SUPPORTING INFORMATION

Cytoplasmic dynein antagonists with improved potency and isoform selectivity

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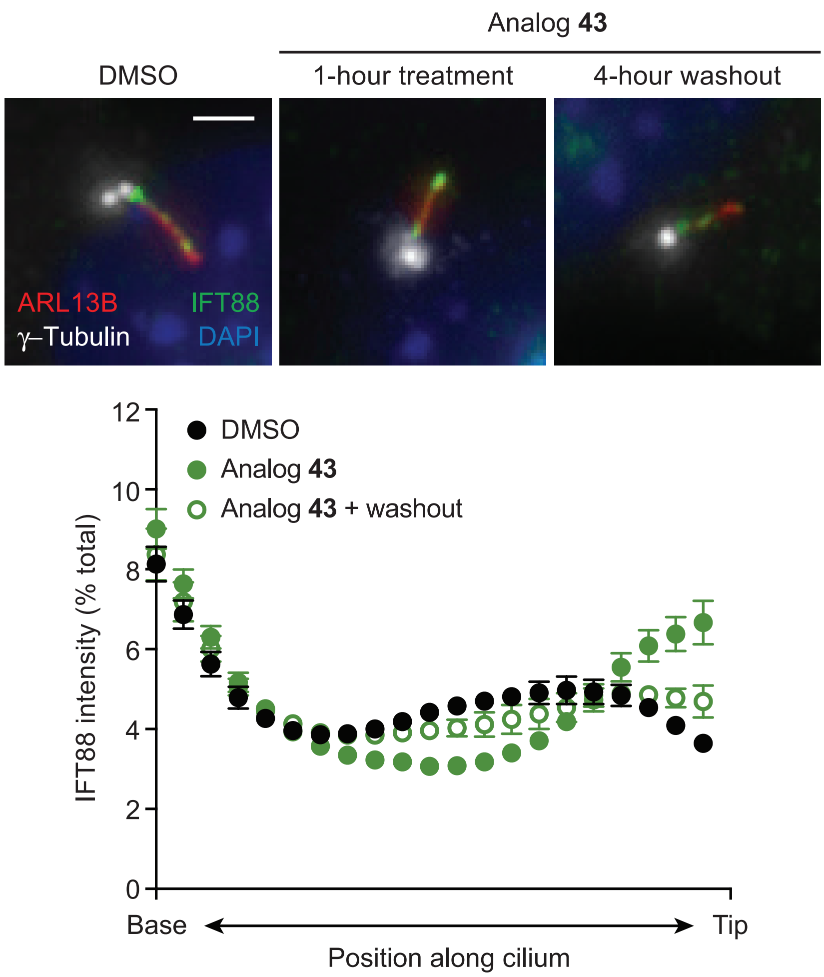
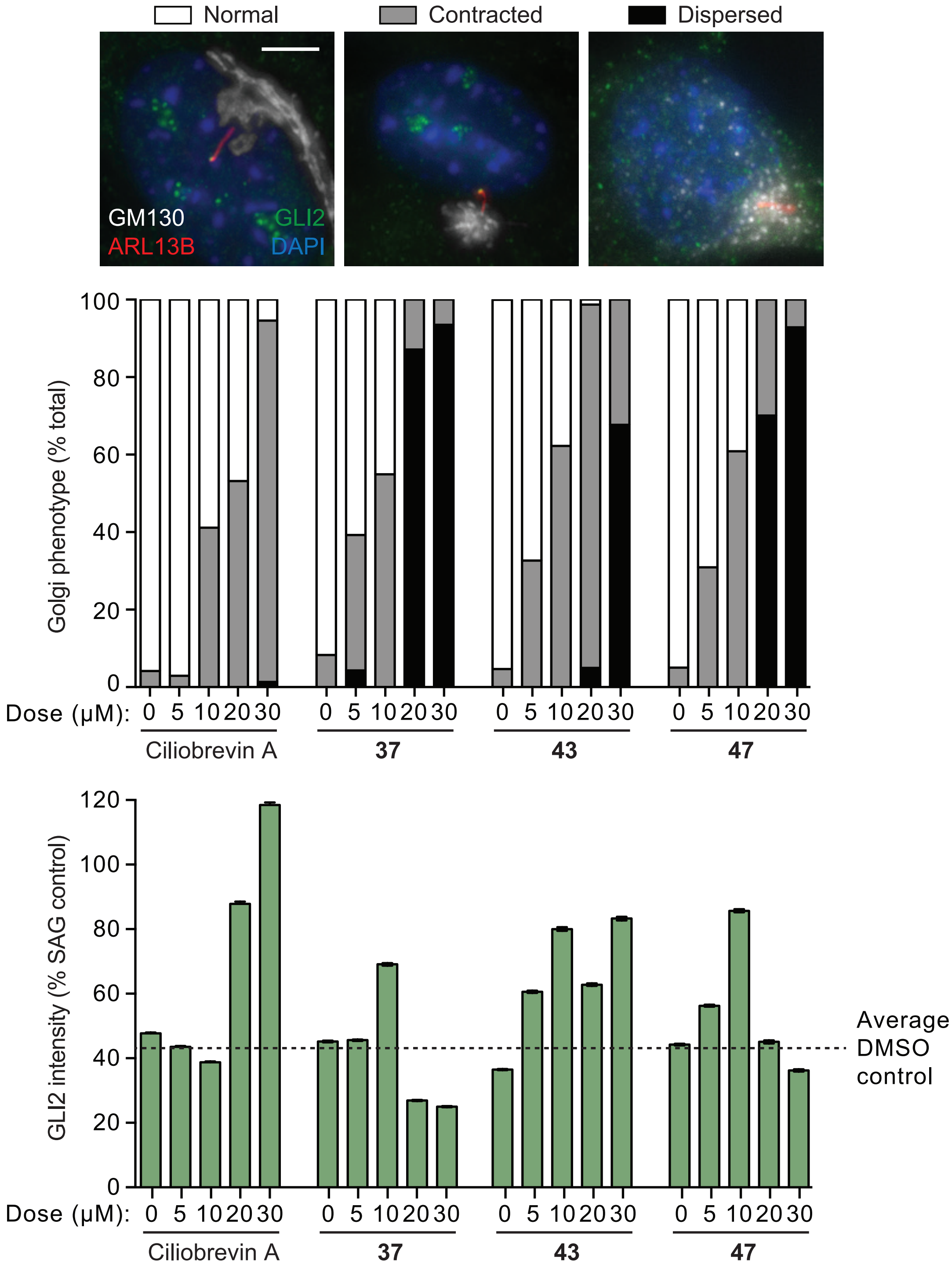
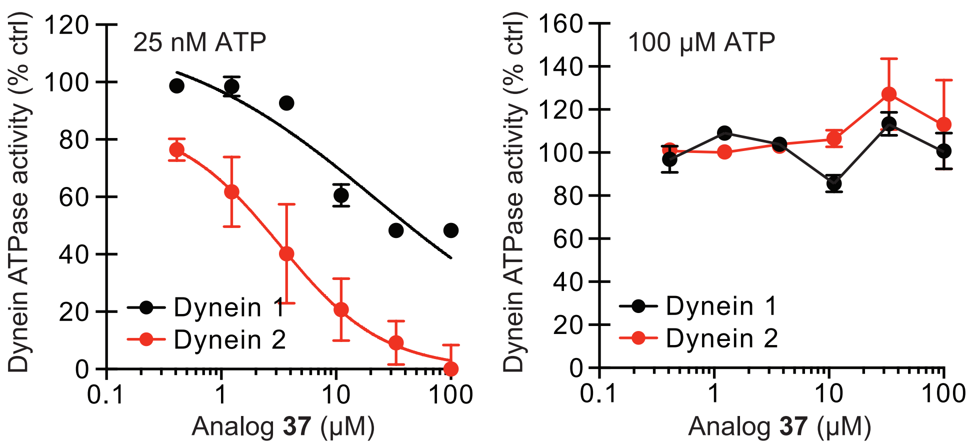


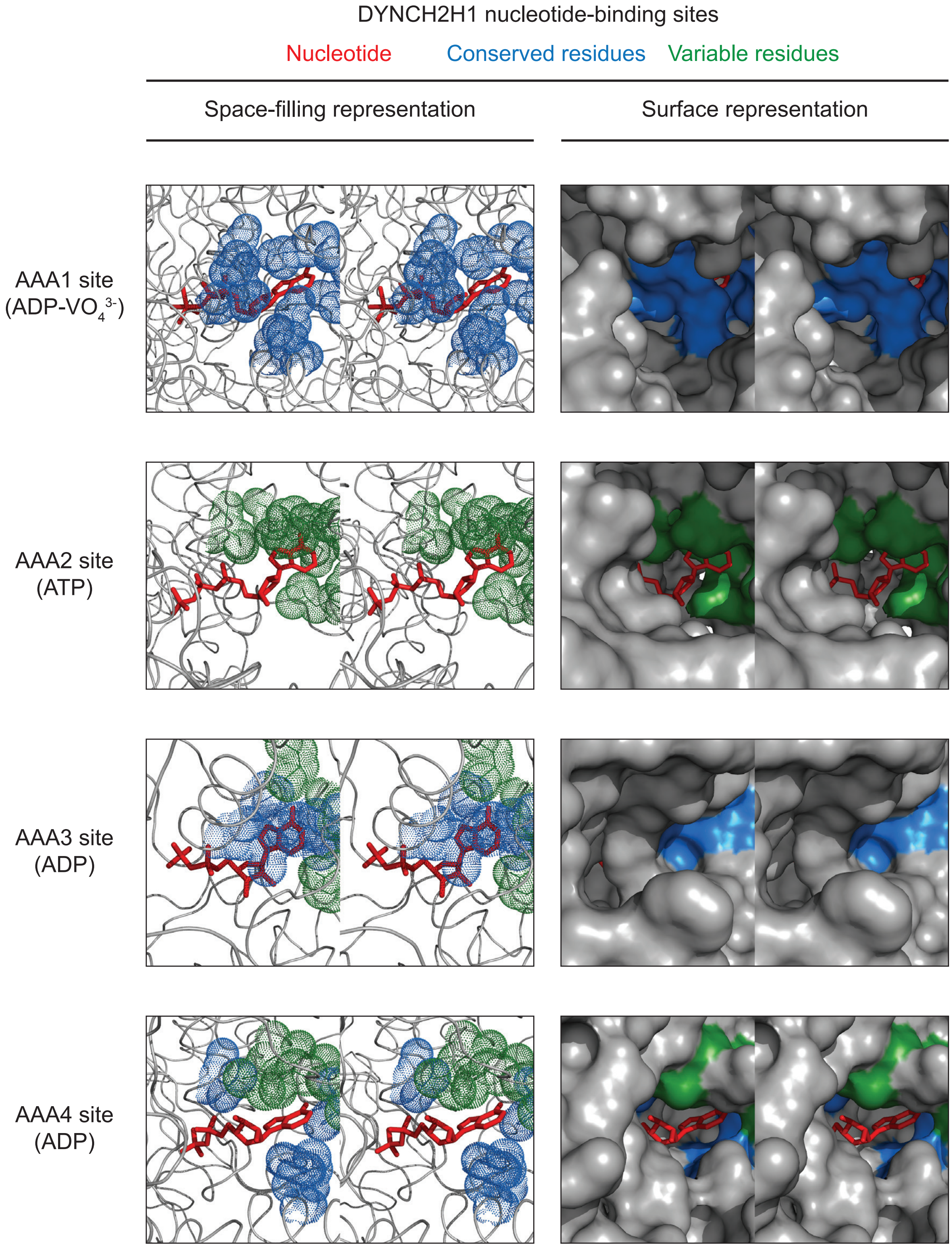
Supplementary Figure 1. C7-benzyl ether ciliobrevins reversibly disrupt IFT88 trafficking. IFT88 distributions along the ciliary axoneme in response to treatment with analog **43** for 1 hour and then compound washout for 4 hours. Representative micrographs of an NIH-3T3 cell-derived line are shown with staining for IFT88, ARL13B (primary cilia), γ-tubulin (basal body), and DNA. Each axoneme was divided into 21 bins, and the IFT88 signal intensity within each bin was normalized to the total ciliary signal using Matlab R2014A (Mathworks). Data represent the average IFT88 signal intensities for 65 cilia ± s.e.m.



Supplementary Figure 2. Effects of C7-benzyl ether ciliobrevins on Golgi morphology and GLI2 trafficking. Representative Golgi organization and GLI2 trafficking phenotypes observed in a serum-starved NIH-3T3 cell-derived line treated with DMSO or ciliobrevins for 4 hours. Cytoplasmic dynein 1 inhibition causes Golgi vesicle dispersion, whereas cytoplasmic dynein 2 blockade causes GLI2 accumulation at the distal tip of primary cilia (ARL13B). Scale bar: 5 μm. Quantitative analyses of the Golgi (top) and GLI2 (bottom) phenotypes are shown. GLI2 signal intensities at the ciliary tip were normalized to that of a SAG-treated control. At least 100 cells were scored for each condition, and GLI2 intensities are shown as average values ± s.e.m of three independent experiments.



Supplementary Figure 3. **ATP concentration-dependent inhibition of cytoplasmic dynein 1 and 2 motor domains by ciliobrevins.** Dose-dependent inhibition of DYNC1H1 (black) and DYNC2H1 (red) motor ATPase activities by ciliobrevin analog **37** at 25 nM and 100 μM ATP concentrations. Data are the average of triplicate samples ± s.e.m.

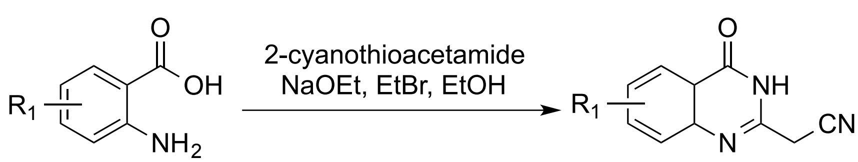


Supplementary Figure 4. Comparison of the nucleotide-binding sites in the cytoplasmic dynein 2 heavy chain. Stereoviews of the nucleotide-binding sites in DYNC2H1, visualized from their most solvent-accessible faces (PDB ID: ARH7). Space-filling (left) and surface (right) renderings are shown, and the linker region N-terminal to the AAA1 domain has been omitted for clarity. Adenine-interacting residues that are conserved between DYNC1H1 and DYNC2H1 are depicted in blue, variable residues in green, and nucleotides in red.

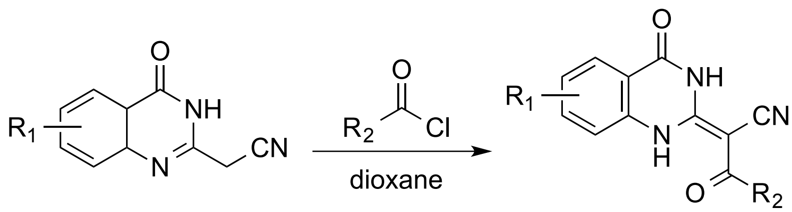
MATERIALS AND METHODS — SYNTHETIC PROCEDURES

Ciliobrevins A (**1**) and D (**2**) and analog **25** were synthesized as previously described.[1, 2] Ciliobrevins **3-19** and **23-31** were prepared using Procedure A, while analogs **20**, **21**, and **22** were obtained through separate routes. Benzyl ether derivatives **32-48** were prepared through Procedure B. Unless otherwise noted, all reactions were performed under a nitrogen atmosphere. THF, DMF, and acetonitrile were distilled from calcium hydride and stored over 4 Å molecular sieves. Other reagents and solvents obtained from commercial sources were used directly without further purification. Purification of products was conducted by flash chromatography on silica gel (EM Science, 70-230 mesh; Sorbtech, 230-400 mesh; or Qingdao Haiyang Chemical, 200-300 mesh). ^{1}H NMR spectra were recorded on Varian or Bruker NMR spectrometers, and chemical shifts are reported in δ units (ppm) using residual solvent as the internal standard. High-resolution mass spectra were obtained on quadrupole time-of-flight (Q-TOF) mass spectrometers utilizing the electrospray ionization method.

<u>Representative procedure A for the synthesis of ciliobrevins 3-19 and 23-31</u>



2-(4-oxo-3,4-dihydroquinazolin-2-yl)acetonitrile derivatives. 2-cyanothioacetamide (200 mg, 2.00 mmol) and bromoethane (165 µL, 2.21 mmol) were added to an ethanolic solution of sodium ethoxide (2.00 mL, 2.20 mmol). The resulting mixture was stirred for 6 h. The corresponding substituted 2-aminobenzoic acid (2.00 mmol) was added, and the reaction was refluxed overnight with stirring. A solid precipitate formed upon cooling of the reaction mixture, which was recovered by vacuum filtration and washed sequentially with ethanol, water, ethanol, and diethyl ether. The solid was then dried to yield the substituted 2-(4-oxo-3,4-dihydroquinazolin-2-yl) acetonitrile. Synthetic yields ranged from 30-50%.



3-oxo-2-(4-oxo-3,4-dihydroquinazolin-2(1*H*)-ylidene)-3-phenylpropanenitrile derivatives. To a solution of 2-(4-oxo-1,4-dihydroquinazolin-2-yl)acetonitrile (92.5 mg, 0.499 mmol) and triethylamine (84 μL, 0.60 mmol) in dioxane (5 mL) was added the corresponding acyl chloride (0.500 mmol), and the resulting mixture was refluxed overnight with stirring. A solid precipitate formed upon cooling of the reaction mixture, which was recovered by vacuum filtration and washed sequentially with methanol, water, methanol, and dichloromethane. The solid was then dried to yield the desired product. Synthetic yields ranged from 30-70%.

^{1}H NMR and HRMS data for ciliobrevins 3-19 and 23-31

3-(2,4-dichlorophenyl)-2-(6-methyl-4-oxo-3,4-dihydroquinazolin-2(1*H*)-ylidene)-3-oxopropanenitrile (3). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.55 (br s, 1H), 7.85 (s, 1H), 7.73-7.75 (m, 2H), 7.65 (d, *J* = 8.4 Hz, 1H), 7.48-7.55 (m, 2H), 2.41 (s, 3H). HRMS (*m/z*) calc. for $C_{18}H_{12}Cl_2N_3O_2$ $[M + H]^+$, 372.0307; observed, 372.0288.

3-(2,4-dichlorophenyl)-2-(7-methyl-4-oxo-3,4-dihydroquinazolin-2(1*H*)-ylidene)-3-oxopropanenitrile (4). ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.46 (br s, 1H), 7.94 (d, *J* = 8.0 Hz, 1H), 7.74 (s, 1H), 7.65 (s, 1H), 7.49-7.55 (m, 2H), 7.29 (d, *J* = 7.9 Hz, 1H), 2.44 (s, 3H). HRMS (*m/z*) calc. for $C_{18}H_{12}Cl_2N_3O_2$ $[M + H]^+$, 372.0307; observed, 372.0305.

3-(2,4-dichlorophenyl)-2-(6-ethyl-4-oxo-3,4-dihydroquinazolin-2(1*H*)-ylidene)-3-oxopropanenitrile (5). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.54 (br s, 1H), 7.87 (s, 1H), 7.70-7.79 (m, 3H), 7.50-7.56 (m, 2H), 2.73 (q, *J* = 7.2 Hz, 2H), 1.22 (t, *J* = 7.2 Hz, 3H). HRMS (*m/z*) calc. for $C_{19}H_{12}Cl_2N_3O_2$ $[M - H]^-$, 384.0307; observed, 384.0309.

3-(2,4-dichlorophenyl)-2-(7-ethyl-4-oxo-3,4-dihydroquinazolin-2(1*H*)-ylidene)-3-oxopropanenitrile (6). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.42 (br s, 1H), 7.95 (d, *J* = 8.0 Hz, 1H), 7.75 (d, *J* = 2.0 Hz, 1H), 7.71 (s, 1H), 7.49-7.56 (m, 2H), 7.33 (d, *J* = 8.4 Hz, 1H), 2.73 (q, *J* = 7.6 Hz, 2H), 1.22 (t, *J* = 7.6 Hz, 3H). HRMS (*m/z*) calc. for $C_{19}H_{12}Cl_2N_3O_2$ $[M - H]^-$, 384.0307; observed, 384.0312.

3-(2,4-dichlorophenyl)-2-(6-isopropyl-4-oxo-3,4-dihydroquinazolin-2(1*H*)-ylidene)-3-oxopropanenitrile (7). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.48 (br s, 1H), 7.89 (d, *J* = 1.6 Hz, 1H), 7.77-7.82 (m, 2H), 7.75 (d, *J* = 1.6 Hz, 1H), 7.49-7.56 (m, 2H), 2.49 (m, 1H), 1.24 (d, *J* = 6.8 Hz, 6H). HRMS (*m/z*) calc. for $C_{20}H_{14}Cl_2N_3O_2$ $[M - H]^-$, 398.0463; observed, 398.0467.

3-(2,4-dichlorophenyl)-2-(7-isopropyl-4-oxo-3,4-dihydroquinazolin-2(1*H*)-ylidene)-3-oxopropanenitrile (8). ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.45 (br s, 1H), 7.96 (d, *J* = 8.1 Hz, 1H), 7.77 (d, *J* = 1.8 Hz, 2H), 7.50-7.58 (m, 2H), 7.41 (dd, *J* = 1.2, 8.1 Hz, 1H), 3.01-3.05 (m, 1H), 1.23 (d, J = 6.9 Hz, 6H). HRMS (*m/z*) calc. for $C_{20}H_{14}Cl_2N_3O_2$ $[M - H]^-$, 398.0463; observed, 398.0468.

2-(6-*tert*-butyl-4-oxo-3,4-dihydroquinazolin-2(1*H*)-ylidene)-3-(2,4-dichlorophenyl)-3-oxo-propanenitrile (9). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (d, J = 2.0 Hz, 1H), 7.88 (dd, J = 2.4, 8.4 Hz, 1H), 7.50 (d, J = 1.6 Hz, 1H), 7.35-7.42 (m, 3H), 1.40 (s, 9H). HRMS (*m/z*) calc. for $C_{21}H_{16}Cl_2N_3O_2$ $[M - H]^-$, 412.0620; observed, 412.0626.

2-(7-*tert*-butyl-4-oxo-3,4-dihydroquinazolin-2(1*H*)-ylidene)-3-(2,4-dichlorophenyl)-3-oxo-propanenitrile (10). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (d, J = 8.4 Hz, 1H), 7.50-7.55 (m, 2H), 7.34-7.42 (m, 3H), 1.40 (s, 9H). HRMS (*m/z*) calc. for $C_{21}H_{16}Cl_2N_3O_2$ $[M - H]^-$, 412.0620; observed, 412.0624.

3-(2,4-dichlorophenyl)-2-(6-methoxy-4-oxo-3,4-dihydroquinazolin-2(1*H*)-ylidene)-3-oxo-propanenitrile (11). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.54 (br s, 1H), 7.80 (d, J = 9.6 Hz, 1H), 7.75 (s, 1H), 7.46-7.56 (m, 4H), 3.86 (s, 3H). HRMS (*m/z*) calculated for $C_{18}H_{12}Cl_2N_3O_3$ $[M + H]^+$: 388.0256; observed: 388.0233.

3-(2,4-dichlorophenyl)-2-(7-methoxy-4-oxo-3,4-dihydroquinazolin-2(1*H*)-ylidene)-3-oxopro-panenitrile (12). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.97 (d, J = 9.0 Hz, 1H), 7.77 (d, J = 2.0 Hz, 1H), 7.56 (dd, J = 8.1, 2.0 Hz, 1H), 7.52 (d, J = 8.1 Hz, 1H), 7.41 (br s, 1H), 7.05 (dd, J = 9.0, 2.2 Hz, 1H), 3.89 (s, 3H). HRMS (*m/z*) calculated for $C_{18}H_{12}Cl_2N_3O_3$ $[M + H]^+$: 388.0256; observed, 388.0241.

3-(2,4-dichlorophenyl)-2-(6-ethoxy-4-oxo-3,4-dihydroquinazolin-2(1*H*)-ylidene)-3-oxopropanenitrile (13). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.50 (s, 1H), 7.61 (d, *J* = 2.0 Hz, 1H), 7.34-7.44 (m, 4H), 7.26 (dd, *J* = 2.8, 8.8 Hz, 1H), 4.04 (q, *J* = 7.2 Hz, 2H), 1.35 (t, *J* = 7.2 Hz, 3H). HRMS (*m/z*) calc. for $C_{19}H_{12}Cl_2N_3O_3$ [M – H]$^-$, 400.0256; observed, 400.0261.

3-(2,4-dichlorophenyl)-2-(7-ethoxy-4-oxo-3,4-dihydroquinazolin-2(1*H*)-ylidene)-3-oxopropanenitrile (14). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.33 (br s, 1H), 7.93 (d, *J* = 8.8 Hz, 1H), 7.74 (d, *J* = 2.0 Hz, 1H), 7.49-7.56 (m, 2H), 7.37 (*s*, 1H), 7.00 (dd, *J* = 2.4, 8.8 Hz, 1H), 4.14 (q, *J* = 7.2 Hz, 2H), 1.38 (t, *J* = 7.2 Hz, 3H). HRMS (*m/z*) calc. for $C_{19}H_{12}Cl_2N_3O_3$ [M – H]$^-$, 400.0256; observed, 400.0258.

3-(2,4-dichlorophenyl)-3-oxo-2-(4-oxo-6-propoxy-3,4-dihydroquinazolin-2(1*H*)-ylidene) propanenitrile (15). ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.54 (br s, 1H), 7.76-7.82 (m, 2H), 7.45-7.57 (m, 4H), 4.04 (t, *J* = 6.6 Hz, 2H), 1.73-1.80 (m, 2H), 0.99 (t, *J* = 7.2 Hz, 2H). HRMS (*m/z*) calc. for $C_{20}H_{14}Cl_2N_3O_3$ [M – H]$^-$, 414.0412; observed, 414.0416.

3-(2,4-dichlorophenyl)-3-oxo-2-(4-oxo-7-propoxy-3,4-dihydroquinazolin-2(1*H*)-ylidene) propanenitrile (16). ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.37 (br s, 1H), 7.93 (d, *J* = 9.0 Hz, 1H), 7.77 (s, 1H), 7.50-7.57 (m, 2H), 7.40 (s, 1H), 7.01 (d, *J* = 8.7 Hz, 1H), 4.04 (d, *J* = 6.6 Hz, 2H), 1.75-1.82 (m, 2H), 0.99 (t, *J* = 7.2 Hz, 3H). HRMS (*m/z*) calc. for $C_{20}H_{14}Cl_2N_3O_3$ [M – H]$^-$, 414.0412; observed, 414.0420.

3-(2,4-dichlorophenyl)-2-(6-isopropoxy-4-oxo-3,4-dihydroquinazolin-2(1*H*)-ylidene)-3-oxo-propanenitrile (17). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.43 (br s, 1H), 7.82 (d, *J* = 9.6 Hz, 1H), 7.75 (s, 1H), 7.69 (s, 1H), 7.49-7.56 (m, 2H), 7.44 (s, 2H), 4.74 (m, 1H), 1.29 (d, *J* = 5.6 Hz, 6H). HRMS (*m/z*) calc. for $C_{20}H_{14}Cl_2N_3O_3$ $[M-H]^-$, 414.0412; observed, 414.0416.

3-(2,4-dichlorophenyl)-2-(7-isopropoxy-4-oxo-3,4-dihydroquinazolin-2(1*H*)-ylidene)-3-oxo-propanenitrile (18). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.33 (br s, 1H), 7.92 (d, *J* = 9.2 Hz, 1H), 7.76 (d, *J* = 2.0 Hz, 1H), 7.50-7.57 (m, 2H), 7.40 (d, *J* = 1.6 Hz, 1H), 6.98 (dd, *J* = 2.4, 8.0 Hz, 1H), 4.71 (m, 1H), 1.34 (d, *J* = 6.0 Hz, 6H). HRMS (*m/z*) calc. for $C_{20}H_{14}Cl_2N_3O_3$ $[M-H]^-$, 414.0412; observed, 414.0420.

(3-(2,4-dichlorophenyl)-2-(6-(dimethylamino)-4-oxo-3,4-dihydroquinazolin-2(1*H*)-ylidene)-3-oxopropanenitrile (19). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.76-7.56 (m, 2H), 7.52 (q, *J* = 1.6, 8.2, 9.9 Hz, 2H), 7.35 (dd, *J* = 2.8, 9.3 Hz, 1H), 7.14 (d, *J* = 2.7 Hz, 1H), 3.00 (s, 3H). HRMS (*m/z*) calc. for $C_{19}H_{15}Cl_2N_4O_2$ $[M+H]^+$, 401.0567; observed, 401.0535.

3-(3,4-dichlorophenyl)-3-oxo-2-(4-oxo-3,4-dihydroquinazolin-2(1*H*)-ylidene)propanenitrile (23). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.07 (d, *J* = 7.8 Hz, 1H), 7.92 (d, *J* = 2.0 Hz, 1H), 7.89 (t, *J* = 7.8 Hz, 1H), 7.86 (t, *J* = 8.3 Hz, 1H), 7.81 (d, *J* = 8.3 Hz, 1H), 7.70 (dd, *J* = 2.0, 8.3 Hz, 1H), 7.48 (t, *J* = 7.8 Hz, 1H). HRMS (*m/z*) calc. for $C_{17}H_{10}Cl_2N_3O_2$ $[M+H]^+$, 358.0145; observed, 358.0133.

3-(3-methoxyphenyl)-3-oxo-2-(4-oxo-3,4-dihydroquinazolin-2(1*H*)-ylidene)propanenitrile (24). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.03 (d, *J* = 7.6 Hz, 1H), 7.87 (d, *J* = 8.0 Hz, 1H), 7.80 (t, *J* = 8.0 Hz, 1H), 7.45 (t, *J* = 8.0 Hz, 1H), 7.39 (d, *J* = 8.0 Hz, 1H), 7.27 (d, *J* = 7.6 Hz, 1H), 7.22 (s, 1H), 7.12 (dd, *J* = 1.6, 8.0 Hz, 1H), 3.80 (s, 3H). HRMS (*m/z*) calc. for $C_{18}H_{14}N_3O_3$ [M + H]$^+$, 320.1035; observed, 320.1029.

3-oxo-2-(4-oxo-3,4-dihydroquinazolin-2(1*H*)-ylidene)-3-(pyridin-3-yl)propanenitrile (26). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.02 (s, 1H), 8.85 (d, *J* = 4.0 Hz, 1H), 8.38 (d, *J* = 8.0 Hz, 1H), 8.05 (d, *J* = 7.6 Hz, 1H), 7.79-7.91 (m, 3H), 7.48 (t, *J* = 7.6 Hz, 1H). HRMS (*m/z*) calc. for $C_{16}H_{11}N_4O_2$ [M + H]$^+$, 291.0882; observed, 291.0865.

3-(2,4-dichlorophenyl)-3-oxo-2-(4-oxo-3,4-dihydrobenzo[*g*]quinazolin-2(1*H*)–ylidene)propanenitrile (27). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.42 (br s, 1H), 8.79 (s, 1H), 8.28 (s, 1H), 8.20 (d, *J* = 8.0 Hz, 1H), 8.02 (d, *J* = 8.4 Hz, 1H), 7.76 (s, 1H), 7.71 (t, *J* = 8.0 Hz, 1H), 7.51-7.61 (m, 3H). HRMS (*m/z*) calc. for $C_{21}H_{12}Cl_2N_3O_2$ [M + H]$^+$, 408.0307; observed, 408.0318.

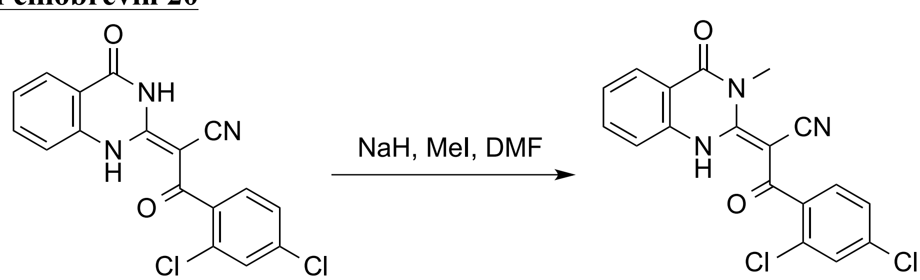
(3-(2,4-dichlorophenyl)-3-oxo-2-(4-oxo-3,4,6,7,8,9-hexahydrobenzo[*g*]quinazolin-2(1*H*)-ylidene)propanenitrile (28). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (s, 1H), 7.49 (s, 1H), 7.30-7.40 (m, 2H), 7.12 (s, 1H), 2.85-2.96-4.40 (m, 4H), 1.85-1.94 (m, 4H). HRMS (*m/z*) calc. for $C_{21}H_{14}Cl_2N_3O_2$ [M – H]$^-$, 410.0463; observed, 410.0469.

3-(2,4-dichlorophenyl)-3-oxo-2-(4-oxo-3,4,7,8-tetrahydro-[1,4]dioxino[2,3-*g*]quinazolin-2(1*H*)-ylidene)propanenitrile (29). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.74 (d, *J* = 2.0 Hz, 1H), 7.53 (d, J =8.0 Hz, 1H), 7.48 (d, J = 8.0 Hz, 1H), 7.43 (s, 1H), 7.34 (s, 1H), 4.33-4.40 (*m*, 4H). HRMS (*m/z*) calc. for $C_{19}H_{10}Cl_2N_3O_4$ $[M-H]^-$, 414.0048; observed, 414.0054.

3-(2,4-dichlorophenyl)-3-oxo-2-(4-oxo-3,4,7,8-tetrahydro-1H-cyclopenta[*g*]quinazolin-2(6*H*)-ylidene)propanenitrile (30). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.44 (br s, 1H), 7.88 (s, 1H), 7.75 (d, J = 1.6 Hz, 1H), 7.69 (s, 1H), 7.49-7.56 (m, 2H), 2.96 (*m*, 4H), 2.06 (*t*, J = 7.6 Hz, 2H). HRMS (*m/z*) calc. for $C_{20}H_{12}Cl_2N_3O_2$ $[M-H]^-$, 396.0307; observed, 396.0305.

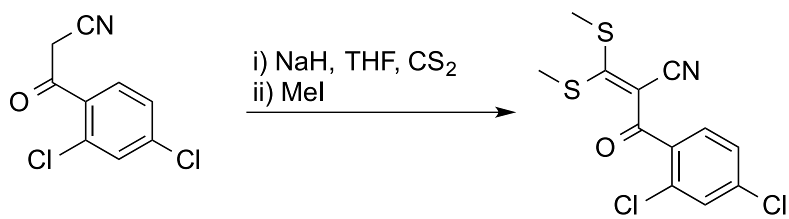
3-(2,4-dichlorophenyl)-3-oxo-2-(4-oxo-3,4-dihydrofuro[3,4-*g*]quinazolin-2(1*H*,6*H*,8*H*)-ylidene)propanenitrile (31). ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.55 (br s, 1H), 8.00 (s, 1H), 7.75-7.77 (m, 2H), 7.52-7.55 (m, 2H), 5.09 (s, 2H), 5.06 (s, 2H). HRMS (*m/z*) calc. for $C_{19}H_{10}Cl_2N_3O_3$ $[M-H]^-$, 398.0099; observed, 398.0102.

<u>**Synthesis of ciliobrevin 20**</u>

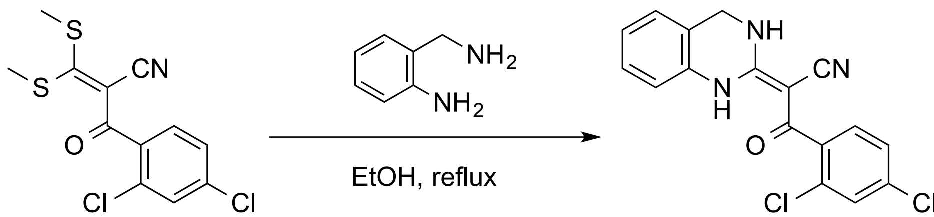


3-(2,4-dichlorophenyl)-2-(3-methyl-4-oxo-3,4-dihydroquinazolin-2(1*H*)-ylidene)-3-oxopropanenitrile (20). To a solution of ciliobrevin A (**1**) (107 mg, 0.299 mmol) in DMF (2 mL) was added sodium hydride (8.6 mg, 0.36 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 40 min. To this solution was added iodomethane (47 mg, 0.33 mmol), and the resulting mixture was stirred at room temperature for 3 h. The reaction was quenched by addition of a saturated solution of $NaHCO_3$ (5 mL), and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were washed with H_2O and brine, then dried over Na_2SO_4. The solvent was removed *in vacuo* and the residue was directly purified through preparative TLC to give the desired product (26 mg, 23%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.71 (s, 1H), 8.26 (d, J = 8.0 Hz, 1H), 7.78 (dd, J = 1.6, 8.0 Hz, 1H), 7.34-7.49 (m, 5H), 3.97 (s, 1H). HRMS (*m/z*) calc. for $C_{18}H_{12}Cl_2N_3O_2$ $[M + H]^+$, 372.0307; observed, 372.0290.

Synthesis of ciliobrevin 21

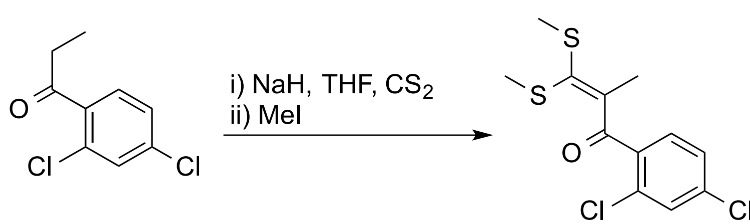


2-(2,4-dichlorobenzoyl)-3,3-bis(methylthio)acrylonitrile. To a stirred solution of 3-(2,4-dichlorophenyl)-3-oxopropanenitrile (1.06 g, 4.95 mmol) in THF (15 mL) at 0 °C was added dry sodium hydride (0.24 g, 10 mmol). The suspension was stirred at 0 °C for 1 h, at which point carbon disulfide (0.403 g, 5.29 mmol) was added, and the reaction was then stirred at room temperature for another 2 h. The resulting red solution was cooled to 0 °C, iodomethane (1.56 g, 11.0 mmol) was added, and the mixture was stirred at room temperature for 18 h. The solvent was then removed *in vacuo*, and the residue was diluted in ether and washed with brine. The aqueous layer was extracted twice with ether, and the combined organic layers were washed twice with 5% sodium thiosulfate, and then brine. The organic layers were dried over $MgSO_4$, filtered and concentrated to give the desired acrylonitrile as a yellow powder (1.08 g, 68%). This product was used in the next step without further purification or characterization.

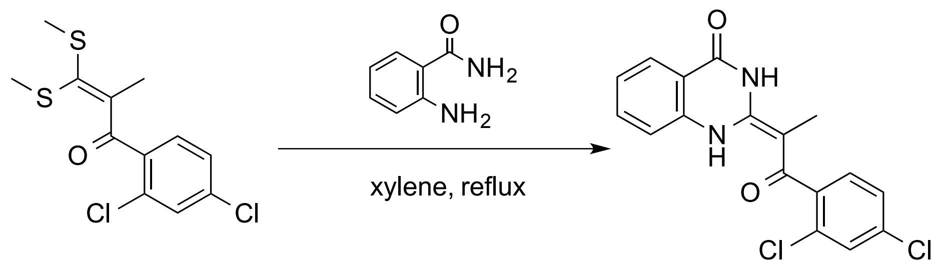


3-(2,4-dichlorophenyl)-2-(3,4-dihydroquinazolin-2(1*H*)-ylidene)-3-oxopropanenitrile (21). A solution of 2-(2,4-dichlorobenzoyl)-3,3-bis(methylthio)acrylonitrile (0.954 g, 3.00 mmol) and 2-aminobenzylamine (366 mg, 3.00 mmol) in ethanol (10 mL) was heated to reflux for 4 h. After cooling the precipitate was filtered. Recrystallization from ethanol gave the desired product (380 mg, 37%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.06 (br s, 1H), 9.58 (br s, 1H), 7.69 (s, 1H), 7.50 (dd, *J* = 1.6, 8.4 Hz, 1H), 7.43 (d, *J* = 8.4 Hz, 1H), 7.09-7.24 (m, 4H), 4.59 (s, 2H). HRMS (*m/z*) calc. for $C_{17}H_{12}Cl_2N_3O$ $[M + H]^+$, 344.0357; observed, 344.0344.

Synthesis of ciliobrevin 22

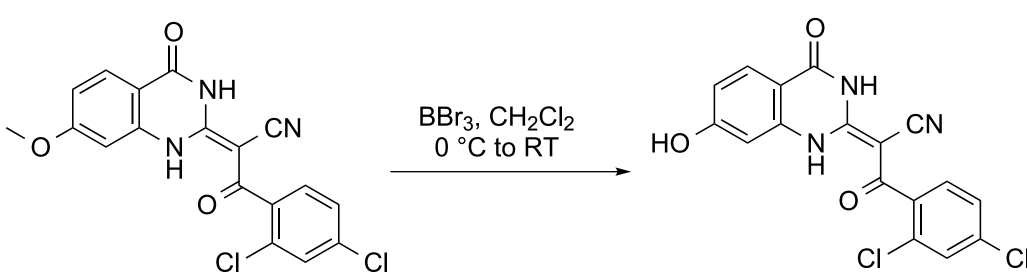


1-(2,4-dichlorophenyl)-2-methyl-3,3-bis(methylthio)prop-2-en-1-one. To a stirred solution of 1-(2,4-dichlorophenyl)propan-1-one (1.02 g, 5.02 mmol) in THF (15 ml) at 0 °C was added dry sodium hydride (0.24 g, 10 mmol). The suspension was stirred at 0 °C for 1 h, at which point carbon disulfide (0.403 g, 5.29 mmol) was added, and the reaction was then stirred at room temperature for another 2 h. The resulting red solution was cooled to 0 °C and iodomethane (1.56 g, 11.0 mmol) was added, and the mixture was stirred at room temperature for 18 h. The solvent was then removed *in vacuo*, and the residue was diluted in ether and washed with brine. The aqueous layer was extracted twice with ether, and the combined organic layers were washed twice with 5% sodium thiosulfate, and then brine. The organic layers were dried over $MgSO_4$, filtered and concentrated to give the desired propenone, which was used in the next step without further purification or characterization.

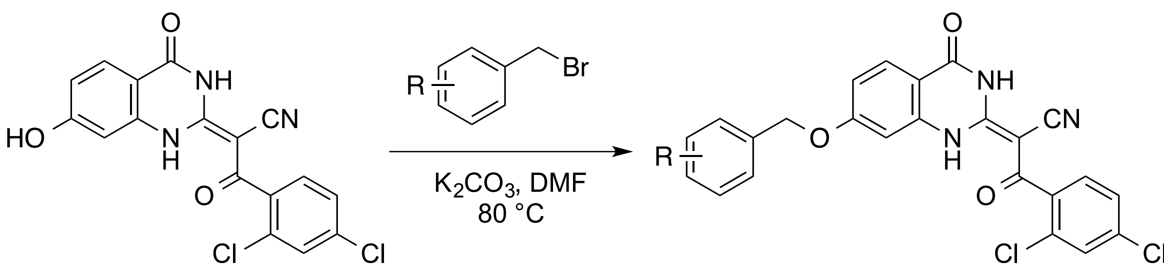


2-(1-(2,4-dichlorophenyl)-1-oxopropan-2-ylidene)-2,3-dihydroquinazolin-4(1*H*)-one (22). A solution of 1-(2,4-dichlorophenyl)-2-methyl-3,3-bis(methylthio) prop-2-en-1-one (307 mg, 1.00 mol) and 2-aminobenzamide (136 mg, 1.00 mmol) in xylene (10 mL) was heated to reflux for 3 h. After cooling, the precipitate was filtered. Recrystallization from ethanol gave the desired quinazolinone as colorless crystals (118 mg, 34%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.65 (s, 1H), 8.44 (s, 1H), 8.15 (d, J = 8.0 Hz, 1H), 7.70 (dt, J = 1.6, 8.0 Hz, 1H), 7.46 (d, J = 2.0 Hz, 1H), 7.23-7.35 (m, 4H), 1.80 (s, 3H). HRMS (*m/z*) calc. for $C_{17}H_{13}Cl_2N_2O_2$ $[M + H]^+$, 347.0354; observed, 347.0351.

<u>Representative procedure B for the synthesis of ciliobrevins 32-48</u>



3-(2,4-dichlorophenyl)-2-(7-hydroxy-4-oxo-3,4-dihydroquinazolin-2(1*H*)-ylidene)-3-oxopro-panenitrile. To a solution of ciliobrevin **12** (104 mg, 0.268 mmol) in dry CH_2Cl_2 (15 mL) was added 1.0 M BBr_3 in CH_2Cl_2 (2.6 mL, 2.6 mmol) at 0 °C. The reaction was allowed to warm to room temperature, continuously stirred for 7 days, and then quenched with water (100 mL). The mixture was then extracted with ethyl acetate (3 x 100 mL), and the organic layers were pooled, washed with brine, and concentrated *in vacuo*. The residue was then purified by silica gel chromatography ($CHCl_3$/MeOH/triethylamine, 8:1:1). Product containing fractions were combined and sequentially washed with 1N HCl, saturated $NaHCO_3$, and brine. Drying over $MgSO_4$ and removal of solvent *in vacuo* yielded the desired hydroxyquinazolinone (79.3 mg, 82%). ^{1}H NMR (500 MHz, CD_3OD) δ 6.88 (d, J = 8.8 Hz, 1H), 6.99 (s, 1H), 7.45 (m, 2H), 7.56 (d, J = 1.7 Hz, 1H), 8.00 (d, J = 8.3 Hz, 1H). HRMS (*m/z*) calc. for $C_{17}H_{10}Cl_2N_3O_3$ $[M + H]^+$, 374.0099; observed, 374.0087.



2-(7-(benzyloxy)-4-oxo-3,4-dihydroquinazolin-2(1*H*)-ylidene)-3-(2,4-dichlorophenyl)-3-oxo-propanenitrile derivatives. To a solution of 3-(2,4-dichlorophenyl)-2-(7-hydroxy-4-oxo-3,4-dihydroquinazolin-2(1*H*)-ylidene)-3-oxopropanenitrile (37 mg, 0.099 mmol) in DMF (4 mL) was added corresponding benzyl bromide (0.15 mmol) and K_2CO_3 (41 mg, 0.30 mmol), and the reaction was heated at 80 °C overnight with stirring. The mixture was then concentrated *in vacuo*, and the resulting residue was purified through flash chromatography (CH_2Cl_2/MeOH, 30:1) to afford the desired product. Synthetic yields ranged from 40-60%.

^{1}H NMR and HRMS data for ciliobrevins 32-48

2-(7-(2-chlorobenzyloxy)-4-oxo-3,4-dihydroquinazolin-2(1*H*)-ylidene)-3-(2,4-dichloro-phenyl)-3-oxopropanenitrile (32). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.42 (br s, 1H), 8.01 (d, *J* = 0.8 Hz, 1H), 7.75-7.43 (m, 8H), 7.16 (m, 1H), 5.30 (s, 2H). HRMS (*m/z*) calc. for $C_{24}H_{14}Cl_3N_3O_3$ $[M - H]^-$, 496.0022; observed, 496.0027.

2-(7-(3-chlorobenzyloxy)-4-oxo-3,4-dihydroquinazolin-2(1*H*)-ylidene)-3-(2,4-dichloro-phenyl)-3-oxopropanenitrile (33). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.36 (br s, 1H), 8.00 (d, *J* = 8.8 Hz, 1H), 7.76 (d, *J* = 1.6 Hz, 1H), 7.56-7.45 (m, 7H), 7.15 (dd, *J* = 2.0, 8.8 Hz, 1H), 5.26 (s, 2H). HRMS (*m/z*) calc. for $C_{24}H_{14}Cl_3N_3O_3$ $[M - H]^-$, 496.0022; observed, 496.0023.

2-(7-(4-chlorobenzyloxy)-4-oxo-3,4-dihydroquinazolin-2(1*H*)-ylidene)-3-(2,4-dichloro-phenyl)-3-oxopropanenitrile (34). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.33 (br s, 1H), 7.99 (d, *J* = 9.2 Hz, 1H), 7.76 (d, *J* = 1.6 Hz, 1H), 7.56-7.47 (m, 7H), 7.13 (dd, *J* = 2.0, 8.8 Hz, 1H), 5.23 (s, 2H). HRMS (*m/z*) calc. for $C_{24}H_{14}Cl_3N_3O_3$ $[M - H]^-$, 496.0022; observed 496.0031.

3-(2,4-dichlorophenyl)-2-(7-(2-methylbenzyloxy)-4-oxo-3,4-dihydroquinazolin-2(1*H*)-ylidene)-3-oxopropanenitrile (35). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.40 (br s, 1H), 8.00 (d, *J* = 8.8 Hz, 1H), 7.76 (d, *J* = 1.6 Hz, 1H), 7.57-7.51 (m, 3H), 7.44 (d, *J* = 7.2 Hz, 1H), 7.30-7.21 (m, 3H), 7.15 (dd, *J* = 2.0, 8.8 Hz, 1H), 5.23 (s, 2H), 2.34 (s, 3H). HRMS (*m/z*) calc. for $C_{25}H_{17}Cl_2N_3O_3$ $[M - H]^-$, 476.0569; observed, 476.0574.

3-(2,4-dichlorophenyl)-2-(7-(3-methylbenzyloxy)-4-oxo-3,4-dihydroquinazolin-2(1*H*)-ylidene)-3-oxopropanenitrile (36). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.34 (br s, 1H), 7.98 (d, *J* = 8.8 Hz, 1H), 7.76 (d, *J* = 1.6 Hz, 1H), 7.56-7.48 (m, 3H), 7.31-7.25 (m, 3H), 7.18 (*d*, *J* = 7.2 Hz, 1H), 7.12 (dd, *J* = 2.4, 8.8 Hz, 1H), 5.18 (s, 2H), 2.32 (s, 3H). HRMS (*m/z*) calc. for $C_{25}H_{17}Cl_2N_3O_3$ $[M - H]^-$, 476.0569; observed, 476.0572.

3-(2,4-dichlorophenyl)-2-(7-(4-methylbenzyloxy)-4-oxo-3,4-dihydroquinazolin-2(1*H*)-ylidene)-3-oxopropanenitrile (37). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.36 (br s, 1H), 7.98 (d, *J* = 8.8 Hz, 1H), 7.76 (d, *J* = 2.0 Hz, 1H), 7.57-7.47 (m, 3H), 7.38 (d, *J* = 8.0 Hz, 2H), 7.24 (d, *J* = 8.0 Hz, 2H), 7.12 (dd, *J* = 2.4, 8.8 Hz, 1H), 5.19 (s, 2H), 2.32 (s, 3H). HRMS (*m/z*) calc. for $C_{25}H_{17}Cl_2N_3O_3$ $[M - H]^-$, 476.0569; observed, 476.0571.

3-(2,4-dichlorophenyl)-2-(7-(2-methoxybenzyloxy)-4-oxo-3,4-dihydroquinazolin-2(1*H*)-ylidene)-3-oxopropanenitrile (38). ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.37 (br s, 1H), 7.96 (s, 1H), 7.77 (s, 1H), 7.55-7.38 (m, 5H), 7.13-7.00 (m, 3H), 5.19 (s, 2H), 3.84 (s, 3H). HRMS (*m/z*) calc. for $C_{25}H_{16}Cl_2N_3O_4$ $[M - H]^-$, 492.0518; observed, 492.0524.

3-(2,4-dichlorophenyl)-2-(7-(3-methoxybenzyloxy)-4-oxo-3,4-dihydroquinazolin-2(1*H*)-ylidene)-3-oxopropanenitrile (39). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.54 (br s, 1H), 7.98 (d, *J* = 8.8 Hz, 1H), 7.75 (s, 1H), 7.53-7.04 (m, 7H), 6.93 (m, 1H), 5.20 (s, 2H), 3.76 (s, 3H). HRMS (*m/z*) calc. for $C_{25}H_{16}Cl_2N_3O_4$ $[M - H]^-$, 492.0518; observed, 492.0520.

3-(2,4-dichlorophenyl)-2-(7-(4-methoxybenzyloxy)-4-oxo-3,4-dihydroquinazolin-2(1*H*)-ylidene)-3-oxopropanenitrile (40). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.36 (br s, 1H), 7.97 (d, J = 8.8 Hz, 1H), 7.75 (s, 1H), 7.56-7.49 (m, 2H), 7.43 (d, J = 8.4 Hz, 3H), 7.08 (d, J = 8.8 Hz, 1H), 6.98 (d, J = 8.8 Hz, 1H), 5.15 (s, 2H), 3.76 (s, 3H). HRMS (*m/z*) calc. for $C_{25}H_{16}Cl_2N_3O_4$ $[M - H]^-$, 492.0518; observed, 492.0523.

2-(((2-(1-cyano-2-(2,4-dichlorophenyl)-2-oxoethylidene)-4-oxo-1,2,3,4-tetrahydroquinazolin-7-yl)oxy)methyl)benzonitrile (41). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.39 (br s, 1H), 8.02 (d, J = 8.8 Hz, 1H), 7.96 (d, J = 7.6 Hz, 1H), 7.78-7.75 (m, 3H), 7.63-7.50 (m, 4H), 7.17 (dd, J = 2.0, 8.8 Hz, 1H), 5.40 (s, 2H). HRMS (*m/z*) calc. for $C_{25}H_{14}Cl_2N_4O_3$ $[M - H]^-$, 487.0365; observed, 487.0369.

3-(((2-(1-cyano-2-(2,4-dichlorophenyl)-2-oxoethylidene)-4-oxo-1,2,3,4-tetrahydroquinazolin-7-yl)oxy)methyl)benzonitrile (42). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.37 (br s, 1H), 8.00-7.97 (m, 2H), 7.87-7.83 (m, 2H), 7.77 (d, J = 1.6 Hz, 1H), 7.67 (t, J = 7.6, 15.2 Hz, 1H), 7.57-7.48 (m, 3H), 7.16 (dd, J = 2.0, 8.8 Hz, 1H), 5.31 (s, 2H). HRMS (*m/z*) calc. for $C_{25}H_{14}Cl_2N_4O_3$ $[M - H]^-$, 487.0365; observed, 487.0370.

4-(((2-(1-cyano-2-(2,4-dichlorophenyl)-2-oxoethylidene)-4-oxo-1,2,3,4-tetrahydroquinazolin-7-yl)oxy)methyl)benzonitrile (43). ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.36 (br s, 1H), 8.00-7.90 (m, 3H), 7.76-7.66 (m, 3H), 7.54-7.47 (m, 3H), 7.15 (d, J = 8.4 Hz, 1H), 5.35 (s, 2H). HRMS (*m/z*) calc. for $C_{25}H_{14}Cl_2N_4O_3$ $[M - H]^-$, 487.0365; observed, 487.0370.

3-(2,4-dichlorophenyl)-3-oxo-2-(4-oxo-7-(pyridin-2-ylmethoxy)-3,4-dihydroquinazolin-2(1*H*)-ylidene)propanenitrile (44). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.41 (br s, 1H), 8.63 (dd, *J* = 0.8, 4.8 Hz, 1H), 8.00 (d, *J* = 8.8 Hz, 1H), 7.90 (m, 1H), 7.76 (d, *J* = 2.0 Hz, 1H), 7.56-7.38 (m, 5H), 7.16 (dd, *J* = 2.4, 8.8 Hz, 1H), 5.32 (s, 2H). HRMS (*m/z*) calc. for $C_{23}H_{14}Cl_2N_4O_3$ $[M - H]^-$, 463.0365; observed, 463.0371.

3-(2,4-dichlorophenyl)-3-oxo-2-(4-oxo-7-(pyridin-3-ylmethoxy)-3,4-dihydroquinazolin-2(1*H*)-ylidene)propanenitrile (45). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.57 (br s, 1H), 8.76 (s, 1H), 8.63 (d, *J* = 4.4 Hz, 1H), 8.01 (m, 2H), 7.75 (s, 1H), 7.55-7.45 (m, 4H), 7.12 (d, *J* = 8.4 Hz, 1H), 5.31 (*s*, 2H). HRMS (*m/z*) calc. for $C_{23}H_{14}Cl_2N_4O_3$ $[M - H]^-$, 463.0365; observed, 463.0372.

3-(2,4-dichlorophenyl)-3-oxo-2-(4-oxo-7-(pyridin-4-ylmethoxy)-3,4-dihydroquinazolin-2(1*H*)-ylidene)propanenitrile (46). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.45 (br s, 1H), 8.66 (d, *J* = 4.4 Hz, 2H), 7.98 (d, *J* = 8.8 Hz, 1H), 7.71 (s, 1H), 7.56-7.46 (m, 4H), 7.24 (s, 1H), 7.08 (d, *J* = 8.4 Hz, 1H), 5.37 (s, 2H). HRMS (*m/z*) calc. for $C_{23}H_{14}Cl_2N_4O_3$ $[M - H]^-$, 463.0365; observed, 463.0372.

2-(7-(Benzo[*d*][1,3]dioxol-5-ylmethoxy)-4-oxo-3,4-dihydroquinazolin-2(1*H*)-ylidene)-3-(2,4-dichlorophenyl)-3-oxopropanenitrile (47). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.33 (br s, 1H), 7.97 (s, 1H), 7.76 (s, 1H), 7.54-7.47 (m, 3H), 7.06-6.97 (m, 4H), 6.03 (s, 2H), 5.11 (s, 2H). HRMS (*m/z*) calc. for $C_{25}H_{15}Cl_2N_3O_5$ $[M - H]^-$, 506.0311; observed, 506.0318.

3-(2,4-dichlorophenyl)-2-(7-((4-(methylsulfonyl)benzyl)oxy)-4-oxo-3,4-dihydroquinazolin-2(1*H*)-ylidene)-3-oxopropanenitrile (48). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.35 (br s, 1H), 8.01-7.97 (m, 3H), 7.76-7.74 (m, 3H), 7.57-7.50 (m, 3H), 7.16 (dd, *J* = 2.4, 8.8 Hz, 1H), 5.39 (s, 2H), 3.23 (s, 3H). HRMS (*m/z*) calc. for $C_{25}H_{17}Cl_2N_3O_5S$ [M – H]$^-$, 540.0188; observed, 540.0193.

MATERIALS AND METHODS — BIOCHEMICAL AND CELLULAR ASSAYS

Generation of stable DYNC1H1- and DYNC2H1-expressing cells

Flp-In T-Rex 293 cells (Invitrogen) were seeded at 2.5 million cells/15-cm plate in DMEM containing 10% (v/v) fetal bovine serum (FBS), 100 U/mL penicillin, and 0.1 mg/mL streptomycin and cultured for 22 h. A 500-μL solution of 0.25 M $CaCl_2$, 9.0 μg pOG44 (Invitrogen), and 1.0 μg of either SBP-SNAP-DYNC1H1 or SBP-SNAP-DYNC2H1 plasmids (which encode the full-length human cytoplasmic dynein 1 and 2 heavy chains with N-terminal streptavidin-binding peptide (SBP) and SNAP tags),[3] was then slowly added to 500 μL HEPES buffered saline (280 mM NaCl, 1.5 mM Na_2HPO_4, 50 mM HEPES, pH 7.0) and allowed to sit for one minute. The resulting turbid solution was added dropwise to one plate of Flp-In T-Rex 293 cells, and the cells were cultured for 48 h with a media change at 24 h. The cells were then split into selection medium (DMEM containing 10% (v/v) FBS, 100 U/mL penicillin, 0.1 mg/mL streptomycin, 10 μg/mL blasticidin, and 100 μg/mL hygromycin B) and cultured to give colonies of stably-transfected cells.

Expression and purification of SBP-SNAP-DYNC1H1 and SBP-SNAP-DYNC2H1

Two hundred 15-cm plates of either SBP-SNAP-DYNC1H1- or SBP-SNAP-DYNC2H1-expressing Flp-In T-Rex 293 cells were cultured to 70% confluency in DMEM containing 10% (v/v) FBS, 100 U/mL penicillin, 0.1 mg/mL streptomycin, 10 μg/mL blasticidin, and 100 μg/mL hygromycin B. Doxycycline (2 μg/mL) was added, and the cells were cultured for an additional 48 h. The cells were then harvested via trypsinization and centrifugation at 200 *g*, resuspended in 200 mL of ice-cold lysis buffer (25 mM HEPES, 50 mM PIPES, 2 mM $MgSO_4$, 0.2 mM EGTA, 0.1% (v/v) Triton X-100, 1 mM DTT, 2 mM PMSF, Complete MiniProtease Inhibitor (Roche), pH 7.5), and sonicated on ice until the cells were completely homogenized. Lysates were cleared by centrifugation at 30,000 *g* for 30 min at 4 °C, and incubated with 5 mL of Streptavidin High Performance Beads (GE Healthcare) for 4 h at 4 °C. The matrix was washed with 100 mL of lysis buffer and added to a 1 cm x 15 cm glass Econo-Column (Bio-Rad), and the SBP-SNAP-DYNC1H1 or SBP-SNAP-DYNC2H1 protein was eluted in 25 x 500-µL aliquots with lysis buffer containing 2 mM biotin. The aliquots were analyzed on a 3-8% Tris-Glycine SDS-PAGE gel (Bio-Rad), and protein-containing fractions were pooled and dialyzed in 1 L of assay buffer (50 mM Tris-HCl, 150 mM KOAc, 2 mM $Mg(OAc)_2$, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, pH 8) overnight. Protein concentrations were quantified using Bradford reagent, and the purified heavy chains were snap-frozen in assay buffer containing 1% (v/v) glycerol using liquid nitrogen and stored at -80 °C.

Expression and purification of 6xHis-DYNC1H1 and GFP-DYNC2H1 motor domains

The motor domains of DYNC1H1 (amino acids 1320-4647) and DYNC2H1 (amino acids 1091-4307) were expressed in insect cells and purified as described below. In the case of the DYNC1H1 motor, an N-terminal 6x-His tag was used for affinity-based purification. Construct preparation was performed as follows: A human *DYNC1H1* cDNA clone (pF1KA0325) was obtained from Kazusa DNA Research Institute, and the coding region that encompasses the motor domain (residues Q1320 - V4647) was amplified and fused with a hexahistidine (His_6)-tag by PCR using Phusion High-Fidelity DNA Polymerase (NEB). The assembled construct was cloned into a pFastBac (Invitrogen) vector using an InFusion HD cloning Kit (Clontech Laboratories).

Purification of the DYNC1H1 motor domain proceeded as follows: An Sf9 cell pellet was resuspended in a buffer containing, 30mM HEPES pH 7.6, 200 mM NaCl, 10 mM imidazole, 1 mM TCEP, 2 mM PMSF, HALT (Thermo Fisher) and complete protease inhibitor cocktail (Roche) and lysed by the addition of Triton X-100 to a final concentration of 0.2% (v/v). The lysate was clarified by centrifugation at 140,000 *g* and then incubated with Ni-NTA beads for 2 h at 4 °C. The beads were washed with 75 bed volumes of lysis buffer, and bound proteins were eluted with buffer containing 30 mM HEPES pH 7.5, 100 mM NaCl, 500 mM imidazole, and 1 mM TCEP. Eluate fractions containing the DYNC1H1 motor domain were diluted into gel-filtration buffer (50 mM Tris HCl pH 7.8, 150 mM KOAc, 2 mM $Mg(OAc)_2$, 1 mM EGTA, 1 mM EDTA, 0.1 mM ATP, and 1 mM DTT) and loaded onto a Mono Q anion exchange column (GE Healthcare). The column was eluted using a salt gradient from 150 to 750 mM KOAc over 20 column volumes. Peak fractions were concentrated using a centrifugal concentrator (Amicon) and subjected to size-exclusion chromatography on a Superose 6 column using gel filtration buffer. Gel filtration revealed a monodisperse peak at an elution volume of 12.2 mL, consistent with the expected molecular weight. Peak fractions were pooled, supplemented with glycerol to a final concentration of 20% (v/v), and snap frozen. The protein yield was ~ 1 mg of DYNC1H1 motor domain per 1 L of Sf9 culture.

The DYNC2H1 motor domain was prepared as described previously.[4] Briefly, the protein was expressed with N-terminal protein A tag and GFP tags separated by a tobacco etch virus (TEV) protease site and purified using IgG-Sepharose. The protein A tag was then proteolytically removed, and gel filtration of the product revealed a monodisperse peak at an elution volume of 12.6 mL, consistent with the expected molecular weight.

DYNC1H1 and DYNC2H1 ATPase assays

The ATPase activities of purified SBP-SNAP-DYNC1H1 and SBP-SNAP-DYNC2H1 proteins were measured through the kinetic hydrolysis of γ-^{32}P ATP. Individual 25-µL reactions were prepared in 96-well plates, each containing assay buffer (50 mM Tris-HCl, 150 mM KOAc, 2 mM $Mg(OAc)_2$, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, pH 8) supplemented with 0.056 µg/mL of SBP-SNAP-DYNC1H1 or SBP-SNAP-DYNC2H1, 0.1% (w/v) BSA, 0.1% (v/v) Triton X-100, and either a ciliobrevin analog or an equivalent amount of DMSO vehicle (4%, v/v). The reactions were initiated by the addition of 1.6 µCi γ-^{32}P ATP (Perkin Elmer; final concentration of 18-26 nM) and incubated at 37 °C for 12 min on a thermocycler. A 2.5-µL aliquot of each reaction was subsequently mixed with 300 µL of charcoal suspension (0.6 M acetic acid, 2.5 mM KH_2PO_4, and 4% (w/v) activated charcoal) and centrifuged to obtain a supernatant containing the hydrolyzed ^{32}P orthophosphate. A 100-µL fraction of this supernatant was added to 1 mL of Ultima Gold scintillation cocktail (Perkin Elmer) and ^{32}P orthophosphate emissions were measured on a Beckman Coulter LS6500 liquid scintillation counter.

The ATPase activities of purified 6xHis-DYNC1H1 and GFP-DYNC2H1 motor domains were assayed in buffer containing 25 mM PIPES pH 7.0, 30 mM KCl, 1 mM EGTA, 5 mM MgCl2, 0.01% (v/v) Triton X-100, 1 mM DTT, and 0.1 mg/mL BSA. For all conditions, the enzymes were incubated with varying concentrations of inhibitor in 2% DMSO for 10 minutes in a volume of 10 µL. Each ATPase reaction was initiated by addition of 2 µL of a 6x ATP stock containing trace γ-^{32}P ATP (6000 Ci/mmol, 10 mCi/mL, Perkin Elmer) and allowed to proceed at room temperature for a time predetermined to lie within the linear range of the assay. For the "low ATP" conditions, final concentrations of 1 nM dynein motor and 25 nM ATP were used, and the reaction was allowed to proceed for 10 minutes. For the "high ATP" conditions, final concentrations of 30 nM dynein and 100 μM ATP were used, and the reaction was allowed to proceed for 30 minutes. Reactions were quenched by the addition of 100 mM EDTA, and 2 μL of each reaction was spotted onto PEI-cellulose thin layer chromatography plates (Millipore). The plates were developed in a glass chamber with a freshly prepared solution of 150 mM formic acid and 150 mM LiCl, dried, exposed to a storage phosphor tray, and scanned on a Typhoon imaging system (GE Healthcare Life Sciences). The fraction of γ-phosphate hydrolyzed in each condition was quantified using ImageJ and normalized to a DMSO control.

Hh signaling assays

Shh-LIGHT2 cells,[5] an NIH-3T3 cell line stably integrated with Gli-dependent firefly luciferase and thymidine kinase promoter-driven *Renilla* luciferase reporters, were cultured in DMEM containing 10% calf serum (CS), 100 U/mL penicillin, 0.1 mg/mL streptomycin, 1 mM sodium pyruvate, 400 μg/mL G418, and 150 μg/mL zeocin. The cells were seeded at 35,000 cells/well in a 96-well plate and treated with individual ciliobrevin analogs or an equivalent amount of DMSO vehicle (1%, v/v) in DMEM containing 0.5% CS and 10% ShhN-conditioned medium.[6] After 30 h, the cells were lysed, and their firefly and *Renilla* luciferase activities were measured using a Dual Luciferase Reporter kit (Promega) and a Veritas microplate luminometer. Dose-response data were curve-fitted with a variable slope, sigmoidal dose-response algorithm using Prism software (GraphPad) to obtain IC_{50} values.

Ciliogenesis assays

Shh-EGFP cells,[7] an NIH-3T3 cell line stably integrated with a Gli-dependent green fluorescent protein reporter, were cultured in DMEM containing 10% CS, 100 U/mL penicillin, 0.1 mg/mL streptomycin, 1 mM sodium pyruvate, and 150 μg/mL zeocin. The cells were seeded onto poly-D-lysine-coated, 12-mm glass coverslips in 24-well plates at a density of 35,000 cells/well and cultured for 40 h. The media was then replaced with DMEM containing 0.5% CS, 100 U/mL penicillin, 0.1 mg/mL streptomycin, 1 mM sodium pyruvate, and either an individual ciliobrevin analog (30 μM) or an equivalent amount of DMSO vehicle (0.3%, v/v). Following a 24-h incubation, the cells were fixed in PBS containing 4% (w/v) paraformaldehyde for 10 min at room temperature, washed 3 x 5 min with PBS, permeabilized with PBS containing 0.3% (v/v) Triton X-100 for 5 min, washed 2 x 5 min with PBS, and then blocked overnight at 4 °C in PBS containing 1% (w/v) BSA and 0.1% (v/v) Triton X-100. The coverslips were next treated overnight at 4 °C with blocking buffer containing mouse monoclonal anti-ARL13B antibody (1:1000; UC-Davis/NIH NeuroMab Facility, 75-287), washed 3 x 5 min with PBS containing 0.1% (v/v) Triton X-100, treated for 1 h at room temperature with blocking buffer containing AlexaFluor 488-conjugated goat polyclonal anti-mouse IgG antibody (1:400 dilution; Invitrogen, A-11029), washed 3 x 5 min with PBS containing 0.1% (v/v) Triton X-100, and mounted onto glass slides with Prolong Gold Antifade reagent containing DAPI (Invitrogen). The immunostained cells were then imaged on a Leica DMI6000B compound microscope equipped with an HC Pan Apochromat CS 20x/0.70 NA oil-immersion objective, a Photometric CoolSNAP HQ CCD camera, and Metamorph software (Molecular Devices).

To quantify primary cilia lengths, the minimum threshold intensity for cilia staining was first established by manual inspection, and ImageJ software was used to quantify the total pixel area of ARL13B-positive pixels equal to or greater than the minimum threshold intensity. The average cilia length was then determined by dividing the number of ARL13B-positive pixels by the number of DAPI-positive nuclei in each field of view, and at least 6 fields of view were analyzed per experimental condition. Approximately 800 cells/condition were analyzed in this manner, although fewer cells were imaged in some cases due to decreased cell viability. Analogs **8**, **12**, **19**, **23**, and **40** could not be analyzed in this assay due to the formation of precipitates that interfered with fluorescence imaging.

<u>IFT88 trafficking assays</u>

Shh-EGFP cells were seeded onto poly-D-lysine-coated, 12-mm glass coverslips in 24-well plates at a density of 125,000 cells/well and cultured for 24 h in the DMEM/10% CS medium described above. The cells were next cultured in DMEM containing 0.5% CS, 100 U/mL penicillin, and 0.1 mg/mL streptomycin for 16 h to promote primary cilia formation, and then transferred into the low-serum medium containing either individual ciliobrevin analogs (50 μM) or an equivalent amount of DMSO vehicle (0.25%, v/v) for 1 h. After compound treatment, the Shh-EGFP cells were fixed in PBS containing 4% (w/v) paraformaldehyde for 8 min at room temperature, washed 1 x with PBS before addition of ice-cold (-20 °C) methanol for 5 min at -20 °C. For wash-out experiments, cells were incubated with low-serum medium after compound treatment for 10 min, and transferred into fresh low-serum medium for an additional 4 h at 37 °C before fixing the cells as described above. Subsequently, cells were washed 1 x with PBS and 2 x with PBS containing 0.1% (v/v) Triton X-100, and blocked for 1 h at room temperature in PBS containing 1% (w/v) BSA and 0.1% (v/v) Triton X-100. The coverslips were subsequently treated with blocking buffer containing mouse monoclonal anti-ARL13B IgG2a antibody (1:3000; UC-Davis/NIH NeuroMab Facility, 75-287) overnight at 4 °C, washed 1 x 5 min in PBS containing 0.1% (v/v) Triton X-100, and then incubated with blocking buffer containing rabbit polyclonal anti-IFT88 antibody (1:70 dilution; ProteinTech

Group, 13967-1-AP) and mouse monoclonal anti-γ-Tubulin IgG1 antibody (1:500 dilution; Sigma, T6557, clone GTU-88, ascites fluid) for 90 min at room temperature. The immunostained cells were washed 4 x 5 min with PBS containing 0.1% (v/v) Triton X-100 and incubated in blocking buffer containing Alexa Fluor 488-conjugated goat polyclonal anti-rabbit IgG antibody (1:300 dilution; Invitrogen, A-11034), Alexa Fluor 594-conjugated goat polyclonal anti-mouse IgG1 antibody (1:500 dilution; Jackson ImmunoResearch, 115-585-205), Alexa Fluor 647-conjugated goat polyclonal anti-mouse IgG2a antibody (1:500 dilution; Jackson ImmunoResearch, 115-605-206) for 1 h at room temperature. Following the secondary antibody incubation, cells were washed 5 x 5 min with PBS containing 0.1% (v/v) Triton X-100. The coverslips were mounted onto glass slides using Prolong Gold Antifade reagent containing DAPI (Invitrogen) to stain nuclei and imaged at 500-nm z intervals on a Zeiss microscope (Axio Imager.M1) using epifluorescent illumination (Lambda XL light source; Sutter Instrument) through a 63x/1.4 NA Plan Apochromat objective. Images were captured with a camera (CoolSNAP HQ^2; Photometrics) using SlideBook software (Intelligent Imaging Innovations).

Quantitative image analyses were conducted using MatLab R2014A (Mathworks). ARL13B staining was used to mask and track the cilia and the γ-tubulin staining was used to orient the cilia from base to tip. The IFT88 signal along each axoneme was analyzed by dividing the total length of the cilium (as measured from the ARL13B staining) in 21 bins, each consisting of a 2-pixel-radius circle. Overlap correction was used to make sure that the summed fluorescence intensities over the 21 bins did not exceed the total ciliary fluorescence signal. The IFT88 signal within each bin was then normalized to the total ciliary signal to determine the fraction of ciliary IFT88 protein localized to each position along the axoneme. IFT88 signals from 70-120 cilia were analyzed from 5 fields of view to obtain traces for each compound, and the experiments were performed in duplicate.

To assess the effects of ciliobrevins on IFT88 movement in real time, murine inner medullary collecting duct (IMCD3) cells stably expressing mNeonGreen-IFT88 were imaged as previously described.[8] The cells were seeded on 25-mm coverslips and serum-starved for 24 h to induce ciliation. Imaging was conducted in phenol red-free media and on a DeltaVision system (Applied Precision) equipped with a PlanApo 603/1.49 NA internal reflection microscopy (TIRF) oil-immersion objective (Olympus). Images were captured with an sCMOS camera (Applied Precision) at 2 Hz, and line scan kymographs were generated by ImageJ. Velocities and frequencies of mNeonGreen-IFT88 foci movements were then quantified from the kymographs.

Mitotic spindle morphology assays

Shh-EGFP cells were seeded onto poly-D-lysine-coated, 12-mm glass coverslips in 24-well plates at a density of 60,000 cells/well and cultured for overnight in the DMEM/10% CS medium described above. The cells were then cultured in the same growth medium containing 15 μM MG132 for 90 min, following by a 30-min incubation with DMEM containing 0.5% CS, 15 μM MG132, and either individual ciliobrevin analogs or an equivalent amount of DMSO vehicle (0.3%, v/v). The Shh-EGFP cells were treated with methanol chilled to -20 °C for 10 min, washed 3 x 5 min with PBS containing 0.1% (v/v) Triton X-100, and blocked for 1 h at room temperature with PBS containing 1% (w/v) BSA and 0.1% (v/v) Triton X-100. The cells were subsequently incubated with blocking buffer containing mouse monoclonal anti-α-tubulin antibody (1:2000 dilution, Sigma-Aldrich, T6199) and rabbit polyclonal anti-γ-tubulin antibody (1:1500 dilution, Sigma-Aldrich, T3559) overnight at 4 °C. The immunostained cells were washed 3 x 5 min with PBS containing 0.1% (v/v) Triton X-100 and incubated with blocking buffer containing Alexa Fluor 488-conjugated goat polyclonal anti-rabbit IgG antibody (1:400 dilution; Invitrogen, A-11034) and Alexa Fluor 594-conjugated goat polyclonal anti-mouse IgG antibody (1:400 dilution; Invitrogen, A-11032) for 1 h at room temperature. The coverslips were washed 3 x 5 min in PBS containing 0.1% (v/v) Triton X-100, mounted onto slides using Prolong Gold Antifade reagent containing DAPI (Invitrogen), and imaged using a Zeiss LSM700 confocal laser-scanning microscope equipped with a Plan Apochromat 63x/1.4 NA oil-immersion objective. At least 200 mitotic spindles were analyzed for each experimental condition.

<u>Golgi dispersion and GLI2 trafficking assays</u>

Shh-EGFP cells were seeded onto poly-D-lysine-coated, 12-mm glass coverslips in 24-well plates at a density of 125,000 cells/well and cultured for 24 h in the DMEM/10% CS medium described above. The cells were next cultured in DMEM containing 0.5% CS, 100 U/mL penicillin, and 0.1 mg/mL streptomycin for 16 h to promote primary cilia formation, and then transferred into the low-serum medium containing either individual ciliobrevin analogs (5, 10, 20, or 30 μM) or an equivalent amount of DMSO vehicle (0.25%, v/v) for 4 h. After compound treatment, the Shh-EGFP cells were fixed in PBS containing 4% (w/v) paraformaldehyde for 10 min at room temperature, washed 2 x 5 min with PBS, permeabilized for 5 min with PBS containing 0.3% (v/v) Triton X-100 and washed 3 x 5 min with PBS. Subsequently, coverslips were blocked for 1 h at room temperature in PBS containing 1% (w/v) BSA and 0.1% (v/v) Triton X-100. The coverslips were subsequently treated with blocking buffer containing mouse monoclonal anti-ARL13B IgG2a antibody (1:3000 dilution; UC-

Davis/NIH NeuroMab Facility, 75-287) and goat polyclonal anti-GLI2 antibody (1:100 dilution; R&D systems, AF3635) overnight at 4 °C, washed 1 x 5 min in PBS containing 0.1% (v/v) Triton X-100, and then incubated with blocking buffer containing rabbit monoclonal anti-GM130 antibody (1:500 dilution; Abcam, ab52647) for 90 min at room temperature. The immunostained cells were washed 4 x 5 min with PBS containing 0.1% (v/v) Triton X-100 and incubated in blocking buffer containing Alexa Fluor 488-conjugated donkey polyclonal anti-goat IgG antibody (1:300 dilution; Invitrogen, A-11055), Alexa Fluor 594-conjugated donkey polyclonal anti-mouse IgG antibody (1:300 dilution; Invitrogen, A21203), Alexa Fluor 647-conjugated donkey polyclonal anti-rabbit IgG antibody (1:300 dilution; Invitrogen, A-31573) for 1 h at room temperature. Following the secondary antibody incubation, cells were washed 5 x 5 min with PBS containing 0.1% (v/v) Triton X-100. The coverslips were mounted onto glass slides using Prolong Gold Antifade reagent containing DAPI (Invitrogen) to stain nuclei and imaged at 500-nm z intervals on a Zeiss microscope (Axio Imager M1) using epifluorescent illumination (Lambda XL light source; Sutter Instrument) and a 63x/1.4 NA Plan Apochromat objective. Images were captured with a CoolSNAP HQ^2 camera (Photometrics) using SlideBook software (Intelligent Imaging Innovations).

Quantitative image analyses were conducted using MatLab R2014A (Mathworks). Golgi dispersal was analyzed by scoring specific morphologies as described in Supplemental Figure 1. ARL13B staining was used to mask and track individual cilia, and GLI2 staining was used to designate the distal tip of each cilium. Each axenome was divided in 21 bins as described above, and the absolute fluorescent GLI2 signal at the ciliary tip (bin 19; maximum GLI2 signal) was averaged over 100-300 cilia analyzed from 4-6 fields of view. The GLI2 signal for each compound was then normalized to that observed in cells treated with the Hh pathway activator SAG. Three independent experiments were analyzed.

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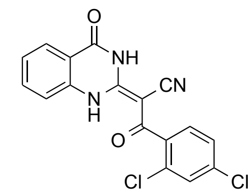

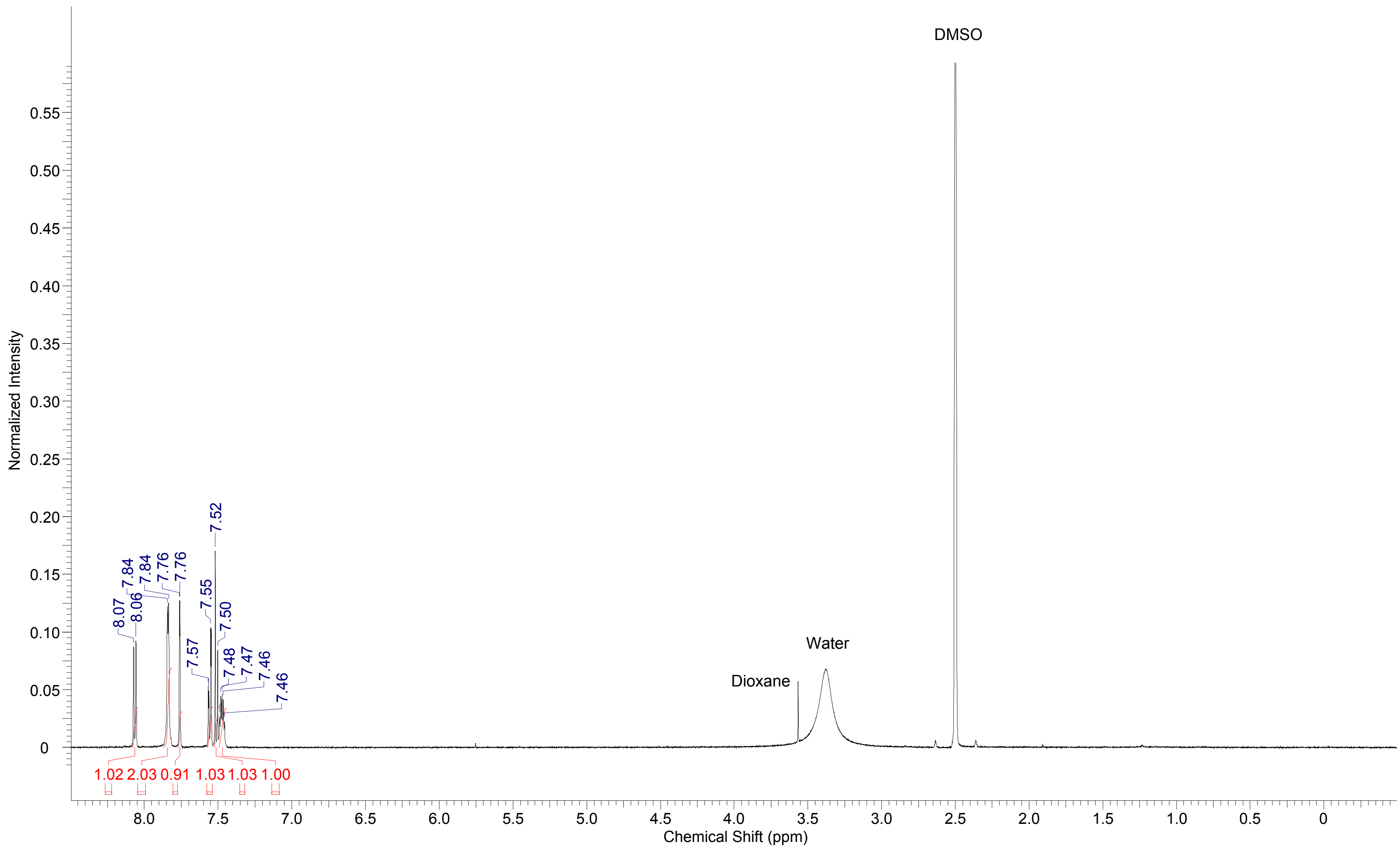

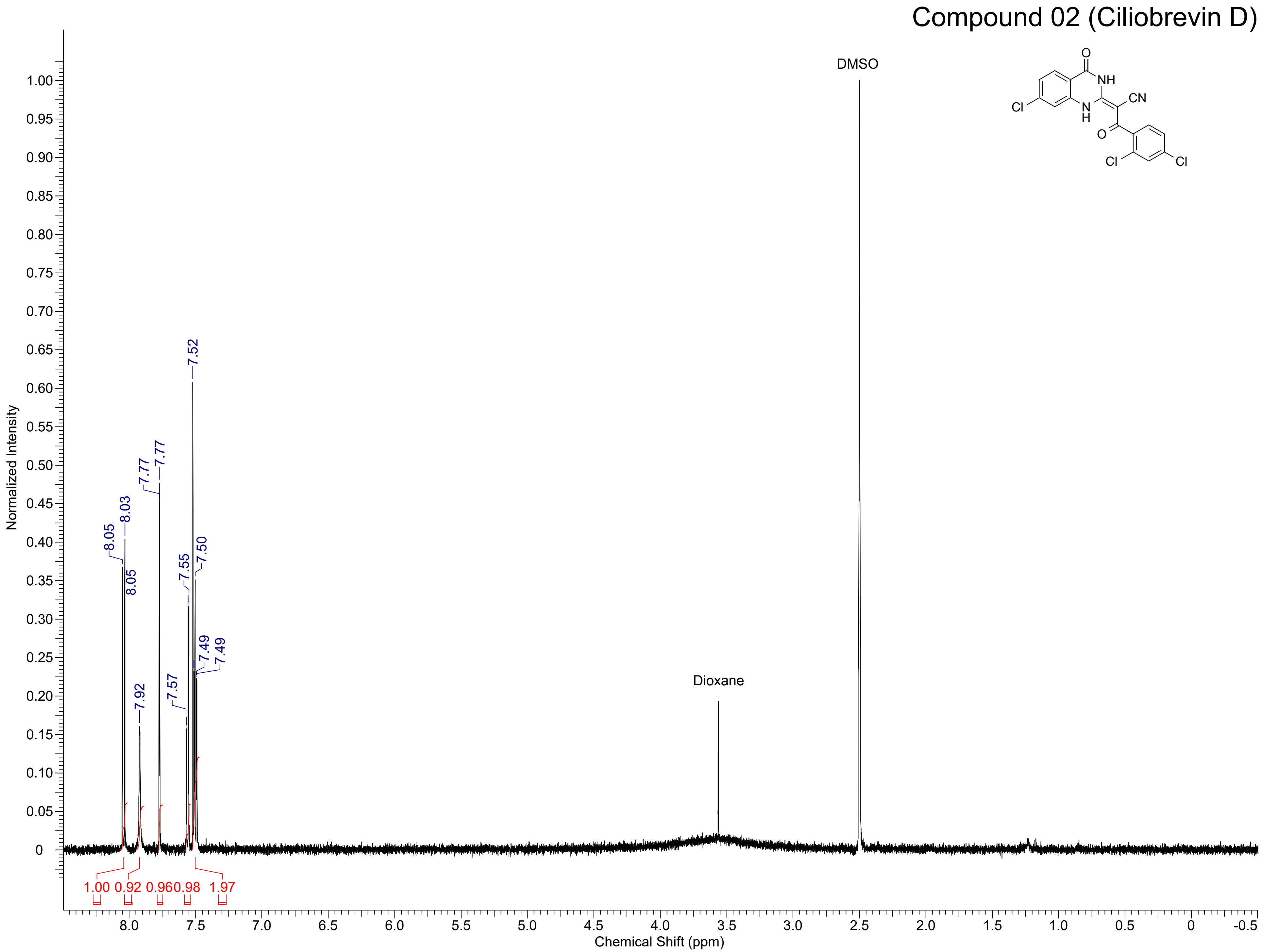
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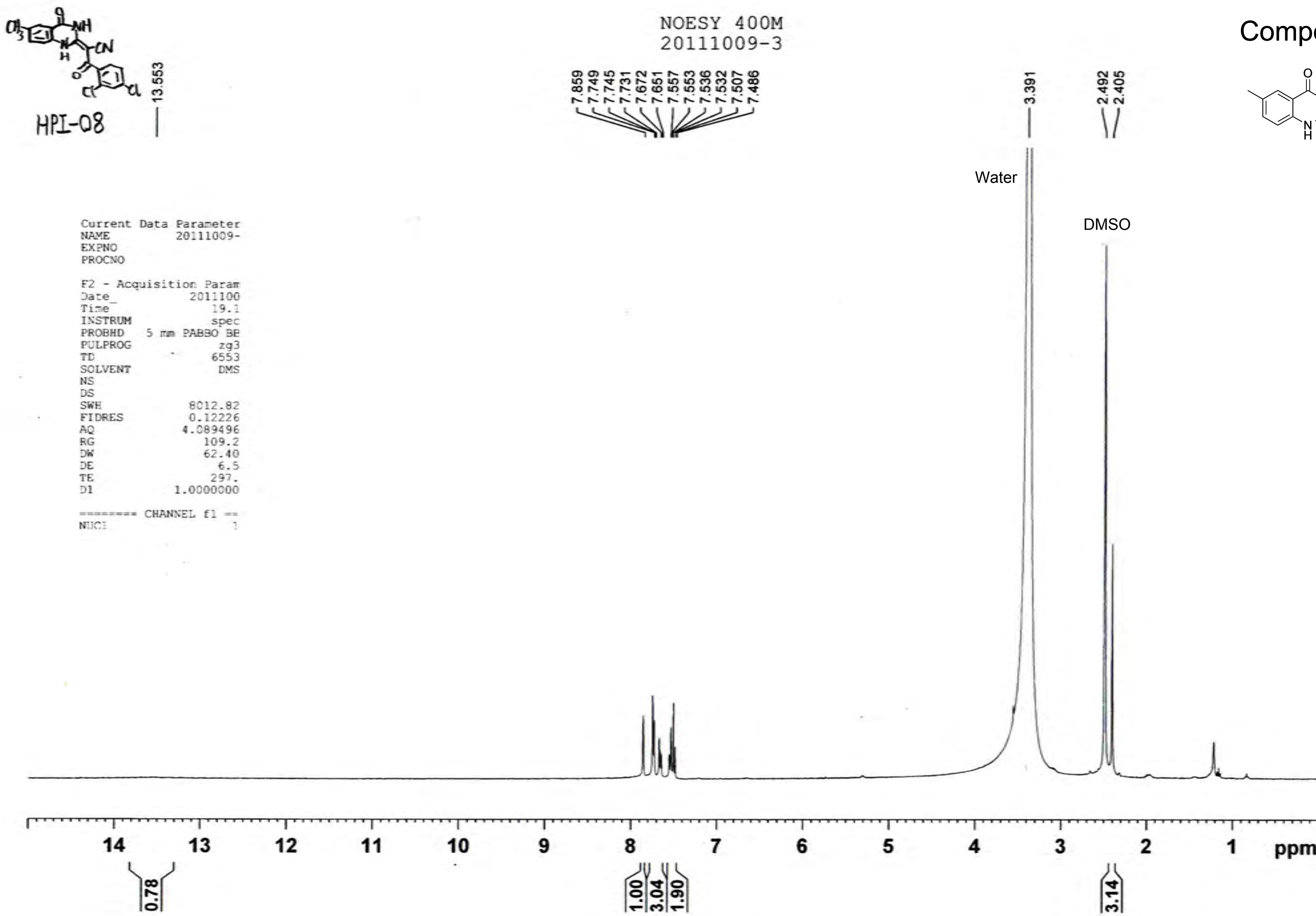
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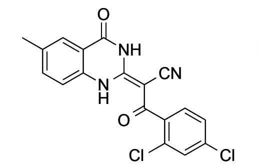
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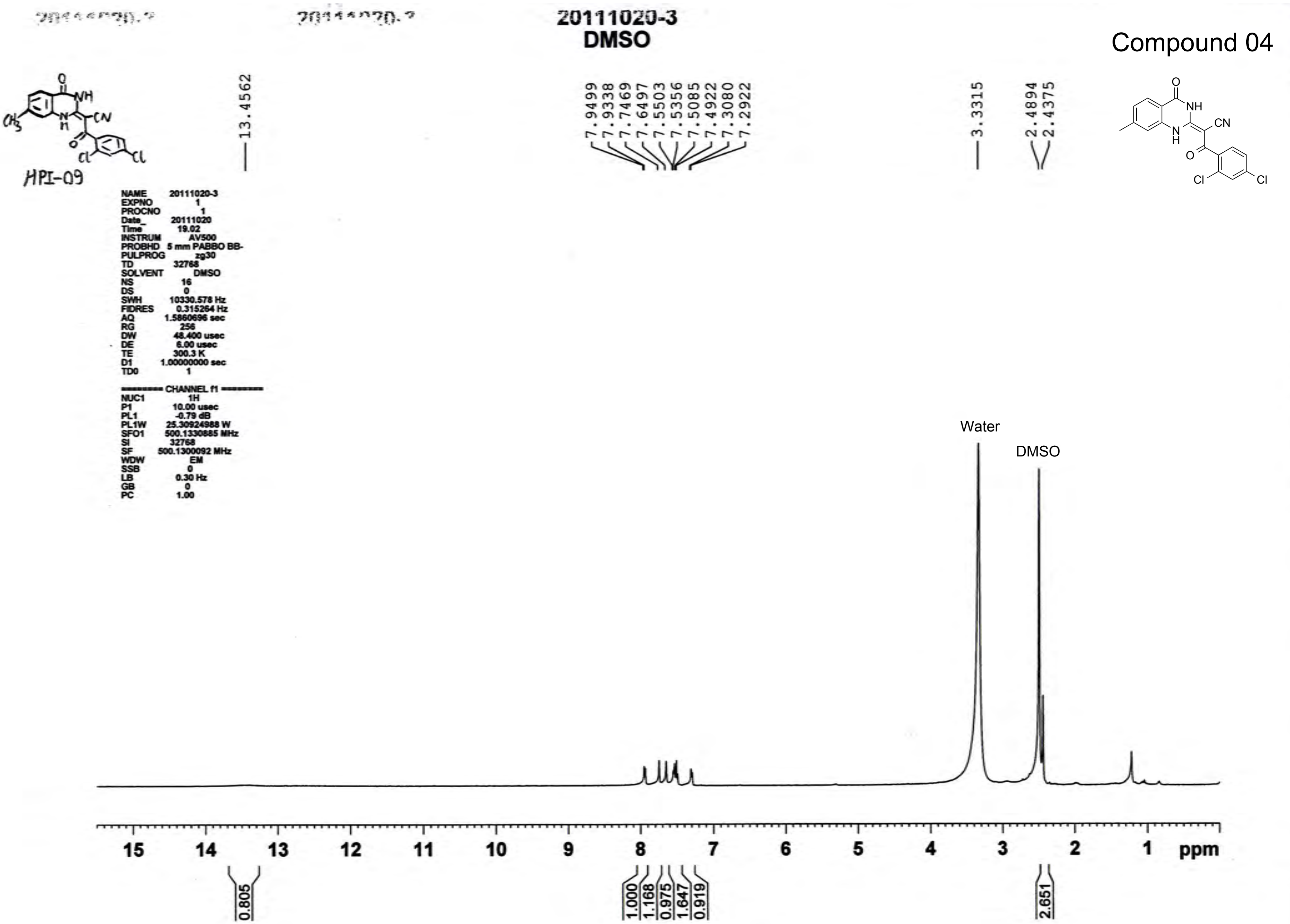
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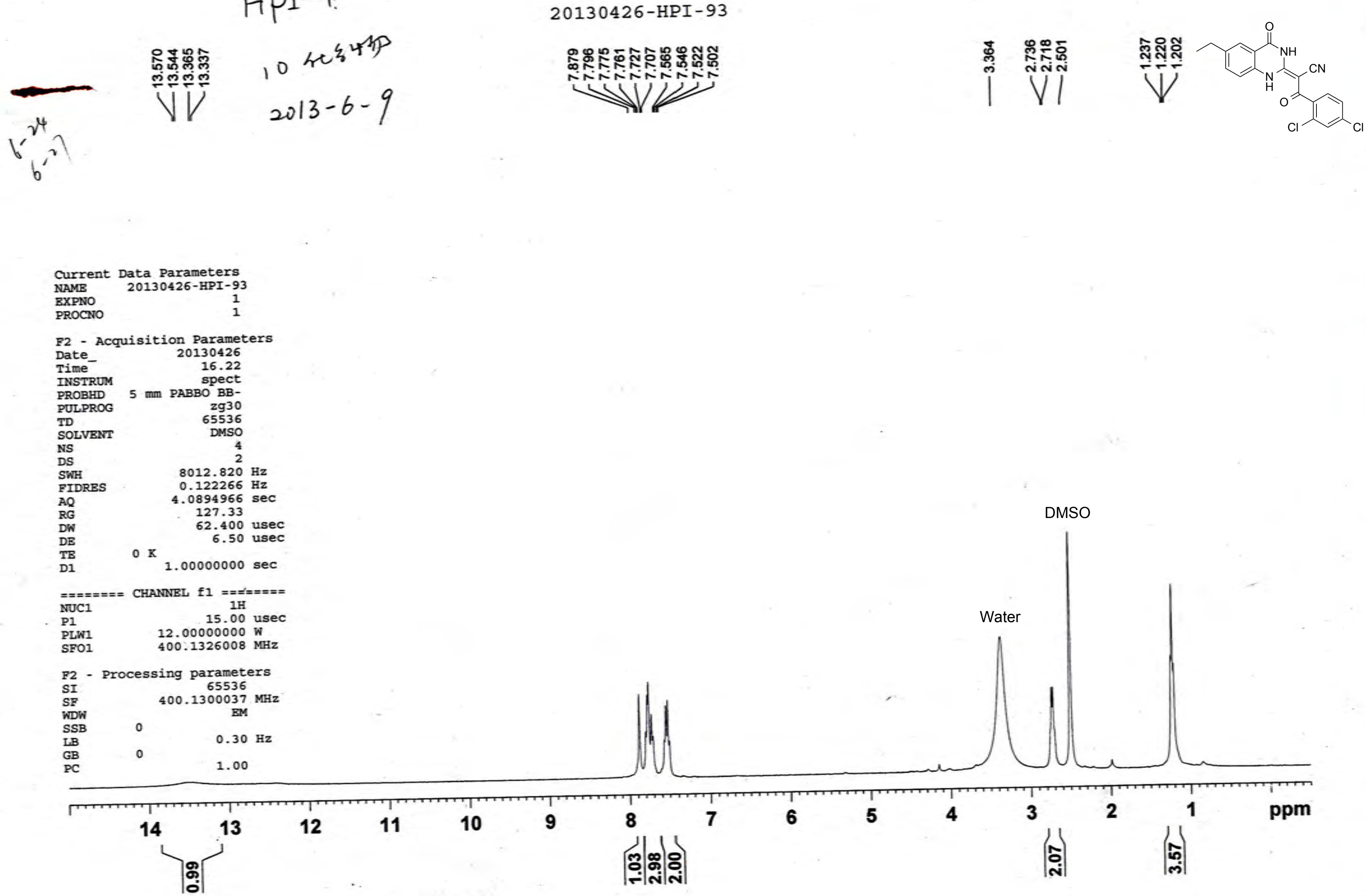

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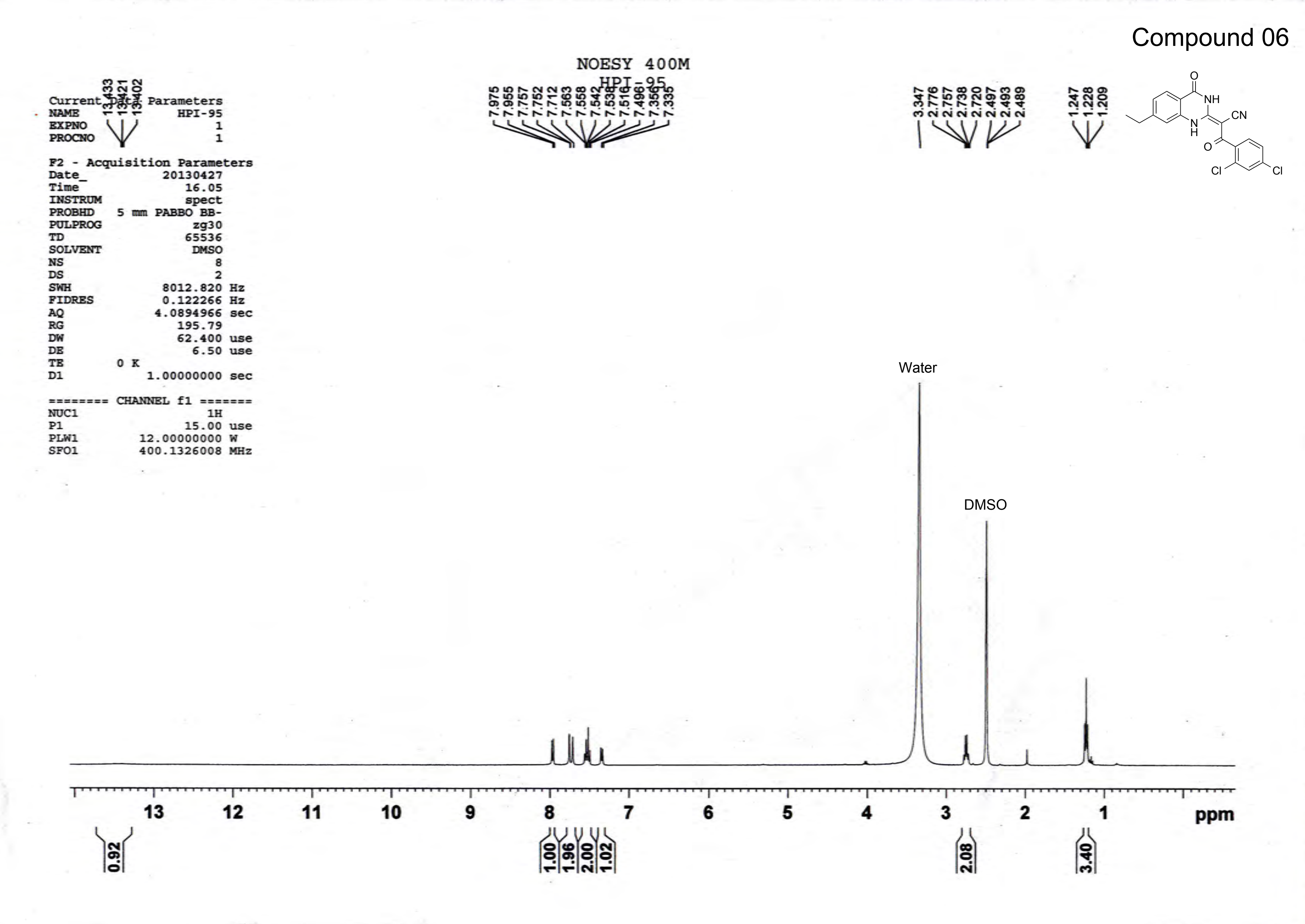
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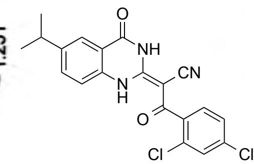
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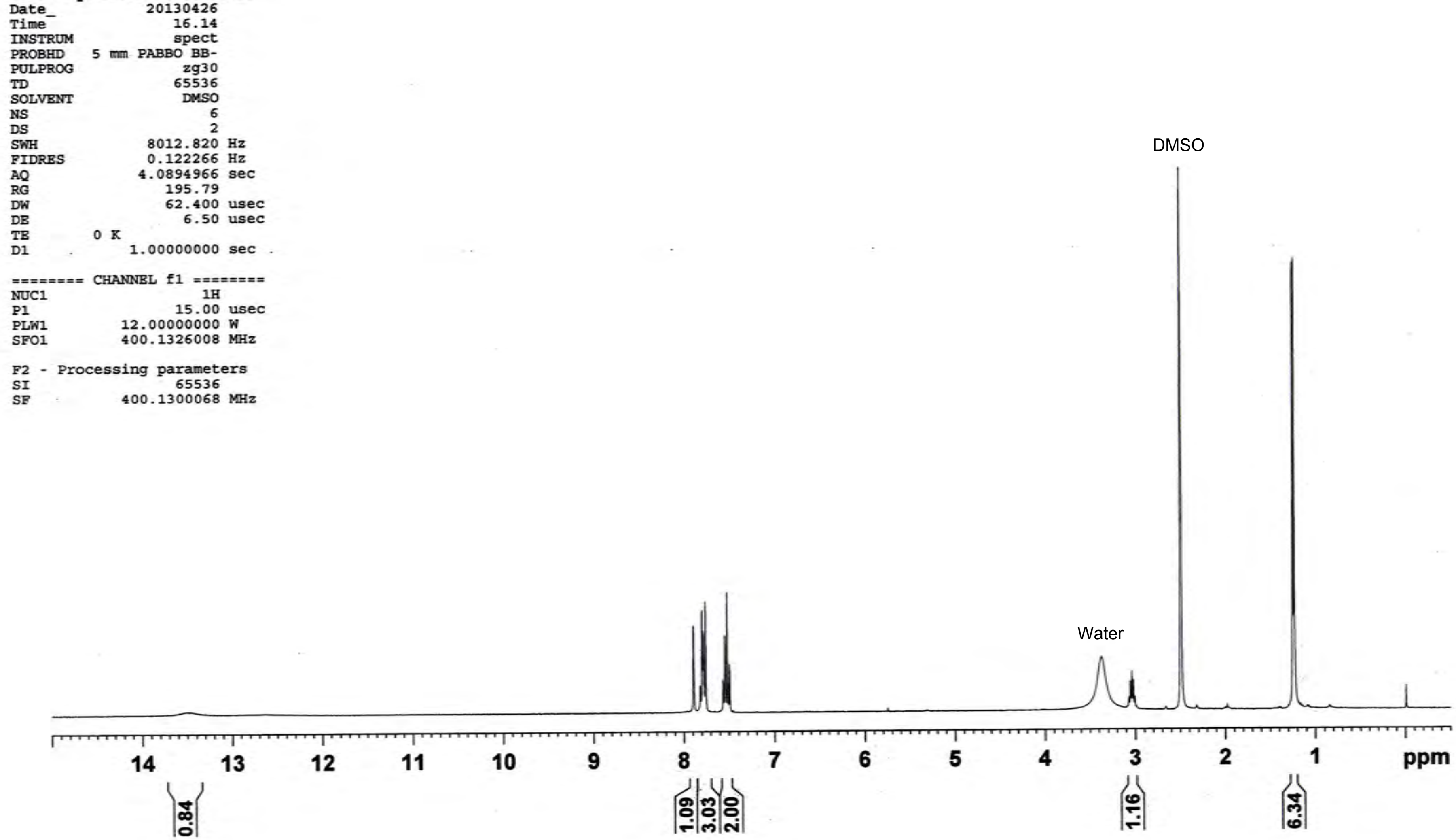
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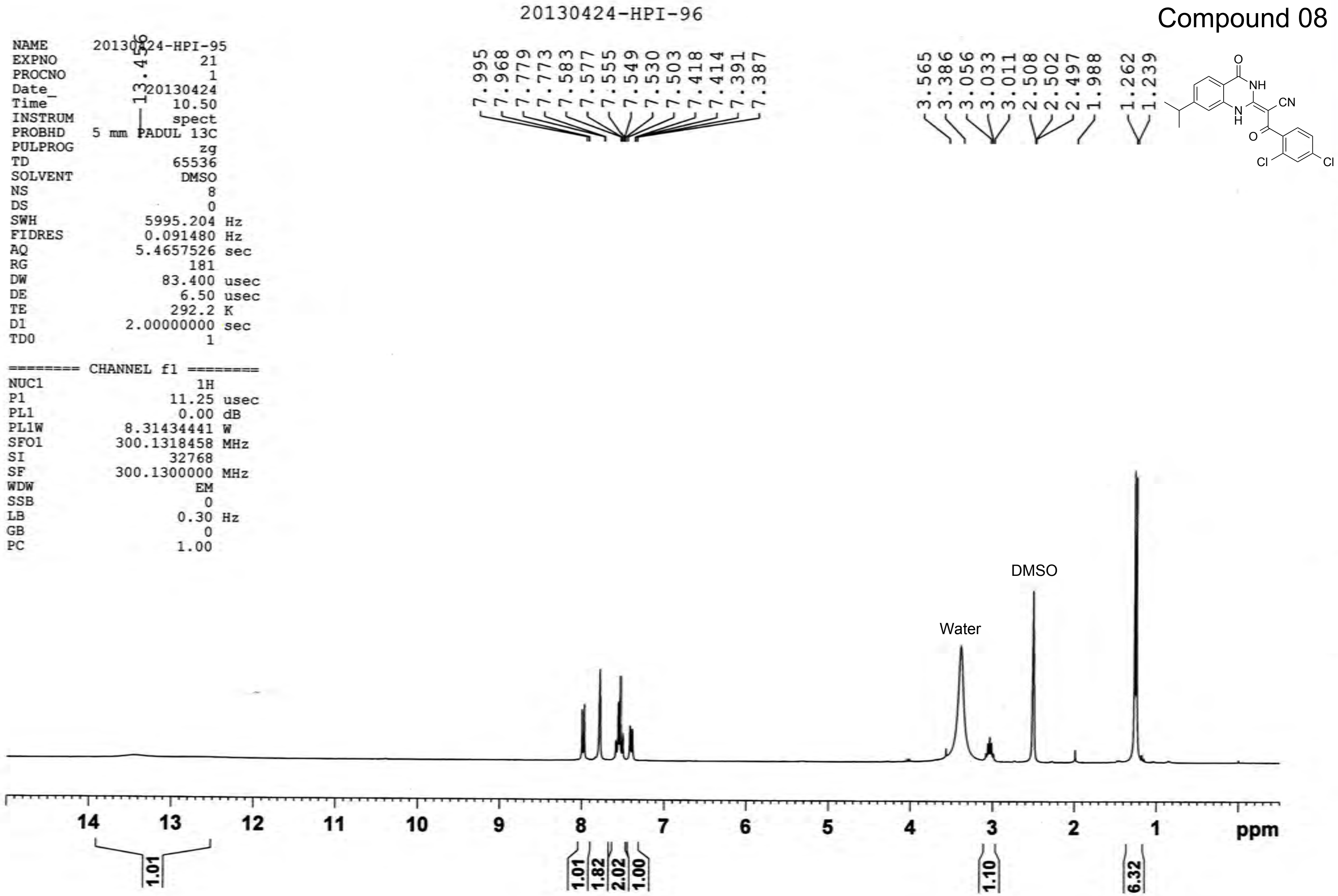
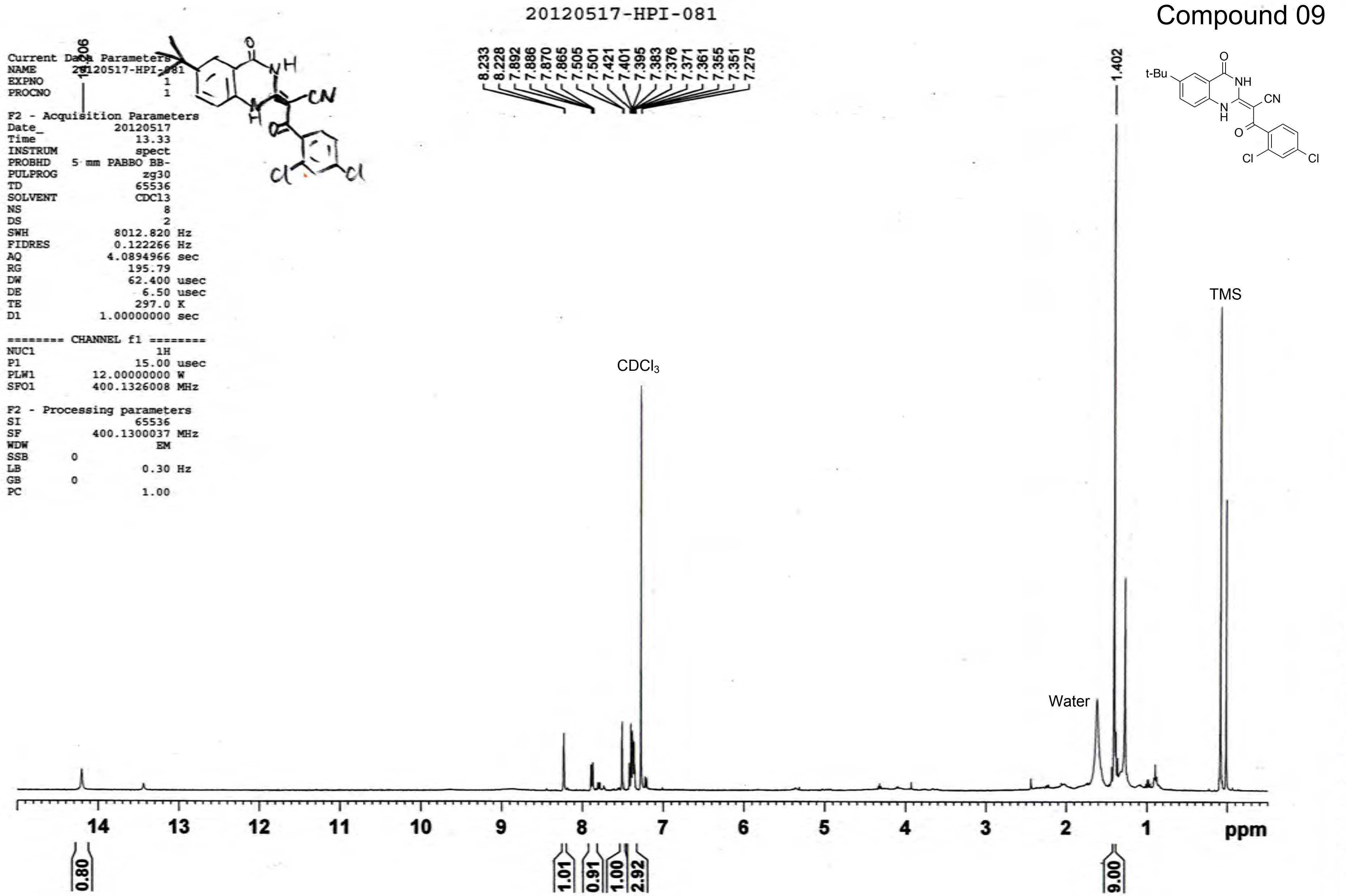
2
1
ppm
0.84
1.09
3.03
2.00
1.16
6.34

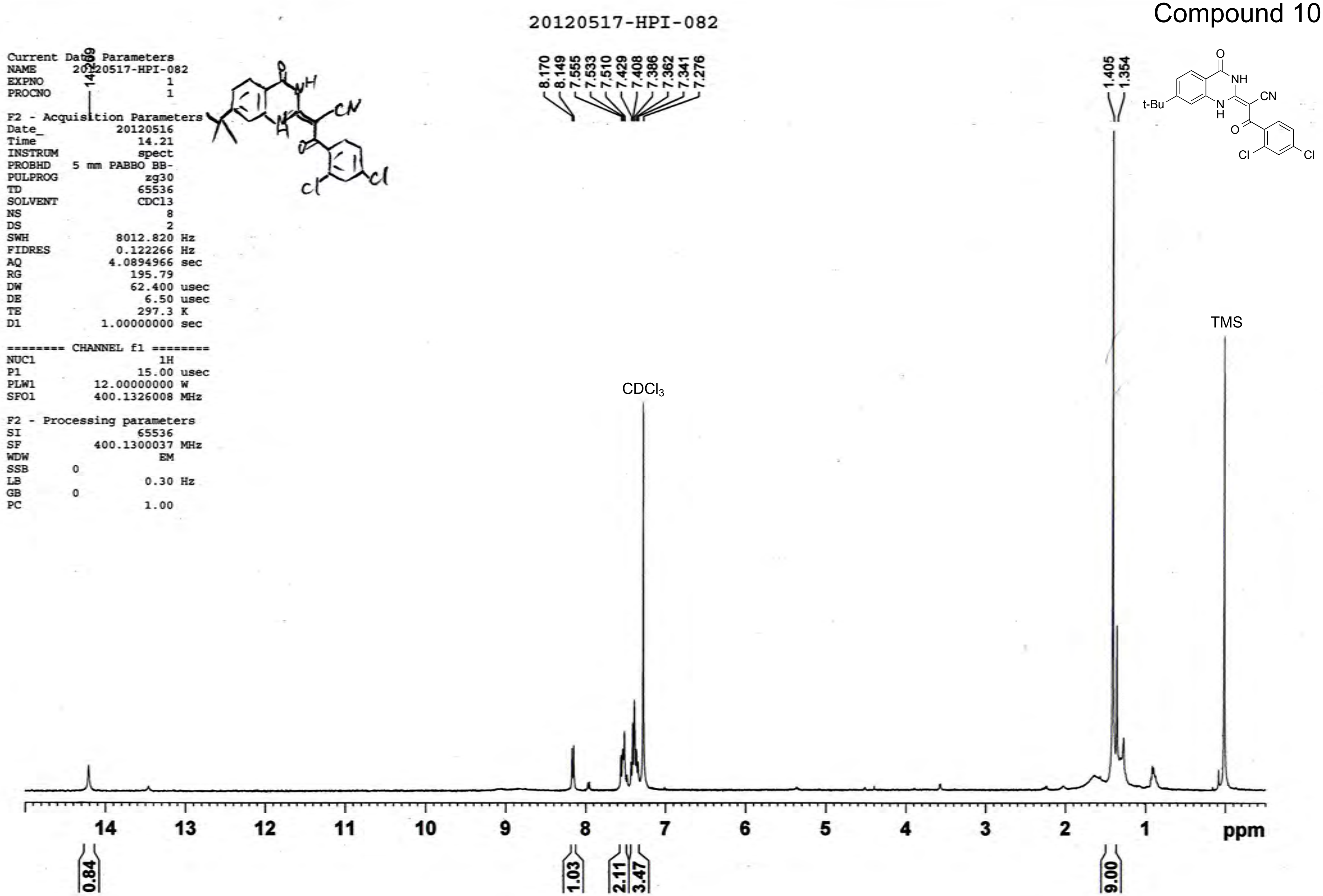
Compound 08


O
NH
CN
N
H
O
Cl
Cl
20130424-HPI-96
NAME 20130424-HPI-95
EXPNO 21
PROCNO 1
Date_ 20130424
Time 10.50
INSTRUM spect
PROBHD 5 mm PADUL 13C
PULPROG zg
TD 65536
SOLVENT DMSO
NS 8
DS 0
SWH 5995.204 Hz
FIDRES 0.091480 Hz
AQ 5.4657526 sec
RG 181
DW 83.400 usec
DE 6.50 usec
TE 292.2 K
D1 2.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 11.25 usec
PL1 0.00 dB
PL1W 8.31434441 W
SFO1 300.1318458 MHz
SI 32768
SF 300.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
13.456
7.995
7.968
7.779
7.773
7.583
7.577
7.555
7.549
7.530
7.503
7.418
7.414
7.391
7.387
3.565
3.386
3.056
3.033
3.011
2.508
2.502
2.497
1.988
1.262
1.239
Water
DMSO
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
1.01
1.01
1.82
2.02
1.00
1.10
6.32

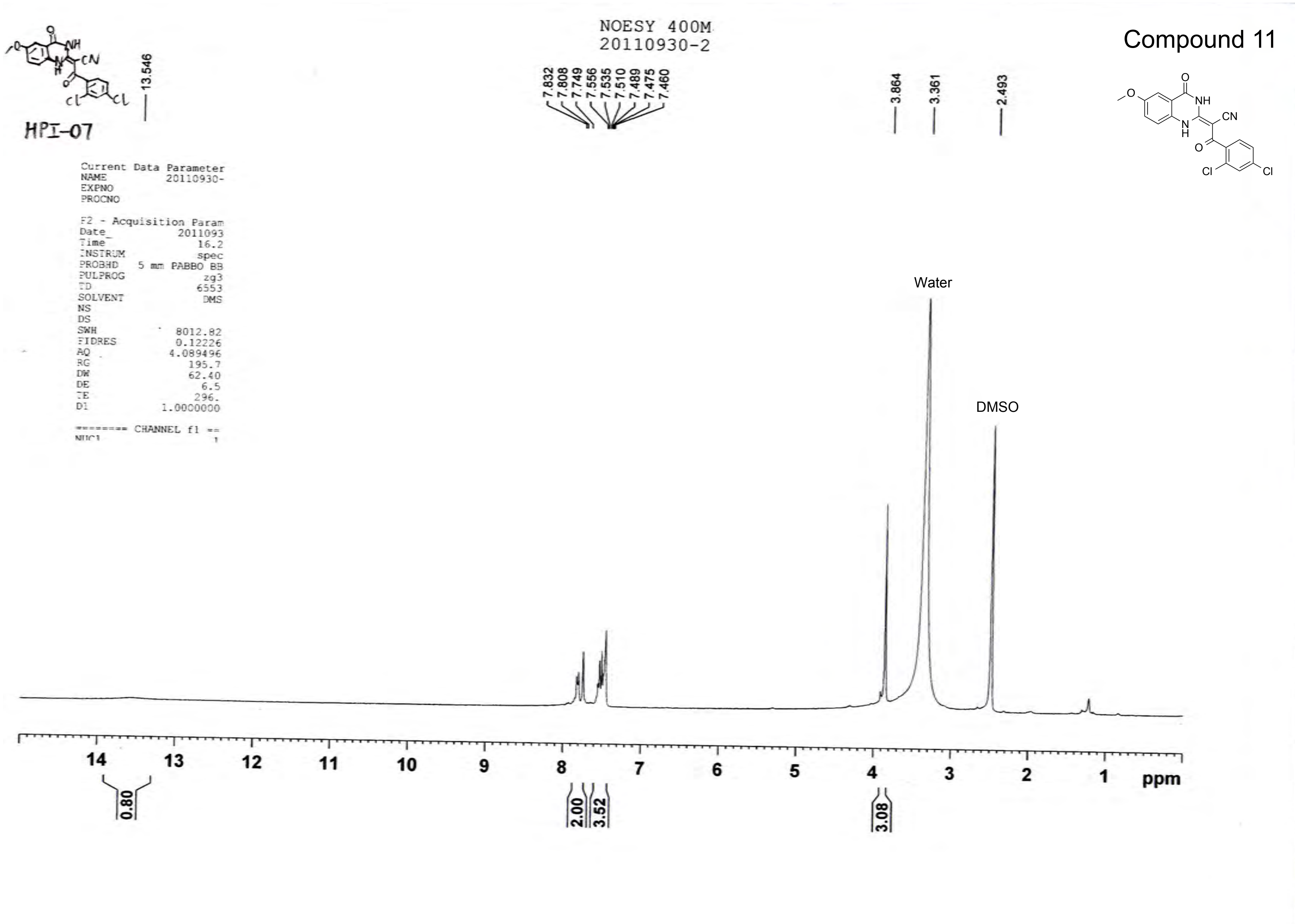
Compound 09

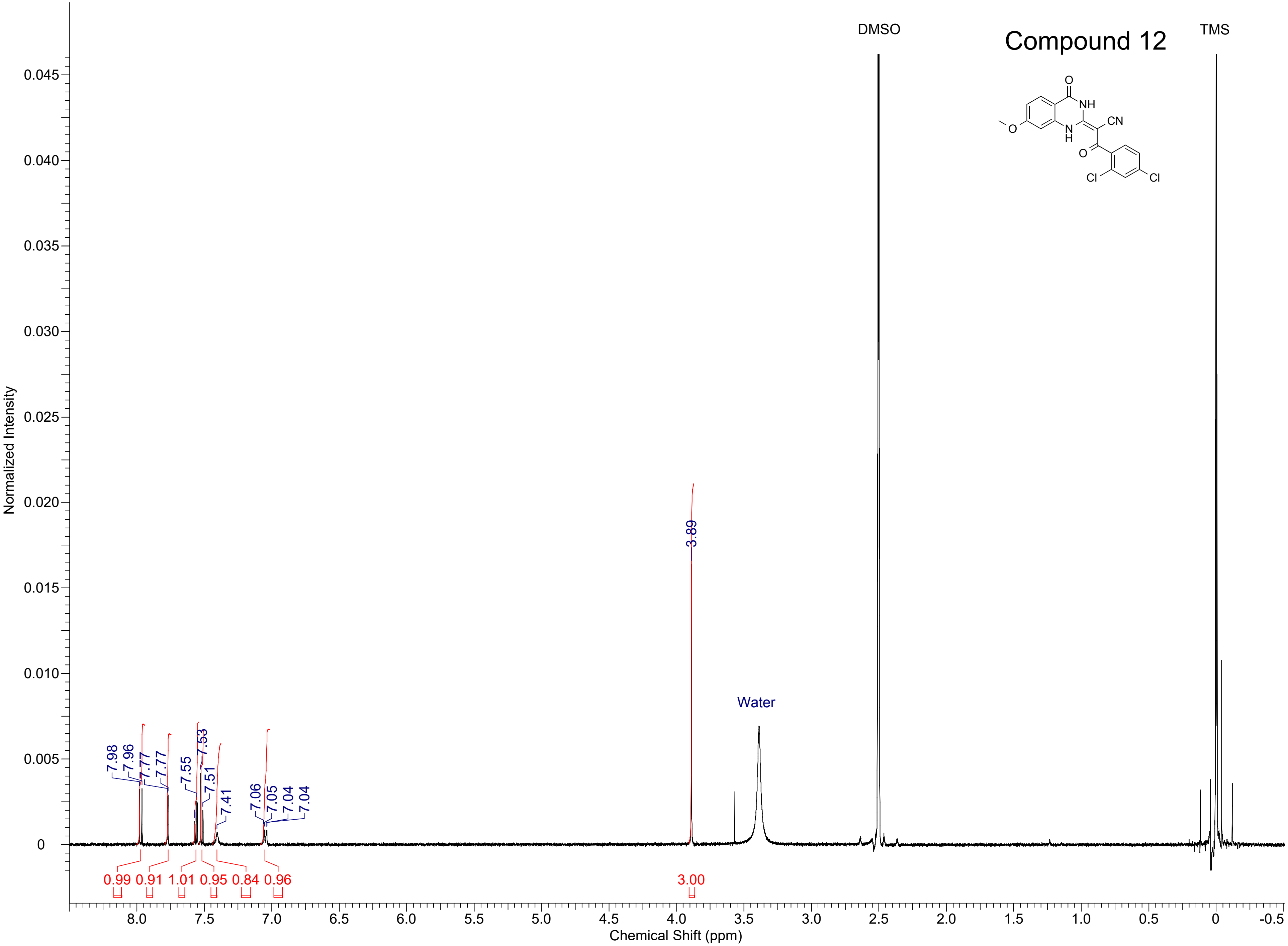

20120517-HPI-081
Current Data Parameters
NAME 20120517-HPI-081
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20120517
Time 13.33
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 8
DS 2
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894966 sec
RG 195.79
DW 62.400 usec
DE 6.50 usec
TE 297.0 K
D1 1.00000000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 15.00 usec
PLW1 12.00000000 W
SFO1 400.1326008 MHz
F2 - Processing parameters
SI 65536
SF 400.1300037 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
14.206
8.233
8.228
7.892
7.886
7.870
7.865
7.505
7.501
7.421
7.401
7.395
7.383
7.376
7.371
7.361
7.355
7.351
7.275
1.402
t-Bu
O
NH
CN
N
H
O
Cl
Cl
CDCl3
Water
TMS
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
0.80
1.01
0.91
1.00
2.92
9.00

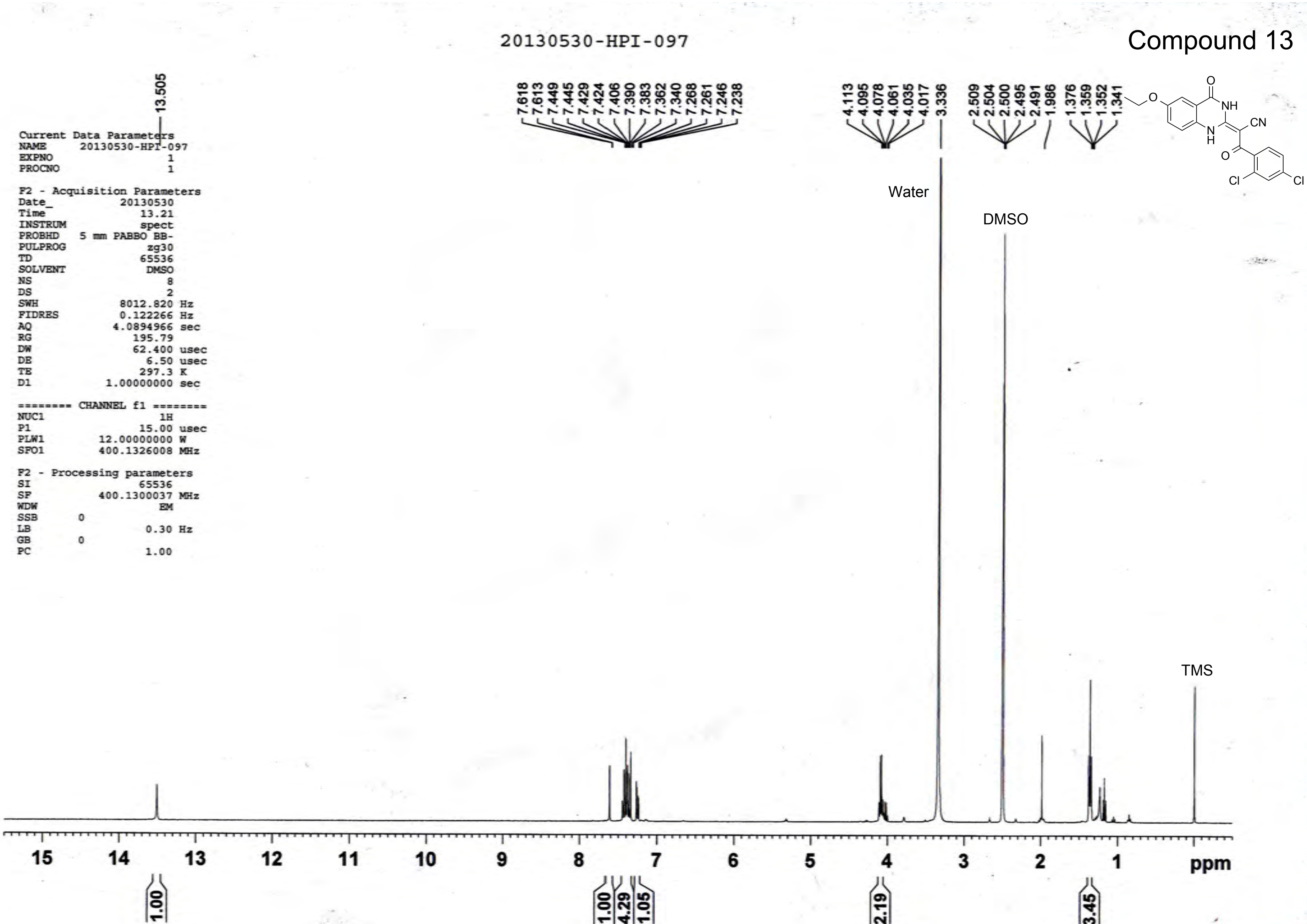
Compound 10



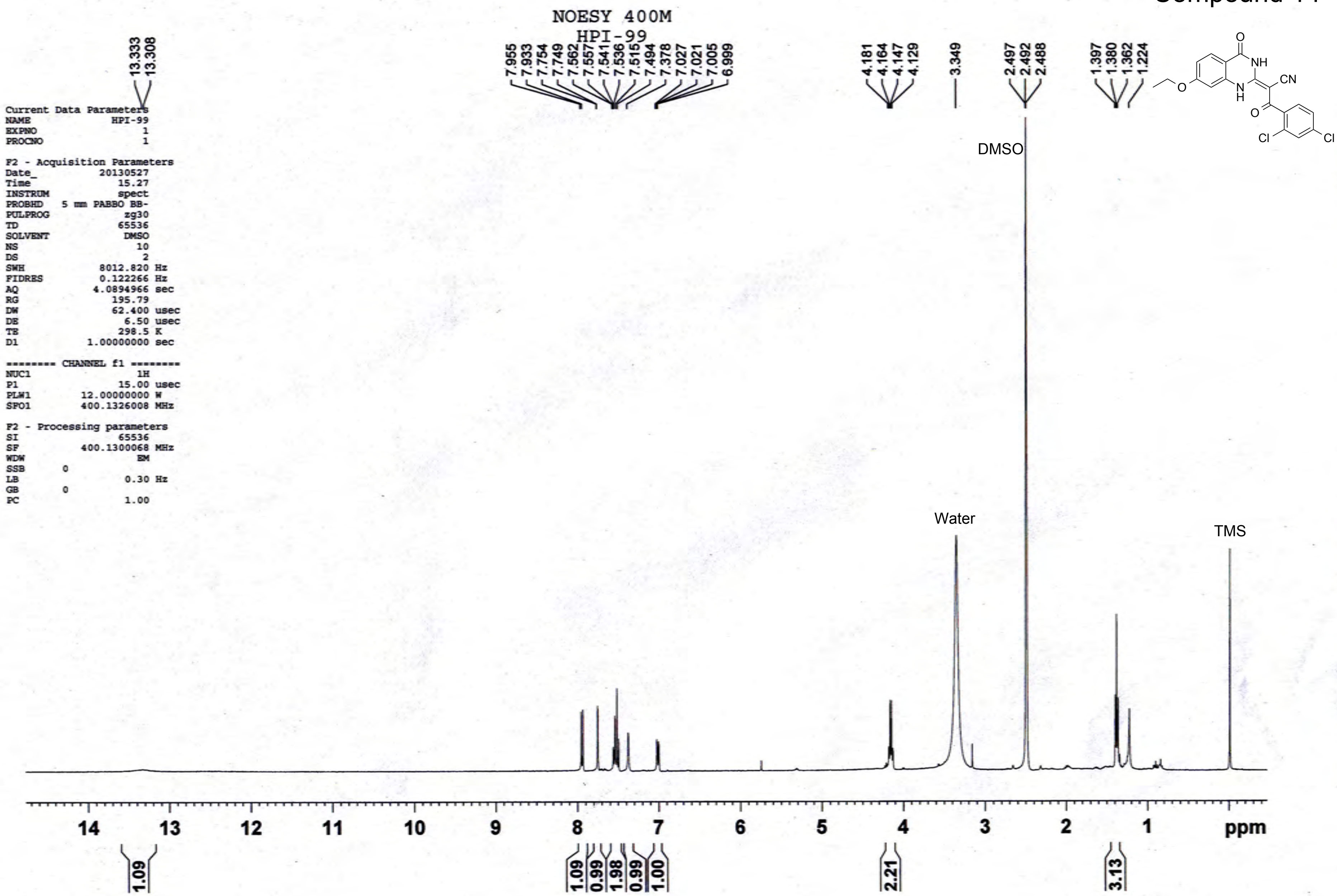
20120517-HPI-082
Current Data Parameters
NAME 20120517-HPI-082
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20120516
Time 14.21
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 8
DS 2
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894966 sec
RG 195.79
DW 62.400 usec
DE 6.50 usec
TE 297.3 K
D1 1.00000000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 15.00 usec
PLW1 12.00000000 W
SFO1 400.1326008 MHz
F2 - Processing parameters
SI 65536
SF 400.1300037 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
8.170
8.149
7.555
7.533
7.510
7.429
7.408
7.386
7.362
7.341
7.276
1.405
1.354
O
NH
CN
t-Bu
N
H
O
Cl
Cl
CDCl3
TMS
14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
0.84
1.03
2.11
3.47
9.00

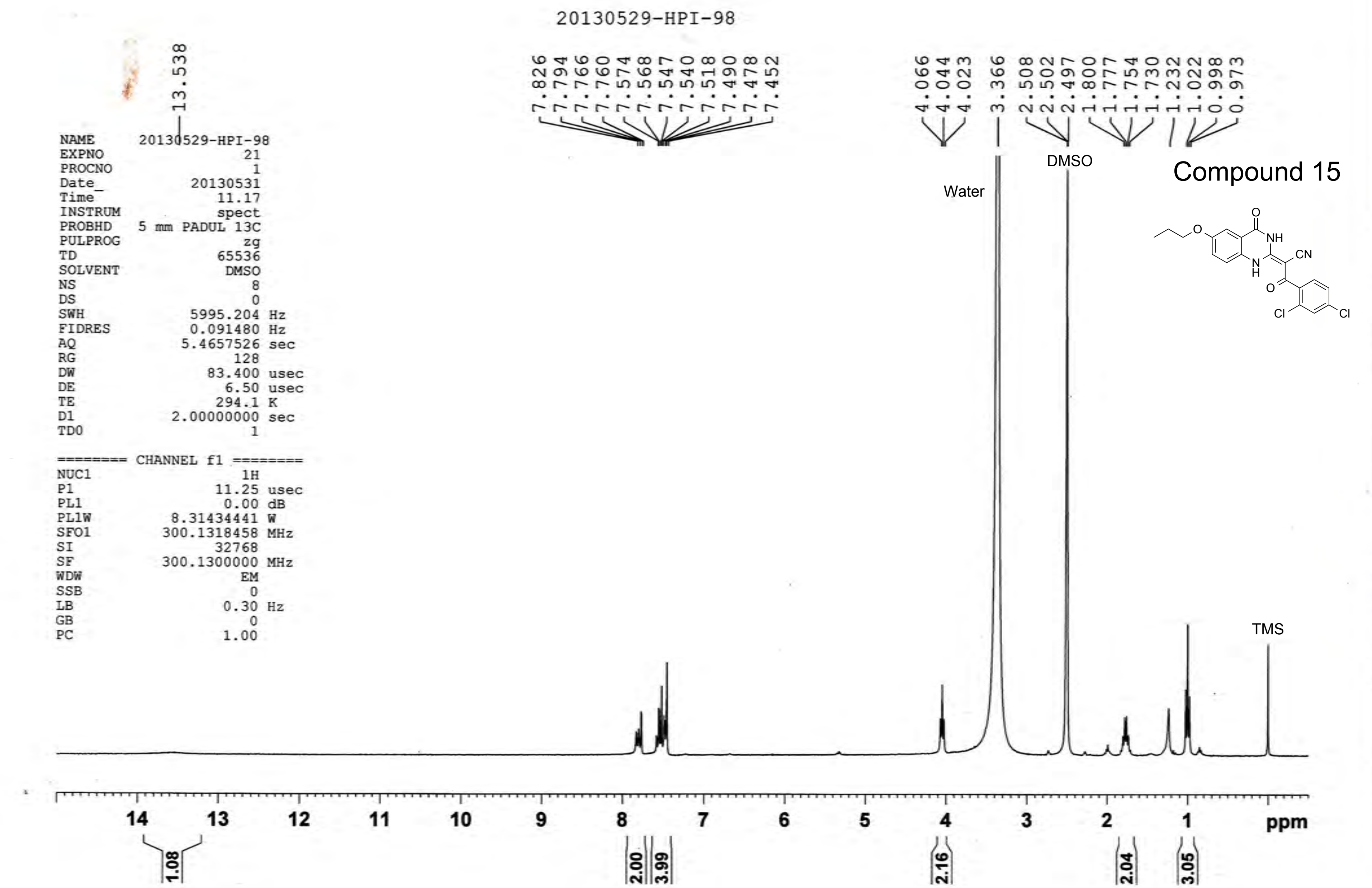

NOESY 400M
20110930-2
Compound 11
HPI-07
13.546
7.832
7.808
7.749
7.556
7.535
7.510
7.489
7.475
7.460
3.864
3.361
2.493
Current Data Parameter
NAME 20110930-
EXPNO
PROCNO
F2 - Acquisition Param
Date_ 2011093
Time 16.2
INSTRUM spec
PROBHD 5 mm PABBO BB
PULPROG zg3
TD 6553
SOLVENT DMS
NS
DS
SWH 8012.82
FIDRES 0.12226
AQ 4.089496
RG 195.7
DW 62.40
DE 6.5
TE 296.
D1 1.0000000
======== CHANNEL f1 ==
Water
DMSO
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
0.80
2.00
3.52
3.08

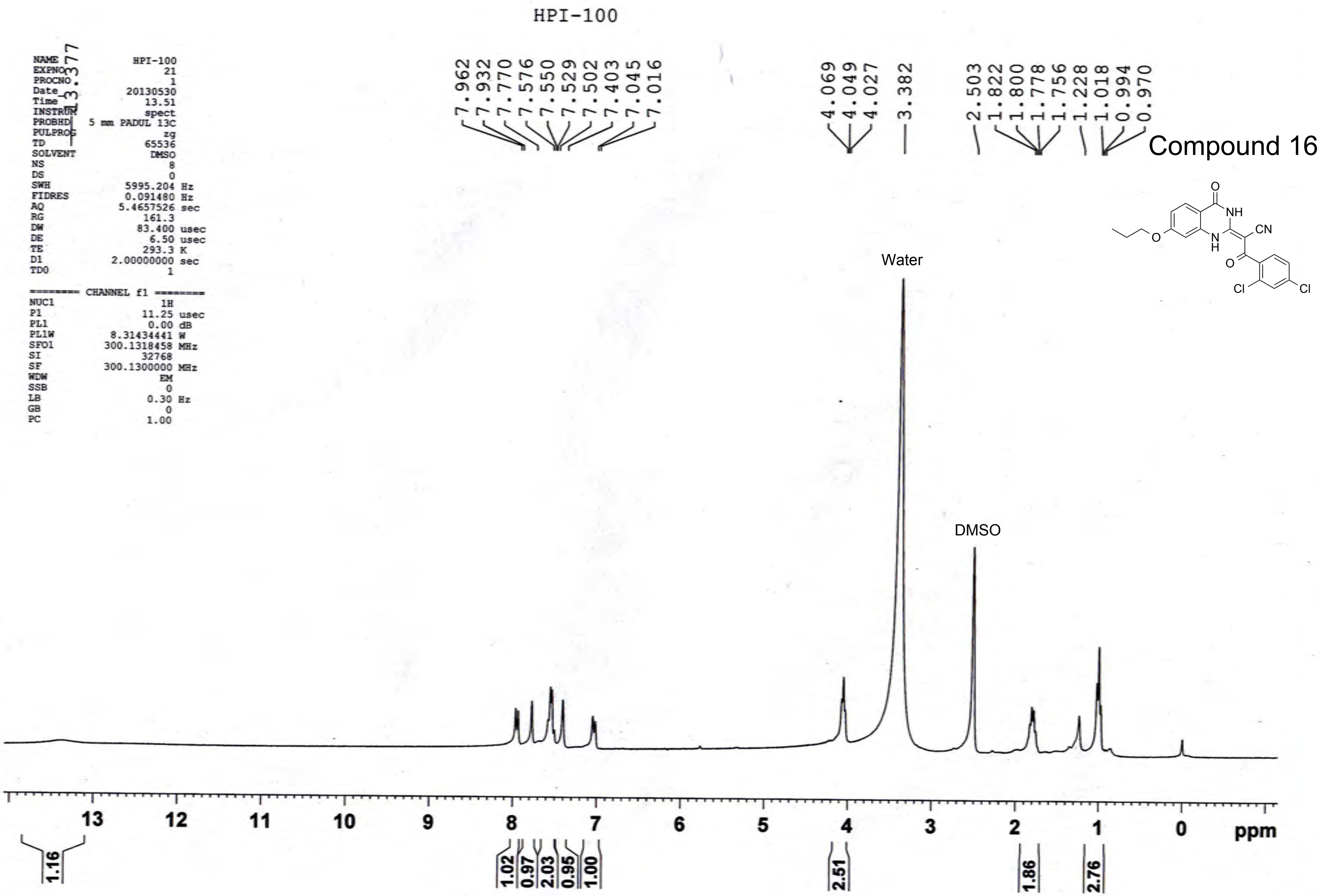

Compound 12
DMSO
TMS
Water
Normalized Intensity
Chemical Shift (ppm)
7.98
7.96
7.77
7.77
7.55
7.53
7.51
7.41
7.06
7.05
7.04
7.04
3.89
0.99
0.91
1.01
0.95
0.84
0.96
3.00
O
NH
CN
O
H
N
O
Cl
Cl


20130530-HPI-097
Compound 13
13.505
Current Data Parameters
NAME 20130530-HPI-097
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20130530
Time 13.21
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 8
DS 2
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894966 sec
RG 195.79
DW 62.400 usec
DE 6.50 usec
TE 297.3 K
D1 1.00000000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 15.00 usec
PLW1 12.00000000 W
SFO1 400.1326008 MHz
F2 - Processing parameters
SI 65536
SF 400.1300037 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
7.618 7.613 7.449 7.445 7.429 7.424 7.406 7.390 7.383 7.362 7.340 7.268 7.261 7.246 7.238
4.113 4.095 4.078 4.061 4.035 4.017
3.336
2.509 2.504 2.500 2.495 2.491
1.986
1.376 1.359 1.352 1.341
Water
DMSO
TMS
O
NH
CN
N
H
O
Cl
Cl
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
1.00
1.00 4.29 1.05
2.19
3.45

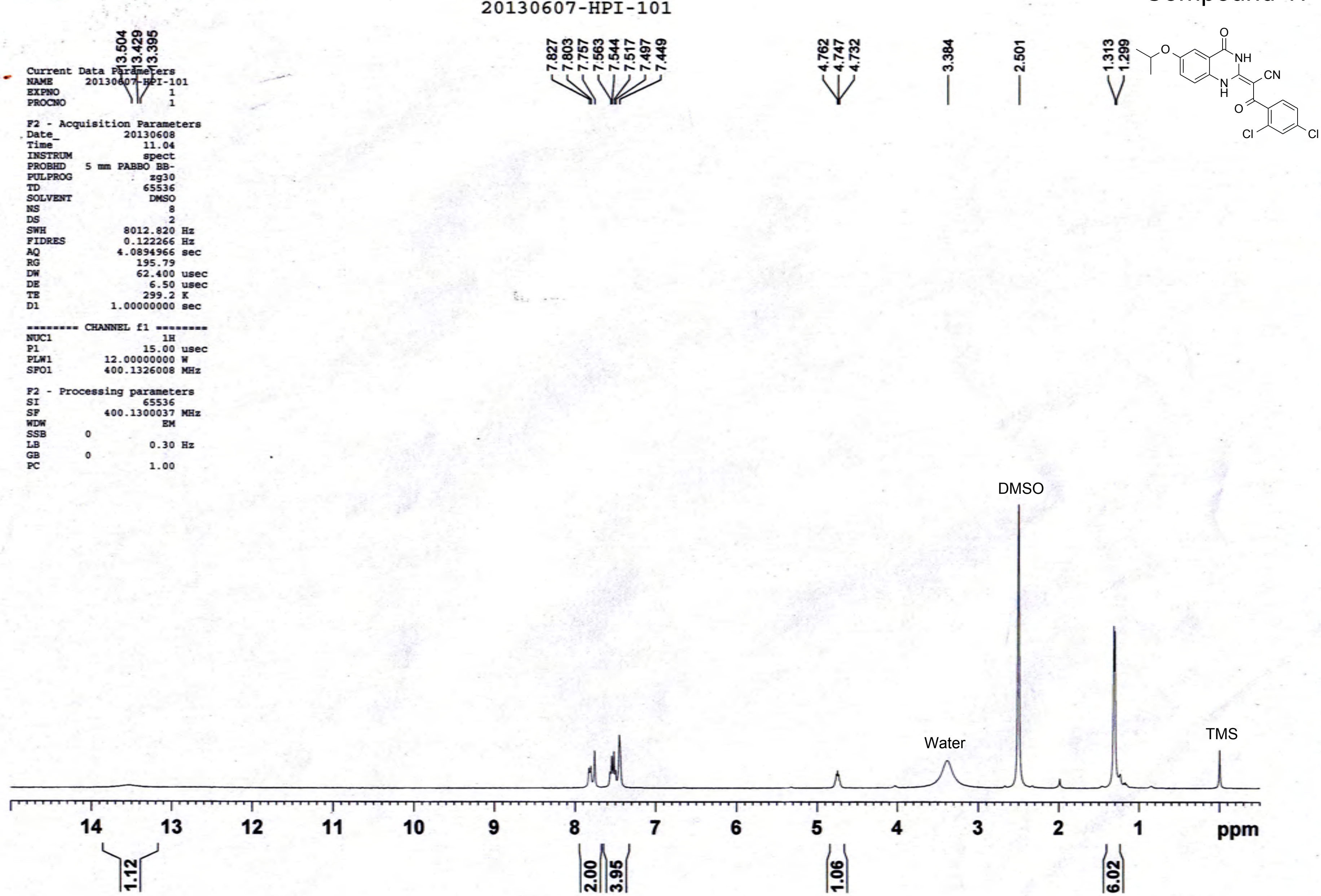
Compound 14


NOESY 400M
HPI-99
13.333
13.308
7.955
7.933
7.754
7.749
7.562
7.557
7.541
7.536
7.515
7.494
7.378
7.027
7.021
7.005
6.999
4.181
4.164
4.147
4.129
3.349
2.497
2.492
2.488
1.397
1.380
1.362
1.224
O
NH
CN
O
N
H
O
Cl
Cl
Current Data Parameters
NAME HPI-99
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20130527
Time 15.27
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 10
DS 2
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894966 sec
RG 195.79
DW 62.400 usec
DE 6.50 usec
TE 298.5 K
D1 1.00000000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 15.00 usec
PLW1 12.00000000 W
SFO1 400.1326008 MHz
F2 - Processing parameters
SI 65536
SF 400.1300068 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
DMSO
Water
TMS
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
1.09
1.09
0.99
1.98
0.99
1.00
2.21
3.13

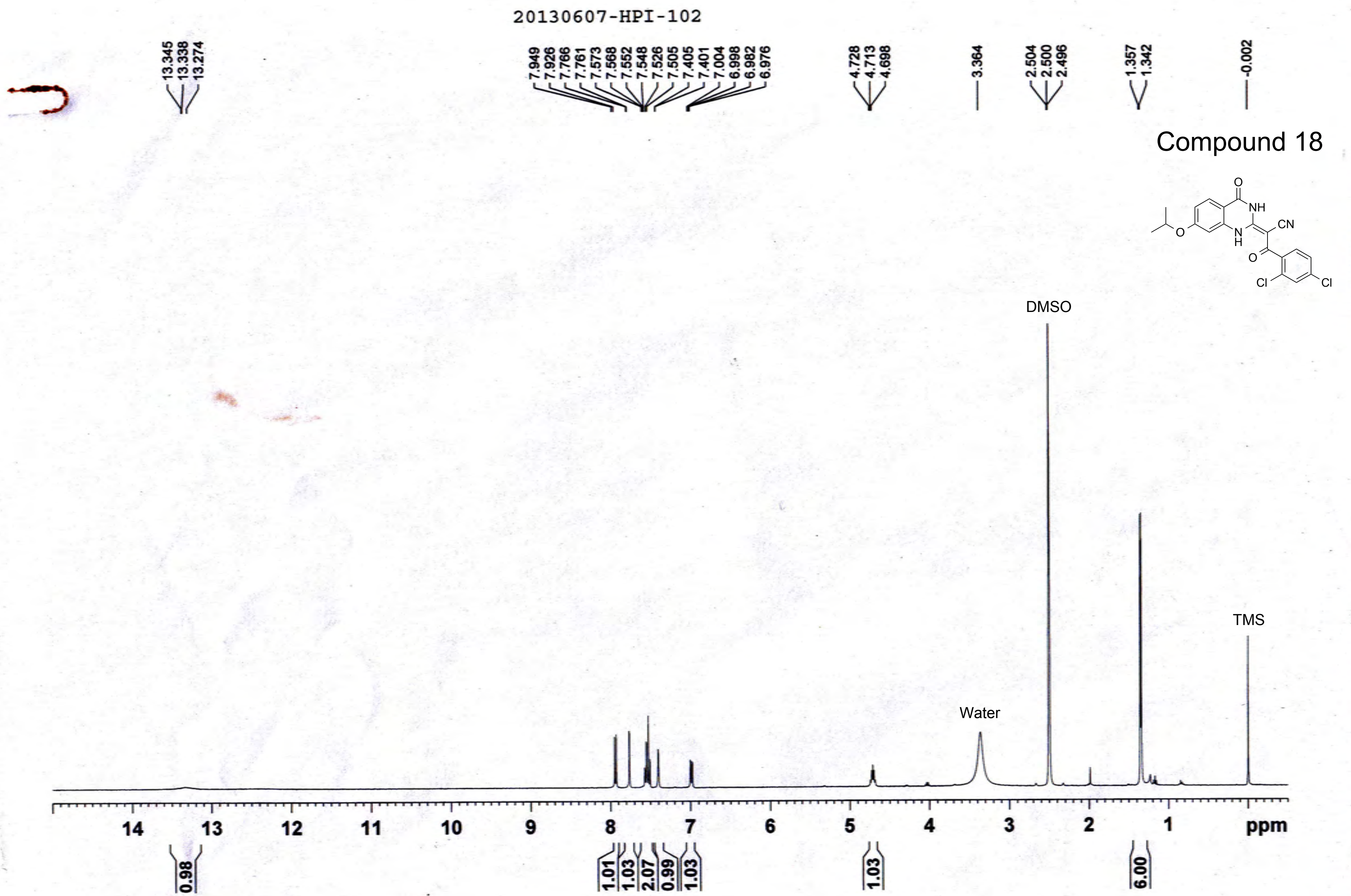

20130529-HPI-98
13.538
7.826
7.794
7.766
7.760
7.574
7.568
7.547
7.540
7.518
7.490
7.478
7.452
4.066
4.044
4.023
3.366
2.508
2.502
2.497
1.800
1.777
1.754
1.730
1.232
1.022
0.998
0.973
NAME 20130529-HPI-98
EXPNO 21
PROCNO 1
Date_ 20130531
Time 11.17
INSTRUM spect
PROBHD 5 mm PADUL 13C
PULPROG zg
TD 65536
SOLVENT DMSO
NS 8
DS 0
SWH 5995.204 Hz
FIDRES 0.091480 Hz
AQ 5.4657526 sec
RG 128
DW 83.400 usec
DE 6.50 usec
TE 294.1 K
D1 2.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 11.25 usec
PL1 0.00 dB
PL1W 8.31434441 W
SFO1 300.1318458 MHz
SI 32768
SF 300.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
Water
DMSO
Compound 15
O
O
NH
CN
N
H
O
Cl
Cl
TMS
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
1.08
2.00
3.99
2.16
2.04
3.05


HPI-100
NAME HPI-100
EXPNO 21
PROCNO 1
Date_ 20130530
Time 13.51
INSTRUM spect
PROBHD 5 mm PADUL 13C
PULPROG zg
TD 65536
SOLVENT DMSO
NS 8
DS 0
SWH 5995.204 Hz
FIDRES 0.091480 Hz
AQ 5.4657526 sec
RG 161.3
DW 83.400 usec
DE 6.50 usec
TE 293.3 K
D1 2.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 11.25 usec
PL1 0.00 dB
PL1W 8.31434441 W
SFO1 300.1318458 MHz
SI 32768
SF 300.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
13.377
7.962
7.932
7.770
7.576
7.550
7.529
7.502
7.403
7.045
7.016
4.069
4.049
4.027
3.382
2.503
1.822
1.800
1.778
1.756
1.228
1.018
0.994
0.970
Compound 16
O
NH
CN
O
N
H
O
Cl
Cl
Water
DMSO
13
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
1.16
1.02
0.97
2.03
0.95
1.00
2.51
1.86
2.76

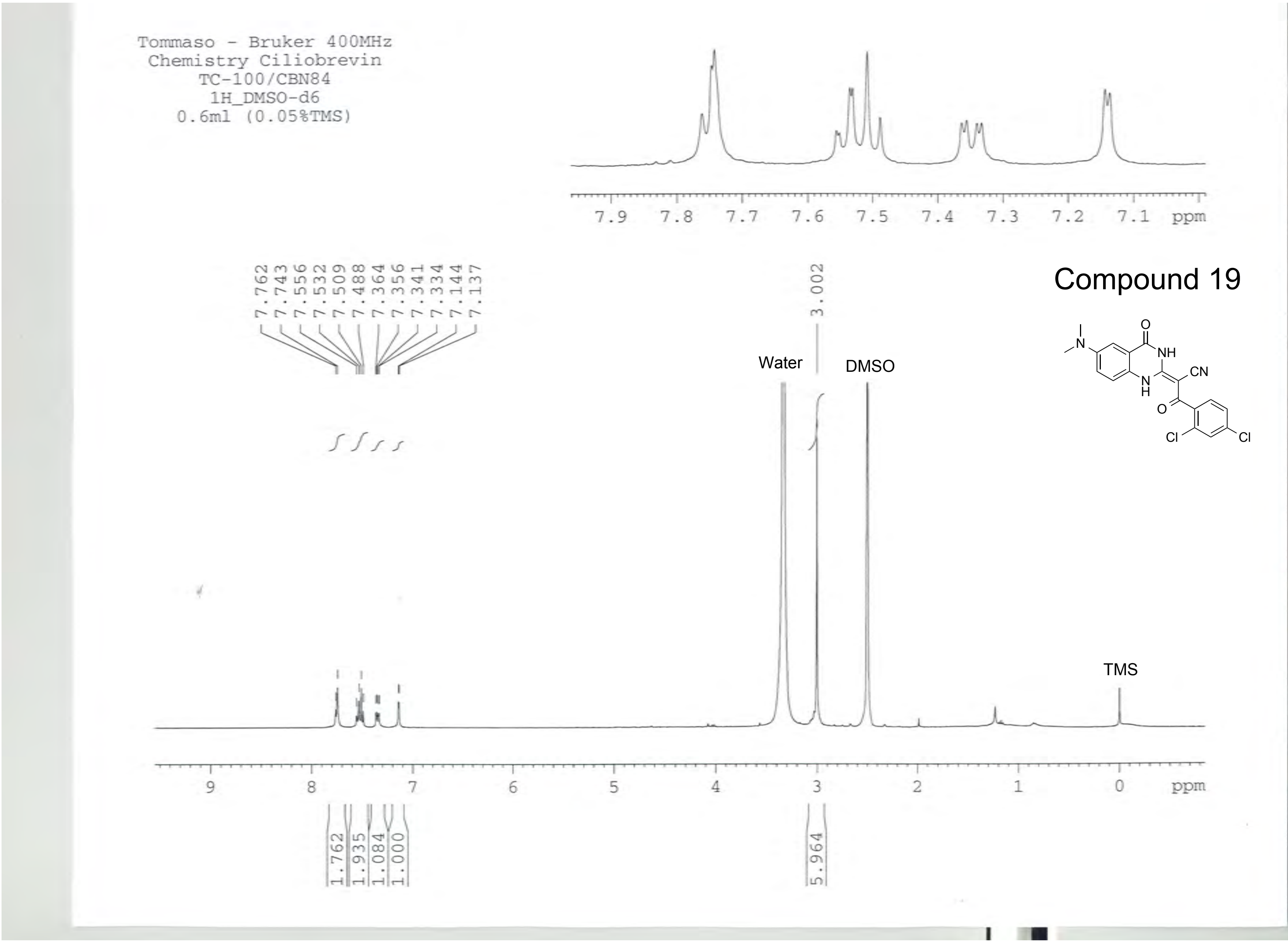
Compound 17

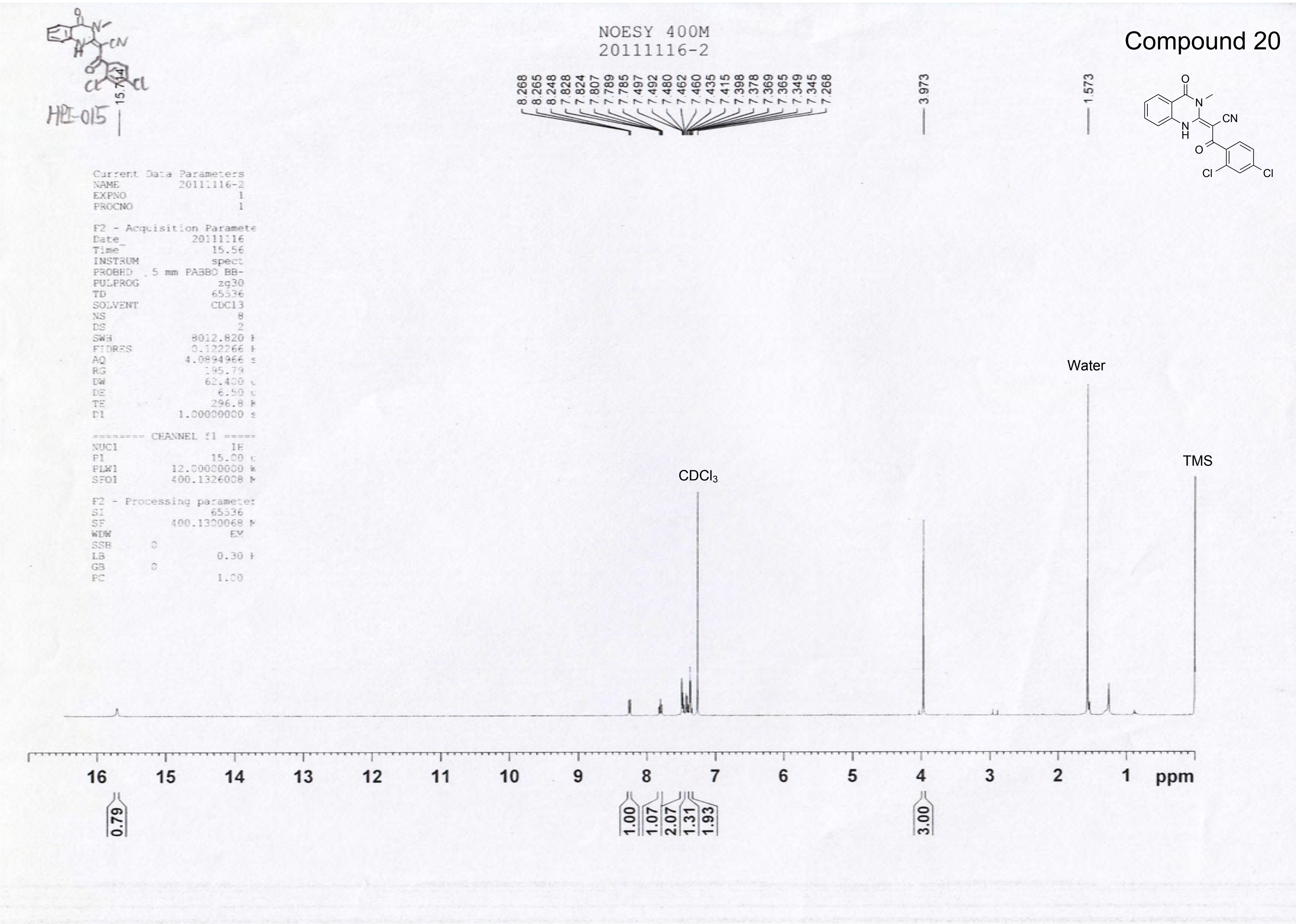


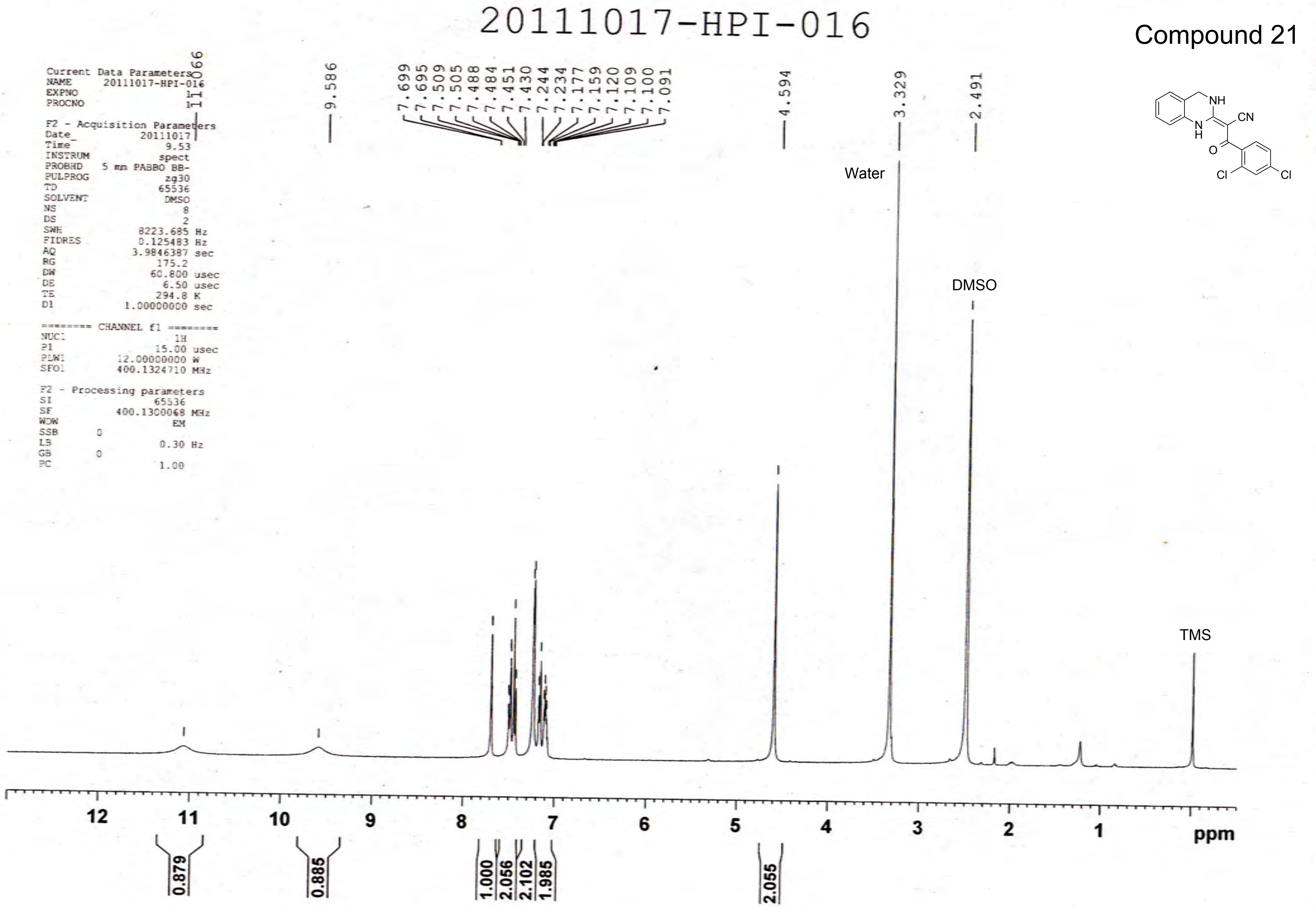
20130607-HPI-101
Current Data Parameters
NAME 20130607-HPI-101
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20130608
Time 11.04
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 8
DS 2
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894966 sec
RG 195.79
DW 62.400 usec
DE 6.50 usec
TE 299.2 K
D1 1.00000000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 15.00 usec
PLW1 12.00000000 W
SFO1 400.1326008 MHz
F2 - Processing parameters
SI 65536
SF 400.1300037 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
13.504
13.429
13.395
7.827
7.803
7.757
7.563
7.544
7.517
7.497
7.449
4.762
4.747
4.732
3.384
2.501
1.313
1.299
O
NH
CN
N
H
O
Cl
Cl
DMSO
Water
TMS
14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
1.12
2.00
3.95
1.06
6.02

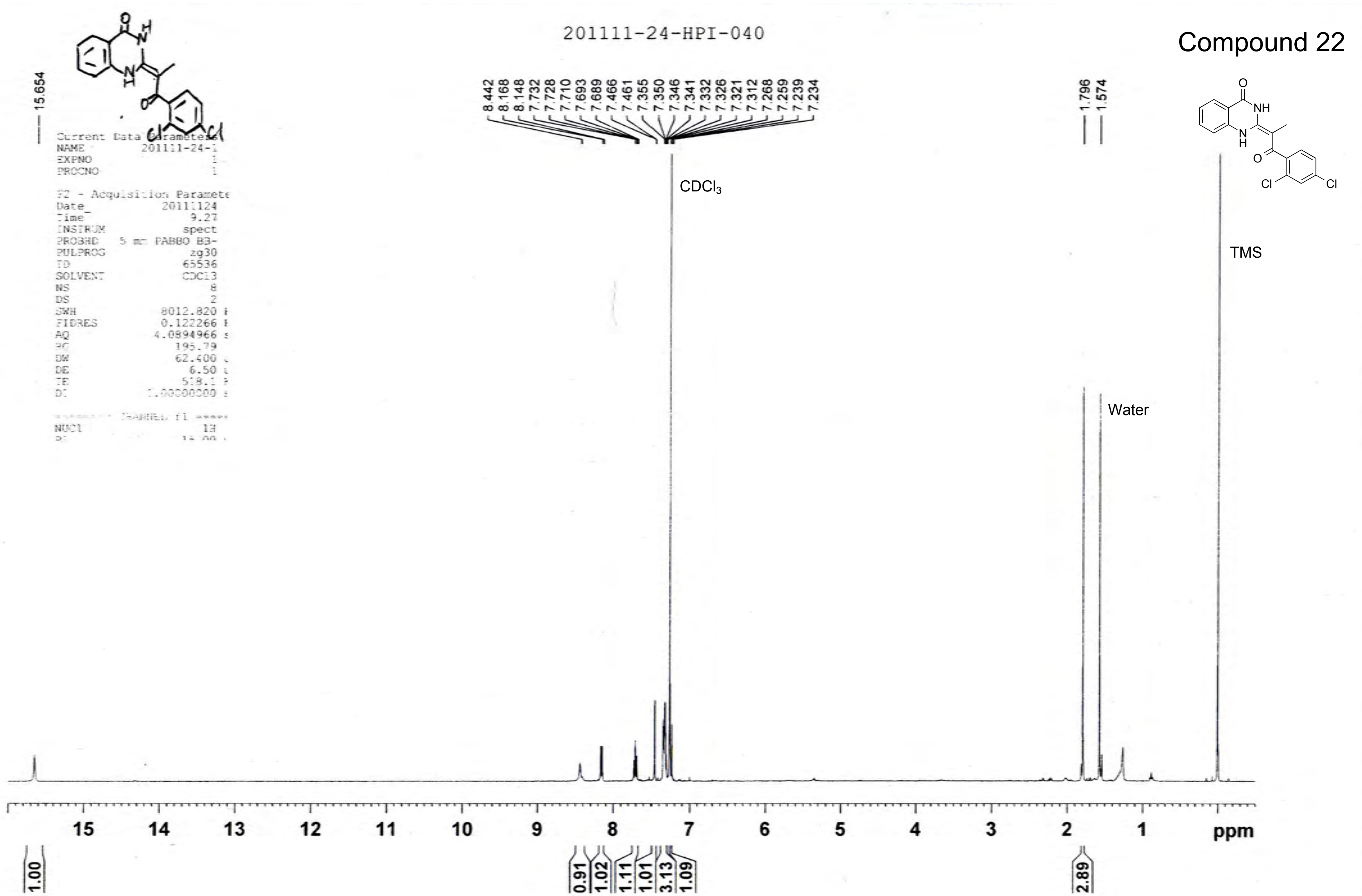


20130607-HPI-102
13.345
13.338
13.274
7.949
7.926
7.766
7.761
7.573
7.568
7.552
7.548
7.526
7.505
7.405
7.401
7.004
6.998
6.982
6.976
4.728
4.713
4.698
3.364
2.504
2.500
2.496
1.357
1.342
-0.002
Compound 18
O
NH
CN
N
H
O
O
Cl
Cl
DMSO
TMS
Water
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
0.98
1.01
1.03
2.07
0.99
1.03
1.03
6.00

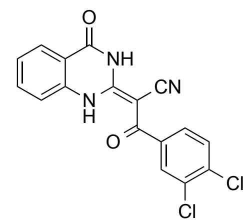

Tommaso - Bruker 400MHz
Chemistry Ciliobrevin
TC-100/CBN84
1H_DMSO-d6
0.6ml (0.05%TMS)
7.762
7.743
7.556
7.532
7.509
7.488
7.364
7.356
7.341
7.334
7.144
7.137
3.002
Water
DMSO
TMS
Compound 19
O
NH
CN
N
H
O
Cl
Cl
7.9 7.8 7.7 7.6 7.5 7.4 7.3 7.2 7.1 ppm
9 8 7 6 5 4 3 2 1 0 ppm
1.762
1.935
1.084
1.000
5.964

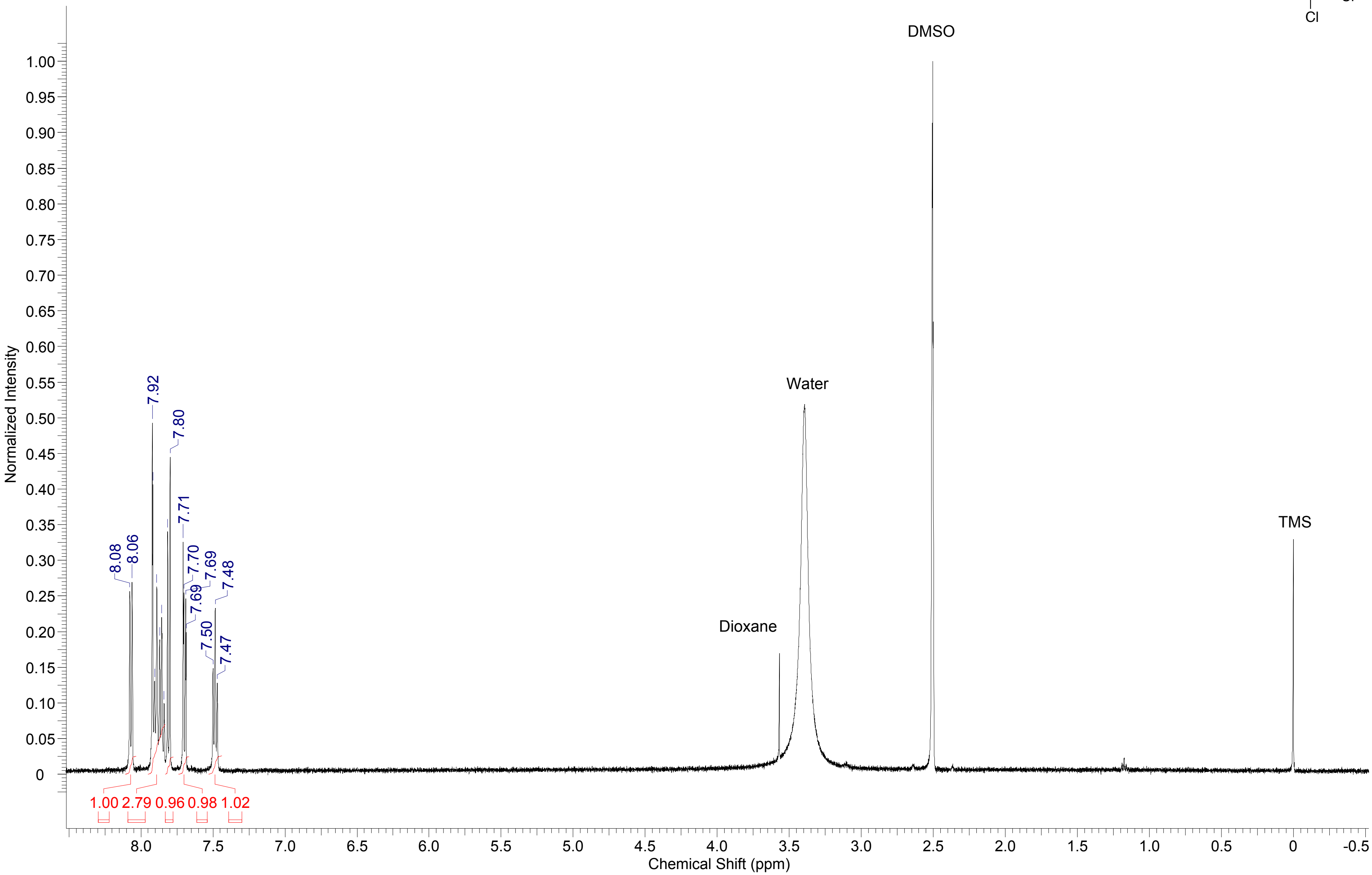

NOESY 400M
20111116-2
Compound 20
HPL-015
15.714
8.268
8.265
8.248
7.828
7.824
7.807
7.789
7.785
7.497
7.492
7.480
7.462
7.460
7.435
7.415
7.398
7.378
7.369
7.365
7.349
7.345
7.268
3.973
1.573
Current Data Parameters
NAME 20111116-2
EXPNO 1
PROCNO 1
F2 - Acquisition Paramete
Date_ 20111116
Time 15.56
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 8
DS 2
SWH 8012.820
FIDRES 0.122266
AQ 4.0894966
RG 195.79
DW 62.400
DE 6.50
TE 296.8
D1 1.00000000
======== CHANNEL f1 =====
NUC1 1H
P1 15.00
PLW1 12.00000000
SFO1 400.1326008
F2 - Processing parameter
SI 65536
SF 400.1300068
WDW EM
SSB 0
LB 0.30
GB 0
PC 1.00
Water
TMS
CDCl3
ppm
0.79
1.00
1.07
2.07
1.31
1.93
3.00


20111017-HPI-016
Compound 21
NH
CN
N
H
O
Cl
Cl
Current Data Parameters
NAME 20111017-HPI-016
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20111017
Time 9.53
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 8
DS 2
SWH 8223.685 Hz
FIDRES 0.125483 Hz
AQ 3.9846387 sec
RG 175.2
DW 60.800 usec
DE 6.50 usec
TE 294.8 K
D1 1.00000000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 15.00 usec
PLW1 12.00000000 W
SFO1 400.1324710 MHz
F2 - Processing parameters
SI 65536
SF 400.1300068 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
11.066
9.586
7.699
7.695
7.509
7.505
7.488
7.484
7.451
7.430
7.244
7.234
7.177
7.159
7.120
7.109
7.100
7.091
4.594
3.329
2.491
Water
DMSO
TMS
12
11
10
9
8
7
6
5
4
3
2
1
ppm
0.879
0.885
1.000
2.056
2.102
1.985
2.055

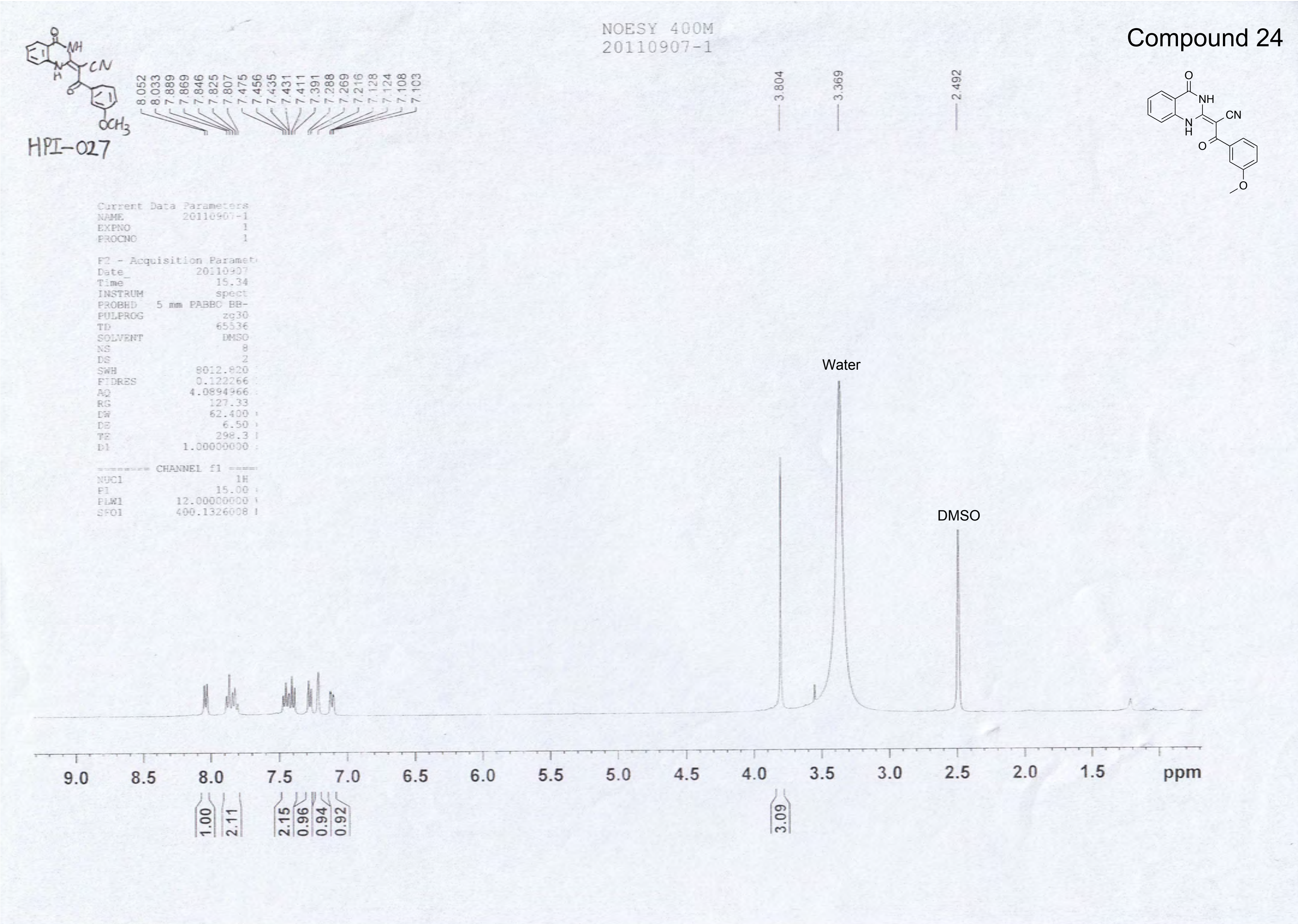

201111-24-HPI-040
Compound 22
15.654
8.442
8.168
8.148
7.732
7.728
7.710
7.693
7.689
7.466
7.461
7.355
7.350
7.346
7.341
7.332
7.326
7.321
7.312
7.268
7.259
7.239
7.234
1.796
1.574
CDCl3
TMS
Water
Current Data Parameters
NAME 201111-24-1
EXPNO 1
PROCNO 1
F2 - Acquisition Paramete
Date_ 20111124
Time 9.27
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 8
DS 2
SWH 8012.820
FIDRES 0.122266
AQ 4.0894966
RG 195.79
DW 62.400
DE 6.50
TE 518.1
NUC1 1H
O
NH
H
Cl
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
1.00
0.91
1.02
1.11
1.01
3.13
1.09
2.89

Compound 23

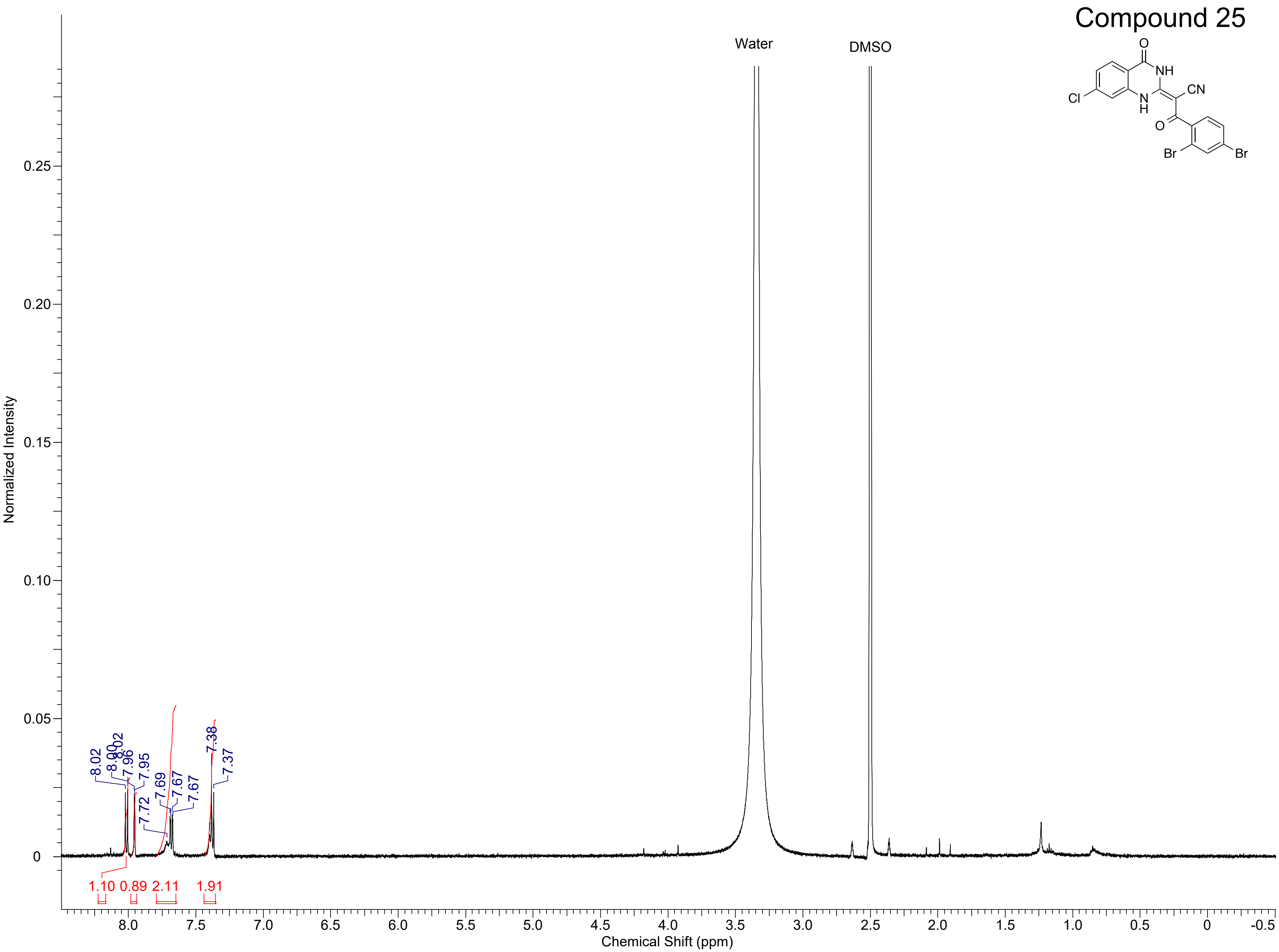



DMSO
Water
Dioxane
TMS
Normalized Intensity
Chemical Shift (ppm)
8.08
8.06
7.92
7.80
7.71
7.70
7.69
7.69
7.50
7.48
7.47
1.00
2.79
0.96
0.98
1.02

Compound 24

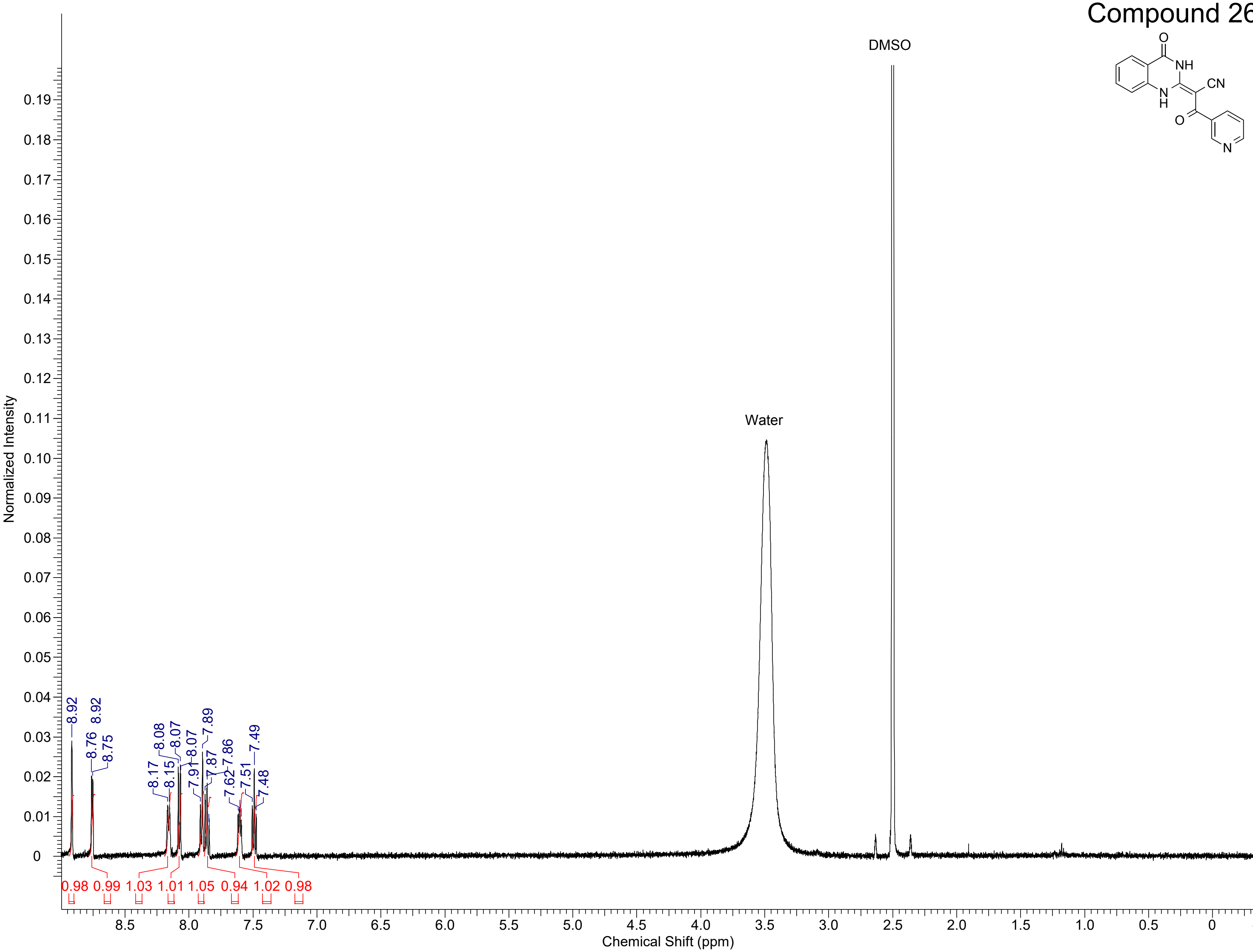


NOESY 400M
20110907-1
HPI-027
8.052
8.033
7.889
7.869
7.846
7.825
7.807
7.475
7.456
7.435
7.431
7.411
7.391
7.288
7.269
7.216
7.128
7.124
7.108
7.103
3.804
3.369
2.492
Current Data Parameters
NAME 20110907-1
EXPNO 1
PROCNO 1
F2 - Acquisition Paramet
Date_ 20110907
Time 15.34
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 8
DS 2
SWH 8012.820
FIDRES 0.122266
AQ 4.0894966
RG 127.33
DW 62.400
DE 6.50
TE 298.3
D1 1.00000000
======== CHANNEL f1 ========
NUC1 1H
P1 15.00
PLW1 12.00000000
SFO1 400.1326008
Water
DMSO
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
1.00
2.11
2.15
0.96
0.94
0.92
3.09

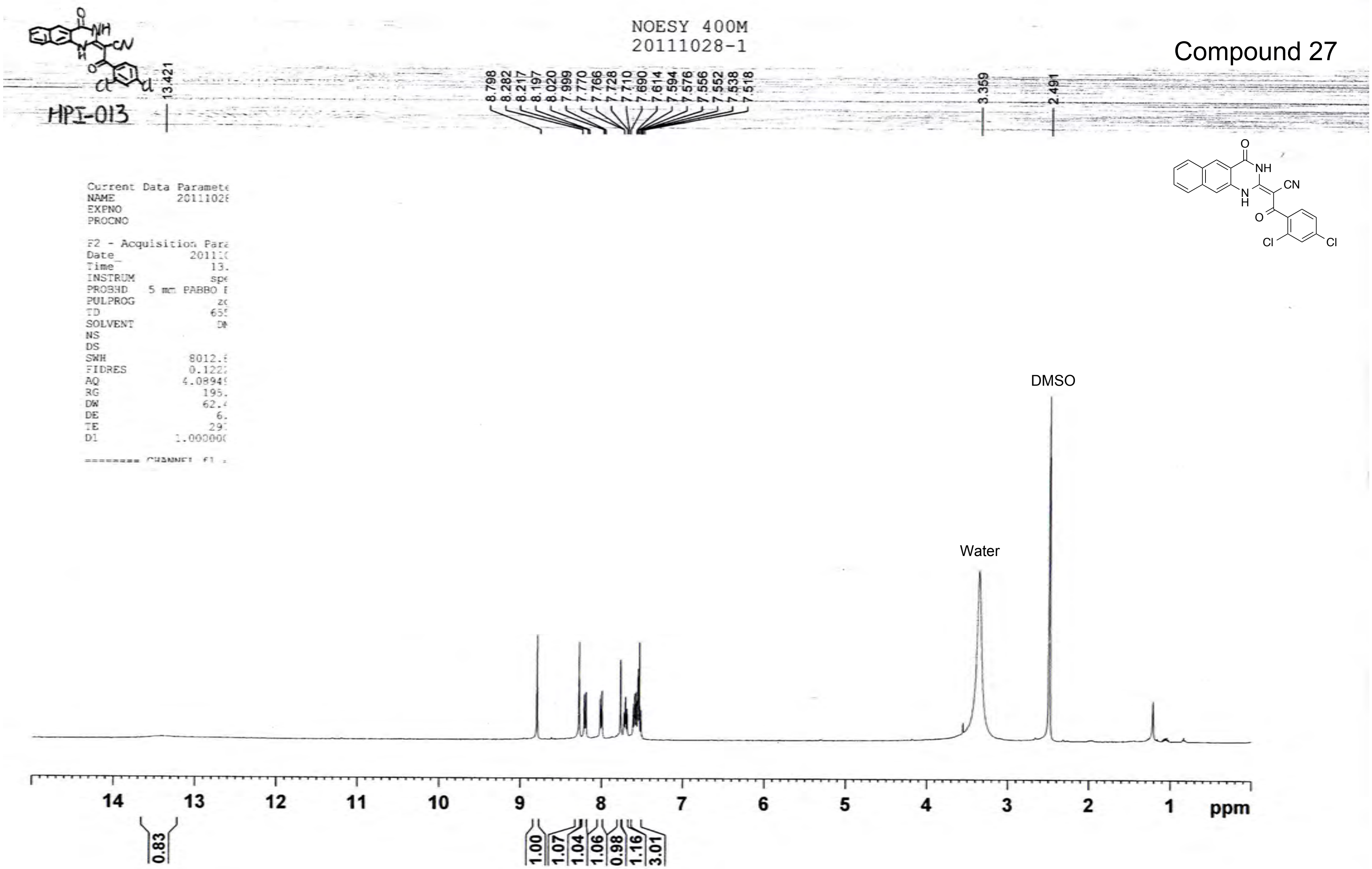


Compound 25
O
NH
CN
Cl
N
H
O
Br
Br
Water
DMSO
Normalized Intensity
0.25
0.20
0.15
0.10
0.05
0
8.02
8.00
8.02
7.96
7.95
7.72
7.69
7.67
7.67
7.38
7.37
1.10
0.89
2.11
1.91
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
-0.5
Chemical Shift (ppm)

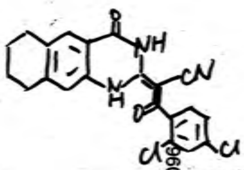
Compound 26

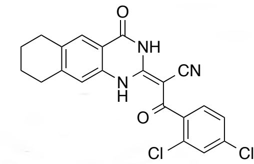

DMSO
Water
Normalized Intensity
Chemical Shift (ppm)
8.92
8.92
8.76
8.75
8.17
8.15
8.08
8.07
8.07
7.91
7.89
7.87
7.86
7.62
7.51
7.49
7.48
0.98
0.99
1.03
1.01
1.05
0.94
1.02
0.98
O
NH
CN
N
H
O
N

Compound 27


NOESY 400M
20111028-1
HPI-013
13.421
8.798
8.282
8.217
8.197
8.020
7.999
7.770
7.766
7.728
7.710
7.690
7.614
7.594
7.576
7.556
7.552
7.538
7.518
3.359
2.491
Current Data Paramete
NAME 2011102
EXPNO
PROCNO
F2 - Acquisition Para
Date_ 20111
Time 13.
INSTRUM spe
PROBHD 5 mm PABBO
PULPROG zc
TD 65
SOLVENT DM
NS
DS
SWH 8012.
FIDRES 0.122
AQ 4.0894
RG 195.
DW 62.
DE 6.
TE 29
D1 1.00000
======== CHANNEL f1
O
NH
CN
N
H
O
Cl
Cl
DMSO
Water
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
0.83
1.00
1.07
1.04
1.06
0.98
1.16
3.01

Compound 28





20120503-HPI-077

14.090

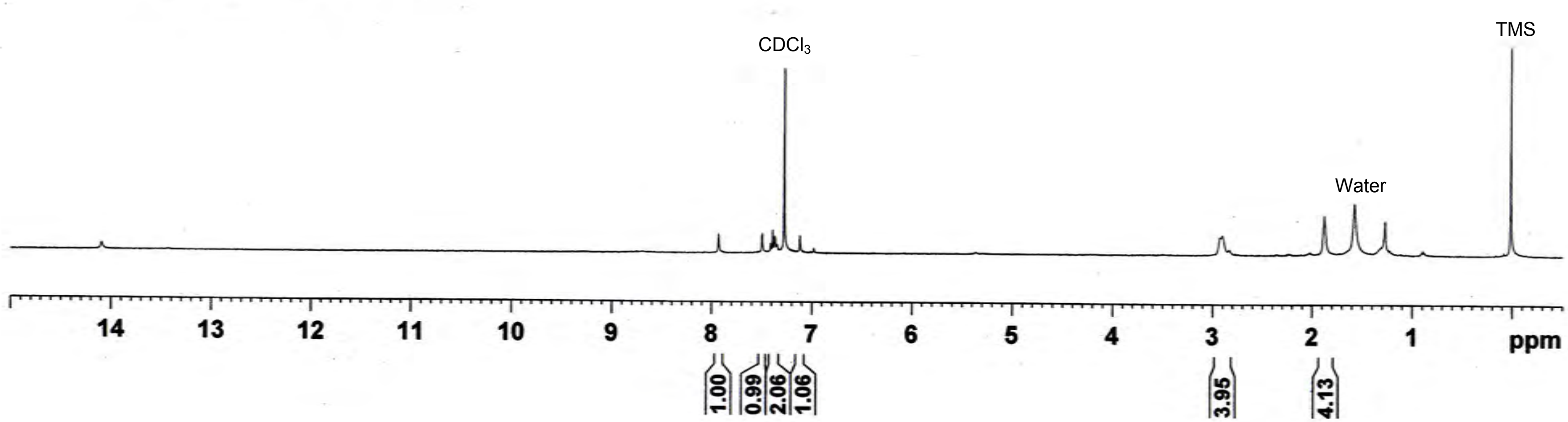
7.930
7.494
7.390
7.367
7.275
7.120

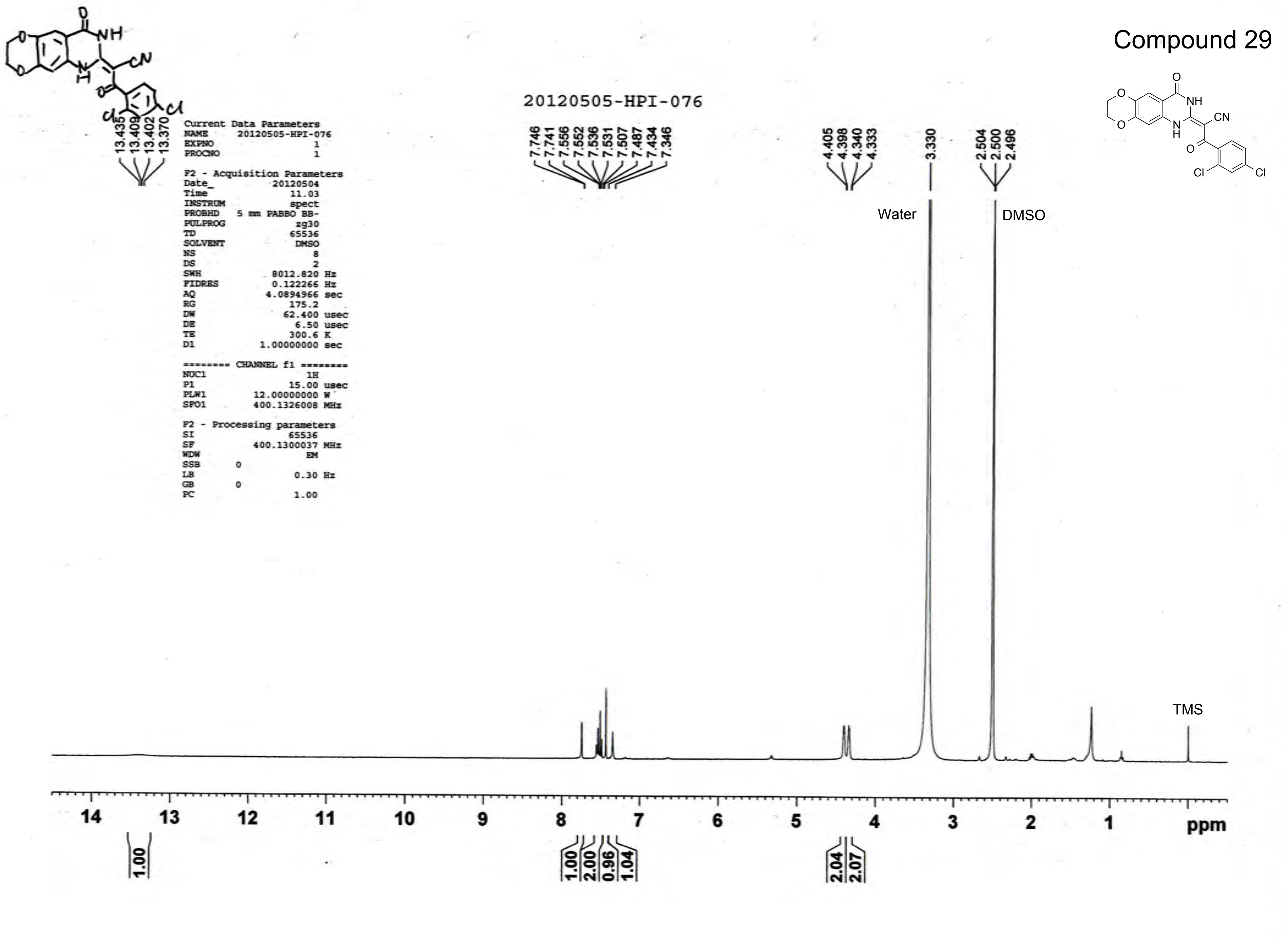
2.904

1.877
1.576
1.270

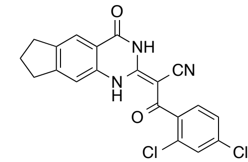
0.015

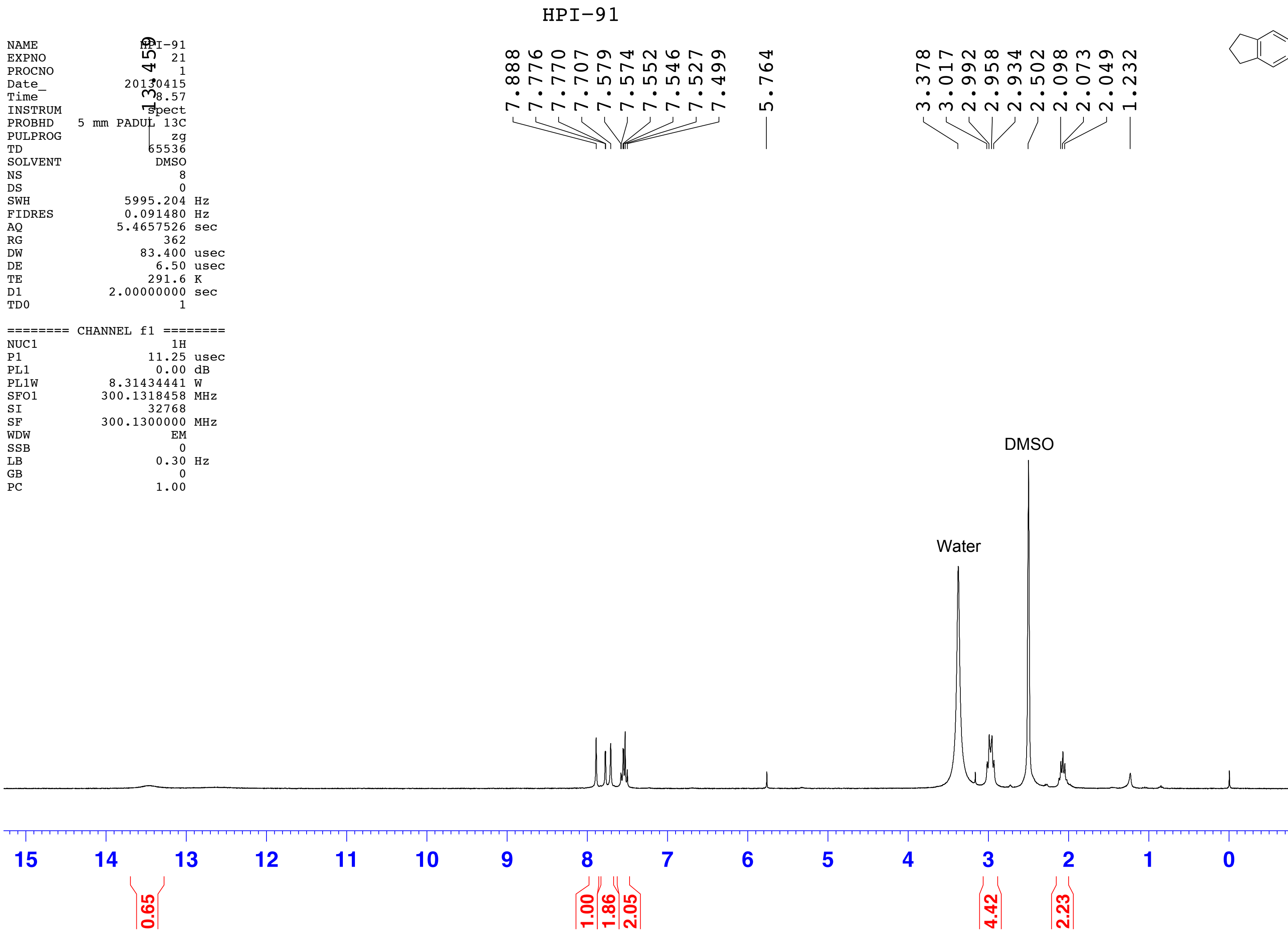
```
Current Data Parameters
NAME       20120503-HPI-077
EXPNO                     1
PROCNO                    1

F2 - Acquisition Parameters
Date_              20120503
Time                   9.05
INSTRUM               spect
PROBHD    5 mm PABBO BB-
PULPROG                zg30
TD                    65536
SOLVENT               CDCl3
NS                        8
DS                        2
SWH             8012.820 Hz
FIDRES          0.122266 Hz
AQ            4.0894966 sec
RG                   195.79
DW               62.400 usec
DE                 6.50 usec
TE                  299.4 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                     1H
P1                15.00 usec
PLW1          12.00000000 W
SFO1         400.1326008 MHz

F2 - Processing parameters
SI                    65536
SF           400.1300037 MHz
WDW                      EM
SSB       0
LB                  0.30 Hz
GB        0
PC                     1.00
```



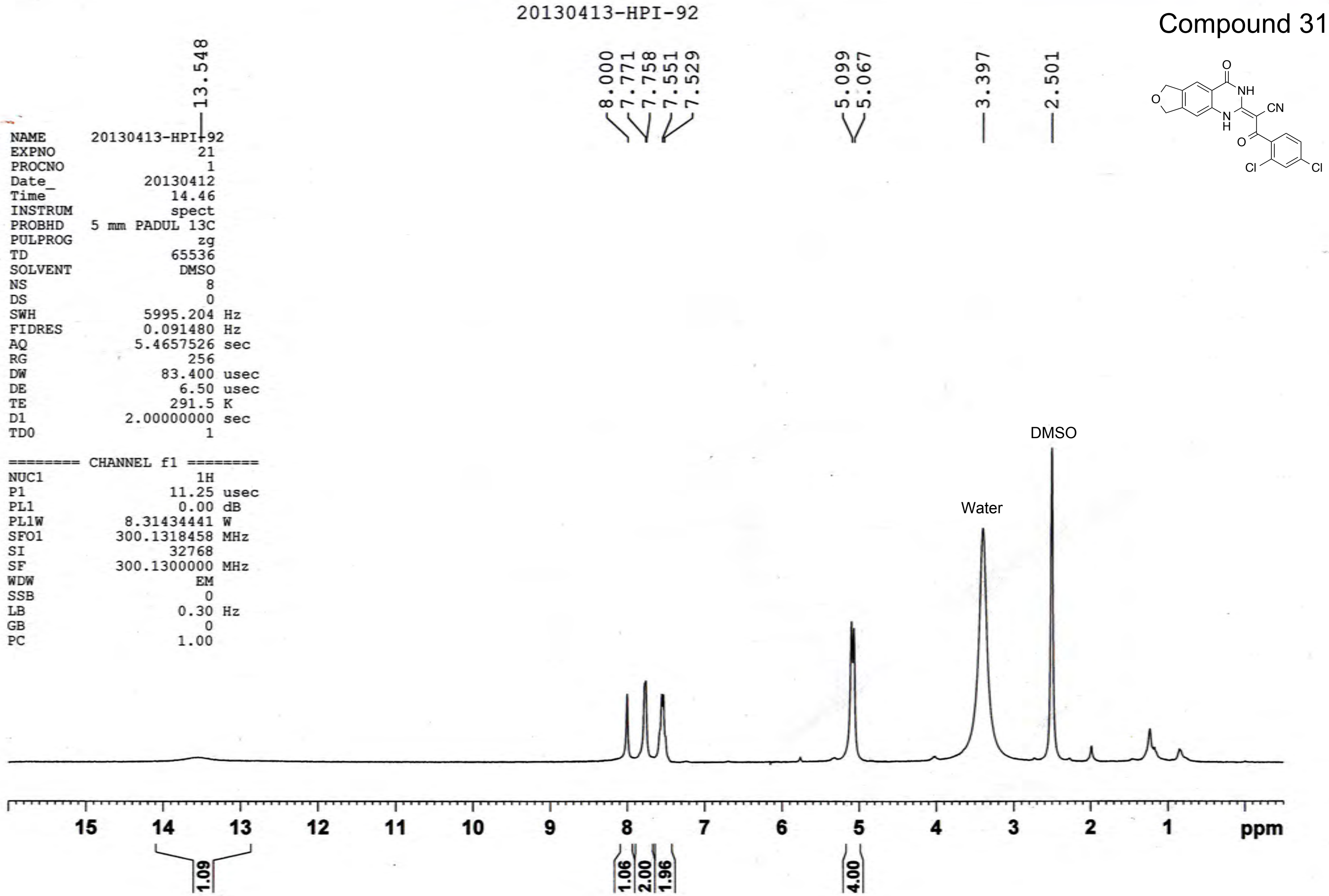

Compound 29
20120505-HPI-076
13.435
13.409
13.402
13.370
Current Data Parameters
NAME 20120505-HPI-076
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20120504
Time 11.03
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 8
DS 2
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894966 sec
RG 175.2
DW 62.400 usec
DE 6.50 usec
TE 300.6 K
D1 1.00000000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 15.00 usec
PLW1 12.00000000 W
SFO1 400.1326008 MHz
F2 - Processing parameters
SI 65536
SF 400.1300037 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
7.746
7.741
7.556
7.552
7.536
7.531
7.507
7.487
7.434
7.346
4.405
4.398
4.340
4.333
3.330
2.504
2.500
2.496
Water
DMSO
TMS
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
1.00
1.00
2.00
0.96
1.04
2.04
2.07
O
NH
CN
N
H
O
Cl
Cl

Compound 30

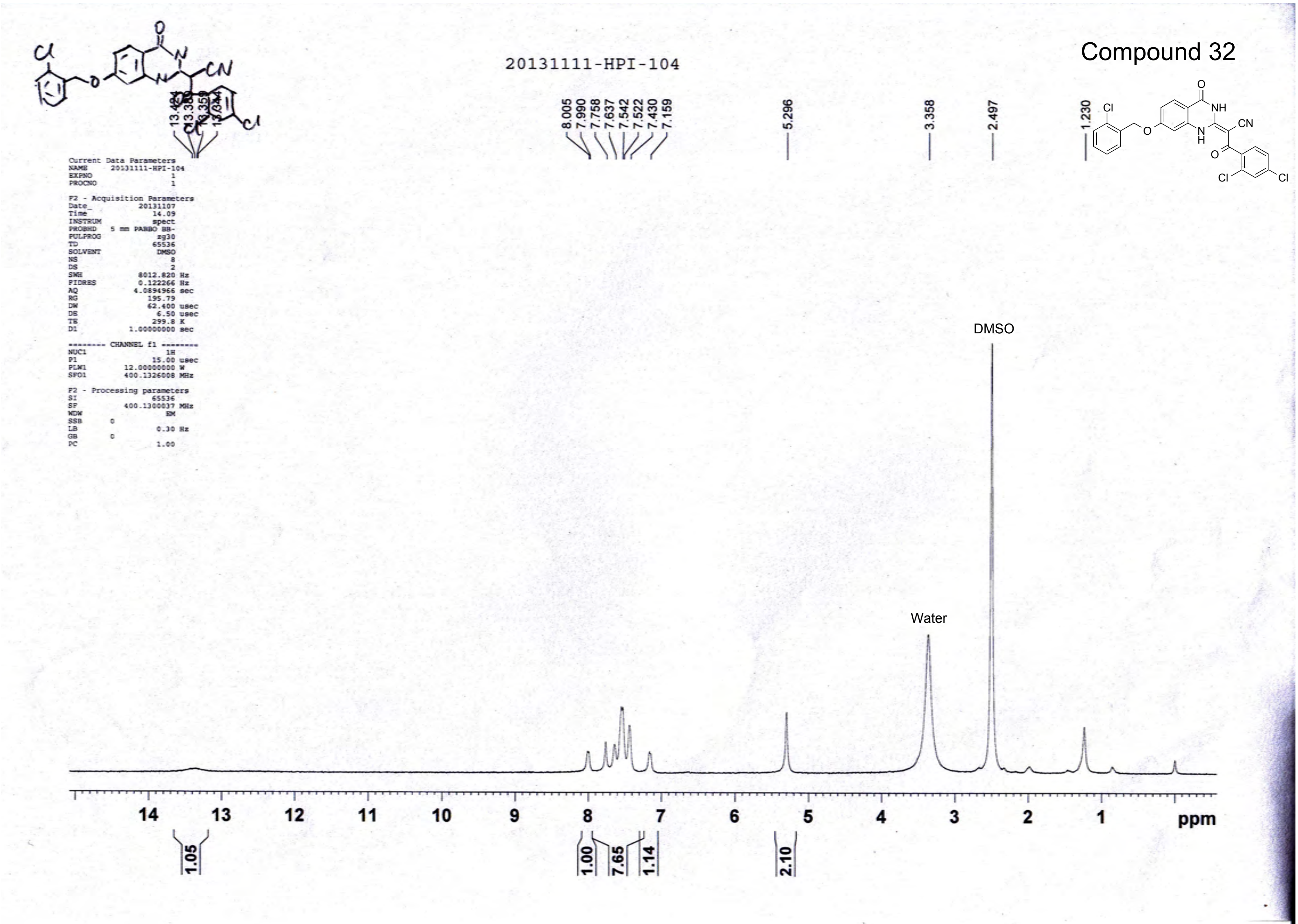

O
NH
CN
N
H
O
Cl
Cl

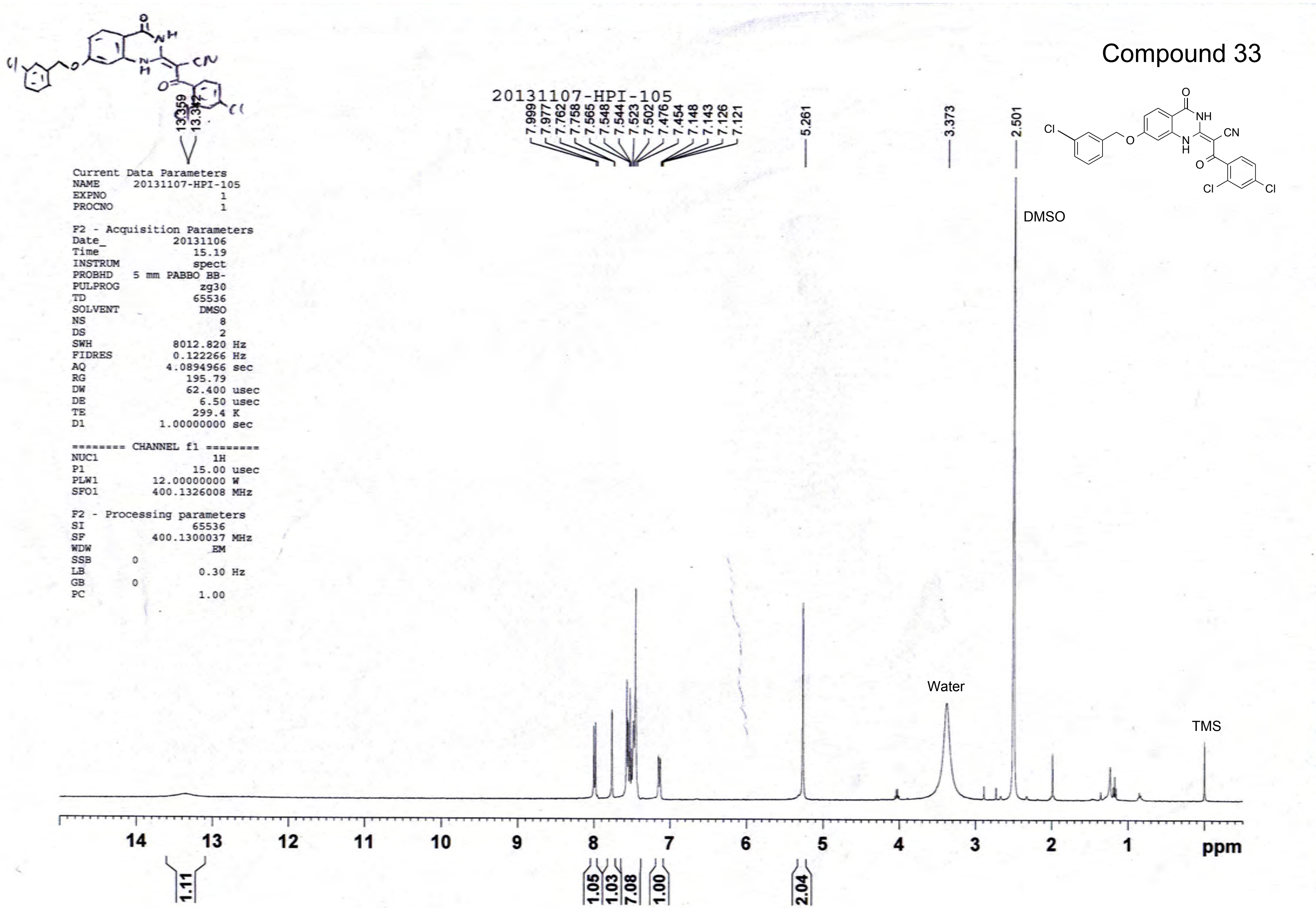

HPI-91
NAME HPI-91
EXPNO 21
PROCNO 1
Date_ 20130415
Time 8.57
INSTRUM spect
PROBHD 5 mm PADUL 13C
PULPROG zg
TD 65536
SOLVENT DMSO
NS 8
DS 0
SWH 5995.204 Hz
FIDRES 0.091480 Hz
AQ 5.4657526 sec
RG 362
DW 83.400 usec
DE 6.50 usec
TE 291.6 K
D1 2.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 11.25 usec
PL1 0.00 dB
PL1W 8.31434441 W
SFO1 300.1318458 MHz
SI 32768
SF 300.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
13.459
7.888
7.776
7.770
7.707
7.579
7.574
7.552
7.546
7.527
7.499
5.764
3.378
3.017
2.992
2.958
2.934
2.502
2.098
2.073
2.049
1.232
Water
DMSO
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
0.65
1.00
1.86
2.05
4.42
2.23

Compound 31

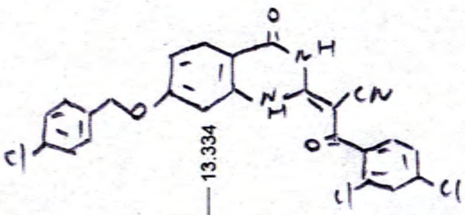


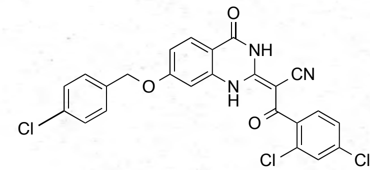
O
NH
CN
O
N
H
O
Cl
Cl
20130413-HPI-92
13.548
8.000
7.771
7.758
7.551
7.529
5.099
5.067
3.397
2.501
NAME 20130413-HPI-92
EXPNO 21
PROCNO 1
Date_ 20130412
Time 14.46
INSTRUM spect
PROBHD 5 mm PADUL 13C
PULPROG zg
TD 65536
SOLVENT DMSO
NS 8
DS 0
SWH 5995.204 Hz
FIDRES 0.091480 Hz
AQ 5.4657526 sec
RG 256
DW 83.400 usec
DE 6.50 usec
TE 291.5 K
D1 2.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 11.25 usec
PL1 0.00 dB
PL1W 8.31434441 W
SFO1 300.1318458 MHz
SI 32768
SF 300.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
DMSO
Water
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
1.09
1.06
2.00
1.96
4.00


Compound 32
20131111-HPI-104
13.401
13.380
13.359
13.344
8.005
7.990
7.758
7.637
7.542
7.522
7.430
7.159
5.296
3.358
2.497
1.230
Current Data Parameters
NAME 20131111-HPI-104
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20131107
Time 14.09
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 8
DS 2
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894966 sec
RG 195.79
DW 62.400 usec
DE 6.50 usec
TE 299.8 K
D1 1.00000000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 15.00 usec
PLW1 12.00000000 W
SFO1 400.1326008 MHz
F2 - Processing parameters
SI 65536
SF 400.1300037 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
DMSO
Water
ppm
1.05
1.00
7.65
1.14
2.10


Compound 33
20131107-HPI-105
13.359
13.342
7.999
7.977
7.762
7.758
7.565
7.548
7.544
7.523
7.502
7.476
7.454
7.148
7.143
7.126
7.121
5.261
3.373
2.501
Current Data Parameters
NAME 20131107-HPI-105
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20131106
Time 15.19
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 8
DS 2
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894966 sec
RG 195.79
DW 62.400 usec
DE 6.50 usec
TE 299.4 K
D1 1.00000000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 15.00 usec
PLW1 12.00000000 W
SFO1 400.1326008 MHz
F2 - Processing parameters
SI 65536
SF 400.1300037 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
DMSO
Water
TMS
ppm
1.11
1.05
1.03
7.08
1.00
2.04

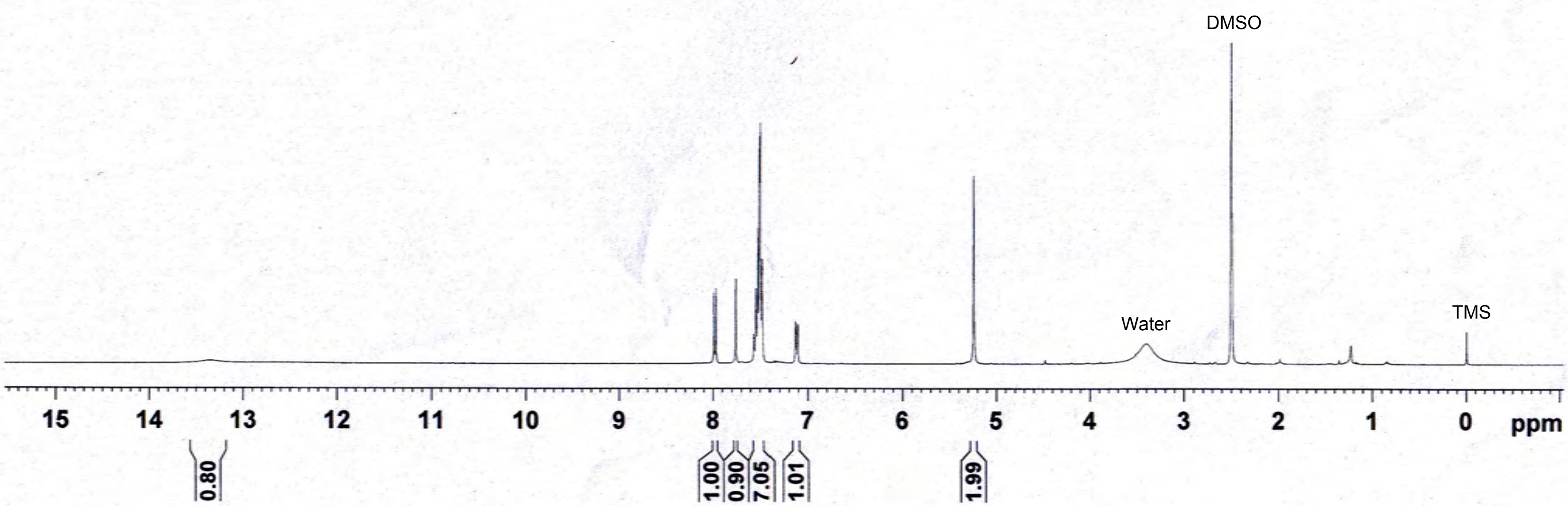
Compound 34



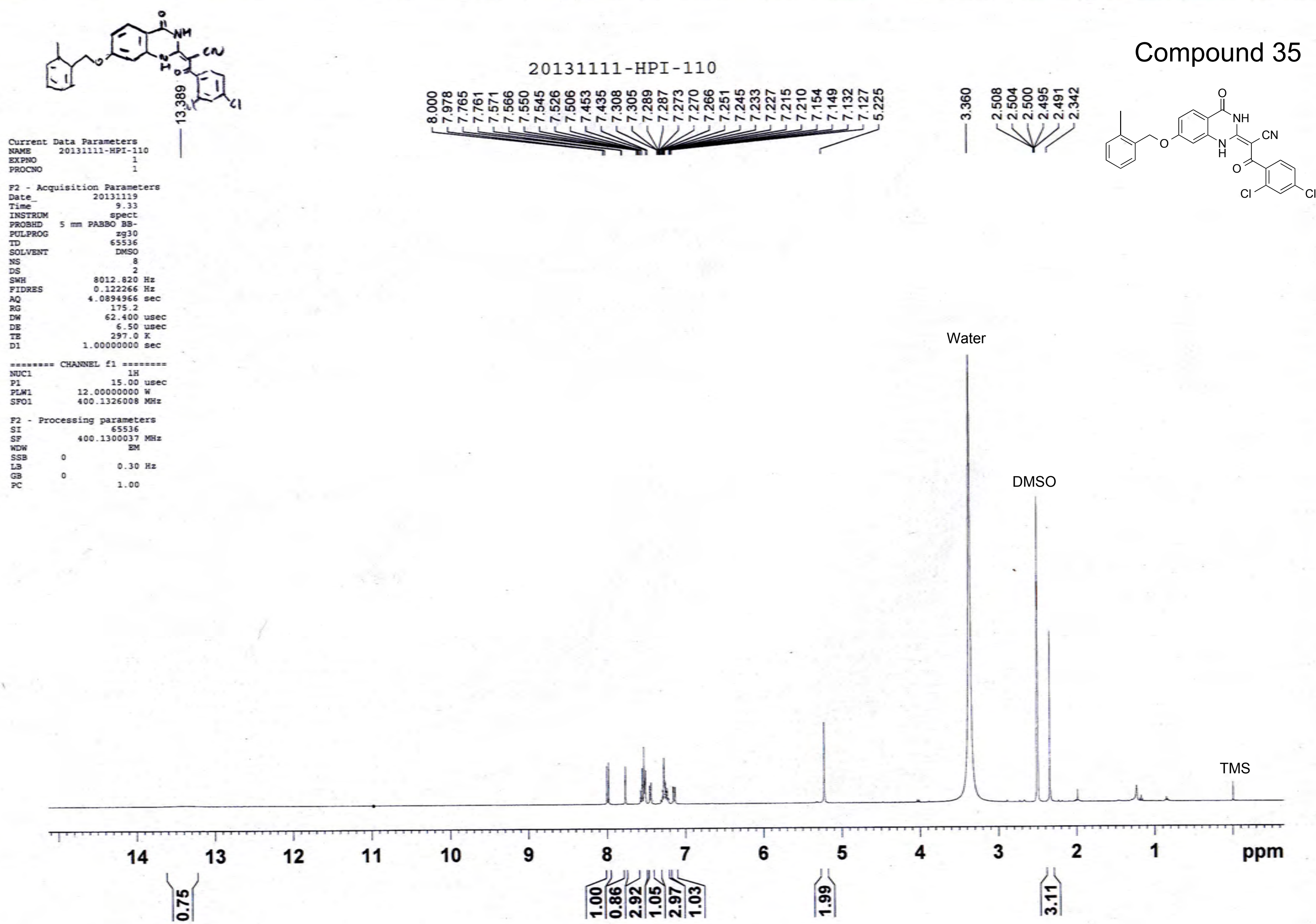


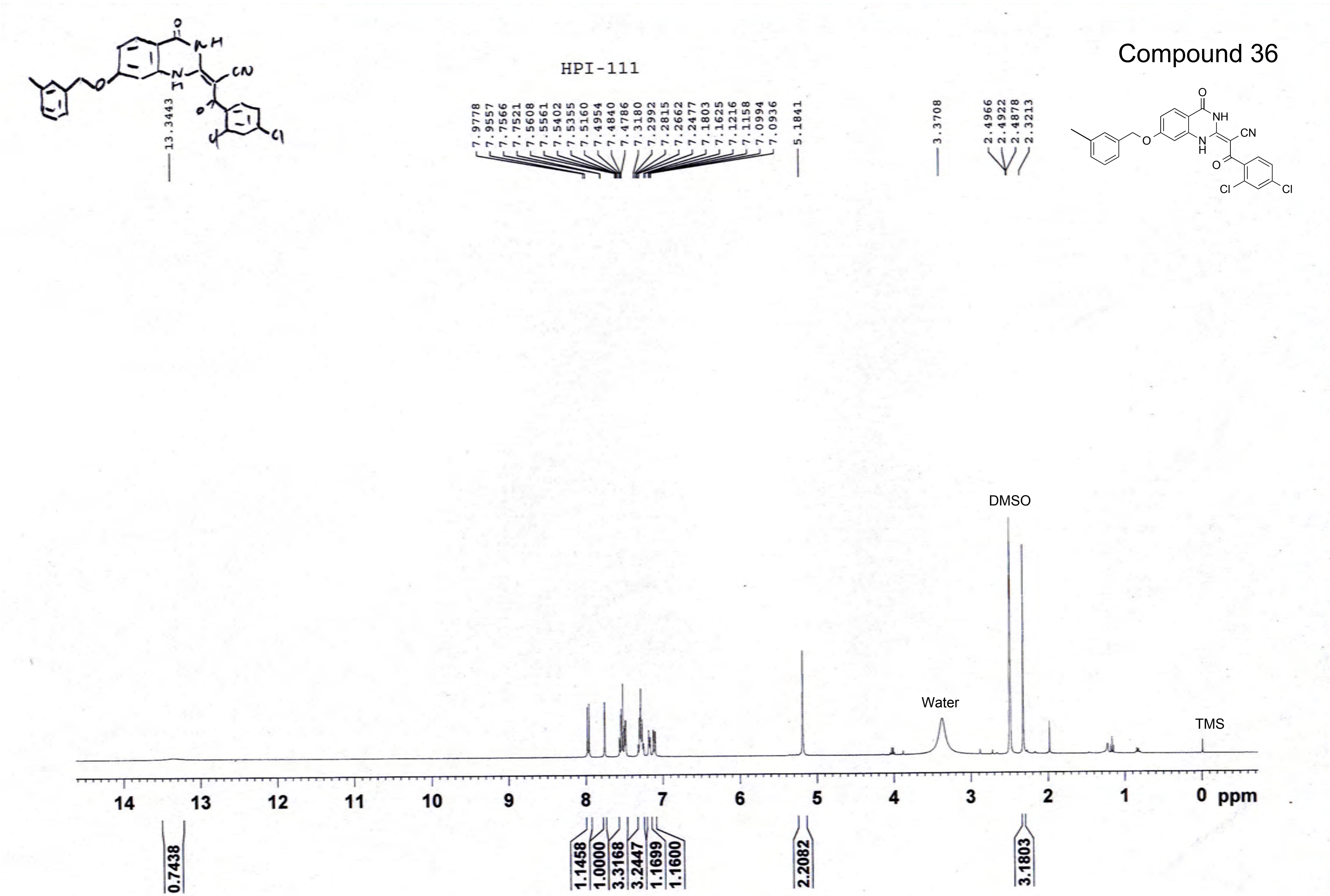
20131109-HPI-106

```
Current Data Parameters
NAME        20131109-HPI-106
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_             20131107
Time                 14.03
INSTRUM              spect
PROBHD   5 mm PABBO BB-
PULPROG               zg30
TD                   65536
SOLVENT               DMSO
NS                       8
DS                       2
SWH               8012.820 Hz
FIDRES            0.122266 Hz
AQ               4.0894966 sec
RG                  195.79
DW                  62.400 usec
DE                    6.50 usec
TE                   299.9 K
D1              1.00000000 sec

======== CHANNEL f1 ========
NUC1                    1H
P1                   15.00 usec
PLW1           12.00000000 W
SFO1          400.1326008 MHz

F2 - Processing parameters
SI                   65536
SF            400.1300037 MHz
WDW                     EM
SSB      0
LB                    0.30 Hz
GB       0
PC                    1.00
```

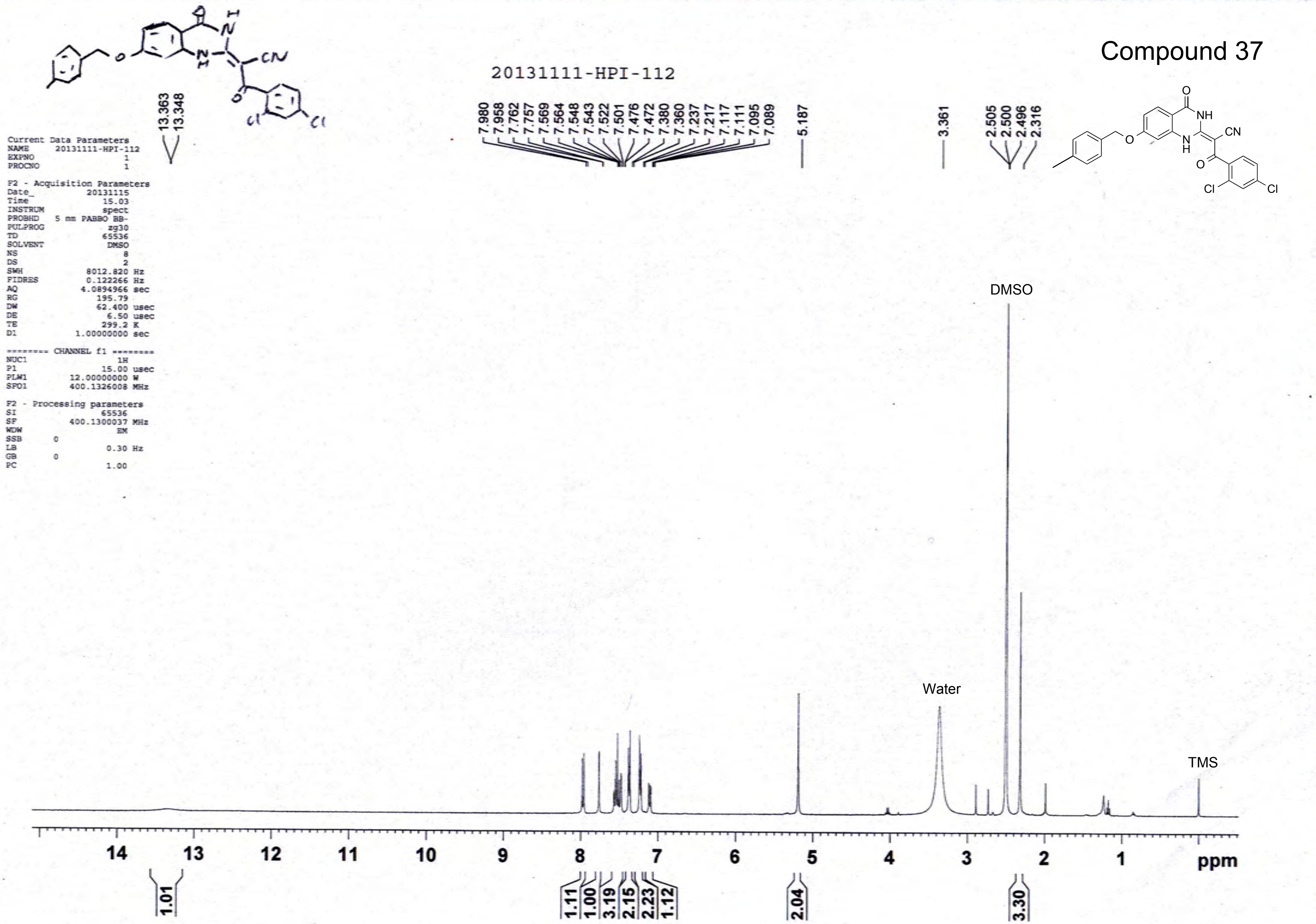


Compound 35

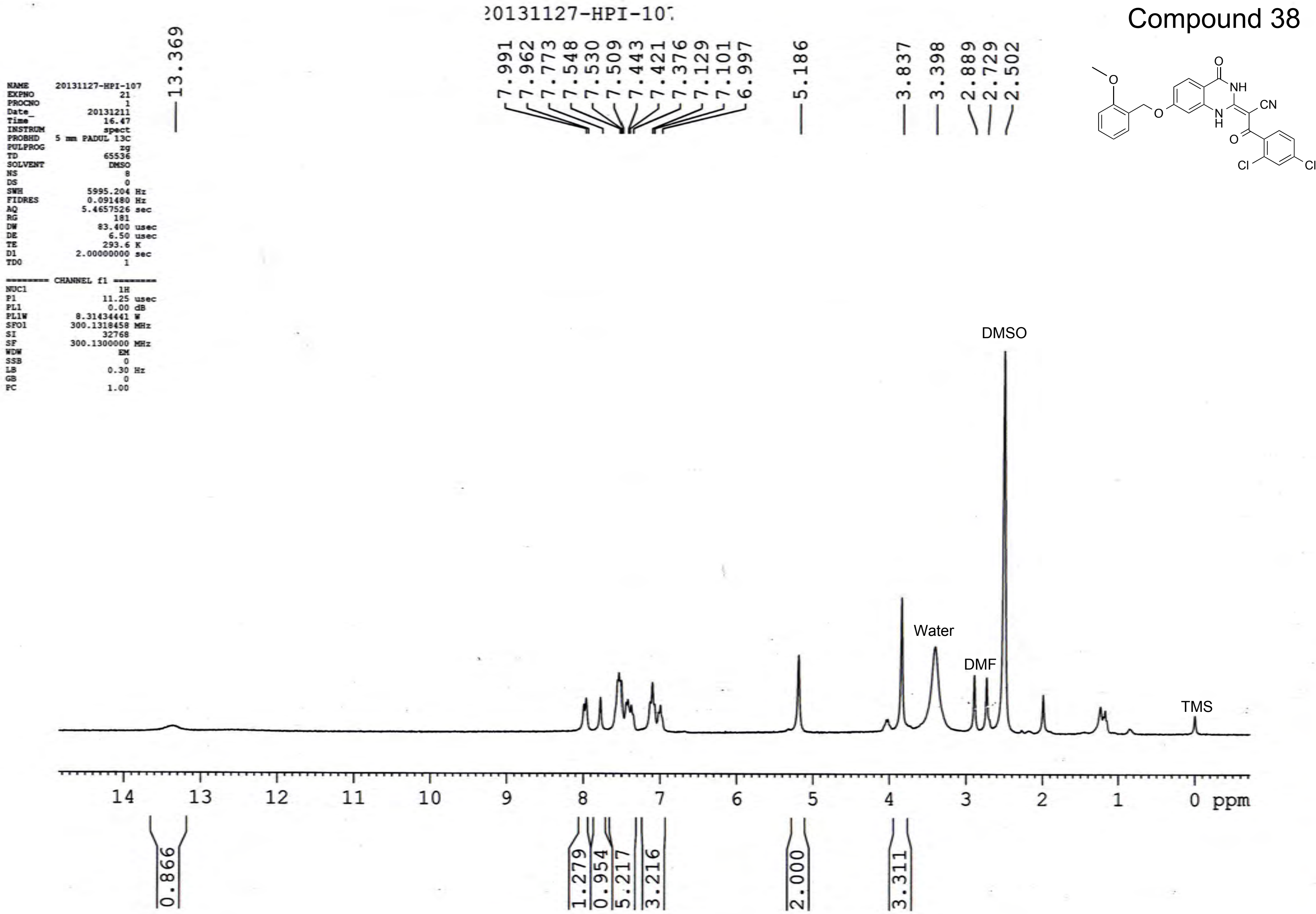

20131111-HPI-110
13.389
8.000
7.978
7.765
7.761
7.571
7.566
7.550
7.545
7.526
7.506
7.453
7.435
7.308
7.305
7.289
7.287
7.273
7.270
7.266
7.251
7.245
7.233
7.227
7.215
7.210
7.154
7.149
7.132
7.127
5.225
3.360
2.508
2.504
2.500
2.495
2.491
2.342
Current Data Parameters
NAME 20131111-HPI-110
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20131119
Time 9.33
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 8
DS 2
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894966 sec
RG 175.2
DW 62.400 usec
DE 6.50 usec
TE 297.0 K
D1 1.00000000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 15.00 usec
PLW1 12.00000000 W
SFO1 400.1326008 MHz
F2 - Processing parameters
SI 65536
SF 400.1300037 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
Water
DMSO
TMS
14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
0.75
1.00
0.86
2.92
1.05
2.97
1.03
1.99
3.11
O
NH
CN
O
N
H
O
Cl
Cl

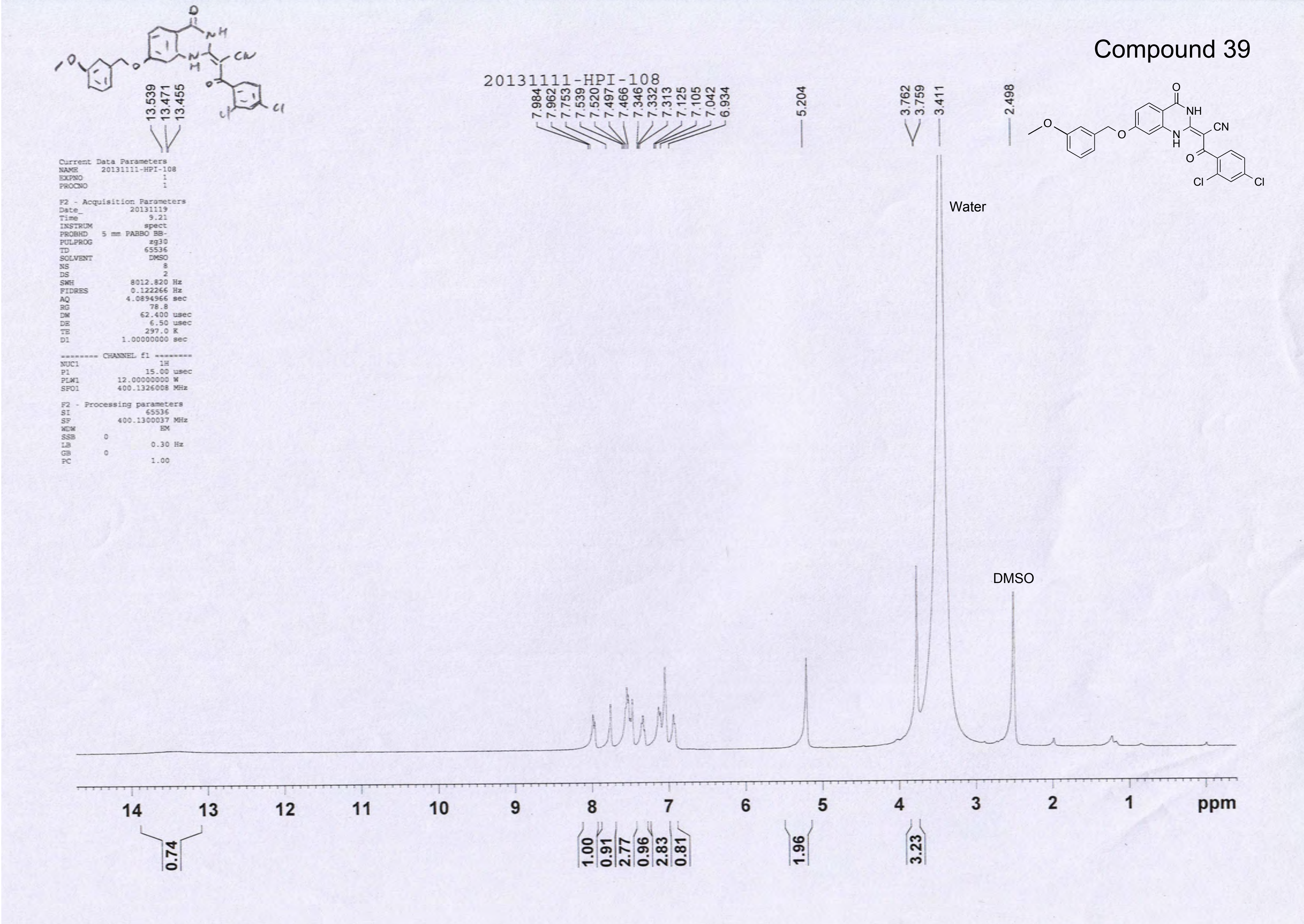

Compound 36
HPI-111
13.3443
7.9778
7.9557
7.7566
7.7521
7.5608
7.5561
7.5402
7.5355
7.5160
7.4954
7.4840
7.4786
7.3180
7.2992
7.2815
7.2662
7.2477
7.1803
7.1625
7.1216
7.1158
7.0994
7.0936
5.1841
3.3708
2.4966
2.4922
2.4878
2.3213
O
NH
CN
N
H
O
O
Cl
Cl
DMSO
Water
TMS
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0 ppm
0.7438
1.1458
1.0000
3.3168
3.2447
1.1699
1.1600
2.2082
3.1803

Compound 37


20131111-HPI-112
Current Data Parameters
NAME 20131111-HPI-112
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20131115
Time 15.03
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 8
DS 2
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894966 sec
RG 195.79
DW 62.400 usec
DE 6.50 usec
TE 299.2 K
D1 1.00000000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 15.00 usec
PLW1 12.00000000 W
SFO1 400.1326008 MHz
F2 - Processing parameters
SI 65536
SF 400.1300037 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
13.363
13.348
7.980
7.958
7.762
7.757
7.569
7.564
7.548
7.543
7.522
7.501
7.476
7.472
7.380
7.360
7.237
7.217
7.117
7.111
7.095
7.089
5.187
3.361
2.505
2.500
2.496
2.316
DMSO
Water
TMS
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
1.01
1.11
1.00
3.19
2.15
2.23
1.12
2.04
3.30

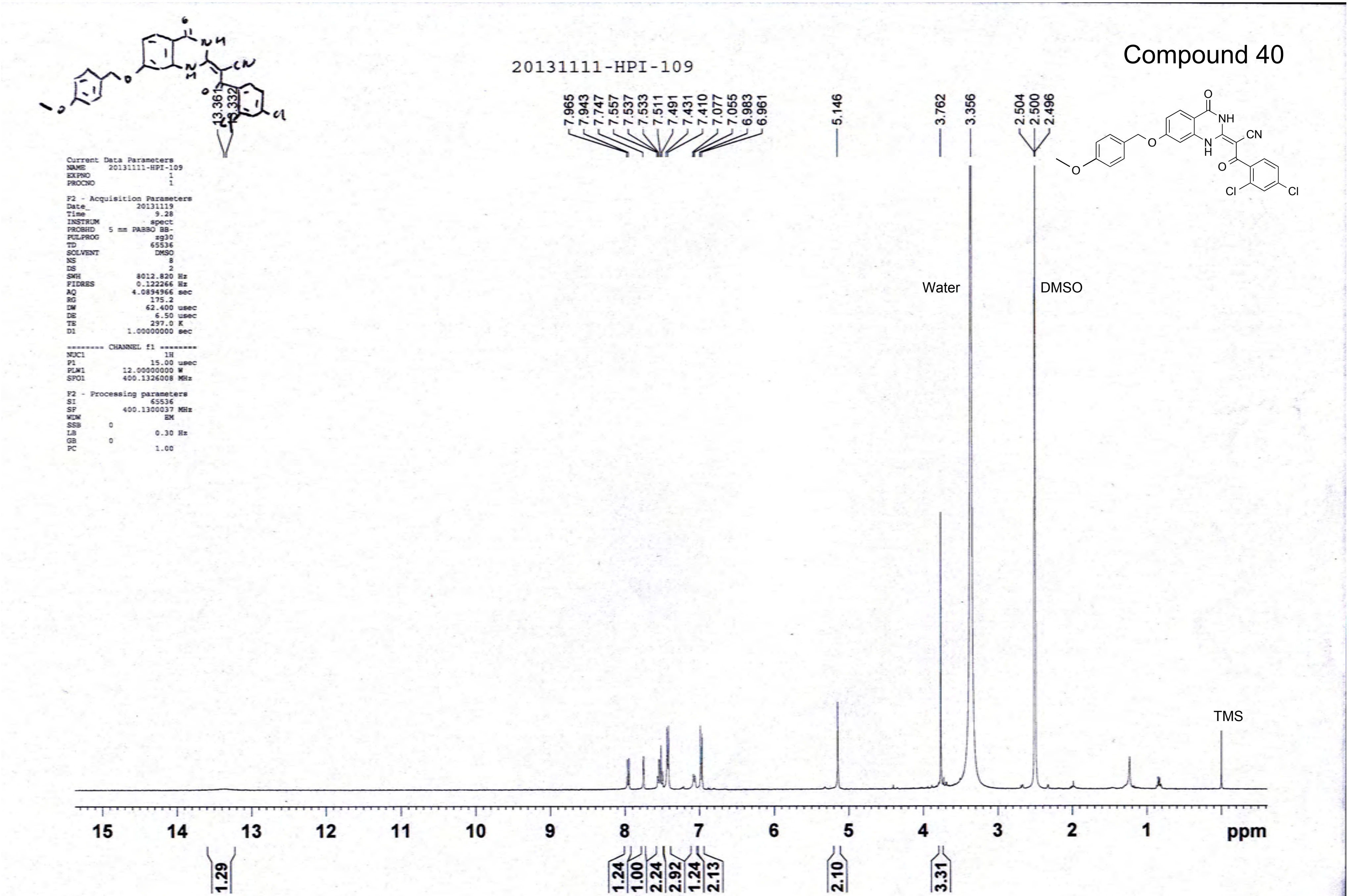
Compound 38


20131127-HPI-107
NAME 20131127-HPI-107
EXPNO 21
PROCNO 1
Date_ 20131211
Time 16.47
INSTRUM spect
PROBHD 5 mm PADUL 13C
PULPROG zg
TD 65536
SOLVENT DMSO
NS 8
DS 0
SWH 5995.204 Hz
FIDRES 0.091480 Hz
AQ 5.4657526 sec
RG 181
DW 83.400 usec
DE 6.50 usec
TE 293.6 K
D1 2.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 11.25 usec
PL1 0.00 dB
PL1W 8.31434441 W
SFO1 300.1318458 MHz
SI 32768
SF 300.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
13.369
7.991
7.962
7.773
7.548
7.530
7.509
7.443
7.421
7.376
7.129
7.101
6.997
5.186
3.837
3.398
2.889
2.729
2.502
DMSO
Water
DMF
TMS
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
0.866
1.279
0.954
5.217
3.216
2.000
3.311
O
NH
CN
N
H
O
O
O
Cl
Cl

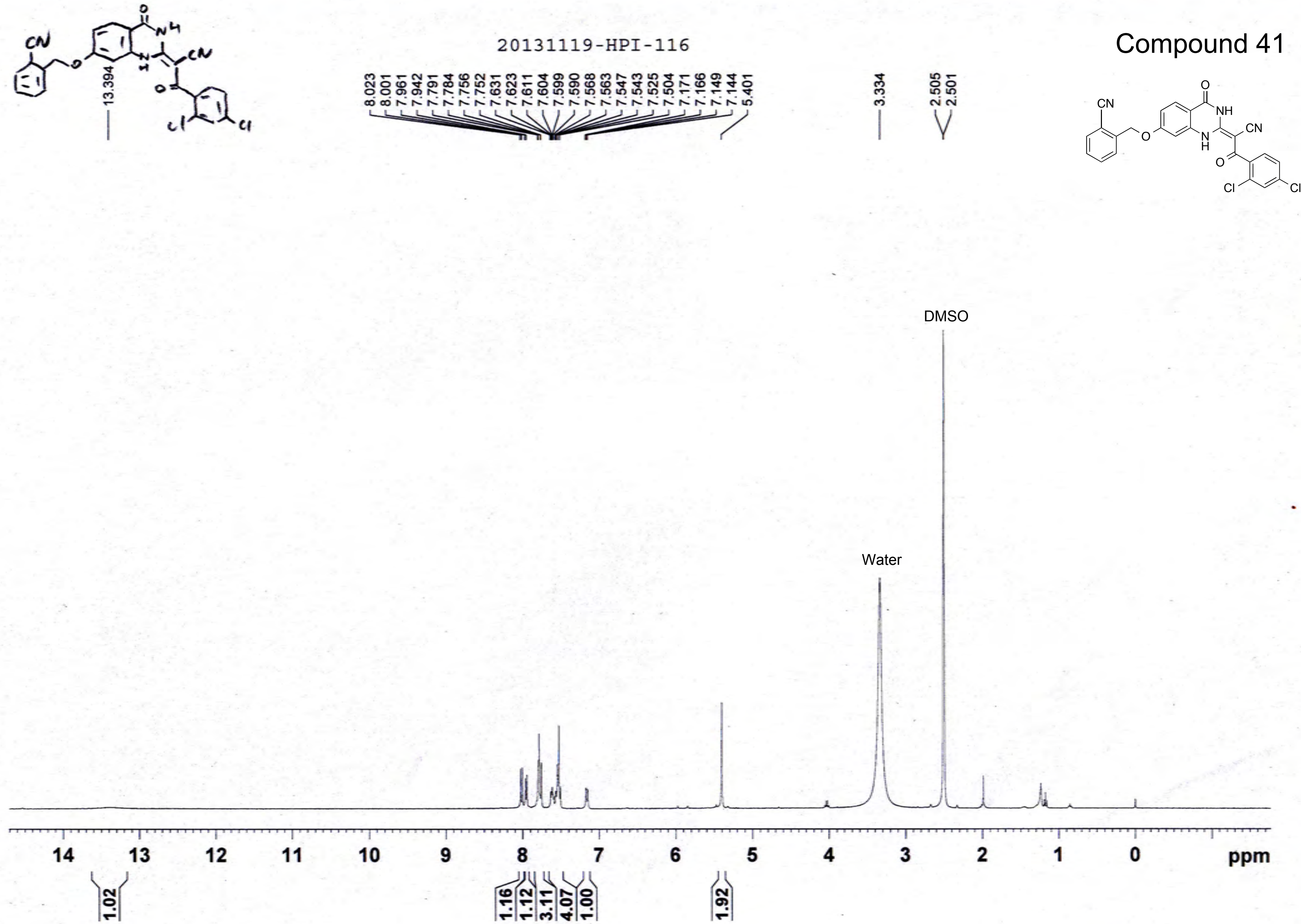


Compound 39
20131111-HPI-108
13.539
13.471
13.455
7.984
7.962
7.753
7.539
7.520
7.497
7.466
7.346
7.332
7.313
7.125
7.105
7.042
6.934
5.204
3.762
3.759
3.411
2.498
Water
DMSO
Current Data Parameters
NAME 20131111-HPI-108
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20131119
Time 9.21
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 8
DS 2
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894966 sec
RG 78.8
DW 62.400 usec
DE 6.50 usec
TE 297.0 K
D1 1.00000000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 15.00 usec
PLW1 12.00000000 W
SFO1 400.1326008 MHz
F2 - Processing parameters
SI 65536
SF 400.1300037 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
0.74
1.00
0.91
2.77
0.96
2.83
0.81
1.96
3.23

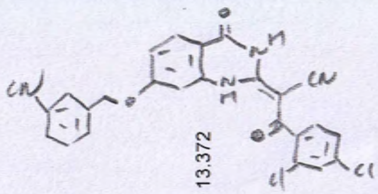
Compound 40


20131111-HPI-109
7.965
7.943
7.747
7.557
7.537
7.533
7.511
7.491
7.431
7.410
7.077
7.055
6.983
6.961
5.146
3.762
3.356
2.504
2.500
2.496
13.361
13.332
Current Data Parameters
NAME 20131111-HPI-109
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20131119
Time 9.28
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 8
DS 2
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894966 sec
RG 175.2
DW 62.400 usec
DE 6.50 usec
TE 297.0 K
D1 1.00000000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 15.00 usec
PLW1 12.00000000 W
SFO1 400.1326008 MHz
F2 - Processing parameters
SI 65536
SF 400.1300037 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
Water
DMSO
TMS
NH
CN
O
H
N
Cl
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
1.29
1.24
1.00
2.24
2.92
1.24
2.13
2.10
3.31

Compound 41

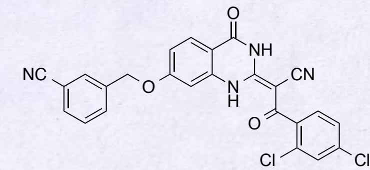


CN
NH
CN
H
Cl
Cl
20131119-HPI-116
13.394
8.023
8.001
7.961
7.942
7.791
7.784
7.756
7.752
7.631
7.623
7.611
7.604
7.599
7.590
7.568
7.563
7.547
7.543
7.525
7.504
7.171
7.166
7.149
7.144
5.401
3.334
2.505
2.501
DMSO
Water
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
1.02
1.16
1.12
3.11
4.07
1.00
1.92

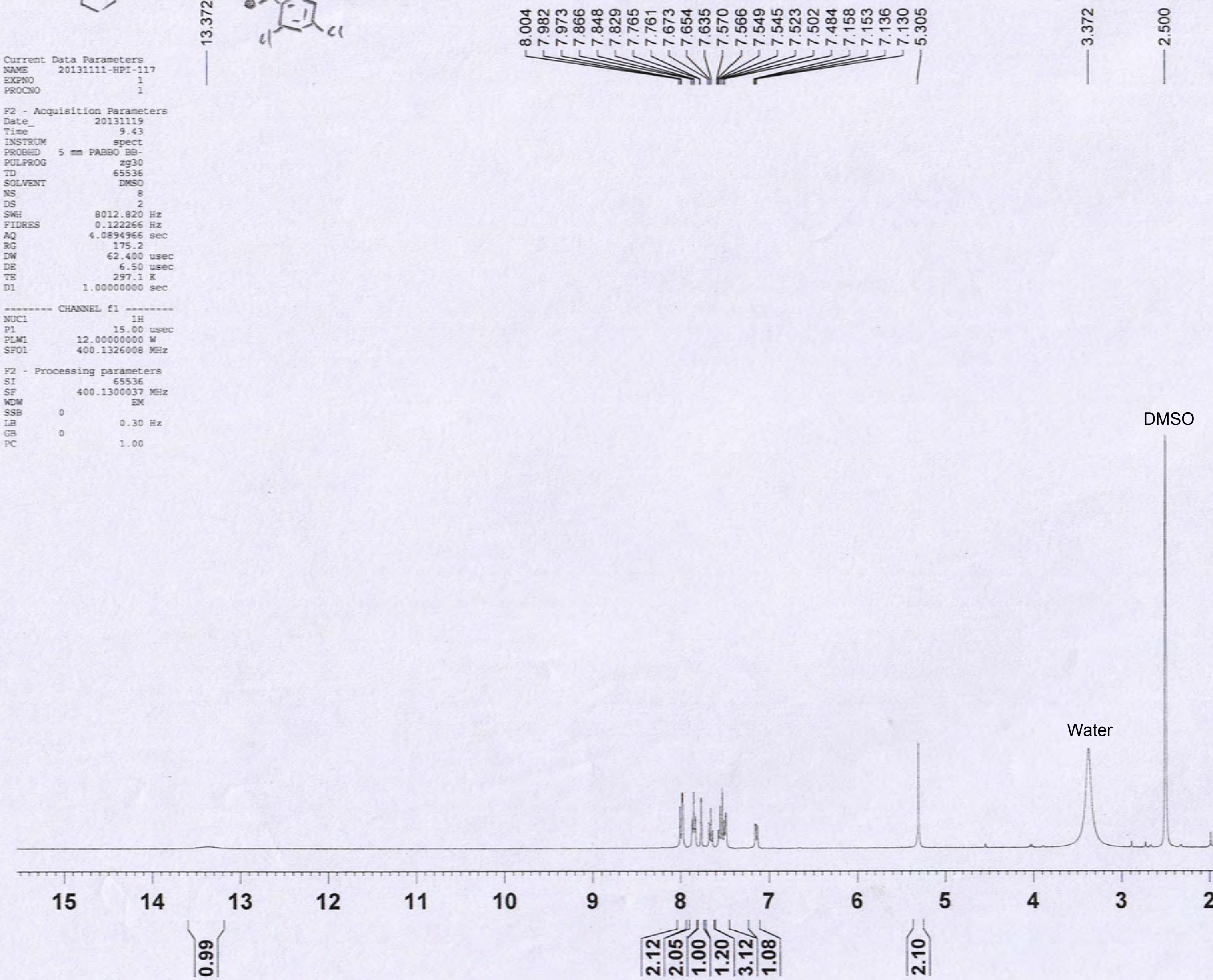


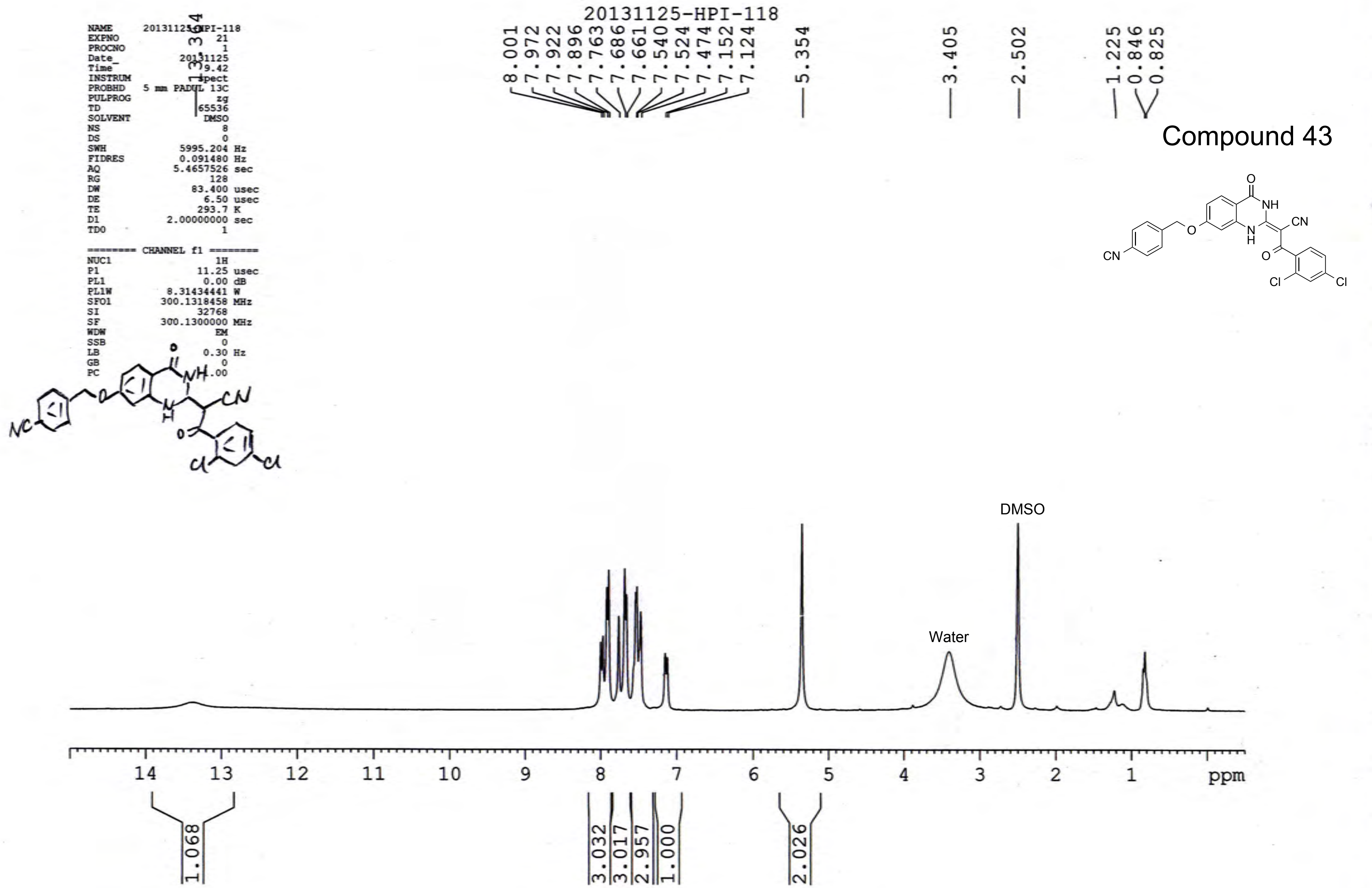
20131111-HPI-117

Compound 42

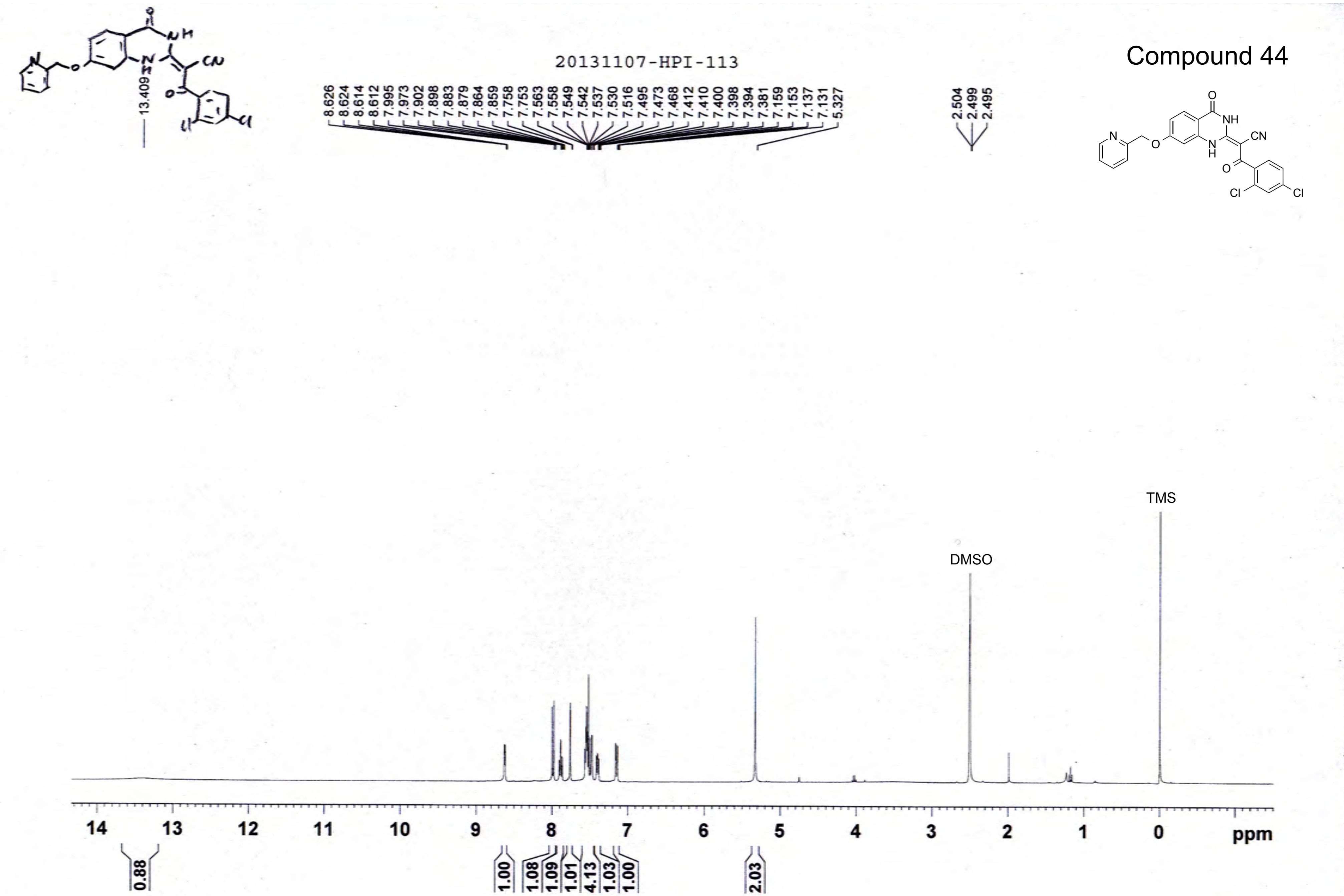



```
Current Data Parameters
NAME      20131111-HPI-117
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_           20131119
Time                9.43
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG             zg30
TD                 65536
SOLVENT             DMSO
NS                     8
DS                     2
SWH             8012.820 Hz
FIDRES          0.122266 Hz
AQ             4.0894966 sec
RG                 175.2
DW                62.400 usec
DE                  6.50 usec
TE                 297.1 K
D1            1.00000000 sec

======== CHANNEL f1 ========
NUC1                  1H
P1                 15.00 usec
PLW1         12.00000000 W
SFO1         400.1326008 MHz

F2 - Processing parameters
SI                 65536
SF           400.1300037 MHz
WDW                   EM
SSB      0
LB                  0.30 Hz
GB       0
PC                  1.00
```

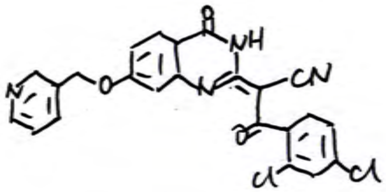



20131125-HPI-118
NAME 20131125-HPI-118
EXPNO 21
PROCNO 1
Date_ 20131125
Time 9.42
INSTRUM spect
PROBHD 5 mm PADUL 13C
PULPROG zg
TD 65536
SOLVENT DMSO
NS 8
DS 0
SWH 5995.204 Hz
FIDRES 0.091480 Hz
AQ 5.4657526 sec
RG 128
DW 83.400 usec
DE 6.50 usec
TE 293.7 K
D1 2.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 11.25 usec
PL1 0.00 dB
PL1W 8.31434441 W
SFO1 300.1318458 MHz
SI 32768
SF 300.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
13.304
8.001
7.972
7.922
7.896
7.763
7.686
7.661
7.540
7.524
7.474
7.152
7.124
5.354
3.405
2.502
1.225
0.846
0.825
Compound 43
O
NH
CN
N
H
O
O
CN
Cl
Cl
NC
Cl
Water
DMSO
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
1.068
3.032
3.017
2.957
1.000
2.026

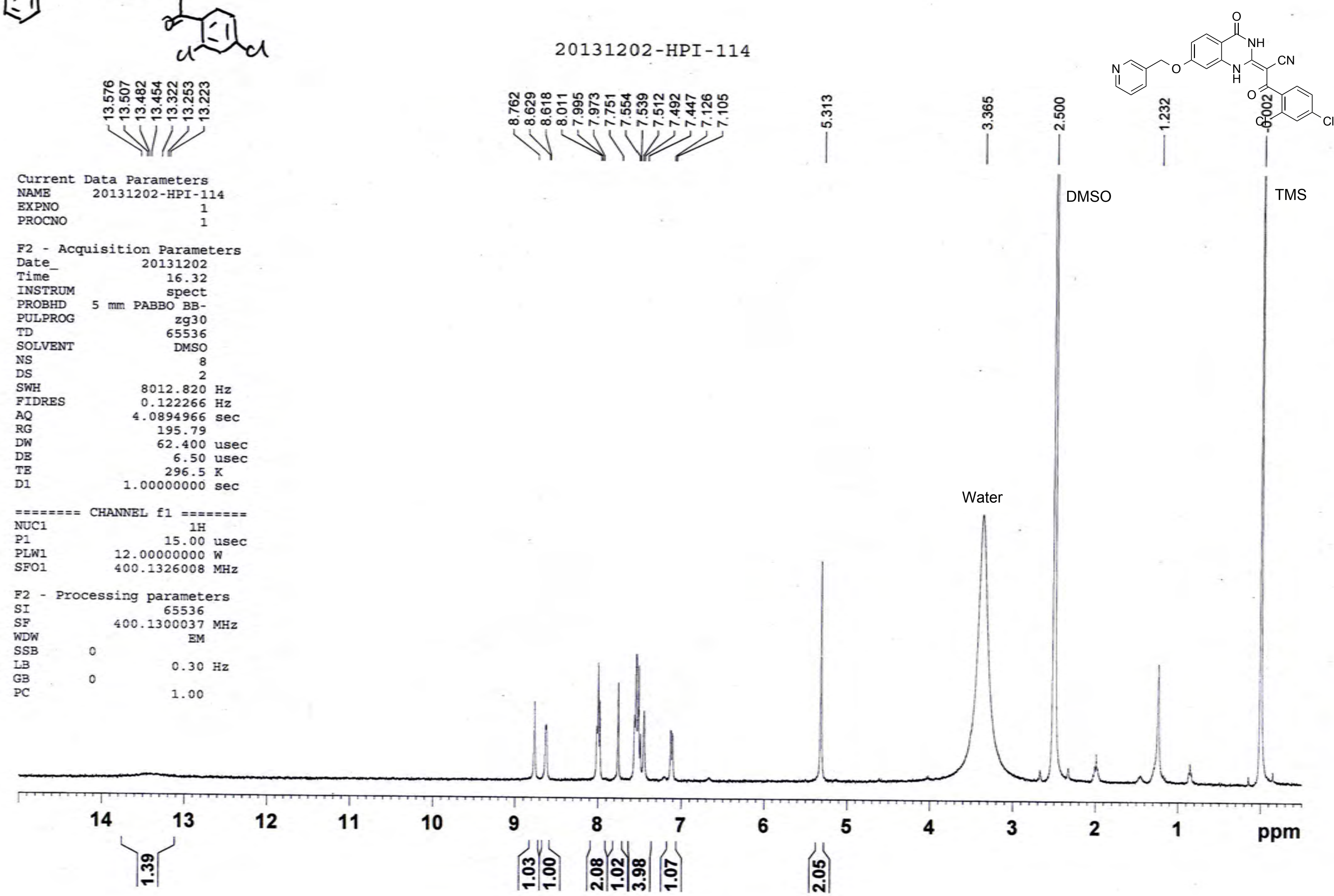
Compound 44



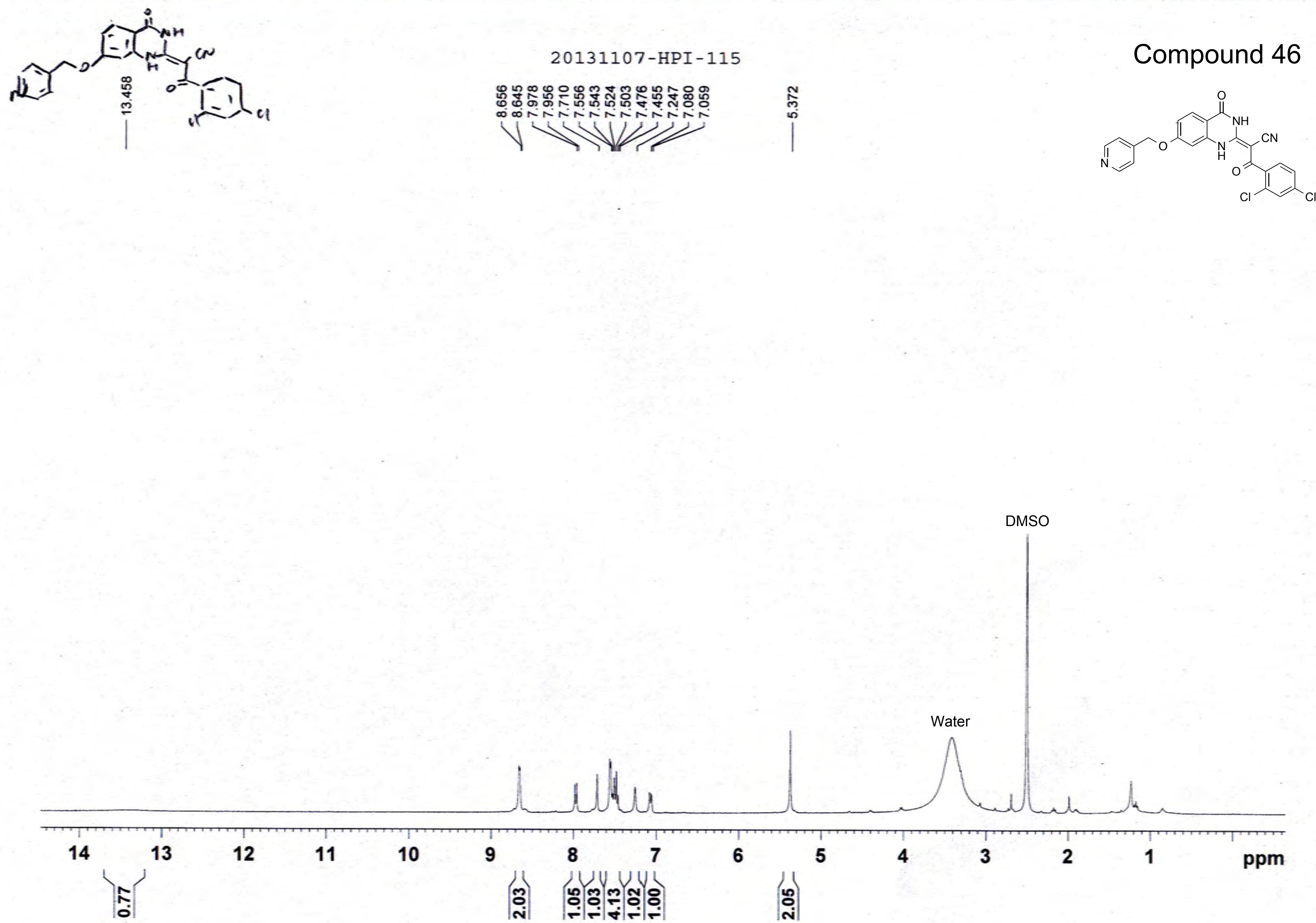
20131107-HPI-113
13.409
8.626
8.624
8.614
8.612
7.995
7.973
7.902
7.898
7.883
7.879
7.864
7.859
7.758
7.753
7.563
7.558
7.549
7.542
7.537
7.530
7.516
7.495
7.473
7.468
7.412
7.410
7.400
7.398
7.394
7.381
7.159
7.153
7.137
7.131
5.327
2.504
2.499
2.495
DMSO
TMS
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
0.88
1.00
1.08
1.09
1.01
4.13
1.03
1.00
2.03



Compound 45

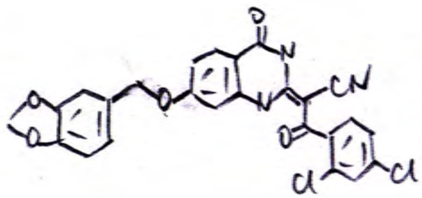

O
NH
CN
N
O
N
H
O
Cl
Cl
20131202-HPI-114
13.576
13.507
13.482
13.454
13.322
13.253
13.223
8.762
8.629
8.618
8.011
7.995
7.973
7.751
7.554
7.539
7.512
7.492
7.447
7.126
7.105
5.313
3.365
2.500
1.232
-0.002
DMSO
TMS
Water
Current Data Parameters
NAME 20131202-HPI-114
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20131202
Time 16.32
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 8
DS 2
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894966 sec
RG 195.79
DW 62.400 usec
DE 6.50 usec
TE 296.5 K
D1 1.00000000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 15.00 usec
PLW1 12.00000000 W
SFO1 400.1326008 MHz
F2 - Processing parameters
SI 65536
SF 400.1300037 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
1.39
1.03
1.00
2.08
1.02
3.98
1.07
2.05

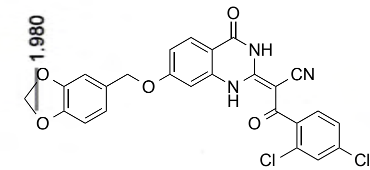
Compound 46


20131107-HPI-115
13.458
8.656
8.645
7.978
7.956
7.710
7.556
7.543
7.524
7.503
7.476
7.455
7.247
7.080
7.059
5.372
Water
DMSO
14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
0.77
2.03
1.05
1.03
4.13
1.02
1.00
2.05

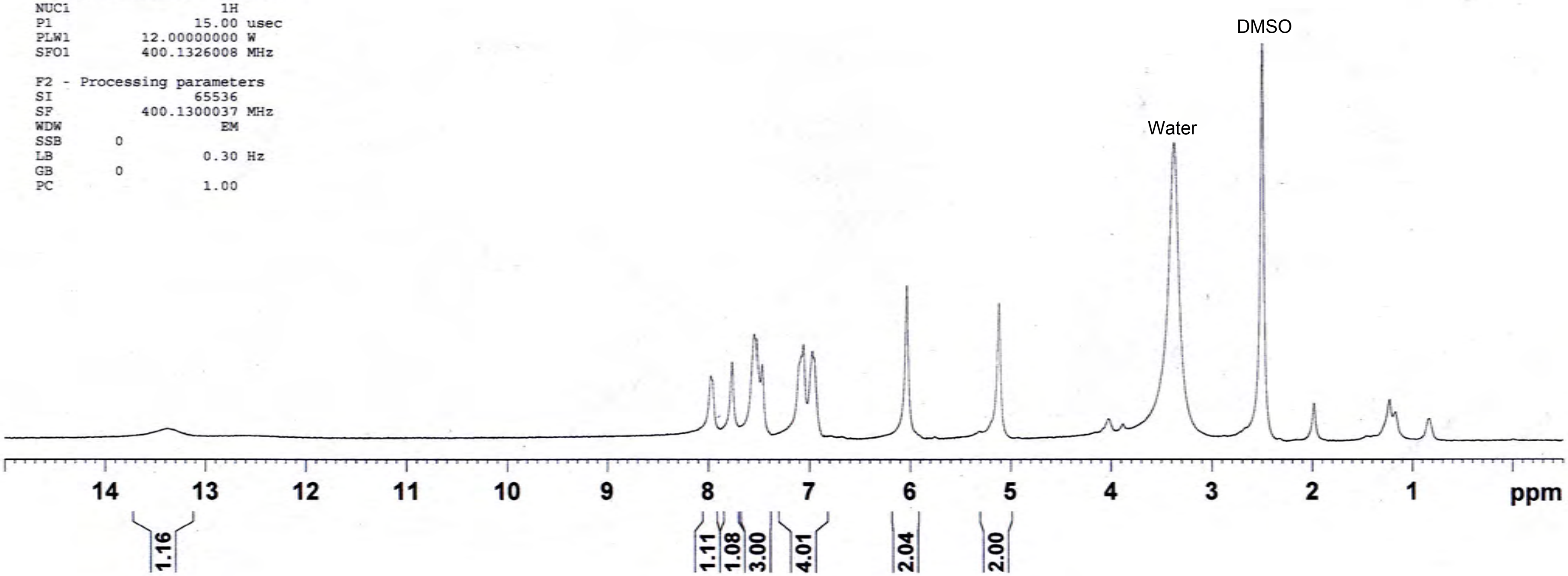
20131126-HPI-121

Compound 47

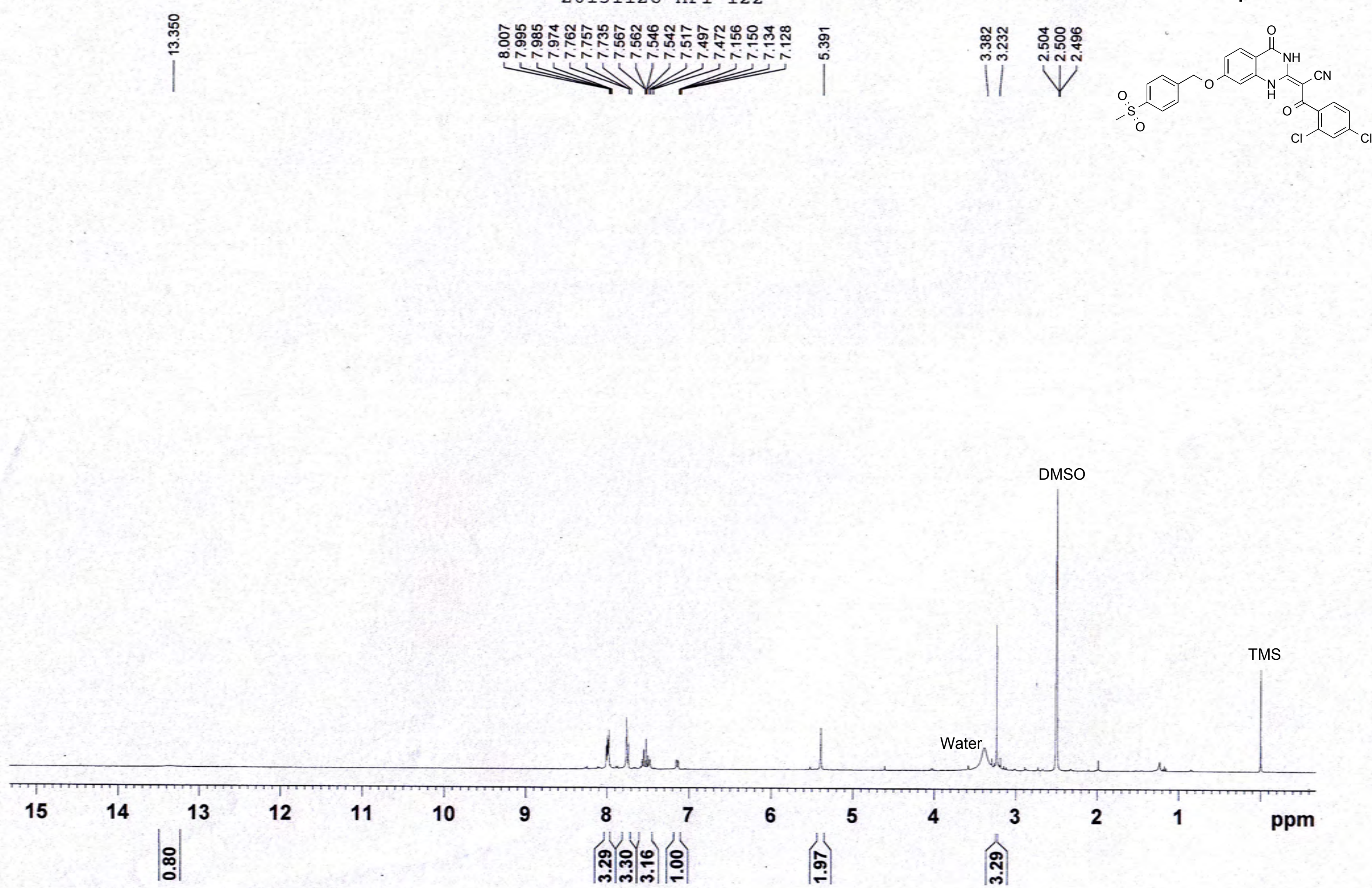





```
Current Data Parameters
NAME      20131126-HPI-121
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_             20131126
Time                 14.56
INSTRUM              spect
PROBHD   5 mm PABBO BB-
PULPROG               zg30
TD                   65536
SOLVENT               DMSO
NS                       8
DS                       2
SWH               8012.820 Hz
FIDRES            0.122266 Hz
AQ               4.0894966 sec
RG                   175.2
DW                  62.400 usec
DE                    6.50 usec
TE                   297.3 K
D1              1.00000000 sec

======== CHANNEL f1 ========
NUC1                    1H
P1                   15.00 usec
PLW1           12.00000000 W
SFO1           400.1326008 MHz

F2 - Processing parameters
SI                   65536
SF             400.1300037 MHz
WDW                     EM
SSB      0
LB                    0.30 Hz
GB       0
PC                    1.00
```



Compound 48



20131126-HPI-122
13.350
8.007
7.995
7.985
7.974
7.762
7.757
7.735
7.567
7.562
7.546
7.542
7.517
7.497
7.472
7.156
7.150
7.134
7.128
5.391
3.382
3.232
2.504
2.500
2.496
O
NH
CN
O
N
H
O
O
S
O
Cl
Cl
DMSO
TMS
Water
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
0.80
3.29
3.30
3.16
1.00
1.97
3.29