Electronic Supporting Information (ESI) for:

Novel Irreversible Peptidic Inhibitors of Transglutaminase 2

*Nicholas J. Cundy[1], Jane Arciszewski[1], Eric W. J. Gates[1], Sydney L. Acton[1], Kyle D. Passley[1], Ernest Awoonor-Williams[1], Elizabeth K. Boyd[1], Nancy Xu[1], Élise Pierson[1], Catalina Ferandez-Ansieta[1], Marie R. Albert[1], Nicole M. R. McNeil[1], Gautam Adhikary[2], Richard L. Eckert[2], Jeffrey W. Keillor[1]**

[1]Department of Chemistry and Biomolecular Sciences, University of Ottawa, Ottawa, Ontario K1N 6N5, Canada

[2]Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, Baltimore, Maryland 21201, United States

Table of Contents

Colorimetric Kinetic Assay of TG2 Inhibition

In order to assay the inhibition of the inhibitors proposed herein, a previously published procedure was adapted.[1–3] In brief, recombinant human transglutaminase 2 was expressed and purified from transformed BL21 *E. coli* as previously described.[4] The activity of the enzyme was monitored using an in-house developed colorimetric substrate namely AL5 (Cbz-Glu(ɣ-*p*-nitrophenylester)-Gly-OH),[5] which upon enzymatic hydrolysis can be monitored by absorbance at 405 nm. Under Kitz and Wilson conditions,[6,7] 125 µL assay buffer (111 mM MOPS, 16 mM $CaCl_2$, pH 6.9) was added to a 1.5-mL Eppendorf tube. To the tube was subsequently added the respective concentration of inhibitor (at least 4 concentrations for each inhibitor) from an aqueous working stock and 5 µL of an AL5 stock in DMSO (resulting in less than 5% DMSO in the final well). A corresponding volume of water was added to the tube to ensure each volume was consistent and the tubes were agitated. From the Eppendorf tube was removed 180 µL which placed in a 96-well polystyrene microplate. To each well was subsequently added 20 µL of prediluted TG2 (50 mU/mL) in assay buffer. The reaction was then monitored using a BioTek Synergy 4 plate reader for 20 min at 25 ˚C. In each inhibition assay a positive control (no inhibitor) and blank (no enzyme) was included and the final assay conditions contained various concentration of inhibitor, 100 µM AL5, 50 mM MOPS, 7.5 mM $CaCl_2$, 5 mU/mL TG2. Each assay was conducted in triplicate. The raw data then had the corresponding blank trial subtracted and the kinetic curves were fit to a one-phase association model to extract the observed first-order rate constants of inactivation using GraphPad Prism. The data were then reanalysed after truncating the kinetic curves to three half-lives. The rate constants were then analysed by a saturation model versus the inhibitor concentration divided by alpha ($\alpha = 1 + [S]/K_M$) to calculate the individual inhibition parameters k_{inact} and K_I. In instances where saturation was not achieved, a linear regression of the lowest 3 concentrations were analysed to acquire the slope, which corresponds to the ratio of k_{inact}/K_I.

Cell Proliferation Assay

SCC-13 cells were harvested, counted, and 1×10^5 cells were plated in standard 35-mm dishes in 3 mL of growth medium. Growth medium consisted of Dulbecco's Modified Eagle Medium (Thermo-Fisher, Frederick, MD) containing 4.5 mg/mL D-glucose, 2 mM L-glutamine, 100 mM sodium pyruvate, and 10% heat-inactivated fetal calf serum (FCS).[8] The cells were permitted to attach for 24 h and then treatment was initiated with inhibitors. The cells were permitted to grow for 48 h after the start of treatment, and the dishes were photographed before the cells were harvested with trypsin to make a single cell suspension for cell counting using a Coulter cell counter. The data are expressed as cell number per square cm of dish surface area.

Matrigel Invasion Assay

Matrigel (250 mg/mL) (BD Biosciences, San Jose, CA) was diluted in 0.01 M Tris-HCl/0.7% NaCl, filter sterilized, and 120 mL was used to coat each BD Biocoat Millicell insert semiporous membrane (1-cm diameter, 8-µm pores). Then each Millicell insert was placed into a well of a standard 24-well cluster plate. Cells (20, 000) were seeded in the upper chamber atop the Matrigel-coated insert membrane in the presence of growth medium containing 1% FCS, while the lower chamber contained growth medium supplemented with 10% FCS. Cell invasion through the Matrigel from the upper to lower chamber was monitored over 0 - 20 h

and detected as the number of cells localized in the lower surface of the insert membrane. To detect cell invasion, the membrane was fixed with 4% paraformaldehyde, incubated with DAPI (Invitrogen, Waltham, MA) to stain cell nuclei which were then visualized on a fluorescent microscope.

General Chemistry Experimental

Where available, commercially available reagents and solvents were purchased from suppliers including Sigma-Aldrich, Oakwood Products, Combi-Blocks, and Fisher Scientific and used without further purification. Thin layer chromatography (TLC) was performed using SiliCycle aluminium backed TLC plates 200 µm thickness with F-254 indicator and visualized using short wave UV light. Flash purification was performed using 230-400 mesh silica gel. All ^{1}H and ^{13}C NMR spectra were acquired at 298 K using a Bruker 400-MHz or 600-MHz spectrometer. ^{1}H- and ^{13}C-NMR spectra were referenced to the indicated deuterated solvent peaks and reported in ppm. Recorded NMR spectra were processed using MestReNova 14.2 – m (multiplet) refers to a resonance assignable to a single or equivalent proton(s) with indistinguishable multiplicity; stack refers to multiple, coincident chemical shift non-equivalent protons. High-resolution mass spectra were obtained using an electrospray ionization source (ESI) and quadrupole time-of-flight (QTOF) analyser or electron impact (EI) magnetic sector mass spectrometer.

General procedures

General procedure 1 – Naphthoylation of an amino acid

A solution of 1-naphthoyl chloride (1.2 eq) in THF (0.4 M) was added dropwise over 10 min to a 0 °C solution of amino acid (1 eq) in $NaOH_{(aq)}$ (2 M, 0.4 M) and the resulting mixture was stirred vigorously. After 3 h, the reaction was determined complete via TLC (10% MeOH in DCM) and the reaction mixture was extracted with Et_2O (20 mL). The two phases were separated and the ethereal portion was set aside. The aqueous was acidified via the addition of $HCl_{(aq)}$ (1 M, 50 mL) before being extracted with EtOAc (3 × 30 mL). The combined organics were washed with brine (100 mL) before being dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude was subjected to flash column chromatography (2.5-5% MeOH in DCM [1% AcOH]) to yield the desired product.

General procedure 2 – EDC HCl and HOBt amide couplings

N-Boc piperizine (1.2 eq), EDC-HCl (1.2 eq), and HOBt (1.0 eq) were added to a solution of carboxylic acid (1.0 eq) in DMF (0.09 M) and the resulting mixture was stirred overnight at room temperature. Upon completion, the reaction mixture was diluted with EtOAc (100 mL) and washed with water (3 x 100 mL), followed by brine (3 x 100 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was purified by flash column chromatography using a gradient elution system of 35-50% EtOAc in hexanes with 1 % triethylamine to yield the product.

General procedure 3 – TFA-mediated *N*-Boc deprotection with basic work up

TFA (10% vol) was added to a solution of *N*-Boc amine in DCM (0.1 M) and the resulting mixture was stirred at rt for 4 h-o/n. The reaction was quenched by slow addition of $NaHCO3_{(aq)}$ (Sat. Soln., 20 mL) before the organics were extracted with EtOAc (100 mL). The organic phase was washed with water (3 x 100 mL) and brine (3 x 100 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The product was used in the following step without further purification.

General procedure 4 – Reaction with an NHS-ester

Triethylamine (1.5 eq) was added to a solution of amine and NHS ester **27** and the resulting solution was stirred at room temperature overnight. The reaction mixture was diluted with DCM (10 mL), washed with 1M $HCl_{(aq)}$ (3 x 10 mL), $NaHCO_{3(aq)}$ (Sat. Soln., 10 mL) and brine (20 mL). The organic was dried over $MgSO_4$, filtered and concentrated under reduced pressure to afford the crude product. The crude product was purified by flash column (1-3% MeOH in DCM) to yield the desired material.

General procedure 5 – HBTU amide couplings with Et_3N as the base

Triethylamine (2.5 eq) and HBTU (1.5 eq) was added to a 0 °C solution of acid (1 eq) in DMF (0.15 M) and the resulting solution was stirred for 0.25 h. *N*-Boc Piperazine (1.5 eq) was then added and the solution was allowed to warm to rt. After stirred overnight the reaction was determined complete via TLC and was quenched via addition of $NaHCO_{3(aq)}$. The quenched reaction mixture was extracted with EtOAc (×3) before the combined organics were washed with water (×4) and brine. The organic was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude was purified by flash column chromatography (1:1 EtOAc:hexanes) to yield the desired compound.

General procedure 6 – TFA-mediated *N*-Boc deprotection

Trifluoroacetic acid (10% vol. eq) was added to a solution of Boc-protected amine (1 eq) in DCM (0.2 M) and the resulting mixture was stirred at rt for 3-4 h. The reaction mixture was concentrated to ~25% of its volume, cooled to 0 °C and Et_2O was added until a solid precipitate persisted – the reaction mixture was cooled to –20 °C overnight to encourage precipitation. The resulting solid was collected via suction filtration to yield the desired TFA salt which was taken forward with no further purification.

General procedure 7 – HBTU amide couplings with DIPEA as the base

Diisopropylethylamine (4 eq) and HBTU (1.5 eq) was added to a 0 °C solution of acid (1.5 eq) in DMF (0.15 M) and the resulting solution was stirred for 0.25 h. *N*-Boc Piperazine (1.5 eq) was then added and the solution was allowed to warm to rt. After stirred overnight the reaction was determined complete via TLC and was quenched via addition of $NaHCO_{3(aq)}$. The quenched reaction mixture was extracted with EtOAc (×3) before the combined organics were washed with water (×4) and brine. The organic was dried over $MgSO_4$, filtered and

concentrated under reduced pressure. The crude was purified by flash column chromatography (4:1 EtOAc:hexanes → 100% EtOAc) to yield the desired compound.

General procedure 8 – Pd/C catalysed hydrogenolysis of a Cbz protecting group

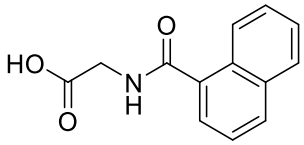
A solution of Cbz-amine (1 eq) and 10% Pd/C (5%) in MeOH (0.1 M) was degassed with $H_{2(g)}$ for 10 min – after degassing the reaction mixture was left under a positive pressure of $H_{2(g)}$ at rt overnight. Upon completion, the reaction mixture was passed through a celite filter to collect the solids. The celite plug was washed with MeOH (3×) and the eluent was concentrated under reduced pressure to yield the desired product with no need for further purification.

General procedure 9 – DMAP-catalysed acryloylation of an amine

Acryloyl chloride (1.3 eq) was added dropwise over 2 min to a 0 °C solution of amine (1 eq), DIPEA (2 eq) and DMAP (0.1 eq) in DCM (0.1 M) – after 0.25 h the reaction mixture was removed from the ice bath and allowed to warm to rt. After 0.75 h the reaction was determined complete via TLC and was quenched via addition of a saturated solution of $NaHCO_{3(aq)}$. The two phases were separated and the organic portion was washed sequentially with $NaHCO_{3(aq)}$, water and brine. The organic portion was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude was subjected to flash column chromatography (100% EtOAc or 1-5% MeOH in EtOAc) to yield the desired acrylamide.

Characterisation data

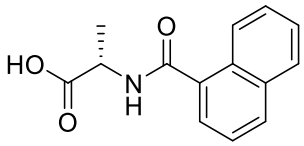
(1-naphthoyl)glycine (1)



General procedure 1 followed to yield **1** (839 mg, 54%) as a white solid.

^{1}H NMR (400 MHz, Methanol-d_4) δ 8.37 – 8.31 (m, 1H), 7.99 (d, J = 8.3 Hz, 1H), 7.95 – 7.89 (m, 1H), 7.70 (dd, J = 7.1, 1.2 Hz, 1H), 7.59 – 7.49 (m, 3H), 4.17 (s, 2H); ^{13}C NMR (101 MHz, Methanol-d_4) δ 172.9, 135.3, 135.1, 131.6, 131.5, 129.3, 127.9, 127.4, 126.5, 126.5, 125.9, 49.6, 49.4, 49.2, 49.0, 48.8, 48.6, 48.4, 42.3; HRMS (ESI-QTOF) m/z: $[M + Na]^+$ Calcd for $C_{13}H_{11}NO_3Na$ 252.0637 found 252.0639

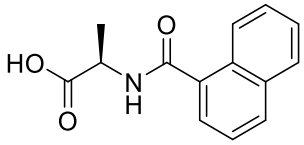
(1-naphthoyl)-L-alanine (2)



General procedure 1 followed to yield **2** (1.68 g, 62%) as a white solid.

^{1}H NMR (400 MHz, Chloroform-d) δ_H 8.27 (d, J = 8.8 Hz, 1H), 7.87 (d, J = 8.4 Hz, 1H), 7.81 (d, J = 7.6 Hz, 1H), 7.60 (d, J = 6.8 Hz, 1H), 7.53–7.44 (m, 2H), 7.40 (t, J = 7.2 Hz, 1H), 6.50–6.42 (br, 1H), 4.05 (q, J = 6.8 Hz, 1H), 1.56 (d, J = 7.2 Hz, 1H); ^{13}C (101 MHz, Chloroform-d) δ_C 175.2, 168.7, 132.6, 132.1, 130.2, 129.1, 127.3, 126.4, 125.5, 124.4, 124.2, 123.6, 47.6, 17.0; HRMS (ESI-QTOF) m/z: $[M + Na]^+$ Calcd for $C_{14}H_{13}NO_3Na$ 266.0793; found 266.0800.

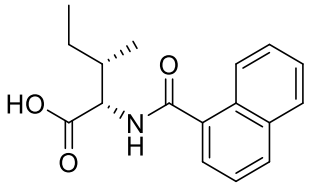
(1-naphthoyl)-D-alanine (3)



General procedure 1 followed to yield **3** (1.50 g, 55%) as a white solid.

^{1}H NMR (400 MHz, Methanol-d_4) δ 8.33 – 8.26 (m, 1H), 7.97 (dt, J = 8.3, 1.1 Hz, 1H), 7.94 – 7.88 (m, 1H), 7.66 (dd, J = 7.1, 1.3 Hz, 1H), 7.58 – 7.49 (m, 3H), 4.69 (q, J = 7.4 Hz, 1H), 1.53 (d, J = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 176.0, 172.4, 135.4, 135.1, 131.5, 131.5, 129.3, 127.9, 127.4, 126.5, 126.4, 125.9, 17.4, 14.4; HRMS (ESI-QTOF) m/z: $[M + Na]^+$ Calcd for $C_{14}H_{13}NO_3Na$ 266.0793; found 266.0788.

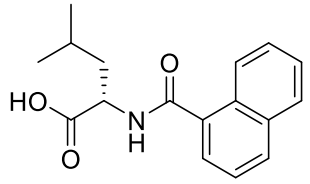
(1-naphthoyl)-L-isoleucine (4)



General procedure 1 followed to yield **4** (475 mg, 40%) as a white solid.

^{1}H NMR (400 MHz, Methanol-d_4) δ 8.21 (dddt, J = 8.3, 3.0, 2.1, 1.1 Hz, 1H), 7.97 (dt, J = 8.3, 1.1 Hz, 1H), 7.95 – 7.88 (m, 1H), 7.64 (dd, J = 7.0, 1.3 Hz, 1H), 7.59 – 7.48 (m, 3H), 4.68 (d, J = 6.0 Hz, 1H), 2.20 – 1.98 (m, 1H), 1.69 – 1.53 (m, 1H), 1.45 – 1.27 (m, 1H), 1.13 – 0.94 (m, 6H); ^{13}C NMR (101 MHz, Methanol-d_4) δ 175.2, 174.9, 172.9, 172.7, 135.7, 135.6, 135.1, 131.5, 131.4, 131.4, 129.3, 127.9, 127.4, 126.4, 125.9, 58.9, 57.5, 38.2, 38.1, 27.6, 26.5, 16.3, 15.4, 12.1, 11.8; HRMS (ESI-QTOF) m/z: $[M + Na]^+$ Calcd for $C_{17}H_{19}NO_3Na$ 308.1263; found 308.1270

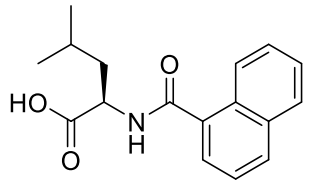
(1-naphthoyl)-L-leucine (5)



General procedure 1 followed to yield **5** (718 mg, 75%) as a white solid.

^{1}H NMR (400 MHz, Methanol-d_4) δ 8.29 – 8.21 (m, 1H), 8.01 – 7.94 (m, 1H), 7.94 – 7.88 (m, 1H), 7.64 (dt, J = 7.1, 1.3 Hz, 1H), 7.53 (dddd, J = 8.2, 6.9, 5.8, 3.1 Hz, 3H), 4.78 – 4.72 (m, 1H), 1.92 – 1.70 (m, 3H), 1.03 (ddd, J = 14.9, 6.4, 1.1 Hz, 6H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 176.0, 172.7, 135.5, 135.1, 131.5, 131.4, 129.3, 127.9, 127.4, 126.4, 126.3, 125.9, 52.7, 49.6, 49.4, 49.2, 49.0, 48.8, 48.6, 48.4, 41.3, 32.7, 26.3, 23.7, 23.4, 21.8, 20.7, 14.4; HRMS (ESI-QTOF) m/z: $[M + Na]^+$ Calcd for $C_{17}H_{19}NO_3Na$ 308.1263; found 308.1265

(1-naphthoyl)-D-leucine (6)

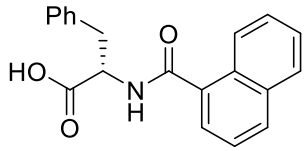


General procedure 1 followed to yield **6** (590 mg, 62%) as a white solid.

^{1}H NMR (400 MHz, Methanol-d_4) δ 8.29 – 8.21 (m, 1H), 7.97 (dt, J = 8.2, 1.0 Hz, 1H), 7.94 – 7.87 (m, 1H), 7.65 (dd, J = 7.0, 1.3 Hz, 1H), 7.59 – 7.48 (m, 3H), 4.75 (dd, J = 9.2, 5.9 Hz, 1H), 1.93 – 1.69 (m, 3H), 1.03 (dd, J = 14.9, 6.4 Hz, 6H); ^{13}C NMR (101 MHz, Methanol-d_4) δ 176.0, 172.7, 135.5, 135.1, 131.5, 131.4, 129.3, 127.9, 127.4, 126.4, 126.3, 125.9, 52.7, 41.3,

26.3, 23.4, 21.8; HRMS (ESI-QTOF) m/z: $[M + Na]^+$ Calcd for $C_{17}H_{19}NO_3Na$ 308.1263; found 308.1269

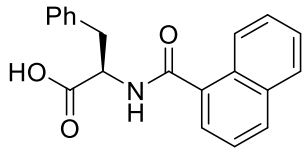
(1-naphthoyl)-L-phenylalanine (7)



General procedure 1 followed to yield **7** (953 mg, 49%) as a white solid.

^{1}H-NMR (400 MHz, Chloroform-*d*) δ 8.15 – 8.09 (m, 1H), 7.90 (d, *J* = 8.2 Hz, 1H), 7.87 – 7.81 (m, 1H), 7.49 (tt, *J* = 6.6, 5.1 Hz, 3H), 7.40 (dd, *J* = 8.2, 7.1 Hz, 1H), 7.34 – 7.27 (m, 3H), 7.24 (dd, *J* = 7.7, 1.8 Hz, 2H), 6.44 (d, *J* = 7.7 Hz, 1H), 5.21 (td, *J* = 7.3, 5.3 Hz, 1H), 3.45 (dd, *J* = 14.1, 5.3 Hz, 1H), 3.21 (dd, *J* = 14.1, 7.1 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.1, 169.9, 135.8, 133.7, 133.2, 131.3, 130.1, 129.5, 129.0, 128.4, 127.5 (d, *J* = 3.7 Hz), 126.6, 125.6, 125.3, 124.8, 53.8, 37.5; HRMS (ESI-QTOF) m/z: $[M + Na]^+$ Calcd for $C_{20}H_{17}NO_3Na$ 342.1106; found 342.1106.

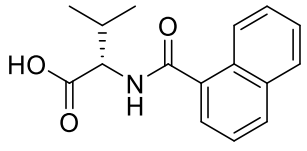
(1-naphthoyl)-D-phenylalanine (8)



General procedure 1 followed to yield **8** (1.22 g, 70%) as a white solid.

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.92 (ddd, *J* = 6.6, 3.2, 0.9 Hz, 1H), 7.86 (ddd, *J* = 8.2, 1.4, 0.7 Hz, 1H), 7.79 (dq, *J* = 8.5, 0.9 Hz, 1H), 5.03 (dd, *J* = 10.6, 4.6 Hz, 1H), 3.42 (dd, *J* = 13.9, 4.6 Hz, 1H), 3.04 (dd, *J* = 14.0, 10.7 Hz, 1H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 174.7, 172.3, 138.9, 135.5, 135.0, 131.3, 131.3, 130.4, 129.6, 129.1, 127.8, 127.8, 127.3, 126.4, 126.1, 125.8, 55.5, 49.6, 49.4, 49.2, 49.0, 48.8, 48.6, 48.4, 38.3, 20.7; HRMS (ESI-QTOF) m/z: $[M + Na]^+$ Calcd for $C_{20}H_{17}NO_3Na$ 342.1106; found 342.1101

(1-naphthoyl)-L-valine (9)

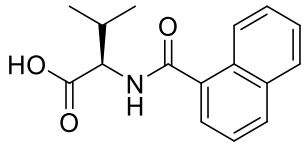


General procedure 1 followed to yield **9** (197 mg, 37%) as a white solid.

^{1}H NMR (400 MHz, Methanol-d_4) δ 8.24 – 8.20 (m, 1H), 7.99 – 7.97 (d, 1H), 7.93 – 7.90 (m, 1H), 7.66 – 7.64 (dd, J = 7.0, 1.1 Hz, 1H), 7.57 – 7.51 (m, 3H), 4.63 – 4.62 (d, J = 6.0 Hz, 1H),

2.36 – 2.28 (dq, J = 13.2, 6.7x(3), 1H), 1.13 – 1.11 (d, J = 6.9 Hz, 3H), 1.07 – 1.05 (d, J = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, Methanol-*d*$_4$) δ 173.5, 171.4, 134.2, 133.7, 130.1, 130.0, 127.9, 126.5, 126.0, 125.0, 124.5, 58.5, 30.2, 18.4, 17.3; HRMS (ESI-QTOF) *m/z* [M + Na]+ Calcd for $C_{16}H_{17}NO_3Na$ 294.1106; found 294.1106.

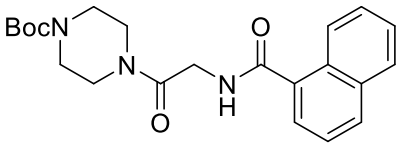
(1-naphthoyl)-D-valine (10)



General procedure 1 followed to yield **10** (305 mg, 26%) as a white solid.

^{1}H NMR (400 MHz, Methanol-*d*$_4$) δ 8.34 (dd, J = 8.2, 1.4 Hz, 1H), 7.95 (d, J = 8.3 Hz, 1H), 7.89 (dd, J = 8.0, 1.7 Hz, 1H), 7.69 (dd, J = 7.1, 1.3 Hz, 1H), 7.60 – 7.46 (m, 3H), 6.45 (d, J = 8.7 Hz, 1H), 4.97 – 4.91 (m, 1H), 2.48 – 2.38 (m, 1H), 1.30 – 1.22 (m, 1H), 1.14 (d, J = 6.9 Hz, 3H), 1.04 (d, J = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, Methanol-*d*$_4$) δ 174.8, 172.8, 135.6, 135.1, 131.5, 131.4, 129.3, 127.9, 127.4, 126.4, 126.4, 125.9, 59.8, 31.6, 19.8, 18.7; HRMS (EI-Magnetic Sector) m/z [M]+ Calcd for $C_{16}H_{17}NO_3$ 271.1208; found 271.1193.

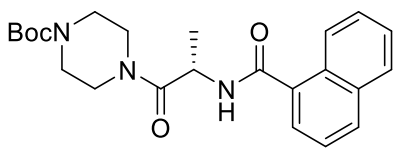
***tert*-Butyl 4-((1-naphthoyl)glycyl)piperazine-1-carboxylate (11)**



General procedure 2 followed to yield **11** (1.17 g, 81%) as a white solid.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.37 – 8.31 (m, 1H), 7.94 – 7.81 (m, 2H), 7.67 (dd, *J* = 7.1, 1.3 Hz, 1H), 7.58 – 7.40 (m, 3H), 4.32 (d, *J* = 3.5 Hz, 2H), 3.62 – 3.35 (m, 8H), 1.47 (s, 9H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 169.5, 169.5, 166.7, 166.7, 154.5, 133.7, 133.7, 133.7, 131.0, 130.2, 128.4, 127.2, 126.5, 125.6, 125.4, 124.8, 124.3, 118.7, 110.5, 80.6, 77.5, 76.8, 45.8, 44.4, 41.9, 41.8, 41.7, 28.4, 8.7; HRMS (ESI-QTOF) m/z: [M + Na]$^+$ Calcd for $C_{22}H_{27}N_3O_4Na$ 420.1899; found 420.1884.

***tert*-Butyl 4-((1-naphthoyl)-L-alanyl)piperazine-1-carboxylate (12)**

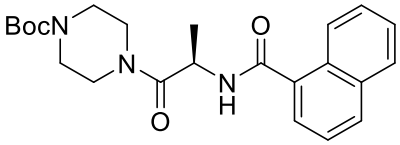


General procedure 2 followed to yield **12** (170 g, 28%) as a white solid.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.37 – 8.30 (m, 1H), 7.96 – 7.84 (m, 2H), 7.66 (dd, *J* = 7.1, 1.3 Hz, 1H), 7.60 – 7.41 (m, 3H), 7.13 (d, *J* = 7.5 Hz, 1H), 5.28 – 5.13 (m, 1H), 3.79 – 3.34 (m, 8H), 1.50 (d, *J* = 15.4 Hz, 12H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 170.9, 168.6, 154.5, 133.6, 133.7, 130.9, 130.2, 128.3, 127.2, 126.4, 125.3, 125.3, 124.7, 80.6, 45.7, 45.4, 42.1, 28.4, 19.2; HRMS (ESI-QTOF) m/z: [M + Na]$^{+}$ Calcd for $C_{23}H_{29}N_3O_4Na$ 434.2056; found 434.2068.

N.B. Compounds 12 and 50 share the same structure but were prepared via different routes.

tert-Butyl 4-((1-naphthoyl)-D-alanyl)piperazine-1-carboxylate (13)

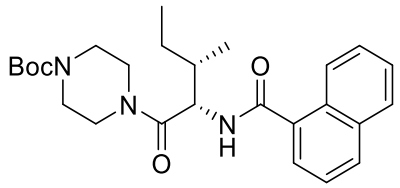


General procedure 2 followed to yield **13** (117 mg, 69%) as a white solid.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.34 (dd, *J* = 8.3, 1.5 Hz, 1H), 7.96 – 7.83 (m, 2H), 7.65 (dd, *J* = 7.1, 1.3 Hz, 1H), 7.60 – 7.41 (m, 3H), 7.13 (d, *J* = 7.5 Hz, 1H), 5.21 (qd, *J* = 6.9, 4.5 Hz, 1H), 3.79 – 3.36 (m, 8H), 1.50 (d, *J* = 14.1 Hz, 12H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 171.1, 168.7, 154.6, 133.9, 133.8, 131.0, 130.3, 128.5, 127.3, 126.5, 125.5, 125.4, 124.8, 80.7, 45.8, 45.7, 45.5, 42.2, 28.5, 19.4; HRMS (ESI-QTOF) m/z: [M + Na]$^{+}$ Calcd for $C_{23}H_{29}N_3O_4Na$ 434.2056; found 434.2057.

N.B. Compounds 13 and 51 share the same structure but were prepared via different routes.

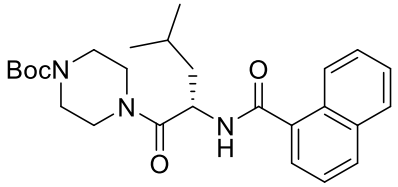
tert-Butyl 4-((1-naphthoyl)-L-isoleucyl)piperazine-1-carboxylate (14)



General procedure 2 followed to yield **14** (537 mg, 69%) as a white solid.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.37 – 8.30 (m, 1H), 7.96 – 7.83 (m, 2H), 7.66 (td, *J* = 7.1, 1.3 Hz, 1H), 7.59 – 7.42 (m, 3H), 6.83 (dd, *J* = 23.1, 8.9 Hz, 1H), 5.34-5.13 (m, 1H), 3.81 – 3.37 (m, 8H), 1.90 – 1.58 (m, 3H), 1.48 (d, *J* = 1.3 Hz, 9H), 1.25 (s, 1H), 1.11 – 1.02 (m, 3H), 0.99 – 0.81 (m, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 170.6, 170.6, 169.4, 154.6, 134.1, 133.9, 131.1, 131.1, 130.3, 128.5, 127.3, 127.3, 126.5, 125.6, 125.5, 125.4, 125.4, 124.8, 124.8, 80.6, 53.3, 52.3, 46.0, 45.8, 42.2, 38.6, 38.4, 28.5, 27.1, 24.4, 16.3, 14.4, 12.2, 11.6; HRMS (ESI-QTOF) m/z: [M + Na]$^{+}$ Calcd for $C_{26}H_{35}N_3O_4Na$ 476.2525; found 476.2511.

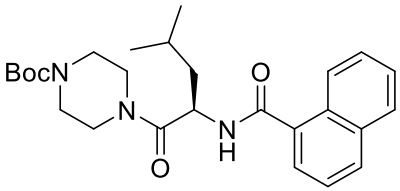
***tert*-Butyl 4-((1-naphthoyl)-L-leucyl)piperazine-1-carboxylate (15)**



General procedure 2 followed to yield **15** (844 mg, 69%) as a white solid.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.37 – 8.30 (m, 1H), 7.92 (dt, *J* = 8.3, 1.1 Hz, 1H), 7.89 – 7.83 (m, 1H), 7.66 (dd, *J* = 7.1, 1.3 Hz, 1H), 7.60 – 7.49 (m, 2H), 7.46 (dd, *J* = 8.3, 7.1 Hz, 1H), 6.81 (d, *J* = 8.7 Hz, 1H), 5.38 – 5.26 (m, 1H), 3.80 – 3.34 (m, 9H), 1.91 – 1.77 (m, 1H), 1.72 – 1.52 (m, 3H), 1.48 (s, 9H), 1.11 (d, *J* = 6.5 Hz, 3H), 0.98 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 171.2, 169.1, 154.6, 133.9, 133.9, 131.1, 130.3, 128.5, 127.3, 126.6, 125.5, 125.5, 124.8, 80.6, 47.8, 45.6, 42.9, 42.2, 28.5, 25.1, 23.6, 22.3; HRMS (ESI-QTOF) m/z: $[M + Na]^+$ Calcd for $C_{26}H_{35}N_3O_4Na$ 434.2525; found 434.2535.

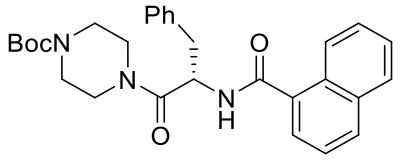
***tert*-Butyl 4-((1-naphthoyl)-D-leucyl)piperazine-1-carboxylate (16)**



General procedure 2 followed to yield **16** (642 mg, 74%) as a white solid.

^{1}H NMR (400 MHz, Methanol-d_4) δ 8.25 – 8.18 (m, 1H), 7.98 (dt, *J* = 8.2, 1.1 Hz, 1H), 7.95 – 7.89 (m, 1H), 7.65 (dd, *J* = 7.1, 1.3 Hz, 1H), 7.59 – 7.49 (m, 2H), 5.19 (dd, *J* = 10.1, 4.5 Hz, 1H), 3.93 – 3.76 (m, 1H), 3.71 – 3.33 (m, 2H), 1.89 – 1.71 (m, 1H), 1.66 – 1.53 (m, **0H**), 1.49 (s, 5H), 1.08 (d, *J* = 6.4 Hz, 2H), 1.01 (d, *J* = 6.5 Hz, 2H); ^{13}C NMR (101 MHz, Methanol-d_4) δ 173.1, 172.3, 156.3, 135.1, 131.6, 131.5, 129.4, 128.0, 127.4, 126.5, 126.3, 125.9, 81.7, 46.7, 43.3, 41.6, 28.6, 26.3, 23.6, 22.1; HRMS (ESI-QTOF) m/z: $[M + Na]^+$ Calcd for $C_{26}H_{35}N_3O_4Na$ 434.2525; found 434.2527.

***tert*-Butyl 4-((1-naphthoyl)-L-phenylalanyl)piperazine-1-carboxylate (17)**



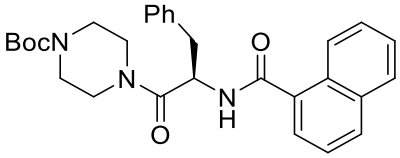
General procedure 2 followed to yield **17** (703 mg, 80%) as a white solid.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.25 – 8.19 (m, 1H), 7.94 – 7.83 (m, 2H), 7.60 (dd, *J* = 7.1, 1.3 Hz, 1H), 7.56 – 7.48 (m, 2H), 7.44 (dd, *J* = 8.3, 7.1 Hz, 1H), 7.35 – 7.24 (m, 5H), 6.98 (d, *J* = 8.2 Hz, 1H), 5.47 (td, *J* = 8.4, 5.9 Hz, 1H), 3.62 – 3.48 (m, 2H), 3.47 – 3.29 (m, 3H),

3.28 – 3.08 (m, 4H), 2.83 (d, *J* = 16.0 Hz, 1H), 1.45 (s, 9H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 170.1, 168.8, 154.5, 136.1, 133.8, 133.7, 131.1, 130.3, 129.8, 128.9, 128.4, 127.5, 127.3, 126.5, 125.5, 125.4, 124.8, 80.5, 50.4, 45.6, 42.1, 40.1, 28.5; HRMS (ESI-QTOF) m/z: [M + Na]$^+$ Calcd for $C_{29}H_{33}N_3O_4Na$ 510.2369; found 510.2351.

N.B. Compounds 17 and 52 share the same structure but were prepared via different routes.

tert-Butyl 4-((1-naphthoyl)-D-phenylalanyl)piperazine-1-carboxylate (18)

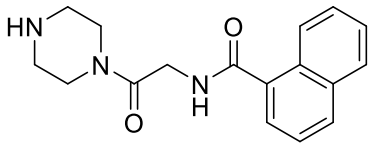


General procedure 2 followed to yield **18** (370 mg, 38%) as a white solid.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.26 – 8.19 (m, 1H), 7.94 – 7.83 (m, 2H), 7.60 (dd, *J* = 7.1, 1.3 Hz, 1H), 7.56 – 7.48 (m, 2H), 7.44 (dd, *J* = 8.2, 7.0 Hz, 1H), 7.36 – 7.27 (m, 5H), 6.95 (d, *J* = 7.6 Hz, 1H), 5.48 (td, *J* = 8.4, 5.9 Hz, 1H), 3.63 – 3.06 (m, 9H), 2.89 – 2.79 (m, 1H), 1.46 (s, 9H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 170.1, 168.8, 154.5, 136.1, 133.8, 133.7, 131.1, 130.3, 129.8, 128.9, 128.4, 127.5, 127.3, 126.6, 125.5, 125.4, 124.8, 80.5, 50.4, 45.6, 42.1, 40.1, 28.5; HRMS (ESI-QTOF) m/z: [M + Na]$^+$ Calcd for $C_{29}H_{33}N_3O_4Na$ 510.2369; found 510.2376.

N.B. Compounds 18 and 53 share the same structure but were prepared via different routes.

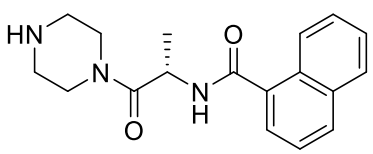
***N*-(2-oxo-2-(piperazin-1-yl)ethyl)-1-naphthamide (19)**



General procedure 3 followed to yield **19** (738 mg, 90%).

^{1}H NMR (400 MHz, Methanol-d_4) δ 8.42 – 8.32 (m, 1H), 8.04 – 7.88 (m, 2H), 7.73 (dd, *J* = 7.1, 1.3 Hz, 1H), 7.61 – 7.48 (m, 3H), 4.33 (s, 2H), 3.59 (dt, *J* = 13.4, 5.2 Hz, 4H), 2.99 – 2.77 (m, 4H); ^{13}C NMR (101 MHz, Methanol-d_4) δ 172.7, 168.9, 135.3, 135.1, 131.7, 131.5, 129.3, 128.0, 127.5, 126.6, 125.9, 49.6, 49.4, 49.2, 49.0, 48.8, 48.6, 48.4, 46.5, 46.4, 46.1, 43.7, 42.3, 31.1; HRMS (ESI-QTOF) m/z: [M + Na]$^+$ Calcd for $C_{17}H_{19}N_3O_2Na$ 320.1375; found 320.1380.

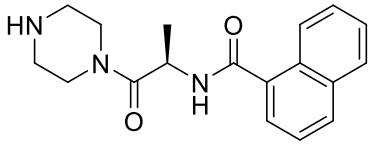
(*S*)-*N*-(1-oxo-1-(piperazin-1-yl)propan-2-yl)-1-naphthamide (20)



General procedure 3 followed to yield **20** (55 mg, 44%).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.31 (dd, *J* = 8.3, 1.4 Hz, 1H), 7.93 – 7.78 (m, 2H), 7.63 (dd, *J* = 7.0, 1.2 Hz, 1H), 7.58 – 7.38 (m, 3H), 7.32 (d, *J* = 7.5 Hz, 1H), 5.14 (p, *J* = 7.0 Hz, 1H), 3.72 – 3.40 (m, 4H), 2.98 – 2.69 (m, 4H), 1.45 (dd, *J* = 6.8, 3.7 Hz, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 170.6, 168.6, 133.9, 133.7, 130.8, 130.2, 128.3, 127.1, 126.4, 125.3, 124.7, 46.5, 46.1, 45.7, 45.6, 43.1, 31.2, 19.1; HRMS (ESI-QTOF) m/z: [M + Na]$^+$ Calcd for $C_{18}H_{21}N_3O_2Na$ 334.1531; found 334.1534.

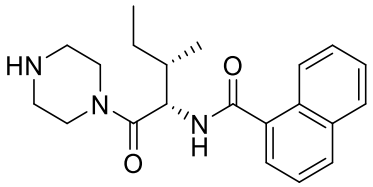
(*R*)-*N*-(1-oxo-1-(piperazin-1-yl)propan-2-yl)-1-naphthamide (21)



General procedure 3 followed to yield **21** (39 mg, 34%).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.33 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.96 – 7.82 (m, 2H), 7.66 (dd, *J* = 7.0, 1.2 Hz, 1H), 7.60 – 7.38 (m, 4H), 7.21 (d, *J* = 7.4 Hz, 1H), 5.18 (p, *J* = 7.0 Hz, 1H), 3.79 – 3.49 (m, 4H), 3.04 – 2.82 (m, 4H), 1.50 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 170.8, 168.85, 134.0, 133.8, 131.0, 130.3, 128.5, 127.3, 126.5, 125.5, 125.4, 124.8, 46.5, 46.1, 45.8, 45.7, 43.0, 31.4, 19.3; HRMS (ESI-QTOF) m/z: [M + Na]$^+$ Calcd for $C_{18}H_{21}N_3O_2Na$ 334.1531; found 334.1520.

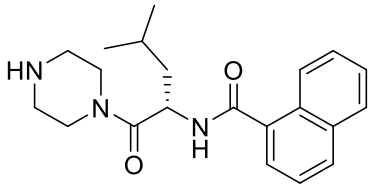
***N*-((*2S,3S*)-3-methyl-1-oxo-1-(piperazin-1-yl)pentan-2-yl)-1-naphthamide (22)**



General procedure 3 followed to yield **22** (235 mg, 59%).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.33 (dd, *J* = 8.4, 3.8 Hz, 1H), 7.98 – 7.80 (m, 2H), 7.67 (td, *J* = 7.0, 1.2 Hz, 1H), 7.61 – 7.36 (m, 4H), 6.92 (dd, *J* = 16.0, 9.0 Hz, 1H), 5.34-5.13 (m, 1H), 3.63 (ddddd, *J* = 42.2, 13.2, 10.5, 6.7, 3.8 Hz, 4H), 3.07 – 2.76 (m, 4H), 1.94 – 1.73 (m, 1H), 1.72 – 1.49 (m, 1H), 1.42 – 1.14 (m, 1H), 1.14 – 0.85 (m, 6H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 170.3, 170.2, 169.4, 169.3, 134.2, 134.1, 133.8, 131.0, 130.9, 130.3, 128.4, 127.4, 127.3, 127.2, 126.6, 126.5, 125.6, 125.5, 125.4, 125.4, 125.3, 124.8, 77.5, 77.2, 76.8, 53.2, 52.1, 47.2, 47.0, 46.5, 46.4, 46.0, 43.3, 38.5, 38.4, 31.3, 27.1, 24.3, 16.3, 14.3, 12.2, 11.6; HRMS (ESI-QTOF) m/z: [M + Na]$^+$ Calcd for $C_{21}H_{27}N_3O_2Na$ 376.2001; found 376.2012.

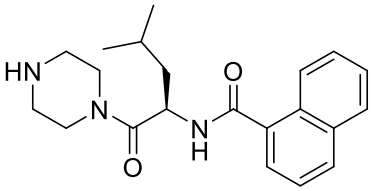
(*S*)-*N*-(4-methyl-1-oxo-1-(piperazin-1-yl)pentan-2-yl)-1-naphthamide (23)



General procedure 3 followed to yield **23** (459 mg, 70%).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.39 – 8.30 (m, 1H), 7.97 – 7.83 (m, 2H), 7.67 (dd, *J* = 7.1, 1.3 Hz, 1H), 7.60 – 7.41 (m, 3H), 6.89 (d, *J* = 8.7 Hz, 1H), 5.37 – 5.27 (m, 1H), 3.78 – 3.46 (m, 4H), 3.06 – 2.79 (m, 4H), 1.87 – 1.79 (m, 1H), 1.73 – 1.50 (m, 2H), 1.11 (d, *J* = 6.5 Hz, 3H), 0.98 (d, *J* = 6.7 Hz, 3H); ^{13}C NMR (101 MHz, Chloroform-d) δ 170.9, 169.1, 134.1, 133.8, 131.0, 130.3, 128.4, 127.3, 126.5, 125.5, 125.4, 124.8, 47.7, 46.9, 46.4, 45.9, 43.4, 43.0, 25.1, 23.67, 22.3; HRMS (ESI-QTOF) m/z: $[M + Na]^+$ Calcd for $C_{21}H_{27}N_3O_2Na$ 376.2001; found 376.1994.

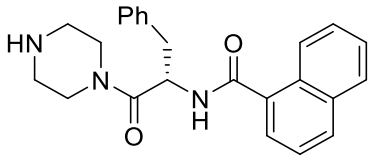
(*R*)-*N*-(4-methyl-1-oxo-1-(piperazin-1-yl)pentan-2-yl)-1-naphthamide (24)



General procedure 3 followed to yield **24** (340 mg, 68%).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.39 – 8.30 (m, 1H), 7.96 – 7.83 (m, 2H), 7.67 (dd, *J* = 7.1, 1.3 Hz, 1H), 7.60 – 7.40 (m, 3H), 6.92 (d, *J* = 8.7 Hz, 1H), 5.38 – 5.24 (m, 1H), 3.69 (ddd, *J* = 12.5, 6.9, 3.9 Hz, 2H), 3.62 – 3.48 (m, 2H), 3.05 – 2.79 (m, 3H), 1.84 (dpd, *J* = 9.1, 6.6, 4.7 Hz, 1H), 1.73 – 1.46 (m, 2H), 1.11 (d, *J* = 6.5 Hz, 3H), 0.98 (d, *J* = 6.7 Hz, 3H); ^{13}C NMR (101 MHz, Chloroform-d) δ 171.0, 169.1, 134.0, 133.8, 131.0, 130.3, 128.4, 127.3, 126.5, 125.5, 125.4, 124.8, 47.7, 46.9, 46.4, 45.9, 43.3, 42.9, 25.1, 23.6, 22.3. HRMS (ESI-QTOF) m/z: $[M + Na]^+$ Calcd for $C_{21}H_{27}N_3O_2Na$ 376.2001; found 376.2018.

(*S*)-*N*-(1-oxo-3-phenyl-1-(piperazin-1-yl)propan-2-yl)-1-naphthamide (25)

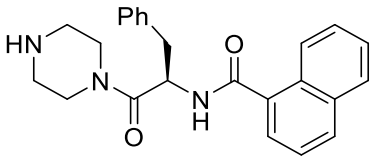


General procedure 3 followed to yield **25** (369 mg, 67%).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.26 – 8.16 (m, 1H), 7.94 – 7.80 (m, 2H), 7.64 – 7.39 (m, 4H), 7.37 – 7.27 (m, 4H), 7.07 (d, *J* = 8.2 Hz, 1H), 5.48 (td, *J* = 8.0, 6.3 Hz, 1H), 3.63 – 3.36 (m, 3H), 3.26 – 3.05 (m, 3H), 2.89 – 2.63 (m, 3H), 2.35 (ddd, *J* = 12.2, 7.0, 3.1 Hz, 1H);

^{13}C NMR (101 MHz, Chloroform-d) δ 169.7, 168.8, 136.2, 133.9, 133.8, 130.9, 130.3, 129.8, 128.7, 128.4, 127.3, 127.2, 126.5, 125.5, 125.4, 124.8, 50.2, 47.0, 45.9, 45.7, 43.3, 40.0; HRMS (ESI-QTOF) m/z: $[M + Na]^+$ Calcd for $C_{24}H_{25}N_3O_2Na$ 410.1844; found 410.1824.

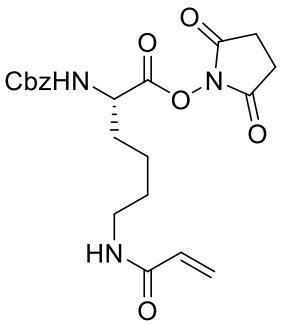
(*R*)-*N*-(1-oxo-3-phenyl-1-(piperazin-1-yl)propan-2-yl)-1-naphthamide (26)



General procedure 3 followed to yield **26** (195 mg, 66%).

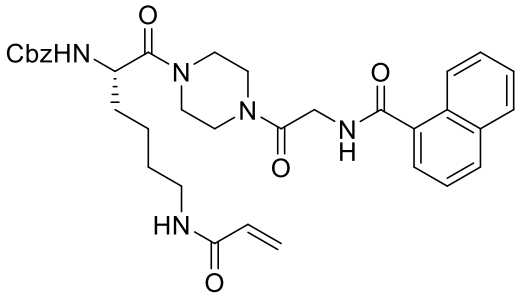
^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.24 – 8.16 (m, 1H), 7.95 – 7.80 (m, 2H), 7.63 – 7.39 (m, 4H), 7.36 – 7.27 (m, 4H), 7.05 (d, *J* = 8.2 Hz, 1H), 5.49 (td, *J* = 8.0, 6.3 Hz, 1H), 3.63 – 3.37 (m, 3H), 3.28 – 3.08 (m, 3H), 2.89 – 2.64 (m, 3H), 2.35 (ddd, *J* = 12.2, 7.0, 3.1 Hz, 1H): ^{13}C NMR (101 MHz, Chloroform-*d*) δ 169.7, 168.8, 136.2, 133.9, 133.8, 131.0, 130.3, 129.8, 128.8, 128.4, 127.3, 127.2, 125.5, 125.4, 124.8, 50.2, 47.0, 46.0, 45.8, 43.3, 40.0; HRMS (ESI-QTOF) m/z: $[M + Na]^+$ Calcd for $C_{24}H_{25}N_3O_2Na$ 410.1844; found 410.1851.

2,5-dioxopyrrolidin-1-yl N^6-acryloyl-N^2-((benzyloxy)carbonyl)-L-lysinate (27)



A solution of acryloyl chloride (1.05 eq) in THF (0.1 M) was added dropwise over 5 min to a 0 °C solution of Cbz-Lys-OH (1 eq) in $NaOH_{(aq)}$ (1 M, 10 mL), the resulting solution was stirred vigorously. After 1 h the reaction was assumed complete and the volatiles were removed under reduced pressure. The remaining aqueous was acidified to pH 2 with 1 M $HCl_{(aq)}$ and extracted with DCM (3 x 50 mL). The organic was dried over $MgSO_4$, filtered and concentrated under reduced pressure yielding the acrylated product as a white solid which was immediately taken forward. The solid was resolubilized in MeCN (0.1 M), *N*-hydroxysuccinimde (1.5 eq) and EDC·HCl (1.5 eq) was added and the resulting solution was stirred at room temperature overnight. The volatiles were removed under reduced pressure and the crude residue was resolubilized in DCM and then washed sequentially with $HCl_{(aq)}$ (1 M), $NaHCO_3$ (Sat. Soln.) and brine. The organic was dried over $MgSO_4$, filtered and concentrated under reduced pressure to yield **27** which was used with no further purification.

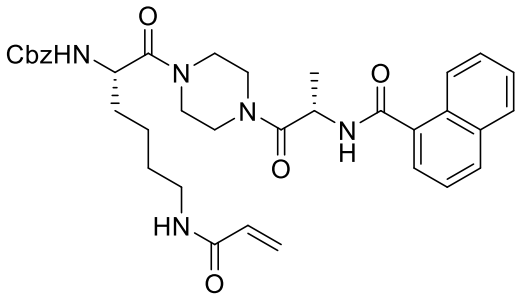
Benzyl (*S*)-(1-(4-((1-naphthoyl)glycyl)piperazin-1-yl)-6-acrylamido-1-oxohexan-2-yl)carbamate (28)



General procedure 4 followed to yield **28** (71 mg, 46%) as a white solid.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.40 – 8.32 (m, 1H), 7.98 – 7.84 (m, 2H), 7.70 (dd, *J* = 7.1, 1.2 Hz, 1H), 7.60 – 7.44 (m, 3H), 7.36 (d, *J* = 4.9 Hz, 5H), 7.13 (d, *J* = 4.7 Hz, 1H), 6.26 (d, *J* = 16.7 Hz, 1H), 6.06 (dd, *J* = 17.0, 10.2 Hz, 1H), 5.78 (dd, *J* = 21.9, 11.8 Hz, 2H), 5.67 – 5.54 (m, 1H), 5.10 (s, 2H), 4.72 – 4.56 (m, 1H), 4.48 – 4.26 (m, 2H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 170.8, 169.6, 166.9, 165.8, 156.3, 136.3, 133.8, 133.6, 131.2, 130.9, 130.3, 128.7, 128.5, 128.4, 128.2, 127.4, 126.6, 126.5, 125.6, 125.5, 124.9, 77.5, 77.2, 76.8, 67.2, 50.5, 45.3, 44.6, 44.3, 41.9, 39.0, 38.9, 29.8, 29.1, 22.3; HRMS (ESI-QTOF) m/z: $[M + Na]^+$ Calcd for $C_{34}H_{39}N_5O_6Na$ 636.2798; found 636.2770.

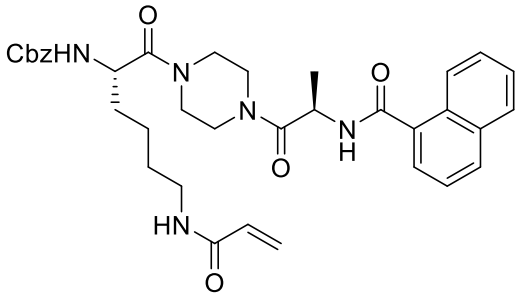
Benzyl ((*S*)-1-(4-((1-naphthoyl)-L-alanyl)piperazin-1-yl)-6-acrylamido-1-oxohexan-2-yl)carbamate (29)



General procedure 4 followed to yield **29** (24 mg, 22%) as a white solid.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.39 – 8.26 (m, 1H), 7.96 – 7.81 (m, 2H), 7.70 – 7.60 (m, 1H), 7.60 – 7.40 (m, 3H), 7.34 (d, *J* = 5.1 Hz, 5H), 7.16 (d, *J* = 7.5 Hz, 1H), 6.30 – 6.18 (m, 1H), 6.12 – 5.93 (m, 2H), 5.85 (dd, *J* = 16.0, 8.3 Hz, 1H), 5.58 (dd, *J* = 10.5, 3.3 Hz, 1H), 5.19 (dd, *J* = 10.2, 4.1 Hz, 1H), 5.08 (s, 2H), 4.63 (td, *J* = 8.4, 4.7 Hz, 1H), 4.00 – 3.02 (m, 10H), 2.09-1.84 (s, 1H), 1.75-1.30 (m, 10H).^{13}C NMR (101 MHz, Chloroform-*d*) δ 171.2, 170.8, 168.9, 168.8, 165.8, 156.3, 136.3, 133.8, 133.7, 131.0, 130.9, 130.2, 128.7, 128.5, 128.4, 128.1, 127.3, 126.6, 126.4, 125.5, 125.4, 124.8, 77.5, 77.2, 76.8, 67.1, 53.6, 50.4, 45.7, 45.3, 42.2, 42.0, 41.9, 38.9, 32.8, 32.6, 29.0, 22.3, 19.3, 19.0; HRMS (ESI-QTOF) m/z: $[M + Na]^+$ Calcd for $C_{35}H_{41}N_5O_6Na$ 650.2955; found 650.2975.

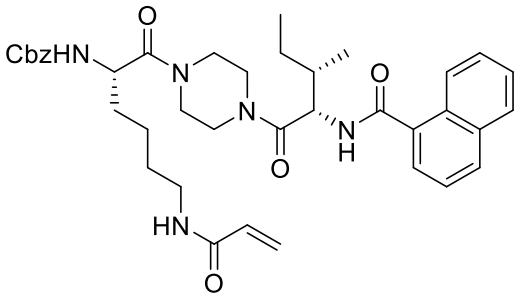
Benzyl ((*S*)-1-(4-((1-naphthoyl)-D-alanyl)piperazin-1-yl)-6-acrylamido-1-oxohexan-2-yl)carbamate (30)



General procedure 4 followed to yield **30** (18 mg, 27%) as a white solid.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.33 (d, *J* = 8.2 Hz, 1H), 7.97 – 7.82 (m, 2H), 7.65 (dd, *J* = 7.1, 1.4 Hz, 1H), 7.60 – 7.41 (m, 3H), 7.34 (d, *J* = 4.0 Hz, 5H), 7.15 (d, *J* = 7.5 Hz, 1H), 6.30 – 6.19 (m, 1H), 6.06 (qd, *J* = 9.6, 4.6 Hz, 1H), 5.88 (dt, *J* = 43.4, 7.1 Hz, 2H), 5.66 – 5.52 (m, 1H), 5.20 (p, *J* = 7.0 Hz, 1H), 5.09 (s, 2H), 4.64 (td, *J* = 8.4, 4.6 Hz, 1H), 4.01 – 3.11 (m, 11H), 1.99 – 1.17 (m, 11H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 171.2, 170.8, 168.9, 165.8, 156.3, 136.3, 133.8, 133.7, 131.1, 130.9, 130.2, 128.7, 128.5, 128.4, 128.2, 127.3, 126.6, 126.5, 125.5, 125.4, 124.8, 77.5, 77.2, 76.8, 67.2, 53.6, 50.4, 45.7, 45.3, 42.2, 41.9, 32.8, 29.0, 22.3, 19.3, 19.0; HRMS (ESI-QTOF) m/z: [M + Na]$^+$ Calcd for $C_{35}H_{41}N_5O_6Na$ 650.2955; found 650.2940.

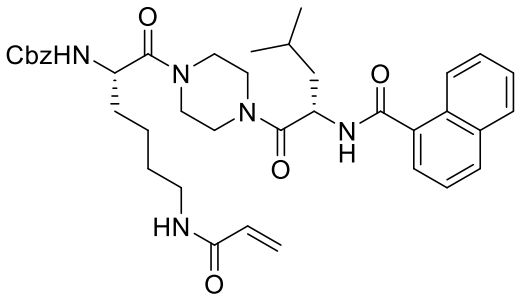
Benzyl ((*S*)-1-(4-((1-naphthoyl)-L-isoleucyl)piperazin-1-yl)-6-acrylamido-1-oxohexan-2-yl)carbamate (31)



General procedure 4 followed to yield **31** (33 mg, 26%) as a white solid.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.32 (td, *J* = 9.5, 8.9, 3.2 Hz, 1H), 7.90 (ddd, *J* = 23.5, 8.0, 1.5 Hz, 2H), 7.66 (ddd, *J* = 9.2, 7.1, 1.3 Hz, 1H), 7.61 – 7.42 (m, 3H), 7.41 – 7.28 (m, 5H), 6.83 (ddd, *J* = 18.5, 8.3, 4.8 Hz, 1H), 6.31 – 6.21 (m, 1H), 6.13 – 5.99 (m, 1H), 5.92 – 5.72 (m, 2H), 5.60 (td, *J* = 10.4, 3.7 Hz, 1H), 5.28 (dd, *J* = 8.7, 4.6 Hz, 1H), 5.10 (d, *J* = 2.3 Hz, 2H), 4.65 (td, *J* = 8.3, 4.5 Hz, 1H), 4.08 – 3.15 (m, 10H), 1.96 – 1.15 (m, 12H), 1.14 – 0.84 (m, 6H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 170.8, 169.5, 165.8, 156.3, 136.3, 134.0, 133.9, 131.1, 130.9, 130.3, 128.7, 128.5, 128.4, 128.2, 127.4, 127.4, 126.6, 126.5, 125.4, 124.8, 77.5, 77.2, 76.8, 67.2, 52.4, 50.4, 45.7, 42.3, 42.1, 39.1, 38.5, 38.3, 38.0, 32.8, 29.0, 27.1, 24.6, 22.3, 16.2, 14.4, 12.1, 11.4; HRMS (ESI-QTOF) m/z: [M + Na]$^+$ Calcd for $C_{38}H_{47}N_5O_6Na$ 692.3424; found 692.3444.

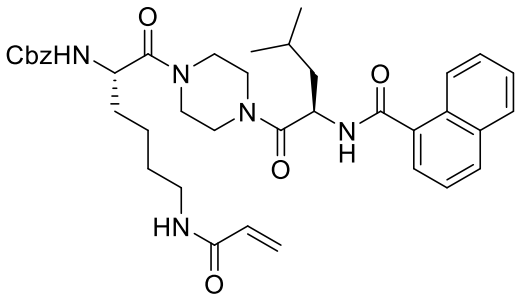
Benzyl ((*S*)-1-(4-((1-naphthoyl)-L-leucyl)piperazin-1-yl)-6-acrylamido-1-oxohexan-2-yl)carbamate (32)



General procedure 4 followed to yield **32** (46 mg, 29%) as a white solid.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.33 (dd, *J* = 8.8, 3.9 Hz, 1H), 7.97 – 7.82 (m, 2H), 7.66 (dt, *J* = 7.1, 1.6 Hz, 1H), 7.61 – 7.42 (m, 3H), 7.35 (q, *J* = 2.6 Hz, 5H), 6.82 (dd, *J* = 8.9, 2.9 Hz, 1H), 6.33 – 6.19 (m, 1H), 6.05 (dt, *J* = 16.0, 10.6 Hz, 1H), 5.92 – 5.71 (m, 2H), 5.60 (t, *J* = 10.1 Hz, 1H), 5.30 (dd, *J* = 9.6, 4.8 Hz, 1H), 5.09 (t, *J* = 2.3 Hz, 2H), 4.65 (d, *J* = 5.9 Hz, 1H), 4.10 – 3.11 (m, 8H), 1.89 – 1.50 (m, 8H), 1.39 (d, *J* = 8.1 Hz, 2H), 1.15 – 0.93 (m, 6H); ^{13}C NMR (101 MHz, Chloroform-*d)* δ 171.4, 170.8, 170.7, 169.4, 169.2, 165.7, 136.3, 133.8, 133.8, 131.1, 131.0, 130.3, 128.7, 128.5, 128.4, 128.2, 127.4, 126.6, 126.4, 125.5, 125.4, 124.8, 77.5, 77.2, 76.8, 67.2, 50.4, 47.8, 45.3, 42.8, 42.5, 42.3, 42.0, 39.0, 32.8, 29.0, 25.1, 23.5, 22.3; HRMS (ESI-QTOF) m/z: $[M + Na]^+$ Calcd for $C_{38}H_{47}N_5O_6Na$ 692.3424; found 692.3425.

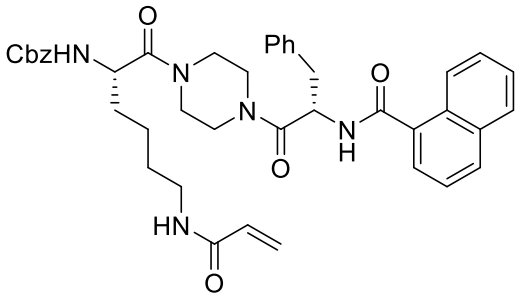
Benzyl ((*S*)-1-(4-((1-naphthoyl)-D-leucyl)piperazin-1-yl)-6-acrylamido-1-oxohexan-2-yl)carbamate (33)



General procedure 4 followed to yield **33** (37 mg, 24%) as a white solid.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.38 – 8.29 (m, 1H), 7.97 – 7.83 (m, 2H), 7.66 (dt, *J* = 7.1, 1.5 Hz, 1H), 7.61 – 7.42 (m, 3H), 7.35 (d, *J* = 3.4 Hz, 5H), 6.82 (d, *J* = 8.7 Hz, 1H), 6.26 (dd, *J* = 17.0, 5.3 Hz, 1H), 6.15 – 5.97 (m, 1H), 5.90 – 5.67 (m, 2H), 5.67 – 5.52 (m, 1H), 5.29 (s, 1H), 5.10 (d, *J* = 2.7 Hz, 2H), 4.65 (q, *J* = 7.5 Hz, 1H), 4.07 – 3.13 (m, 8H), 1.91 – 1.33 (m, 10H), 1.17 – 0.91 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.4, 170.8, 169.3, 165.7, 156.3, 136.3, 133.8, 133.8, 131.1, 131.0, 130.3, 128.7, 128.5, 128.4, 128.2, 127.4, 126.6, 126.4, 125.5, 125.4, 124.8, 77.5, 77.2, 76.8, 67.2, 50.4, 47.8, 45.7, 45.4, 42.5, 42.3, 42.0, 39.1, 33.0, 29.0, 25.1, 23.5, 22.3; HRMS (ESI-QTOF) m/z: $[M + Na]^+$ Calcd for Calcd $C_{38}H_{47}N_5O_6Na$ 692.3424; found 692.3425.

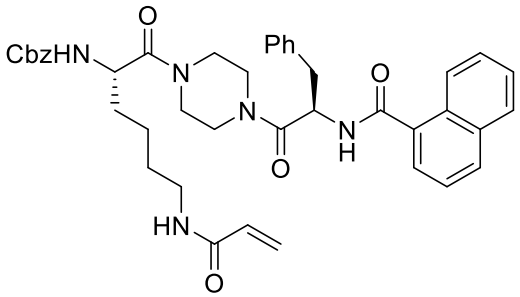
Benzyl ((*S*)-1-(4-((1-naphthoyl)-L-phenylalanyl)piperazin-1-yl)-6-acrylamido-1-oxohexan-2-yl)carbamate (34)



General procedure 4 followed to yield **34** (42 mg, 32%) as a white solid.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.27 – 8.17 (m, 1H), 7.97 – 7.81 (m, 2H), 7.60 (ddd, *J* = 7.2, 3.6, 1.3 Hz, 1H), 7.57 – 7.41 (m, 3H), 7.40 – 7.26 (m, 10H), 7.07 – 6.93 (m, 1H), 6.33 – 6.19 (m, 1H), 6.16 – 5.98 (m, 1H), 5.86 (s, 1H), 5.80 – 5.68 (m, 1H), 5.61 (ddt, *J* = 13.7, 10.1, 1.7 Hz, 1H), 5.44 (d, *J* = 7.9 Hz, 1H), 5.08 (d, *J* = 9.5 Hz, 2H), 4.65 – 4.45 (m, 1H), 3.83 – 2.98 (m, 12H), 1.81 (s, 2H), 1.68 – 1.29 (m, 6H);^{13}C NMR (101 MHz, $CDCl_3$) δ 170.5, 170.3, 169.1, 165.8, 156.3, 136.3, 136.1, 133.8, 133.6, 131.2, 130.9, 130.3, 129.9, 129.8, 129.7, 128.9, 128.7, 128.5, 128.4, 128.2, 127.6, 127.3, 126.6, 126.5, 125.5, 125.4, 124.8, 77.5, 77.2, 76.8, 67.2, 50.3, 45.4, 45.1, 41.8, 41.7, 40.1, 39.9, 39.1, 32.9, 29.0, 22.4; HRMS (ESI-QTOF) m/z: [M + Na]$^+$ Calcd for $C_{41}H_{45}N_5O_6Na$ 726.3268; found 726.3248.

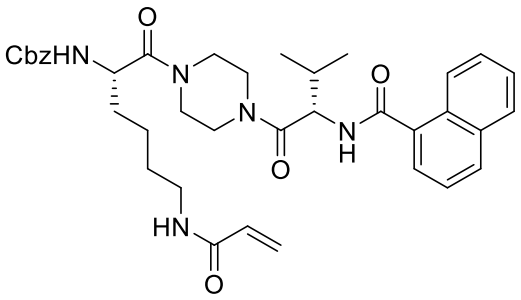
Benzyl ((*S*)-1-(4-((1-naphthoyl)-D-phenylalanyl)piperazin-1-yl)-6-acrylamido-1-oxohexan-2-yl)carbamate (35)



General procedure 4 followed to yield **35** (60 mg, 46%) as a white solid.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.27 – 8.17 (m, 1H), 7.96 – 7.81 (m, 2H), 7.64 – 7.41 (m, 4H), 7.40 – 7.27 (Stack, 10H), 7.00 (q, *J* = 10.8, 8.9 Hz, 1H), 6.33 – 6.19 (m, 1H), 6.15 – 5.98 (m, 1H), 5.91 – 5.69 (m, 2H), 5.61 (ddt, *J* = 16.3, 10.2, 1.6 Hz, 1H), 5.45 (q, *J* = 7.8, 7.4 Hz, 1H), 5.08 (d, *J* = 9.0 Hz, 2H), 4.64 – 4.46 (m, 1H), 3.83 – 2.98 (Stack, 12H), 2.04 – 1.29 (m, 8H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 170.5, 170.3, 168.9, 165.7, 156.3, 136.3, 136.0, 133.8, 133.6, 131.1, 130.9, 130.2, 129.9, 129.7, 128.9, 128.7, 128.5, 128.4, 128.2, 127.6, 127.3, 126.6, 126.5, 125.5, 125.4, 124.8, 77.5, 77.2, 76.8, 67.2, 45.9, 45.4, 45.1, 41.8, 41.7, 40.0, 39.0, 32.9, 29.0, 22.3; HRMS (ESI-QTOF) m/z: [M + Na]$^+$ Calcd for $C_{41}H_{45}N_5O_6Na$ 726.3268; found 726.3260.

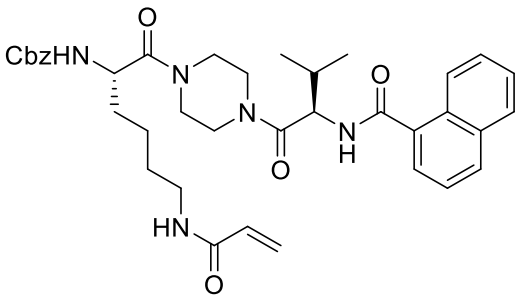
Benzyl ((*S*)-1-(4-((1-naphthoyl)-L-valyl)piperazin-1-yl)-6-acrylamido-1-oxohexan-2-yl)carbamate (36)



General procedure 2 followed to yield **36** (20 mg, 20%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (d, *J* = 7.9 Hz, 1H), 7.92 (d, *J* = 8.2 Hz, 1H), 7.86 (dd, *J* = 7.6, 1.8 Hz, 1H), 7.72 – 7.62 (m, 1H), 7.58 – 7.49 (m, 2H), 7.45 (t, *J* = 7.7 Hz, 1H), 7.40 – 7.26 (m, 5H), 6.95 (d, *J* = 4.5 Hz, 1H), 6.29 – 6.19 (m, 1H), 6.13 – 5.99 (m, 2H), 5.91 – 5.82 (m, 1H), 5.63 – 5.53 (m, 1H), 5.17 – 5.03 (m, 3H), 4.68 – 4.58 (m, 1H), 3.99 – 3.22 (m, 10H), 2.18 – 2.07 (m, 1H), 1.73 – 1.47 (m, 4H), 1.42 – 1.32 (m, 2H), 1.16 – 1.04 (m, 3H), 1.03 – 0.98 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.78, 170.56, 169.64, 169.52, 165.73, 156.28, 136.33, 133.89, 133.81, 131.06, 130.94, 130.28, 128.64, 128.45, 128.32, 128.13, 127.33, 126.55, 126.37, 125.38, 125.35, 124.78, 67.09, 53.98, 50.39, 45.71, 42.20, 42.02, 39.00, 32.84, 31.65, 28.97, 22.29, 19.93, 17.68. HRMS (ESI-QTOF) m/z: $[M + Na]^+$ Calcd for $C_{37}H_{45}N_5O_6Na$ 678.786; found 678.3268.

Benzyl ((*S*)-1-(4-((1-naphthoyl)-D-valyl)piperazin-1-yl)-6-acrylamido-1-oxohexan-2-yl)carbamate (37)

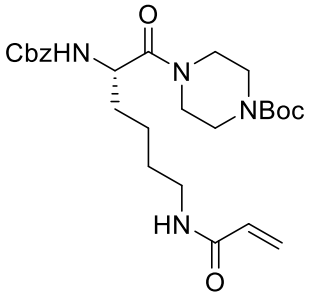


General procedure 2 followed to yield **37** (27 mg, 28%) as a white solid.

^{1}H NMR (400MHz, $CDCl_3$) δ 8.36 – 8.33 (d, J = 7.9 Hz, 1H), 7.96 – 7.93 (d, J = 8.3 Hz, 1H), 7.90 – 7.87 (dd, J = 7.5, 1.5, 1H), 7.69 – 7.67 (ddd, J = 7.1, 2.8, 1.3 Hz, 1H), 7.59 – 7.52 (dp, J = 6.5, 1.6 Hz, 2H), 7.50 – 7.46 (t, J = 7.7 Hz, 1H), 7.39 – 7.31 (m, 5H), 6.90 – 6.88 (dd, J = 8.2, 3.0 Hz, 1H), 6.29 – 6.25 (d, J = 16.9, 1H), 6.12 – 6.02 (m, J = 8.0 Hz, 1H), 5.89 – 5.73 (m, J = 8.0 Hz, 2H), 5.65 – 5.59 (dt, J = 9.7, 4.6 Hz, 1H), 5.19 – 5.09 (m, 3H), 4.69 – 4.64 (dt, J = 7.9, 4.6 Hz, 1H), 4.16 – 3.23 (m, 11H), 2.18 – 2.10 (m, J = 6.4 Hz, 1H), 1.80 – 1.34 (m, 8H), 1.17 – 1.11 (m, J = 7.2 Hz, 3H), 1.04 – 1.02 (m, J = 6.5 Hz, 3H); ^{13}C NMR (101MHz, $CDCl_3$)

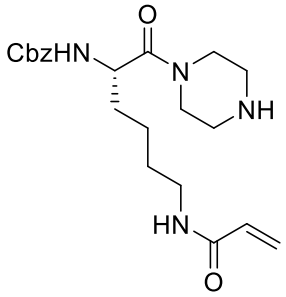
δ 170.6, 170.5, 169.4, 165.6, 156.3, 136.1, 133.8, 133.7, 130.9, 130.8, 130.2, 128.5, 128.3, 128.2, 128.0, 127.2, 126.4, 126.2, 125.3, 125.2, 124.7, 67.0, 53.8, 50.2, 45.6, 42.2, 41.9, 38.9, 32.8, 31.6, 29.7, 28.9, 22.2, 19.8, 17.5; HRMS (ESI-QTOF) m/z: $[M + Na]^+$ Calcd for $C_{37}H_{45}N_5O_6Na$ 678.786; found 678.3268.

tert-Butyl 4-(N^6-acryloyl-N^2-((benzyloxy)carbonyl)-L-lysyl)piperazine-1-carboxylate (38)



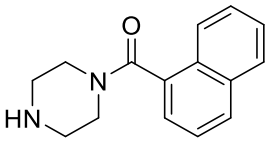
Cbz-Lys-OH (502 mg, 1.78 mmol) was dissolved in 1M $NaOH_{(aq)}$ (2 mL) and cooled to 0°C. Acryloyl chloride (0.14 mL, 1.78 mmol) in THF (0.86 mL) was added dropwise over 20 min. The reaction was stirred for 10 min afterwards for a total mixing time of 30 min at 0°C. The reaction mixture was quenched with brine and acidified to pH 1 with 1M HCl. The organics were extracted with EtOAc (3 x 20 mL) and the combined organics were washed with $NaHCO_3$ (60 mL) and brine (60 mL). The oranics were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The acid product was then dissolved in DCM, followed by the addition of EDC·HCl, HOBt and DIPEA. The reaction mixture was stirred at room temperature for 30 min. Boc-Piperazine (629 mg, 3.38 mmol) was added to the reaction mixture and stirred at room temperature for 16 h. The reaction mixture was then washed with 1M HCl twice, $NaHCO_3$, and brine. It was then dehydrated with anhydrous magnesium sulfate, filtered and concentrated to afford the crude product as a yellowy oil. The crude product was purified by flash column chromatography (elution with gradient 80:20 ethyl acetate:hexanes to 100% ethyl acetate) to afford 119 mg (13%) of the desired product **38** as a yellowy oil. ^1H NMR (400MHz, $CDCl_3$) δ 7.37 – 7.29 (m, 5H), δ 6.28 – 6.23 (dd, J = 17.0, 1.3 Hz, 1H), δ 6.11 – 6.04 (dd, J = 17.0, 10.3 Hz, 1H), δ 5.96 (br, 1H), δ 5.84 – 5.81 (d, J = 8.2 Hz, 1H), δ 5.61 – 5.58 (dd, J = 10.3, 0.9 Hz, 1H), δ 5.08 (s, 2H), δ 4.65 – 4.60 (ddd, J = 8.1, 8.0, 4.5 Hz, 1H) δ 3.71 – 3.24 (m, 11H), δ 1.62 – 1.50 (m, J = 7.2 Hz, 3H), δ 1.47 (s, 9H), δ 1.43 – 1.36 (m, J = 7.4 Hz, 2H); ^{13}C NMR (101MHz, $CDCl_3$) δ 170.4, 165.6, 156.2, 154.4, 136.3, 130.9, 128.5, 128.2, 128.0, 126.2, 80.4, 66.9, 50.2, 45.3, 41.9, 39.0, 32.8, 28.8, 28.3, 22.1; HRMS (ESI-QTOF) m/z $[M + Na]^+$ Calcd for $C_{26}H_{38}N_4O_6Na$ 525.2689; found 525.2674.

Benzyl (*S*)-(6-acrylamido-1-oxo-1-(piperazin-1-yl)hexan-2-yl)carbamate (39)



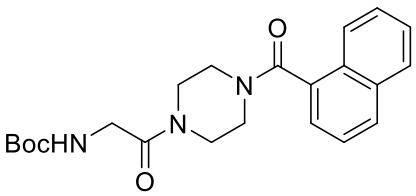
General procedure 3 followed to yield **39** (94 mg) as a yellow oil.

Naphthalen-1-yl(piperazin-1-yl)methanone (40)



Prepared via a previously reported procedure.[1]

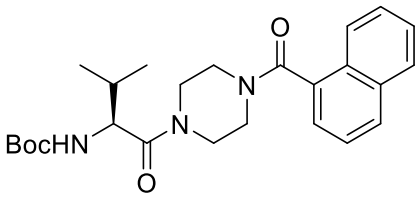
***tert*-Butyl (2-(4-(1-naphthoyl)piperazin-1-yl)-2-oxoethyl)carbamate (41)**



General procedure 2 followed to yield **41** (177 mg, 61 %) as a white solid.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.94 – 7.85 (m, 2H), 7.83 – 7.77 (m, 1H), 7.57 – 7.47 (Stack, 3H), 7.42 (dd, J = 7.0, 1.2 Hz, 1H), 5.44 (s, 1H), 3.69 – 4.07 (m, 5H), 3.35 – 3.68 (m, 2H), 3.22 (m, 3H), 1.45 (s, 9H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 169.6, 167.2, 155.8, 133.5, 133.2, 129.7, 129.5, 128.6, 127.3, 126.7, 125.2, 124.4, 124.0, 79.9, 46.9, 46.7, 44.8, 44.4, 42.4, 42.2, 41.9, 41.6, 41.5, 28.3; HRMS (ESI-QTOF) m/z [M + Na]$^{+}$ Calcd for $C_{22}H_{27}N_3O_4Na$ 420.1899; found 420.1914.

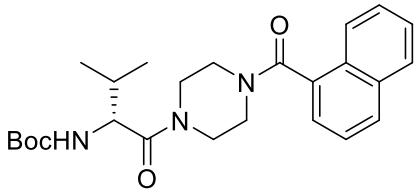
***tert*-butyl (*S*)-(1-(4-(1-naphthoyl)piperazin-1-yl)-3-methyl-1-oxobutan-2-yl)carbamate (42)**



General procedure 2 followed to yield **42** (142 mg, 54%) as a white solid.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.89 – 7.84 (m, 2H), 7.83 – 7.75 (m, 1H), 7.53 – 7.48 (m, 1H), 7.45 (d, *J* = 8.1 Hz, 1H), 7.39 (d, *J* = 7.1 Hz, 1H), 5.31 (s, 1H), 3.99 – 3.25 (m, 6H), 3.22 – 3.14 (m, 2H), 1.97 – 1.79 (m, 1H), 1.42 (Stack, 12H), 1.37 (d, *J* = 8.8 Hz, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 171.1, 169.7, 155.9, 133.5, 133.4, 129.6, 128.6, 127.3, 126.7, 125.2, 124.5, 124.0, 79.7, 60.4, 54.9, 42.0, 31.5, 28.4, 19.7, 17.3; HRMS (ESI-QTOF) m/z [M + Na]$^+$ Calcd for $C_{25}H_{33}N_3O_4Na$ 462.2369; found 462.2365.

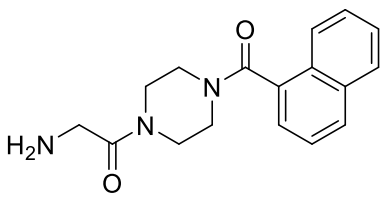
***tert*-Butyl (*R*)-(1-(4-(1-naphthoyl)piperazin-1-yl)-3-methyl-1-oxobutan-2-yl)carbamate (43)**



General procedure 2 followed to yield **43** (102 mg, 52%) as a white solid.

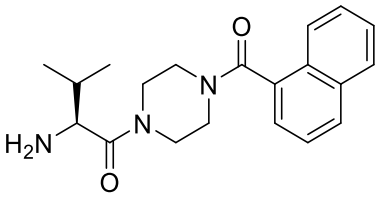
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.89 – 7.84 (m, 2H), 7.80 (s, 2H), 7.51 (d, *J* = 4.1 Hz, 2H), 7.47 (d, *J* = 8.0 Hz, 1H), 7.40 (d, *J* = 6.8 Hz, 1H), 5.30 (d, *J* = 9.0 Hz, 1H), 3.98 – 3.26 (m, 6H), 3.24 – 3.13 (m, 2H), 1.45 – 1.40 (Stack, 12H), 1.38 (d, *J* = 8.6 Hz, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 171.0, 169.7, 155.9, 133.6, 133.5, 129.6, 128.7, 127.3, 126.7, 125.2, 124.5, 124.0, 79.7, 60.4, 54.9, 42.0, 31.5, 28.4, 19.7, 17.3; HRMS (ESI-QTOF) m/z [M + Na]+ Calcd for $C_{25}H_{33}N_3O_4Na$ 462.2369; found 462.2357.

1-(4-(1-naphthoyl)piperazin-1-yl)-2-aminoethan-1-one (44)



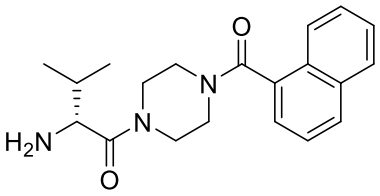
General procedure 3 followed to yield **44** (41 mg) as a viscous yellow oil.

(*S*)-1-(4-(1-naphthoyl)piperazin-1-yl)-2-amino-3-methylbutan-1-one (45)



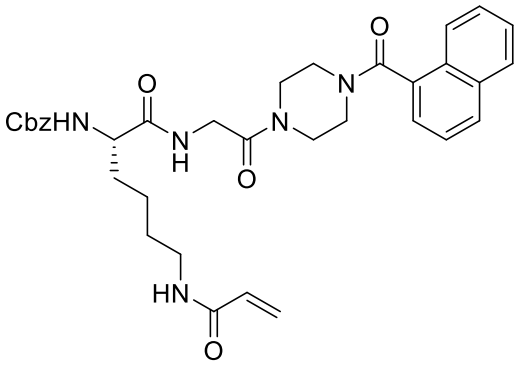
General procedure 3 followed to yield **45** (76 mg) as a white solid.

(*R*)-1-(4-(1-naphthoyl)piperazin-1-yl)-2-amino-3-methylbutan-1-one (46)



General procedure 3 followed to yield **46** (55 mg) as a white solid.

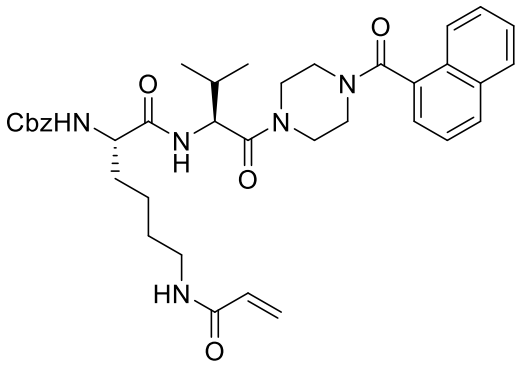
Benzyl (*S*)-(1-((2-(4-(1-naphthoyl)piperazin-1-yl)-2-oxoethyl)amino)-6-acrylamido-1-oxohexan-2-yl)carbamate (47)



General procedure 4 followed to yield **47** (30 mg, 48%) as a pale pink solid.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.93 – 7.86 (Stack, 2H), 7.82 – 7.76 (m, 1H), 7.57 – 7.46 (Stack, 3H), 7.41 (dd, J = 7.0, 1.2 Hz, 1H), 7.36 – 7.25 (Stack, 5H), 7.14 (s, 1H), 6.23 (d, J = 16.9 Hz, 1H), 6.11 – 5.95 (m, 2H), 5.76 (d, J = 7.9 Hz, 1H), 5.54 (t, J = 8.4 Hz, 1H), 5.13 - 5.00 (Stack, 2H), 4.30 - 3.71 (Stack, 6H), 3.68 - 3.47 (Stack, 3H), 3.34 - 3.11 (Stack, 5H), 1.95 - 1.31 (Stack, 7H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 172.1, 169.9, 166.8, 165.9, 156.4, 136.3, 133.6, 133.3, 130.9, 129.8, 129.6, 128.8, 128.6, 128.3, 128.2, 127.5, 126.8, 126.5, 125.3, 124.5, 124.1, 67.1, 54.8, 46.8, 45.0, 44.5, 42.6, 42.1, 41.7, 41.6, 41.3, 38.9, 32.3, 29.8, 29.0, 22.5; HRMS (ESI-QTOF) m/z [M + Na]$^{+}$ Calcd for $C_{34}H_{39}N_4O_6Na$ 636.2798; found 636.2813.

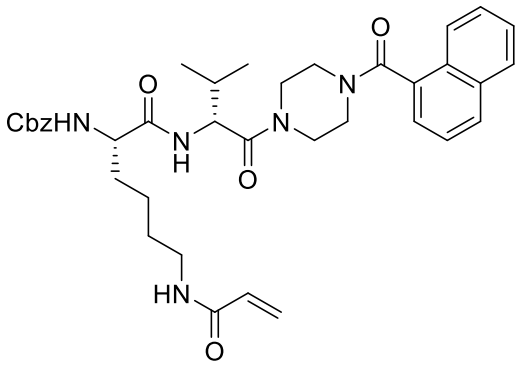
Benzyl ((*S*)-1-(((*S*)-1-(4-(1-naphthoyl)piperazin-1-yl)-3-methyl-1-oxobutan-2-yl)amino)-6-acrylamido-1-oxohexan-2-yl)carbamate (48)



General procedure 4 followed to yield **48** (95 mg, 67%) as a white solid.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.91 – 7.85 (Stack, 2H), 7.79 (m, 1H), 7.54 – 7.50 (m, 1H), 7.50z – 7.45 (m, 2H), 7.40 (d, *J* = 6.9 Hz, 2H), 7.31 (Stack, 5H), 6.96 (s, 1H), 6.23 – 6.17 (m, 2H), 6.08 (s, 1H), 5.74 (dd, 2H), 5.52 (s, 2H), 5.06 (t, *J* = 13.0 Hz, 2H), 4.26 – 3.12 (Stack, 10H), 2.06 – 1.87 (m, 1H), 1.83 – 1.72 (m, 1H), 1.71 – 1.63 (m, 1H), 1.54 – 1.43 (m, 3H), 1.37 – 1.28 (m, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 172.0, 170.4, 169.7, 165.9, 156.4, 136.3, 133.6, 133.4, 130.9, 129.7, 128.7, 128.6, 128.3, 128.1, 127.3, 126.8, 126.5, 125.3, 124.5, 124.1, 67.1, 54.9, 53.8, 47.3, 46.9, 45.9, 42.0, 41.7, 38.8, 31.3, 28.9, 22.5, 19.7, 17.7; HRMS (ESI-QTOF) m/z [M + Na]$^+$ Calcd for $C_{37}H_{45}O_6N_5Na$ 678.3268; found 678.3251.

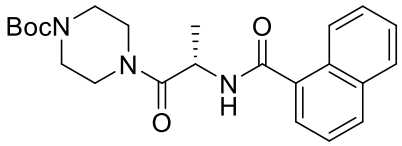
Benzyl ((*S*)-1-(((*R*)-1-(4-(1-naphthoyl)piperazin-1-yl)-3-methyl-1-oxobutan-2-yl)amino)-6-acrylamido-1-oxohexan-2-yl)carbamate (49)



General procedure 4 followed to yield **49** (73 mg, 70%) as a white solid.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.90 – 7.83 (Stack, 2H), 7.82 – 7.75 (m, 1H), 7.54 – 7.44 (Stack, 2H), 7.40 (d, *J* = 7.1 Hz, 1H), 7.32 – 7.27 (Stack, 5H), 7.16 (s, 1H), 6.22 – 6.16 (Stack, 2H), 6.03 (s, 1H), 5.90 – 5.80 (m, 1H), 5.53 (s, 1H), 5.05 (s, 2H), 4.31 – 4.15 (m, 1H), 4.13 – 3.10 (Stack, 10H), 2.04 – 1.87 (m, 1H), 1.81 (d, *J* = 14.5 Hz, 1H), 1.63 (dq, *J* = 15.3, 7.5, 7.0 Hz, 1H), 1.46 (s, 3H), 1.31 (s, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 172.0, 170.4, 169.7, 165.8, 156.3, 136.3, 133.6, 133.4, 130.9, 129.7, 128.7, 128.6, 128.2, 128.1, 127.3, 126.7, 126.3, 125.3, 124.5, 124.0, 67.0, 54.9, 53.6, 47.3, 46.9, 45.9, 42.6, 42.0, 41.7, 38.9, 32.6, 32.5, 31.5, 29.0, 22.6, 19.7, 17.7; HRMS (ESI-QTOF) m/z [M + Na]$^+$ Calcd for $C_{37}H_{45}O_6N_5Na$ 678.3268; found 678.3255.

***tert*-Butyl 4-((1-naphthoyl)-L-alanyl)piperazine-1-carboxylate (50)**

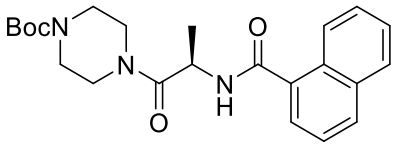


General procedure 5 followed to yield **50** (1.11 g, 39%) as a white solid.

^{1}H NMR (400 MHz, Chloroform-*d*) δ_H 8.27 (d, *J* = 8.4 Hz, 1H), 7.86 (d, *J* = 8.0 Hz, 1H), 7.81 (dd, *J* = 7.6, 1.6 Hz, 1H), 7.59 (dd, *J* = 7.2, 1.2 Hz, 1H), 7.52–7.43 (m, 2H), 7.39 (t, *J* = 8.0 Hz, 1H), 5.14 (quint, *J* = 7.2 Hz, 1H), 3.69–3.33 (m, 8H), 1.45 (d, *J* = 6.8 Hz, 3H), 1.42 (s, 9H); ^{13}C (101 MHz, Chloroform-*d*) δ_C 169.9, 167.6, 153.4, 132.7, 132.6, 129.9, 129.1, 127.3, 126.2, 125.4, 124.3, 124.2, 123.7, 79.5, 44.7, 44.3, 41.1, 27.4, 18.2; HRMS (ESI-QTOF) m/z: [M + Na]$^+$ Calcd for $C_{23}H_{29}N_3O_4Na$ 434.2056; found 434.2049.

N.B. Compounds 50 and 12 share the same structure but were prepared via different routes.

***tert*-Butyl 4-((1-naphthoyl)-D-alanyl)piperazine-1-carboxylate (51)**

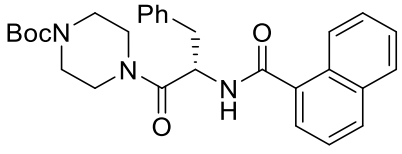


General procedure 5 followed to yield **51** (1.12 g, 47%) as a white solid.

^{1}H-NMR (400 MHz, Chloroform-*d*) 8.34 (d, *J* = 8.2 Hz, 1H), 7.93 (d, *J* = 8.3 Hz, 1H), 7.89 – 7.85 (m, 1H), 7.66 (dd, *J* = 7.1, 1.2 Hz, 1H), 7.54 (Stack, 2H), 7.46 (dd, *J* = 8.2, 7.1 Hz, 1H), 7.14 (d, *J* = 7.4 Hz, 1H), 5.21 (p, *J* = 6.9 Hz, 1H), 3.77 – 3.38 (Stack, 8H), 1.52 (d, *J* = 6.8 Hz, 3H), 1.49 (s, 9H); ^{13}C-NMR (101 MHz, Chloroform-*d*) 171.0, 168.7, 154.6, 133.9, 133.8, 131.0, 130.3, 128.5, 127.3, 126.5, 125.5, 125.4, 124.8, 80.7, 45.8, 45.5, 42.2, 28.5, 19.4; HRMS (ESI-QTOF) m/z: [M + Na]$^+$ Calcd for $C_{23}H_{29}N_3O_4Na$ 434.2056; found 434.2045.

N.B. Compounds 51 and 13 share the same structure but were prepared via different routes.

***tert*-Butyl 4-((1-naphthoyl)-L-phenylalanyl)piperazine-1-carboxylate (52)**



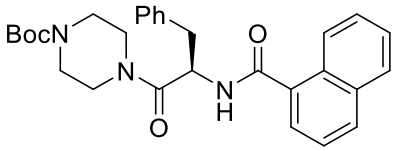
General procedure 5 followed to yield **52** (1.06 g, 82%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 – 8.18 (m, 1H), 7.92 – 7.87 (m, 1H), 7.84 (dt, *J* = 7.8, 2.6 Hz, 1H), 7.59 (dd, *J* = 7.1, 1.3 Hz, 1H), 7.54 – 7.47 (Stack, 2H), 7.42 (dd, *J* = 8.2, 7.0 Hz, 1H), 7.33 – 7.27 (Stack, 2H), 7.25 – 7.18 (Stack, 3H), 5.46 (td, *J* = 8.5, 6.0 Hz, 1H), 3.53 – 3.28

(Stack, 4H), 3.23 – 3.06 (Stack, 4H), 2.79 (ddd, *J* = 12.7, 6.3, 2.8 Hz, 1H), 1.45 (s, 9H). ^{13}C-NMR δ (101 MHz, Chloroform-*d*) 170.0, 168.8, 154.4, 136.1, 133.7, 133.7, 130.9, 130.2, 129.7, 128.8, 128.3, 127.4, 127.2, 126.4, 125.4, 125.4, 124.7, 80.4, 50.3, 45.6, 41.9, 39.9, 28.4; HRMS (ESI-QTOF) m/z: $[M + Na]^+$ Calcd for $C_{29}H_{33}N_3O_4Na$ 510.2360; found 510.2343.

N.B. Compounds 52 and 17 share the same structure but were prepared via different routes.

tert-Butyl 4-((1-naphthoyl)-D-phenylalanyl)piperazine-1-carboxylate (53)

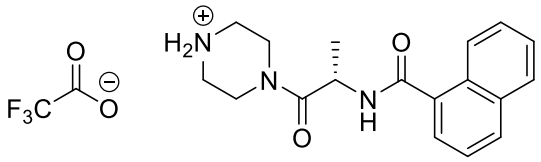


General procedure 5 followed to yield **53** (815 mg, 45%) as a white solid.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.25 – 8.18 (m, 1H), 7.94 – 7.82 (m, 2H), 7.60 (dd, *J* = 7.1, 1.2 Hz, 1H), 7.51 (td, *J* = 6.2, 3.4 Hz, 2H), 7.44 (dd, *J* = 8.2, 7.1 Hz, 1H), 7.35 – 7.27 (m, 5H), 7.02 (d, *J* = 8.2 Hz, 1H), 5.47 (td, *J* = 8.4, 5.9 Hz, 1H), 3.61 – 3.06 (m, 8H), 2.82 (ddd, *J* = 13.1, 7.1, 3.2 Hz, 1H), 1.78 (s, 1H), 1.45 (s, 9H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 170.0, 168.8, 154.5, 136.1, 133.8, 133.7, 131.0, 130.3, 129.7, 128.8, 128.4, 127.5, 127.3, 126.5, 125.5, 125.4, 124.8, 80.5, 77.5, 77.2, 76.8, 50.4, 45.6, 42.0, 40.1, 28.5; HRMS (ESI-QTOF) m/z: $[M + Na]^+$ Calcd for $C_{29}H_{33}N_3O_4Na$ 510.2369; found 510.2365.

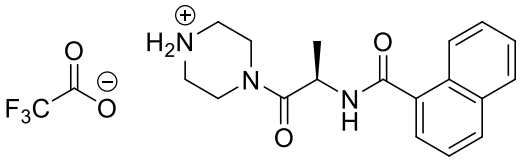
N.B. Compounds 53 and 18 share the same structure but were prepared via different routes.

4-((1-naphthoyl)-L-alanyl)piperazin-1-ium trifluoroacetate (54)



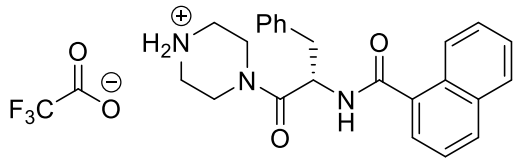
General procedure 6 followed to yield **54** (1.04 g) as a white solid. The product was carried forward without any further purification or characterization.

4-((1-naphthoyl)-D-alanyl)piperazin-1-ium trifluoroacetate (55)



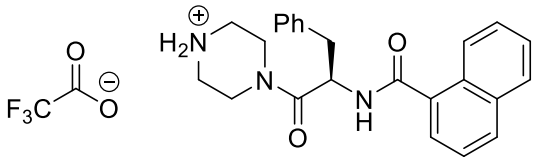
General procedure 6 followed to yield **55** (1.08 g) as a white solid. The product was carried forward without any further purification or characterization.

4-((1-naphthoyl)-L-phenylalanyl)piperazin-1-ium trifluoroacetate (56)



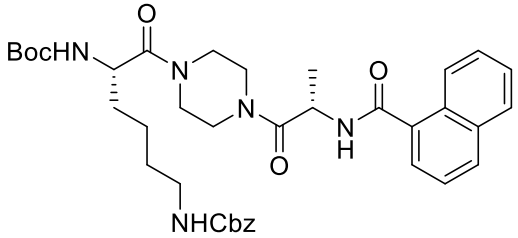
General procedure 6 followed to yield **56** (990 mg) as a white solid. The product was carried forward without any further purification or characterization.

4-((1-naphthoyl)-D-phenylalanyl)piperazin-1-ium trifluoroacetate (57)



General procedure 6 followed to yield **57** (1.08 g) as a white solid. The product was carried forward without any further purification or characterization.

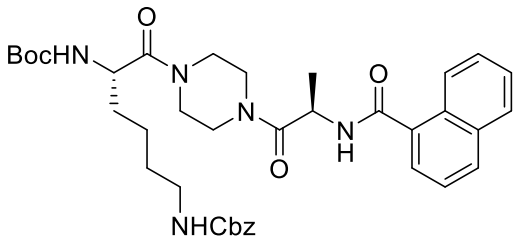
Benzyl *tert*-butyl ((*S*)-6-(4-((1-naphthoyl)-L-alanyl)piperazin-1-yl)-6-oxohexane-1,5-diyl)dicarbamate (58)



General procedure 7 followed to yield **58** (1.01 g, 65%) as a white solid.

^{1}H NMR (400 MHz, Chloroform-*d*) δ_H 8.27 (d, *J* = 8.4 Hz, 1H), 7.86 (d, *J* = 8.4 Hz, 1H), 7.81 (dd, *J* = 7.6, 1.6 Hz, 1H), 7.59 (dt, *J* = 6.8, 1.6 Hz, 1H), 7.53–7.43 (m, 2H), 7.40 (tt, *J* = 7.6, 0.8 Hz, 1H), 7.33–7.23 (m, 5H), 5.31–5.25 (br, 1H), 5.15 (t, *J* = 7.2 Hz, 1H), 5.02 (s, 2H), 4.81–4.74 (br, 1H), 4.56–4.47 (br, 1H), 4.05 (q, *J* = 7.2 Hz, 1H), 3.80–3.26 (m, 8H), 3.20–3.06 (m, 2H), 1.56–1.33 (m, 18H); ^{13}C (101 MHz, Chloroform-*d*) δ_C 171.2, 171.1, 168.8, 156.6, 155.7, 136.7, 133.9, 133.8, 131.1, 130.3, 128.7, 128.5, 128.2, 128.1, 127.3, 126.6, 125.5, 125.4, 124.8, 66.8, 49.9, 45.8, 45.3, 42.4, 42.2, 40.7, 33.0, 29.6, 28.5, 22.4, 19.4; HRMS (ESI-QTOF) m/z: $[M + Na]^+$ Calcd for $C_{37}H_{47}N_5O_7Na$ 696.3373; found 696.3360.

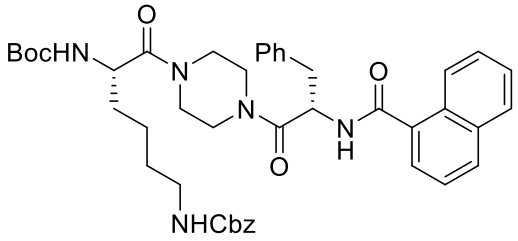
Benzyl *tert*-butyl ((*S*)-6-(4-((1-naphthoyl)-D-alanyl)piperazin-1-yl)-6-oxohexane-1,5-diyl)dicarbamate (59)



General procedure 7 followed to yield **59** (965 mg, 61%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (d, *J* = 8.2 Hz, 1H), 7.93 (d, *J* = 8.3 Hz, 1H), 7.87 (dd, *J* = 7.6, 1.7 Hz, 1H), 7.65 (dt, *J* = 7.0, 1.5 Hz, 1H), 7.60 – 7.50 (Stack, 2H), 7.49 – 7.43 (m, 1H), 7.40 – 7.29 (Stack, 5H), 7.11 (d, *J* = 7.9 Hz, 1H), 5.38 (d, *J* = 8.6 Hz, 1H), 5.21 (p, *J* = 6.9 Hz, 1H), 5.07 (d, *J* = 3.4 Hz, 2H), 4.90 (s, 1H), 4.58 (td, *J* = 8.5, 4.6 Hz, 1H), 3.93 (s, 1H), 3.83 – 3.32 (Stack, 7H), 3.17 (d, *J* = 8.0 Hz, 2H), 1.78 (s, 2H), 1.60 – 1.48 (Stack, 6H), 1.43 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.2, 168.8, 168.8, 156.6, 155.7, 140.4, 136.7, 133.8, 133.8, 131.1, 130.3, 128.6, 128.5, 127.3, 126.6, 125.5, 125.4, 124.8, 82.9, 80.1, 66.7, 49.9, 45.7, 45.3, 40.7, 33.1, 29.6, 28.5, 22.4, 19.4. (Coincident carbon resonances identified via 2D NMR experiments); HRMS (ESI-QTOF) m/z: $[M + Na]^+$ Calcd for $C_{37}H_{47}N_5O_7Na$ 696.3373; found 696.3383.

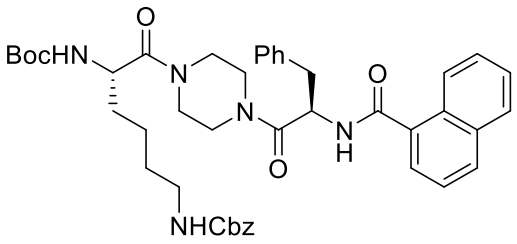
Benzyl *tert*-butyl ((*S*)-6-(4-((1-naphthoyl)-L-phenylalanyl)piperazin-1-yl)-6-oxohexane-1,5-diyl)dicarbamate (60)



General procedure 7 followed to yield **60** (1.16 g, 79%) as a white solid.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.24 (dd, *J* = 6.4, 3.4 Hz, 1H), 7.92 (d, *J* = 8.2 Hz, 1H), 7.89 – 7.85 (m, 1H), 7.61 (d, *J* = 7.0 Hz, 1H), 7.53 (qd, *J* = 6.9, 3.5 Hz, 2H), 7.44 (dd, *J* = 8.3, 7.1 Hz, 1H), 7.38 – 7.27 (m, 8H), 6.97 (q, *J* = 8.3, 7.6 Hz, 1H), 5.50 – 5.42 (m, 1H), 5.35 (t, *J* = 12.4 Hz, 1H), 5.15 – 5.04 (m, 2H), 4.88 (s, 1H), 4.49 (d, *J* = 24.7 Hz, 1H), 3.76 (d, *J* = 11.3 Hz, 1H), 3.71 – 3.30 (m, 3H), 3.29 – 3.06 (m, 3H), 1.78 (s, 3H), 1.65 – 1.46 (Stack, 4H), 1.46 – 1.38 (m, 9H), 1.36 (Stack, 4H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 170.9, 170.2, 168.8, 156.6, 155.7, 136.6, 133.8, 133.6, 131.2, 130.3, 129.9, 129.7, 128.9, 128.6, 128.5, 128.2, 127.6, 127.4, 126.6, 125.5, 125.4, 124.8, 80.2, 66.8, 49.8, 45.1, 41.9, 40.7, 33.1, 29.6, 28.5, 22.3; HRMS (ESI-QTOF) m/z: $[M + Na]^+$ Calcd for $C_{43}H_{51}N_5O_7Na$ 772.3686; found 772.3717.

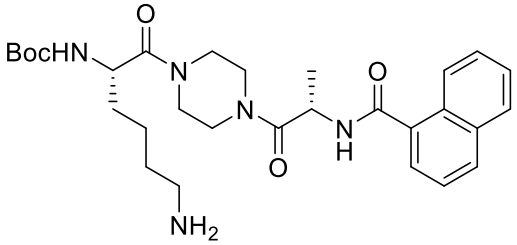
Benzyl *tert*-butyl ((*S*)-6-(4-((1-naphthoyl)-D-phenylalanyl)piperazin-1-yl)-6-oxohexane-1,5-diyl)dicarbamate (61)



General procedure 7 followed to yield **61** (1.33 g, 82%) as a white solid.

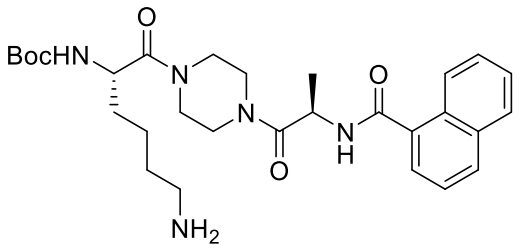
^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.27 – 8.20 (m, 1H), 7.94 – 7.90 (m, 1H), 7.89 – 7.84 (m, 1H), 7.61 (dd, *J* = 7.1, 1.2 Hz, 1H), 7.53 (qd, *J* = 6.9, 3.5 Hz, 2H), 7.44 (td, *J* = 7.6, 7.0, 1.1 Hz, 1H), 7.32 (dddd, *J* = 17.3, 10.0, 8.0, 3.1 Hz, 7H), 6.98 (q, *J* = 8.4, 7.5 Hz, 1H), 5.52 – 5.32 (m, 1H), 5.08 (dd, *J* = 11.9, 4.9 Hz, 2H), 4.89 (s, 1H), 3.82 – 2.88 (m, 5H), 1.73 (s, 2H), 1.68 – 1.29 (m, 13H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 170.9, 170.2, 168.7, 156.6, 155.7, 136.7, 136.3, 133.8, 133.6, 131.2, 130.3, 129.9, 129.8, 129.7, 128.9, 128.6, 128.5, 128.2, 127.6, 127.3, 126.6, 125.5, 125.4, 124.8, 80.1, 77.5, 77.2, 76.8, 66.8, 60.5, 50.4, 49.8, 45.8, 45.5, 45.1, 42.1, 41.9, 41.7, 40.0, 33.1, 29.6, 28.5, 22.3, 14.3; HRMS (ESI-QTOF) m/z: $[M + Na]^+$ Calcd for $C_{43}H_{51}N_5O_7Na$ 772.3686; found 772.3682.

***tert*-Butyl ((*S*)-1-(4-((1-naphthoyl)-L-alanyl)piperazin-1-yl)-6-amino-1-oxohexan-2-yl)carbamate (62)**



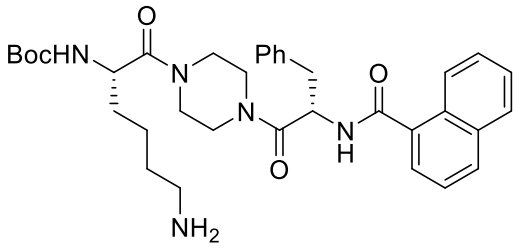
General procedure 8 followed to yield **62** (407 mg) as a colourless oil. The product was carried forward without any further purification or characterization.

***tert*-Butyl ((S)-1-(4-((1-naphthoyl)-D-alanyl)piperazin-1-yl)-6-amino-1-oxohexan-2-yl)carbamate (63)**



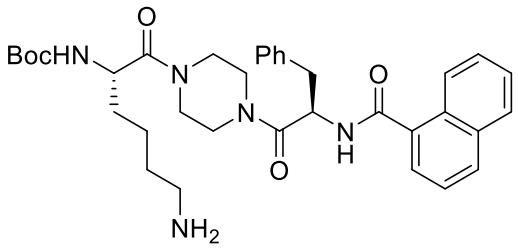
General procedure 8 followed to yield **63** (748 mg) as a colourless oil. The product was carried forward without any further purification or characterization.

***tert*-Butyl ((S)-1-(4-((1-naphthoyl)-L-phenylalanyl)piperazin-1-yl)-6-amino-1-oxohexan-2-yl)carbamate (64)**



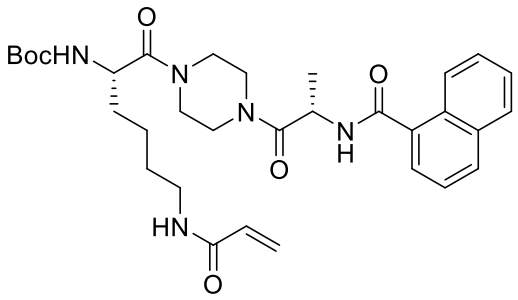
General procedure 8 followed to yield **64** (901 mg) as a colourless oil. The product was carried forward without any further purification or characterization.

***tert*-Butyl ((*S*)-1-(4-((1-naphthoyl)-D-phenylalanyl)piperazin-1-yl)-6-amino-1-oxohexan-2-yl)carbamate (65)**



General procedure 8 followed to yield **65** (1.08 g) as a colourless oil. The product was carried forward without any further purification or characterization.

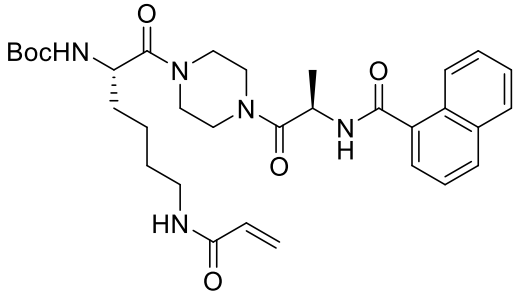
***tert*-Butyl ((*S*)-1-(4-((1-naphthoyl)-L-alanyl)piperazin-1-yl)-6-acrylamido-1-oxohexan-2-yl)carbamate (66)**



General procedure 9 followed to yield **66** (277 mg, 63%) as a colourless oil.

^{1}H NMR (400 MHz, Chloroform-*d*) δ_H 8.27 (d, *J* = 8.0 Hz, 1H), 7.87 (d, *J* = 8.0 Hz, 1H), 7.81 (dd, *J* = 7.6, 1.6 Hz, 1H), 7.59 (d, *J* = 7.2 Hz, 1H), 7.53–7.43 (m, 2H), 7.40 (t, *J* = 7.6 Hz, 1H), 7.07–6.99 (br, 1H), 6.21 (dd, *J* = 16.8, 1.2 Hz, 1H), 6.07–5.96 (m, 1H), 5.81–5.69 (br, 1H), 5.61–5.52 (m, 1H), 5.35 (d, *J* = 4.2 Hz, 1H), 5.15 (quint, *J* = 7.2 Hz, 1H), 4.05 (q, *J* = 7.2 Hz, 1H), 3.82–3.17 (m, 10H), 1.50–1.16 (m, 18H); ^{13}C (101 MHz, Chloroform-*d*) δ_C 174.3, 174.1, 169.9, 164.5, 154.5, 132.6, 132.5, 129.8, 129.6, 129.0, 127.2, 126.1, 125.3, 124.2, 124.1, 123.6, 78.8, 59.2, 48.7, 44.5, 31.8, 27.8, 27.1, 21.2, 19.9, 18.1, 13.0; HRMS (ESI-QTOF) m/z: [M + Na]$^+$ Calcd for $C_{32}H_{43}N_5O_6Na$ 616.3111; found 616.3130.

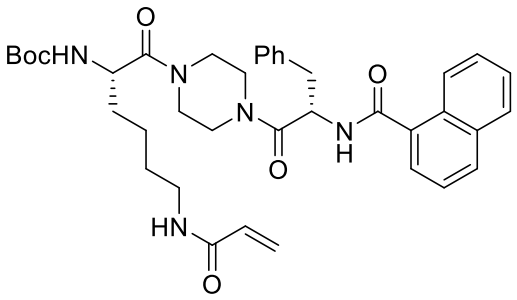
***tert*-Butyl ((*S*)-1-(4-((1-naphthoyl)-D-alanyl)piperazin-1-yl)-6-acrylamido-1-oxohexan-2-yl)carbamate (67)**



General procedure 9 followed to yield **67** (570 mg, 71%) as a colourless oil.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.33 (d, *J* = 8.2 Hz, 1H), 7.93 (d, *J* = 8.3 Hz, 1H), 7.90 – 7.85 (m, 1H), 7.66 (d, *J* = 7.0 Hz, 1H), 7.60 – 7.50 (m, 3H), 7.46 (t, *J* = 7.7 Hz, 1H), 7.09 (d, *J* = 7.3 Hz, 1H), 6.27 (dd, *J* = 16.9, 1.7 Hz, 1H), 6.08 (dq, *J* = 16.8, 9.3, 8.1 Hz, 1H), 5.81 (s, 1H), 5.63 (t, *J* = 9.2 Hz, 1H), 5.41 (d, *J* = 8.5 Hz, 1H), 5.22 (p, *J* = 6.9 Hz, 1H), 4.59 (q, *J* = 7.7 Hz, 1H), 3.97 (d, *J* = 12.8 Hz, 1H), 3.66 (tdd, *J* = 54.6, 20.6, 11.2 Hz, 6H), 3.45 – 3.21 (m, 3H), 1.71 (s, 4H), 1.63 – 1.48 (m, 4H), 1.44 (Stack, 11H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 171.2, 171.1, 165.7, 162.9, 153.8, 137.2, 135.6, 133.8, 131.1, 130.9, 130.3, 128.5, 127.4, 126.6, 125.5, 125.4, 124.8, 100.2, 80.1, 49.6, 45.8, 42.2, 33.3, 29.1, 28.5, 22.9, 22.5; HRMS (ESI-QTOF) m/z: [M + Na]$^+$ Calcd for $C_{32}H_{43}N_5O_6Na$ 616.3111; found 616.3130.

***tert*-Butyl ((*S*)-1-(4-((1-naphthoyl)-L-phenylalanyl)piperazin-1-yl)-6-acrylamido-1-oxohexan-2-yl)carbamate (68)**

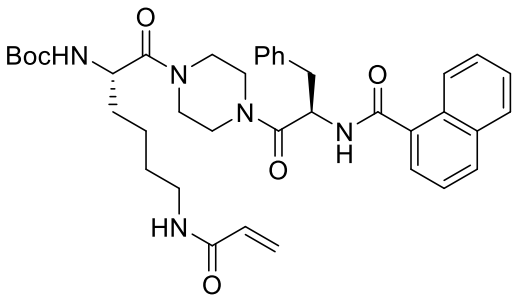


General procedure 9 followed to yield **68** (667 mg, 68%) as a colourless oil.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.26 – 8.20 (m, 1H), 7.93 (d, *J* = 8.2 Hz, 1H), 7.88 – 7.85 (m, 1H), 7.61 (dd, *J* = 7.4, 2.9 Hz, 1H), 7.53 (hept, *J* = 5.0 Hz, 2H), 7.45 (t, *J* = 7.7 Hz, 1H), 7.38 – 7.27 (m, 5H), 6.93 (t, *J* = 8.1 Hz, 1H), 6.34 – 6.22 (m, 1H), 6.17 – 6.00 (m, 1H), 5.82 (s, 1H), 5.68 – 5.57 (m, 1H), 5.53 – 5.33 (m, 2H), 4.57 – 4.42 (m, 1H), 3.83 – 2.89 (m, 11H), 1.71 (s, 3H), 1.67 – 1.48 (m, 1H), 1.46 – 1.41 (m, 9H), 1.38 (t, *J* = 7.0 Hz, 1H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 170.2, 168.9, 165.8, 164.3, 155.8, 133.8, 133.6, 131.2, 130.3, 129.9, 129.8, 129.7, 128.5, 127.6, 127.5, 127.4, 126.6, 126.5, 125.5, 124.8, 111.8, 79.4, 50.4, 50.3, 49.7, 41.9, 41.7, 29.0, 28.5, 22.5*; HRMS (ESI-QTOF) m/z: [M + Na]$^+$ Calcd for $C_{38}H_{47}N_5O_6Na$ 692.3424; found 692.3407.

*Two carbon resonances are missing, 2D-NMR experiments suggest they fall coincident with other carbon resonances.

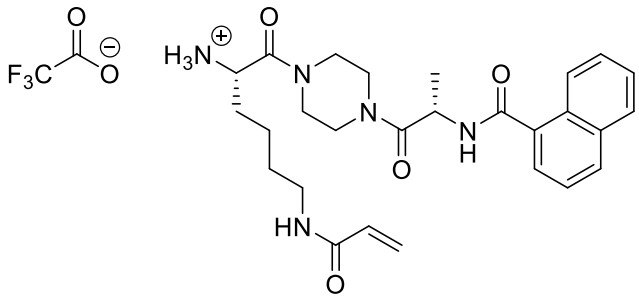
***tert*-Butyl ((*S*)-1-(4-((1-naphthoyl)-D-phenylalanyl)piperazin-1-yl)-6-acrylamido-1-oxohexan-2-yl)carbamate (69)**



General procedure 9 followed to yield **69** (552 mg, 46%) as a colourless oil.

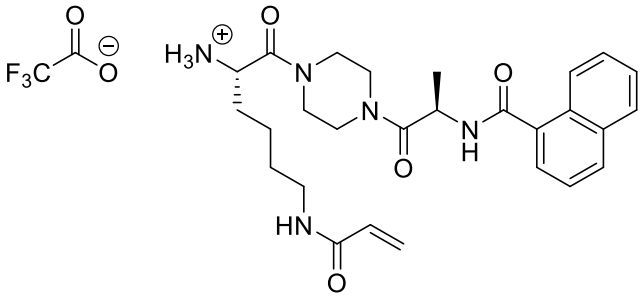
^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.27 – 8.19 (m, 1H), 7.92 (dd, *J* = 8.3, 1.2 Hz, 1H), 7.89 – 7.83 (m, 1H), 7.61 (dq, *J* = 7.3, 1.6 Hz, 1H), 7.57 – 7.48 (m, 2H), 7.48 – 7.40 (m, 1H), 7.37 – 7.27 (m, 5H), 6.97 (q, *J* = 10.1, 8.5 Hz, 1H), 6.35 – 6.21 (m, 1H), 6.17 – 6.00 (m, 1H), 5.85 (s, 1H), 5.62 (ddt, *J* = 16.2, 10.3, 1.6 Hz, 1H), 5.53 – 5.34 (m, 2H), 4.58 – 4.42 (m, 1H), 3.83 – 2.87 (Stack, 12H), 1.91 (s, 1H), 1.68 – 1.30 (Stack, 11H); HRMS (ESI-QTOF) m/z: [M + Na]$^+$ Calcd for $C_{38}H_{47}N_5O_6Na$ 692.3424; found 692.3432.

(*S*)-1-(4-((1-naphthoyl)-L-alanyl)piperazin-1-yl)-6-acrylamido-1-oxohexan-2-aminium trifluoroacetate (70)



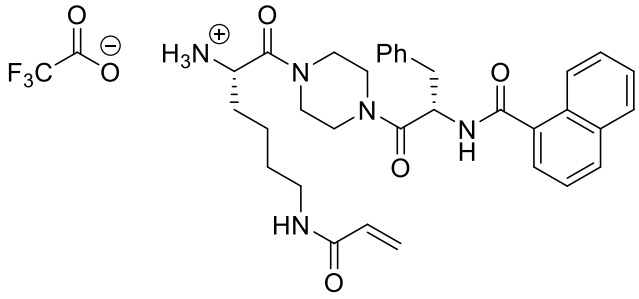
General procedure 6 followed to yield **70** (261 mg) as a white solid. The product was carried forward without any further purification or characterization.

(*S*)-1-(4-((1-naphthoyl)-D-alanyl)piperazin-1-yl)-6-acrylamido-1-oxohexan-2-aminium trifluoroacetate (71)



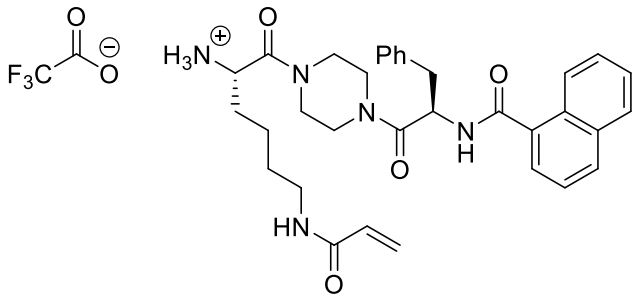
General procedure 6 followed to yield **71** (541 mg) as a white solid. The product was carried forward without any further purification or characterization.

(*S*)-1-(4-((1-naphthoyl)-L-phenylalanyl)piperazin-1-yl)-6-acrylamido-1-oxohexan-2-aminium trifluoroacetate (72)



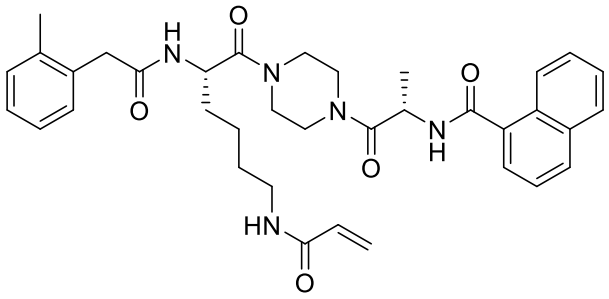
General procedure 6 followed to yield **72** (558 mg) as a white solid. The product was carried forward without any further purification or characterization.

(*S*)-1-(4-((1-naphthoyl)-D-phenylalanyl)piperazin-1-yl)-6-acrylamido-1-oxohexan-2-aminium trifluoroacetate (73)



General procedure 6 followed to yield **73** (486 mg) as a white solid. The product was carried forward without any further purification or characterization.

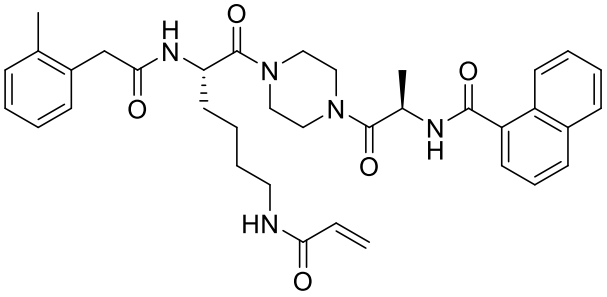
***N*-((*S*)-1-(4-(*N*^{6}-acryloyl-*N*^{2}-(2-(*o*-tolyl)acetyl)-L-lysyl)piperazin-1-yl)-1-oxopropan-2-yl)-1-naphthamide (74)**



General procedure 7 followed to yield **74** (27 mg, 37%) as a white solid.

^{1}H-NMR (400 MHz, Chloroform-*d*) δ_H 8.25 (d, *J* = 8.4 Hz, 1H), 7.86 (d, *J* = 8.0 Hz, 1H), 7.80 (d, *J* = 7.2 Hz, 1H), 7.58 (d, *J* = 7.2 Hz, 1H), 7.47 (quint, *J* = 8.0 Hz, 2H), 7.39 (t, *J* = 7.6 Hz, 1H), 7.16–7.10 (m, 4H), 6.36 (d, *J* = 8.0 Hz, 1H), 6.19 (d, *J* = 16.8 Hz, 1H), 6.02–5.90 (m, 2H), 5.57–5.49 (m, 1H), 4.88–4.79 (m, 1H), 3.93–3.31 (m, 12H), 2.23 (s, 3H), 1.66–1.35 (m, 9H); ^{13}C-NMR (101 MHz, Chloroform-*d*) δ_C 171.3, 171.2, 170.5, 168.8, 165.9, 137.0, 133.8, 133.7, 133.0, 131.1, 130.9, 130.5, 130.2, 128.5, 128.0, 127.9, 127.3, 126.8, 126.6, 126.5, 125.5, 125.3, 124.8, 144.0, 60.5, 48.4, 45.7, 41.6, 28.6, 22.2, 21.2, 19.7, 19.3, 14.3; HRMS (ESI-QTOF) m/z: $[M + Na]^+$ Calcd for $C_{36}H_{43}N_5O_5Na$ 648.3162; found 648.3171.

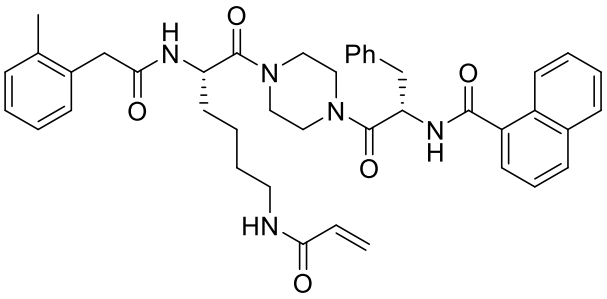
***N*-((*R*)-1-(4-(*N*^{6}-acryloyl-*N*^{2}-(2-(*o*-tolyl)acetyl)-L-lysyl)piperazin-1-yl)-1-oxopropan-2-yl)-1-naphthamide (75)**



General procedure 7 followed to yield **75** (78 mg, 76%) as a white solid.

^{1}H-NMR (400 MHz, Chloroform-*d*) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (d, *J* = 8.2 Hz, 1H), 7.92 (d, *J* = 8.3 Hz, 1H), 7.86 (dd, *J* = 7.6, 1.7 Hz, 1H), 7.65 (dt, *J* = 7.1, 1.0 Hz, 1H), 7.53 (Stack, 2H), 7.45 (dd, *J* = 8.2, 7.1 Hz, 1H), 7.23 – 7.15 (Stack, 5H), 7.11 (dd, *J* = 7.5, 2.3 Hz, 1H), 6.43 (d, *J* = 8.1 Hz, 1H), 6.25 (dd, *J* = 16.9, 1.8 Hz, 1H), 6.09 – 5.97 (Stack, 2H), 5.60 (dd, *J* = 10.6, 7.0 Hz, 1H), 5.20 (h, *J* = 6.6 Hz, 1H), 4.91 (td, *J* = 8.3, 4.2 Hz, 1H), 3.91 (td, *J* = 18.2, 17.0, 8.5 Hz, 1H), 3.79 – 3.41 (Stack, 9H), 3.28 (dd, *J* = 19.3, 9.2 Hz, 2H), 2.29 (d, *J* = 2.3 Hz, 3H), 1.74 – 1.60 (Stack, 2H), 1.49 (d, *J* = 6.0 Hz, 2H), 1.25 (s, 3H); ^{13}C-NMR (101 MHz, Chloroform-*d*) δ 171.2, 170.5, 168.9, 165.9, 137.0, 133.8, 133.7, 133.0, 131.1, 130.9, 130.5, 130.2, 128.5, 128.0, 127.3, 126.8, 126.6, 126.5, 125.5, 125.3, 124.8, 77.4, 48.4, 45.7, 45.2, 42.2, 42.1, 41.6, 39.0, 38.8, 32.7, 32.5, 31.9, 29.8, 28.6, 22.8, 22.2, 19.7, 19.3, 14.3. (Some resonances not observed due to falling coincident with other resonances); HRMS (ESI-QTOF) m/z: $[M + Na]^+$ Calcd for $C_{36}H_{43}N_5O_5Na$ 648.3162; found 648.3167.

***N*-((*S*)-1-(4-(*N*^{6}-acryloyl-*N*^{2}-(2-(*o*-tolyl)acetyl)-L-lysyl)piperazin-1-yl)-1-oxo-3-phenylpropan-2-yl)-1-naphthamide (76)**

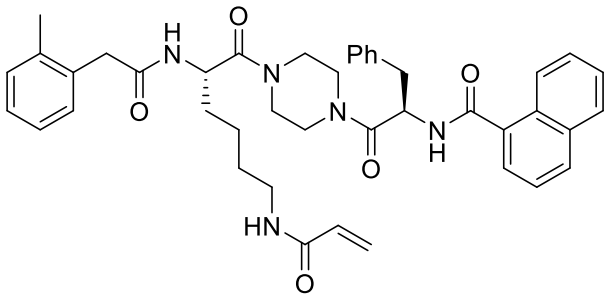


General procedure 7 followed to yield **76** (95 mg, 92%) as a white solid.

^{1}H-NMR (400 MHz, Chloroform-*d*) □ 8.21 (dt, *J* = 8.2, 3.4 Hz, 1H), 7.91 (d, *J* = 8.2 Hz, 1H), 7.88 – 7.83 (m, 1H), 7.60 (d, *J* = 7.0 Hz, 1H), 7.52 (Stack, 2H), 7.43 (t, *J* = 7.7 Hz, 1H), 7.35 – 7.23 (Stack, 5H), 7.23 – 7.14 (Stack, 4H), 7.05 (q, *J* = 8.1, 7.5 Hz, 1H), 6.51 – 6.37 (m, 1H), 6.32 – 6.20 (m, 1H), 6.12 – 5.96 (m, 1H), 5.64 – 5.54 (m, 1H), 5.49 – 5.37 (m, 1H), 4.90 – 4.73 (m, 1H), 3.47 – 3.16 (m, 3H), 3.15 – 2.97 (m, 1H) 2.30 – 2.25 (m, 3H), 1.63 – 1.38 (Stack, 5H), 1.31 – 1.19 (m, 2H); ^{13}C-NMR (101 MHz, Chloroform-*d*) δ 171.0, 170.2, 168.9, 165.9, 137.0,

136.1, 133.8, 133.5, 133.0, 131.1, 130.9, 130.9, 130.5, 130.2, 129.9, 129.7, 129.6, 128.9, 128.4, 128.0, 127.6, 127.3, 126.7, 126.6, 126.3, 125.5, 125.4, 124.8, 53.7, 50.4, 48.3, 45.7, 45.4, 45.0, 42.0, 41.6, 40.1, 39.8, 39.0, 38.7, 32.6, 28.5, 22.2, 19.7, 18.7, 17.5, 12.0. (Some resonances not observed due to falling coincident with other resonances); HRMS (ESI-QTOF) m/z: $[M + Na]^+$ Calcd for $C_{42}H_{47}N_5O_5Na$ 724.3475; found 724.3449.

_N_-((_R_)-1-(4-(_N_^{6}-acryloyl-_N_^{2}-(2-(_o_-tolyl)acetyl)-L-lysyl)piperazin-1-yl)-1-oxo-3-phenylpropan-2-yl)-1-naphthamide (77)

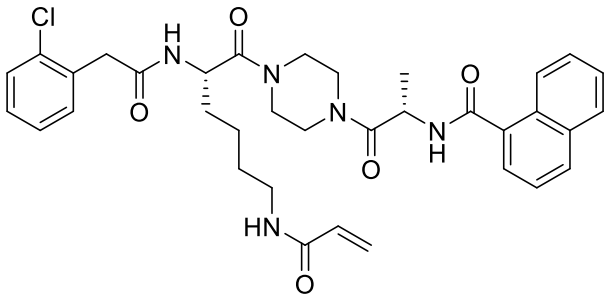


General procedure 7 followed to yield **77** (58 mg, 57%) as a white solid.

^{1}H-NMR (600 MHz, $CDCl_3$) δ 8.23 – 8.18 (m, 1H), 7.91 (d, *J* = 8.2 Hz, 1H), 7.85 (dd, *J* = 5.9, 3.8 Hz, 1H), 7.60 (d, *J* = 6.9 Hz, 1H), 7.55 – 7.49 (Stack, 2H), 7.43 (t, *J* = 7.6 Hz, 1H), 7.34 – 7.29 (Stack, 2H), 7.29 – 7.25 (d, *J* = 6.0 Hz, 3H), 7.22 – 7.15 (Stack, 4H), 7.12 – 7.04 (m, 1H), 6.48 – 6.40 (m, 1H), 6.29 – 6.21 (m, 1H), 6.12 – 5.98 (Stack, 2H), 5.63 – 5.55 (m, 1H), 5.43 (dtt, *J* = 12.3, 8.7, 6.0 Hz, 1H), 4.81 (dtt, *J* = 34.4, 7.9, 4.0 Hz, 1H), 3.73 – 2.89 (Stack, 14H), 2.29 – 2.25 (m, 3H), 1.63 – 1.40 (Stack, 4H), 1.30 – 1.20 (m, 4H); ^{13}C-NMR (151 MHz, $CDCl_3$) δ 171.0, 171.0, 170.2, 170.2, 169.1, 169.0, 168.9, 165.9, 165.8, 137.0, 136.2, 136.1, 136.0, 136.0, 133.8, 133.5, 133.0, 133.0, 131.1, 130.9, 130.9, 130.9, 130.5, 130.2, 129.9, 129.7, 129.6, 129.6, 128.9, 128.9, 128.4, 128.0 (d, *J* = 2.4 Hz), 127.6, 127.6, 127.5, 127.4, 127.3, 126.7, 126.6, 126.3, 125.5, 125.4 (d, *J* = 2.9 Hz), 124.7, 50.5, 50.4, 50.3, 50.3, 48.3, 48.3, 48.2, 45.6, 45.3, 45.0, 45.0, 44.8, 42.1, 42.0, 41.8, 41.7, 41.6, 41.6, 41.6, 40.1, 40.0, 39.9, 39.8, 39.1, 39.0, 39.0, 38.9, 32.6, 29.8, 28.5, 28.5, 22.2, 22.2, 19.7; HRMS (ESI-QTOF) m/z: $[M + Na]^+$ Calcd for $C_{42}H_{47}N_5O_5Na$ 724.3475; found 724.3482.

*Use of a high-field, 600 MHz NMR spectrometer, rotamers were clearly observable throughout ^{13}C-NMR spectrum and some examples in the ^{1}H-NMR. All resonances have been picked, reported and correlated using 2D-NMR HSQC and HMBC experiments.

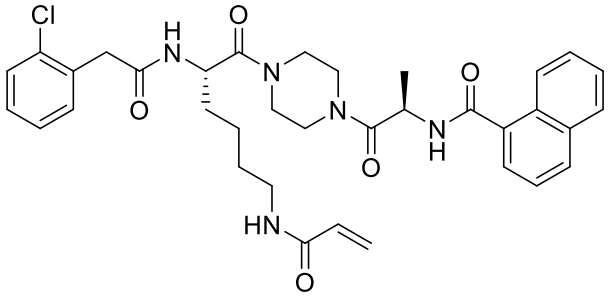
***N*-((*S*)-1-(4-(*N*^{6}-acryloyl-*N*^{2}-(2-(2-chlorophenyl)acetyl)-L-lysyl)piperazin-1-yl)-1-oxopropan-2-yl)-1-naphthamide (78)**



General procedure 7 followed to yield **78** (32 mg, 43%) as a white solid.

^{1}H-NMR (400 MHz, Chloroform-*d*) δ_H 8.26 (d, *J* = 8.4 Hz, 1H), 7.86 (d, *J* = 8.0 Hz, 1H), 7.80 (dd, *J* = 8.4, 1.6 Hz, 1H), 7.58 (d, *J* = 7.2 Hz, 1H), 7.51–7.42 (m, 2H), 7.38 (t, *J* = 7.6 Hz, 1H), 7.35–7.30 (m, 1H), 7.28–7.23 (m, 1H), 7.22–7.15 (m, 2H), 7.07 (d, *J* = 7.6 Hz, 1H), 6.57 (d, *J* = 8.0 Hz, 1H), 6.17 (dd, *J* = 16.8, 1.2 Hz, 1H), 6.02–5.90 (m, 2H), 5.54–5.47 (m, 1H), 5.18–5.08 (br, 1H), 4.89–4.81 (m, 1H), 3.67–3.12 (m, 12H), 1.52–1.22 (m, 9H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ_C 171.2, 170.5, 169.7, 165.8, 162.6, 134.5, 133.8, 133.7, 132.8, 131.9, 131.1, 131.0, 130.2, 129.9, 129.2, 128.5, 127.5, 127.3, 126.5, 126.2, 125.5, 125.3, 124.8, 60.5, 48.5, 45.7, 42.2, 41.5, 36.6, 28.6, 22.2, 21.2, 14.3; HRMS (ESI-QTOF) m/z: $[M + Na]^+$ Calcd for $C_{35}H_{40}N_5O_5{}^{35}ClNa$ 668.2616; found 668.2634.

***N*-((*R*)-1-(4-(*N*^{6}-acryloyl-*N*^{2}-(2-(2-chlorophenyl)acetyl)-L-lysyl)piperazin-1-yl)-1-oxopropan-2-yl)-1-naphthamide (79)**

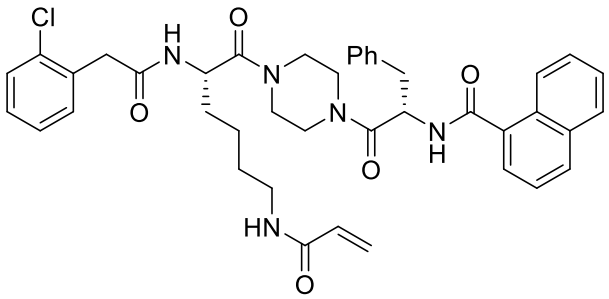


General procedure 7 followed to yield **79** (59 mg, 55%) as a white solid.

^{1}H-NMR (400 MHz, Chloroform-*d*) □ 8.33 (d, *J* = 8.2 Hz, 1H), 7.93 (d, *J* = 8.3 Hz, 1H), 7.87 (dd, *J* = 7.6, 1.8 Hz, 1H), 7.65 (dt, *J* = 7.1, 1.0 Hz, 1H), 7.59 – 7.49 (Stack, 2H), 7.50 – 7.43 (m, 1H), 7.43 – 7.38 (m, 1H), 7.37 – 7.31 (m, 1H), 7.30 – 7.25 (Stack, 4H), 7.08 (d, *J* = 7.5 Hz, 1H), 6.56 (d, *J* = 8.0 Hz, 1H), 6.25 (dd, *J* = 17.0, 1.6 Hz, 1H), 6.01 (Stack, 2H), 5.63 – 5.54 (m, 1H), 5.20 (t, *J* = 7.3 Hz, 1H), 4.93 (td, *J* = 8.4, 4.2 Hz, 1H), 3.94 (d, *J* = 11.6 Hz, 1H), 3.85 – 3.42 (Stack, 8H), 3.38 – 3.19 (m, 2H), 1.84 (s, 3H), 1.67 (s, 6H), 1.55 – 1.47 (Stack, 4H); ^{13}C-NMR (101 MHz, Chloroform-*d*) □ 171.2, 170.5, 165.9, 162.7, 134.5, 133.8, 132.8, 131.9, 131.1, 130.9, 130.3, 129.9, 129.5, 129.3, 128.5, 127.6, 127.4, 126.6, 125.5, 125.4, 124.8, 48.5, 45.7, 41.6, 39.1, 28.6, 22.2, 19.3. (Some resonances not observed due to falling coincident with

other resonances – Identified via 2D-NMR experiments); HRMS (ESI-QTOF) m/z: $[M + Na]^+$ Calcd for $C_{35}H_{40}N_5O_5{}^{35}ClNa$ 668.2616; found 688.2623.

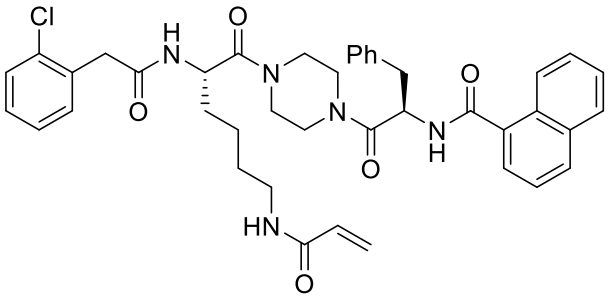
_N_-((_S_)-1-(4-(_N_^{6}-acryloyl-_N_^{2}-(2-(2-chlorophenyl)acetyl)-L-lysyl)piperazin-1-yl)-1-oxo-3-phenylpropan-2-yl)-1-naphthamide (80)



General procedure 7 followed to yield **80** (80 mg, 76%) as a white solid.

^{1}H-NMR (400 MHz, Chloroform-*d*) δ 8.21 (q, *J* = 4.3 Hz, 1H), 7.91 (d, *J* = 8.2 Hz, 1H), 7.88 – 7.83 (m, 1H), 7.59 (dt, *J* = 7.1, 1.7 Hz, 1H), 7.51 (Stack, 2H), 7.43 (dd, *J* = 8.3, 7.0 Hz, 1H), 7.38 (qd, *J* = 3.5, 1.8 Hz, 1H), 7.34 – 7.20 (Stack, 9H), 7.08 (td, *J* = 8.4, 5.6 Hz, 1H), 6.67 – 6.58 (m, 1H), 6.30 – 6.18 (m, 1H), 6.15 – 5.93 (Stack, 2H), 5.60 – 5.51 (m, 1H), 5.48 – 5.36 (m, 1H), 4.90 – 4.74 (m, 1H), 3.70 (d, *J* = 7.4 Hz, 2H), 3.61 – 2.99 (m, 11H), 2.12 (m, 1H), 1.64 – 1.42 (Stack, 4H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 170.2, 170.2, 169.8, 169.0, 165.8, 136.2, 136.1, 136.0, 134.5, 133.7, 133.5, 132.8, 131.8, 131.1, 131.0, 131.0, 130.2, 129.9, 129.7, 129.6, 129.2, 128.9, 128.4, 127.5, 127.3, 126.5, 126.2, 125.5, 125.4, 124.7, 77.5, 50.4, 48.4, 45.6, 45.3, 45.0, 41.7, 41.4, 39.1, 39.0, 38.7, 32.7, 28.5, 22.2. (Some resonances not observed due to falling coincident with other resonances); HRMS (ESI-QTOF) m/z: $[M + Na]^+$ Calcd for $C_{41}H_{44}N_5O_5{}^{35}ClNa$ 744.2929; found 744.2929.

_N_-((_R_)-1-(4-(_N_^{6}-acryloyl-_N_^{2}-(2-(2-chlorophenyl)acetyl)-L-lysyl)piperazin-1-yl)-1-oxo-3-phenylpropan-2-yl)-1-naphthamide (81)

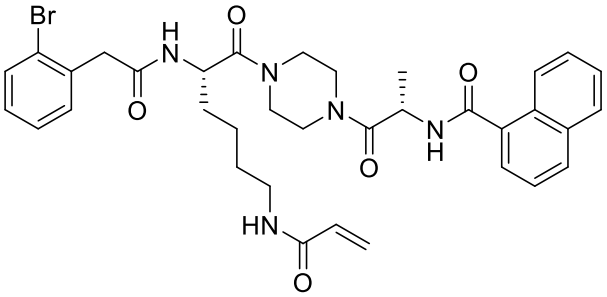


General procedure 7 followed to yield **81** (23 mg, 22%) as a white solid.

^{1}H-NMR (400 MHz, Chloroform-*d*) δ 8.26 – 8.17 (m, 1H), 7.92 (d, *J* = 8.3 Hz, 1H), 7.89 – 7.82 (m, 1H), 7.60 (dt, *J* = 7.2, 1.7 Hz, 1H), 7.57 – 7.48 (Stack, 2H), 7.47 – 7.37 (Stack, 2H),

7.35 – 7.28 (Stack, 5H), 7.02 (q, *J* = 7.0, 6.0 Hz, 1H), 6.63 – 6.52 (m, 1H), 6.31 – 6.19 (m, 1H), 6.08 – 5.92 (m, 2H), 5.57 (td, *J* = 11.2, 10.2, 3.2 Hz, 1H), 5.44 (q, *J* = 7.7 Hz, 1H), 4.90 – 4.75 (m, 1H), 3.78 – 2.86 (Stack, 15H), 1.67 – 1.42 (Stack, 5H), 1.40 – 1.28 (Stack, 3H). ^{13}C-NMR (101 MHz, Chloroform-*d*) δ 170.3, 170.2, 169.8, 169.8, 165.8, 134.5, 133.8, 133.5, 132.8, 131.9, 131.2, 131.0, 130.2, 129.9, 130.0 – 129.6 (Stack), 129.2, 128.9, 128.5, 127.5, 127.3, 126.6, 126.3, 125.5, 125.4, 124.8, 114.0, 60.5, 50.4, 48.5, 48.4, 45.7, 45.4, 45.0, 42.0, 41.8, 41.7, 41.5, 32.8, 29.8, 28.5, 28.5, 22.2; HRMS (ESI-QTOF) m/z: $[M + Na]^+$ Calcd for $C_{41}H_{44}{}^{35}ClN_5O_5Na$ 744.2929; found 744.2921.

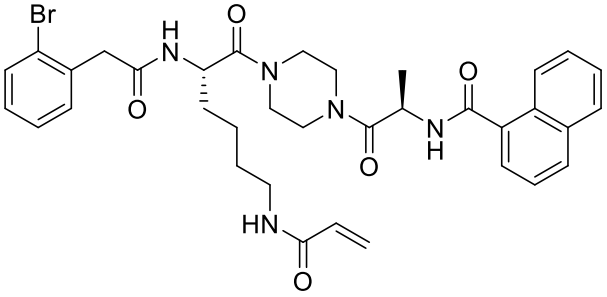
_N_-((_S_)-1-(4-(_N_^{6}-acryloyl-_N_^{2}-(2-(2-bromophenyl)acetyl)-L-lysyl)piperazin-1-yl)-1-oxopropan-2-yl)-1-naphthamide (82)



General procedure 7 followed to yield **82** (37 mg, 47%) as a white solid.

^{1}H-NMR (400 MHz, Chloroform-*d*) δ_H 8.26 (d, *J* = 8.0 Hz, 1H), 7.86 (d, *J* = 8.4 Hz, 1H), 7.80 (d, *J* = 7.6 Hz, 1H), 7.58 (d, *J* = 6.8 Hz, 1H), 7.54–7.42 (m, 3H), 7.39 (t, *J* = 7.6 Hz, 1H), 7.29–7.22 (m, 2H), 7.14–7.02 (m, 2H), 6.18 (d, *J* = 16.8 Hz, 1H), 6.02–5.90 (m, 2H), 5.56–5.47 (m, 1H), 5.18–5.08 (br, 1H), 4.90–4.81 (br, 1H), 3.72–3.12 (m, 12H), 1.56–1.22 (m, 9H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ_C 171.2, 170.4, 169.7, 168.8, 165.9, 134.5, 133.8, 133.7, 133.2, 131.9, 131.1, 130.9, 130.2, 129.4, 128.5, 128.2, 127.3, 126.6, 126.4, 125.5, 125.3, 125.0, 124.8, 60.5, 53.6, 48.5, 45.7, 44.0, 42.2, 28.6, 22.2, 19.3, 14.3; HRMS (ESI-QTOF) m/z: $[M + Na]^+$ Calcd for $C_{35}H_{40}N_5O_5{}^{79}BrNa$ 712.2111; found 712.2095.

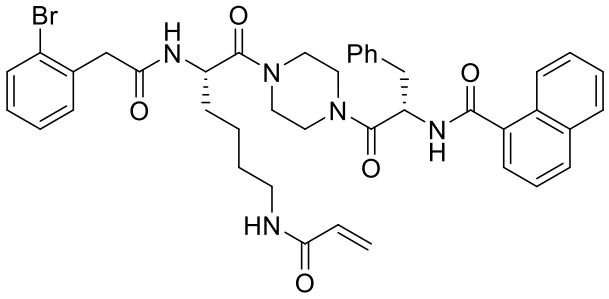
_N_-((_R_)-1-(4-(_N_^{6}-acryloyl-_N_^{2}-(2-(2-bromophenyl)acetyl)-L-lysyl)piperazin-1-yl)-1-oxopropan-2-yl)-1-naphthamide (83)



General procedure 7 followed to yield **83** (75 mg, 66%) as a white solid.

^{1}H-NMR (400 MHz, Chloroform-*d*) □ 8.32 (d, *J* = 8.2 Hz, 1H), 7.92 (d, *J* = 8.3 Hz, 1H), 7.86 (dd, *J* = 7.4, 1.8 Hz, 1H), 7.65 (dt, *J* = 7.0, 1.0 Hz, 1H), 7.61 – 7.56 (Stack, 2H), 7.56 – 7.49 (Stack, 2H), 7.48 – 7.42 (m, 1H), 7.36 – 7.29 (m, 2H), 7.17 (ddd, *J* = 8.0, 6.4, 2.7 Hz, 1H), 7.10 (d, *J* = 7.5 Hz, 1H), 6.57 (d, *J* = 7.8 Hz, 1H), 6.25 (dd, *J* = 16.9, 1.7 Hz, 1H), 6.08 – 5.95 (Stack, 2H), 5.57 (dd, *J* = 10.6, 4.2 Hz, 1H), 5.19 (d, *J* = 7.7 Hz, 1H), 4.93 (td, *J* = 8.4, 4.2 Hz, 1H), 3.93 (dt, *J* = 14.9, 5.4 Hz, 1H), 3.74 – 3.40 (Stack, 9H), 3.36 – 3.20 (m, 3H), 1.93 (s, 1H), 1.73 – 1.63 (m, 1H), 1.63 – 1.46 (Stack, 6H); ^{13}C-NMR (101 MHz, Chloroform-*d*) δ 171.2, 170.4, 169.8, 168.8, 165.9, 137.7, 136.2, 134.6, 133.8, 133.7, 133.2, 131.9, 131.1, 130.9, 130.2, 129.4, 128.5, 128.2, 127.4, 126.6, 126.4, 125.5, 125.4, 125.1, 124.8, 48.5, 45.7, 45.3, 44.0, 42.2, 39.0, 38.1, 28.6, 22.2, 19.4. (Some resonances not observed due to falling coincident with other resonances); HRMS (ESI-QTOF) m/z: [M + Na]$^+$ Calcd for $C_{35}H_{40}N_5O_5{}^{79}BrNa$ 712.2111; found 712.2091.

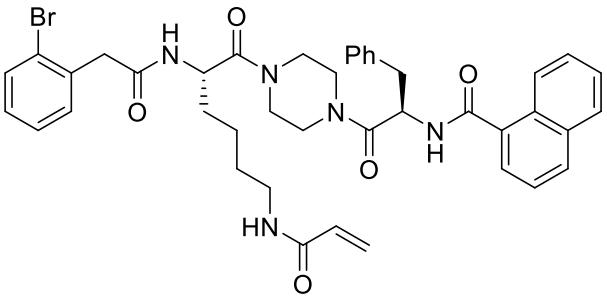
_N_-((_S_)-1-(4-(_N_^{6}-acryloyl-_N_^{2}-(2-(2-bromophenyl)acetyl)-L-lysyl)piperazin-1-yl)-1-oxo-3-phenylpropan-2-yl)-1-naphthamide (84)



General procedure 7 followed to yield **84** (95 mg, 85%) as a white solid.

^{1}H-NMR (400 MHz, Chloroform-*d*) □ 8.24 – 8.17 (m, 1H), 7.91 (d, *J* = 8.2 Hz, 1H), 7.88 – 7.81 (m, 1H), 7.62 – 7.54 (Stack, 2H), 7.53 – 7.48 (Stack, 2H), 7.42 (td, *J* = 7.6, 7.0, 1.1 Hz, 1H), 7.34 – 7.22 (Stack, 8H), 7.20 – 7.08 (Stack, 2H), 6.68 – 6.58 (m, 1H), 6.30 – 6.19 (m, 1H), 6.12 (dd, *J* = 13.9, 4.8 Hz, 1H), 6.08 – 5.94 (m, 1H), 5.62 – 5.51 (m, 1H), 5.43 (td, *J* = 8.5, 6.0 Hz, 1H), 4.82 (dtd, *J* = 24.2, 8.4, 4.1 Hz, 1H), 3.71 (d, *J* = 7.6 Hz, 3H), 3.63 – 2.99 (Stack, 12H), 2.70 – 2.45 (m, 1H), 1.67 – 1.43 (Stack, 4H); ^{13}C-NMR (101 MHz, Chloroform-*d*) □ 170.2, 169.7, 168.9, 165.8, 136.0, 134.5, 133.7, 133.5, 133.2, 131.8, 131.1, 131.0, 130.2, 129.8, 129.7, 129.6, 129.4, 128.9, 128.4, 128.1, 127.6, 127.3, 126.5, 126.2, 125.5, 125.4, 125.0, 124.7, 50.3, 48.4, 45.6, 45.3, 45.0, 43.9, 42.0, 41.6, 39.8, 38.7, 32.7, 28.5, 22.2. (Some resonances not observed due to falling coincident with other resonances); HRMS (ESI-QTOF) m/z: [M + Na]$^+$ Calcd for $C_{41}H_{44}N_5O_5{}^{79}BrNa$ 788.2424; found 788.2435.

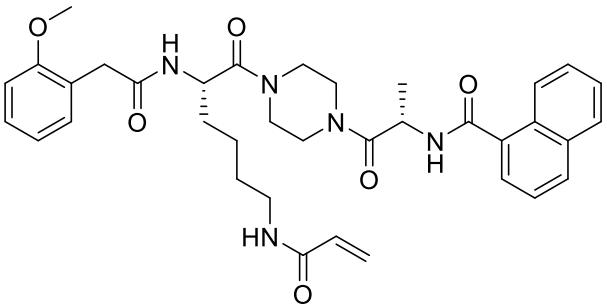
***N*-((*R*)-1-(4-(N^6-acryloyl-N^2-(2-(2-bromophenyl)acetyl)-L-lysyl)piperazin-1-yl)-1-oxo-3-phenylpropan-2-yl)-1-naphthamide (85)**



General procedure 7 followed to yield **85** (37 mg, 33%) as a white solid.

^{1}H-NMR (400 MHz, Chloroform-*d*) δ 8.25 – 8.18 (m, 1H), 7.92 (d, *J* = 8.2 Hz, 1H), 7.89 – 7.82 (m, 1H), 7.62 – 7.55 (Stack, 2H), 7.55 – 7.50 (Stack, 2H), 7.44 (dd, *J* = 8.2, 7.1 Hz, 1H), 7.36 – 7.27 (Stack, 6H), 7.21 – 7.10 (m, 1H), 7.01 (d, *J* = 4.3 Hz, 1H), 6.56 (td, *J* = 10.9, 9.2, 5.8 Hz, 1H), 6.32 – 6.20 (m, 1H), 6.08 – 5.94 (m, 2H), 5.63 – 5.53 (m, 1H), 5.44 (h, *J* = 7.5, 6.9 Hz, 1H), 4.92 – 4.75 (m, 1H), 3.80 – 2.86 (Stack, 15H), 1.64 – 1.46 (m, 5H), 1.37 – 1.21 (Stack, 3H); ^{13}C-NMR (101 MHz, Chloroform-*d*) δ 170.3, 170.2, 169.7, 169.7, 165.8, 134.5, 133.8, 133.5, 133.2, 131.9, 131.2, 130.2, 129.9, 129.7 (d, *J* = 9.8 Hz), 129.4, 128.9 (d, *J* = 2.6 Hz), 128.5, 128.2, 127.3, 126.6, 126.3, 125.5, 125.4, 125.1, 124.8, 114.0, 50.4, 48.4, 45.0, 44.0, 41.8 (d, *J* = 11.7 Hz), 39.1, 34.8, 32.8, 31.7, 28.5, 25.4, 22.8, 14.3; HRMS (ESI-QTOF) m/z: $[M + Na]^+$ Calcd for $C_{41}H_{44}{}^{79}BrN_5O_5Na$ 788.2424; found 788.2449

***N*-((*S*)-1-(4-(N^6-acryloyl-N^2-(2-(2-methoxyphenyl)acetyl)-L-lysyl)piperazin-1-yl)-1-oxopropan-2-yl)-1-naphthamide (86)**

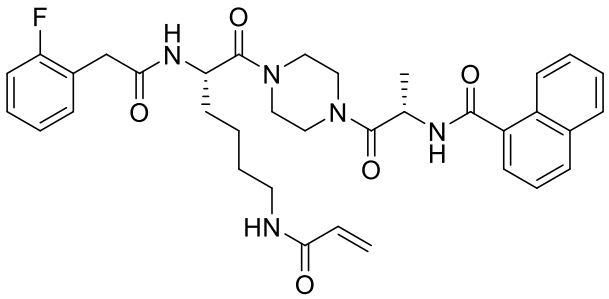


General procedure 7 followed to yield **86** (118 mg, 86%) as a white solid.

^{1}H-NMR (400 MHz, Chloroform-*d*) δ_H 8.28 (d, *J* = 7.6 Hz, 1H), 7.87 (d, *J* = 8.4 Hz, 1H), 7.82 (d, *J* = 7.6 Hz, 1H), 7.60 (d, *J* = 6.8 Hz, 1H), 7.55–7.44 (m, 2H), 7.34 (t, *J* = 7.6 Hz, 1H), 7.02 (d, *J* = 6.4 Hz, 1H), 6.93–6.83 (m, 2H), 6.79 (d, *J* = 7.6 Hz, 1H), 6.26–6.15 (m, 2H), 6.07–5.95 (m, 1H), 5.52 (d, *J* = 9.6 Hz, 1H), 5.20–5.08 (m, 1H), 4.89–4.78 (m, 1H), 3.89–3.09 (m, 17H), 1.54–1.12 (m, 9H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ_C 171.3, 171.1, 170.5, 168.8, 165.8, 157.1, 133.7, 133.6, 131.2, 131.1, 131.0, 130.2, 129.0, 128.4, 127.2, 126.5, 126.0, 125.4, 125.3, 123.3,

121.1, 114.0, 110.7, 55.4, 48.2, 45.6, 45.4, 45.2, 42.0, 41.8, 39.0, 38.8, 32.6, 28.4, 22.0, 19.1; HRMS (ESI-QTOF) m/z: $[M + Na]^+$ Calcd for $C_{36}H_{43}N_5O_6Na$ 664.3093; found 664.3111.

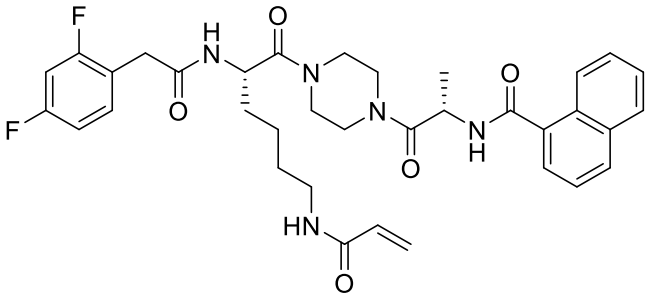
_N_-((_S_)-1-(4-(_N_^{6}-acryloyl-_N_^{2}-(2-(2-fluorophenyl)acetyl)-L-lysyl)piperazin-1-yl)-1-oxopropan-2-yl)-1-naphthamide (87)



General procedure 7 followed to yield **87** (41 mg, 60%) as a white solid.

^{1}H-NMR (400 MHz, Chloroform-*d*) δ_H 8.26 (d, *J* = 8.0 Hz, 1H), 7.85 (d, *J* = 8.4 Hz, 1H), 7.80 (d, *J* = 7.6 Hz, 1H), 7.58 (d, *J* = 7.2 Hz, 1H), 7.52–7.42 (m, 1H), 7.38 (t, *J* = 7.6 Hz, 1H), 7.10–6.97 (m, 3H), 6.65–6.55 (m, 1H), 6.18 (dd, *J* = 17.2, 1.2 Hz, 1H), 6.02–5.86 (m, 2H), 5.56–5.47 (m, 1H), 5.19–5.08 (br, 1H), 4.90–4.79 (m, 1H), 4.08–3.11 (m, 14H), 1.57–1.20 (m, 9H); ^{13}C-NMR (101 MHz, Chloroform-*d*) δ_C 171.2, 170.5, 165.9, 162.3, 159.9, 133.8, 133.7, 131.8, 131.7, 131.1, 131.0, 130.3, 129.6, 129.5, 128.5, 127.3, 126.6, 126.3, 125.5, 125.4, 124.8, 124.7, 124.6, 48.6, 45.8, 45.3, 45.2, 42.2, 41.9, 39.0, 36.9, 36.9, 28.7, 22.2, 19.3; HRMS (ESI-QTOF) m/z: $[M + Na]^+$ Calcd for $C_{35}H_{40}N_5O_5FNa$ 652.2941; found 652.2911.

_N_-((_S_)-1-(4-(_N_^{6}-acryloyl-_N_^{2}-(2-(2,4-difluorophenyl)acetyl)-L-lysyl)piperazin-1-yl)-1-oxopropan-2-yl)-1-naphthamide (88)



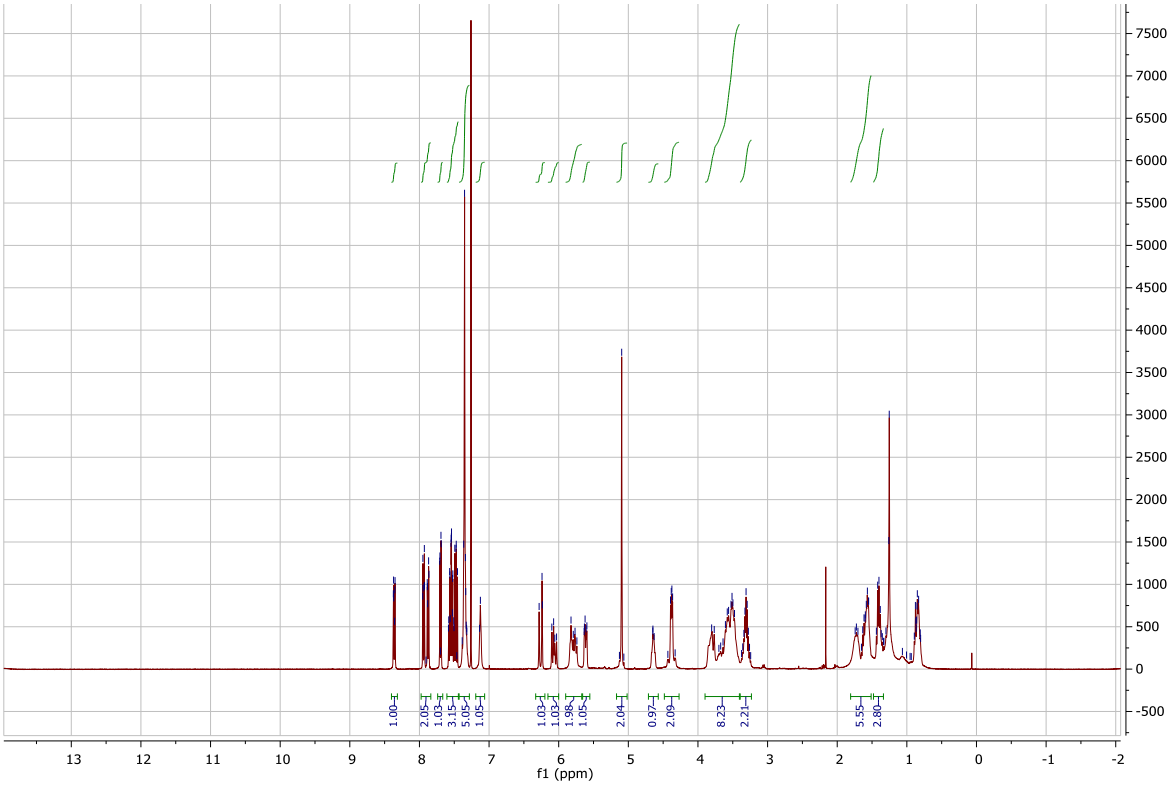
General procedure 7 followed to yield **88** (63 mg, 91%) as a white solid.

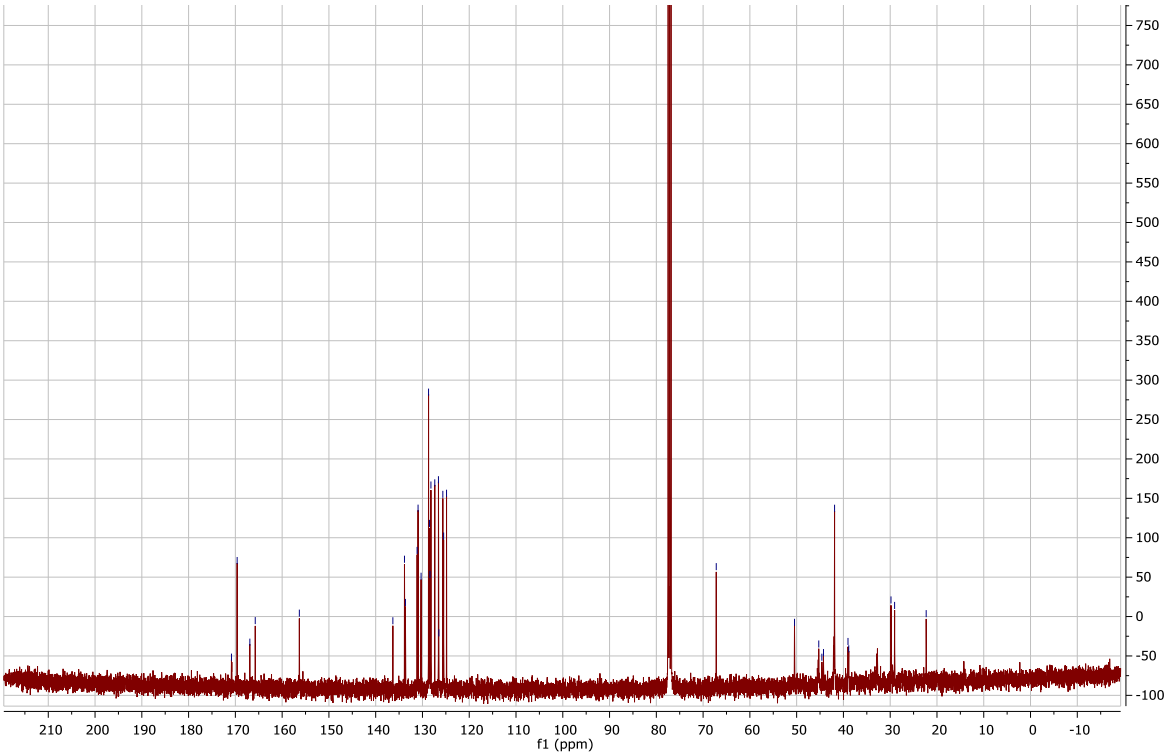
^{1}H-NMR (400 MHz, Chloroform-*d*) δ_H 8.30 (d, *J* = 8.0 Hz, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 7.84 (d, *J* = 7.6 Hz, 1H), 7.62 (d, *J* = 7.2 Hz, 1H), 7.51 (quint, *J* = 8.0 Hz, 2H), 7.42 (t, *J* = 7.6 Hz, 1H), 7.16–7.06 (m, 1H), 6.88–6.77 (m, 2H), 6.22 (d, *J* = 16.8 Hz, 1H), 6.06–5.92 (m, 1H), 5.60–5.52 (m, 1H), 5.23–5.12 (m, 1H), 4.92–4.83 (m, 1H), 3.98–3.17 (m, 13H), 1.73–1.20 (m, 10H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ_C 171.1, 170.4, 169.6, 168.7, 165.7, 133.7, 133.6, 132.4,

132.3, 132.2, 132.1, 131.0, 130.8, 130.1, 128.4, 127.2, 126.4, 126.2, 125.4, 125.2, 124.7, 111.7, 111.5, 48.6, 45.6, 45.2, 42.1, 38.8, 38.7, 38.6, 36.0, 32.6, 28.6, 22.0, 19.1; HRMS (ESI-QTOF) m/z: $[M + Na]^+$ Calcd for $C_{35}H_{39}N_5O_5F_2Na$ 670.2817; found 670.2817.

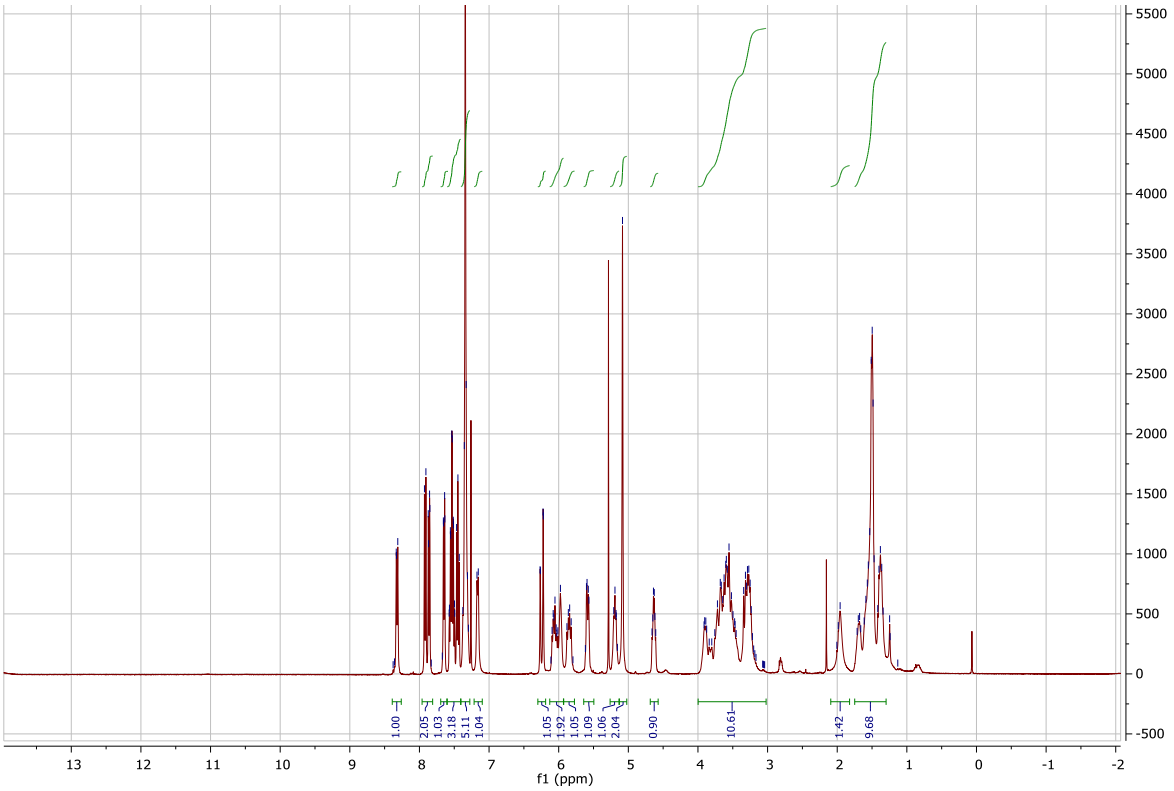
NMR Spectra

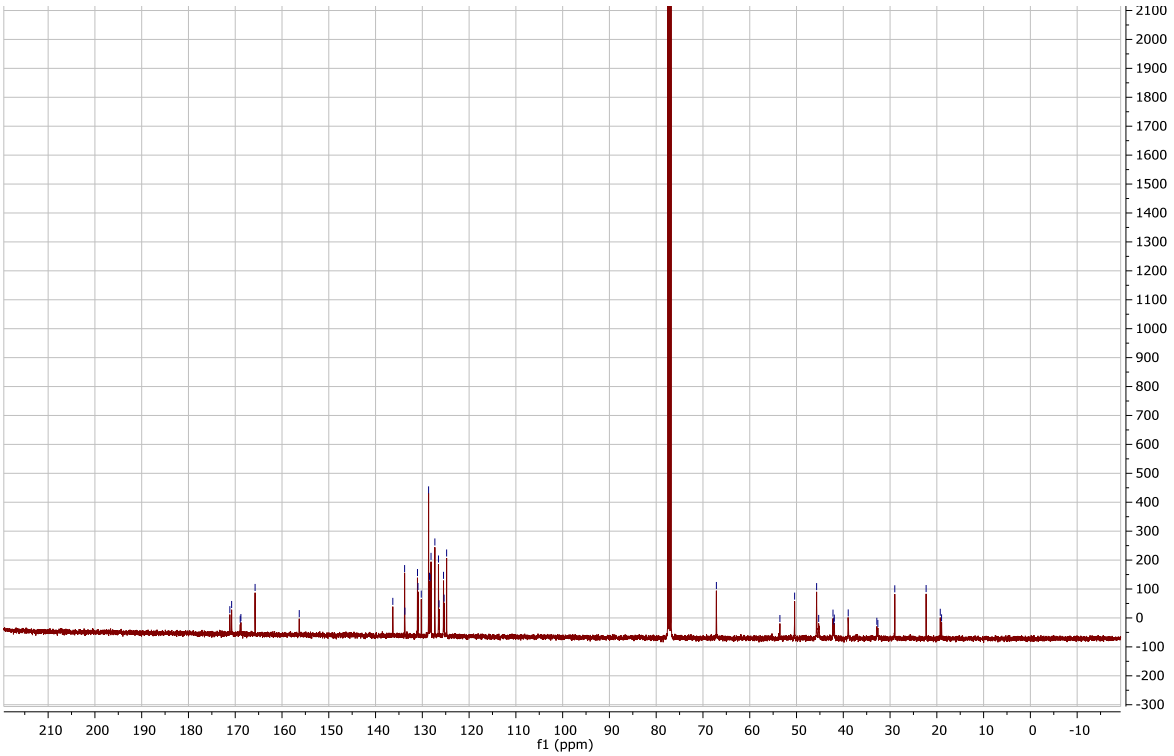
Compound 28 – ^{1}H and ^{13}C NMR Spectra



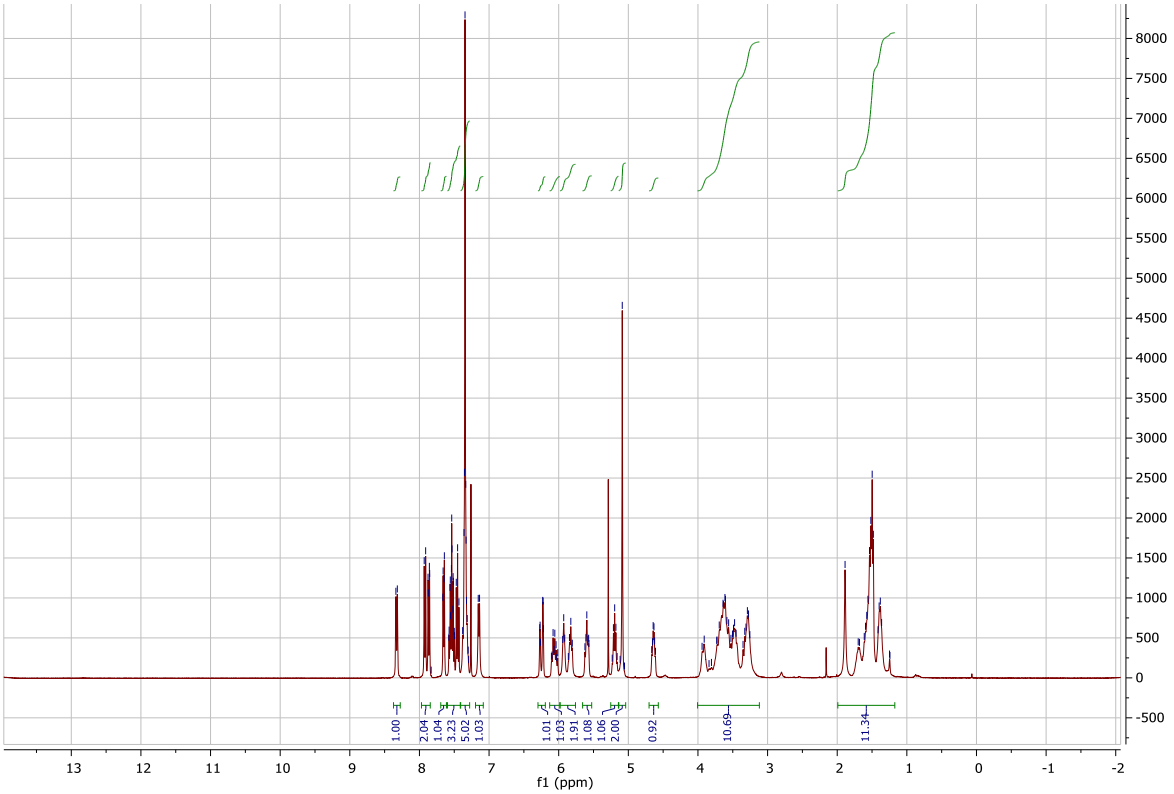


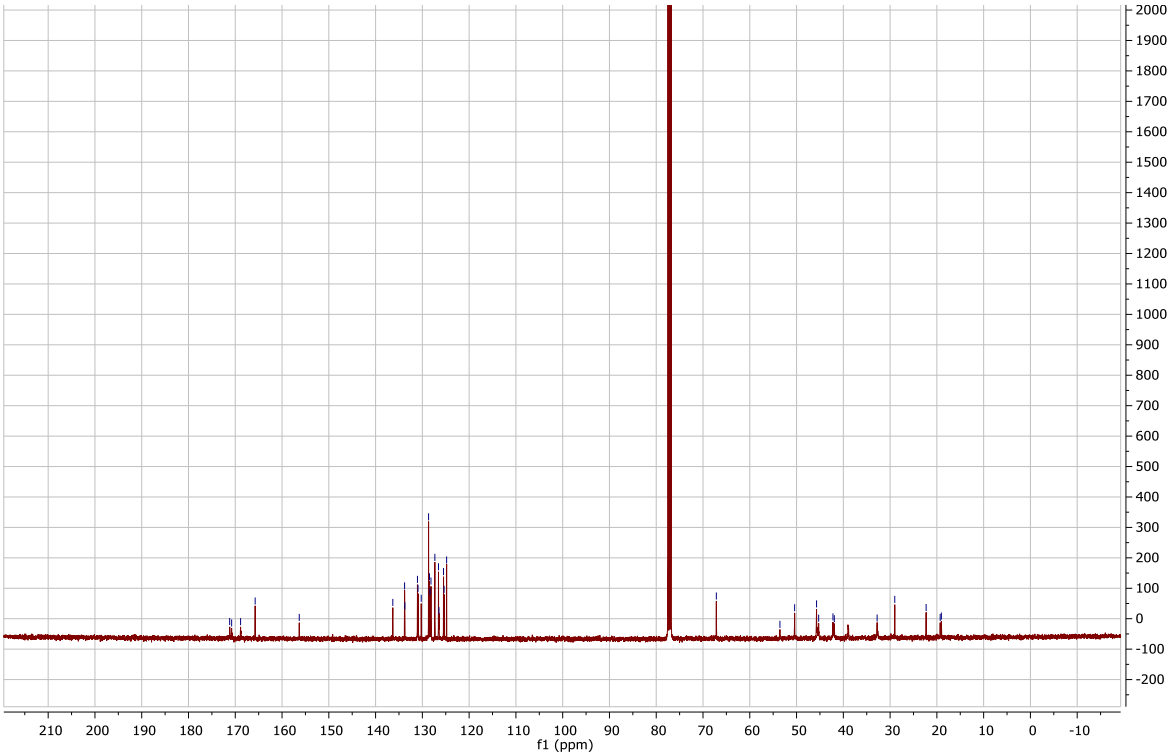
Compound 29 – ^{1}H and ^{13}C NMR Spectra



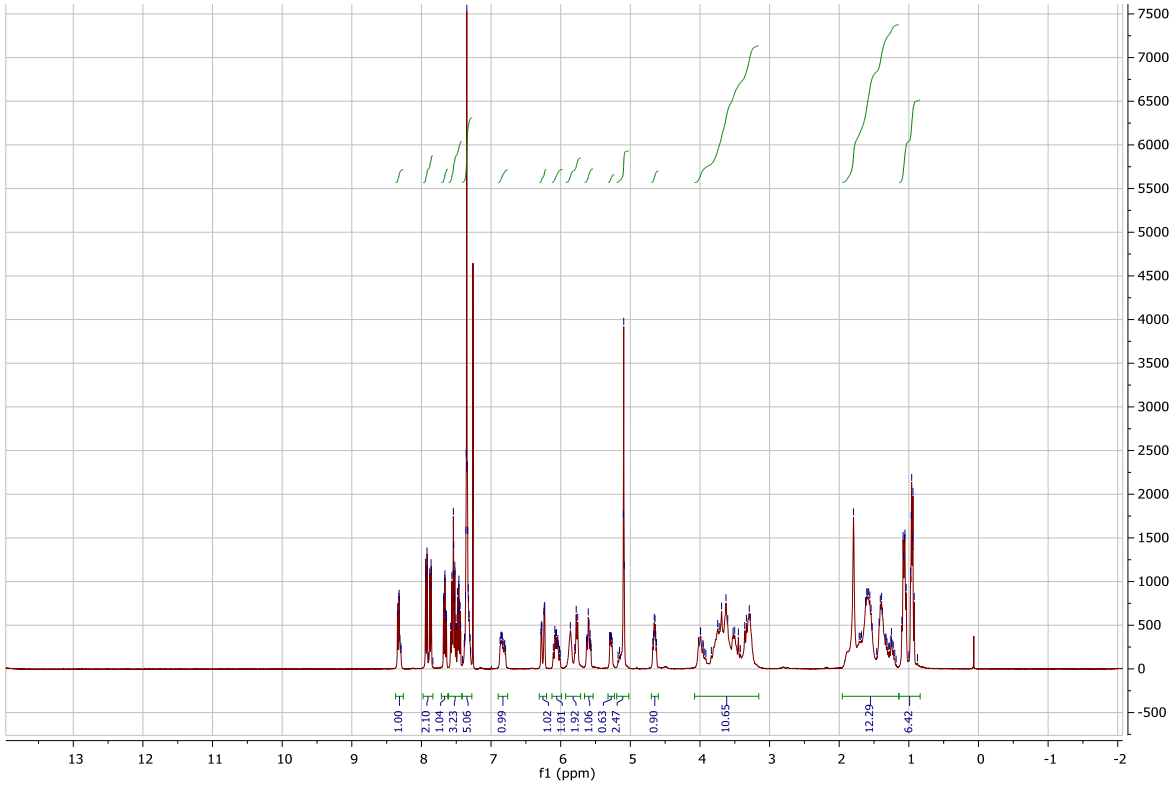


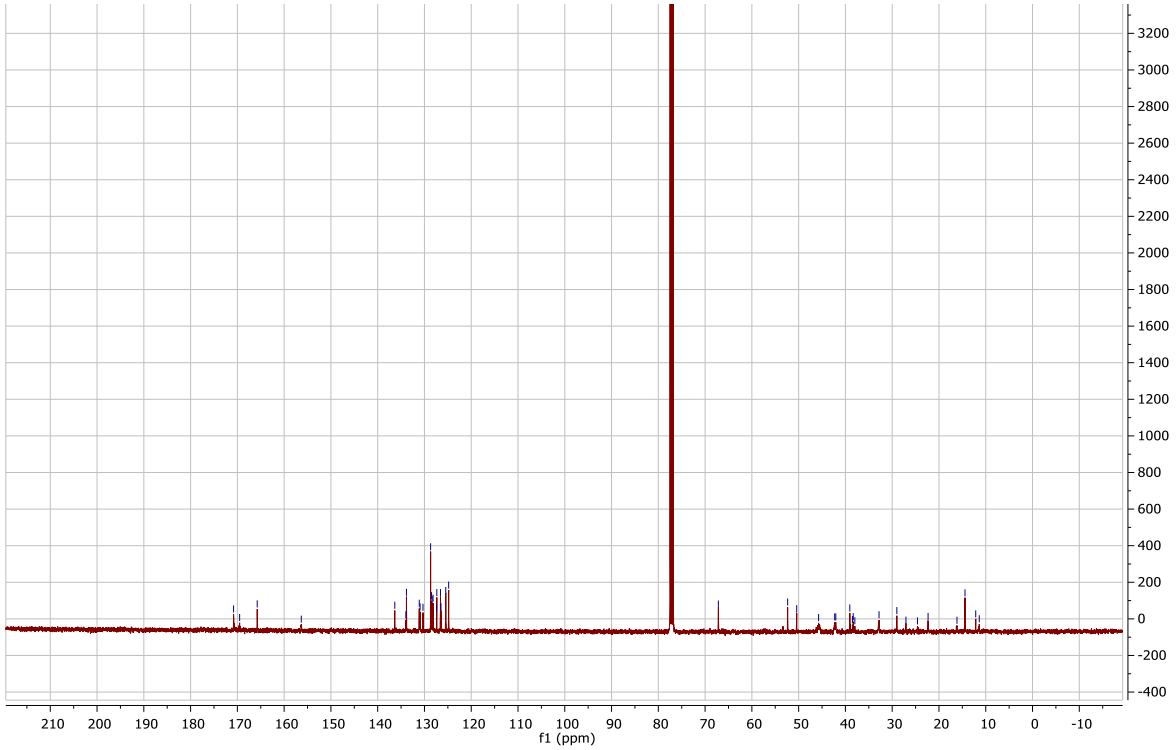
Compound 30 – ^{1}H and ^{13}C NMR Spectra



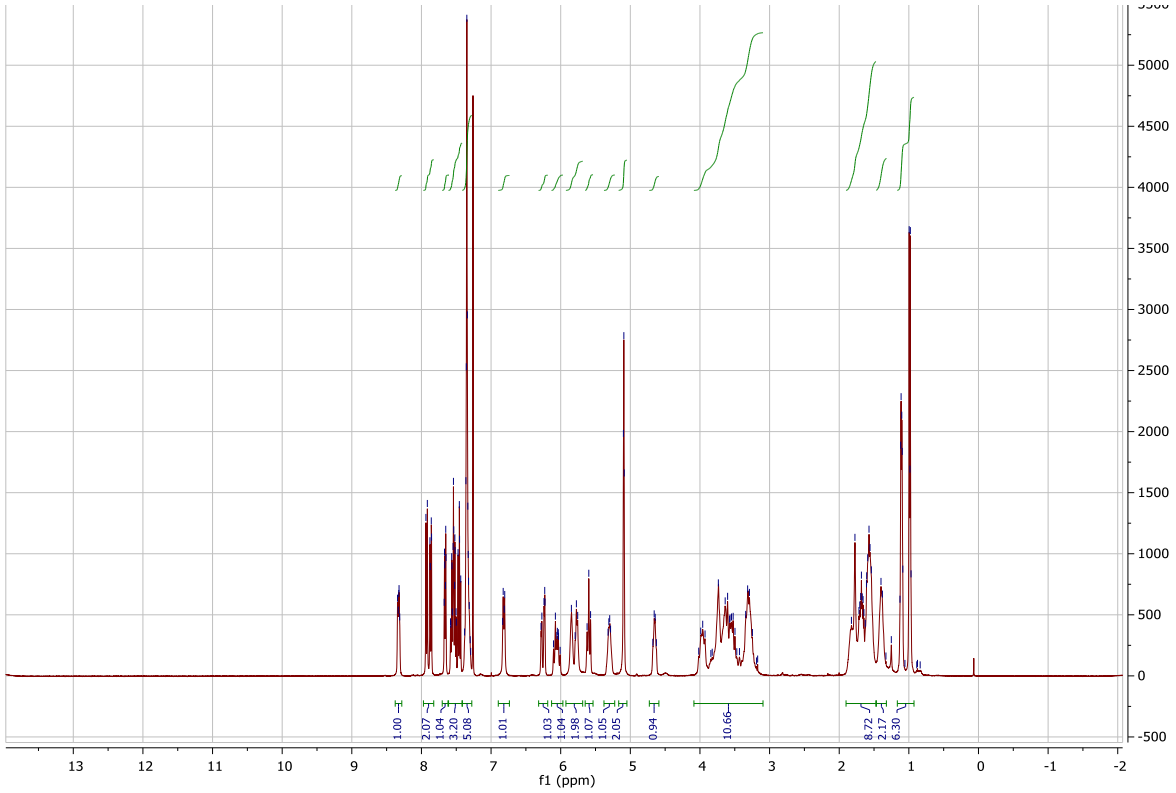


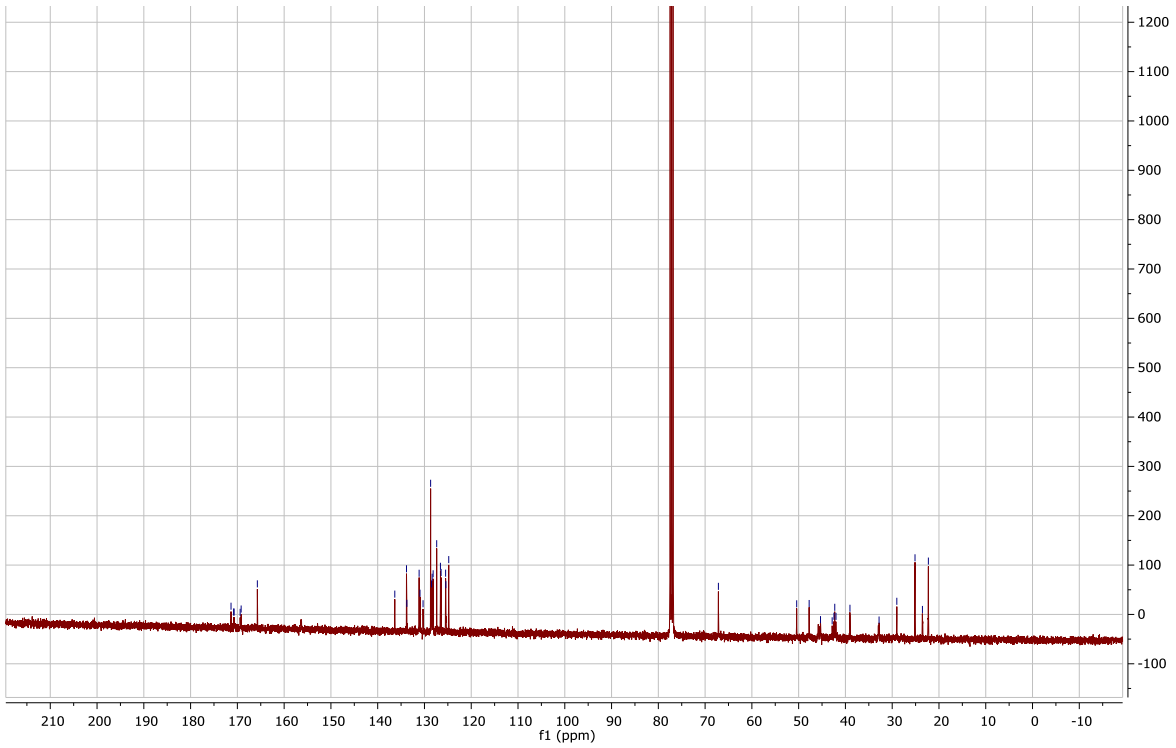
Compound 31 – ^{1}H and ^{13}C NMR Spectra



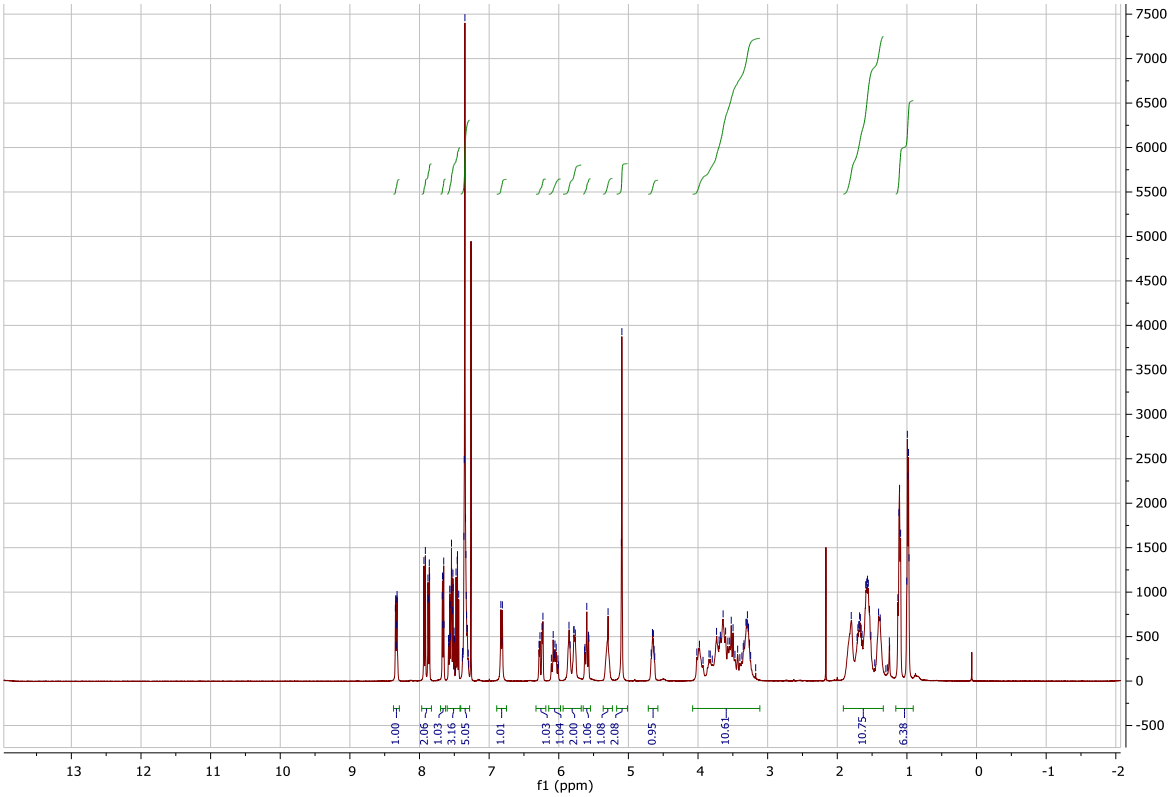


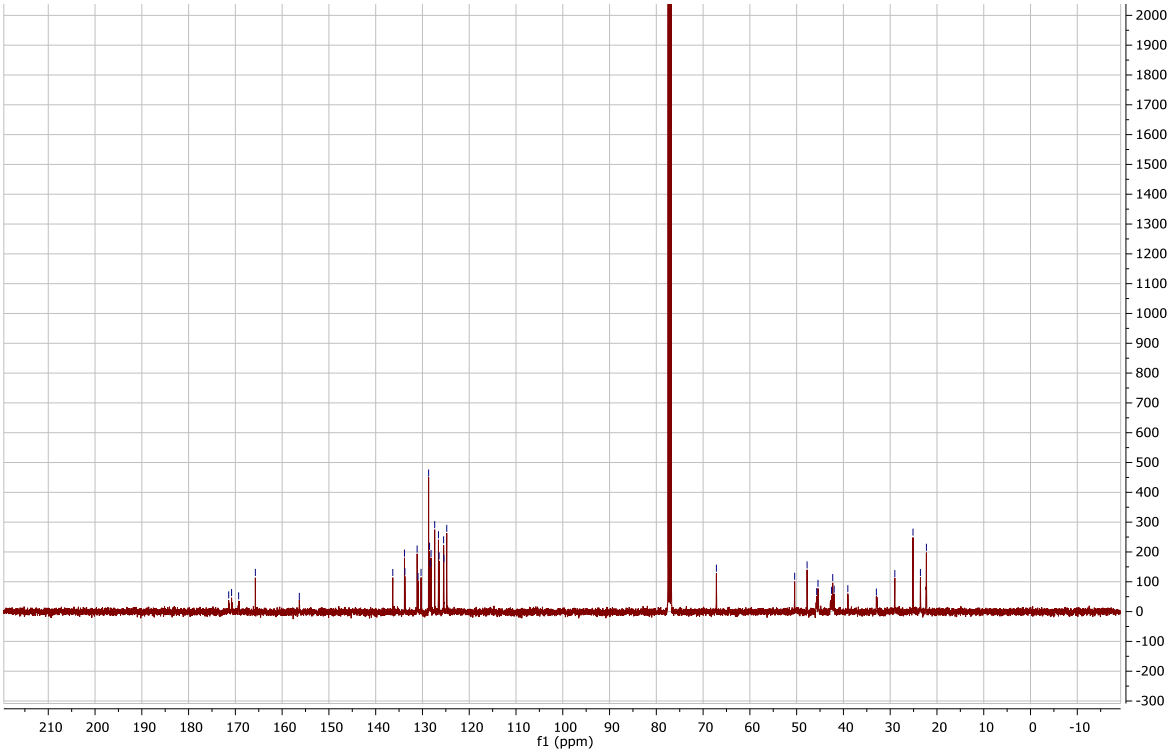
Compound 32 – ^{1}H and ^{13}C NMR Spectra



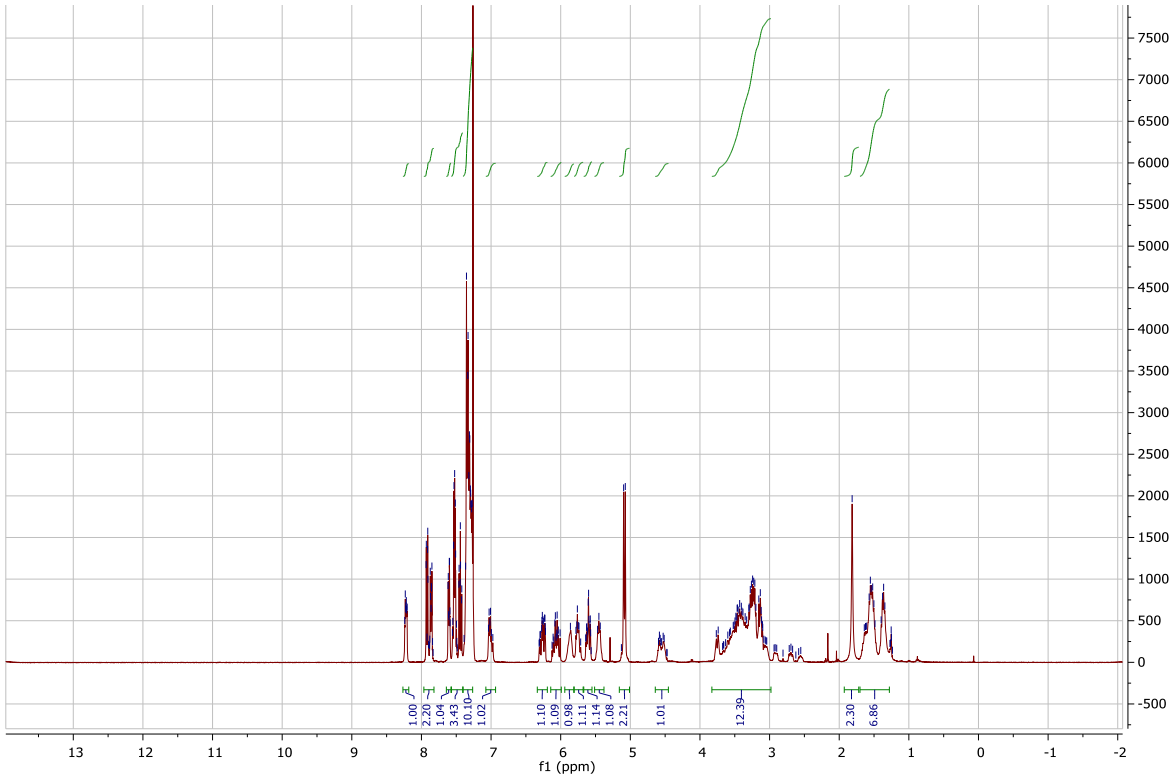


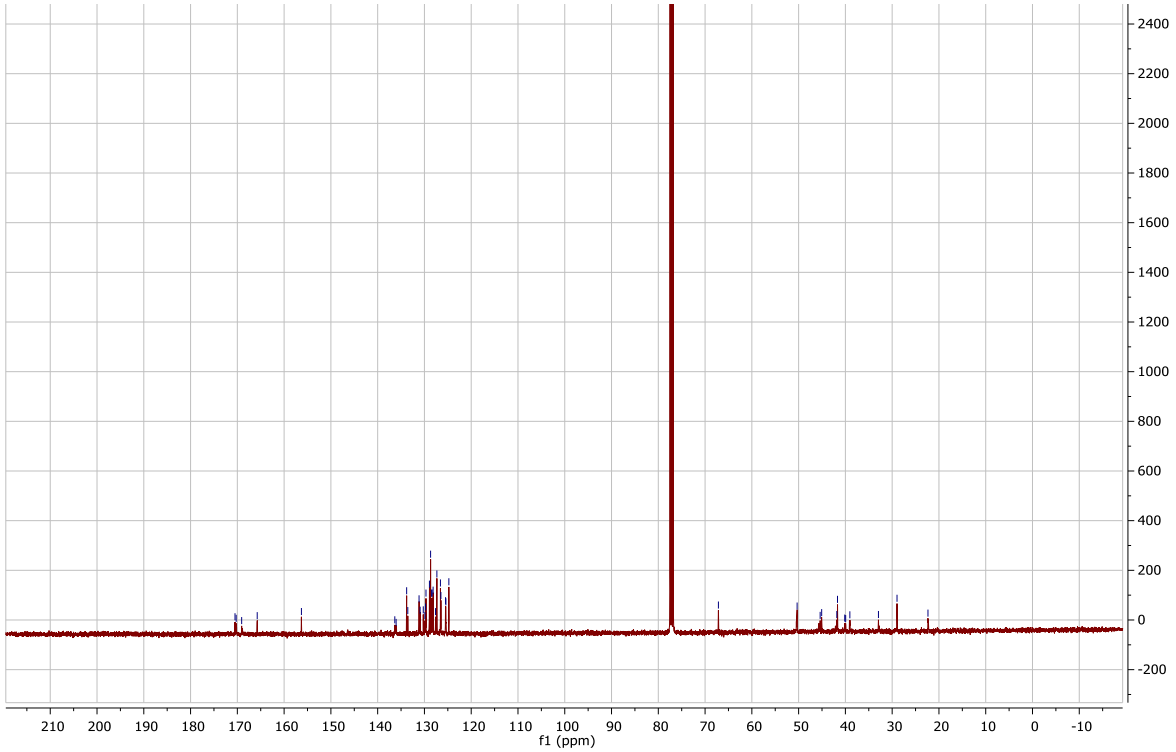
Compound 33 – ^{1}H and ^{13}C NMR Spectra



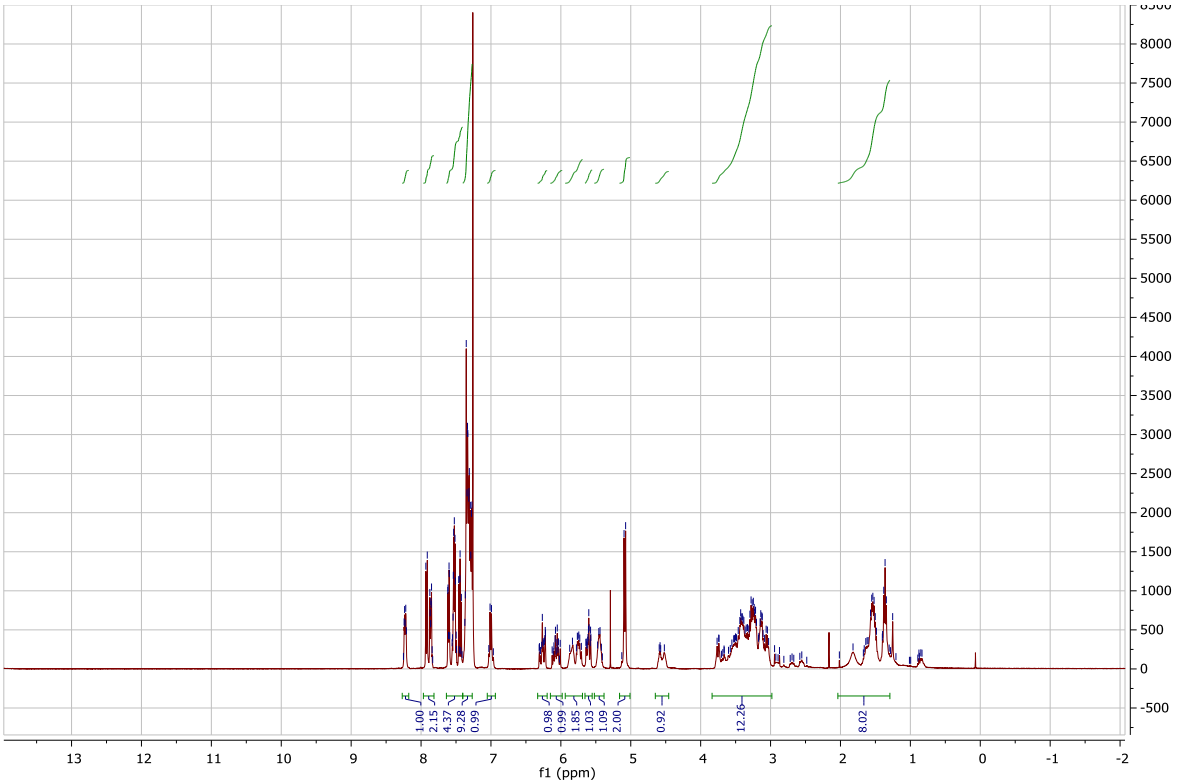


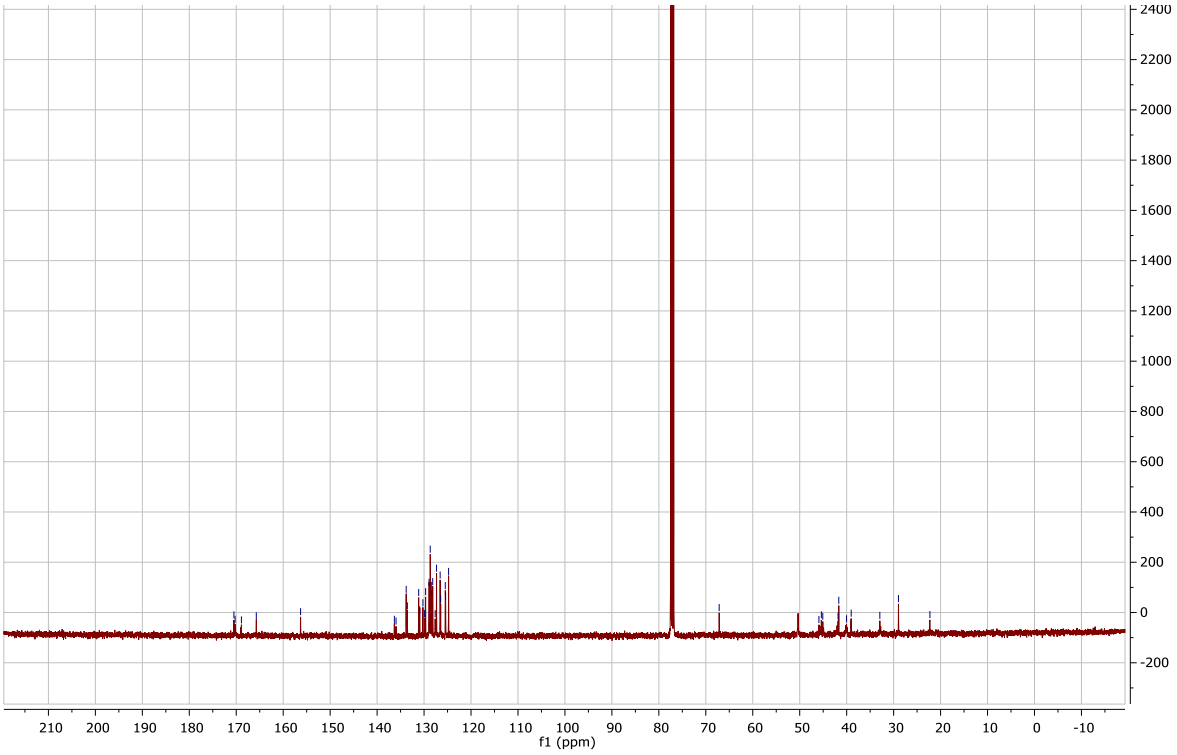
Compound 34 – ^{1}H and ^{13}C NMR Spectra



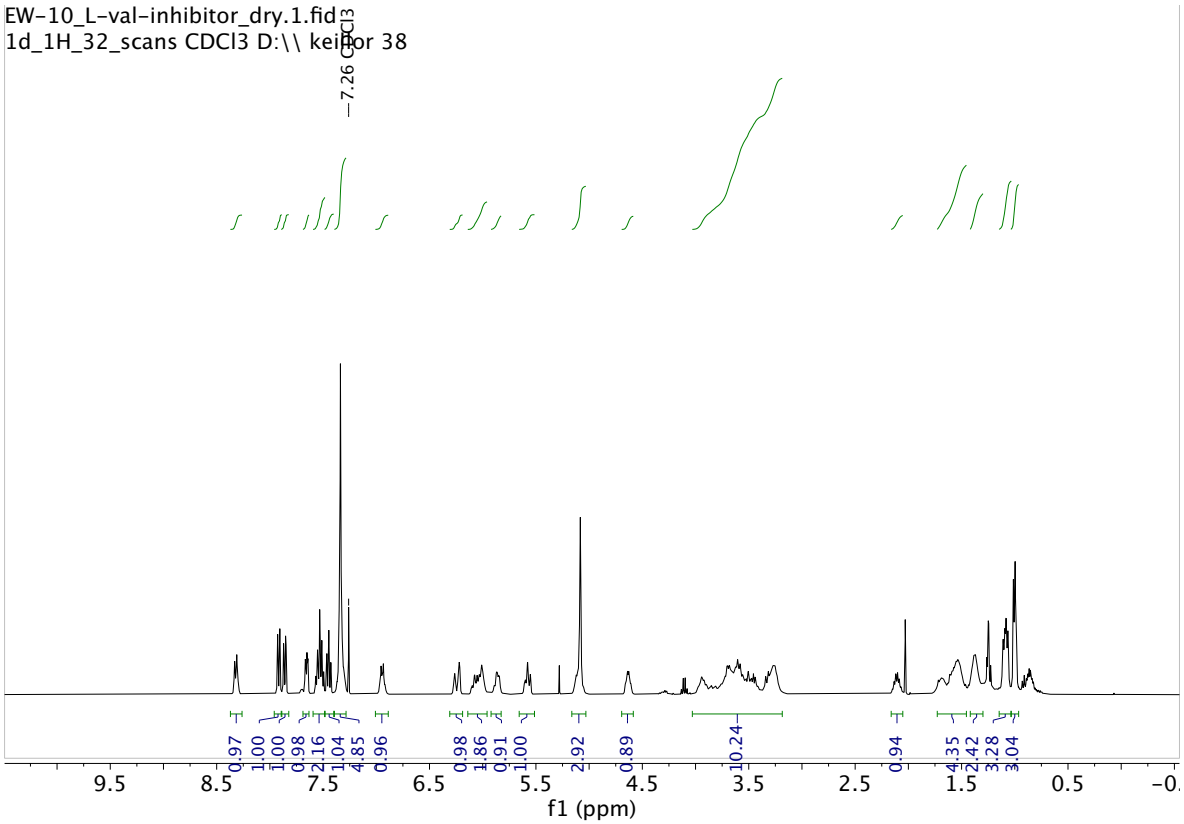


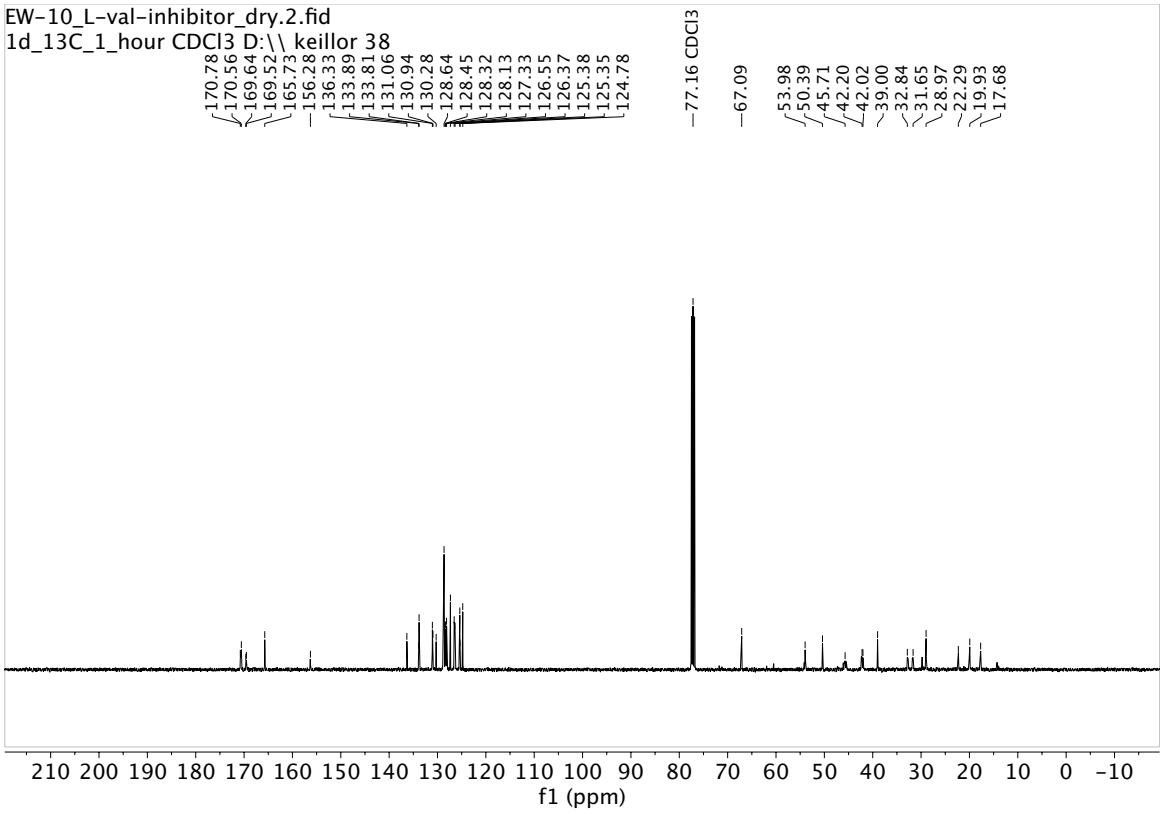
Compound 35 (aka JA38) – ^{1}H and ^{13}C NMR Spectra



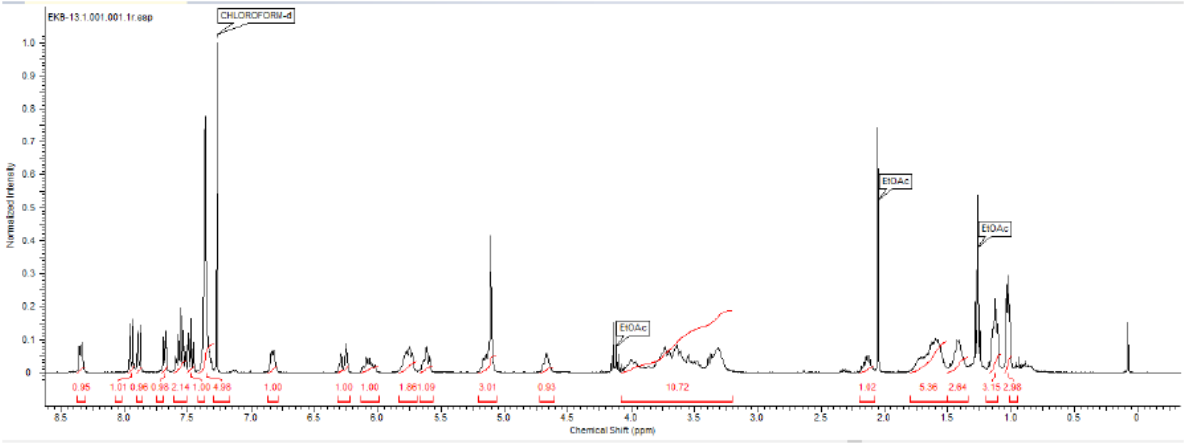


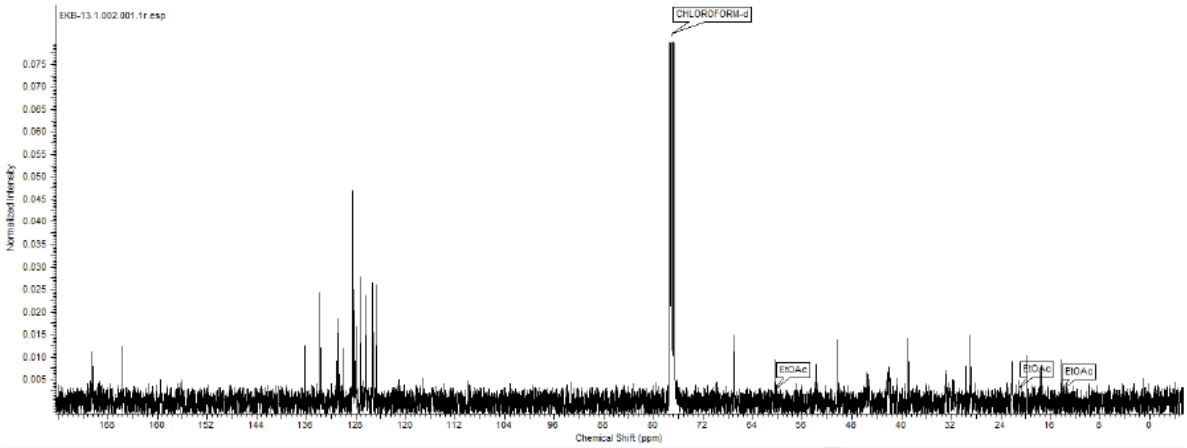
Compound 36 – ^{1}H and ^{13}C NMR Spectra



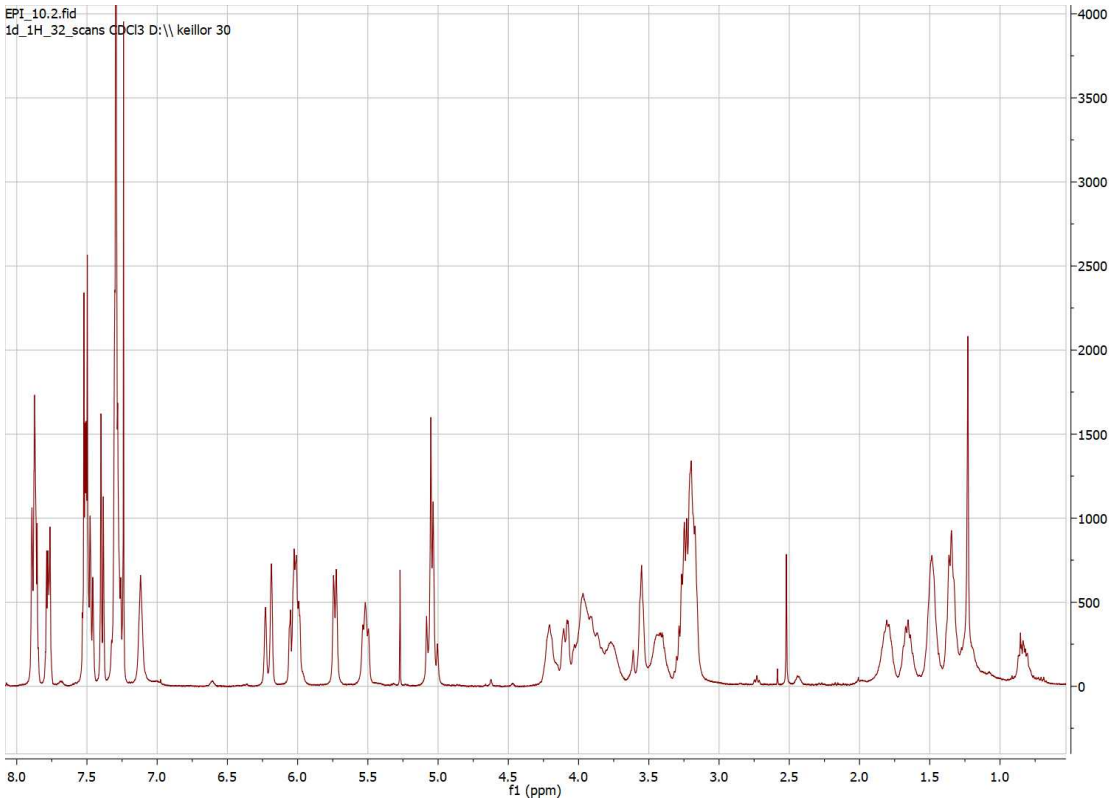


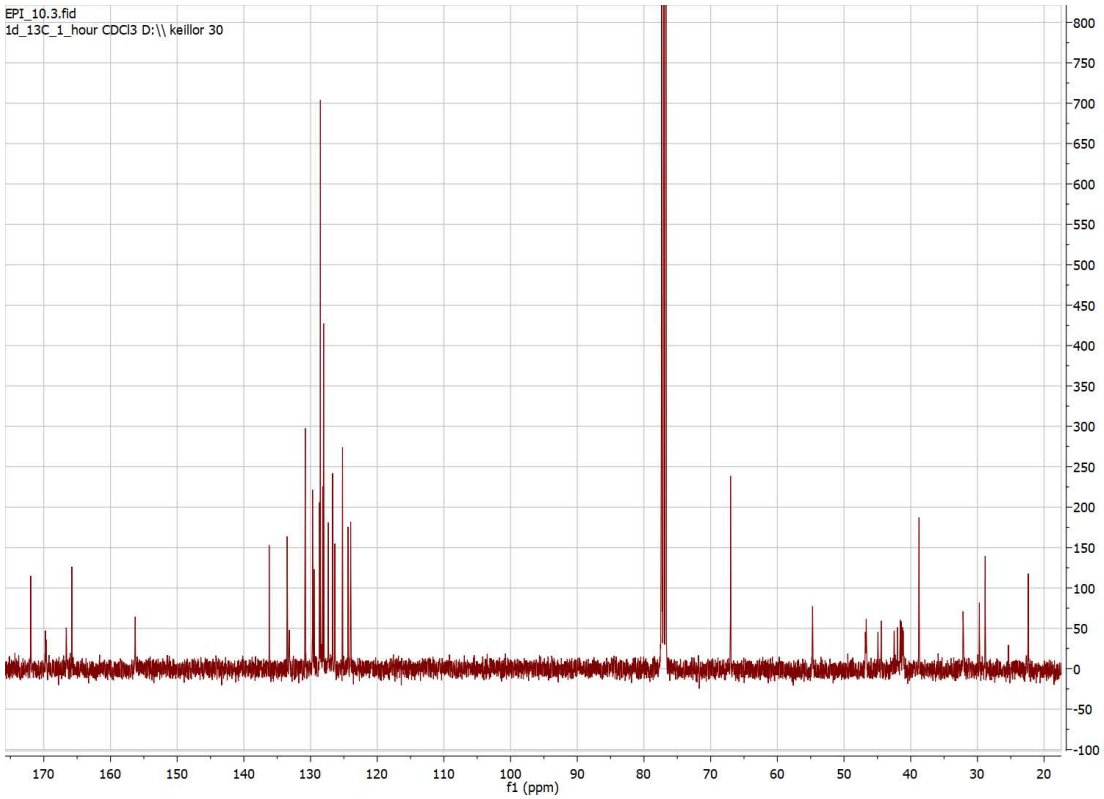
Compound 37 – ^{1}H and ^{13}C NMR Spectra



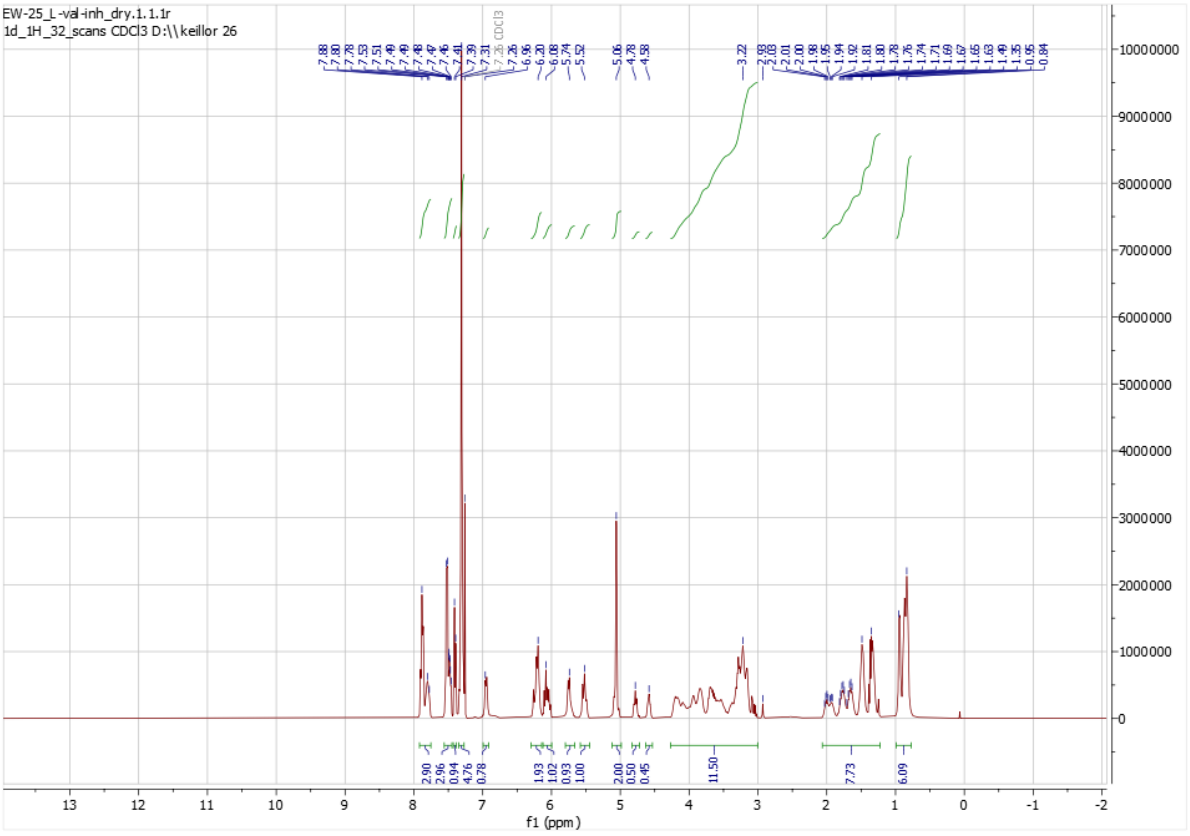


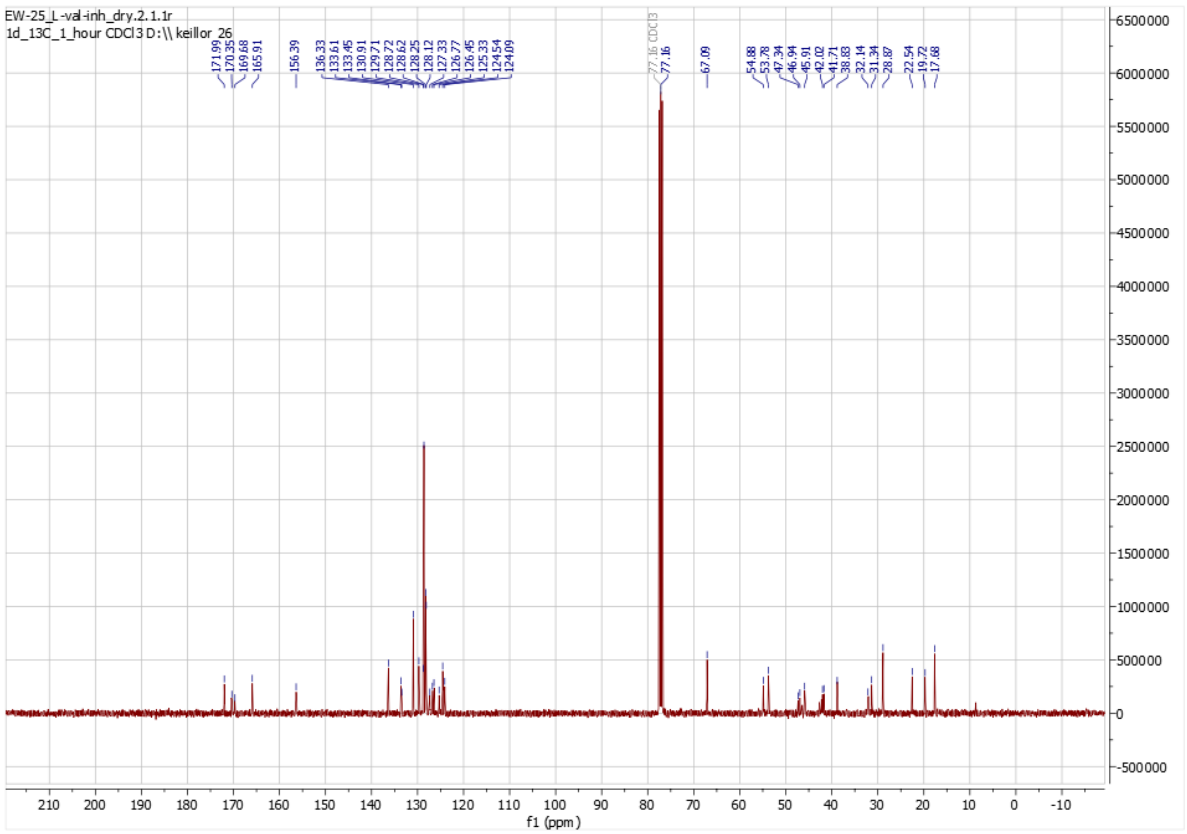
Compound 47 – ^{1}H and ^{13}C NMR Spectra



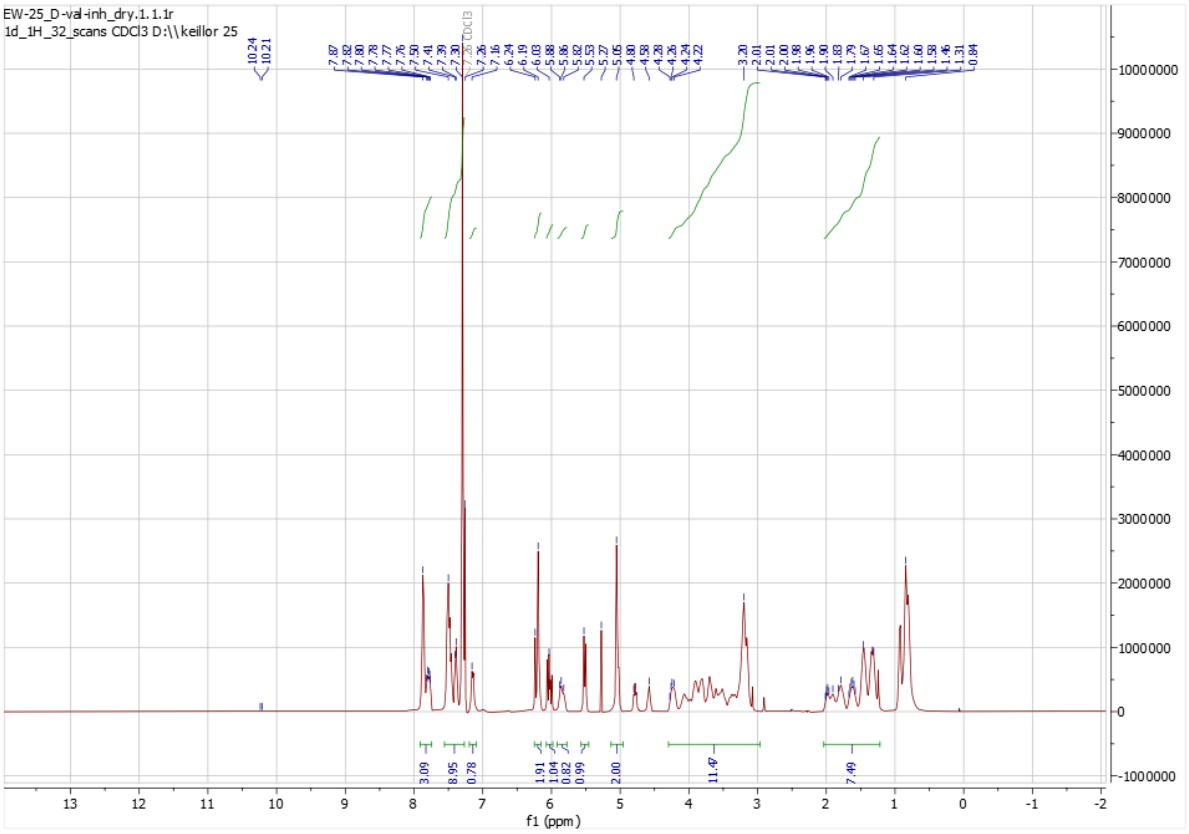


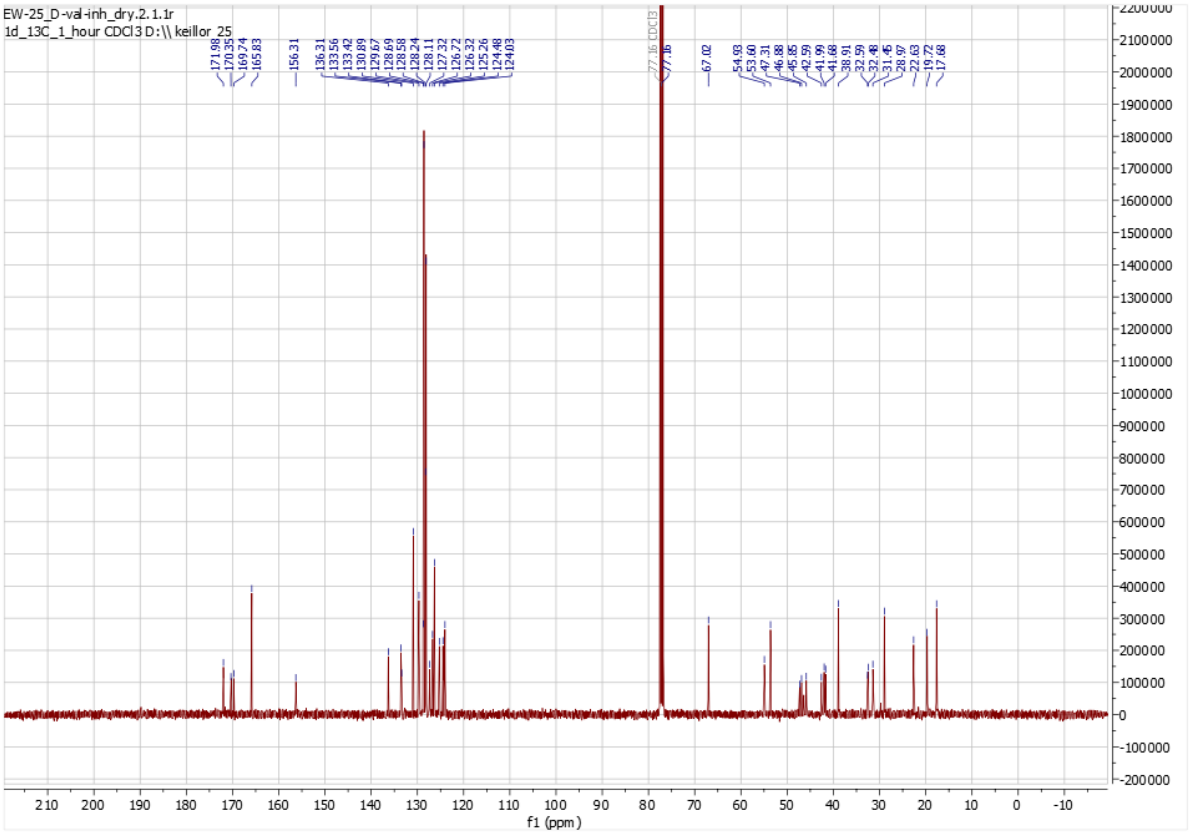
Compound 48 – ^{1}H and ^{13}C NMR Spectra



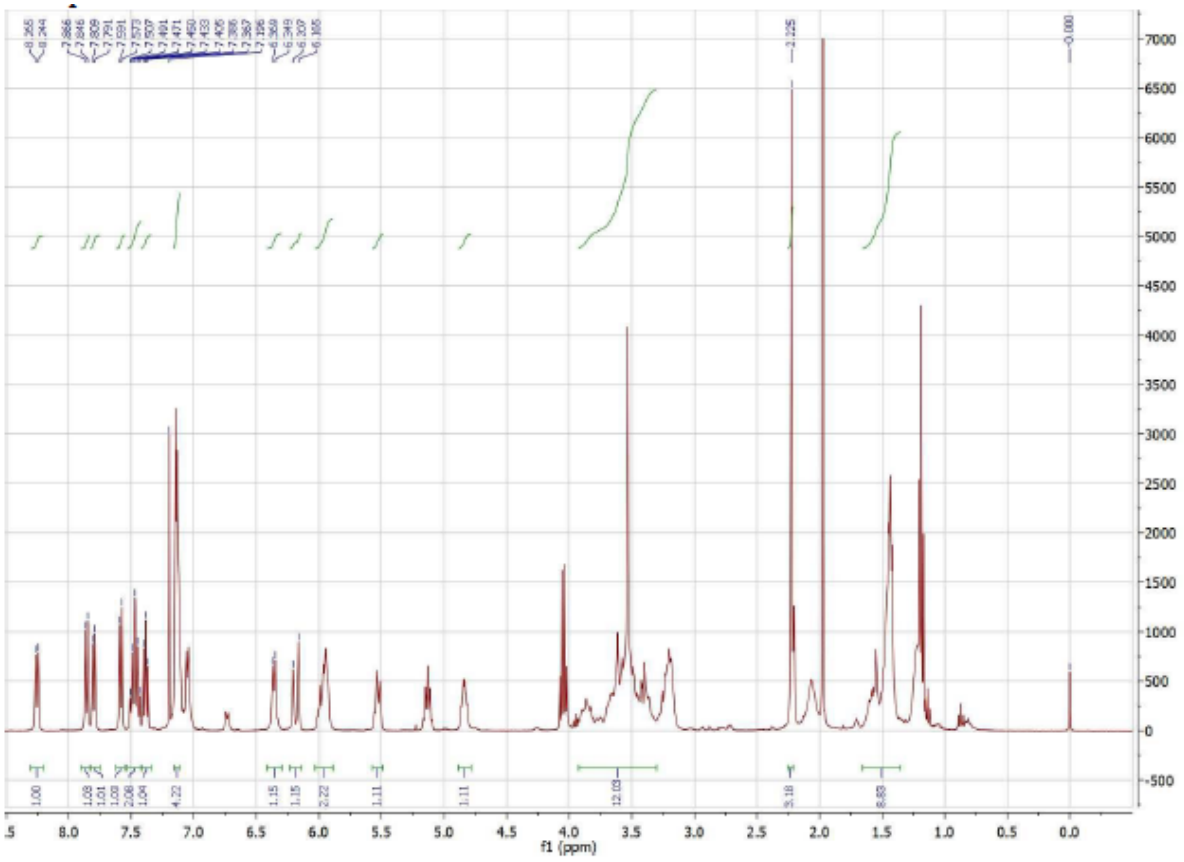


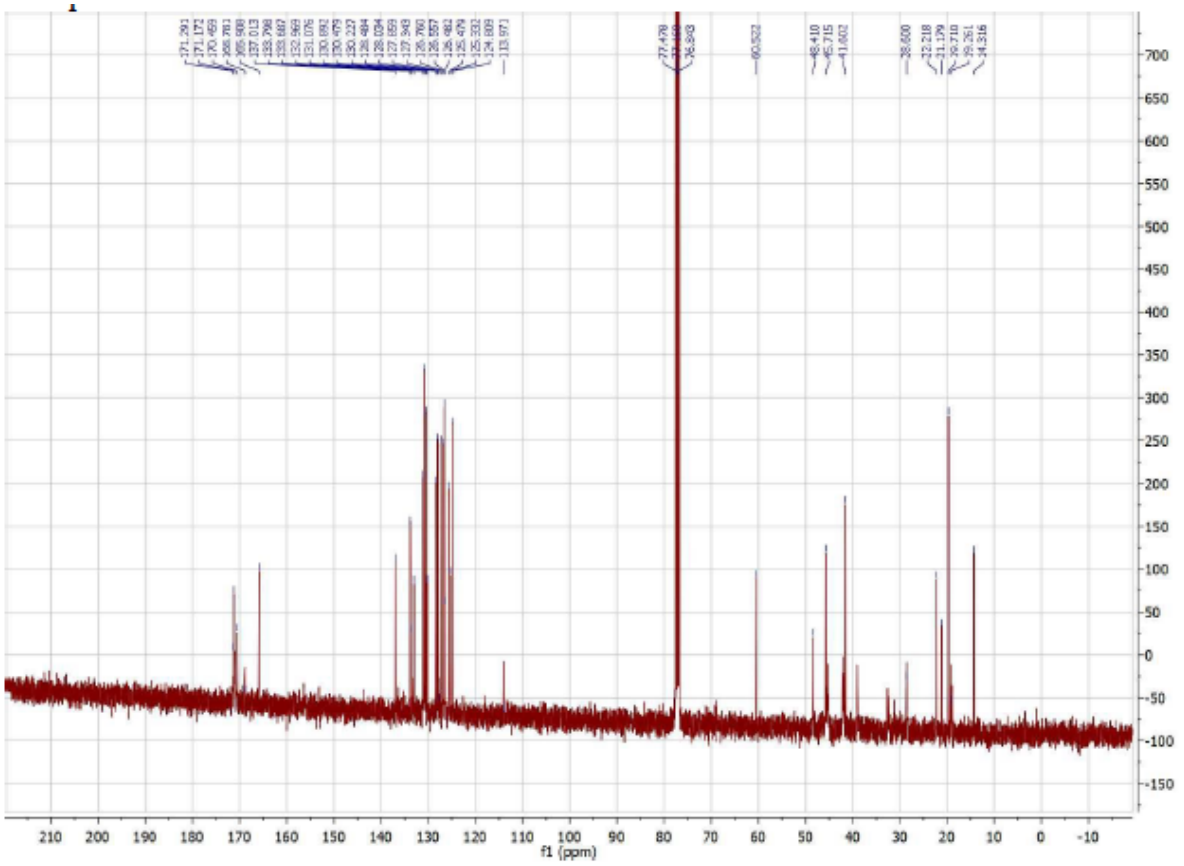
Compound 49 – ^{1}H and ^{13}C NMR Spectra



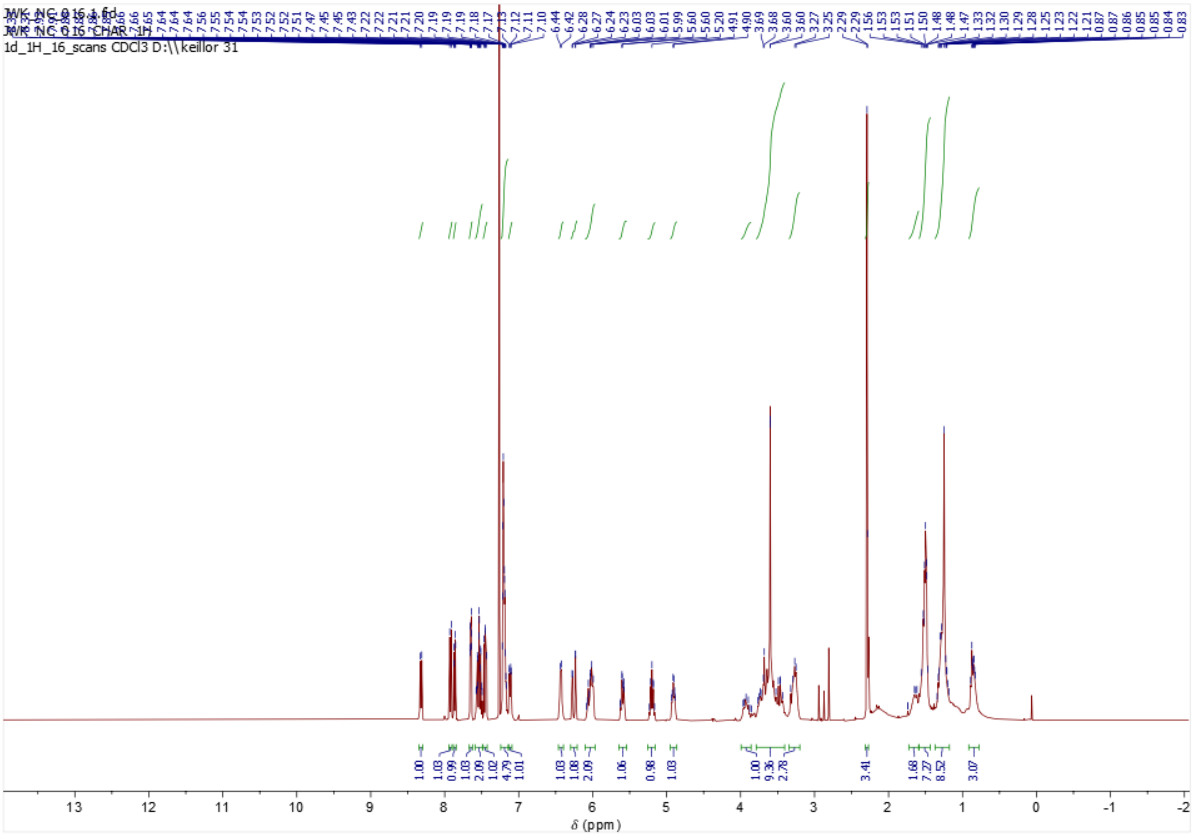


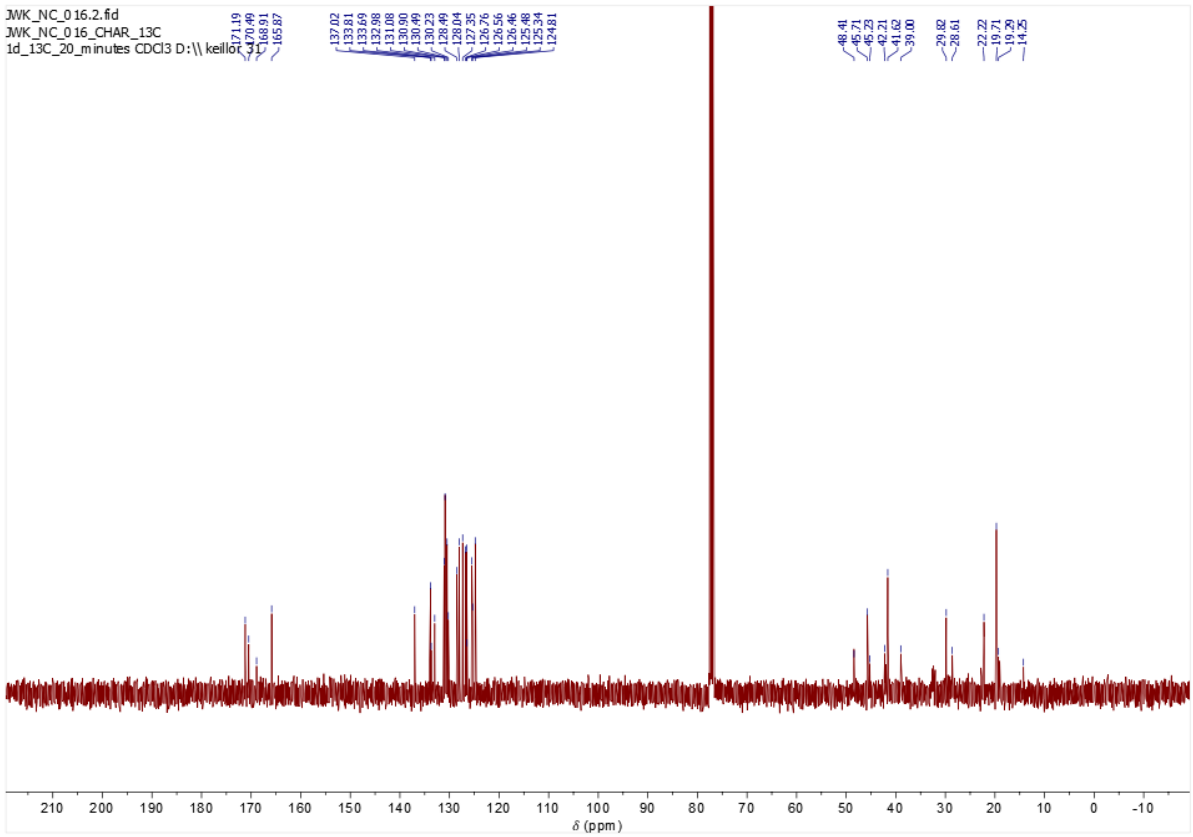
Compound 74 – ^{1}H and ^{13}C NMR Spectra



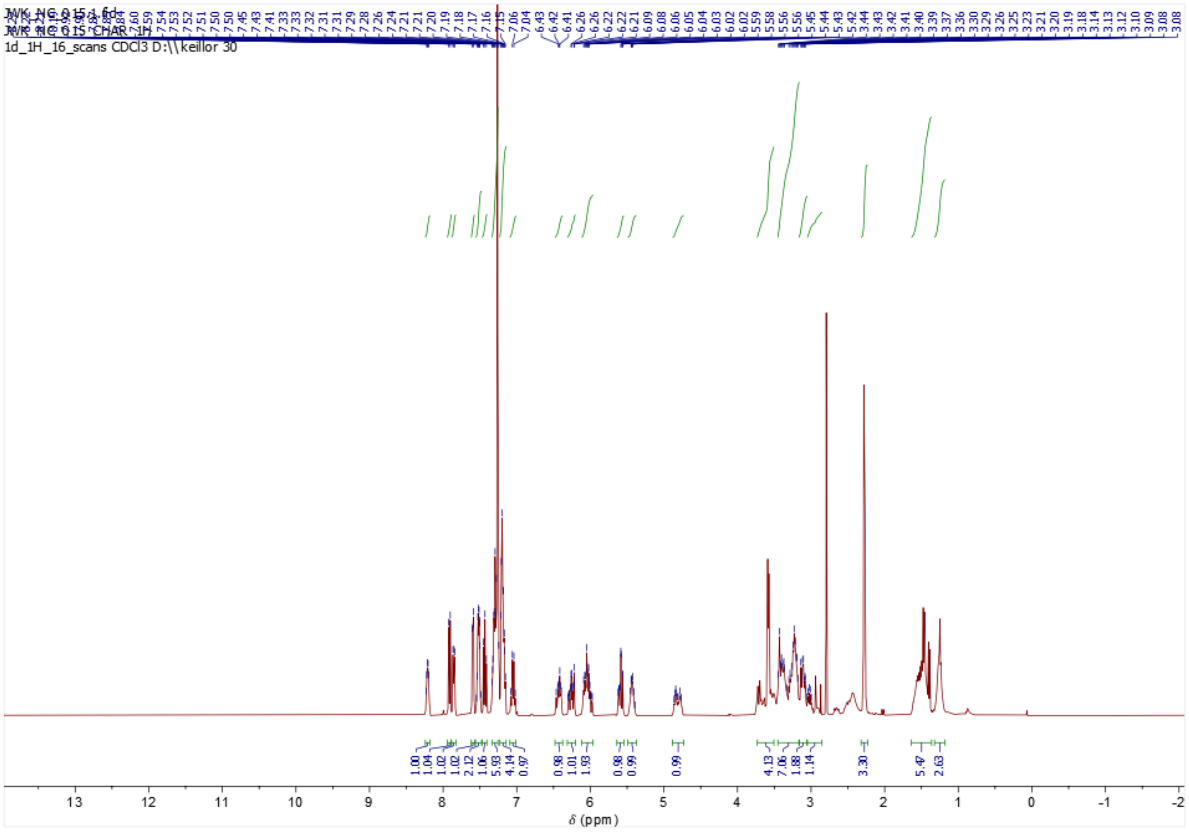


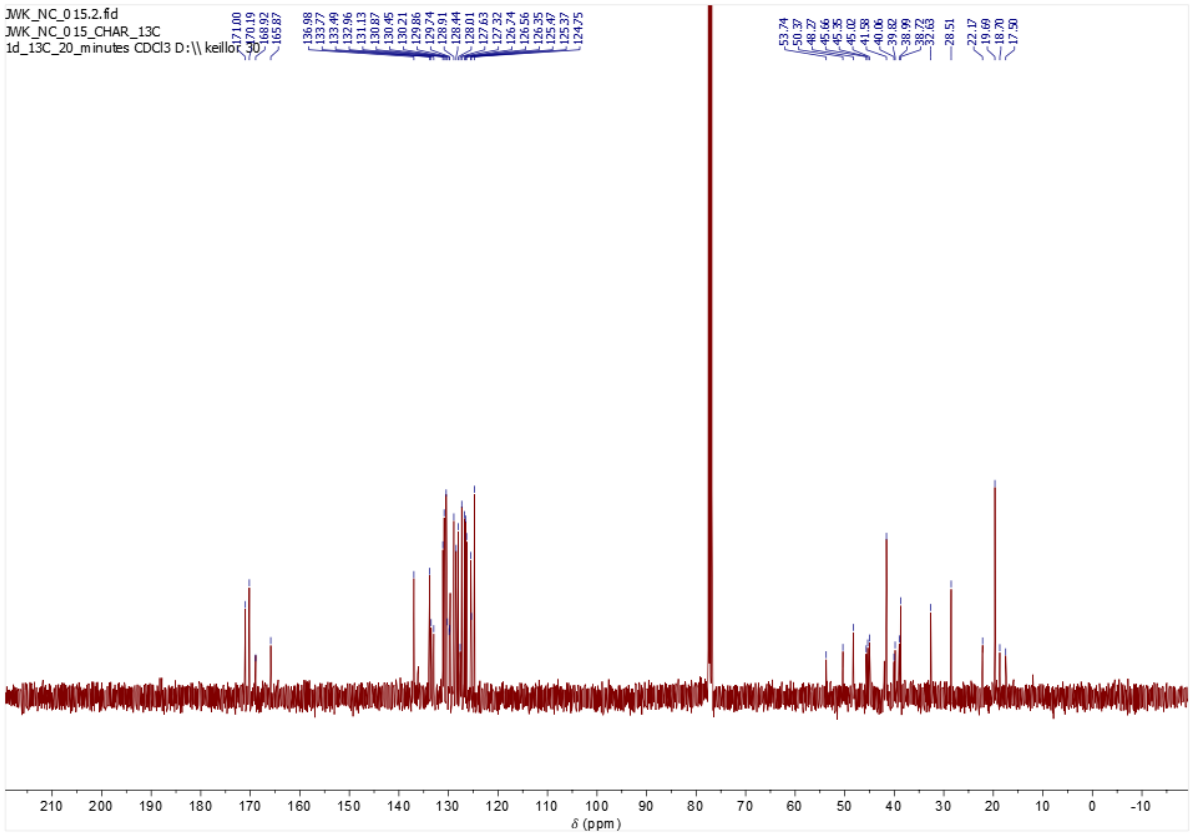
Compound 75 – ^{1}H and ^{13}C NMR Spectra



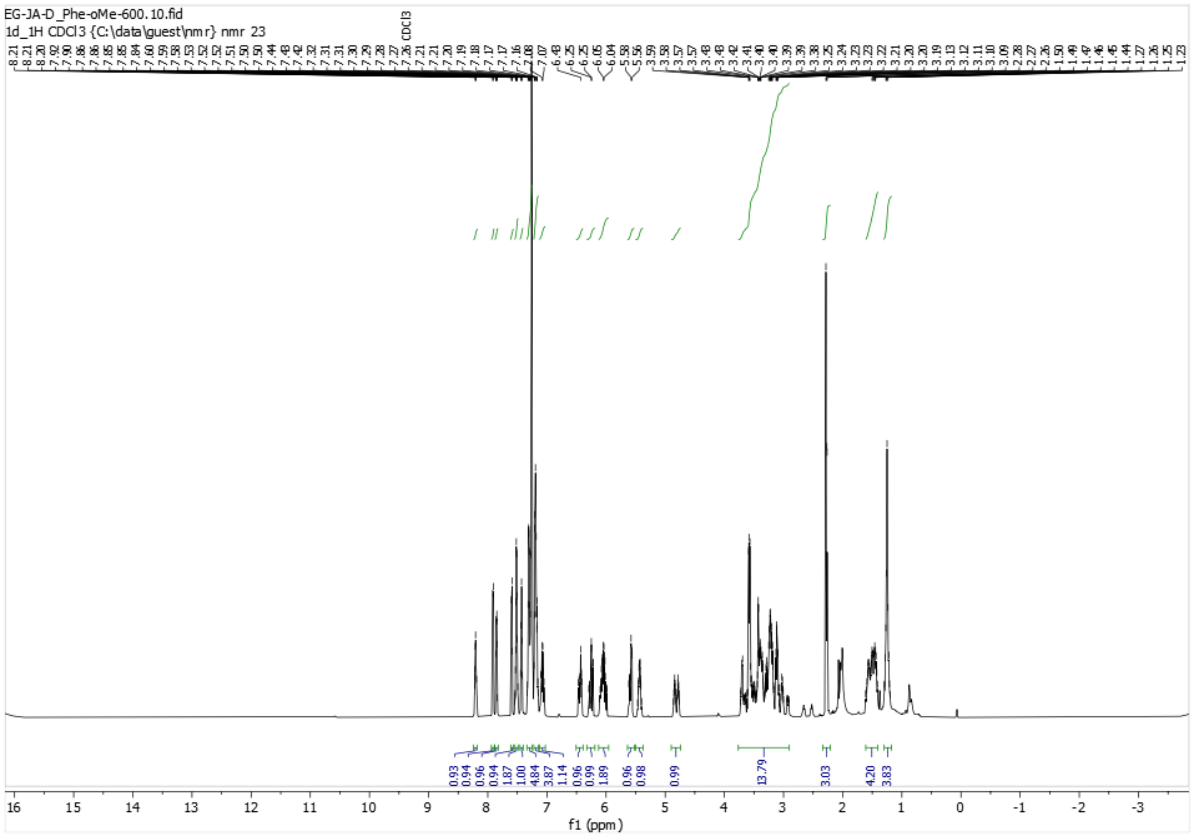


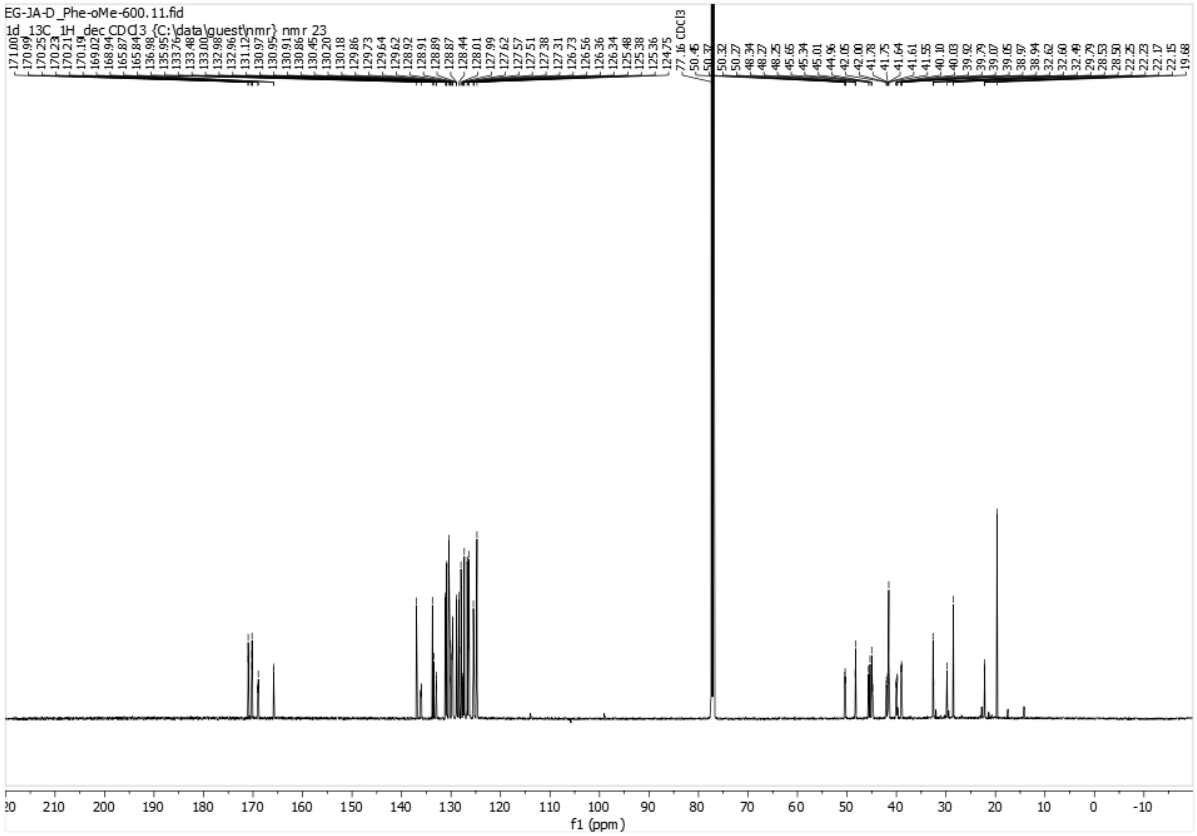
Compound 76 – ^{1}H and ^{13}C NMR Spectra



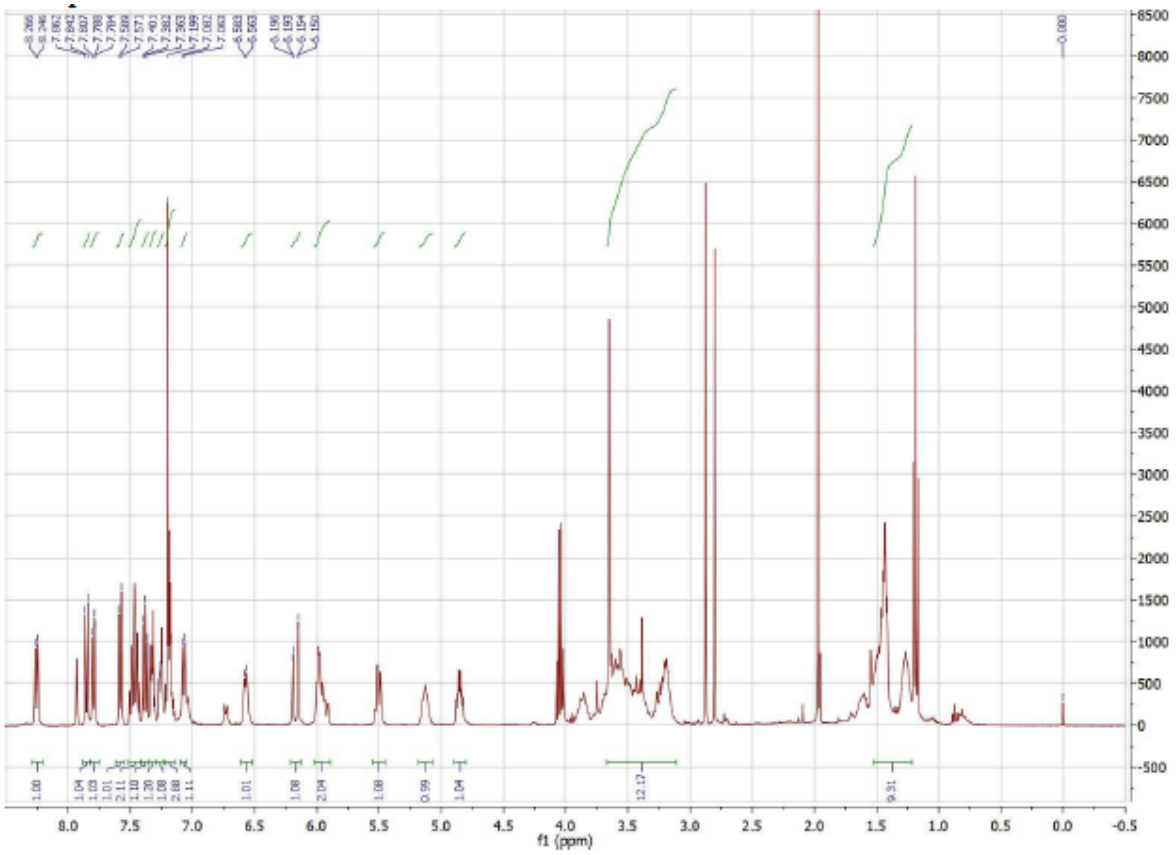


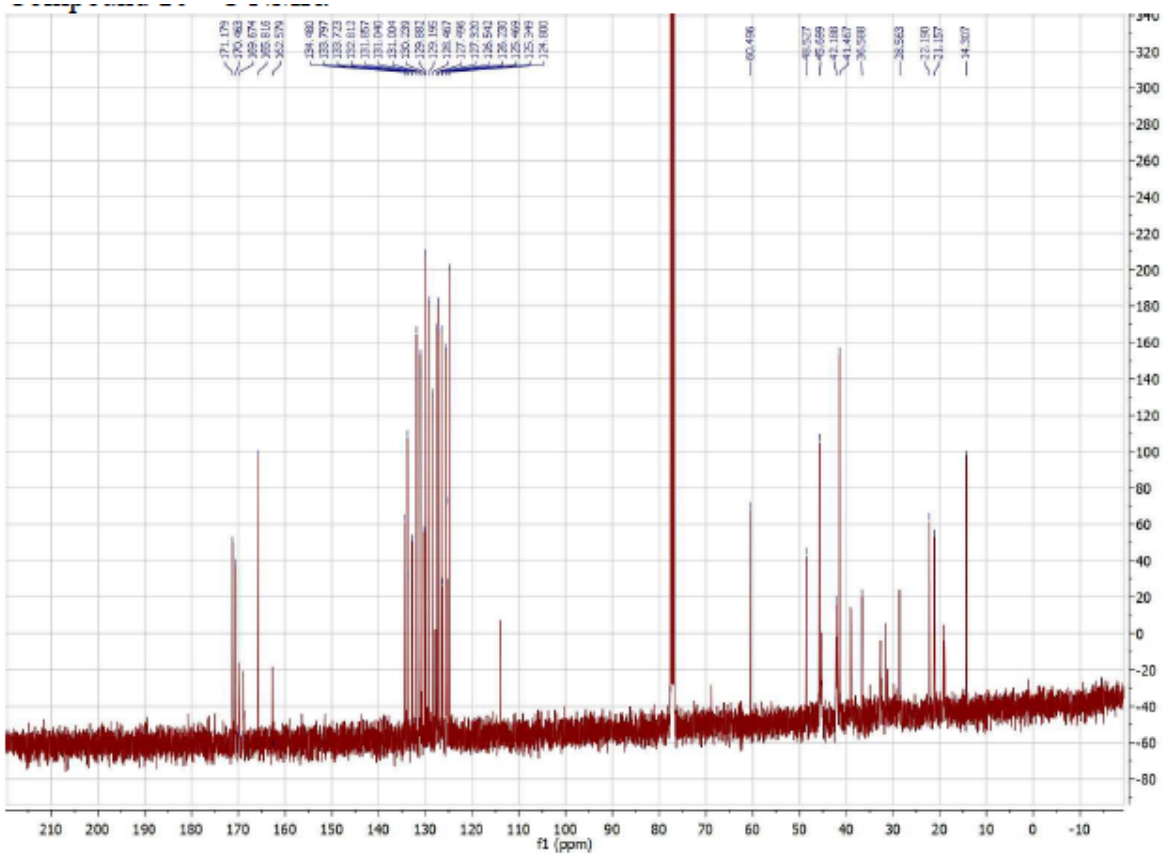
Compound 77 – ^{1}H and ^{13}C NMR Spectra



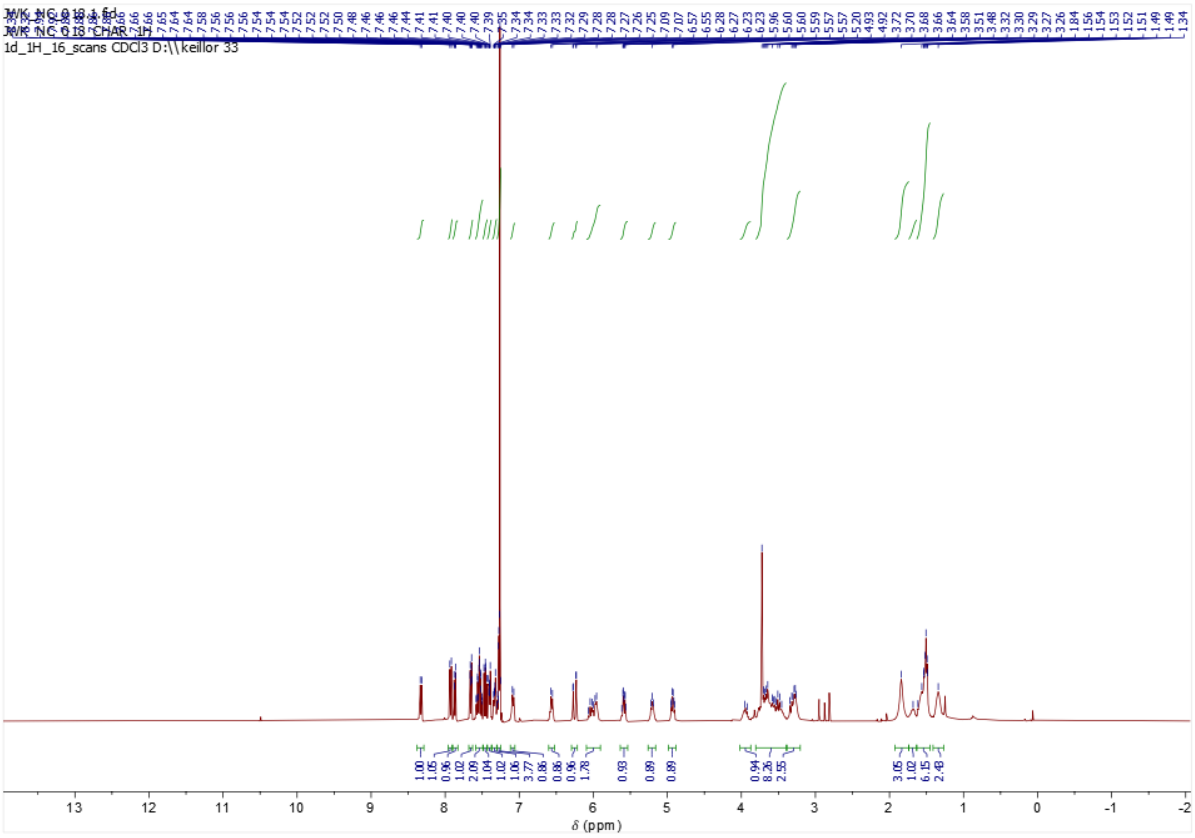


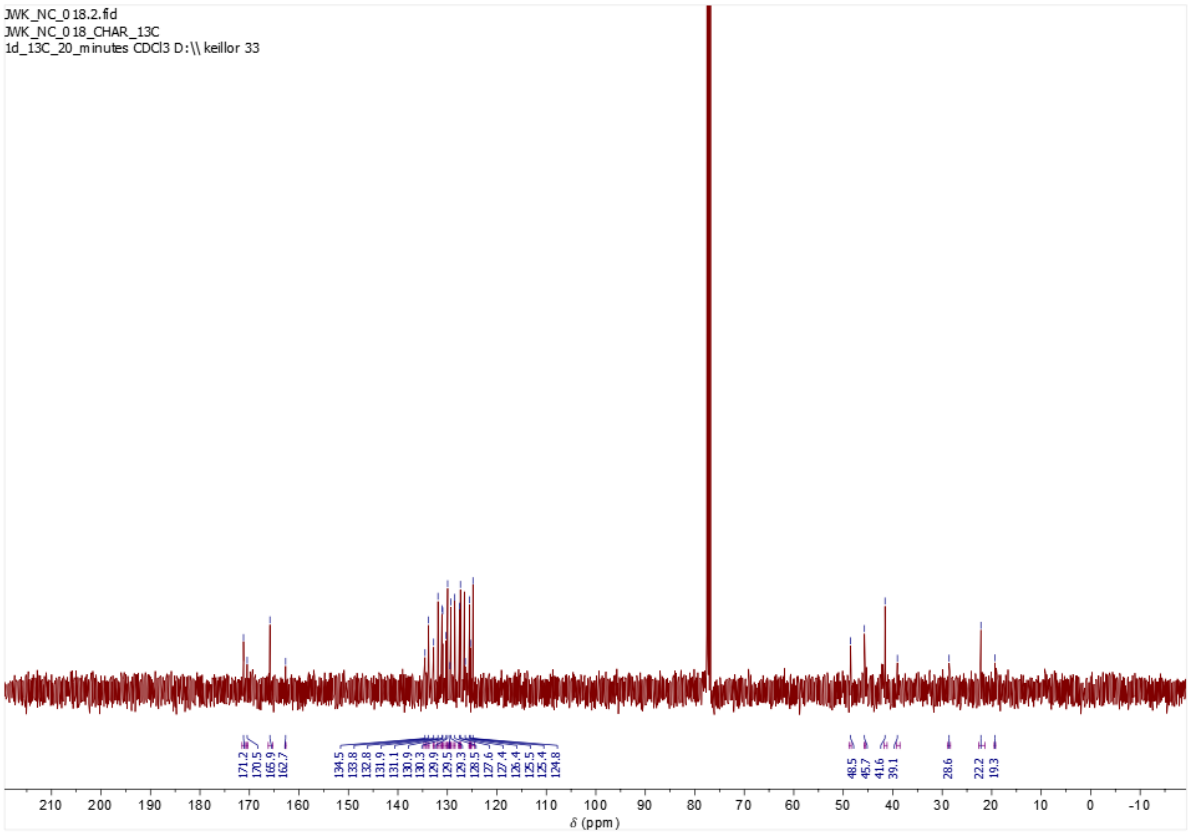
Compound 78 – ^{1}H and ^{13}C NMR Spectra



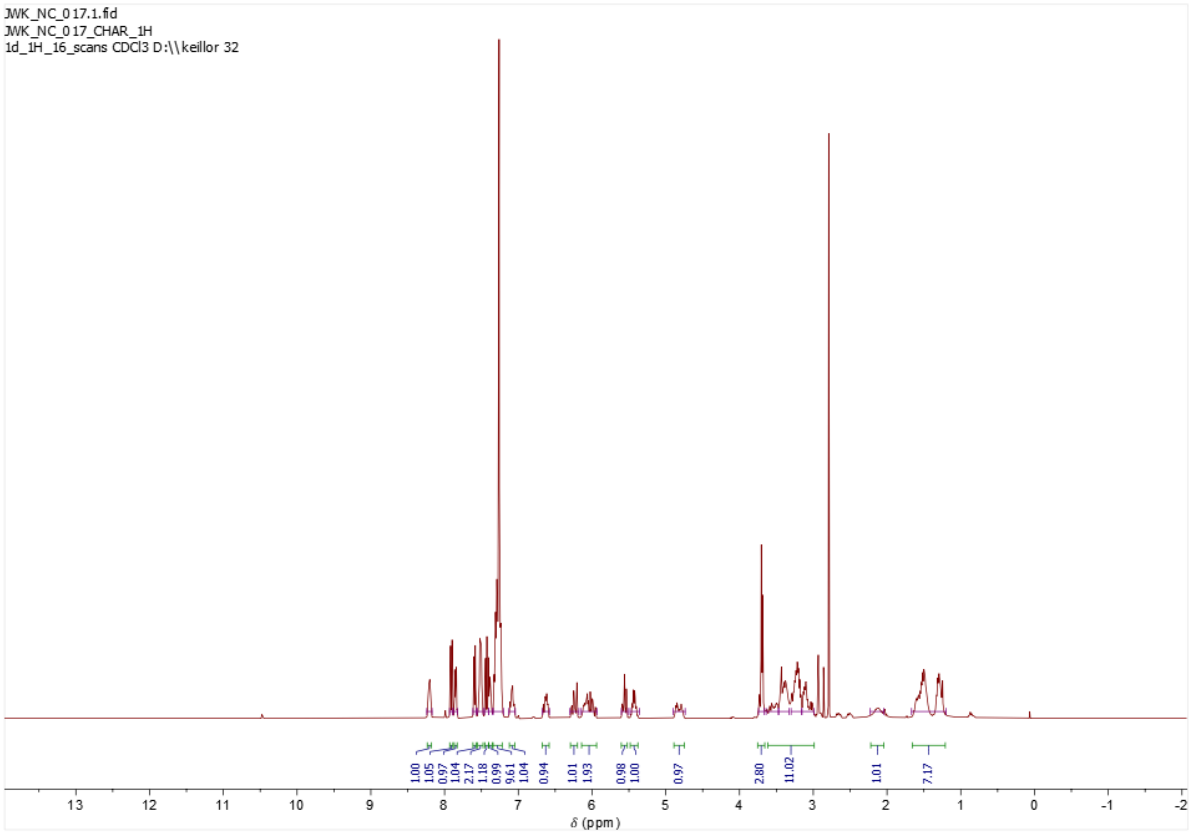


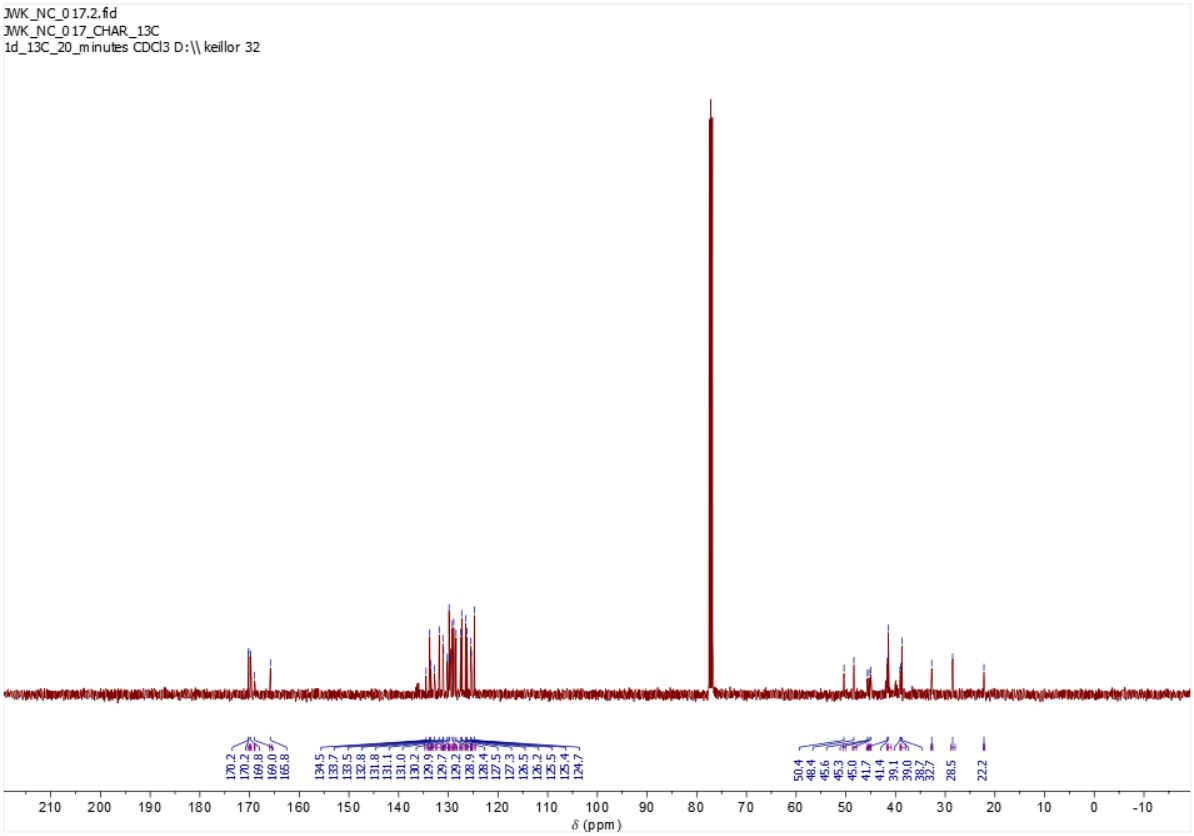
Compound 79 – ^{1}H and ^{13}C NMR Spectra



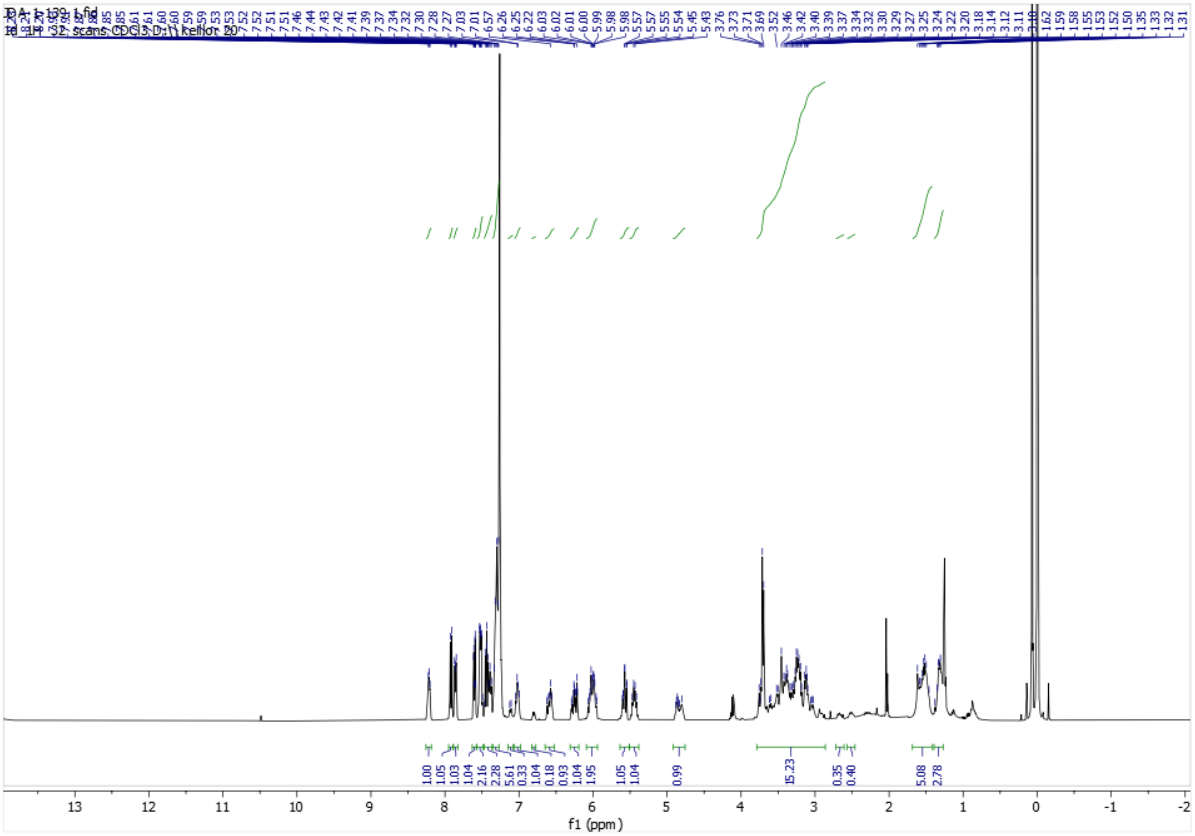


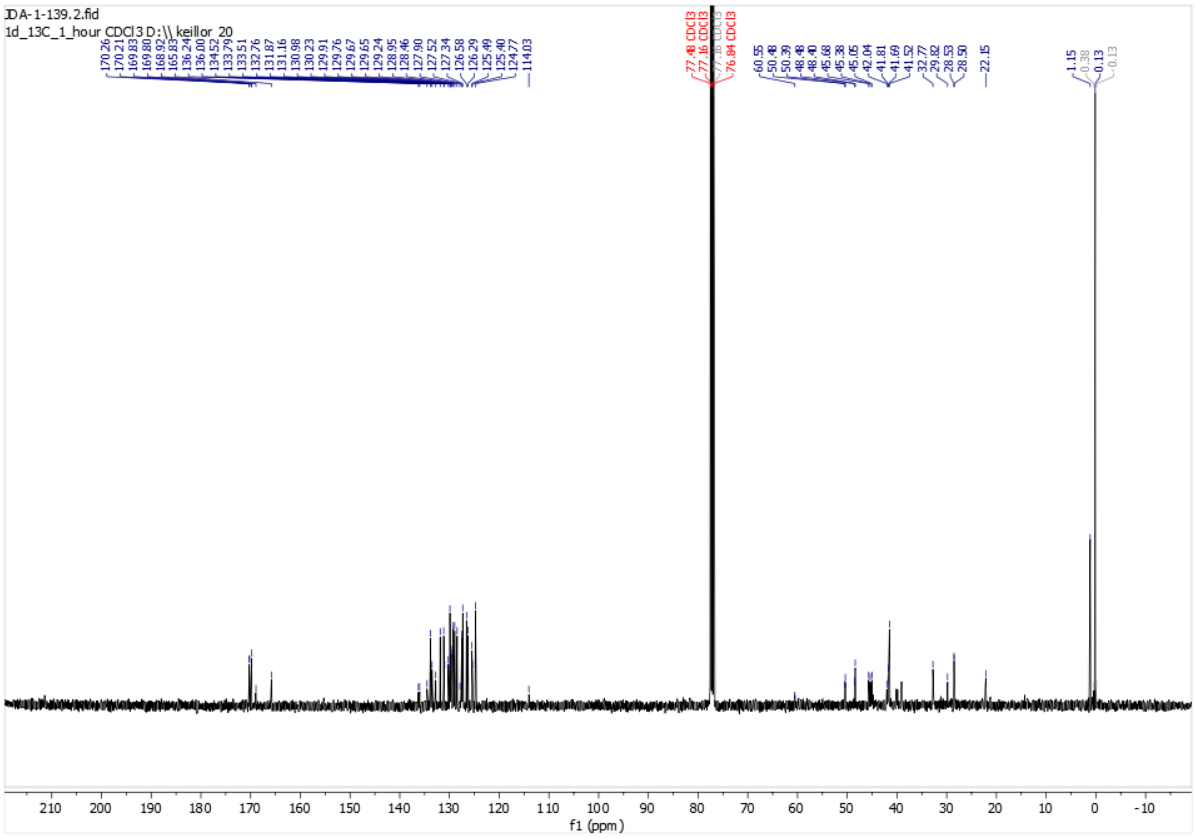
Compound 80 – ^{1}H and ^{13}C NMR Spectra



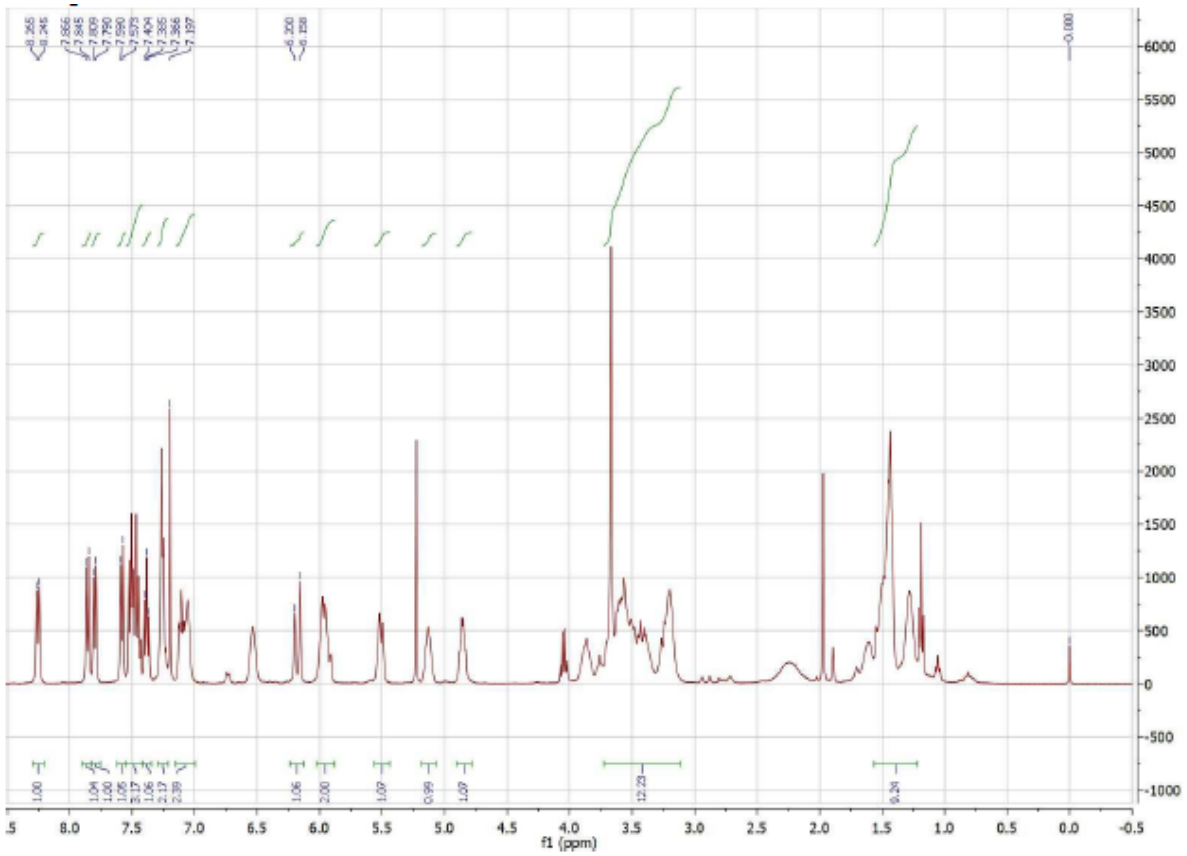


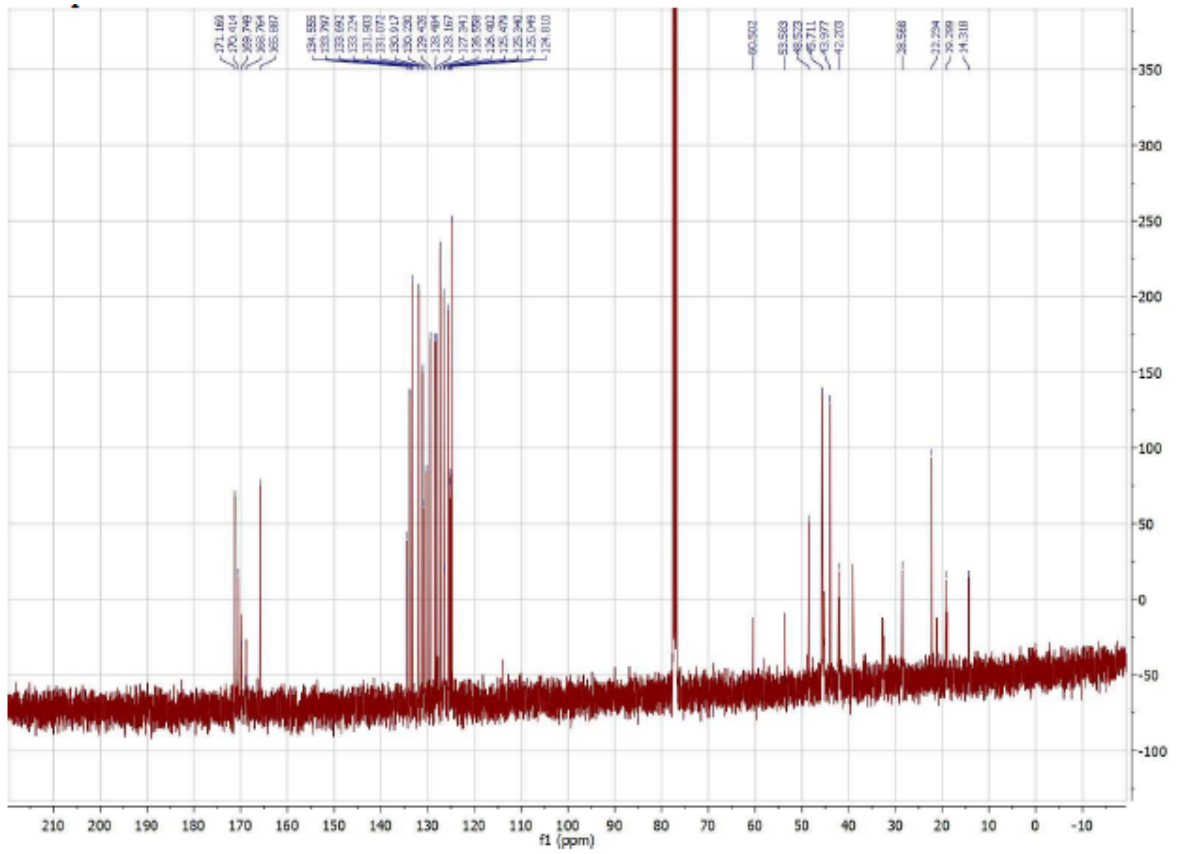
Compound 81 – ^{1}H and ^{13}C NMR Spectra



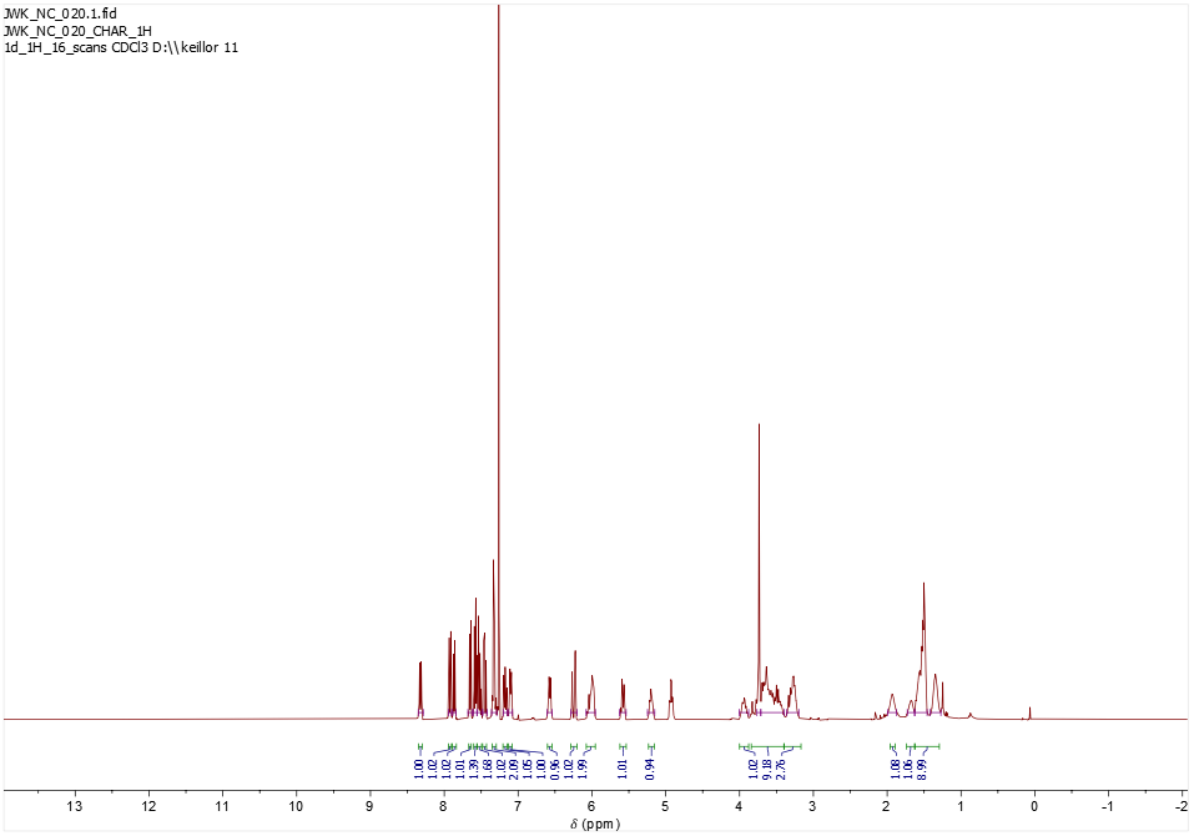


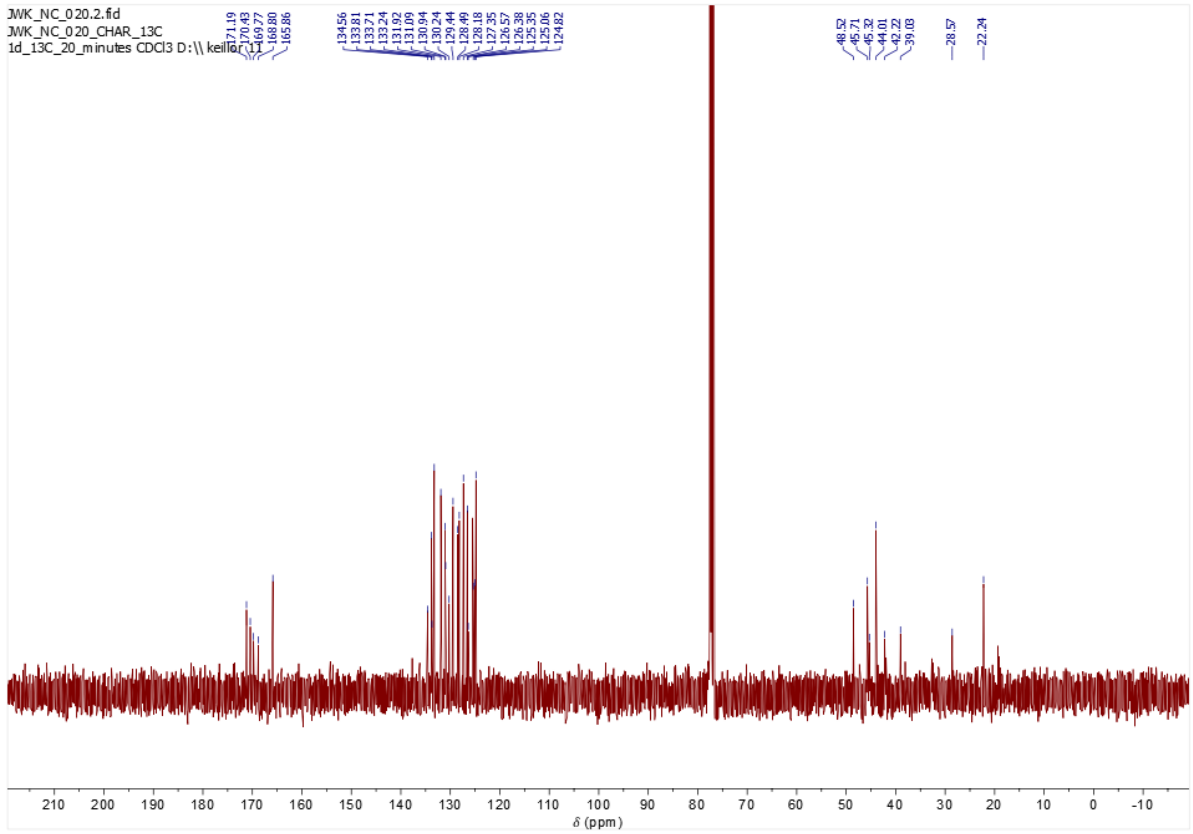
Compound 82 – ^{1}H and ^{13}C NMR Spectra



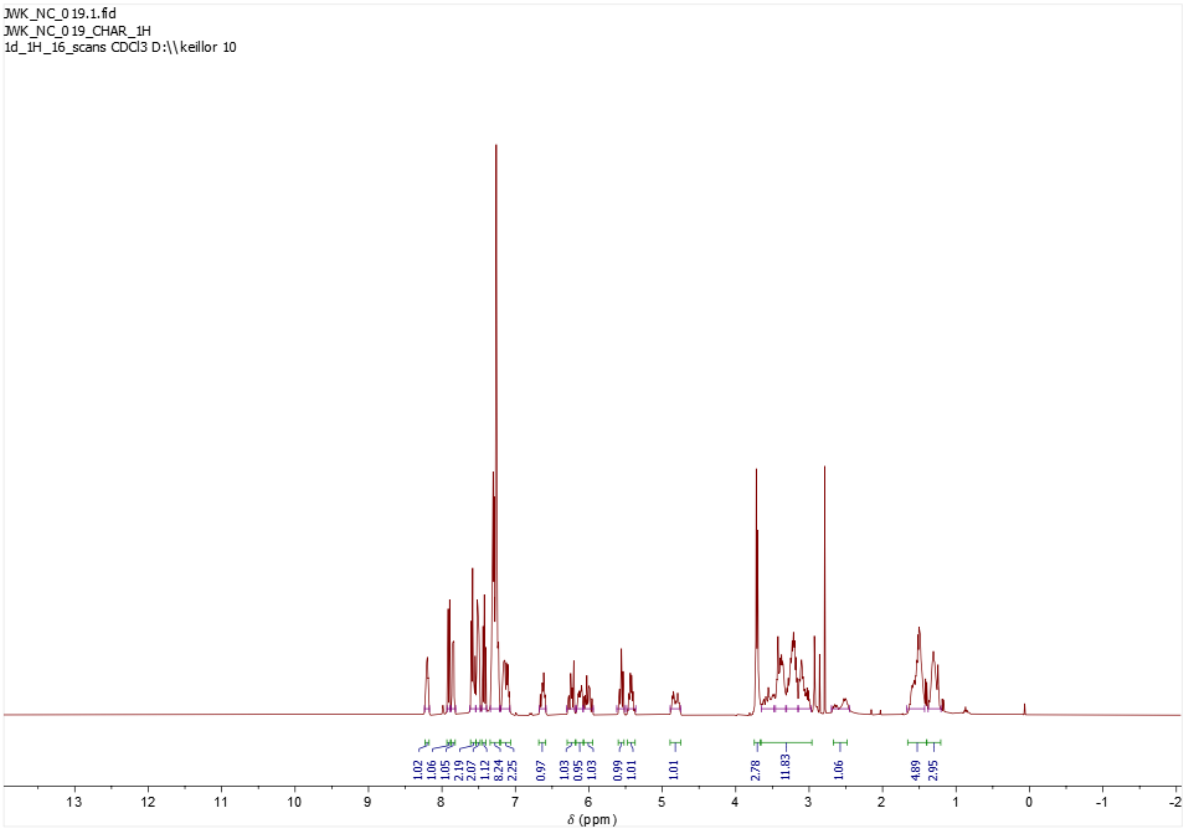


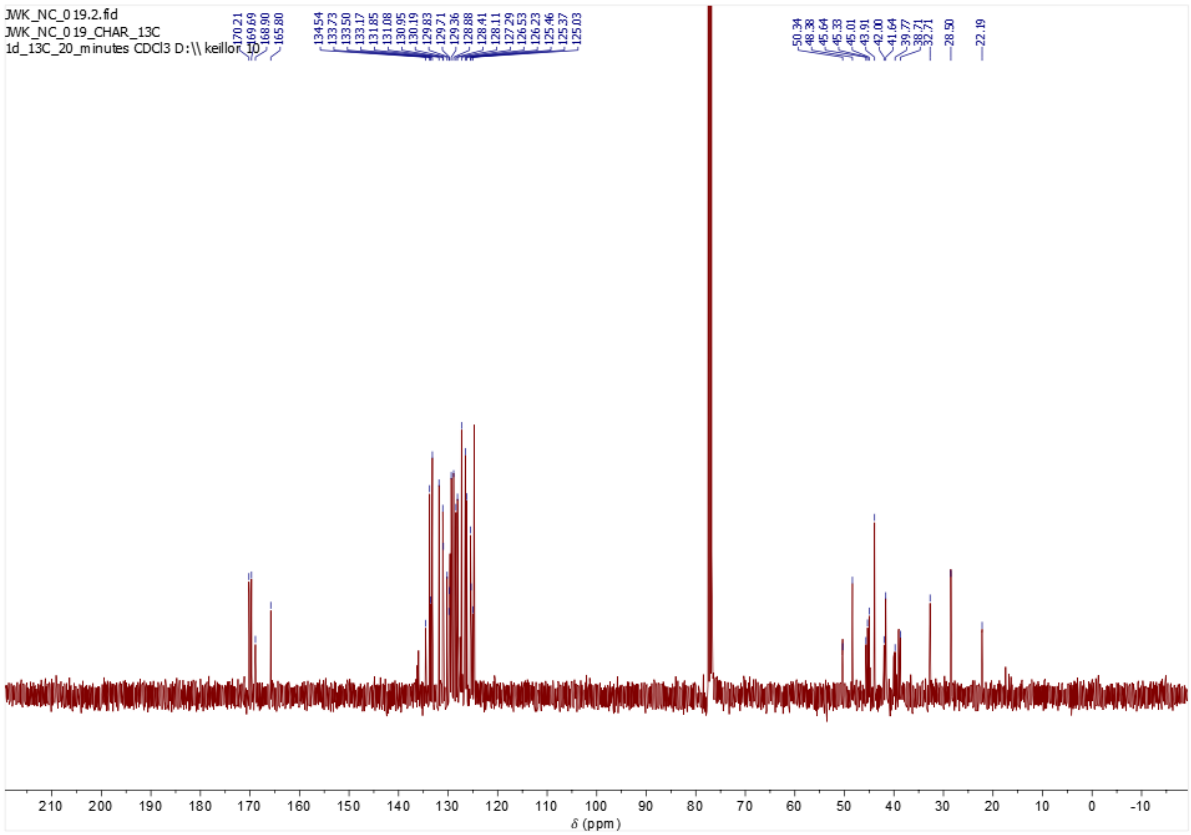
Compound 83 – ^{1}H and ^{13}C NMR Spectra



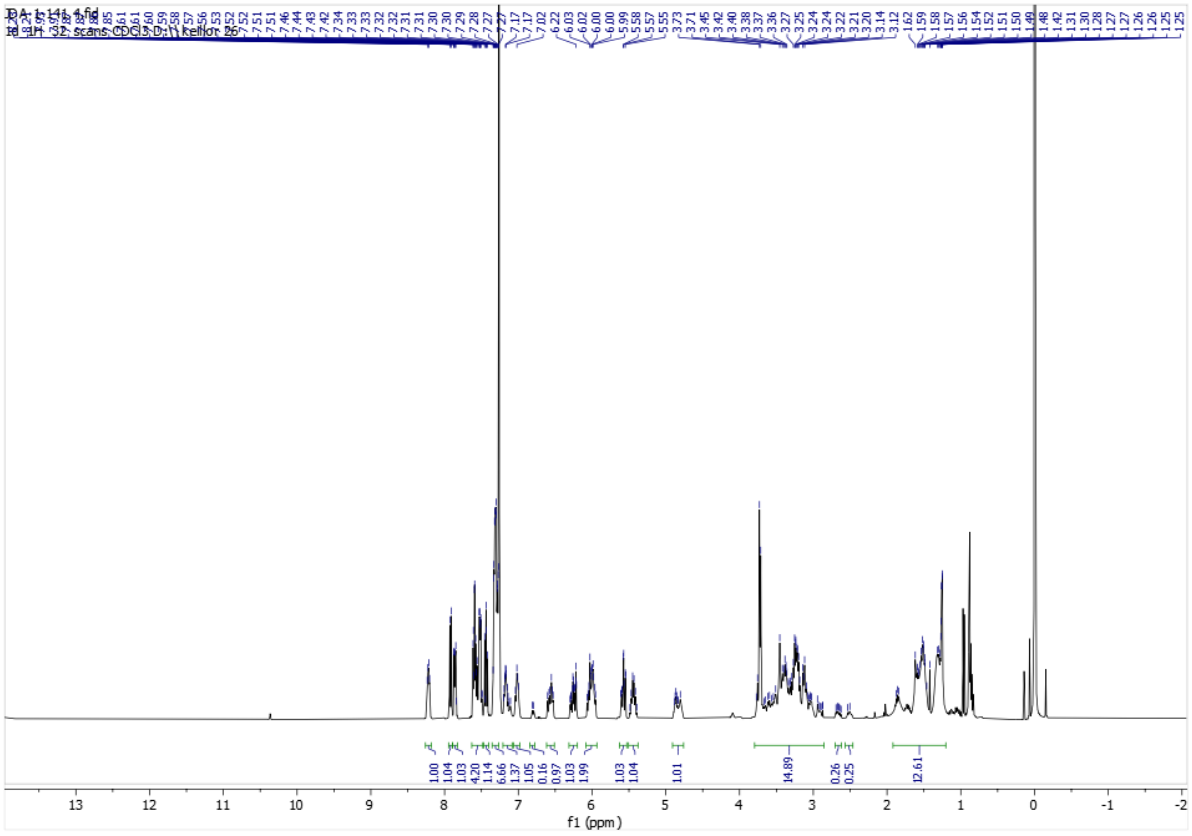


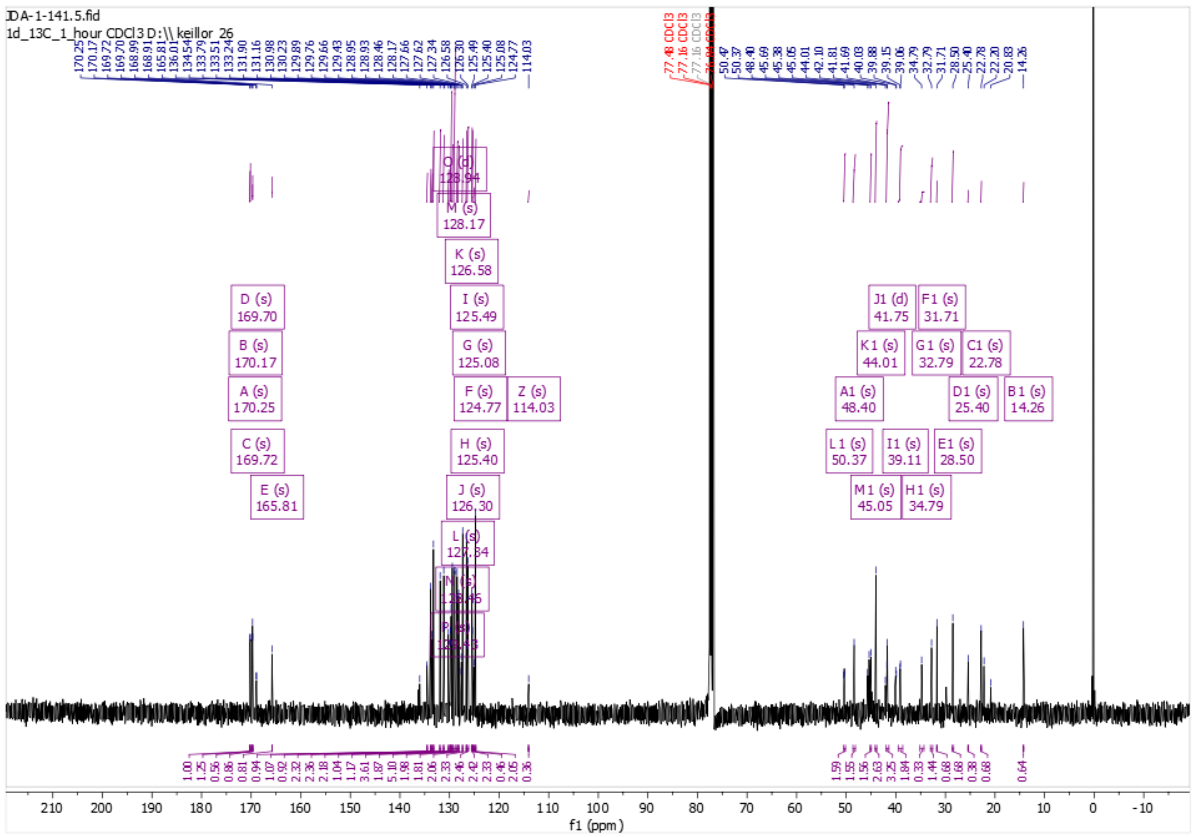
Compound 84 – ^{1}H and ^{13}C NMR Spectra



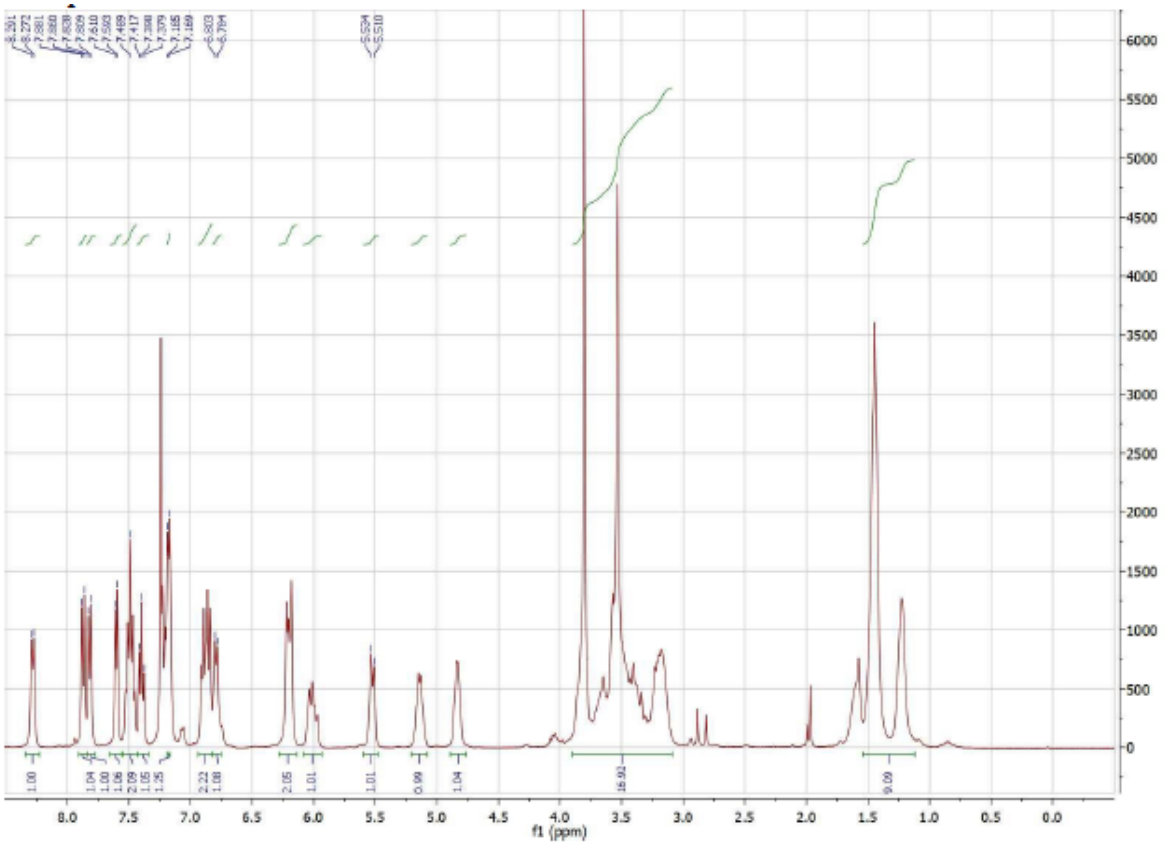


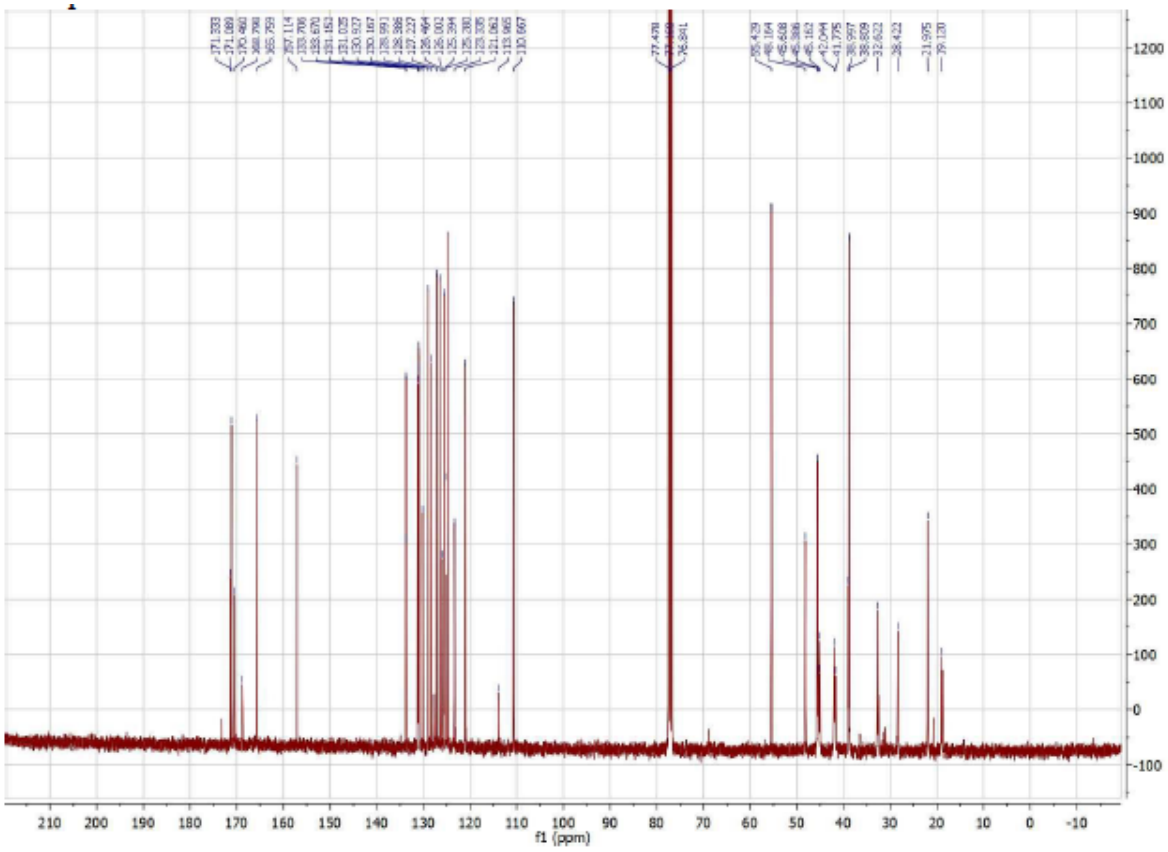
Compound 85 – ^{1}H and ^{13}C NMR Spectra



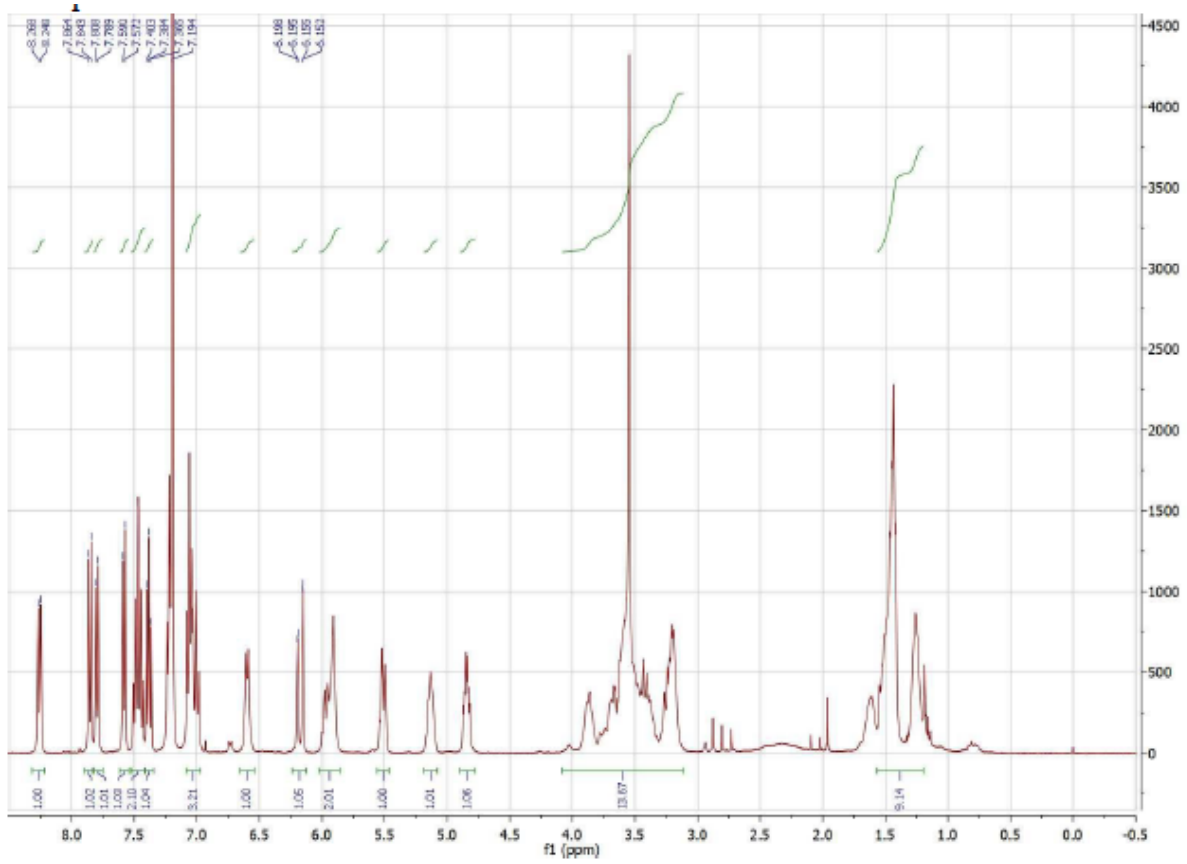


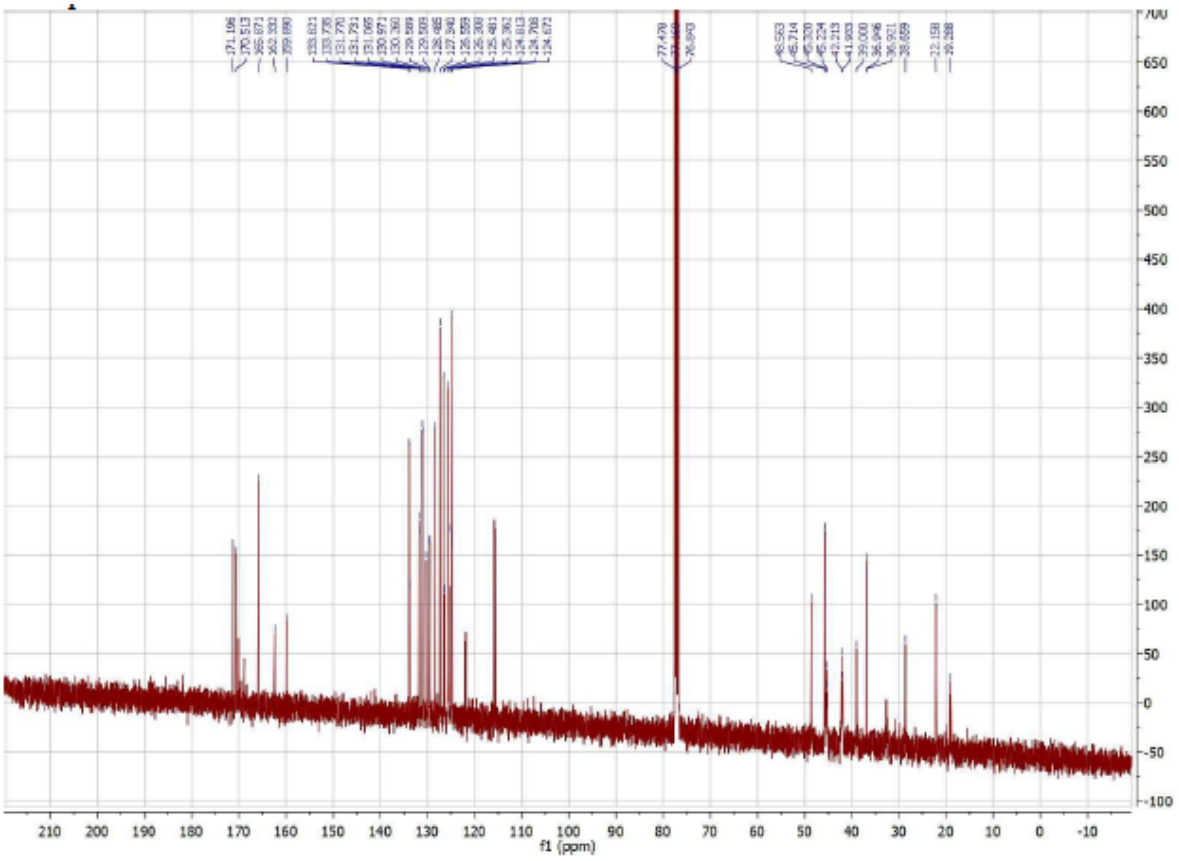
Compound 86 – ^{1}H and ^{13}C NMR Spectra



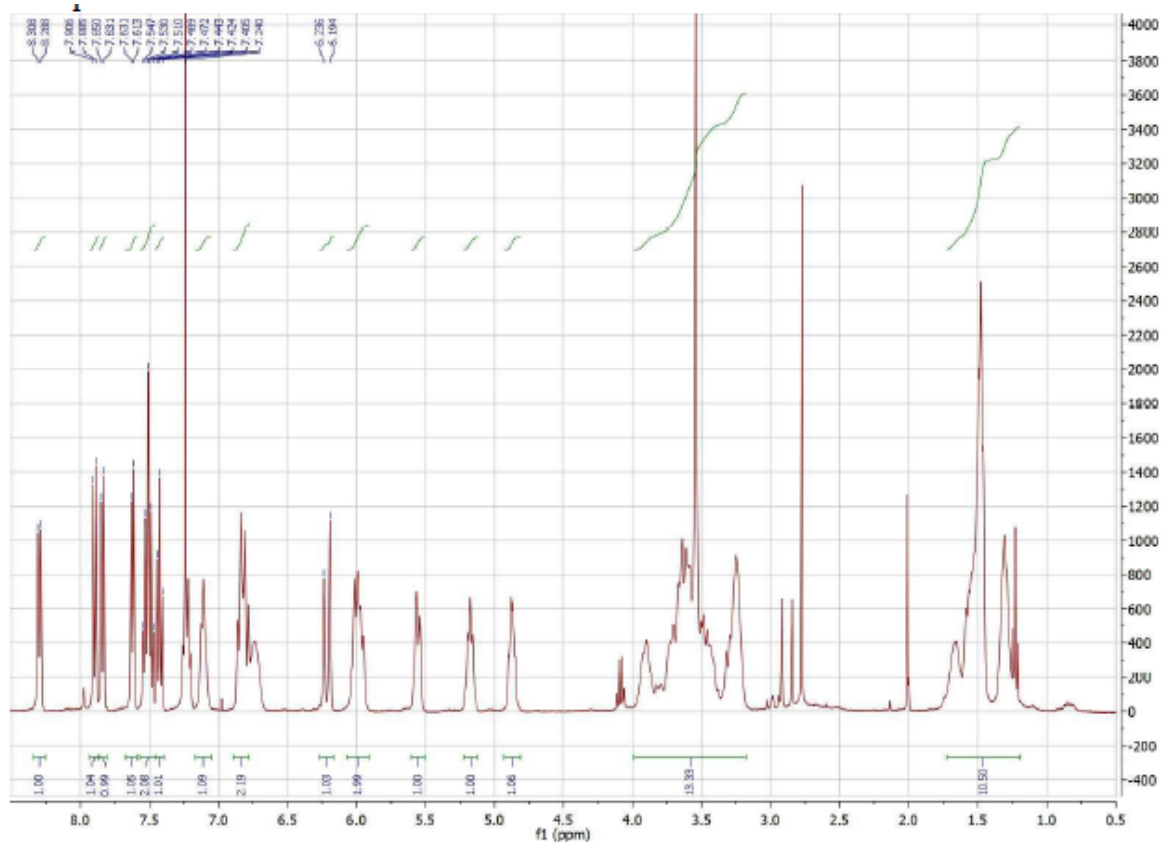


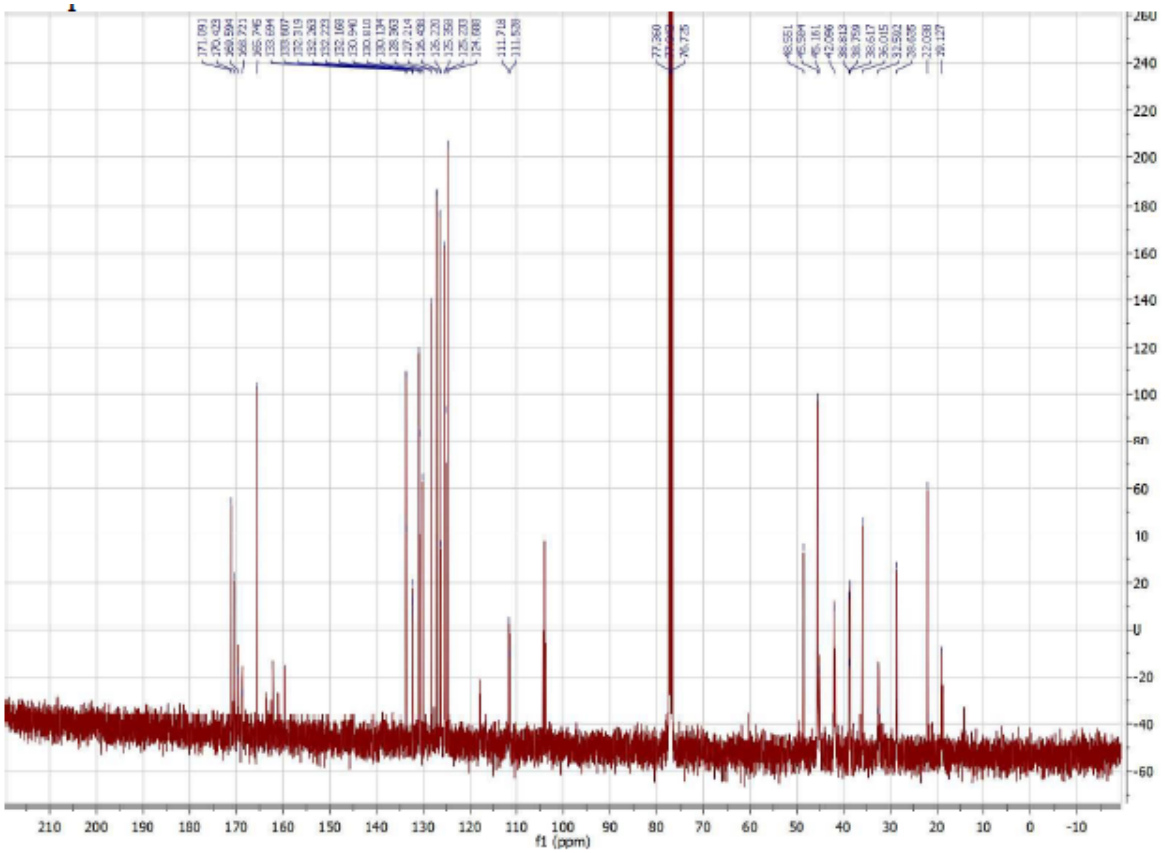
Compound 87 – ^{1}H and ^{13}C NMR Spectra





Compound 88 – ^{1}H and ^{13}C NMR Spectra





References

1 N. M. R. McNeil, E. W. J. Gates, N. Firoozi, N. J. Cundy, J. Leccese, S. Eisinga, J. D. A. Tyndall, G. Adhikary, R. L. Eckert and J. W. Keillor, *Eur J Med Chem*, 2022, **232**, 114172.

2 A. M. M. Rangaswamy, P. Navals, E. W. J. Gates, S. Shad, S. K. I. Watt and J. W. Keillor, *RSC Med Chem*, 2022, **13**, 413–428.

3 A. Akbar, N. M. R. McNeil, M. R. Albert, V. Ta, G. Adhikary, K. Bourgeois, R. L. Eckert and J. W. Keillor, *J Med Chem*, 2017, **60**, 7910–7927.

4 I. Roy, O. Smith, C. M. Clouthier and J. W. Keillor, *Protein Expr Purif*, 2013, **87**, 41–46.

5 A. Leblanc, C. Gravel, J. Labelle and J. W. Keillor, *Biochemistry*, 2001, **40**, 8335–8342.

6 R. Kitz and I. B. Wilson, *J Biol Chem*, 1962, **237**, 3245–3249.

7 S. R. Stone and J. Hofsteenge, *Biochemical Journal*, 1985, **230**, 497–502.

8 G. Adhikary, D. Grun, C. Kerr, S. Balasubramanian, E. A. Rorke, M. Vemuri, S. Boucher, J. R. Bickenbach, T. Hornyak, W. Xu, M. L. Fisher and R. L. Eckert, *PLoS One*, 2013, **8**, e84324.