

Supplementary Information for

**Diastereoselective Synthesis and Biological Evaluation
of Enantiomerically Pure Tricyclic Indolines**

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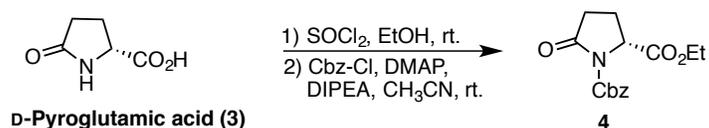
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I. Compound Synthesis and Characterization

Unless otherwise noted, reagents were obtained commercially and used without further purification. Toluene and tetrahydrofuran (THF) were distilled from sodium under a nitrogen atmosphere. Thin-layer chromatography (TLC) analysis of reaction mixtures was performed on Dynamicadsorbents silica gel F-254 TLC plates. Flash chromatography was carried out on Zeoprep 60 ECO silica gel. ¹H and ¹³C NMR spectra were recorded on Varian INOVA (400, 500 MHz) and Bruker Avance-III (300 MHz) spectrometers. ¹H NMR and ¹³C NMR are referenced to the residual solvent peak at 7.26 ppm and 77.16 ppm (CDCl₃) respectively. Mass spectral and analytical data were obtained with a PE SCIEX/ABI API QSTAR Pulsar iHybrid LC/MS/MS (Applied Biosystems) operated by the Central Analytical Laboratory, University of Colorado at Boulder. Optical rotations were measured on a JASCO P-1030 and are reported as averages of ten data points.

General procedure for the preparation of alkyne-tethered indoles

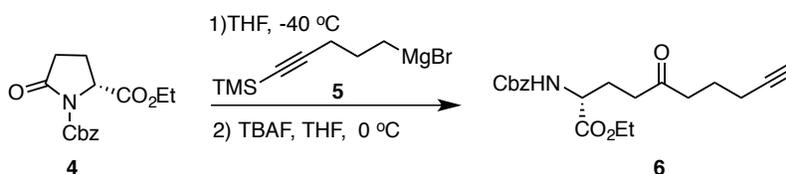
(The synthesis of **10** was carried out from D-pyrroglutamic acid (shown) and its enantiomer from L-pyrroglutamic acid.)



A suspension of D-pyrroglutamic acid (1.27 g, 10 mmol, 1.0 eq.) was prepared in 4 mL of anhydrous ethanol under argon. Thionylchloride (0.87 mL, 12 mmol, 1.2 eq.) was added dropwise at 0 °C. The reaction was then allowed to slowly warm to room temperature, forming a clear yellow solution overnight. The solvent was removed by rotary evaporator and the residue dissolved in 20 mL of ethyl acetate and stirred over excess potassium carbonate for 20 minutes then filtered and dried over sodium sulfate. The solution was passed through a short plug of silica, rinsed with ethyl acetate, and solvent removed before continuing to the next step.

The crude intermediate (0.80 g, 5.1 mmol, 1.0 eq.) was dissolved in 20 mL of acetonitrile in the presence of 4-dimethylaminopyridine (312 mg, 2.6 mmol, 0.5 eq.) and diisopropylethylamine (1.27 mL, 7.7 mmol, 1.5 eq.) at 0 °C. Carboxybenzylchloride (1.1 mL, 7.7 mmol, 1.5 eq.) was

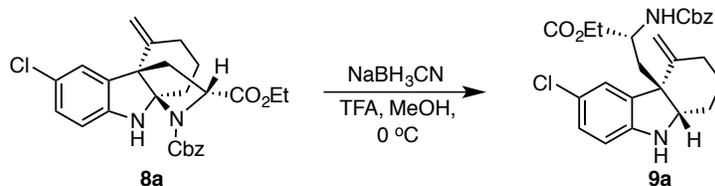
added dropwise and the reaction left to stir and slowly warm to room temperature overnight. Ethyl acetate was added to dilute the solution, then it was washed with brine and the organic phase dried over sodium sulfate and solvent removed. The dark orange residue was purified by silica column chromatography (gradient elution hexanes:ethyl acetate 2:1 to 1:1 v/v) yielding compound **4** as a yellow oil (757 mg, 51%).



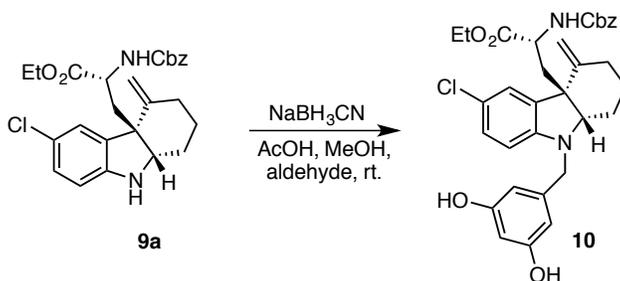
Magnesium (360 mg, 15 mmol, 1.3 eq.) was placed in a flask with stir bar and heated with a heat gun to remove water. A few crystals of elemental iodine were added as well as 8 mL of diethyl ether, then the flask was sealed under argon and stirred vigorously for several minutes before adding a few drops of the alkyl bromide. The reaction was gently heated to reflux with a heat gun until the reaction initiated, indicated by a color change from red/purple to colorless. The remaining alkyl bromide (2.53 g, 11.54 mmol, 1.0 eq.) was added dropwise to prepare the Grignard reagent **5** which was used in the next step immediately.

Compound **4** (1.94 g, 6.66 mmol, 1.0 eq.) was dissolved in 30 mL of tetrahydrofuran and cooled to -40 °C under argon. The Grignard solution prepared previously was added dropwise then the solution stirred for 2 hours before being quenched by dropwise addition of acetic acid/methanol (1:1 v/v, 2 mL). The mixture was diluted in ethyl acetate and extracted with water, saturated sodium bicarbonate solution, then brine. The organic phase was dried over sodium sulfate and solvent removed.

The crude material from the previous step was dissolved in 15 mL of tetrahydrofuran and cooled to 0 °C under argon. Tetra-*n*-butylammonium fluoride (8 mL [1 M], 8 mmol, 1.2 eq.) was added dropwise and the mixture left to stir for 1 hour over ice before being quenched by the addition of water. The mixture was extracted with ethyl acetate, washed with brine, and dried over sodium sulfate. The solution was concentrated then the residue purified by silica column chromatography (gradient elution hexanes:ethyl acetate 5:1 to 3:1) yielding **6** as a yellow oil (6.4 g, 56% for two steps).

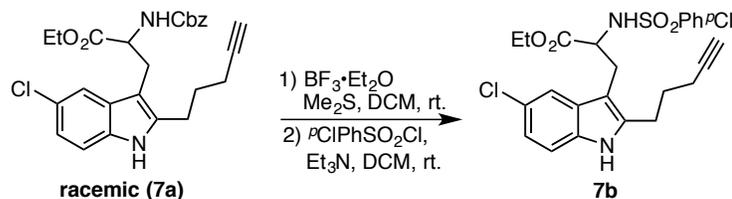


A solution of the tetracyclic indoline **8a** (10 mg, 0.021 mmol, 1.0 eq.) in 2.0 mL of anhydrous methanol was prepared with trifluoroacetic acid (4 μL , 0.042 mmol, 2.0 eq.) and sodium cyanoborohydride (5.4 mg, 0.086 mmol, 4.0 eq.) at $0\text{ }^\circ\text{C}$ under argon. The mixture was stirred for 1 hour at room temperature and monitored by TLC. The solvent was removed and the residue dissolved in ethyl acetate and washed with saturated sodium bicarbonate then brine and the organic phase dried over sodium sulfate. The solution was filtered through celite and the solvent removed. The crude residue was purified by silica column chromatography (100% ethyl acetate) yielding the ring-opened product **9a** (7 mg, 70%) as a clear oil.



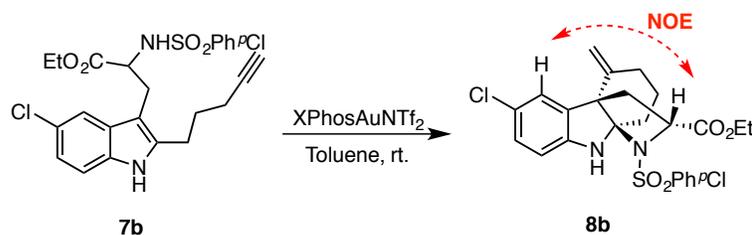
The ring-opened indoline **9a** (7 mg, 0.15 mmol, 1.0 eq.) was dissolved in 1.0 mL of methanol with sodium cyanoborohydride (4 mg, 0.06 mmol, 4.0 eq.), glacial acetic acid (2 μL , 0.03 mmol, 2.0 eq.), and 3,5-dihydroxybenzaldehyde (2.5 mg, 0.018 mmol, 1.2 eq.). The reaction was left to stir overnight and monitored by TLC until starting material was consumed. Additional sodium cyanoborohydride was needed to push the reaction to completion in some cases. [Product purified by prepared TLC to remove regioisomers 2:1 hexanes:ethyl acetate] The reaction was worked up as in the previous step and purified by preparative TLC (2:1 hexanes:ethyl acetate) affording the biological probe **10** as a colorless oil (4 mg, 45%).

Characterization of relative configurations of cyclic product (from racemic pyroglutamic acid)



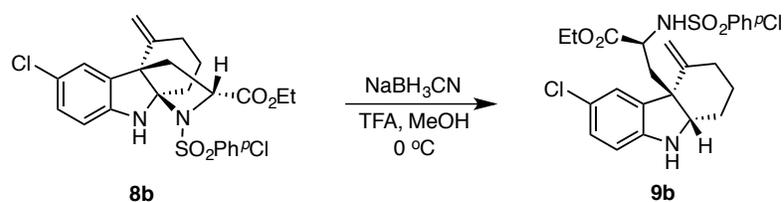
A solution of **7a** (70 mg, 0.15 mmol, 1.0 eq.) was prepared in anhydrous dichloromethane with boron trifluoride diethyl etherate (0.19 mL, 1.5 mmol, 10 eq.) and dimethyl sulfide (0.3 mL, 4.05 mmol, 27 eq.) was added dropwise at 0 °C under argon. The resulting mixture was stirred at room temperature overnight. When TLC showed no remaining starting material, the mixture was quenched with saturated sodium bicarbonate solution and extracted with dichloromethane three times. The combined organic phases were washed with brine, dried over sodium sulfate, filtered with celite, and solvent removed to yield a crude product which was used in the next step.

The crude material (30 mg, 0.09 mmol, 1.0 eq.) was dissolved in 8 mL of anhydrous dichloromethane. Triethyl amine (37 μ L, 0.27 mmol, 3.0 eq.) and 4-chlorobenzenesulfonyl chloride (23 mg, 0.11 mmol, 1.2 eq.) were added dropwise at 0 °C then the reaction was left to stir and slowly warm to room temperature overnight. The reaction was quenched and extracted as in the previous step and the residue purified by silica column chromatography (gradient elution hexanes:ethyl acetate 6:1 to 5:1 to 4:1) to yield the modified indole **7b** as a colorless oil (16 mg, 34%).



The indole **7b** (26 mg, 0.051 mmol, 1.0 eq.) was dissolved in 6.0 mL of toluene. The catalyst XPhosAuNTf₂ (5 mg, 0.005 mmol, 0.1 eq.) was added then allowed to stir at room temperature for 2 hours. The solvent was removed and the residue purified by silica column chromatography

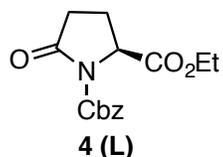
(gradient elution hexanes:ethyl acetate 8:1 to 7:1 to 6:1) affording the tetracyclic product **8b** as a colorless oil (18 mg, 69%).



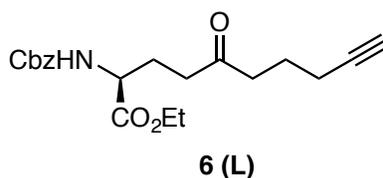
The ring opening procedure was followed as before (see **8a** to **9a**) with the same workup. The product **9b** was purified by silica column chromatography (gradient elution hexanes:ethyl acetate 6:1 to 5:1 to 4:1), The product **9b** was collected as an colorless oil (14 mg, 78%).

Characterization data (from L-pyroglutamic acid)

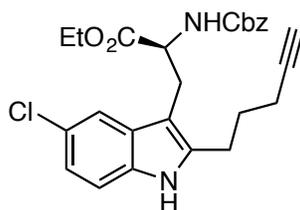
All characterization standards were prepared from L-pyroglutamic acid. "L" is used to declare the starting material of the representative characterization standards for clarity. **7b**, **8b**, and **9b** were characterized as racemic mixtures.



$[\alpha]_D^{20} = -40.1^\circ$ (c 0.82, CHCl_3); $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 7.40 – 7.28 (m, 5H), 5.33 – 5.18 (m, 2H), 4.65 (dd, $J = 9.5, 2.7$ Hz, 1H), 4.13 (qd, $J = 7.1, 3.0$ Hz, 2H), 2.64 (ddd, $J = 17.5, 10.5, 9.4$ Hz, 1H), 2.50 (ddd, $J = 17.6, 9.3, 3.3$ Hz, 1H), 2.34 (ddt, $J = 13.4, 10.4, 9.4$ Hz, 1H), 2.06 (dddd, $J = 13.4, 9.5, 3.3, 2.7$ Hz, 1H), 1.18 (t, $J = 7.1$ Hz, 3H); $^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ 173.05, 171.07, 150.94, 135.05, 128.63, 128.52, 128.24, 68.39, 61.90, 58.83, 31.10, 21.91, 14.09. MS (ESI) m/z calculated $[\text{M}+\text{Na}]^+$ 314.1, found 314.1. HRMS (ESI) m/z calculated for $\text{C}_{15}\text{H}_{17}\text{NNaO}_5$ $[\text{M}+\text{Na}]^+$ 314.1004, found 314.1004.

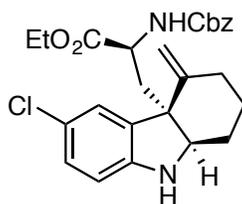


$[\alpha]_D^{20} = +7.9^\circ$ (c 1.03, CHCl_3); $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 7.34 – 7.27 (m, 5H), 5.52 (d, $J = 8.2$ Hz, 1H), 5.07 (d, $J = 2.3$ Hz, 2H), 4.30 (td, $J = 8.4, 4.9$ Hz, 1H), 4.20 – 4.12 (m, 2H), 2.59 – 2.39 (m, 4H), 2.18 (td, $J = 6.9, 2.6$ Hz, 2H), 2.15 – 2.08 (m, 1H), 1.95 (t, $J = 2.7$ Hz, 1H), 1.93 – 1.83 (m, 1H), 1.74 (p, $J = 7.1$ Hz, 2H), 1.24 (t, $J = 7.2$ Hz, 3H); $^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ 208.93, 172.06, 156.01, 136.24, 128.52, 128.19, 128.11, 83.53, 69.20, 66.97, 61.62, 53.38, 41.11, 38.47, 26.39, 22.13, 17.70, 14.15; MS (ESI) m/z calculated $[\text{M}+\text{Na}]^+$ 382.2, found 382.1, HRMS (ESI) m/z calculated for $\text{C}_{20}\text{H}_{25}\text{NNaO}_5$ $[\text{M}+\text{Na}]^+$ 382.1630, found 382.1631.



7a (L)

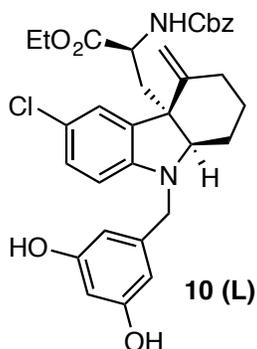
$[\alpha]_D^{20} = +33.3^\circ$ (c 0.92, CHCl_3); m. p. 110-115 °C; $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 8.08 (s, 1H), 7.41 (d, $J = 1.9$ Hz, 1H), 7.39 – 7.27 (m, 5H), 7.15 (d, $J = 8.5$ Hz, 1H), 7.05 (dd, $J = 8.6, 2.0$ Hz, 1H), 5.34 (d, $J = 8.3$ Hz, 1H), 5.09 (s, 2H), 4.63 (dt, $J = 8.3, 5.7$ Hz, 1H), 4.23 – 3.99 (m, 2H), 3.22 (d, $J = 5.8$ Hz, 2H), 2.80 (t, $J = 7.5$ Hz, 2H), 2.17 (dt, $J = 6.9, 3.2$ Hz, 2H), 2.05 (m, $J = 3.0$ Hz, 1H), 1.89 – 1.74 (m, 2H), 1.19 (t, $J = 7.1$ Hz, 3H); $^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ 172.01, 155.70, 137.86, 136.35, 133.77, 129.91, 128.64, 128.29, 128.25, 125.38, 121.78, 117.84, 111.54, 106.19, 83.63, 69.92, 67.09, 61.86, 54.69, 28.12, 27.45, 24.64, 17.90, 14.05; MS (ESI) m/z calculated for $[\text{M}+\text{H}]^+$ 467.2, found 467.1, HRMS (ESI) m/z calculated for $\text{C}_{26}\text{H}_{28}\text{ClN}_2\text{O}_4$ $[\text{M}+\text{H}]^+$ 467.1738, found 467.1729.



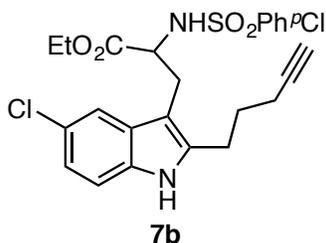
9a (L)

$[\alpha]_D^{20} = +67.2^\circ$ (c 0.53, CHCl_3); $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 7.40 – 7.28 (m, 5H), 7.08 – 6.98 (m, 2H), 6.58 (d, $J = 8.2$ Hz, 1H), 5.14 (d, $J = 9.2$ Hz, 1H), 5.08 (s, 2H), 4.89 (s, 1H), 4.65 (s, 1H), 4.39 (d, $J = 7.8$ Hz, 1H), 4.18 – 4.07 (m, 1H), 4.05 – 3.95 (m, 1H), 3.90 (s, 1H), 2.31 (dd, J

= 15.1, 4.6 Hz, 1H), 2.24 – 2.06 (m, 3H), 1.79 – 1.67 (m, 3H), 1.59 – 1.54 (s, 1H), 1.24 (t, J = 8.0 Hz, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 172.78, 155.70, 149.87, 148.78, 136.37, 134.80, 128.65, 128.33, 128.21, 127.91, 125.01, 123.44, 112.52, 111.44, 67.20, 64.45, 61.79, 53.02, 52.02, 37.42, 32.30, 29.85, 27.49, 22.03, 14.14; MS (ESI) m/z calculated for [M+H]⁺ 469.2, found 469.2, HRMS (ESI) m/z calculated for C₂₆H₃₀ClN₂O₄ [M+H]⁺ 469.1894, found 469.1884.

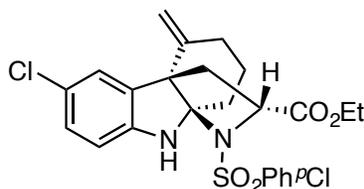


[α]_D²⁰ = +29.2° (c 0.42, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.35 – 7.21 (m, 5H), 7.03 – 6.94 (m, 2H), 6.38 – 6.14 (m, 6H), 5.29 (d, J = 9.4 Hz, 1H), 5.10 – 4.98 (m, 2H), 4.90 (s, 1H), 4.76 (s, 1H), 4.60 – 4.52 (m, 1H), 4.40 (d, J = 16.7 Hz, 1H), 4.17 – 3.97 (m, 3H), 3.90 (t, J = 4.4 Hz, 1H), 2.47 – 2.37 (m, 1H), 2.19 – 2.09 (m, 3H), 1.74 – 1.40 (m, 4H), 1.25 (t, J = 8.0 Hz, 3H); ¹³C NMR (101 MHz, cdcl₃) δ 172.92, 157.53, 156.32, 149.82, 149.53, 140.02, 135.73, 133.27, 128.65, 128.43, 128.32, 128.19, 124.67, 121.90, 111.94, 108.51, 106.76, 101.91, 67.69, 66.51, 62.22, 52.49, 52.27, 47.17, 37.91, 32.40, 29.84, 23.84, 21.26, 14.06; MS (ESI) m/z calculated for [M+H]⁺ 591.2, found 591.2, HRMS (ESI) m/z calculated for C₃₃H₃₆ClN₂O₆ [M+H]⁺ 591.2262, found 591.2257.



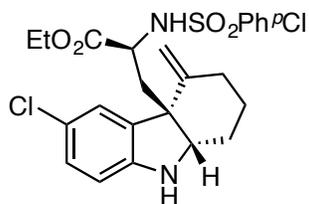
¹H NMR (500 MHz, CDCl₃) δ 7.91 (s, 1H), 7.55 (d, J = 8.6 Hz, 2H), 7.28 (d, J = 8.6 Hz, 2H), 7.25 (d, J = 1.9 Hz, 1H), 7.16 (d, J = 8.5 Hz, 1H), 7.07 (dd, J = 8.5, 2.0 Hz, 1H), 5.16 (d, J = 9.4 Hz, 1H), 4.17 – 4.11 (m, 1H), 4.05 – 3.91 (m, 2H), 3.17 (dd, J = 14.8, 5.7 Hz, 1H), 3.07 (dd, J = 14.8, 6.9 Hz, 1H), 2.95 – 2.81 (m, 2H), 2.24 (td, J = 6.7, 2.7 Hz, 2H), 2.10 (t, J = 2.6 Hz, 1H),

1.86 (p, J = 7.1 Hz, 2H), 1.12 (t, J = 7.2 Hz, 3H); MS (ESI) m/z calculated for $[M+H]^+$ 507.1, found 507.0, HRMS (ESI) m/z calculated for $C_{24}H_{24}Cl_2N_2NaO_4S$ $[M+Na]^+$ 529.0732, found 529.0731.



8b

1H NMR (500 MHz, $CDCl_3$) δ 7.56 (d, J = 8.6 Hz, 2H), 7.31 (d, J = 8.6 Hz, 2H), 6.99 (d, J = 2.1 Hz, 1H), 6.88 (dd, J = 8.3, 2.1 Hz, 1H), 6.13 (d, J = 8.2 Hz, 1H), 4.96 (s, 1H), 4.92 (s, 1H), 4.67 (s, 1H), 4.26 – 4.14 (m, 2H), 4.07 (t, J = 8.1 Hz, 1H), 2.56 (t, J = 4.1 Hz, 1H), 2.53 (d, J = 8.2 Hz, 2H), 2.43 (ddd, J = 14.6, 12.1, 4.2 Hz, 1H), 2.20 (dd, J = 7.5, 4.8 Hz, 2H), 1.79 – 1.72 (m, 1H), 1.51 – 1.42 (m, 1H), 1.29 (t, J = 7.1 Hz, 3H); MS (ESI) m/z calculated for $[M+H]^+$ 507.1, found 507.0, HRMS (ESI) m/z calculated for $C_{24}H_{25}Cl_2N_2O_4S$ $[M+H]^+$ 507.0912, found 507.0912.



9b

1H NMR (500 MHz, $CDCl_3$) δ 7.68 (d, J = 8.7 Hz, 2H), 7.42 (d, J = 8.7 Hz, 1H), 7.03 (dd, J = 8.3, 2.2 Hz, 1H), 6.96 (d, J = 2.1 Hz, 1H), 6.59 (d, J = 8.3 Hz, 1H), 5.22 (d, J = 10.8 Hz, 1H), 4.82 (s, 1H), 4.50 (s, 1H), 4.09 (t, J = 5.0 Hz, 1H), 4.00 (ddd, J = 10.8, 7.6, 4.3 Hz, 1H), 3.80 – 3.73 (m, 2H), 2.22 (dt, J = 13.7, 4.3 Hz, 1H), 2.18 – 2.12 (m, 2H), 2.10 – 2.07 (m, 1H), 1.84 – 1.78 (m, 2H), 1.77 – 1.68 (m, 1H), 1.65 – 1.61 (m, 1H), 1.09 (t, J = 7.2 Hz, 3H); MS (ESI) m/z calculated for $[M+H]^+$ 509.1, found 509.1, HRMS (ESI) m/z calculated for $C_{24}H_{27}Cl_2N_2O_4S$ $[M+H]^+$ 509.1069, found 509.1067.

II. Biological Evaluation of Enantiomerically Pure Tricyclic Indolines

Bacterial Strains: MRSA strain BAA-44 was a gift from the laboratory of Daniel Feldheim. MRSA strains BAA-1720 and ATCC-33592 were purchased from ATCC (<http://www.atcc.org>). MRSA Strain NR-100 was provided by the Network on Antimicrobial Resistance in Staphylococcus aureus (NARSA) for distribution by BEI Resources, NIAID, NIH: a.k.a. Staphylococcus aureus, Strain COL, NR-45906.

Biological Evaluation: The minimal inhibitory concentration (MIC) was determined as described in the manuscript. The minimal re-sensitizing concentration (MRC) was by modifying the MIC protocol with the addition of the antibiotics cefazolin or the combination therapy amoxicillin/clavulanic acid. These were included at the same sub-lethal concentration in every well tested such that the final concentrations would be 8 µg/mL for cefazolin and 4/2 µg/mL for amoxicillin/clavulanic acid. Plates were prepared such that the highest compound concentration tested was 64 µg/mL of **9b**. No inhibition was observed.

Supplemental Table 1

Compound	MIC	MRC ^a	MRC ^b
	BAA-44	BAA-44	BAA-44
1 (racemic)	>64	4	8
9b (racemic)	>64	>64	>64

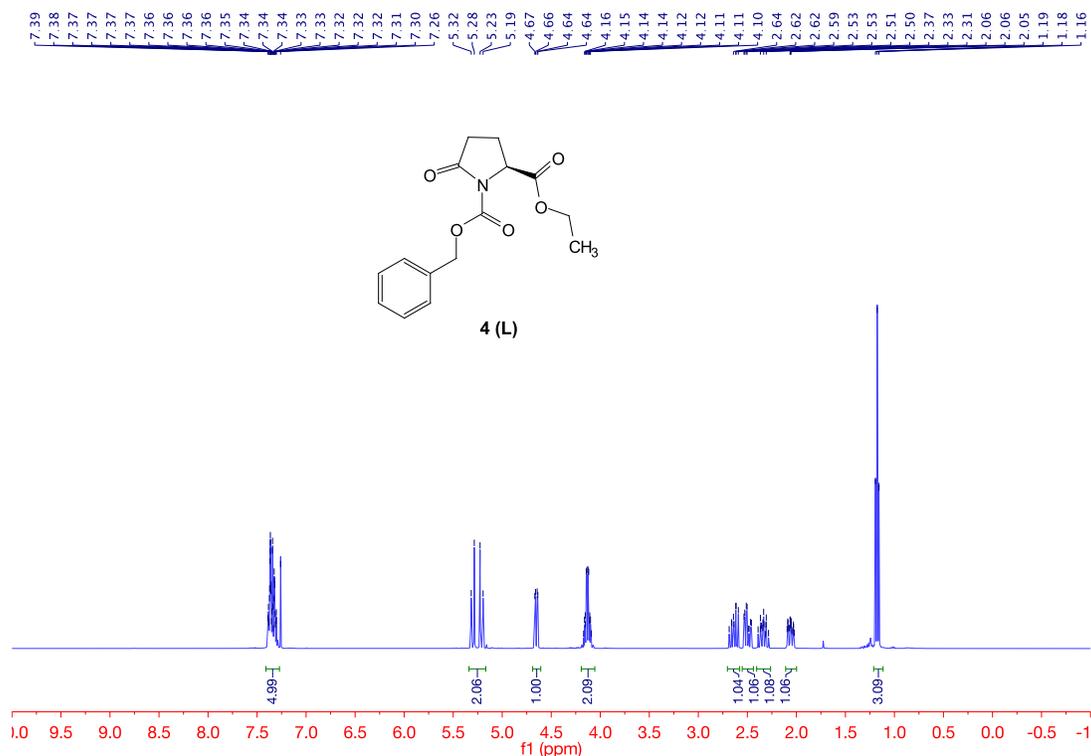
All values are reported in µg/mL. ^aCefazolin. ^bAmoxicillin/clavulanic acid

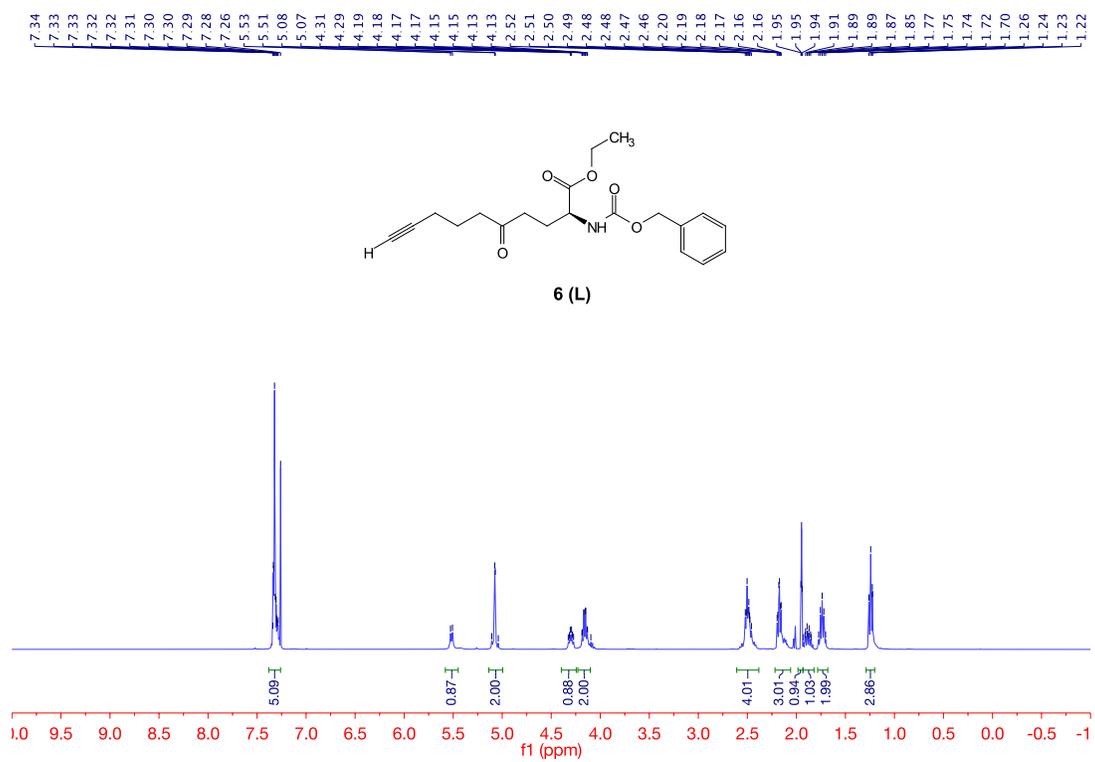
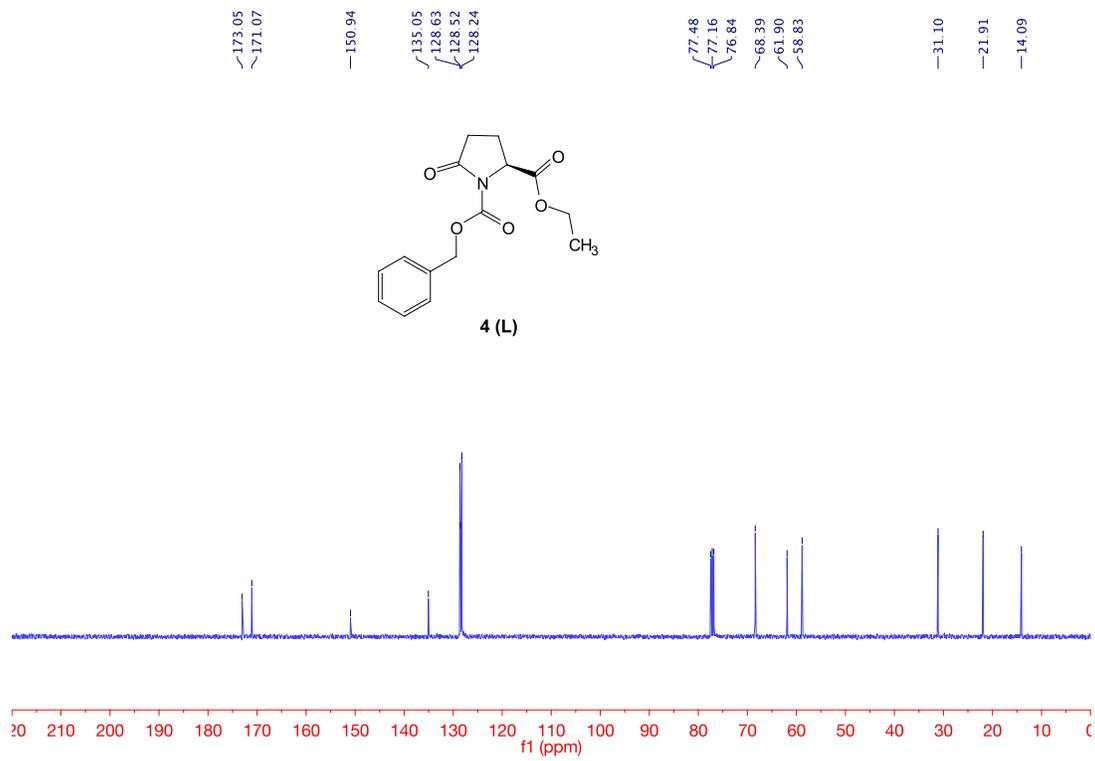
The mammalian cytotoxicity of **10** (racemic) was evaluated in human cervical adenocarcinoma HeLa cells using a Promega cell viability assay (CellTiter-Glo luminescent cell viability assay kit). HeLa cells in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS) and 1% penicillin/streptomycin, were seeded (20,000 cells/well) on white, cell-culture-treated, 96-well plates (Corning 3917) to a volume of 99 µL in each well. After incubation at 37 °C in 5% CO₂/95% air for 16 hours, 1 µL of **10** in DMSO was added to each well from a two-fold serial dilution masterplate. The final concentration range tested was 0.5–32 µg/mL. Plates were incubated as before for 24 hours and then brought to room temperature over 30 minutes. 100 µL of CellTiter-Glo reagent (Promega) was added to each well and mixed for 2 minutes on an orbital shaker. The luminescent signal was stabilized by incubation for 10 minutes at room temperature. Luminescence was recorded with an Envision

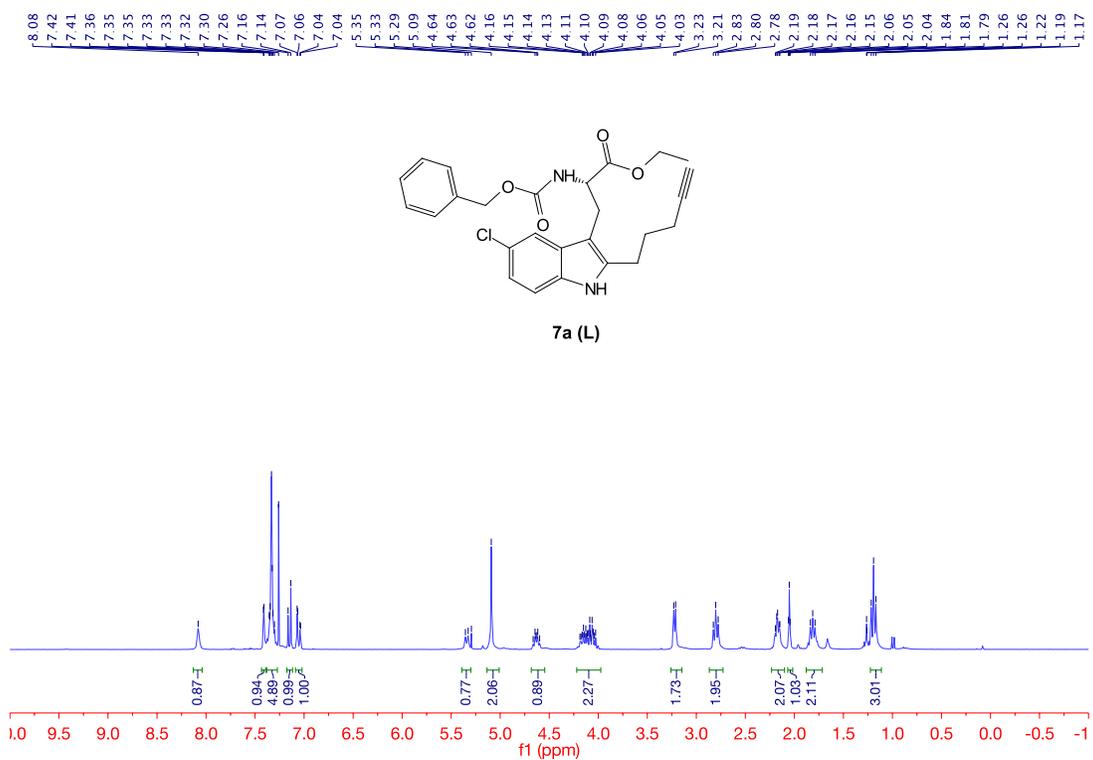
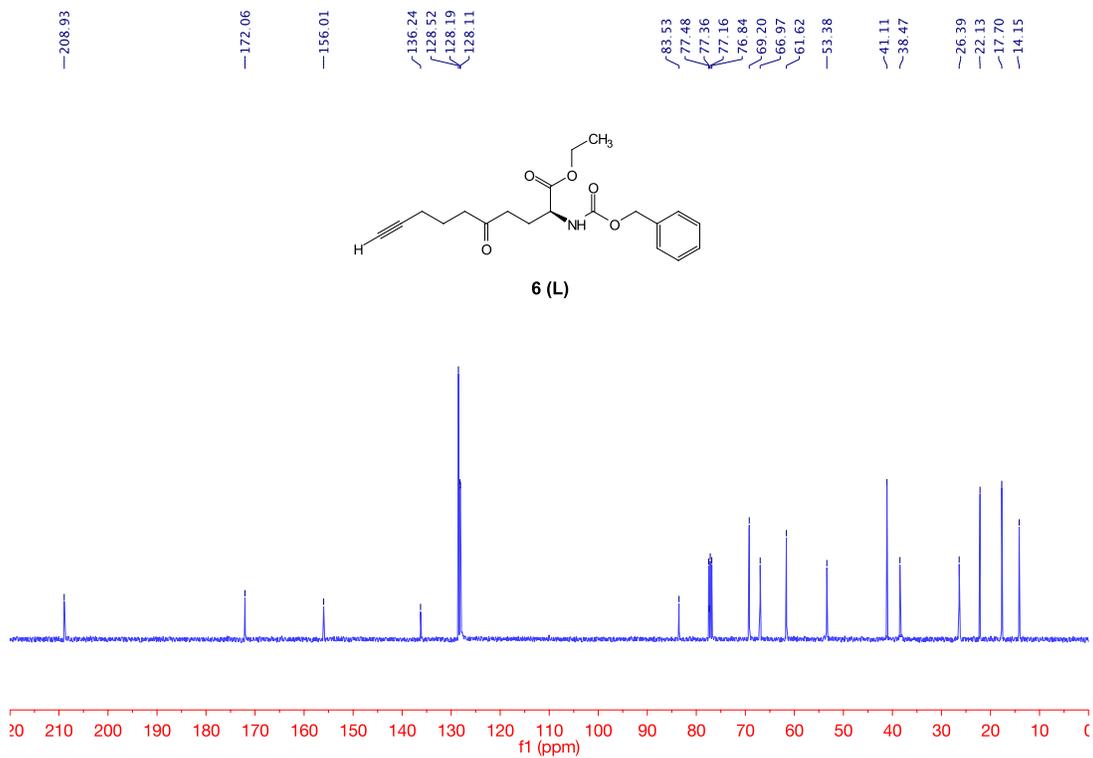
Multilabel Plate Reader (PerkinElmer). Results were confirmed by testing in triplicate. The GI50 was calculated by fitting data using KaleidaGraph (v4.1.1; Synergy Software).

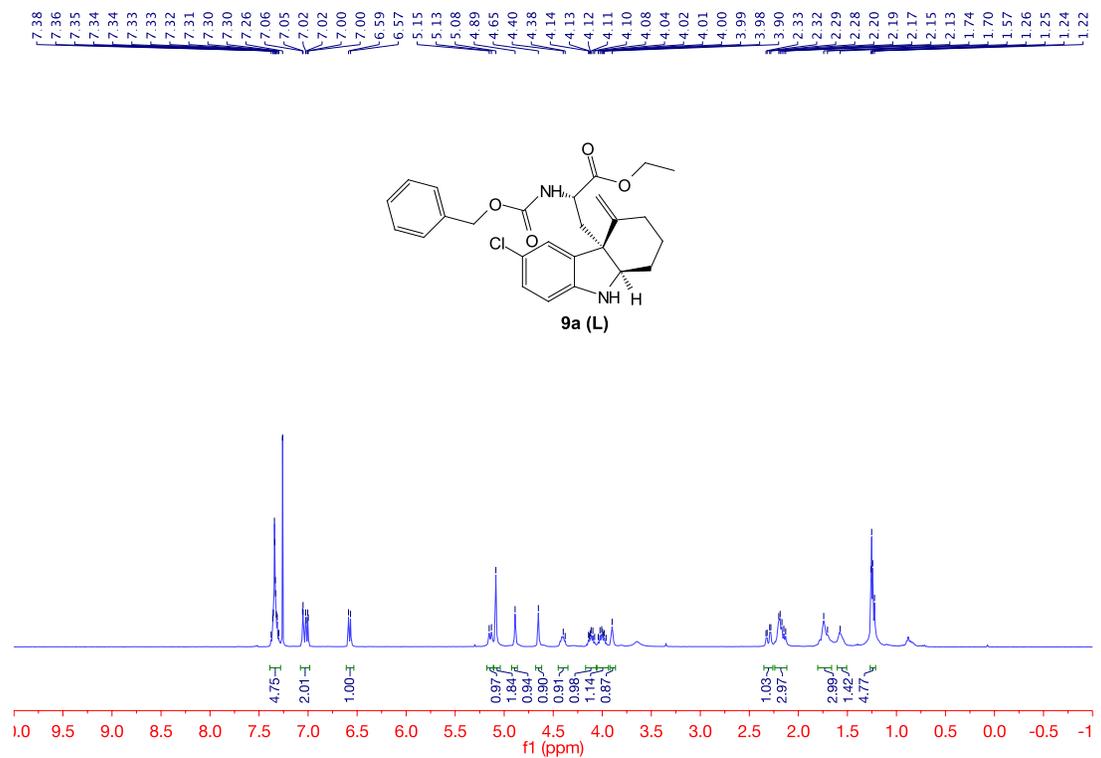
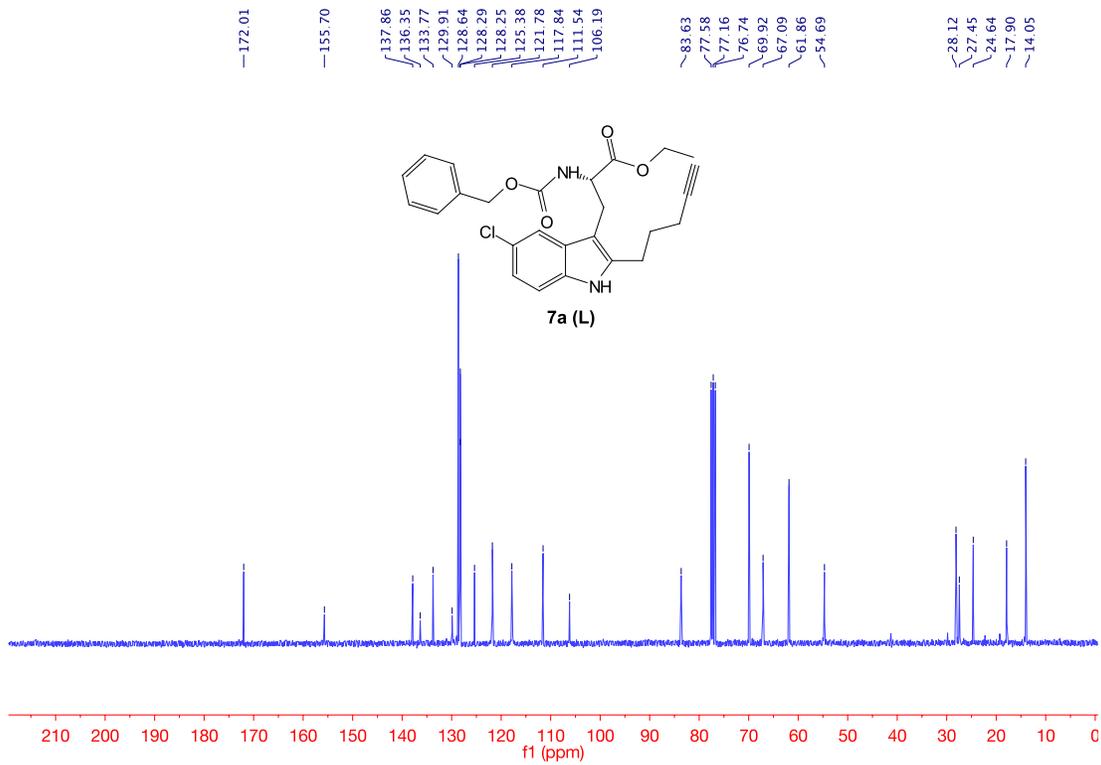
III. NMR Spectra

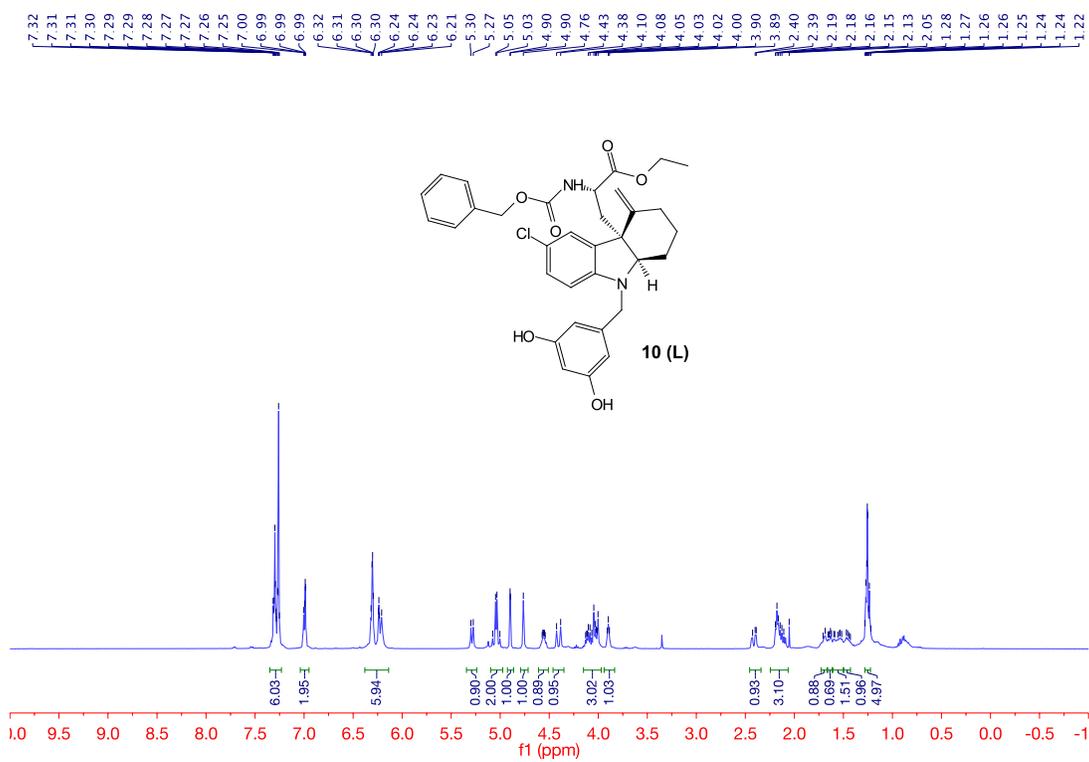
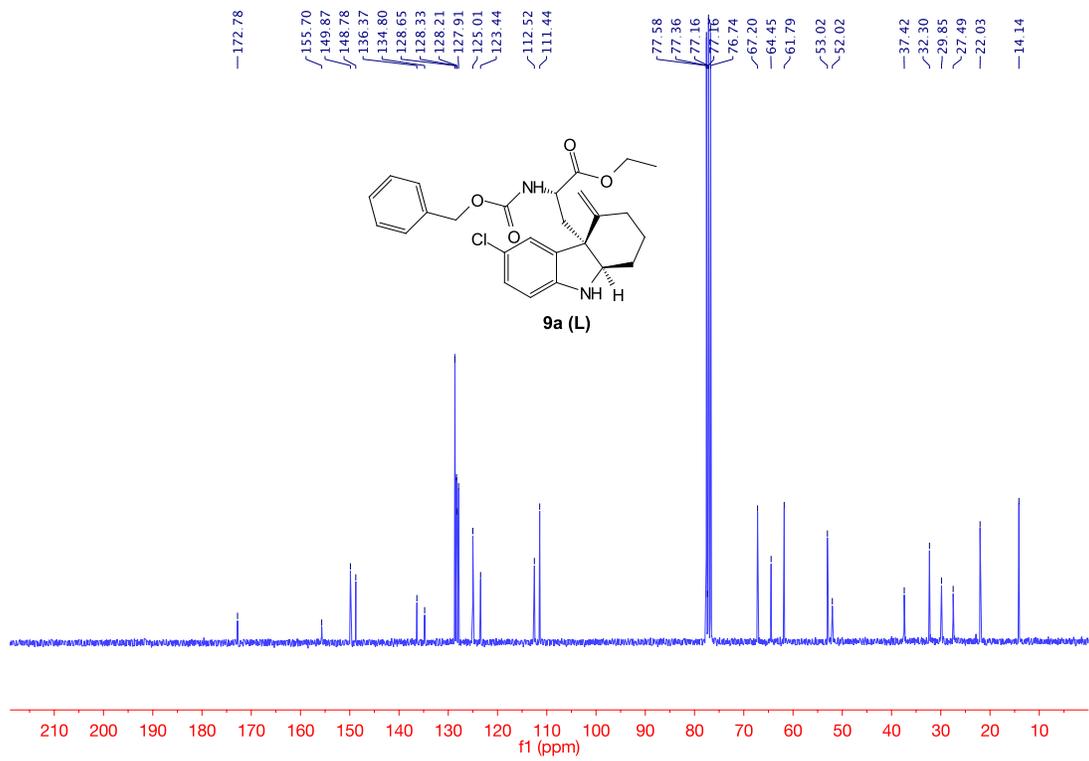
The NMR spectra of **8a** are not included because the spectra were too complex to be deciphered. **8b** was characterized as a mixture of isomers. Purification of the subsequent ring-opened products **9a** and **9b** was greatly simplified, yielding pure samples for NMR analysis. The NOESY of **8b** was used to elucidate the relative stereochemistry of the major isomer of the cyclization step.

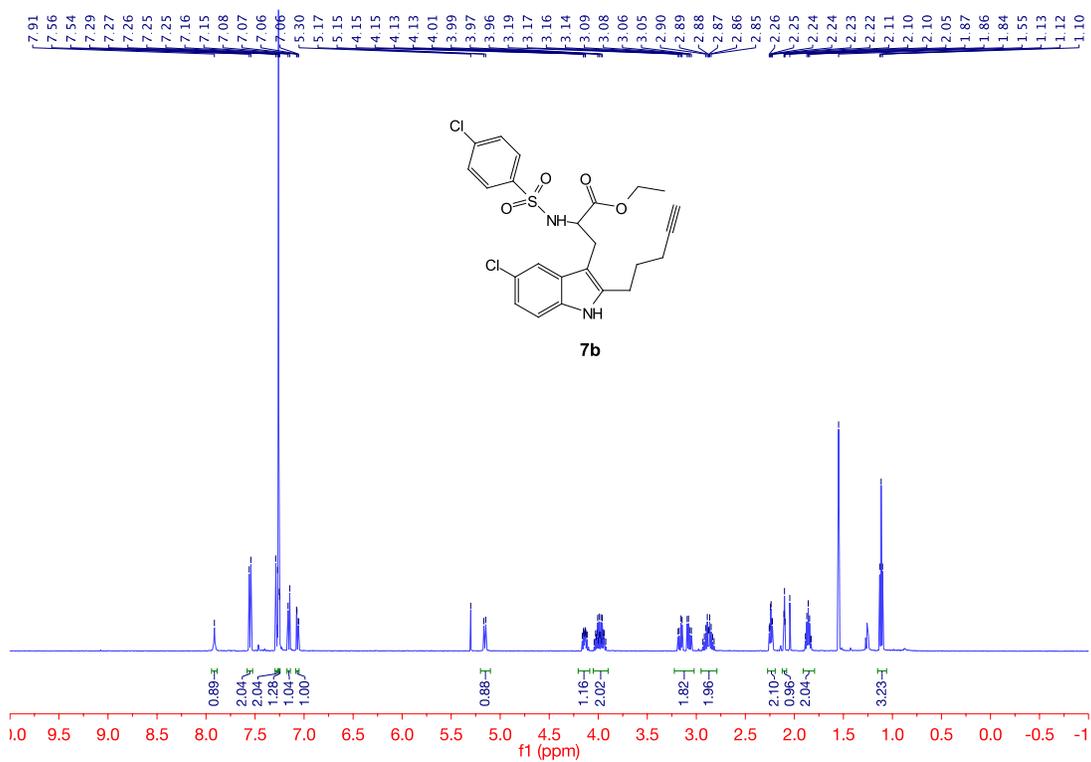
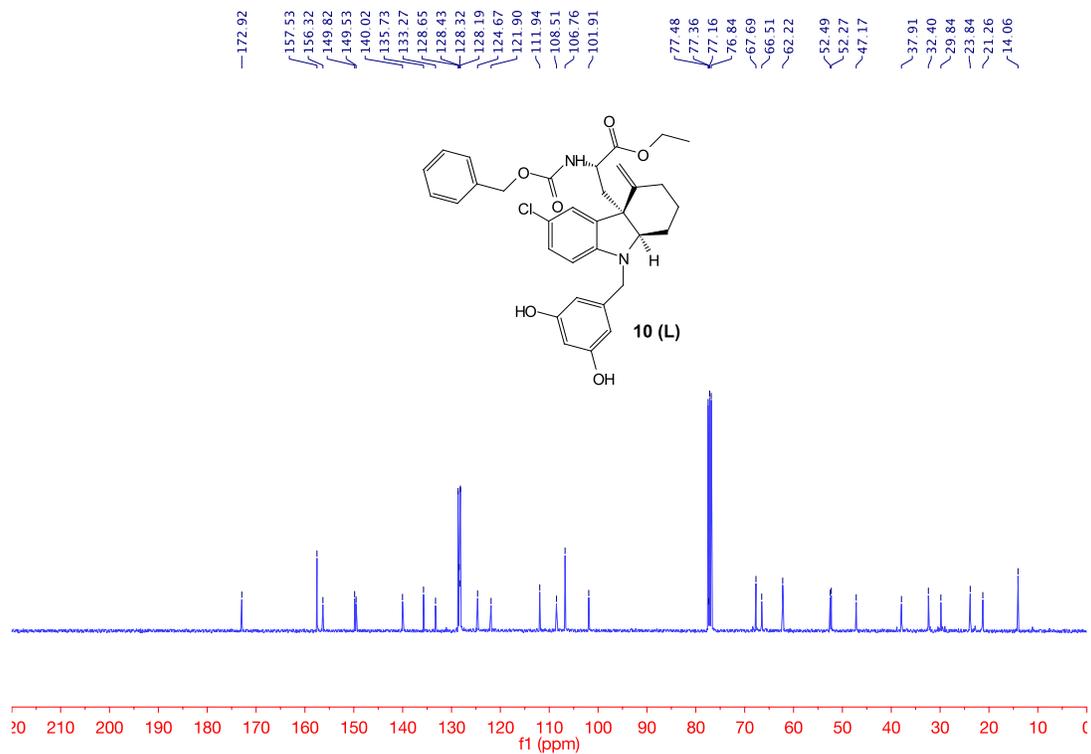


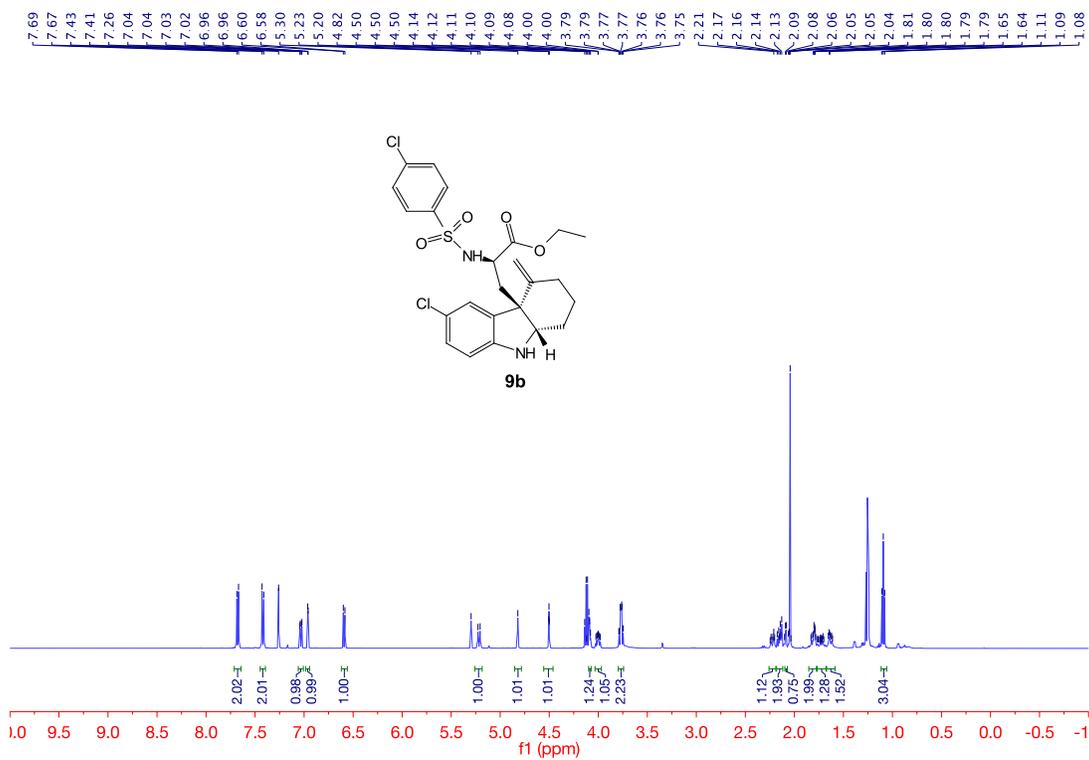
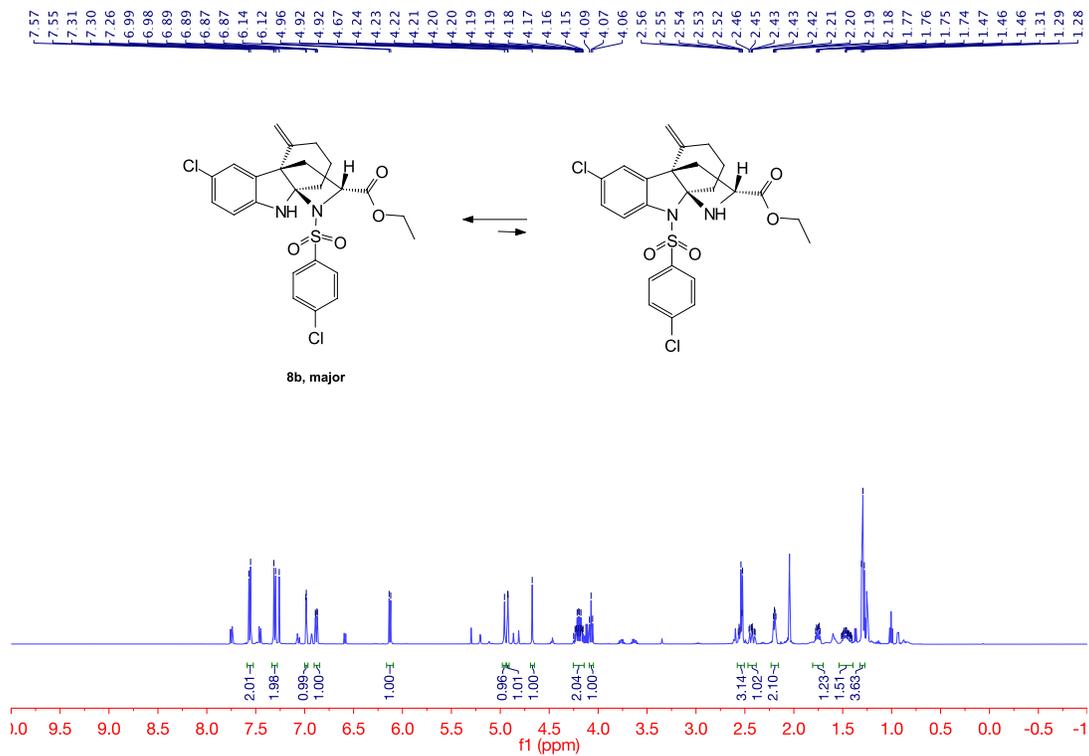


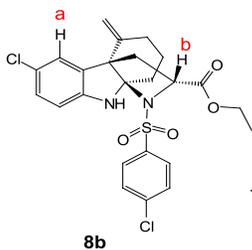




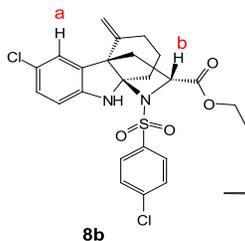
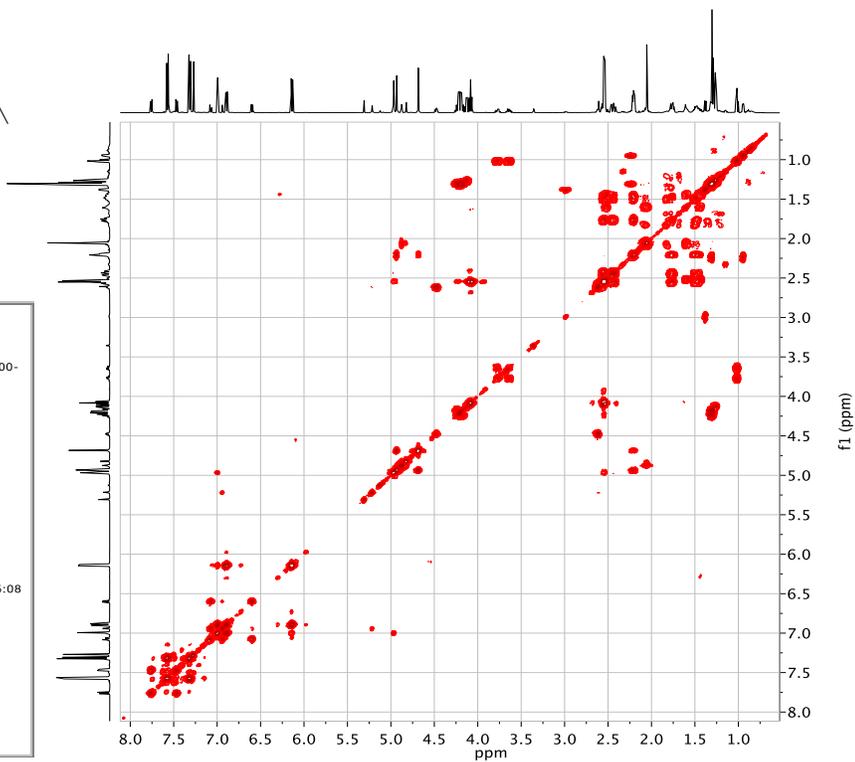








Parameter	Value
1 Data File Name	X:/ wang/ Wei/ WH-1-182-2-Bio500-gCOSY.fid/ fid
2 Solvent	cdcl3
3 Pulse Sequence	gCOSY
4 Number of Scans	2
5 Receiver Gain	58
6 Relaxation Delay	1.0000
7 Pulse Width	6.7000
8 Acquisition Date	2016-03-31T17:05:08
9 Mixing Time	0.5
10 Spectrometer Frequency	(499.60, 499.60)
11 Acquired Size	(569, 384)
12 Spectral Size	(1024, 1024)
13 Nucleus	(1H, 1H)



Parameter	Value
1 Data File Name	X:/ wang/ Wei/ WH-1-182-2-Bio500-NOESY.fid/ fid
2 Solvent	cdcl3
3 Pulse Sequence	NOESY
4 Number of Scans	8
5 Receiver Gain	58
6 Relaxation Delay	1.0000
7 Pulse Width	6.7000
8 Acquisition Date	2016-03-31T16:50:10
9 Mixing Time	0.4
10 Spectrometer Frequency	(499.60, 499.60)
11 Acquired Size	(569, 200)
12 Spectral Size	(1024, 1024)
13 Nucleus	(1H, 1H)

