Supporting Information

Fluorescence switchable probes based on a molecular rotor for selective proteins and small molecules detection

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Materials and apparatus: Chemicals and peptide coupling reagents were purchased from Sigma-Aldrich, Alfa Aesar, TCI, Advanced Chemtech and used without further purification. Solvents (DMF, DCM, hexane, ethyl acetate and methanol) were from Sigma-Aldrich and TCI and used without further treatment and distillation. Besides hCAII which was expressed and purified in our laboraotry, all other proteins used in the selectivity test were purchased from Sigma-Aldrich. Thin layer chromatography (TLC) was performed on TLC-aluminum sheets (Silica gel 60 F254, Merck). Flash column chromatography was performed with silica gel (230-400 mesh, Merck). ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on Bruker Avance II 400 with chemical shifts (δ) reported in ppm relative to the solvent residual signals of CD_3OD (3.30 ppm), $CDCl_3$ (7.24 ppm), DMSO-d6 (2.49 ppm) and coupling constants reported in Hz. HPLC analysis and purification were performed with an analytical column (EC 150/4.6 Nucleosil 300-5 C18, Macherey-Nagel) and a semi-preparative column (VP 150/21 Nucleosil 300-5 C18, Machrey-Nagel). Fluorescence spectra were recorded using Hitachi F-4500 fluorescence spectrophotometer and TECAN Infinite M200Pro. High resolution mass spectra (HRMS) were recorded on HPLC/MS-MS (Varian 901-FTMS).

hCAII protein expression and purification: hCAII vector pET51b_hCAII was transformed and expressed with N-terminal His-tag in *E.coli* strain BL21. Bacterial cultures in LB medium were grown at 37˚C to an OD_{600} of 1.0. Expression of the HCAII protein was induced by the addition of 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG). The bacteria were grown for an additional 16 hours at 18 ˚C and harvested by centrifugation. They were lysed by sonication and the insoluble protein and cell debris were removed by centrifugation. The protein was purified with Ni-NTA (Qiagen) according to the instructions of the suppliers. The purified protein was snap frozen in liquid nitrogen and stored in HEPES buffer (50 mM HEPES, 50 mM NaCl, pH = 7) at -78 °C until further use. The protein concentration was determined using Thermo Scientific

Pierce™ BCA Protein Assay Kit.

Protein detection with fluorescence switchable probe in microtiter plates: Fluorescence probes were dissolved in DMSO to obtain 10 mM stock solutions. 5 μM fluorescent probe and indicated concentration of target proteins in 100 μL of PBS buffer (1% DMSO, 137 mM NaCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.2) were incubated for 10 minutes (120 minutes for thrombin) in 96-well plate. The fluorescence spectra were measured on TECAN Infinite M200Pro.

Fluorescence lifetime measurements: For time-resolved measurements, the excitation laser wavelength was 400 nm and the power used was less than 1 mW. The laser system is a femto second mode-locked Ti:sapphire laser generating a pulse train (82 MHz, 800 nm) of which the second-harmonic pulses are generated with a nonlinear crystals (BBO, type I). Picosecond time-resolved fluorescence was measured by time-correlated single-photon counting (TCSPC). The samples were placed in a cuvette, and the fluorescence was filtered through a bandpass filter (±10 nm) and detected by a multichannel plate photomultiplier (MCP-PMT, Hamamatsu). The instrument response function was set at 40 ps at fwhm. The polarization of laser beam was kept at the magic angle with respect to the detection position. We obtained the time constants from the experimental curves by deconvoluting a biexponential function against the instrument response function with fwhm ~40 ps (assuming Gaussian function).

Fluorescence imaging of transfected HeLa Cells: The cells were maintained in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin. 1.5×10^4 cells were seeded in 8-well chamber slides and cultured overnight at 37 °C in air with 5% CO_2. Cells were then transfected by using X-treme GENE HP DNA transfection reagent (Roche Applied Science) according to the manufacturer's protocol. Thirty hours after transfection, the cells were washed with DMEM supplemented with 10% FBS twice and cultured overnight. For nucleus staining,

the cells were washed twice with Opti-MEM and stained with 0.25 μM Hoechst 34580. After incubating for thirty minutes, excess Hoechst 34580 was removed. For the detection of cell surface hCAII, the cells were treated with 0.25 μM of probe **1** prepared in Opti-MEM (1.0 % DMSO (v/v)). The fluorescence imaging was carried out by using Laser Scanning Confocal Microscope (LSM 700, Zeiss, Germany) without any washing steps. For TO channel, the images were taken by using 488 nm laser and the emission was collected from 540-640 nm. For Hoechst 34580, we used 405 nm laser and SP490 emission filter.

Polypeptide sequence of the recombinant hCAII protein

MASWSHPQFE KGADDDDKVP AGGMSHHWGY GKHNGPEHWH KDFPIAKGER

>>----------hCAII---------->

QSPVDIDTHT AKYDPSLKPL SVSYDQATSL RILNNGHAFN VEFDDSQDKA

>----------hCAII----------

VLKGGPLDGT YRLIQFHFHW GSLDGQGSEH TVDKKKYAAE LHLVHWNTKY

>----------hCAII----------

GDFGKAVQQP DGLAVLGIFL KVGSAKPGLQ KVVDVLDSIK TKGKSADFTN

>----------hCAII----------

FDPRGLLPES LDYWTYPGSL TTPPLLECVT WIVLKEPISV SSEQVLKFRK

>----------hCAII----------

LNFNGEGEPE ELMVDNWRPA QPLKNRQIKA SFKRAPGFSS ISAHHHHHHH

>----------hCAII---------->> >>--His-tag-->

HHH

--->>

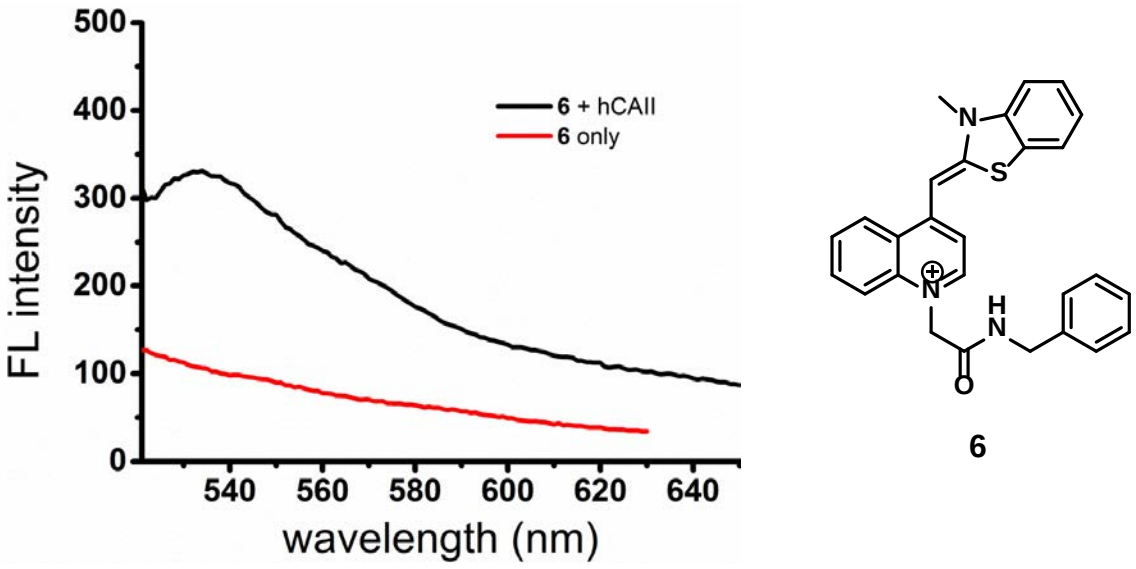


Figure S1. Fluorescence spectra of 5 μM **6** in the absence and presence of 6 μM hCAII in PBS buffer (1% DMSO).

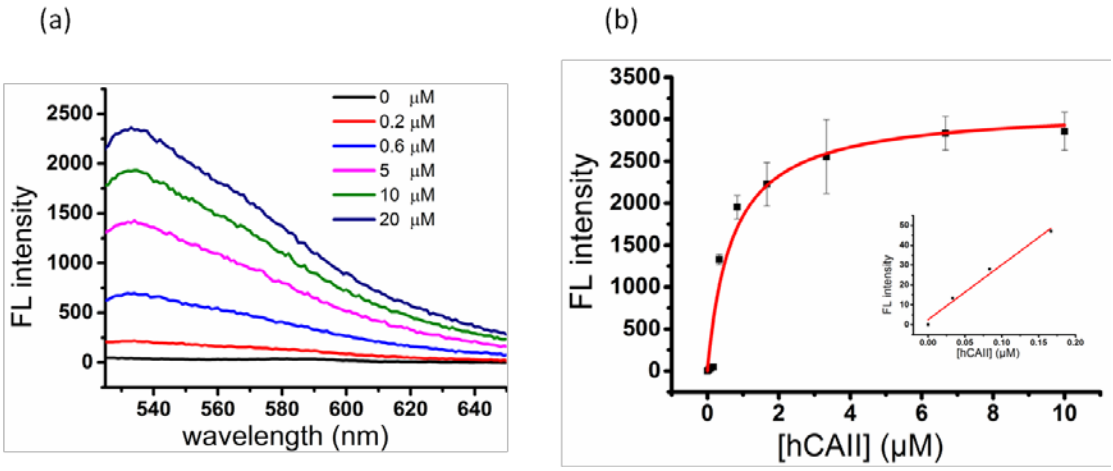


Figure S2. (a) Fluorescence spectra to increasing concentrations of hCAII with probe **1**. (b) Fluorescence response of **1** (0.5 μM) with increasing concentration of hCAII in PBS buffer. The fluorescence titration curve was fitted on 1:1 binding isotherm model to give K_d = 0.69 μM. The error bar was calculated from three independent experiments. The inset showst the linear range for the detection of hCAII which was estimated to be from 0.1 μM to 0.6 μM.

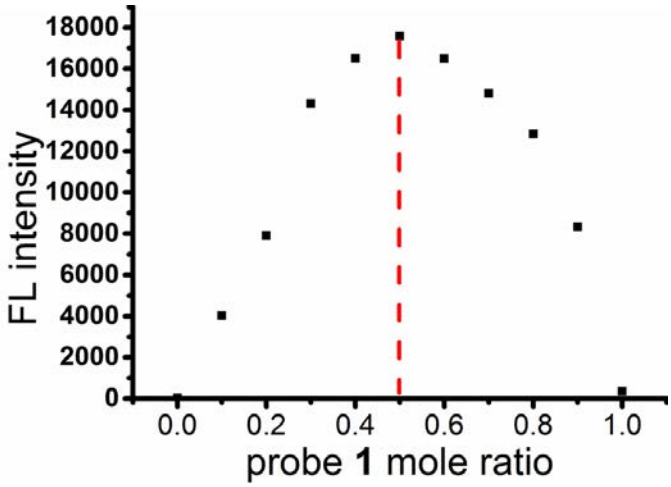


Figure S3. Job's plot analysis of probe **1** with hCAII.

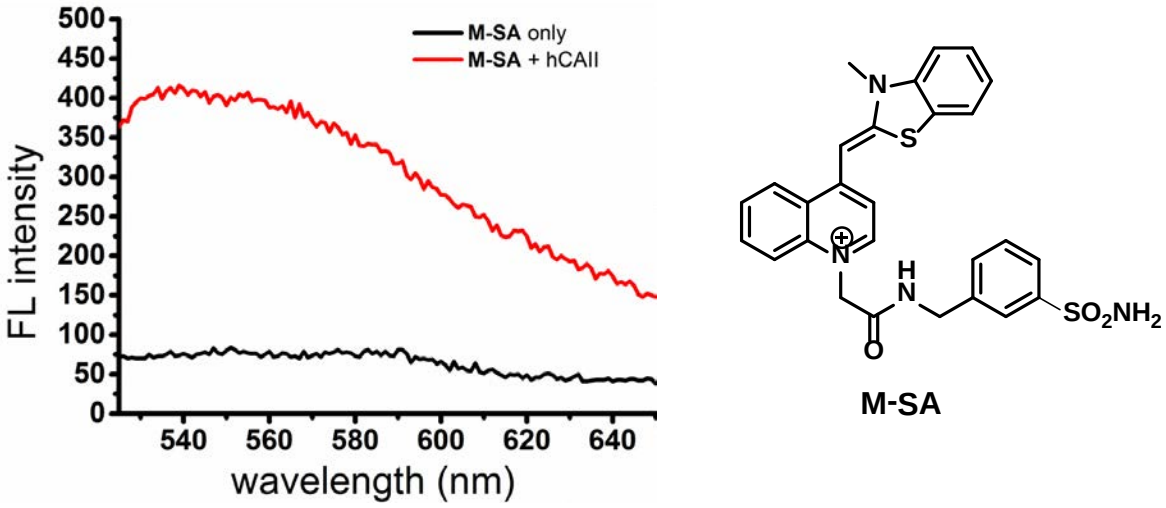


Figure S4. Fluorescence spectra of 5 μM **M-SA** in the absence and presence of 6 μM hCAII in PBS buffer (1% DMSO).

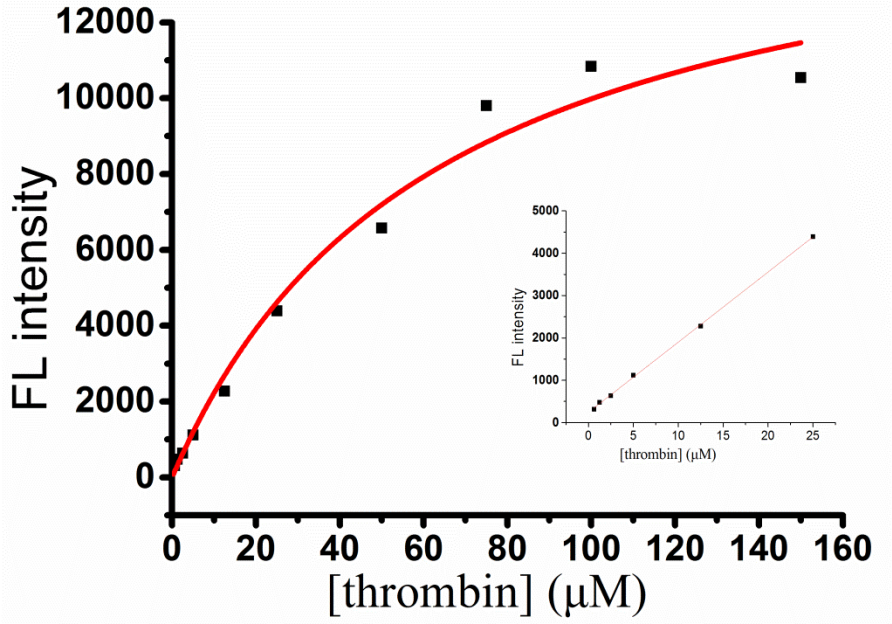


Figure S5. Fluorescence response to increasing concentrations of thrombin with probe **3**. The inset shows the thrombin detection linear range from 0-25 μM.

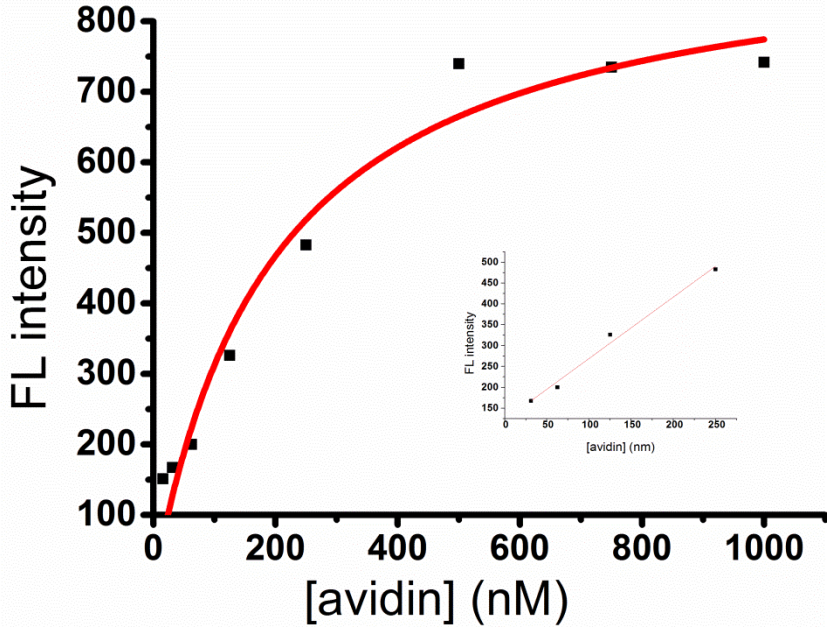


Figure S6. Fluorescence response to increasing concentrations of avidin with probe **4**. The inset shows the avidin detection linear range from 25 nM to 250 nM.

Table S1. Identification of hCAII inhibitors with probe **1**.

Inhibitors	(C_{50}, μM)
acetazolamide	0.66
furosemide	16.44
indapamide	4.15
clopamide	16.19
sulfanilamide	20.14
sulfapyridine (1mM)	N/A
sulfadiazine (1mM)	N/A
sulfamethazine (1mM)	N/A
toluenesulfonic acid (1mM)	N/A
phenylboric acid (1mM)	N/A

N/A : No fluorescence decrease was observed.

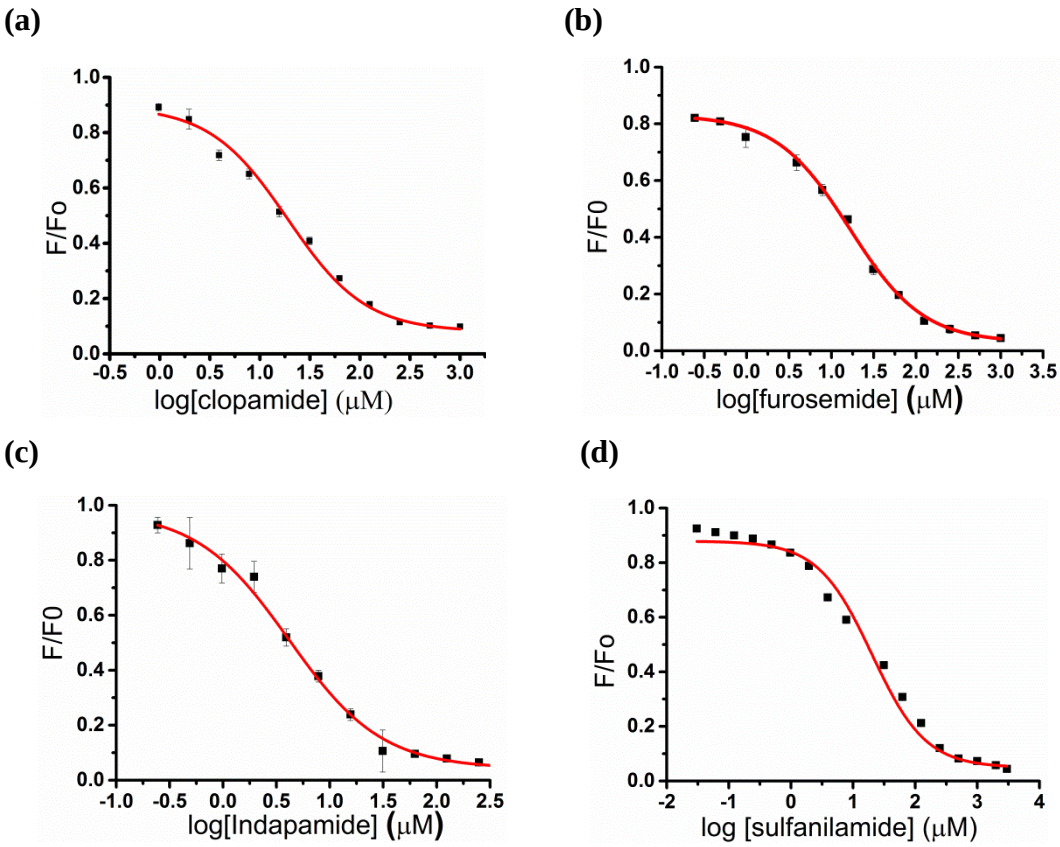


Figure S7. Dose-response curve of 5 μM probe **1**-hCAII mixture against different concentrations of sulfonamide drugs. (a) clopamide, (b) furosemide, (c) indapamide and (d) sulfanilamide. The error bar was calculated from three independent experiments.

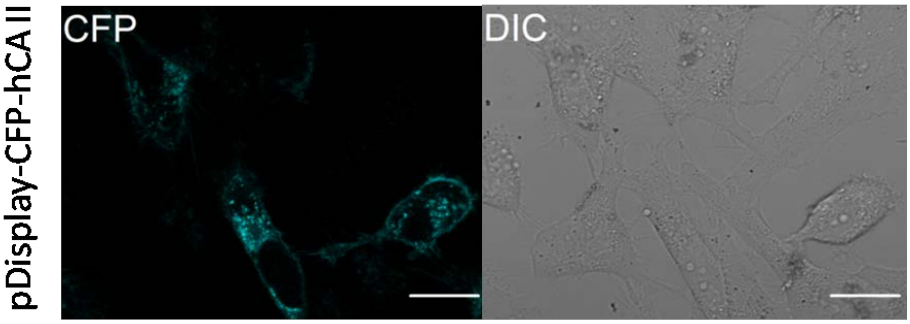


Figure S8. Fluorescence image of recombinant CFP-hCAII protein expressed on the Hela cell surface. Strong fluorescence was observed in intracellular secretory pathways along with the CFP-hCAII fluorescence on the cell surface. CFP = cyan fluorescent protein.

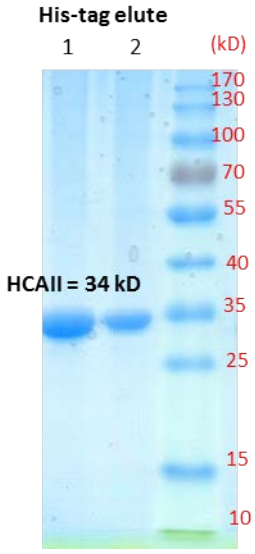
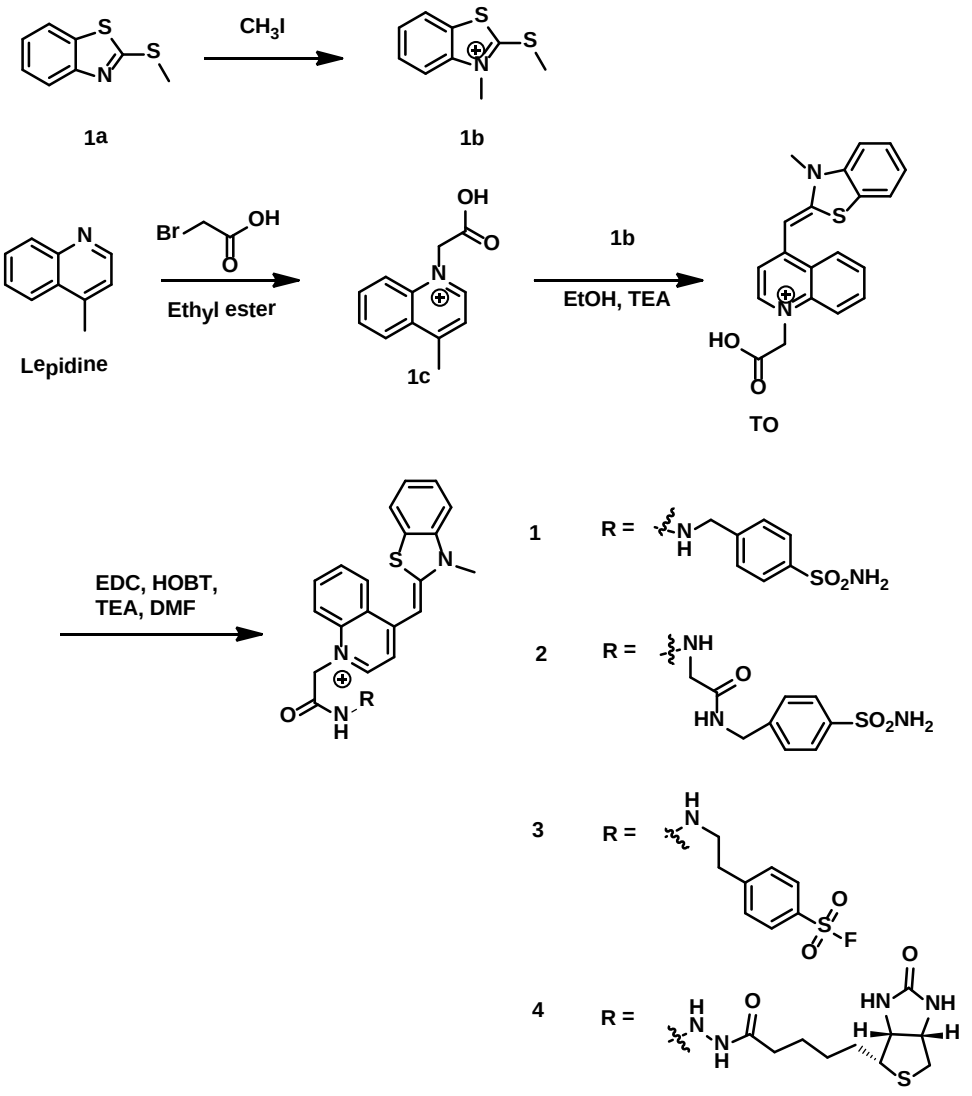


Figure S9. The SDS-PAGE gel shows the purity of hCAII protein after His-tag purification.



Scheme S1. Synthesis of TO-based fluorescence switchable probes.

Preparation of 3-methyl-2-(methylthio)benzo[d]thiazol-3-ium (1b)

To 2-(methylthio)benzo[d]thiazole (1 g, 5.52 mmol) in a round bottom flask was added methyl iodide (1.03 mL, 16.53 mmol) at room temperature. The reaction mixture was refluxed at 100 °C for 15 hr. The heavy white precipitate was filtered and washed with ether to give compound **1b** in 85% yield (920 mg). **^{1}H NMR** (400 MHz, DMSO-d6): δ 8.40 (d, *J* = 8.3 Hz, 1H), 8.19 (d, *J* = 8.3 Hz, 1H), 7.83 (t, *J* = 7.5 Hz, 1H), 7.71 (t, *J* = 7.6 Hz, 1H), 4.10 (s, 3H), 3.12 (s, 3H) ppm; **^{13}C NMR** (101 MHz, DMSO-d6): δ 181.10, 142.44, 129.10, 128.16, 126.92, 123.94, 115.66, 36.56,

18.23 ppm; **HRMS** (ESI): calcd for $C_9H_{10}NS_2^+$ $[M]^+$: 196.0249, found: 196.0251.

Preparation of 1-(carboxymethyl)-4-methylquinolinium (**1c**)

To an ethyl acetate solution of bromoacetic acid was added Lepidine (641.7 μL, 3.5 mmole), followed by stirring the reaction mixture overnight. The white precipitate was filtered and washed with ether to give compound **1c** in 45% yield (319 mg). **^{1}H NMR** (400 MHz, CD_3OD): δ 9.22 (d, *J* = 6.1 Hz, 1H), 8.60 (d, *J* = 8.6 Hz, 1H), 8.33 (d, *J* = 9.0 Hz, 1H), 8.29 – 8.21 (m, 1H), 8.14 – 7.98 (m, 2H), 5.92 (s, 2H), 3.11 (s, 3H) ppm; **HRMS** (ESI): calcd for $C_{12}H_{12}NO_2^+$ $[M]^+$: 202.0863, found: 202.0864.

The NMR data were in agreement with the literature values.[1]

Preparation of TO

To an ethanol solution (1 mL) of compound **1b** (150 mg, 0.74 mmol) and **1c** (150 mg, 0.76 mmol) was added triethylamine (310 μL, 2.22 mmol) at 60°C under N_2. When color of the reaction turned deep red, sufficient amount of NaI was added. After removing the solvent, the crude mixture was purified by flash column (DCM/MeOH = 4:1) to give compound **TO** in 80% yield (207 mg). **^{1}H NMR** (400 MHz, DMSO-d6): δ 8.77 (s, 1H), 8.56 (d, *J* = 6.8 Hz, 1H), 8.01 (d, *J* = 8.2 Hz, 1H), 7.95 – 7.91 (m, 1H), 7.85 (d, *J* = 9.1 Hz, 1H), 7.77-7.67 (m, 2H), 7.57 (t, *J* = 7.8 Hz, 1H), 7.38 (d, *J* = 7.2 Hz, 2H), 6.90 (s, 1H), 4.87 (s, 2H), 3.98 (s, 3H) ppm; **^{13}C NMR** (125 MHz, DMSO-d6): δ 168.13, 159.22, 148.46, 145.27, 140.50, 138.06, 132.73, 128.06, 126.56, 125.44, 124.20, 124.03, 123.61, 122.83, 118.52, 112.72, 107.85, 87.58, 58.87 ppm; HRMS (ESI): calcd for $C_{20}H_{17}N_2O_2S^+$ $[M]^+$: 349.1005, found: 349.1007.

Preparation of compound 1

To a solution of **TO** (20 mg, 0.057 mmol) in DMF (2 mL) was added 4-(aminomethyl)benzenesulfonamide (19.15 mg,0.086 mmole), HOBT (17.46 mg, 0.114 mmole), EDC.HCl (21.85 mg, 0.114 mmole) and triethylamine (79.45 μL, 0.57 mmole) at room temperature and the mixture was stirred overnight. The solvent was removed in vacuo and the crude mixture was purified by reversed-phase HPLC to give product **1** as a red solid in 15 % yield. **^{1}H NMR** (400 MHz, DMSO-d6) : δ 8.79 (d, *J* = 9.5 Hz, 1H), 8.52 (d, *J* = 7.3 Hz, 1H), 8.07 (d, *J* = 8.3 Hz, 1H), 7.96 (m, 1H), 7.85 – 7.60 (m, 6H), 7.47 – 7.34 (m, 4H), 6.98 (s, 1H), 5.39 (s, 2H), 4.43 (s, 2H), 4.05 (s, 3H) ppm; **^{13}C NMR** (100 MHz, DMSO-d6) : δ 165.87, 160.75, 148.74, 145.53, 142.84, 140.49, 137.84, 133.22, 128.27, 127.60, 126.69, 125.70, 124.75, 124.07, 122.94, 117.42, 113.27, 107.51, 88.69, 55.83, 42.12, 33.98 ppm; **HRMS** (ESI): calcd for $C_{27}H_{25}N_4O_3S_2^+$ $[M]^+$: 517.1363, found: 517.1364.

Preparation of compound 2

Based on the synthetic procedure for compound **1,** compound **2** was obtained as a red solid in 30 % yield. **^{1}H NMR** (400 MHz, CD_3OD) : δ 8.61 (d, *J* = 8.4 Hz, 1H), 8.27 (d, *J* = 7.1 Hz, 1H), 7.92 (d, *J* = 7.9 Hz, 1H), 7.85 – 7.57 (m, 7H), 7.50 – 7.35 (m, 4H), 6.96 (s, 1H), 5.34 (s, 2H), 4.47 (s, 2H), 4.03 (s, 3H), 4.00 (s, 2H) ppm; **HRMS** (ESI): calcd for $C_{29}H_{28}N_5O_4S_2^+$ $[M]^+$: 574.1577, found: 574.1578.

Preparation of compound 3

To a solution of **TO** (20 mg, 0.057 mmol) in DMF (2 mL) was added 4-(2-Aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF) (11.38 mg, 0.0475 mmole), PyBOP (49.43 mg, 0.095 mmole) and *N*,*N*-Diisopropylethylamine (13.24 μL, 0.095 mmole) at room temperature

and the reaction mixture was stirred overnight. The solvent was removed in vacuo and the crude mixture was purified by reversed-phase HPLC to give product **3** as a red solid in 47 % yield. **^{1}H NMR** (400 MHz, DMSO-d6): δ 8.52 (d, *J* = 8.6 Hz, 1H), 8.40 (s, 1H), 8.20 (d, *J* = 7.4 Hz, 1H), 7.86 – 7.76 (m, 2H), 7.67 – 7.29 (m, 7H), 7.23 – 7.08 (m, 2H), 6.71 (s, 1H), 4.98 (s, 2H), 3.79 (s, 3H), 2.68 (t, *J* = 6.6 Hz, 4H) ppm; **^{13}C NMR** (100 MHz, DMSO-d6): δ 165.61, 149.23, 148.77, 145.56, 140.55, 137.72, 133.18, 130.85, 128.51, 126.69, 125.80, 124.84, 124.23, 123.90, 123.00, 117.26, 113.33, 107.52, 94.04, 88.71, 55.82, 42.76, 34.93, 34.03 ppm; **HRMS** (ESI): calcd for $C_{28}H_{25}FN_3O_3S_2^+$ $[M]^+$: 534.1316, found: 534.1314.

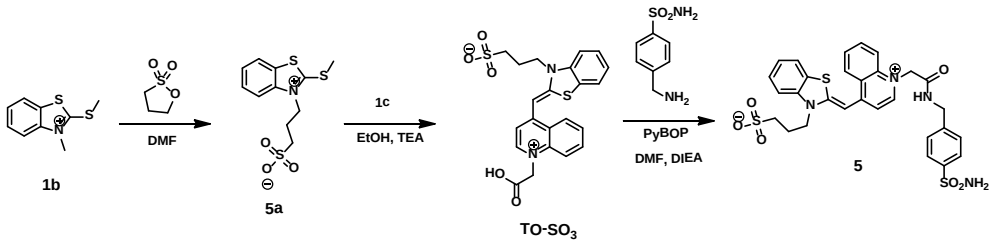
Preparation of compound 4

Based on the synthetic procedure for compound **1,** compound **4** was obtained as a red solid in 13 % yield. **^{1}H NMR** (400 MHz, CD_3OD) : δ 8.61 (d, *J* = 8.1 Hz, 1H), 8.30 (d, *J* = 7.1 Hz, 1H), 7.93-7.84 (m, 2H), 7.70 (m, 4H), 7.51-7.28 (m, 3H), 5.36 (s, 2H), 4.39 (s, 1H), 4.18 (s, 1H), 4.02 (s, 3H), 3.19-3.11 (m, 2H), 2.92 – 2.83 (m, 2H), 2.65 (d, *J* = 12.5 Hz, 1H), 2.37 – 2.25 (m, 2H), 1.72-1.29 (m, 6H) ppm; **^{13}C NMR** (100 MHz, DMSO-d6): δ 171.81, 165.70, 163.38, 161.61, 141.16, 137.80, 133.90, 127.40, 126.41, 125.51, 124.52, 123.65, 114.01, 108.14, 89.47, 79.50, 61.70, 59.85, 56.05, 53.36, 43.64, 34.70, 33.54, 28.72, 25.67 ppm; **HRMS** (ESI): calcd for $C_{30}H_{33}N_6O_3S_2^+$ $[M]^+$: 589.2050, found: 589.2048.

Preparation of compound 6

Based on the synthetic procedure for compound **1,** compound **6** was obtained as a red solid in 13 % yield; **^{1}H NMR** (400 MHz, CD_3OD): δ 8.66 (d, *J* = 8.7 Hz, 1H), 8.32 (d, *J* = 7.3 Hz, 1H), 7.94-7.86 (m, 2H), 7.72 (d, *J* = 8.6 Hz, 3H), 7.64 (t, *J* = 7.9 Hz, 1H), 7.50-7.43 (m, 2H), 7.34 – 7.28 (m, 4H), 6.99 (s, 1H), 5.30 (s, 2H), 4.45 (s, 2H), 4.05 (s, 3H) ppm; **HRMS** (ESI): calcd for

$C_{27}H_{24}N_3OS^+$ $[M]^+$: 438.1635, found: 438.1633.



Scheme 2. Synthesis of compound **5.**

Preparation of 5a

A mixture of 3-methyl-2-(methylthio)benzo[d]thiazol-3-ium and propanesultone in 2 mL DMF was heated overnight in an oil bath at 115°C. The reaction mixture was cooled to room temperature, wash several times with ethyl ether and dried to give product **5a** in 55% yield (500 mg). ^{1}H NMR (400 MHz, CD_3OD): δ 8.23 (dd, *J* = 13.5, 8.3 Hz, 2H), 7.88-7.83 (m, 1H), 7.73 (t, *J* = 7.8 Hz, 1H), 4.91 – 4.86 (m, 2H), 3.14 (s, 3H), 3.01 (t, *J* = 6.7 Hz, 2H), 2.43 – 2.32 (m, 2H) ppm; **^{13}C NMR** (100 MHz, CD_3OD) δ 130.92, 128.61, 124.72, 116.71, 50.12 , 24.00, 18.51 ppm; **HRMS** (ESI): calcd for $C_{11}H_{13}NO_3S_3$ $[M+H]^+$: 304.0136, found: 304.0128.

Preparation of TO-SO_3

Compound **1a** (50 mg, 0.24 mmol) and compound **5a** (77.07 mg, 0.25 mmol) was suspended in dry ethanol (1 mL), and stirred under N_2 at 60°C, followed by triethylamine (310 μL, 2.22 mmol). The mixture was allowed to cool to room temperature and the precipitation was induced by addition of ether. The crude solid was suspended in acetone/Et_2O (7:10) for 1 hour, then collect via vacuum filtration followed by washing with Et_2O to give compound **TO-SO_3** in 50% yield (56 mg). **^{1}H NMR** (400 MHz, DMSO-d6) δ 9.07 (d, *J* = 8.6 Hz, 1H), 8.51 (d, *J* = 7.2 Hz, 1H),

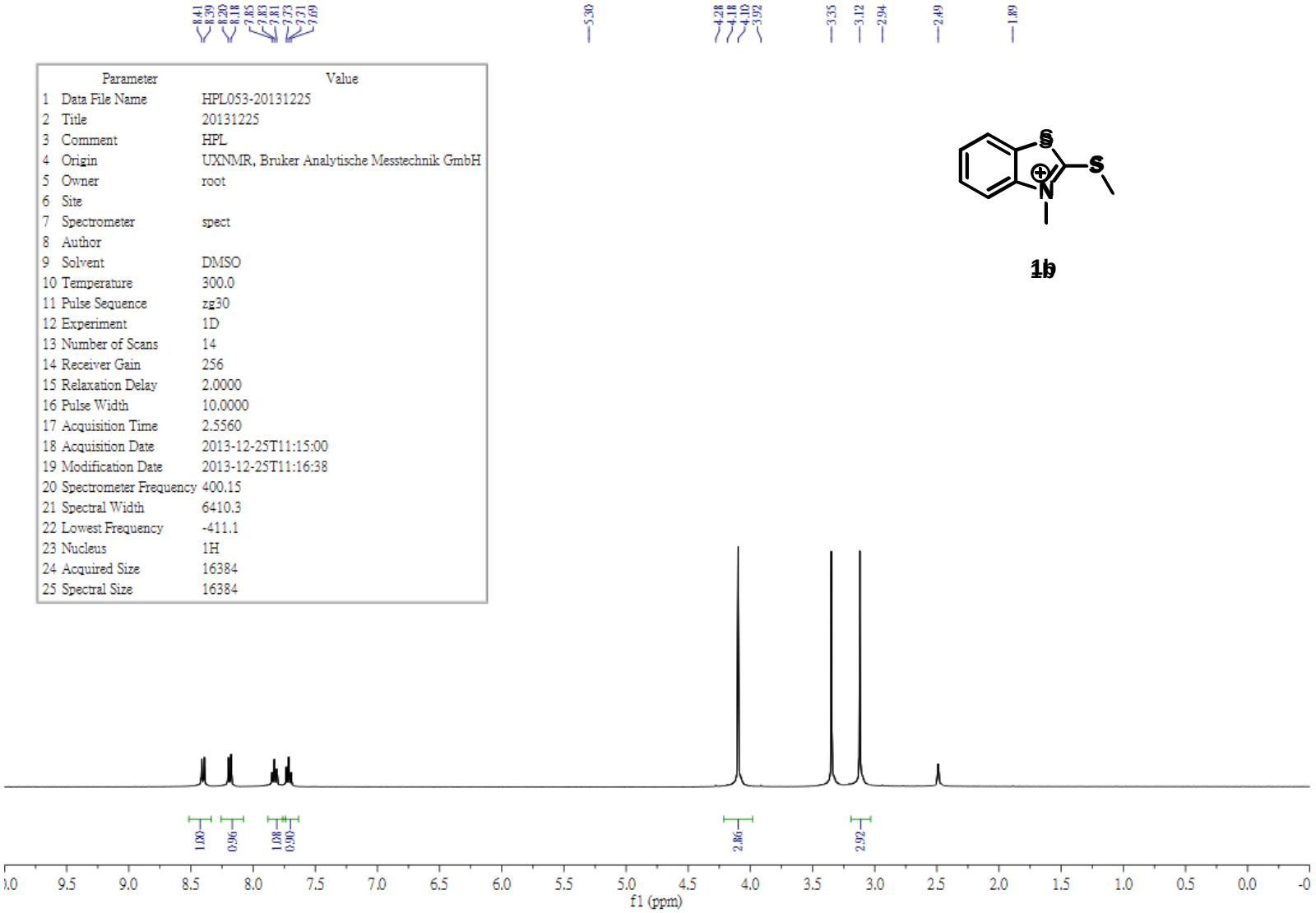
8.03 (d, *J* = 7.9 Hz, 1H), 7.93 – 7.79 (m, 3H), 7.68-7.57 (m, 2H), 7.44-7.34 (m, 2H), 7.19 (s, 1H), 5.25 (s, 2H), 4.81 (s, 2H), 2.69 (t, *J* = 5.3 Hz, 2H), 2.13 (m, 2H) ppm. **HRMS** (ESI): calcd for $C_{22}H_{20}N_2O_5S_2$ $[M+H]^+$: 457.0892, found: 457.0886.

Preparation of compound 5

Based on the synthetic procedure for compound **3,** compound **5** was obtained as a red solid in 63 % yield. **^{1}H NMR** (400 MHz, DMSO-d6) δ 9.08 (d, *J* = 7.4 Hz, 1H), 8.50 (d, *J* = 7.3 Hz, 1H), 8.04 (d, *J* = 7.9 Hz, 1H), 7.99 – 7.80 (m, 2H), 7.79-7.49 (m, 5H), 7.43-7.36 (m, 3H), 7.30 (s, 1H), 7.20 (s, 1H), 5.35 (s, 2H), 4.81 (s, 2H), 4.40 (s, 2H), 2.66 (t, *J* = 6.5 Hz, 2H), 2.11 (m, 2H) ppm; **^{13}C NMR** (125 MHz, DMSO-d6) δ 165.86, 160.02, 149.24, 145.39, 142.78, 139.91, 137.75, 133.15, 128.34, 127.63, 126.90, 126.80, 125.70, 124.70, 124.23, 122.95, 117.05, 113.21, 107.66, 88.66, 55.92, 47.54, 42.12, 30.71, 23.26, 21.11 ppm; **HRMS** (ESI): calcd for $C_{33}H_{36}N_5O_4S_2^+$ $[M]^+$: 624.1171, found: 624.1171.

References

1. Hara, Y.; Fujii, T.; Kashida, H.; Sekiguchi, K.; Liang, X.; Niwa, K.; Takase, T.; Yoshida, Y.; Asanuma, H. *Angew. Chem. Int. Ed.* **2010**, *49*, 5502-5506.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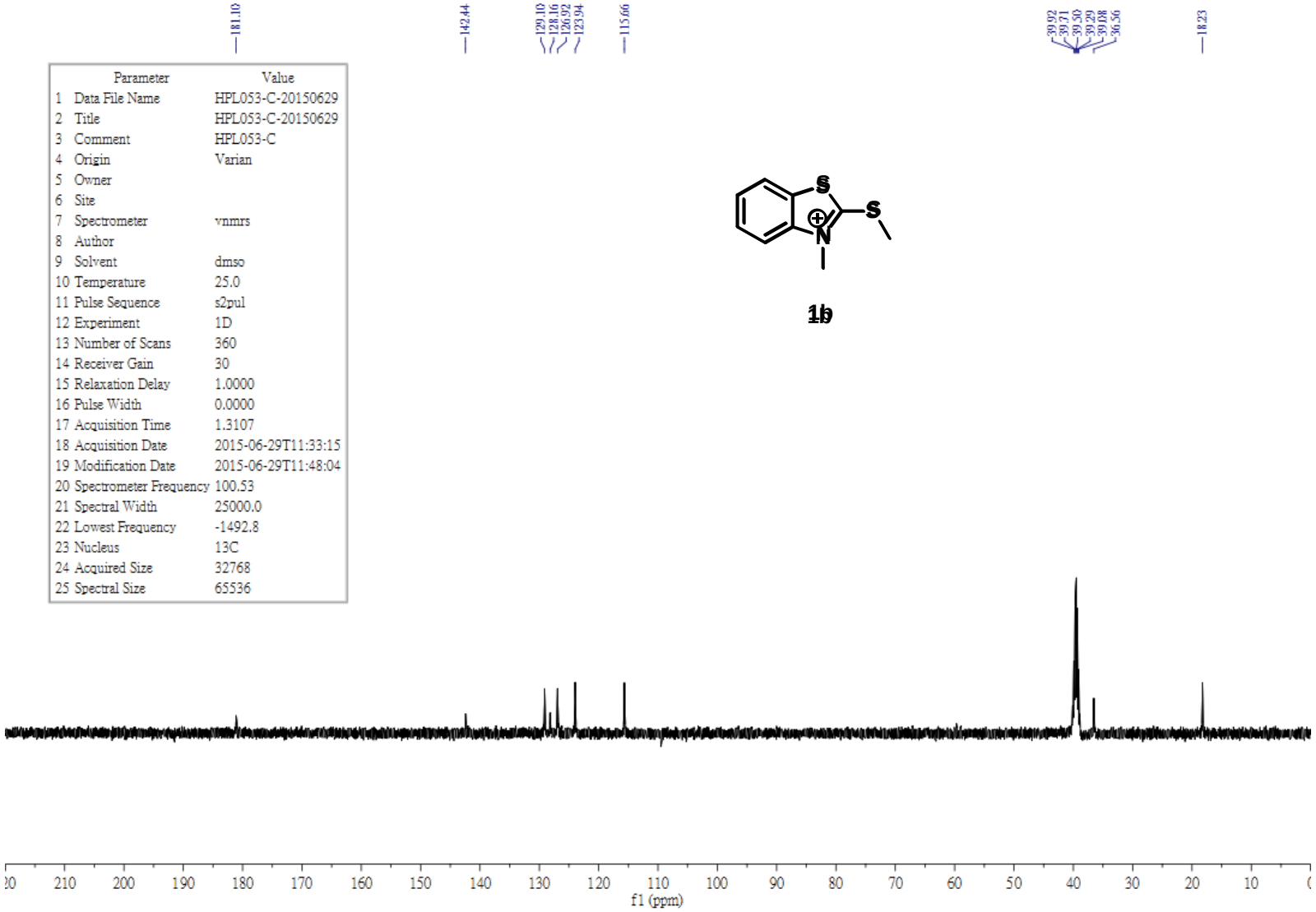
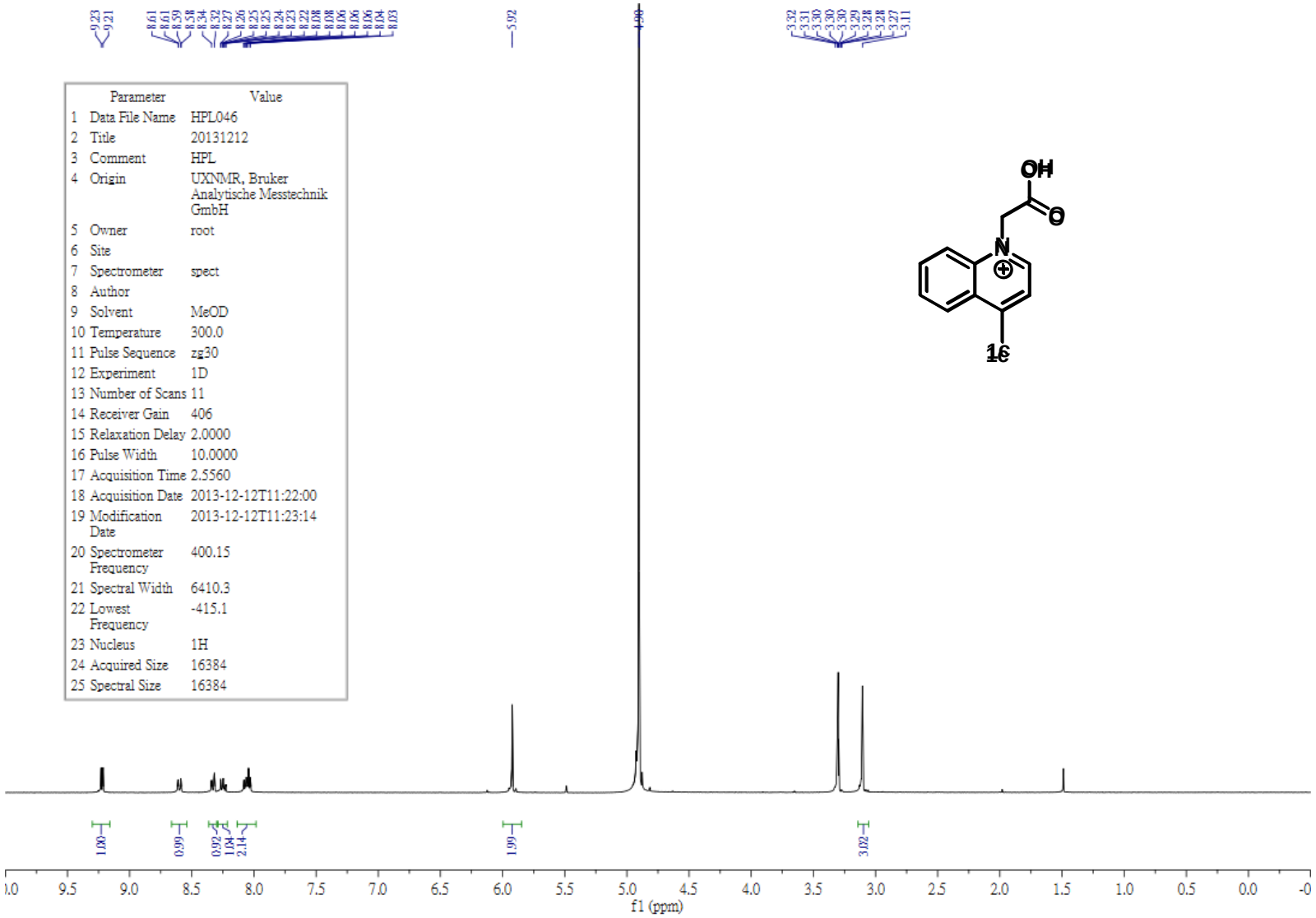
f1 (ppm)

1b

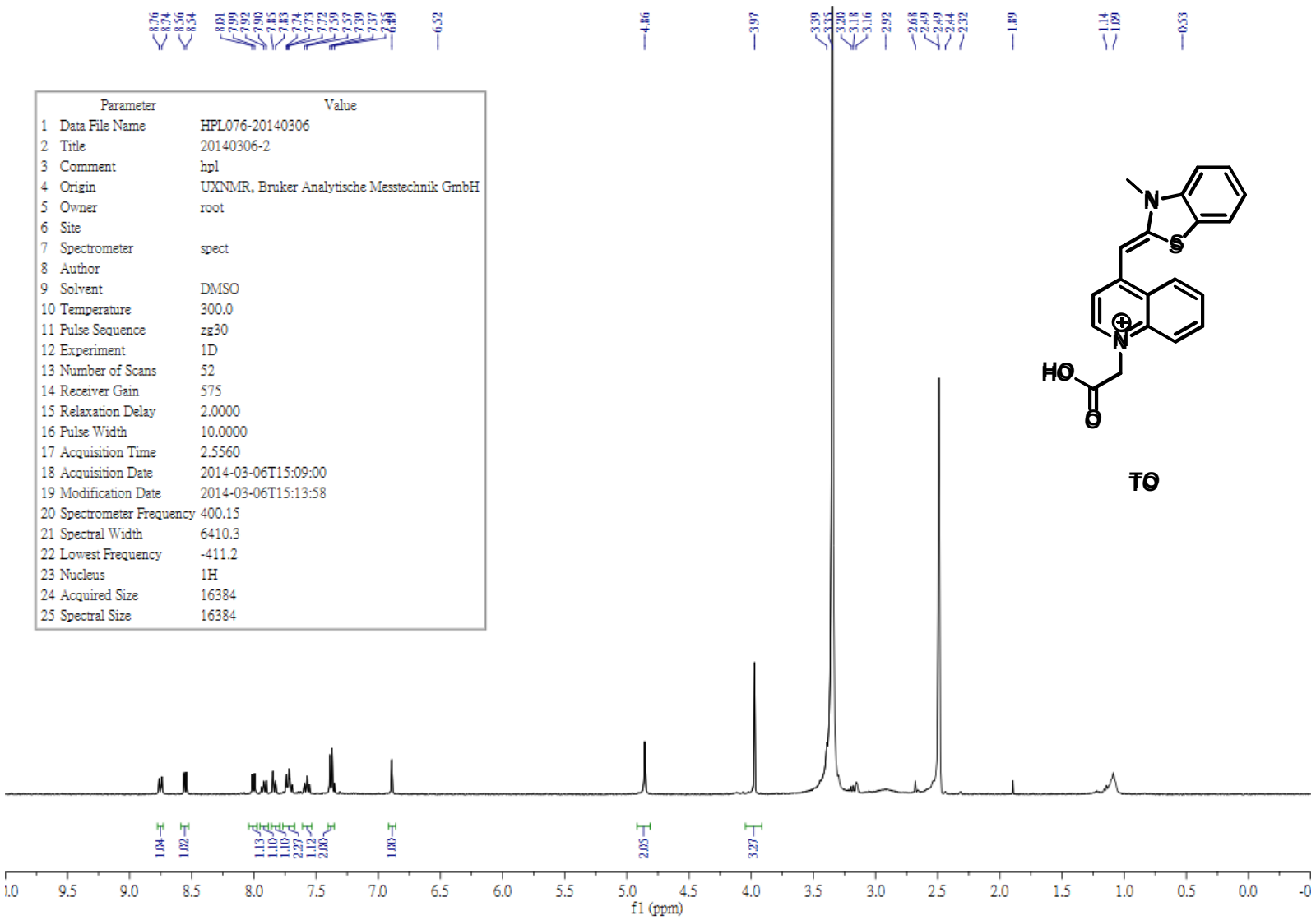


	Parameter	Value
1	Data File Name	HPL053-C-20150629
2	Title	HPL053-C-20150629
3	Comment	HPL053-C
4	Origin	Varian
5	Owner	
6	Site	
7	Spectrometer	vnmrs
8	Author	
9	Solvent	dmso
10	Temperature	25.0
11	Pulse Sequence	s2pul
12	Experiment	1D
13	Number of Scans	360
14	Receiver Gain	30
15	Relaxation Delay	1.0000
16	Pulse Width	0.0000
17	Acquisition Time	1.3107
18	Acquisition Date	2015-06-29T11:33:15
19	Modification Date	2015-06-29T11:48:04
20	Spectrometer Frequency	100.53
21	Spectral Width	25000.0
22	Lowest Frequency	-1492.8
23	Nucleus	13C
24	Acquired Size	32768
25	Spectral Size	65536

1b

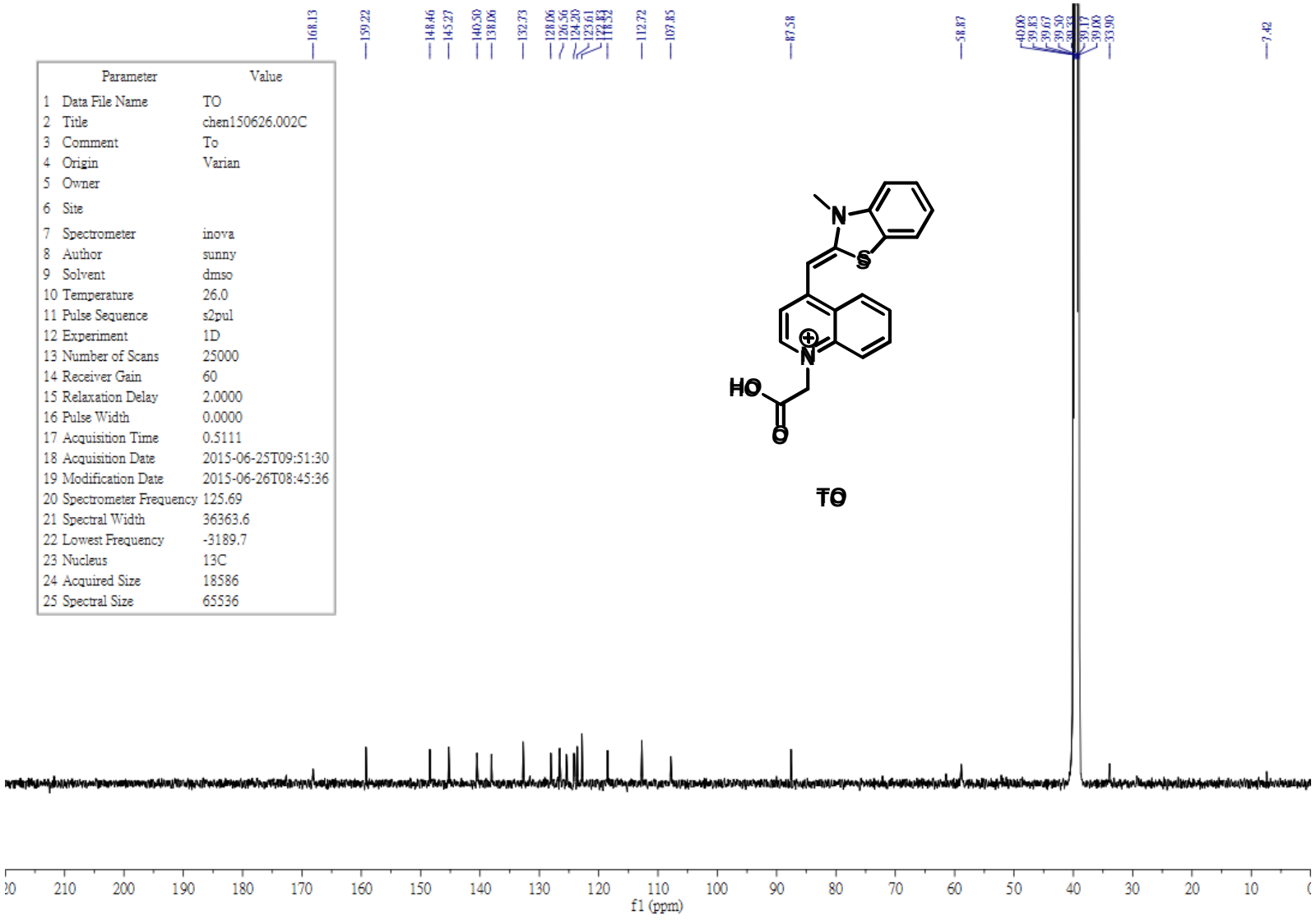


	Parameter	Value
1	Data File Name	HPL046
2	Title	20131212
3	Comment	HPL
4	Origin	UXNMR, Bruker Analytische Messtechnik GmbH
5	Owner	root
6	Site	
7	Spectrometer	spect
8	Author	
9	Solvent	MeOD
10	Temperature	300.0
11	Pulse Sequence	zg30
12	Experiment	1D
13	Number of Scans	11
14	Receiver Gain	406
15	Relaxation Delay	2.0000
16	Pulse Width	10.0000
17	Acquisition Time	2.5560
18	Acquisition Date	2013-12-12T11:22:00
19	Modification Date	2013-12-12T11:23:14
20	Spectrometer Frequency	400.15
21	Spectral Width	6410.3
22	Lowest Frequency	-415.1
23	Nucleus	1H
24	Acquired Size	16384
25	Spectral Size	16384

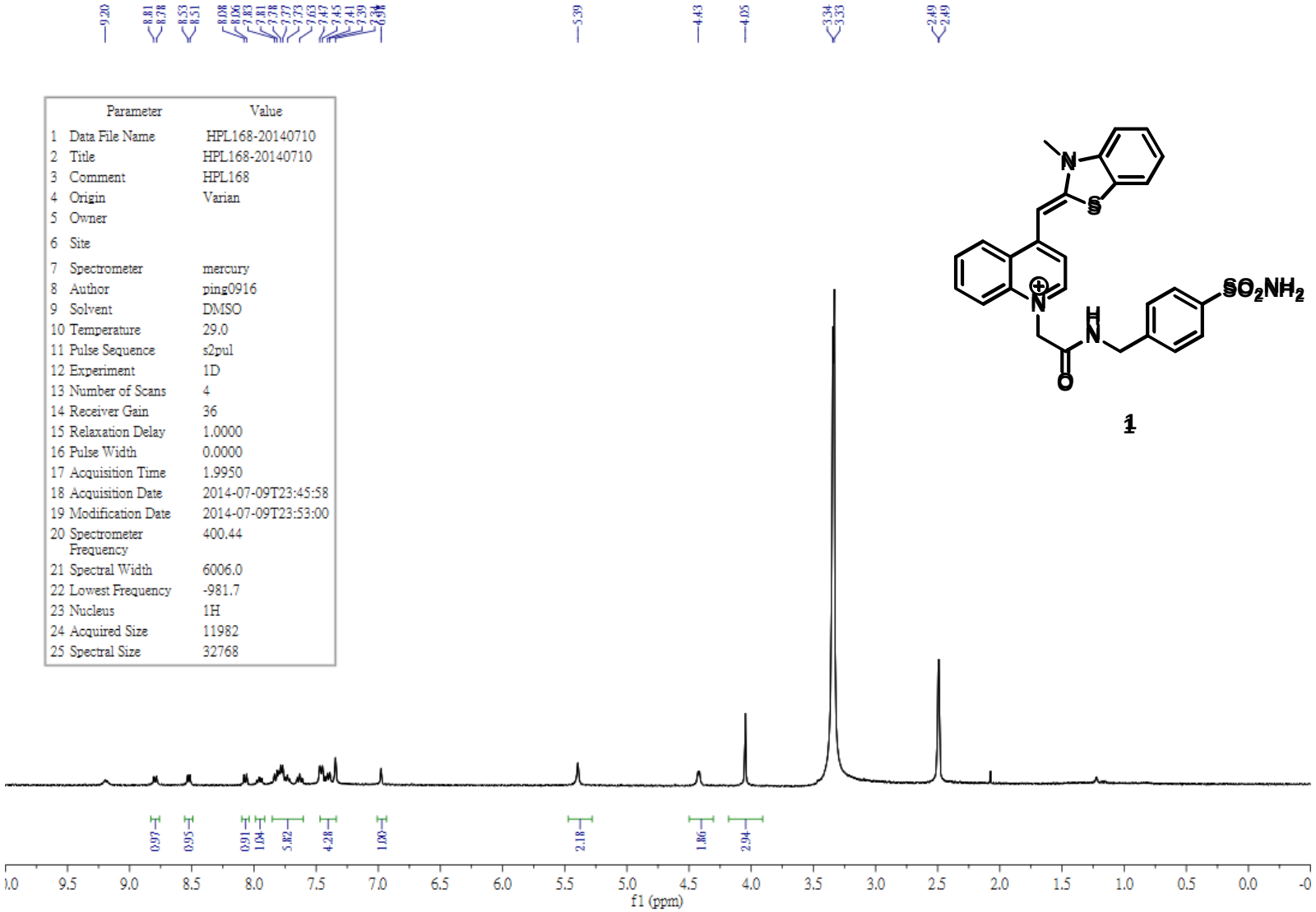


	Parameter	Value
1	Data File Name	HPL076-20140306
2	Title	20140306-2
3	Comment	hpl
4	Origin	UXNMR, Bruker Analytische Messtechnik GmbH
5	Owner	root
6	Site	
7	Spectrometer	spect
8	Author	
9	Solvent	DMSO
10	Temperature	300.0
11	Pulse Sequence	zg30
12	Experiment	1D
13	Number of Scans	52
14	Receiver Gain	575
15	Relaxation Delay	2.0000
16	Pulse Width	10.0000
17	Acquisition Time	2.5560
18	Acquisition Date	2014-03-06T15:09:00
19	Modification Date	2014-03-06T15:13:58
20	Spectrometer Frequency	400.15
21	Spectral Width	6410.3
22	Lowest Frequency	-411.2
23	Nucleus	1H
24	Acquired Size	16384
25	Spectral Size	16384

TO



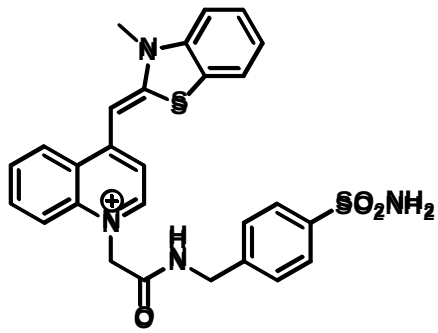
	Parameter	Value
1	Data File Name	TO
2	Title	chen150626.002C
3	Comment	To
4	Origin	Varian
5	Owner	
6	Site	
7	Spectrometer	inova
8	Author	sunny
9	Solvent	dmso
10	Temperature	26.0
11	Pulse Sequence	s2pul
12	Experiment	1D
13	Number of Scans	25000
14	Receiver Gain	60
15	Relaxation Delay	2.0000
16	Pulse Width	0.0000
17	Acquisition Time	0.5111
18	Acquisition Date	2015-06-25T09:51:30
19	Modification Date	2015-06-26T08:45:36
20	Spectrometer Frequency	125.69
21	Spectral Width	36363.6
22	Lowest Frequency	-3189.7
23	Nucleus	13C
24	Acquired Size	18586
25	Spectral Size	65536


9.20
8.81
8.78
8.53
8.51
8.08
8.06
7.83
7.81
7.78
7.77
7.73
7.63
7.47
7.45
7.41
7.39
5.39
4.43
4.05
3.34
3.33
2.49
2.49
Parameter Value
1 Data File Name HPL168-20140710
2 Title HPL168-20140710
3 Comment HPL168
4 Origin Varian
5 Owner
6 Site
7 Spectrometer mercury
8 Author ping0916
9 Solvent DMSO
10 Temperature 29.0
11 Pulse Sequence s2pul
12 Experiment 1D
13 Number of Scans 4
14 Receiver Gain 36
15 Relaxation Delay 1.0000
16 Pulse Width 0.0000
17 Acquisition Time 1.9950
18 Acquisition Date 2014-07-09T23:45:58
19 Modification Date 2014-07-09T23:53:00
20 Spectrometer Frequency 400.44
21 Spectral Width 6006.0
22 Lowest Frequency -981.7
23 Nucleus 1H
24 Acquired Size 11982
25 Spectral Size 32768
N
S
N
H
N
O
SO2NH2
0.97
0.95
0.91
1.04
5.82
4.28
1.00
2.18
1.86
2.94
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
f1 (ppm)

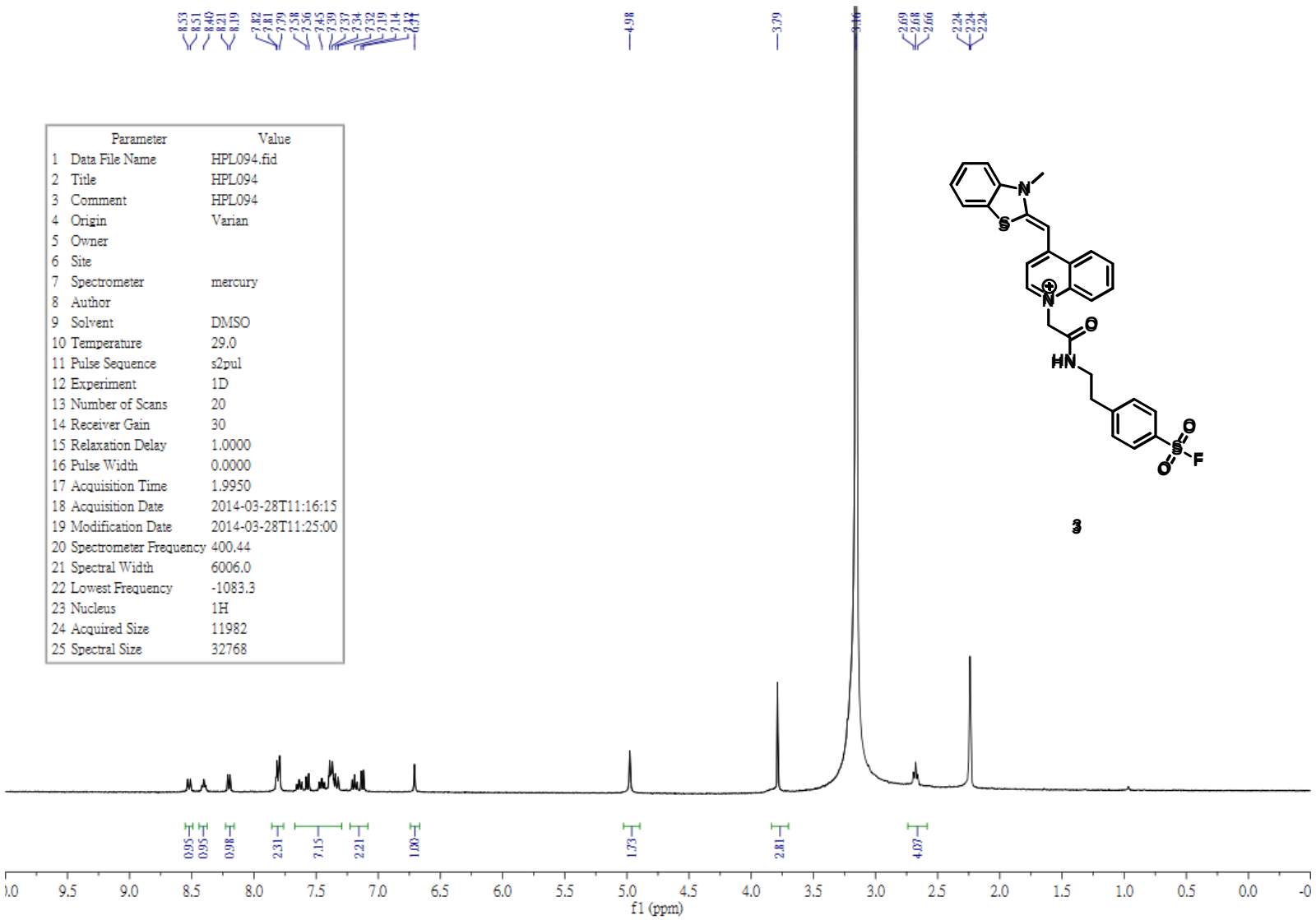
1

HPL168-C-20140710
HPL168-C

	Parameter	Value
1	Data File Name	HPL168-C-20140710
2	Title	HPL168-C-20140710
3	Comment	HPL168-C
4	Origin	Varian
5	Owner	
6	Site	
7	Spectrometer	mercury
8	Author	ping0916
9	Solvent	DMSO
10	Temperature	29.0
11	Pulse Sequence	s2pul
12	Experiment	1D
13	Number of Scans	27536
14	Receiver Gain	32
15	Relaxation Delay	0.0000
16	Pulse Width	0.0000
17	Acquisition Time	1.1994
18	Acquisition Date	2014-07-09T23:46:41
19	Modification Date	2014-07-10T09:01:00
20	Spectrometer Frequency	100.70
21	Spectral Width	25000.0
22	Lowest Frequency	-3038.0
23	Nucleus	13C
24	Acquired Size	29984
25	Spectral Size	65536

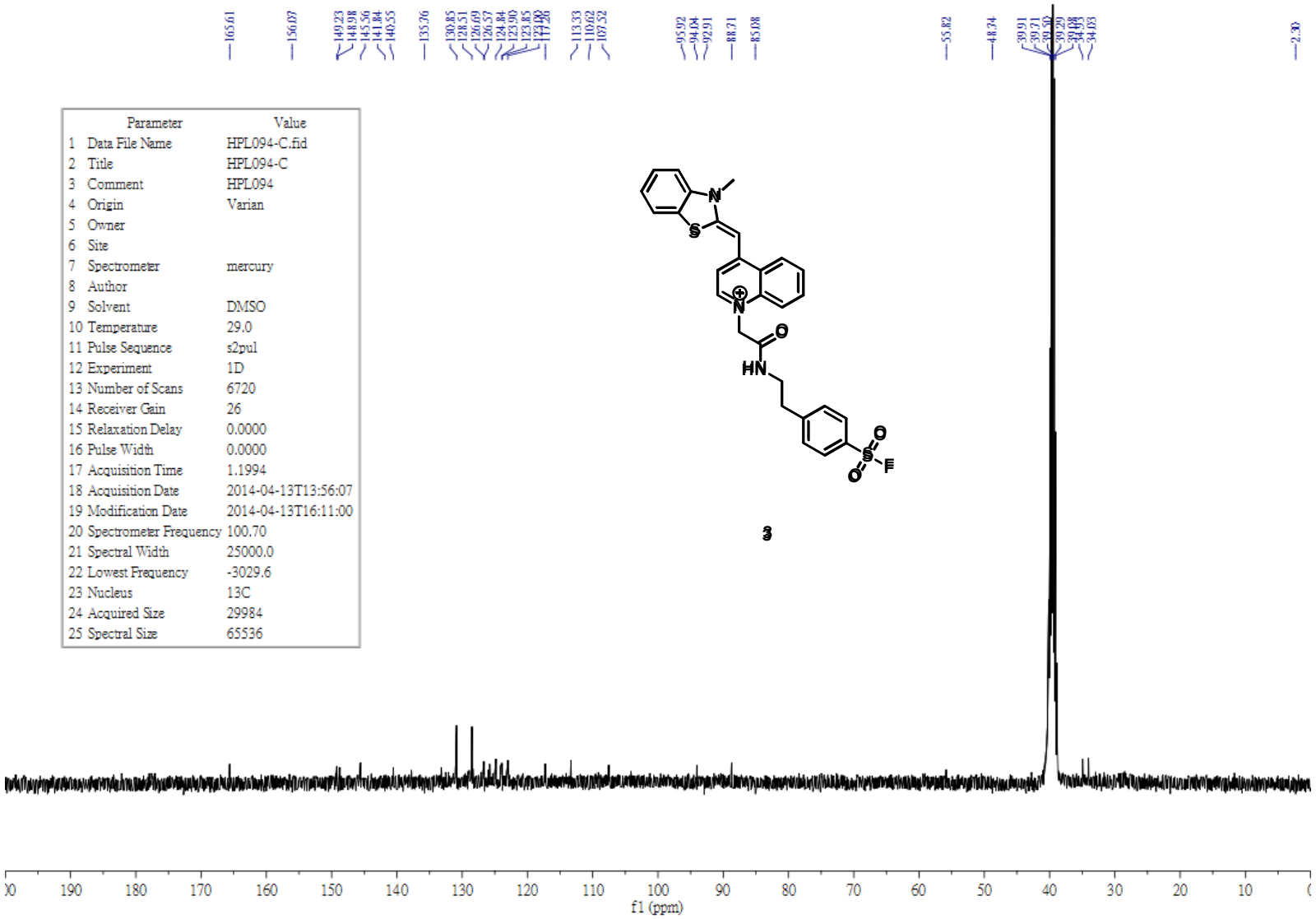


1



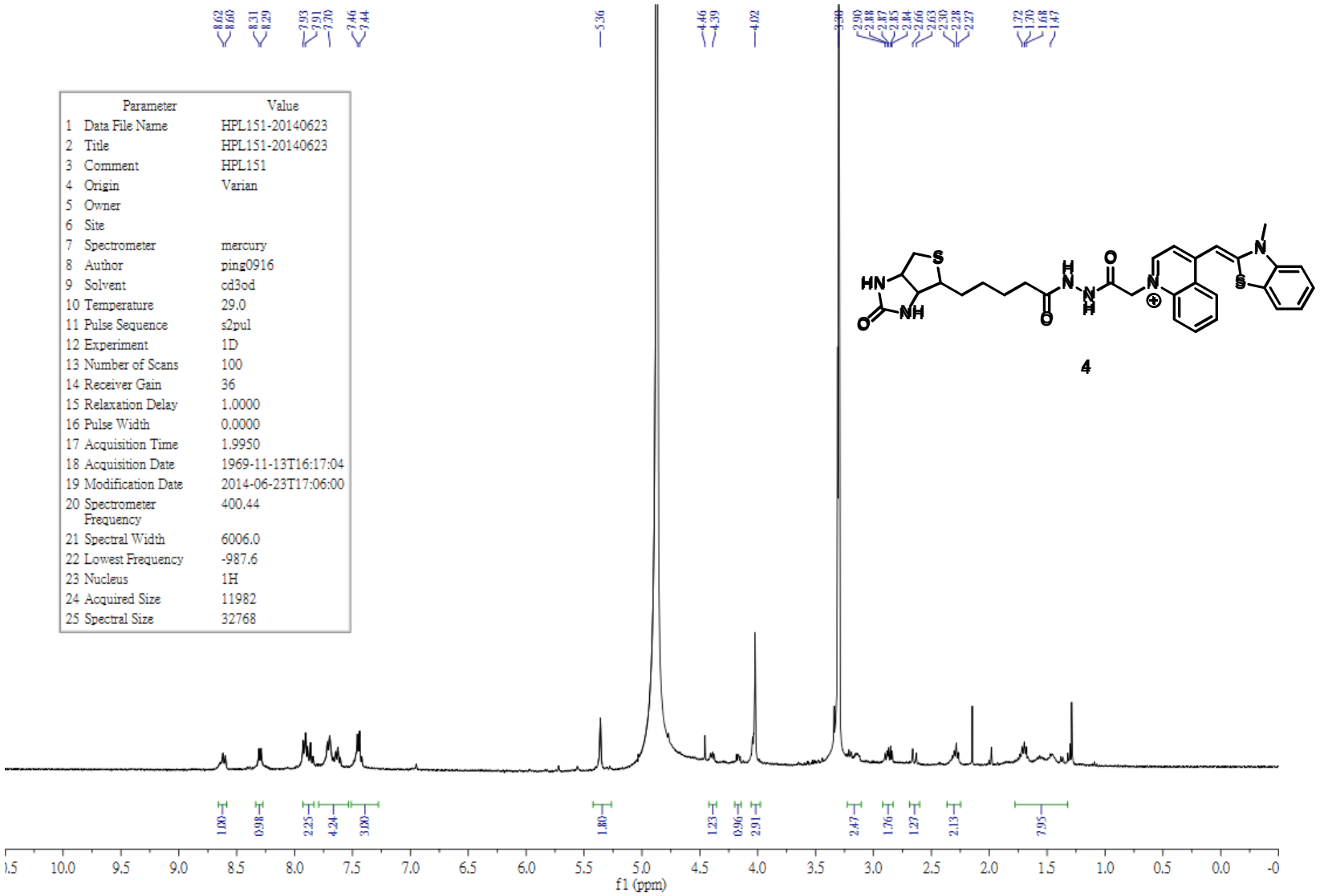
Parameter	Value
1 Data File Name	HPL094.fid
2 Title	HPL094
3 Comment	HPL094
4 Origin	Varian
5 Owner	
6 Site	
7 Spectrometer	mercury
8 Author	
9 Solvent	DMSO
10 Temperature	29.0
11 Pulse Sequence	s2pul
12 Experiment	1D
13 Number of Scans	20
14 Receiver Gain	30
15 Relaxation Delay	1.0000
16 Pulse Width	0.0000
17 Acquisition Time	1.9950
18 Acquisition Date	2014-03-28T11:16:15
19 Modification Date	2014-03-28T11:25:00
20 Spectrometer Frequency	400.44
21 Spectral Width	6006.0
22 Lowest Frequency	-1083.3
23 Nucleus	1H
24 Acquired Size	11982
25 Spectral Size	32768

3

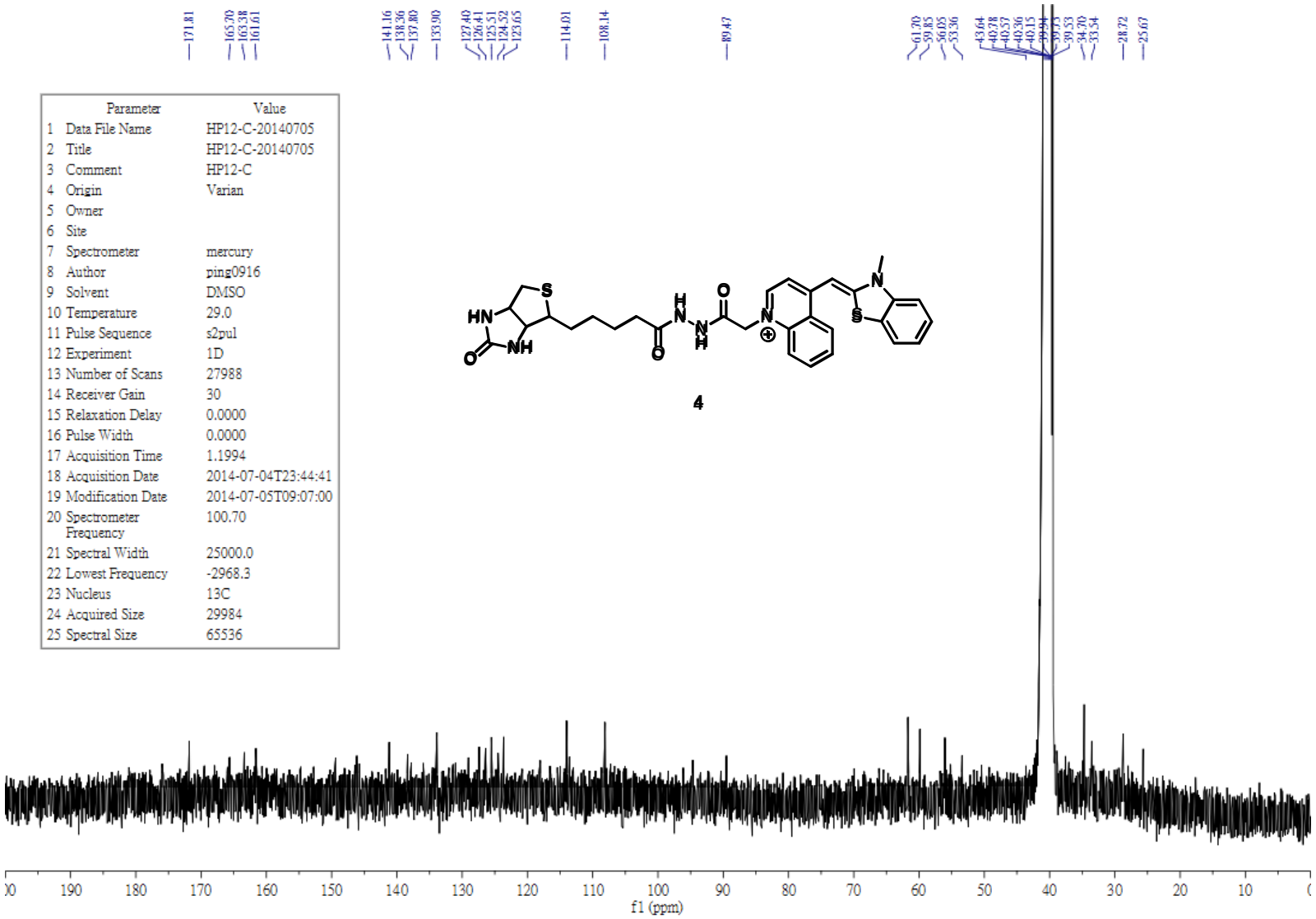


	Parameter	Value
1	Data File Name	HPL094-C.fid
2	Title	HPL094-C
3	Comment	HPL094
4	Origin	Varian
5	Owner	
6	Site	
7	Spectrometer	mercury
8	Author	
9	Solvent	DMSO
10	Temperature	29.0
11	Pulse Sequence	s2pul
12	Experiment	1D
13	Number of Scans	6720
14	Receiver Gain	26
15	Relaxation Delay	0.0000
16	Pulse Width	0.0000
17	Acquisition Time	1.1994
18	Acquisition Date	2014-04-13T13:56:07
19	Modification Date	2014-04-13T16:11:00
20	Spectrometer Frequency	100.70
21	Spectral Width	25000.0
22	Lowest Frequency	-3029.6
23	Nucleus	13C
24	Acquired Size	29984
25	Spectral Size	65536

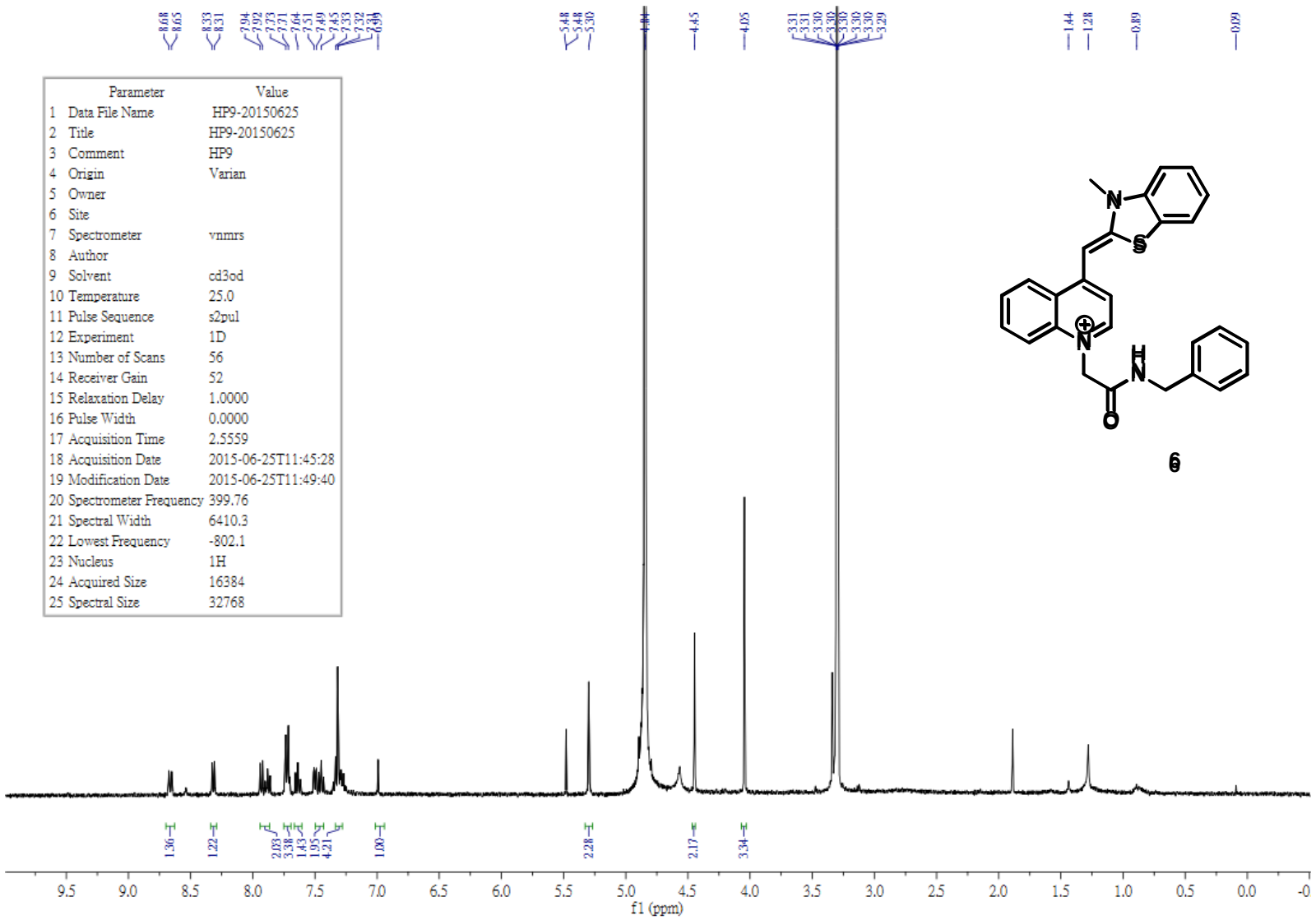
3


8.62
8.60
8.31
8.29
7.93
7.91
7.70
7.46
7.44
5.36
4.46
4.39
4.02
3.00
2.90
2.88
2.87
2.85
2.84
2.66
2.63
2.30
2.28
2.27
1.72
1.70
1.68
1.47
Parameter Value
1 Data File Name HPL151-20140623
2 Title HPL151-20140623
3 Comment HPL151
4 Origin Varian
5 Owner
6 Site
7 Spectrometer mercury
8 Author ping0916
9 Solvent cd3od
10 Temperature 29.0
11 Pulse Sequence s2pul
12 Experiment 1D
13 Number of Scans 100
14 Receiver Gain 36
15 Relaxation Delay 1.0000
16 Pulse Width 0.0000
17 Acquisition Time 1.9950
18 Acquisition Date 1969-11-13T16:17:04
19 Modification Date 2014-06-23T17:06:00
20 Spectrometer Frequency 400.44
21 Spectral Width 6006.0
22 Lowest Frequency -987.6
23 Nucleus 1H
24 Acquired Size 11982
25 Spectral Size 32768
1.00
0.98
2.25
4.24
3.00
1.89
1.23
0.96
2.91
2.47
1.76
1.27
2.13
7.95
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
f1 (ppm)

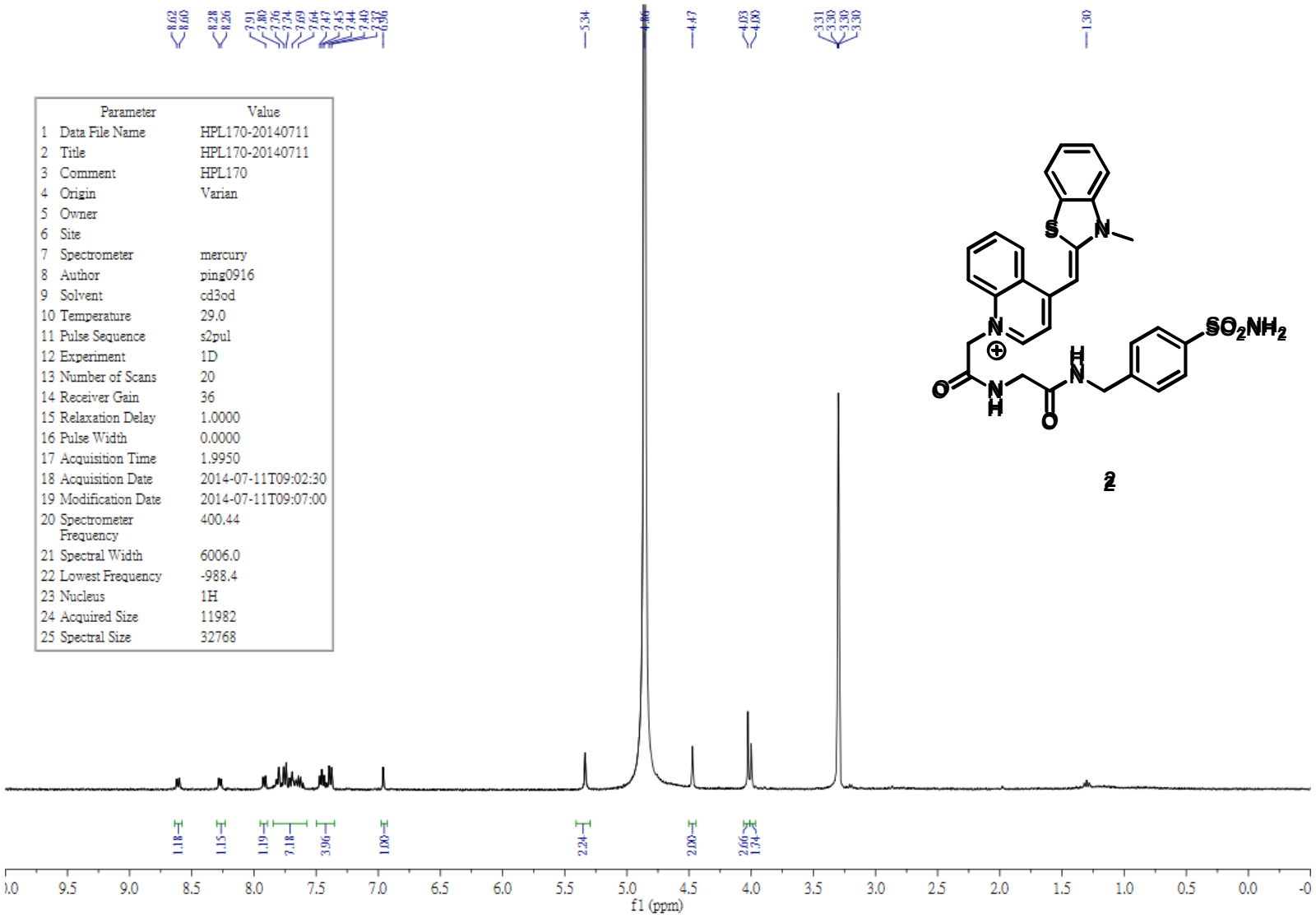
4



	Parameter	Value
1	Data File Name	HP12-C-20140705
2	Title	HP12-C-20140705
3	Comment	HP12-C
4	Origin	Varian
5	Owner	
6	Site	
7	Spectrometer	mercury
8	Author	ping0916
9	Solvent	DMSO
10	Temperature	29.0
11	Pulse Sequence	s2pul
12	Experiment	1D
13	Number of Scans	27988
14	Receiver Gain	30
15	Relaxation Delay	0.0000
16	Pulse Width	0.0000
17	Acquisition Time	1.1994
18	Acquisition Date	2014-07-04T23:44:41
19	Modification Date	2014-07-05T09:07:00
20	Spectrometer Frequency	100.70
21	Spectral Width	25000.0
22	Lowest Frequency	-2968.3
23	Nucleus	13C
24	Acquired Size	29984
25	Spectral Size	65536

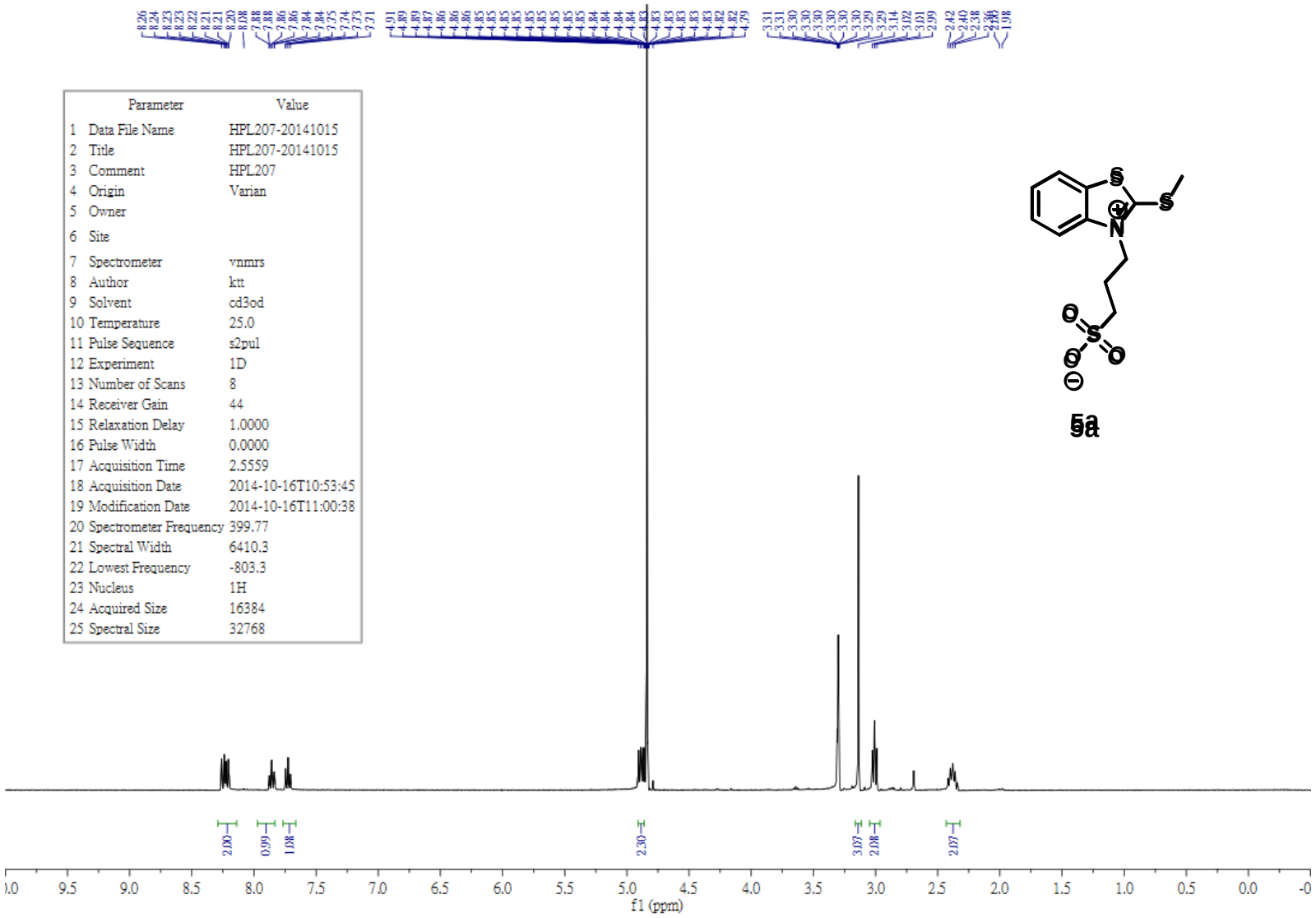

8.68
8.65
8.33
8.31
7.94
7.92
7.73
7.71
7.64
7.51
7.49
7.45
7.33
7.32
7.31
6.99
5.48
5.48
5.30
4.45
4.05
3.31
3.31
3.30
3.30
3.30
3.30
3.30
3.29
1.44
1.28
0.89
0.09
Parameter Value
1 Data File Name HP9-20150625
2 Title HP9-20150625
3 Comment HP9
4 Origin Varian
5 Owner
6 Site
7 Spectrometer vnmrs
8 Author
9 Solvent cd3od
10 Temperature 25.0
11 Pulse Sequence s2pul
12 Experiment 1D
13 Number of Scans 56
14 Receiver Gain 52
15 Relaxation Delay 1.0000
16 Pulse Width 0.0000
17 Acquisition Time 2.5559
18 Acquisition Date 2015-06-25T11:45:28
19 Modification Date 2015-06-25T11:49:40
20 Spectrometer Frequency 399.76
21 Spectral Width 6410.3
22 Lowest Frequency -802.1
23 Nucleus 1H
24 Acquired Size 16384
25 Spectral Size 32768
1.36
1.22
2.03
3.38
1.43
1.95
4.21
1.00
2.28
2.17
3.34
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
f1 (ppm)

6

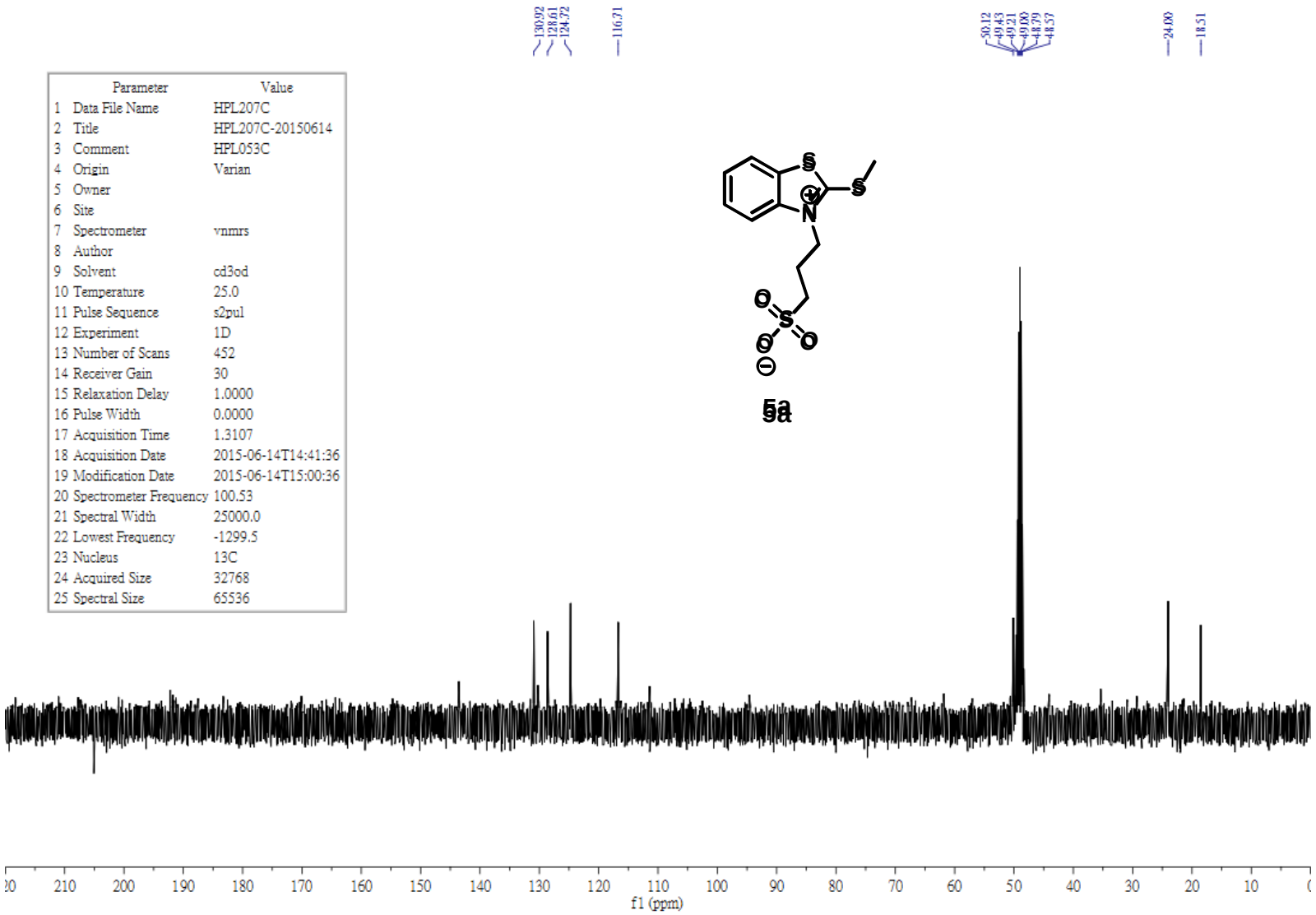


	Parameter	Value
1	Data File Name	HPL170-20140711
2	Title	HPL170-20140711
3	Comment	HPL170
4	Origin	Varian
5	Owner	
6	Site	
7	Spectrometer	mercury
8	Author	ping0916
9	Solvent	cd3od
10	Temperature	29.0
11	Pulse Sequence	s2pul
12	Experiment	1D
13	Number of Scans	20
14	Receiver Gain	36
15	Relaxation Delay	1.0000
16	Pulse Width	0.0000
17	Acquisition Time	1.9950
18	Acquisition Date	2014-07-11T09:02:30
19	Modification Date	2014-07-11T09:07:00
20	Spectrometer Frequency	400.44
21	Spectral Width	6006.0
22	Lowest Frequency	-988.4
23	Nucleus	1H
24	Acquired Size	11982
25	Spectral Size	32768

2

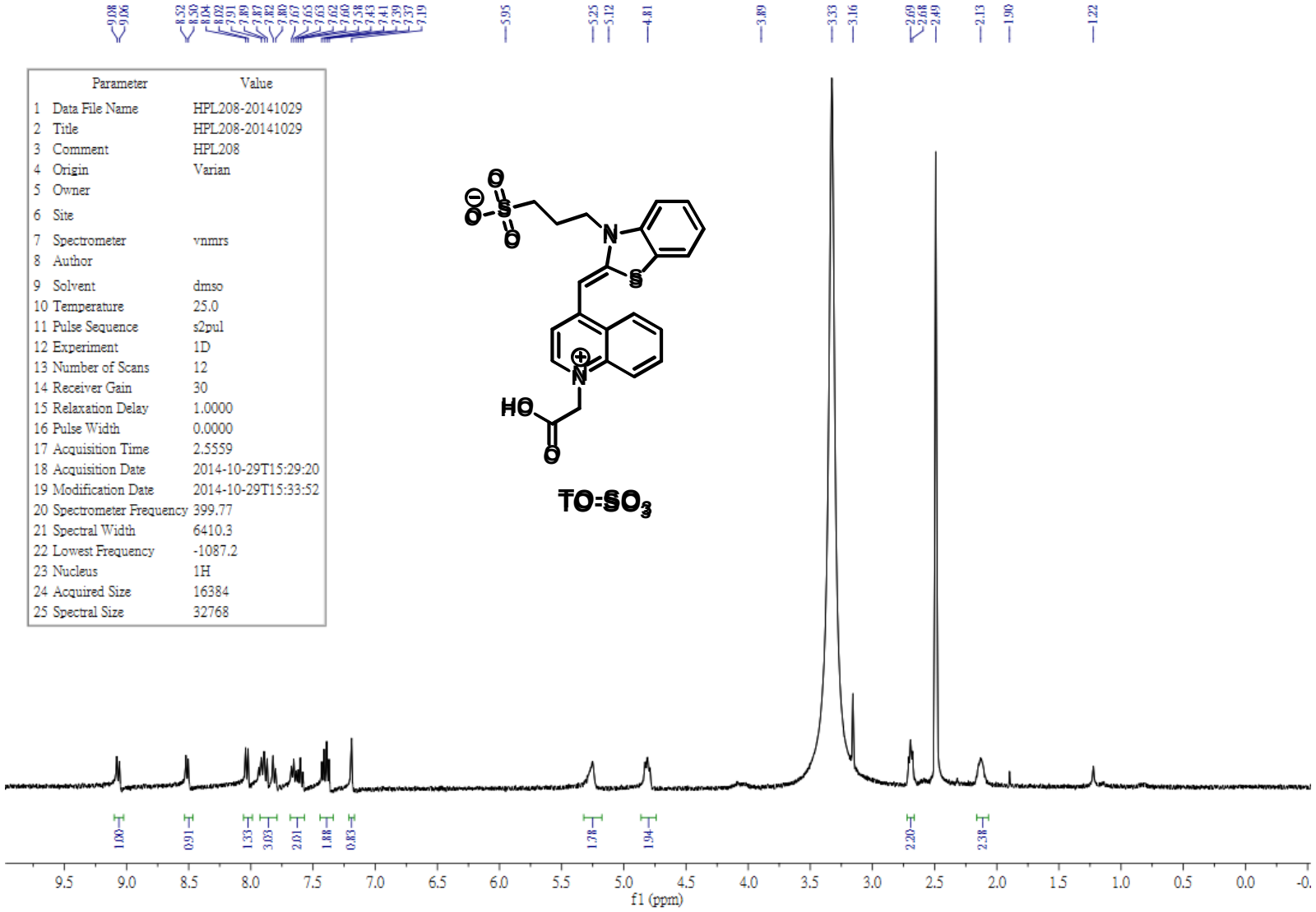

8.26 8.24 8.23 8.23 8.22 8.21 8.21 8.20 8.08 7.88 7.88 7.86 7.86 7.84 7.84 7.75 7.74 7.73 7.71
4.91 4.89 4.89 4.87 4.86 4.86 4.86 4.85 4.85 4.85 4.85 4.85 4.85 4.85 4.84 4.84 4.84 4.84 4.83 4.83 4.83 4.83 4.83 4.83 4.82 4.82 4.79
3.31 3.31 3.30 3.30 3.30 3.30 3.30 3.30 3.30 3.29 3.29 3.14 3.02 3.01 2.99
2.42 2.40 2.38 2.36 1.98
Parameter Value
1 Data File Name HPL207-20141015
2 Title HPL207-20141015
3 Comment HPL207
4 Origin Varian
5 Owner
6 Site
7 Spectrometer vnmrs
8 Author ktt
9 Solvent cd3od
10 Temperature 25.0
11 Pulse Sequence s2pul
12 Experiment 1D
13 Number of Scans 8
14 Receiver Gain 44
15 Relaxation Delay 1.0000
16 Pulse Width 0.0000
17 Acquisition Time 2.5559
18 Acquisition Date 2014-10-16T10:53:45
19 Modification Date 2014-10-16T11:00:38
20 Spectrometer Frequency 399.77
21 Spectral Width 6410.3
22 Lowest Frequency -803.3
23 Nucleus 1H
24 Acquired Size 16384
25 Spectral Size 32768
2.03 0.99 1.08 2.30 3.07 2.08 2.07
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
f1 (ppm)

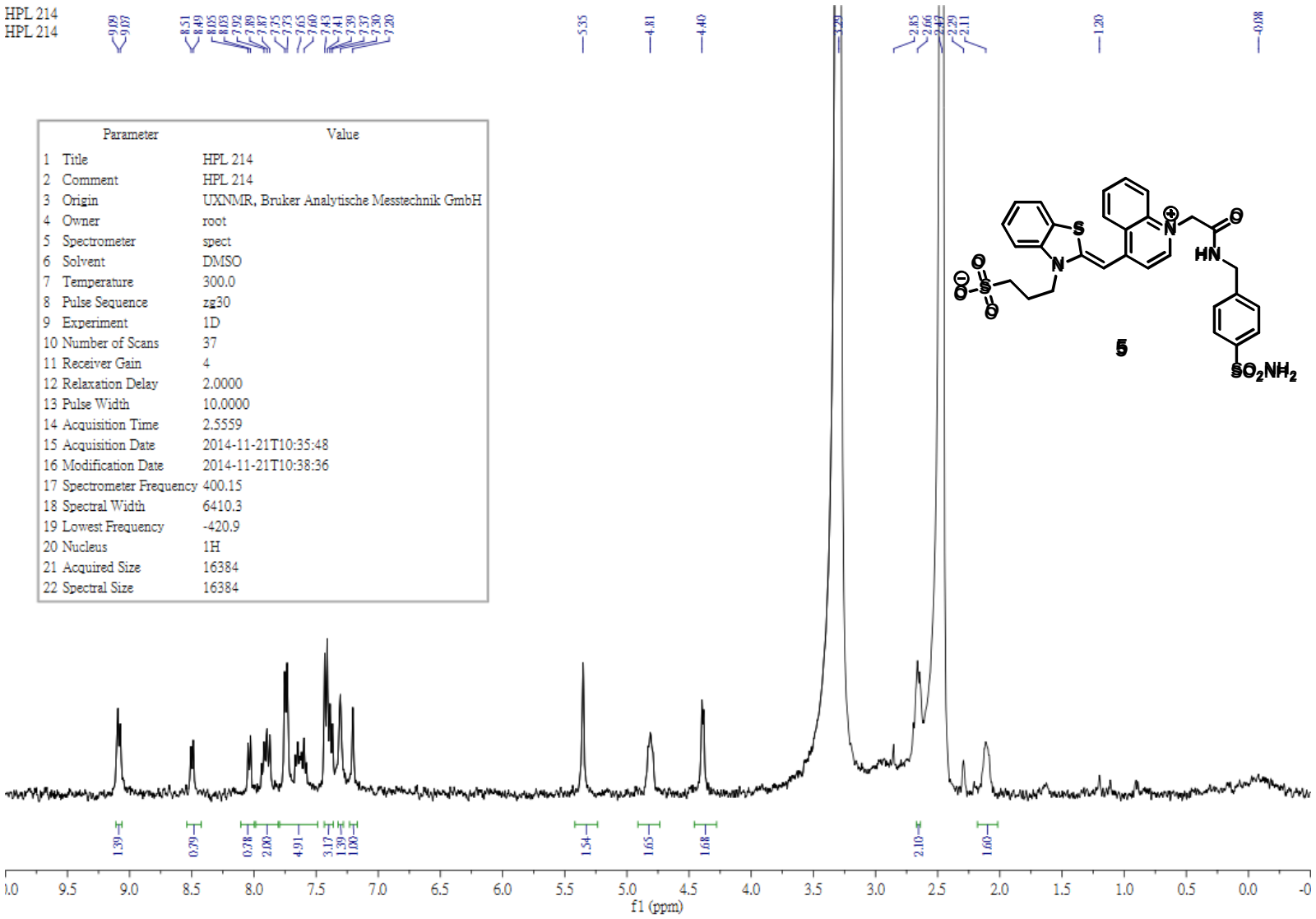
5a

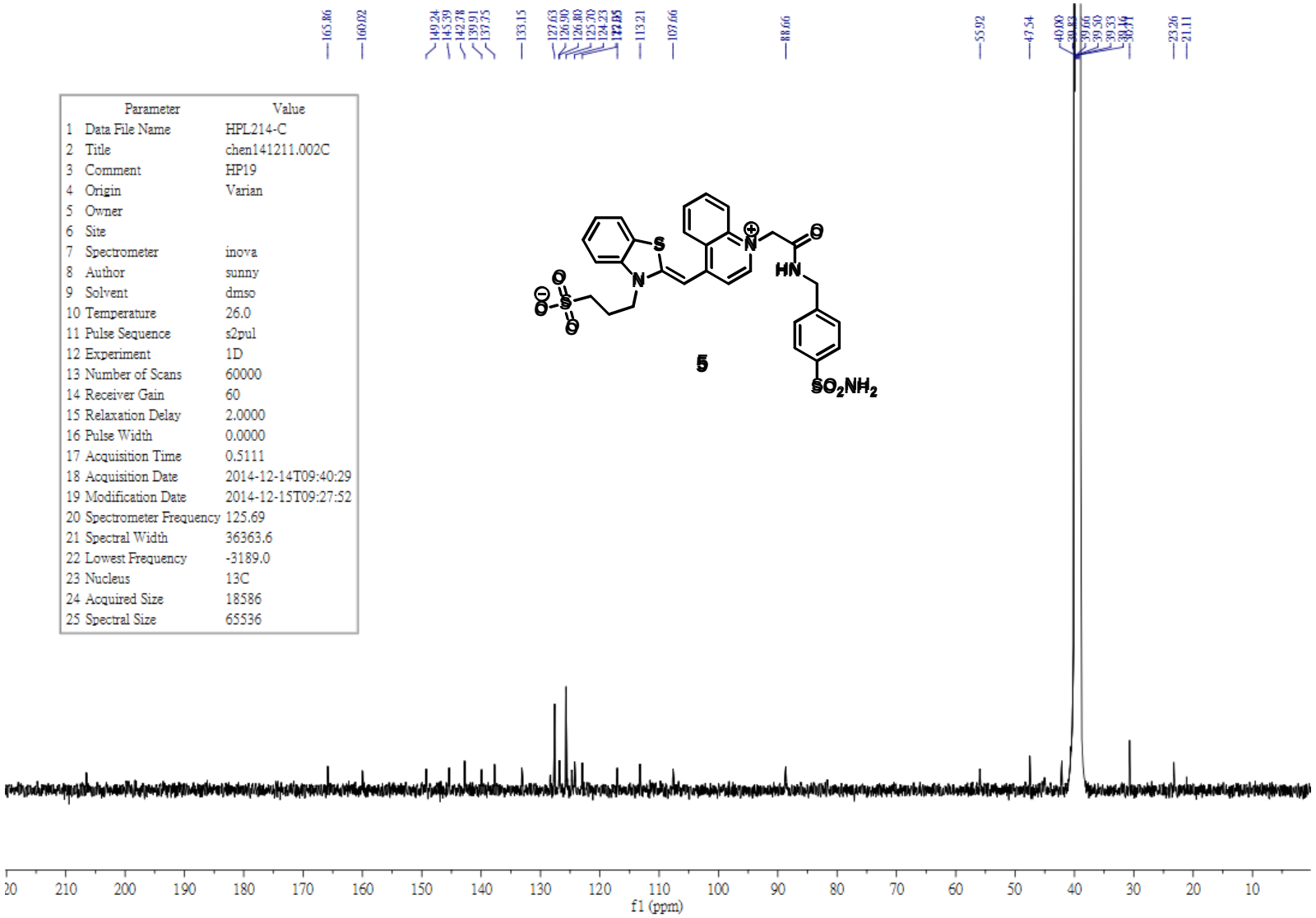


	Parameter	Value
1	Data File Name	HPL207C
2	Title	HPL207C-20150614
3	Comment	HPL053C
4	Origin	Varian
5	Owner	
6	Site	
7	Spectrometer	vnmrs
8	Author	
9	Solvent	od3od
10	Temperature	25.0
11	Pulse Sequence	s2pul
12	Experiment	1D
13	Number of Scans	452
14	Receiver Gain	30
15	Relaxation Delay	1.0000
16	Pulse Width	0.0000
17	Acquisition Time	1.3107
18	Acquisition Date	2015-06-14T14:41:36
19	Modification Date	2015-06-14T15:00:36
20	Spectrometer Frequency	100.53
21	Spectral Width	25000.0
22	Lowest Frequency	-1299.5
23	Nucleus	13C
24	Acquired Size	32768
25	Spectral Size	65536

5a


9.08
9.06
8.52
8.50
8.04
8.02
7.91
7.89
7.87
7.82
7.80
7.67
7.65
7.63
7.62
7.60
7.58
7.43
7.41
7.39
7.37
7.19
5.95
5.25
5.12
4.81
3.89
3.33
3.16
2.69
2.68
2.49
2.13
1.90
1.22
Parameter Value
1 Data File Name HPL208-20141029
2 Title HPL208-20141029
3 Comment HPL208
4 Origin Varian
5 Owner
6 Site
7 Spectrometer vnmrs
8 Author
9 Solvent dmso
10 Temperature 25.0
11 Pulse Sequence s2pul
12 Experiment 1D
13 Number of Scans 12
14 Receiver Gain 30
15 Relaxation Delay 1.0000
16 Pulse Width 0.0000
17 Acquisition Time 2.5559
18 Acquisition Date 2014-10-29T15:29:20
19 Modification Date 2014-10-29T15:33:52
20 Spectrometer Frequency 399.77
21 Spectral Width 6410.3
22 Lowest Frequency -1087.2
23 Nucleus 1H
24 Acquired Size 16384
25 Spectral Size 32768
HO
TO-SO3
1.00
0.91
1.33
3.03
2.01
1.88
0.83
1.78
1.94
2.20
2.38
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
f1 (ppm)


HPL 214
HPL 214
9.09
9.07
8.51
8.49
8.05
8.03
7.92
7.89
7.87
7.75
7.73
7.65
7.60
7.43
7.41
7.39
7.37
7.30
7.20
5.35
4.81
4.40
3.20
2.85
2.66
2.42
2.29
2.11
1.20
0.08
Parameter Value
1 Title HPL 214
2 Comment HPL 214
3 Origin UXNMR, Bruker Analytische Messtechnik GmbH
4 Owner root
5 Spectrometer spect
6 Solvent DMSO
7 Temperature 300.0
8 Pulse Sequence zg30
9 Experiment 1D
10 Number of Scans 37
11 Receiver Gain 4
12 Relaxation Delay 2.0000
13 Pulse Width 10.0000
14 Acquisition Time 2.5559
15 Acquisition Date 2014-11-21T10:35:48
16 Modification Date 2014-11-21T10:38:36
17 Spectrometer Frequency 400.15
18 Spectral Width 6410.3
19 Lowest Frequency -420.9
20 Nucleus 1H
21 Acquired Size 16384
22 Spectral Size 16384
5
1.39
0.79
0.78
2.00
4.91
3.17
1.39
1.08
1.54
1.65
1.68
2.10
1.60
f1 (ppm)



	Parameter	Value
1	Data File Name	HPL214-C
2	Title	chen141211.002C
3	Comment	HP19
4	Origin	Varian
5	Owner	
6	Site	
7	Spectrometer	inova
8	Author	sunny
9	Solvent	dmso
10	Temperature	26.0
11	Pulse Sequence	s2pul
12	Experiment	1D
13	Number of Scans	60000
14	Receiver Gain	60
15	Relaxation Delay	2.0000
16	Pulse Width	0.0000
17	Acquisition Time	0.5111
18	Acquisition Date	2014-12-14T09:40:29
19	Modification Date	2014-12-15T09:27:52
20	Spectrometer Frequency	125.69
21	Spectral Width	36363.6
22	Lowest Frequency	-3189.0
23	Nucleus	13C
24	Acquired Size	18586
25	Spectral Size	65536