Supporting Information

Brominated luciferins are versatile bioluminescent probes

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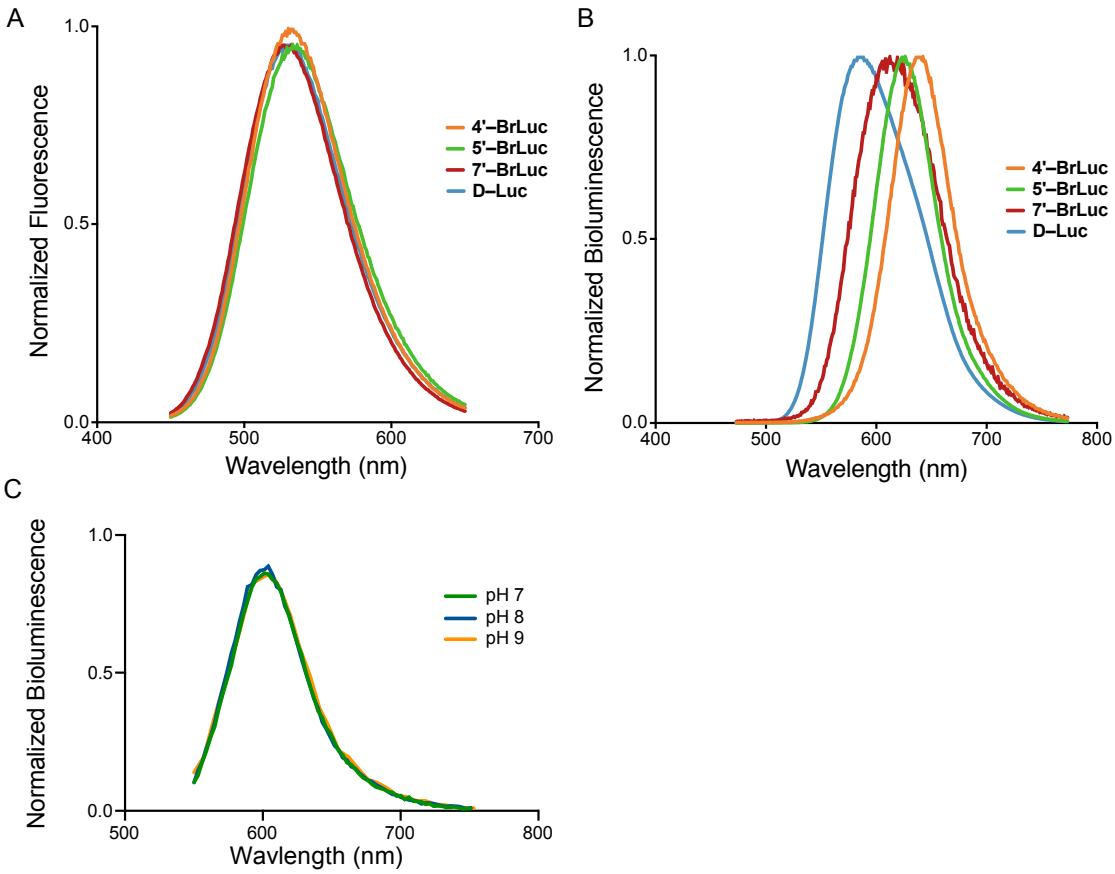


Figure S1. Optical analyses of luciferin analogs. (A) Fluorescence spectra of **D–Luc** and brominated analogs using 365 nm excitation light (pH 7.6). (B) Bioluminescence spectra of **D–Luc** and the brominated analogs at pH 7.6. (C) Bioluminescence spectra of **5'–BrLuc** at different pH values.

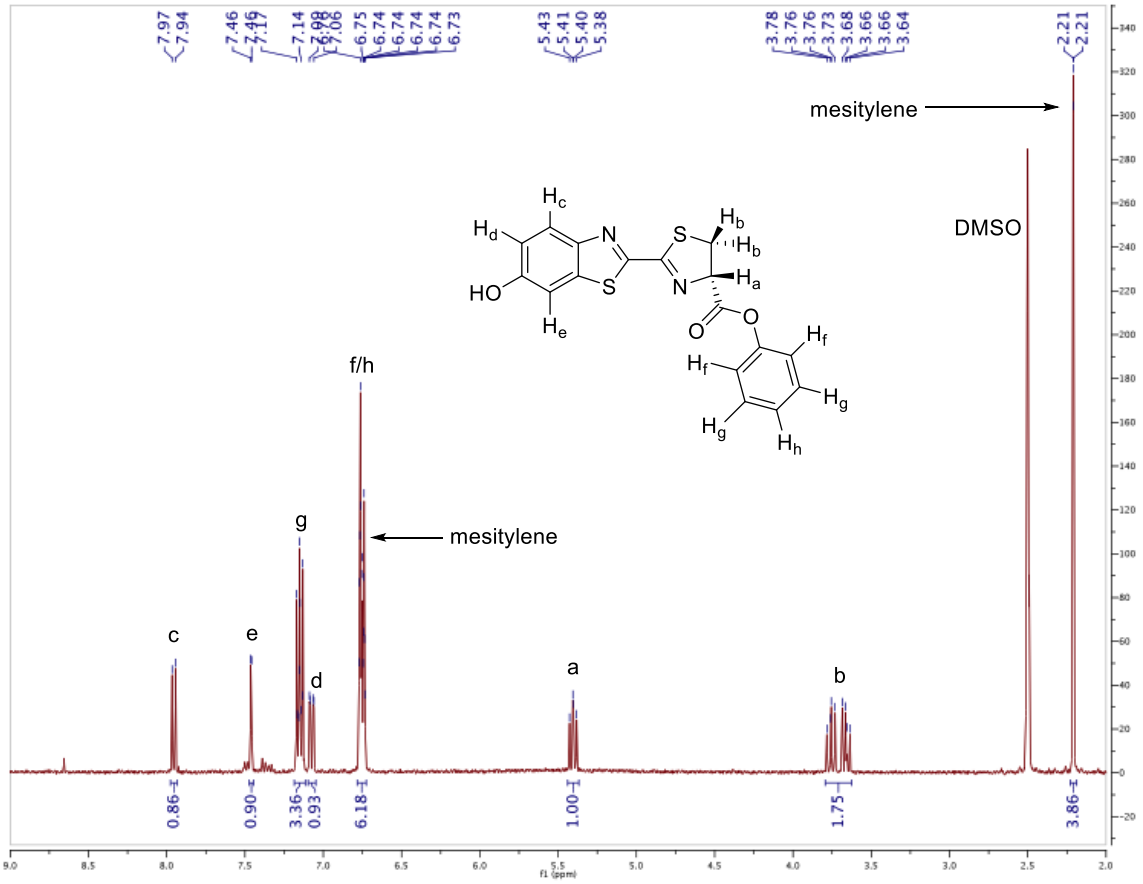


Figure S2. Representative ^{1}H NMR spectrum of a luciferin phenyl ester prepared as described in the *General Experimental Procedures* section.

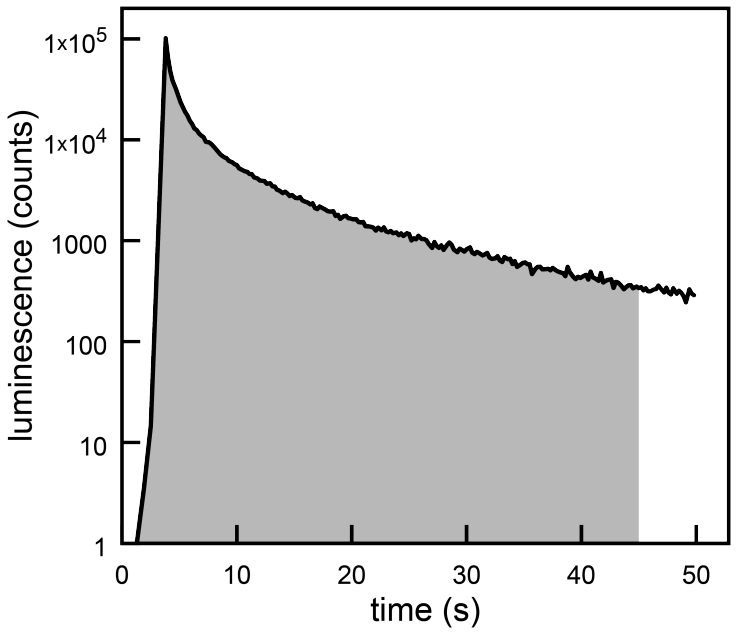


Figure S3. Representative luminescence data following the addition of base to the phenyl ester of **D–Luc** (as described in the *General Experimental Procedures* section). The shaded area denotes the region used for trapezoidal integration.

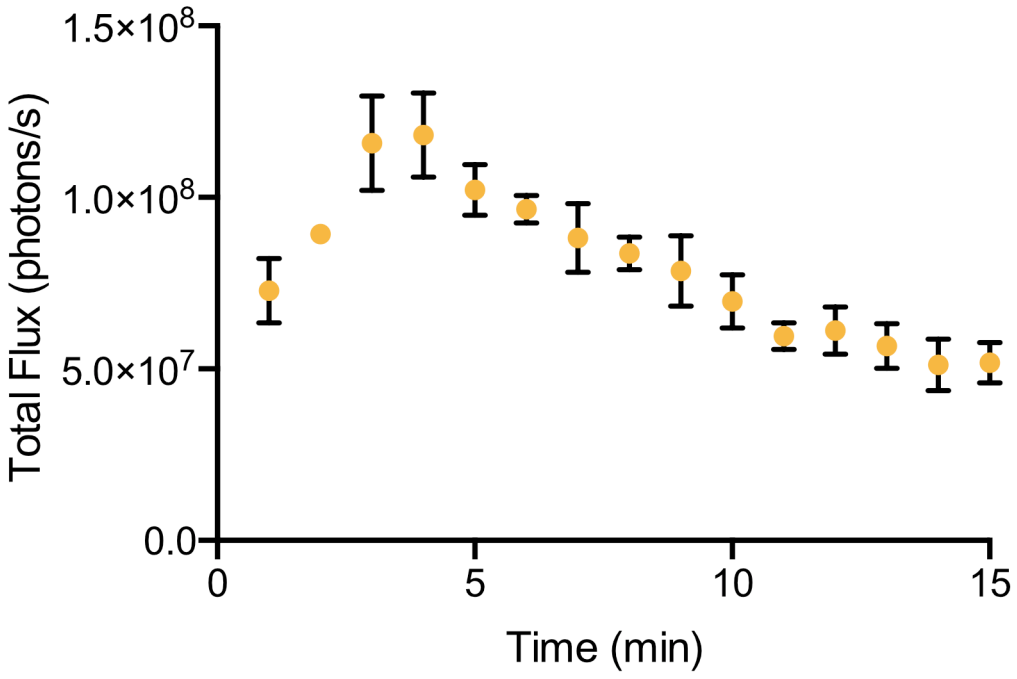


Figure S4. Luciferin analog **5′–BrLuc** (100 µL of a 1 mM solution) was administered (i.v.) into a luciferase transgenic mouse, and bioluminescence images were acquired. Photon flux values were integrated over the entire animal, and plotted as total flux (shown). Data are representative of n = 3 independent experiments.

General Experimental Procedures

Expression and purification of Fluc

Firefly luciferase was expressed and purified as previously described.[1]

Bioluminescence kinetic measurements

Measurements were acquired on a Tecan F200 Pro injection port luminometer with a neutral density filter. Reactions were performed in black 96-well flat-bottom plates (Grenier). Bioluminescence buffer[2] (93.5 μL of 20 mM Tris-HCl pH 7.6, 2 mM $MgSO_4$, 2 mM ATP, 0.1 mM EDTA, 1 mM TCEP, 0.5 mg/mL BSA) was added to each well, followed by coenzyme A (0.5 μL of a 100 mM solution) and luciferin substrate (1 μL of a 0.01-100 mM solution in DMSO). The luminescence from each well was measured for 30 s prior to the addition of Fluc (5 μL of a 1 mg/mL solution in bioluminescence buffer). Luminescence was then recorded every 0.1 s over a 1-min period. Samples were analyzed in triplicate and multiple runs were performed. The emission maxima were determined by averaging the largest photon outputs from five independent runs. K_m and relative k_{cat} values were determined using nonlinear regression analyses and robust fit outlier removal in GraphPad Prism (version 6.0f for Macintosh, GraphPad Software).

Bioluminescence imaging (in vitro)

Imaging was performed using an IVIS Lumina (Xenogen) system equipped with a cooled CCD camera. Reactions were performed in black 96-well flat-bottom plates (Grenier). Bioluminescence buffer (93.5 μL) was added to each well, along with coenzyme A (0.5 μL of a 100 mM solution) and luciferin substrate (1 μL of a 0.5-100

mM solution in DMSO). To initiate photon production, Fluc (5 μL of a 1 mg/mL solution in bioluminescence buffer) was added to each well. The plate was then briefly agitated and placed in the IVIS instrument. The bioluminescent output was recorded every 5-30 s over a 45-75 min time period. Measurements were performed in triplicate.

Bioluminescence imaging (in cellulo)

HEK293 cells stably expressing Fluc (provided by the Contag Lab, Stanford) were grown in DMEM supplemented with fetal bovine serum (FBS, 10%) penicillin (10 U/mL), and streptomycin (10 μg/mL). The cells were cultured in a water-saturated CO_2 (5%) incubator at 37 ºC. Imaging was performed using an IVIS Lumina (Xenogen) system equipped with a heated stage (37 ºC) and a cooled CCD camera. Reactions were performed in black 96-well flat-bottom plates (Grenier) with 100,000 cells per well. Luciferin (50 μL of 2X stock in PBS, pH 7.4) was added to each well, and bioluminescence images were acquired as above.

Bioluminescence imaging (in vivo)

Pathogen free luciferase-expressing transgenic mice (B6;FVB-*Ptprc*^{a} Tg(CAG-luc,-GFP)L2G85Chco *Thy1*^{a}/J) or FVB mice were obtained from the Jackson Laboratory. The mice were housed in University of California, Irvine's animal care facility and provided access to food and water *ad libitum*. All procedures were approved by the Institutional Animal Care and Use Committee at UC Irvine (protocol #2011-2987 to J.A.P.). FVB mice were innoculated with luciferase-epressing DB7 cells (10^6) in the right flank. Luciferin solutions were formulated using the potassium salt of the desired

luciferin and sterile PBS (Dulbecco's Phosphate-Buffered Saline, ThermoFisher). Mice were anesthetized with isoflurane (2% in 1 L/min of O_2), and were injected i.v. (tail vein) or i.p. (intraperitoneal) with 100 μL of luciferin solutions. Bioluminescent images were acquired using the IVIS Lumina system (PerkinElmer). Images were acquired every minute for 15 min (10 second exposure per image). Images were analyzed using Living Image software.

Bioluminescence emission spectra

Emission spectra for D-luciferin and all analogs were recorded on a FluoroMax-4 spectrometer (Horiba Jobin-Yvon). Luciferin (10 μL of an 10 mM solution in bioluminescence buffer, pH 7-9) and Fluc (10 μL of a 1 mg/mL solution in bioluminescence buffer) along with coenzyme A (5 μL of a 100 mM solution) were placed in a 10 mm path length quartz cuvette (1 mL total volume). Emission data were collected over 450-750 nm (1 nm intervals) at room temperature. The acquisition times were 0.1 s/wavelength. The spectra were then normalized to D-luciferin and plotted.

General chemiluminescence procedure

Phenyl esters of each luciferin analog were prepared following the basic procedure of Kim *et al*.[3] In brief, the potassium salt of each luciferin (6.0 μmol) was added to an oven-dried, two-dram vial containing a small stir bar. Deuterated dimethylsulfoxide (0.55 mL) containing a mesitylene internal standard (0.275 μL) was then added, and the luciferin was dissolved with stirring (5 min). Phenylchloroformate (0.76 μL, 6.0 μmol) was subsequently added, and a brief color change was observed in

most cases. The solutions were stirred for an additional 5 min. A portion of each solution (5 μL) was reserved, and the remainder was added to an NMR tube for analysis. The NMR sample was kept at ambient temperature until luminometer measurements were acquired (see below). At that point, the NMR sample was frozen (-73 °C) to preserve the contents of the tube. At a later time, the tube was thawed and a ^{1}H NMR spectrum was immediately acquired (2 scans, 20 s relaxation delay). The concentration of the luciferin phenyl ester was determined via comparison to the internal standard (Figure S2).

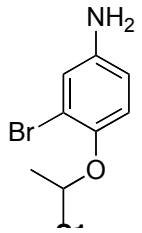
The reserved portion of each luciferin ester solution was diluted to 0.5 mL with anhydrous DMSO, and 50 μL of this solution was added to six wells of a black 96-well flat-bottom plate (Greiner). Chemiluminescence values were acquired on a Tecan Infinite F200 PRO plate-reading luminometer. Data were acquired for 1.5 s prior to injection of potassium phenoxide solution (50 μL of a of 0.1 M solution). The phenoxide solution was prepared via dissolution of potassium *tert*-butoxide (112 mg) and phenol (94 mg) in anhydrous dimethylsulfoxide (10 mL) with stirring (30 min). The total volume in each well was 100 μL. After the addition of base, luminescence data were collected for an additional 50 s (100 ms integration times were used). Relative luminescence yields were determined via trapezoidal integration of the data (Figure S2).

Synthetic experimental procedures

All reactions were performed in flame- or oven-dried glassware under positive pressure of nitrogen or argon unless otherwise noted. Dichloromethane, dimethylacetamide, *N*,*N*-dimethylformamide, triethylamine, and toluene were dried by columns packed with activated alumina on a solvent purification system. Anhydrous pyridine and DMSO were purchased from Acros Organics in AcroSeal™ bottles. All reagents were used as purchased without further purification. 4,5-Dichloro-1,2,3-dithiazol-1-ium chloride (Appel's salt) was synthesized according to a published procedure[4] and stored in a desiccator. Thin layer chromatography (TLC) was performed on Merck 60 F_{254} pre-coated silica gel plates, and TLC plates were visualized with UV light and ninhydrin stain when appropriate. Flash-column chromatography was performed using silica gel (60 Å, 230-240 mesh, Merck KGA). NMR spectra were recorded with Bruker Advanced spectrometers using deuterated solvents. ^{1}H NMR spectra were recorded at 400 or 500 MHz as indicated. ^{13}C NMR spectra were recorded at 125 MHz. ^{1}H NMR data are reported in the following order: chemical shift (δ ppm), multiplicity, coupling constant (Hz), and integration. ^{13}C NMR data are reported in terms of chemical shift. Infrared spectra were recorded using a Thermo Scientific iD5 ATR infrared spectrophotometer. High-resolution mass spectra were obtained from the UC Irvine Mass Spectrometry Facility. The abbreviations used can be found in the document *JOC Standard Abbreviations and Acronyms*.

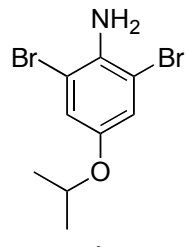
Synthetic procedures

3-Bromo-4-isopropoxyaniline (S1)



Following the general method of Shen and Driver,[5] to a flask containing 2-bromo-1-isopropoxy-4-nitrobenzene[6] were added iron filings (0.15 g, 560 μmol), acetone (3 mL) and water (10 mL). Glacial acetic acid (1 mL) was then added, and the mixture was heated at reflux for 3 h. The mixture was then diluted with ethyl acetate (20 mL) and washed with saturated sodium carbonate (2 x 20 mL), ammonium chloride (2 x 20 mL), and brine (1 x 20 mL). The organic layer was then dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified *via* flash-column chromatography (eluting with 8:2 hexanes:ethyl acetate) to yield **S1** (72 mg, 57%) as a brown oil. The spectra matched those reported previously.[7]

2,6-Dibromo-4-isopropoxyaniline (1)

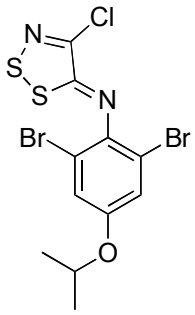


Following the general procedure of Popeney and Guan,[8] to a solution of 4-isopropoxy aniline (1.20 g, 7.90 mmol) in CH_2Cl_2 (66 mL) and methanol (22 mL) was added calcium carbonate (3.03 g, 30.0 mmol), followed by benzyltrimethylammonium tribromide (6.53 g, 16.0 mmol). The reaction was stirred at room temperature for 2 h. The reaction was then quenched with 1 M $Na_2S_2O_3$ and washed with 1 M $Na_2S_2O_3$ (2 x 100 mL), water (2 x 100 mL) and brine (1 x 100 mL). The organic layer was dried with $MgSO_4$, then filtered and concentrated *in vacuo*. The crude material was purified by flash-column chromatography (eluting with 8:2 hexanes:ethyl acetate) to afford **1** (1.0 g, 43%) as an orange oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.03 (s, 2H), 4.34 (septet, J = 6.1 Hz, 1H), 1.29 (d, J = 6.1

Hz, 6H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 150.2, 136.4, 120.9, 109.0, 72.0, 22.0; HRMS (CI) *m/z* calcd for $C_9H_{12}Br_2NO$ $[M+H]^+$ 309.9265, found 309.9274.

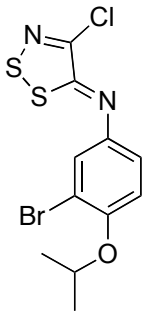
Representative procedure for the synthesis of Appel's salt adducts (2, 4, 6)

(Z)-2,6-Dibromo-N-(4-chloro-5H-1,2,3-dithiazol-5-ylidene)-4-isopropoxyaniline (2)



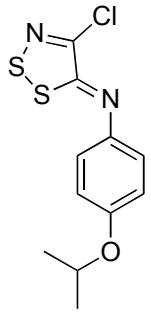
Following the general method of Michaelidou and Koutentis,[9] to a flask of **1** (0.94 g, 3.3 mmol) under argon was added Appel's salt (0.83 g, 4.0 mmol), followed by anhydrous CH_2Cl_2 (15 mL) and anhydrous pyridine (0.59 mL, 7.3 mmol). The reaction mixture was stirred at room temperature for 2 h, then loaded onto silica gel and purified *via* flash-column chromatography (eluting with 8:2 hexanes:ethyl acetate). Compound **2** (1.3 g, 91%) was isolated as a brown oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.33 (s, 2H), 4.66 (septet, J = 6.0 Hz, 1H), 1.23 (d, J = 6.0 Hz, 6H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 164.8, 155.8, 146.7, 142.8, 120.3, 113.6, 71.3, 22.0; HRMS (ESI-TOF)$^+$ *m/z* calcd for $C_{11}H_{10}Br_2ClN_2OS_2$ $[M+H]^+$ 442.8290, found 442.8295.

(Z)-3-Bromo-N-(4-chloro-5H-1,2,3-dithiazol-5-ylidene)-4-isopropoxyaniline (4)



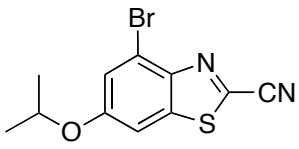
Compound **4** was isolated as a brown oil (0.92 g, 87%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.52, (s, 1H), 7.28 (s, 2H), 4.72 (septet, J = 4.8 Hz, 1H), 1.34 (d, J = 4.8 Hz, 6H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 159.2, 152.5, 147.5, 144.5, 125.2, 121.0, 116.6, 113.3, 72.1, 22,3; HRMS (CI) *m/z* calcd for $C_{11}H_{11}BrClN_2OS_2$ $[M+H]^+$ 364.9185, found 364.9189.

(Z)-N-(4-chloro-5H-1,2,3-dithiazol-5-ylidene)-4-isopropoxyaniline (6)



Compound **6** was isolated as a brown oil (2.84 g, 99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.20 (d, J = 8.9 Hz, 2H), 7.01 (d, J = 8.9 Hz, 2H), 4.61 (septet, J = 6.0 Hz, 1H), 1.25 (d, J = 6.0 Hz, 6H); ^{13}C NMR (500 MHz, DMSO-d_6) δ 156.6, 156.4, 147.8, 142.9, 122.3, 116.9, 70.0, 22.3; HRMS (ESI-TOF)$^{+}$ *m/z* calcd for $C_{11}H_{12}ClN_2OS_2$ $[M+H]^{+}$ 287.0080, found 287.0084.

Representative procedure for the fragmentation and cyclization of Appel's salt adducts (3, 5, 8)



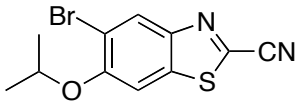
4-Bromo-6-isopropoxybenzo[*d*]thiazole-2-carbonitrile (3)

Following the general procedure of Micaelidou and Koutentis,[9] a flask containing **2** (0.28 g, 640 μmol) was flushed with dry nitrogen and charged with CH_2Cl_2 (5 mL). The flask was cooled to 0 °C in an ice bath and DBU (0.29 mL, 1.9 mmol) was added. The reaction mixture was stirred for 5 min, then adsorbed to silica gel. The adsorbed material was rinsed with hexanes, and then eluted with 7:3 hexanes:ethyl acetate. The isolated thioamide was used immediately in the next reaction. (Note: this compound degrades quickly).

Following the general procedure of Inamoto and coworkers,[10] to a flask containing (2,6-dibromo-4-isopropoxyphenyl)carbamothioyl cyanide (0.24 g, 640 μmol based on crude yield from previous step), was added palladium(II) chloride (11 mg, 64 μmol), copper(I) iodide (60 mg, 320 μmol), and tetrabutyl ammonium bromide (0.43 g,

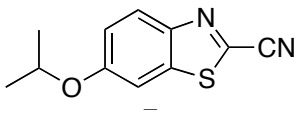
1.3 mmol). The flask was flushed with dry nitrogen, and then DMF (8 mL) and DMSO (8 mL) were added. The reaction was heated at 125 °C for 2 h. The mixture was then diluted with ethyl acetate (40 mL) and washed with 1 M $NaHSO_4$ (1 x 40 mL), water (3 x 40 mL), ammonium chloride (1 x 40 mL) and brine. The organic layers were combined and then dried with $MgSO_4$, and concentrated *in vacuo*. The concentrate was purified via flash-column chromatography (eluting with 9:1 hexanes:ethyl acetate) to yield **3** (33 mg, 6.7% over two steps) as a brown solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45, (d, J = 2.3 Hz, 1H), 7.29 (d, J = 2.3 Hz, 1H), 4.64 (septet, J = 6.0 Hz, 1H), 1.40 (d, J = 6.0 Hz, 6H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 159.0, 145.2, 137.7, 133.6, 122.9, 119.2, 112.8, 104.3, 71.7, 21.9; HRMS (ESI-TOF)$^+$ *m/z* calcd for $C_{11}H_{10}BrN_2OS$ $[M+H]^+$ 350.9779, found 350.9783.

5-Bromo-6-isopropoxybenzo[d]thiazole-2-carbonitrile (5)



Compound **5** was isolated as a brown solid (79 mg, 4%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (s, 1H), 7.36 (s, 1H), 4.69 (septet, J = 6.0 Hz, 1H), 1.47, (d, J = 6.0 Hz, 6H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 155.2, 146.9, 136.1, 134.4, 129.3, 115.9, 113.0, 104.4, 73.0, 21.8; HRMS (CI) *m/z* calcd for $C_{11}H_{10}BrN_2OS$ $[M+H]^+$ 296.9697, found 296.9694.

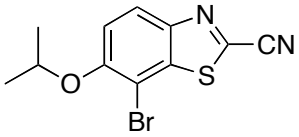
6-Isopropoxybenzo[d]thiazole-2-carbonitrile (7)



Compound **7** was isolated as a brown solid (48 mg, 3%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, J = 9.1 Hz, 1H), 7.34 (d, J = 2.4 Hz, 1H), 7.20 (dd, J = 9.1, 2.5 Hz, 1H), 4.66 (septet, J = 5.3 Hz, 1H), 1.41 (d, J =

5.3 Hz, 1H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 158.9, 146.7, 137.5, 126.0, 119.6, 113.3, 104.9, 71.1, 21.9; HRMS (CI) *m/z* calcd for $C_{11}H_{11}N_2OS$ $[M+H]^+$ 236.0858, found 236.0864.

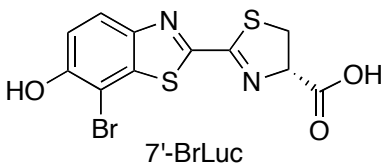
7-Bromo-6-isopropoxybenzo[d]thiazole-2-carbonitrile (8)



To a flask of **7** (0.13 g, 60 μmol) was added *N*-bromosuccinimide (0.16 g, 92 μmol), followed by CH_3CN (15 mL). The reaction mixture was stirred for 12 h, then extracted with ethyl acetate (30 mL). The combined organic layers were washed with water (3 x 60 mL) and brine (1 x 60 mL), then dried with $MgSO_4$, filtered, and concentrated *in vacuo* to yield **8** (0.14 g, 75%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (d, J = 11.4 Hz, 1H), 7.60 (d, J = 11.5 Hz, 1H), 4.88 (septet, J = 6.0 Hz, 1H), 1.31 (d, J = 6.0 Hz, 6H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 155.3, 145.7, 134.2, 124.7, 116.4, 113.0, 103.3, 73.7, 22.2; HRMS (CI) *m/z* calcd for $C_{11}H_{10}BrN_2OS$ $[M+H]^+$ 296.9697, found 296.9696.

Representative procedure for the formation of brominated luciferins (7′–BrLuc, 4′–BrLuc, 5′–BrLuc)

(S)-2-(7-Bromo-6-hydroxybenzo[d]thiazol-2-yl)-4,5-dihydrothiazole-4-carboxylic acid (7′–BrLuc)

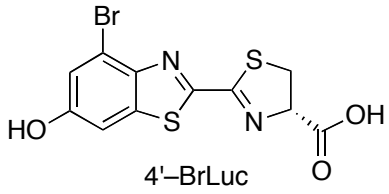


To a flask of **8** (52 mg, 18 μmol) was added anhydrous CH_2Cl_2 (5 mL), followed by a 1.0 M solution of BCl_3 in hexanes (1.06 mmol, 1.06 mL, added slowly). The mixture was stirred at room temperature under nitrogen for 24 h. The reaction was then

quenched with a saturated solution of ammonium chloride (10 mL) and extracted with ethyl acetate (30 mL). The organic layers were combined and washed with saturated ammonium chloride (2 x 30 mL) and brine (1 x 30 mL). The organic layer was then dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The concentrate was purified via flash-column chromatography (eluting with 1:1 hexanes:ethyl acetate) to yield the deprotected intermediate which was used immediately in the following step.

The isolated material (33 mg, 130 μmol, based on the crude isolated yield) was dissolved in degassed methanol (2 mL) and D-cysteine (24 mg, 140 μmol) in degassed 0.05 M phosphate buffer (pH 8.0) was added. The mixture was stirred at room temperature under nitrogen, overnight. The mixture was then acidified with 1 M $NaHSO_4$ (10 mL) and extracted with ethyl acetate (20 mL). The combined organic layers were washed with saturated ammonium chloride (2 x 20 mL) and brine (1 x 20 mL), then dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield **7′–BrLuc** (41 mg, 65% over two steps) as a yellow solid. Note: this compound was treated with anhydrous K_2CO_3 (1.0 equiv.) in water and lyophilized for chemiluminescence assays. ^{1}H NMR (500 MHz, D_2O) δ 7.56 (d, J = 8.9 Hz, 1H), 6.89 (d, J = 8.9 Hz, 1H), 5.17 (m, 1H), 3.76 (m, 1H), 3.55 (m, 1H); ^{13}C NMR (500 MHz, D_2O) δ 180.6, 168.4, 164.4, 157.0, 144.6, 142.9, 125.7, 123.4, 103.7, 82.7, 39.1. Note: high resolution mass spectrometry was not successful due to multiple fragmentation pathways.[11]

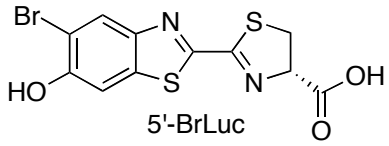
(S)-2-(4-Bromo-6-hydroxybenzo[d]thiazol-2-yl)-4,5-dihydrothiazole-4-carboxylic acid (4′–BrLuc)



4'–BrLuc

Compound **4′–BrLuc** was isolated as a yellow solid (14 mg, 17% over two steps). ^{1}H NMR (500 MHz, D_2O) δ 7.01 (m, 2H), 5.21 (m, 1H), 3.84 (m, 1H), 3.64 (m, 1H); ^{13}C NMR (500 MHz, D_2O) δ 180.4, 168.3, 169.8, 159.3, 146.7, 139.9, 123.3, 119.2, 109.0, 82.8, 39.3. Note: high resolution mass spectrometry was not successful due to multiple fragmentation pathways.[11]

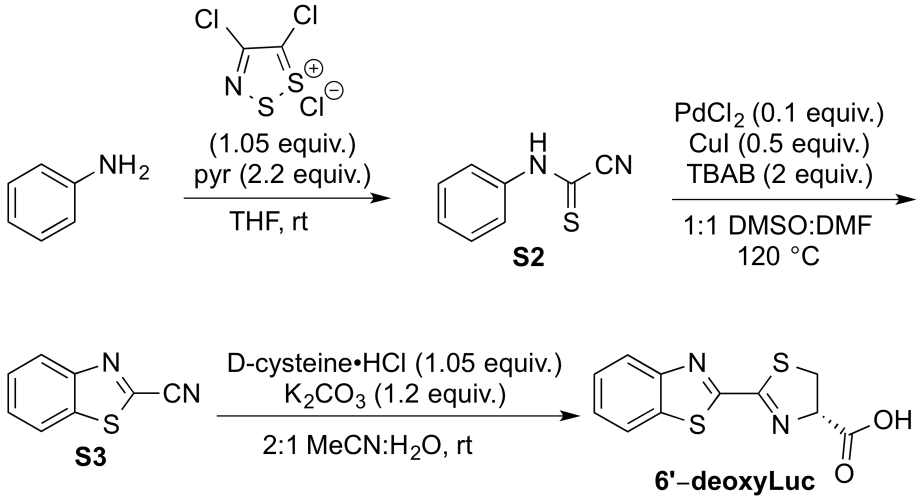
(S)-2-(5-Bromo-6-hydroxybenzo[d]thiazol-2-yl)-4,5-dihydrothiazole-4-carboxylic acid (5′–BrLuc)



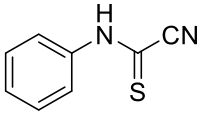
5'-BrLuc

Compound **5′–BrLuc** was isolated as a yellow solid (16 mg, 36% over two steps). ^{1}H NMR (500 MHz, D_2O) δ 7.81 (s, 1H), 7.12 (s, 1H), 5.20 (apparent t, J = 8.6 Hz, 1H), 3.81 (m, 1H), 3.60 (m, 1H); ^{13}C NMR (500 MHz, D_2O) δ 180.0, 168.2, 160.4, 158.8, 147.4, 139.0, 129.1, 116.2, 110.1, 82.8, 39.1. Note: high resolution mass spectrometry was not successful due to multiple fragmentation pathways.[11]

Scheme S1: Synthesis of **6′–deoxyLuc**.

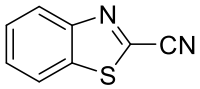


Phenylcarbamothioyl cyanide (S2)



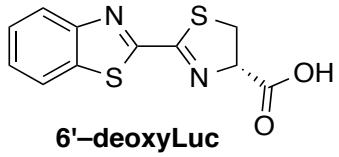
A dry, nitrogen-purged round bottom flask containing a stir bar and anhydrous tetrahydrofuran (30 mL) was charged with aniline (0.91 mL, 10 mmol), and Appel's salt (2.19 g, 10.5 mmol). The resulting solution was stirred at room temperature for 40 min and pyridine (1.66 mL, 20.5 mmol) was subsequently added. When the starting material was completely consumed (by TLC), a solution of sodium thiosulfate pentahydrate (3.2 g, 20 mmol) in 15 mL water and CH_3CN (15 mL) was added. The solution was stirred, and when complete consumption of the intermediate was observed by TLC, the reaction mixture was diluted with ethyl acetate, washed with saturated $NaHSO_4$, and dried with $MgSO_4$. The mixture was filtered, concentrated *in vacuo* and purified by flash column chromatography (eluting with 10% ethyl acetate in hexanes) to yield **S2** (0.75 g, 46%) as a brown solid. Note: high resolution mass spectrometry was not obtained for this compound due to its multiple fragmentation pathways. ^{1}H NMR (500 MHz, $CDCl_3$, mixture of tautomers) δ 9.51 (s, 1H), 7.78 (d, J = 8.3 Hz, 1H), 7.57 – 7.28 (m, 4H), 1.75 (s, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.8, 162.0, 137.0, 136.9, 130.2, 129.5, 129.0, 128.5, 122.9, 122.6, 113.7, 112.1.

Benzothiazole-2-carbonitrile (S3)



A dry, nitrogen-purged round bottom flask containing a stir bar, anhydrous DMSO (100 mL), and anhydrous DMF (100 mL) was charged with **S2** (720 mg, 4.5 mmol), palladium(II) chloride (64 mg, 0.45 mmol), copper(I) iodide (420 mg, 2.2 mmol), and tetrabutylammonium bromide (2.9 g, 9.0 mmol). The flask was fitted with a condenser and heated at 120 ºC under nitrogen for 8 h. The solution was then cooled and quenched with water. Ethyl acetate was added and the layers were separated. The aqueous layer was extracted twice with ethyl acetate. The organic layers were then combined and washed with water (6 x 150 mL) and brine (2 x 100 mL). The organics were combined, and dried with $MgSO_4$. The mixture was filtered, concentrated *in vacuo* and purified by flash column chromatography (eluting with 10% ethyl acetate in hexanes) to yield **S3** (228 mg, 32%) as a taupe solid. Spectra matched those previously reported.[12]

6′–deoxyLuc

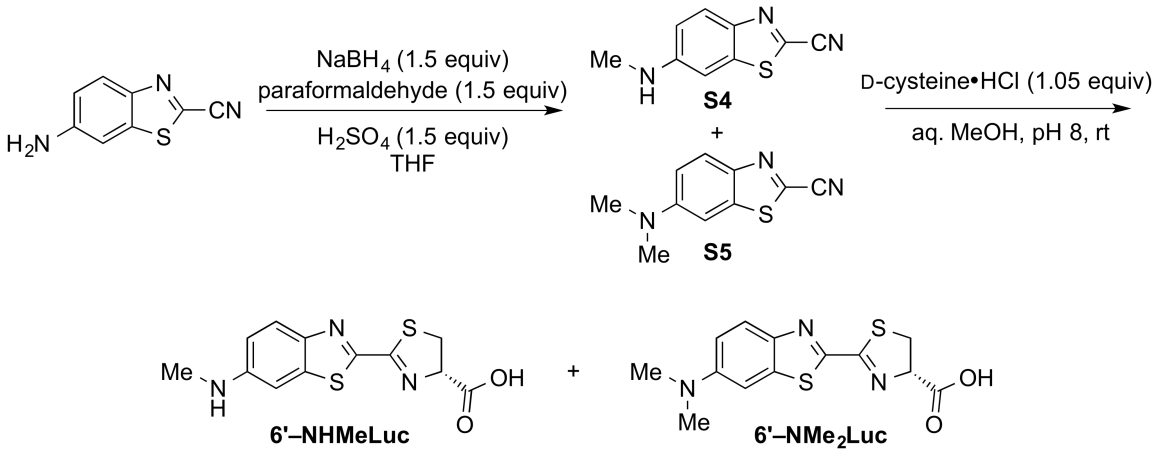


6'–deoxyLuc

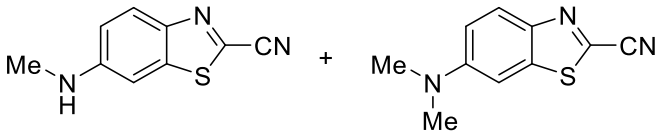
S3 (222 mg, 1.38 mmol) was dissolved in CH_3CN (6 mL) and a solution of K_2CO_3 (228 mg, 1.70 mmol), D-cysteine hydrochloride monohydrate (252 mg, 1.50 mmol), and water (3 mL) was added dropwise with stirring. After 45 min, the volatile organics were removed *in vacuo*, and hydrochloric acid (1 M) was added until the solution was acidic. A cream colored precipitate was observed. The solid was collected by filtration, washed with water, and dried under high vacuum to afford **6′–deoxyLuc** (319 mg, 88%) as an off

white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.16 (ddd, J = 28.6, 7.7, 0.5 Hz, 1H), 7.57 (dtd, J = 19.7, 7.4, 1.2 Hz, 1H), 4.94 (app t, J = 9.0 Hz, 1H), 3.77 (dd, J = 10.4, 8.2 Hz, 1H), 3.51 (t, J = 10.0 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.9, 162.2, 159.4, 152.7, 135.1, 126.9, 126.9, 123.8, 122.7, 84.0, 36.3; HRMS (ESI^+) calcd for $C_{11}H_8N_2O_2S_2Na$ $[M + Na]^+$ 324.9484, found 324.9479.

Scheme S2: Synthesis of **6′–NHMeLuc** and **6′–NMe$_2$Luc**.



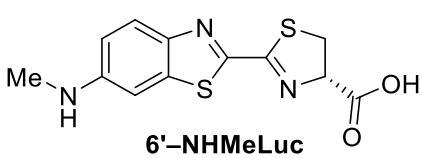
6-(Methylamino)benzo[*d*]thiazole-2-carbonitrile (S4) and 6-(Dimethylamino)benzo[*d*]thiazole-2-carbonitrile (S5)



Following a previously reported procedure,[2] 6-aminobenzo[*d*]thiazole-2-carbonitrile (88 mg, 0.50 mmol) and $NaBH_4$ (29 mg, 0.77 mmol) were dissolved in THF (10 mL). In a separate dried flask, paraformaldehyde (23 mg, 0.77 mmol) was suspended in dry THF (10 mL), and H_2SO_4 (40 μL, 0.77 mmol) was added. The two solutions were stirred separately at room temperature for 15 min. The carbonitrile solution was then added to the paraformaldehyde solution dropwise via syringe. The resulting mixture was stirred under nitrogen for 1 h, then an additional equivalent of $NaBH_4$ was added. After 1 h, the reaction mixture was basified with a 1.8 M solution of KOH (20 mL) and ethyl acetate (20 mL) was added. The layers were separated and the aqueous layer was extracted with additional ethyl acetate (2 x 10 mL). The combined organic layers were washed with

brine (1 x 20 mL), and dried over $MgSO_4$. The mixture was then filtered and concentrated *in vacuo*. The crude material ws purified by flash column chromatography (eluting with 30% ethyl acetate in hexanes) to yield a mixture of **S4** (31 mg, 33%), and **S5** (17 mg, 16%) as orange-red solids. Spectra matched those previously reported.[2]

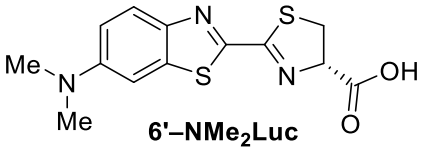
6′–NHMeLuc



6'–NHMeLuc

6′–NHMeLuc was prepared from **S4** via a previously published method.[2] Spectra matched those previously reported.[2]

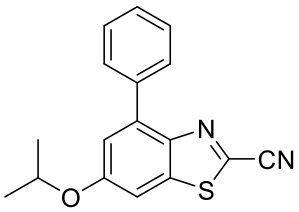
6′–NMe_2Luc



6'–NMe_2Luc

6′–NMe_2Luc was prepared from **S5** as previously reported.[2] Spectra matched those previously reported.[2]

Stille cross-coupling of 5 with phenyltributylstannane



A 15 mL pressure tube containing lithium chloride (9 mg, 200 μmol) was flame dried under vacuum. After cooling, **5** (55.0 mg, 168 μmol), and palladium tetrakis (43 mg, 37 μmol) were added. The flask was evacuated and flushed with nitrogen, and dioxane (2.5 mL) was added against positive flow, followed by phenyltributylstannane (63 μL, 200 μmol). The mixture was heated at 120 °C for 6 h. The mixture was cooled and washed with water (3 times, 3 mL). The organics were then dried over magnesium sulfate, filtered, and

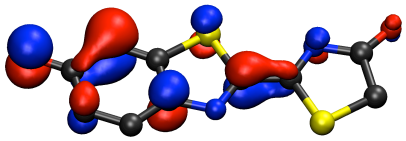
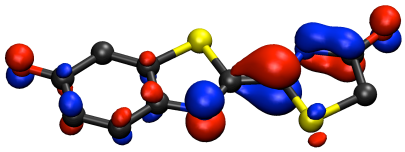
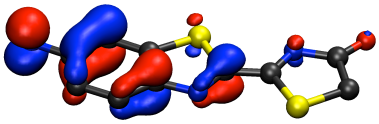
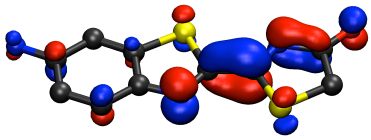
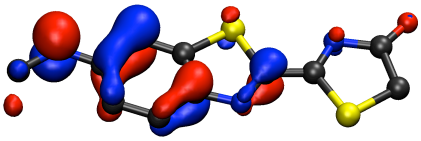
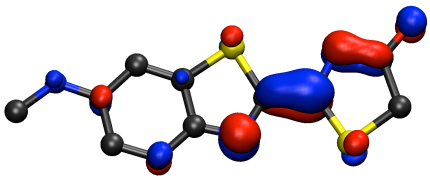
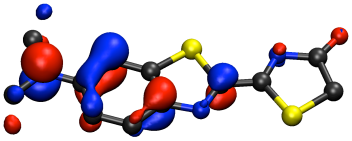
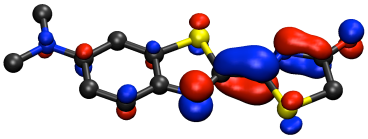
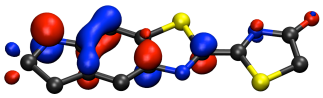
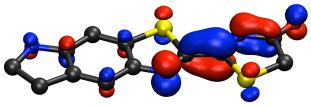
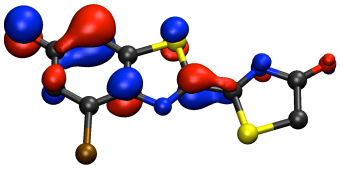
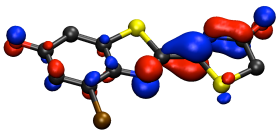
concentrated via rotary evaporation. The crude mixture was purified via preparative TLC (eluting with 30% ethyl acetate in hexanes) to provide **9** (17 mg, 31%). ^{1}H NMR (500 MHz, CDCl3) δ 7.82 – 7.77 (m, 2H), 7.55 – 7.49 (m, 2H), 7.49 – 7.43 (m, 1H), 7.31 (dd, *J* = 17.7, 3.1 Hz, 1H), 4.71 (dt, *J* = 15.1, 7.5 Hz, 1H), 1.44 (d, *J* = 7.6 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 158.8, 144.8, 139.4, 138.6, 137.4, 132.5, 129.6, 128.6, 128.5, 119.2, 113.5, 104.0, 71.1, 22.0. HRMS (ESI^+) calcd. for $C_{17}H_{14}N_2OSNa$ $[M + Na]^+$ 317.0724, found 317.0731.

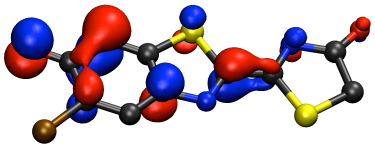
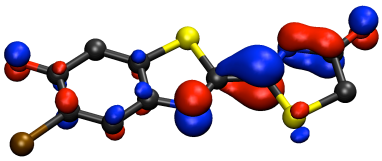
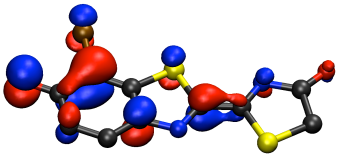
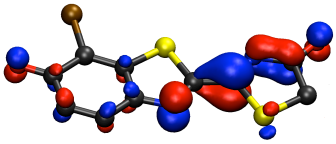
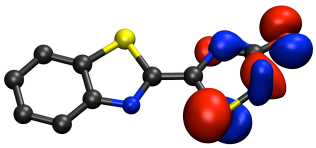
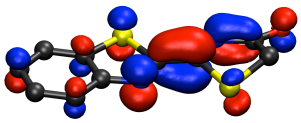
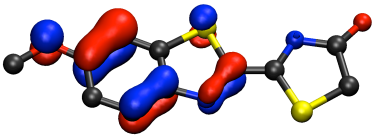
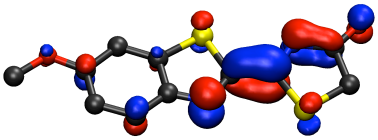
Computational Details

Density functional theory (DFT) structural optimizations of the singlet ground state S_0 and the first singlet excited state (S_1) were performed using the hybrid-GGA functional, PBE0[14] in the gas phase. Basis sets of double-zeta quality with polarization and diffuse functions[15] (def2-SVPD) were necessary to bind the additional electron of the anionic structures. Analytical force constant calculations[16] for the ground state and numerical force constant calculations were performed to verify minima by the absence of imaginary vibrational modes. Constrained excited state geometry optimizations were performed by fixing the out-of-plane bending angle of the carbon-carbon single bond connecting the two thiazoline rings to 0° and adjusting the torsional angle between the nitrogens in each ring. All calculations were performed with the quantum chemistry package TURBOMOLE.[17,18]

Molecular orbitals involved in the emission of luciferin analogs.

For each luciferin analogue, we report the molecular orbitals involved in the emission "de-excitation" from the S_1 excited state geometry. All orbitals are plotted with a contour value of 0.05au. The primary contribution to the excitation is a HOMO to LUMO transition that tends to have pi-pi character originating on the anionic oxygen attached to the benzothiazole with the transition dipole moment pointing towards the thiazoline motif. In the case of luciferin that are electron poor at the 6' position of the benzothiazole, e.g. **6'–deoxyLuc**, no such character is observed and corroborates the notion that an electron rich moiety is necessary for strong emission.

Compound	HOMO	LUMO
D-Luc		
6'–aminoLuc		
6'–MeNHLH$_2$		
6'–Me$_2$NLH$_2$		
CycLuc1		
4'–BrLuc		

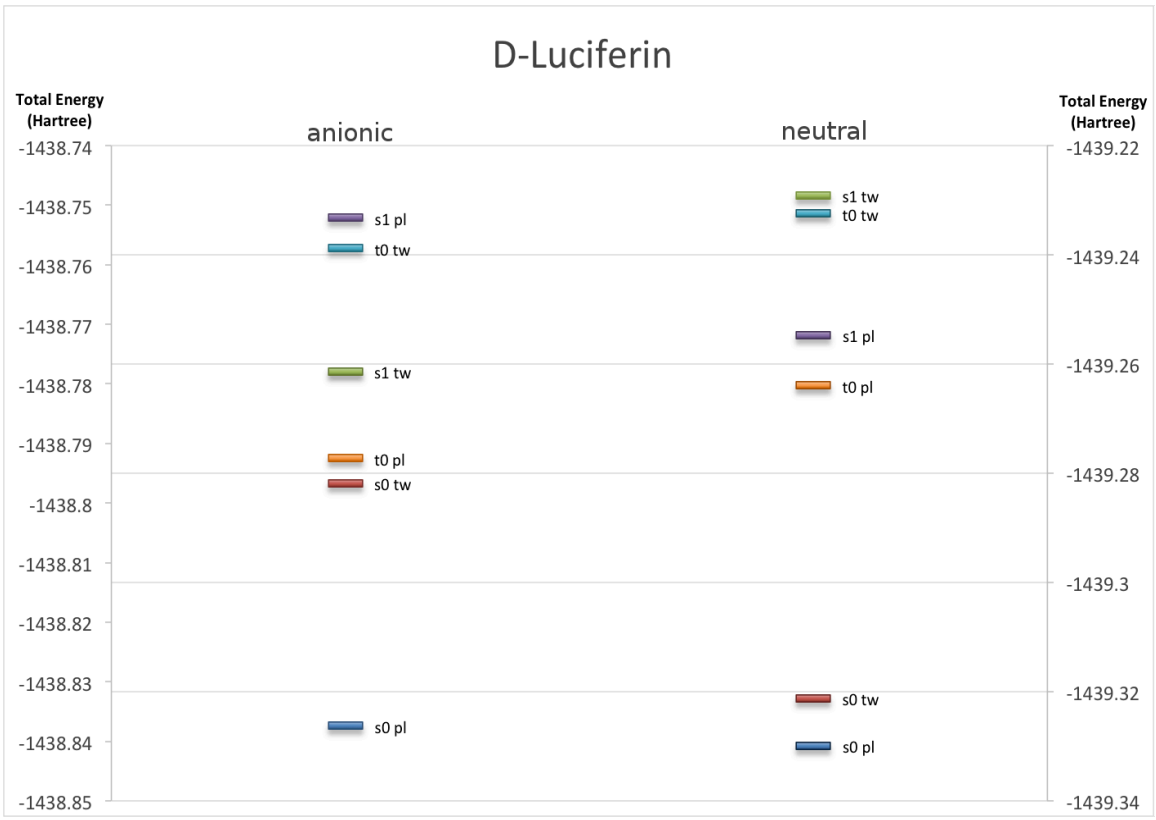
5'–BrLuc		
7'–BrLuc		
6'–deoxyLuc		
6'–methoxyLuc		

Ordering of relevant spin and geometric states

In order to reconcile the differences in experimental and computational results for the brominated luciferin series, and **6'–methoxyLuc**, the ground and excited electronic states were studied in both the planar and twisted geometric states of the neutral and anionic species; both singlet and triplet states were included to help rationalize possible intruder states that may be responsible for the quenching of the chemiluminescence. Electronic states were computed at the geometry of the first singlet excited state, which was confirmed to be a

minimum by numerical frequency analysis. In the case of neutral **4'–BrLuc**, no twisted S_1 minimum was found. This picture gives relative energetics of the different electronic states at the assumed emissive nuclear configuration.

For native **D-luc**, both the twisted and the planar S1 state are relatively isolated from other states. Given that our model can reasonably predict the emission strength of native luciferin, the isolated nature of the S_1 state helps to establish a baseline by demonstrating that it is less probable that there is some other electronic state lower in energy that can be accessed. In the case of the anionic **7'–BrLuc**, one observes a very small separation between the planar S_1 state and the twisted triplet ground state, which could account for the considerable decrease in experimentally measured emission. The planar S_1 state for the other brominated luciferins seems to be modestly isolated, but there could be a distribution of protonated and deprotonated luciferin whose different accessible electronic states could cause interference. Considering **6'–methoxyLuc**, there seem to be no nearby electronic states, but there is a low-lying planar triplet state that could act as a channel for nonradiative decay. The overall environment in solution is complex compared to our model and many factors besides those described here could be in effect; however, electronic states in both the planar and twisted configurations are definitely accessible under photoexcitation and provide a reasonable explanation for the quenching of chemiluminescence.


D-Luciferin
Total Energy (Hartree)
anionic
neutral
Total Energy (Hartree)
-1438.74
-1438.75
-1438.76
-1438.77
-1438.78
-1438.79
-1438.8
-1438.81
-1438.82
-1438.83
-1438.84
-1438.85
-1439.22
-1439.24
-1439.26
-1439.28
-1439.3
-1439.32
-1439.34
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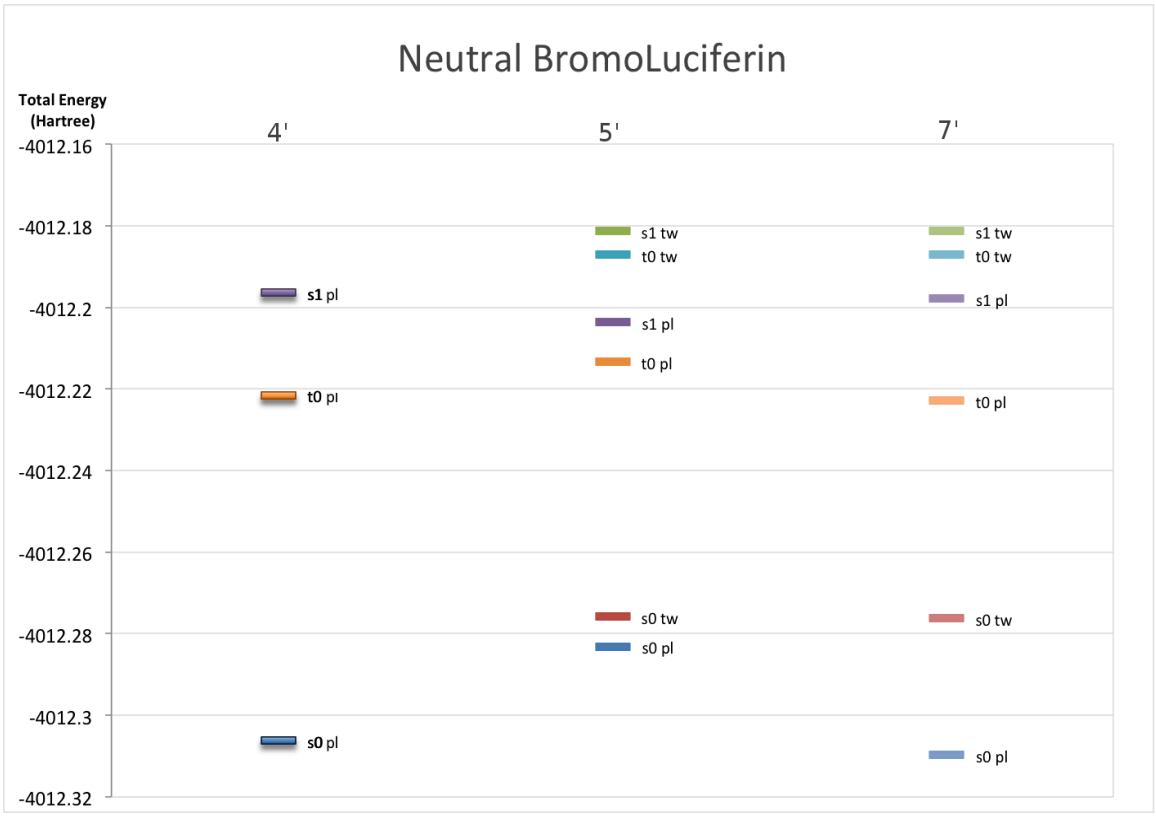
s0 pl


Neutral BromoLuciferin
Total Energy (Hartree)
4'
5'
7'
-4012.16
-4012.18
-4012.2
-4012.22
-4012.24
-4012.26
-4012.28
-4012.3
-4012.32
s1 pl
t0 pl
s0 pl
s1 tw
t0 tw
s1 pl
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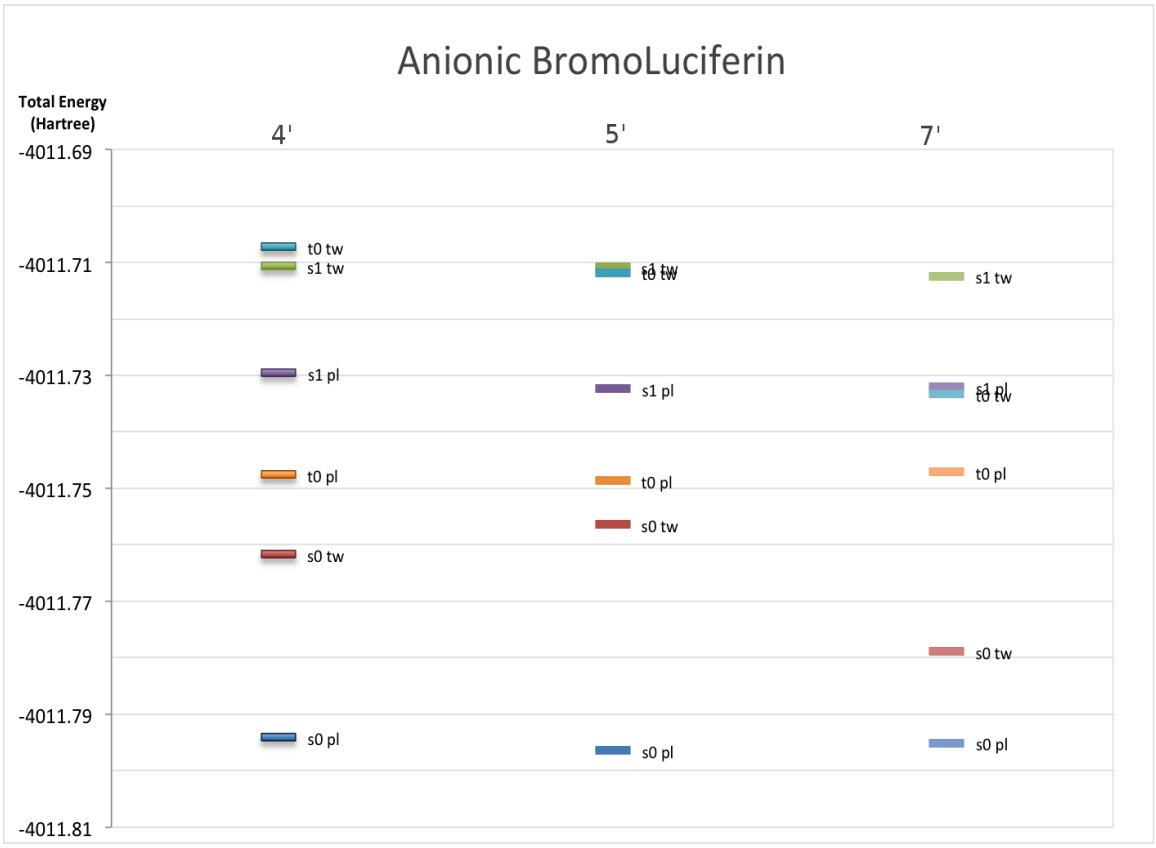
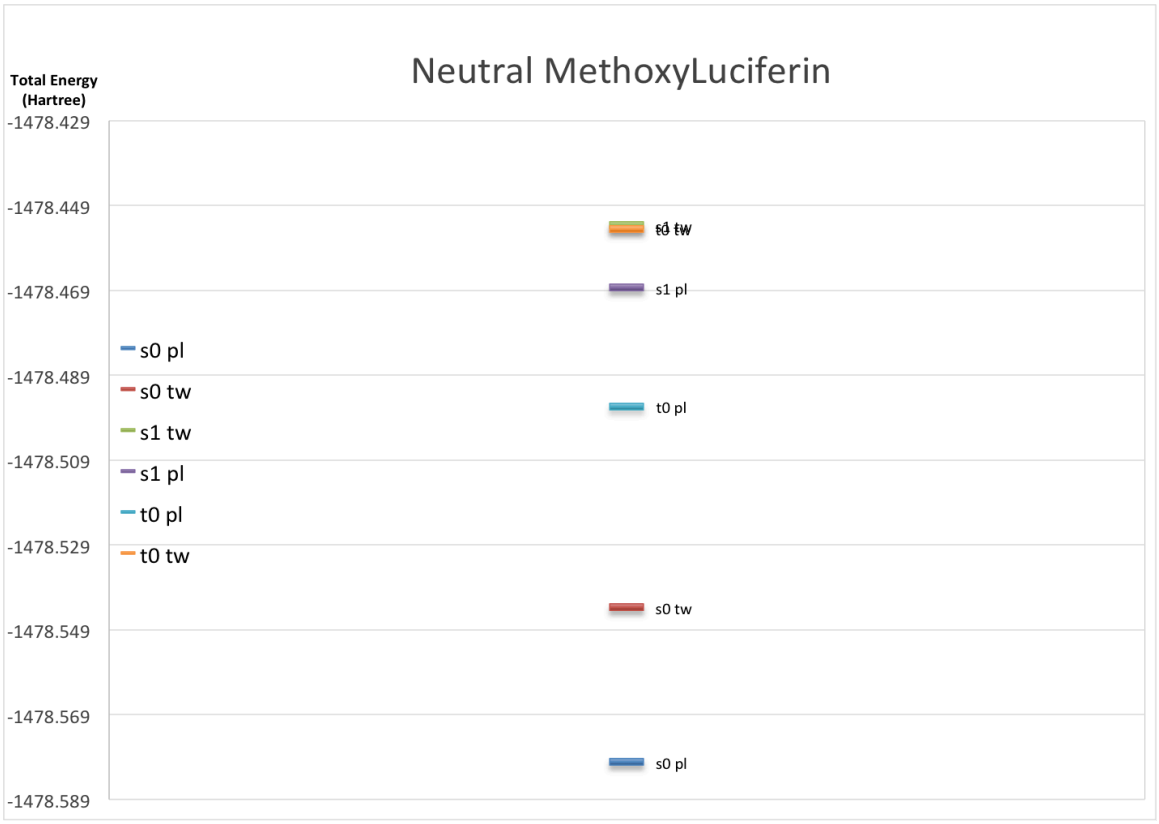
t0 pl
s0 tw
s0 pl


Anionic BromoLuciferin
Total Energy (Hartree)
-4011.69
-4011.71
-4011.73
-4011.75
-4011.77
-4011.79
-4011.81
4'
5'
7'
t0 tw
s1 tw
s1 pl
t0 pl
s0 tw
s0 pl


Neutral MethoxyLuciferin
Total Energy (Hartree)
-1478.429
-1478.449
-1478.469
-1478.489
-1478.509
-1478.529
-1478.549
-1478.569
-1478.589
s0 pl
s0 tw
s1 tw
s1 pl
t0 pl
t0 tw

D-Luc

S0 Energy = -1438.843074343

C -1.2813373 4.2620165 0.9672691
C -0.5639216 3.0090061 1.0785824
C 0.8064982 3.0073406 1.1731258
C 1.5866350 4.2210546 1.1679940
C 0.8995049 5.4641204 1.0601380
C -0.4599930 5.4801449 0.9650070
S 1.9038716 1.6557876 1.3136942
C 3.2596334 2.7958787 1.3508706
N 2.9068526 4.0653135 1.2658870
C 4.5972422 2.3599630 1.4611518
S 5.8813591 3.5980542 1.4854097
N 4.9702370 1.1080088 1.5469792
C 6.3234754 0.9454358 1.6461948
C 7.1002788 2.2773794 1.6302748
O 6.9126056 -0.1140081 1.7399920
O -2.5220026 4.3248825 0.8775392
H -1.0078312 6.4213743 0.8805400
H 1.4886068 6.3838958 1.0560990
H -1.1523024 2.0906698 1.0827860
H 7.7967781 2.2782298 0.7806943
H 7.6821334 2.3670627 2.5575835

S1 Energy = -1438.837364170

C -1.3077364 4.2411522 0.9742593
C -0.5251260 3.0144489 1.0853816
C 0.8732071 3.0787400 1.1717981
C 1.5811853 4.2982977 1.1571969
C 0.8343348 5.4909973 1.0477456
C -0.5449927 5.4702381 0.9599381
S 1.9763984 1.7519350 1.3077014
C 3.3014484 2.9069091 1.3384698
N 2.9533467 4.1697306 1.2536181
C 4.6384353 2.4025237 1.4553801
S 5.9626301 3.5791753 1.4991833
N 4.9516785 1.1310834 1.5337424
C 6.2915119 0.9031962 1.6420835
C 7.1250283 2.2021387 1.6445183
O 6.8391147 -0.1862002 1.7311552
O -2.5568109 4.1882638 0.8988577
H -1.1121422 6.3987922 0.8751294
H 1.3781167 6.4389700 1.0335322
H -1.0664676 2.0675599 1.0986238
H 7.8317080 2.1872338 0.8022538
H 7.7034552 2.2664248 2.5772437

6'–aminoLuc

S0 Energy = -1419.531486458

C -4.4714246 0.6994008 -0.0531430
C -3.3692188 1.5614488 -0.0974595
C -4.2658215 -0.7069461 0.0061684
C -3.0009735 -1.2492622 0.0244840
C -2.0921667 1.0063512 -0.0803884
C 0.2163665 0.2382510 -0.0579573
S -0.5584490 1.8136621 -0.1235692
C 1.6607835 0.1366511 -0.0622214
C 3.7786309 0.8331079 -0.1041887
C 4.0076765 -0.6855881 -0.0437758
S 2.3821488 -1.4681121 -0.0009116
H 4.5888990 -0.9326787 0.8545634
H 4.5767526 -1.0051463 -0.9269342
N 2.4274703 1.1729047 -0.1094693
N -0.5788406 -0.7855947 -0.0088251
C -1.8823847 -0.3974068 -0.0192836
H -3.5184629 2.6409029 -0.1456867
H -2.8488274 -2.3279379 0.0711795
H -5.1385883 -1.3622747 0.0351306
O 4.6868831 1.6208778 -0.1420452
N -5.7511204 1.1900916 -0.1014747
H -5.9101560 2.1741553 0.0461581
H -6.5173909 0.5846211 0.1462701

S1 Energy = -1419.527675377

C -4.4803789 0.6851627 -0.0178088
C -3.3266056 1.5285508 -0.0610676
C -4.3249978 -0.7152743 0.0290246
C -3.0598503 -1.2789556 0.0336751
C -2.0683707 0.9377477 -0.0550975
C 0.2198206 0.1264484 -0.0523619
S -0.5154815 1.7232744 -0.1001641
C 1.6501356 0.0722123 -0.0638300
C 3.7302659 0.8922127 -0.1104362
C 4.0432301 -0.6194627 -0.0588742
S 2.4601755 -1.4950325 -0.0164827
H 4.6396163 -0.8437868 0.8364763
H 4.6276427 -0.9073218 -0.9438741
N 2.3864681 1.1573965 -0.1079582
N -0.6099309 -0.9016371 -0.0076986

C -1.9050211 -0.4652489 -0.0080012
H -3.4498270 2.6122753 -0.0984732
H -2.9332101 -2.3617129 0.0698932
H -5.2111955 -1.3504931 0.0610567
O 4.6238033 1.7152787 -0.1476353
N -5.7079766 1.2648930 -0.0238185
H -5.8180548 2.2669642 -0.0557480
H -6.5484712 0.7079876 0.0058242

6'–MeNHLH$_2$

S0 Energy = -1458.761653271
C -4.4872976 0.6696379 -0.0489409
C -3.3824353 1.5327374 -0.1473878
C -4.2720884 -0.7304882 0.0981780
C -2.9995231 -1.2591416 0.1461942
C -2.1052672 0.9900004 -0.0985360
C 0.2091789 0.2348838 -0.0417864
S -0.5742369 1.8011763 -0.1969441
C 1.6531610 0.1391059 -0.0510849
C 3.7681740 0.8399187 -0.1370161
C 4.0035162 -0.6744100 -0.0183228
S 2.3812831 -1.4597322 0.0727991
H 4.5957110 -0.8835664 0.8824064
H 4.5639884 -1.0267934 -0.8945450
N 2.4162632 1.1749752 -0.1482248
N -0.5804386 -0.7877926 0.0730776
C -1.8861072 -0.4084055 0.0475822
H -3.5376057 2.6067408 -0.2618215
H -2.8411273 -2.3321072 0.2603661
H -5.1294213 -1.3979398 0.1745117
O 4.6738481 1.6285687 -0.2099286
N -5.7542279 1.1765487 -0.0942315
C -6.9503067 0.3858882 -0.0248385
H -5.8519107 2.1722670 -0.2066495
H -7.0154443 -0.3411765 -0.8521188
H -7.8189738 1.0492230 -0.0928716
H -7.0241598 -0.1697600 0.9249636

S1 Energy = -1458.758090104
C -4.4940452 0.6138937 -0.0458721
C -3.3500759 1.4627083 -0.1364094
C -4.3265676 -0.7776759 0.0912730
C -3.0503608 -1.3241779 0.1393935
C -2.0836445 0.8894190 -0.0874074
C 0.2132737 0.1040229 -0.0300020
S -0.5432916 1.6872872 -0.1817513
C 1.6443727 0.0696163 -0.0445610
C 3.7108312 0.9173472 -0.1637966
C 4.0492132 -0.5815949 -0.0108331
S 2.4807752 -1.4770004 0.1024370
H 4.6543218 -0.7338128 0.8936643
H 4.6333114 -0.9197773 -0.8779575
N 2.3633530 1.1606597 -0.1676050
N -0.6032185 -0.9254865 0.0826763
C -1.9065876 -0.5057360 0.0515846
H -3.4831241 2.5410358 -0.2431797
H -2.9132055 -2.4009690 0.2454444
H -5.1955703 -1.4295292 0.1595461
O 4.5903845 1.7502028 -0.2662265
N -5.7219068 1.2035500 -0.0986606
C -6.9633152 0.4926620 -0.0284835
H -5.7527764 2.2078640 -0.1987465
H -7.0532381 -0.2347449 -0.8530727
H -7.7923929 1.2032438 -0.0971100
H -7.0519632 -0.0626493 0.9204859

6'–Me$_2$NLH$_2$

S0 Energy = -1497.990421989
C -4.4861974 0.7491604 -0.0910281
C -3.3611606 1.5968276 -0.1029254
C -4.2810704 -0.6653136 -0.0660703
C -3.0199584 -1.2164225 -0.0494197
C -2.0926843 1.0261819 -0.0865954
C 0.2120281 0.2425126 -0.0606940
S -0.5521773 1.8244503 -0.0965605
C 1.6546717 0.1319701 -0.0515171
C 3.7772472 0.8162629 -0.0572887
C 3.9971041 -0.7046679 -0.0201194
S 2.3667505 -1.4782345 -0.0106258
H 4.5657277 -0.9700488 0.8810927
H 4.5755926 -1.0121816 -0.9014106
N 2.4291049 1.1640647 -0.0717082
N -0.5910980 -0.7771687 -0.0435917
C -1.8908190 -0.3788969 -0.0578753
H -3.4715258 2.6777862 -0.1257107
H -2.8823335 -2.2979416 -0.0301953
H -5.1408846 -1.3315481 -0.0599715
O 4.6912395 1.5987041 -0.0718205
N -5.7553110 1.2633106 -0.1035751
C -6.9004261 0.3869323 -0.1106124
H -6.9294967 -0.2594303 0.7820583
H -6.9190932 -0.2614487 -1.0026626

H -7.8138926 0.9887326 -0.1156257
C -5.9470514 2.6908924 -0.1349602
H -5.5047395 3.1446984 -1.0383141
H -5.4994370 3.1821005 0.7455521
H -7.0177921 2.9136213 -0.1359343

S1 Energy = -1497.987259200
C -4.4966845 0.7060184 -0.0951344
C -3.3379064 1.5324563 -0.1030681
C -4.3377266 -0.7006779 -0.0714968
C -3.0750048 -1.2718974 -0.0554425
C -2.0812256 0.9327865 -0.0856622
C 0.2064057 0.1197652 -0.0591166
S -0.5299475 1.7200821 -0.0907584
C 1.6380950 0.0676805 -0.0498289
C 3.7165129 0.8948966 -0.0549151
C 4.0342412 -0.6157478 -0.0211218
S 2.4534342 -1.4965669 -0.0141354
H 4.6189543 -0.8505449 0.8791625
H 4.6322186 -0.8879359 -0.9019022
N 2.3720938 1.1552380 -0.0672201
N -0.6200908 -0.9069333 -0.0462647
C -1.9188294 -0.4680740 -0.0610814
H -3.4222004 2.6165555 -0.1228028
H -2.9600356 -2.3561791 -0.0384609
H -5.2079783 -1.3521217 -0.0662337
O 4.6074021 1.7221357 -0.0680827
N -5.7431889 1.2882624 -0.1110534
C -6.9170887 0.4558623 -0.1048516
H -6.9465014 -0.1815247 0.7945456
H -6.9379717 -0.2073221 -0.9857761
H -7.8124491 1.0821284 -0.1182652
C -5.8948431 2.7218790 -0.1370610
H -5.4332203 3.1604881 -1.0369026
H -5.4356909 3.1923887 0.7476793
H -6.9584563 2.9718076 -0.1428581

CycLuc1
S0 Energy = -1496.809364595
C -4.5332049 0.8700070 0.1884754
C -3.4533613 1.7437545 0.1266118
C -4.3651522 -0.5455850 0.2133999
C -3.1167863 -1.1115332 0.1776570
C -2.1843145 1.1564577 0.0896171
C 0.1120877 0.3549146 0.0035415
S -0.6418683 1.9392121 0.0022762
C 1.5514649 0.2322211 -0.0585663
C 3.6773770 0.8961032 -0.1735707
C 3.8849595 -0.6267986 -0.1410293
S 2.2501354 -1.3848422 -0.0472983
H 4.4949910 -0.8940398 0.7320955
H 4.4162729 -0.9430566 -1.0485217
N 2.3340119 1.2567174 -0.1214294
N -0.6993542 -0.6578692 0.0621271
C -1.9952502 -0.2539636 0.1118390
H -3.5905528 2.8247393 0.1037956
H -2.9647913 -2.1914208 0.1969222
O 4.5971562 1.6694522 -0.2374958
N -5.8720096 1.1721768 0.2532084
C -6.6725631 -0.0121654 -0.0344857
H -6.2017164 2.0817157 -0.0338749
C -5.7307858 -1.1718301 0.3314280
H -6.9422776 -0.0552014 -1.1051920
H -7.6014056 -0.0146429 0.5505426
H -5.8687522 -2.0462145 -0.3170772
H -5.9041856 -1.4993389 1.3695036

S1 Energy = -1496.805965042
C -4.5188303 0.8448987 0.0649123
C -3.4057626 1.7038943 -0.0120338
C -4.3933374 -0.5645303 0.1453505
C -3.1441156 -1.1508470 0.1515579
C -2.1554606 1.0885034 -0.0049445
C 0.1249134 0.2489555 -0.0108181
S -0.5914225 1.8572849 -0.0896190
C 1.5596392 0.1717704 -0.0422211
C 3.6502853 0.9576060 -0.1443735
C 3.9407244 -0.5561388 -0.0564846
S 2.3452184 -1.4049256 0.0351680
H 4.5484552 -0.7654240 0.8349124
H 4.5062645 -0.8768773 -0.9425104
N 2.3087500 1.2431367 -0.1275050
N -0.7045317 -0.7689543 0.0710004
C -2.0034705 -0.3225669 0.0759388
H -3.5234551 2.7860514 -0.0737316
H -3.0096882 -2.2313761 0.2125619
O 4.5534279 1.7670832 -0.2187222
N -5.8340155 1.1946670 0.0757037
C -6.7191482 0.0498699 0.1600770
H -6.1648151 2.1465673 0.0261097
C -5.7705014 -1.1687788 0.2125729
H -7.3916321 0.0217248 -0.7129199

H -7.3574459 0.1257051 1.0554184
H -5.9546457 -1.8571504 -0.6255961
H -5.9152757 -1.7511800 1.1346954

4'–BrLuc

S0 Energy = -4011.790393777

O -6.8448625 3.5066263 0.0000277
C -5.7550987 2.8962422 -0.0001237
C -4.4743511 3.5689170 -0.0003715
C -3.3128202 2.8399157 -0.0005227
C -3.2717813 1.3958513 -0.0004524
C -4.5269237 0.7361945 -0.0002101
C -5.7085744 1.4350660 -0.0000525
S -1.6680617 3.4417652 -0.0008231
C -1.0904728 1.7475828 -0.0008238
N -2.0679365 0.8190991 -0.0006187
C 0.2372386 1.4631867 -0.0010187
S 0.7687657 -0.3190377 -0.0010166
N 1.2835062 2.2978614 -0.0012224
C 2.4933628 1.7402297 -0.0013884
C 2.4823412 0.1899125 -0.0013115
H 3.0104887 -0.1843149 0.8905541
H 3.0102020 -0.1844092 -0.8933036
O 3.5877124 2.3056246 -0.0015908
H -4.4739261 4.6604285 -0.0004295
H -6.6651359 0.9136911 0.0001339
Br -4.5378820 -1.1629725 -0.0001056

S1 Energy = -4011.710603849723

O -6.7829107 3.6274877 -0.0000102
C -5.7500840 2.9238810 -0.0001261
C -4.4317689 3.5471242 -0.0003866
C -3.2788420 2.7497812 -0.0005274
C -3.3110194 1.3393784 -0.0004453
C -4.5919987 0.7416342 -0.0001902
C -5.7608189 1.4779501 -0.0000238
S -1.6406106 3.3008724 -0.0008041
C -1.1150913 1.6245661 -0.0007993
N -2.0771422 0.7305339 -0.0006072
C 0.2923759 1.3577569 -0.0010236
S 0.8186614 -0.3320233 -0.0011378
N 1.2191917 2.2868683 -0.0011642
C 2.4891504 1.7898465 -0.0013885
C 2.5314880 0.2469192 -0.0013424
H 3.0703789 -0.1065825 0.8892589
H 3.0702231 -0.1066770 -0.8920082
O 3.5235144 2.4402342 -0.0015856
H -4.3849815 4.6365284 -0.0004720
H -6.7312604 0.9838975 0.0001866
Br -4.6826752 -1.1525073 -0.0000731

5'–BrLuc

S0 Energy = -4011.801518284

O -6.7891639 3.5566622 -0.0032265
C -5.7123659 2.9455578 -0.0020496
C -4.4263046 3.6087163 -0.0048395
C -3.2568236 2.8885796 -0.0034073
C -3.2398164 1.4495888 0.0009261
C -4.4786221 0.7552349 0.0037855
C -5.6411938 1.4692353 0.0023466
S -1.6061438 3.4546740 -0.0061729
C -1.0610640 1.7704864 -0.0014388
N -2.0314378 0.8797553 0.0018765
C 0.3135098 1.4331837 -0.0013169
S 0.7500242 -0.2931721 0.0036376
N 1.2894669 2.3021346 -0.0046127
C 2.5322110 1.7265635 -0.0037090
C 2.4881321 0.1859963 0.0010092
H 3.0086956 -0.1874802 0.8932456
H 3.0074937 -0.1929026 -0.8896401
O 3.5923193 2.3188898 -0.0062399
H -4.4424913 4.6989112 -0.0081132
Br -7.2975886 0.5503267 0.0061108
H -4.4737861 -0.3346915 0.0070588

S1 Energy = -4011.796387194

O -6.7437212 3.6772546 -0.0031658
C -5.7186254 2.9697383 -0.0018696
C -4.3954213 3.5818131 -0.0044834
C -3.2377910 2.7950392 -0.0030318
C -3.2846579 1.3868124 0.0009242
C -4.5476541 0.7623570 0.0035154
C -5.7058689 1.5174540 0.0022117
S -1.5935900 3.3370327 -0.0058902
C -1.0840590 1.6554509 -0.0015344
N -2.0491522 0.7671677 0.0016384
C 0.3244271 1.3753295 -0.0014447
S 0.8310340 -0.3204403 0.0041211
N 1.2604039 2.2930692 -0.0052328
C 2.5258156 1.7815085 -0.0041678
C 2.5505003 0.2384339 0.0013366
H 3.0856052 -0.1180970 0.8930933

H 3.0847145 -0.1245141 -0.8883520
O 3.5668260 2.4206560 -0.0070476
H -4.3611156 4.6716393 -0.0076101
Br -7.3876280 0.6456411 0.0056586
H -4.5949914 -0.3270960 0.0065609

7'–BrLuc
S0 Energy = -4011.759897560
O -6.8117381 3.4595796 0.0029335
C -5.7216478 2.8707448 0.0013385
C -4.4348731 3.5452103 0.0045764
C -3.2531015 2.8403188 0.0026474
C -3.2340551 1.4008789 -0.0027133
C -4.4737326 0.7034700 -0.0060358
C -5.6422345 1.4048733 -0.0040951
S -1.6157756 3.4241695 0.0058077
C -1.0583751 1.7446135 -0.0001276
N -2.0207710 0.8437951 -0.0040662
C 0.3182677 1.4165633 -0.0005116
S 0.7643403 -0.3082045 -0.0066373
N 1.2897944 2.2900858 0.0033428
C 2.5355001 1.7211731 0.0020153
C 2.4996733 0.1802397 -0.0037318
H 3.0212415 -0.1961605 0.8866843
H 3.0223923 -0.1895294 -0.8962514
O 3.5926833 2.3185410 0.0048960
Br -4.4145662 5.4330358 0.0116693
H -4.4573940 -0.3881333 -0.0100862
H -6.6069278 0.8930852 -0.0065650

S1 Energy = -4011.795056788
O -6.7843515 3.5880324 0.0033296
C -5.7447304 2.9044070 0.0014106
C -4.4133815 3.5294764 0.0045355
C -3.2306306 2.7536740 0.0023402
C -3.2794805 1.3475690 -0.0029263
C -4.5444281 0.7235825 -0.0060107
C -5.7124689 1.4607122 -0.0039673
S -1.5979460 3.3016099 0.0053605
C -1.0783132 1.6198572 -0.0006018
N -2.0425238 0.7318175 -0.0044872
C 0.3286271 1.3500736 -0.0007245
S 0.8529965 -0.3429053 -0.0066833
N 1.2604692 2.2750099 0.0033161
C 2.5278859 1.7751634 0.0022345
C 2.5671773 0.2317636 -0.0034626
H 3.1050927 -0.1254000 0.8863331
H 3.1061519 -0.1188499 -0.8952208
O 3.5658623 2.4214073 0.0053212
Br -4.3248798 5.3856081 0.0115190
H -4.5783246 -0.3683564 -0.0101170
H -6.6841035 0.9640973 -0.0064089

6'–deoxyLuc
S0 Energy = -1364.262556246
C -3.8887853 0.5411853 0.0180407
C -3.9813083 -0.8641396 0.0333510
C -2.8472751 -1.6632313 0.0353918
H -4.9657325 -1.3351363 0.0428777
C -2.6586667 1.1731746 0.0050638
H -4.8026377 1.1368910 0.0154811
C -1.4949478 0.3864087 0.0077234
H -2.5681963 2.2595490 -0.0081928
C -1.6014809 -1.0284963 0.0224105
H -2.9301269 -2.7502593 0.0459045
N -0.2139126 0.8614673 -0.0042680
S -0.0171715 -1.7323308 0.0190287
C 0.6414379 -0.1116984 -0.0006608
C 2.0829149 0.0893377 -0.0129804
S 2.6897696 1.7383480 -0.0324891
N 2.9128232 -0.8948249 -0.0102107
C 4.3652767 1.0675144 -0.0392240
C 4.2416239 -0.4637484 -0.0226020
H 4.9265511 1.3996161 0.8443366
H 4.9118806 1.3813354 -0.9385757
O 5.1979637 -1.1909620 -0.0204063

S1 Energy = -1364.230020612
C -3.8559532 0.5712146 0.0087144
C -3.9801107 -0.8276326 -0.0767330
C -2.8552328 -1.6466446 -0.0950343
H -4.9723293 -1.2785667 -0.1279337
C -2.6136292 1.1765583 0.0796964
H -4.7565146 1.1873864 0.0202942
C -1.4550570 0.3728865 0.0672685
H -2.5053782 2.2595641 0.1460202
C -1.6012735 -1.0435992 -0.0229363
H -2.9574920 -2.7302281 -0.1616933
N -0.1826011 0.8234438 0.1372677
S -0.0334010 -1.7961254 -0.0189254
C 0.6835525 -0.1825684 0.0994154
C 2.0575145 0.0104766 0.1765614

S 2.7004163 1.6696698 0.3979463
N 3.0050565 -0.9447297 0.1866544
C 4.2720697 1.0967426 -0.1928708
C 4.2224603 -0.4725259 0.1487035
H 5.1188655 1.6225679 0.2616548
H 4.3679470 1.0850764 -1.2904330
O 5.3410903 -0.9529663 0.2563626

6'–methoxyLuc

S0 Energy = -1478.585905861
C -1.9457970 4.8111594 0.6845139
C -0.6014930 5.0305282 0.4054565
C -2.5278098 3.6288309 0.2233707
C -1.7700652 2.6829162 -0.5062453
C -0.4334305 2.9108234 -0.7785140
C 0.1743274 4.0905754 -0.3264205
H -2.2352423 1.7647298 -0.8604312
S 0.3912284 6.3856428 0.8378426
N 1.4763897 4.4471802 -0.5197342
C 1.7296041 5.5970213 0.0209326
C 3.0327088 6.2358887 -0.0217056
N 3.2560264 7.3808234 0.5256109
S 4.3402938 5.4041219 -0.8533971
C 5.4209665 6.7867227 -0.4324256
C 4.5770564 7.7980662 0.3590192
H 5.8164506 7.2677286 -1.3369697
H 6.2664107 6.4530192 0.1838765
O 5.0296732 8.8297438 0.7783827
H 0.1572773 2.1868500 -1.3402255
H -2.5554427 5.5199750 1.2436690
O -3.8308072 3.4632503 0.5189803
C -4.4987251 2.3008076 0.0942555
H -5.5301848 2.3930159 0.4477822
H -4.0472212 1.3953547 0.5305478
H -4.4999112 2.2162513 -1.0043316

S1 Energy = -1478.580085938
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C -0.5388785 4.9784218 0.3602200
C -2.5226898 3.5843639 0.1975980
C -1.8064591 2.6305781 -0.5233447
C -0.4547536 2.8521572 -0.8025455
C 0.2029577 4.0230497 -0.3696471
H -2.2836720 1.7170192 -0.8717882
S 0.4740507 6.3254358 0.7825550
N 1.5078183 4.3379954 -0.5857000
C 1.8023033 5.5113490 -0.0448518
C 3.0585498 6.1795059 -0.0584263
N 3.2276935 7.3498519 0.5172052
S 4.4434269 5.4342124 -0.8657924
C 5.4314980 6.8726580 -0.3890814
C 4.5033062 7.8273186 0.3954081
H 5.8282793 7.3874346 -1.2750250
H 6.2774763 6.5748132 0.2457066
O 4.9219653 8.8806493 0.8346122
H 0.1181310 2.1142760 -1.3659913
H -2.4780628 5.5069822 1.2143548
O -3.8099637 3.4991991 0.5308661
C -4.5550478 2.3653572 0.1431859
H -5.5683465 2.5186056 0.5234362
H -4.1262099 1.4509222 0.5814733
H -4.5780176 2.2726754 -0.9536606

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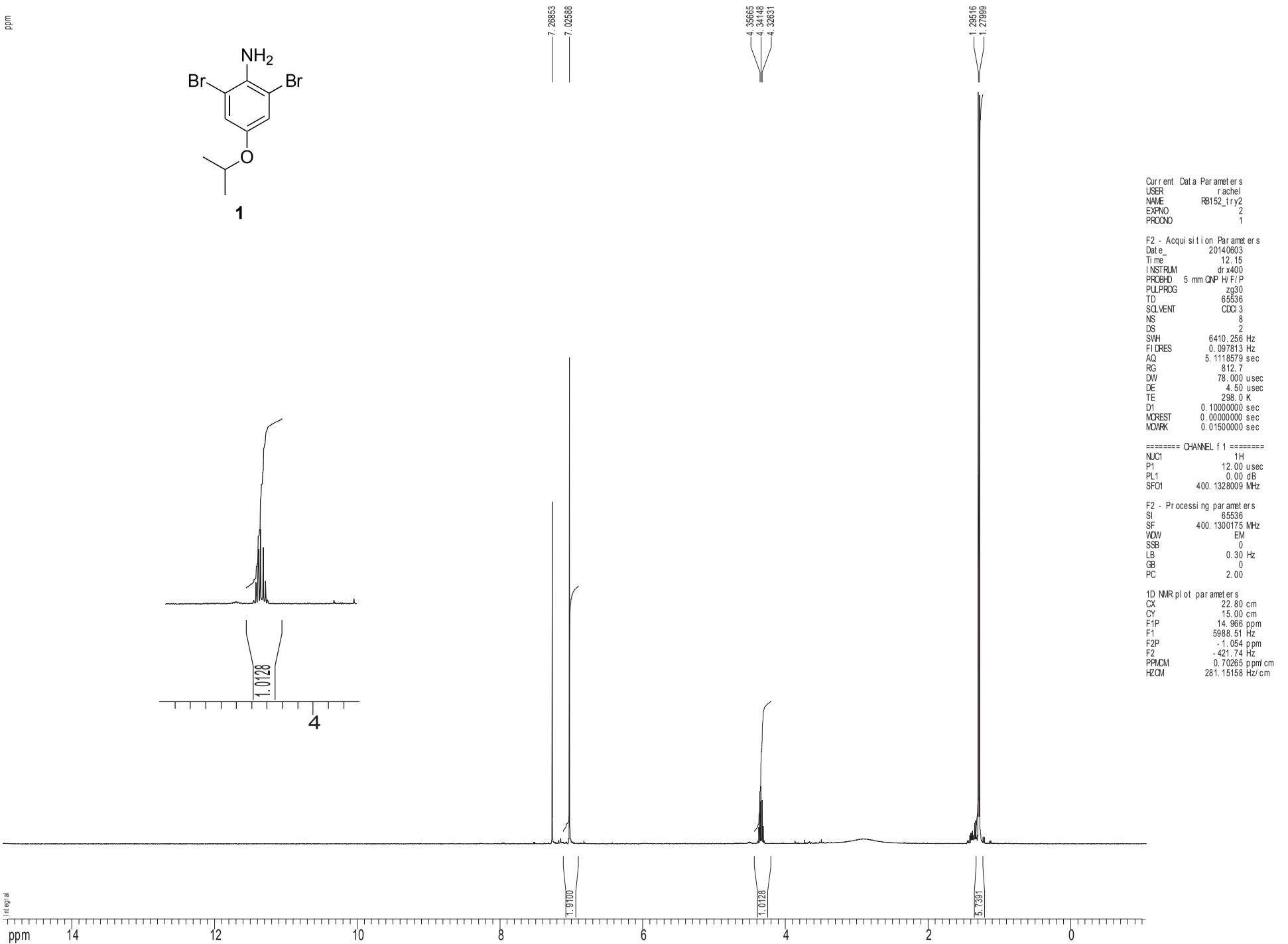
1H spectrum



Current Data Parameters
USER rachel
NAME RB152_try2
EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
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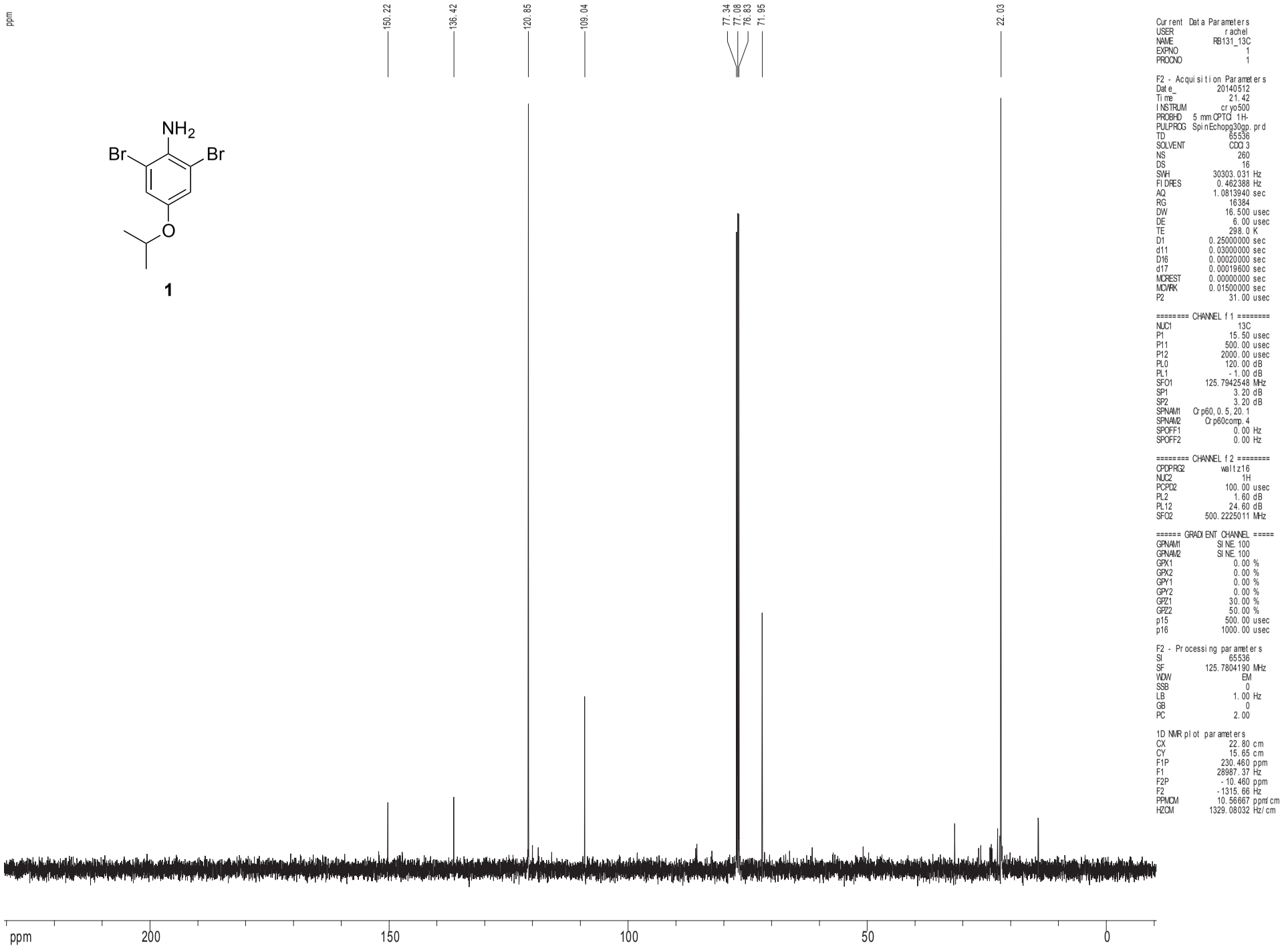
INSTRUM drx400
PROBHD 5 mm QNP H/F/P
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 8
DS 2
SWH 6410.256 Hz
FIDRES 0.097813 Hz
AQ 5.1118579 sec
RG 812.7
DW 78.000 usec
DE 4.50 usec
TE 298.0 K
D1 0.10000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec

======== CHANNEL f1 ========
NUC1 1H
P1 12.00 usec
PL1 0.00 dB
SFO1 400.1328009 MHz

F2 - Processing parameters
SI 65536
SF 400.1300175 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 2.00

1D NMR plot parameters
CX 22.80 cm
CY 15.00 cm
F1P 14.966 ppm
F1 5988.51 Hz
F2P -1.054 ppm
F2 -421.74 Hz
PPMCM 0.70265 ppm/cm
HZCM 281.15158 Hz/cm

Z-restored spin-echo 13C spectrum with 1H decoupling



Current Data Parameters
USER rachel
NAME RB131_13C
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
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Time 21.42
INSTRUM cryo500
PROBHD 5 mm CPTCI 1H-
PULPROG SpinEchopg30gp.prd
TD 65536
SOLVENT CDCl3
NS 260
DS 16
SWH 30303.031 Hz
FIDRES 0.462388 Hz
AQ 1.0813940 sec
RG 16384
DW 16.500 usec
DE 6.00 usec
TE 298.0 K
D1 0.25000000 sec
d11 0.03000000 sec
D16 0.00020000 sec
d17 0.00019600 sec
MOREST 0.00000000 sec
MOWRK 0.01500000 sec
P2 31.00 usec

======== CHANNEL f1 ========
NUC1 13C
P1 15.50 usec
P11 500.00 usec
P12 2000.00 usec
PL0 120.00 dB
PL1 -1.00 dB
SFO1 125.7942548 MHz
SP1 3.20 dB
SP2 3.20 dB
SPNAM1 Crp60,0.5,20.1
SPNAM2 Crp60comp.4
SPOFF1 0.00 Hz
SPOFF2 0.00 Hz

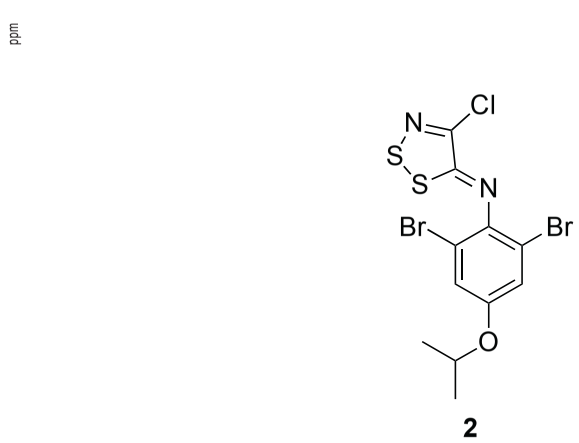
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 100.00 usec
PL2 1.60 dB
PL12 24.60 dB
SFO2 500.2225011 MHz

====== GRADIENT CHANNEL =====
GPNAM1 SINE.100
GPNAM2 SINE.100
GPX1 0.00 %
GPX2 0.00 %
GPY1 0.00 %
GPY2 0.00 %
GPZ1 30.00 %
GPZ2 50.00 %
p15 500.00 usec
p16 1000.00 usec

F2 - Processing parameters
SI 65536
SF 125.7804190 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 2.00

1D NMR plot parameters
CX 22.80 cm
CY 15.65 cm
F1P 230.460 ppm
F1 28987.37 Hz
F2P -10.460 ppm
F2 -1315.66 Hz
PPMCM 10.56667 ppm/cm
HZCM 1329.08032 Hz/cm

1H spectrum



2

Current Data Parameters
USER rachel
NAME rb255_pump2
EXPNO 1
PROCNO 1

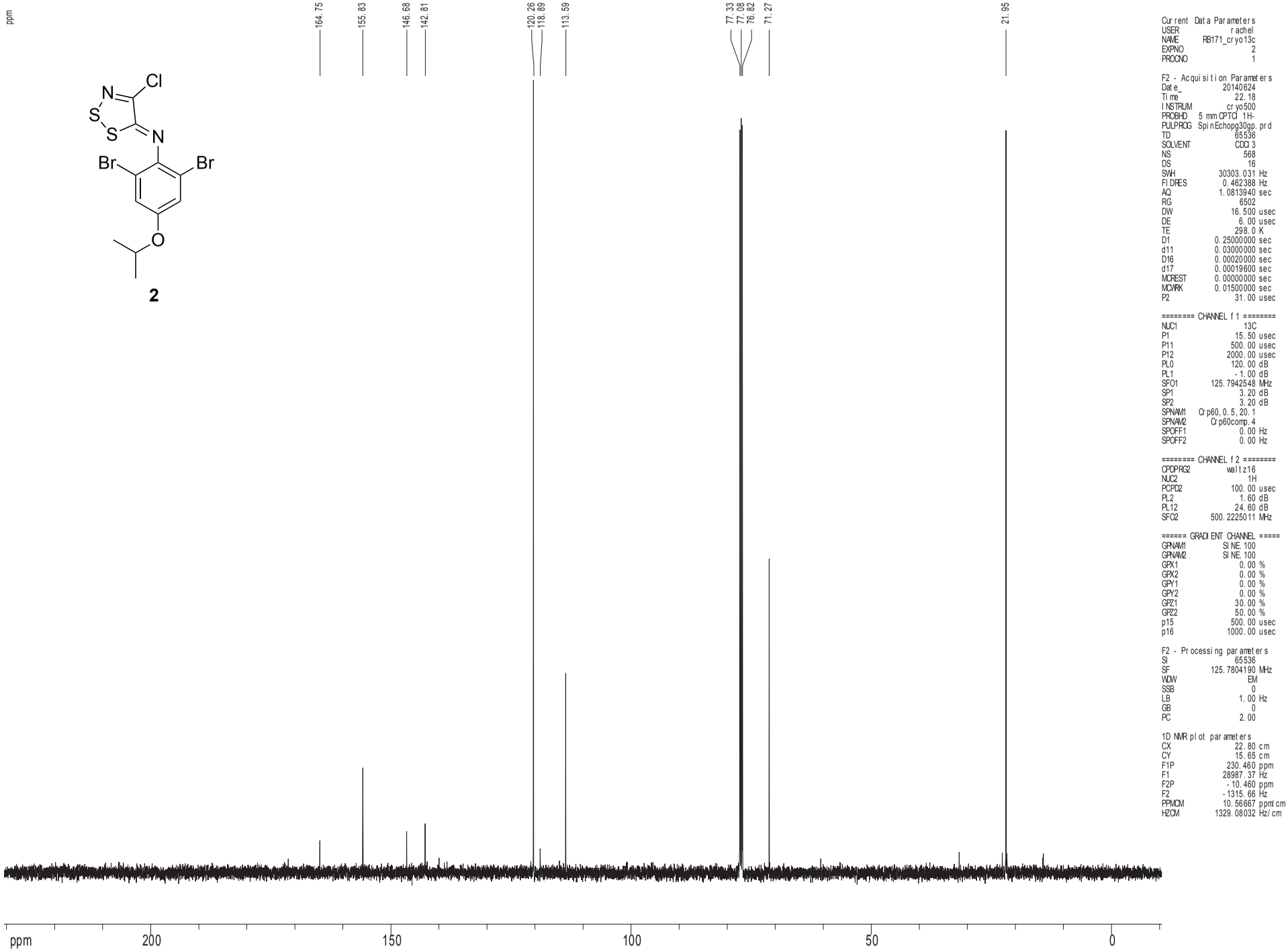
F2 - Acquisition Parameters
Date_ 20141001
Time 10.52
INSTRUM drx400
PROBHD 5 mm QNP H/F/P
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 8
DS 2
SWH 6410.256 Hz
FIDRES 0.097813 Hz
AQ 5.1118579 sec
RG 1024
DW 78.000 usec
DE 4.50 usec
TE 297.9 K
D1 0.10000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec

======== CHANNEL f1 ========
NUC1 1H
P1 12.00 usec
PL1 0.00 dB
SFO1 400.1328009 MHz

F2 - Processing parameters
SI 65536
SF 400.1300175 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 2.00

1D NMR plot parameters
CX 22.80 cm
CY 15.00 cm
F1P 14.966 ppm
F1 5988.51 Hz
F2P -1.054 ppm
F2 -421.74 Hz
PPMCM 0.70265 ppm/cm
HZCM 281.15158 Hz/cm

Z-restored spin-echo 13C spectrum with 1H decoupling



Current Data Parameters
USER rachel
NAME RB171_cryo13c
EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
Date_ 20140624
Time 22.18
INSTRUM cryo500
PROBHD 5 mm CPTCI 1H-
PULPROG SpinEchopg30gp.prd
TD 65536
SOLVENT CDCl3
NS 568
DS 16
SWH 30303.031 Hz
FIDRES 0.462388 Hz
AQ 1.0813940 sec
RG 6502
DW 16.500 usec
DE 6.00 usec
TE 298.0 K
D1 0.25000000 sec
d11 0.03000000 sec
D16 0.00020000 sec
d17 0.00019600 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
P2 31.00 usec

======== CHANNEL f1 ========
NUC1 13C
P1 15.50 usec
P11 500.00 usec
P12 2000.00 usec
PL0 120.00 dB
PL1 -1.00 dB
SFO1 125.7942548 MHz
SP1 3.20 dB
SP2 3.20 dB
SPNAM1 Crp60,0.5,20.1
SPNAM2 Crp60comp.4
SPOFF1 0.00 Hz
SPOFF2 0.00 Hz

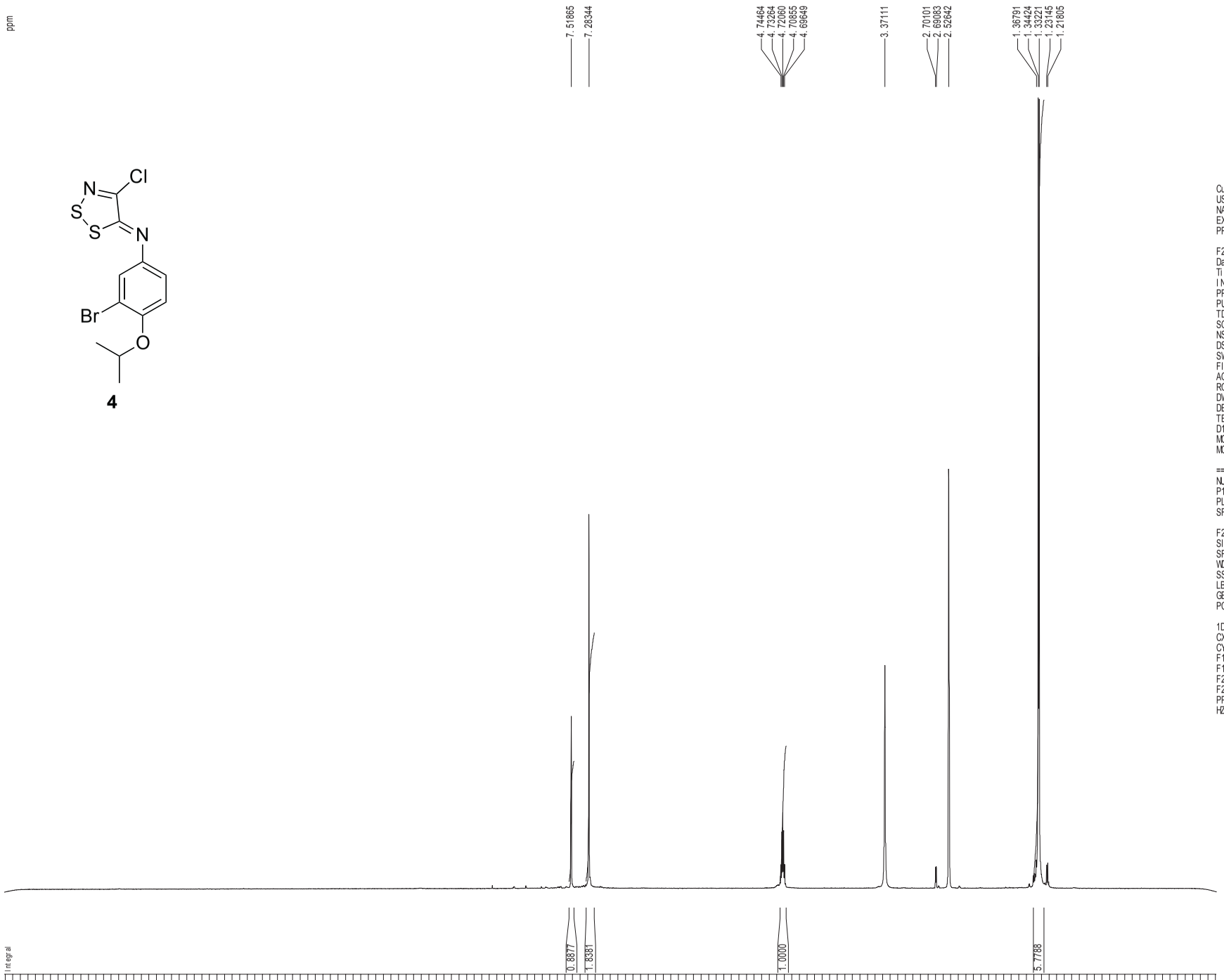
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 100.00 usec
PL2 1.60 dB
PL12 24.60 dB
SFO2 500.2225011 MHz

====== GRADIENT CHANNEL =====
GPNAM1 SINE.100
GPNAM2 SINE.100
GPX1 0.00 %
GPX2 0.00 %
GPY1 0.00 %
GPY2 0.00 %
GPZ1 30.00 %
GPZ2 50.00 %
p15 500.00 usec
p16 1000.00 usec

F2 - Processing parameters
SI 65536
SF 125.7804190 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 2.00

1D NMR plot parameters
CX 22.80 cm
CY 15.65 cm
F1P 230.460 ppm
F1 28987.37 Hz
F2P -10.460 ppm
F2 -1315.66 Hz
PPMCM 10.56667 ppm/cm
HZCM 1329.08032 Hz/cm

1H spectrum



4

Current Data Parameters
USER rachel
NAME RB282_1hcryo
EXPNO 2
PROCNO 1

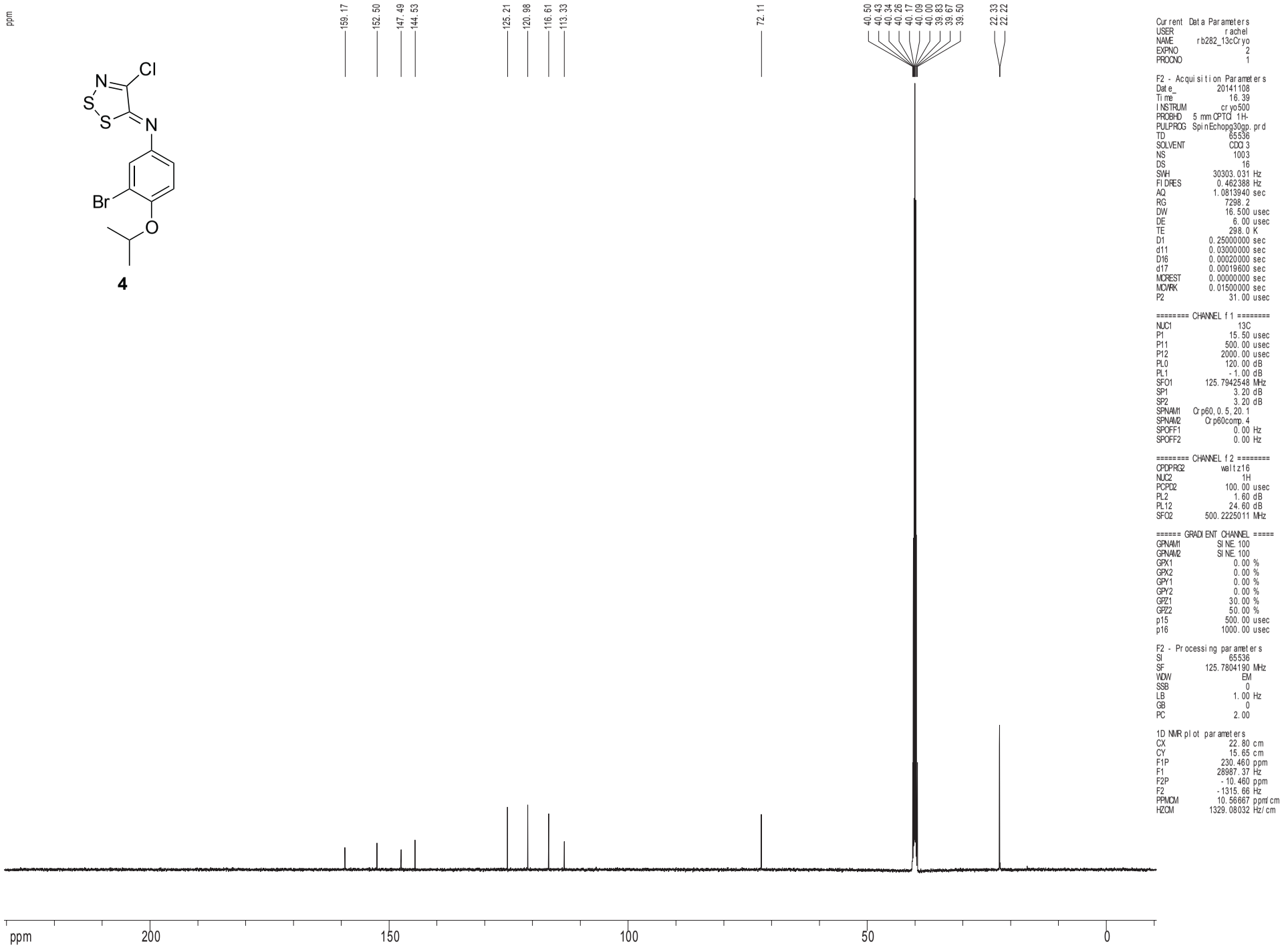
F2 - Acquisition Parameters
Date_ 20141108
Time 16.33
INSTRUM cryo500
PROBHD 5 mm CPTCI 1H-
PULPROG zg30
TD 81728
SOLVENT DMSO
NS 8
DS 2
SWH 8012.820 Hz
FIDRES 0.098043 Hz
AQ 5.0998774 sec
RG 16
DW 62.400 usec
DE 6.00 usec
TE 298.0 K
D1 0.10000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec

======== CHANNEL f1 ========
NUC1 1H
P1 7.50 usec
PL1 1.60 dB
SFO1 500.2235015 MHz

F2 - Processing parameters
SI 65536
SF 500.2200000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 4.00

1D NMR plot parameters
CX 22.80 cm
CY 15.00 cm
F1P 15.009 ppm
F1 7507.95 Hz
F2P -1.009 ppm
F2 -504.87 Hz
PPMCM 0.70257 ppm/cm
HZCM 351.43951 Hz/cm

Z-restored spin-echo 13C spectrum with 1H decoupling



Current Data Parameters
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NAME rb282_13cCryo
EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
Date_ 20141108
Time 16.39
INSTRUM cryo500
PROBHD 5 mm CPTCI 1H-
PULPROG SpinEchopg30gp.prd
TD 65536
SOLVENT CDCl3
NS 1003
DS 16
SWH 30303.031 Hz
FIDRES 0.462388 Hz
AQ 1.0813940 sec
RG 7298.2
DW 16.500 usec
DE 6.00 usec
TE 298.0 K
D1 0.25000000 sec
d11 0.03000000 sec
D16 0.00020000 sec
d17 0.00019600 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
P2 31.00 usec

======== CHANNEL f1 ========
NUC1 13C
P1 15.50 usec
P11 500.00 usec
P12 2000.00 usec
PL0 120.00 dB
PL1 -1.00 dB
SFO1 125.7942548 MHz
SP1 3.20 dB
SP2 3.20 dB
SPNAM1 Crp60,0.5,20.1
SPNAM2 Crp60comp.4
SPOFF1 0.00 Hz
SPOFF2 0.00 Hz

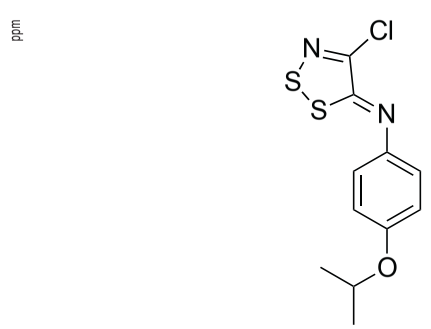
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 100.00 usec
PL2 1.60 dB
PL12 24.60 dB
SFO2 500.2225011 MHz

====== GRADIENT CHANNEL =====
GPNAM1 SINE.100
GPNAM2 SINE.100
GPX1 0.00 %
GPX2 0.00 %
GPY1 0.00 %
GPY2 0.00 %
GPZ1 30.00 %
GPZ2 50.00 %
p15 500.00 usec
p16 1000.00 usec

F2 - Processing parameters
SI 65536
SF 125.7804190 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 2.00

1D NMR plot parameters
CX 22.80 cm
CY 15.65 cm
F1P 230.460 ppm
F1 28987.37 Hz
F2P -10.460 ppm
F2 -1315.66 Hz
PPMCM 10.56667 ppm/cm
HZCM 1329.08032 Hz/cm

1H spectrum



6

Current Data Parameters
USER rachel
NAME rb257_dmso
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
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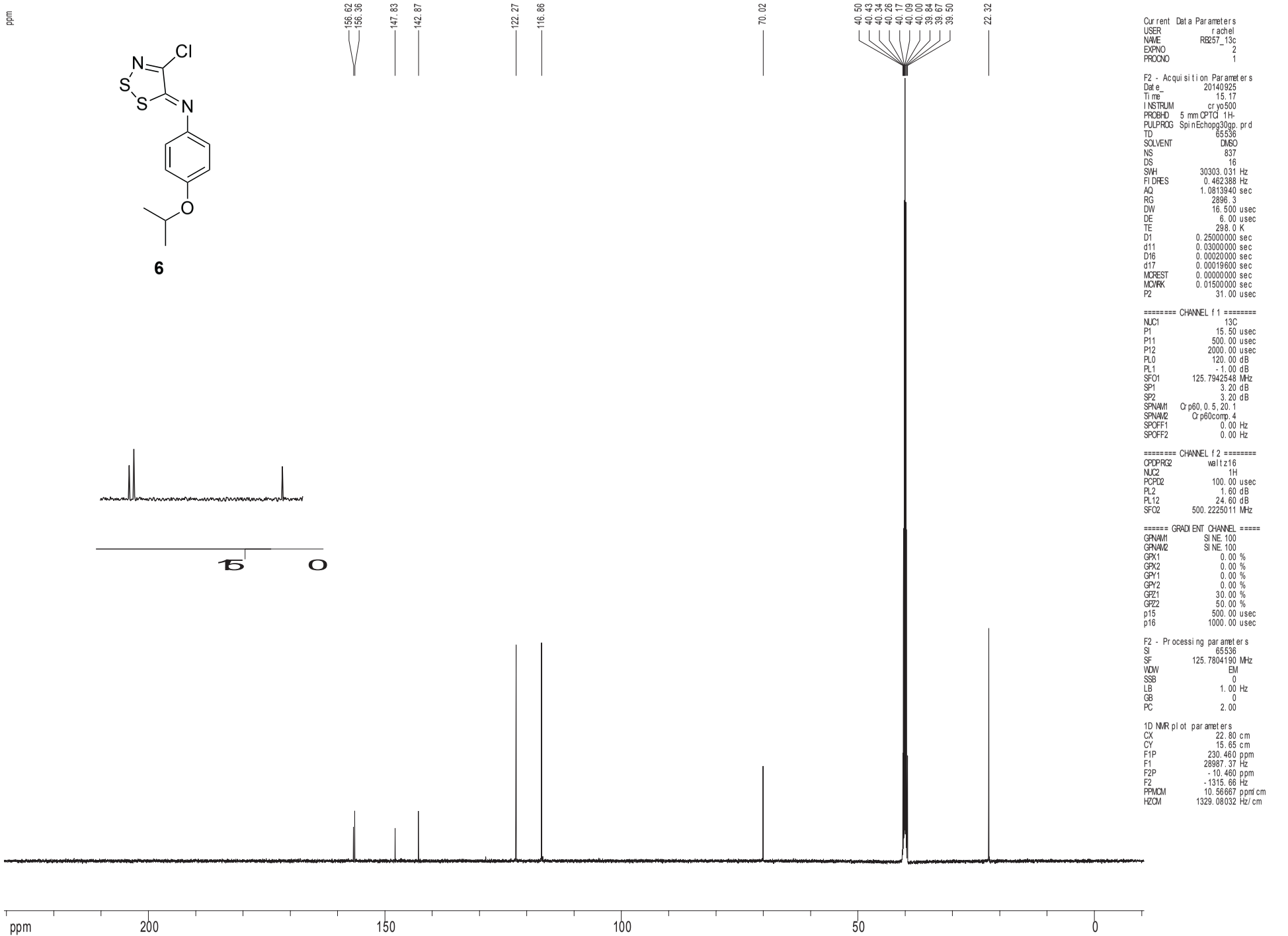
Time 13.33
INSTRUM drx400
PROBHD 5 mm QNP H/F/P
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 8
DS 2
SWH 6410.256 Hz
FIDRES 0.097813 Hz
AQ 5.1118579 sec
RG 645.1
DW 78.000 usec
DE 4.50 usec
TE 298.0 K
D1 0.10000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec

======== CHANNEL f1 ========
NUC1 1H
P1 12.00 usec
PL1 0.00 dB
SFO1 400.1328009 MHz

F2 - Processing parameters
SI 65536
SF 400.1300175 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 2.00

1D NMR plot parameters
CX 22.80 cm
CY 15.00 cm
F1P 14.966 ppm
F1 5988.51 Hz
F2P -1.054 ppm
F2 -421.74 Hz
PPMCM 0.70265 ppm/cm
HZCM 281.15158 Hz/cm

Z-restored spin-echo 13C spectrum with 1H decoupling



Current Data Parameters
USER rachel
NAME RB257_13c
EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
Date_ 20140925
Time 15.17
INSTRUM cryo500
PROBHD 5 mm CPTCI 1H-
PULPROG SpinEchopg30gp.prd
TD 65536
SOLVENT DMSO
NS 837
DS 16
SWH 30303.031 Hz
FIDRES 0.462388 Hz
AQ 1.0813940 sec
RG 2896.3
DW 16.500 usec
DE 6.00 usec
TE 298.0 K
D1 0.25000000 sec
d11 0.03000000 sec
D16 0.00020000 sec
d17 0.00019600 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
P2 31.00 usec

======== CHANNEL f1 ========
NUC1 13C
P1 15.50 usec
P11 500.00 usec
P12 2000.00 usec
PL0 120.00 dB
PL1 -1.00 dB
SFO1 125.7942548 MHz
SP1 3.20 dB
SP2 3.20 dB
SPNAM1 Crp60,0.5,20.1
SPNAM2 Crp60comp.4
SPOFF1 0.00 Hz
SPOFF2 0.00 Hz

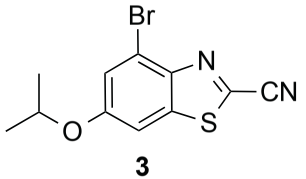
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 100.00 usec
PL2 1.60 dB
PL12 24.60 dB
SFO2 500.2225011 MHz

====== GRADIENT CHANNEL =====
GPNAM1 SINE.100
GPNAM2 SINE.100
GPX1 0.00 %
GPX2 0.00 %
GPY1 0.00 %
GPY2 0.00 %
GPZ1 30.00 %
GPZ2 50.00 %
p15 500.00 usec
p16 1000.00 usec

F2 - Processing parameters
SI 65536
SF 125.7804190 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 2.00

1D NMR plot parameters
CX 22.80 cm
CY 15.65 cm
F1P 230.460 ppm
F1 28987.37 Hz
F2P -10.460 ppm
F2 -1315.66 Hz
PPMCM 10.56667 ppm/cm
HZCM 1329.08032 Hz/cm

1H spectrum



3

Current Data Parameters
USER rachel
NAME RB156
EXPNO 1
PROCNO 1

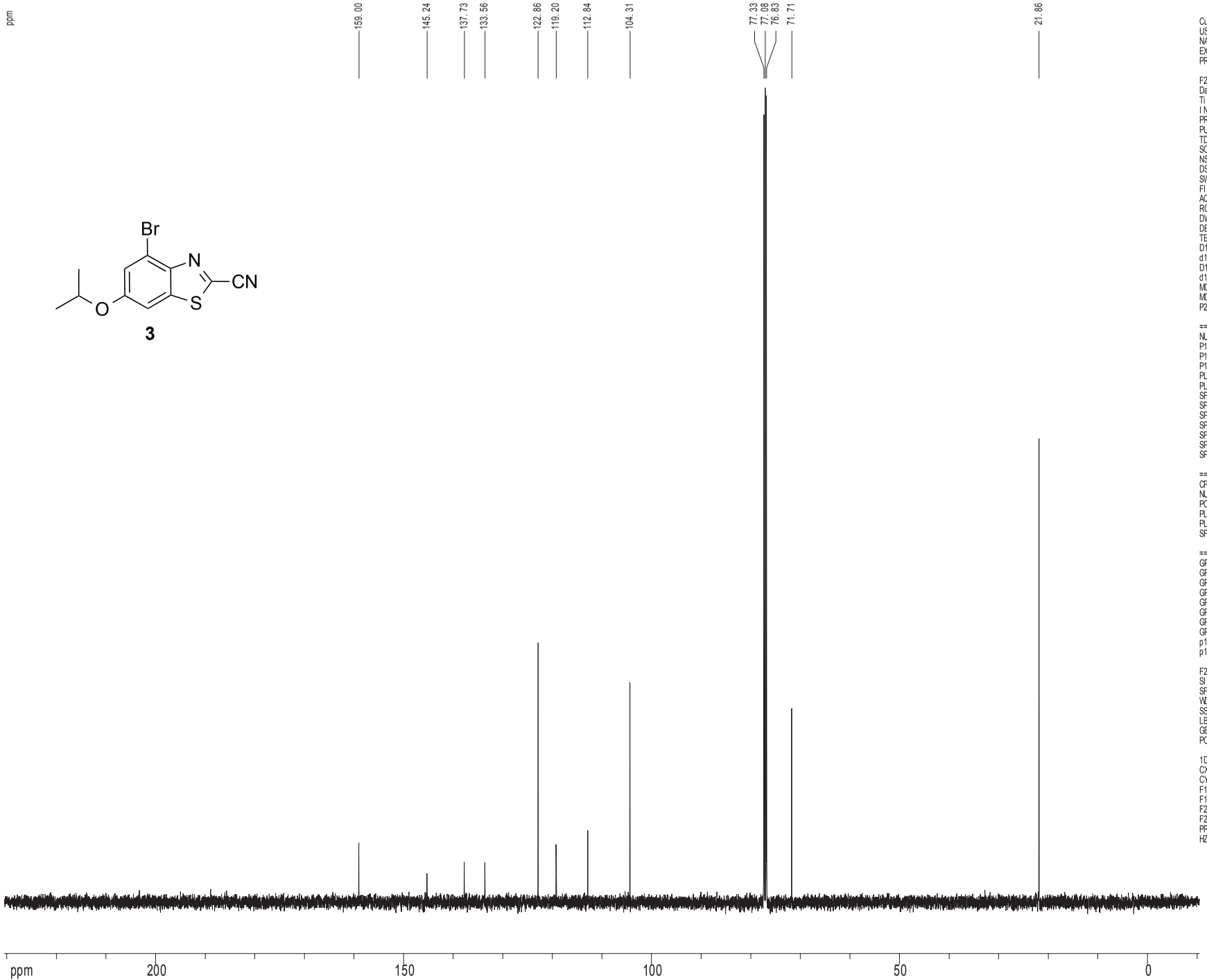
F2 - Acquisition Parameters
Date_ 20140605
Time 10.16
INSTRUM drx400
PROBHD 5 mm QNP H/F/P
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 8
DS 2
SWH 6410.256 Hz
FIDRES 0.097813 Hz
AQ 5.1118579 sec
RG 812.7
DW 78.000 usec
DE 4.50 usec
TE 298.0 K
D1 0.10000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec

======== CHANNEL f1 ========
NUC1 1H
P1 12.00 usec
PL1 0.00 dB
SFO1 400.1328009 MHz

F2 - Processing parameters
SI 65536
SF 400.1300175 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 2.00

1D NMR plot parameters
CX 22.80 cm
CY 15.00 cm
F1P 14.966 ppm
F1 5988.51 Hz
F2P -1.054 ppm
F2 -421.74 Hz
PPMCM 0.70265 ppm/cm
HZCM 281.15158 Hz/cm

Z-restored spin-echo 13C spectrum with 1H decoupling



Current Data Parameters
USER rachel
NAME Rb156_cryo13c
EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
Date_ 20140605
Time 11.13
INSTRUM cryo500
PROBHD 5 mm CPTCI 1H-
PULPROG SpinEchopg30gp.prd
TD 65536
SOLVENT CDCl3
NS 613
DS 16
SWH 30303.031 Hz
FIDRES 0.462388 Hz
AQ 1.0813940 sec
RG 6502
DW 16.500 usec
DE 6.00 usec
TE 298.0 K
D1 0.25000000 sec
d11 0.03000000 sec
D16 0.00020000 sec
d17 0.00019600 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
P2 31.00 usec

======== CHANNEL f1 ========
NUC1 13C
P1 15.50 usec
P11 500.00 usec
P12 2000.00 usec
PL0 120.00 dB
PL1 -1.00 dB
SFO1 125.7942548 MHz
SP1 3.20 dB
SP2 3.20 dB
SPNAM1 Crp60,0.5,20.1
SPNAM2 Crp60comp.4
SPOFF1 0.00 Hz
SPOFF2 0.00 Hz

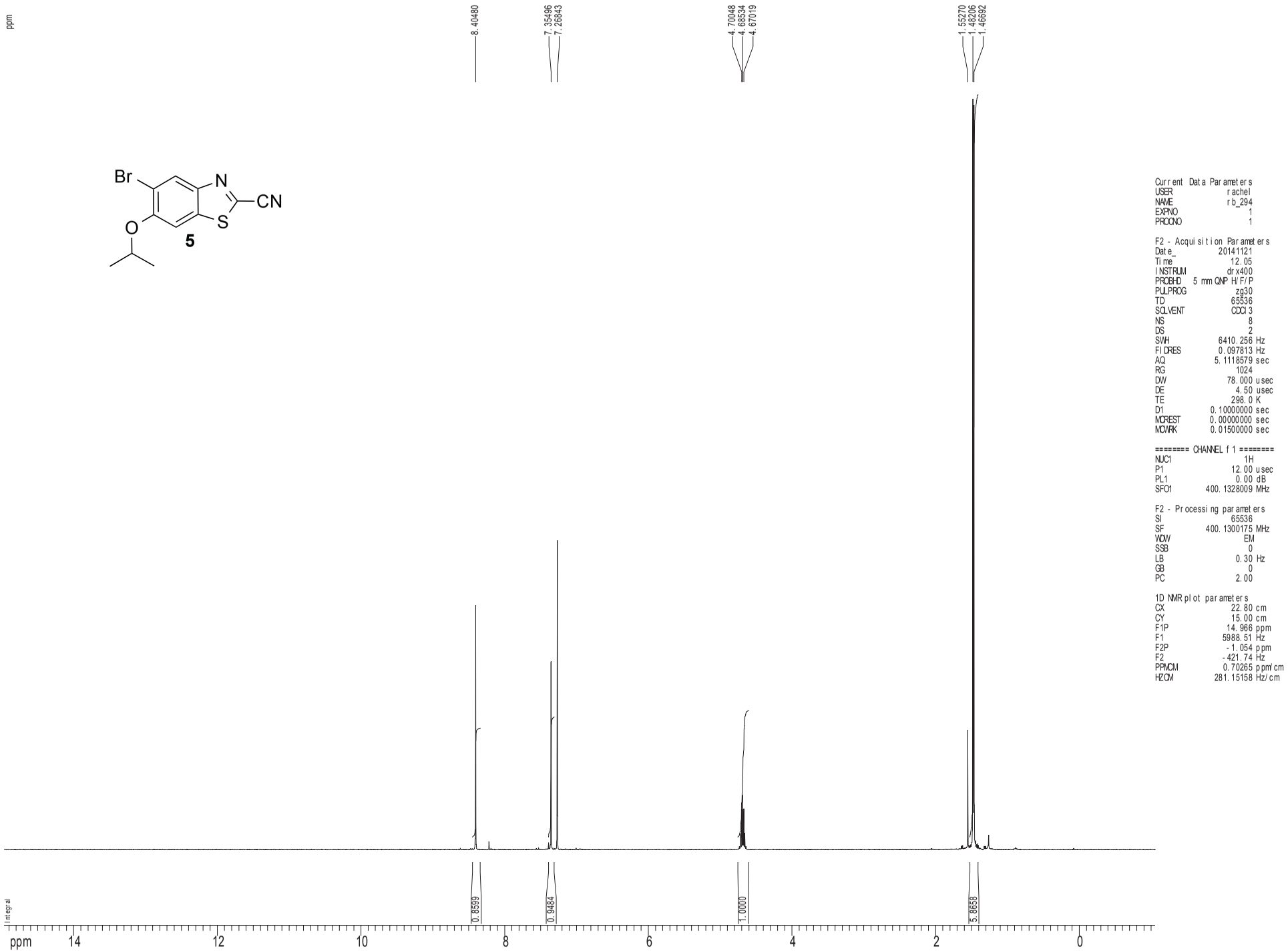
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 100.00 usec
PL2 1.60 dB
PL12 24.60 dB
SFO2 500.2225011 MHz

====== GRADIENT CHANNEL =====
GPNAM1 SINE.100
GPNAM2 SINE.100
GPX1 0.00 %
GPX2 0.00 %
GPY1 0.00 %
GPY2 0.00 %
GPZ1 30.00 %
GPZ2 50.00 %
p15 500.00 usec
p16 1000.00 usec

F2 - Processing parameters
SI 65536
SF 125.7804190 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 2.00

1D NMR plot parameters
CX 22.80 cm
CY 15.65 cm
F1P 230.460 ppm
F1 28987.37 Hz
F2P -10.460 ppm
F2 -1315.66 Hz
PPMCM 10.56667 ppm/cm
HZCM 1329.08032 Hz/cm

1H spectrum



Current Data Parameters
USER rachel
NAME rb_294
EXPNO 1
PROCNO 1

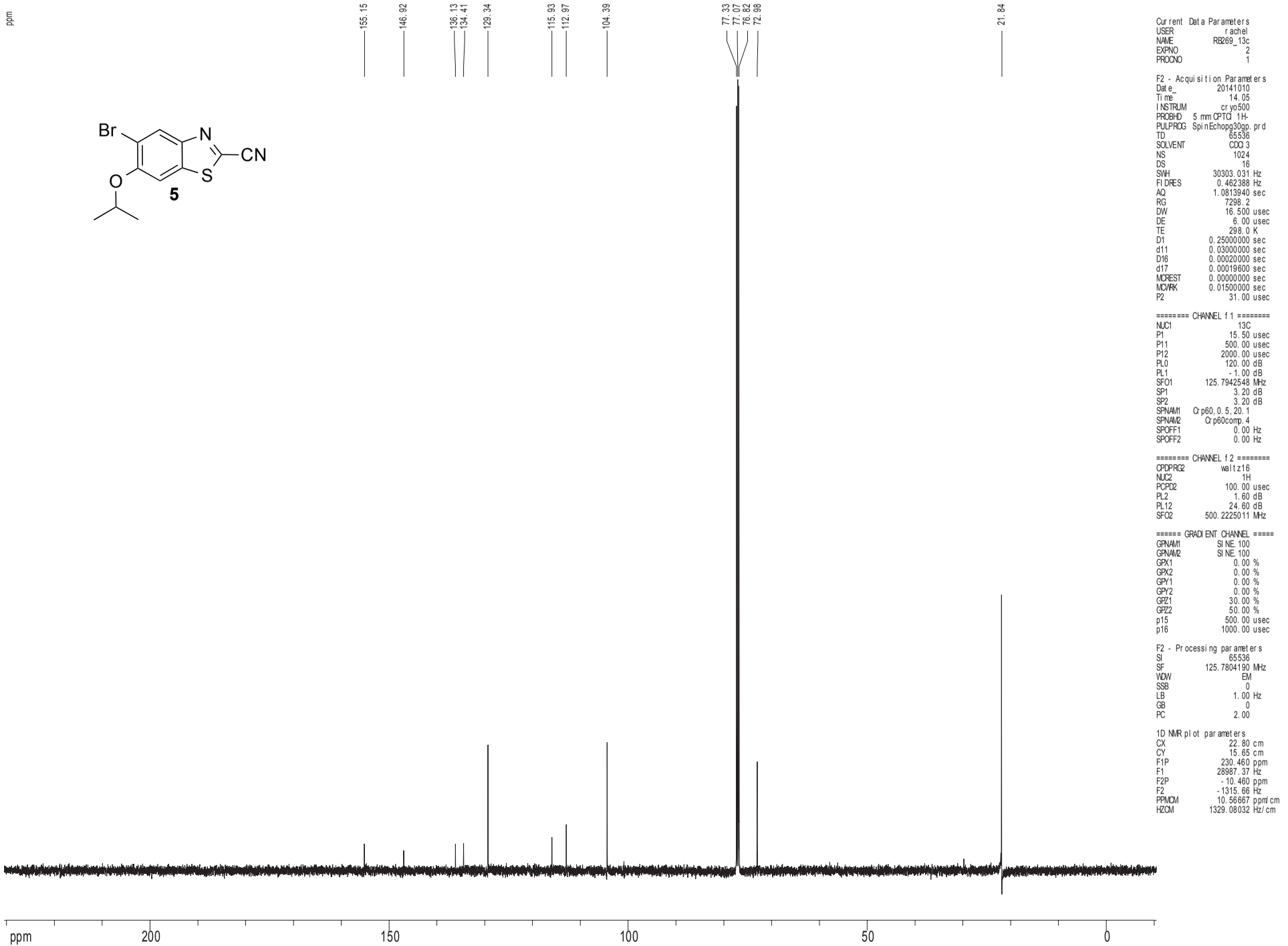
F2 - Acquisition Parameters
Date_ 20141121
Time 12.05
INSTRUM drx400
PROBHD 5 mm QNP H/F/P
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 8
DS 2
SWH 6410.256 Hz
FIDRES 0.097813 Hz
AQ 5.1118579 sec
RG 1024
DW 78.000 usec
DE 4.50 usec
TE 298.0 K
D1 0.10000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec

======== CHANNEL f1 ========
NUC1 1H
P1 12.00 usec
PL1 0.00 dB
SFO1 400.1328009 MHz

F2 - Processing parameters
SI 65536
SF 400.1300175 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 2.00

1D NMR plot parameters
CX 22.80 cm
CY 15.00 cm
F1P 14.966 ppm
F1 5988.51 Hz
F2P -1.054 ppm
F2 -421.74 Hz
PPMCM 0.70265 ppm/cm
HZCM 281.15158 Hz/cm

Z-restored spin-echo 13C spectrum with 1H decoupling



Current Data Parameters
USER rachel
NAME RB269_13c
EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
Date_ 20141010
Time 14.05
INSTRUM cryo500
PROBHD 5 mm CPTCI 1H-
PULPROG SpinEchopg30gp.prd
TD 65536
SOLVENT CDCl3
NS 1024
DS 16
SWH 30303.031 Hz
FIDRES 0.462388 Hz
AQ 1.0813940 sec
RG 7298.2
DW 16.500 usec
DE 6.00 usec
TE 298.0 K
D1 0.25000000 sec
d11 0.03000000 sec
D16 0.00020000 sec
d17 0.00019600 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
P2 31.00 usec

======== CHANNEL f1 ========
NUC1 13C
P1 15.50 usec
P11 500.00 usec
P12 2000.00 usec
PL0 120.00 dB
PL1 -1.00 dB
SFO1 125.7942548 MHz
SP1 3.20 dB
SP2 3.20 dB
SPNAM1 Crp60,0.5,20.1
SPNAM2 Crp60comp.4
SPOFF1 0.00 Hz
SPOFF2 0.00 Hz

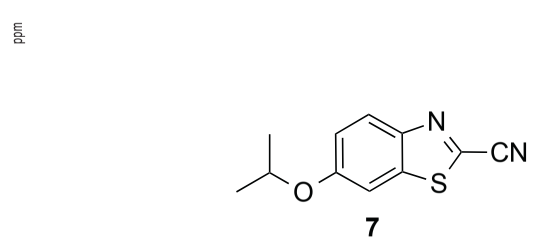
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 100.00 usec
PL2 1.60 dB
PL12 24.60 dB
SFO2 500.2225011 MHz

====== GRADIENT CHANNEL =====
GPNAM1 SINE.100
GPNAM2 SINE.100
GPX1 0.00 %
GPX2 0.00 %
GPY1 0.00 %
GPY2 0.00 %
GPZ1 30.00 %
GPZ2 50.00 %
p15 500.00 usec
p16 1000.00 usec

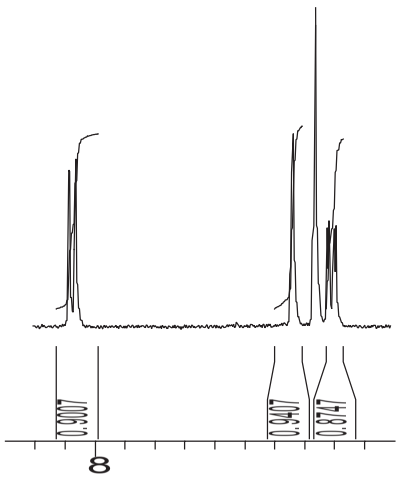
F2 - Processing parameters
SI 65536
SF 125.7804190 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 2.00

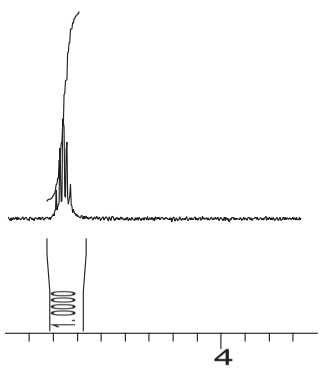
1D NMR plot parameters
CX 22.80 cm
CY 15.65 cm
F1P 230.460 ppm
F1 28987.37 Hz
F2P -10.460 ppm
F2 -1315.66 Hz
PPMCM 10.56667 ppm/cm
HZCM 1329.08032 Hz/cm

1H spectrum



7





Current Data Parameters
USER rachel
NAME rb259_try4
EXPNO 1
PROCNO 1

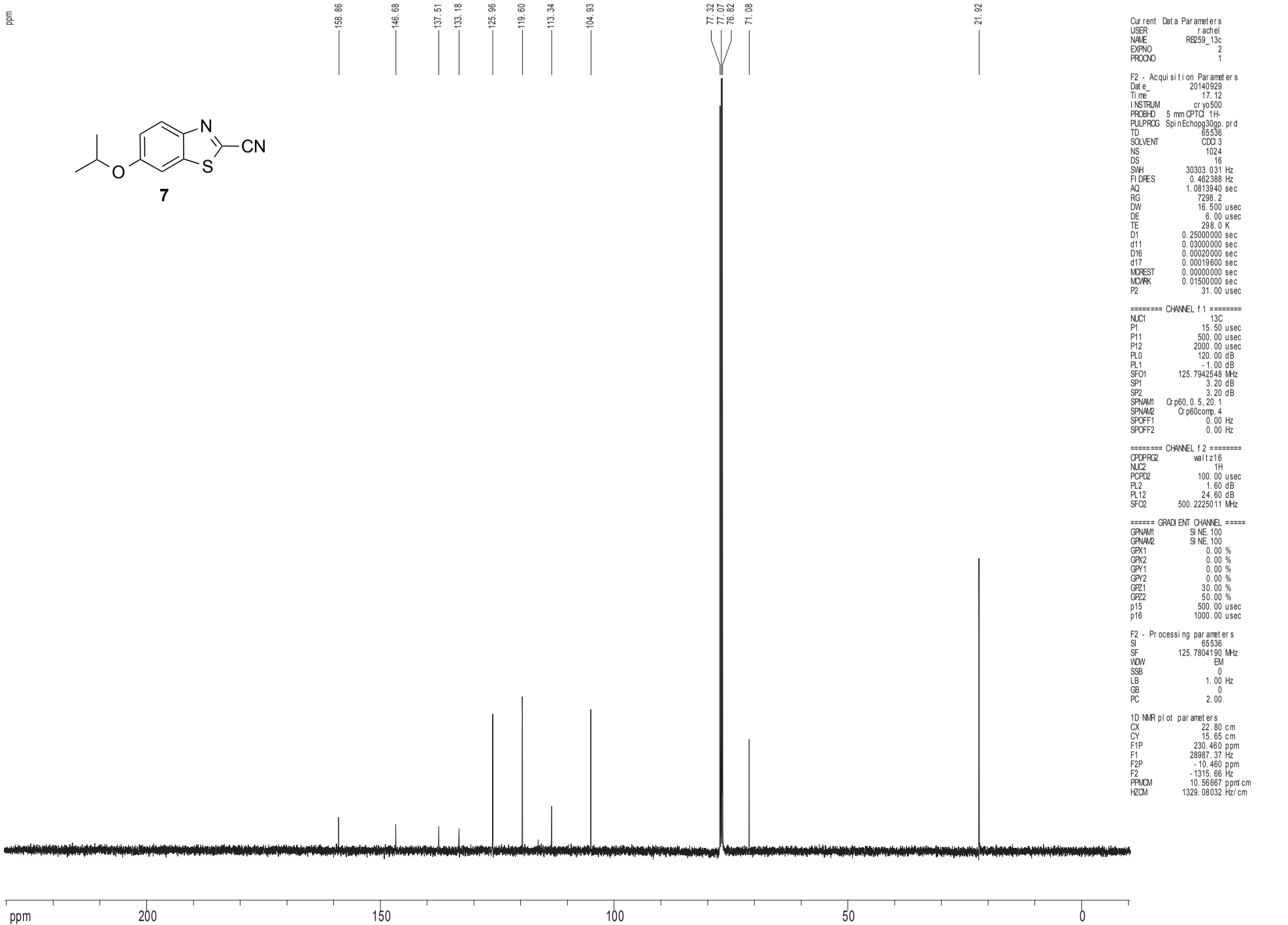
F2 - Acquisition Parameters
Date_ 20141016
Time 14.57
INSTRUM drx400
PROBHD 5 mm QNP H/F/P
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 8
DS 2
SWH 6410.256 Hz
FIDRES 0.097813 Hz
AQ 5.1118579 sec
RG 1290.2
DW 78.000 usec
DE 4.50 usec
TE 298.0 K
D1 0.10000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec

======== CHANNEL f1 ========
NUC1 1H
P1 12.00 usec
PL1 0.00 dB
SFO1 400.1328009 MHz

F2 - Processing parameters
SI 65536
SF 400.1300175 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 2.00

1D NMR plot parameters
CX 22.80 cm
CY 15.00 cm
F1P 14.966 ppm
F1 5988.51 Hz
F2P -1.054 ppm
F2 -421.74 Hz
PPMCM 0.70265 ppm/cm
HZCM 281.15158 Hz/cm

Z-restored spin-echo 13C spectrum with 1H decoupling



Current Data Parameters
USER rachel
NAME RB259_13c
EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
Date_ 20140929
Time 17.12
INSTRUM cryo500
PROBHD 5 mm CPTCI 1H-
PULPROG SpinEchopg30gp.prd
TD 65536
SOLVENT CDCl3
NS 1024
DS 16
SWH 30303.031 Hz
FIDRES 0.462388 Hz
AQ 1.0813940 sec
RG 7298.2
DW 16.500 usec
DE 6.00 usec
TE 298.0 K
D1 0.25000000 sec
d11 0.03000000 sec
D16 0.00020000 sec
d17 0.00019600 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
P2 31.00 usec

======== CHANNEL f1 ========
NUC1 13C
P1 15.50 usec
P11 500.00 usec
P12 2000.00 usec
PL0 120.00 dB
PL1 -1.00 dB
SFO1 125.7942548 MHz
SP1 3.20 dB
SP2 3.20 dB
SPNAM1 Crp60,0.5,20.1
SPNAM2 Crp60comp.4
SPOFF1 0.00 Hz
SPOFF2 0.00 Hz

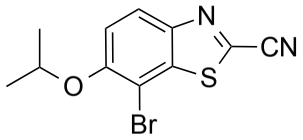
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 100.00 usec
PL2 1.60 dB
PL12 24.60 dB
SFO2 500.2225011 MHz

====== GRADIENT CHANNEL =====
GPNAM1 SINE.100
GPNAM2 SINE.100
GPX1 0.00 %
GPX2 0.00 %
GPY1 0.00 %
GPY2 0.00 %
GPZ1 30.00 %
GPZ2 50.00 %
p15 500.00 usec
p16 1000.00 usec

F2 - Processing parameters
SI 65536
SF 125.7804190 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 2.00

1D NMR plot parameters
CX 22.80 cm
CY 15.65 cm
F1P 230.460 ppm
F1 28987.37 Hz
F2P -10.460 ppm
F2 -1315.66 Hz
PPMCM 10.56667 ppm/cm
HZCM 1329.08032 Hz/cm

1H spectrum



8

Current Data Parameters
USER rachel
NAME rb261_dmso
EXPNO 1
PROCNO 1

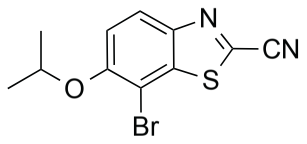
F2 - Acquisition Parameters
Date_ 20141007
Time 16.55
INSTRUM drx400
PROBHD 5 mm QNP H/F/P
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 8
DS 2
SWH 6410.256 Hz
FIDRES 0.097813 Hz
AQ 5.1118579 sec
RG 1149.4
DW 78.000 usec
DE 4.50 usec
TE 298.0 K
D1 0.10000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec

======== CHANNEL f1 ========
NUC1 1H
P1 12.00 usec
PL1 0.00 dB
SFO1 400.1328009 MHz

F2 - Processing parameters
SI 65536
SF 400.1300175 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 2.00

1D NMR plot parameters
CX 22.80 cm
CY 15.00 cm
F1P 14.966 ppm
F1 5988.51 Hz
F2P -1.054 ppm
F2 -421.74 Hz
PPMCM 0.70265 ppm/cm
HZCM 281.15158 Hz/cm

Z-restored spin-echo 13C spectrum with 1H decoupling



8

Current Data Parameters
USER rachel
NAME RB190_cryo13c
EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
Date_ 20140712
Time 19.28
INSTRUM cryo500
PROBHD 5 mm CPTCI 1H-
PULPROG SpinEchopg30gp.prd
TD 65536
SOLVENT CDCl3
NS 1024
DS 16
SWH 30303.031 Hz
FIDRES 0.462388 Hz
AQ 1.0813940 sec
RG 7298.2
DW 16.500 usec
DE 6.00 usec
TE 298.0 K
D1 0.25000000 sec
d11 0.03000000 sec
D16 0.00020000 sec
d17 0.00019600 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
P2 31.00 usec

======== CHANNEL f1 ========
NUC1 13C
P1 15.50 usec
P11 500.00 usec
P12 2000.00 usec
PL0 120.00 dB
PL1 -1.00 dB
SFO1 125.7942548 MHz
SP1 3.20 dB
SP2 3.20 dB
SPNAM1 Crp60,0.5,20.1
SPNAM2 Crp60comp.4
SPOFF1 0.00 Hz
SPOFF2 0.00 Hz

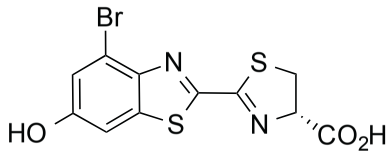
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 100.00 usec
PL2 1.60 dB
PL12 24.60 dB
SFO2 500.2225011 MHz

====== GRADIENT CHANNEL =====
GPNAM1 SINE.100
GPNAM2 SINE.100
GPX1 0.00 %
GPX2 0.00 %
GPY1 0.00 %
GPY2 0.00 %
GPZ1 30.00 %
GPZ2 50.00 %
p15 500.00 usec
p16 1000.00 usec

F2 - Processing parameters
SI 65536
SF 125.7804190 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 2.00

1D NMR plot parameters
CX 22.80 cm
CY 15.65 cm
F1P 230.460 ppm
F1 28987.37 Hz
F2P -10.460 ppm
F2 -1315.66 Hz
PPMCM 10.56667 ppm/cm
HZCM 1329.08032 Hz/cm

1H spectrum



4′–BrLuc

Current Data Parameters
USER rachel
NAME rb4xbromoluciferin_cryo
EXPNO 1
PROCNO 1

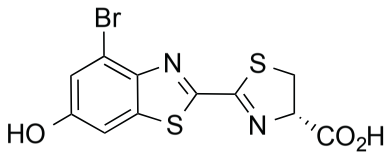
F2 - Acquisition Parameters
Date_ 20141117
Time 19.28
INSTRUM cryo500
PROBHD 5 mm CPTCI 1H-
PULPROG zg30
TD 81728
SOLVENT D2O
NS 8
DS 2
SWH 8012.820 Hz
FIDRES 0.098043 Hz
AQ 5.0998774 sec
RG 16
DW 62.400 usec
DE 6.00 usec
TE 298.0 K
D1 0.10000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec

======= CHANNEL f1 =======
NUC1 1H
P1 7.50 usec
PL1 1.60 dB
SFO1 500.2235015 MHz

F2 - Processing parameters
SI 65536
SF 500.2200000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 4.00

1D NMR plot parameters
CX 22.80 cm
CY 15.00 cm
F1P 15.009 ppm
F1 7507.95 Hz
F2P -1.009 ppm
F2 -504.87 Hz
PPMCM 0.70257 ppm/cm
HZCM 351.43951 Hz/cm

Z-restored spin-echo 13C spectrum with 1H decoupling



4′–BrLuc

```
Current Data Parameters
USER                rachel
NAME        rb4xbromoluciferin_cryo
EXPNO                    2
PROCNO                   1

F2 - Acquisition Parameters
Date_              20141117
Time                 19.39
INSTRUM              cryo500
PROBHD    5 mm CPTCI 1H-
PULPROG   SpinEchopg30gp.prd
TD                   65536
SOLVENT              CDCl3
NS                     489
DS                      16
SWH              30303.031 Hz
FIDRES           0.462388 Hz
AQ               1.0813940 sec
RG                   13004
DW                 16.500 usec
DE                  6.00 usec
TE                 298.0 K
D1               0.25000000 sec
d11              0.03000000 sec
D16              0.00020000 sec
d17              0.00019600 sec
MCREST           0.00000000 sec
MCWRK            0.01500000 sec
P2                  33.10 usec

======== CHANNEL f1 ========
NUC1                  13C
P1                 16.55 usec
P11               500.00 usec
P12              2000.00 usec
PL0               120.00 dB
PL1                -1.00 dB
SFO1          125.7942548 MHz
SP1                 2.70 dB
SP2                 2.70 dB
SPNAM1     Crp60,0.5,20.1
SPNAM2      Crp60comp.4
SPOFF1              0.00 Hz
SPOFF2              0.00 Hz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                  1H
PCPD2             100.00 usec
PL2                 1.60 dB
PL12               24.50 dB
SFO2          500.2225011 MHz

====== GRADIENT CHANNEL =====
GPNAM1          SINE.100
GPNAM2          SINE.100
GPX1                0.00 %
GPX2                0.00 %
GPY1                0.00 %
GPY2                0.00 %
GPZ1               30.00 %
GPZ2               50.00 %
p15               500.00 usec
p16              1000.00 usec

F2 - Processing parameters
SI                   65536
SF            125.7800839 MHz
WDW                    EM
SSB                     0
LB                   1.00 Hz
GB                      0
PC                   2.00

1D NMR plot parameters
CX                  22.80 cm
CY                  15.65 cm
F1P               233.125 ppm
F1                29322.47 Hz
F2P                -7.796 ppm
F2                -980.56 Hz
PPMCM            10.56670 ppm/cm
HZCM             1329.08032 Hz/cm
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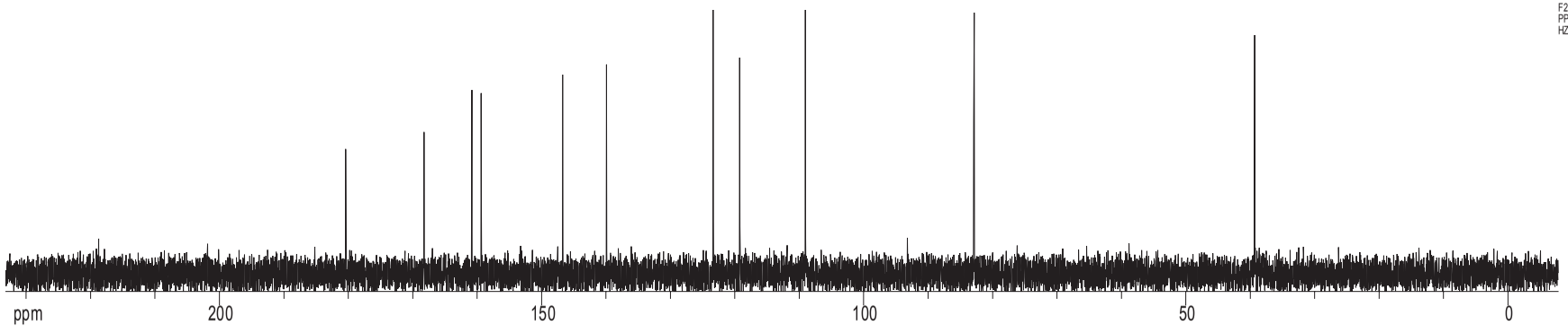
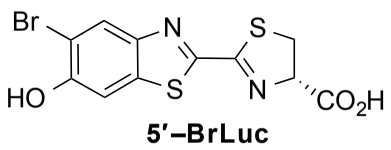
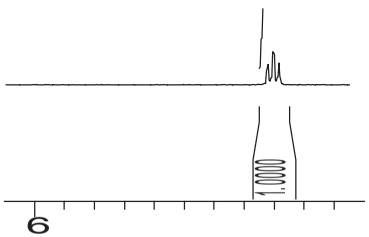


1H spectrum



5′–BrLuc



Current Data Parameters
USER rachel
NAME rl_5bromo
EXPNO 1
PROCNO 1

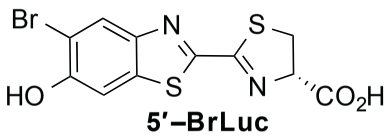
F2 - Acquisition Parameters
Date_ 20150118
Time 17.11
INSTRUM cryo500
PROBHD 5 mm CPTCI 1H-
PULPROG zg30
TD 81728
SOLVENT D2O
NS 8
DS 2
SWH 8012.820 Hz
FIDRES 0.098043 Hz
AQ 5.0998774 sec
RG 6.3
DW 62.400 usec
DE 6.00 usec
TE 298.0 K
D1 0.10000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec

======== CHANNEL f1 ========
NUC1 1H
P1 7.50 usec
PL1 1.60 dB
SFO1 500.2235015 MHz

F2 - Processing parameters
SI 65536
SF 500.2200000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 4.00

1D NMR plot parameters
CX 22.80 cm
CY 15.00 cm
F1P 15.009 ppm
F1 7507.95 Hz
F2P -1.009 ppm
F2 -504.87 Hz
PPMCM 0.70257 ppm/cm
HZCM 351.43951 Hz/cm

Z-restored spin-echo 13C spectrum with 1H decoupling



5′–BrLuc

```
Current Data Parameters
USER                rachel
NAME                rl_5bromp
EXPNO                    2
PROCNO                   1

F2 - Acquisition Parameters
Date_               20150118
Time                  17.18
INSTRUM             cryo500
PROBHD    5 mm CPTCI 1H-
PULPROG   SpinEchopg30gp.prd
TD                    65536
SOLVENT               CDCl3
NS                     1024
DS                       16
SWH                30303.031 Hz
FIDRES             0.462388 Hz
AQ                 1.0813940 sec
RG                     13004
DW                   16.500 usec
DE                    6.00 usec
TE                   298.0 K
D1                 0.25000000 sec
d11                0.03000000 sec
D16                0.00020000 sec
d17                0.00019600 sec
MCREST             0.00000000 sec
MCWRK              0.01500000 sec
P2                    33.10 usec

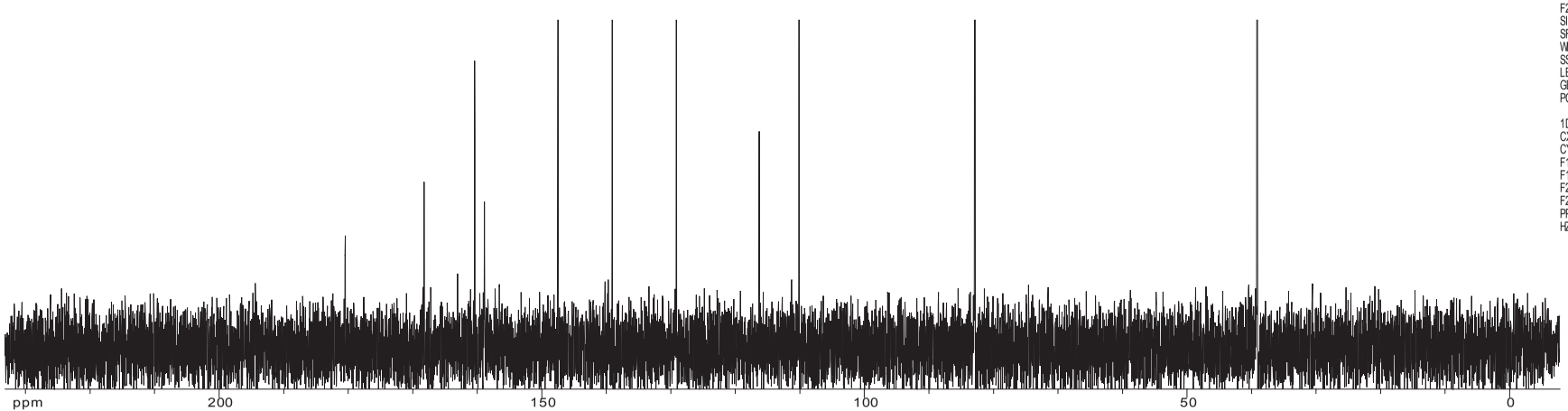
======== CHANNEL f1 ========
NUC1                     13C
P1                    16.55 usec
P11                  500.00 usec
P12                 2000.00 usec
PL0                  120.00 dB
PL1                   -1.00 dB
SFO1             125.7942548 MHz
SP1                   2.70 dB
SP2                   2.70 dB
SPNAM1    Crp60,0.5,20.1
SPNAM2    Crp60comp.4
SPOFF1                0.00 Hz
SPOFF2                0.00 Hz

======== CHANNEL f2 ========
CPDPRG2             waltz16
NUC2                    1H
PCPD2               100.00 usec
PL2                   1.60 dB
PL12                 24.50 dB
SFO2             500.2225011 MHz

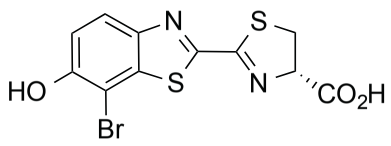
====== GRADIENT CHANNEL =====
GPNAM1              SINE.100
GPNAM2              SINE.100
GPX1                  0.00 %
GPX2                  0.00 %
GPY1                  0.00 %
GPY2                  0.00 %
GPZ1                 30.00 %
GPZ2                 50.00 %
p15                 500.00 usec
p16                1000.00 usec

F2 - Processing parameters
SI                    65536
SF               125.7800839 MHz
WDW                     EM
SSB                      0
LB                    1.00 Hz
GB                       0
PC                    2.00

1D NMR plot parameters
CX                   22.80 cm
CY                   15.65 cm
F1P                 233.125 ppm
F1                29322.47 Hz
F2P                  -7.796 ppm
F2                 -980.56 Hz
PPMCM              10.56670 ppm/cm
HZCM             1329.08032 Hz/cm
```



1H spectrum



7′–BrLuc

Current Data Parameters
USER rachel
NAME rb7brompluciferin_cryo
EXPNO 1
PROCNO 1

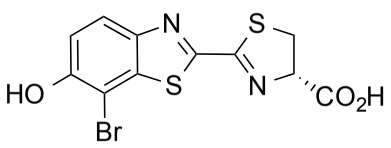
F2 - Acquisition Parameters
Date_ 20141117
Time 19.01
INSTRUM cryo500
PROBHD 5 mm CPTCI 1H-
PULPROG zg30
TD 81728
SOLVENT D2O
NS 8
DS 2
SWH 8012.820 Hz
FIDRES 0.098043 Hz
AQ 5.0998774 sec
RG 12.7
DW 62.400 usec
DE 6.00 usec
TE 298.0 K
D1 0.10000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec

======== CHANNEL f1 ========
NUC1 1H
P1 7.50 usec
PL1 1.60 dB
SFO1 500.2235015 MHz

F2 - Processing parameters
SI 65536
SF 500.2200000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 4.00

1D NMR plot parameters
CX 22.80 cm
CY 15.00 cm
F1P 15.009 ppm
F1 7507.95 Hz
F2P -1.009 ppm
F2 -504.87 Hz
PPMCM 0.70257 ppm/cm
HZCM 351.43951 Hz/cm

Z-restored spin-echo 13C spectrum with 1H decoupling



7′–BrLuc

```
Current Data Parameters
USER                rachel
NAME        rb7brombuciferin_cryo
EXPNO                    2
PROCNO                   1

F2 - Acquisition Parameters
Date_              20141117
Time                 19.03
INSTRUM            cryo500
PROBHD   5 mm CPTCI 1H-
PULPROG  SpinEchopg30gp.prd
TD                   65536
SOLVENT              CDCl3
NS                     883
DS                      16
SWH              30303.031 Hz
FIDRES           0.462388 Hz
AQ               1.0813940 sec
RG                    8192
DW                 16.500 usec
DE                  6.00 usec
TE                298.0 K
D1               0.25000000 sec
d11              0.03000000 sec
D16              0.00020000 sec
d17              0.00019600 sec
MCREST           0.00000000 sec
MCWRK            0.01500000 sec
P2                  33.10 usec

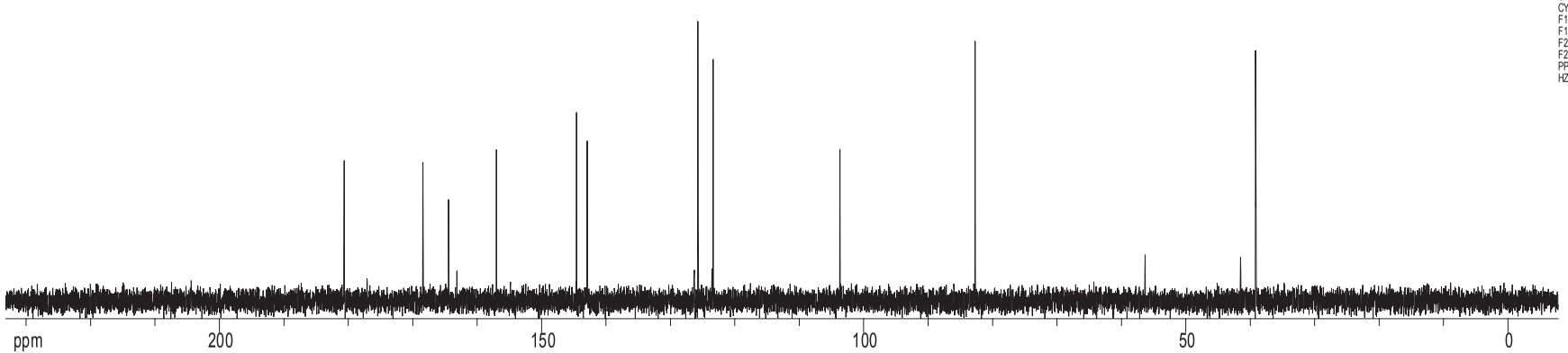
======== CHANNEL f1 ========
NUC1                   13C
P1                  16.55 usec
P11                500.00 usec
P12               2000.00 usec
PL0                120.00 dB
PL1                 -1.00 dB
SFO1          125.7942548 MHz
SP1                  2.70 dB
SP2                  2.70 dB
SPNAM1     Crp60,0.5,20.1
SPNAM2     Crp60comp.4
SPOFF1               0.00 Hz
SPOFF2               0.00 Hz

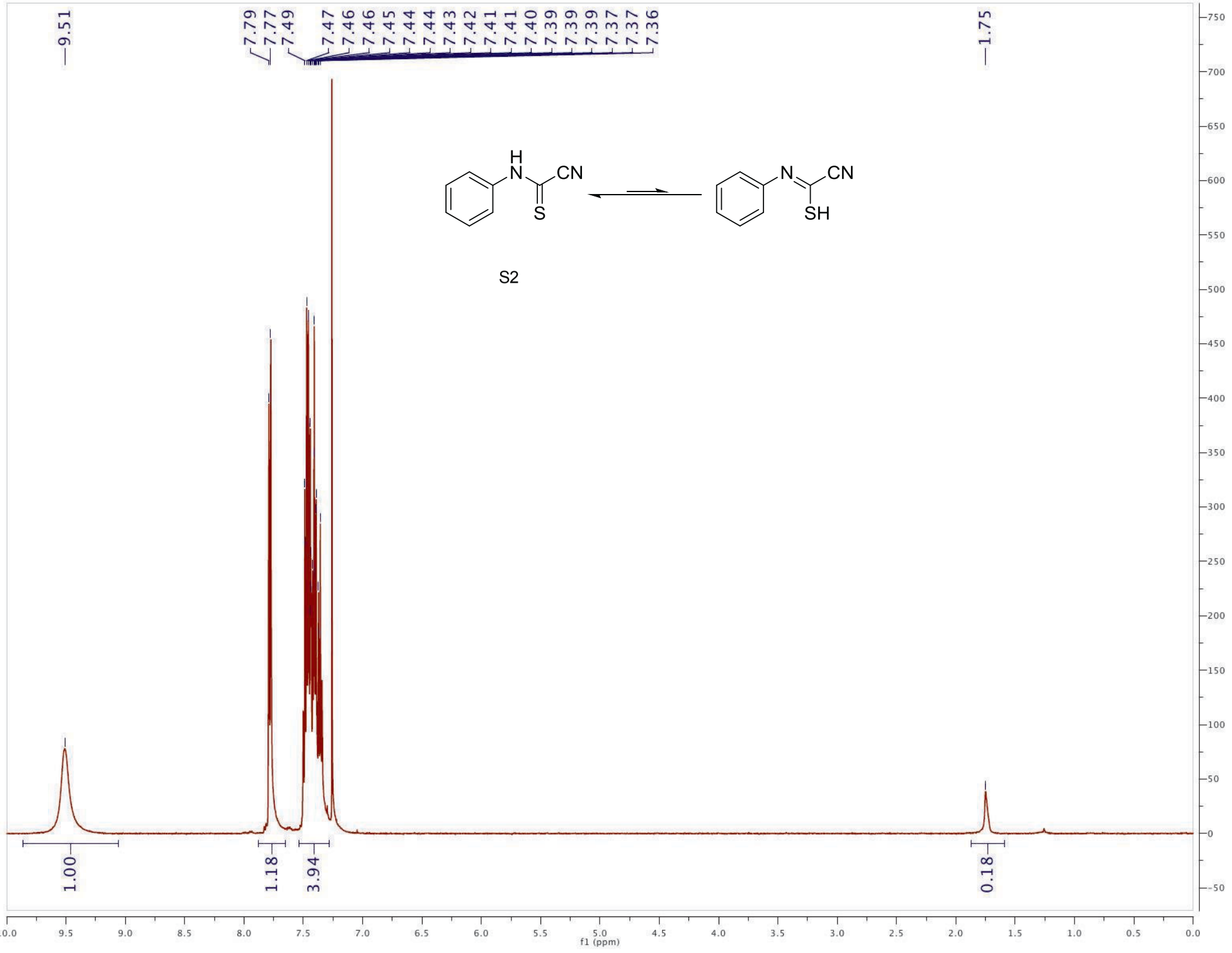
======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                   1H
PCPD2              100.00 usec
PL2                 1.60 dB
PL12                24.50 dB
SFO2          500.2225011 MHz

====== GRADIENT CHANNEL =====
GPNAM1           SINE.100
GPNAM2           SINE.100
GPX1                 0.00 %
GPX2                 0.00 %
GPY1                 0.00 %
GPY2                 0.00 %
GPZ1                30.00 %
GPZ2                50.00 %
p15                500.00 usec
p16               1000.00 usec

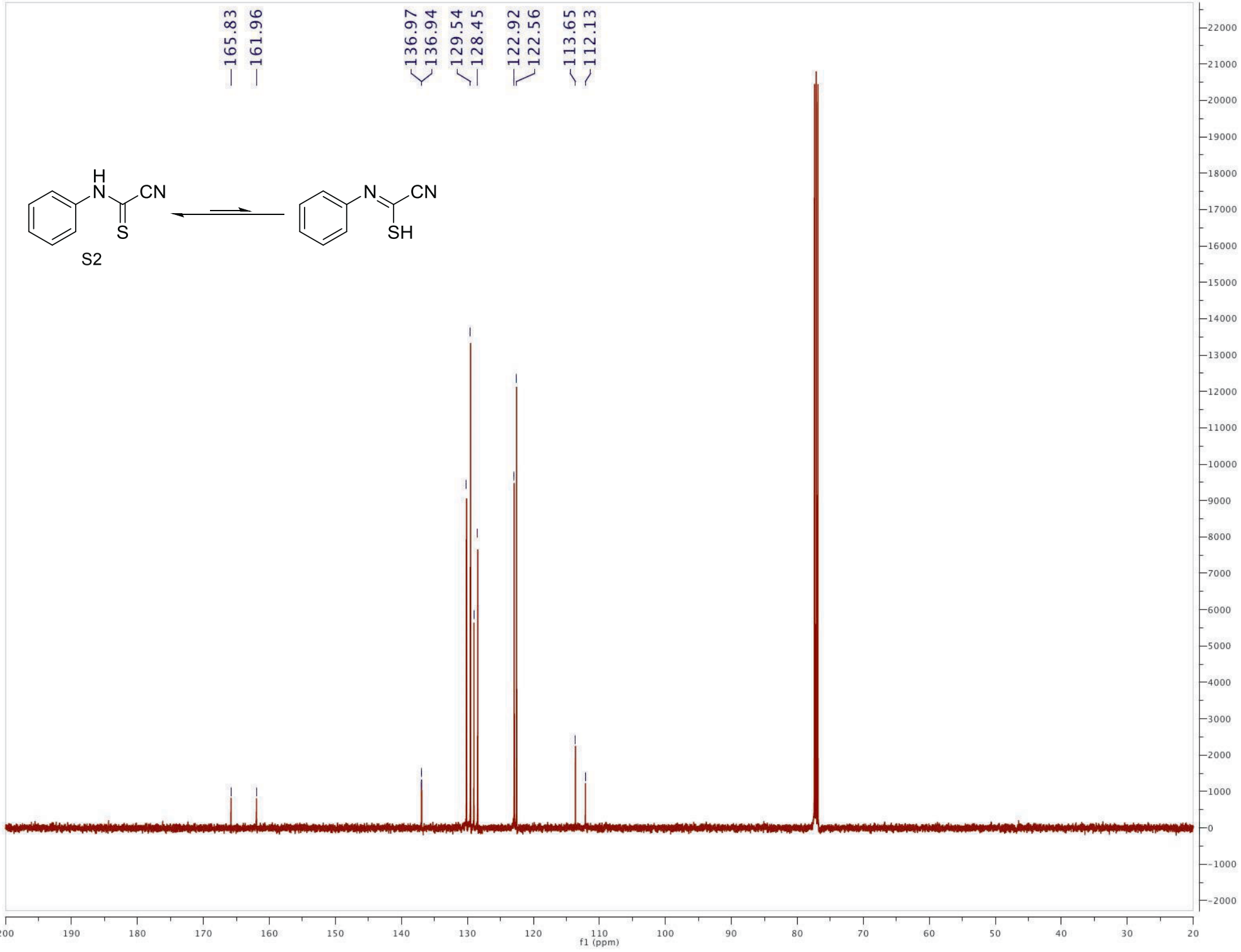
F2 - Processing parameters
SI                   65536
SF            125.7800839 MHz
WDW                    EM
SSB                    0
LB                   1.00 Hz
GB                     0
PC                   2.00

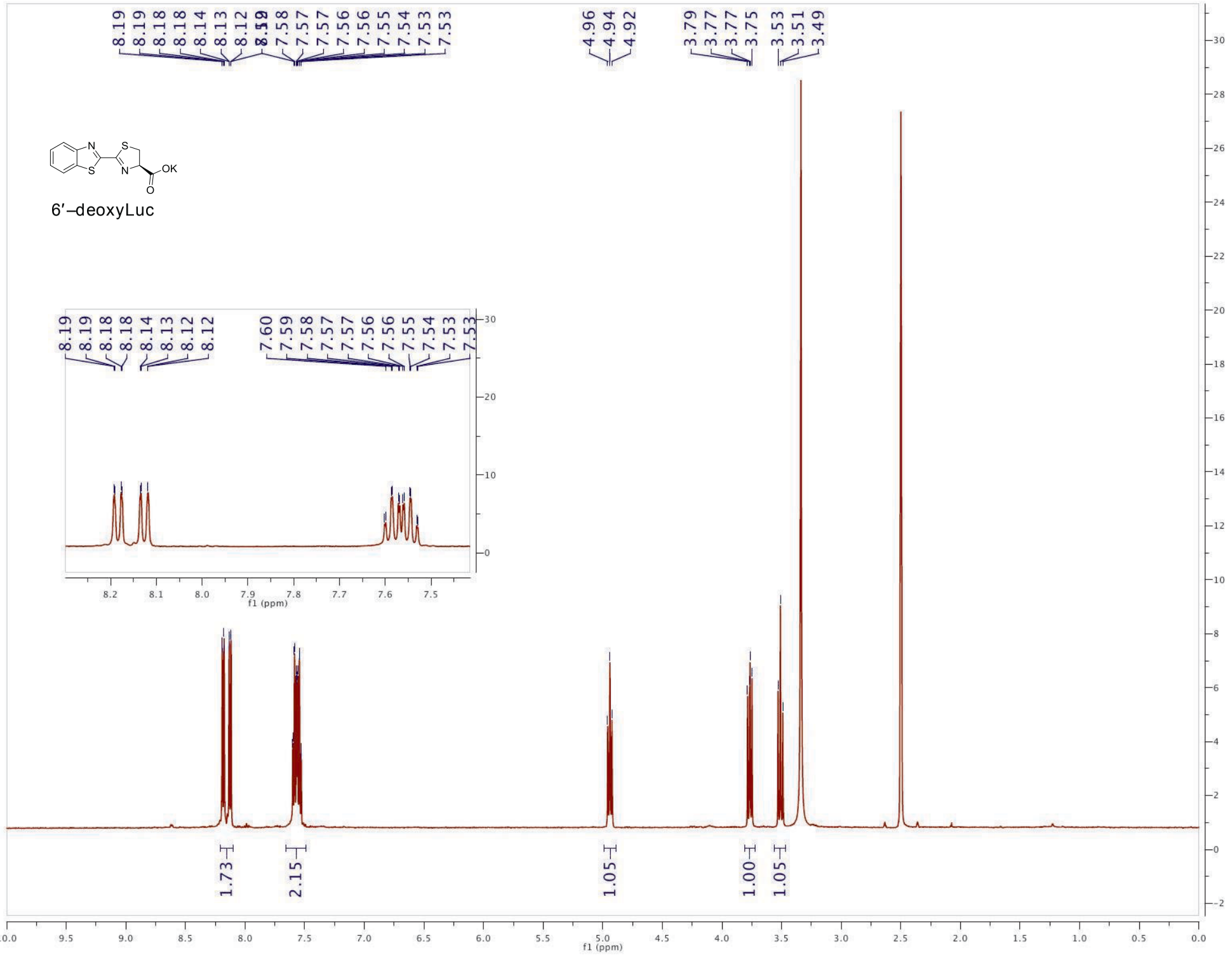
1D NMR plot parameters
CX                  22.80 cm
CY                  15.65 cm
F1P               233.125 ppm
F1              29322.47 Hz
F2P                -7.796 ppm
F2               -980.56 Hz
PPMCM            10.56670 ppm/cm
HZCM             1329.08032 Hz/cm
```

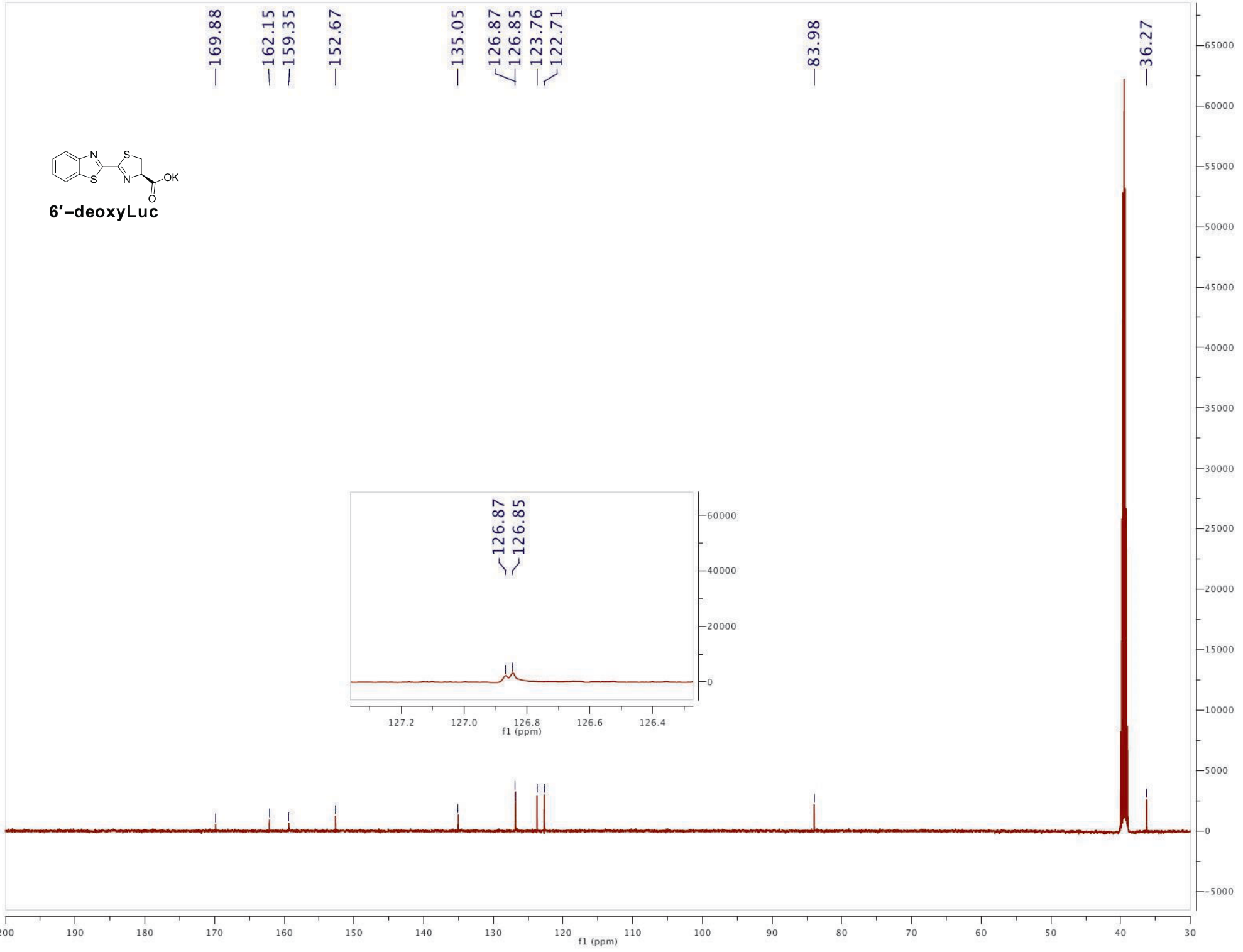


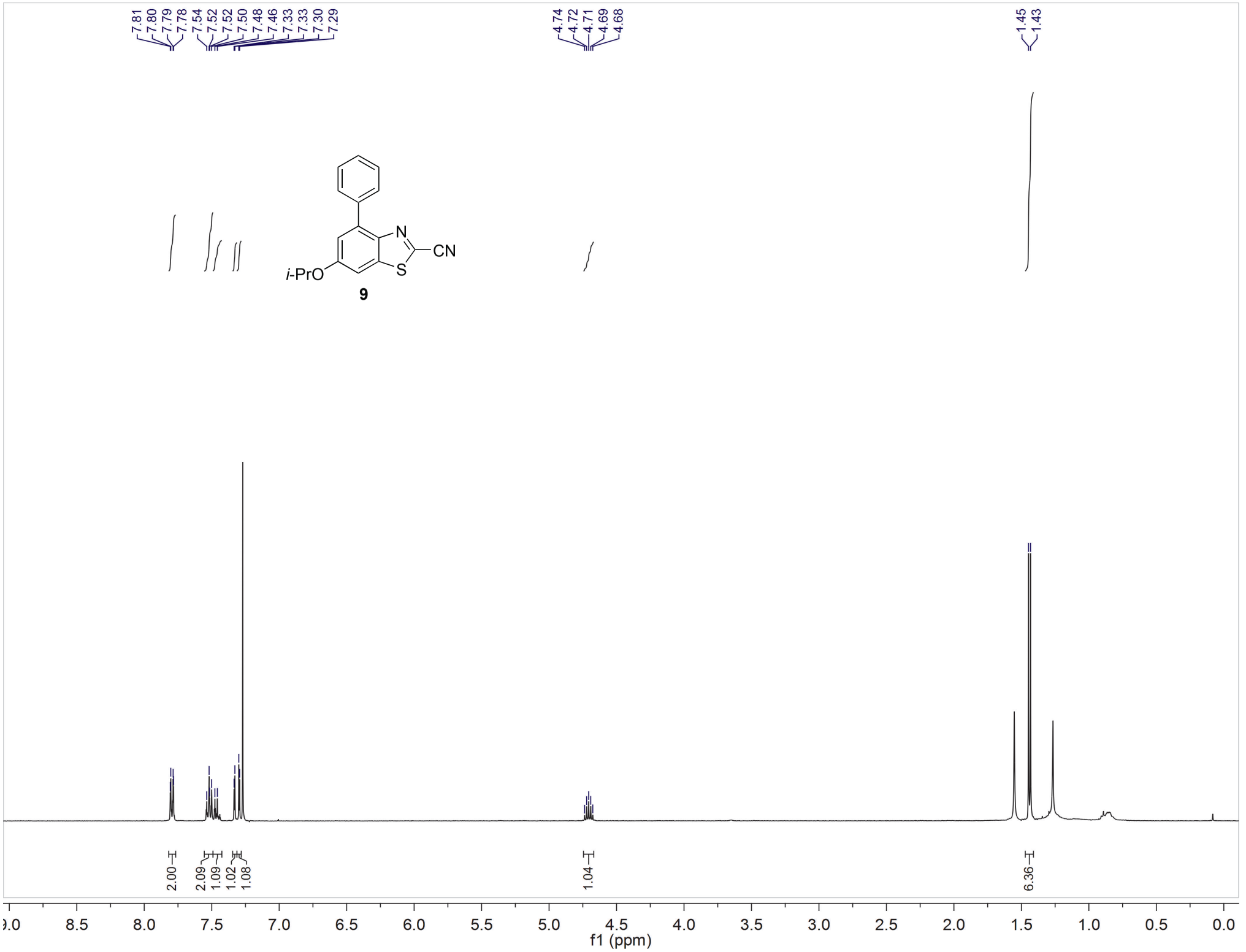

9.51
7.79
7.77
7.49
7.47
7.46
7.46
7.45
7.44
7.44
7.43
7.42
7.41
7.41
7.40
7.39
7.39
7.39
7.37
7.37
7.36
1.75
H
N
CN
S
N
CN
SH
1.00
1.18
3.94
0.18
f1 (ppm)

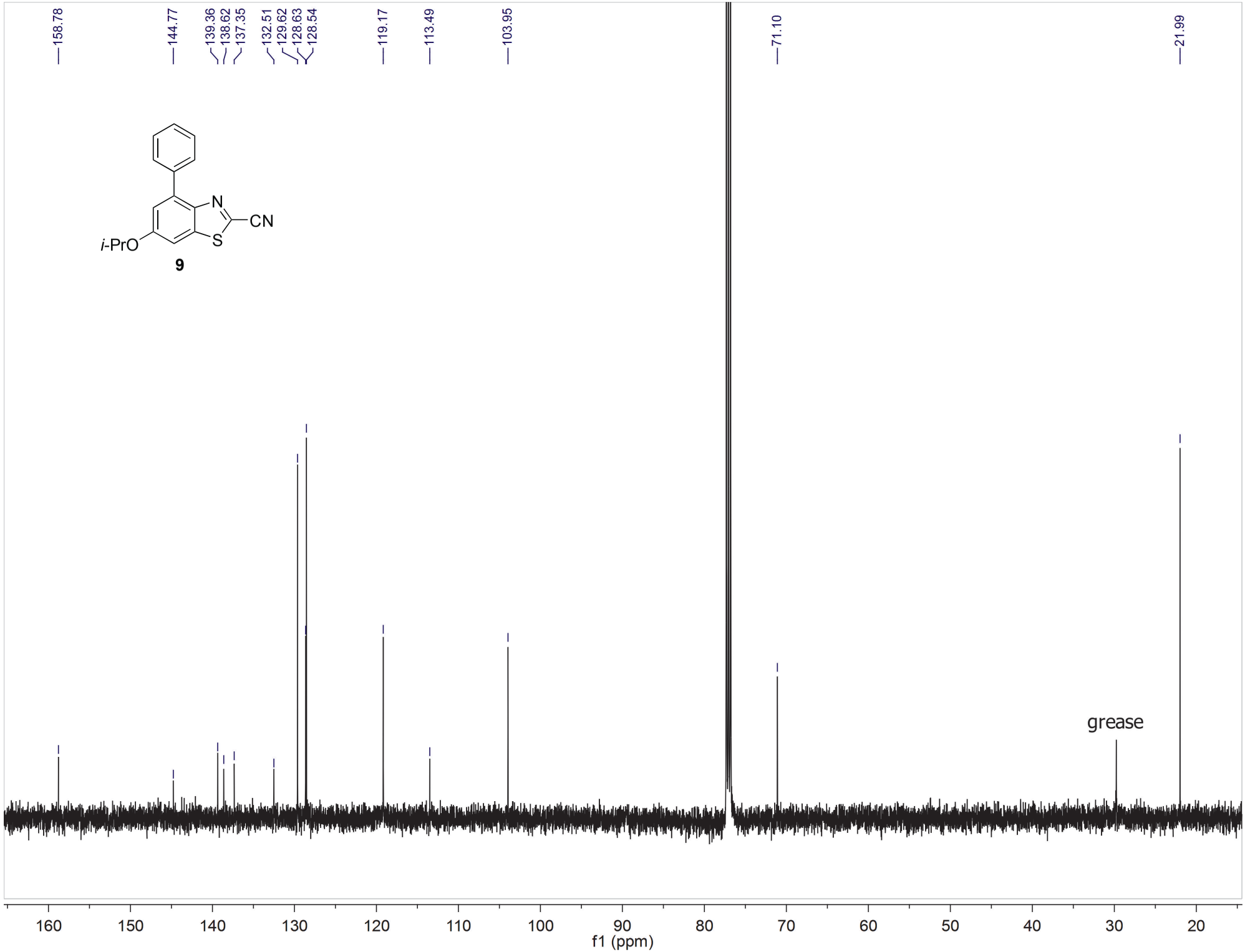
S2

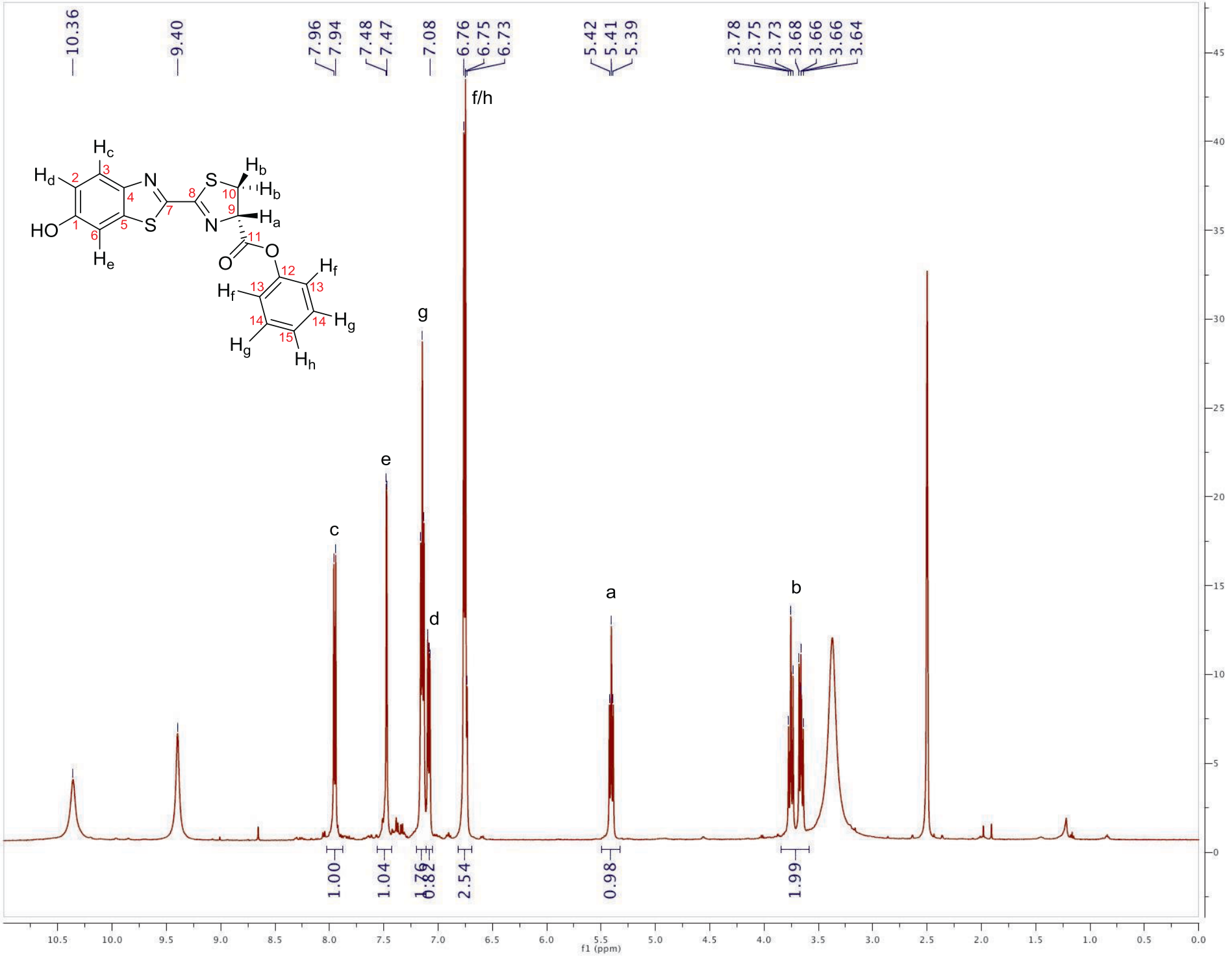

165.83
161.96
136.97
136.94
129.54
128.45
122.92
122.56
113.65
112.13
H
N
CN
S
S2
N
CN
SH
22000
21000
20000
19000
18000
17000
16000
15000
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
-2000
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)

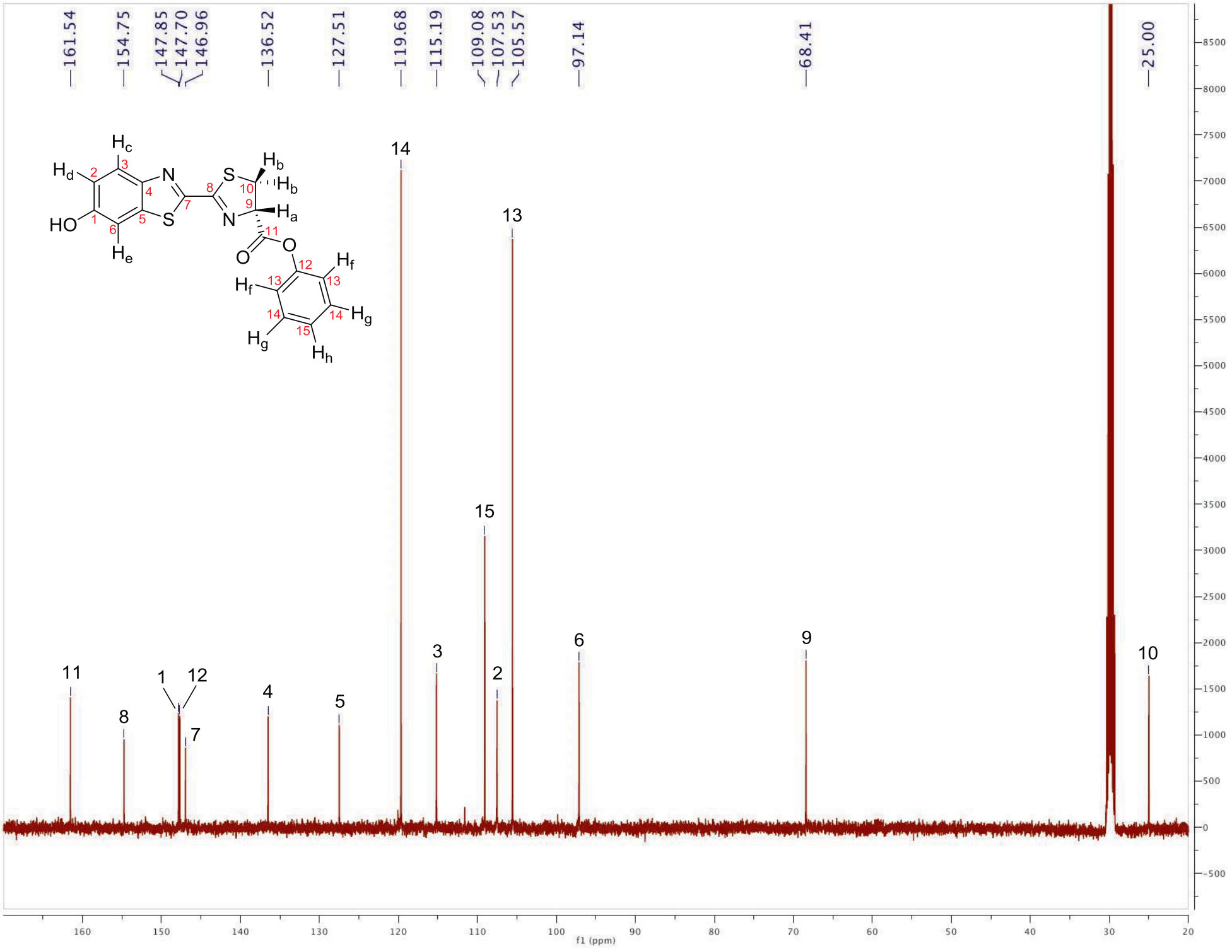

8.19
8.19
8.18
8.18
8.14
8.13
8.12
7.58
7.57
7.57
7.56
7.56
7.55
7.54
7.53
7.53
4.96
4.94
4.92
3.79
3.77
3.77
3.75
3.53
3.51
3.49
N
S
N
S
OK
O
6′–deoxyLuc
7.60
7.59
7.58
7.57
7.57
7.56
7.56
7.55
7.54
7.53
7.53
f1 (ppm)
1.73
2.15
1.05
1.00
1.05
f1 (ppm)


169.88
162.15
159.35
152.67
135.05
126.87
126.85
123.76
122.71
83.98
36.27
N
S
N
S
OK
O
6′–deoxyLuc
126.87
126.85
f1 (ppm)
f1 (ppm)

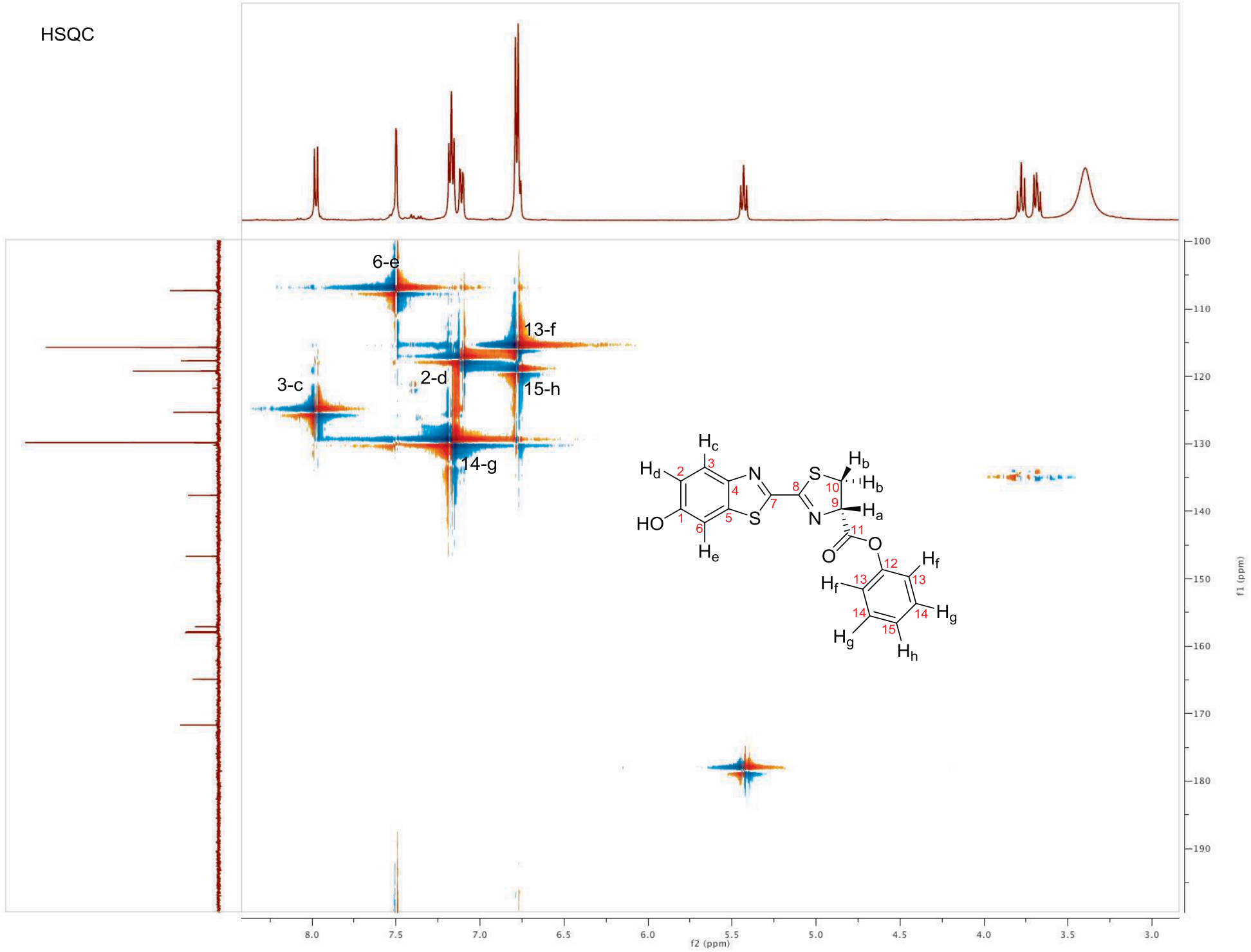

7.81
7.80
7.79
7.78
7.54
7.52
7.52
7.50
7.48
7.46
7.33
7.30
7.29
4.74
4.72
4.71
4.69
4.68
1.45
1.43
N
CN
S
i-PrO
9
2.00
2.09
1.09
1.02
1.08
1.04
6.36
f1 (ppm)


158.78
144.77
139.36
138.62
137.35
132.51
129.62
128.63
128.54
119.17
113.49
103.95
71.10
21.99
N
CN
S
i-PrO
9
grease
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)

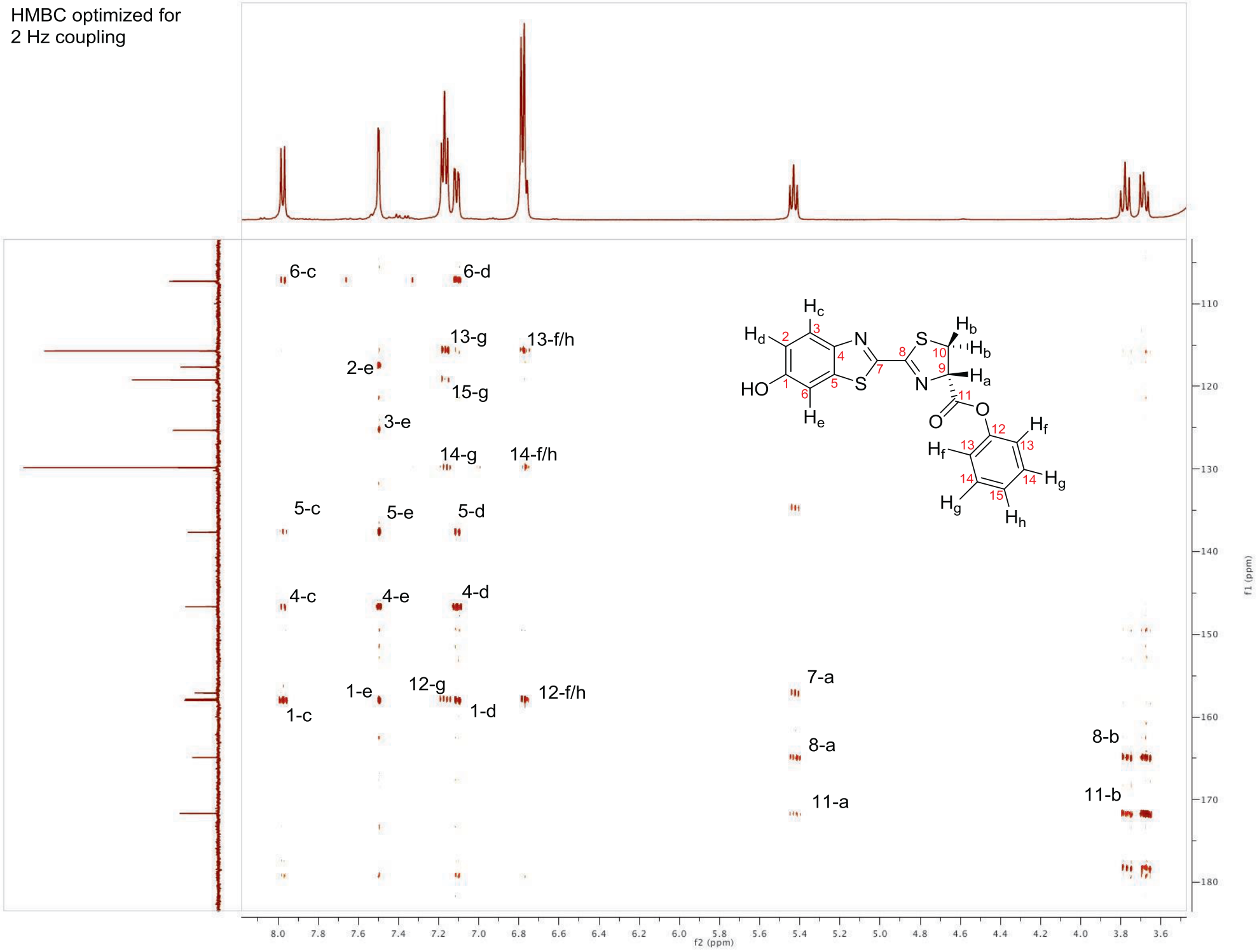

10.36
9.40
7.96
7.94
7.48
7.47
7.08
6.76
6.75
6.73
5.42
5.41
5.39
3.78
3.75
3.73
3.68
3.66
3.66
3.64
f/h
g
e
c
d
a
b
1.00
1.04
1.76
0.82
2.54
0.98
1.99
f1 (ppm)

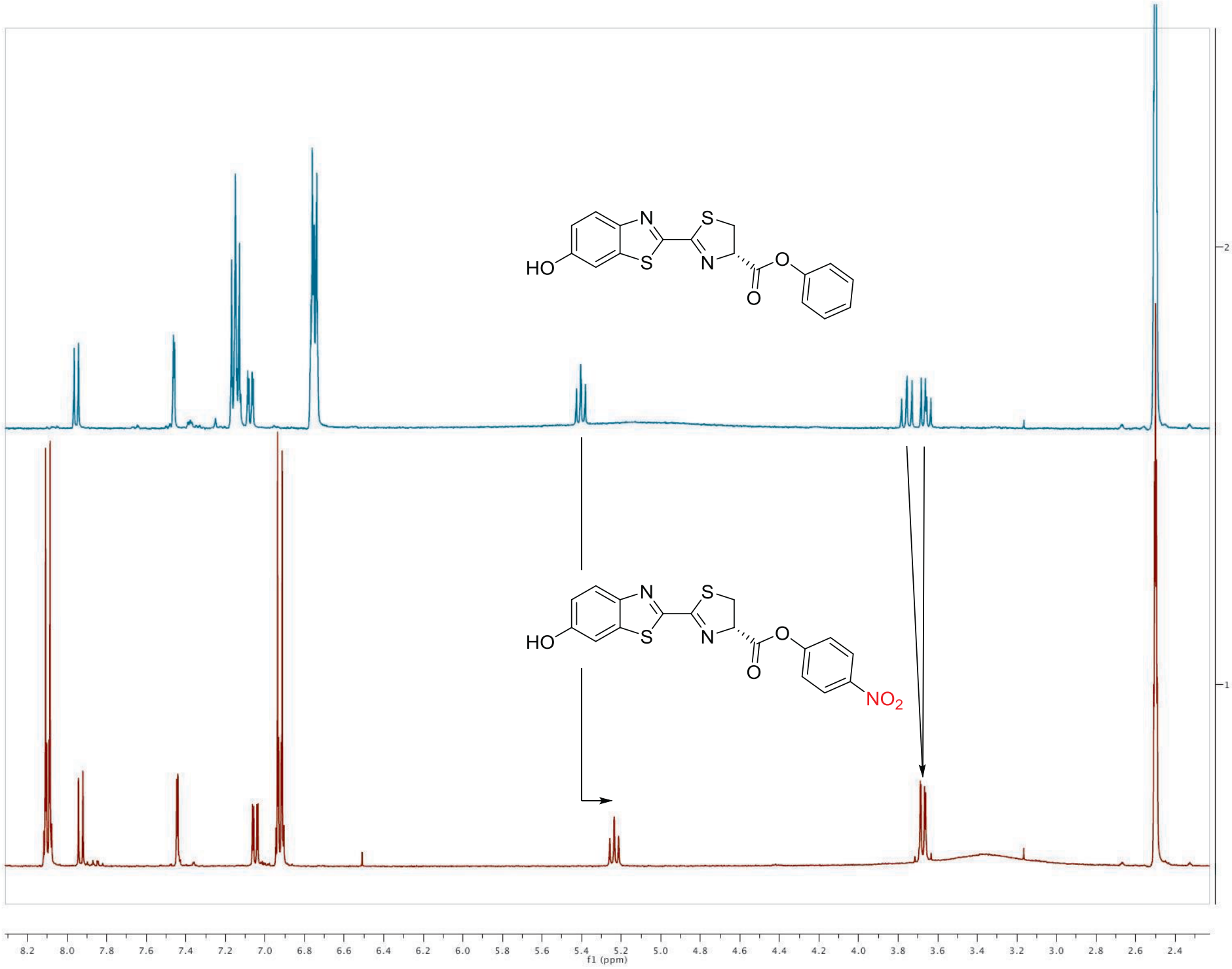

161.54
154.75
147.85
147.70
146.96
136.52
127.51
119.68
115.19
109.08
107.53
105.57
97.14
68.41
25.00
HO
N
S
O
11
12
13
14
15
1
2
3
4
5
6
7
8
9
10
f1 (ppm)

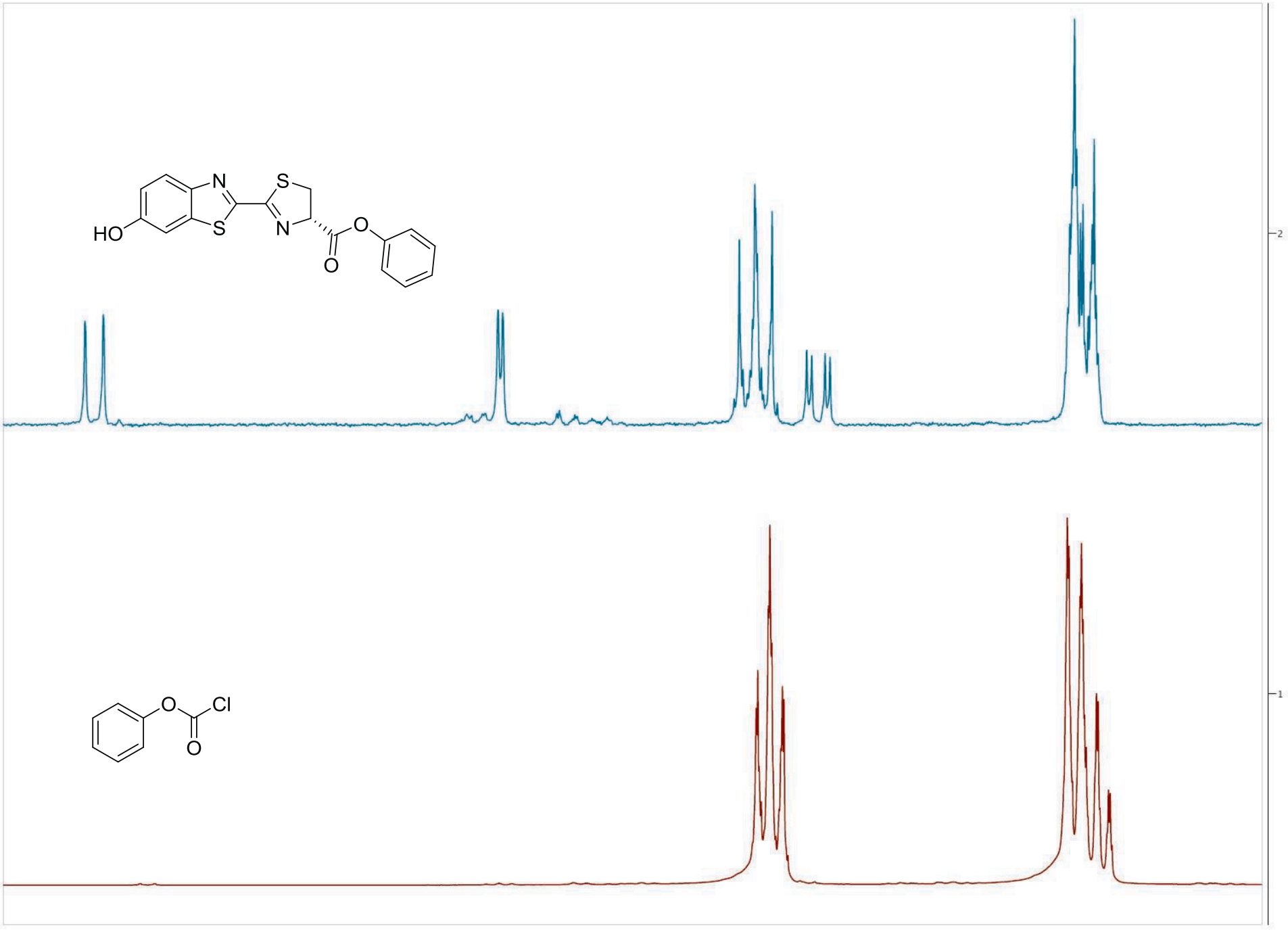
HSQC



HMBC optimized for
2 Hz coupling




HO
N
S
S
N
O
O
HO
N
S
S
N
O
O
NO2
f1 (ppm)


HO
N
S
S
N
O
O
O
Cl
O
2
1
8.0
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
7.1
7.0
6.9
6.8
6.7
6.6
f1 (ppm)