Simultaneous Structure-Activity Studies and Arming of Natural Products by CH Amination Reveal Cellular Targets of Eupalmerin Acetate

Jing Li,[†, ≠] Justin S. Cisar,[‡] Congying Zhou,[†] Brunilda A. Vera,[§] Howard Williams,[†] Abimael D. Rodriguez,[§] Benjamin F. Cravatt,[‡] Daniel Romo[†,≠]*

[†]Department of Chemistry and the [≠]Natural Products LINCHPIN Laboratory, Texas A&M University, P.O. Box 30012, College Station, TX 77842-3012, USA

[‡]The Skaggs Institute for Chemical Biology and Department of Chemical Physiology, and The Scripps Research Institute Molecular Screening Center, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037

[§]Department of Chemistry, University of Puerto Rico, P.O. Box 23346, UPR Station, San Juan, 00931-3346, Puerto Rico

Supplementary Information

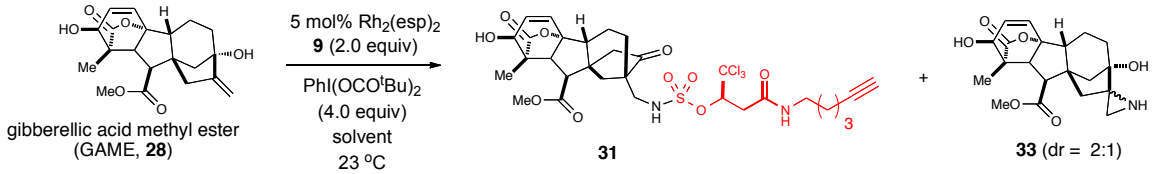
General Procedures

(*R*)-(-)-3-Hydroxy-4,4,4-trichlorobutyric β-lactone (**7**) was purchased from Aldrich and used as received. Hex-5-yn-1-amine (**6**) was synthesized according to the procedure described by Ravoo and Reinhoudt.[1] All other natural products were either commercially available or obtained from collaborators. All non-aqueous reactions were performed under a nitrogen atmosphere in oven-dried glassware. Toluene, benzene, diethyl ether, acetonitrile, and methylene chloride were purified by passing through activated alumina columns. Tetrahydrofuran was freshly distilled over sodium and benzophenone. Methanol was freshly distilled from Mg turnings. Triethylamine was distilled from calcium hydride and *i*-Pr_2NEt was distilled from potassium hydroxide prior to use. Yields refered to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials, unless otherwise stated. Reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. Reactions were monitored by (high performance liquid chromatorgraphy/mass spectrometry) HPLC/MS and thin layer chromatography (TLC). LC/MS was carried out using an ion trap HPLC/MS instrument using a C-18 50 x 2.10 mm 3 micron column, eluenting with gradient 5% acetonitrile 95% water to 100% acetonitrile in 15.8 min, detecting with UV 250 nm, PDA 190-400 nm and MS ion trap (ionization modes are APCI (+) and (-) or ESI (+) and (-), scan range 100-2100). Thin-layer chromatography (TLC) was carried out on 0.25 mm E. Merck silica gel plates (60F-254) using UV light for visualization and either an ethanolic solution of phosphomolybdic acid and cerium sulfate or an ethanolic solution of para-anisaldehyde with heat. Flash column chromatography was performed with 60Å Silica Gel (230-400 mesh) as stationary phase using a gradient solvent system (EtOAc/hexanes as eluent unless indicated otherwise). Preparative thin-layer chromatography (PTLC) separations were carried out on 0.25 or 0.50 mm E. Merck silica gel plates (60F-254). ^{1}H NMR chemical shifts were measured at 300 or 500 MHz and referenced relative to trace amounts of chloroform (δ 7.26) and were reported in parts per million (δ, ppm). Coupling constants (*J*) were reported in Hertz (Hz), with multiplicity reported following usual convention: s, singlet; d, doublet; t,

[1] Dorota I. Rozkiewicz, Dominik Jan´czewski, Willem Verboom, Bart Jan Ravoo, and David N. Reinhoudt *Angew. Chem. Int. Ed.* **2006**, *45*, 5292-5296.

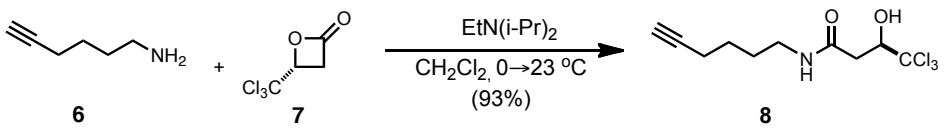
triplet; q, quadruplet; dd, doublet of doublets; ddd, doublet of doublet of doublets; dddd, doublet of doublet of doublet of doublets; dt, doublet of triplets; dq, doublet of quartets; m, multiplet; br s, broad singlet. ^{13}C NMR spectra were measured at 75 MHz or 125 MHz and referenced relative to residual chloroform (δ 77.16) and were reported in ppm. High resolution mass spectra (HRMS) were obtained through the Center for Chemical Characterization and Analysis (Texas A&M University) using MALDI (matrix-assisted laser-desorption ionization) or ESI (electrospray ionization).

Table S1: CH amination/azirdination optimization using gibberellic acid methyl ester (GAME) as substrate

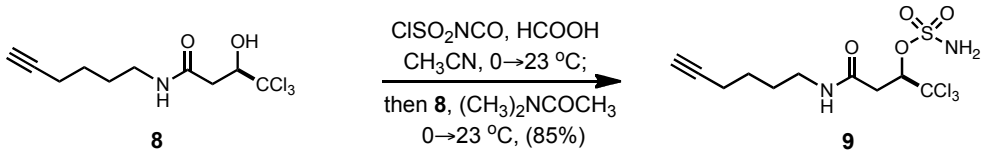


NP form	Nitrene (eq.)	Oxidant (eq.)	Catalyst (eq.)	Slovent (M)	Temp (oC)	Time (h)	Product (31) Yield	SM recovery	Comments
Crystal	*1.0*	2.0	0.05	Benzene 0.125 M	25	18	24.1%	61.7%	
Crystal	*2.0*	4.0	0.05	same	25	18	27.6%	55.0%	
Crystal	*3.0*	6.0	0.05	same	25	18	31.8%	19.4%	
Crystal	2.0	4.0	*0.01*	same	25	18	25.1%	57.2%	
Crystal	2.0	4.0	*0.10*	same	25	18	24.6%	48.3%	
Crystal	2.0	4.0	0.05	same	*40*	18	25.4%	31.1%	
Crystal	2.0	4.0	0.05	same	25	*0.5*	37.3%	51.1%	
Crystal	2.0	4.0	*0.10*	same	25	*0.5*	33.1%	57.2%	
Crystal	2.0	4.0	*0.10*	same	25	0.5	31.2%	62.2%	on silica gel
Amorphous	2.0	4.0	0.05	same	25	18	51.1%	27.0%	
Amorphous	2.0	4.0	0.05	same	25	0.5	55.0%	44.4%	
Amorphous	2.0	4.0	0.05	same	25	0.5	34.5%	55.6%	Add catalyst portion wise
Amorphous	2.0	*2.4*	0.05	same	25	0.5	33.7%	65.0%	
Amorphous	2.0	4.0	0.05	CH_2Cl_2 0.125 M	25	0.5	29.3%	28.9%	Comp. **33** 40.3%
Amorphous	2.0	4.0	0.05	Benzene 0.025 M	25	0.5	38.7%	58.3%	
Amorphous	2.0	4.0	0.05	0.125 M 	25	0.5	6.4%	66.6%	Comp. **33** 22.9%

Preparation of alkynyl sulfamate nitrene precursor 9.



(*R*)-4,4,4-trichloro-N-(hex-5-yn-1-yl)-3-hydroxybutanamide (8). *N,N'*-diisopropylethyl amine (1.56 ml, 9 mmol) was added to a solution of (*R*)-(–)-3-Hydroxy-4,4,4-trichlorobutyric β-lactone (2.5 g, 13.5 mmol) in CH_2Cl_2 (30 mL) and cooled to 0 ^oC. A solution of hex-5-yn-1-amine (0.87 g, 9 mmol) in CH_2Cl_2 (10 mL) was added dropwise to the above solution at 0 ^oC. The reaction mixture was stirred at 0 ^oC for 1 h, then at ambient temperature (23 ^oC) for an additional 1 h. After the reaction was judged complete by TLC, the solvent was removed by evaporation and the residue was purified by flash column chromatography (EtOAc/hexane: 20-40%) to give amide **8** (2.4 g, 8.4 mmol, 93% yield) as a colorless oil. ^1H NMR (300 MHz, $CDCl_3$): δ 6.42 (t, *J* = 5.4 Hz, 1H), 5.48 (br s, 1H), 4.54 (d, *J* = 9.3 Hz, 1H), 3.26 (ddd, *J* = 12.9, 6.9, 2.4 Hz, 2H), 2.92 (dd, *J* = 15.0, 2.4 Hz, 1H), 2.56 (dd, *J* = 14.7, 9.3 Hz, 1H), 2.19 (dt, *J* = 6.6, 2.7 Hz, 2H), 1.95 (t, *J* = 2.4 Hz, 1H), 1.65-1.50 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.0, 102.3, 83.9, 79.7, 68.9, 39.2, 37.8, 28.3, 25.5, 18.0; HRMS (ESI+): *m/z* calcd. for $C_{10}H_{15}Cl_3NO_2$ [M+H] 286.0168, found 286.0173.

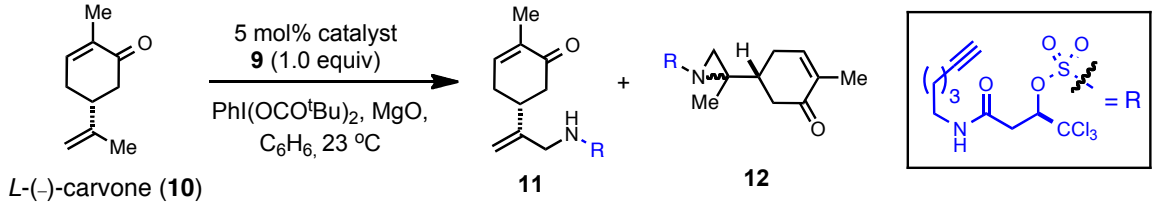


8 **9**

(*R*)-1,1,1-trichloro-4-(hex-5-yn-1-ylamino)-4-oxobutan-2-yl sulfamate (9). A solution of HCO_2H (0.35 mL, 9.2 mmol) in CH_3CN (3 mL) was added to a solution of $ClSO_2NCO$ (0.8 mL, 9.2 mmol) in CH_3CN (5 mL) over 10 min and the mixture was stirred at ambient temperature (23 ^oC) for 8 h. A solution of alcohol **8** (1.32 g, 4.6 mmol) in dimethylacetamide (DMA, 6 mL) was then added to the reaction mixture at 0 ^oC and the resulting solution was stirred at 23 ^oC for 2 h. Upon completion of the reaction, it was quenched with water (20 mL) and extracted with Et_2O (50 mL). The separated aqueous layer was extracted with Et_2O (2 x 100 mL) and the combined organic layers were washed with water (5 x 50 mL), dried over $MgSO_4$, and concentrated. The residue was purified by flash column chromatography (EtOAc/hexane: 20-40%) to give sulfamate **9** (1.4 g, 3.9 mmol, 90% yield) as a colorless solid. ^1H NMR (500 MHz, $CDCl_3$) δ 5.97 (br s, 1H), 5.74 (s, 2H), 5.51 (dd, *J* = 8.5, 2.0 Hz, 1H), 3.32 (dd, *J* = 13.0, 7.0 Hz, 2H), 3.17 (dd, *J* = 16.5, 2.0 Hz, 1H), 2.92 (dd, *J* = 16.5, 9.0 Hz, 1H), 2.24 (dt, *J* = 7.0, 2.5 Hz, 2H),

1.98 (t, J = 2.5 Hz, 1H), 1.69-1.63 (m, 2H), 1.59-1.53 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.0, 98.5, 85.9, 84.0, 69.2, 39.7, 38.2, 28.4, 25.6, 18.1; HRMS (ESI+): *m/z* calcd. for $C_{10}H_{16}Cl_3N_2O_4S$ [M+H] 364.9896, found 364.9890.

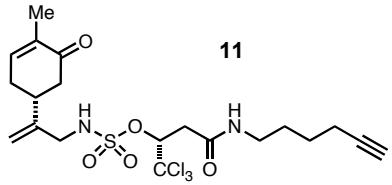
General procedure for the survey of catalysts for C-H amination versus aziridination of (*S*)-carvone.



L-(–)-carvone (**10**)

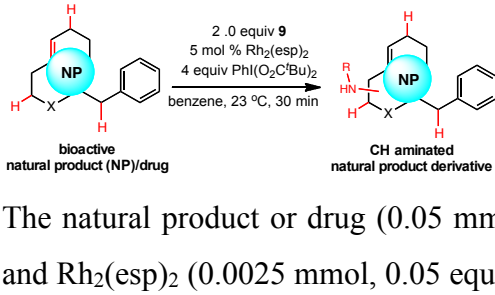
Sulfamate **9** (36.5 mg, 0.1 mmol), $Rh_2(esp)_2$ (3.5 mg, 0.005 mmol), and MgO (10 mg, 0.25 mmol) were mixed in benzene (0.4 mL) and (-)-carvone (16 mL, 0.1 mmol) was added in one portion. The resulting mixture was stirred at 23 °C for 30 min to give a homogeneous suspension. A solution of $PhI(O_2C^tBu)_2$ (81 mg, 0.20 mmol) in benzene (0.8 mL) was slowly added to the reaction mixture via syringe pump over 4 h. Upon complete addition, the mixture was stirred at 23 °C for 10 h. The suspension was filtered through a short pad of Celite and the filtrate was concentrated. The residue was purified by flash column chromatography (EtOAc/hexane, 20-45%) to give CH amination product **11** (5.1 mg, 0.01 mmol, 10% yield) and aziridine **12** (8.2 mg, 0.016 mmol, 16% yield, dr = 1.4:1), both isolated as colorless oils.

(*R*)-1,1,1-trichloro-4-(hex-5-yn-1-ylamino)-4-oxobutan-2-yl (2-((*R*)-4-methyl-5-oxocyclohex-3-en-1-yl)allyl)sulfamate 11 (10% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.74 (br s, 1H), 5.87 (t, J = 5.0 Hz, 1H), 5.75 (br s, 1H), 5.48 (dd, J = 8.0, 2.0 Hz, 1H), 5.14 (s, 1H), 5.00 (s, 1H), 3.88 (dd, J = 14.5, 7.5 Hz, 1H), 3.63 (dd, J = 14.5, 4.5 Hz, 1H), 3.39-3.34 (m, 1H), 3.26-3.20 (m, 1H), 3.15 (dd, J = 16.5, 2.0 Hz, 1H), 2.89-2.84 (m, 2H), 2.62-2.55 (m, 2H), 2.40 (dd, J = 15.5, 12.5 Hz, 1H), 2.29-2.25 (m, 1H), 2.24 (dt, J = 7.0, 2.5 Hz, 2H), 1.98 (t, J = 2.5 Hz, 1H), 1.79 (s, 3H), 1.69-1.62 (m, 2H), 1.62-1.54 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 199.2, 168.6,



145.9, 144.3, 135.8, 113.5, 98.7, 85.5, 83.9, 69.2, 47.9, 43.0, 39.8, 38.3, 31.5, 29.9, 28.5, 25.7, 18.2, 15.8; HRMS (ESI+): *m/z* calcd. for $C_{20}H_{28}Cl_3N_2OS$ [M+H] 513.0785, found 513.0795.

(*R*)-1,1,1-trichloro-4-(hex-5-yn-1-ylamino)-4-oxobutan-2-yl 2-methyl-2-((*R*)-4-methyl-5-oxocyclohex-3-en-1-yl)aziridine-1-sulfonate 12 (dr = 1.4:1, 16% yield). Major diastereomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.78 (d, *J* = 5.0 Hz, 1H), 5.79 (t, *J* = 5.0 Hz, 1H), 5.57 (br s, 2H), 3.39-3.31 (m, 1H), 3.19-3.14 (m, 1H), 3.09 (dd, *J* = 16.0, 4.0 Hz, 1H), 2.85 (dd, *J* = 16.0, 5.5 Hz, 1H), 2.70 (s, 1H), 2.53-2.49 (m, 1H), 2.44 (s, 1H), 2.37-2.31 (m, 2H), 2.25-2.20 (m, 1H), 2.21 (dt, *J* = 6.5, 2.5 Hz, 2H), 1.96 (t, *J* = 2.5 Hz, 1H), 1.77 (t, *J* = 1.5 Hz, 3H), 1.68-1.61 (m, 2H), 1.65 (s, 3H), 1.59-1.51 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 198.3, 167.1, 144.5, 135.6, 98.4, 86.3, 84.1, 69.0, 51.1, 43.1, 41.3, 40.4, 39.8, 39.6, 28.6, 28.2, 25.8, 18.2, 16.4, 15.8; HRMS (ESI+): *m/z* calcd. for $C_{20}H_{28}Cl_3N_2OS$ [M+H] 513.0785, found 513.0788.



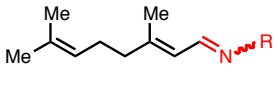
12

Representative procedure for CH amination, aziridination, and N-amination of complex natural products.

The natural product or drug (0.05 mmol, 1.0 equiv), sulfamate **9** (0.1 mmol, 2.0 equiv), and $Rh_2(esp)_2$ (0.0025 mmol, 0.05 equiv) were mixed in a small vial and dried under high vacuum for 10 min before being purged with N_2. Benzene (0.4 mL) was added to give a green suspension (0.125 M), which was stirred at 23 °C under N_2 for 10 min. $PhI(O_2C^tBu)_2$ (0.2 mmol, 4.0 equiv) was then added in one portion and the reaction mixture was vigorously stirred at 23 °C for 30 min. The crude reaction mixture was loaded on a silica gel column directly and purified by flash column chromatography (eluting with gradient EtOAc/hexane or $MeOH/CH_2Cl_2$) to isolate the desired product(s).

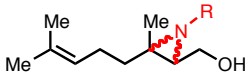
Note 1: This is a general procedure for natural products or drugs available in relatively large quantity (> 0.05 mmol). For sample-limited natural products, the reaction could be performed on ~1-5 mg scale (~4-20 μmol) using 0.2 mL of solvent (reaction concentration, 20 mM) which still gives reasonable conversion. Amounts of all other reagents were reduced accordingly. See procedure for EuPAyne performed on 5 mg below for an example. Note 2: If the natural product or drug is volatile, it should be added to the reaction vial after the 10 min evacuation process.

(*R*)-1,1,1-trichloro-4-(hex-5-yn-1-ylamino)-4-oxobutan-2-yl ((*E*)-3,7-dimethylocta-2,6-dien-1-ylidene)sulfamate 13a (*E*/*Z* = 8:1, 55% yield). Major isomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.92 (d, *J* = 17.0 Hz, 1H), 6.21 (dt, *J* = 17.0, 2.0 Hz, 1H), 5.71 (m, 1H), 5.64 (dd, *J* = 10.5, 6.0 Hz, 1H), 5.05 (tq, *J* = 9.5, 2.5 Hz, 1H), 3.41-3.30 (m, 1H), 3.27-3.16 (m, 1H), 3.09 (dd, *J* = 26.5, 6.0 Hz, 1H), 2.84 (dd, *J* = 27.0, 10.5 Hz, 1H), 2.37-2.32 (m, 2H), 2.26-2.19 (m, 4H), 2.17 (d, *J* = 2.5 Hz, 3H), 1.96 (t, *J* = 4.0 Hz, 1H), 1.69 (d, *J* = 2.0 Hz, 3H), 1.67-1.52 (m, 4H), 1.61 (d, *J* = 1.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.9, 171.3, 167.2, 133.5, 123.0, 122.2, 98.3, 86.4, 84.0, 69.0, 41.5, 39.6 (2C), 28.5, 26.0, 25.8 (2C), 19.0, 18.2, 17.9; HRMS (ESI+): *m/z* calcd. for $C_{20}H_{30}Cl_3N_2O_4S$ [M+H] 499.0992, found 499.0997.



13a

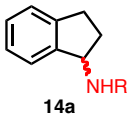
(*R*)-1,1,1-trichloro-4-(hex-5-yn-1-ylamino)-4-oxobutan-2-yl 3-(hydroxymethyl)-2-methyl-2-(4-methylpent-3-en-1-yl)aziridine-1-sulfonate 13b (dr = 1.2:1, 37% yield, separable diastereomers). Major isomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.74 (m, 1H), 5.67 (t, *J* = 5.5 Hz, 1H), 5.09 (t, *J* = 7.5 Hz, 1H), 3.87 (br s, 1H), 3.79 (d, *J* = 13.0 Hz, 1H), 3.58 (dd, *J* = 12.5, 7.5 Hz, 1H), 3.33 (dt, *J* = 20.0, 7.0 Hz, 1H), 3.26 (dt, *J* = 19.5, 6.0 Hz, 1H), 3.13 (t, *J* = 2.5 Hz, 1H), 3.11 (dd, *J* = 16.0, 4.5 Hz, 1H), 3.02 (dd, *J* = 16.0, 6.0 Hz, 1H), 2.26-2.22 (m, 1H), 2.23 (dt, *J* = 7.0, 3.0 Hz, 1H), 2.17-2.10 (m, 1H), 1.97 (t, *J* = 3.0 Hz, 1H), 1.73-1.64 (m, 3H), 1.70 (s, 3H), 1.62 (s, 3H), 1.63-1.54 (m, 3H), 1.32 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.2, 133.2, 122.6, 98.5, 85.8, 83.9, 69.1, 60.6, 56.5, 54.9, 39.7, 39.5, 36.0, 28.4, 25.8, 25.7, 25.3, 18.2, 18.1, 17.9; HRMS (ESI+): *m/z* calcd. for



13b

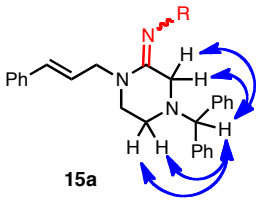
$C_{20}H_{32}Cl_3N_2O_5S$ $[M+H]^+$ 517.1098, found 517.1094. Minor isomer: ^1H NMR (500 MHz, $CDCl_3$) δ 5.82 (dd, *J* = 5.5, 4.0 Hz, 1H), 5.71 (t, *J* = 5.0 Hz, 1H), 5.07 (t, *J* = 5.5 Hz, 1H), 3.90 (ddd, *J* = 12.5, 6.5, 4.0 Hz, 1H), 3.65 (ddd, *J* = 13.0, 8.0, 5.5 Hz, 1H), 3.46 (t, *J* = 6.0 Hz, 1H), 3.38-3.25 (m, 2H), 3.12 (dd, *J* = 16.0, 3.5 Hz, 1H), 3.06 (dd, *J* = 8.0, 3.5 Hz, 1H), 2.95 (dd, *J* = 16.5, 5.5 Hz, 1H), 2.26-2.21 (m, 3H), 2.20-2.09 (m, 2H), 1.96 (t, *J* = 3.0 Hz, 1H), 1.79-1.75 (m, 1H), 1.69 (s, 3H), 1.68-1.63 (m, 2H), 1.61 (s, 3H), 1.60-1.54 (m, 2H), 1.35 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.1, 133.2, 122.5, 98.4, 85.8, 84.0, 69.0, 60.5, 55.7, 55.3, 39.8, 39.5, 35.5, 28.4, 25.8, 25.7, 25.2, 18.2, 18.2, 17.9; HRMS (ESI+): *m/z* calcd. for $C_{20}H_{31}Cl_3N_2O_5SNa$ $[M+Na]^+$ 539.0917, found 539.0915.

(*R*)-1,1,1-trichloro-4-(hex-5-yn-1-ylamino)-4-oxobutan-2-yl (2,3-dihydro-1H-inden-1-yl)sulfamate 14a (dr = 1.5:1, 48% yield) Major diastereomer: ^1H NMR (500 MHz, $CDCl_3$) δ 7.47-7.45 (m, 1H), 7.26-7.19 (m, 3H), 6.00 (t, *J* = 5.5 Hz, 1H), 5.85 (d, *J* = 9.0 Hz, 1H), 5.57 (dt, *J* = 9.0, 1.5 Hz, 1H), 5.09-5.02 (m, 1H), 3.21-3.10 (m, 2H), 3.04-2.80 (m, 4H), 2.66-2.58 (m, 1H), 2.16-2.12 (m, 2H), 1.93 (t, *J* = 3.0 Hz, 1H), 1.91-1.85 (m, 1H), 1.58-1.45 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.5, 142.7, 141.5, 128.5, 127.1, 124.8, 124.5, 98.8, 85.6, 83.9, 69.0, 60.3, 39.6, 38.1, 34.2, 30.1, 28.3, 25.6, 18.1; HRMS (ESI+): calcd. for $C_{19}H_{24}Cl_3N_2O_4S$ [M+H] 481.0522, found 481.0507.



14a

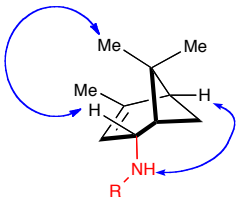
(*R*)-1,1,1-trichloro-4-(hex-5-yn-1-ylamino)-4-oxobutan-2-yl)oxy)sulfonyl)amino substituted cinnarizine 15a (*E*/*Z* = 2:1, 21% yield). Major isomer: ^1H NMR (500 MHz, $CDCl_3$) δ 7.47-7.15 (m, 15H), 6.57 (d, *J* = 16.0 Hz, 1H), 6.20 (dt, *J* = 9.7, 7.0 Hz, 1H), 5.72 (t, *J* = 5.5 Hz, 1H), 5.39 (t, *J* = 5.0 Hz, 1H), 4.41 (s, 1H), 4.33 (d, *J* = 6.5 Hz, 2H), 3.97 (d, *J* = 18.5 Hz, 1H), 3.77 (d, *J* = 18.5 Hz, 1H), 3.52-3.47 (m, 1H), 3.46-3.41 (m, 1H), 3.33-3.24 (m, 1H), 3.22-3.15 (m, 1H), 2.91 (dd, *J* = 15.5, 5.0 Hz, 1H), 2.76-2.72 (m, 1H), 2.68 (dd, *J* = 15.5, 5.0 Hz, 1H), 2.64-2.56 (m, 1H), 2.20 (dt, *J* = 7.0, 2.5 Hz, 2H), 1.96 (t, *J* = 2.5 Hz, 1H), 1.64-1.50 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 184.3, 168.0, 165.2, 140.7, 140.6, 135.9, 135.5, 129.1 (2C), 129.0 (2C), 128.8 (2C), 128.5, 127.9 (4C), 127.8, 126.8 (2C), 121.2, 99.1, 84.7, 84.3, 74.8, 68.9, 53.5, 52.4, 47.9, 46.8, 40.4, 38.6,



15a

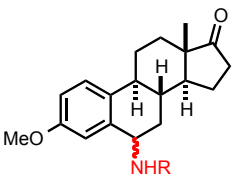
28.5, 25.8, 18.2; HRMS (MALDI+): *m/z* calcd. for $C_{36}H_{39}Cl_3N_4O_4SNa$ [M+Na] 751.1649, found 751.1656. The attachment point of imine was determined by NOESY analysis.

(*R*)-1,1,1-trichloro-4-(hex-5-yn-1-ylamino)-4-oxobutan-2-yl)oxy)sulfonyl)amino substituted α-pinene 16a (dr >19:1, 21% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.73 (br s, 1H), 5.60 (d, *J* = 8.7 Hz, 1H), 5.56 (dd, *J* = 8.1, 2.1 Hz, 1H), 5.28 (br s, 1H), 4.19 (m, 1H), 3.46-3.37 (m, 1H), 3.29-3.21 (m, 1H), 3.19 (dd, *J* = 16.8, 2.1 Hz, 1H), 2.91 (dd, *J* = 16.5, 8.1 Hz, 1H), 2.35 (m, 2H), 2.26 (dt, *J* = 6.6, 2.7 Hz, 2H), 2.05 (dt, *J* = 5.4, 1.2 Hz, 1H), 2.00 (t, *J* = 2.7 Hz, 1H), 1.73 (t, *J* = 1.8 Hz, 3H), 1.70-1.54 (m, 4H), 1.34 (s, 3H), 1.23 (d, *J* = 8.7 Hz, 1H), 0.90 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 168.4, 149.8, 115.8, 98.9, 85.3, 83.9, 69.1, 55.8, 47.2, 46.0, 44.6, 39.7, 38.4, 28.8, 28.5, 26.4, 25.8, 23.0, 20.6, 18.2; HRMS (ESI+): *m/z* calcd. for $C_{20}H_{29}Cl_3N_2O_4SLi$ [M+Li] 505.1074, found 505.1061. The stereochemistry of the single diastereomer obtained was determined by NOESY analysis.



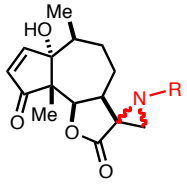
16a

(*R*)-1,1,1-trichloro-4-(hex-5-yn-1-ylamino)-4-oxobutan-2-yl)oxy)sulfonyl)amino substituted β-estrone 3-methyl ether 17a (dr = 4:1, 40% yield). Major isomer: ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.24 (d, *J* = 9.0 Hz, 1H), 7.01 (d, *J* = 2.5 Hz, 1H), 6.86 (dd, *J* = 8.5, 2.5 Hz, 1H), 5.82 (d, *J* = 7.5 Hz, 1H), 5.73 (t, *J* = 5.5 Hz, 1H), 5.55 (dd, *J* = 8.0, 2.5 Hz, 1H), 4.85 (t, *J* = 5.0 Hz, 1H), 3.81 (s, 3H), 3.32-3.21 (m, 2H), 3.10 (dd, *J* = 16, 2.5 Hz, 1H), 2.85 (dd, *J* = 16, 8.0 Hz, 1H), 2.52-2.39 (m, 3H), 2.22-2.12 (m, 4H), 2.09-2.02 (m, 1H), 1.98-1.96 (m, 1H), 1.94 (t, *J* = 2.5 Hz, 1H), 1.82-1.76 (m, 1H), 1.70-1.49 (m, 9H), 0.92 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 220.4, 167.9, 158.4, 135.4, 132.4, 126.7, 115.4, 114.5, 98.9, 85.9, 84.0, 69.1, 55.6, 53.4, 50.0, 48.2, 44.0, 39.7, 39.0, 36.0, 33.3 (2C), 31.6, 28.4, 25.7, 25.7, 21.6, 18.2, 14.1; HRMS (ESI-): *m/z* calcd. for $C_{29}H_{36}Cl_3N_2O_6S$ $[M-H]^-$ 645.1360, found 645.1309.



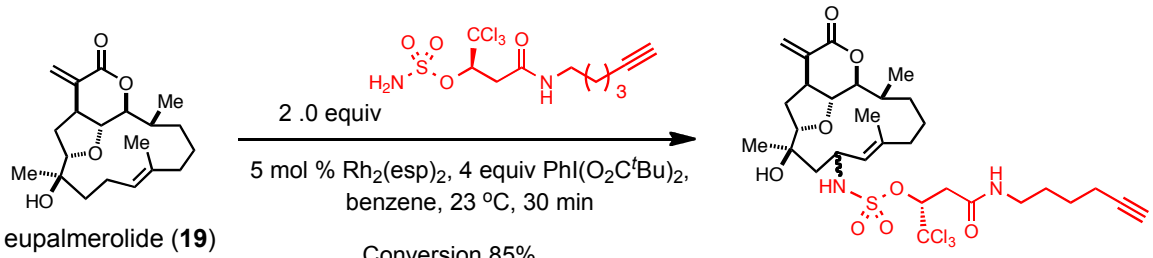
17a

(*R*)-1,1,1-trichloro-4-(hex-5-yn-1-ylamino)-4-oxobutan-2-yl)oxy)sulfonyl)amino substituted parthenin 18a (dr = 1:1, 8% yield). One isomer: ^{1}H NMR (500 MHz, CD_3OD) δ 7.63 (d, *J* = 5.5 Hz, 1H), 7.14 (dd, *J* = 14.5, 0.5 Hz, 1H), 6.59 (dd, *J* = 14.5, 0.5 Hz, 1H), 6.17 (d, *J* = 6.0 Hz, 1H), 5.76 (dd, *J* = 6.5, 4.0 Hz, 1H), 5.13 (d, *J* = 6.5 Hz, 1H), 3.46-3.39 (m, 1H), 3.26-3.20 (m, 1H), 3.18-3.16 (m, 1H), 3.14 (dd, *J* = 9.5, 5.5 Hz, 1H), 3.12 (s, 1H), 3.01 (s, 1H), 2.97 (dd, *J* = 16.0, 6.5 Hz, 1H), 2.37-2.29 (m, 1H), 2.26-2.19 (m, 5H), 1.70-1.60 (m, 3H), 1.58-1.51 (m, 3H), 1.35 (s, 3H), 1.14 (d, *J* = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 213.0, 172.5, 169.3, 166.2, 143.9, 131.9, 125.6, 99.3, 88.6, 85.0, 81.5, 69.7, 60.1, 42.8, 41.4, 40.4, 39.6, 38.2, 31.9, 29.2, 27.0, 25.5, 19.8, 18.7, 18.0; HRMS (MALDI+): *m/z* calcd. for $C_{25}H_{31}Cl_3N_2O_8SNa$ [M+Na] 647.0757, found 647.0748.



18a

CH amination of eupalmerolide:

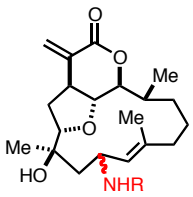


eupalmerolide (**19**)

19a (dr 1:1, 52%)

(*R*)-1,1,1-trichloro-4-(hex-5-yn-1-ylamino)-4-oxobutan-2-yl)oxy)sulfonyl)amino substituted eupalmerolide (19a): Eupalmerolide (1.3 mg, 3.9 μmol, 1.0 equiv), sulfamate **9** (2.8 mg, 7.8 μmol, 2.0 equiv) were mixed in a small vial and dried under high vacuum for 10 min before being purged with N_2. $Rh_2(esp)_2$ (1.9 mg) was mixed with 2 mL of Benzene and 0.2 mL of such suspension (containing catalyst 0.15 mg, 0.19 μmol, 0.05 equiv) was added to the reaction mixture to give a green suspension (0.02 M), which was stirred at 23 °C under N_2 for 10 min. $PhI(O_2C^tBu)_2$ (6.3 mg, 15.6 μmol, 4.0 equiv) was then added in one portion and the reaction mixture was vigorously stirred at 23 °C for 30 min. The crude reaction mixture was loaded on a silica gel column directly and purified by flash column chromatography eluting with gradient EtOAc/hexane to

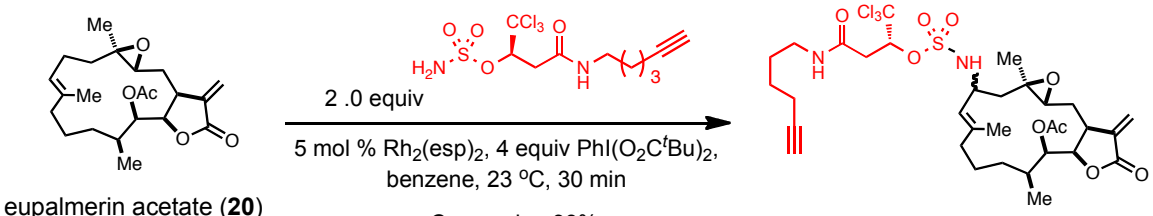
isolate the desired product **19a** as colorless oil (1.4 mg, 52% yield, d.r. = 1:1) and un-reacted eupalmerolide was also recovered as colorless oil (0.2 mg, 15%).



19a

One diastereomer of undetermined relative stereochemistry could be isolated pure: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.45 (s, 1H), 6.17 (br s, 1H), 5.60 (br s, 1H), 5.50 (s, 1H), 5.48 (dd, *J* = 8.5, 3.0 Hz, 1H), 5.30-5.26 (m, 1H), 4.60-4.55 (m, 1H), 4.36 (dd, *J* = 10.0, 5.5 Hz, 1H), 4.00 (dd, *J* = 10.0, 4.0 Hz, 1H), 3.87 (dd, *J* = 12.0, 5.5 Hz, 1H), 3.37 (dd, *J* = 13.0, 6.5 Hz, 1H), 3.28 (dd, *J* = 13.0, 6.5 Hz, 1H), 3.05 (dd, *J* = 13.0, 2.0 Hz, 1H), 2.77 (dd, *J* = 16.5, 7.0 Hz, 1H), 2.79-2.74 (m, 1H), 2.27-2.23 (m, 1H), 2.24 (dt, *J* = 7.0, 1.5 Hz, 2H), 2.17-2.13 (m, 1H), 2.10-2.01 (m, 2H), 1.98 (t, *J* = 1.5 Hz, 1H), 1.94-1.88 (m, 2H), 1.83-1.80 (m, 1H), 1.75-1.72 (m, 1H), 1.70-1.64 (m, 2H), 1.65 (s, 3H), 1.59-1.51 (m, 2H), 1.31 (s, 3H), 1.35-1.25 (m, 2H), 1.10 (d, *J* = 6.0 Hz, 3H), 0.89-0.85 (m, 1H); HRMS (MALDI+): *m/z* calcd. for $C_{30}H_{43}Cl_3N_2O_8SNa$ [M+Na] 719.1697, found 719.1680. The attachment point of the sulfamate side chain was determined by COSY analysis (*vide infra*).

CH amination of EuPA:

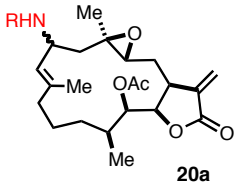


eupalmerin acetate (**20**) (**EuPA**)

20a (dr 1:1, 28%, **EuPAyne**)

(*R*)-1,1,1-trichloro-4-(hex-5-yn-1-ylamino)-4-oxobutan-2-yl)oxy)sulfonyl)amino substituted substituted eupalmerin (EuPAyne, 20a): EuPA (5.0 mg, 13.3 μmol, 1.0 equiv), sulfamate **9** (9.7 mg, 26.6 μmol, 2.0 equiv), and $Rh_2(esp)_2$ (0.5 mg, 0.66 μmol, 0.05 equiv) were mixed in a small vial and dried under high vacuum for 10 min before being purged with N_2. 0.4 mL of Benzene was added to the reaction mixture to give a green suspension (0.03 M), which was stirred at 23 °C under N_2 for 10 min. $PhI(O_2C^tBu)_2$ (21.6 mg, 53.2 μmol, 4.0 equiv) was then added in one portion and the reaction mixture was vigorously stirred at 23 °C for 45 min. The crude reaction mixture was loaded on a

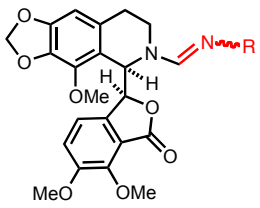
silica gel column directly and purified by flash column chromatography eluting with gradient EtOAc/hexane to isolate the desired product **20a** as light yellow oil (2.7 mg, 28% yield, d.r. = 1:1) and un-reacted eupalmerolide was also recovered as light yellow oil (3.1 mg, 62%).



20a

One isomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.09 (d, *J* = 3.0 Hz, 1H), 5.70 (br s, 1H), 5.50 (br s, 1H), 5.44 (dd, *J* = 8.0, 2.5 Hz, 1H), 5.29 (d, *J* = 3.5 Hz, 1H), 5.16 (d, *J* = 10.0 Hz, 1H), 5.04 (d, *J* = 9.0 Hz, 1H), 4.86 (d, *J* = 7.5 Hz, 1H), 4.55-4.47 (m, 1H), 3.36-3.28 (m, 3H), 3.10 (dd, *J* = 16.0, 2.0 Hz, 1H), 2.84 (dd, *J* = 16.5, 8.0 Hz, 1H), 2.81 (d, *J* = 5.0 Hz, 1H), 2.47 (dd, *J* = 13.0, 4.0 Hz, 1H), 2.24 (dt, *J* = 7.0, 3.0 Hz, 2H), 2.21-2.16 (m, 2H), 2.03-1.98 (m, 1H), 1.97 (t, *J* = 3.0 Hz, 1H), 1.89 (s, 3H), 1.89-1.84 (m, 1H), 1.79-1.75 (m, 1H), 1.77 (s, 3H), 1.69-1.65 (m, 3H), 1.59-1.55 (m, 2H), 1.49-1.44 (m, 1H), 1.38 (s, 3H), 1.33-1.25 (m, 3H), 0.81 (d, *J* = 7.0 Hz, 3H); HRMS (MALDI+): *m/z* calcd. for $C_{32}H_{45}Cl_3N_2O_9SNa$ [M+Na] 761.1803, found 761.1838. The attachment point of sulfamate side chain was determined by COSY analysis (*vide infra*).

(*R*)-1,1,1-trichloro-4-(hex-5-yn-1-ylamino)-4-oxobutan-2-yl (((*R*)-5-((S)-4,5-dimethoxy-3-oxo-1,3-dihydroisobenzofuran-1-yl)-4-methoxy-7,8-dihydro-[1,3]dioxolo[4,5-g]isoquinolin-6(5H)-yl)methylene)sulfamate 21a (unseparable mixture, *E*/*Z* = 1.3:1, 21% yield).

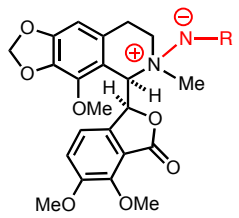


21a

Major isomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.36 (s, 1H), 7.06 (d, *J* = 8.5 Hz, 1H), 6.39 (s, 1H), 6.38 (t, *J* = 5.5 Hz, 1H), 6.31 (d, *J* = 8.5 Hz, 1H), 6.00 (dd, *J* = 4.5, 1.5 Hz, 2H), 5.96 (d, *J* = 4.5 Hz, 1H), 5.48 (dd, *J* = 6.0, 3.5 Hz, 1H), 5.30 (d, *J* = 4.5 Hz, 1H), 4.08 (s, 6H), 4.12-4.00 (m, 1H), 3.88 (s, 3H), 3.42-3.36 (m, 1H), 3.33-3.26 (m, 1H), 3.06 (dd, *J* = 16.0, 4.0 Hz, 1H), 2.89 (dd, *J* = 16.0, 6.0 Hz, 1H), 2.78-2.71 (m, 1H), 2.59-2.54 (m, 1H), 2.34-2.28 (m, 1H), 2.23 (dt, *J* = 6.5, 2.5 Hz, 2H), 1.94 (t, *J* = 2.5 Hz, 1H), 1.72-1.66 (m, 2H), 1.62-1.57 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.9, 166.9, 162.0, 153.1, 150.0, 148.8, 139.5, 137.9, 134.4, 128.8, 118.9, 118.1, 117.9, 112.6, 102.9, 101.3, 98.9, 84.8, 84.2, 78.5, 68.6, 62.7, 59.8, 59.2, 56.8, 40.1, 39.5, 39.4, 28.4, 26.7, 25.7, 18.1; HRMS (MALDI+): *m/z* calcd. for $C_{32}H_{34}Cl_3N_3O_{11}SNa$ [M+Na] 796.0871, found 796.0836; Minor isomer: ^{1}H NMR

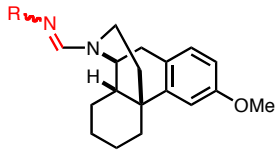
(500 MHz, $CDCl_3$) δ 8.29 (s, 1H), 7.13 (d, *J* = 8.0 Hz, 1H), 6.72 (d, *J* = 8.5 Hz, 1H), 6.36 (s, 1H), 6.15 (d, *J* = 3.5 Hz, 1H), 6.07 (t, *J* = 5.5 Hz, 1H), 5.94 (d, *J* = 3.0 Hz, 1H), 5.92 (dd, *J* = 6.0, 1.5 Hz, 2H), 5.64 (t, *J* = 4.5 Hz, 1H), 4.04 (s, 3H), 3.89 (s, 3H), 3.81 (s, 3H), 3.44-3.40 (m, 1H), 3.34-3.30 (m, 1H), 3.18-3.13 (m, 1H), 3.16-3.13 (m, 1H), 3.10 (dd, *J* = 6.5, 5.0 Hz, 2H), 2.93-2.88 (m, 1H), 2.84-2.79 (m, 1H), 2.18 (dt, *J* = 7.0, 2.5 Hz, 2H), 1.87 (t, *J* = 2.5 Hz, 1H), 1.62-1.57 (m, 2H), 1.54-1.49 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.9, 167.1, 161.6, 152.8, 149.6, 148.2, 139.7, 139.1, 134.1, 129.3, 119.0, 118.4, 118.1, 112.3, 102.6, 101.1, 98.7, 85.4, 84.0, 79.5, 68.6, 62.3, 59.3, 56.8, 53.2, 46.8, 39.6, 39.1, 28.5, 28.4, 25.6, 18.0.

(*R*)-((*S*)-6,7-dimethoxy-3-((*R*)-4-methoxy-6-methyl-5,6,7,8-tetrahydro-[1,3]dioxolo[4,5-g]isoquinolin-5-yl)isobenzofuran-1(3H)-one-1-ium-1-yl)(((1,1,1-trichloro-4-(hex-5-yn-1-ylamino)-4-oxobutan-2-yl)oxy)sulfonyl)amide 21b (37% yield). ^1H NMR (500 MHz, $CDCl_3$) δ 7.59 (d, *J* = 8.5 Hz, 1H), 7.30 (d, *J* = 8.5 Hz, 1H), 6.90 (s, 1H), 6.34 (s, 1H), 6.17 (br s, 1H), 5.97 (d, *J* = 1.5 Hz, 1H), 5.84 (d, *J* = 1.0 Hz, 1H), 5.81 (d, *J* = 1.0 Hz, 1H), 5.43 (t, *J* = 4.5 Hz, 1H), 4.15-4.07 (m, 1H), 3.98 (s, 3H), 3.91 (s, 3H), 3.84-3.78 (m, 1H), 3.45 (s, 3H), 3.39-3.30 (m, 3H), 3.20 (s, 3H), 3.11-3.01 (m, 3H), 2.22 (dt, *J* = 7.0, 2.5 Hz, 2H), 1.95 (t, *J* = 2.5 Hz, 1H), 1.69-1.57 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.4, 166.8, 152.6, 150.5, 147.6, 139.7, 139.5, 133.6, 126.3, 119.5, 119.2, 117.6, 109.2, 102.1, 101.2, 99.6, 84.2 (2C), 75.6, 71.6, 68.6, 62.2, 60.3, 58.4, 57.0, 51.6, 40.5, 39.4, 28.4, 25.7, 25.4, 18.1; HRMS (MALDI+): *m/z* calcd. for $C_{32}H_{36}Cl_3N_3O_{11}SNa$ [M+Na] 798.1027, found 798.1020.



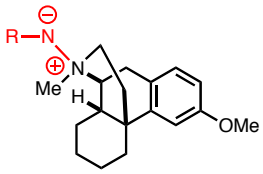
21b

(*R*)-1,1,1-trichloro-4-(hex-5-yn-1-ylamino)-4-oxobutan-2-yl (((4b*S*,8a*S*,9*S*)-3-methoxy-6,7,8,8a,9,10-hexahydro-5H-9,4b-(epiminoethano)phenanthren-11-yl)methylene)sulfamate 22a (*E*/*Z* = 2:1, 27% yield). Major isomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.20 (s, 1H), 7.04 (dd, *J* = 5.0, 2.5 Hz, 1H), 6.84 (t, *J* = 3.0 Hz, 1H), 6.76 (dd, *J* = 8.5, 2.5 Hz, 1H), 5.70 (t, *J* = 5.5 Hz, 1H), 5.37 (t, *J* = 5.0 Hz, 1H), 4.22 (dd, *J* = 13.5, 4.5 Hz, 1H), 3.80 (s, 3H), 3.76 (t, *J* = 4.5 Hz, 1H), 3.33-3.28 (m, 1H), 3.23 (dd, *J* = 18.5, 6.5 Hz, 1H), 3.12-3.07 (m, 1H), 3.01 (dd, *J* = 16.0, 4.5 Hz, 1H), 2.91 (d, *J* = 17.0 Hz, 1H), 2.88 (dd, *J* = 16.0, 4.5 Hz, 1H), 2.63 (dt, *J* = 13.5, 4.0 Hz, 1H), 2.40 (d, *J* = 13.0 Hz, 1H), 2.18 (dt, *J* = 7.0, 2.5 Hz, 2H), 1.94 (t, *J* = 2.5 Hz, 1H), 1.83 (dt, *J* = 12.5, 3.5 Hz, 1H), 1.71-1.67 (m, 1H), 1.65-1.56 (m, 5H), 1.52-1.47 (m, 5H), 1.40-1.33 (m, 1H), 1.12 (dd, *J* = 12.5, 3.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.9, 159.2, 159.1, 139.7, 129.3, 126.7, 112.0, 111.6, 99.1, 84.4, 84.1, 68.9, 60.0, 55.4, 44.6, 40.7, 40.0, 39.4, 39.3, 38.2, 36.2, 31.5, 28.5, 26.2, 26.1, 25.8, 21.9, 18.2; HRMS (MALDI+): *m/z* calcd. for $C_{28}H_{37}Cl_3N_3O_5S$ [M+H] 632.1513, found 632.1505.



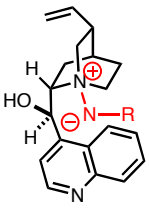
22a

(*R*)-((4b*S*,8a*S*,9*S*)-3-methoxy-11-methyl-6,7,8,8a,9,10-hexahydro-5H-9,4b-(epiminoethano)phenanthrene-1-ium-1-yl)(((1,1,1-trichloro-4-(hex-5-yn-1-ylamino)-4-oxobutan-2-yl)oxy)sulfonyl)amide 22b (42% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.05 (d, *J* = 8.5 Hz, 1H), 6.85 (t, *J* = 3.0 Hz, 1H), 6.78 (dd, *J* = 8.0, 2.0 Hz, 1H), 6.49 (t, *J* = 5.0 Hz, 1H), 5.28 (t, *J* = 4.5 Hz, 1H), 3.98 (br s, 1H), 3.80 (s, 3H), 3.70 (dt, *J* = 12.0, 2.0 Hz, 1H), 3.56 (s, 3H), 3.34-3.27 (m, 2H), 3.23 (dd, *J* = 19.5, 6.5 Hz, 1H), 3.10 (d, *J* = 20.0 Hz, 1H), 3.05 (t, *J* = 5.0 Hz, 2H), 3.09-3.01 (m, 1H), 2.78 (dt, *J* = 13.0, 3.5 Hz, 1H), 2.55 (dt, *J* = 13.0, 3.5 Hz, 1H), 2.38 (d, *J* = 14.0 Hz, 1H), 2.23 (dt, *J* = 7.0, 2.5 Hz, 2H), 1.94 (t, *J* = 2.5 Hz, 1H), 1.71-1.64 (m, 3H), 1.63-1.50 (m, 5H), 1.45-1.37 (m, 1H), 1.31-1.26 (m, 1H), 1.02 (dq, *J* = 12.5, 4.0 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.6, 159.5, 139.9, 128.8, 124.2, 112.1, 111.4, 99.8, 84.1 (2C), 77.2, 72.9, 68.6, 60.7, 55.3, 54.3, 41.1, 39.3, 37.3, 36.9, 36.0, 35.1, 28.4, 27.3, 26.3, 25.8, 25.7, 21.8, 18.1; HRMS (MALDI+): *m/z* calcd. for $C_{28}H_{39}Cl_3N_3O_5S$ [M+H] 634.1670, found 634.1662.

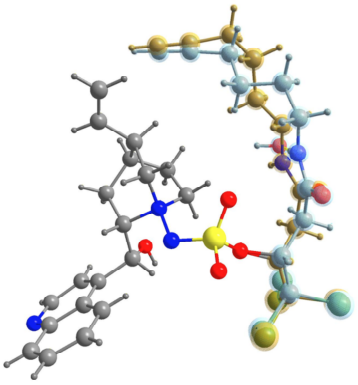


22b

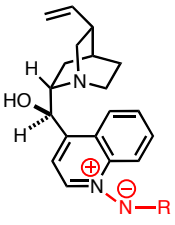
(*R*)-((*R*)-quinolin-4-yl((1S,2S,4S,5R)-5-vinylquinuclidin-2-yl)methanol-1-ium-1-yl)(((1,1,1-trichloro-4-(hex-5-yn-1-ylamino)-4-oxobutan-2-yl)oxy)sulfonyl)amide 23a (19% yield). The material was recrystallized with EtOAc/hexanes to give colorless crystals, which were suitable for analysis by X-ray further confirming its structure: m.p. = 152.6 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.93 (d, *J* = 5.0 Hz, 1H), 8.29 (d, *J* = 8.5 Hz, 1H), 8.14 (d, *J* = 8.5 Hz, 1H), 7.76 (t, *J* = 7.5 Hz, 1H), 7.74 (d, *J* = 5.0 Hz, 1H), 7.65 (t, *J* = 7.5 Hz, 1H), 6.99 (br s, 1H), 6.13 (br s, 1H), 5.56 (ddd, *J* = 18.0, 11.0, 6.5 Hz, 1H), 5.42 (t, *J* = 4.5 Hz, 1H), 5.05 (d, *J* = 18.0 Hz, 1H), 5.02 (d, *J* = 11.0 Hz, 1H), 4.65-4.59 (m, 2H), 4.25-4.21 (m, 1H), 3.99 (br s, 1H), 3.70 (t, *J* = 9.0 Hz, 1H), 3.51-3.48 (m, 1H), 3.29-3.22 (m, 2H), 3.21-3.14 (m, 1H), 3.01 (dd, *J* = 15.5, 4.5 Hz, 1H), 2.82 (br s, 1H), 2.35-2.22 (m, 2H), 2.12 (t, *J* = 6.5 Hz, 2H), 2.11 (br s, 1H), 2.05-1.96 (m, 1H), 1.90 (br s, 1H), 1.62-1.55 (m, 2H), 1.51-1.45 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.2, 150.1, 148.1, 146.1, 137.0, 130.3, 129.6, 127.6, 124.7, 123.1, 118.9, 117.2, 99.8, 83.96, 83.94, 73.2, 68.7, 67.3, 64.9, 55.3, 39.9, 39.5, 39.4, 28.2, 26.5, 26.3, 25.6, 20.7, 18.0; HRMS (MALDI+): *m/z* calcd. for $C_{29}H_{35}Cl_3N_4O_5SNa$ [M+Na] 679.1285, found 679.1274.



23a



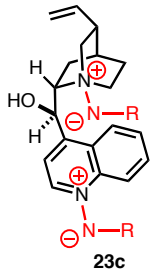
(*R*)-((*R*)-quinolin-4-yl((1*S*,2*S*,4*S*,5*R*)-5-vinylquinuclidin-2-yl)methanol-1-ium-1-yl)(((1,1,1-trichloro-4-(hex-5-yn-1-ylamino)-4-oxobutan-2-yl)oxy)sulfonyl)amide 23b (37% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.00 (d, *J* = 6.5 Hz, 1H), 8.92 (d, *J* = 8.5 Hz, 1H), 8.37 (d, *J* = 8.0 Hz, 1H), 7.95-7.90 (m, 2H), 7.72 (t, *J* = 7.0 Hz, 1H), 6.30 (br s, 1H), 5.81 (br s, 1H), 5.12 (ddd, *J* = 17.0, 10.5, 3.0 Hz, 1H), 5.08 (t, *J* = 4.5 Hz, 1H), 5.00 (d, *J* = 11.5 Hz, 1H), 4.98 (d, *J* = 4.5 Hz, 1H), 3.53 (br s, 1H), 3.32-3.20 (m, 2H), 3.22-3.18 (m, 1H), 3.15-3.05 (m, 1H), 2.95 (dd, *J* = 16.0, 12.0 Hz, 1H), 2.81 (dd, *J* = 15.5, 5.5 Hz, 1H), 2.78-2.70 (m, 2H), 2.40-2.35 (m, 1H), 2.21 (dt, *J* = 7.0, 2.5 Hz, 2H),



23b

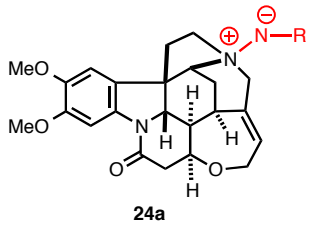
1.95 (t, *J* = 2.5 Hz, 1H), 1.90-1.78 (m, 1H), 1.80-1.70 (m, 2H), 1.69-1.51 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.3, 156.8, 147.5, 140.4, 140.2, 133.8, 129.9, 127.4, 124.6, 122.0, 119.4, 115.7, 99.5, 84.3, 84.0, 70.8, 68.9, 61.1, 56.1, 43.4, 40.1, 39.6, 39.0, 28.4, 27.5, 26.0, 25.8, 21.4, 18.2; HRMS (MALDI+): *m/z* calcd. for $C_{29}H_{36}Cl_3N_4O_5S$ [M+H] 657.1465, found 657.1451.

Bis-(*R*)-((*R*)-quinolin-4-yl((1*S*,2*S*,4*S*,5*R*)-5-vinylquinuclidin-2-yl)methanol-1-ium-1-yl)(((1,1,1-trichloro-4-(hex-5-yn-1-ylamino)-4-oxobutan-2-yl)oxy)sulfonyl)amide 23c (6% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.13 (d, *J* = 6.5 Hz, 1H), 9.03 (d, *J* = 9.5 Hz, 1H), 8.66 (d, *J* = 9.0 Hz, 1H), 8.16 (d, *J* = 6.5 Hz, 1H), 8.03 (dd, *J* = 9.5, 7.5 Hz, 1H), 7.90 (dd, *J* = 9.0, 7.5 Hz, 1H), 7.10 (br s, 1H), 6.26 (t, *J* = 5.0 Hz, 1H), 6.04 (t, *J* = 5.0 Hz, 1H), 5.75 (br s, 1H), 5.60 (ddd, *J* = 17.5, 10.5, 6.0 Hz, 1H), 5.29 (t, *J* = 4.5 Hz, 1H), 5.14 (t, *J* = 4.5 Hz, 1H), 5.08 (d, *J* = 10.5 Hz, 1H), 5.07 (d, *J* = 17.5 Hz, 1H), 4.61-4.55 (m, 1H), 4.51 (dd, *J* = 12.5, 11.0 Hz, 1H), 4.12-4.07 (m, 1H), 3.67-3.62 (m, 1H), 3.52-3.48 (m, 1H), 3.32-3.27 (m, 3H), 3.24-3.16 (m, 1H), 3.13 (dd, *J* = 16.0, 4.0 Hz, 1H), 2.99 (dd, *J* = 16.0, 5.0 Hz, 1H), 2.94 (dd, *J* = 16.0, 4.0 Hz, 1H), 2.86-2.82 (m, 1H), 2.78 (dd, *J* = 16.0, 5.0 Hz, 1H), 2.41-2.35 (m, 1H), 2.30-2.25 (m, 1H), 2.23 (dt, *J* = 7.0, 2.5 Hz, 2H), 2.19 (dt, *J* = 7.0, 2.5 Hz, 2H), 2.09 (br s, 1H), 2.02-1.98 (m, 1H), 1.97 (t, *J* = 2.0 Hz, 1H), 1.95 (t, *J* = 2.5 Hz, 1H), 1.70-1.52 (m, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.83, 167.95, 154.79, 147.50, 140.32, 136.59, 134.06, 130.62, 126.85, 124.87, 121.90, 119.65, 117.54, 99.61, 99.40, 84.22, 84.01, 83.94, 83.82, 73.59, 68.86, 68.74, 67.16, 64.92, 55.95, 39.87 (2C), 39.73, 39.44, 39.41, 28.27, 28.26, 26.32, 26.19, 25.70, 25.65, 20.18, 18.07, 18.05; HRMS (ESI+): *m/z* calcd. for $C_{39}H_{49}Cl_6N_6O_9S_2$ [M+H] 1019.1128, found 1019.1115.



23c

(*R*)-((4a*R*,4a1*R*,5a*S*,8a*R*,8a1*S*,15a*S*)-10,11-dimethoxy-4a1,5,5a,7,8,8a1,15,15a-octahydro-2H-4,6-methanoindolo[3,2,1-ij]oxepino[2,3,4-de]pyrrolo[2,3-h]quinolin-14(4aH)-one-1-ium-1-yl)(((1,1,1-trichloro-4-(hex-5-yn-1-ylamino)-4-oxobutan-2-yl)oxy)sulfonyl)amide 24a (28% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (s, 1H), 6.89 (s, 1H), 6.35 (m, 1H), 6.20 (t, *J* = 5.5 Hz, 1H), 5.37 (t, *J* = 4.5 Hz, 1H), 4.80 (s, 1H), 4.65 (dd, *J*

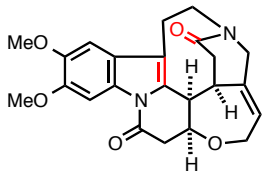


24a

= 12.5, 7.0 Hz, 1H), 4.57 (d, *J* = 13.5 Hz, 1H), 4.34 (dt, *J* = 8.5, 2.5 Hz, 1H), 4.29 (d, *J* = 13.5 Hz, 1H), 4.27 (dd, *J* = 13.5, 7.0 Hz, 1H), 4.08 (dd, *J* = 14.0, 5.5 Hz, 1H), 3.97 (d, *J* = 10.5 Hz, 1H), 3.90 (s, 3H), 3.89 (s, 3H), 3.72 (dt, *J* = 13.5, 5.5 Hz, 1H), 3.33-3.28 (m, 3H), 3.14 (dd, *J* = 17.5, 8.5 Hz, 1H), 3.05 (dd, *J* = 4.5, 1.5 Hz, 2H), 2.82 (dt, *J* = 15.5, 4.0 Hz, 1H), 2.67 (dd, *J* = 17.5, 2.5 Hz, 1H), 2.59 (dt, *J* = 14.0, 7.5 Hz, 1H), 2.22 (dt, *J* = 7.0, 2.5 Hz, 1H), 2.04 (dd, *J* = 13.5, 5.5 Hz, 1H), 1.94 (t, *J* = 2.5 Hz, 1H), 1.73 (d, *J* = 15.5 Hz, 1H), 1.69-1.63 (m, 2H), 1.61-1.55 (m, 2H), 1.39 (dt, *J* = 10.5, 2.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.5 (2C), 150.6, 147.1, 135.8, 135.7, 134.4, 119.0, 105.2, 101.2, 99.9, 84.5, 84.3, 81.4, 77.6, 68.8, 68.0, 64.3, 63.8, 59.0, 56.9, 56.4, 52.5, 47.4, 42.3, 40.7, 39.5 (2C), 30.3, 28.5, 25.9, 25.7, 18.2; HRMS (MALDI+): *m/z* calcd. for $C_{33}H_{40}Cl_3N_4O_8S$ [M+H] 757.1625, found 757.1622. The stereochemistry of **16a** was determined by NOESY analysis.

(7a*S*,15b*R*,15c*R*)-12,13-dimethoxy-7a,8,15b,15c-tetrahydro-1H-3,15-ethanopyrido[4'',3'':4',5']oxepino[3',2':3,4]pyrido[1,2-a]indole-2,9(4H,6H)-dione

24b (21% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.94 (s, 1H), 6.84 (s, 1H), 6.08-6.06 (m, 1H), 4.63 (ddd, *J* = 13.5, 13.0, 2.5 Hz, 1H), 4.32 (dd, *J* = 14.0, 7.0 Hz, 1H), 4.25 (dd, *J* = 15.5, 2.0 Hz, 1H), 4.20 (d, *J* = 6.0 Hz, 1H), 4.16 (ddd, *J* = 14.0, 5.0, 2.0 Hz, 1H), 3.92 (s, 3H), 3.91 (s, 3H), 3.92-3.90 (m, 1H), 3.57 (d, *J* = 6.5 Hz, 1H), 3.36 (s, 1H), 3.23 (dd, *J* = 13.0, 2.5 Hz, 1H), 3.23 (dd, *J* = 19.0, 6.0 Hz, 1H), 3.24-3.20 (m, 1H), 2.92 (dd, *J* =15.0, 6.5 Hz, 1H), 2.88-2.82 (m, 1H), 2.83 (d, *J* = 19.0 Hz, 1H), 2.54 (dd, *J* = 15.0, 1.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.8, 167.9, 148.1, 146.9, 145.0, 130.1, 129.5, 124.6, 123.2, 117.6, 100.4, 99.6, 83.8, 67.9, 56.3 (2C), 52.8, 49.2, 48.7, 42.3, 41.4, 39.5, 25.6; HRMS (ESI+): *m/z* calcd. for $C_{23}H_{24}N_2O_5Na$ [M+Na] 431.1583, found 431.1581.



24b

Table S2: NMR assignments of compound **24b**

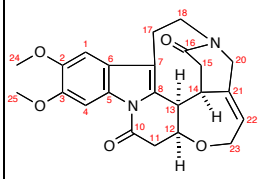
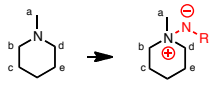
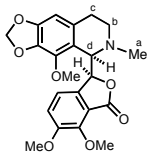
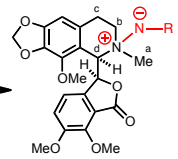
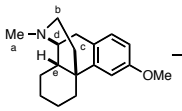
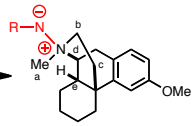
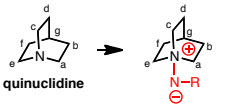
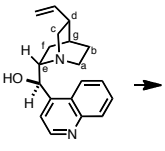
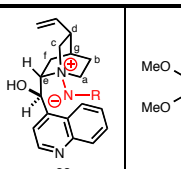
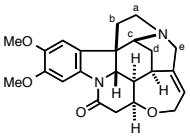
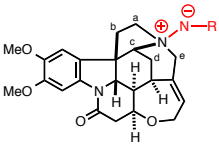
	C	^{13}C (ppm)	^{1}H (ppm)
 **24b**	1	100.42	6.84 (s, 1H)
	2	146.87	
	3	148.09	
	4	99.64	7.94 (s, 1H)
	5	129.47	
	6	124.64	
	7	117.59	
	8	130.10	
	10	167.94	
	11	42.25	3.23 (dd, *J* = 19.0, 6.0 Hz, 1H), 2.83 (d, *J* = 19.0 Hz, 1H)
	12	83.75	4.20 (d, *J* = 6.0 Hz, 1H)
	13	48.65	3.36 (s, 1H)
	14	41.42	3.57 (d, *J* = 6.5 Hz, 1H)
	15	39.47	2.92 (dd, *J* =15.0, 6.5 Hz, 1H), 2.54 (dd, *J* = 15.0, 1.5 Hz, 1H)
	16	174.84	
	17	25.62	3.24-3.20 (m, 1H), 2.88-2.82 (m, 1H)
	18	49.23	4.63 (ddd, *J* = 13.5, 13.0, 2.5 Hz, 1H), 3.23 (dd, *J* = 13.0, 2.5 Hz, 1H)
	20	52.81	4.25 (dd, *J* = 15.5, 2.0 Hz, 1H), 3.92-3.90 (m, 1H)
	21	145.04	
	22	123.19	6.08-6.06 (m, 1H)
	23	67.91	4.32 (dd, *J* = 14.0, 7.0 Hz, 1H), 4.16 (ddd, *J* = 14.0, 5.0, 2.0 Hz, 1H)
	24	56.26	3.91 (s, 3H)
	25	56.30	3.92 (s, 3H)

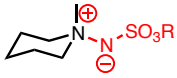
Table S3: A comparison between inner salts of simpler model compounds (2-methylpiperidine and quiniculidine) and *N*-aminated complex natural products.

NMR (ppm)	No	 1-methylpiperidine		 (S,R)-noscapine	 21b	 dextromethorphan	 22b
^{1}H	a	2.23$^{\Delta}$	3.47$^{\delta}$	2.53$^{\Delta}$	3.40$^{\delta}$	2.40$^{\Delta}$	3.56$^{\delta}$
	b	2.32$^{\Delta}$ 2.32$^{\Delta}$	3.92$^{\delta}$ 3.09$^{\delta}$	2.58$^{\Delta}$ 2.34$^{\Delta}$	4.09$^{\delta}$ 3.83$^{\delta}$	2.45$^{\Delta}$ 2.08$^{\Delta}$	3.70$^{\delta}$ 2.80$^{\delta}$
	c	1.57 1.57	2.39 1.63	2.34 1.98	3.30 3.05	1.75 1.39	2.55 1.30
	d	2.32$^{\Delta}$ 2.32$^{\Delta}$	3.92$^{\delta}$ 3.09$^{\delta}$	4.37$^{\Delta}$ _	5.93$^{\delta}$ _	2.82$^{\Delta}$ _	3.95$^{\delta}$ _
	e	1.57 1.57	2.39 1.63	_ _	_ _	1.84 _	3.06 _
^{13}C	a	46.9$^{\Delta}$	56.0$^{\delta}$	46.1$^{\Delta}$	57.0$^{\delta}$	42.8$^{\Delta}$	54.3$^{\delta}$
	b	56.7$^{\Delta}$	66.97$^{\delta}$	49.8$^{\Delta}$	60.3$^{\delta}$	47.4$^{\Delta}$	60.7$^{\delta}$
	c	26.3	20.90	27.8	25.4	41.9	36.9
	d	56.7$^{\Delta}$	66.92$^{\delta}$	60.7$^{\Delta}$	71.6$^{\delta}$	58.1$^{\Delta}$	72.9$^{\delta}$
	e	26.3	20.78	_	_	45.2	37.3

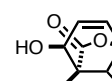
NMR (ppm)	No	 quinuclidine		 cinchonidine	 23a	 brucine	 24a
^{1}H	a	2.87$^{\Delta}$ 2.87$^{\Delta}$	3.87$^{\delta}$ 3.87$^{\delta}$	2.58$^{\Delta}$ 3.46$^{\Delta}$	4.17$^{\delta}$ 4.61$^{\delta}$	3.14$^{\Delta}$ 2.83$^{\Delta}$	4.65$^{\delta}$ 3.72$^{\delta}$
	b	1.53 1.53	2.03 2.03	1.73 1.48	2.35 1.99	1.84 1.84	2.58 2.03
	c	2.87$^{\Delta}$ 2.87$^{\Delta}$	3.87$^{\delta}$ 3.87$^{\delta}$	3.03$^{\Delta}$ 2.62$^{\Delta}$	4.58$^{\delta}$ 3.48$^{\delta}$	3.83$^{\Delta}$ _	4.80$^{\delta}$ _
	d	1.53 1.53	2.03 2.03	_ _	_ _	2.33 1.43	2.82 1.72
	e	2.87$^{\Delta}$ 2.87$^{\Delta}$	3.87$^{\delta}$ 3.87$^{\delta}$	-- 3.09$^{\Delta}$	-- 3.69$^{\delta}$	2.68$^{\Delta}$ 3.67$^{\Delta}$	4.29$^{\delta}$ 4.57$^{\delta}$
	f	1.53 1.53	2.03 2.03	1.73 1.48	2.29 1.47	_ _	_ _
	g	1.73	2.16	1.78	2.04	_	_
^{13}C	a	47.9$^{\Delta}$	60.1$^{\delta}$	43.3$^{\Delta}$	55.9$^{\delta}$	52.7$^{\Delta}$	63.8$^{\delta}$
	b	26.8	25.7	27.8	25.7	26.8	39.5
	c	47.9$^{\Delta}$	60.1$^{\delta}$	57.1$^{\Delta}$	64.8$^{\delta}$	60.4$^{\Delta}$	81.4$^{\delta}$
	d	26.8	25.7	_	_	31.6	25.7
	e	47.9$^{\Delta}$	60.1$^{\delta}$	60.5$^{\Delta}$	67.1$^{\delta}$	50.2$^{\Delta}$	68.0$^{\delta}$
	f	26.8	25.7	21.7	20.6	_	_
	g	20.9	19.6	28.0	26.5	_	_

(*R*)-(1-methylpiperidine-1-ium-1-yl)(((1,1,1-trichloro-4-(hex-5-yn-1-ylamino)-4-oxobutan-2-yl)oxy)sulfonyl)amide (22% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.41 (br s, 1H), 5.24 (t, *J* = 4.5 Hz, 1H), 3.98-3.91 (m, 2H), 3.48 (s, 3H), 3.35-3.25 (m, 2H), 3.14-3.05 (m, 2H), 3.02 (t, *J* = 4.5 Hz, 2H), 2.44-2.35 (m, 2H), 2.22 (dt, *J* = 7.0, 3.0 Hz, 2H),

1.94 (t, *J* = 3.0 Hz, 1H), 1.83-1.80 (m, 1H), 1.71-1.56 (m, 6H), 1.49-1.43 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.5, 99.7, 84.2, 84.0, 68.6, 67.0, 66.9, 56.0, 40.9, 39.2, 28.4, 25.7, 21.5, 20.9, 20.8, 18.1; HRMS (ESI+): *m/z* calcd. for $C_{16}H_{27}Cl_3N_3O_4S$ $[M+H]^+$ 462.0788, found 462.0809.



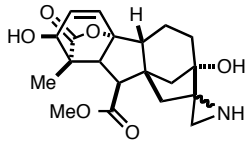
(*R*)-quinuclidine-1-ium-1-yl(((1,1,1-trichloro-4-(hex-5-yn-1-ylamino)-4-oxobutan-2-yl)oxy)sulfonyl)amide (72% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.37 (br s, 1H), 5.22 (t, *J* = 4.5 Hz, 1H), 4.12-3.78 (m, 6H), 3.35-3.26 (m, 2H), 3.01 (ddd, *J* = 20.5, 16.0, 5.0 Hz, 2H), 2.21 (dt, *J* = 7.0, 2.5 Hz, 2H), 2.17-2.14 (m, 1H), 2.04-201 (m, 6H), 1.94 (t, *J* = 3.0 Hz, 1H), 1.68-1.55 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.5, 99.7, 84.3, 84.2, 68.5, 60.1 (3C), 40.8, 39.3, 28.3, 25.7, 25.6 (3C), 19.6, 18.1; HRMS (ESI+): *m/z* calcd. for $C_{17}H_{26}Cl_3N_3O_4SLi$ $[M+Li]^+$ 474.0788, found 474.0774.



(1*S*,2*S*,4a*R*,4b*R*,9a*S*,10*S*)-methyl 7-((*R*)-1,1,1-trichloro-4-(hex-5-yn-1-ylamino)-4-oxobutan-2-yl methylsulfamate)-2-hydroxy-1-methyl-8,13-dioxo-1,2,4b,5,6,7,8,9,10,10a-decahydro-4a,1-(epoxymethano)-7,9a-methanobenzo[α]azulene-10-carboxylate 31 (55% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.41 (d, *J* = 9.0 Hz, 1H), 6.00 (t, *J* = 6.5 Hz, 1H), 5.94 (dd, *J* = 9.0, 3.5 Hz, 1H), 5.73 (t, *J* = 5.5 Hz, 1H), 5.46 (dd, *J* = 7.5, 2.5 Hz, 1H), 4.22 (dd, *J* = 7.5, 4.0 Hz, 1H), 3.76 (s, 3H), 3.40-3.34 (m, 1H), 3.34-3.25 (m, 2H), 3.26 (d, *J* = 7.0 Hz, 1H), 3.17 (dd, *J* = 13.5, 5.5 Hz, 1H), 3.12 (dd, *J* = 16.5, 2.5 Hz, 1H), 2.98 (dd, *J* = 19.0, 3.5 Hz, 1H), 2.83 (dd, *J* = 16.5, 7.5 Hz, 1H), 2.74 (d, *J* = 7.0 Hz, 1H), 2.24 (dt, *J* = 7.0, 2.5 Hz, 2H), 2.21-2.16 (m, 1H), 2.18 (d, *J* = 19.0 Hz, 1H), 2.07-2.01 (m, 2H), 1.98 (t, *J* = 2.5 Hz, 1H), 1.77 (dd, *J* = 12.0, 4.0 Hz, 1H), 1.73 (br s, 1H), 1.70-1.62 (m, 3H), 1.60-1.32 (m, 4H), 1.33 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 216.6, 178.4, 173.0, 168.2, 133.2, 131.4, 98.7, 89.3, 85.4, 84.0, 70.8, 69.1, 55.2, 54.9, 52.7, 52.6, 51.2, 50.7, 50.1, 49.8, 47.1, 43.5, 39.7, 38.8, 31.3, 28.4, 25.7, 18.8, 18.2, 14.5; HRMS (MALDI+): *m/z* calcd. for $C_{30}H_{37}Cl_3N_3O_{10}SNa$ [M+Na] 745.1125, found 745.1135.

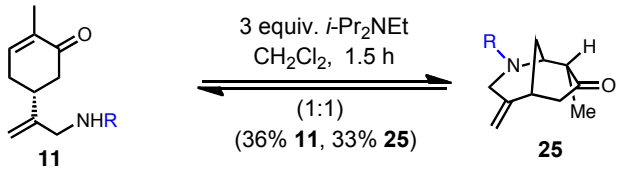
31

(1*S*,2*S*,4a*R*,4b*R*,7*S*,9a*S*,10*S*)-methyl 2,7-dihydroxy-1-methyl-13-oxo-2,4b,5,6,7,9,10,10a-octahydro-1H-spiro[4a,1-(epoxymethano)-7,9a-methanobenzo[α]azulene-8,2'-aziridine]-10-carboxylate 32 (dr = 2:1 separable diastereomers) Major isomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.19 (dd, *J* = 9.5, 1.0 Hz, 1H), 5.92 (dd, *J* = 9.5, 3.5 Hz, 1H), 4.16 (d, *J* = 1.5 Hz, 1H), 3.74 (s, 3H), 3.17 (d, *J* = 10.5 Hz, 1H), 2.93 (d, *J* = 4.5 Hz, 1H), 2.85 (d, *J* = 4.5 Hz, 1H), 2.84 (d, *J* = 6.0 Hz, 1H), 2.34 (br s, 1H), 2.27-2.20 (m, 1H), 2.14 (dd, *J* = 11.5, 2.0 Hz, 1H), 2.03 (dd, *J* = 14, 6.5 Hz, 1H), 1.98-1.93 (m, 4H), 1.77-1.65 (m, 4H), 1.25 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 178.3, 172.6, 132.8, 132.7, 90.4, 73.3, 70.0, 68.0, 53.7, 53.1, 52.8, 52.5, 51.7, 49.9, 49.5, 43.5, 42.7, 33.6, 17.3, 14.5; HRMS (ESI+): calcd. for $C_{20}H_{26}NO_6$ [M+H] 376.1760, found 376.1762. Minor isomer: ^{1}H NMR (500 MHz, $CDCl_3$+CD_3OD) δ 6.23 (d, *J* = 9.0 Hz, 1H), 5.81 (dd, *J* = 9.0, 3.5 Hz, 1H), 3.98 (d, *J* = 4.0 Hz, 1H), 3.65 (s, 3H), 3.14 (d, *J* = 10.5 Hz, 1H), 3.02 (d, *J* = 10.5 Hz, 1H), 2.65 (d, *J* = 6.5 Hz, 1H), 2.64 (d, *J* = 7.0 Hz, 1H), 2.08-2.03 (m, 2H), 1.99 (dd, *J* = 8.5, 5.0 Hz, 1H), 1.97-1.80 (m, 3H), 1.73-1.70 (m, 1H), 1.56 (dd, *J* = 13.5, 3.5 Hz, 1H), 1.37 (dd, *J* = 14, 3.5 Hz, 1H), 1.12 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$+CD_3OD) δ 179.6, 172.8, 133.1, 131.9, 91.0, 74.9, 69.2, 67.5, 53.7, 52.8, 52.1, 52.0, 51.4, 50.9, 50.5, 44.3, 43.1, 31.6, 16.9, 14.2.



33

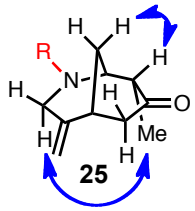
Equilibration between compounds 11 and 25:



11 **25**

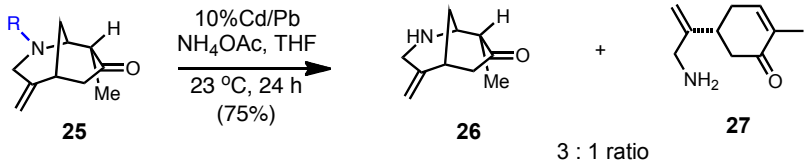
Compound **11** (6 mg, 0.012 mmol) was dissolved in CH_2Cl_2 (1.0 mL) and *i*-Pr_2NEt (4.8 μL, 3.0 equiv.) was added. The resulting mixture was stirred at ambient temperature (23 ^{o}C) and reaction progress was followed by TLC. After 1.5 h stirring, the reaction mixture was purified directly by flash chromatography (EtOAc/Hexane 35%) to afford the starting material **11** (2.2 mg, 0.004 mmol, 36% yield) and its bridged derivative **25** (2.0 mg, 0.004 mmol, 33% yield). Both were isolated as colorless oil.

(1*S*,5*R*,8*R*)-(R)-1,1,1-trichloro-4-(hex-5-yn-1-ylamino)-4-oxobutan-2-yl 8-methyl-4-methylene-7-oxo-2-azabicyclo[3.3.1]nonane-2-sulfonate 25. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.63 (m, 1H), 5.53 (t, *J* = 4.5 Hz, 1H), 4.95 (s, 1H) 4.91 (s, 1H), 4.52 (s, 1H),



4.22 (d, *J* = 16.5 Hz, 1H), 3.47 (d, *J* = 17.0 Hz, 1H), 3.35-3.29 (m, 2H), 3.10 (s, 1H), 3.06 (dd, *J* = 16.0, 4.0 Hz, 1H), 2.80 (dd, *J* = 16.5, 5.0 Hz, 1H), 2.67 (dd, *J* = 16.0, 5.5 Hz, 1H), 2.62-2.56 (m, 1H), 2.52 (d, *J* = 16.0 Hz, 1H), 2.32 (dd, *J* = 13.5, 3.0 Hz, 1H), 2.23 (dt, *J* = 7.0, 2.5 Hz, 2H), 2.12 (d, *J* = 13.5 Hz, 1H), 1.96 (t, *J* = 2.5 Hz, 1H), 1.68-1.53 (m, 4H), 1.27 (d, *J* = 6.5Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 210.1, 167.3, 141.7, 112.8, 98.7, 84.7, 84.2, 68.9, 58.1, 48.9, 47.4, 46.4, 39.9, 39.6, 39.2, 33.6, 28.6, 25.8, 18.2, 11.6; HRMS (MALDI+): *m/z* calcd. for $C_{20}H_{28}Cl_3N_2O_5S$ [M+H] 513.0785, found 513.0802. The stereochemistry of **25** was confirmed by NOESY analysis.

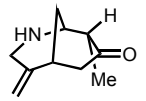
Cleavage of sulfamate side chain to give amines 26 and 27:



Sulfamate **25** (15 mg, 0.03 mmol, 1.0 equiv.) was dissolved in THF (0.5 mL), and mixed with *aq.* NH_4OAc solution (1M, 0.5 mL) and 28 mg of 10% Cd/Pb couple[2] was added in one portion and the suspension was vigorously stirred for 2 h. Additional 10% Cd/Pb couple (17 mg) was added and the mixture was vigorously stirred overnight. The mixture was filled through a short pad of Celite and eluted with MeOH (5 x 1 mL). The filtrate was concentrated and the residue was purified by flash column chromatography (eluting with $MeOH/CH_2Cl_2$) to isolate the product (4 mg, 0.02 mmol, 75% yield) as a colorless oil, which was identified as a 3:1 mixture of compound **26** and **27**.

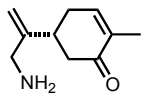
(1*S*,5*R*,8*R*)-8-methyl-4-methylene-2-azabicyclo[3.3.1]nonan-7-one 26 (Major product). ^{1}H NMR (500 MHz, CD_3OD) δ 4.79 (dd, *J* = 4.5, 1.0 Hz, 2H), 4.34 (br s, 1H), 4.10 (d, *J* = 16.5 Hz, 1H), 3.25 (d, *J* = 17.0 Hz, 1H), 3.03 (br s, 1H), 2.78 (dd, *J* = 16.0,

[2] Q. Dong, C. E. Anderson, M. A. Ciufolini *Tetrahedron Lett.* **1995**, *36*, 5681-5682.



26

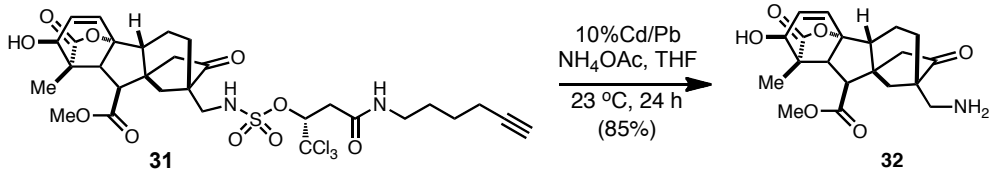
6.0 Hz, 1H), 2.67-2.63 (m, 1H), 2.52 (ddd, *J* = 13.5, 7.0, 4.0 Hz, 1H), 2.33 (dt, *J* = 15.5, 2.5 Hz, 1H), 2.01 (dt, *J* = 13.0, 2.5 Hz, 1H), 1.18 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 215.3, 147.2, 109.8, 57.7, 50.5, 49.0, 46.5, 41.4, 33.2, 11.9; LRMS (APCI+): *m/z* calcd. for $C_{10}H_{16}NO$ [M+H] 166.1, found 166.1.



27

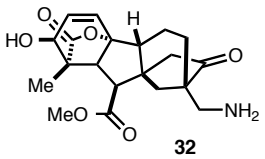
(*R*)-5-(3-aminoprop-1-en-2-yl)-2-methylcyclohex-2-enone 27 (Minor product). ^{1}H NMR (500 MHz, CD_3OD) δ 6.88 (dd, *J* = 5.5, 1.0 Hz, 1H), 5.12 (s, 1H), 4.92 (s, 1H), 3.63 (d, *J* = 3.0 Hz, 2H), 2.96-2.90 (m, 1H), 2.60-2.56 (m, 2H), 2.51-2.45 (m, 1H), 2.40-2.36 (m, 1H), 1.74 (d, *J* = 1.0 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 202.2, 150.1, 147.5, 136.0, 111.7, 48.4, 44.2, 40.1, 32.8, 15.7.

Cleavage of sulfamate side chain to give primary amine derivative of gibberelic acid derivative 32:



31 **32**

Sulfamate **31** (22 mg, 0.03 mmol, 1.0 equiv.) was dissolved in THF (0.5 mL). *aq*. NH_4OAc solution (1M, 0.5 mL) and 28 mg of 10% Cd/Pb couple were added in one portion and the suspension was stirred vigorously at room temperature (23 ^{o}C) for 2 h. An additional portion of 10% Cd/Pb couple (17 mg) was added and the mixture was vigorously stirred overnight. The mixture was filled through a short pad of Celite and eluted with MeOH (5 x 1 mL). The filtrate was concentrated and the residue was purified by flash column chromatography (eluting with $MeOH/CH_2Cl_2$) to isolate the product **32** (9.6 mg, 0.026 mmol, 85% yield) as colorless oil.

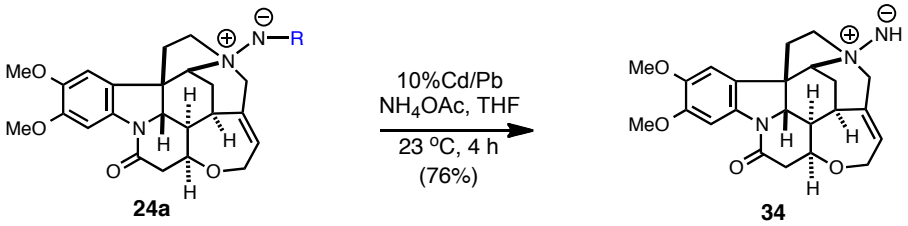


32

(1*S*,2*S*,4a*R*,4b*R*,9a*S*,10*S*)-methyl 7-(aminomethyl)-2-hydroxy-1-methyl-8,13-dioxo-1,2,4b,5,6,7,8,9,10,10a-decahydro-4a,1-(epoxymethano)-7,9a-methanobenzo[α]azulene-10-carboxylate 32. ^{1}H NMR (500 MHz, CD_3OD) δ 6.47 (d, *J* = 10.0 Hz, 1H), 5.90 (dd, *J* = 9.5, 3.5

Hz, 1H), 4.05 (d, *J* = 3.5 Hz, 1H), 3.76 (s, 3H), 3.29 (d, *J* = 7.0 Hz, 1H), 3.12 (d, *J* = 13.0 Hz, 1H), 3.00 (d, *J* = 13.0 Hz, 1H), 2.82 (d, *J* = 7.5 Hz, 1H), 2.77 (dd, *J* = 19.0, 3.5 Hz, 1H), 2.28-2.23 (m, 3H), 2.10-2.06 (m, 1H), 2.74 (dd, *J* = 11.5, 3.5 Hz, 1H), 1.63-1.60 (m, 2H), 1.47 (dt, *J* = 13.0, 6.5 Hz, 1H), 1.25 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 219.8, 180.9, 174.6, 134.7, 131.7, 91.3, 71.4, 56.5 (2C), 54.0, 52.6, 52.5, 51.9, 51.3, 50.9, 47.3, 44.4, 33.2, 19.8, 14.7; HRMS (MALDI+): *m/z* calcd. for $C_{20}H_{26}NO_6$ [M+H] 376.1754, found 376.1768.

Cleavage of sulfamate side chain to give *N*-amino brucine 34:



Inner salt **24a** (1.1 mg, 0.0015 mmol, 1.0 equiv.) was dissolved in THF (0.05 mL). *aq.* NH_4OAc solution (1M, 0.05 mL) and 2 mg of 10% Cd/Pb couple were added in one portion and the suspension was vigorously stirred at room temperature (23 °C) for 2 h. Additional 10% Cd/Pb couple (2 mg) was added and the mixture was vigorously stirred for additional 2 h. The mixture was filled through a short pad of Celite, rinsed with MeOH (5 x 1 mL). The filtrate was concentrated and the residue was purified by prep. TLC purification (developed with 5% MeOH/CH_2Cl_2), and the major UV absorption band was collected and extracted with 25% of MeOH/CH_2Cl_2. A colorless oil (449 μg, 1.1 μmol, 76% yield) was obtained after evaporation of solvents.

((4a*R*,4a1*R*,5a*S*,6*S*,8a*S*,8a1*S*,15a*S*)-10,11-dimethoxy-14-oxo-2,4a,4a1,5,5a,6,7,8,8a1,14,15,15a-dodecahydro-4,6-methanoindolo[3,2,1-*ij*]oxepino[2,3,4-*de*]pyrrolo[2,3-*h*]quinolin-6-ium-6-yl)amide 34. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.59 (br s, 1H), 7.76 (d, *J* = 1.0 Hz, 1H), 7.20 (d, *J* = 1.0 Hz, 1H), 6.45 (br s, 1H), 5.11 (dd, *J* = 12.5, 7.0 Hz, 1H), 5.07 (br s, 1H), 4.77 (d, *J* = 13.5 Hz, 1H), 4.40 (d, *J* = 14.0 Hz, 1H), 4.35 (d, *J* = 8.5 Hz, 1H), 4.30 (dd, *J* = 14.0, 7.0 Hz, 1H), 4.10 (dd, *J* = 13.5, 5.5 Hz, 1H), 4.00 (d, *J* = 10.5 Hz, 1H), 3.96 (s, 3H), 3.91 (s, 3H), 3.73 (dt, *J* = 13.5,

5.5 Hz, 1H), 3.34 (br s, 1H), 3.16 (dd, *J* = 17.5, 9.0 Hz, 1H), 2.94 (d, *J* = 15.5 Hz, 1H), 2.83 (dt, *J* = 14.5, 7.5 Hz, 1H), 2.68 (d, *J* = 17.5 Hz, 1H), 2.10 (dd, *J* = 14.0, 4.5 Hz, 1H), 1.80 (d, *J* = 15.0 Hz, 1H), 1.44 (d, *J* = 10.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.2, 150.3, 147.1, 137.2, 135.2, 132.8, 118.4, 105.3, 100.7, 78.5, 77.5, 66.5, 64.2, 61.3, 58.8, 56.6, 56.3, 52.3, 47.2, 42.1, 39.2, 30.1, 25.4; HRMS (ESI+): *m/z* calcd. for $C_{23}H_{28}N_3O_4$ [M+H] 410.2080, found 410.2063.

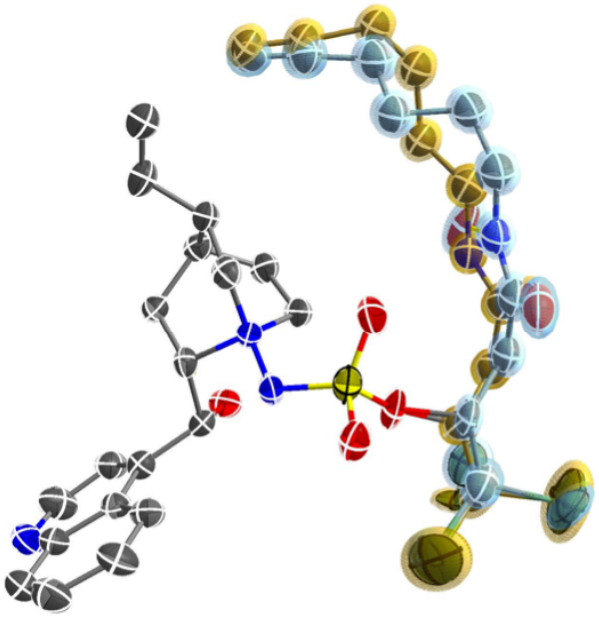


Figure S1. ORTEP Representation of Single Crystal X-ray Structure of (R)-((R)-quinolin-4-yl((1S,2S,4S,5R)-5-vinylquinuclidin-2-yl)methanol-1-ium-1-yl)(((1,1,1-trichloro-4-(hex-5-yn-1-ylamino)-4-oxobutan-2-yl)oxy)sulfonyl)amide 23a (CCDC Registry # 930772)

CheckCif Alerts and Responses:_vrf_PLAT374_ALERT_2_A; PROBLEM: long N - N Bond (> 1.45 Angstrom) RESPONSE: N+-N(-) single bond; _vrf_PLAT327_ALERT_2_B; PROBLEM : Check for Possibly Missing H on sp3? Carbon .... >C21_3 RESPONSE : Disordered amine-alkane chain : CIFCHECK program error all H atoms present ; _vrf_PLAT327_ALERT_2_B; PROBLEM : Check for Possibly Missing H on sp3? Carbon .... <C21_4 RESPONSE : Disordered amine-alkane chain : CIFCHECK program error all H atoms present ; _vrf_PLAT420_ALERT_2_B ; PROBLEM : D-H Without Acceptor *O1W - *H1WA ... ? RESPONSE : Disordered amine-alkane chain : H-bond to amine ; _vrf_PLAT420_ALERT_2_B ; PROBLEM : D-H Without Acceptor *O1W - *H1WB ... ? RESPONSE : Disordered amine-alkane chain : H-bond to amine

Cell culture

HL-60 cells (ATCC) were grown in RPMI 1640 (Mediatech) supplemented with 10% fetal bovine serum (FBS) (Gemini). Cells were passaged six times in RPMI-1640 minus L-Lysine and L-Arginine (Thermo) supplemented 10% dialyzed FBS (Gemini) and 100 mg / L [$^{13}C_6$,$^{15}N_4$] L- Arginine-HCl and [$^{13}C_6$,$^{15}N_2$] L-Lysine-HCl (Aldrich) or L-Arginine-HCl and L-Lysine-HCl (Sigma) and cell aliquots were frozen and replaced periodically. HEK293T cells were grown in DMEM supplemented with 10% FBS.

EuPAyne labeling

EuPA and EuPAyne were dissolved in DMSO to make 50 mM stock solutions and stored at -80 °C. Immediately before labeling experiments, the EuPA stock solution was added to serum free RPMI media to make a 150 μM solution and the EuPAyne stock solution was added to serum free RPMI to make a 50 μM solution. In the **control experiments**, 9E6 heavy and light cells in 9 mL were treated with 1 mL of the 50 μM EuPAyne RPMI/DMSO solution (5 μM EuPAyne final concentration) and incubated at 37 °C for 30 min. In the **competition experiments**, the light cells (8E6 in 8 mL) were pretreated with 1 mL of 150 μM EuPA. After incubation at 37 °C for 30 min, 1 mL of 50 μM EuPAyne was added (giving final 5 μM EuPAyne and 15 μM EuPA), and the cells were incubated for an addition 30 min at 37 °C. Heavy cells (8E6 cells in 8 mL) were treated with 1 mL of 0.1% DMSO in serum free RPMI for 30 min at 37 °C. EuPAyne (1 mL of 50 μM dmso/RPMI solution) was then added and the cells were incubated at 37 °C for an additional 30 min. Following labeling, cells were collected by centrifugation (500x g) and washed with PBS (2 x 10 mL) and stored as pellets at -80 °C.

Lysate preparation, click chemistry, and enrichment

Frozen cell pellets were resuspended in 65 μL PBS and sonicated. Protein concentrations were determined using the BCA protein assay on a microplate reader. For fluorescent gel-based analysis, 50 μg of lysate (1 mg/mL) was mixed with 25 mM rhodamine-azide, 1 mM Tris(2-carboxyethyl)phosphine (TCEP, Sigma-Aldrich), 100 mM Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA) (Sigma-Aldrich), and 1 mM $CuSO_4$ in PBS in a

1.5 mL plastic tube at room temperature. After 1 hour, samples were mixed with SDS sample loading buffer and loaded (10 µg) without boiling on a 4-20% gradient tris-glycine gel (Invitrogen, 150 Volts for 90 min) and imaged on a Hitachi FMBIO-II flatbed fluorescence scanner. For proteomic studies, 1 mg of both heavy and light proteomes were mixed equally in a 1:1 ratio and adjusted to 2 mL in PBS. 20 µL 1 mM $CuSO_4$, 60 µL 100 µM TBTA, 20 µL 1 mM TCEP, and 40 µL 10 mM biotin-azide were added. After mixing for 1.5 hr, 2 mL MeOH and 0.5 mL $CHCl_3$ were added, shaken, and centrifuged at 4000x g for 10 min at room temperature yielding a protein interphase between aqueous and organic layers. The top and bottom layers were aspirated, leaving the protein interphase. To the protein interphase 2 mL MeOH was added and the mixture was sonicated and 2 mL PBS and 0.5 mL $CHCl_3$ were added. The mixture was shaken and then centrifuged at 4000x g. The top and bottom phases were aspirated leaving the protein interphase. The protein interphase was then washed with cold methanol and solubilized in 6 M urea in PBS containing 0.5% SDS (500 µL final volume). The solution was treated with 10 mM neutralized TCEP for 30 minutes at 37 °C and followed with treatment with 20 mM iodoacetamide for 30 minutes at room temperature. The sample was diluted 10x in PBS and SDS was added to a final concentration of 0.2%. Streptavidin beads (Thermo) (100 mL slurry) were added and rotated at room temperature for 2 hours. Beads were transferred to Bio-spin filters (BioRad) and coupled to a vacuum manifold and washed with 10 x 1 mL 1% SDS in PBS, then 20 x 1 mL PBS. Beads were transferred to screw-top eppendorf tubes and resuspended in 2 M urea/PBS supplemented with 1 mM calcium chloride and sequence grade porcine trypsin (Promega) for overnight digestion at 37°C. The eluant was collected the following day and acidified with 5% formic acid.

Mass spectrometry

Mass spectrometry was performed using a Thermo Orbitrap Velos mass spectrometer. Peptides were eluted using a 5-step MudPIT protocol (using 0%, 25%, 50%, 80%, and 100% salt bumps of 500 mM aqueous ammonium acetate, each step followed by an increasing gradient of aqueous acetonitrile/0.1% formic acid) and data were collected in data-dependent acquisition mode with dynamic exclusion turned on (60 s, repeat of 1).

Specifically, one full MS (MS1) scan (400-1800 m/z) was followed by 7 MS2 scans of the most abundant ions. The MS2 spectra data were extracted from the raw file using RAW Xtractor (version 1.9.1; publicly available at http://fields.scripps.edu/?q=content/download). ProLuCID searches allowed for variable oxidation of methionine (+15.9949), static modification of cysteine residues (+57.0215 due to alkylation), and no enzyme specificity. Each data set was independently searched with light and heavy params files; for the light search, all other amino acids were left at default masses; for the heavy search, static modifications on lysine (8.0142) and arginine (10.0082) were specified. The precursor ion mass tolerance was set to 50 ppm and the fragment ion mass tolerance was left at the default assignment of 0. The data was searched using a human reverse-concatenated non-redundant (gene-centric) FASTA database using the ProLuCID (http://fields.scripps.edu/prolucid/index.html). The resulting MS2 spectra matches were assembled into protein identifications and filtered using DTASelect (version 2.0.47) with the --trypstat option, which applies different statistical models for the analysis of tryptic, half-tryptic, non-tryptic peptides. Redundant peptide identifications common between multiple proteins were allowed, but the database was restricted to a single consensus splice variant. SILAC ratios were quantified using in-house software.[3] The program was updated to identify cases where complete inhibition could not be quantified based on light/heavy peak pairs due the absence of a MS1 signal from either the heavy or light sample. In order to identify these cases, all single MS1 chromatographic peaks (from either the light or the heavy sample) were identified within a retention time window. Next, these peaks were aligned with the corresponding sequence ProLuCID /DTASelect identification and the charge state and monoisotopic mass were validated using the "envelope correlation score" filter.[3] Finally, the candidate peak was cross-checked to ensure there was no corresponding (heavy or light) peak co-eluting around the same retention time window. Only after all these conditions are met, the peptide was assigned as the case of complete inhibition with an artificial threshold ratio of 20. The described control and competition experiments were performed in duplicate. Ratios reported were the mean ratio of peptides for each given protein. Valid

[3] Weerapana E, Wang C, Simon GM, Richter F, Khare S, Dillon MB, et al. Quantitative reactivity profiling predicts functional cysteines in proteomes *Nature* **2010**, *468*, 790-795

ratios for at least 2 peptides per protein were required in order for the protein to be considered quantified (see Supplemental Table S2).

Plasmids

The template for DRL1 were obtained from HL-60 cDNA using the forward primer (GCATGAATTCATGTCGGACATCG; ECOR1 cut site in red) and reverse primer (GCATCTCGAGCTGGTCTCCAAGTC; XhoI cut site in red). The template for CYB5B were obtained from HL-60 cDNA using the forward primer (GCATGAATTCATGTCCGGTTCAATGG; ECOR1 cut site in red) and reverse primer (GCATCTCGAGGGAGGATTTGCTTTCC; XhoI cut site in red). Each amplified cDNA was subcloned into the expression plasmid pcDNA3.1(+)-myc-His A (Invitrogen). Human TBXAS1 cDNA was obtained from OpenBiosystems in the pCMV-SPORT6 expression vector.

Validation of EuPA labeling events

HEK293T cells were grown to 70% confluency in 10 cm dishes in DMEM (supplemented with 10% FCS). Cells were transfectred using Fugene HD and 5 µg vector or empty vector control ("mock") using the manufacturers protocols. After 24 hrs, the cells were trypsanized and replated in duplicate in 6-well dishes. After an additional 24 hrs, the media was aspirated, and the cells were treated with EuPA (15 µM) or DMSO. After 30 min at 37 °C, EuPAyne (5 µM) was added and allowed to react for an additional 30 min at 37 °C. Cells were harvested, centrifuged (1400 g x 4 min) and the cell pellet wash washed twice with cold PBS. Cell pellets were sonicated and adjusted to 1 mg/mL using the BCA Assay. Conjugation to rhodamine-azide and imaging was performed as described above. Western blotting was performed using anti-TBXAS1 (1:1000 Cayman Chemical) or anti-Myc (1:1000 Invitrogen) using 100 µg sample.

Uniprot Accession	Description	Gene Symbol	MW kDa	Rep 1	Rep 2	Ave. Ratio	S.E.M.	Rep 1	Rep 2	Ave. Ratio	S.E.M.
Q9BUN8	DERL1 Derlin-1	DERL1	28.8/ 26.4	0.96	0.9	0.93	0.03	20	0	20.00	0.00
O43169	CYB5B Cytochrome b5 type B	CYB5B	16.3	0.96	0.91	0.94	0.03	20	20	20.00	0.00
Q5TFE4	NT5DC1 5-nucleotidase domain-containing protein 1	NT5DC1	51.8	0.87	0.94	0.91	0.04	14.32	20	17.16	2.84
P50416	CPT1A Carnitine O-palmitoyltransferase 1, liver isoform	CPT1A	88.4/ 86.2	1.04	0.93	0.99	0.06	20	11.59	15.80	4.21
O15533	TAPBP Tapasin	TAPBP	43.9/ 53.9	1	0.82	0.91	0.09	10.39	20	15.20	4.81
P24557	TBXAS1 Thromboxane-A synthase	TBXAS1	60.5	0.97	0.85	0.91	0.06	20	8.86	14.43	5.57
Q03518	TAP1 Antigen peptide transporter 1	TAP1	87.2	0.95	0.93	0.94	0.01	8.16	20	14.08	5.92
Q9NQC3	RTN4 Reticulon-4	RTN4	129.9 /40.3 /22.0	0.99	0.91	0.95	0.04	9.91	9.04	9.48	0.44
P04150	NR3C1 Glucocorticoid receptor	NR3C1	85.6/ 64.8	0.8	0.86	0.83	0.03	4.35	12.84	8.60	4.25
Q9Y6K0	CEPT1 Choline/ethanolaminephospho transferase 1	CEPT1	46.6	0.98	0.9	0.94	0.04	7.5	0	3.75	3.75
Q5VV42	CDKAL1 CDK5 regulatory subunit-associated protein 1-like	CDKAL1	65.1/ 54.7/ 11.0	0.76	0.51	0.64	0.13	12.07	2.72	7.40	4.68
O95197	RTN3 Reticulon-3	RTN3	112.6 /26.4	0.98	0.96	0.97	0.01	6.79	6.85	6.82	0.03
P16615	ATP2A2 Sarcoplasmic/endoplasmic reticulum calcium ATPase	ATP2A2	114/ 112	1	0.84	0.92	0.08	8.56	3.5	6.03	2.53
Q8WV74	NUDT8 Nucleoside diphosphate-linked moiety X motif 8	NUDT8	25.4/ 15	0.87	0.74	0.81	0.07	5.12	4.33	4.73	0.40

Table S4. Chemoproteomic list of high affinity EuPA targets. Ratios reported are the mean ratio of peptides for each given protein. Proteins were only considered if > 2 peptides were quantified. Targets with signals > 5-fold higher in EuPAyne (15 μM) compared to EuPAyne + 3X EuPA (Competition experiment) are shown. A value of 20 was given as an upper limit ratio for peptides were completely competed with EuPA. See methods below for “control experiment” and “competition experiment” descriptions.

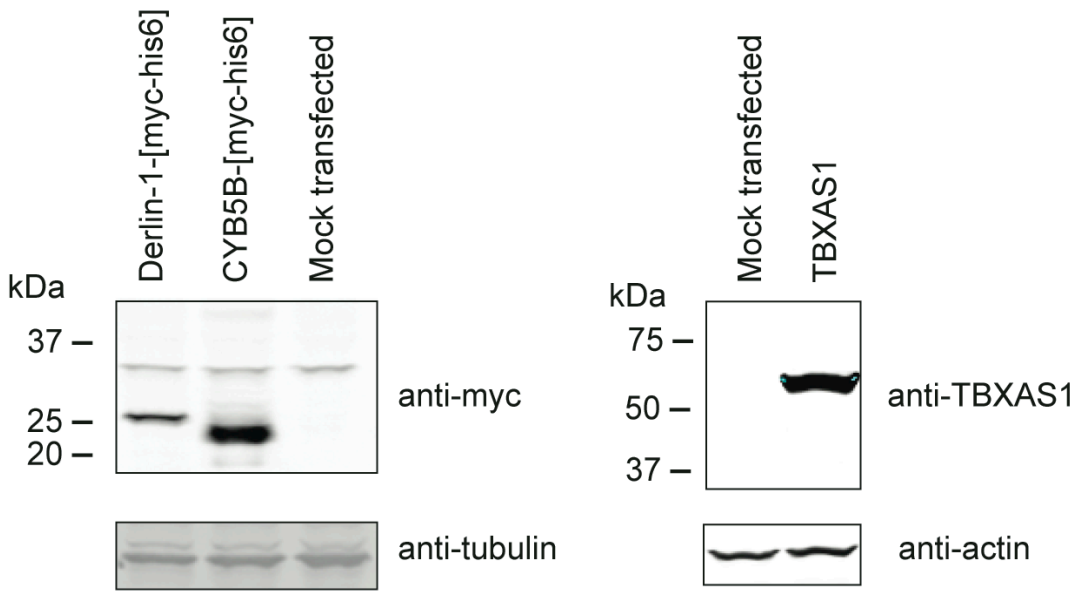


Figure S2A. Western blot from HEK293T cells overexpressing myc-His_6 tagged Derlin-1 or myc-His_6 tagged CYB5B or TBXAS1.

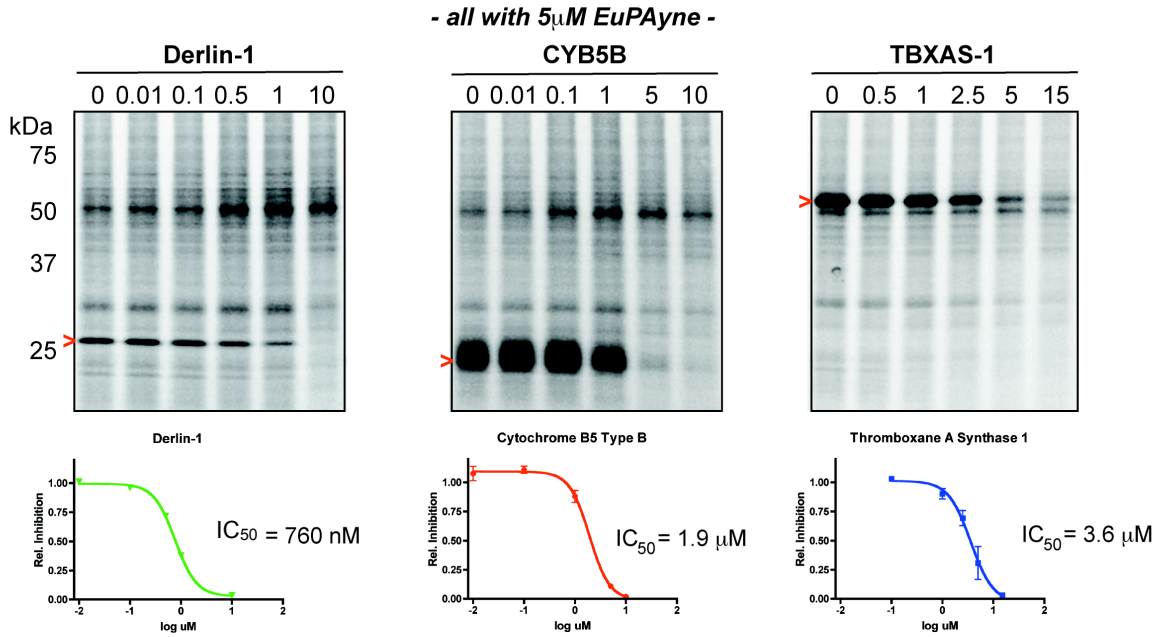
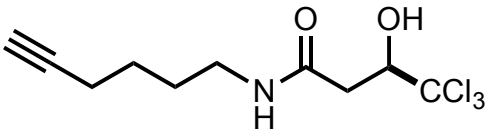
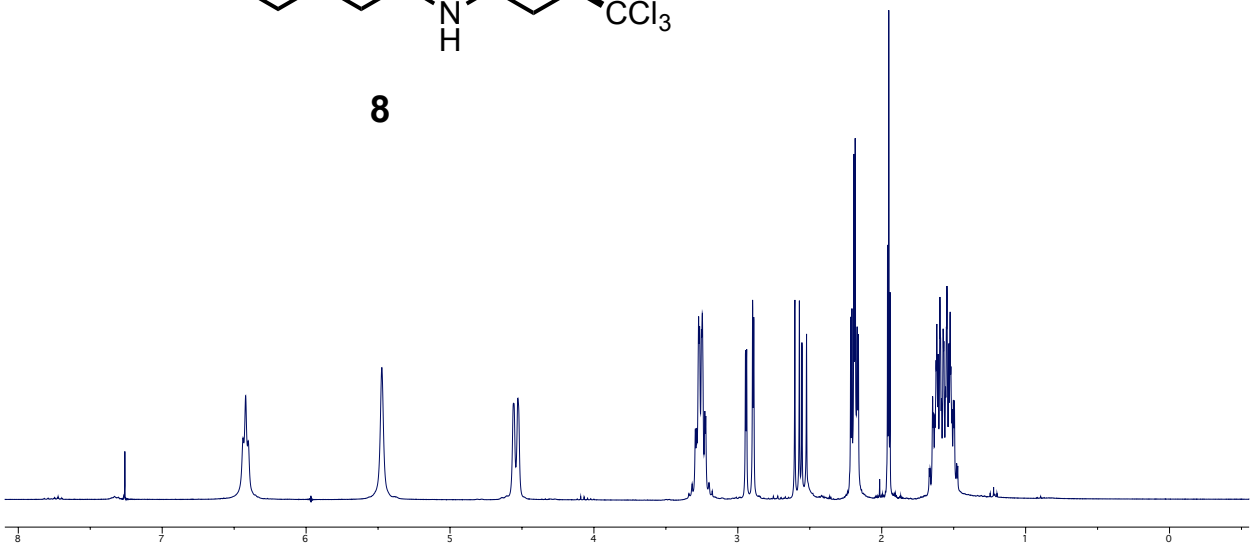
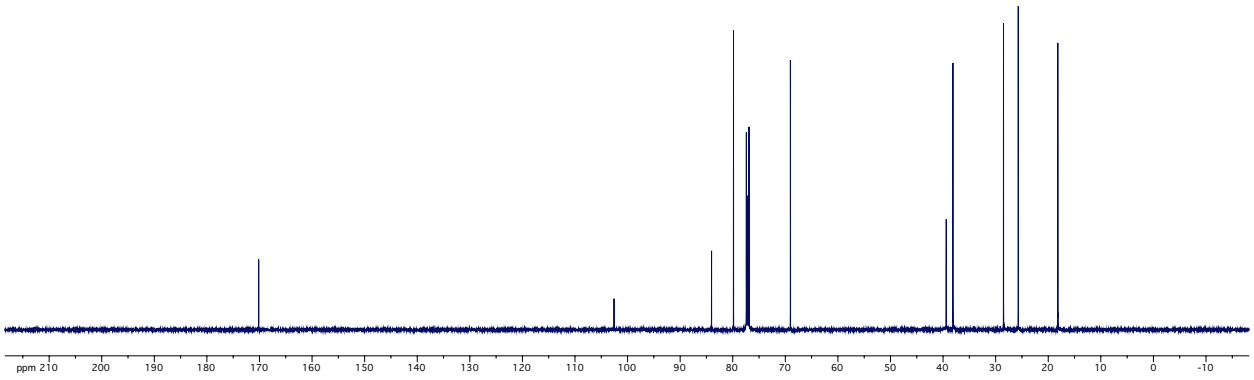


Figure S2B. *In situ* EuPAyne (5 μM) labeling of 293T cells overexpressing target proteins competed with varying concentrations (0–10 μM EuPA) and quantitation of labeling IC_{50}. In-gel fluorescence scanning depicted in grayscale.

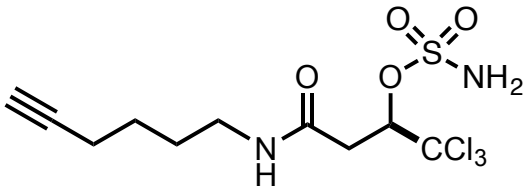


8

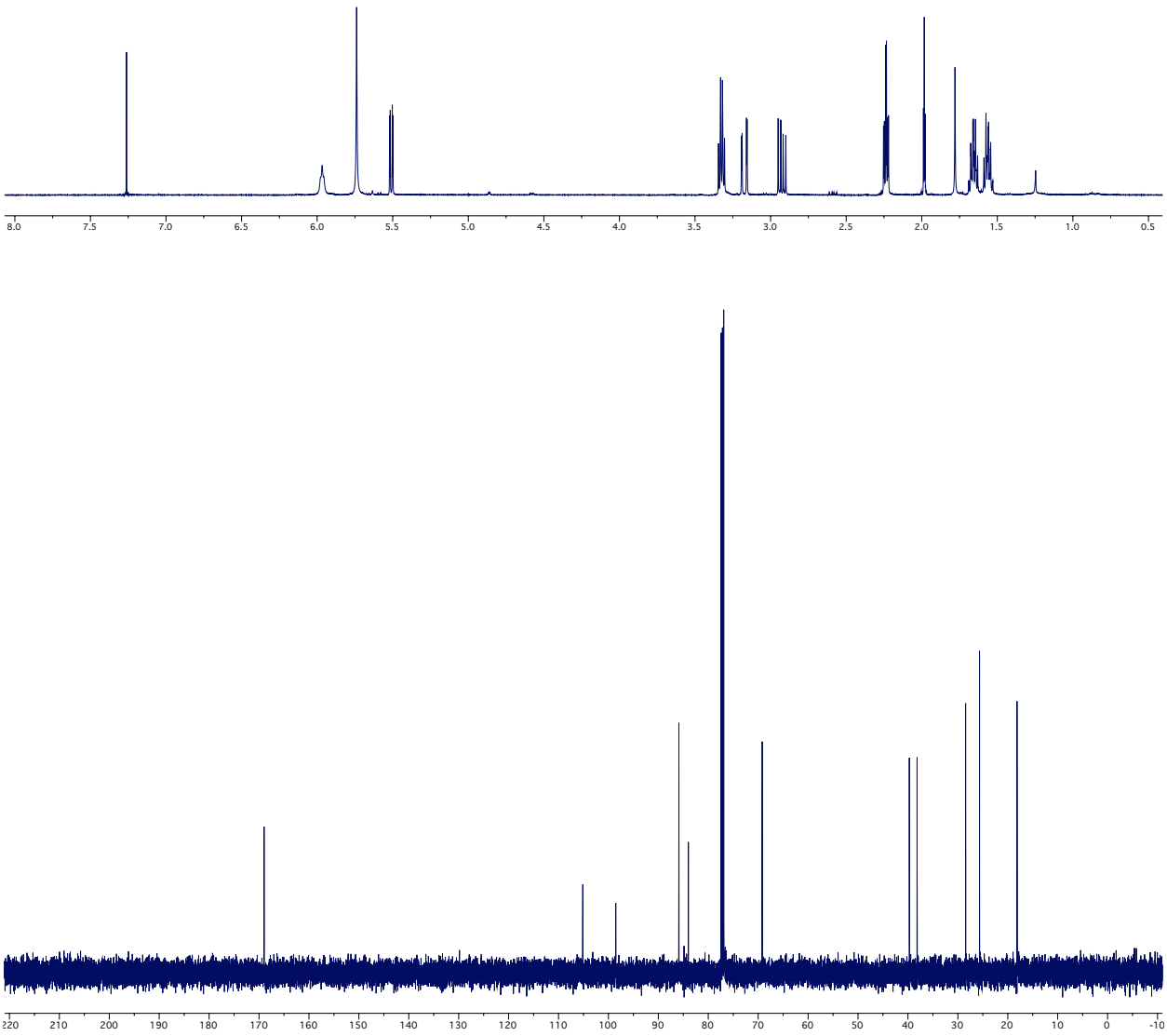




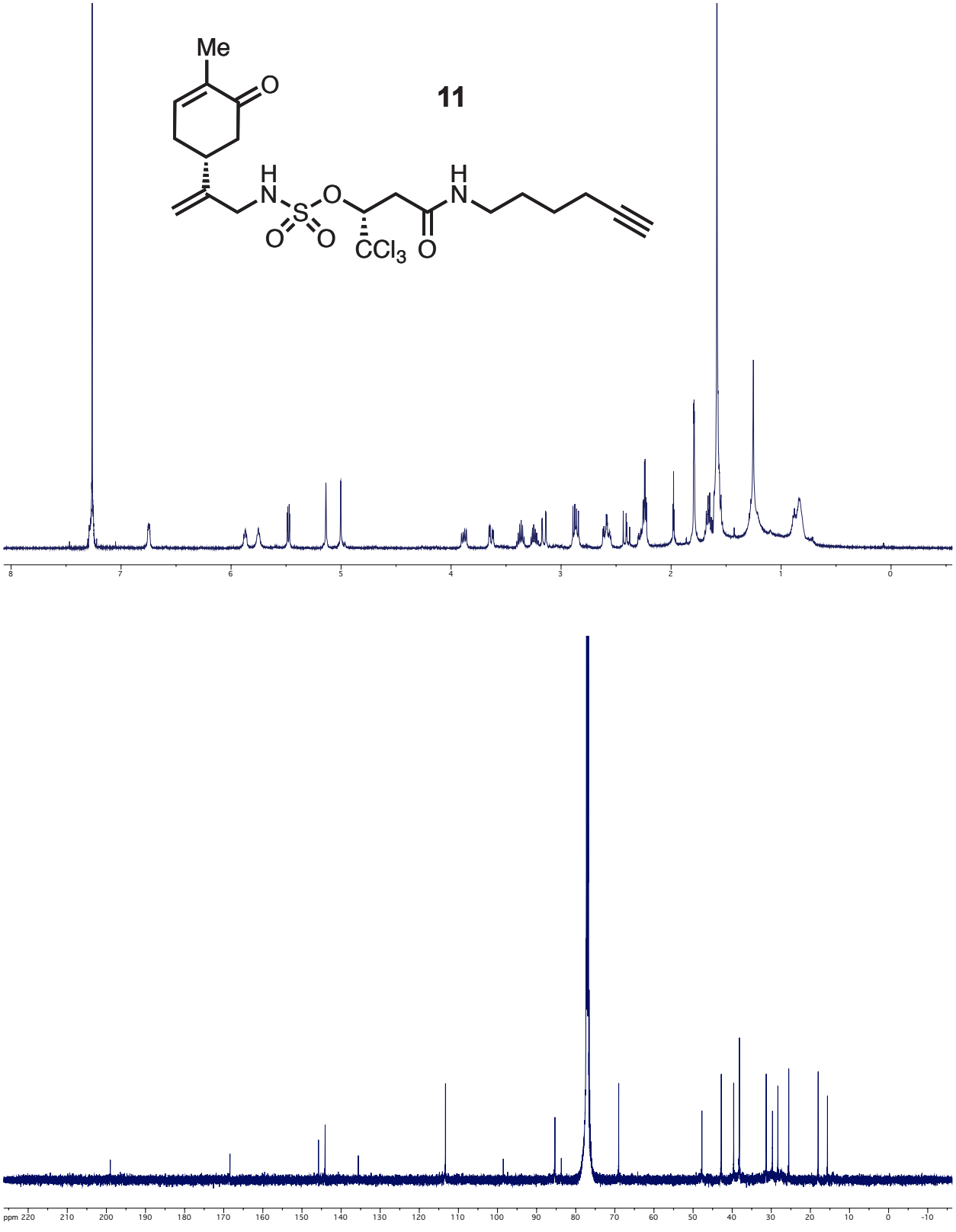
^{1}H (300 MHz) and ^{13}C (125 MHz) NMR of **8** in $CDCl_3$



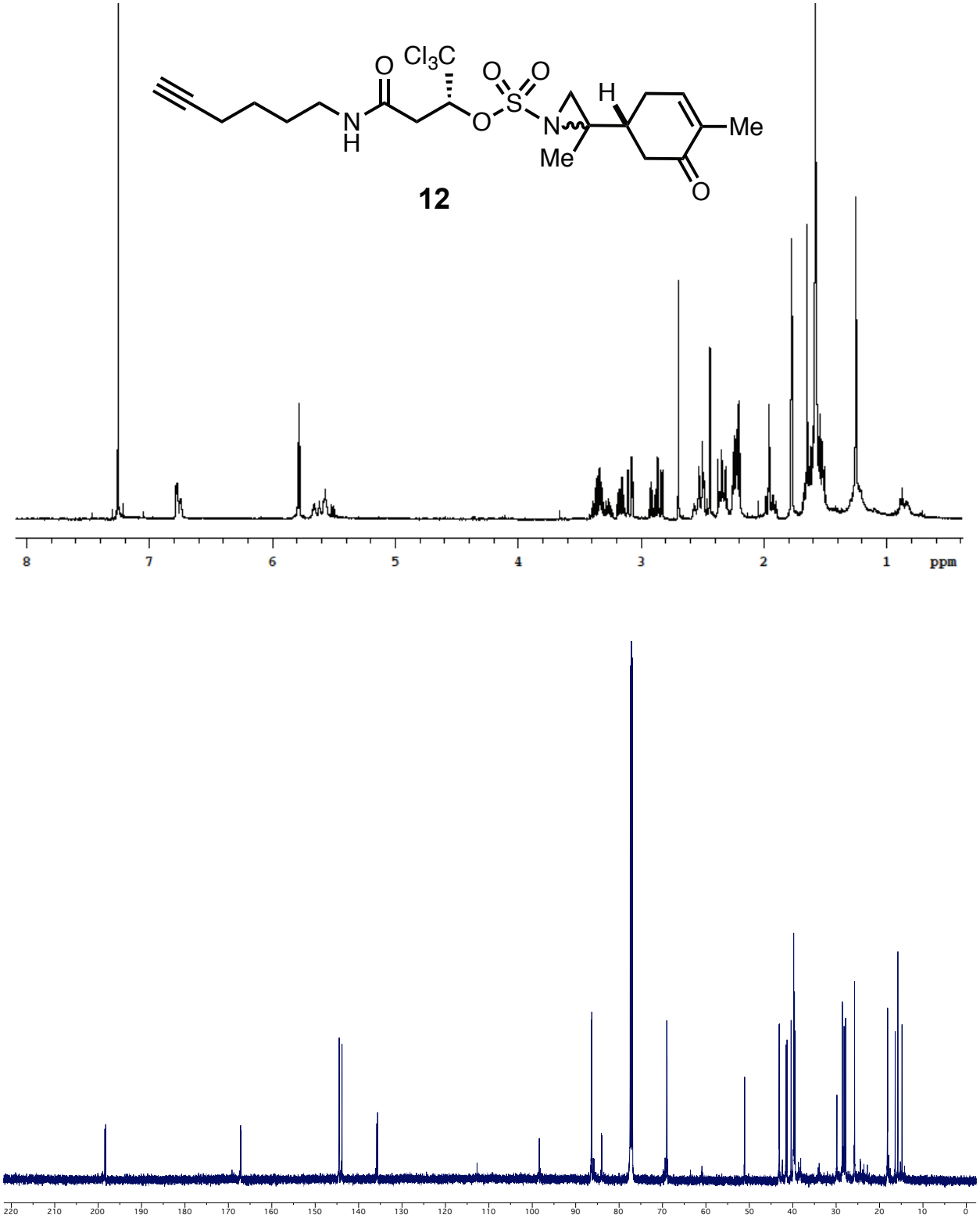
9



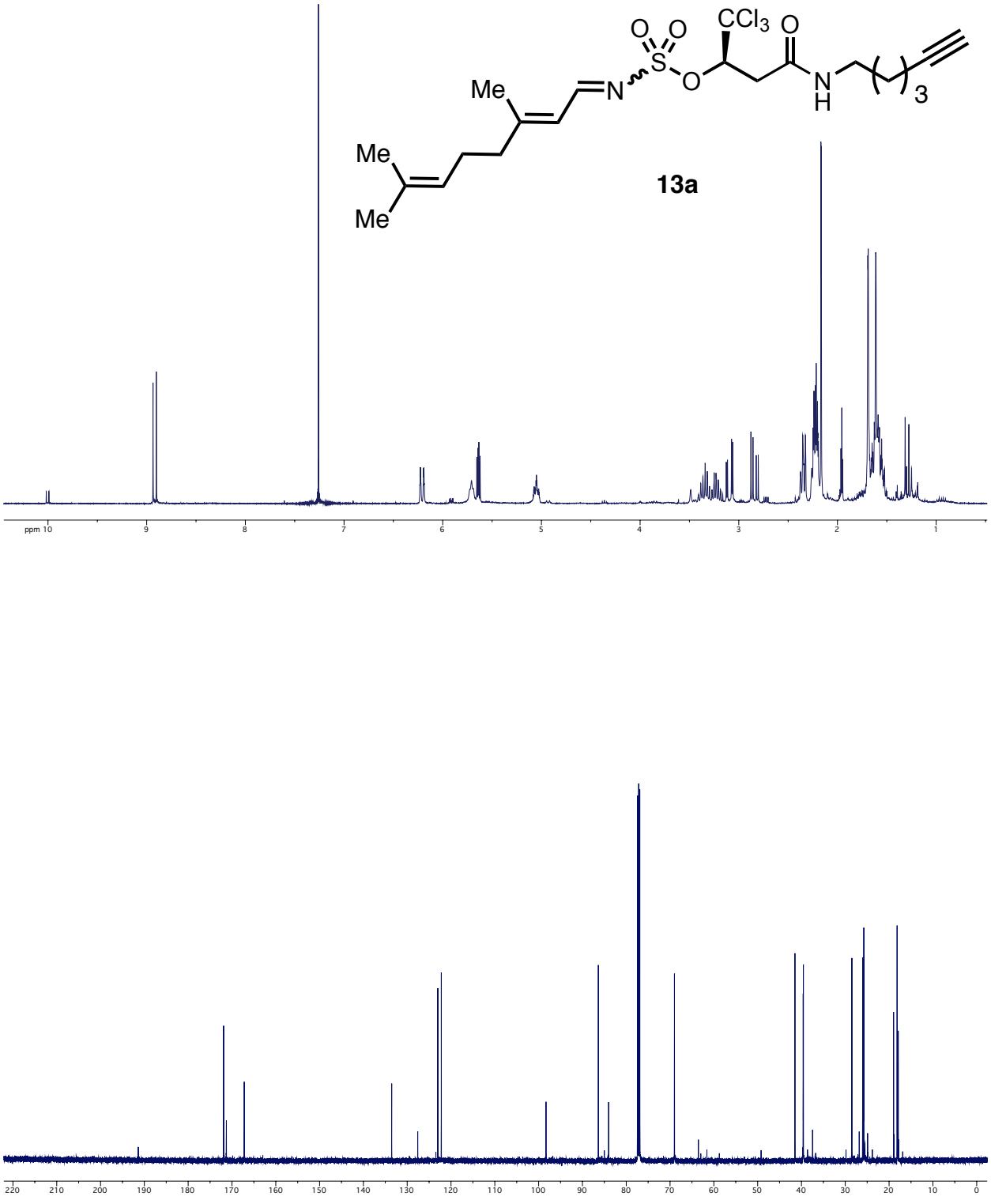
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR of **9** in $CDCl_3$



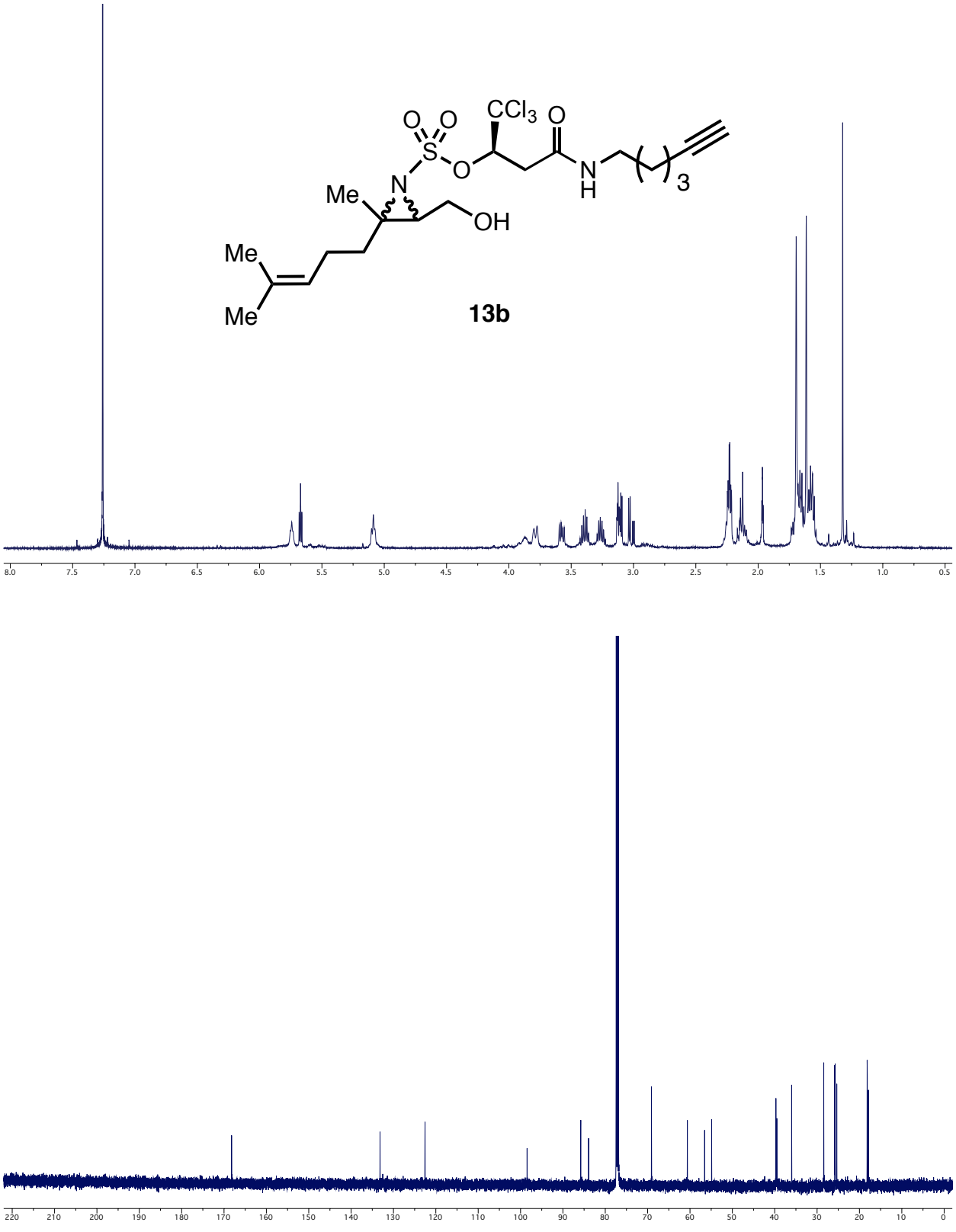
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR of **11** in $CDCl_3$



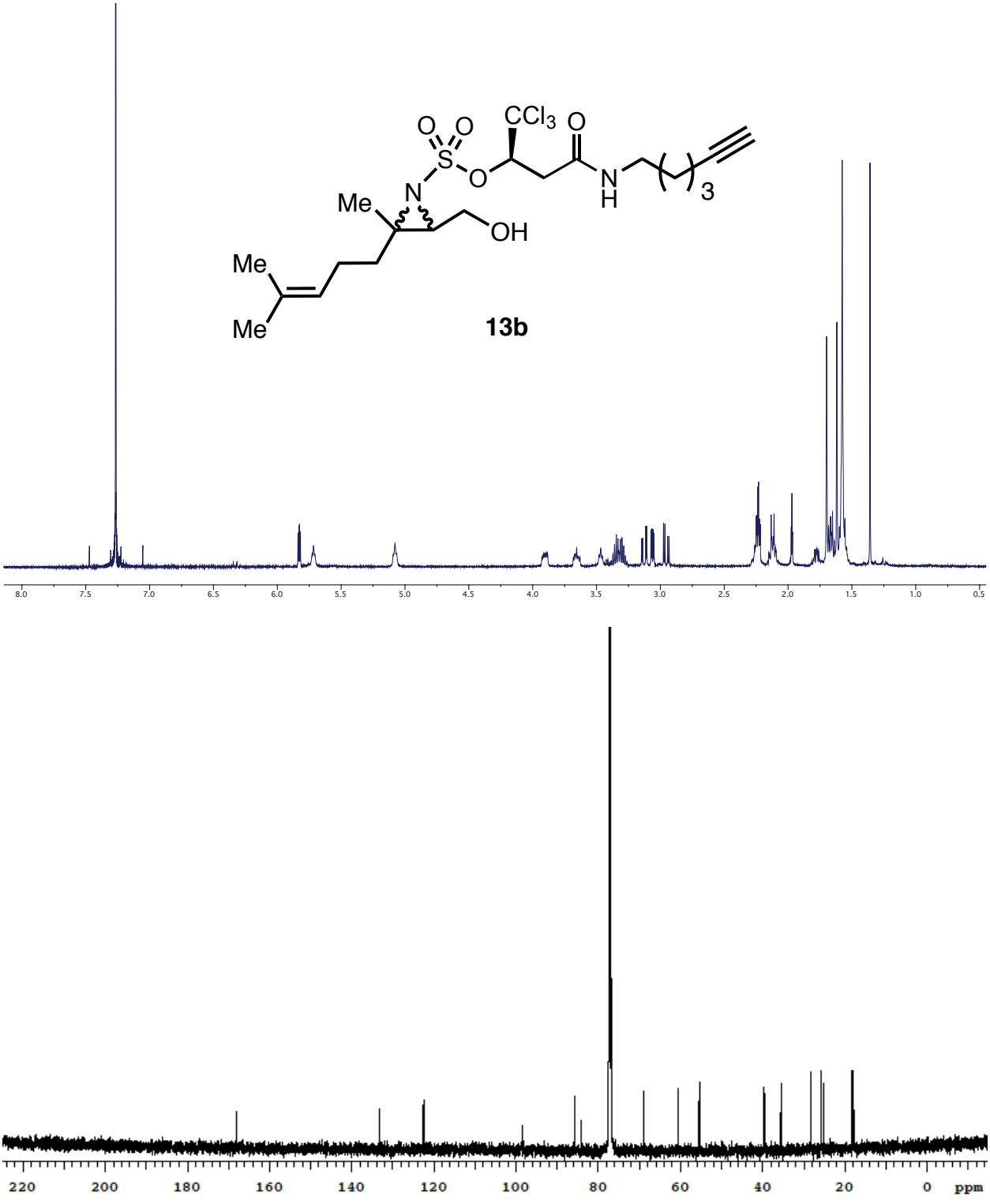
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR of **12** (1.4:1 mixture of two diastereomers) in $CDCl_3$



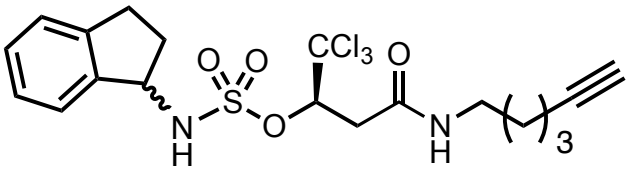
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR of **13a** (8:1 mixture of E/Z isomers) in $CDCl_3$



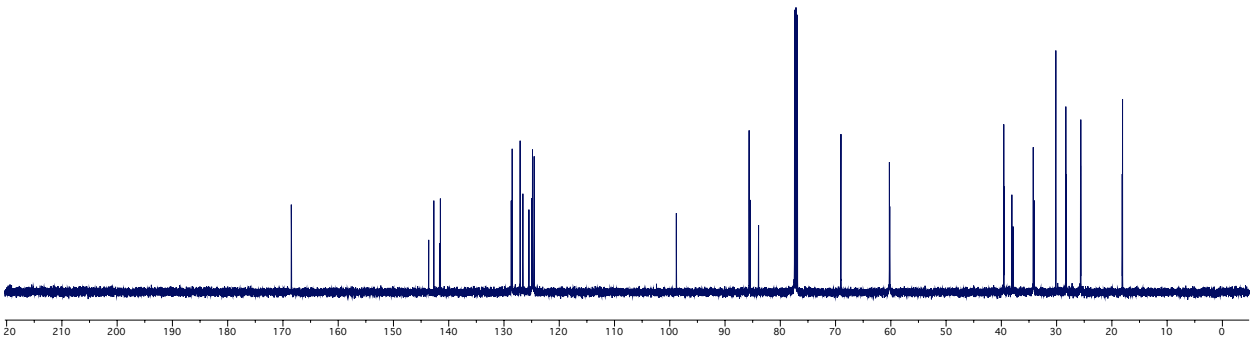
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR of **13b** (Major isomer) in $CDCl_3$



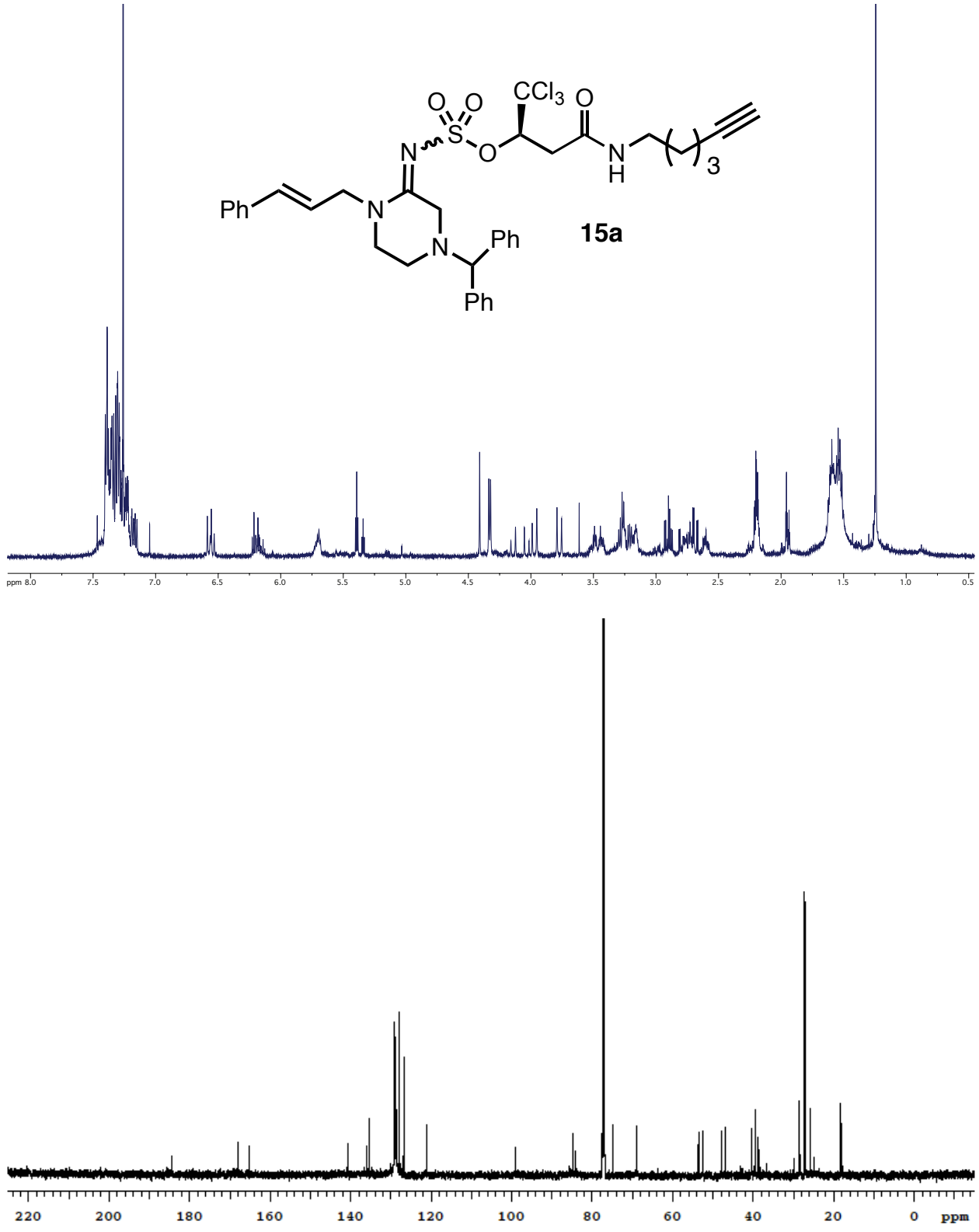
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR of **13b** (Minor isomer) in $CDCl_3$



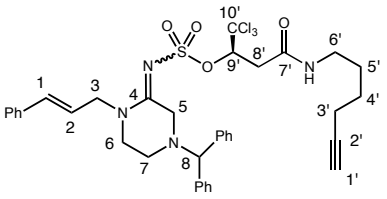
14a



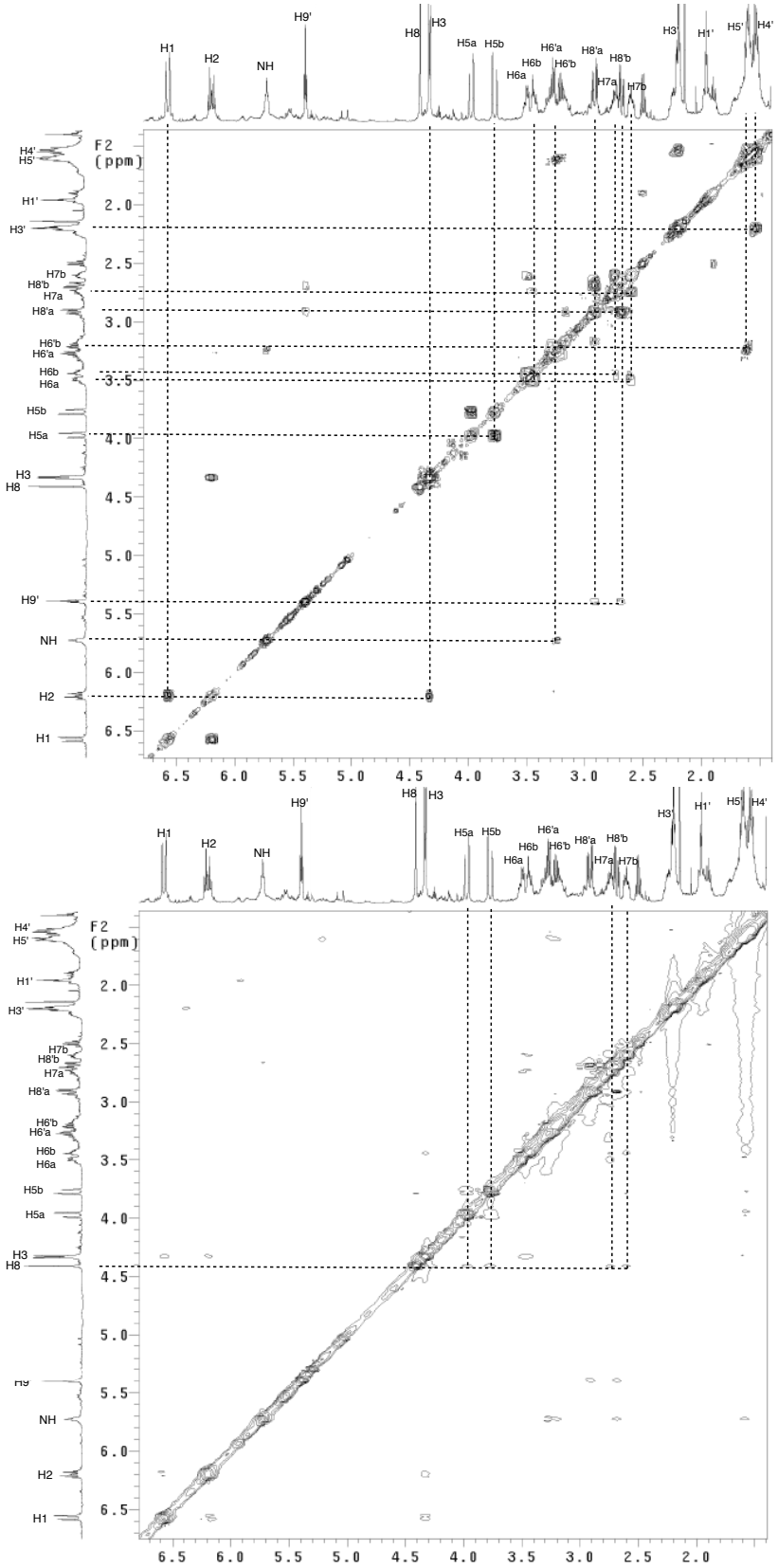
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR of **14a** (1.5:1 mixture of two diastereomers) in $CDCl_3$

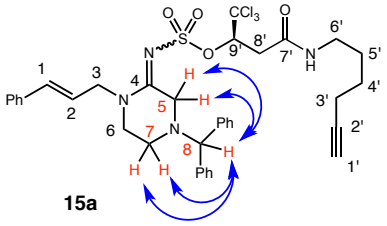


^{1}H (500 MHz) and ^{13}C (125 MHz) NMR of **15a** (2:1 mixture of E/Z isomers) in $CDCl_3$



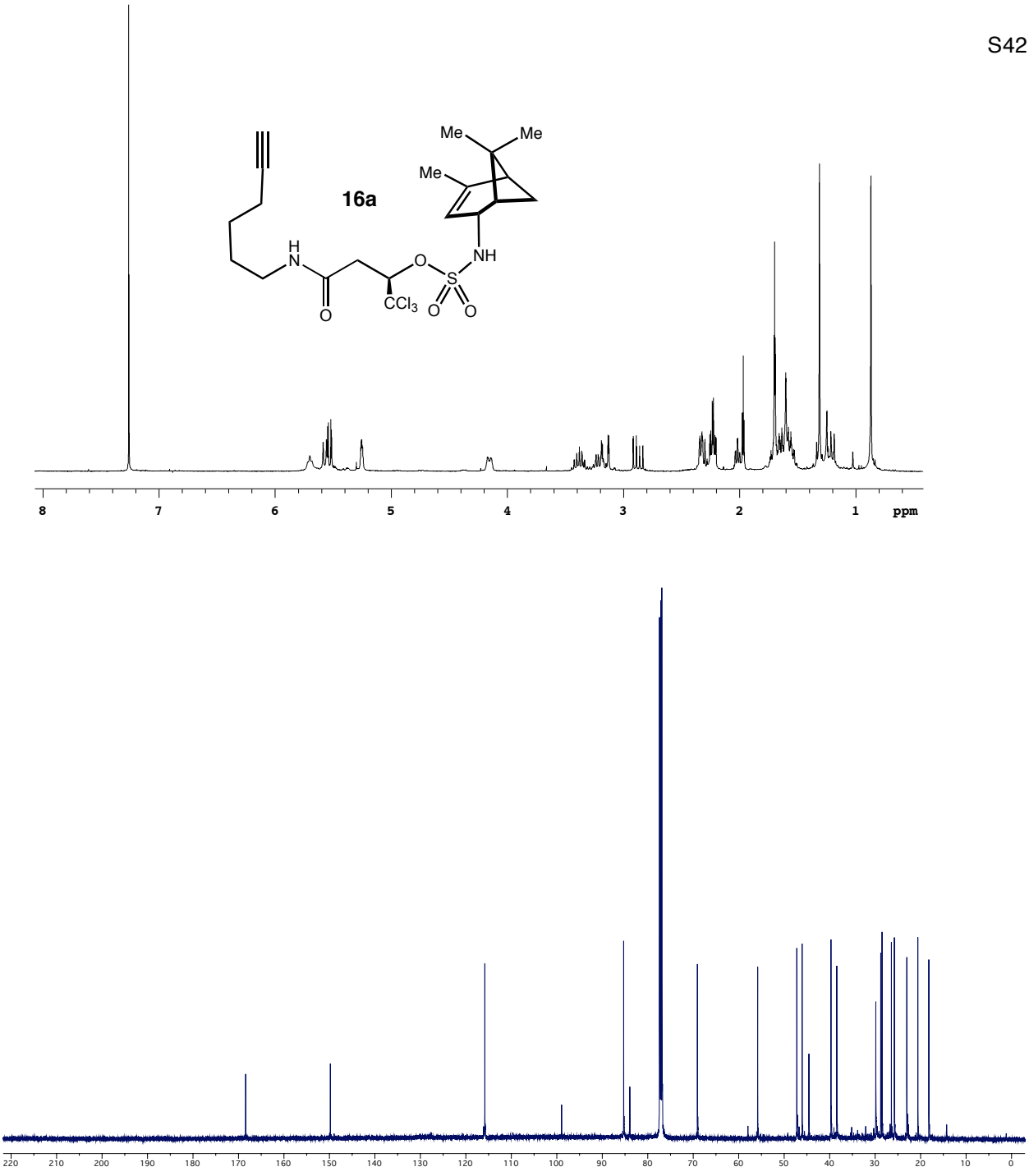
15a



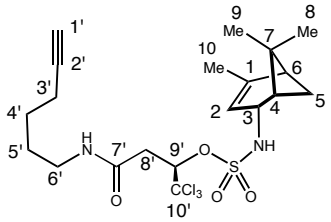


15a

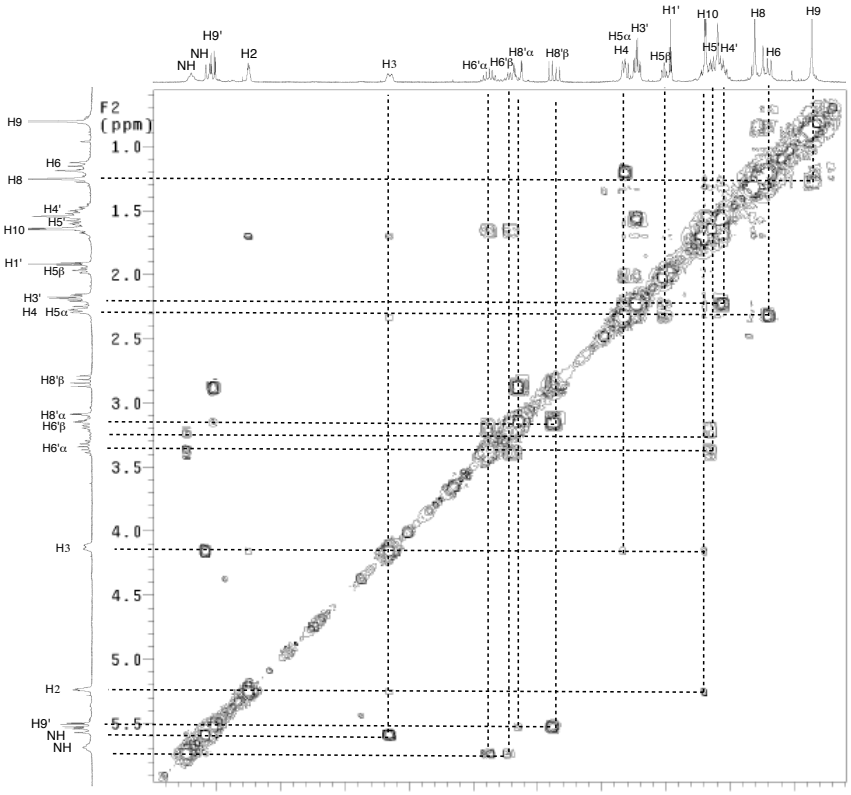
COSY and NOESY (500 MHz) NMR of **15a** in $CDCl_3$[41]

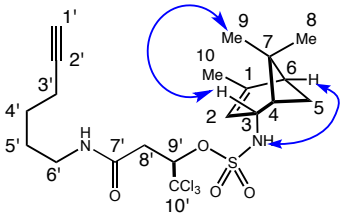


^{1}H (500 MHz) and ^{13}C (125 MHz) NMR of **16a** in $CDCl_3$

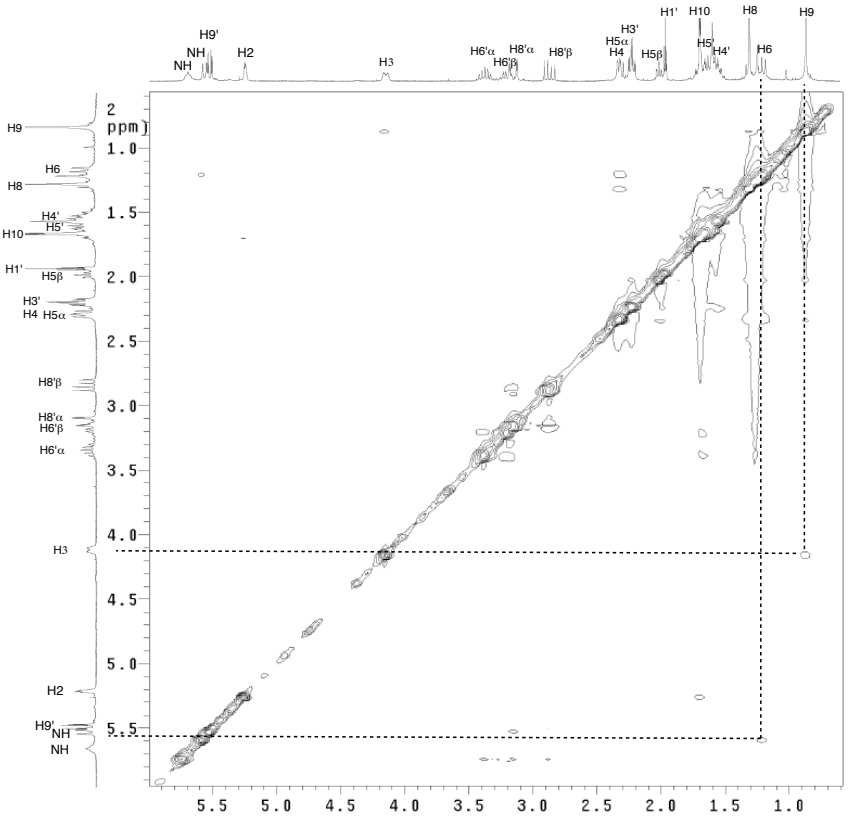


16a

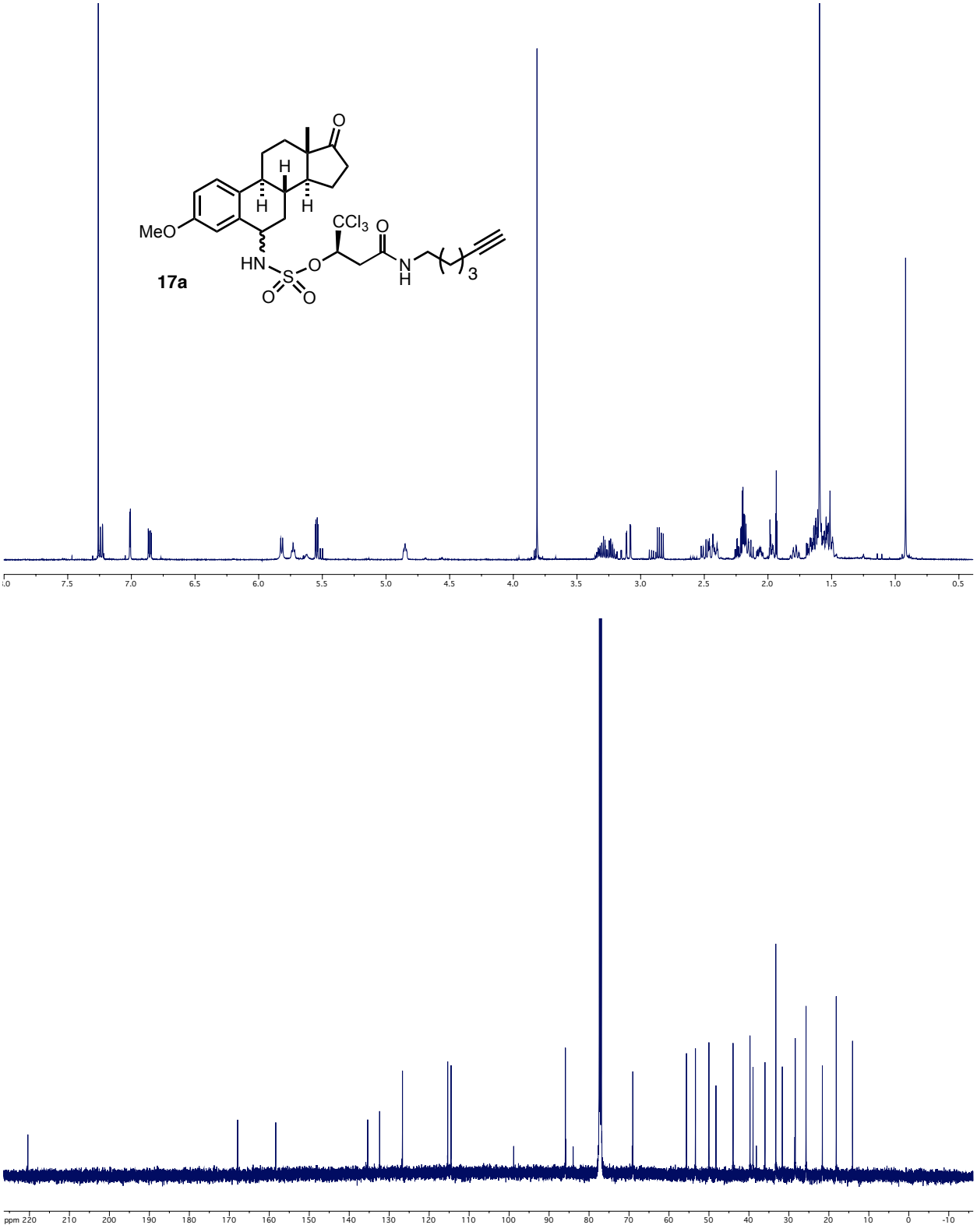




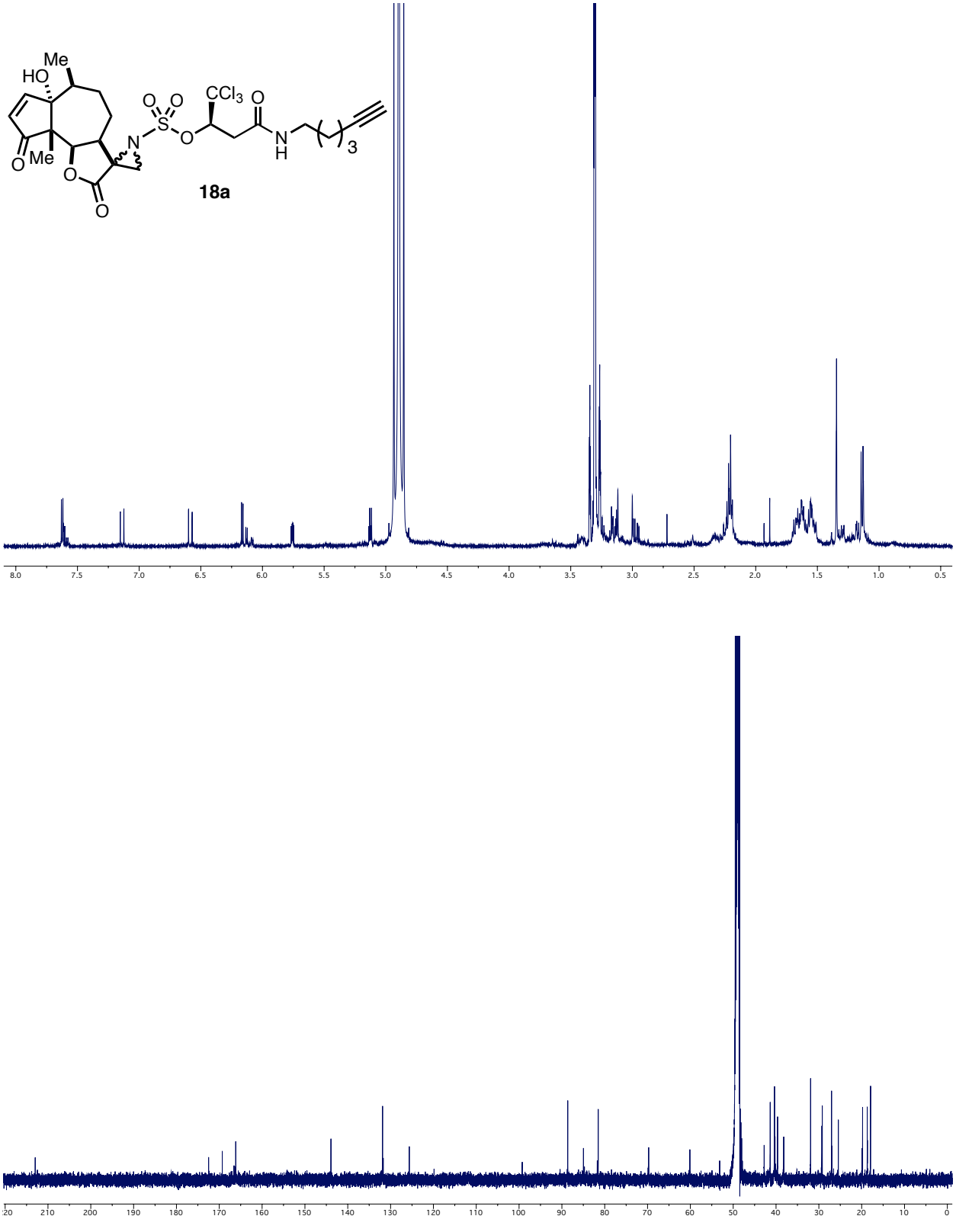
16a



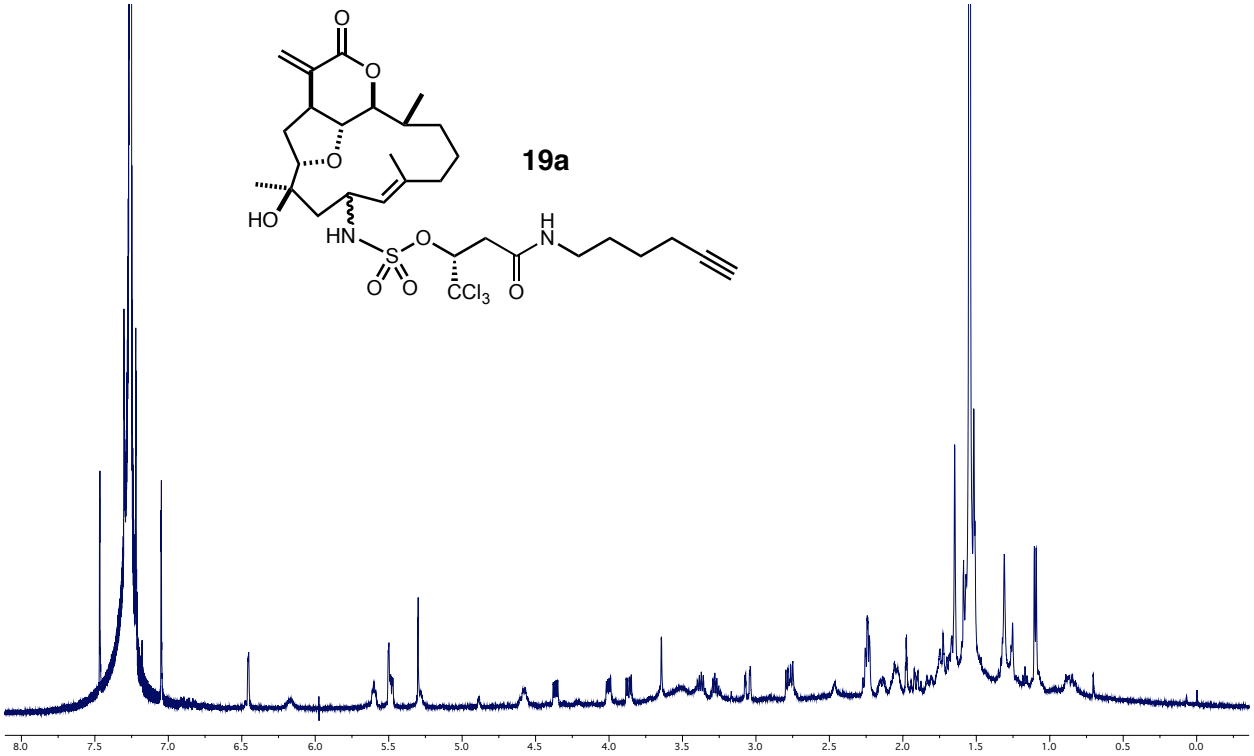
COSY and NOESY (500 MHz) NMR of **16a** in $CDCl_3$

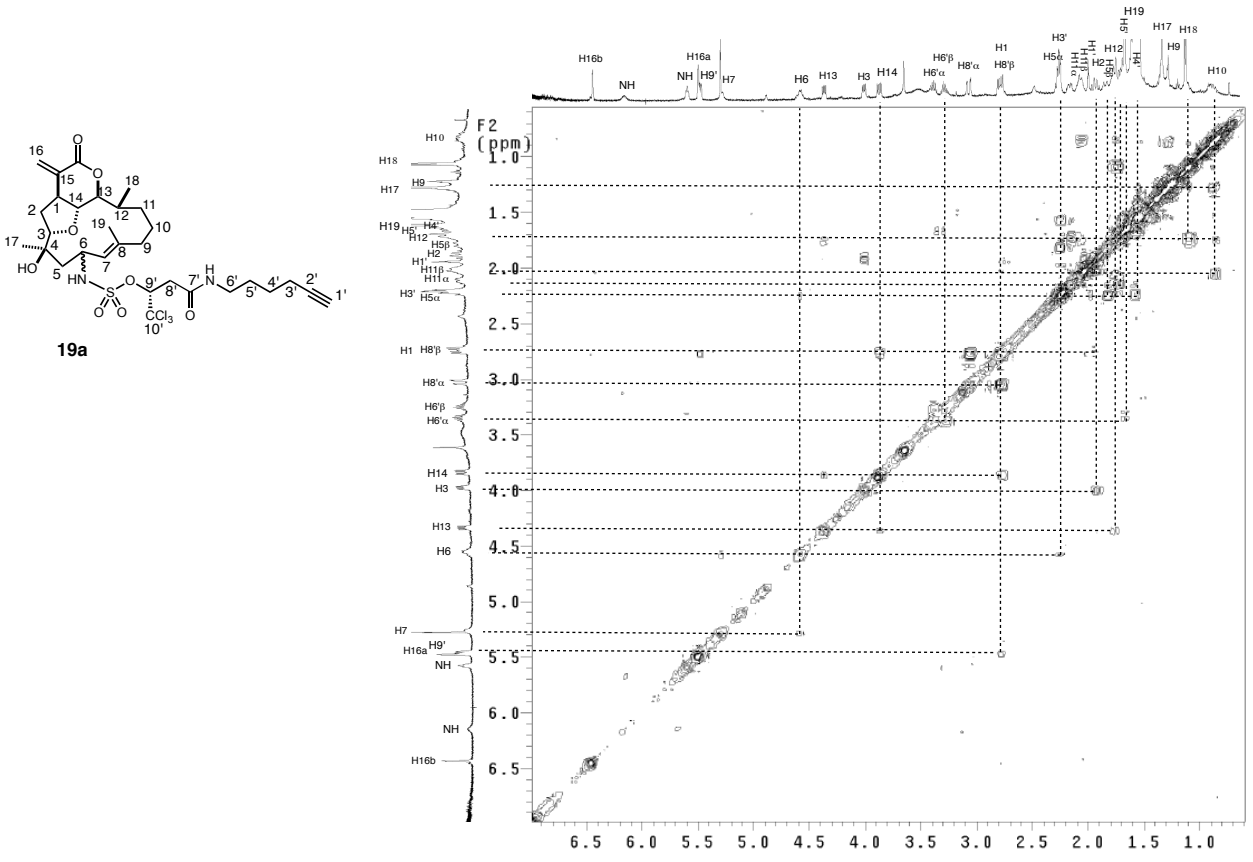


^{1}H (500 MHz) and ^{13}C (125 MHz) NMR of **17a** (4:1 mixture of diastereomers) in $CDCl_3$

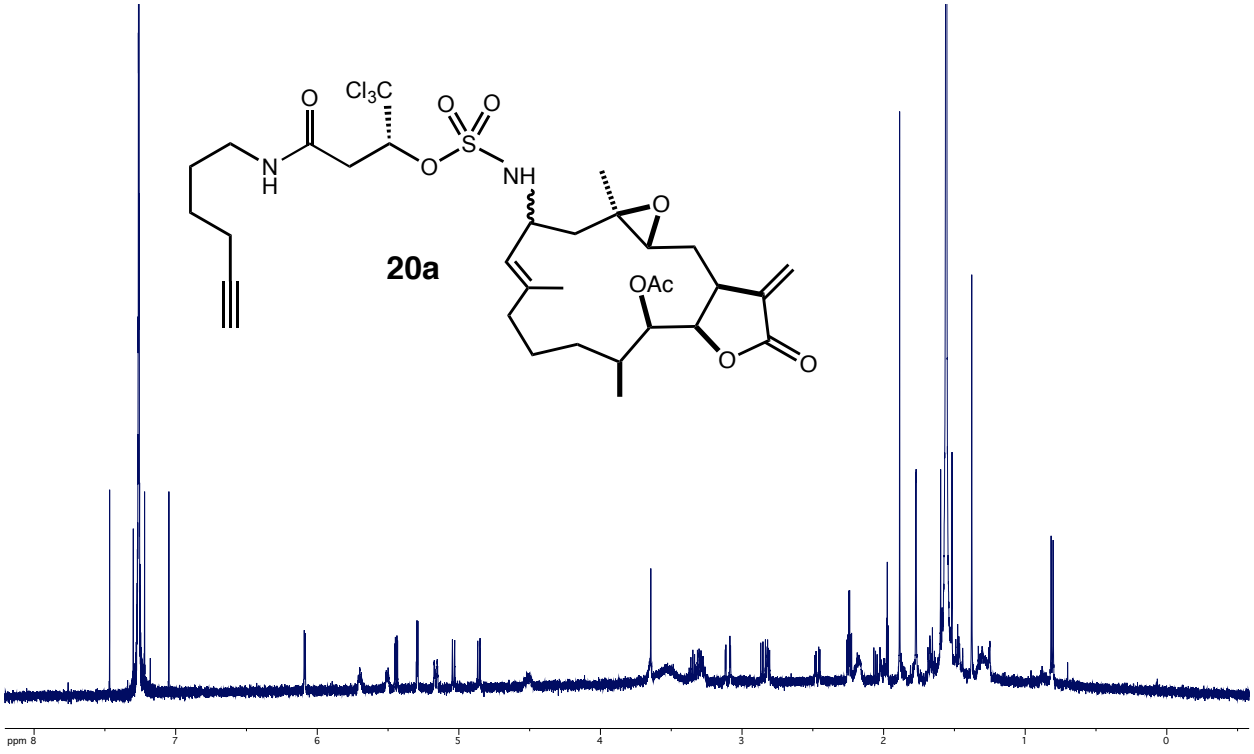


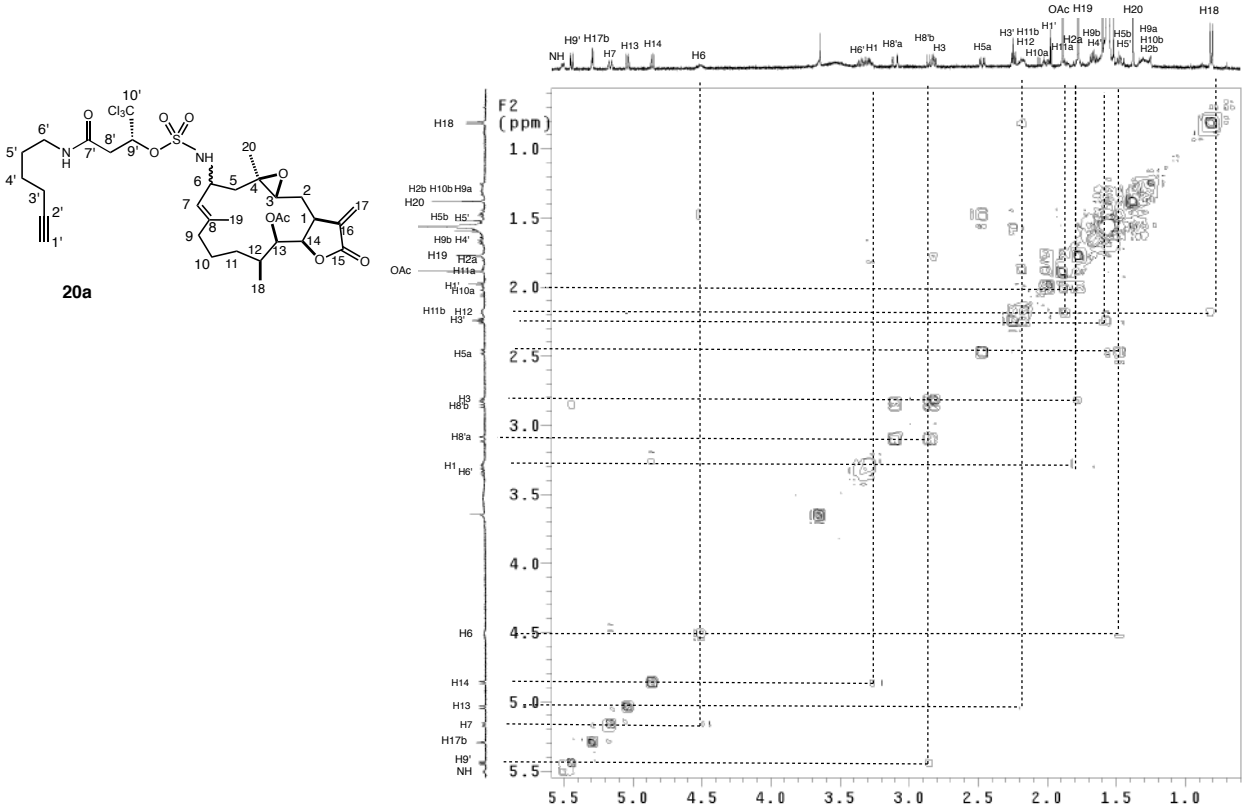
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR of **18a** (one diastereomer) in CD_3OD



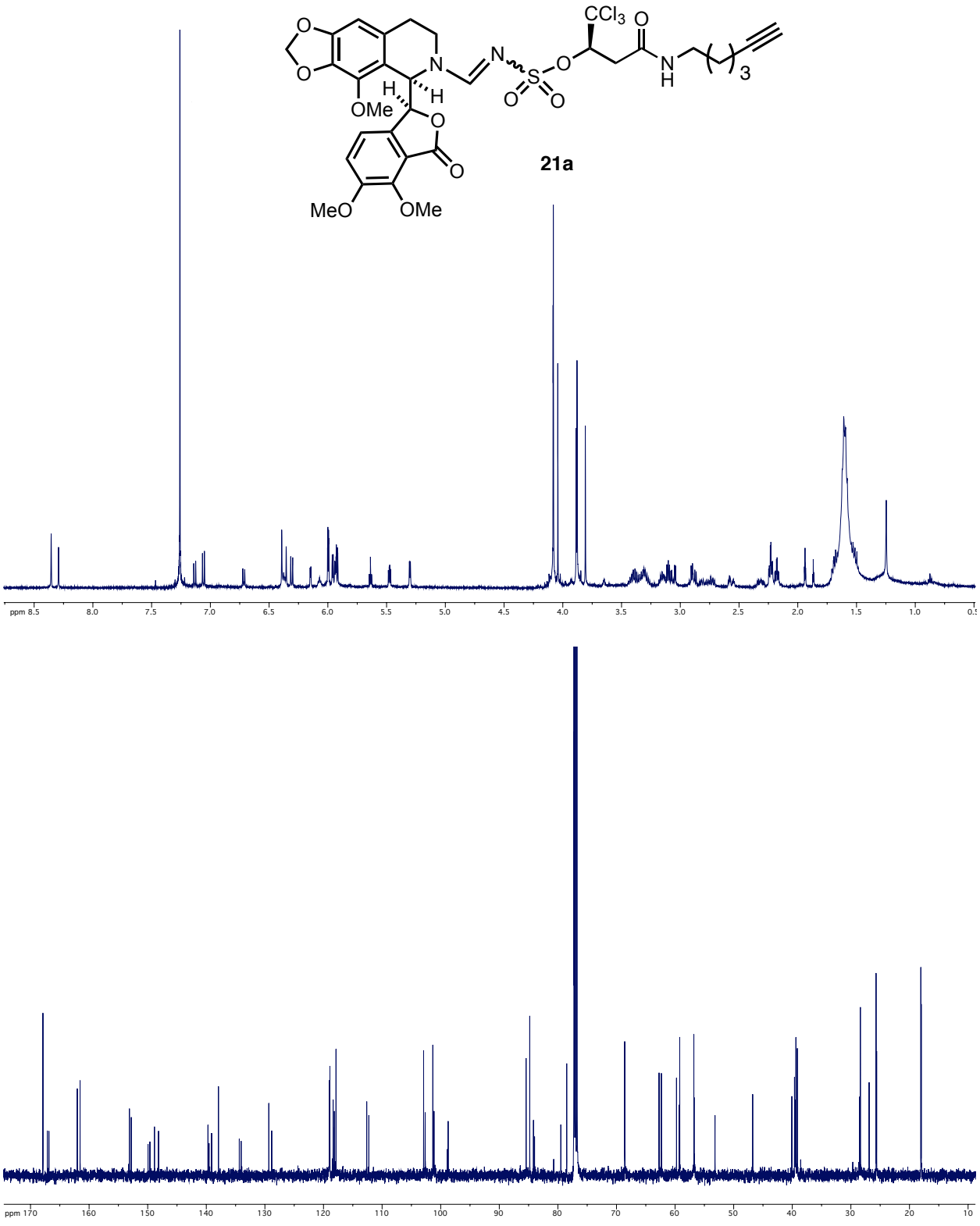


^{1}H (500 MHz) and COSY NMR of **19a** (one diastereomer) in $CDCl_3$

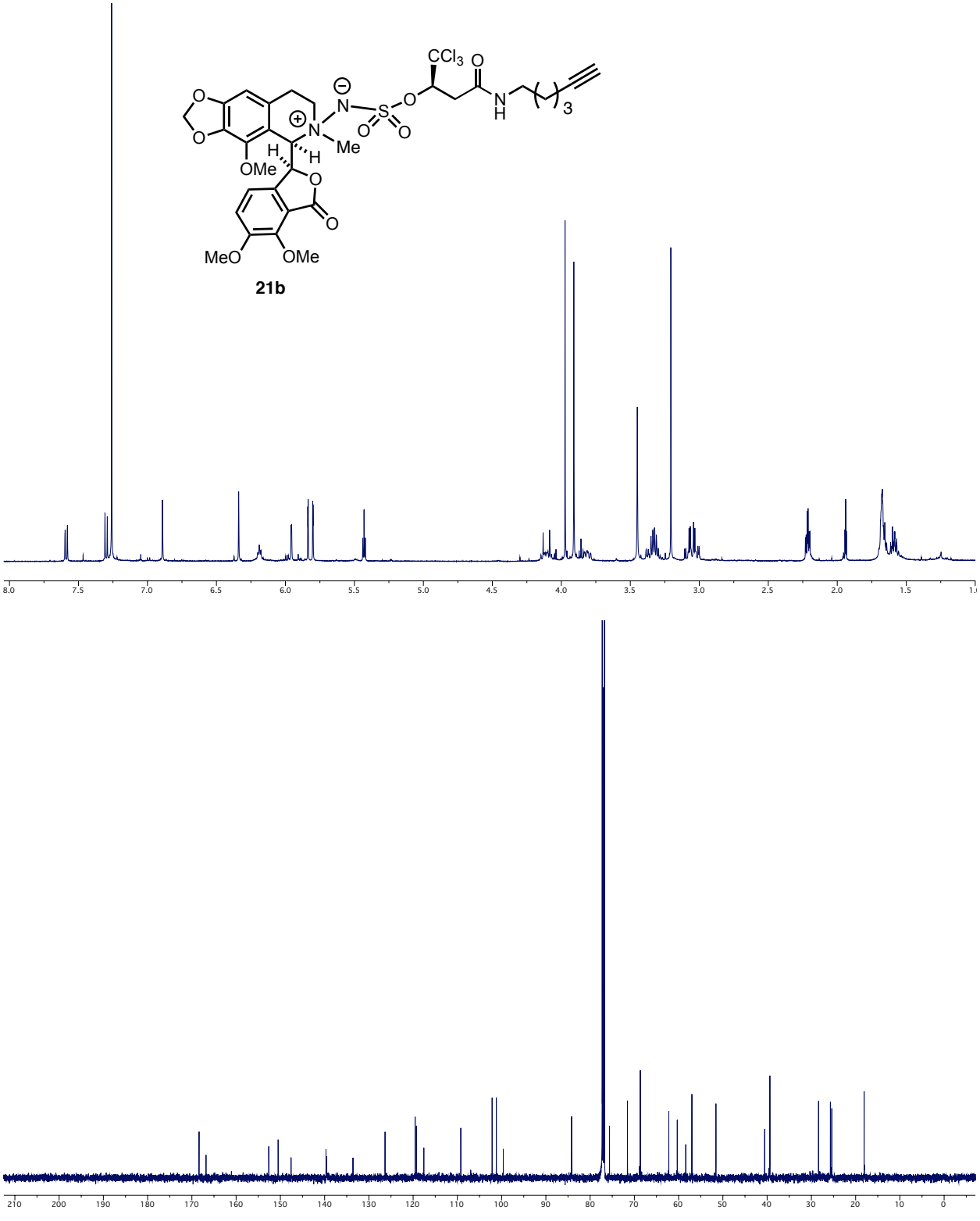




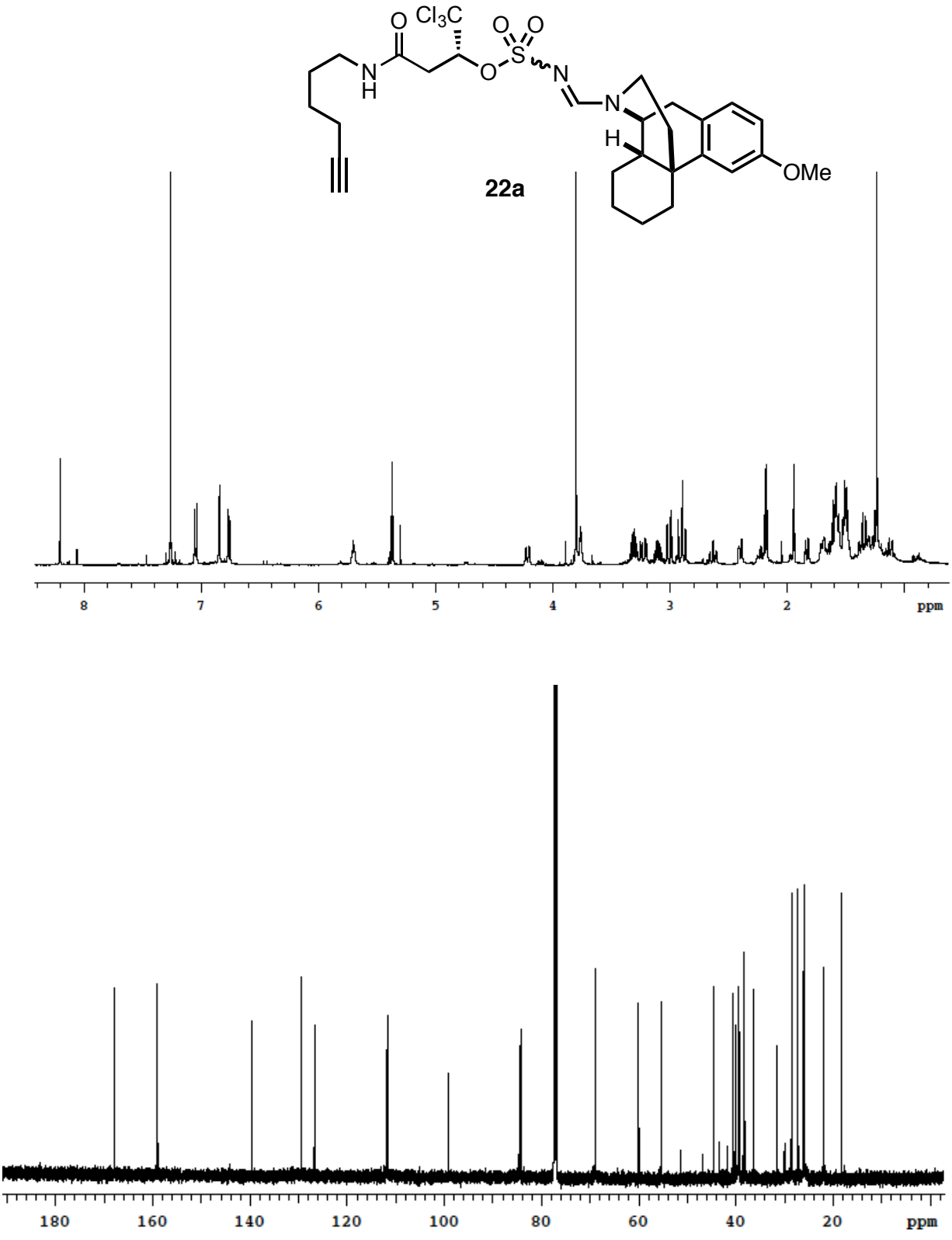
^{1}H (500 MHz) and COSY NMR of **20a** (one diastereomer) in $CDCl_3$



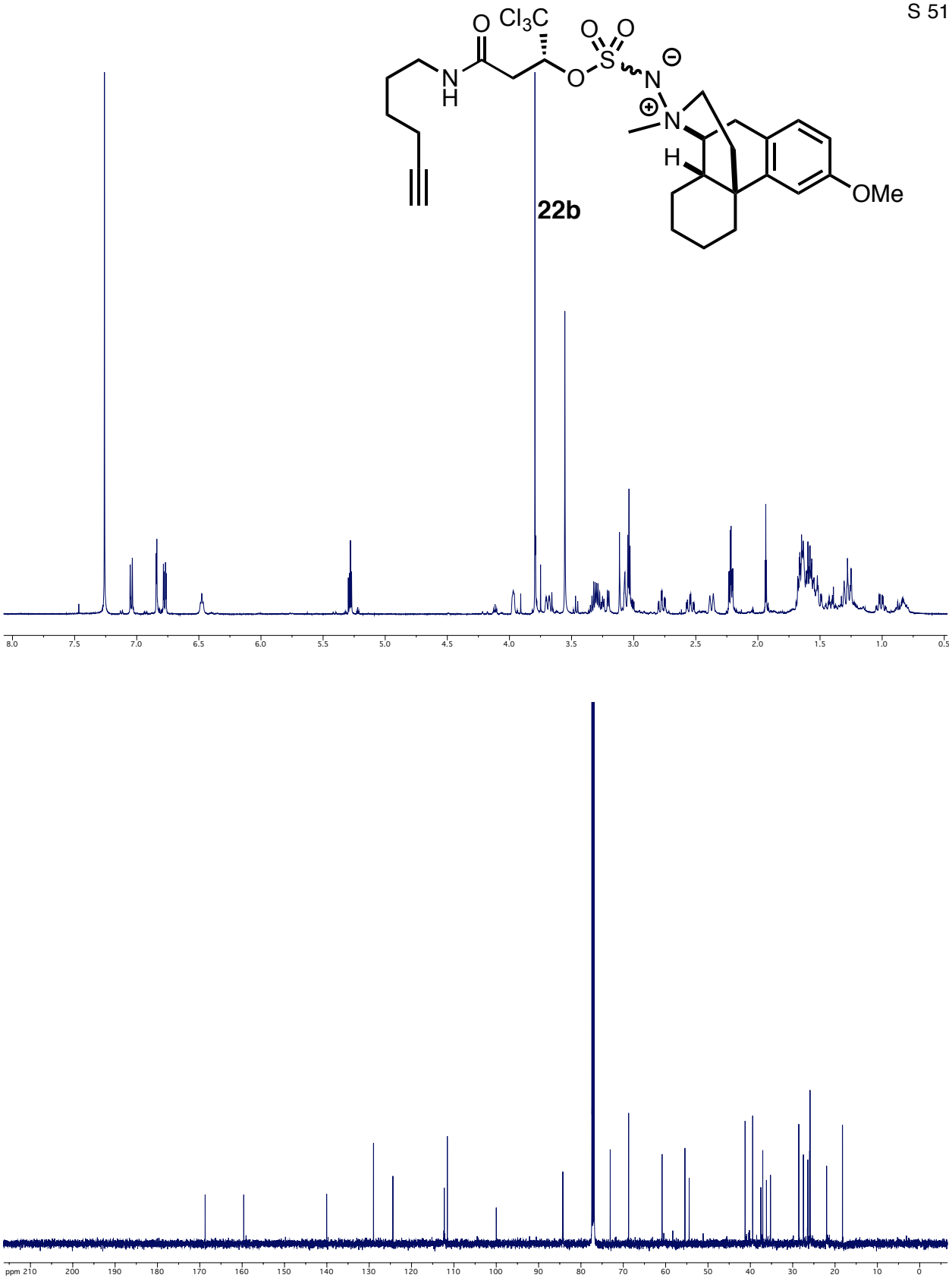
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR of **21a** (1.3:1 mixture of E/Z isomers) in $CDCl_3$



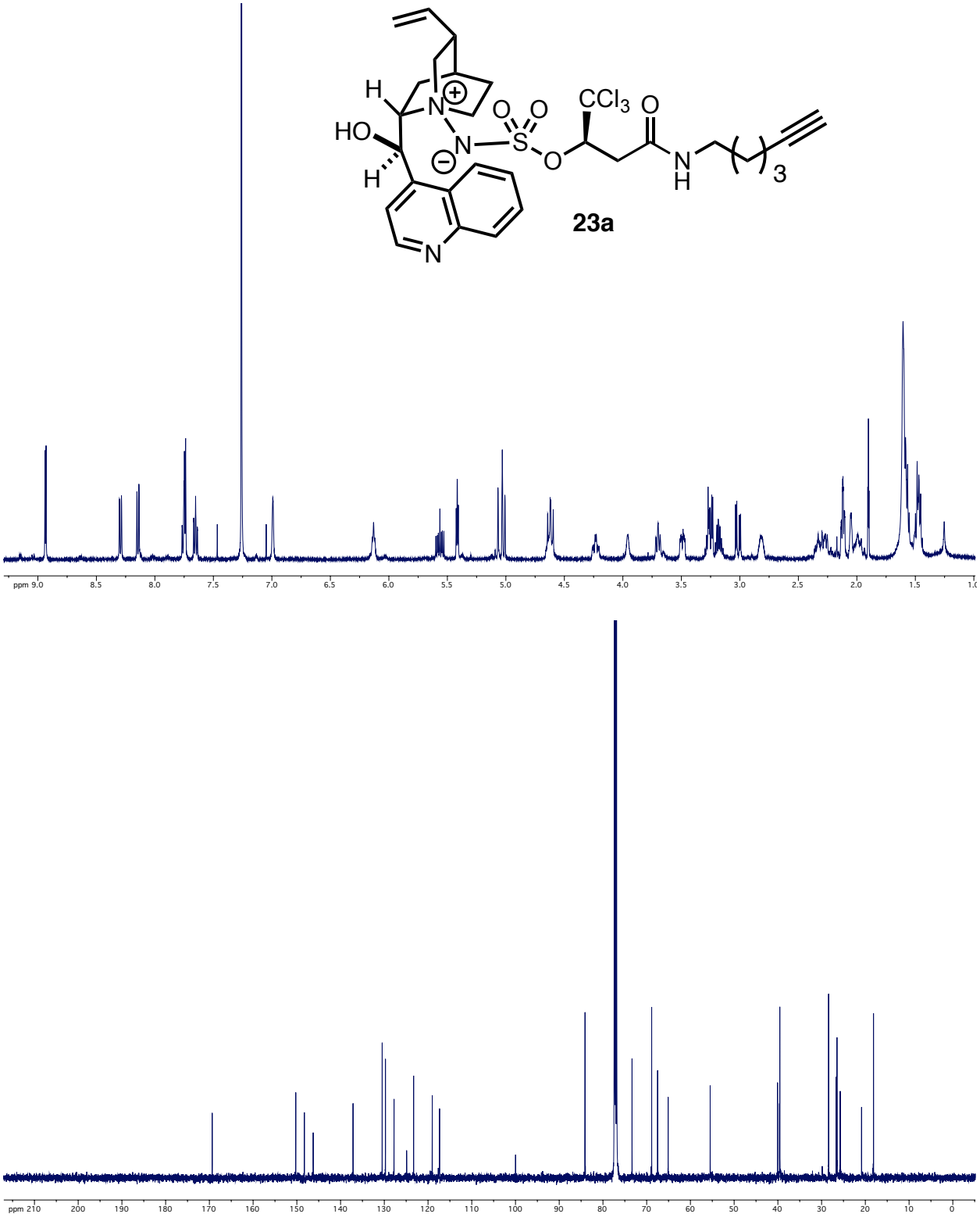
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR of **21b** in $CDCl_3$



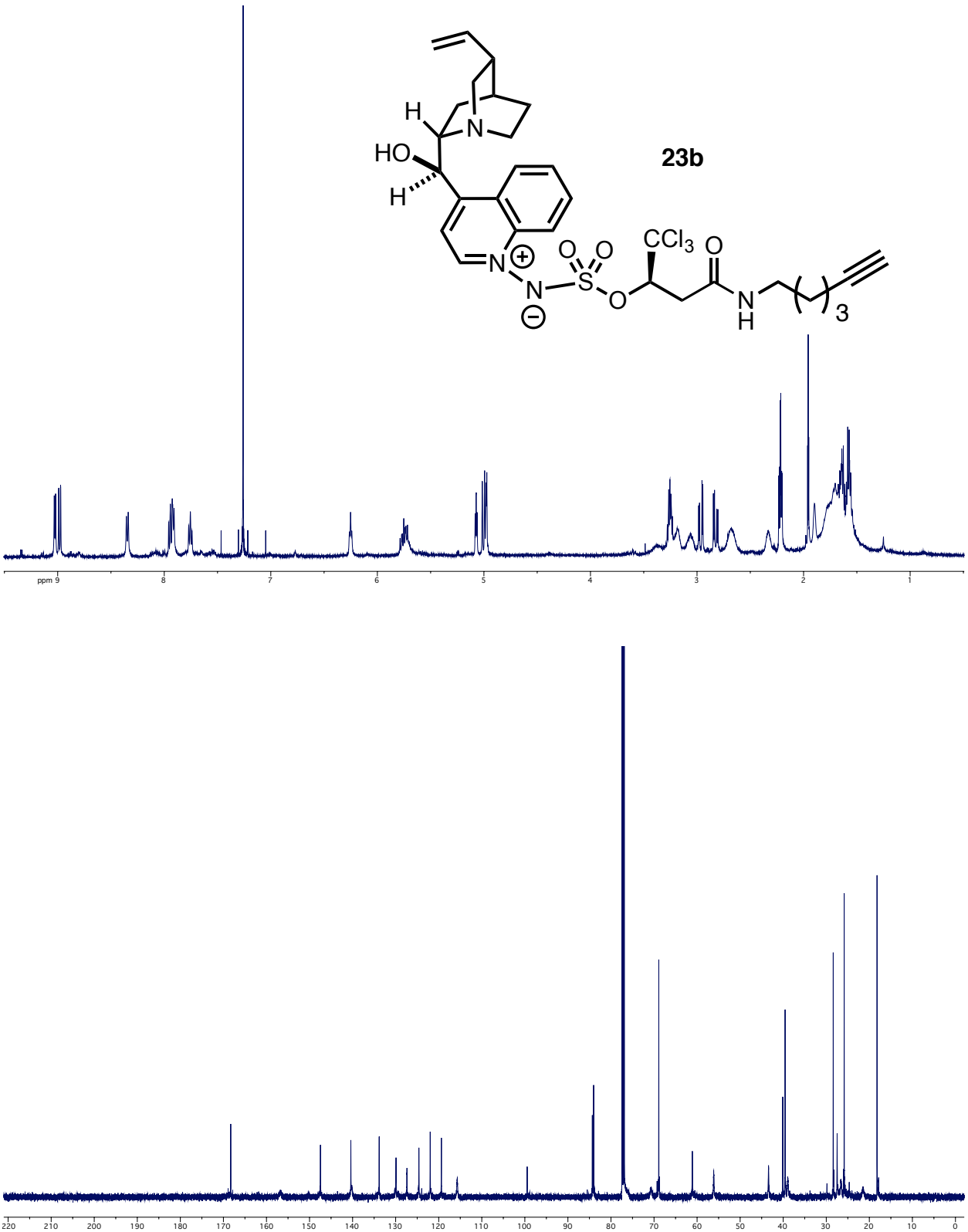
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR of **22a** (major diastereomer) in $CDCl_3$



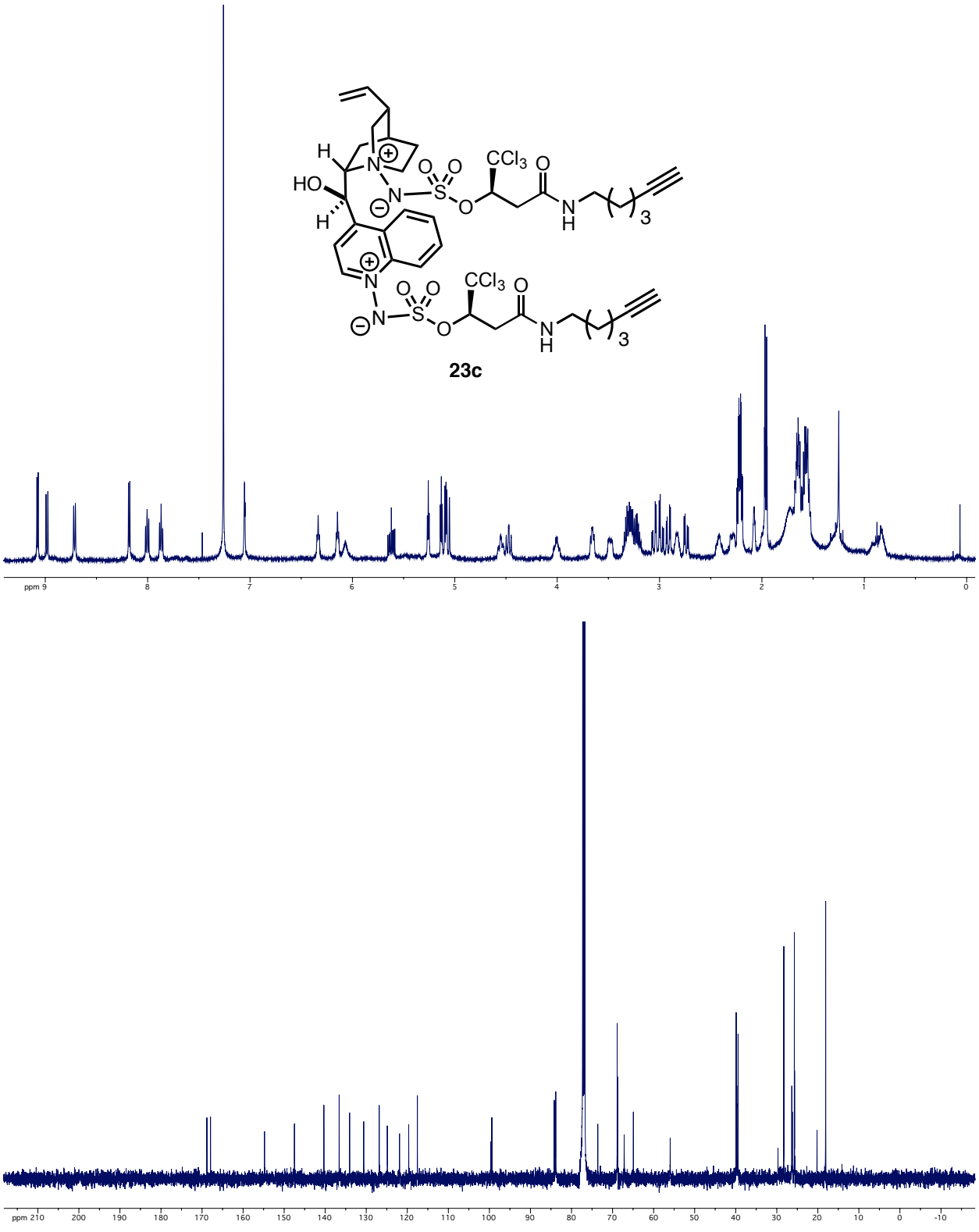
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR of **22b** in $CDCl_3$



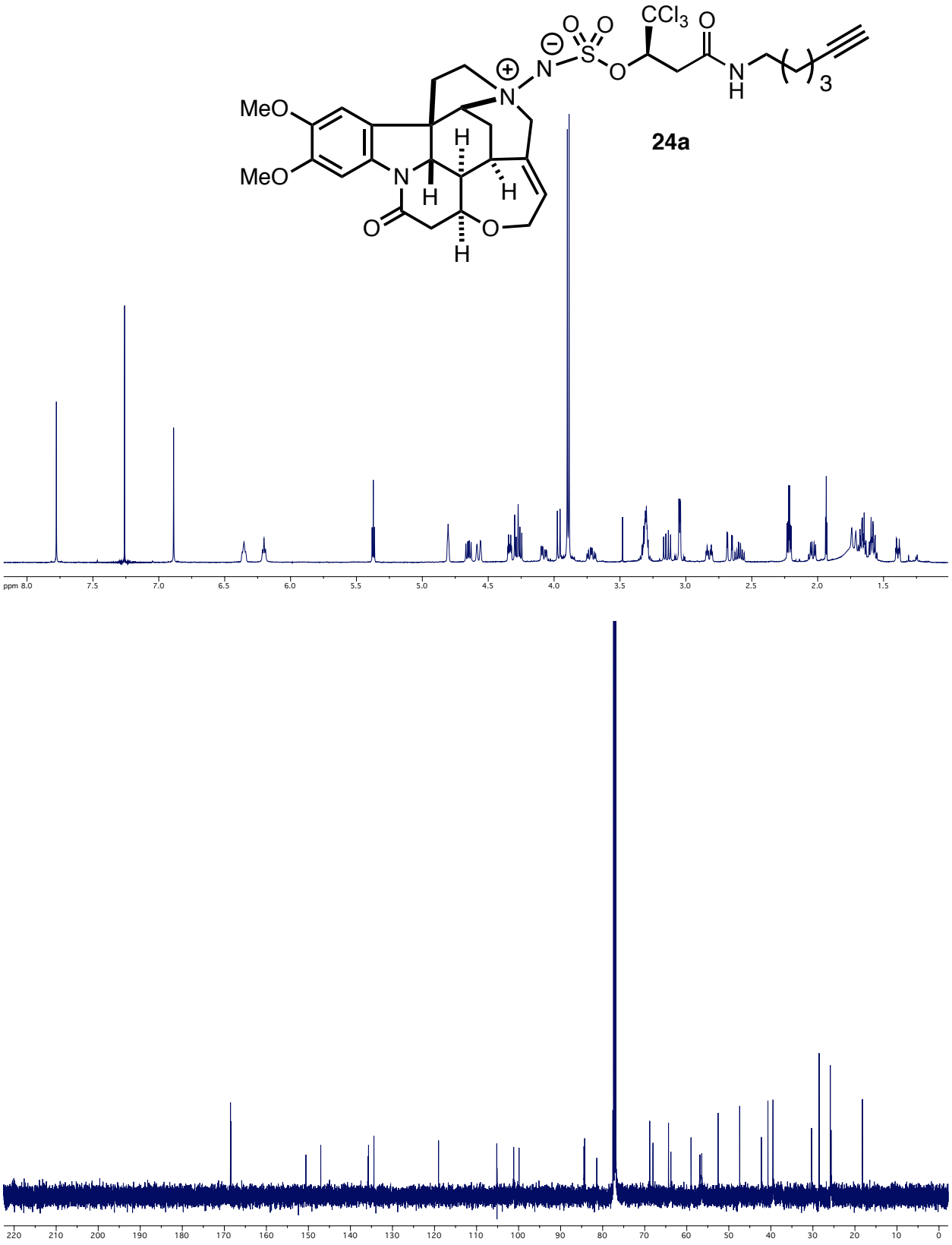
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR of **23a** in $CDCl_3$



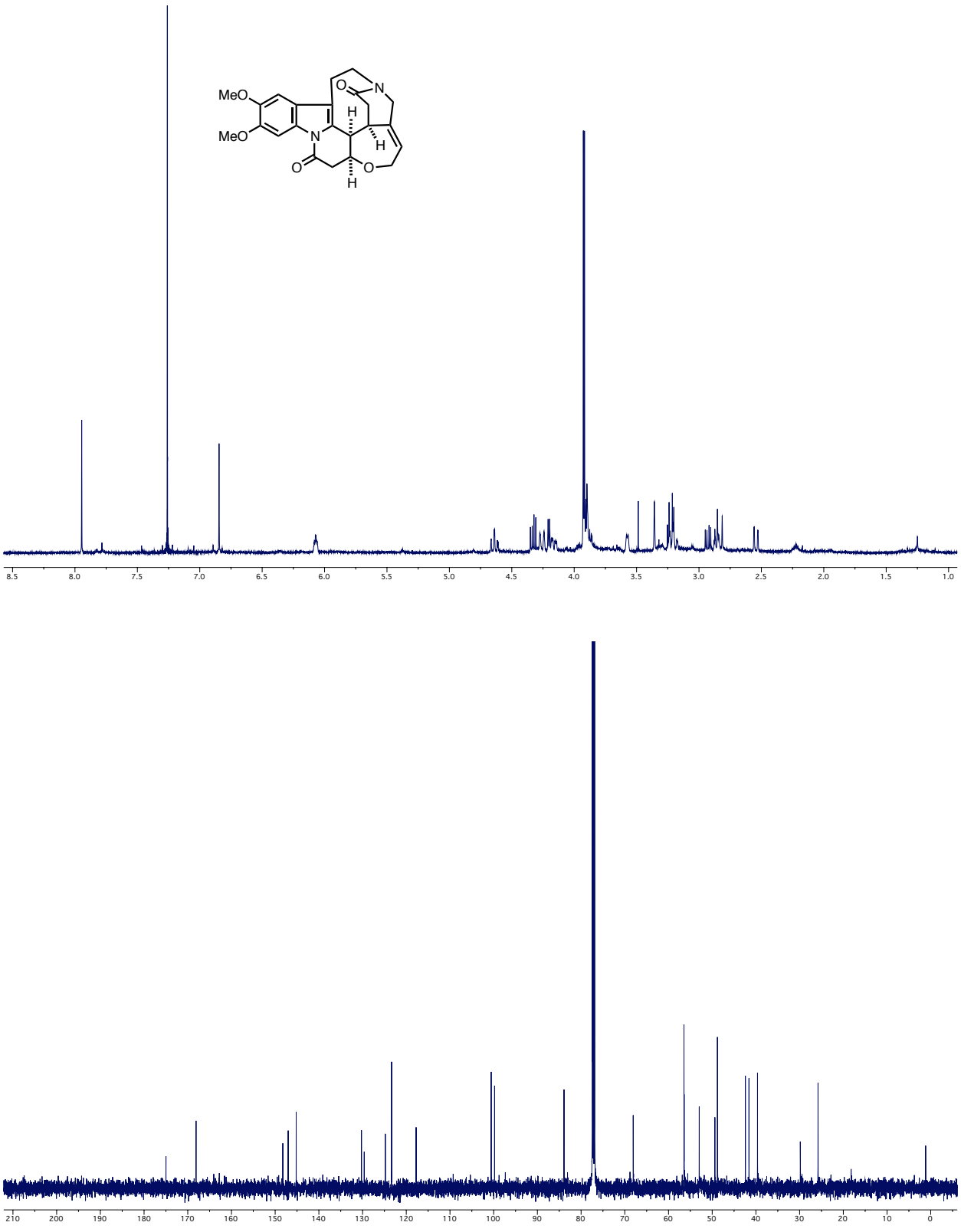
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR of **23b** in $CDCl_3$



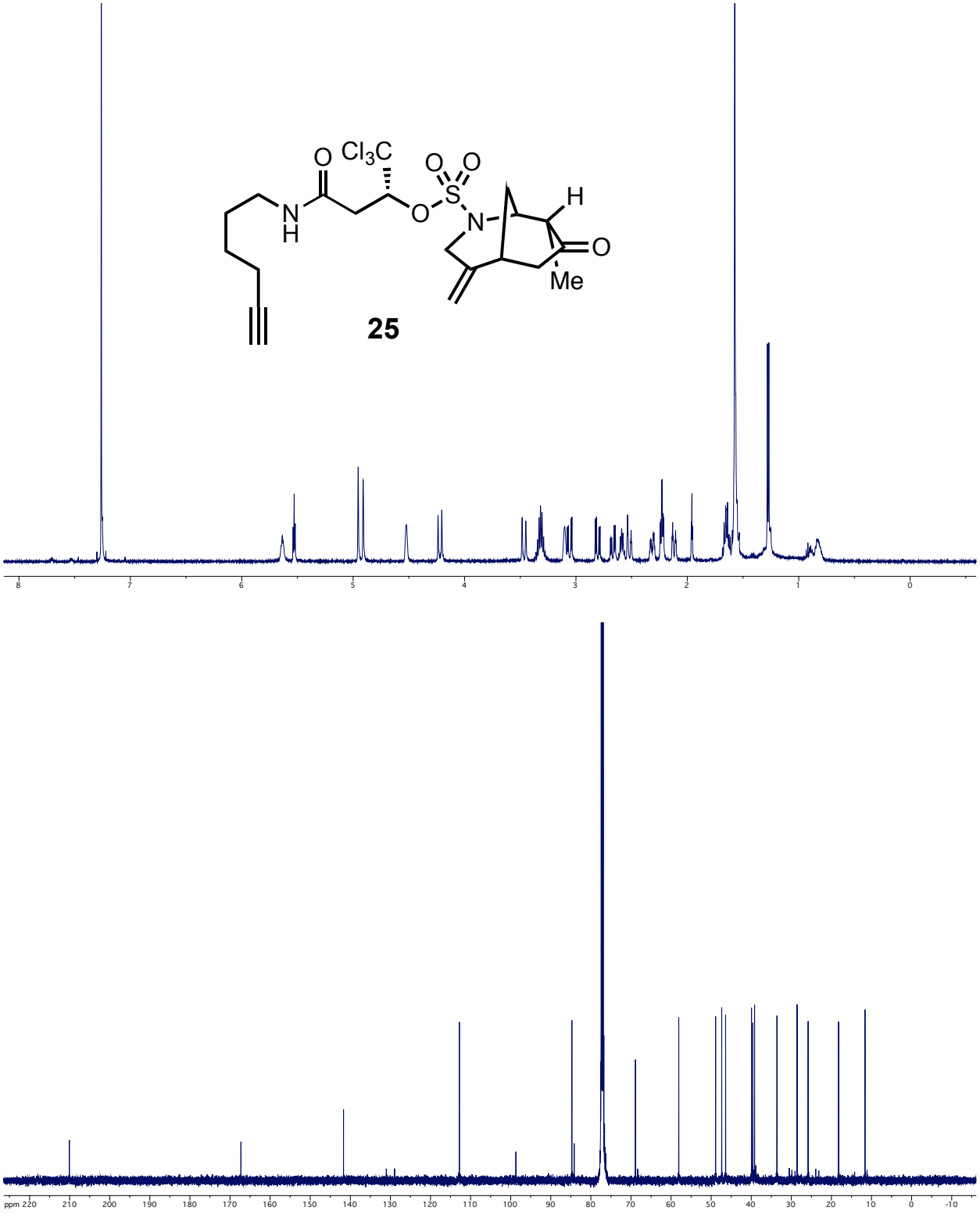
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR of **23c** in $CDCl_3$



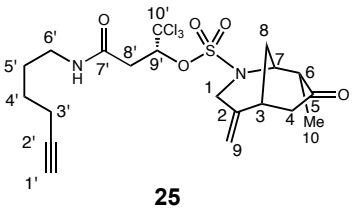
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR of **24a** in $CDCl_3$



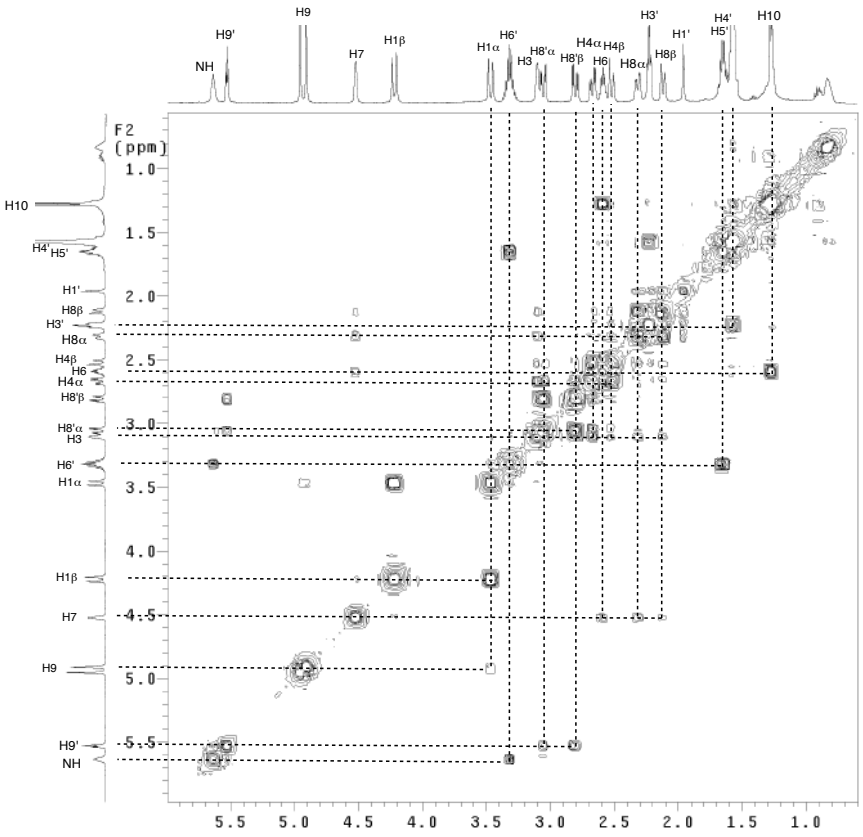
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR of **24b** in $CDCl_3$

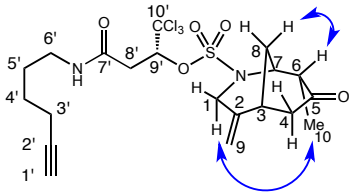


^{1}H (500 MHz) and ^{13}C (125 MHz) NMR of **25** in $CDCl_3$



25





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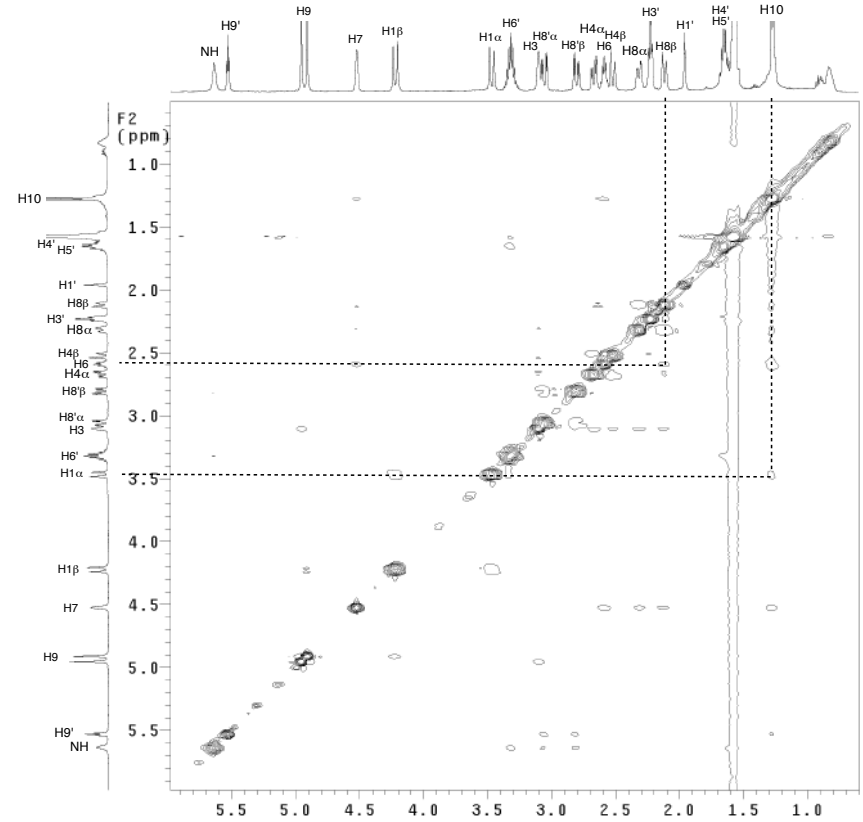
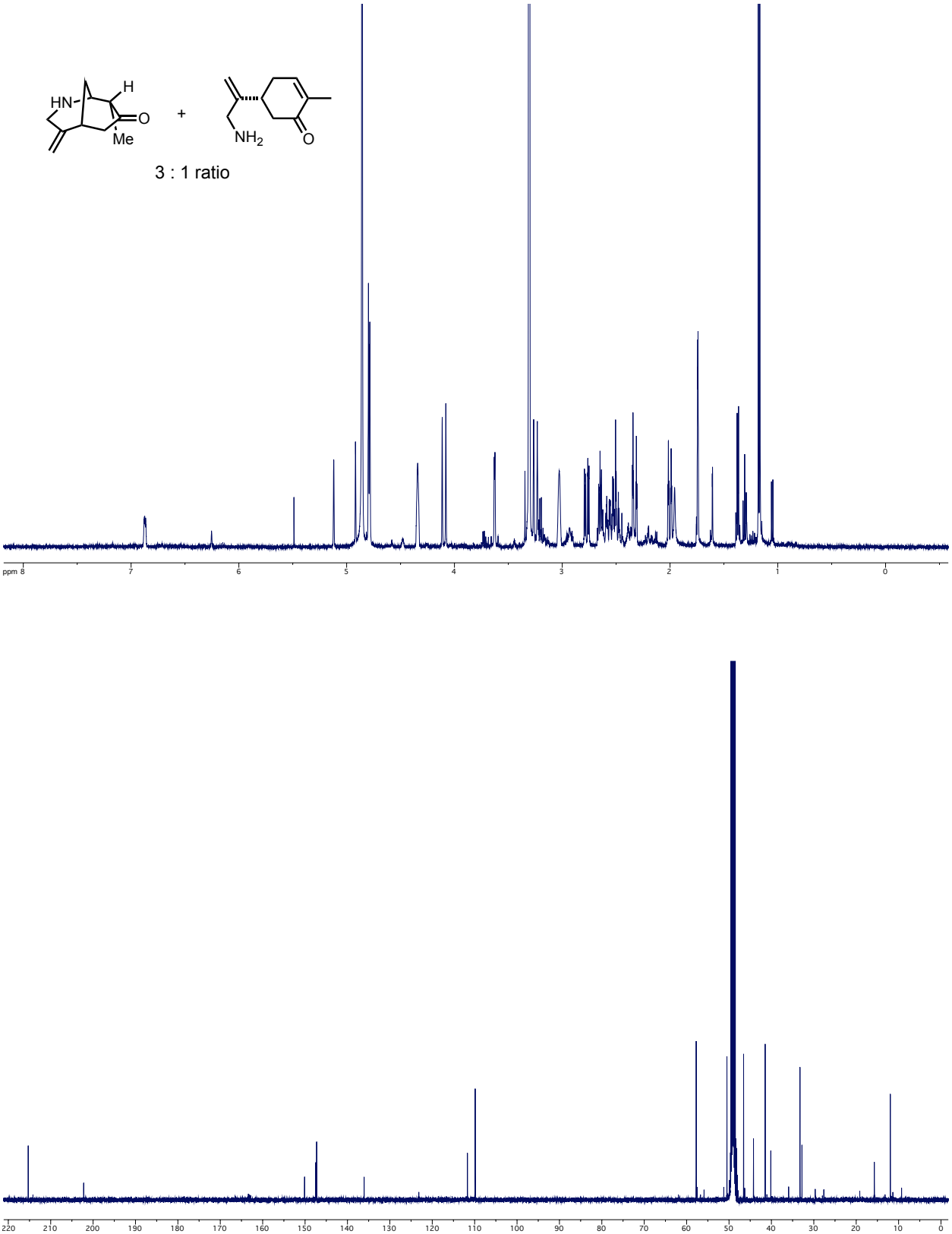
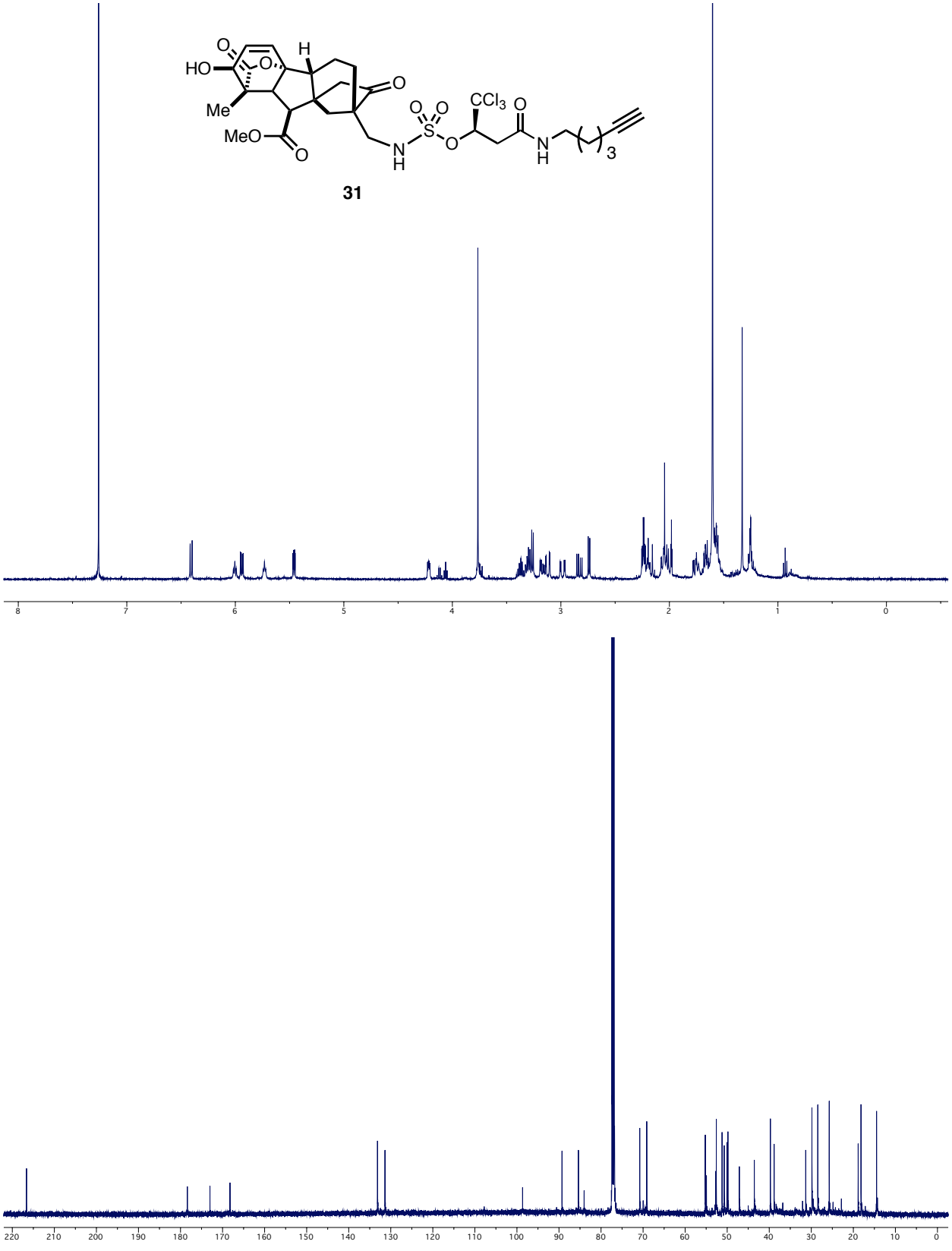


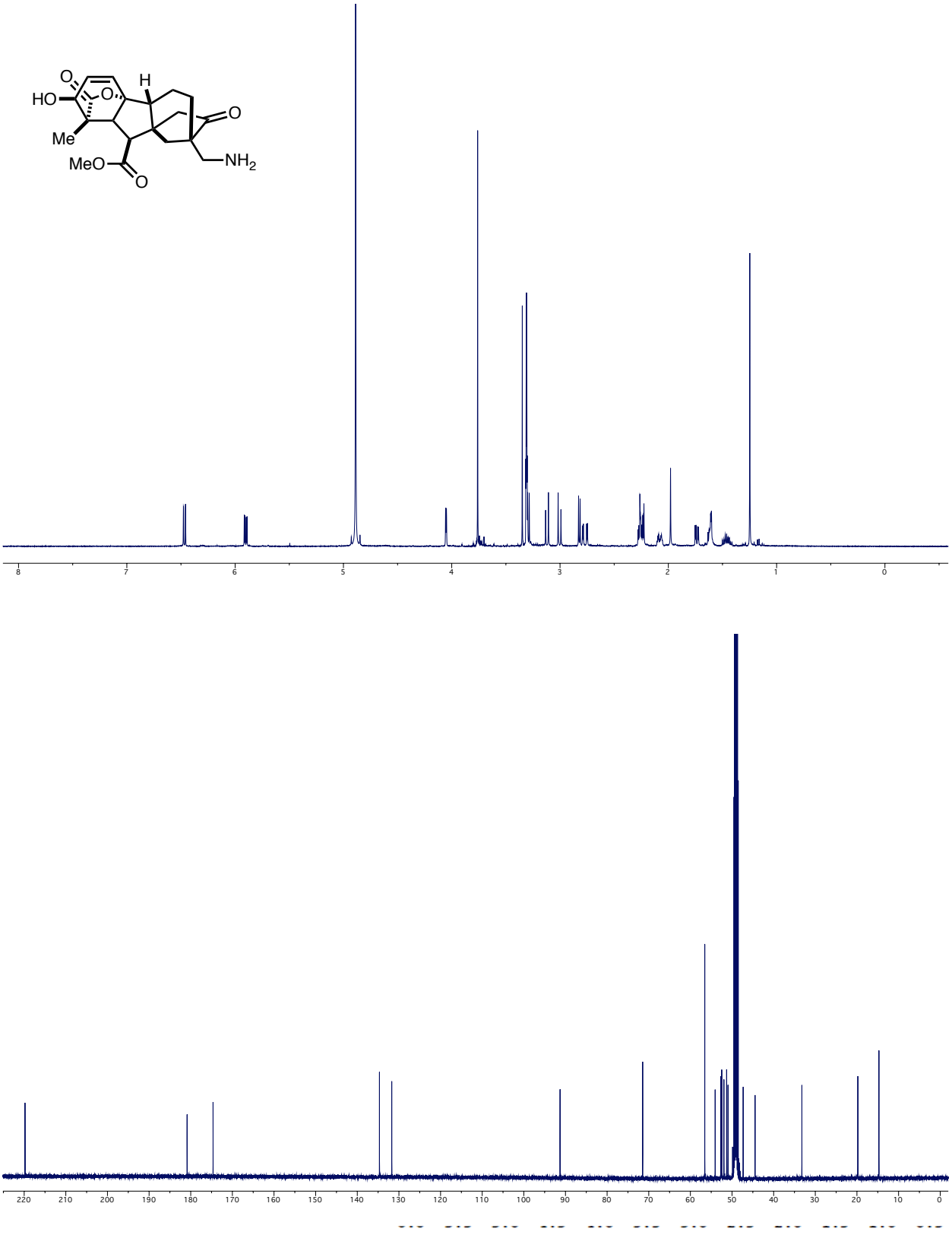
COSY and NOESY (500 MHz) NMR of **25** in $CDCl_3$



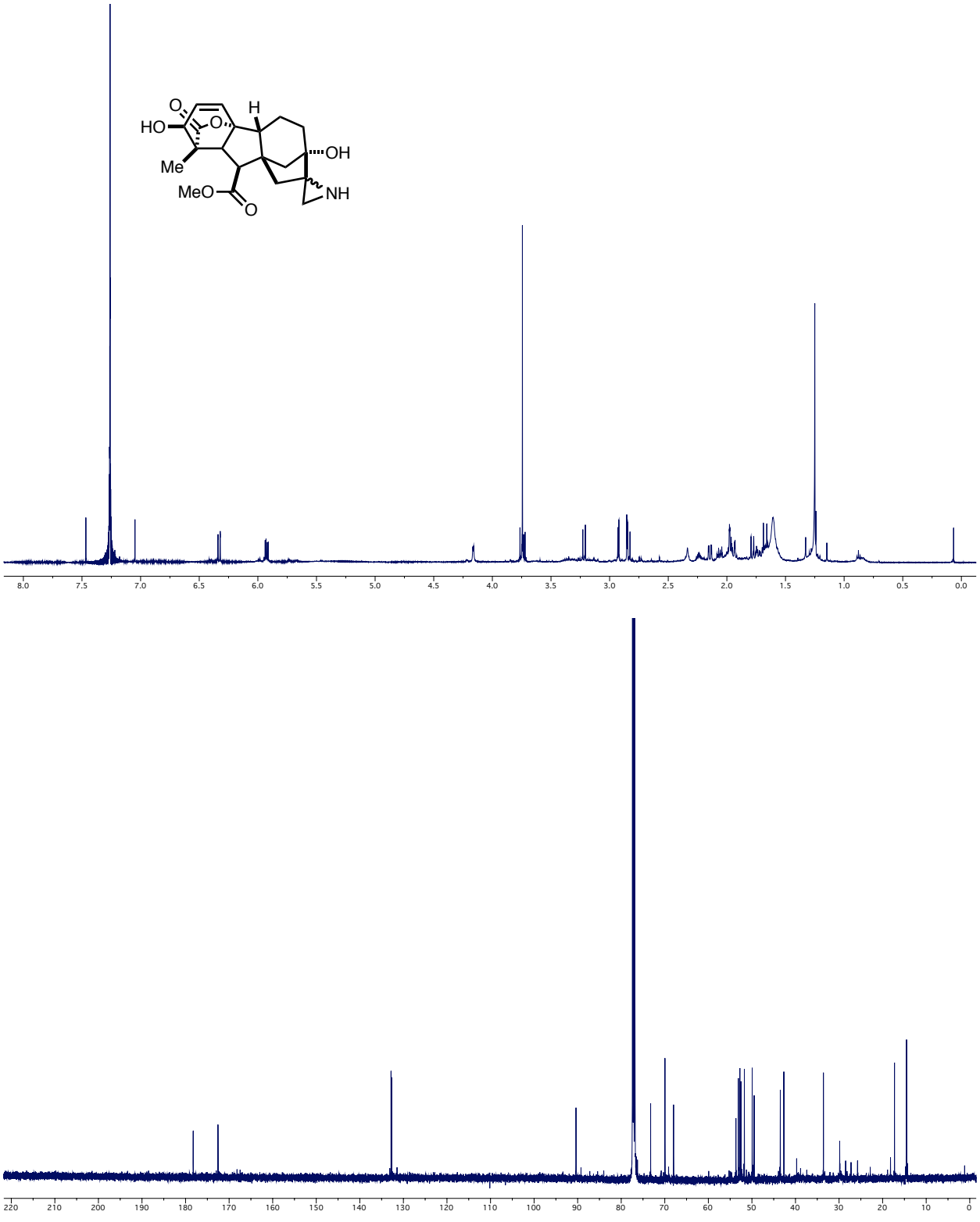
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR of **26**/**27** (3:1 ratio) in CD_3OD



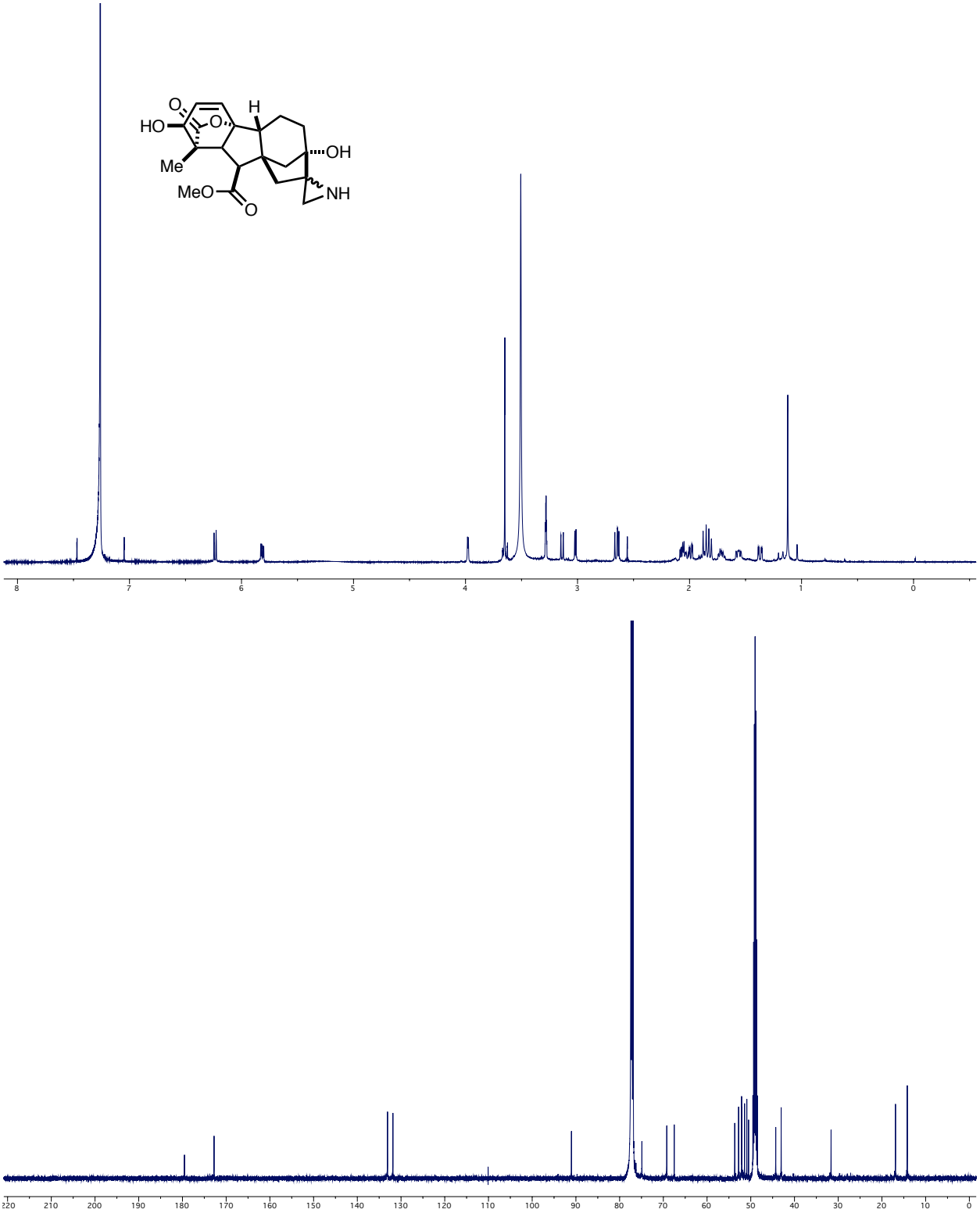
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR of **31** in $CDCl_3$



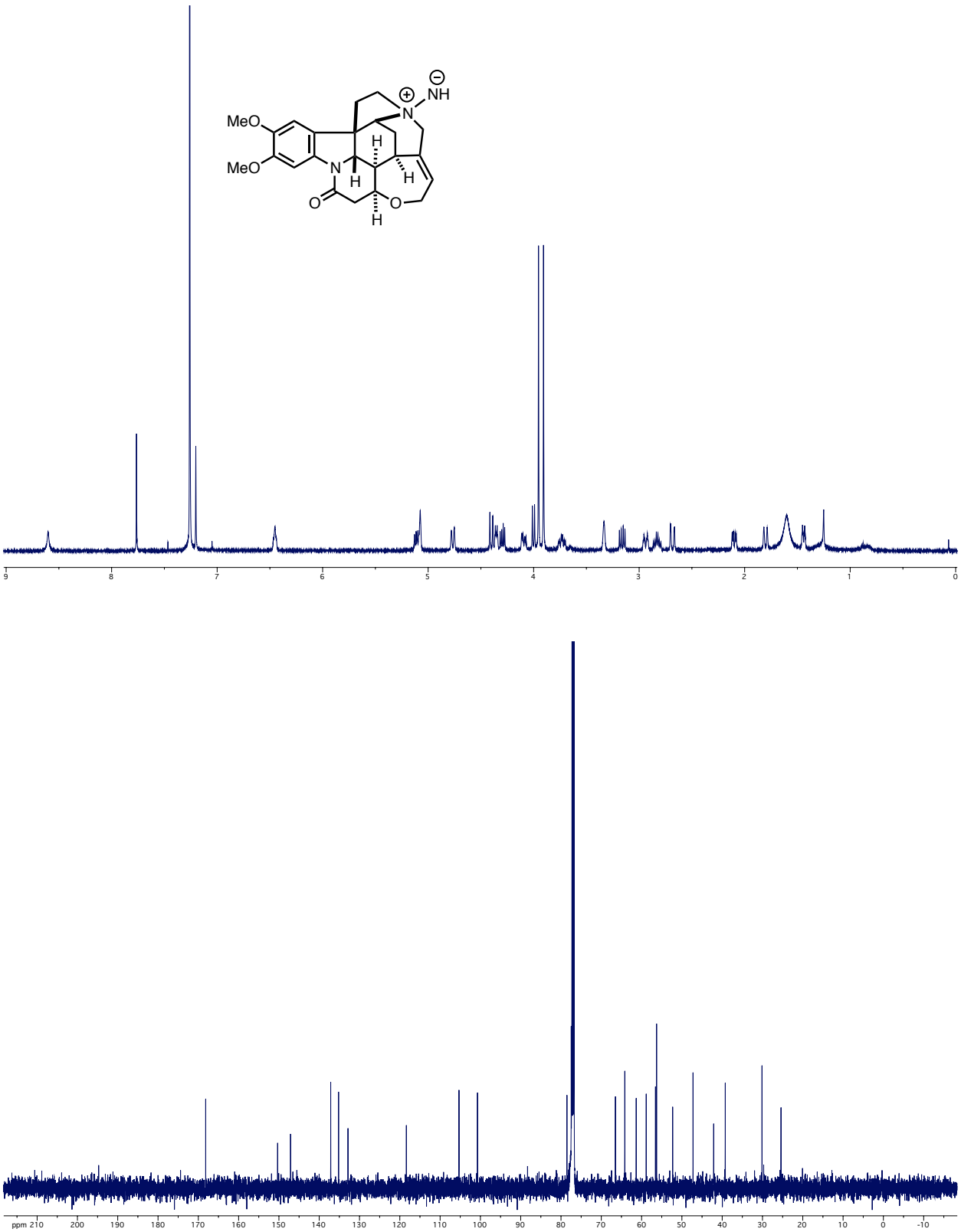
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR of **32** in CD_3OD



^{1}H (500 MHz) and ^{13}C (125 MHz) NMR of **33** (major isomer) in $CDCl_3$



^{1}H (500 MHz) and ^{13}C (125 MHz) NMR of **33** (minor isomer) in $CDCl_3$ + CD_3OD



^{1}H (500 MHz) and ^{13}C (125 MHz) NMR of **34** in $CDCl_3$