SUPPORTING INFORMATION

Fluorescent Probe Encapsulated in SNAP-tag Protein Cavity to Eliminate Nonspecific Fluorescence and Increase Detection Sensitivity

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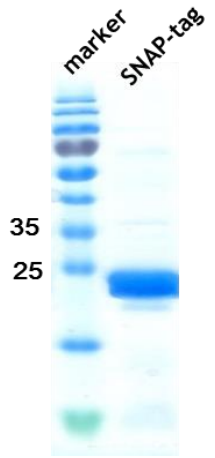
Materials and instruments

Chemicals and reagents were purchased from Sigma-Aldrich, TCI and Acros and were used without further purification. All solvents were used after appropriate distillation or purification. Sodium sulfide nonahydrate, nitroreductase (*E. coli*) and β-nicotinamide adenine dinucleotide disodium salt hydrate (NADH) were purchased from Sigma-Aldrich. Ultrapure water was collected from a Milli-Q reference water purification system. Besides the SNAP-tag protein which was expressed and purified in our laboratory, all other proteins used in the selectivity test were purchased from Sigma-Aldrich. PBS buffer (0.9 mM KCl, 2.67 mM KH_2PO_4, 138 mM NaCl, 8.1 mM Na_2HPO_4) was diluted 10-times from commercially available concentrates supplied by Amersco. 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). HPLC analysis was performed with analytical column (EC 150/4.6 Nucleosil 300-5 C18, Macherey-Nagel). Products were purified by semi-preparative column (VP 150/21 Nucleosil 300-5 C18, Macherey-Nagel). ^{1}H, and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on Bruker DMX-400, Varian Mercury-400 and Varian Unity Inova-500 with ^{1}H chemical shifts (δ) reported in ppm relative to the solvent residual signals of $CDCl_3$ (7.24 ppm), CD_3OD (3.30 ppm), d-DMSO (2.49 ppm). ^{13}C chemical shifts (δ) were reported in ppm relative to the solvent residual signals of d-DMSO (39.5 ppm). Coupling constants were reported in Hz. Absorption spectra were recorded on Hitachi U-3310 spectrophotometer. Fluorescence spectra were recorded using Hitachi F-4500 fluorescence spectrophotometer. High resolution mass spectra (HRMS) were recorded on Varian 901-FTMS. In-gel fluorescence was carried out by using Ettan DIGE imager (GE healthcare).

SNAP-tag protein expression and purification

Plasmid pET51b-SNAP-tag with C-terminal His-tag was transformed to *E. coli* strain BL21. The bacteria was cultured at 37 °C in LB broth containing 100 μg/mL ampicillin to OD_{600} of 1.2. Protein expression was induced by the addition of 1 mM IPTG. After 16 h at 18 °C, the cultures were harvested by centrifugation. The cells were lysed by sonication and insoluble protein and cell debris were removed by centrifugation. The SNAP-tag protein were then purified by Ni-NTA. The purified proteins were concentrated and transferred in PBS buffer using Amicon® Ultra centrifugal filters. The proteins were snap frozen in liquid nitrogen before being stored at -

78 °C. The concentration of the proteins was determined using BCA assay. Protein purity was checked by SDS-PAGE and stained either by Instant Blue or Coomassie Blue. (SNAP-tag: 23 kD)



Polypeptide sequence of recombinant SNAP-tag protein

MASWSHPQFE KGADDDDKVP MDKDCEMKRT TLDSPLGKLE LSGCEQGLHE

>>----------SNAP---------->

IIFLGKGTSA ADAVEVPAPA AVLGGPEPLM QATAWLNAYF HQPEAIEEFP

>----------SNAP----------> VPALHHPVFQ QESFTRQVLW KLLKVVKFGE VISYSHLAAL AGNPAATAAV

>----------SNAP----------> KTALSGNPVP ILIPCHRVVQ GDLDVGGYEG GLAVKEWLLA HEGHRLGKPG

>----------SNAP----------> LGTSRAPGFS SISAHHHHHH HHHH

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

General procedure for nitroreductase detection

All measurements were carried out in 0.05 M Tris buffer (pH 7.4) solution containing 1% DMSO. Appropriate concentrations of NTRase and NADH (final concentration 500 μM) in Tris buffer were added to a microcentrifuge tube. 1 mM **BGSBD-NO_2** stock solution in DMSO was diluted 100-fold in the microcentrifuge tube to obtain a final concentration of 10 μM. The mixture was incubated at 37 °C for 1 hour for enzymatic reaction. Subsequently, SNAP-tag was added to the mixture (final concentration 12.5 μM) and incubated at 37 °C for 0.5 hour for the

conjugation with the fluorescence probe. Fluorescence spectra were recorded in the range from 460 to 700 nm with λ_{ex} = 440 nm for **BGSBD-NO_2** from a xenon lamp.

General procedure for hydrogen sulfide detection

All experiments were carried out in pH 7.4 degas PBS solution containing 1% DMSO. In a microcentrifuge tube, 10 μM fluorescence probe bearing azide moiety was mixed with 12.5 μM SNAP-tag in PBS buffer and the mixture was incubated at 37 °C for 0.5 hour to form SNAP-tag conjugated fluorescence probe. Appropriate concentration of H_2S (prepared by dissolving $Na_2S.9H_2O$ in degas aqueous solution) was added to the mixtures and incubated at 37 °C for 2.5 hours. Fluorescence spectra were recorded in the range from 460 to 700 nm with λ_{ex} = 440 nm for **BGSBD-N_3** and **BGNAPH-N_3** and in the range from 415 to 650 nm with λ_{ex} = 383 nm for **BGCCA-N_3** from a xenon lamp.

General procedure for H_2S and NTRase recovery experiment in 10% blood plasma

Blood samples from healthy volunteers were drawn by a professional medical officer and collected in BD Vacutainer® PSTTM II tubes (BD Diagnostics, cat. no. 367376). The tubes were gently inverted 8-10 times immediately after collection to mix lithium heparin anticoagulant. The tube was centrifuged at 1300 g for 10 minutes and the supernatant was transferred in polypropylene tube using a Pasteur pipette. The plasma samples were stored at -20 °C freezer. 10% plasma was prepared by mixing plasma with 90% volume of Tris buffer (NTRase detection) or PBS (H_2S detection).

For the analysis of NTRase in 10% plasma, 1 mM **BGSBD-NO_2** stock solution in DMSO was diluted 100-fold in a microcentrifuge tube containing 10% plasma to obtain a final concentration of 10 μM. 1 μg/mL NTRase and NADH (final concentration 500 μM) were then spiked into the microcentrifuge tube. The mixture was incubated at 37 °C for 1.5 hours. Subsequently, SNAP-tag was added to the mixture (final concentration 12.5 μM) and incubated at 37 °C for 0.5 hour. Fluorescence spectra were recorded in the range from 460 to 700 nm with λ_{ex} = 440 nm for **BGSBD-NO_2** from a xenon lamp. To determine the percent recovery, the fluorescence intensity obtained in 10% plasma was interpolated to the calibration curve obtained in Tris buffer.

For the analysis of H_2S in 10% plasma, 25 μM H_2S was spiked into a microcentrifuge tube containing 10% plasma and 10 μM SNAP-tag conjugated **BGSBD-N_3** or **BGNAPH-N_3**. The

mixture was incubated at 37 °C for 2.5 hours. Fluorescence spectra were recorded in the range from 460 to 700 nm with λ_{ex} = 440 nm from a xenon lamp. The percent recovery was determined by interpolating the fluorescence intensity obtained in 10% plasma to the calibration curve obtained in PBS buffer.

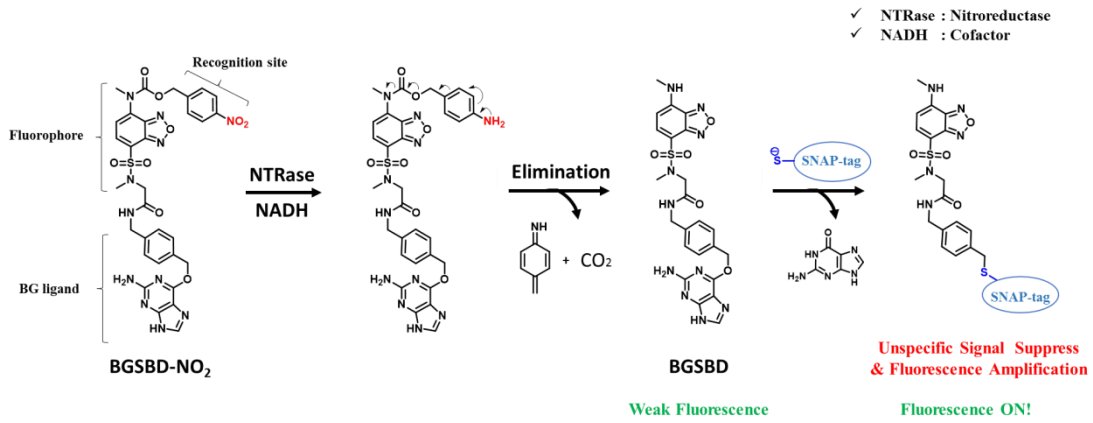


Figure S1. The fluorescence turn-on mechanism of **BGSBD-NO_2** upon reaction with NTRase in the presence of cofactor NADH and SNAP-tag protein.

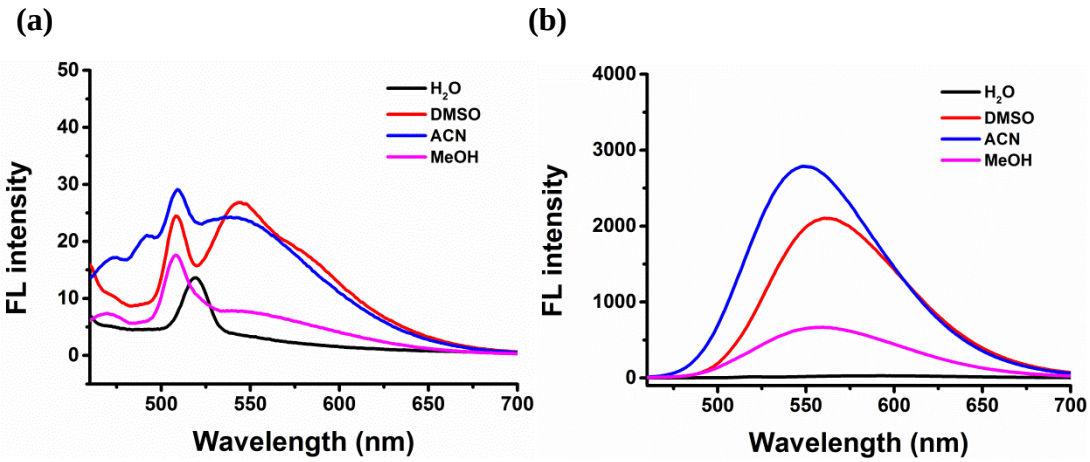


Figure S2. (a) Fluorescence spectra of **BGSBD-NO_2** in different solvents. Because **BGSBD-NO_2** bears an electron-withdrawing carbamate group, it displays weak fluorescence even in hydrophobic solvents. (b) Fluorescence spectra of **BGSBD** in different solvents. **BGSBD** shows blueshifted and strong emission in hydrophobic solvents such as DMSO and ACN.

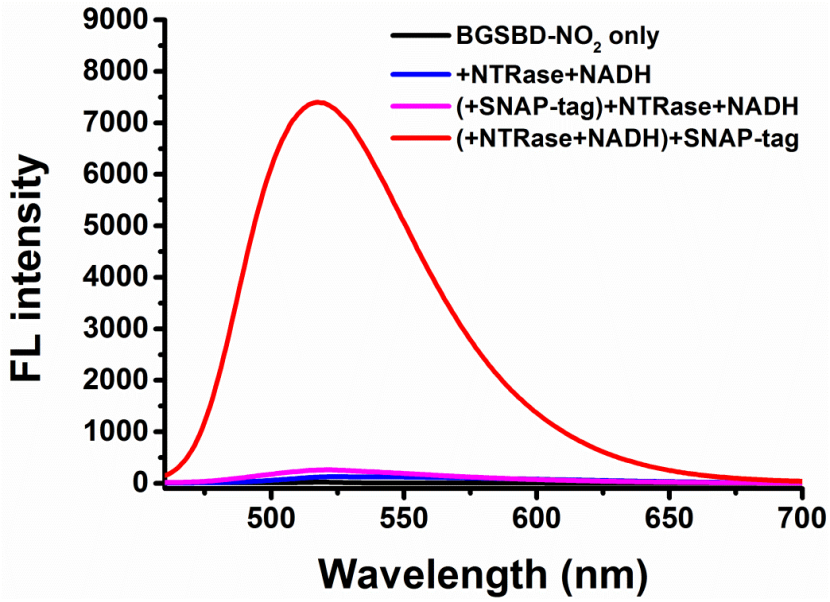


Figure S3. Fluorescence amplified detection of NTRase employing different detection schemes. Lower fluorecence amplification ratio was obtained when SNAP-tag conjugated **BGSBD-NO_2** was used directly for NTRase detection (pink line). Stronger fluorescence can be obtained when **BGSBD-NO_2** was reacted initially with NTRase, followed by the addition of SNAP-tag (red line).

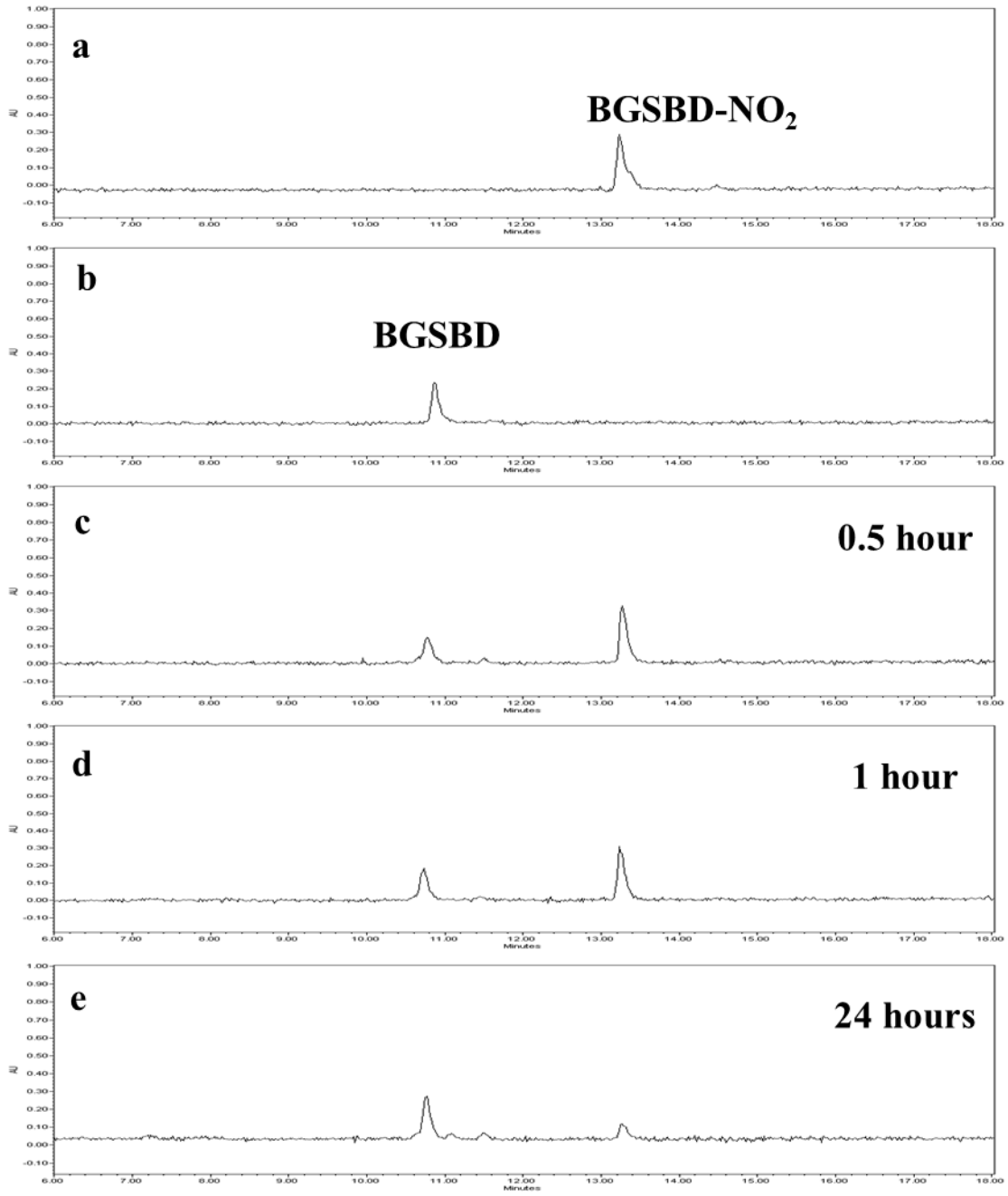
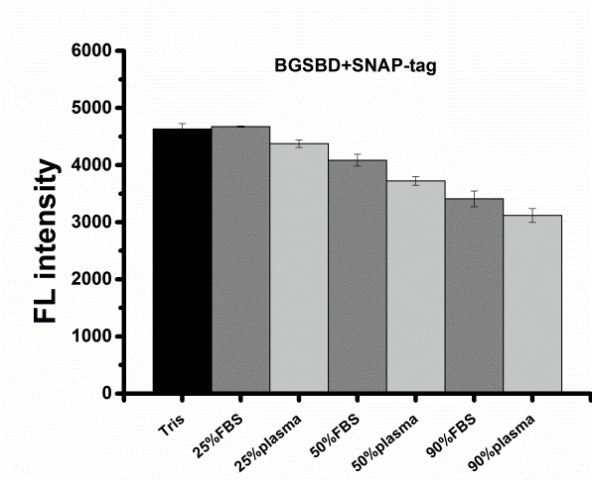


Figure S4. HPLC traces of (a) 100 μM **BGSBD-NO_2**, (b) 100 μM **BGSBD**, (c), (d), and (e) 100 μM **BGSBD-NO_2** mixed with 1.5 μg/mL NTR in the presence of 500 μM NADH for 0.5, 1 and 24 hours, respectively.

(a)



(b)

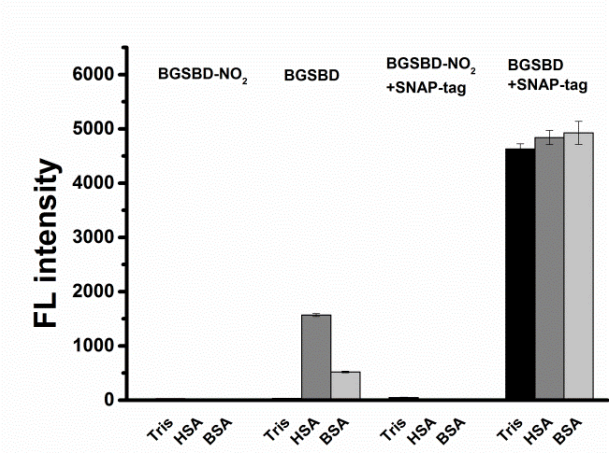


Figure S5. (a) Fluorescence intensity of 10 μM SNAP-tag conjugated **BGSBD** in Tris, 25%, 50%, 90% FBS and plasma, respectively. The fluorescence of SNAP-tag shielded **BGSBD** decrease in high percentage of FBS and plasma which are probably due to the self-absorption quenching by high concentration of proteins. (b) Fluorescence intensity of 10 μM **BGSBD-NO_2**, **BGSBD**, SNAP-tag conjugated **BGSBD-NO_2** and **BGSBD** in Tris buffer, 250 μM HSA and 250 μM BSA.

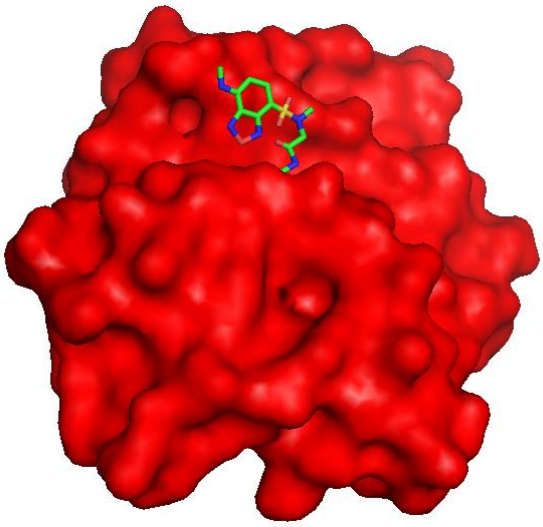
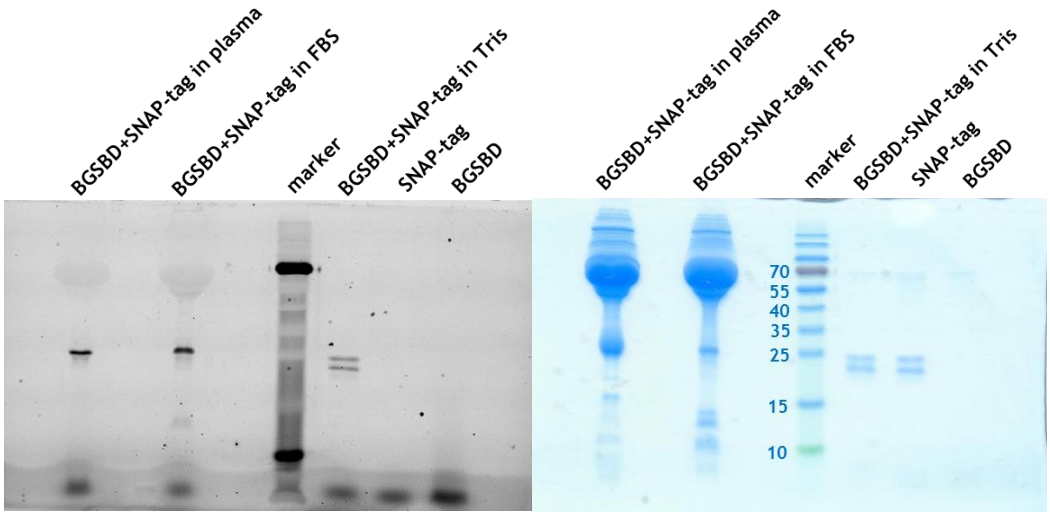


Figure S6. Molecular model of SNAP-tag-**BGSBD** complex. The model was generated using SNAP-tag crystal structure (PDB: 3L00) and Pymol software.

(a)



(b)

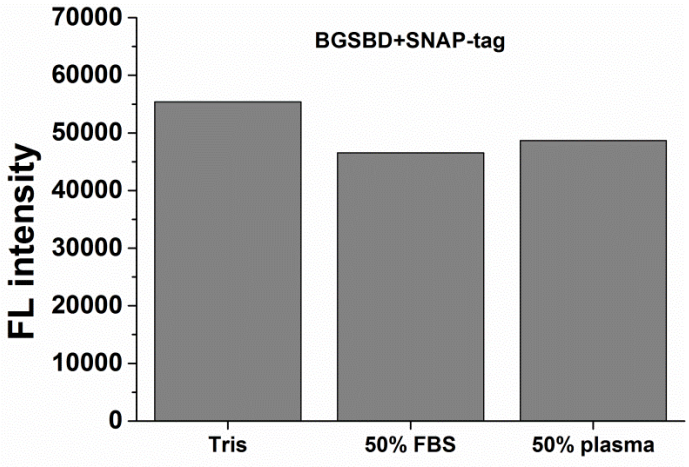


Figure S7. (a) Investigation of SNAP-tag labeling efficiency with **BGSBD** in 50% FBS and plasma by SDS-PAGE. The gel was fluorescently scanned (left), followed by staining with Instant Blue (right). The molecular weight of the recombinant SNAP-tag is about 23 kDa. (b) Fluorescence intensity of SNAP-tag conjugated **BGSBD** bands were calculated pixel-by-pixel by Image J software.

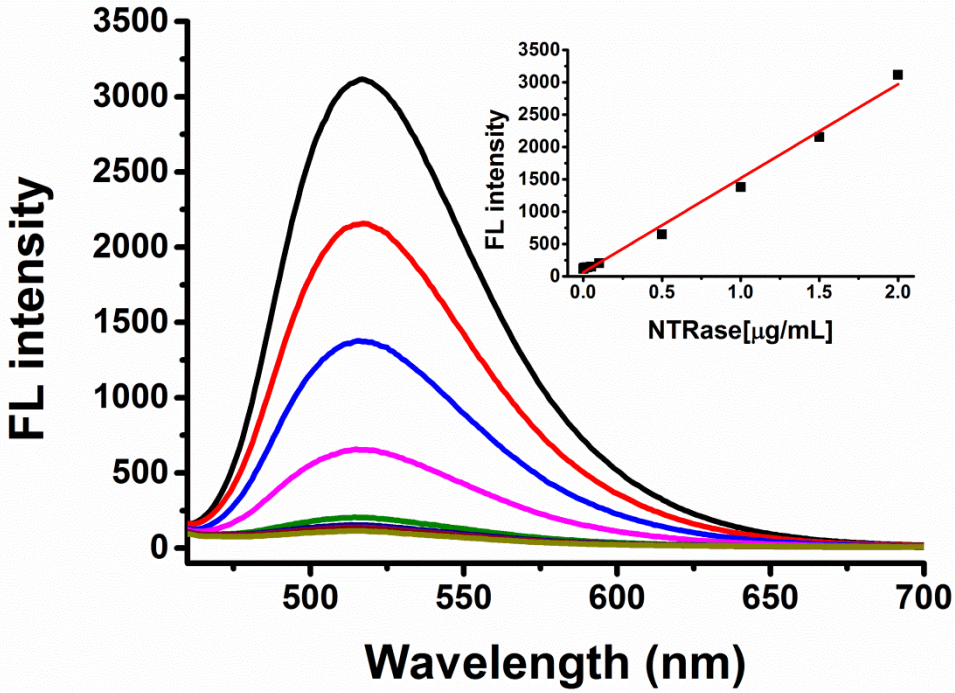


Figure S8. Fluorescence spectra of 10 μM **BGSBD-NO_2** with increasing concentrations of NTRase in 10% blood plasma. The fluorescence was amplified with 12.5 μM SNAP-tag. The inset shows that the fluorescence response was linear in the range of 0 - 2 μg/mL NTRase and the LOD was estimated to be about 11 ng/mL NTRase. The linear equation is y = 1441.8x + 69.0 and the correlation coefficient R^2 = 0.9923.

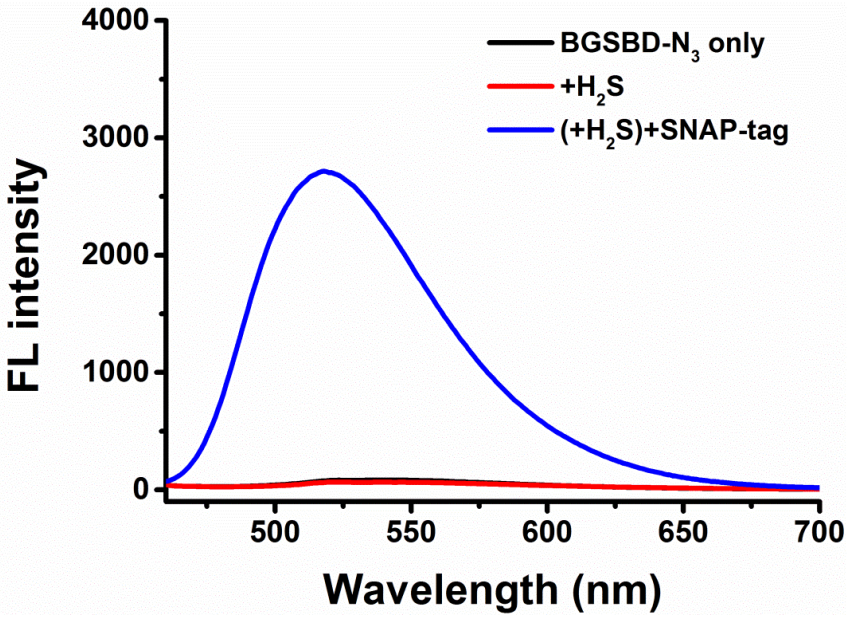


Figure S9. Fluorescence spectra of 10 μM SNAP-tag conjugated **BGSBD-N_3** with 500 μM H_2S. The fluorescence spectra of 10 μM free **BGSBD-N_3** in the absence and presence of 500 μM H_2S were included for comparison.

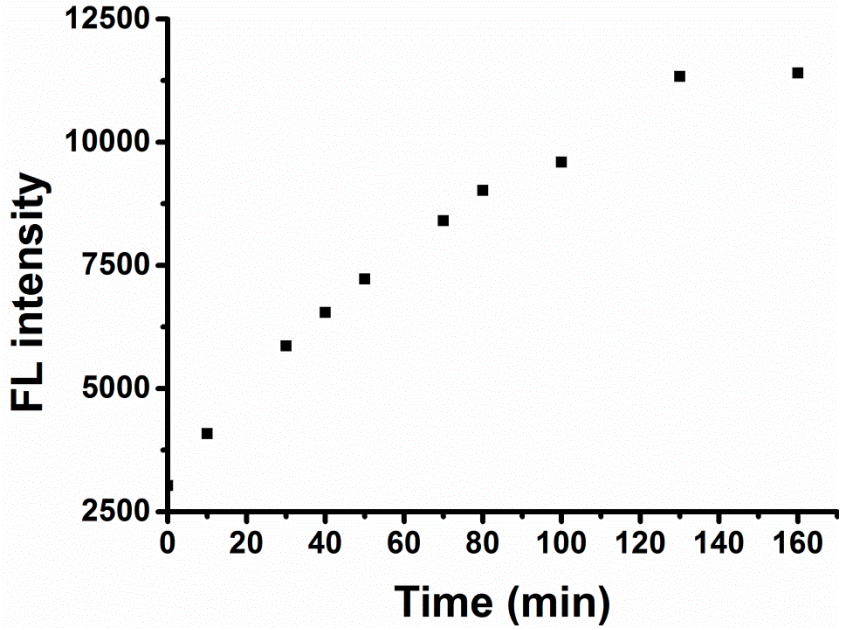


Figure S10. Reaction time course of 10 μM SNAP-tag conjugated **BGSBD-N_3** with 500 μM H_2S at 37°C.

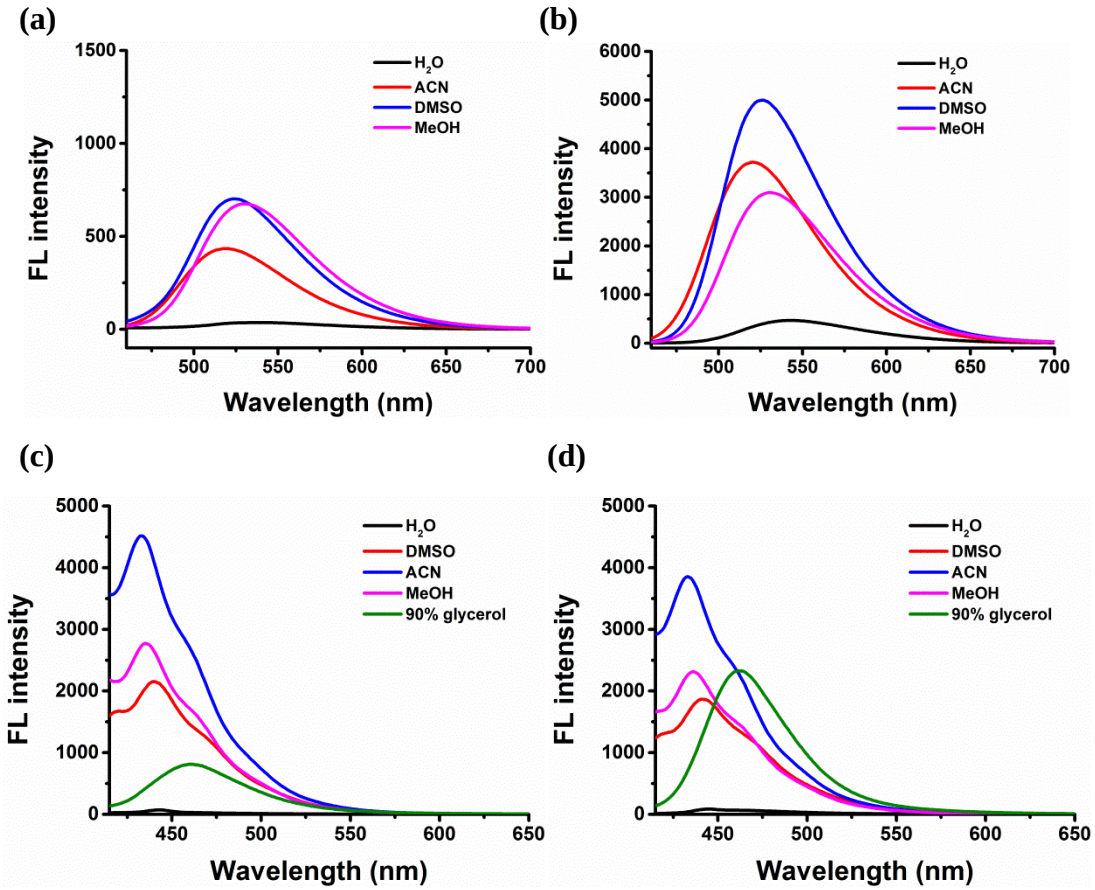


Figure S11. Fluorescence spectra of (a) **BGNAPH-N_3**, (b) **BGNAPH**, (c) **BGCCA-N_3** and (d) **BGCCA** in H_2O, ACN, DMSO, MeOH and 90% glycerol. **BGNAPH-N_3** and **BGNAPH** exhibit strong environment-sensitive effects which show weak fluorescence in aqueous solution but strong emission in hydrophobic solvents. **BGCCA-N_3** and **BGCCA** are fluorescent molecular rotor which show strong emission in hydrophobic and viscous solvents.

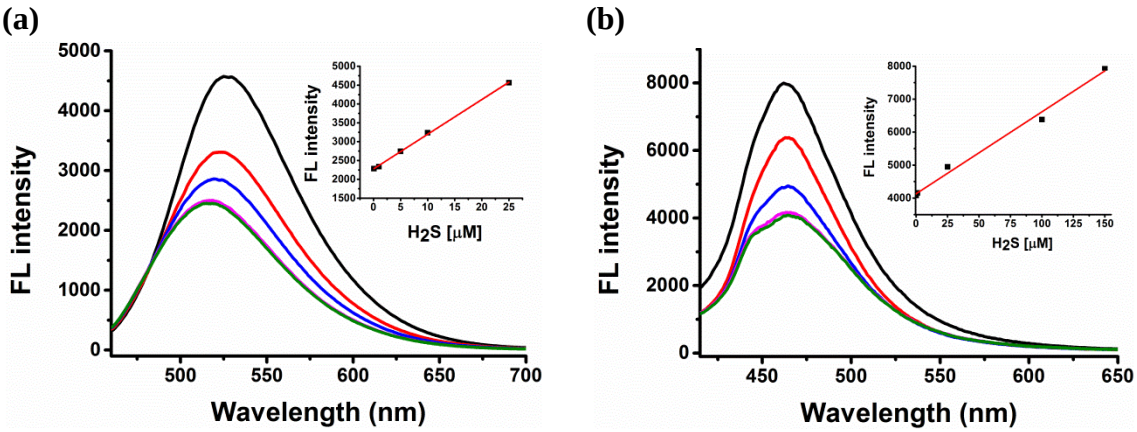


Figure S12. (a) Fluorescence spectra of 10 μM SNAP-tag conjugated **BGNAPH-N_3** with increasing concentrations of H_2S in PBS buffer. The inset shows that the fluorescence response was linear in the range of 0.1 - 25 μM H_2S in degas PBS and the LOD was determined to be about 3.9 μM H_2S. The linear equation is y = 92.125x + 2274 and the correlation coefficient R^2 = 0.9992. (b) Fluorescence spectra of 10 μM SNAP-tag conjugated **BGCCA-N_3** with increasing concentrations of H_2S in PBS buffer. The inset shows that the fluorescence response was linear in the range of 0.1 - 150 μM H_2S in degas PBS and the LOD was estimated to be about 10 μM H_2S. The linear equation is y = 24.592x + 4143 and the correlation coefficient R^2 = 0.9907.

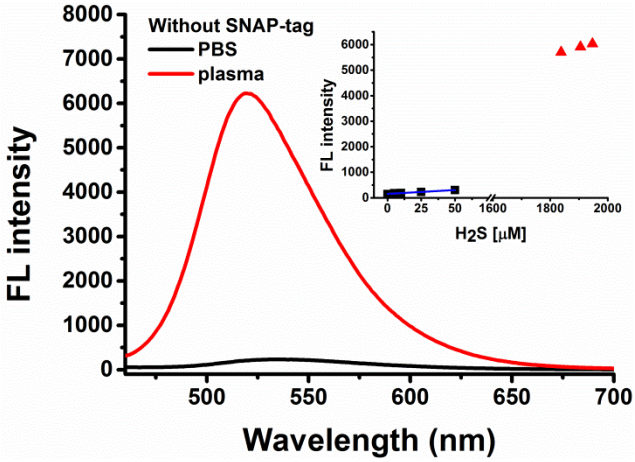


Figure S13. Fluorescence spectra of 25 μM H_2S in PBS buffer and 10% plasma as analyzed by 10 μM free **BGNAPH-N_3**. The inset shows the fluorescence intensity (red triangles) obtained in 10% plasma and the calibration curve created in Tris buffer with 10 μM free **BGNAPH-N_3**. In all the measurements, the fluorescence intensity was obtained by subtracting the FBS and plasma background fluorescence from the original spectra.

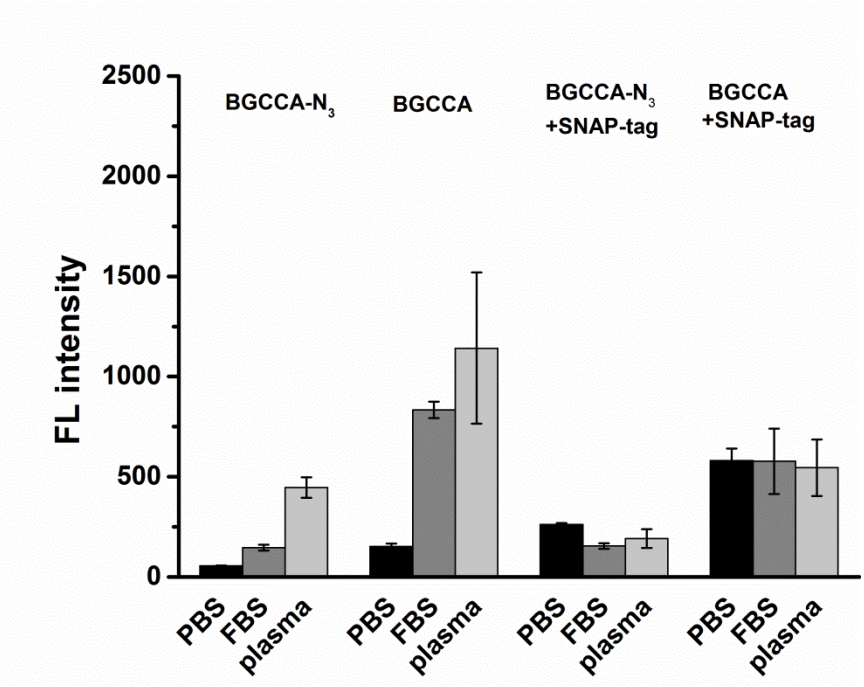


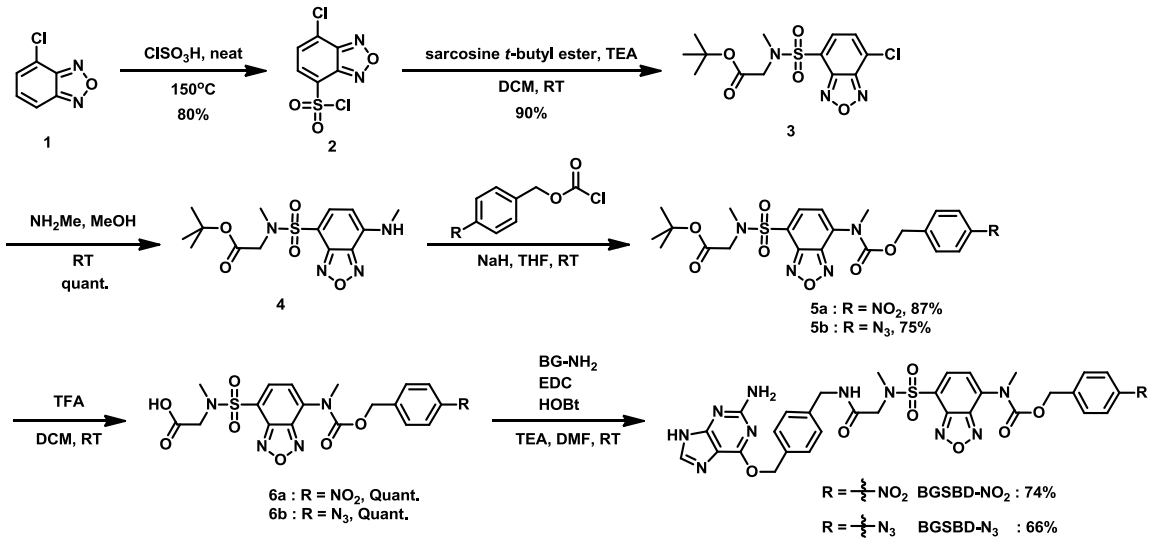
Figure S14. Fluorescence intensity of 10 μM **BGCCA-N_3**, **BGCCA**, SNAP-tag conjugated **BGCCA-N_3** and **BGCCA** in PBS buffer, 25% FBS and 25% human blood plasma. The fluorescence intensity was obtained by subtracting the FBS and plasma background fluorescence from the original spectra. Analysis condition: 10 μM free probes or SNAP-tag conjugated probes were added to PBS buffer, 25% plasma and FBS samples (v/v, diluted with Tris buffer). Fluorescence spectra were recorded after the mixtures were incubated at 37°C for 30 minutes. Error bars were calculated from three independent measurements.

Table S1. Summary of NTRase fluorescent probes

Name	LOD	Dynamic range	Reference
Cy7-1	1.14ng/mL	0.15-0.45 μg/mL	*J. Am. Chem. Soc.* **2015**, 137, 6407.
Probe1	0.27ng/mL	15-300 ng/mL	*Anal. Chem*. **2013**, 85, 3926.
NCL	0.15ug/mL	--	*PloS one* **2015**, 10, e0131037.
Probe1	20ng/mL	0.05-0.9 μg/mL	*Anal. Chem*. **2015**, 87, 11832.
BGSBD-NO_2+SNAP-tag	1ng/mL	0-2 μg/mL	*This Paper*

Table S2. Summary of H_2S fluorescent probes

Name	LOD	Dynamic range	Reference
MeRho-Az	86 nM	0-15 μM	*J. Am. Chem. Soc.* **2015,** 137, 10216
PI-N3	0.879μM	1-7.9μM	*Org. Biomol. Chem.* **2012**, 10, 9683.
SF1/SF2	5-10uM	--	*J. Am. Chem. Soc.* **2011**, 133, 10078.
HSN1/HSN2	5-10/1-5uM	--	*Chem. Commun.* **2012**, 48, 4767.
DNS-Az	1uM	--	*Angew. Chem. Int. Ed.* **2011**, 50, 9672.
Cy-N3	0.08uM	0-100 μM	*Chem. Commun.* **2012**, 48, 2852
CCLS-1/ CCLS-2	0.7±0.3/ 4.6±2.0uM	x_1 - 50 μM/ x_2 – 250 μM	*J. Am. Chem. Soc.* **2013**, 135, 16697
Probe 4	0.259 μM	2 – 10 μM	*Org. Biomol.Chem.* **2013**, 11, 8166.
cpGFP-Tyr66pAzF	10 μM	<50μM	*J. Am. Chem. Soc.* **2012**, 134, 9589.
FS1	5–10 μM	--	*Chem. Commun.* **2012,** 48, 8395.
L1Cu	1.7 μM	2.5-15μM	*Dalton Trans.* **2012,** 41, 19, 5799.
BGSBD-N_3+SNAP-tag	3.3 μM	0-250 μM	*This Paper*



Scheme S1. Synthesis of SBD-based fluorescence probes **BGSBD-NO_2** and **BGSBD-N_3**.

Synthesis of compound 2

Chlorosulfonic acid (6 mL, 90 mmol) was added dropwise to compound **1** (2 g, 12.9 mmol) in a round bottom flask at 0 °C. The reaction mixture was stirred at 0 °C for additional 1.5 hours and then heated at 150 °C for 6 hours. The reaction mixture was poured into ice water and extracted with 50 mL dichloromethane (3x). The combined organic layer was washed with 50 mL 1 M HCl (2x) and 30 mL brine (1x). The organic layer was concentrated under reduced pressure to give the pure desired product **2** as a white powder. Compound **2** was used in the next step without further purification. **Yield** = 80 % (2.61 g); $\mathbf{R_f}$ = 0.42 (EA : Hex = 1 : 4); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.16 (d, *J* = 7.5 Hz, 1H), 7.64 (d, *J* = 7.5 Hz, 1H) ppm.

Synthesis of compound 3

To a stirred solution of compound **2** (20 mg, 79 μmol) and sarcosine *t*-butyl ester hydrochloride (17 mg, 95 μmol) in dichloromethane (1 mL) was added Et_3N (22 μL, 158 μmol) at room temperature. The resulting mixture was stirred for 10 minutes at room temperature. The reaction mixture was extracted with 50 mL 1 M HCl (2x) and 30 mL brine (1x). The combined organic layer was dried over $MgSO_4$, filtered and concentrated. The product was used in the next step without further purification. **Yield** = 90% (26 mg); $\mathbf{R_f}$ = 0.36 (EA : Hex = 1 : 4); **^{1}H NMR** (600 MHz, $CDCl_3$): δ 7.90 (d, *J* = 7.3 Hz, 1H), 7.49 (d, *J* = 7.3 Hz, 1H), 4.14 (s, 2H), 3.02 (s, 3H), 1.28 (s, 9H) ppm; **^{13}C NMR** (150 MHz, $CDCl_3$): δ 167.22, 148.84, 145.73, 133.21, 129.01,

127.64, 82.53, 51.71, 35.73, 29.65, 27.80 ppm.

Synthesis of compound 4

Methylamine (40% in methanol, 147 μL, 1.5 mmol) was added to compound **3** (50 mg, 138 μmol) in a round bottom flask at room temperature. The resulting mixture was stirred for 1 hour at room temperature. Excess methylamine was removed under reduced pressure and the crude was redissolved in 50 mL ethyl acetate. The organic layer was extracted with 50 mL 1 M HCl (2x) and 30 mL brine (1x). The combined organic layer was dried over $MgSO_4$, filtered and concentrated to give the desired product **4** as a yellow powder. Product **4** was used in the next step without further purification. **Yield** = quantitative (48 mg); $\mathbf{R_f}$ = 0.26 (EA : Hex = 1 : 4); **^{1}H NMR** (600 MHz, $CDCl_3$): δ 7.88 (d, *J* = 7.9 Hz, 1H), 6.06 (d, *J* = 7.9 Hz, 1H), 5.68 (d, *J* = 4.6 Hz, 1H), 4.09 (s, 2H), 3.09 (d, *J* = 5.2 Hz, 3H), 2.96 (s, 3H), 1.32 (s, 9H) ppm; **^{13}C NMR** (150 MHz, $CDCl_3$): δ 167.84, 145.99, 144.38, 141.38, 138.40, 112.14, 98.43, 81.95, 51.86, 35.45, 30.03, 27.88 ppm; **HRMS** (ESI): m/z calc. for $C_{16}H_{19}N_6O_3S$ 375.1239 $[M+H]^+$, found 375.1225 $[M+H]^+$.

Synthesis of compound 5a

To NaH (13 mg, 0.54 mmol, 60% in oil) in dry THF (2 mL) solution at 0 °C was added compound **4** (100 mg, 0.28 mmol) in dry THF (3 mL). After 10 minutes, 4-nitrobenzyl chloroformate (90 mg, 0.42 mmol) in dry THF (3 mL) was added slowly and the reaction mixture was stirred at room temperature for 10 minutes. Water (10 mL) was added and the aqueous phase was extracted with EtOAc (3X15 mL). The combined organic layers were dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography to afford a yellow powder. **Yield** = 87% (130 mg); $\mathbf{R_f}$ = 0.35 (EA : Hex = 1 : 1); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.19 (d, *J* = 8.8 Hz, 2H), 7.97 (d, *J* = 8.0 Hz, 1H), 7.45 (d, *J* = 8.8 Hz, 2H), 7.37 (d, *J* = 8.0 Hz, 1H), 5.28 (s, 2H), 4.16 (s, 2H), 3.55 (s, 2H), 3.04 (s, 3H), 1.29 (s, 9H) ppm; **^{13}C NMR** (125 MHz, $CDCl_3$): δ 167.27, 154.04, 147.69, 146.74, 146.14, 142.65, 135.36, 133.81, 128.35, 125.91, 124.57, 123.74, 82.29, 66.87, 51.64, 37.66, 35.68, 27.74 ppm; **HRMS** (ESI): m/z calc. for $C_{22}H_{25}N_5NaO_9S$ $[M+Na]^+$ 558.1265, found 558.1276 $[M+Na]^+$.

Synthesis of compound 6a

To a stirred solution of **5a** (20 mg, 37 μmol) in dichloromethane (1 mL) was added trifluoroacetic acid (77 μL, 1 mmol) at room temperature. The reaction mixture was stirred for 2 hours at room temperature. Excess trifluoroacetic acid was coevaporated with 5 mL toluene under reduced pressure. The product was redissolved in 5 mL ACN/H_2O (1:1) and lyophilized to give a yellow powder. The product **6a** was used in the next step without further purification. **Yield** = quantitative (18 mg).

Synthesis of compound BDSBD-NO_2

To a stirred solution of **6a** (18 mg, 37 μmol), EDC·HCl (9 mg, 45 μmol), HOBt·H_2O (7 mg, 45 μmol) and Et_3N (26 μL, 185 μmol) in DMF (1 mL) was added BG-NH_2 (12 mg, 45 μmol) at room temperature. The resulting mixture was stirred overnight at room temperature. The crude was purified by reverse phase preparative HPLC to give the desired product **BDSBD-NO_2** as a yellow powder after lyophilization. BG-NH_2 was prepared as previously reported.[1] **Yield** = 74% (20 mg); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 8.57 (t, *J* = 5.6 Hz, 1H), 8.36 (s, 1H), 8.17 (d, *J* = 8.8 Hz, 2H), 8.10 (d, *J* = 7.6 Hz, 1H), 7.66 (d, *J* = 7.6 Hz, 1H), 7.54 (d, *J* = 8.8 Hz, 2H), 7.46 (d, *J* = 8.0 Hz, 2H), 7.20 (d, *J* = 8.0 Hz, 2H), 5.50 (s, 2H), 5.30 (s, 2H), 4.16 (d, *J* = 5.6 Hz, 2H), 4.03 (s, 2H), 3.45 (s, 3H), 2.96 (s, 3H) ppm; **^{13}C NMR** (125 MHz, DMSO-d_6): δ 167.23, 159.09, 158.57, 158.41, 158.14, 154.15, 153.84, 147.18, 147.03, 146.25, 143.82, 139.44, 135.65, 135.14, 134.54, 128.92, 128.39, 127.40, 125.97, 124.37, 123.65, 67.86, 66.48, 51.85, 41.95, 36.31 ppm; **HRMS (ESI)**: m/z calc. for $C_{31}H_{30}N_{11}O_9S$ $[M+H]^+$ 732.1943, found 732.1953 $[M+H]^+$.

Synthesis of compound 5b

To NaH (13 mg, 0.54 mmol, 60% in oil) in dry THF (2 mL) solution at 0 °C was added compound **4** (100 mg, 0.28 mmol) in dry THF (3 mL). After 10 minutes, 4-azidobenzyl chloroformate (89 mg, 0.42 mmol) in dry THF (3 mL) was added slowly and the reaction mixture was stirred at room temperature for 10 minutes. Water (10 mL) was added and the aqueous phase was extracted with EtOAc (3X15 mL). The combined organic layers were dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography to give yellow powder. 4-azidobenzyl carbonochloridate

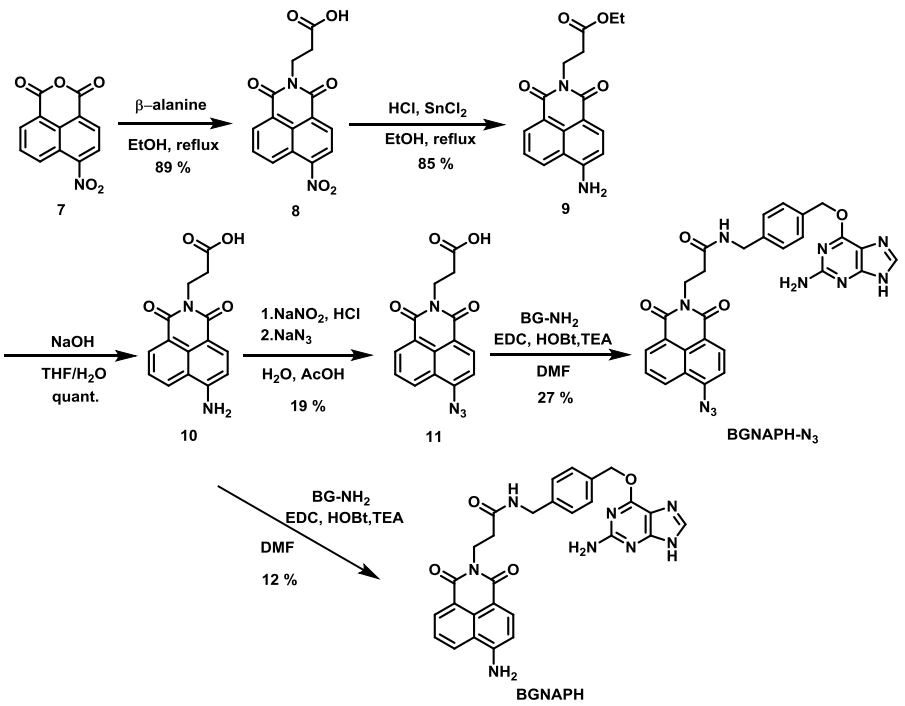
was prepared as previously reported.[2,3] **Yield** = 75% (112 mg); $\mathbf{R_f}$ = 0.57 (EA : Hex = 1 : 1); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.95 (d, *J* = 7.6 Hz, 1H), 7.35 (d, *J* = 7.6 Hz, 1H), 7.30 (d, *J* = 8.4 Hz, 2H), 6.99 (d, *J* = 8.4 Hz, 2H), 5.16 (s, 2H), 4.15 (s, 2H), 3.53 (s, 3H), 3.03 (s, 3H), 1.30 (s, 9H) ppm; **HRMS** (ESI): m/z calc. for $C_{22}H_{25}N_7NaO_7S$ $[M+Na]^+$ 554.1428, found 554.1435 $[M+Na]^+$.

Synthesis of compound 6b

To a stirred solution of **5b** (20 mg, 38 μmol) in dichloromethane (1 mL) was added trifluoroacetic acid (77 μL, 1 mmol) at room temperature. The reaction mixture was stirred for 2 hours at room temperature. Excess trifluoroacetic acid was coevaporated with 5 mL toluene under reduced pressure. The product was redissolved in 5 mL ACN/H_2O (1:1) and lyophized to give a yellow powder. The product **6b** was used in the next step without further purification. **Yield** = quantitative (18 mg).

Synthesis of compound BGSBD-N_3

To a stirred solution of **6b** (18 mg, 38 μmol), EDC·HCl (9 mg, 46 μmol), HOBt·H_2O (7 mg, 46 μmol) and Et_3N (26 μL, 190 μmol) in DMF (1 mL) was added BG-NH_2 (12 mg, 46 μmol) at room temperature. The resulting mixture was stirred overnight at room temperature. The crude was purified by reverse phase preparative HPLC to give the desired product **BGSBD-N_3** as a yellow powder after lyophilization. **Yield** = 66% (18 mg); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 8.57 (t, *J* = 6.0 Hz, 1H), 8.43 (s, 1H), 8.08 (d, *J* = 7.6 Hz, 1H), 7.61 (d, *J* = 7.6 Hz, 1H), 7.48 (d, *J* = 8.0 Hz, 2H), 7.33 (d, *J* = 7.6 Hz, 2H), 7.22 (d, *J* = 8.0 Hz, 2H), 7.06 (d, *J* = 7.6 Hz, 2H), 5.51 (s, 2H), 5.13 (s, 2H), 4.17 (d, *J* = 6 Hz, 2H), 4.02 (s, 2H), 3.41 (s, 3H), 2.94 (s, 3H) ppm; **^{13}C NMR** (125 MHz, DMSO-d_6): δ 167.19, 158.95, 158.37, 158.15, 154.00, 153.79, 146.97, 146.16, 140.69, 139.55, 139.33, 135.68, 135.32, 134.35, 132.82, 129.85, 128.96, 127.41, 125.61, 124.03, 119.19, 68.04, 67.17, 51.88, 41.93, 37.40, 36.26 ppm; **HRMS (ESI)**: m/z calc. for $C_{31}H_{30}N_{13}O_7S$ $[M+H]^+$ 728.2106, found 728.2121 $[M+H]^+$.



Scheme S2. Synthesis of **BGNAPH-N_3** and **BGNAPH**.

Synthesis of compound 8

4-Nitro-1,8-naphthalic anhydride (2.0 g, 8.2 mmol) and 3-aminopropionic acid (0.9 g, 10.1 mmol) were refluxed in ethanol (50 mL) for 8 hours until reaction was completed. The cooled mixture was diluted with water and the precipitated solid was collected by filtration to afford white microcrystal compound **8**. **Yield** = 89 % (2.3 g); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 8.69 (d, *J* = 8.4 Hz, 1H), 8.66 – 8.58 (m, 2H), 8.54 (d, *J* = 8.0 Hz, 1H), 8.08 (t, *J* = 8.0 Hz, 1H), 4.25 (t, *J* = 7.6, 2H), 2.61 (t, *J* = 7.6, 2H). **^{13}C NMR** (100 MHz, DMSO-d6): δ 172.39, 162.68, 161.89, 148.95, 131.61, 130.04, 129.54, 128.70, 128.10, 126.37, 124.20, 122.57, 122.50, 36.00, 31.95 ppm; **HRMS** (ESI): m/z calc. for $C_{15}H_{10}N_2O_6$ 314.0460 $[M+H]^-$, found 313.0457 $[M+H]^-$.

Synthesis of compound 9

A solution of ompound **8** (1.0 g, 3.2 mmol) and stannous chloride (3.0 g, 16 mmol) in ethanol (25 mL) was refluxed for 3 hours with hydrochloric acid (1.5 mL) till the reaction was completed. The mixture was poured into 100 mL water and then the precipitated solid was filtered out. The crude products **9** were purified by column chromatography (eluent: ethyl acetate/hexane = 2/1) to give a light yellow solid. **Yield** = 85 % (0.84 g); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 8.60 (d, *J* =

8.4 Hz, 1H), 8.41 (d, *J* = 7.2 Hz, 1H), 8.18 (d, *J* = 8.4 Hz, 1H), 7.64 (dd, *J* = 8.4, 7.2 Hz, 1H), 7.46 (s, 2H), 6.83 (d, *J* = 8.4 Hz, 1H), 4.24 (t, *J* = 7.5 Hz, 2H), 4.02 (q, *J* = 7.1 Hz, 2H), 2.59 (t, *J* = 7.5 Hz, 2H), 1.10 (t, *J* = 7.1 Hz, 3H); **^{13}C NMR** (100 MHz, DMSO-d6): δ 171.02, 163.69, 162.73, 152.80, 133.94, 130.98, 129.69, 129.36, 123.88, 121.59, 119.32, 108.27, 107.39, 60.10, 35.36, 32.52, 13.95 ppm; **HRMS** (ESI): m/z calc. for $C_{17}H_{16}N_2O_4$ 313.1188 $[M+H]^+$, found 313.1191 $[M+H]^+$.

Synthesis of compound 10

Sodium hydroxide (0.8 g, 20 mmol) was dissolved in H_2O (5 mL) and added dropwise to compound **9** (0.5 g, 1.6 mmol) which was dissolved in THF and H_2O solution in ice bath. The resulting mixture was stirred at room temperature for 18 hours. Then the solution was neutralized with 10% hydrochloric acid and lyophilized to afford compound **10** as an orange solid. **Yield** = quantitative; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 8.65 (d, *J* = 8.4 Hz, 1H), 8.39 (d, *J* = 7.2 Hz, 1H), 8.15 (d, *J* = 7.2 Hz, 1H), 7.62 (dd, *J* = 8.4, 7.2 Hz, 1H), 7.53 (s, 2H), 6.84 (d, *J* = 8.4 Hz, 1H), 4.12 (t, *J* = 8.6 Hz, 2H), 2.21 (d, *J* = 8.6 Hz, 2H) ppm; **^{13}C NMR** (100 MHz, DMSO-d_6): δ 175.13, 163.69, 162.87, 152.70, 133.79, 130.84, 129.63, 129.36, 123.85, 121.88, 119.36, 108.08, 107.61, 37.54, 36.21 ppm; **HRMS** (ESI): m/z calc. for $C_{15}H_{12}N_2O_4$ 307.6094 $[M+H]^+$, found 307.6087 $[M+H]^+$.

Synthesis of compound BGNAPH

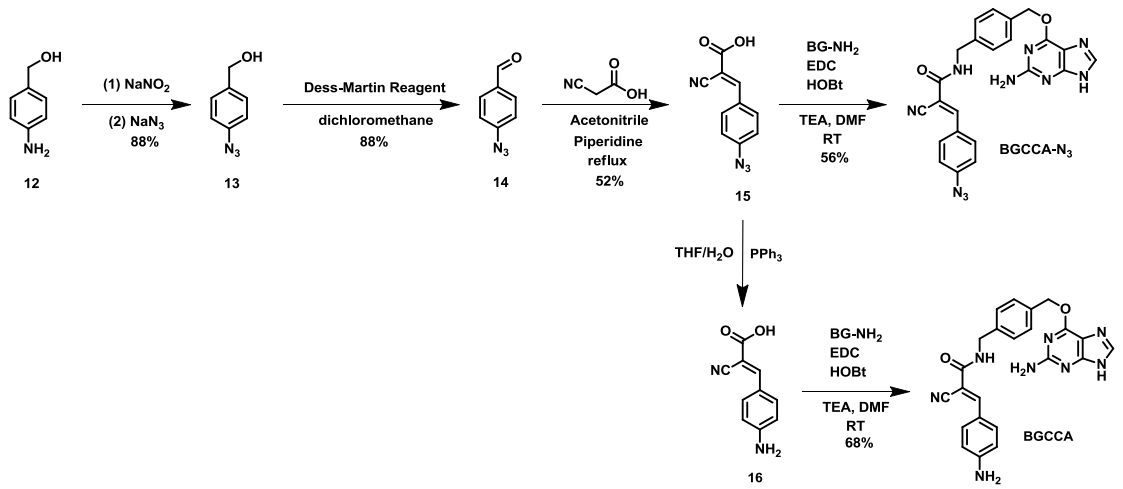
To a stirred solution of **11a** (20 mg, 70 μmol), EDC·HCl (41 mg, 214 μmol), HOBt·H_2O (29 mg, 215 μmol) and Et_3N (99 μL, 712 μmol) in DMF (1 mL) was added BG-NH_2 (25 mg, 92 μmol) at room temperature. The resulting mixture was stirred at 40 °C for 18 hours. The crude product was purified by reverse phase preparative HPLC to give the desired product **BGNAPH** as a yellow powder after lyophilization. **Yield** = 12 % (4.5 mg); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 8.60 (d, *J* = 8.4 Hz, 1H), 8.47 (m, 2H), 8.41 (d, *J* = 7.2 Hz, 1H), 8.18 (d, *J* = 8.4 Hz, 1H), 7.64 (dd, *J* = 8.4, 7.2 Hz, 1H), 7.45 (d, *J* = 7.8 Hz, 2H), 7.27 (d, *J* = 7.8 Hz, 2H), 6.83 (d, *J* = 8.4 Hz, 1H), 5.51 (s, 2H), 4.24 (m, 4H) ppm; **^{13}C NMR** (125 MHz, DMSO-d_6): δ 170.01, 163.70, 162.78, 158.84, 157.89, 152.76, 139.95, 133.97, 131.01, 129.73, 129.33, 128.99, 128.84, 127.49, 123.98, 121.79, 119.37, 108.15, 107.54, 68.10, 42.35, 41.82, 36.06, 33.74 ppm; **HRMS** (ESI): m/z calc. for $C_{28}H_{24}N_8O_4$ $[M+H]^+$ 537.1998, found 537.1997 $[M+H]^+$.

Synthesis of compound 11

To a stirred solution of compound **10** (200 mg, 0.64 mmol) in 5 M hydrochloric acid (2 mL) was added acetic acid (2 mL). To this solution, sodium nitrite (180 mg, 2.60 mmol) dissolved in 10 mL of water was added dropwise within 15 mins in ice bath. The solution was vigorous stirred for 18 hours at room temperature. The solution was cooled to 0 ºC and sodium azide (110 mg, 1.7 mmol) was batch added in. Then the solution was stirred for 3 hours at room temperature. The reaction solution was poured into saturated aqueous $NaHCO_3$ and extracted with dichloromethane. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by reverse phase preparative HPLC to give the desired product **11** as pale yellow powder. **Yield** = 19 % (71 mg); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 8.52 (dd, *J* = 7.2, 1.2 Hz, 1H), 8.47 (d, *J* = 8.0 Hz, 1H), 8.43 (dd, *J* = 8.4, 1.2 Hz, 1H), 7.86 (dd, *J* = 8.4, 7.2 Hz, 1H), 7.75 (d, *J* = 8.0 Hz, 1H), 4.24 (t, *J* = 7.6 Hz, 2H), 2.58 (t, *J* = 7.6 Hz, 2H) ppm; **^{13}C NMR** (100 MHz, DMSO-d6): δ 172.45, 163.09, 162.61, 142.87, 131.57, 131.49, 128.38, 128.27, 127.27, 123.49, 122.08, 118.08, 115.92, 35.72, 32.17 ppm; **HRMS** (ESI): m/z calc. for $C_{15}H_{10}N_4O_4$ $[M+Na]^+$ 333.0599, found 333.0598 $[M+Na]^+$.

Synthesis of compound BGNAPH-N_3

To a stirred solution of **BGNAPH-N_3** (15 mg, 53 μmol), EDC·HCl (35 mg, 183 μmol), HOBt·H_2O (25 mg, 185 μmol) and Et_3N (70 μL, 504 μmol) in DMF (1 mL) was added BG-NH_2 (18 mg, 67 μmol) at room temperature. The resulting mixture was stirred for 18 hours at room temperature. The crude product was purified by reverse phase preparative HPLC to give the desired product **BGNAPH-N_3 as pale yellow powder** after lyophilization. **Yield** = 27% (8 mg). **^{1}H NMR** (400 MHz, DMSO-d_6): δ 8.52 (d, *J* = 7.2, 1H), 8.46 (d, *J* = 8.4, 1H), 8.40 (d, *J* = 8.4, 1H), 8.10 (s, 1H), 7.85 (dd, *J* = 8.4, 7.2 Hz, 1H), 7.74 (d, *J* = 8.4 Hz, 1H), 7.39 (d, *J* = 8.0 Hz, 2H), 7.23 (d, *J* = 8.0 Hz, 2H), 5.46 (s, 2H), 4.25 (m, 4H) ppm; **^{13}C NMR** (125 MHz, DMSO-d_6): δ 169.88, 163.18, 162.72, 159.23, 158.06, 157.81, 155.43, 142.91, 139.39, 138.96, 134.901, 131.64, 131.57, 128.50, 128.41, 127.33, 123.59, 122.27, 118.28, 116.01, 66.89, 41.88, 36.55, 33.61 ppm; **HRMS** (ESI): m/z calc. for $C_{28}H_{22}N_{10}O_4$ $[M+H]^+$ 563.1903, found 563.1905 $[M+H]^+$.



Scheme S3. Synthesis of **BGCCA-N_3** and **BGCCA**.

Synthesis of compound 13

4-Aminobenzylalcohol (1000 mg, 8.12 mmol) was dissolved in hydrochloric acid (5 mL, 5 M). To this solution, sodium nitrite (840 mg, 12.18 mmol) dissolved in 20 mL of water was dropwise added within 30 mins. The solution was vigorous stirred in ice-cold water. Sodium azide (2100 mg, 32.3 mmol) was batch added in. The resulting solution was stirred at room temperature overnight. The reaction was monitored by TLC. After the completion of reaction, the reaction solution was poured into saturated aqueous $NaHCO_3$ and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by silica gel chromatography to obtain the pure product **13** as yellow oil; **Yield** = 88% (1290 mg); **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 7.34 (d, J = 8.0 Hz, 2H), 7.01 (d, J = 8.0 Hz, 2H), 4.65 (s, 2H) ppm; **^{13}C-NMR** (100 MHz, CDCl3): δ = 138.69, 137.29, 128.06, 118.57, 63.66 ppm; **HRMS** (EI): m/z calc. for $C_7H_7N_3O$ $[M]^+$ 149.0589, Found 149.0585 $[M]^+$.

Synthesis of compound 14

Compound **13** (150 mg, 1.01 mmol) was dissolved in 15 mL dry CH_2Cl_2. Dess-Martin reagent (640 mg, 1.51 mmol) was added and the mixture was stirred for 2 hours at room temperature, at which point oxidation was completed. The mixture was diluted with EtOAc (60 mL), washed with saturated $Na_2S_2O_3$ (10 mL), saturated aqueous $NaHCO_3$ (10 mL), and brine. Then organic layer was dried with Na_2SO_4 and concentrated under reduced pressure. The crude product was

purified by silica gel column chromatography using Hexane/EtOAc (10:1, v:v) as eluent to afford **14** as yellow oil. **Yield** = 88% (130 mg); **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 9.82 (s, 1H), 7.76 (d, *J* = 8.8 Hz, 2H), 7.03 (d, *J* = 8.8 Hz, 2H) ppm; **^{13}C-NMR** (100 MHz, $CDCl_3$): δ 119.15, 131.18 (2C), 132.95, 145.86, 190.20 ppm; **HRMS** (EI): m/z calc. for $C_7H_5N_3O$ $[M]^+$ 147.0433, Found 147.0428 $[M]^+$.

Synthesis of compound 15

To a solution of **14** (0.1 g, 0.68 mmol) and cyano acetic acid (0.12 g, 1.36 mmol) in ethanol (2 mL), pyrrolidine (0.15 g, 2.04 mmol) was added with stirring and stirring was continued at room temperature for 1 hour. The reaction mixture was concentrated under vacuum and the residue obtained was extracted using water and ethyl acetate. The organic layer was collected, dried with Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography to afford the product as a yellow solid. **Yield** = 52 % (76 mg); **^{1}H-NMR** (400 MHz, DMSO-d_6): δ 8.22 (s, 1H), 8.04 (d, *J* = 8.8 Hz, 2H), 7.20 (d, *J* = 8.8 Hz, 2H) ppm; **^{13}C-NMR** (100 MHz, DMSO-d_6): δ 94.16, 107.46, 111.28, 120.21, 124.44, 136.92, 145.01, 155.58 ppm; **HRMS** (ESI): m/z calc. for $C_{10}H_6N_4O_2$ $[M]^+$ 214.0491, found 214.0493 $[M]^+$.

Synthesis of compound BGCCA-N_3

To a 10 mL reaction flask containing **15** (10 mg, 0.047 mmol), BG-NH_2 (15 mg, 0.056 mmol), and PyBOP (36.4 mg, 0.07 mmol) in DMF was added DIPEA (0.2 mmole) at room temperature. The reaction mixture was stirred at room temperature overnight. The crude was purified by reverse phase preparative HPLC to give the desired product **BGCCA-N_3** as a yellow powder after lyophilization. **Yield** = 56% (12 mg); **^{1}H-NMR** (400 MHz, DMSO-d_6): δ 9.03 (s, 1H), 8.40 (s, 1H), 8.17 (s, 1H), 7.99 (d, *J* = 8.4 Hz, 2H), 7.50 (d, *J* = 7.6 Hz, 2H), 7.36 (d, *J* = 7.6 Hz, 2H), 7.29 (d, *J* = 8.4 Hz, 2H), 5.51 (s, 2H), 4.41 (s, 2H) ppm; **^{13}C-NMR** (100 MHz, DMSO-d_6): δ 67.94, 104.90, 116.53, 119.98, 127.56, 128.51, 128.93, 132.08, 134.38, 139.26 ,140.60, 143.55 ,149.73, 153.71, 158.18 ,158.87, 161.15 ppm; **HRMS** (ESI): m/z calc. for $C_{23}H_{18}N_{10}O_2$ $[M+H]^+$ 467.1687, found 467.1685 $[M+H]^+$.

Synthesis of compound 16

To a solution of **15** (10 mg, 0.047 mmol) in 1 mL H_2O/THF (10/90), triphenylphosphine (74 mg, 0.28 mmol) was added with stirring and stirring was continued at room temperature overnight. The product was used in the next step without further purification. **^{1}H-NMR** (400 MHz, DMSO-d_6): δ 8.05 (s, 1H), 7.82 (d, *J* = 8.8 Hz, 2H), 6.69 (d, *J* = 8.8 Hz, 2H) ppm; **^{13}C-NMR** (100 MHz, DMSO-d_6): δ 92.64, 113.47, 117.96, 118.46, 134.09, 154.03, 154.70, 164.91 ppm; **HRMS** (ESI): m/z calc. for $C_{10}H_8N_2O_2$ $[M+H]^+$ 189.0659, found 189.0657 $[M+H]^+$.

Synthesis of compound BGCCA

To a 10 mL reaction flask containing **16** (10 mg, 0.053 mmol), BG-NH_2 (21.5 mg, 0.08 mmol), and PyBOP (41.5 mg, 0.08 mmol) in DMF was added DIPEA (0.2 mmol) at room temperature. The reaction mixture was stirred at room temperature overnight. The crude was purified by reverse phase preparative HPLC to give the desired product **BGCCA-NH_2** as a yellow powder after lyophilization. **Yield** = 68% (16mg); **^{1}H-NMR** (400 MHz, DMSO-d_6): δ 8.69 (s, 1H), 8.52 (s, 1H), 7.92 (s, 1H), 7.73 (d, *J* = 8.0 Hz, 2H), 7.50 (d, *J* = 8.0 Hz, 2H), 7.33 (d, *J* = 8.0 Hz, 2H), 6.64 (d, *J* = 8.0 Hz, 2H), 5.53 (s, 2H), 4.38(s, 2H) ppm; **^{13}C-NMR** (100 MHz, DMSO-d_6): δ 68.40, 95.73, 113.22, 113.54, 118.30, 118.80, 127.52, 127.88, 129.00, 132.75 ,133.34, 133.96 ,139.94, 141.30, 150.95 ,153.17, 153.94, 158.80, 162.43 ppm; **HRMS** (ESI): m/z calc. for $C_{23}H_{20}N_8O_2$ $[M+H]^+$ 441.1782, found 441.1783 $[M+H]^+$.

1. A. Keppler, S. Gendreizig, T. Gronemeyer, H. Pick, H. Vogel, K. Johnsson, *Nat. Biotechnol.* **2003**, *21*, 86.
2. B. Chen, P. Wang, Q. Jin and X. Tang, *Org. Biomol. Chem.*, **2014**, *12*, 5629.
3. S. K. Bae, C. H. Heo, D. J. Choi, D. Sen, E. H. Joe, B. R. Cho and H. M. Kim, *J. Am. Chem. Soc.*, **2013**, *135*, 9915.

YS51-20151222
YS51

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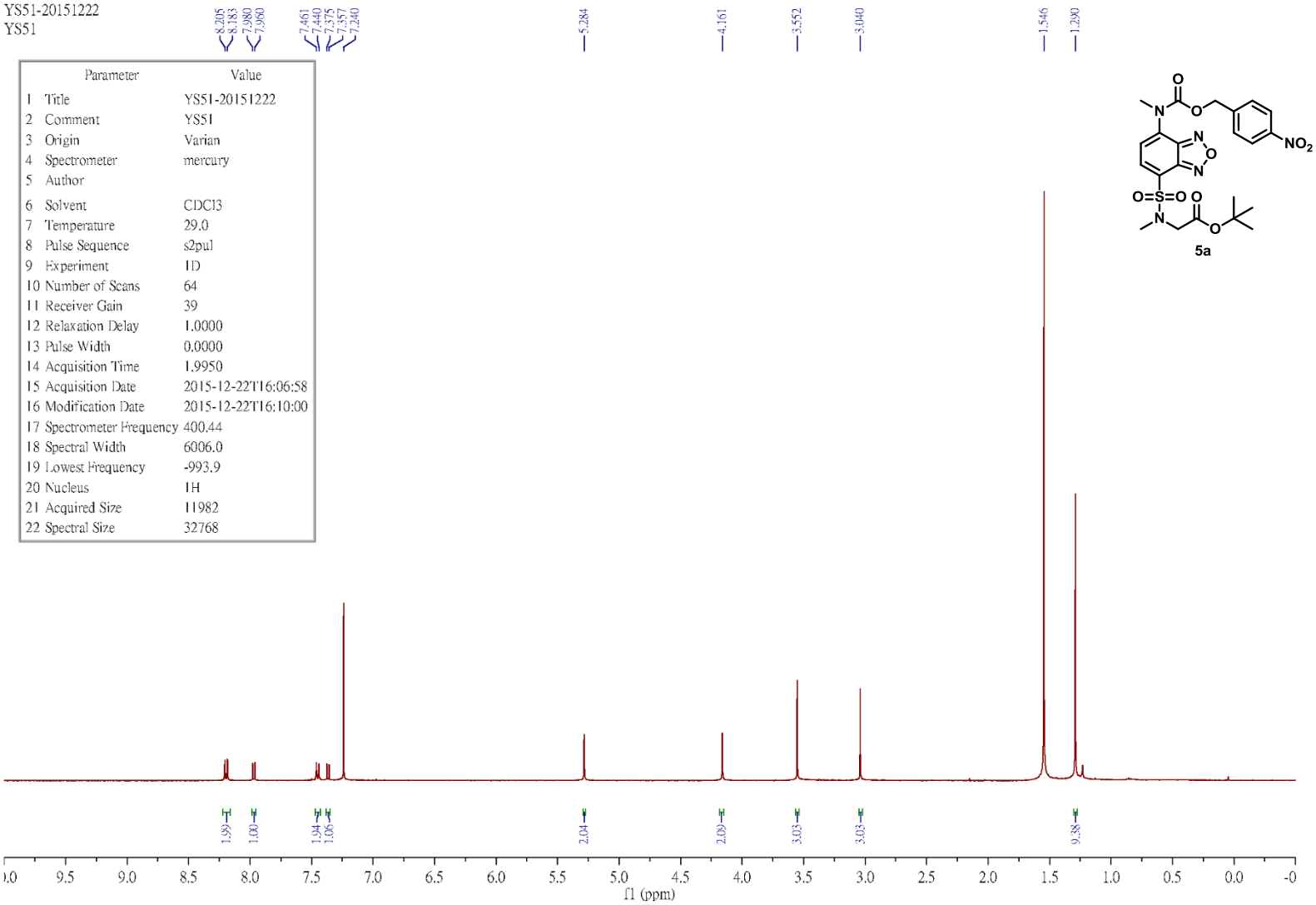

5a

YS51
YS 51

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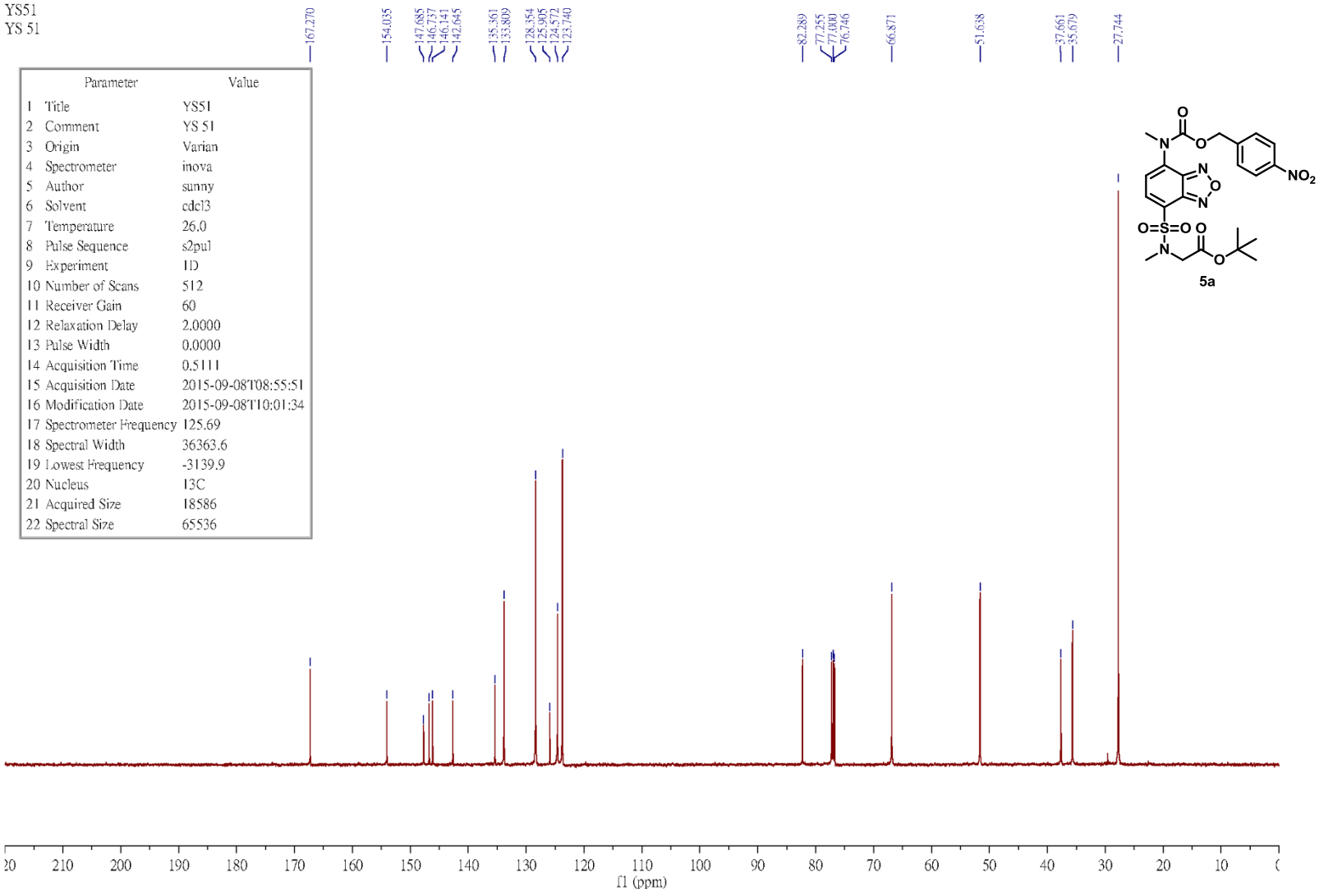

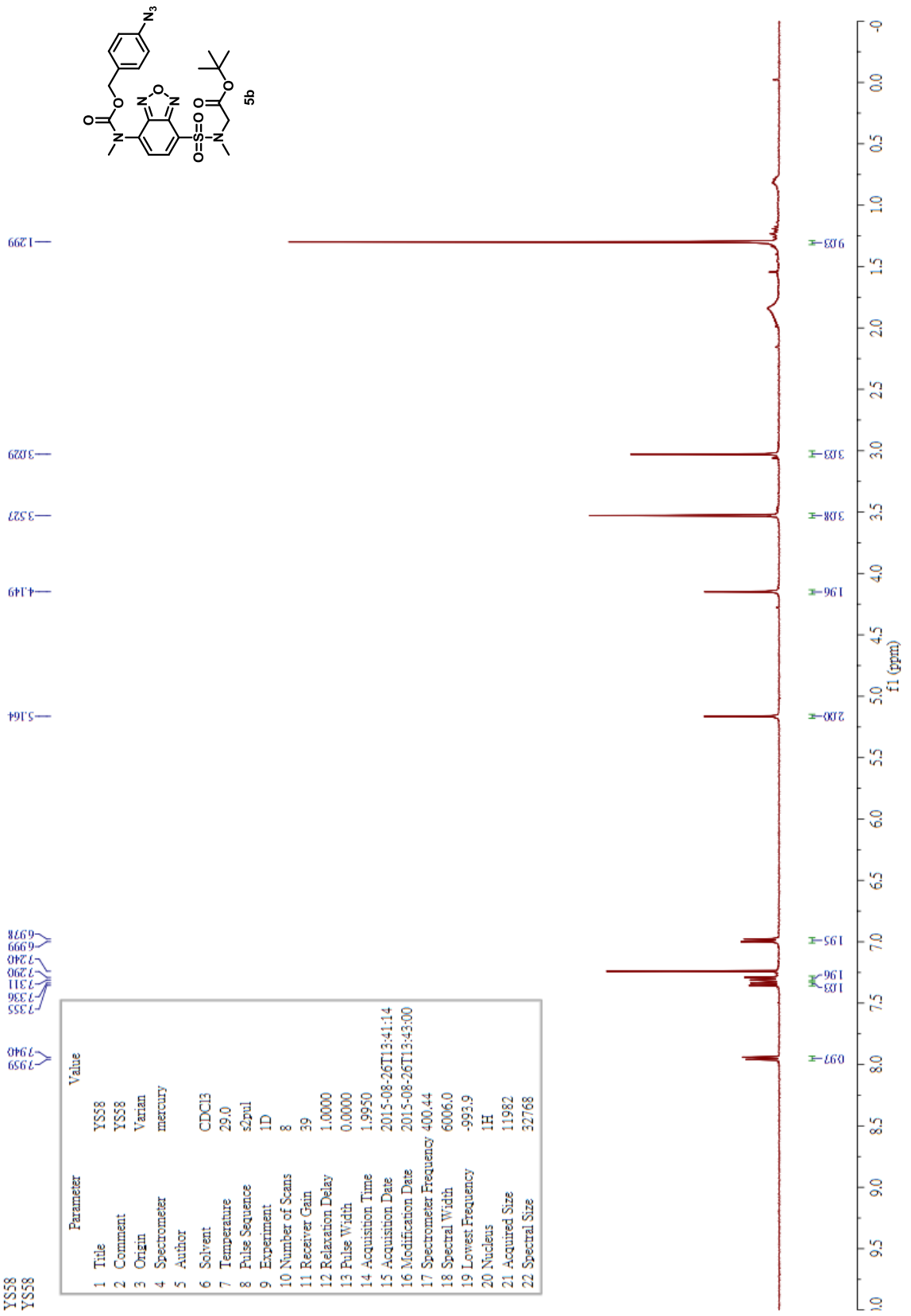
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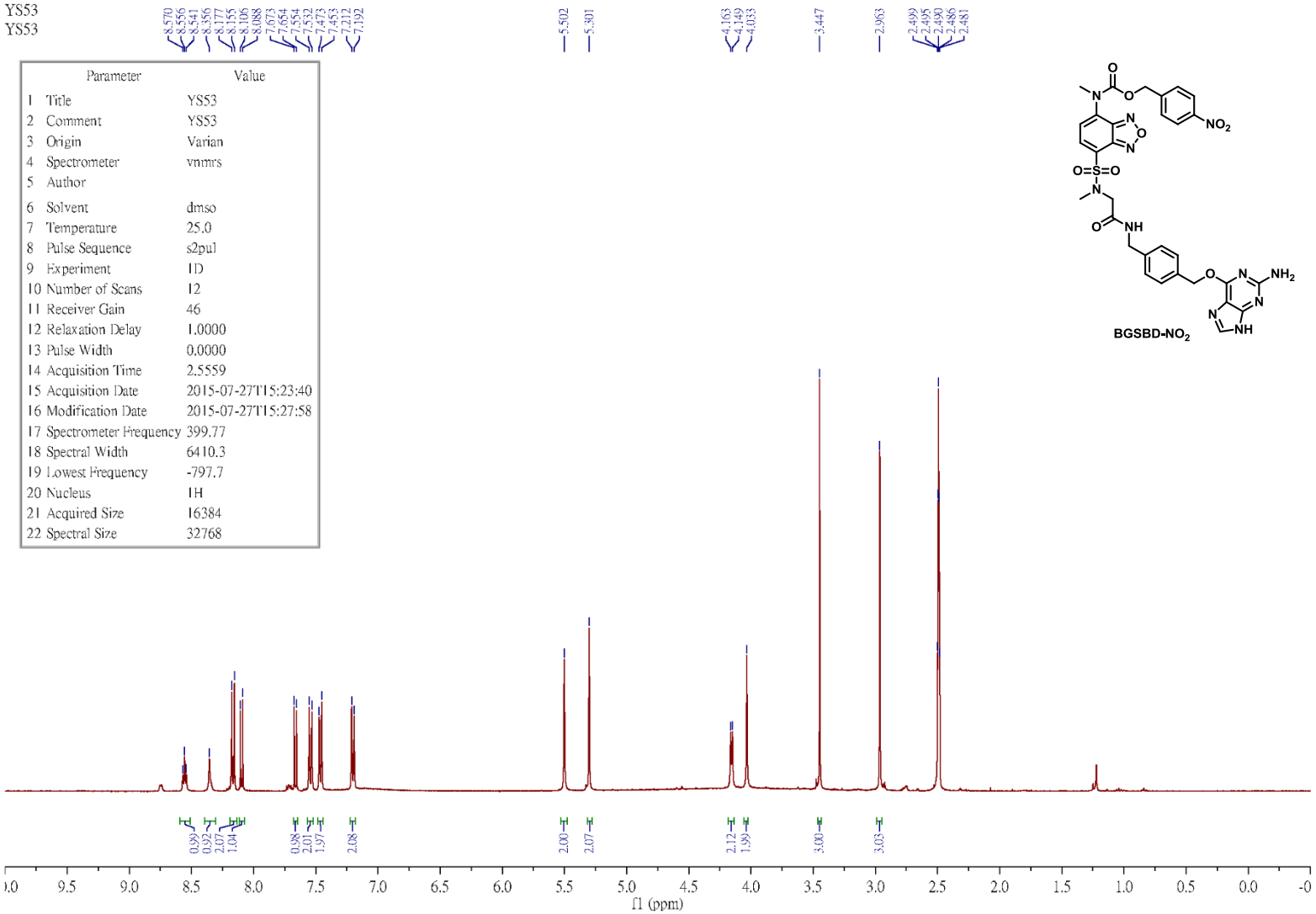
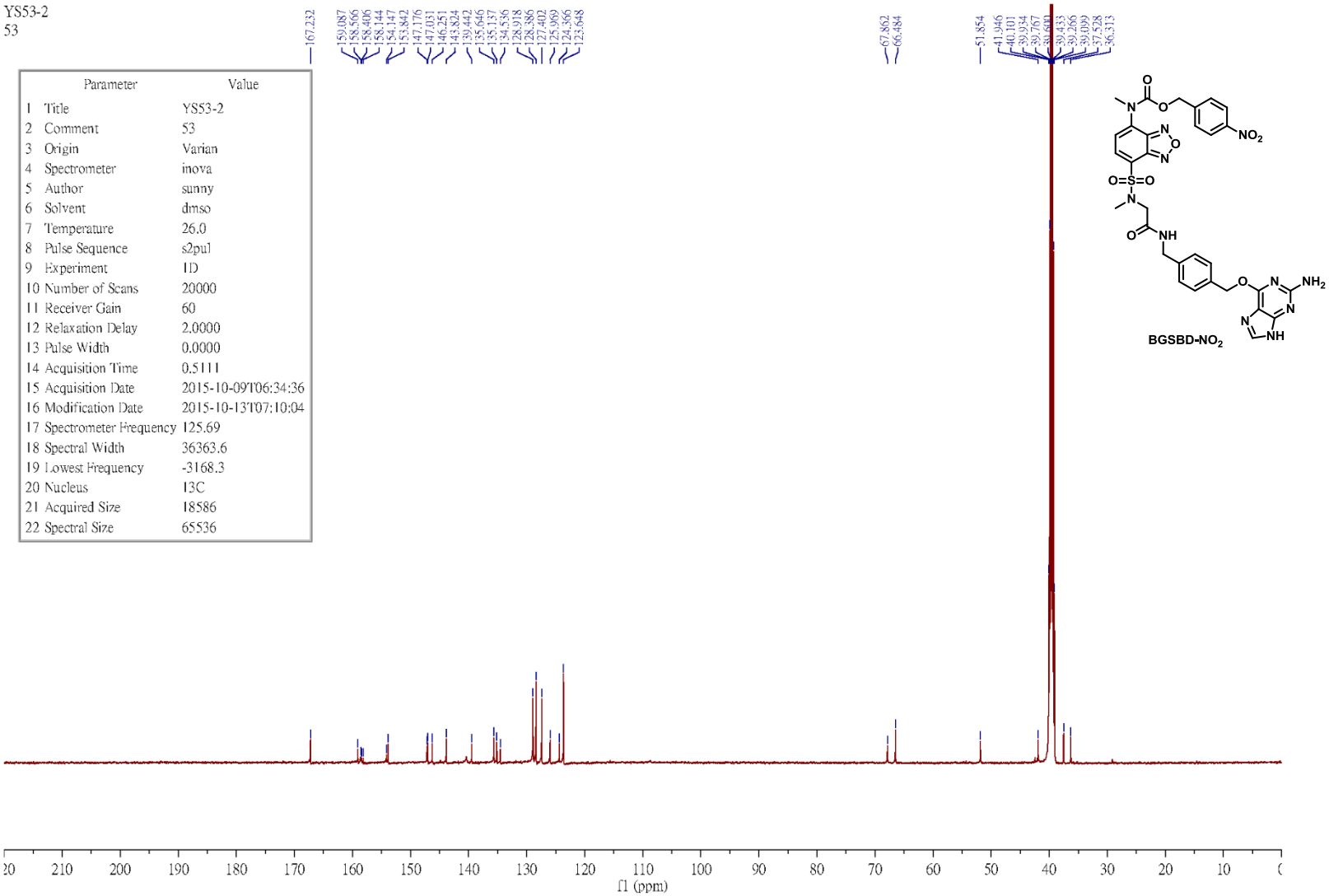
17 Spectrometer Frequency 400.44
18 Spectral Width 6006.0
19 Lowest Frequency -993.9
20 Nucleus 1H
21 Acquired Size 11982
22 Spectral Size 32768
5b
f1 (ppm)


YS53
YS53
8.570
8.556
8.541
8.356
8.177
8.155
8.106
8.088
7.673
7.654
7.554
7.532
7.473
7.453
7.212
7.192
5.502
5.301
4.163
4.149
4.033
3.447
2.963
2.499
2.495
2.490
2.486
2.481
Parameter Value
1 Title YS53
2 Comment YS53
3 Origin Varian
4 Spectrometer vnmrs
5 Author
6 Solvent dmso
7 Temperature 25.0
8 Pulse Sequence s2pul
9 Experiment 1D
10 Number of Scans 12
11 Receiver Gain 46
12 Relaxation Delay 1.0000
13 Pulse Width 0.0000
14 Acquisition Time 2.5559
15 Acquisition Date 2015-07-27T15:23:40
16 Modification Date 2015-07-27T15:27:58
17 Spectrometer Frequency 399.77
18 Spectral Width 6410.3
19 Lowest Frequency -797.7
20 Nucleus 1H
21 Acquired Size 16384
22 Spectral Size 32768
BGSBD-NO2
0.99
0.92
2.07
1.04
0.98
2.01
1.97
2.08
2.00
2.07
2.12
1.99
3.00
3.03
f1 (ppm)

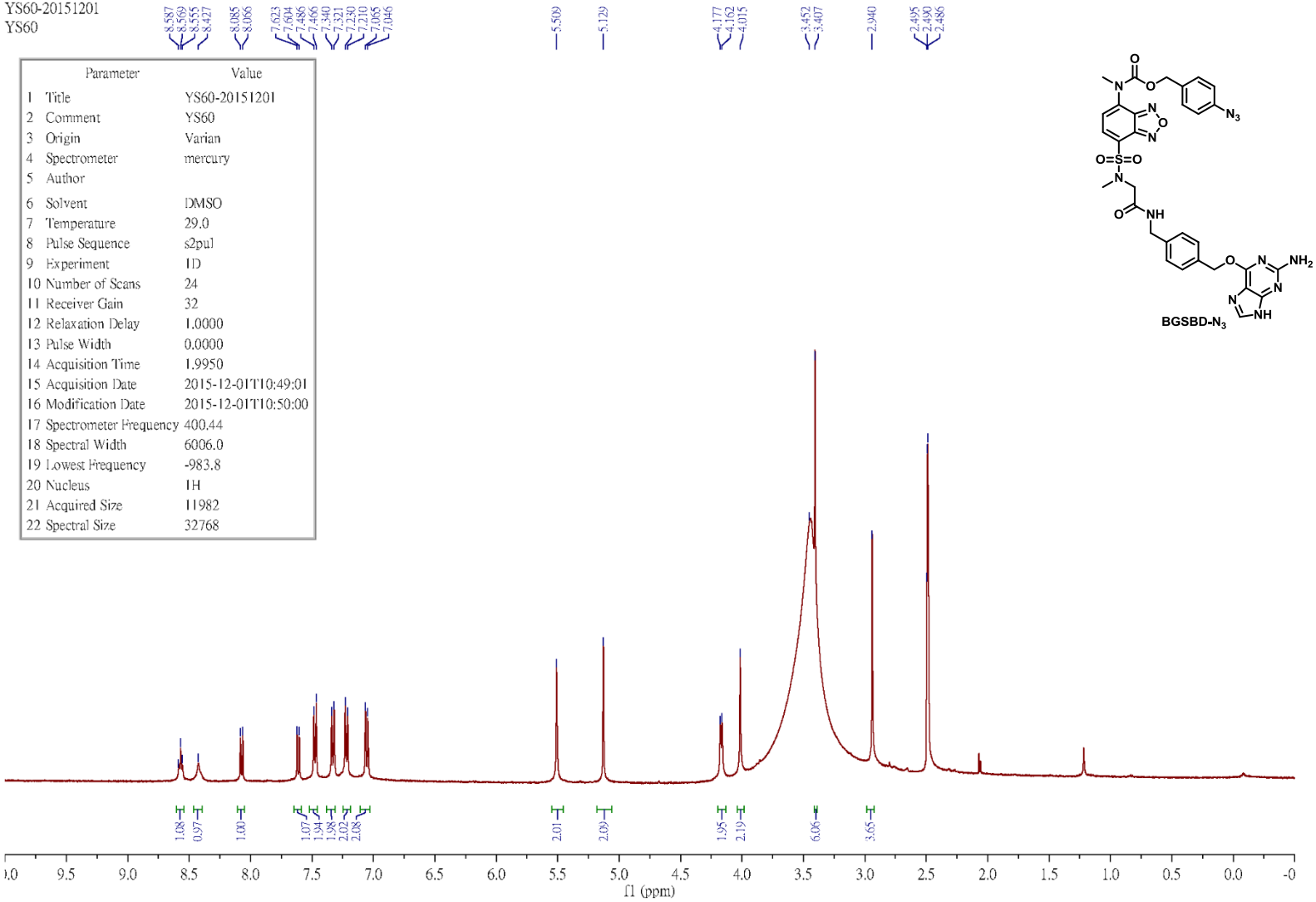
YS53-2
53

	Parameter	Value
1	Title	YS53-2
2	Comment	53
3	Origin	Varian
4	Spectrometer	inova
5	Author	sunny
6	Solvent	dmso
7	Temperature	26.0
8	Pulse Sequence	s2pul
9	Experiment	1D
10	Number of Scans	20000
11	Receiver Gain	60
12	Relaxation Delay	2.0000
13	Pulse Width	0.0000
14	Acquisition Time	0.5111
15	Acquisition Date	2015-10-09T06:34:36
16	Modification Date	2015-10-13T07:10:04
17	Spectrometer Frequency	125.69
18	Spectral Width	36363.6
19	Lowest Frequency	-3168.3
20	Nucleus	13C
21	Acquired Size	18586
22	Spectral Size	65536



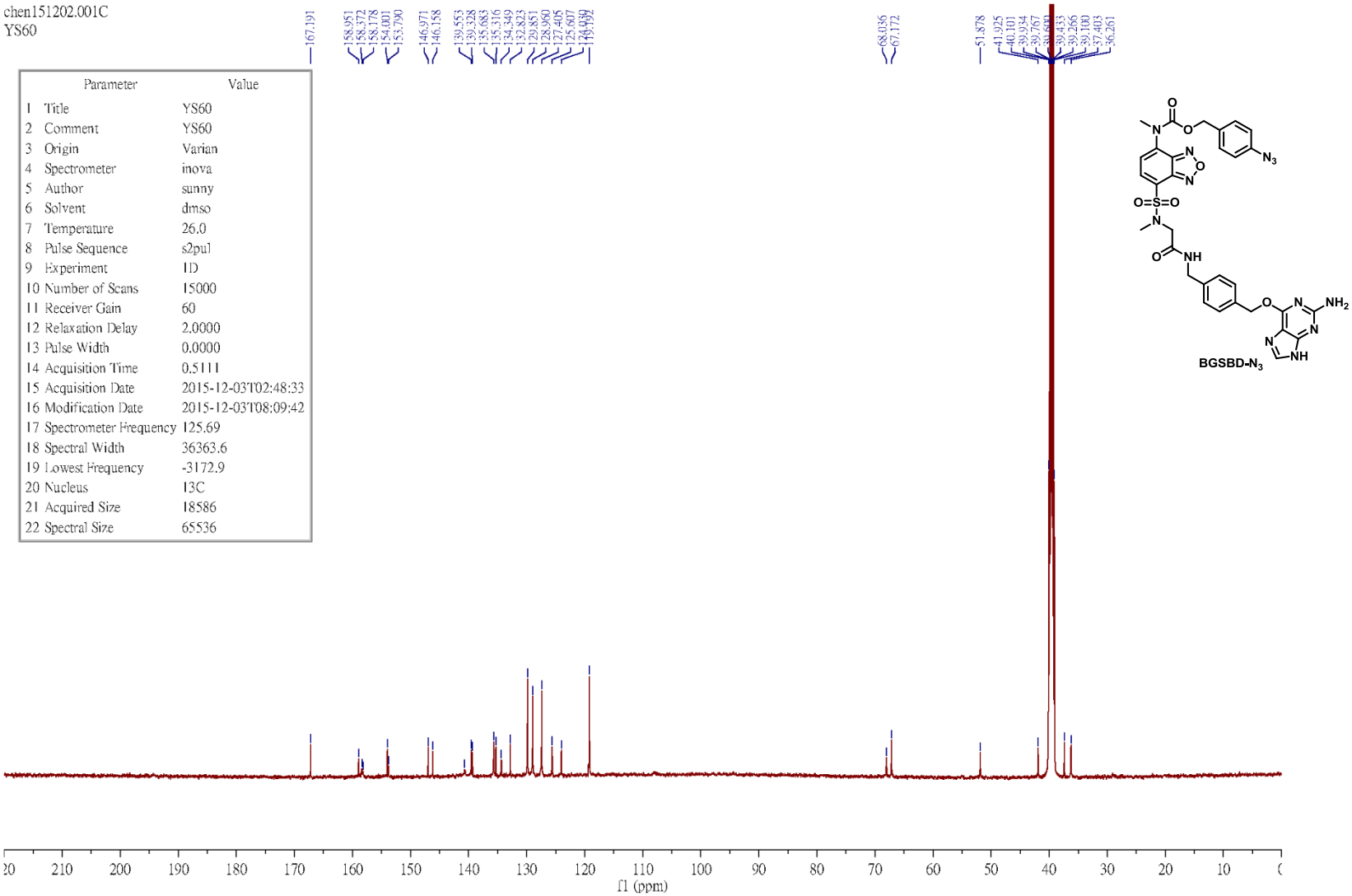
YS60-20151201
YS60

	Parameter	Value
1	Title	YS60-20151201
2	Comment	YS60
3	Origin	Varian
4	Spectrometer	mercury
5	Author	
6	Solvent	DMSO
7	Temperature	29.0
8	Pulse Sequence	s2pul
9	Experiment	1D
10	Number of Scans	24
11	Receiver Gain	32
12	Relaxation Delay	1.0000
13	Pulse Width	0.0000
14	Acquisition Time	1.9950
15	Acquisition Date	2015-12-01T10:49:01
16	Modification Date	2015-12-01T10:50:00
17	Spectrometer Frequency	400.44
18	Spectral Width	6006.0
19	Lowest Frequency	-983.8
20	Nucleus	1H
21	Acquired Size	11982
22	Spectral Size	32768



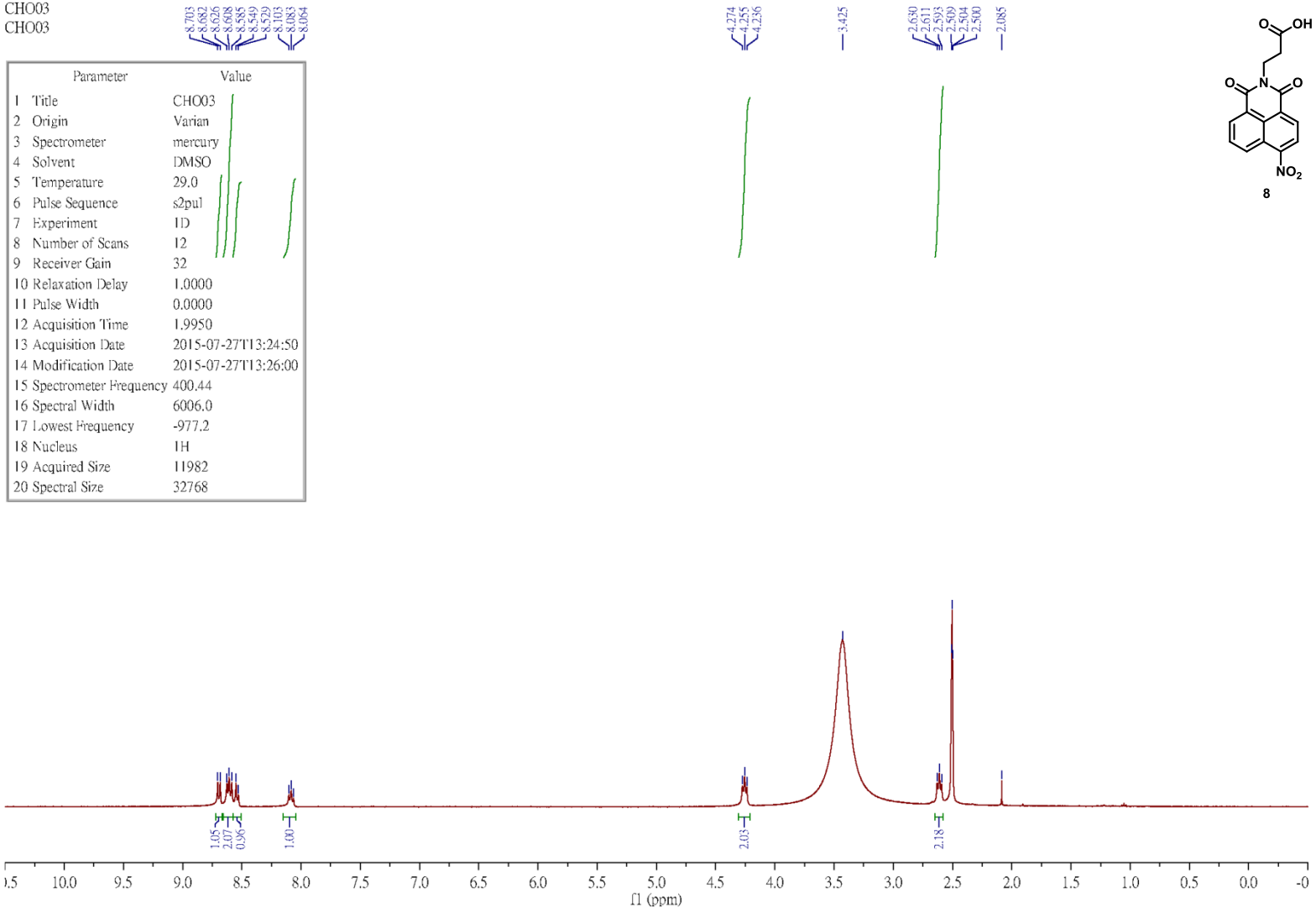
chen151202.001C
YS60

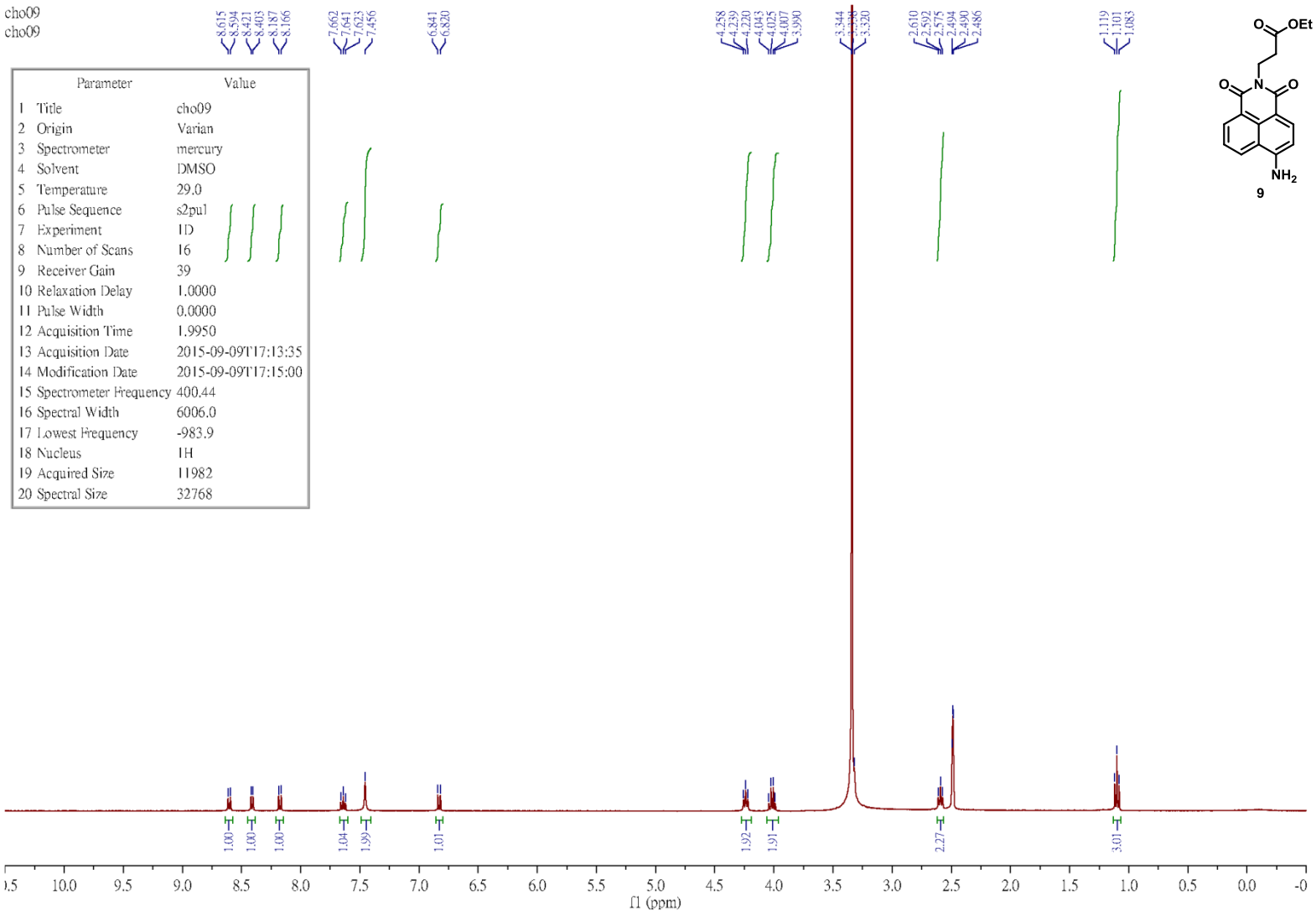
	Parameter	Value
1	Title	YS60
2	Comment	YS60
3	Origin	Varian
4	Spectrometer	inova
5	Author	sunny
6	Solvent	dmso
7	Temperature	26.0
8	Pulse Sequence	s2pul
9	Experiment	1D
10	Number of Scans	15000
11	Receiver Gain	60
12	Relaxation Delay	2.0000
13	Pulse Width	0.0000
14	Acquisition Time	0.5111
15	Acquisition Date	2015-12-03T02:48:33
16	Modification Date	2015-12-03T08:09:42
17	Spectrometer Frequency	125.69
18	Spectral Width	36363.6
19	Lowest Frequency	-3172.9
20	Nucleus	13C
21	Acquired Size	18586
22	Spectral Size	65536

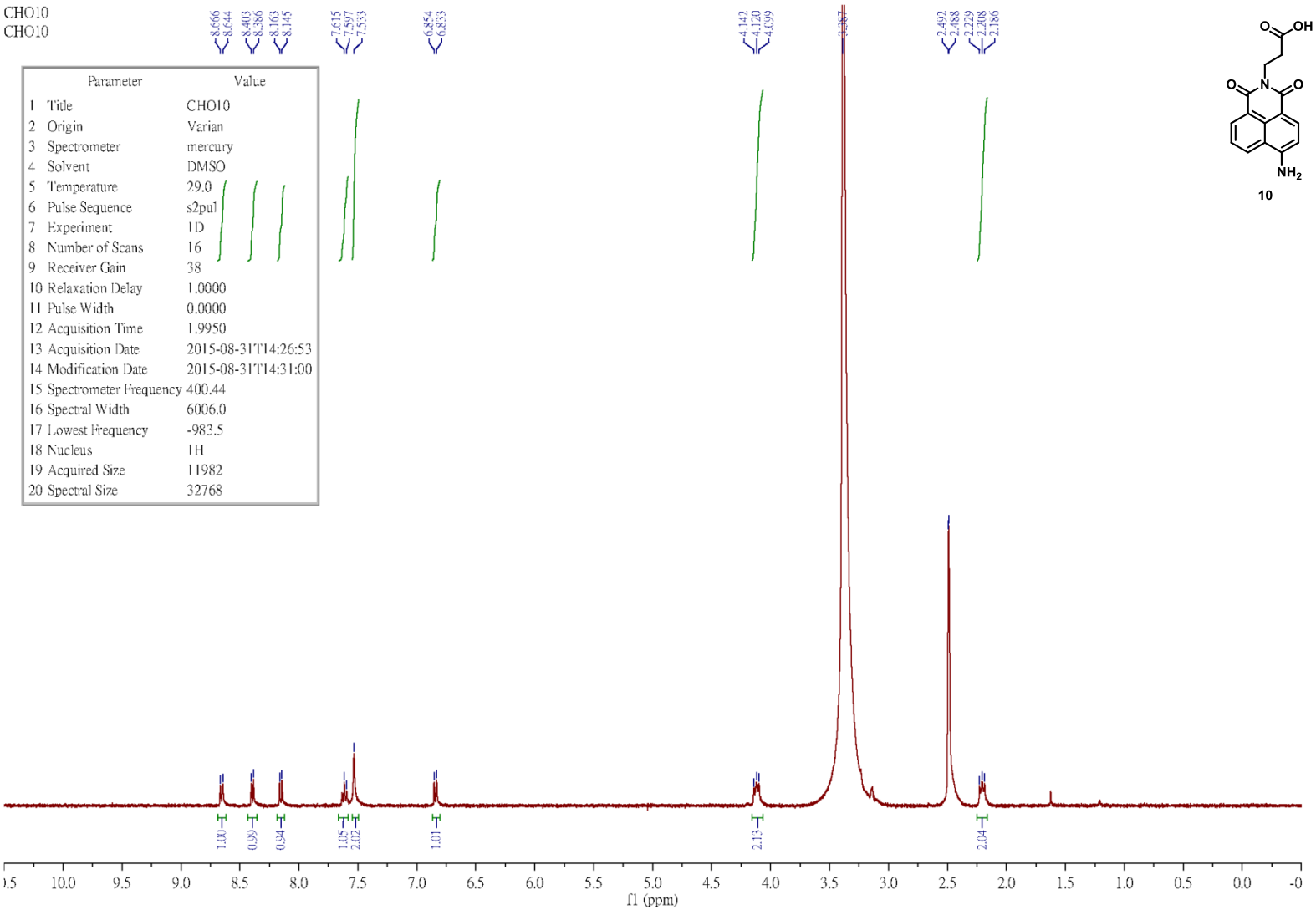


CHO03
CHO03

	Parameter	Value
1	Title	CHO03
2	Origin	Varian
3	Spectrometer	mercury
4	Solvent	DMSO
5	Temperature	29.0
6	Pulse Sequence	s2pul
7	Experiment	1D
8	Number of Scans	12
9	Receiver Gain	32
10	Relaxation Delay	1.0000
11	Pulse Width	0.0000
12	Acquisition Time	1.9950
13	Acquisition Date	2015-07-27T13:24:50
14	Modification Date	2015-07-27T13:26:00
15	Spectrometer Frequency	400.44
16	Spectral Width	6006.0
17	Lowest Frequency	-977.2
18	Nucleus	1H
19	Acquired Size	11982
20	Spectral Size	32768



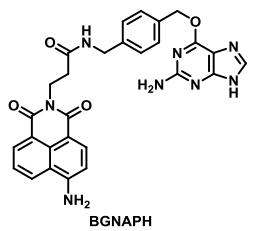

cho09
cho09
8.615
8.594
8.421
8.403
8.187
8.166
7.662
7.641
7.623
7.456
6.841
6.820
4.258
4.239
4.220
4.043
4.025
4.007
3.990
3.344
3.320
2.610
2.592
2.575
2.494
2.490
2.486
1.119
1.101
1.083
Parameter Value
1 Title cho09
2 Origin Varian
3 Spectrometer mercury
4 Solvent DMSO
5 Temperature 29.0
6 Pulse Sequence s2pul
7 Experiment 1D
8 Number of Scans 16
9 Receiver Gain 39
10 Relaxation Delay 1.0000
11 Pulse Width 0.0000
12 Acquisition Time 1.9950
13 Acquisition Date 2015-09-09T17:13:35
14 Modification Date 2015-09-09T17:15:00
15 Spectrometer Frequency 400.44
16 Spectral Width 6006.0
17 Lowest Frequency -983.9
18 Nucleus 1H
19 Acquired Size 11982
20 Spectral Size 32768
O
OEt
N
O
O
NH2
9
1.00
1.00
1.00
1.04
1.99
1.01
1.92
1.91
2.27
3.01
f1 (ppm)

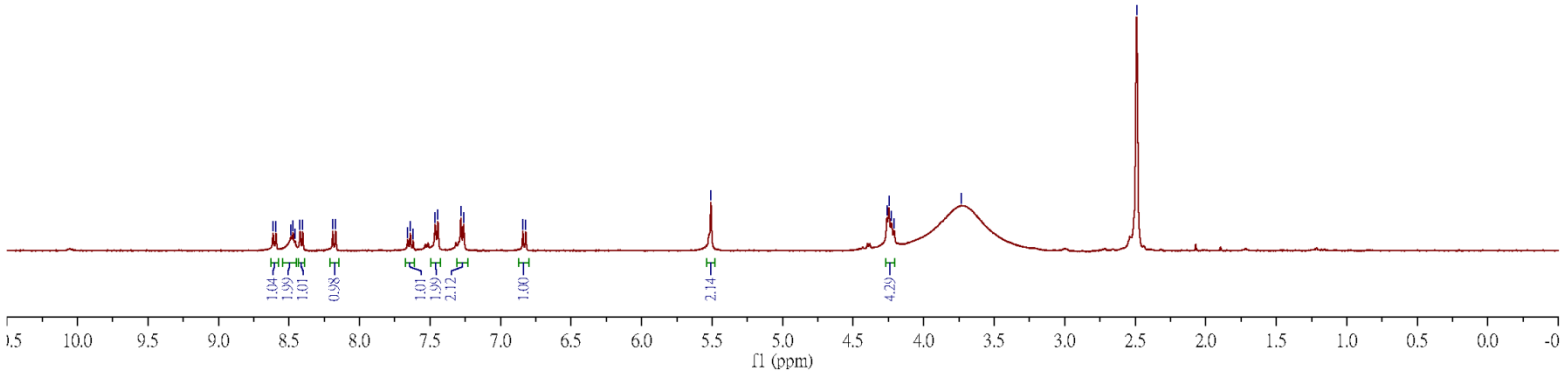

CHO10
CHO10
8.666
8.644
8.403
8.386
8.163
8.145
7.615
7.597
7.533
6.854
6.833
4.142
4.120
4.099
3.387
2.492
2.488
2.229
2.208
2.186
Parameter Value
1 Title CHO10
2 Origin Varian
3 Spectrometer mercury
4 Solvent DMSO
5 Temperature 29.0
6 Pulse Sequence s2pul
7 Experiment 1D
8 Number of Scans 16
9 Receiver Gain 38
10 Relaxation Delay 1.0000
11 Pulse Width 0.0000
12 Acquisition Time 1.9950
13 Acquisition Date 2015-08-31T14:26:53
14 Modification Date 2015-08-31T14:31:00
15 Spectrometer Frequency 400.44
16 Spectral Width 6006.0
17 Lowest Frequency -983.5
18 Nucleus 1H
19 Acquired Size 11982
20 Spectral Size 32768
1.00
0.99
0.94
1.05
2.02
1.01
2.13
2.04
f1 (ppm)
10

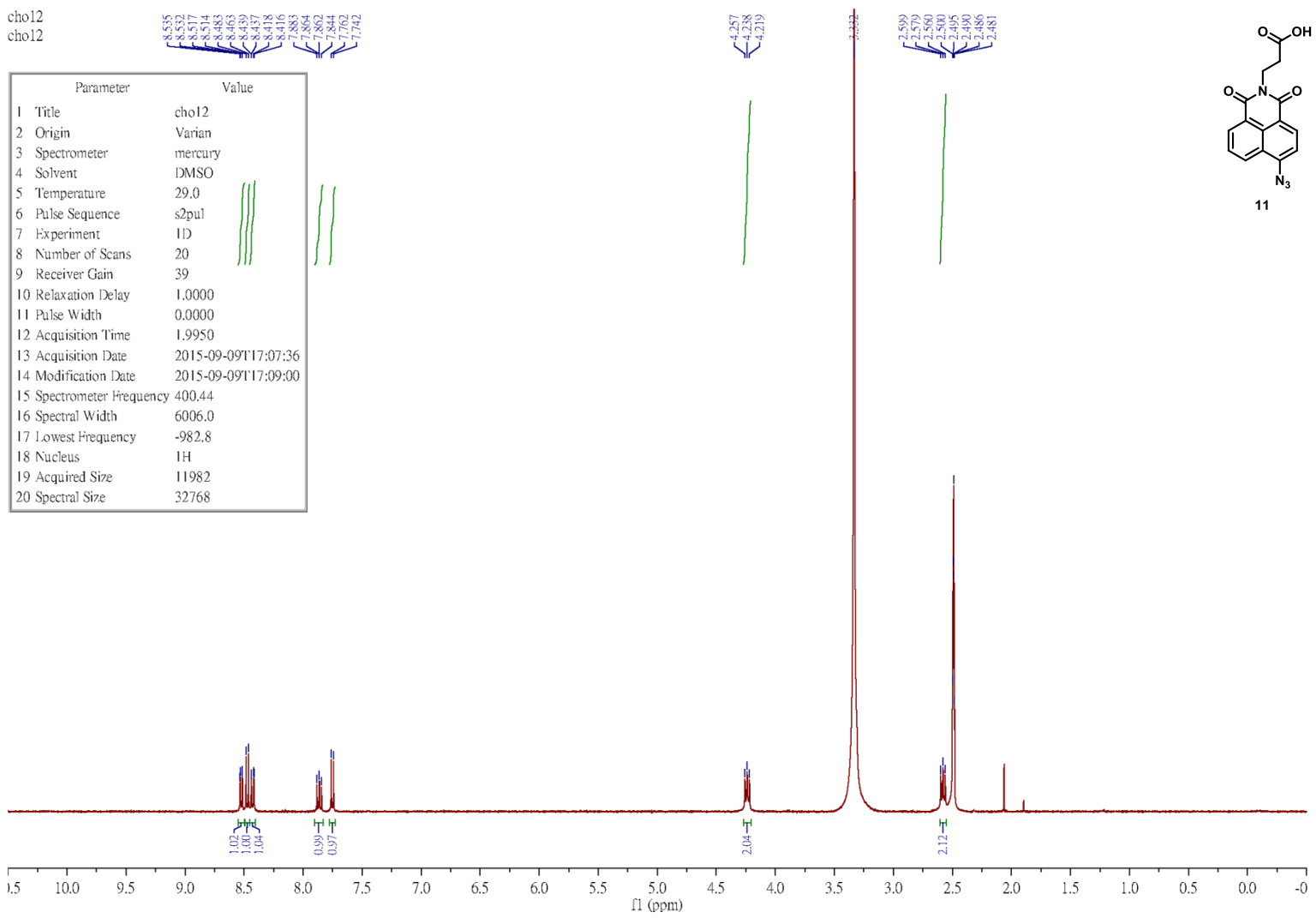
160128-DMSO-CHO11-H
160128-DMSO-CHO11-H

8.613 8.592 8.486 8.472 8.457 8.421 8.403 8.189 8.169 7.660 7.639 7.619 7.464 7.444 7.282 7.262 6.842 6.821 5.508 4.260 4.247 4.230 4.210 3.734 2.490

Parameter	Value
1 Title	CHO11-H
2 Comment	CHO11-H
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	42
11 Receiver Gain	4
12 Relaxation Delay	2.0000
13 Pulse Width	10.0000
14 Acquisition Time	2.5559
15 Acquisition Date	2016-01-28T13:49:17
16 Modification Date	2016-01-28T13:52:22
17 Spectrometer Frequency	400.15
18 Spectral Width	6410.3
19 Lowest Frequency	-410.9
20 Nucleus	1H
21 Acquired Size	16384
22 Spectral Size	32768

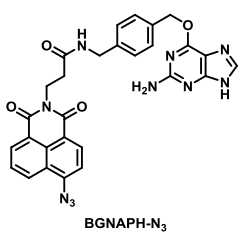


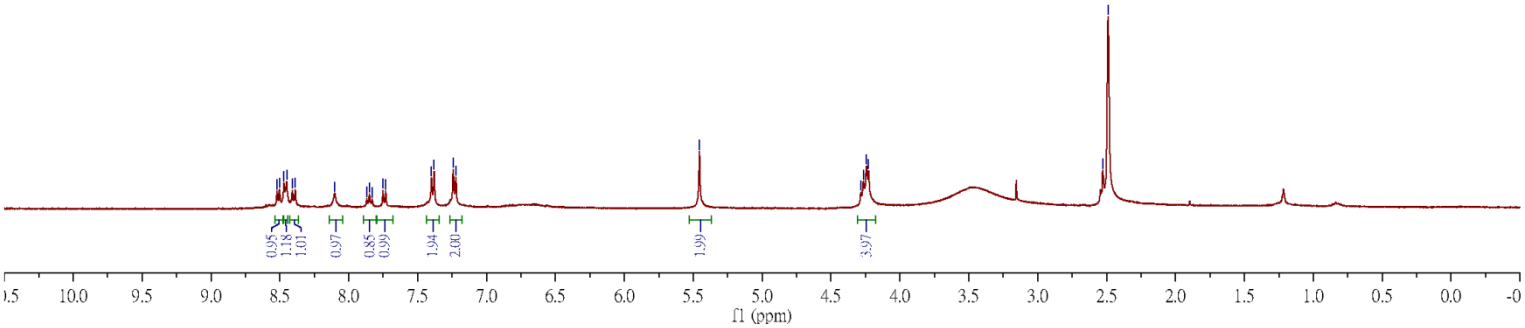


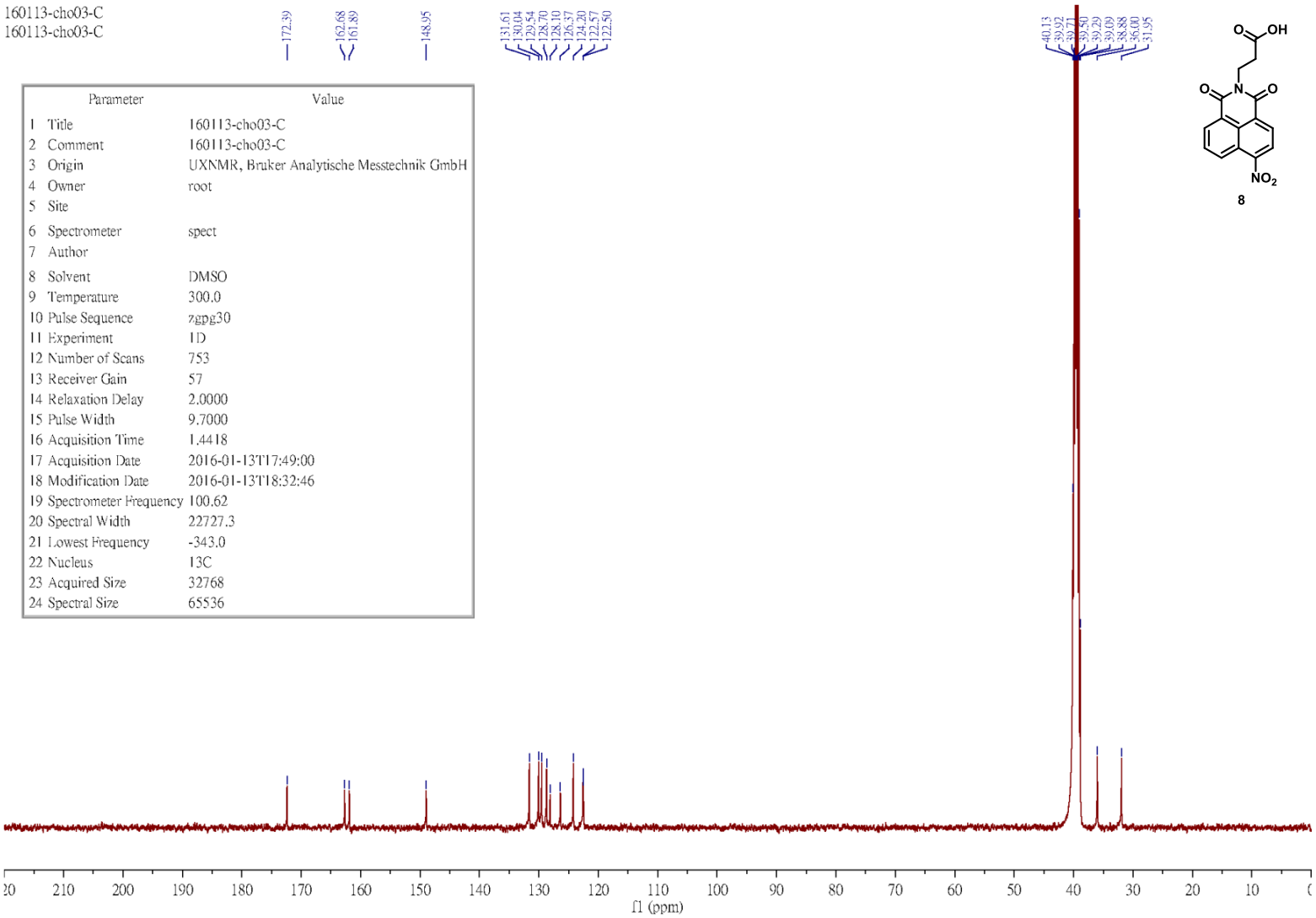

cho12
cho12
8.535
8.532
8.517
8.514
8.483
8.463
8.439
8.437
8.418
8.416
7.883
7.864
7.862
7.844
7.762
7.742
4.257
4.238
4.219
2.332
2.599
2.579
2.560
2.500
2.495
2.490
2.486
2.481
Parameter Value
1 Title cho12
2 Origin Varian
3 Spectrometer mercury
4 Solvent DMSO
5 Temperature 29.0
6 Pulse Sequence s2pul
7 Experiment 1D
8 Number of Scans 20
9 Receiver Gain 39
10 Relaxation Delay 1.0000
11 Pulse Width 0.0000
12 Acquisition Time 1.9950
13 Acquisition Date 2015-09-09T17:07:36
14 Modification Date 2015-09-09T17:09:00
15 Spectrometer Frequency 400.44
16 Spectral Width 6006.0
17 Lowest Frequency -982.8
18 Nucleus 1H
19 Acquired Size 11982
20 Spectral Size 32768
O
OH
O
N
O
N3
11
1.02
1.00
1.04
0.99
0.97
2.04
2.12
f1 (ppm)

CHO13-2
CHO13-2

Parameter	Value
1 Title	CHO13-2
2 Comment	CHO13-2
3 Origin	Varian
4 Owner	
5 Site	
6 Spectrometer	mercury
7 Author	
8 Solvent	DMSO
9 Temperature	29.0
10 Pulse Sequence	s2pul
11 Experiment	1D
12 Number of Scans	20
13 Receiver Gain	39
14 Relaxation Delay	1.0000
15 Pulse Width	0.0000
16 Acquisition Time	1.9950
17 Acquisition Date	2015-10-15T14:45:27
18 Modification Date	2015-10-15T14:46:00
19 Spectrometer Frequency	400.44
20 Spectral Width	6006.0
21 Lowest Frequency	-982.8
22 Nucleus	1H
23 Acquired Size	11982
24 Spectral Size	32768





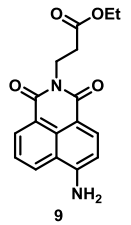


Parameter	Value
1 Title	160113-cho03-C
2 Comment	160113-cho03-C
3 Origin	UXNMR, Bruker Analytische Messtechnik GmbH
4 Owner	root
5 Site	
6 Spectrometer	spect
7 Author	
8 Solvent	DMSO
9 Temperature	300.0
10 Pulse Sequence	zgpg30
11 Experiment	1D
12 Number of Scans	753
13 Receiver Gain	57
14 Relaxation Delay	2.0000
15 Pulse Width	9.7000
16 Acquisition Time	1.4418
17 Acquisition Date	2016-01-13T17:49:00
18 Modification Date	2016-01-13T18:32:46
19 Spectrometer Frequency	100.62
20 Spectral Width	22727.3
21 Lowest Frequency	-343.0
22 Nucleus	13C
23 Acquired Size	32768
24 Spectral Size	65536

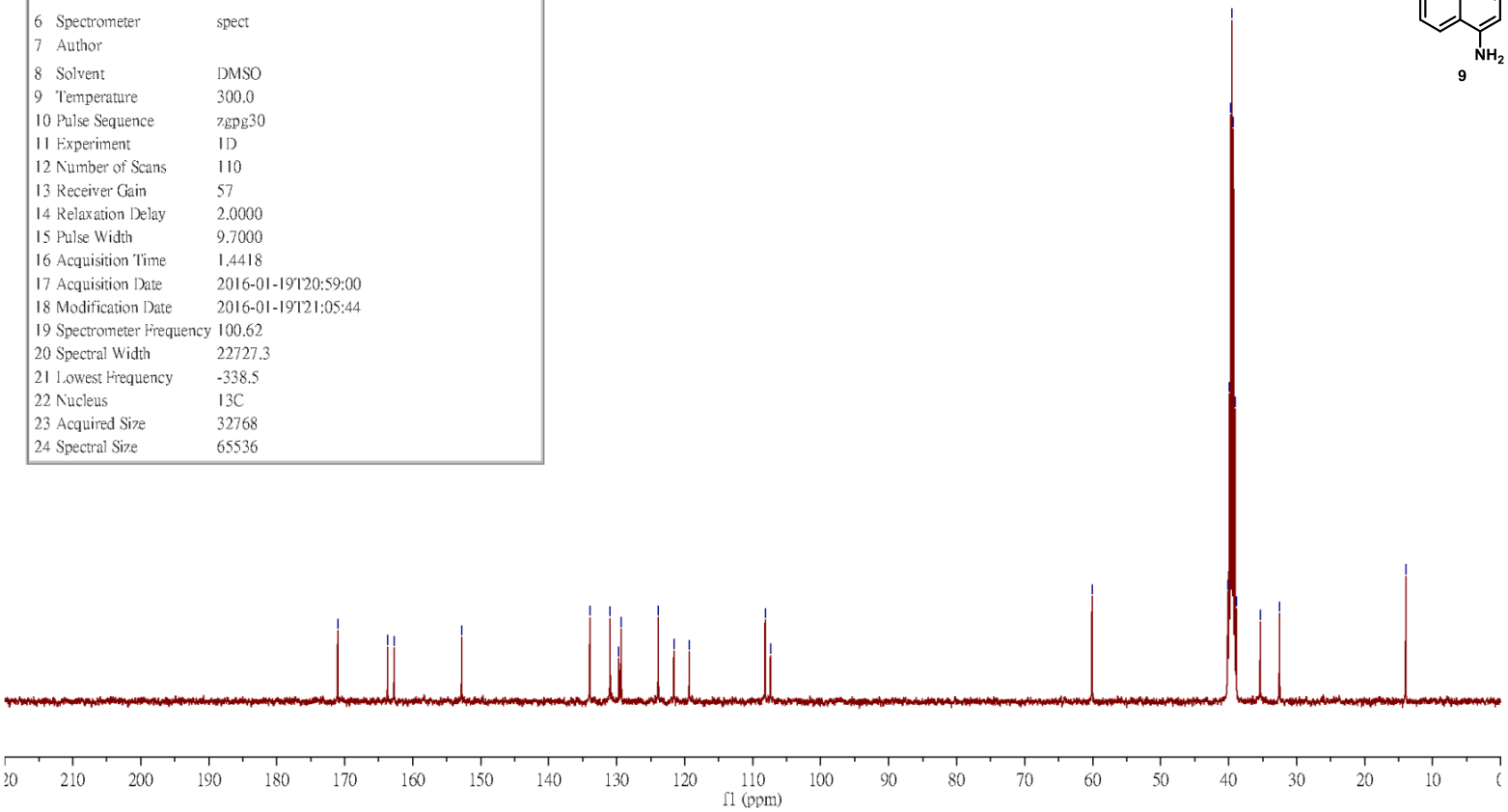
160119-CHO09-C
160119-CHO09-C

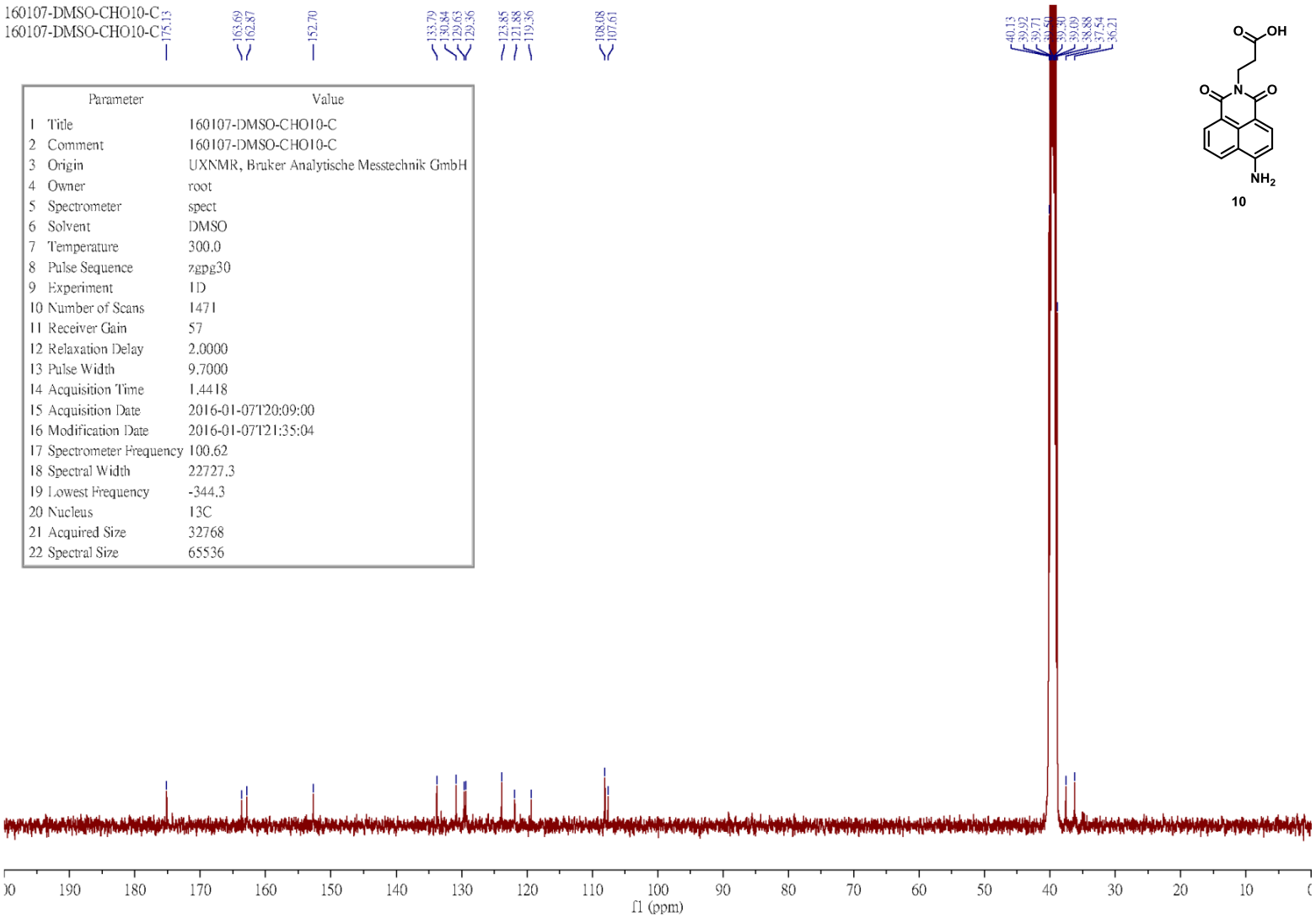
171.02
163.69
162.73
152.80
133.94
130.98
129.69
129.36
123.88
121.59
119.32
108.17
107.39
60.10
40.13
39.92
39.71
39.50
39.29
39.09
38.88
55.36
32.52
13.96

Parameter	Value
1 Title	160119-CHO09-C
2 Comment	160119-CHO09-C
3 Origin	UXNMR, Bruker Analytische Messtechnik GmbH
4 Owner	root
5 Site	
6 Spectrometer	spect
7 Author	
8 Solvent	DMSO
9 Temperature	300.0
10 Pulse Sequence	zgpg30
11 Experiment	1D
12 Number of Scans	110
13 Receiver Gain	57
14 Relaxation Delay	2.0000
15 Pulse Width	9.7000
16 Acquisition Time	1.4418
17 Acquisition Date	2016-01-19T20:59:00
18 Modification Date	2016-01-19T21:05:44
19 Spectrometer Frequency	100.62
20 Spectral Width	22727.3
21 Lowest Frequency	-338.5
22 Nucleus	13C
23 Acquired Size	32768
24 Spectral Size	65536

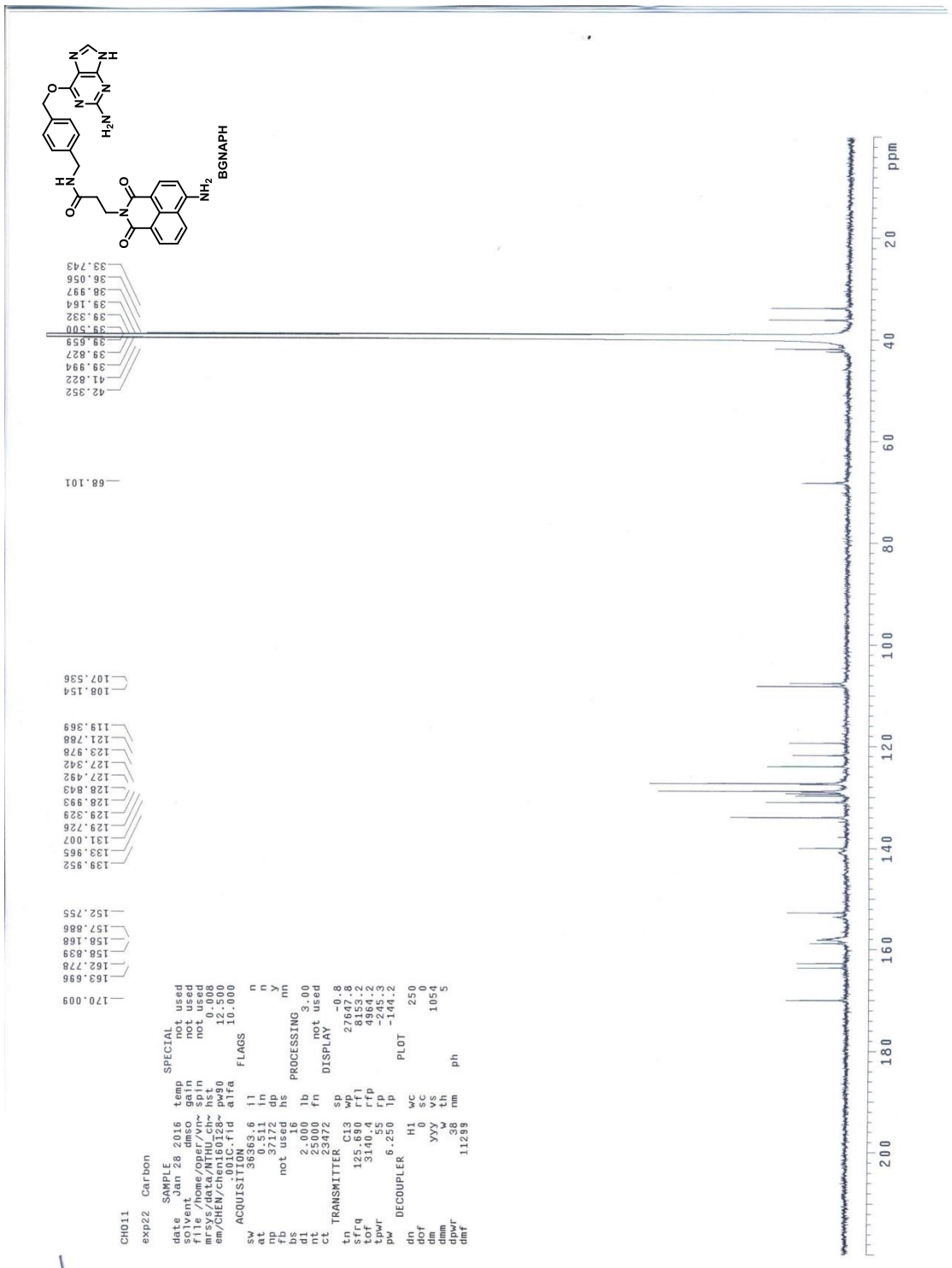


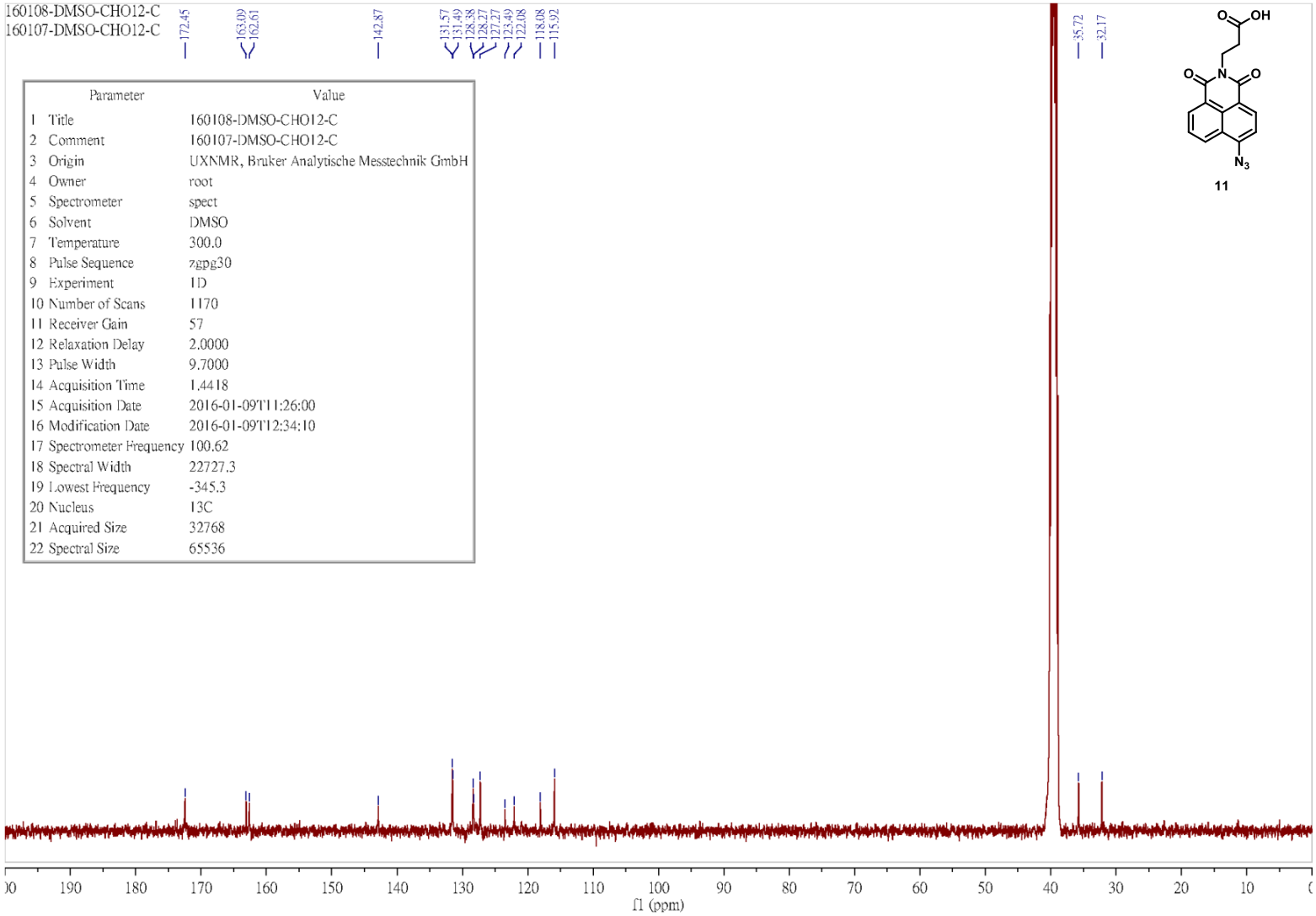
9



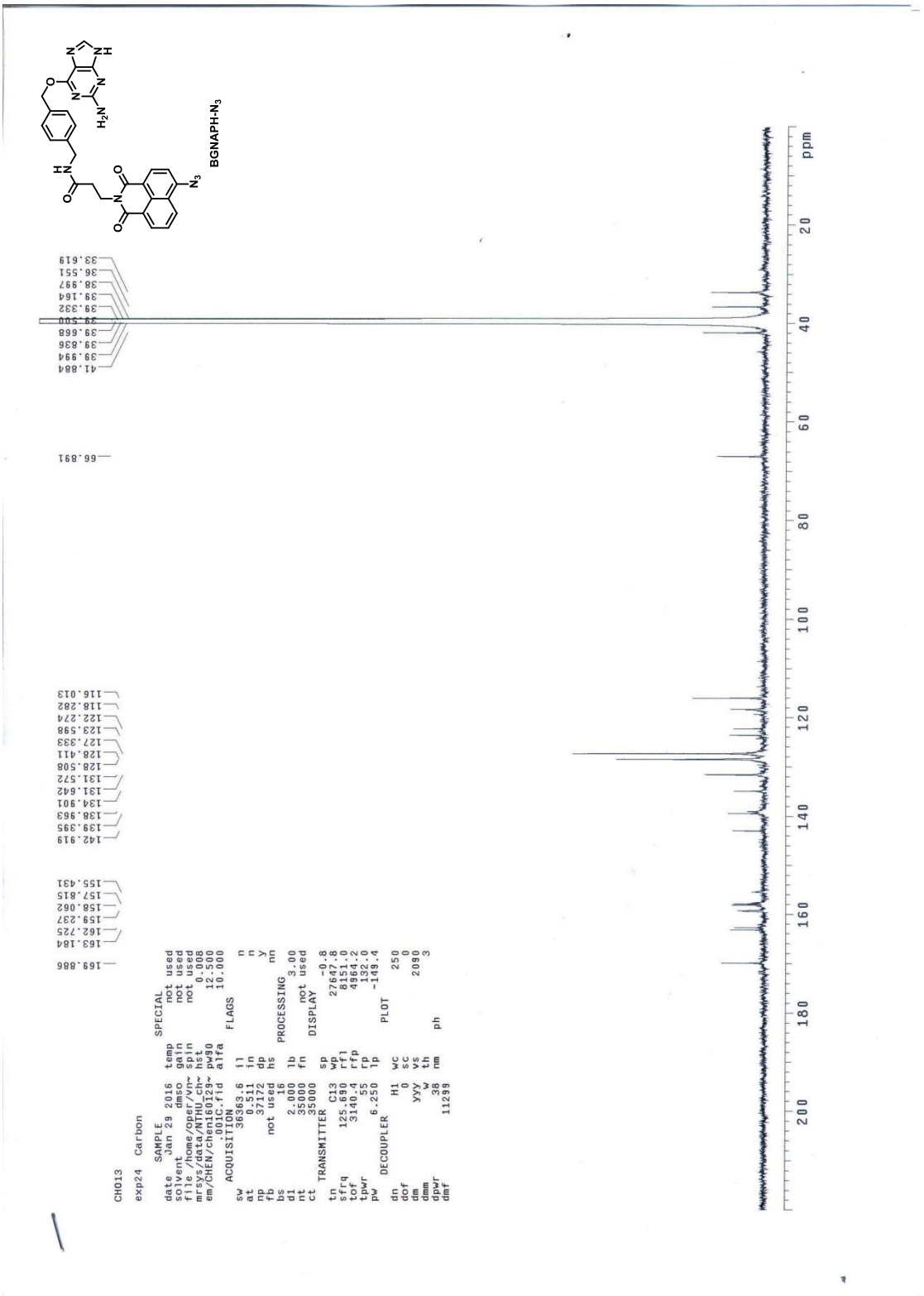


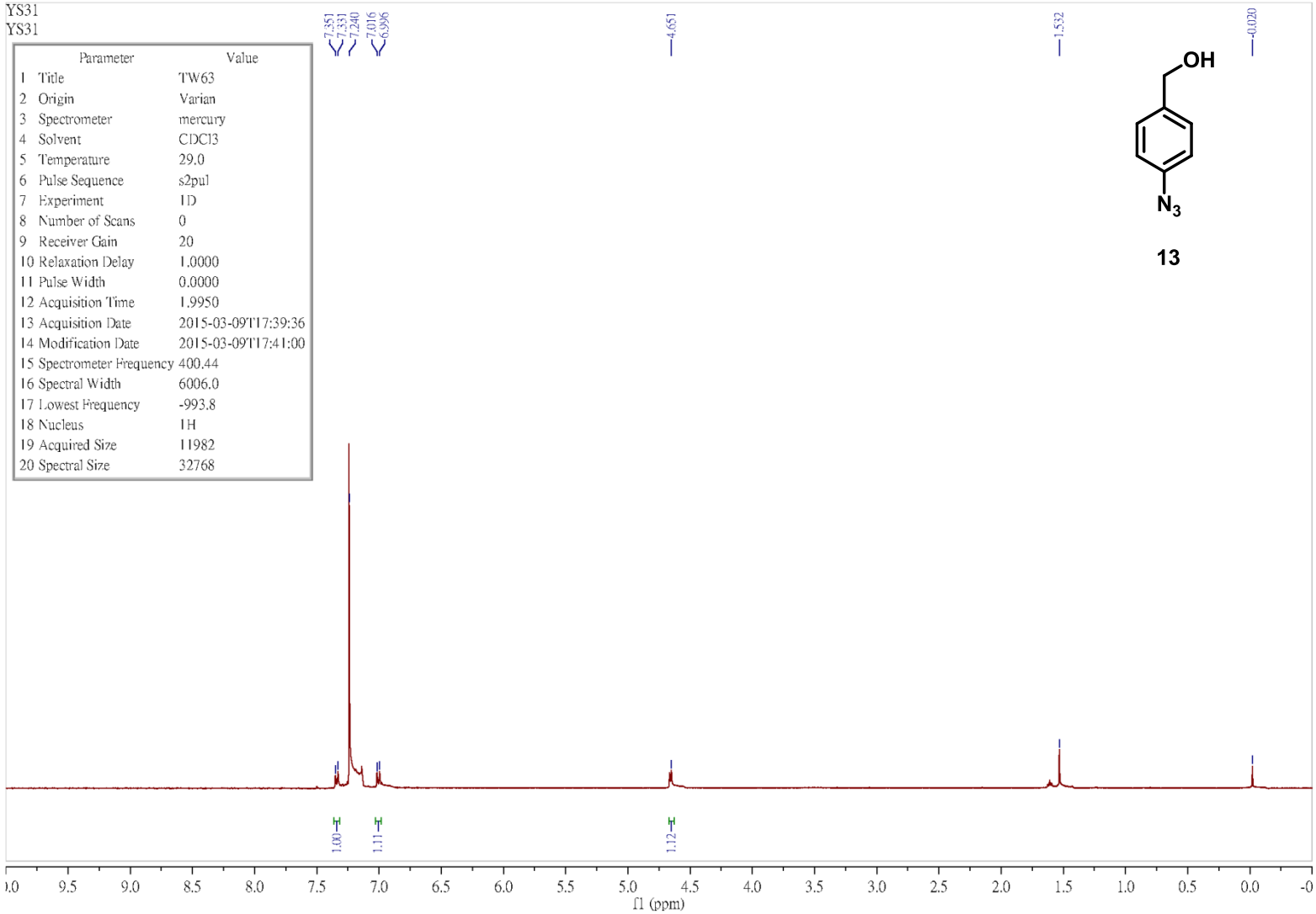
Parameter	Value
1 Title	160107-DMSO-CHO10-C
2 Comment	160107-DMSO-CHO10-C
3 Origin	UXNMR, Bruker Analytische Messtechnik GmbH
4 Owner	root
5 Spectrometer	spect
6 Solvent	DMSO
7 Temperature	300.0
8 Pulse Sequence	zgpg30
9 Experiment	1D
10 Number of Scans	1471
11 Receiver Gain	57
12 Relaxation Delay	2.0000
13 Pulse Width	9.7000
14 Acquisition Time	1.4418
15 Acquisition Date	2016-01-07T20:09:00
16 Modification Date	2016-01-07T21:35:04
17 Spectrometer Frequency	100.62
18 Spectral Width	22727.3
19 Lowest Frequency	-344.3
20 Nucleus	13C
21 Acquired Size	32768
22 Spectral Size	65536


BGNAPH
CHO11
exp22 Carbon
SAMPLE
date Jan 28 2016
solvent dmso
file /home/oper/vnmrsys/data/NTHU_ch-em/CHEN/chen160128-.001C.fid
ACQUISITION
sw 36363.6
at 0.511
np 37172
fb not used
bs 16
d1 2.000
nt 25000
ct 23472
TRANSMITTER
tn C13
sfrq 125.690
tof 3140.4
tpwr 55
pw 6.250
DECOUPLER
dn H1
dof 0
dm yyy
dmm w
dpwr 38
dmf 11299
temp not used
gain not used
spin not used
hst 0.000
pw90 12.500
alfa 10.000
FLAGS
il n
in n
dp y
hs nn
PROCESSING
lb 3.00
fn not used
DISPLAY
sp -0.8
wp 27647.8
rf1 8153.2
rfp 4964.2
rp -245.3
lp -144.2
PLOT
wc 250
sc 0
vs 1054
th 5
ph
nm
170.009
163.696
162.778
158.839
158.168
157.886
152.755
139.952
133.965
131.007
129.726
129.329
128.993
128.843
127.492
127.342
123.978
121.788
119.369
108.154
107.536
68.101
42.352
41.822
39.994
39.827
39.659
39.500
39.332
39.164
38.997
36.056
33.743
200
180
160
140
120
100
80
60
40
20
ppm



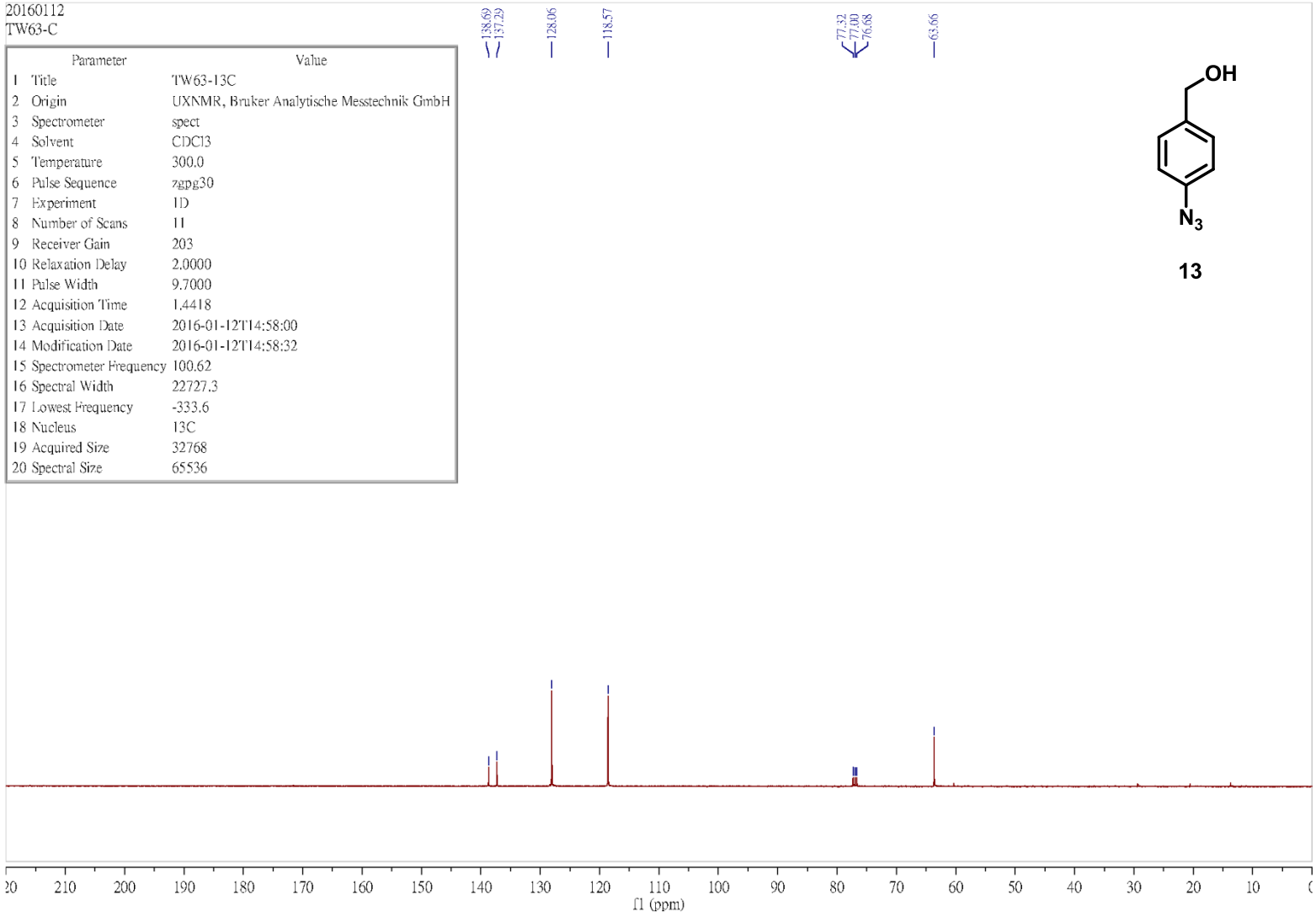
Parameter	Value
1 Title	160108-DMSO-CHO12-C
2 Comment	160107-DMSO-CHO12-C
3 Origin	UXNMR, Bruker Analytische Messtechnik GmbH
4 Owner	root
5 Spectrometer	spect
6 Solvent	DMSO
7 Temperature	300.0
8 Pulse Sequence	zgpg30
9 Experiment	1D
10 Number of Scans	1170
11 Receiver Gain	57
12 Relaxation Delay	2.0000
13 Pulse Width	9.7000
14 Acquisition Time	1.4418
15 Acquisition Date	2016-01-09T11:26:00
16 Modification Date	2016-01-09T12:34:10
17 Spectrometer Frequency	100.62
18 Spectral Width	22727.3
19 Lowest Frequency	-345.3
20 Nucleus	13C
21 Acquired Size	32768
22 Spectral Size	65536

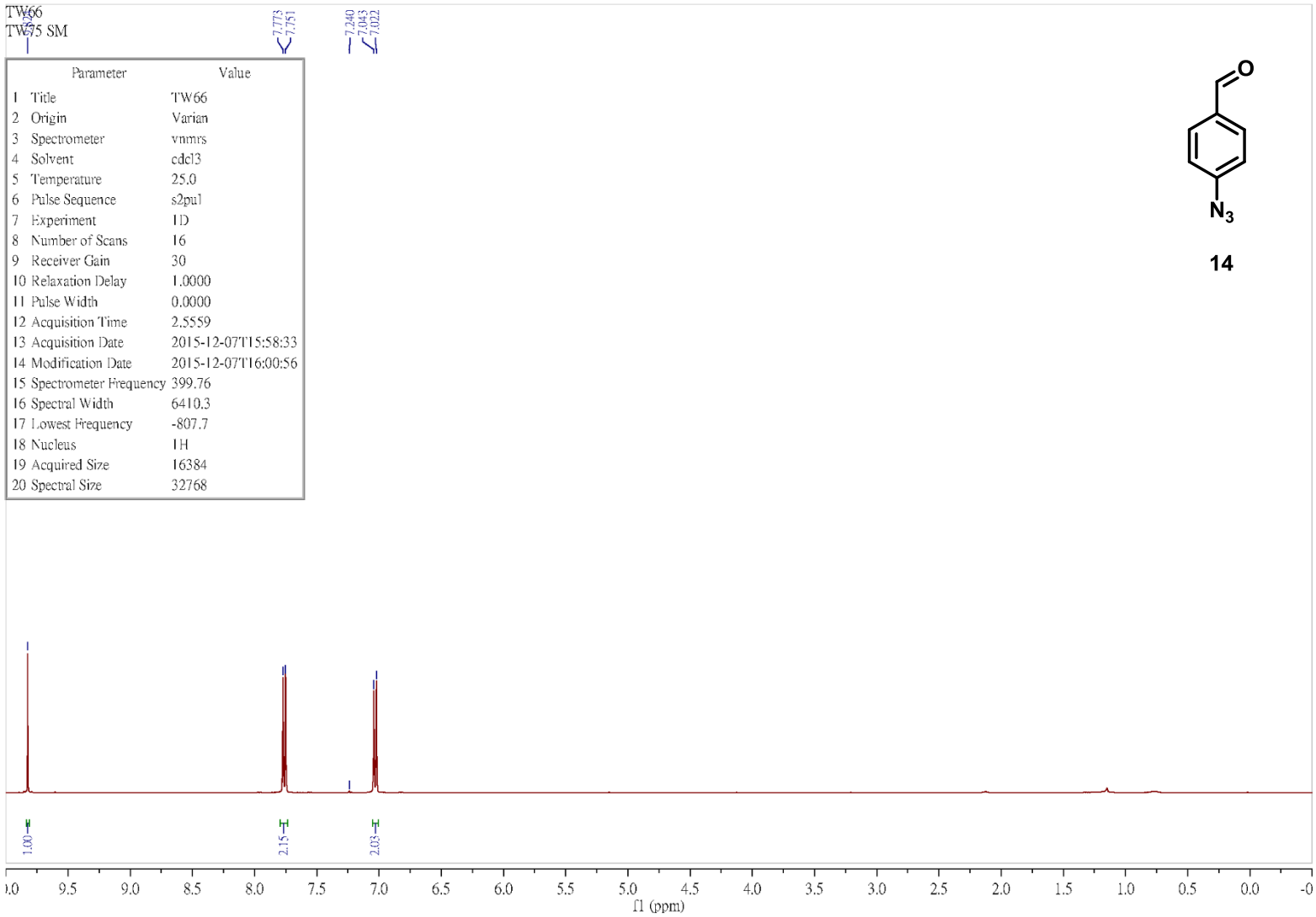

BGNAPH-N3
ppm

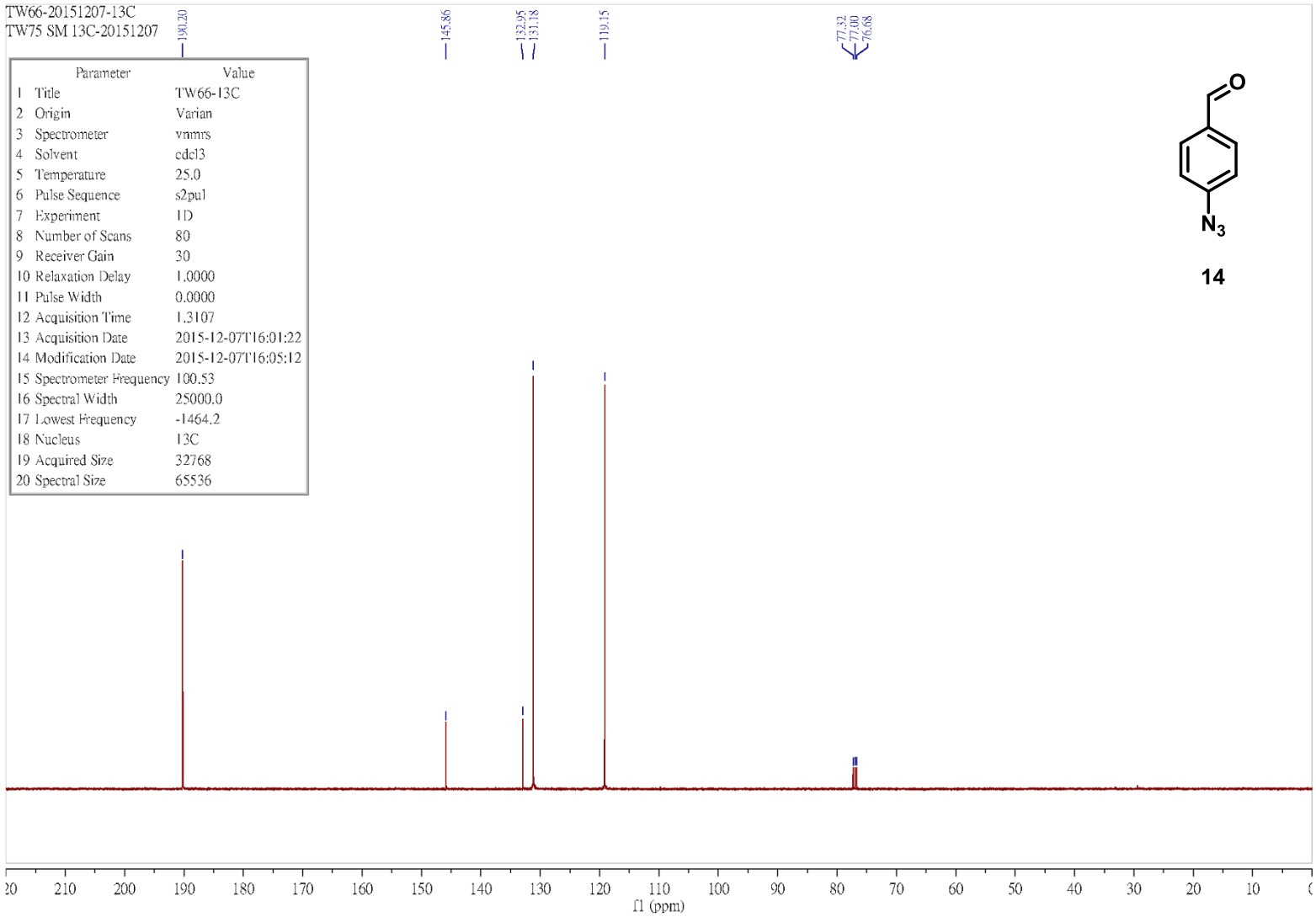

YS31
YS31
Parameter Value
1 Title TW63
2 Origin Varian
3 Spectrometer mercury
4 Solvent CDCl3
5 Temperature 29.0
6 Pulse Sequence s2pul
7 Experiment 1D
8 Number of Scans 0
9 Receiver Gain 20
10 Relaxation Delay 1.0000
11 Pulse Width 0.0000
12 Acquisition Time 1.9950
13 Acquisition Date 2015-03-09T17:39:36
14 Modification Date 2015-03-09T17:41:00
15 Spectrometer Frequency 400.44
16 Spectral Width 6006.0
17 Lowest Frequency -993.8
18 Nucleus 1H
19 Acquired Size 11982
20 Spectral Size 32768
7.351
7.331
7.240
7.016
6.996
4.651
1.532
0.020
OH
N3
13
1.00
1.11
1.12
f1 (ppm)

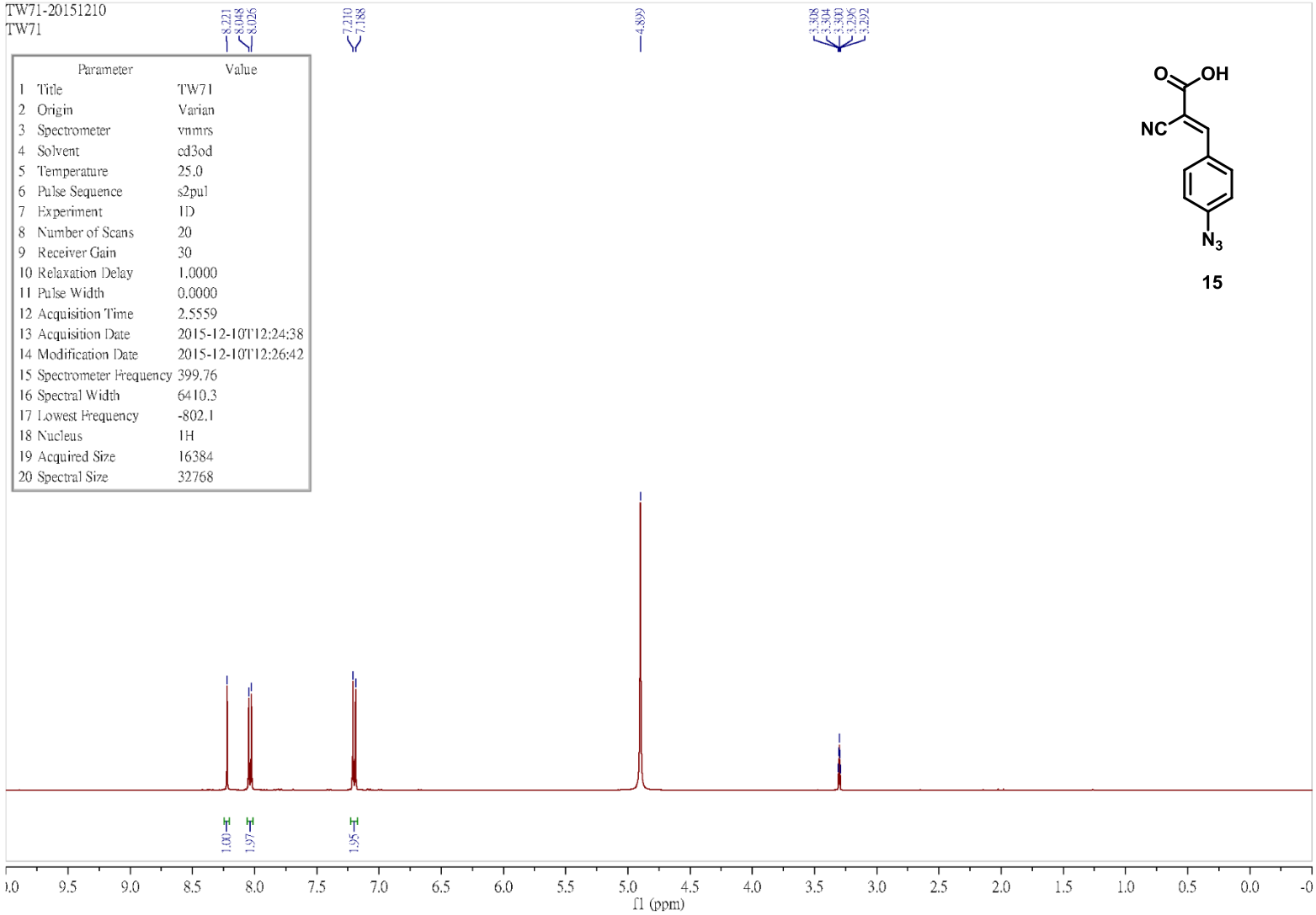
20160112
TW63-C

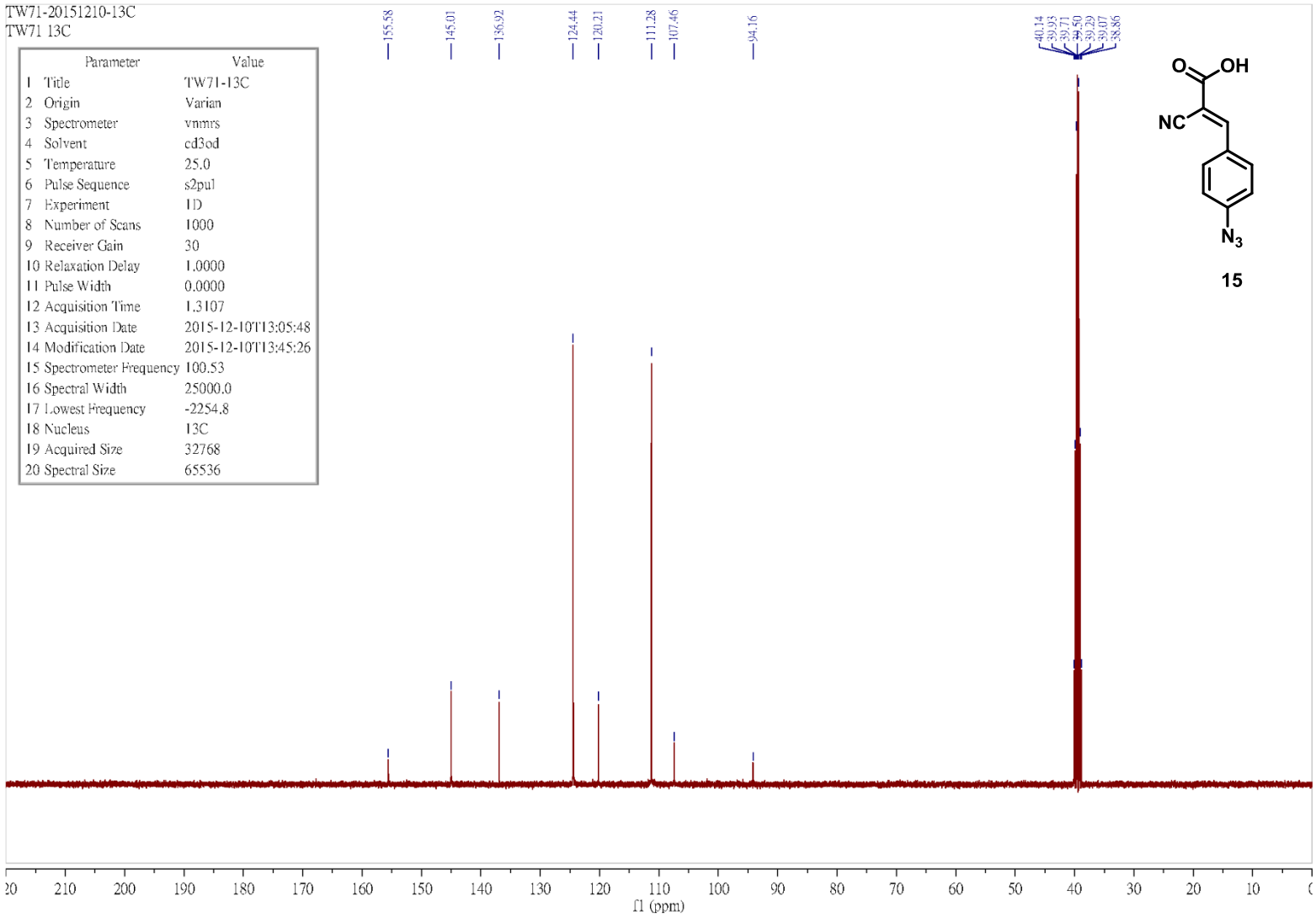
	Parameter	Value
1	Title	TW63-13C
2	Origin	UXNMR, Bruker Analytische Messtechnik GmbH
3	Spectrometer	spect
4	Solvent	CDCl3
5	Temperature	300.0
6	Pulse Sequence	zgpg30
7	Experiment	1D
8	Number of Scans	11
9	Receiver Gain	203
10	Relaxation Delay	2.0000
11	Pulse Width	9.7000
12	Acquisition Time	1.4418
13	Acquisition Date	2016-01-12T14:58:00
14	Modification Date	2016-01-12T14:58:32
15	Spectrometer Frequency	100.62
16	Spectral Width	22727.3
17	Lowest Frequency	-333.6
18	Nucleus	13C
19	Acquired Size	32768
20	Spectral Size	65536

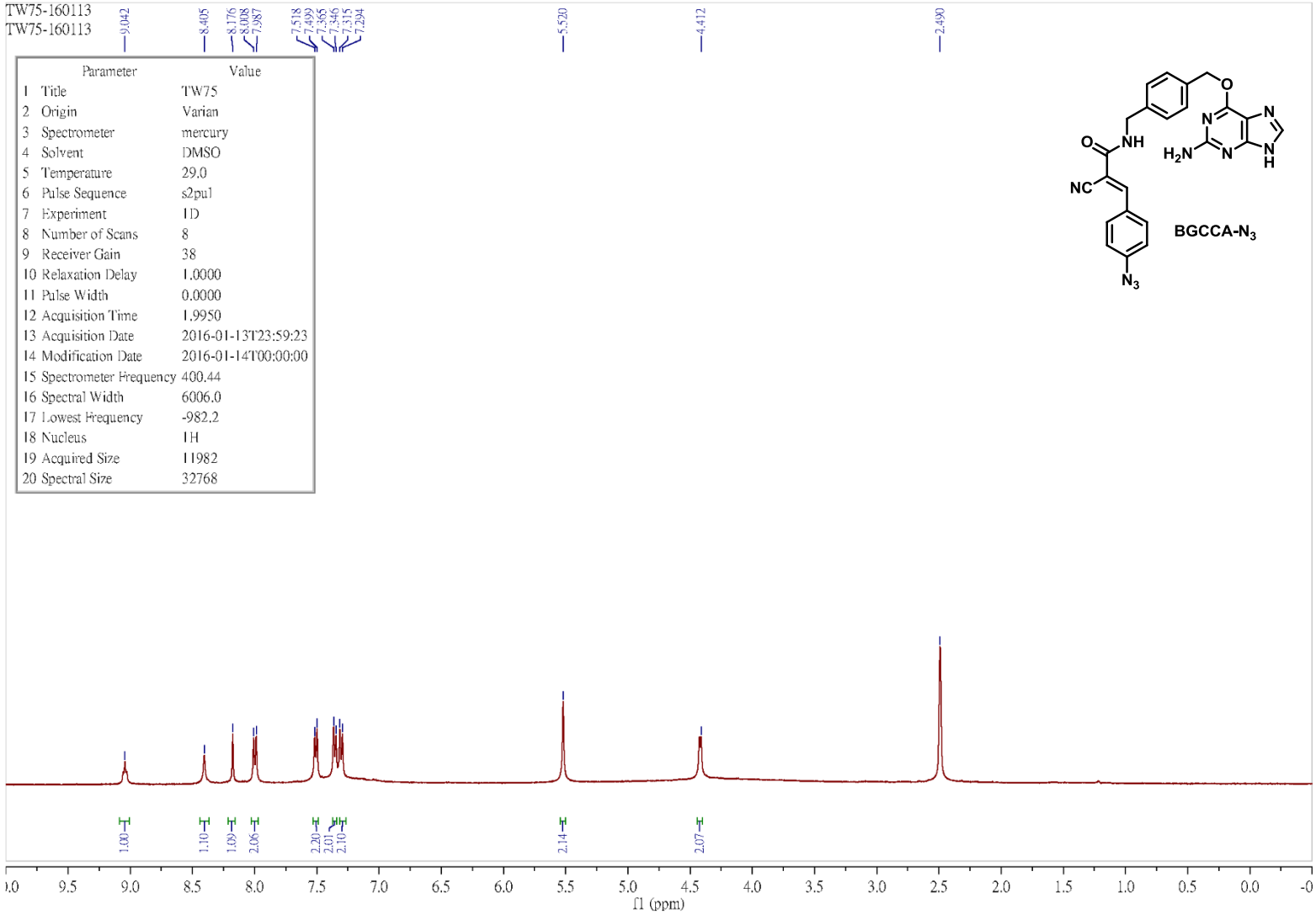


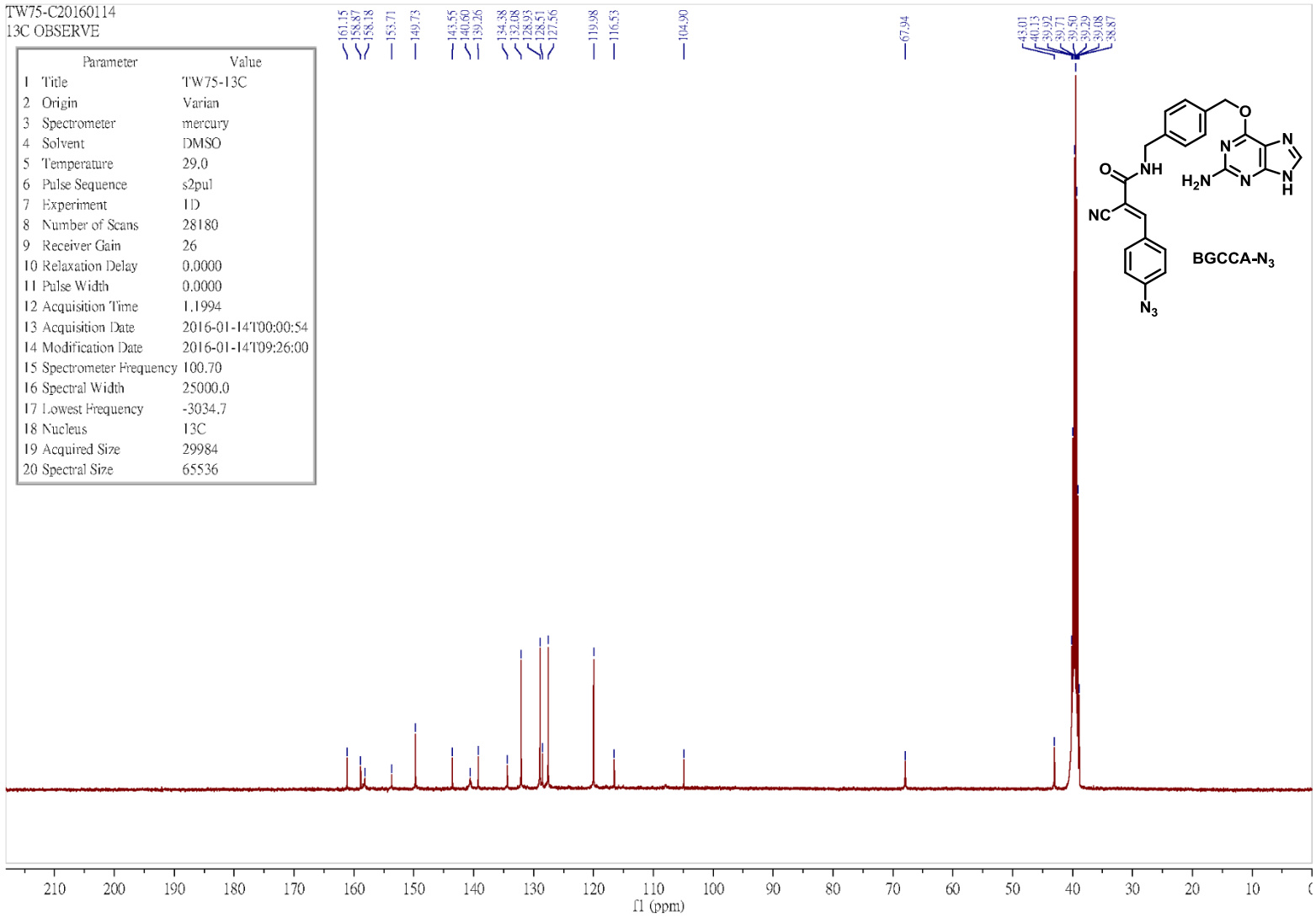

TW66
TW75 SM
7.773
7.751
7.240
7.043
7.022
Parameter Value
1 Title TW66
2 Origin Varian
3 Spectrometer vnmrs
4 Solvent cdcl3
5 Temperature 25.0
6 Pulse Sequence s2pul
7 Experiment 1D
8 Number of Scans 16
9 Receiver Gain 30
10 Relaxation Delay 1.0000
11 Pulse Width 0.0000
12 Acquisition Time 2.5559
13 Acquisition Date 2015-12-07T15:58:33
14 Modification Date 2015-12-07T16:00:56
15 Spectrometer Frequency 399.76
16 Spectral Width 6410.3
17 Lowest Frequency -807.7
18 Nucleus 1H
19 Acquired Size 16384
20 Spectral Size 32768
O
N3
14
1.00
2.15
2.03
f1 (ppm)

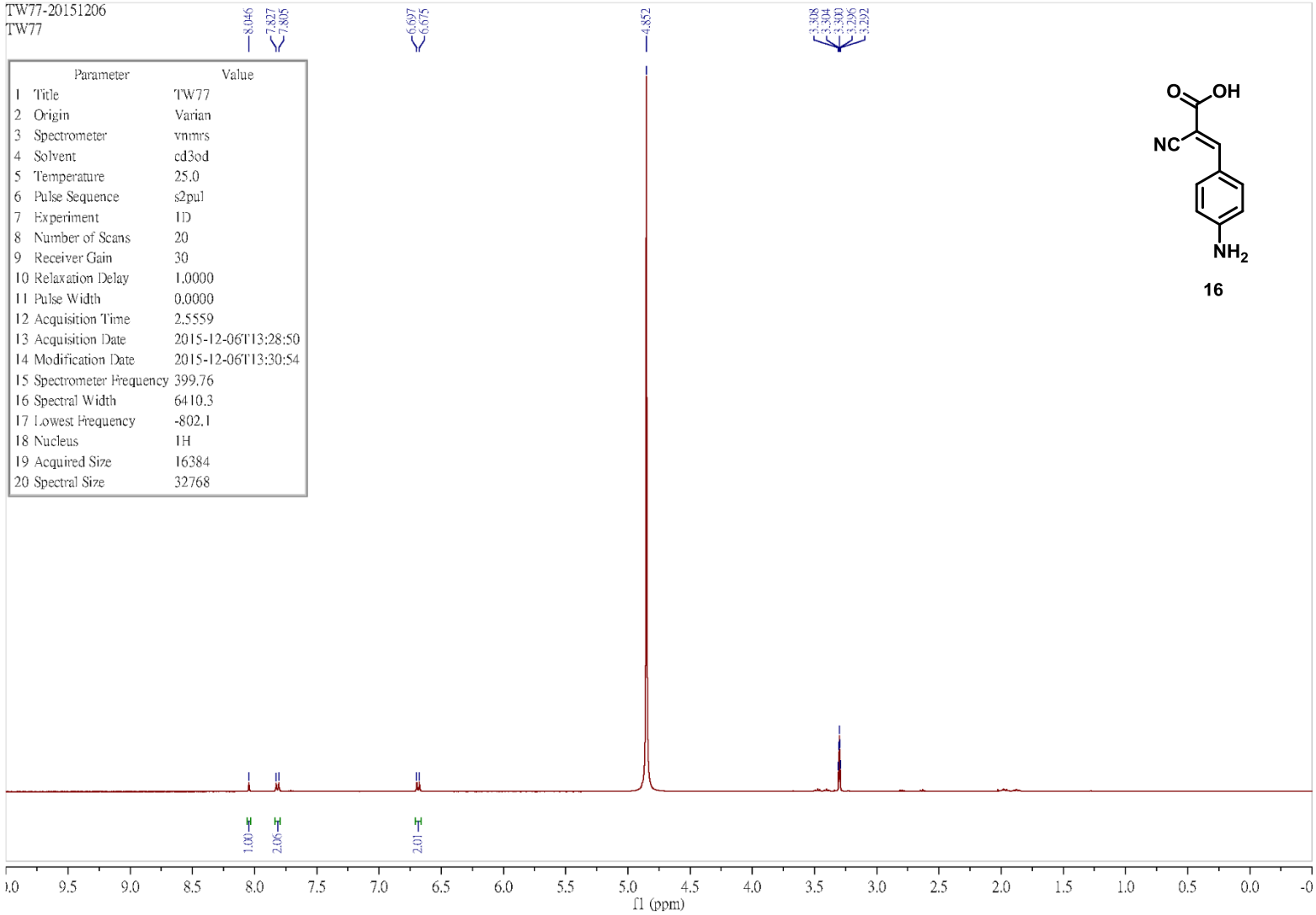

TW66-20151207-13C
TW75 SM 13C-20151207
190.20
145.86
132.95
131.18
119.15
77.32
77.00
76.68
Parameter Value
1 Title TW66-13C
2 Origin Varian
3 Spectrometer vnmrs
4 Solvent cdcl3
5 Temperature 25.0
6 Pulse Sequence s2pul
7 Experiment 1D
8 Number of Scans 80
9 Receiver Gain 30
10 Relaxation Delay 1.0000
11 Pulse Width 0.0000
12 Acquisition Time 1.3107
13 Acquisition Date 2015-12-07T16:01:22
14 Modification Date 2015-12-07T16:05:12
15 Spectrometer Frequency 100.53
16 Spectral Width 25000.0
17 Lowest Frequency -1464.2
18 Nucleus 13C
19 Acquired Size 32768
20 Spectral Size 65536
O
N3
14
f1 (ppm)

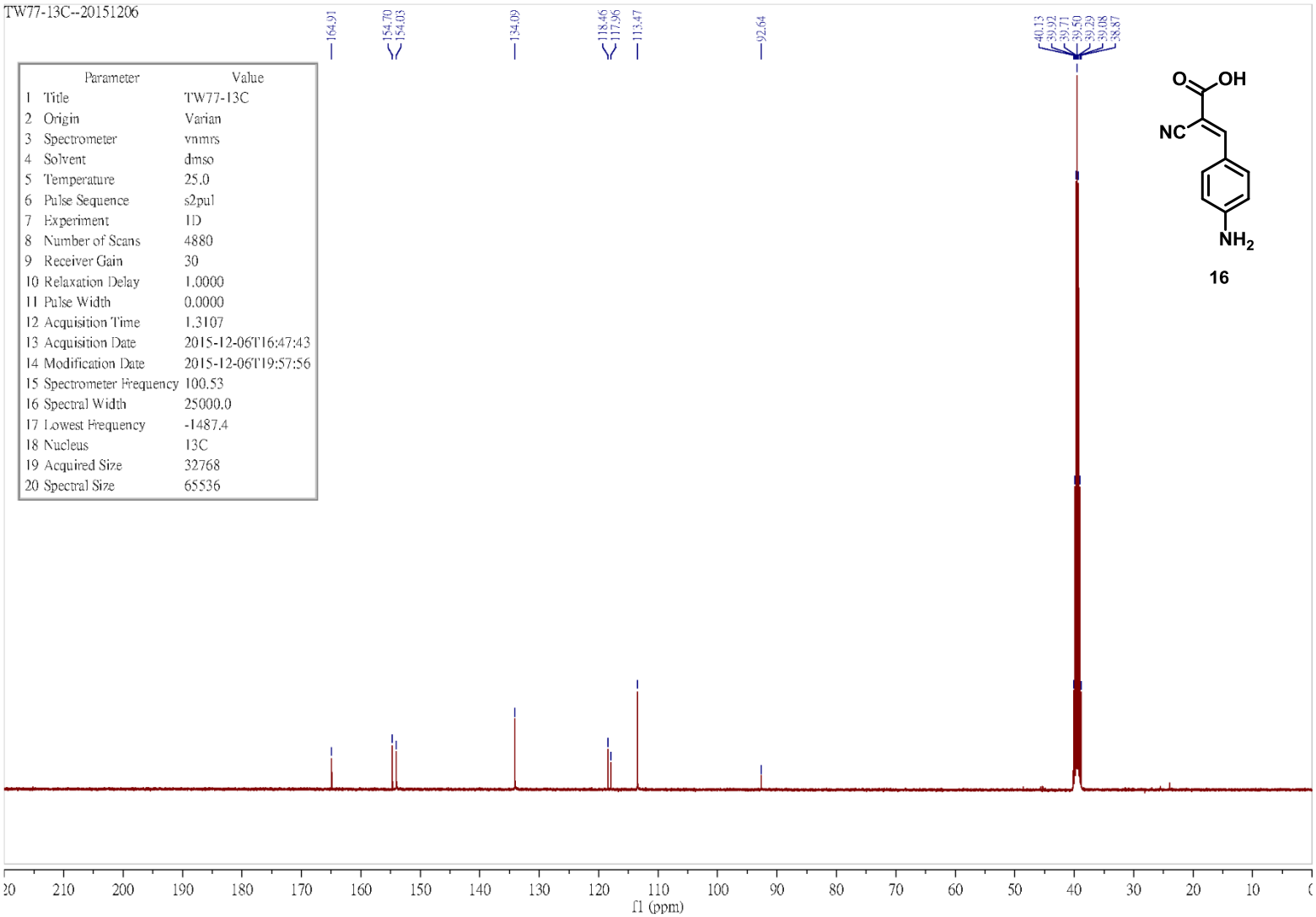

TW71-20151210
TW71
8.221
8.048
8.026
7.210
7.188
4.899
3.308
3.304
3.300
3.296
3.292
Parameter Value
1 Title TW71
2 Origin Varian
3 Spectrometer vnmrs
4 Solvent cd3od
5 Temperature 25.0
6 Pulse Sequence s2pul
7 Experiment 1D
8 Number of Scans 20
9 Receiver Gain 30
10 Relaxation Delay 1.0000
11 Pulse Width 0.0000
12 Acquisition Time 2.5559
13 Acquisition Date 2015-12-10T12:24:38
14 Modification Date 2015-12-10T12:26:42
15 Spectrometer Frequency 399.76
16 Spectral Width 6410.3
17 Lowest Frequency -802.1
18 Nucleus 1H
19 Acquired Size 16384
20 Spectral Size 32768
O
OH
NC
N3
15
1.00
1.97
1.95
f1 (ppm)


TW71-20151210-13C
TW71 13C
Parameter Value
1 Title TW71-13C
2 Origin Varian
3 Spectrometer vnmrs
4 Solvent cd3od
5 Temperature 25.0
6 Pulse Sequence s2pul
7 Experiment 1D
8 Number of Scans 1000
9 Receiver Gain 30
10 Relaxation Delay 1.0000
11 Pulse Width 0.0000
12 Acquisition Time 1.3107
13 Acquisition Date 2015-12-10T13:05:48
14 Modification Date 2015-12-10T13:45:26
15 Spectrometer Frequency 100.53
16 Spectral Width 25000.0
17 Lowest Frequency -2254.8
18 Nucleus 13C
19 Acquired Size 32768
20 Spectral Size 65536
155.58
145.01
136.92
124.44
120.21
111.28
107.46
94.16
40.14
39.93
39.71
39.50
39.29
39.07
38.86
O
OH
NC
N3
15
f1 (ppm)

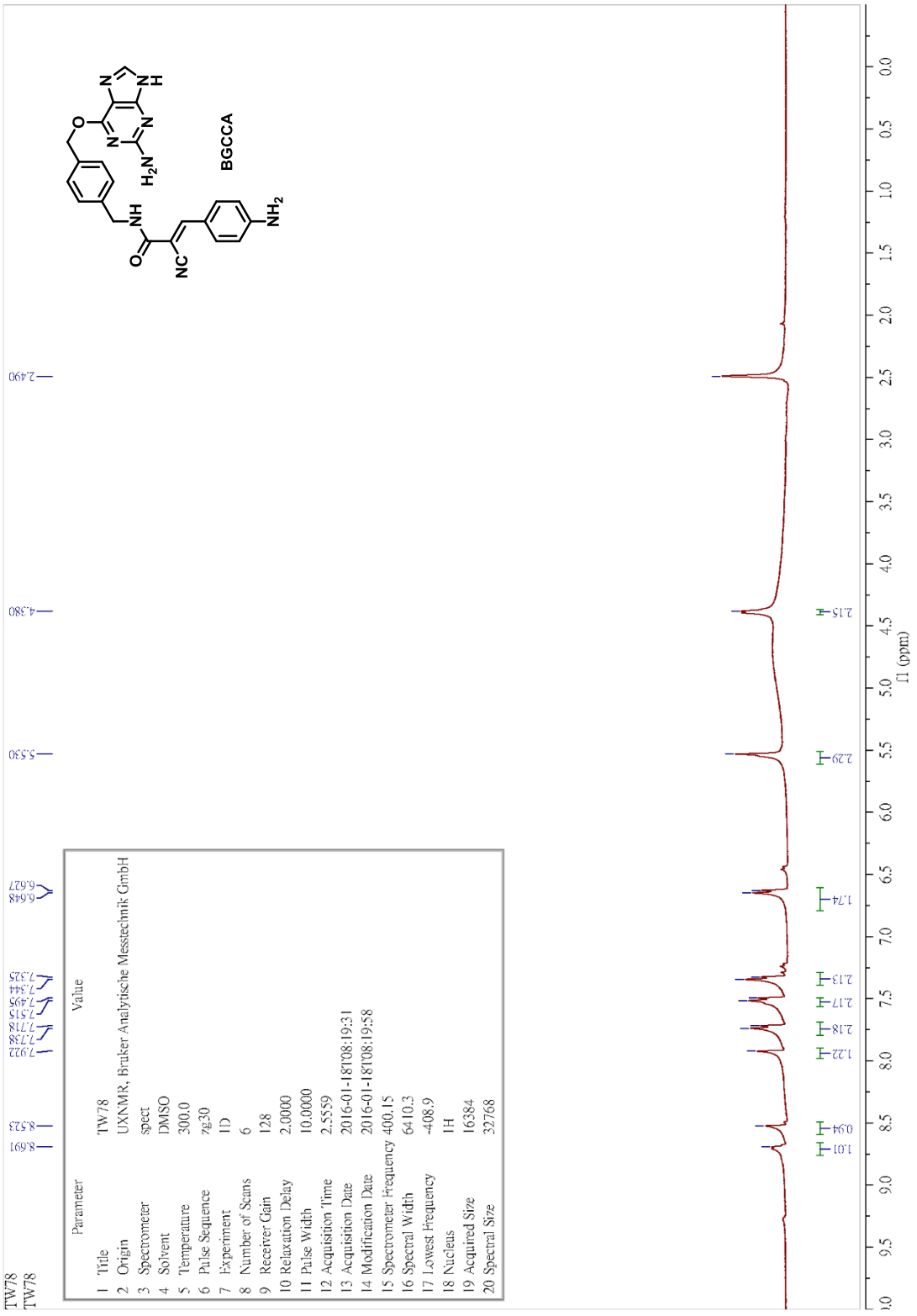

TW75-160113
TW75-160113
9.042
8.405
8.176
8.008
7.987
7.518
7.499
7.365
7.346
7.315
7.294
5.520
4.412
2.490
Parameter Value
1 Title TW75
2 Origin Varian
3 Spectrometer mercury
4 Solvent DMSO
5 Temperature 29.0
6 Pulse Sequence s2pul
7 Experiment 1D
8 Number of Scans 8
9 Receiver Gain 38
10 Relaxation Delay 1.0000
11 Pulse Width 0.0000
12 Acquisition Time 1.9950
13 Acquisition Date 2016-01-13T23:59:23
14 Modification Date 2016-01-14T00:00:00
15 Spectrometer Frequency 400.44
16 Spectral Width 6006.0
17 Lowest Frequency -982.2
18 Nucleus 1H
19 Acquired Size 11982
20 Spectral Size 32768
O
NH
NC
N
H2N
N
N
H
N3
BGCCA-N3
1.00
1.10
1.09
2.06
2.20
2.01
2.10
2.14
2.07
f1 (ppm)


TW75-C20160114
13C OBSERVE
Parameter Value
1 Title TW75-13C
2 Origin Varian
3 Spectrometer mercury
4 Solvent DMSO
5 Temperature 29.0
6 Pulse Sequence s2pul
7 Experiment 1D
8 Number of Scans 28180
9 Receiver Gain 26
10 Relaxation Delay 0.0000
11 Pulse Width 0.0000
12 Acquisition Time 1.1994
13 Acquisition Date 2016-01-14T00:00:54
14 Modification Date 2016-01-14T09:26:00
15 Spectrometer Frequency 100.70
16 Spectral Width 25000.0
17 Lowest Frequency -3034.7
18 Nucleus 13C
19 Acquired Size 29984
20 Spectral Size 65536
161.15
158.87
158.18
153.71
149.73
143.55
140.60
139.26
134.38
132.08
128.93
128.51
127.56
119.98
116.53
104.90
67.94
43.01
40.13
39.92
39.71
39.50
39.29
39.08
38.87
BGCCA-N3
f1 (ppm)

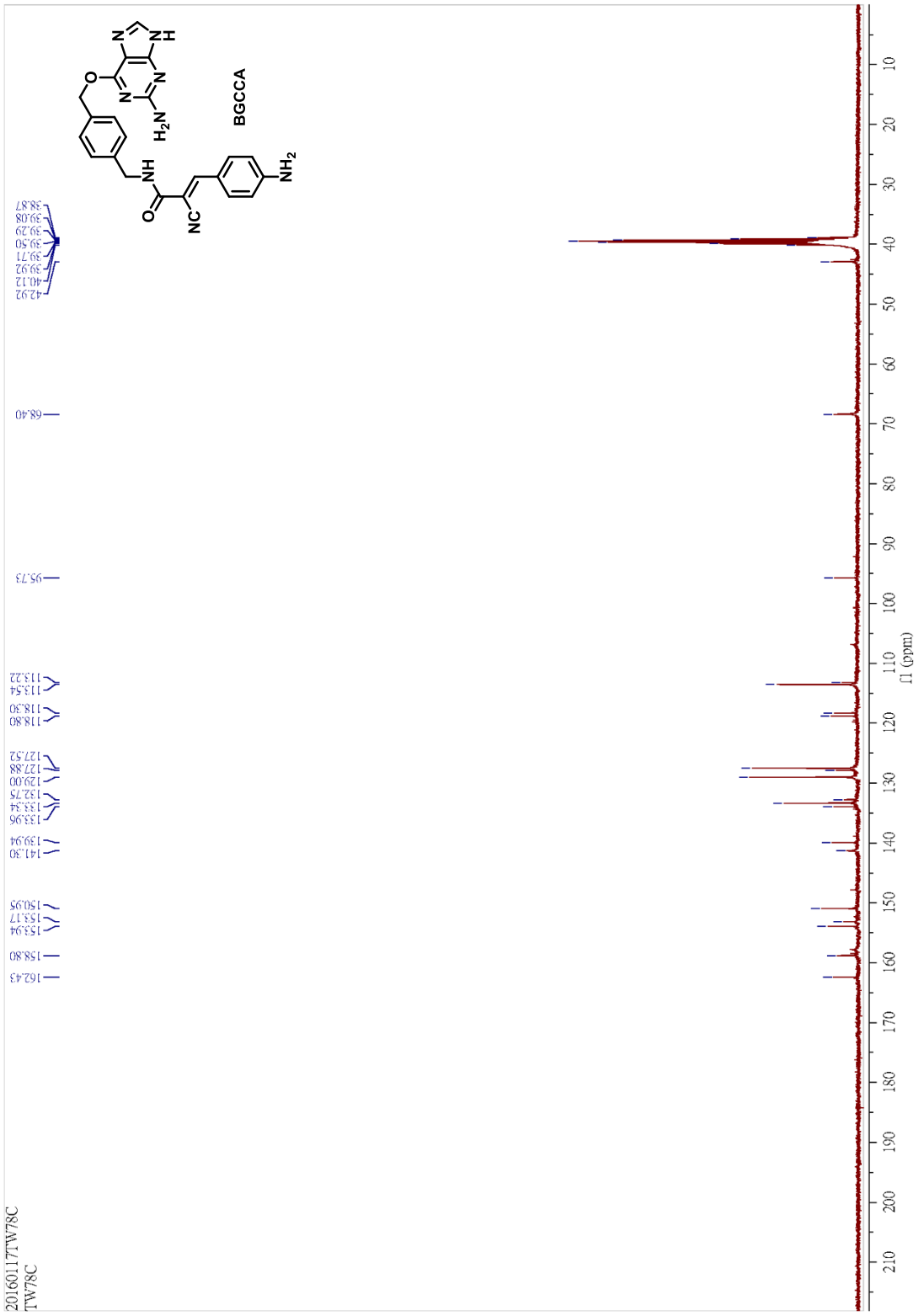

TW77-20151206
TW77
8.046
7.827
7.805
6.697
6.675
4.852
3.308
3.304
3.300
3.296
3.292
Parameter Value
1 Title TW77
2 Origin Varian
3 Spectrometer vnmrs
4 Solvent cd3od
5 Temperature 25.0
6 Pulse Sequence s2pul
7 Experiment 1D
8 Number of Scans 20
9 Receiver Gain 30
10 Relaxation Delay 1.0000
11 Pulse Width 0.0000
12 Acquisition Time 2.5559
13 Acquisition Date 2015-12-06T13:28:50
14 Modification Date 2015-12-06T13:30:54
15 Spectrometer Frequency 399.76
16 Spectral Width 6410.3
17 Lowest Frequency -802.1
18 Nucleus 1H
19 Acquired Size 16384
20 Spectral Size 32768
O
OH
NC
NH2
16
1.00
2.06
2.01
f1 (ppm)



	Parameter	Value
1	Title	TW77-13C
2	Origin	Varian
3	Spectrometer	vnmrs
4	Solvent	dmso
5	Temperature	25.0
6	Pulse Sequence	s2pul
7	Experiment	1D
8	Number of Scans	4880
9	Receiver Gain	30
10	Relaxation Delay	1.0000
11	Pulse Width	0.0000
12	Acquisition Time	1.3107
13	Acquisition Date	2015-12-06T16:47:43
14	Modification Date	2015-12-06T19:57:56
15	Spectrometer Frequency	100.53
16	Spectral Width	25000.0
17	Lowest Frequency	-1487.4
18	Nucleus	13C
19	Acquired Size	32768
20	Spectral Size	65536


TW78
TW78
Parameter Value
1 Title TW78
2 Origin UXNMR, Bruker Analytische Messtechnik GmbH
3 Spectrometer spect
4 Solvent DMSO
5 Temperature 300.0
6 Pulse Sequence zg30
7 Experiment 1D
8 Number of Scans 6
9 Receiver Gain 128
10 Relaxation Delay 2.0000
11 Pulse Width 10.0000
12 Acquisition Time 2.5559
13 Acquisition Date 2016-01-18T08:19:31
14 Modification Date 2016-01-18T08:19:58
15 Spectrometer Frequency 400.15
16 Spectral Width 6410.3
17 Lowest Frequency -408.9
18 Nucleus 1H
19 Acquired Size 16384
20 Spectral Size 32768
8.691
8.523
7.922
7.738
7.718
7.515
7.495
7.344
7.325
6.648
6.627
5.530
4.380
2.490
BGCCA
10.01
0.94
1.74
2.13
2.17
2.18
1.22
2.15
2.29
f1 (ppm)

1


BGCCA
20160117TW78C
TW78C
f1 (ppm)