Supporting Information

Mollenyne A, a Long-Chain Chlorodibromohydrin Amide from the Sponge *Spirastrella mollis*.

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Experimental Section

General Experimental Procedures. UV spectra were measured on a Jasco J600 double-beam UV-vis spectrometer using spectroscopic grade solvents (Fluka) and a 1 mm quartz cell with 50 nm/min scan rate and 1 nm slit. LR ESI mass spectra were obtained on a ThermoElectron MSQ single quad mass spectrometer coupled to an Accela UPLC. ^{1}H, ^{13}C, and 2D NMR spectra were recorded on a Bruker Avance III DRX-600 (600 MHz, ^{1}H, 1.7 mm CPTCI probe) or Varian Xsense (500 MHz) spectrometers in CD_3OD or CD_3CN referenced to residual solvent signals as internal standards [δ_H CHD_2OH 3.31 ppm; δ_C 49.00 ppm and δ_H CHD_2CN 1.94 ppm; δ_C 1.32 ppm]. Preparative HPLC was carried out using dual Dynamax Model SD-200 pumps, with UV–detection (Pharmacia LKB UV-1 detector operating at 254 nm). Semi–preparative HPLC was carried out using either a Gilson model 302 pump equipped with tandem detectors—UV−visible (ISCO model UA-5, λ 254 nm) and refractive index (Waters R401)—or a Rainin HPXL dual-pump with split flow (7:1) between two detectors—a Jasco CD-2095 UV-CD and an ESA model 301 evaporative light scattering detector (ELSD). HPLC grade solvents were used for HPLC (EMD Chemicals). TLC was performed on silica gel coated 0.25 mm aluminum backed plates (Whatman AL SIL G/UV) with visualized by heating with vanillin-H_2SO_4-EtOH.

Animal Material. *Spirastrella mollis* was collected in June 2008 in Plana Cays, Bahamas (22° 36.459' N, 73° 33.755' W) at a depth of 30 m using scuba, and kept in EtOH at –20 ˚C until extraction. A voucher sample is archived at UCSD.

Extraction and Isolation. A frozen sample of *Spirastrella mollis* (wet wt. 712 g, dry extracted wt. 192 g) was cut into pieces and extracted with 2:1 MeOH/CH_2Cl_2 (4 x 1.5 L, 23 ˚C, overnight). These extracts were combined and the solvent evaporated. The crude extract was partitioned between EtOAc (4 x 1 L) and water (3 L). The organic layer was dried and partitioned between hexane (3 x 0.5 L) and 9:1 MeOH/H_2O (1.5 L). The hexane layers were combined and back extracted with 9:1 MeOH/H_2O (2 x 0.5 L). The combined aqueous MeOH layers were adjusted to 1:1 MeOH/H_2O and extracted with CH_2Cl_2 (4 x 1 L). The CH_2Cl_2 layer was separated and the solvent evaporated to give a brown gum (1.084 g) which was subjected to C_{18} flash chromatography (1:9 to 9:1 MeOH/H_2O, then MeOH) to give ten fractions. The sixth fraction (219.3 mg) was subjected to preparative reversed phase HPLC (1:9 CH_3CN/H_2O to CH_3CN gradient over 40 min) to give four fractions. The second prep HPLC fraction (12.2 mg) was subjected to two rounds of semipreparative reversed phase HPLC (23:27 CH_3CN/H_2O + 0.025 % TFA) to give 0.5 mg of **1** (2.6 x 10^{-6} % based on wet wt.).

Mollenyne A (1): colorless glass; UV (MeOH) λ_{max} 261 nm, 275 nm; CD (MeOH) λ 276 nm ($\Delta\varepsilon$ –2.7); ^{1}H NMR, ^{13}C NMR, see Table 1. HRESIMS *m/z* 629.0888 $[M+H]^+$ (calcd for $C_{26}H_{36}O_2N_4Br_2Cl$ 629.0888).

Hydrogenation of 1 to bromochloro-compound 7: Mollenyne A (**1**, 250 μg) in MeOH (0.25 mL) was treated with Pd/C (10%, 30 μg) under an H_2 (1 atm) for 3 days until most of the starting material had been consumed. The solution was filtered thru a PTFE membrane filter (0.45 μ), and the solvent was evaporated under a stream of N_2. The material was purified by HPLC (column: Phenomenex, Luna, C18 (2), 5μ, 10 x 250 mm; flow rate: 2 mLmin^{-1} mobile phase: 10-100% CH_3CN/H_2O+0.1% TFA over 45 minutes) to give the fully saturated bromochloro-compound **7** (200 μg). ^{1}H NMR (600 MHz, $CD_3OD/CDCl_3$): δ 4.42 (ddd, *J* = 8.8, 5.3, 1.7 Hz, 1H), 3.87 (dd, *J* = 9.4, 1.7 Hz, 1H), 3.79 (td, *J* = 9.4, 2.2 Hz, 1H), 3.15 (t, *J* = 7.1 Hz, 2H), 3.12 (t, *J* = 7.1 Hz, 2H), 2.17 (t, *J* = 7.6 Hz, 2H), 2.05–2.00 (m, 1H), 1.94–1.88 (m, 1H), 1.76–1.70 (m, 1H), 1.64–1.55 (m, 4H), 1.54–1.46 (m, 4H), 1.42–1.20 (m, 23H), 0.85 (t, *J* = 7.0 Hz, 3H); HRESIMS *m/z* 567.3031 $[M+H]^+$ (calcd for $C_{26}H_{53}BrClN_4O_2$ 567.3035).

Chloroepoxide (8): Compound **7** (50 μg) was dissolved in MeOH (0.5 mL), and potassium carbonate (150 μg) was added and the mixture was stirred at room temperature for 1 hour. The reaction was neutralized with a solution of 95:5 MeOH/AcOH, and the solvent was evaporated under a stream of N_2. The mixture was subjected to HPLC (column: Phenomenex, Luna, C18 (2), 5μ, 10 x 250 mm; flow rate: 2 mLmin^{-1} mobile phase: 10-100% CH_3CN/H_2O+0.1% TFA over 45 minutes) to give the chloroepoxide **8** (~30 μg). ^{1}H NMR (600 MHz, $CD_3OD/CDCl_3$): δ 3.54 (ddd, *J* = 8.0, 8.0, 4.0 Hz, 1H), 3.18 (t, *J* = 7.7 Hz, 2H), 3.16 (t, *J* = 7.7 Hz, 2H), 2.90 (ddd, *J* = 5.6, 5.6, 2.0 Hz, 1H), 2.86 (dd, *J* = 8.0, 2.0 Hz, 1H), 2.18 (t, *J* = 7.7 Hz, 2H), 1.93–1.88 (m, 1H),1.79–1.72 (m, 1H), 1.65–1.27 (m, 32H), 0.90 (t, *J* = 7.1 Hz, 3H) HRESIMS *m/z* 487.3776 $[M+H]^+$ (calcd for $C_{26}H_{52}Cl\ N_4O_2$ 487.3773).

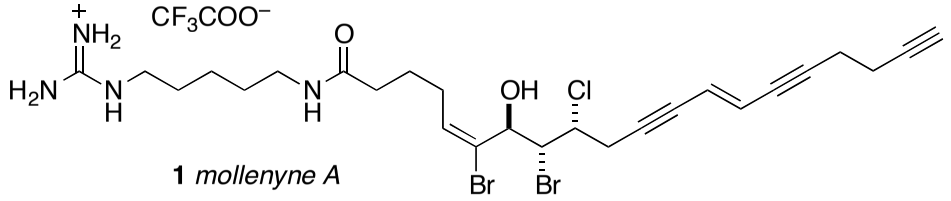
Mollenyne A Benzoate Ester (9): Mollenyne A (**1**, 50 μg) was stirred with excess benzoyl chloride (5 μL) in pyridine for 2 hours at room temperature. The solvent was evaporated under a stream of N2, then by high vacuum. The material was purified by gradient HPLC (column: Phenomenex, Luna, C18 (2), 5μ, 10 x 250 mm; flow rate: 2 mLmin^{-1} mobile phase: 10-100% CH_3CN/H_2O+0.1% TFA over 45 minutes), then by isocratic HPLC (column: Phenomenex, Synergi Hydro-RP, 4μ, 10 x 250 mm; flow rate: 2 mLmin^{-1} mobile phase: 17:8 CH_3CN/H_2O+0.1%) to give mollenyne A benzoate **9** (18 μg). UV (MeOH) λ_{max} 235 nm, 261 nm, 275 nm; CD (MeOH) λ 227 nm (Δε +6.1), 260 nm (Δε +7.5), 275 nm (Δε +6.5); HRESIMS *m/z* 733.1146 $[M+H]^+$ (calcd for $C_{33}H_{40}Br_2ClN_4O_3$ 733.1150).

Preparation of mollenyne A cinnamate derivative (10): Mollenyne A (**1**, 50 mg) was stirred with excess *p*-methoxycinnamoyl chloride (1 mg) in pyridine (250 mL) for 4 hours at room temperature. The solvent was evaporated under a stream of N_2, then by high vacuum. The material was purified by gradient HPLC (column: Phenomenex, Luna, C18 (2), 5μ, 10 x 250 mm; flow rate: 2 mLmin^{-1} mobile phase: 10-100% CH_3CN/H_2O+0.1% TFA over 45 minutes), then by isocratic HPLC (column: Phenomenex, Synergi Hydro-RP, 4μ, 10 x 250 mm; flow rate: 2 mLmin^{-1} mobile phase: 17:8 CH_3CN/H_2O+0.1%) to give mollenyne A cinnamate ester **10** (17 μg). UV (MeOH) λ_{max} 262 nm, 275, 313; CD (MeOH) λ 262 nm (Δε –6.6), 290 nm (Δε +10.8), 296 nm (Δε +10.8), 307 nm (Δε +10.7), 312 nm (Δε +10.7); HRESIMS *m/z* 789.1401 $[M+H]^+$ (calcd for $C_{36}H_{44}Br_2ClN_4O_4$ 789.1412).

Table S1. ^{1}H (600 MHz) and ^{13}C NMR for mollenyne A (**1**) (CD_3CN).

No.	δ_H, m (*J* in Hz)	δ_C[a]	COSY	HMBC[b]
1		174.1, C		
2	2.14, m	35.7, CH_2	3	1, 3, 4
3	1.75, m	25.4, CH_2	2, 4	1, 2, 4, 5
	1.66, m			
4	2.27, m	29.5, CH_2	3, 5	2, 3, 5, 6
	2.13, m			
5	6.07, dd (9.4, 6.5)	137.3, CH	4	3, 6, 7
6		128.0, C		
7	4.71, d (9.7)	70.0, CH	8	5, 6, 8, 9
8	4.52, dd (9.7, 1.6)	59.0[c], CH	7	6, 7, 9, 10
9	4.59, ddd (8.0, 7.0, 1.6)	59.2[c], CH	10	7, 8, 10, 11
10	3.00, ddd (17.2, 7.0, 2.0)	29.5, CH_2	9, 13	8, 9, 11, 12, 13
	2.93, ddd (17.2, 8.0, 2.0)			
11		90.5, C		
12		82.1, C		
13	5.95, dt (16.1, 2.0)	122.1, CH	10, 14	
14	6.01, dt (16.1, 2.0)	120.5, CH	13, 17	
15		80.0, C		
16		95.0, C		
17	2.55, td (7.0, 2.0)	19.9, CH_2	14, 18	13, 14, 15, 16, 18, 19
18	2.39, td (7.0, 2.6)	18.7, CH_2	17, 20	16, 17, 19, 20
19		83.6, C		
20	2.26, d (2.6)	70.5, CH	18	18
NH	8.23, brs			
1’	3.13 (m)	39.1, CH_2	2’	1, 2’, 3’
2’	1.47, p (7.3)	29.2, CH_2	1’, 3’	1’, 3’, 4’
3’	1.34, p (7.3)	24.1, CH_2	2’, 4’	1’, 2’, 4’, 5’
4’	1.56, p (7.3)	28.3, CH_2	3’, 5’	2’, 3’, 5’
5’	3.08 (m)	41.9, CH_2	4’	3’, 4’, 6’
NH	6.86, brs			
6’		158.7, C		

[a] Determined from HSQC. [b] HMBC correlations, optimized for J_{CH}= 8 Hz. Correlations are from H→C.



1 *mollenyne A*

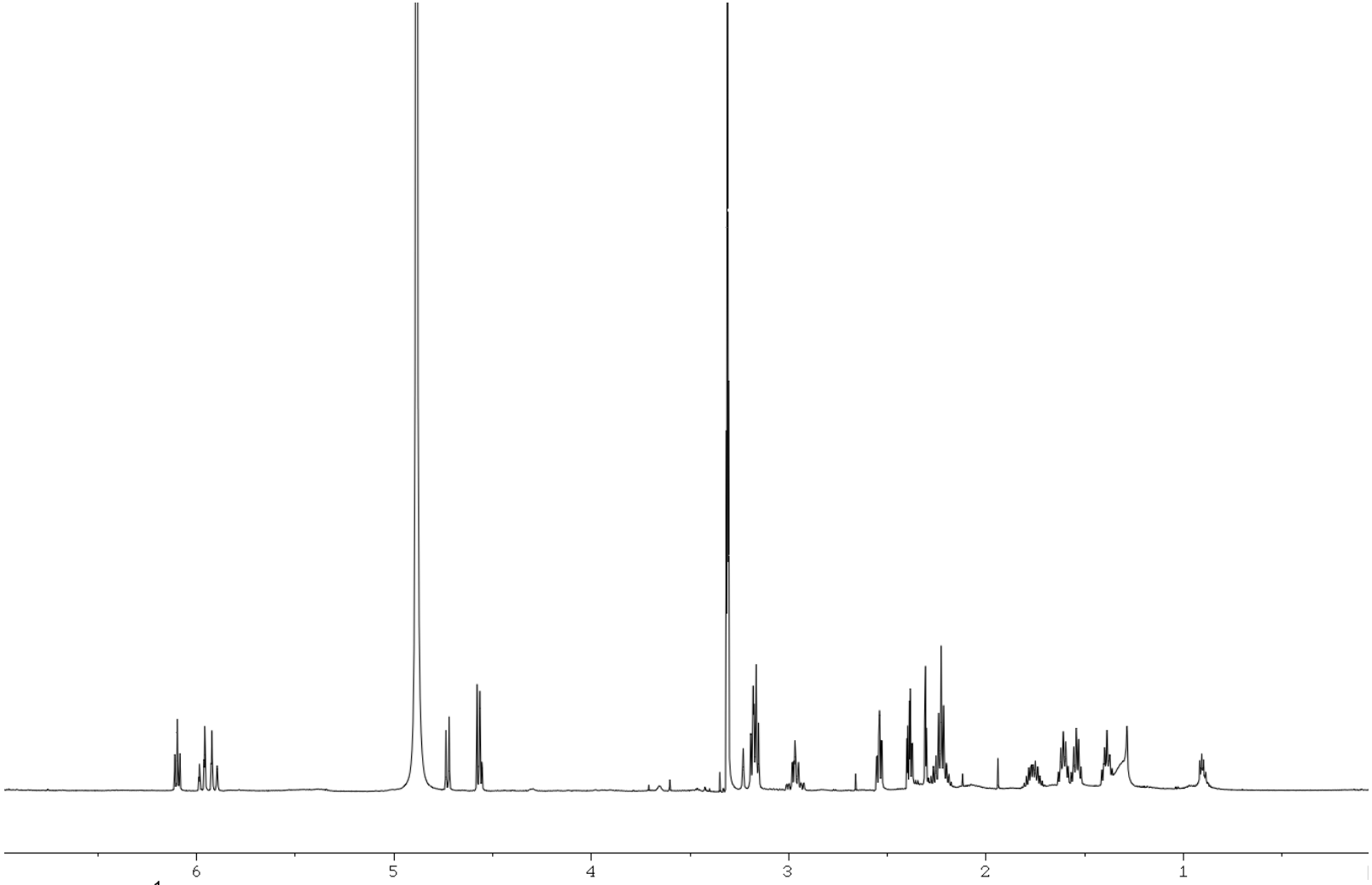


Figure S1. ^{1}H NMR spectrum of molleneyne A (**1**) (600 MHz, MeOH-d_4).

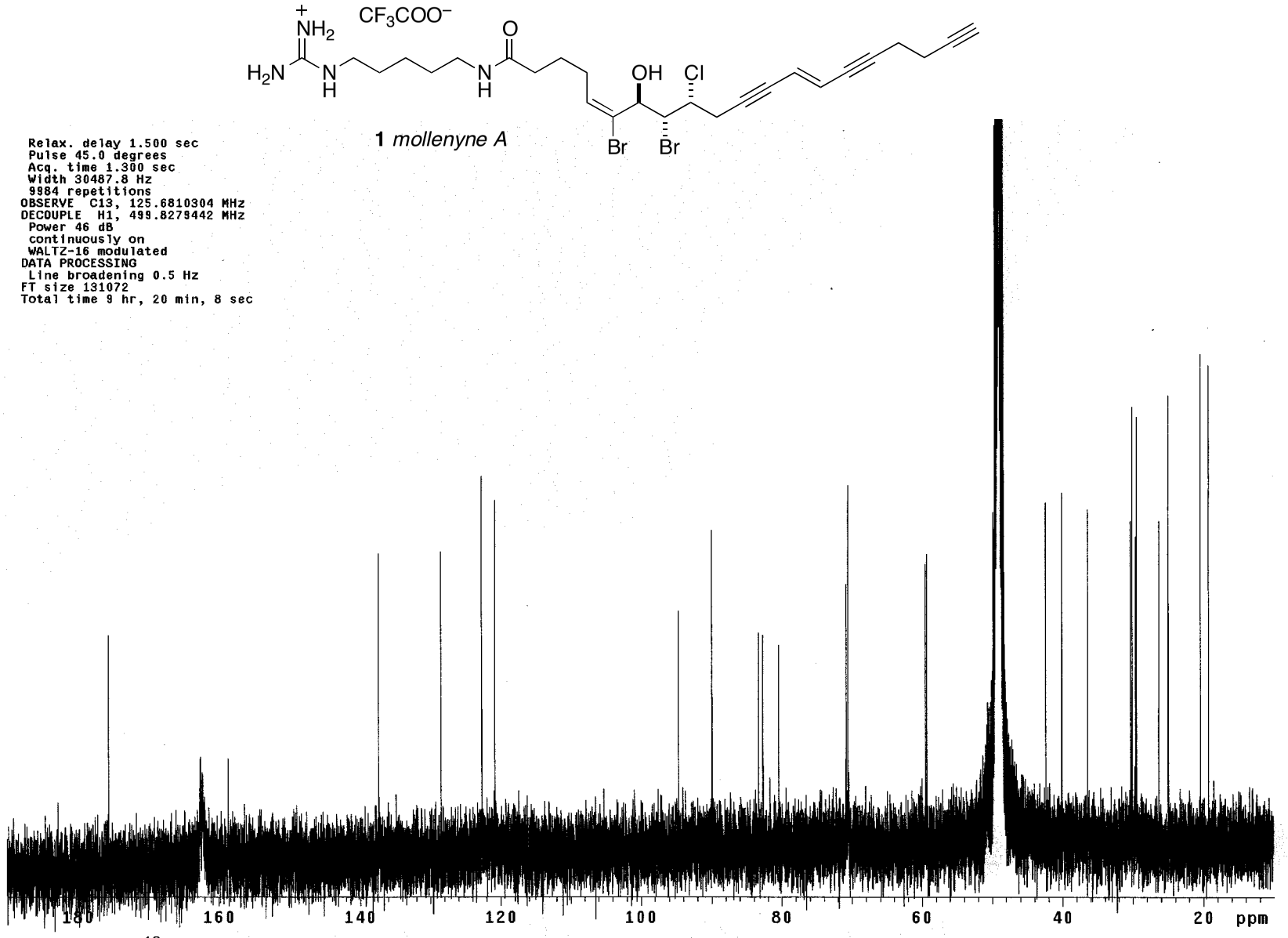


Figure S2. ^{13}C NMR spectrum of molleneyne A (**1**) (125 MHz, MeOH-d_4).

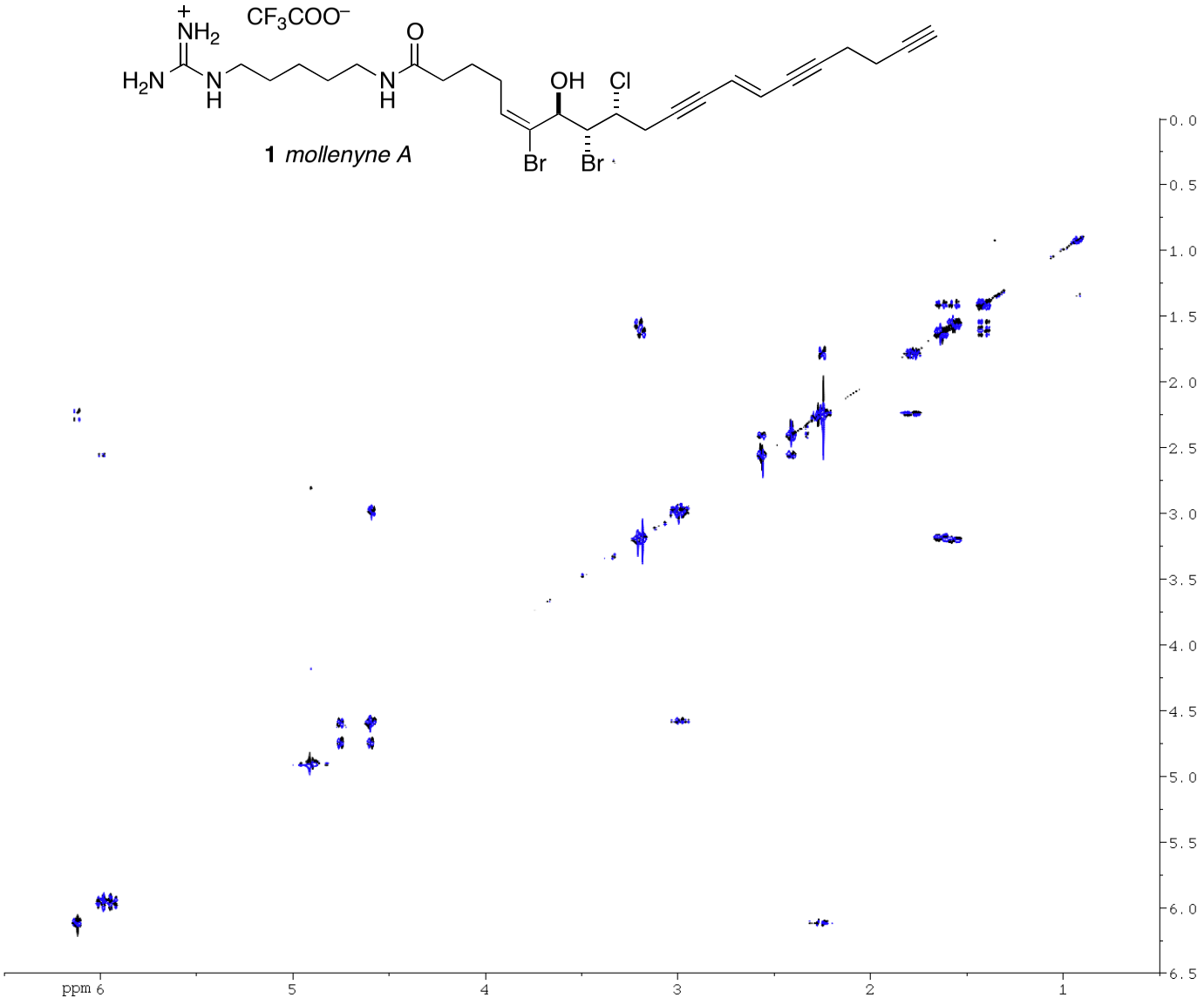
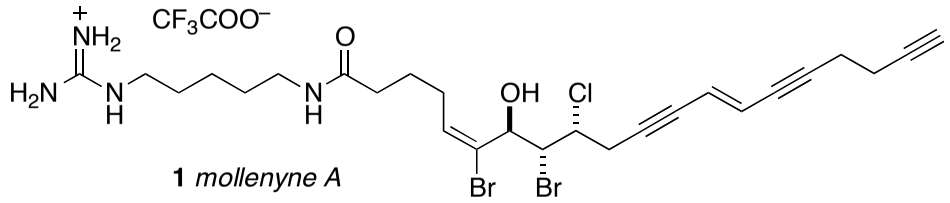


Figure S3. DQF–COSY spectrum of molleneyne A (**1**) (600 MHz, MeOH-d_4).



1 *mollenyne A*

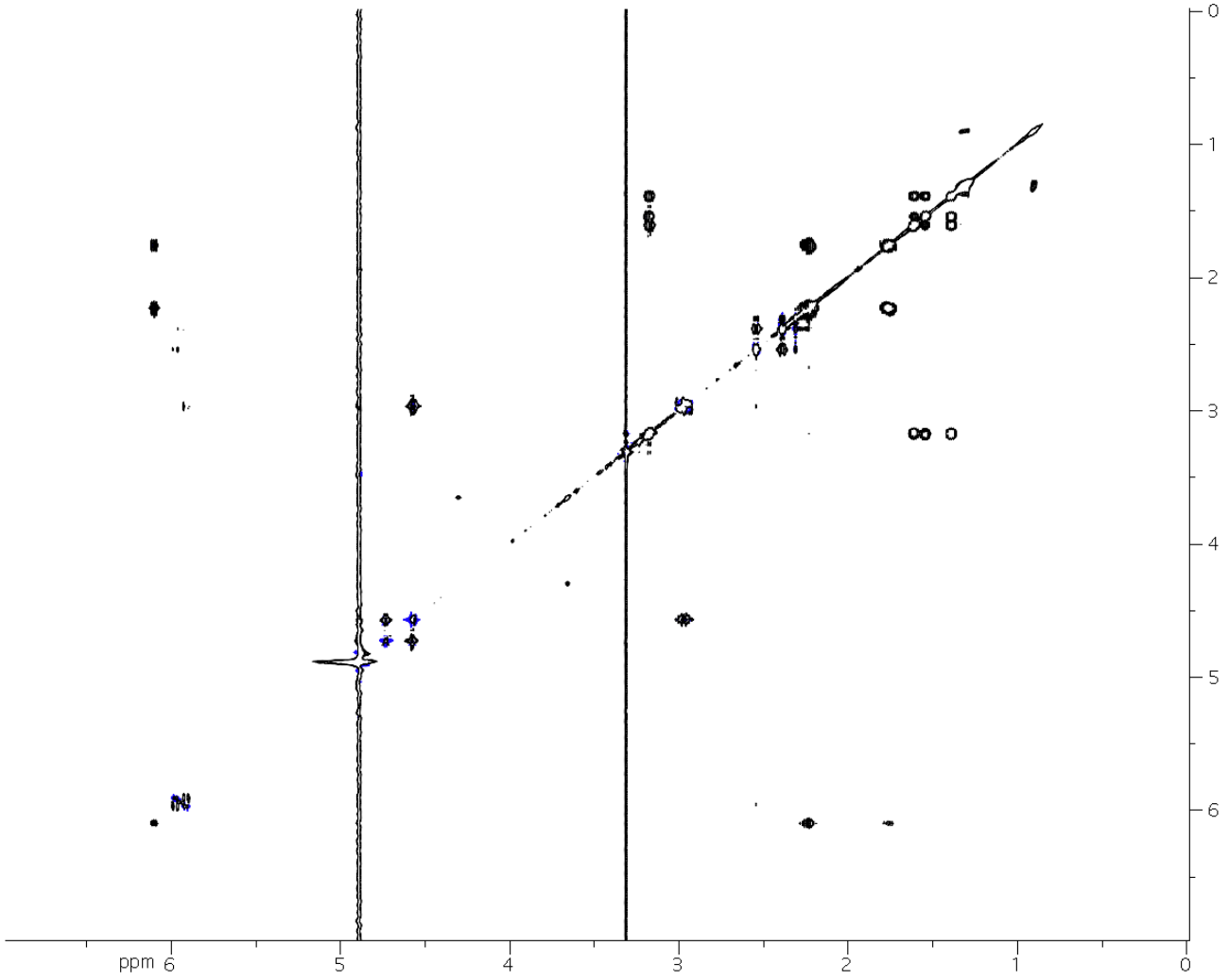


Figure S4. TOCSY spectrum of molleneyne A (**1**) (600 MHz, MeOH-d_4).

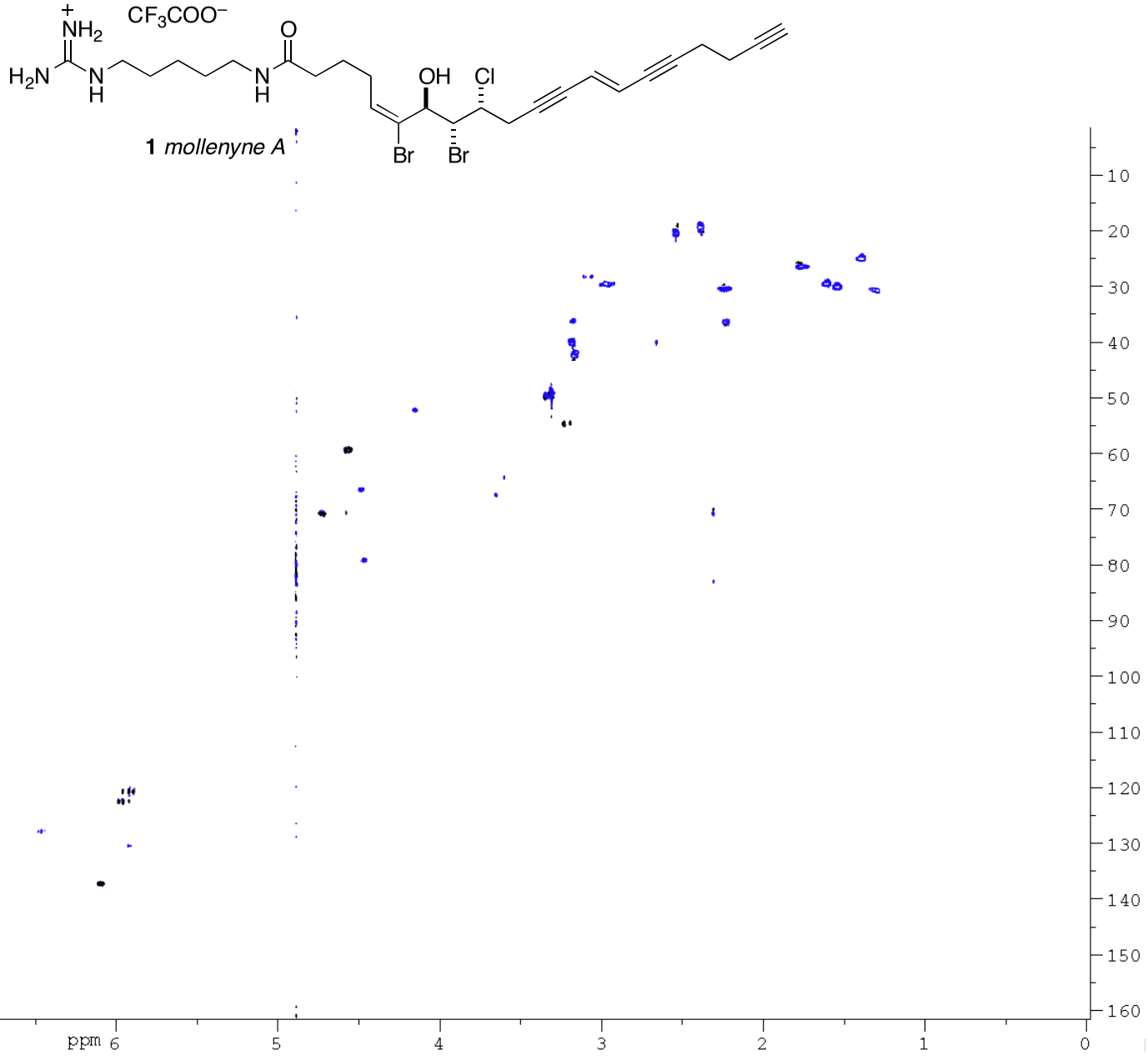


Figure S5. gHSQC spectrum of molleneyne A (**1**) (600 MHz, MeOH-d_4).

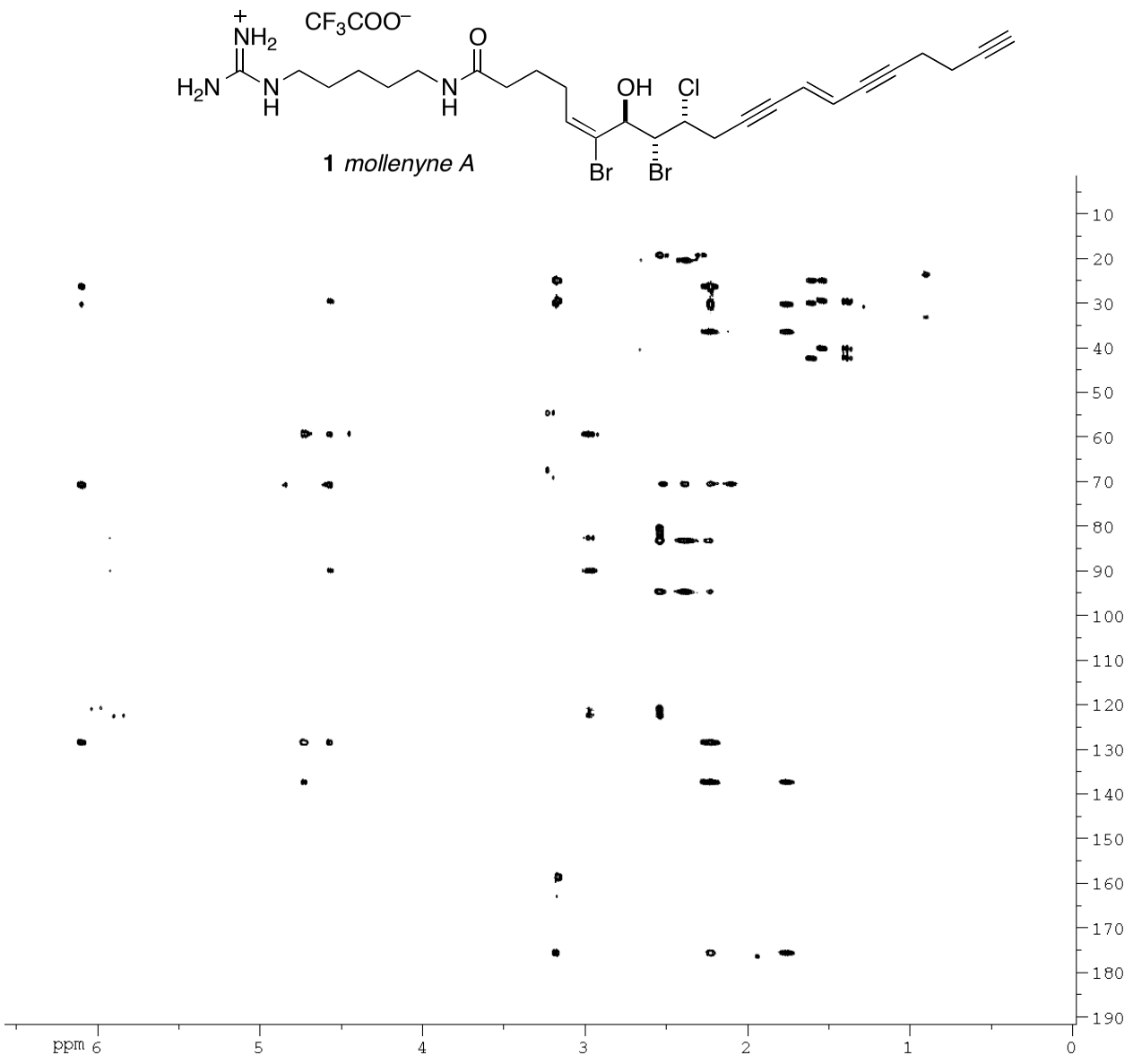


Figure S6. HMBC spectrum of molleneyne A (**1**) (600 MHz, MeOH-d_4, $^{n}J_{HC}$=8Hz).

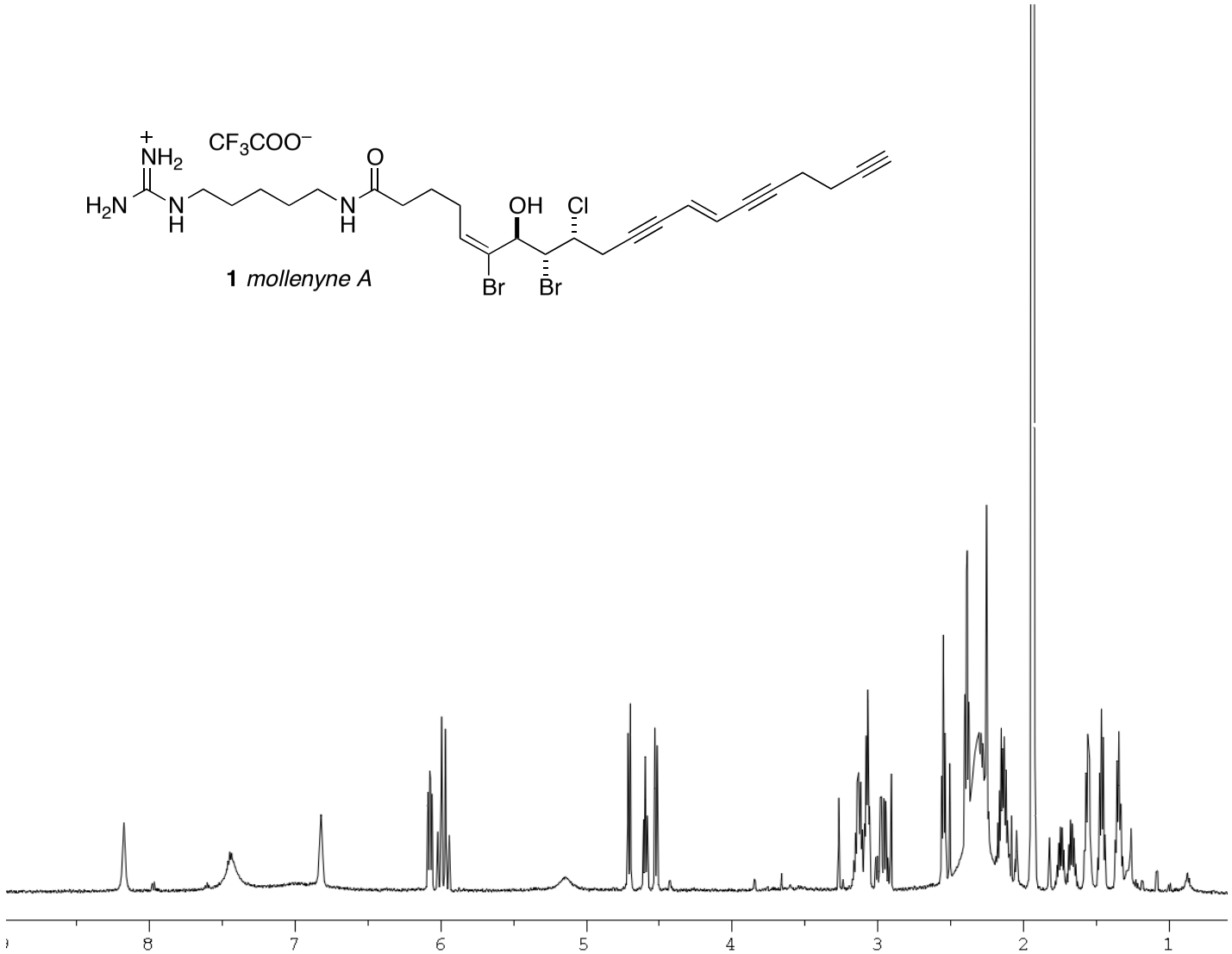


Figure S7. ^{1}H NMR spectrum of molleneyne A (**1**) (600 MHz, CD_3CN).

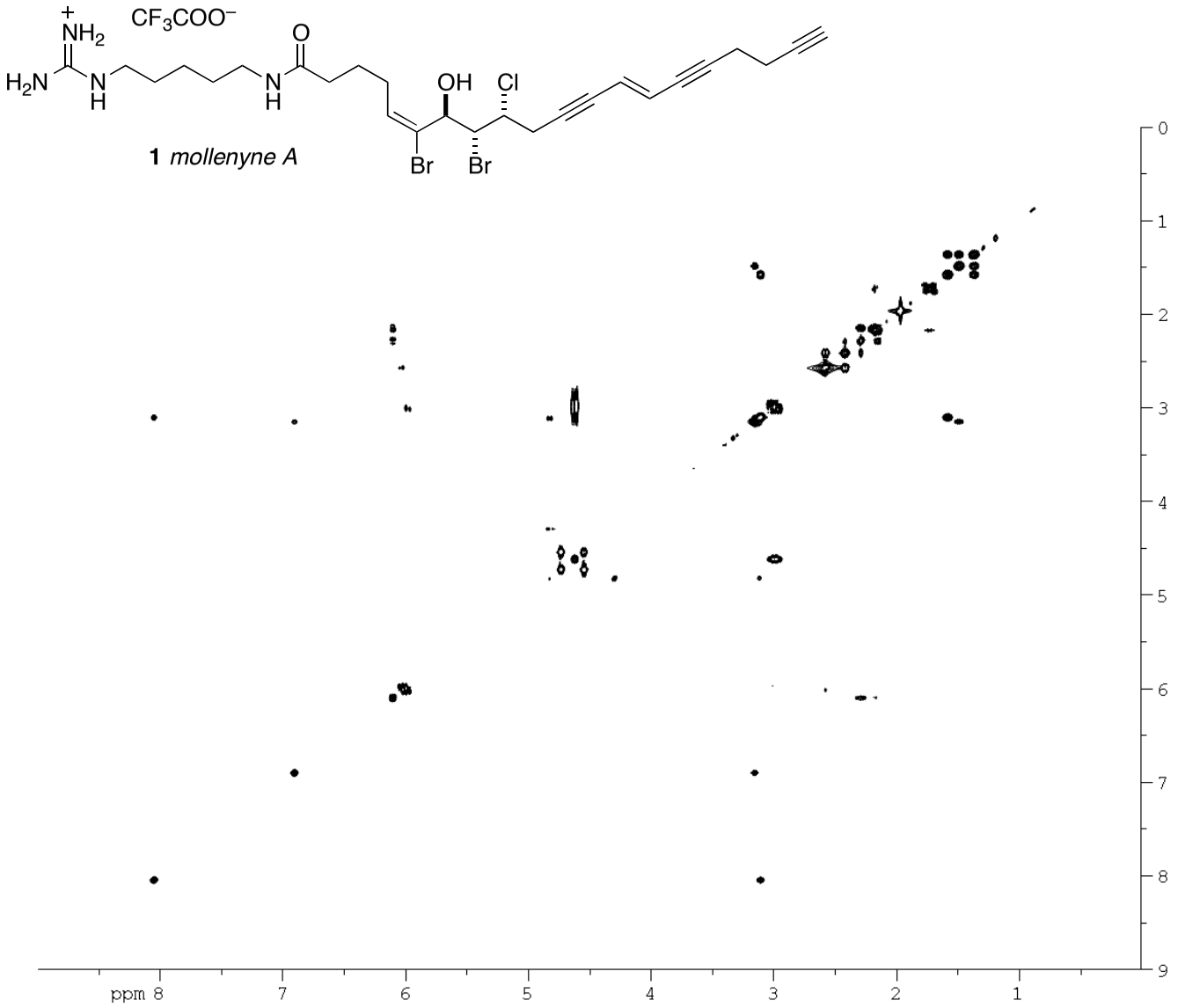
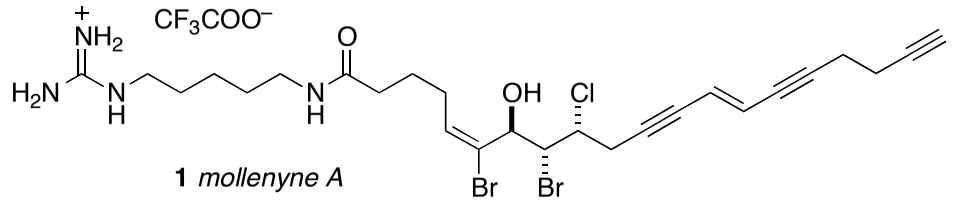


Figure S8. gCOSY spectrum of molleneyne A (**1**) (600 MHz, CD_3CN).



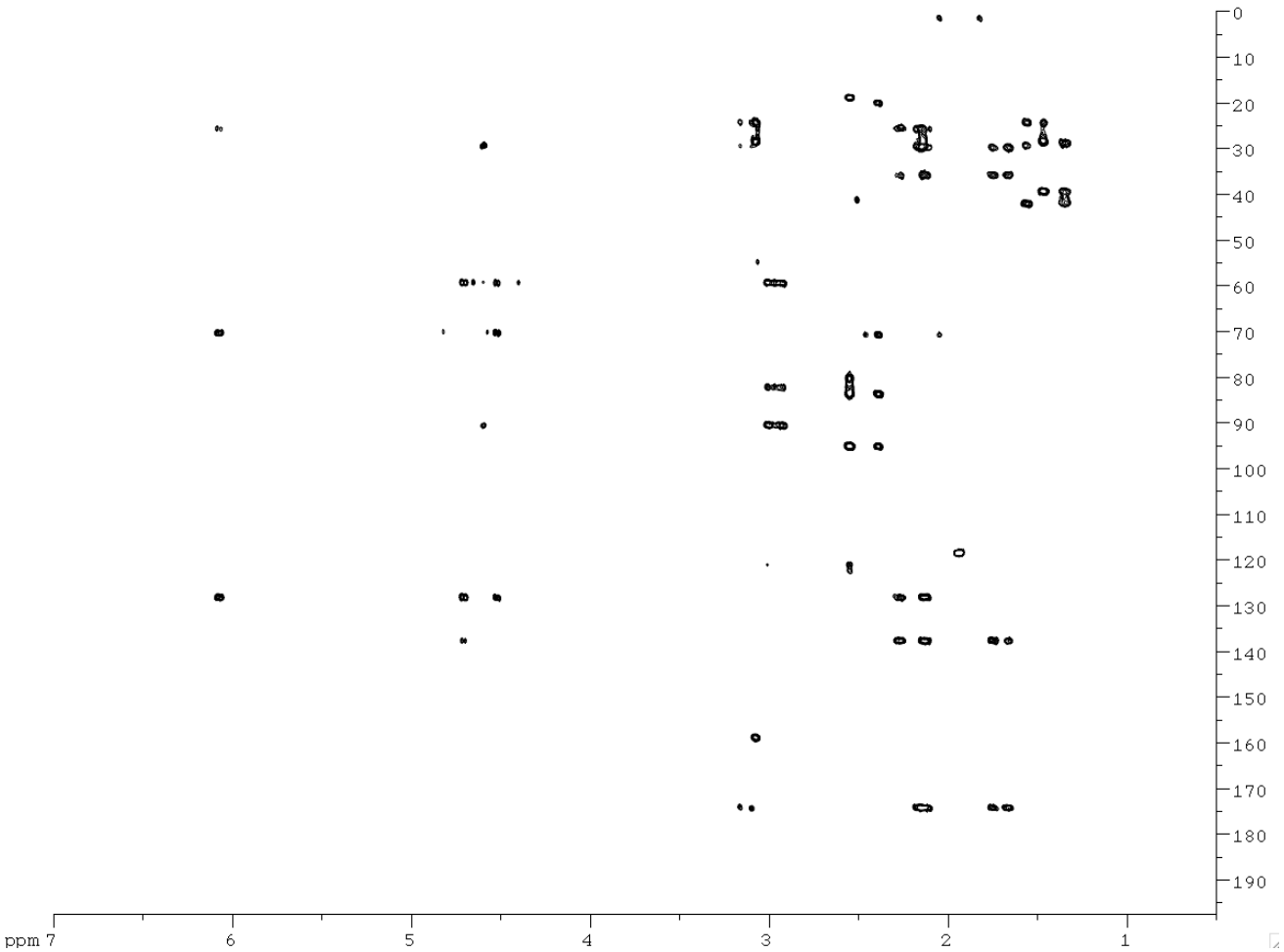
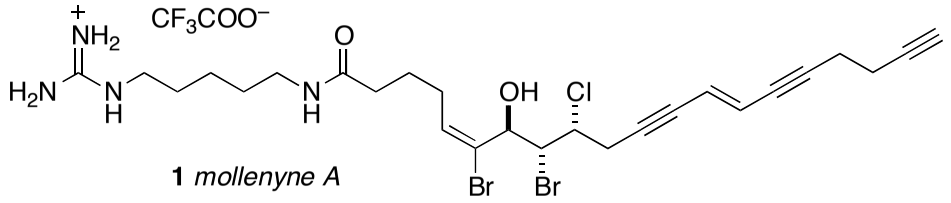


Figure S9. gHMBC spectrum of molleneyne A (**1**) (600 MHz, CD_3CN, $^{n}J_{HC}$ = 8Hz).



1 *mollenyne A*

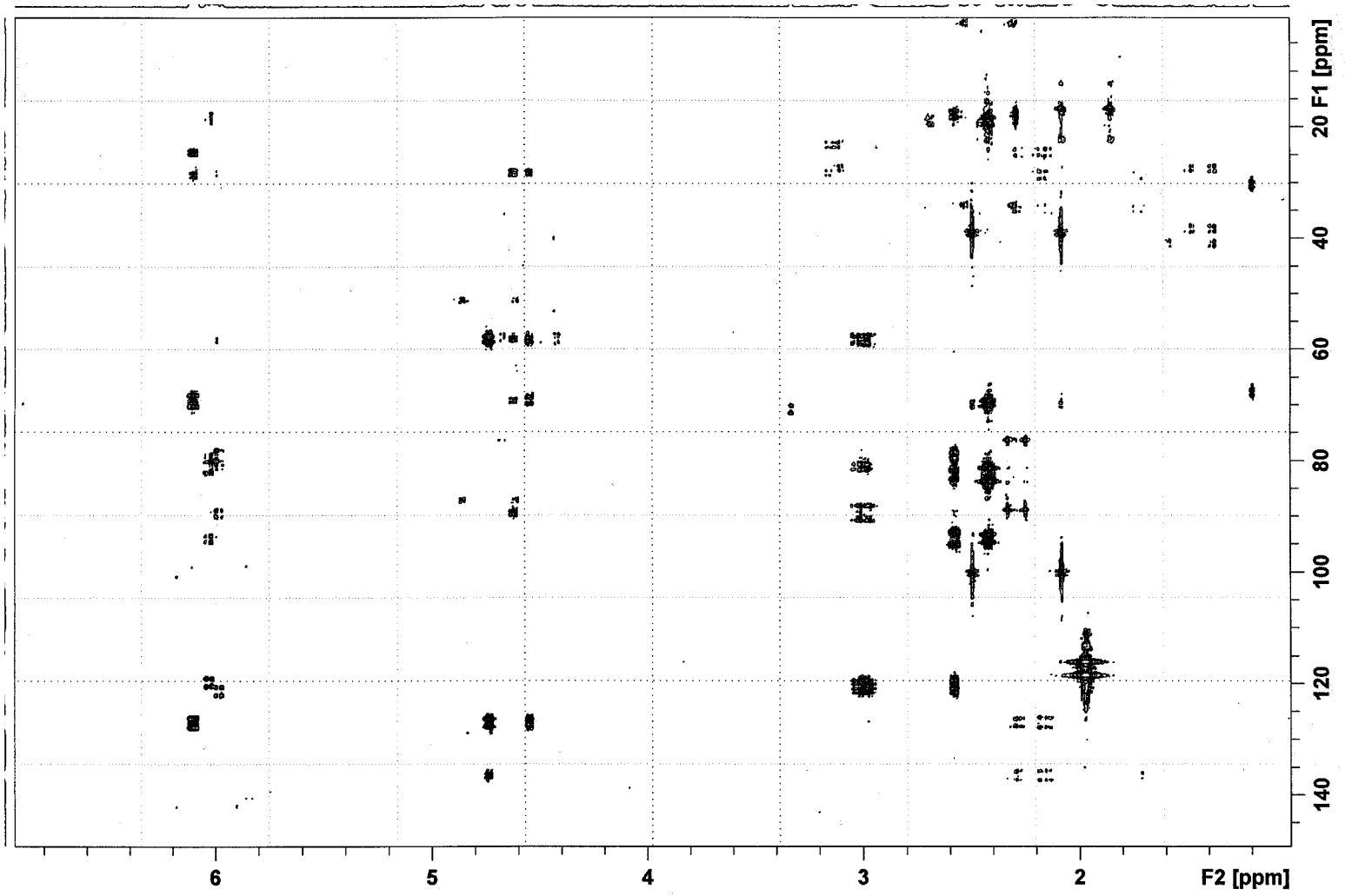


Figure S10. *J*–HMBC spectrum of molleneyne A (**1**) (600 MHz, CD_3CN, $^{n}J_{HC}$ = 3Hz, J_{scale} = 37).

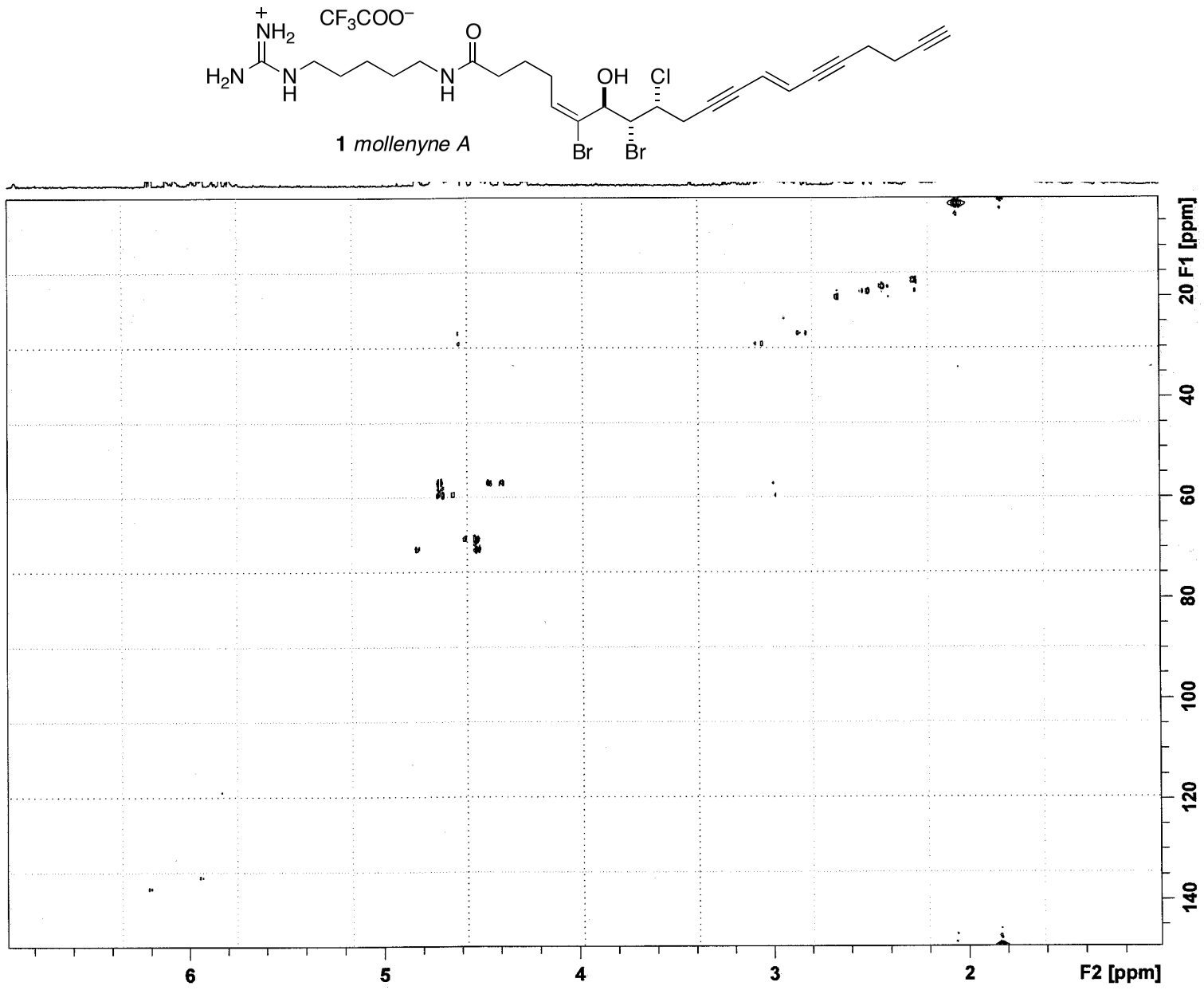


Figure S11. HSQC–HECADE spectrum of molleneyne A (**1**) (600 MHz, CD_3CN, t_{mix} = 60ms).

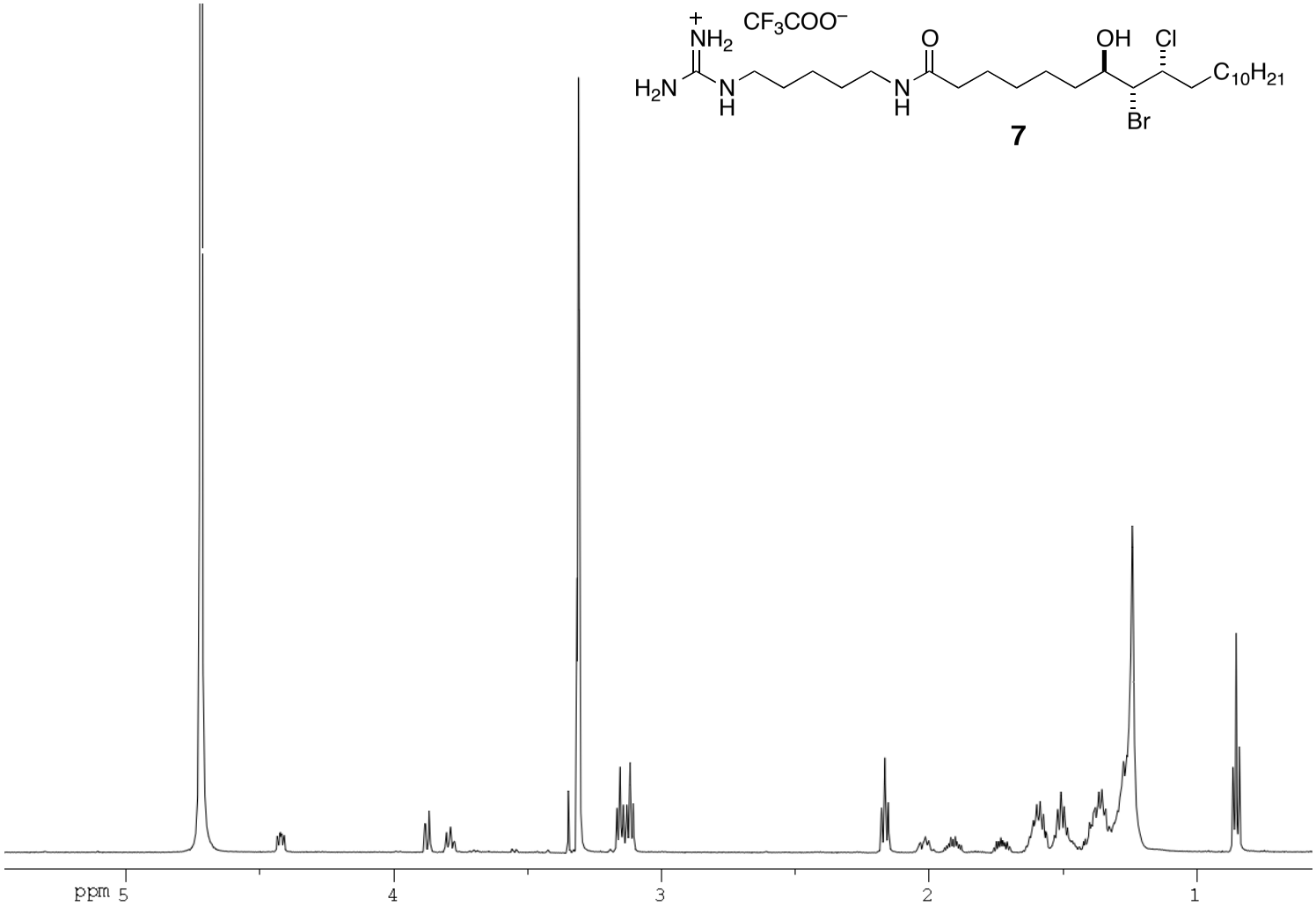


Figure S12. ^{1}H NMR spectrum of hydrogenation product **7** (600 MHz, 1:1 MeOH-d_4/$CDCl_3$).

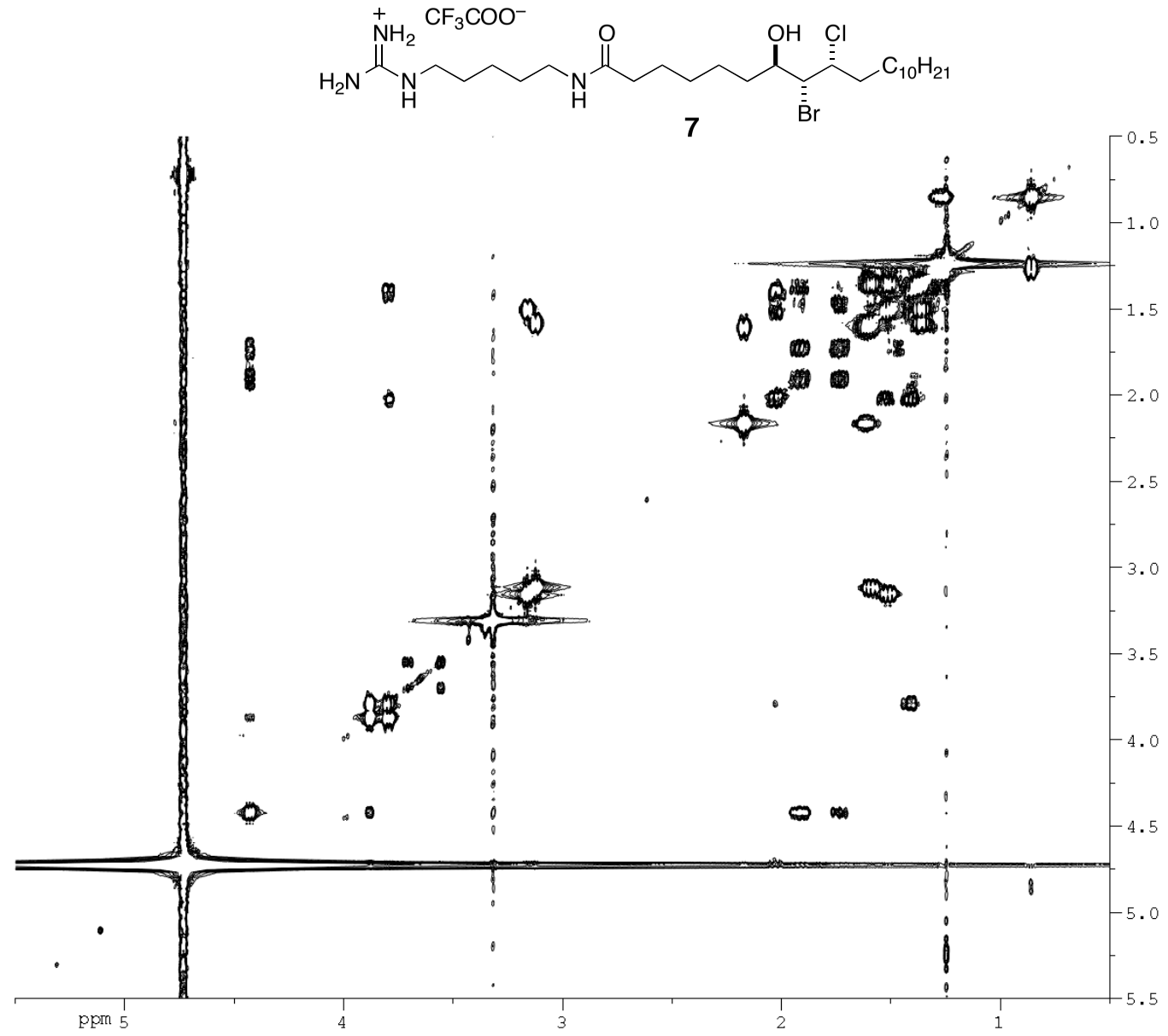
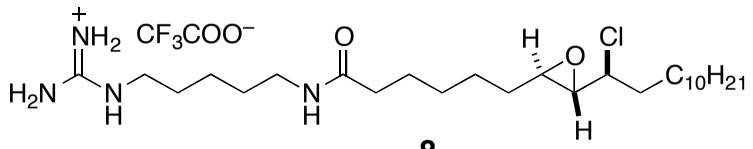


Figure S13. gCOSY spectrum of hydrogenation product **7** (600 MHz, 1:1 MeOH-d_4/$CDCl_3$).



8

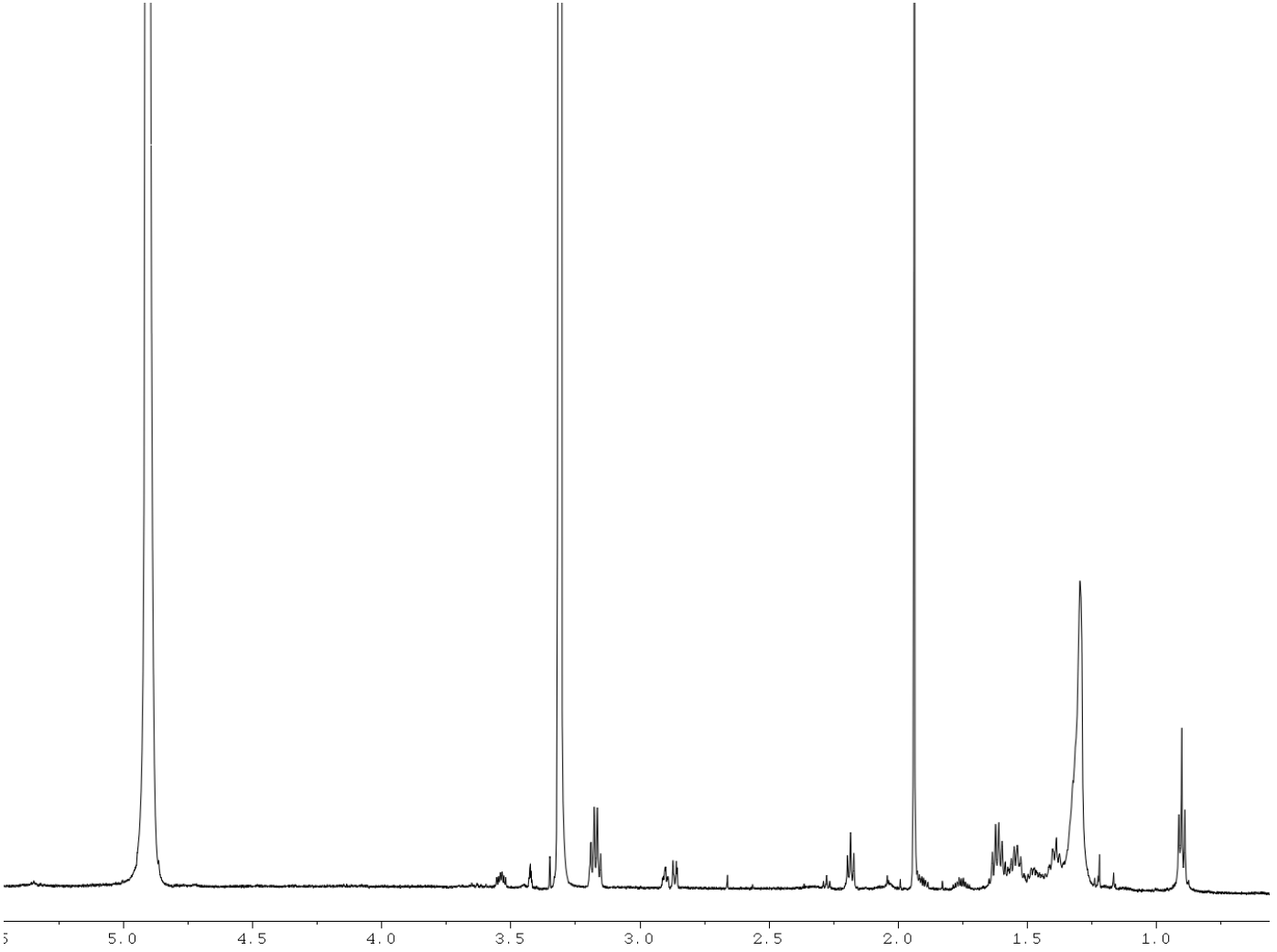


Figure S14. ^{1}H NMR spectrum of epoxide **8** (600 MHz, MeOH-d_4).

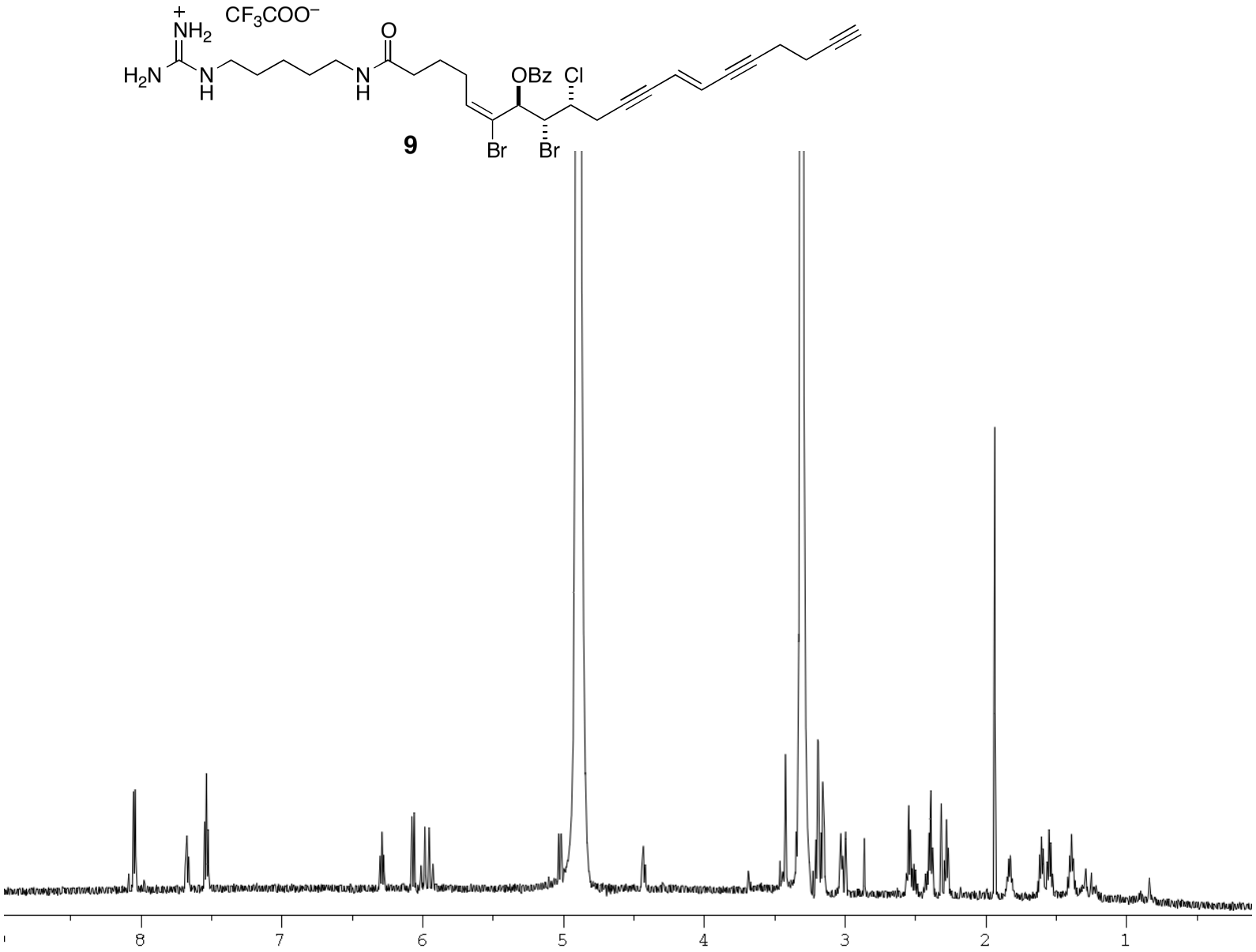


Figure S15. ^{1}H NMR spectrum of benzoate **9** (600 MHz, MeOH-d_4).

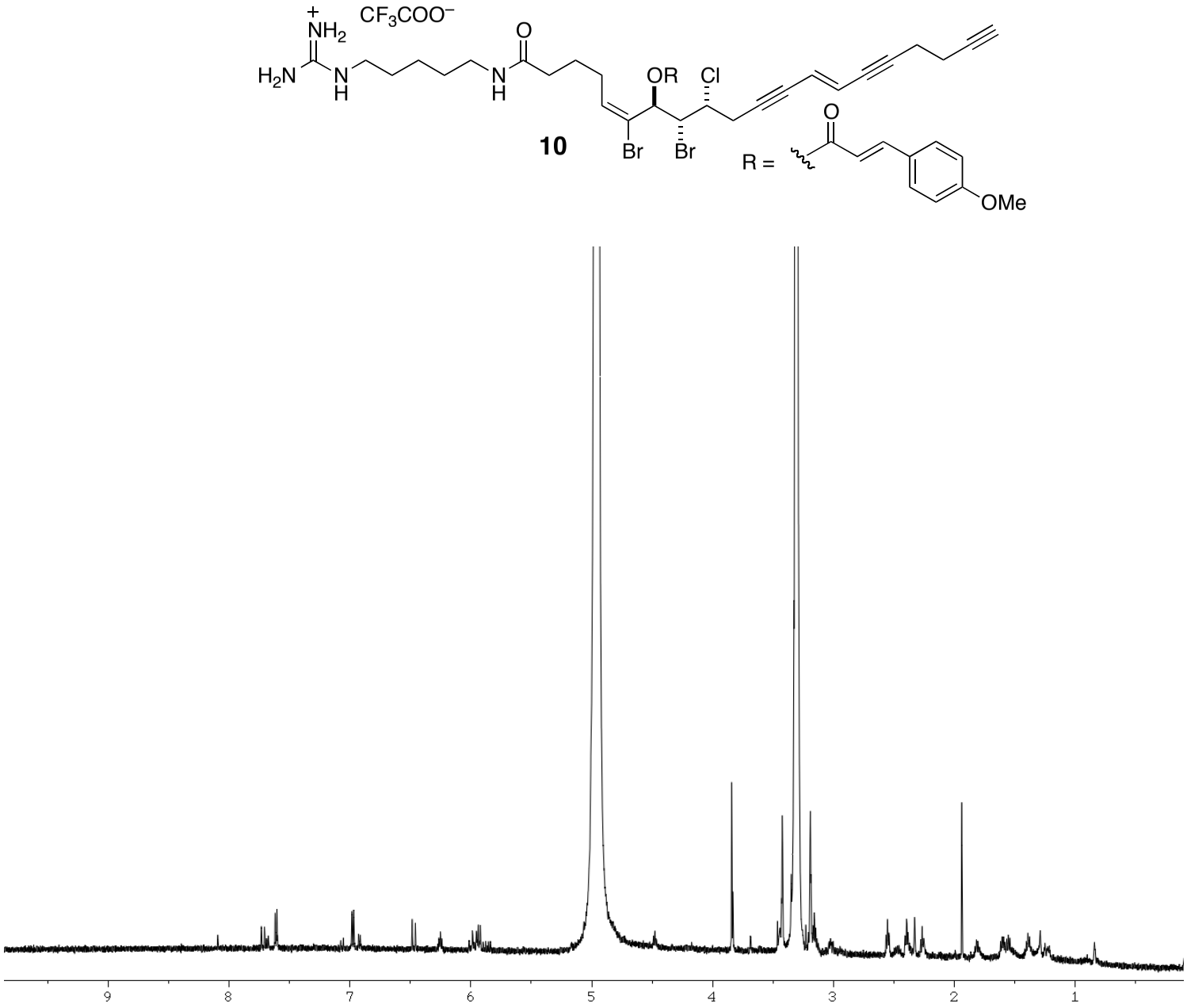


Figure S16. ^{1}H NMR spectrum of *p*-methoxycinnamate**10** (600 MHz, MeOH-d_4).