Supporting Information

Comparison of the deacylase and deacetylase activity of zinc-dependent HDACs

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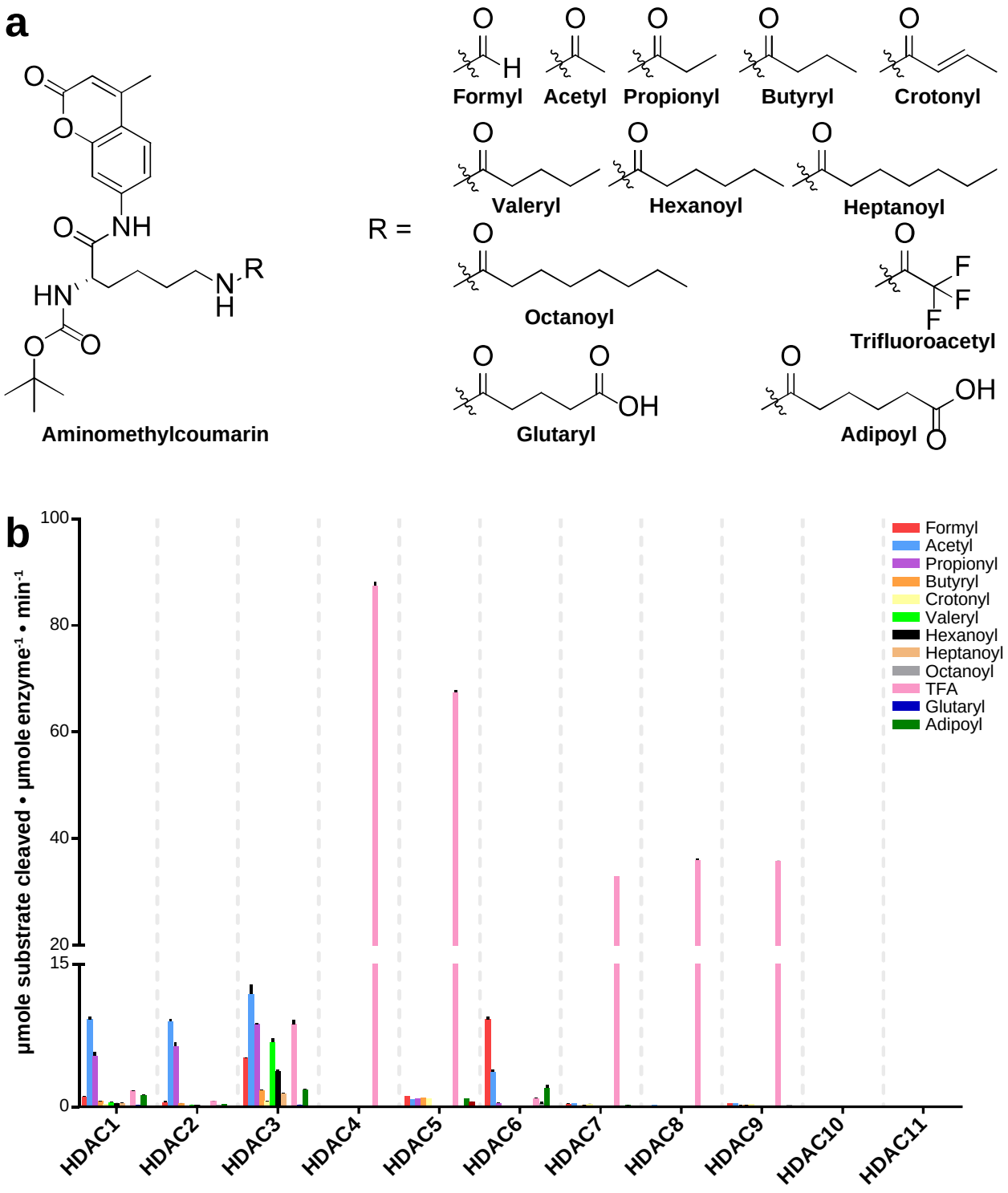
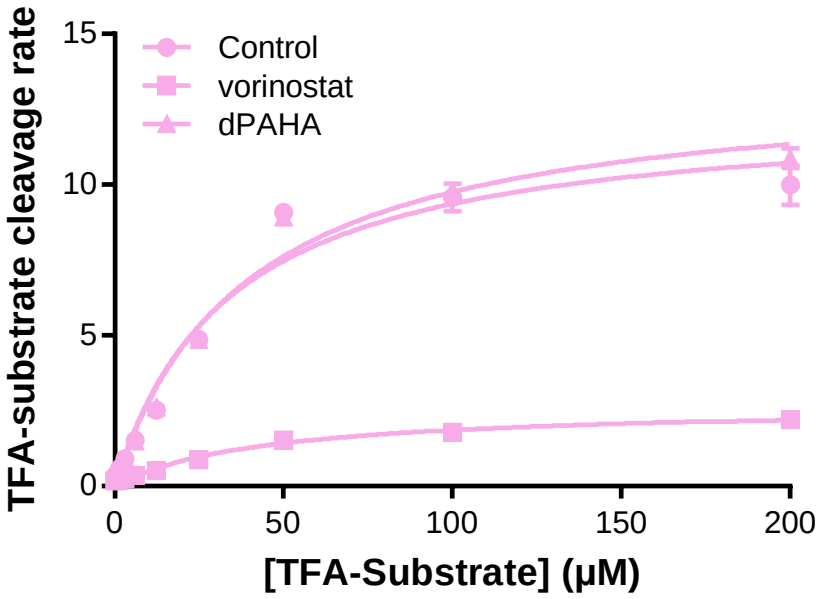
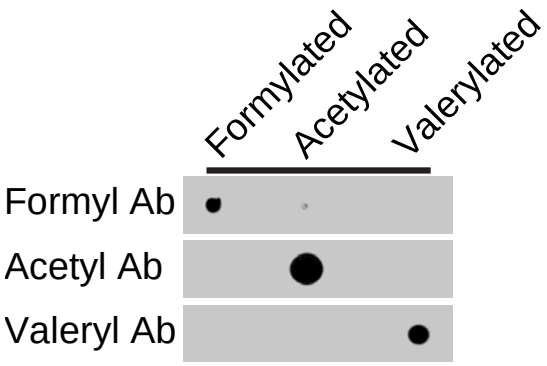


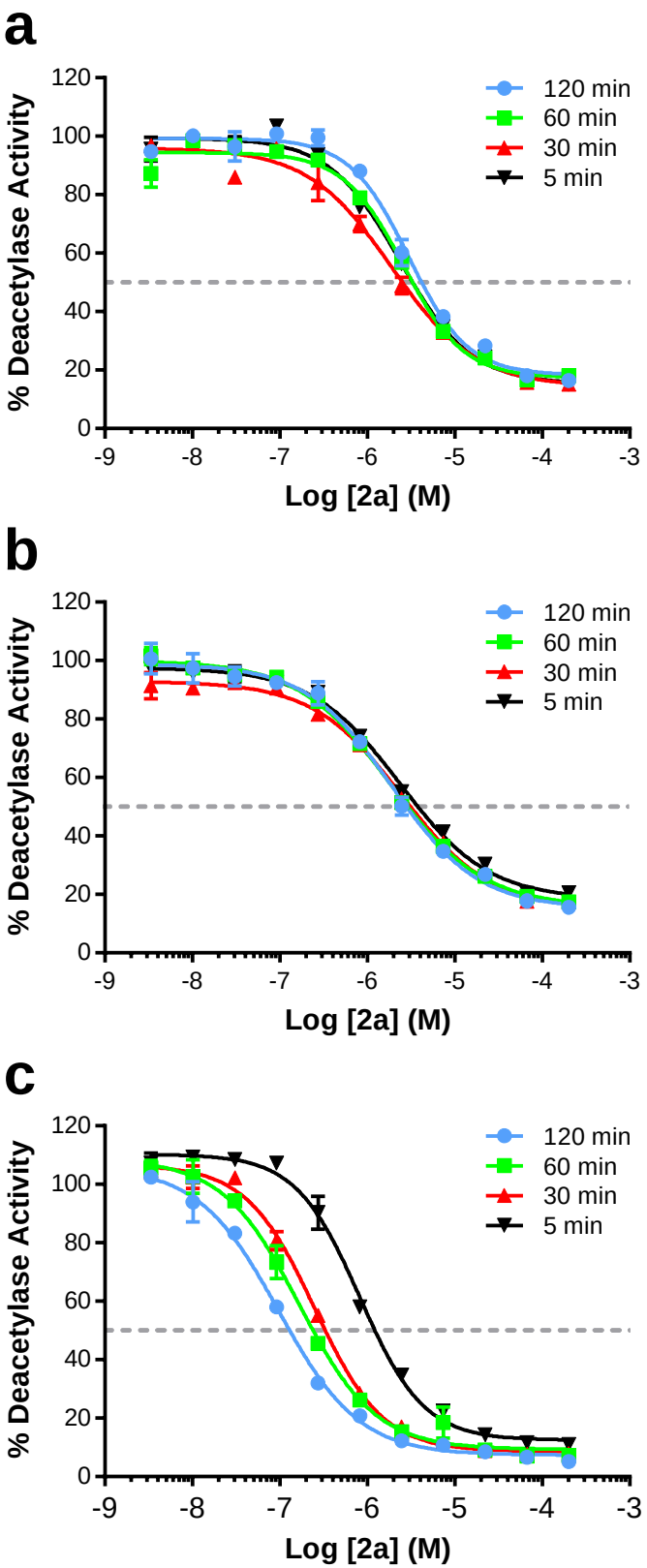
Supplementary Figure 1 a) Chemical structures of 12 acyl-based substrates synthesized for kinetic profiling purposes. **b)** All synthesized acyl-substrate data against zinc-dependent HDAC enzymes. n = 3; error bars are S.E.M.



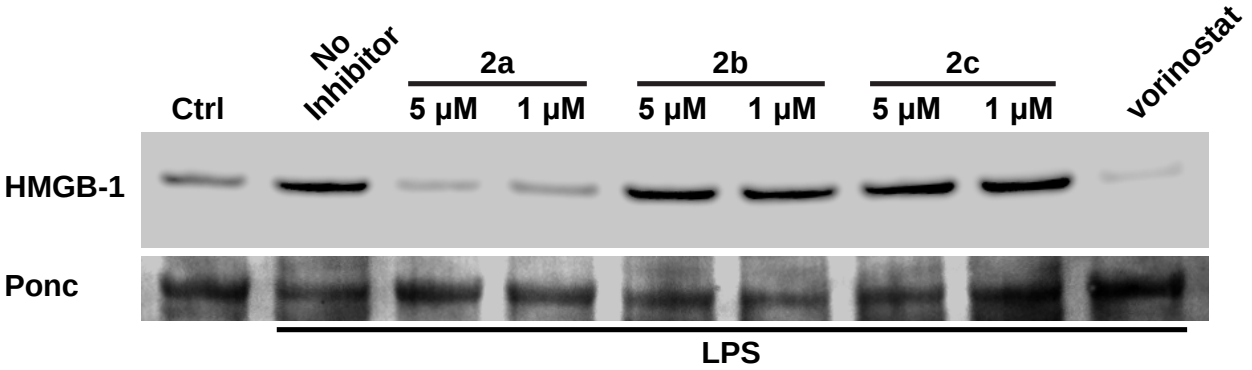
Supplementary Figure 2 V_{max} comparison of HDAC3 with TFA-substrate. Y-axis units: nM · min^{-1}. 1 μM for vorinostat and dPAHA. 2 hour incubation with inhibitors, 2 hour incubation with substrate. Dunnett's multiple comparisons test yields p-value of 0.6208 comparing dPAHA to control. p-value of 0.0002 comparing vorinostat to control. n = 3; error bars are S.E.M.



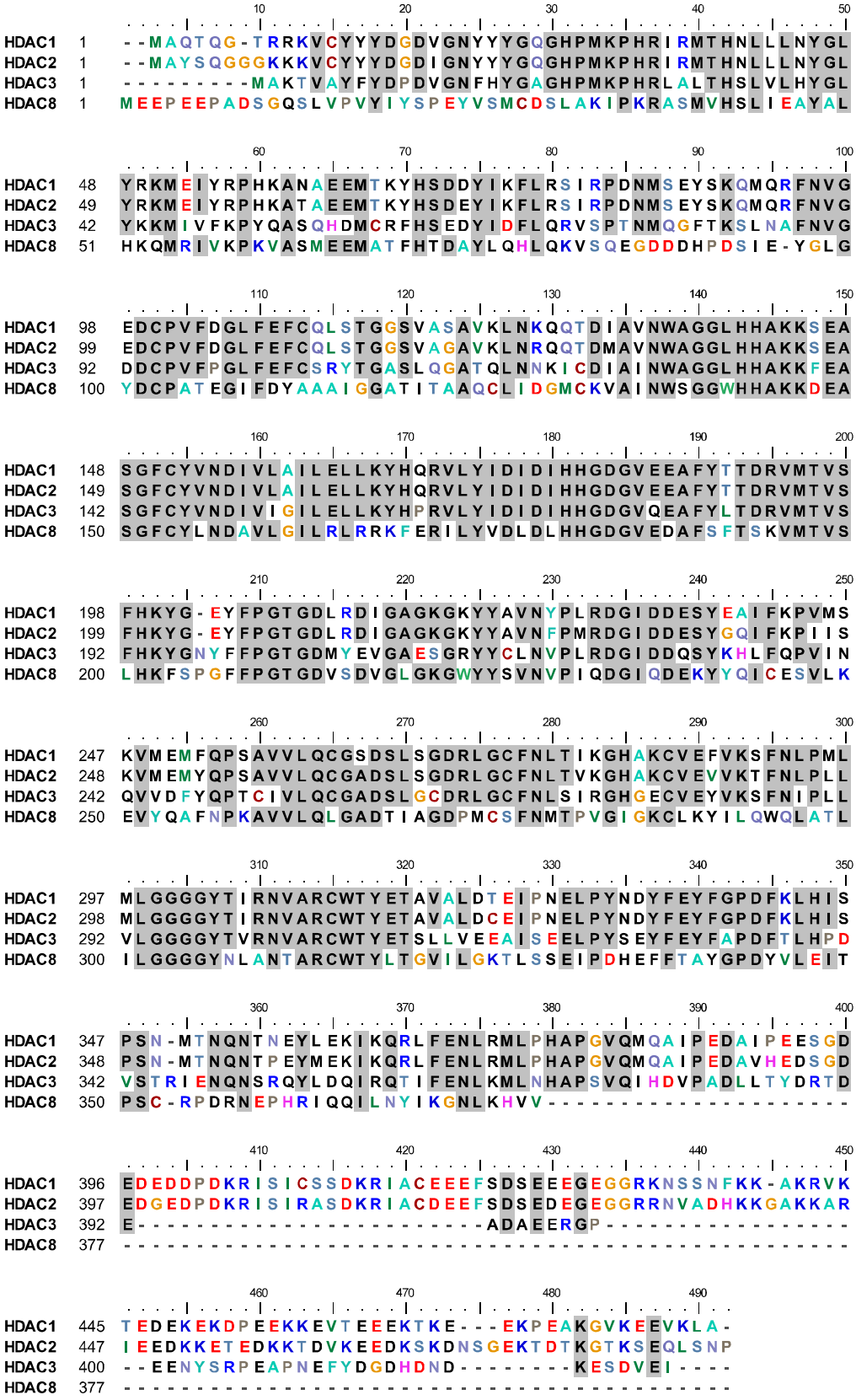
Supplementary Figure 3 Antibody Cross Reactivity Comparison with Acylated Bovine Serum Albumin. Solutions (10 mg/mL) of acylated BSA were dotted onto nitrocellulose (0.5 µL) and incubated with various antibodies. There is little appreciable cross-reactivity between all used antibodies indicating a high level of specificity toward advertised target. Representative blot, n = 2.



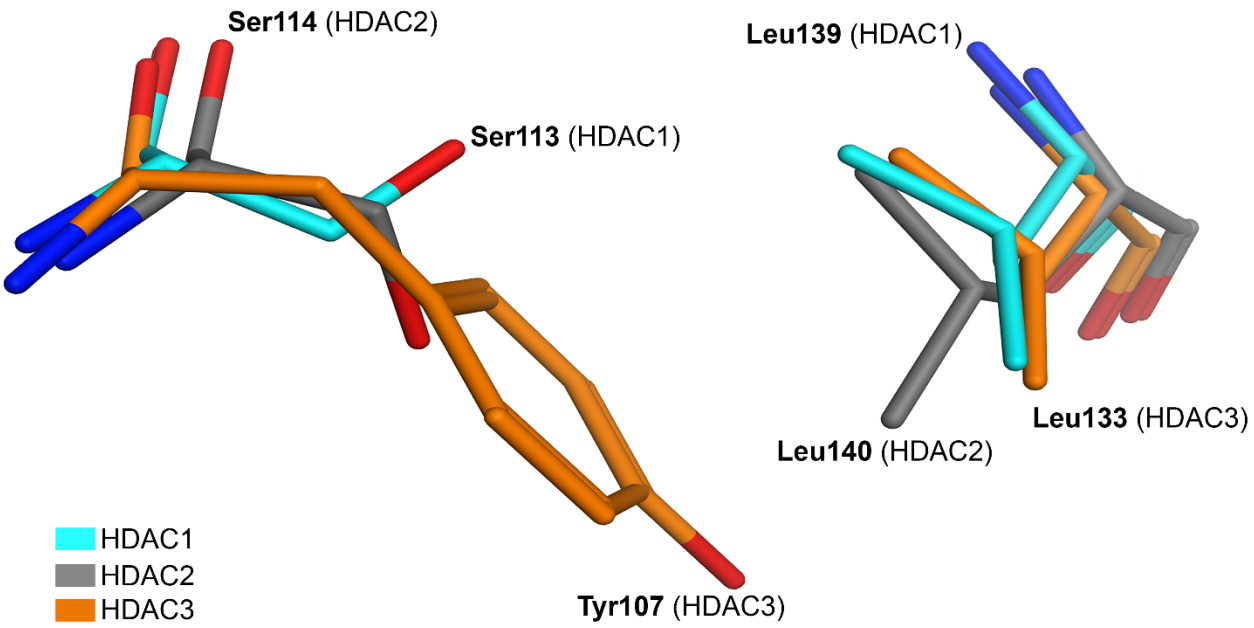
Supplementary Figure 4 Time dependent Kinetics of HDACs 1-3 vs **2a**. **a)** HDAC1 **b)** HDAC2 **c)** HDAC3. **2a** shows time-dependent inhibition toward HDAC3 but not HDACs 1 or 2 at 30°C. n = 2; error bars are S.E.M.



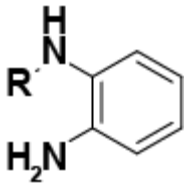
Supplementary Figure 5 Western blot analysis of RAW264.7 cells. HMGB-1 secretion monitoring with ponceau stain as loading control. Cells were treated for six hours. 1 µM vorinostat used. Representative blot of n = 2 experiments.

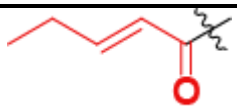
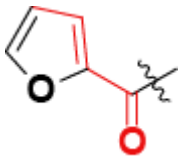
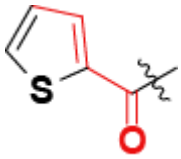
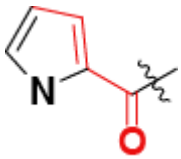
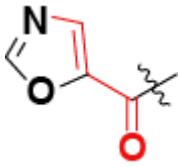
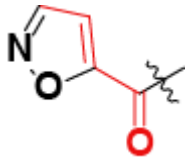
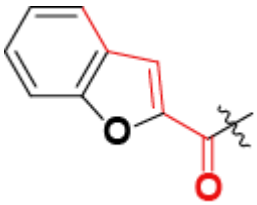


Supplementary Figure 6 Alignment of Class I HDACs.



Supplementary Figure 7 Crystal structure overlay of HDACs 1-3. Leu133 of HDAC3 and Leu139 of HDAC1 possess a very similar conformation and intramolecular assembly. HDAC2, however, does demonstrate different geometry as previously reported. PDB files: 5ICN, 3MAX, and 4A69 for HDACs 1-3 respectively.



Name	R-Group	HDAC1 IC_{50}	HDAC3 IC_{50}
1		16.7 μM	4.00 μM
1a		3.20 μM	0.721 μM
1b		5.31 μM	1.43 μM
1c		6.92 μM	1.80 μM
1d		2.12 μM	0.418 μM
1e		4.11 μM	1.37 μM
1f		0.357 μM	0.209 μM

Supplementary Table 1. Mean values of n = 3 experiments, S.E.M. for all values < 10% of mean.

Supplementary Methods and reagents

Figure generation

All figures were created using Adobe Illustrator CC or Adobe Photoshop CC. Graphs were generated using GraphPad Prism 7.0. Microsoft Word and Powerpoint 2016 were used for tables and illustrations. Molecular modeling pictures were captured in M.O.E. 2014. Chemical structures were created using ChemDraw 14.0 Alignment was performed in Bioedit 7.2.5.

Human HDAC Isozymes

All human HDAC isozymes were purchased from BPS biosciences and of >90% purity as determined via Coomassie staining with the exception of HDACs 4 and 7 at 65% and 70% pure respectively. All isozymes were generated from baculovirus expression systems in Sf9 insect cells. HDAC1 Lot: 140113. HDAC2 Lot: 110922G. HDAC3 Lot: 120524. HDAC4 Lot: 130115. HDAC5 Lot: 100414. HDAC6 Lot: 140110-G20mM. HDAC7 Lot: 90402. HDAC8 Lot: 110913. HDAC9 Lot: 91020-D. HDAC10 Lot: 101011. HDAC11 Lot: 141104.

Deacetylation and Deacylation Assays

Each isozyme of HDAC was tested for its ability to remove acyl-PTMs at 50 µM substrate concentration and 2ng of HDAC enzymes with the homogeneous fluorescence release HDAC deacetylase assay over two hours. For K_m determination, HDAC substrates were titrated against HDACs 1, 2, 3, and 6 (2 ng enzyme for HDAC3/NCoR2, 10 ng for HDAC1 and 2, and 20 ng for HDAC6). For HDAC3/NCoR2 and HDAC6 over-expression and knockdown lysates, 25 µg of total lysates were used with 50 µM of acetyl-substrate and 25 µg of lysates were used with 100 µM of the valeryl-substrate.

Individual IC_{50} values of HDAC inhibitors for each HDAC isozyme were measured by incubating purified recombinant enzymes with 12 point serial diluted inhibitor concentrations. The

deacetylase activity of HDACs 1, 2, 3, 6, and 10 was measured by assaying enzyme activity against acetyl-substrate, and Class IIa (HDAC4, 5, 7, and 9) and HDAC8 enzyme activity was measured using TFA-substrate. The inhibitors were pre-incubated with each enzyme for two hours at 30^{o}C, and then the substrate was added and further incubated for two hours. The deacylated lysine-AMC is sensitive to protease cleavage by trypsin, yielding free AMC; the amount of free AMC was determined by Tecan M200 at EX=360 nm EM=460 nm. The absolute deacylated lysine concentration was determined using a standard curve defined by free AMC under the same condition.

Cell Culture, Transfection, and Treatment

Hek293 cells were cultured in ATCC recommended conditions. For transfection, cells were plated at 70% confluence in 20 mL in T75 tissue culture flask in media without antibiotics. The HDAC over-expression plasmids were transfected with lipofectamine LTX with Plus reagent, according to the manufacturer's instruction, for 48 hours. For HDAC knockdown experiments, siRNAs were transfected and the cells were harvested after 24 hours. For the LPS treatment, RAW246.7 cells were cultured at 500,000 cells/mL for 24 hours. The culture media was refreshed and HDAC inhibitors were added three hours before the LPS (200 ng/mL) challenge. For the timed NF-κB p65 activation, the cells were harvested at indicated time points and nuclear and cytosolic lysates were isolated. For the NO production, 50 µL of media were collected and the media NO concentrations were determined using Griess reagents. For HMGB-1 secretion, the cell media was collected and concentrated using Amicon Ultra-4 spin column. The concentrated lysates were then mixed with 4x LDS loading buffer and run on a 4-12% polyacrylamide gel. The HMGB-1 levels were determined using HMGB-1 monoclonal antibody (Abcam, ab18256). Cell viability is monitored using non-toxic CellTiter-Blue® Cell Viability Assay based on resazurin conversion into a fluorescent end product resorufin by viable cell mitochondria as described by the supplier.

Western Blot and Cellular Lysate Preparation

Acetylated NF-κB p65(Ac-K122/123) and acetylated p53(Ac-K382) antibodies were purchased from Signalway Biotechnology and Cell Signaling Technology. Formyl- and valeryl-lysine antibodies were purchased from GeneTex. Acetyl-lysine antibody was purchased from ImmuneChem. All other antibodies were purchased from Santa Cruz Biotechnology. Formyl- and valeryl-lysine antibodies were demonstrated to be >50,000 fold selective for their respective acyl group via ELISA testing done by the manufacturer. HDAC-inhibitor-treated cells were harvested under indicated conditions. For HDAC inhibitor treatments, the cells were treated with HDAC inhibitors and harvested. The cells were lysed with RIPA buffer and loaded with LDS loading buffer into 4-12% SDS-PAGE gels (Novex, Invitrogen). The proteins were then transferred onto nitrocellulose membranes. The membranes were blocked for 1 hour with 5% milk or BSA. For over-expression and siRNA experiments, the cells were lysed in 1% triton buffer without SDS to preserve their maximum HDAC activity. The lysates were used for activity assays and diluted with 3xLDS buffer for Western blot analysis.

Acylation of Fatty Acid Free Bovine Serum

Fatty Acid Free Bovine Serum Albumin was purchased from Sigma. 10 mg was reacted with 1 mL of Valeryl Chloride (Sigma) or Acetic Anhydride (Acros) overnight at room temperature. These solutions were spun at 4°C at 15,000G for 15 minutes. The supernatant aspirated and the pelleted BSA resuspended in 1 mL of methanol. These washing and centrifugation steps were repeated 4 additional times. Residual volatiles were removed via lyophilization and the resulting dried pellet was resuspended in 1 mL of deionized water.

33.3 mg of Fatty Acid Free Bovine Serum Albumin was suspended in 5 mL of 99% Formic Acid (Sigma). The solution was raised to 65°C. 1.2 mL of Acetic Anhydride (Acros) was slowly dropped in via injection over 30 minutes. The solution was allowed to stir for 5 additional minutes before

quenching with 1.5 mL of distilled ice water. Volatiles were removed under reduced pressure at room temperature. Residual solution was removed via lyophilization. The resulting pellet was resuspended in 3.3 mL of distilled water. This methodology was adapted from du Vigneaud, Dorfmann, and Loring (1932).

Cross Sensitivity Verification of Formyl-, Acetyl-, and Valeryl-Lysine Antibodies.

0.5 µL of each solution (10 mg/mL) was dotted onto nitrocellulose using a 0.25 µL – 2 µL pipette fitted with 10 µL pipette tip. After the cellulose was dried, it was transferred into a 5% (w/v) solution of Bovine Serum Albumin. 1:1000 (v/v) of respective primary antibody was added and allowed to rock overnight at room temperature. The cellulose was washed several times and appropriate secondary antibody was added (1:1000 (v/v)). This solution was rocked for 1 hour at room temperature before washing and imaging. Imaging was performed on GE ImageQuant LAS 4000.

Statistical Analysis

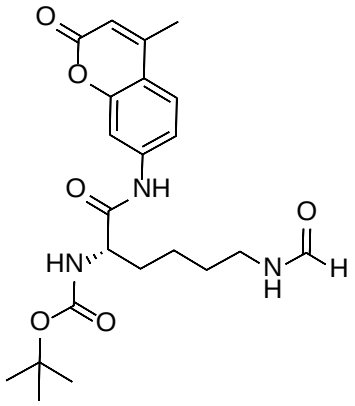
All analyses were performed by GraphPad Prism. All calculations were Dunnett's Multiple Comparisons with a significance threshold set to 0.01.

Alignment of Class I HDACs

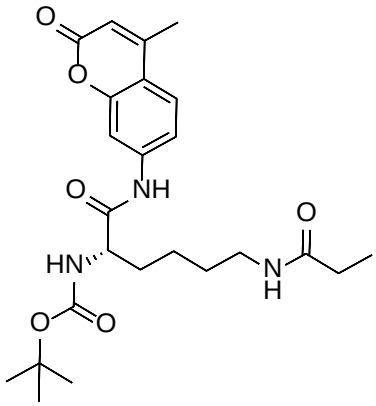
Sequence alignments were performed using ClustalW with the BLOSOM62 similarity matrix.

Supplementary Synthetic Methods and Characterization

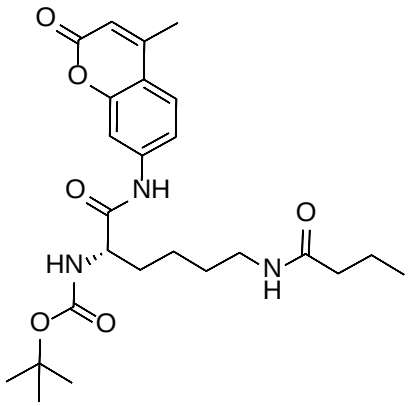
Acylated substrates synthesis:



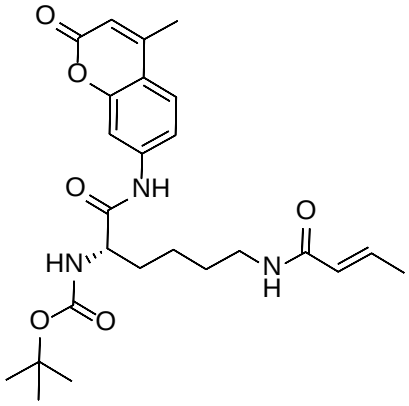
Formyl-substrate. Nα-(tert-Butoxycarbonyl)-L-Lysine-7-amido-4-methylcoumarin (100mg, 0.25 mmole) was dissolved in Dimethylformamide with catalytic amount of DIPEA. **Formyl-substrate** was generated by adding 2,2,2-Trifluoroethyl Formate (128 mg, 1 mmole 4 equiv) and reacted for 12 hours. The reaction mixture was injected directly into Combiflash instrument in HPLC format to obtain the desired product. 1H NMR (400 MHz, DMSO): δ 10.43 (s, 1H), 7.99 (s, 2H), 7.78 (d, 1H, J = 2 Hz), 7.73 (d, 1H, J = 8 Hz), 7.50 (d, 1H, J = 8 Hz), 7.13 (d, 1H, J = 8 Hz), 6.27 (s, 1H), 4.06 (m, 1H), 3.07 (m, 2H), 2.41 (s, 3H), 1.66-1.60 (m, 2H), 1.42-1.39 (m, 11H), 1.30-1.27 (m, 2H); 13C HSQC (400MHz, 100MHz, DMSO): δ 126.4, 115.7, 112.7, 106.1, 55.7, 37.4, 31.7, 29.3, 28.7, 28.6, 18.5. [(m+H^+)/z = 432.1]



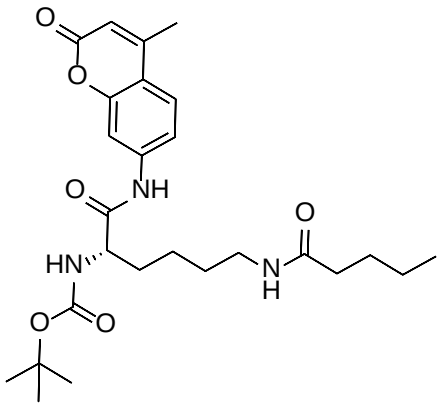
Propionyl-substrate. Nα-(tert-Butoxycarbonyl)-L-Lysine-7-amido-4-methylcoumarin (100mg, 0.25mmole) was dissolved in Dimethylformamide with catalytic amount of DIPEA. **Propionyl-substrate** was generated by adding propionyl chloride (92mg, 1mmole 4equiv) and reacted for 12 hours. The reaction mixture was injected directly into Combiflash instrument in HPLC format to obtain the desired product. 41% yield. 1H NMR (400 MHz, DMSO): δ 10.42 (s, 1H), 7.79 (d, 1H, J = 2 Hz), 7.75-7.71 (m, 2H), 7.72 (d, 1H, J = 8 Hz), 7.11 (d, 1H, J = 8 Hz), 6.28 (s, 1H), 4.08-4.03 (m, 1H), 3.05-3.00 (m, 2H), 2.41 (s, 3H), 2.07-2.01 (q, 2H, J = 8 Hz), 1.69-1.60 (m, 2H), 1.42-1.37 (m, 11H), 1.32-1.28 (m, 2H), 0.97 (t, 3H, J = 8 Hz); 13C HSQC (400MHz, 100MHz, DMSO): δ 126.4, 115.7, 112.7, 106.1, 55.7, 38.6, 31.7, 29.0, 28.7, 28.6, 23.5, 18.5, 10.5. LC/MS: [(m+H^+)/z = 460.2]



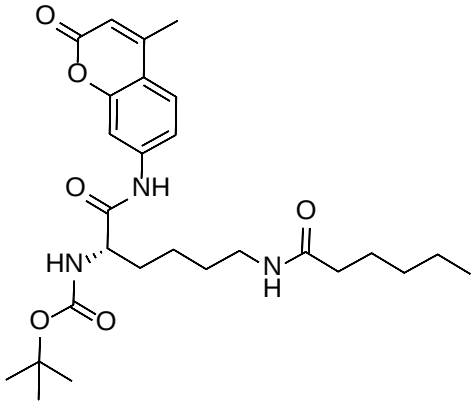
Butyryl-substrate. Nα-(tert-Butoxycarbonyl)-L-Lysine-7-amido-4-methylcoumarin (100mg, 0.25 mmole) was dissolved in Dimethylformamide with catalytic amount of DIPEA. **Butyryl-substrate** was generated by adding butyryl chloride (107mg, 1 mmole 4 equiv) and reacted for 12 hours. The reaction mixture was injected directly into Combiflash instrument in HPLC format to obtain the desired product. 1H NMR (400 MHz, DMSO): δ 10.42 (s, 1H), 7.79 (s, 1H), 7.75-7.73 (m, 2H), 7.50 (d, 1H, J = 8 Hz), 7.11 (d, 1H, J = 8 Hz), 6.28 (s, 1H), 4.07-4.04 (m, 1H), 3.05-3.01 (m, 2H), 2.41 (s, 3H), 2.00 (t, 2H, J = 7 Hz), 1.69-1.55 (m, 2H), 1.53-1.45 (m, 2H), 1.44-1.40 (m, 11), 1.31-1.28 (m, 2H), 0.82 (t, 3H, J = 8 Hz); 13C HSQC (400MHz, 100MHz, DMSO): δ 126.4, 115.7, 112.7, 106.1, 55.7, 38.5, 37.9, 31.7, 28.7, 28.6, 23.5, 19.2, 18.5, 14.2. LC/MS: [(m+H^+)/z = 474.1]



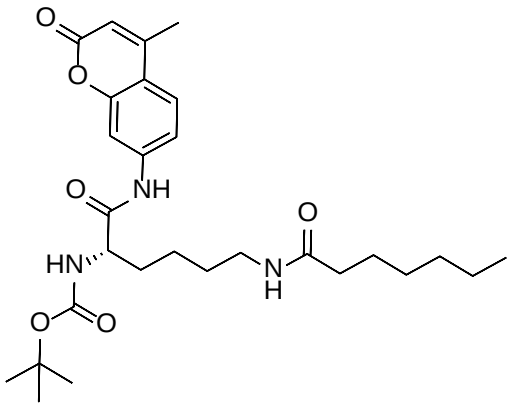
Crotonyl-substrate. Nα-(tert-Butoxycarbonyl)-L-Lysine-7-amido-4-methylcoumarin (100mg, 0.25 mmole) was dissolved in Dimethylformamide with catalytic amount of DIPEA. **Crotonyl-substrate** was generated by adding crotonyl anhydride (105mg, 1 mmole 4 equiv) and reacted for 12 hours. The reaction mixture was injected directly into Combiflash instrument in HPLC format to obtain the desired product. 1H NMR (400 MHz, DMSO): δ 10.42 (s, 1H), 7.86 (t, 1H, J = 6 Hz), 7.78 (s, 1H), 7.73 (d, 1H, J = 8 Hz), 7.50 (d, 1H, J = 8 Hz), 7.12 (d, 1H, J = 8 Hz), 6.61-6.55 (m, 1H), 6.27 (s, 1H), 5.87 (d, 1H, J = 11 Hz), 4.07-4.04 (m, 1H), 3.12-3.07 (m, 2H), 2.41 (s, 3H), 1.77 (d, 3H, J = 7 Hz), 1.67-1.60 (m, 2H), 1.45-1.39 (m, 11H), 1.32-1.29 (m, 2H); 13C HSQC (400MHz, 100MHz, DMSO): δ 137.8, 126.5, 126.4, 115.7, 112.7, 106.1, 55.8, 31.7, 29.4, 28.7, 28.6, 23.5, 18.5, 17.8. [(m+H^+)/z = 572.3]



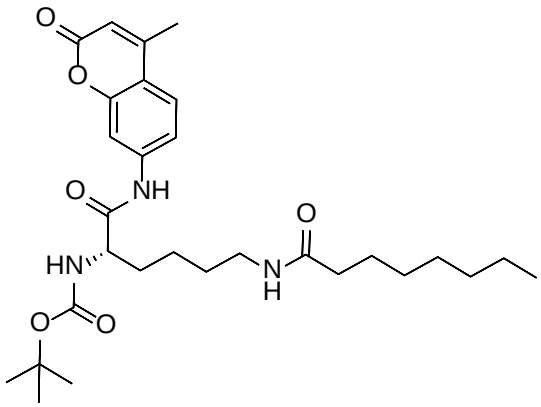
Valeryl-substrate. Nα-(tert-Butoxycarbonyl)-L-Lysine-7-amido-4-methylcoumarin (100mg, 0.25 mmole) was dissolved in Dimethylformamide with catalytic amount of DIPEA. **Valeryl-substrate** was generated by adding valeryl chloride (121mg, 1 mmole 4 equiv) and reacted for 12 hours. The reaction mixture was injected directly into Combiflash instrument in HPLC format to obtain the desired product. 1H NMR (400 MHz, DMSO): δ 10.43 (s, 1H), 7.79 (d, 1H, J = 2 Hz), 7.74-7.73 (m, 2H), 7.51 (d, 1H, J = 9 Hz), 7.11 (d, 1H, J = 8 Hz), 6.28 (s, 1H), 4.08-4.03 (m, 1H), 3.04-3.00 (m, 2H), 2.41 (s, 3H), 2.02 (t, 2H, J = 8 Hz), 1.65-1.62 (m, 2H), 1.47-1.40 (m, 13H), 1.29-1.22 (m, 4H), 0.84 (t, 3H, J= 8 Hz); 13C HSQC (400MHz, 100MHz, DMSO): δ 126.4, 115.7, 112.7, 106.1, 55.7, 38.6, 35.6, 31.7, 27.9, 28.8, 28.7, 23.5, 22.3, 18.5, 14.2. [(m+H^+)/z = 488.3]



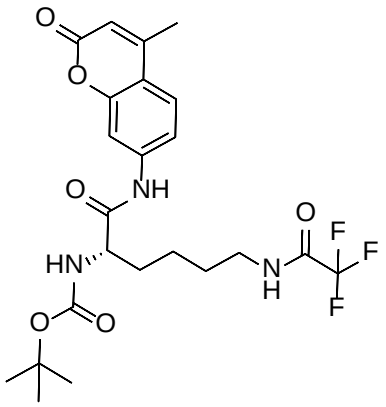
Hexanoyl-substrate. Nα-(tert-Butoxycarbonyl)-L-Lysine-7-amido-4-methylcoumarin (100mg, 0.25 mmole) was dissolved in Dimethylformamide with catalytic amount of DIPEA. **Hexanoyl-substrate** was generated by adding hexanoyl chloride (135mg, 1 mmole 4 equiv) and reacted for 12 hours. The reaction mixture was injected directly into Combiflash instrument in HPLC format to obtain the desired product. 1H NMR (400 MHz, DMSO): δ 10.42 (s, 1H), 7.79 (d, 1H, J = 1 Hz), 7.75-7.73 (m, 2H), 7.50 (d, 1H, J = 9 Hz), 7.11 (d, 1H, J = 8 Hz), 6.28 (s, 1H), 4.07-4.04 (m, 1H), 3.04-3.00 (m, 2H), 2.41 (s, 3H), 2.01 (t, 2H, J = 8 Hz), 1.67-1.60 (m, 2H), 1.45-1.39 (m, 13H), 1.29-1.18 (m, 6H), 0.84 (t, 3H, J = 7 Hz); 13C HSQC (400MHz, 100MHz, DMSO): δ 126.4, 115.7, 112.7, 106.0, 55.7, 38.5, 35.9, 31.7, 31.4, 28.7, 28.5, 23.5, 22.3, 18.5, 14.3. [(m+H^+)/z = 502.2]



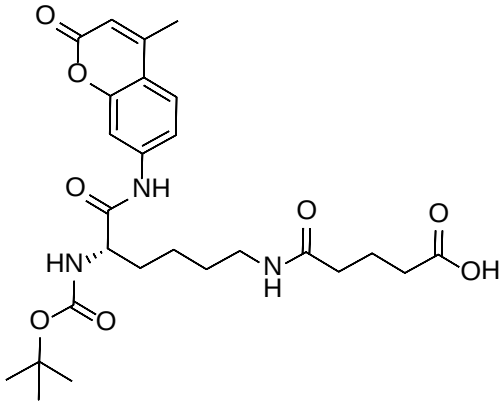
Heptanoyl-substrate. Nα-(tert-Butoxycarbonyl)-L-Lysine-7-amido-4-methylcoumarin (100mg, 0.25 mmole) was dissolved in Dimethylformamide with catalytic amount of DIPEA. **Heptanoyl-substrate** was generated by adding Heptanoyl Chloride (149mg, 1 mmole 4 equiv) and reacted for 12 hours. The reaction mixture was injected directly into Combiflash instrument in HPLC format to obtain the desired product. 1H NMR (400 MHz, DMSO): δ 10.42 (s, 1H), 7.79 (d, 1H, J = 2 Hz), 7.75-7.73 (m, 2H), 7.50 (d, 1H, J = 8 Hz), 7.10 (d, 1H, J = 8 Hz), 6.28 (s, 1H), 4.08-4.03 (m, 1H), 3.04-3.00 (m, 2H), 2.41 (s, 3H), 2.01 (t, 2H, J = 8 Hz), 1.69-1.61 (m, 2H), 1.44-1.39 (m, 13H), 1.29-1.20 (m, 8H), 0.85 (t, 3H, J = 7 Hz); 13C HSQC (400MHz, 100MHz, DMSO): δ 126.4, 115.8, 112.8, 106.1, 55.7, 38.5, 35.9, 31.7, 31.4, 28.8, 28.7, 28.5, 25.7, 23.4, 22.5, 18.5, 14.4. [$(m+H^+)/z$ = 516.4]



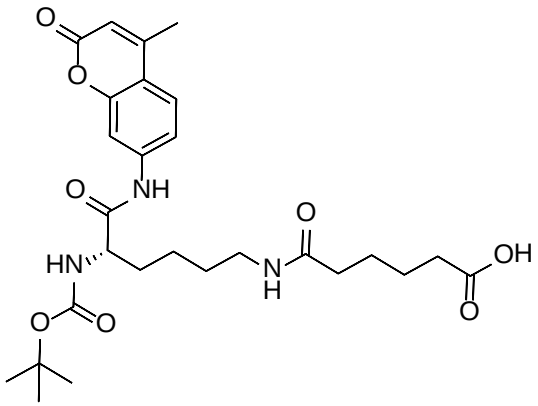
Octanoyl-substrate. Nα-(tert-Butoxycarbonyl)-L-Lysine-7-amido-4-methylcoumarin (100mg, 0.25 mmole) was dissolved in Dimethylformamide with catalytic amount of DIPEA. **Octanoyl-substrate** was generated by adding Octanoyl Chloride (163mg, 1 mmole 4 equiv) and reacted for 12 hours. The reaction mixture was injected directly into Combiflash instrument in HPLC format to obtain the desired product. 1H NMR (400 MHz, DMSO): δ 10.42 (s, 1H), 7.79 (d, 1H, J = 2 Hz), 7.75-7.73 (m, 2H), 7.50 (d, 1H, J = 8 Hz), 7.10 (d, 1H, J = 8 Hz), 6.28 (s, 1H), 4.07-4.03 (m, 1H), 3.04-3.00 (m, 2H), 2.41 (s, 3H), 2.01 (t, 2H, J = 8 Hz), 1.64-1.60 (m, 2H), 1.46-1.39 (m, 13H), 1.29-1.21 (m, 10H), 0.87-0.83 (m, 3H); 13C HSQC (400MHz, 100MHz, DMSO): δ 126.3, 115.7, 112.7, 106.1, 55.7, 38.6, 35.9, 31.7, 31.6, 29.4, 28.9, 28.7, 28.5, 25.8, 23.4, 22.5, 18.5, 14.4. [$(m+H^+)/z$ = 530.2]



TFA-substrate. Nα-(tert-Butoxycarbonyl)-L-Lysine-7-amido-4-methylcoumarin (100mg, 0.25 mmole) was dissolved in Dimethylformamide with catalytic amount of DIPEA. **TFA-substrate** was generated by adding trifluoroacetic anhydride (210mg, 1 mmole 4 equiv) and reacted for 12 hours. The reaction mixture was injected directly into Combiflash instrument in HPLC format to obtain the desired product. 1H NMR (400 MHz, DMSO): δ 10.46 (s, 1H), 9.42 (t, 1H, J = 2 Hz), 7.78 (d, 1H, J = 2 Hz), 7.74 (d, 1H, J = 9 Hz), 7.50 (d, 1H, J = 9 Hz), 7.14 (d, 1H, J = 8 Hz), 6.28 (s, 1H), 4.09-4.05 (m, 1H), 3.20-3.16 (m, 2H), 2.41 (s, 3H), 1.69-1.60 (m, 2H), 1.55-1.46 (m, 2H), 1.39 (s, 9H), 1.34-1.25 (m, 2H); 13C HSQC (400MHz, 100MHz, DMSO): δ 126.5, 115.7, 112.8, 106.1, 55.6, 39.5, 31.6, 28.7, 28.5, 28.4, 18.5. [$(m+H^+)/z$ = 500.2]

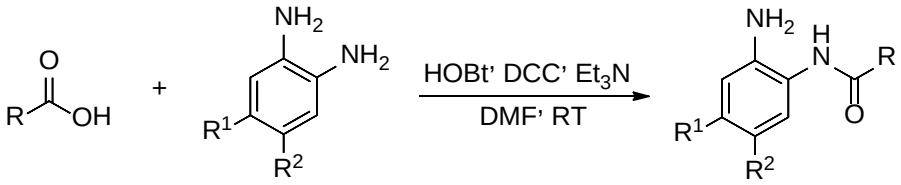


Glutaryl-substrate. Nα-(tert-Butoxycarbonyl)-L-Lysine-7-amido-4-methylcoumarin (100mg, 0.25 mmole) was dissolved in Dimethylformamide with catalytic amount of DIPEA. **Glutaryl-substrate** was generated by adding glutaric anhydride (114mg, 1 mmole 4 equiv) and reacted for 12 hours. The reaction mixture was injected directly into Combiflash instrument in HPLC format to obtain the desired product. 1H NMR (400 MHz, DMSO): δ 10.43 (s, 1H), 7.81-7.78 (m, 2H), 7.73 (d, 1H, J = 9 Hz), 7.50 (d, 1H, J = 9 Hz), 7.11 (d, 1H, J = 8 Hz), 6.27 (s, 1H), 4.08-4.03 (m, 1H), 3.34-3.01 (m, 2H), 2.41 (s, 3H), 2.18 (t, 2H, J = 8 Hz), 2.07 (t, 2H, J = 8 Hz), 1.73-1.60 (m, 4H), 1.45-1.38 (m, 13H); 13C HSQC (400MHz, 100MHz, DMSO): δ 126.4, 115.7, 112.7, 106.1, 55.7, 38.6, 35.0, 33.6, 31.7, 29.4, 28.7, 28.6, 21.2, 18.5. [$(m+H^+)/z$ = 518.2]



Adipoyl-substrate, Nα-(tert-Butoxycarbonyl)-L-Lysine-7-amido-4-methylcoumarin (100mg, 0.25mmole) was dissolved in Dimethylformamide (DMF), 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), Hydroxybenzotriazole (HOBt), and adipic acid (1:1:1 equiv.) with catalytic amount of DIPEA were dissolved in DMF. **Adipoyl-substrate** was generated by dripping Boc-Lys-AMC solution slowly into the activated adipic acid solution (four equiv.) and reacted for 2 hours. The reaction mixture was stopped by adding water into the reaction and injected directly into Combiflash instrument in HPLC format to obtain the desired product. 1H NMR (400 MHz, DMSO): δ 10.46 (s, 1H), 7.79-7.77 (m, 2H), 7.74 (d, 1H, J = 9 Hz), 7.51 (d, 1H, J = 9 Hz), 7.11 (d, 1H, J = 6 Hz), 6.28 (s, 1H), 4.06-4.04 (m, 1H), 3.04-3.01 (m, 2H), 2.41 (s, 3H), 2.18 (t, 2H, J = 7Hz), 2.03 (t, 2H, J = 7 Hz), 1.69-1.59 (m, 2H), 1.41-1.45 (m, 4H), 1.39 (s, 9H), 1.30-1.28 (m, 4H); 13C HSQC (400MHz, 100MHz, DMSO): δ 126.4, 115.7, 112.7, 106.1, 55.8, 38.6, 35.6, 34.0, 31.7, 29.4, 28.7, 28.6, 24.8, 23.6, 18.5. [$(m+H^+)/z$ = 532.2]

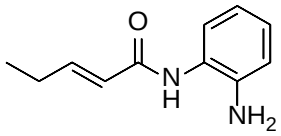
HDAC3 Inhibitors synthesis



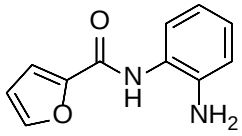
RA_1 **RA_2**

Carboxylic acid **RA_1** (2 mmol, 1 equiv), HOBt (4 mmol, 2 equiv), and DCC (4 mmol, 2 equiv) were dissolved in 50 mL DMF, to which was added Et_3N (4mmol, 2 equiv), and the resulting solution was stirred at room temperature for 30 mins. *o*-Phenylenediamine **RA_2**(2 mmol, 1 equiv) was then added to the solution, and the mixture was stirred overnight. Pour the mixture into 100 mL water, and extract it with 250 mL ethyl acetate. The organic phase was washed with saturated $NaHCO_3$ and brine successively, and it was then dried over $MgSO_4$ and filtered. The filtrates were concentrated under vacuum, and the residue was purified by Combiflash instrument to obtain the desired product.

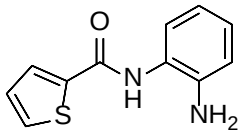
HDAC3 Inhibitor Characterization



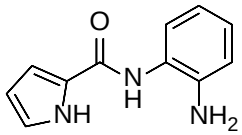
Compound **1**, (E)-N-(2-aminophenyl)pent-2-enamide, white solid, 28% yield. ^{1}H NMR (400 MHz, DMSO): δ 9.23 (s, 1H), 7.24-7.22 (d, *J* = 7.6 Hz, 1H), 6.92-6.78 (m, 2H), 6.73 (dd, J_1 = 8.0 Hz, J_2 = 0.8 Hz, 1H), 6.55 (td, J_1 = 7.2 Hz, J_2 = 1.6 Hz, 1H), 6.15 (d, *J* = 15.2 Hz, 1H), 4.85 (br, 1H), 2.23-2.17 (m, 2H), 1.03 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, DMSO) δ 172.2, 164.1, 145.9, 142.2, 126.2, 125.4, 123.8, 116.8, 116.5, 25.0, 12.9. LC/MS: [(m+H$^+$)/z = 191.25].



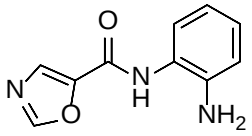
Compound **1a**, N-(2-aminophenyl)furan-2-carboxamide, white solid, 70% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.20 (br, 1H), 7.45-7.44 (m, 1H), 7.34 (d, *J* = 8.0 Hz, 1H), 7.18 (d, *J* = 3.2 Hz, 1H), 7.04-7.01 (m, 1H), 6.80-6.76 (m, 2H), 6.52-6.51 (m, 1H), 3.91 (br, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.6, 147.6, 144.5, 140.8, 127.2, 125.3, 123.6, 119.5, 118.1, 115.3, 112.4. LC/MS: [(m+H$^+$)/z = 203.33].



Compound **1b**, N-(2-aminophenyl)thiophene-2-carboxamide, white solid, 62% yield. ^{1}H NMR (400 MHz, DMSO): δ 9.74 (s, 1H), 7.98-7.97 (m, 1H), 7.79 (dd, J_1 = 4.8 Hz, J_2 = 1.2 Hz, 1H), 7.21-7.19 (m, 1H), 7.13-7.11 (m, 1H), 7.01-6.97 (m, 1H), 6.78 (dd, J_1 = 8.0 Hz, J_2 = 0.8 Hz, 1H), 6.63-6.59 (m, 1H), 5.50-3.50 (br, 2H); ^{13}C NMR (100 MHz, DMSO) δ 160.5, 143.6, 140.3, 131.8, 129.6, 128.5, 127.4, 127.3, 123.2, 117.0, 116.7. LC/MS: [(m+H$^+$)/z = 219.19].

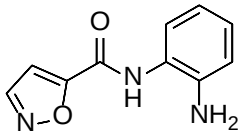


Compound **1c**, N-(2-aminophenyl)-1H-pyrrole-2-carboxamide, white solid, 31% yield. ^{1}H NMR (400 MHz, DMSO): δ 11.6 (s, 1H), 9.32 (s, 1H), 7.17-7.14 (m, 1H), 7.02-6.93 (m, 4H), 6.80-6.78 (m, 1H), 6.63-6.59 (m, 2H), 6.19-6.17 (m, 1H), 4.86 (br, 2H). ^{13}C NMR (100 MHz, DMSO) δ 159.8, 143.4, 126.9, 126.6, 126.4, 123.9, 122.5, 117.0, 116.7, 111.7, 109.3. LC/MS: [(m+H$^+$)/z = 202.23].

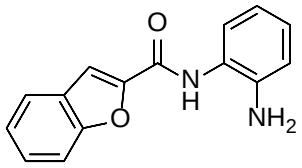


Compound **1d**, N-(2-aminophenyl)oxazole-5-carboxamide, white solid, 44% yield. ^{1}H NMR (400 MHz, DMSO): δ 9.80 (s, 1H), 8.59 (s, 1H), 7.93 (s, 1H), 7.12-7.10 (m, 1H), 7.01-6.97 (m, 1H),

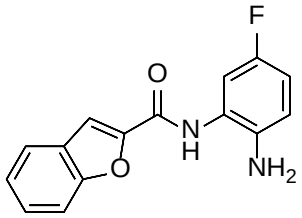
6.78-6.76 (m, 1H), 6.61 (m, 1H), 4.94 (br, 2H). ^{13}C NMR (100 MHz, DMSO) δ 155.7, 154.0, 145.8, 143.8, 130.1, 127.5, 127.4, 122.0, 116.7, 116.5. LC/MS: [(m+H$^+$)/z = 204.19].



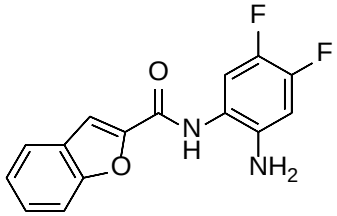
Compound **1e**, N-(2-aminophenyl)isoxazole-5-carboxamide, white solid, 22% yield. ^{1}H NMR (400 MHz, DMSO): δ 10.08 (s, 1H), 8.77 (d, *J* = 1.6 Hz, 1H), 7.20 (d, *J* = 1.6 Hz, 1H), 7.13-7.10 (m, 1H), 7.02-6.98 (m, 1H), 6.79-6.76 (m, 1H), 6.61-6.57 (m, 1H), 4.99 (br, 2H). ^{13}C NMR (100 MHz, DMSO) δ 163.2, 154.9, 152.1, 144.0, 127.8, 127.5, 121.7, 116.6, 116.5, 106.9. LC/MS: [(m+H$^+$)/z = 204.17].



Compound **1f**, N-(2-aminophenyl)benzofuran-2-carboxamide, white solid, 75% yield. ^{1}H NMR (400 MHz, DMSO): δ 9.94 (s, 1H), 7.81 (d, *J* = 8.0 Hz, 1H), 7.73-7.69 (m, 2H), 7.51-7.47 (m, 1H), 7.37-7.33 (m, 1H), 7.23-7.21 (m, 1H), 7.03-6.99 (m, 1H), 6.84-6.81 (m, 1H), 6.65 (m, 1H), 4.98 (br, 2H). ^{13}C NMR (100 MHz, DMSO) δ 157.4, 154.8, 149.4, 143.6, 127.6, 127.4(127.4), 127.2, 124.2, 123.3, 122.7, 116.9, 116.7, 112.3, 110.7. LC/MS: [(m+H$^+$)/z = 253.23].

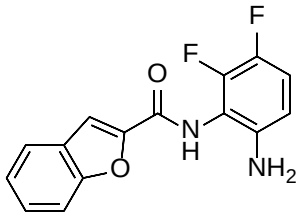


Compound **2**, N-(2-amino-5-fluorophenyl)benzofuran-2-carboxamide, white solid, 80% yield. ^{1}H NMR (400 MHz, DMSO): δ 9.88 (s, 1H), 7.83-7.81 (m, 1H), 7.74-7.70 (m, 2H), 7.52-7.48 (m, 1H), 7.39-7.35 (m, 1H), 7.20-7.16 (m, 1H), 6.62-6.58 (m, 1H), 6.42-6.38 (m, 1H), 5.35 (s, 2H). ^{13}C NMR (100 MHz, DMSO) δ 161.8 (d, *J* = 239 Hz), 157.7, 154.8, 149.5, 146.1 (d, *J* = 13.3 Hz), 129.2 (d, *J* = 11.7 Hz), 127.6, 127.4, 124.2, 123.3, 118.6, 112.3, 110.6, 102.6 (d, *J* = 23.4 Hz), 102.0 (d, *J* = 25.1 Hz). ^{19}F NMR (400 MHz, DMSO): δ -115.9. LC/MS: [(m+H$^+$)/z = 271.17].

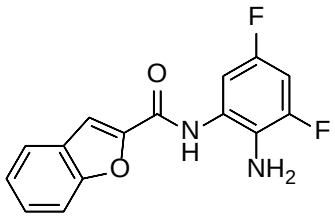


Compound **2a**, N-(2-amino-4,5-difluorophenyl)benzofuran-2-carboxamide, white solid, 78% yield. ^{1}H NMR (400 MHz, DMSO): δ 9.89 (br, 1H), 7.83-7.81 (m, 1H), 7.73-7.70 (m, 2H), 7.52-7.48 (m, 1H), 7.38-7.28 (m, 2H), 6.77-6.72 (m, 1H), 5.19 (s, 2H). ^{13}C NMR (100 MHz, DMSO) δ 157.6,

154.8, 149.2, 141.4, 141.3, 127.6, 127.5, 124.3, 123.3, 118.1, 116.0, 115.8, 112.3, 111.0, 103.6, 103.4. ^{19}F NMR (400 MHz, DMSO): δ -140.9, -153.8. LC/MS: [(m+H$^+$)/z = 289.08]

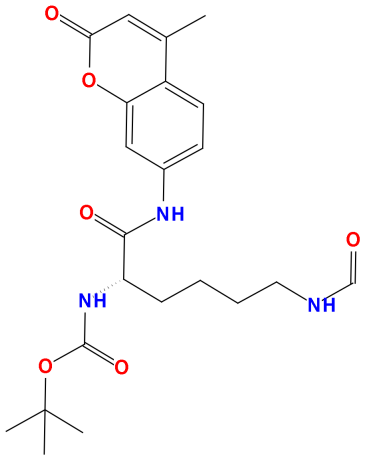


Compound **2b**, N-(6-amino-2,3-difluorophenyl)benzofuran-2-carboxamide, white solid, 66% yield. ^{1}H NMR (400 MHz, DMSO): δ 10.0 (s, 1H), 7.83 (d, J = 3.86 Hz, 1H), 7.2 (d, J = 5.40 Hz, 2H), 7.51 (t, J = 2.74 Hz, 1H), 7.37 (t, J = 2.75 Hz, 1H), 7.02-6.98 (m, 1H), 6.58 (q, J = 6.85 Hz, 1H), 5.41 (s, 2H). ^{13}C NMR (100 MHz, DMSO): δ 157.8, 154.8, 149.3, 140.5, 138.2, 135.0, 127.6, 127.5, 124.2, 123.3, 119.9, 112.3, 110.8, 102.7, 102.5. ^{19}F NMR (400 MHz, DMSO): δ -141.5, -158.2. LC/MS [(m+H$^+$)/z = 289.08].

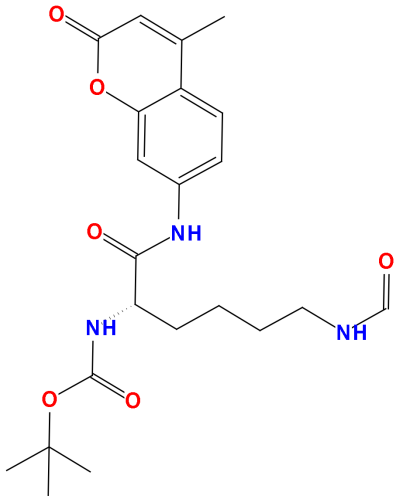


Compound **2c**, N-(2-amino-3,5-difluorophenyl)benzofuran-2-carboxamide, light orange solid, 54% yield. ^{1}H NMR (400 MHz, DMSO): δ 9.72 (s, 1H), 7.82 (d, J = 3.72 Hz, 1H), 7.72-7.70 (m, 2H), 7.50 (t, J = 5.66 Hz, 1H), 7.37 (t, J = 5.66 Hz, 1H), 6.39-6.33 (m, 2H), 5.73 (s, 1H). ^{13}C NMR (100 MHz, DMSO): δ 163.0, 160.8, 158.1, 154.8, 149.2, 127.6, 127.5, 124.2, 123.3, 112.3, 110.8, 106.0, 105.8, 96.8, 90.9. ^{19}F NMR (400 MHz, DMSO): δ -112.7, -117.2. LC/MS [(m+H$^+$)/z = 289.08].

1H NMR of **Formyl-substrate**



HSQC of **Formyl-substrate**

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ZGOPTNS

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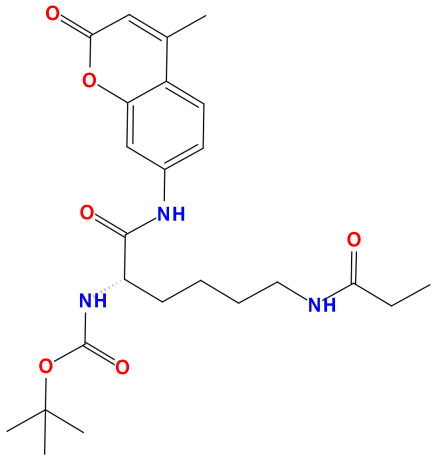
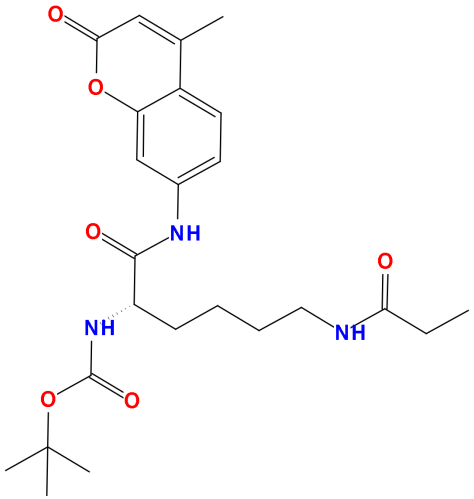
```

1H NMR of **Propionyl-substrate**



HSQC of **Propionyl-substrate**




```
F2 - Acquisition Parameters
Date_              20150831
Time                 16.31
INSTRUM              spect
PROBHD    5 mm PABBI 1H/
PULPROG       hsqcetgpsi2
TD                   1024
SOLVENT              DMSO
NS                      2
DS                      0
SWH              5330.490 Hz
FIDRES           5.205557 Hz
AQ             0.0960512 sec
RG                   2050
DW                 93.800 usec
DE                  6.50 usec
TE                 298.0 K
CNST2         145.0000000
D0             0.00000300 sec
D1             1.50000000 sec
D4             0.00172414 sec
D11            0.03000000 sec
D16            0.00020000 sec
D24            0.00086207 sec
IN0            0.00003000 sec
ZGOPTNS

======== CHANNEL f1 ========
NUC1                   1H
P1                   7.80 usec
P2                  15.60 usec
P28               1000.00 usec
PLW1           9.86279964 W
SFO1          400.0724053 MHz

======== CHANNEL f2 ========
CPDPRG[2               garp
NUC2                   13C
P3                  13.00 usec
P4                  26.00 usec
PCPD2               80.00 usec
PLW2         102.08999634 W
PLW12          2.69589996 W
SFO2          100.6052242 MHz

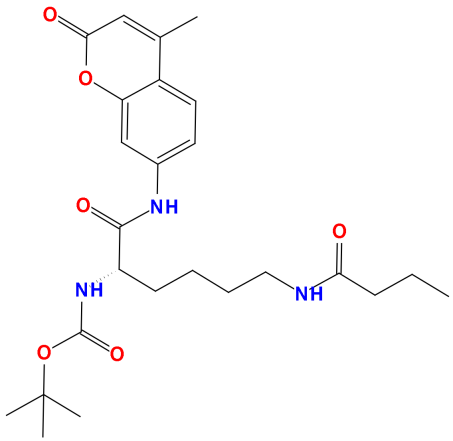
====== GRADIENT CHANNEL =====
GPNAM[1]       SMSQ10.100
GPNAM[2]       SMSQ10.100
GPNAM[3]       SMSQ10.100
GPNAM[4]       SMSQ10.100
GPZ1                80.00 %
GPZ2                20.10 %
GPZ3                11.00 %
GPZ4                -5.00 %
P16               1000.00 usec
P19                600.00 usec

F1 - Acquisition parameters
TD                     256
SFO1            100.6052 MHz
FIDRES         130.197311 Hz
SW               165.650 ppm
FnMODE     Echo-Antiecho

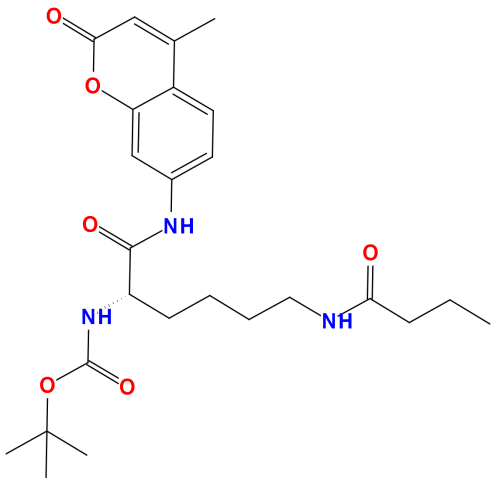
F2 - Processing parameters
SI                    1024
SF              400.0700000 MHz
WDW                   QSINE
SSB                       2
LB         0 Hz
GB         0
PC                     1.00

F1 - Processing parameters
SI                    1024
MC2         echo-antiecho
SF              100.5976820 MHz
WDW                   QSINE
SSB                       2
LB         0 Hz
GB         0
```

1H NMR of **Butyryl-substrate**



HSQC of **Butyryl-substrate**




```
F2 - Acquisition Parameters
Date_           20150901
Time               15.28
INSTRUM            spect
PROBHD   5 mm PABBI 1H/
PULPROG     hsqcetgpsi2
TD                 1024
SOLVENT            DMSO
NS                    2
DS                    0
SWH           6602.113 Hz
FIDRES        6.447376 Hz
AQ           0.0775509 sec
RG                 2050
DW              75.733 usec
DE               6.50 usec
TE              298.0 K
CNST2       145.0000000
D0           0.00000300 sec
D1           1.50000000 sec
D4           0.00172414 sec
D11          0.03000000 sec
D16          0.00020000 sec
D24          0.00086207 sec
IN0          0.00003000 sec
ZGOPTNS

======== CHANNEL f1 ========
NUC1                1H
P1               7.80 usec
P2              15.60 usec
P28           1000.00 usec
PLW1         9.86279964 W
SFO1        400.0724053 MHz

======== CHANNEL f2 ========
CPDPRG[2            garp
NUC2                13C
P3              13.00 usec
P4              26.00 usec
PCPD2           80.00 usec
PLW2       102.08999634 W
PLW12        2.69589996 W
SFO2        100.6052242 MHz

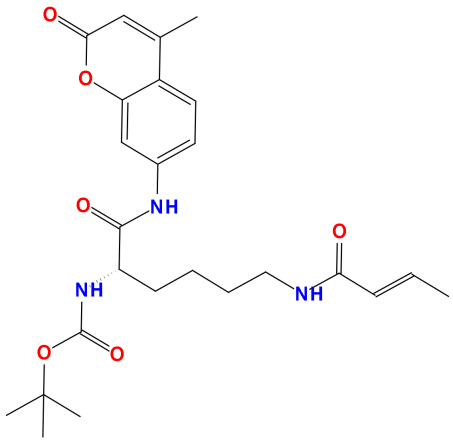
====== GRADIENT CHANNEL =====
GPNAM[1]     SMSQ10.100
GPNAM[2]     SMSQ10.100
GPNAM[3]     SMSQ10.100
GPNAM[4]     SMSQ10.100
GPZ1            80.00 %
GPZ2            20.10 %
GPZ3            11.00 %
GPZ4            -5.00 %
P16           1000.00 usec
P19            600.00 usec

F1 - Acquisition parameters
TD                  256
SFO1          100.6052 MHz
FIDRES       130.197311 Hz
SW             165.650 ppm
FnMODE    Echo-Antiecho

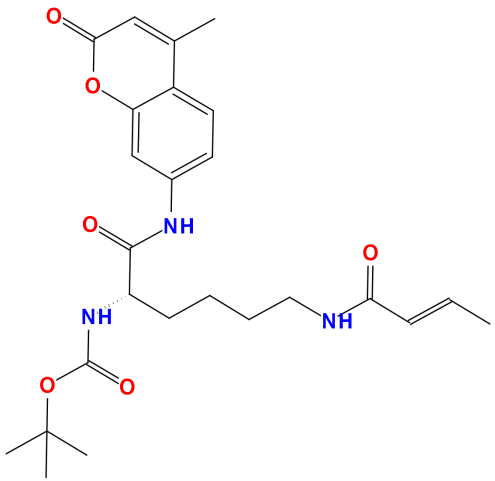
F2 - Processing parameters
SI                 1024
SF          400.0700000 MHz
WDW               QSINE
SSB                   2
LB       0 Hz
GB       0
PC                 1.00

F1 - Processing parameters
SI                 1024
MC2       echo-antiecho
SF          100.5976820 MHz
WDW               QSINE
SSB                   2
LB       0 Hz
GB       0
```

1H NMR of **Crotonyl-substrate**



HSQC of **Crotonyl-substrate**




```
F2 - Acquisition Parameters
Date_              20150831
Time                 16.50
INSTRUM              spect
PROBHD   5 mm PABBI 1H/
PULPROG      hsqcetgpsi2
TD                   1024
SOLVENT               DMSO
NS                       2
DS                       0
SWH               5330.490 Hz
FIDRES               5.205557 Hz
AQ                0.0960512 sec
RG                    2050
DW                  93.800 usec
DE                   6.50 usec
TE                  298.0 K
CNST2          145.0000000
D0              0.00000300 sec
D1              1.50000000 sec
D4              0.00172414 sec
D11             0.03000000 sec
D16             0.00020000 sec
D24             0.00086207 sec
IN0             0.00003000 sec
ZGOPTNS

======== CHANNEL f1 ========
NUC1                    1H
P1                   7.80 usec
P2                  15.60 usec
P28               1000.00 usec
PLW1           9.86279964 W
SFO1          400.0724053 MHz

======== CHANNEL f2 ========
CPDPRG[2              garp
NUC2                   13C
P3                  13.00 usec
P4                  26.00 usec
PCPD2               80.00 usec
PLW2         102.08999634 W
PLW12          2.69589996 W
SFO2          100.6052242 MHz

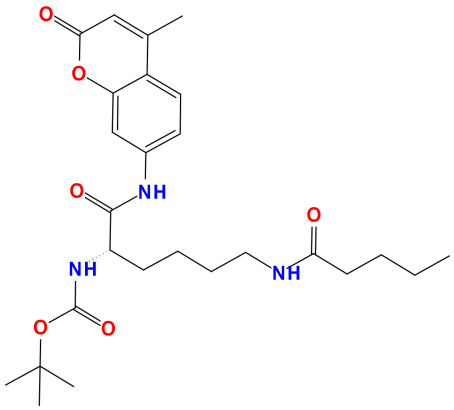
====== GRADIENT CHANNEL =====
GPNAM[1]       SMSQ10.100
GPNAM[2]       SMSQ10.100
GPNAM[3]       SMSQ10.100
GPNAM[4]       SMSQ10.100
GPZ1                80.00 %
GPZ2                20.10 %
GPZ3                11.00 %
GPZ4                -5.00 %
P16               1000.00 usec
P19                600.00 usec

F1 - Acquisition parameters
TD                     256
SFO1            100.6052 MHz
FIDRES         130.197311 Hz
SW                165.650 ppm
FnMODE     Echo-Antiecho

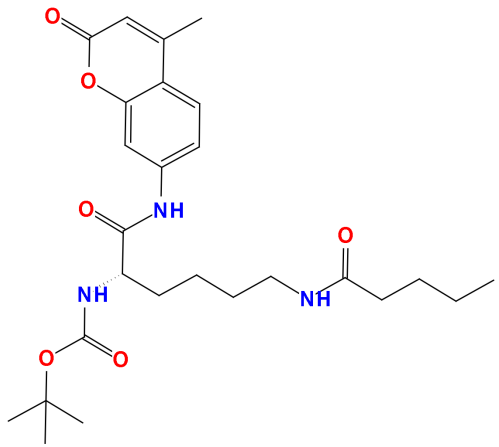
F2 - Processing parameters
SI                    1024
SF           400.0700000 MHz
WDW                  QSINE
SSB                      2
LB         0 Hz
GB         0
PC                    1.00

F1 - Processing parameters
SI                    1024
MC2         echo-antiecho
SF           100.5976820 MHz
WDW                  QSINE
SSB                      2
LB         0 Hz
GB         0
```

1H NMR of **Valeryl-substrate**



HSQC of **Valeryl-substrate**




```
F2 - Acquisition Parameters
Date_           20150901
Time              15.07
INSTRUM           spect
PROBHD   5 mm PABBI 1H/
PULPROG     hsqcetgpsi2
TD                1024
SOLVENT           DMSO
NS                   2
DS                   0
SWH           6602.113 Hz
FIDRES        6.447376 Hz
AQ           0.0775509 sec
RG                2050
DW              75.733 usec
DE               6.50 usec
TE              298.0 K
CNST2       145.0000000
D0           0.00000300 sec
D1           1.50000000 sec
D4           0.00172414 sec
D11          0.03000000 sec
D16          0.00020000 sec
D24          0.00086207 sec
IN0          0.00003000 sec
ZGOPTNS

======== CHANNEL f1 ========
NUC1                1H
P1                7.80 usec
P2               15.60 usec
P28            1000.00 usec
PLW1         9.86279964 W
SFO1        400.0724053 MHz

======== CHANNEL f2 ========
CPDPRG[2            garp
NUC2                13C
P3               13.00 usec
P4               26.00 usec
PCPD2            80.00 usec
PLW2        102.08999634 W
PLW12         2.69589996 W
SFO2         100.6052242 MHz

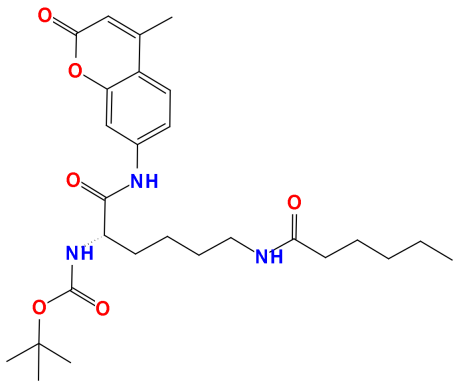
====== GRADIENT CHANNEL =====
GPNAM[1]      SMSQ10.100
GPNAM[2]      SMSQ10.100
GPNAM[3]      SMSQ10.100
GPNAM[4]      SMSQ10.100
GPZ1             80.00 %
GPZ2             20.10 %
GPZ3             11.00 %
GPZ4             -5.00 %
P16            1000.00 usec
P19             600.00 usec

F1 - Acquisition parameters
TD                  256
SFO1           100.6052 MHz
FIDRES       130.197311 Hz
SW             165.650 ppm
FnMODE    Echo-Antiecho

F2 - Processing parameters
SI                 1024
SF           400.0700000 MHz
WDW                QSINE
SSB                    2
LB       0 Hz
GB       0
PC                 1.00

F1 - Processing parameters
SI                 1024
MC2        echo-antiecho
SF           100.5976820 MHz
WDW                QSINE
SSB                    2
LB       0 Hz
GB       0
```

1H NMR of **Hexanoyl-substrate**

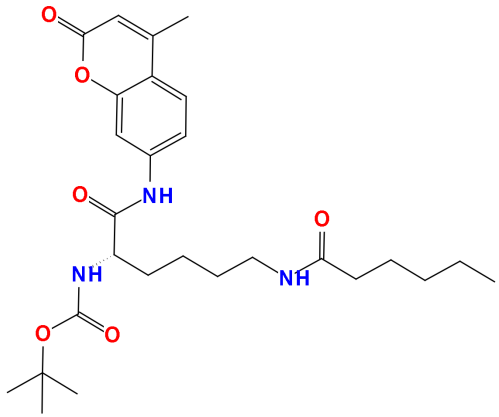



```
F2 - Acquisition Parameters
Date_            20150901
Time                15.46
INSTRUM             spect
PROBHD    5 mm PABBI 1H/
PULPROG              zg30
TD                  30046
SOLVENT              DMSO
NS                       8
DS                       0
SWH              8012.820 Hz
FIDRES           0.266685 Hz
AQ              1.8748704 sec
RG                     181
DW                62.400 usec
DE                 6.50 usec
TE                 298.0 K
D1           1.00000000 sec
TD0                      1

======== CHANNEL f1 ========
NUC1                   1H
P1                  7.80 usec
PLW1          9.86279964 W
SFO1         400.0724688 MHz

F2 - Processing parameters
SI                   65536
SF           400.0700000 MHz
WDW                     EM
SSB       0
LB                  0.30 Hz
GB        0
PC                  1.00
```

HSQC of **Hexanoyl-substrate**




```
F2 - Acquisition Parameters
Date_           20150901
Time              15.51
INSTRUM           spect
PROBHD   5 mm PABBI 1H/
PULPROG     hsqcetgpsi2
TD                1024
SOLVENT           DMSO
NS                   2
DS                   0
SWH            6602.113 Hz
FIDRES         6.447376 Hz
AQ           0.0775509 sec
RG                2050
DW              75.733 usec
DE               6.50 usec
TE              298.0 K
CNST2       145.0000000
D0           0.00000300 sec
D1           1.50000000 sec
D4           0.00172414 sec
D11          0.03000000 sec
D16          0.00020000 sec
D24          0.00086207 sec
IN0          0.00003000 sec
ZGOPTNS

======== CHANNEL f1 ========
NUC1                1H
P1               7.80 usec
P2              15.60 usec
P28           1000.00 usec
PLW1         9.86279964 W
SFO1       400.0724053 MHz

======== CHANNEL f2 ========
CPDPRG[2            garp
NUC2                13C
P3              13.00 usec
P4              26.00 usec
PCPD2           80.00 usec
PLW2       102.08999634 W
PLW12        2.69589996 W
SFO2       100.6052242 MHz

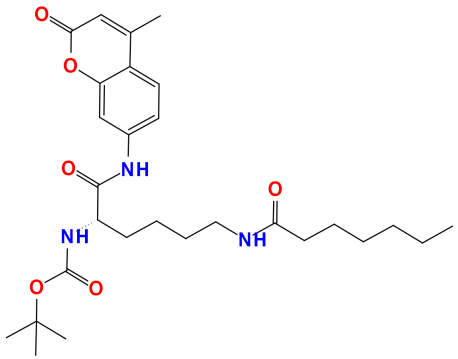
====== GRADIENT CHANNEL =====
GPNAM[1]      SMSQ10.100
GPNAM[2]      SMSQ10.100
GPNAM[3]      SMSQ10.100
GPNAM[4]      SMSQ10.100
GPZ1             80.00 %
GPZ2             20.10 %
GPZ3             11.00 %
GPZ4             -5.00 %
P16           1000.00 usec
P19            600.00 usec

F1 - Acquisition parameters
TD                  256
SFO1          100.6052 MHz
FIDRES       130.197311 Hz
SW            165.650 ppm
FnMODE    Echo-Antiecho

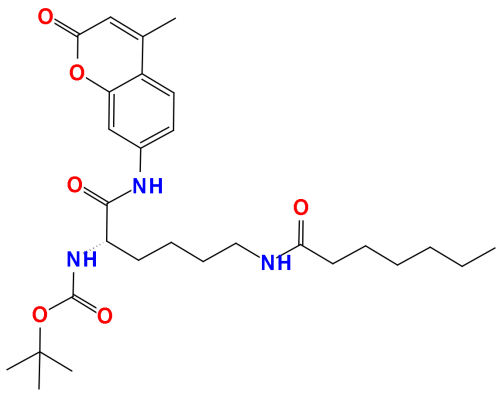
F2 - Processing parameters
SI                 1024
SF           400.0700000 MHz
WDW                QSINE
SSB                    2
LB       0 Hz
GB       0
PC                 1.00

F1 - Processing parameters
SI                 1024
MC2        echo-antiecho
SF           100.5976820 MHz
WDW                QSINE
SSB                    2
LB       0 Hz
GB       0
```

1H NMR of **Heptanoyl-substrate**



HSQC of **Heptanoyl-substrate**




```
F2 - Acquisition Parameters
Date_           20150902
Time              17.09
INSTRUM           spect
PROBHD   5 mm PABBI 1H/
PULPROG     hsqcetgpsi2
TD                1024
SOLVENT           DMSO
NS                   2
DS                   0
SWH           6602.113 Hz
FIDRES        6.447376 Hz
AQ            0.0775509 sec
RG                2050
DW              75.733 usec
DE               6.50 usec
TE              298.0 K
CNST2       145.0000000
D0           0.00000300 sec
D1           1.50000000 sec
D4           0.00172414 sec
D11          0.03000000 sec
D16          0.00020000 sec
D24          0.00086207 sec
IN0          0.00003000 sec
ZGOPTNS

======== CHANNEL f1 ========
NUC1                1H
P1                7.80 usec
P2               15.60 usec
P28            1000.00 usec
PLW1         9.86279964 W
SFO1        400.0724053 MHz

======== CHANNEL f2 ========
CPDPRG[2            garp
NUC2                13C
P3               13.00 usec
P4               26.00 usec
PCPD2            80.00 usec
PLW2       102.08999634 W
PLW12        2.69589996 W
SFO2        100.6052242 MHz

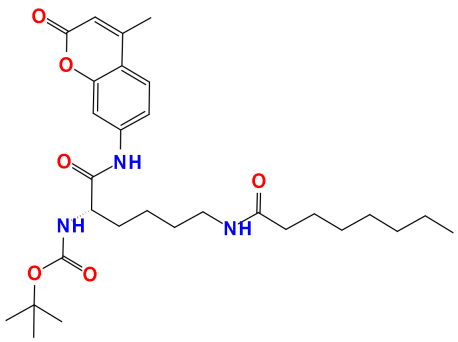
====== GRADIENT CHANNEL =====
GPNAM[1]      SMSQ10.100
GPNAM[2]      SMSQ10.100
GPNAM[3]      SMSQ10.100
GPNAM[4]      SMSQ10.100
GPZ1             80.00 %
GPZ2             20.10 %
GPZ3             11.00 %
GPZ4             -5.00 %
P16            1000.00 usec
P19             600.00 usec

F1 - Acquisition parameters
TD                  256
SFO1           100.6052 MHz
FIDRES       130.197311 Hz
SW             165.650 ppm
FnMODE    Echo-Antiecho

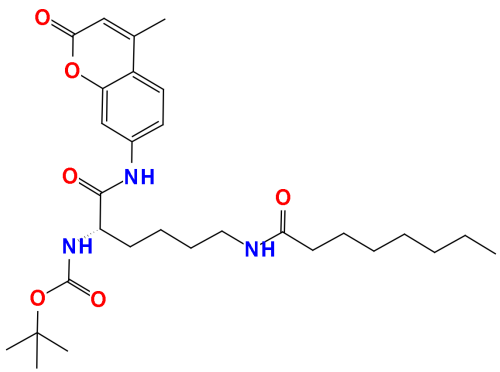
F2 - Processing parameters
SI                 1024
SF          400.0700000 MHz
WDW                QSINE
SSB                    2
LB       0 Hz
GB       0
PC                 1.00

F1 - Processing parameters
SI                 1024
MC2        echo-antiecho
SF           100.5976820 MHz
WDW                QSINE
SSB                    2
LB       0 Hz
GB       0
```

1H NMR of **Octanoyl-substrate**



HSQC of **Octanoyl-substrate**




```
F2 - Acquisition Parameters
Date_           20150902
Time              15.45
INSTRUM           spect
PROBHD   5 mm PABBI 1H/
PULPROG     hsqcetgpsi2
TD                1024
SOLVENT           DMSO
NS                   2
DS                   0
SWH           6602.113 Hz
FIDRES        6.447376 Hz
AQ           0.0775509 sec
RG                2050
DW              75.733 usec
DE               6.50 usec
TE              298.0 K
CNST2       145.0000000
D0           0.00000300 sec
D1           1.50000000 sec
D4           0.00172414 sec
D11          0.03000000 sec
D16          0.00020000 sec
D24          0.00086207 sec
IN0          0.00003000 sec
ZGOPTNS

======== CHANNEL f1 ========
NUC1                1H
P1               7.80 usec
P2              15.60 usec
P28           1000.00 usec
PLW1         9.86279964 W
SFO1        400.0724053 MHz

======== CHANNEL f2 ========
CPDPRG[2            garp
NUC2                13C
P3              13.00 usec
P4              26.00 usec
PCPD2           80.00 usec
PLW2       102.08999634 W
PLW12        2.69589996 W
SFO2        100.6052242 MHz

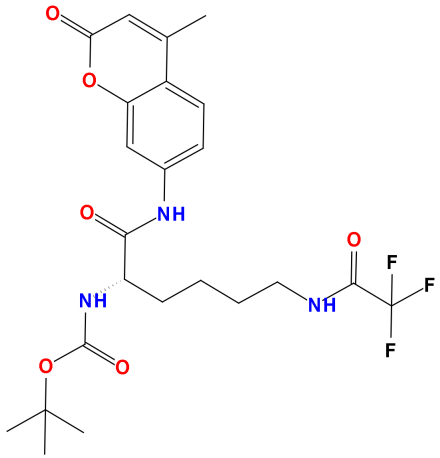
====== GRADIENT CHANNEL =====
GPNAM[1]      SMSQ10.100
GPNAM[2]      SMSQ10.100
GPNAM[3]      SMSQ10.100
GPNAM[4]      SMSQ10.100
GPZ1             80.00 %
GPZ2             20.10 %
GPZ3             11.00 %
GPZ4             -5.00 %
P16           1000.00 usec
P19            600.00 usec

F1 - Acquisition parameters
TD                  256
SFO1           100.6052 MHz
FIDRES       130.197311 Hz
SW             165.650 ppm
FnMODE    Echo-Antiecho

F2 - Processing parameters
SI                 1024
SF          400.0700000 MHz
WDW               QSINE
SSB                   2
LB       0 Hz
GB       0
PC                 1.00

F1 - Processing parameters
SI                 1024
MC2        echo-antiecho
SF           100.5976820 MHz
WDW               QSINE
SSB                   2
LB       0 Hz
GB       0
```

1H NMR of **TFA-substrate**

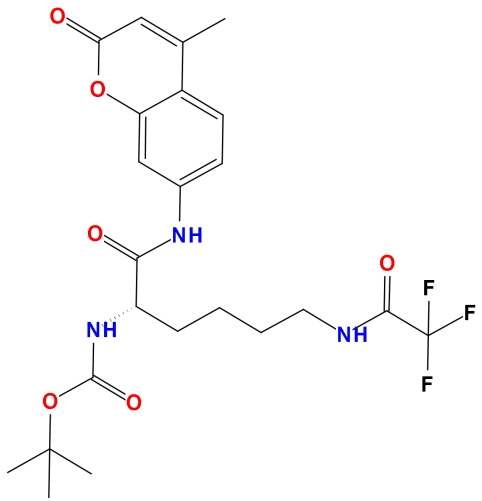


```
F2 - Acquisition Parameters
Date_           20150901
Time              16.31
INSTRUM           spect
PROBHD   5 mm PABBI 1H/
PULPROG           zg30
TD               30046
SOLVENT           DMSO
NS                   8
DS                   0
SWH            8012.820 Hz
FIDRES         0.266685 Hz
AQ             1.8748704 sec
RG                 181
DW              62.400 usec
DE               6.50 usec
TE               298.0 K
D1          1.00000000 sec
TD0                  1

======== CHANNEL f1 ========
NUC1                1H
P1               7.80 usec
PLW1         9.86279964 W
SFO1        400.0724688 MHz

F2 - Processing parameters
SI                65536
SF          400.0700000 MHz
WDW                 EM
SSB       0
LB              0.30 Hz
GB        0
PC              1.00
```

HSQC of **TFA-substrate**




```
F2 - Acquisition Parameters
Date_           20150901
Time              16.35
INSTRUM           spect
PROBHD  5 mm PABBI 1H/
PULPROG     hsqcetgpsi2
TD                1024
SOLVENT           DMSO
NS                   2
DS                   0
SWH            6602.113 Hz
FIDRES         6.447376 Hz
AQ           0.0775509 sec
RG                2050
DW              75.733 usec
DE               6.50 usec
TE              298.0 K
CNST2       145.0000000
D0           0.00000300 sec
D1           1.50000000 sec
D4           0.00172414 sec
D11          0.03000000 sec
D16          0.00020000 sec
D24          0.00086207 sec
IN0          0.00003000 sec
ZGOPTNS

======== CHANNEL f1 ========
NUC1                1H
P1               7.80 usec
P2              15.60 usec
P28           1000.00 usec
PLW1         9.86279964 W
SFO1        400.0724053 MHz

======== CHANNEL f2 ========
CPDPRG[2            garp
NUC2                13C
P3              13.00 usec
P4              26.00 usec
PCPD2           80.00 usec
PLW2        102.08999634 W
PLW12         2.69589996 W
SFO2         100.6052242 MHz

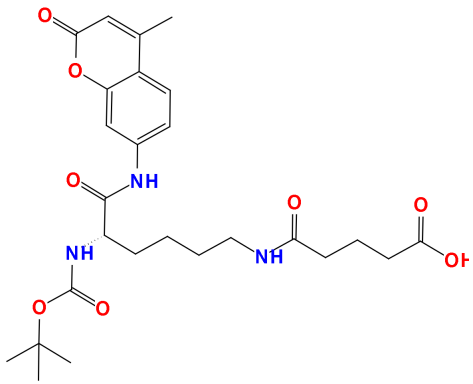
====== GRADIENT CHANNEL =====
GPNAM[1]      SMSQ10.100
GPNAM[2]      SMSQ10.100
GPNAM[3]      SMSQ10.100
GPNAM[4]      SMSQ10.100
GPZ1             80.00 %
GPZ2             20.10 %
GPZ3             11.00 %
GPZ4             -5.00 %
P16           1000.00 usec
P19            600.00 usec

F1 - Acquisition parameters
TD                  256
SFO1          100.6052 MHz
FIDRES       130.197311 Hz
SW             165.650 ppm
FnMODE    Echo-Antiecho

F2 - Processing parameters
SI                 1024
SF           400.0700000 MHz
WDW                QSINE
SSB                    2
LB       0 Hz
GB       0
PC                 1.00

F1 - Processing parameters
SI                 1024
MC2        echo-antiecho
SF           100.5976820 MHz
WDW                QSINE
SSB                    2
LB       0 Hz
GB       0
```

1H NMR of **Glutaryl-substrate**

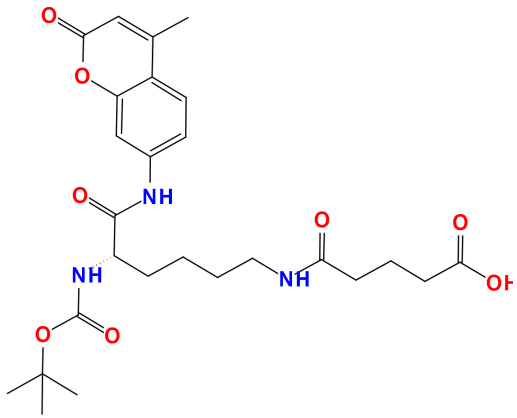


```
F2 - Acquisition Parameters
Date_            20150902
Time               16.03
INSTRUM            spect
PROBHD   5 mm PABBI 1H/
PULPROG             zg30
TD                 30046
SOLVENT             DMSO
NS                     8
DS                     0
SWH             8012.820 Hz
FIDRES          0.266685 Hz
AQ              1.8748704 sec
RG                  71.8
DW                62.400 usec
DE                 6.50 usec
TE                298.0 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                 7.80 usec
PLW1          9.86279964 W
SFO1        400.0724688 MHz

F2 - Processing parameters
SI                 65536
SF          400.0700000 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

HSQC of **Glutaryl-substrate**




```
F2 - Acquisition Parameters
Date_           20150902
Time              16.06
INSTRUM           spect
PROBHD   5 mm PABBI 1H/
PULPROG     hsqcetgpsi2
TD                1024
SOLVENT           DMSO
NS                   2
DS                   0
SWH           6602.113 Hz
FIDRES        6.447376 Hz
AQ            0.0775509 sec
RG                2050
DW              75.733 usec
DE               6.50 usec
TE              298.0 K
CNST2       145.0000000
D0           0.00000300 sec
D1           1.50000000 sec
D4           0.00172414 sec
D11          0.03000000 sec
D16          0.00020000 sec
D24          0.00086207 sec
IN0          0.00003000 sec
ZGOPTNS

======== CHANNEL f1 ========
NUC1                1H
P1               7.80 usec
P2              15.60 usec
P28           1000.00 usec
PLW1         9.86279964 W
SFO1        400.0724053 MHz

======== CHANNEL f2 ========
CPDPRG[2            garp
NUC2                13C
P3              13.00 usec
P4              26.00 usec
PCPD2           80.00 usec
PLW2       102.08999634 W
PLW12        2.69589996 W
SFO2        100.6052242 MHz

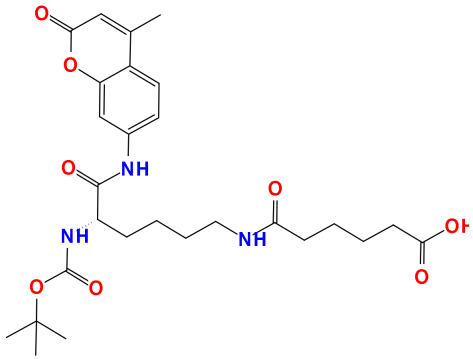
====== GRADIENT CHANNEL =====
GPNAM[1]      SMSQ10.100
GPNAM[2]      SMSQ10.100
GPNAM[3]      SMSQ10.100
GPNAM[4]      SMSQ10.100
GPZ1             80.00 %
GPZ2             20.10 %
GPZ3             11.00 %
GPZ4             -5.00 %
P16           1000.00 usec
P19            600.00 usec

F1 - Acquisition parameters
TD                  256
SFO1          100.6052 MHz
FIDRES       130.197311 Hz
SW            165.650 ppm
FnMODE    Echo-Antiecho

F2 - Processing parameters
SI                 1024
SF          400.0700000 MHz
WDW                QSINE
SSB                    2
LB       0 Hz
GB       0
PC                 1.00

F1 - Processing parameters
SI                 1024
MC2        echo-antiecho
SF          100.5976820 MHz
WDW                QSINE
SSB                    2
LB       0 Hz
GB       0
```

1H NMR of **Adipoyl-substrate**

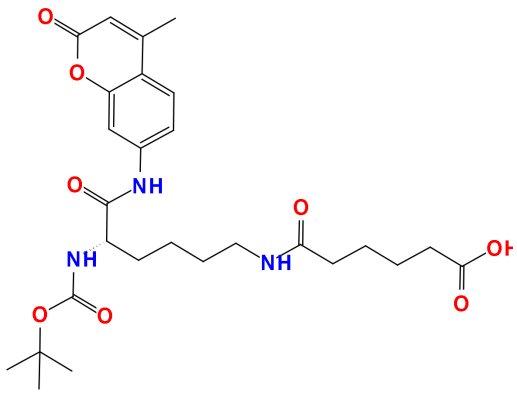


```
F2 - Acquisition Parameters
Date_            20150902
Time               16.24
INSTRUM            spect
PROBHD   5 mm PABBI 1H/
PULPROG             zg30
TD                 30046
SOLVENT             DMSO
NS                     8
DS                     0
SWH             8012.820 Hz
FIDRES          0.266685 Hz
AQ              1.8748704 sec
RG                   101
DW                62.400 usec
DE                 6.50 usec
TE                298.0 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                  7.80 usec
PLW1          9.86279964 W
SFO1        400.0724688 MHz

F2 - Processing parameters
SI                 65536
SF          400.0700000 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

HSQC of **Adipoyl-substrate**




```
F2 - Acquisition Parameters
Date_           20150901
Time              16.35
INSTRUM           spect
PROBHD   5 mm PABBI 1H/
PULPROG     hsqcetgpsi2
TD                1024
SOLVENT           DMSO
NS                   2
DS                   0
SWH            6602.113 Hz
FIDRES         6.447376 Hz
AQ             0.0775509 sec
RG                2050
DW               75.733 usec
DE                6.50 usec
TE               298.0 K
CNST2       145.0000000
D0           0.00000300 sec
D1           1.50000000 sec
D4           0.00172414 sec
D11          0.03000000 sec
D16          0.00020000 sec
D24          0.00086207 sec
IN0          0.00003000 sec
ZGOPTNS

======== CHANNEL f1 ========
NUC1                1H
P1                7.80 usec
P2               15.60 usec
P28            1000.00 usec
PLW1         9.86279964 W
SFO1        400.0724053 MHz

======== CHANNEL f2 ========
CPDPRG[2            garp
NUC2                13C
P3               13.00 usec
P4               26.00 usec
PCPD2            80.00 usec
PLW2       102.08999634 W
PLW12        2.69589996 W
SFO2        100.6052242 MHz

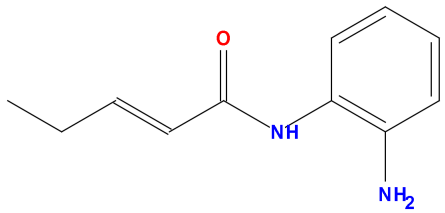
====== GRADIENT CHANNEL =====
GPNAM[1]      SMSQ10.100
GPNAM[2]      SMSQ10.100
GPNAM[3]      SMSQ10.100
GPNAM[4]      SMSQ10.100
GPZ1             80.00 %
GPZ2             20.10 %
GPZ3             11.00 %
GPZ4             -5.00 %
P16            1000.00 usec
P19             600.00 usec

F1 - Acquisition parameters
TD                 256
SFO1          100.6052 MHz
FIDRES       130.197311 Hz
SW             165.650 ppm
FnMODE    Echo-Antiecho

F2 - Processing parameters
SI                1024
SF          400.0700000 MHz
WDW               QSINE
SSB                  2
LB       0 Hz
GB       0
PC                1.00

F1 - Processing parameters
SI                1024
MC2       echo-antiecho
SF          100.5976820 MHz
WDW               QSINE
SSB                  2
LB       0 Hz
GB       0
```

1H NMR of **1**



9.25 7.26 7.24 6.94 6.94 6.92 6.90 6.90 6.89 6.89 6.88 6.88 6.87 6.85 6.84 6.83 6.81 6.80 6.76 6.76 6.74 6.74 6.60 6.59 6.58 6.57 6.56 6.55 6.19 6.15 4.87

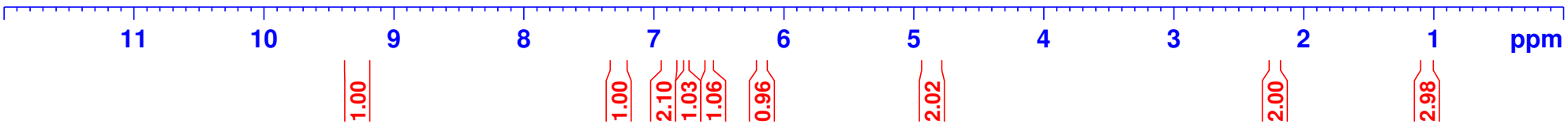
2.24 2.22 2.21

1.07 1.05 1.04

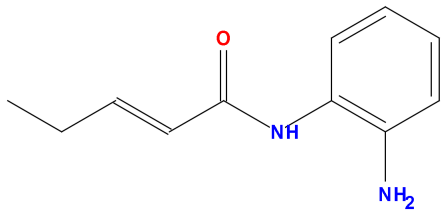
F2 - Acquisition Parameters
Date_ 20141007
Time 14.57
INSTRUM spect
PROBHD 5 mm PABBI 1H/
PULPROG zg30
TD 30046
SOLVENT DMSO
NS 8
DS 0
SWH 8012.820 Hz
FIDRES 0.266685 Hz
AQ 1.8748704 sec
RG 22.6
DW 62.400 usec
DE 6.50 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 1H
P1 7.80 usec
PLW1 9.86279964 W
SFO1 400.0724688 MHz

F2 - Processing parameters
SI 65536
SF 400.0700000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



13C NMR of **1**



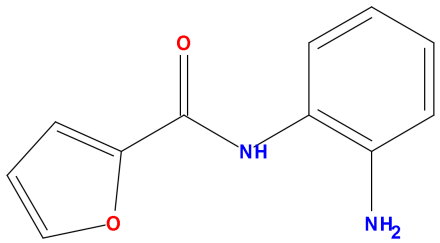

```
F2 - Acquisition Parameters
Date_           20141007
Time              15.11
INSTRUM           spect
PROBHD   5 mm PABBI 1H/
PULPROG          zgpg30
TD                30046
SOLVENT             DMSO
NS                  128
DS                    8
SWH           25252.525 Hz
FIDRES         0.840462 Hz
AQ            0.5949108 sec
RG                 1290
DW               19.800 usec
DE                6.50 usec
TE               298.0 K
D1           4.00000000 sec
D11          0.03000000 sec
TD0                  1

======== CHANNEL f1 ========
NUC1                13C
P1                13.70 usec
PLW1       102.08999634 W
SFO1        100.6077418 MHz

======== CHANNEL f2 ========
CPDPRG[2            garp
NUC2                1H
PCPD2             90.00 usec
PLW2          6.30959988 W
PLW12         0.07789600 W
PLW13         0.06309600 W
SFO2         400.0718803 MHz

F2 - Processing parameters
SI                65536
SF           100.5976820 MHz
WDW                 EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```

1H NMR of **1a**



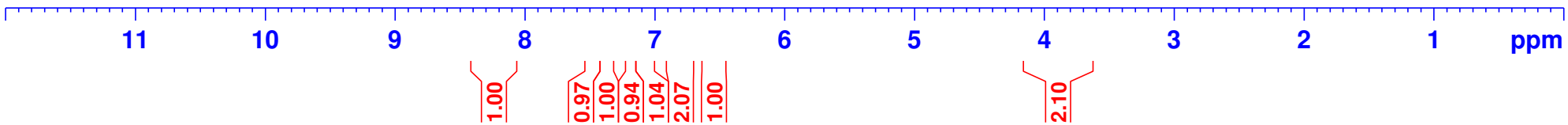
8.23 7.47 7.47 7.35 7.33 7.19 7.18 7.09 7.08 7.07 7.06 7.05 7.04 6.83 6.81 6.79 6.52 6.52 6.51 6.51

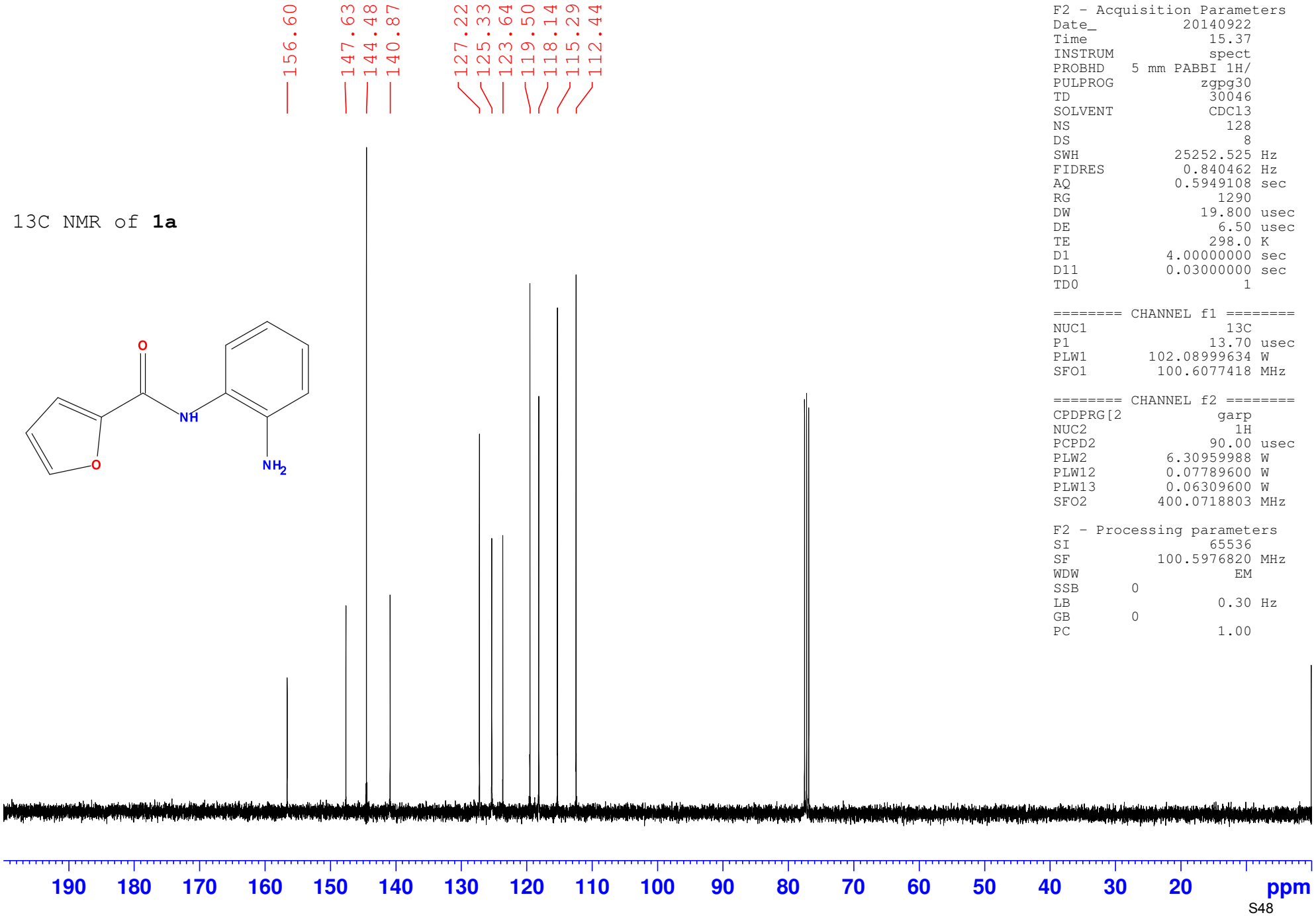
3.94

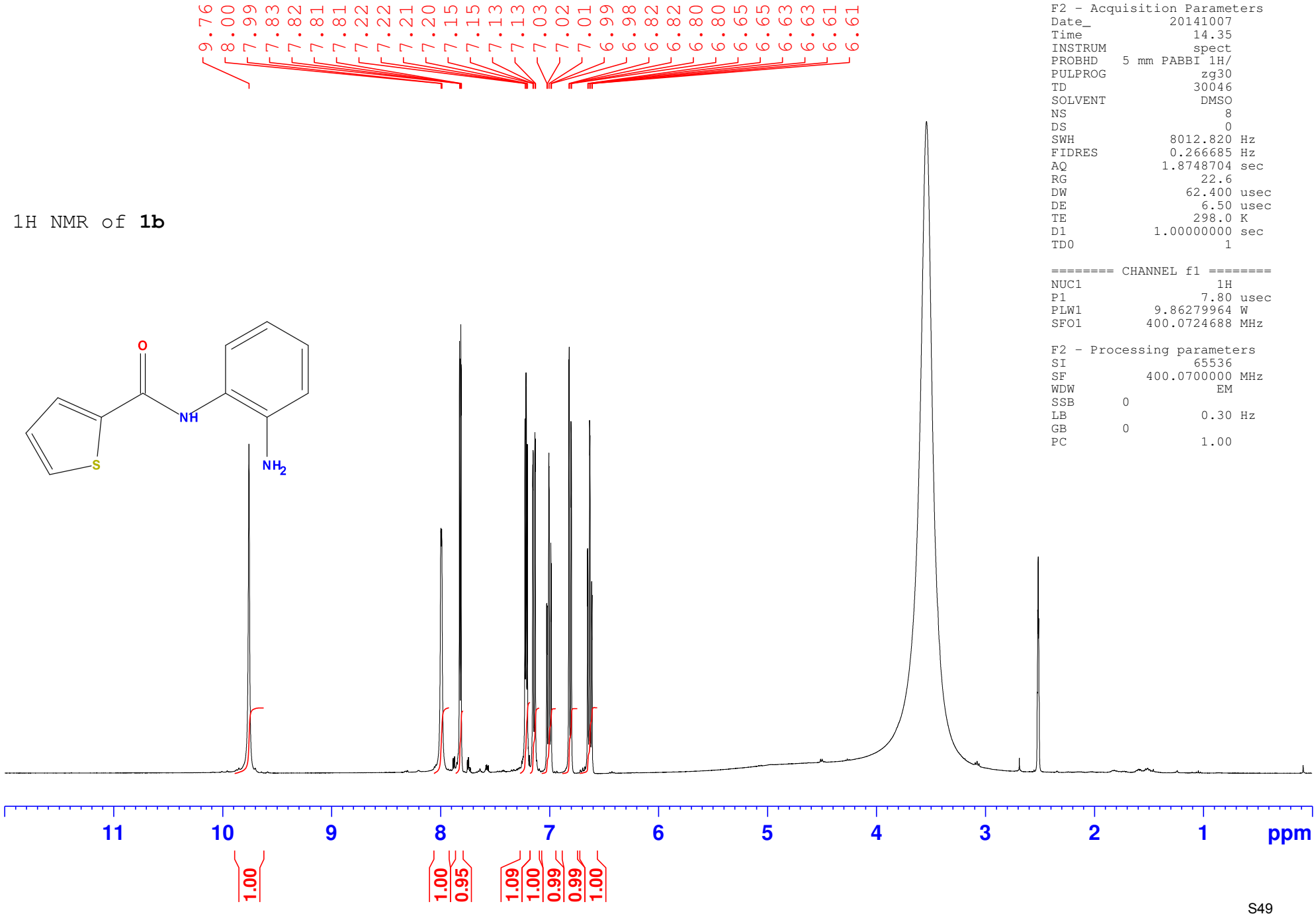
```
F2 - Acquisition Parameters
Date_            20140922
Time               15.21
INSTRUM            spect
PROBHD   5 mm PABBI 1H/
PULPROG             zg30
TD                 30046
SOLVENT            CDCl3
NS                     8
DS                     0
SWH             8012.820 Hz
FIDRES          0.266685 Hz
AQ             1.8748704 sec
RG                  20.2
DW                62.400 usec
DE                 6.50 usec
TE                298.0 K
D1           1.00000000 sec
TD0                    1

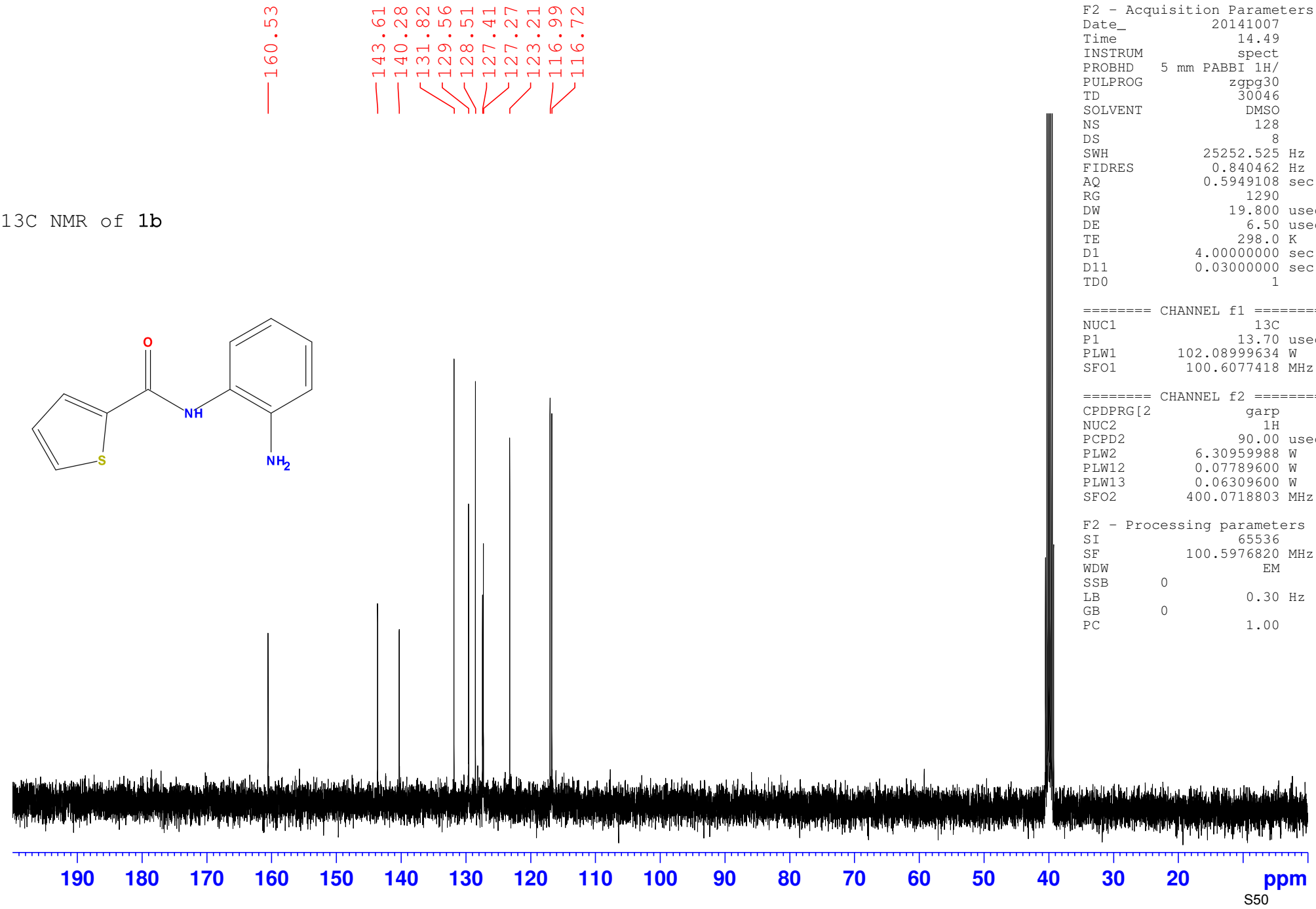
======== CHANNEL f1 ========
NUC1                  1H
P1                  7.80 usec
PLW1          9.86279964 W
SFO1        400.0724688 MHz

F2 - Processing parameters
SI                 65536
SF          400.0700000 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```




156.60
147.63
144.48
140.87
127.22
125.33
123.64
119.50
118.14
115.29
112.44
13C NMR of 1a
O
NH
O
NH2
F2 - Acquisition Parameters
Date_ 20140922
Time 15.37
INSTRUM spect
PROBHD 5 mm PABBI 1H/
PULPROG zgpg30
TD 30046
SOLVENT CDCl3
NS 128
DS 8
SWH 25252.525 Hz
FIDRES 0.840462 Hz
AQ 0.5949108 sec
RG 1290
DW 19.800 usec
DE 6.50 usec
TE 298.0 K
D1 4.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 13.70 usec
PLW1 102.08999634 W
SFO1 100.6077418 MHz
======== CHANNEL f2 ========
CPDPRG[2 garp
NUC2 1H
PCPD2 90.00 usec
PLW2 6.30959988 W
PLW12 0.07789600 W
PLW13 0.06309600 W
SFO2 400.0718803 MHz
F2 - Processing parameters
SI 65536
SF 100.5976820 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm


9.76
8.00
7.99
7.83
7.82
7.81
7.81
7.22
7.22
7.21
7.20
7.15
7.15
7.13
7.13
7.03
7.02
7.01
6.99
6.98
6.82
6.82
6.80
6.80
6.65
6.65
6.63
6.63
6.61
6.61
1H NMR of 1b
O
NH
NH2
S
F2 - Acquisition Parameters
Date_ 20141007
Time 14.35
INSTRUM spect
PROBHD 5 mm PABBI 1H/
PULPROG zg30
TD 30046
SOLVENT DMSO
NS 8
DS 0
SWH 8012.820 Hz
FIDRES 0.266685 Hz
AQ 1.8748704 sec
RG 22.6
DW 62.400 usec
DE 6.50 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 7.80 usec
PLW1 9.86279964 W
SFO1 400.0724688 MHz
F2 - Processing parameters
SI 65536
SF 400.0700000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
11
10
9
8
7
6
5
4
3
2
1
ppm
1.00
1.00
0.95
1.09
1.00
0.99
0.99
1.00

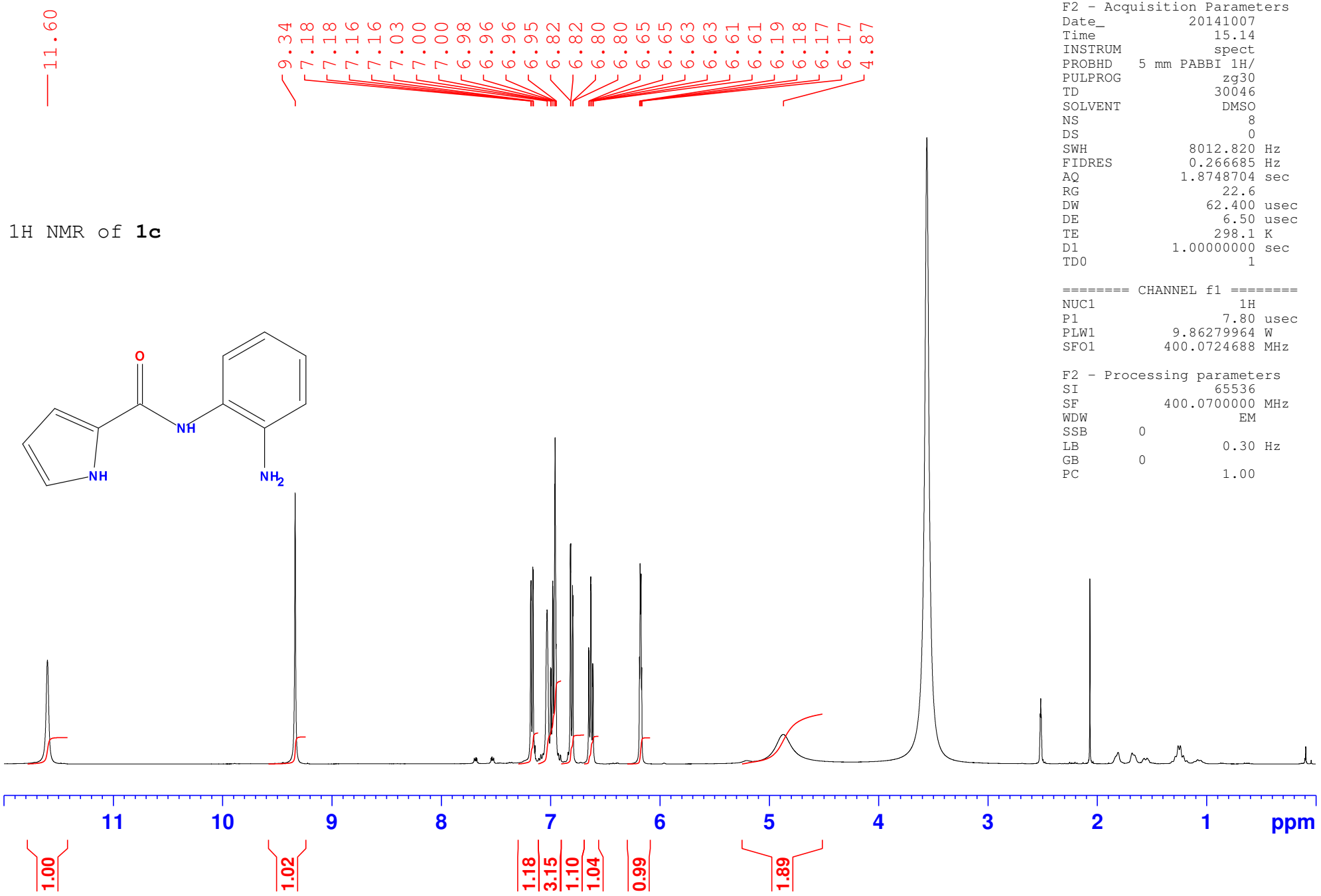



```
F2 - Acquisition Parameters
Date_           20141007
Time               14.49
INSTRUM            spect
PROBHD   5 mm PABBI 1H/
PULPROG          zgpg30
TD                30046
SOLVENT            DMSO
NS                   128
DS                     8
SWH          25252.525 Hz
FIDRES        0.840462 Hz
AQ           0.5949108 sec
RG                  1290
DW             19.800 usec
DE              6.50 usec
TE                298.0 K
D1          4.00000000 sec
D11         0.03000000 sec
TD0                    1

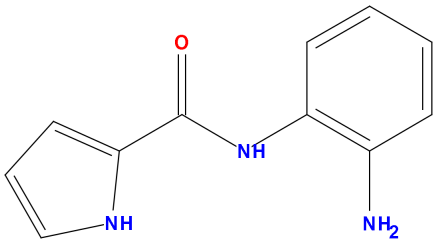
======== CHANNEL f1 ========
NUC1                13C
P1               13.70 usec
PLW1       102.08999634 W
SFO1        100.6077418 MHz

======== CHANNEL f2 ========
CPDPRG[2            garp
NUC2                 1H
PCPD2             90.00 usec
PLW2         6.30959988 W
PLW12        0.07789600 W
PLW13        0.06309600 W
SFO2        400.0718803 MHz

F2 - Processing parameters
SI                 65536
SF          100.5976820 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```


11.60
9.34
7.18
7.18
7.16
7.16
7.03
7.00
7.00
6.98
6.96
6.96
6.95
6.82
6.82
6.80
6.80
6.65
6.65
6.63
6.63
6.61
6.61
6.19
6.18
6.17
6.17
4.87
1H NMR of 1c
O
NH
NH
NH2
F2 - Acquisition Parameters
Date_ 20141007
Time 15.14
INSTRUM spect
PROBHD 5 mm PABBI 1H/
PULPROG zg30
TD 30046
SOLVENT DMSO
NS 8
DS 0
SWH 8012.820 Hz
FIDRES 0.266685 Hz
AQ 1.8748704 sec
RG 22.6
DW 62.400 usec
DE 6.50 usec
TE 298.1 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 7.80 usec
PLW1 9.86279964 W
SFO1 400.0724688 MHz
F2 - Processing parameters
SI 65536
SF 400.0700000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
11
10
9
8
7
6
5
4
3
2
1
ppm
1.00
1.02
1.18
3.15
1.10
1.04
0.99
1.89

13C NMR of **1c**



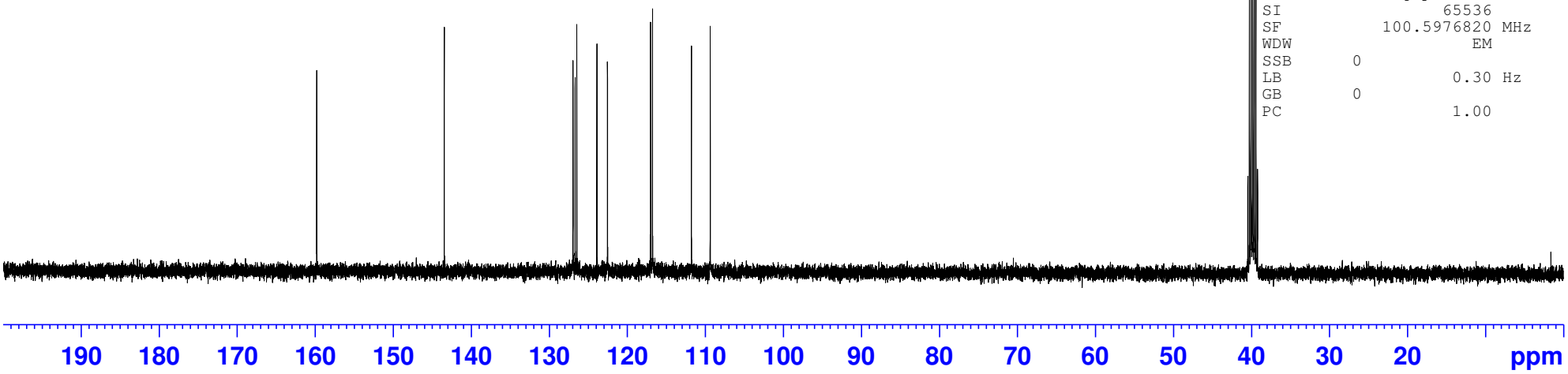
159.80
143.45
126.95
126.67
126.45
123.88
122.53
117.00
116.75
111.76
109.35

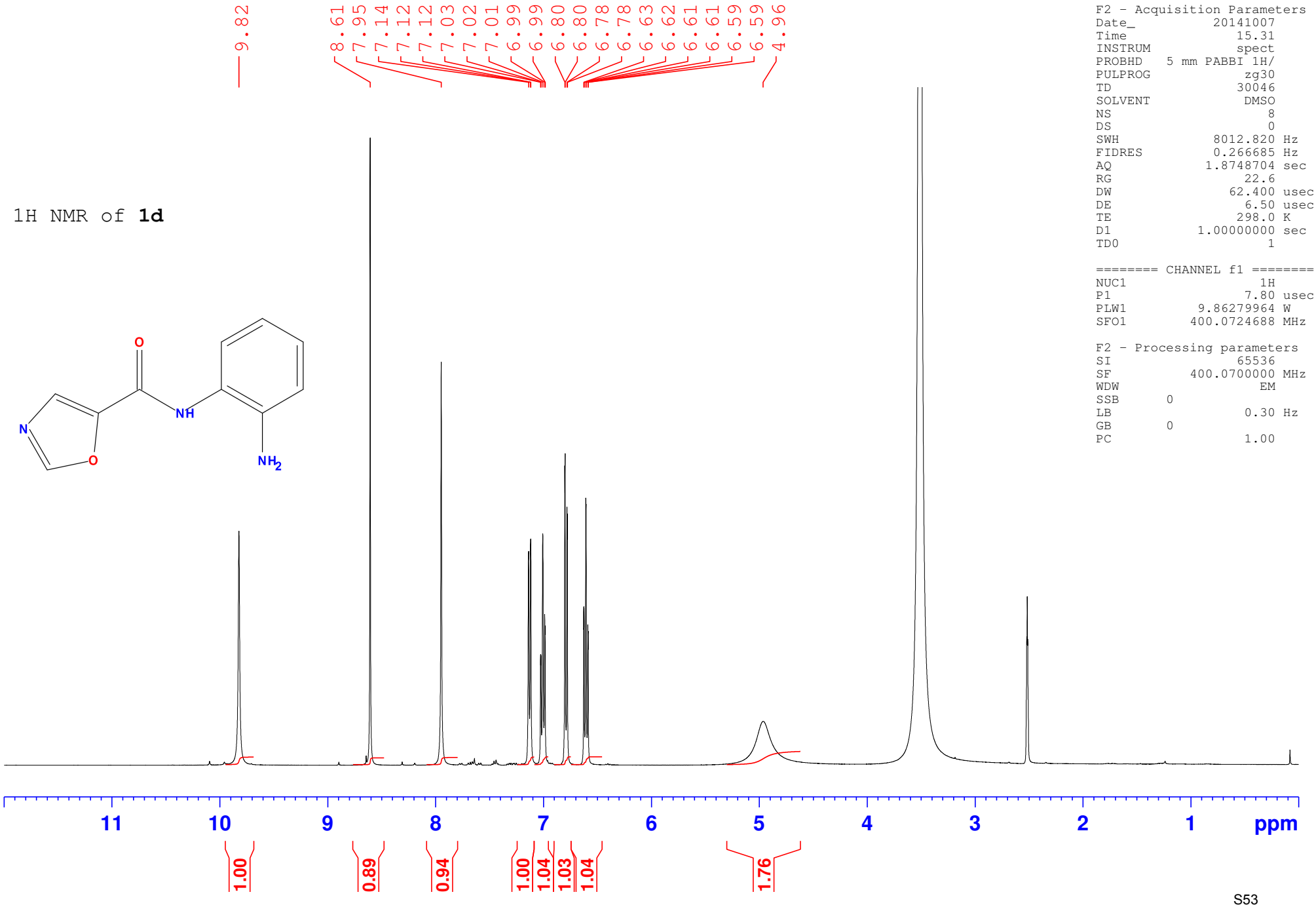
```
F2 - Acquisition Parameters
Date_           20141007
Time               15.28
INSTRUM            spect
PROBHD   5 mm PABBI 1H/
PULPROG          zgpg30
TD                30046
SOLVENT             DMSO
NS                    128
DS                      8
SWH           25252.525 Hz
FIDRES         0.840462 Hz
AQ             0.5949108 sec
RG                 1290
DW                19.800 usec
DE                 6.50 usec
TE                298.0 K
D1           4.00000000 sec
D11          0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                13C
P1                13.70 usec
PLW1       102.08999634 W
SFO1        100.6077418 MHz

======== CHANNEL f2 ========
CPDPRG[2            garp
NUC2                 1H
PCPD2             90.00 usec
PLW2          6.30959988 W
PLW12         0.07789600 W
PLW13         0.06309600 W
SFO2        400.0718803 MHz

F2 - Processing parameters
SI                 65536
SF          100.5976820 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```





F2 - Acquisition Parameters

Date_	20141007
Time	15.31
INSTRUM	spect
PROBHD	5 mm PABBI 1H/
PULPROG	zg30
TD	30046
SOLVENT	DMSO
NS	8
DS	0
SWH	8012.820 Hz
FIDRES	0.266685 Hz
AQ	1.8748704 sec
RG	22.6
DW	62.400 usec
DE	6.50 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1

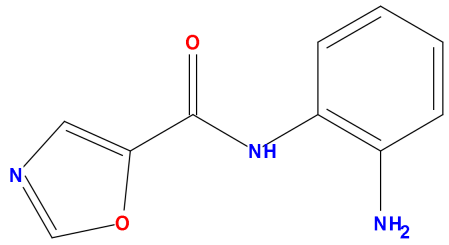
======== CHANNEL f1 ========

NUC1	1H
P1	7.80 usec
PLW1	9.86279964 W
SFO1	400.0724688 MHz

F2 - Processing parameters

SI	65536
SF	400.0700000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

13C NMR of **1d**



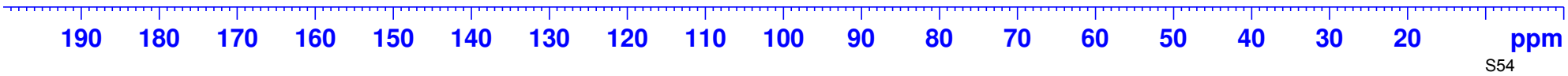
155.79
153.97
145.88
143.86
130.14
127.58
127.46
122.05
116.72
116.51

```
F2 - Acquisition Parameters
Date_           20141007
Time               15.45
INSTRUM            spect
PROBHD   5 mm PABBI 1H/
PULPROG          zgpg30
TD                30046
SOLVENT             DMSO
NS                    128
DS                      8
SWH           25252.525 Hz
FIDRES         0.840462 Hz
AQ             0.5949108 sec
RG                 1290
DW                19.800 usec
DE                 6.50 usec
TE                298.0 K
D1           4.00000000 sec
D11          0.03000000 sec
TD0                   1

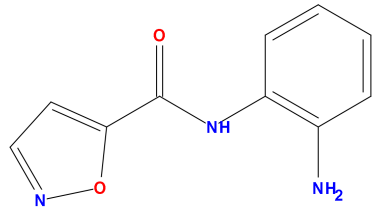
======== CHANNEL f1 ========
NUC1                 13C
P1                 13.70 usec
PLW1       102.08999634 W
SFO1        100.6077418 MHz

======== CHANNEL f2 ========
CPDPRG[2             garp
NUC2                 1H
PCPD2              90.00 usec
PLW2          6.30959988 W
PLW12         0.07789600 W
PLW13         0.06309600 W
SFO2         400.0718803 MHz

F2 - Processing parameters
SI                 65536
SF          100.5976820 MHz
WDW                  EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```



1H NMR of **1e**



10.09
8.78 8.78 7.22 7.22 7.14 7.14 7.12 7.12 7.04 7.04 7.02 7.00 7.00 6.80 6.80 6.78 6.78 6.63 6.62 6.61 6.60 6.59 6.59 5.00

F2 - Acquisition Parameters

Date_	20141007
Time	15.49
INSTRUM	spect
PROBHD	5 mm PABBI 1H/
PULPROG	zg30
TD	30046
SOLVENT	DMSO
NS	8
DS	0
SWH	8012.820 Hz
FIDRES	0.266685 Hz
AQ	1.8748704 sec
RG	22.6
DW	62.400 usec
DE	6.50 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1

======== CHANNEL f1 ========

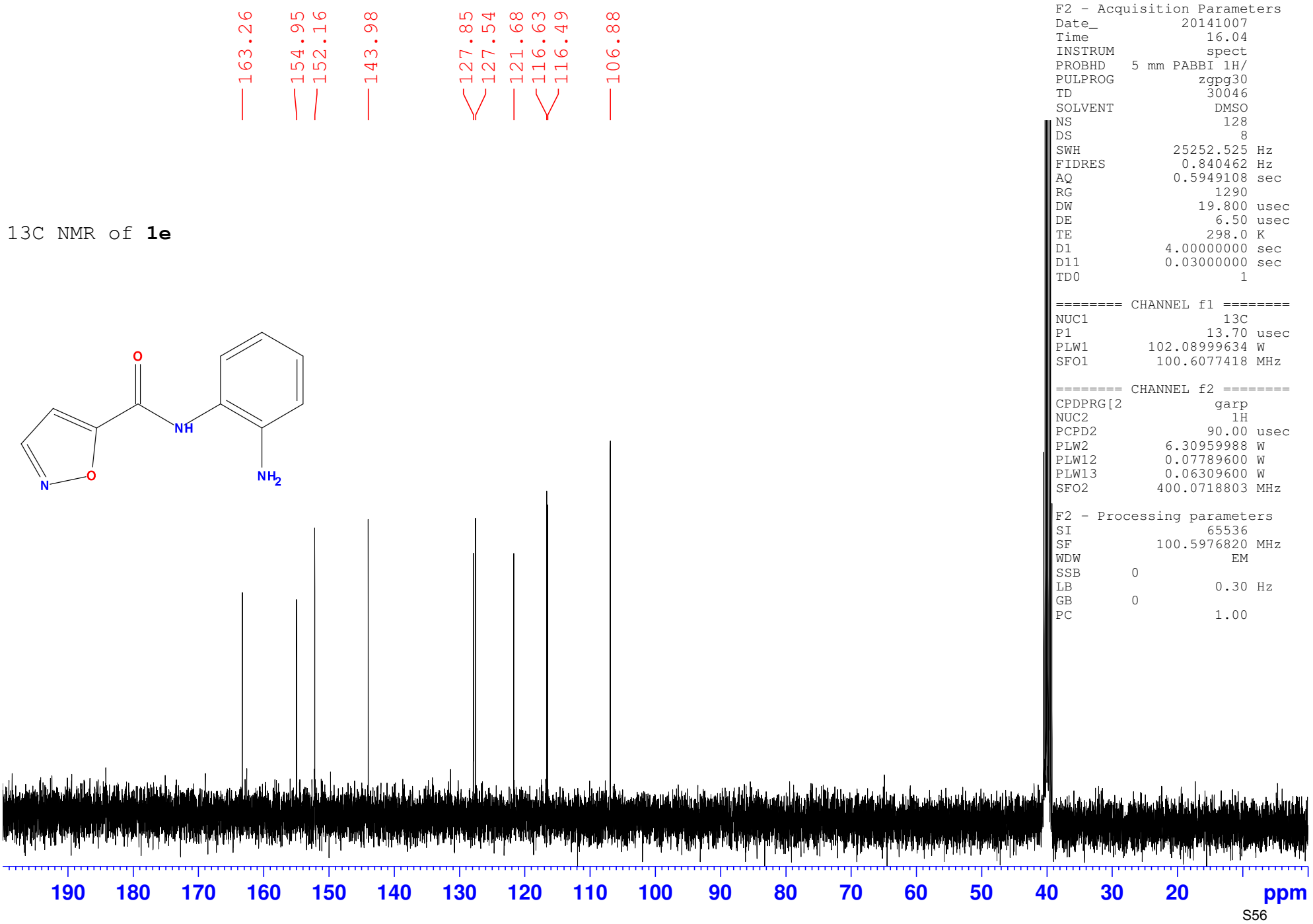
NUC1	1H
P1	7.80 usec
PLW1	9.86279964 W
SFO1	400.0724688 MHz

F2 - Processing parameters

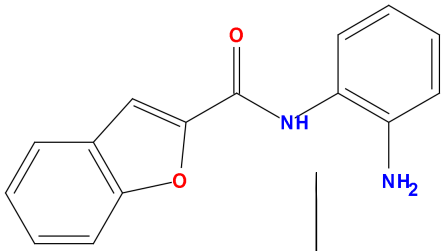
SI	65536
SF	400.0700000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

11 10 9 8 7 6 5 4 3 2 1 ppm

1.00 0.97 1.02 1.08 1.11 1.11 1.09 2.05


163.26
154.95
152.16
143.98
127.85
127.54
121.68
116.63
116.49
106.88
13C NMR of 1e
O
NH
N
O
NH2
F2 - Acquisition Parameters
Date_ 20141007
Time 16.04
INSTRUM spect
PROBHD 5 mm PABBI 1H/
PULPROG zgpg30
TD 30046
SOLVENT DMSO
NS 128
DS 8
SWH 25252.525 Hz
FIDRES 0.840462 Hz
AQ 0.5949108 sec
RG 1290
DW 19.800 usec
DE 6.50 usec
TE 298.0 K
D1 4.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 13.70 usec
PLW1 102.08999634 W
SFO1 100.6077418 MHz
======== CHANNEL f2 ========
CPDPRG[2 garp
NUC2 1H
PCPD2 90.00 usec
PLW2 6.30959988 W
PLW12 0.07789600 W
PLW13 0.06309600 W
SFO2 400.0718803 MHz
F2 - Processing parameters
SI 65536
SF 100.5976820 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

1H NMR of **1f**

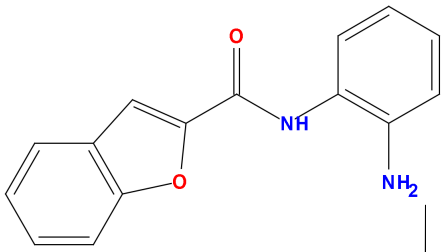



```
F2 - Acquisition Parameters
Date_            20141008
Time                16.32
INSTRUM             spect
PROBHD   5 mm PABBI 1H/
PULPROG              zg30
TD                  30046
SOLVENT              DMSO
NS                       8
DS                       0
SWH             8012.820 Hz
FIDRES          0.266685 Hz
AQ             1.8748704 sec
RG                    22.6
DW                62.400 usec
DE                 6.50 usec
TE                298.0 K
D1            1.00000000 sec
TD0                      1

======== CHANNEL f1 ========
NUC1                    1H
P1                   7.80 usec
PLW1          9.86279964 W
SFO1         400.0724688 MHz

F2 - Processing parameters
SI                   65536
SF           400.0700000 MHz
WDW                     EM
SSB       0
LB                   0.30 Hz
GB        0
PC                   1.00
```

13C NMR of **1f**



157.46
154.85
149.45
143.66
127.66
127.46
127.40
127.21
124.26
123.30
122.72
116.92
116.75
112.37
110.72

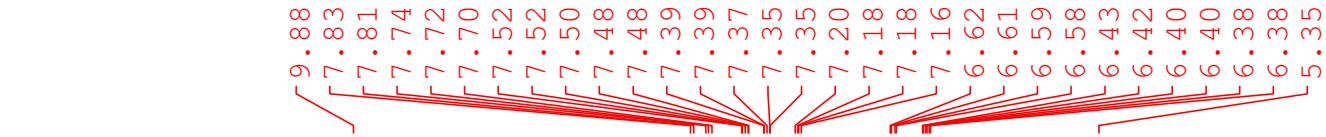

```
F2 - Acquisition Parameters
Date_           20141008
Time               16.45
INSTRUM            spect
PROBHD   5 mm PABBI 1H/
PULPROG          zgpg30
TD                30046
SOLVENT            DMSO
NS                   128
DS                     8
SWH            25252.525 Hz
FIDRES          0.840462 Hz
AQ             0.5949108 sec
RG                  1290
DW                19.800 usec
DE                 6.50 usec
TE                298.0 K
D1           4.00000000 sec
D11          0.03000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                13C
P1                13.70 usec
PLW1       102.08999634 W
SFO1        100.6077418 MHz

======== CHANNEL f2 ========
CPDPRG[2            garp
NUC2                1H
PCPD2             90.00 usec
PLW2          6.30959988 W
PLW12         0.07789600 W
PLW13         0.06309600 W
SFO2         400.0718803 MHz

F2 - Processing parameters
SI                 65536
SF           100.5976820 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

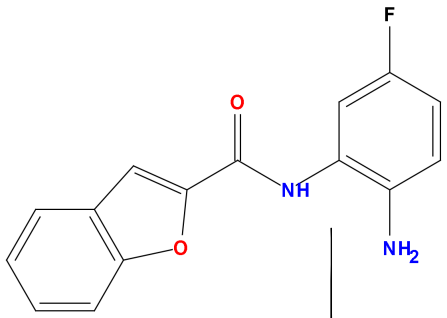
1H NMR of **2**

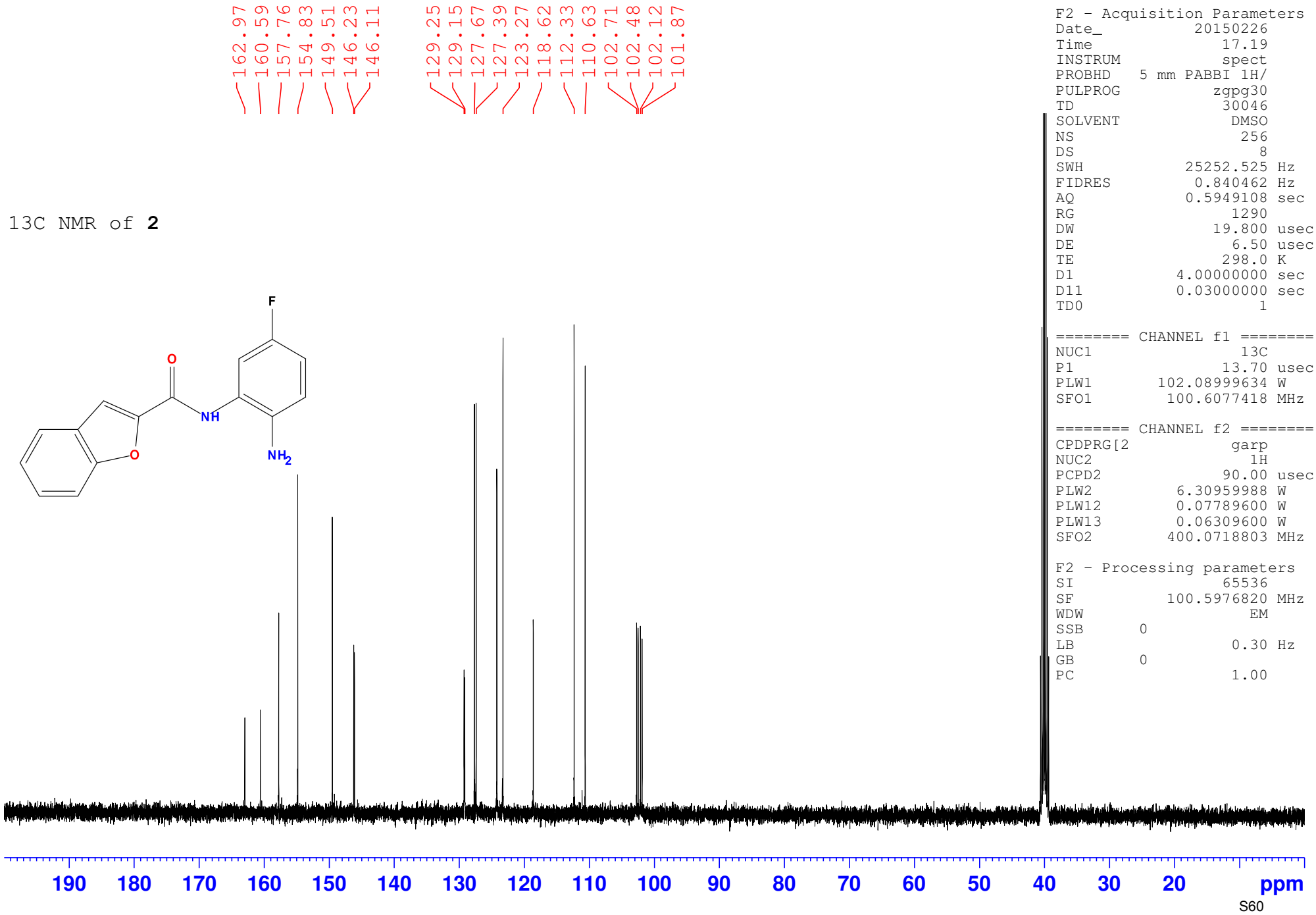



```
F2 - Acquisition Parameters
Date_           20150226
Time               16.55
INSTRUM            spect
PROBHD   5 mm PABBI 1H/
PULPROG            zg30
TD                30046
SOLVENT            DMSO
NS                    8
DS                    0
SWH            8012.820 Hz
FIDRES         0.266685 Hz
AQ           1.8748704 sec
RG                 22.6
DW               62.400 usec
DE                6.50 usec
TE               298.0 K
D1          1.00000000 sec
TD0                   1

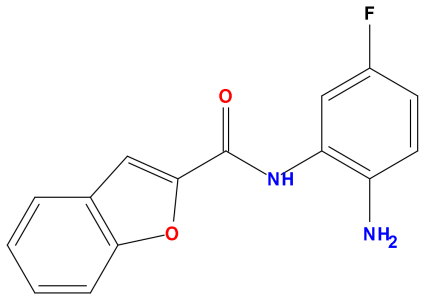
======== CHANNEL f1 ========
NUC1                1H
P1                7.80 usec
PLW1         9.86279964 W
SFO1        400.0724688 MHz

F2 - Processing parameters
SI                65536
SF          400.0700000 MHz
WDW                 EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```




162.97
160.59
157.76
154.83
149.51
146.23
146.11
129.25
129.15
127.67
127.39
123.27
118.62
112.33
110.63
102.71
102.48
102.12
101.87
13C NMR of 2
F
O
NH
O
NH2
F2 - Acquisition Parameters
Date_ 20150226
Time 17.19
INSTRUM spect
PROBHD 5 mm PABBI 1H/
PULPROG zgpg30
TD 30046
SOLVENT DMSO
NS 256
DS 8
SWH 25252.525 Hz
FIDRES 0.840462 Hz
AQ 0.5949108 sec
RG 1290
DW 19.800 usec
DE 6.50 usec
TE 298.0 K
D1 4.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 13.70 usec
PLW1 102.08999634 W
SFO1 100.6077418 MHz
======== CHANNEL f2 ========
CPDPRG[2 garp
NUC2 1H
PCPD2 90.00 usec
PLW2 6.30959988 W
PLW12 0.07789600 W
PLW13 0.06309600 W
SFO2 400.0718803 MHz
F2 - Processing parameters
SI 65536
SF 100.5976820 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

19F NMR of **2**

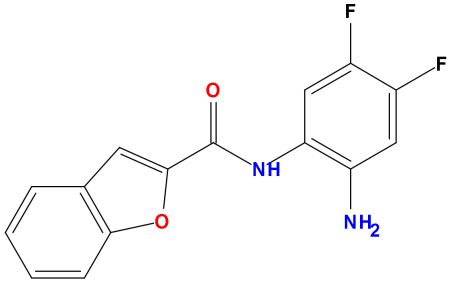


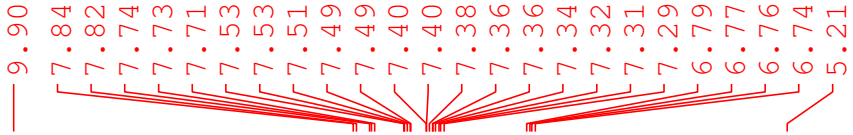

```
F2 - Acquisition Parameters
Date_           20150414
Time               10.34
INSTRUM            spect
PROBHD   5 mm PABBI 1H/
PULPROG              zg
TD                32768
SOLVENT            DMSO
NS                    16
DS                     0
SWH           15000.000 Hz
FIDRES         0.457764 Hz
AQ             1.0922667 sec
RG                   256
DW                33.333 usec
DE                 6.50 usec
TE                298.0 K
D1           2.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                 19F
P1                 18.50 usec
PLW1          2.51188588 W
SFO1         376.3967370 MHz

F2 - Processing parameters
SI                 16384
SF           376.4419100 MHz
WDW                  EM
SSB       0
LB                 2.00 Hz
GB        0
PC                 1.00
```

1H NMR of **2a**

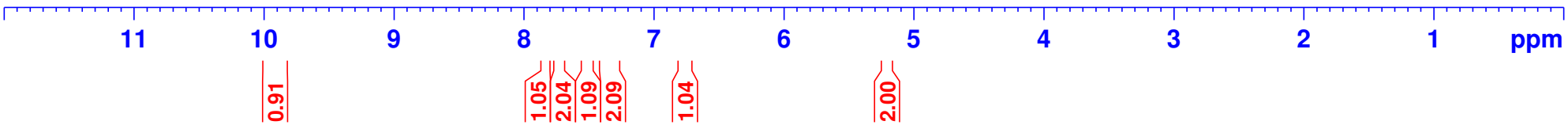




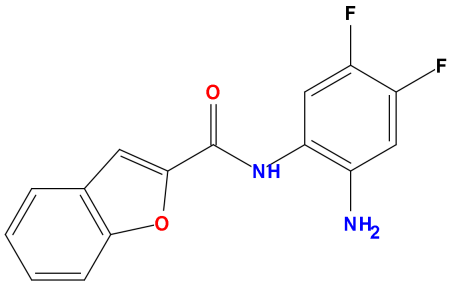

```
F2 - Acquisition Parameters
Date_           20150226
Time               15.58
INSTRUM            spect
PROBHD   5 mm PABBI 1H/
PULPROG             zg30
TD                 30046
SOLVENT             DMSO
NS                     8
DS                     0
SWH             8012.820 Hz
FIDRES          0.266685 Hz
AQ             1.8748704 sec
RG                   114
DW                62.400 usec
DE                 6.50 usec
TE                297.9 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                 7.80 usec
PLW1          9.86279964 W
SFO1         400.0724688 MHz

F2 - Processing parameters
SI                 65536
SF           400.0700000 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```



13C NMR of **2a**



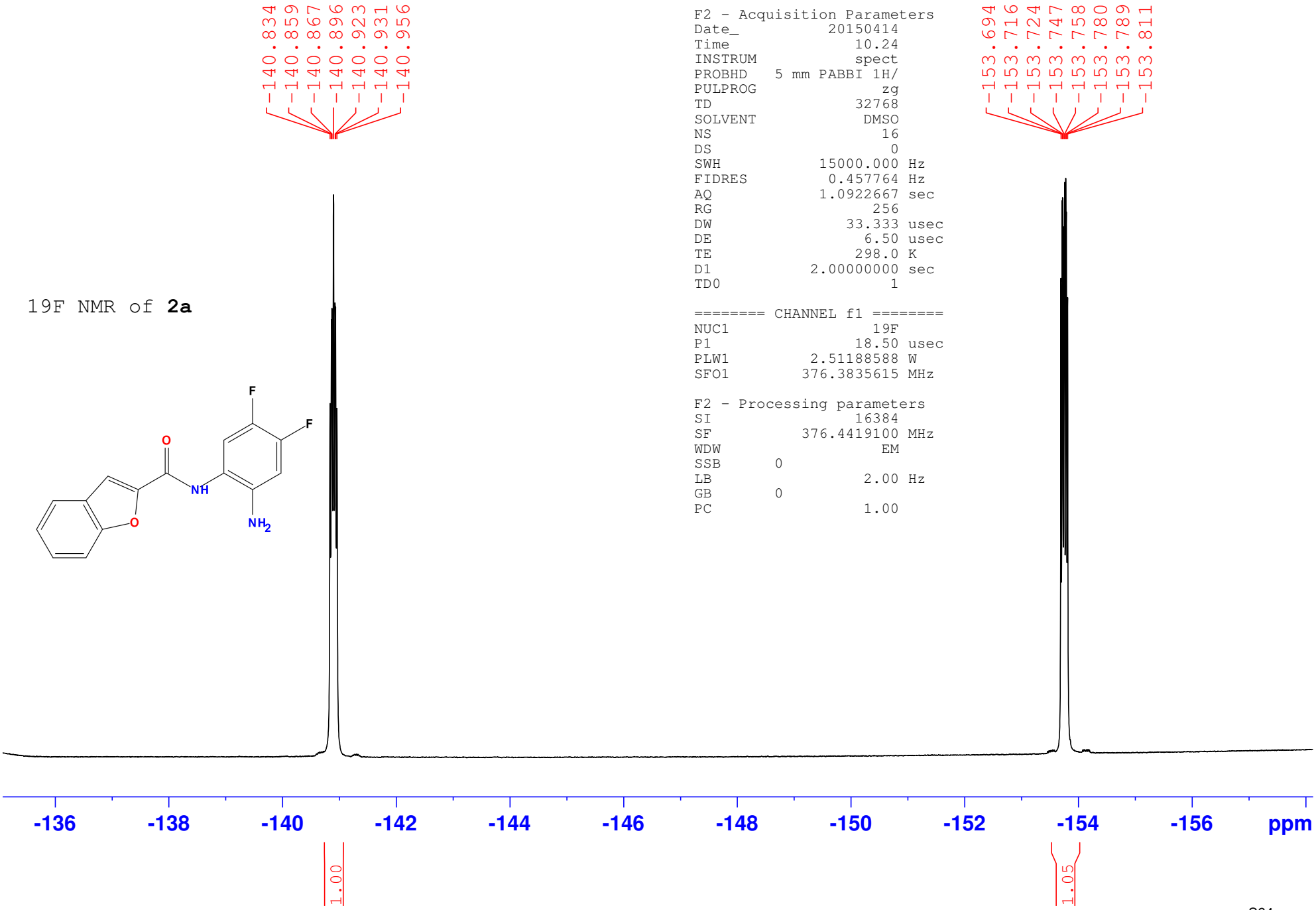
157.59
154.85
149.17
141.43
141.35
127.60
127.56
124.29
123.36
118.07
115.99
115.80
112.37
110.99
103.65
103.45

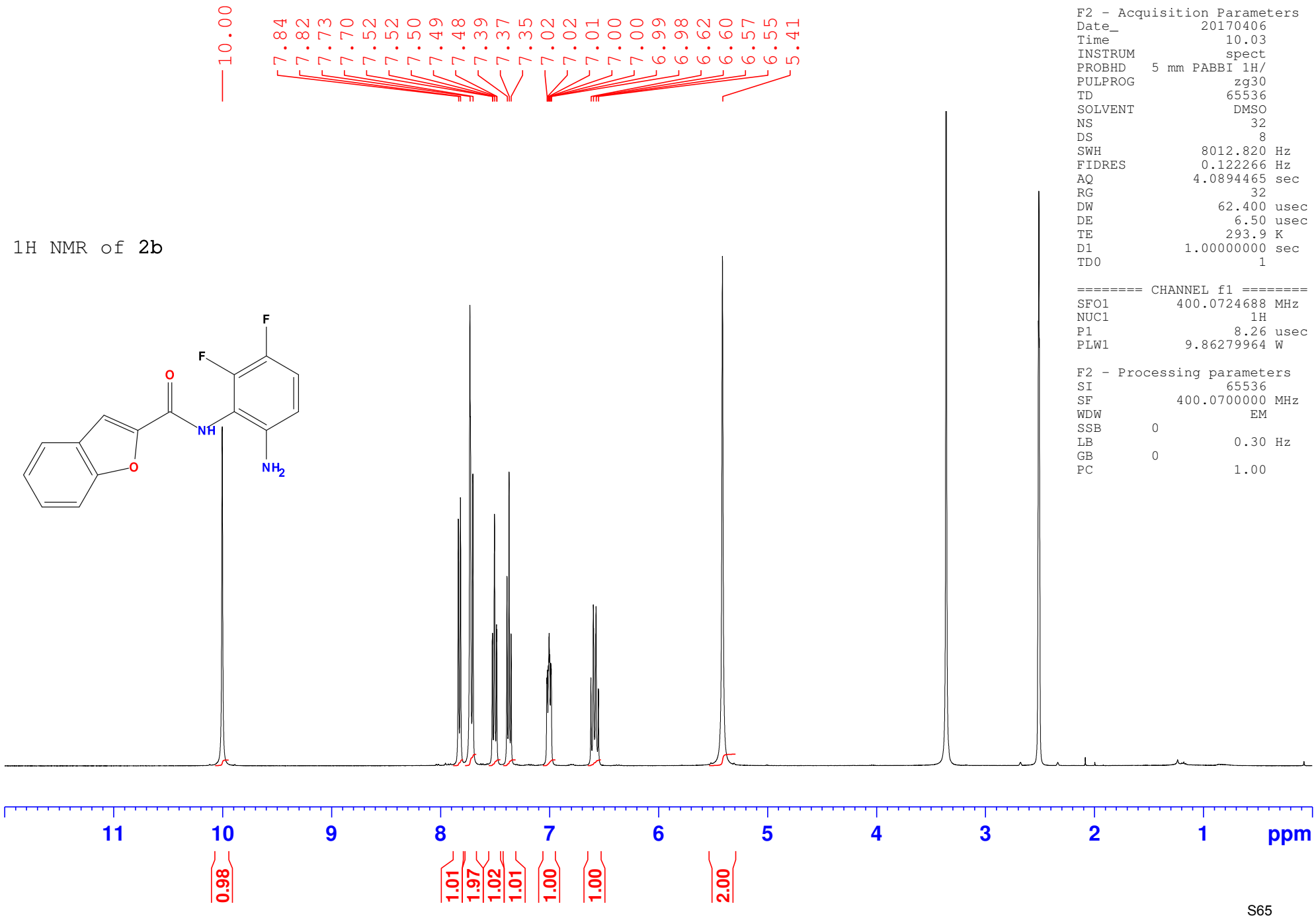
```
F2 - Acquisition Parameters
Date_              20150226
Time                 16.22
INSTRUM              spect
PROBHD    5 mm PABBI 1H/
PULPROG             zgpg30
TD                   30046
SOLVENT               DMSO
NS                      256
DS                        8
SWH              25252.525 Hz
FIDRES            0.840462 Hz
AQ                0.5949108 sec
RG                     1290
DW                   19.800 usec
DE                    6.50 usec
TE                   298.0 K
D1              4.00000000 sec
D11             0.03000000 sec
TD0                       1

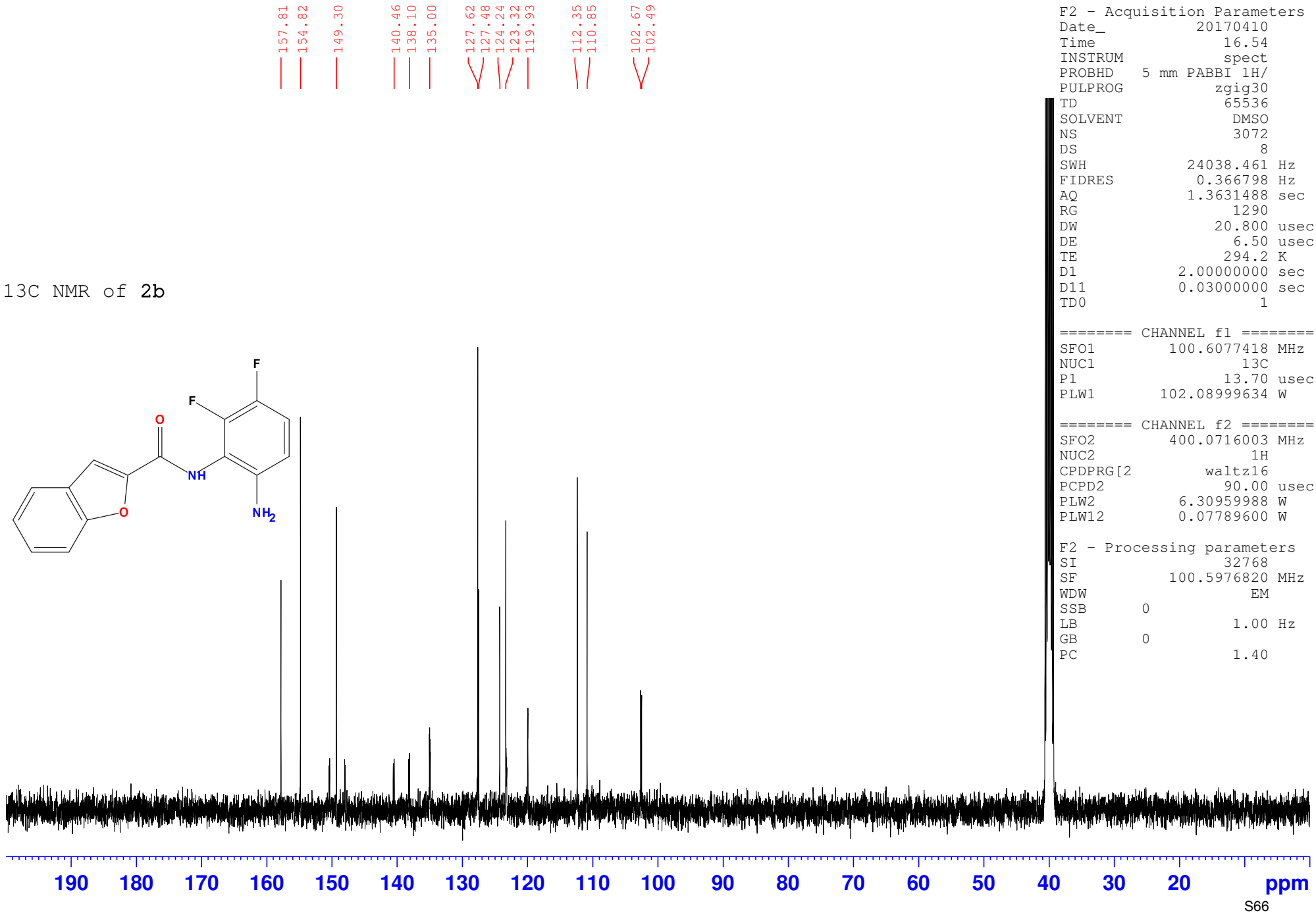
======== CHANNEL f1 ========
NUC1                    13C
P1                   13.70 usec
PLW1         102.08999634 W
SFO1          100.6077418 MHz

======== CHANNEL f2 ========
CPDPRG[2                garp
NUC2                     1H
PCPD2                90.00 usec
PLW2           6.30959988 W
PLW12          0.07789600 W
PLW13          0.06309600 W
SFO2          400.0718803 MHz

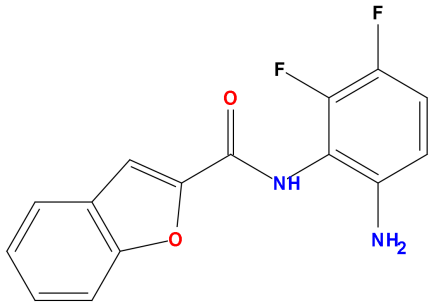
F2 - Processing parameters
SI                    65536
SF             100.5976820 MHz
WDW                      EM
SSB       0
LB                   0.30 Hz
GB        0
PC                    1.00
```


-140.834
-140.859
-140.867
-140.896
-140.923
-140.931
-140.956
-153.694
-153.716
-153.724
-153.747
-153.758
-153.780
-153.789
-153.811
F2 - Acquisition Parameters
Date_ 20150414
Time 10.24
INSTRUM spect
PROBHD 5 mm PABBI 1H/
PULPROG zg
TD 32768
SOLVENT DMSO
NS 16
DS 0
SWH 15000.000 Hz
FIDRES 0.457764 Hz
AQ 1.0922667 sec
RG 256
DW 33.333 usec
DE 6.50 usec
TE 298.0 K
D1 2.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 19F
P1 18.50 usec
PLW1 2.51188588 W
SFO1 376.3835615 MHz
F2 - Processing parameters
SI 16384
SF 376.4419100 MHz
WDW EM
SSB 0
LB 2.00 Hz
GB 0
PC 1.00
19F NMR of 2a
F
F
O
NH
O
NH2
-136
-138
-140
-142
-144
-146
-148
-150
-152
-154
-156
ppm
1.00
1.05


1H NMR of 2b
10.00
7.84
7.82
7.73
7.70
7.52
7.52
7.50
7.49
7.48
7.39
7.37
7.35
7.02
7.02
7.01
7.00
7.00
6.99
6.98
6.62
6.60
6.57
6.55
5.41
F
F
O
NH
O
NH2
F2 - Acquisition Parameters
Date_ 20170406
Time 10.03
INSTRUM spect
PROBHD 5 mm PABBI 1H/
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 32
DS 8
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894465 sec
RG 32
DW 62.400 usec
DE 6.50 usec
TE 293.9 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.0724688 MHz
NUC1 1H
P1 8.26 usec
PLW1 9.86279964 W
F2 - Processing parameters
SI 65536
SF 400.0700000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
11
10
9
8
7
6
5
4
3
2
1
ppm
0.98
1.01
1.97
1.02
1.01
1.00
1.00
2.00


157.81
154.82
149.30
140.46
138.10
135.00
127.62
127.48
124.24
123.32
119.93
112.35
110.85
102.67
102.49
13C NMR of 2b
F
F
O
NH
O
NH2
F2 - Acquisition Parameters
Date_ 20170410
Time 16.54
INSTRUM spect
PROBHD 5 mm PABBI 1H/
PULPROG zgig30
TD 65536
SOLVENT DMSO
NS 3072
DS 8
SWH 24038.461 Hz
FIDRES 0.366798 Hz
AQ 1.3631488 sec
RG 1290
DW 20.800 usec
DE 6.50 usec
TE 294.2 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6077418 MHz
NUC1 13C
P1 13.70 usec
PLW1 102.08999634 W
======== CHANNEL f2 ========
SFO2 400.0716003 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 90.00 usec
PLW2 6.30959988 W
PLW12 0.07789600 W
F2 - Processing parameters
SI 32768
SF 100.5976820 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

19F NMR of **2b**



-141.51
-141.55

-158.14
-158.16
-158.20
-158.22

F2 - Acquisition Parameters

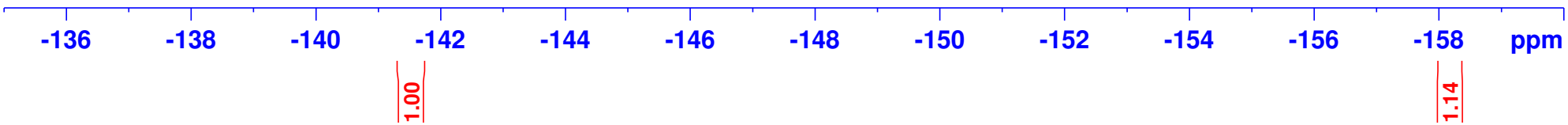
Date_	20170410
Time	11.23
INSTRUM	spect
PROBHD	5 mm PABBO BB/
PULPROG	zgflqn
TD	131072
SOLVENT	DMSO
NS	32
DS	4
SWH	89285.711 Hz
FIDRES	0.681196 Hz
AQ	0.7340032 sec
RG	202.75
DW	5.600 usec
DE	6.50 usec
TE	300.0 K
D1	1.00000000 sec
TD0	1

======== CHANNEL f1 ========

SFO1	376.4607164 MHz
NUC1	19F
P1	15.00 usec
PLW1	19.00000000 W

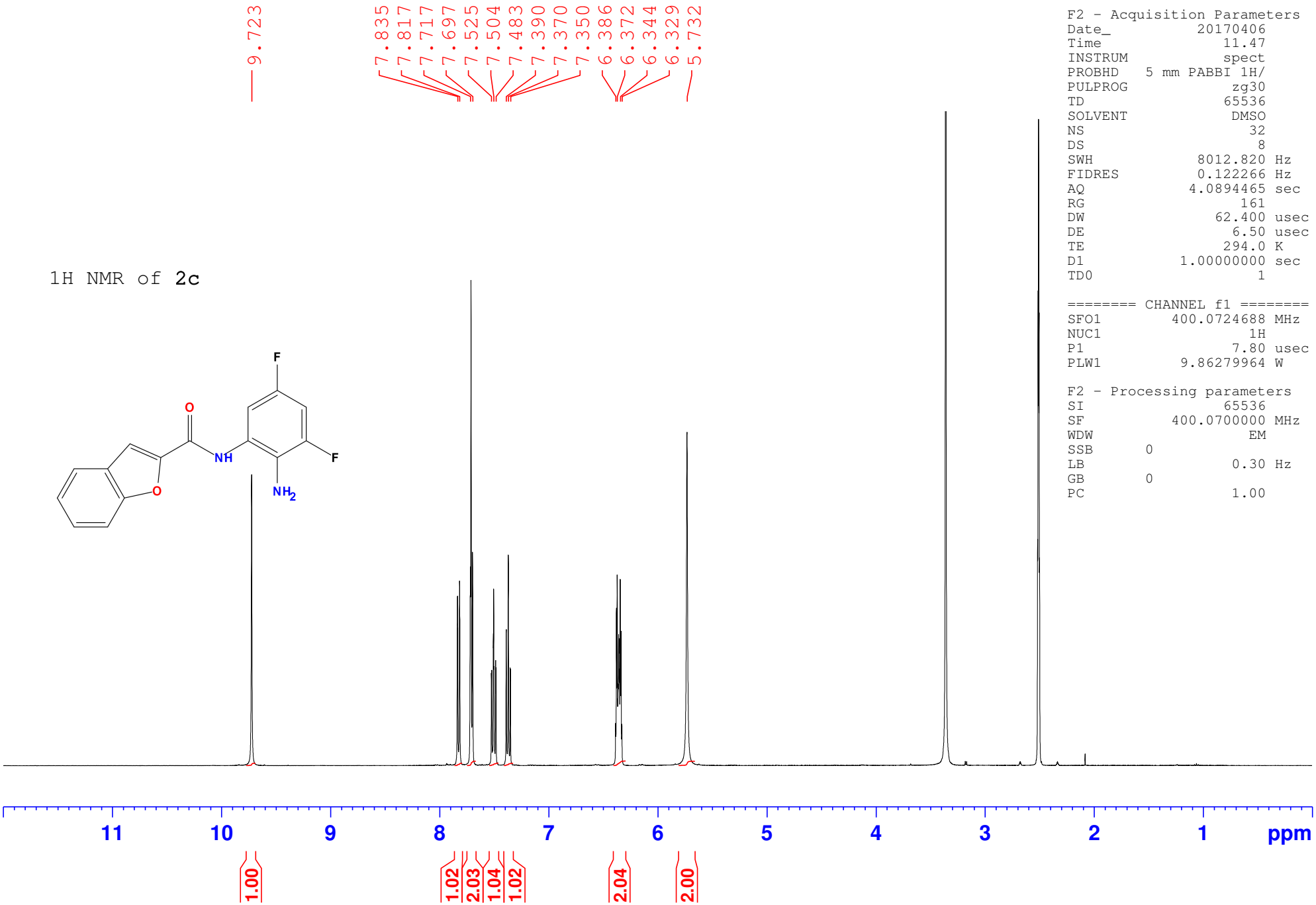
F2 - Processing parameters

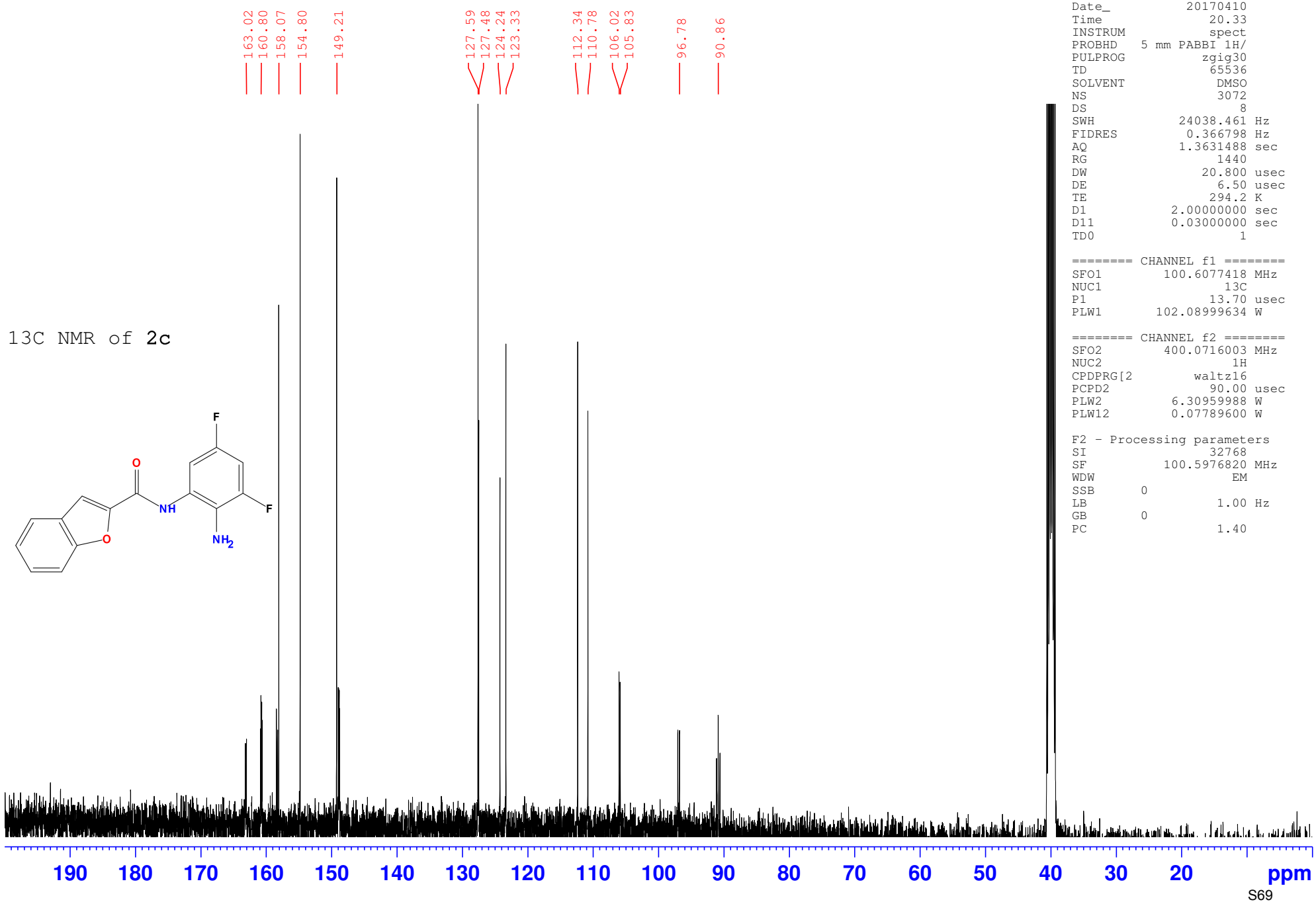
SI	131072
SF	376.4983662 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



1.00

1.14


9.723
7.835
7.817
7.717
7.697
7.525
7.504
7.483
7.390
7.370
7.350
6.386
6.372
6.344
6.329
5.732
1H NMR of 2c
F
O
NH
F
O
NH2
F2 - Acquisition Parameters
Date_ 20170406
Time 11.47
INSTRUM spect
PROBHD 5 mm PABBI 1H/
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 32
DS 8
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894465 sec
RG 161
DW 62.400 usec
DE 6.50 usec
TE 294.0 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.0724688 MHz
NUC1 1H
P1 7.80 usec
PLW1 9.86279964 W
F2 - Processing parameters
SI 65536
SF 400.0700000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
11
10
9
8
7
6
5
4
3
2
1
ppm
1.00
1.02
2.03
1.04
1.02
2.04
2.00



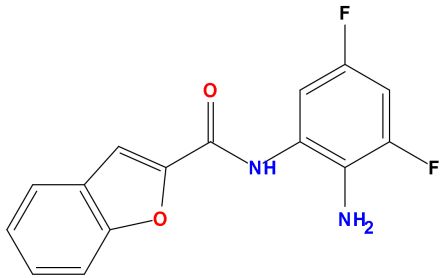

```
F2 - Acquisition Parameters
Date_           20170410
Time              20.33
INSTRUM           spect
PROBHD   5 mm PABBI 1H/
PULPROG          zgig30
TD                65536
SOLVENT            DMSO
NS                 3072
DS                    8
SWH           24038.461 Hz
FIDRES         0.366798 Hz
AQ            1.3631488 sec
RG                 1440
DW               20.800 usec
DE                6.50 usec
TE               294.2 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1        100.6077418 MHz
NUC1               13C
P1               13.70 usec
PLW1       102.08999634 W

======== CHANNEL f2 ========
SFO2        400.0716003 MHz
NUC2               1H
CPDPRG[2         waltz16
PCPD2             90.00 usec
PLW2          6.30959988 W
PLW12         0.07789600 W

F2 - Processing parameters
SI                32768
SF          100.5976820 MHz
WDW                EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

19F NMR of **2c**



-112.66
-112.69
-112.71
-112.74

-117.19
-117.21
-117.23

F2 - Acquisition Parameters

Date_	20170410
Time	11.25
INSTRUM	spect
PROBHD	5 mm PABBO BB/
PULPROG	zgflqn
TD	131072
SOLVENT	DMSO
NS	32
DS	4
SWH	89285.711 Hz
FIDRES	0.681196 Hz
AQ	0.7340032 sec
RG	202.75
DW	5.600 usec
DE	6.50 usec
TE	300.0 K
D1	1.00000000 sec
TD0	1

======== CHANNEL f1 ========

SFO1	376.4607164 MHz
NUC1	19F
P1	15.00 usec
PLW1	19.00000000 W

F2 - Processing parameters

SI	131072
SF	376.4983662 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00