Regioselective Radical Alkylation of Arenes Using Evolved Photoenzymes

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Part I. General Information:

Unless otherwise noted, all chemicals and reagents for chemical reactions were obtained from commercial suppliers and used as received (Sigma-Aldrich, Oakwood Chemical, Combi-Blocks, Chem-Impex, New England Biolabs (NEB) and Acros Chemicals). GDH-105 was purchased from Codexis® as cell free lysate and used as received. Silica gel chromatography purifications were carried out using AMD Silica Gel 60. ^{1}H-, ^{13}C-, and ^{19}F- NMR spectra were recorded on a Bruker UltraShield Plus (500 MHz, 125 MHz, and 282 MHz respectively) instrument, and are internally referenced to residual proton signals in $CDCl_3$ (7.26 ppm). Data for ^{1}H-NMR are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, brs = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublet, dt = doublet of triplet, ddd = doublet of doublet of doublet), coupling constant (Hz), and integration. Data for ^{13}C NMR are reported in terms of chemical shift relative to $CDCl_3$ (77 ppm). High resolution mass spectra (HRMS) were obtained on a Thermo Fisher Scientific DART Mass Spectrometer. IR spectra were recorded on a Bruker Tensor II Infrared Spectrometer and peaks are reported in terms of frequency of absorption (cm^{-1}).

Chromatography. Analytical high-performance liquid chromatography (HPLC) was carried out using an Agilent 1260 Infinity LCMS System and an Agilent 1260 Infinity LC system using XSelect Premier HSS T3 VanGuard FIT Column, 100Å, 2.5 µm, 4.6 x 100 mm. Semi-preparative HPLC was carried out using an Agilent 1260 Infinity LC system with the Phenomenex Synergi 4 μm Hydro-RP 80 Å LC column 250 x 10 mm.

LED Lamps.

The cyan LEDs lamps were constructed in house from Chanzon High Power 50 W Cyan LED Chips(497 nm/1500 mA/DC30-34 V/50 W, measured photon flux = 12,000 mM/m2s) (Amazon 1DGL-JC-50W-490) powered by Mean Well HLG-320H-C1750A power supplies (320 W/183 V/1750 mA). Each LED chip was secured to a Nagulagu cooling aluminum LED heatsink equipped with a 12 V fan (Amazon B01K1Z6VP6).

Cloning. pET22b(+) and pET15a were used as a cloning and expression vector for all enzymes described in this study. Genes for 'ene' reductase enzymes were purchased as gBlocks from IDT and cloned using Gibson Cloning.[43] All genes were cloned between the NdeI and XhoI restriction sites and contained an N-terminal (GluER) or C-terminal (NostocER) 6xHis tag. Cloning for each construct was carried out using BL21 *E. coli*.

Protein Expression and Purification. Enzymes used in purified protein experiments were expressed in BL21(DE3) *E. coli* cultures transformed with plasmid encoding ERED variants. Transformed glycerol stocks were used to initiate 10 mL overnight cultures (37 °C, 250 rpm). Expression cultures (500 mL of Turbo Broth with ampicillin (100 µg/ml final concentration) in a

2L flask) were inoculated with 1-2 ml of the overnight culture and grown to OD_{600} = 0.6 (37 ºC, 250 rpm). Once the cell cultures reached an OD of 0.6 they were chilled on ice for 15 minutes prior to the addition of IPTG. For AspER, expression was induced with 0.1 mM IPTG (24 h 25 °C 250 rpm). Following expression, cells were pelleted and frozen at -80 °C for storage. GluER T36A was expressed using the addition of 4% (v/v) auto inducing mix (sterile filtered mixture of 1.25% glucose, 5% lactose and 15% glycerol).The pellets were kept at -80 °C for at least 24 hrs before thawing for purification. For purification, frozen cells were thawed in ice-cold water and resuspended in buffer A (for GluER: 50 mM TEOA 25 mM imidazole pH 7.0, for all other proteins reported herein: 20 mM KPi, 300 mM NaCl, 30 mM imidazole, pH 7.0). Lysozyme (1 mg/mL), DNAse (0.1 mg/mL), FMN (1 mg/mL), and PMSF (1 mg/mL, added as a 35 mg/mL solution in absolute ethanol) were added to the resuspended cells, followed by shaking at room temperature for 30 minutes. The resuspended cells were disrupted by sonication (2 x 4 min, output control 5, 35% duty cycle; Sonicator QSonica Q500 Ultra Sonicator). To pellet insoluble material, lysates were centrifuged at 14,000 x g for 1.5 h at 4 °C. Proteins were purified using a nickel NTA column (5 mL HisTrap HP, GE Healthcare, Piscataway, NJ) using an AKTAStart purifier FPLC system (GE healthcare). ERED enzymes were eluted with 100 % buffer B (for all proteins reported herein: 20 mM KPi, 300 mM NaCl, 250 mM imidazole pH 7.0) over 5 column volumes. Fractions containing enzyme were pooled, concentrated, and subjected to three exchanges with no-imidazole buffer (for GluER: 50 mM triethanolamine (TEOA), 10% glycerol, pH=7.0, for all other EREDs reported: 20 mM KPi, 300 mM NaCl, pH 7.0) to remove excess salt and imidazole. Concentrated (1.0-1.5 mM) proteins were aliquoted, flash-frozen in liquid N_2, and stored at -80 °C until later use. Protein concentration was determined by absorbance at λ_{max} for oxidized flavin with calculated extinction coefficients. All proteins other than GluER, GluER mutants and AspER were used as aliquots pre-expressed and purified according to the procedures detailed in previously published work from the Hyster lab.[44]

Determination of Extinction Coefficients. Extinction coefficients for ERED enzymes were calculated based on the extinction coefficient (12.2×10^{-3} $M^{-1}cm^{-1}$ at 464 nm) for free FMN released after protein denaturation.[45] (i). Extinction coefficient for GluER T36A: $\varepsilon_{464} = 11.4 \times 10^{-3}$ $M^{-1}cm^{-1}$, PagER: $\varepsilon_{456} = 12.3 \times 10^{-3}$ M^{-1} cm^{-1}, AspER: $\varepsilon_{464} = 11.4 \times 10^{-3}$ M^{-1} cm^{-1}

Preparation of PagER Lysate. The cells were resuspended in 50 mM TEOA pH = 7 and frozen. They were then thawed and lysozyme (1 mg/mL), DNAse (0.1 mg/mL), FMN (1 mg/mL), and PMSF (1 mg/mL, was added as a 35 mg/mL solution in absolute ethanol) were added to the resuspended cells, followed by shaking at room temperature for 30 minutes. The resuspended cells were disrupted by sonication (2 x 4 min, output control 5, 35% duty cycle; Sonicator QSonica Q500 Ultra Sonicator). To pellet insoluble material, lysates were centrifuged twice at 14,000 x g for 1 h each at 4 °C. Lysates were aliquoted into 50 mL Falcon tubes (5 mL each) and flash-frozen in liquid N_2. The frozen lysates were lyophilized for 24 h at room temperature. On average, 5 g of lysate was obtained from 4 L Turbo Broth expression media (Induced with autoinducing mix). The lysates were quantified by Stain-Free SDS page gel with a standard curve of purified GluER T36A of a known concentration.

Part II. DNA and Protein Sequences Information:

GluER T36A

DNA Sequence

ATGCCGACCCTTTTCGACCCCATCGATTTCGGACCTATCCACGCCAAGAATCGTATCGTCA
TGTCCCCCCTGACTCGCGGTCGCGCTGACAAAGAGGCGGTTCCAGCTCCCATTATGGCTG
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Protein Sequence

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PagER

DNA Sequence

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Protein Sequence
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NostocER Y219F

DNA Sequence
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Protein Sequence
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OYE1

DNA Sequence
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Protein Sequence
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DKK

MorB

DNA Sequence

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Protein Sequence
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YersER

DNA Sequence
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Protein Sequence
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NCR

DNA Sequence
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Protein Sequence
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AspER

DNA Sequence
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AGGTCTGGATGTGGCTCTGGTCGGCCGTGGGTTCCAGAAAGATCCAGGCTTGGCCTGGAC
TTTTGCCCAACACTTGGATGTTGAAATTGCAATGGCCTCCCAAATTCGTTGGGGCTTCACTC
GCCGAGGAGGAACTCCCTATATTGACCCGAAAGCGTACAAGGAGTCTATTTTTGAACTCGA
GCACCACCACCACCACCACTGA

Protein Sequence
MALPDVENTPAAGIPYFTPAQNPPAGTAANPQTSGNAVPKLYTPLTVRGVTFHNRLGLAPLCQYSA
EDGHMTDYHIAHLGGIAQRGPGLMMIEATSVSPEGRITPQDVGLWKDSQIAPMKRVIDFVHSQSQ
KIGVQIAHAGRKASNIAPWLMNKGIVATEKVGGWPDRVIGPSTVPFHETFPTPKAMTKDDIEQFKR
DWFDACKRAIAAGADFIEIHNAHGYLLSSFLSPSSNTRTDEYGGSFENRIRLSLEIAQVTRDAVGPNV
PVFLRVSATDWIEETLPEESWKLSDSVRFAEALAAQGAIDLIDVSSGGVHAAQKIKSGPAFQAPFAV
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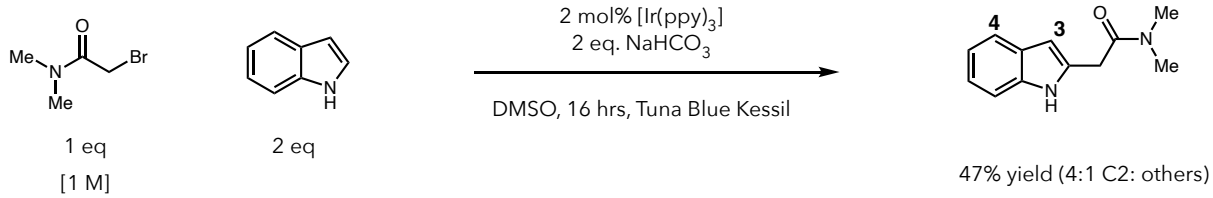
RWGFTRRGGTPYIDPKAYKESIFE

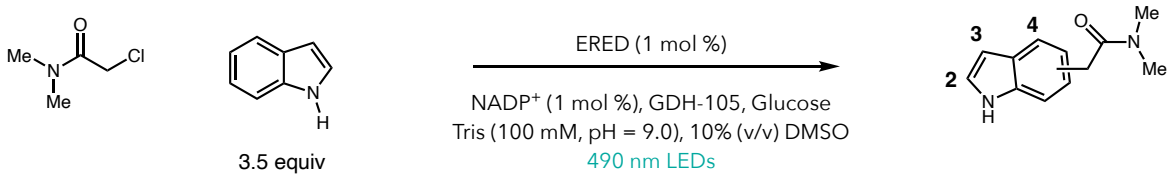
Part III. Experimental Procedures:



Supplemental Figure 1: Photoredox procedure for indole alkylation

The procedure was adapted from O'Brien et. al.[46] 2 equiv, of indole, 2 equiv of $NaHCO_3$, and 2 mol% of $Ir(ppy)_3$ were added to a shell vial with a stir bar and a septa was put on.. The solids were degassed thoroughly. Degassed, dry DMSO was added to the vial such that the final concentration of α-bromodimethylacetamide would be 1M. α-bromodimethylacetamide was then added and the solution was degassed with nitrogen. The vial was then sealed and placed under a 34W Tuna Blue Kessil for 16 hours. The reaction became solid and the solids were dissolved d_6-DMSO so that a crude regioisomeric ratio was taken. The crude reaction was then columned over silica gel to yield the C2 regioisomer as the major regioisomer.

Supplemental Table 1: EREDs screened



ERED	C2 yield (%)	C3 yield (%)	C4 yield (%)
GluER T36A	21%	10%	19%
NostocER Y219F	40%	3%	9%
OYE1	<1%	4%	n.d.
MorB	15%	<1%	2%
YersER	16%	2%	5%
NCR	14%	7%	2%
AspER	50%	<1%	<1%
GluER T36A 6x EtOAc extractions	29%	22%	32%

Reaction conditions: α-chloroamide (2 mg, 20 μmol), 3.5 equiv. of arene (70 μmol), 1 mol% ERED, 1 mol% $NADP^+$, 1 mg GDH and 6 equiv of glucose (120 μmol) was irradiated with Cyan LEDs for 24 hours. Yield of each regioisomer was determined by NMR using 1,3,5-trimethoxybenzene as standard.

Part IV. Directed Evolution Information:

DNA preparation of site saturated mutagenesis libraries

Site saturation mutagenesis primers were designed and mixed using the 22 codon trick described in Kille et al.[47] The primer mix was then used in a standard PCR reaction with an extension time of 3 minutes and 30 seconds with Phusion® polymerase from NEB with the purified parent plasmid as the template DNA. The PCR product was digested with DpnI to cleave the DNA and gel purified. A Thermo Scientific™ NanoDrop™ One Microvolume UV-Vis Spectrophotometer was used to check the DNA concentration of the purified PCR product. 50 ng – 100 ng of the purified PCR product was recircularized using NEBuilder® HiFi DNA assembly. The recircularized DNA was then transformed into BL21(DE3) E. coli electrocompetent cells and plated on to LB Aga plates containing ampicillin at (100 μg/mL). Concurrently, the parent DNA was transformed to ensure all wells in the plates started from similar growth phases.

Protein Expression in 96 well plates for site-saturated mutagenesis.

A 96 deep-well plate was prepared containing 500 uL of LB + ampicillin (100 μg/mL). Each well was inoculated with a single colony from the LB Agar plates with the colonies of BL21(DE3) *E. coli* containing the site saturated mutagenesis DNA with sterile toothpicks. A well containing a single colony with the parent DNA was placed throughout the plate (A2, B4, C6, D8, E10, F12, G2, H4). This was to identify the ratio of products under the plate conditions as well as a baseline yield. The deep well plate was shaken at 30°C and 250 rpm overnight. The next day 50 μL of the overnight culture was used to inoculate another 96 deep well plate where each well contains 950 uL of Terrific Broth + ampicillin (100 μg/mL) + 4% (v/v) auto inducing mix (sterile filtered mixture of 1.25% glucose, 5% lactose and 15% glycerol). The overnight culture was also used to create a glycerol stock by mixing 50 μL of overnight culture with 50 μL of 50% (v/v) glycerol. This plate (the expression plate) was shaken at 30°C and 250 rpm for 24 hours. After 24 hours the cells in the plate were centrifuged at 4000 x g for 10 minutes. The supernatant was removed and the pelleted cells in the plate were frozen at -80°C until use.

Lysis and Screening for indole and quinoline alkylation with GluER T36A mutants

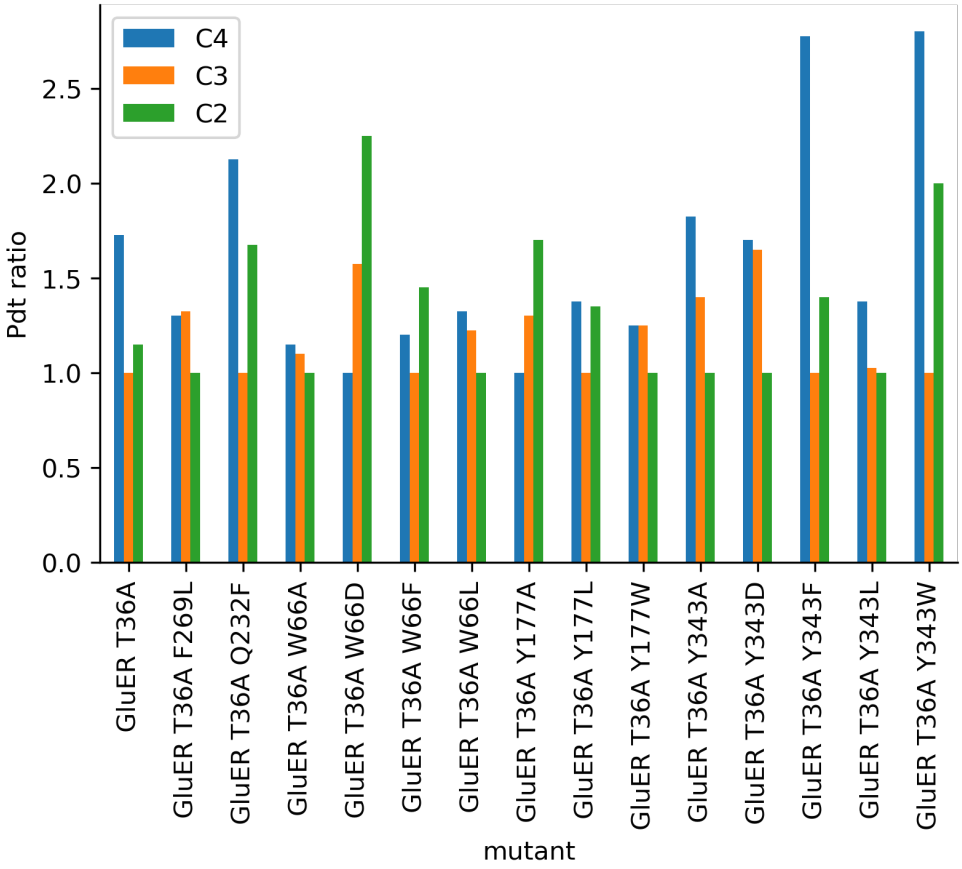
The frozen expression plate was allowed to come to room temperature. Then the pellet in each well was resuspended in 100 μL of lysis buffer (100 mM Tris pH 9 for indole reaction and 100 mM KP_i pH 8 for quinoline reaction) containing 1 mg/mL lysozyme, 0.1 mg/mL DNAse and 200 mM PMSF). The pellets were resuspended and shaken at 37° C for 30 minutes. The lysate was spun down at 4000 x g for 10 minutes to remove cellular debris . 100 uL of the supernatant was removed to a clear bottom, white microtiter plate.

The microtiter plate was brought into the Coy Chamber and 100 uL of turnover mix was added to each well (3.72 mg/mL glucose, 0.67 mg/mL GDH-105, 0.2 mg/mL FMN, 0.29 mg/mL NADP+). 0.5 mg of chloroamide was added to each well using 5 uL of DMSO. 1.86 mg of indole was added using 5 uL of DMSO. The plate was sealed using adhesive film and removed from the Coy Chamber. The plate was then irradiated for 16 hours using a Lumidox 96-well Cyan LED array with active cooling base from Analytical Sales and Services Inc. The reaction mixture

was moved to a 96 deep-well plate and quenched using 1000 uL of ACN and allowed to either shake for 1 hour at room temperature or sit in a 4°C fridge for 4 hours. The deep well plate was centrifuged at 4000 x g for 20 minutes to remove precipitated protein. 200 μL of the supernatant was filtered through a Millipore 96-well plate into a clear shallow-well 96 well microtiter plate. The regioisomeric ratio and relative yield compared to control were assessed using a LC using the XSelect Premier HSS T3 VanGuard FIT Column, 100Å, 2.5 µm, 4.6 x 100 mm (40-95% ACN in H_2O for 4 minutes). Promising wells were identified using a simple data filtration function coded in Python (data was filtered for yield greater than or equal to the control and having a better product area/undesired product area than the control well). An overnight culture was seeded from the glycerol stock of that well to have the DNA extracted and sequenced. The wells were then expressed using the standard GluER T36A protocol in 2L baffled flasks and purified using the standard procedure for hit confirmation using the general procedure for the biocatalytic alkylation of heterocycles.

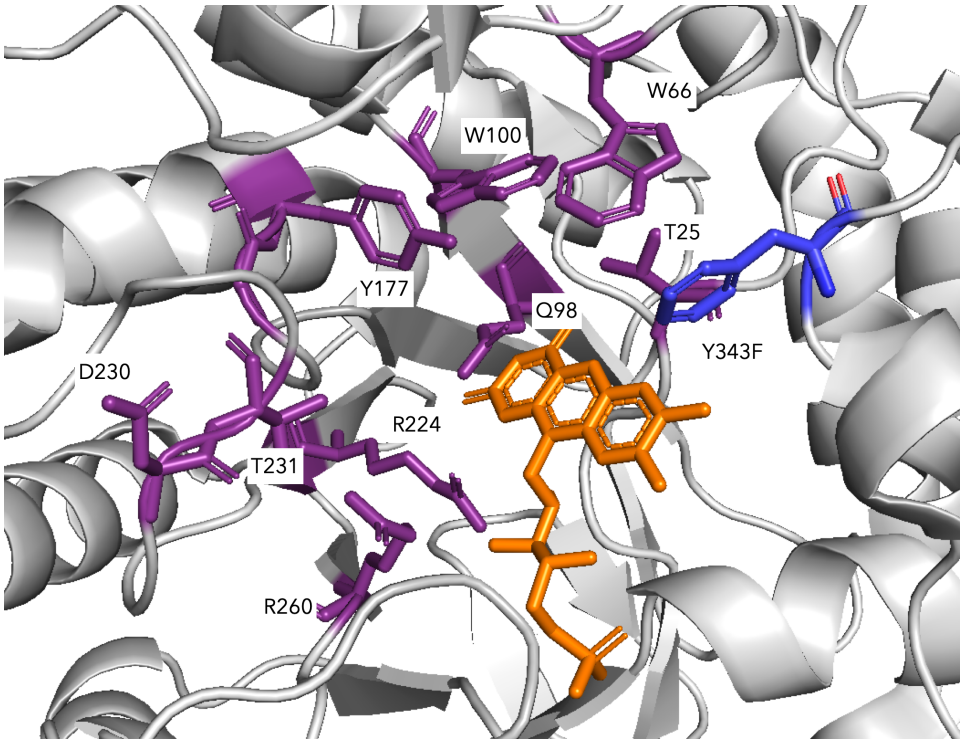
Maximum Diversity Plate

The Maximum Diversity plate was grown from the glycerol stock that was prepared as described in Nicholls et. al.[48] It was expressed using the same protein expression protocol, lysis, and screening protocol as the site saturation mutagenesis plates. GluER T36A Y343F was identified as the best mutant from this plate and was used as the parent round. Further verification was found using purified protein from shaken flask expression.

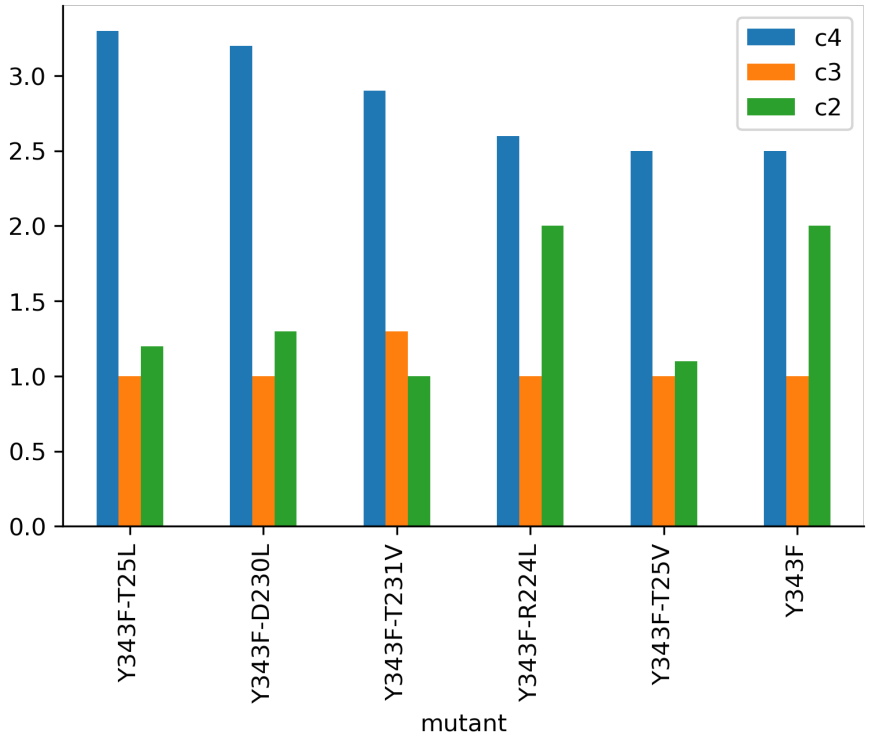


Supplemental Figure 2: Ratio Results from Maximum Diversity Plate

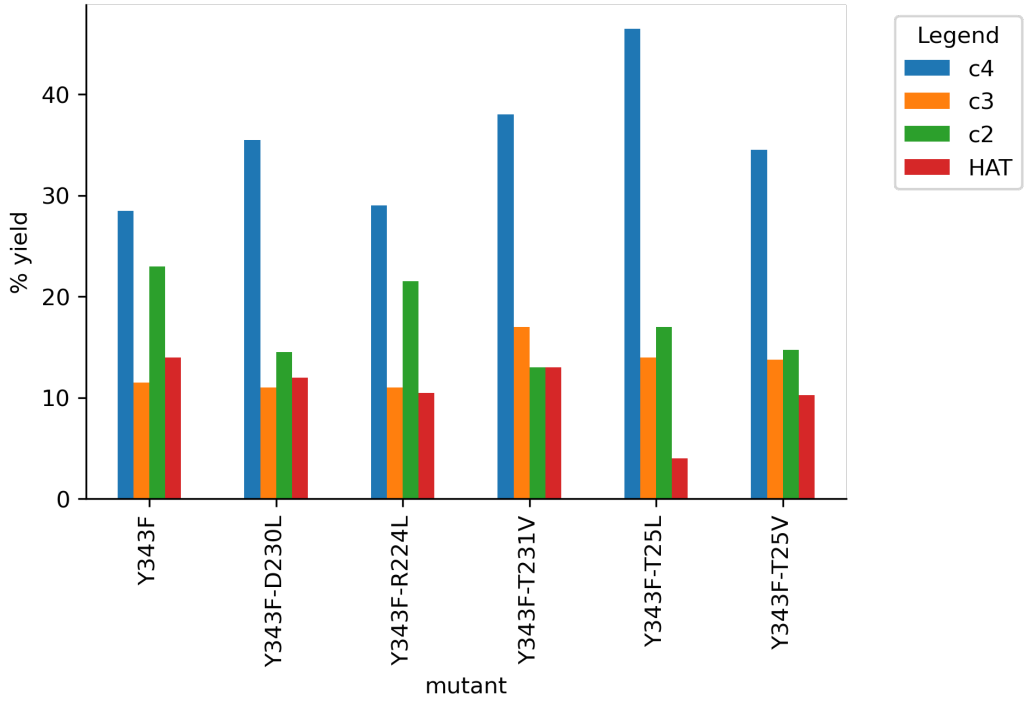
Site Saturation Mutagenesis for Indole Alkylation **Round 1**



Template	Sites Targeted	Hits Identified
GluER T36A Y343F	R224, Y177, T231, D230, W100, Q98, W66, G176, R260, T25	T25L, T231V, D230L, T25V, R224L

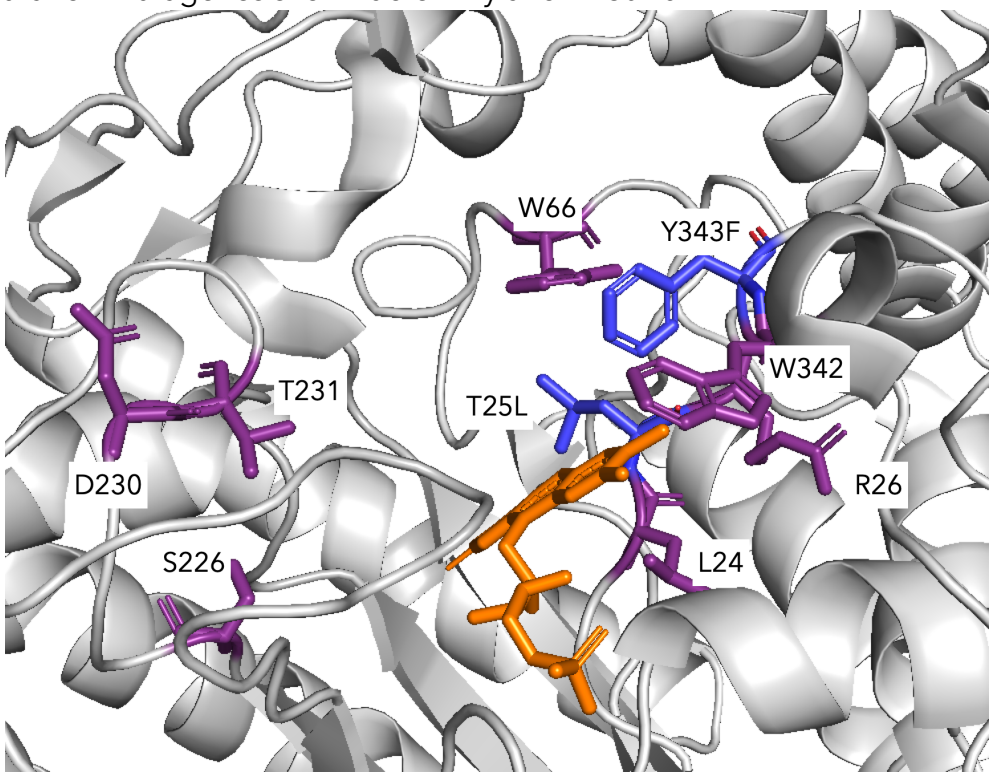


Supplemental Figure 3: Round 1 Hit Ratios Summary

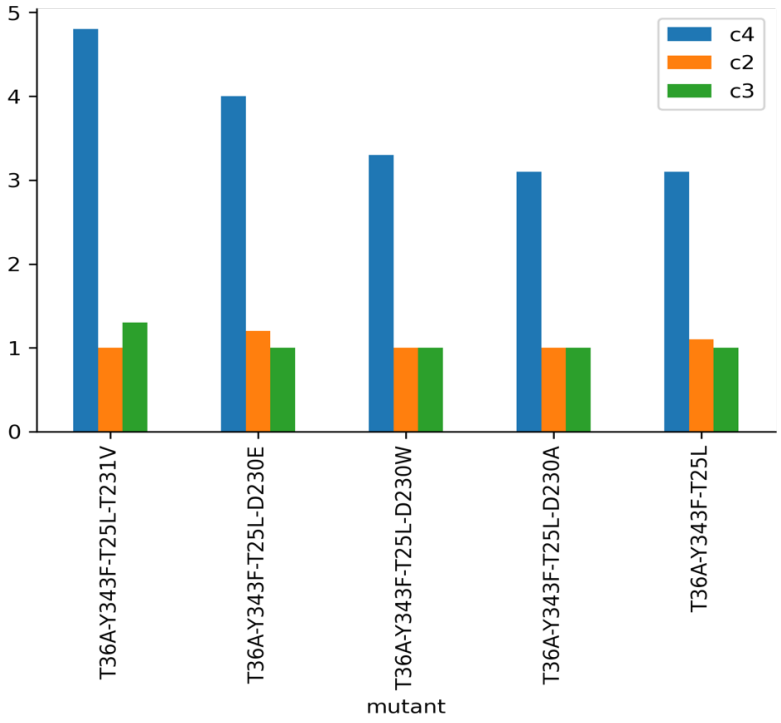


Supplemental Figure 4: Round 1 Hit Product Yield Summary

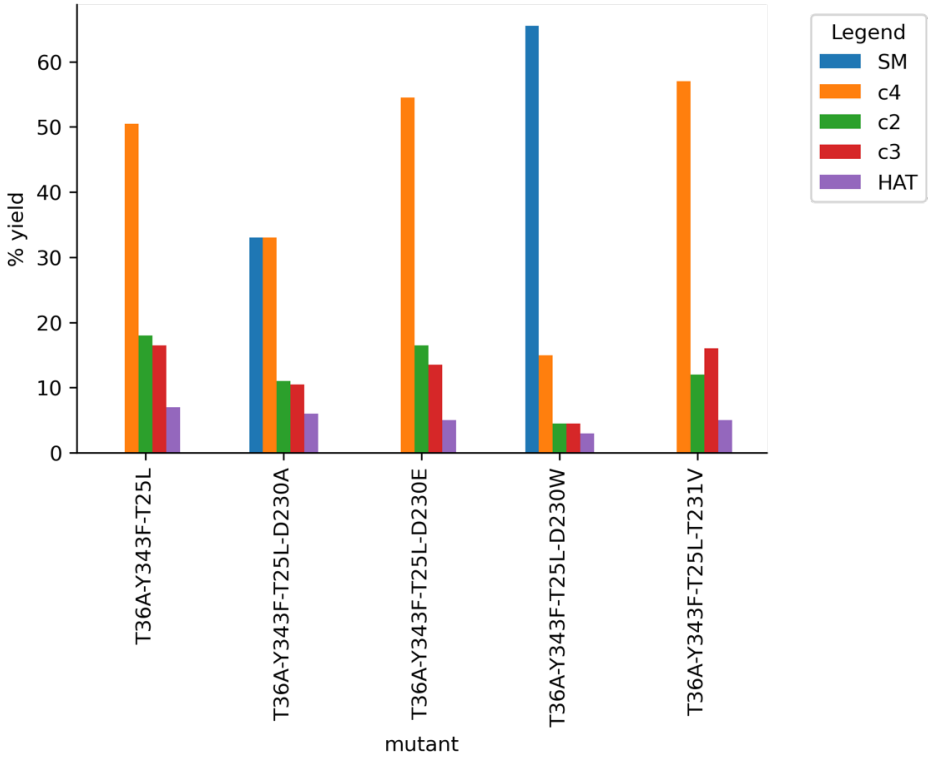
Site Saturation Mutagenesis for Indole Alkylation **Round 2**



Template	Sites Targeted	Hits Identified
GluER T36A Y343F T25L	T231, S226, R26, L24, D230, W66, W342	T231V, D230W, D230E, D230A

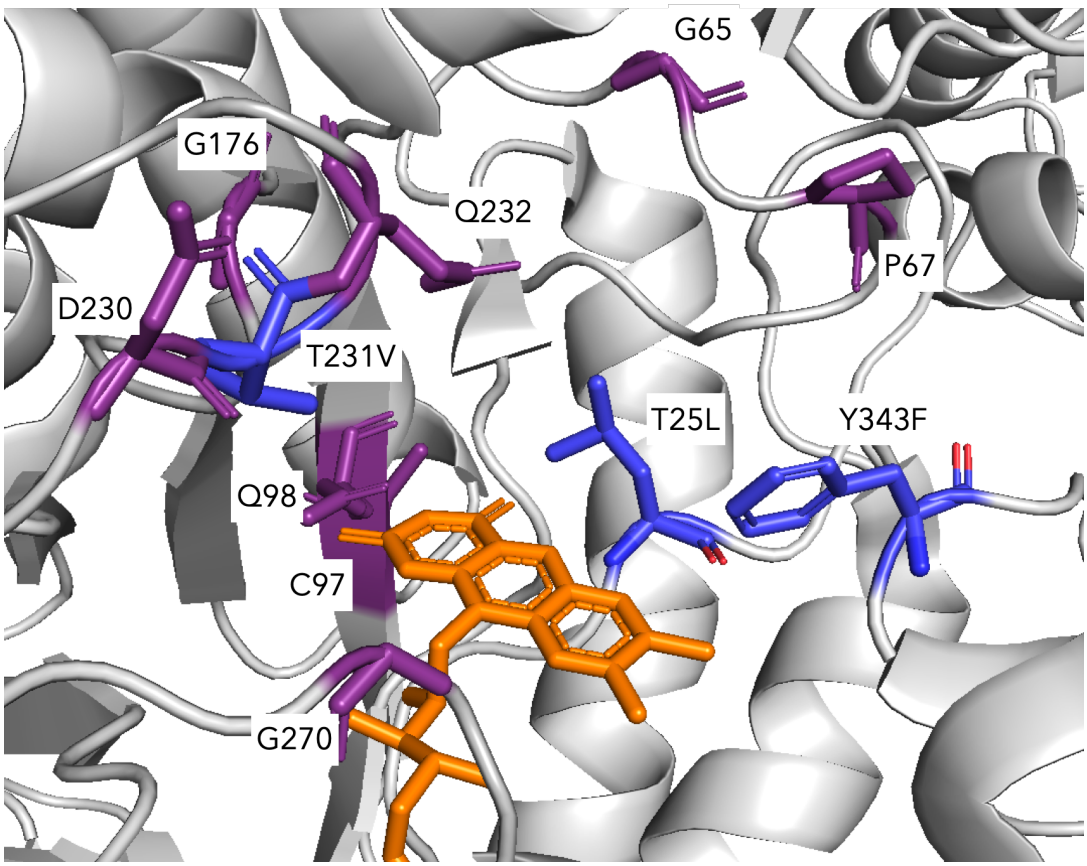


Supplemental Figure 5: Round 2 Hit Ratios Summary

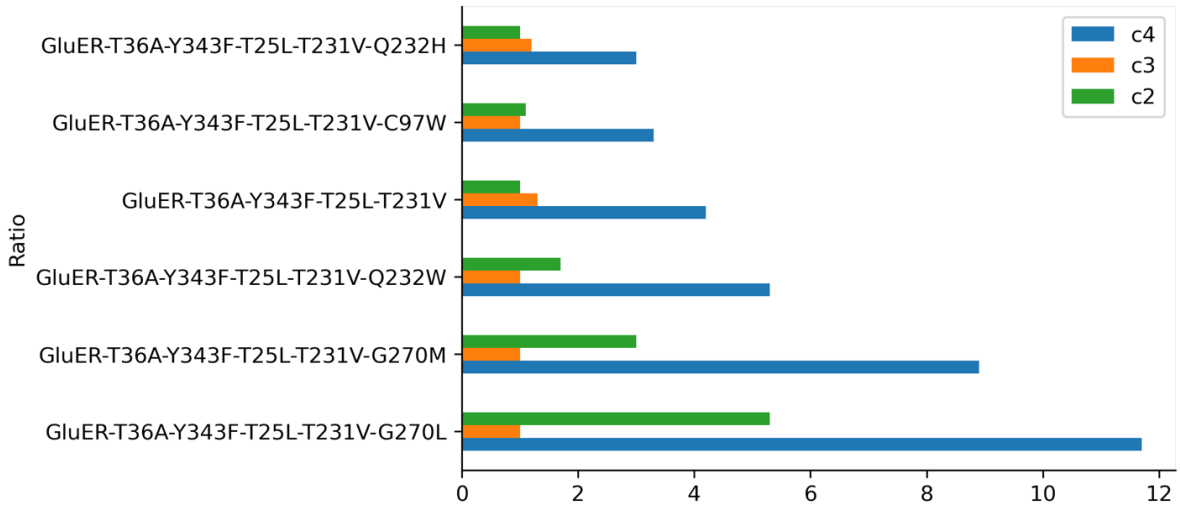


Supplemental Figure 6: Round 2 Hit Yields Summary

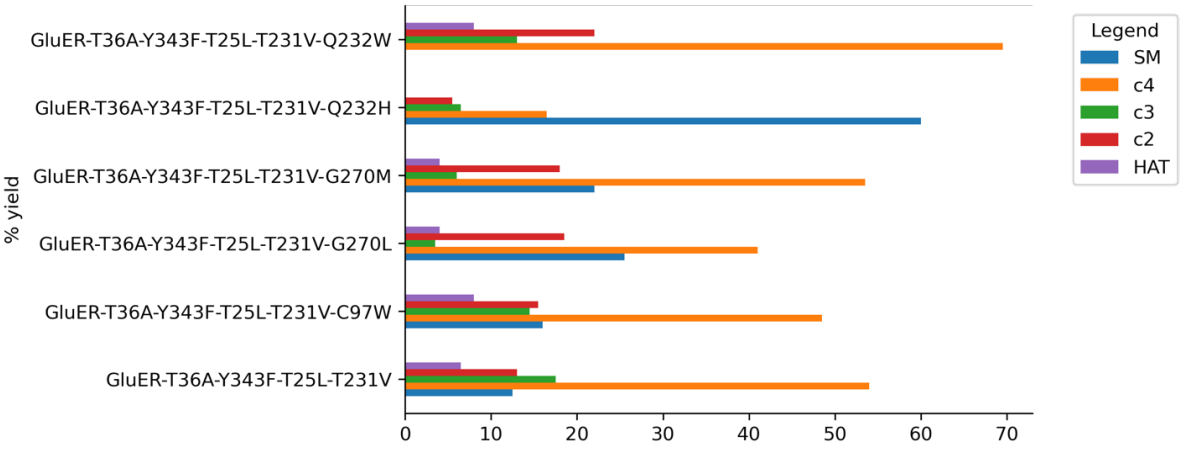
Site Saturation Mutagenesis for Indole Alkylation **Round 3**



Template	Sites Targeted	Hits Identified
GluER T36A Y343F T25L T231V	G65, G176, C97, G270, P67, Q98, D230, Q232, R228,	G270L, G270M, Q232W, C97W, Q232H

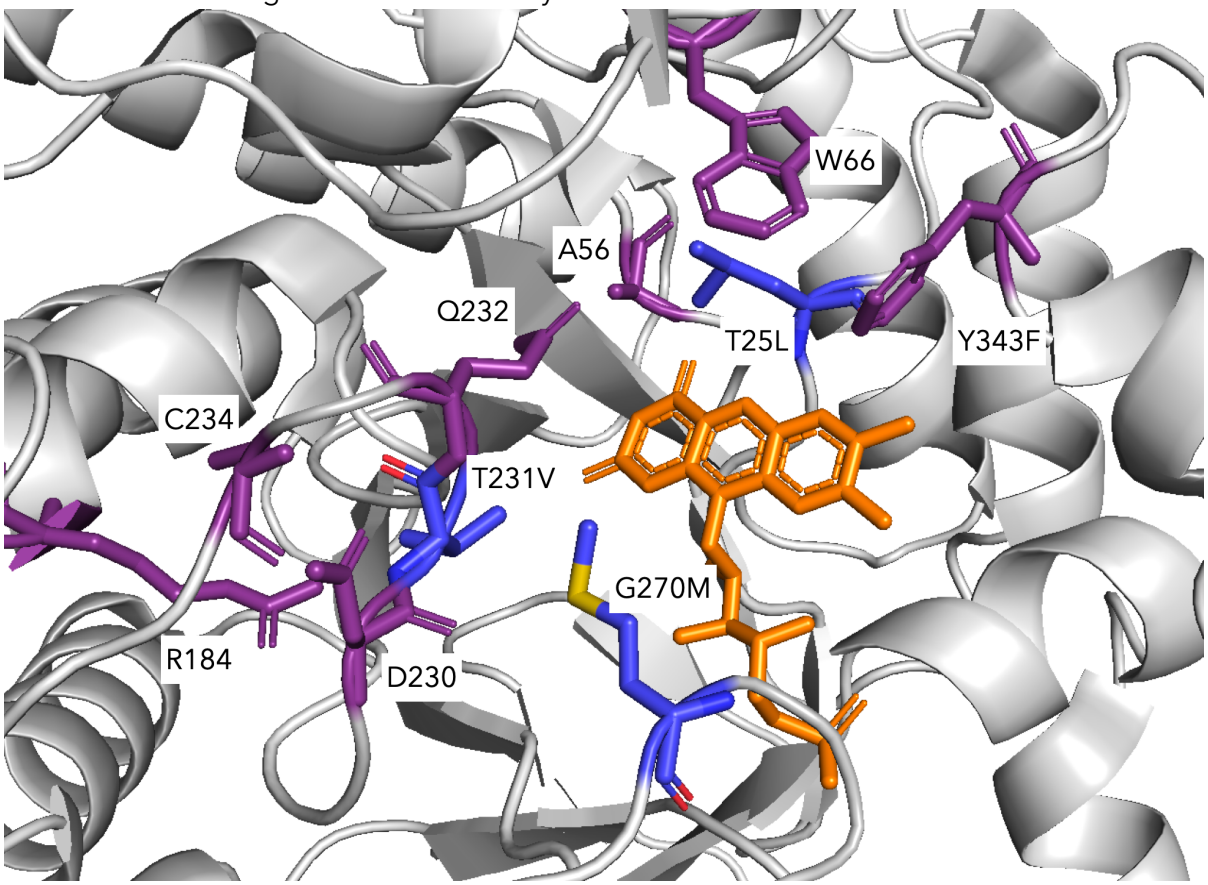


Supplemental Figure 7: Round 3 Hit Ratios Summary

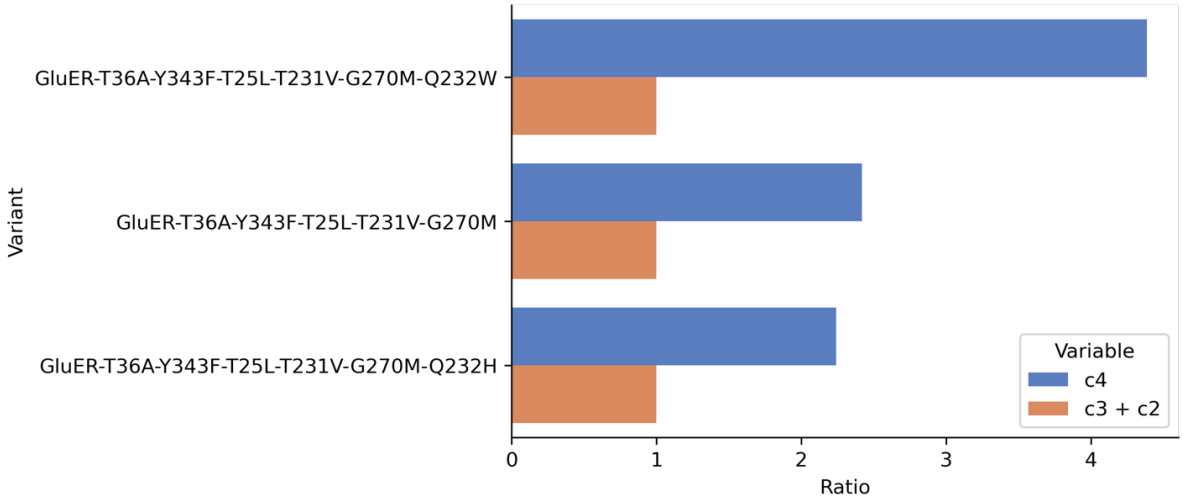


Supplemental Figure 8: Round 3 Hit Yields Summary

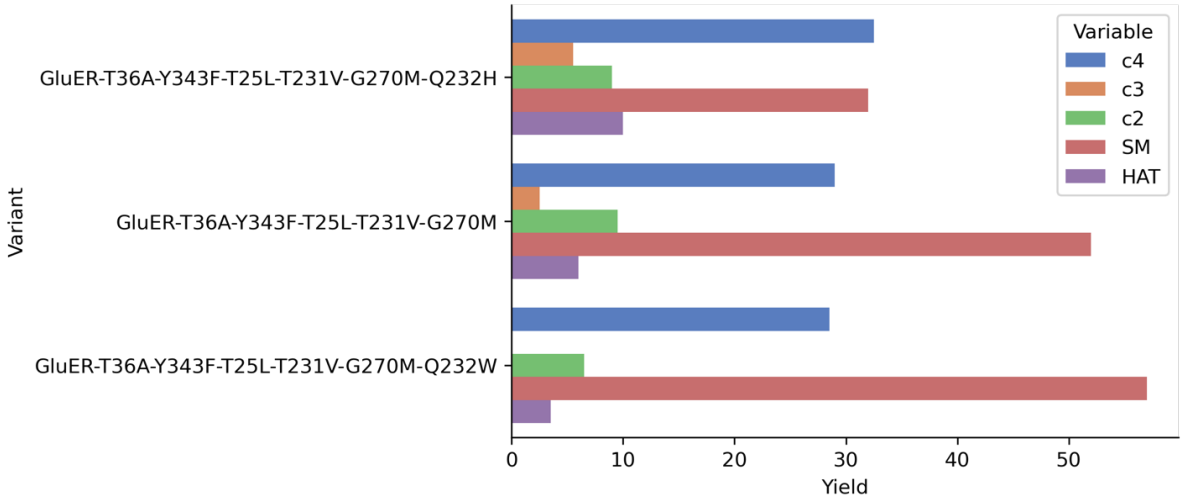
Site Saturation Mutagenesis for Indole Alkylation **Round 4**



Template	Sites Targeted	Hits Identified
GluER T36A Y343F T25L T231V G270M	Q232, F343, D230, R184, A56, W66	G270L, G270M, Q232W, C97W, Q232H

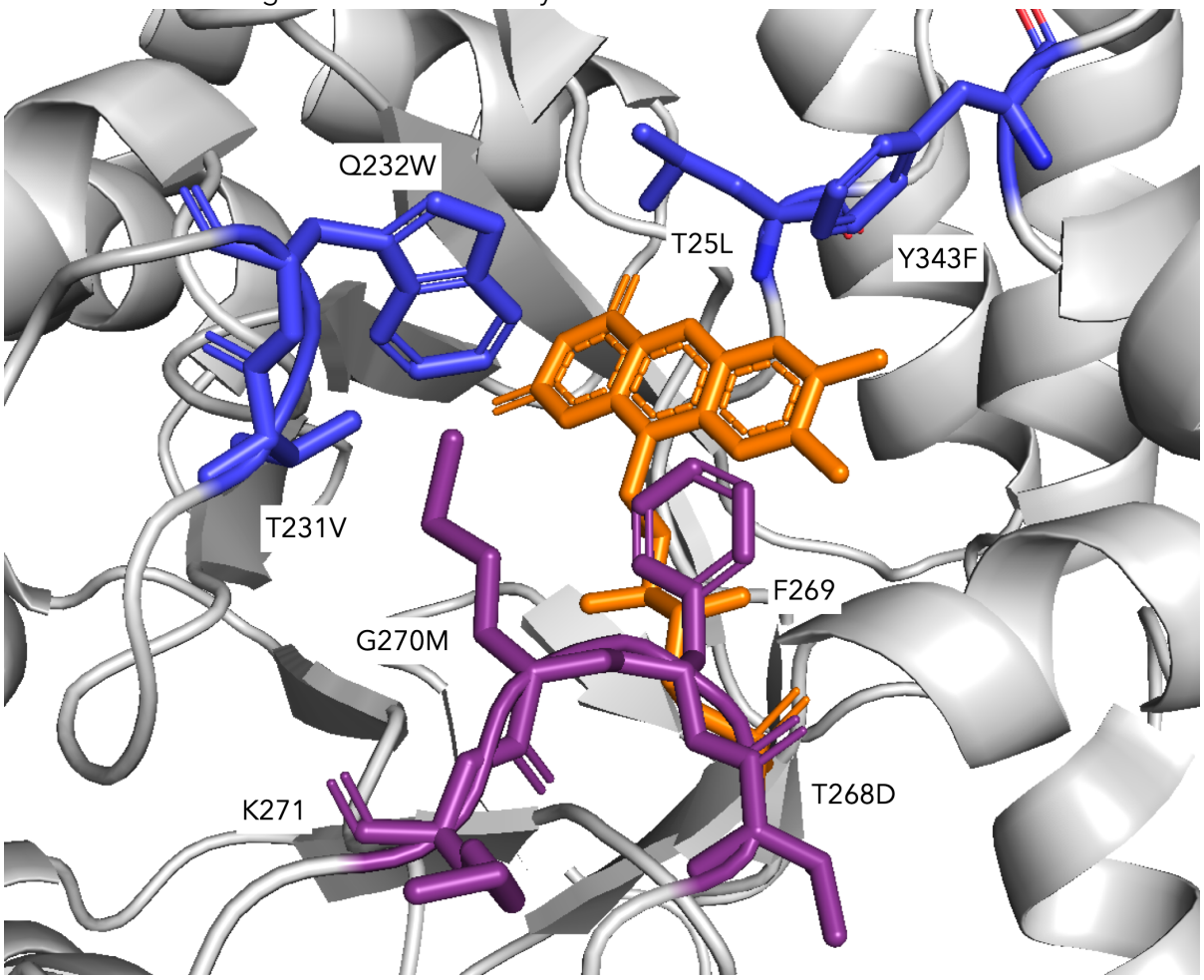


Supplemental Figure 9: Round 4 Hit Ratios Summary

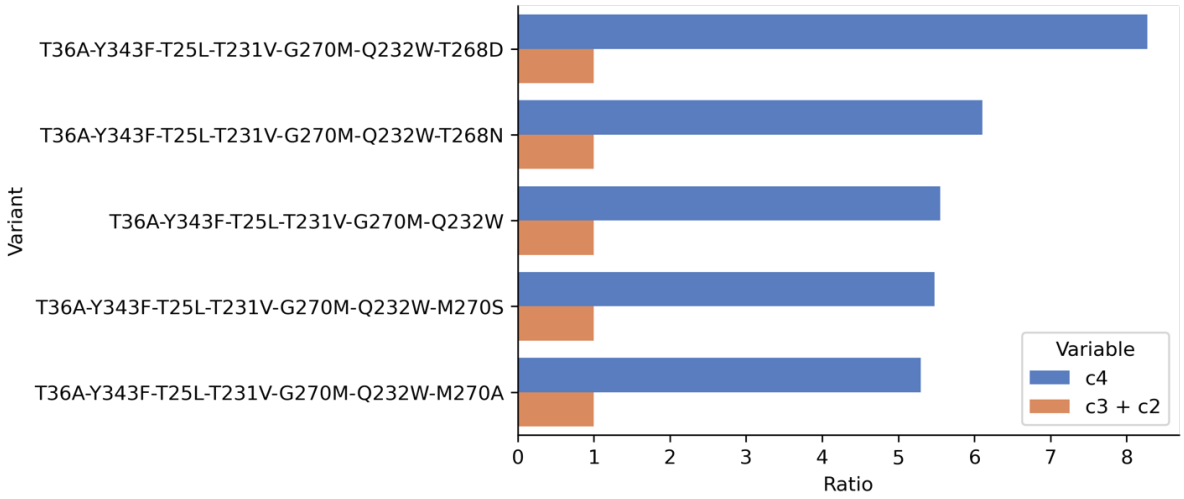


Supplemental Figure 10: Round 4 Hit Yields Summary

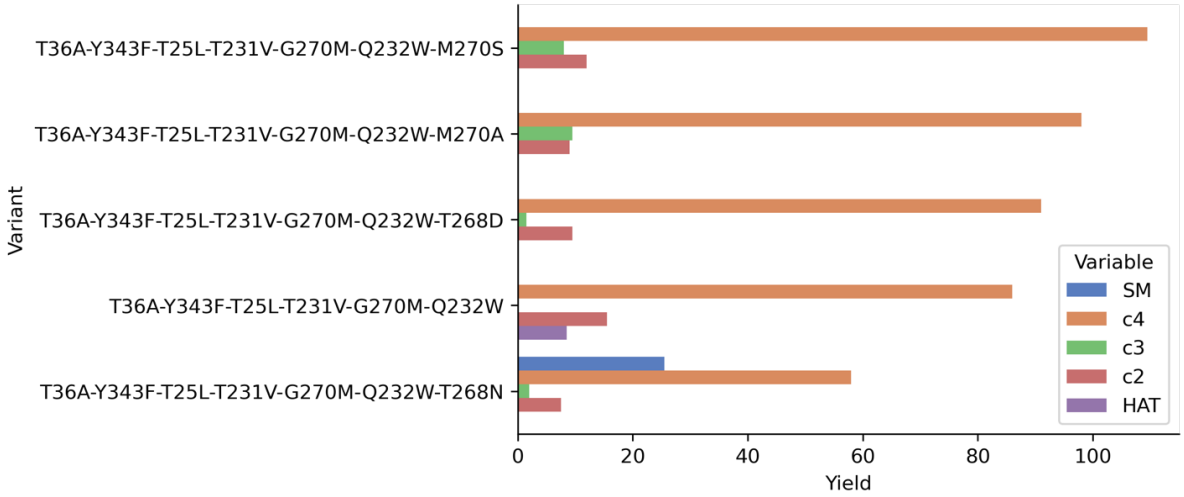
Site Saturation Mutagenesis for Indole Alkylation **Round 5**



Template	Sites Targeted	Hits Identified
GluER T36A Y343F T25L T231V G270M Q232W	M270, F269, T268, K271	M270S, M270A, T268N, T268D

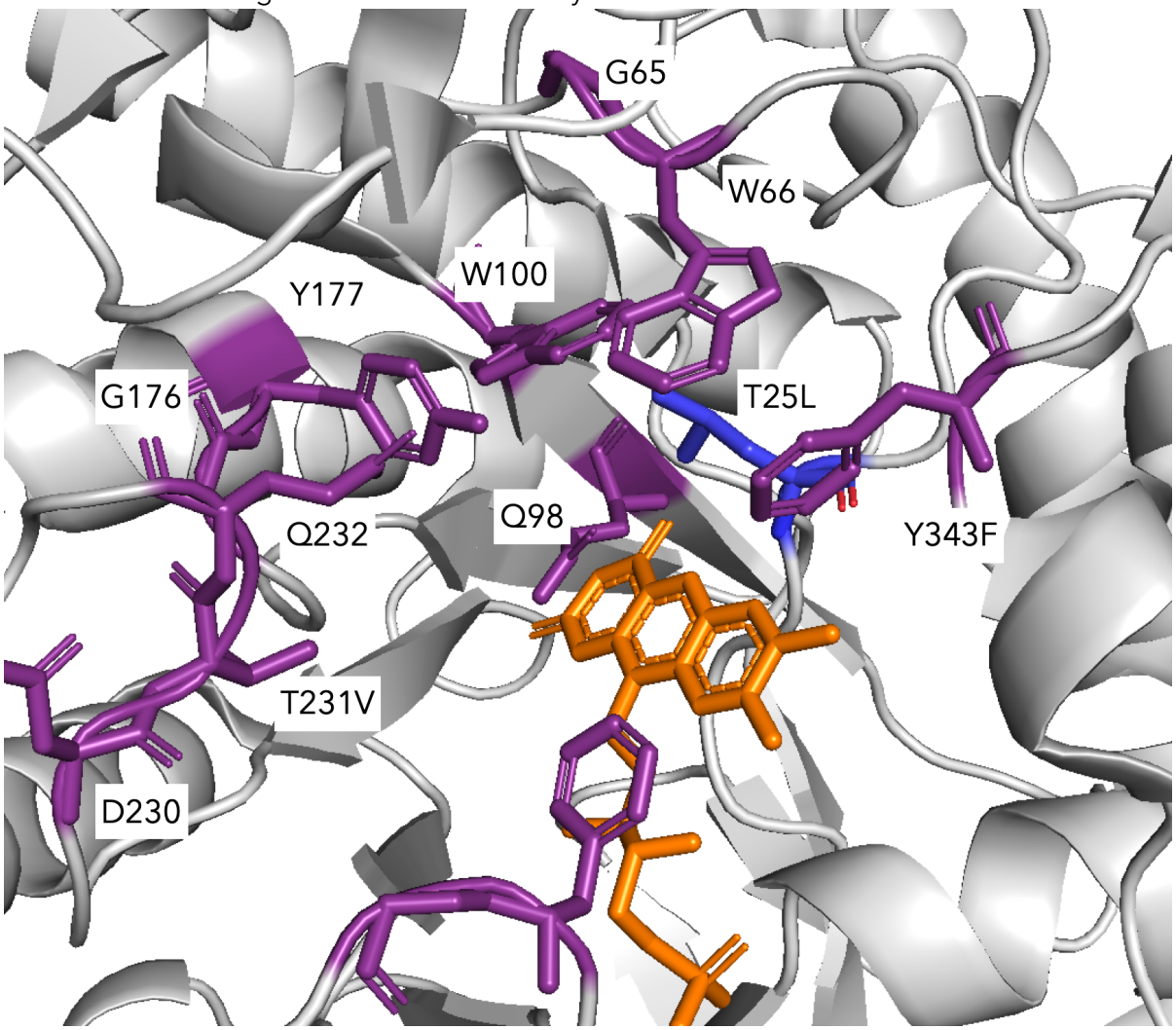


Supplemental Figure 11: Round 4 Hit Ratios Summary

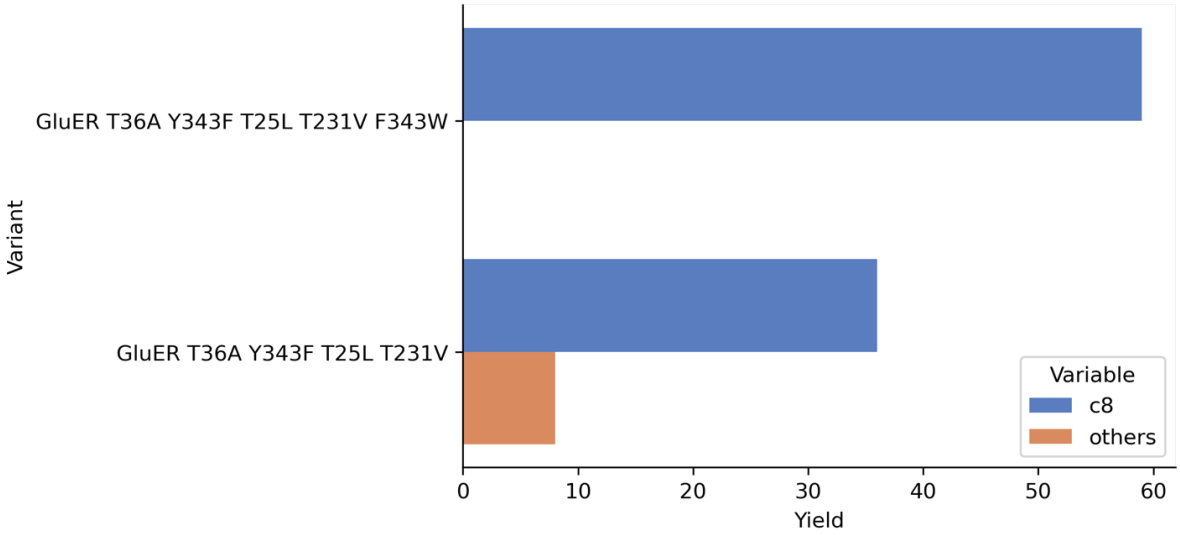


Supplemental Figure 12: Round 4 Hit Yields Summary

Site Saturation Mutagenesis for Quinoline Alkylation **Round 1**



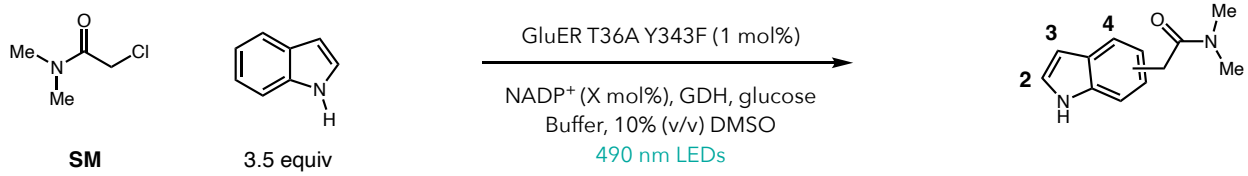
Template	Sites Targeted	Hits Identified
GluER T36A Y343F T25L T231V	W66, G176, G270, Q232, T231, D230, F343, F269, I179, Y177, G65, Q98, W100	F343W



Supplemental Figure 13: Quinoline Hit Yield Summary

Part V. Reaction Condition Optimization:

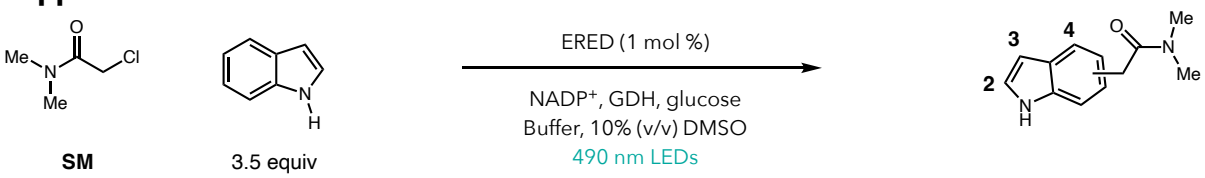
Supplemental Table 2: NADP⁺ loading with GluER T36A Y343F



NADP⁺ mol%	SM yield (%)	C2 % yield	C3 % yield	C4 % yield
1%	n.d.	42%	15%	37%
2%	n.d.	34%	18%	33%
3%	n.d.	25%	15%	30%
4%	n.d.	25%	16%	32%
5%	n.d.	24%	16%	33%
10%	55%	7%	10%	18%

Reaction conditions: α-chloroamide (2 mg, 20 μmol), 3.5 equiv. of arene (70 μmol), 1 mol% GluER T36A Y343F, X mol% NADP⁺, 1 mg GDH and 6 equiv of glucose (120 μmol) was irradiated with Cyan LEDS for 24 hours. Yield of each regioisomer was determined by NMR using 1,3,5-trimethoxybenzene as standard.

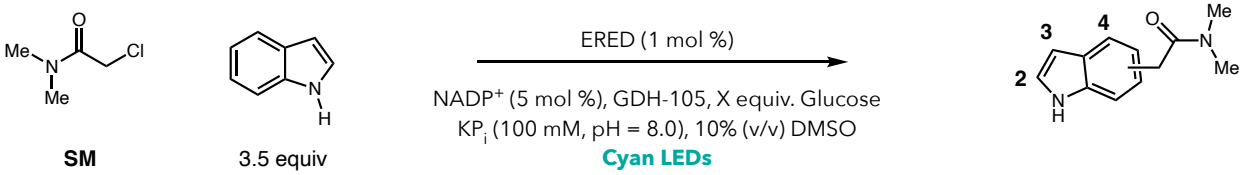
Supplemental Table 3: Buffer Screen across EREDs



ERED	Buffer	SM yield (%)	C2 % yield	C3 % yield	C4 % yield
GluER T36A	100 mM Tris, pH 9	n.d.	24%	18%	32%
GluER T36A	100 mM KP_i, pH 8	30%	14%	9%	14%
GluER-R5	100 mM Tris, pH 9	n.d.	12%	n.d.	62%
GluER-R5	100 mM KP_i, pH 8	n.d.	12%	n.d.	69%
AspER	100 mM Tris, pH 9	48%	48%	n.d.	n.d.
AspER	100 mM KP_i, pH 8	79%	7%	n.d.	n.d.

Reaction conditions: α-chloroamide (2 mg, 20 μmol), 3.5 equiv. of arene (70 μmol), 1 mol% ERED, 1 mol% $NADP^+$, 1 mg GDH and 6 equiv of glucose (120 μmol) was irradiated with Cyan LEDS for 24 hours. Yield of each regioisomer was determined by NMR using 1,3,5-trimethoxybenzene as standard.

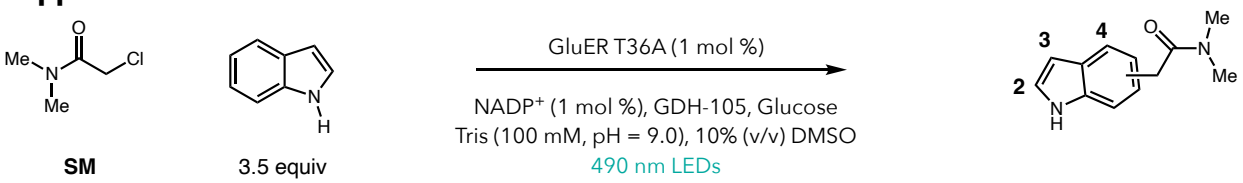
Supplemental Table 4: Glucose loading screen



ERED	Eq. Glucose	SM % yield	C2 % yield	C3 % yield	C4 % yield
PagER	6	n.d.	9%	n.d.	91%
PagER	0.5	n.d.	9%	n.d.	91%
GluER T36A	6	<5%	32%	20%	25%
GluER T36A	0.5[a]	<5%	26%	13%	22%

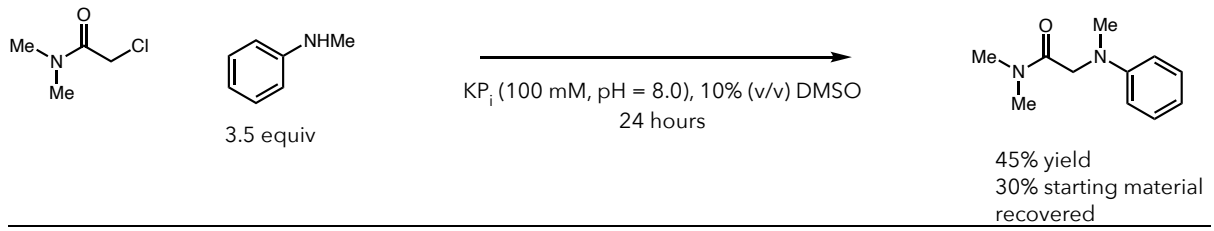
Reaction conditions: α-chloroamide (2 mg, 20 μmol), 3.5 equiv. of arene (70 μmol), 1 mol% ERED, 5 mol% $NADP^+$, 1 mg GDH and 6 or 0.5 equiv of glucose (120 μmol) was irradiated with Cyan LEDS for 24 hours. Yield of each regioisomer was determined by NMR using 1,3,5-trimethoxybenzene as standard. Reactions with GluER T36A used 1 mol% $NADP^+$

Supplemental Table 5: Control Reations with GluER T36A



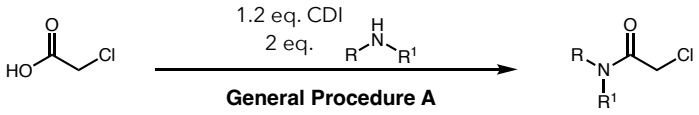
Condition	SM yield (%)	C2 % yield	C3 % yield	C4 % yield
standard	n.d.	24%	18%	32%
1 mol% FMN	98%	n.d.	n.d.	n.d.
no NADP+	65%	20%	4%	8%
no GDH	66%	16%	2%	5%
no glucose	72%	18%	4%	7%
no light	>99%	n.d.	n.d.	n.d.

Reaction conditions: α-chloroamide (2 mg, 20 μmol), 3.5 equiv. of arene (70 μmol), 1 mol% ERED, 1 mol% $NADP^+$, 1 mg GDH and 6 equiv of glucose (120 μmol) was irradiated with Cyan LEDS for 24 hours. Yield of each regioisomer was determined by NMR using 1,3,5-trimethoxybenzene as standard.



Supplemental Figure 14: Background N-alkylation of N-methylaniline

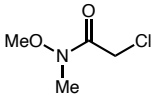
Part VI. Starting Materials Synthesis and Characterization:



General Procedure A

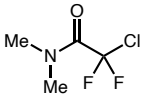
Starting materials were synthesized using a procedure in Page et. al,.[49] To a flame dried flask and stir bar, dry DCM was added. 1.2 eq of CDI and the starting acid were added and the reaction was stirred for 1 hour. The amine was added and the reaction was allowed to stir overnight. The reaction mixture was poured into a separatory funnel containing saturated sodium bicarbonate and more DCM. The DCM layer was separated and then washed with 10% HCl. The organic layer was then washed with brine, dried over sodium sulfate and concentrated *in vacuo* to afford the pure chloroamide.

2-chloro-N-methoxy-N-methylacetamide (4)



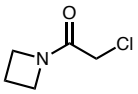
Synthesized using general procedure A. All spectra match reported values.[49]

2-chloro-2,2-difluoro-N,N-dimethylacetamide (5)



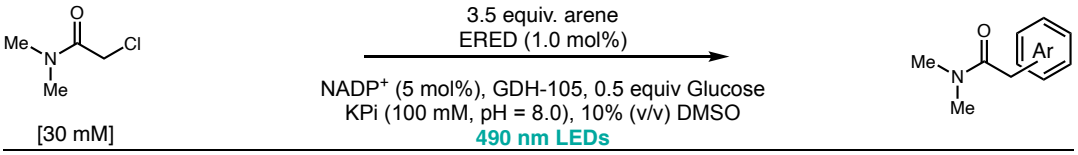
Synthesized using general procedure A. All spectra match reported values.[49]

1-(azetidin-1-yl)-2-chloroethan-1-one (6)



Synthesized using General Procedure A to produce a clear oil. All spectra match reported values.[50]

Part VII. General Procedure for the Biocatalytic Alkylation of Arenes:



General Procedure for NMR Yield:

All reactions were set up in a Coy® Chamber. Reactions were run with 20 umol of chloroamide starting material at a final substrate concentration of 30 mM in duplicate. A shell vial with a magnetic cross stir bar was charged with a "master mix" solution of 0.5 equivalents of (D)-glucose and 1 mg of GDH/ 20 μmol of substrate dissolved in 100 mM Tris pH 9 or 100 mM KP_i pH 8 in a volume such that the final concentration of substrate would be 30 mM after addition of all the other reagents. d_6-DMSO (deuterated DMSO allowed for simplified NMR analysis) was added to the shell vial such that it would be 10% (v/v). Then 140 uL of a 5 mg/mL solution of NADP+ was added. Finally, 1 mol% of ERED was added. Neat chloroamide was then added to the shell vial, followed by arene. Then the arene was added. Both reagents were added neat if the density was known. If the density of either of the substrates was not known, they were added as stock solutions in d_6-DMSO. The vial was sealed with a rubber septa and brought out of the Coy® Chamber where it is placed on a stir plate at 300 rpm under a fan and irradiated with cyan LEDs (50 W Chanzon high power LED chip, λ_{max}= 490 nm, measured photon flux = 12,000 mM/m^2 s) for 24 hours.

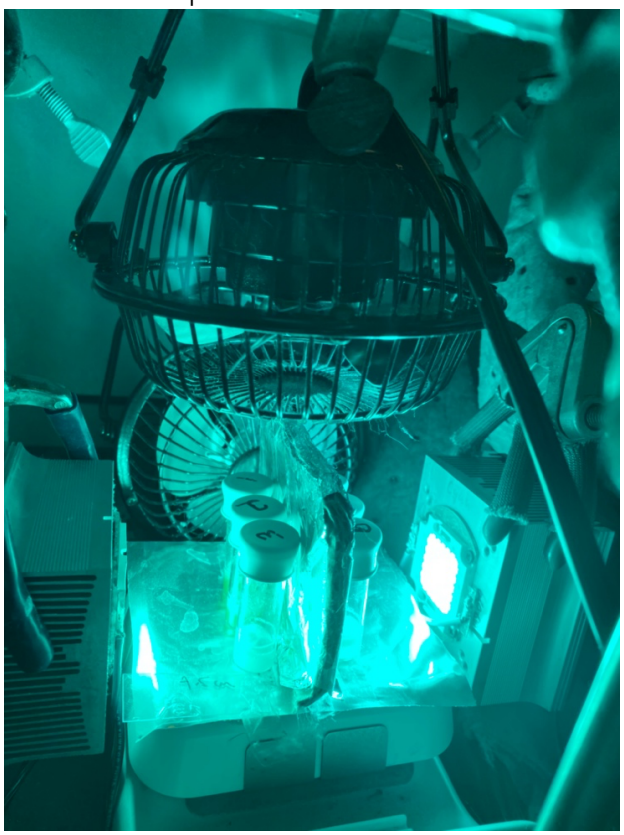
After 24 hours, 6.667 μmol of internal standard (1,3,5-trimethoxybenzene) was added from a stock solution in ethyl acetate.

Workup 1: Then an equal volume of ethyl acetate was added and the reactions were stirred for 30 minutes. They were centrifuged at 2000 x g for 2 minutes and organic layer was removed. The extraction process was repeated 6 times. The organic layers were pooled and concentrated *in vacuo* and then resuspended in $CDCl_3$ and concentrated *in vacuo* again. The dried crude mixture was resuspended in $CDCl_3$ and analyzed by ^{1}H-NMR for yield calculation, where the singlet at δ = 6.06 ppm corresponding to the three aromatic protons of 1,3,5-trimethoxybenzene was set to an integration of 1.00. Integration of varying signals of different product could be used to calculate an effective yield. The regioisomeric ratio was determined relative to the trimethoxybenzene standard.

Workup 2: 1 mL of ACN was added to crash out protein and the water was removed using a Genovac EZ-Plus 2 on the aqueous setting at 45°. The dried crude mixture was resuspended in $CDCl_3$ and analyzed by ^{1}H-NMR for yield calculation, where the singlet at δ = 6.06 ppm corresponding to the three aromatic protons of 1,3,5-trimethoxybenzene was set to an integration of 1.00. Integration of varying signals of different product could be used to

calculate an effective yield. The regioisomeric ratio was determined relative to the trimethoxybenzene standard.

Photo: NMR yield scale reaction set up



General Procedure for isolated yield:

All reactions were set up in an anaerobic Coy® Chamber. They were run with 200 umol of chloroamide starting material at a final substrate concentration of 30 mM. A 25 mL pear-shaped flask with a stir bar was charged with 0.5 equivalents of (D)-glucose, 20 mg of GDH and 5 mol% of NADP+. The solids were brought into the Coy Chamber and dissolved in 100 mM KP_i pH 8 in a volume such that the final concentration of substrate would be 30 mM after addition of all the other reagents. DMSO was added to the flask such that it would be 10% (v/v) of the final volume. Finally, 1 mol% of ERED was added. If the arene or alkyl halide was a solid, it was weighed and put in the flask beforehand. Both reagents were added neat under anaerobic conditions if it was a liquid and the density was known. The vial was sealed with a rubber septa and brought out of the Coy® Chamber where it is placed on a stir plate at 600

rpm under a fan and immediately irradiated with cyan LEDs (50 W Chanzon high power LED chip, λ_{max}= 490 nm, measured photon flux = 12,000 mM/m^2 s) for 24 hours.

Workup was performed as follows: 66.7 umol of trimethoxybenzene was added to the contents of the reaction flask and they were poured into a 125 mL Erlenmeyer flask containing 50 mL of water and 50 mL of ethyl acetate. This was stirred vigorously for 45 minutes, after which time the biphasic mixture was filtered through a thick pad of Celite® to remove precipitated protein. This pad was washed with ethyl acetate (2 x 25 mL). The filtrate was poured into a separatory funnel and the dichloromethane layer was collected. The aqueous layer was extracted with dichloromethane (4 x 50 mL) (without any emulsion-related difficulties), and the combined organic layers were dried with anhydrous sodium sulfate and concentrated. A crude NMR yield was taken before purification. The crude residue was purified using silica gel chromatography (20% – 80% EtOAc in toluene). Fractions containing product were combined and concentrated to yield the pure coupled product. If further purification was necessary, a reverse phase column was run. To separate out the regioisomers for purification, semi-preparative HPLC was used.

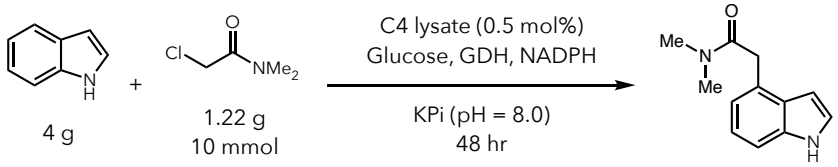
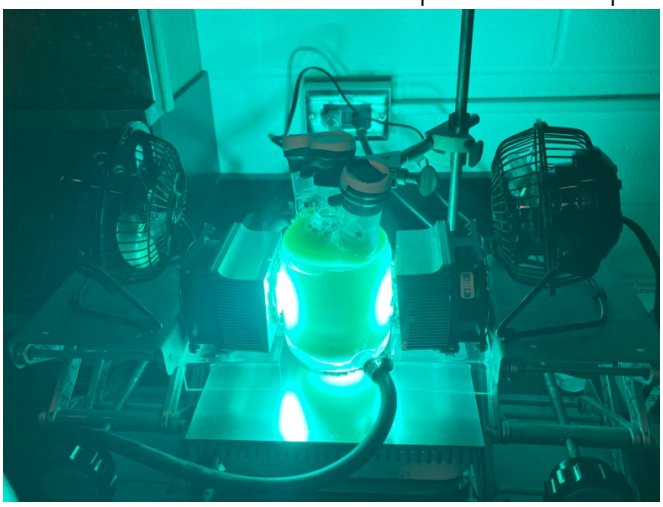
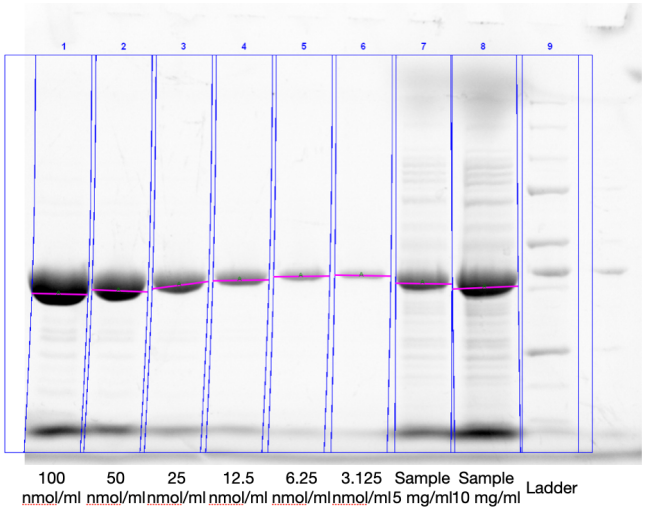
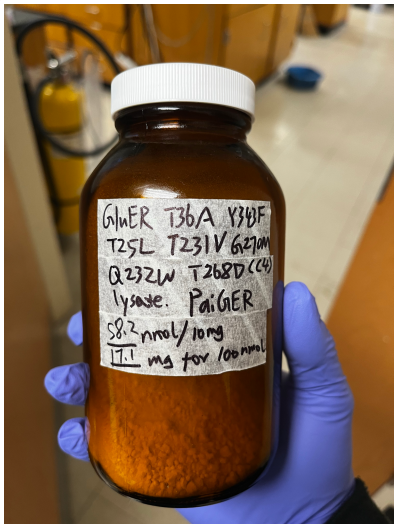


Photo: Gram scale reaction set up and isolated product

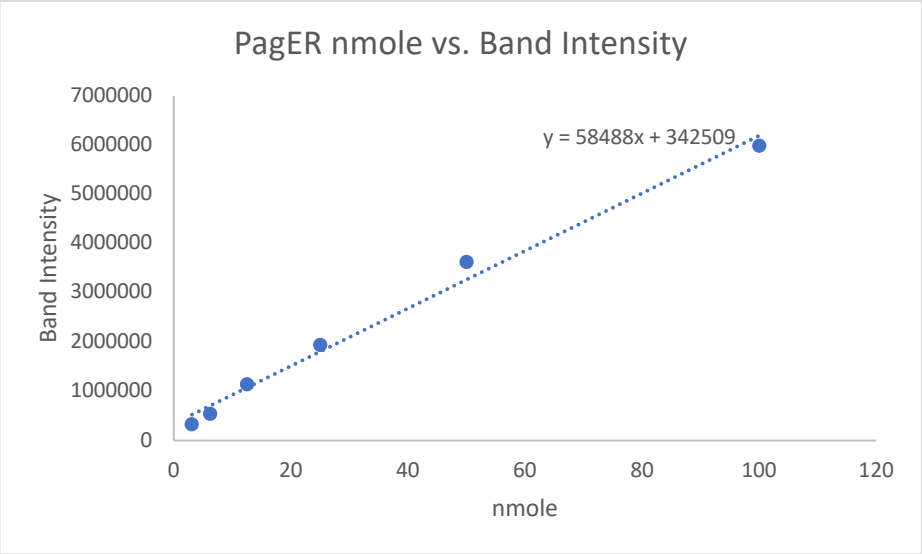


Lysate standard curve and enzyme concentration for the gram scale reaction:





PagER Lysate Standard Curve:



General Procedure for gram scale reaction:

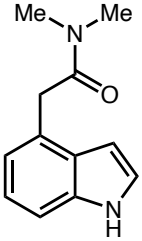
Gram scale reaction was set up in an anaerobic three neck jacketed flask. They were run with 10 mmol of chloroamide starting material at a final substrate concentration of 30 mM. A 250 mL three neck jacket flask with a stir bar was charged with 8.55 g of PagER lysate (0.5 mol% at 17.1 mg/100 nmol), 4 g of indole (3.5 equiv), 1.8 g of (D)-glucose (1.1 equiv), 500 mg of GDH and 350 mg of $NADP^+$ (0.5 equiv). The solids were sealed with rubber septa, vacuumed and refilled with nitrogen gas three times. Then they were dissolved in 100 mM degassed KP_i pH 8 in a volume such that the final concentration of substrate would be 30 mM through cannula transferring. Upon completion, chloroamide was added via syringe neat. DMSO was added to the flask slowly such that it would be 10% (v/v) of the final volume. The jacketed flask was connected with water cooling line (tap water, rt) and brought on a stir plate at 400 rpm under a fan and immediately irradiated with cyan LEDs (2 x 100 W Chanzon high power LED chip from the side and 1 x 50 W Chanzon high power LED chip, λ_{max}= 490 nm, measured photon flux = 12,000 mM/m^2 s) for 48 hours.

Workup was performed as follows: the reaction media was poured into a 2L Erlenmeyer flask containing 600 mL of MeCN. Enzymes were immediately crashed out and this was stirred vigorously for 20 minutes, after which time the heterogenous mixture was filtered through a thick pad of Celite® to remove precipitated protein. This pad was washed with MeCN (3 x 100 mL). The filtrate was poured into a round bottom flask and most of the MeCN was removed in vacuo. Then the remaining aqueous mixture was transferred into a separatory funnel and the dichloromethane layer was collected. The aqueous layer was extracted with dichloromethane (4 x 300 mL) (without any emulsion-related difficulties), and the combined organic layers were dried with anhydrous sodium sulfate and concentrated. Excess DMSO was distilled off under vacuum and then the crude residue was purified using silica gel chromatography (50% – 100%

EtOAc in toluene). Fractions containing product were combined and concentrated to yield the pure isolated product 2-(1H-indol-4-yl)-N,N-dimethylacetamide (1.05 g, 52% isolated yield)

Part VIII. Product Characterization:

2-(1H-indol-4-yl)-N,N-dimethylacetamide (**3a)**



The product standard was isolated using the isolated scale biocatalytic procedure using PagER (75% isolated yield, 9:1 C4: others). It was further purified by semi-preparative HPLC (40-95% MeCN over 18 minutes) to give the pure product as a white solid. Spectra match reported values.[51]

NMR yield:

Trial 1: 91% yield C4, 10% yield C2, <5% yield C3 (9:1 C4: others)
Trial 2: 88% yield, 9% yield C2, < 5% yield C3 (9:1 C4: others)

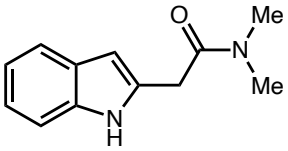
^{1}H NMR (500 MHz, $CDCl_3$): δ 8.39 (s, 1H), 7.30 (d, J = 8.1 Hz, 1H), 7.20 (t, J = 2.9 Hz, 1H), 7.14 (t, J = 7.7 Hz, 1H), 6.98 (d, J = 7.2 Hz, 1H), 6.65 (dd, J = 2.2, 1.1 Hz, 1H), 4.01 (s, 2H), 2.97 (d, J = 11.5 Hz, 6H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 171.5, 136.0, 127.4, 127.2, 124.3, 122.3, 119.6, 110.0, 101.0, 39.5, 38.0, 35.8.

IR (cm^{-1}) : 3257, 2923, 1630, 1501, 1397, 1342, 755

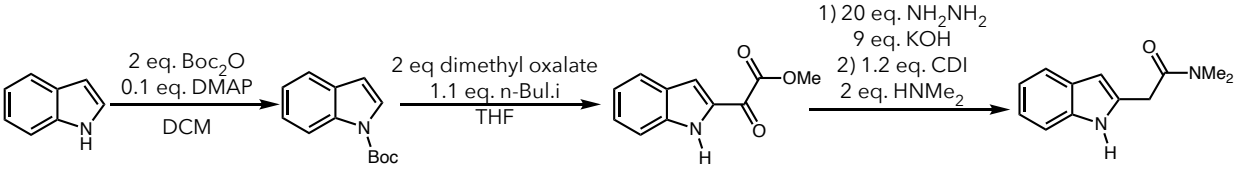
HRMS: calc'd $[M+H]^+$: 203.117886, found 203.11751

2-(1H-indol-2-yl)-N,N-dimethylacetamide (**3b)**



The product standard was synthesized using the following procedure. The assay yields were determined using the NMR scale biocatalytic procedure with 1.0 mol% AspER except no $NADP^+$, GDH-105 or glucose was added and the reaction was run in 100 mM Tricine pH 9.

Synthetic Roadmap:



Procedure 1: Indole (1 equiv) and DMAP (0.1 equiv) were added to a round bottom flask and dissolved in DCM. Boc_2O (2 equiv) was added in portions and the solution was left to stir overnight. The reaction mixture was washed with brine, dried over sodium sulfate and concentrated *in vacuo*. The crude product was purified using silica gel chromatography (99% yield).

Procedure 2: Using a modified procedure from Hlasta et, al[52], 1-Boc indole was added to a flamed dried flask charged with a magnetic stir bar. The flask was placed under inert atmosphere. Dry THF was then added and the flask was cooled to -78°C. 1.1 equiv of *t*-butyllithium was then added dropwise and the solution was allowed to stir at -78°C for 30 minutes. Dimethyl oxalate (2 equiv) was placed in a flame dried vial and dissolved in dry THF and then added to the reaction flask dropwise. The reaction mixture was left to warm up to room temperature overnight. The reaction was quenched with water and transferred to a separatory funnel. The aqueous layer was extracted three times with ethyl acetate and then dried over sodium sulfate. The organic layer was then concentrated and purified using silica gel chromatography. During the purification process, the indole was deprotected to yield pure methyl 2-(1H-indol-2-yl)-2-oxoacetate (45% yield).

Procedure 3: The procedure used was modified from Bennasar et. al.[53] methyl 2-(1H-indol-2-yl)-2-oxoacetate, hydrazine hydrate (20 equiv, 80% solution in water) and potassium hydroxide (9 equiv) were added to a flask with a stir bar and dissolved in ethanol. The solution was refluxed under an inert atmosphere for 5 hours. The ethanol and hydrazine was distilled off carefully and the residue was then heated 180°C for 30 minutes. The crude residue was dissolved in water and acid was added. The aqueous layer was extracted with ethyl acetate 3 times. The organic layers were collected and dried over sodium sulfate and then concentrated

in vacuo. The crude acid was then used directly in General Procedure A to produce the product standard as a pink solid in 83% yield over two steps.

NMR yield:

Trial 1: 52% yield
Trial 2: 48% yield

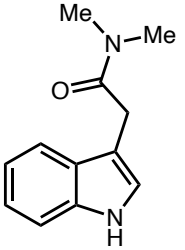
^{1}H NMR (500 MHz, $CDCl_3$): δ 9.09 (s, 1H), 7.54 (d, J = 7.8 Hz, 1H), 7.33 (d, J = 8.1 Hz, 1H), 7.14 (ddd, J = 8.1, 7.0, 1.2 Hz, 1H), 7.10 – 7.02 (m, 1H), 6.30 (s, 1H), 3.89 (s, 2H), 3.11 (s, 3H), 2.98 (s, 3H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 170.0, 136.3, 131.8, 128.2, 121.5, 119.9, 119.7, 110.9, 100.9, 37.9, 35.7, 33.0.

IR (cm^{-1}) : 3260, 2923, 2852, 1633, 1494, 1455, 1339, 1229, 743

HRMS: calc'd $[M+H]^+$: 203.117886, found 203.11751

2-(1H-indol-3-yl)-N,N-dimethylacetamide (**3c)**



The product standard was synthesized using General Procedure A from commercially available starting materials. All spectra match reported values.[54]

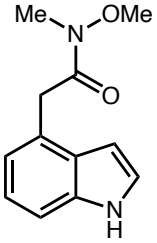
^{1}H NMR (500 MHz, $CDCl_3$): δ 8.19 (s, 1H), 7.65 (d, J = 7.9 Hz, 1H), 7.36 (dd, J = 8.1, 1.0 Hz, 1H), 7.22 – 7.17 (m, 1H), 7.17 – 7.08 (m, 2H), 3.83 (d, J = 1.1 Hz, 2H), 3.03 (s, 3H), 2.98 (s, 3H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 171.7, 136.3, 127.4, 122.6, 122.3, 119.8, 119.0, 111.3, 109.5, 38.0, 35.8, 31.4.

IR (cm^{-1}) : 3245, 2926, 1628, 1457, 1261, 742

HRMS: calc'd $[M+H]^+$: 203.117886, found 203.11751

2-(1H-indol-4-yl)-N-methoxy-N-methylacetamide (**12a**)



The product standard was isolated using the isolated scale biocatalytic procedure using PagER (67% isolated yield, 3.7:1 C4: others). It was further purified by semi-preparative HPLC (40-95% MeCN over 18 minutes) to give the pure product as a white solid.

NMR yield:

Trial 1: 50% yield C4 11% yield C2 ~<5% yield C3
Trial 2: 54% yield C4 11% yield C2 ~<5% yield C3

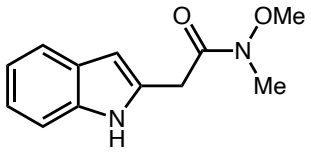
^{1}H NMR (500 MHz, $CDCl_3$): δ 8.36 (s, 1H), 7.27 (d, J = 8.5 Hz, 1H), 7.22 – 7.08 (m, 2H), 7.04 (d, J = 7.1 Hz, 1H), 6.65 (dt, J = 4.4, 1.6 Hz, 1H), 4.07 (s, 2H), 3.54 (s, 3H), 3.20 (s, 3H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 172.8, 135.9, 127.8, 127.1, 124.2, 122.1, 122.1, 120.5, 110.1, 101.1, 61.4, 37.6, 32.4.

IR (cm^{-1}): 3286, 3052, 2935, 1636, 1416, 1340, 1174, 751

HRMS: calc'd $[M+H]^+$: 219.112806, found 219.11247

2-(1H-indol-2-yl)-N-methoxy-N-methylacetamide (**12b**)



The product standard was isolated using the isolated scale biocatalytic procedure using PagER (67% isolated yield, 3.7:1 C4:C2+C3). It was further purified by semi-preparative HPLC (40-95% MeCN over 18 minutes) to give the pure product as a white solid.

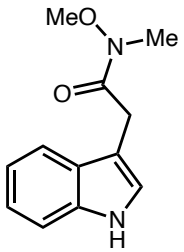
^{1}H NMR (500 MHz, $CDCl_3$): δ 9.00 (s, 1H), 7.54 (dd, J = 7.8, 1.1 Hz, 1H), 7.34 (dd, J = 8.0, 1.0 Hz, 1H), 7.14 (ddd, J = 8.1, 7.0, 1.2 Hz, 1H), 7.07 (td, J = 7.5, 1.1 Hz, 1H), 6.35 (dd, J = 2.1, 1.1 Hz, 1H), 3.99 (s, 2H), 3.73 (s, 3H), 3.24 (s, 3H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 171.1, 136.4, 131.9, 128.3, 121.7, 120.1, 119.8, 111.0, 101.6, 61.8, 32.3, 31.3.

IR (cm^{-1}): 3302, 3053, 2934, 1645, 1456, 1421, 1386, 1297, 748

HRMS: calc'd $[M+H]^+$: 219.112806, found 219.11247

2-(1H-indol-3-yl)-N-methoxy-N-methylacetamide (**12c**)



The product standard was synthesized using General Procedure A from commercially available starting materials. The pure product is a off-white solid.

IR (cm^{-1}): 3285, 2933, 1636, 1458, 1421, 1174, 1096, 1000, 741

HRMS: calc'd $[M+H]^+$: 219.112806, found 219.11247

2,2-difluoro-2-(1H-indol-4-yl)-N,N-dimethylacetamide (**13)**

The product standard was isolated using the isolated scale biocatalytic procedure using 1.5 mol% of PagER (45% isolated yield).

NMR yield:

Trial 1: 48% yield
Trial 2: 54% yield

^{1}H NMR (500 MHz, CDCl3): δ 8.79 (s, 1H), 7.51 (d, J = 8.1 Hz, 1H), 7.30 (d, J = 7.4 Hz, 1H), 7.20 (t, J = 7.8 Hz, 1H), 6.79 (s, 1H), 3.05 (s, 3H), 2.90 (s, 3H).

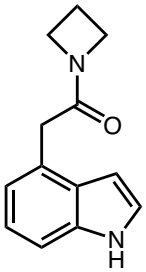
^{13}C NMR (126 MHz, CDCl3): δ 164.2, 136.7, 125.9, 124.8, 124.4, 121.3, 117.3, 114.2, 114.2, 114.2, 101.9, 101.8, 101.8, 37.6, 37.1.

^{19}F NMR (282 MHz, $CDCl_3$): δ -93.9 (s).

IR (cm^{-1}): 3280, 1656, 1403, 1342, 1221, 1169, 1078, 898, 757

HRMS: calc'd $[M+H]^+$: 239.099046, found 239.09839

1-(azetidin-1-yl)-2-(1H-indol-4-yl)ethan-1-one (**14a)**



The product standard was isolated using the isolated scale biocatalytic procedure using 1 mol% of PagER (43% isolated yield 5:1 C4:C2). It was purified using semi-prep HPLC (30 to 75% ACN in H2O over 23 minutes) for NMR analysis to give the C4 adduct as a white solid.

NMR yield:

Trial 1: 32% yield C4, 6% yield C2 (5:1 C4:C2)
Trial 2: 32% yield C4, 7% yield C2 (5:1 C4: C2)

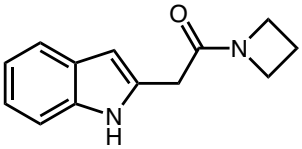
^{1}H NMR (500 MHz, $CDCl_3$): δ 8.50 (s, 1H), 7.29 (d, J = 8.1 Hz, 1H), 7.18 (t, J = 2.9 Hz, 1H), 7.13 (t, J = 7.6 Hz, 1H), 7.01 (d, J = 7.2 Hz, 1H), 6.65 (t, J = 2.5 Hz, 1H), 4.01 (dt, J = 10.8, 7.7 Hz, 4H), 3.77 (s, 2H), 2.15 (p, J = 7.7 Hz, 2H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 171.2, 136.0, 127.7, 126.7, 124.3, 122.1, 120.2, 110.1, 101.1, 50.9, 48.3, 37.9, 15.3.

IR (cm^{-1}): 3265, 2922, 2853, 1626, 1464, 1441, 722

HRMS: calc'd $[M+H]^+$: 215.117886, found 215.11757

1-(azetidin-1-yl)-2-(1H-indol-2-yl)ethan-1-one (**14b)**



The product standard was synthesized using the isolated scale biocatalytic procedure.

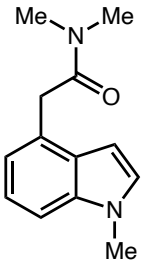
^{1}H NMR (500 MHz, $CDCl_3$): δ 9.10 (s, 1H), 7.53 (d, J = 7.8 Hz, 1H), 7.34 (d, J = 8.1 Hz, 1H), 7.19 – 7.02 (m, 2H), 6.30 – 6.24 (m, 1H), 4.25 (t, J = 7.7 Hz, 2H), 4.05 (t, J = 7.8 Hz, 2H), 3.60 (s, 2H), 2.27 (p, J = 7.7 Hz, 2H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 169.6, 136.4, 131.6, 128.3, 121.7, 120.0, 119.8, 111.1, 101.0, 50.7, 48.3, 30.9, 15.1.

IR (cm^{-1}): 3257, 3051, 2950, 2879, 1633, 1455, 1430, 1297, 800

HRMS: calc'd $[M+H]^+$: 215.117886, found 215.11757

N,N-dimethyl-2-(1-methyl-1H-indol-4-yl)acetamide (**15a**)



The product standard was isolated using the isolated scale biocatalytic procedure using 1.0 mol% of GluER-T36A-Y343F-T25L-T231V-G270M-Q232W (40% isolated yield).

NMR yield:

Trial 1: 38% yield C4, 8% yield C2 (5:1 C4:others)
Trial 2: 36% yield C4, 7% yield C2 (5:1 C4:others)

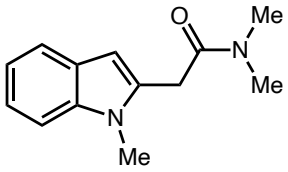
NMR: ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.24 (d, J = 8.2 Hz, 1H), 7.17 (d, J = 8.1 Hz, 1H), 7.06 (s, 1H), 6.98 (d, J = 7.1 Hz, 1H), 6.59 (d, J = 3.1 Hz, 1H), 3.99 (s, 2H), 3.79 (s, 3H), 2.96 (d, J = 16.1 Hz, 6H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 171.5, 136.9, 128.9, 128.0, 127.3, 121.9, 119.1, 108.1, 99.4, 39.5, 38.0, 35.8, 33.1.

IR (cm^{-1}): 2931, 1638, 1496, 1394, 1239, 1132, 745

HRMS: calc'd $[M+H]^+$: 217.133536, found 217.13312

N,N-dimethyl-2-(1-methyl-1H-indol-2-yl)acetamide (**15b**)



The product standard was isolated using the isolated scale biocatalytic procedure using 1.0 mol% of GluER-T36A-Y343F-T25L-T231V-G270M-Q232W (40% isolated yield). The spectra match the values from synthesis of the authentic standard using Procedure 2 and 3 from the synthesis of N,N-dimethyl-2-(1H-indol-2-yl)acetamide (17% overall yield).

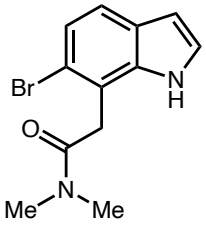
^{1}H NMR (500 MHz, $CDCl_3$): δ 7.54 (d, J = 7.9 Hz, 1H), 7.29 (d, J = 8.2 Hz, 1H), 7.19 (dd, J = 8.3, 7.0 Hz, 1H), 7.11 – 7.04 (m, 1H), 6.32 (s, 1H), 3.89 (s, 2H), 3.76 (s, 3H), 3.16 – 2.92 (m, 6H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 169.3, 137.9, 133.8, 127.7, 121.3, 120.2, 119.6, 109.3, 101.3, 38.0, 36.0, 33.7, 30.2.

IR (cm^{-1}): 2929, 1645, 1456, 1394, 1132, 771, 748

HRMS: calc'd $[M+H]^+$: 217.133536, found 217.13312

2-(6-bromo-1H-indol-7-yl)-N,N-dimethylacetamide (**16a**)



The product standard was isolated using the isolated scale biocatalytic procedure using 1.5 mol% of PagER (50% isolated yield).

NMR yield:

Trial 1: 43% yield C7, 7% yield C4 (6:1 C7:others)
Trial 2: 49% yield C7, 8% yield C4 (6:1 C7:others)

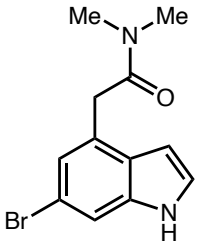
^{1}H NMR (500 MHz, d_6-DMSO): δ 11.22 (s, 1H), 7.46 (dd, J = 1.7, 0.9 Hz, 1H), 7.34 (dd, J = 3.2, 2.4 Hz, 1H), 6.96 (d, J = 1.7 Hz, 1H), 6.51 (ddd, J = 3.1, 2.0, 0.9 Hz, 1H), 3.89 (s, 2H), 2.99 (s, 3H), 2.84 (s, 3H)

^{13}C NMR (126 MHz, d_6-DMSO): δ 169.8, 136.6, 129.5, 126.7, 125.9, 121.6, 113.4, 112.3, 100.2, 37.5, 37.2, 35.0.

IR (cm^{-1}): 3235, 2921, 2851, 1630, 1418, 1395, 1140, 858, 812

HRMS: calc'd $[M+H]^+$: 281.028406, found 281.02794

2-(6-bromo-1H-indol-4-yl)-N,N-dimethylacetamide (**16b**)



The product standard was isolated using the isolated scale biocatalytic procedure using 1.5 mol% of PagER (50% isolated yield).

NMR yield:

Trial 1: 43% yield C7, 7% yield C4 (6:1 C7:others)
Trial 2: 49% yield C7, 8% yield C4 (6:1 C7:others)

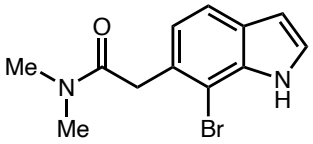
^{1}H NMR (500 MHz, d_6-DMSO) δ 11.12 (s, 1H), 7.60 (s, 1H), 7.42 (s, 1H), 7.38 – 7.34 (m, 1H), 6.40 (s, 1H), 3.80 (s, 2H), 3.06 (s, 3H), 2.87 (s, 3H).

^{13}C NMR (126 MHz, d_6-DMSO) δ 169.9, 135.6, 127.3, 126.6, 125.6, 122.1, 117.0, 114.5, 100.9, 39.8, 37.0, 35.1.

IR (cm^{-1}): 3245, 2932, 1636, 1397, 1297, 1138, 766

HRMS: calc'd $[M+H]^+$: 281.028406, found 281.02794

2-(7-bromo-1H-indol-6-yl)-N,N-dimethylacetamide (**17a)**



The product standard was isolated using the isolated scale biocatalytic procedure using 1 mol% of PagER (17% isolated yield).

NMR yield:

Trial 1: 23% yield C6, 17% yield C4 (1:1 C4:C6)
Trial 2: 23% yield C6, 15% yield C4 (1:1 C4: C6)

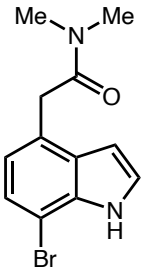
^{1}H NMR (500 MHz, $CDCl_3$): δ 8.39 (s, 1H), 7.52 (d, J = 8.0 Hz, 1H), 7.22 (t, J = 2.8 Hz, 1H), 7.05 (d, J = 8.1 Hz, 1H), 6.59 (dd, J = 3.1, 2.2 Hz, 1H), 3.95 (s, 2H), 3.01 (d, J = 7.4 Hz, 6H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 171.0, 135.3, 128.0, 127.9, 124.9, 122.0, 120.1, 106.7, 103.9, 40.5, 37.7, 35.9.

IR (cm^{-1}): 3255, 2922, 1636, 1508, 1397, 1137, 797, 727

HRMS: calc'd $[M+H]^+$: 281.028406, found 281.02791

2-(7-bromo-1H-indol-4-yl)-N,N-dimethylacetamide **(17b)**



The product standard was isolated using the isolated scale biocatalytic procedure using 1 mol% of PagER (17% isolated yield 1:1 C4:C6). It was purified using semi-prep HPLC (30 to 65% ACN in H2O over 25 minutes) for NMR analysis to give the C4 adduct as a white solid.

NMR yield:

Trial 1: 23% yield C4, 17% yield C6 (1:1 C4:C6)
Trial 2: 23% yield C4, 15% yield C6 (1:1 C4: C6)

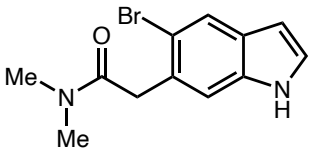
^{1}H NMR (500 MHz, $CDCl_3$): δ 7.29 (d, J = 7.8 Hz, 1H), 7.26 (d, J = 5.7 Hz, 1H), 6.87 (d, J = 7.8 Hz, 1H), 6.72 (dd, J = 3.3, 2.2 Hz, 1H), 3.95 (s, 2H), 2.97 (d, J = 4.9 Hz, 6H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 171.0, 134.6, 128.6, 126.8, 124.9, 124.5, 121.0, 103.4, 102.4, 102.4, 39.0, 38.0, 35.8.

IR (cm^{-1}): 3235, 2923, 2852, 1632, 1504, 1396, 1119, 790, 727

HRMS: calc'd $[M+H]^+$: 281.028406, found 281.02791

2-(5-bromo-1H-indol-6-yl)-N,N-dimethylacetamide (**18a)**



The product standard was isolated using the isolated scale biocatalytic procedure using 1.0 mol% of PagER (41% isolated yield).

NMR yield:

Trial 1: 31% yield C6, 7% yield C7, 10% C2 (2:1 C6:others)
Trial 2: 30% yield C7, 6% yield C7, 10% C2 (2:1 C6:others)

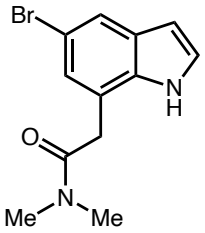
NMR: ^{1}H NMR (500 MHz, DMSO) δ 11.23 (s, 1H), 7.33 (m, 1 H), 7.22 (s, 2H), 6.48 – 6.41 (m, 1H), 4.00 (s, 2H), 3.12 (s, 3H), 2.84 (s, 3H).

^{13}C NMR (126 MHz, DMSO) δ 168.9, 134.6, 129.9, 127.3, 126.6, 124.3, 114.5, 111.6, 100.6, 37.6, 37.0, 35.1.

IR (cm^{-1}): 3233, 2920, 1627, 1496, 1454, 1425, 1026, 820, 763

HRMS: calc'd $[M+H]^{+}$: 281.028406, found 281.02828

2-(5-bromo-1H-indol-7-yl)-N,N-dimethylacetamide (**18b)**



The product standard was isolated using the isolated scale biocatalytic procedure using 1.0 mol% of PagER (41% isolated yield).

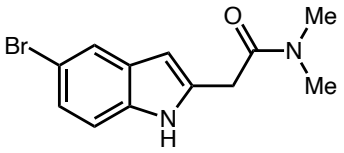
^{1}H NMR (500 MHz, CDCl3) δ 8.73 (s, 1H), 7.79 (s, 1H), 7.34 (s, 1H), 7.12 (dd, J = 3.2, 2.5 Hz, 1H), 6.39 (ddd, J = 3.1, 2.0, 0.9 Hz, 1H), 3.93 (s, 2H), 3.04 (s, 6H)

^{13}C NMR (126 MHz, CDCl3) δ 171.7 135.5, 128.7, 127.5, 125.8, 124.2, 115.4, 112.8, 101.6, 41.3, 37.9, 35.9

IR (cm^{-1}): 3232, 2922, 1627, 1479, 1396, 1301, 1258, 764

HRMS: calc'd $[M+H]^{+}$: 281.028406, found 281.02828

2-(5-bromo-1H-indol-2-yl)-N,N-dimethylacetamide (**18c**)



The product standard was isolated using the isolated scale biocatalytic procedure using 1.0 mol% of PagER (41% isolated yield).

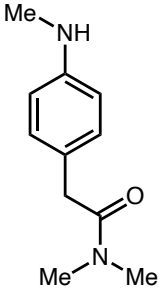
^{1}H NMR (500 MHz, $CDCl_3$): δ 9.25 (s, 1H), 7.65 (dt, J = 1.7, 0.7 Hz, 1H), 7.24 – 7.16 (m, 2H), 6.23 (dd, J = 2.0, 0.9 Hz, 1H), 3.87 (s, 2H), 3.12 (s, 3H), 2.99 (s, 3H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 169.9, 135.0, 133.4, 130.0, 124.4, 122.5, 113.0, 112.5, 100.6, 38.0, 35.9, 32.5.

IR (cm^{-1}): 3233, 2920, 1627, 1425, 1026, 763

HRMS: calc'd $[M+H]^+$: 281.028406, found 281.02828

N,N-dimethyl-2-(4-(methylamino)phenyl)acetamide **(19a)**



The product standard was isolated using the isolated scale biocatalytic procedure using 1 mol% of GluER T36A T25L (45% isolated yield).

NMR yield:

Trial 1: 57% yield para, 6% yield ortho (10:1 para: ortho)
Trial 2: 57% yield C4, 6% yield ortho (10:1 para : others)

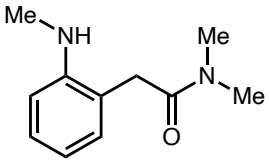
^{1}H NMR (500 MHz, $CDCl_3$): δ 7.07 (d, J = 8.4 Hz, 2H), 6.57 (d, J = 8.5 Hz, 2H), 3.60 (s, 2H), 2.98 (s, 3H), 2.94 (s, 3H), 2.81 (s, 3H)

^{13}C NMR (126 MHz, $CDCl_3$): δ 172.0, 148.2, 129.6, 123.6, 112.8, 40.4, 37.8, 35.8, 31.0.

IR (cm^{-1}): 3352, 2925, 1614, 1522, 1394, 1262, 1182, 817, 793

HRMS: calc'd $[M+H]^+$: 193.133536, found 193.133334

N,N-dimethyl-2-(2-(methylamino)phenyl)acetamide (**19b)**



The product standard was isolated using the isolated scale biocatalytic procedure using 1 mol% of GluER T36A W66F (35% isolated yield including N-alkylation product).

NMR yield using GluER T36A W66F:

Trial 1: 12% yield ortho, 21% yield para and N-alkylation (0.6:1 ortho:others)
Trial 2: 11% yield ortho, 19% yield para and N-alkylation (0.6:1 ortho: others)

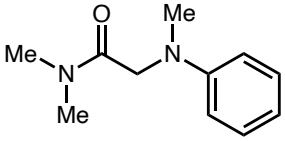
^{1}H NMR (500 MHz, $CDCl_3$): δ 7.19 (td, J = 7.7, 1.6 Hz, 1H), 7.06 – 7.01 (m, 1H), 6.64 (m, 2H), 3.63 (s, 2H), 3.14 (s, 3H), 2.93 (s, 3H), 2.85 (s, 3H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 171.2, 149.2, 130.6, 128.4, 119.2, 116.2, 110.3, 38.8, 38.1, 35.9, 30.4.

IR (cm^{-1}): 3053, 2925, 1706, 1614, 1470, 1346, 1091

HRMS: calc'd $[M+H]^+$: 193.133536, found 193.133334

N,N-dimethyl-2-(methyl(phenyl)amino)acetamide (**19c**)



The product standard was isolated using the isolated scale biocatalytic procedure using 1 mol% of NostocER Y219F (74% isolated yield). This happens non-enzymatically in buffer.

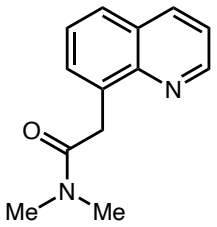
^{1}H NMR (500 MHz, $CDCl_3$): δ 7.22 (dd, J = 8.8, 7.2 Hz, 2H), 6.76 – 6.64 (m, 3H), 4.11 (s, 2H), 3.04 (d, J = 3.4 Hz, 6H), 2.97 (s, 3H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 169.6, 149.6, 129.3, 117.3, 112.6, 54.5, 39.5, 36.5, 35.9.

IR (cm^{-1}): 2924, 1652, 1598, 1371, 1216, 1144, 1114, 748, 691

HRMS: calc'd $[M+H]^+$: 193.133536, found 193.133334

N,N-dimethyl-2-(quinolin-8-yl)acetamide **(25a)**



The product standard was isolated using the isolated scale biocatalytic procedure using 1 mol% of Q2 (21% isolated yield only one regioisomer).

NMR yield:

Trial 1: 64%
Trial 2: 66%

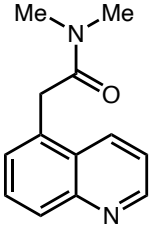
^{1}H NMR (500 MHz, $CDCl_3$): δ 8.90 (dd, J = 4.2, 1.8 Hz, 1H), 8.14 (dd, J = 8.2, 1.8 Hz, 1H), 7.73 (dd, J = 8.2, 1.5 Hz, 1H), 7.71 – 7.68 (m, 1H), 7.54 – 7.47 (m, 1H), 7.40 (dd, J = 8.3, 4.2 Hz, 1H), 4.41 (s, 2H), 3.11 (s, 3H), 3.00 (s, 3H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 172.1, 149.6, 146.5, 136.5, 134.6, 129.8, 128.5, 127.1, 126.6, 121.2, 38.0, 35.8, 35.7.

IR (cm^{-1}): 2925, 1636, 1522, 1497, 1393, 1131, 812, 784

HRMS: calc'd $[M+H]^+$: 215.117886, found 215. 11752

N,N-dimethyl-2-(quinolin-5-yl)acetamide (**25b)**



The product standard was isolated using the isolated scale biocatalytic procedure using 100mM KP_i pH 6 and 1 mol% of GluER T36A Y343F (46% isolated yield 1.8:1:1.4 C5:C4:C8).

NMR yield:

Trial 1: 48% (21% C5, 12% C4, 15% C8) (0.7 C5: others)
Trial 2: 45% (18% C5, 12% C4, 15% C8) (0.7 C5: others)

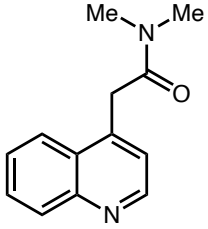
^{1}H NMR (500 MHz, $CDCl_3$): δ 8.93 (dd, J = 4.3, 1.6 Hz, 1H), 8.42 – 8.36 (m, 1H), 8.04 (d, J = 8.5 Hz, 1H), 7.65 (dd, J = 8.5, 7.1 Hz, 1H), 7.45 – 7.39 (m, 1H), 7.38 (d, J = 1.1 Hz, 1H), 4.14 (s, 2H), 3.09 (s, 3H), 3.01 (s, 3H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 170.6, 150.3, 148.8, 132.7, 132.1, 129.3, 129.1, 127.7, 127.5, 121.3, 38.2, 38.0, 36.0.

IR (cm^{-1}): 2925, 1641, 1501, 1264, 1136, 1058, 804

HRMS: calc'd $[M+H]^+$: 215.117886, found 215. 11752

N,N-dimethyl-2-(quinolin-4-yl)acetamide (**25c**)



The product standard was isolated using the isolated scale biocatalytic procedure using 100mM KP_i pH 6 and 1 mol% of GluER T36A Y343F (46% isolated yield 1.8:1:1.4 C5:C4:C8).

NMR yield:

Trial 1: 48% (21% C5, 12% C4, 15% C8) (0.7 C5: others)
Trial 2: 45% (18% C5, 12% C4, 15% C8) (0.7 C5: others)

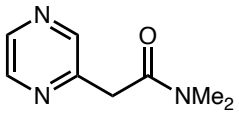
^{1}H NMR (500 MHz, $CDCl_3$): δ 8.85 (d, J = 4.4 Hz, 1H), 8.14 (dd, J = 8.5, 1.4 Hz, 1H), 7.98 (dd, J = 8.6, 1.5 Hz, 1H), 7.73 (ddd, J = 8.4, 6.9, 1.4 Hz, 1H), 7.59 (ddd, J = 8.3, 6.9, 1.4 Hz, 1H), 7.25 (d, 1H), 4.16 (s, 2H), 3.05 (d, J = 10.5 Hz, 6H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 169.7, 150.3, 148.5, 141.5, 130.5, 129.5, 127.7, 127.0, 123.5, 121.5, 38.0, 37.8, 35.9.

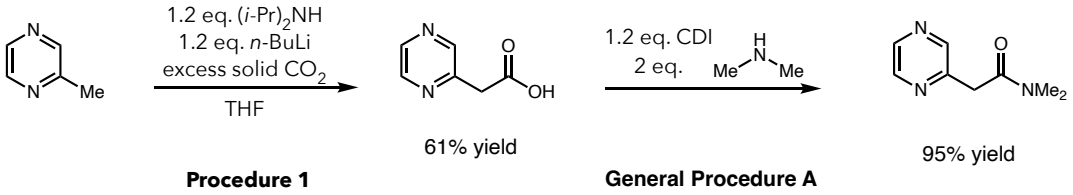
IR (cm^{-1}): 2924, 1641, 1507, 1393, 1262, 1136, 810, 782, 756

HRMS: calc'd $[M+H]^+$: 215.117886, found 215. 11752

N,N-dimethyl-2-(pyrazin-2-yl)acetamide **(26)**



The product standard synthesized via the following method:



Procedure 1: Using a modified procedure from Nizamov et. al[55], dry THF was added to a flame dried flask charged with a magnetic stir bar and placed under inert atmosphere. It was cooled to -78°C Diisopropylamine (1.2 equiv) was added and then *n*-butyllithium (2.5M in hexanes, 1.2 equiv) was added slowly and the reaction mixture was allowed to stir at -78°C for 20 minutes. 2-methylpyrazine was added dropwise and the reaction was stirred for 1 hour at -78°C. Dry ice was placed into a syringe with a long needle and the needle was placed inside the reaction flask to sparge it with CO_2 for quenching. After 10 minutes, dry ice was directly added to the reaction flask. The cooling bath was removed and the reaction mixture was allowed to warm up at room temperature. Water was then added. The liquid phases were separated. The aqueous phase was adjusted to pH 3 and re-extracted five times with ethyl acetate. The organic extracts were pooled, dried over sodium sulfate and concentrated *in vacuo* to yield 2-(pyrazin-2-yl)acetic acid in 61% yield.

General Procedure A was then used with dimethylamine to yield the product standard in 95% yield

NMR yield:

Trial 1: 45%
Trial 2: 41%

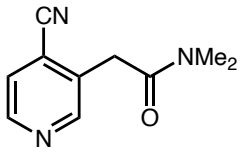
^{1}H NMR (500 MHz, $CDCl_3$): δ 8.56 (s, 1H), 8.46 – 8.37 (m, 2H), 3.87 (s, 2H), 3.07 (s, 3H), 2.93 (s, 3H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 169.0, 151.8, 145.7, 143.9, 142.8, 40.7, 37.8, 35.6.

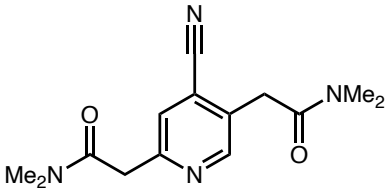
IR (cm^{-1}): 2929, 1637, 1496, 1395, 1141, 1019, 725

HRMS: calc'd $[M+H]^+$: 166.097486, found 166.09695

2-(4-cyanopyridin-3-yl)-N,N-dimethylacetamide (**27**)



The product standard was isolated using the isolated scale biocatalytic procedure using 1 mol% of Q2 (14% yield). It was inseparable from the doubly alkylated adduct. The proposed structure for the doubly alkylated adduct is below.



NMR yield:

Trial 1: 40% C3 adduct, 11% di-alkylated product
Trial 2: 35% C3 adduct, 9% di-alkylated product

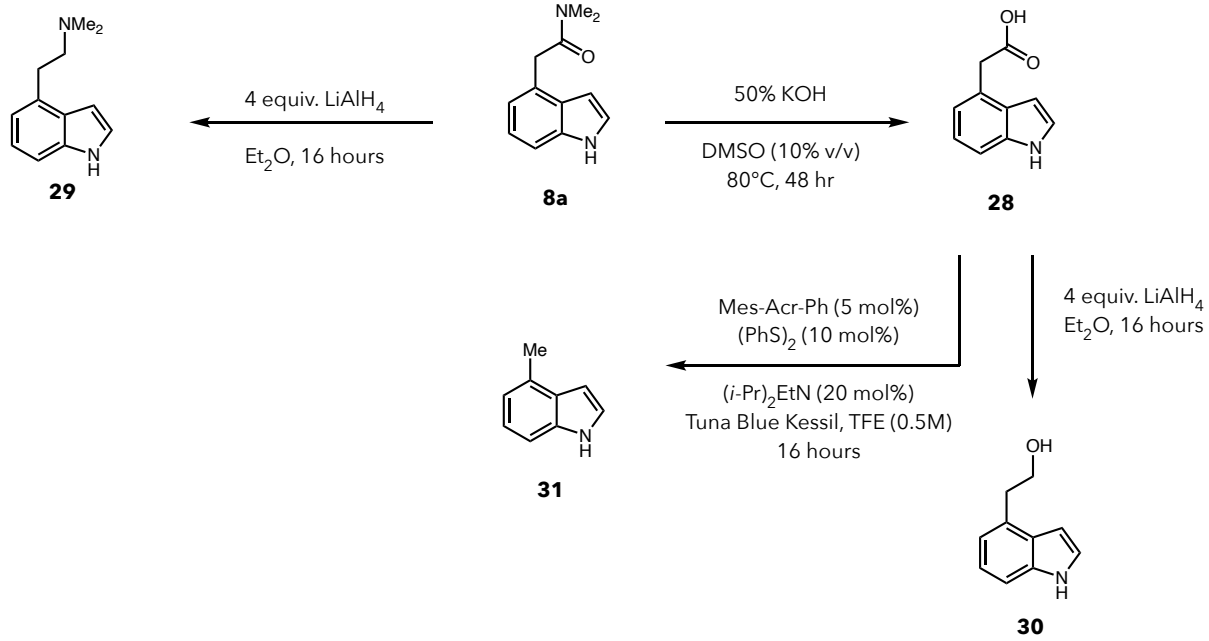
^{1}H NMR (500 MHz, $CDCl_3$): δ 8.71 (s, 1H), 8.68 (d, J = 5.0 Hz, 1H), 7.51 (d, J = 5.0 Hz, 1H), 3.89 (s, 2H), 3.18 (s, 3H), 3.02 (s, 3H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 167.9, 152.3, 148.9, 133.4, 125.2, 121.6, 115.8, 37.6, 36.1, 36.0.

IR (cm^{-1}): 2921, 2233, 1645, 1506, 1396, 1263, 1138, 840

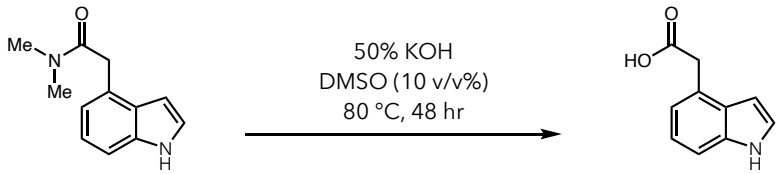
HRMS: calc'd $[M+H]^+$: 190.099286, found 190.09722

Part IX. Product Derivatization and Characterization:



Supplemental Figure 15: Product Derivatization Map

Synthesis of 2-(1*H*-indol-4-yl)acetic acid (**28):**

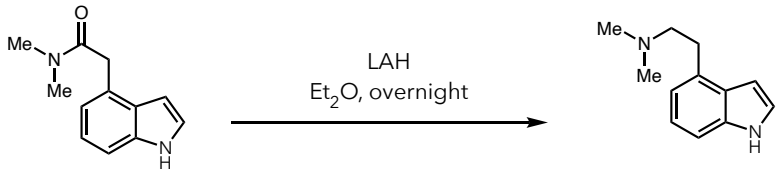


In a round bottle flask equipped with a stir bar was charged with the amide product (1 mmol, 203 mg) from gram scale reaction. The flask was sealed with a septum, evacuated, and backfilled with dry nitrogen. Aqueous degassed KOH (50%, 20 mL) and DMSO (10% v/v) were added via syringe. The reaction mixture was allowed to stir at 80°C overnight. Upon completion as determined by TLC analysis, the flask was cooled to at 0°C and concentrated HCl (12M) was added (until pH = 1). The aqueous layer was extracted with EtOAc (45 mL x 3). The combined organic layers were dried with Na_2SO_4 and concentrated with the aid of a rotary evaporator without further purification (>99% yield). All spectra match reported values.[56]

^{1}H NMR (500 MHz, Acetone) δ 10.20 (s, 1H), 7.34 – 7.23 (m, 2H), 7.04 (t, J = 7.7 Hz, 1H), 6.95 (d, J = 7.1 Hz, 1H), 6.56 (t, J = 2.7 Hz, 1H), 3.85 (s, 2H).

^{13}C NMR (126 MHz, Acetone) δ 172.9, 137.0, 128.6, 127.0, 125.2, 121.9, 120.6, 120.6, 110.9, 100.8, 39.3.

Synthesis of 2-(1*H*-indol-4-yl)-N,N-dimethylethan-1-amine (**29):**

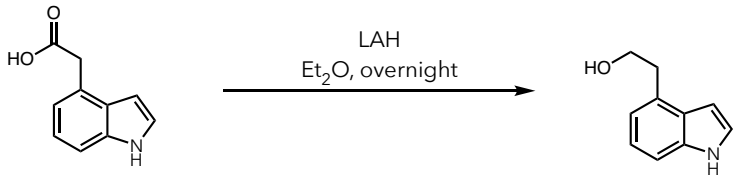


In an oven-dried round bottle flask equipped with a stir bar was charged with lithium aluminum hydride (LAH, 4.0 equiv.). Anhydrous Et_2O (0.25 M) was added via syringe. Dimethyl amide derived from enzymatic reaction (1.0 equiv.) was then added (Caution: gas evolution observed). The reaction mixture was stirred at rt overnight. Upon completion as determined via TLC analysis, aqueous NaOH (10%) was added dropwise to quench the reaction until white gel was formed (Caution: gas evolution observed). The resulting solution was filtered, concentrated with the aid of a rotary evaporator. The filtered product produced a white solid. (82% yield). All spectra match reported values.[51]

^{1}H NMR (500 MHz, CDCl3) δ 9.00 (s, 1H), 7.40 – 7.31 (m, 1H), 7.27 (d, J = 2.8 Hz, 1H), 7.24 (d, J = 7.8 Hz, 1H), 7.07 (d, J = 7.1 Hz, 1H), 6.71 (ddd, J = 3.2, 2.1, 1.0 Hz, 1H), 3.28 – 3.20 (m, 2H), 2.89 – 2.79 (m, 2H), 2.51 (s, 7H).

^{13}C NMR (126 MHz, CDCl3) δ 136.0, 132.3, 127.4, 124.1, 122.1, 119.4, 109.4, 100.6, 60.6, 45.4, 32.0.

Synthesis of 2-(1*H*-indol-4-yl)ethan-1-ol (**30):**

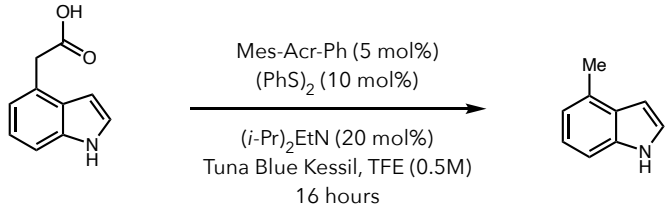


In an oven-dried round bottle flask equipped with a stir bar was charged with lithium aluminum hydride (LAH, 4.0 equiv.). Anhydrous Et_2O (0.25 M) was added via syringe. Carboxylic acid (1.0 equiv.) derived from enzymatic reaction was then added (Caution:

gas evolution observed). The reaction mixture was stirred at rt overnight. Upon completion as determined via TLC analysis, aqueous NaOH (10%) was added dropwise to quench the reaction until white gel was formed (Caution: gas evolution observed). The resulting solution was filtered, concentrated with the aid of a rotary evaporator. The filtered product was a pure oil. (90% yield) All spectra match reported values.[57]

^{1}H NMR (500 MHz, CDCl3) δ 8.26 (s, 1H), 7.30 (d, J = 8.2 Hz, 1H), 7.23 – 7.19 (m, 1H), 7.19 – 7.12 (m, 1H), 6.99 (d, J = 7.1 Hz, 1H), 6.62 (ddd, J = 3.2, 2.2, 1.1 Hz, 1H), 3.99 (t, J = 6.5 Hz, 2H), 3.19 (t, J = 6.5 Hz, 2H).
^{13}C NMR (126 MHz, CDCl3) δ 136.0, 130.5, 127.7, 124.1, 122.3, 120.3, 109.8, 101.0, 63.2, 37.0.

Synthesis of 4-methylindole (**31):**



4-methylindole (31) was synthesized using a modified procedure from Griffin et. al.[58] To a flame-dried one dram round bottom flask (25 ml) equipped with a magnetic stir bar was added the carboxylate acid derived from enzymatic reaction (1 equiv., 130 mg, 0.74 mmol), Mes- Acr-Ph (5 mol%), and diphenyl disulfide (10 mol%). The flask was sealed with a septum, evacuated, and backfilled with dry nitrogen and sparged trifluoroethanol was added to achieve a concentration of 0.5 M with respect to acid substrate. N,N-diisopropylethylamine (20 mol%), was added, and the flask was sealed with a robber septum and electric tape. The reaction was irradiated with 450 nm Kessil lamps and stirred at ambient temperature from 24 hours. Upon completion, the solvent was removed in vacuo and the product was further purified by flash chromatography to yellow pale yellow stinky smell product (26% yield). All spectra match reported values.[59]

^{1}H NMR (500 MHz, CDCl3) δ 8.13 (s, 1H), 7.25 (d, J = 8.0 Hz, 1H), 7.20 (t, J = 2.8 Hz, 1H), 7.13 (t, J = 7.6 Hz, 1H), 6.96 – 6.92 (m, 1H), 6.59 (ddd, J = 3.2, 2.1, 1.0 Hz, 1H), 2.59 (s, 3H).

^{13}C NMR (126 MHz, CDCl3) δ 135.6, 130.4, 127.9, 123.6, 122.2, 120.1, 108.7, 101.2, 19.0.

Part X. Charge Transfer Complex Studies

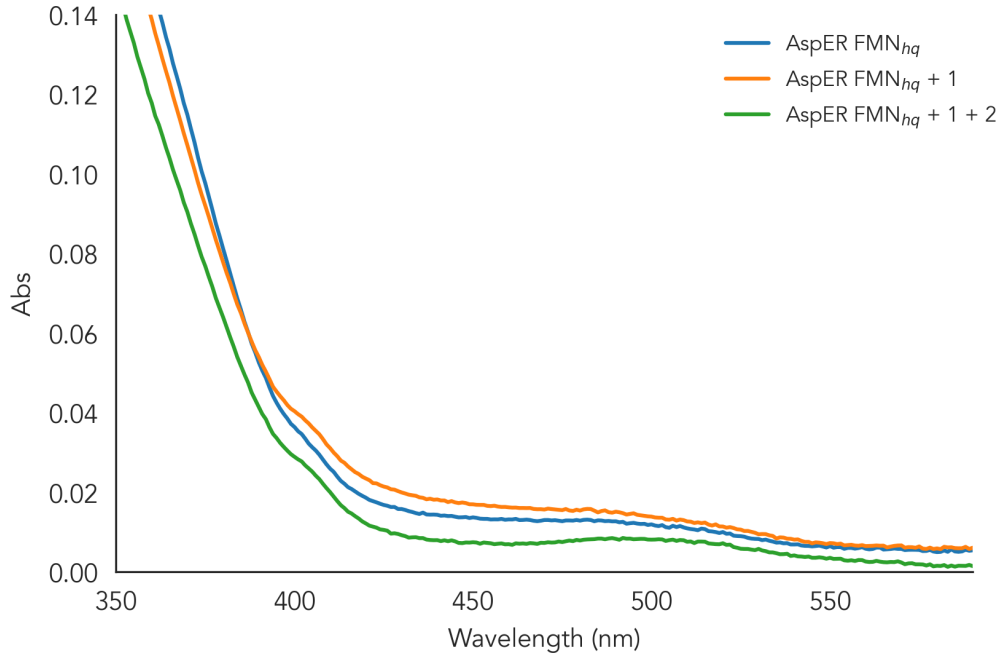
Identification of Charge Transfer Complexes in Proteins Used in this Study

All samples were prepared in an MBraun glovebox with O_2 level less than 5 ppm. Cuvettes were specially designed with a GL18 cap to maintain oxygen free conditions for the duration of the experiment.

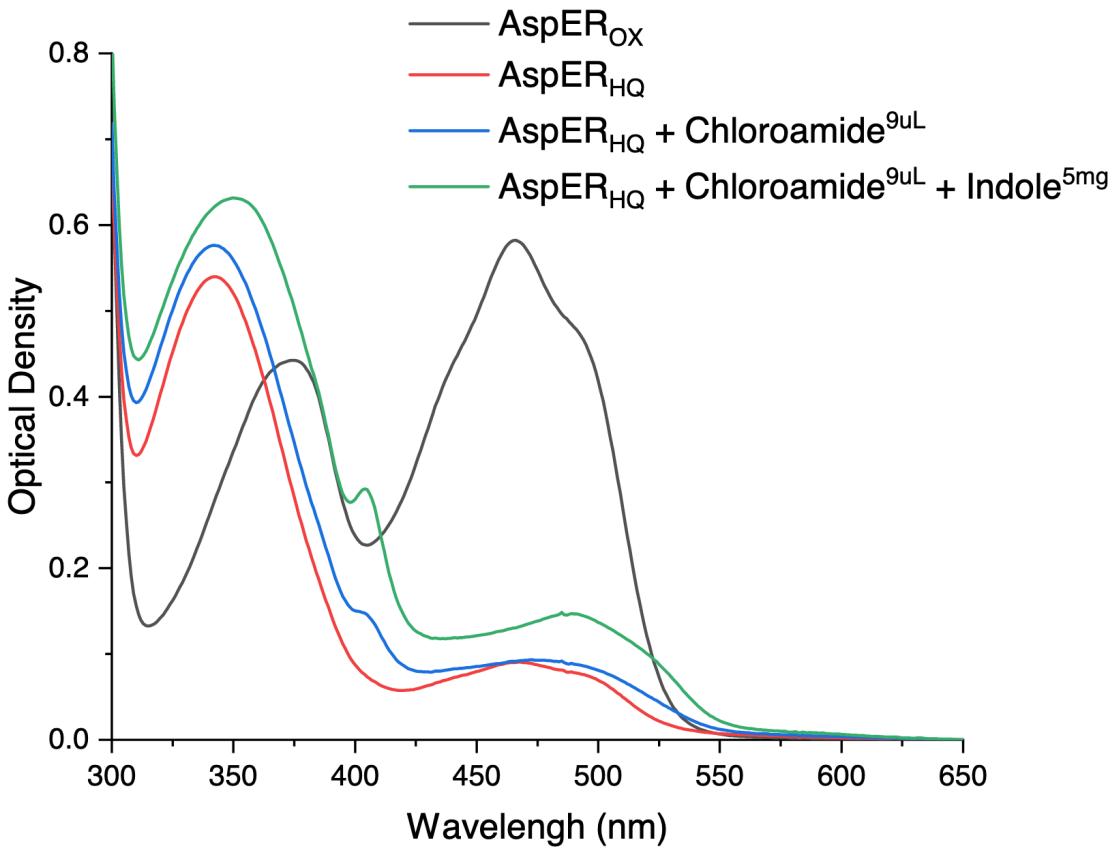
To generate the charge-transfer state in solution 200 nmol of ERED is diluted to a final volume of 1.0 mL in KP_i buffer (pH = 9.0) and absorption spectra is taken. The cuvette is taken back into the MBraun and 120 nmol (1.2 equiv) sodium dithionite is added to generate FMN_{hq} and an absorption spectrum is taken 9 uL or 18 uL of substrate **1** is added (3800:1 mol ratio substrate:enzyme) to the solution and an absorption spectrum is taken, showing a weak new absorptive feature at ~500 nm At this stage, if the scatter has increased (this was noticed for mutants of GluER T36A), the solution was filtered through cellulose acetate syringe filters. Then the solution was removed from the cuvette in the MBraun and placed in a vial, 8.2 mg of indole was added as a solution in 50 uL of degassed THF and the solution was filtered through a cellulose acetate syringe filter again into the cuvette to remove any insoluble indole. The final absorption spectrum was then taken. A strong new absorptive feature at 360 nm and 480 nm appeared for PagER (Supplemental Figure 18, red trace). The mixture is then removed from the cuvette and placed into Amicon Ultra Centrifugal Filters (0.5 mL, 10 kDa cutoff) and centrifuged in the MBraun at 12500 rpm for 3 minutes to remove small molecules. The protein is resuspended in KP_i buffer and the centrifugal process is repeated 3 times. Then the resuspended protein is transferred back to the cuvette and a spectrum is taken to show recovered FMN_{ox} (Supplemental Figure 18, purple trace). This indicates ground state electron transfer occurs with PagER after addition of both substrates. When this centrifugation step was repeated with the solution containing GluER T36A Y343F T25L T231V G270M FMN_{hq}, substrate 1 and indole, the FMN_{hq} was recovered (Supplemental Figure 25), suggesting the protein's ability to catalyze ground state electron transfer comes from subsequent mutations. If the same experiment is repeated with PagER but it is centrifuged after the chloroamide is added, the FMN_{hq} is recovered, suggesting the arene is necessary for ground state reduction of the chloroamide (Supplemental Figure 19). Due to the increased scatter from protein aggregation when indole was added when a CT state study was conducted with GluER T36A Y343F T25L T231V G270M Q232W, the spectra observed when both substrates are added is inconclusive (Supplemental Figure 26). However, due to the ability of GluER T36A Y343F T25L T231V G270M Q232W to catalyze the coupling with light irradiation, it is likely this protein can also do ground state electron transfer.

Due to the inability to reduce AspER with sodium dithionite (Supplemental Figure 16, blue trace), stoichiometric NADPH was used as the reductant and the procedure was repeated as detailed above except using 5 mg of indole instead of 8.2 mgs. Full reduction was still not possible (Supplemental Figure 17, red trace), however the spectral features upon addition of substrate are more pronounced (Supplemental Figure 17, blue and green traces). Due to the incomplete reduction using this method, it is impossible to comment on the absorptivity of the CT state, however it suggests a CT state pathway is available to AspER.

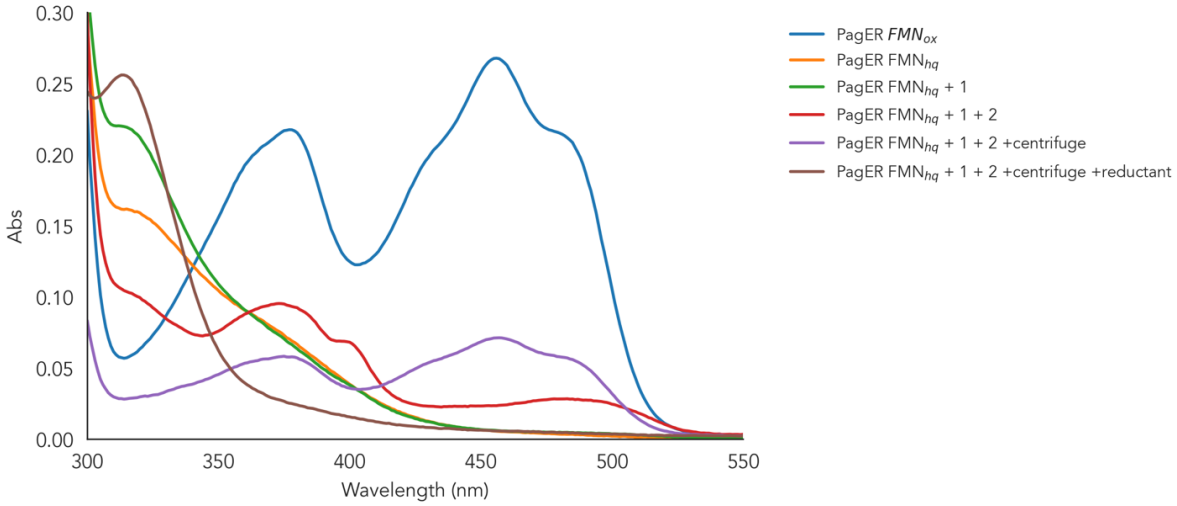
The CT state for all mutants and AspER are shown in Supplemental Figures 16-28. No CT state is observed with free FMN (Supplemental Figure 29).



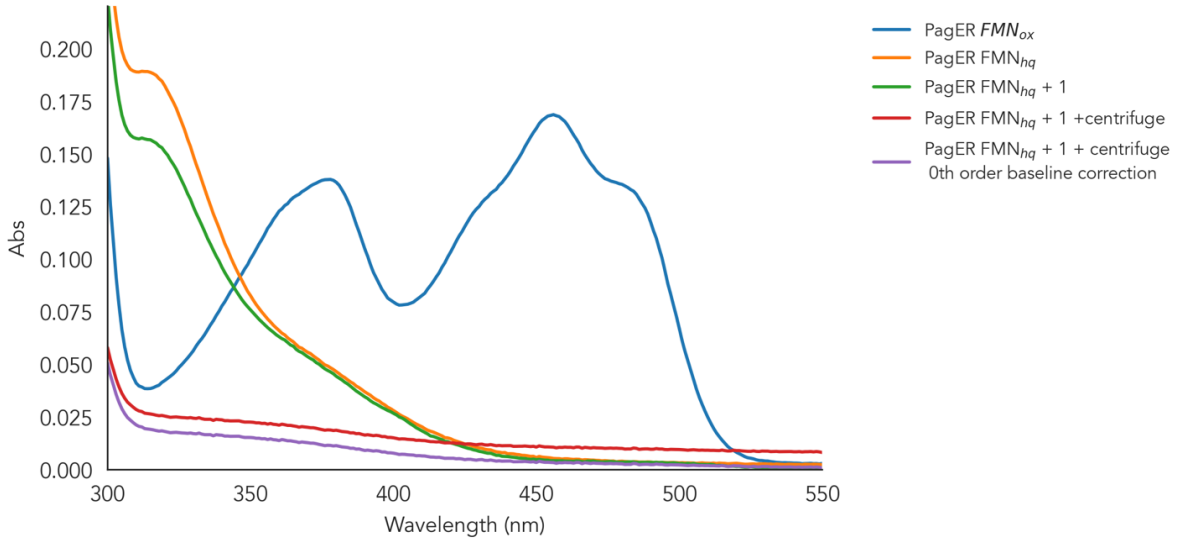
Supplemental Figure 16: Charge Transfer Studies with AspER using sodium dithionite



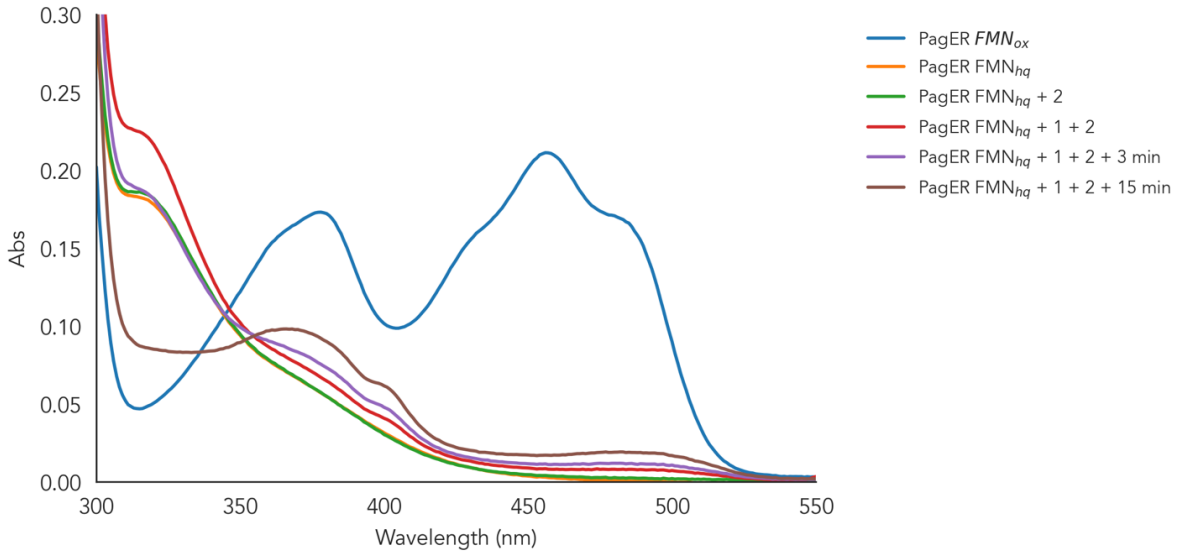
Supplemental Figure 17: Charge Transfer Studies with AspER using NADPH



Supplemental Figure 18: Charge Transfer Studies with PagER with filtration to remove small molecules



Supplemental Figure 19: Charge Transfer Studies with PagER and chloroamide 1 with filtration to remove small molecules



Supplemental Figure 20: Charge Transfer Studies with PagER with timepoints

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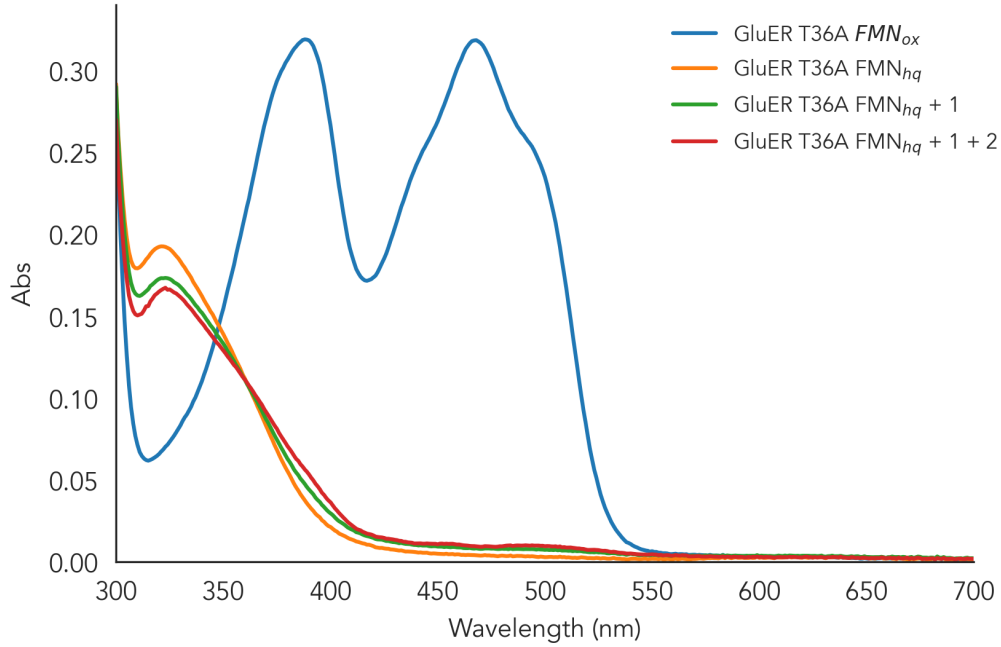
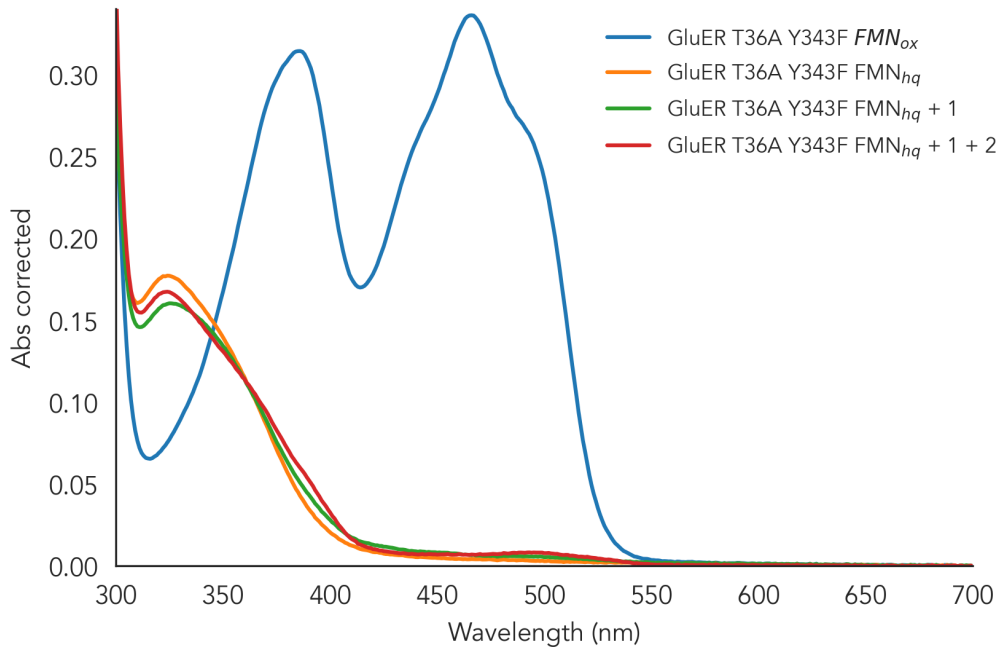
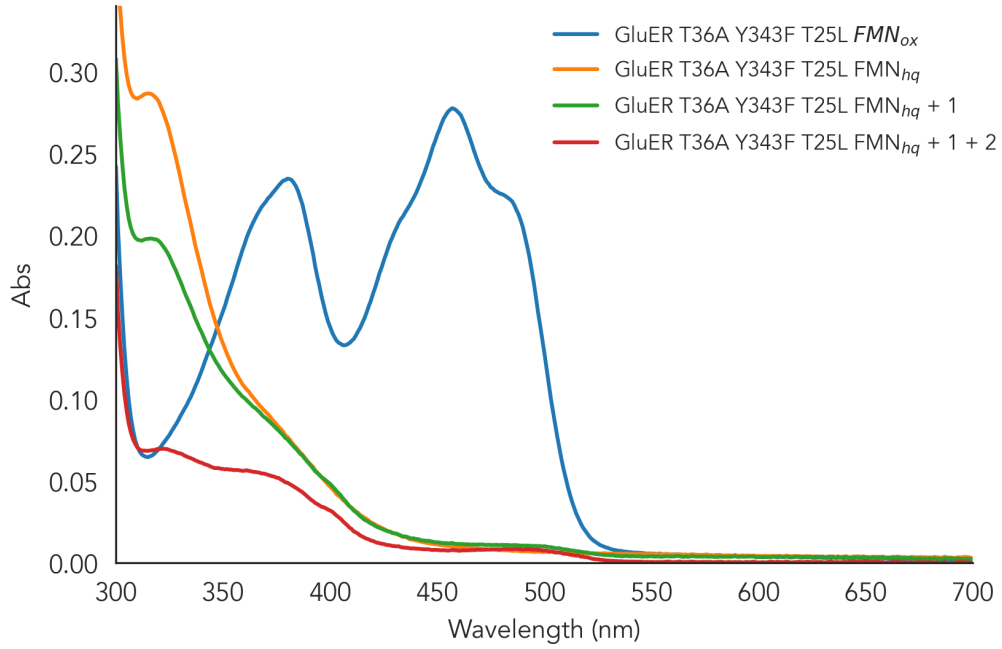


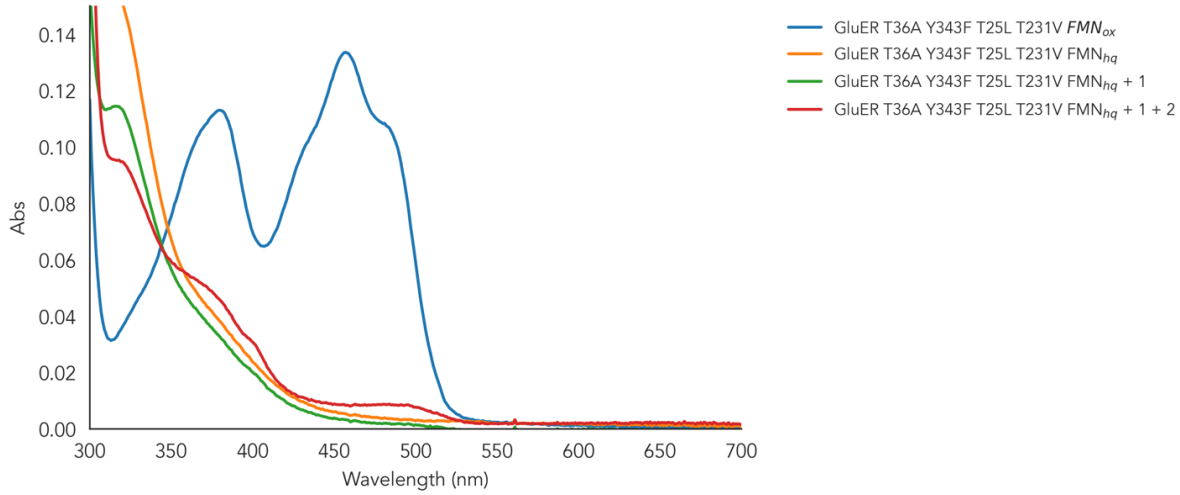
Supplemental Figure 21: Charge Transfer Studies with GluER T36A



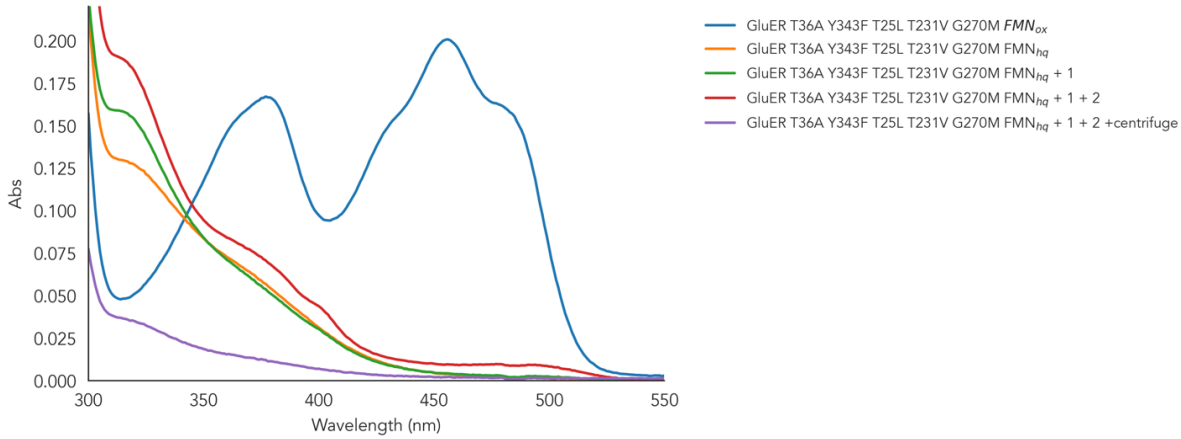
Supplemental Figure 22: Charge Transfer Studies with GluER T36A Y343F



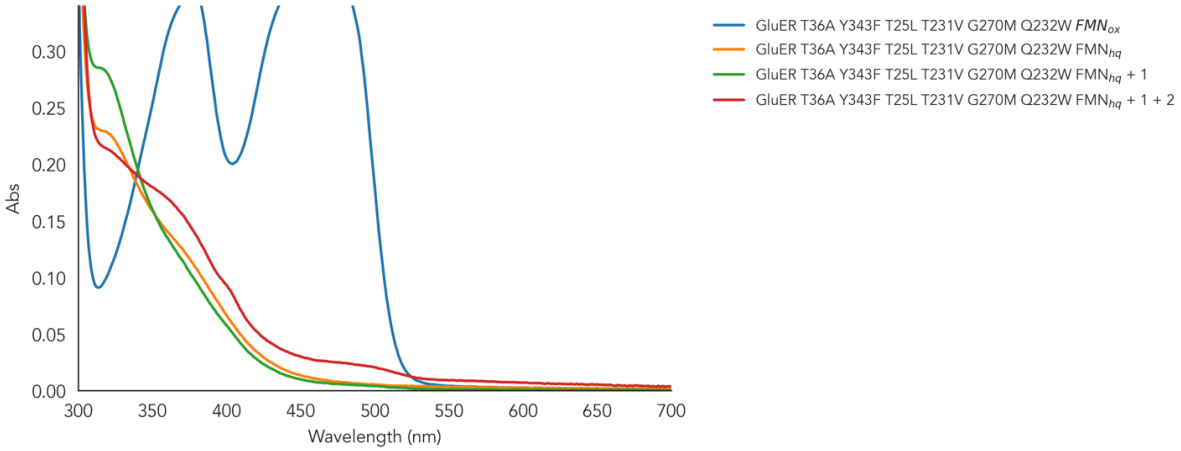
Supplemental Figure 23: Charge Transfer Studies with GluER T36A Y343F T25L



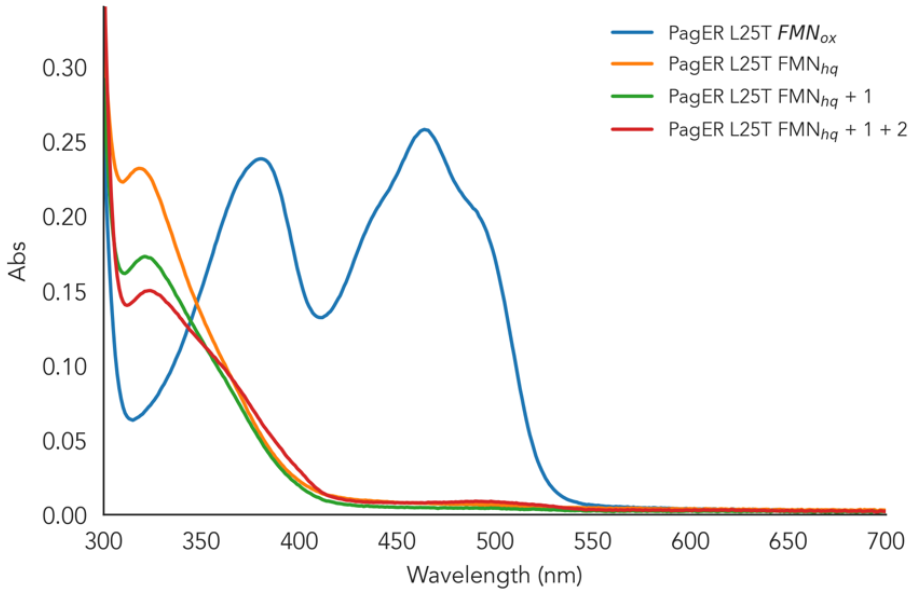
Supplemental Figure 24: Charge Transfer Studies with GluER T36A Y343F T25L T231V



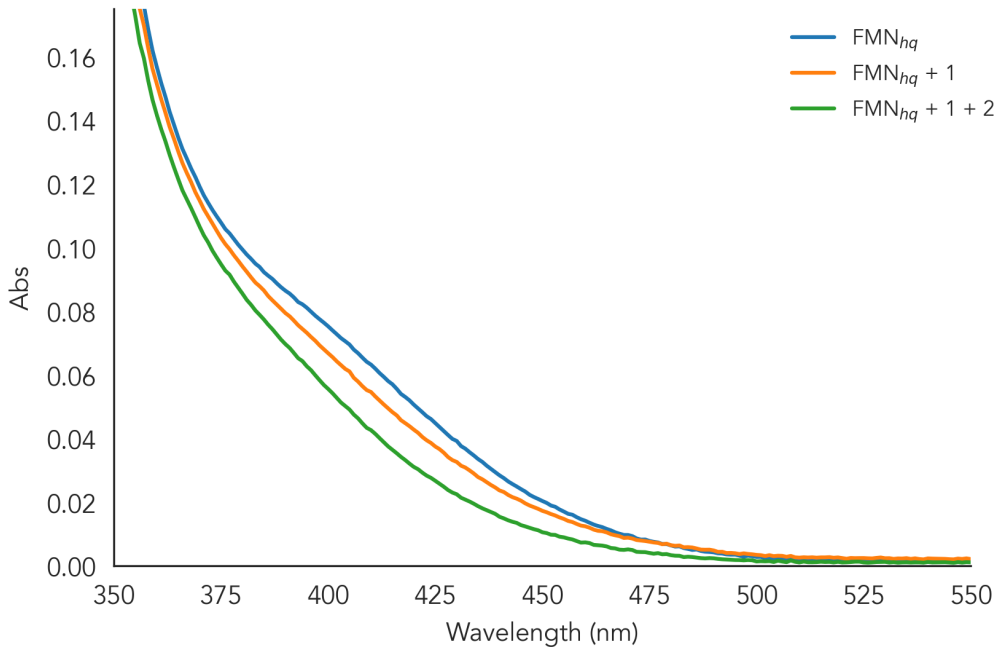
Supplemental Figure 25: Charge Transfer Studies with GluER T36A Y343F T25L T231V G270M and centrifugation



Supplemental Figure 26: Charge Transfer Studies with GluER T36A Y343F T25L T231V G270M Q232W



Supplemental Figure 27: Charge Transfer Studies with PagER L25T



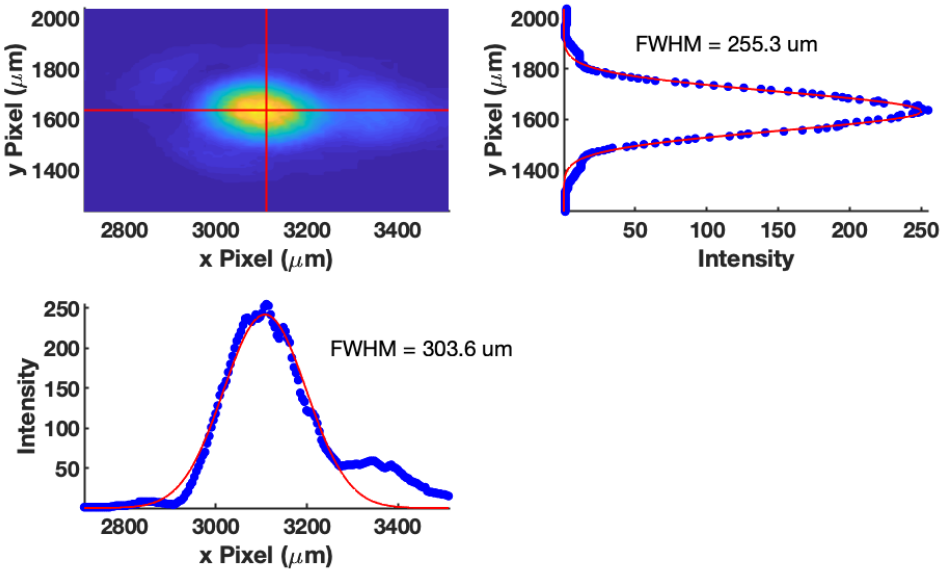
Supplemental Figure 28: Charge Transfer Studies with FMN

Part XI: Transient Absorption Spectroscopy for AspER

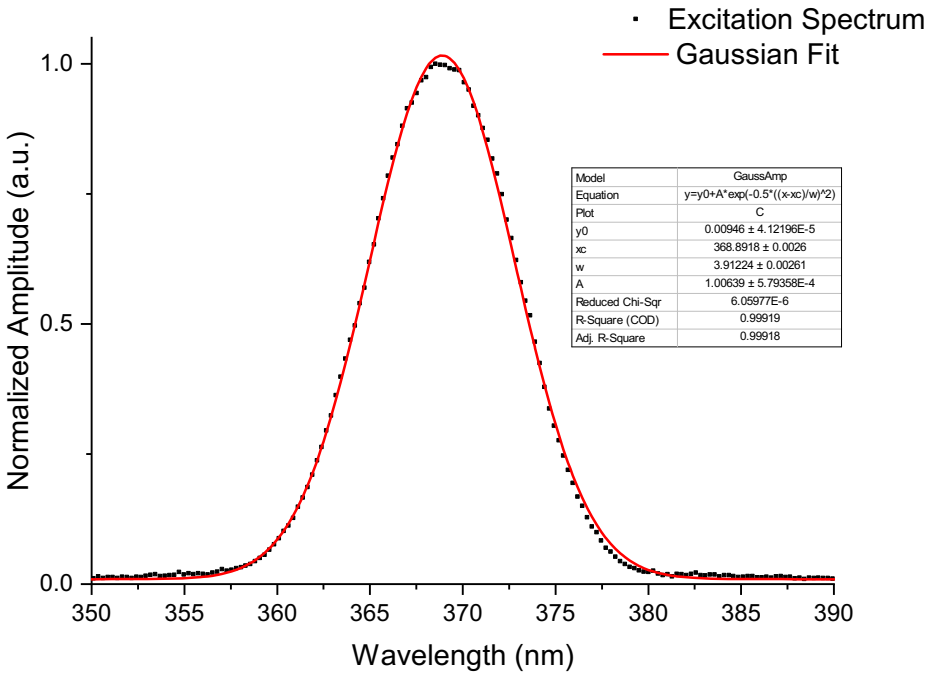
The samples were prepared in 100mM Tris buffer pH 9 with two equivalents of glucose, 1 mol% of NADP+, and 1 mg of desalted and lyophilized GDH to mimic the conditions of the reaction. Substrates were added with 5mg of indole in 50uL of THF and 4uL of neat chloramide. The total sample volume was kept at 1004uL or 1000uL absent of chloramide. The transient absorption samples were excited at 370 nm with 250nJ of energy. The samples were continuously stirred and translated during the duration of the experiment.

A sequential model for fitting of each iteration of AspER hydroquinone was employed. In the presence of the turnover system, AspER exhibits a tri-exponential decay with lifetimes of 3 ps, 42 ps, and 475 ps. The longest lifetime is attributed to $NADP^+$/H + FMNhq interactions as it was not observed when sodium dithionite was used as a reductant for the spectroscopy. Upon addition of the indole, a slight change in lifetime is observed with slight increases in the two shortest time components and a decrease in the longest but spectrally similar to the spectroscopy conducted in the absence of substrate.

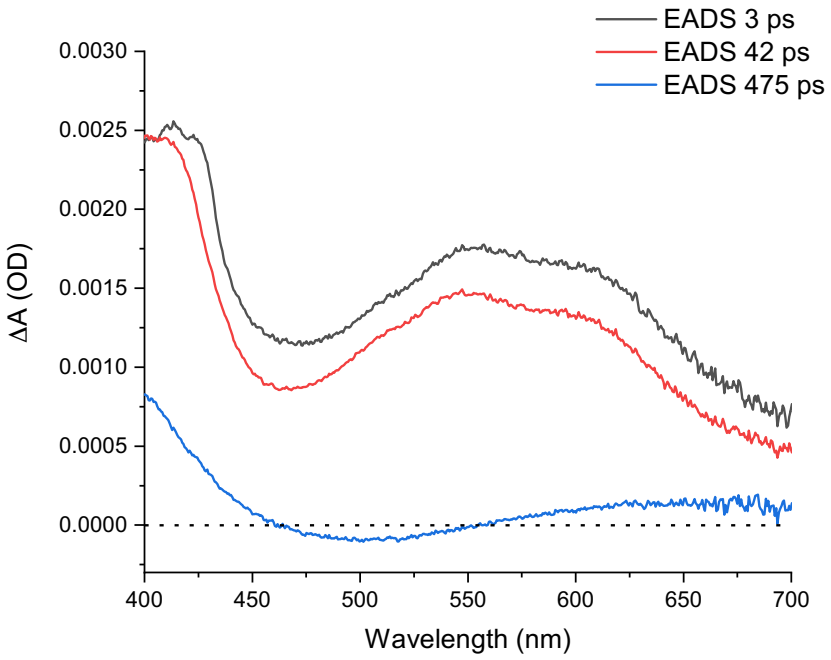
More significant changes were observed with the addition of the chloramide. The results indicate that HAT is not a favored pathway. Instead, we observe a decay back to the ground state and there is no formation of any oxidized flavin or long-lived transient spectrum. The lifetimes are similar to those observed when only indole is present. Spectrally they are shifted, possibly indicating when the chloroamide is in the active site, it prefers back electron transfer over mesolytic cleavage. It is only upon the addition of both components, we see a drastic change in both lifetime and spectrum. The first component decays in 21 ps and it can be attributed to the absorption spectrum of the neutral semiquinone. The mesolytic cleavage is not observed indicating that the chloride ejection step and bond formation to the indole is almost concerted, whereby an electron is returned to the neutral flavin semiquinone reforming the anionic hydroquinone within this timescale. The second component of 323 ps is observed in all samples which is attributed to the possible FMNhq + $NADP^+$/H interaction. Finally, a long-time bleach is observed at 450nm indicating the loss of the charge-transfer complex formed between the chloroamide, indole, and flavin hydroquinone.



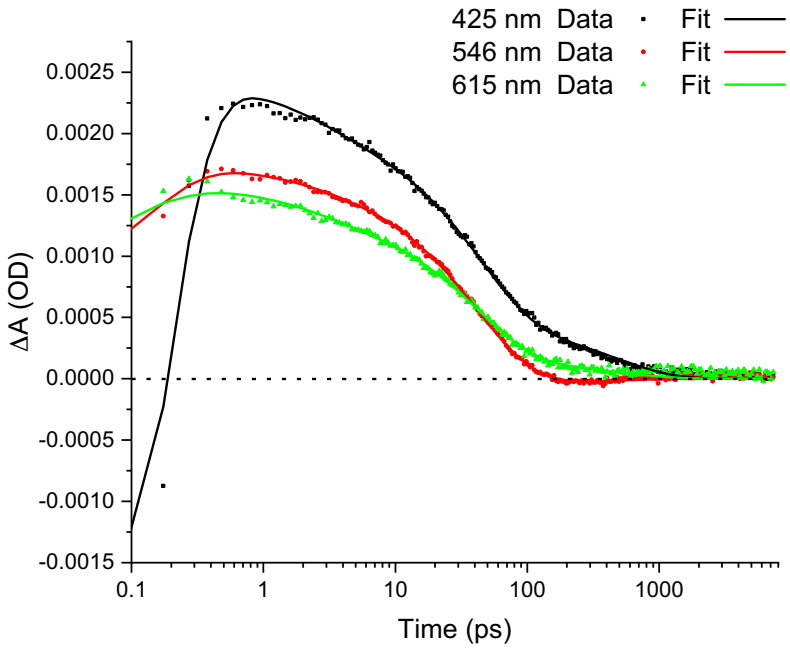
Supplemental Figure 29: Excitation beam profile fit with gaussian shape to extract the full-width half maximum (FWHM).



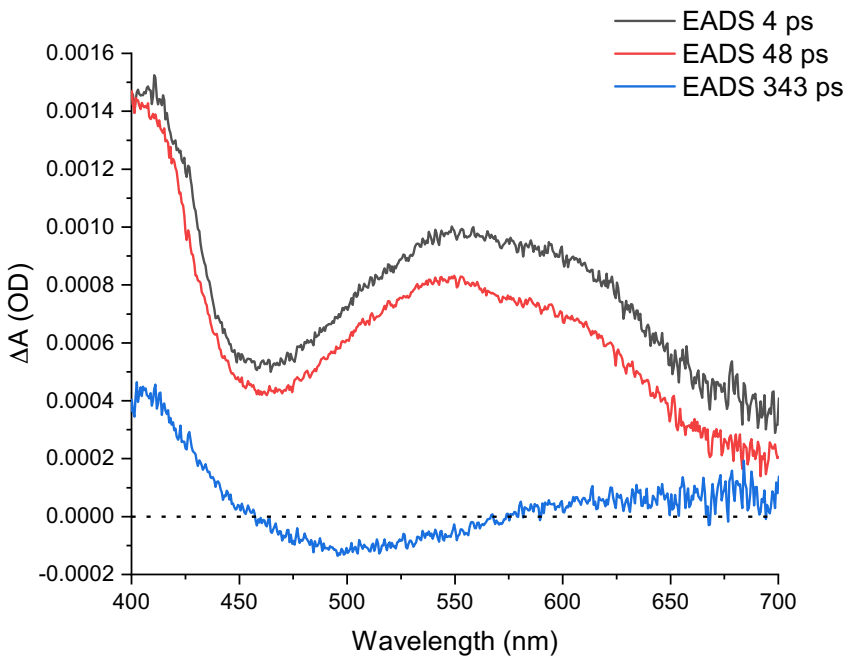
Supplemental Figure 30: Gaussian Fit to excitation spectrum



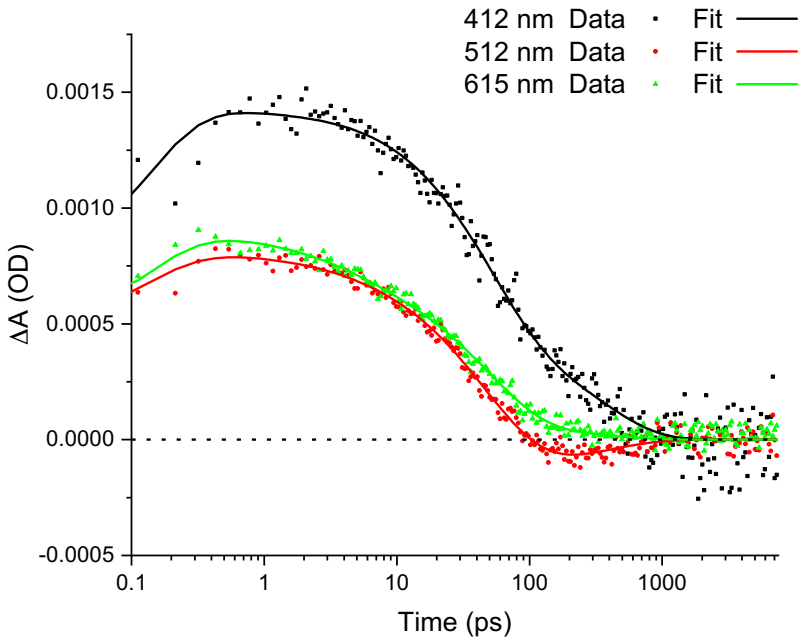
Supplemental Figure 31: AspER hydroquinone global analysis



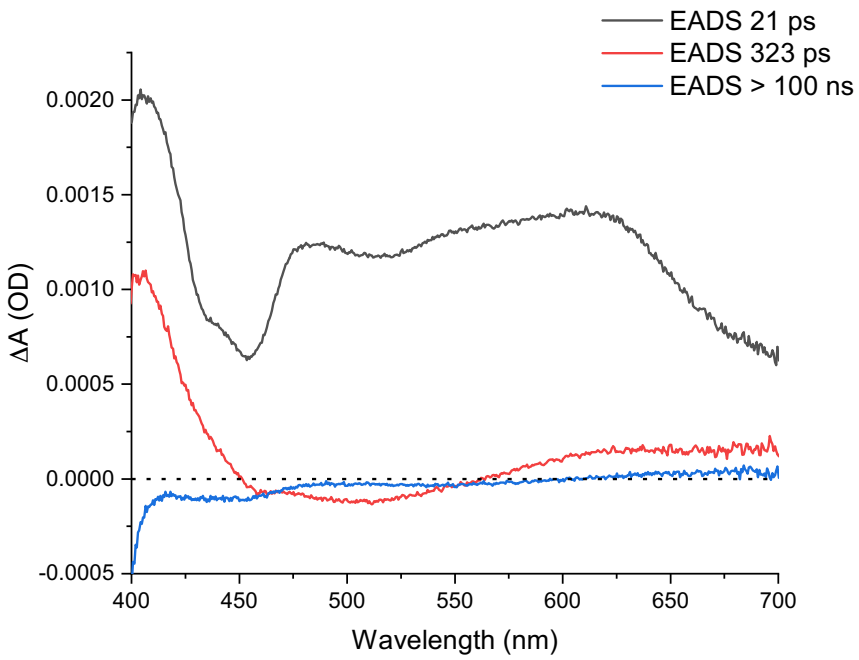
Supplemental Figure 32: AspER hydroquinone global analysis fits at selected wavelengths



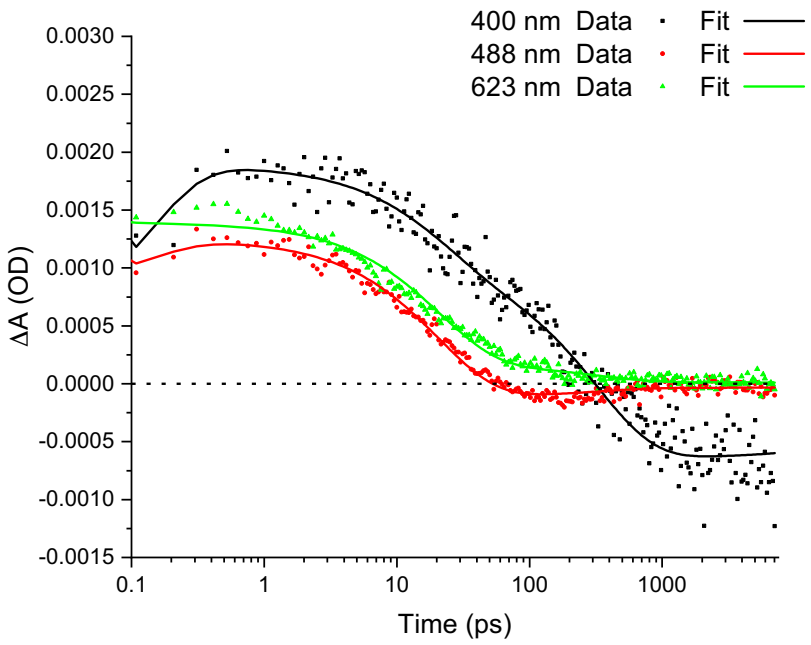
Supplemental Figure 33: AspER hydroquinone with indole global analysis



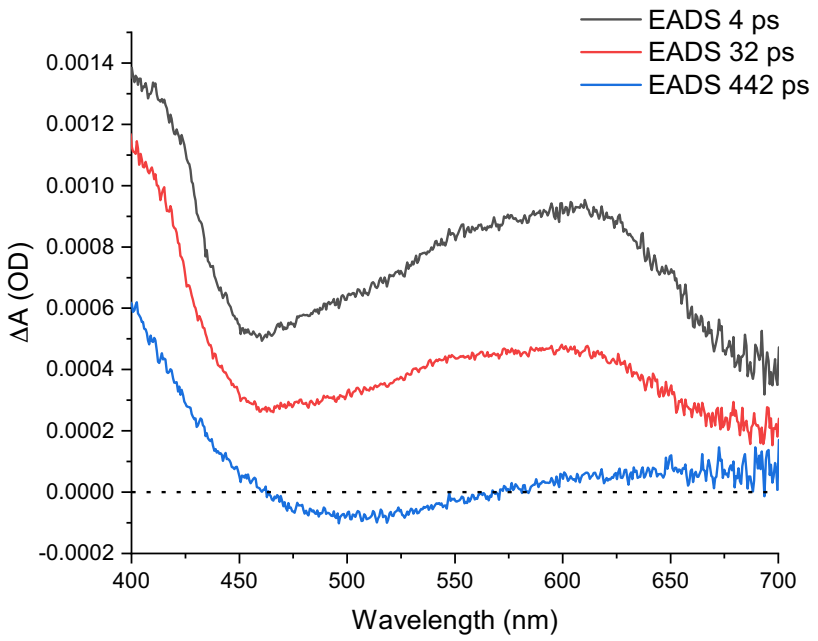
Supplemental Figure 34: AspER hydroquinone with indole global analysis fits at selected wavelengths



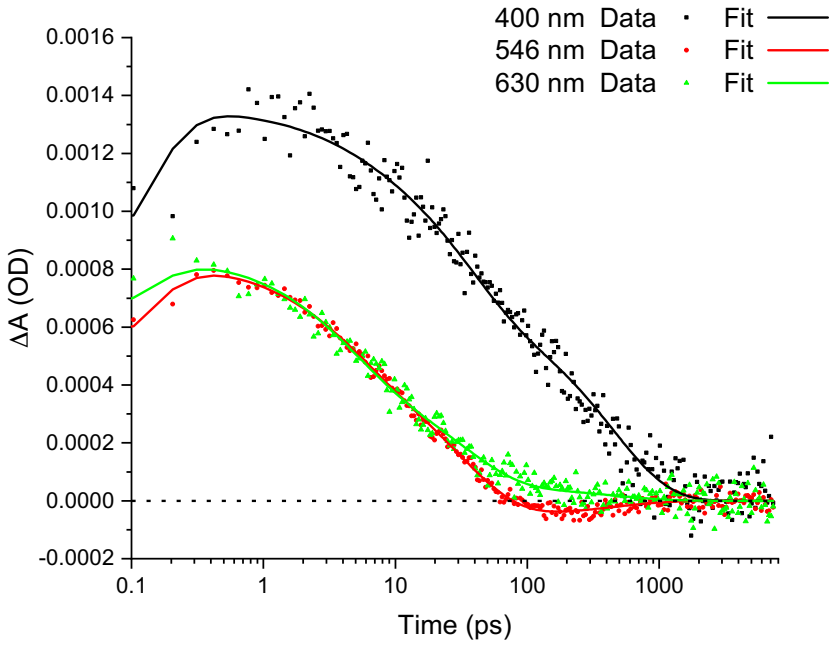
Supplemental Figure 35: AspER hydroquinone with amide and indole global analysis



Supplemental Figure 36: AspER hydroquinone with amide and indole global analysis fits at selected wavelengths



Supplemental Figure 37: AspER hydroquinone with amide global analysis



Supplemental Figure 38: AspER hydroquinone with amide global analysis fits at selected wavelengths

Part XII: Mechanistic Studies

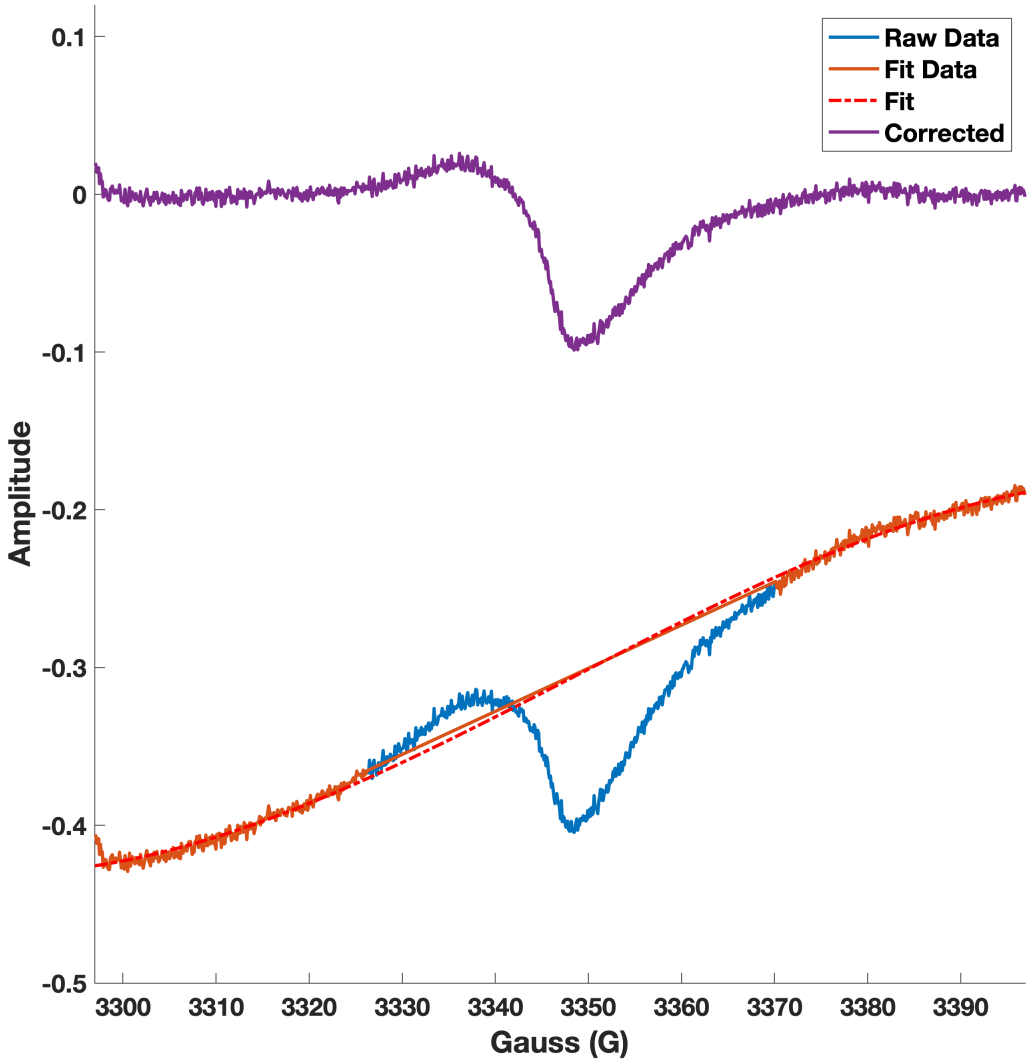
Electron Paramagnetic Resonance (EPR) Procedure:

All samples were prepared in an MBraun glovebox with O_2 level less than 0.1 ppm. Wilmad Precision Quartz EPR tubes were used.

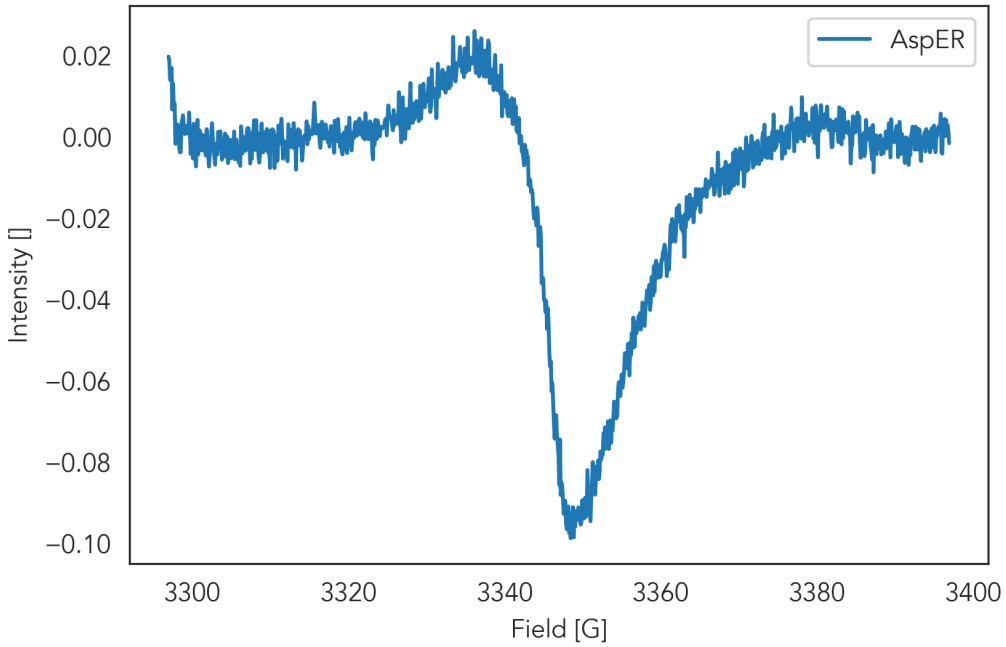
200 uL of a 0.3 mM solution was prepared in 100 mM Tris pH 9 and added to the EPR tube under strict anaerobic conditions. The EPR tube was capped and wrapped in parafilm to decrease oxygen exposure. The tubes were then irradiated with cyan LEDs (50 W Chanzon high power LED chip, λ_{max}= 490 nm, measured photon flux = 12,000 mM/m^2 s) for 30 minutes. A fan was used to maintain room temperature during the irradiation. After 30 minutes, the tubes were flash frozen using liquid nitrogen. All experiments were performed with a Bruker EMX plus spectrometer cooled with liquid helium to 8K, with continuous wave X-band frequency of 9.38Ghz and a time constant of 5.12ms and conversion time of 60ms. The microwave cavity was tuned for each sample using the Bruker Xenon software. The lowest possible microwave power 0.2 μW was used.

EPR Baseline correction

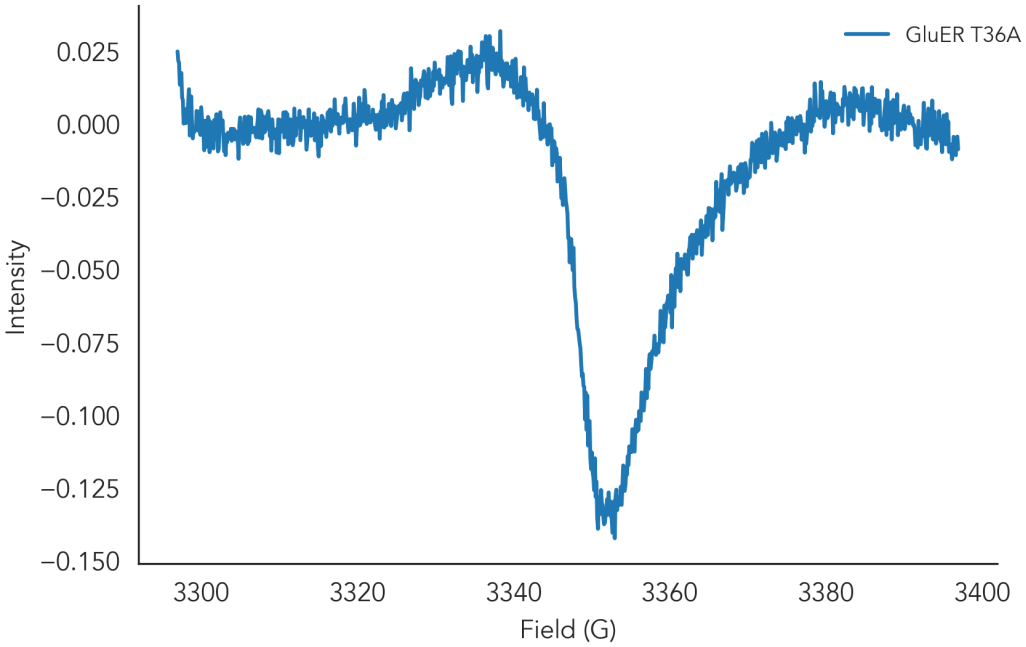
The EPR spectra were baseline corrected using the procedure outlined in Dellalunga et.al.[61] The signal was fit with a third order polynomial with the radical signal (3326 – 3370 Gauss) ignored in the fitting procedure. The subsequent fits were then subtracted from the data. The spectra pre and post fit are shown in Supplemental Figure 39.



Supplemental Figure 39: Raw Data Fit and Baseline Correction

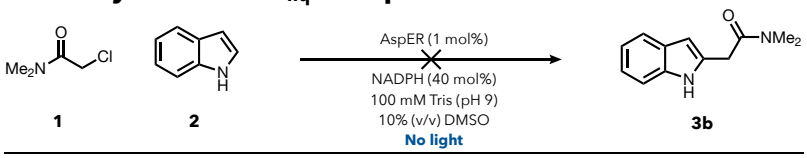


Supplemental Figure 40: EPR spectra of AspER semiquinone



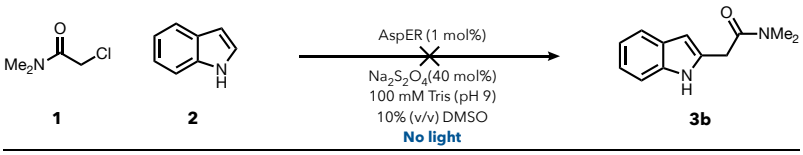
Supplemental Figure 41: EPR spectra of GluER T36A semiquinone

Ground State Reactivity of the FMN_{hq} in AspER



To determine if the ground state hydroquinone is the active species in AspER a reaction was set up using NADPH as the reductant in a mBraun glovebox with oxygen below 0.1 ppm. Reactions were run with 10 umol of chloroamide starting material at a final substrate concentration of 30 mM. A shell vial wrapped in tin foil to prevent any light irradiation was charged with a magnetic cross stir bar and a volume of 100 mM Tris pH 9 such that final concentration would be 30 mM after addition of all the other reagents. DMSO was added to the shell vial such that it would be 10% (v/v). Then 149 μL of a 20 mg/mL solution of NADPH was added. Finally, 1 mol% of ERED was added. Neat chloroamide was then added to the shell vial, followed by indole (4.1 mg/10 uL of DMSO). The vial was sealed with a rubber septa and brought out of the mBraun where it is placed on a stir plate at 300 rpm for 24 hours. After 24 hours, 1.75 mL of acetonitrile and 100 μL of a 2m mg/mL solution of 1,3,5-tribromobenzene was added to the reaction vial and the solution was centrifuged at 16,800 xg for 10 minutes and filtered through a kimwipe plug for LCMS analysis. No alkylated indole product was detected.

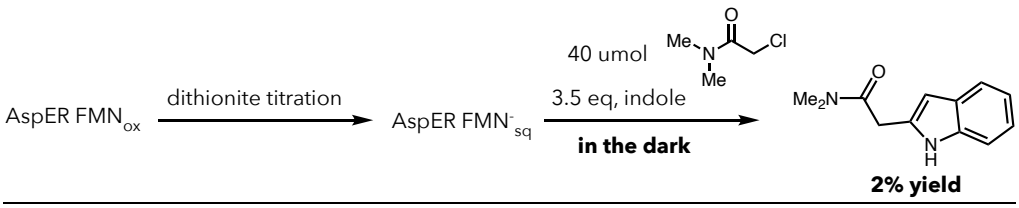
Dithionite Reactivity in AspER



1 2 3b

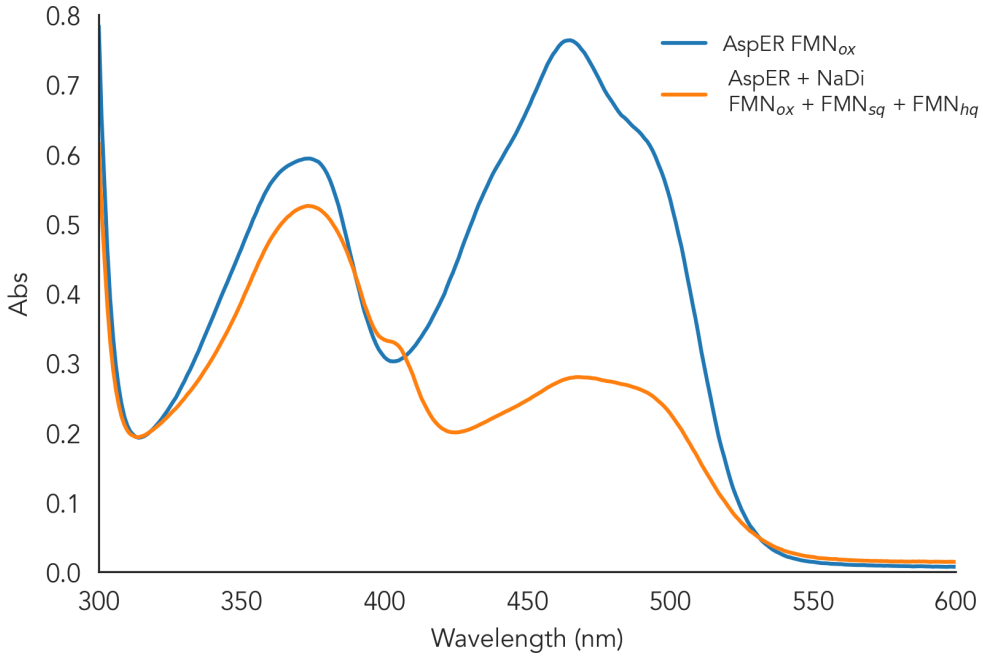
To determine if dithionite can catalyze the reaction by reducing the substrate in AspER's active site a reaction was set up using sodium dithionite as the reductant in a mBraun glovebox with oxygen below 0.1 ppm. Reactions were run with 20 umol of chloroamide starting material at a final substrate concentration of 30 mM. A shell vial wrapped in tin foil to prevent any light irradiation was charged with a magnetic cross stir bar and a volume of 100 mM Tris pH 9 such that final concentration would be 30 mM after addition of all the other reagents. THF was added to the shell vial such that it would be 10% (v/v). Then X μL of a 5 mg/mL solution of sodium dithionite was added. Finally, 1 mol% of ERED was added. Neat chloroamide was then added to the shell vial, followed by indole (8.2 mg/20 uL of THF). The vial was sealed with a rubber septa and brought out of the mBraun where it is placed on a stir plate at 300 rpm for 24 hours. After 24 hours, the standard NMR analysis work up procedure was followed to see no yield of the alkylated product.

Dithionite Titration and substrate addition

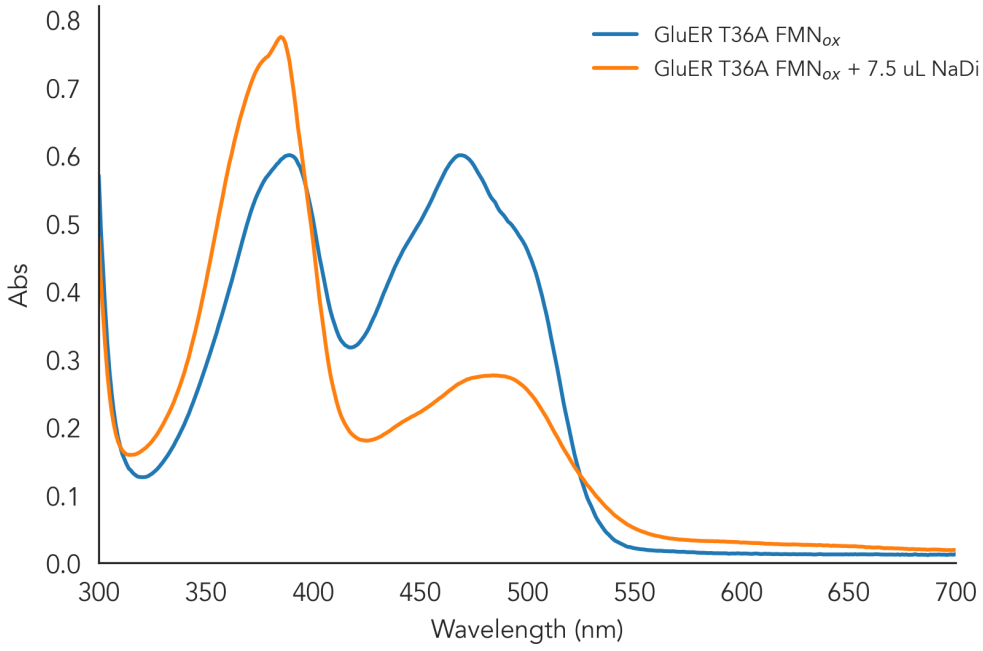


All samples were prepared in an MBraun glovebox with O_2 level less than 0.1 ppm. Cuvettes were specially designed with a GL18 cap to maintain oxygen free conditions for the duration of the experiment.

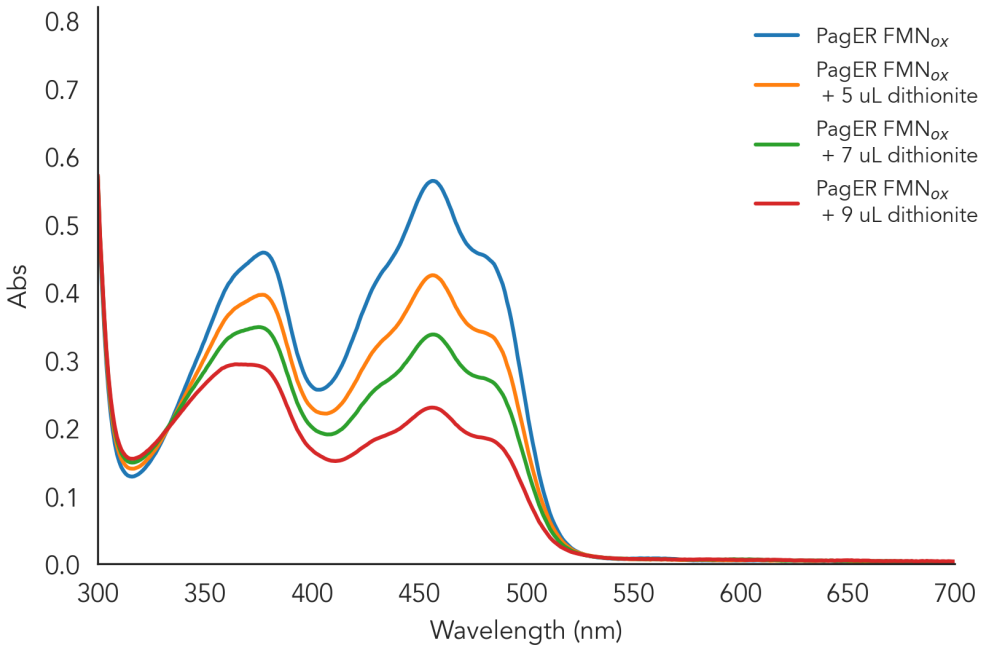
400 nmole of protein was added to 100 mM Tris buffer pH 9 such that the final volume was 1.2 mL. This solution was put into an anaerobic cuvette with a pathlength of 2 mm. The cuvette was sealed an a spectra was taken of the oxidized flavin. The cuvette was brought back into the mBraun and dithionite (5 mg/mL solution) was added carefully to generate the semiquinone. Qualitatively, semiquinone formation can be monitored through color change (reddish-brown for GluER T36A and orange-ish red for AspER). Semiquinone formation was measured using a UV-Vis. Typically 7-8 μL was added. Once the protein was determined to be in mostly the semiquinone state, it was brought back into the mBraun and split into two shell vials wrapped in foil (600 uL of the solution into each vial). 20 μmol of chloroamide 1 and indole (8.2 mg/20 uL DMSO) were added and a septa was placed. The solutions were allowed to sit in the dark for 48 hours. One vial was worked up using the standard NMR workup (workup 1) for the biocatalytic alkylation to reveal the C2 product was produced in 2% yield. The NMR mixture was then dried and subjected to LCMS analysis against the authentic standard to confirm the correct C2 product mass and retention time were observed. The same procedure was repeated with GluER T36A (Supplemental Figure 42) except only LCMS analysis was obtained to show the C2 product was now formed with the most product area [1.3 :1 C2:(C3+C4)] by integration. PagER cannot access the semiquinone using this method (Supplemental Figure 43).



Supplemental Figure 42: Dithionite Titration for AspER

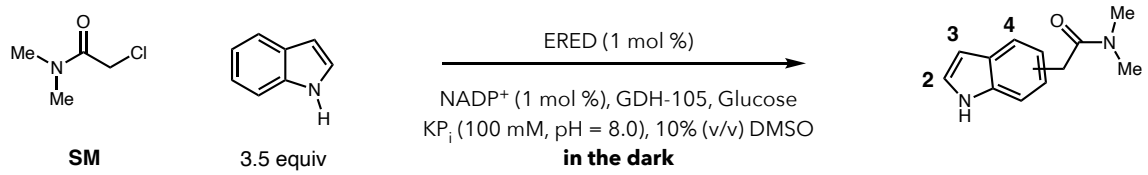


Supplemental Figure 43: Dithionite Titration for GluER T36A



Supplemental Figure 44: Dithionite Titration for PagER

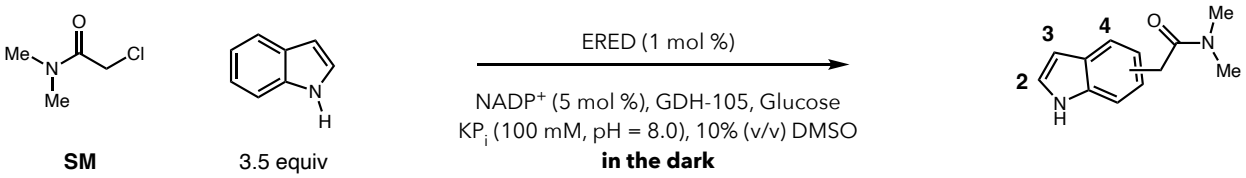
Procedure to determine ground state reactivity of GluER T36A mutants



All reactions were set up in a Coy® Chamber. Reactions were run with 20 umol of chloroamide starting material at a final substrate concentration of 30 mM. A shell vial was wrapped in tin foil with a magnetic cross stir bar was charged with a "master mix" solution of 6 equivalents of (D)-glucose dissolved in 100 mM Tris pH 9 in a volume such that the final concentration of substrate would be 30 mM after addition of all the other reagents. d_6-DMSO (deuterated DMSO allowed for simplified NMR analysis) was added to the shell vial such that the final concentration would be 10% (v/v). To this solution, 200 uL of a 5 mg/mL GDH solution was added. Then 28 uL for GluEr T36A and 140 uL for all other mutants of a 5 mg/mL solution of $NADP^+$ was added. Finally, 1 mol% of ERED was added. Neat chloroamide was then added to the shell vial. Then indole was added as a stock solution in DMSO. The vial was immediately sealed with a rubber septa to prevent light from getting into solution once the substrates were added. It was then brought out of the Coy® Chamber where it is placed on a stir plate at 300 rpm in a dark room.

After 24 hours, 6.667 μmol of internal standard (1,3,5-trimethoxybenzene) was added from a stock solution in ethyl acetate. Then an equal volume of ethyl acetate was added and the reactions were stirred for 30 minutes. They were centrifuged at 2000 x g for 2 minutes and organic layer removed. The extraction process was repeated 6 times. The organic layers were pooled and concentrated *in vacuo* and then resuspended in $CDCl_3$ and concentrated *in vacuo* again. The dried crude mixture was resuspended in $CDCl_3$ and analyzed by ^{1}H-NMR for yield calculation, where the singlet at δ = 6.06 ppm corresponding to the three aromatic protons of 1,3,5-trimethoxybenzene was set to an integration of 1.00. Integration of varying signals of different product could be used to calculate an effective yield. The regioisomeric ratio was determined relative to the trimethoxybenzene standard.

Supplemental Table 6: No light control with GluER T36A mutants



SM 3.5 equiv **in the dark**

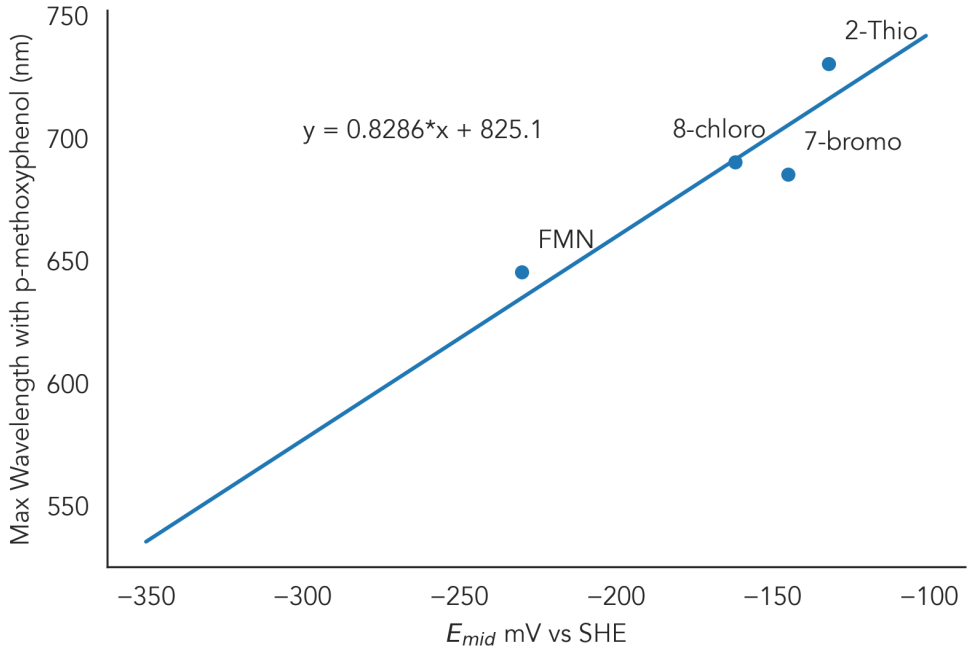
	ERED	SM % yield	C2 % yield	C3 % yield	C4 % yield
	GluER T36A[a]	85%	n.d.	n.d.	n.d.
	+ Y343F	81%	n.d.	n.d.	n.d.
	+ T25L	79%	n.d.	n.d.	n.d.
	+T231V	68%	n.d.	n.d.	n.d.
	+G270M	64%	n.d.	n.d.	<1%
	+Q232W	67%	2%	n.d.	8%
[PagER]	+T268D	67%	2%	n.d.	14%
	-T25L	71%	n.d.	n.d.	n.d.

Reaction conditions: α-chloroamide (2 mg, 20 μmol), 3.5 equiv. of arene (70 μmol), 1 mol% ERED, 5 mol% $NADP^+$, 1 mg GDH and 0.5 equiv of glucose (10 μmol) was irradiated with Cyan LEDS for 24 hours. Yield of each regioisomer was determined by NMR using 1,3,5-trimethoxybenzene as standard. [a]The reaction was carried out with 1 mol% $NADP^+$

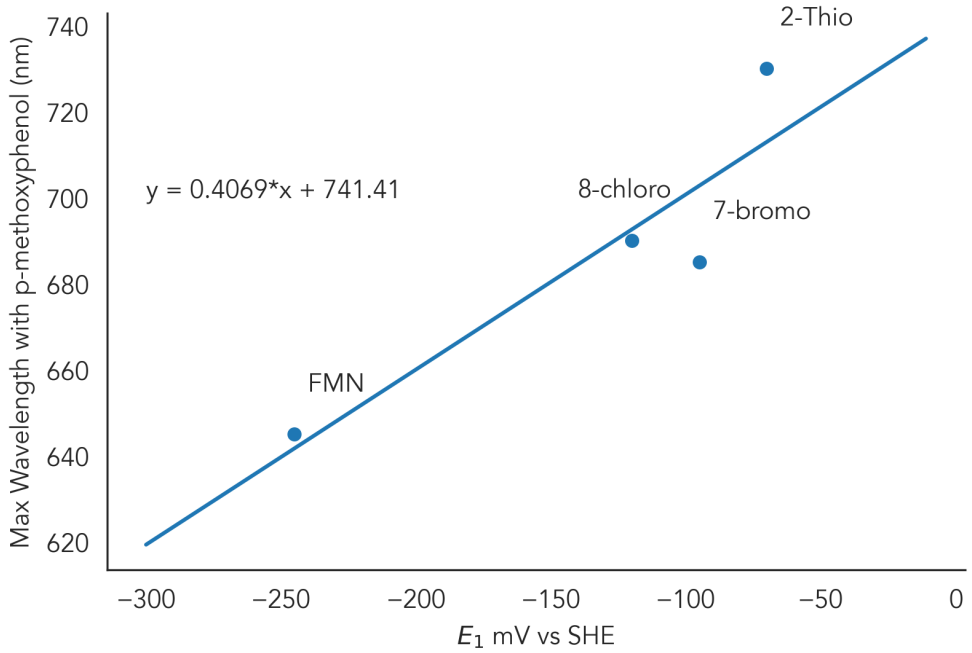
Procedure for estimating the redox potential in protein bound flavin

Using a modified procedure from Stewart et. al.[62] 50 nmole of protein was diluted to 100 umol in 100 mM KP_i pH 8 and added to a quartz cuvette. A UV-Vis spectra was taken of oxidized flavin (all spectra are presented in Supplemental Figure 47). Then p-methoxyphenol was added from a 200 mM stock solution in ethanol such that final concentration would be 5 mM. Another UV-Vis spectra was taken of the CT state produced (all CT states are presented in Supplemental Figure 48). The wavelength of the local maxima of the CT state was identified and used to calculate the redox potential of the FMN_{ox}/FMN_{sq} (E_1) redox couple and the midpoint potential (E_{mid}), the average of the FMN_{ox}/FMN_{sq} and the FMN_{sq}/FMN_{hq} redox couples, using a linear relationship identified by Stewart et. al (Supplemental Figure 45 and 46). The midpoint and FMN_{ox}/FMN_{sq} for each step of the C4 indole evolutionary campaign are presented in Supplemental Figure 49 and 50 respectively.

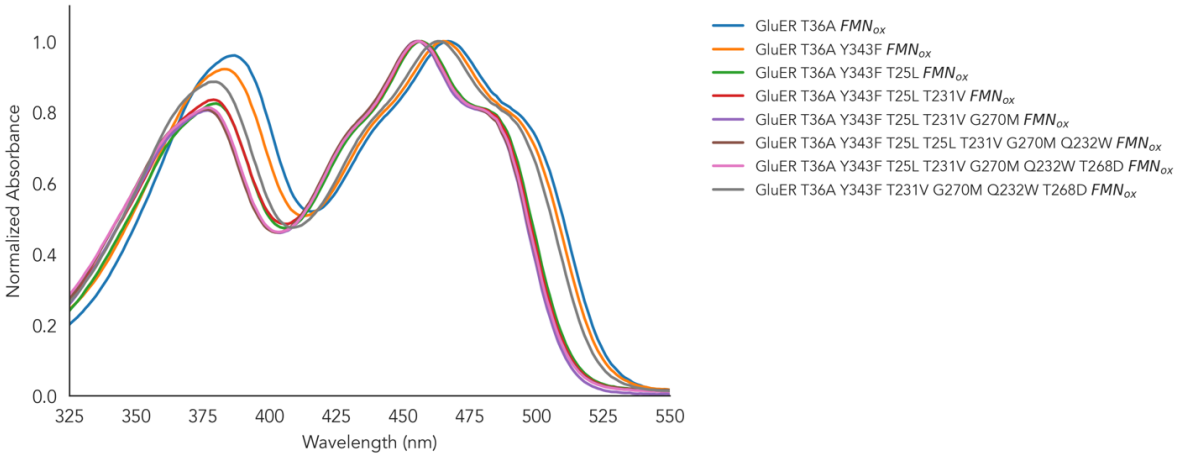
From the midpoint potential and the E_1 potential, we can conclude the potential of both the FMN_{ox}/FMN_{sq} and the FMN_{sq}/FMN_{hq} was lowered significantly to make a more reducing flavin hydroquinone and flavin semiquinone. We believe this has two benefits in the alkylation of indole. Massey observed lower FMN_{ox}/FMN_{sq} potentials corresponded with decreased semiquinone stability. PagER cannot be photoreduced at the same rate as GluER T36A, which means it has less access to the semiquinone. Attempts to generate the semiquinone with PagER using dithionite titration were also unsuccessful. By not accessing the semiquinone in solution, the C2 selective semiquinone pathway for alkylation cannot be accessed and less C2 product is formed. Additionally, lowering the FMN_{hq} reduction potential can be correlated to a decrease in the ionization potential of the FMN_{hq}. Lowering the ionization potential decreases the band gap of the CT state formed to do ground state electron transfer.



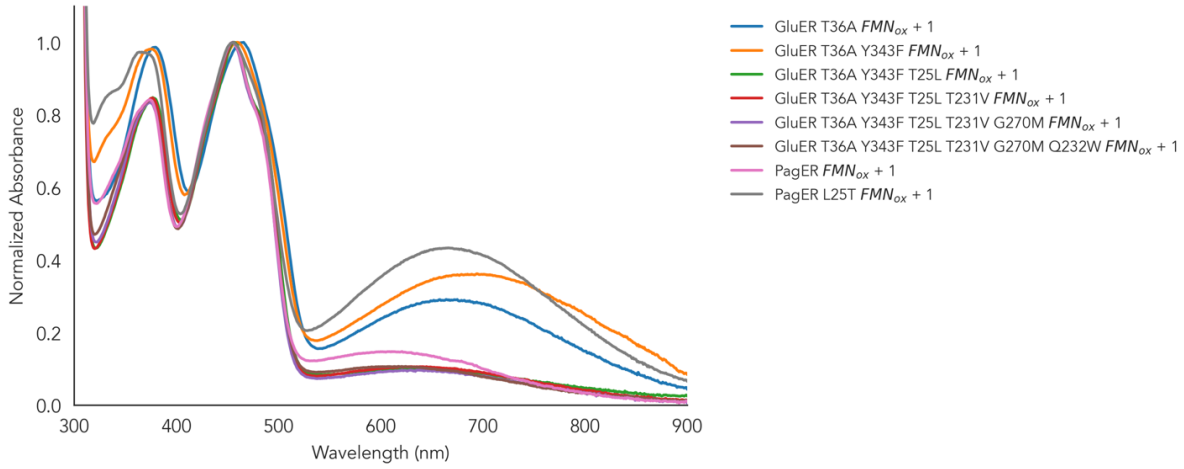
Supplemental Figure 45: Relationship between midpoint potential and λ_{maz} of the CT state between FMN_{ox} and *p*-methoxyphenol



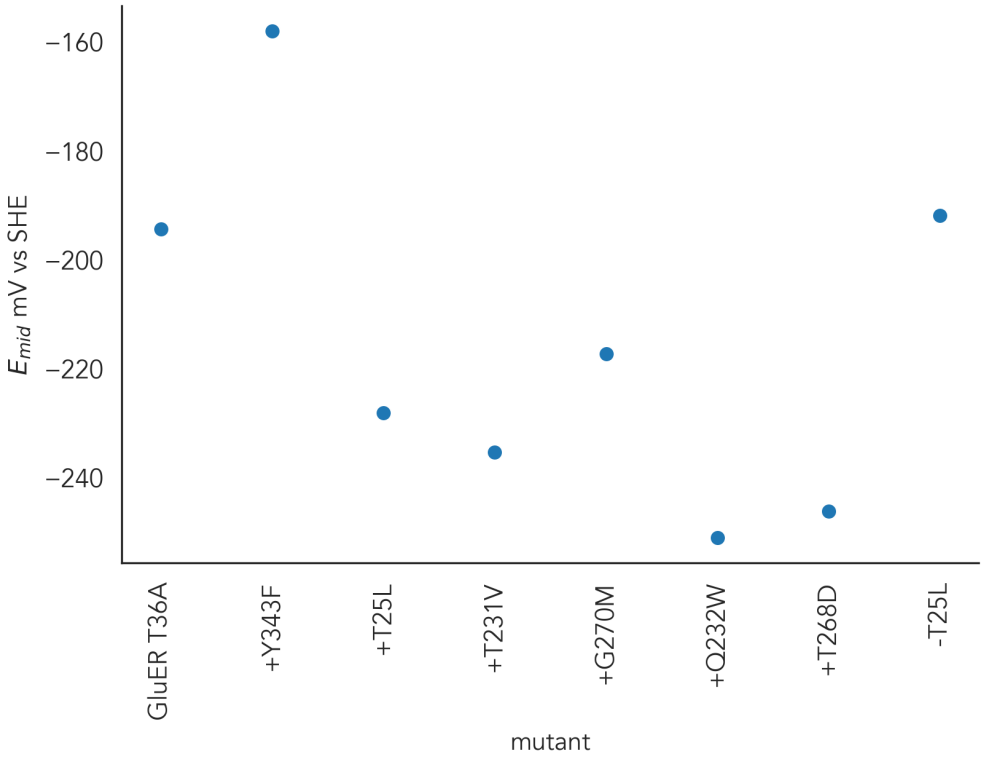
Supplemental Figure 46: Relationship between E_1 and λ_{maz} of the CT state between FMN_{ox} and *p*-methoxyphenol



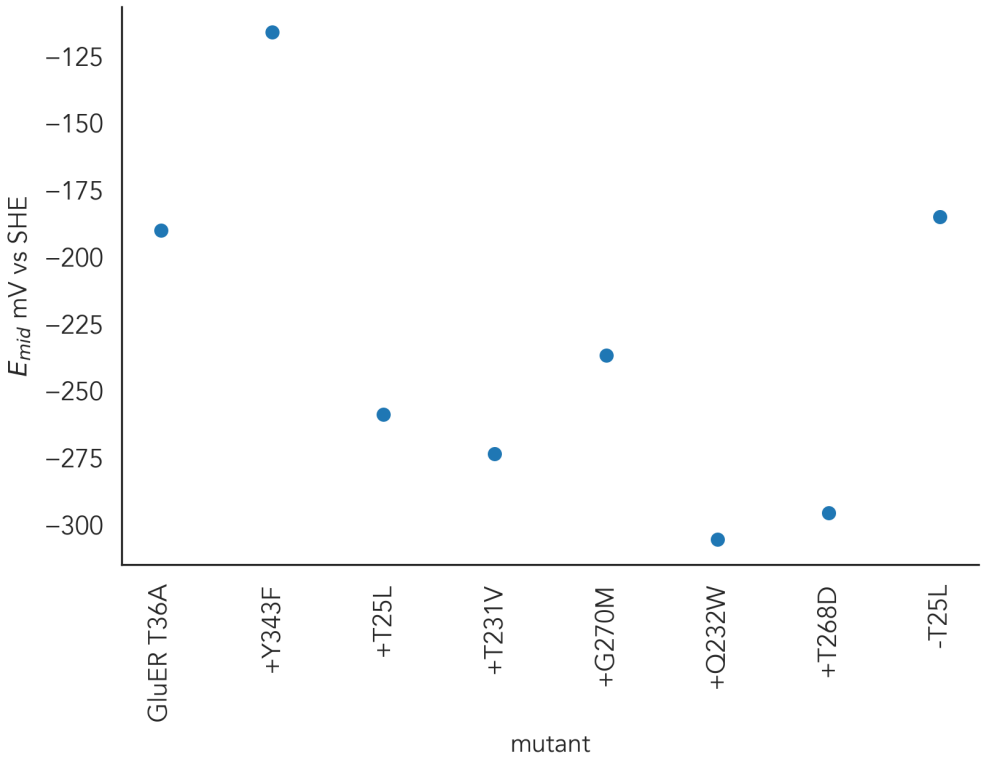
Supplemental Figure 47: Oxidized Flavin Traces



Supplemental Figure 48: CT state with FMN_{ox} and *p*-methoxyphenol



Supplemental Figure 49: Midpoint potential of mutants

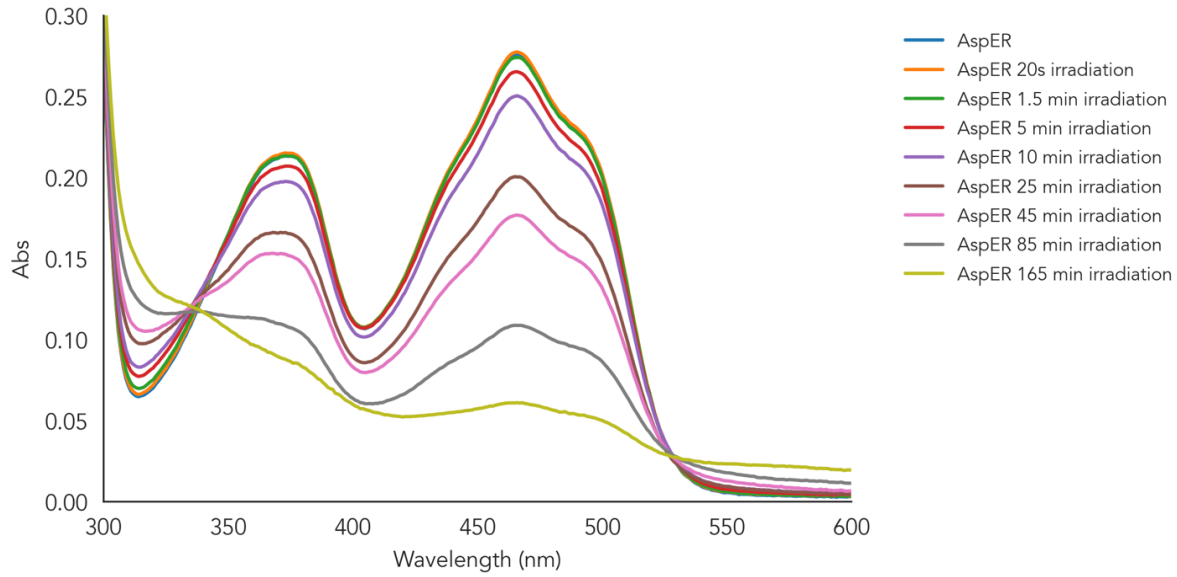


Supplemental Figure 50: E_1 of all mutants

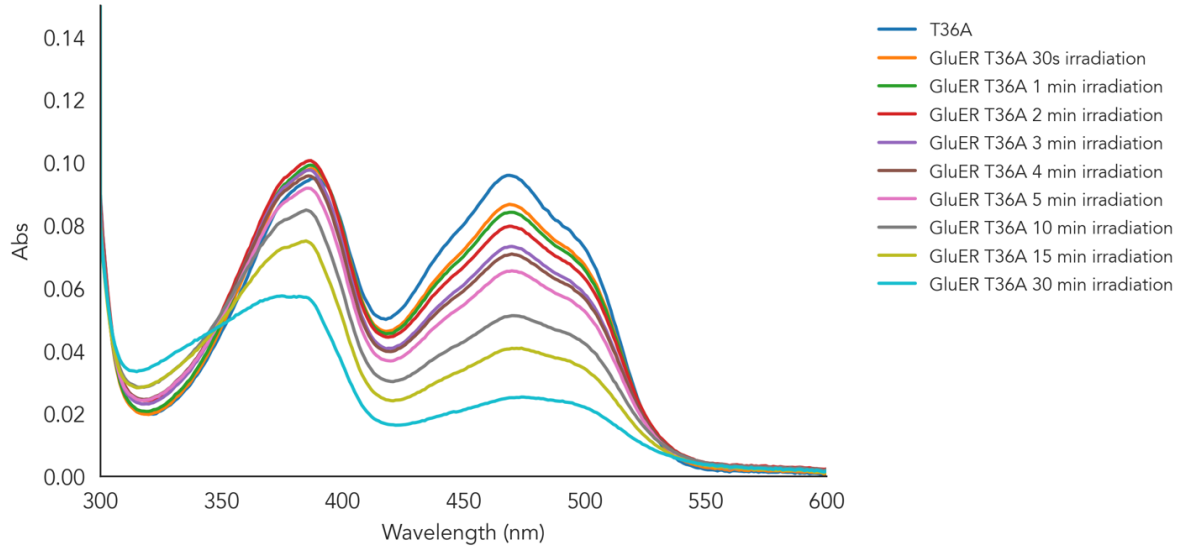
Photoreduction Time Course Procedure

All samples were prepared in an MBraun glovebox with O_2 level less than 5 ppm. Cuvettes were specially designed with a GL18 cap to maintain oxygen free conditions for the duration of the experiment.

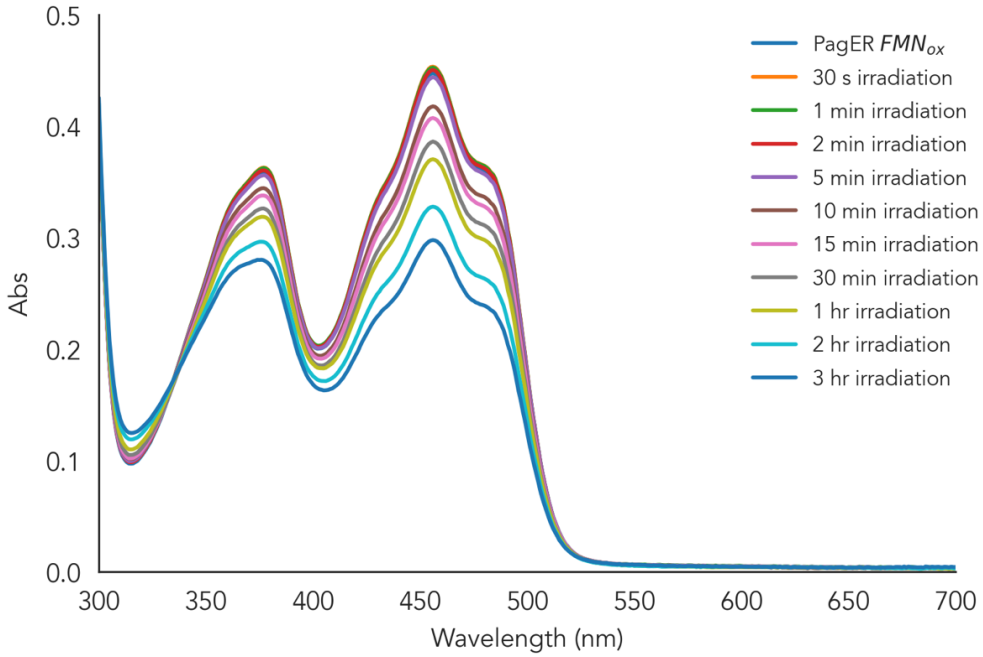
200 nmole of ERED was added to 1-2 mL of buffer (100 mM Tris pH 9) and added to the cuvette in the mBraun. The cuvette was sealed to the outside atmosphere and a spectrum was taken. The solution in the cuvette was then irradiated with cyan LEDs (50 W Chanzon high power LED chip, λ_{max}= 490 nm, measured photon flux = 12,000 mM/m^2 s). A fan was used to maintain room temperature during the irradiation. At fixed time points, the cuvette was removed from the light and a spectrum was taken to monitor the process of photoreduction. The photoirradiation time courses for GluER T36A, PagER and AspER are shown in Supplemental Figures 51-53.



Supplemental Figure 51: AspER Photoreduction Time Course

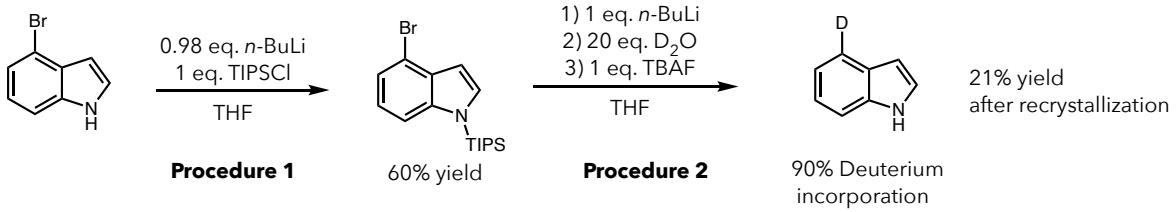


Supplemental Figure 52: GluER T36A Photoreduction Time Course



Supplemental Figure 53: PagER Photoreduction Time Course

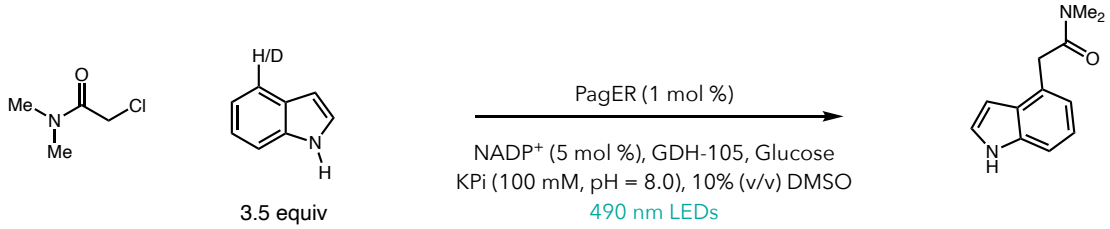
Procedure for the preparation of 4-D indole



Procedure 1: Indole was added to a flame dried flask and stir bar and then dissolved in dry THF and placed under inert atmosphere. The flask was cooled to -78°C and *n*-BuLi (0.98 eq.) was added dropwise. The flask was stirred for 30 minutes and 1 equivalent of TIPSCl was added. The flask was allowed to come to room temperature while stirring and was then quenched with water and poured into a separatory funnel containing ether and water. The aqueous layer was extracted three times with ether. The organic layers were pooled and dried over sodium sulfate and then concentrated *in vacuo*. The crude residue was columned (20% Hexanes/Ethyl Acetate) and the fractions were collected and concentrated to yield pure N-TIPS-4-bromoindole as a white solid (60% yield).

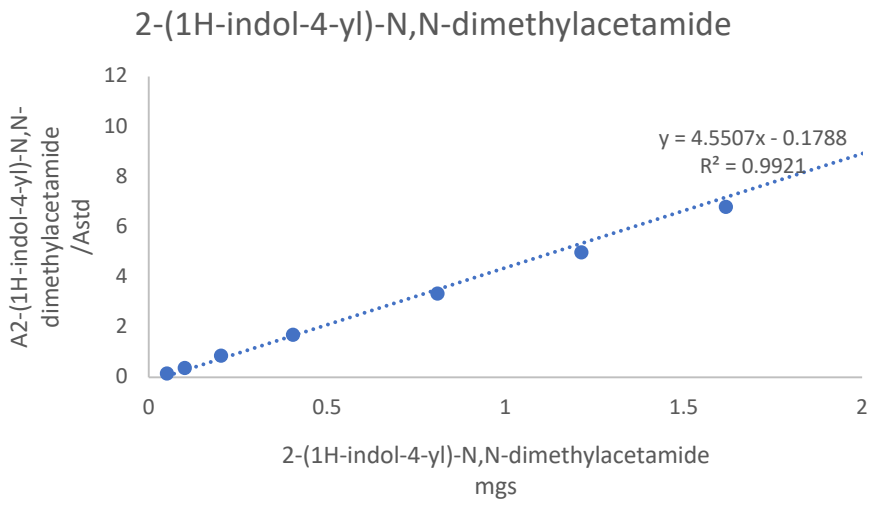
Procedure 2: N-TIPS-4-bromoindole was added to a flame dried flask charged with a magnetic stir bar. Dry THF was added and the flask was placed under inert atmosphere and then cooled to -78°C. 1 equivalent of *n*-BuLi was added over several minutes. After complete addition, the flask was allowed to stir for 5 minutes before 20 equivalents of D_2O were added. The flask was warmed to room temperature. The reaction mixture was diluted with more water and ether, separated and the aqueous layer was extracted three times with ether. The organic extracts were pooled, dried over sodium sulfate and then concentrated *in vacuo*. The crude residue was columned to yield a mixture of 4-bromoindole and 4-deuteroindole. 4-deuteroindole was recrystallized from this mixture using cold hexanes to yield pure 4-deuteroindole (21% yield, 90% deuterium incorporation). All spectra match reported values.

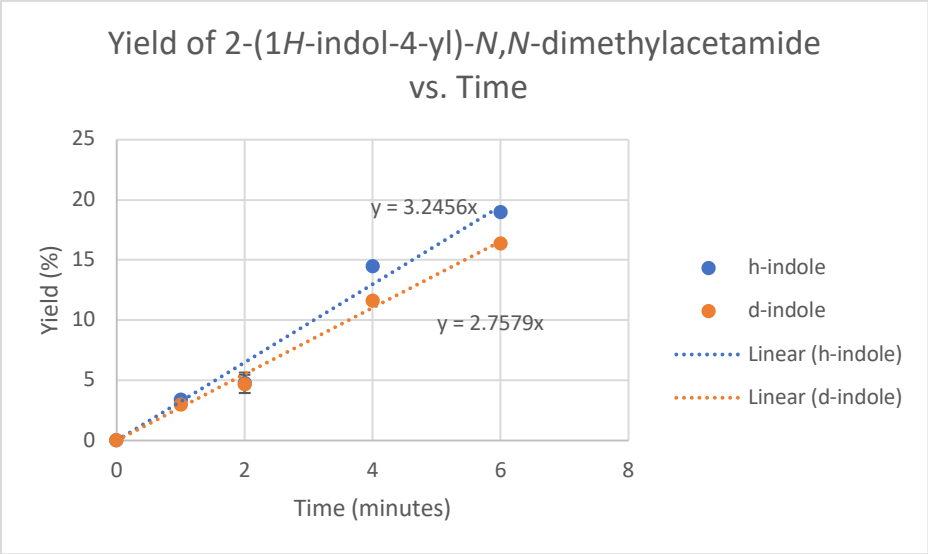
Kinetic Assay to determine KIE:



To determine if the oxidation of the indoyl radical is the rate determining step in PagER kinetic assays were conducted. All timepoints were taken in duplicate. Reactions were run in a Coy Chamber with 10 umol of chloroamide starting material at a final substrate concentration of 30 mM. Shell vials were charged with magnetic cross stir bars. A mastermix solution was added containing 0.5 equivalents of glucose, 1 mg of GDH, 5 mol% of $NADP^+$ and 1 mol% PagER in 300 uL of 100 mM KP_i pH 8. DMSO was added to the shell vial such that it would be 10% (v/v). Neat chloroamide was then added to the shell vial, followed by indole (4.1 mg/10 uL of DMSO). The vials were sealed with a rubber septa and brought out of the Coy Chamber where it is placed on a stir plate at 300 rpm. At each timepoijnt, 1.75 mL of acetonitrile and 100 μL of a 2m mg/mL solution of 1,3,5-tribromobenzene was added to the reaction vial and the solution was centrifuged at 16,800 xg for 10 minutes and filtered through a kimwipe plug for LCMS analysis. The standard curve to determine the amount of product form is below.

Standard Curve for 2-(1H-indol-4-yl)-N,N-dimethylacetamide





Supplemental Figure 54: Time Course for PagER using H/D-indole

Part XIII. Small Molecule and Protein Crystallography:

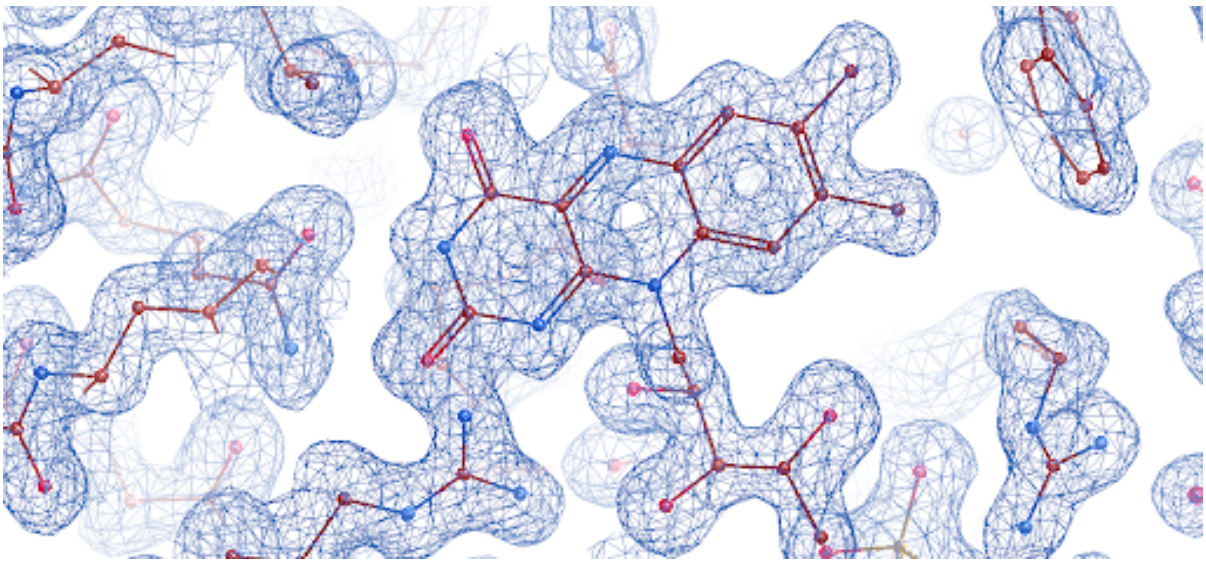
PagER was expressed in BL21 pET22b *E. coli* cells and purified using a His-Trap HP 5mL affinity chromatography column (GE Life Sciences). The proteins were then subjected to buffer exchange and concentrated to a final concentration of 1.00 mM, approximately 40 mg/mL. Protein sample was then dialyzed into a 50 mM TEOA buffer before concentrating to a final concentration of 25 ug/uL. Initial crystallization screens for GluER were carried out using Crystal Screen 2 in hanging drop vapor-diffusion crystallization trays (Hampton Research) as well as with the help of the Hauptman-Woodward Institute.[63] Following optimization, a single crystal for PagER grew in 0.1 M Tris, 0.1 M Potassium Bromide, and 20-25% (w/v) PEG 4000 using sitting drop trays (Hampton Research). Initial crystal appeared after 7 days.
Diffraction data for T36A GluER was obtained at the ID7B2 (FlexX) beamline of CHESS using 0.9202 Å wavelength at 100 K to a maximum resolution of 0.9 Å. All data was integrated with the program DIALS and scaled with the program AIMLESS.[64, 65] Crystal data was analyzed for defects with program XTRIAGE and identified radiation damage, significant twinning (50% -h,-k,l) and low completeness in the 0.9-1.2 Å range. The dataset was then trimmed and scaled to 1.5 Å to account for radiation damage and resolution completeness; twinning was accounted for in subsequent refinement steps. The structure for PagER was determined by the method of molecular replacement using the PDB entry 6MYW (GluER T36A) and the program PHASER.[66] There were three molecules in the asymmetric unit of PagER. Electron density was clearly visible for most of the C-terminus (a.a. 316-356) with few exceptions that are otherwise supported by excellent fitting of adjacent, bonded atoms to the electron density map. Secondary and tertiary structure remained largely unchanged from GluER T36A with the exception of a few residues in and adjacent to the active site. Electron density for Flavin Mononucleotide (FMN) is also well supported by electron density in all NCS copies. COOT was used for model building, while structure refinement was carried out with Phenix, incorporating restrained anisotropic B-factor and occupancy refinement for protein and FMN co-factor atoms in the final stages.[67, 68] The final model for PagER has solid agreement with the data with an R-factor of 26% and an R-free of 27% for 8912 atoms. Ramachandran statistics for PagER show 97% (7921) of residues favored, 2% (370) allowed, and less than 1% (5) as outliers. The structure was uploaded to the Protein Databank under

*Outliers are well supported by electron density and NCS constraints.

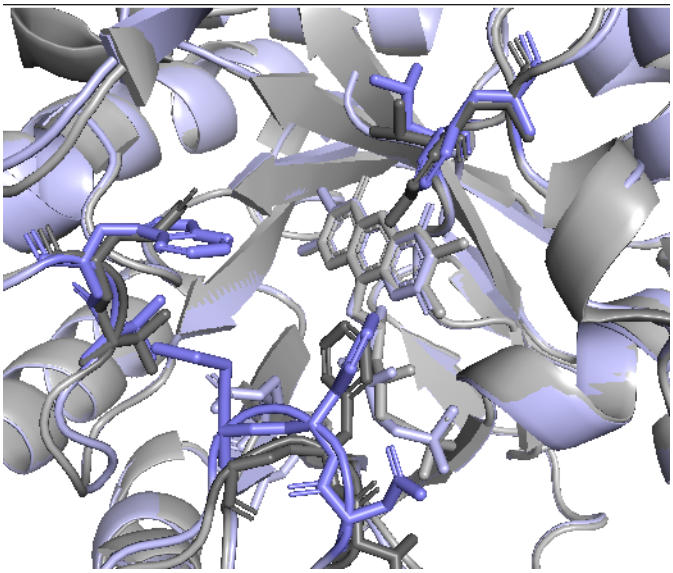
Supplemental Table 7. Data collection and refinement statistics for PagER

	PagER PDB: 8FW1
Data collection	
Space group	P1
Cell dimensions	
a, *b*, *c* (Å)	50.165, 51.849, 144.935
α, β, γ (°)	90.51, 90.11, 114.86
Wavelength (Å)	0.9202
Resolution range (Å)	72.46-1.50
Total observations	504333
Total unique observations	205104
R_{merge}	0.097 (0.309)
R_{pim}	0.077 (0.239)
$I / \sigma I$	7.9 (0.6)
Completeness (%)	96.61 (93.80)
Multiplicity	2.5 (2.3)
Refinement	
Resolution range (Å)	45.51-1.50
Reflections (total)	203975
Reflections (test)	2021
R_{work} / R_{free}	0.2675/0.2728
No. atoms	8912
Protein	8355
Ligand/ion	90
Water	467
B-Factors	16.16
R.M.S.-Deviations	
Bonds(Å)	0.03
Angles(°)	0.76
Ramachandran Statistics	
Favored (%)	94.81
Allowed (%)	4.44
Outliers (%)	0.74
Rotamer Outliers (%)	2.45
Clashscore	29

* Numbers in parentheses represent values for highest resolution shell



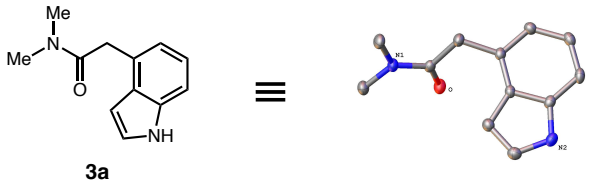
Supplemental Figure 55. Electron density map (2Fo-Fc) for PagER. The quality of the maps is high with FMN location well supported by electron density.



Supplemental Figure 56. Overlay of PagER (blue) and GluER T36A (grey).

Small Molecule X-Ray Crystallographic Data

Low-temperature X-ray diffraction data for **Rjjc1**, **Rjjc2** and **Rjjc3** were collected on a Rigaku XtaLAB Synergy diffractometer coupled to a Rigaku Hypix detector with Cu Kα radiation (λ = 1.54184 Å), from a PhotonJet micro-focus X-ray source at 100 K. The diffraction images were processed and scaled using the CrysAlisPro software.[69] The structures were solved through intrinsic phasing using SHELXT[70] and refined against F^2 on all data by full-matrix least squares with SHELXL[71] following established refinement strategies.[72] All non-hydrogen atoms were refined anisotropically. All hydrogen atoms bound to carbon were included in the model at geometrically calculated positions and refined using a riding model. Hydrogen atoms bound to nitrogen were located in the difference Fourier synthesis and subsequently refined semi-freely with the help of distance restraints. The isotropic displacement parameters of all hydrogen atoms were fixed to 1.2 times the Ueq value of the atoms they are linked to (1.5 times for methyl groups). Details of the data quality and a summary of the residual values of the refinements are listed in Tables 8-10.



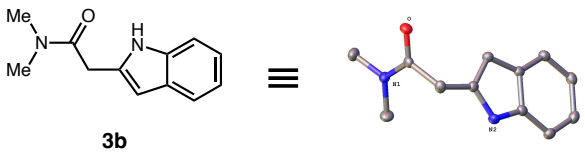
3a

CCDC 2237629

Supplementary Table 8. Crystal data and structure refinement for compound 3a.

Identification code	rjjc3_abs
Crystal data	
Chemical formula	$C_{12}H_{14}N_2O$
M_r	202.25
Crystal system, space group	Monoclinic, $P2_1$
Temperature (K)	102
a, *b*, *c* (Å)	7.1001 (1), 8.5854 (2), 8.5676 (2)
b (°)	96.381 (2)
V (Å^3)	519.02 (2)
Z	2
Radiation type	Cu *K*a
m (mm^{-1})	0.67
Crystal size (mm)	0.19 × 0.04 × 0.04
Data collection	
Diffractometer	XtaLAB Synergy, Dualflex, HyPix
Absorption correction	Gaussian *CrysAlis PRO* 1.171.42.58a (Rigaku Oxford Diffraction, 2022) Numerical absorption correction based on gaussian integration over a multifaceted crystal model Empirical absorption correction using spherical

	harmonics, implemented in SCALE3 ABSPACK scaling algorithm.
T_{min}, T_{max}	0.798, 1.000
No. of measured, independent and observed [$I > 2s(I)$] reflections	10537, 2103, 2029
R_{int}	0.051
$(\sin q/l)_{max}$ ($Å^{-1}$)	0.634
Refinement	
$R[F^2 > 2s(F^2)]$, $wR(F^2)$, S	0.034, 0.090, 1.08
No. of reflections	2103
No. of parameters	141
No. of restraints	2
H-atom treatment	H atoms treated by a mixture of independent and constrained refinement
$D\rho_{max}$, $D\rho_{min}$ (e $Å^{-3}$)	0.15, -0.18
Absolute structure	Flack x determined using 855 quotients [(I+)-(I-)]/[(I+)+(I-)] (Parsons, Flack and Wagner, Acta Cryst. B69 (2013) 249-259).
Absolute structure parameter	-0.01 (15)



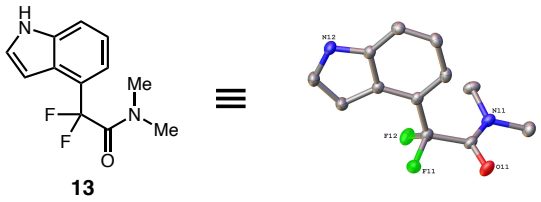
3b

CCDC 2237628

Supplementary Table 9. Crystal data and structure refinement for compound 3b.

Identification code	rjjc2_abs
Crystal data	
Chemical formula	$C_{12}H_{14}N_2O$
M_r	202.25
Crystal system, space group	Orthorhombic, *Pbca*
Temperature (K)	102
a, *b*, *c* (Å)	12.7799 (2), 11.8103 (2), 13.7400 (2)
V (Å^3)	2073.84 (6)
Z	8
Radiation type	Cu *K*a
m (mm^{-1})	0.67
Crystal size (mm)	0.08 × 0.04 × 0.04
Data collection	
Diffractometer	XtaLAB Synergy, Dualflex, HyPix
Absorption correction	Gaussian *CrysAlis PRO* 1.171.42.58a (Rigaku Oxford Diffraction, 2022) Numerical absorption correction based on gaussian integration over a multifaceted crystal model Empirical absorption correction using spherical harmonics, implemented in SCALE3 ABSPACK scaling algorithm.
T_{min}, T_{max}	0.887, 1.000
No. of measured, independent and observed [I > 2s(I)] reflections	12433, 2209, 2015

R_{int}	0.035
$(\sin q/l)_{max}$ ($Å^{-1}$)	0.634
Refinement	
$R[F^2 > 2s(F^2)]$, $wR(F^2)$, S	0.037, 0.097, 1.06
No. of reflections	2209
No. of parameters	141
No. of restraints	1
H-atom treatment	H atoms treated by a mixture of independent and constrained refinement
$D\rho_{max}$, $D\rho_{min}$ (e $Å^{-3}$)	0.26, -0.21



CCDC 2237627

Supplementary Table 10. Crystal data and structure refinement for compound 13.

Identification code	rjjc1_abs
Crystal data	
Chemical formula	$C_{12}H_{12}F_2N_2O$
M_r	238.24
Crystal system, space group	Monoclinic, $P2_1/c$
Temperature (K)	101
a, *b*, *c* (Å)	13.3477 (1), 13.5372 (1), 13.7530 (1)
b (°)	111.309 (1)
V (Å^3)	2315.14 (3)
Z	8
Radiation type	Cu *K*a
m (mm^{-1})	0.94
Crystal size (mm)	0.13 × 0.1 × 0.08
Data collection	
Diffractometer	XtaLAB Synergy, Dualflex, HyPix
Absorption correction	Gaussian *CrysAlis PRO* 1.171.42.58a (Rigaku Oxford Diffraction, 2022) Numerical absorption correction based on gaussian integration over a multifaceted crystal model Empirical absorption correction using spherical

	harmonics, implemented in SCALE3 ABSPACK scaling algorithm.
T_{min}, T_{max}	0.696, 1.000
No. of measured, independent and observed [$I > 2s(I)$] reflections	27663, 4937, 4509
R_{int}	0.034
$(\sin q/l)_{max}$ ($Å^{-1}$)	0.635
Refinement	
$R[F^2 > 2s(F^2)]$, $wR(F^2)$, S	0.036, 0.096, 1.06
No. of reflections	4937
No. of parameters	317
No. of restraints	2
H-atom treatment	H atoms treated by a mixture of independent and constrained refinement
$D\rho_{max}$, $D\rho_{min}$ (e $Å^{-3}$)	0.32, -0.24

Part XIV. References:

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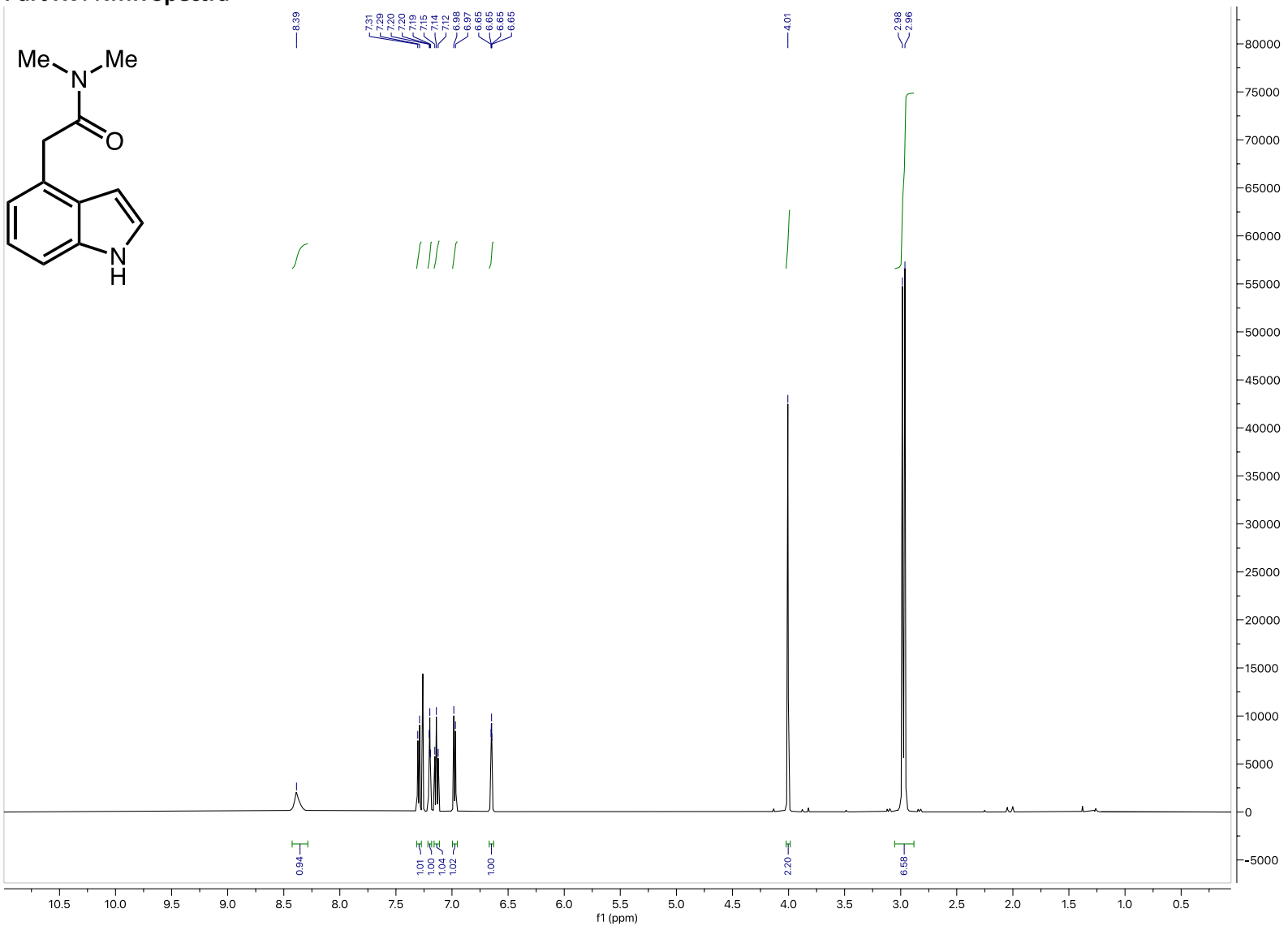
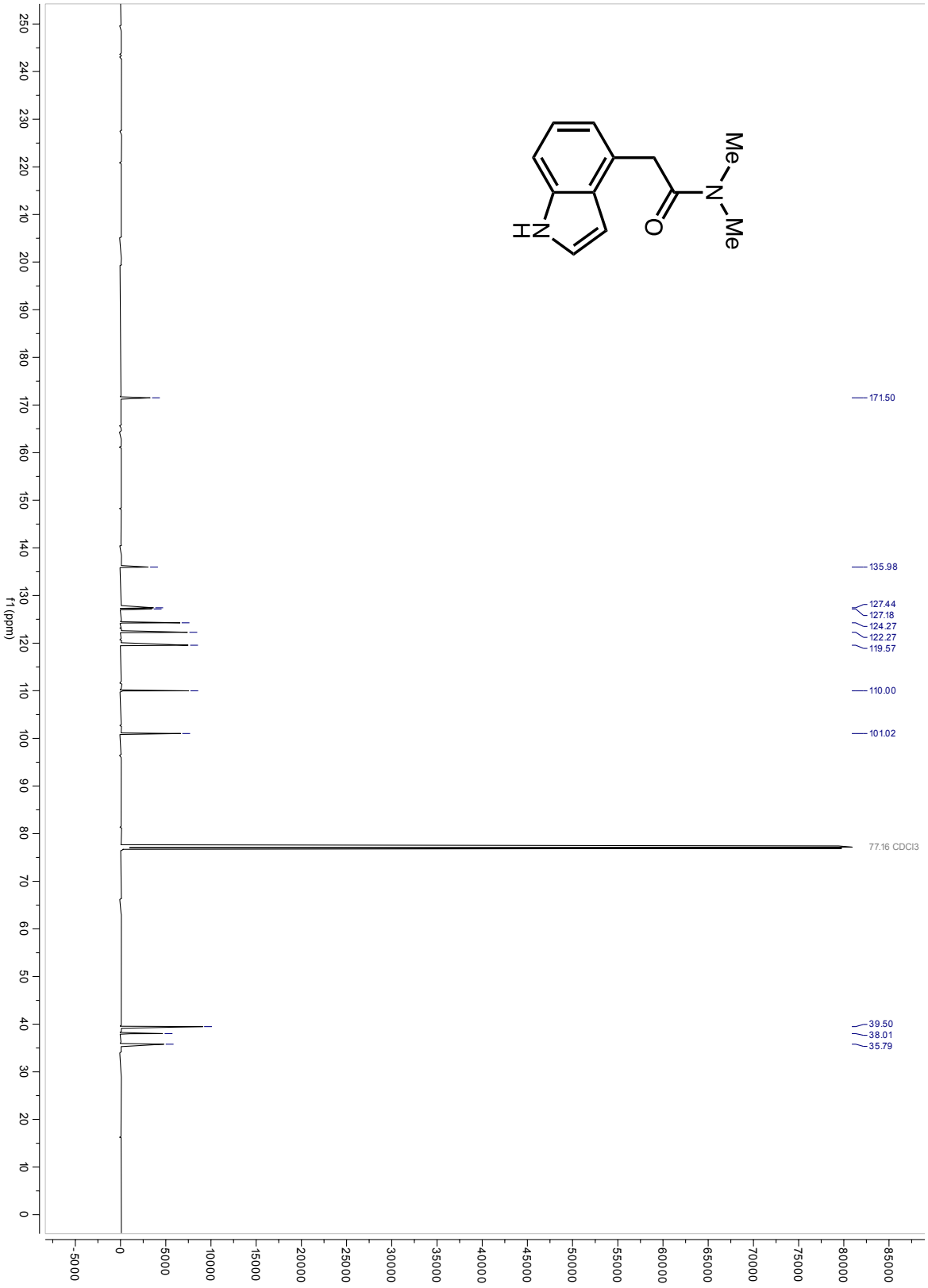
Part XV: NMR Spectra

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38.01
35.79
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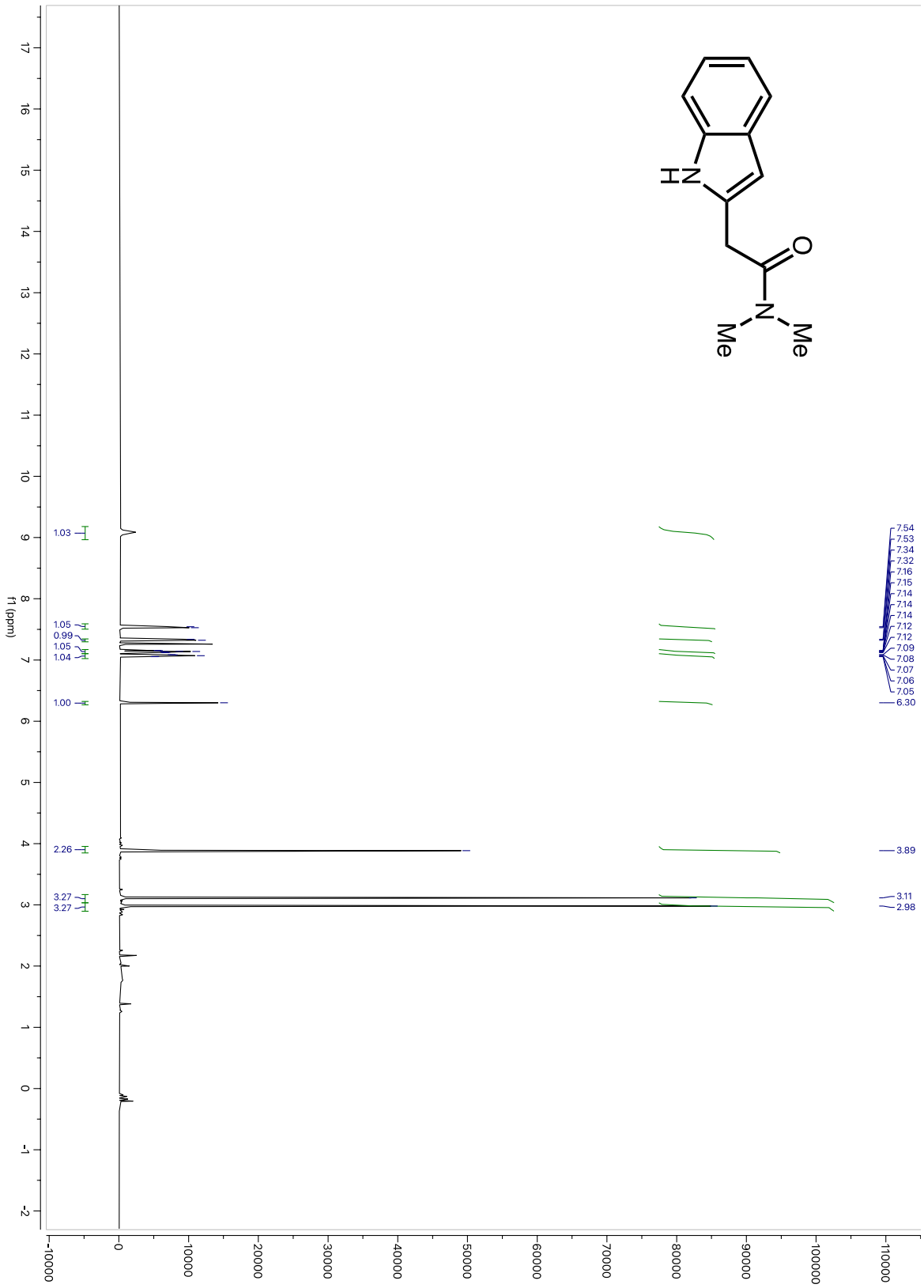
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-1
-2
-10000
0
10000
20000
30000
40000
50000
60000
70000
80000
90000
100000
110000
1.03
1.05
0.99
1.05
1.04
1.00
2.26
3.27
3.27
7.54
7.53
7.34
7.32
7.16
7.15
7.14
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7.12
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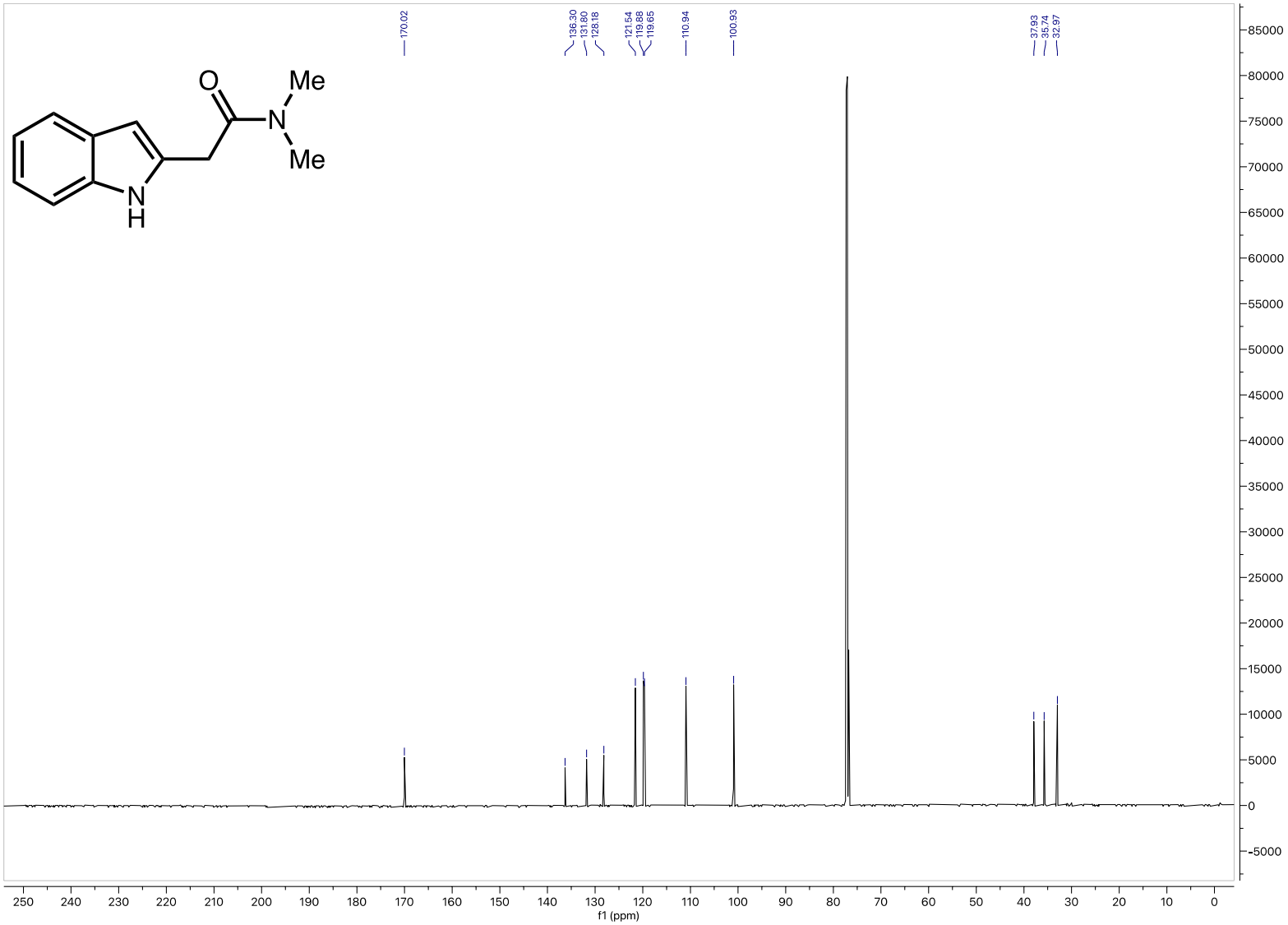
Me
Me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70000
65000
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55000
50000
45000
40000
35000
30000
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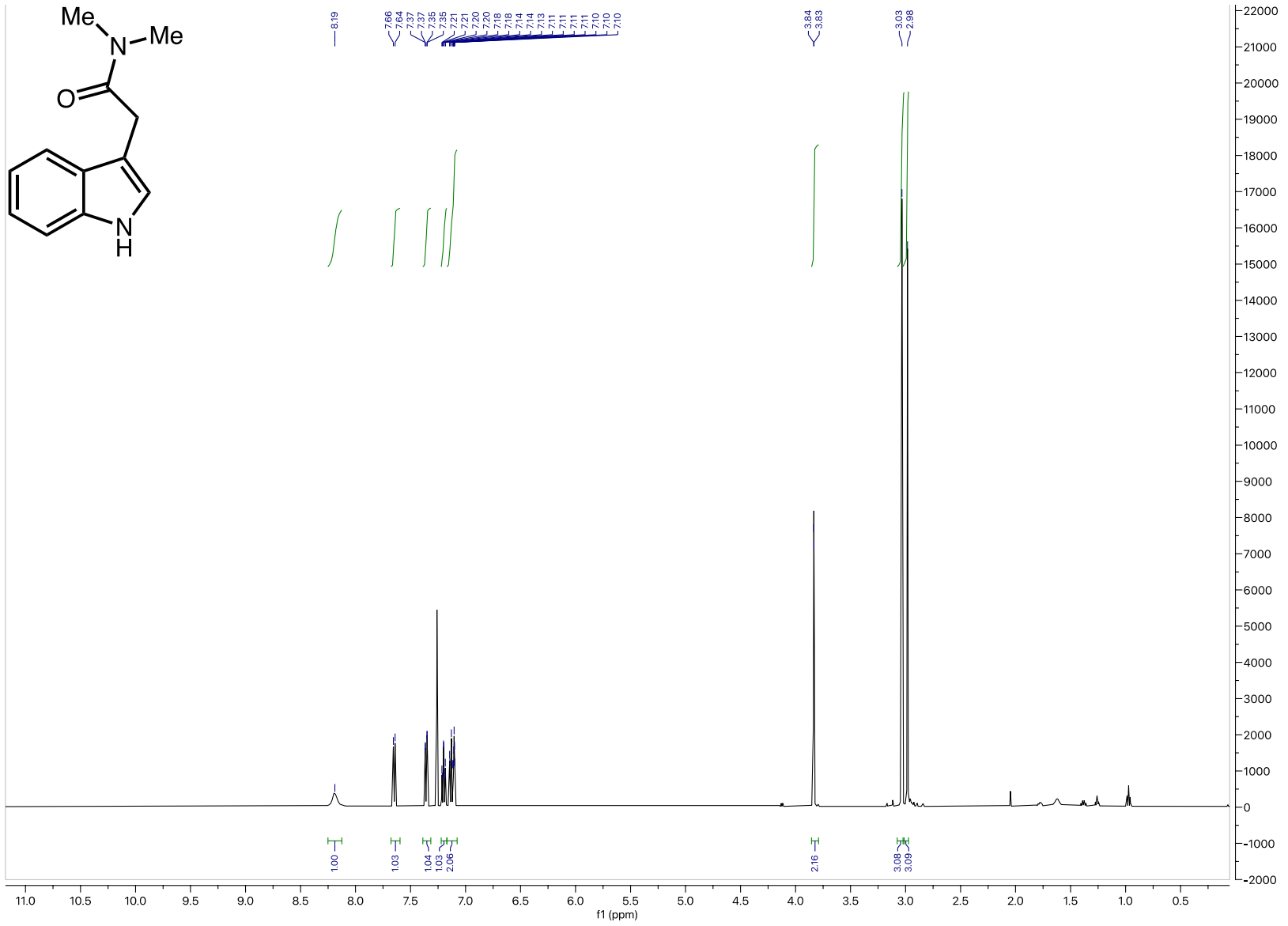
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7.11
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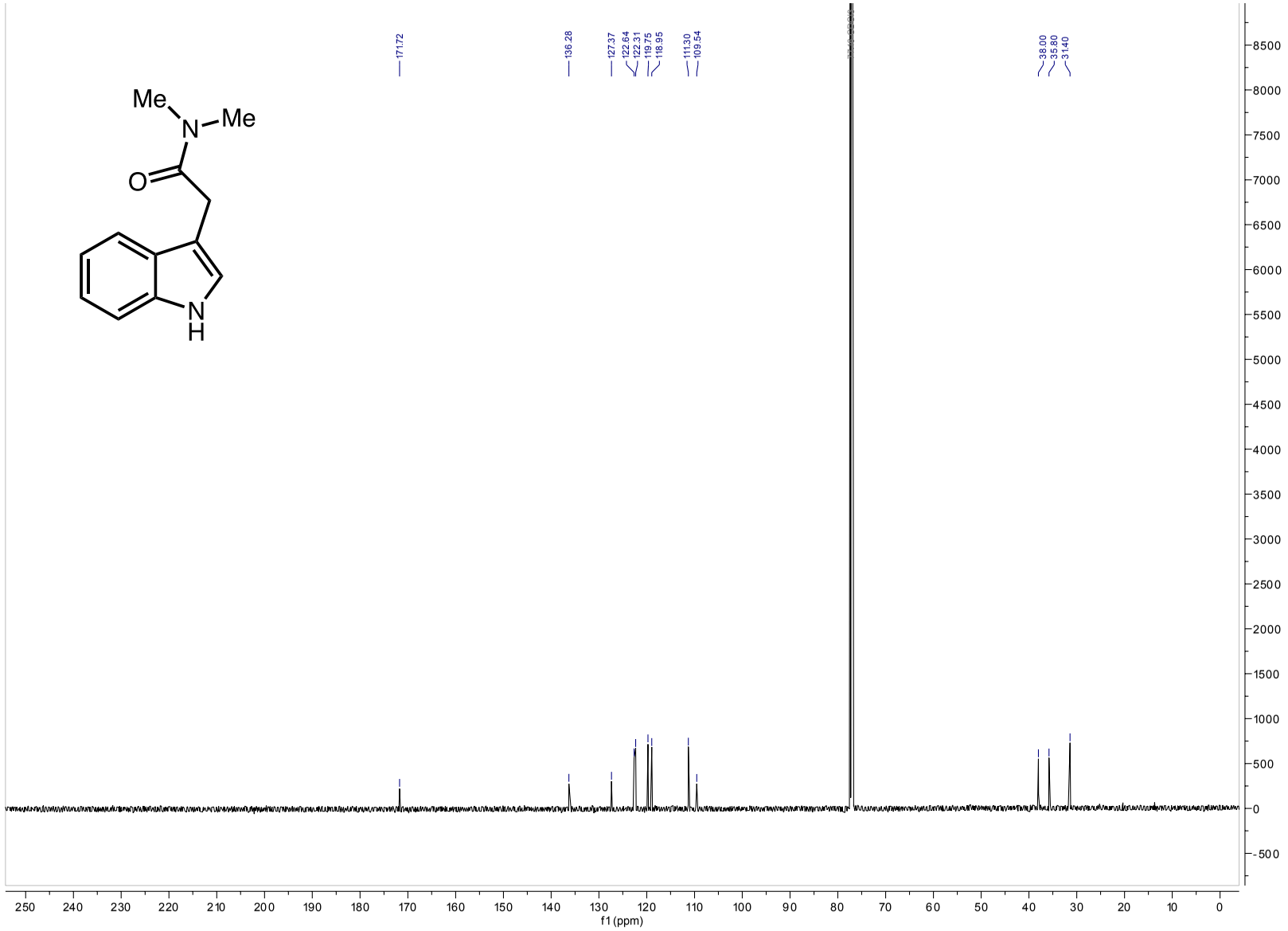
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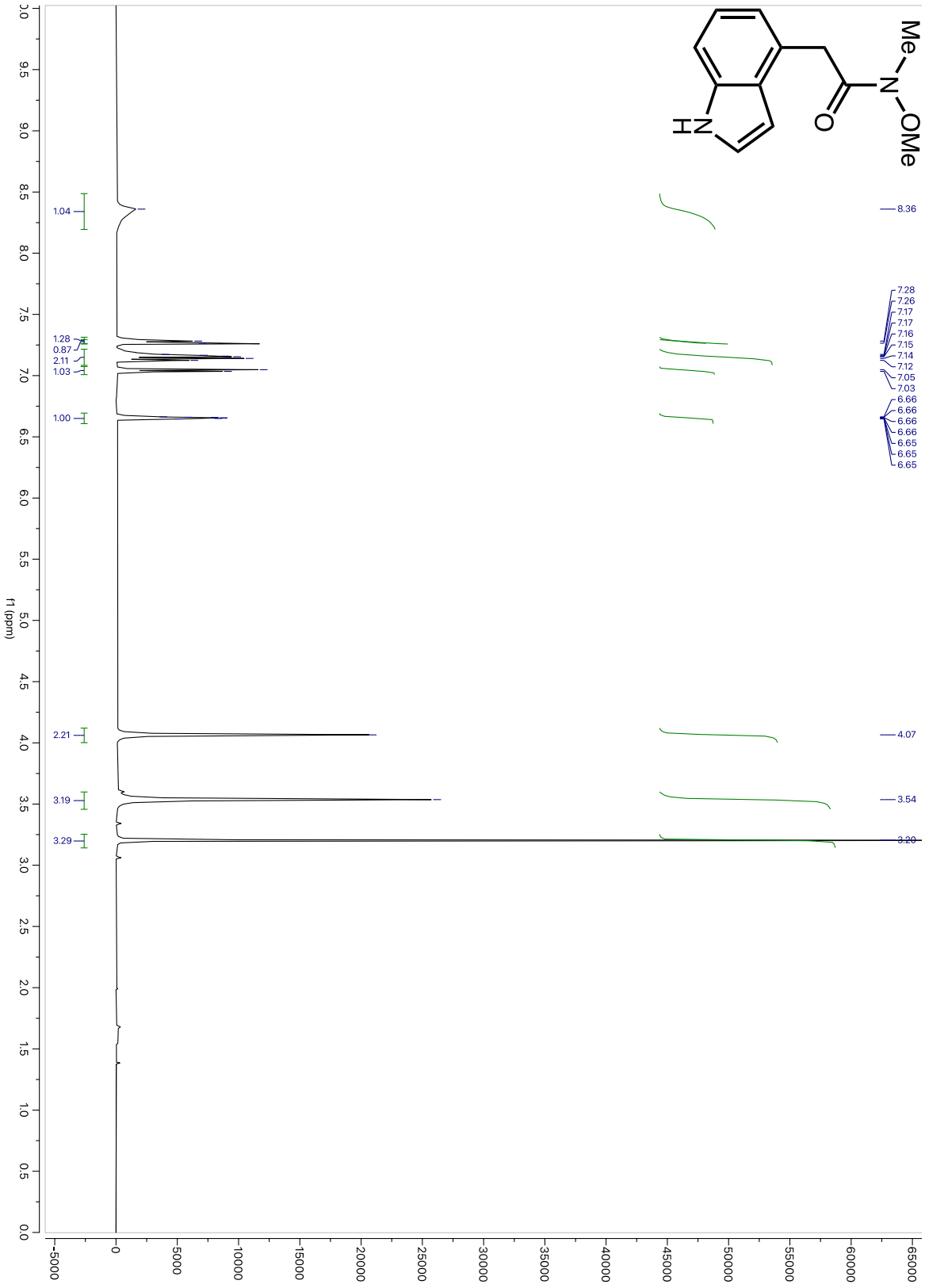
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7.17
7.16
7.15
7.14
7.12
7.05
7.03
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6.66
6.66
6.65
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4.07
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3.20
f1 (ppm)

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O
H
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CDCl3
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32.39
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140
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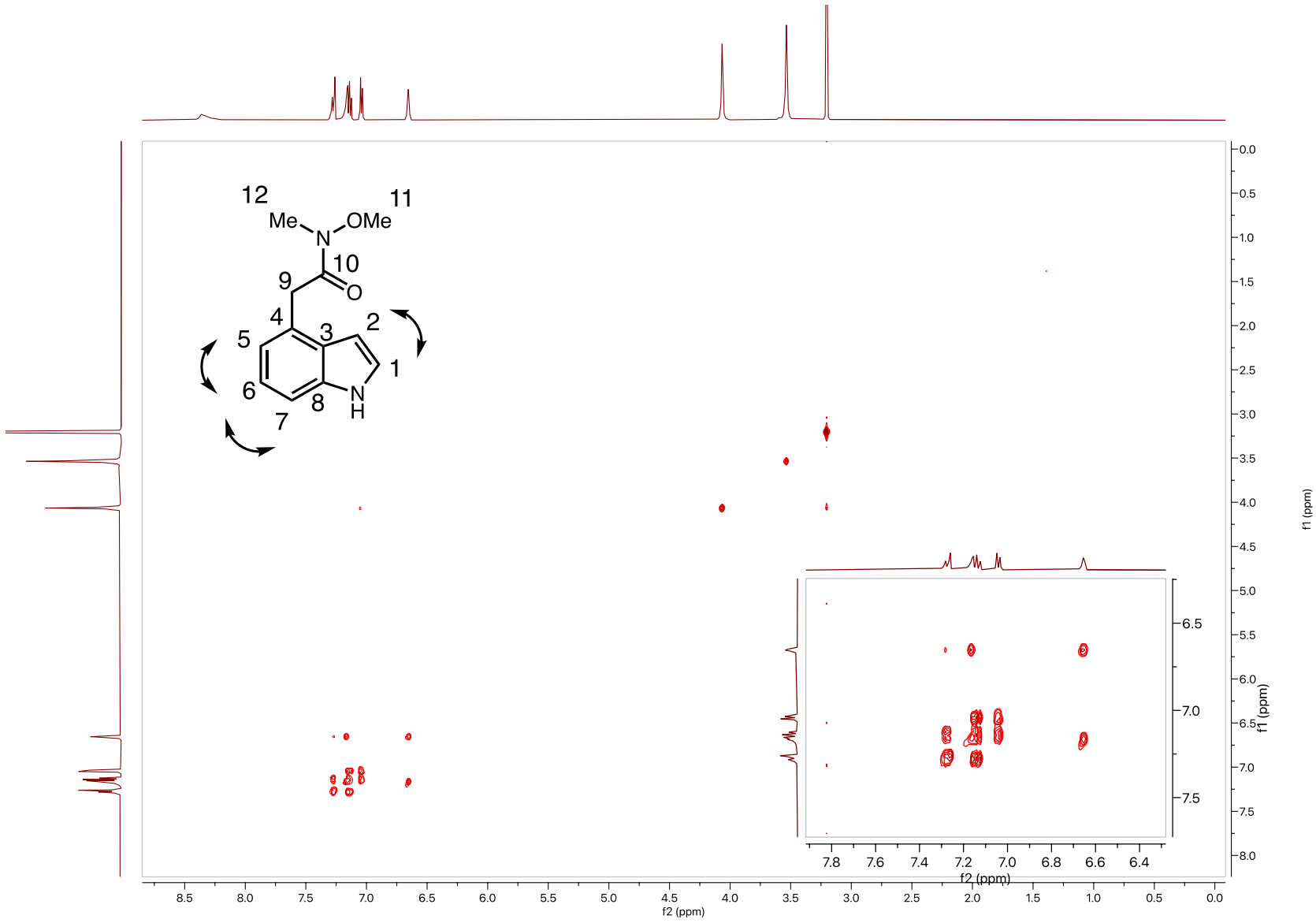
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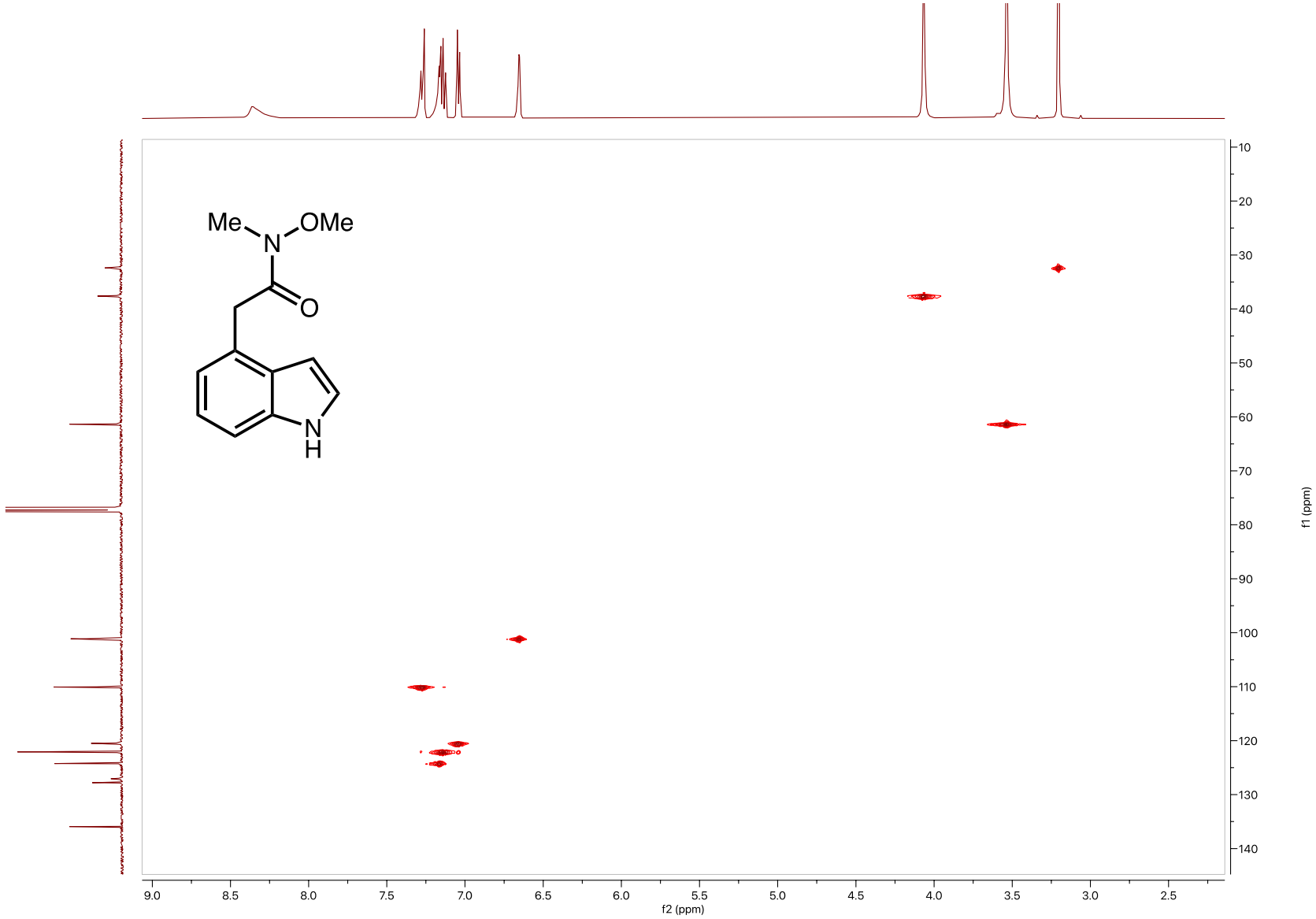
f1 (ppm)
f2 (ppm)
f1 (ppm)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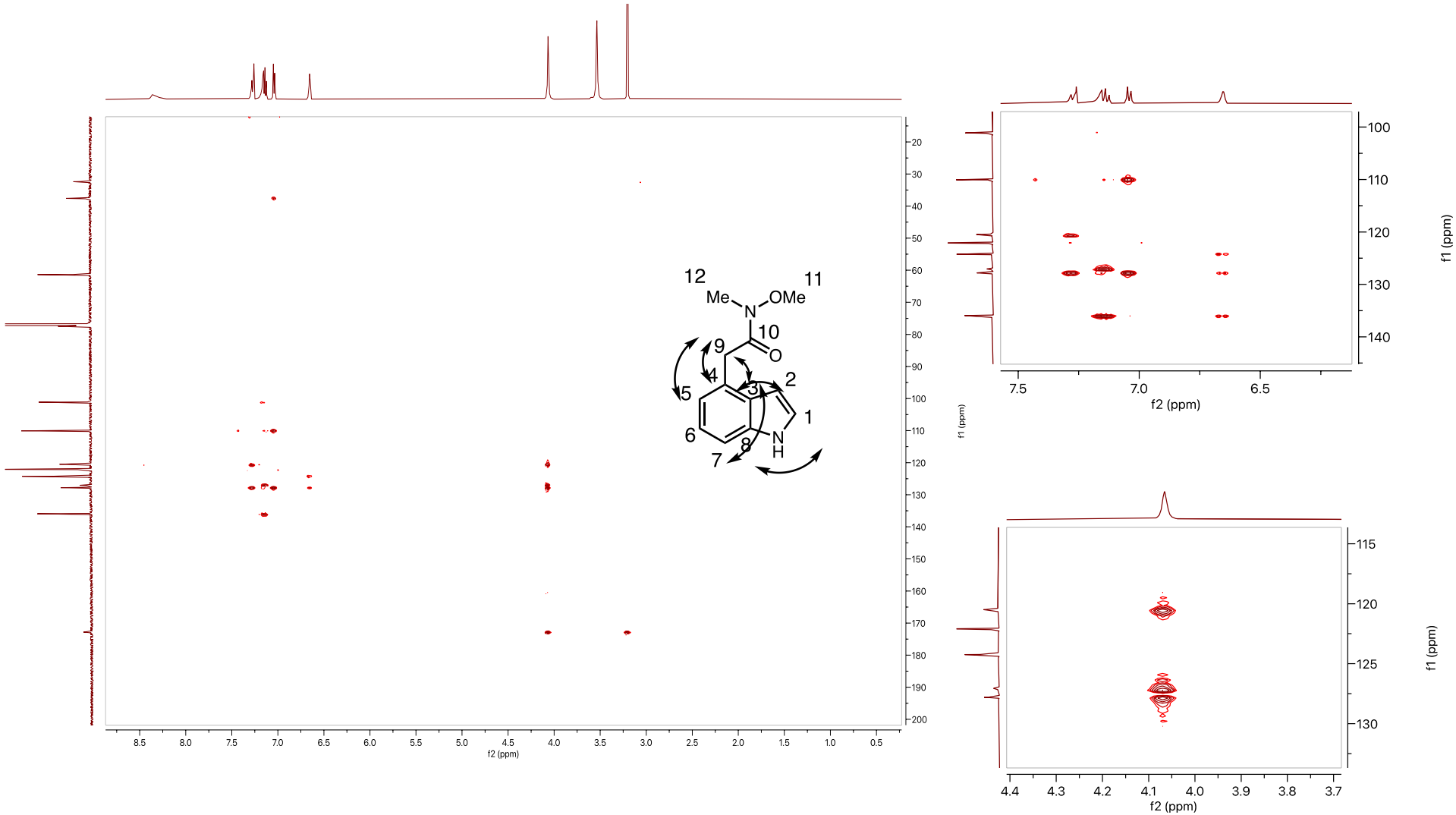
f1 (ppm)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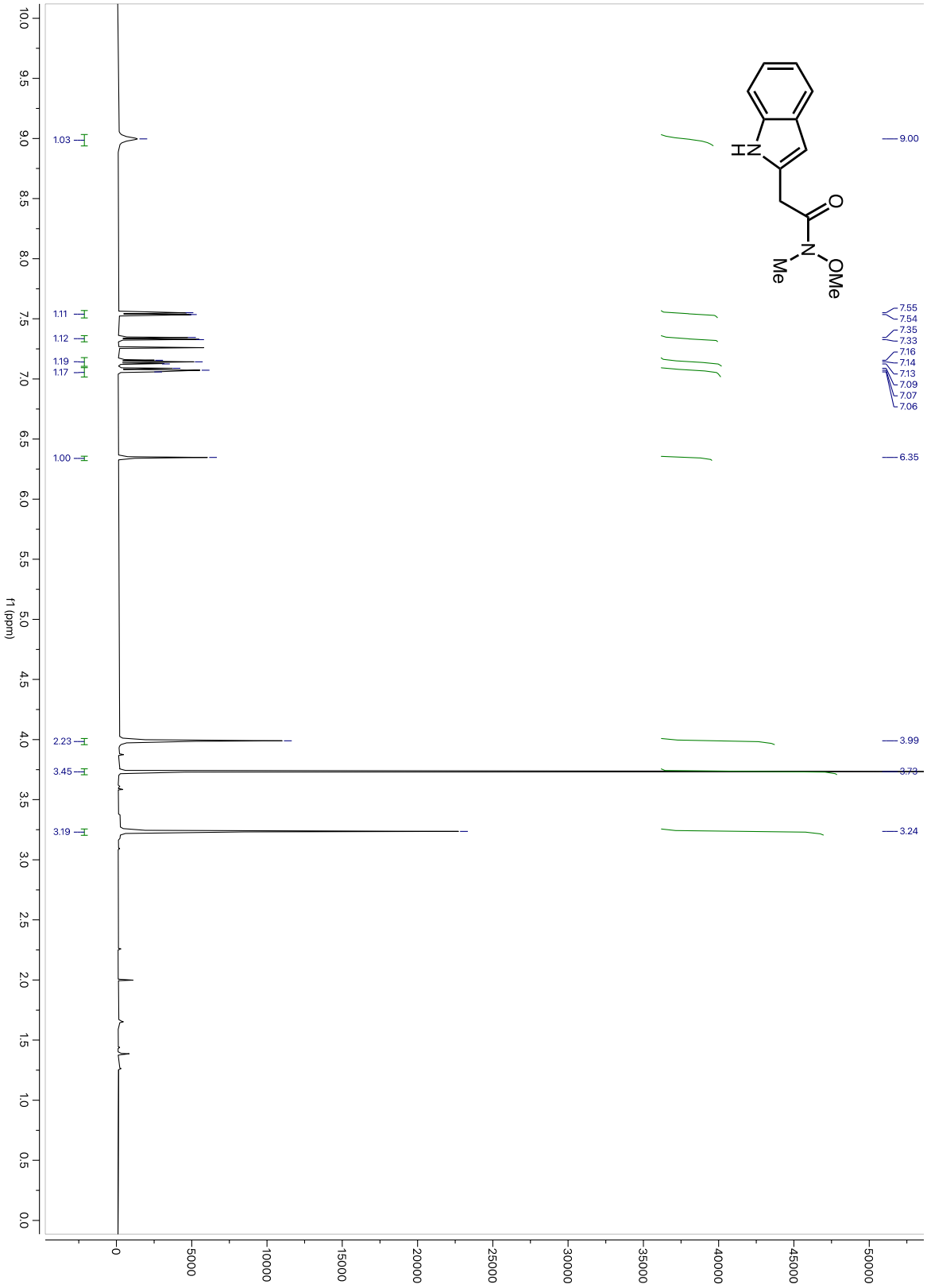
f1 (ppm)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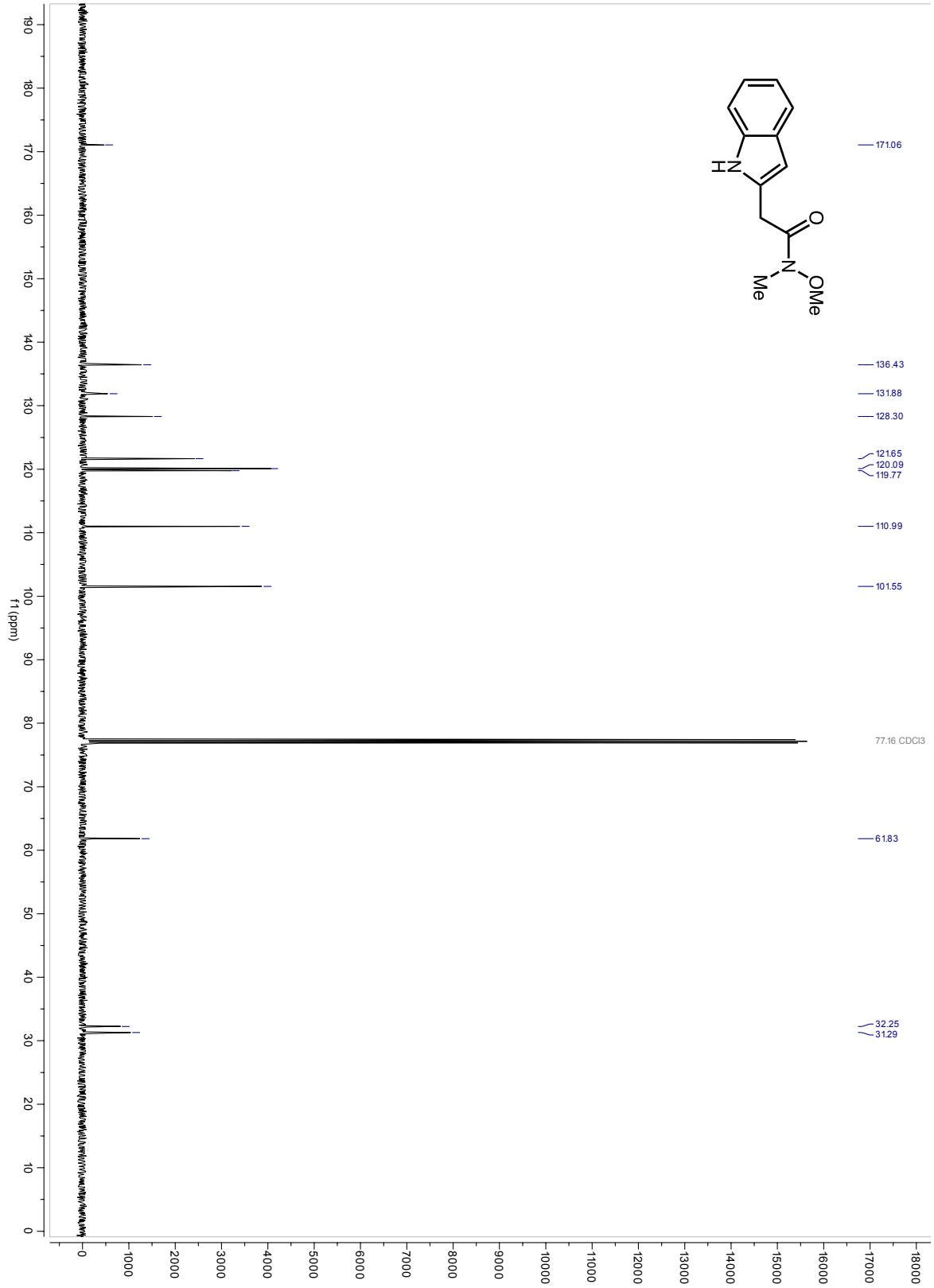
N
Me
OMe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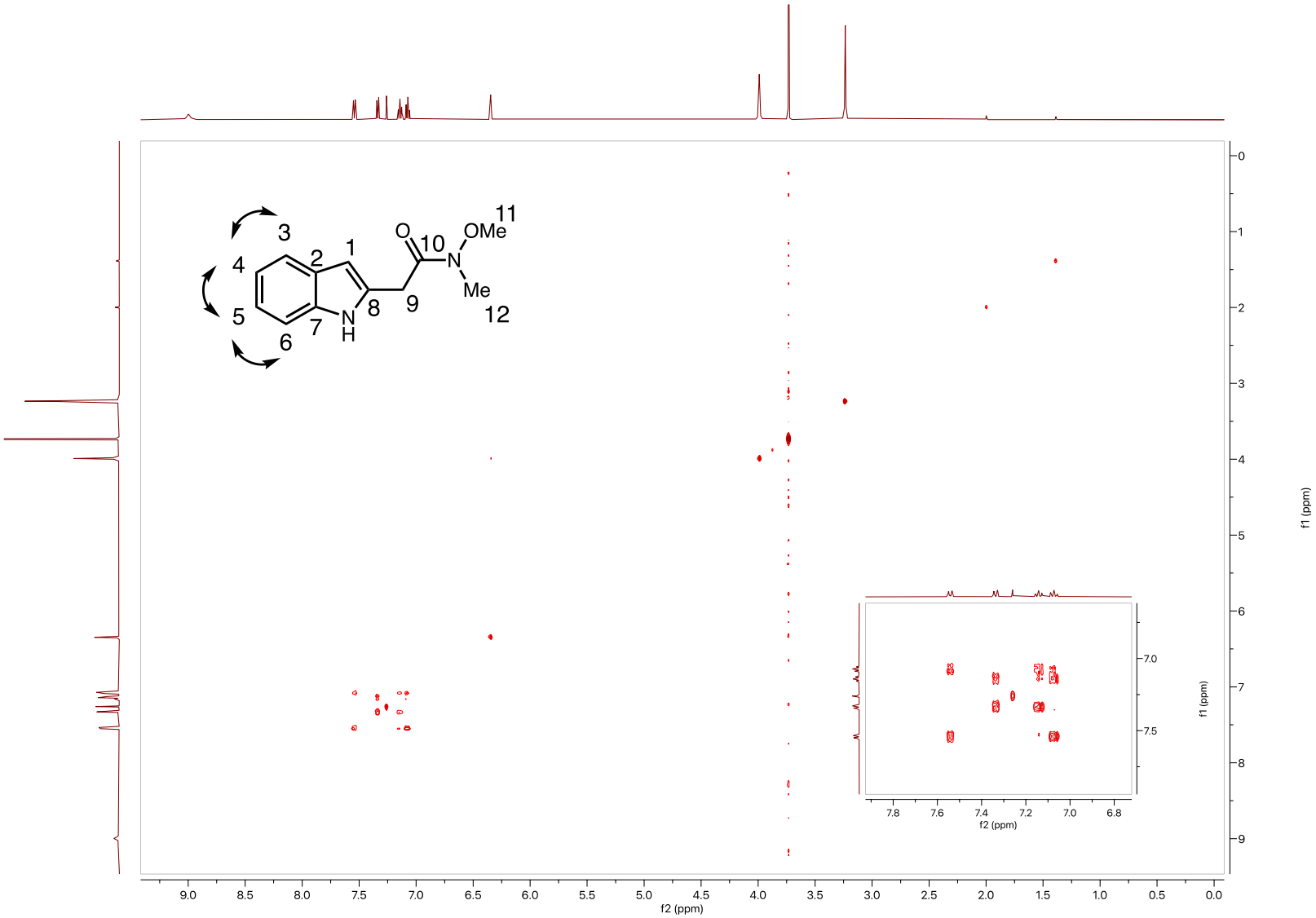
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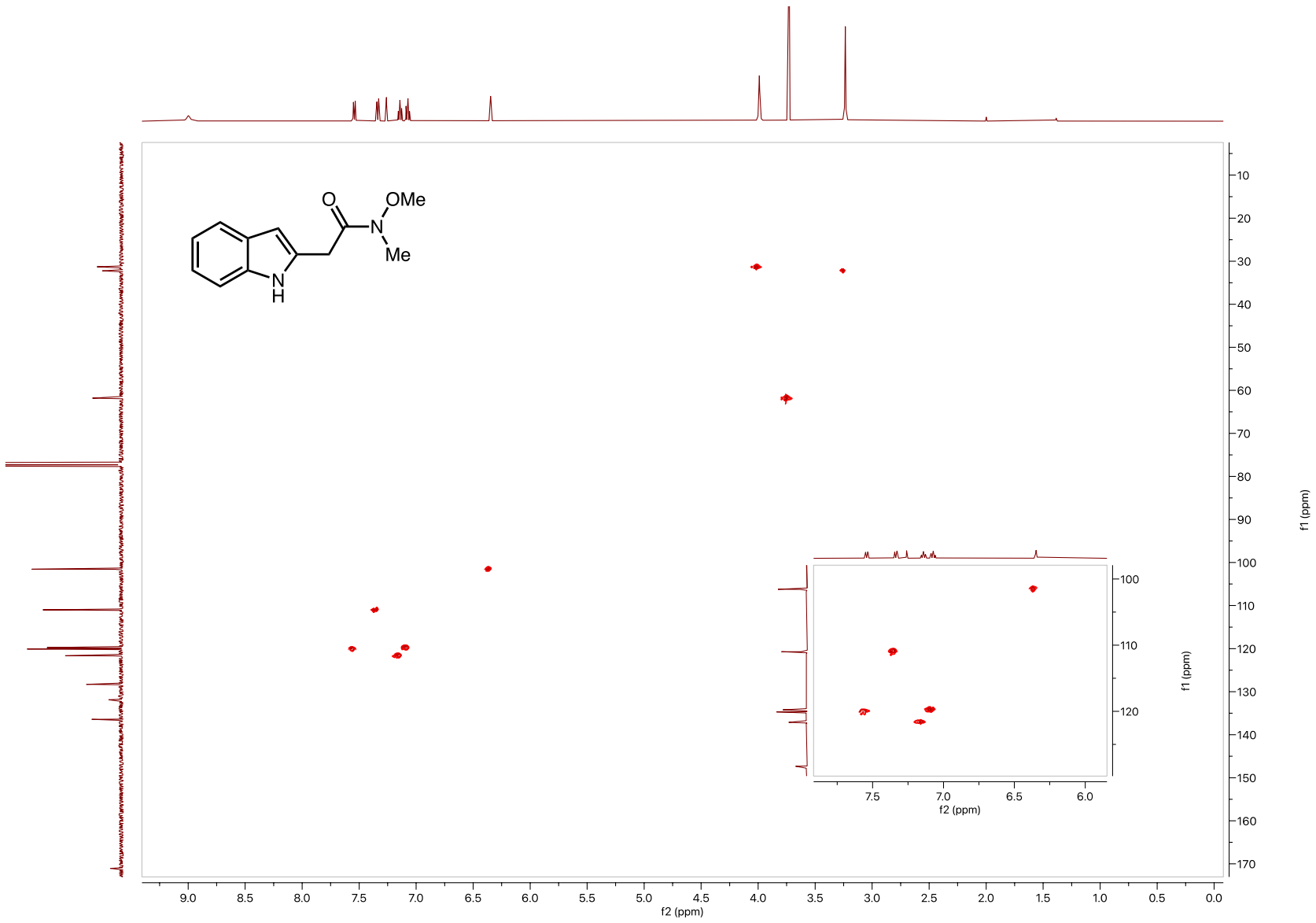
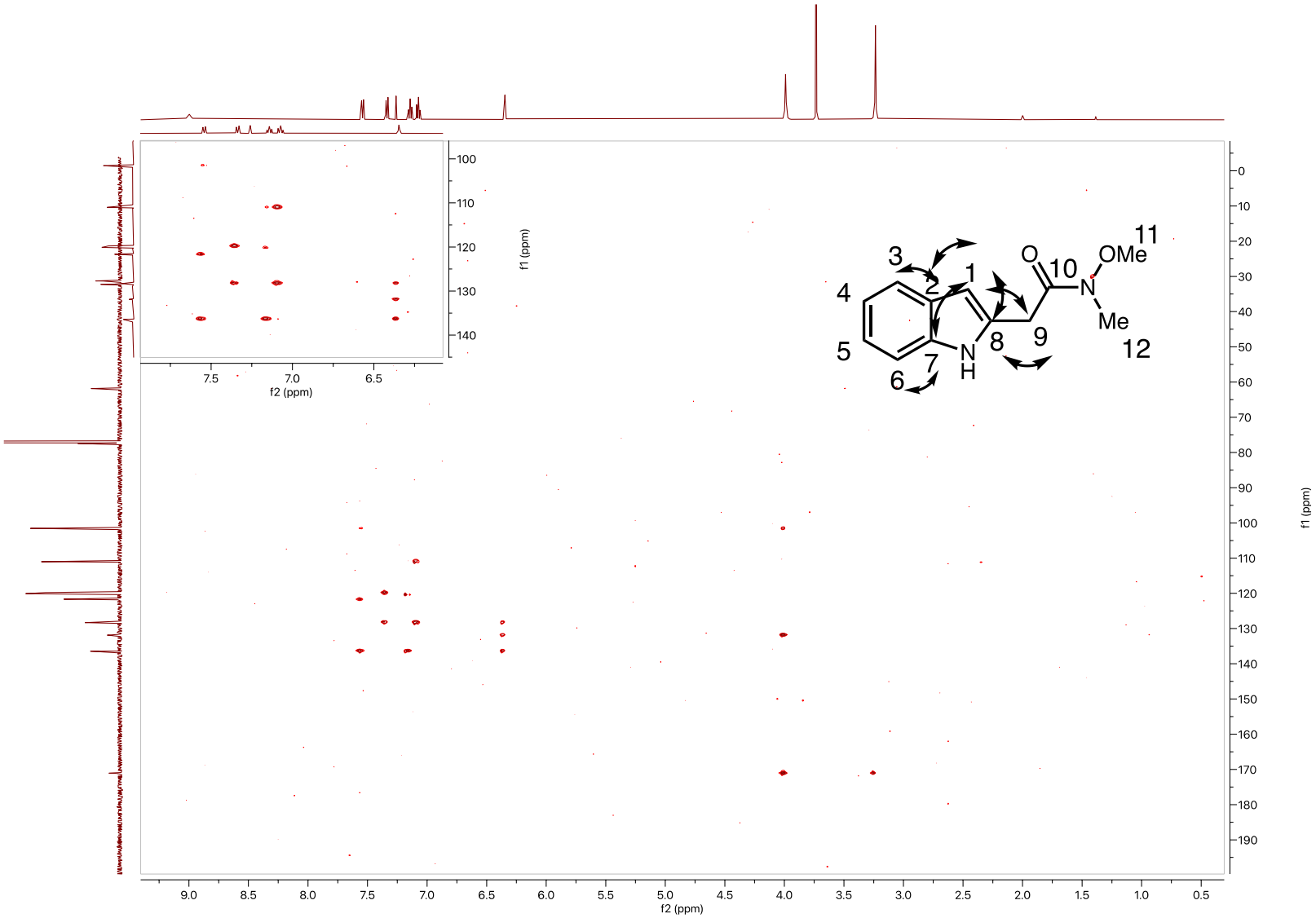
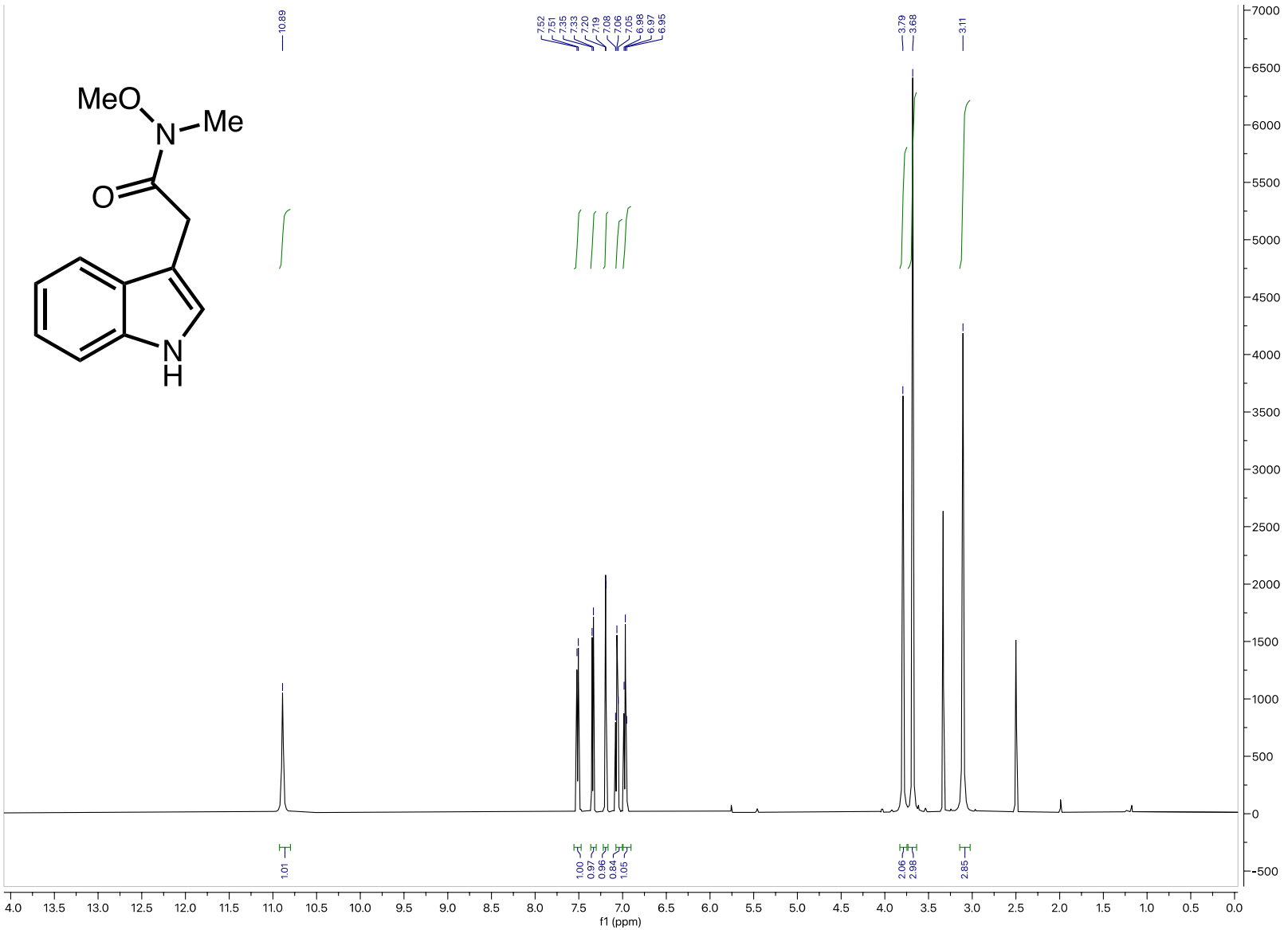
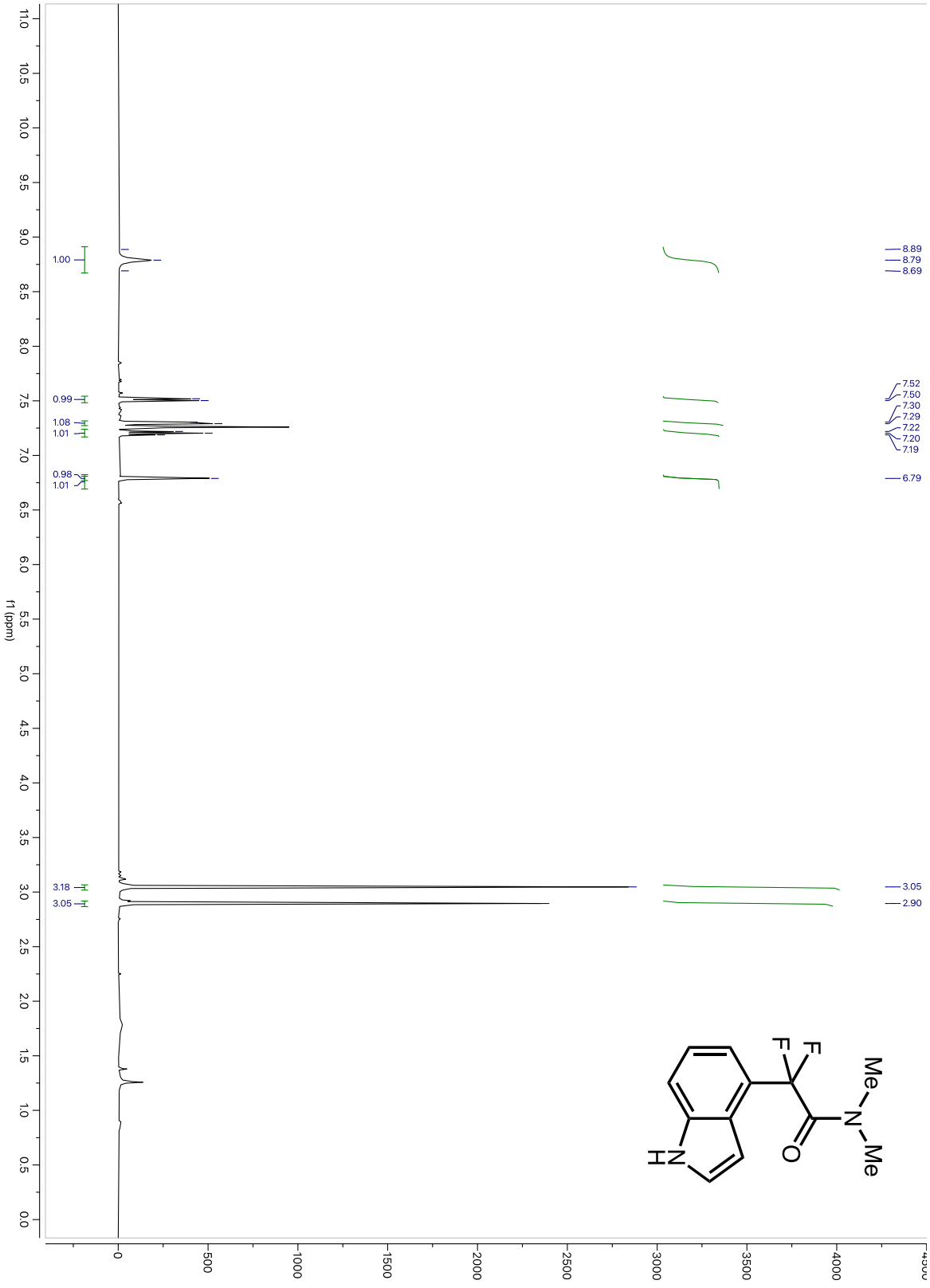
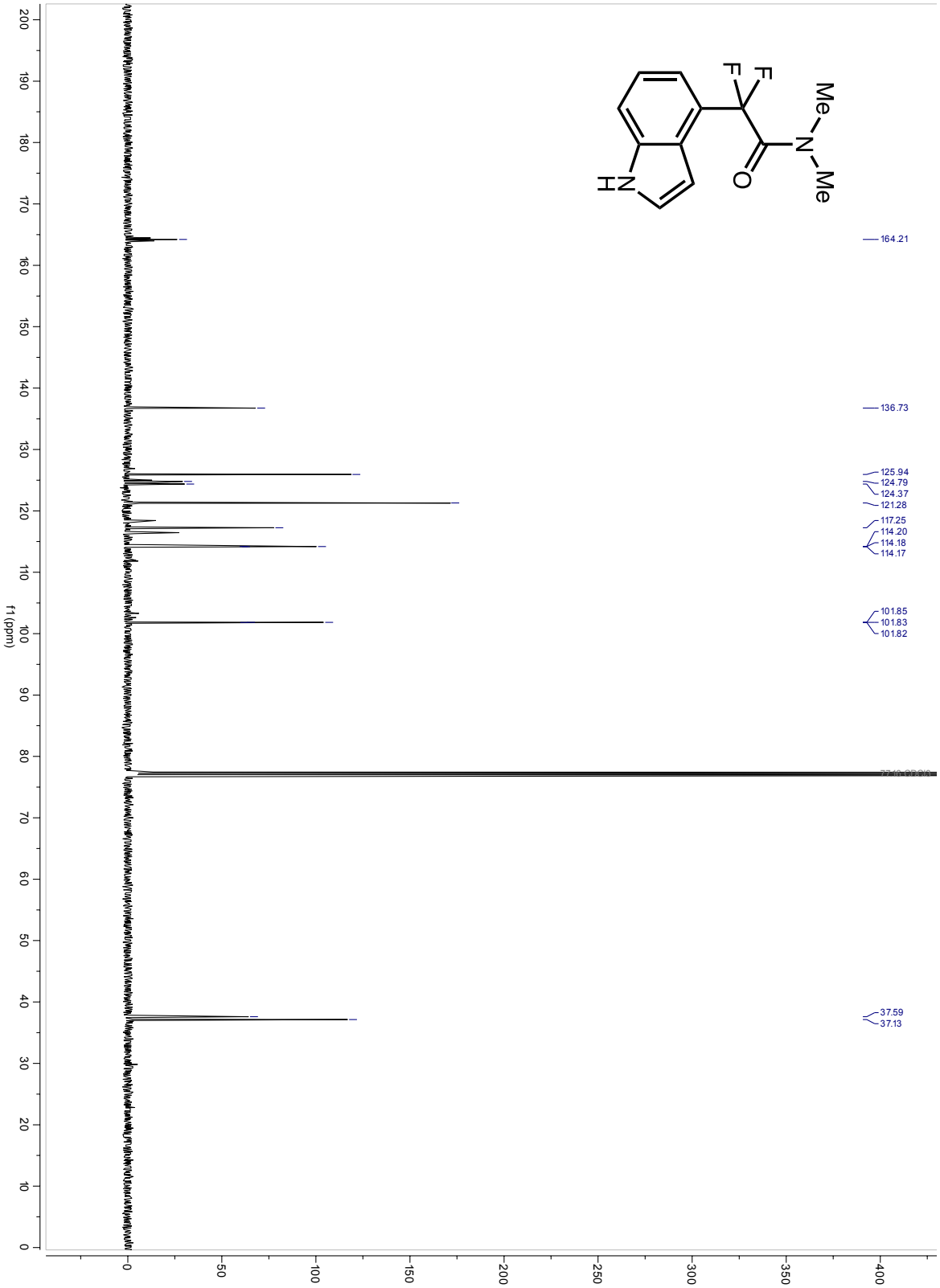
f2 (ppm)
f1 (ppm)
f2 (ppm)
f1 (ppm)

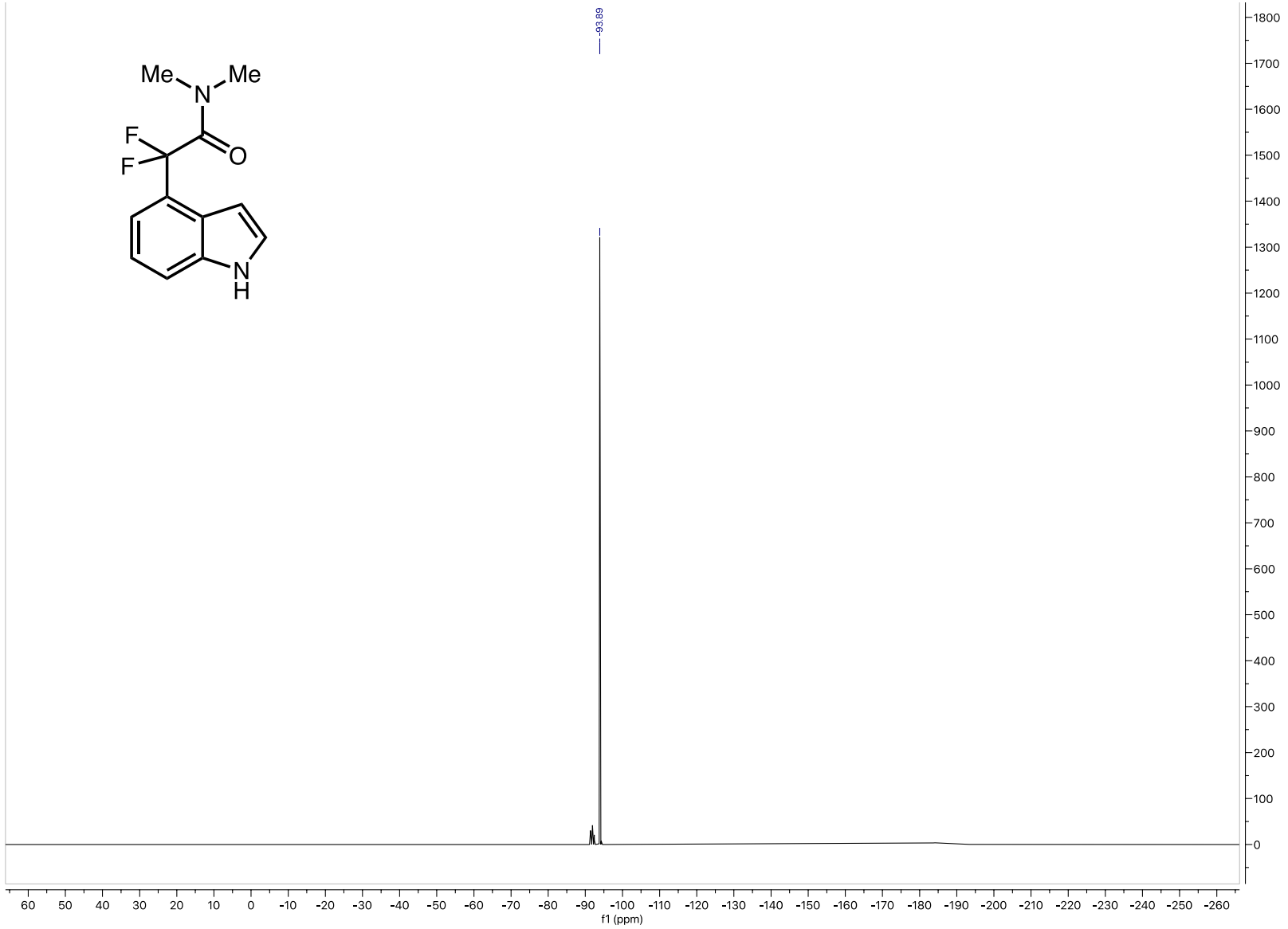

O
OMe
N
Me
N
H
f2 (ppm)
f1 (ppm)
f2 (ppm)
f1 (ppm)

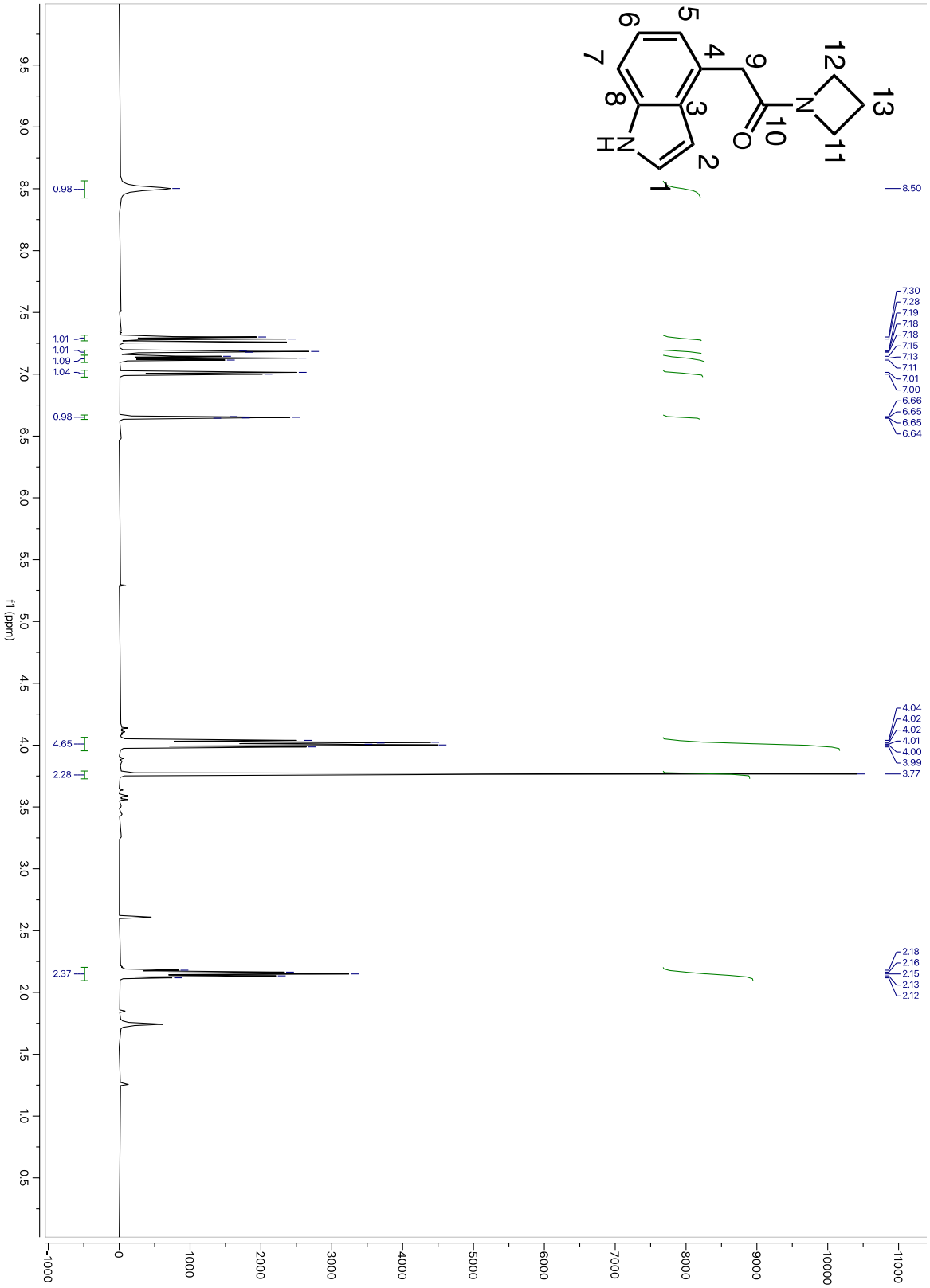

f2 (ppm)
f1 (ppm)
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
100
110
120
130
140
7.5
7.0
6.5
f2 (ppm)
f1 (ppm)
3
2
1
4
5
6
7
N
H
8
9
O
10
N
OMe
11
Me
12

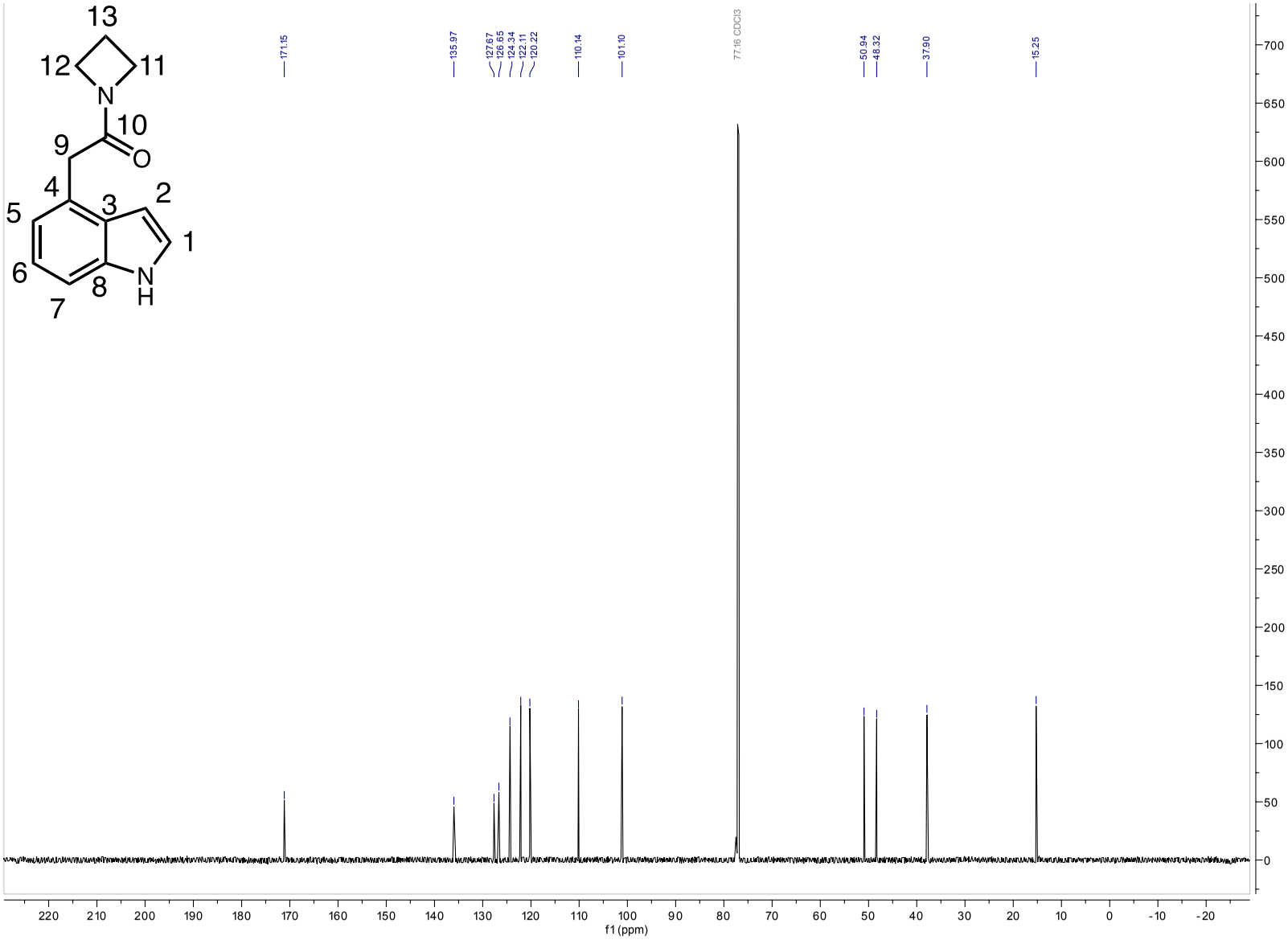

MeO
N
Me
O
N
H
10.89
7.52
7.51
7.35
7.33
7.20
7.19
7.08
7.06
7.05
6.98
6.97
6.95
3.79
3.68
3.11
1.01
1.00
0.97
0.96
0.84
1.05
2.06
2.98
2.85
f1 (ppm)

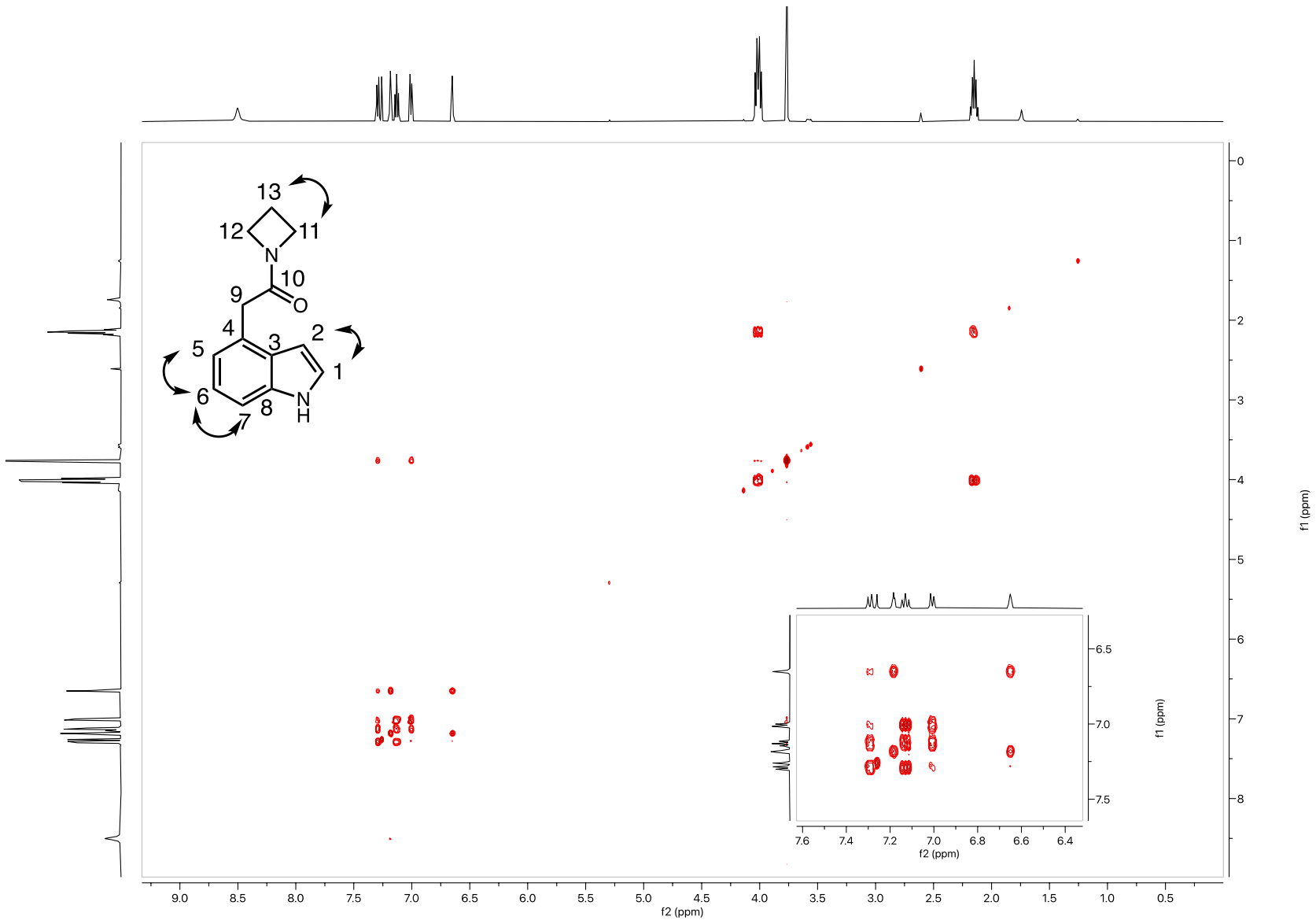

f1 (ppm)
1.00
0.99
1.08
1.01
0.98
1.01
3.18
3.05
8.89
8.79
8.69
7.52
7.50
7.30
7.29
7.22
7.20
7.19
6.79
3.05
2.90
F
F
Me
N
Me
O
H
N

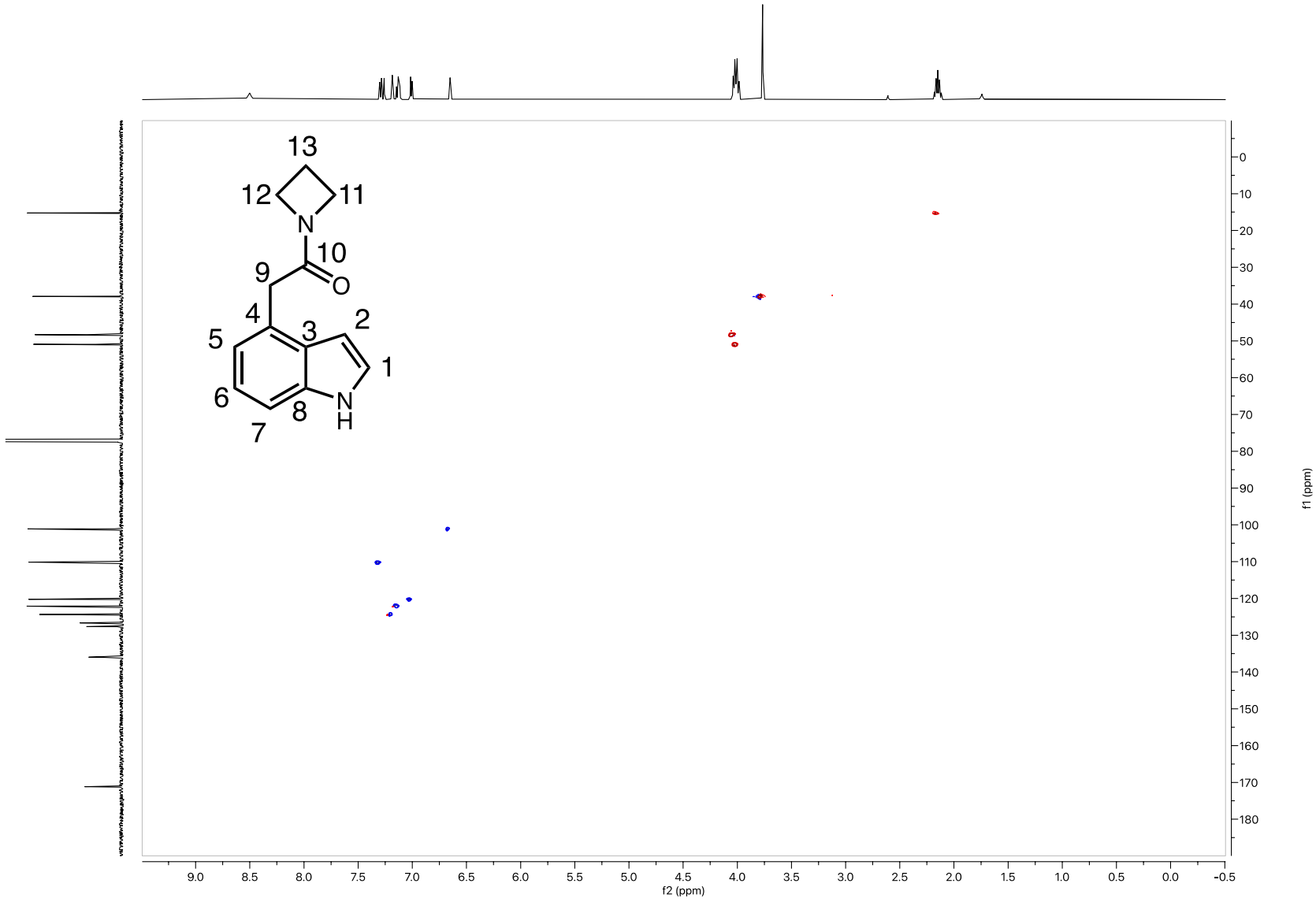

F
F
Me
N
Me
O
H
N
164.21
136.73
125.94
124.79
124.37
121.28
117.25
114.20
114.18
114.17
101.85
101.83
101.82
37.59
37.13
f1 (ppm)

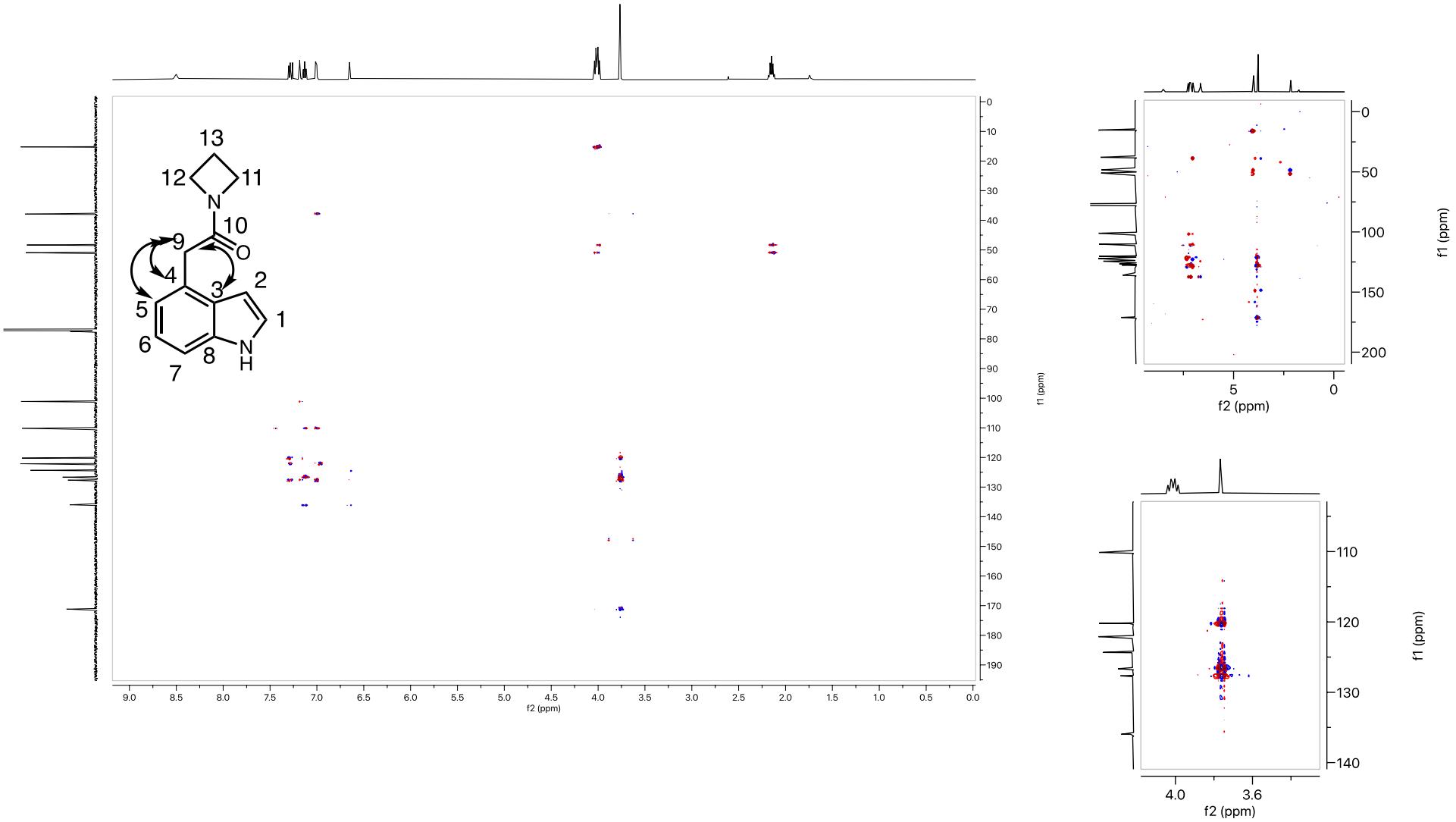

-93.89
Me
N
Me
F
F
O
N
H
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
60 50 40 30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210 -220 -230 -240 -250 -260
f1 (ppm)

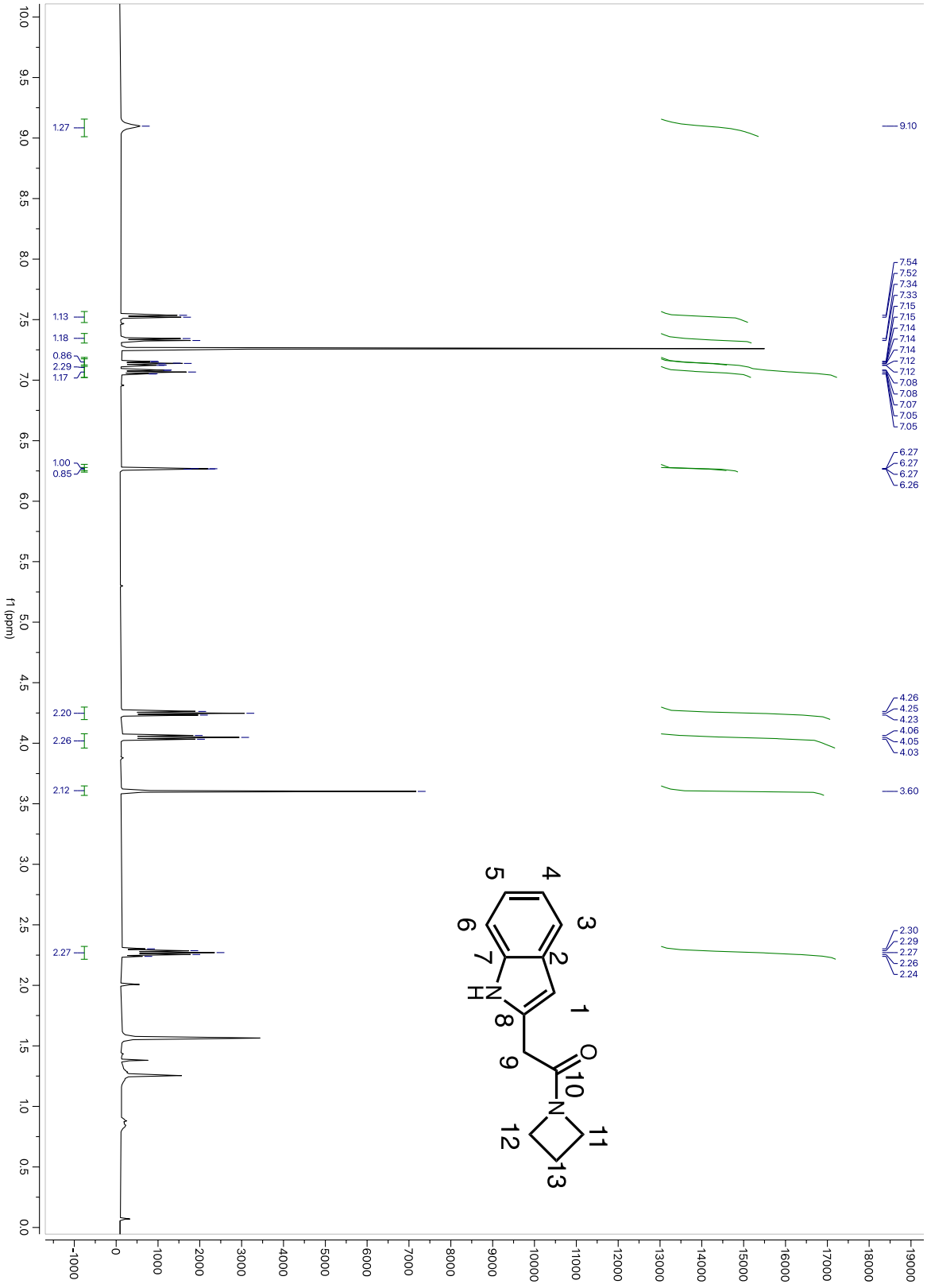

f1 (ppm)
0.98
1.01
1.01
1.09
1.04
0.98
4.65
2.28
2.37
8.50
7.30
7.28
7.19
7.18
7.18
7.15
7.13
7.11
7.01
7.00
6.66
6.65
6.65
6.64
4.04
4.02
4.02
4.01
4.00
3.99
3.77
2.18
2.16
2.15
2.13
2.12

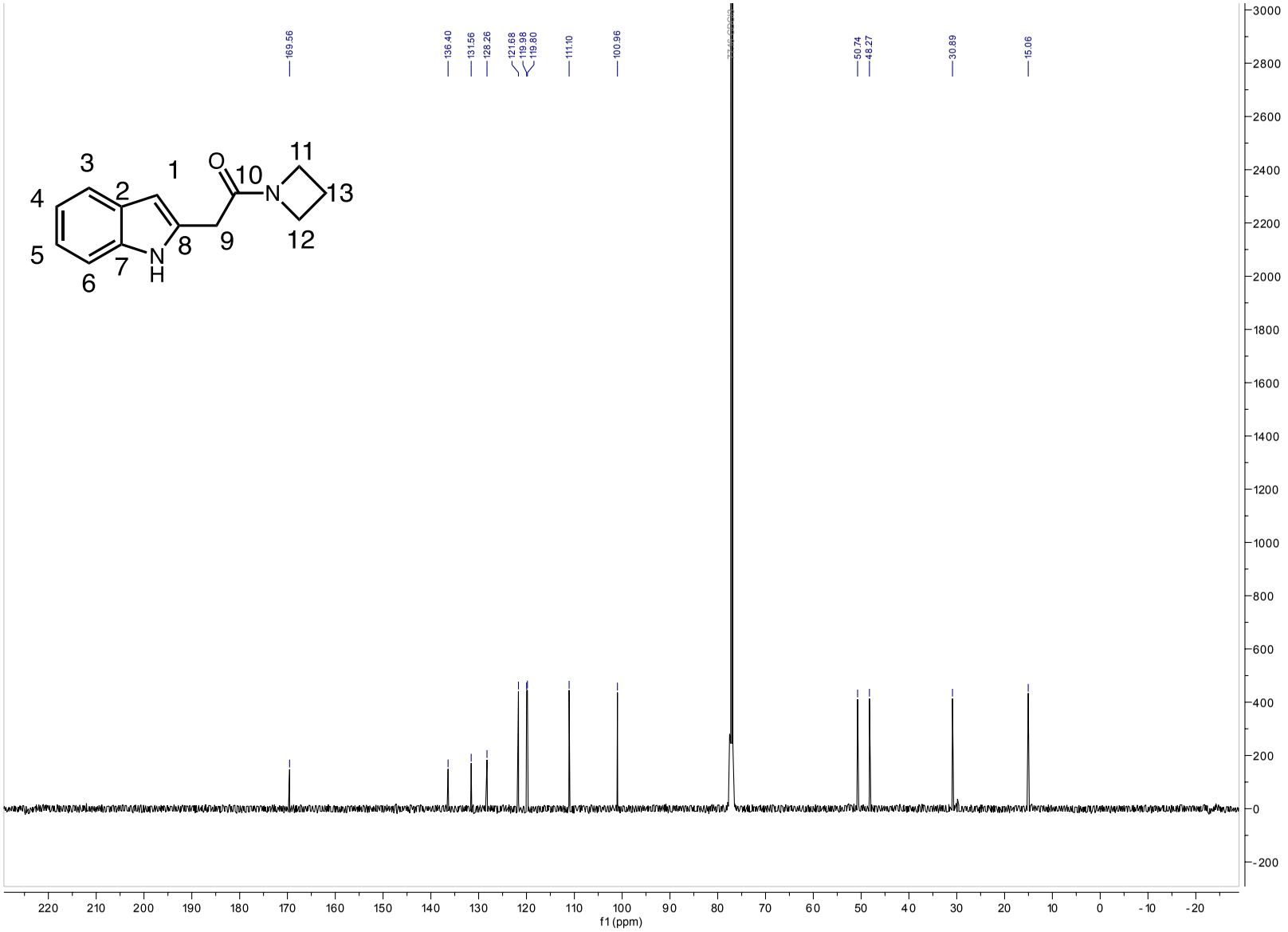

13
12
11
N
10
9
O
4
3
2
5
1
6
8
N
H
7
171.15
135.97
127.67
126.65
124.34
122.11
120.22
110.14
101.10
77.16 CDCl3
50.94
48.32
37.90
15.25
700
650
600
550
500
450
400
350
300
250
200
150
100
50
0
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
f1 (ppm)

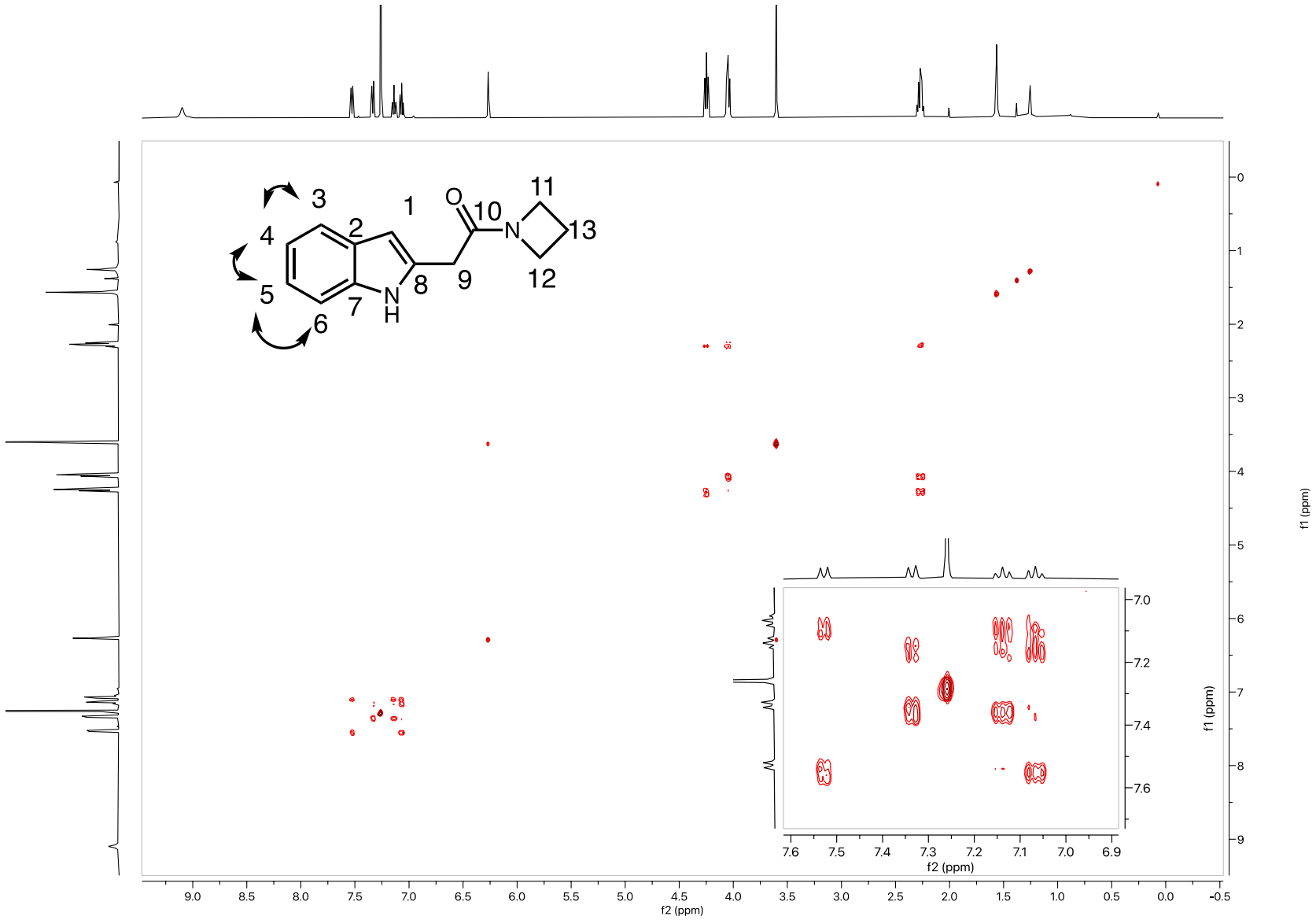

13
12
11
N
10
9
O
4
3
2
5
1
6
8
N
H
7
f2 (ppm)
f1 (ppm)
f2 (ppm)
f1 (ppm)

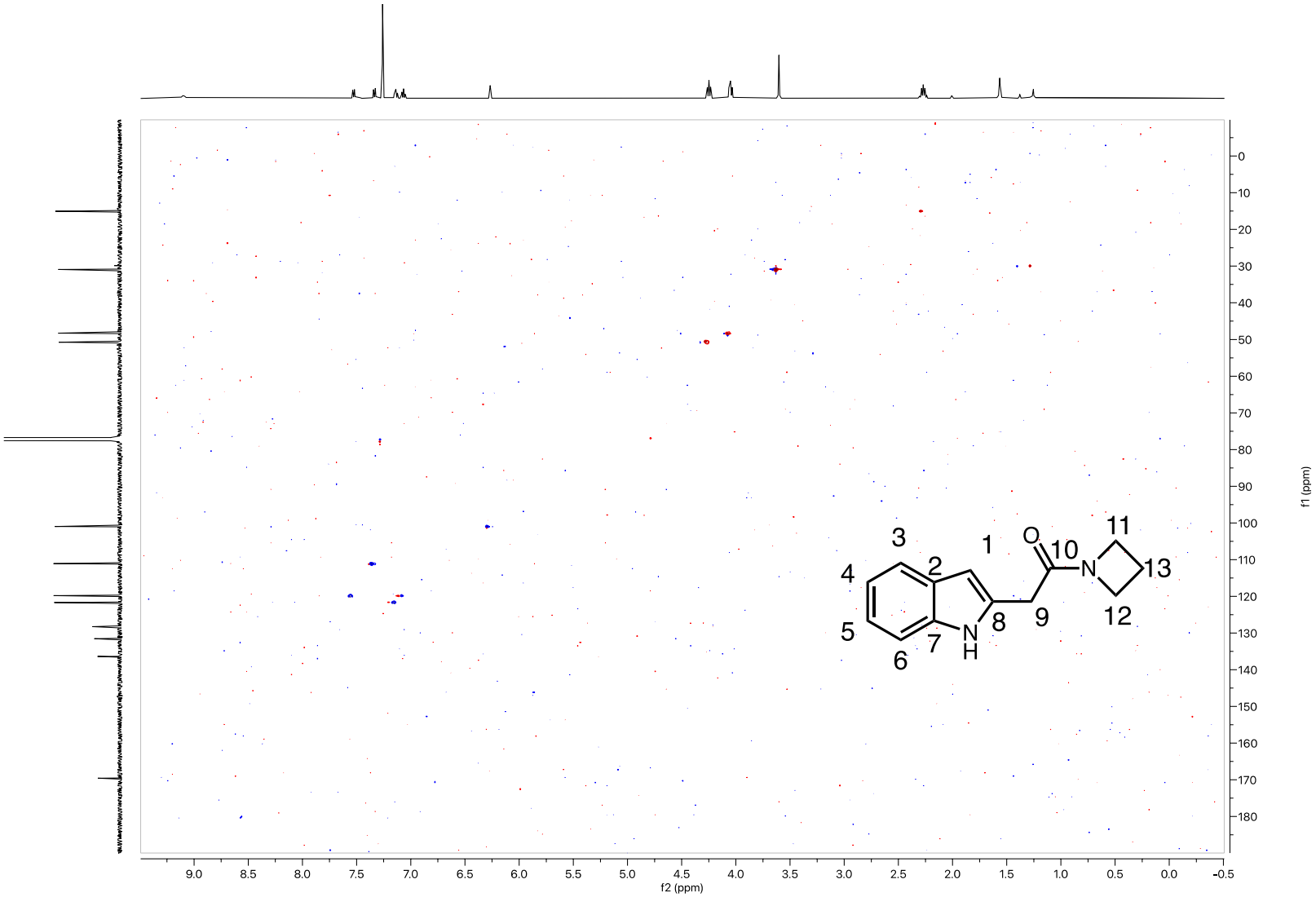

13
12
11
N
10
9
O
4
3
2
5
1
6
8
N
H
7
f2 (ppm)
f1 (ppm)

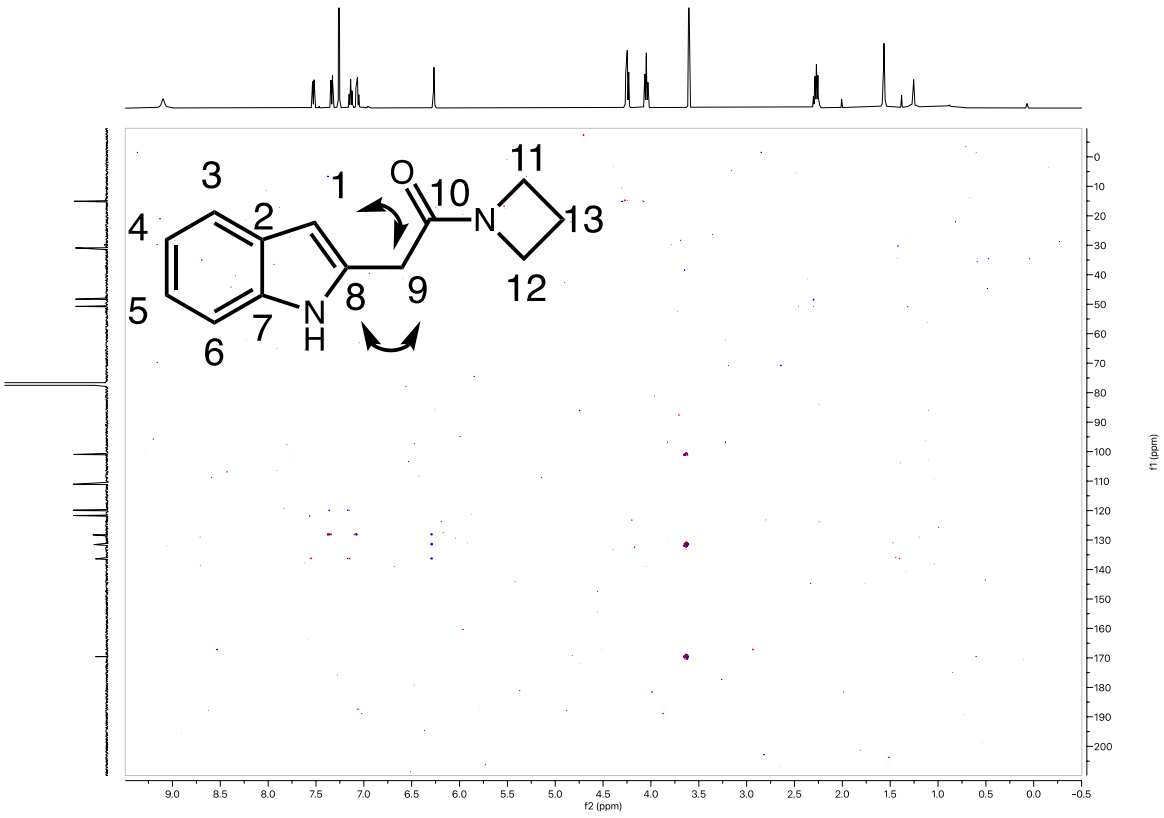

13
12
11
N
10
O
9
4
3
2
1
5
6
8
N
H
7
f2 (ppm)
f1 (ppm)
f2 (ppm)
f1 (ppm)
f2 (ppm)
f1 (ppm)

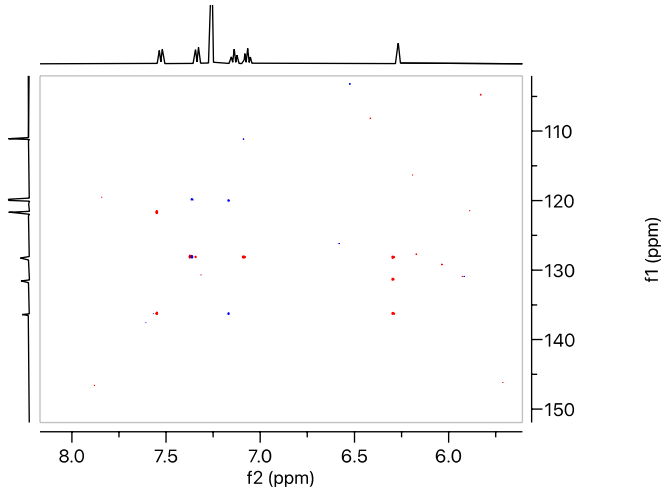

f1 (ppm)
1.27
1.13
1.18
0.86
2.29
1.17
1.00
0.85
2.20
2.26
2.12
2.27
9.10
7.54
7.52
7.34
7.33
7.15
7.15
7.14
7.14
7.14
7.12
7.12
7.08
7.08
7.07
7.05
7.05
6.27
6.27
6.27
6.26
4.26
4.25
4.23
4.06
4.05
4.03
3.60
2.30
2.29
2.27
2.26
2.24
O
N
H
N

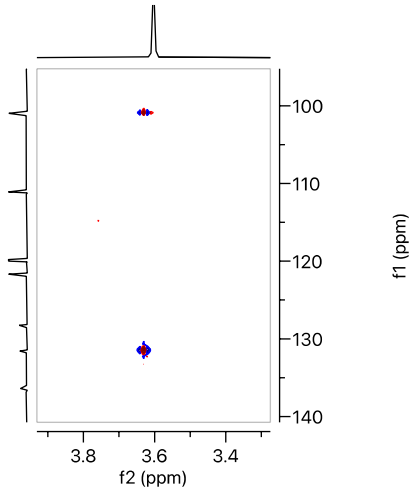

169.56
136.40
131.56
128.26
121.68
119.98
119.80
111.10
100.96
50.74
48.27
30.89
15.06
1
2
3
4
5
6
7
8
9
10
11
12
13
O
N
H
f1 (ppm)

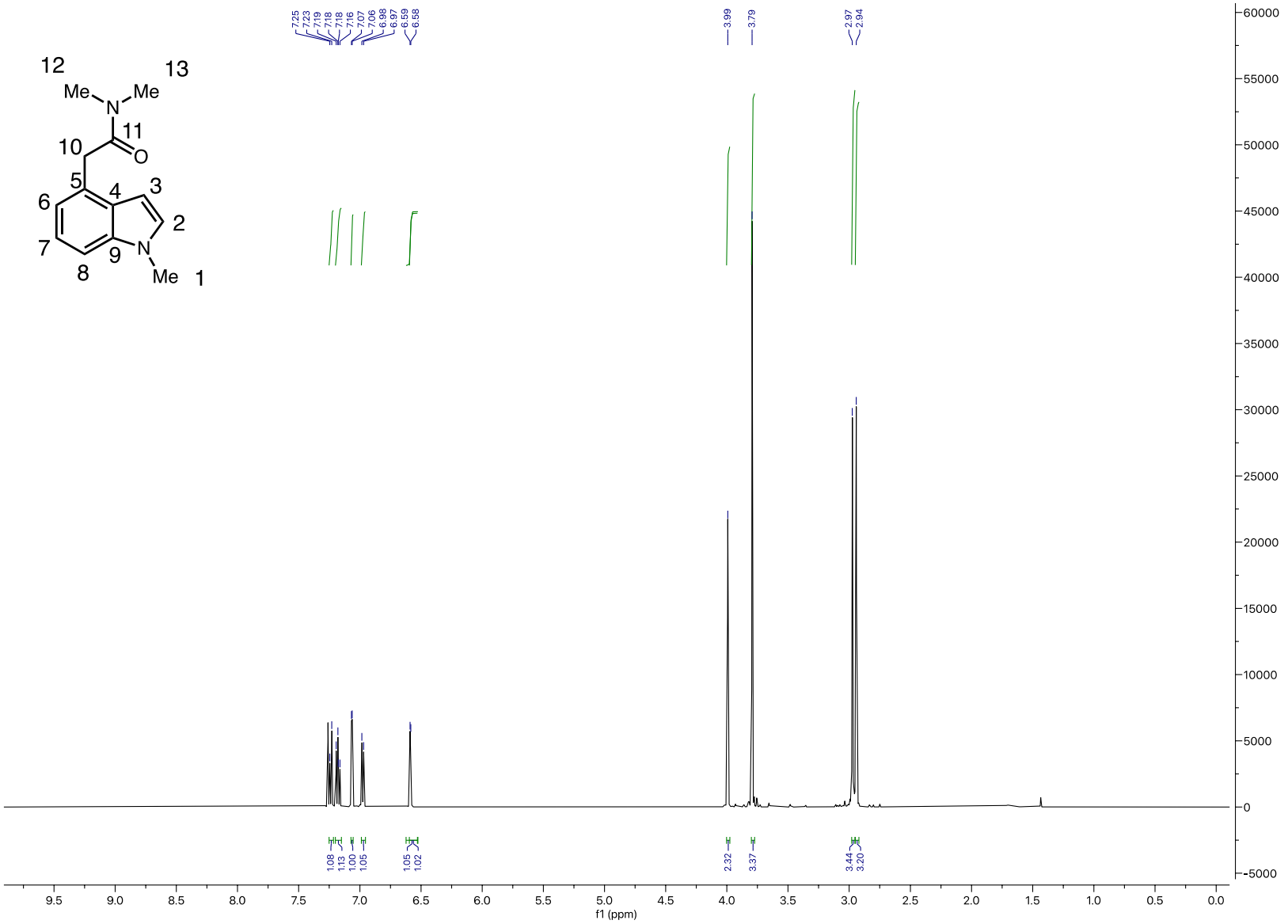

3
4
5
6
2
1
O
10
11
N
13
12
8
9
7
N
H
f2 (ppm)
f1 (ppm)
7.6
7.5
7.4
7.3
7.2
7.1
7.0
6.9
7.0
7.2
7.4
7.6

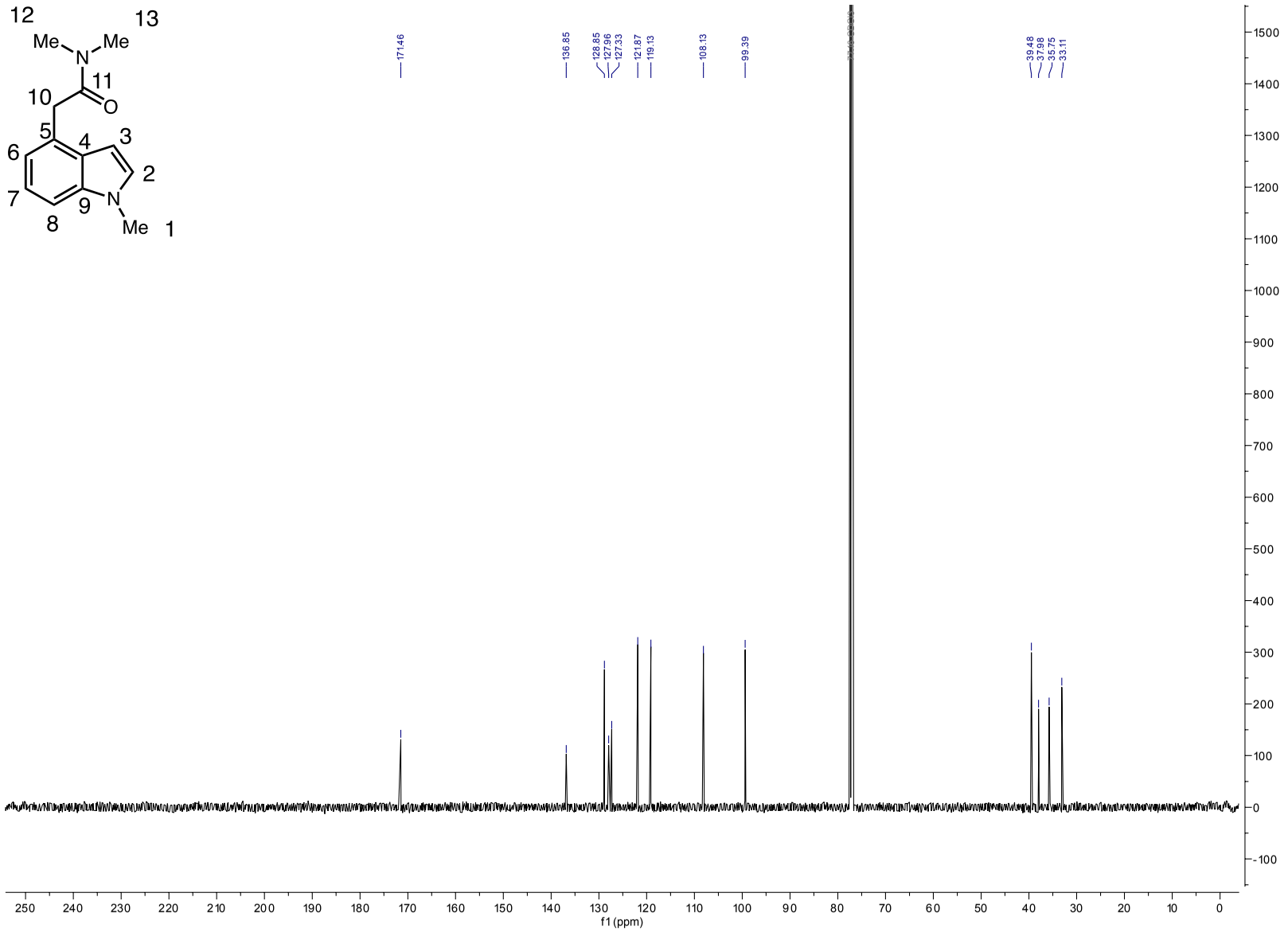

f2 (ppm)
f1 (ppm)
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
1
2
3
4
5
6
7
8
9
10
11
12
13
N
H
O
N

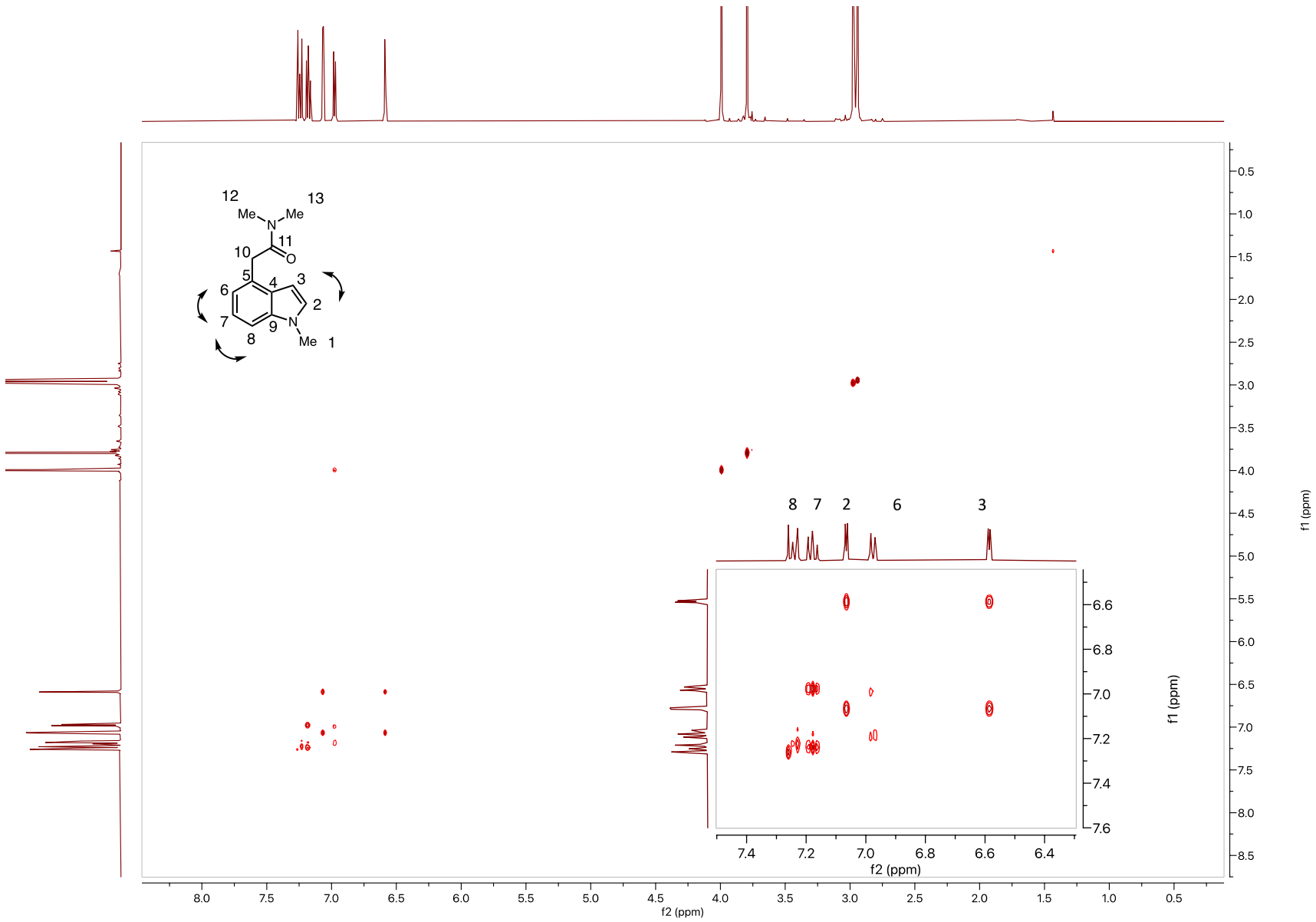

3
4
5
6
2
7
N
H
1
O
8
9
10
N
11
12
13
f2 (ppm)
f1 (ppm)

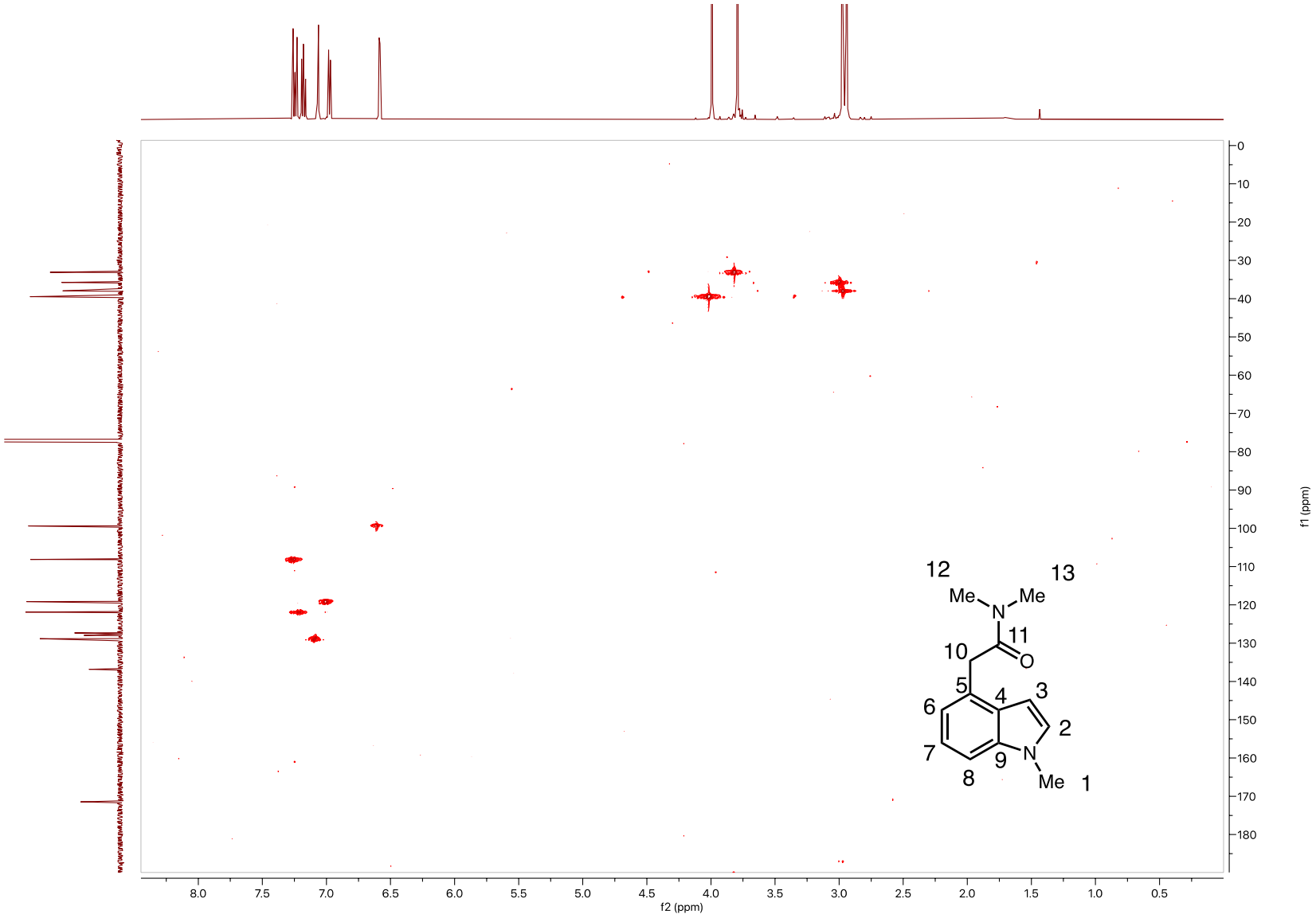

f2 (ppm)
f1 (ppm)

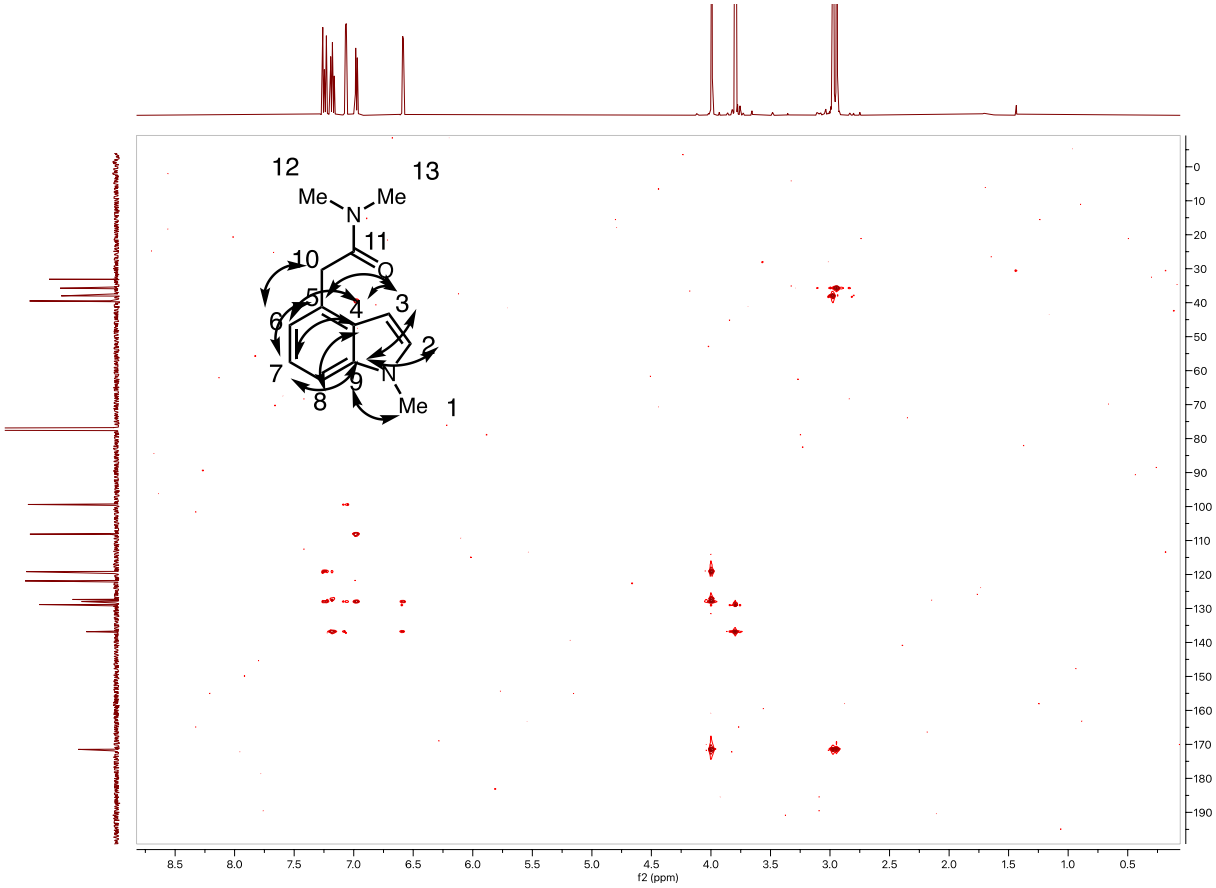

f2 (ppm)
f1 (ppm)

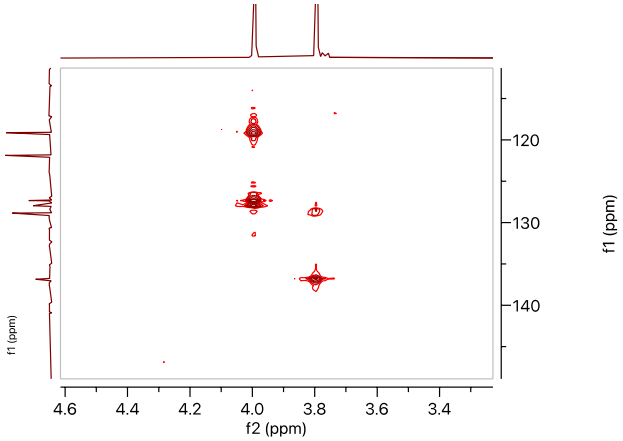

12
13
Me
N
Me
11
10
O
5
4
3
6
2
7
9
N
8
Me
1
7.25
7.23
7.19
7.18
7.18
7.16
7.07
7.06
6.98
6.97
6.59
6.58
3.99
3.79
2.97
2.94
1.08
1.13
1.00
1.05
1.05
1.02
2.32
3.37
3.44
3.20
60000
55000
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
-5000
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

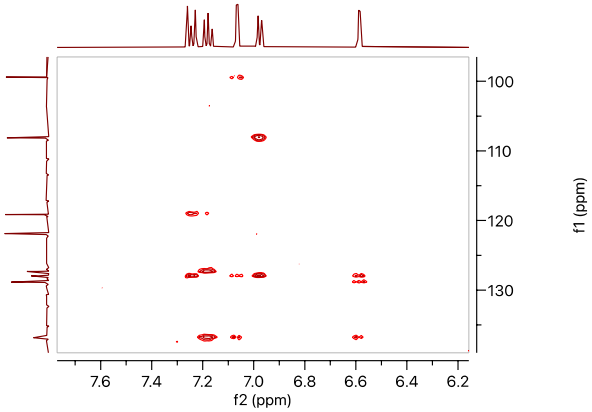

12
13
Me
N
Me
11
10
O
5
4
3
6
2
7
9
N
8
Me
1
171.46
136.85
128.85
127.96
127.33
121.87
119.13
108.13
99.39
39.48
37.98
35.75
33.11
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
250
240
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

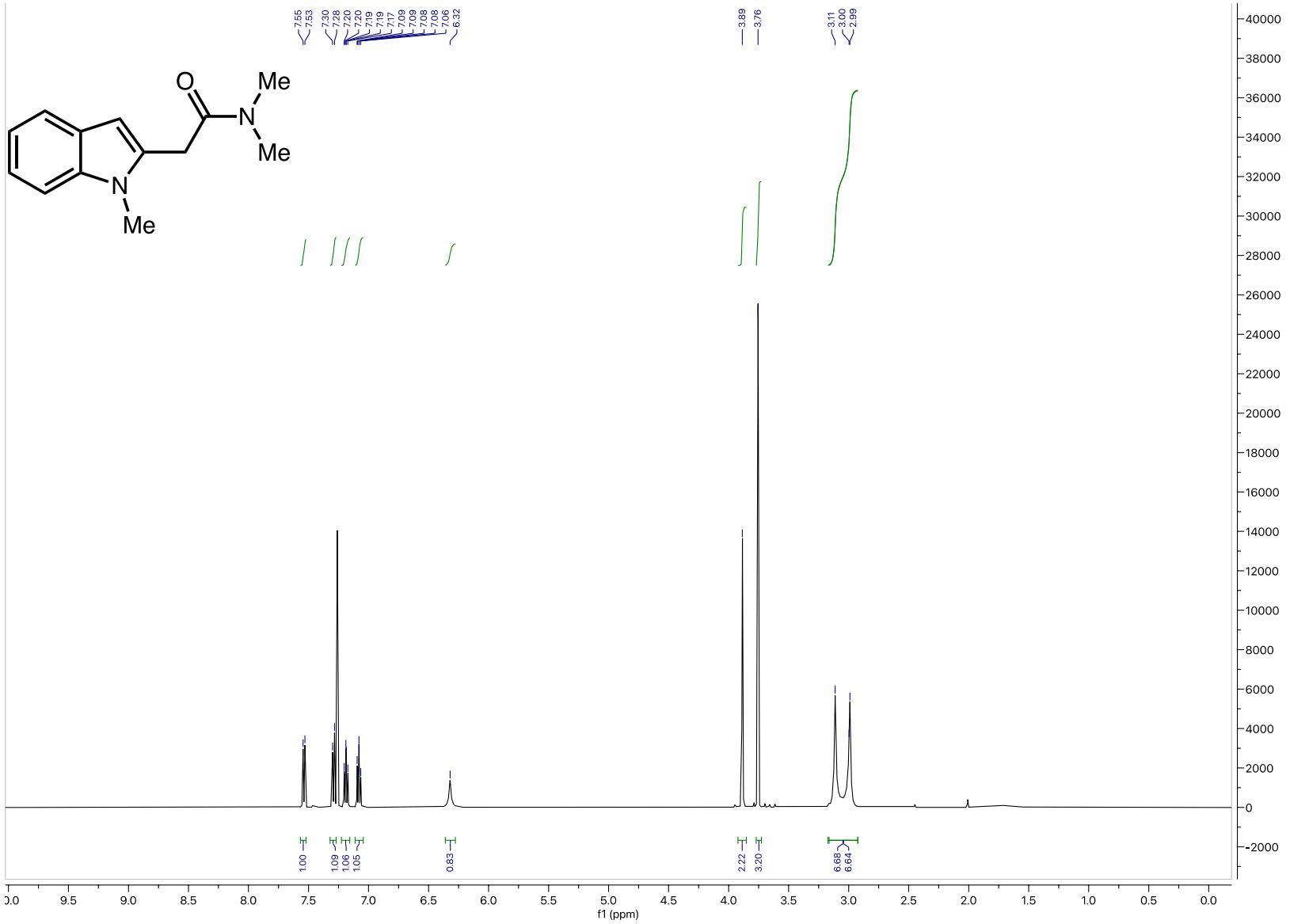

12
13
Me
N
Me
11
10
O
5
4
3
6
2
7
9
N
8
Me
1
8 7 2 6 3
6.6
6.8
7.0
7.2
7.4
7.6
7.4 7.2 7.0 6.8 6.6 6.4
f2 (ppm)
f1 (ppm)
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f2 (ppm)
0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5
f1 (ppm)

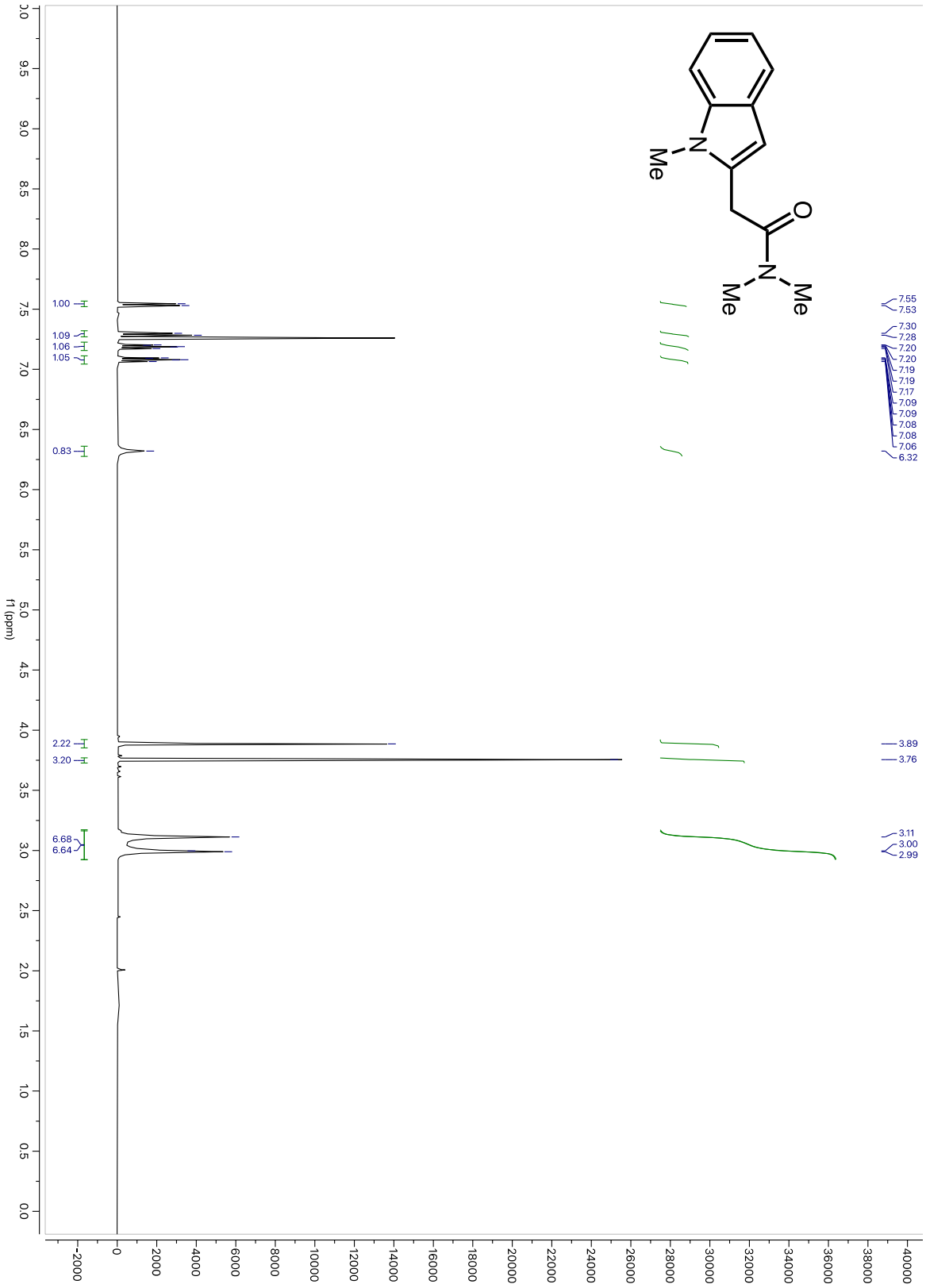

f2 (ppm)
f1 (ppm)
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
12
13
Me
N
Me
11
10
O
5
4
3
6
2
7
9
N
8
Me
1

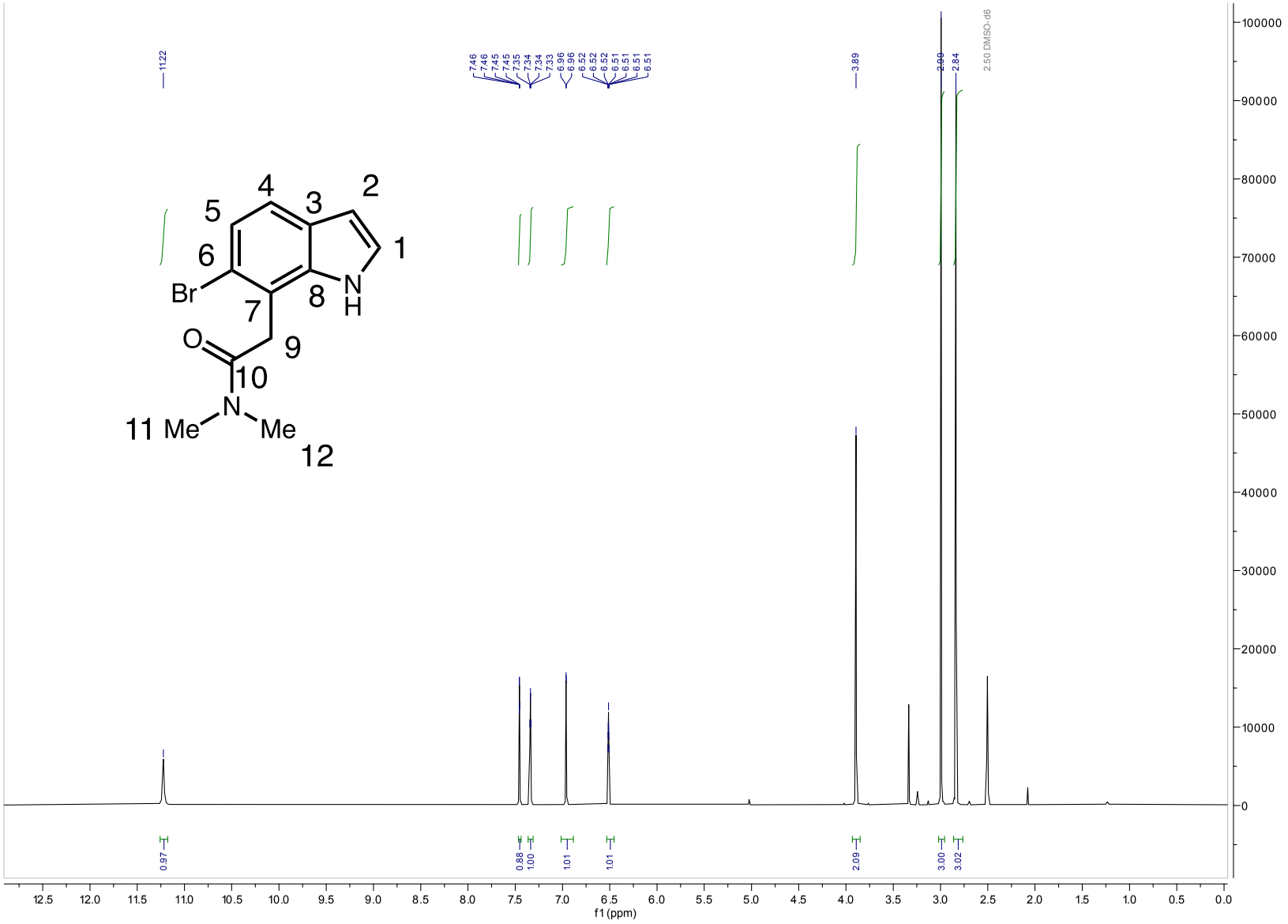

12
13
Me
N
Me
11
10
O
5
4
3
6
2
7
8
9
N
Me
1
f2 (ppm)

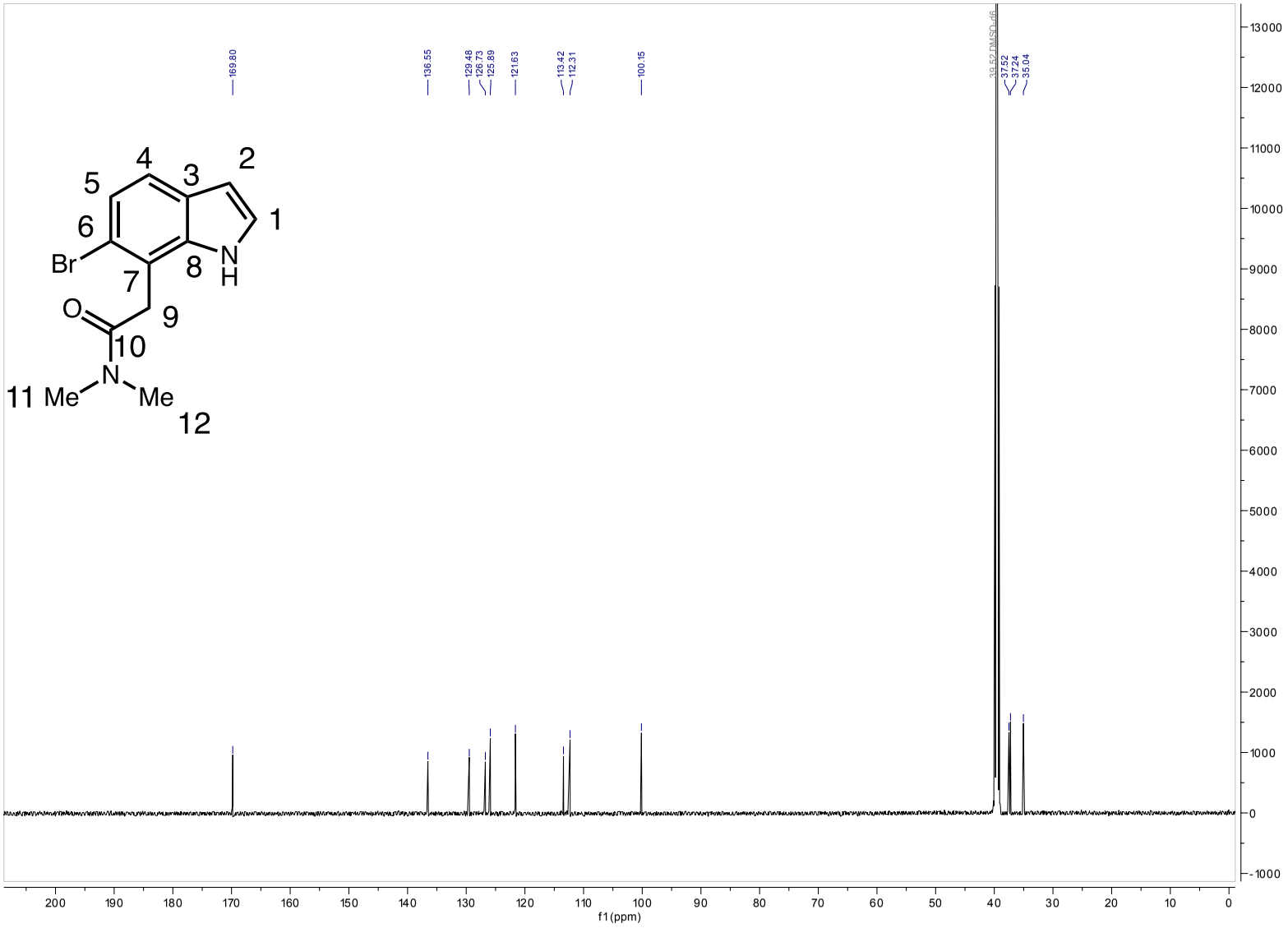

f1 (ppm)
f2 (ppm)

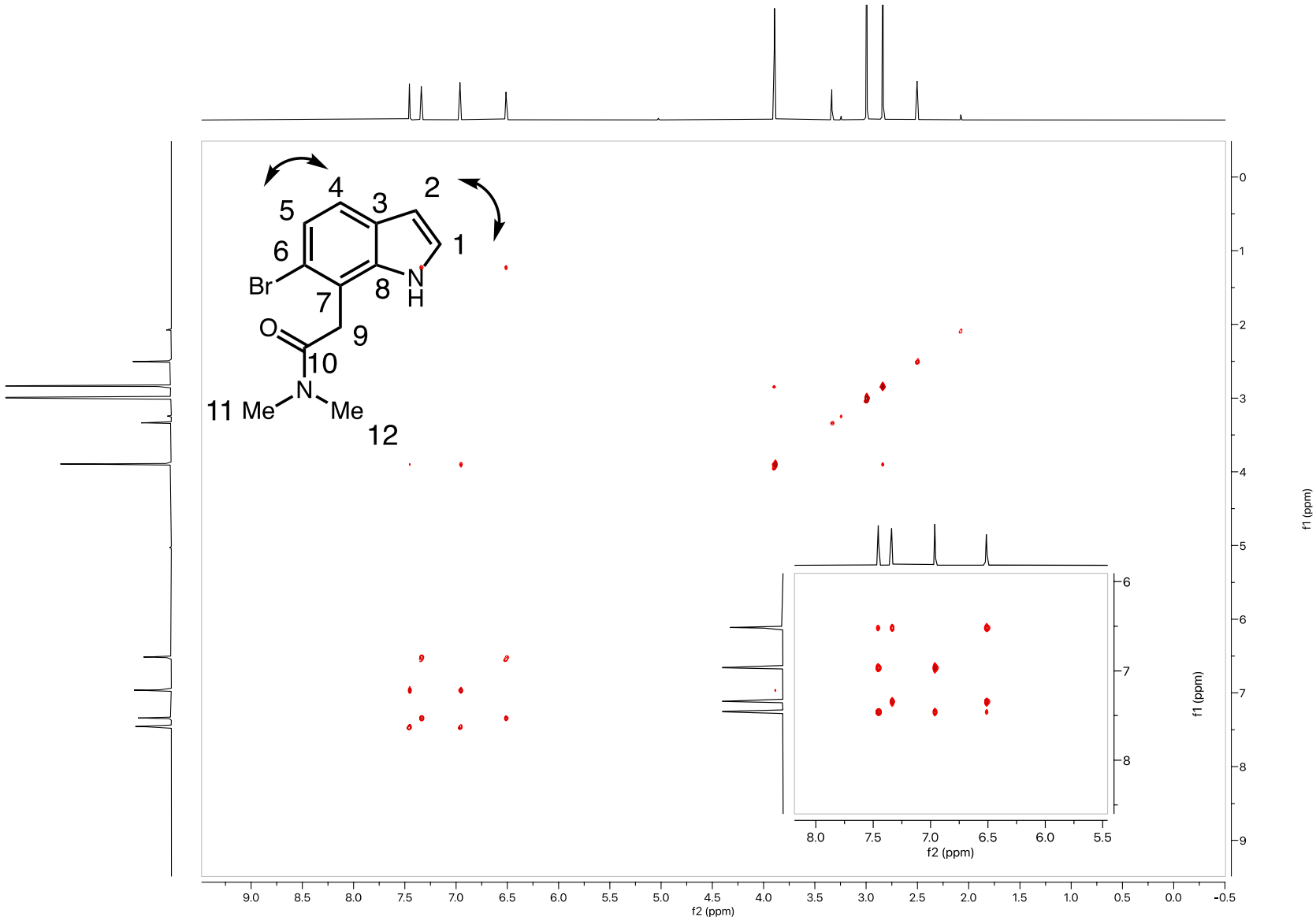

f1 (ppm)
f2 (ppm)

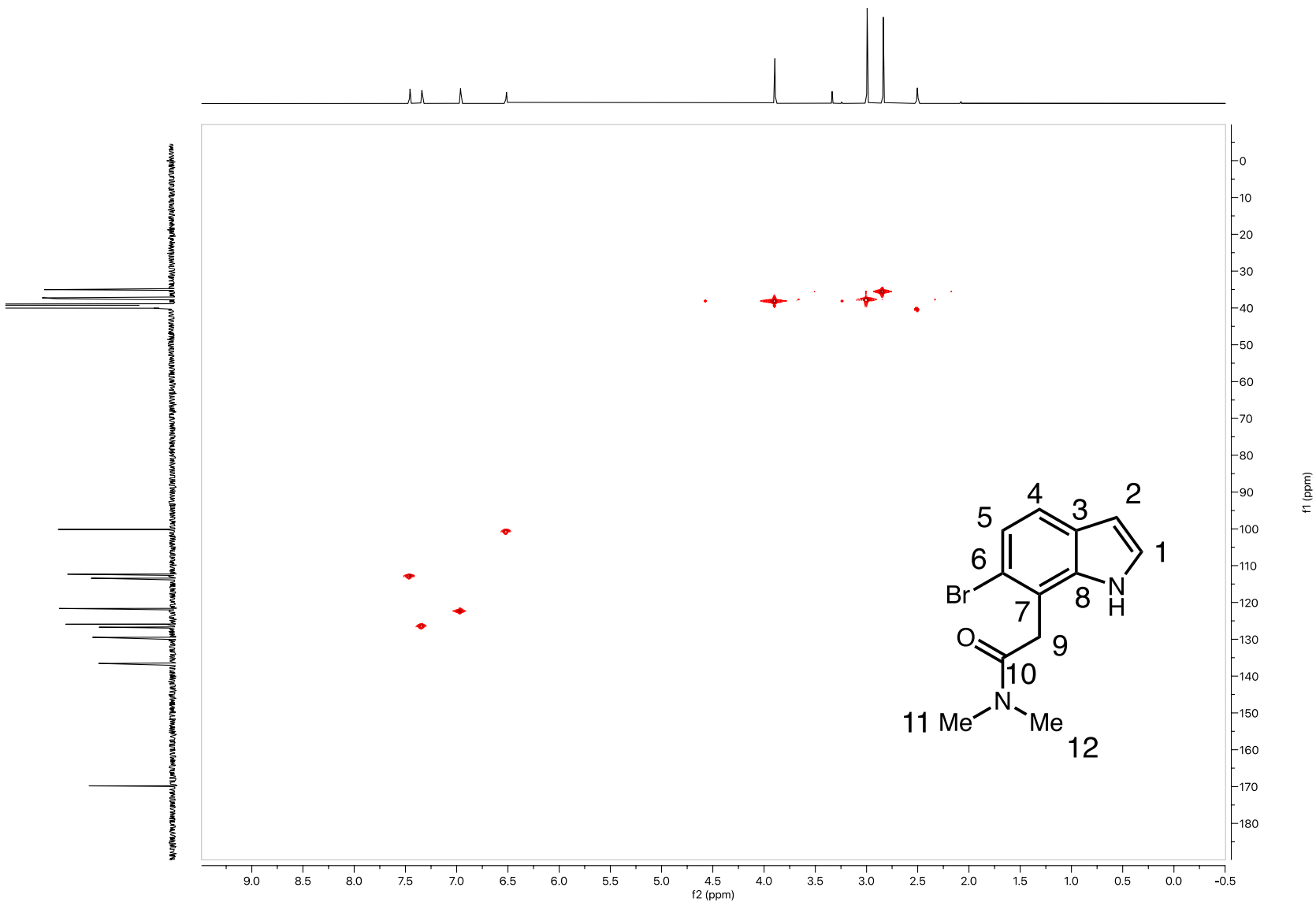

7.55
7.53
7.30
7.28
7.20
7.20
7.19
7.19
7.17
7.09
7.09
7.08
7.08
7.06
6.32
3.89
3.76
3.11
3.00
2.99
O
Me
N
Me
N
Me
1.00
1.09
1.06
1.05
0.83
2.22
3.20
6.68
6.64
f1 (ppm)

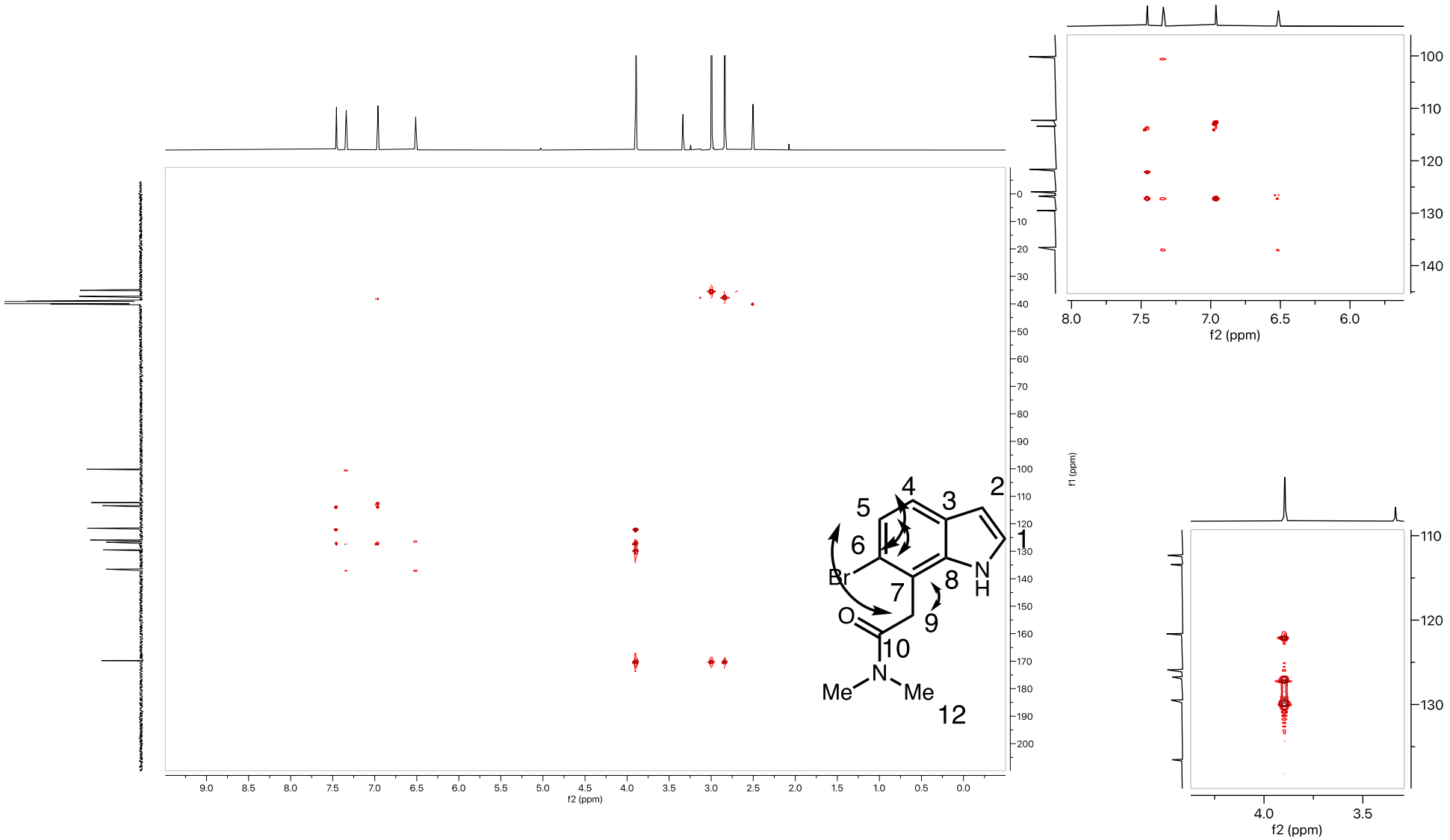

f1 (ppm)
1.00
1.09
1.06
1.05
0.83
2.22
3.20
6.68
6.64
7.55
7.53
7.30
7.28
7.20
7.20
7.19
7.19
7.17
7.09
7.09
7.08
7.08
7.06
6.32
3.89
3.76
3.11
3.00
2.99
N
Me
O
N
Me
Me

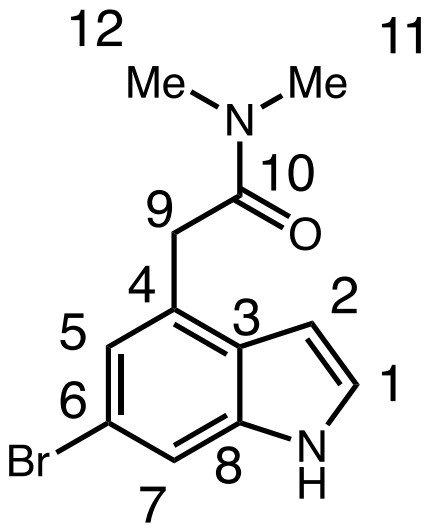

11.22
7.46
7.46
7.45
7.45
7.35
7.34
7.34
7.33
6.96
6.96
6.52
6.52
6.52
6.51
6.51
6.51
6.51
3.89
2.00
2.84
2.50 DMSO-d6
4
3
2
5
1
6
Br
8
N
H
7
O
9
10
N
11 Me
Me
12
0.97
0.88
1.00
1.01
1.01
2.09
3.00
3.02
f1 (ppm)


169.80
136.55
129.48
126.73
125.89
121.63
113.42
112.31
100.15
39.52 DMSO-d6
37.52
37.24
35.04
Br
N
H
O
N
Me
Me
f1 (ppm)

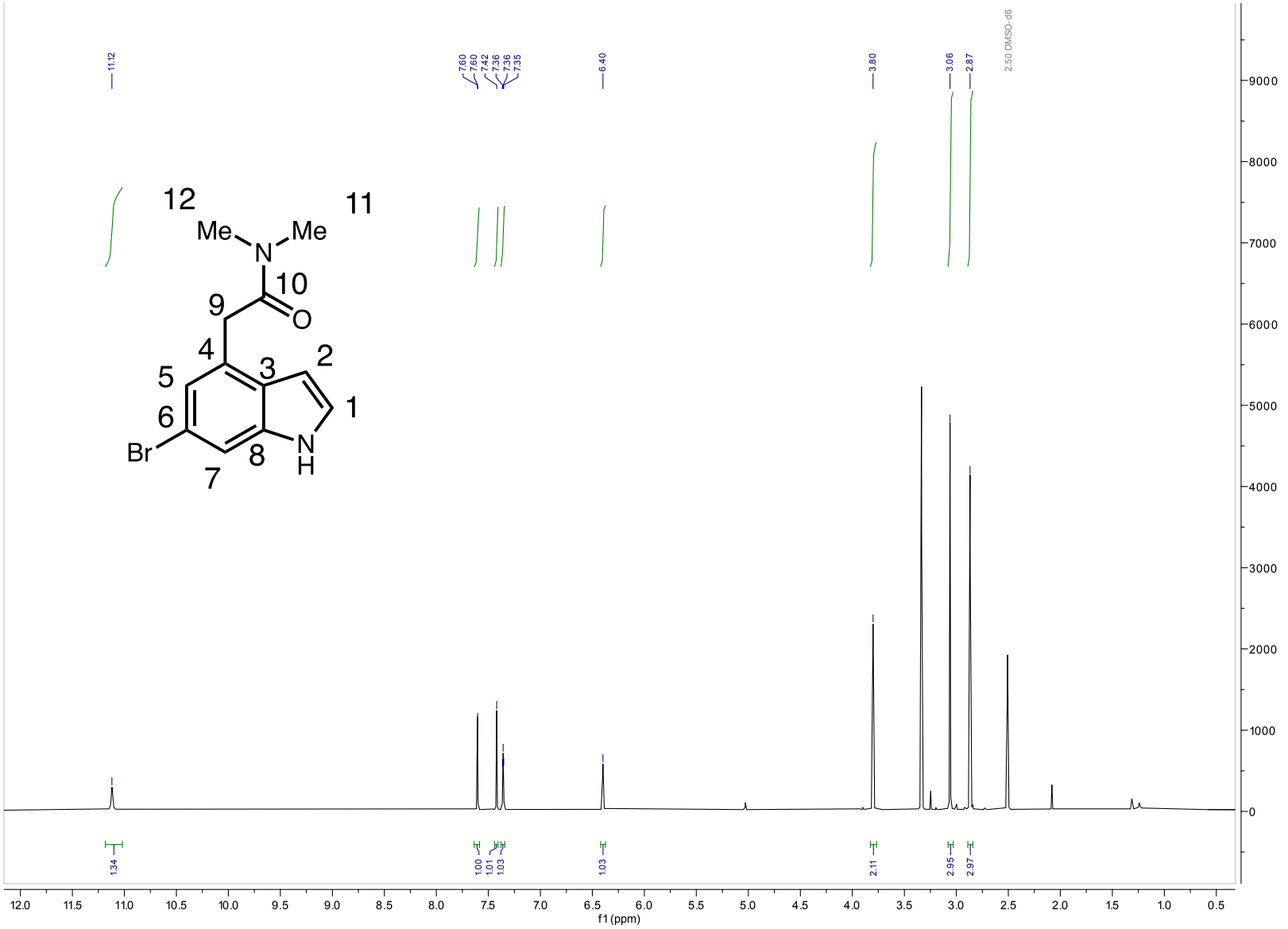

4
5
3
2
6
1
Br
8
N
H
7
O
9
10
N
11 Me
Me
12
f2 (ppm)
f1 (ppm)
f2 (ppm)
f1 (ppm)

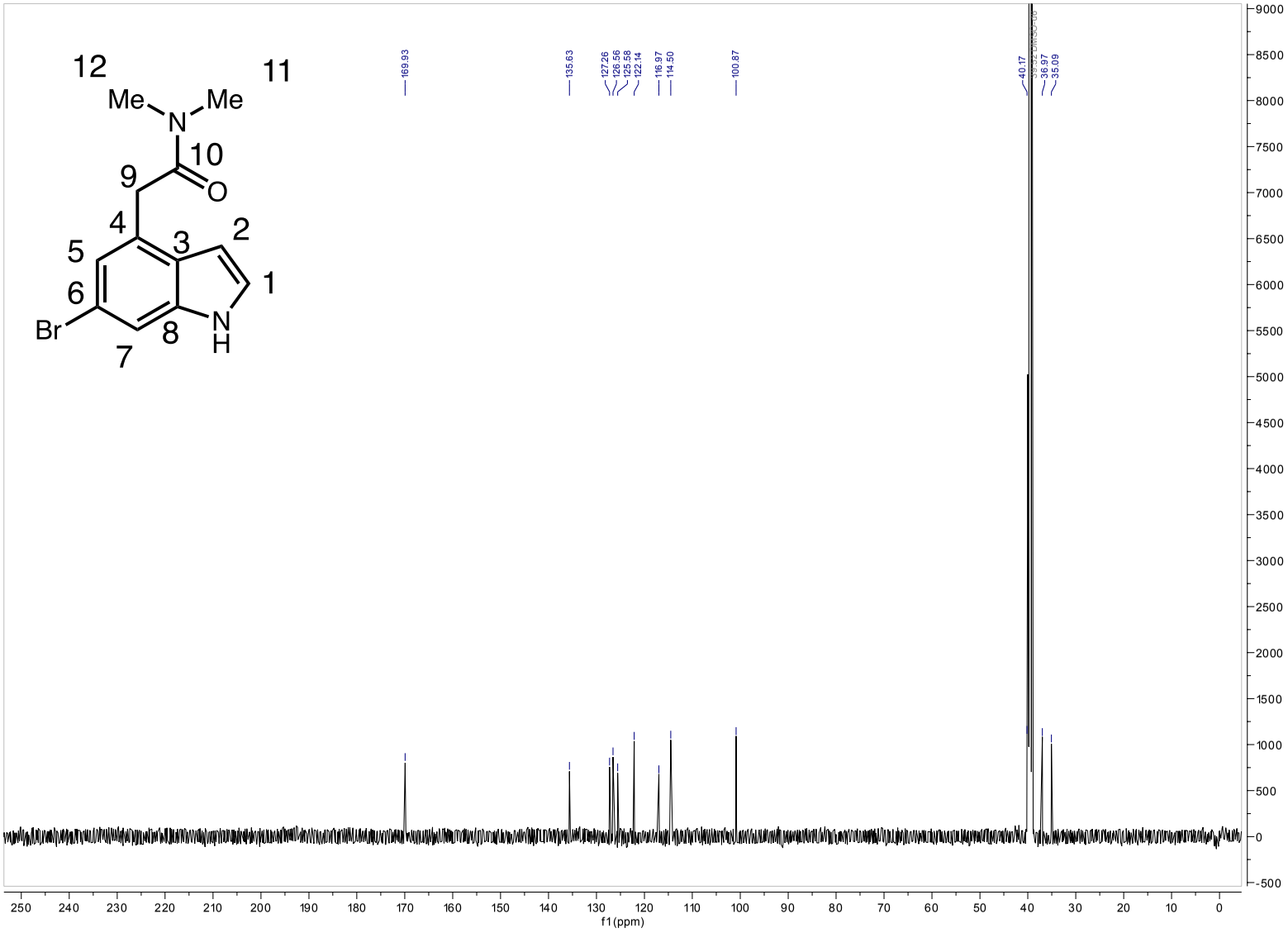

f2 (ppm)
f1 (ppm)
Br
N
H
O
N
Me
Me
1
2
3
4
5
6
7
8
9
10
11
12

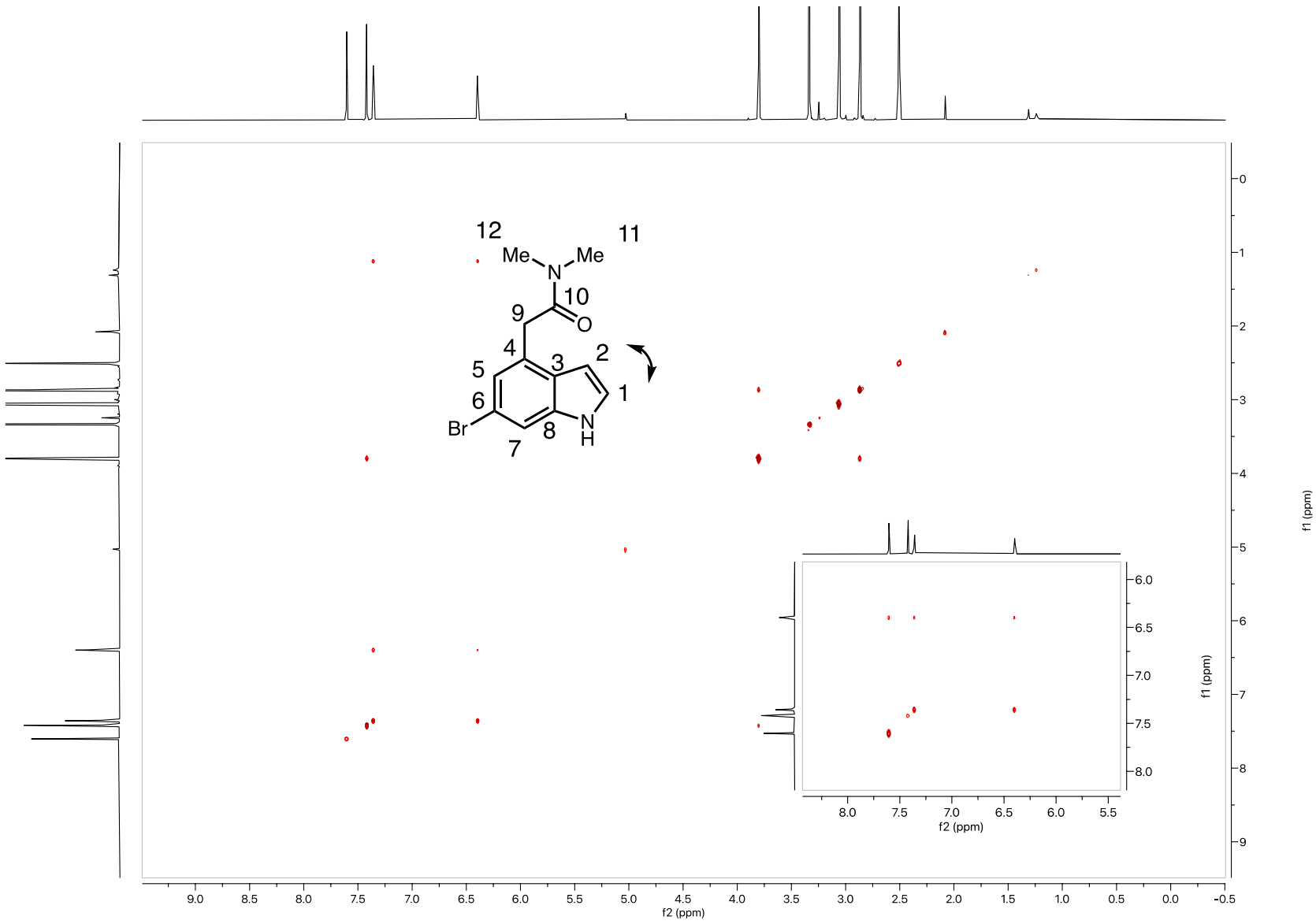

f2 (ppm)
f1 (ppm)
Br
O
N
H
Me
Me
12

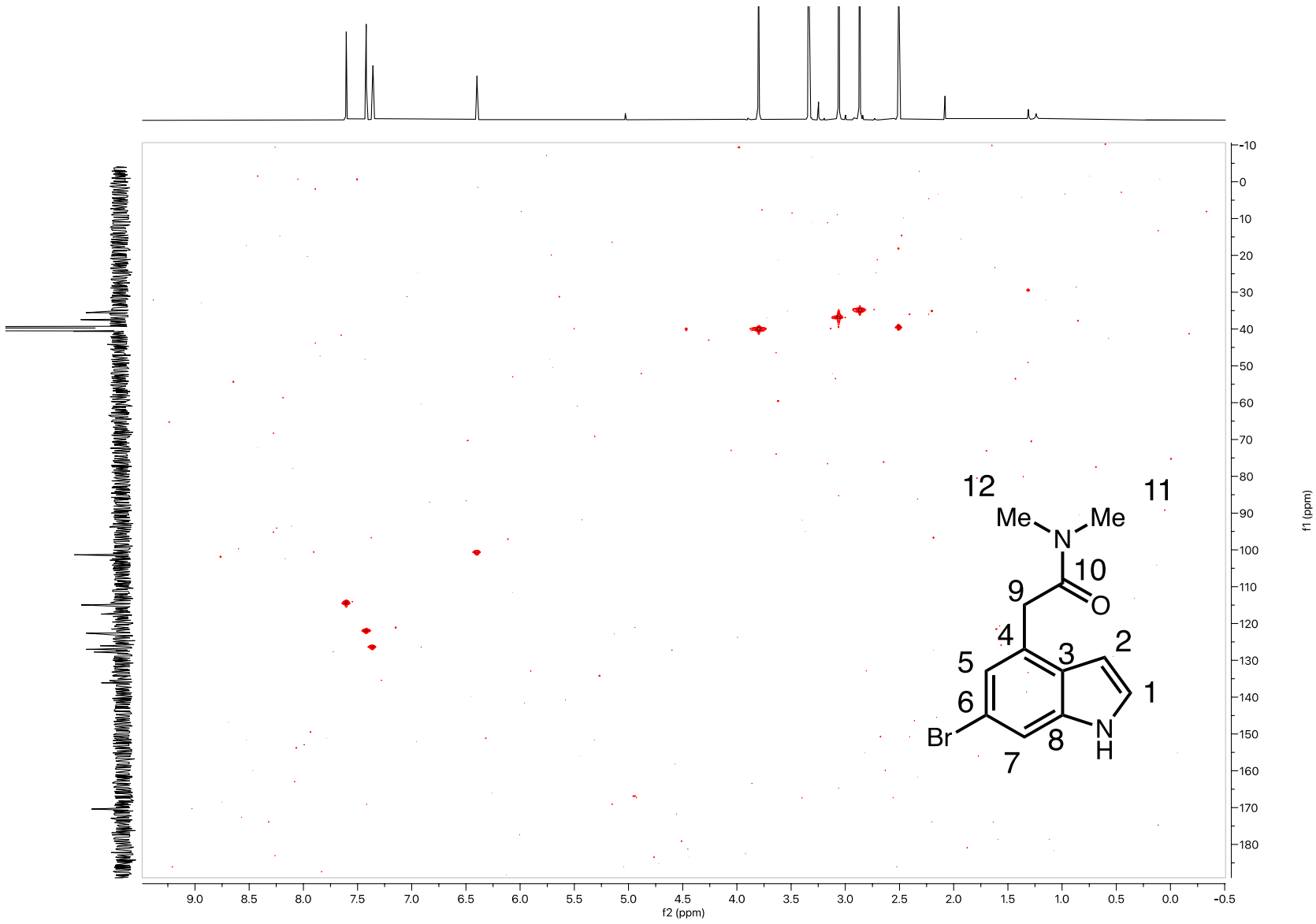


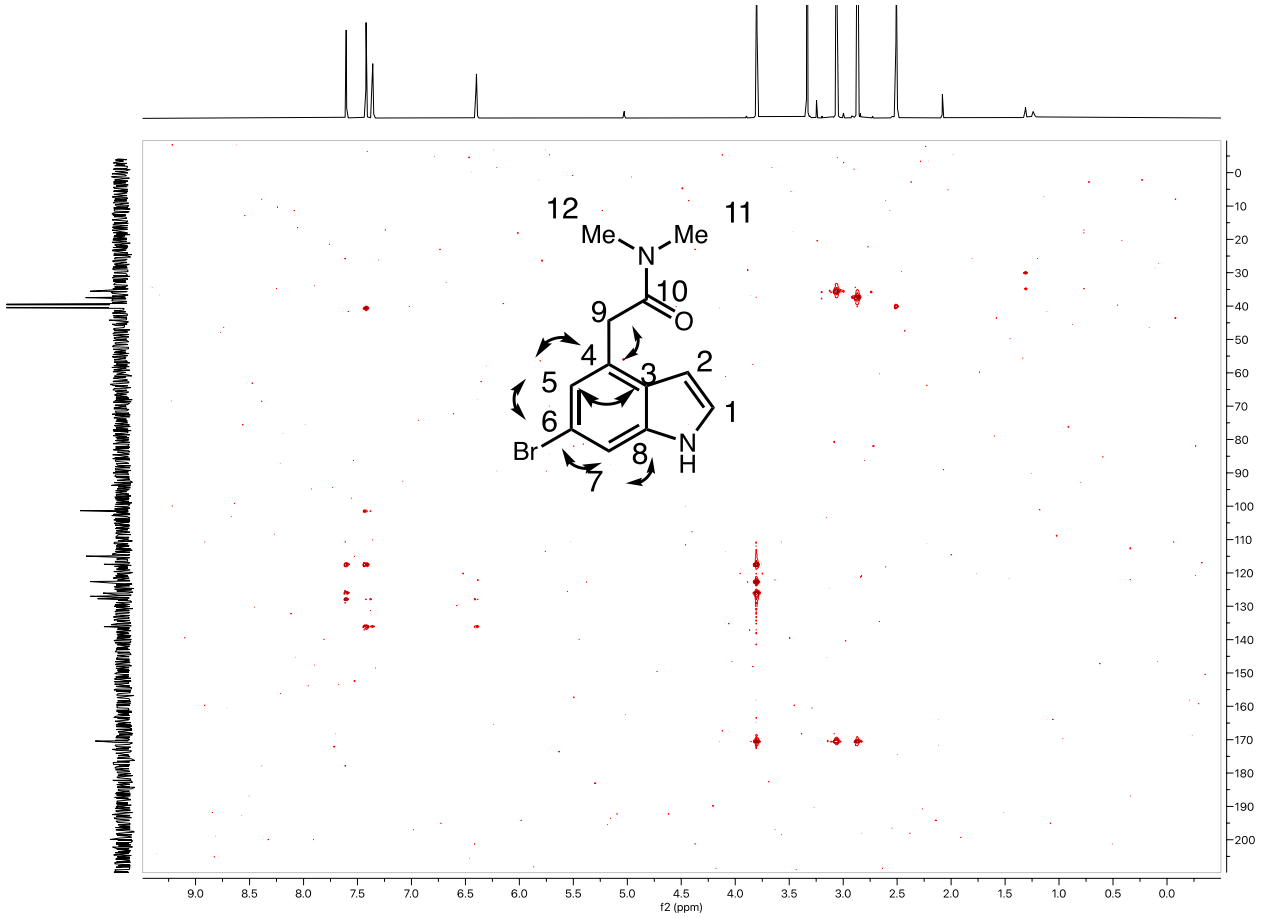
Position	δ_H multiplet (J in Hz)	δ_C
1	7.34, m	126.56
2	6.40, m	100.87
3		127.26
4		125.58
5	7.60	114.50
6		116.97
7	7.42	122.40
8		135.63
9	3.89, s	39.81
10		169.93
11, 12	2.99,2.84	36.97,35.09

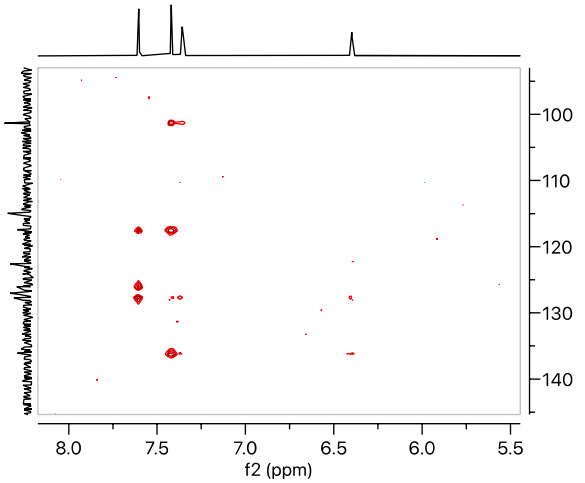

11.12
7.60
7.60
7.42
7.36
7.36
7.35
6.40
3.80
3.06
2.87
2.50 DMSO-d6
12
11
Me
N
Me
10
9
O
4
3
2
5
1
6
Br
8
N
H
7
1.34
1.00
1.01
1.03
1.03
2.11
2.95
2.97
f1 (ppm)

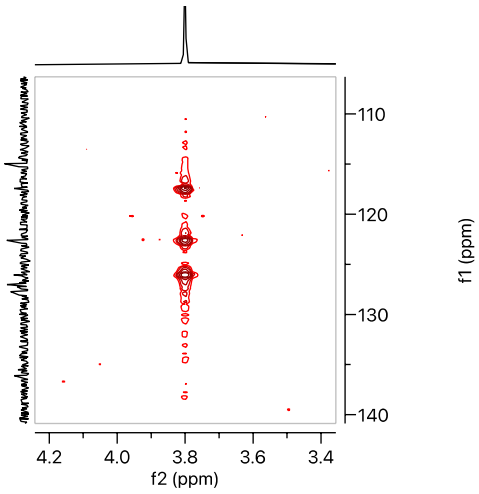

12
11
Me
N
Me
10
9
O
4
3
2
5
1
6
Br
8
N
H
7
169.93
135.63
127.26
126.56
125.58
122.14
116.97
114.50
100.87
40.17
36.97
35.09
9000
8500
8000
7500
7000
6500
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
250 240 230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

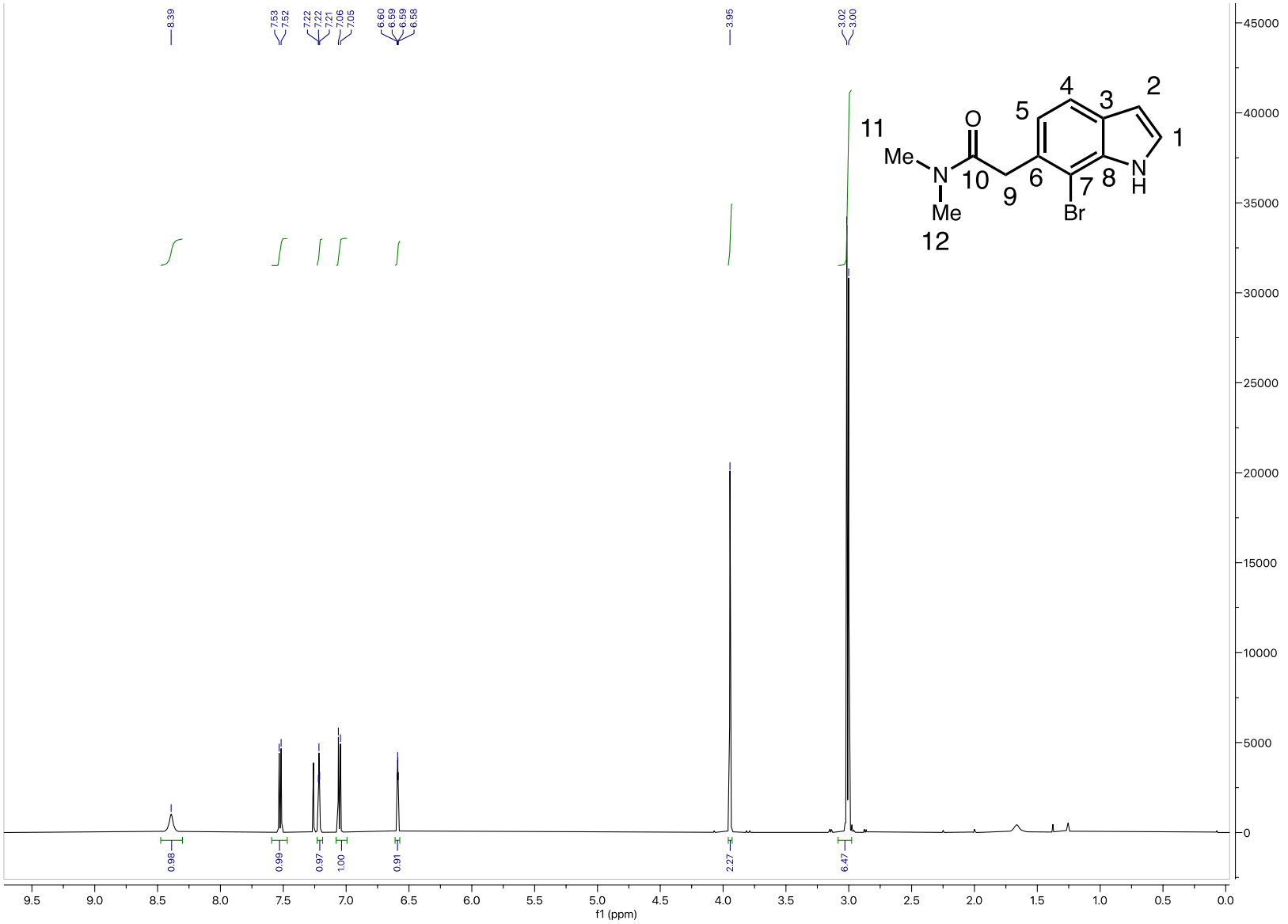

12
11
Me
N
Me
10
9
O
4
3
2
5
1
6
Br
8
N
H
7
f2 (ppm)
f1 (ppm)
f2 (ppm)
f1 (ppm)

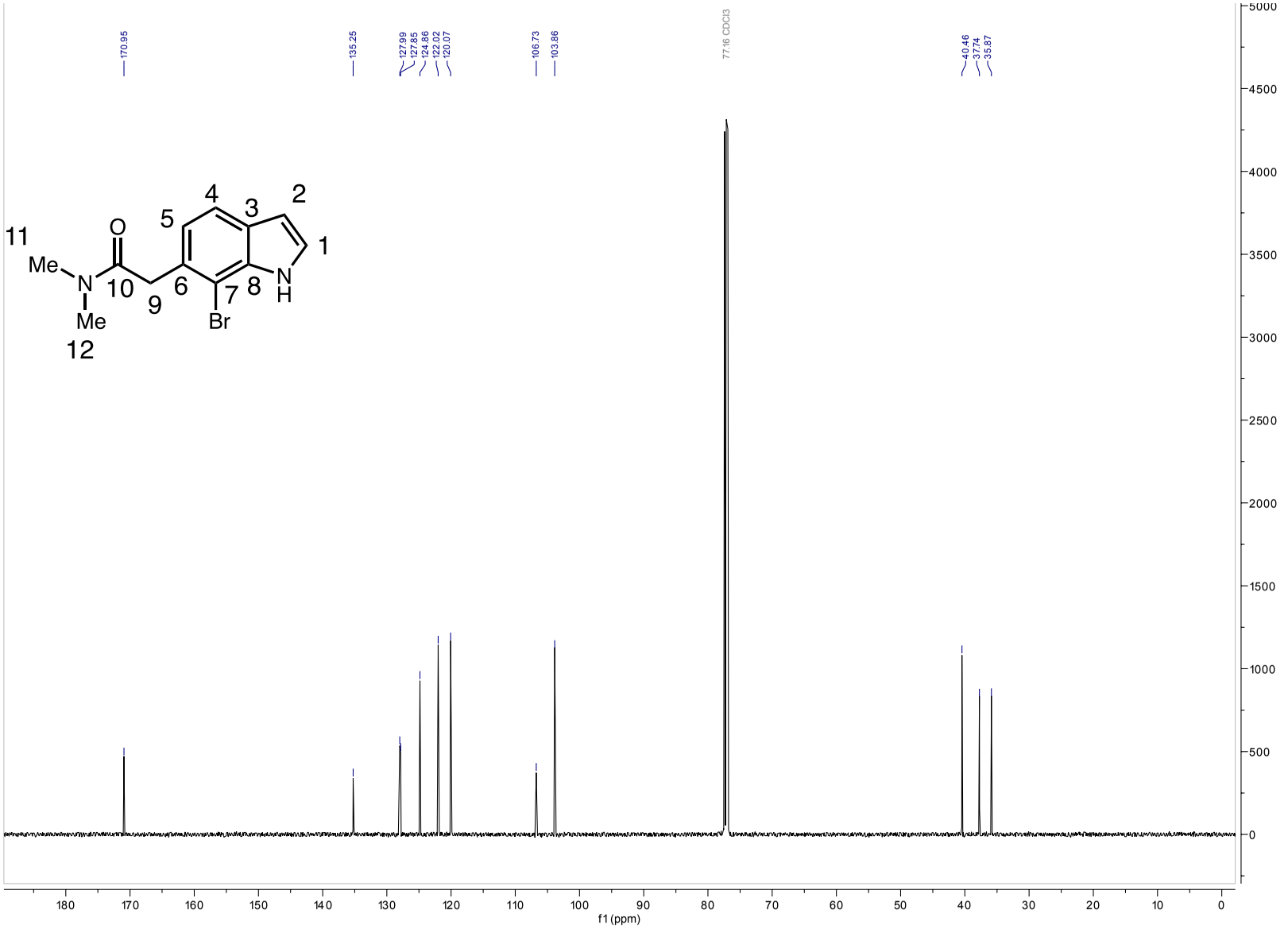

f2 (ppm)
f1 (ppm)
12
11
Me
N
Me
10
9
O
4
3
2
5
1
6
Br
8
N
H
7

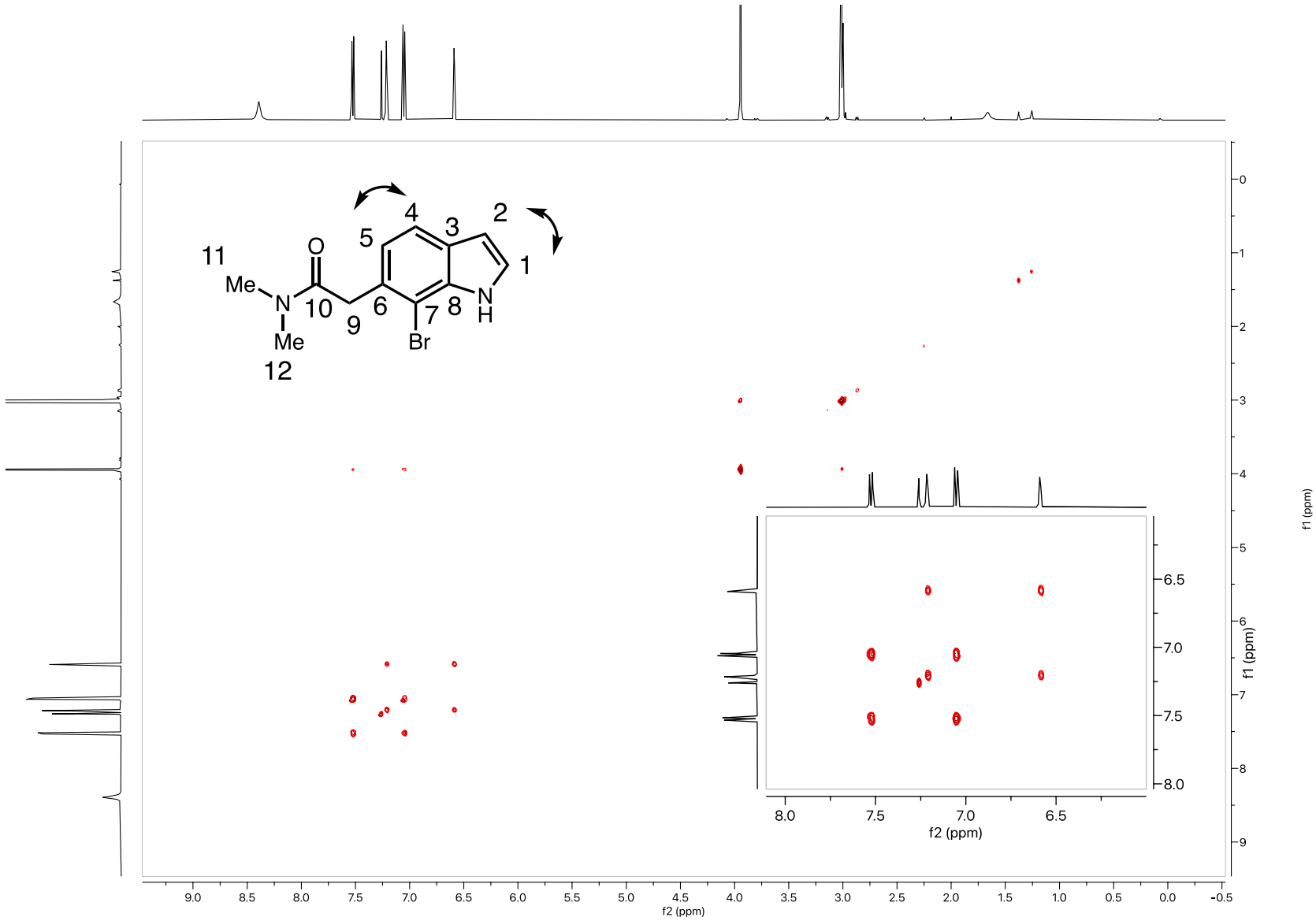

12
Me
N
Me
11
10
O
9
4
3
2
5
1
6
Br
8
N
H
7
f2 (ppm)

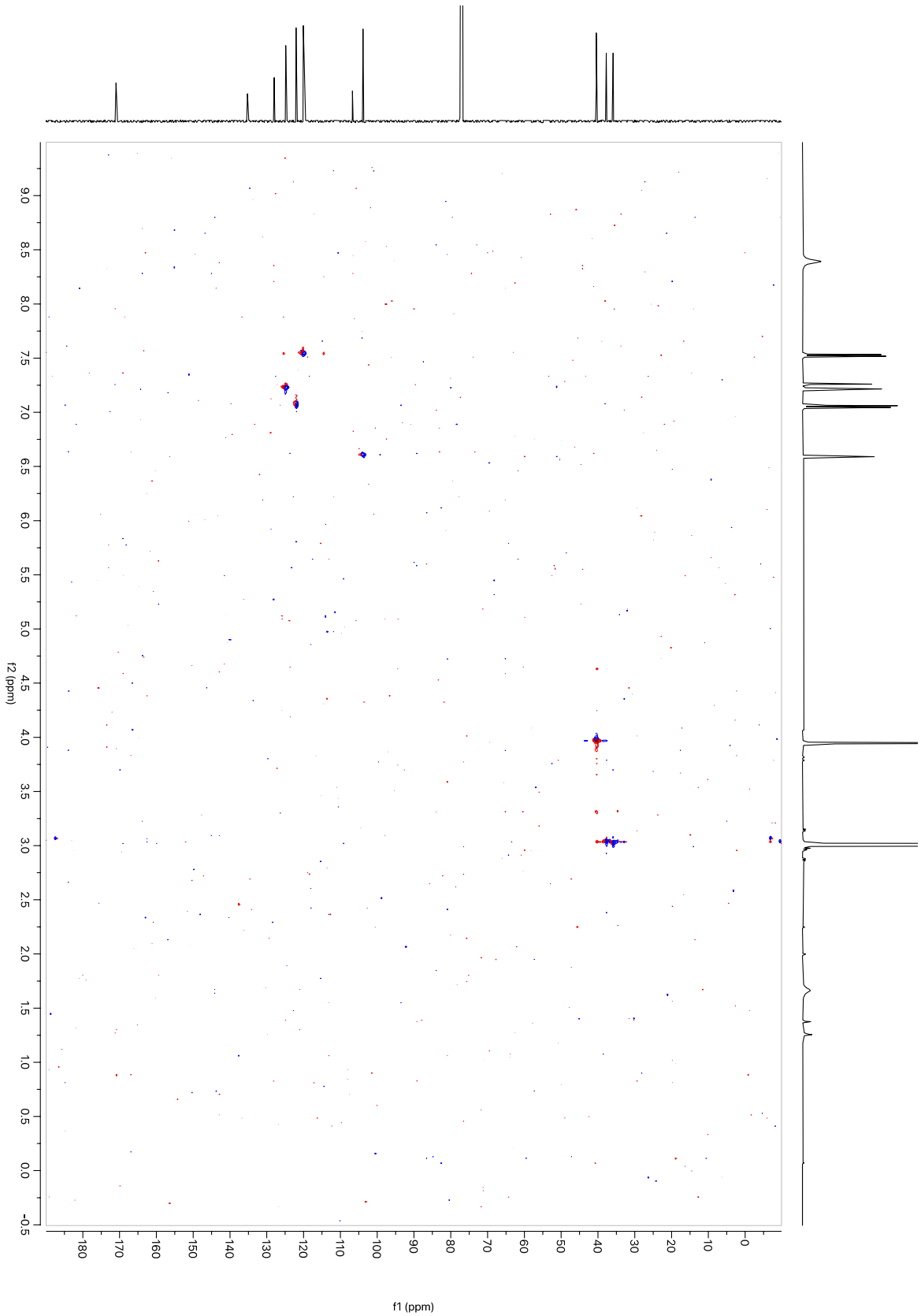

f2 (ppm)
f1 (ppm)

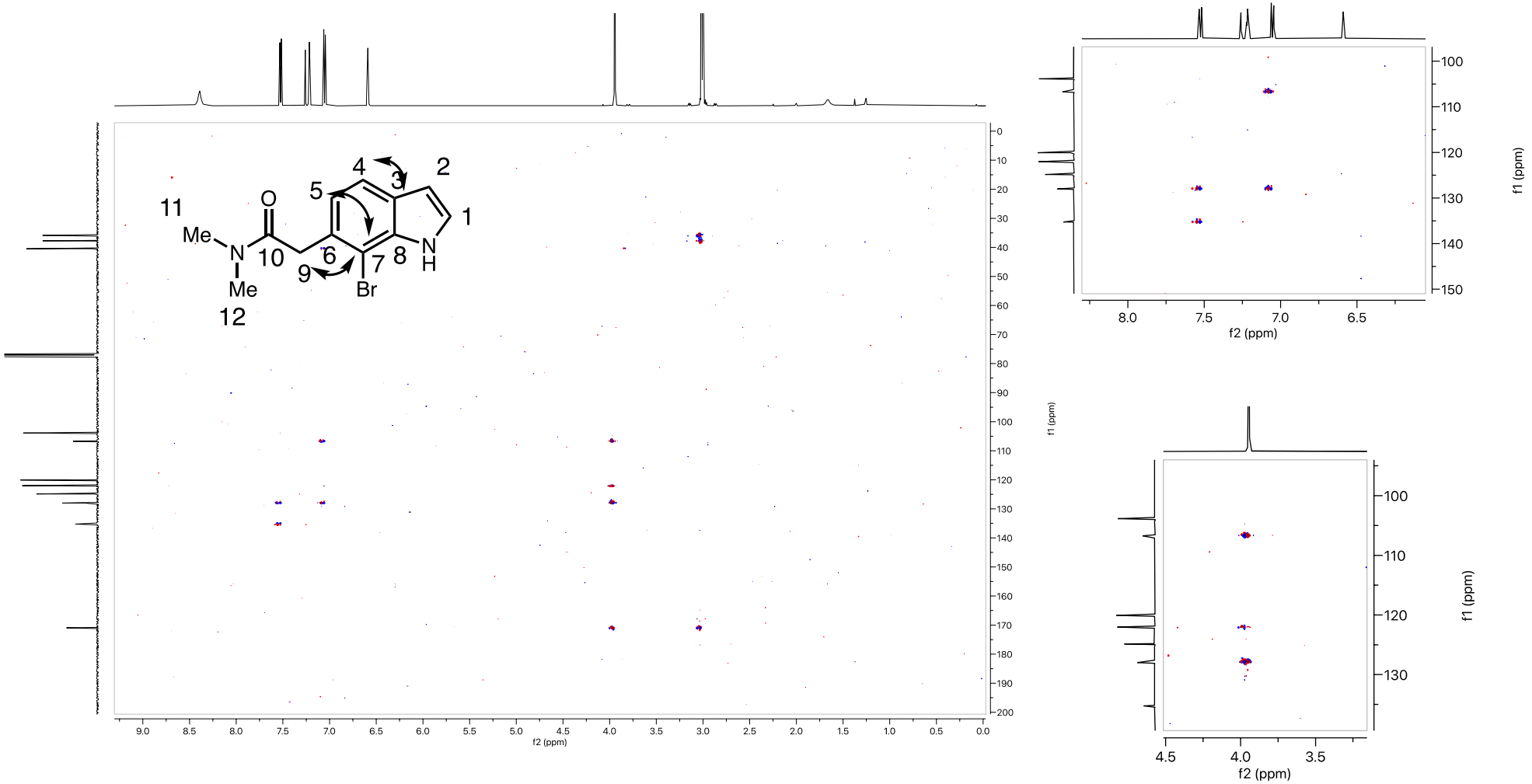

f2 (ppm)
f1 (ppm)

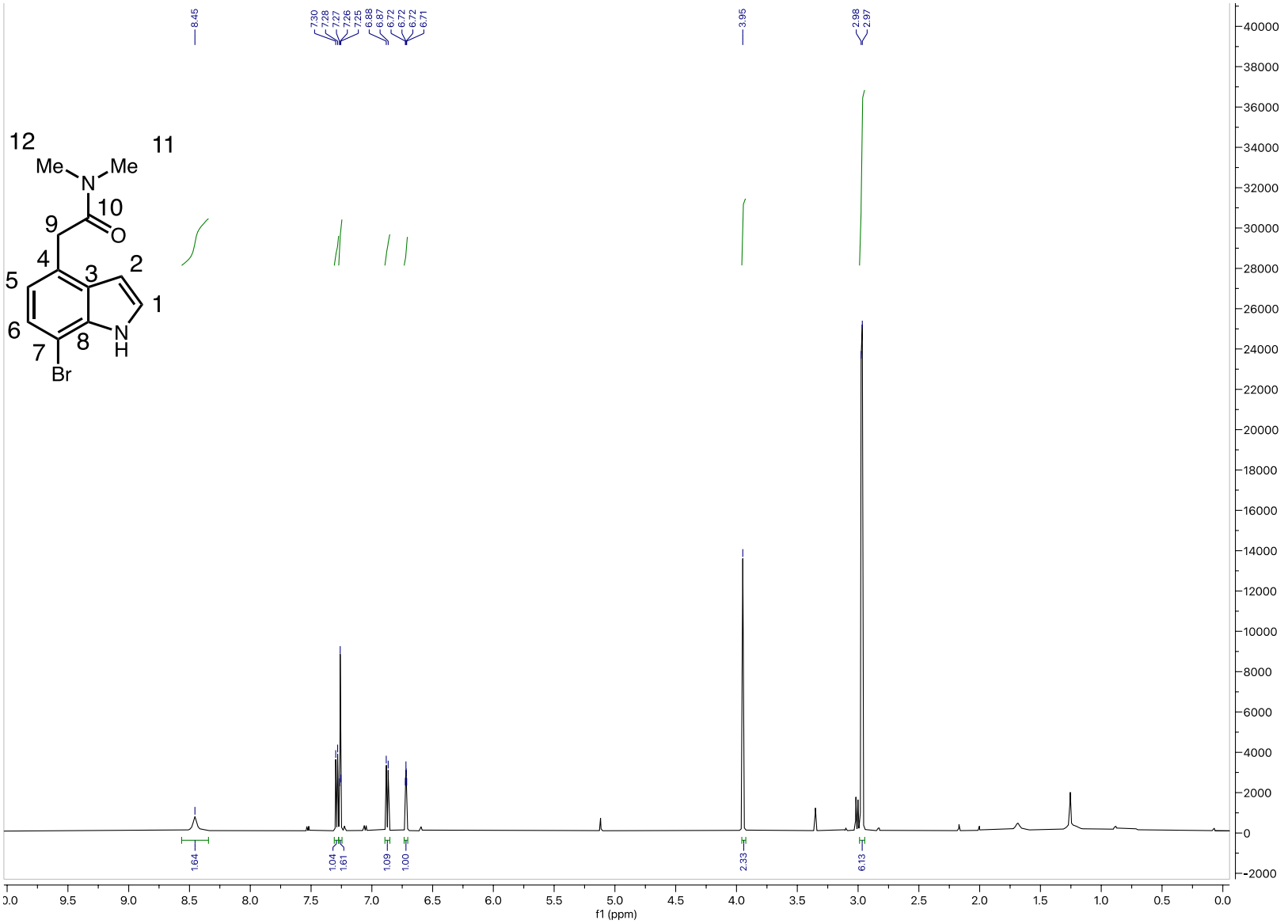

8.39
7.53
7.52
7.22
7.22
7.21
7.06
7.05
6.60
6.59
6.59
6.58
3.95
3.02
3.00
0.98
0.99
0.97
1.00
0.91
2.27
6.47
f1 (ppm)
Me
N
Me
O
Br
N
H
1
2
3
4
5
6
7
8
9
10
11
12

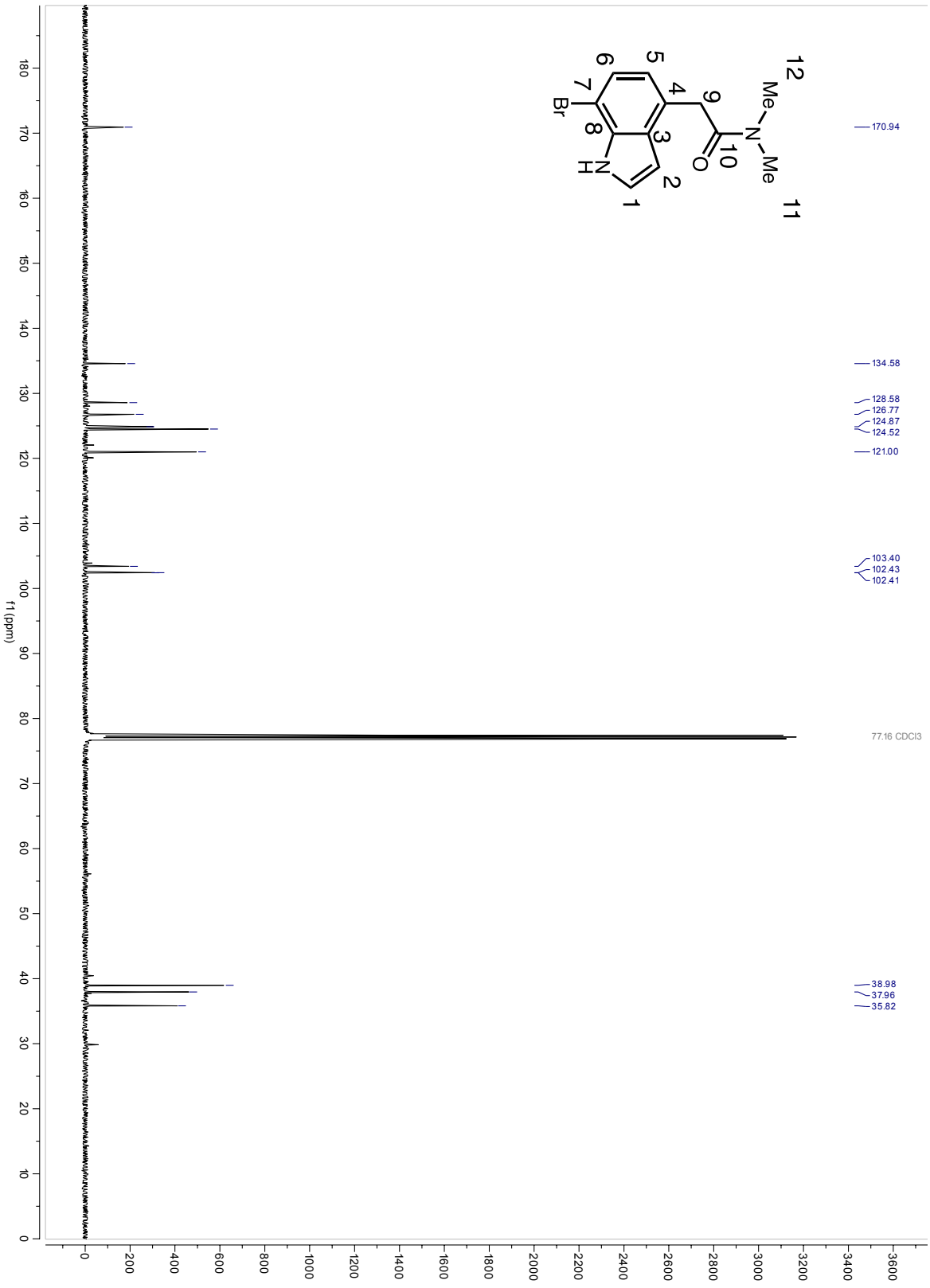

170.95
135.25
127.99
127.85
124.66
122.02
120.07
106.73
103.86
77.16 CDCl3
40.46
37.74
35.87
O
Me
N
Me
Br
H
N
1
2
3
4
5
6
7
8
9
10
11
12
f1 (ppm)

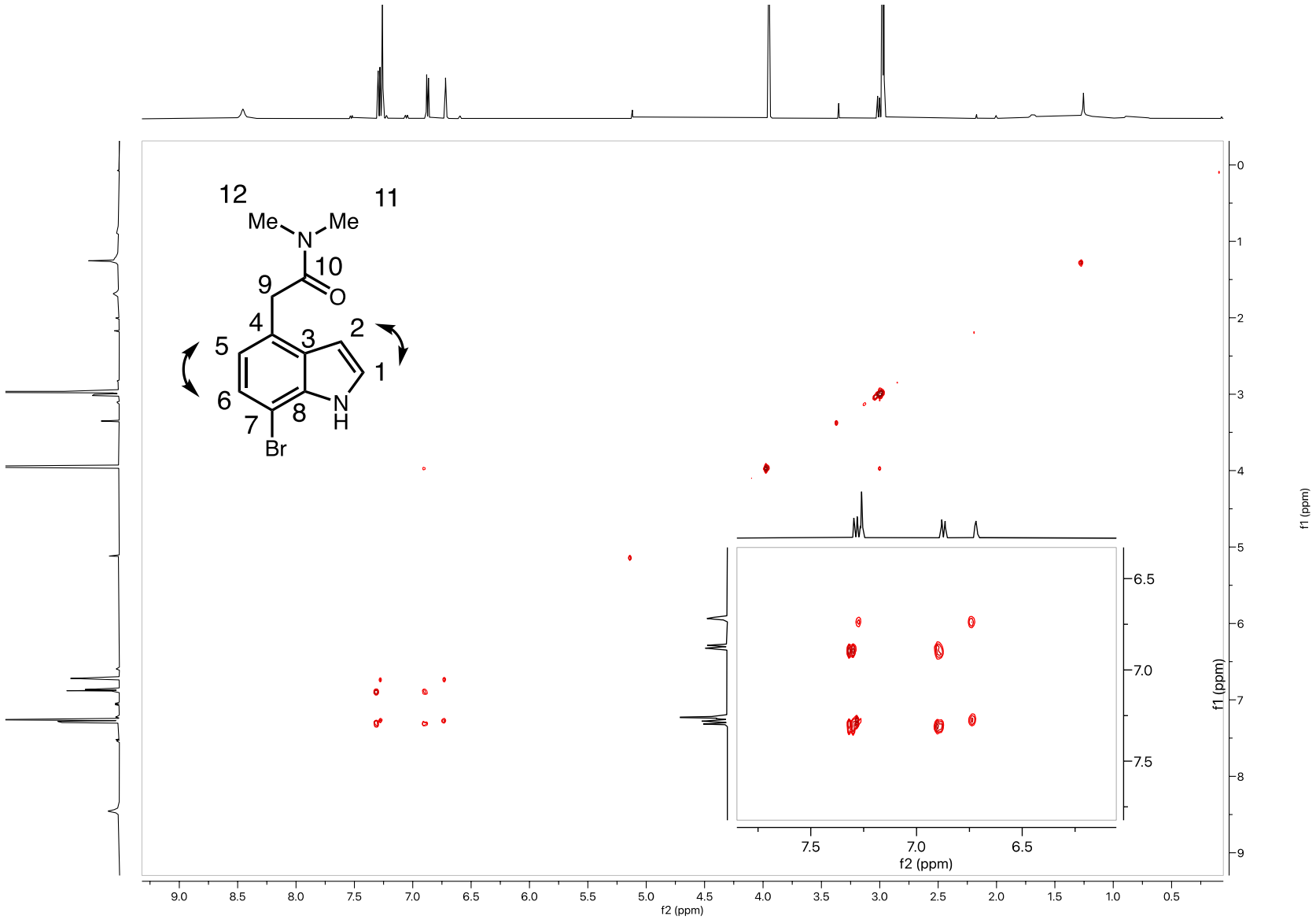

11
Me
N
Me
12
O
10
9
6
5
4
3
2
1
7
8
N
H
Br
f2 (ppm)
f1 (ppm)
f2 (ppm)
f1 (ppm)

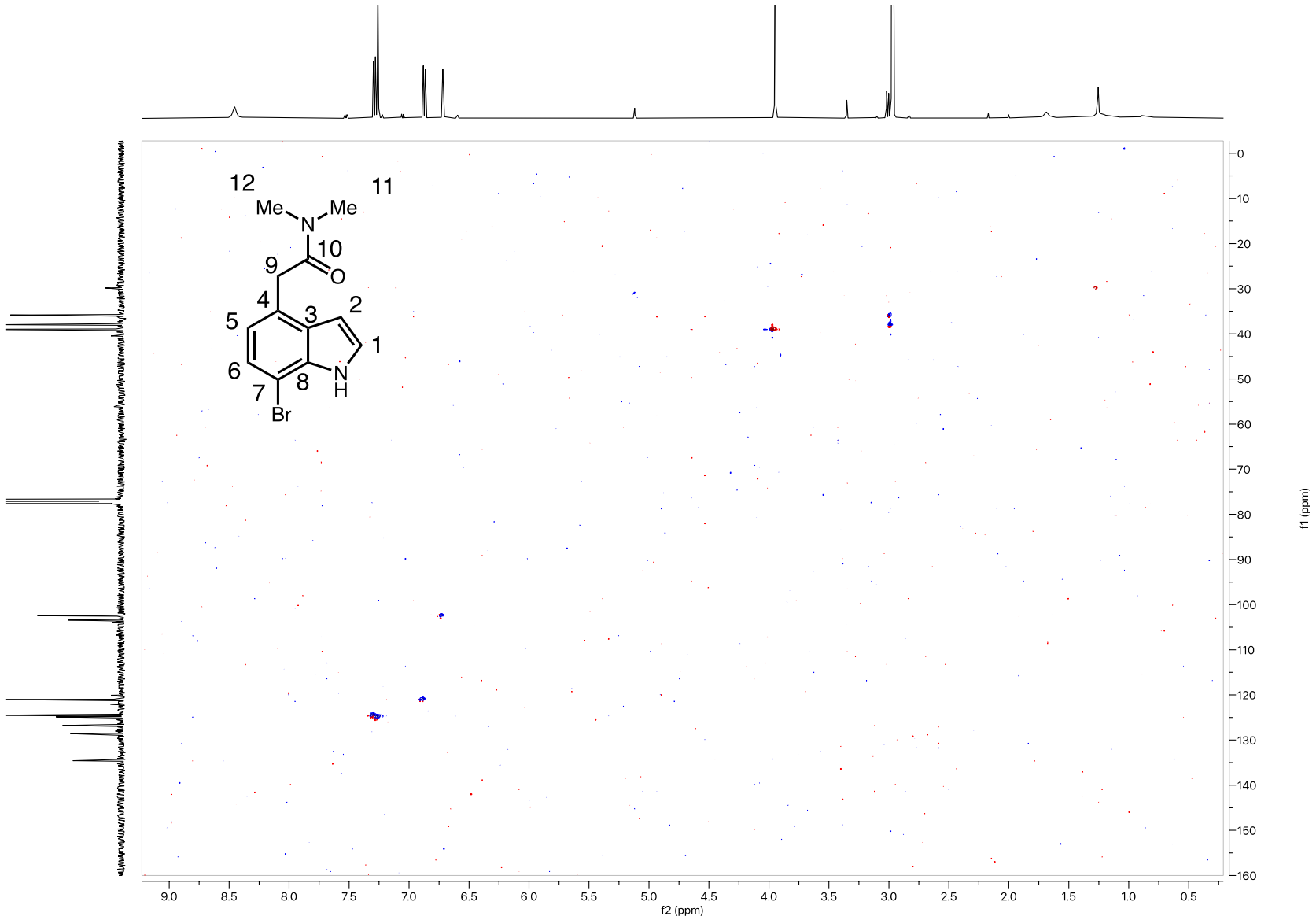

f2 (ppm)
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

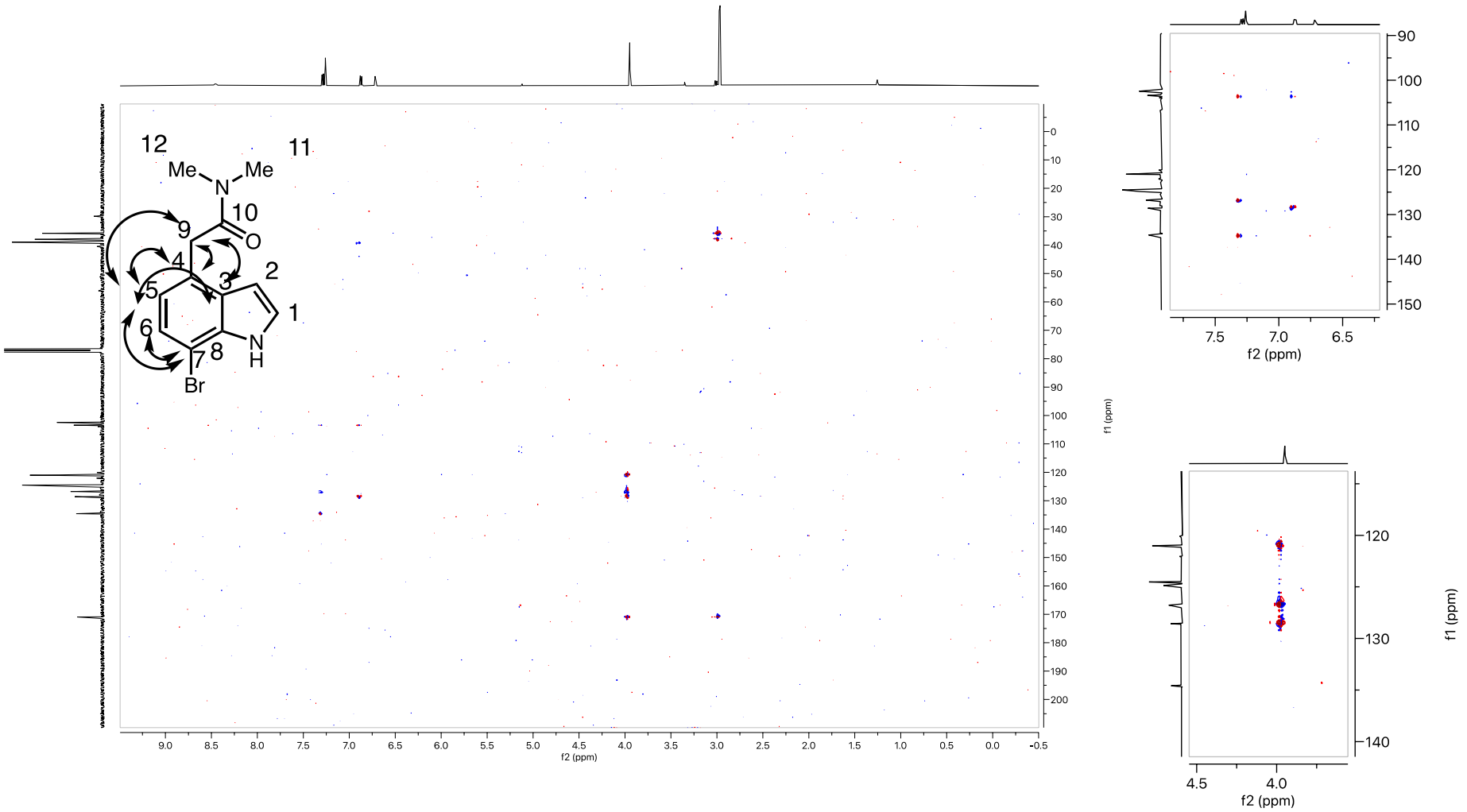

11
Me
N
Me
12
O
10
5
4
3
2
1
6
9
7
8
N
H
Br
f2 (ppm)
f1 (ppm)
f2 (ppm)
f1 (ppm)
f2 (ppm)
f1 (ppm)

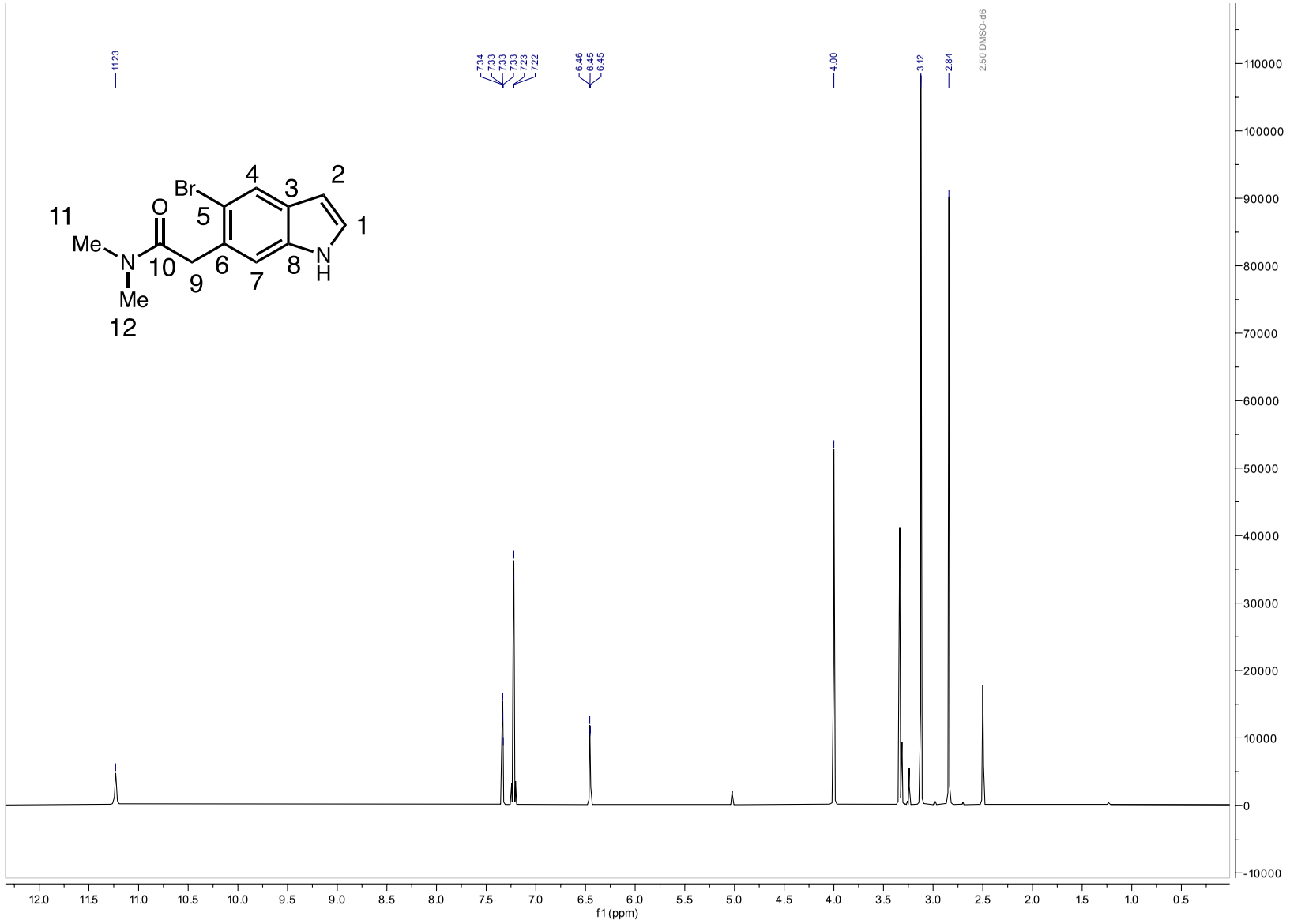

8.45
7.30
7.28
7.27
7.26
7.25
6.88
6.87
6.72
6.72
6.72
6.71
3.95
2.98
2.97
12
11
Me
N
Me
10
9
O
4
3
2
5
1
6
8
N
H
7
Br
1.64
1.04
1.61
1.09
1.00
2.33
6.13
f1 (ppm)

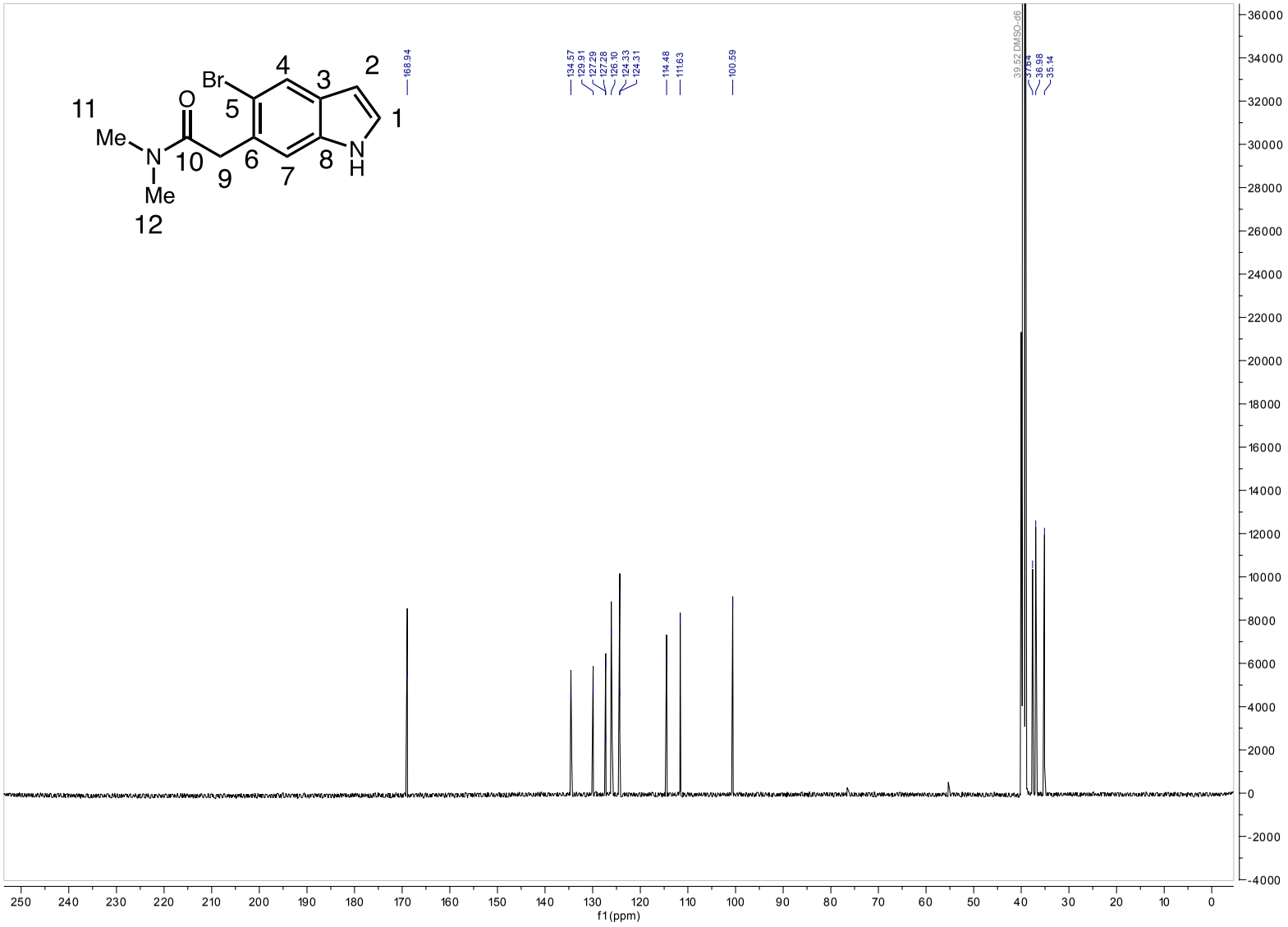

Br
Me
Me
O
H
170.94
134.58
128.58
126.77
124.87
124.52
121.00
103.40
102.43
102.41
77.16 CDCl3
38.98
37.96
35.82
f1 (ppm)

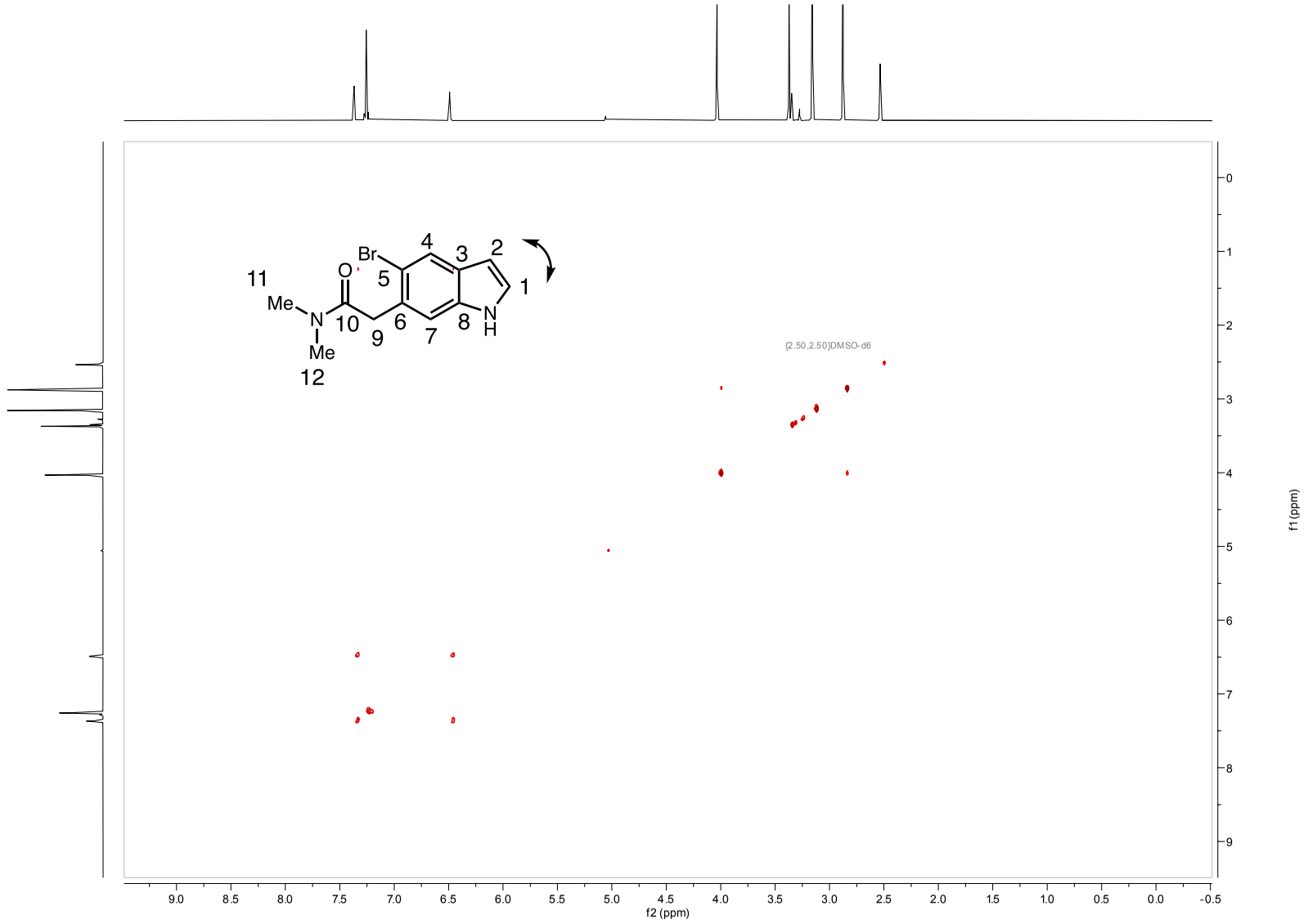

12
11
Me
N
Me
10
9
O
4
3
2
5
1
6
8
N
H
7
Br
f2 (ppm)
f1 (ppm)
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
1
2
3
4
5
6
7
8
9
6.5
7.0
7.5

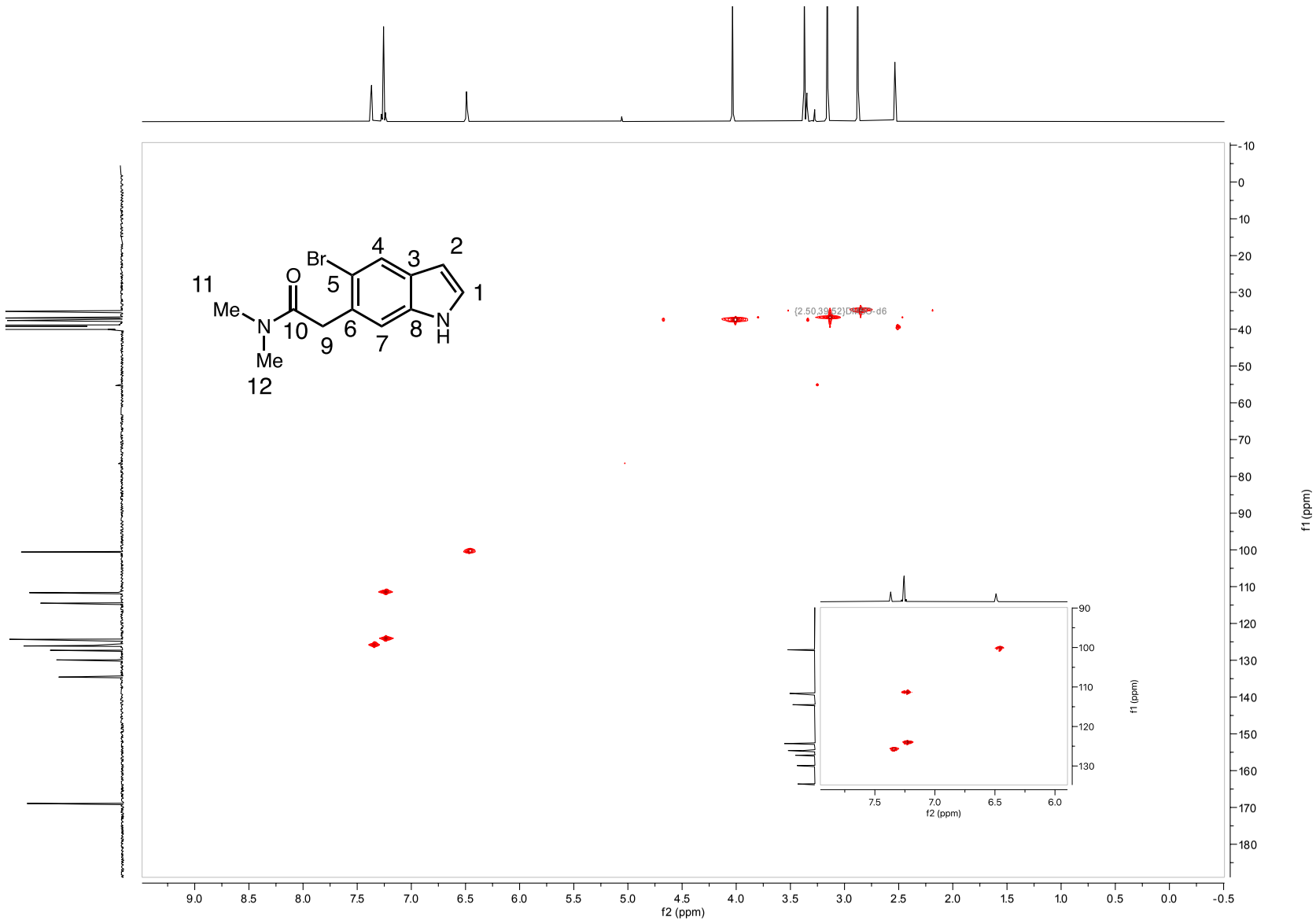

12
11
Me
N
Me
10
9
O
4
3
2
5
1
6
8
N
H
7
Br
f2 (ppm)
f1 (ppm)

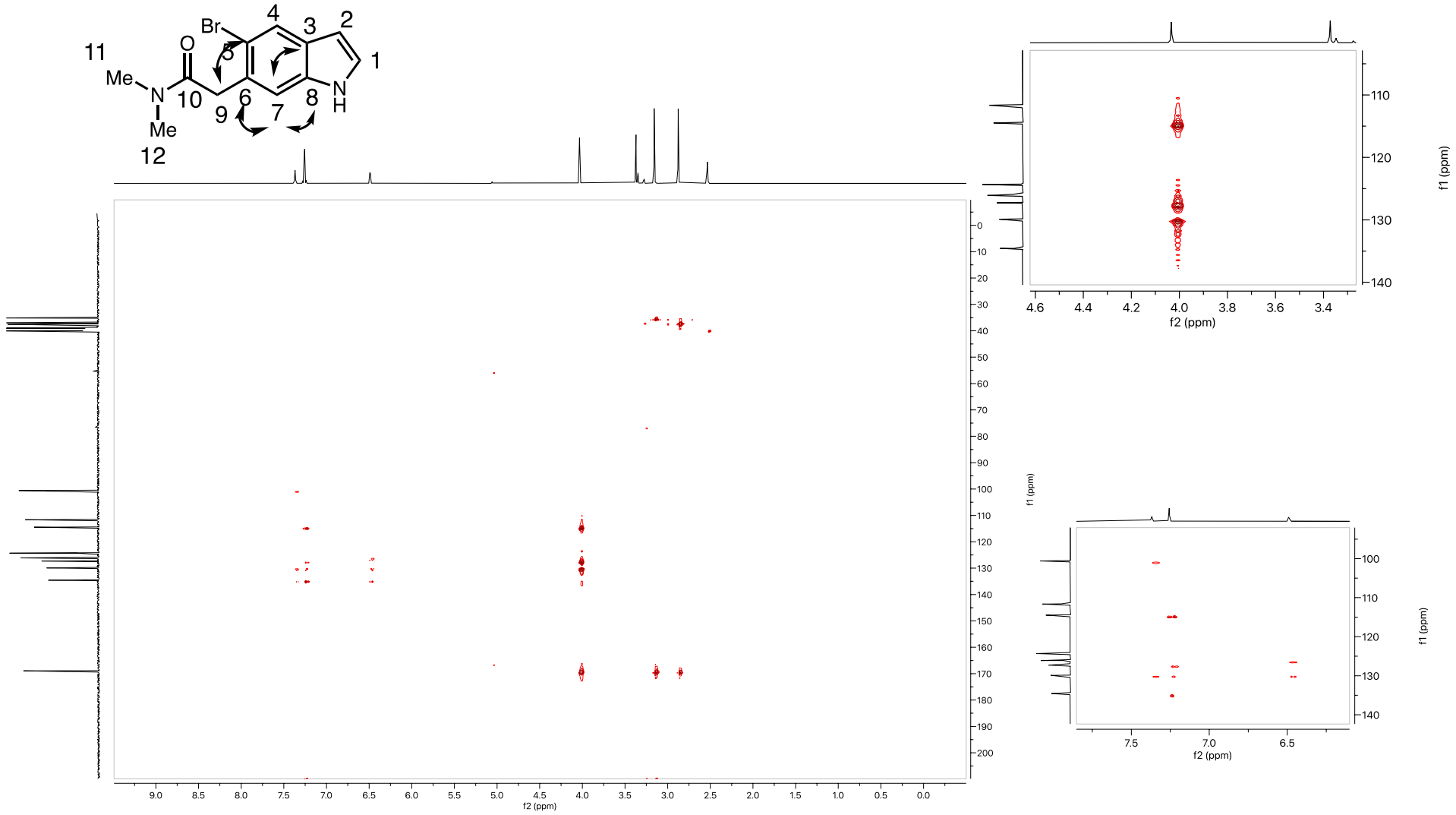

12
11
Me
N
Me
10
O
9
4
3
2
5
1
6
N
H
8
7
Br
f2 (ppm)
f1 (ppm)
f2 (ppm)
f1 (ppm)
f2 (ppm)
f1 (ppm)

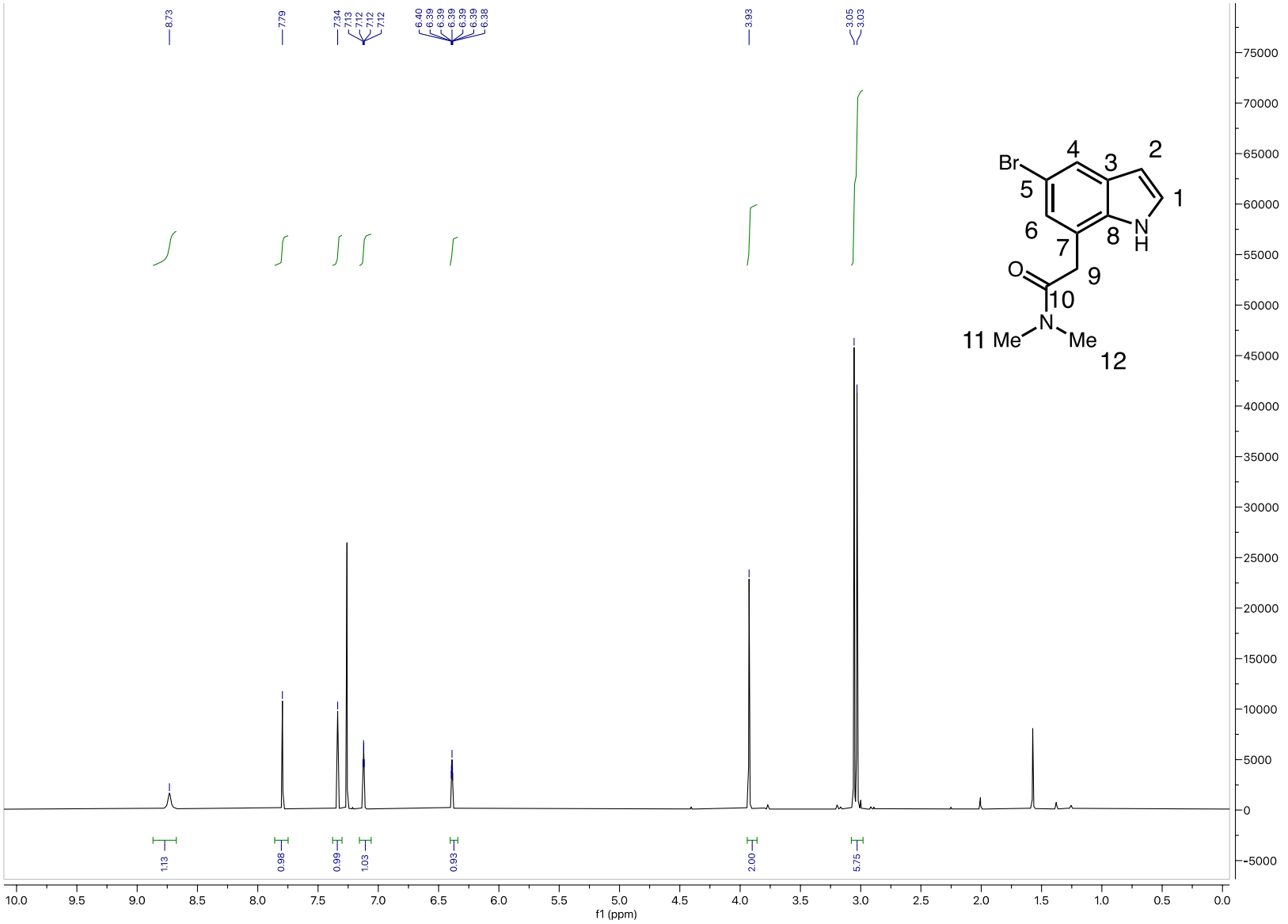

11.23
7.34
7.33
7.33
7.33
7.23
7.22
6.46
6.45
6.45
4.00
3.12
2.84
2.50 DMSO-d6
Br
O
N
Me
Me
N
H
1
2
3
4
5
6
7
8
9
10
11
12
f1 (ppm)

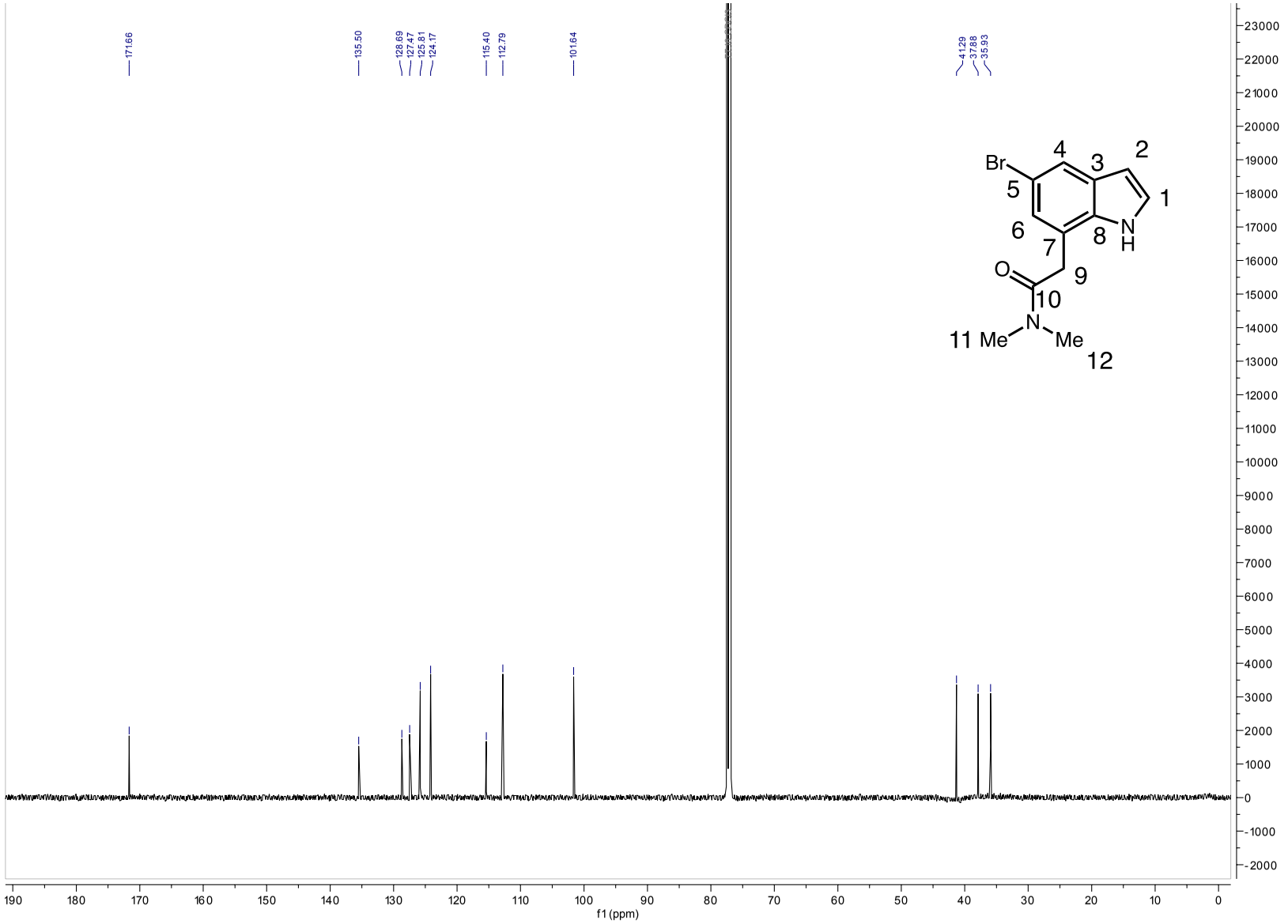

Br
O
Me
N
Me
11
12
10
9
6
7
5
4
3
2
1
8
N
H
168.94
134.57
129.91
127.29
127.28
126.10
124.33
124.31
114.48
111.63
100.59
39.52 DMSO-d6
37.64
36.98
35.14
f1 (ppm)

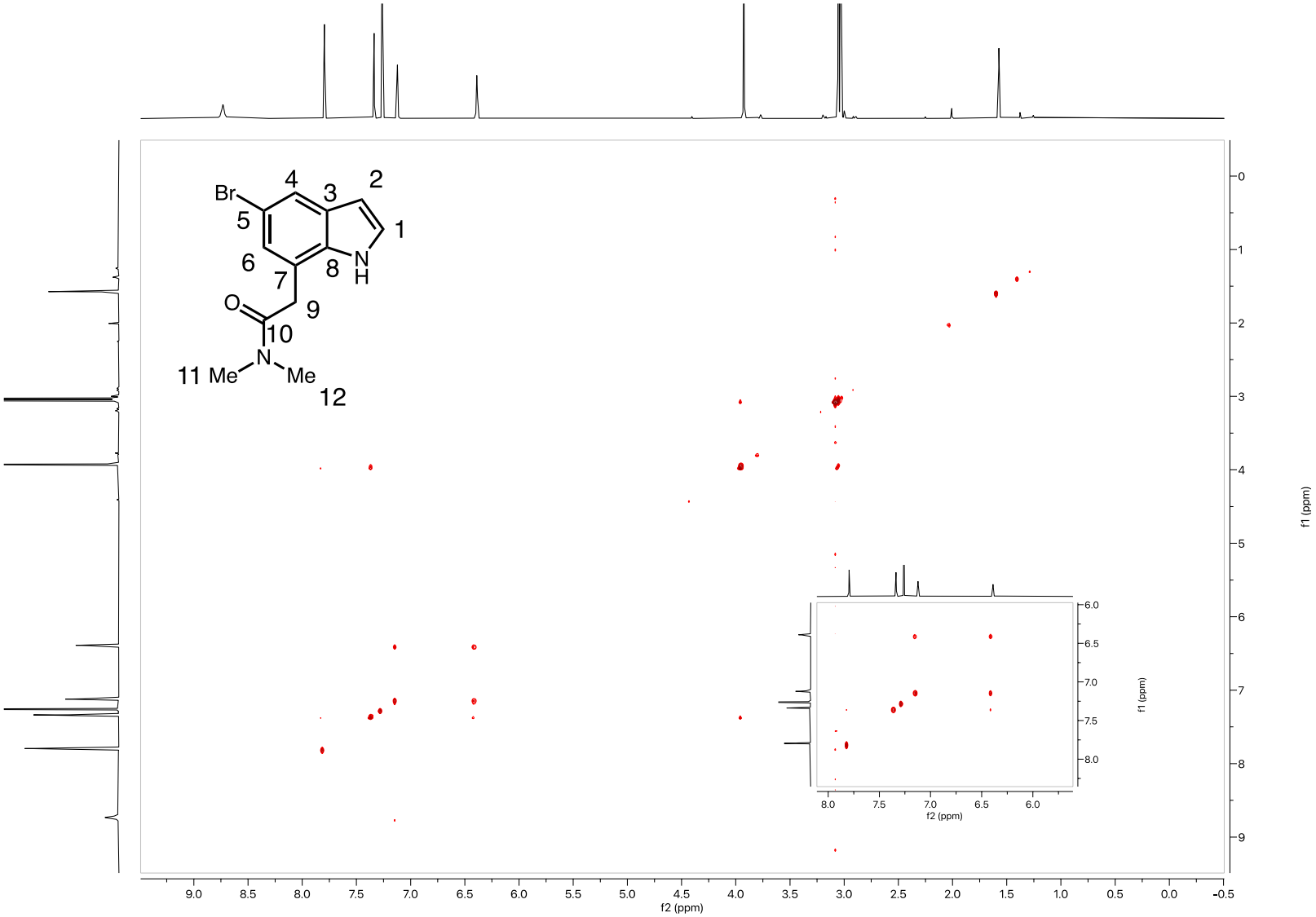

Br
O
N
Me
Me
N
H
1
2
3
4
5
6
7
8
9
10
11
12
{2.50,2.50}DMSO-d6
f2 (ppm)
f1 (ppm)

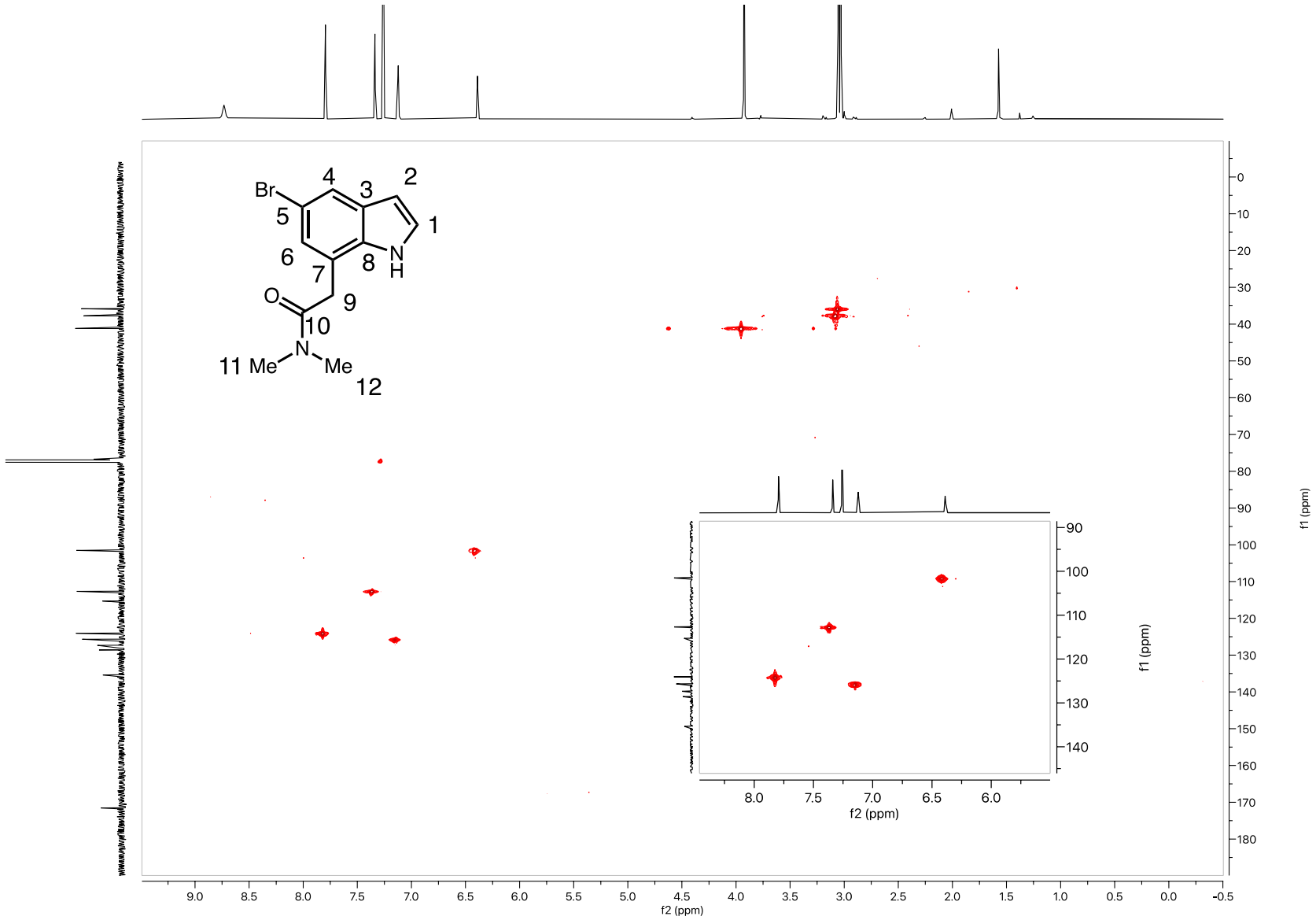

Br
O
Me
N
Me
H
f2 (ppm)
f1 (ppm)

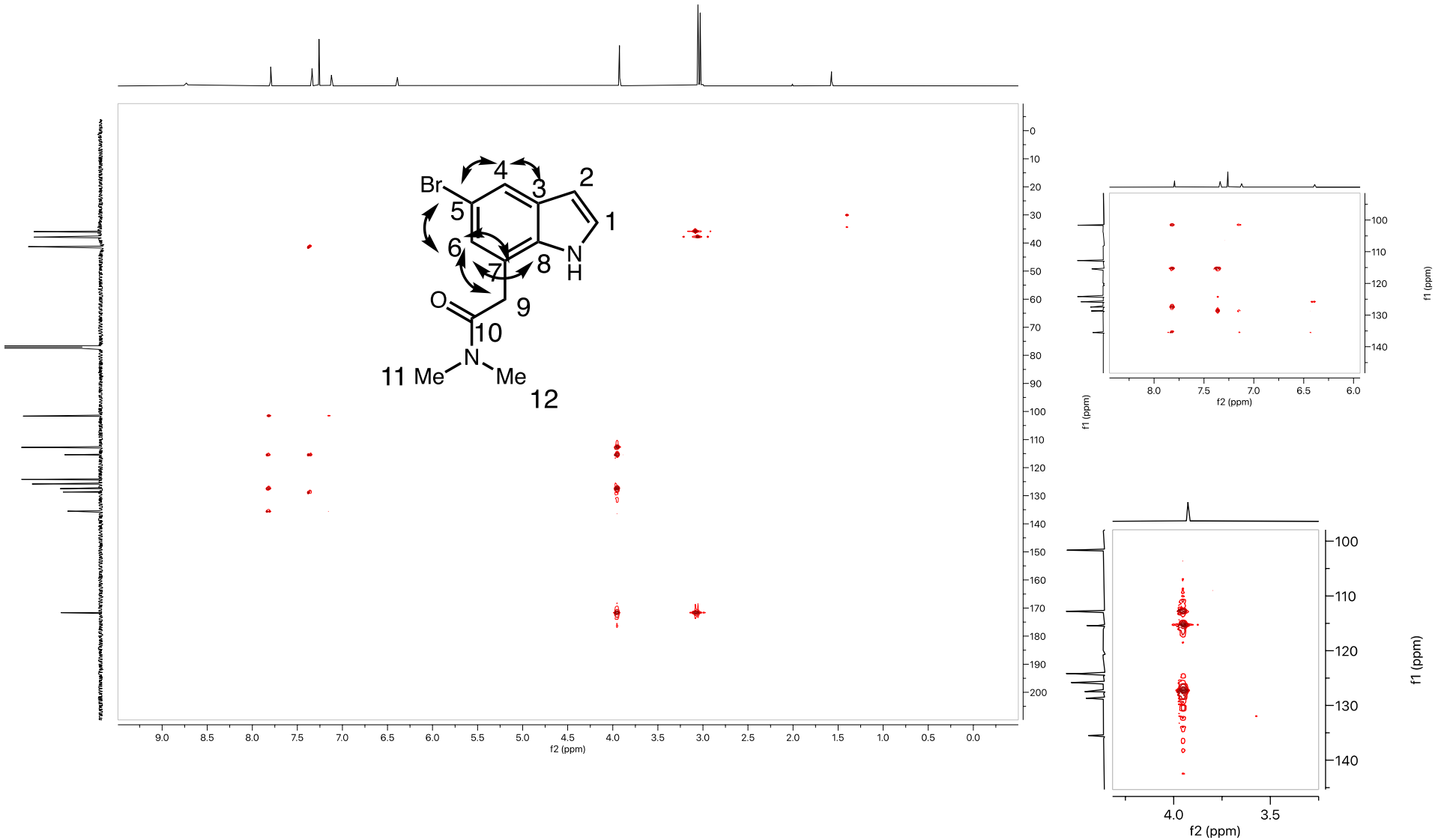

Br
O
N
H
Me
Me
1
2
3
4
5
6
7
8
9
10
11
12
f2 (ppm)
f1 (ppm)

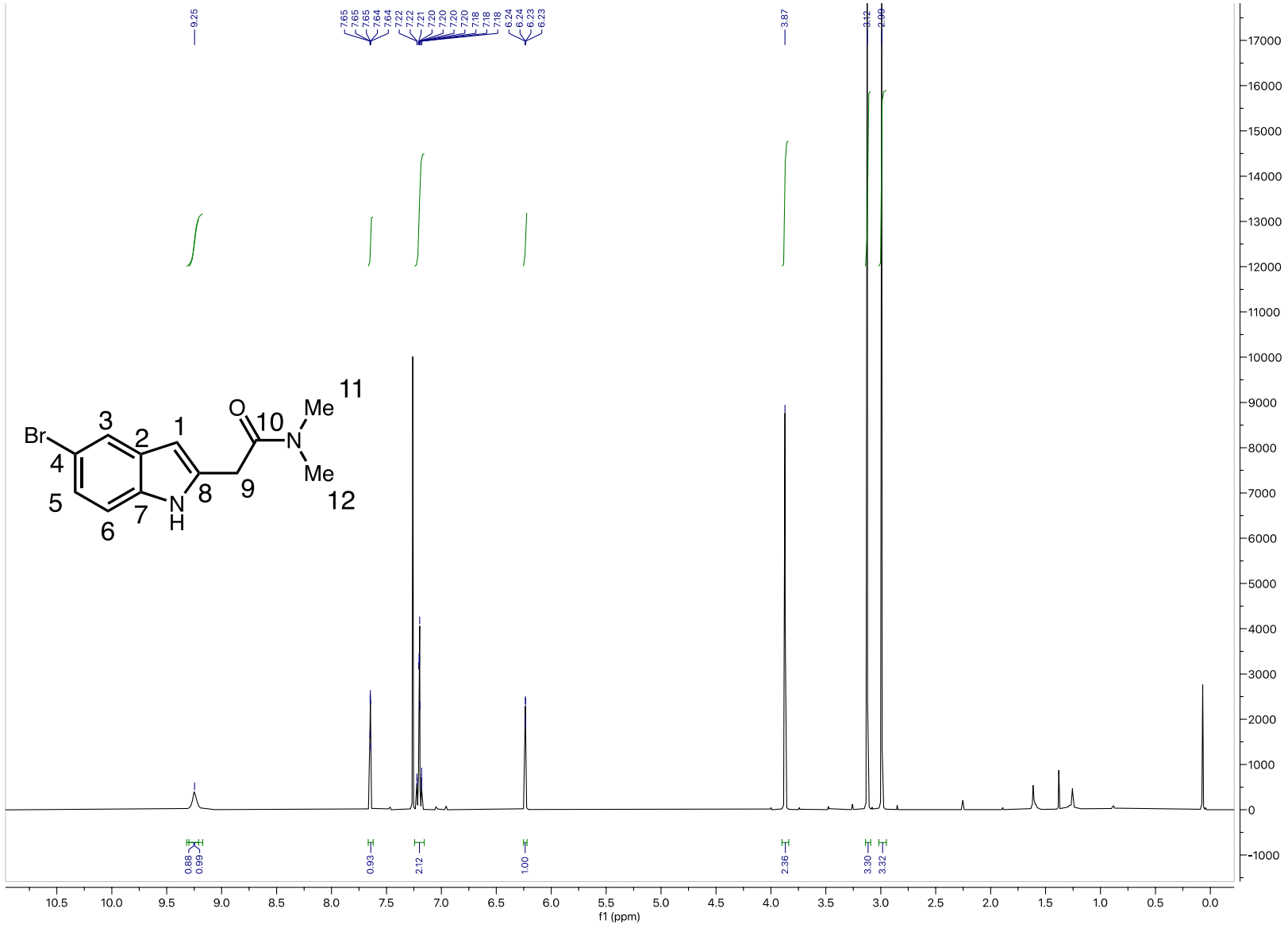

8.73
7.79
7.34
7.13
7.12
7.12
7.12
6.40
6.39
6.39
6.39
6.39
6.39
6.38
3.93
3.05
3.03
Br
1
2
3
4
5
6
7
8
9
10
11 Me
Me
12
N
H
O
1.13
0.98
0.99
1.03
0.93
2.00
5.75
f1 (ppm)

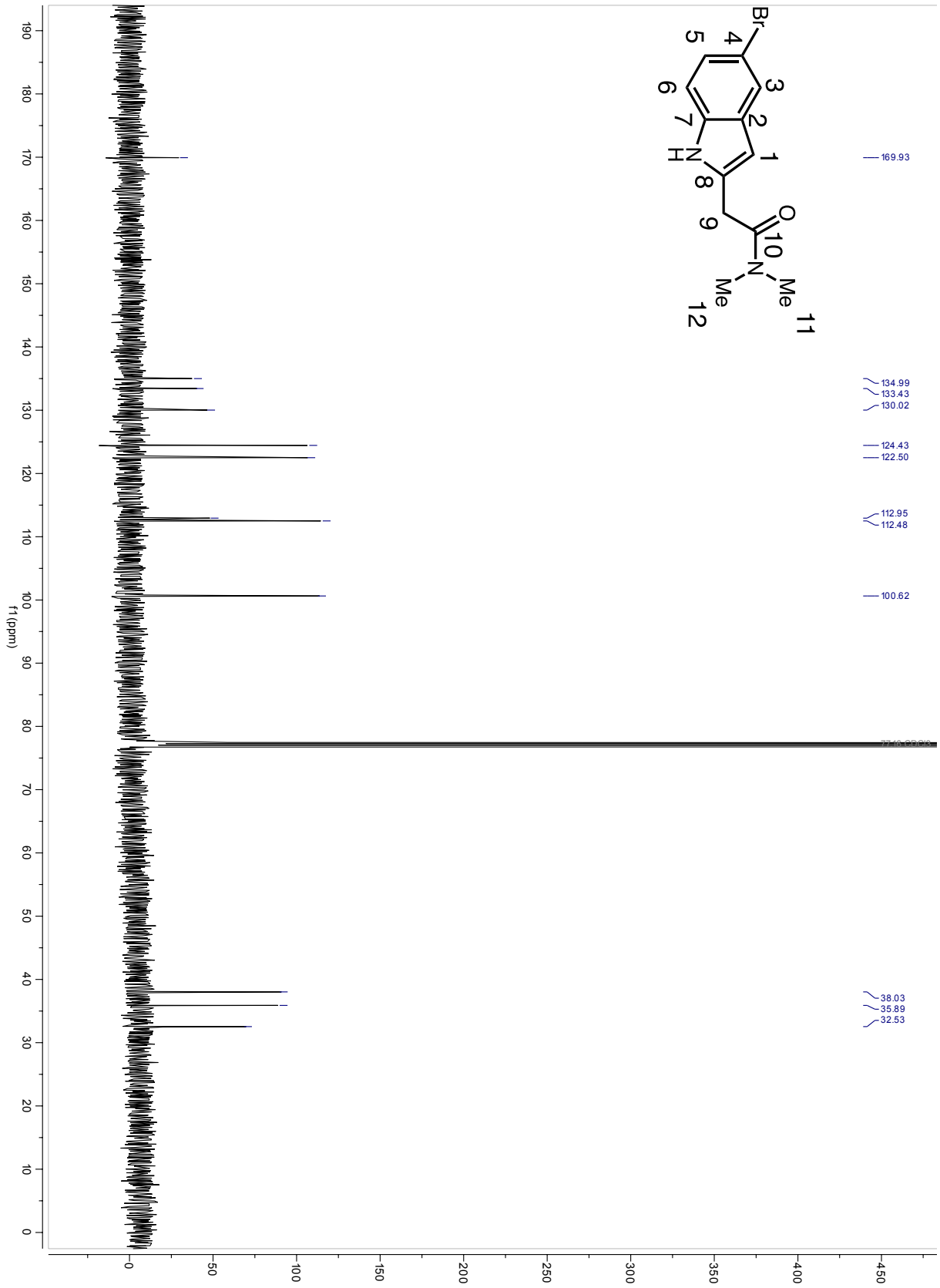

171.66
135.50
128.69
127.47
125.81
124.17
115.40
112.79
101.64
41.29
37.88
35.93
Br
1
2
3
4
5
6
7
8
9
10
11 Me
Me
12
N
H
O
f1 (ppm)

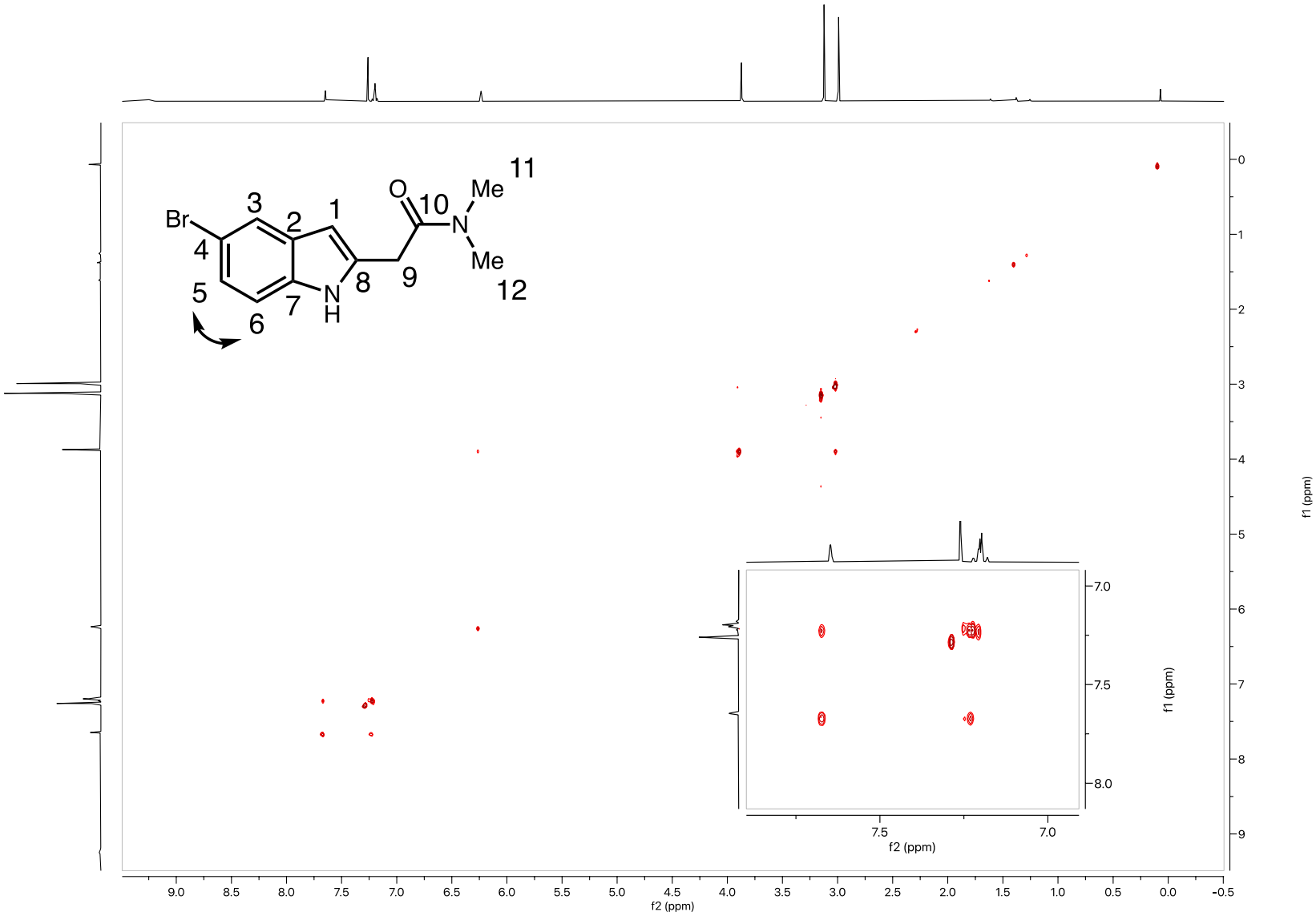

Br
4
3
2
5
1
6
8
N
H
7
9
O
10
11 Me
N
Me
12
f2 (ppm)
f1 (ppm)
f2 (ppm)
f1 (ppm)

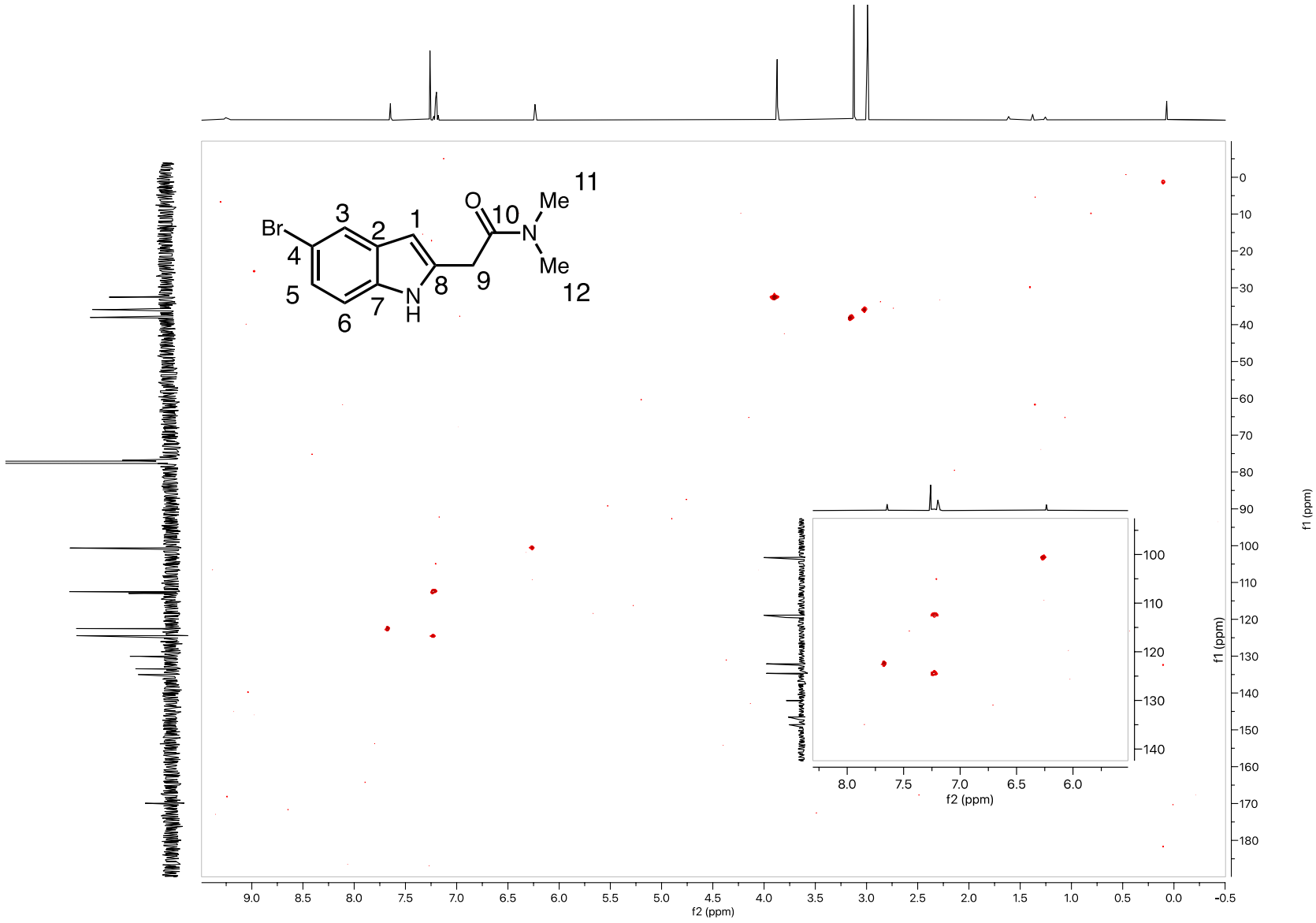

Br
4
3
2
5
1
6
7
8
N
H
O
9
10
N
11 Me
Me
12
f2 (ppm)
f1 (ppm)
f2 (ppm)
f1 (ppm)

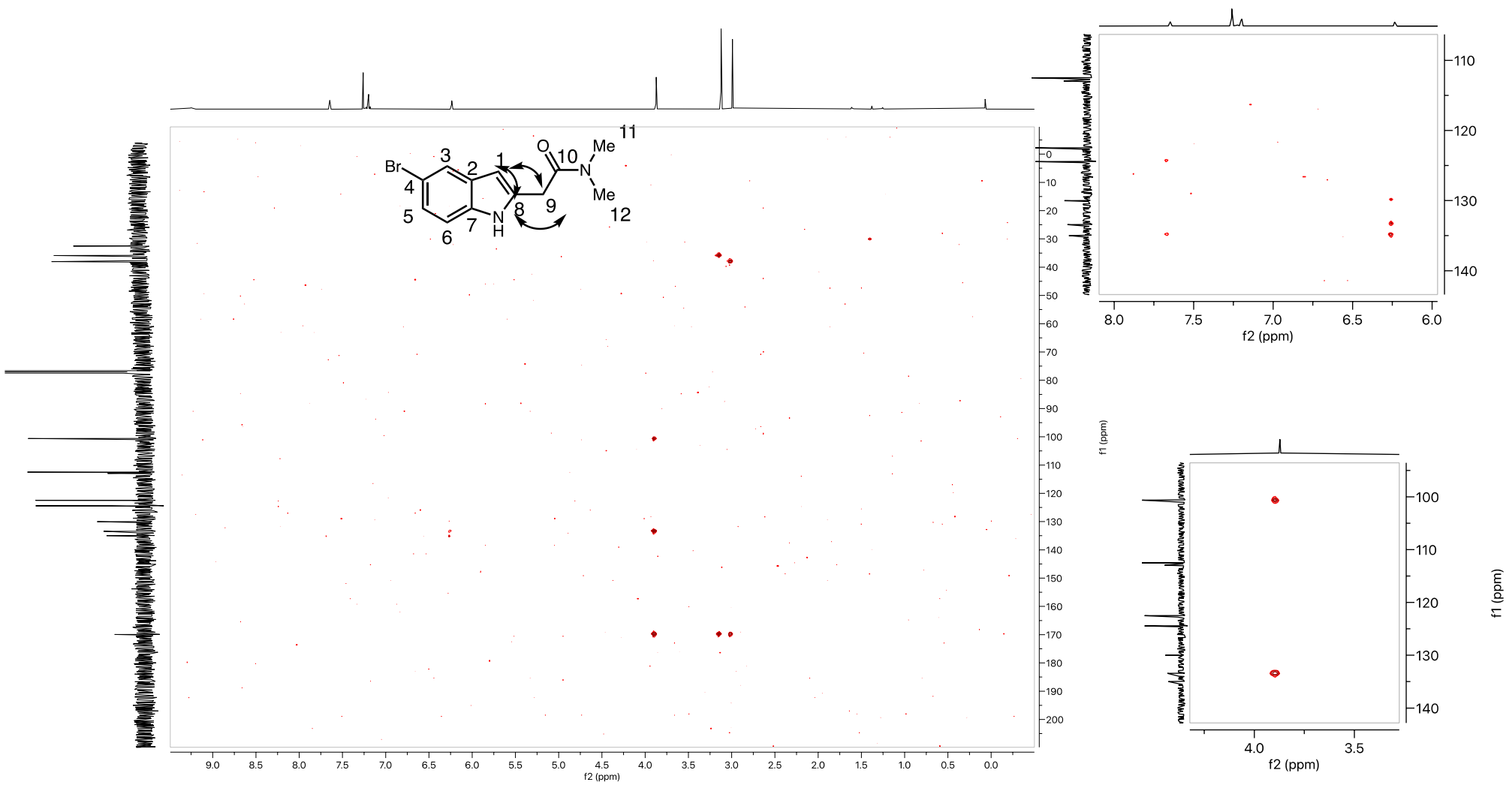

Br
1
2
3
4
5
6
7
8
N
H
9
O
10
N
11 Me
Me
12
f2 (ppm)
f1 (ppm)

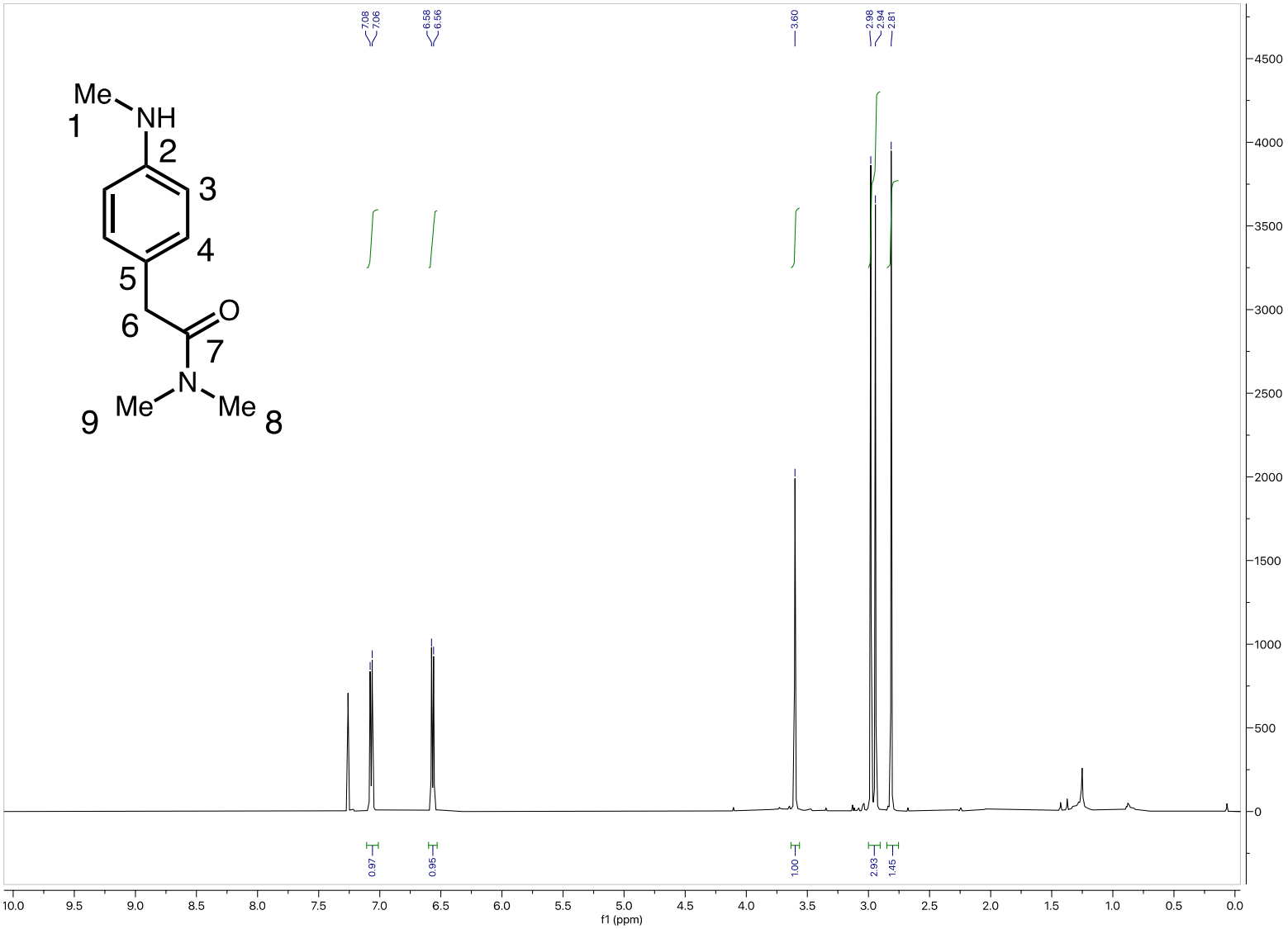

9.25
7.65
7.65
7.65
7.64
7.64
7.22
7.22
7.21
7.20
7.20
7.20
7.20
7.18
7.18
7.18
6.24
6.24
6.23
6.23
3.87
3.12
2.99
Br
1
2
3
4
5
6
7
8
9
10
11
12
N
H
O
Me
Me
0.88
0.99
0.93
2.12
1.00
2.36
3.30
3.32
f1 (ppm)

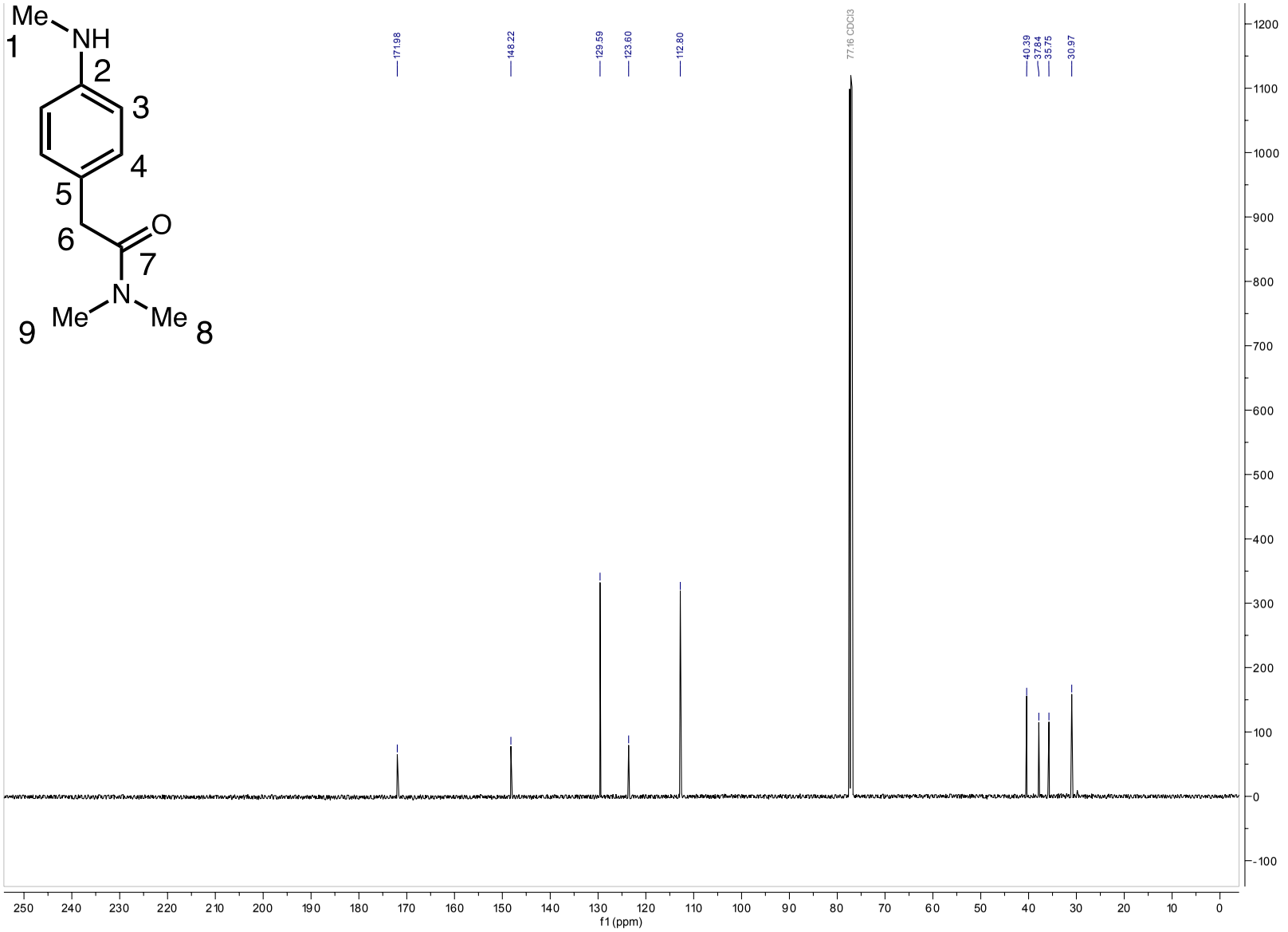

Br
1
2
3
4
5
6
7
N
H
8
9
O
10
N
Me
Me
11
12
169.93
134.99
133.43
130.02
124.43
122.50
112.95
112.48
100.62
38.03
35.89
32.53
f1 (ppm)

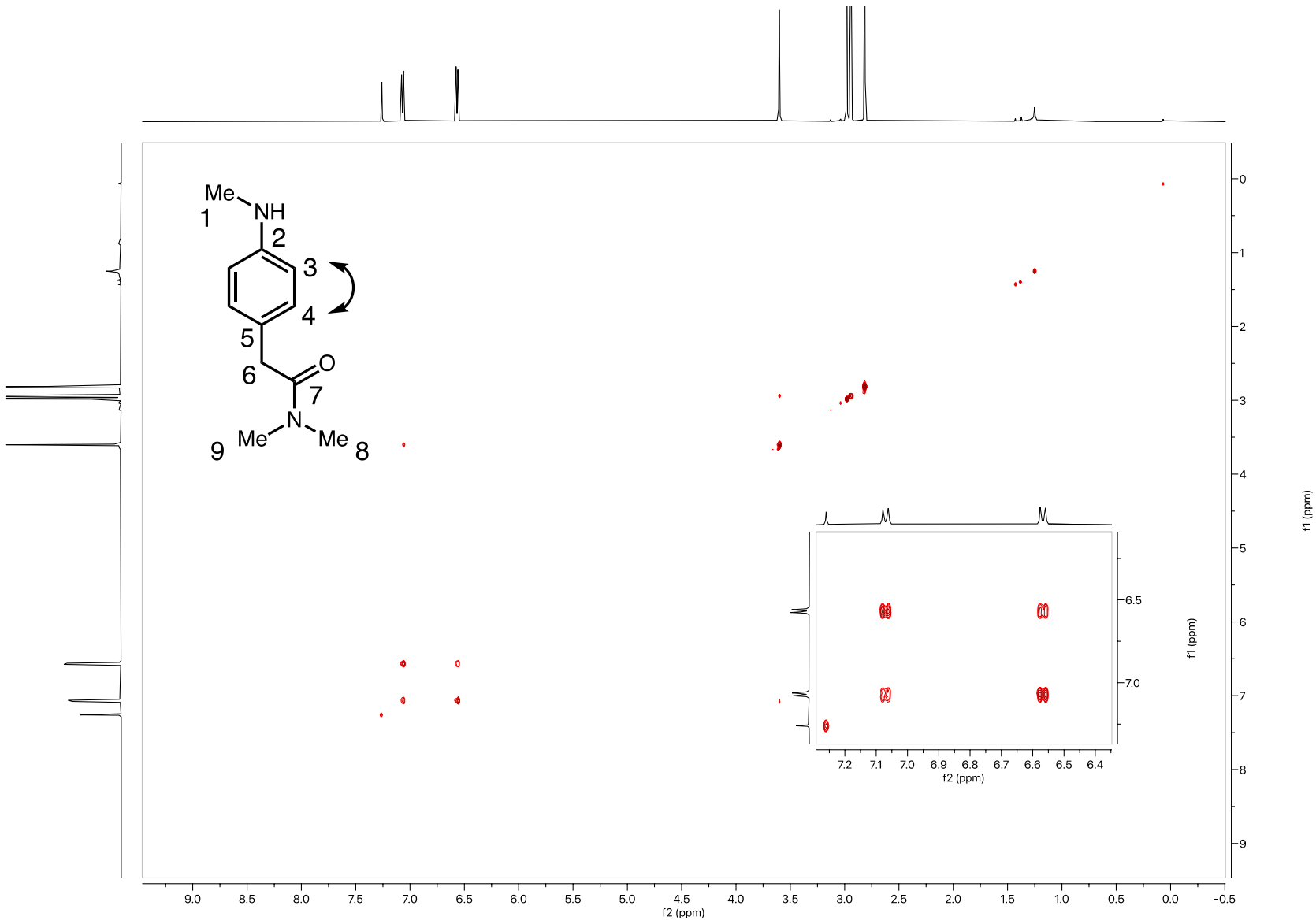

Br
1
2
3
4
5
6
7
8
9
10
11
12
O
N
Me
Me
H
f2 (ppm)
f1 (ppm)

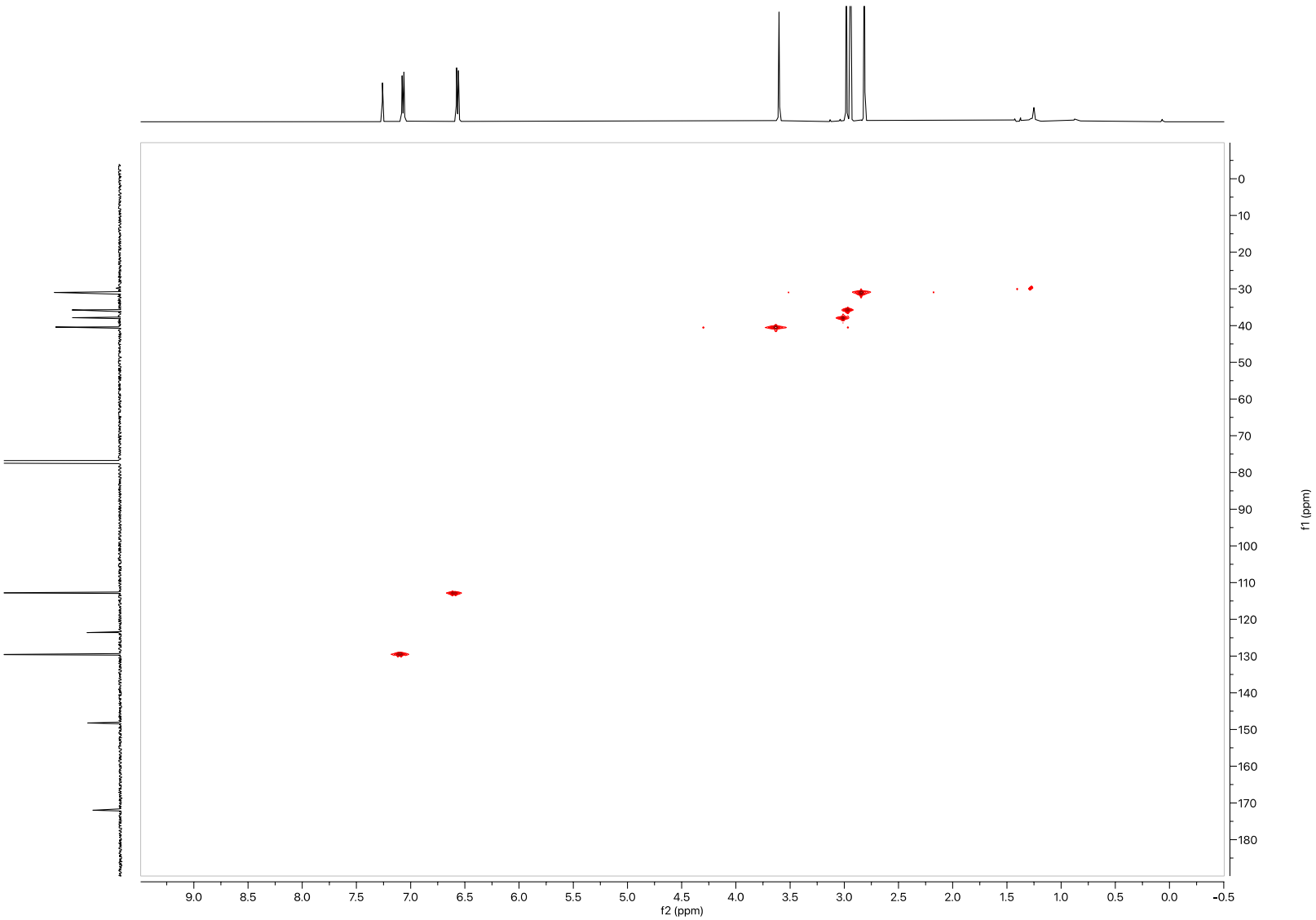

Br
O
Me
Me
N
H
f2 (ppm)
f1 (ppm)

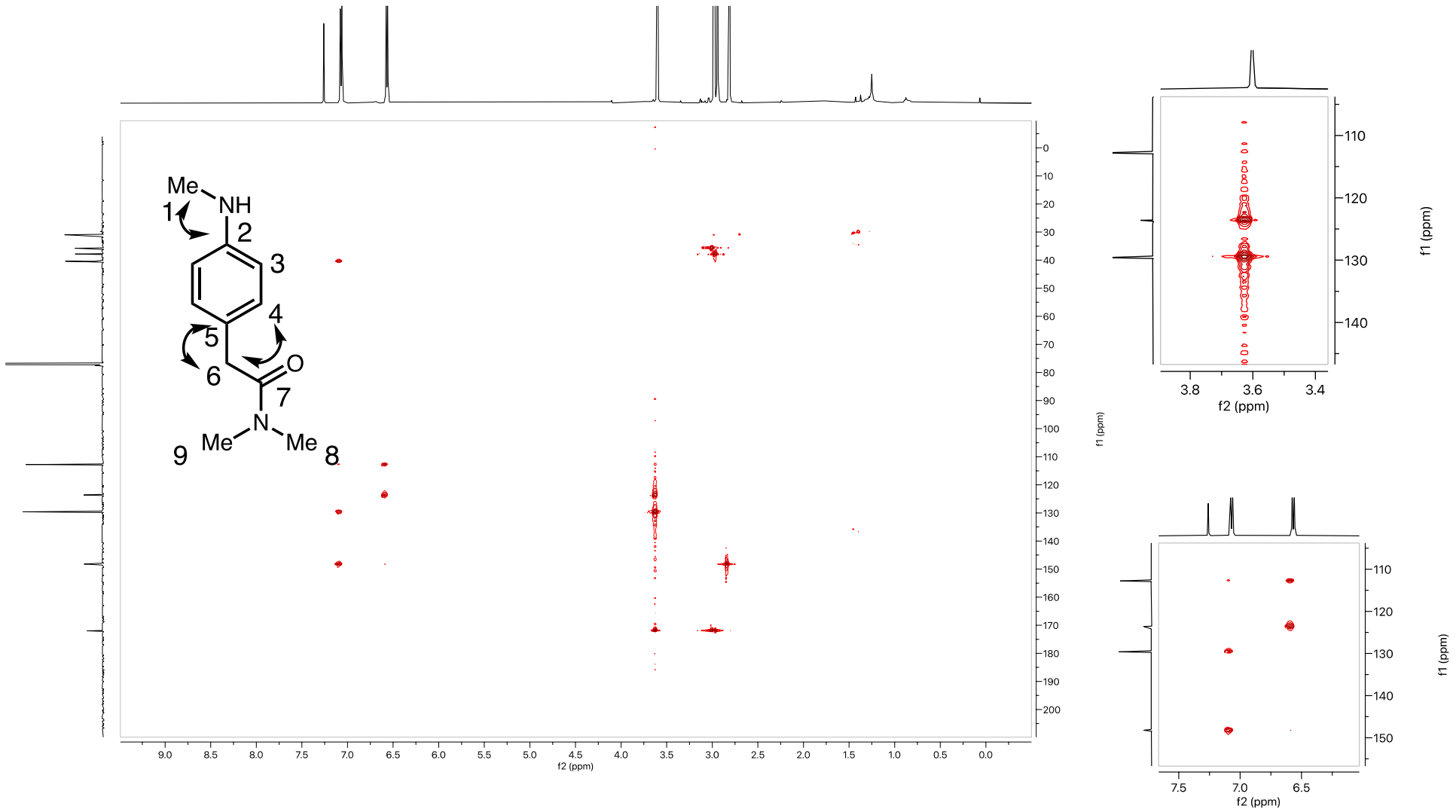

Br
1
2
3
4
5
6
7
8
9
10
11
12
N
H
O
Me
Me
f2 (ppm)
f1 (ppm)

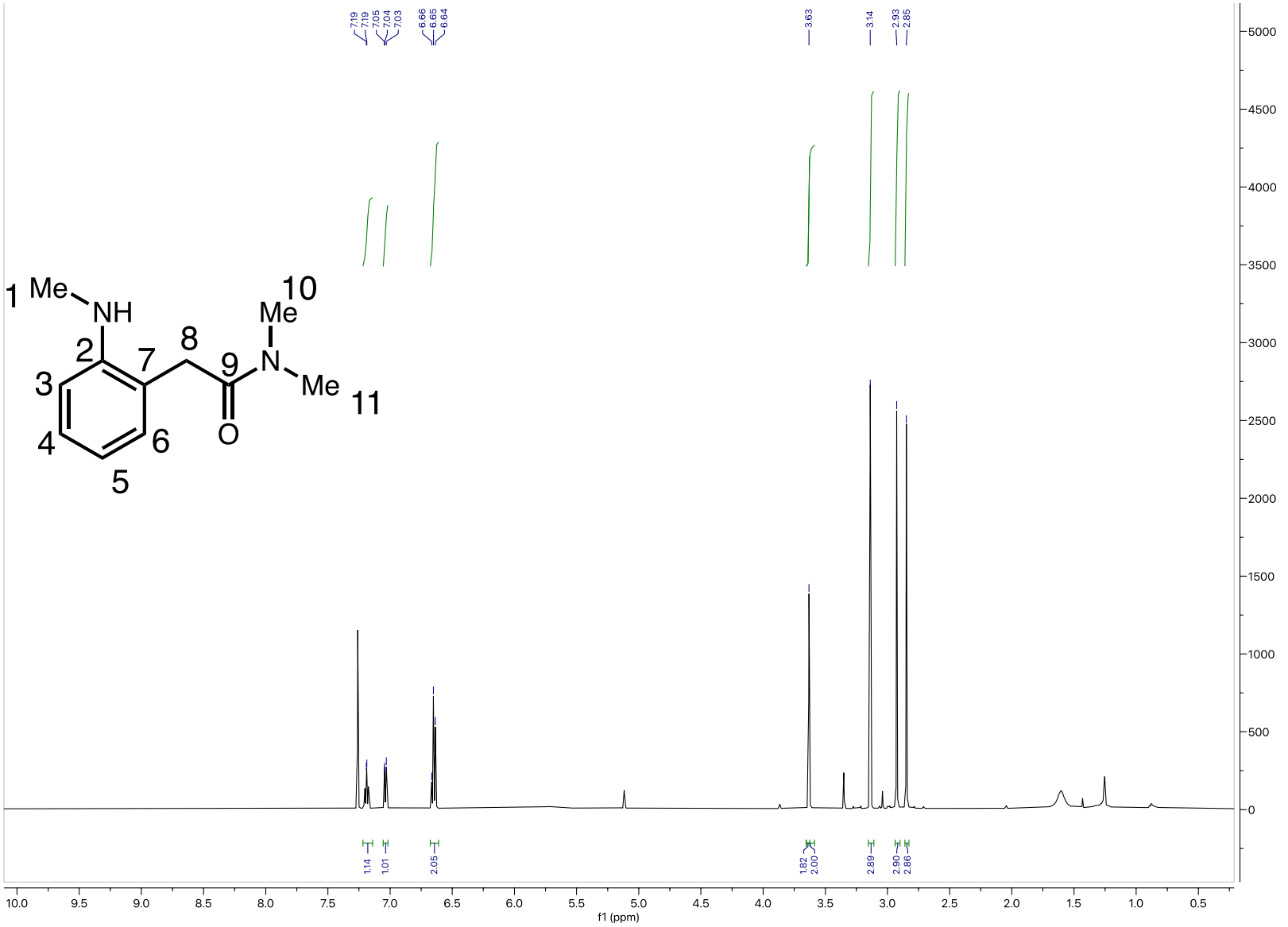

7.08
7.06
6.58
6.56
3.60
2.98
2.94
2.81
Me
NH
1
2
3
4
5
6
O
7
N
Me
Me
9
8
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0.97
0.95
1.00
2.93
1.45
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

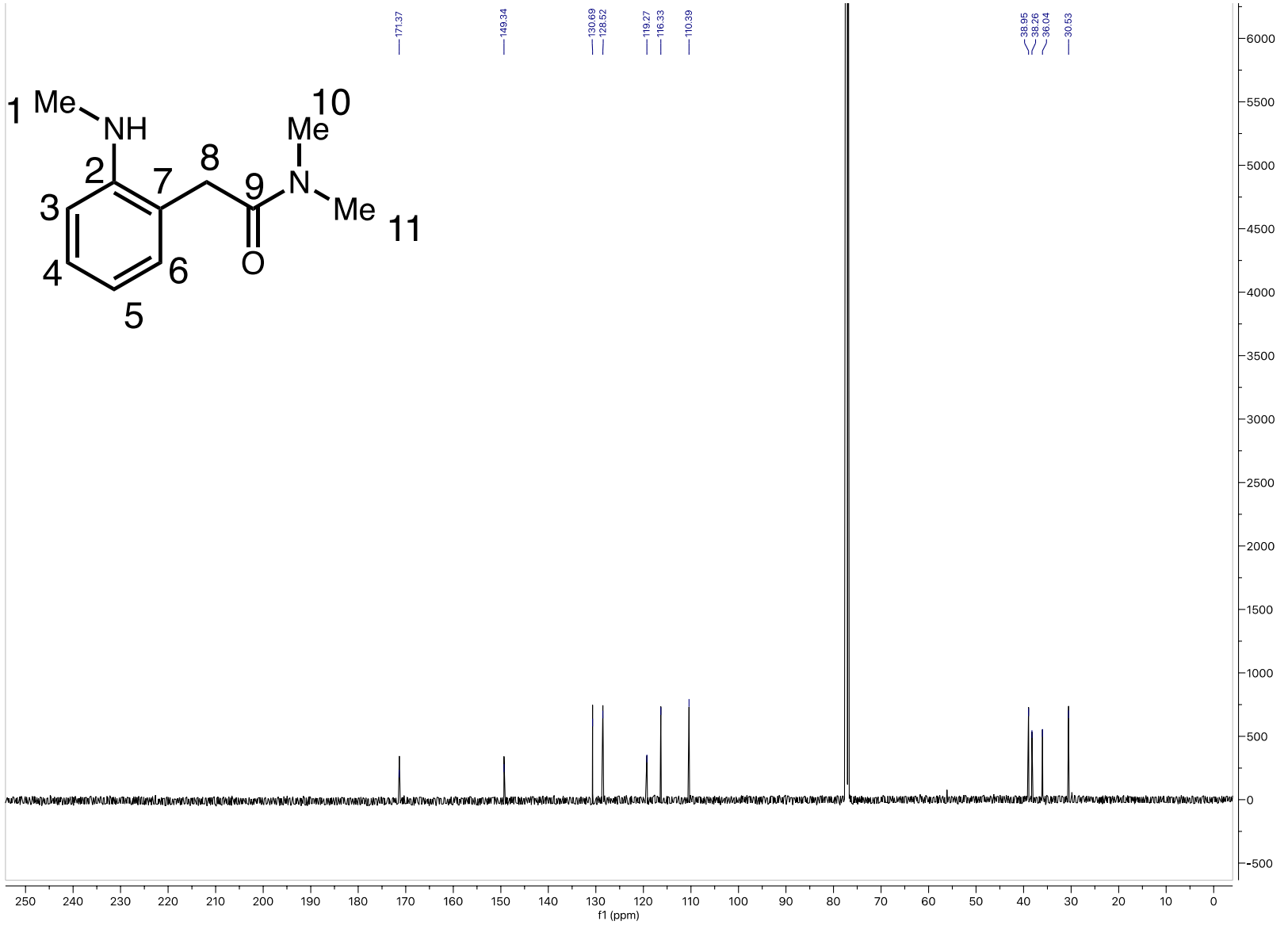

Me
NH
1
2
3
4
5
6
O
7
N
Me
Me
9
8
171.98
148.22
129.59
123.60
112.80
77.16 CDCl3
40.39
37.84
35.75
30.97
f1 (ppm)

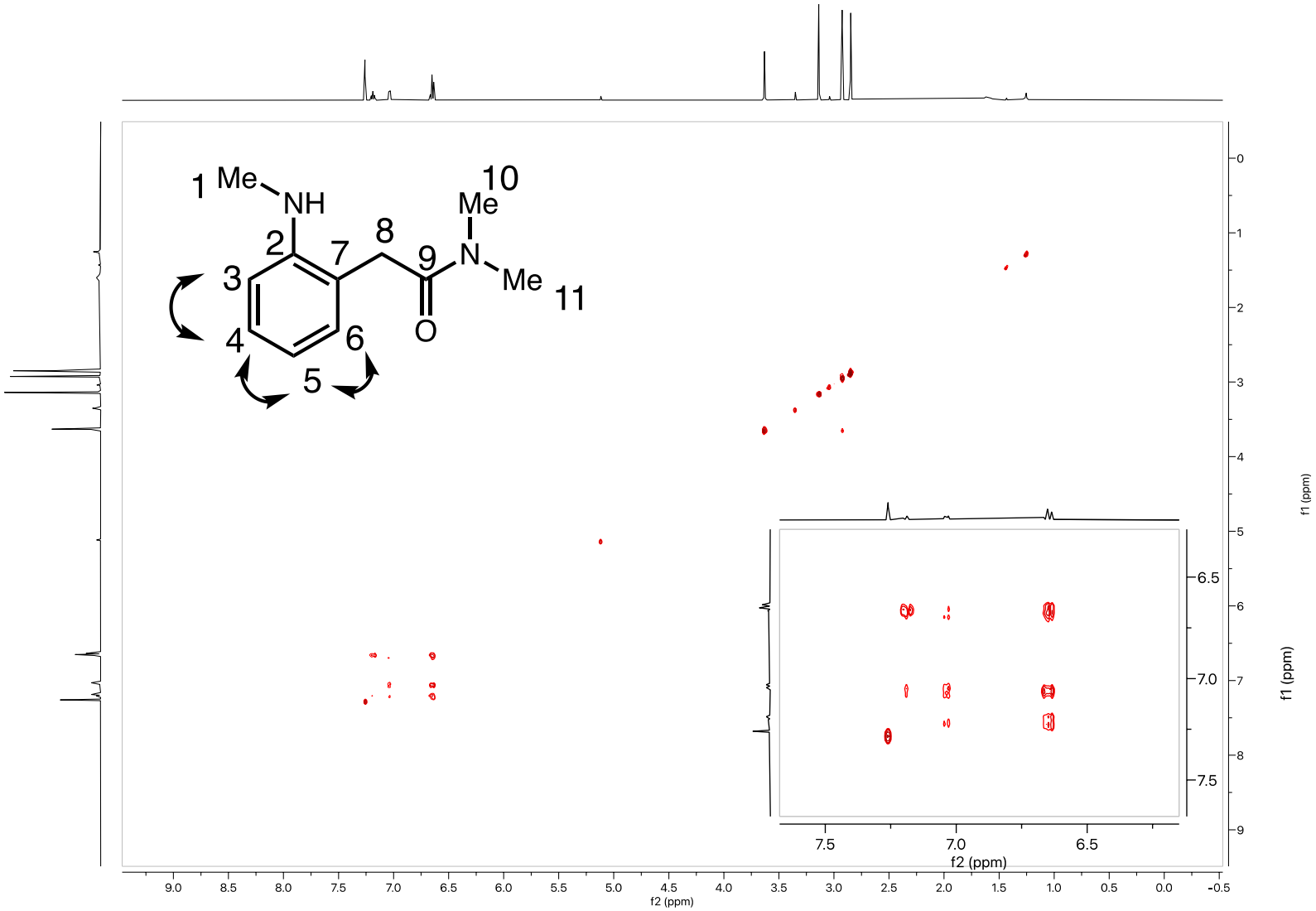

Me
NH
1
2
3
4
5
6
O
7
N
Me
Me
9
8
f2 (ppm)
f1 (ppm)
f2 (ppm)
f1 (ppm)

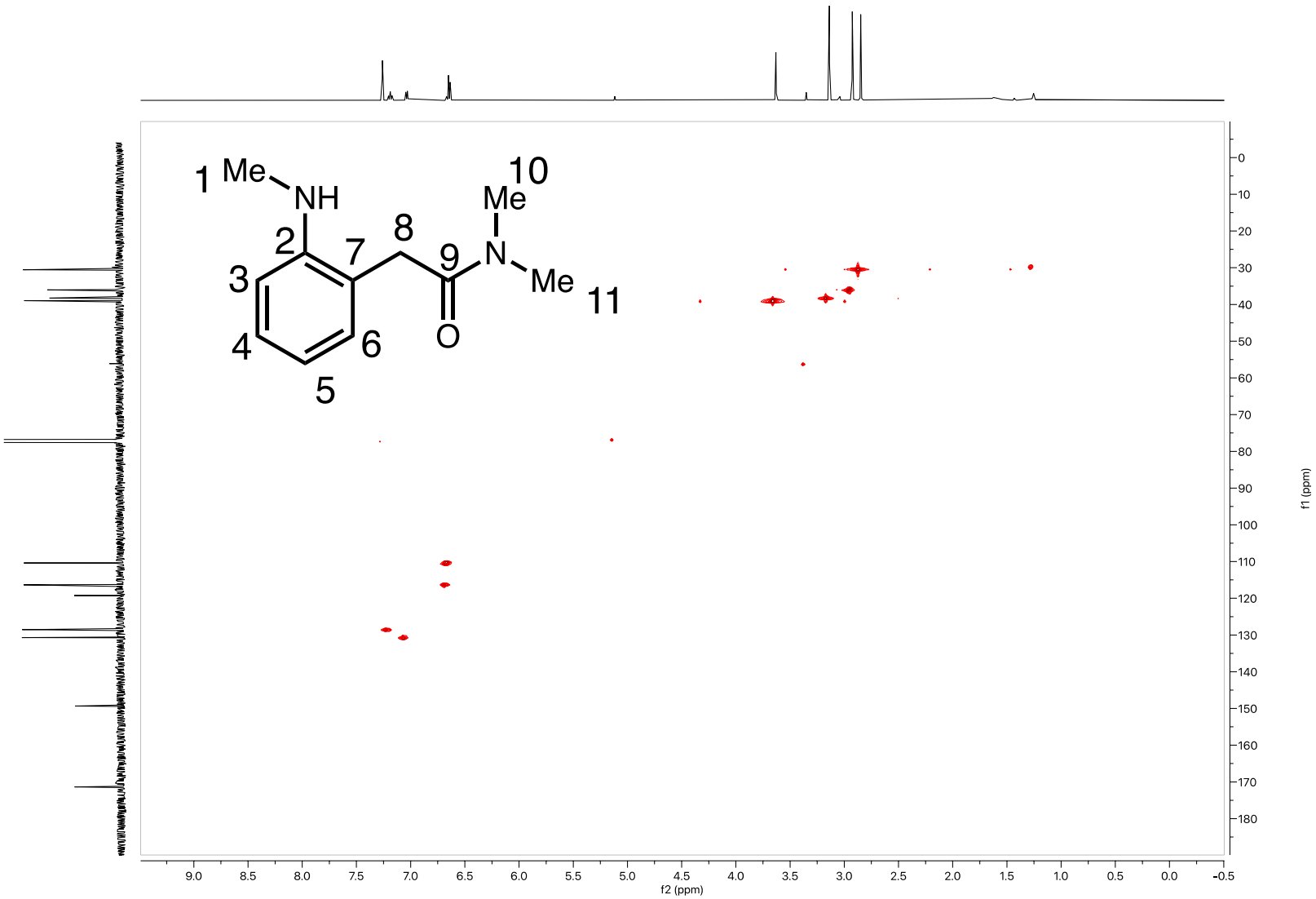

9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f2 (ppm)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
f1 (ppm)

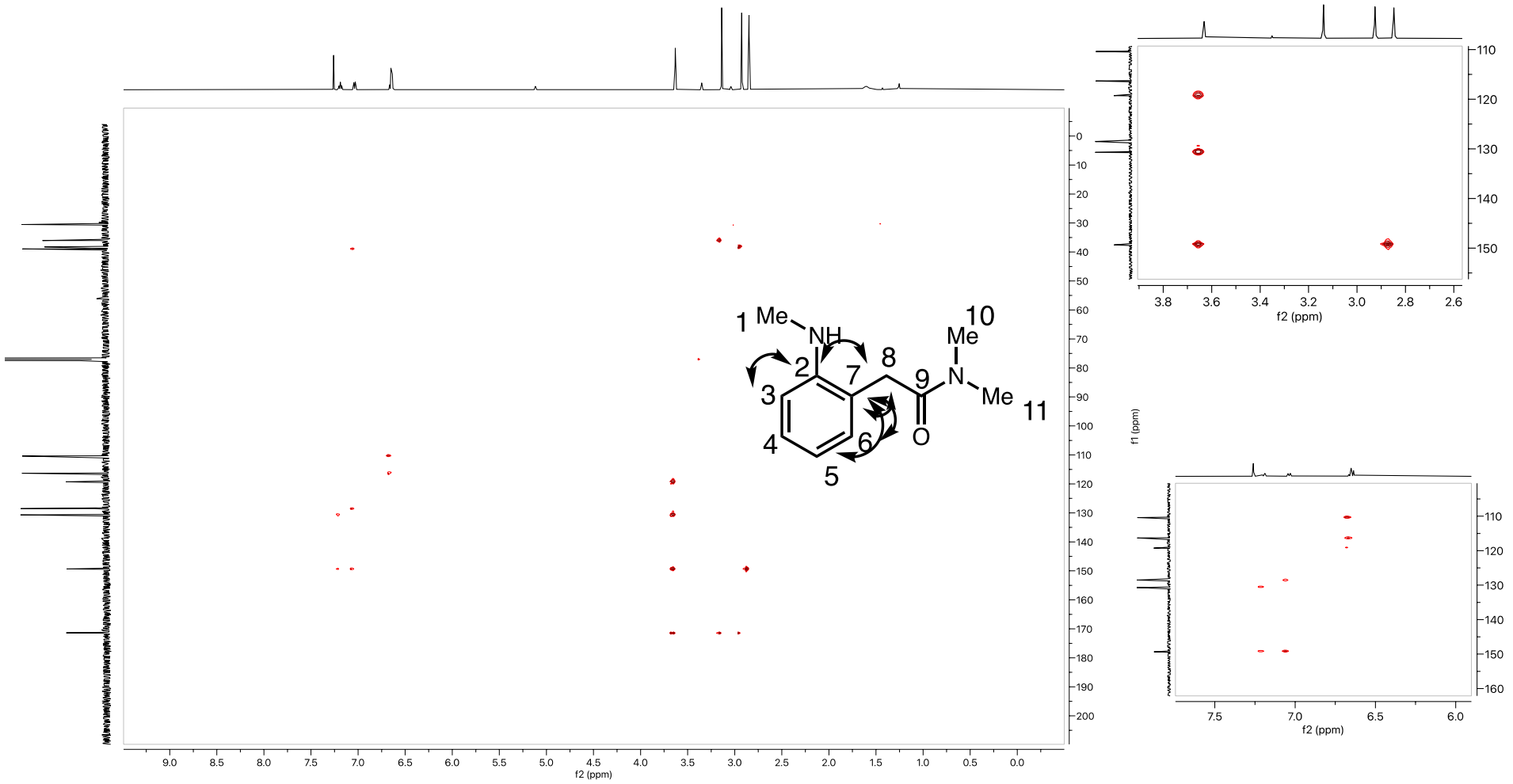

Me
NH
1
2
3
4
5
6
O
7
N
Me
Me
9
8
f2 (ppm)
f1 (ppm)
f2 (ppm)
f1 (ppm)
f2 (ppm)
f1 (ppm)

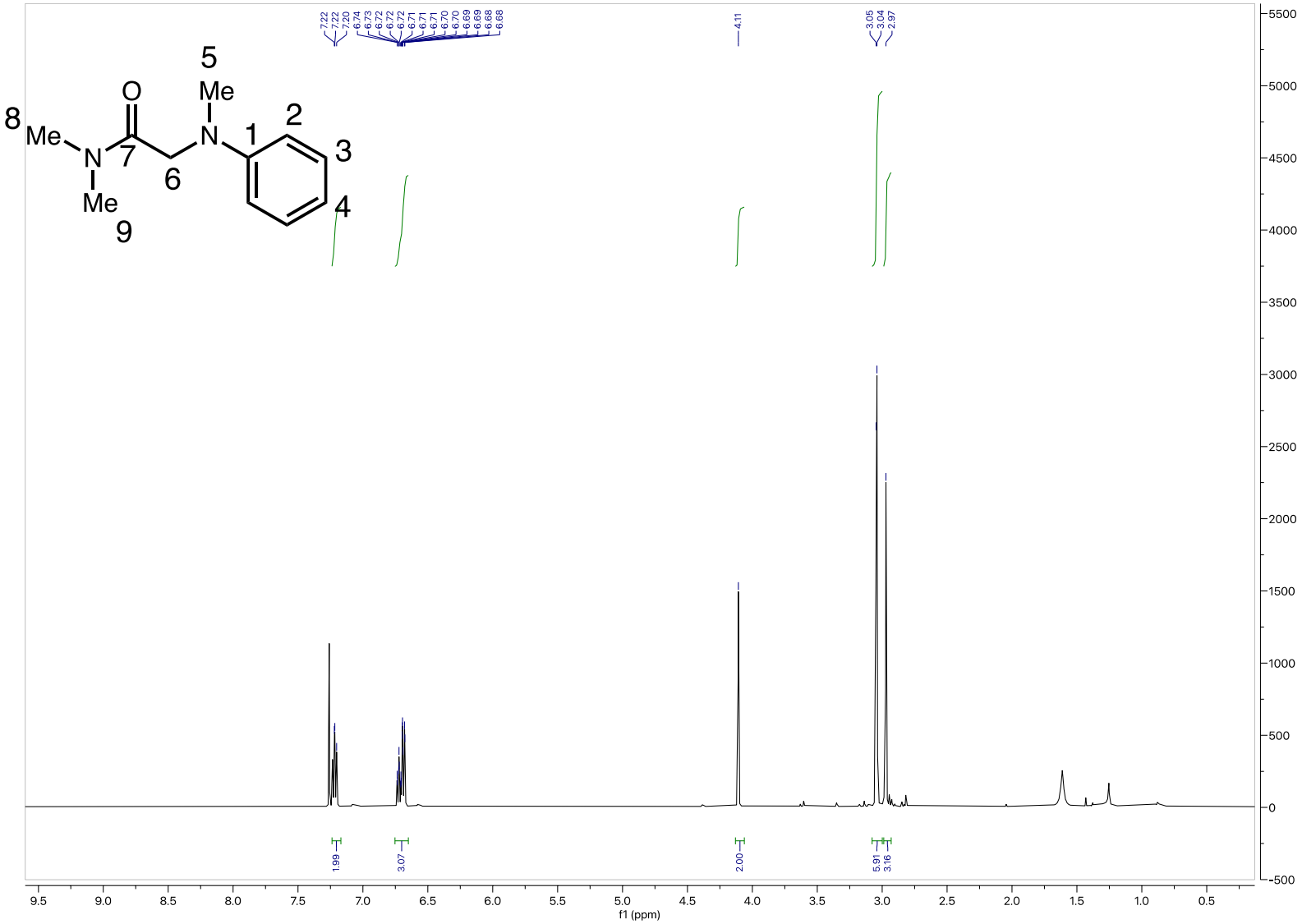

7.19
7.19
7.05
7.04
7.03
6.66
6.65
6.64
3.63
3.14
2.93
2.85
1 Me
NH
2
3
4
5
6
7
8
9
10
Me
N
Me
11
Me
O
1.14
1.01
2.05
1.82
2.00
2.89
2.90
2.86
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

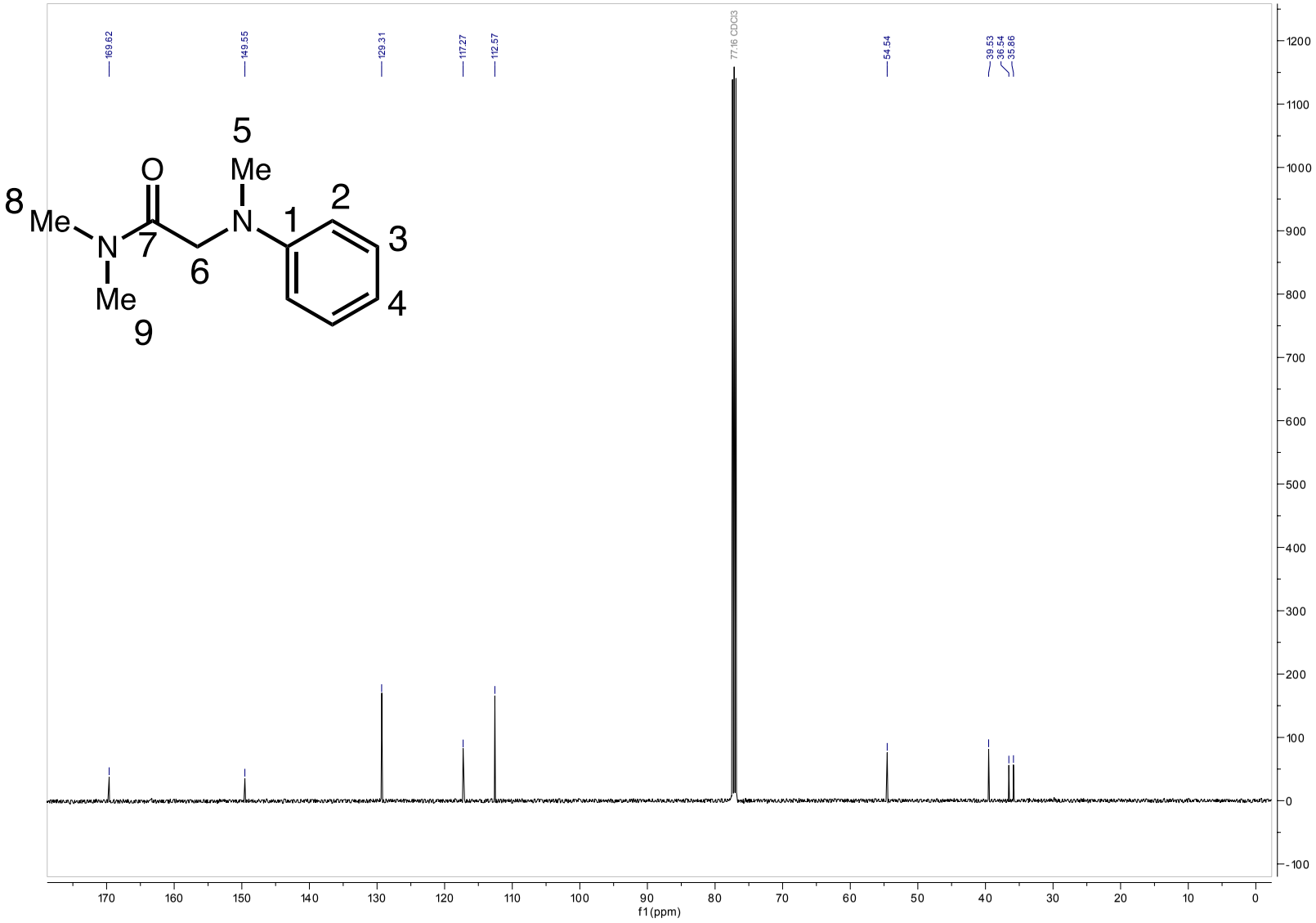

171.37
149.34
130.69
128.52
119.27
116.33
110.39
38.95
38.26
36.04
30.53
1
Me
NH
2
3
4
5
6
7
8
9
N
10
Me
Me
11
O
f1 (ppm)

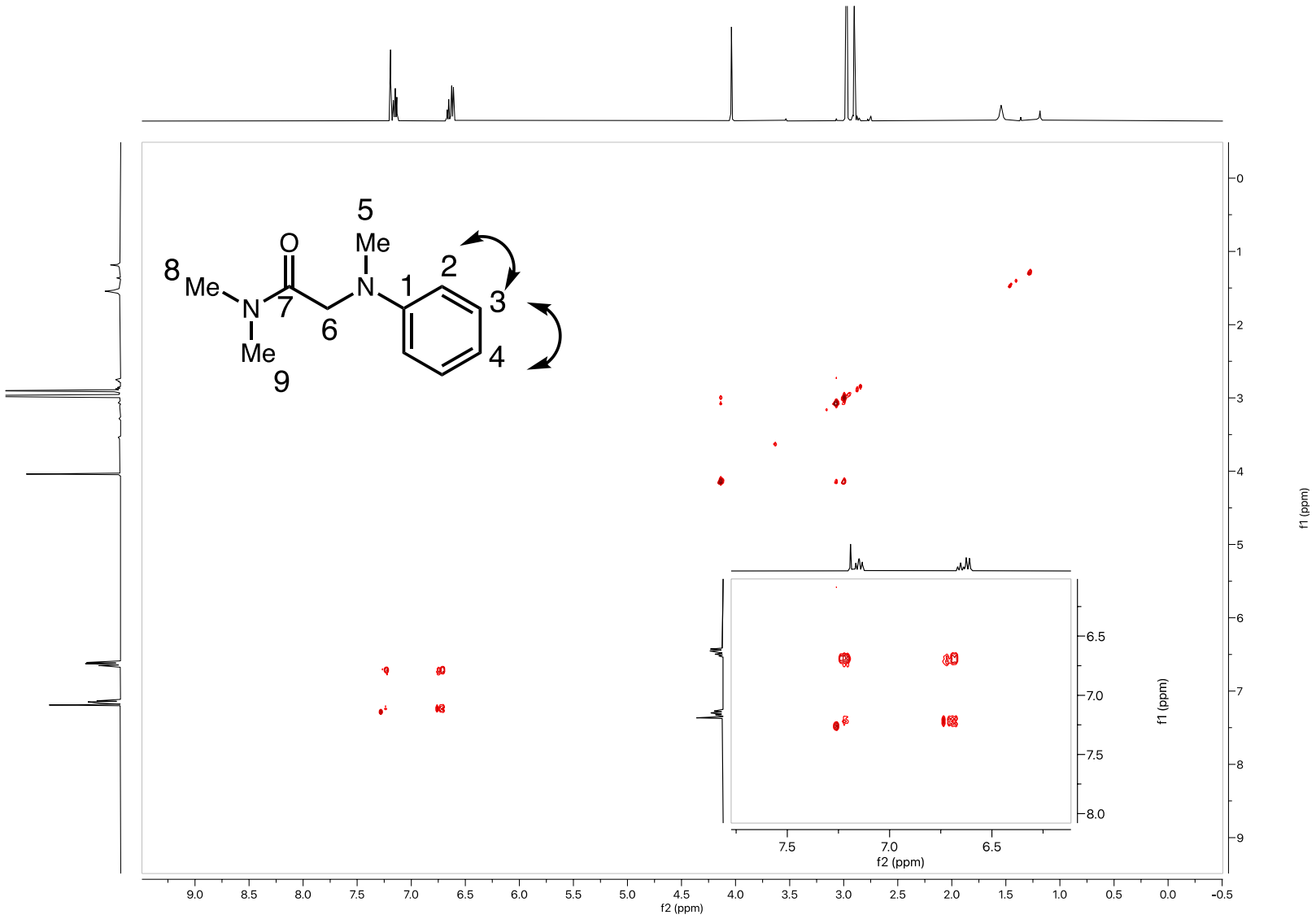

1 Me
NH
2
7
8
10
Me
9
N
Me
11
3
4
6
O
5
f2 (ppm)
f1 (ppm)

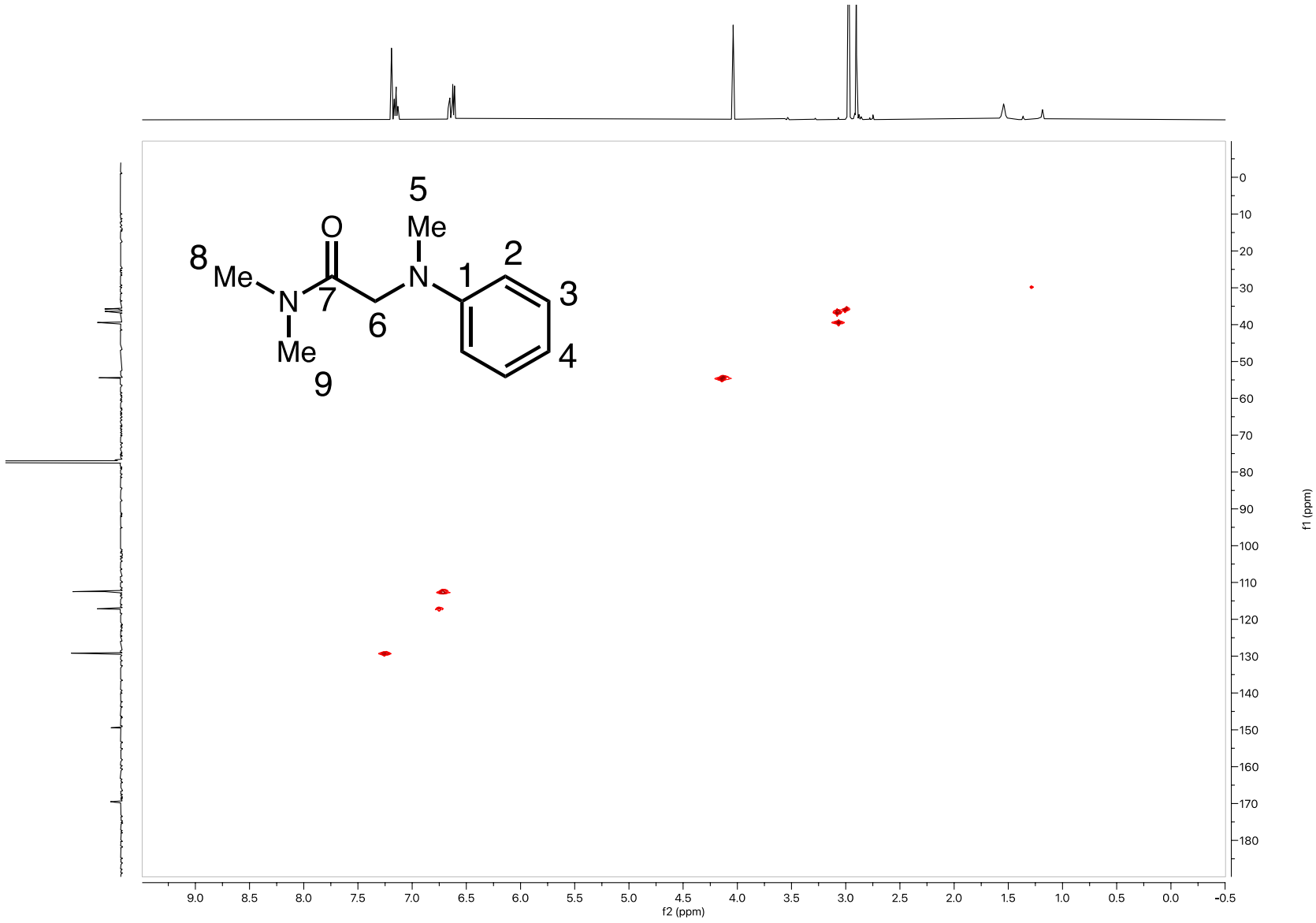

1
Me
NH
2
3
4
5
6
7
8
9
10
Me
N
Me
11
O
f2 (ppm)
f1 (ppm)

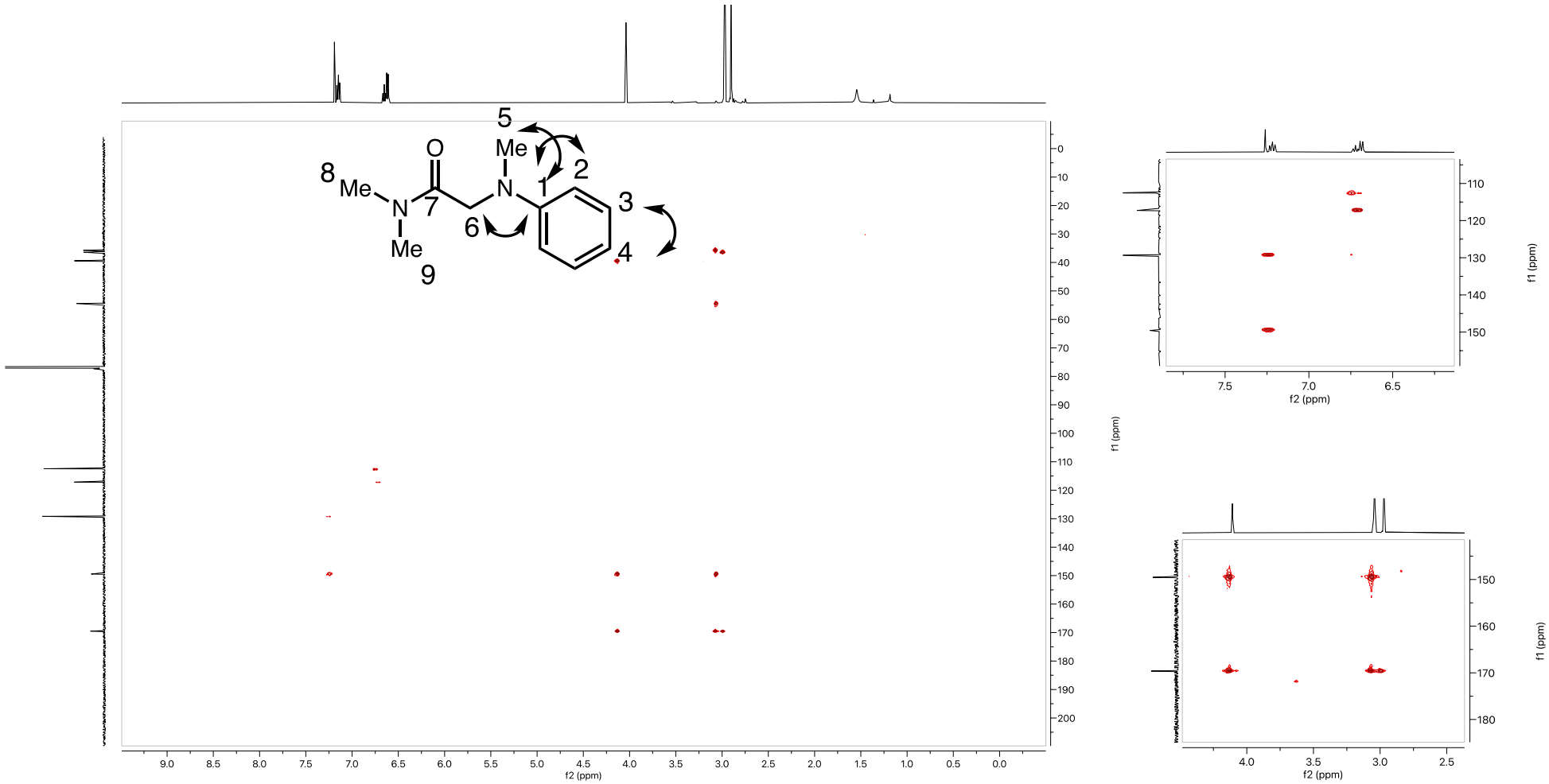

1
Me
NH
2
3
4
5
6
7
8
9
10
Me
N
Me
11
O
f2 (ppm)
f1 (ppm)
f2 (ppm)
f2 (ppm)

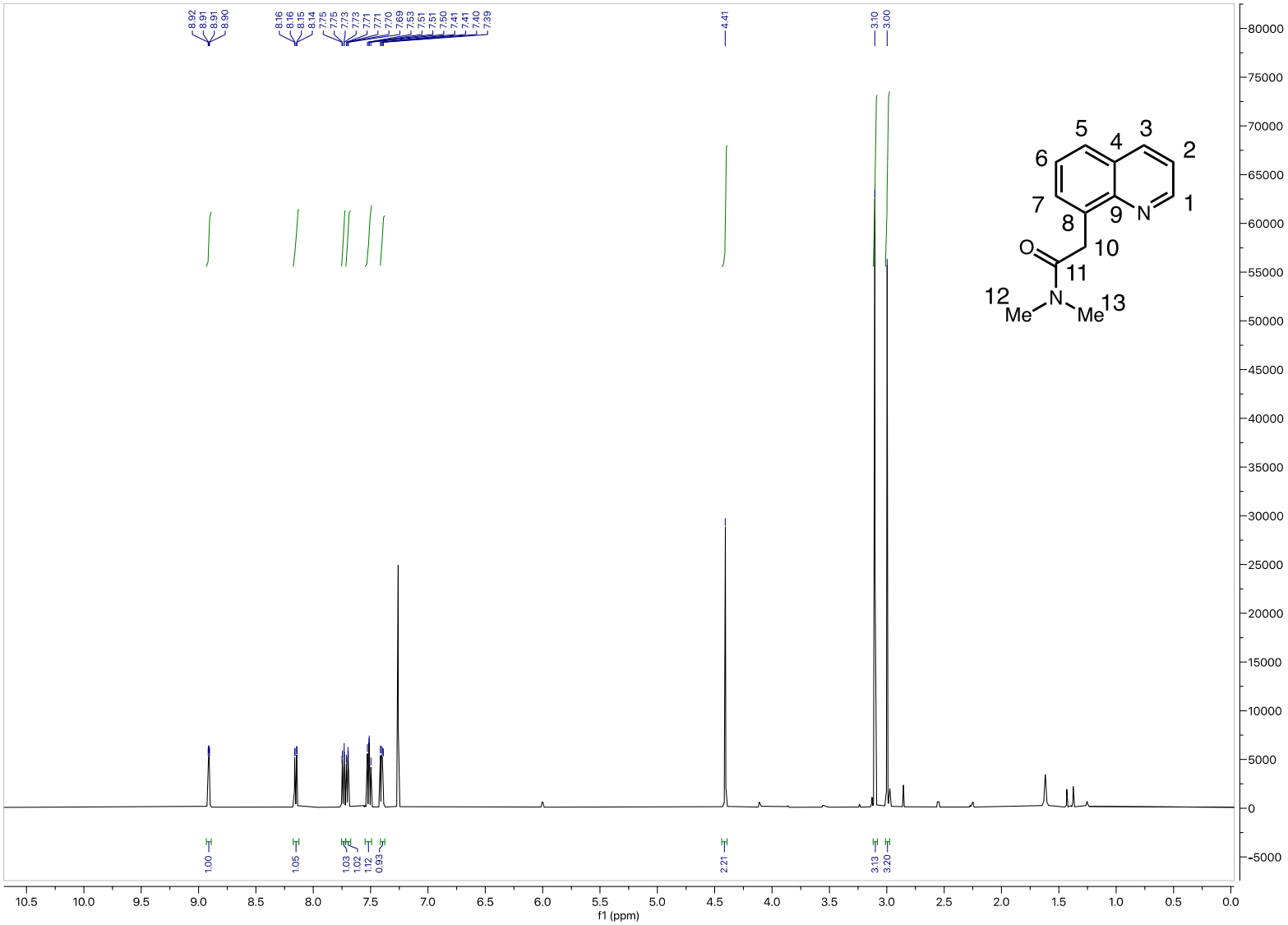

7.22
7.22
7.20
6.74
6.73
6.72
6.72
6.72
6.71
6.71
6.71
6.70
6.70
6.69
6.69
6.68
6.68
4.11
3.05
3.04
2.97
5
Me
O
8
Me
N
7
N
1
2
3
6
Me
4
9
1.99
3.07
2.00
5.91
3.16
f1 (ppm)

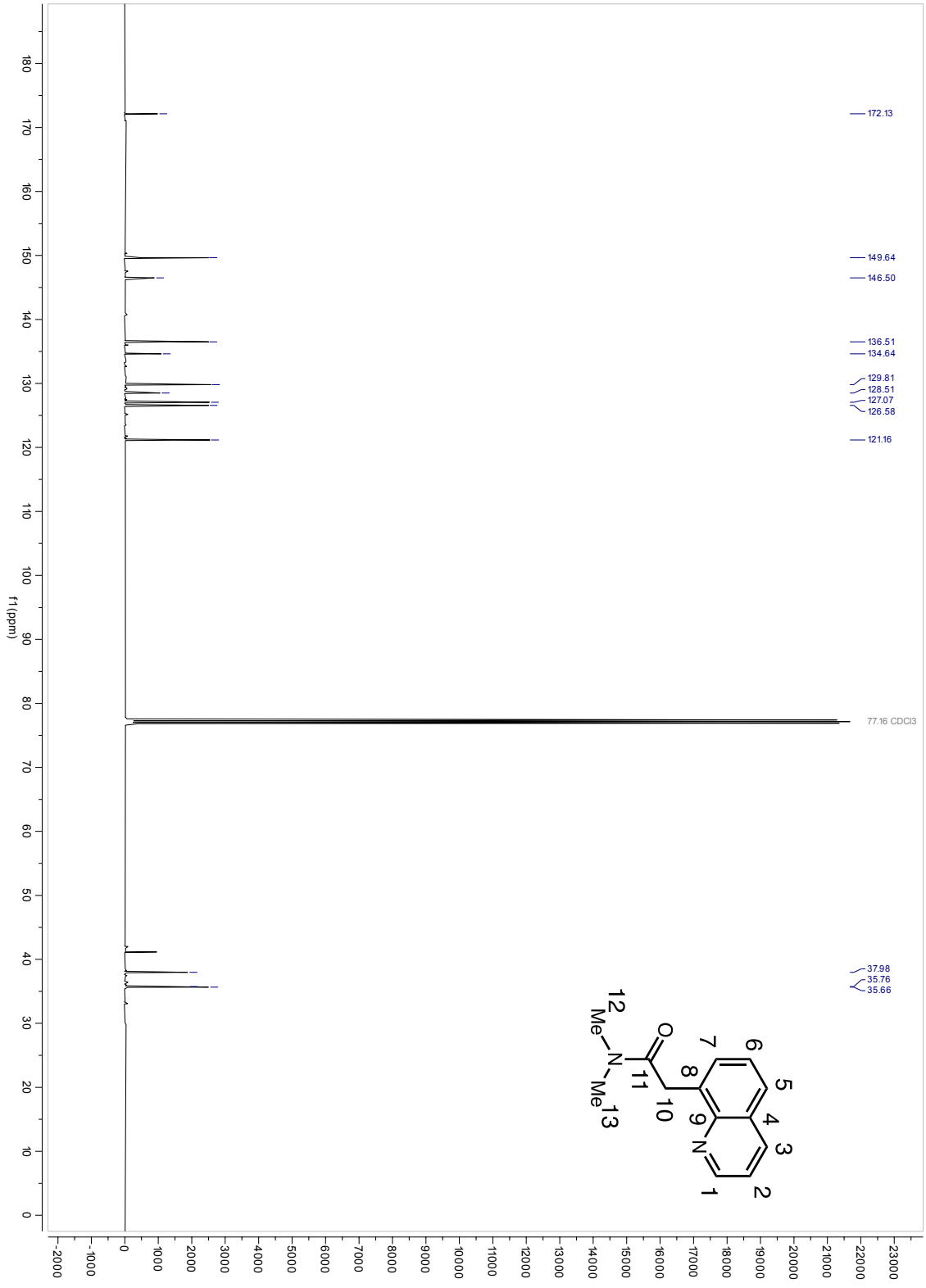

169.62
149.55
129.31
117.27
112.57
77.16 CDCl3
54.54
39.53
36.54
35.86
O
5
Me
8
Me
N
7
N
1
2
3
6
Me
4
9
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

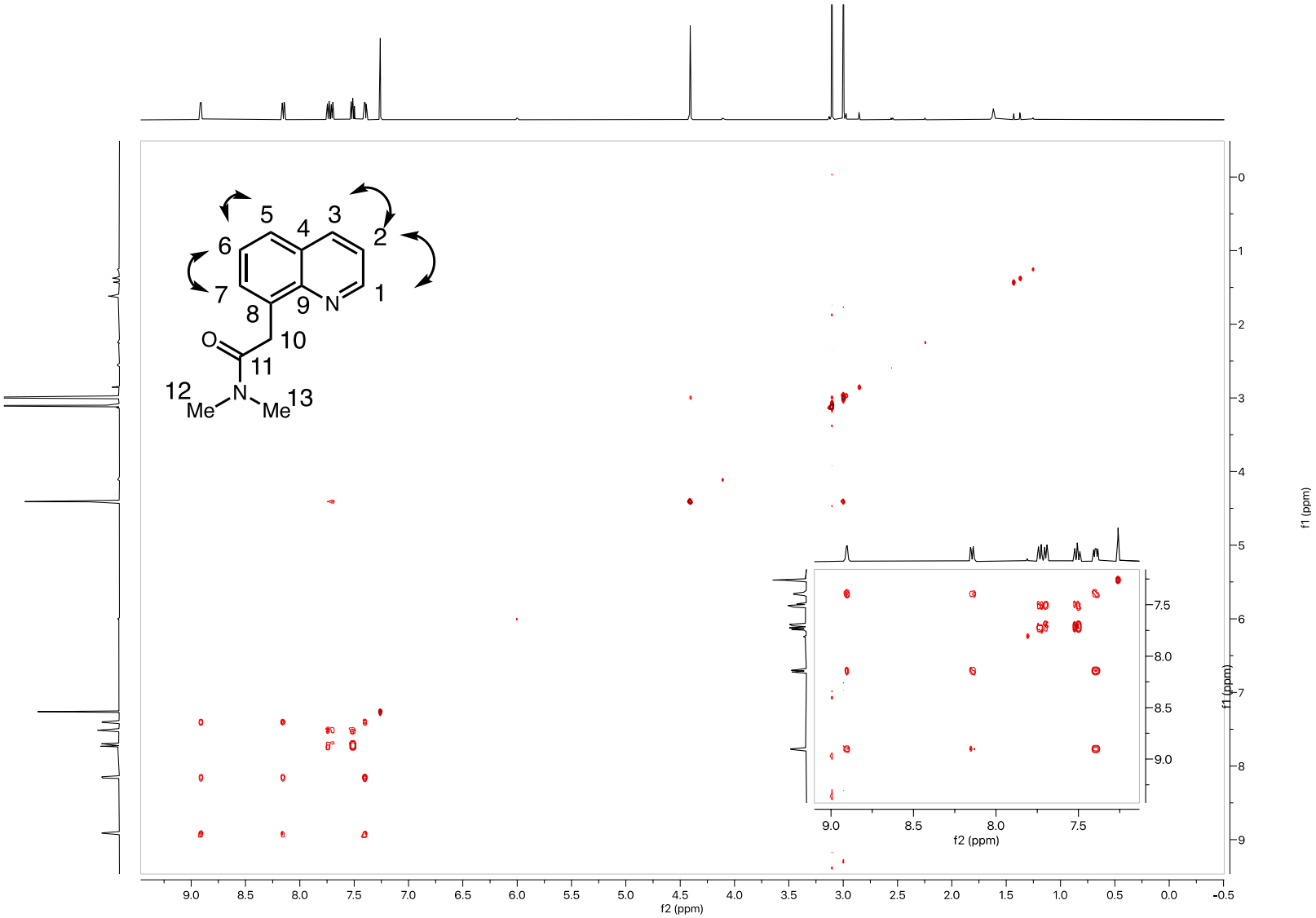

5
Me
O
8
Me
N
7
N
1
2
3
6
Me
4
9
f2 (ppm)
f1 (ppm)
f2 (ppm)
f1 (ppm)

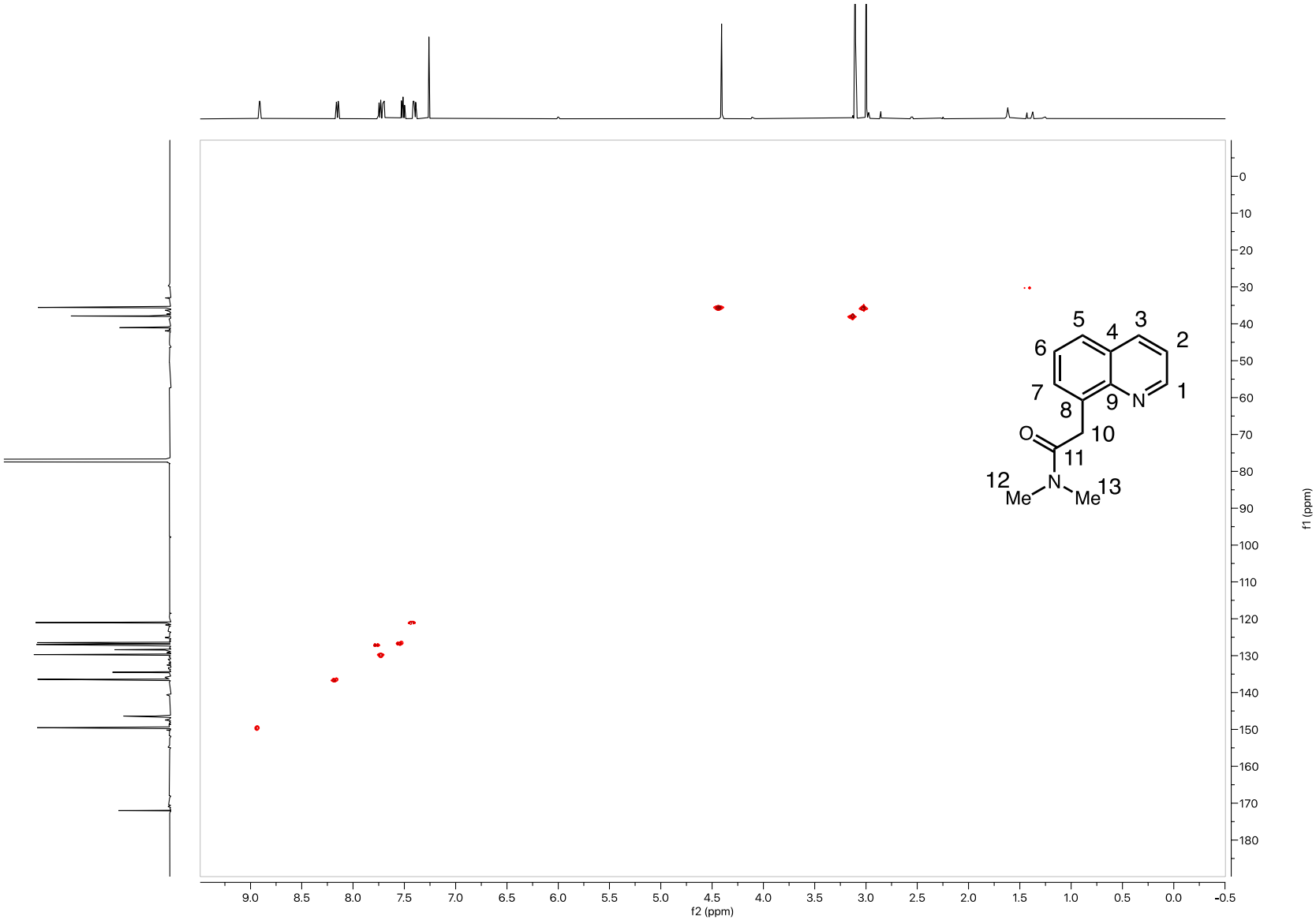

5
Me
O
8
Me
N
7
N
1
2
3
6
Me
4
9
f2 (ppm)
f1 (ppm)

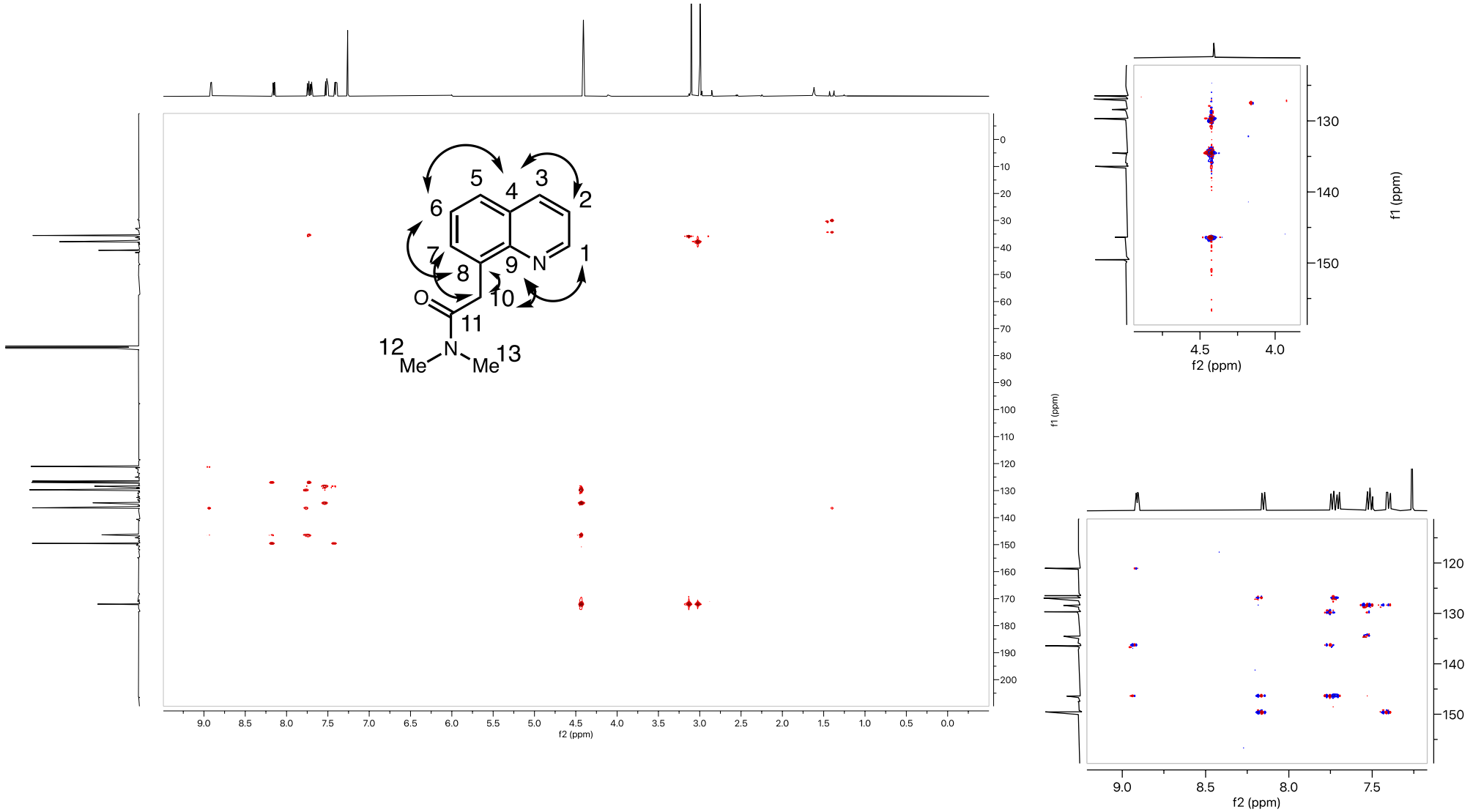

O
Me
8
Me
N
7
N
1
2
3
4
5
6
Me
9
f2 (ppm)
f1 (ppm)
f2 (ppm)
f1 (ppm)
f2 (ppm)
f1 (ppm)

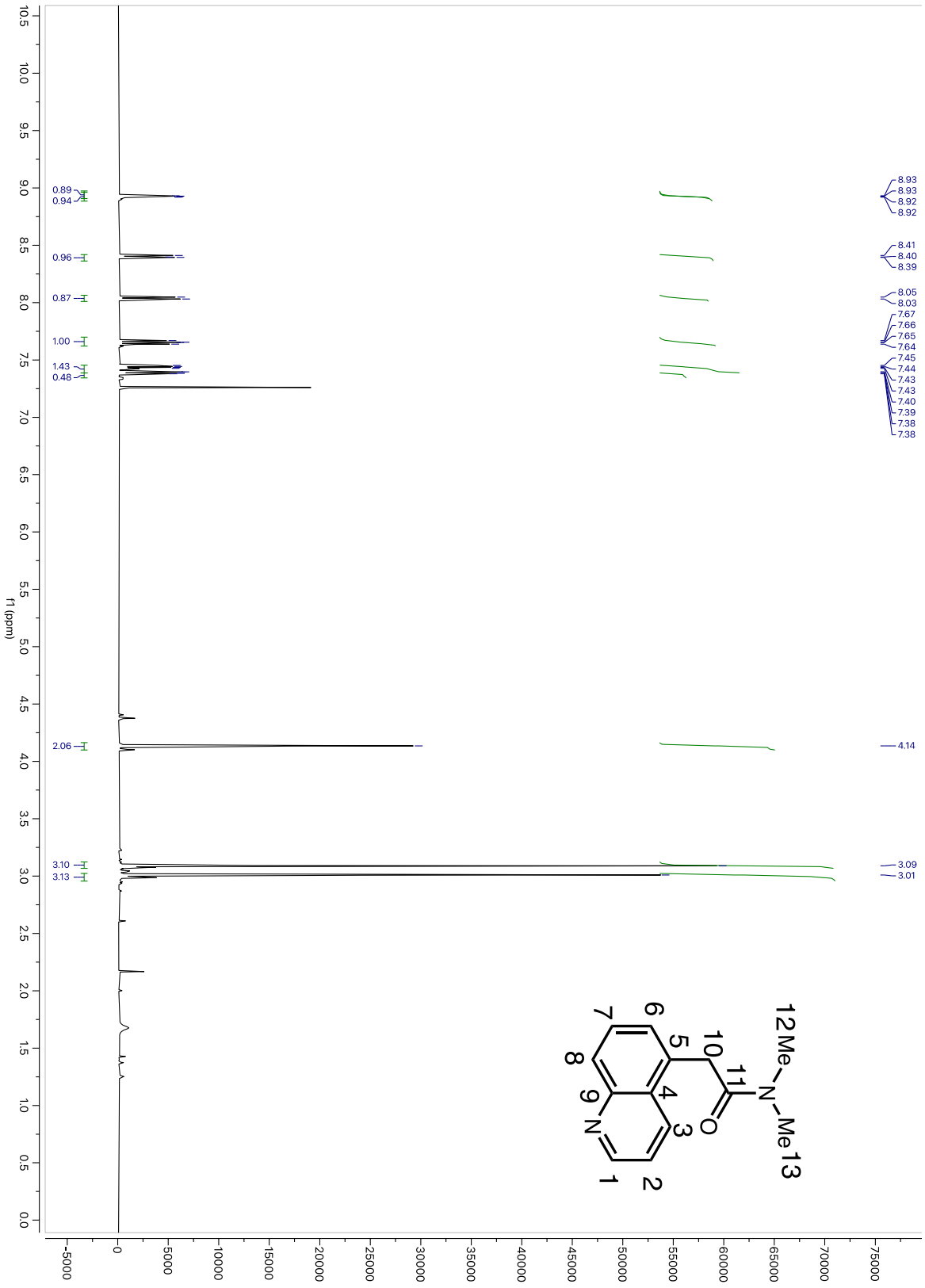

8.92
8.91
8.91
8.90
8.16
8.16
8.15
8.14
7.75
7.75
7.73
7.73
7.71
7.71
7.70
7.69
7.53
7.51
7.51
7.50
7.41
7.41
7.40
7.39
4.41
3.10
3.00
1.00
1.05
1.03
1.02
1.12
0.93
2.21
3.13
3.20
f1 (ppm)

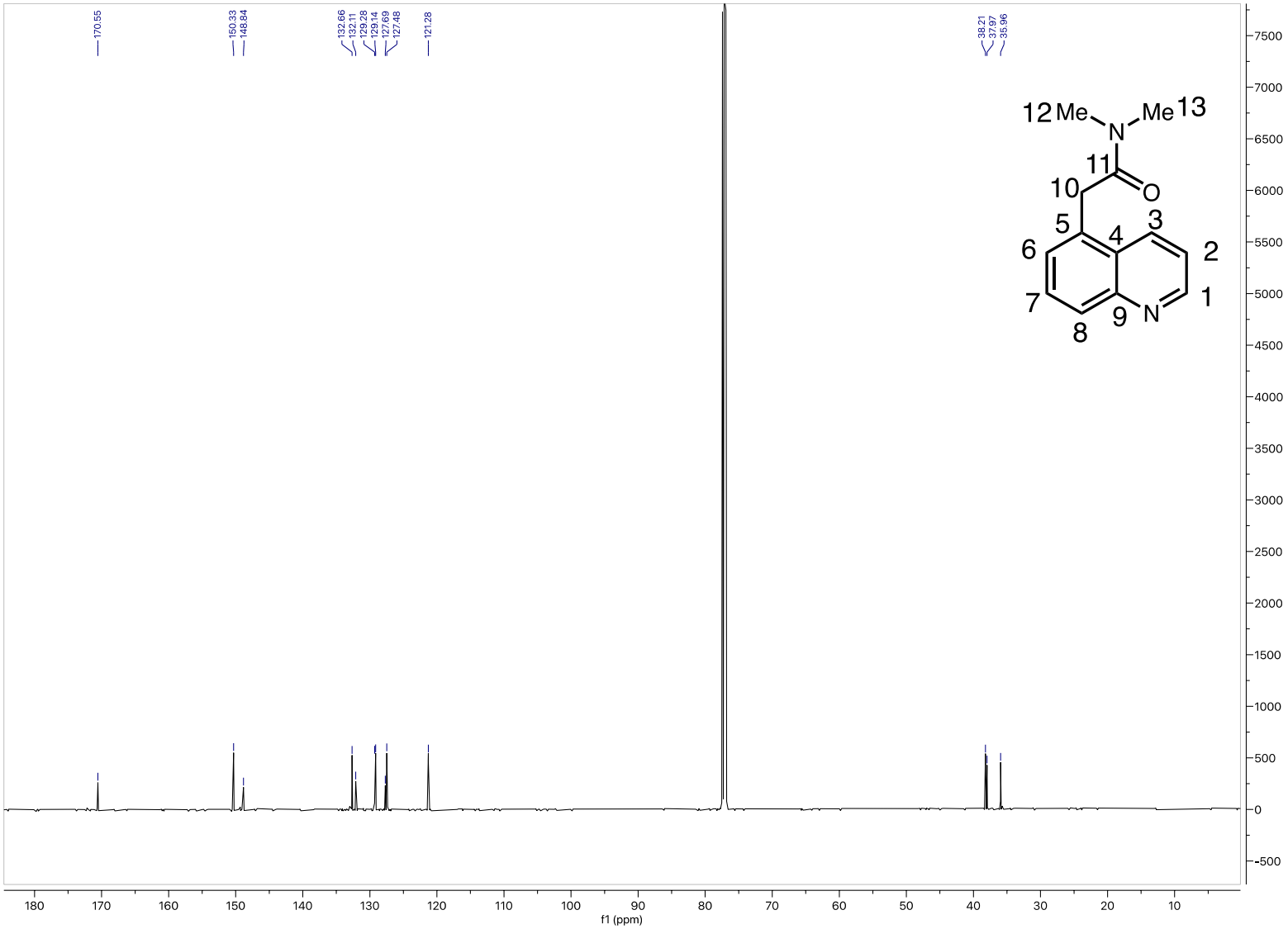

172.13
149.64
146.50
136.51
134.64
129.81
128.51
127.07
126.58
121.16
77.16 CDCl3
37.98
35.76
35.66
f1(ppm)
O
N
Me
Me
1
2
3
4
5
6
7
8
9
10
11
12
13

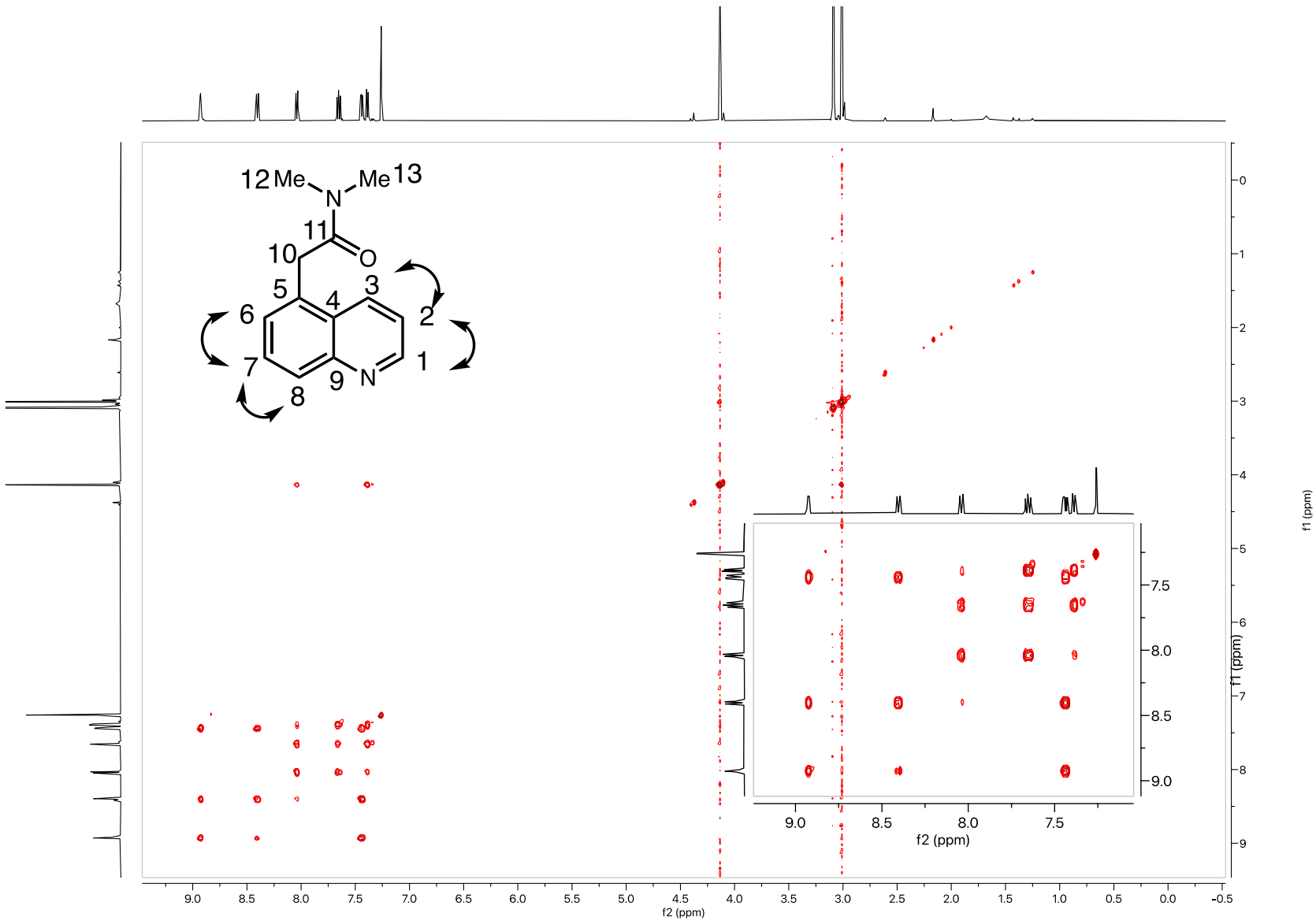

5
4
3
2
6
7
8
9
N
1
O
10
11
12
Me
N
Me
13
f2 (ppm)
f1 (ppm)

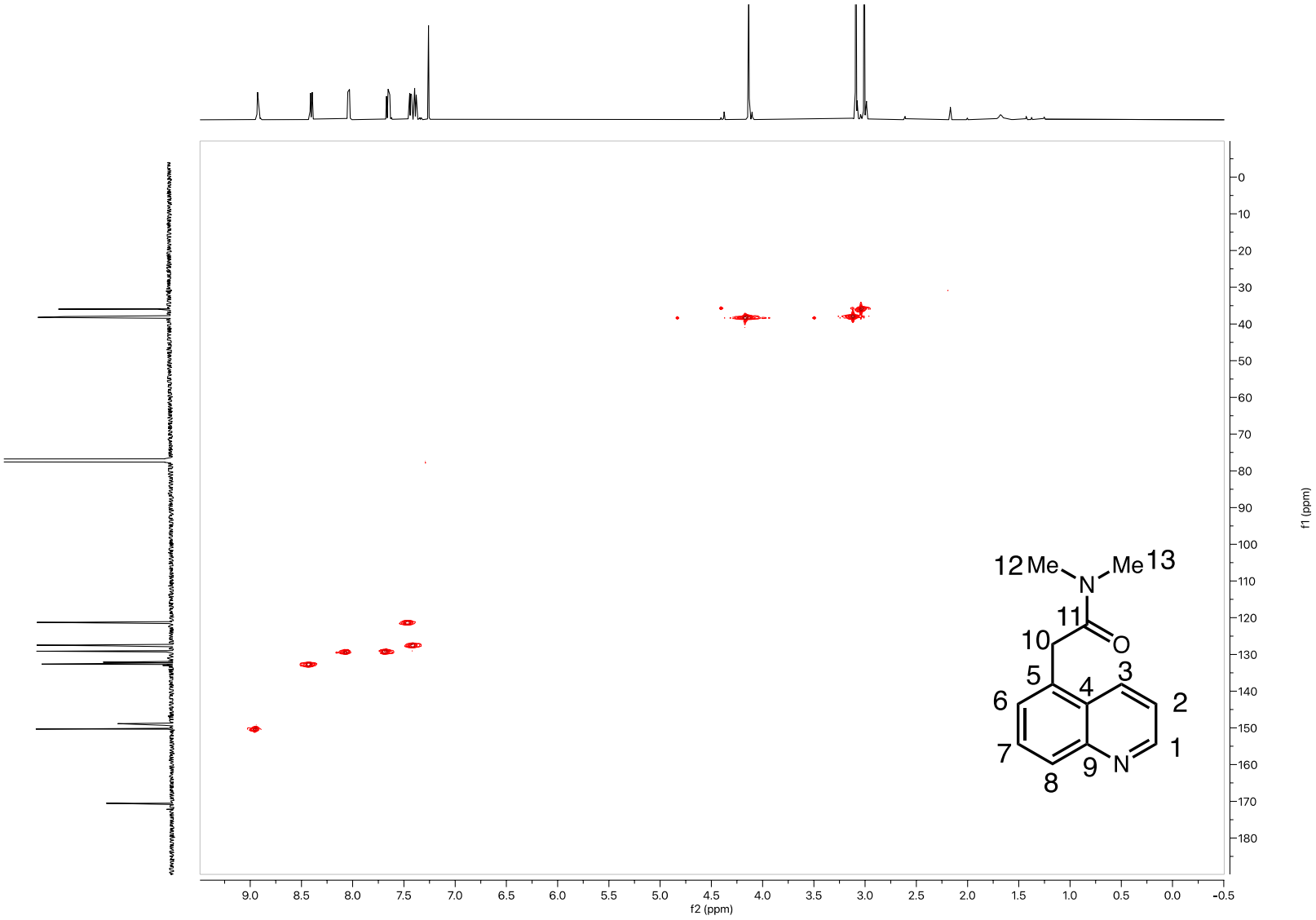

f2 (ppm)
f1 (ppm)
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
1
2
3
4
5
6
7
8
9
N
10
O
11
12
Me
N
Me
13

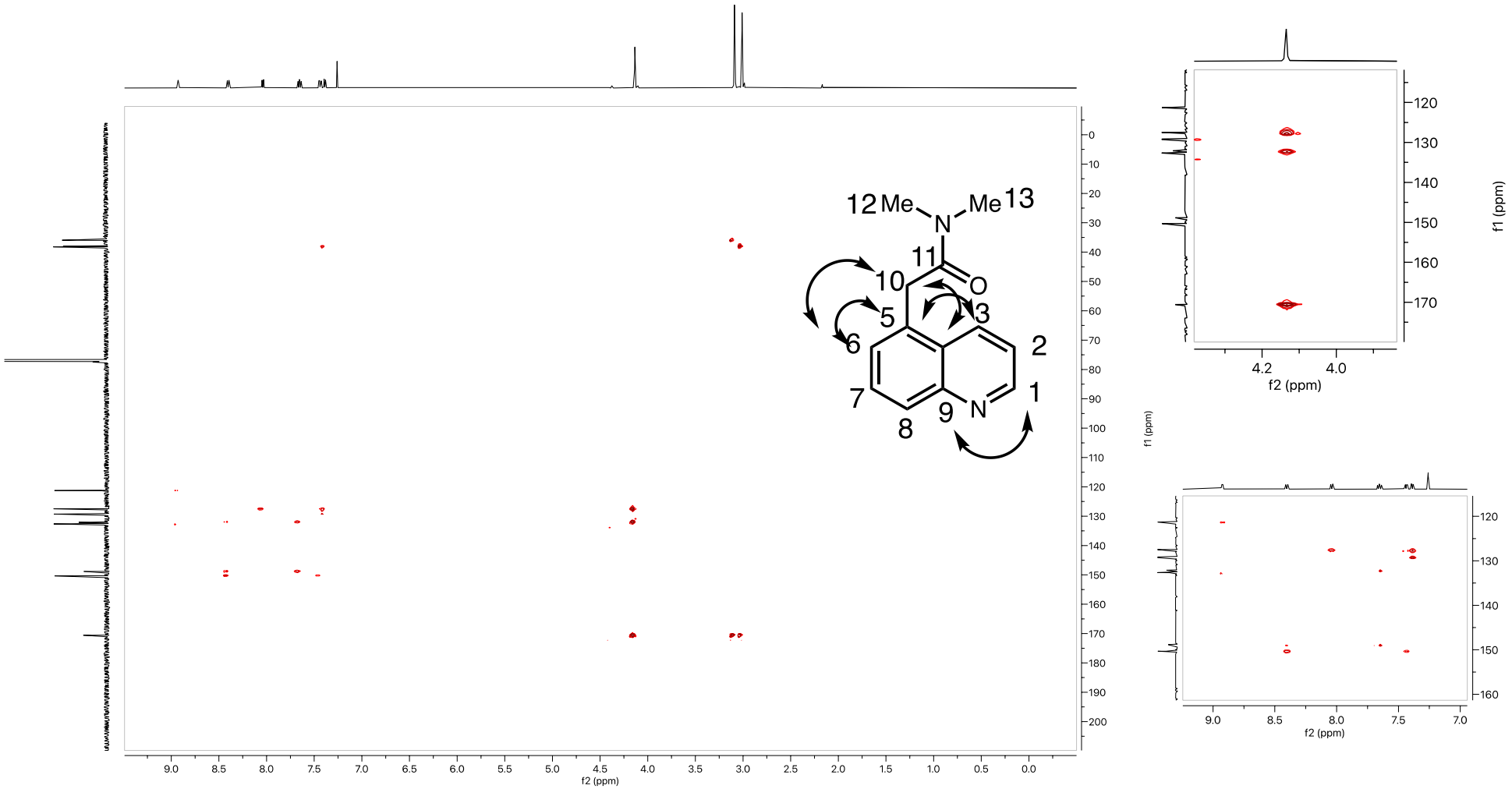

5
4
3
2
6
1
7
9
N
8
O
10
11
12
Me
N
Me
13
f2 (ppm)
f1 (ppm)
f2 (ppm)
f1 (ppm)
f2 (ppm)

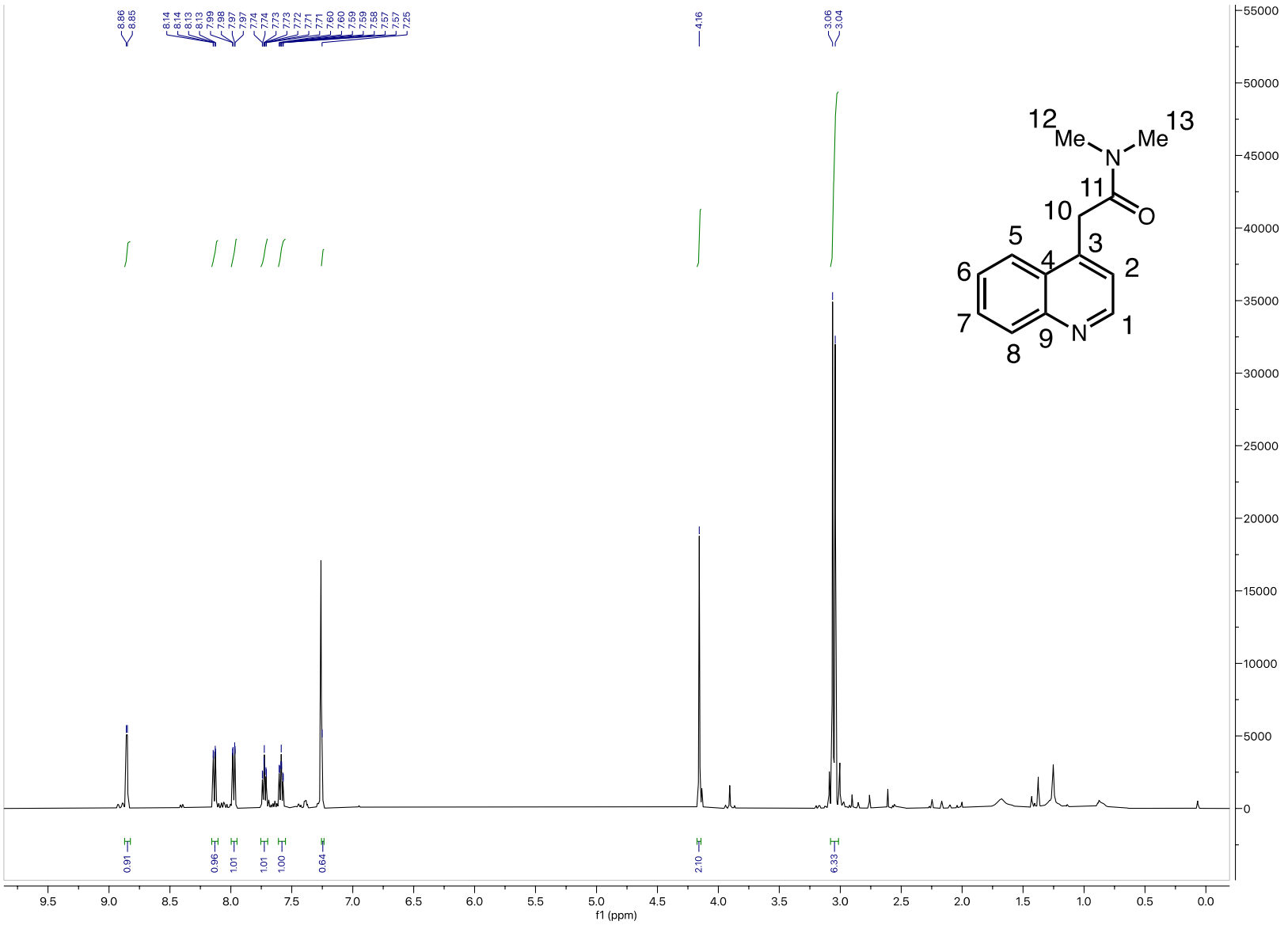

f1 (ppm)
0.89
0.94
0.96
0.87
1.00
1.43
0.48
2.06
3.10
3.13
8.93
8.93
8.92
8.92
8.41
8.40
8.39
8.05
8.03
7.67
7.66
7.65
7.64
7.45
7.44
7.43
7.43
7.40
7.39
7.38
7.38
4.14
3.09
3.01
12Me
N
Me13
11
10
O
1
2
3
4
5
6
7
8
9
N

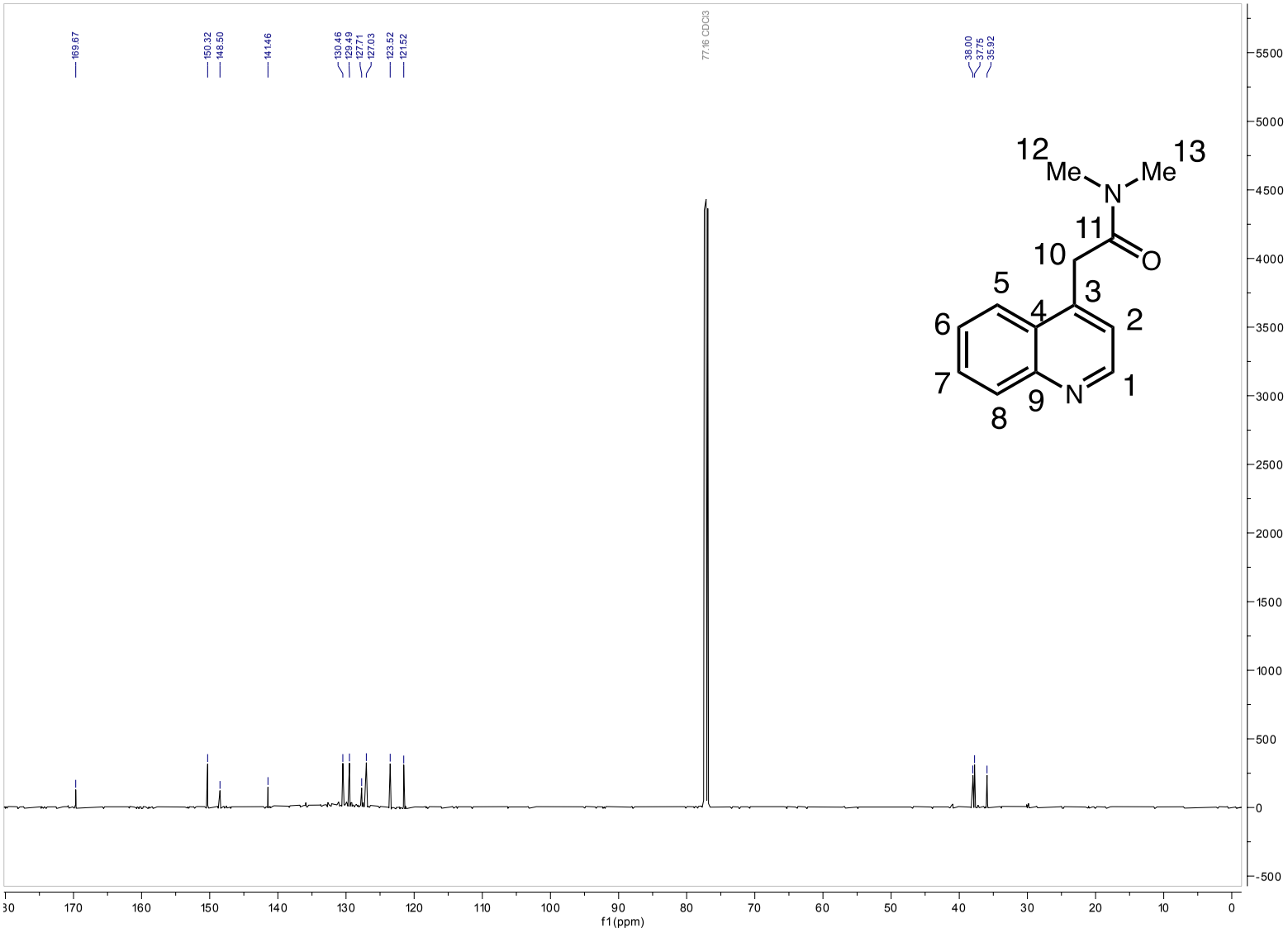

170.55
150.33
148.84
132.66
132.11
129.28
129.14
127.69
127.48
121.28
38.21
37.97
35.96
12 Me
Me 13
N
11
10
O
5
4
3
6
2
7
1
8
9
N
f1 (ppm)

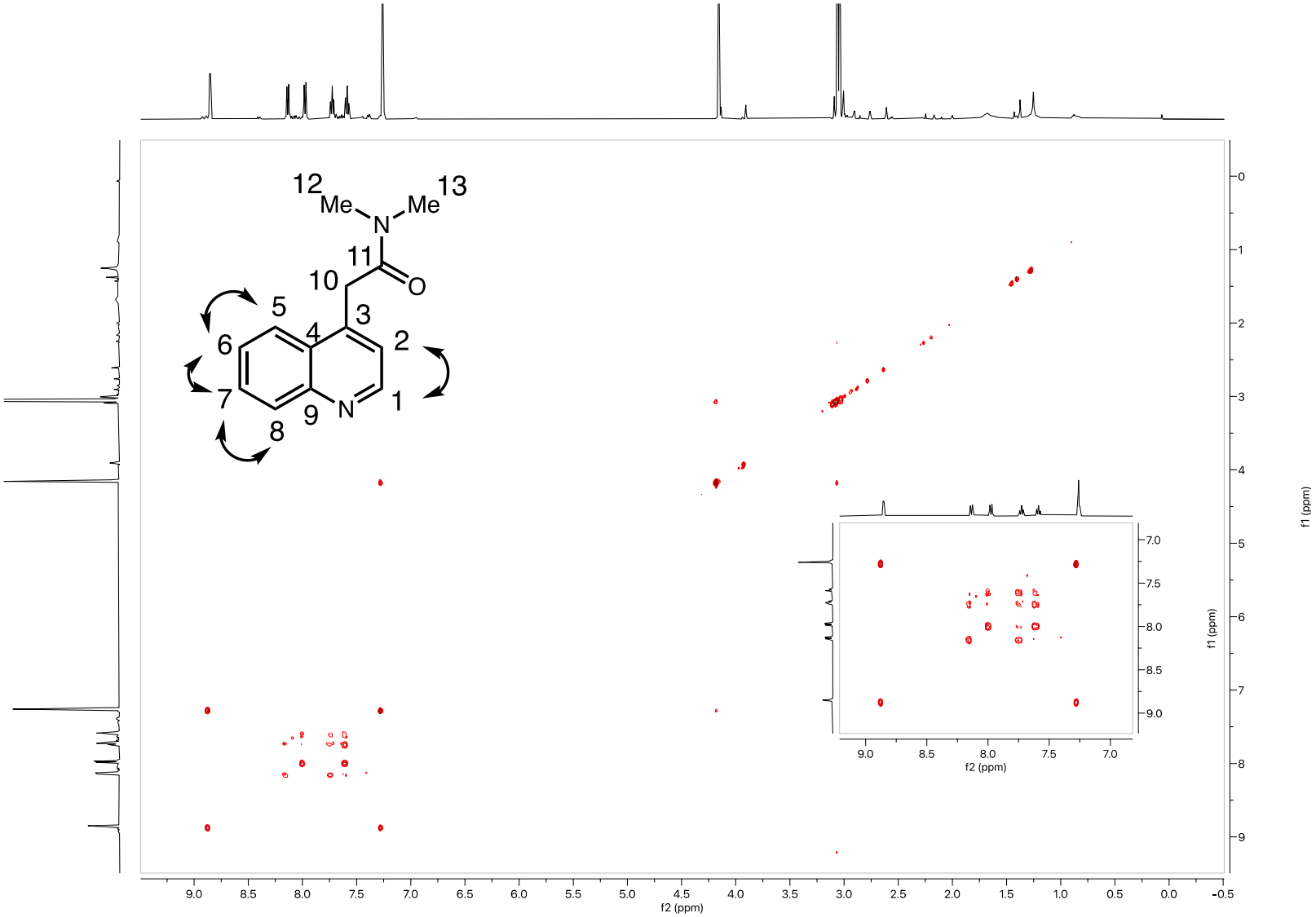

12Me
Me13
N
11
10
O
5
4
3
6
2
7
1
9
N
8
f2 (ppm)
f1 (ppm)

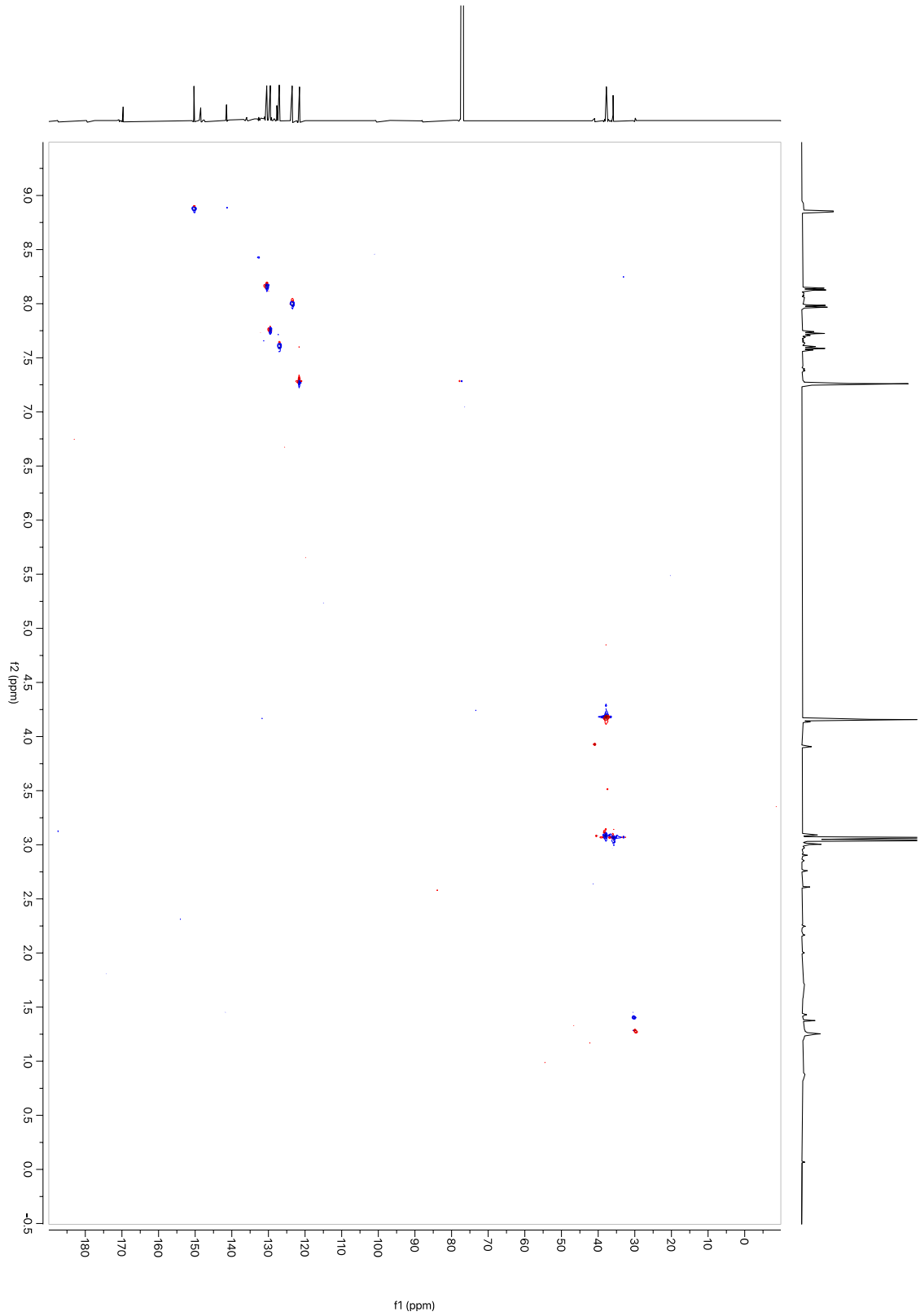

f2 (ppm)
f1 (ppm)
12 Me
Me 13
N
11
10
O
5
4
3
6
2
7
1
9
N
8

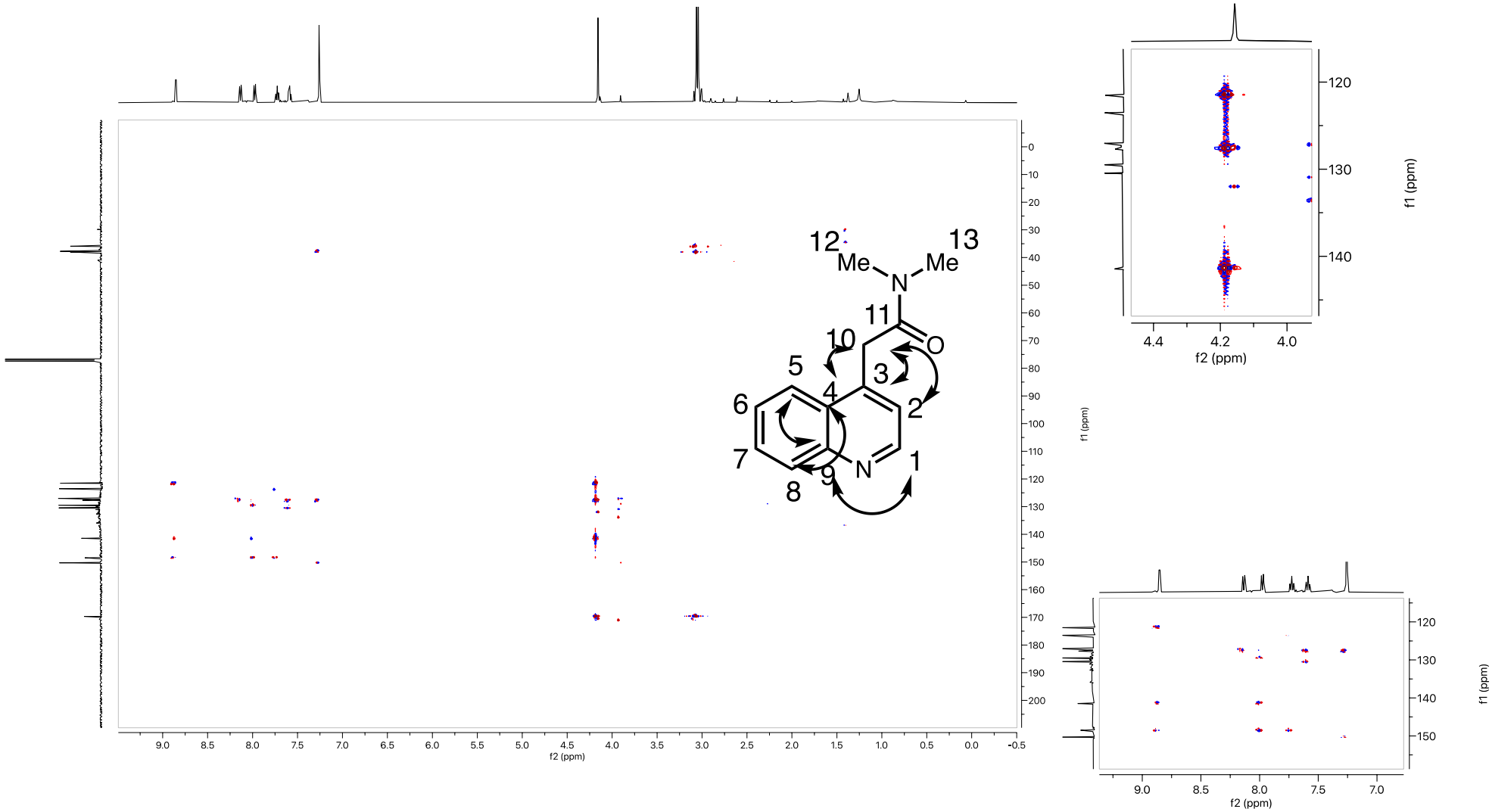

12 Me
Me 13
N
11
10
O
5
3
6
2
7
1
9
N
8
f2 (ppm)
f1 (ppm)
4.2
4.0
f2 (ppm)
f1 (ppm)
9.0
8.5
8.0
7.5
7.0
f2 (ppm)

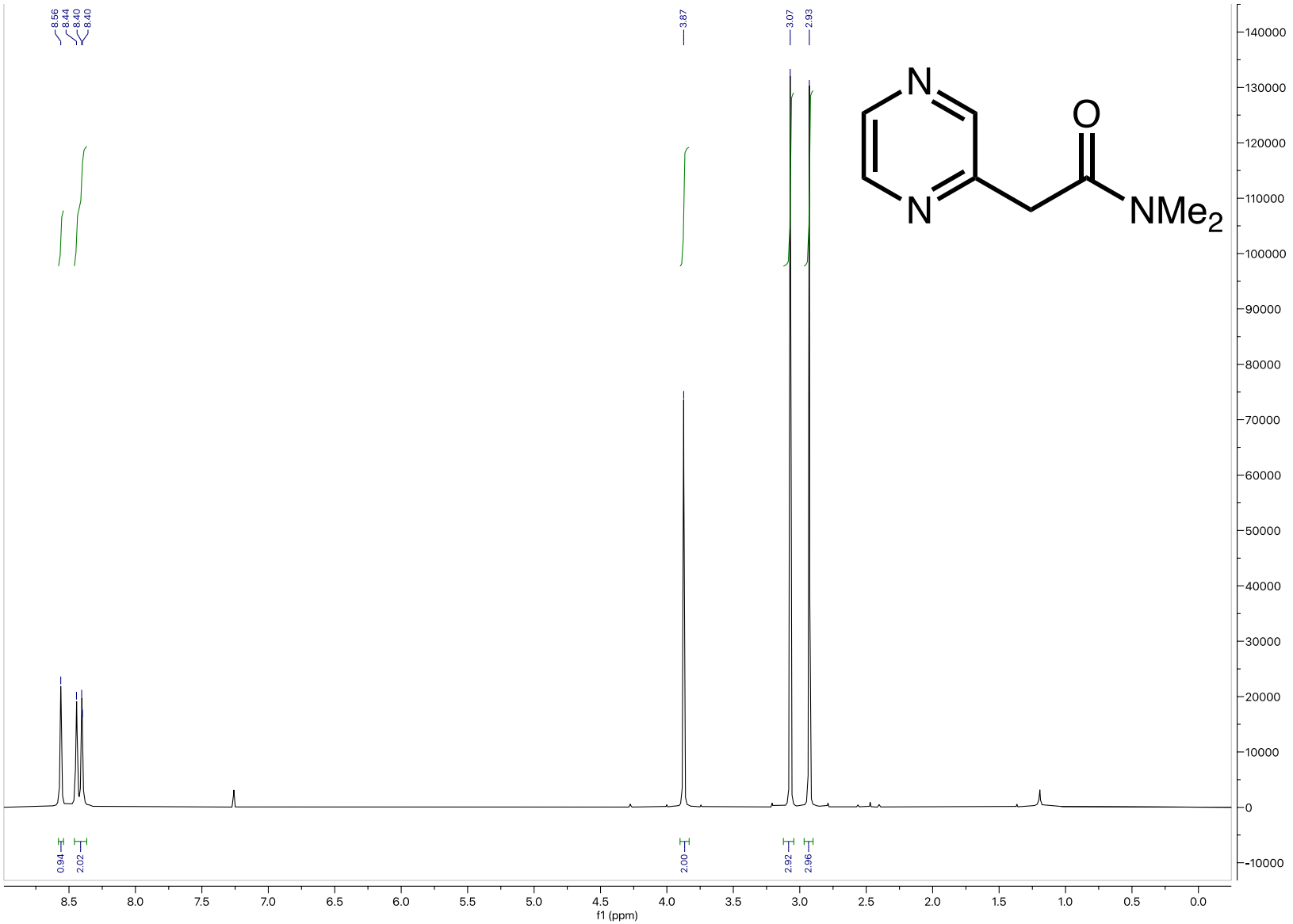

8.86
8.85
8.14
8.14
8.13
8.13
7.99
7.98
7.97
7.97
7.74
7.74
7.73
7.73
7.72
7.71
7.71
7.60
7.60
7.59
7.59
7.58
7.57
7.57
7.25
4.16
3.06
3.04
12
Me
N
Me
13
11
10
O
5
3
4
2
6
7
9
N
1
8
0.91
0.96
1.01
1.01
1.00
0.64
2.10
6.33
f1 (ppm)

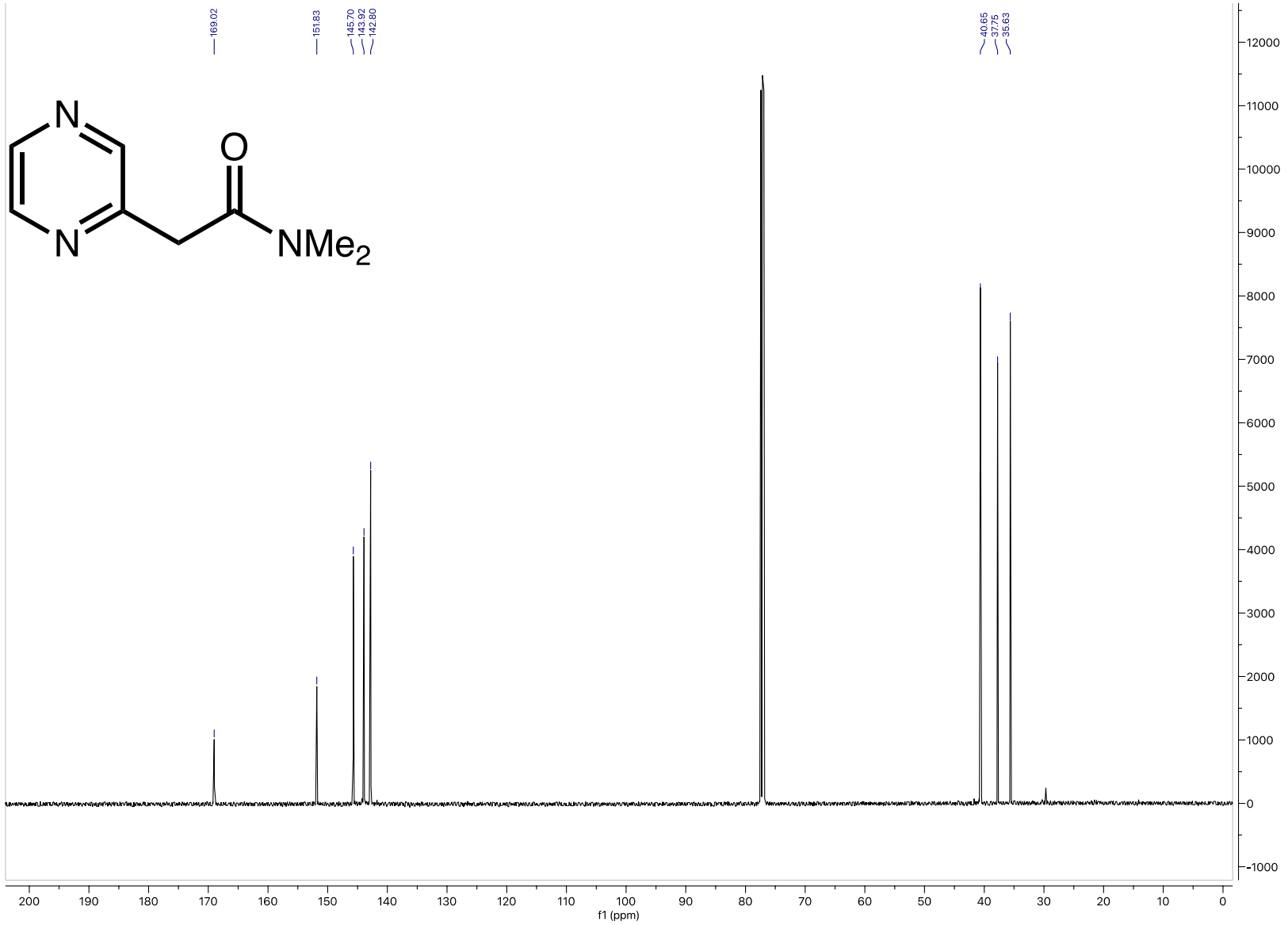

169.67
150.32
148.50
141.46
130.46
129.49
127.71
127.03
123.52
121.52
77.16 CDCl3
38.00
37.75
35.92
12
Me
N
Me
13
11
10
O
5
3
4
6
2
7
1
9
N
8
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
f1 (ppm)

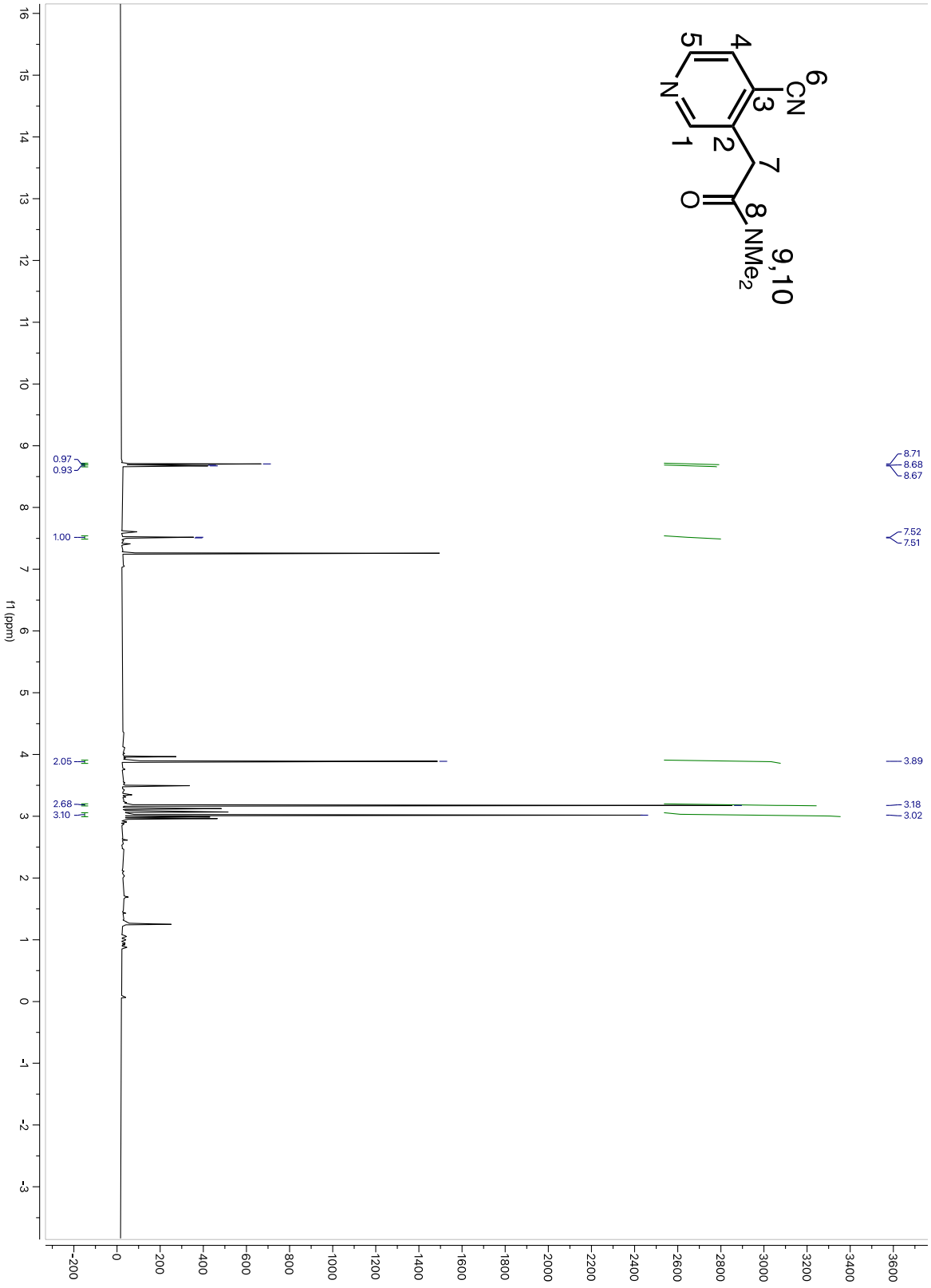

12
Me
N
Me
13
11
10
O
5
3
4
6
2
7
9
N
1
8
f2 (ppm)
f1 (ppm)
f2 (ppm)
f1 (ppm)

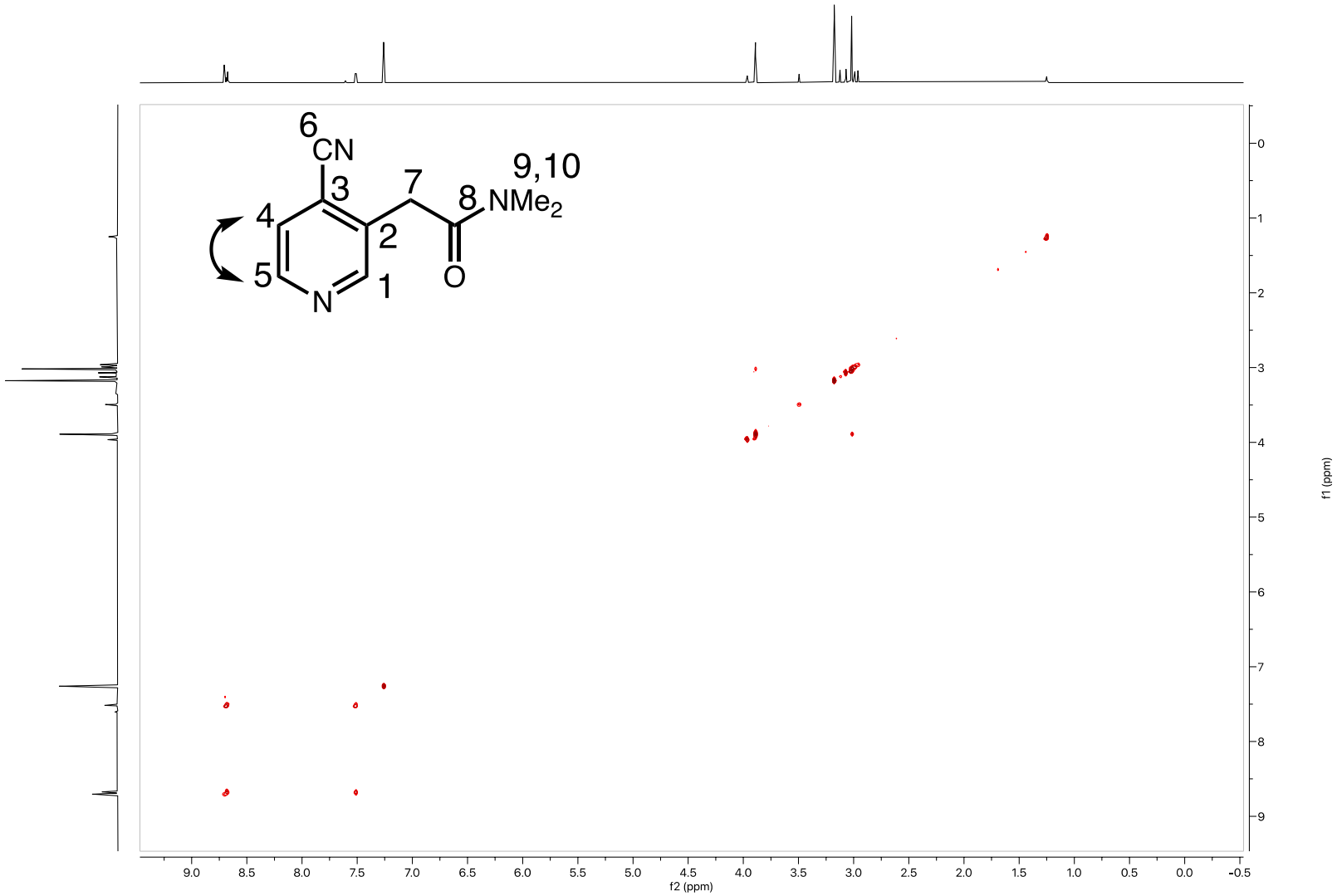

f2 (ppm)
f1 (ppm)

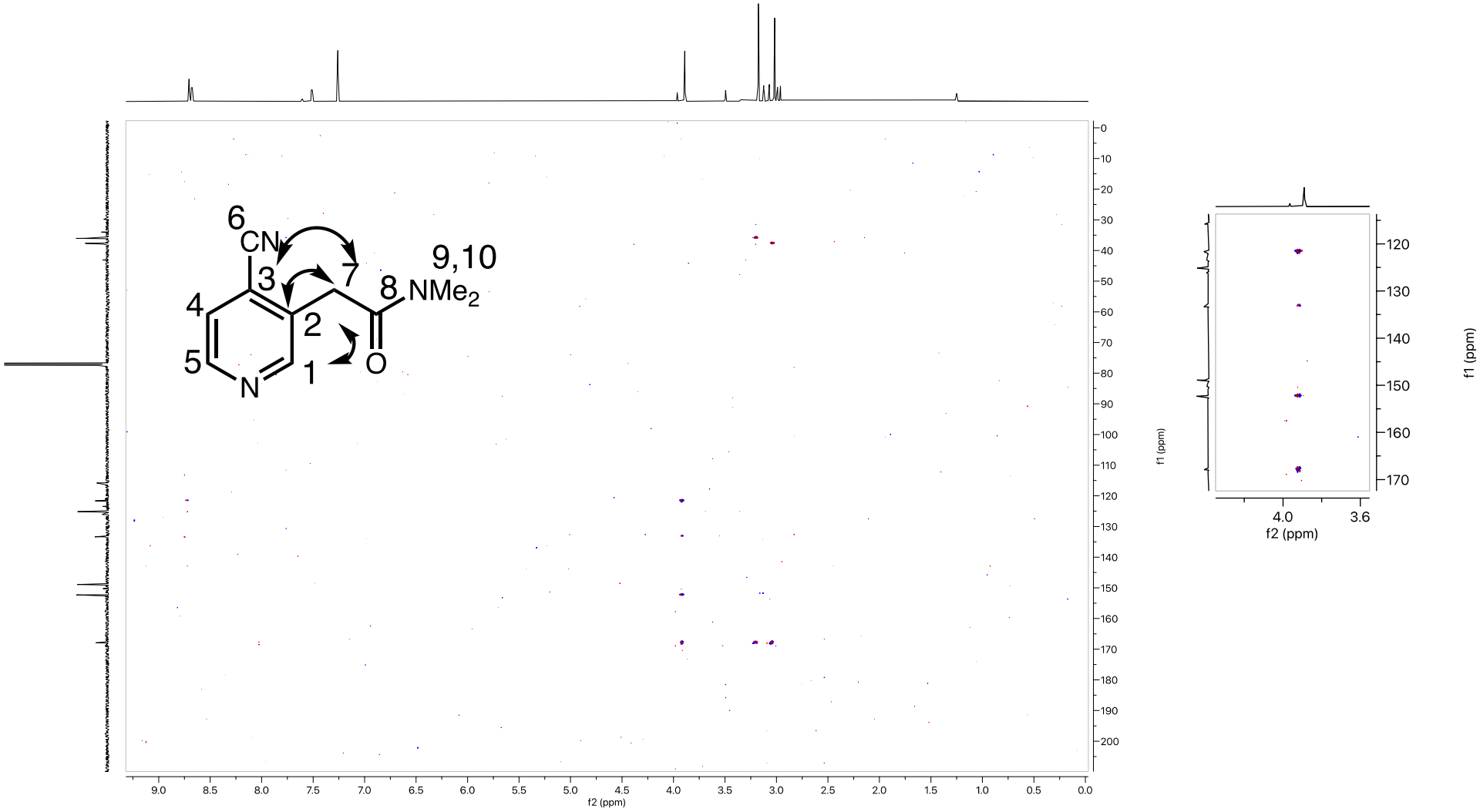

12
Me
N
Me
13
11
10
O
5
3
4
2
6
7
N
1
8
9
f2 (ppm)
f1 (ppm)
f2 (ppm)
f1 (ppm)
f2 (ppm)
f1 (ppm)

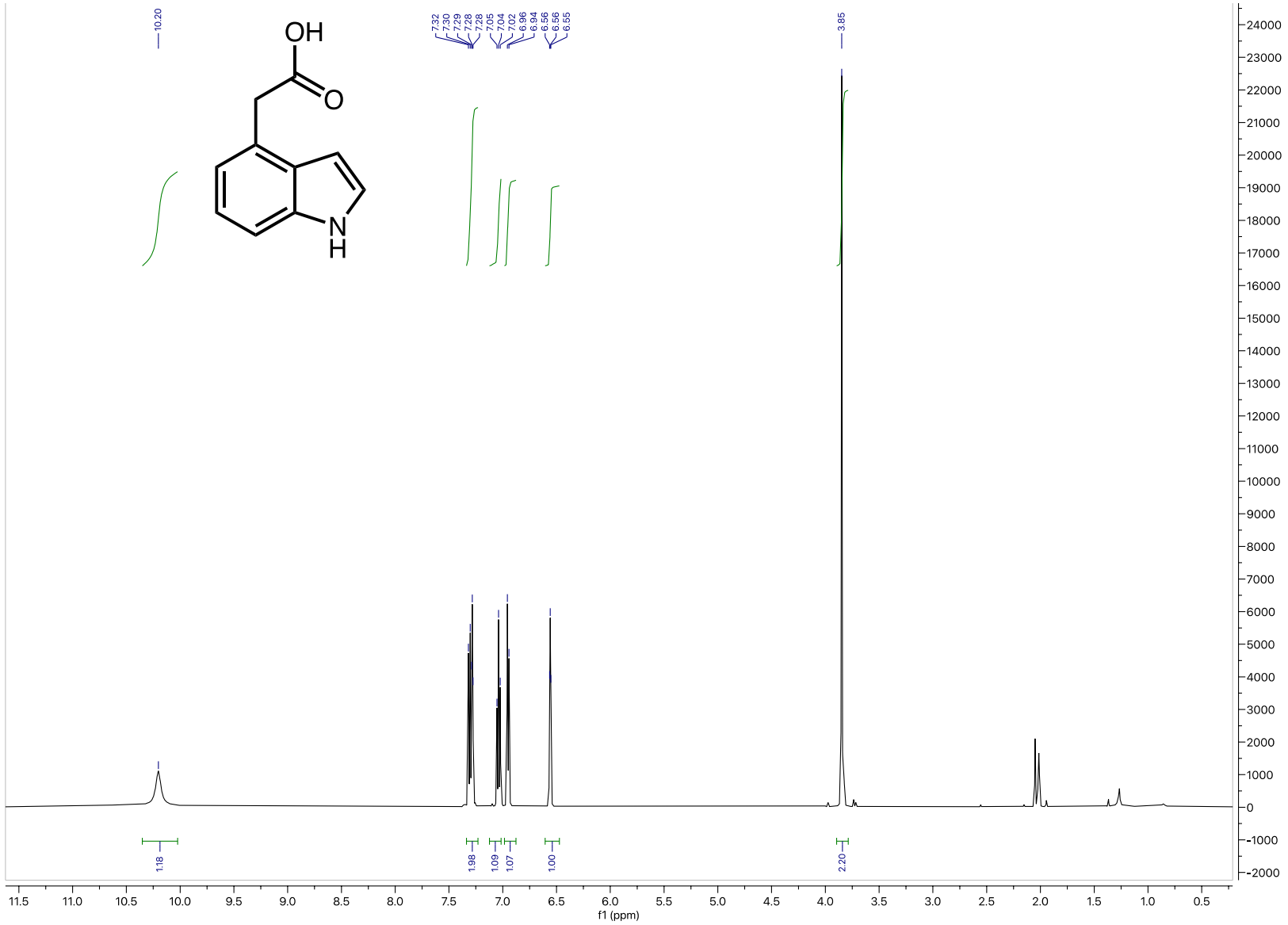

8.56
8.44
8.40
8.40
3.87
3.07
2.93
N
N
O
NMe2
0.94
2.02
2.00
2.92
2.96
f1 (ppm)

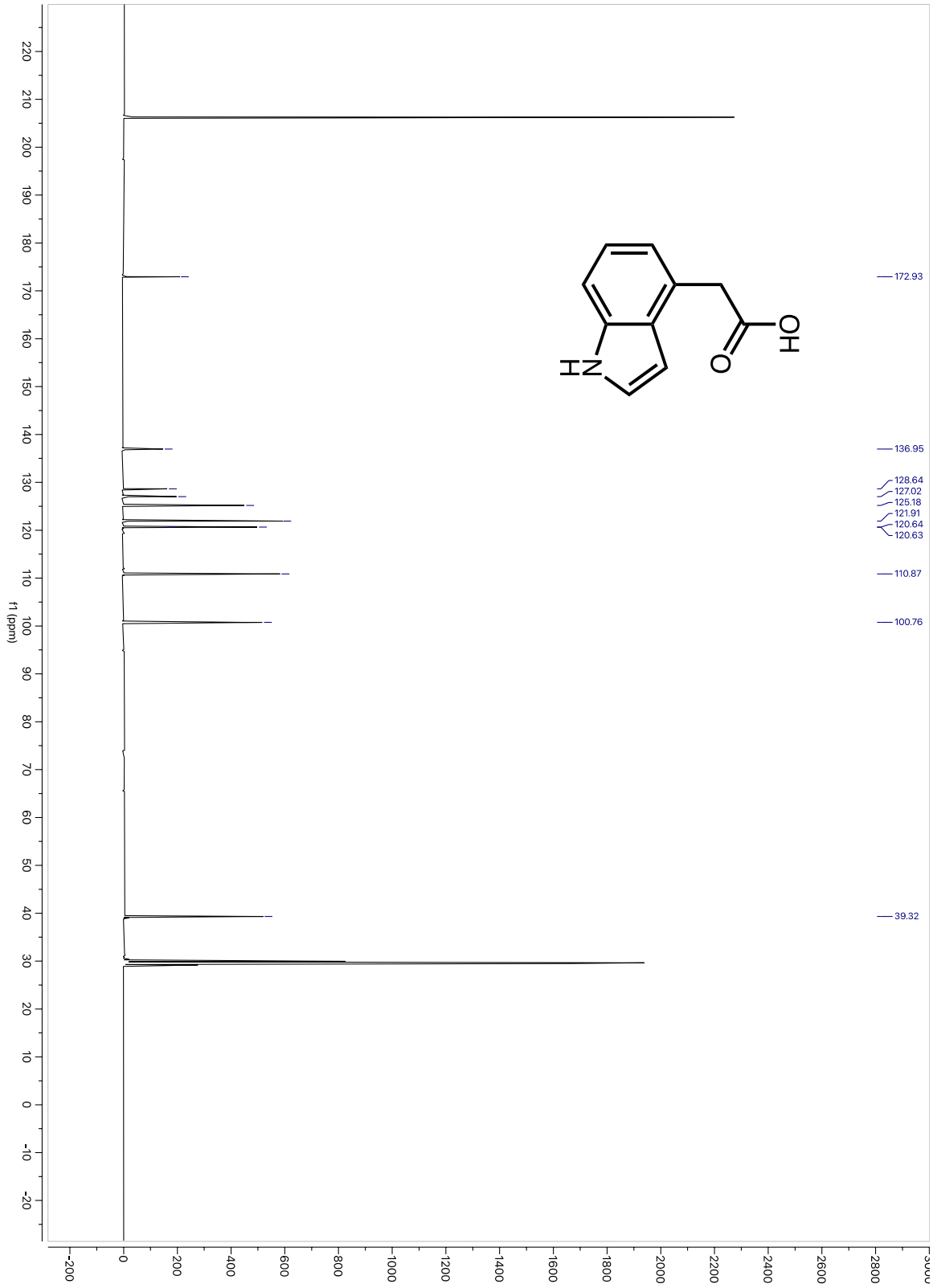

169.02
151.83
145.70
143.92
142.80
40.65
37.75
35.63
N
O
N
NMe2
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

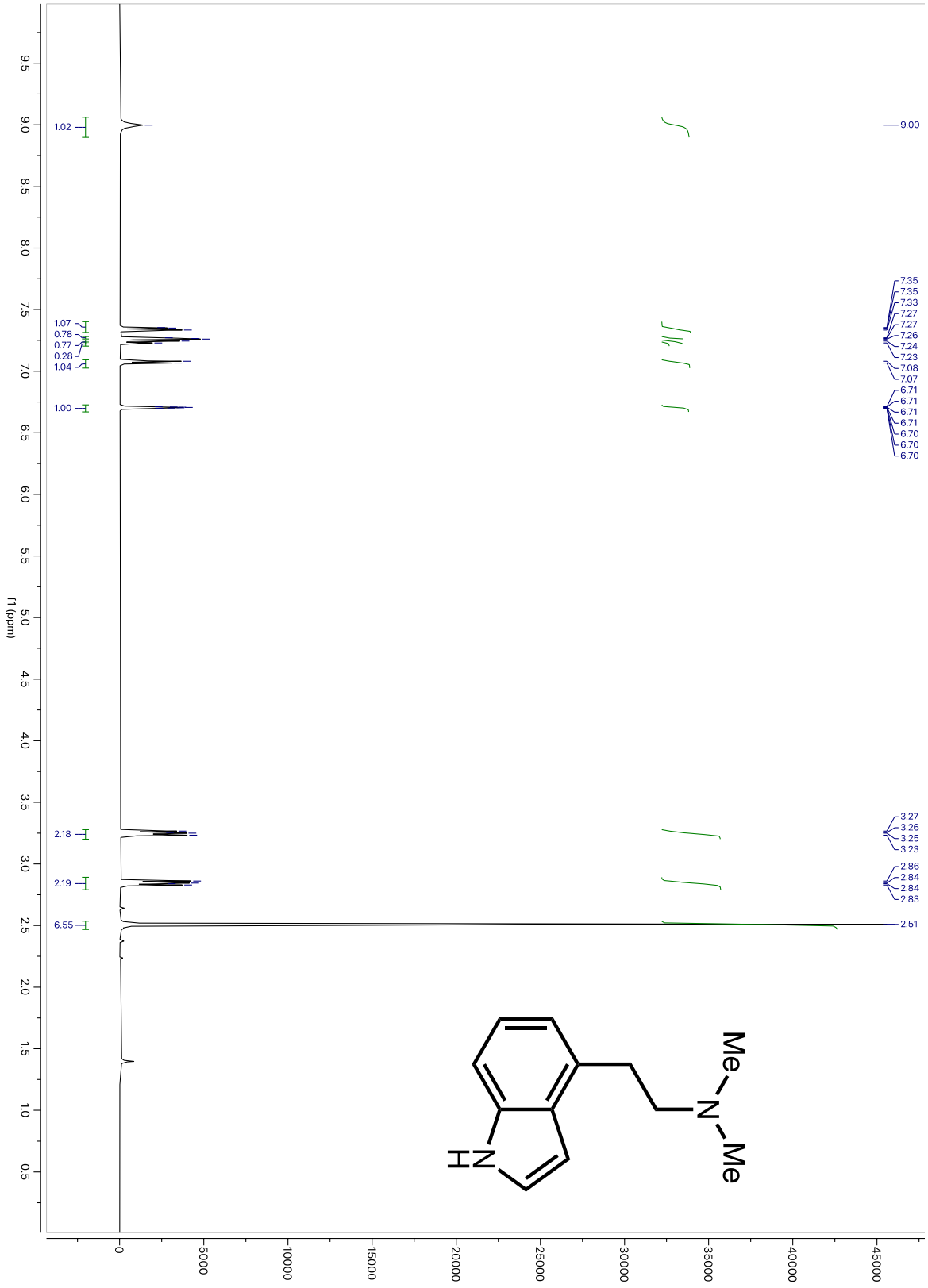

f1 (ppm)
0.97
0.93
1.00
2.05
2.68
3.10
8.71
8.68
8.67
7.52
7.51
3.89
3.18
3.02
N
CN
O
NMe2
1
2
3
4
5
6
7
8
9,10

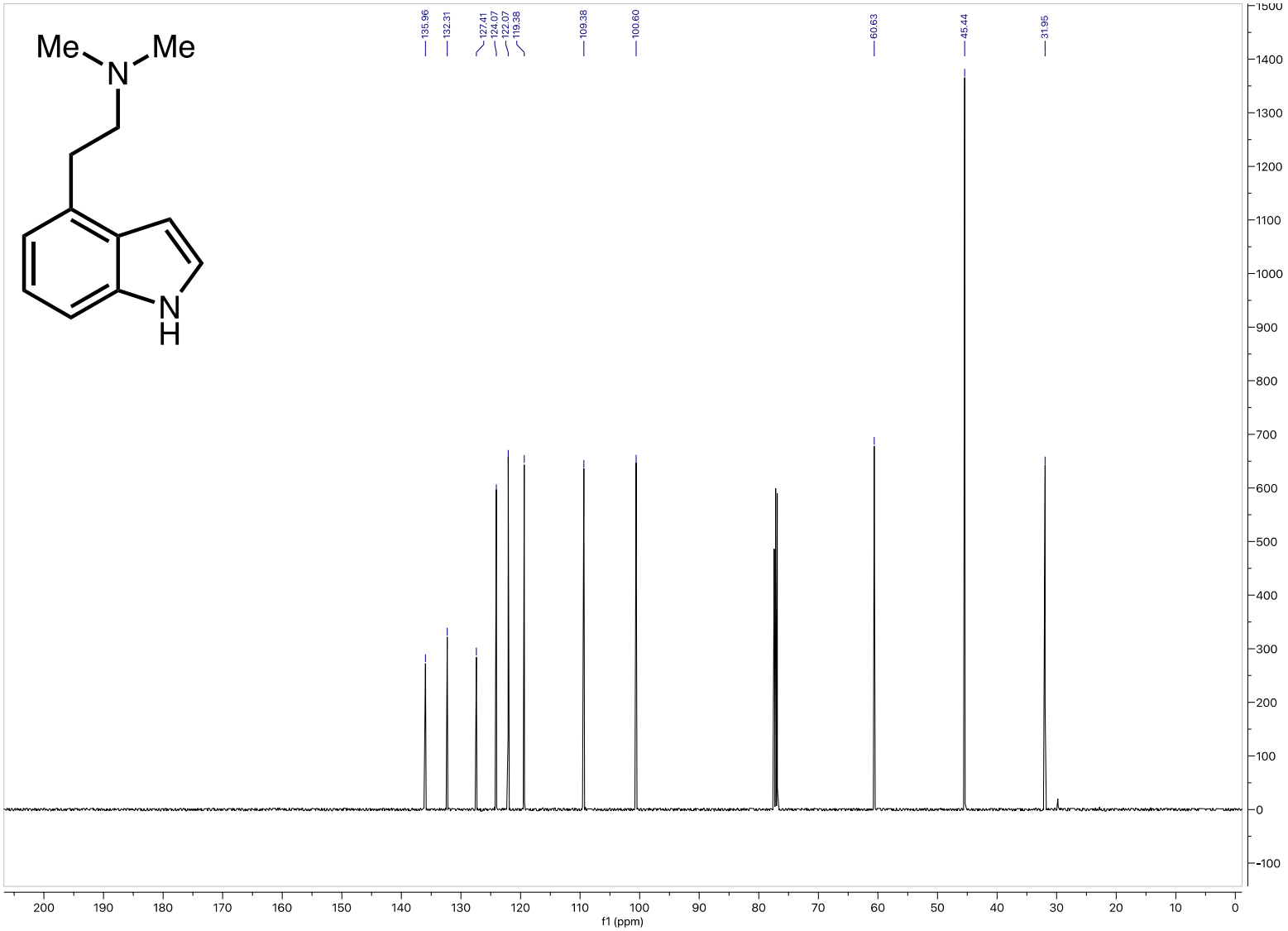

6
CN
3
7
8
9,10
NMe2
4
2
5
1
N
O
f2 (ppm)
f1 (ppm)

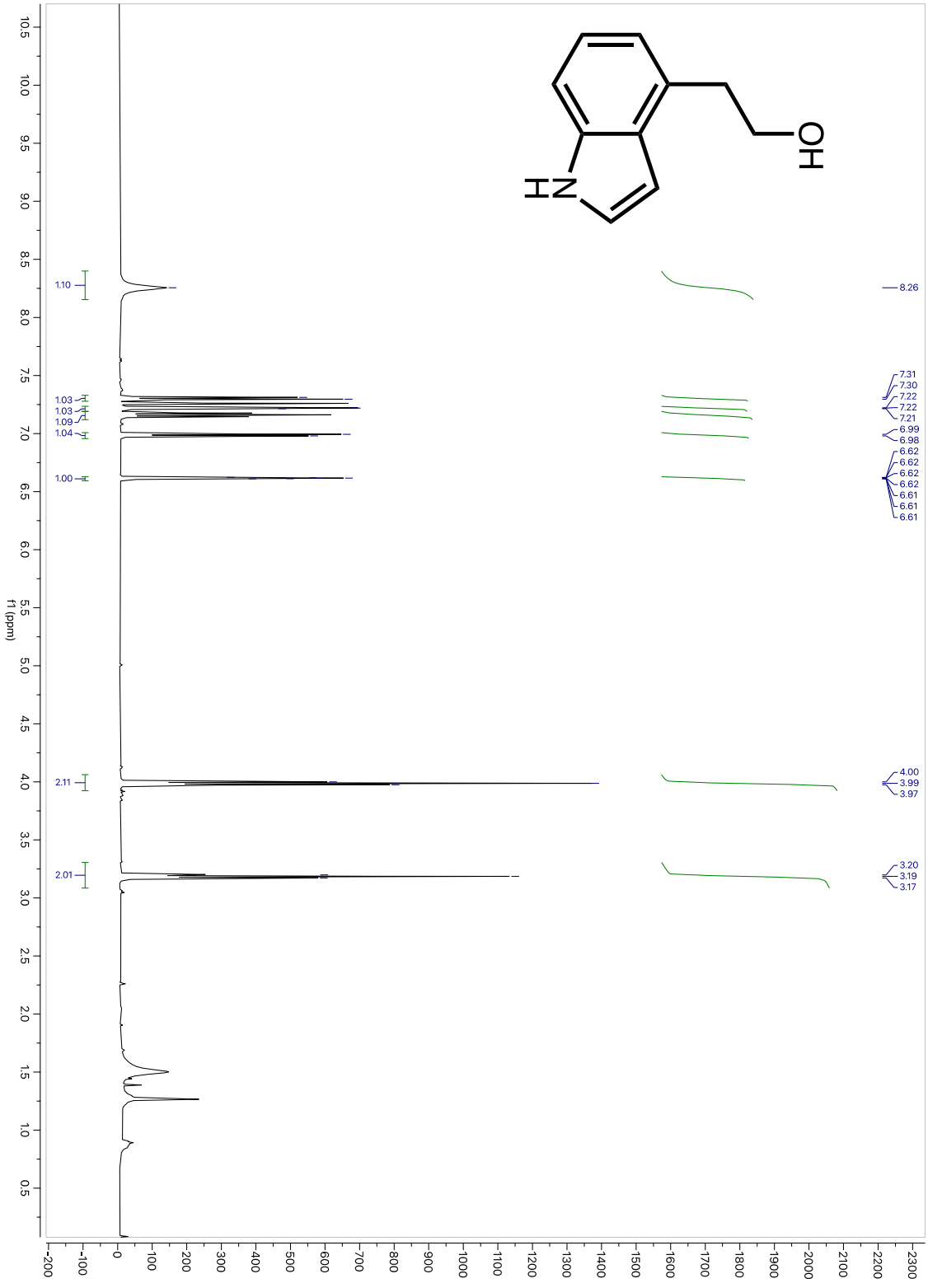

6
CN
3
7
8
NMe2
9,10
4
2
5
1
N
O
f2 (ppm)
f1 (ppm)
4.0
3.6
f2 (ppm)
f1 (ppm)

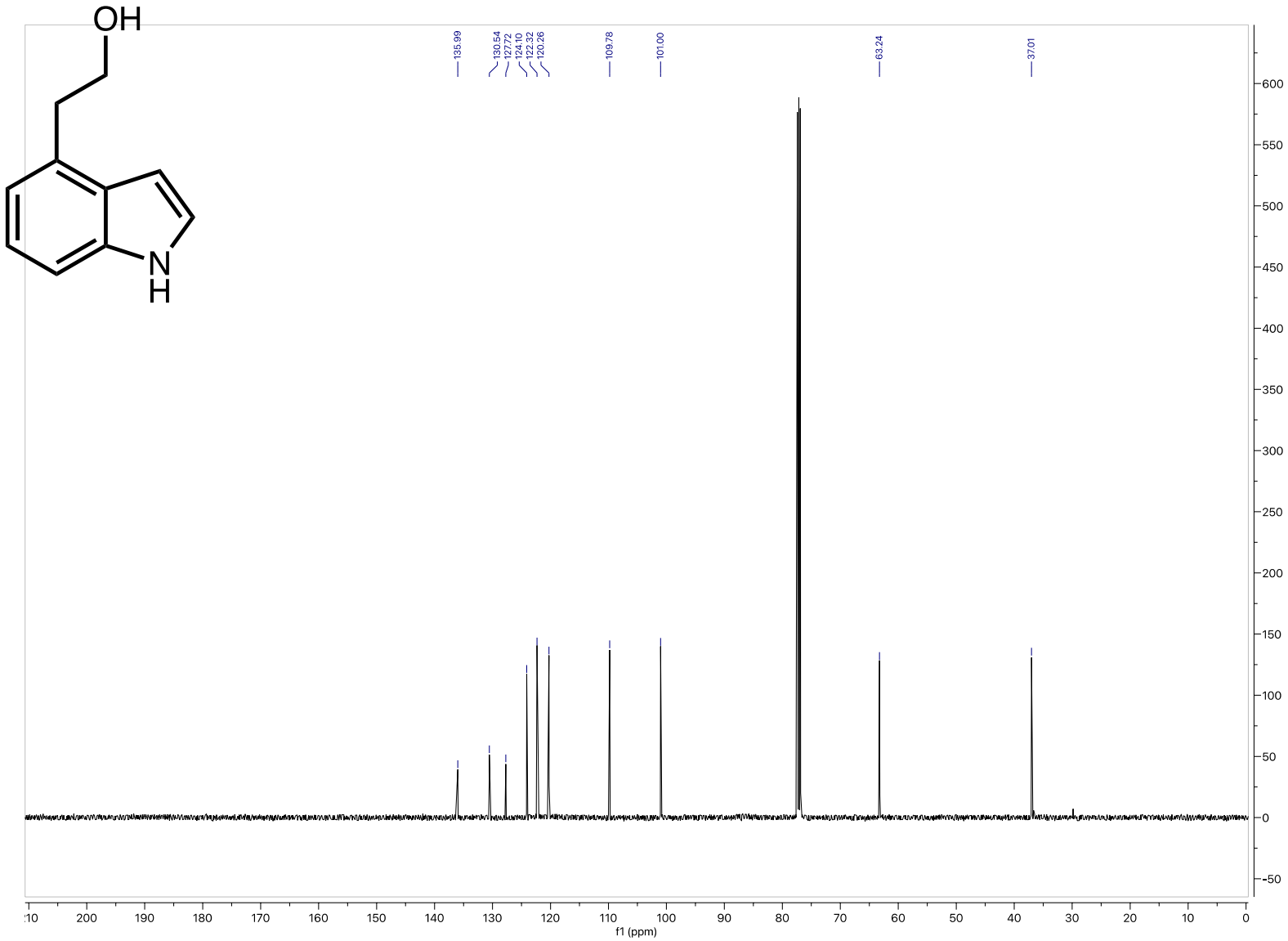

10.20
7.32
7.30
7.29
7.28
7.28
7.05
7.04
7.02
6.96
6.94
6.56
6.56
6.55
3.85
OH
O
N
H
1.18
1.98
1.09
1.07
1.00
2.20
f1 (ppm)

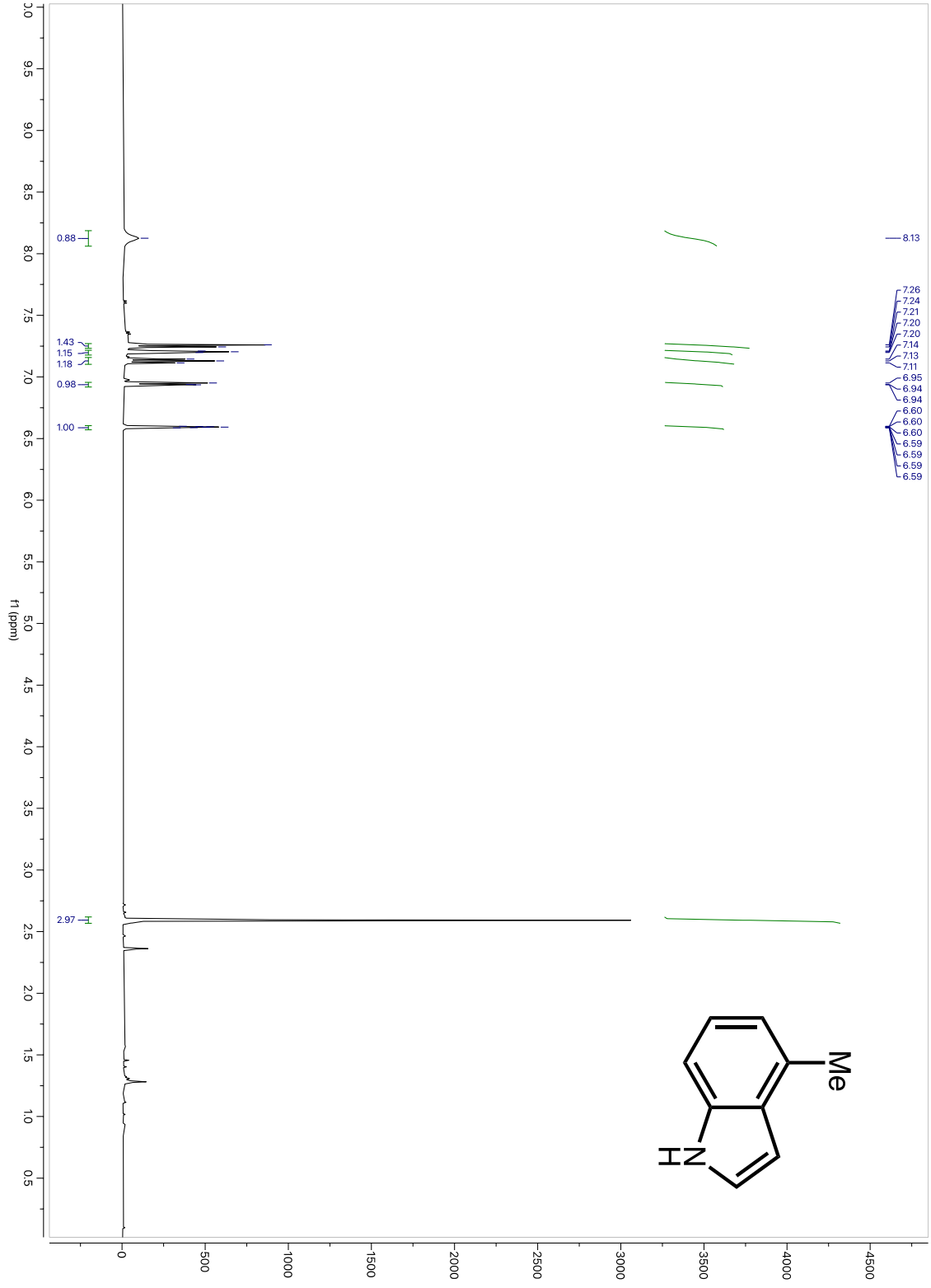

f1 (ppm)
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
-200
0
200
400
600
800
1000
1200
1400
1600
1800
2000
2200
2400
2600
2800
172.93
136.95
128.64
127.02
125.18
121.91
120.64
120.63
110.87
100.76
39.32
HN
O
OH

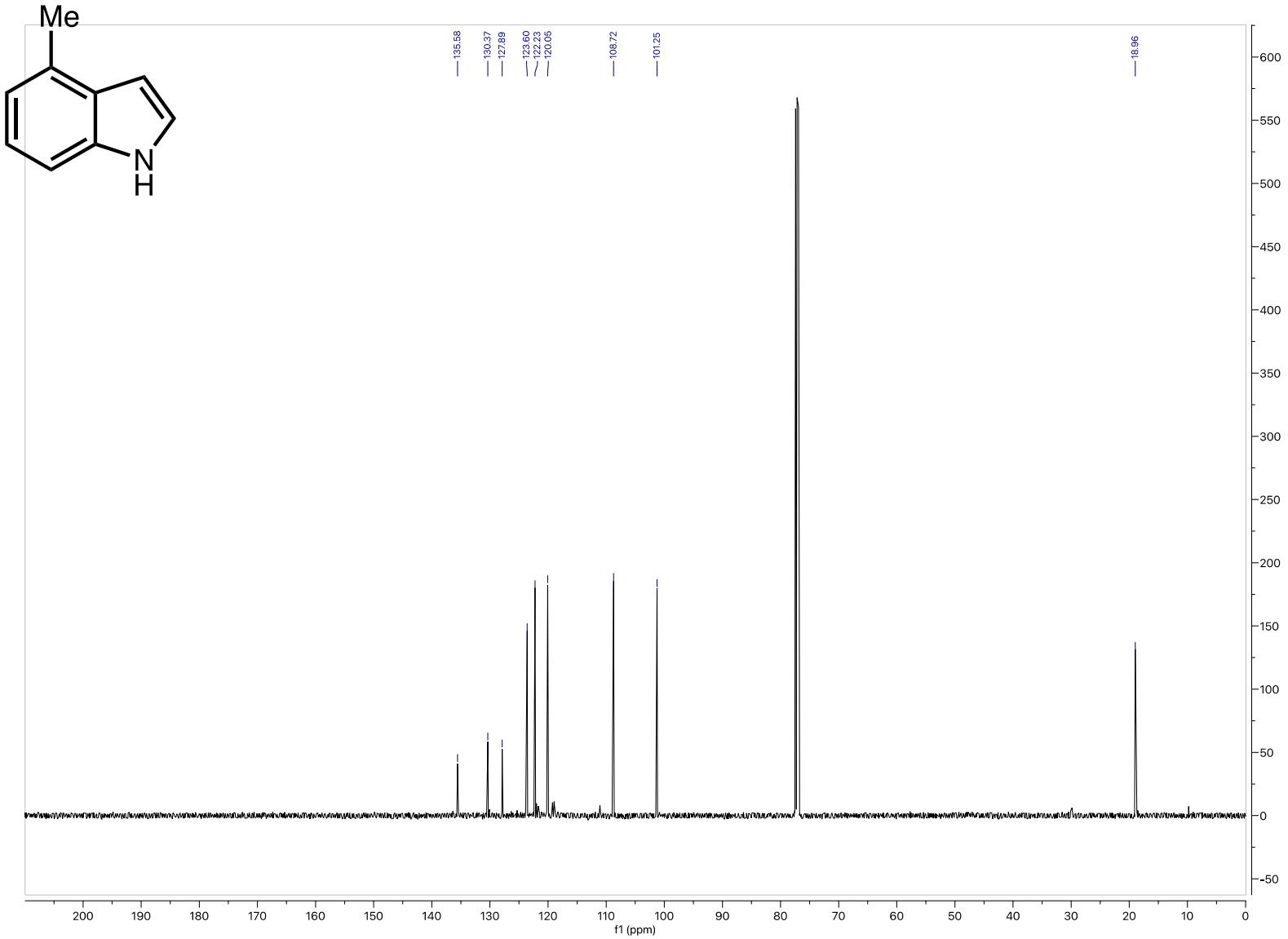

f1 (ppm)
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
1.02
1.07
0.78
0.77
0.28
1.04
1.00
2.18
2.19
6.55
9.00
7.35
7.35
7.33
7.27
7.27
7.26
7.24
7.23
7.08
7.07
6.71
6.71
6.71
6.71
6.70
6.70
6.70
3.27
3.26
3.25
3.23
2.86
2.84
2.84
2.83
2.51
0
5000
10000
15000
20000
25000
30000
35000
40000
45000
Me
N
Me
H
N


Me
N
Me
N
H
135.96
132.31
127.41
124.07
122.07
119.38
109.38
100.60
60.63
45.44
31.95
f1 (ppm)


HN
OH
1.10
1.03
1.03
1.09
1.04
1.00
2.11
2.01
8.26
7.31
7.30
7.22
7.22
7.21
6.99
6.98
6.62
6.62
6.62
6.62
6.61
6.61
6.61
4.00
3.99
3.97
3.20
3.19
3.17
f1 (ppm)


OH
N
H
135.99
130.54
127.72
124.10
122.32
120.26
109.78
101.00
63.24
37.01
600
550
500
450
400
350
300
250
200
150
100
50
0
-50
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


f1 (ppm)
0.88
1.43
1.15
1.18
0.98
1.00
2.97
8.13
7.26
7.24
7.21
7.20
7.20
7.14
7.13
7.11
6.95
6.94
6.94
6.60
6.60
6.60
6.59
6.59
6.59
6.59
Me
HN


Me
N
H
135.58
130.37
127.89
123.60
122.23
120.05
108.72
101.25
18.96
600
550
500
450
400
350
300
250
200
150
100
50
0
-50
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)