Supplementary information

Biocompatible sulfonium-based covalent probes for endogenous tubulin fluorescence nanoscopy in live and fixed cells

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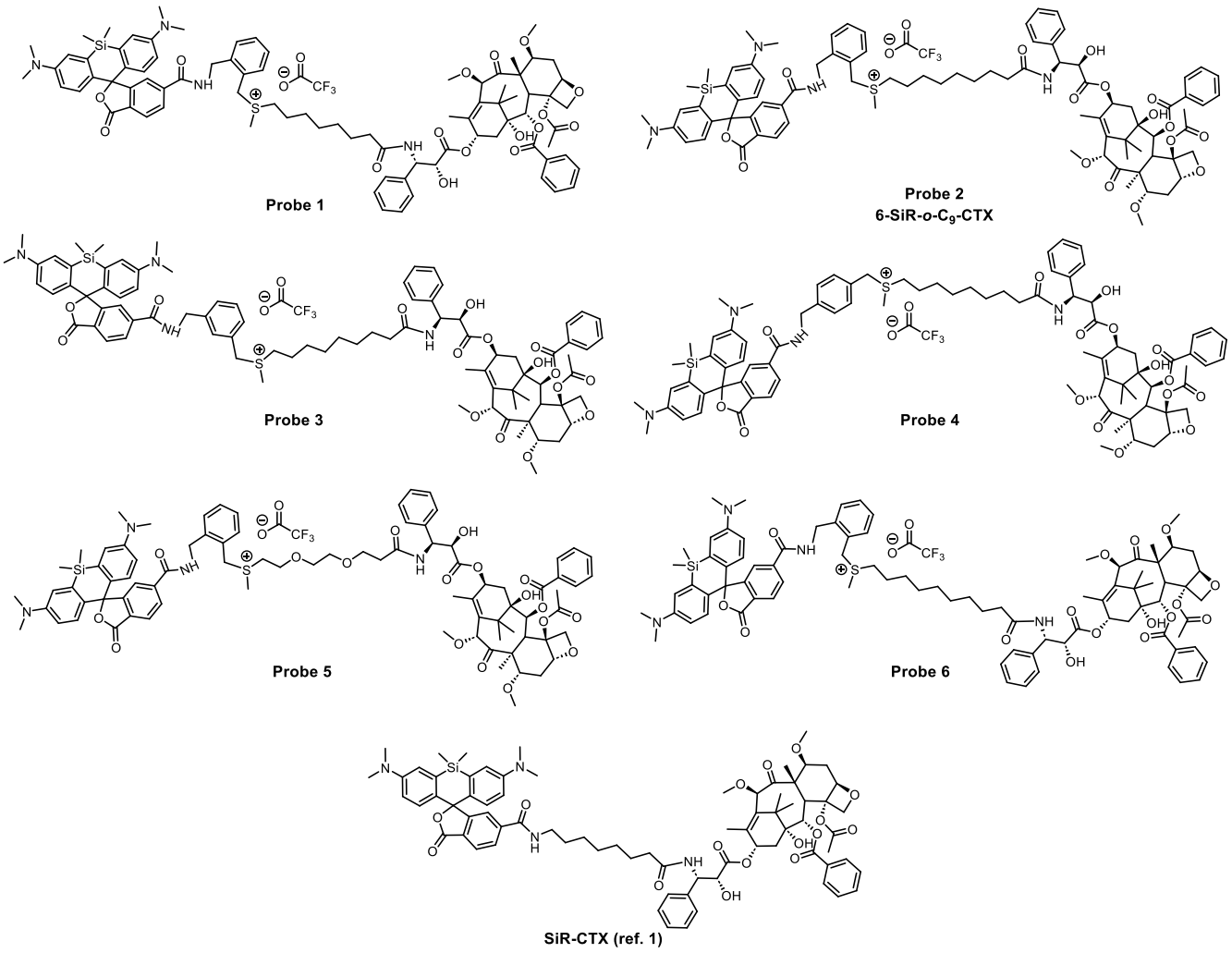
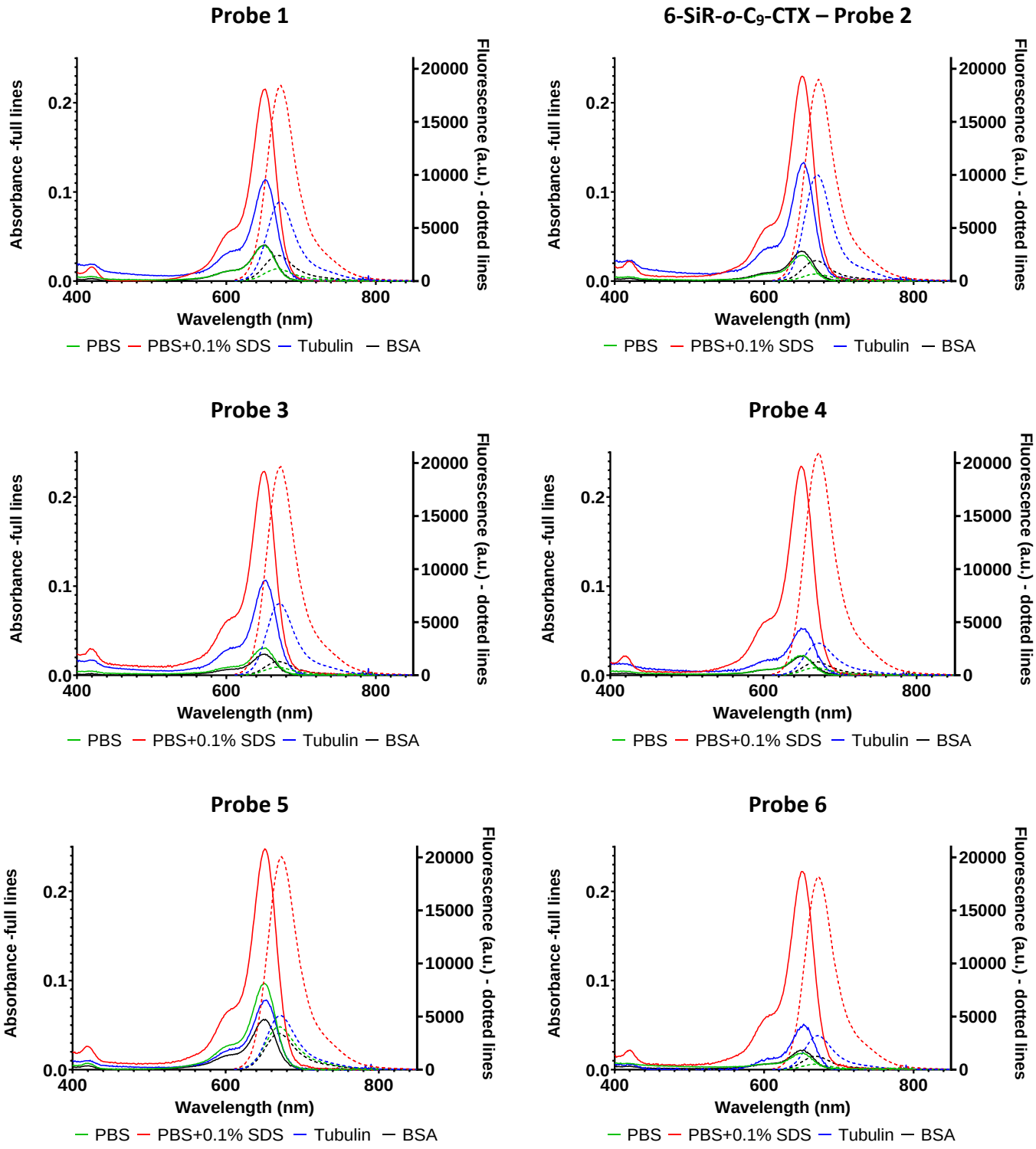
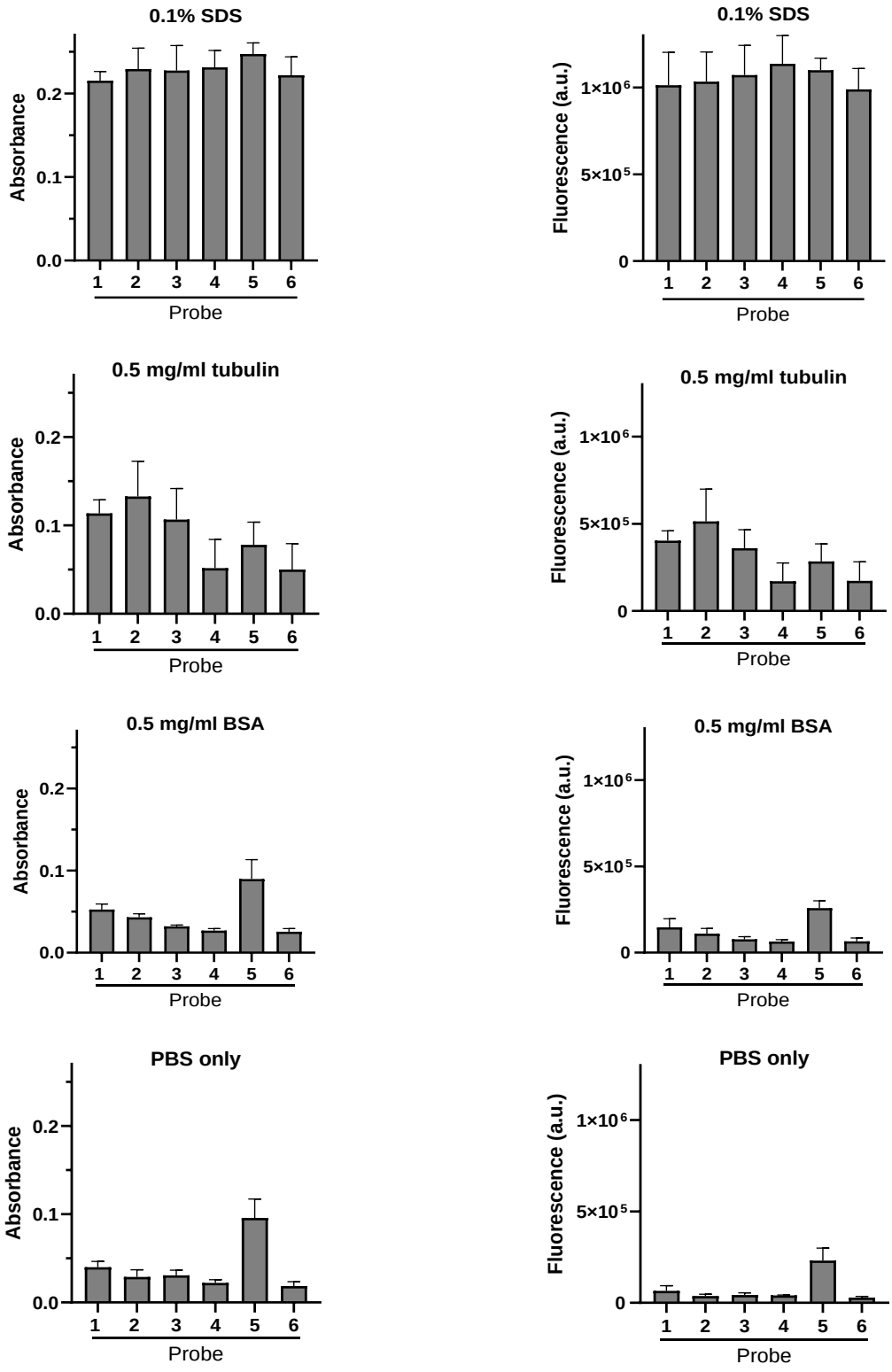
Supplementary Figures



Supplementary Figure 1. Detailed structures of the covalent probes and non-covalent tubulin probe SiR-CTX[1]

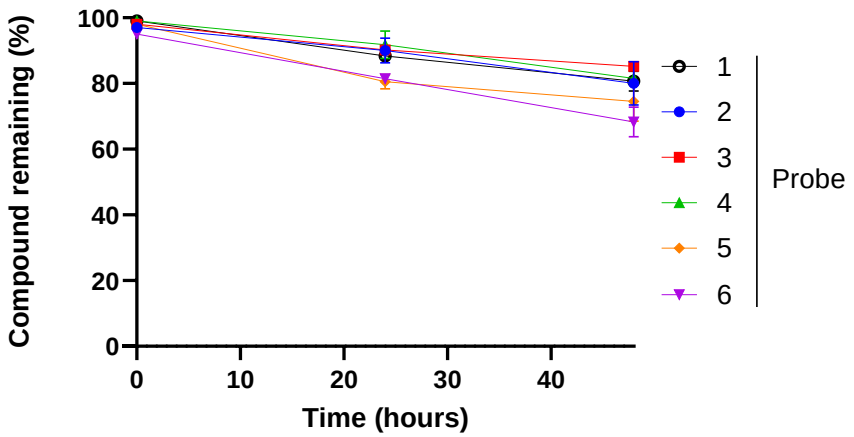


Supplementary Figure 2. Absorption and emission spectra of covalent tubulin probes. Spectra were recorded after incubating 5 µM probes with 0.5 mg/mL tubulin in General Tubulin Buffer (Blue), 0.5 mg/mL BSA in PBS (Black), in PBS (Green), or in PBS+0.1% SDS (Red) at 37°C for 4h. Spectra are represented as averages of three independently repeated experiments (N=3).

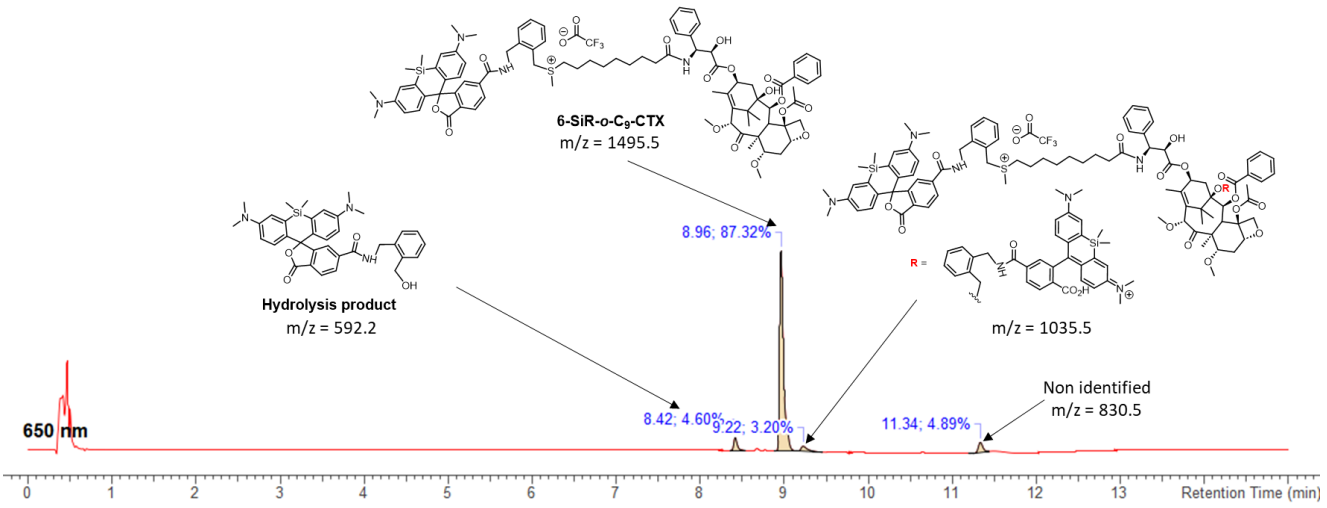


Supplementary Figure 3. Absorbance and fluorescence emission of covalent tubulin probes in different conditions. Spectra were recorded after incubating 5 µM probes with 0.5 mg/mL tubulin in General Tubulin Buffer, 0.5 mg/mL BSA in PBS and in 0.1% SDS in PBS, only in PBS at 37°C for 4h. Absorbance at the maximum (652 nm) and integration of fluorescence spectra (between 610 and 850 nm) are represented as averages of three independently repeated experiments (N=3) with standard deviations.

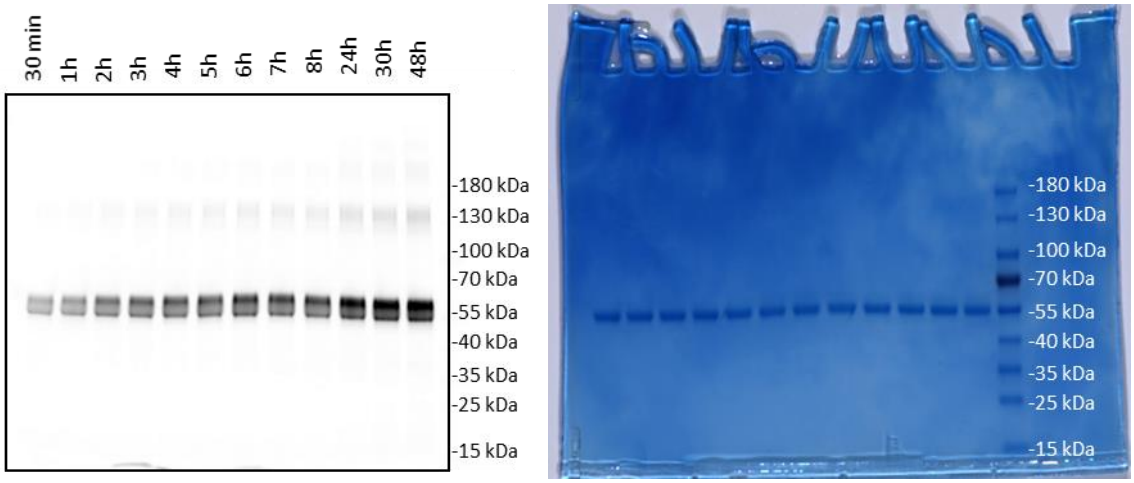
a)



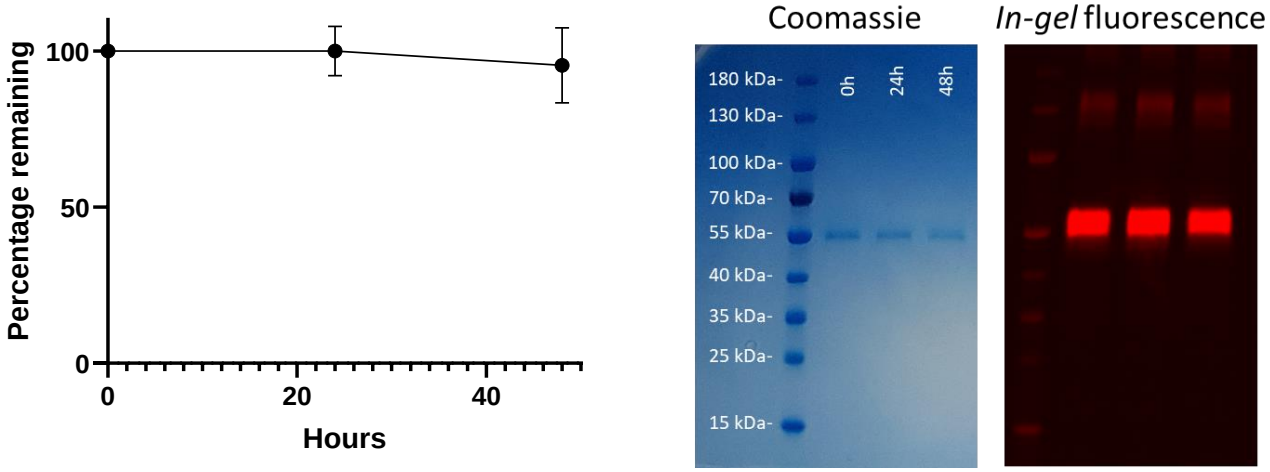
b)



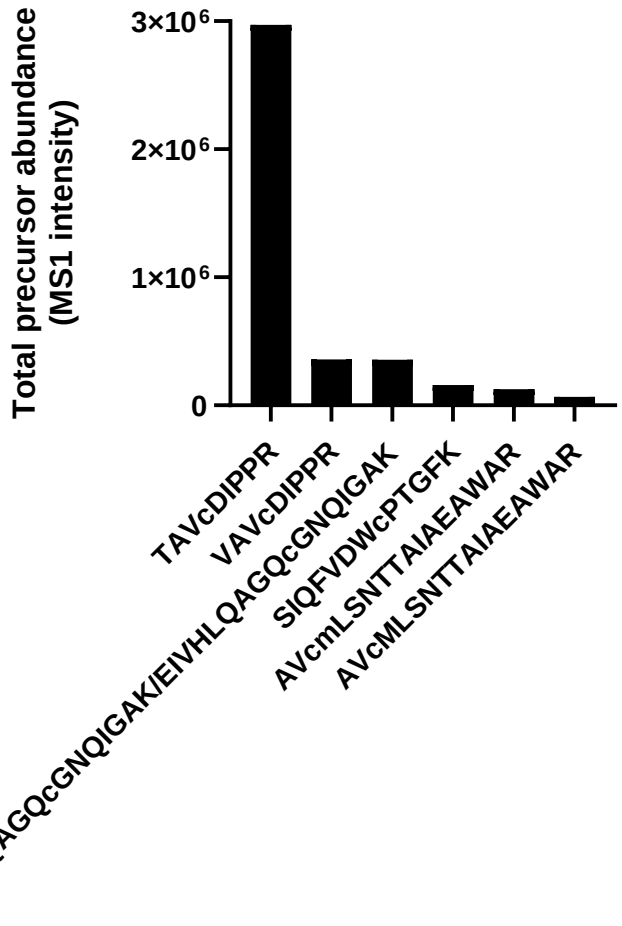
Supplementary Figure 4. Stability of covalent tubulin probes in PBS pH=7.4 at 37°C. Solution of covalent probes (20 µM in PBS) were heated at 37°C in the dark. Solutions were analyzed after 0, 24h and 48h by LC/MS. (**a**) The percentage of compound remaining over time according is plotted. (**b**) Examples of degradation products observed after 48h.



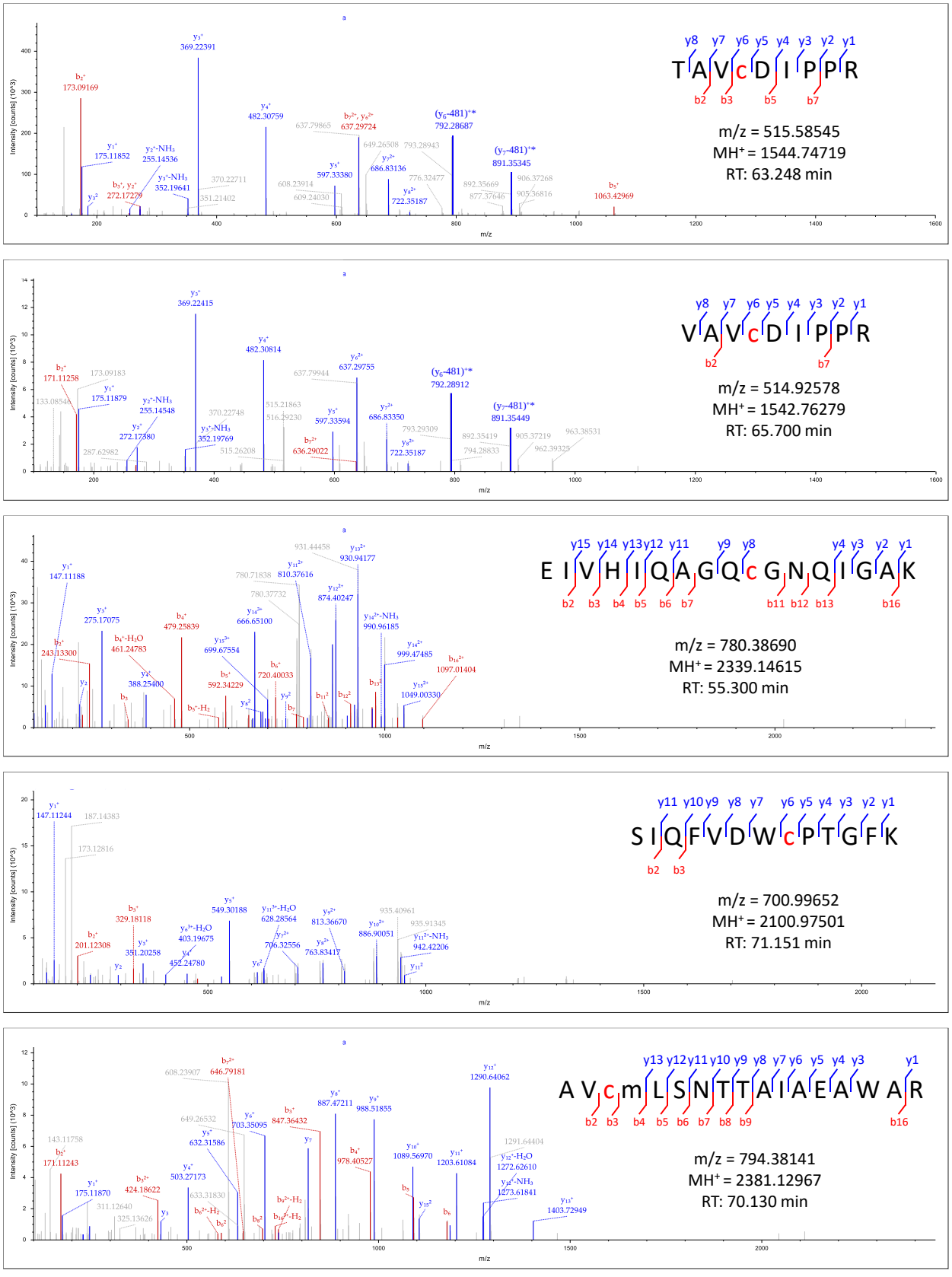
Supplementary Figure 5. Full representative *in-gel* fluorescence and Coomassie staining for kinetic (Figure 4a&b).



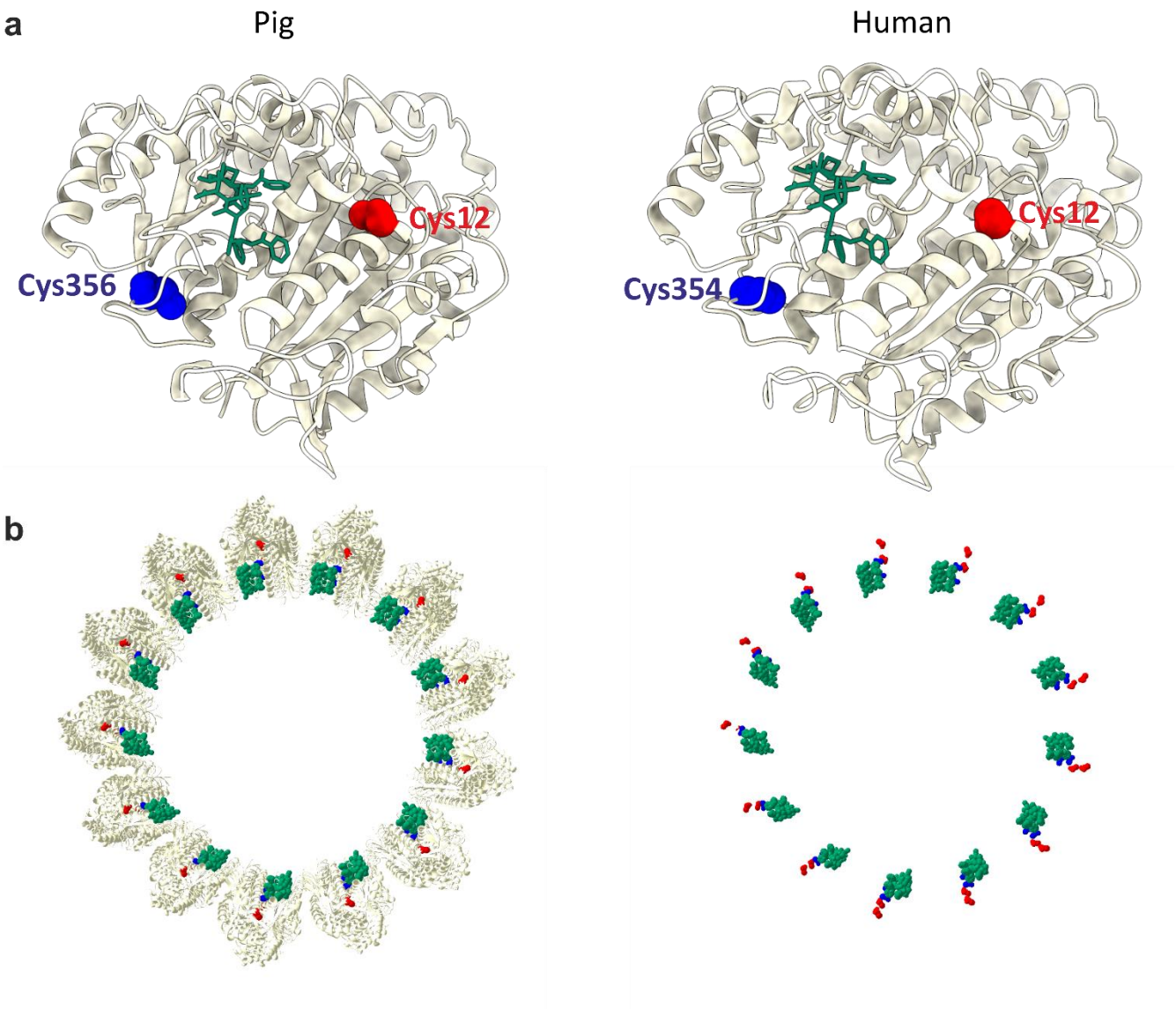
Supplementary Figure 6. Stability of the covalent bond between the dye and tubulin in PBS pH=7.4 at 37°C. The experiment was performed in triplicate (N=3) to obtain mean and standard deviation values (shown as error bars).



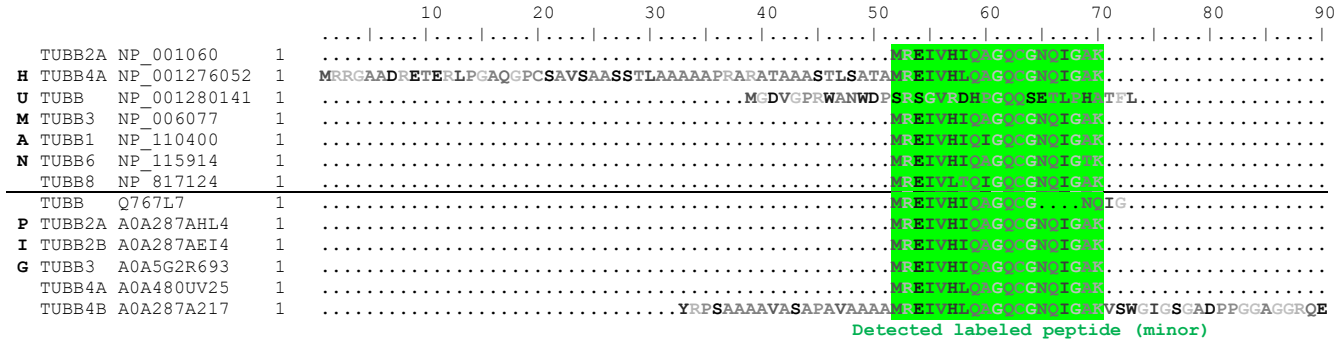
Supplementary Figure 7. Identified labeled peptides after *in-gel* analysis of SiR-labeled tubulin. c is the modified cysteine. m is an oxidized methionine.

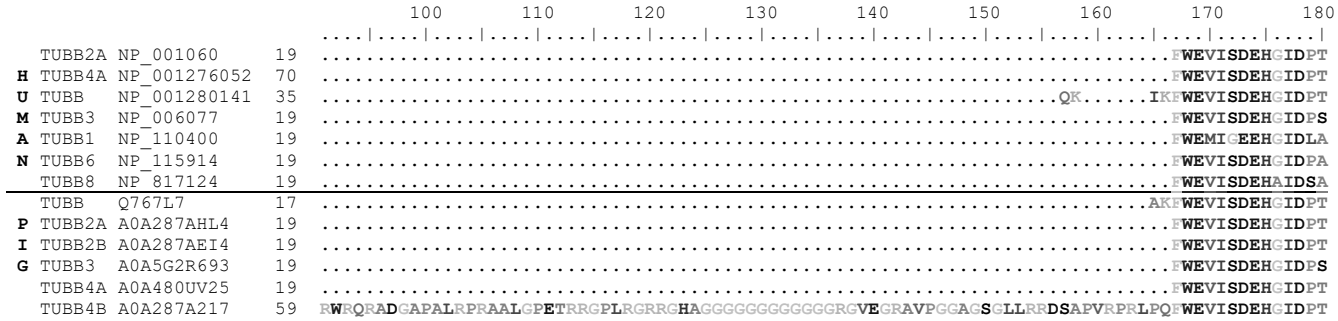


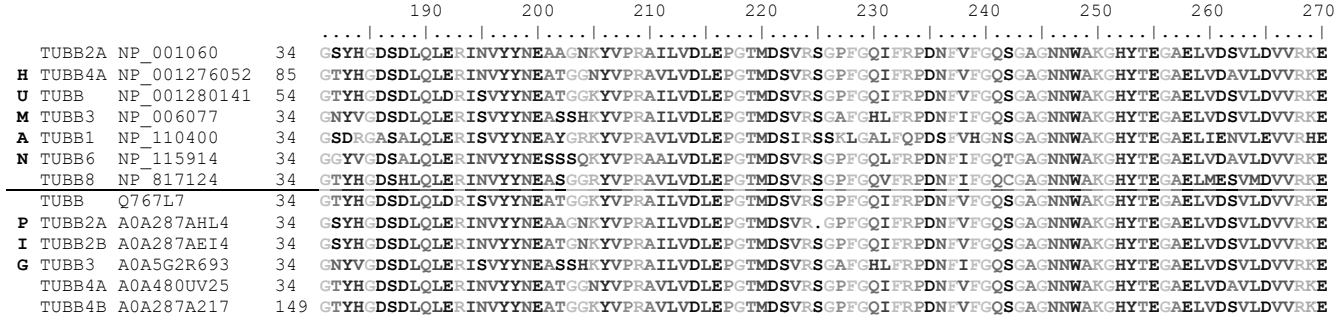
Supplementary Figure 8. MS spectra of identified labeled peptides. c is the modified cysteine. m is an oxidized methionine. Ions marked with a star (*) correspond to fragment ions with a mass loss of 481 Da, which may be caused by fragmentation of the label.

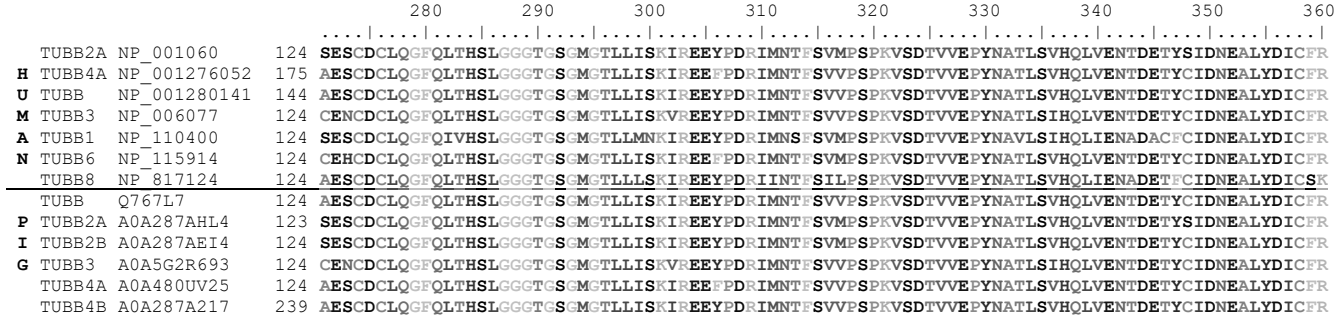


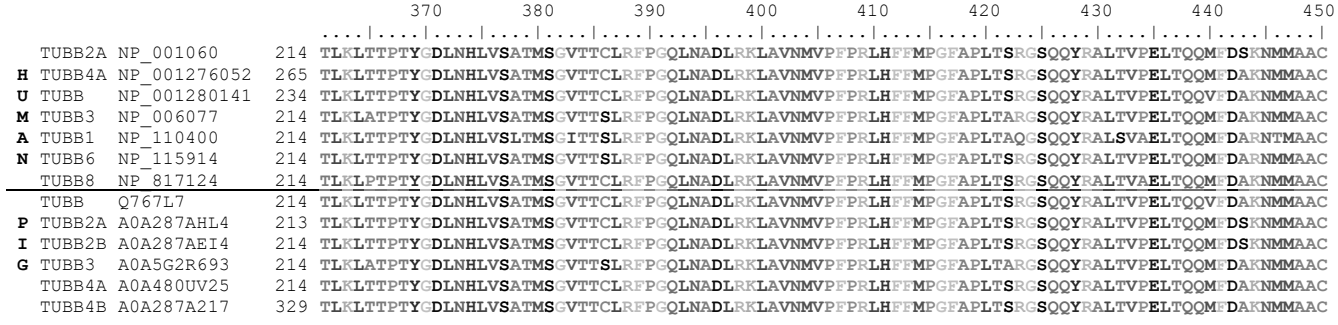
Supplementary Figure 9. Structural models of tubulin taxane binding sites and labeled Cys residues. (**a**) Comparison of cryo-EM β-tubulin complex with taxol from pig (PDB: 5SYF) and model of human β-tubulin complex with taxol (model is based on cryo-EM structure PDB: 6E7C). (**b**) Microtubule-taxol complex model (PDB: 5SYF). Left panel shows top view of microtubule composed of tubulin dimers, taxol and the main labeling site (Cys356). Right panel shows same view as on left, but protein molecules are omitted. Protein molecule is shown in white, Taxol is green, the main labeling site (Cys356 or Cys354) is highlighted in blue, and the minor labeling site (Cys12) in red. GTP, GDP and Mg^{2+} molecules are omitted for clarity.

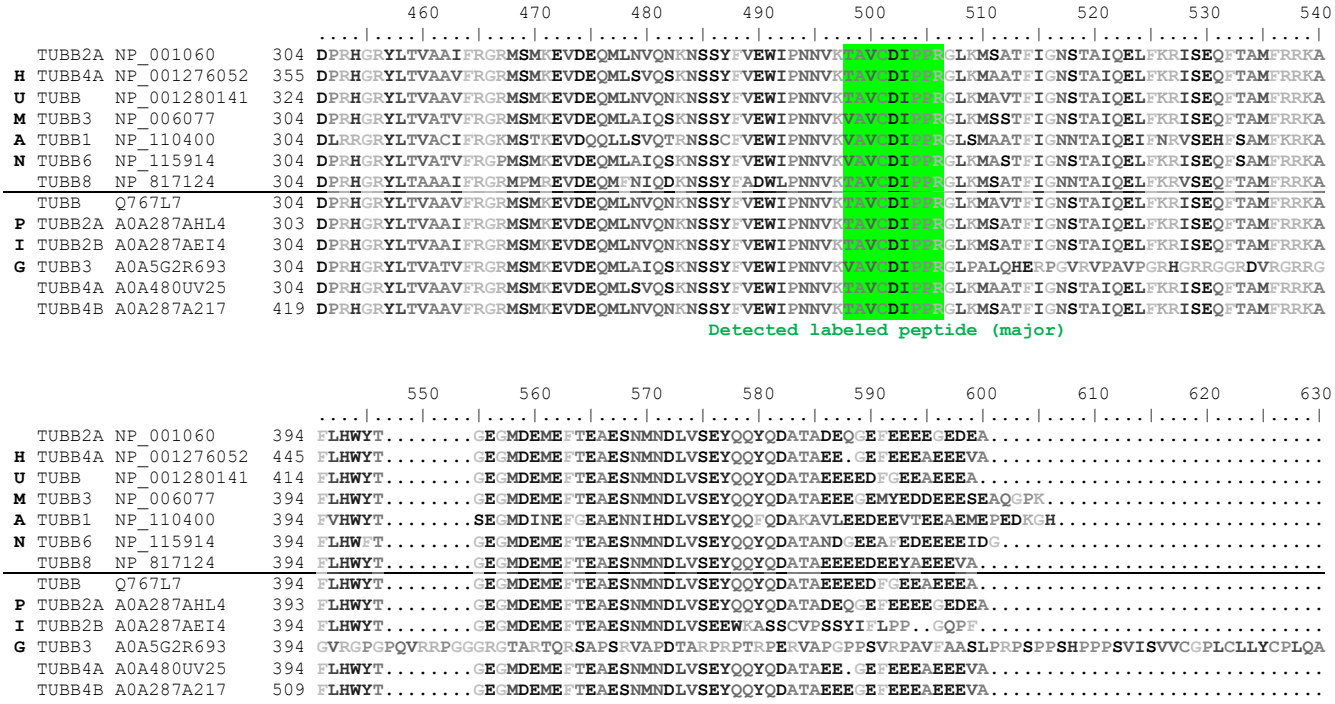

Detected labeled peptide (minor)



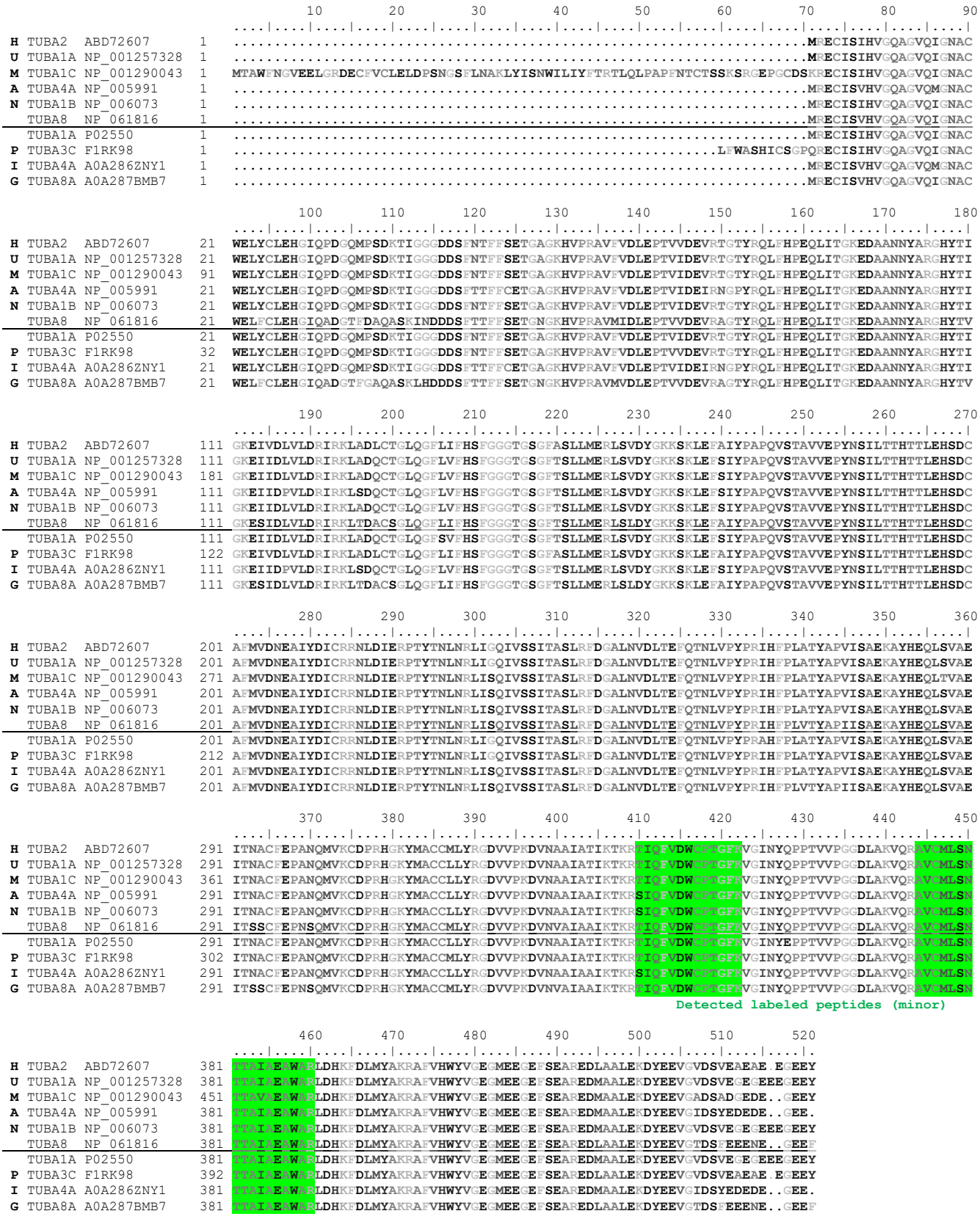




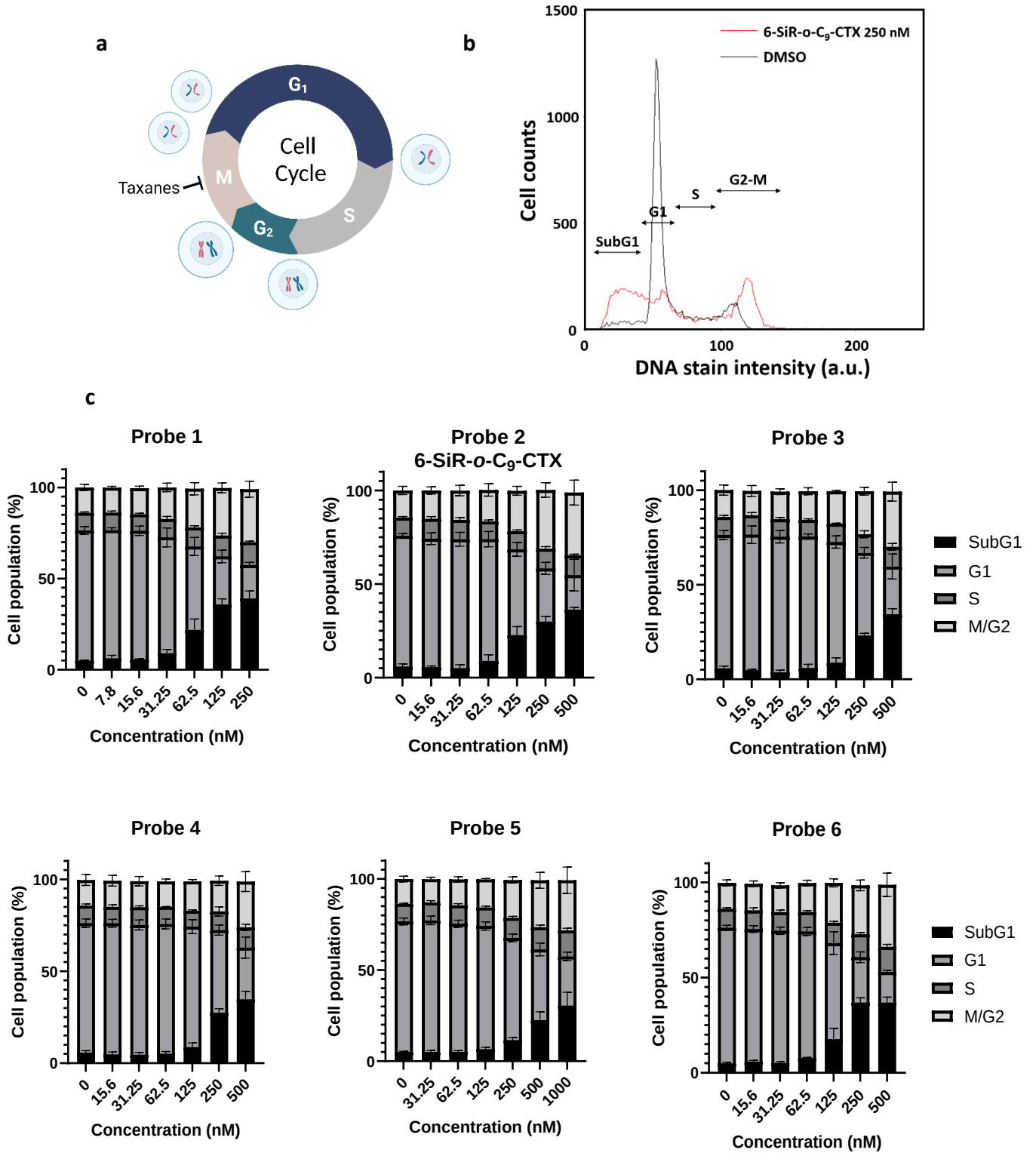




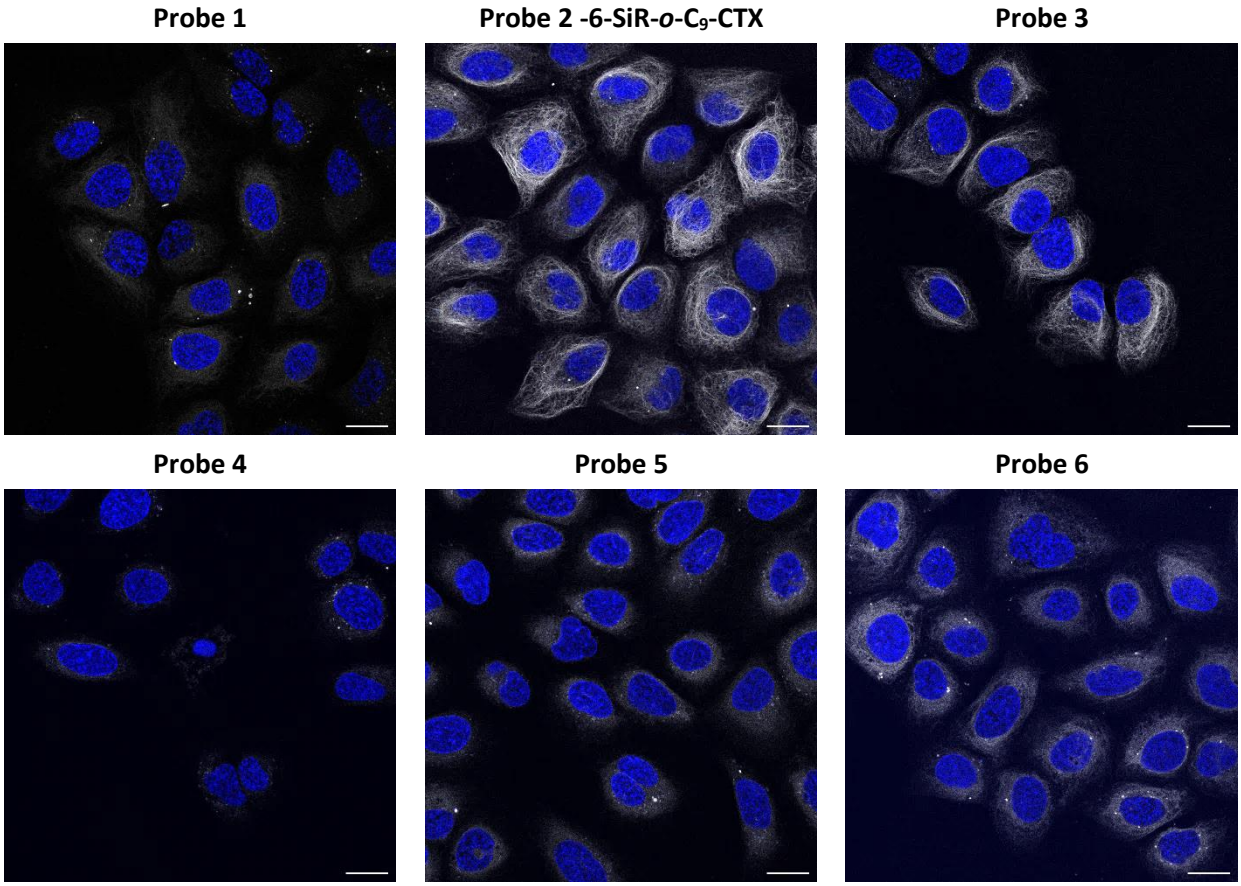
Supplementary Figure 10. Sequence alignment of β-tubulin isotypes from human (Homo sapiens) and pig (Sus scrofa). Labeled peptides detected in the tubulin sample are marked green.



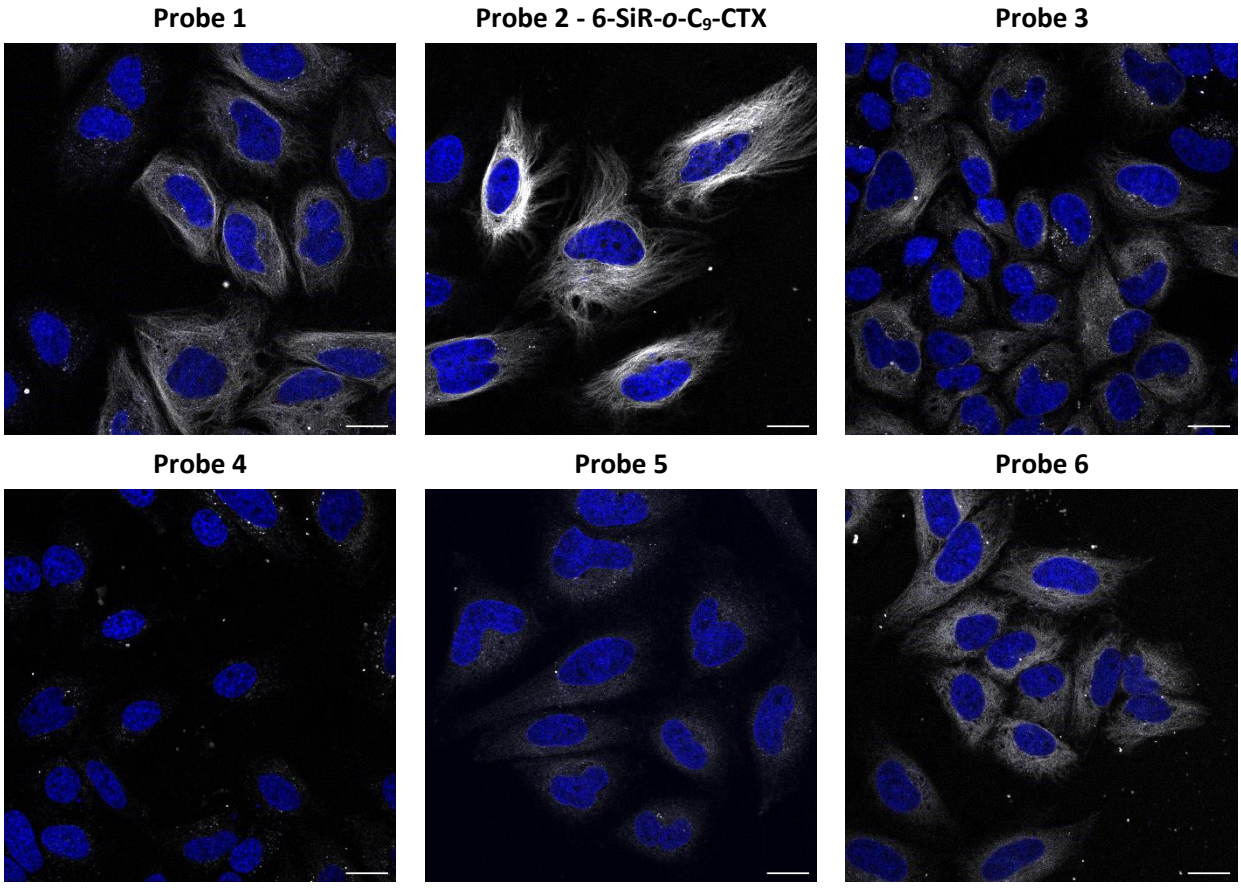
Supplementary Figure 11. Sequence alignment of α-tubulin isotypes from human (Homo sapiens) and pig (Sus scrofa). Labeled peptides detected in the tubulin sample are marked green.



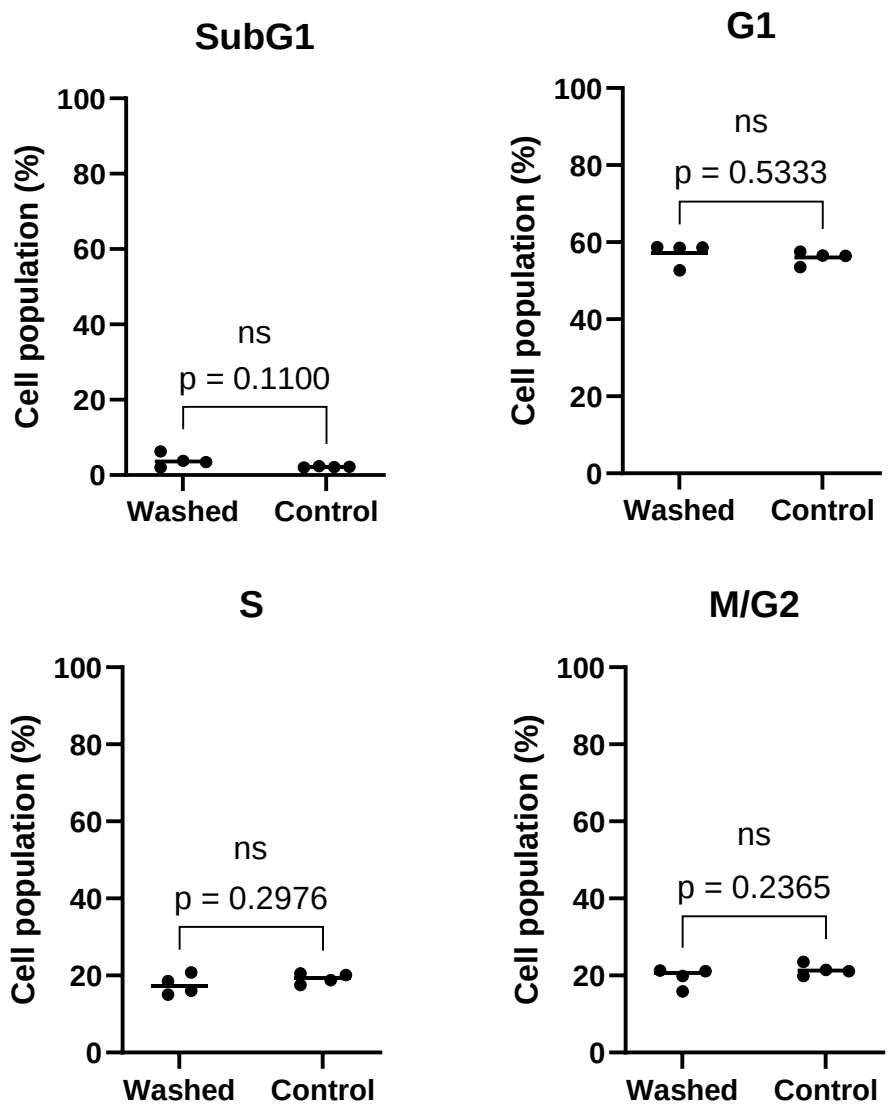
Supplementary Figure 12. Cell cycle perturbation induced by covalent tubulin probes. (**a**) Cytotoxicity of taxanes results from the inhibition of the cycle at the stage of mitosis. Created with BioRender.com. (**b**) Representative histogram of DNA content distribution in HeLa cells treated with DMSO or 250 nM of **6-SiR-*o*-C$_9$-CTX** for 24h. The cell cycle phases are identified by the amount of DNA per cell. (**c**) Cytotoxicity measurements of tubulin probes. Experimental data are averages of three independent experiments (N=3) and presented as means with standard deviations.



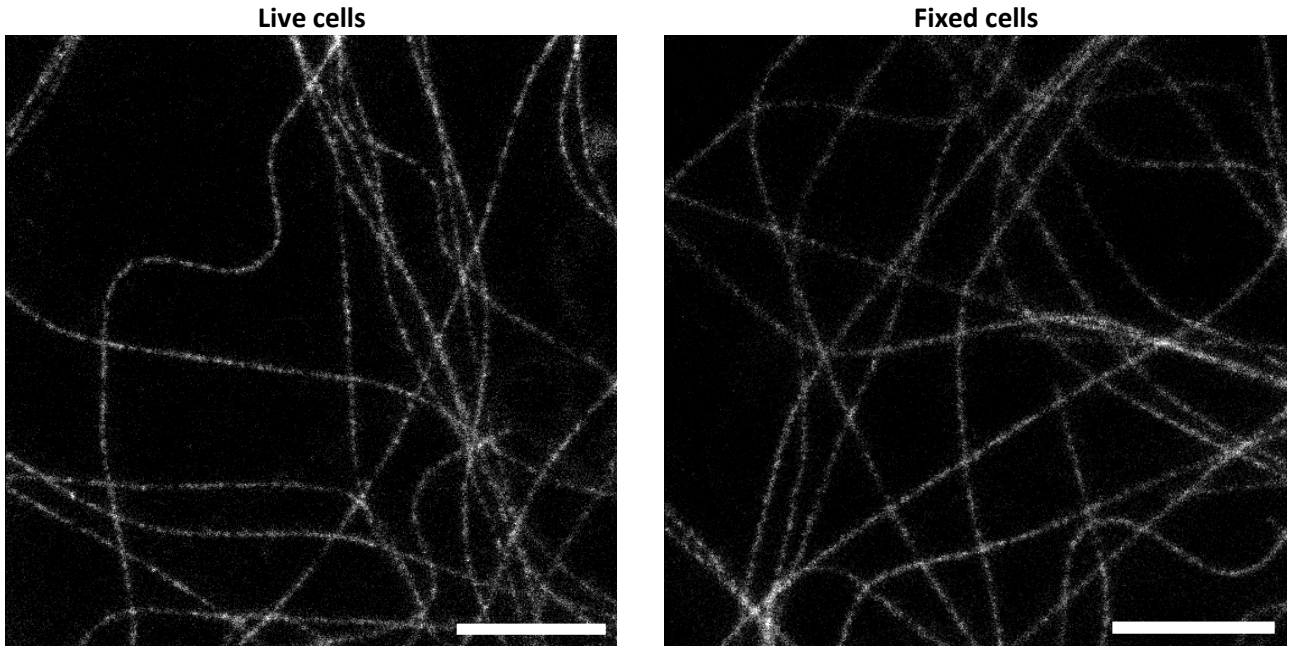
Supplementary Figure 13. Live U-2 OS cells stained with probes (1 µM in OptiMEM), verapamil (10 µM) and Hoechst 33342 (1 µg/mL) for 4h. Confocal microscopy images were acquired using LEICA SP8. Gray channel (λ_{ex}= 633 nm, λ_{em}= 650-710 nm) corresponds to probe staining and blue channel (λ_{ex}= 405 nm, λ_{em}= 415-480 nm) corresponds to Hoechst 33342 staining. Scale bar = 20 µm.



Supplementary Figure 14. Live HeLa CCL cells stained with probes (1 µM in OptiMEM) and Hoechst 33342 (1 µg/mL) for 4h. Confocal microscopy images were acquired using LEICA SP8. Gray channel (λ_{ex}= 633 nm, λ_{em}= 650-710 nm) corresponds to probe staining and blue channel (λ_{ex}= 405 nm, λ_{em}= 415-480 nm) corresponds to Hoechst 33342 staining. Scale bar = 20 µm.



Supplementary Figure 15. Statistical analysis associated with the cell cycle analysis of washing experiment. Data from four different experiments (N=4). The line corresponds to the mean and each dot corresponds to the result of one single experiment. Unpaired t test was performed between the 2 conditions.



Supplementary Figure 16. Representative images used to measure apparent microtubule FWHM. Human dermal fibroblasts cells incubated with **6-SiR-*o*-C_9-CTX** (1 µM in OptiMEM) for 4h, imaged live or after fixation with glutaraldehyde. Images acquired with Abberior Expert Line. Scale bar = 2 µm.

Supplementary Tables

	$\lambda_{abs,max}$ (nm)	$\lambda_{em,max}$ (nm)	QY PBS+0.1 % SDS (%)	Fluorescence lifetime PBS+0.1 % SDS (ns)	Percentage dye open Tubulin/PBS+0.1% SDS (%)	FI increase Tubulin/PBS
Probe 1	652	672	59.1 ± 0.5	3.94 ± 0.01	40.0 ± 2.6	7.0 ± 2.2
Probe 2 – 6-SiR-*o*-C_9-CTX			59.3 ± 1.4	3.93 ± 0.01	49.0 ± 10.8	13.7 ± 1.3
Probe 3			60.3 ± 3.2	3.94 ± 0.00	33.3 ± 5.0	8.8 ± 2.8
Probe 4			59.4 ± 1.3	3.95 ± 0.01	14.3 ± 8.0	4.1 ± 2.4
Probe 5			59.4 ± 1.1	3.93 ± 0.02	25.7 ± 9.0	1.3 ± 0.5
Probe 6			58.9 ± 1.2	3.90 ± 0.01	17.0 ± 10.1	5.6 ± 2.9

Supplementary Table 1. Photophysical properties of the probes (1-6). Experimental data are averages of three independent experiments (N=3) and presented as means with standard deviations.

Labeled peptide	Tubulin isotypes and isoforms	Total precursor abundance (MS1 intensity)
TAVcDIPPR	TUBB2A, TUBB2B, TUBB4A, TUBB4B, TUBB	$2{,}97.10^6$
VAVcDIPPR	TUBB3	$3{,}59.10^5$
EIVHIQAGQcGNQIGAK	TUBB2A, TUBB2B, TUBB, TUBB3	$3{,}57.10^5$
EIVHLQAGQcGNQIGAK	TUBB4A, TUBB4B	
SIQFVDWcPTGFK	LOC100158003, LOC100127131, TUBA4A	$1{,}59.10^5$
AVcmLSNTTAIAEAWAR	LOC100158003, LOC100127131, TUBA3C, TUBA1A, TUBA4A, TUBA8	$1{,}25.10^5$
AVcMLSNTTAIAEAWAR	LOC100158003, LOC100127131, TUBA3C, TUBA1A, TUBA4A, TUBA8	$6{,}63.10^4$

Supplementary Table 2. Identified labeled peptides in the sample. Reacted Cys residue is marked in red. m is an oxidized methionine.

Probe name	Cytotoxicity threshold (nM)
Probe 1	31.25
Probe 2 – 6-SiR-*o*-C_9-CTX	125
Probe 3	250
Probe 4	250
Probe 5	250
Probe 6	125

Supplementary Table 3. Cytotoxicity threshold of the probes (1-6). The indicated probe concentration represents the lowest concentration tested, at which a cytotoxicity effect was observed.

Figure	Probe	Cell line	Microscope	Objective	Excitation (%)/ STED (%)	Pixel dwell time (µs)	Pixel size (nm)	Emission (nm)	Comment
Fig. 3a	1,2,3,4,5,6	H. fibro	Leica TCS SP8	63x 1.40	633 (1)	0.6	90	650-710	
Supplementary Fig. 13	1,2,3,4,5,6	U-2 OS							
Supplementary Fig. 14	1,2,3,4,5,6	HeLa CCL							
Fig. 3b	2	U-2 OS HeLa CCL							
Fig. 4b	2, SiR-CTX	U-2 OS							
Fig. 5a	2	HeLa CCL (live)	Abberior Expert Line	100x 1.40	640 (10)/775(25)	10	20	650-720	
		HeLa CCL (fixed)			640 (5)/775(60)	4	20	650-720	Line accum.: 2
		U-2 OS (live)			640 (10)/775(25)	10	20	650-720	
		U-2 OS (fixed)			640 (10)/775(70)	4	20	650-720	Line accum.: 2
		H. fibro (live)			640 (17)/ 775(40)	3	20	650-720	
Fig. 5b	2	H. fibro (fixed)	Abberior Expert Line	100x 1.40	640 (10)/775(70)	4	20	650-720	Line accum.: 2
Fig. 5d	2	H. fibro (fixed)	Abberior Expert Line	100x 1.40	640 (10)/775(75)	1	15	650-720	Line accum.: 3
Supplementary 16	2	H. fibro (live)			640 (15)/775(75)	1	15	650-720	Line accum.: 3
Movie 2	2	H. fibro (live)	Abberior Facility Line	60x 1.40	640 (5)/775(10)	5	30	660-750	One frame every 25 sec

Supplementary Table 4. Acquisition parameters for microscopy images

Supplementary Methods

Determination of absolute quantum yields

All reported absolute fluorescence quantum yield values (Φ) were measured using a Quantaurus-QY spectrometer (model C11374-01, Hamamatsu Photonics). This instrument uses an integrating sphere to determine photons absorbed and emitted by a sample. Measurements were carried out using dilute samples in air saturated solvents at 25°C at concentrations ranging from 10^{-6} to 10^{-7} M (A < 0.1 as indicated in the user's manual) and by using 3 mL quartz cuvettes (Hamamatsu Photonics Art. No. A10095-02) provided by the instrument supplier. The fluorescence quantum yields were measured in PBS buffer containing 0.1% SDS. Reported values are averages (N = 3) with standard deviation.

Determination of fluorescence lifetimes

The fluorescence decay characteristics of the solution samples in PBS containing 0.1% SDS at concentrations ranging from 10^{-6} to 10^{-7} M were recorded using a fluorescence lifetime measurement system (Quantaurus-Tau, Hamamatsu Photonics) in 3 mL high performance quartz glass cuvettes (Hellma Analytics Art. No. 101-10-K-40). The decay profile was registered for 53 ns interval after excitation and the experiment was continued until 10 000 peak count was reached. The instrument response function was obtained by using diluted LUDOX® TM-50 colloidal silica (Sigma Aldrich, # 420778). The analysis of the obtained fluorescence decay profile was performed using the instrument software. Reported values are averages (N = 3) with standard deviation.

Maintenance and preparation of the cells

Human primary dermal fibroblasts (Lonza, #CC-2511) were cultured in high-glucose DMEM (Thermo Fisher, #31053044) with 10% FBS (Thermo Fisher, #10082147) supplemented with 1 mM Sodium pyruvate (Sigma, #S8636), 1% GlutaMax (Thermo Fisher, #35050038) and 1% Penicillin-Streptomycin (Sigma, #P0781) in a humidified 5% CO_2 incubator at 37 °C. The cells were split every 3-4 days or at confluence.

HeLa (ATCC, CCL-2) cells were cultured in high-glucose DMEM (Thermo Fisher, #31966047) with 10% FBS (BioSELL, #S0615) supplemented with 1% Penicillin-Streptomycin (Sigma, #P0781) in a humidified 5% CO_2 incubator at 37 °C. The cells were split every 3-4 days or at confluence.

U-2 OS cells (ATCC, HTB-96) were cultured in McCoys 5A medium (Thermo Fisher, #16600082) with 10% FBS (BioSELL, #S0615) supplemented with 1 mM Sodium pyruvate (Sigma, #S8636) and 1% of Penicillin-Streptomycin (Sigma #P0781) in a humidified 5% CO_2 incubator at 37 °C. The cells were split every 3-4 days or at confluence.

Confocal and STED microscopy experiments were performed using µ-Slide 8 Well Glass Bottom dishes (Ibidi, #80827).

Chemical Synthesis of the building blocks

Reagents were purchased as reagent-grade (from Sigma-Aldrich, BLD Pharm, Enamine, abcr, TCI or Aaron Chem) and used without further purification. 6-SiR-CO_2H was synthesized according to published procedure.[2] Flash chromatographies were carried out on Biotage® Selekt with Biotage® Sfär Silica HC (20 µm) columns.

NMR spectra were recorded at 25 °C with an Agilent 400-MR spectrometer at 400 MHz (^1H) and 101 MHz (^{13}C). Chemical shifts (δ), which are expressed in part per million (ppm), were determined relative to residual non-deuterated solvent as an internal reference: $CDCl_3$ (^{13}C NMR: δ = 77.16 ppm; ^1H NMR: δ = 7.26 ppm) and $(CD_3)_2SO$ (^{13}C NMR: δ = 39.52 ppm; ^1H NMR: δ = 2.50 ppm) or with an external reference (^{19}F: δ = 0 ppm for $CFCl_3$). Multiplicities of signals are described as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet or overlap of non-equivalent resonances; br = broad signal. Coupling constants (*J*) are given in Hz.

ESI-MS were recorded on a Varian 500-MS spectrometer (Agilent). ESI-HRMS were recorded on a MICROTOF spectrometer (Bruker) equipped with ESI ion source (Apollo) and direct injector with LC autosampler Agilent RR 1200.

Analytical LC–MS analysis was performed on an Agilent 1260 Infinity II LC/MS system equipped with an Autosampler (G7129A), Binary Pump (G7112B), Diode Array Detector WR (G7115A), Fluorescence Detector Spectra and Single Quadrupole MSD XT (G6135B). Analysis was done by using a Ascentis® Express AQ-C18 UHPLC Column 2 µm, 5 cm x 2.1 mm with Mobile phase A: 25 mM $HCOONH_4$ (pH = 3.5) aqueous buffer/ Mobile Phase B: MeOH and the following gradient (Flow: 0.4 mL/min, 40°C):

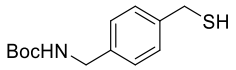
Time (min)	%B
0	40
2	40
8	100
9	100
10	40
15	40

Preparative HPLC was performed on a combined Agilent1260/1290 Infinity II preparative system equipped with a 1290 Infinity II open-bed sampler (G7169B)/fraction collector (G7159B), 1260 Infinity II multiple wavelength detector (G7165A) and with:

Device A: 1260 Infinity II preparative binary pump (G7161A) and Agilent 5 Prep-C_{18}, 5 µm, 100 x 50 mm preparative column.

Device B: 1290 Infinity II preparative binary pump (G7161B) and Agilent Pursuit 10 C_{18}, 10 µm, 250 X 50 mm preparative column.

Compound *p*-7, *tert*-butyl (4-(mercaptomethyl)benzyl)carbamate :



Chemical Formula: $C_{13}H_{19}NO_2S$
Molecular Weight: 253,36

***tert*-Butyl-4-(bromomethyl)benzylcarbamate (2.5 g, 8.32 mmol, 1.0 eq.)** and **thiourea (634 mg, 8.32 mmol, 1.0 eq.)** were refluxed together in **EtOH (21 mL, 0.4M)** for 2h.Water (1 mL) and **NaOH (664 mg, 16.6 mmol, 2.0 eq.)** were added. After 1h at 80°C, the mixture was diluted in water, the pH was lower to 2-3 using aqueous HCl 1M. The aqueous layer was extracted twice with AcOEt. Organic layers were combined, washed with brine and dried over Na_2SO_4. The crude was purified by flash chromatography on silica gel (120g, Hexanes/AcOEt from 90:10 to 60:40) to give the expected product as **a white solid (1.63 g, 6.44 mmol, η = 77 %**).

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.28 (d, *J* = 8.0 Hz, 2H), 7.23 (d, *J* = 8.0 Hz, 2H), 4.81 (br s, 1H), 4.29 (br s, 2H), 3.72 (d, *J* = 7.5 Hz, 2H), 1.74 (t, *J* = 7.5 Hz, 1H), 1.46 (s, 9H).

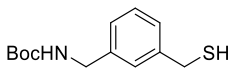
^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) = 156.0 (C_q), 140.4 (C_q), 137.9 (C_q), 128.4 (CH), 127.9 (CH), 79.7 (C_q), 44.5 (CH_2), 28.8 (CH_2), 28.6 (CH_3).

ESI-MS, positive mode : m/z = 276.1 $[M+Na]^+$

ESI-MS, negative mode : m/z = 252.1 $[M-H]^-$

HRMS (ESI) calculated for $C_{13}H_{19}NO_2SNa^+$ $[M+Na]^+$: 276.1029, found: 276.1031.

Compound *m*-7, *tert*-butyl (3-(mercaptomethyl)benzyl)carbamate:



Chemical Formula: $C_{13}H_{19}NO_2S$
Molecular Weight: 253,36

***tert*-Butyl-3-(bromomethyl)benzylcarbamate (2.0 g, 6.66 mmol, 1.0 eq.)** and **thiourea (507 mg, 6.66 mmol, 1.0 eq.)** were refluxed together in **EtOH (17 mL, 0.4 M)** for 2h. **Water (1 mL)** and **NaOH (533 mg, 13.3 mmol, 2.0 eq.)** were added. After 1h at 80°C, the mixture was diluted in water, the pH was lower to 2-3 using aqueous HCl 1M. The aqueous layer was extracted twice with AcOEt. Organic layers were combined, washed with brine and dried over Na_2SO_4. The crude was purified by flash chromatography on silica gel (50 g, Hexanes/AcOEt from 100:0 to 80:20) to give the expected product as **white crystals (1450 mg, 5.72 mmol, η=86%).**

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.35 – 7.17 (m, 3H), 7.17 – 7.13 (m, 1H), 4.90 (s, 1H), 4.27 (s, 2H), 3.70 (d, *J* = 7.5 Hz, 2H), 1.75 (t, *J* = 7.5 Hz, 1H), 1.45 (s, 9H).

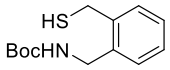
^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) = 156.0 (C_q), 141.5 (C_q), 139.5 (C_q), 129.0 (CH), 127.1 (CH), 127.0 (CH), 126.2 (CH), 79.6 (C_q), 44.7 (CH_2), 28.9 (CH_2), 28.5 (CH_3).

ESI-MS, positive mode : m/z = 276.1 $[M+Na]^+$

ESI-MS, negative mode : m/z = 252.1 $[M-H]^-$

HRMS (ESI) calculated for $C_{13}H_{20}NO_2S^+$ $[M+H]^+$: 254.1209, found: 254.1213.

Compound *o*-7, *tert*-butyl (2-(mercaptomethyl)benzyl)carbamate:



Chemical Formula: $C_{13}H_{19}NO_2S$
Molecular Weight: 253,36

***tert*-Butyl-2-(bromomethyl)benzylcarbamate (2.0 g, 6.66 mmol, 1.0 eq.)** and **thiourea (507 mg, 6.66 mmol, 1.0 eq.)** were refluxed together in **EtOH (17 mL, 0.4M)** for 2h. Water (1 mL) **and NaOH (533 mg, 13.3 mmol, 2.0 eq.)** were added. After 1h at 80°C, the mixture was diluted in water, the pH was lower to 2-3 using aqueous HCl 1M. The aqueous layer was extracted twice with AcOEt. Organic layers were combined, washed with brine and dried over Na_2SO_4. The crude was purified by flash chromatography on silica gel (80 g, Hexanes/AcOEt from 100:0 to 80:20) to give the expected product as **white crystals (1500 mg, 5.92 mmol, η=90%).**

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.46 – 7.15 (m, 4H), 4.96 (s, 1H), 4.42 (s, 2H), 3.78 (d, *J* = 7.0 Hz, 2H), 1.79 (t, *J* = 7.0 Hz, 1H), 1.47 (s, 9H).

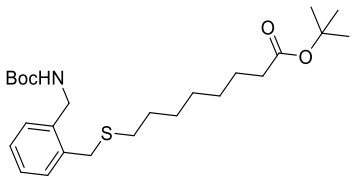
^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) = 155.8 (C_q), 139.2 (C_q), 136.3 (C_q), 129.5 (CH), 129.2 (CH), 128.1 (CH), 127.8 (CH), 79.7 (C_q), 42.1 (CH_2), 28.5 (CH_3), 26.1 (CH_2).

ESI-MS, positive mode : m/z = 276.1 $[M+Na]^+$

ESI-MS, negative mode : m/z = 252.1 $[M-H]^-$

HRMS (ESI) calculated for $C_{13}H_{20}NO_2S^+$ $[M+H]^+$: 254.1209, found: 254.1219.

Compound 8:



Chemical Formula: $C_{25}H_{41}NO_4S$
Molecular Weight: 451,66

Under Ar atmosphere, ***o*-7 (300 mg, 1.18 mmol, 1.0 eq.)** and **potassium carbonate (546 mg, 2.37 mmol, 2.0 eq.)** were mixed together in **dry DMF (12 mL, 0.1M). *Tert*-butyl 8-bromooctanoate (497 mg, 1.78 mmol, 1.5 eq.)** was added and the solution was stirred at rt. After 18h, water (25 mL) and AcOEt (30 mL) were added. The aqueous layer was extracted

twice with AcOEt. Organic layers were combined, was washed with brine and dried over Na_2SO_4. The crude was purified by flash column chromatography on silica gel (10g, Hexanes/AcOEt from 100:0 to 85:15) to give the expected product as a colorless oil **(493 mg, 1.09 mmol, η = 92%)**.

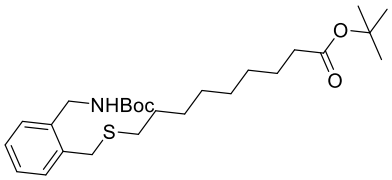
^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.34 – 7.30 (m, 1H), 7.25 – 7.17 (m, 3H), 5.08 (s, 1H), 4.41 (s, 2H), 3.73 (s, 2H), 2.49 – 2.40 (m, 2H), 2.18 (t, *J* = 7.5 Hz, 2H), 1.63 – 1.51 (m, 4H), 1.44 (s, 9H), 1.43 (s, 9H), 1.36 – 1.20 (m, 6H).

^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) = 173.3 (C_q), 155.9 (C_q), 137.2 (C_q), 136.1 (C_q), 130.3 (CH), 129.3 (CH), 127.7 (CH), 127.5 (CH), 80.0 (C_q), 79.5 (C_q), 42.1 (CH_2), 35.6 (CH_2), 33.9 (CH_2), 29.3 (CH_2), 29.0 (CH_2), 29.0 (CH_2), 28.8 (CH_2), 28.5 (CH_3), 28.2 (CH_3), 25.1 (CH_2).

ESI-MS, positive mode : m/z = 452.2 $[M+H]^+$

HRMS (ESI) calculated for $C_{25}H_{42}NO_4S^+$ $[M+H]^+$: 452.2829, found: 452.2841.

Compound 9:



Chemical Formula: $C_{26}H_{43}NO_4S$
Molecular Weight: 465,69

Under Ar atmosphere, ***o*-7 (150 mg, 0.59 mmol, 1.0 eq.)** and **potassium carbonate (163 mg, 1.18 mmol, 2.0 eq.)** were mixed together in **dry DMF (6 mL, 0.1M). *Tert*-butyl 9-bromononanoate (249 mg, 0.89 mmol, 1.5 eq.)** was added and the solution was stirred at rt for 24h. AcOEt was added (20 mL). The organic layer was washed once with brine and dried over Na_2SO_4. The crude was purified by flash column chromatography on silica gel (10g, Hexanes/AcOEt from 100:0 to 85:15) to give the expected product as a colorless oil **(217 mg, 0.592 mmol, η = 79%)**.

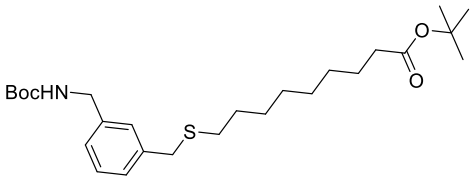
^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.35 – 7.31 (m, 1H), 7.26 – 7.17 (m, 3H), 5.06 (s, 1H), 4.42 (s, 2H), 3.74 (s, 2H), 2.49 – 2.42 (m, 2H), 2.19 (t, J = 7.5 Hz, 2H), 1.62 – 1.53 (m, 4H), 1.45 (s, 9H), 1.44 (s, 9H), 1.38 – 1.23 (m, 8H).

^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) = 173.4 (C_q), 155.9 (C_q), 137.3 (C_q), 136.2 (C_q), 130.4 (CH), 129.4 (CH), 127.8 (CH), 127.5 (CH), 80.0 (C_q), 79.6 (C_q), 42.2 (CH_2), 35.7 (CH_2), 33.9 (CH_2), 32.1 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 29.0, (CH_2) 28.6 (CH_3), 28.3 (CH_3), 25.2 (CH_2).

ESI-MS, positive mode : m/z = 466.2 $[M+H]^+$

HRMS (ESI) calculated for $C_{26}H_{44}NO_4S^+$ $[M+H]^+$: 466.2986, found: 466.2988.

Compound 10:



Chemical Formula: $C_{26}H_{43}NO_4S$
Molecular Weight: 465,69

Under Ar atmosphere, ***m*-7 (300 mg, 1.18 mmol, 1.0 eq.)** and **potassium carbonate (163 mg, 1.18 mmol, 2.0 eq.)** were mixed together in **dry DMF (6 mL, 0.1M). Tert-butyl 9-bromononanoate (249 mg, 0.79 mmol, 1.5 eq.)** was added and the solution was stirred at rt for 24h. AcOEt was added (20 mL). The organic layer was washed once with brine and dried over Na_2SO_4. The organic layer was washed once with brine and dried over Na_2SO_4. The crude was purified by flash column

chromatography on silica gel (10g, Hexanes/AcOEt from 100:0 to 85:15) to give the expected product as a colorless oil **(256 mg, η = 93%)**.

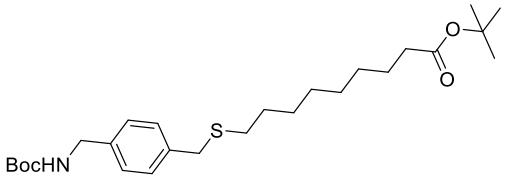
^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.27 – 7.11 (m, 4H), 4.89 (s, 1H), 4.27 (s, 2H), 3.66 (s, 2H), 2.42 – 2.35 (m, 2H), 2.17 (t, J = 7.5 Hz, 2H), 1.60 – 1.49 (m, 4H), 1.44 (s, 9H), 1.42 (s, 9H), 1.38 – 1.21 (m, 8H).

^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) = 173.3 (C_q), 156.0 (C_q), 139.3 (C_q), 139.1 (C_q), 128.8 (CH), 127.9 (CH), 127.9 (CH), 126.1 (CH), 80.0 (C_q), 79.6 (C_q), 44.7 (CH_2), 36.3 (CH_2), 35.7 (CH_2), 31.6 (CH_2), 29.2 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 29.1 (CH_2), 28.9 (CH_2), 28.5 (CH_3), 28.2 (CH_3), 25.1 (CH_2).

ESI-MS, positive mode : m/z = 466.3 $[M+H]^+$

HRMS (ESI) calculated for $C_{26}H_{44}NO_4S^+$ $[M+H]^+$: 466.2986, found: 466.2992.

Compound 11:



Chemical Formula: $C_{26}H_{43}NO_4S$
Molecular Weight: 465,69

Under Ar atmosphere, ***p*-7 (150 mg, 0.59 mmol, 1.0 eq.)** and **potassium carbonate (163 mg, 1.18 mmol, 2.0 eq.)** were mixed together in **dry DMF (6 mL, 0.1M). Tert-butyl 9-bromononanoate (249 mg, 0.89 mmol, 1.5 eq.)** was added and the solution was stirred at rt for 24h. AcOEt was added (20 mL). The organic layer was washed once with brine and dried over Na_2SO_4. The crude was purified by flash column chromatography on silica gel (10g, Hexanes/AcOEt from 100:0 to 80:20) to give the expected product as a colorless oil **(227 mg, 0.59 mmol, η = 82%)**.

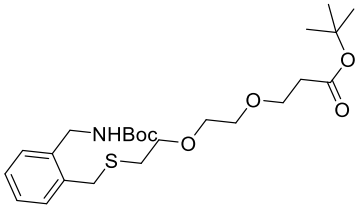
^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.22 (d, *J* = 8.0 Hz, 2H), 7.17 (d, *J* = 8.0 Hz, 2H), 4.95 (s, 1H), 4.24 (s, 2H), 3.63 (s, 2H), 2.38 – 2.31 (m, 2H), 2.15 (t, *J* = 7.5 Hz, 2H), 1.56 – 1.47 (m, 4H), 1.42 (s, 9H), 1.41 (s, 9H), 1.32 – 1.20 (m, 8H).

^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) = 173.2 (C_q), 155.9 (C_q), 137.7 (C_q), 137.6 (C_q), 129.0 (CH), 127.5 (CH), 79.9 (C_q), 79.4 (C_q), 44.4 (CH_2), 35.9 (CH_2), 35.6 (CH_2), 31.3 (CH_2), 29.1 (CH_2), 29.1 (CH_2), 29.0 (CH_2), 29.0 (CH_2), 28.8 (CH_2), 28.4 (CH_3), 28.1 (CH_3), 25.0 (CH_2).

ESI-MS, positive mode : m/z = 466.2 $[M+H]^+$

HRMS (ESI) calculated for $C_{26}H_{43}NO_4SNa^+$ $[M+Na]^+$: 488.2805, found: 488.2804.

Compound 12:



Chemical Formula: $C_{24}H_{39}NO_6S$
Molecular Weight: 469,63

Under Ar atmosphere, ***o*-7 (100 mg, 0.39 mmol, 1.0 eq.)** and **potassium carbonate (108 mg, 0.78 mmol, 2.0 eq.)** were mixed together in **dry DMF (4 mL, 0.1M). Bromo-PEG_2- *tert*-butyl ester (129 mg, 0.43 mmol, 1.1 eq.)** was added and the solution was stirred at rt for 18h. AcOEt (20 mL) was added. The organic layer was washed twice with brine and dried over Na_2SO_4. The crude was purified by flash column chromatography on silica gel (10g, Hexanes/AcOEt from 70:30 to 0:100) to give the expected product as a colorless oil **(140 mg, 0.30 mmol, η = 76 %)**.

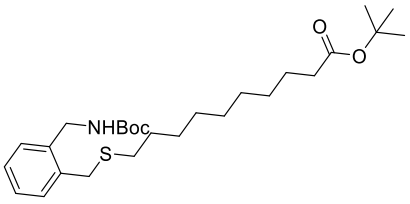
^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.36 – 7.32 (m, 1H), 7.27 – 7.17 (m, 3H), 4.43 (d, *J* = 3.5 Hz, 2H), 3.84 (s, 2H), 3.71 (t, *J* = 6.5 Hz, 2H), 3.65 – 3.56 (m, 6H), 2.66 (t, *J* = 6.5 Hz, 2H), 2.50 (t, *J* = 6.5 Hz, 2H), 1.46 (s, 9H), 1.44 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) = 171.0 (C_q), 155.9 (C_q), 137.4 (C_q), 136.0 (C_q), 130.5 (CH), 129.5 (CH), 127.9 (CH), 127.6 (CH), 80.7 (C_q), 79.7 (C_q, from HMBC), 71.2 (CH_2), 70.5 (CH_2), 70.4 (CH_2), 67.0 (CH_2), 42.1 (CH_2), 36.4 (CH_2), 34.3 (CH_2), 31.3 (CH_2), 28.6 (CH_3), 28.2 (CH_3).

ESI-MS, positive mode : m/z = 470.2 $[M+H]^+$

HRMS (ESI) calculated for $C_{24}H_{39}NO_6SNa^+$ $[M+Na]^+$: 492.2390, found: 492.2392.

Compound 13:



Chemical Formula: $C_{27}H_{45}NO_4S$
Molecular Weight: 479,72

Under Ar atmosphere, ***o*-7 (100 mg, 0.40 mmol, 1.0 eq.)** and **potassium carbonate (109 mg, 0.79 mmol, 2.0 eq.)** were mixed together in **dry DMF (4 mL, 0.1M). Tert-butyl 10-bromodecanoate (147 mg, 0.47 mmol, 1.2 eq.)** was added and the solution was stirred at rt for 24h. AcOEt (20 mL) was added. The organic layer was washed once with brine and dried over Na_2SO_4. The crude was purified by flash column chromatography on silica gel (10g, Hexanes/AcOEt from 100:0 to 80:20) to give the expected product as a colorless oil **(133 mg, 0.27 mmol, η = 70%)**.

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.34 – 7.31 (m, 1H), 7.25 – 7.16 (m, 3H), 5.05 (s, 1H), 4.42 (s, 2H), 3.74 (s, 2H), 2.48 – 2.42 (m, 2H), 2.21 – 2.15 (m, 2H), 1.61 – 1.51 (m, 4H), 1.45 (s, 9H), 1.43 (s, 9H), 1.36 – 1.23 (m, 10H).

^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) = 173.4 (C_q), 155.9 (C_q), 137.2 (C_q), 136.2 (C_q), 130.4 (CH), 129.4 (CH), 127.8 (CH), 127.5 (CH), 80.0 (C_q), 79.6 (C_q), 42.2 (CH_2), 35.7 (CH_2), 33.9 (CH_2), 32.1 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 29.0 (CH_2), 28.6 (CH_3), 28.2 (CH_3), 25.2 (CH_2).

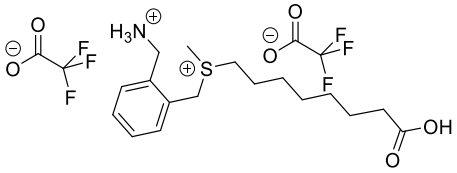
ESI-MS, positive mode : m/z = 480.3 $[M+H]^+$

HRMS (ESI) calculated for $C_{27}H_{46}NO_4S^+$ $[M+H]^+$: 480.3142, found: 480.3142.

General procedure for sulfonium synthesis:

The thioether (1.0 eq) was solubilized in **a HCOOH/AcOH mixture (1:1, 0.5M)**. **Methyl trifluoromethanesulfonate (3.0 eq.)** was added and the solution was stirred at rt for 1h. The mixture was diluted in acetonitrile and purified by reverse-phase HPLC (Device A, H_2O+0.1%TFA/ACN, linear gradient from 95:5 to 0:100, 40 mL/min). Solvents were removed under vacuum. As this type of compounds is highly hygroscopic, the final product was dissolved in a precise amount of **DMSO-d_6.** Concentration of the samples and therefore yields were determined by ^{1}H-NMR with an external standard. The products were kept in DMSO-d_6 (stored with preactivated powdered 4Å molecular sieves) and use in the final step without any further treatments.

Compound 14:



Chemical Formula: $C_{21}H_{29}F_6NO_6S$
Molecular Weight: 537,51

The general procedure starting with **8 (70 mg, 0.155 mmol, 1.0 eq.)** gave the expected product as **a colorless oil (0.140 mmol, η = 90 %).**

^{1}H NMR (400 MHz, DMSO-d_6): δ(ppm) = 8.53 (s, 3H), 7.63 (dd, *J* = 7.5, 1.5 Hz, 1H), 7.58 – 7.44 (m, 3H), 4.92 (d, *J* = 13.0 Hz, 1H), 4.83 (d, *J* = 13.0 Hz, 1H), 4.27 – 4.23 (m, 2H), 3.40 – 3.29 (m, 2H), 2.87 (s, 3H), 2.18 (t, *J* = 7.5 Hz, 2H), 1.74 – 1.63 (m, 1H), 1.60 – 1.44 (m, 3H), 1.36 – 1.20 (m, 6H).

^{13}C NMR (101 MHz, DMSO-d_6): δ(ppm) = 174.5 (C_q), 158.8 (q, J_F = 34.2 Hz), 134.4 (C_q), 132.3 (CH), 131.0 (CH), 130.2 (CH), 129.5 (CH), 127.8 (C_q), 116.4 (q, J_F = 294.5 Hz), 42.8 (CH_2), 40.9 (CH_2), 38.9 (CH_2), 33.7 (CH_2), 28.2 (CH_2), 28.0 (CH_2), 27.7 (CH_2), 24.4 (CH_2), 23.4 (CH_2), 22.0 (CH_3).

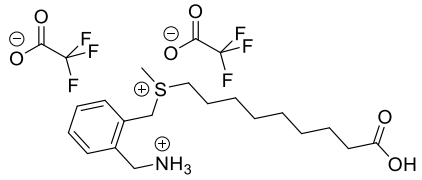
^{19}F NMR (376 MHz, DMSO-d_6): δ(ppm) = -74.5.

ESI-MS, positive mode: m/z = 310.1 $[M\text{-}H\text{-}2CF_3CO_2]^+$

ESI-MS, negative mode: m/z = 308.1 $[M\text{-}2H\text{-}2CF_3CO_2]^-$

HRMS (ESI) calculated for $C_{17}H_{28}NO_2S^+$ $[M\text{-}H\text{-}2CF_3CO_2]^+$: 310.1835, found: 310.1836.

Compound 15:



Chemical Formula: $C_{22}H_{31}F_6NO_6S$
Molecular Weight: 551,54

The general procedure starting with **9 (100 mg, 0.215 mmol, 1.0 eq.)** gave the expected product as **a colorless oil (0.210 mmol, η = 98 %).**

^{1}H NMR (400 MHz, DMSO-d_6): δ(ppm) = 8.55 (s, 3H, NH), 7.62 (dd, *J* = 7.5, 1.5 Hz, 1H), 7.57 – 7.44 (m, 3H), 4.92 (d, *J* = 13.0 Hz, 1H), 4.83 (d, *J* = 13.0 Hz, 1H), 4.24 (t, *J* = 5.5 Hz, 2H), 3.38 – 3.29 (m, 2H), 2.86 (s, 3H), 2.18 (t, *J* = 7.5 Hz, 2H), 1.76 – 1.60 (m, 1H), 1.59 - 1.43 (m, 3H), 1.34 – 1.19 (m, 8H).

^{13}C NMR (101 MHz, DMSO-d_6): δ(ppm) = 174.6 (C_q), 158.8 (q, J_F = 34.3 Hz), 134.5 (C_q), 132.3 (CH), 131.0 (CH), 130.2 (CH), 129.5 (CH), 127.8 (C_q), 116.5 (q, J_F = 294.7 Hz), 42.9 (CH_2), 40.9 (CH_2), 38.9 (CH_2), 33.8 (CH_2), 28.5 (CH_2), 28.4 (CH_2), 28.2 (CH_2), 27.8 (CH_2), 24.5 (CH_2), 23.4 (CH_2), 22.0 (CH_3).

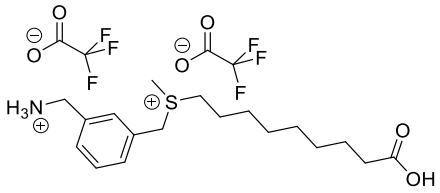
^{19}F NMR (376 MHz, DMSO-d_6): δ(ppm) = -74.5.

ESI-MS, positive mode: m/z = 324.2 $[M\text{-}H\text{-}2CF_3CO_2]^+$

ESI-MS, negative mode: m/z = 322.2 $[M\text{-}2H\text{-}2CF_3CO_2]^-$

HRMS (ESI) calculated for $C_{18}H_{30}NO_2S^+$ $[M\text{-}H\text{-}2CF_3CO_2]^+$: 324.1992, found: 324.1993.

Compound 16:



Chemical Formula: $C_{22}H_{31}F_6NO_6S$
Molecular Weight: 551,54

The general procedure starting with **10 (100 mg, 0.215 mmol, 1.0 eq.)** gave the expected product as **a colorless oil (0.210 mmol, η = 98 %).**

^{1}H NMR (400 MHz, DMSO-d_6): δ(ppm) = 8.49 (s, 3H), 7.61 – 7.48 (m, 4H), 4.78 (d, *J* = 13.0 Hz, 1H), 4.69 (d, *J* = 13.0 Hz, 1H), 4.08 (q, *J* = 5.5 Hz, 2H), 3.33 – 3.20 (m, 2H), 2.81 (s, 3H), 2.19 (t, *J* = 7.5 Hz, 2H), 1.78 – 1.59 (m, 2H), 1.55 – 1.42 (m, 2H), 1.40 – 1.20 (m, 8H).

^{13}C NMR (101 MHz, DMSO-d_6): δ(ppm) = 174.6 (C_q), 158.8 (q, J_F = 35.1 Hz), 135.4 (C_q), 131.2 (CH), 130.9 (CH), 130.3 (CH), 129.7 (CH), 128.8 (C_q), 116.2 (q, J_F = 293.1 Hz), 44.4 (CH_2), 42.1 (CH_2), 40.6 (CH_2), 33.8 (CH_2), 28.5 (CH_2), 28.5 (CH_2), 28.2 (CH_2), 27.8 (CH_2), 24.6 (CH_2), 23.4 (CH_2), 21.6 (CH_3).

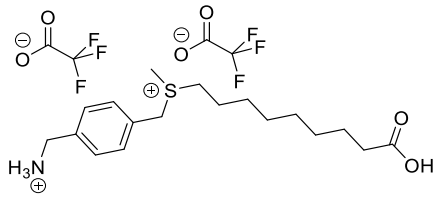
^{19}F NMR (376 MHz, DMSO-d_6): δ(ppm) = -74.7.

ESI-MS, positive mode : m/z = 324.2 $[M\text{-}H\text{-}2CF_3CO_2]^+$

ESI-MS, negative mode : m/z = 322.2 $[M\text{-}2H\text{-}2CF_3CO_2]^-$

HRMS (ESI) calculated for $C_{18}H_{30}NO_2S^+$ $[M\text{-}H\text{-}2CF_3CO_2]^+$: 324.1992, found: 324.1992.

Compound 17:



Chemical Formula: $C_{22}H_{31}F_6NO_6S$
Molecular Weight: 551,54

The general procedure starting with **11 (100 mg, 0.215 mmol, 1.0 eq.)** gave the expected product as **a colorless oil (0.196 mmol, η = 91 %).**

^{1}H NMR (400 MHz, DMSO-d_6): δ(ppm) = 8.49 (s, 3H), 7.62 – 7.48 (m, 4H), 4.78 (d, *J* = 13.0 Hz, 1H), 4.71 (d, *J* = 13.0 Hz, 1H), 4.08 (q, *J* = 5.5 Hz, 2H), 3.32 – 3.17 (m, 2H), 2.80 (s, 3H), 2.19 (t, *J* = 7.5 Hz, 2H), 1.78 – 1.59 (m, 2H), 1.52 – 1.45 (m, 2H), 1.38 – 1.17 (m, 8H).

^{13}C NMR (101 MHz, DMSO-d_6): δ(ppm) = 174.6 (C_q), 158.8 (q, J_F = 34.7 Hz), 135.7 (C_q), 130.9 (CH), 129.8 (CH), 128.7 (C_q), 116.3 (q, J_F = 293.8 Hz), 44.3 (CH_2), 42.0 (CH_2), 40.6 (CH_2), 33.8 (CH_2), 28.5 (CH_2), 28.5 (CH_2), 28.2 (CH_2), 27.8 (CH_2), 24.6 (CH_2), 23.3 (CH_2), 21.7 (CH_3).

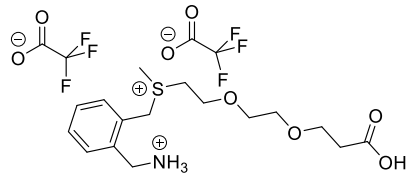
^{19}F NMR (376 MHz, DMSO-d_6): δ(ppm) = -74,7.

ESI-MS, positive mode: m/z = 324.1 $[M\text{-}H\text{-}2CF_3CO_2]^+$

ESI-MS, negative mode: m/z = 322.1 $[M\text{-}2H\text{-}2CF_3CO_2]^-$

HRMS (ESI) calculated for $C_{18}H_{30}NO_2S^+$ $[M\text{-}H\text{-}2CF_3CO_2]^+$: 324.1992, found: 324.1999.

Compound 18:



Chemical Formula: $C_{20}H_{27}F_6NO_8S$
Molecular Weight: 555,49

The general procedure starting with **12 (75 mg, 0.160 mmol, 1.0 eq.)** gave the expected product as **a colorless oil (0.088 mmol, η = 55 %).**

^{1}H NMR (400 MHz, DMSO-d_6): δ(ppm) = 8.47 (s, 3H), 7.65 – 7.60 (m, 1H), 7.59 – 7.44 (m, 3H), 4.93 (d, *J* = 13.0 Hz, 1H), 4.83 (d, *J* = 13.0 Hz, 1H), 4.23 (q, *J* = 5.5 Hz, 2H), 3.91 – 3.78 (m, 2H), 3.71 – 3.43 (m, 8H), 2.88 (s, 3H), 2.43 (t, *J* = 6.0 Hz, 2H).

^{13}C NMR (101 MHz, DMSO-d_6): δ(ppm) = 172.7 (C_q), 158.6 (q, J_F = 35.0 Hz, C_q), 134.5 (C_q), 132.4 (CH), 130.8 (CH), 130.3 (CH), 129.5 (CH), 127.6 (C_q), 116.1 (q, J_F = 294.9 Hz, C_q), 69.7 (CH_2), 69.3 (CH_2), 66.3 (CH_2), 64.4 (CH_2), 43.1 (CH_2), 41.6 (CH_2), 38.8 (CH_2), 34.8 (CH_2), 22.5 (CH_3).

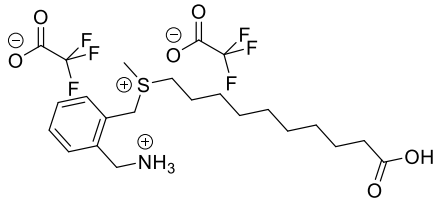
^{19}F NMR (376 MHz, DMSO-d_6): δ(ppm) = -74.6.

ESI-MS, positive mode: m/z = 328.1 $[M\text{-}H\text{-}2CF_3CO_2]^+$

ESI-MS, negative mode: m/z = 326.1 $[M\text{-}2H\text{-}2CF_3CO_2]^-$

HRMS (ESI) calculated for $C_{16}H_{26}NO_4S^+$ $[M\text{-}H\text{-}2CF_3CO_2]^+$: 325.1577, found: 325.1582.

Compound 19:



Chemical Formula: $C_{23}H_{33}F_6NO_6S$
Molecular Weight: 565,56

The general procedure starting with **13 (40 mg, 0.083 mmol, 1.0 eq.)** gave the expected product as **a colorless oil (0.080 mmol, η = 96 %).**

^{1}H NMR (400 MHz, DMSO-d_6): δ(ppm) = 8.52 (s, 3H), 7.66 – 7.59 (m, 1H), 7.59 – 7.42 (m, 3H), 4.90 (d, *J* = 13.0 Hz, 1H), 4.82 (d, *J* = 13.0 Hz, 1H), 4.24 (s, 2H), 3.39 – 3.28 (m, 2H), 2.86 (s, 3H), 2.18 (t, *J* = 7.5 Hz, 2H), 1.73 – 1.63 (m, 1H), 1.60 – 1.42 (m, 3H), 1.36 – 1.28 (m, 2H), 1.27 – 1.20 (m, 8H).

^{13}C NMR (101 MHz, DMSO-d_6): δ(ppm) = 174.5 (C_q), 158.6 (q, J_F = 34.9 Hz), 134.3 (C_q), 132.3 (CH), 130.9 (CH), 130.2 (CH), 129.4 (CH), 127.7 (C_q), 116.2 (q, J_F = 293.6 Hz), 42.7 (CH_2), 40.8 (CH_2), 38.8 (CH_2), 33.7 (CH_2), 28.6 (CH_2), 28.5 (CH_2), 28.5 (CH_2), 28.2 (CH_2), 27.8 (CH_2), 24.5 (CH_2), 23.3 (CH_2), 21.9 (CH_3).

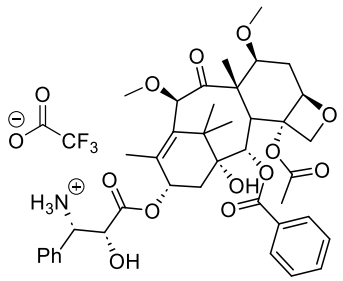
^{19}F NMR (376 MHz, DMSO-d_6): δ(ppm) = -74.6.

ESI-MS, positive mode: m/z = 338.2 $[M\text{-}H\text{-}2CF_3CO_2]^+$

ESI-MS, negative mode: m/z = 336.2 $[M\text{-}2H\text{-}2CF_3CO_2]^-$

HRMS (ESI) calculated for $C_{19}H_{32}NO_2S^+$ $[M\text{-}H\text{-}2CF_3CO_2]^+$: 338.2148, found: 338.2147.

CTX-NH_2.CF_3OCO_2H:



Chemical Formula: $C_{42}H_{50}F_3NO_{14}$
Molecular Weight: 849,85

A solution of **cabazitaxel (1 g, 1.20 mmol, 1.0 eq.)** in **95% formic acid (5 mL)** was stirred at room temperature for 4h. Reaction progress was monitored by analytical HPLC. Once reaction was complete, formic acid was evaporated on rotary evaporator. The residue was solubilized in a H_2O/ACN mixture and purified by reverse-phase HPLC (Device B, H_2O+0.1%TFA/ACN, linear gradient 70:30 to 0:100, 150 mL/min). The fractions containing the product were lyophilized to obtain the expected compound as a white powder **(660 mg, 0.78 mmol, η = 65 %)**.

^{1}H NMR (400 MHz, DMSO-d_6): δ(ppm) = 8.71 (s, 3H, NH_3), 7.94 – 7.89 (m, 2H), 7.76 – 7.71 (m, 1H), 7.67 – 7.62 (m, 2H), 7.53 – 7.42 (m, 4H), 7.35 – 7.30 (m, 1H), 5.84 (td, *J* = 9.0, 2.0 Hz, 1H), 5.34 (d, *J* = 7.0 Hz, 1H), 4.93 (dd, *J* = 9.5, 2.0 Hz, 1H), 4.67 (s, 1H), 4.49 – 4.38 (m, 2H), 4.01 – 3.96 (m, 2H), 3.72 (dd, *J* = 10.5, 6.5 Hz, 1H), 3.55 (d, *J* = 7.0 Hz, 1H), 3.29 (s, 3H), 3.20 (s, 3H), 2.67 – 2.60 (m, 1H), 2.08 (s, 3H), 1.80 (s, 3H), 1.68 (dd, *J* = 15.5, 9.0 Hz, 1H), 1.57 – 1.42 (m, 5H), 0.97 (s, 3H), 0.94 (s, 3H).

^{13}C NMR (101 MHz, DMSO-d_6): δ(ppm) = 204.7 (C_q), 171.2 (C_q), 169.8 (C_q), 165.1 (C_q), 158.3 (q, J_F = 32.0 Hz), 137.9 (C_q), 135.1 (C_q), 133.5 (CH), 133.4 (C_q), 129.9 (C_q), 129.5 (CH), 129.3 (CH), 128.9 (CH), 128.7 (CH), 128.0 (CH), 117.0 (q, J_F = 296.5 Hz), 83.2 (CH), 82.0 (CH), 80.3 (C_q), 80.2 (CH), 76.8 (C_q), 75.2 (CH_2), 74.3 (CH), 73.0 (CH), 70.2 (CH), 57.0 (CH), 56.7 (CH_3), 56.7 (CH_3), 56.0 (C_q), 46.4 (CH), 42.9 (C_q), 34.7 (CH_2), 31.7 (CH_2), 26.7 (CH_3), 22.5 (CH_3), 21.2 (CH_3), 14.0 (CH_3), 10.2 (CH_3).

^{19}F NMR (376 MHz, DMSO-d_6): δ(ppm) = -73.9.

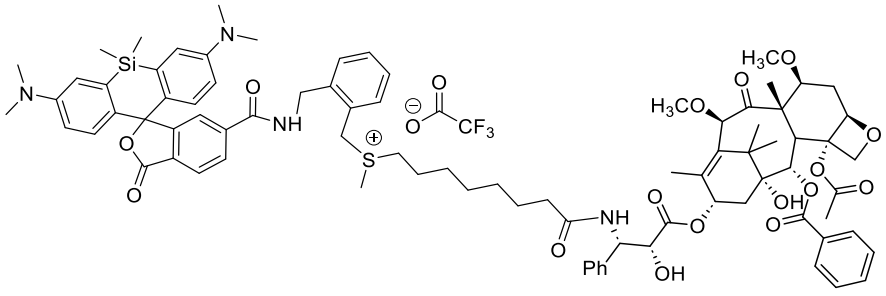
ESI-MS, positive mode: m/z = 736.2 [M-$CF_3OCO_2^-$]

ESI-MS, negative mode: m/z = 780.30 [M-CF_3OCO_2H + $HCOO^-$]

HRMS (ESI) calculated for $C_{40}H_{50}NO_{12}^+$ [M-CF_3CO_2]$^+$: 736.3328, found: 736.3347.

Characterization of the final probes

Probe 1 :



Exact Mass: 1481,67

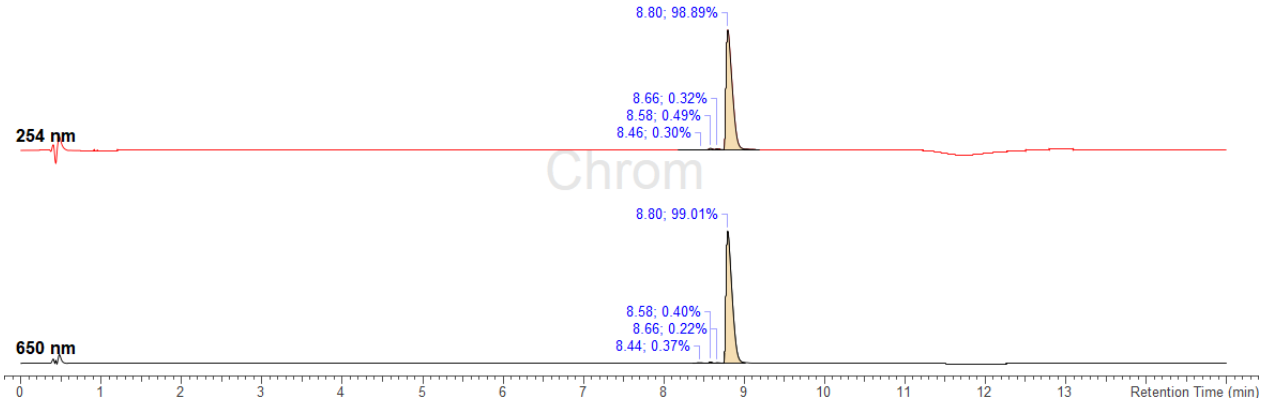
According to the general procedure, **6-SiR-CO_2H** (2.0 mg, 4.23 µmol), sulfonium **14** (13.8 µL -of a 0.4M solution in DMSO-,5.50 µmol) and **CTX-NH_2.CF_3CO_2H** (10.8 mg, 12.7 µmol) led to the expected product as a blue powder (2.46 µmol, **η = 58 %**).

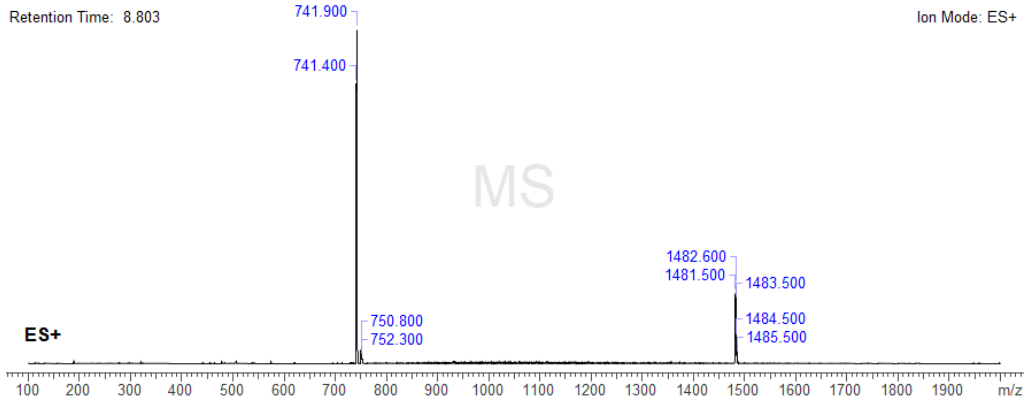
^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.33 (t, *J* = 5.5 Hz, 1H, NH), 8.36 (d, *J* = 9.0 Hz, 1H, NH), 8.14 (dd, *J* = 8.0, 1.5 Hz, 1H, H_{Ar}), 8.07 (d, *J* = 8.0 Hz, 1H, H_{Ar}), 8.00 – 7.96 (m, 2H, H_{Ar}), 7.72 – 7.66 (m, 2H, H_{Ar}), 7.62 – 7.57 (m, 2H, H_{Ar}), 7.46 – 7.31 (m, 8H, H_{Ar}), 7.24 – 7.20 (m, 1H, H_{Ar}), 7.04 (s, 2H, H_{Ar}), 6.67 -6.62 (m, 4H, H_{Ar}), 5.96 – 5.91 (m, 1H, CH), 5.39 (d, *J* = 7.0 Hz, 1H, CH), 5.32 – 5.27 (m, 1H, CH), 4.98 – 4.94 (m, 1H, CH), 4.86 (d, *J* = 13.0 Hz, 1H, 1H from CH_2), 4.76 (d, *J* = 13.0 Hz, 1H, 1H from CH_2), 4.71 (s, 1H, CH), 4.56 (d, *J* = 5.5 Hz, 2H, CH_2), 4.42 (d, *J* = 5.5 Hz, 1H, CH), 4.03 (s, 2H, CH_2), 3.76 – 3.73 (dd, *J* = 10.5, 6.5 Hz, 1H, CH), 3.63 (d, *J* = 7.0 Hz, 1H, CH), 3.33 – 3.26 (m, 5H, CH_3 + CH_2), 3.21 (s, 3H, CH_3), 2.93 (s, 12H, 4*CH_3), 2.84 (s, 3H, CH_3), 2.68 – 2.63 (m, 1H, 1H from CH_2), 2.25 (s, 3H, CH_3), 2.19 – 2.11 (td, *J* = 7.0, 4.0 Hz, 2H, CH_2), 2.00 – 1.94 (m, 1H, 1H from CH_2), 1.90 – 1.81 (m, 4H, CH_3 +1H from CH_2), 1.60 – 1.41 (m, 8H, 1*CH_3 + 2*CH_2 + 1H from CH_2), 1.24 – 1.13 (m, 6H, 3*CH_2), 1.02 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 0.62 (s, 3H, CH_3), 0.52 (s, 3H, CH_3).

^{19}F NMR (376 MHz, DMSO-d_6): δ(ppm) = -74,6.

ESI-MS, positive mode: m/z = 1481.5 $[M\text{-}CF_3CO_2^-]^+$

HRMS (ESI) calculated for $C_{84}H_{101}N_4O_{16}SSi^+$ $[M\text{-}CF_3CO_2^-]^+$: 1481.6697, found: 1481.6697.



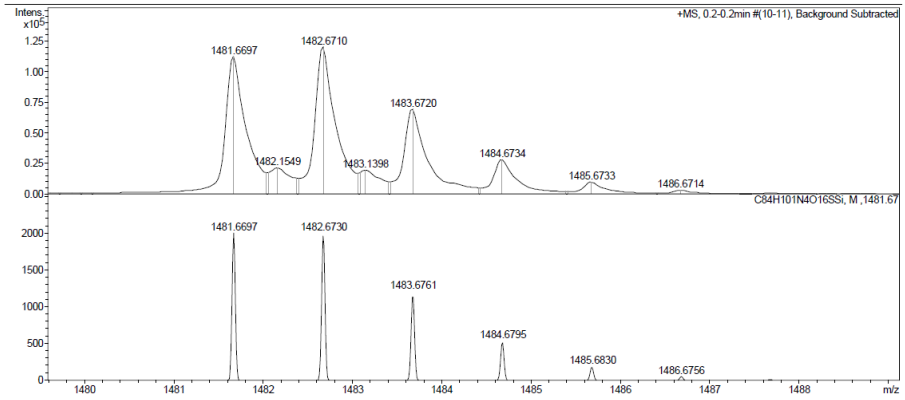


Analysis Info

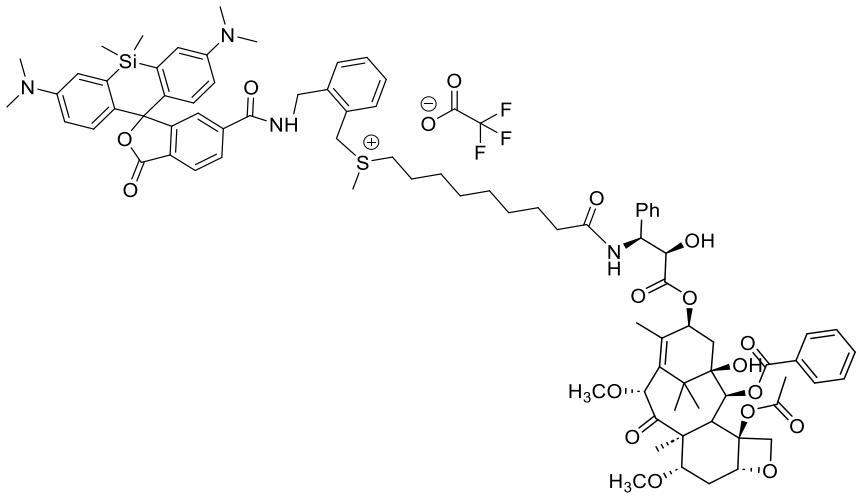
Analysis Name	Z:\Data\2024\2409\sam040924\MA230_30_01_127436.d	Acquisition Date	04.09.2024 23:01:49
Method	hystar_p.m	Operator	BDAL@DE
Sample Name	MA230	Instrument / Ser#	micrOTOF 10237
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active			Set Dry Heater	180 °C
Scan Begin	50 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source



Probe 2 - 6-SiR-*o*-C_9-CTX:



Exact Mass: 1495,69

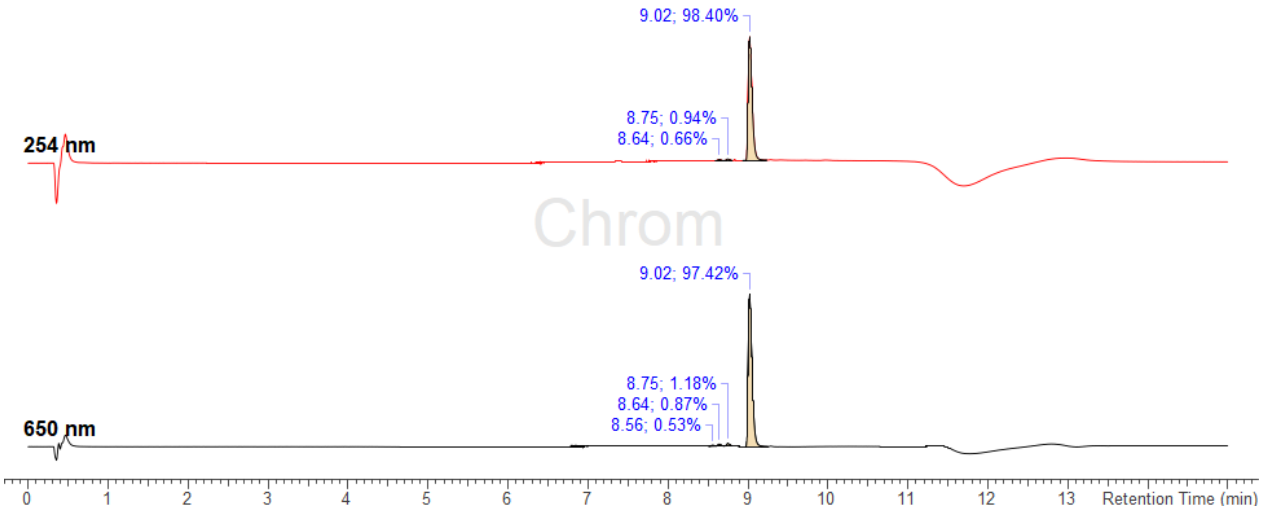
According to the general procedure, **6-SiR-CO_2H** (3.0 mg, 6.35 µmol), sulfonium **15** (27.5 µL -of a 0.3M solution in DMSO-, 8.26 µmol) and **CTX-NH_2.CF_3CO_2H** (16.2 mg, 19.0 µmol) led to the expected product as a blue powder (3.24 µmol, **η = 51 %**).

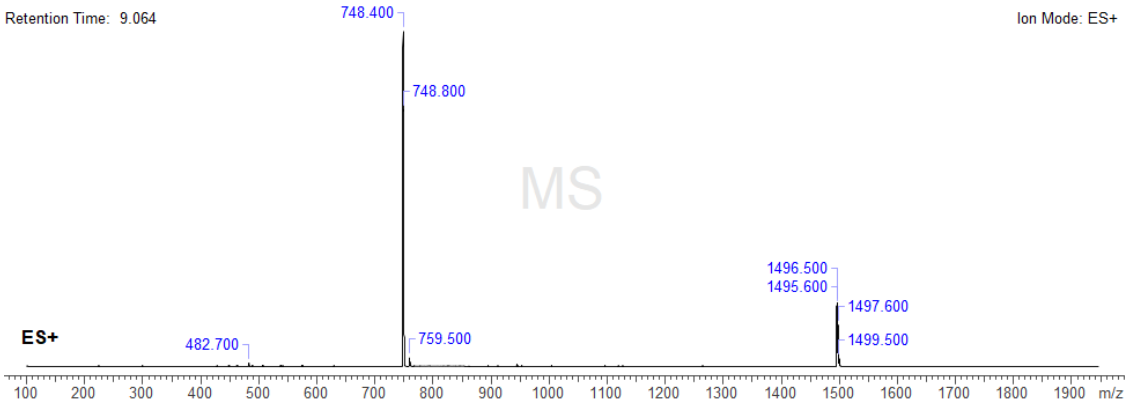
^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.34 (t, *J* = 5.5 Hz, 1H, NH), 8.36 (d, *J* = 9.0 Hz, 1H, NH), 8.14 (dd, *J* = 8.0, 1.5 Hz, 1H, H_{Ar}), 8.07 (d, *J* = 8.0 Hz, 1H, H_{Ar}), 8.00 – 7.96 (m, 2H, H_{Ar}), 7.72 – 7.66 (m, 2H, H_{Ar}), 7.62 – 7.57 (m, 2H, H_{Ar}), 7.48 – 7.28 (m, 8H, H_{Ar}), 7.24 – 7.20 (m, 1H, H_{Ar}), 7.04 (s, 2H, H_{Ar}), 6.66 -6.64 (m, 4H, H_{Ar}), 5.96 – 5.91 (m, 1H, CH), 5.40 (d, *J* = 7.0 Hz, 1H, CH), 5.30 (dd, *J* = 9.0, 5.5 Hz, 1H, CH), 4.96 (dd, *J* = 10.0, 2.0 Hz, 1H, CH), 4.86 (d, *J* = 13.0 Hz, 1H, 1H from CH_2), 4.76 (d, *J* = 13.0 Hz, 1H, 1H from CH_2), 4.71 (s, 1H, CH), 4.56 (d, *J* = 5.5 Hz, 2H, CH_2), 4.43 (d, *J* = 5.5 Hz, 1H, CH), 4.03 (s, 2H, CH_2), 3.76 – 3.73 (m, 1H, CH, from HSQC), 3.63 (d, *J* = 7.0 Hz, 1H, CH), 3.33 – 3.27 (m, 5H, CH_3 + CH_2), 3.21 (s, 3H, CH_3), 2.93 (s, 12H, 4*CH_3), 2.84 (s, 3H, CH_3), 2.71 – 2.61 (m, 1H, 1H from CH_2), 2.25 (s, 3H, CH_3), 2.19 – 2.11 (m, 2H, CH_2), 2.01 – 1.94 (m, 1H, 1H from CH_2), 1.91 – 1.81 (m, 4H, CH_3 +1H from CH_2), 1.64 – 1.42 (m, 8H, 1*CH_3 + 2*CH_2 + 1H from CH_2), 1.24 – 1.13 (m, 8H, 4*CH_2), 1.03 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 0.62 (s, 3H, CH_3), 0.52 (s, 3H, CH_3).

^{19}F NMR (376 MHz, DMSO-d_6): δ(ppm) = -74,6.

ESI-MS, positive mode: m/z = 1495.5 $[M\text{-}CF_3CO_2^-]^+$

HRMS (ESI) calculated for $C_{85}H_{103}N_4O_{16}SSi^+$ $[M\text{-}CF_3CO_2^-]^+$: 1495.6854, found: 1495.6874.



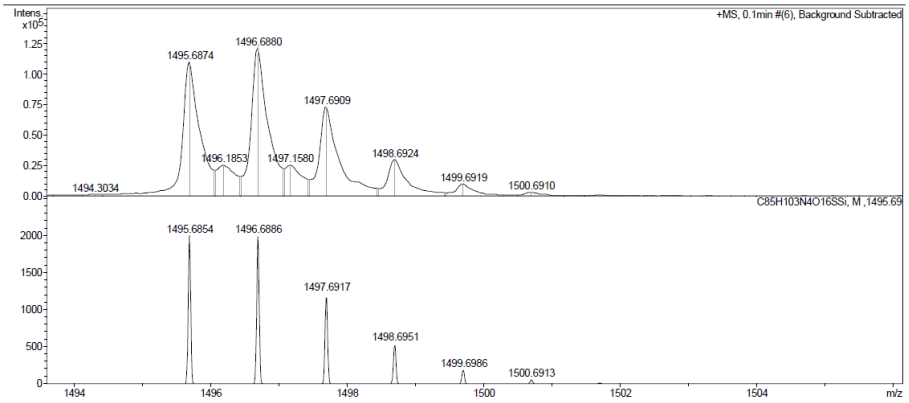


Analysis Info

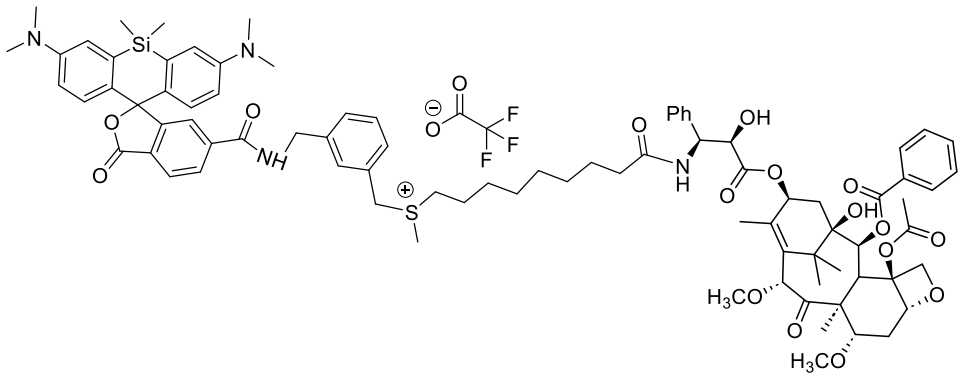
Analysis Name	Z:\Data\2024\2409\sam040924\MA280_26_01_127432.d	Acquisition Date	04.09.2024 22:43:15
Method	hystar_p.m	Operator	BDAL@DE
Sample Name	MA280	Instrument / Ser#	micrOTOF 10237
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active			Set Dry Heater	180 °C
Scan Begin	50 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source



Probe 3 :



Exact Mass: 1495,69

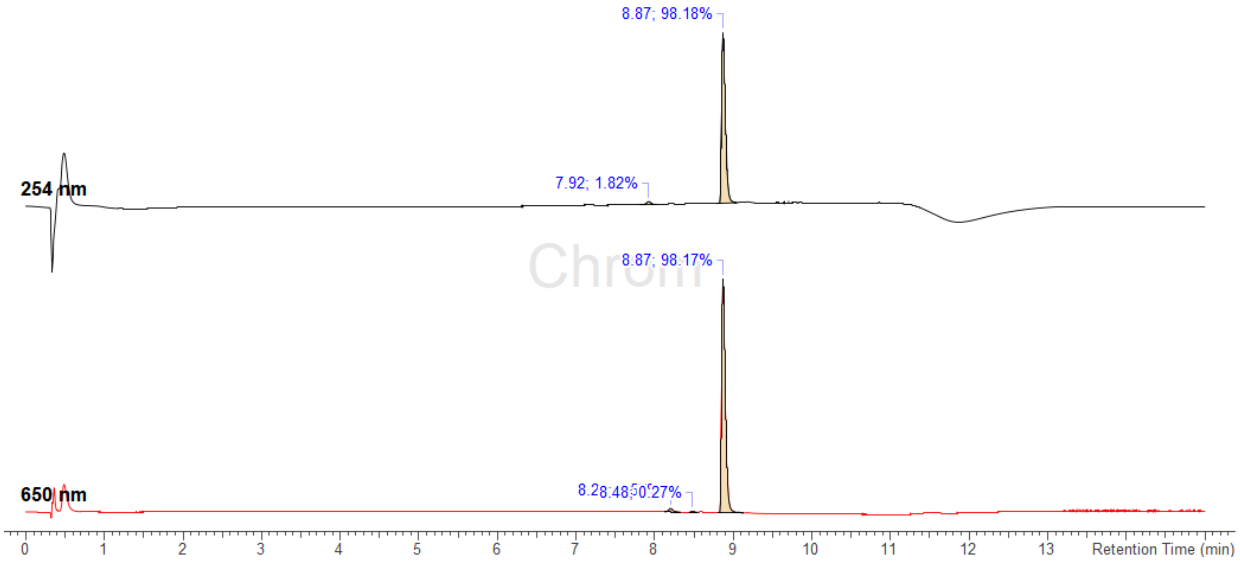
According to the general procedure, **6-SiR-CO_2H** (2.0 mg, 4.23 µmol), sulfonium **16** (18.3 µL -of a 0.3M solution in DMSO-,5.50 µmol) and **CTX-NH_2.CF_3CO_2H** (10.8 mg, 12.7 µmol) led to the expected product as a blue powder (2.29 µmol, **η = 54 %**).

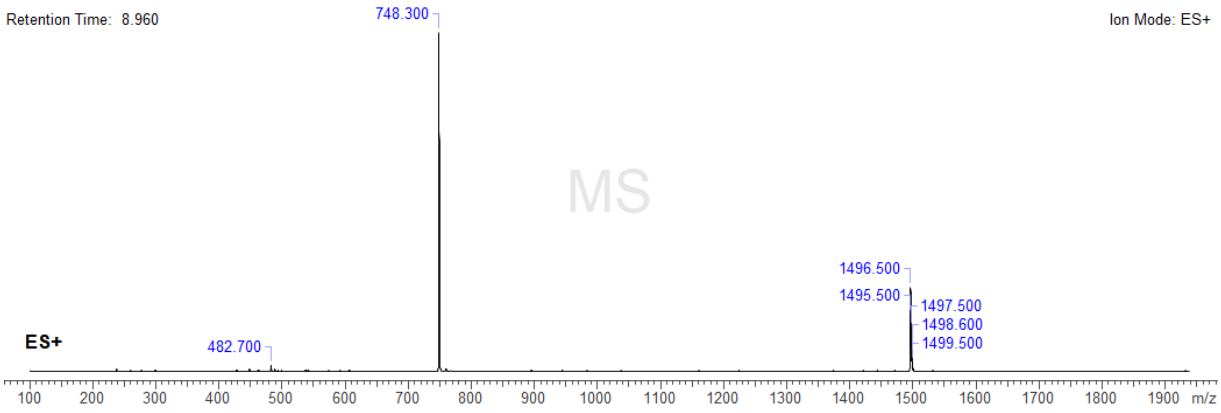
^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 9.35 (t, *J* = 6.0 Hz, 1H, NH), 8.36 (d, *J* = 9.0 Hz, 1H, NH), 8.13 (dd, *J* = 8.0, 1.5 Hz, 1H, H_{Ar}), 8.06 (d, *J* = 8.0 Hz, 1H, H_{Ar}), 8.01 – 7.95 (m, 2H, H_{Ar}), 7.72 – 7.65 (m, 2H, H_{Ar}), 7.62 – 7.56 (m, 2H, H_{Ar}), 7.42 – 7.31 (m, 8H, H_{Ar}), 7.24 – 7.20 (m, 1H, H_{Ar}), 7.03 (s, 2H, H_{Ar}), 6.66 -6.64 (m, 4H, H_{Ar}), 5.96 – 5.91 (m, 1H, CH), 5.40 (d, *J* = 7.0 Hz, 1H, CH), 5.30 (dd, *J* = 9.0, 5.5 Hz, 1H, CH), 4.98 – 4.95 (m, 1H, CH), 4.71 (s, 1H, CH), 4.69 – 4.57 (m, 2H, CH_2), 4.46 – 4.42 (m, 3H, 1*CH + 1*CH_2), 4.03 (s, 2H, CH_2), 3.76 (dd, *J* = 10.5, 6.5 Hz, 1H, CH), 3.63 (d, *J* = 7.0 Hz, 1H, CH), 3.31 (s, 3H, CH_3), 3.21 (s, 3H, CH_3), 3.20 – 3.12 (m, 2H, CH_2), 2.92 (s, 12H, 4*CH_3), 2.72 (s, 3H, CH_3), 2.70 – 2.60 (m, 1H, 1H from CH_2), 2.26 (s, 3H, CH_3), 2.22 – 2.08 (m, 2H, CH_2), 2.02 – 1.95 (m, 1H, CH_2, 1H from CH_2), 1.90 – 1.83 (m, 4H, 1*CH_3+1H from CH_2), 1.63 – 1.41 (m, 8H, 1*CH_3 + 2*CH_2 + 1H from CH_2), 1.25 – 1.10 (m, 8H, 4*CH_2), 1.03 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 0.63 (s, 3H, CH_3), 0.52 (s, 3H, CH_3).

^{19}F NMR (376 MHz, DMSO-d_6): δ(ppm) = -74,5.

ESI-MS, positive mode: m/z = 1495.6 $[M\text{-}CF_3CO_2^-]^+$

HRMS (ESI) calculated for $C_{85}H_{103}N_4O_{16}SSi^+$ $[M\text{-}CF_3CO_2^-]^+$: 1495.6854, found: 1495.6861.



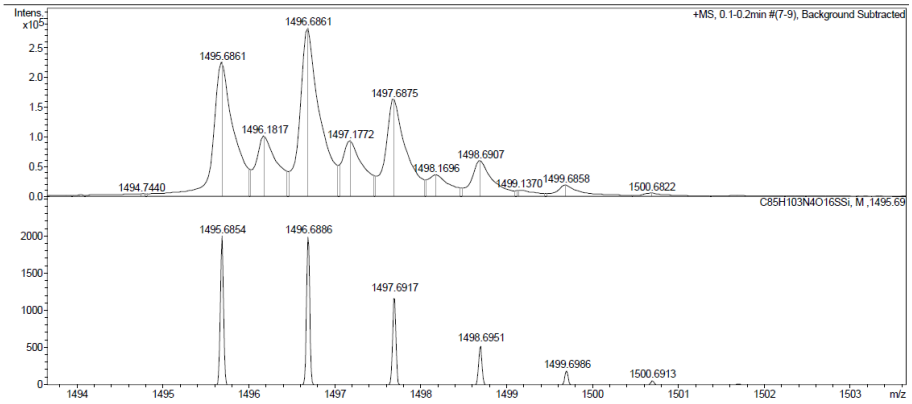


Analysis Info

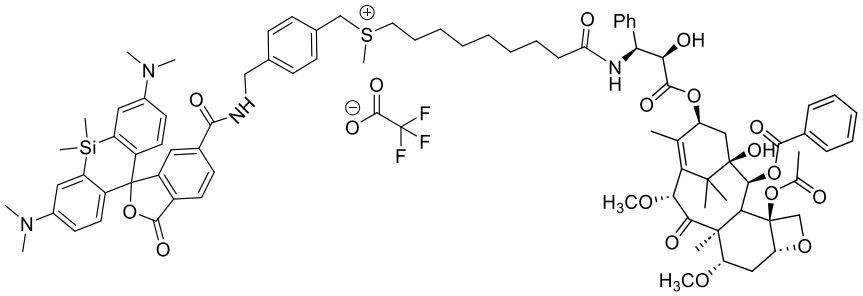
Analysis Name	Z:\Data\2024\2409\sam040924\MA278_31_01_127437.d	Acquisition Date	04.09.2024 23:06:27
Method	hystar_p.m	Operator	BDAL@DE
Sample Name	MA278	Instrument / Ser#	micrOTOF 10237
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active			Set Dry Heater	180 °C
Scan Begin	50 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source



Probe 4 :



Exact Mass: 1495,68

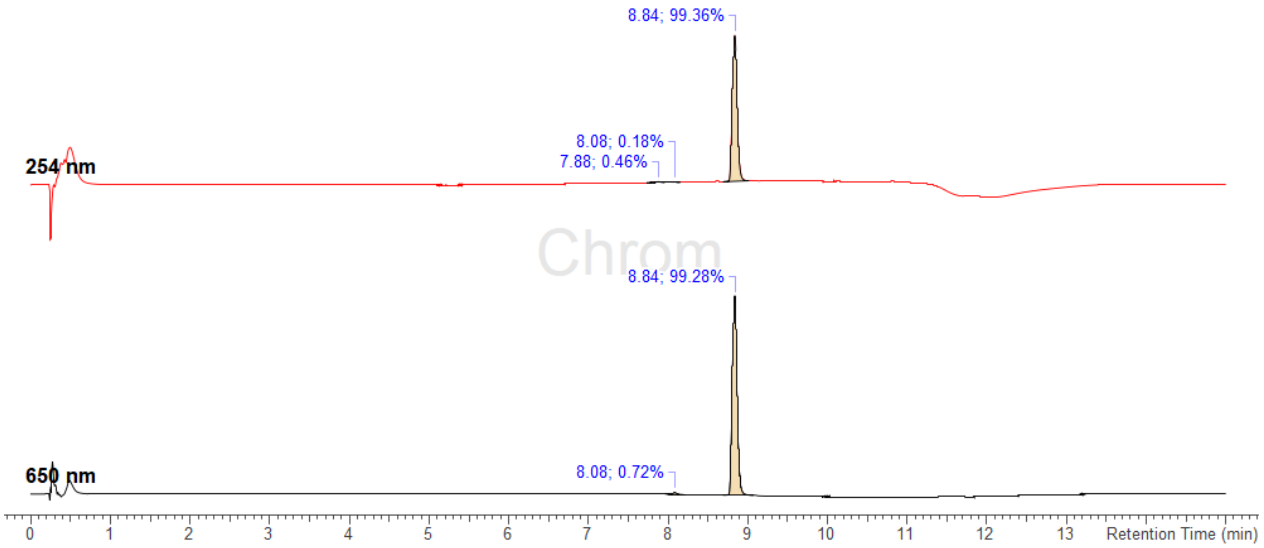
According to the general procedure, **6-SiR-CO_2H** (2.0 mg, 4.23 µmol), sulfonium **17** (19.6 µL -of a 0.28M solution in DMSO-,5.50 µmol) and **CTX-NH_2.CF_3CO_2H** (10.8 mg, 12.7 µmol) led to the expected product as a blue powder (2.46 µmol, **η = 58 %**).

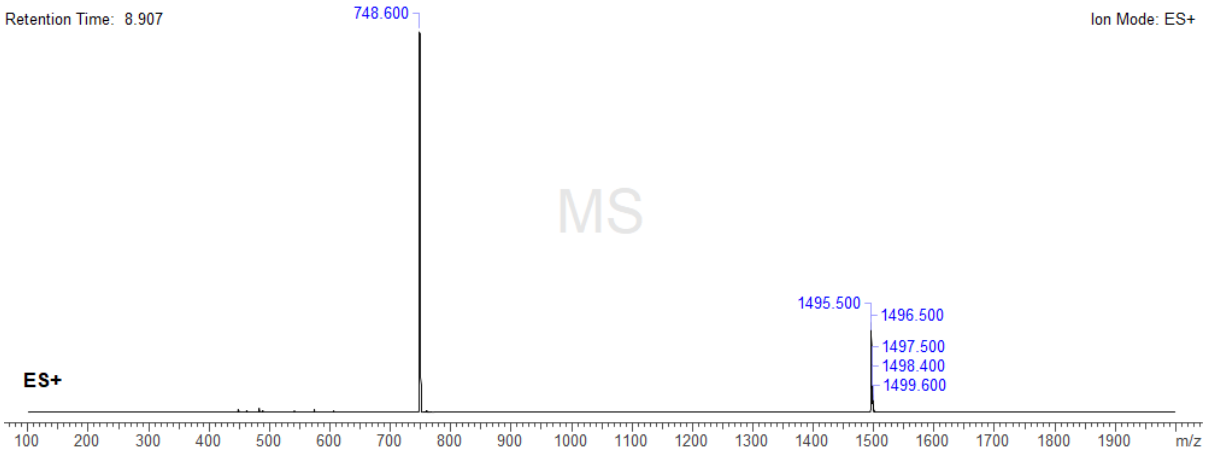
^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 9.35 (t, *J* = 6.0 Hz, 1H, NH), 8.36 (d, *J* = 9.0 Hz, 1H, NH), 8.15 – 8.11 (m, 1H, H_{Ar}), 8.06 (d, *J* = 8.0 Hz, 1H, H_{Ar}), 8.00 – 7.96 (m, 2H, H_{Ar}), 7.70 – 7.66 (m, 2H, H_{Ar}), 7.62 – 7.56 (m, 2H, H_{Ar}), 7.42 – 7.31 (m, 8H, H_{Ar}), 7.25 – 7.20 (m, 1H, H_{Ar}), 7.03 (s, 2H, H_{Ar}), 6.68 -6.63 (m, 4H, H_{Ar}), 5.99 – 5.89 (m, 1H, CH),5.40 (d, *J* = 7.0 Hz, 1H, CH), 5.30 (dd, *J* = 9.0, 5.5 Hz, 1H, CH), 4.96 (dd, *J* = 9.5, 2.0 Hz, 1H, CH), 4.71 (s, 1H, CH), 4.67 (d, *J* = 13.0 Hz, 1H, 1H from CH_2), 4.59 (d, *J* = 13.0 Hz, 1H, 1H from CH_2), 4.46 (d, *J* = 6.0 Hz, 2H, CH_2), 4.43 (d, *J* = 5.5 Hz, 1H, CH), 4.03 (s, 2H, CH_2), 3.76 (dd, *J* = 10.5, 6.5 Hz, 1H, CH), 3.63 (d, *J* = 7.0 Hz, 1H, CH), 3.31 (s, 3H, CH_3), 3.23 – 3.13 (m, 5H, 1*CH_2 + 1*CH_3), 2.93 (s, 12H, 4*CH_3), 2.73 (s, 3H, CH_3), 2.67 – 2.62 (m, 1H, 1H from CH_2), 2.26 (s, 3H, CH_3), 2.20 – 2.12 (m, 2H, CH_2), 2.00 – 1.95 (m, 1H, 1H from CH_2), 1.90 – 1.81 (m, 4H, 1*CH_3 + 1H from CH_2),1.64 – 1.44 (m, 8H, 1*CH_3 + 2*CH_2 + 1H from CH_2), 1.27 – 1.14 (m, 8H, 4*CH_2), 1.03 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 0.63 (s, 3H, CH_3), 0.52 (s, 3H, CH_3).

^{19}F NMR (376 MHz, DMSO-d_6): δ(ppm) = -74,5.

ESI-MS, positive mode: m/z = 1495.5 $[M\text{-}CF_3CO_2^-]^+$

HRMS (ESI) calculated for $C_{85}H_{103}N_4O_{16}SSi^+$ $[M\text{-}CF_3CO_2^-]^+$: 1495.6854, found: 1495.6857.



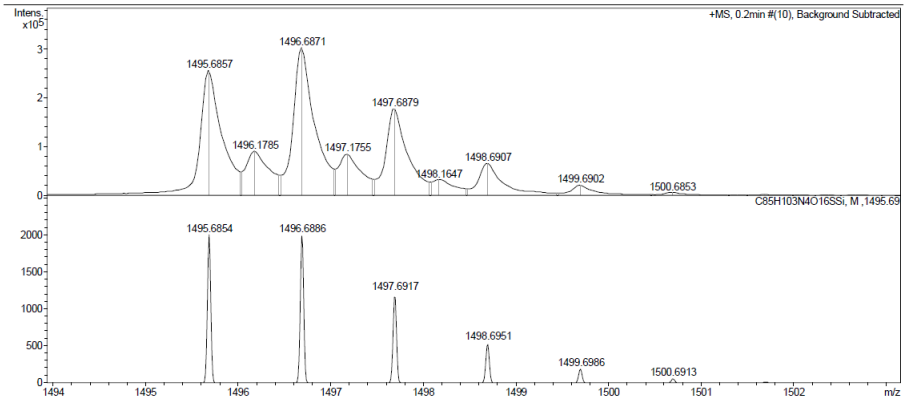


Analysis Info

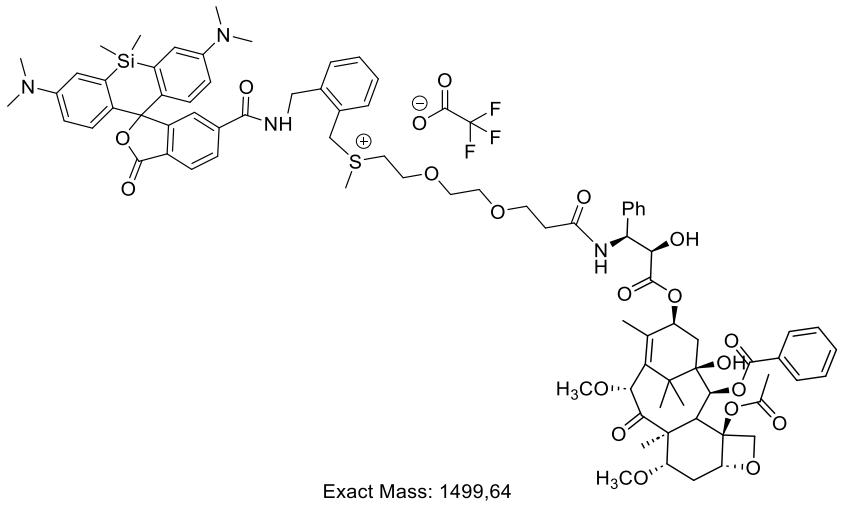
Analysis Name	Z:\Data\2024\2409\sam040924\MA279_29_01_127435.d	Acquisition Date	04.09.2024 22:57:09
Method	hystar_p.m	Operator	BDAL@DE
Sample Name	MA279	Instrument / Ser#	micrOTOF 10237
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active			Set Dry Heater	180 °C
Scan Begin	50 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source



Probe 5 :



Exact Mass: 1499,64

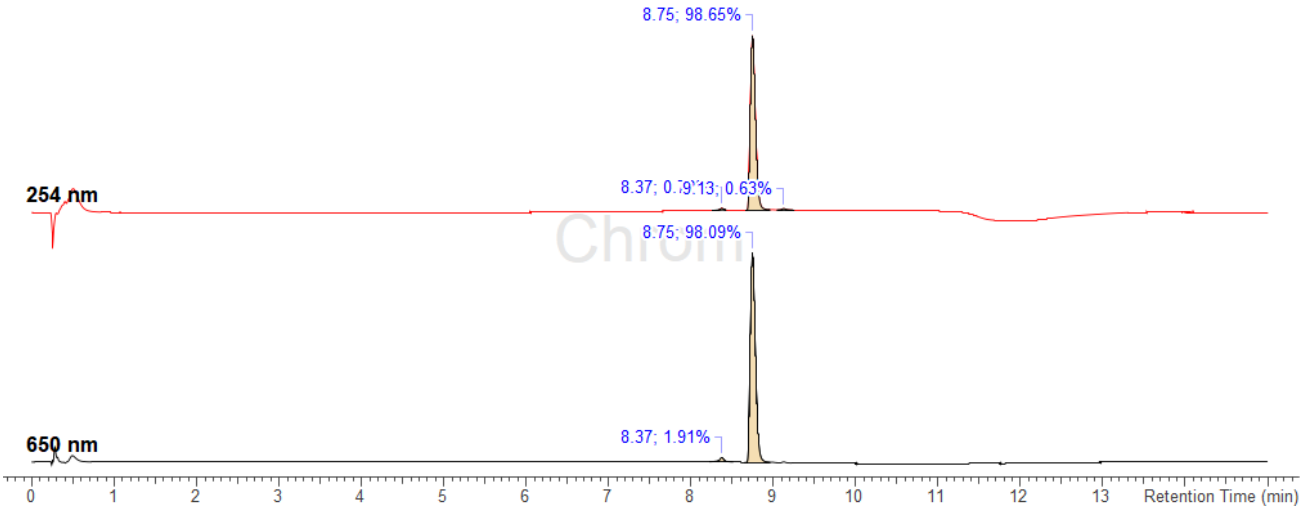
According to the general procedure, **6-SiR-CO_2H** (2.0 mg, 4.23 µmol), sulfonium **18** (37.7 µL -of a 0.146 M solution in DMSO-,5.50 µmol) and **CTX-$NH_2.CF_3CO_2H$** (10.8 mg, 12.7 µmol) led to the expected product as a blue powder (2.26 µmol, **η = 53 %**).

^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 9.34 (t, *J* = 5.5 Hz, 1H, NH), 8.46 (d, *J* = 9.0 Hz, 1H, NH), 8.13 (dd, *J* = 8.0, 1.5 Hz, 1H, H_{Ar}), 8.07 (d, *J* = 8.0 Hz, 1H, H_{Ar}), 8.00 – 7.96 (m, 2H, H_{Ar}), 7.72 – 7.65 (m, 2H, H_{Ar}), 7.62 – 7.56 (m, 2H, H_{Ar}), 7.45 – 7.40 (m, 3H, H_{Ar}), 7.38 – 7.29 (m, 5H, H_{Ar}), 7.23 – 7.18 (m, 1H, H_{Ar}), 7.03 (s, 2H, H_{Ar}), 6.64 (s, 4H, H_{Ar}), 5.97 – 5.92 (m, 1H, CH), 5.39 (d, *J* = 7.0 Hz, 1H, CH), 5.28 (dd, *J* = 9.0, 5.5 Hz, 1H, CH), 4.97 – 4.94 (m, 1H, CH), 4.91 (d, *J* = 13.0 Hz, 1H, 1H from CH_2), 4.77 (d, *J* = 13.0 Hz, 1H, 1H from CH_2), 4.70 (s, 1H, CH), 4.55 (d, *J* = 5.5 Hz, 2H, CH_2), 4.43 (d, *J* = 6.0 Hz, 1H, CH), 4.05 – 4.00 (m, 2H, CH_2), 3.84 – 3.68 (m, 3H, CH_2 + CH), 3.63 (d, *J* = 7.0 Hz, 1H, CH), 3.57 – 3.41 (m, 8H, 4*CH_2), 3.30 (s, 3H, CH_3), 3.21 (s, 3H, CH_3), 2.92 (s, 12H, 4*CH_3), 2.83 (s, 3H, CH_3), 2.69 – 2.61 (m, 1H, 1H from CH_2), 2.45 – 2.38 (m, 2H, CH_2), 2.23 (s, 3H, CH_3), 1.96 (dd, *J* = 15.0, 9.0 Hz, 1H, 1H from CH_2), 1.85 – 1.79 (m, 4H, 1*CH_3 + 1H from CH_2), 1.54 – 1.46 (m, 4H, 1*CH_3 + 1H from CH_2), 1.02 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 0.62 (s, 3H, CH_3), 0.52 (s, 3H, CH_3).

^{19}F NMR (376 MHz, DMSO-d_6): δ(ppm) = -74.5.

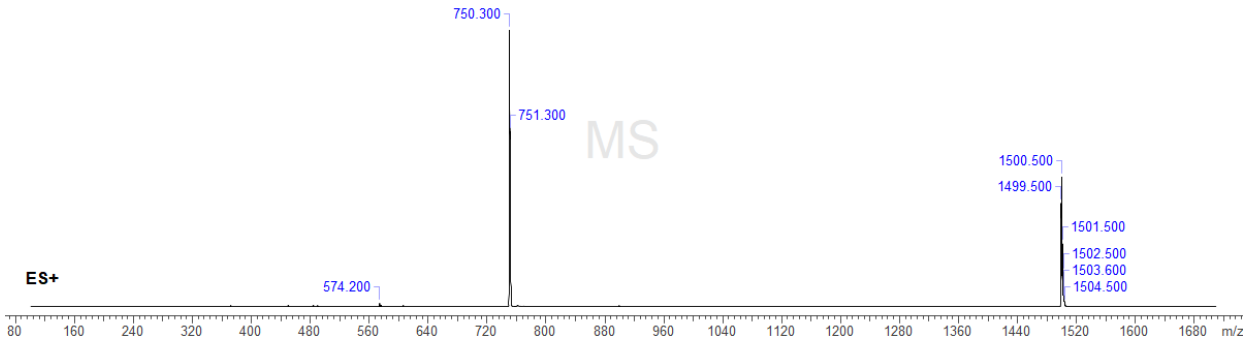
ESI-MS, positive mode: m/z = 1499.6 $[M\text{-}CF_3CO_2^-]^+$

HRMS (ESI) calculated for $C_{83}H_{99}N_4O_{18}SSi^+$ $[M\text{-}CF_3CO_2^-]^+$: 1499.6439, found: 1499.6442.



Retention Time: 8.829

Ion Mode: ES+

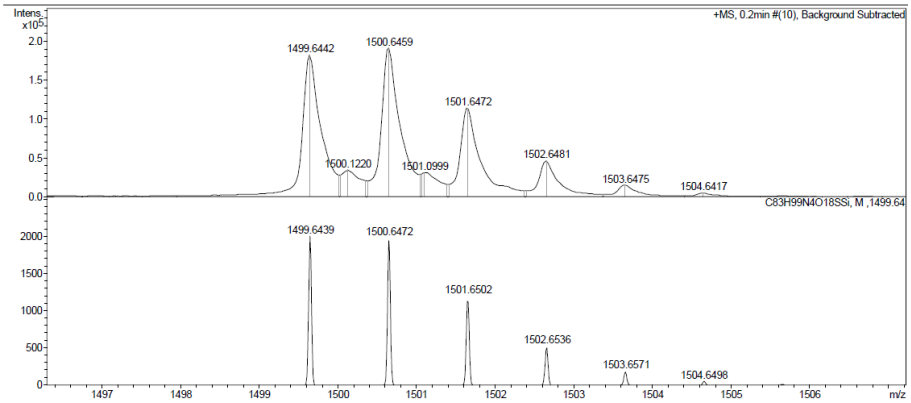


Analysis Info

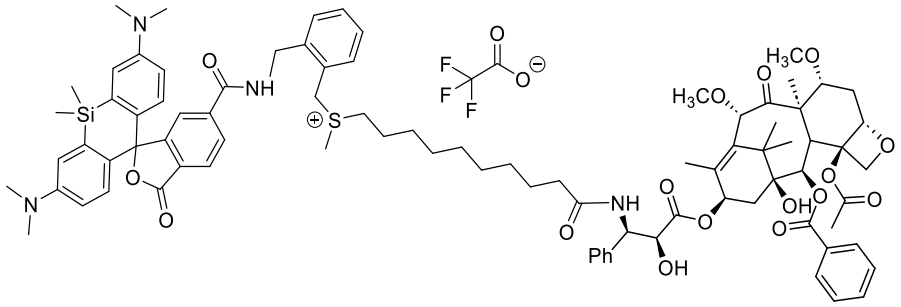
Analysis Name	Z:\Data\2024\2409\sam040924\MA244_27_01_127433.d	Acquisition Date	04.09.2024 22:47:54
Method	hystar_p.m	Operator	BDAL@DE
Sample Name	MA244	Instrument / Ser#	micrOTOF 10237
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active			Set Dry Heater	180 °C
Scan Begin	50 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source



Probe 6 :



Exact Mass: 1509,70

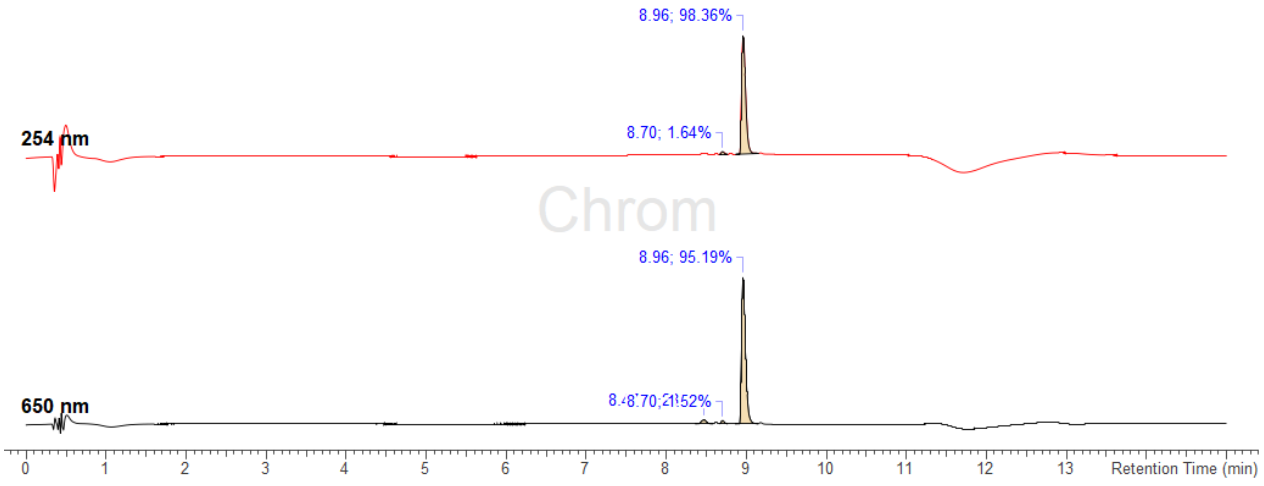
According to the general procedure, **6-SiR-CO_2H** (2.0 mg, 4.23 µmol), sulfonium **18** (71.4 µL -of a 77 mM solution in DMSO-,5.50 µmol) and **CTX-NH_2.CF_3CO_2H** (10.8 mg, 12.7 µmol) led to the expected product as a blue powder (1.48 µmol, **η = 35 %**).

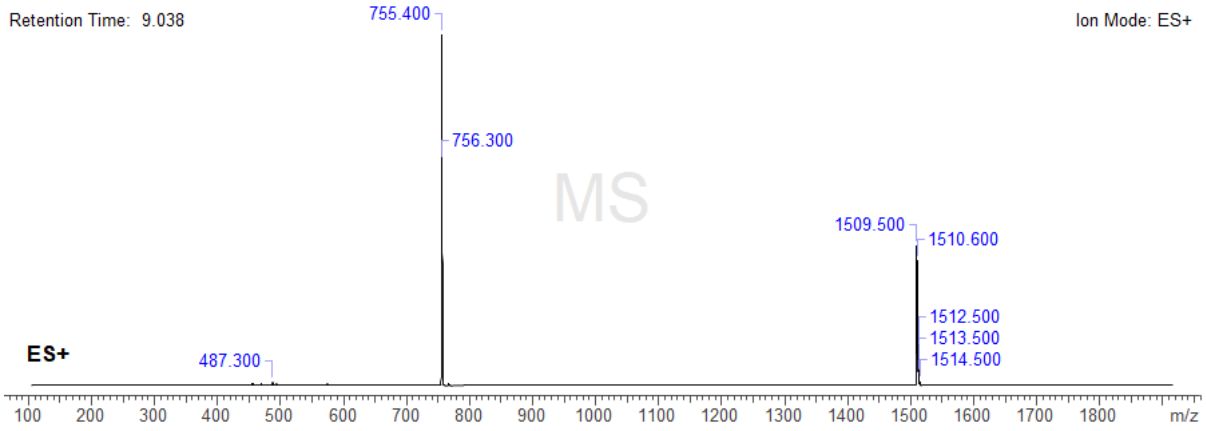
^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 9.33 (t, *J* = 5.5 Hz, 1H, NH), 8.35 (d, *J* = 9.0 Hz, 1H, NH), 8.13 (dd, *J* = 8.0, 1.5 Hz, 1H, , H_{Ar}), 8.06 (d, *J* = 8.0 Hz, 1H, H_{Ar}), 8.00 – 7.96 (m, 2H, , H_{Ar}), 7.72 – 7.66 (m, 2H, H_{Ar}), 7.62 – 7.57 (m, 2H,H_{Ar}), 7.46 – 7.40 (m, 3H, H_{Ar}), 7.39 – 7.31 (m, 5H, H_{Ar}), 7.24 – 7.20 (m, 1H, H_{Ar}), 7.02 (s, 2H, H_{Ar}), 6.63 (s, 4H, H_{Ar}), 5.96 -5.91 (m, 1H, CH), 5.40 (d, *J* = 7.0 Hz, 1H, CH), 5.32 – 5.28 (m, 1H, CH), 4.98 – 4.94 (m, 1H, CH), 4.86 (d, *J* = 13.0 Hz, 1H, 1H from CH_2), 4.76 (d, *J* = 13.0 Hz, 1H, 1H from CH_2), 4.71 (s, 1H, CH), 4.64 (s, 1H, OH), 4.57 – 4.54 (m, 2H, CH_2), 4.43 (d, *J* = 5.5 Hz, 1H, CH), 4.03 (s, 2H, CH_2), 3.76 (dd, *J* = 10.5, 6.5 Hz, 1H, CH), 3.63 (d, *J* = 7.0 Hz, 1H, CH), 3.33 – 3.27 (m, 5H, 1*CH_2 + 1*CH_3), 3.21 (s, 3H, CH_3), 2.92 (s, 12H, 4*CH_3), 2.84 (s, 3H, CH_3), 2.69 – 2.64 (m, 1H, 1H from CH_2), 2.26 (s, 3H, CH_3), 2.19 – 2.11 (m, 2H, CH_2), 2.01 – 1.95 (m, 1H, 1H from CH_2), 1.92 – 1.82 (m, 4H, 1*CH_3 + 1H from CH_2), 1.64 – 1.40 (m, 8H, 1*CH_3 + 2*CH_2 + 1H from CH_2), 1.23 – 1.13 (m, 10H, 5*CH_2), 1.03 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 0.62 (s, 3H, CH_3), 0.52 (s, 3H, CH_3).

^{19}F NMR (376 MHz, DMSO-d_6): δ(ppm) = -74,3.

ESI-MS, positive mode: m/z = 1509.5 $[M\text{-}CF_3CO_2^-]^+$

HRMS (ESI) calculated for $C_{86}H_{105}N_4O_{16}SSi^+$ $[M\text{-}CF_3CO_2^-]^+$: 1509.7010, found: 1509.7014.



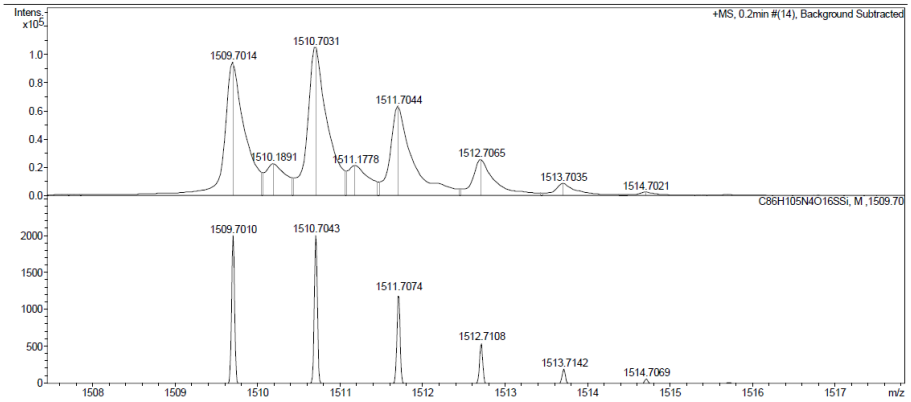


Analysis Info

Analysis Name	Z:\Data\2024\2409\sam040924\MA282_28_01_127434.d	Acquisition Date	04.09.2024 22:52:31
Method	hystar_p.m	Operator	BDAL@DE
Sample Name	MA282	Instrument / Ser#	micrOTOF 10237
Comment			

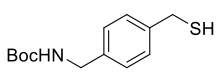
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active			Set Dry Heater	180 °C
Scan Begin	50 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source

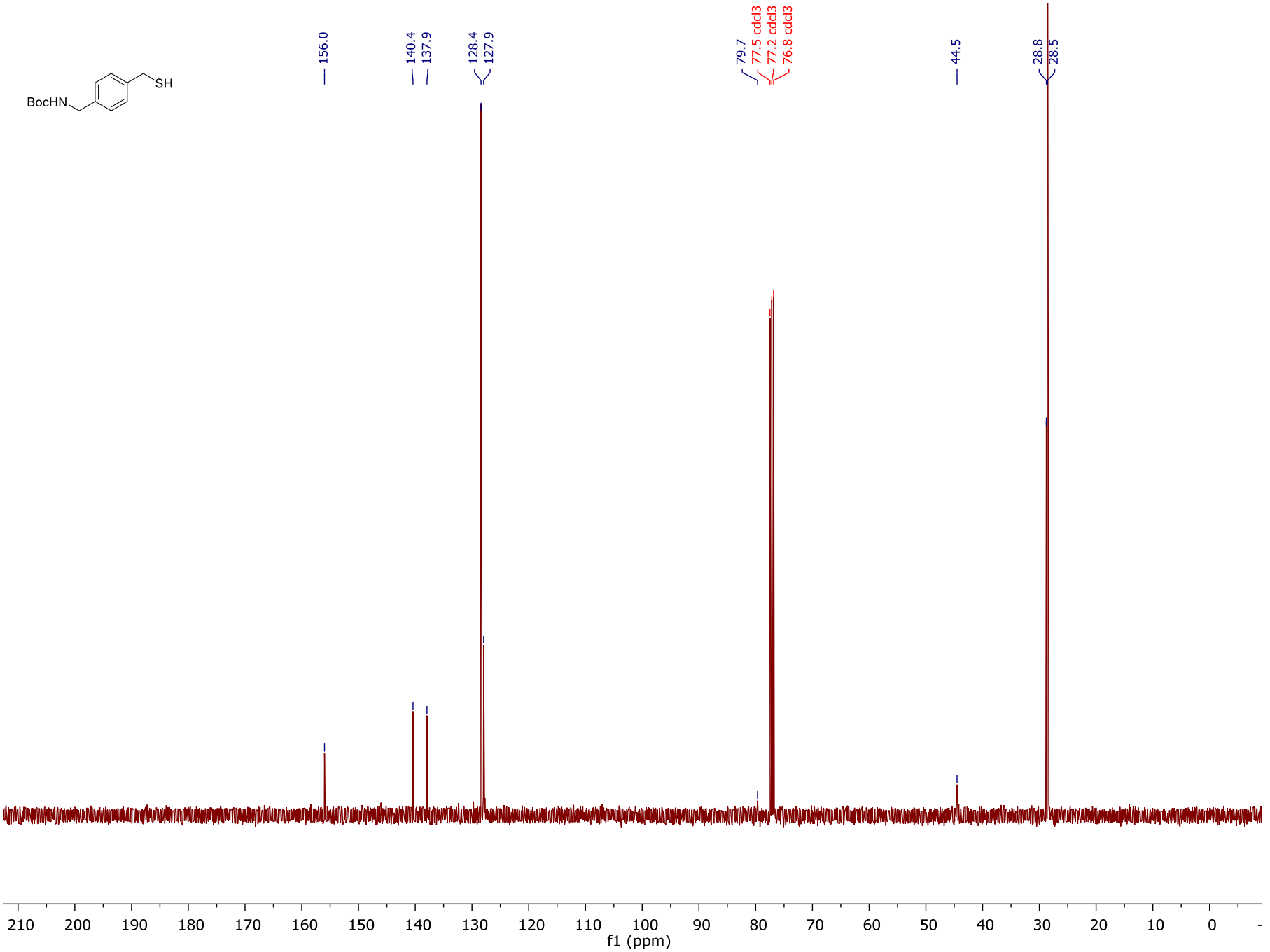


NMR spectra

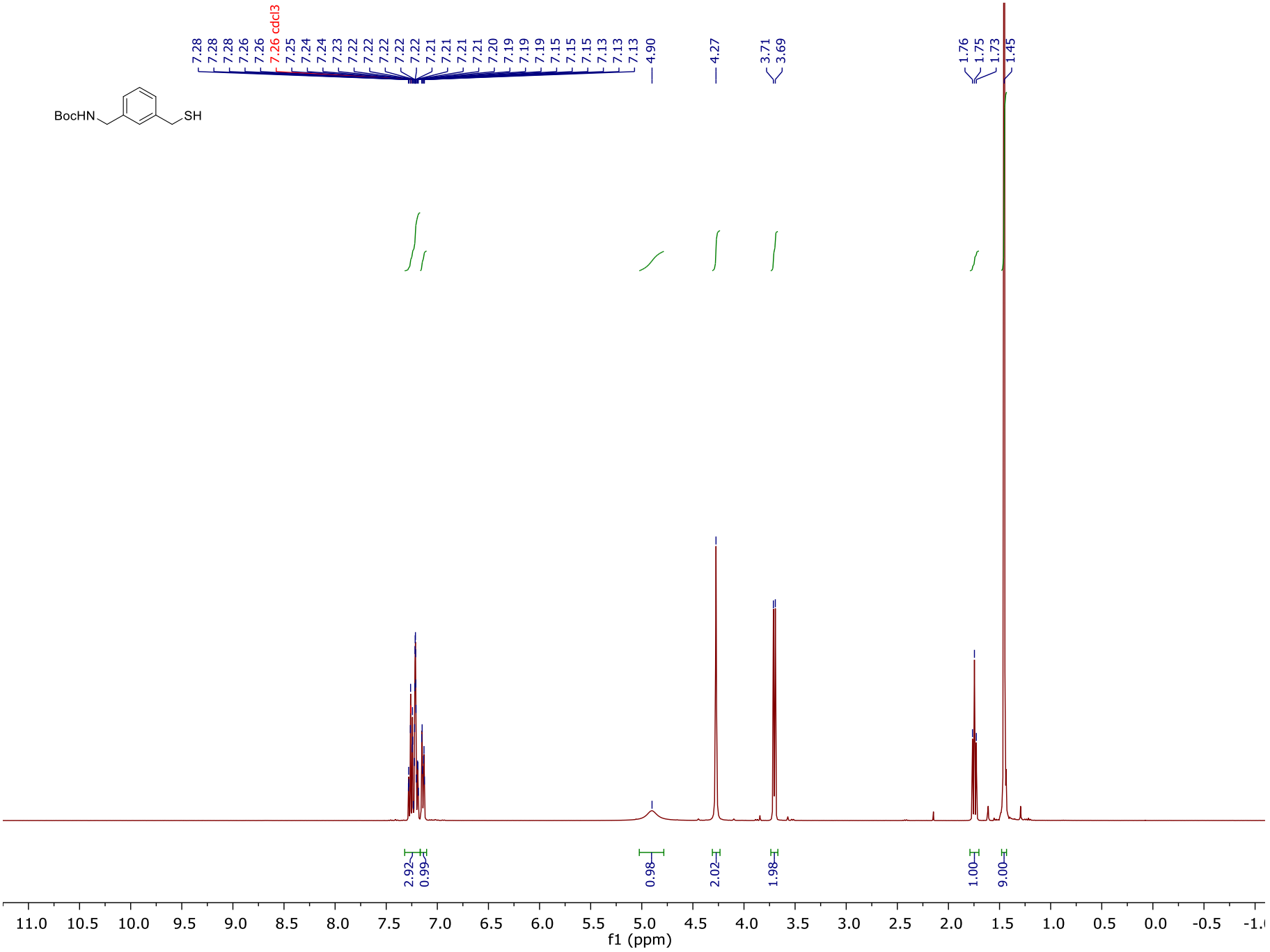
^{1}H NMR of ***p*-7:**



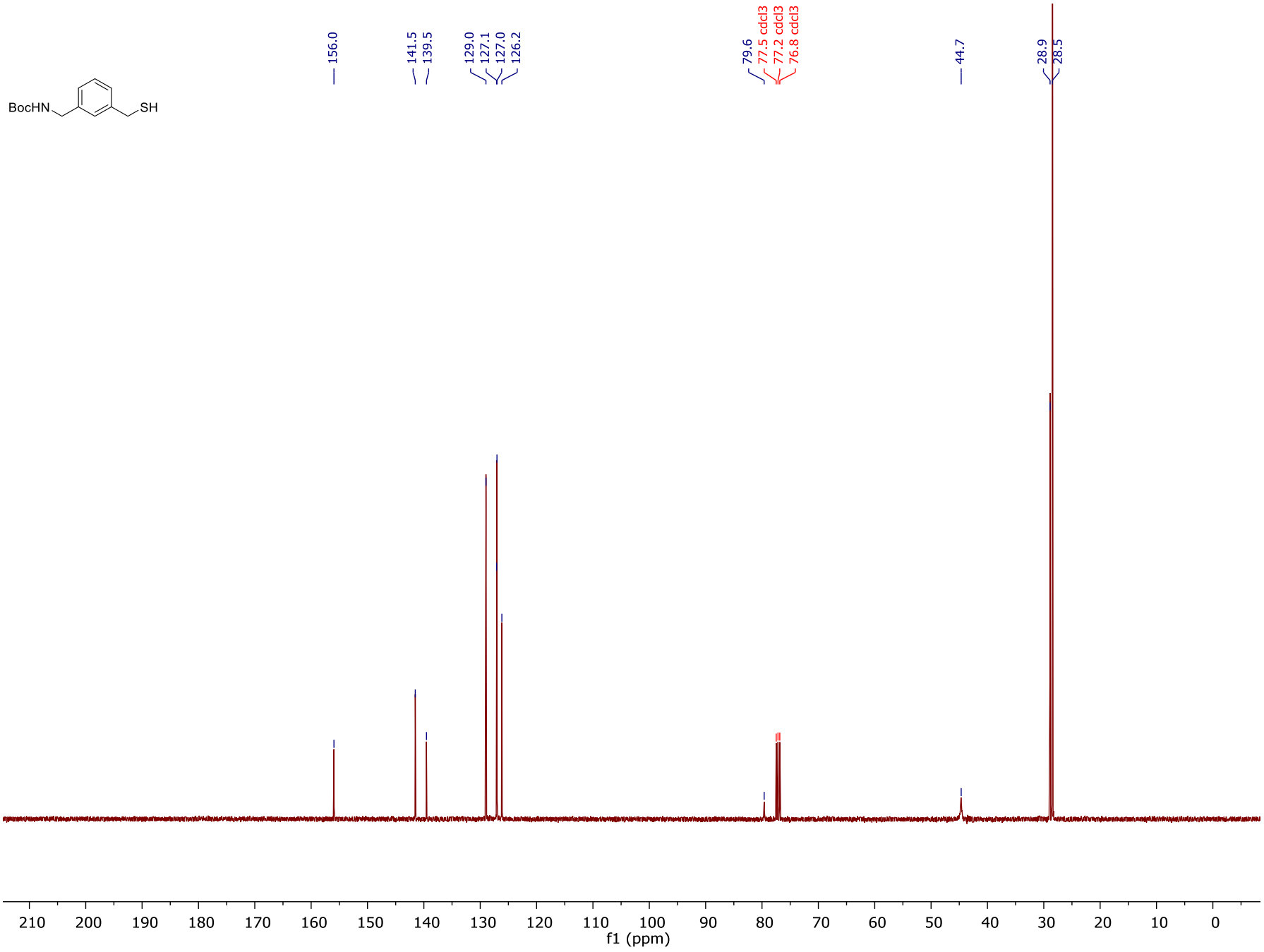
^{13}C NMR of ***p*-7**:


BocHN
SH
156.0
140.4
137.9
128.4
127.9
79.7
77.5 cdcl3
77.2 cdcl3
76.8 cdcl3
44.5
28.8
28.5
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -
f1 (ppm)

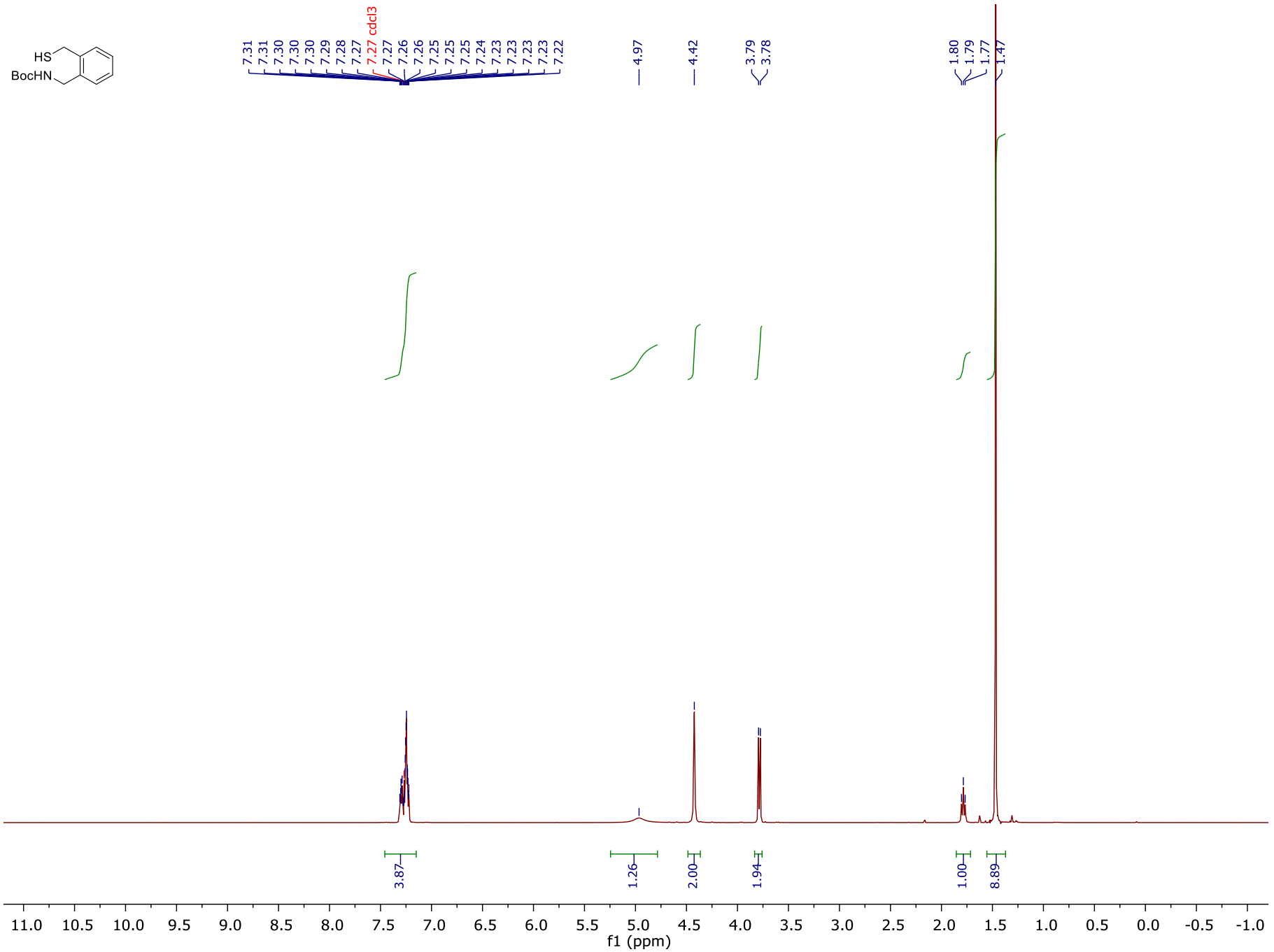
^{1}H NMR of ***m*-7**:


BocHN
SH
7.28
7.28
7.28
7.26
7.26
7.26 cdcl3
7.25
7.24
7.24
7.23
7.22
7.22
7.22
7.22
7.21
7.21
7.21
7.21
7.20
7.19
7.19
7.19
7.15
7.15
7.15
7.13
7.13
7.13
4.90
4.27
3.71
3.69
1.76
1.75
1.73
1.45
2.92
0.99
0.98
2.02
1.98
1.00
9.00
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

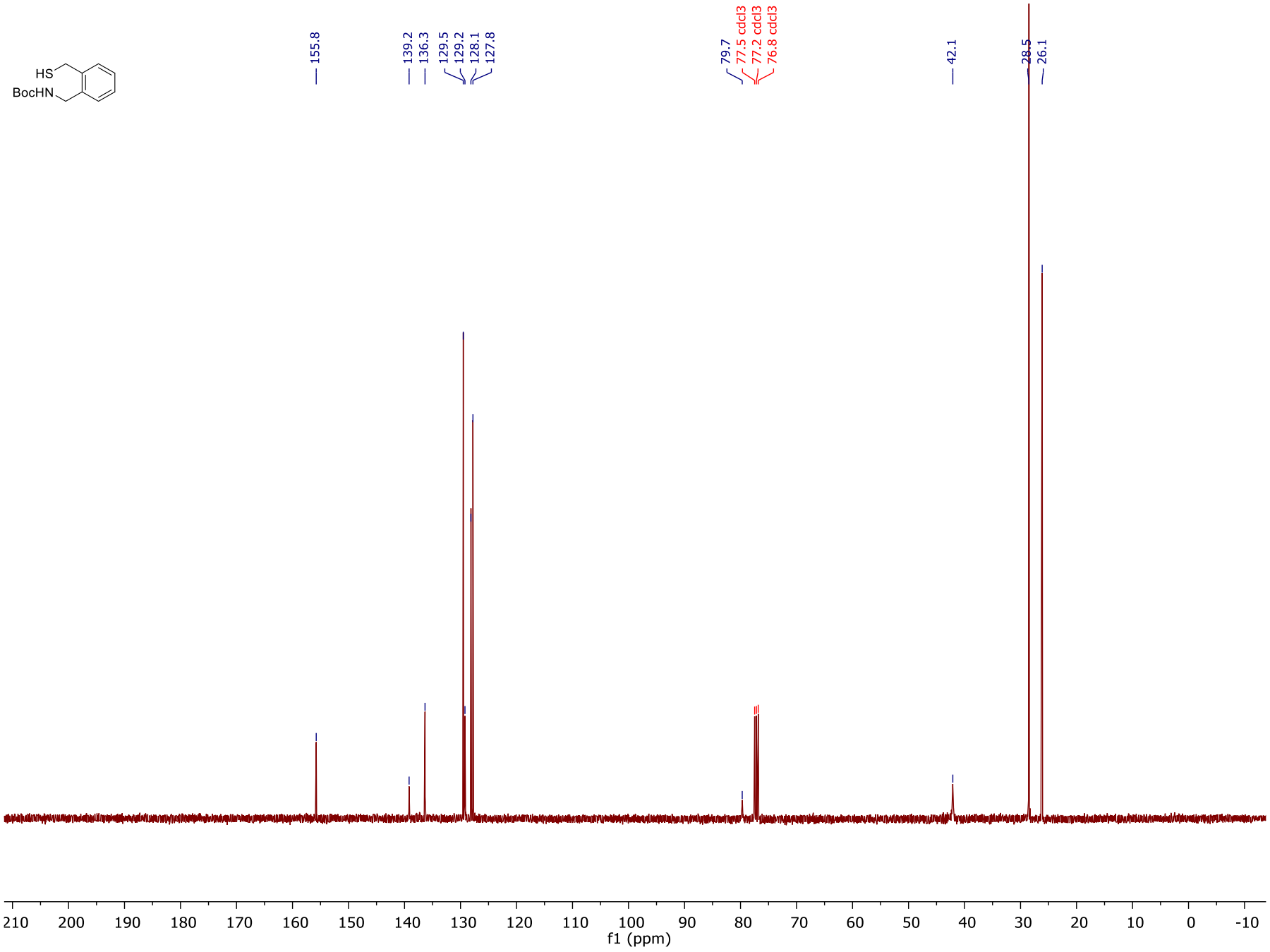
^{13}C NMR of ***m*-7**:


BocHN
SH
156.0
141.5
139.5
129.0
127.1
127.0
126.2
79.6
77.5 cdcl3
77.2 cdcl3
76.8 cdcl3
44.7
28.9
28.5
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

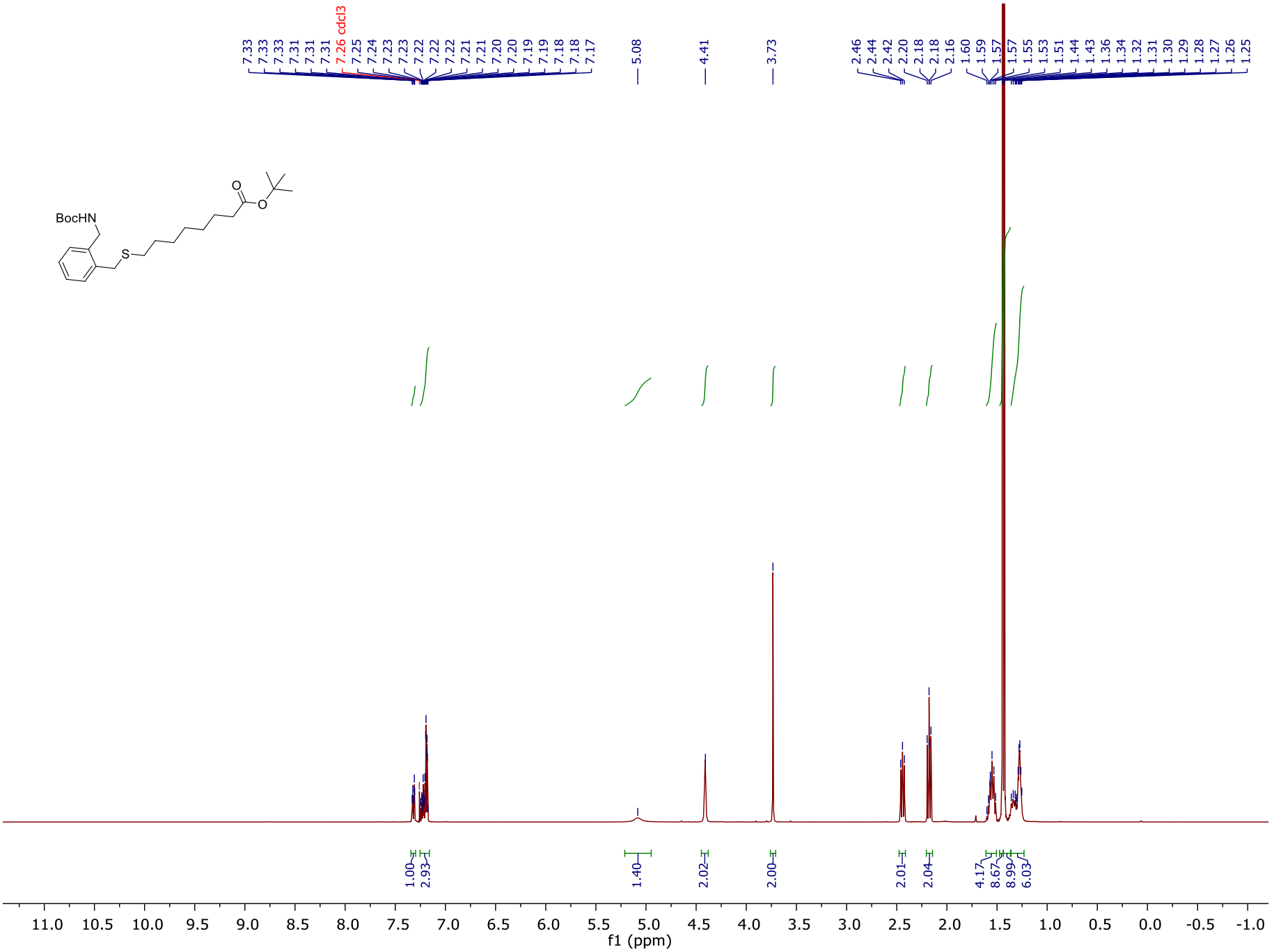
^{1}H NMR of ***o*-7:**


HS
BocHN
7.31
7.31
7.30
7.30
7.30
7.29
7.28
7.27
7.27 cdcl3
7.27
7.26
7.26
7.25
7.25
7.25
7.24
7.23
7.23
7.23
7.23
7.22
4.97
4.42
3.79
3.78
1.80
1.79
1.77
1.47
3.87
1.26
2.00
1.94
1.00
8.89
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)

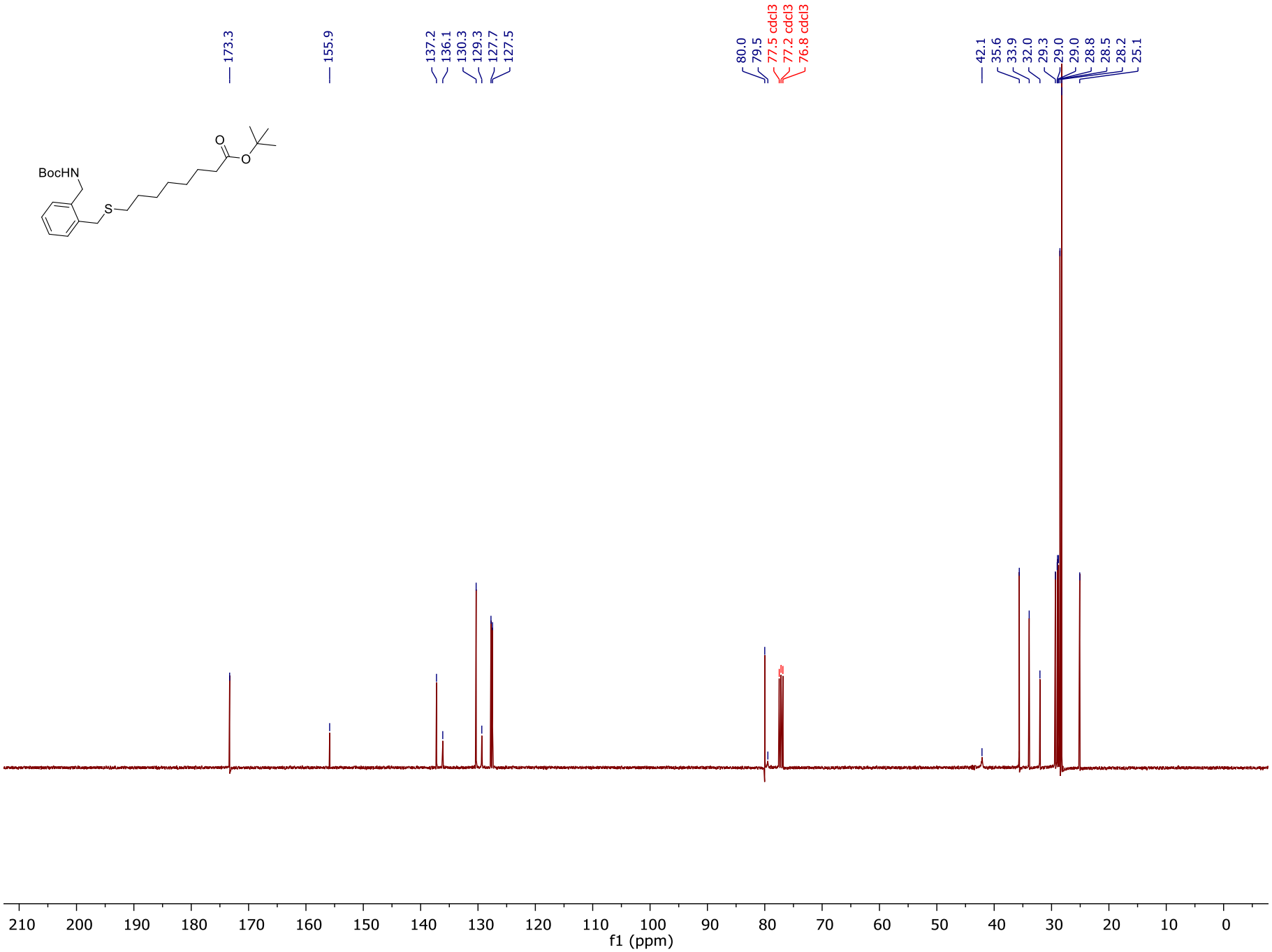
^{13}C NMR of ***o*-7**:


HS
BocHN
155.8
139.2
136.3
129.5
129.2
128.1
127.8
79.7
77.5 cdcl3
77.2 cdcl3
76.8 cdcl3
42.1
28.5
26.1
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

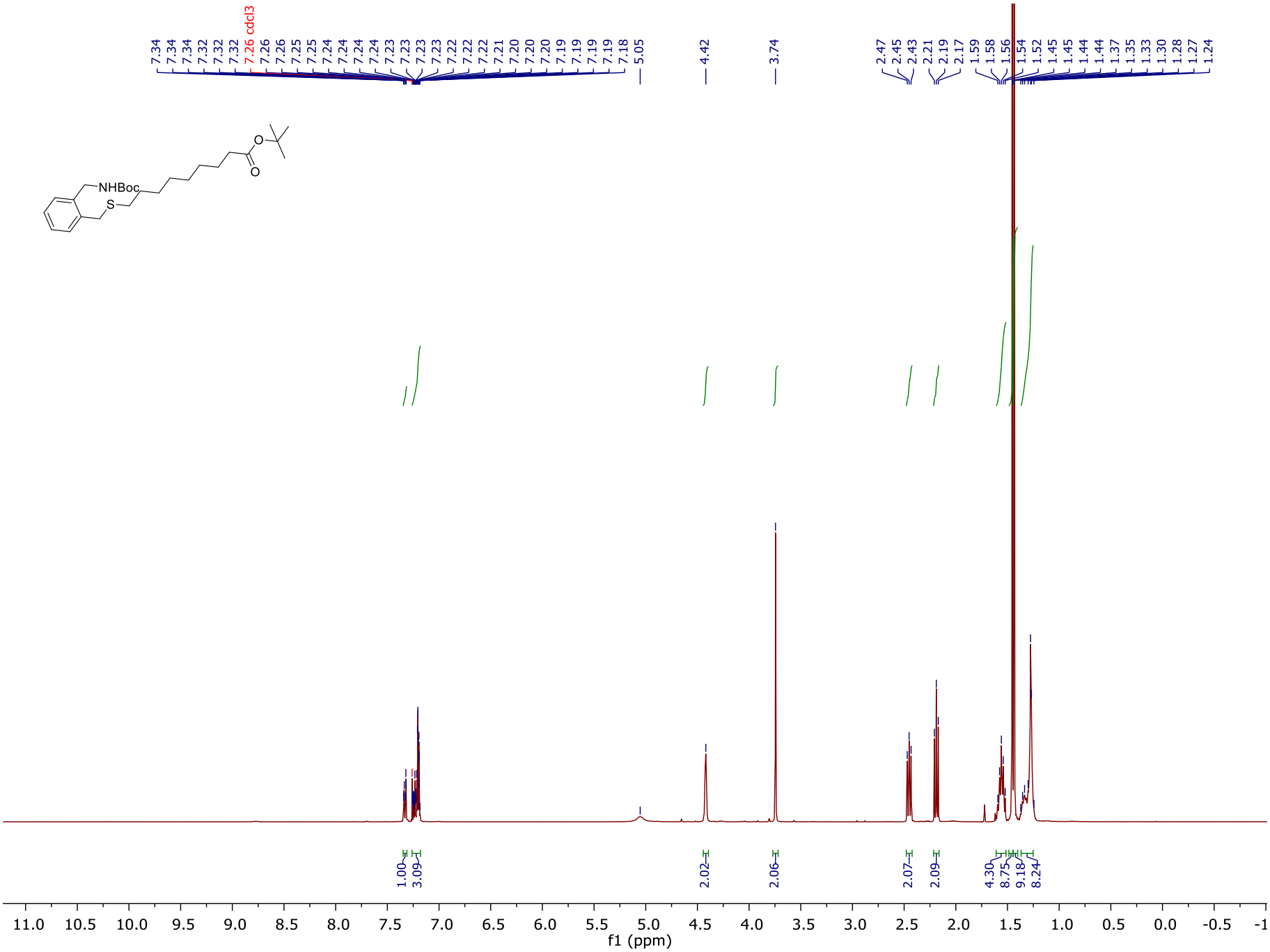
^{1}H NMR of **8:**


7.33 7.33 7.33 7.31 7.31 7.31 7.26 cdcl3 7.25 7.24 7.23 7.23 7.22 7.22 7.22 7.21 7.21 7.20 7.20 7.19 7.19 7.18 7.18 7.17
5.08
4.41
3.73
2.46 2.44 2.42 2.20 2.18 2.18 2.16 1.60 1.59 1.57 1.57 1.55 1.53 1.51 1.44 1.43 1.36 1.34 1.32 1.31 1.30 1.29 1.28 1.27 1.26 1.25
BocHN
O
O
S
1.00 2.93 1.40 2.02 2.00 2.01 2.04 4.17 8.67 8.99 6.03
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)

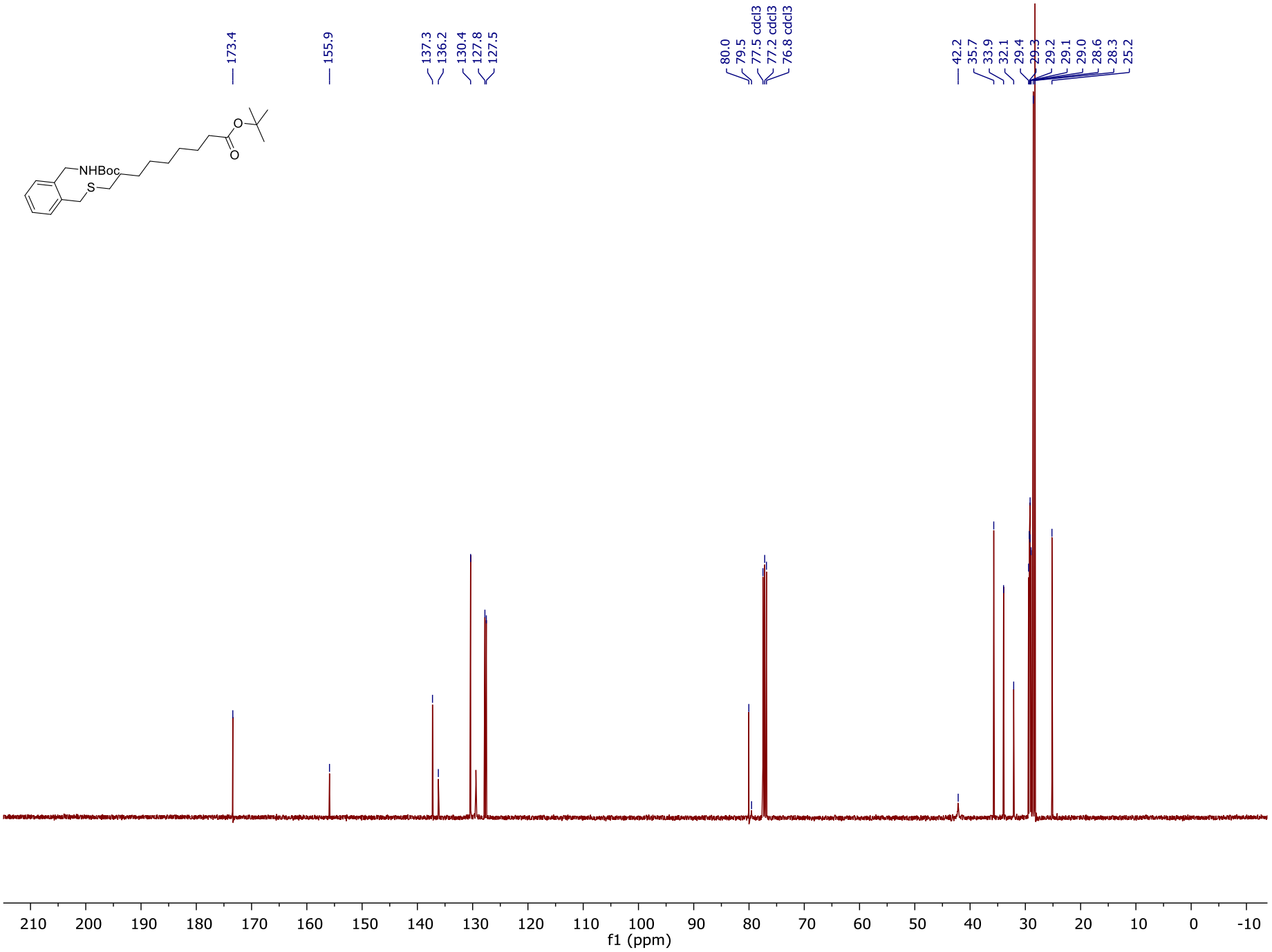
^{13}C NMR of **8:**


173.3
155.9
137.2
136.1
130.3
129.3
127.7
127.5
80.0
79.5
77.5 cdcl3
77.2 cdcl3
76.8 cdcl3
42.1
35.6
33.9
32.0
29.3
29.0
29.0
28.8
28.5
28.2
25.1
BocHN
O
O
S
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

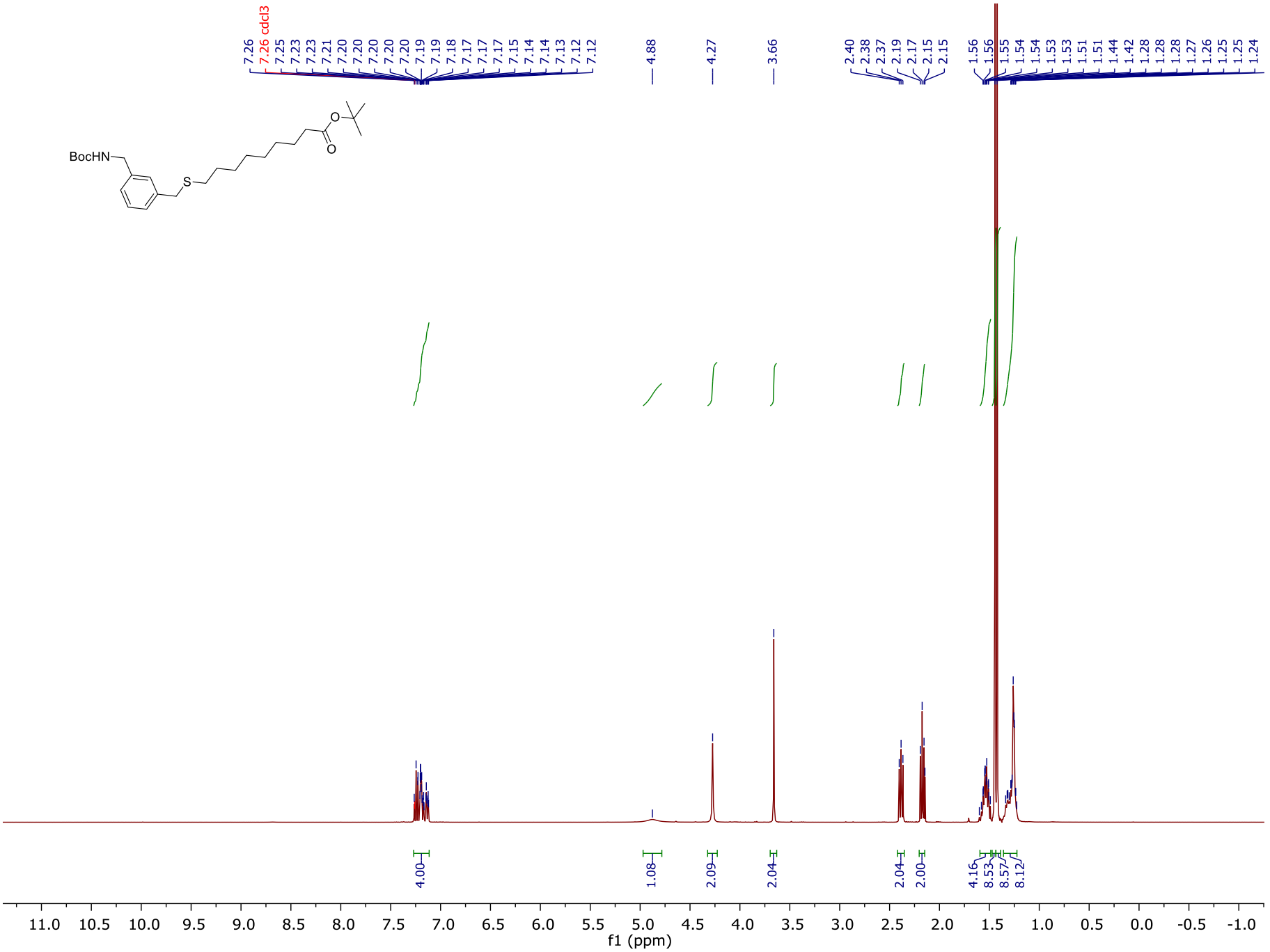
^{1}H NMR of **9:**


7.34 7.34 7.34 7.32 7.32 7.32 7.26 cdcl3 7.26 7.26 7.25 7.25 7.24 7.24 7.24 7.24 7.23 7.23 7.23 7.23 7.22 7.22 7.22 7.21 7.20 7.20 7.20 7.19 7.19 7.19 7.19 7.18 5.05 4.42 3.74 2.47 2.45 2.43 2.21 2.19 2.17 1.59 1.58 1.56 1.54 1.52 1.45 1.45 1.44 1.44 1.37 1.35 1.33 1.30 1.28 1.27 1.24
NHBoc
S
O
O
1.00 3.09 2.02 2.06 2.07 2.09 4.30 8.75 9.18 8.24
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1
f1 (ppm)

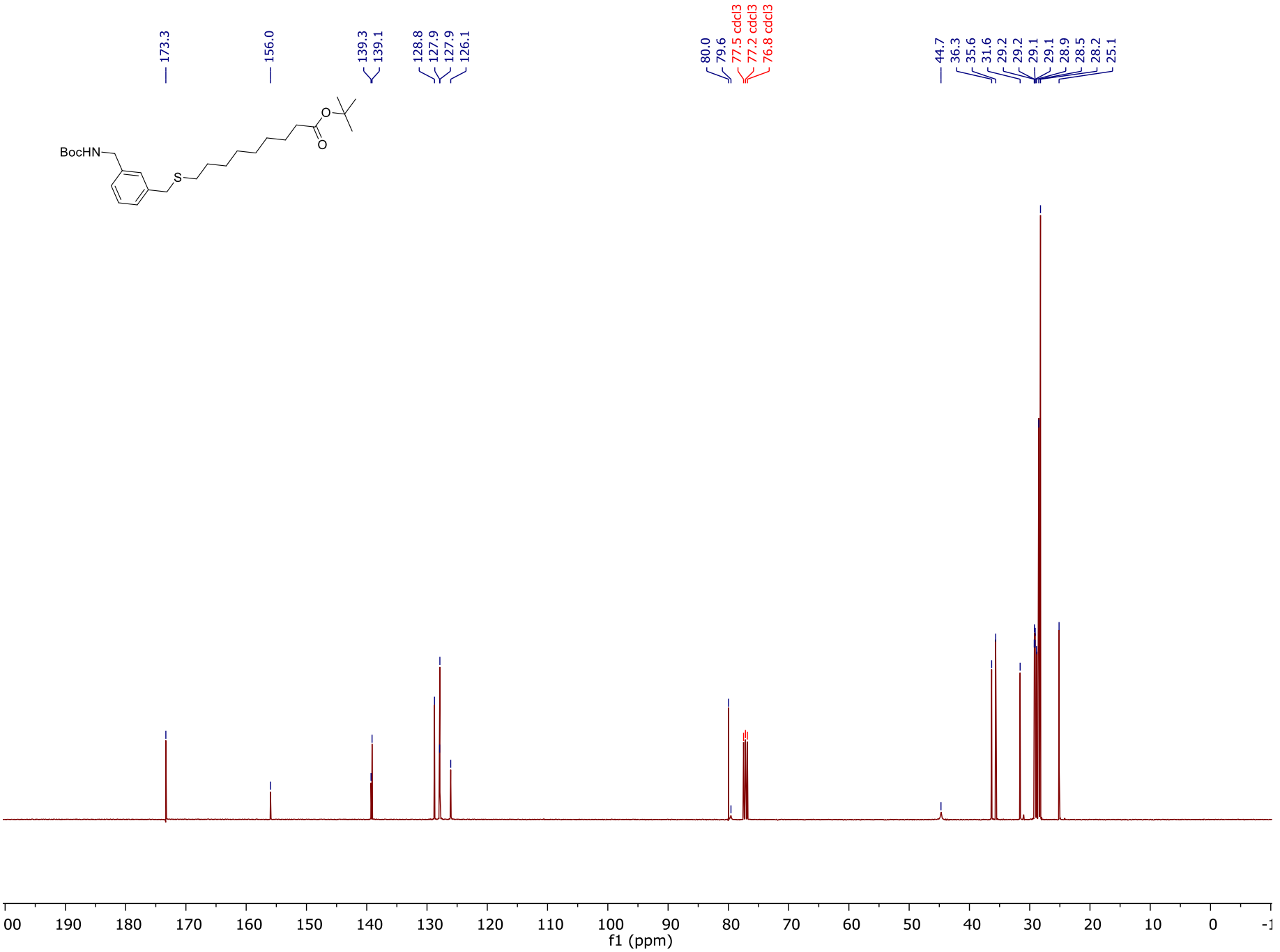
^{13}C NMR of **9:**


173.4
155.9
137.3
136.2
130.4
127.8
127.5
80.0
79.5
77.5 cdcl3
77.2 cdcl3
76.8 cdcl3
42.2
35.7
33.9
32.1
29.4
29.3
29.2
29.1
29.0
28.6
28.3
25.2
NHBoc
S
O
O
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

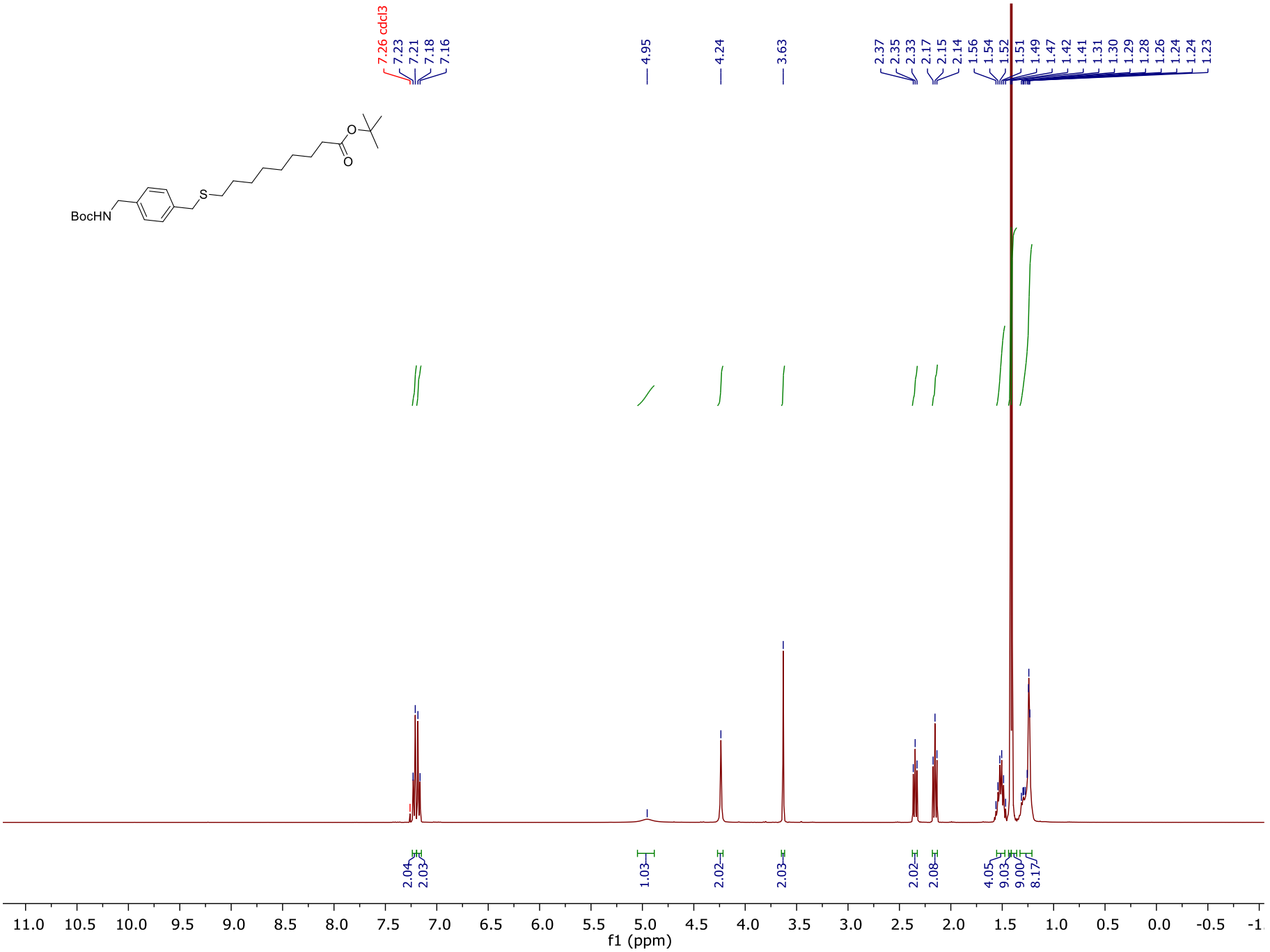
^{1}H NMR of **10:**


7.26
7.26 cdcl3
7.25
7.23
7.23
7.21
7.20
7.20
7.20
7.20
7.20
7.19
7.19
7.18
7.17
7.17
7.17
7.15
7.14
7.14
7.13
7.12
7.12
4.88
4.27
3.66
2.40
2.38
2.37
2.19
2.17
2.15
2.15
1.56
1.56
1.55
1.54
1.54
1.53
1.53
1.51
1.51
1.44
1.42
1.28
1.28
1.28
1.27
1.26
1.25
1.25
1.24
BocHN
S
O
O
4.00
1.08
2.09
2.04
2.04
2.00
4.16
8.53
8.57
8.12
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
f1 (ppm)

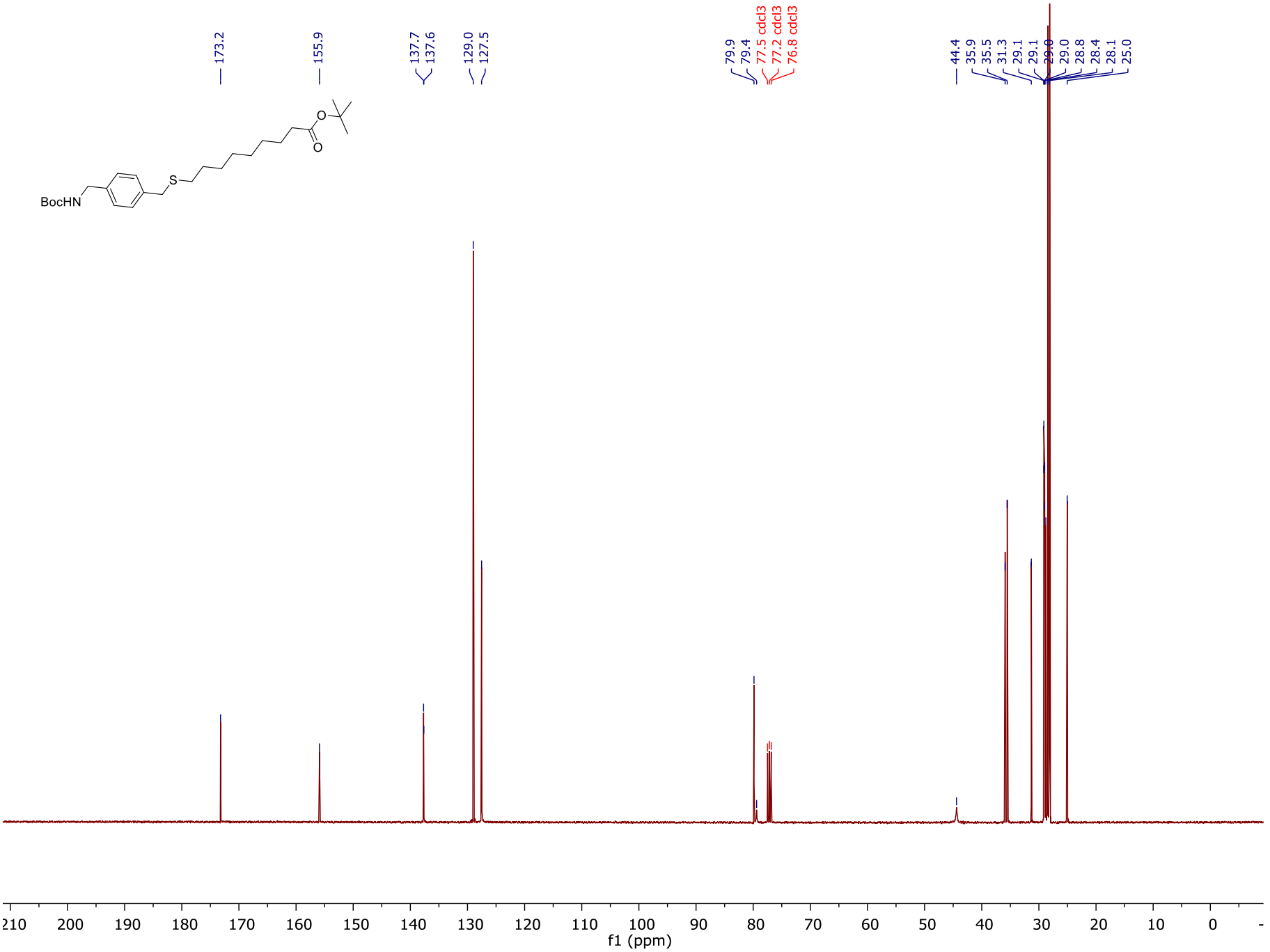
^{13}C NMR of **10:**


173.3
156.0
139.3
139.1
128.8
127.9
127.9
126.1
80.0
79.6
77.5 cdcl3
77.2 cdcl3
76.8 cdcl3
44.7
36.3
35.6
31.6
29.2
29.2
29.1
29.1
28.9
28.5
28.2
25.1
BocHN
S
O
O
f1 (ppm)

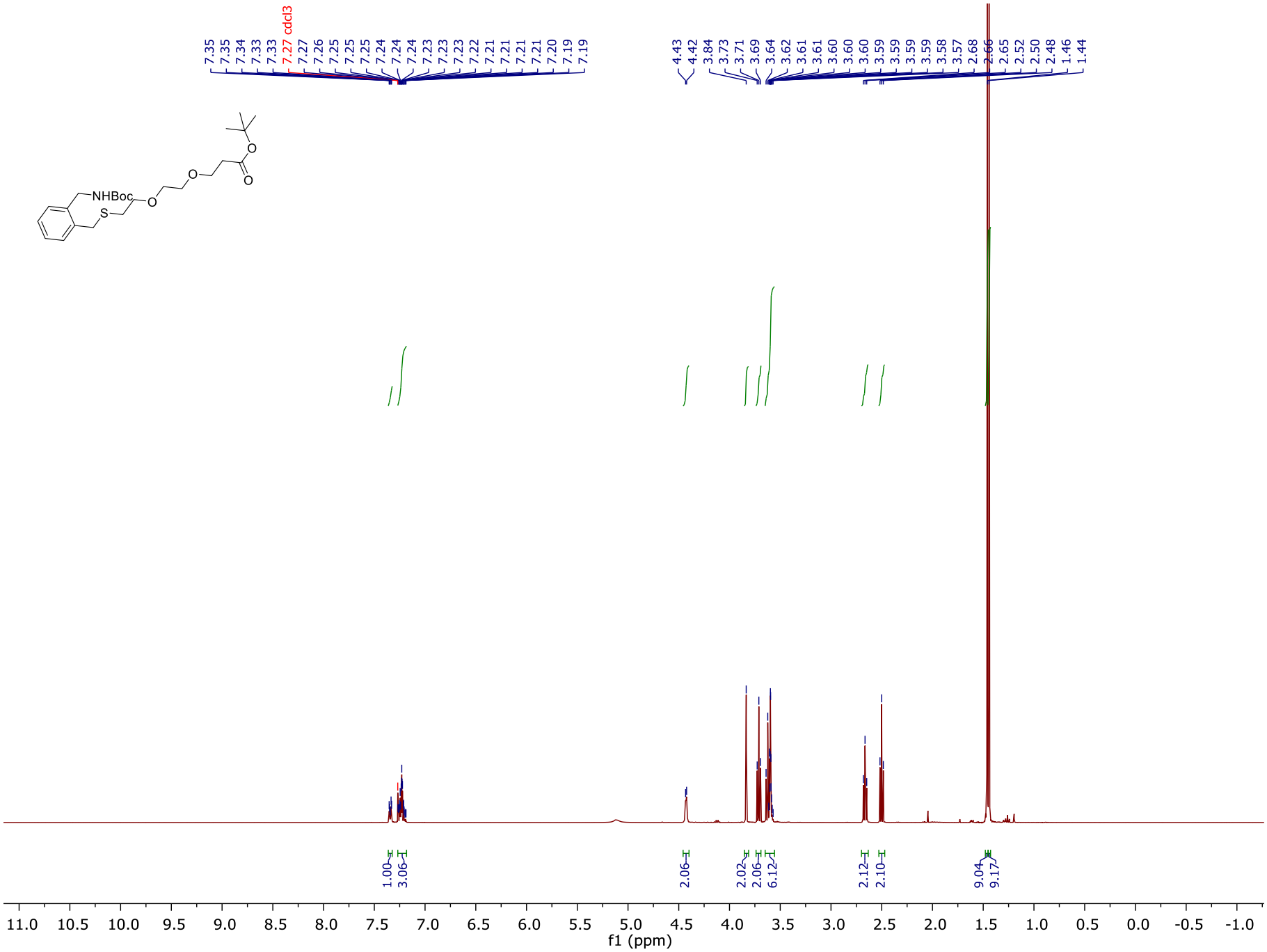
^{1}H NMR of **11***:*


7.26 cdcl3
7.23
7.21
7.18
7.16
4.95
4.24
3.63
2.37
2.35
2.33
2.17
2.15
2.14
1.56
1.54
1.52
1.51
1.49
1.47
1.42
1.41
1.31
1.30
1.29
1.28
1.26
1.24
1.24
1.23
BocHN
S
O
O
2.04
2.03
1.03
2.02
2.03
2.02
2.08
4.05
9.03
9.00
8.17
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

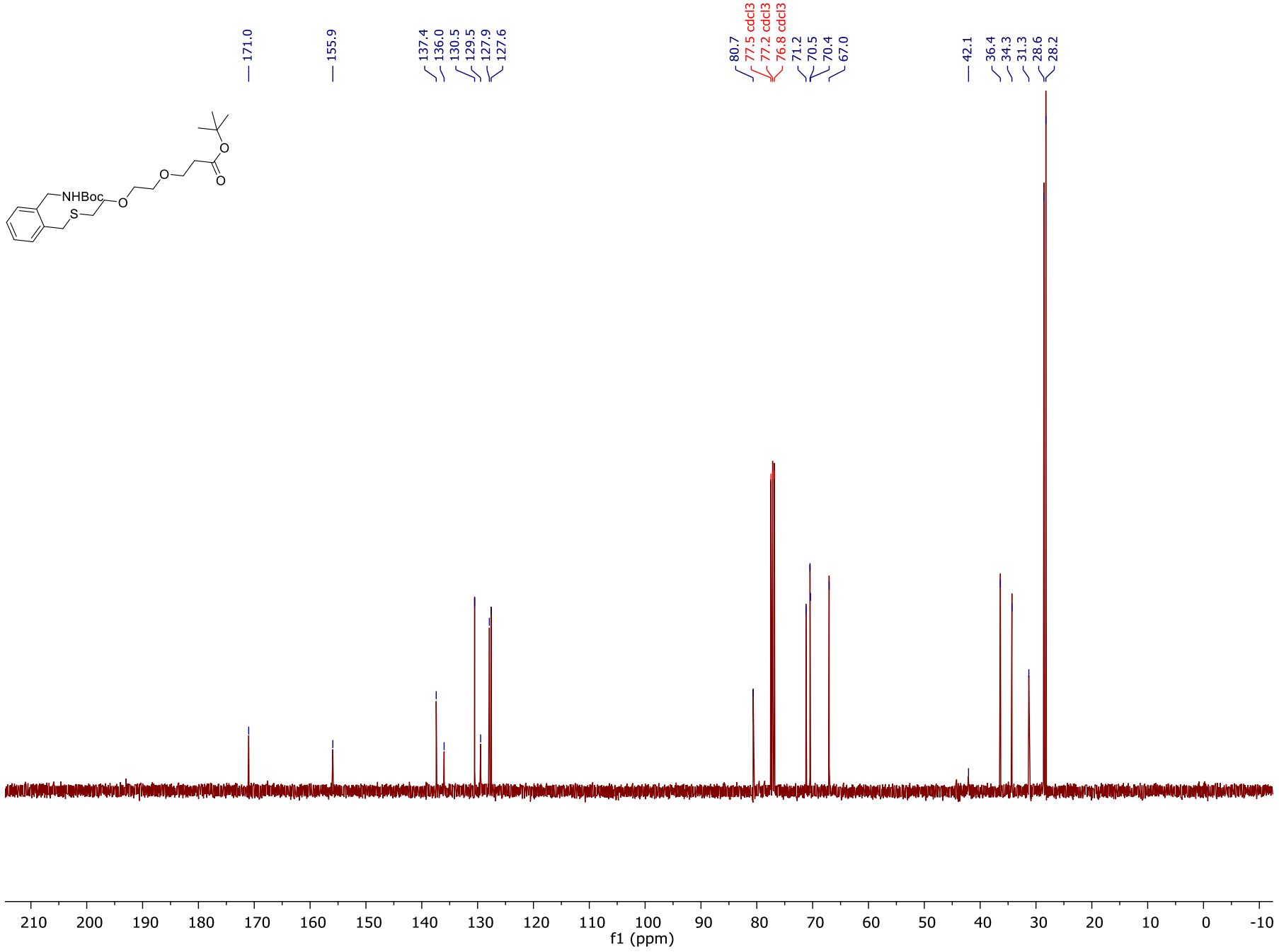
^{13}C NMR of **11:**


173.2
155.9
137.7
137.6
129.0
127.5
79.9
79.4
77.5 cdcl3
77.2 cdcl3
76.8 cdcl3
44.4
35.9
35.5
31.3
29.1
29.1
29.0
29.0
28.8
28.4
28.1
25.0
BocHN
S
O
O
f1 (ppm)

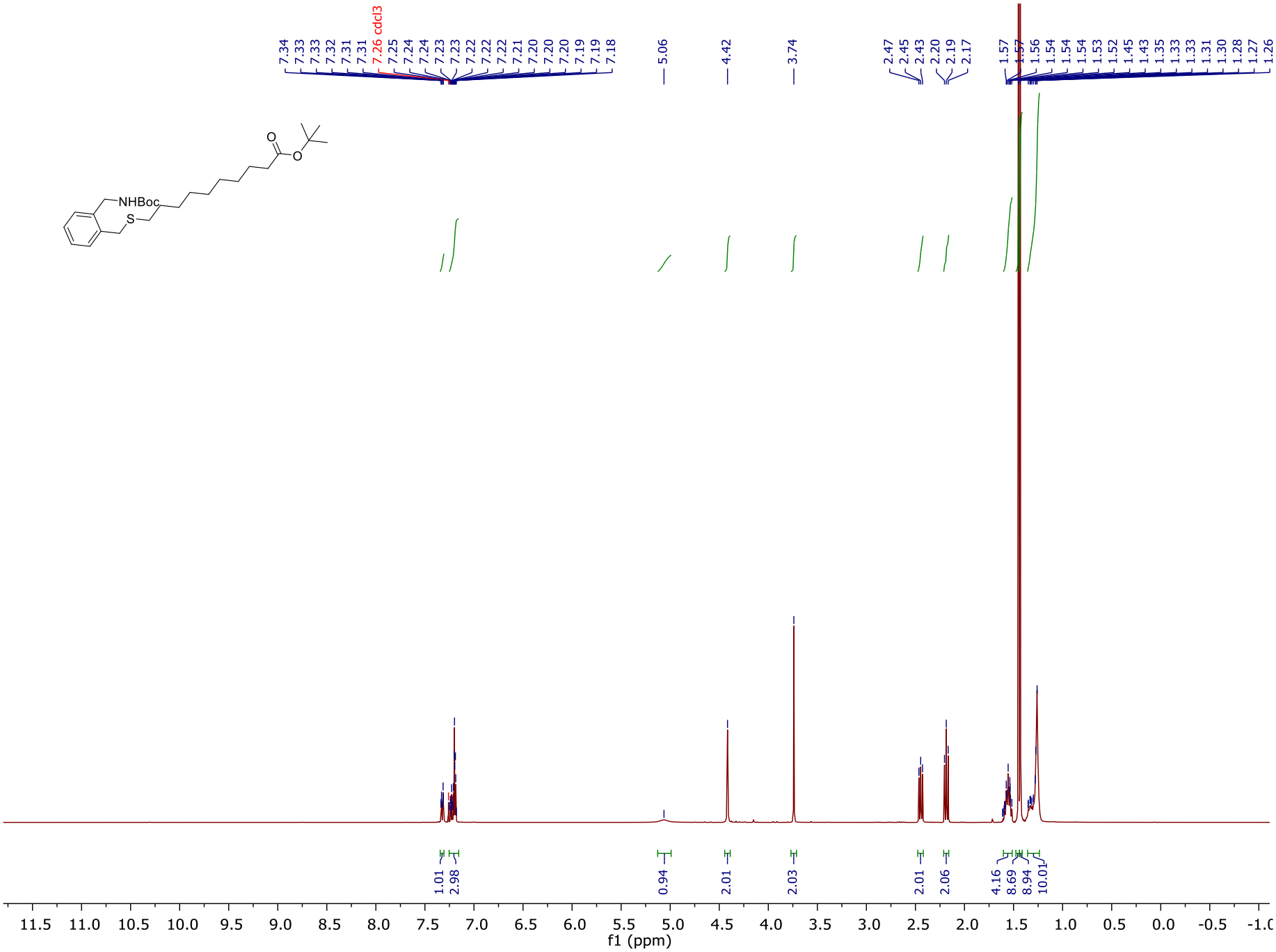
^{1}H NMR of **12:**


7.35 7.35 7.34 7.33 7.33 7.27 cdcl3 7.27 7.26 7.25 7.25 7.24 7.24 7.24 7.23 7.23 7.23 7.22 7.21 7.21 7.21 7.20 7.19 7.19
4.43 4.42 3.84 3.73 3.71 3.69 3.64 3.62 3.61 3.61 3.60 3.60 3.60 3.59 3.59 3.59 3.59 3.58 3.57 2.68 2.66 2.65 2.52 2.50 2.48 1.46 1.44
NHBoc
S
O
O
O
O
O
1.00 3.06 2.06 2.02 2.06 6.12 2.12 2.10 9.04 9.17
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)

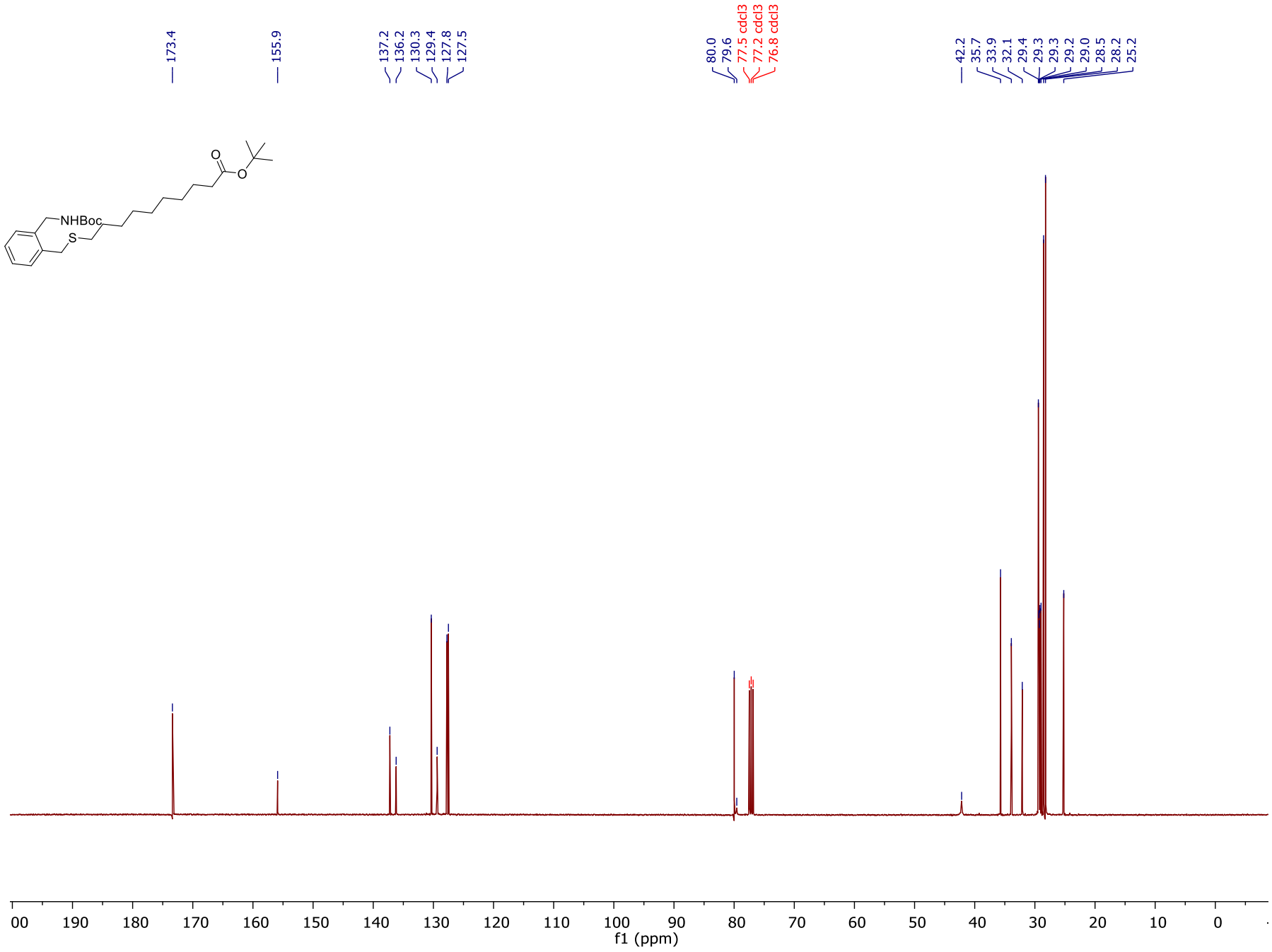
^{13}C NMR of **12:**


171.0
155.9
137.4
136.0
130.5
129.5
127.9
127.6
80.7
77.5 cdcl3
77.2 cdcl3
76.8 cdcl3
71.2
70.5
70.4
67.0
42.1
36.4
34.3
31.3
28.6
28.2
NHBoc
S
O
O
O
O
O
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

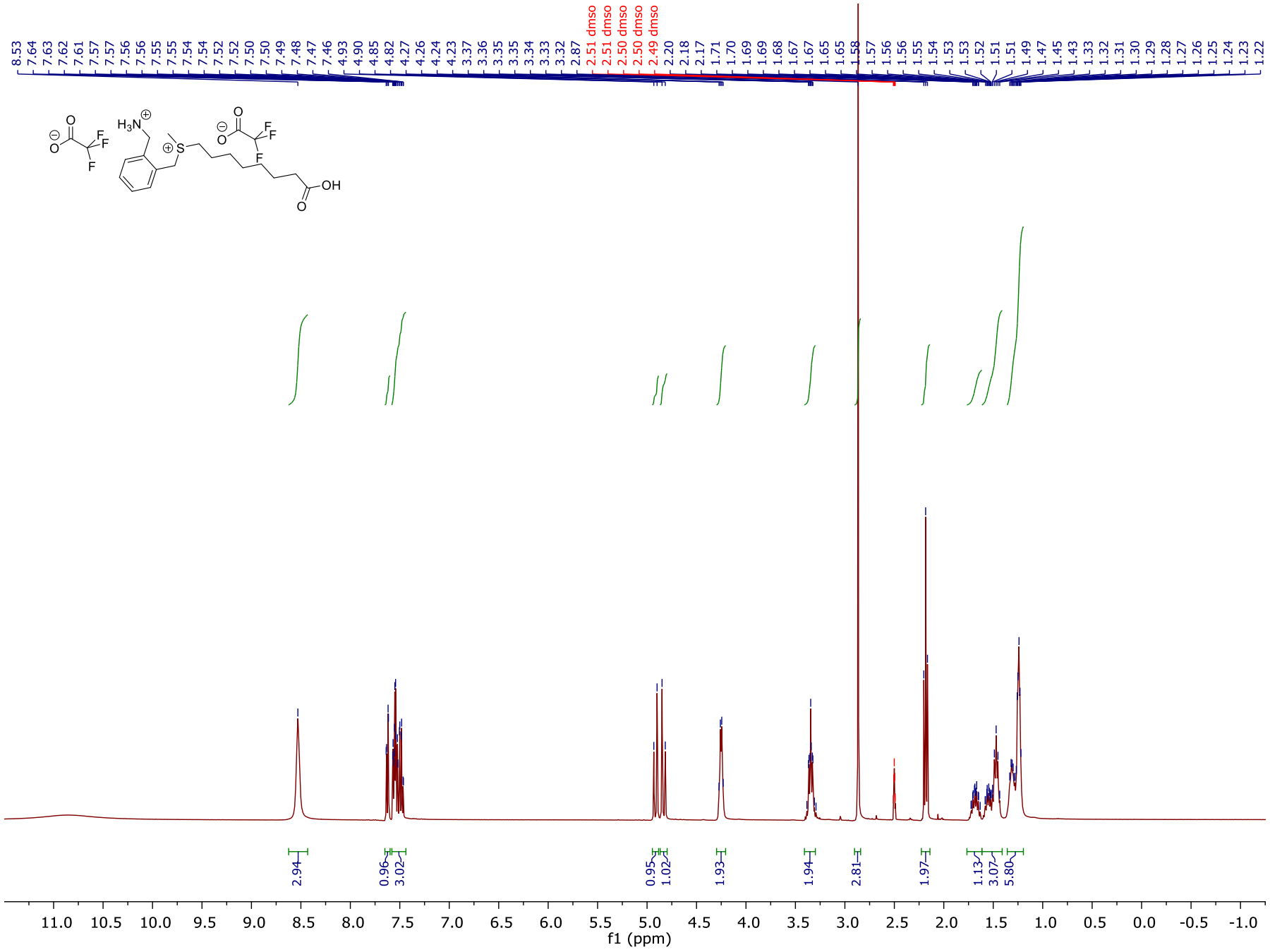
^{1}H NMR of **13:**


7.34
7.33
7.33
7.32
7.31
7.31
7.26 cdcl3
7.25
7.24
7.24
7.23
7.23
7.22
7.22
7.22
7.21
7.20
7.20
7.20
7.19
7.19
7.18
5.06
4.42
3.74
2.47
2.45
2.43
2.20
2.19
2.17
1.57
1.57
1.56
1.54
1.54
1.54
1.53
1.52
1.45
1.43
1.35
1.33
1.33
1.31
1.30
1.28
1.27
1.26
NHBoc
S
O
O
1.01
2.98
0.94
2.01
2.03
2.01
2.06
4.16
8.69
8.94
10.01
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

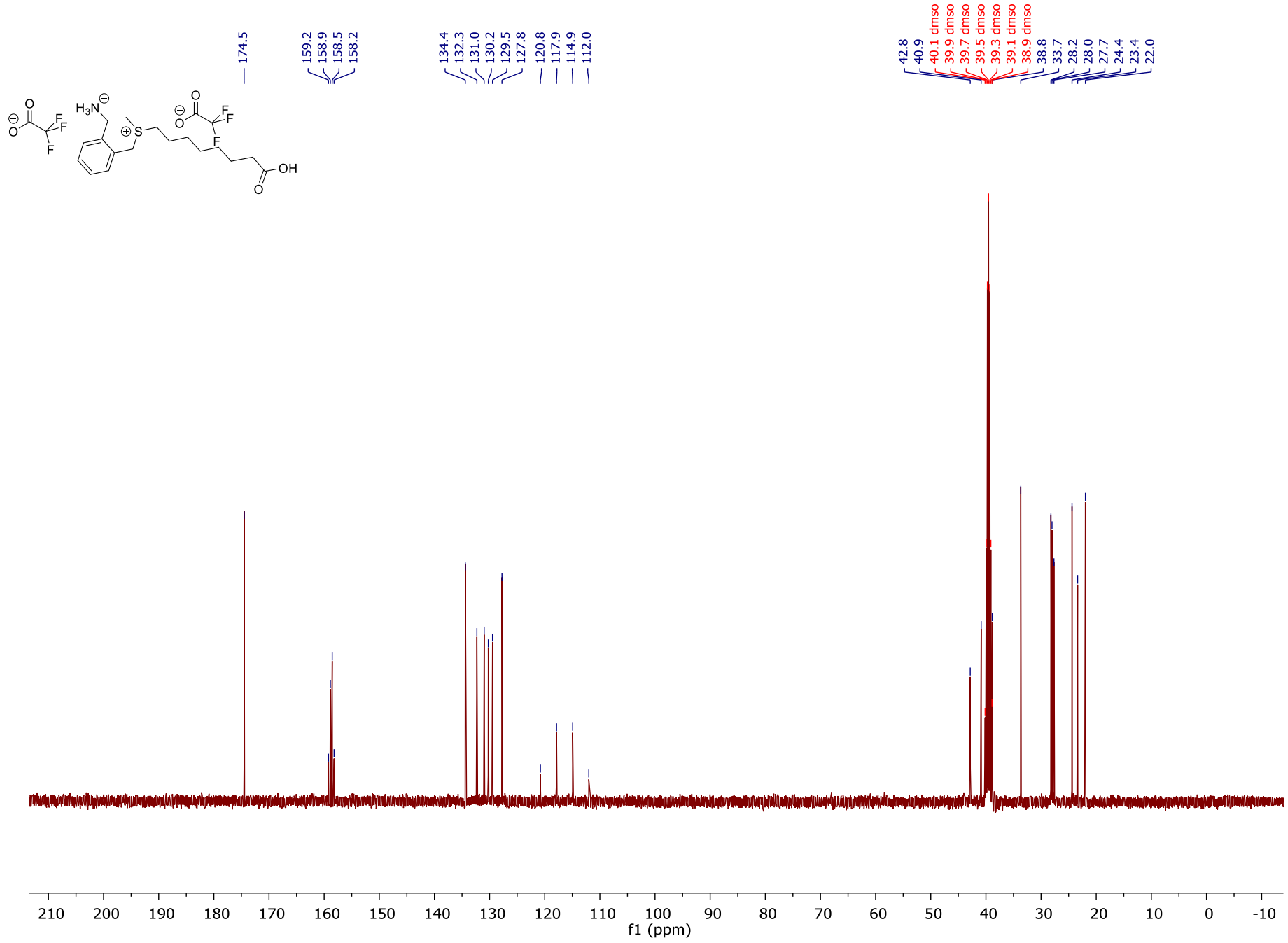
^{13}C NMR of **13:**


173.4
155.9
137.2
136.2
130.3
129.4
127.8
127.5
80.0
79.6
77.5 cdcl3
77.2 cdcl3
76.8 cdcl3
42.2
35.7
33.9
32.1
29.4
29.3
29.3
29.2
29.0
28.5
28.2
25.2
NHBoc
S
O
O
f1 (ppm)

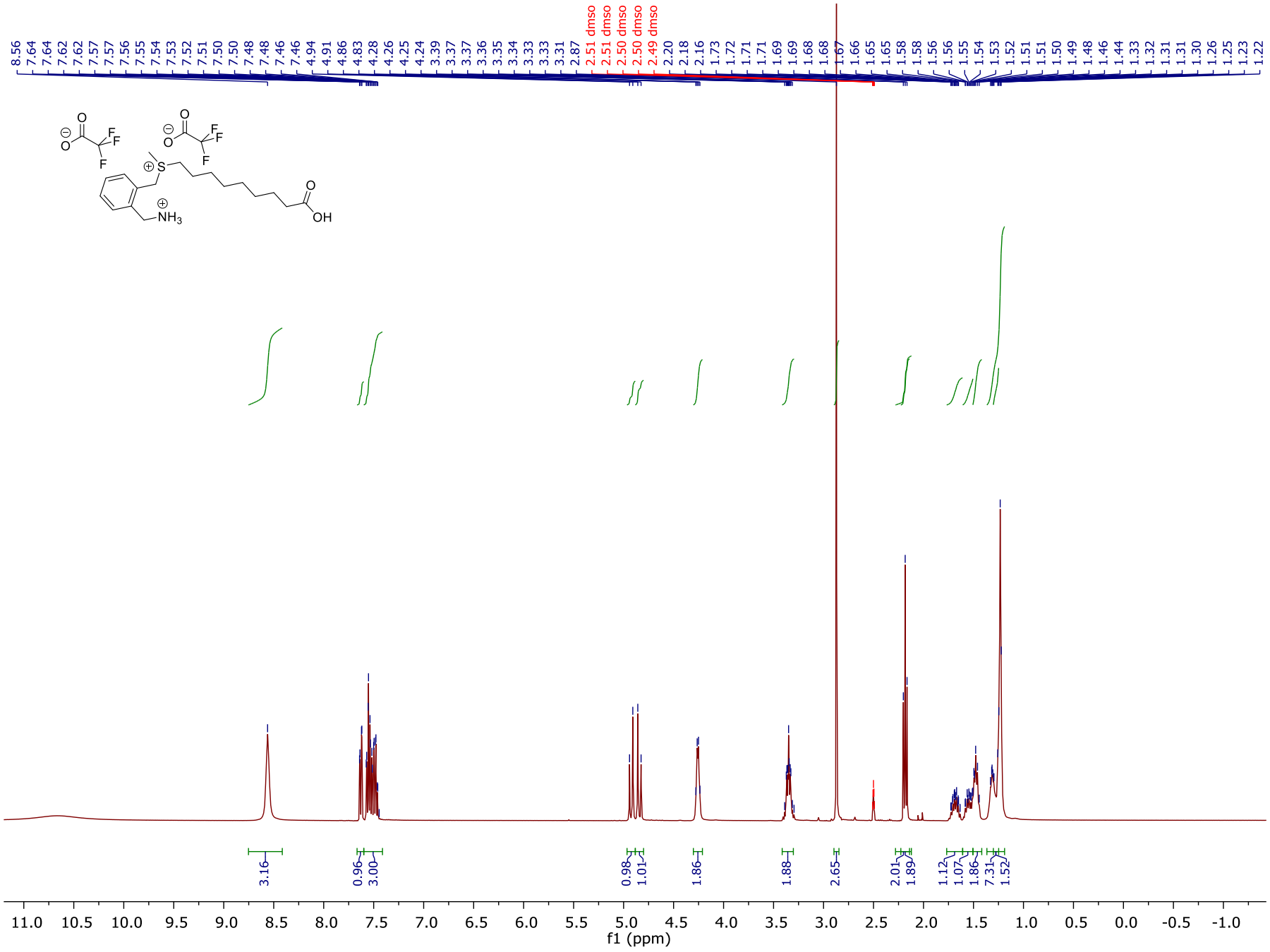
^{1}H NMR of **14:**


8.53 7.64 7.63 7.62 7.61 7.57 7.57 7.56 7.56 7.55 7.55 7.54 7.54 7.52 7.52 7.50 7.50 7.49 7.48 7.47 7.46 4.93 4.90 4.85 4.82 4.27 4.26 4.24 4.23 3.37 3.36 3.35 3.35 3.34 3.33 3.32 2.87 2.51 dmso 2.51 dmso 2.50 dmso 2.50 dmso 2.49 dmso 2.20 2.18 2.17 1.71 1.70 1.69 1.69 1.68 1.67 1.67 1.65 1.65 1.58 1.57 1.56 1.56 1.55 1.54 1.53 1.53 1.52 1.51 1.51 1.49 1.47 1.45 1.43 1.33 1.32 1.31 1.30 1.29 1.28 1.27 1.26 1.25 1.24 1.23 1.22
2.94 0.96 3.02 0.95 1.02 1.93 1.94 2.81 1.97 1.13 3.07 5.80
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)

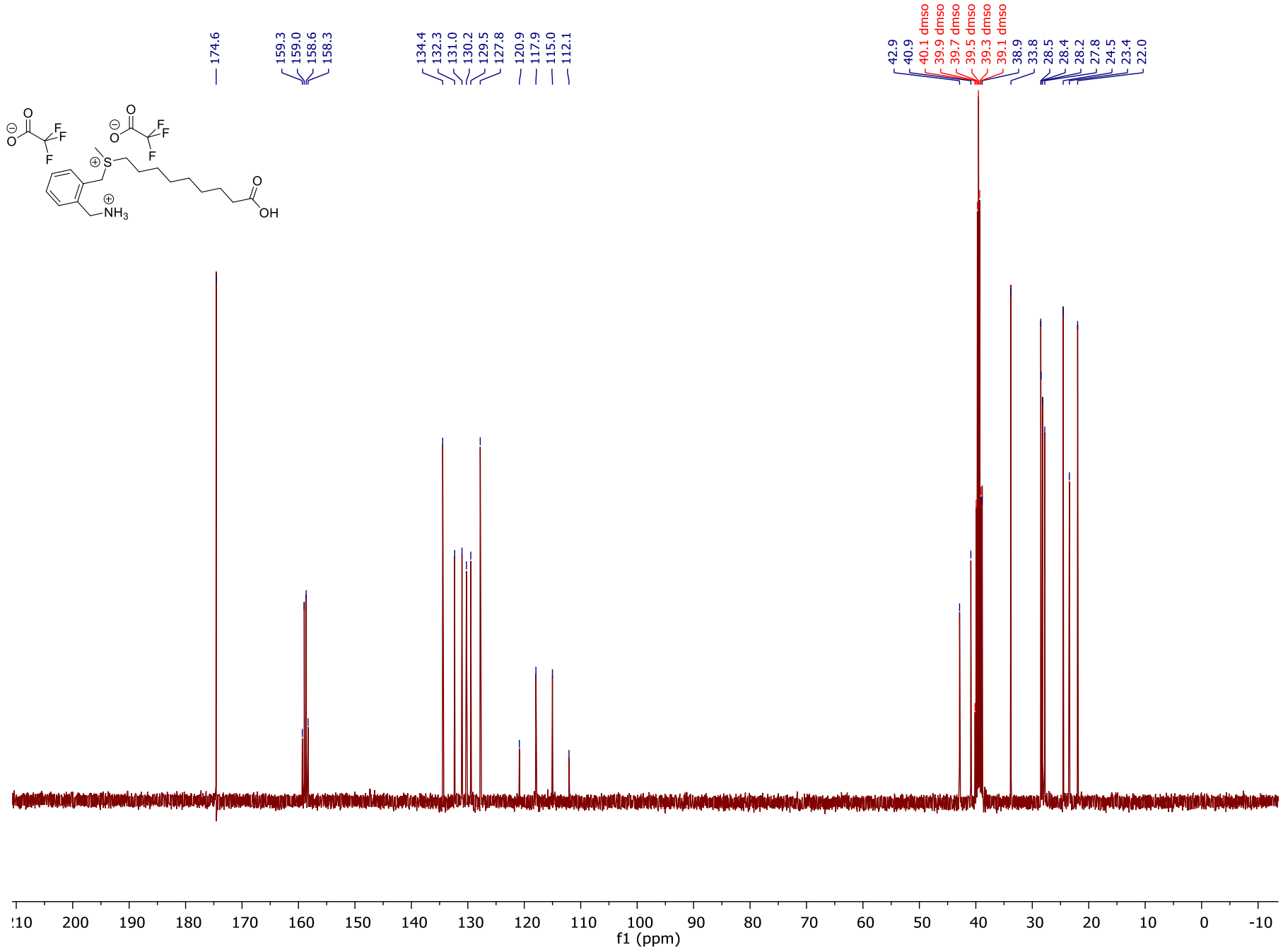
^{13}C NMR of **14:**


174.5
159.2
158.9
158.5
158.2
134.4
132.3
131.0
130.2
129.5
127.8
120.8
117.9
114.9
112.0
42.8
40.9
40.1 dmso
39.9 dmso
39.7 dmso
39.5 dmso
39.3 dmso
39.1 dmso
38.9 dmso
38.8
33.7
28.2
28.0
27.7
24.4
23.4
22.0
f1 (ppm)

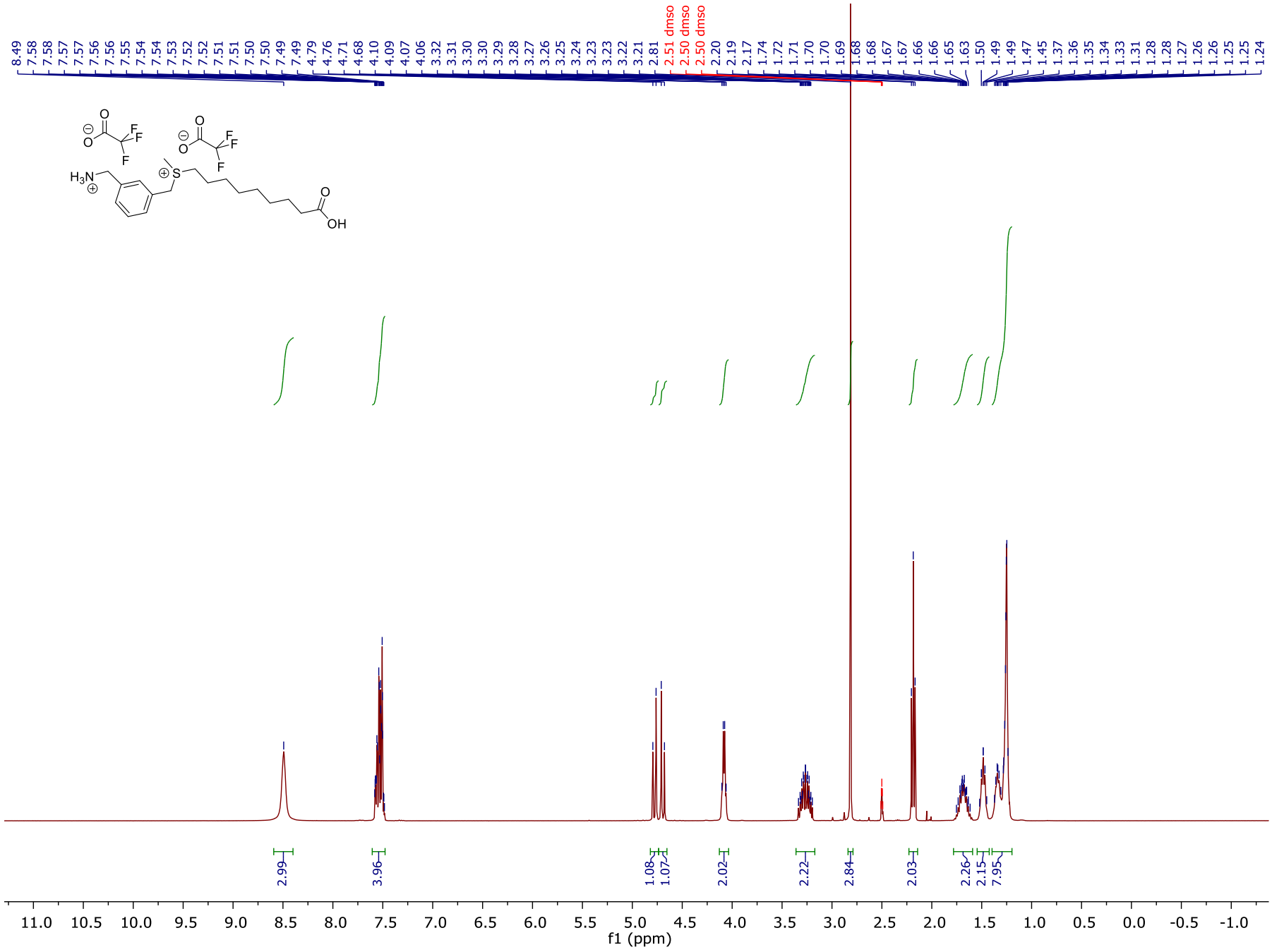
^{1}H NMR of **15:**


8.56 7.64 7.64 7.62 7.62 7.57 7.57 7.56 7.55 7.54 7.53 7.52 7.51 7.50 7.50 7.48 7.48 7.46 7.46 4.94 4.91 4.86 4.83 4.28 4.26 4.25 4.24 3.39 3.37 3.37 3.36 3.35 3.34 3.33 3.33 3.31 2.87 2.51 dmso 2.51 dmso 2.50 dmso 2.50 dmso 2.49 dmso 2.20 2.18 2.16 1.73 1.72 1.71 1.71 1.69 1.69 1.68 1.68 1.67 1.66 1.65 1.65 1.58 1.58 1.56 1.56 1.55 1.54 1.53 1.52 1.51 1.51 1.50 1.49 1.48 1.46 1.44 1.33 1.32 1.31 1.31 1.30 1.26 1.25 1.23 1.22
3.16 0.96 3.00 0.98 1.01 1.86 1.88 2.65 2.01 1.89 1.12 1.07 1.86 7.31 1.52
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)

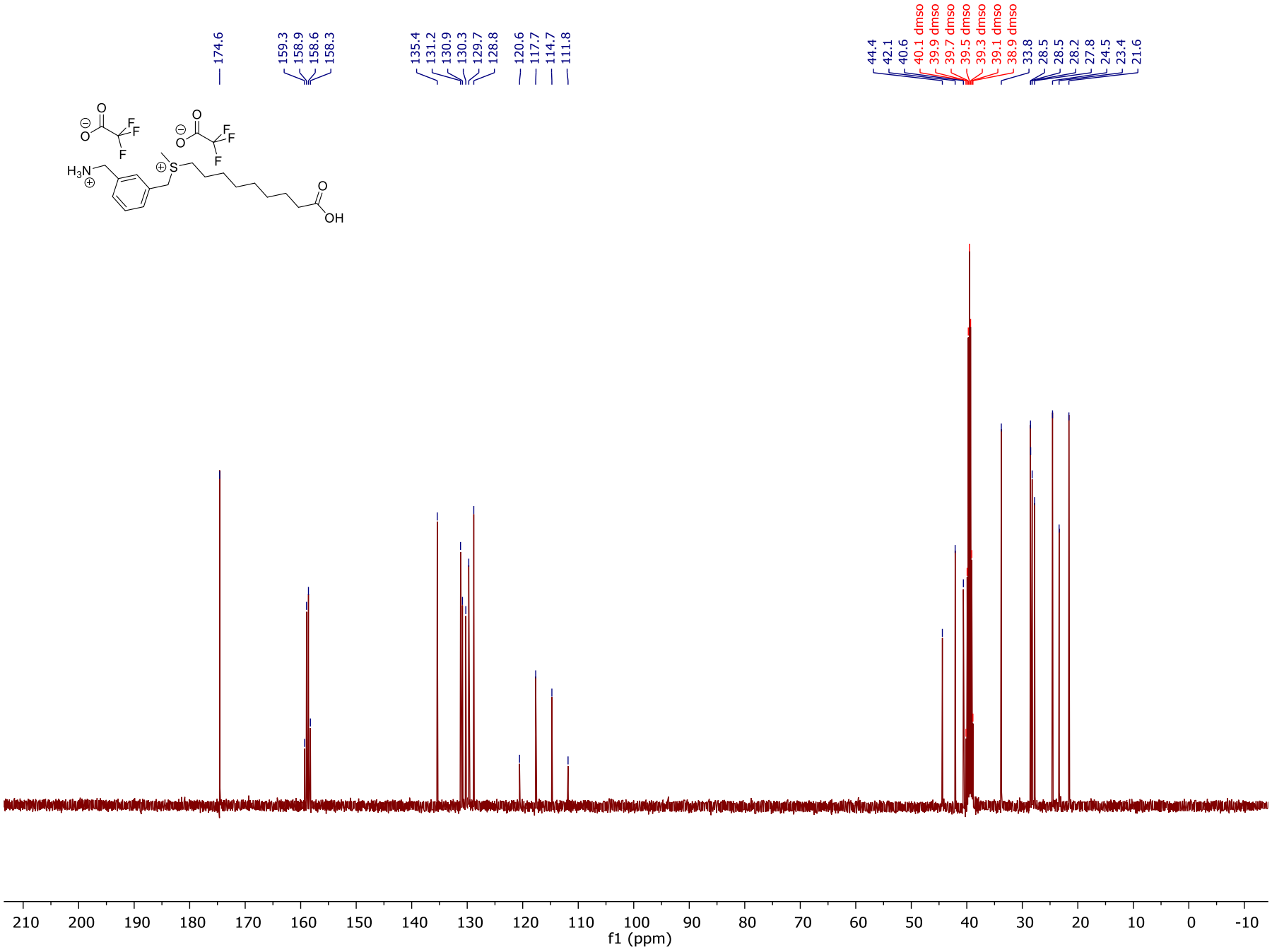
^{13}C NMR of **15:**


174.6
159.3
159.0
158.6
158.3
134.4
132.3
131.0
130.2
129.5
127.8
120.9
117.9
115.0
112.1
42.9
40.9
40.1 dmso
39.9 dmso
39.7 dmso
39.5 dmso
39.3 dmso
39.1 dmso
38.9
33.8
28.5
28.4
28.2
27.8
24.5
23.4
22.0
f1 (ppm)

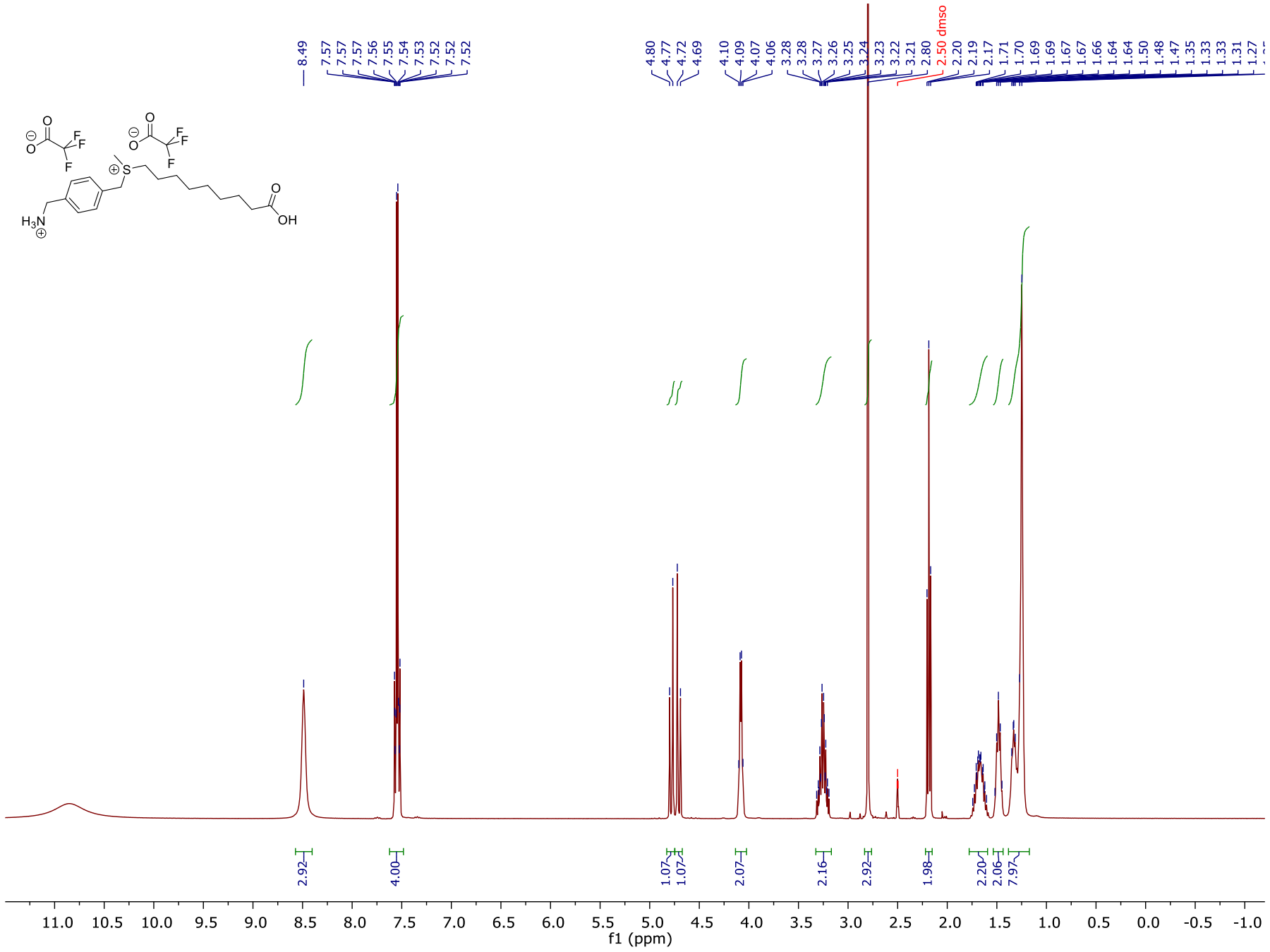
^{1}H NMR of **16:**


8.49 7.58 7.58 7.57 7.57 7.56 7.56 7.55 7.54 7.54 7.53 7.52 7.52 7.51 7.51 7.50 7.50 7.49 7.49 4.79 4.76 4.71 4.68 4.10 4.09 4.07 4.06 3.32 3.31 3.30 3.30 3.29 3.28 3.27 3.26 3.25 3.24 3.23 3.23 3.22 3.21 2.81 2.51 dmso 2.50 dmso 2.50 dmso 2.20 2.19 2.17 1.74 1.72 1.71 1.70 1.70 1.69 1.68 1.68 1.67 1.67 1.66 1.66 1.65 1.63 1.50 1.49 1.49 1.47 1.45 1.37 1.36 1.35 1.34 1.33 1.31 1.28 1.28 1.27 1.26 1.26 1.25 1.25 1.24
2.99 3.96 1.08 1.07 2.02 2.22 2.84 2.03 2.26 2.15 7.95
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)

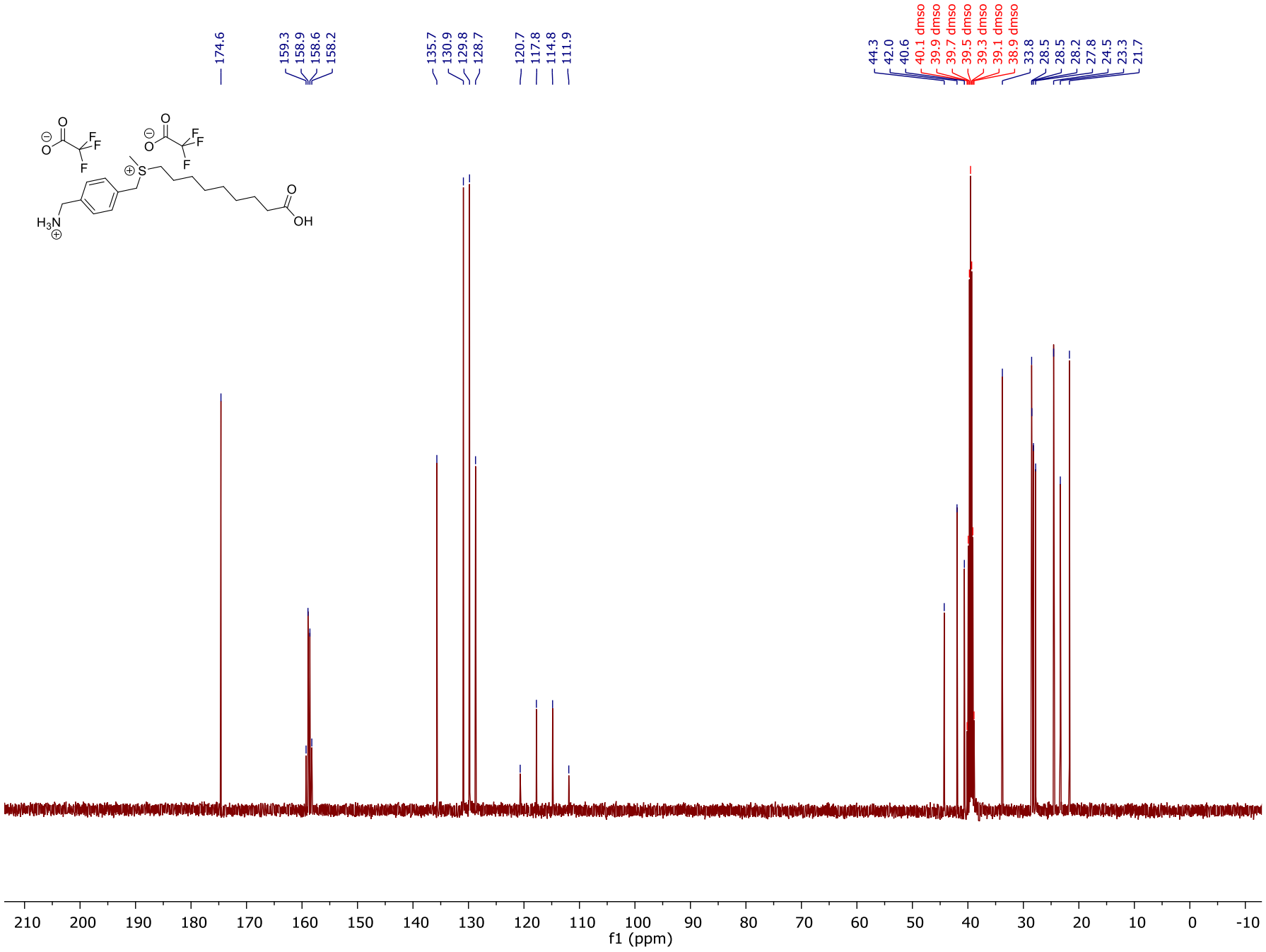
^{13}C NMR of **16:**


174.6
159.3
158.9
158.6
158.3
135.4
131.2
130.9
130.3
129.7
128.8
120.6
117.7
114.7
111.8
44.4
42.1
40.6
40.1 dmso
39.9 dmso
39.7 dmso
39.5 dmso
39.3 dmso
39.1 dmso
38.9 dmso
33.8
28.5
28.5
28.2
27.8
24.5
23.4
21.6
f1 (ppm)

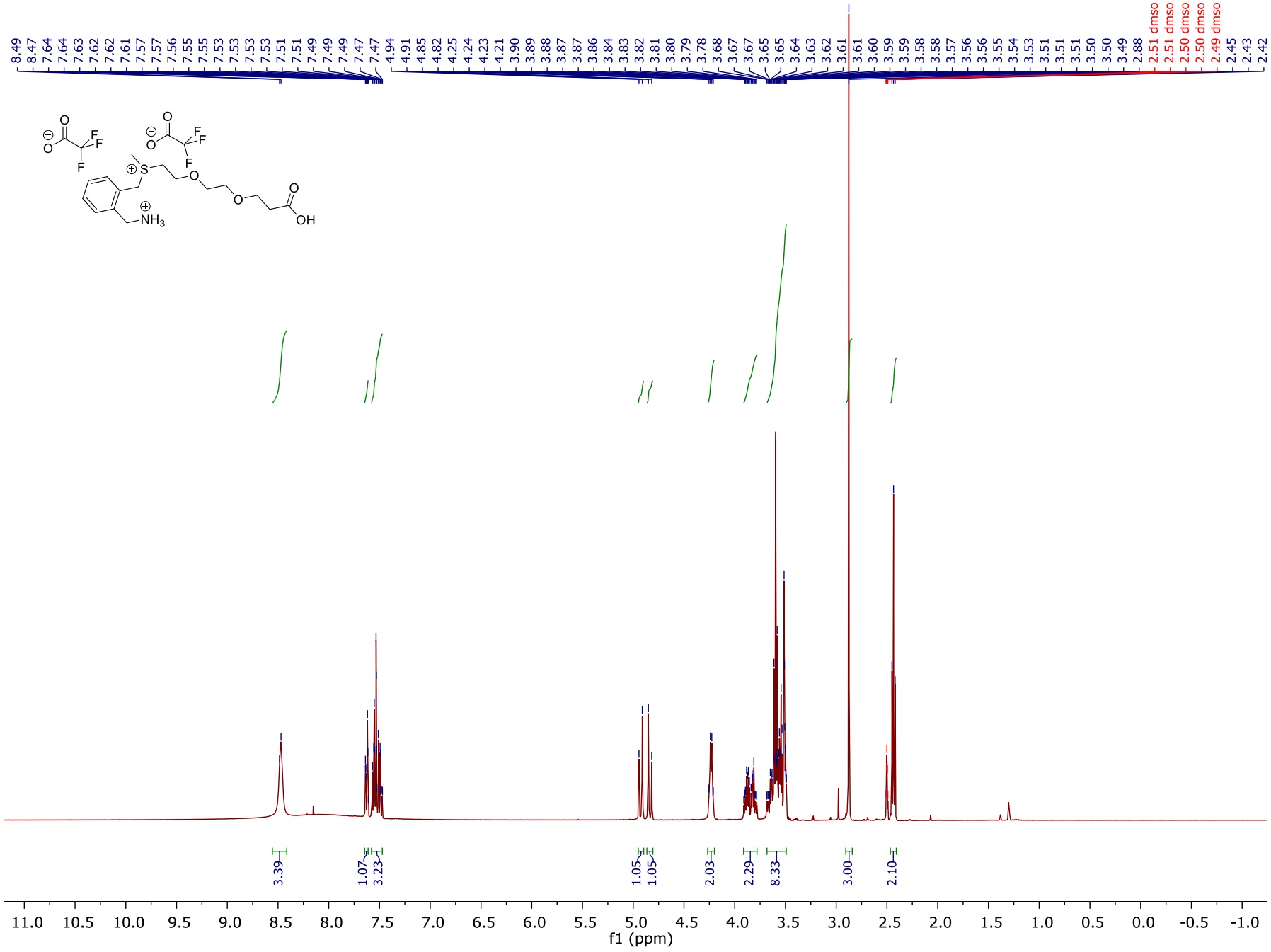
^{1}H NMR of **17:**


8.49
7.57
7.57
7.57
7.56
7.55
7.54
7.53
7.52
7.52
7.52
4.80
4.77
4.72
4.69
4.10
4.09
4.07
4.06
3.28
3.28
3.27
3.26
3.25
3.24
3.23
3.22
3.21
2.80
2.50 dmso
2.20
2.19
2.17
1.71
1.70
1.69
1.69
1.67
1.67
1.66
1.64
1.64
1.50
1.48
1.47
1.35
1.33
1.33
1.31
1.27
2.92
4.00
1.07
1.07
2.07
2.16
2.92
1.98
2.20
2.06
7.97
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
f1 (ppm)

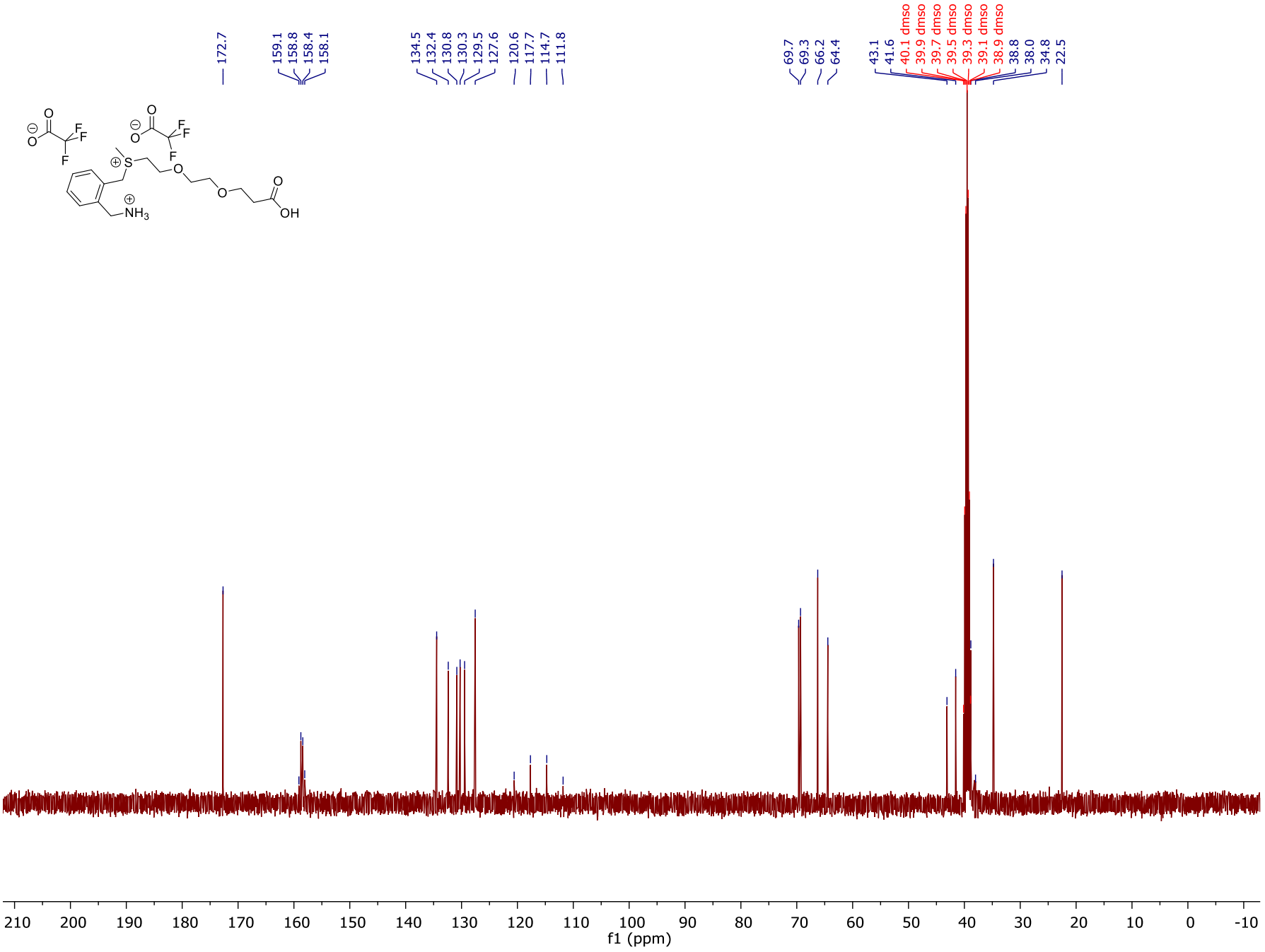
^{13}C NMR of **17:**


174.6
159.3
158.9
158.6
158.2
135.7
130.9
129.8
128.7
120.7
117.8
114.8
111.9
44.3
42.0
40.6
40.1 dmso
39.9 dmso
39.7 dmso
39.5 dmso
39.3 dmso
39.1 dmso
38.9 dmso
33.8
28.5
28.5
28.2
27.8
24.5
23.3
21.7
f1 (ppm)

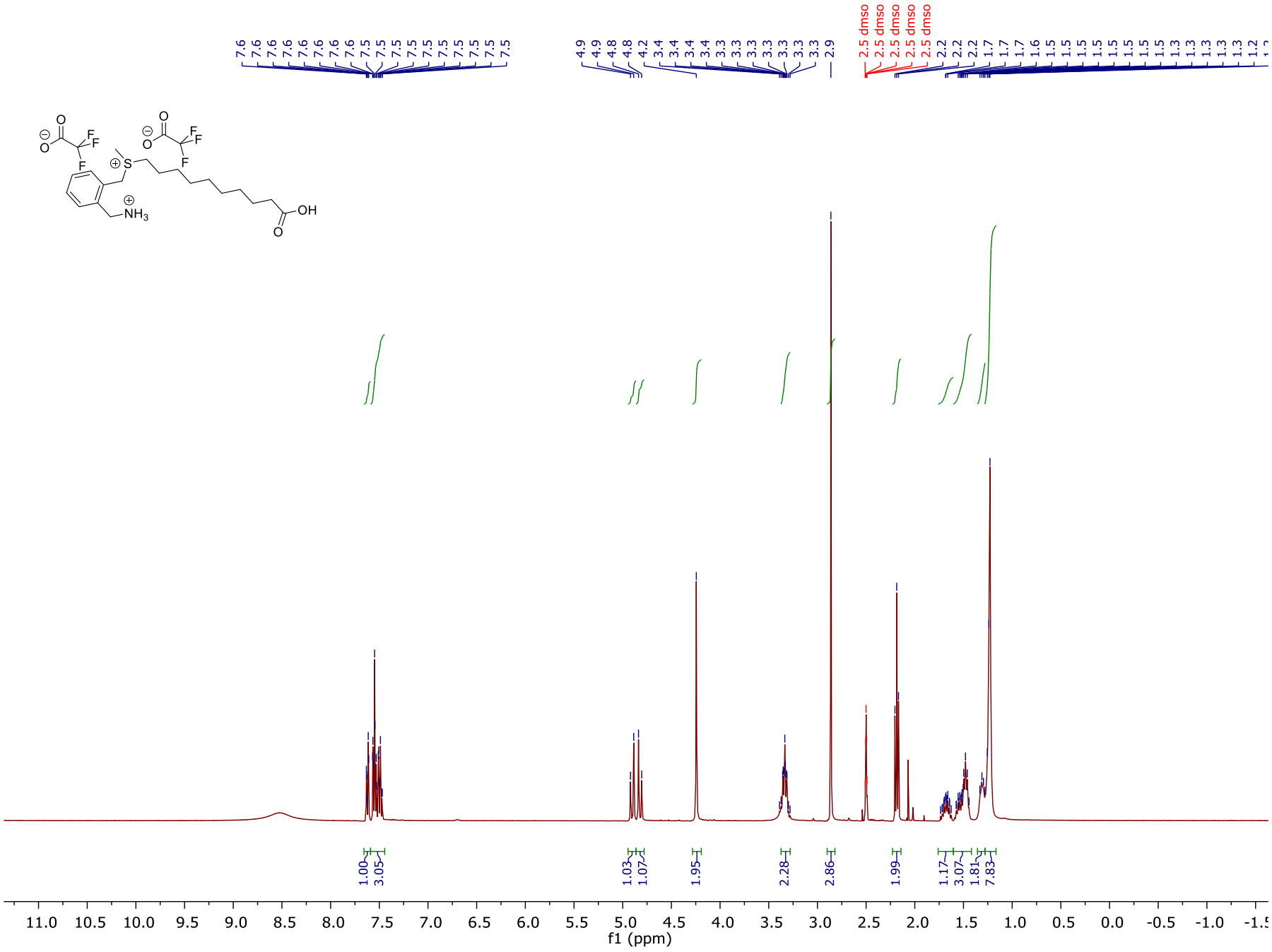
^{1}H NMR of **18:**


8.49 8.47 7.64 7.64 7.63 7.62 7.62 7.61 7.57 7.57 7.56 7.55 7.55 7.53 7.53 7.53 7.53 7.51 7.51 7.49 7.49 7.47 7.47 4.94 4.91 4.85 4.82 4.25 4.24 4.23 4.21 3.90 3.89 3.88 3.87 3.87 3.86 3.84 3.83 3.82 3.81 3.80 3.79 3.78 3.68 3.67 3.67 3.65 3.65 3.64 3.63 3.62 3.61 3.61 3.60 3.59 3.59 3.58 3.58 3.57 3.56 3.56 3.55 3.54 3.53 3.51 3.51 3.51 3.50 3.50 3.49 2.88 2.51 dmso 2.51 dmso 2.50 dmso 2.50 dmso 2.49 dmso 2.45 2.43 2.42
3.39 1.07 3.23 1.05 1.05 2.03 2.29 8.33 3.00 2.10
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)

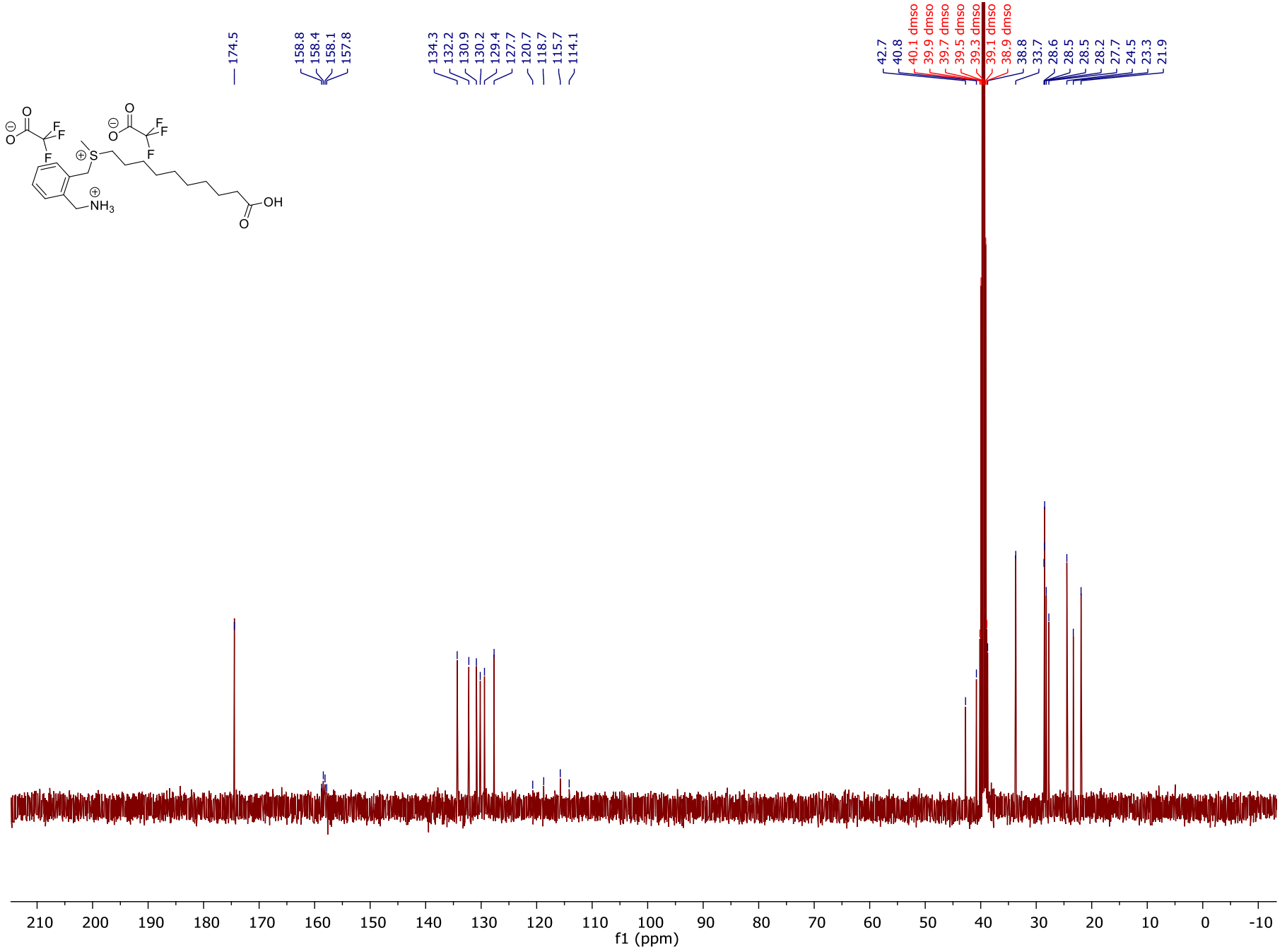
^{13}C NMR of **18:**


172.7
159.1
158.8
158.4
158.1
134.5
132.4
130.8
130.3
129.5
127.6
120.6
117.7
114.7
111.8
69.7
69.3
66.2
64.4
43.1
41.6
40.1 dmso
39.9 dmso
39.7 dmso
39.5 dmso
39.3 dmso
39.1 dmso
38.9 dmso
38.8
38.0
34.8
22.5
f1 (ppm)

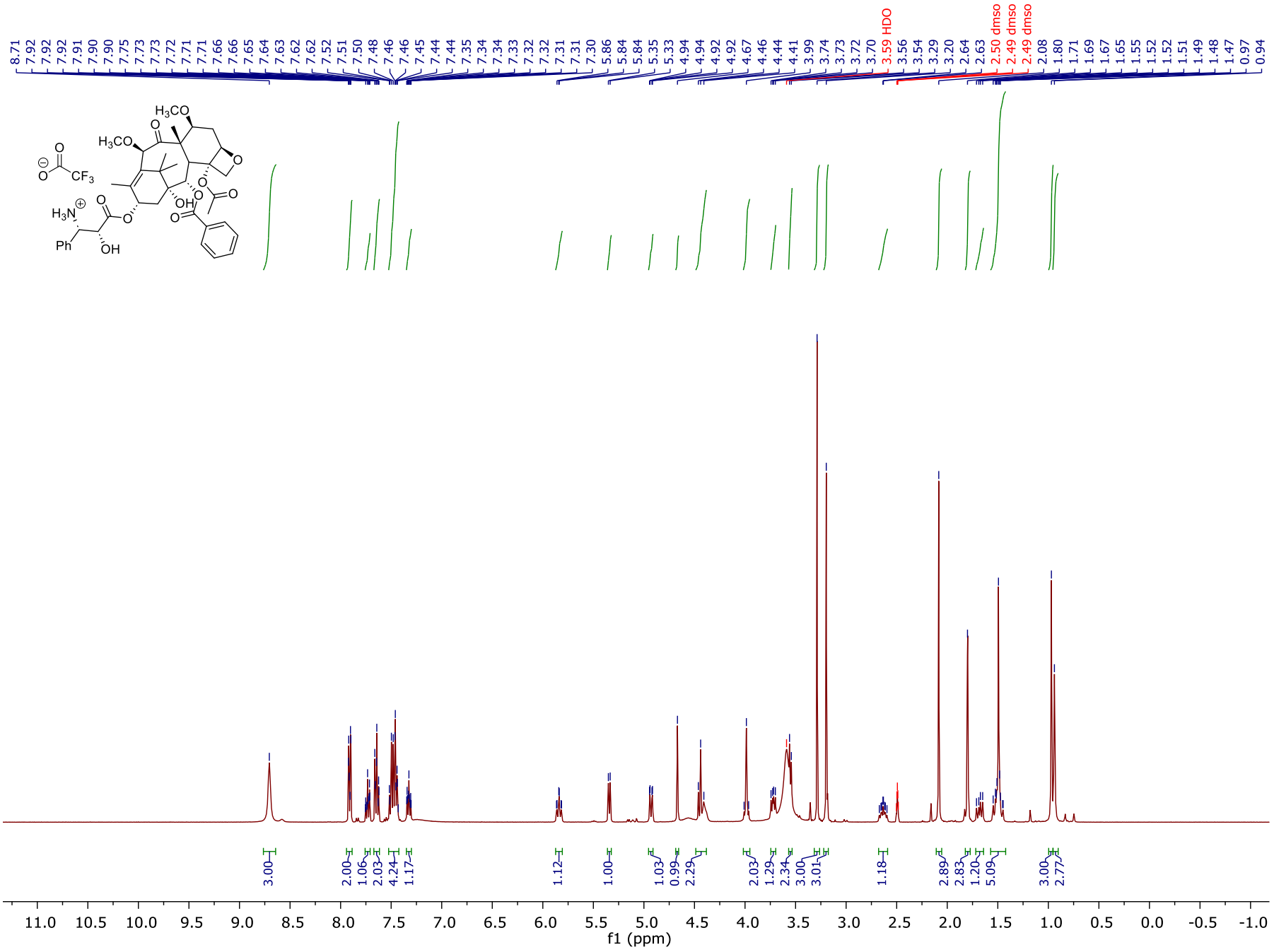
^{1}H NMR of **19:**


7.6 7.6 7.6 7.6 7.6 7.6 7.6 7.5 7.5 7.5 7.5 7.5 7.5 7.5 7.5 7.5
4.9 4.9 4.8 4.8 4.2 3.4 3.4 3.4 3.3 3.3 3.3 3.3 3.3 3.3 3.3 2.9
2.5 dmso 2.5 dmso 2.5 dmso 2.5 dmso 2.5 dmso 2.2 2.2 2.2 1.7 1.7 1.6 1.5 1.5 1.5 1.5 1.5 1.5 1.5 1.3 1.3 1.3 1.3 1.2
1.00 3.05 1.03 1.07 1.95 2.28 2.86 1.99 1.17 3.07 1.81 7.83
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)

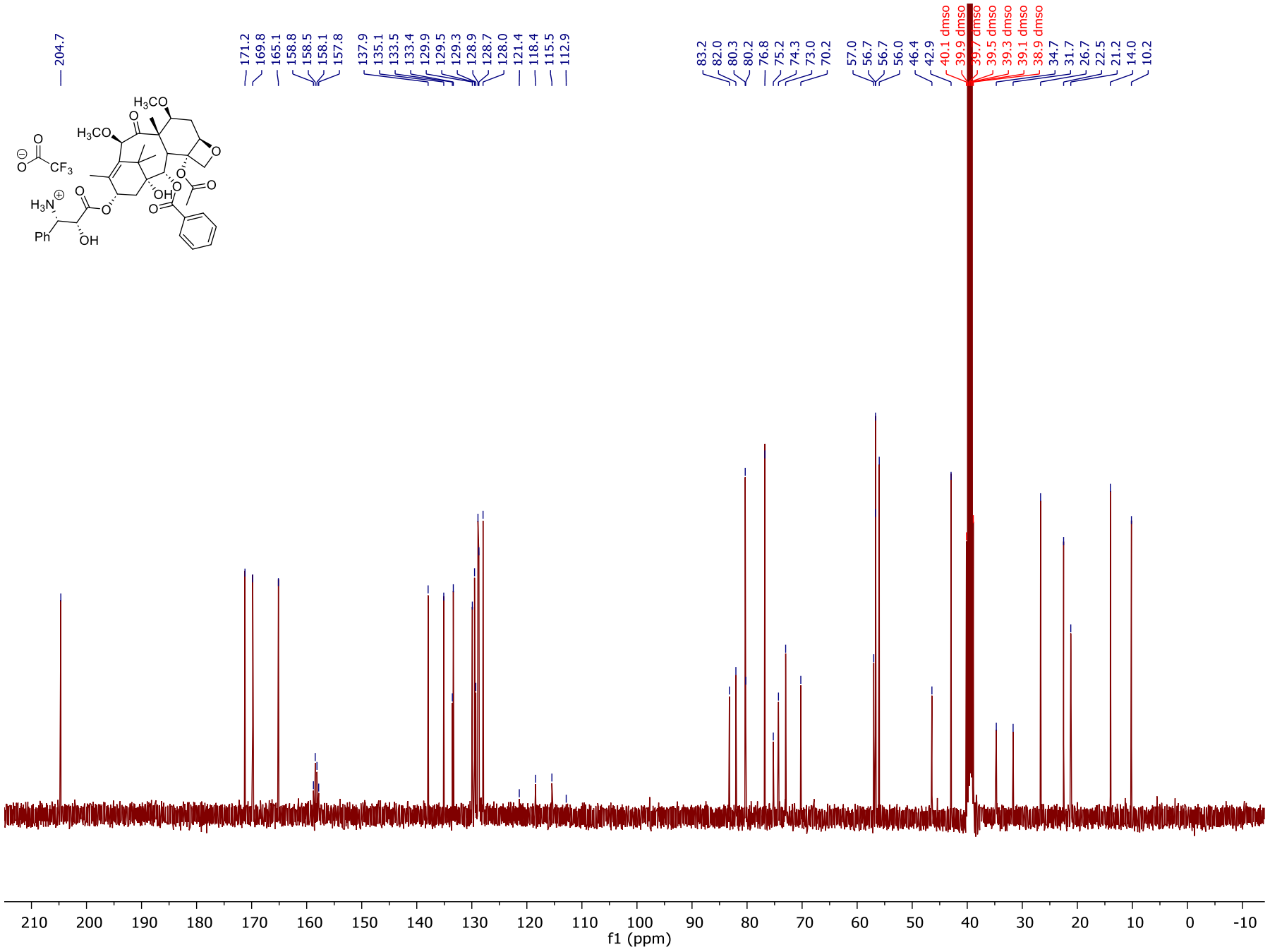
^{13}C NMR of **19:**


174.5
158.8
158.4
158.1
157.8
134.3
132.2
130.9
130.2
129.4
127.7
120.7
118.7
115.7
114.1
42.7
40.8
40.1 dmso
39.9 dmso
39.7 dmso
39.5 dmso
39.3 dmso
39.1 dmso
38.9 dmso
38.8
33.7
28.6
28.5
28.5
28.2
27.7
24.5
23.3
21.9
f1 (ppm)

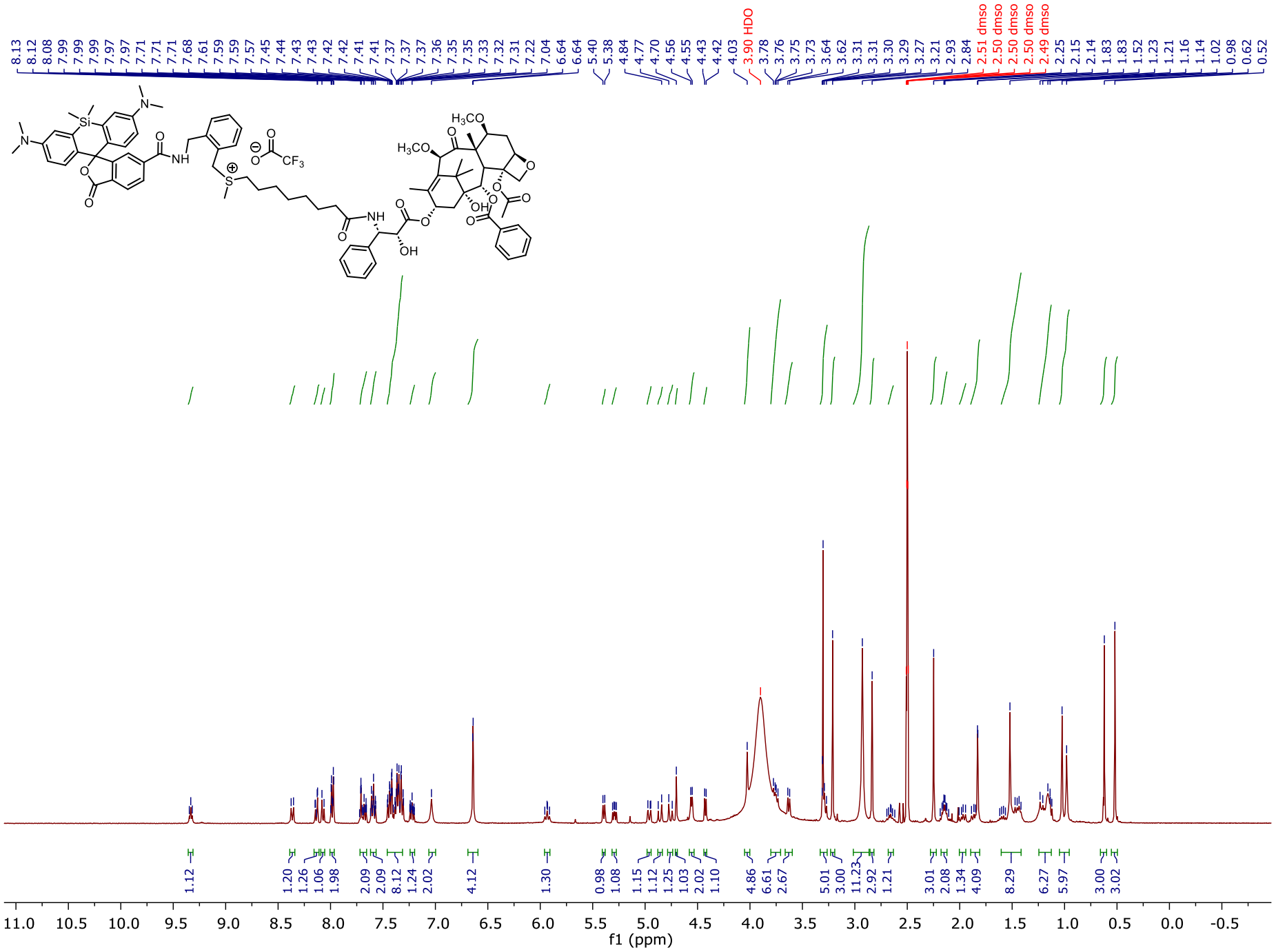
^{1}H NMR of ***CTX-NH$_3$-CF$_3$CO$_2$:***



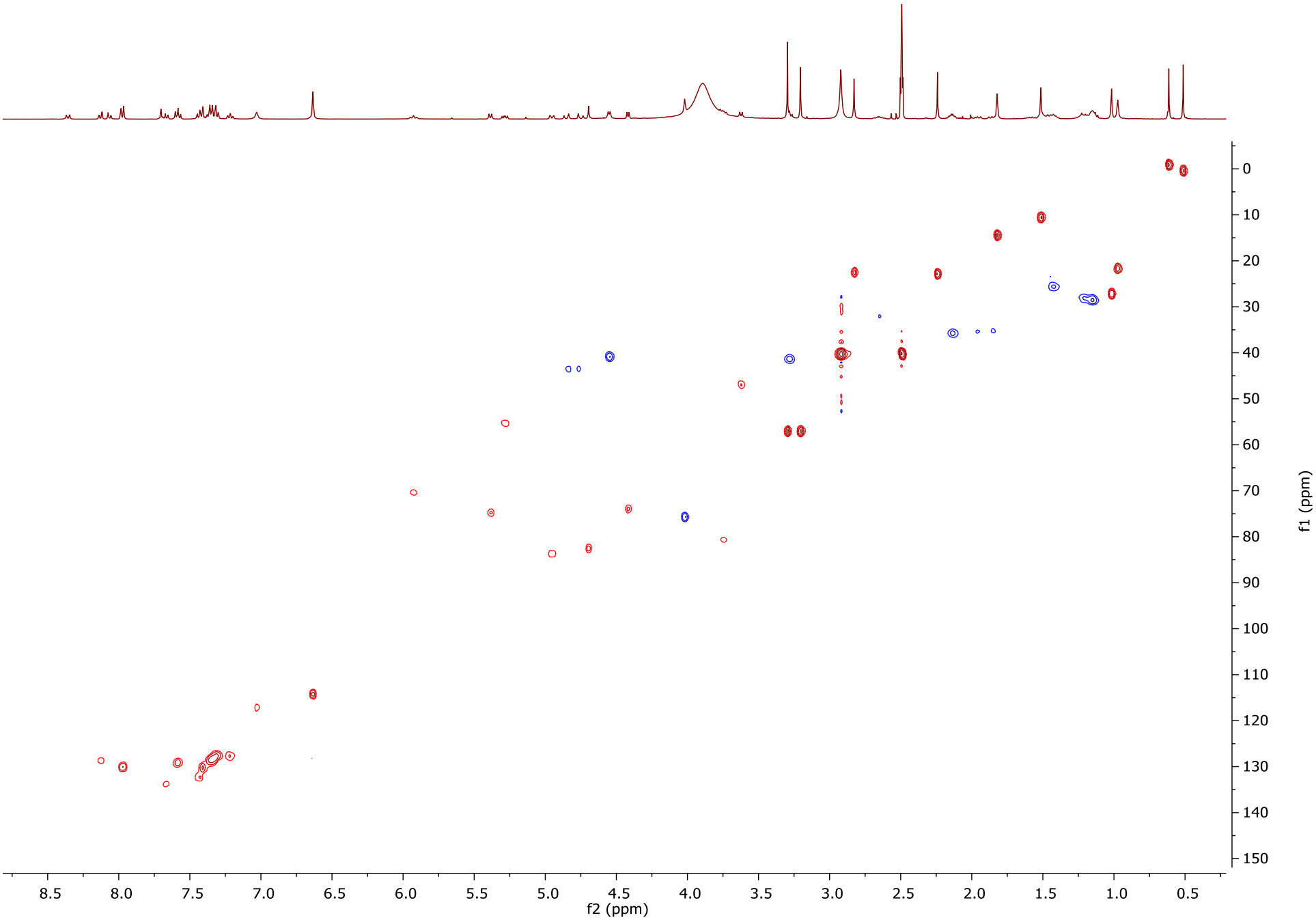
^{13}C NMR of ***CTX-NH$_3$-CF$_3$CO$_2$:***



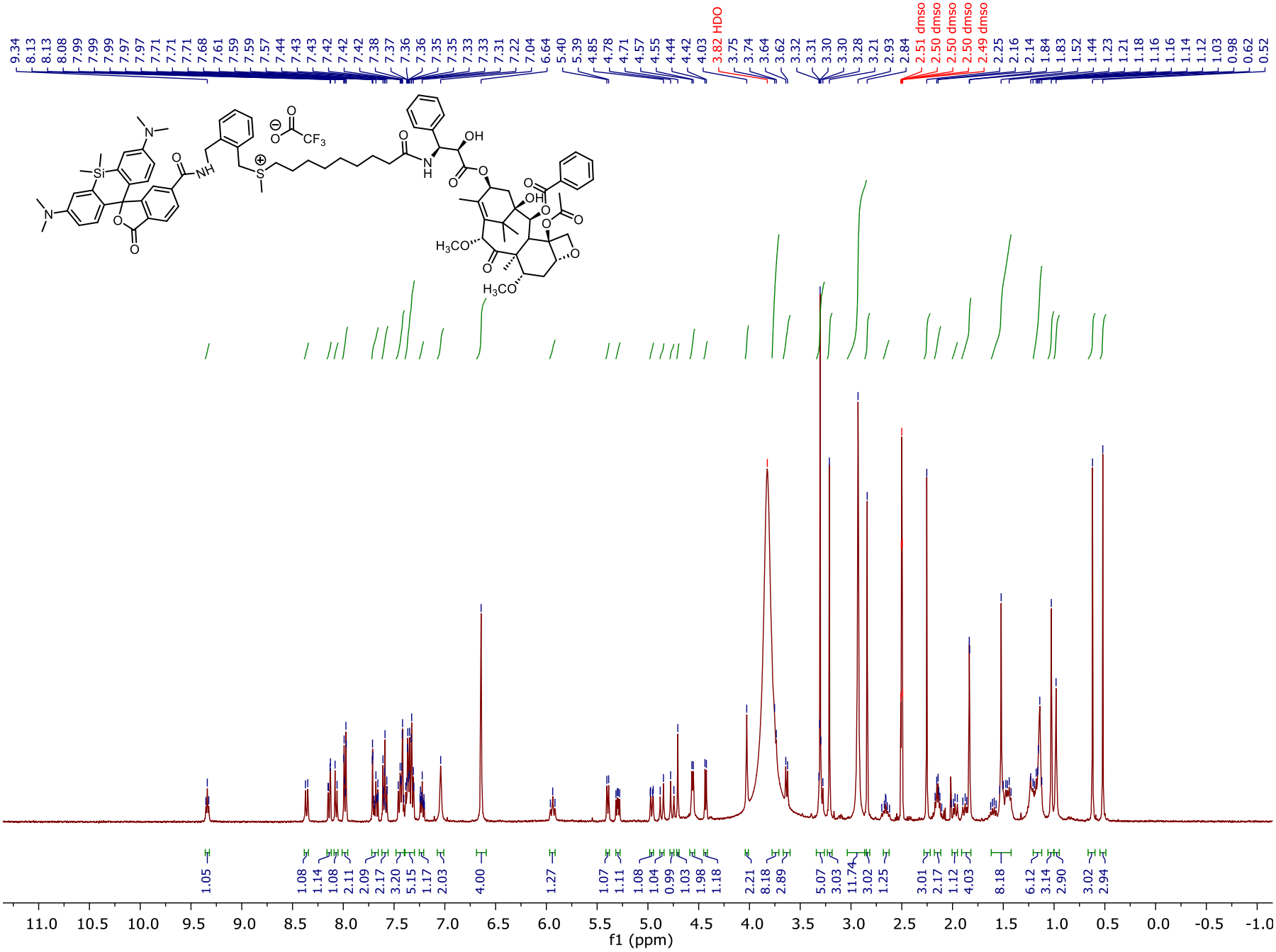
^{1}H NMR of **Probe 1:**



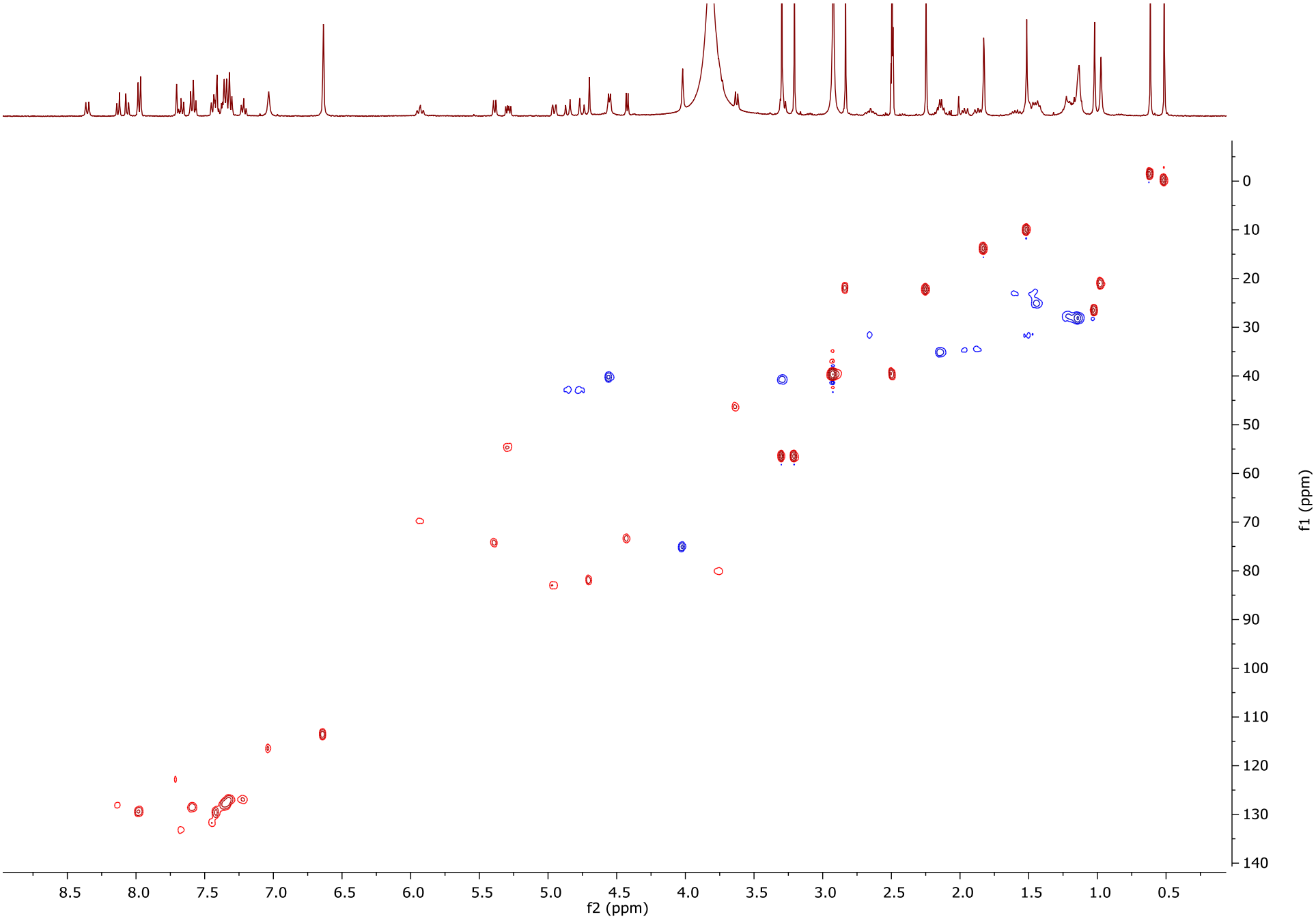
HSQC of **Probe 1:**



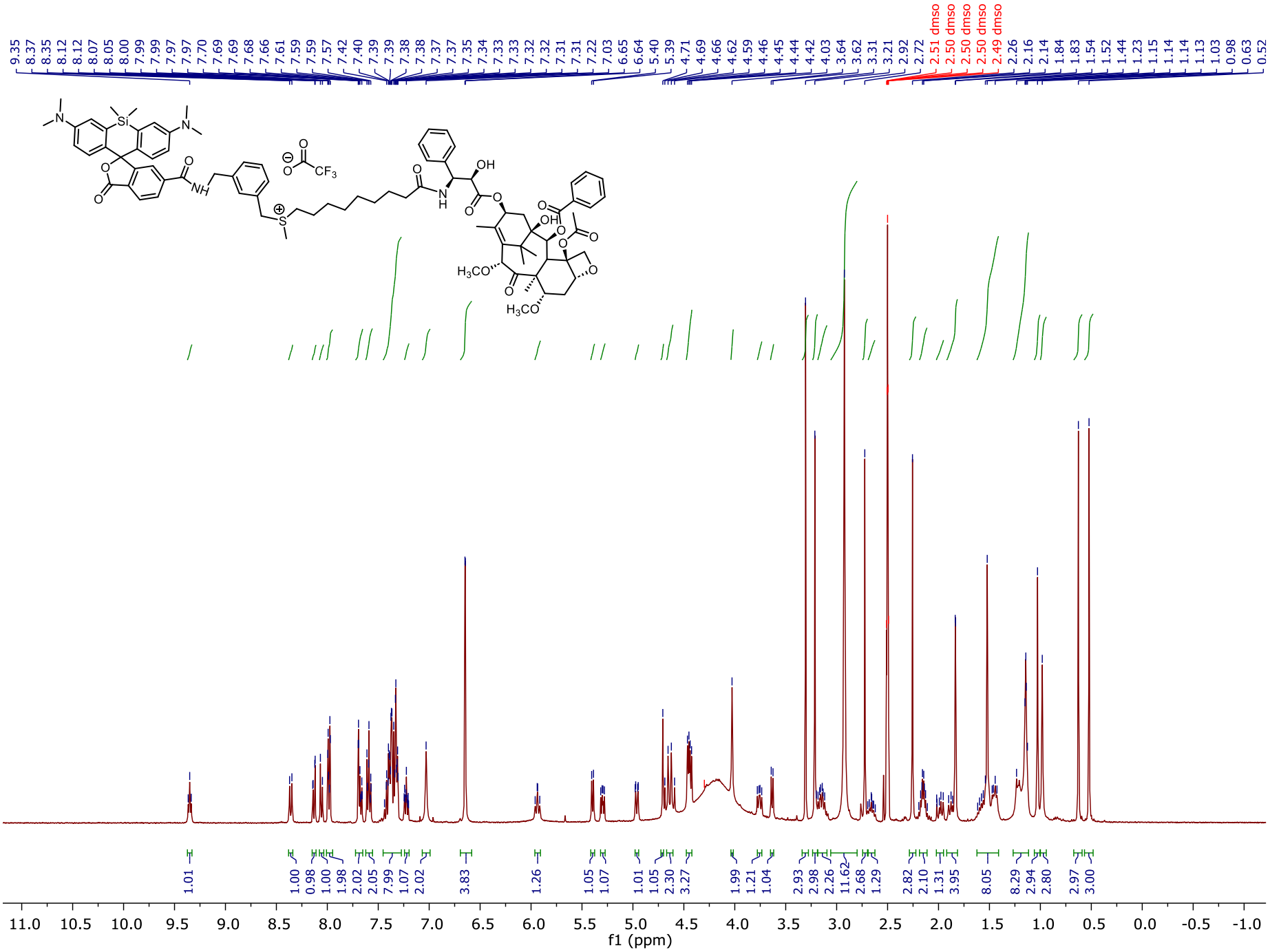
^{1}H NMR of **Probe 2 - 6-SiR-*o*-C$_9$-CTX:**



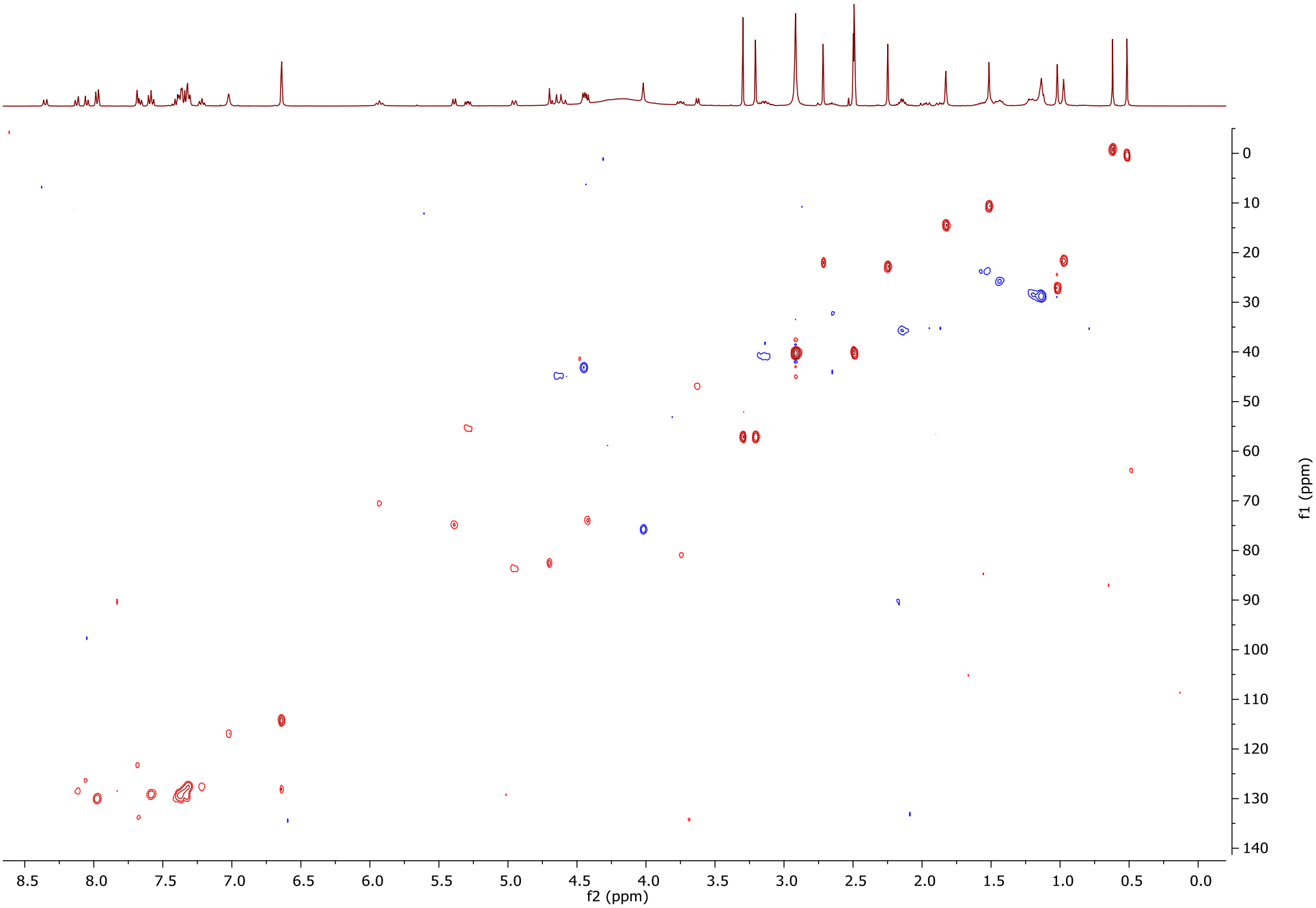
HSQC of **Probe 2 - 6-SiR-*o*-C$_9$-CTX:**



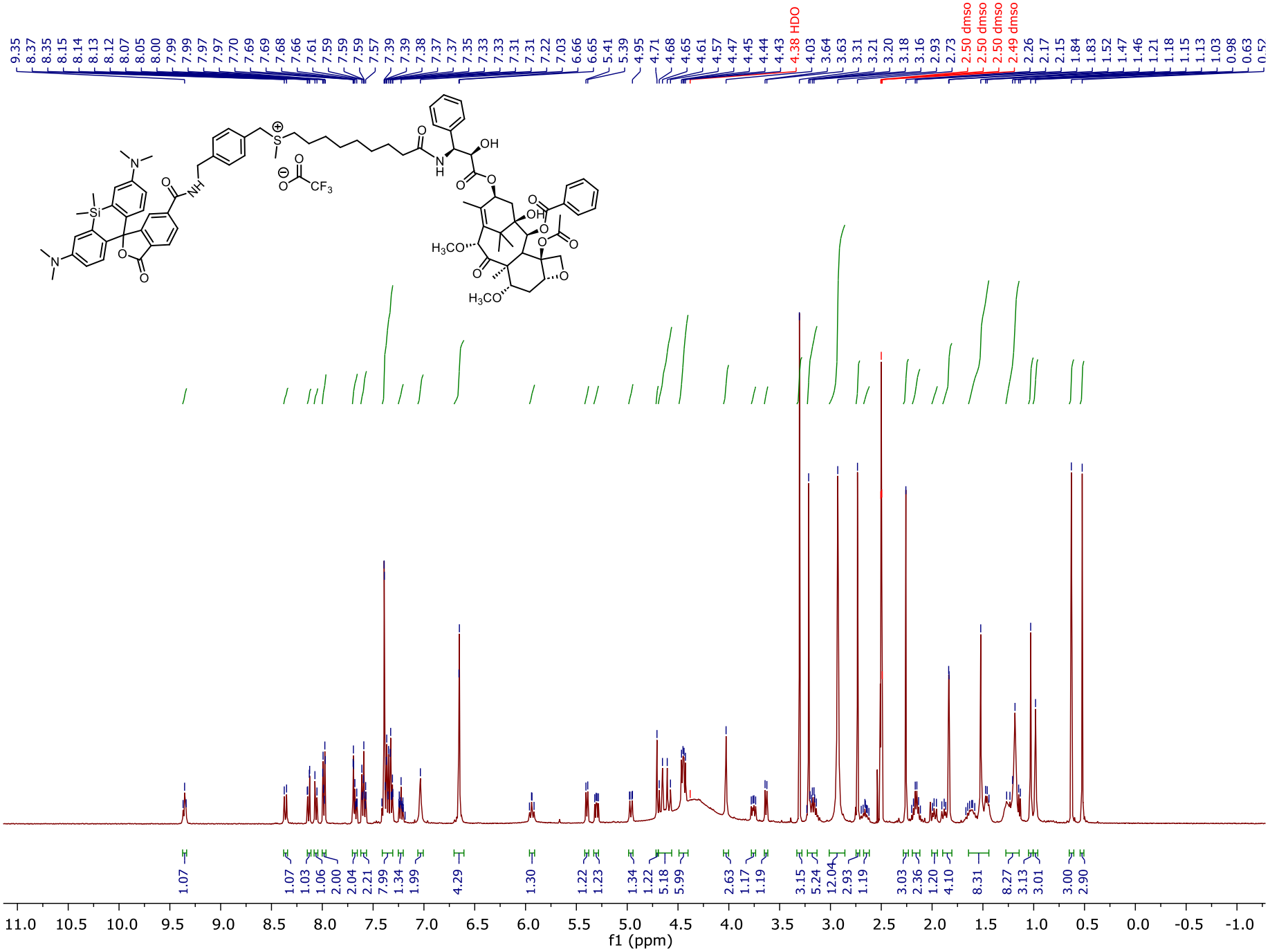
^{1}H NMR of **Probe 3:**



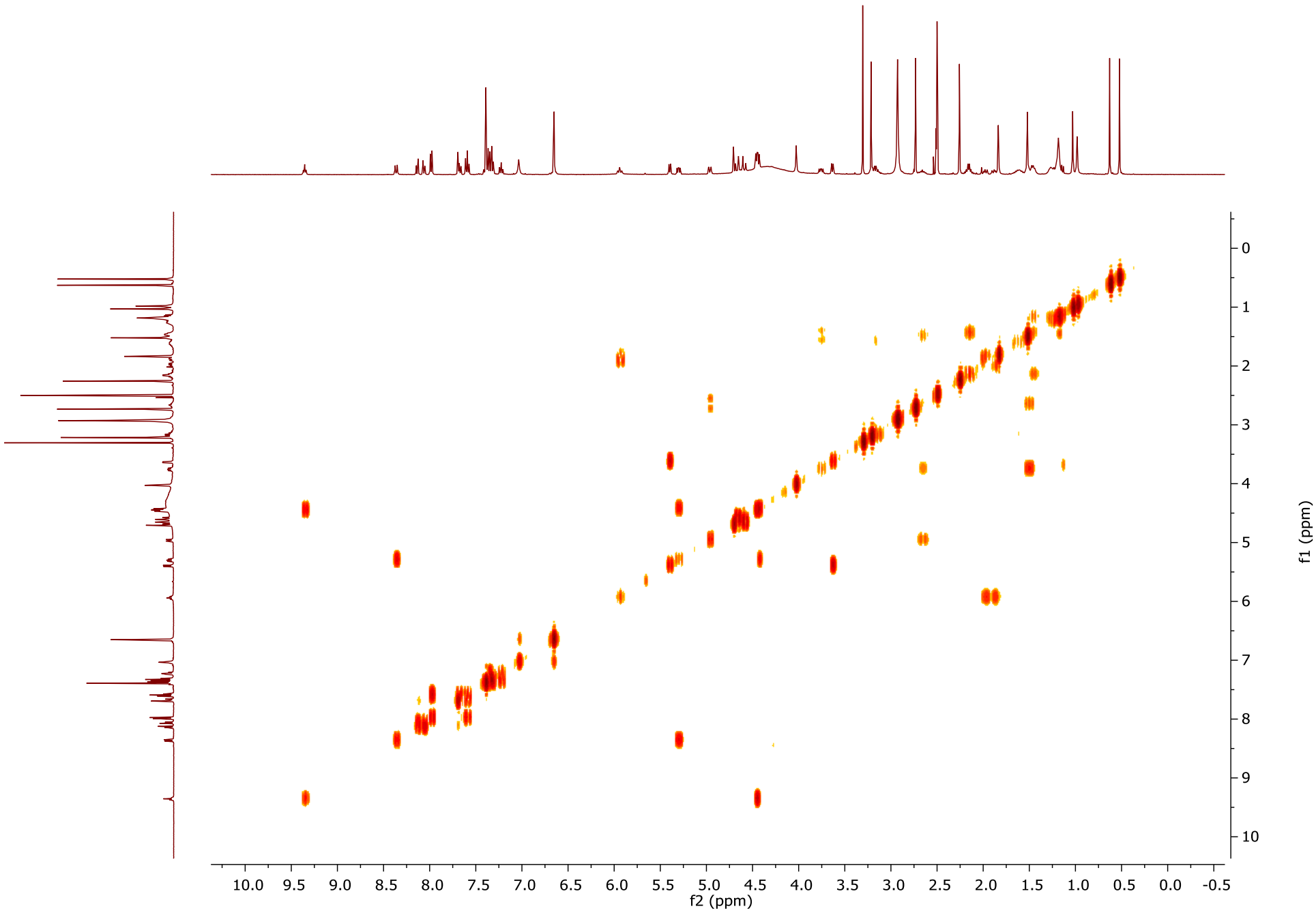
HSQC of **Probe 3:**



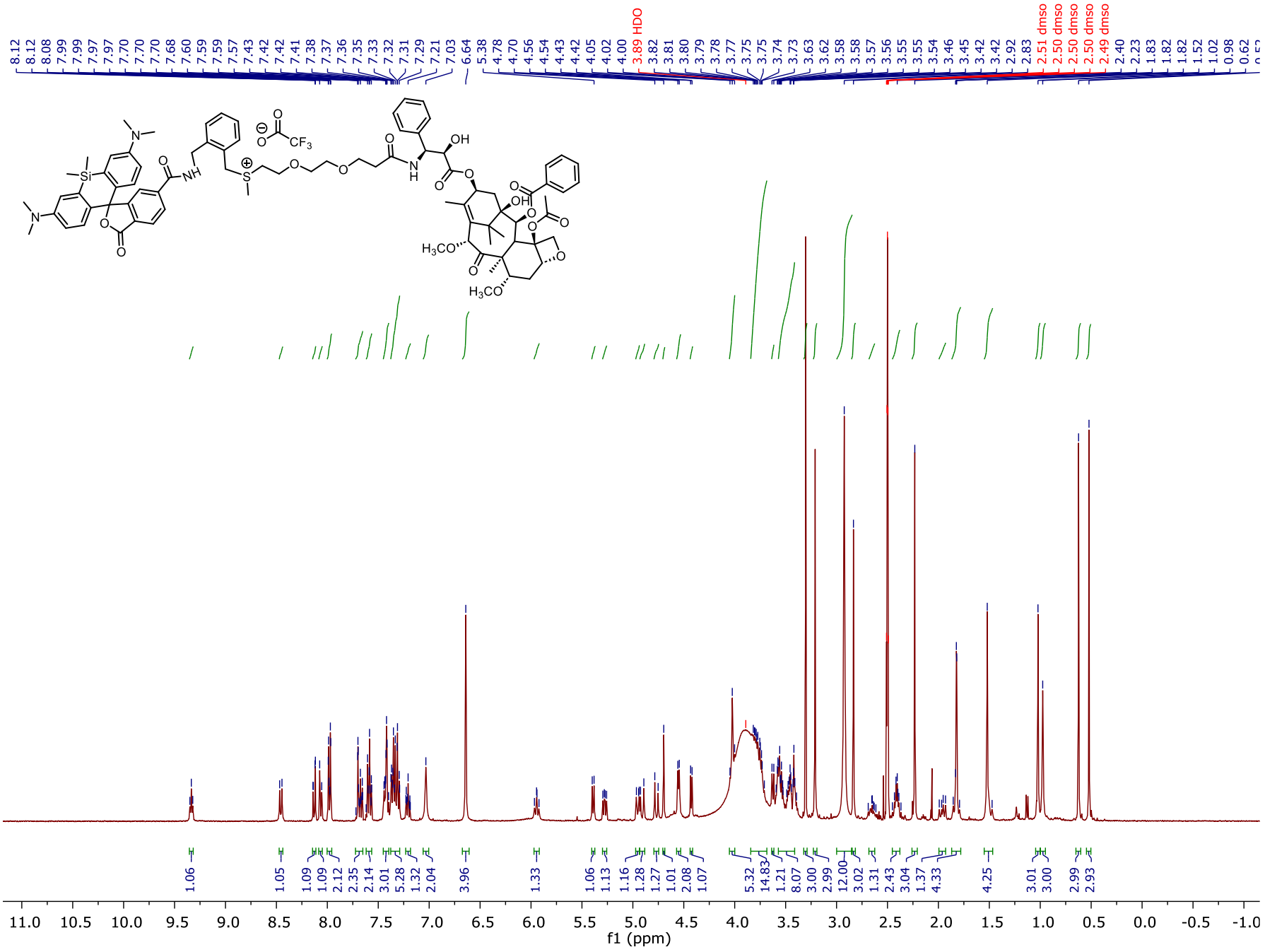
^{1}H NMR of **Probe 4:**



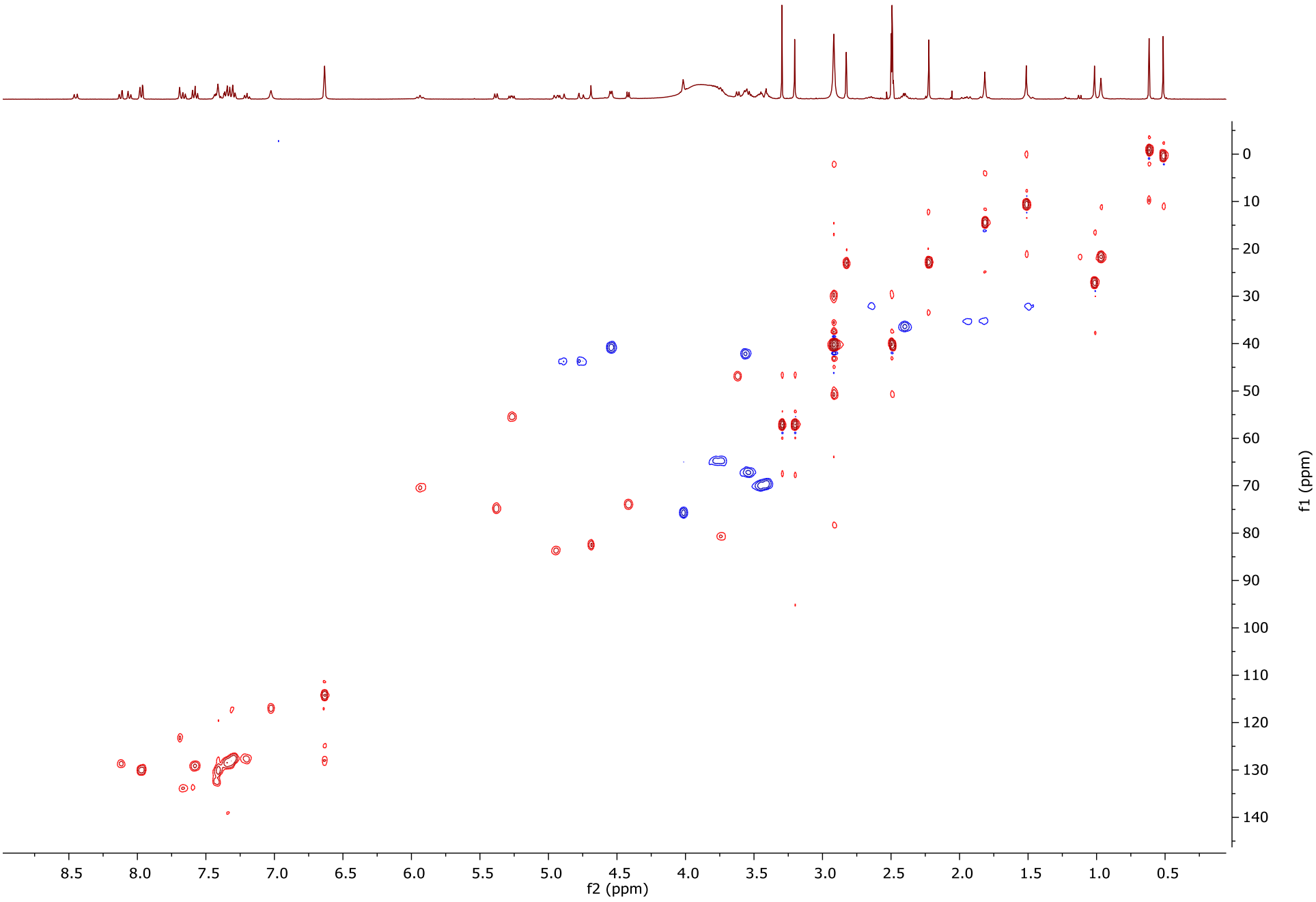
COSY of **Probe 4:**



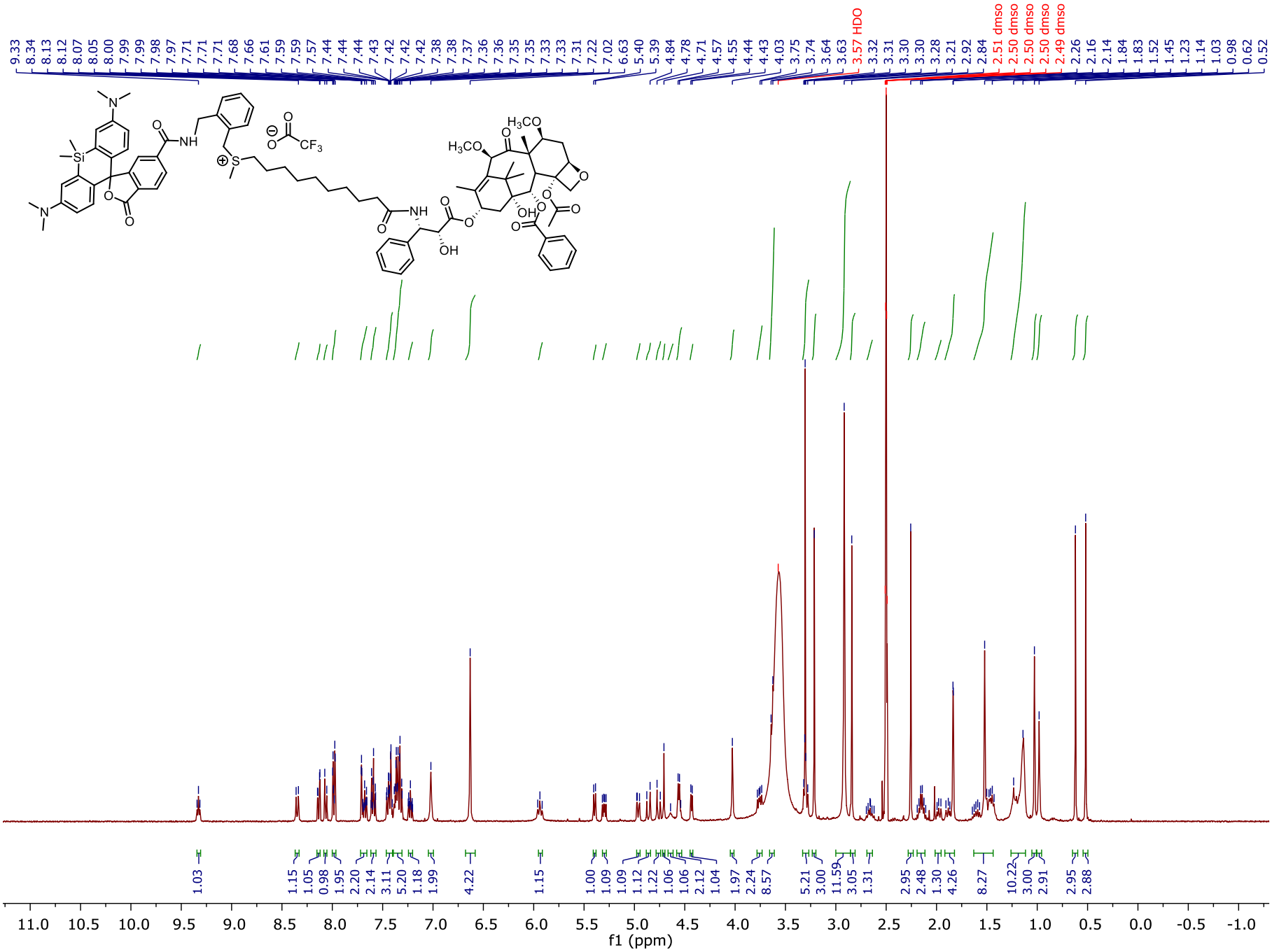
^{1}H NMR of **Probe 5:**



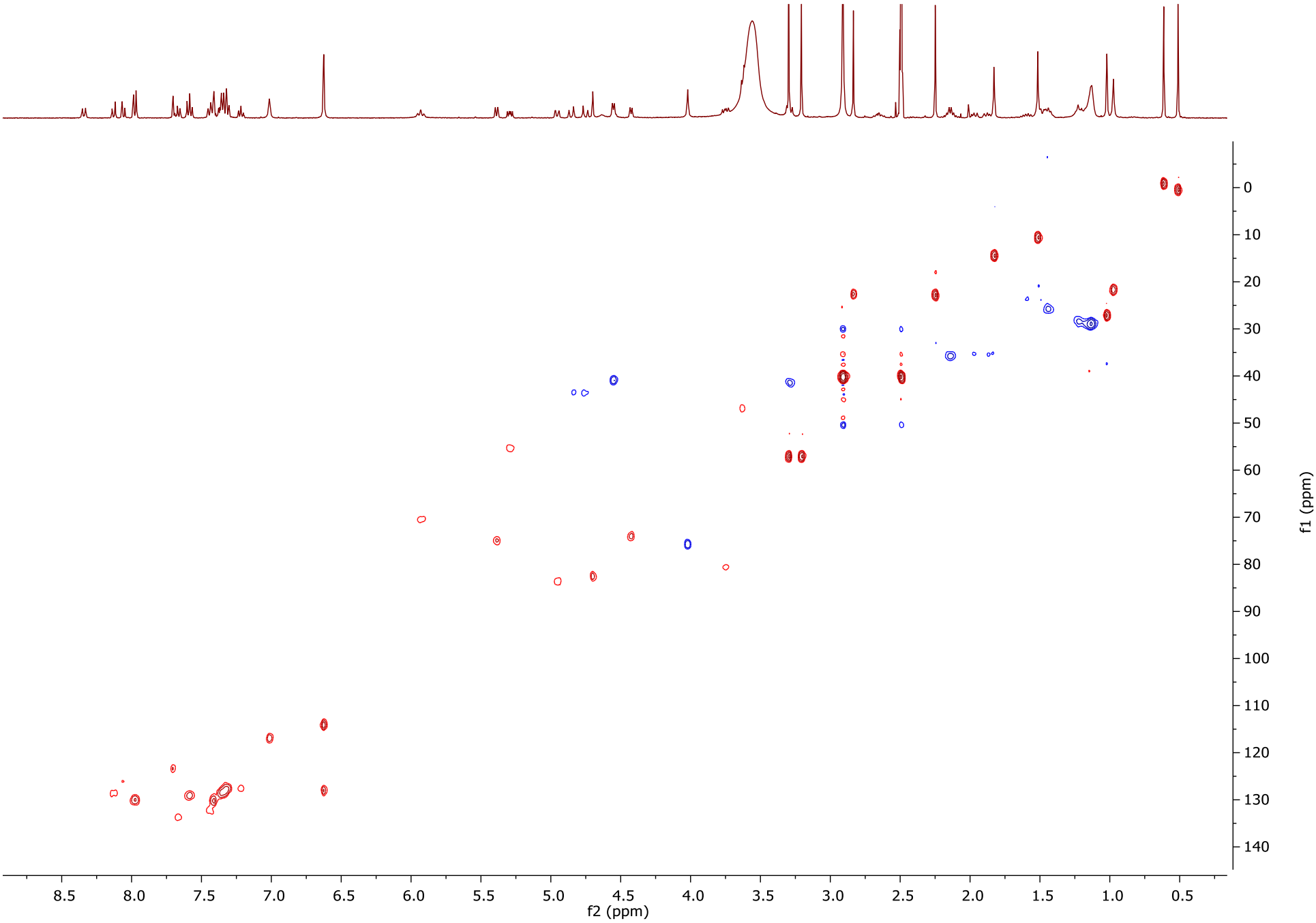
HSQC of **Probe 5:**



^{1}H NMR of **Probe 6:**



HSQC of **Probe 6:**



References

1 Lukinavičius, G. *et al.* Fluorescent dyes and probes for super-resolution microscopy of microtubules and tracheoles in living cells and tissues. *Chem Sci* **9**, 3324-3334, doi:10.1039/c7sc05334g (2018).

2 Bucevicius, J., Keller-Findeisen, J., Gilat, T., Hell, S. W. & Lukinavicius, G. Rhodamine-Hoechst positional isomers for highly efficient staining of heterochromatin. *Chem Sci* **10**, 1962-1970, doi:10.1039/c8sc05082a (2019).