Electronic Supplementary Material (ESI) for Chemical Communications

Supplementary Material

The genetic incorporation of thirteen novel non-canonical amino acids

Alfred Tuley, Yane-Shih Wang, Xinqiang Fang, Yadagiri Kurra, Yohannes Rezenom, and Wenshe Liu

Department of Chemistry, Texas A&M University, College Station, TX 77843
E-mail: wliu@chem.tamu.edu

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1. Superfolder Green Fluorescent Protein Expression

The constructs used to incorporate compounds **1**-**4** in this study and corresponding protein purification and characterization are identical to previous reports.[1,2] For compounds **5**-**15**, BL21(DE3) *E. coli* cells containing pEVOL-PylT-pylRS-PylRSN346A/C348A (Cm^r) and pET-PylT-sfGFP-S2TAG' (Amp^r) vectors were grown in 500 mL of LB media with ampicillin (100 μg/mL) and chloramphenicol (34 μg/mL) until the OD_{600} reached 1.0-1.3, at which point the cells were pelleted at 4,000 r.p.m. for 20 min, then washed and resuspended in 30 mL H_2O. The resuspended cells were added as 5 mL aliquots to 45 mL of minimal media (33.7 mM Na_2HPO_4, 22 mM KH_2PO_4, 8.6 mM NaCl, 9.4 mM NH_4Cl, 1 mM $MgSO_4$, 0.3 mM $CaCl_2$, 1% glycerol) supplemented with 2 mM non-canonical amino acid (NAA), 1 mM IPTG, and 0.2% arabinose, at which point protein expression was allowed to occur for 12 h. The cells were then pelleted at 4,000 r.p.m. for 20 min, resuspended in lysis buffer (50 mM NaH_2PO_4, 300 mM NaCl, pH 8) and lysed via sonication. The crude lysate was then centrifuged at 10,000 r.p.m. for 1 hour, and the supernatant was treated with imidazole to a final concentration of 10 mM. Next, the supernatant was subsequently incubated with Ni^{2+}-NTA resin for 1 hour at 4 ºC. The resin was washed with lysis buffer containing 10 mM imidazole (3x column volume) and 20 mM imidazole (3x column volume), then eluted with elution buffer (50 mM NaH_2PO_4, 300 mM NaCl, 500 mM imidazole, pH 8). The protein was dialyzed against 10 mM Tris buffer, and if necessary, the proteins were concentrated using Amicon Ultracel-10k centrifugal filter units. Purity of the proteins was confirmed via 15% SDS-PAGE and ESI-MS analysis. Yields were determined using a commercially available BCA protein assay kit (Thermo Scientific).

2. sfGFP-2 Protein Labeling

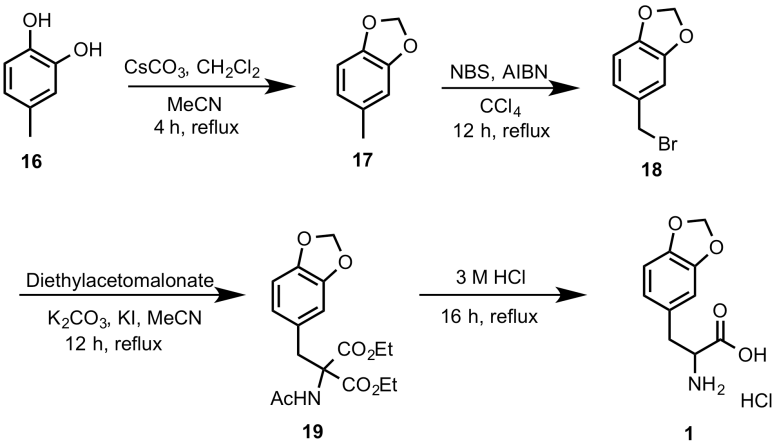
To 16 μl of 1X Phosphate buffered saline (PBS) was added 2 μl sfGFP-**2** (3 mg/mL, 1 μM) and 2 μl Dibenzylcyclooctyne (DBCO) dye (25 mM, DMSO stock solution)[3] and was allowed to react at room temperature overnight. The protein was then precipitated with 180 μl of methanol and left at -20 ºC for 1 h. Next, the precipitated protein was centrifuged for 5 min, 14,000 r.p.m. and washed twice with 100% methanol. Finally, the residue was resuspended in 20 μl H_2O and subjected to SDS-PAGE analysis. The control experiment was identical except 4 μl sfGFP-**3** (5 mg/mL, 0.9 μM) was added to 14 μl PBS, followed by 2 μl DBCO dye.

3. ESI-MS Analysis of Intact Proteins

Nanoelectrospray ionization in positive mode was performed using an Applied Biosystems QSTAR Pulsar (Concord, ON, Canada) equipped with a nanoelectrospray ion source. Solution was flowed at 700 nL/min through a 50 μm ID fused-silica capillary that was tapered at the tip. Electrospray needle voltage was held at 2100 V.

4. Organic Synthesis

Reactions were carried out using oven-dried glassware and under an atmosphere of argon, where appropriate. Reagents were purchased and used without further purification. NMR spectra were obtained with Inova 300 and Mercury 300 MHz instruments.



4.1 2-amino-3-(benzo[*d*][1,3]dioxol-5-yl)propanoic acid hydrochloride (1)

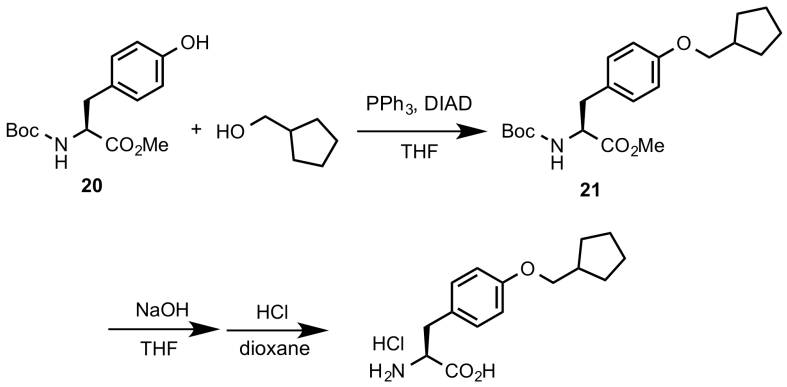
To a solution of catechol **16** (5 g, 8.06 mmol) in MeCN (25 mL) was added Cs_2CO_3 (3.93g 12.09 mmol), followed by CH_2Cl_2 (5.21 mL, 8.06 mmol). The resulting mixture was refluxed for 4 hours. After cooling to room temperature, the reaction mixture was concentrated and applied to column chromatography, yielding the acetal **17** in 40% yield (2.12 g).[4] ^{1}HNMR (300 MHz, $CDCl_3$) δ 6.71(m, 3H), 5.93(s, 2H), 2.27(s, 3H).

The compound **17** (2.0 g, 14.7 mmol) was dissolved in CCl_4 (15 mL), followed by addition of NBS (3.12 g, 17.62 mmol) and AIBN (0.337 g, 2.05 mmol). The mixture was heated to reflux for 12 h under the protection of argon. After cooling, the precipitate was removed via filtration and the filtrate was concentrated and dried under vacuum to afford the known compound **18**,[5] which was used directly in the next step without further purification.

To a solution of compound **18** (3.5 g, 16.27 mmol) in anhydrous acetonitrile (15 mL) was added diethyl 2-acetamidomalonate (3.88 g, 17.88 mmol), K_2CO_3 (4.49 g, 32.53 mmol) and KI (1.0 g, 15.38 mmol). The resulting mixture was heated to reflux for 12 h under the protection of argon, then cooled to room temperature. The solid was filtered, the solvent was removed under reduced pressure, and the residue was purified via column chromatograph with hexanes/ethyl acetate (3:1 v/v) as eluent to give the pure product **19** (60% yield, 3.42 g). ^{1}HNMR (300 MHz, $CDCl_3$) δ 6.69 (d, J = 8.2Hz, 1 H), 6.57(d, J = 7.9 Hz, 1 H), 6.47 (s, 1H), 5.92 (s, 2H), 4.30-4.22 (m, 4H), 3.56 (s, 2H), 2.04 (s, 3H), 1.32-1.23 (m, 6H).

A suspension of compound **19** (3.0 g, 8.54 mmol) in 3 M HCl (91.16 mL, 32 eq.) was heated to reflux for 16 h before cooling to room temperature. Water was evaporated under reduced pressure and the solid was collected. The solid was washed with Et_2O (10 mL × 3) and then dried under vacuum to afford compound **1** as a solid (68% yield, 1.42 g). ^{1}H NMR (300 MHz, CD_3OD) δ 6.78-6.66 (m, 3H), 5.85 (s, 2H), 4.14 (t, J = 6.6 Hz, 1 H), 3.09 (ABq, J = 15.3, 6.6 Hz, 2 H). ^{13}C NMR (75 MHz, CD_3OD) δ 169.8, 148.1, 147.3, 127.6, 122.6, 109.1, 108.2, 101.1, 53.9, 39.5.

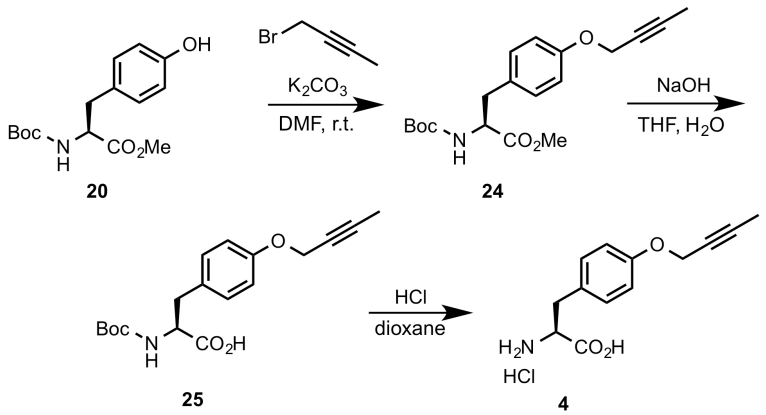
4.2 Alkylation of Tyrosine



4.2.1 (*S*)-2-amino-3-(4-(cyclopentylmethoxy)phenyl)propanoic acid hydrochloride (3)

N-Boc-*o*-methyl-L-tyrosine (**20**) (1.3 g, 4.4 mmol), cyclopentylmethanol (0.57 mL, 5.28 mmol), and triphenylphosphine (1.73 g, 6.6 mmol) were added to a round bottom flask, then the atmosphere evacuated and replaced with argon. THF (10 mL) was then added and the reaction mixture was cooled to 0 ºC, at which point DEAD (1.3 mL, 6.6 mmol) was added dropwise and stirred overnight. Upon completion, the reaction mixture was concentrated and purified via column chromatography (gradient elution, 25% to 50% ethyl acetate/hexanes) to give the product **21** in 78% yield (1.3 g).

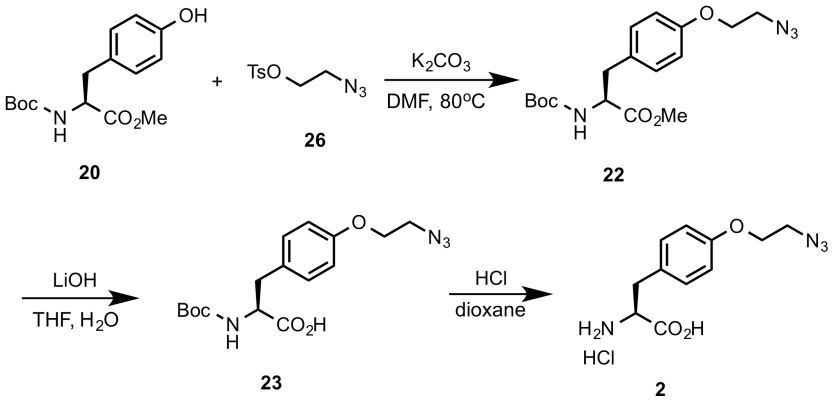
This compound was deprotected using the previously described procedure;[1] treatment with 5 mL 1 M NaOH/THF for two hours, followed by Boc deprotection in 5 mL 4 M HCl/dioxane to give the free amino **3** acid in 97% yield (898 mg), two steps. ^{1}H NMR (300 MHz, CD_3OD) δ 7.16 (d, J = 8.4 Hz, 2 H), 6.87 (d, J = 8.7, 6.9 Hz, 2 H), 4.15 (t, J = 5.4 Hz, 1 H), 3.80 (d, J = 6.9 Hz, 2 H), 3.14 (dd, J = 7.8, 5.4 Hz, 2 H), 2.31 (m, 1 H), 1.82 (m, 2 H), 1.61 (m, 4 H), 1.36 (m, 2 H). ^{13}C NMR (75 MHz, CD_3OD) δ 169.9, 158.9, 130.0, 125.6, 114.6, 71.8, 53.8, 38.9, 35.0, 28.9, 24.9.



4.2.2 (*S*)-2-amino-3-(4-(but-2-yn-1-yloxy)phenyl)propanoic acid hydrochloride (4)

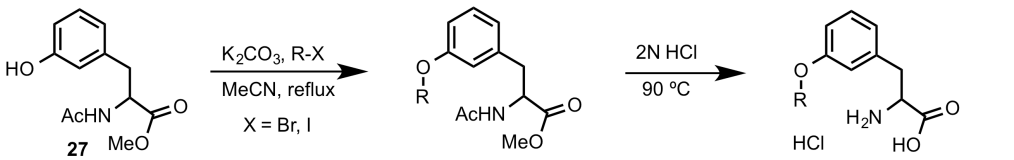
N-Boc-*o*-methyl-L-tyrosine (1 g, 3.39 mmol) was treated with 3-bromo-2-propyne (0.36 mL, 4.06 mmol) and potassium carbonate (1.4 g, 10.2 mmol) in DMF and left to

react overnight at room temperature. The reaction mixture was then diluted with ethyl acetate and washed with 3 M HCl. The organic layers were then dried, concentrated, and purified via flash chromatography (gradient elution, 25% to 50% ethyl acetate/hexanes) to afford the protected azide **24** in 30% yield (370 mg). Deprotection using the protocol listed above gave the title compound **4** in 44% yield (274 mg). ^{1}H NMR (300 MHz, CD_3OD) δ 7.10 (d, J = 8.7 Hz, 2 H), 6.83 (d, J = 8.7 Hz, 2 H), 4.53 (m, 2 H), 4.08 (q, J = 7.5 Hz, 1 H), 3.02 (dd, J = 14.7, 7.5 Hz, 2 H), 1.68 (s, 3 H). ^{13}C NMR (75 MHz, CD_3OD) δ 168.1, 155.8, 128.4, 124.6, 113.3, 81.0, 72.0, 53.9, 52.0, 33.2.



4.2.3 (*S*)-2-amino-3-(4-(2-azidoethoxy)phenyl)propanoic acid hydrochloride (2)

N-Boc-*o*-methyl-L-tyrosine **20** (1.8 g, 6.09 mmol) was treated with compound **26** (1.8 g, 7.31 mmol) and potassium carbonate (3.3 g, 24.4 mmol) in DMF (10 mL) and the reaction was heated to 80 ºC. When the reaction was complete based on TLC, the reaction mixture was worked up as usual and purified via flash chromatography (gradient elution, 25% to 50% ethyl acetate/hexanes) to yield compound **22**. Deprotection was achieved using 20 mL 1 M LiOH, followed by treatment with 5 mL 4 M HCl/Dioxane to afford free amino acid **2** (50%, two steps, 873 mg). ^{1}H NMR (300 MHz, D_2O) δ 7.16 (d, J = 8.4 Hz, 2 H), 6.88 (d, J = 8.4 Hz, 2 H), 4.11 (m, 3 H), 3.50 (m, 2 H), 3.11 (m, J = 7.2, 6.9, 5.4 Hz, 2 H). ^{13}C NMR (75 MHz, D_2O) δ 169.8, 158.1, 130.2, 126.4, 114.7, 67.0, 53.8, 49.8, 35.0.



4.3 Representative Procedure for *m*-Tyrosine Alkylation

4.3.1 Methyl 2-acetamido-3-(3-ethoxyphenyl)propanoate (28)

To a solution of *m*-tyrosine[6] **27** (1.5 g, 6.32 mmol) in DMF (12.64 mL) was added K_2CO_3 (2.84 g, 20.55 mmol), followed by ethyl iodide (0.76 mL, 9.48 mmol). The

reaction was stirred at room temperature for 18 h, then quenched with 16 mL H_2O and 79 mL ethyl acetate. The organic layer was extracted three times with 40 mL H_2O, then dried with sodium sulfate and concentrated. Column chromatography (gradient elution, 50 to 75% ethyl acetate/hexanes) afforded the compound as a yellow, crystalline solid (72% yield, 1.2 g). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.17 (t, *J* = 7.8 Hz, 1H), 6.76 (dd, *J* = 8.1, 5.7 Hz, 1 H), 6.66 (m, 2 H), 6.04 (d, *J* = 7.5 Hz, 1 H), 4.85 (dt, *J* = 7.8, 5.7 Hz, 1 H), 3.97 (q, *J* = 6.9 Hz, 2 H), 3.71 (s, 3 H), 3.07 (m, *J* = 8.1, 5.7 Hz, 2 H), 1.97 (s, 3 H), 1.38 (t, *J* = 6.9 Hz, 3 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.2, 169.7, 159.2, 137.3, 129.6, 121.5, 115.6, 113.2, 63.4, 53.1, 52.4, 37.9, 23.3, 14.9.

4.3.2 Methyl 2-acetamido-3-(3-propoxyphenyl)propanoate (29)

Synthesized according to the general procedure with *m*-tyrosine (1.5 g, 6.32 mmol), DMF (12.64 mL), K_2CO_3 (2.84g, 20.55 mmol), and n-propyl bromide (0.86 mL, 9.48 mmol) to yield a yellow, crystalline solid, (68% yield, 1.2 g). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.18 (t, *J* = 8.1 Hz, 1 H), 6.79 (m, *J* = 5.7 Hz, 1 H), 6.66 (m, 2H), 5.88 (d, *J* = 7.8 Hz, 1 H), 4.87 (dt, *J* = 7.8, 5.7 Hz, 1H), 3.88 (t, *J* = 6.6 Hz, 2 H), 3.74 (s, 3 H), 3.09 (m, *J* = 8.1, 5.7 Hz, 2 H), 1.99 (s, 3H), 1.79 (sx, *J* = 7.2 Hz, 2 H), 1.03 (t, *J* = 7.2 Hz, 3 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.1, 169.7, 159.3, 137.3, 129.6, 121.4, 115.6, 113.2, 69.5, 53.1, 52.4, 37.9, 23.2, 22.6, 10.6.

4.3.3 Methyl 2-acetamido-3-(3-butoxyphenyl)propanoate (30)

Synthesized according to the general procedure with *m*-tyrosine (1.0 g, 4.21 mmol), DMF (8.42 mL), K_2CO_3 (1.89g, 13.69 mmol), and n-butyl iodide (0.72 mL, 6.32 mmol) to yield a yellow, crystalline solid (65% yield, 0.8 g). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.19 (t, *J* = 7.8 Hz, 1 H), 6.77 (d, *J* = 7.2 Hz, 1 H), 6.64 (m, 2 H), 5.87 (d, *J* = 6.9 Hz, 1 H), 4.87 (dt, *J* = 7.8, 5.4 Hz, 1 H), 3.92 (t, *J* = 6.6 Hz, 2 H), 3.74 (s, 3 H), 3.09 (dd, *J* = 8.1, 5.7 Hz, 2 H), 1.99 (s, 3 H), 1.76 (p, *J* = 6.9, 6.6, 6.3 Hz, 2 H), 1.46 (m, 2 H), 0.97 (t, *J* = 7.2 Hz, 3 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.2, 169.7, 159.4, 137.3, 129.6, 121.4, 115.6, 113.2, 67.7, 53.1, 52.4, 37.9, 31.4, 23.2, 19.3, 13.9.

4.3.4 Methyl 2-acetamido-3-(3-(pentyloxy)phenyl)propanoate (31)

Synthesized according to the general procedure with *m*-tyrosine (1.5 g, 6.32 mmol), DMF (12.64 mL), K_2CO_3 (2.84g, 20.55 mmol), and n-pentyl bromide (1.18 mL, 9.48 mmol) to yield a yellow, crystalline solid (72% yield, 1.4 g). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.19 (t, *J* = 8.1 Hz, 1 H), 6.78 (m, 1 H), 6.63 (m, 2H), 5.87 (d, *J* = 6.9 Hz, 1 H), 4.82 (dt, *J* = 7.8, 5.7 Hz, 1H), 3.91 (t, *J* = 6.6 Hz, 2 H), 3.74 (s, 3 H), 3.10 (dq, *J* = 8.1, 6.0 Hz, 2 H), 1.99 (s, 3H), 1.77 (p, *J* = 7.2, 6.9, 6.6 Hz, 2 H), 1.39 (m, 4 H), 0.93 (t, *J* = 7.2 Hz, 3 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.1, 169.7, 159.2, 137.3, 129.5, 121.2, 115.5, 113.0, 67.8, 53.1, 52.3, 37.8, 28.9, 28.2, 23.0, 22.4, 14.0.

4.4.5 Methyl 2-acetamido-3-(3-(hexyloxy)phenyl)propanoate (32)

Synthesized according to the general procedure with *m*-tyrosine (1.0 g, 4.21 mmol), DMF (8.42 mL), K_2CO_3 (1.89g, 13.69 mmol), and 1-iodohexane (0.93 mL, 6.32 mmol) to yield a yellow, crystalline solid (44% yield, 0.6 g). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.18 (t, *J* = 8.1 Hz, 1 H), 6.77 (dd, *J* = 8.1, 5.7 Hz, 1 H), 6.63 (m, 2H), 5.88 (d, *J* = 7.2 Hz, 1 H), 4.87 (dt, *J* = 7.8, 5.7 Hz, 1H), 3.91 (t, *J* = 6.6 Hz, 1 H), 3.74 (s, 3 H), 3.09 (m, *J*

= 8.1, 5.7 Hz, 2 H), 1.99 (s, 3H), 1.77 (p, *J* = 8.1, 6.6 Hz, 2 H), 1.44 (m, 4 H), 1.33 (m, 4H), 0.91 (t, *J* = 7.2 Hz, 3 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.2, 169.7, 159.4, 137.3, 129.6, 121.4, 115.6, 113.2, 68.0, 53.1, 52.4, 37.9, 31.7, 29.3, 25.8, 23.3, 22.7, 14.1.

4.5 Representative Procedure for *meta*-alkoxy Phenylalanine Deprotection:

4.5.1 2-amino-3-(3-ethoxyphenyl)propanoic acid hydrochloride (6)

A round bottomed flask charged with compound **28** (1.1 g, 4.15 mmol) was treated with 14.3 mL 2 M HCl, and the resulting suspension was heated to 100 ºC, which was refluxed overnight until complete consumption of starting material as observed on TLC. The reaction mixture was then cooled to room temperature and concentrated *in vacuo*. If necessary, the compound was resubjected to the reaction conditions due to the persistence of methyl ester and/or N-acyl amide signals in ^{1}H and ^{13}C NMR. The title compound was obtained as a white solid in 45% yield (463 mg). ^{1}H NMR (300 MHz, D_2O) δ 7.35 (t, *J* = 7.8 Hz, 1 H), 6.93 (m, 3 H), 4.31 (t, *J* = 7.5 Hz 1 H), 4.11 (q, *J* = 6.9 Hz, 2 H), 3.24 (dq, *J* = 14.7, 5.7 Hz, 2 H), 1.37 (t, *J* = 6.9 Hz, 3 H). ^{13}C NMR (75 MHz, D_2O) δ 171.4, 158.3, 135.6, 130.4, 122.1, 115.5, 114.1, 64.2, 54.0, 35.5, 13.8.

4.5.2 2-amino-3-(3-propoxyphenyl)propanoic acid hydrochloride (7)

Prepared according to the general procedure using compound **29** (1.0 g, 3.58 mmol) and 12.34 mL 2 M HCl. Obtained as a white solid in 43% yield (402 mg). ^{1}H NMR (300 MHz, D_2O) δ 7.34 (t, *J* = 7.5 Hz, 1 H), 6.90 (m, 3 H), 4.29 (t, *J* = 5.4 Hz, 1 H), 3.97 (t, *J* = 6.6 Hz), 3.21 (dd, *J* = 14.7, 5.7 Hz, 2 H), 1.73 (sx, *J* = 7.5, 7.2, 6.9, 6.6 Hz, 2 H), 0.96 (t, *J* = 7.5, 3 H). ^{13}C NMR (75 MHz, D_2O) δ 171.3, 158.5, 135.5, 130.3, 122.0, 115.5, 114.1, 70.1, 53.9, 35.4, 21.7, 9.5.

4.5.3 2-amino-3-(3-butoxyphenyl)propanoic acid hydrochloride (8)

Prepared according to the general procedure using compound **30** (0.8 g, 2.73 mmol) in 9.4 mL 2 M HCl to obtain the title compound in 30% yield (222 mg). ^{1}H NMR (300 MHz, DMSO) δ 8.53 (s, 2 H), 7.21 (t, *J* = 7.8 Hz, 1 H), 6.89 (s, 1 H), 6.82 (d, *J* = 7.8 Hz, 2 H), 4.14 (t, *J* = 5.4 Hz, 1 H), 3.94 (t, *J* = 6.6 Hz, 2 H), 3.12 (d, *J* = 6.0 Hz, 2 H), 1.69 (p, *J* = 7.5, 6.9, 6.6 Hz, 2 H), 1.45 (sx, *J* = 7.5, 7.2 Hz, 2 H), 0.93 (t, *J* = 7.2 Hz, 3 H). ^{13}C NMR (75 MHz, DMSO) δ 170.2, 158.7, 136.4, 129.5, 121.5, 115.6, 113.1, 66.9, 53.1, 35.5, 30.8, 18.8, 13.7.

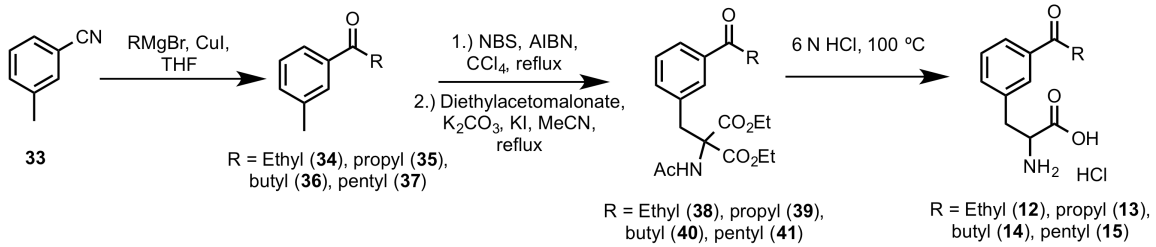
4.5.4 2-amino-3-(3-(pentyloxy)phenyl)propanoic acid hydrochloride (9)

Prepared according to the general procedure using compound **31** (1.0 g, 3.25 mmol) in 11.2 mL 2 M HCl to obtain the title compound in 61% yield (570 mg). ^{1}H NMR (300 MHz, DMSO) δ 8.49 (s, 2 H), 7.21 (t, *J* = 7.5 Hz, 1 H), 6.88 (s, 1 H), 6.82 (d, *J* = 8.1 Hz, 2 H), 4.14 (t, *J* = 6.0 Hz, 1 H), 3.94 (t, *J* = 6.6 Hz, 1 H), 3.11 (d, *J* = 6.0 Hz, 2 H), 1.71 (p, *J* = 6.9, 6.6, 6.3 Hz, 2 H), 1.36 (m, 4 H), 0.89 (t, *J* = 6.6 Hz, 3 H). ^{13}C NMR (75 MHz, DMSO) δ 170.2, 158.7, 136.4, 129.5, 121.6, 115.6, 113.1, 67.2, 53.0, 35.6, 28.4, 27.7, 21.9, 13.9.

4.5.5 2-amino-3-(3-(hexyloxy)phenyl)propanoic acid hydrochloride (10)

Prepared according to the general procedure using compound **32** (0.6 g, 1.87 mmol) in 6.44 mL 2 M HCl to yield the compound as a white solid in 65% yield (367

mg). ^{1}H NMR (300 MHz, DMSO) δ 8.48 (s, 3 H), 7.21 (t, *J* = 7.8 Hz, 1 H), 6.88 (s, 1 H), 6.82 (d, *J* = 7.8 Hz, 2 H), 4.13 (m, *J* = 5.1, 4.5 Hz, 1 H), 3.93 (t, *J* = 6.6 Hz, 2 H), 3.10 (d, *J* = 6.3 Hz, 2 H), 1.69 (p, *J* = 7.8, 6.6 Hz, 2 H), 1.41 (m, 2 H), 1.29 (m, 4 H), 0.88 (t, *J* = 6.6 Hz, 3 H). ^{13}C NMR (75 MHz, DMSO) δ 170.2, 158.7, 136.3, 129.5, 121.5, 115.6, 113.1, 67.2, 53.0, 35.6, 31.0, 28.7, 25.2, 22.1, 13.9.



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R = Ethyl (**34**), propyl (**35**), butyl (**36**), pentyl (**37**)

R = Ethyl (**38**), propyl (**39**), butyl (**40**), pentyl (**41**)

R = Ethyl (**12**), propyl (**13**), butyl (**14**), pentyl (**15**)

4.6 Representative Procedure for Grignard Addition to *m*-Tolunitrile

4.6.1 1-(*m*-tolyl)propan-1-one (34)

Ethyl magnesium bromide (1 M/THF, 85.36 mL, 85.36 mmol) was added dropwise to a solution of *m*-tolunitrile (10.25 mL, 85.36 mmol) and copper (I) iodide (40.64 mg, 0.213 mmol) in anhydrous THF (170.72 mL) under argon. After stirring for 22 h, the reaction was quenched with approx. 5 mL 1 M HCl at 0 ºC and stirred for 4 h, allowing the reaction to warm to room temperature. The resulting layers were separated and the organic layer was dried with $MgSO_4$, filtered, and concentrated. The crude, yellow oil was purified via silica gel chromatography (gradient, 0 to 10% EtOAc/Hex) to afford the known compound[7] **34** in 94% yield (9.4 g) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (m, 1H), 7.38 (m, 3H), 2.99 (q, *J* = 7.2 Hz, 2H), 2.41 (s, 3H), 1.22 (t, *J* = 7.2 Hz, 3H).

4.6.2 1-(*m*-tolyl)butan-1-one (35)

Prepared according to the general procedure with Propylmagnesium bromide (2 M/THF, 42.68 mL, 85.36 mmol), *m*-tolunitrile (10.25 mL, 85.36 mmol), and CuI (40.64 mg, 0.213 mmol) to yield the known compound[8] in 96 % yield (13.24 g). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77 (s, 2H), 7.36 (s, 2H), 2.94 (t, *J* = 7.2 Hz, 2H), 2.41 (s 3H), 1.76 (sx, *J* = 7.2 Hz, 2H), 1.00 (t, *J* = 7.2 Hz, 3H).

4.6.3 1-(*m*-tolyl)pentan-1-one (36)

Prepared according to the general procedure with butylmagnesium chloride (2 M/THF, 42.68 mL, 85.36 mmol), *m*-tolunitrile (10.25 mL, 85.36 mmol), and CuI (40.64 mg, 0.213 mmol) to yield the known compound[9] in 76% yield (11.43 g). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (m, 2H), 7.34 (m, 2H), 2.95 (t, *J* = 7.2 Hz, 2H), 2.41 (s, 3H), 1.71 (p, *J* = 7.5 Hz, 2H), 1.41 (sx, *J* = 7.2 Hz, 2H), 0.95 (t, *J* = 7.5 Hz, 3H).

4.6.4 1-(*m*-tolyl)hexan-1-one (37)

Prepared according to the general procedure with Pentylmagnesium bromide (1 M/THF, 42.68 mL, 85.36 mmol), *m*-tolunitrile (10.25 mL, 85.36 mmol), and CuI (40.64 mg, 0.213 mmol) to yield the known compound[10] in 94% yield (15.3 g). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74 (m, 1H), 7.46 (s, 1H), 7.37 (m, 2H), 2.94 (t, *J* = 6.9 Hz, 2H), 2.41 (s, 3H), 1.73 (p, *J* = 7.2 Hz, 2H), 1.35 (m, 4H), 0.91 (t, *J* = 6.6 Hz, 3H).

4.7 Representative Procedure for the Synthesis of Protected Ketones

4.7.1 Diethyl 2-acetamido-2-(3-propionylbenzyl)malonate (38)

To a solution of *m*-Keto toluene **34** (8.0 g, 53.98 mmol) in CCl_4 (134.95 mL) was added NBS (10.57 g, 59.38 mmol) and AIBN (2.67 g, 16.19 mmol), and the resulting suspension was refluxed overnight. Upon completion, the reaction was filtered and concentrated, and the resulting crude material was subjected to the next step without further purification.

A round-bottom flask was charged with brominated **34** (4.24 g, 19.09 mmol), diethyl 2-acetamidomalonate (3.73 g, 17.18 mmol), K_2CO_3 (5.28 g, 38.18 mmol), and KI (3.17 g, 19.09 mmol), followed by 119.3 mL of MeCN, and the resulting suspension was heated to reflux and stirred overnight. Upon completion, the reaction was cooled to room temperature, filtered with celite, and concentrated. Purification via silica gel chromatography (gradient elution, 0 to 30% EtOAc/Hex) afforded **38** as a yellow solid in 58% yield (3.6 g). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (d, J = 7.8 Hz, 1 H), 7.64 (s, 1 H), 7.36 (t, J = 7.8, 7.5 Hz, 1 H), 7.20 (d, J = 8.4 Hz, 1 H), 6.52 (s, 1 H), 4.28 (q, J = 7.2, 6.9 Hz, 4 H), 2.96 (q, J = 7.2 Hz, 2 H), 2.05 (s, 3 H), 1.31 (t, J = 7.2 Hz, 6 H), 1.21 (t, J = 7.2 Hz, 3 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 200.5, 169.3, 167.4, 137.0, 136.0, 134.5, 129.3, 128.6, 127.1, 67.2, 63.0, 37.7, 31.9, 23.1, 14.1, 8.3.

4.7.2 Diethyl 2-acetamido-2-(3-butyrylbenzyl)malonate (39)

Synthesized according to the general procedure with ketone **35** (8.0 g, 49.31 mmol), NBS (9.65 g, 54.24 mmol), AIBN (2.43 g, 14.79 mmol), and 123.28 mL CCl_4. The crude product was subjected to the next step without further purification.

Malonate synthesis was performed according to the general procedure using brominated **35** (5.73 g, 23.75 mmol), diethyl 2-acetamidomalonate (4.64 g, 21.37 mmol), K_2CO_3 (6.56 g, 47.5 mmol), KI (3.94 g, 23.75 mmol), and 148 mL MeCN. 37% yield, 2.9 g. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.82 (d, J = 7.8 Hz, 1 H), 7.63 (s, 1 H), 7.34 (t, J = 7.5 Hz, 1 H), 7.20 (d, J = 7.2 Hz, 1 H), 6.52 (s, 1 H), 4.28 (q, J = 7.2, 6.9 Hz, 4 H), 2.89 (t, J = 7.2 Hz, 2 H), 2.05 (s, 3 H), 1.75 (sx, J = 7.5, 7.2 Hz, 2 H), 1.31 (t, J = 7.2 Hz, 6 H), 0.99 (t, J = 7.5 Hz, 3 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 200.1, 169.3, 167.4, 137.2, 136.0, 134.5, 129.4, 128.6, 127.1, 67.2, 63.0, 40.6, 37.7, 23.1, 17.8, 14.1, 14.0.

4.7.3 Diethyl 2-acetamido-2-(3-pentanoylbenzyl)malonate (40)

Synthesized according to the general procedure with ketone **36** (9.5 g, 53.9 mmol), NBS (10.55 g, 59.29 mmol), AIBN (2.66 g, 16.17 mmol), and 134.75 mL CCl_4. The crude product was subjected to the next step without further purification.

Malonate synthesis was performed according to the general procedure using brominated **36** (6.26 g, 24.53 mmol), diethyl 2-acetamidomalonate (4.79 g, 22.08 mmol), K_2CO_3 (6.78 g, 49.07 mmol), KI (4.07 g, 24.53 mmol), and 153.3 mL MeCN. 51% yield as a yellow solid (4.4 g). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.82 (d, J = 8.1 Hz, 1 H), 7.62 (s, 1 H), 7.36 (t, J = 7.8 Hz, 1 H), 6.52 (s, 1 H), 4.28 (q, J = 7.2 Hz, 4 H), 3.7 (s, 2 H), 2.92

(t, J = 7.5 Hz, 2 H), 2.05 (s, 3 H), 1.70 (p, J = 7.8, 7.5, 7.2 Hz, 2 H), 1.38 (p, J = 7.8, 7.5, 7.2 Hz, 2 H), 1.31 (t, J = 6.9, 7.2 Hz, 6 H), 0.95 (t, J = 7.2 Hz, 3 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 200.2, 169.3, 167.4, 137.2, 135.9, 134.5, 129.3, 128.6, 127.1, 67.2, 62.9, 38.4, 37.7, 26.4, 23.1, 22.5, 14.0.

4.7.4 Diethyl 2-acetamido-2-(3-hexanoylbenzyl)malonate (41)

Synthesized according to the general procedure with ketone **37** (10.0 g, 52.55 mmol), NBS (10.3 g, 57.81 mmol), AIBN (2.59 g, 15.77 mmol), and 131.38 mL CCl_4. The crude product was subjected to the next step without further purification.

Malonate synthesis was performed according to the general procedure using brominated **37** (5.73 g, 23.75 mmol), diethyl 2-acetamidomalonate (4.52 g, 20.79 mmol), K_2CO_3 (6.39 g, 46.21 mmol), KI (3.84 g, 23.11 mmol), and 144.44 mL MeCN. 37% yield as a yellow solid (3.1 g). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.82 (d, J = 6.6 Hz, 1 H), 7.62 (s, 1 H), 7.36 (t, J = 7.5 Hz, 1 H), 7.20 (d, J = 7.5 Hz, 1 H), 6.52 (s, 1 H), 4.28 (q, J = 7.2 Hz, 4 H), 3.71 (s, 2 H), 2.91 (t, J = 7.5 Hz, 2 H), 2.05 (s, 3 H), 1.72 (m, 2 H), 1.34 (m, 4 H), 1.31 (m, 6 H), 0.91 (t, J = 6.6 Hz, 3 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 200.2, 169.4, 167.4, 137.2, 135.9, 134.5, 129.3, 128.6, 127.1, 67.2, 62.9, 38.7, 37.7, 31.6, 24.0, 23.1, 22.6, 14.1.

4.8 Representative Procedure for Malonate Deprotection

4.8.1 2-amino-3-(3-propionylphenyl)propanoic acid hydrochloride (12)

A suspension of **38** (1.0 g, 2.75 mmol) in 6 M HCl was refluxed overnight, until disappearance of protecting groups was verified via ^{1}H NMR. The resulting solution was concentrated *in vacuo* to yield **12** as a yellow solid (36% yield, 256 mg). ^{1}H NMR (300 MHz, D_2O) δ 7.93 (d, J = 6.9 Hz, 1 H), 7.87 (s, 1 H), 7.55 (m, 2 H), 4.16 (t, J = 7.2 Hz, 1 H), 3.30 (dq, J = 14.1, 5.7 Hz, 2 H), 3.09 (q, J = 7.2 Hz, 2 H), 1.14 (t, J = 6.9 Hz, 3 H). ^{13}C NMR (75 MHz, D_2O) δ 206.4, 172.4, 136.8, 135.1, 134.4, 129.3, 128.7, 127.5, 54.8, 35.7, 31.9, 7.5.

4.8.2 2-amino-3-(3-butyrylphenyl)propanoic acid hydrochloride (13)

Synthesized according to the representative procedure using **39** (1.0 g, 2.65 mmol) in 9.14 mL 6 M HCl. 80% yield, 576 mg. ^{1}H NMR (300 MHz, D_2O) δ 7.95 (d, J = 7.2 Hz, 1 H), 7.85 (s, 1 H), 7.53 (m, 2 H), 4.28 (t, J = 7.2 Hz, 1 H), 3.31 (dq, J = 14.7, 5.7 Hz, 2 H), 3.02 (t, J = 7.2 Hz, 2 H), 1.66 (q, J = 7.2 Hz, 2 H), 0.92 (t, J = 7.2 Hz, 3 H). ^{13}C NMR (75 MHz, D_2O) δ 206.1, 171.5, 136.9, 134.8, 134.5, 129.4, 128.8, 127.8, 54.2, 40.4, 35.4, 17.6, 12.8.

4.8.3 2-amino-3-(3-pentanoylphenyl)propanoic acid hydrochloride (14)

Synthesized according to the representative procedure using **40** (1.08 g, 2.75 mmol) in 9.48 mL 6 M HCl. 29% yield, 200 mg. ^{1}H NMR 7.96 (dt, J = 7.2 Hz, 1 H), 7.87 (s, 1 H), 7.58 (m, 2 H), 4.31 (t, J = 7.2 Hz, 1 H), 3.35 (dq, J = 14.7, 5.7 Hz, 2 H), 3.07 (t, J = 7.2 Hz, 2 H), 1.65 (p, J = 7.2 Hz, 2 H), 1.34 (sx, J = 7.5, 7.2 Hz, 2 H), 0.91 (t, J = 7.2 Hz, 3 H). ^{13}C NMR (75 MHz, D_2O) δ 206.2, 171.5, 136.9, 134.7, 134.5, 129.4, 128.8, 127.8, 54.2, 38.3, 35.4, 26.2, 21.6, 13.0.

4.8.4 2-amino-3-(3-hexanoylphenyl)propanoic acid hydrochloride (15)

Synthesized according to the representative procedure using **41** (1.03 g, 2.55 mmol) in 8.79 mL 6 M HCl. 34% yield, 229 mg. ^{1}H NMR (300 MHz, D_2O) δ 7.95 (d, *J* = 7.2 Hz, 1 H), 7.87 (s, 1 H), 7.57 (m, 2 H), 4.22 (t, *J* = 7.2 Hz, 1 H), 3.33 (dq, *J* = 14.7, 7.8 Hz, 2 H), 3.08 (t, *J* = 7.2 Hz, 2 H), 1.68 (m, 2 H), 1.33 (m, 4 H), 0.86 (m, 3 H). ^{13}C NMR (75 MHz, D_2O) δ 205.2, 181.6, 139.1 136.3, 134.6, 128.8, 128.7, 126.3, 57.3, 40.9, 30.7, 23.8, 21.8, 13.3.

5. sfGFPS2TAG' Protein Sequence

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X denotes an amber stop codon for NAA incorporation in this study.

Compounds **5-15** were expressed using the sequence provided above. sfGFP expression of **1-4** used a sequence described previously.[1,2]

References

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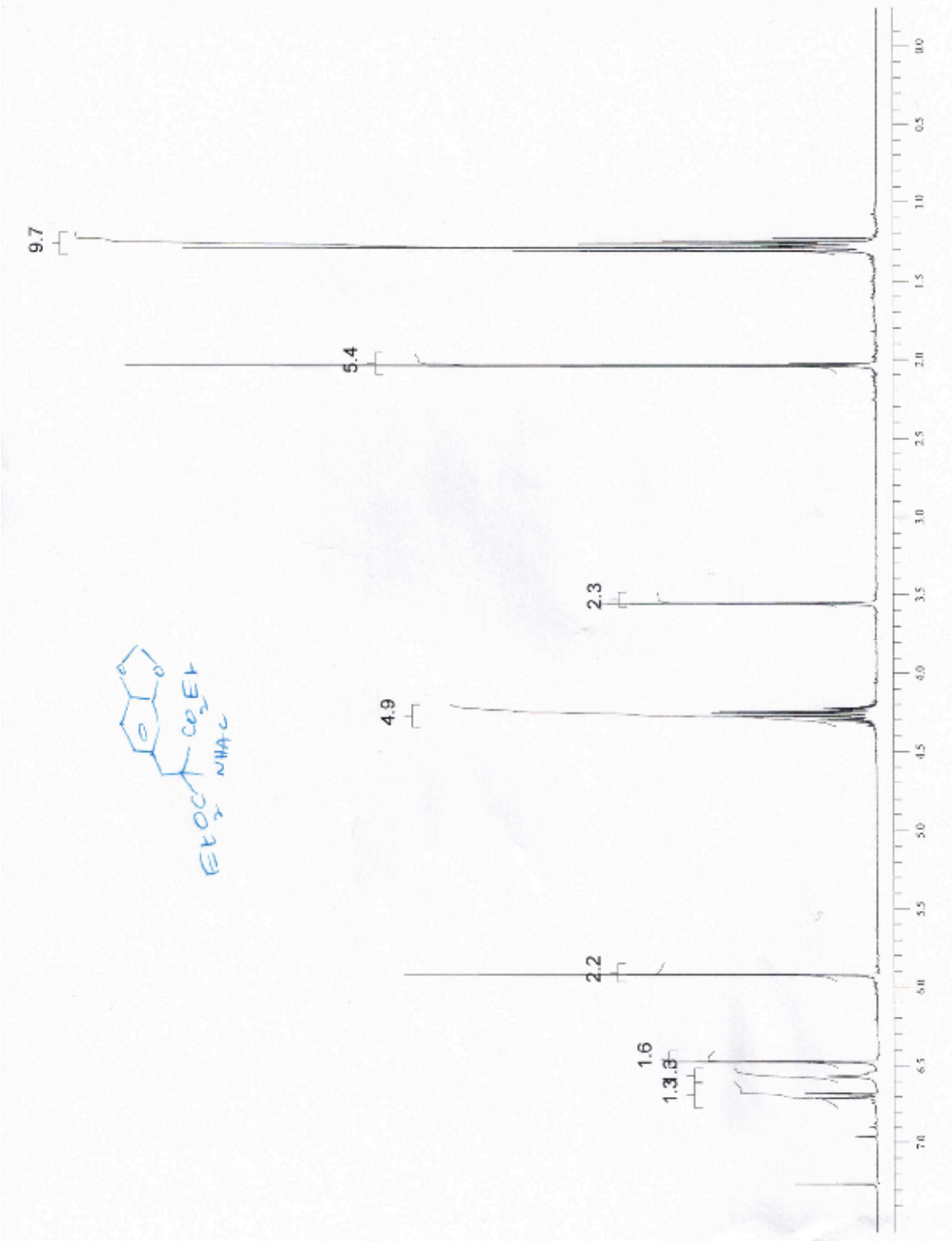
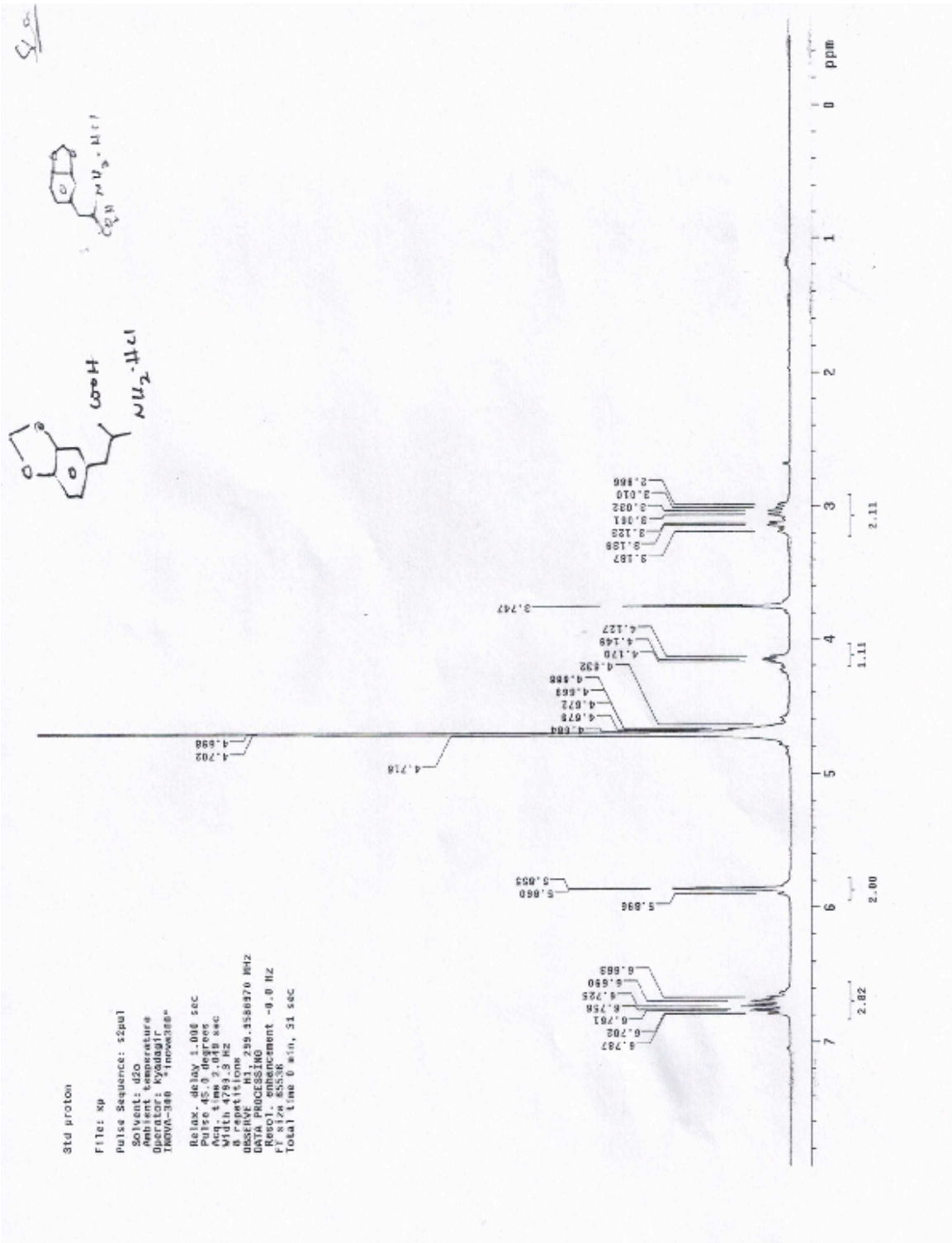
6. NMR Data

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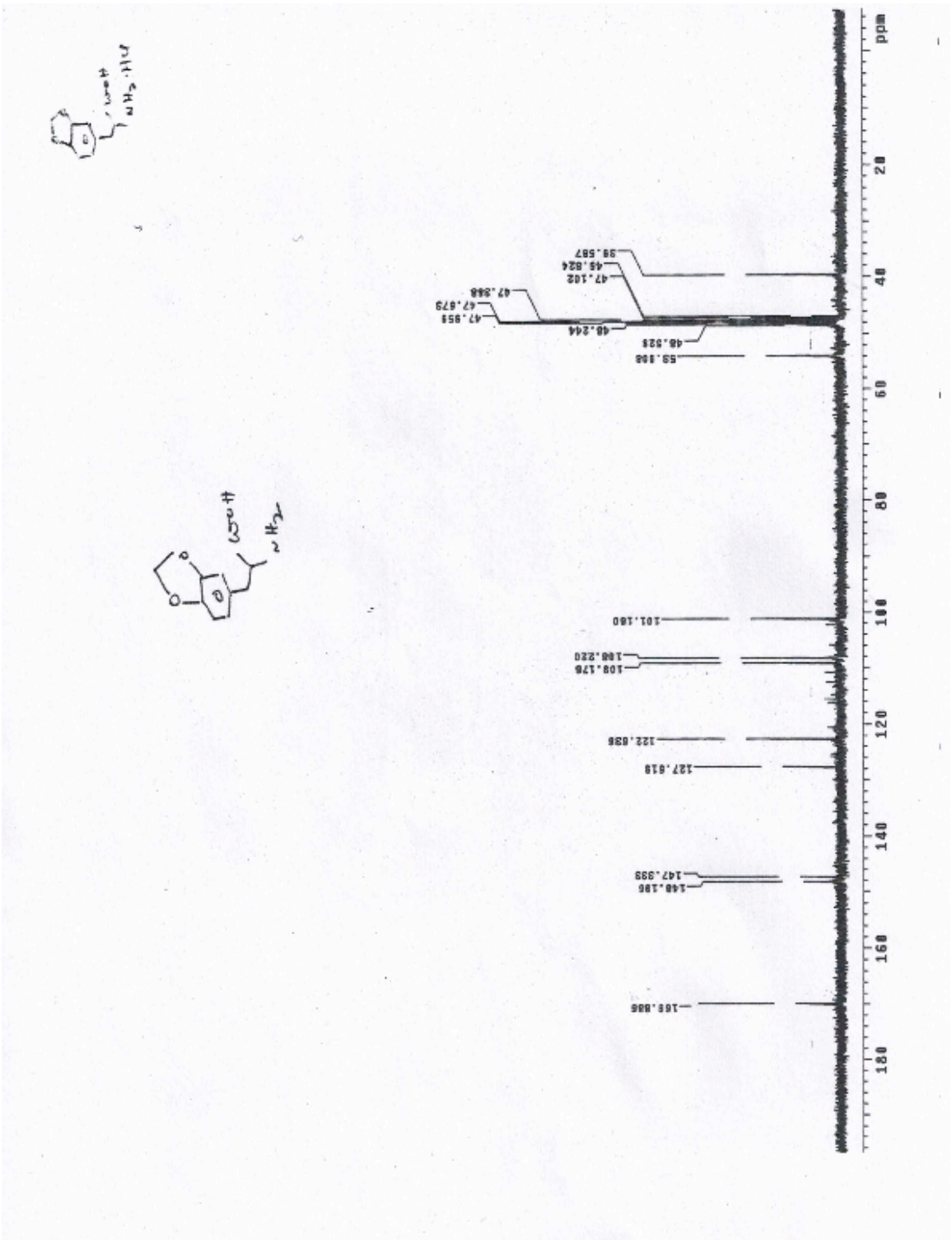
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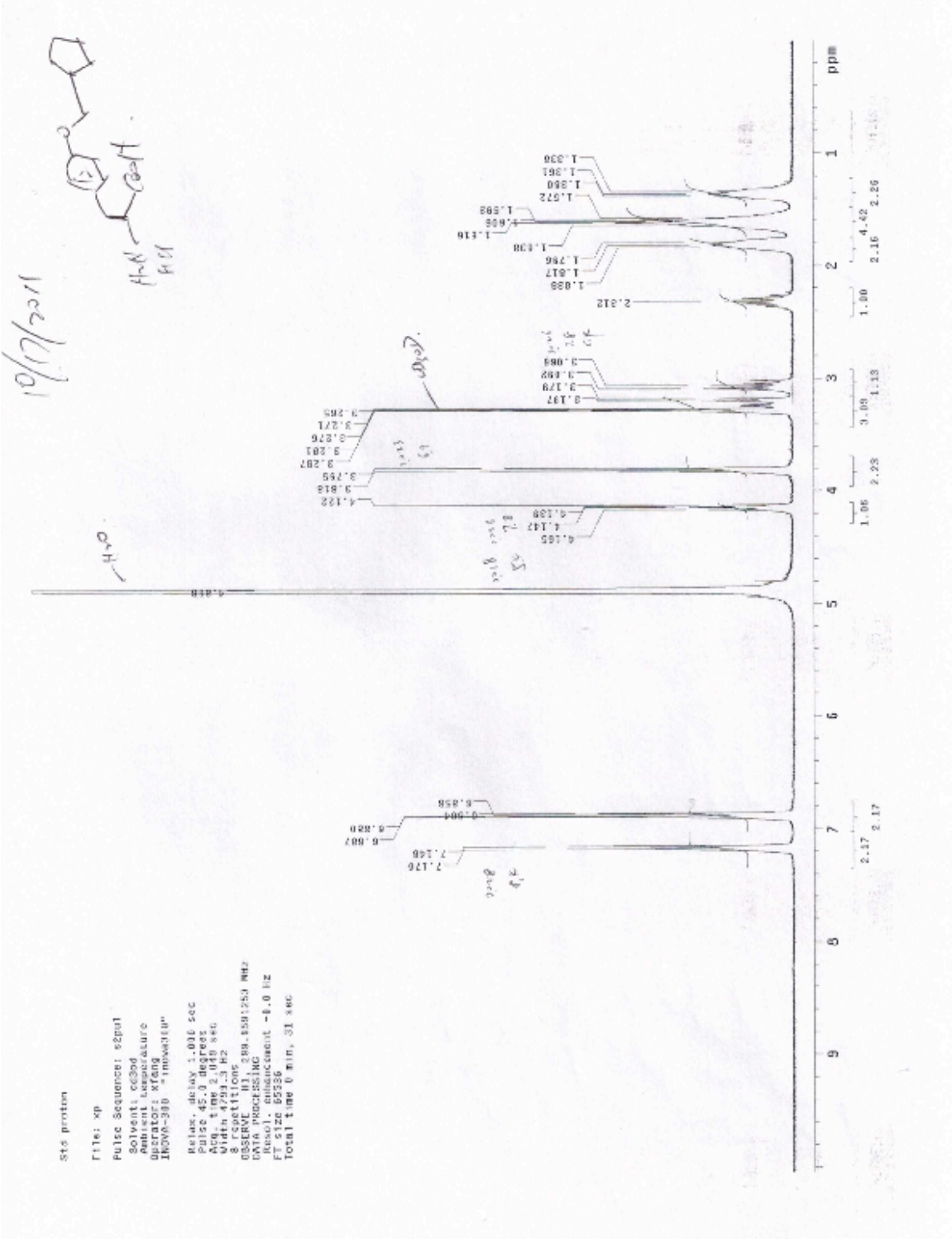
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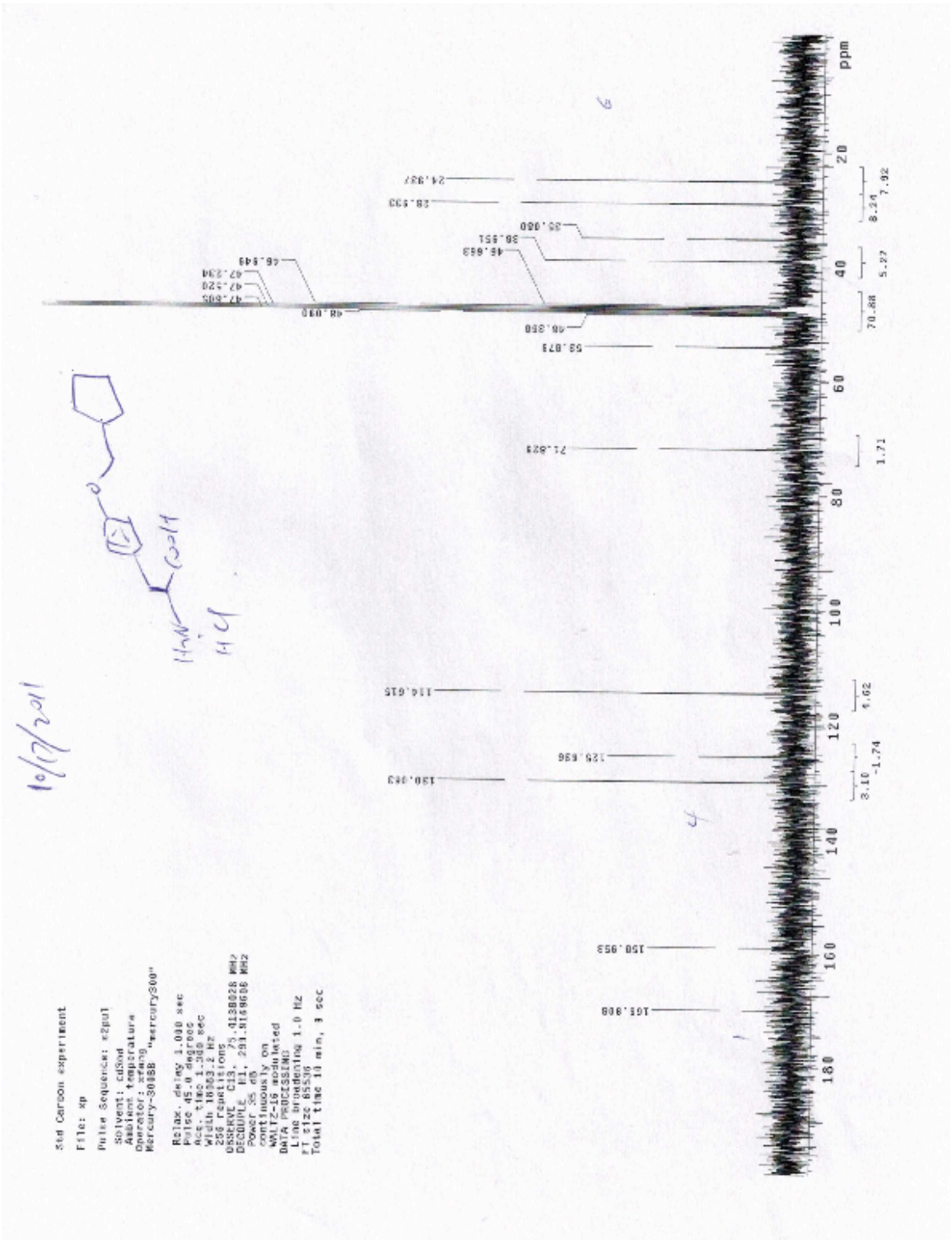
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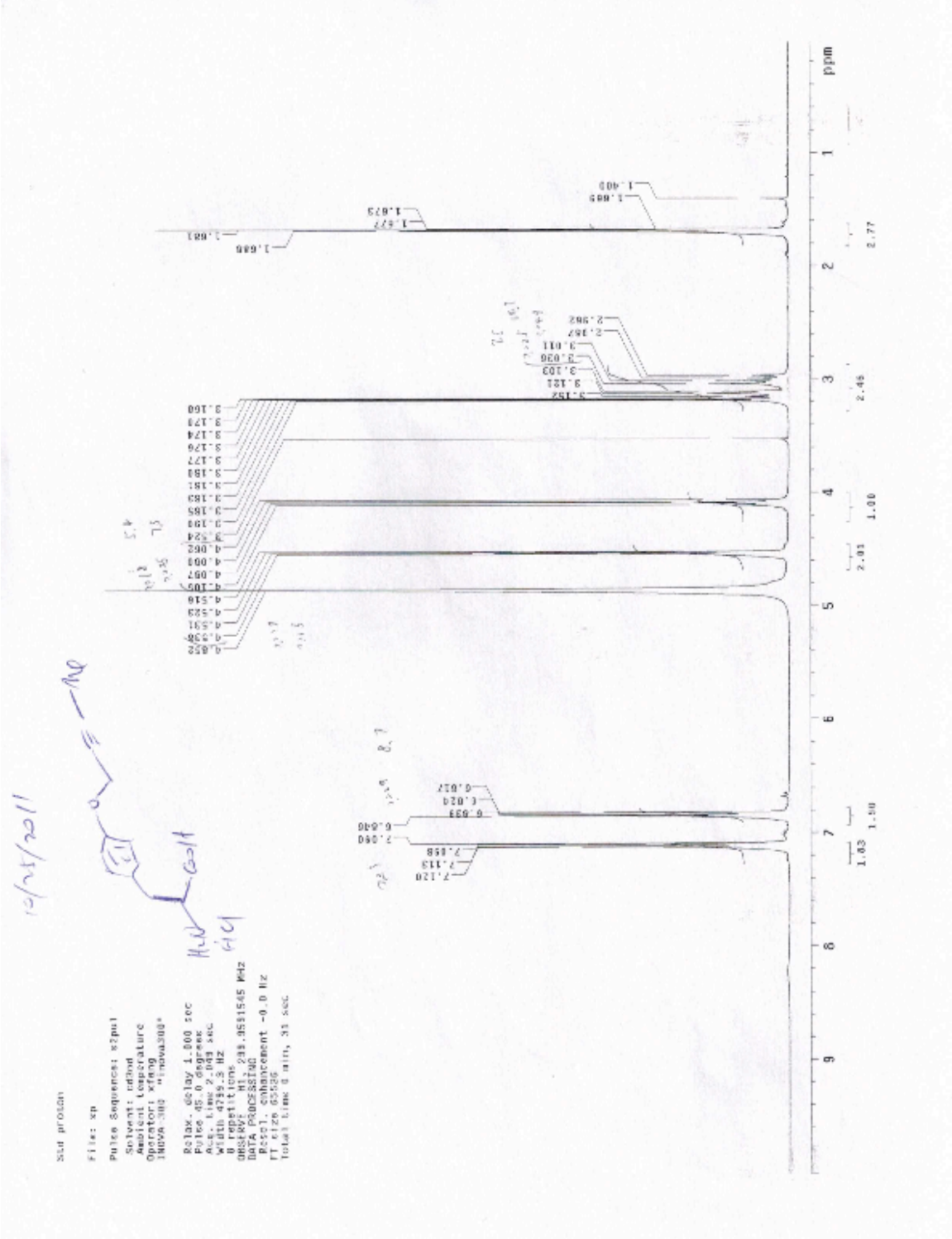
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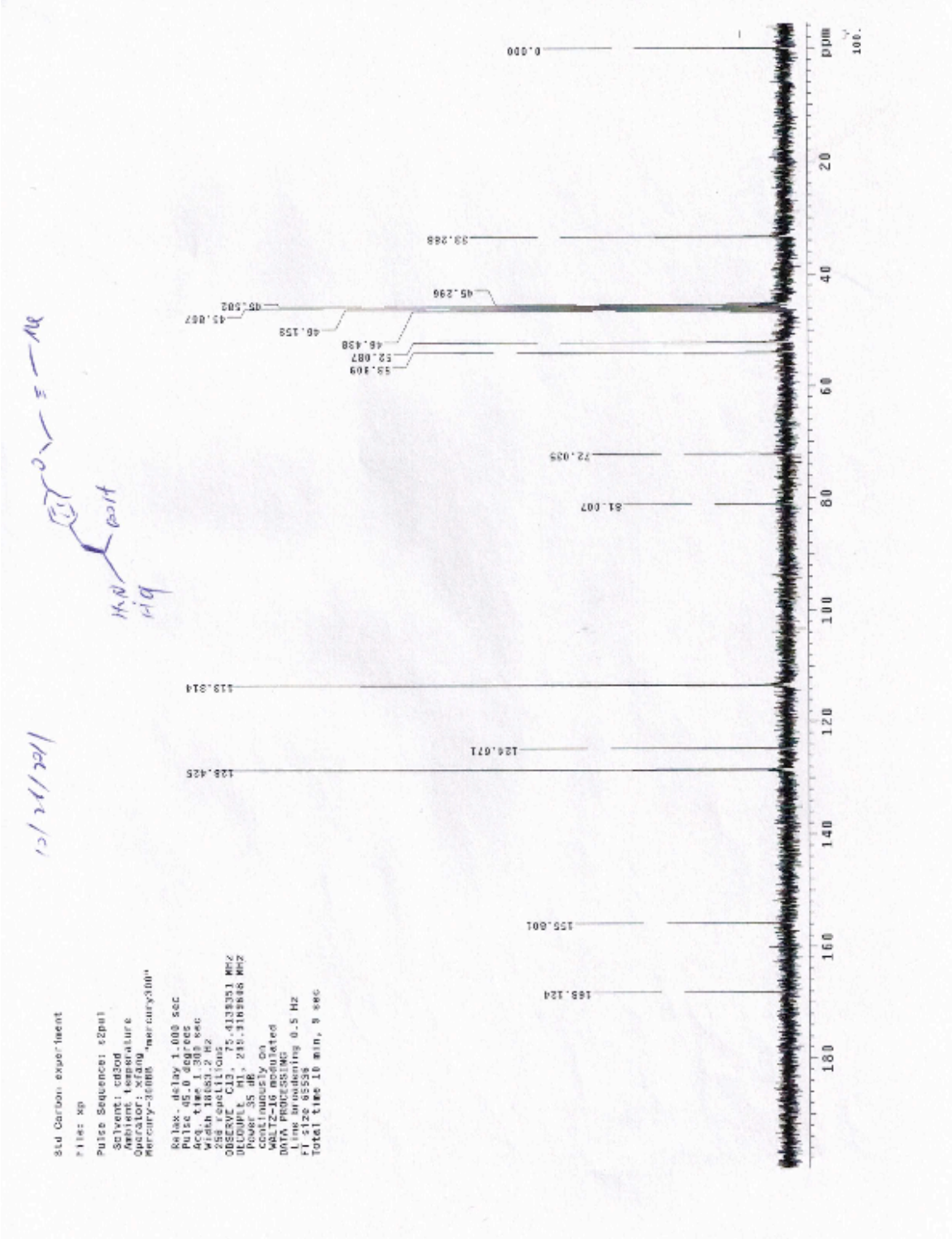
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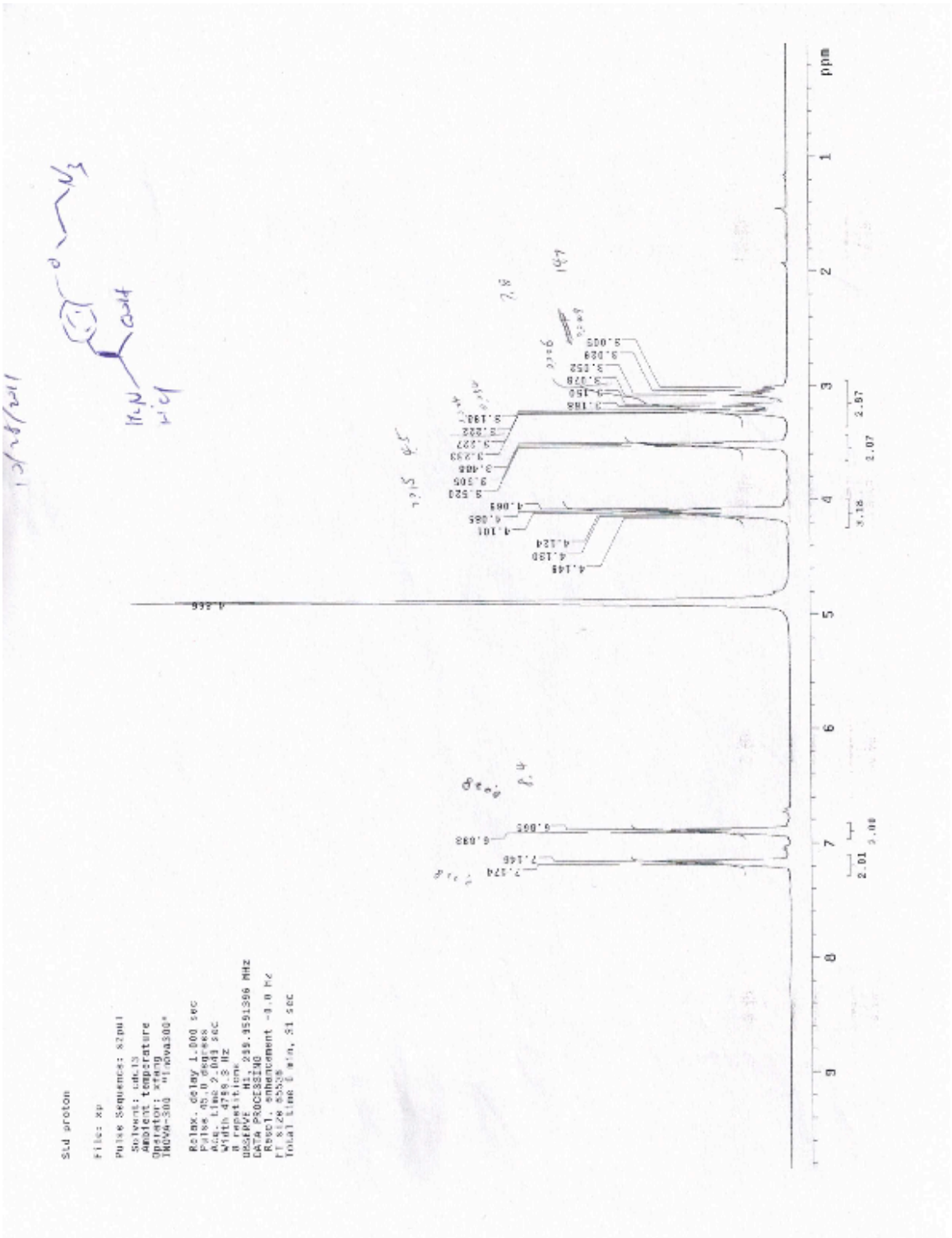
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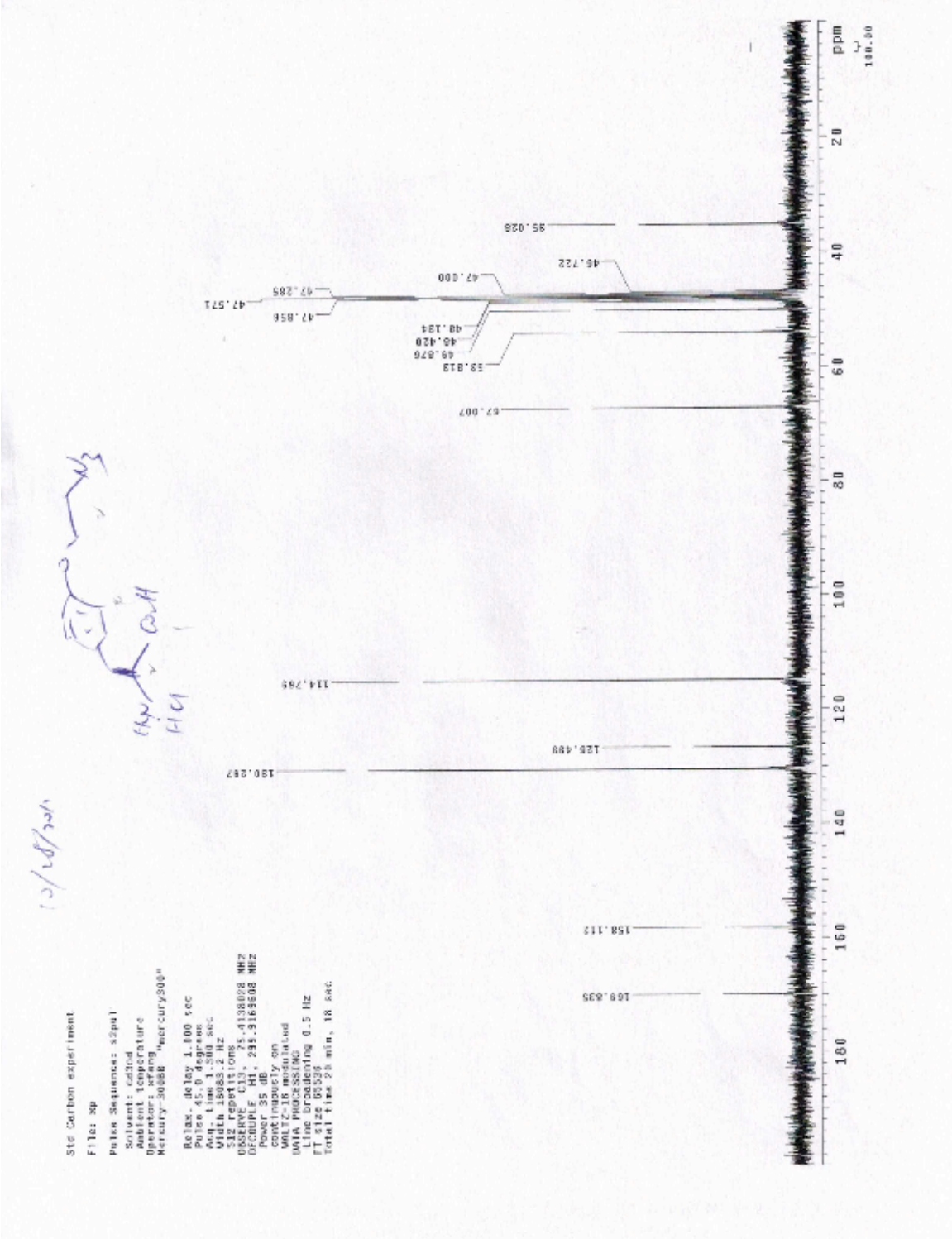
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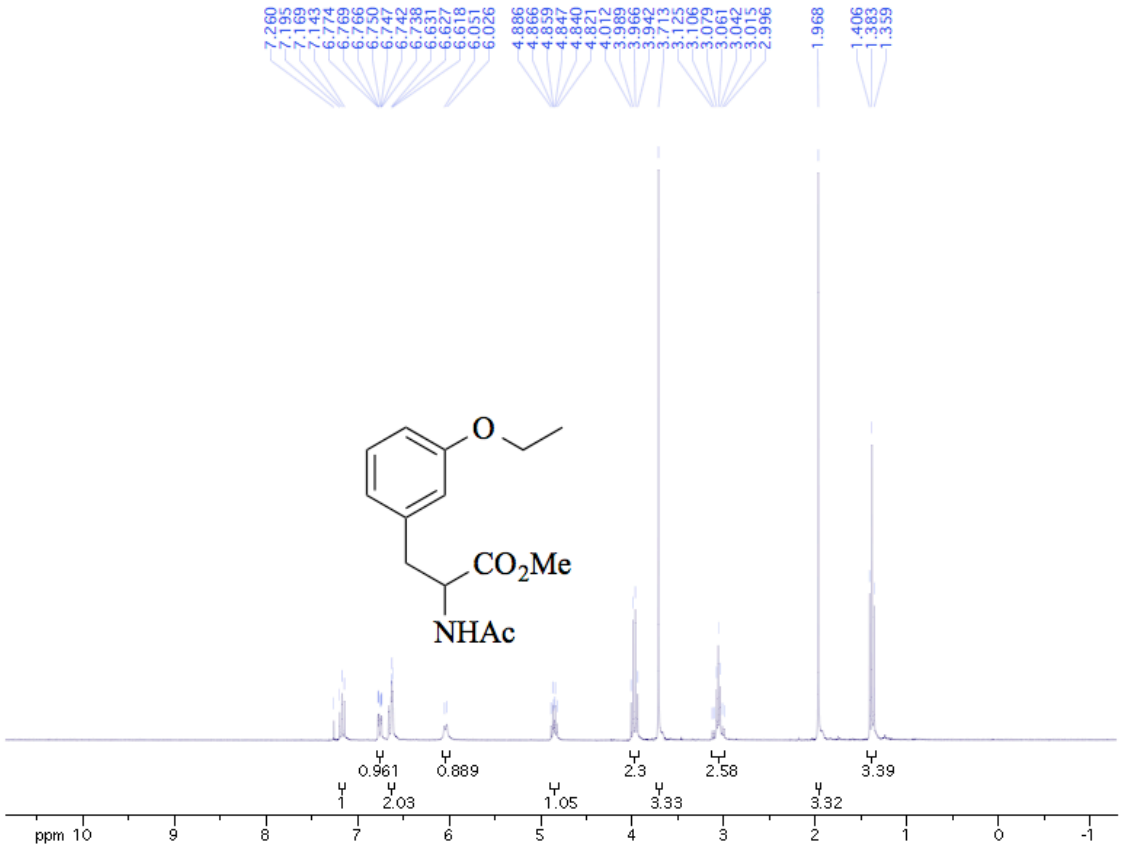
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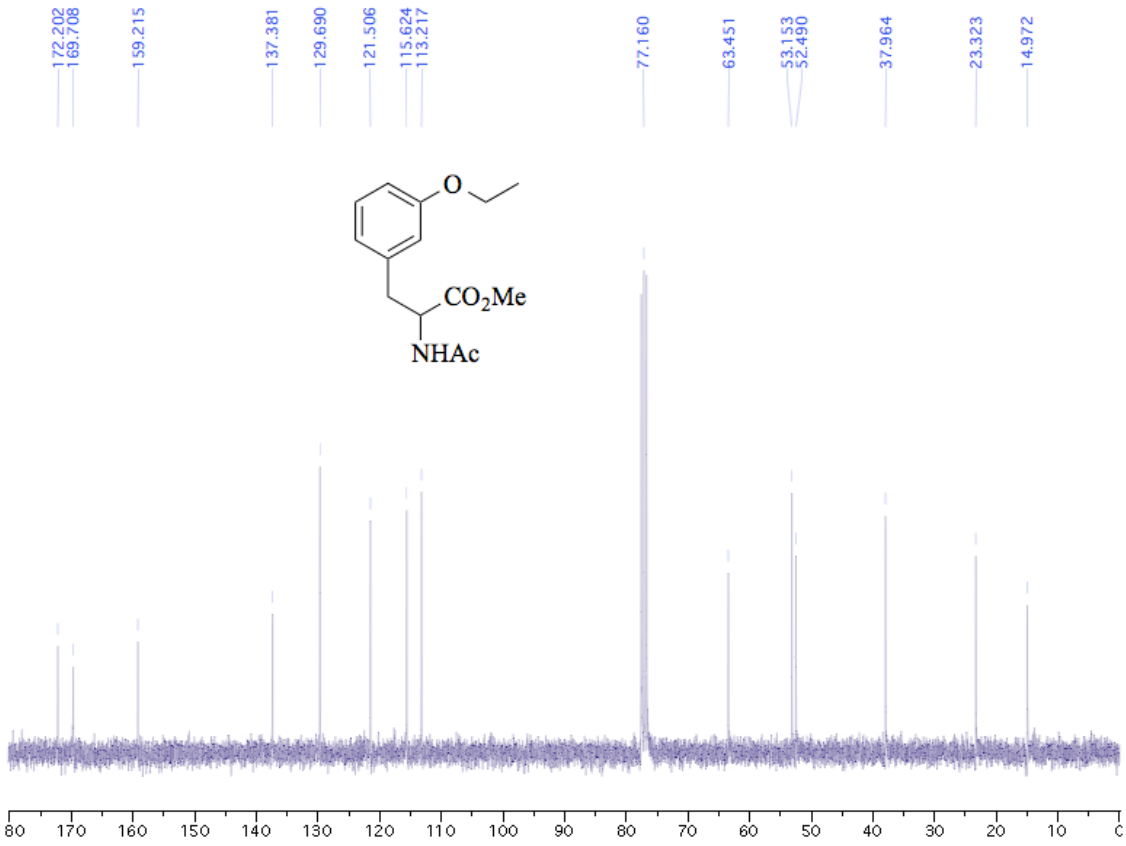
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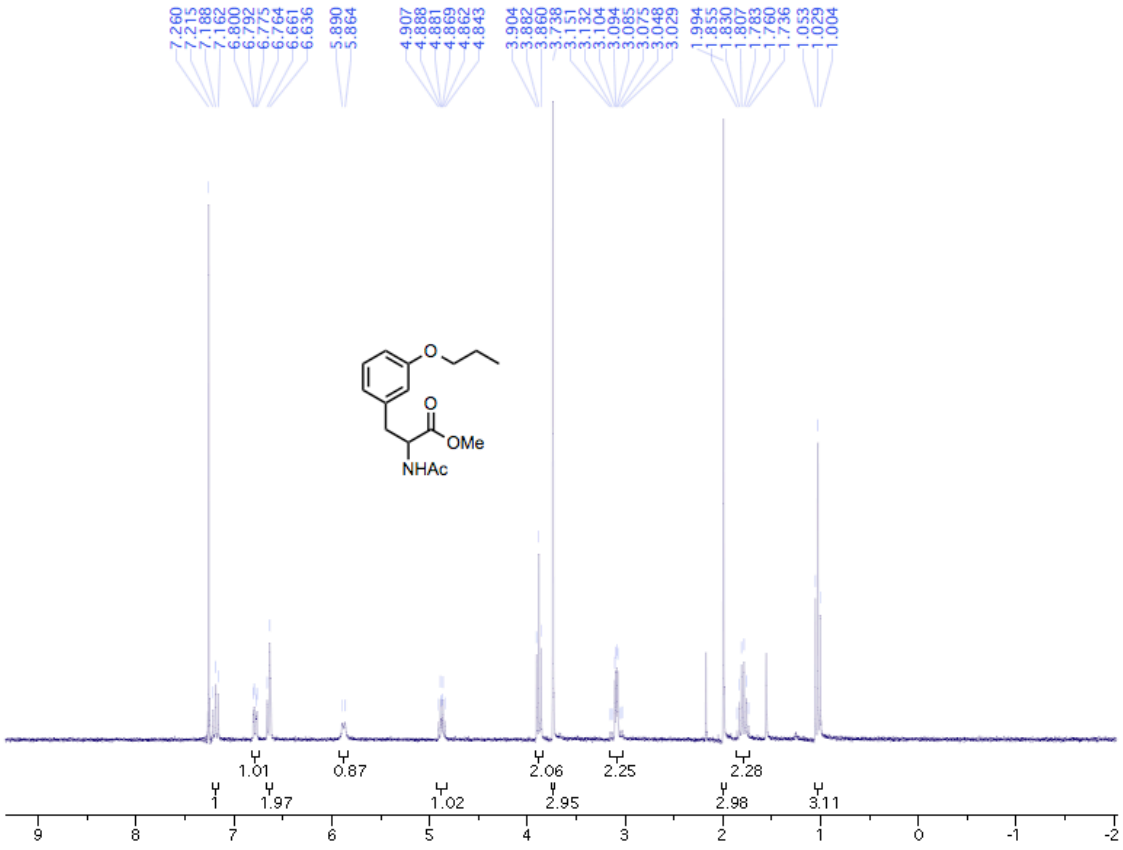
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1.004
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O
OMe
NHAc
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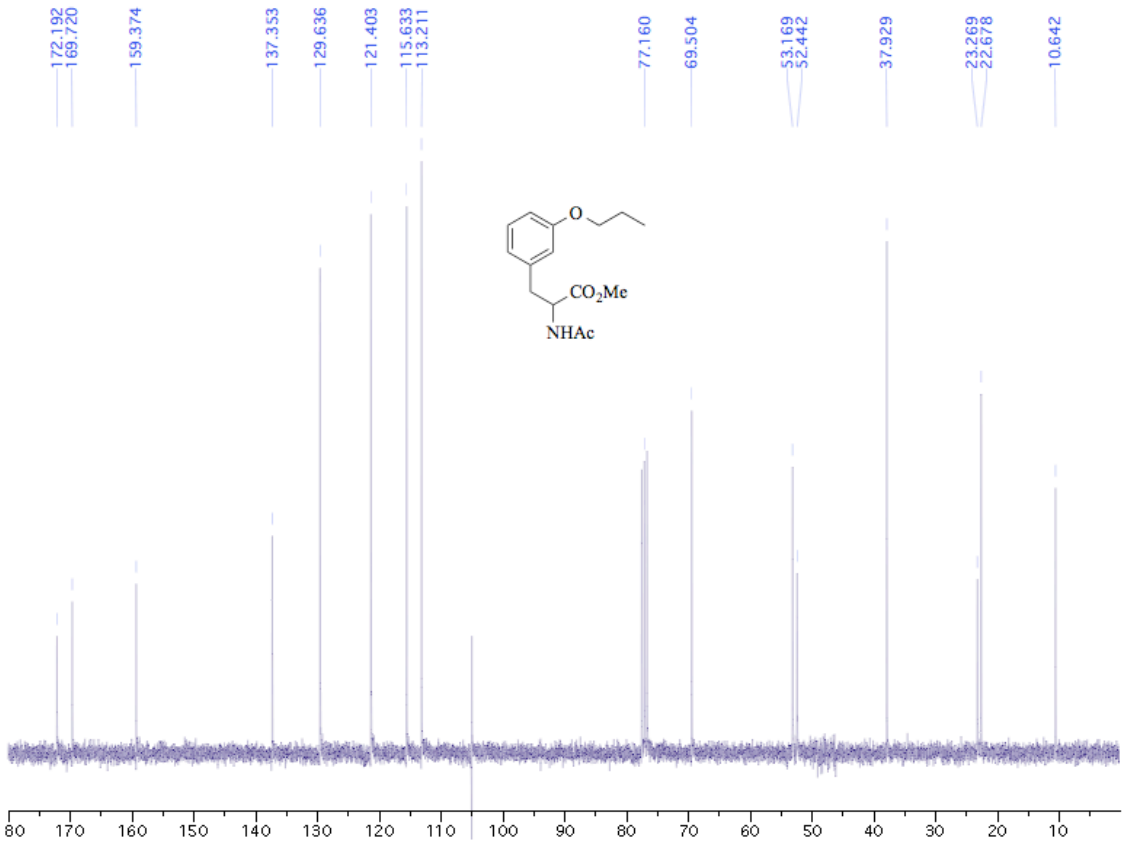

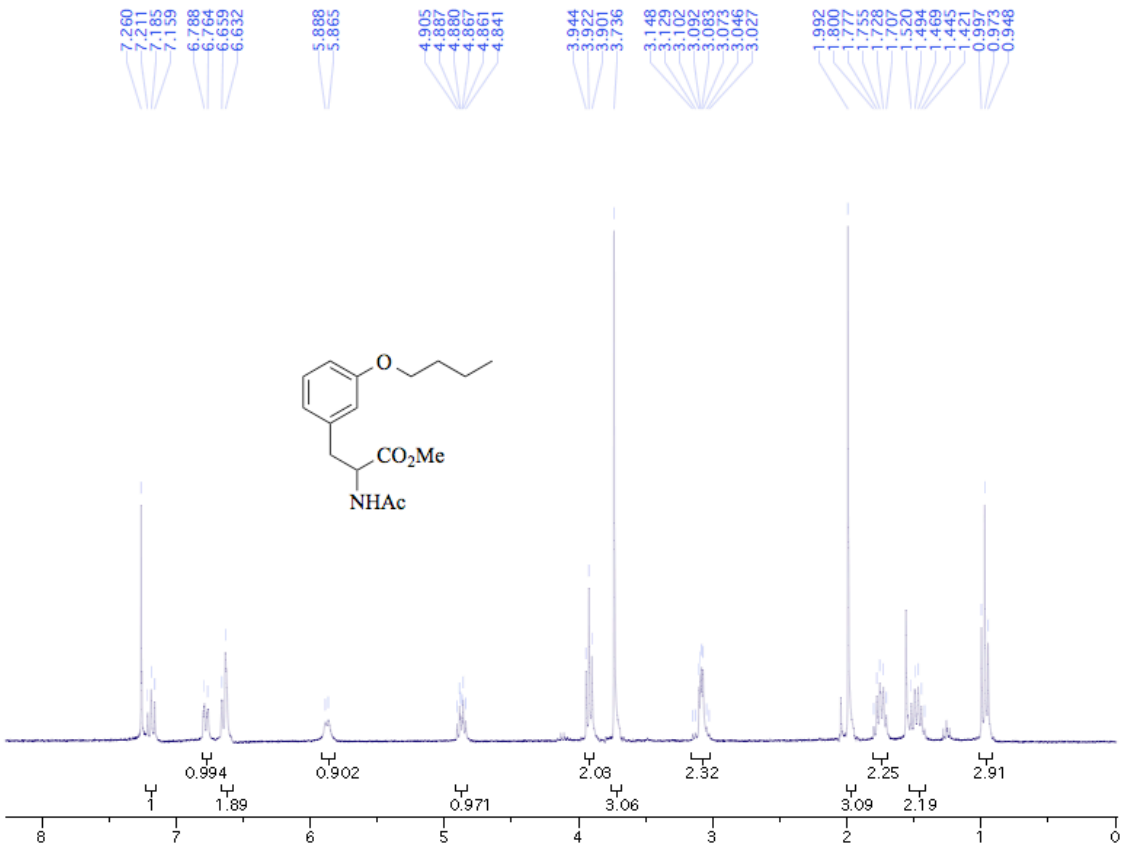
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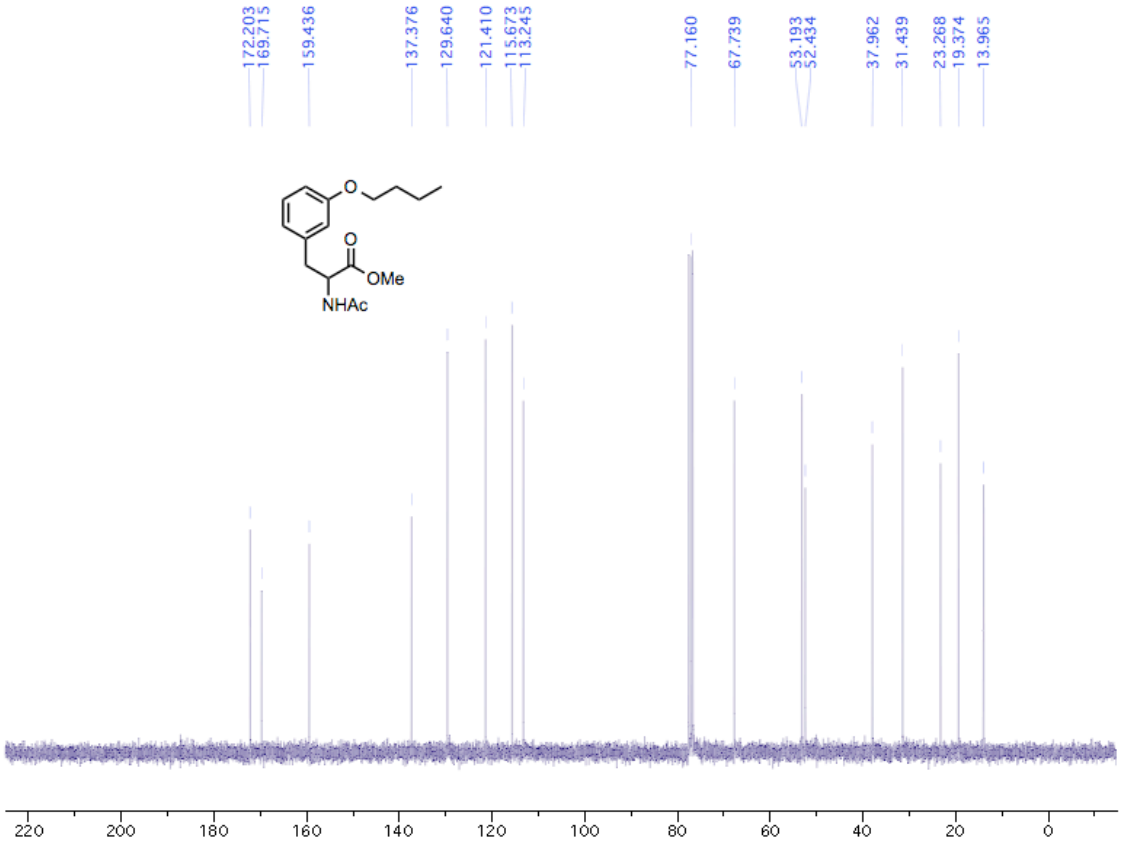

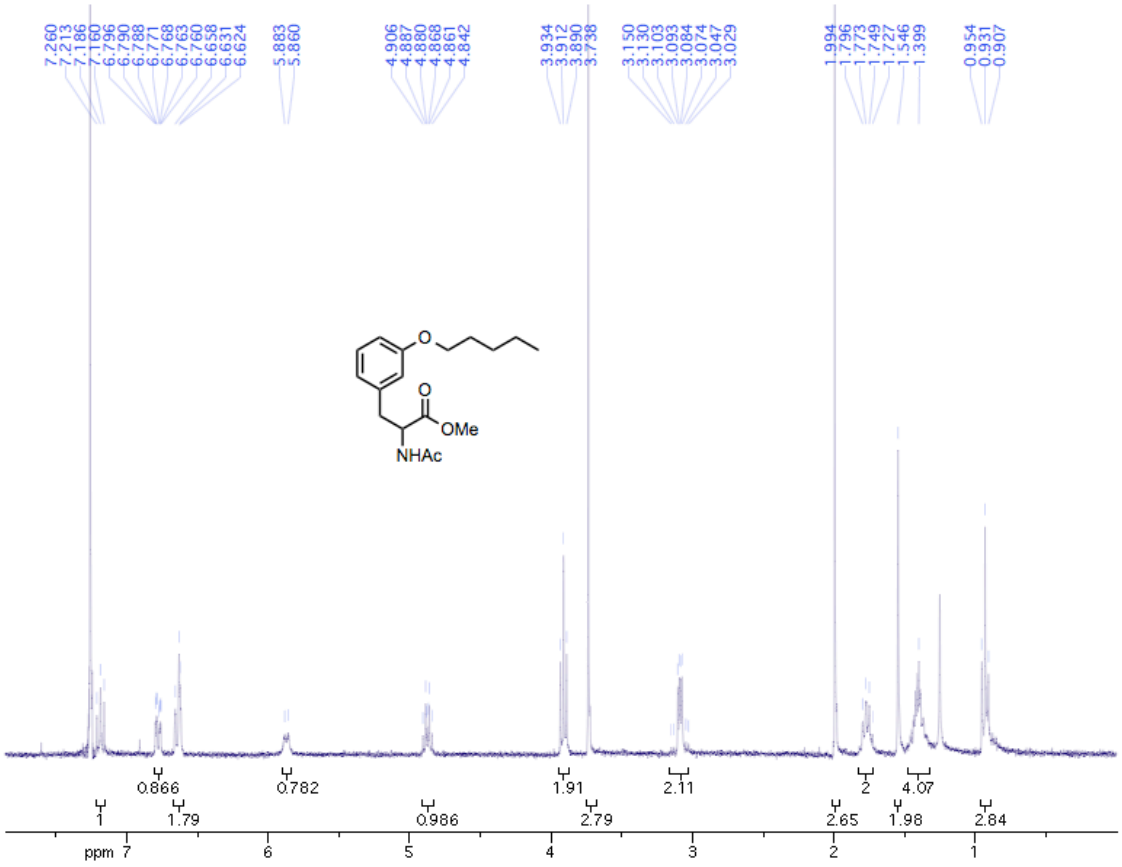
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6.631
6.624
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3.738
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3.130
3.103
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3.074
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1.399
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O
OMe
NHAc
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0.782
1.91
2.11
2
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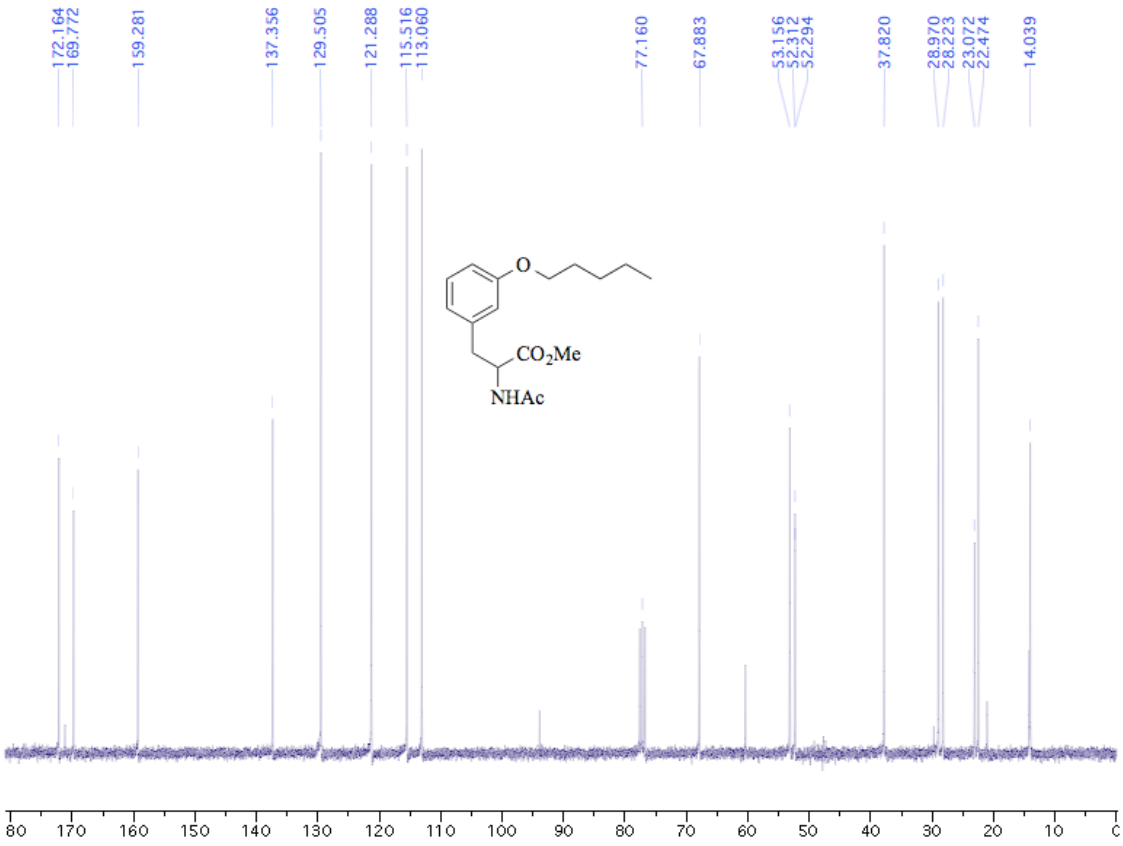

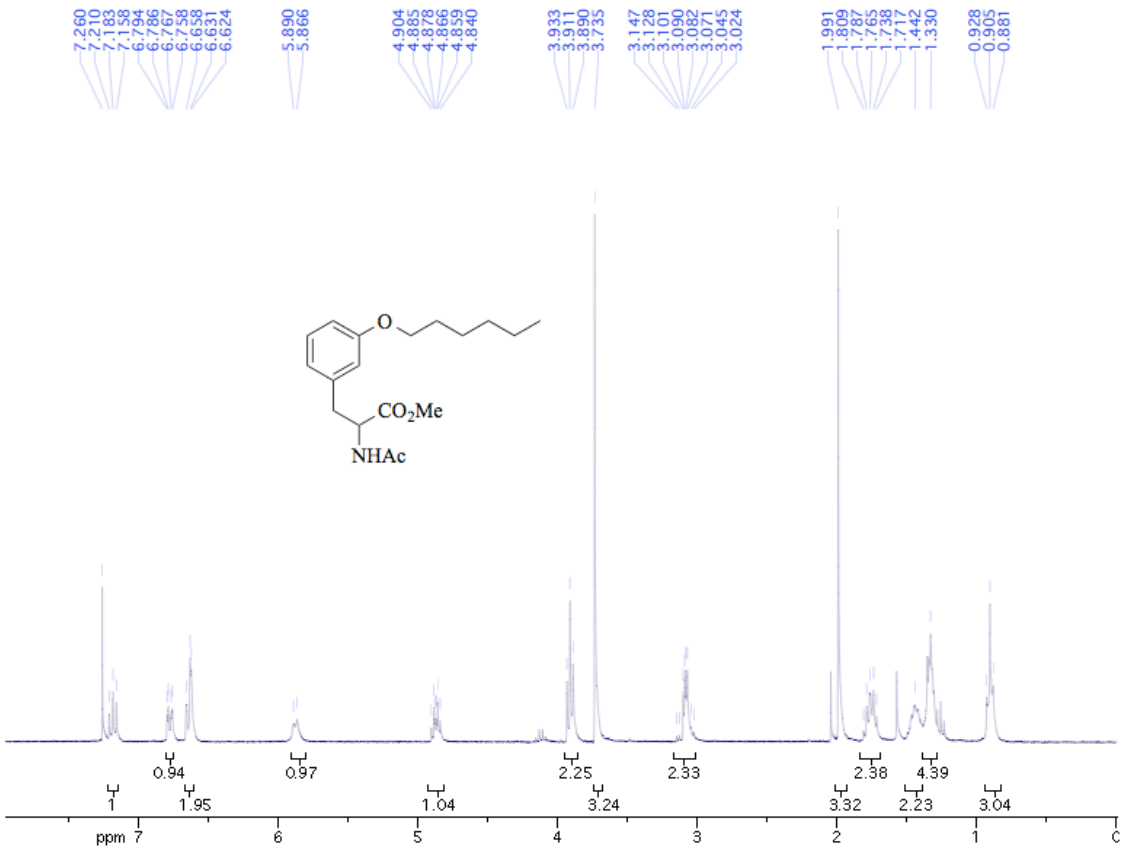
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4.885
4.878
4.866
4.859
4.840
3.933
3.911
3.890
3.735
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3.090
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1.442
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0.905
0.881
O
CO2Me
NHAc
0.94
0.97
2.25
2.33
2.38
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3.24
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3
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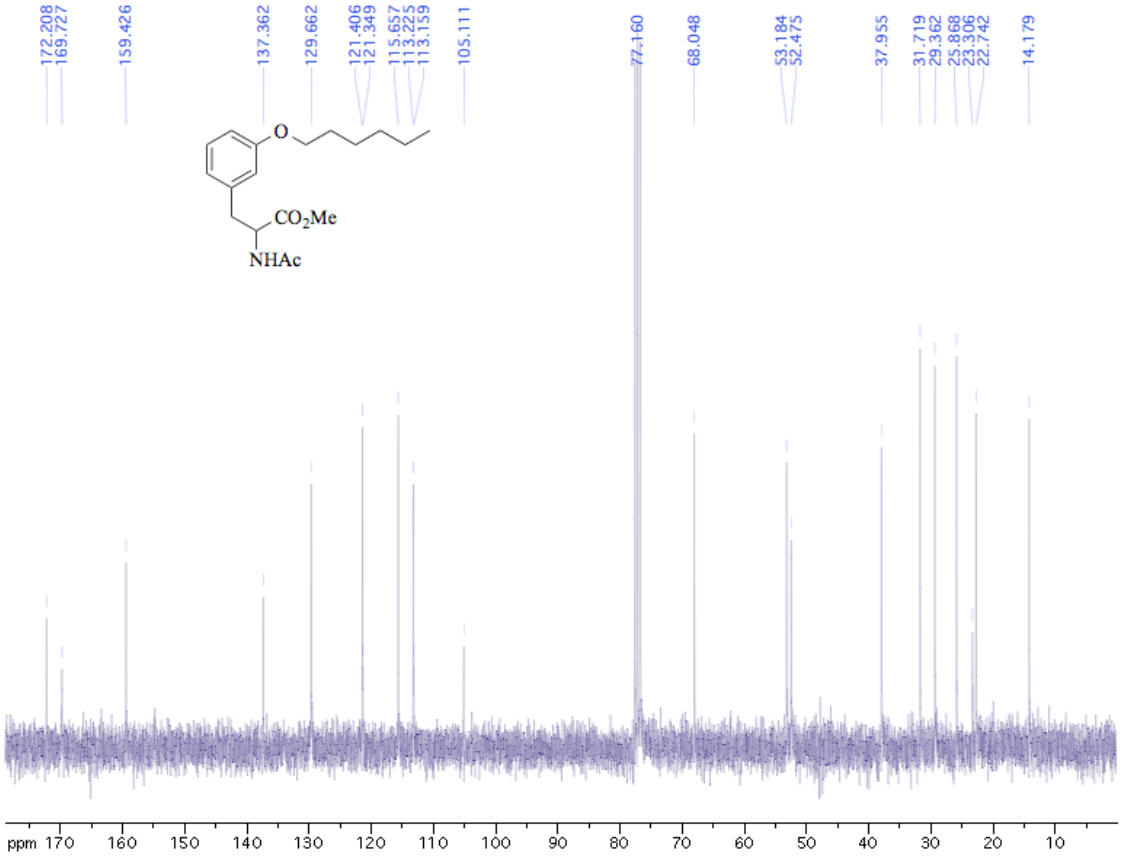
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53.184
52.475
37.955
31.719
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CO2Me
NHAc
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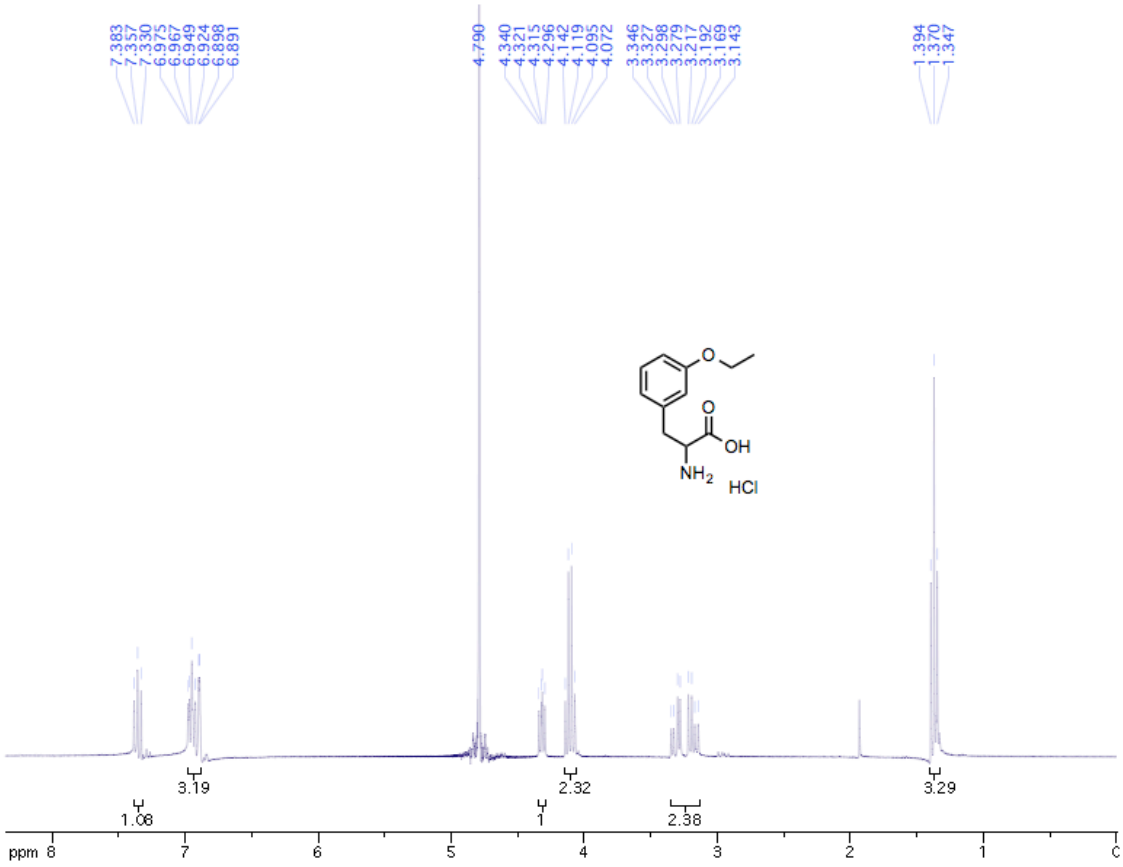

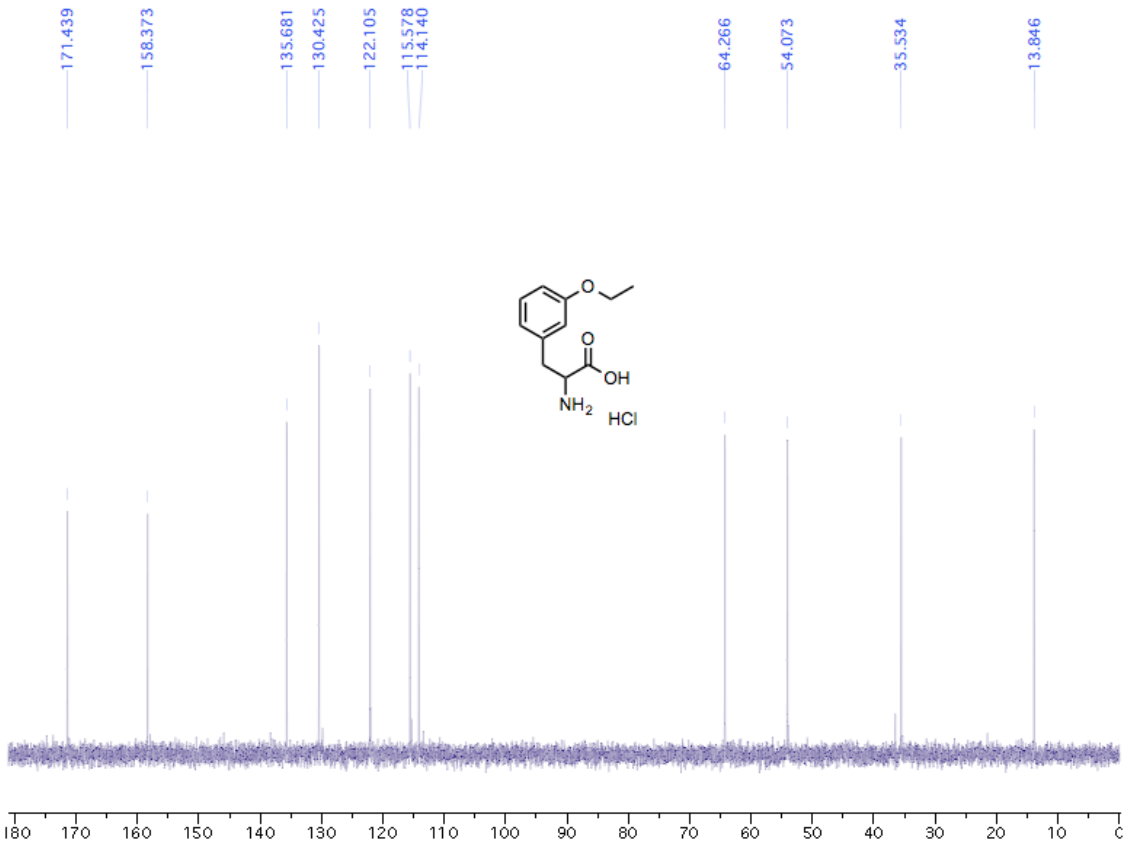

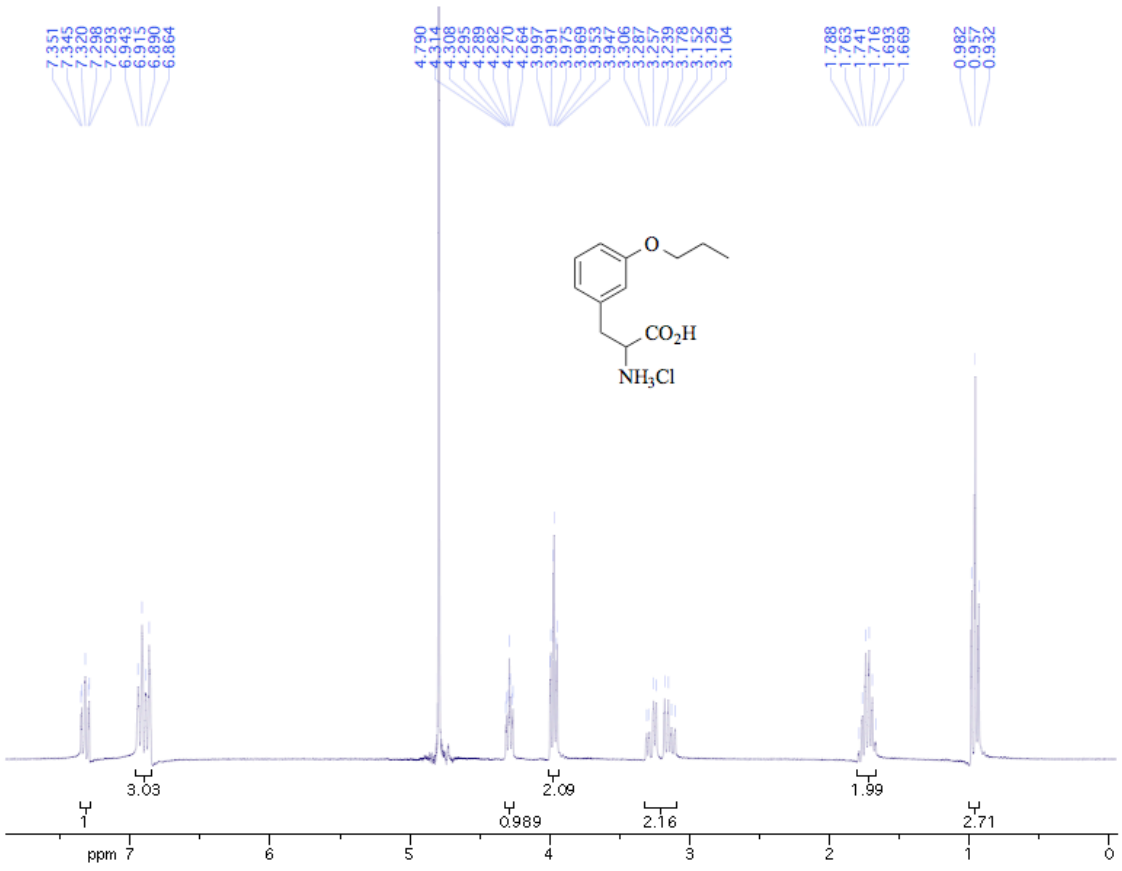

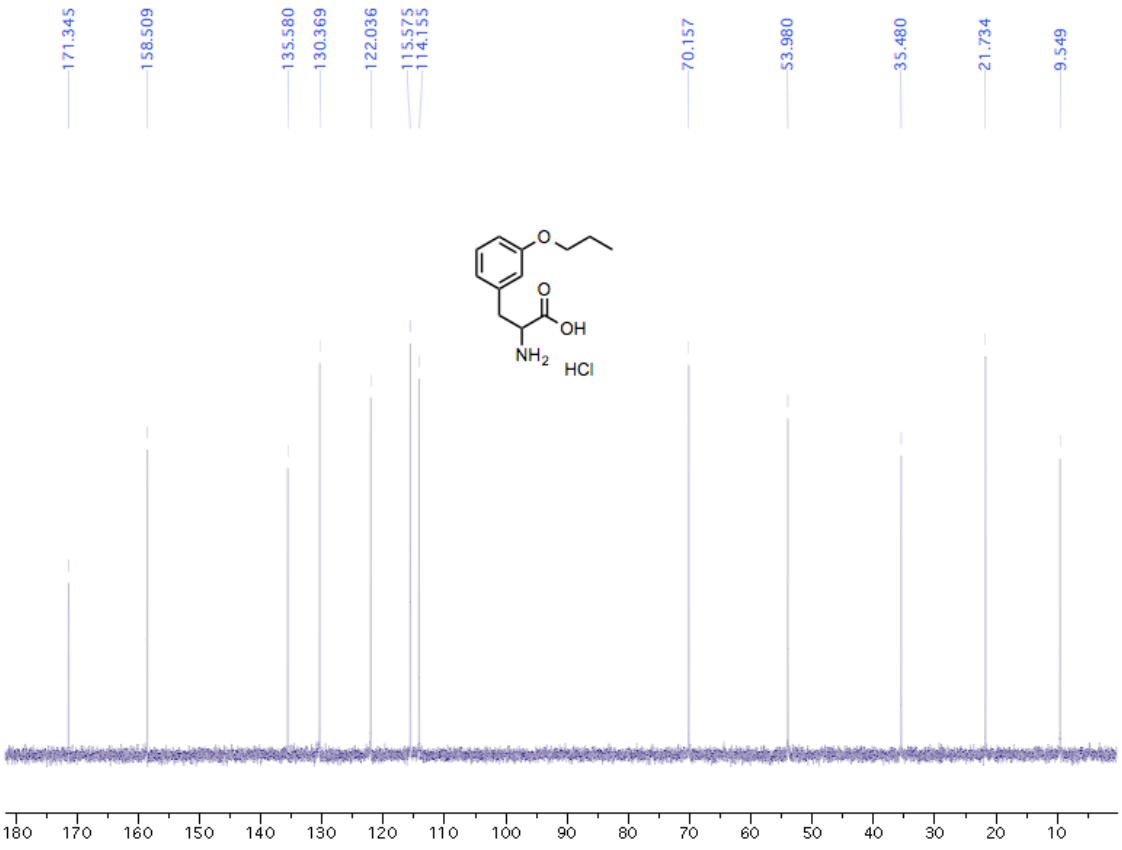

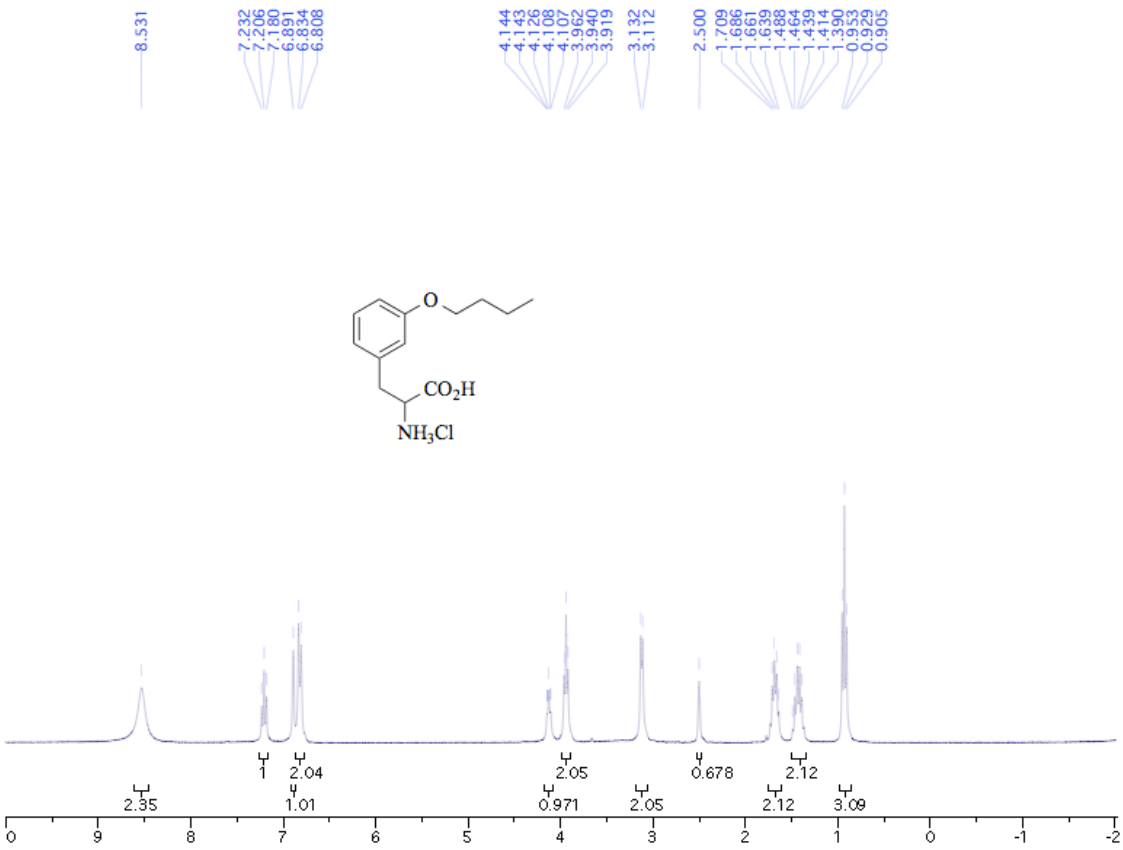
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6.808
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4.143
4.126
4.108
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3.940
3.919
3.132
3.112
2.500
1.709
1.686
1.661
1.639
1.488
1.464
1.439
1.414
1.390
0.953
0.929
0.905
O
CO2H
NH3Cl
1
2.04
2.05
0.678
2.12
2.35
1.01
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3.09
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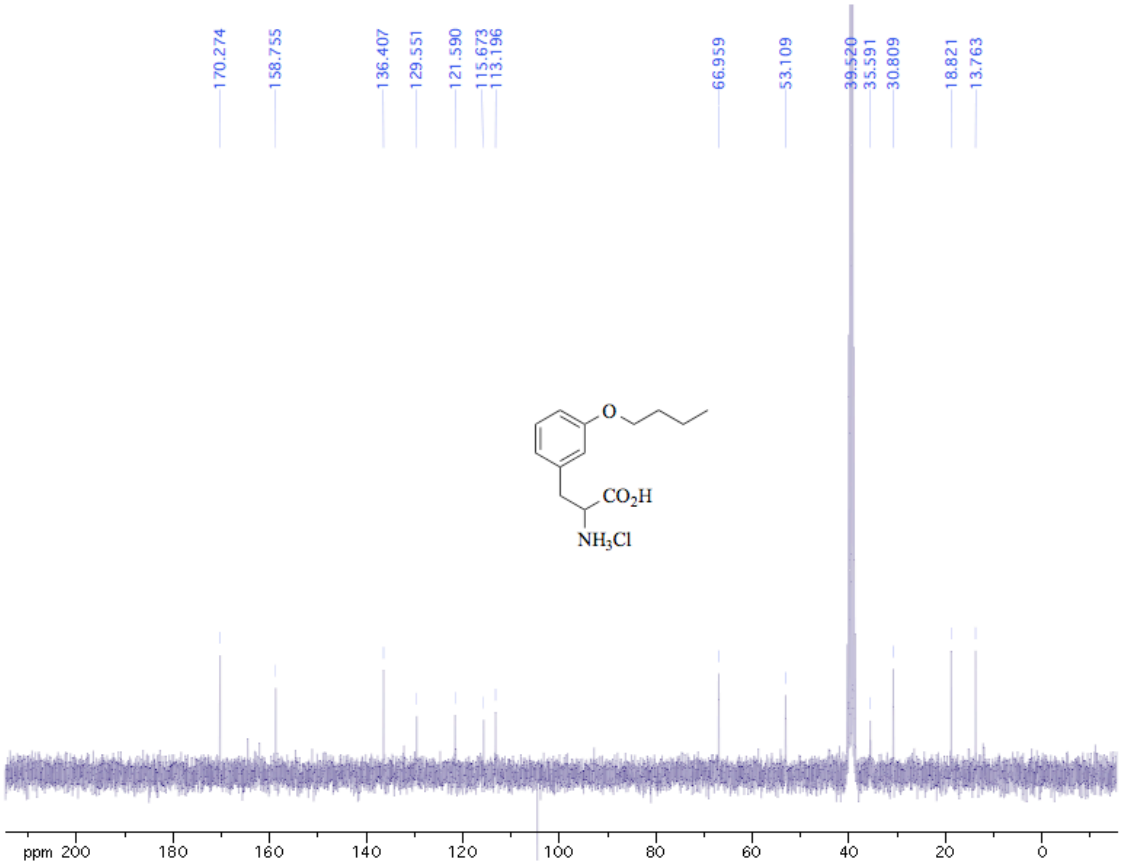

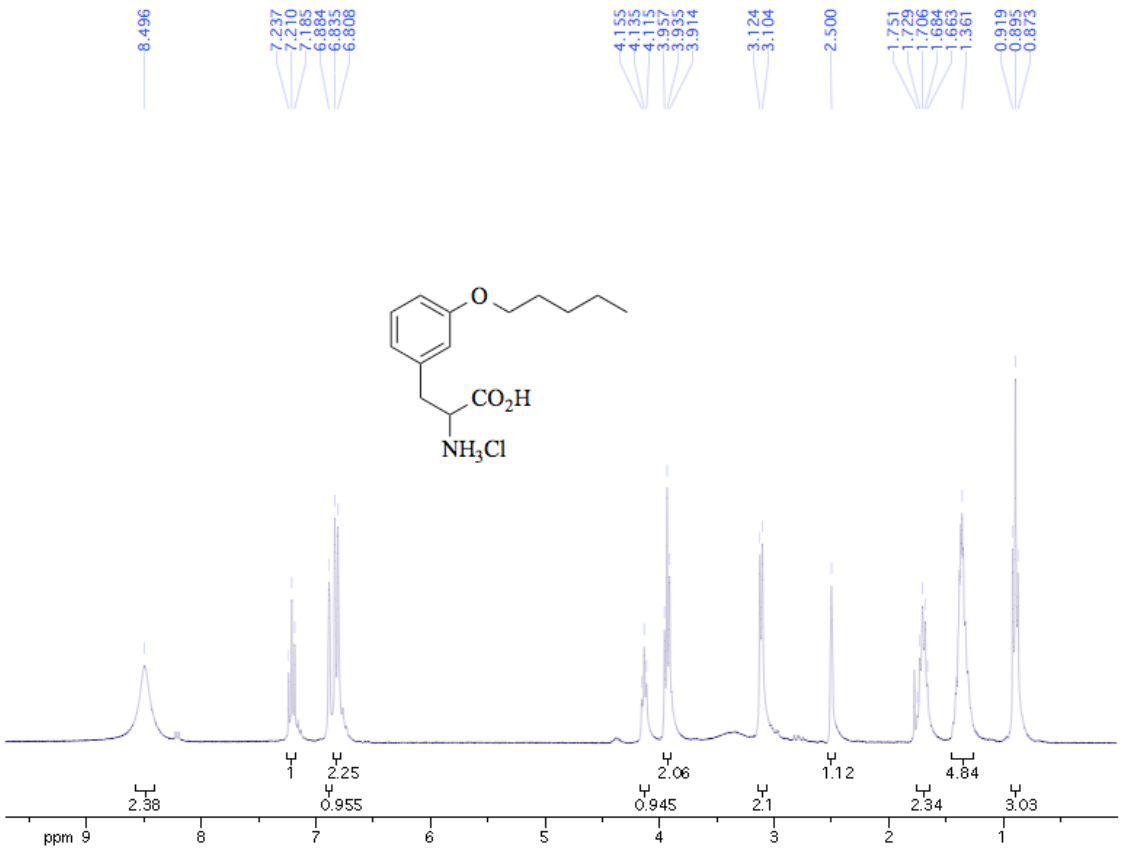

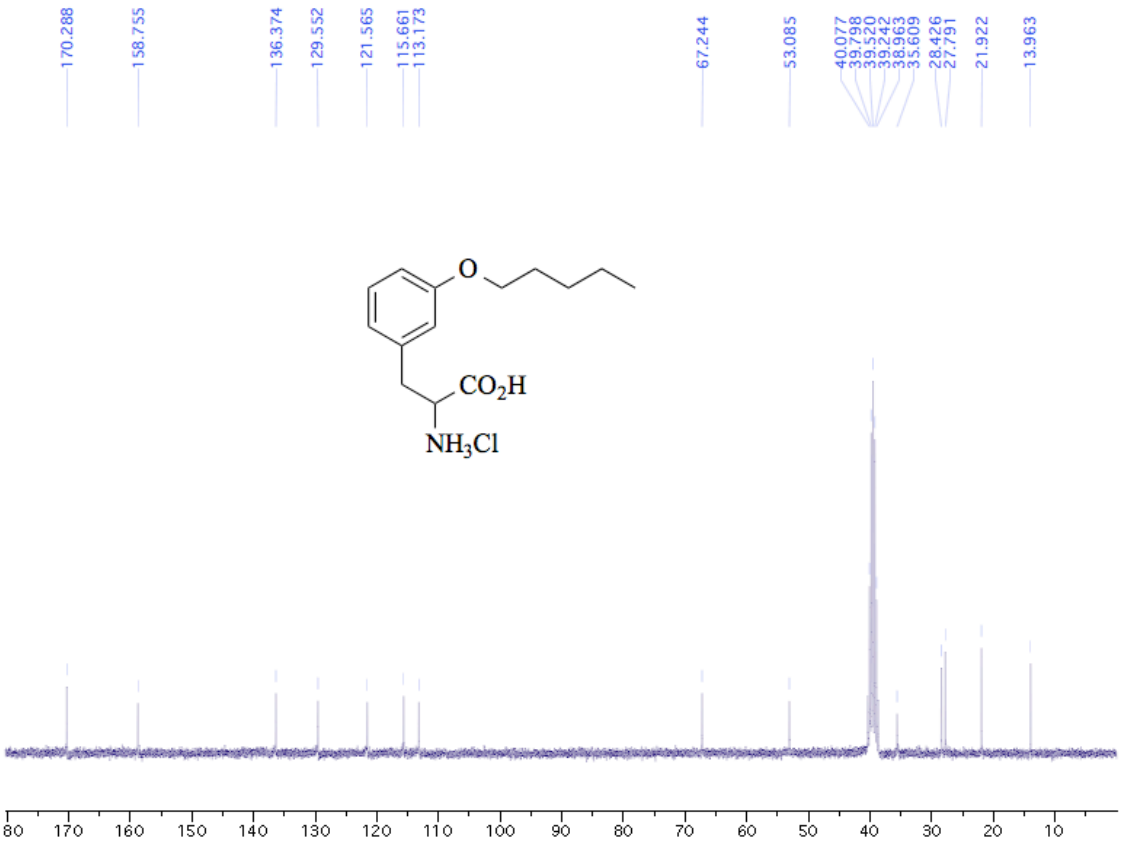
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67.244
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39.798
39.520
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38.963
35.609
28.426
27.791
21.922
13.963
O
CO2H
NH3Cl
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160
150
140
130
120
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80
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50
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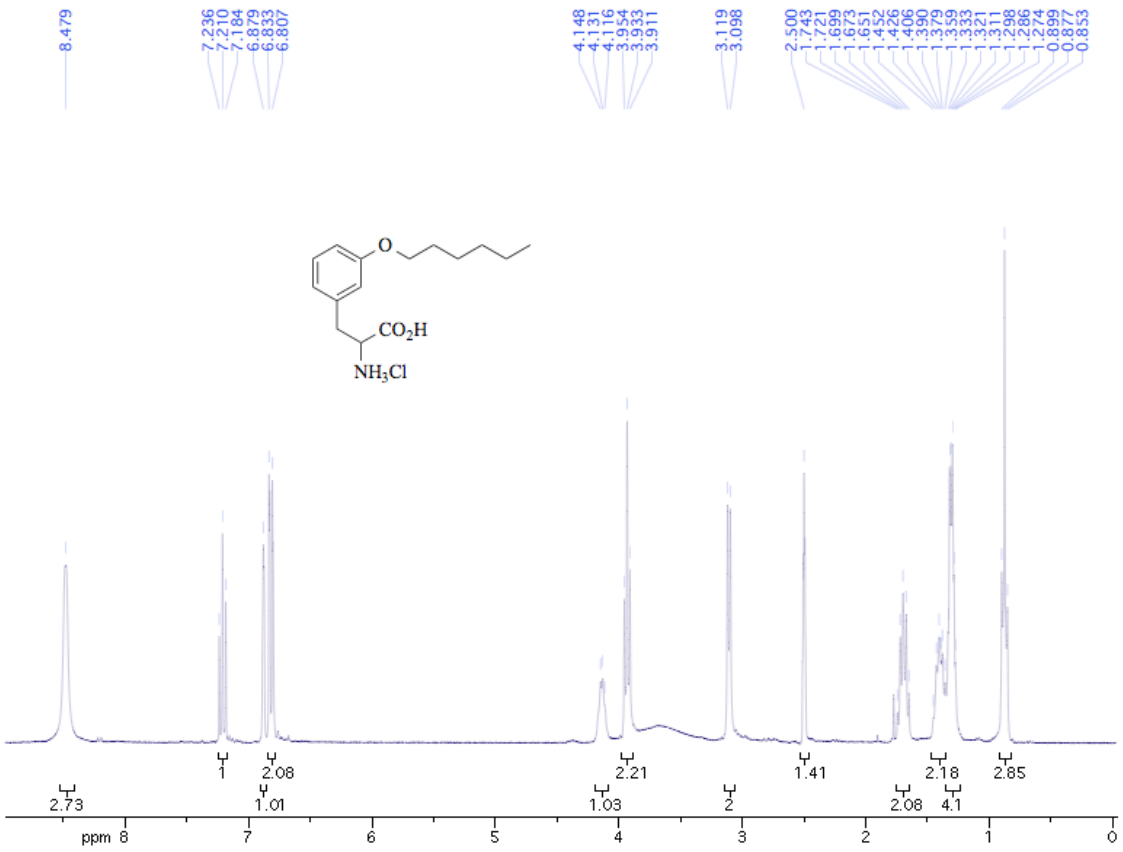
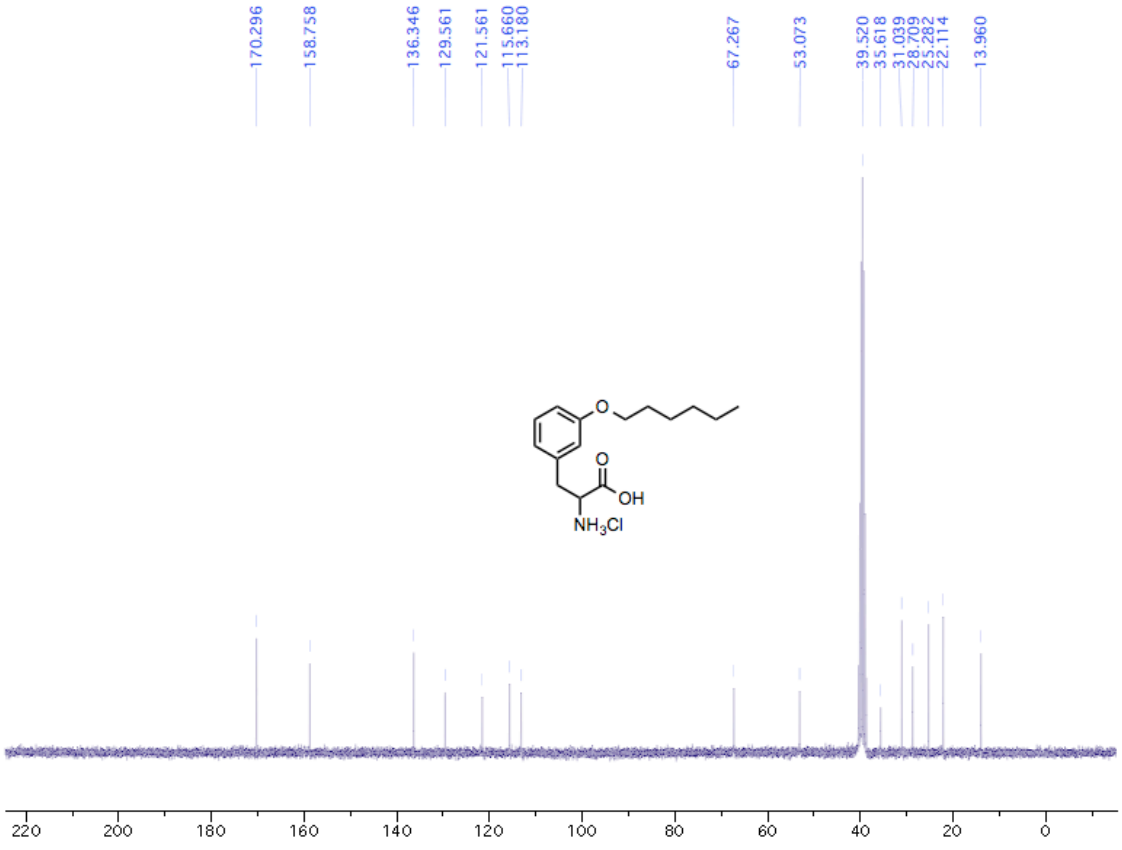
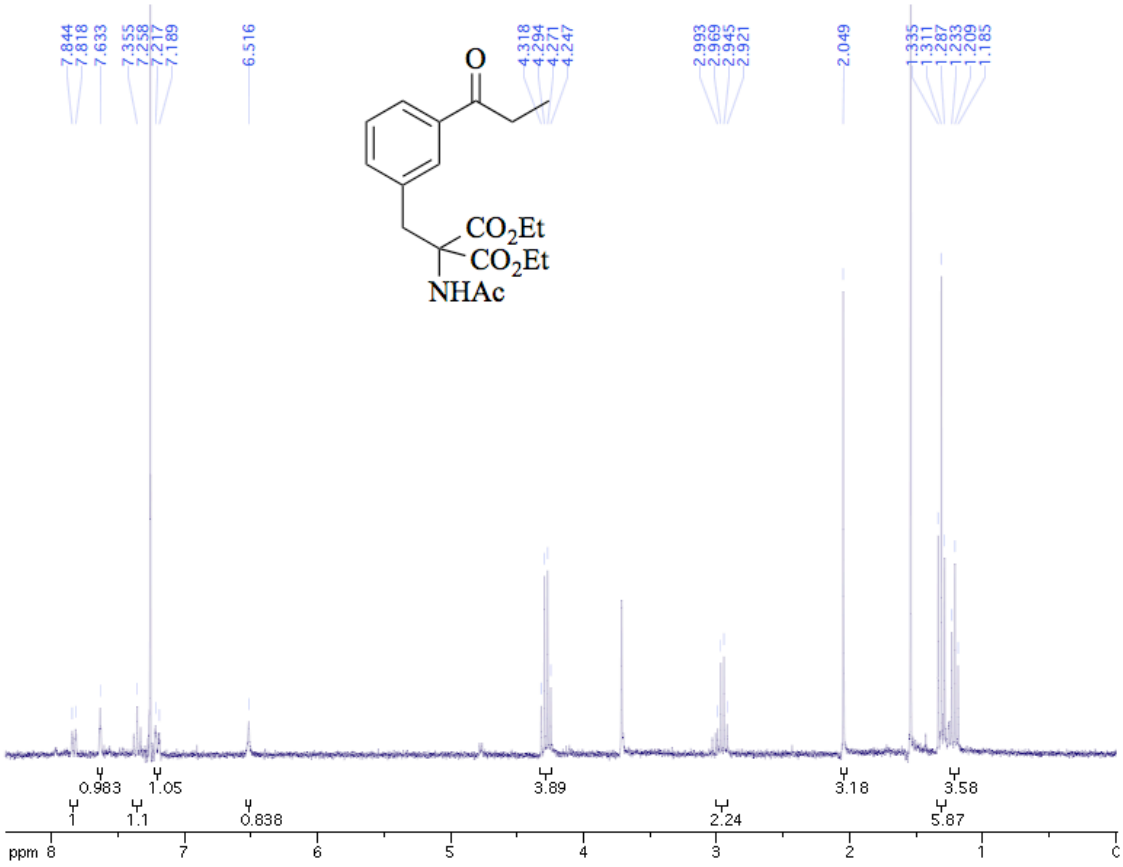
30
20
10



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4.294
4.271
4.247
2.993
2.969
2.945
2.921
2.049
1.335
1.311
1.287
1.233
1.209
1.185
O
CO2Et
CO2Et
NHAc
0.983
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3.89
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1.1
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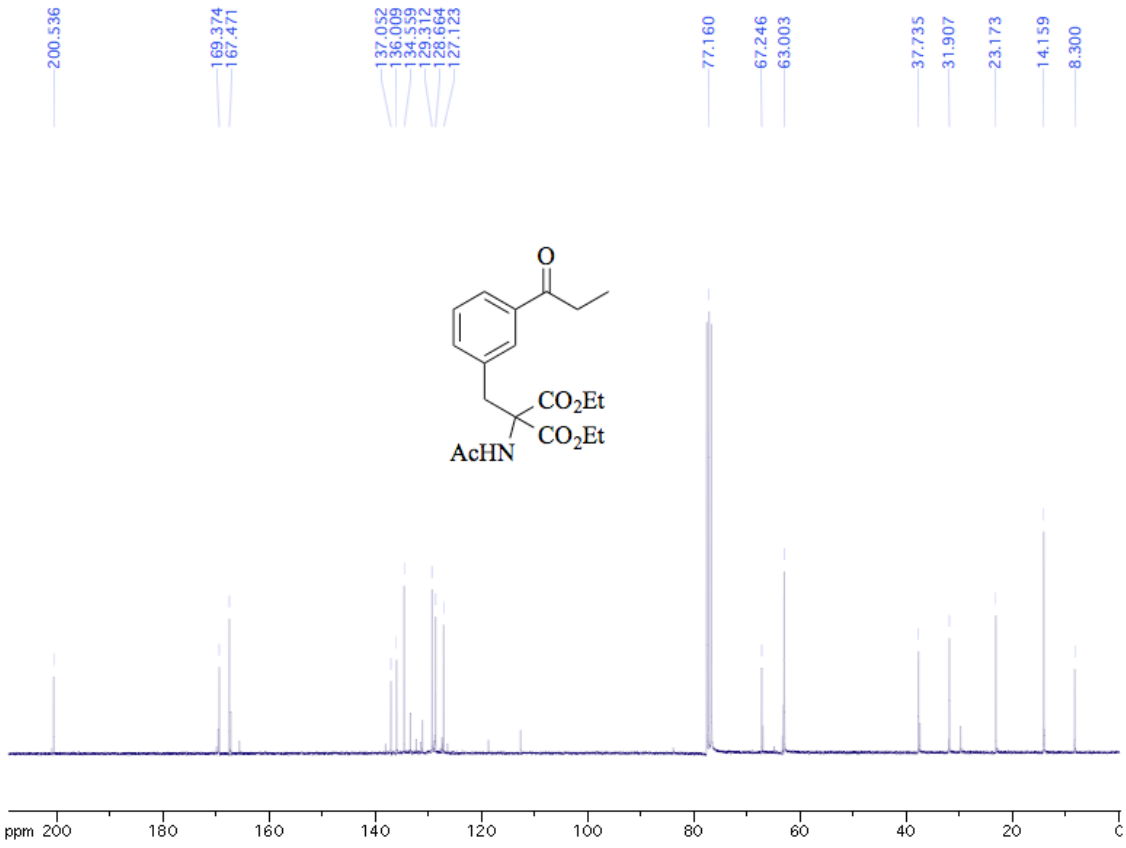
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77.160
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37.735
31.907
23.173
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CO2Et
CO2Et
AcHN
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140
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60
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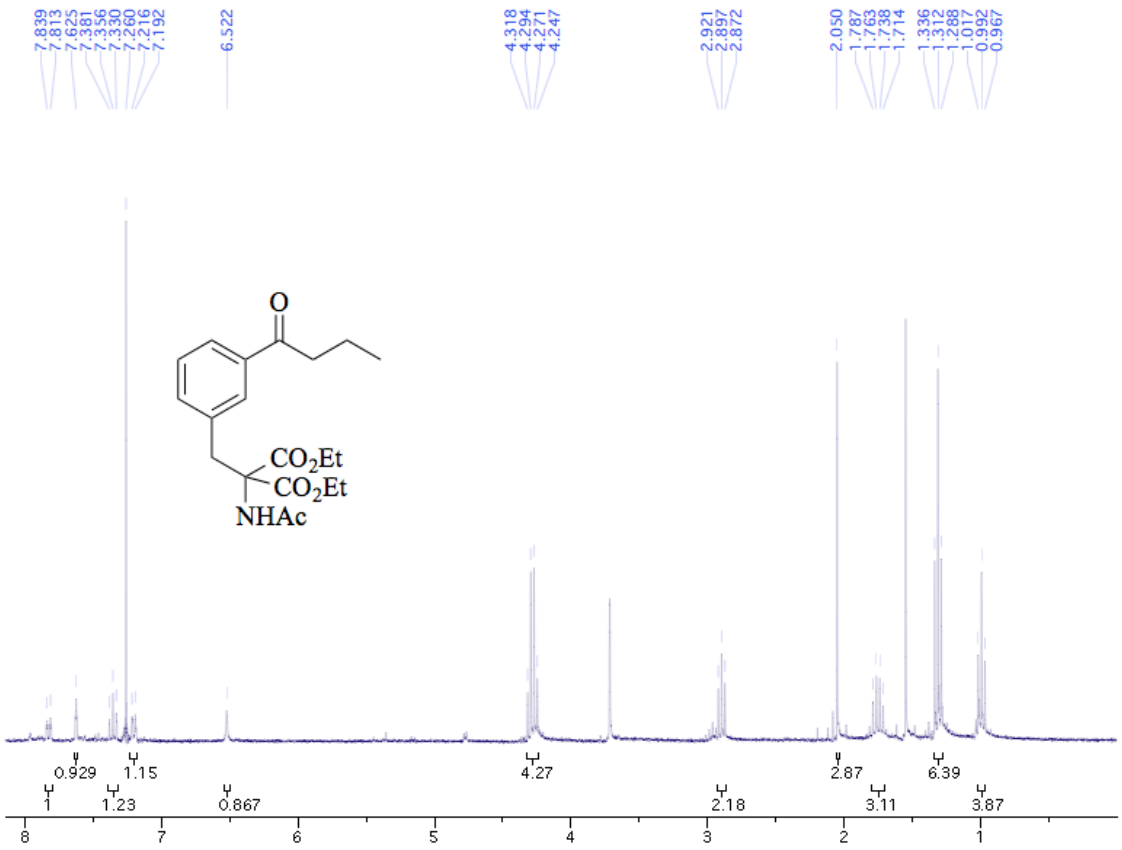
0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4.271
4.247
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2.897
2.872
2.050
1.787
1.763
1.738
1.714
1.336
1.312
1.288
1.017
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0.967
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CO2Et
NHAc
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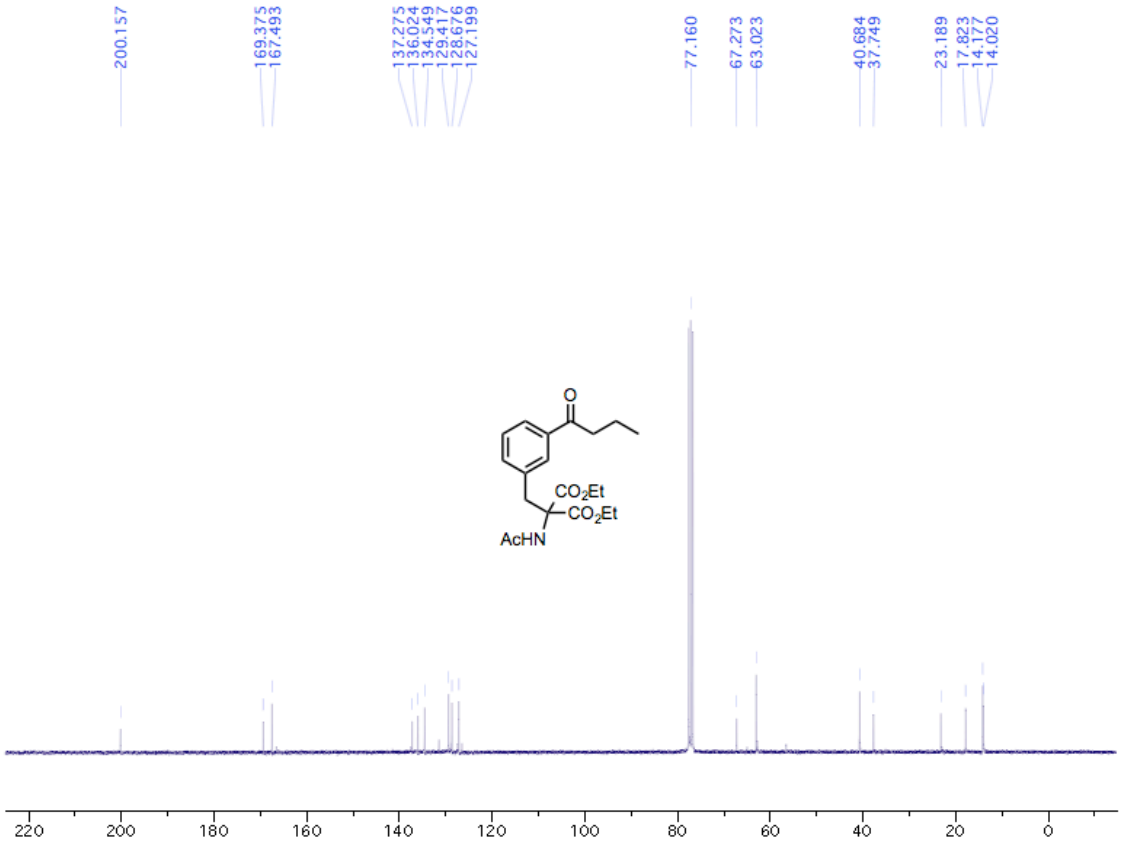
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67.273
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40.684
37.749
23.189
17.823
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CO2Et
AcHN
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140
120
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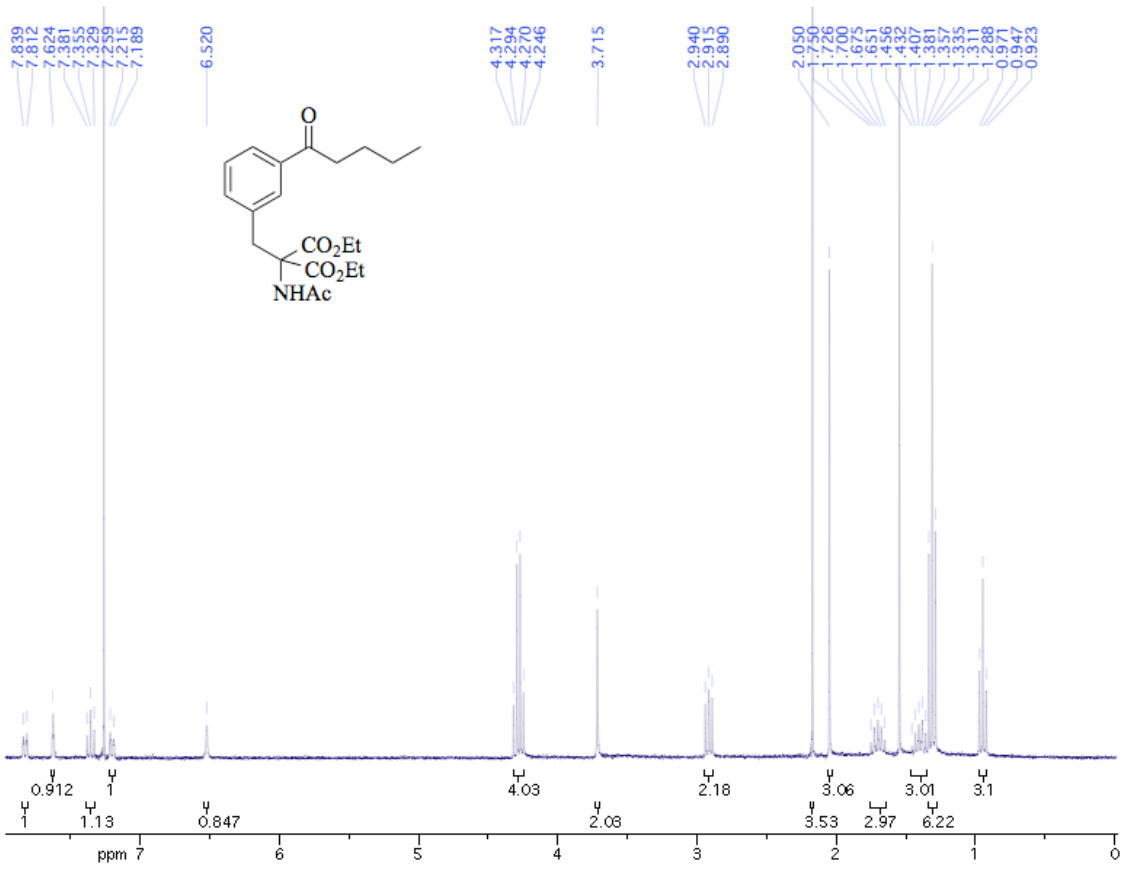
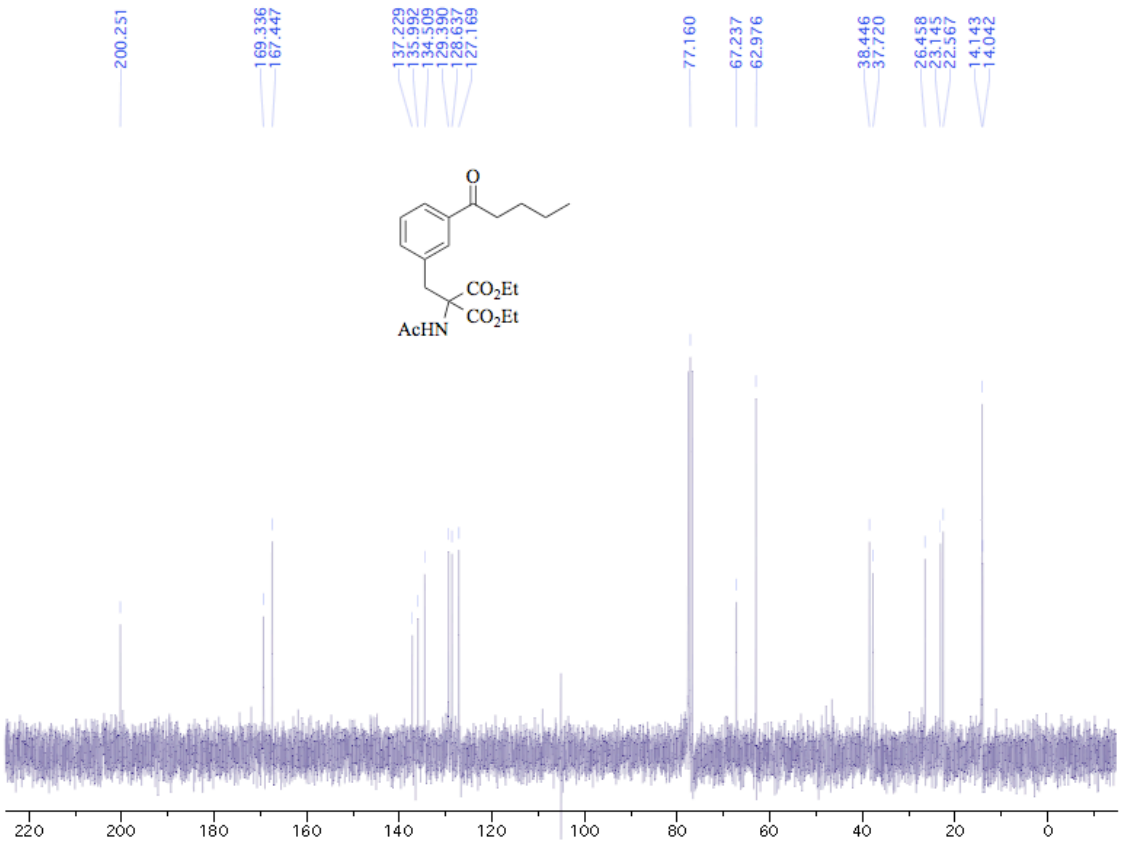
80
60
40
20
0

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38.446
37.720
26.458
23.145
22.567
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CO2Et
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140
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20
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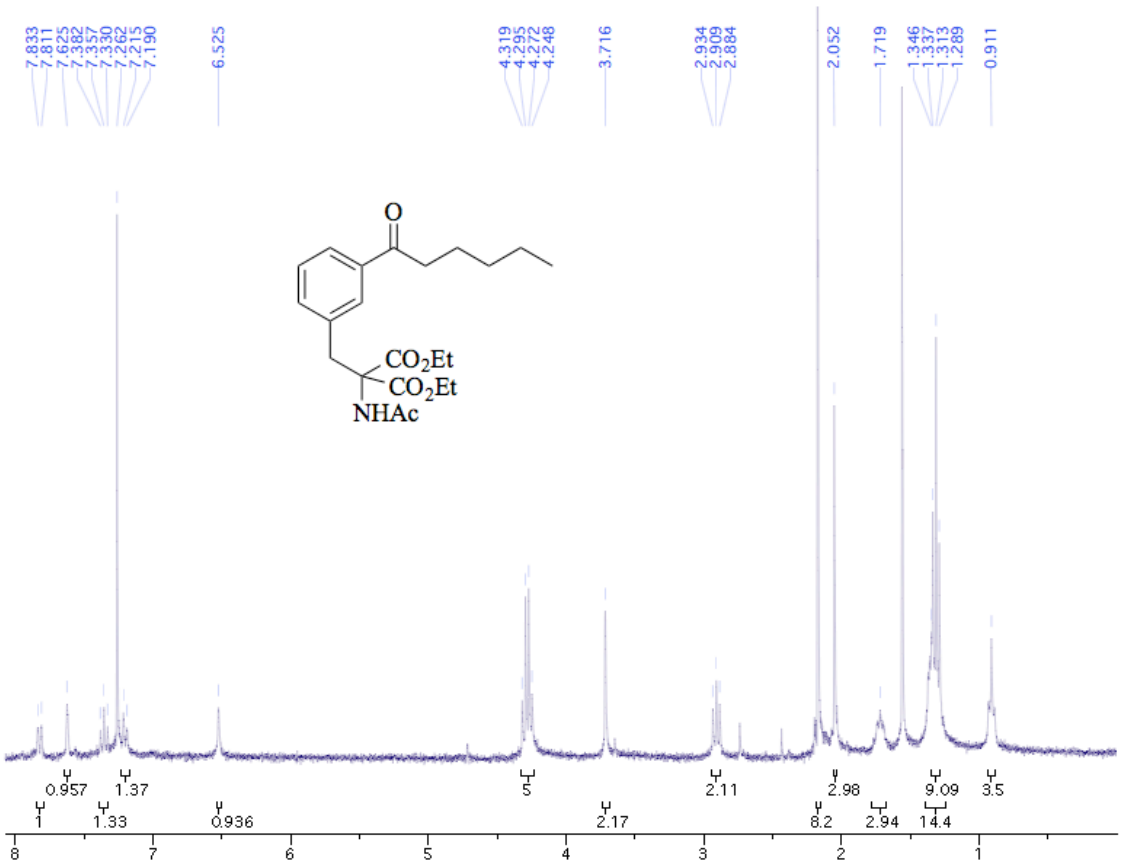
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4.295
4.272
4.248
3.716
2.934
2.909
2.884
2.052
1.719
1.346
1.337
1.313
1.289
0.911
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CO2Et
NHAc
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5
2.11
2.98
9.09
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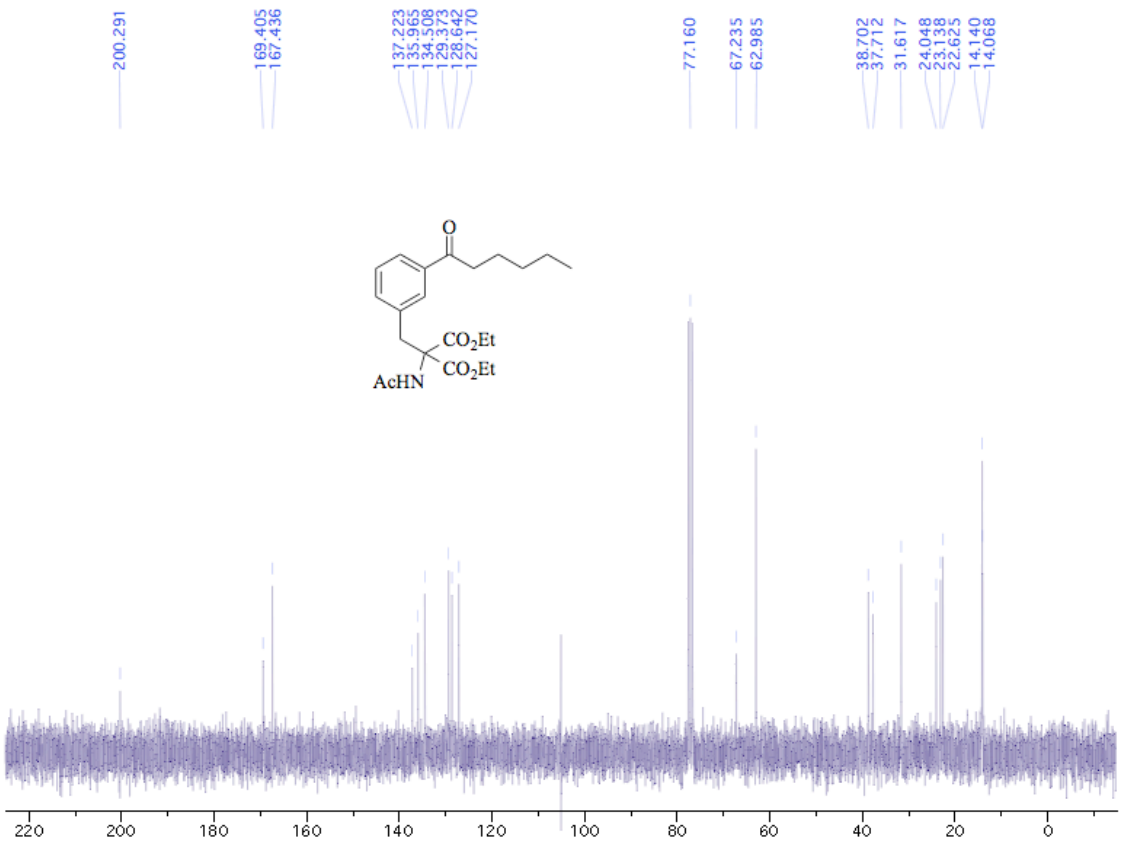
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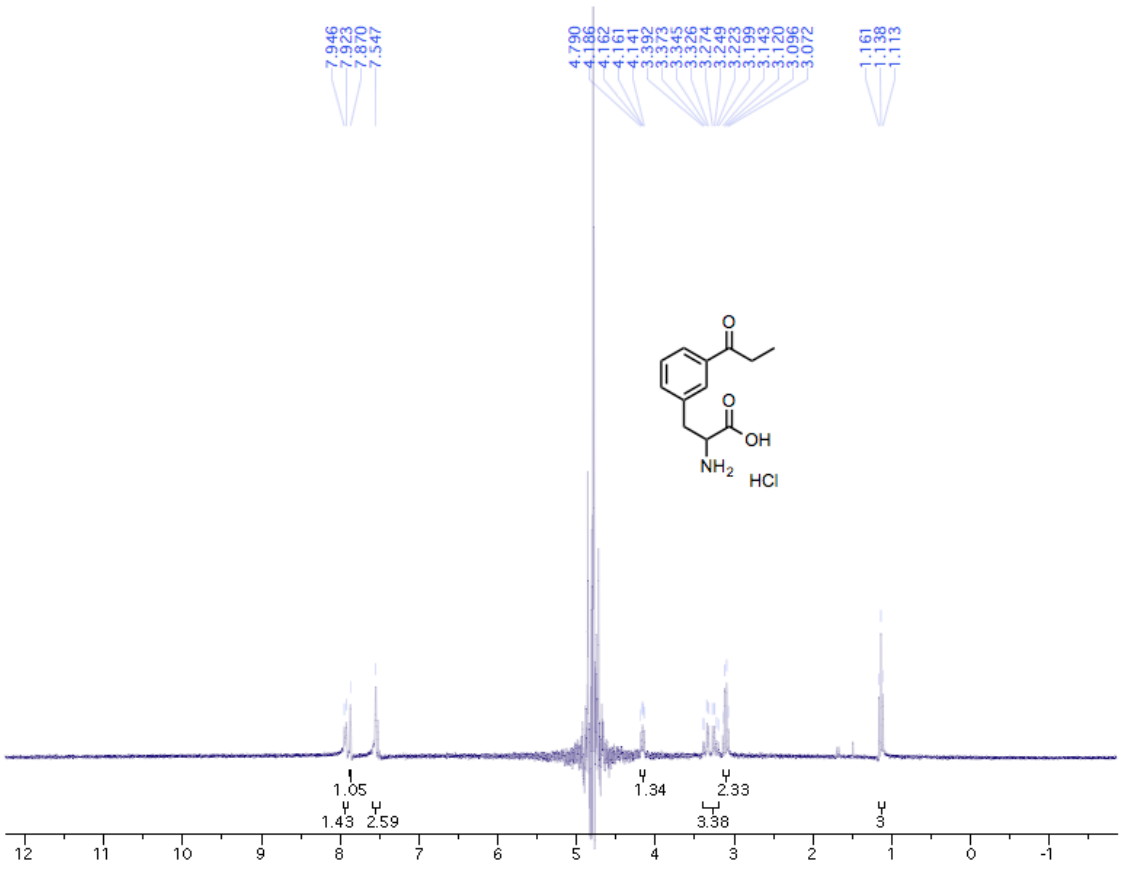
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3.249
3.223
3.199
3.143
3.120
3.096
3.072
1.161
1.138
1.113
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O
OH
NH2
HCl
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1.43
2.59
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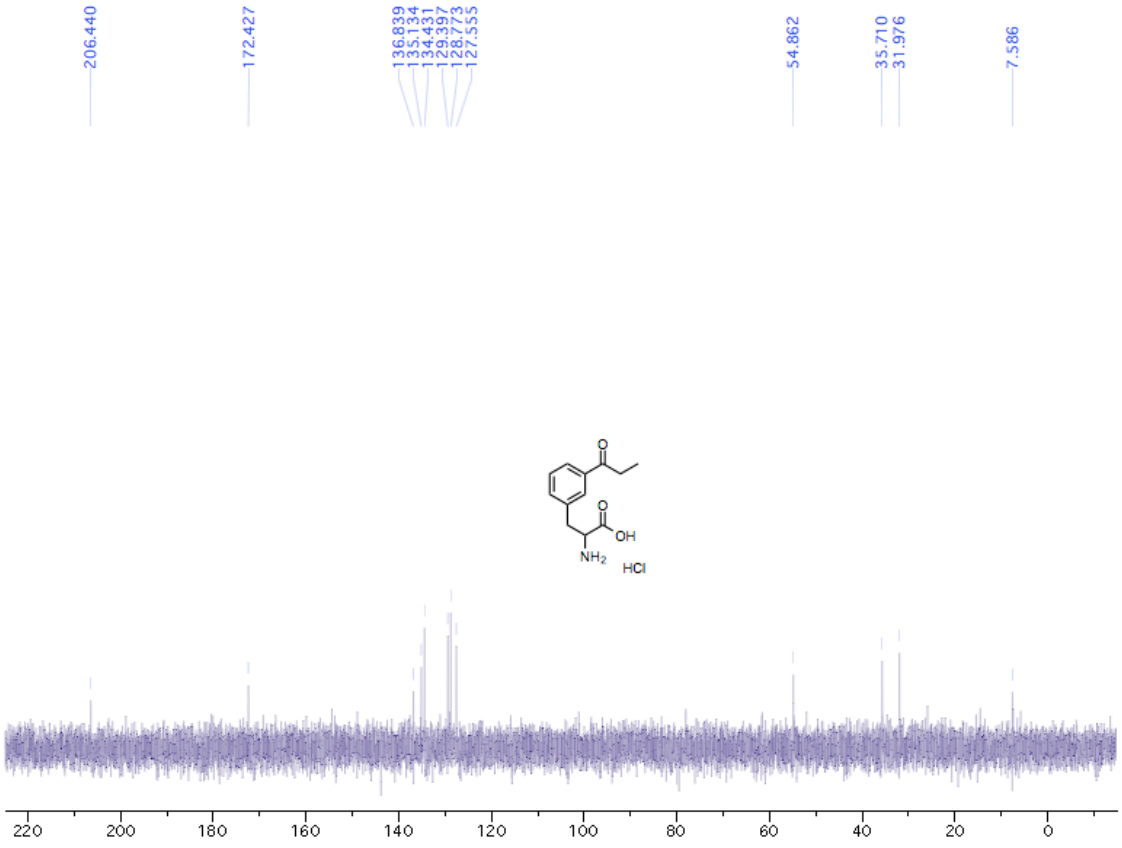
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O
OH
NH2
HCl
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120
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20
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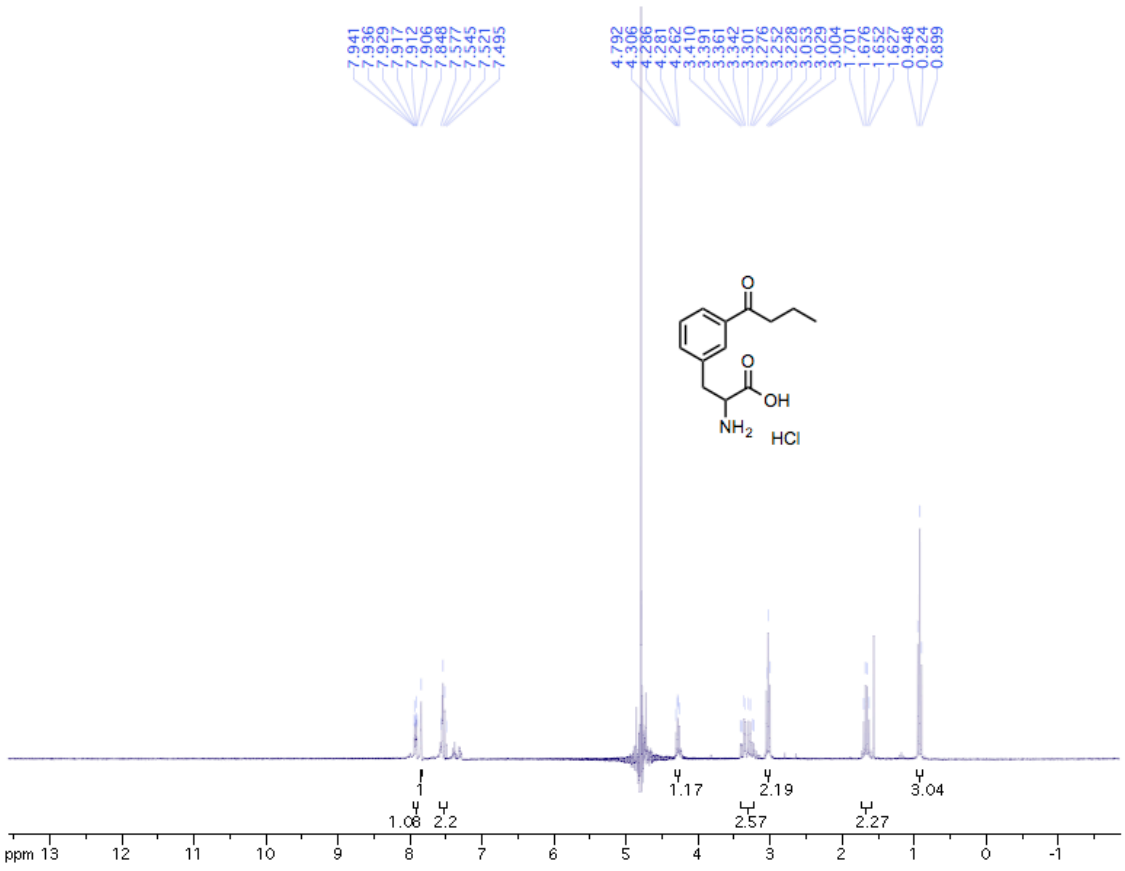
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3.301
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3.053
3.029
3.004
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1.676
1.652
1.627
0.948
0.924
0.899
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O
OH
NH2
HCl
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1.08
2.2
1.17
2.19
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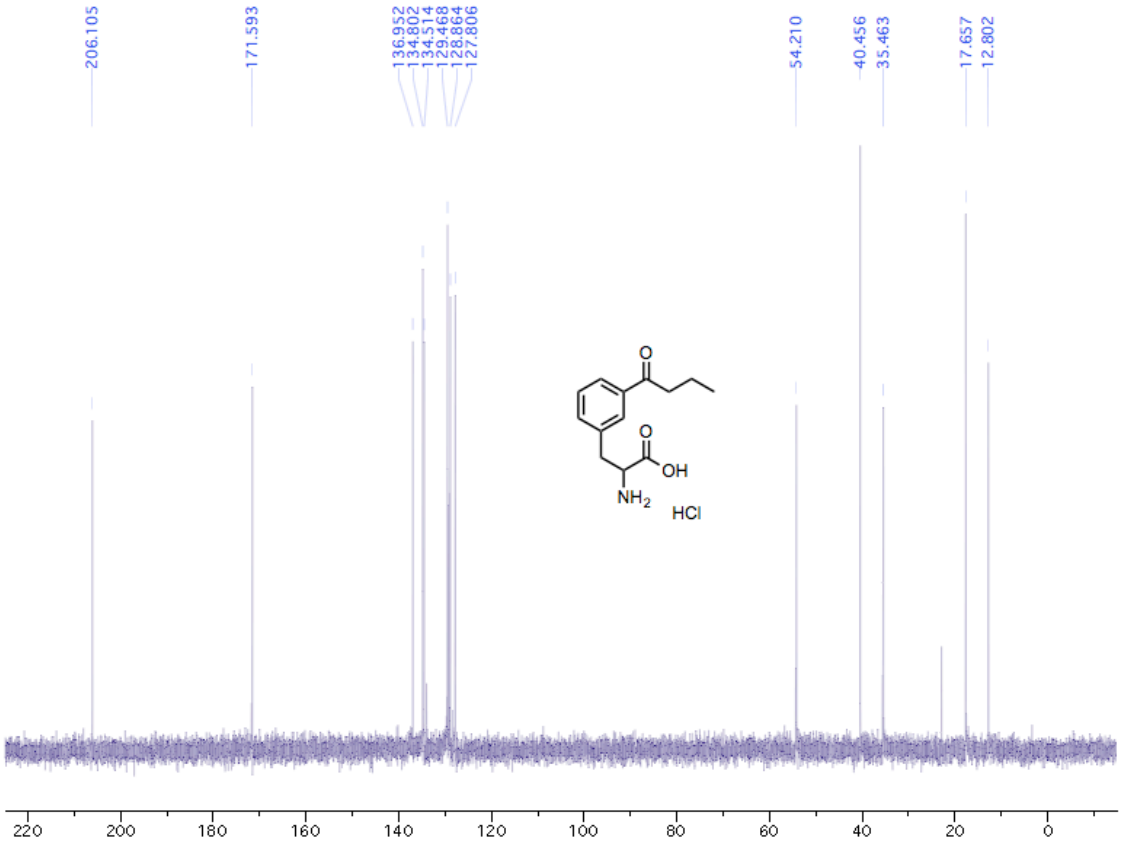
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OH
NH2
HCl
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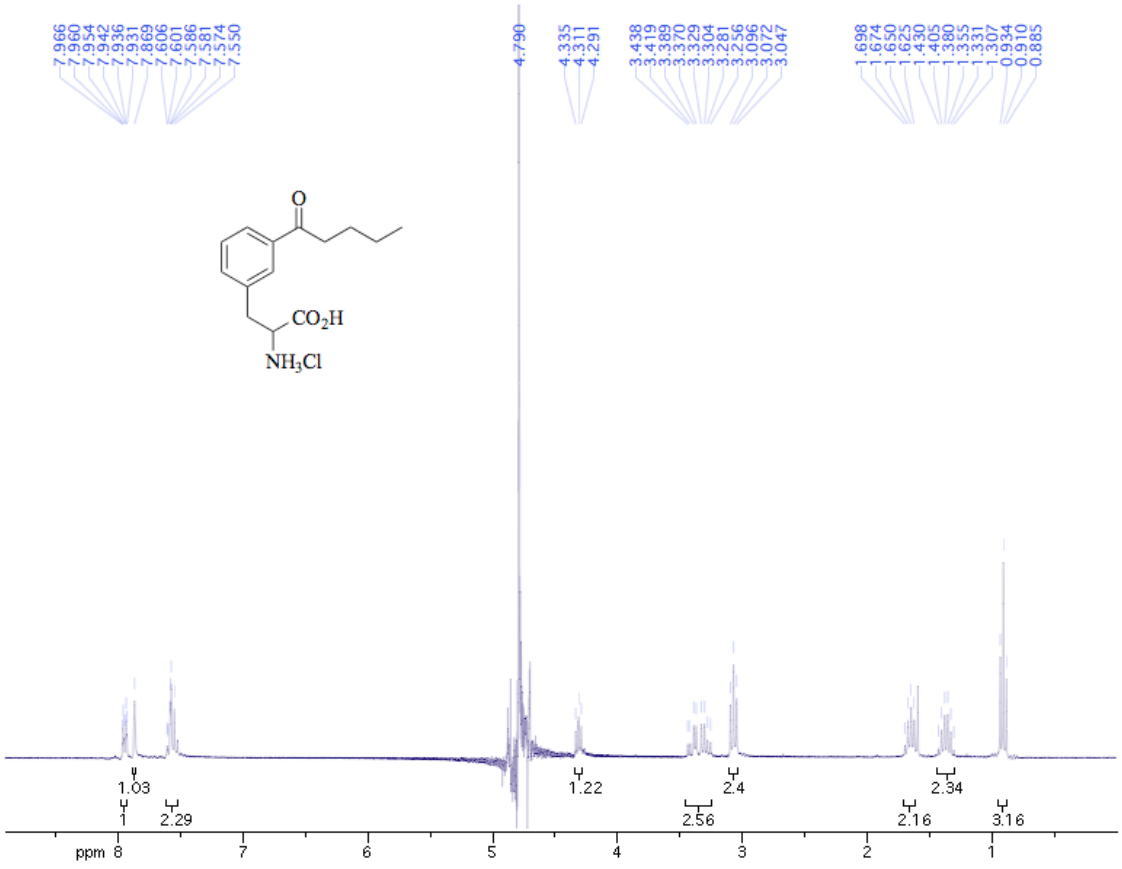
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7.550
4.790
4.335
4.311
4.291
3.438
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0.910
0.885
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CO2H
NH3Cl
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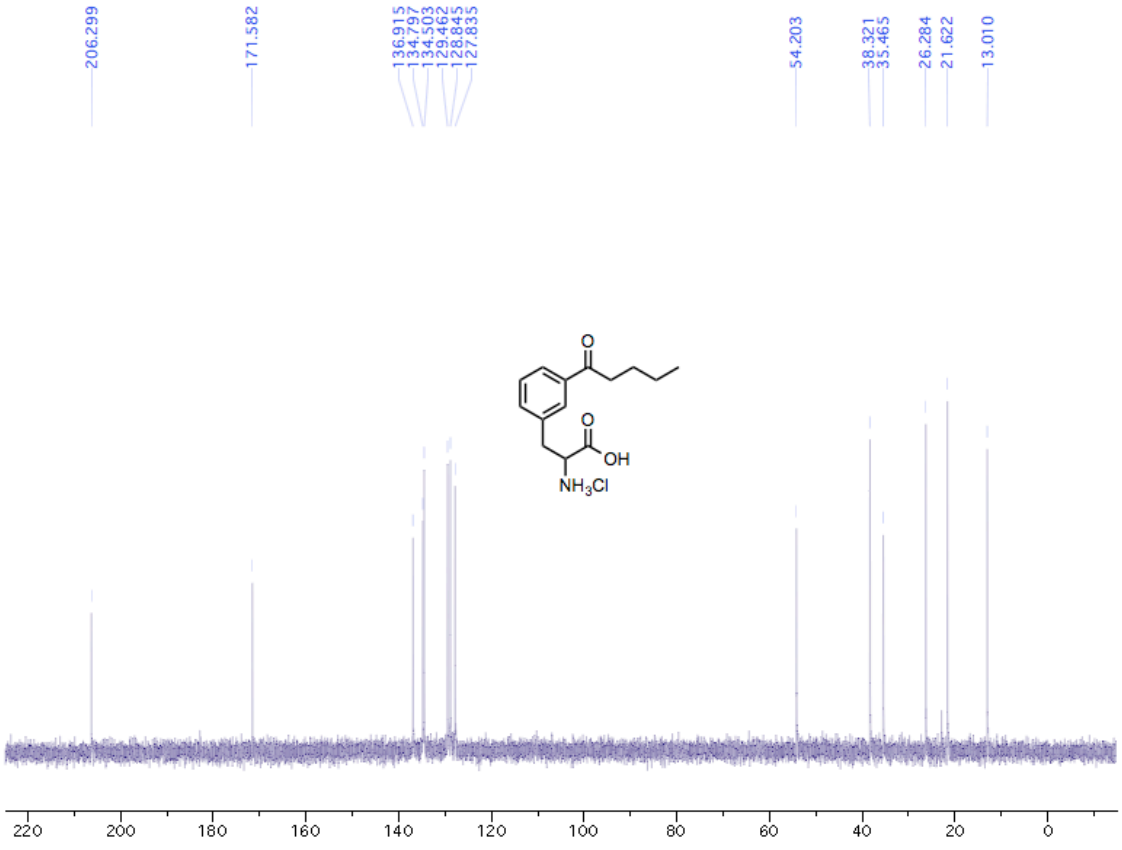
2.29
1.22
2.4
2.56
2.34
2.16
3.16
ppm 8
7
6
5
4
3
2
1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171.582
136.915
134.797
134.503
129.462
128.845
127.835
54.203
38.321
35.465
26.284
21.622
13.010
O
O
OH
NH3Cl
220
200
180
160
140
120
100
80
60
40
20
0