Volume	5445.2(8) $Å^3$	
Z	6	
Density (calculated)	1.532 Mg/m^3	
Absorption coefficient	4.593 mm^{-1}	
F(000)	2550	
Crystal size	0.22 x 0.14 x 0.12 mm^3	
Crystal color, habit	clear colourless block	
Theta range for data collection	1.615 to 72.538°.	
Index ranges	-18<=h<=18, -18<=k<=18, -33<=l<=33	
Reflections collected	51813	
Independent reflections	7165 [R(int) = 0.0547]	
Completeness to theta = 67.679°	100.0 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.3246 and 0.1867	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	7165 / 3 / 452	
Goodness-of-fit on F^2	1.051	
Final R indices [I>2sigma(I)]	R1 = 0.0385, wR2 = 0.1045	
R indices (all data)	R1 = 0.0396, wR2 = 0.1059	
Absolute structure parameter	0.011(6)	
Extinction coefficient	n/a	
Largest diff. peak and hole	1.323 and -0.713 $e.Å^{-3}$	

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for baran736. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
Br(1)	4144(1)	10144(1)	3028(1)	46(1)
Br(2)	-272(1)	13874(1)	3315(1)	44(1)
O(1)	-2231(4)	7758(4)	5303(2)	43(1)
O(2)	-1519(4)	8815(4)	4670(2)	45(1)
O(3)	3016(3)	8020(3)	6059(2)	34(1)
O(4)	2771(5)	6427(4)	6086(2)	50(1)
O(5)	1852(4)	10861(3)	3725(1)	33(1)
O(6)	1406(3)	9913(3)	5534(1)	31(1)
N(1)	3185(4)	8912(4)	5150(2)	29(1)
N(2)	1665(4)	11191(4)	4512(2)	26(1)
N(3)	359(4)	9141(4)	4887(2)	28(1)
C(1)	-4937(9)	6712(8)	5164(5)	77(3)
C(2)	-4077(6)	6909(6)	5341(3)	51(2)
C(3)	-3133(6)	7875(6)	5245(4)	49(2)
C(4)	-1453(5)	8339(5)	5006(2)	32(1)
C(5)	-478(5)	8338(5)	5151(2)	28(1)
C(6)	-552(5)	7294(5)	5059(2)	31(1)
C(7)	357(5)	7288(4)	5278(2)	30(1)
C(8)	302(5)	6868(4)	5738(2)	31(1)
C(9)	1171(5)	7064(5)	5993(2)	33(1)
C(10)	2120(5)	7698(5)	5783(2)	31(1)
C(11)	3241(5)	7297(5)	6213(2)	34(1)
C(12)	4135(5)	7742(5)	6554(2)	37(1)
C(13)	4334(7)	6887(7)	6715(4)	57(2)
C(14)	5086(7)	8577(8)	6296(4)	67(2)
C(15)	3852(9)	8150(9)	7004(3)	67(3)
C(16)	2215(4)	8133(4)	5319(2)	27(1)
C(17)	1308(5)	7865(5)	5065(2)	29(1)
C(18)	3545(5)	9920(5)	5392(2)	31(1)
C(19)	3803(4)	10710(4)	4973(2)	29(1)
C(20)	3755(4)	10109(4)	4527(2)	29(1)

C(21)	4011(5)	10452(5)	4046(2)	34(1)
C(22)	3876(5)	9729(5)	3695(2)	34(1)
C(23)	3508(5)	8708(5)	3807(2)	34(1)
C(24)	3253(5)	8383(5)	4291(2)	35(1)
C(25)	3369(4)	9082(4)	4646(2)	27(1)
C(26)	3115(4)	11204(4)	4930(2)	28(1)
C(27)	2044(4)	10550(4)	4718(2)	25(1)
C(28)	1606(4)	11294(4)	4029(2)	25(1)
C(29)	1197(5)	11977(4)	3871(2)	28(1)
C(30)	1085(5)	12643(5)	4179(2)	36(1)
C(31)	666(6)	13213(5)	4019(3)	39(1)
C(32)	356(5)	13122(5)	3536(2)	35(1)
C(33)	482(6)	12502(6)	3214(3)	44(2)
C(34)	908(6)	11932(6)	3379(2)	42(2)
C(35)	1236(4)	9833(4)	5094(2)	25(1)
Cl(1)	1510(3)	5676(2)	4620(1)	93(1)
Cl(2)	1168(2)	3992(2)	5266(1)	51(1)
C(36)	1277(9)	5205(7)	5214(3)	61(2)

Table 3. Bond lengths [Å] and angles [°] for baran736.

Br(1)-C(22)	1.905(6)	C(19)-C(26)	1.562(8)
Br(2)-C(32)	1.912(6)	C(20)-C(21)	1.396(9)
O(1)-C(3)	1.473(9)	C(20)-C(25)	1.400(8)
O(1)-C(4)	1.338(8)	C(21)-C(22)	1.393(9)
O(2)-C(4)	1.203(9)	C(22)-C(23)	1.392(10)
O(3)-C(10)	1.411(7)	C(23)-C(24)	1.398(9)
O(3)-C(11)	1.368(8)	C(24)-C(25)	1.382(9)
O(4)-C(11)	1.195(9)	C(26)-C(27)	1.532(8)
O(5)-C(28)	1.227(7)	C(27)-C(35)	1.550(8)
O(6)-C(35)	1.225(7)	C(28)-C(29)	1.511(8)
N(1)-C(16)	1.426(8)	C(29)-C(30)	1.388(9)
N(1)-C(18)	1.495(8)	C(29)-C(34)	1.406(9)
N(1)-C(25)	1.406(8)	C(30)-C(31)	1.375(9)
N(2)-C(27)	1.467(7)	C(31)-C(32)	1.385(10)
N(2)-C(28)	1.340(7)	C(32)-C(33)	1.370(10)
N(3)-C(5)	1.438(8)	C(33)-C(34)	1.388(10)
N(3)-C(35)	1.339(8)	Cl(1)-C(36)	1.739(10)
C(1)-C(2)	1.279(14)	Cl(2)-C(36)	1.769(9)
C(2)-C(3)	1.472(11)		
C(4)-C(5)	1.531(8)	C(4)-O(1)-C(3)	114.9(6)
C(5)-C(6)	1.550(8)	C(11)-O(3)-C(10)	118.1(5)
C(6)-C(7)	1.508(8)	C(16)-N(1)-C(18)	113.8(5)
C(7)-C(8)	1.392(9)	C(25)-N(1)-C(16)	119.8(5)
C(7)-C(17)	1.386(9)	C(25)-N(1)-C(18)	108.0(5)
C(8)-C(9)	1.386(9)	C(28)-N(2)-C(27)	121.8(5)
C(9)-C(10)	1.394(10)	C(35)-N(3)-C(5)	124.2(5)
C(10)-C(16)	1.404(8)	C(1)-C(2)-C(3)	122.4(9)
C(11)-C(12)	1.498(9)	C(2)-C(3)-O(1)	111.1(6)
C(12)-C(13)	1.535(10)	O(1)-C(4)-C(5)	112.1(5)
C(12)-C(14)	1.534(12)	O(2)-C(4)-O(1)	123.2(6)
C(12)-C(15)	1.533(12)	O(2)-C(4)-C(5)	124.6(6)
C(16)-C(17)	1.406(8)	N(3)-C(5)-C(4)	108.3(5)
C(18)-C(19)	1.559(8)	N(3)-C(5)-C(6)	111.5(5)
C(19)-C(20)	1.505(8)	C(4)-C(5)-C(6)	112.5(5)

C(7)-C(6)-C(5)	110.0(5)	C(24)-C(25)-N(1)	127.6(6)
C(8)-C(7)-C(6)	121.0(5)	C(24)-C(25)-C(20)	120.8(6)
C(17)-C(7)-C(6)	119.7(5)	C(27)-C(26)-C(19)	117.7(4)
C(17)-C(7)-C(8)	118.3(6)	N(2)-C(27)-C(26)	110.9(4)
C(9)-C(8)-C(7)	121.6(6)	N(2)-C(27)-C(35)	106.5(4)
C(8)-C(9)-C(10)	118.9(6)	C(26)-C(27)-C(35)	114.4(4)
C(9)-C(10)-O(3)	120.1(5)	O(5)-C(28)-N(2)	123.3(5)
C(9)-C(10)-C(16)	121.7(5)	O(5)-C(28)-C(29)	120.8(5)
C(16)-C(10)-O(3)	117.8(6)	N(2)-C(28)-C(29)	115.8(5)
O(3)-C(11)-C(12)	111.3(5)	C(30)-C(29)-C(28)	124.5(5)
O(4)-C(11)-O(3)	123.0(6)	C(30)-C(29)-C(34)	117.8(6)
O(4)-C(11)-C(12)	125.7(6)	C(34)-C(29)-C(28)	117.7(5)
C(11)-C(12)-C(13)	108.3(6)	C(31)-C(30)-C(29)	121.8(6)
C(11)-C(12)-C(14)	110.5(6)	C(30)-C(31)-C(32)	118.7(6)
C(11)-C(12)-C(15)	108.0(6)	C(31)-C(32)-Br(2)	119.2(5)
C(14)-C(12)-C(13)	109.2(7)	C(33)-C(32)-Br(2)	119.2(5)
C(15)-C(12)-C(13)	108.9(7)	C(33)-C(32)-C(31)	121.7(6)
C(15)-C(12)-C(14)	112.0(8)	C(32)-C(33)-C(34)	119.0(6)
C(10)-C(16)-N(1)	120.2(5)	C(33)-C(34)-C(29)	120.8(7)
C(10)-C(16)-C(17)	117.0(5)	O(6)-C(35)-N(3)	124.7(5)
C(17)-C(16)-N(1)	122.3(5)	O(6)-C(35)-C(27)	121.9(5)
C(7)-C(17)-C(16)	122.3(5)	N(3)-C(35)-C(27)	113.4(4)
N(1)-C(18)-C(19)	106.5(5)	Cl(1)-C(36)-Cl(2)	113.3(5)
C(18)-C(19)-C(26)	116.9(5)		
C(20)-C(19)-C(18)	102.3(5)		
C(20)-C(19)-C(26)	112.8(5)		
C(21)-C(20)-C(19)	128.3(5)		
C(21)-C(20)-C(25)	121.1(6)		
C(25)-C(20)-C(19)	110.6(5)		
C(22)-C(21)-C(20)	116.9(6)		
C(21)-C(22)-Br(1)	118.5(5)		
C(23)-C(22)-Br(1)	118.7(5)		
C(23)-C(22)-C(21)	122.8(6)		
C(22)-C(23)-C(24)	119.2(6)		
C(25)-C(24)-C(23)	119.1(6)		
C(20)-C(25)-N(1)	111.5(5)		

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for baran736. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + ...\ + 2\ h\ k\ a^*\ b^*\ U^{12}]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
Br(1)	52(1)	54(1)	28(1)	1(1)	6(1)	22(1)
Br(2)	48(1)	41(1)	52(1)	7(1)	-4(1)	28(1)
O(1)	35(2)	44(3)	51(3)	6(2)	10(2)	21(2)
O(2)	49(3)	70(3)	29(2)	1(2)	-2(2)	40(3)
O(3)	36(2)	28(2)	36(2)	0(2)	-11(2)	15(2)
O(4)	55(3)	39(3)	62(3)	-7(2)	-24(3)	27(2)
O(5)	50(3)	35(2)	21(2)	-4(2)	-4(2)	26(2)
O(6)	34(2)	36(2)	21(2)	1(2)	-3(2)	17(2)
N(1)	30(2)	31(2)	28(2)	2(2)	-1(2)	17(2)
N(2)	36(3)	33(2)	18(2)	-1(2)	0(2)	23(2)
N(3)	31(2)	34(2)	20(2)	-2(2)	-5(2)	18(2)
C(1)	62(6)	51(5)	116(10)	-2(5)	-6(6)	27(5)
C(2)	40(4)	45(4)	60(5)	5(3)	9(3)	15(3)
C(3)	32(3)	37(4)	77(5)	4(3)	6(3)	16(3)
C(4)	34(3)	38(3)	27(3)	-9(2)	-2(2)	21(3)
C(5)	30(3)	36(3)	19(2)	-2(2)	-2(2)	18(2)
C(6)	36(3)	32(3)	21(3)	0(2)	0(2)	14(2)
C(7)	34(3)	29(3)	23(3)	-3(2)	-5(2)	13(2)
C(8)	35(3)	29(3)	29(3)	-1(2)	-4(2)	16(2)
C(9)	44(3)	31(3)	25(3)	-1(2)	-6(2)	20(3)
C(10)	39(3)	33(3)	27(3)	-3(2)	-9(2)	24(3)
C(11)	36(3)	40(3)	31(3)	1(2)	-5(2)	23(3)
C(12)	39(3)	39(3)	34(3)	3(3)	-10(3)	21(3)
C(13)	55(4)	52(4)	71(6)	8(4)	-18(4)	33(4)
C(14)	41(4)	66(6)	81(6)	15(5)	-2(4)	18(4)
C(15)	83(6)	88(7)	50(5)	-29(5)	-33(5)	56(6)
C(16)	27(3)	24(2)	31(3)	0(2)	0(2)	14(2)
C(17)	37(3)	31(3)	23(3)	1(2)	-3(2)	22(2)
C(18)	32(3)	32(3)	29(3)	-1(2)	-7(2)	15(2)
C(19)	27(2)	30(3)	29(3)	0(2)	-2(2)	13(2)
C(20)	26(3)	32(3)	28(3)	0(2)	-3(2)	14(2)

C(21)	28(3)	37(3)	35(3)	4(2)	2(2)	15(2)
C(22)	30(3)	42(3)	29(3)	0(2)	3(2)	18(3)
C(23)	33(3)	41(3)	30(3)	-5(2)	2(2)	19(3)
C(24)	36(3)	27(3)	41(3)	-3(2)	-4(3)	15(3)
C(25)	22(2)	32(3)	25(3)	-4(2)	-4(2)	12(2)
C(26)	32(3)	26(2)	26(3)	0(2)	0(2)	15(2)
C(27)	31(3)	26(2)	22(3)	-1(2)	-2(2)	18(2)
C(28)	29(3)	23(2)	22(3)	-2(2)	-3(2)	12(2)
C(29)	34(3)	26(3)	21(2)	5(2)	2(2)	15(2)
C(30)	46(3)	42(3)	30(3)	-3(3)	0(2)	29(3)
C(31)	52(4)	40(3)	33(3)	3(3)	1(3)	29(3)
C(32)	37(3)	31(3)	39(3)	5(2)	-5(3)	19(3)
C(33)	54(4)	51(4)	32(3)	-2(3)	-11(3)	31(3)
C(34)	60(4)	48(4)	27(3)	-4(3)	-11(3)	33(3)
C(35)	29(3)	30(3)	19(3)	0(2)	-2(2)	18(2)
Cl(1)	137(3)	48(1)	74(2)	5(1)	4(2)	31(1)
Cl(2)	76(1)	56(1)	35(1)	-4(1)	-2(1)	44(1)
C(36)	85(6)	56(5)	51(5)	-13(4)	-12(4)	42(5)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$x 10^3) for baran736.

	x	y	z	U(eq)
H(2)	1560(60)	11550(50)	4730(20)	32
H(3)	240(60)	9140(60)	4574(8)	33
H(1A)	-4983	7197	4962	92
H(1B)	-5531	6079	5235	92
H(2A)	-4046	6415	5542	61
H(3A)	-3109	8493	5157	59
H(5)	-360	8493	5508	33
H(6A)	-1185	6746	5208	37
H(6B)	-579	7165	4703	37
H(8)	-347	6436	5879	37
H(9)	1120	6771	6306	40
H(13A)	3744	6371	6897	85
H(13B)	4939	7170	6924	85
H(13C)	4446	6573	6426	85
H(14A)	5212	8299	5997	100
H(14B)	5675	8820	6514	100
H(14C)	4979	9145	6211	100
H(15A)	3652	8645	6901	101
H(15B)	4440	8482	7224	101
H(15C)	3282	7585	7175	101
H(17)	1349	8088	4737	35
H(18A)	4157	10106	5593	37
H(18B)	3007	9894	5606	37
H(19)	4523	11269	5017	35
H(21)	4267	11146	3962	41
H(23)	3431	8238	3558	41
H(24)	3003	7690	4375	42
H(26A)	3046	11429	5261	33
H(26B)	3479	11822	4725	33
H(27)	2080	10123	4449	30

H(30)	1304	12706	4509	43
H(31)	590	13660	4236	47
H(33)	281	12463	2882	52
H(34)	1006	11507	3157	51
H(36A)	639	5161	5332	73
H(36B)	1837	5690	5428	73

Table 6. Hydrogen bonds for baran736 [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
N(2)-H(2)...O(5)#1	0.875(15)	2.15(3)	2.977(6)	158(7)
N(3)-H(3)...O(6)#2	0.874(15)	2.10(4)	2.918(6)	155(7)

Symmetry transformations used to generate equivalent atoms:

#1 x-y+1,x+1,z+1/6 #2 y-1,-x+y,z-1/6

Tryptorubin biosynthesis

Bioinformatic analysis

Genome sequences were analyzed using Geneious 11.1.3, antiSMASH5.0 [37] and PRISM3. [38] The genome sequences of *Streptomyces* sp. CLI2509 (CP021118.1), *Streptomyces* sp. SPB78 (GG657742.1) and *Streptomyces* sp. Tu6071 (NZ_AFHJ00000000.1) were retrieved from GenBank, translated in all six reading frames and screened for the core peptide sequence (AWYIWY). The genomes of tryptorubin producers[12] were compared to closely related *Streptomyces* species as determined by 16S rRNA homology to verify that the putative *trp* BGC is only present in tryptorubin producers. Homologous gene clusters were identified by Blast-search of the P450 protein sequence and manual annotation of the precursor gene. Homologous precursor and P450 sequences were retrieved from GenBank and MUSCLE aligned. In the case of P450s, characterized P450s not involved in tryptorubin biosynthesis were retrieved from GenBank and aligned with the tryptorubin associated P450s. A maximum likelihood phylogenetic tree (bootstrap replicas 1000) was constructed from the P450 alignment using Geneious and visualized using iTOL.[39] Transcription start sites were determined using BPROM. Genome neighborhood diagrams were generated using the EFI Genome Neighborhood tool.

Cultivation, Extraction and Analysis

Xanthomonas sp. Leaf148 was inoculated into ISP2 medium (6 x 1 L) and cultured in 2.5 L Ultra-High-Yield baffled flasks at 28 ºC and 220 rpm shaking for 6 days with HP20 beads (4% by weight). Cultures were subsequently filtered, the beads washed with water and methanol and subsequently extracted with acetone overnight. Acetone was removed under reduced pressure, the extract resuspended in methanol and subjected to liquid chromatography-high resolution electrospray ionization tandem mass spectrometry using an Agilent 1290 Infinity HPLC connected to an Agilent 6530 Accurate-Mass Q-TOF mass spectrometer. The crude extract was separated by reversed-phase HPLC (Phenomenex Kinetex 2.6 µm, C18 100 A) column (100 x 2.1 mm), a flow rate of 0.3 mL and a water to acetonitrile gradient over 22 mins (0-2 min 10% ACN, 2-18 min 10-100 % ACN, 18-22 min 100% ACN). The mass spectrometer was operated in positive mode, the mass range was set from 500 to 100 Da and the collision energy from 20-50. Raw data were converted into mzxml format and subjected to molecular network analysis using GNPS[40]. The data was filtered by removing all MS/MS peaks within +/- 17 Da of the

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precursor *m/z*. MS/MS spectra were window filtered by choosing only the top 6 peaks in the +/- 50 Da window throughout the spectrum. The data was then clustered with MS-Cluster with a parent mass tolerance of 2.0 Da and a MS/MS fragment ion tolerance of 0.5 Da to create consensus spectra. A network was then created where edges were filtered to have a cosine score above 0.5 and more than 4 matched peaks. The resulting network was visualized using Cytoscape 3.7.1.

Table S14: Proteins encoded in the tryptorubin (*trp*) BGC including surrounding genes, deduced protein functions and accession numbers.

protein name	residues	function	similarity/ identity (%)	closest homolog	accession number
	103	4 α-hydroxytetrahydrobiopterin dehydratase	99/99	*Streptomyces* sp. GZWMJZ-114	WP_129284629.1
	238	SAM dependent methyltransferase	100/99	*Streptomyces* sp. SolWspMP-sol7th	WP_093854812.1
	261	oxidoreductase	99/99	*Streptomyces* sp. SolWspMP-sol7th	WP_093854814.1
	607	Long chain fatty acid CoA ligase	99/99	*Streptomyces* sp. SolWspMP-sol7th	WP_093854811.1
TrpA	26	precursor peptide (hypothetical protein)	69/69	*Streptomyces misionensis*	SEB93745.1
TrpB	348	cytochrome P450	99/99	*Streptomyces* sp. LcepLS	WP_093582223.1
	192	N-acetyl transferase	92/92	*Streptomyces* sp. SolWspMP-sol7th	WP_093854810.1

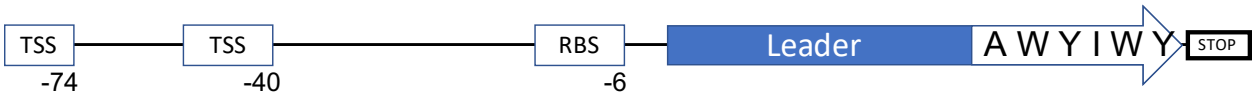

Figure S17: Graphical representation of *in-silico* analyses of the previously unannotated *trpA* gene. *trpA* putatively encodes the tryptorubin precursor peptide, is directly followed by a stop codon and has the typical features of a prokaryotic gene including a ribosomal binding site (RBS, Shine-Dalgarno sequence) and two elements required to initiate transcription (TSS, transcription start site).

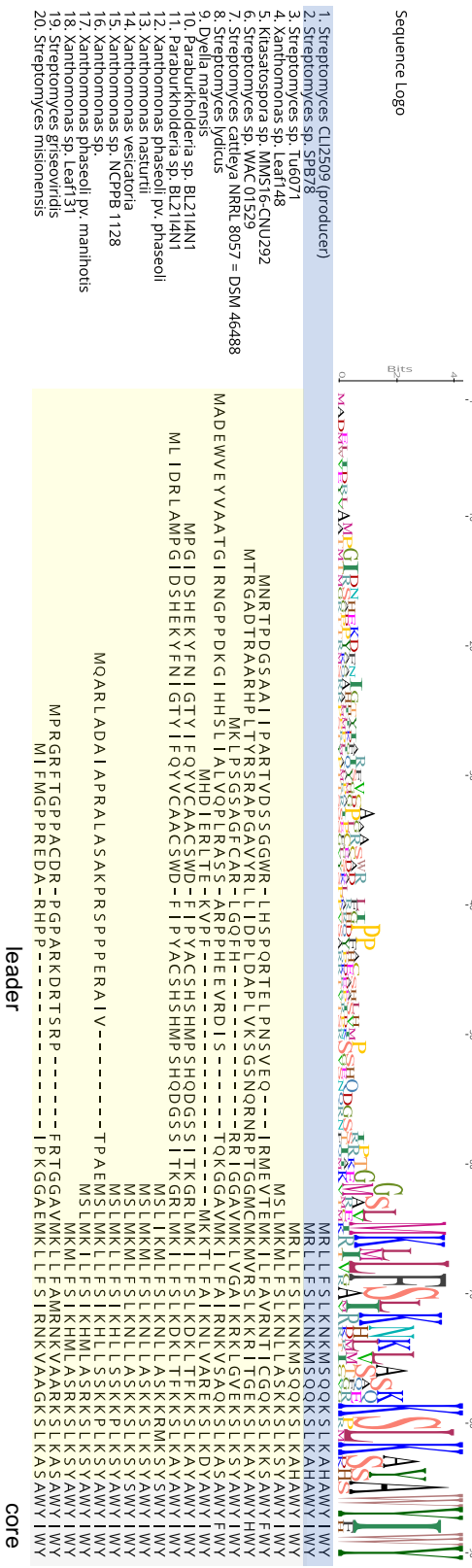

Figure S18: Selection of MUSCLE aligned tryptorubin-like precursor peptides. Dark blue bar highlights previously reported tryptorubin producers. Yellow: leader peptide; grey: core peptide.

Figure S19, see Supplementary Datafile S19. Caption: Genome neighborhood analysis of putative tryptorubin-like peptide producers. TrpB was used as query for the analysis. Cytochrome P450s are highlighted in red. Since TrpA homologs are usually not annotated as proteins, most homologs are missed in the Genome Neighborhood Diagram. The diagram shows the wide variety of putative tryptorubin-like peptide producers and suggests that the minimal *trp* BGC consist of only the precursor gene *trpA* and its associated gene, *trpB*, encoding a cytochrome P450. No other gene is conserved in other putative *trp* BGCs.

Figure S20, see Supplementary Datafile S20. Caption: Phylogenetic analysis of 550 cytochrome P450 amino acid sequences. The top 50 hits from the genome neighborhood analysis were compared to a representative group of 500 P450 enzymes associated with bacterial secondary metabolism, retrieved from the antiSMASH database. The top 250 hits from both smCoG hit queries (SMCOG1007 and SMCOG1034) were selected, MUSCLE aligned with the P450s from tryptorubin-like BGCs and a maximum likelihood phylogenetic tree with 1000 bootstrap replicas computed. The *trp*-encoded P450 group together to form a unique phylogenetic clade of enzymes that are likely responsible for the unusual C-C and C-N bridge formations.

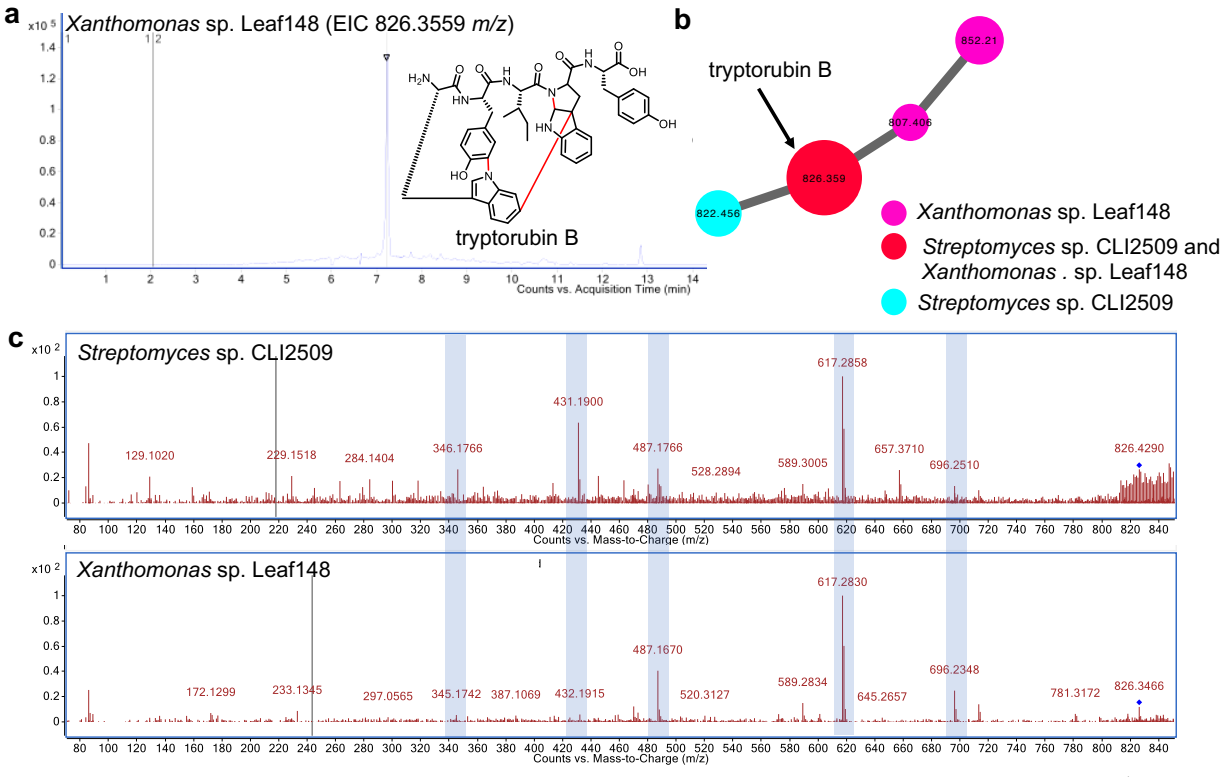

Figure S21: *Xanthomonas* sp. Leaf148 produces a tryptorubin-like peptide. a) Extracted ion chromatogram of *m/z* 826.3559 $[M+H]^+$ (tryptorubin B). b) Detail of molecular network representation of data-dependent MS/MS data of *Xanthomonas* sp. Leaf148 and *Streptomyces* sp. CLI2509 (known tryptorubin B producer) extracts. Nodes represent molecules that share MS/MS fragmentation patterns and interconnecting edges

indicate structural similarity based on common MS/MS features. The detection of a metabolite with a *m/z* of 826.359 in both the reported tryptorubin B producer and *Xanthomonas* sp. Leaf148 indicates that *Xanthomonas* sp. Leaf148 produces a tryptorubin-like metabolite. c) Comparison of MS/MS spectra from both producers. Blue bars highlight characteristic high-intensity fragment ions common for both MS/MS spectra. Although the fragmentation pattern and associated *m/z* values are in excellent agreement, the retention times differ by 3%, which could indicate a slight structural difference.