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Development of HC-258, a covalent acrylamide TEAD inhibitor that reduces gene expression and cell migration

Ahmed Fnaiche,^{‡a} Hwai-Chien Chan,^{‡a} Alexis Paquin,^a Narjara González Suárez,^a Victoria Vu,^b Fengling Li,^b Abdellah Allali-Hassani,^b Michelle Ada Cao,^{b,d} Magdalena M. Szewczyk,^b Albina Bolotokova,^b Frédéric Allemand,^c Muriel Gelin,^c Dalia Barsyte-Lovejoy,^b Vijayaratnam Santhakumar,^b Masoud Vedadi,^{d,e} Jean-François Guichou,^c Borhane Annabi,^a and Alexandre Gagnon*^a

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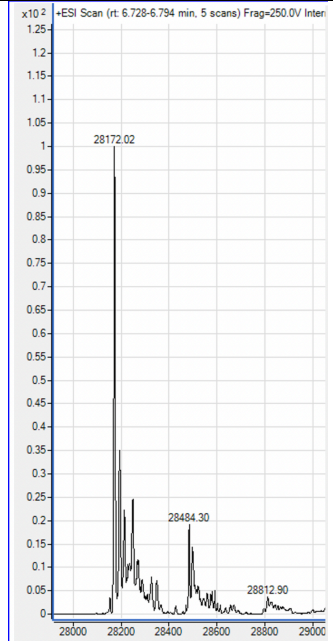
1. Biological evaluation

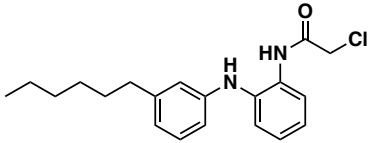
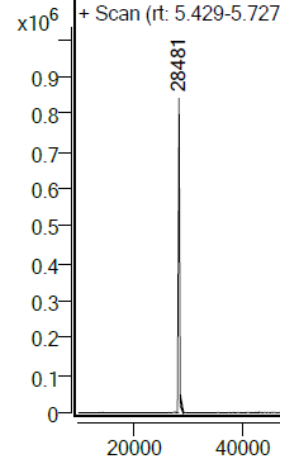
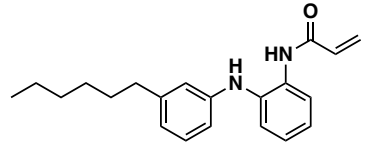
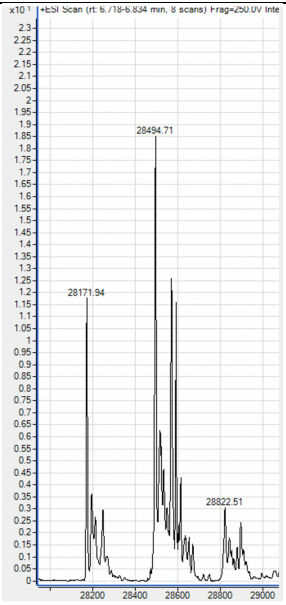
1a) Fluorescence polarization displacement assay using bodipy-PA

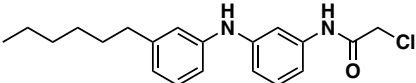
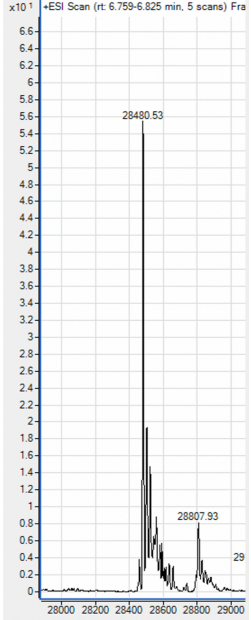
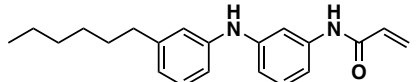
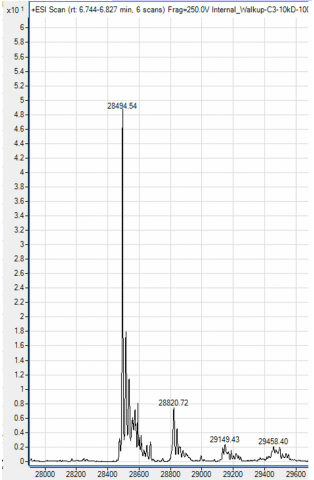
All FP experiments were performed in a total assay volume of 20 μ L per well in 384-well black polypropylene PCR plates (PCR-384-BK, Axygen, Tewksbury, MA) using a fluorescent bodipy-PA and TEAD4. Fluorescence polarization (FP) was measured after 30 min of incubation at room temperature, using a BioTek Synergy 4 (BioTek, Winooski, VT) with excitation and emission wavelengths of 485 nm and 528 nm, respectively. All experiments were performed in triplicate ($n = 3$), and plotted values are the average of three replicates \pm standard deviation. The FP values were blank subtracted and were presented as the percentage of control (FP %). Data were visualized using GraphPad Prism software 8.3.0 (GraphPad, La Jolla, CA).

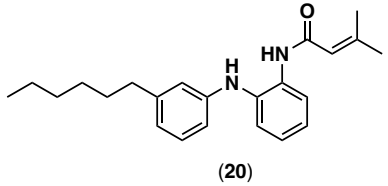
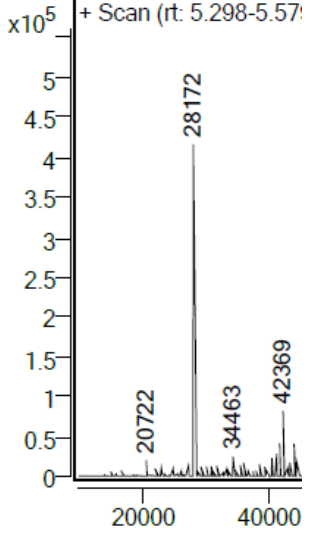
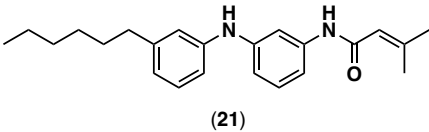
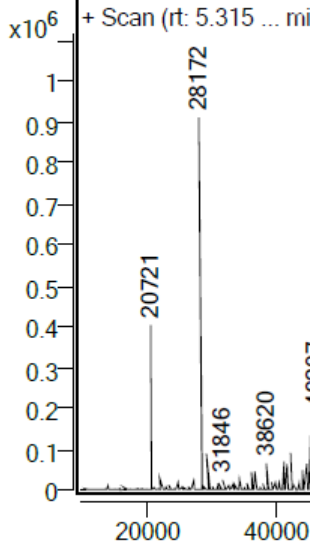
1b) High resolution mass spectrometry experiment for determination of covalent adduct formation between TEAD4 and selected compounds

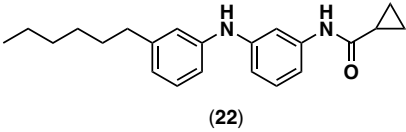
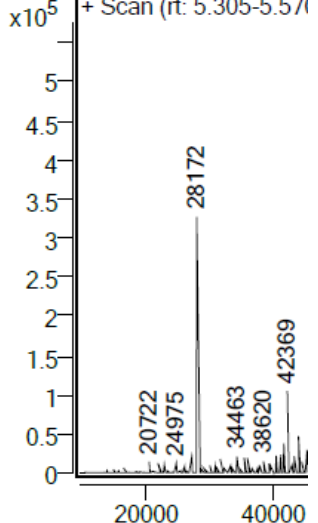
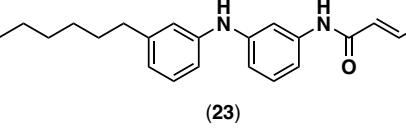
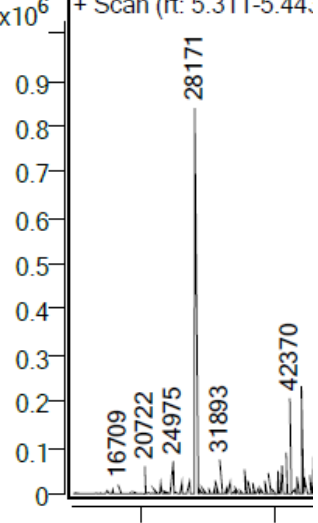
TEAD4 (final concentration of 15 μ M) was mixed with 0.3 mM of each compound and incubated for 2 hr at RT before being quenched by adding 0.1% trifluoroacetic acid. The resulting samples were separated on a HPLC column with 5-95% acetonitrile in water as eluent and the MS data were analyzed using an Agilent LC/MSD Time-of-Flight Mass Spectrometer equipped with an electrospray ionization source.

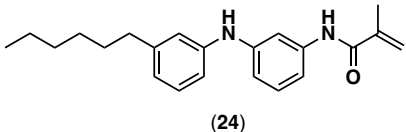
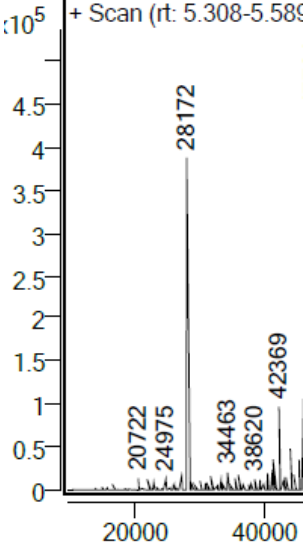
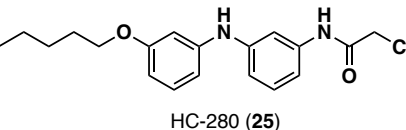
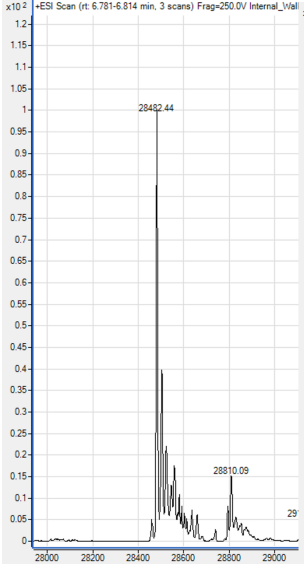
Table S1: HRMS results from incubation of TEAD4 with selected compounds					
Compound's structure and number	Compound molecular weight (g/mol)	Targeted mass: TEAD4 + compound (g/mol)	Observed mass (g/mol)	Result	Covalent adduct formed?
TEAD4 alone	28,172.02	28,172.02	28,172.02	 <p>The mass spectrum shows relative intensity on the y-axis (scaled by 10²) and m/z on the x-axis. The base peak is at m/z 28172.02. Other labeled peaks are at m/z 28484.30 and 28812.90. The x-axis ranges from 28000 to 29000, and the y-axis ranges from 0 to 1.25.</p>	Not applicable

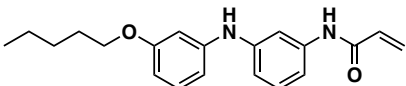
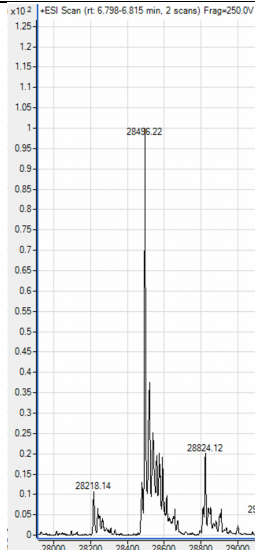
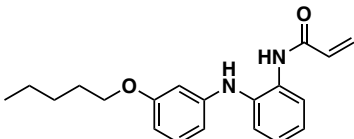
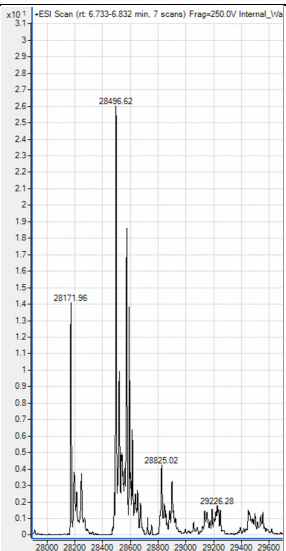
 <p>HC-322 (16)</p>	344.1655	28,480.20	28,481		Complete formation of covalent adduct
 <p>HC-320 (17)</p>	322.2045	28,494.22	28,494.71		Partial formation of covalent adduct

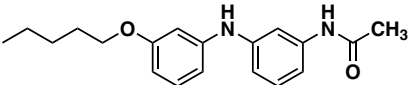
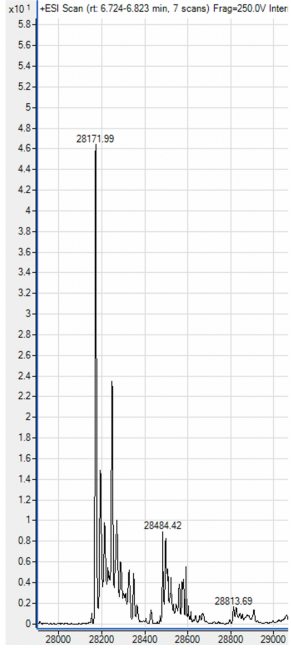
 <p>HC-312 (18)</p>	344.1655	28,480.20	28,480.53		Complete formation of covalent adduct
 <p>HC-310 (19)</p>	322.2045	28,494.22	28,494.54		Complete formation of covalent adduct

 <p>(20)</p>	350.2358	28,522.25	28,172	 <p>+ Scan (rt: 5.298-5.57)</p>	No covalent adduct formed
 <p>(21)</p>	350.2358	28,522.25	28,172	 <p>+ Scan (rt: 5.315 ... mi</p>	No covalent adduct formed

 <p>(22)</p>	336.2202	28,508.24	28,172		No covalent adduct formed
 <p>(23)</p>	336.2202	28,508.24	28,171		No covalent adduct formed

 <p>(24)</p>	336.2202	28,508.24	28,172	 <p>+ Scan (rt: 5.308-5.586)</p>	No covalent adduct formed
 <p>HC-280 (25)</p>	346.1448	28,482.18	28,482.44	 <p>ESI Scan (rt: 6.781-6.814 min, 3 scans) Frag=250 DV Internal_Val</p>	Complete formation of covalent adduct

 <p>HC-258 (26)</p>	324.1838	28,496.20	28,496.22		Complete formation of covalent adduct
 <p>AF-864 (27)</p>	324.1838	28,496.20	28,496.62		Partial formation of covalent adduct

 <p>AF-857 (28)</p>	312.1838	28,496.20	28,171.99	 <p>ESI Scan (rt: 6.724-6.823 min, 7 scans) Frag+250.0V Inter</p>	No covalent adduct formed
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1c) Differential static light scattering (DSLS)

Binding of the compounds to TEAD4 was evaluated by monitoring the effect of compounds on the thermal stability of the protein using differential static light scattering (DSLS, StarGazer, Harbinger). TEAD4 was used at final concentration of 0.2 mg/mL in a buffer consisting of 0.1 M HEPES pH 7.5 and 150 mM NaCl. This method assesses ligand binding through ligand induced stability where protein aggregation due to denaturation is measured while heating the sample from 25 to 85 °C at 1 °C/min in a 50 µL volume (covered with 50 µL of mineral oil to prevent evaporation) in a clear-bottom 384-well plate (from Nunc). Aggregation was monitored by the increase of scattered light using CCD camera detection. Pixel intensities were integrated using image analysis software, plotted against temperature and data were then fitted to a Boltzmann sigmoid function to obtain the aggregation temperature (T_{agg}) from the midpoint of the transition.

1d) Total RNA isolation, cDNA synthesis and real-time quantitative PCR

Total RNA was extracted from treated MDA-MB-231 cell monolayers using TriZol reagent (Life Technologies, Gaithersburg, MD). For cDNA synthesis, 2 µg of total RNA were reverse transcribed using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster S16 City, CA), and cDNA was stored at -80°C. Gene expression was quantified by real-time quantitative PCR using iQ SYBR Green Supermix (Bio-Rad, Hercules, CA). DNA amplification was carried out using an Icyler iQ5 (Bio-Rad, Hercules, CA) and product detection was performed by measuring binding of the fluorescent dye SYBR Green I to double stranded DNA. The QuantiTect primer sets were obtained from Qiagen (Valencia, CA): CTGF (Hs_CTGF_1_SG, QT00052899), Cyr61 (Hs_CYR61_1_SG, QT00003451), AXL (Hs_AXL_1_SG, QT00067725), NF2 (Hs_NF2_va.1_SG, QT01004934), PPIA (Hs_PPIA_4_SG, QT01866137), and GAPDH (Hs_GAPDH_1_SG, QT00079247). The relative quantities of target gene mRNA compared against two internal controls, GAPDH and PPIA mRNA, were measured by following a Δ CT method employing an amplification plot (fluorescence signal vs. cycle number). The difference (Δ CT) between the mean values in the triplicate samples of target gene and those of GAPDH and PPIA mRNAs were calculated by CFX Manager™ (Bio-Rad, Hercules, CA) and the relative quantified value (RQV) was expressed as $2^{-\Delta$ CT.

1e) Chemotactic cell migration assay

Cell migration assays were carried out using the Real-Time Cell Analyzer (RTCA) Dual-Plate (DP) Instrument of the xCELLigence system (Roche Diagnostics, Basel, Switzerland). Human triple negative MDA-MB-231 breast cancer cells were seeded in 6-well plates at 200,000 cells/well. The

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next day, growth media was replaced by negative media (NM) supplemented or not with the 10 μ M of the test compounds (LEM41, AF-208, AF-2112 and AF-851) or DMSO used as vehicle control. Cells were kept for 48 hours, after which the viability was determined by 0.4% Trypan Blue exclusion assay and ranged from 73-98%. Then, cells were placed onto CIM Plates 16 (Roche Diagnostics) for the migration assessment in response to NM or positive media (PM, NM supplemented with 2% FBS). These migration plates have transwells with a pore size of 8 μ m and a gold electrode arrays on their bottom side of the membrane, providing real-time data acquisition of cell migration. Prior to cell seeding, the underside of the wells from the upper chamber were coated with 25 μ L of 0.15% gelatin in PBS and incubated for 1 hour at 37°C. Cell migration was continuously monitored for 8 hours. The impedance values were measured by the RTCA DP Instrument software and were expressed as Normalized Cell Migration Index. Each experiment was performed in quadruplicates.

1f) NanoBret assay

Method: HEK293T cells plated in 96-well white plastic plates (Greiner) were transfected with 1ng C-terminally tagged TEAD1-NanoLuc and 30ng C-terminally tagged YAP-HaloTag using XtremeGENE™ HP DNA Transfection Reagent (Sigma), following the manufacturer's instructions. The next day, the media was replaced with DMEM (no phenol red) supplemented with 4% FBS, penicillin (100 U/ml) and streptomycin (100 μ g/ml) in the presence or absence of compounds and HaloTag® NanoBRET™ 618 Ligand (Promega) for 4 h. Next, NanoBRET™ Nano-Glo® Substrate (Promega) solution was added to each well. Donor emission at 450 nm (filter, 450 nm/band-pass 80 nm) and acceptor emission at 618 nm (filter, 610 nm/long-pass) was measured within 10 min of substrate addition using a CLARIOstar microplate reader (Mandel). mBU values were calculated by subtracting the mean of 618/460 signal from cells without a NanoBRET™ 618 Ligand \times 1,000 from the mean of 618/460 signal from cells with a NanoBRET™ 618 Ligand \times 1,000.

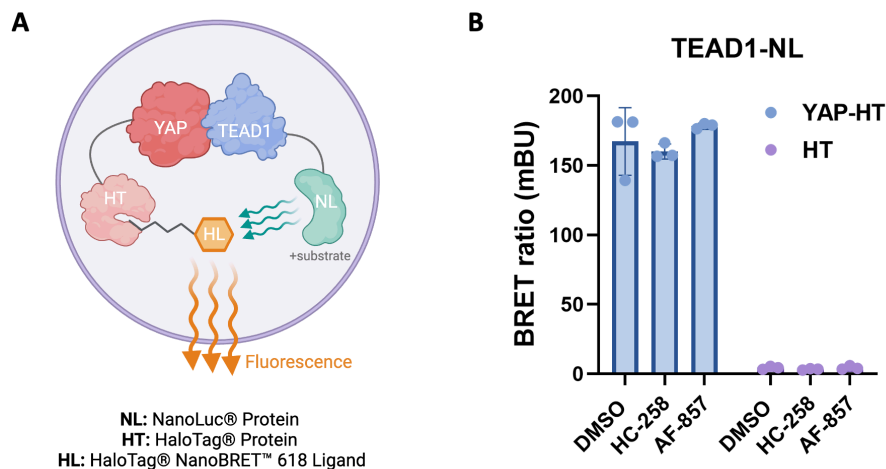


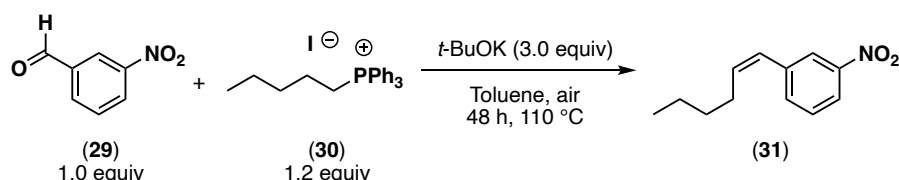
Figure S1. NanoBRET protein-protein interaction assay to determine compound inhibition of YAP-TEAD1 interaction. **A.** Schematic of TEAD1-tagged NanoLuciferase (NanoLuc, NL, donor) and C-terminally tagged YAP-haloalkane dehalogenase (HaloTag, HT) irreversibly bound to a cell-permeable 618 nm fluorochrome (HaloLigand, HL, acceptor). **B.** BRET ratio of YAP-TEAD1 interaction or negative control HT-TEAD1 in cells treated with compounds (10 μ M). Data are from three experiments (n=3).

2. Syntheses

Unless otherwise stated, all reactions were performed under argon in non-flame dried glassware. Commercial reagents were used without further purification. Anhydrous solvents were obtained using an encapsulated solvent purification system and were further dried over 4 Å molecular sieves. The evolution of reactions was monitored by analytical thin-layer chromatography (TLC) using silica gel 60 F254 precoated plates visualized by ultraviolet radiation (254 nm). Flash chromatography was performed employing 230-400 mesh silica using the indicated solvent system according to standard techniques. $^1\text{H-NMR}$ spectra were recorded on a BrukerAvance-III 300 MHz, 500 MHz or 600 MHz. $^{13}\text{C-NMR}$ spectra were recorded on a BrukerAvance-III 75 MHz, 126 MHz or 151 MHz spectrometer. Chemical shifts for $^1\text{H-NMR}$ spectra are recorded in parts per million from tetramethylsilane using the residual non-deuterated solvent resonance as the internal standard (chloroform- d , δ 7.26 ppm; methanol- d_4 , δ 3.31 ppm; dimethylsulfoxide- d_6 , δ 2.50 ppm; acetone- d_6 , δ 29.84 ppm). Data is reported as follow: chemical shift, multiplicity (s = singlet, s(br) = broad singlet, d = doublet, t = triplet, q = quartet, quint = quintet, sext = sextet, sept = septet, m = multiplet, dd = doublet of doublet, dt = doublet of triplet, ddd = doublet of doublet of doublet), coupling constant J in Hz and integration. Chemical shifts for $^{13}\text{C-NMR}$ spectra are recorded in parts per million from tetramethylsilane the residual non-deuterated solvent resonance as the internal standard (chloroform- d , δ 77.16 ppm; methanol- d_4 , δ 49.00 ppm; dimethylsulfoxide- d_6 , δ 39.52 ppm; acetone- d_6 , δ 2.05 ppm). Compound purity was assessed on an Agilent 1260 infinity HPLC

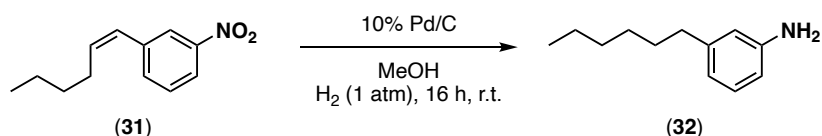
system equipped with an Agilent Eclipse Plus C18 (3.5 μ M, 4.6 x 100 mm) column using a 20-minute gradient method (0 to 100% MeCN + 0.06% TFA in water + 0.06% TFA; the absorbance was measured at 254 nm). Purity is greater than 95% for all final compounds. HRMS were performed on a TOF LCMS analyzer using the electrospray (ESI) mode.

(Z)-1-Nitro-3-(hex-1-en-1-yl)benzene (31)



To a suspension of 3-nitrobenzaldehyde (29) (2.2 g, 14.6 mmol) and potassium *tert*-butoxide (4.9 g, 43.7 mmol) in toluene (80.0 mL) was added pentyltriphenylphosphonium iodide (30) (8.0 g, 17.4 mmol) at room temperature under air atmosphere. The yellow suspension was heated at reflux for 48 hours, cooled down to room temperature and the solvent was evaporated under vacuum. The residue was diluted in 30 mL of CH₂Cl₂ and the organic phase was washed with water (3 x 50 mL), brine (1 x 50 mL), dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The crude orange material was purified by flash column chromatography (1% to 10% EtOAc/Hexanes) to provide the desired compound (Z)-1-nitro-3-(hex-1-en-1-yl)benzene (31) (2.91g, 97%) as a yellow oil. This compound has been previously reported.¹ *R*_f = 0.58 (10% EtOAc/Hexane); ¹H NMR (300 MHz, CDCl₃) δ 8.15 (dt, *J* = 17.2, 1.7 Hz, 1H), 8.09–8.00 (m, 1H), 7.64–7.54 (m, 1H), 7.52–7.41 (m, 1H), 6.43 (d, *J* = 12.9 Hz, 1H), 5.83 (dt, *J* = 11.7, 7.4 Hz, 1H), 2.36–2.26 (m, 2H), 1.51–1.42 (m, 2H), 1.42–1.31 (m, 2H), 0.90 (t, *J* = 7.2 Hz, 3H).

3-Hexylaniline (32)

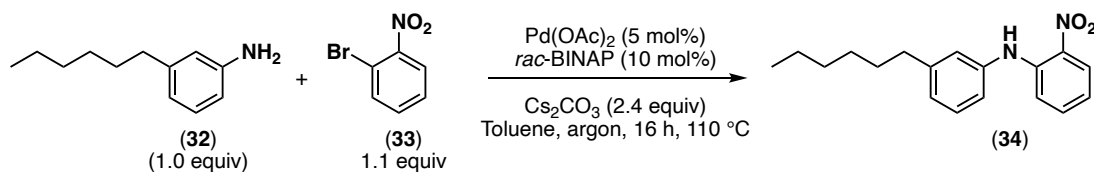


CAUTION: Palladium activated by hydrogen is extremely flammable. During the filtration on celite, the celite should never come to dryness. Failing to do this could result in a fire.

¹ Mélin, L.; Abdullayev, S.; Fnaiche, A.; Vu, V.; González Suárez, N.; Zeng, H.; Szewczyk, M. M.; Li, F.; Senisterra, G.; Allali-Hassani, A.; Chau, I.; Dong, A.; Woo, S.; Annabi, B.; Halabelian, L.; LaPlante, S. R.; Vedadi, M.; Baryte-Lovejoy, D.; Santhakumar, V.; Gagnon, A. Development of LM98, a small-molecule TEAD inhibitor derived from flufenamic acid. *ChemMedChem* **2021**, *16*, 2982-3002.

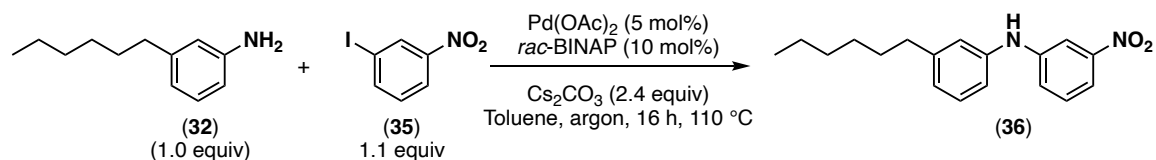
To a solution of (*Z*)-1-nitro-3-(hex-1-en-1-yl)benzene (**31**) (2.91 g, 14.2 mmol) in MeOH (10 mL) was added Pd/C 10% (151 mg). The reaction vessel was evacuated under vacuum and filled with hydrogen. The cycle was repeated twice, and the suspension was stirred at room temperature for 16 h under H₂ atmosphere. The mixture was evacuated under vacuum, filled back with argon, filtered over a plug of celite, rinsed with CH₂Cl₂ and concentrated under reduced pressure to afford 3-hexylaniline (**32**) (2.51 g, 99%) as a yellow oil which was used directly in the next step. This compound has been previously reported.¹ *R*_f = 0.29 (100% CH₂Cl₂); ¹H NMR (300 MHz, CDCl₃) δ 7.21 (t, *J* = 8.1 Hz, 1H), 6.75 (d, *J* = 7.5 Hz, 1H), 6.64 – 6.58 (m, 2H), 3.80 (s, 2H), 2.67 (t, *J* = 8.1 Hz, 2H), 1.81 – 1.69 (m, 2H), 1.57 – 1.40 (m, 6H), 1.08 (t, *J* = 6.4 Hz, 3H).

N-(3-Hexylphenyl)-2-nitroaniline (**34**)



To a solution of 3-hexylaniline (**32**) (600 mg, 3.38 mmol) in dry toluene (7 mL) was added 1-bromo-2-nitrobenzene (**33**) (752 mg, 3.72 mmol), cesium carbonate (2.65 g, 8.13 mmol) and a freshly prepared solution of Pd(OAc)₂/*rac*-BINAP in dry toluene. This solution was obtained by stirring palladium(II) acetate (38 mg, 0.17 mmol) and *rac*-BINAP (211 mg, 0.33 mmol) in dry toluene (3 mL) for 15 minutes with argon bubbling through the mixture. The main reaction mixture was heated at 110 °C for 16 h, cooled down to room temperature, filtered over a plug of celite, and concentrated under reduced pressure. Purification by flash column chromatography (100% CH₂Cl₂) provided *N*-(3-hexylphenyl)-2-nitroaniline (**34**) (952 mg, 94%) as an orange oil. *R*_f = 0.65 (100% CH₂Cl₂); ¹H NMR (300 MHz, CDCl₃) δ 9.50 (s, 1H), 8.20 (dd, *J* = 8.6, 1.5 Hz, 1H), 7.40 – 7.28 (m, 2H), 7.23 (d, *J* = 8.6 Hz, 1H), 7.13 – 7.03 (m, 3H), 6.80 – 6.72 (m, 1H), 2.64 (t, *J* = 7.5 Hz, 2H), 1.73 – 1.58 (m, 2H), 1.43 – 1.24 (m, 6H), 0.91 (t, *J* = 6.5 Hz, 3H). HRMS (ESI-TOF) calcd. for [C₁₈H₂₂N₂O₂ + H]⁺: 299.17540, found 299.17516.

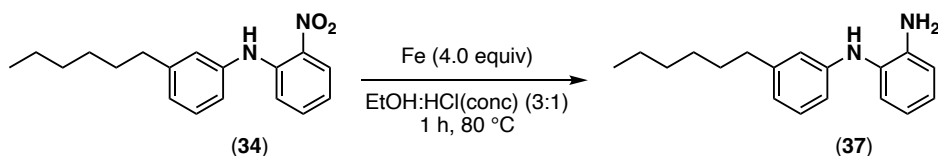
3-Hexyl-*N*-(3-nitrophenyl)aniline (**36**)



To a solution of 3-hexylaniline (**32**) (624 mg, 3.52 mmol) in dry toluene (7 mL) was added 1-iodo-3-nitrobenzene (**35**) (964 mg, 3.87 mmol), cesium carbonate (2.75 g, 8.44 mmol) and a freshly

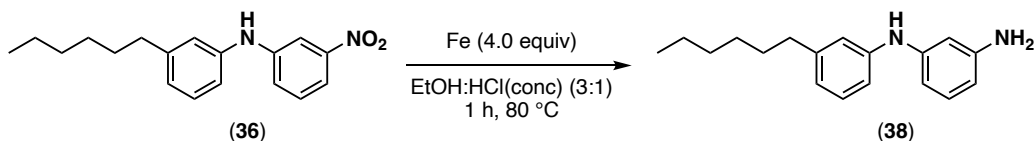
prepared solution of Pd(OAc)₂/*rac*-BINAP in dry toluene. This solution was obtained by stirring palladium(II) acetate (40 mg, 0.18 mmol) and (*rac*)-BINAP (219 mg, 0.35 mmol) in dry toluene (3 mL) for 15 min with argon bubbling through the mixture. The main reaction mixture was heated at 110 °C for 16 h, cooled down to room temperature, filtered over a plug of celite and concentrated under reduced pressure. Purification by flash column chromatography (100% CH₂Cl₂) provided 3-hexyl-*N*-(3-nitrophenyl)aniline (**36**) (1.04 g, 99%) as a red oil. *R*_f = 0.62 (100% CH₂Cl₂); ¹H NMR (300 MHz, CDCl₃) δ 7.86 (t, *J* = 2.2 Hz, 1H), 7.70 (ddd, *J* = 8.0, 2.0, 0.9 Hz, 1H), 7.38 (t, *J* = 8.1 Hz, 1H), 7.30 – 7.25 (m, 2H), 7.02 – 6.97 (m, 2H), 6.93 (d, *J* = 7.5 Hz, 1H), 5.97 (s, 1H), 2.65 – 2.58 (m, 2H), 1.65 (dt, *J* = 15.2, 7.6 Hz, 2H), 1.37 (dd, *J* = 12.4, 4.7 Hz, 6H), 0.91 (t, *J* = 6.5 Hz, 3H). HRMS (ESI-TOF) calcd. for [C₁₈H₂₂N₂O₂ + H]⁺: 299.17540, found 299.17476.

*N*¹-(3-Hexylphenyl)benzene-1,2-diamine (**37**)



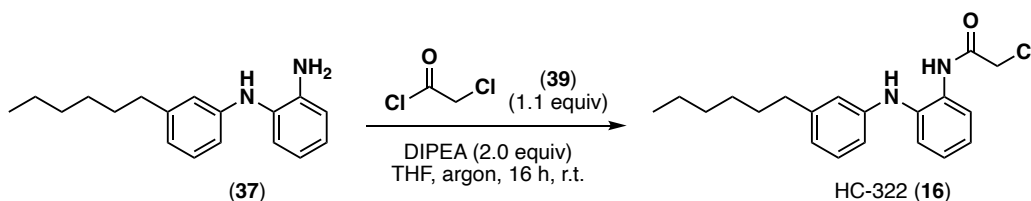
Metallic iron (713 mg, 12.8 mmol) was added to a solution of *N*-(3-hexylphenyl)-2-nitroaniline (**34**) (952 mg, 3.19 mmol) in 16 mL 3:1 EtOH/HCl(conc). After heating at 80 °C for 1 h, the reaction mixture was cooled down to room temperature and quenched with a slow addition of saturated aqueous solution of NaHCO₃ (50 mL). The aqueous phase was extracted with EtOAc (3 x 50 mL). The combined organic phases were washed with water (1 x 50 mL), brine (1 x 50 mL), dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The crude material was purified by flash column chromatography (1% to 10% EtOAc/Hexanes) to provide *N*¹-(3-hexylphenyl)benzene-1,2-diamine (**37**) (659 mg, 77%) as a brown dark oil. *R*_f = 0.86 (40% EtOAc/Hexane); ¹H NMR (300 MHz, CDCl₃) δ 7.15 (t, *J* = 7.6 Hz, 2H), 7.04 (td, *J* = 7.8, 1.4 Hz, 1H), 6.86 – 6.74 (m, 2H), 6.70 (d, *J* = 7.6 Hz, 1H), 6.67 – 6.57 (m, 2H), 5.17 (s, 1H), 3.77 (s, 2H), 2.56 (t, *J* = 7.6 Hz, 2H), 1.67 – 1.56 (m, 2H), 1.43 – 1.27 (m, 6H), 0.93 (t, *J* = 6.7 Hz, 3H). HRMS (ESI-TOF) calcd. for [C₁₈H₂₄N₂ + H]⁺: 269.20123, found 269.20167.

*N*¹-(3-Hexylphenyl)benzene-1,3-diamine (**38**)



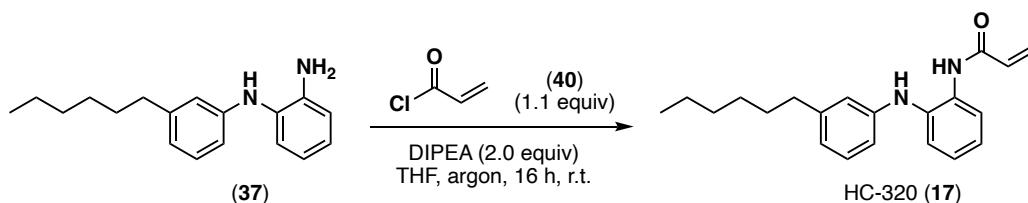
Metallic iron (764 mg, 13.7 mmol) was added to a solution of 3-hexyl-*N*-(3-nitrophenyl)aniline (**36**) (1.02 g, 3.42 mmol) in 16 mL 3:1 EtOH/HCl(conc). After heating at 80 °C for 1 h, the reaction mixture was cooled down to room temperature and quenched with a slow addition of saturated aqueous solution of NaHCO₃ (50 mL). The aqueous phase was extracted with EtOAc (3 x 50 mL). Combined organic phases were washed with water (1 x 50 mL), brine (1 x 50 mL), dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The crude material was purified by flash column chromatography (1% to 10% EtOAc/Hexanes) to provide *N*¹-(3-hexylphenyl)benzene-1,3-diamine (**38**) (584 mg, 64%) as a dark yellow oil. *R*_f = 0.82 (40% EtOAc/Hexanes); ¹H NMR (300 MHz, CDCl₃) δ 7.21 – 7.14 (m, 1H), 7.05 (t, *J* = 7.9 Hz, 1H), 6.92 (d, *J* = 6.3 Hz, 2H), 6.77 (d, *J* = 7.5 Hz, 1H), 6.47 (dd, *J* = 8.0, 2.0 Hz, 1H), 6.41 (t, *J* = 2.1 Hz, 1H), 6.30 – 6.24 (m, 1H), 5.59 (br. s, 1H), 3.61 (br. s, 2H), 2.63 – 2.49 (m, 2H), 1.71 – 1.49 (m, 2H), 1.35 (dd, *J* = 20.4, 4.4 Hz, 6H), 0.90 (t, *J* = 6.6 Hz, 3H). HRMS (ESI-TOF) calcd. for [C₁₈H₂₄N₂ + H]⁺: 269.20123, found 269.20159.

2-Chloro-*N*-(2-((3-hexylphenyl)amino)phenyl)acetamide (**16**) (HC-322)



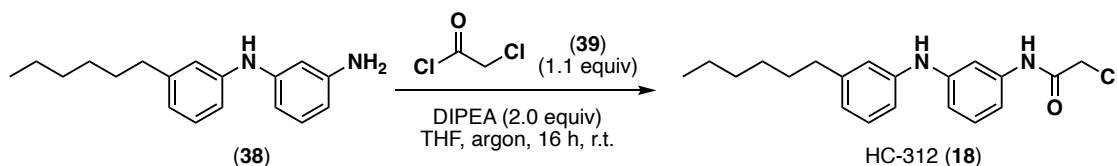
To a solution of *N*¹-(3-hexylphenyl)benzene-1,2-diamine (**37**) (70 mg, 0.26 mmol) and *N,N*-diisopropylethylamine (DIPEA) (90 μL, 0.52 mmol) in anhydrous THF (3 mL) was added a solution of chloroacetyl chloride (**39**) (23 μL, 0.29 mmol) in anhydrous THF (3 mL) at room temperature. The solution was stirred at room temperature for 16 hours under argon atmosphere. The solvent was evaporated under vacuum and the residue was purified by flash chromatography (100% CH₂Cl₂) to furnish 2-chloro-*N*-(2-((3-hexylphenyl)amino)phenyl)acetamide (HC-322: **16**) (51 mg, 57%) as a yellow oil. *R*_f = 0.29 (100% CH₂Cl₂); ¹H NMR (300 MHz, CDCl₃) δ 8.69 (s, 1H), 8.02 – 7.93 (m, 1H), 7.30 – 7.24 (m, 1H), 7.20 – 7.14 (m, 2H), 7.12 (d, *J* = 7.7 Hz, 1H), 6.73 (d, *J* = 7.6 Hz, 1H), 6.60 (d, *J* = 8.7 Hz, 2H), 5.50 (s, 1H), 4.12 (s, 2H), 2.52 (t, *J* = 7.8 Hz, 2H), 1.64 – 1.49 (m, 2H), 1.38 – 1.21 (m, 6H), 0.95 – 0.81 (m, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 164.4, 144.6, 144.4, 134.3, 131.0, 129.4, 126.3, 124.9, 124.0, 122.5, 121.1, 116.8, 113.9, 43.1, 36.1, 31.9, 31.5, 29.1, 22.8, 14.2. HRMS (ESI-TOF) calcd. for [C₂₀H₂₅ClN₂O + H]⁺: 345.17282, found 345.17332.

***N*-2-((3-Hexylphenyl)amino)phenylacrylamide (17) (HC-320)**



To a solution of *N*¹-(3-hexylphenyl)benzene-1,2-diamine (37) (70 mg, 0.26 mmol) and *N,N*-diisopropylethylamine (DIPEA) (91 μ L, 0.52 mmol) in anhydrous THF (3 mL) was added a solution of acryloyl chloride (40) (23 μ L, 0.29 mmol) in anhydrous THF (3 mL) at room temperature. The solution was stirred at room temperature for 16 hours under argon atmosphere. The solvent was evaporated under vacuum and the residue was purified by flash chromatography (100% CH₂Cl₂) to furnish *N*-2-((3-hexylphenyl)amino)phenylacrylamide (HC-320: 17) (47 mg, 56%) as a yellow oil. R_f = 0.28 (100% CH₂Cl₂); ¹H NMR (300 MHz, CDCl₃) δ 7.96 (d, J = 29.8 Hz, 2H), 7.35 – 7.21 (m, 1H), 7.16 – 7.06 (m, 3H), 6.72 (d, J = 7.5 Hz, 1H), 6.62 (d, J = 7.6 Hz, 2H), 6.32 (d, J = 16.4 Hz, 1H), 6.18 (dd, J = 16.9, 10.0 Hz, 1H), 5.69 (dd, J = 10.0, 1.5 Hz, 2H), 2.52 (t, J = 8.0, 7.5 Hz, 2H), 1.64 – 1.50 (m, 2H), 1.36 – 1.24 (m, 6H), 0.94 – 0.82 (m, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 164.1, 144.7, 144.6, 134.2, 131.9, 131.2, 129.3, 127.7, 125.7, 124.7, 123.8, 122.6, 120.8, 116.6, 113.8, 36.1, 31.8, 31.5, 29.1, 22.7, 14.2; HRMS (ESI-TOF) calcd. for [C₂₁H₂₆N₂O + H]⁺: 323.2118, found 323.2125.

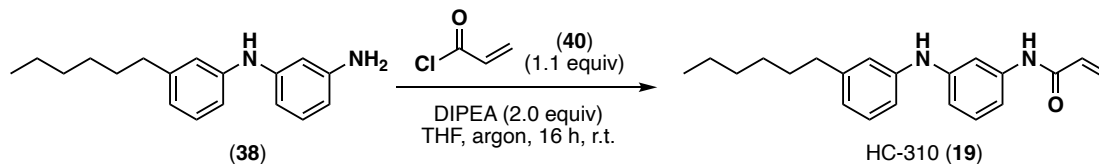
2-Chloro-*N*-3-((3-hexylphenyl)amino)phenylacetamide (18) (HC-312)



To a solution of *N*¹-(3-hexylphenyl)benzene-1,3-diamine (38) (70 mg, 0.26 mmol) and *N,N*-diisopropylethylamine (DIPEA) (90 μ L, 0.52 mmol) in anhydrous THF (3 mL) was added a solution of chloroacetyl chloride (39) (23 μ L, 0.29 mmol) in anhydrous THF (3 mL) at room temperature. The solution was stirred at room temperature for 16 hours under argon atmosphere. The solvent was evaporated under vacuum and the residue was purified by flash chromatography (100% CH₂Cl₂) to furnish 2-chloro-*N*-3-((3-hexylphenyl)amino)phenylacetamide (HC-312: 18) (80 mg, 89%) as a yellow oil. R_f = 0.43 (100% CH₂Cl₂); ¹H NMR (300 MHz, CDCl₃) δ 8.27 (s, 1H), 7.35 (d, J = 1.9 Hz, 1H), 7.25 – 7.15 (m, 2H), 7.05 – 6.86 (m, 4H), 6.82 (d, J = 7.5 Hz, 1H), 5.83 (s, 1H), 4.14 (s, 2H), 2.58 (t, J = 7.8 Hz, 2H), 1.70 – 1.56 (m, 2H), 1.43 – 1.26 (m, 6H), 0.99 – 0.86 (m, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 164.0, 144.6, 144.4, 142.3, 137.8, 129.9, 129.2,

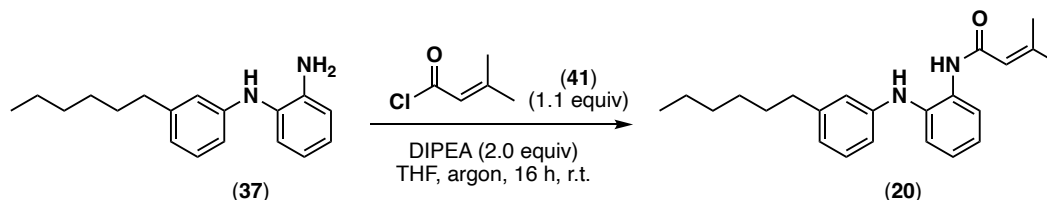
122.0, 119.0, 116.0, 113.5, 112.0, 108.8, 43.0, 36.0, 31.8, 31.4, 29.1, 22.7, 14.2; **HRMS (ESI-TOF)** calcd. for $[C_{20}H_{25}ClN_2O + H]^+$: 345.1728, found 345.1730.

***N*-(3-((3-Hexylphenyl)amino)phenyl)acrylamide (19) (HC-310)**



To a solution of *N*¹-(3-hexylphenyl)benzene-1,3-diamine (**38**) (70 mg, 0.26 mmol) and *N,N*-diisopropylethylamine (DIPEA) (90 μ L, 0.52 mmol) in anhydrous THF (3 mL) was added a solution of acryloyl chloride (**40**) (23 μ L, 0.29 mmol) in anhydrous THF (3 mL) at room temperature. The solution was stirred at room temperature for 16 hours under argon atmosphere. The solvent was evaporated under vacuum and the residue was purified by flash chromatography (1% to 30% EtOAc/Hexanes) to furnish *N*-(3-((3-hexylphenyl)amino)phenyl)acrylamide (HC-310: **19**) (84 mg, 99%) as a yellow oil. $R_f = 0.35$ (30% EtOAc/Hexanes); $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.99 (s, 1H), 7.41 (s, 1H), 7.19 (t, $J = 7.8$ Hz, 2H), 7.08 (d, $J = 7.6$ Hz, 1H), 6.97 – 6.85 (m, 3H), 6.80 (d, $J = 7.5$ Hz, 1H), 6.47 – 6.39 (m, 1H), 6.22 (dd, $J = 16.9, 10.2$ Hz, 1H), 5.73 (dd, $J = 9.9, 1.4$ Hz, 2H), 2.57 (t, $J = 7.6$ Hz, 2H), 1.68 – 1.52 (m, 2H), 1.40 – 1.23 (m, 6H), 0.93 (t, $J = 6.5$ Hz, 3H); $^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ 163.9, 144.4, 142.5, 138.9, 131.4, 129.8, 129.2, 127.8, 121.8, 118.9, 116.0, 113.0, 112.1, 109.0, 60.6, 36.1, 31.8, 31.5, 29.1, 22.7, 14.2; **HRMS (ESI-TOF)** calcd. for $[C_{21}H_{26}N_2O + H]^+$: 323.2118, found 323.2122.

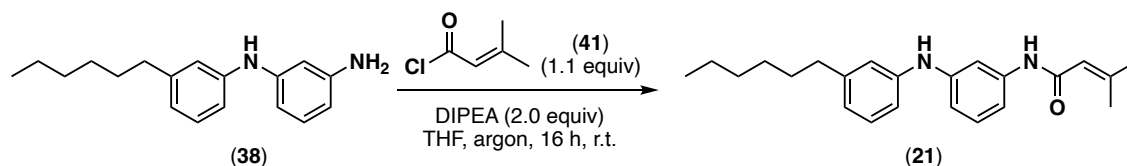
***N*-(2-((3-Hexylphenyl)amino)phenyl)-3-methylbut-2-enamide (20)**



To a solution of *N*¹-(3-hexylphenyl)benzene-1,2-diamine (**37**) (70 mg, 0.26 mmol) and *N,N*-diisopropylethylamine (DIPEA) (91 μ L, 0.52 mmol) in anhydrous THF (3 mL) was added a solution of 3,3-dimethylacryloyl chloride (**41**) (32 μ L, 0.29 mmol) in anhydrous THF (3 mL) at room temperature. The solution was stirred at room for 16 hours under argon atmosphere. The solvent was evaporated under vacuum and the residue was purified by flash chromatography (100% CH_2Cl_2) to furnish *N*-(2-((3-hexylphenyl)amino)phenyl)-3-methylbut-2-enamide (**20**) (65 mg, 71%) as a yellow oil. $R_f = 0.38$ (100% CH_2Cl_2); $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.95 (s, 1H), 7.65

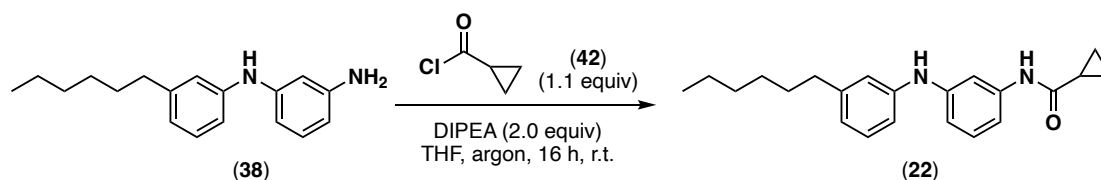
(s, 1H), 7.30 – 7.20 (m, 1H), 7.14 (d, $J = 7.5$ Hz, 1H), 7.11 – 7.04 (m, 2H), 6.72 (d, $J = 7.6$ Hz, 1H), 6.64 (d, $J = 7.3$ Hz, 2H), 5.74 – 5.63 (m, 2H), 2.53 (t, $J = 7.8$ Hz, 2H), 2.20 (d, $J = 1.1$ Hz, 3H), 1.85 (s, 3H), 1.65 – 1.50 (m, 2H), 1.39 – 1.24 (m, 6H), 0.97 – 0.83 (m, 3H); ^{13}C NMR (75 MHz, CDCl_3) δ 165.6, 153.4, 144.9, 144.5, 134.2, 132.3, 129.2, 125.2, 124.5, 123.6, 122.5, 120.5, 118.7, 116.6, 113.8, 36.1, 31.8, 31.5, 29.1, 27.4, 22.7, 20.1, 14.2. HRMS (ESI-TOF) calcd. for $[\text{C}_{23}\text{H}_{30}\text{N}_2\text{O} + \text{H}]^+$: 351.24309, found 351.24401.

N-(3-((3-Hexylphenyl)amino)phenyl)-3-methylbut-2-enamide (21)



To a solution of *N*¹-(3-hexylphenyl)benzene-1,3-diamine (**38**) (70 mg, 0.26 mmol) and *N,N*-diisopropylethylamine (DIPEA) (91 μL , 0.52 mmol) in anhydrous THF (3 mL) was added a solution of 3,3-dimethylacryloyl chloride (**41**) (32 μL , 0.29 mmol) in anhydrous THF (3 mL) at room temperature. The solution was stirred at room for 16 hours under argon atmosphere. The solvent was evaporated under vacuum. The residue was purified by flash chromatography (1% to 30% EtOAc/Hexanes) to furnish *N*-(3-((3-hexylphenyl)amino)phenyl)-3-methylbut-2-enamide (**21**) (79 mg, 87%) as a yellow oil. $R_f = 0.49$ (30% EtOAc/Hexanes); ^1H NMR (300 MHz, CDCl_3) δ 7.54 (s, 1H), 7.35 (s, 1H), 7.20 – 7.10 (m, 2H), 7.02 (d, $J = 7.6$ Hz, 1H), 6.93 – 6.86 (m, 2H), 6.85 – 6.80 (m, 2H), 6.77 (d, $J = 7.5$ Hz, 1H), 5.88 – 5.62 (m, 2H), 2.55 (t, $J = 7.5$ Hz, 2H), 2.21 (s, 3H), 1.86 (s, 3H), 1.67 – 1.54 (m, 2H), 1.41 – 1.28 (m, 6H), 1.00 – 0.84 (m, 2H); ^{13}C NMR (75 MHz, CDCl_3) δ 165.3, 153.1, 144.3, 144.2, 142.7, 139.4, 129.6, 129.1, 121.5, 118.9, 118.6, 115.7, 112.5, 112.0, 109.0, 36.1, 31.8, 31.4, 29.1, 27.4, 22.7, 20.0, 14.2. HRMS (ESI-TOF) calcd. for $[\text{C}_{23}\text{H}_{30}\text{N}_2\text{O} + \text{H}]^+$: 351.24309, found 351.24410.

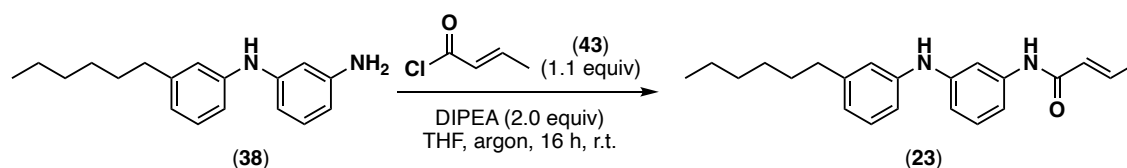
N-(3-((3-Hexylphenyl)amino)phenyl)cyclopropanecarboxamide (22)



To a solution of *N*¹-(3-hexylphenyl)benzene-1,3-diamine (**38**) (70 mg, 0.26 mmol) and *N,N*-diisopropylethylamine (DIPEA) (90 μL , 0.52 mmol) in anhydrous THF (3 mL) was added a solution of cyclopropanecarbonyl chloride (**42**) (26 μL , 0.29 mmol) in anhydrous THF (3 mL) at

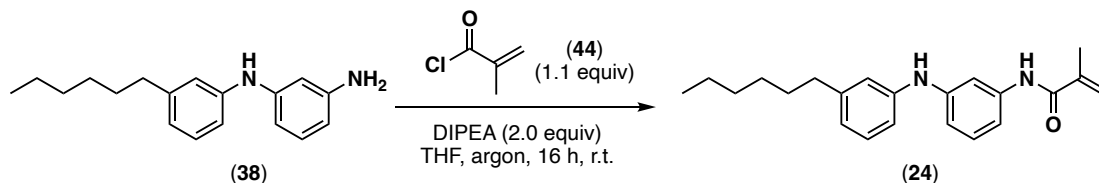
room temperature. The solution was stirred at room for 16 hours under argon atmosphere. The solvent was evaporated under vacuum and the residue was purified by flash chromatography (1% to 30% EtOAc/Hexanes) to furnish *N*-(3-((3-hexylphenyl)amino)phenyl)cyclopropanecarboxamide (**22**) (58 mg, 66%) as a yellow oil. $R_f = 0.58$ (30% EtOAc/Hexanes); $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.86 (s, 1H), 7.30 (s, 1H), 7.21 – 7.09 (m, 2H), 6.98 (d, $J = 7.7$ Hz, 1H), 6.90 (d, $J = 7.9$ Hz, 1H), 6.87 (s, 1H), 6.82 (d, $J = 9.1$ Hz, 1H), 6.77 (d, $J = 7.6$ Hz, 1H), 5.89 (br. s, 1H), 2.55 (t, $J = 7.6$ Hz, 2H), 1.68 – 1.55 (m, 2H), 1.55 – 1.45 (m, 1H), 1.41 – 1.28 (m, 6H), 1.11 – 1.02 (m, 2H), 0.95 – 0.86 (m, 3H), 0.84 – 0.75 (m, 2H); $^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ 172.3, 144.4, 142.6, 139.3, 129.7, 129.1, 121.7, 118.8, 115.8, 112.5, 111.9, 108.9, 36.1, 31.8, 31.5, 29.1, 22.7, 15.8, 14.2, 9.0, 8.0; **HRMS (ESI-TOF)** calcd. for $[\text{C}_{22}\text{H}_{28}\text{N}_2\text{O} + \text{H}]^+$: 337.2274, found 337.2278.

(*E*)-*N*-(3-((3-Hexylphenyl)amino)phenyl)but-2-enamide (23**)**



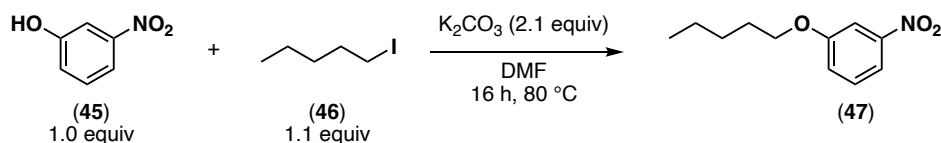
To a solution of *N*¹-(3-hexylphenyl)benzene-1,3-diamine (**38**) (70 mg, 0.26 mmol) and *N,N*-diisopropylethylamine (DIPEA) (91 μL , 0.52 mmol) in anhydrous THF (3 mL) was added a solution of crotonoyl chloride (**43**) (27 μL , 0.29 mmol) in anhydrous THF (3 mL) at room temperature. The solution was stirred at room for 16 hours under argon atmosphere. The solvent was evaporated under vacuum and the residue was purified by flash chromatography (1% to 30% EtOAc/Hexanes) to furnish (*E*)-*N*-(3-((3-hexylphenyl)amino)phenyl)but-2-enamide (**23**) (19 mg, 22%) as a yellow oil. $R_f = 0.41$ (30% EtOAc/Hexanes); $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.36 (br. s, 2H), 7.16 (t, $J = 8.1$ Hz, 2H), 7.04 – 6.86 (m, 4H), 6.83 (dd, $J = 7.9, 1.4$ Hz, 1H), 6.77 (d, $J = 7.5$ Hz, 1H), 6.00 – 5.89 (m, 1H), 5.75 (br. s, 1H), 2.55 (t, $J = 7.6$ Hz, 2H), 1.88 (dd, $J = 6.9, 1.5$ Hz, 3H), 1.66 – 1.53 (m, 2H), 1.40 – 1.23 (m, 6H), 0.94 – 0.83 (m, 3H); $^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ 144.5, 142.6, 141.7, 139.2, 132.3, 129.8, 129.2, 125.7, 121.8, 118.9, 115.9, 112.8, 77.2, 36.1, 31.9, 31.5, 29.2, 22.7, 18.0, 14.2. **HRMS (ESI-TOF)** calcd. for $[\text{C}_{22}\text{H}_{28}\text{N}_2\text{O} + \text{H}]^+$: 337.22744, found 337.22827.

***N*-3-((3-hexylphenyl)amino)phenylmethacrylamide (24)**



To a solution of *N*-(3-hexylphenyl)benzene-1,3-diamine (**38**) (70 mg, 0.26 mmol) and *N,N*-diisopropylethylamine (DIPEA) (91 μ L, 0.52 mmol) in anhydrous THF (3 mL) was added a solution of methacryloyl chloride (**44**) (27 μ L, 0.29 mmol) in anhydrous THF (3 mL) at room temperature. The solution was stirred at room for 16 hours under argon atmosphere. The solvent was evaporated under vacuum and the residue was purified by flash chromatography (100% CH_2Cl_2) to furnish *N*-3-((3-hexylphenyl)amino)phenylmethacrylamide (**24**) (67 mg, 76%) as a yellow oil. $R_f = 0.19$ (100% CH_2Cl_2); $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.66 (s, 1H), 7.39 (t, $J = 2.0$ Hz, 1H), 7.18 (t, $J = 7.9$ Hz, 2H), 7.06 – 6.99 (m, 1H), 6.96 – 6.88 (m, 2H), 6.88 – 6.82 (m, 1H), 6.78 (d, $J = 7.5$ Hz, 1H), 5.84 – 5.73 (m, 2H), 5.43 (s, 1H), 2.57 (t, $J = 7.7$ Hz, 2H), 2.04 (s, 3H), 1.68 – 1.55 (m, 2H), 1.42 – 1.25 (m, 6H), 0.97 – 0.85 (m, 3H); $^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ 166.8, 144.4, 144.4, 142.5, 141.0, 138.9, 129.7, 129.2, 121.7, 119.8, 118.8, 115.9, 113.0, 112.1, 109.0, 36.1, 31.8, 31.5, 29.1, 22.7, 18.8, 14.2. **HRMS (ESI-TOF)** calcd. for $[\text{C}_{22}\text{H}_{28}\text{N}_2\text{O} + \text{H}]^+$: 337.22744, found 337.22863.

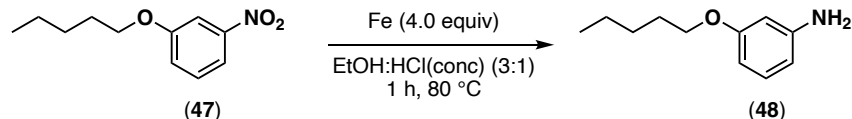
1-Nitro-3-(pentyloxy)benzene (47)



To a solution of 3-nitrophenol (**45**) (840 mg, 6.04 mmol) in DMF (3.5 mL) was added potassium carbonate (1.75 g, 12.7 mmol). The reaction mixture was stirred at 80 $^\circ\text{C}$ for 1 hour, whereupon 1-iodopentane (**46**) (867 μ L, 6.64 mmol) was added. The reaction was stirred at 80 $^\circ\text{C}$ for 16 hours, cooled down to room temperature and diluted with EtOAc (50 mL) and water (50 mL). The phases were separated and the aqueous phase was extracted with EtOAc (3 x 20 mL). The combined organic phases were dried over Na_2SO_4 , filtered, and concentrated under reduced pressure. Purification by flash column chromatography (1% to 20% EtOAc/Hexanes) provided the desired compound 1-nitro-3-(pentyloxy)benzene (**47**) (1.10 g, 87 %) as a yellow oil. This compound has been previously reported.¹ $R_f = 0.59$ (20% EtOAc/Hexanes); $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.83

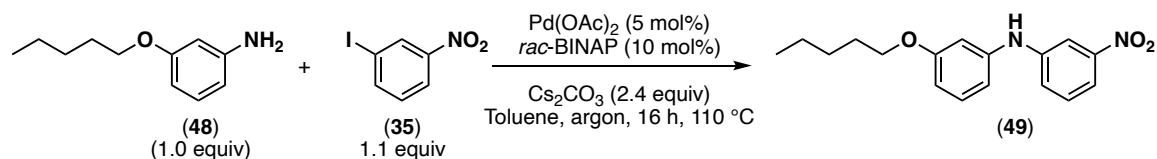
– 7.77 (m, 1H), 7.71 (s, 1H), 7.41 (t, $J = 8.2$ Hz, 1H), 7.21 (dd, $J = 8.3, 2.5$ Hz, 1H), 4.03 (t, $J = 6.5$ Hz, 2H), 1.82 (q, 2H), 1.52 – 1.33 (m, 4H), 0.94 (t, $J = 7.0$ Hz, 3H).

3-(Pentyloxy)aniline (48)



Metallic iron (1.17 g, 21.0 mmol) was added to a solution of 1-nitro-3-(pentyloxy)benzene (**47**) (1.10 g, 5.26 mmol) in 20 mL 3:1 EtOH/HCl(conc). After heating at 80 °C for 1 hour, the reaction mixture was cooled down to room temperature and quenched with a slow addition of saturated aqueous solution of NaHCO₃ (50 mL). The aqueous phase was extracted with EtOAc (3 x 50 mL). The combined organic phases were washed with water (1 x 50 mL), brine (1 x 50 mL), dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The crude material was purified by flash column chromatography (1% to 20% EtOAc/Hexanes) to provide 3-(pentyloxy)aniline (**48**) (801 mg, 85%) as a dark brown oil. $R_f = 0.29$ (20% EtOAc/Hexanes); ¹H NMR (300 MHz, CDCl₃) δ 7.05 (t, $J = 8.0$ Hz, 1H), 6.35 – 6.20 (m, 3H), 3.92 (t, $J = 6.6$ Hz, 2H), 3.68 (s, 2H), 1.77 (p, $J = 6.7$ Hz, 2H), 1.52 – 1.33 (m, 4H), 0.94 (t, $J = 7.0$ Hz, 3H). HRMS (ESI-TOF) calcd. for [C₁₁H₁₇NO + H]⁺: 180.1383, found 180.1385.

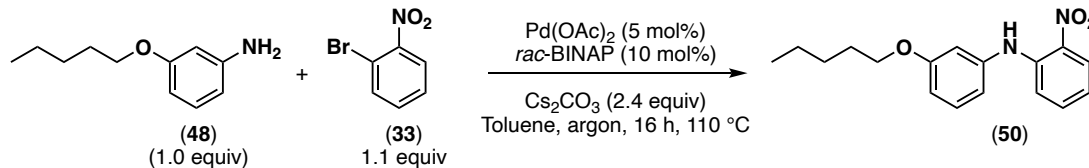
3-Nitro-*N*-(3-(pentyloxy)phenyl)aniline (49)



To a solution of 3-(pentyloxy)aniline (**48**) (600 mg, 3.35 mmol) in dry toluene (7 mL) was added the 1-iodo-3-nitrobenzene (**35**) (917 mg, 3.68 mmol), cesium carbonate (2.62 g, 8.04 mmol) and a freshly prepared solution of Pd(OAc)₂/*rac*-BINAP in dry toluene. This solution was obtained by stirring palladium(II) acetate (38 mg, 0.17 mmol) and (*rac*)-BINAP (208 mg, 0.33 mmol) in dry toluene (3 mL) for 15 minutes with argon bubbling through the mixture. The main reaction mixture was heated at 110 °C for 16 hours, cooled down to room temperature, filtered over a plug of celite and concentrated under reduced pressure. Purification by flash column chromatography (1% to 5% EtOAc/Hexanes) provided 3-nitro-*N*-(3-(pentyloxy)phenyl)aniline (**49**) (1.0 g, 99%) as a red oil. $R_f = 0.21$ (10% EtOAc/Hexanes); ¹H NMR (300 MHz, CDCl₃) δ 7.86 (t, $J = 2.2$ Hz, 1H), 7.70 (ddd, $J = 7.9, 2.1, 1.2$ Hz, 1H), 7.37 (t, $J = 8.0$ Hz, 1H), 7.30 (ddd, $J = 8.1, 2.2, 1.2$ Hz, 1H), 7.22 (d, $J = 8.0$ Hz, 1H), 6.74 – 6.68 (m, 1H), 6.67 (t, $J = 2.2$ Hz, 1H), 6.61 (dd, $J = 8.3, 1.8$ Hz, 1H),

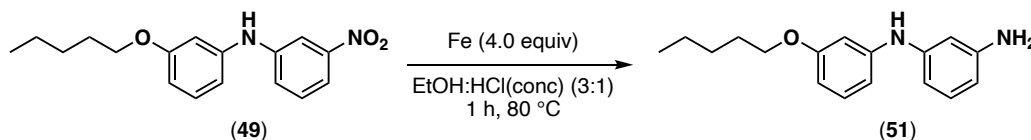
5.92 (s, 1H), 3.94 (t, $J = 6.6$ Hz, 2H), 1.87 – 1.69 (m, 2H), 1.50 – 1.33 (m, 4H), 0.93 (t, $J = 7.0$ Hz, 3H). **HRMS (ESI-TOF)** calcd. for $[C_{17}H_{20}N_2O_3 + H]^+$: 301.15467, found 301.15424.

2-Nitro-*N*-(3-(pentyloxy)phenyl)aniline (**50**)



To a solution of 3-(pentyloxy)aniline (**48**) (600 mg, 3.35 mmol) in dry toluene (7 mL) was added the 1-bromo-2-nitrobenzene (**33**) (744 mg, 3.68 mmol), cesium carbonate (2.62 g, 8.04 mmol) and a freshly prepared solution of Pd(OAc)₂/*rac*-BINAP in dry toluene. This solution was obtained by stirring palladium(II) acetate (38 mg, 0.17 mmol) and (*rac*)-BINAP (208 mg, 0.33 mmol) in dry toluene (3 mL) for 15 minutes with argon bubbling through the mixture. The main reaction mixture was heated at 110 °C for 16 hours, cooled down to room temperature, filtered over a plug of celite and concentrated under reduced pressure. Purification by flash column chromatography (1% to 5% EtOAc/Hexanes) provided 2-nitro-*N*-(3-(pentyloxy)phenyl)aniline (**50**) (987 mg, 98%) as an orange oil. $R_f = 0.54$ (10% EtOAc/Hexanes); ¹H NMR (300 MHz, CDCl₃) δ 9.47 (s, 1H), 8.18 (dd, $J = 8.5, 1.4$ Hz, 1H), 7.37 (d, $J = 6.9$ Hz, 1H), 7.30 (ddd, $J = 8.1, 4.4, 1.9$ Hz, 2H), 6.89 – 6.72 (m, 4H), 3.97 (t, $J = 6.5$ Hz, 2H), 1.87 – 1.75 (m, 2H), 1.55 – 1.35 (m, 4H), 0.97 (t, $J = 7.1$ Hz, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 160.3, 142.8, 139.8, 135.6, 133.2, 130.3, 126.5, 117.5, 116.3, 116.0, 111.6, 110.4, 68.1, 28.9, 28.2, 22.4, 14.0. **HRMS (ESI-TOF)** calcd. for $[C_{17}H_{20}N_2O_3 + H]^+$: 301.15467, found 301.15452.

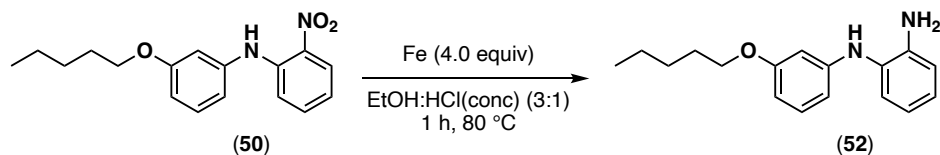
*N*¹-(3-(Pentyloxy)phenyl)benzene-1,3-diamine (**51**)



Metallic iron (231 mg, 4.1 mmol) was added to a solution of 3-nitro-*N*-(3-(pentyloxy)phenyl)aniline (**49**) (311 mg, 1.03 mmol) in 4 mL of 3:1 EtOH/HCl(conc). After heating at 80 °C for 1 hour, the reaction mixture was cooled down to room temperature and quenched with a slow addition of saturated aqueous solution of NaHCO₃ (30 mL). The aqueous phase was extracted with EtOAc (3 x 30 mL). The combined organic phases were washed with water (1 x 30 mL), brine (1 x 30 mL), dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The crude material was purified by flash column chromatography (100% CH₂Cl₂) to provide *N*¹-(3-

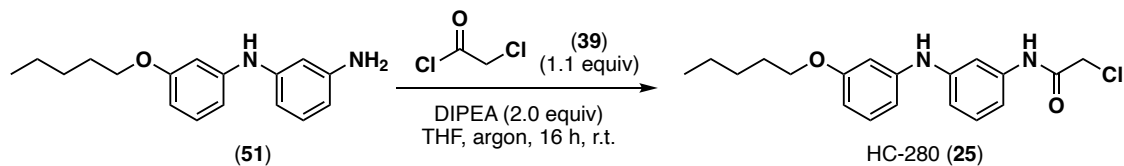
(pentyloxy)phenyl)benzene-1,3-diamine (**51**) (137 mg, 49%) as a dark oil. $R_f = 0.28$ (100% CH_2Cl_2); $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.16 (t, $J = 8.4$ Hz, 1H), 7.06 (t, $J = 7.9$ Hz, 1H), 6.69 – 6.62 (m, 2H), 6.53 – 6.46 (m, 2H), 6.42 (t, $J = 2.0$ Hz, 1H), 6.28 (dd, $J = 7.9, 1.5$ Hz, 1H), 3.94 (t, $J = 6.6$ Hz, 2H), 3.55 (s, 2H), 1.84 – 1.73 (m, 2H), 1.51 – 1.33 (m, 4H), 0.96 (t, $J = 7.0$ Hz, 3H). **HRMS** (ESI-TOF) calcd. for $[\text{C}_{17}\text{H}_{22}\text{N}_2\text{O} + \text{H}]^+$: 271.18049, found 271.18139.

N^1 -(3-(Pentyloxy)phenyl)benzene-1,2-diamine (**52**)



Metallic iron (165 mg, 2.95 mmol) was added to a solution of 2-nitro- N -(3-(pentyloxy)phenyl)aniline (**50**) (221 mg, 0.74 mmol) in 4 mL 3:1 EtOH/HCl(conc). After heating at 80 °C for 1 hour, the reaction mixture was cooled down to room temperature and quenched with a slow addition of saturated aqueous solution of NaHCO_3 (30 mL). The aqueous phase was extracted with EtOAc (3 x 30 mL). The combined organic phases were washed with water (1 x 30 mL), brine (1 x 30 mL), dried over Na_2SO_4 , filtered, and concentrated under reduced pressure. The crude material was purified by flash column chromatography (100% CH_2Cl_2) to provide N^1 -(3-(pentyloxy)phenyl)benzene-1,2-diamine (**52**) (175 mg, 88%) as a dark red oil. $R_f = 0.42$ (100% CH_2Cl_2); $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.16 – 7.08 (m, 2H), 7.04 (t, $J = 7.2$ Hz, 1H), 6.78 (dd, $J = 13.4, 7.1$ Hz, 2H), 6.40 (dd, $J = 8.2, 1.4$ Hz, 1H), 6.37 – 6.28 (m, 2H), 5.18 (s, 1H), 3.91 (t, $J = 6.6$ Hz, 2H), 3.78 (s, 2H), 1.82 – 1.70 (m, 2H), 1.52 – 1.33 (m, 4H), 0.94 (t, $J = 6.9$ Hz, 3H). **HRMS** (ESI-TOF) calcd. for $[\text{C}_{17}\text{H}_{22}\text{N}_2\text{O} + \text{H}]^+$: 271.18049, found 271.18069.

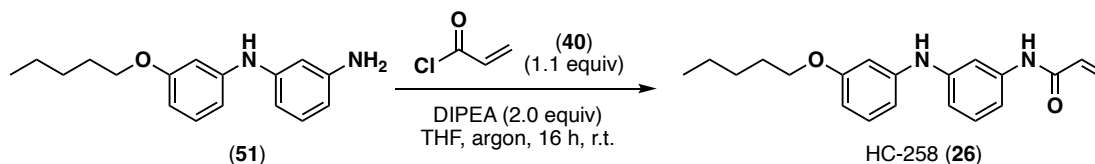
2-Chloro- N -(3-((3-(pentyloxy)phenyl)amino)phenyl)acetamide (**25**) (HC-280)



To a solution of N^1 -(3-(pentyloxy)phenyl)benzene-1,3-diamine (**51**) (60 mg, 0.22 mmol) and N,N -diisopropylethylamine (DIPEA) (77 μL , 0.44 mmol) in anhydrous THF (3 mL) was added a solution of chloroacetyl chloride (**39**) (19 μL , 0.24 mmol) in anhydrous THF (3 mL) at room temperature. The solution was stirred at room for 16 hours under argon atmosphere. The solvent was evaporated under vacuum and the residue was purified by flash chromatography (1% to 30% EtOAc/Hexanes) to furnish 2-chloro- N -(3-((3-(pentyloxy)phenyl)amino)phenyl)acetamide (HC-

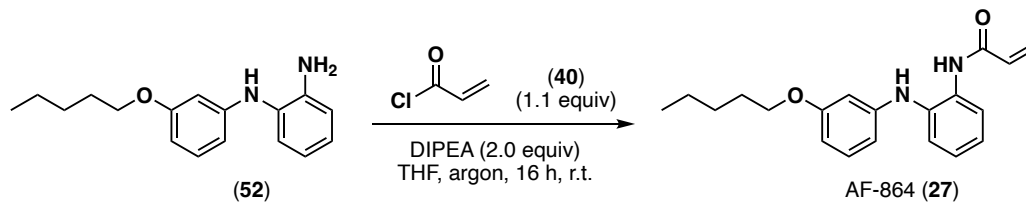
280: **25**) (73 mg, 96%) as a yellow oil. $R_f = 0.38$ (30% EtOAc/Hexanes); $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 8.19 (s, 1H), 7.37 (t, $J = 2.0$ Hz, 1H), 7.19 (dt, $J = 14.4, 8.2$ Hz, 2H), 6.99 (dd, $J = 7.9, 1.2$ Hz, 1H), 6.90 (dd, $J = 8.1, 1.6$ Hz, 1H), 6.68 – 6.62 (dd, $J = 4.8, 2.5$ Hz, 2H), 6.51 (dd, $J = 7.5, 1.7$ Hz, 1H), 5.81 (s, 1H), 4.16 (s, 2H), 3.93 (t, $J = 6.6$ Hz, 2H), 1.87 – 1.67 (m, 2H), 1.53 – 1.30 (m, 4H), 0.93 (t, $J = 7.0$ Hz, 3H); $^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ 163.9, 160.4, 144.2, 143.9, 137.8, 130.2, 130.0, 114.3, 112.4, 110.9, 109.4, 107.7, 104.8, 68.1, 43.0, 29.1, 28.3, 22.6, 14.1; **HRMS** (ESI-TOF) calcd. for $[\text{C}_{19}\text{H}_{23}\text{ClN}_2\text{O}_2 + \text{H}]^+$: 347.1521, found 347.1519.

N-(3-((3-(Pentyloxy)phenyl)amino)phenyl)acrylamide (**26**) (HC-258)



To a solution of *N*¹-(3-(pentyloxy)phenyl)benzene-1,3-diamine (**51**) (64 mg, 0.24 mmol) and *N,N*-diisopropylethylamine (DIPEA) (84 μL , 0.48 mmol) in anhydrous THF (2 mL) was added a solution of acryloyl chloride (**40**) (21 μL , 0.26 mmol) in anhydrous THF (2 mL) at room temperature. The solution was stirred at room for 16 hours under argon atmosphere. The solvent was evaporated under vacuum and the residue was purified by flash chromatography (1% to 30% EtOAc/Hexanes) to furnish *N*-(3-((3-(pentyloxy)phenyl)amino)phenyl)acrylamide (HC-258: **26**) (78 mg, 73%) as a yellow oil. $R_f = 0.29$ (30% EtOAc/Hexanes) $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.88 (s, 1H), 7.62 (s, 1H), 7.20 – 7.11 (m, 2H), 7.05 (d, $J = 7.5$ Hz, 1H), 6.85 (d, $J = 7.9$ Hz, 1H), 6.64 – 6.60 (m, 2H), 6.50–6.44 (m, 1H), 6.43 – 6.38 (m, 1H), 6.29 – 6.20 (m, 1H), 5.73 (dd, $J = 10.0, 1.1$ Hz, 1H), 3.92 (t, $J = 6.6$ Hz, 2H), 2.05 (s, 1H), 1.83 – 1.68 (m, 2H), 1.50 – 1.28 (m, 4H), 0.92 (t, $J = 6.9$ Hz, 3H). $^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ 163.9, 160.3, 144.0, 144.0, 138.9, 131.3, 130.1, 129.8, 127.9, 113.7, 112.5 110.8, 109.5, 107.4, 104.7, 68.1, 29.1, 28.3, 22.6, 14.1; **HRMS** (ESI-TOF) calcd. for $[\text{C}_{20}\text{H}_{24}\text{N}_2\text{O}_2 + \text{H}]^+$: 325.1916, found 325.1911.

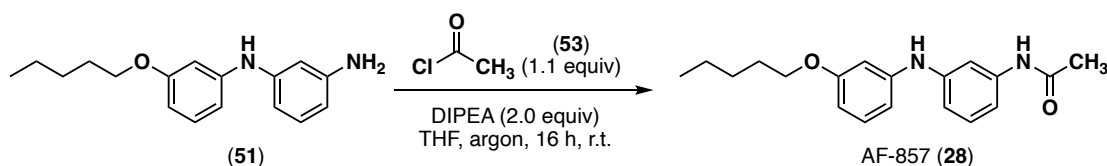
N-(2-((3-(Pentyloxy)phenyl)amino)phenyl)acrylamide (**27**) (AF-864)



To a solution of *N*¹-(3-(pentyloxy)phenyl)benzene-1,2-diamine (**52**) (80 mg, 0.30 mmol) and *N,N*-diisopropylethylamine (DIPEA) (105 μL , 0.60 mmol) in anhydrous THF (2 mL) was added a

solution of acryloyl chloride (**40**) (26 μ L, 0.33 mmol) in anhydrous THF (2 mL) at room temperature. The solution was stirred at room for 16 hours under argon atmosphere. The solvent was evaporated under vacuum and the residue was purified by flash chromatography (1% to 30% EtOAc/Hexanes) to furnish *N*-(3-((3-(pentyloxy)phenyl)amino)phenyl)acetamide (AF-864: **27**) (72 mg, 74%) as a yellow oil. R_f = 0.35 (30% EtOAc/Hexanes); $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 8.07 (d, J = 6.6 Hz, 1H), 7.81 (br. s, 1H), 7.32 – 7.27 (m, 1H), 7.21 – 7.03 (m, 3H), 6.45 – 6.25 (m, 4H), 6.24 – 6.12 (m, 1H), 5.71 (d, J = 10.5 Hz, 1H), 5.54 (br. s, 1H), 3.88 (t, J = 6.5 Hz, 2H), 1.81 – 1.68 (m, 2H), 1.46 – 1.28 (m, 4H), 0.91 (t, J = 6.8 Hz, 3H); **HRMS (ESI-TOF)** calcd. for $[\text{C}_{20}\text{H}_{24}\text{N}_2\text{O}_2 + \text{H}]^+$: 325.1916, found 325.1912.

N-(3-((3-(Pentyloxy)phenyl)amino)phenyl)acetamide (**28**) (AF-857)



To a solution of *N*¹-(3-(pentyloxy)phenyl)benzene-1,3-diamine (**51**) (16 mg, 0.06 mmol) and *N,N*-diisopropylethylamine (DIPEA) (21 μ L, 0.12 mmol) in anhydrous THF (3 mL) was added a solution of acetyl chloride (**53**) (5 μ L, 0.07 mmol) in anhydrous THF (3 mL) at room temperature. The solution was stirred at room for 16 hours under argon atmosphere. The solvent was evaporated under vacuum and the residue was purified by flash chromatography (1% to 20% EtOAc/Hexanes) to furnish *N*-(3-((3-(pentyloxy)phenyl)amino)phenyl)acetamide (AF-857: **28**) (11 mg, 59%) as a yellow oil. R_f = 0.50 (20% EtOAc/Hexanes); $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.92 – 7.84 (m, 1H), 7.64 (br. s, 1H), 7.31 – 7.27 (m, 1H), 7.18 – 7.05 (m, 3H), 6.40 (dd, J = 16.8, 8.0 Hz, 2H), 6.33 (s, 1H), 5.65 (br. s, 1H), 3.88 (t, J = 6.6 Hz, 2H), 2.13 (s, 3H), 1.73 (dd, J = 13.9, 6.8 Hz, 2H), 1.47 – 1.30 (m, 4H), 0.92 (t, J = 7.0 Hz, 3H); **HRMS (ESI-TOF)** calcd. for $[\text{C}_{19}\text{H}_{24}\text{N}_2\text{O}_2 + \text{H}]^+$: 313.1911, found 313.1916.

3. Protein expression and purification for crystallization studies

The cDNA coding the human TEAD2 sequence (residue 217 to 447) was ordered to Integrated DNA Technologies. This target protein was cloned in frame into a pDBHis-MBP between *Nde*I and *Xho*I sites to give the pDBHis-MBP-TEAD2 plasmid. In this construct TEAD2 is fused with a (his)₆-MBP N-terminal tag. This N-terminal tag is followed by the HRV 3C (3C) protease recognition site (Leu-Glu-Val-Leu-Phe-Gln/Gly-Pro). Specific cleavage occurs between Gln and Gly, with Gly-Pro remaining at TEAD2 protein N terminus. The pDBHis-MBP-TEAD2 plasmid

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was transformed in *Escherichia coli* BL21λDE3 cells and grown on LB agar plate with 50 μg/mL kanamycin at 37°C overnight. Transformed cells were grown at 37°C in LB media, supplemented with 50 μg/mL kanamycin, until 0.6 OD₂₈₀, then the culture temperature was decreased to 16°C and production was induced with 0.2 mM of IPTG overnight. Cells were harvested by 20 min centrifugation at 6000 x g at 4 °C. The pellet was resuspended in 20 mM Tris-HCl pH 7.5, 600 mM NaCl and 2 mM 2-mercaptoethanol (buffer A) and stored at -80 °C. Cells were supplemented with a Complete® EDTA free tablet (Roche, Meylan, France) lysed by sonication, insoluble proteins and cell debris were sedimented by centrifugation at 40.000 x g at 4 °C for 30 min. Supernatant was supplemented with imidazole to a 10 mM final concentration, filtered through 0.45 μm filters and loaded onto an affinity column (1 mL His Trap FF, GE Lifescience), equilibrated with buffer B (buffer A + 10 mM imidazole). Columns were washed with 20 column volumes of buffer B and proteins eluted with a linear 0–100% gradient of buffer C (buffer A + 300 mM imidazole). The peak fractions were analyzed by SDS-PAGE. Fractions containing tagged TEAD2-MBP were pooled. These fractions were dialyzed overnight at 4 °C against a conservation buffer containing 20 mM Tris pH 7.5, 600 mM NaCl, 2 mM DTT (Buffer D) and at the same time the (his)6-MBP tag was separated from TEAD2 by 3C cleavage (ratio protein/protease 1000/1 w/w). Uncleaved protein, (his)6-MBP tag and 3C protease were removed by binding onto gravity column containing 2.5 mL Ni-sepharose and 0.5 mL Glutathione sepharose (3C protease being GST-Tagged). Purified TEAD2 did not bind this column and was directly collected in the flow through. Purified protein was analyzed by SDS-PAGE, concentrated and injected onto gel filtration column (Superdex 75 Hiload 16/60 GE Lifescience) equilibrated with buffer E (20 mM Tris pH 7.5, 100 mM NaCl, 2 mM MgCl₂, 2 mM TCEP and 5% glycerol). The peak fractions were analyzed by SDS-PAGE and the fractions containing pure TEAD2 were pooled.

4. Crystallization and structure determination

Crystals of hTEAD2₂₁₇₋₄₄₇ were grown at 20 °C using the hanging-drop vapor-diffusion method with a reservoir solution containing 0.1 M HEPES (pH 7.2) and 2.8 M sodium formate. The crystals were cryo-protected with reservoir solution supplemented with 25% glycerol and then flash-cooled in liquid nitrogen. X-ray diffraction data were collected at the ESRF Synchrotron in Grenoble, France, on beamline ID30A-1 (DOI10.15151/ESRF-ES-1171220113).² Data were integrated and

² Bowler, M. W.; Nurizzo, D.; Barrett, R.; Beteva, A.; Bodin, M.; Caserotto, H.; Delageniere, S.; Dobias, F.; Flot, D.; Giraud, T.; Guichard, N.; Guijarro, M.; Lentini, M.; Leonard, G.; McSweeney, S.; Oskarsson, M.; Schmidt, W.; Snigirev, A.; von Stetten, D.; Surr, J.; Svensson, O.; Theveneau, P.; Mueller-Dieckmann, C. MASSIF-1: A beamline dedicated to the fully

Fnaiche, A.; Chan, H.-C. *et al.* Development of HC-258, a covalent acrylamide TEAD inhibitor that reduces gene expression and cell migration.

processed using XDS.³ The crystals belong to the space group C2 with two monomers in the asymmetric unit. The structures were solved by molecular replacement using PDB entry 6S60 as the search model. Bound ligands were manually identified and fitted into Fo–Fc electron density using Coot.⁴ Files CIF format for ligand were generated using Grade Server (<http://grade.globalphasing.org/cgi-bin/grade/server.cgi>). The structures were refined by rounds of rebuilding in Coot and refinement using Refmac from the CCP4 suite.⁵ Data collection and refinement statistics for crystal structures are presented in Table S2.

automatic characterisation and data collection from crystals of biological macromolecules *J. Sync. Rad.* **2015**, *22*, 1540–1547.

³ Kabsch, W. X. D. S. *Acta Crystallogr. D Biol. Crystallogr.* **2010**, *66* (Pt 2), 125–132.

⁴ Emsley, P.; Cowtan, K. Coot: Model-Building Tools for Molecular Graphics. *Acta Crystallogr. D Biol. Crystallogr.* **2004**, *60* (Pt 12 Pt 1), 2126–2132.

⁵ Hough, M. A.; Wilson, K. S. From Crystal to Structure with CCP4. *Acta Crystallogr. Sect. Struct. Biol.* **2018**, *74* (Pt 2), 67.

Table S2. Data collection and refinement statistics (molecular replacement)

	TEAD-HC258 (26)	TEAD-AF864 (27)
(PDB accession code)	8PUY	8PUX
Data collection		
Space group	C2	C2
Cell dimensions		
<i>a, b, c</i> (Å)	122.69 61.60 79.70	122.31 61.61 79.70
α, β, γ (°)	90 117.5 90	90 117.4 90
Resolution (Å)	48.38-2.20 (2.28-2.20)*	54.28 -2.05 (2.12-2.05)*
<i>R</i> _{merge}	0.043(0.620)	0.028 (0.418)
<i>R</i> _{means}	0.053 (0.758)	0.039 (0.591)
<i>R</i> _{pim}	0.030 (0.430)	0.028 (0.418)
<i>I</i> / σ <i>I</i>	12.63 (1.61)	11.63 (1.24)
Completeness (%)	96.52 (98.31)	99.73 (99.39)
Redundancy	2.8 (2.8)	1.9 (1.8)
CC _{1/2}	0.997 (0.687)	0.999 (0.907)
CC*	0.999 (0.901)	1.000 (0.975)
Wilson B-factor	60.05	47.14
Refinement		
Resolution (Å)	2.20	2.05
No. reflections	73715 (7347)	64076 (6040)
Unique reflections	26066 (2617)	33152 (3290)
<i>R</i> _{work} / <i>R</i> _{free}	22.06/25.20	21.95/26.05
No. atoms		
Protein	3313	3339
Ligand	39	38
Water	11	41
<i>B</i> -factors		
Protein	76.45	64.48
Ligand	84.58	76.28
Water	64.50	57.55
R.m.s. deviations		
Bond lengths (Å)	0.013	0.013
Bond angles (°)	1.75	1.77

*Values in parentheses are for highest-resolution shell.

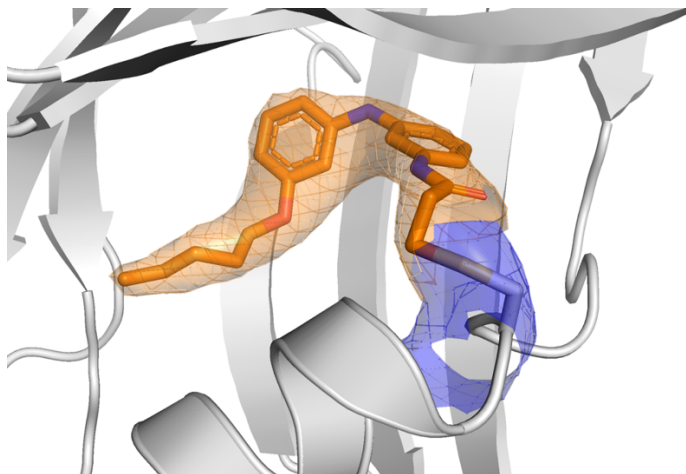


Figure S2. Co-crystal structure of the compound **HC258 (26)** with TEAD2 (PDB code: 8PUY). TEAD2 is represented in cartoon format and the compounds in stick format. The blue and orange mesh and surface represent the electron density maps ($2F_o - F_c$ omit map contoured at 1.0σ) of Cysteine380 and HC258 respectively.

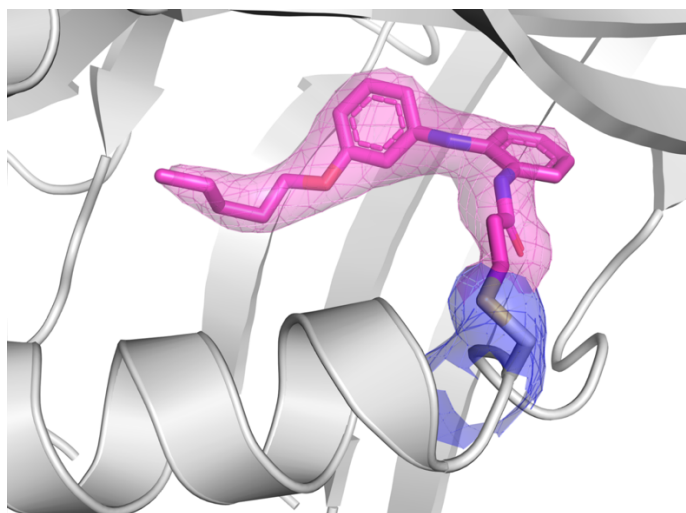
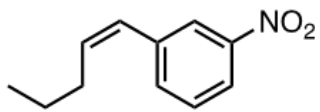


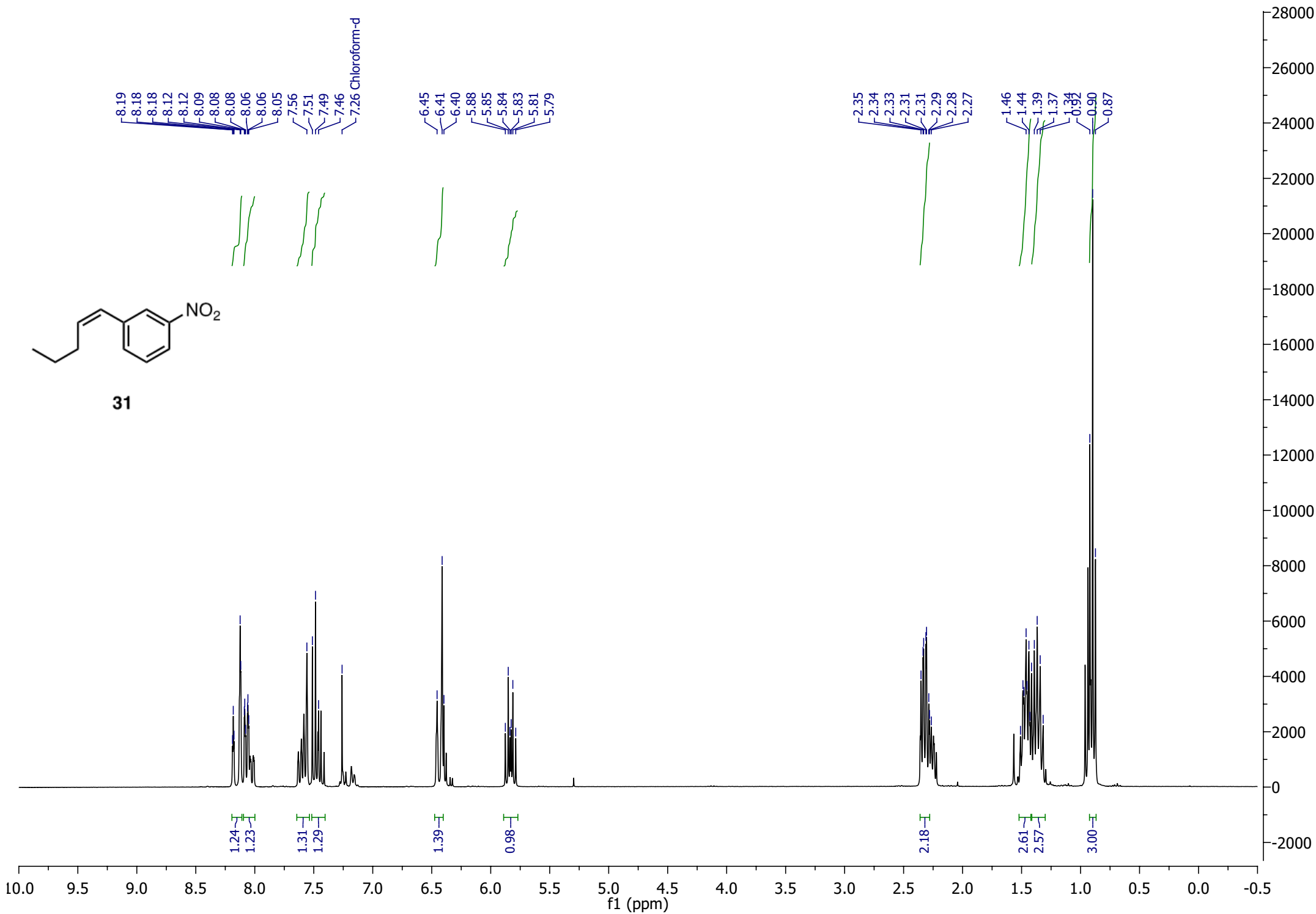
Figure S3. Co-crystal structure of the compound **AF864 (27)** with TEAD2 (PDB code: 8PUX). TEAD2 is represented in cartoon format and the compounds in stick format. The blue and purple mesh and surface represent the electron density maps ($2F_o - F_c$ omit map contoured at 1.0σ) of Cysteine380 and AF264 respectively.

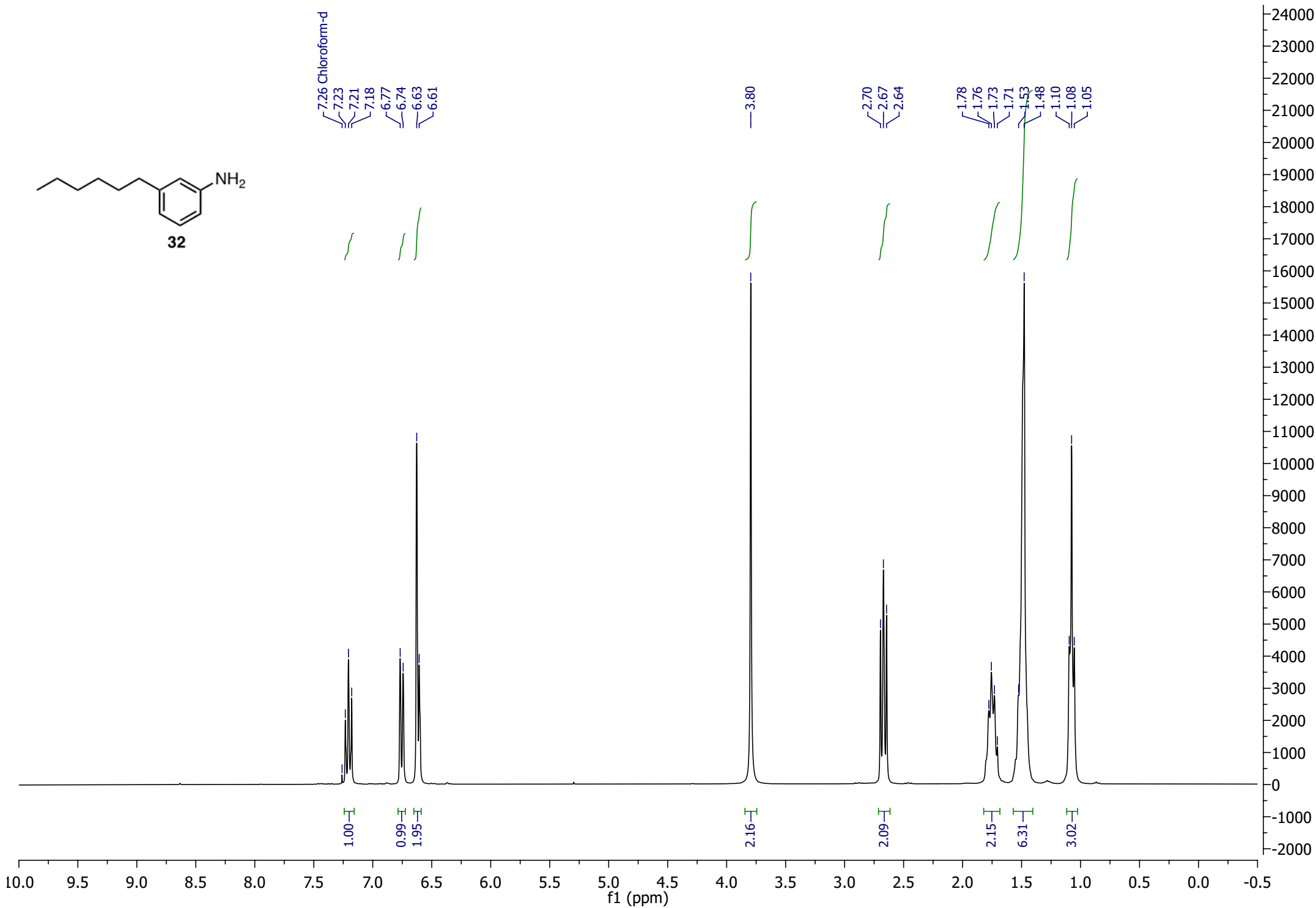
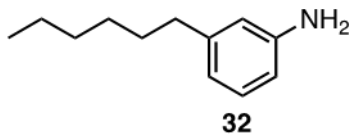
Fnaiche, A.; Chan, H.-C. *et al.* Development of HC-258, a covalent acrylamide TEAD inhibitor that reduces gene expression and cell migration.

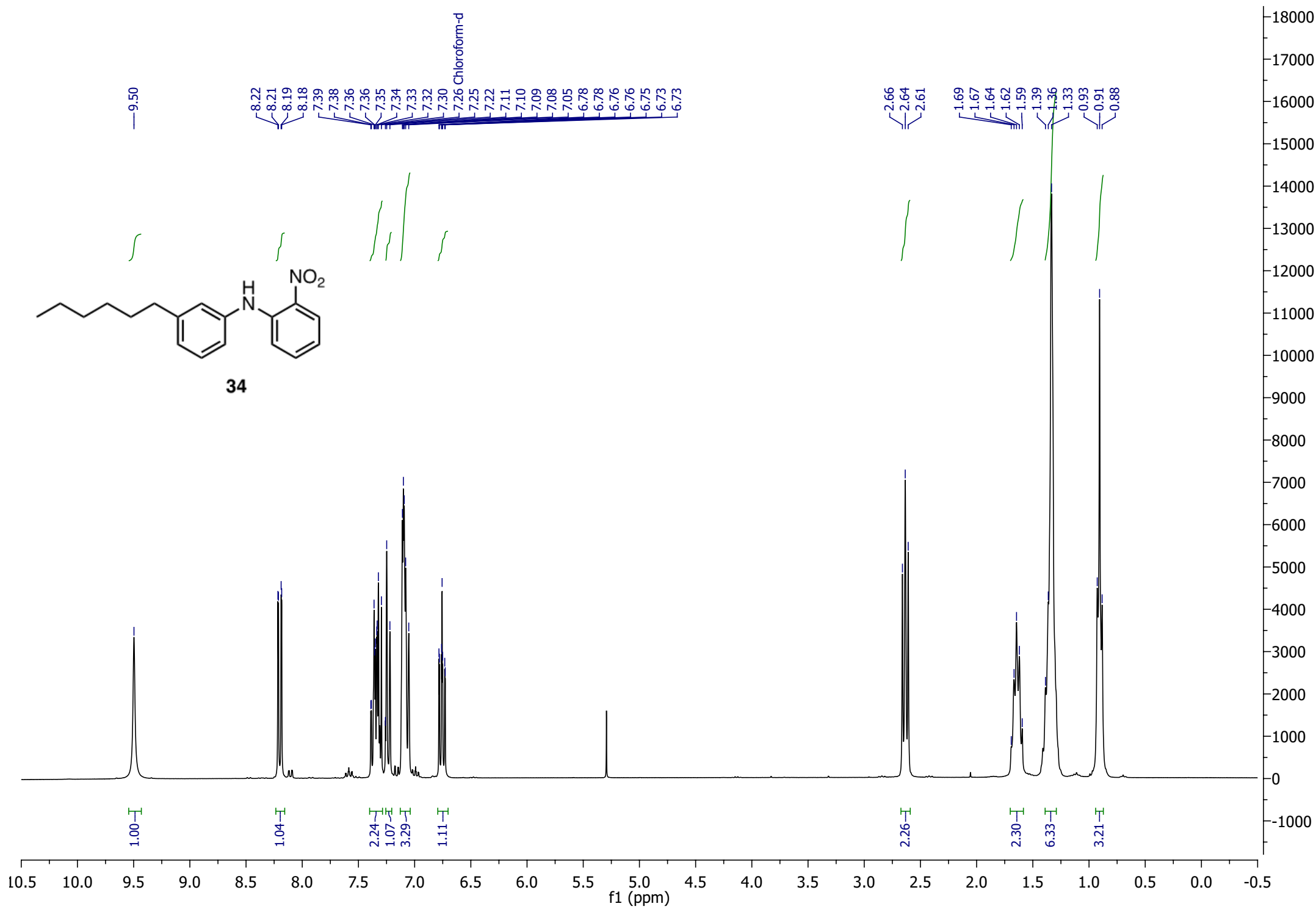
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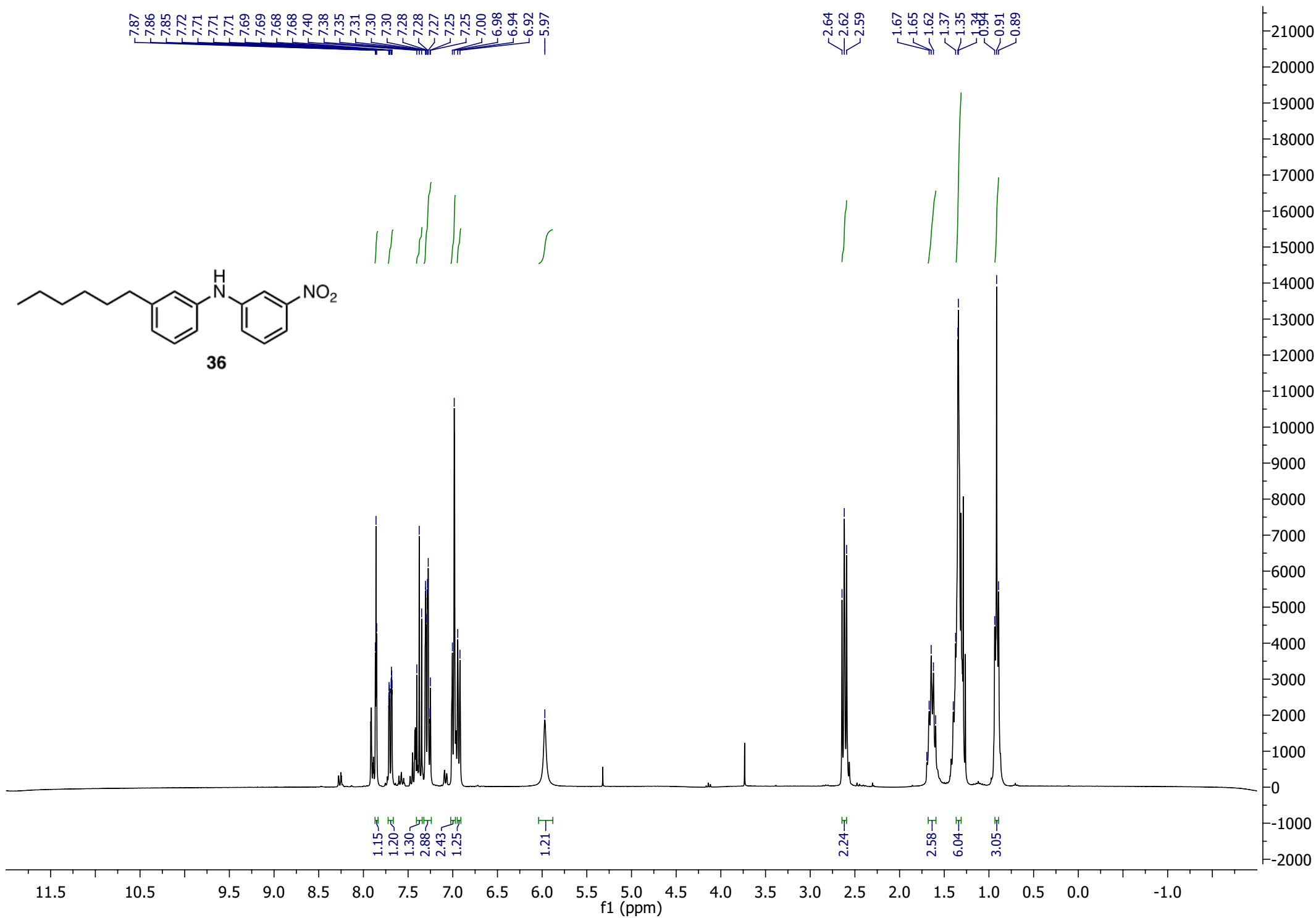


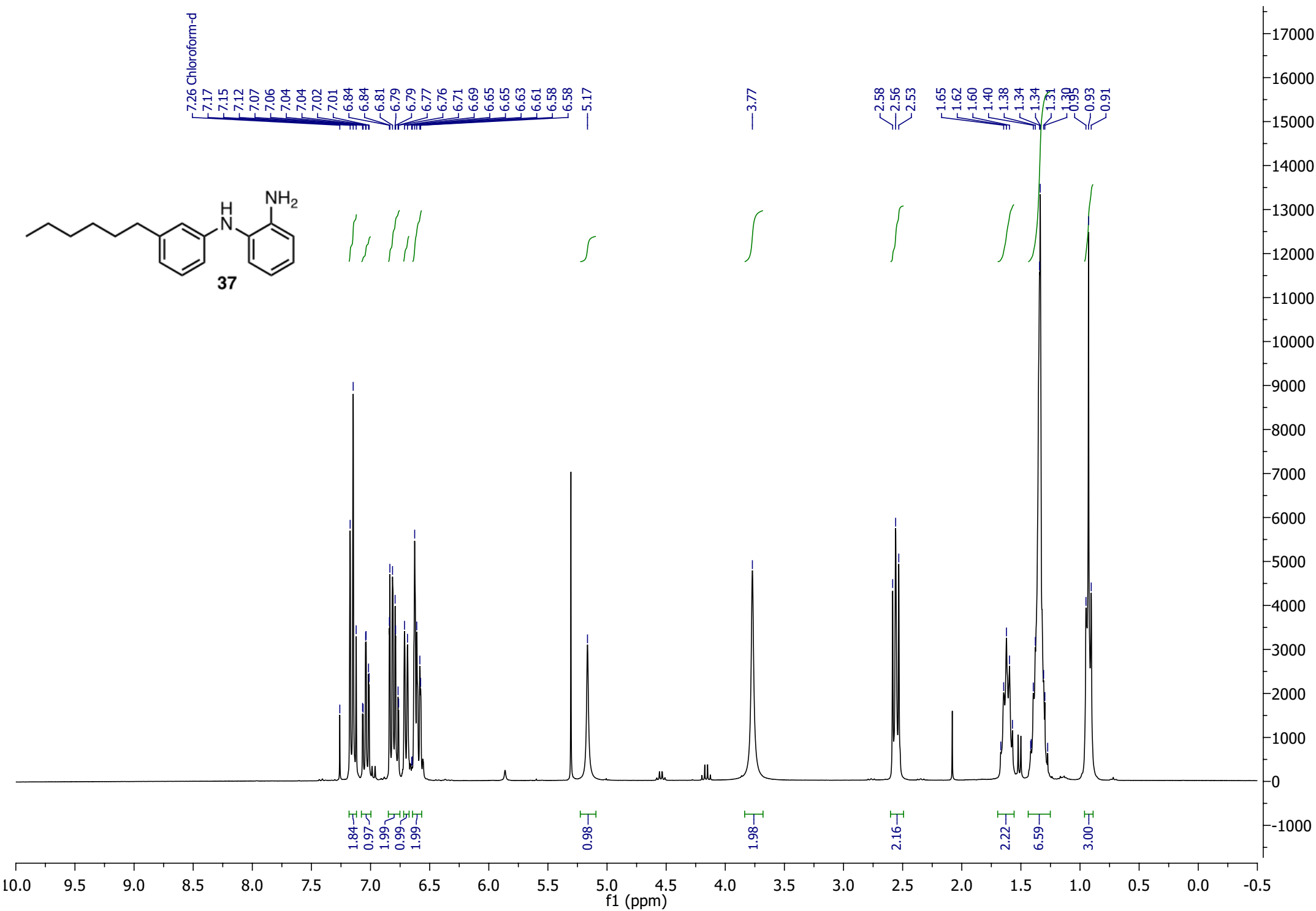
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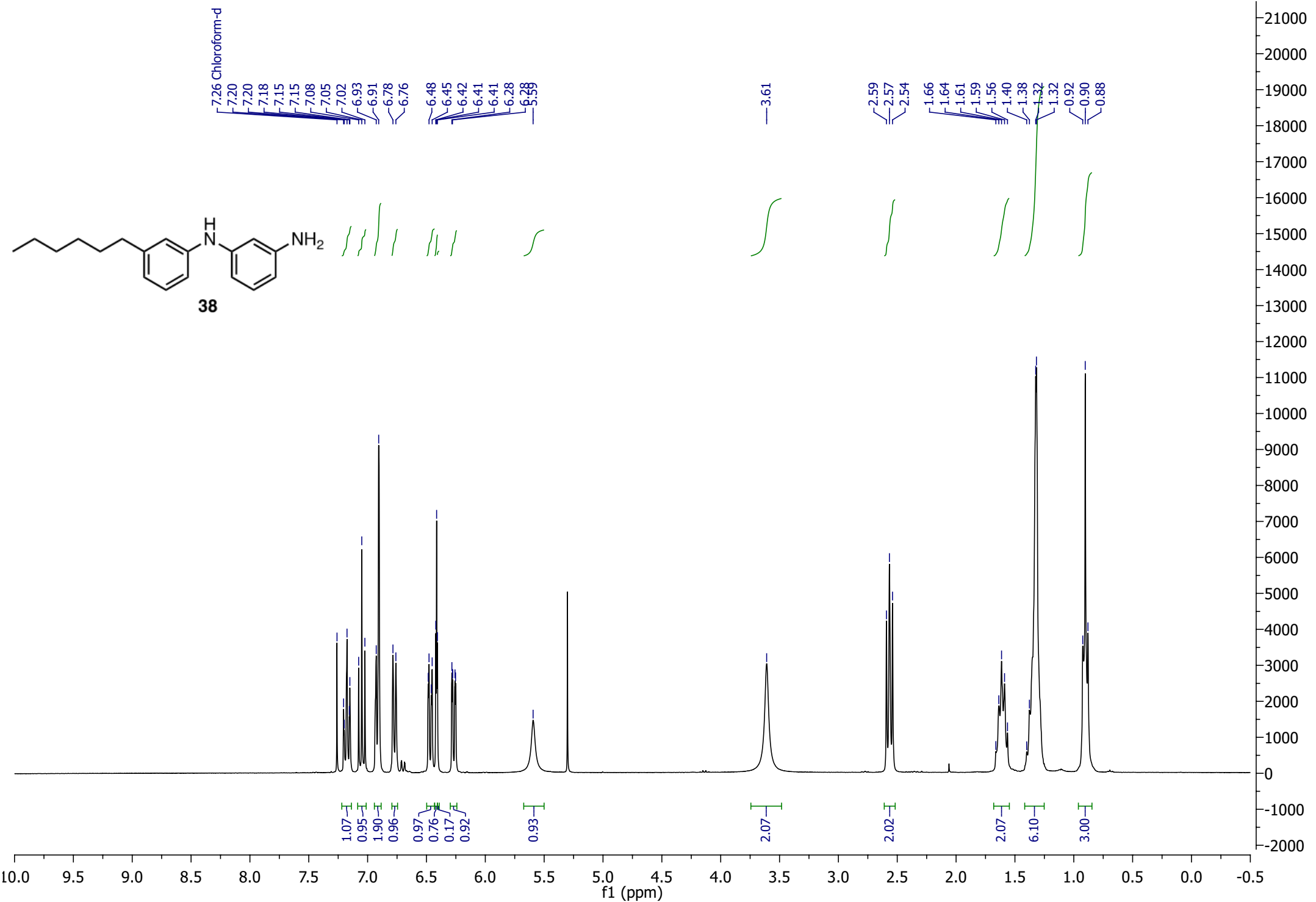


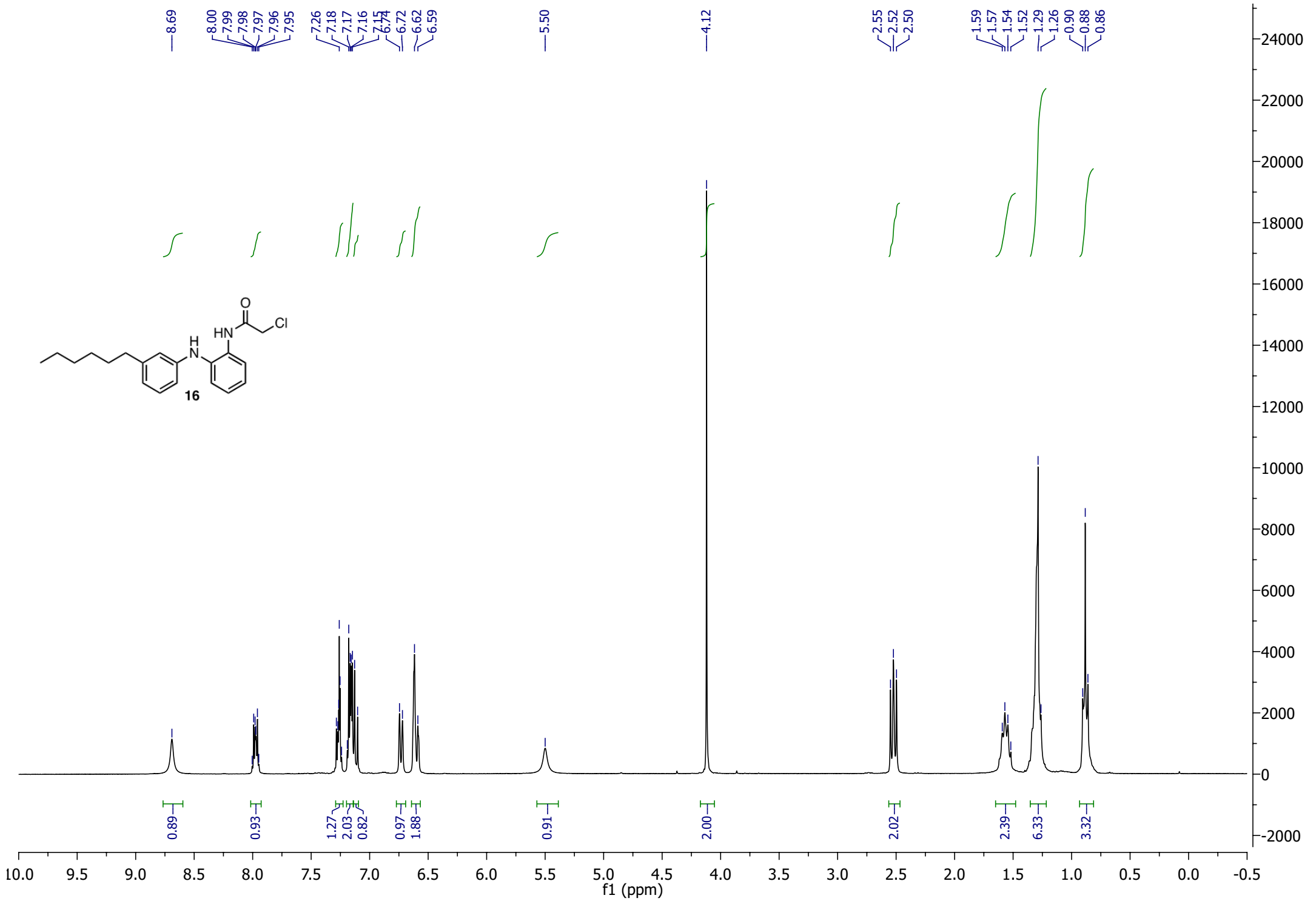
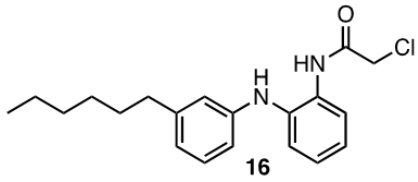


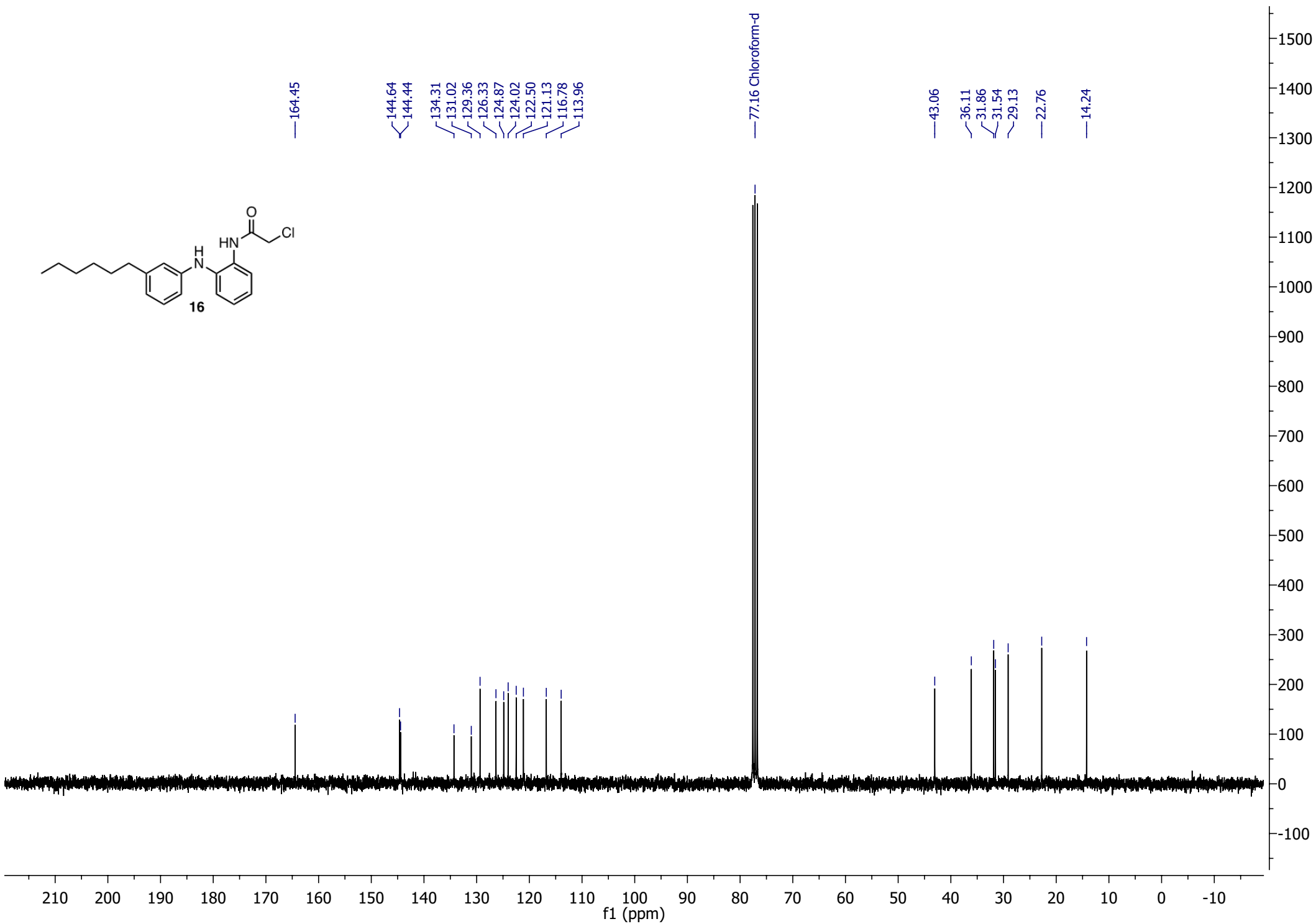
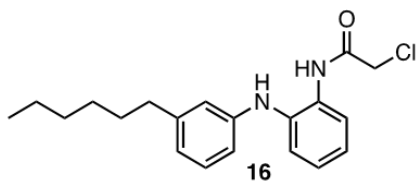


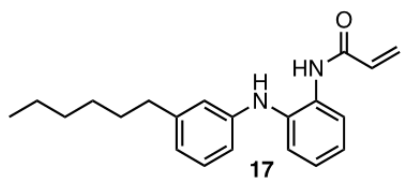












8.01
7.91
7.28
7.26
7.25
6.73
6.71
6.63
6.61
6.29
6.23
6.19
6.14
5.70
5.67

2.54
2.52
2.49
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1.57
1.54
1.52
1.36
1.34
1.30
1.29
0.91
0.89
0.87



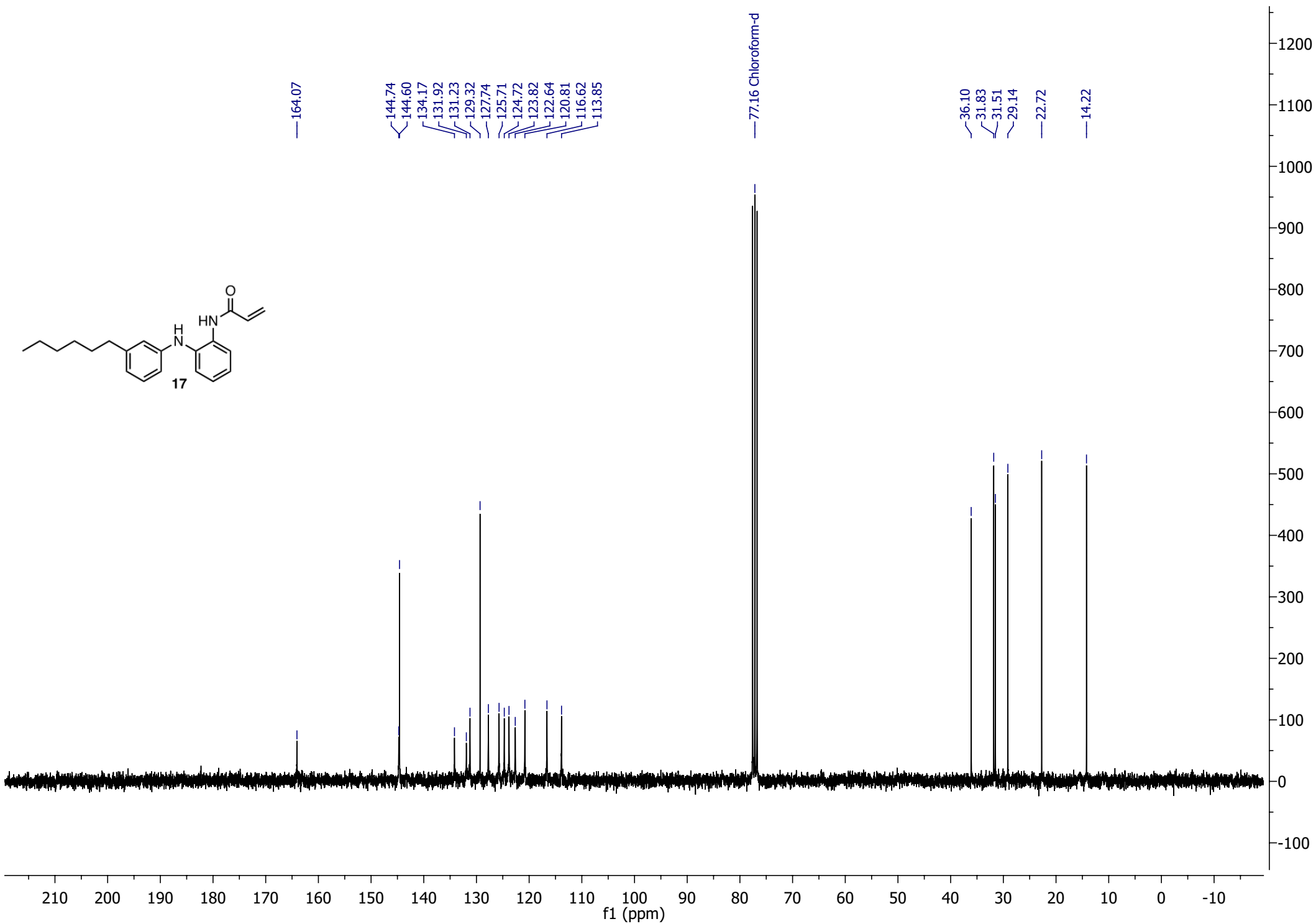
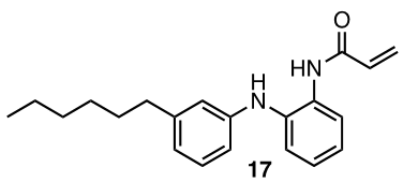
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3.24
1.12
2.09
1.06
1.11
2.11

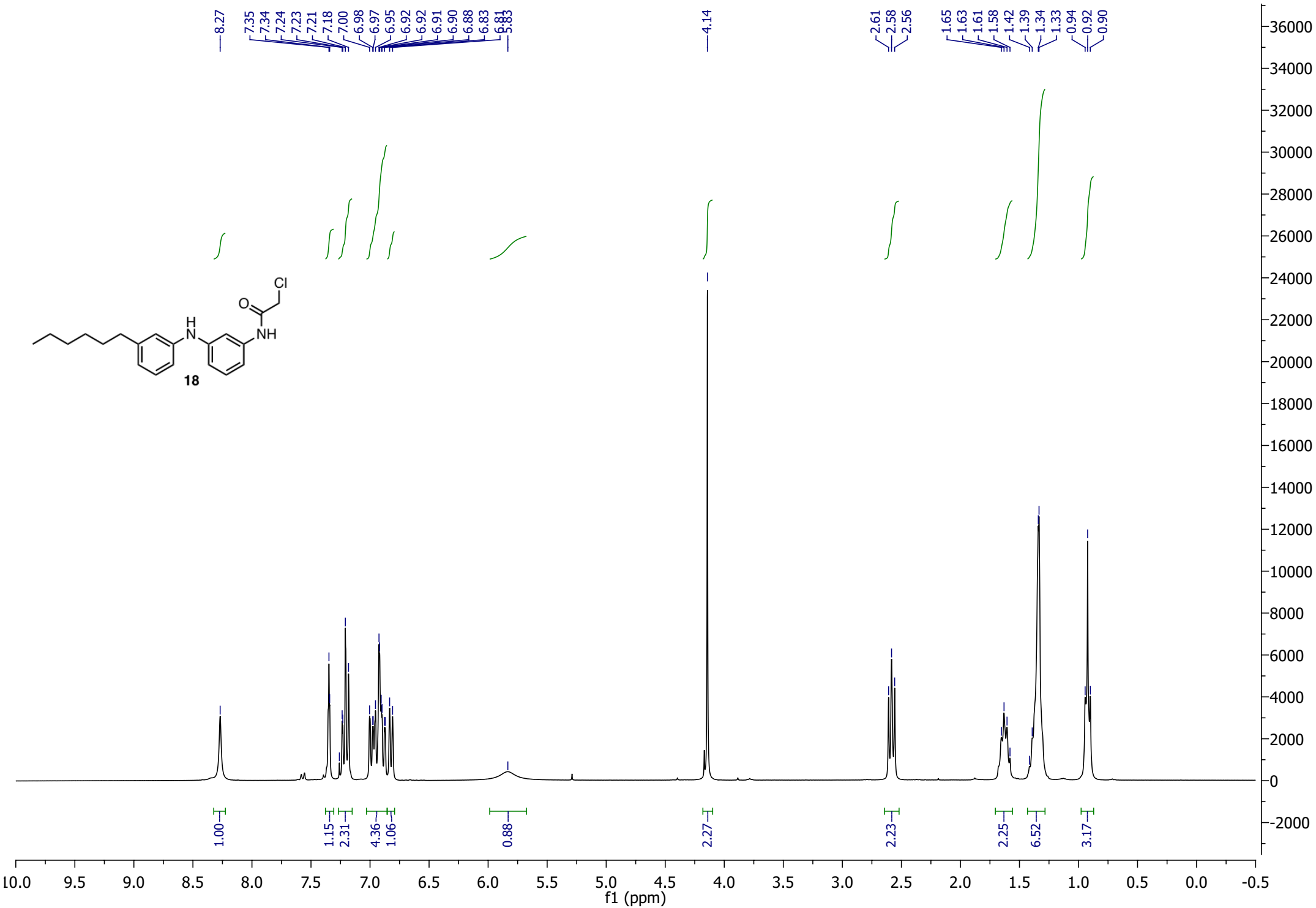
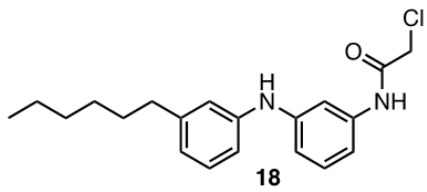
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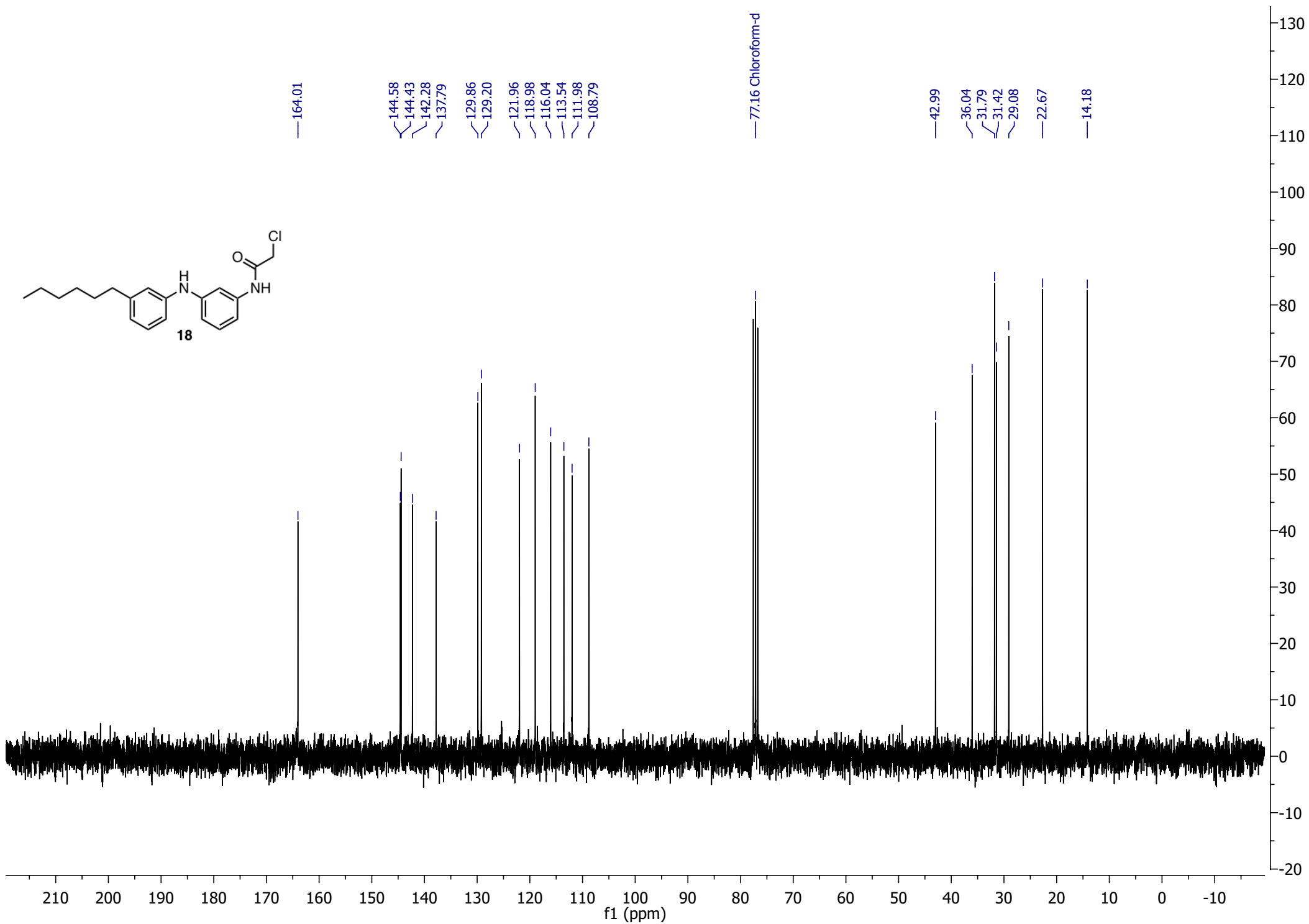
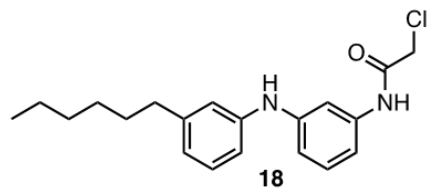
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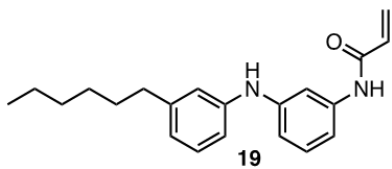
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20000
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15000
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
-2000









7.99
7.21
7.19
7.16
6.94
6.92
6.90
6.89
6.82
6.79
6.41
6.40
6.35
6.31
5.75
5.74
5.71
5.71

2.60
2.57
2.55

1.65
1.63
1.60
1.58
1.34
0.95
0.93
0.90

0.96

1.00

2.33

1.05

3.34

1.19

1.10

1.17

2.05

2.35

2.33

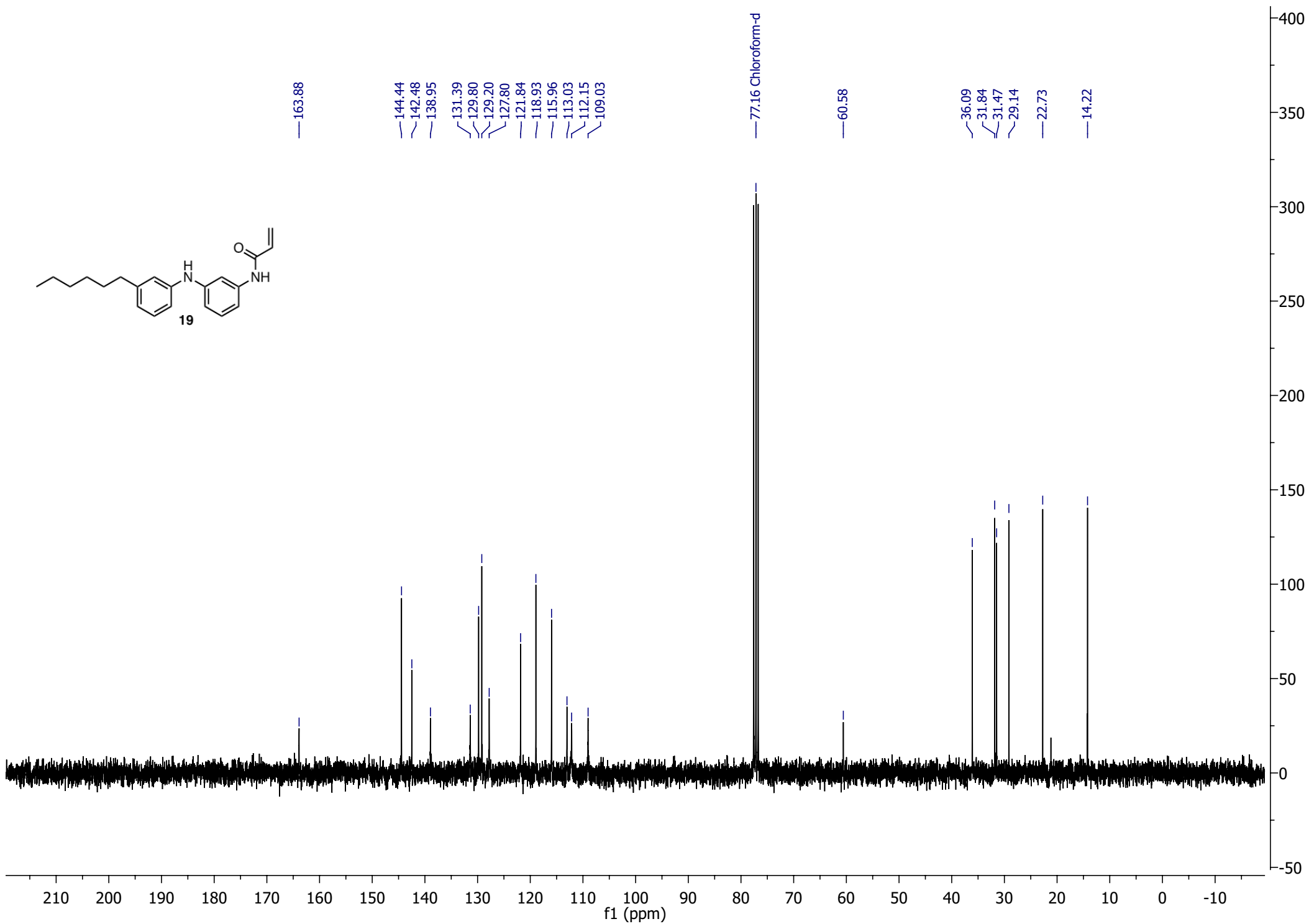
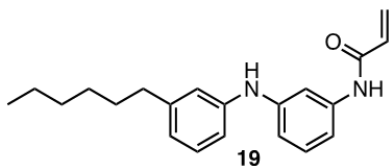
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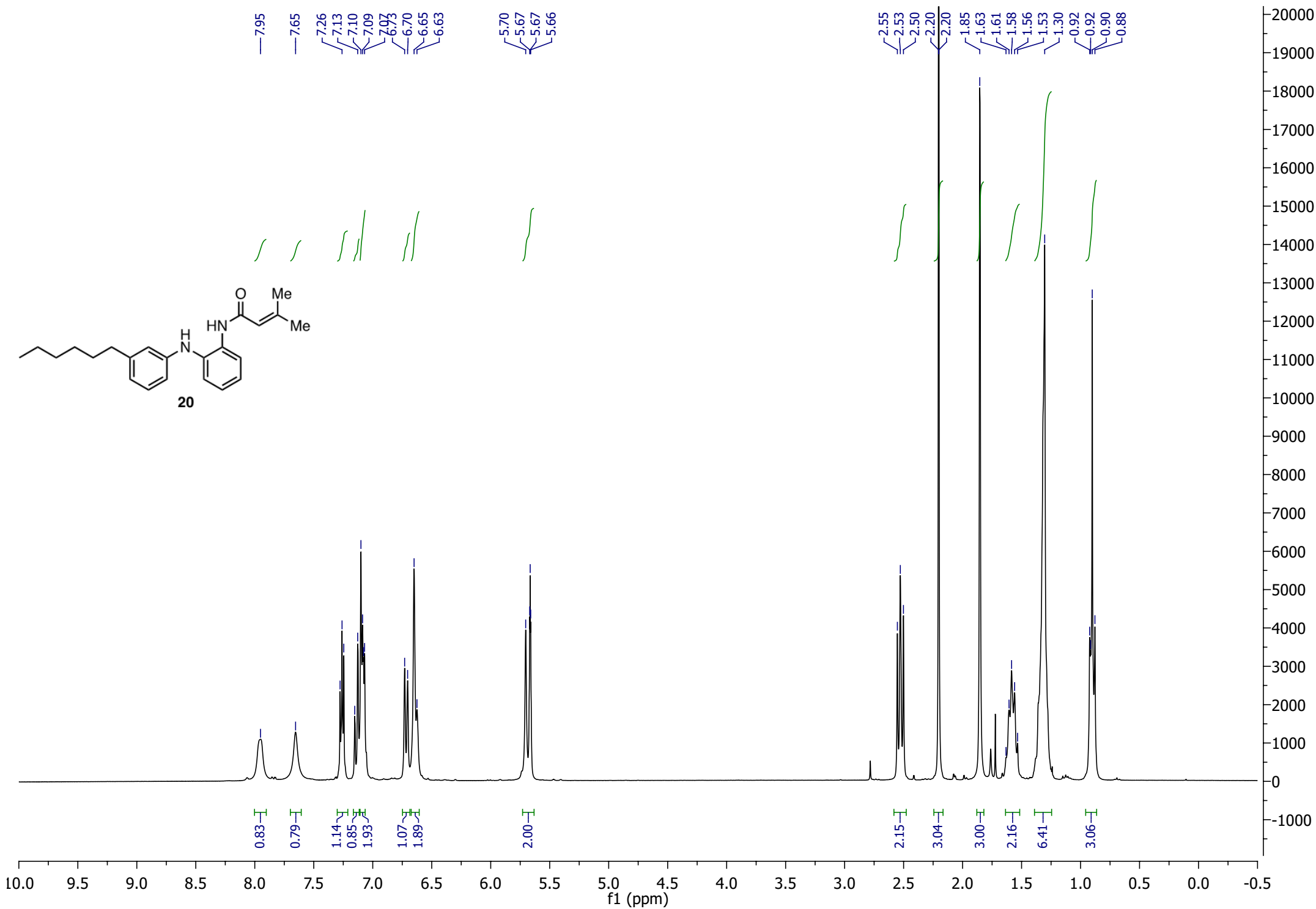
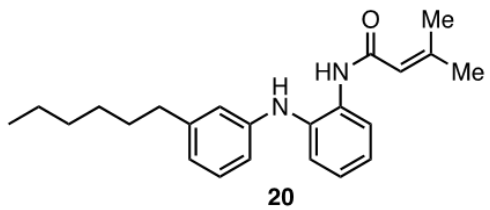
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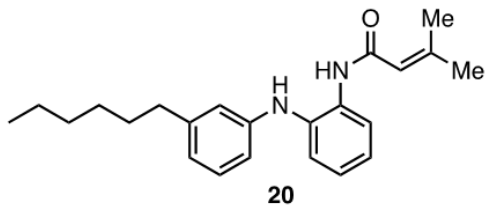
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f1 (ppm)

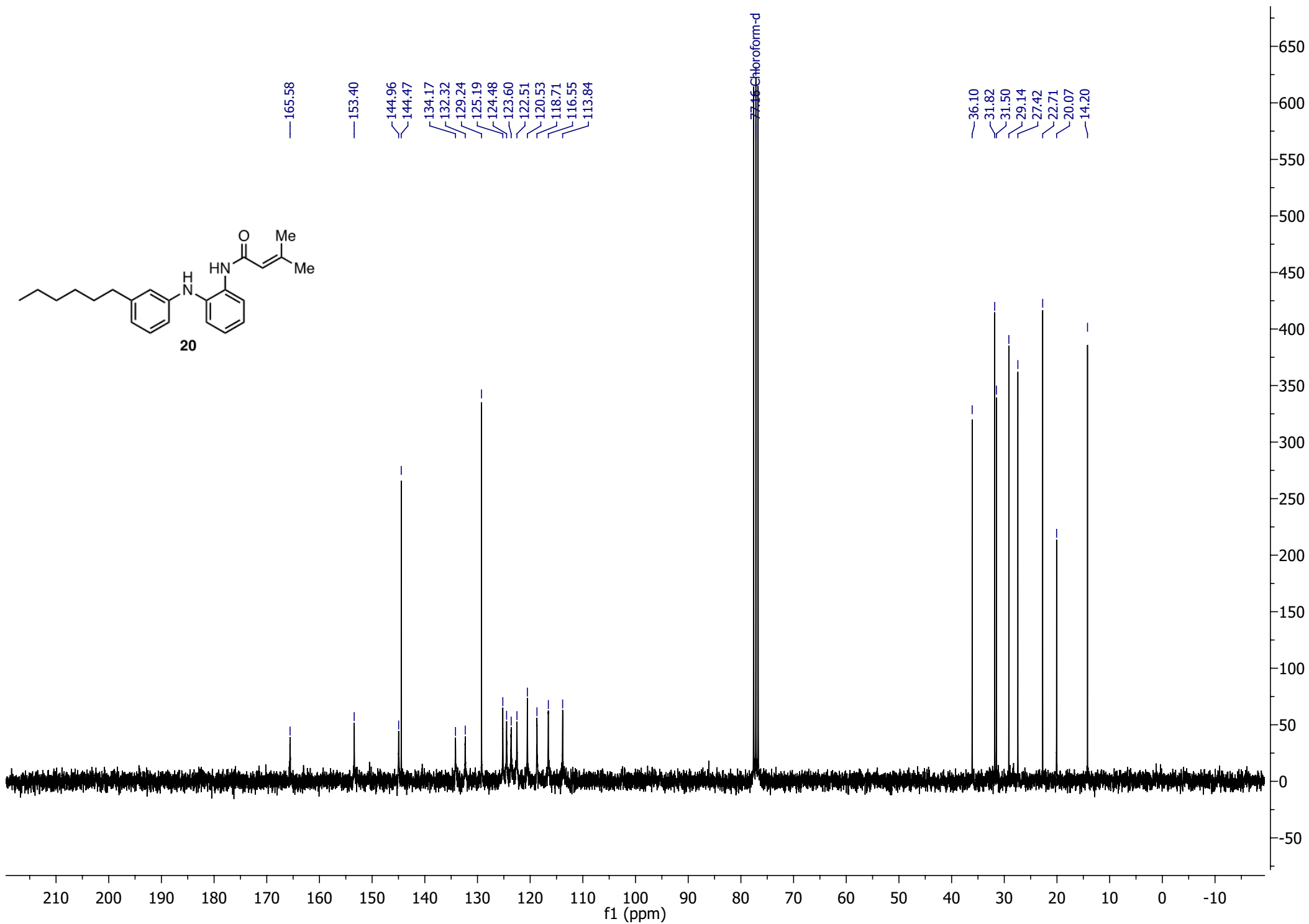
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15000
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000

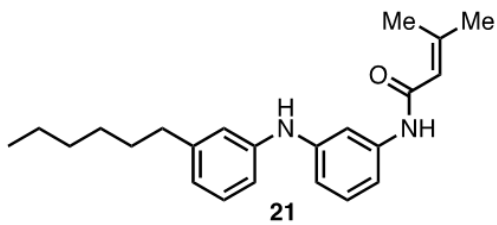






20





7.54
7.35
7.18
7.17
7.16
7.15
7.13
7.12
7.03
7.00
6.91
6.88
6.84
6.84
6.82
6.81
6.78
6.76
5.72

2.58
2.55
2.53
2.22
2.21
2.06
1.86
1.61
1.32
1.32
1.30
0.93
0.92
0.91
0.89

1.00
0.81
2.20
0.90
2.19
1.12
1.09

1.75

2.19

3.38

3.27

2.26

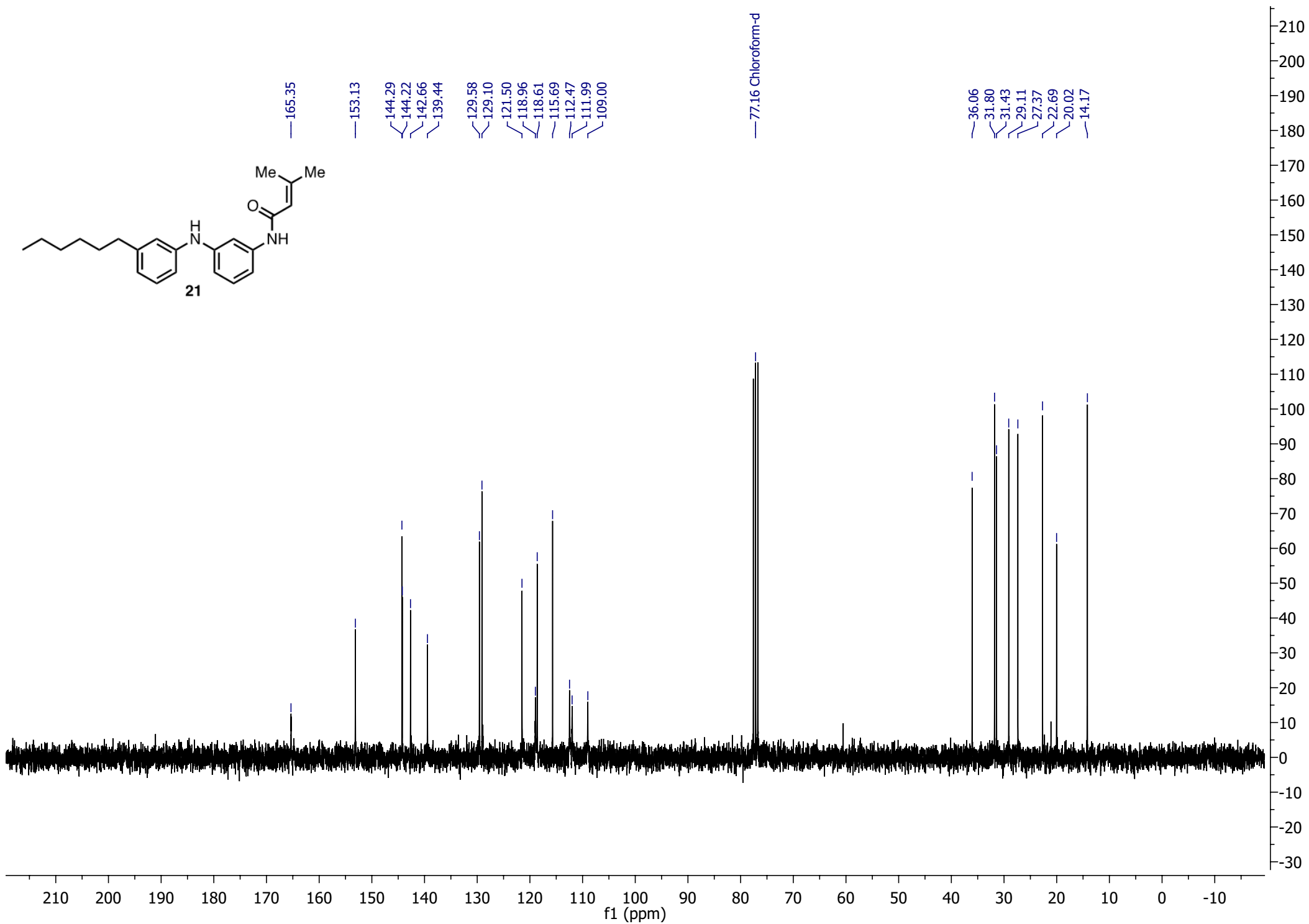
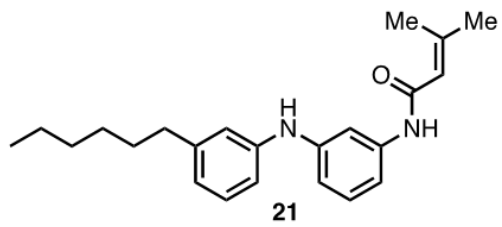
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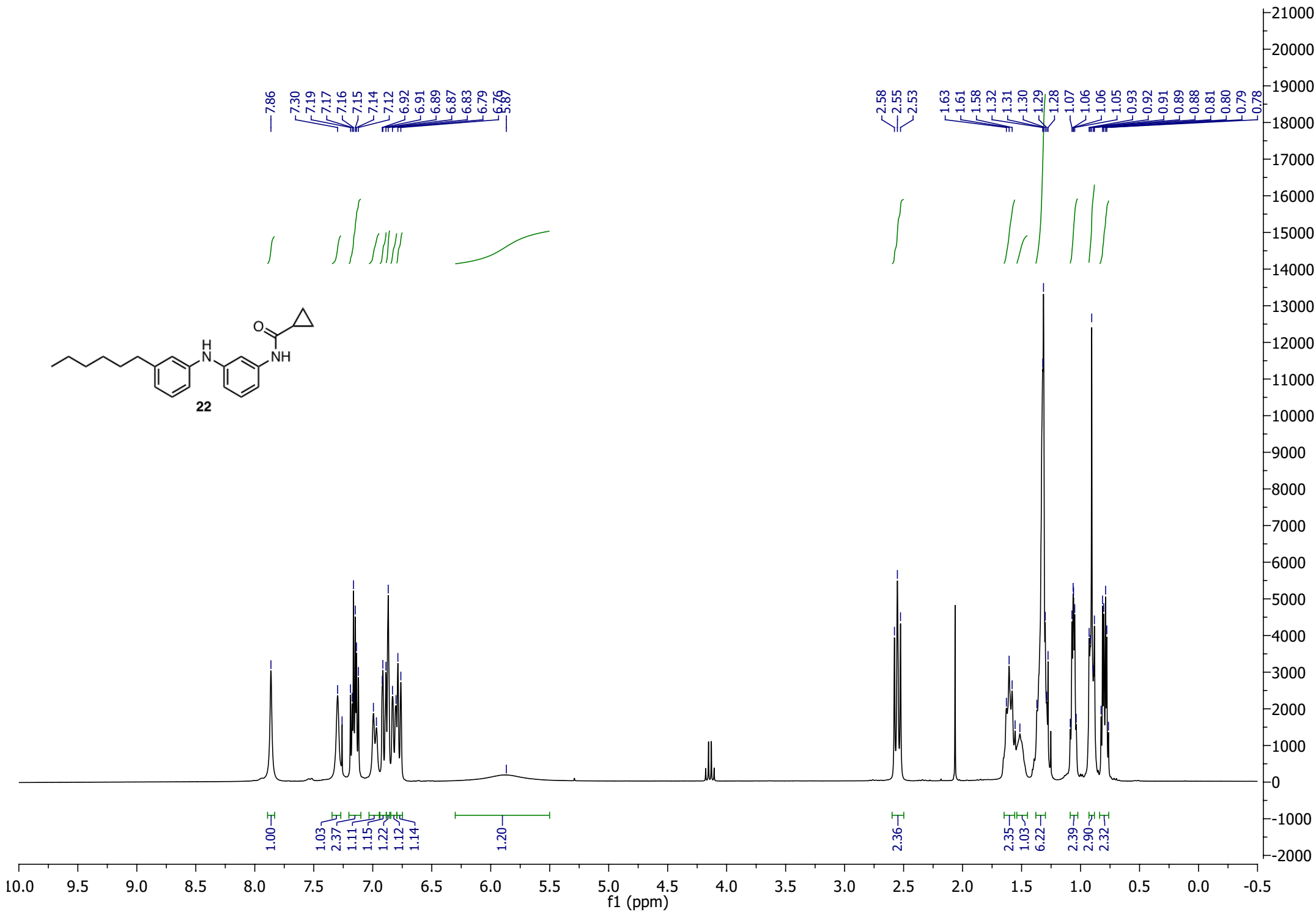
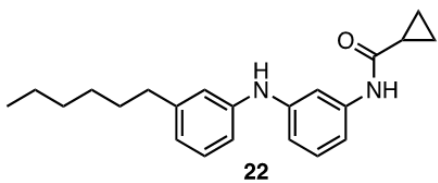
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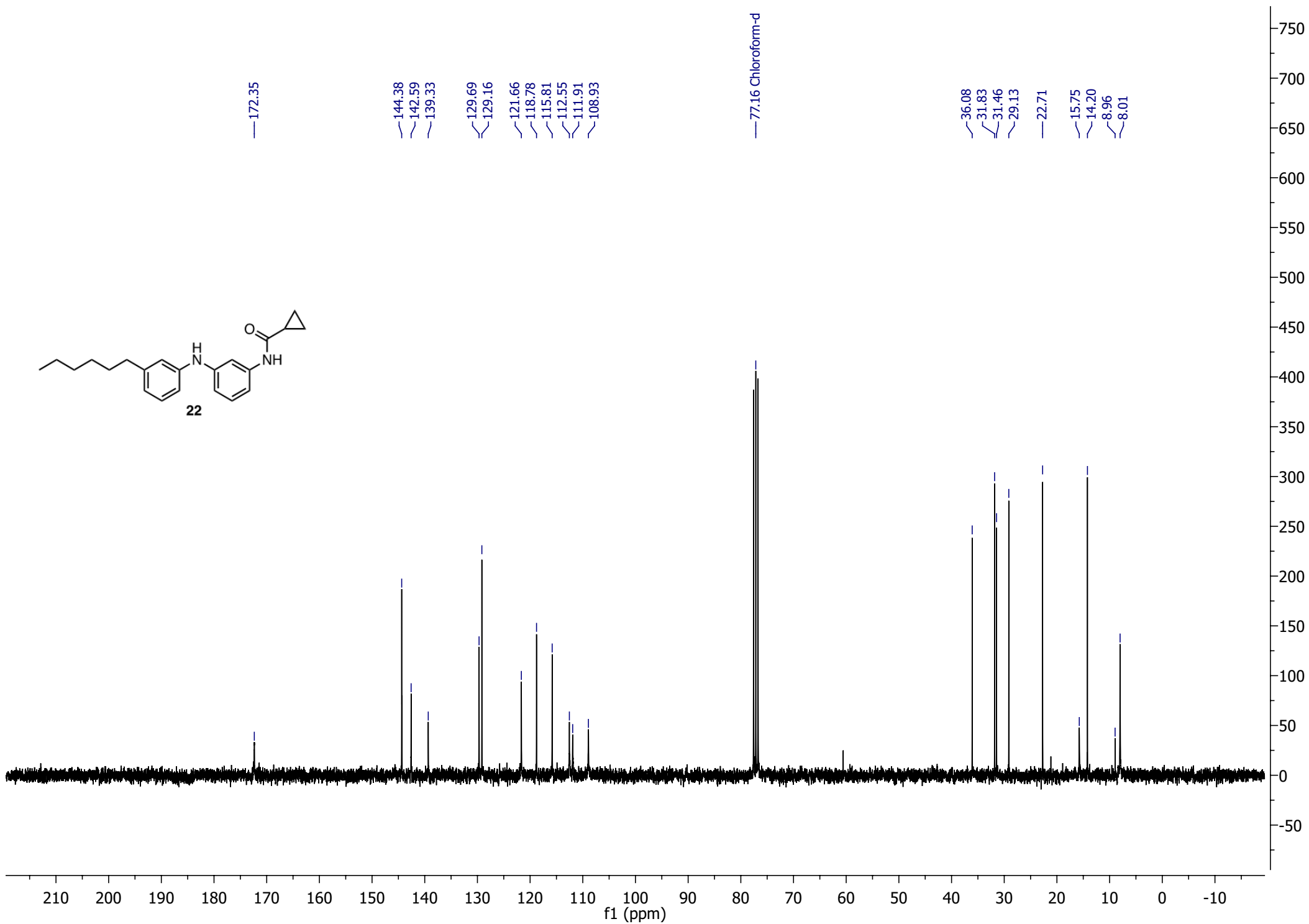
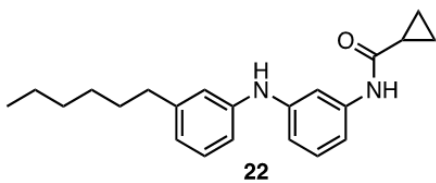
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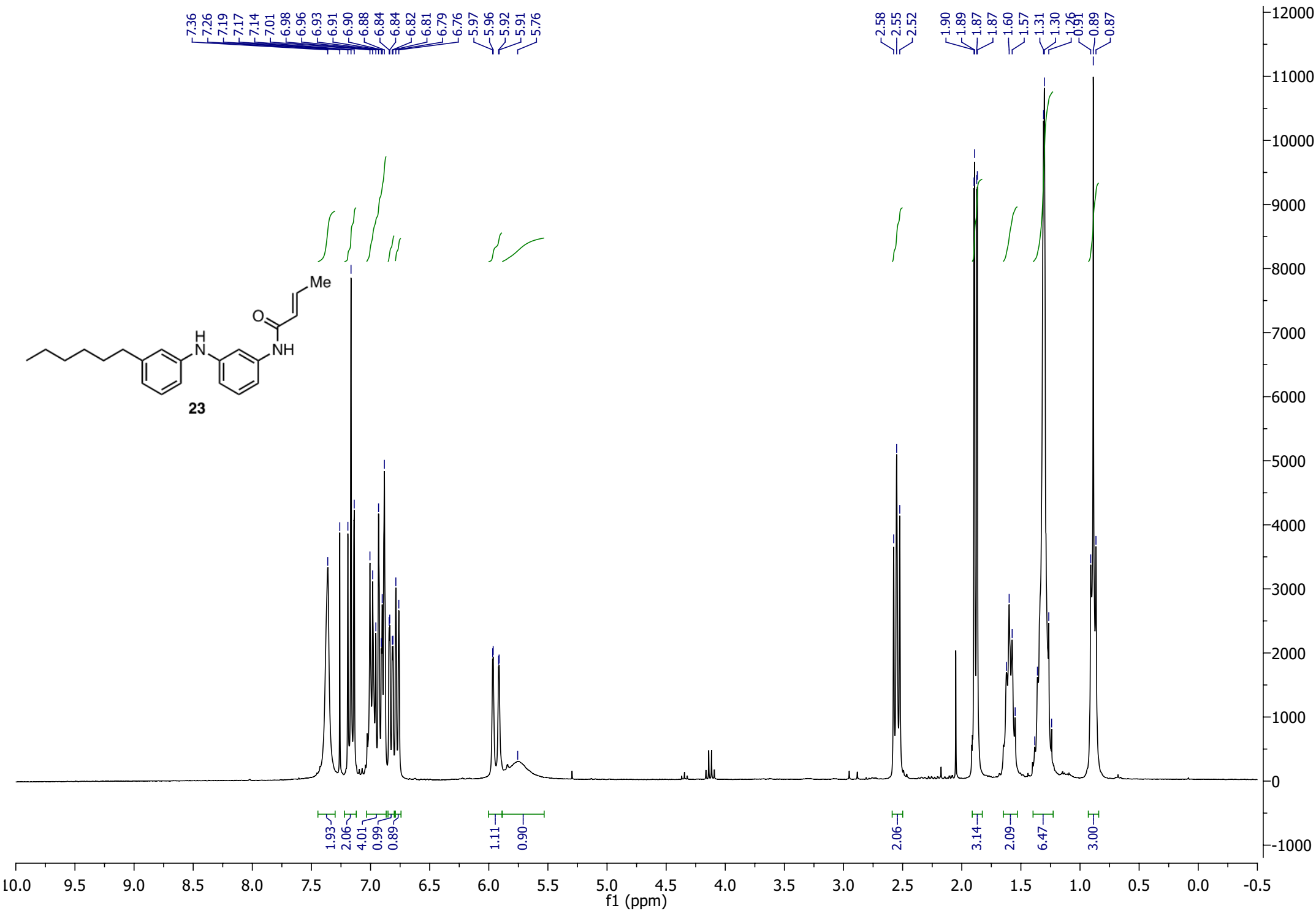
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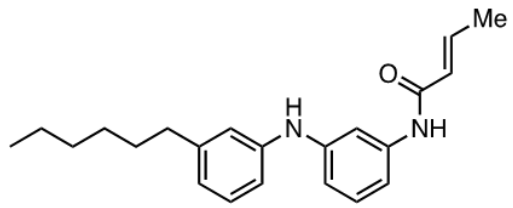
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6000
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2000
1000
0
-1000



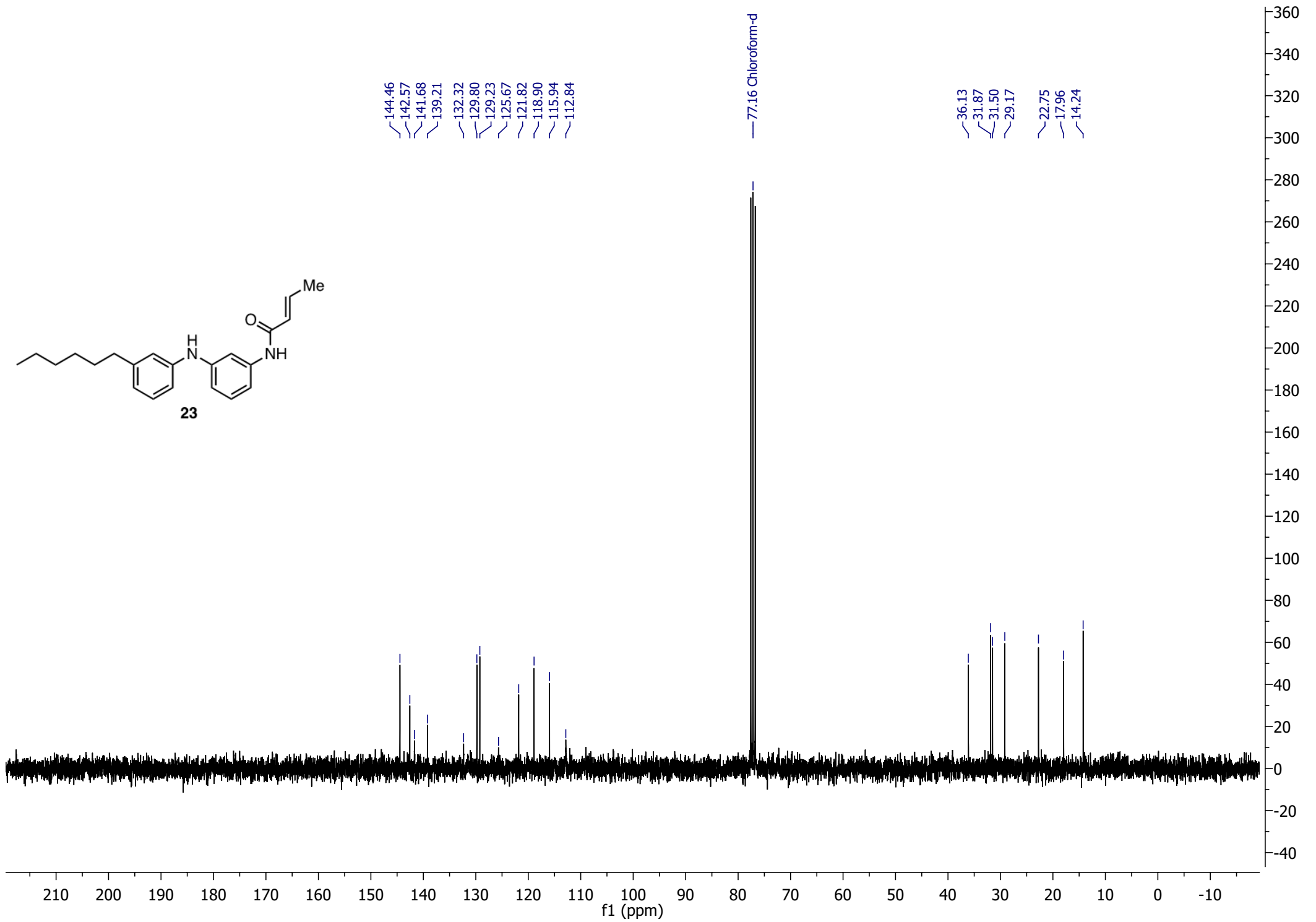


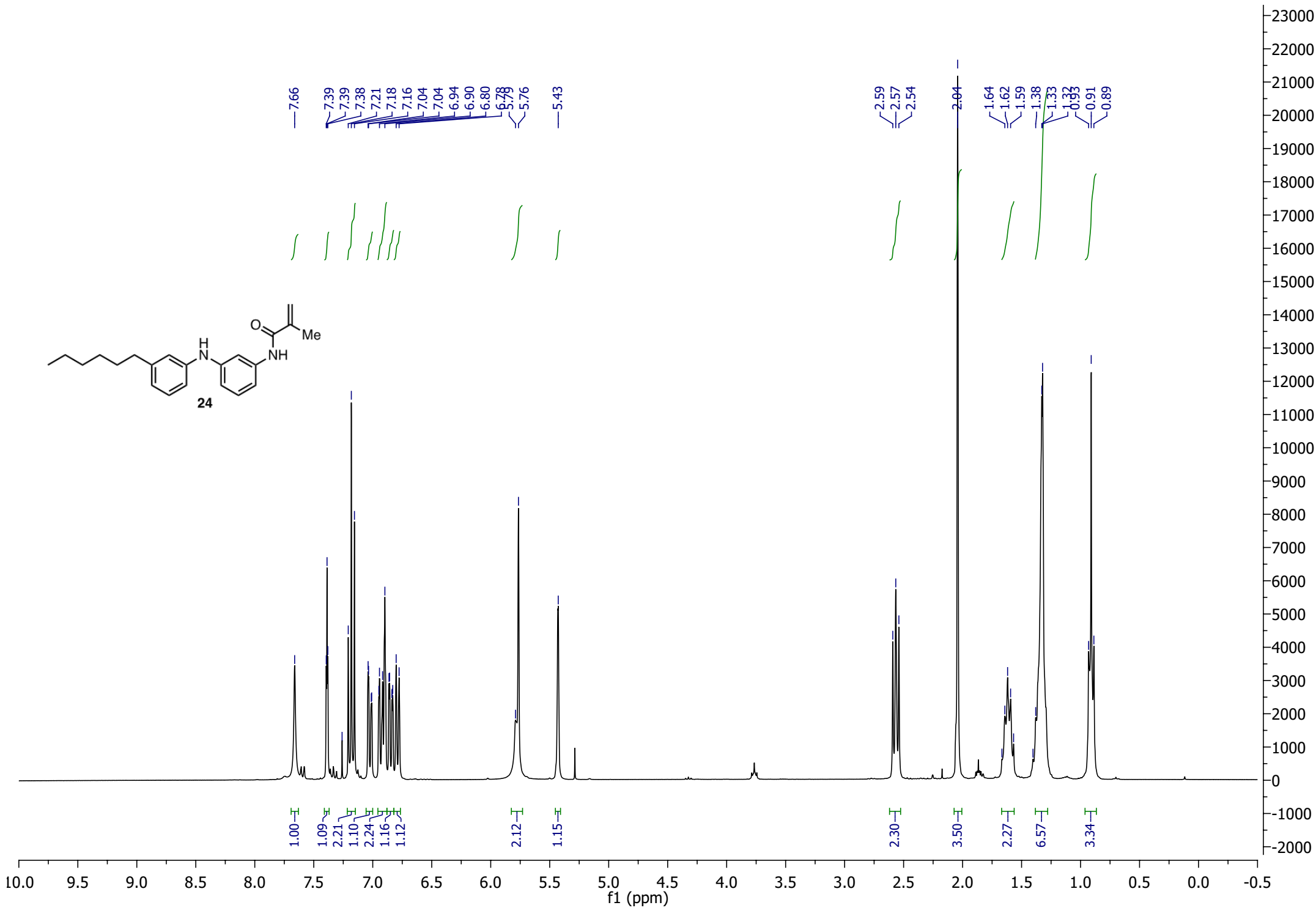
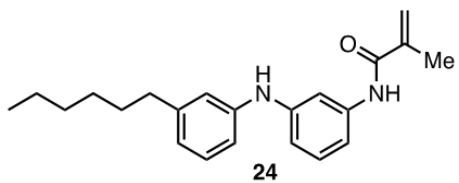


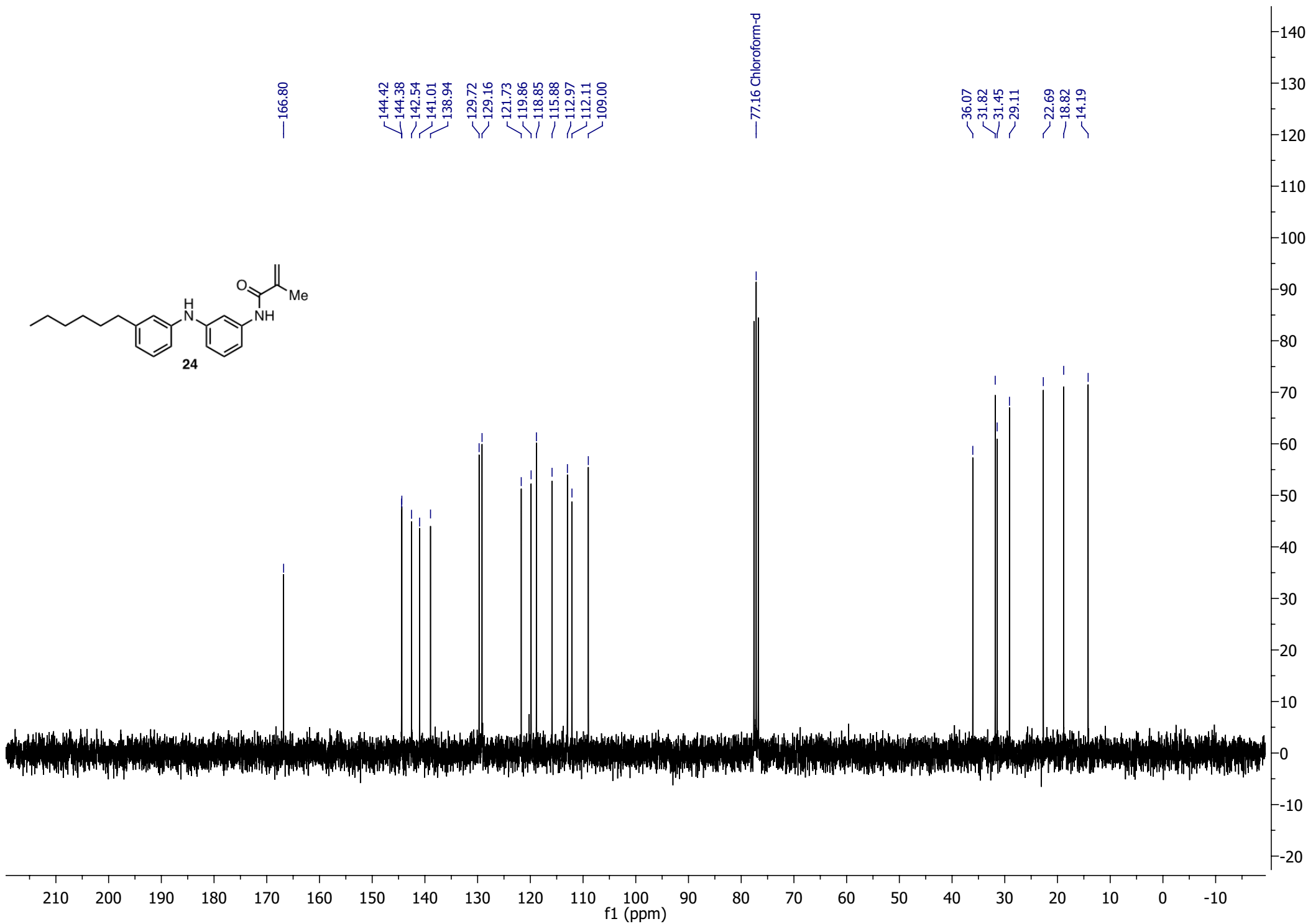
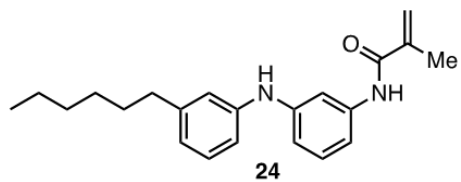


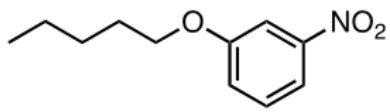


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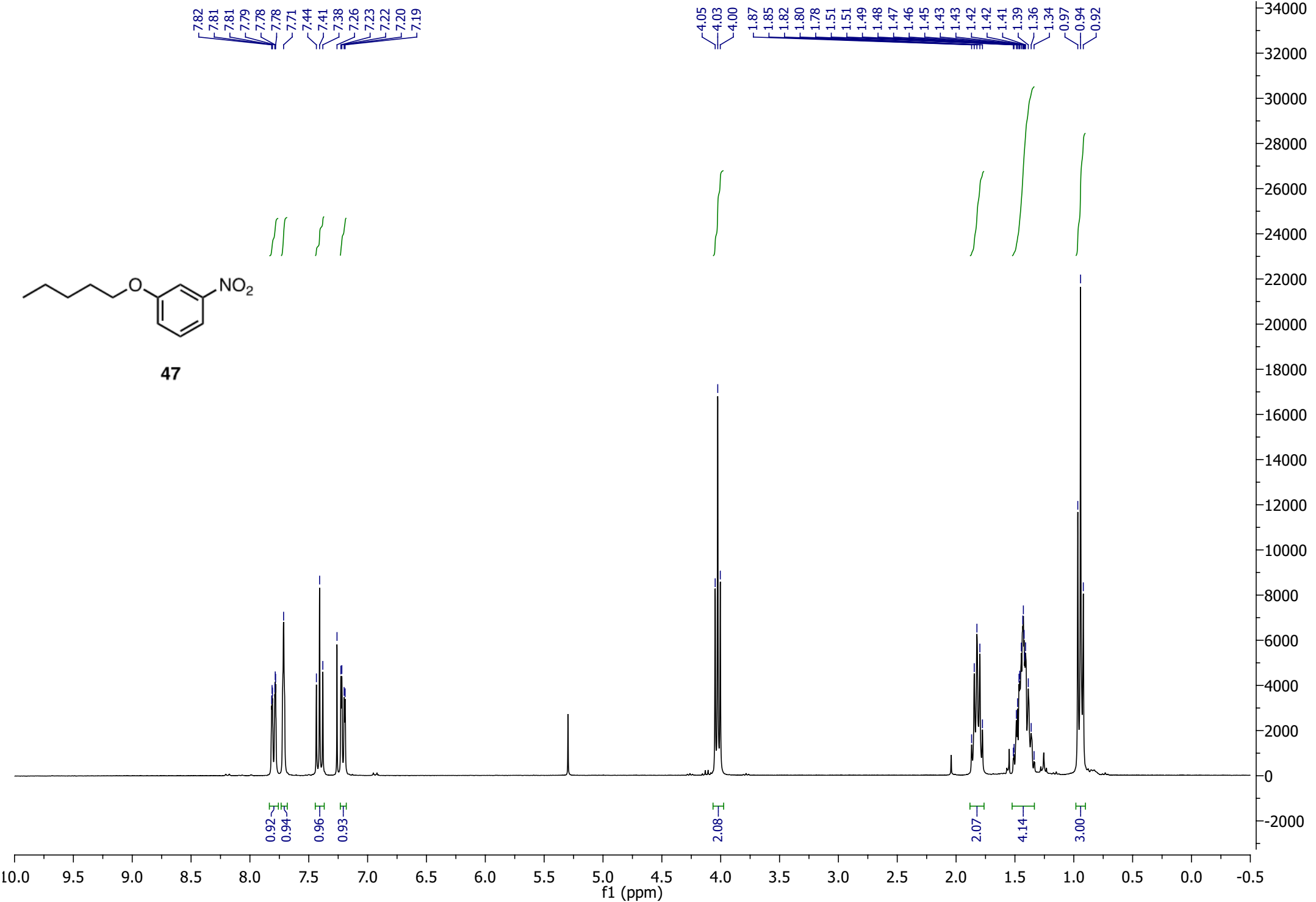


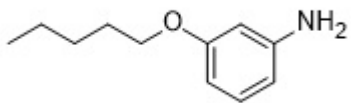






47





48

7.26 Chloroform-d

7.08
7.06
7.03

6.35
6.32
6.30
6.27
6.26

3.94
3.92
3.89
3.68

1.82
1.80
1.77
1.75
1.73
1.46
1.45
1.43
1.42
1.41
1.40
1.38
1.35
0.96
0.94
0.92

0.94

2.85

2.00

1.93

2.01

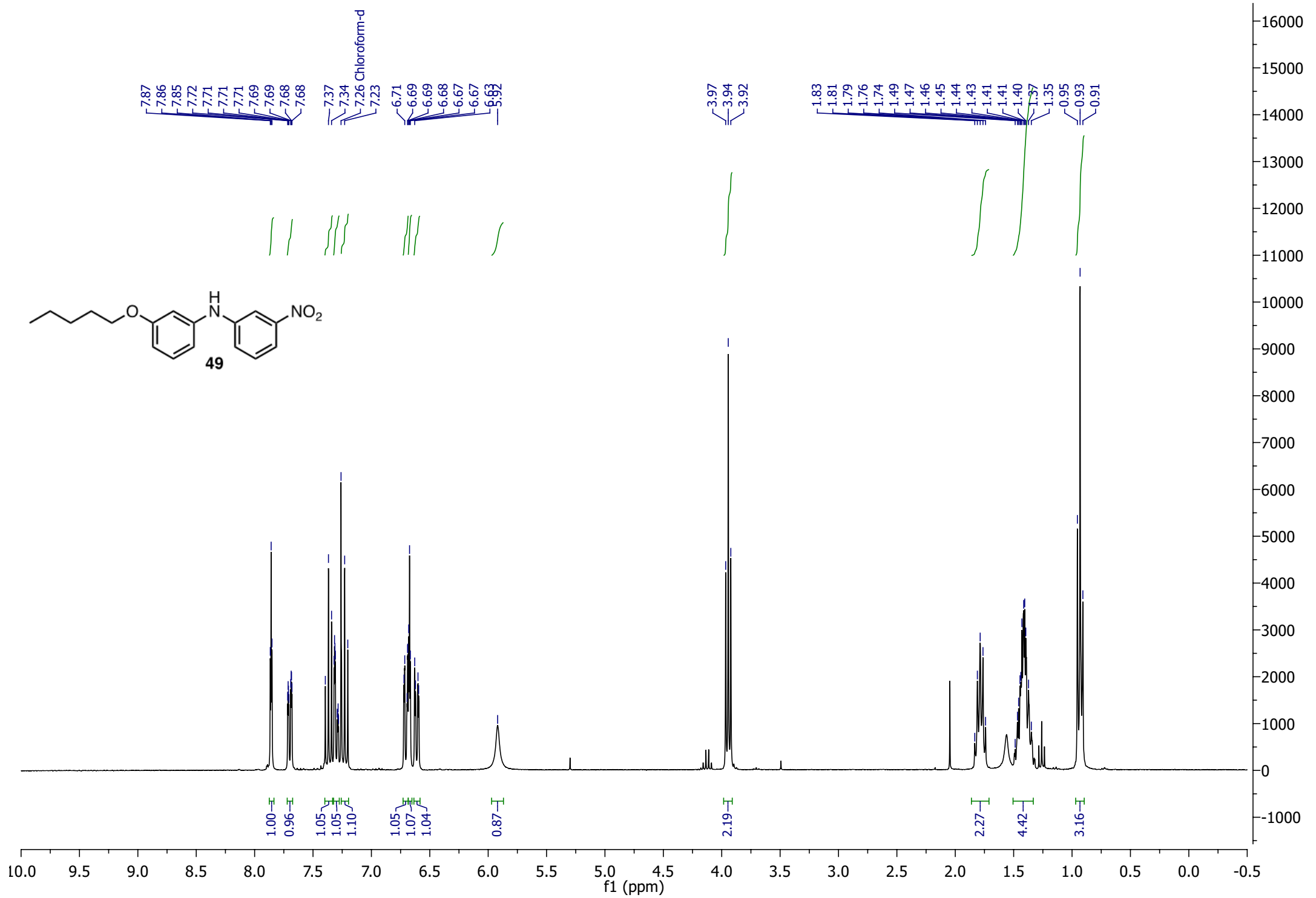
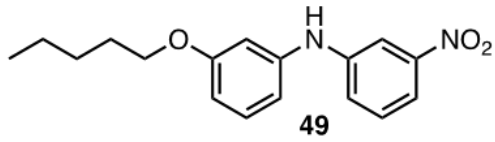
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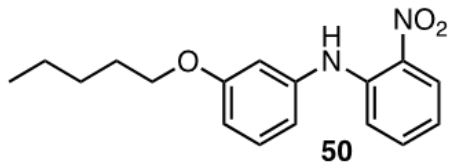
3.00

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f1 (ppm)

50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
-5000





9.47

8.20
8.19
8.17
8.17

7.32
7.31
7.30
7.29

6.88
6.84
6.83

6.80
6.77
6.75
6.75

4.00
3.97
3.95

1.86
1.84
1.82
1.80
1.77
1.53
1.52
1.50
1.49
1.48
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0.94

1.00

1.05

1.08
2.23

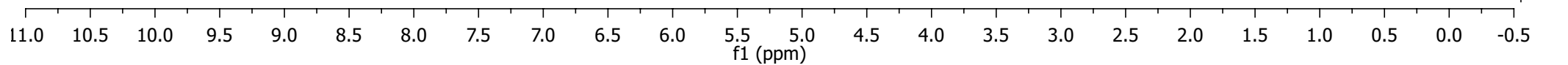
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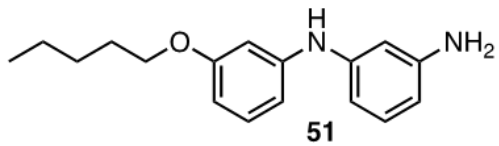
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2.23

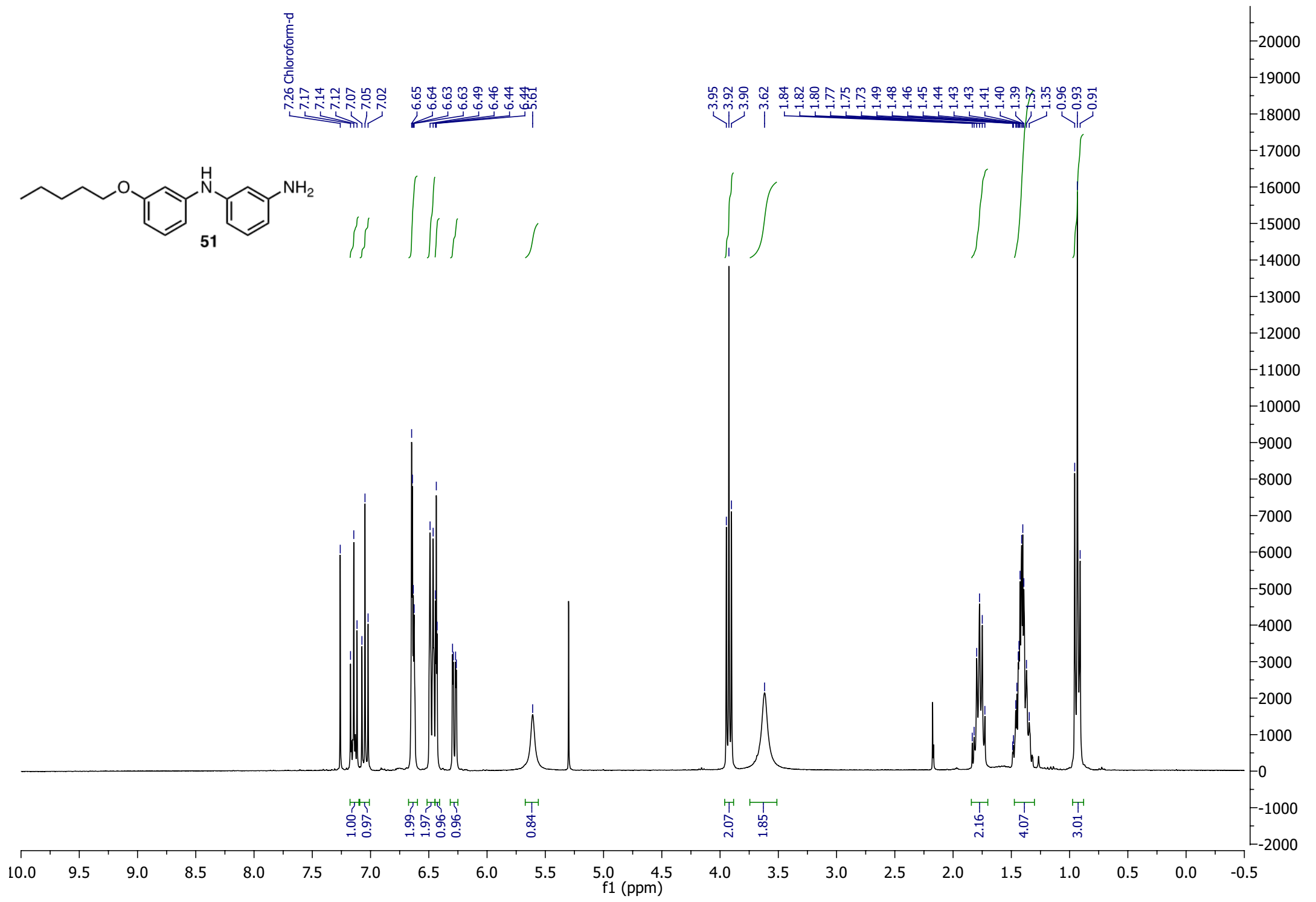
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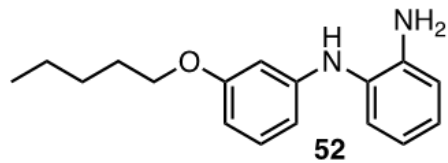
3.27





7.26 Chloroform-d
7.17
7.14
7.12
7.07
7.05
7.02
6.65
6.64
6.63
6.63
6.49
6.46
6.44
6.44
5.61
3.95
3.92
3.90
3.62
1.84
1.82
1.80
1.77
1.75
1.73
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1.46
1.45
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1.43
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1.41
1.40
1.39
1.37
1.35
0.96
0.93
0.91



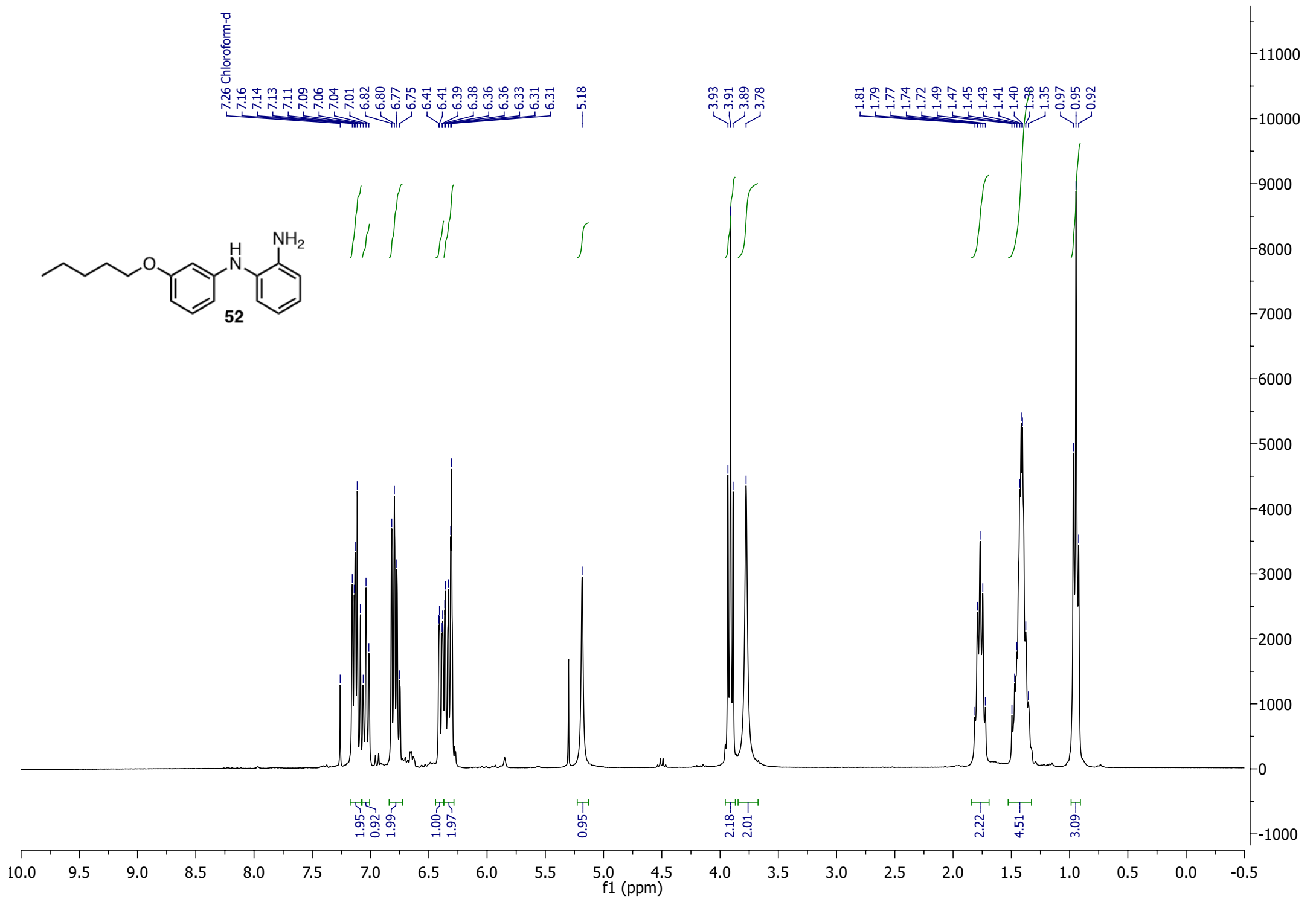


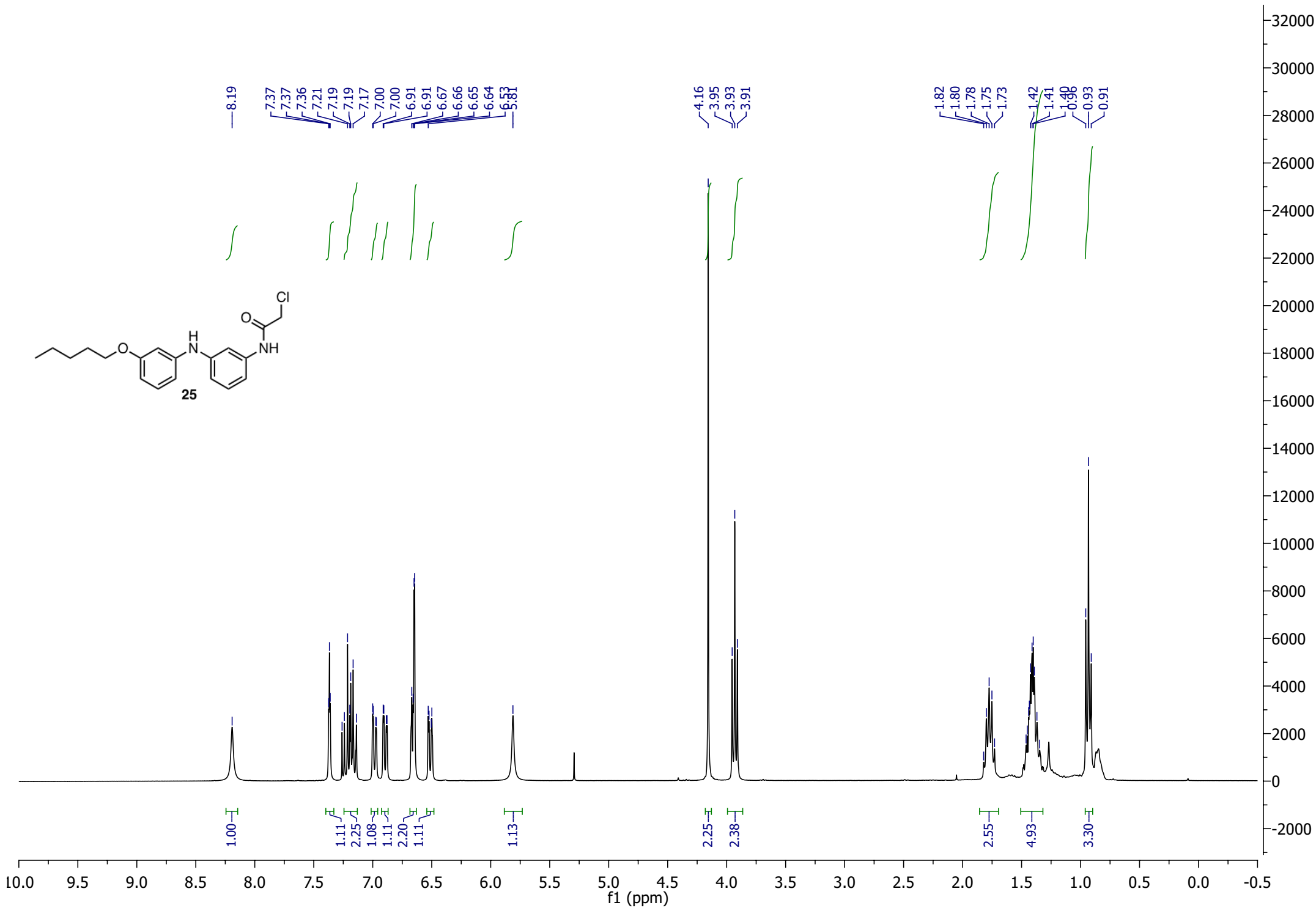
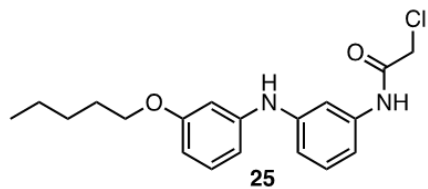
Chloroform-d

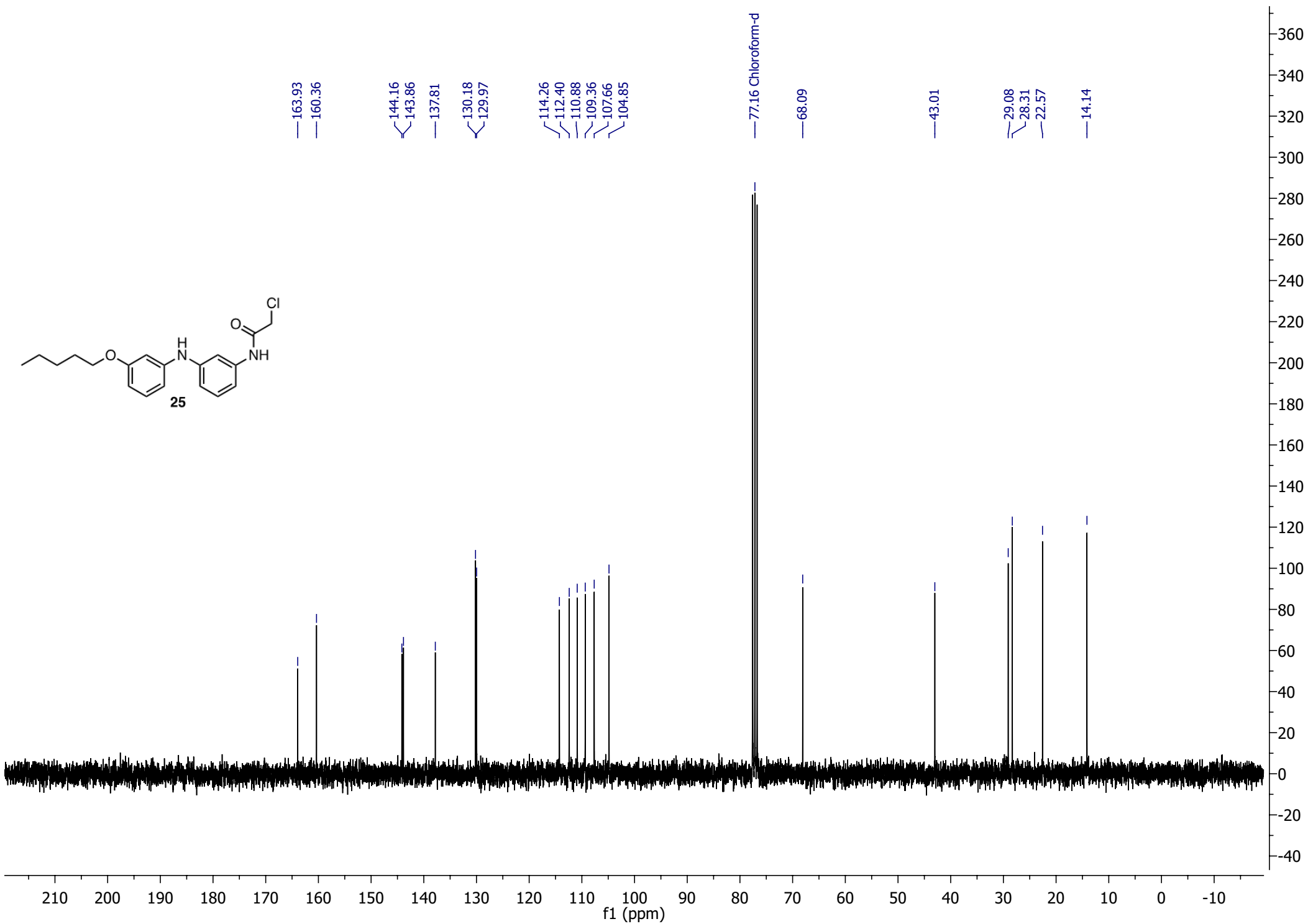
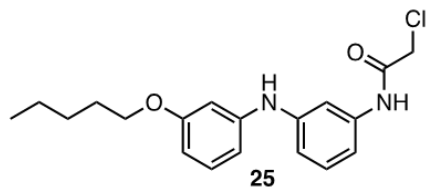
7.26	7.16	7.14	7.13	7.11	7.09	7.06	7.04	7.01	6.82	6.80	6.77	6.75	6.41	6.41	6.39	6.38	6.36	6.36	6.33	6.31	6.31	5.18
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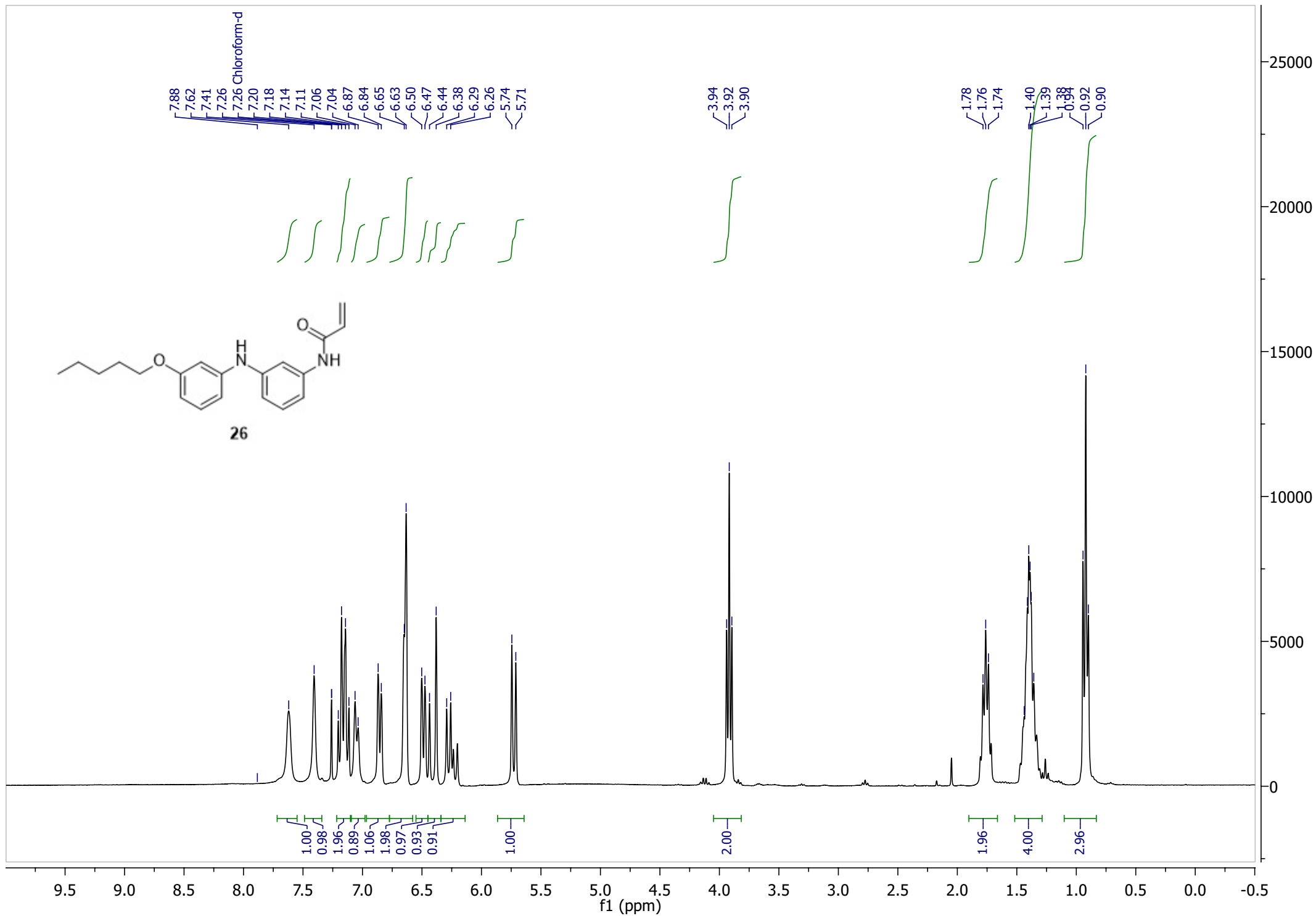
3.93	3.91	3.89	3.78
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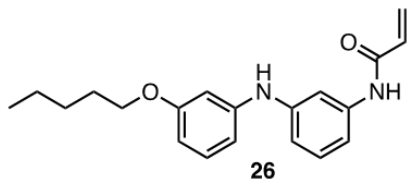
1.81	1.79	1.77	1.74	1.72	1.49	1.47	1.45	1.43	1.41	1.40	1.38	1.35	0.97	0.95	0.92
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