Electronic Supporting Information

Fluorescent p53 Helix Mimetics Pairing Anticancer and Bioimaging Properties

Sintu Karmakar,[a] Mimasha Mallik,[b,c,†] Sushree Sulava,[b,c,†] Unnati Modi,[d,†] Suryanarayana Allu,[e] Shruti Sangwan,[e] Srinu Tothadi,[f] J Prakasha Reddy,[g] Rajesh Vasita,[d] Ashwini K. Nangia,[e] Debasmita Pankaj Alone,[b,c] Panchami Prabhakaran[a,*]

[a]School of Chemical Sciences, Central University of Gujarat, Gandhinagar, India-382030.

[b]School of Biological Sciences, Molecular Genetics Laboratory, National Institute of Science Education and Research (NISER), Odisha, India-752050.

[c]Homi Bhabha National Institute (HBNI), Training School Complex, Anushaktinagar, Mumbai, India-400094

[d]School of Life Sciences, Central University of Gujarat, Gandhinagar, India-382030.

[e]School of Chemistry, University of Hyderabad, Prof. C. R. Rao Road, Hyderabad, India-500046.
[f]Analytical and Environmental Sciences Division and Centralized Instrumentation Facility, CSIR-Central Salt and Marine Chemicals Research Institute, Bhavnagar, India-364002.

[g]School of Applied Materials Sciences, Central University of Gujarat, Gandhinagar, India-382030.

1. General Remarks

The chemicals and solvents were purchased from the following commercial sources. Ethyl acetate (EtOAc), hexane, dichloromethane (DCM), acetonitrile (ACN), acetone, *N*,*N*-dimethylformamide (DMF), methanol, diethyl ether, glacial acetic acid, tetrahydrofuran (THF) and urea from Rankem, India; 2-hydroxy-4-nitrobenzoic acid, 3-nitrophthalic acid, 2-(bromomethyl)naphthalene, Ghosez's reagent, and lithium hydroxide monohydrate ($LiOH.H_2O$) from Sigma Aldrich; stannous chloride dihydrate ($SnCl_2.2H_2O$), potassium carbonate (K_2CO_3), thionyl chloride ($SOCl_2$), triethyl amine (TEA), isobutyl bromide, *N*-(*tert*-butoxycarbonyl)-L-phenylalanine (Boc-Phe-OH) and benzotriazol-1-yloxytripyrrolidino phosphonium hexafluorophosphate (PyBOP) from SRL Chemical, India; allyl bromide from TCI Chemicals, India; *N*-(*tert*-butoxycarbonyl)-L-leucine monohydrate (Boc-Leu-$OH.H_2O$) from Glr Innovations; benzyl bromide, *N*,*N*-diisopropylethylamine (DIPEA) and 200-400 mesh silica gel from Lobachemie, India. Tripple negative breast cancer cell line (MDA-MB 231), Lung adenocarcinoma cell line (A549), HEK-293 (Human Embryonic Kidney) and U87MG (Human Malignant Glioblastoma) were procured from NCCS (National Centre for Cell Science), Pune, India. Dulbecco's modified eagle medium with high glucose, Dulbecco's modified eagle medium: Nutrient mixture F12 (DMEM: F12; 1:1), Fetal Bovine Serum (FBS), 0.25% Trypsin –EDTA, and 100X Antibiotic-Antimycotic solution were purchased from Gibco® [ThermoFisher Scientific]. 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) availed from Sigma Aldrich and 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)–2H-tetrazolium (MTS) purchased from Promega, USA. 7-AAD (7-Aminoactinomycin D) nuclear stain was purchased from Invitrogen (component of SelectFX® Nuclear labelling kit for fixed cells). Analytical thin-layer chromatography was done on precoated silica gel plates (TLC silicagel $60F_{254}$, Merck). Column chromatographic purifications were done with 200-400 mesh silica gel. NMR spectra were taken in $CDCl_3$ or DMSO-d_6 on Bruker 500-MHz ultra shield plus NMR spectrometer. All the chemical shifts are reported in δ ppm downfield to TMS. $CDCl_3$ was taken as a reference at 7.26 for proton and 77.0 for carbon or DMSO-d_6 at 2.50 for proton and 39.50 for carbon. NMR peak multiplicities are given as singlet (s), broad singlet (bs), doublet (d), triplet (t), doublet of doublet (dd), triplet of triplet (tt), doublet of doublet of doublet (ddd), doublet of doublet of triplet (ddt), quartet (q), and multiplet (m). Melting point was determined using Stuart SMP40 automatic melting point apparatus. Fourier Transform Infrared Spectroscopy (FT-IR) was done using PerkinElmer SP-65 instrument. Matrix-Assisted Laser Desorption Ionization time-of-flight (MALDI-TOF) was recorded on Bruker Autoflex speed using matrix 2,3-dihydroxybenzoic acid (DHB) or α-Cyano-4-hydroxycinnamic acid (CHCA). HRMS data was recorded by Electrospray ionization (ESI) on Xevo XS QTOF mass spectrometer waters ACQUITY UHPLC Milford USA. Analytical RP-HPLC was done using PerkinElmer Flexer FX-6 instrument with HC-C18 PerkinElmer column having 250 × 4.6 mm ID and 0.5 µm porous silica outer shell. Preparative HPLC was done using Shimadzu-Nexera preparative HPLC with C18 column having 20 × 250 mm ID and particle size 5 µm. Fluorescence studies were done using Shimadzu RF-6000 Spectro fluorophotometer in methanol using a standard quartz cuvette of 2 mL capacity. Scanning electron microscope (SEM) studies were carried out using Carl Zeiss EVO18 SEM instrument.

2. Methods and Procedures

2.1 General Synthetic Procedures

Procedure A: *O*-Alkylation/*N*-Alkylation
In a round bottom flask, the compound (1 equiv.) and anhydrous potassium carbonate (K_2CO_3, 1.5 equiv.) were taken in dry *N*,*N*-dimethylformamide (DMF) under nitrogen atmosphere. Alkyl halide (1.2 equiv.) was added to the mixture. The reaction mixture was stirred at room temperature for 12 h and progress of the reaction was monitored by TLC. The reaction mixture was diluted by ethyl acetate (EtOAc) and neutralized using 2N potassium bisulphate ($KHSO_4$) solution. The organic layer was extracted, washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to yield product. It was further purified through column chromatography and used for next step.

Procedure B: Ester hydrolysis
Ester hydrolysis was done by dissolving the ester in tetrahydrofuran (THF)-water (v/v) mixture (2:1) with lithium hydroxide monohydrate ($LiOH.H_2O$, 2 equiv.) and stirred the reaction mixture at room temperature for 12 h. THF was completely removed, neutralized by 2N $KHSO_4$ solution and ethyl acetate was added to the mixture to extract the product. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, concentrated under reduced pressure to give the acid. The product was taken for the next step without further purification.

Procedure C: Nitro reduction with $SnCl_2$
To the substrate in EtOAc, stannous chloride dihydrate ($SnCl_2.2H_2O$, 5 equiv.) was added and the mixture was stirred at 50 °C for 24 h (the reaction did not progress at room temperature because of low solubility). The progress of the reaction was monitored using TLC and staining was done with ninhydrin solution. After completion of the reaction, EtOAc was added to the reaction mixture and basified with saturated $NaHCO_3$ solution. The product in ethyl acetate was extracted and washed with brine and dried over anhydrous Na_2SO_4. The amine obtained after removal of solvent and drying under vacuum was used for the next step.

Procedure D: Nitro reduction with H_2/Pd-C
To the stirred solution of the substrate in EtOAc, 10% Pd-C (w/w) in ethyl acetate was added to the solution. H_2 filled balloon pressure was applied to the reaction mixture and it was allowed to stir at room temperature. After 12 h TLC indicates the complete conversion of the reactant to the product, then the reaction mixture was filtered through celite. The filtrate was dried over anhydrous Na_2SO_4. After removal of solvent and drying under vacuum yielded desired product.

Procedure E: Coupling reaction with $SOCl_2$
To a solution of acid (1 .2 equiv.) in dry DCM under nitrogen atmosphere at room temperature, was added cat. amount DMF followed by $SOCl_2$ (2 equiv.). The reaction mixture was allowed to stir at room temperature for 2h. DCM was evaporated, and the acid chloride was obtained after drying under high vacuum for an hour. To a mixture of amine (1 equiv.) and triethyl amine (2 equiv.) taken in dry DCM at 0 °C under nitrogen atmosphere, was added dropwise the acid chloride in dry DCM. After 30 min, the mixture was allowed to stir at room temperature for 12 h. DCM was added to the reaction mixture and washed with 2N $KHSO_4$ solution, saturated $NaHCO_3$ solution and brine. The organic layer was collected, dried over anhydrous Na_2SO_4, concentrated under reduced pressure. The crude product obtained was purified through precipitation (EtOAc or EtOAc/Et_2O).

Procedure F: Coupling reaction with PyBOP

Amine (1 equiv.) was added to a solution containing boc protected amino acid (1.2 equiv.), PyBOP (1.5 equiv.) and *N*,*N*-Diisopropylethylamine (DIPEA) (1.5 equiv.) in dry DCM at 0 °C under inert atmosphere. After 15 min reaction, the mixture was allowed to stir at room temperature and then refluxed at 40 °C for 12 h. DCM was added, and the reaction mixture was washed with 2N $KHSO_4$ solution followed by saturated $NaHCO_3$ solution and brine. The organic layer was dried over anhydrous Na_2SO_4, concentrated to obtain desired yield and the product obtained in this step was further purified through precipitation method for further use.

Procedure G: Boc-deprotection

A round bottom flask containing the compound in saturated solution of HCl in acetonitrile (ACN) was stoppered and stirred for 1-2 h (reaction time depends on the substrate) at 0 °C. The progress of the reaction was monitored using TLC and staining was done with ninhydrin solution. After completion, acetonitrile was completely removed, washed repeatedly with diethyl ether, and dried under reduced pressure. The product obtained in this step was either purified through RP-HPLC chromatography or precipitation. The purity of the compound was assessed by analytical RP-HPLC.

Procedure H: Coupling reaction with Ghosez's reagent

1-Chloro-*N*,*N*,2-trimethyl-1-propenylamine (Ghosez's reagent) (1.5 equiv.) was added dropwise to a solution containing dimer acid (1 equiv.) in DCM at 0 °C under inert atmosphere. After 1 h, DCM was completely evaporated, the mixture was dried in high vacuum for another 1 h. Meantime DIPEA (1.5 equiv.) was added to the solution containing amine (1.2 equiv.) in DCM at 0 °C under inert atmosphere. The dried acid was then dissolved in DCM, added dropwise to the amine solution at 0 °C under inert atmosphere and the reaction mixture was allowed to stir at room temperature after 30 min. The progress of the reaction was monitored using TLC. After 12 h the reaction mixture was washed with saturated $NaHCO_3$ solution followed by 2N $KHSO_4$ solution and brine. The organic layer was dried over anhydrous Na_2SO_4, concentrated to obtain desired yield and the product obtained in this step was further purified through precipitation method for further use.

2.2 Experimental procedures

a) Cell culture

HEK-293 (Human Embryonic Kidney) cells and U87MG (Human Malignant Glioblastoma) cells were cultured in T75 flasks in a complete medium containing DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% fetal bovine serum (FBS) (P40-48500, PAN Biotech) and a 1.0% antibiotic mixture of penicillin-streptomycin (A001, Himedia) and amphotericin (A011, Himedia). The cells were maintained at 37°C and 5.0% CO_2 in an incubator. Subsequently, they were split using 0.25% trypsin (TCL048, Himedia) once they reached 70–80% confluency and subcultured for further passages.

MDA-MB-231 and A549 cells were cultured in Dulbecco's modified eagle medium with high glucose and Dulbecco's modified eagle medium: Nutrient mixture F12 (DMEM: F12; 1:1) respectively, supplied with 10% Fetal Bovine Serum (FBS). Cells were cultured at 37˚C and 5% CO_2. Post reaching 70-80 % confluency, cells were trypsinized and counted according to the experimental cell number requirement and accordingly seeded in the desired culture vessel.

b) Anti-cancer activity assessment of helix mimetics using MTT assay

MTT assay was used to gauge the anticancer activity of **SK17**, **SK18**, **SK19**, **SK20,** and **SK21** In a 96-well culture plate, 8 x 10^3 MDA-MB-231 and A549 cells were plated in each well of two different culture plates. Post 24 hours cells were treated with complete medium containing individually and attaining various working concentrations (1, 10, 25, 50, 100, 250 and 500

μM). Here, the cells cultured in complete media without helix mimetic served as the experimental control. After 24 hours of incubation, the culture media was replaced with 100 μL of working concentration of MTT (0.5 mg/mL in PBS) further incubated at 37 ºC for three hours in the incubator. The formazan crystals thus developed were solubilized in DMSO and spectrophotometrically measured at 570 nm (BioTek Synergy H1). The percentage cell viability of each group was calculated with respect to its control and plotted in the graphical form using GraphPad Prism 8 software (where n=3).

c) Anti-cancer activity assessment of helix mimetic using MTS assay

Cell viability of HEK-293 cells and U87MG were assessed using the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)–2H-tetrazolium (MTS) reagent Cells were seeded in 9c)6-well plates at a density of 5000 cells/well in 100 μL of medium and incubated overnight at 37°C and 5.0% CO_2. Stock solutions of the compounds were prepared by dissolving in DMSO (TC185, Himedia) which were further diluted in DMEM to yield the working range of concentration. The cells were exposed to the drug (treatment)/vehicle (control) so that the final DMSO concentration per well was 0.1% and then incubated for 24 h at 37°C and 5.0% CO_2. Following treatment, 20μL of MTS reagent was added to each well, and the plate was incubated for 3 hours at 37 °C and 5.0% CO_2. Subsequently, the optical density was measured at 490 nm using a microplate reader (Varioskan Flash, Thermofisher Scientific, USA). The percentage viability of the cells was calculated and plotted against the respective compound concentrations, with each experiment conducted thrice.

d) Cell internalization study using Confocal Laser Scanning Microscope (CLSM)

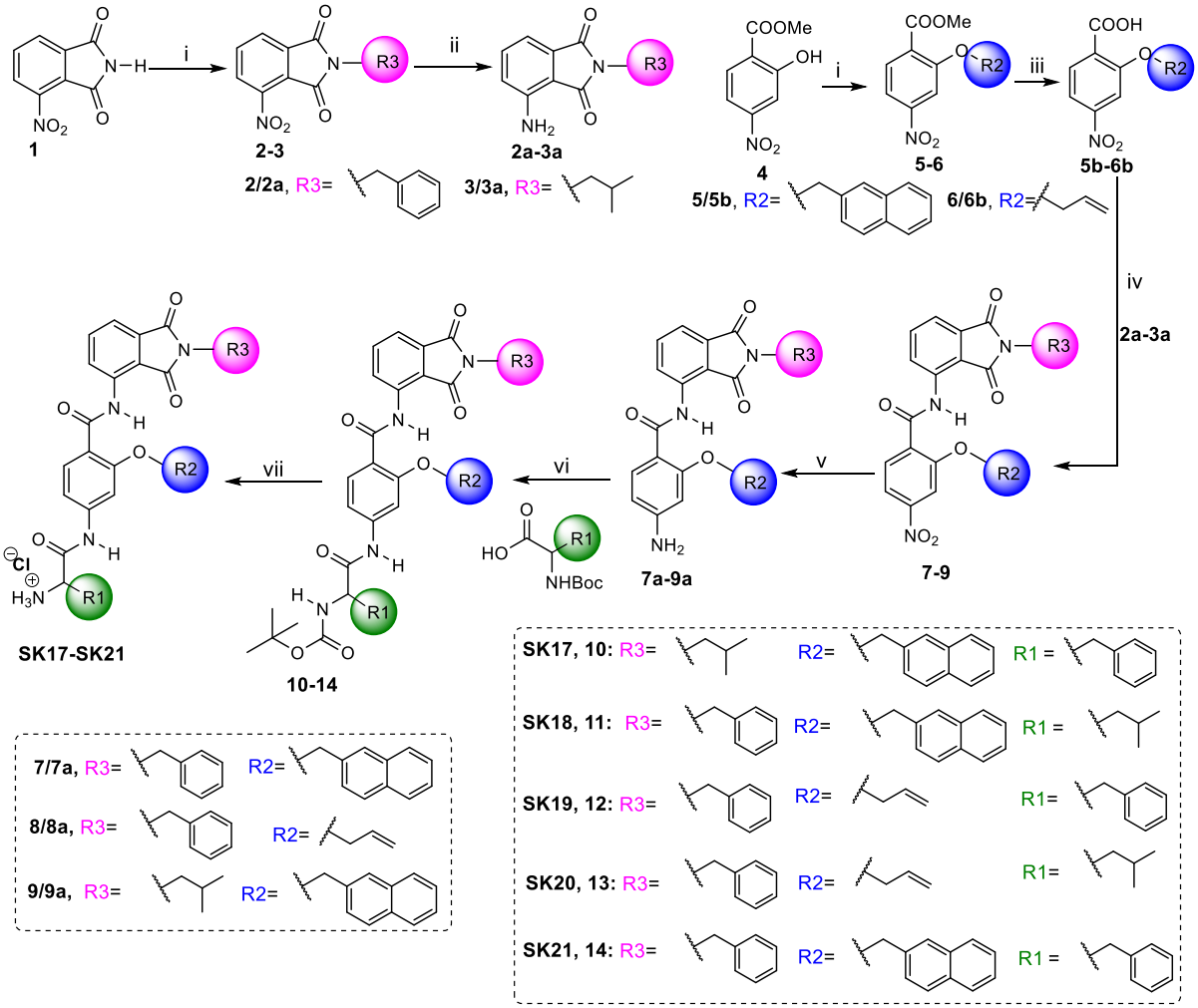
The helix mimetic internalization/interaction with the cell was analysed by localizing the fluorescence. After 24 hours of culture, MDA-MB-231 and A549 cells were treated with culture media containing compound individually for 2 hours. Post treatment, cells were fixed with 4% Paraformaldehyde. The cytoplasm and as nucleus were stained with Phalloidin-FITC for 60 mins and 7-AAD for 30 mins sequentially. The internalized mimetics were traced using Confocal Laser Scanning Microscope (CLSM) 780 by Zeiss using camera mode selection in light path, subjecting to Filter set 49 (Exc.- 365 and Emm. – 445-450), Filter set 10 (Exc.- 450-490 and Emm. – 515-565), and Filter set 20 (Exc.- 546/12 and Emm. – 575-640). Further, the mean fluorescence intensity for the molecules was derived through the Zen 2010 image processing software from the profile of each molecule groups fluorescence images (n = 3). The mean fluorescence intensity was plotted using GraphPad Prism 8 software (where n = 3). For statistical analysis, Student's T-Test (unpaired) was applied where *, **, *** and **** signifies P value < 0.05, < 0.01, < 0.001, and < 0.0001 respectively, where n = 3.

e) Live cell imaging

Both HEK-293 and U87MG cells were seeded in 6-well plates at a density of 8000 cells/well in 2000 μl medium on 0.11 x 18 mm diameter coverslips (TCP018, Himedia) and incubated overnight at 37˚C and 5.0% CO_2. Subsequently, compounds were added to the cells at a concentration of 25 μM and incubated at 37˚C and 5.0% CO_2 for 2 h, while the control was prepared with 0.1% DMSO. After two hours of incubation, live cell imaging was performed at 475 nm using an automated fluorescence microscope (BX63, Olympus, Japan) and the images were analysed using Image J software.

2.3 Synthesis

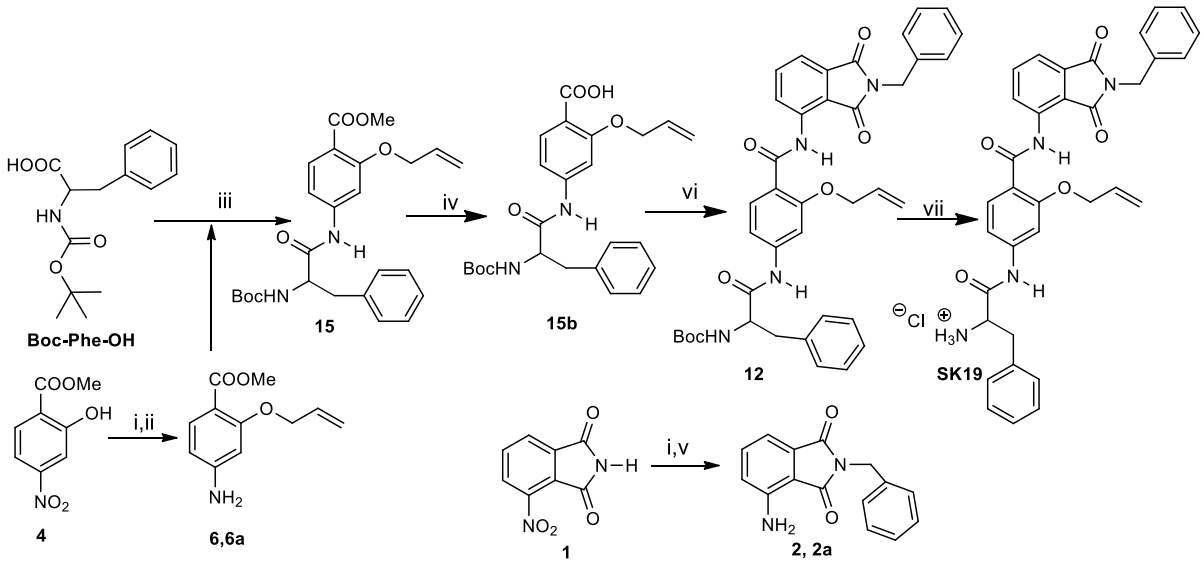
Synthetic Schemes:



Scheme S1. Synthesis of helix mimetics **SK17-SK21** (top-to-bottom approach).

Reagents and conditions: i) alkyl halide (1.2 equiv.), K_2CO_3 (1.5 equiv.), DMF, rt; ii) H_2 (balloon pressure), Pd-C, EtOAc, rt; iii) $LiOH.H_2O$ (2 equiv.), THF:water = 2:1(v/v), rt; iv) (a) acid (1.2 equiv.), $SOCl_2$ (2 equiv.), DMF (cat. amount), DCM (b), amine (1 equiv.), TEA (2 equiv.), DCM; v) $SnCl_2.2H_2O$ (5 equiv.), ethyl acetate, 50 °C**; vi) acid (1.2 equiv.), amine (1 equiv.), PyBOP (1.5 equiv.), DCM, DIPEA (1.5 equiv.), 50 °C; vii) saturated HCl in ACN, 0 °C.

** The reaction at room temperature did not proceed due to poor solubility in ethyl acetate.



Scheme S2. Synthesis of helix mimetic **SK19** via bottom-to-top approach.

Reagents and conditions: i) alkyl halide (1.2 equiv.), K_2CO_3 (1.5 equiv.), DMF, rt; ii) $SnCl_2.2H_2O$ (5 equiv.), ethyl acetate, rt; iii) acid (1.2 equiv.), amine (1 equiv.), PyBOP (1.5 equiv.), DCM, DIPEA (1.5 equiv.), rt; iv) acid (1 equiv.), $LiOH.H_2O$ (2 equiv.), THF: water = 2:1(v/v), rt; v) H_2 (balloon pressure), Pd-C, rt; vi) Ghosez's reagent (1.5 equiv.), DCM, DIPEA, rt; vii) saturated HCl in ACN, 0 °C

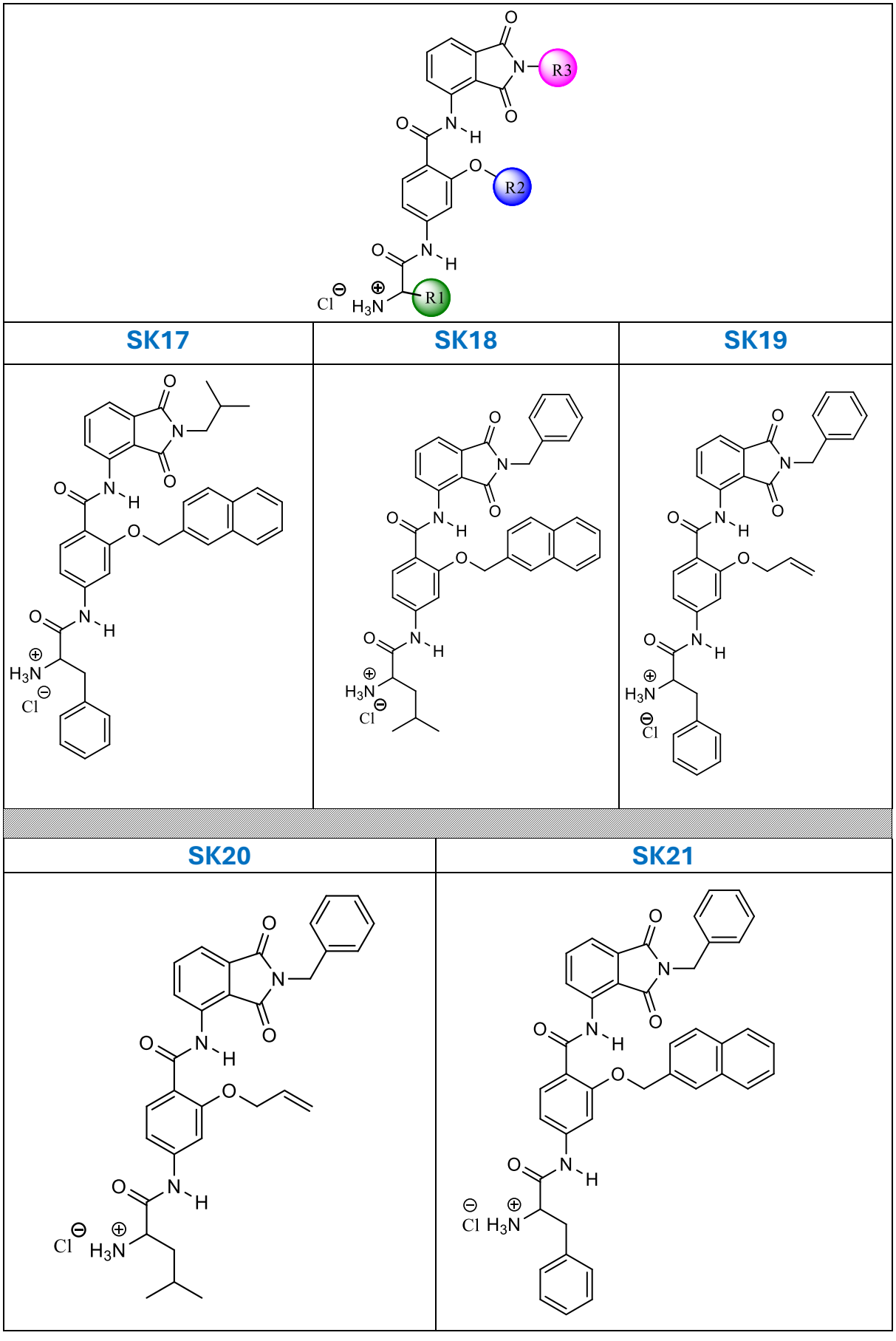
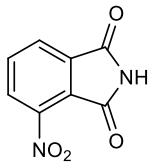


Figure S1. Structures of helix mimetics **SK17-SK21**.

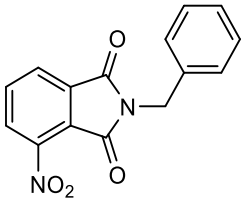
4-Nitroisoindoline-1,3-dione (1)[1]



3-nitrophthalic acid (5 g, 23.68 mmol 1 equiv.), glacial acetic acid (20 mL) and urea (1.43 g, 23.68 mmol, 1 equiv.). White solid (4.04 g, 89%); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 11.77 (s, 1H), 8.27–8.25 (d, *J* = 7.98 Hz, 1H), 8.12–8.11 (d, *J* = 7.06 Hz, 1H), 8.05–8.02 (t, *J* = 7.75 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 167.2, 164.6, 144.4, 136.1, 134.6, 128.1, 126.8, 123.9.

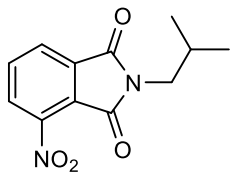
2-Benzyl-4-nitroisoindoline-1,3-dione (2)[2]

General procedure A; compound **1** (2 g, 10.41 mmol, 1 equiv.), DMF (10 mL), K_2CO_3 (2.16 g, 15.61 mmol, 1.5 equiv.), benzyl bromide (1.5 mL, 12.49 mmol, 1.2 equiv.). White Solid (2.70 g, 92%), m.p: 143–144 °C, R_f = 0.43 (eluent: EtOAc/ hexane, 30:70 v/v) ; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 8.30–8.28 (d, *J* = 8.04 Hz, 1H), 8.20–8.18 (d, *J* = 7.40 Hz, 1H), 8.08–8.04 (t, *J* = 7.69 Hz, 1H), 7.34–7.33 (m, 4H), 7.27 (bs, 1H), 4.77 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 165.9, 163.3, 144.4, 136.3, 136.2, 133.6, 128.6, 128.5, 127.61, 127.57, 127.1, 123.2, 41.4; IR (KBr) ν (cm^{-1}): 1721, 1659, 1540, 1391; ESI-MS calcd. For $C_{15}H_{11}N_2O_4$, $[M+H]^+$ *m/z*: 283.0713, found, 283.0721.



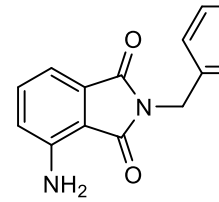
2-Isobutyl-4-nitroisoindoline-1,3-dione (3)

General procedure A; compound **1** (2 g, 10.41 mmol, 1 equiv.), DMF (10 mL), K_2CO_3 (2.16 g, 15.61 mmol, 1.5 equiv.), isobutyl bromide (1.3 mL, 12.49 mmol, 1.2 equiv.), pale yellow solid (2.45 g, 95%), m.p: 65–67 °C, R_f = 0.50 (eluent: EtOAc/ hexane, 30:70 v/v); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.11–8.09 (m,1H), 7.93–7.90 (t, *J* = 7.91 Hz, 1H), 3.52–3.51 (d, *J* = 7.38 Hz, 2H), 2.15–2.07 (m, 1H), 0.93–0.92 (d, *J* = 6.72 Hz, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 166.1, 163.1, 145.0, 135.2, 134.0, 128.4, 126.9, 123.6, 45.9, 27.7, 20.0; IR (KBr) ν (cm^{-1}): 1777, 1719, 1538, 1442, 1351; ESI-MS calcd. For $C_{12}H_{13}N_2O_4$, $[M+H]^+$ *m/z*: 249.0870, found, 249.0876.



4-Amino-2-benzylisoindoline-1,3-dione (2a)[3]

General procedure D; compound **2** (2 g, 7.08 mmol, 1 equiv.), Pd-C (0.2 g), greenish yellow solid, (1.75 g, 98%), m.p: 145–146 °C, R_f = 0.53 (eluent: EtOAc/ hexane, 40:60 v/v); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 7.43 (bs, 1H), 6.98 (d, *J* = 6.04 Hz, 2H), 6.51 (s, 2H), 4.69 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 169.2, 167.9, 146.7, 137.2, 135.3, 132.3, 128.6, 127.39, 127.38, 121.6, 110.9, 108.8, 40.4; IR (KBr) ν (cm^{-1}): 3476, 3355, 1691, 1634, 1403, 1330; ESI–MS calcd. for $C_{15}H_{13}N_2O_2$ $[M+H]^+$ *m/z*: 253. 0972, found, 253.1043.



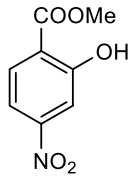
4-Amino-2-isobutylisoindoline-1,3-dione (3a)

General procedure D; compound **3** (2 g, 8.05 mmol, 1 equiv.), Pd-C (0.2 g), greenish yellow solid, (1.70 g, 97%), m.p: 105–108 °C, R_f = 0.60 (eluent: EtOAc/ hexane, 40:60 v/v); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.40–7.37 (dd, *J* = 8.27, 7.17 Hz, 1H), 7.13–7.12 (d, *J* = 7.09 Hz, 1H), 6.85–6.83 (d, *J* = 8.28 Hz, 1H), 4.97 (bs, 2H), 3.44–3.42 (d, *J* = 7.38 Hz, 2H), 2.16–2.02 (m, 1H), 0.93–0.92 (d, *J* = 6.73 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.5, 168.9, 145.1, 135.0, 132.7, 120.9, 112.6, 111.3, 44.9, 27.8, 20.1; IR (KBr) ν (cm^{-1}): 3447,

3317, 1687, 1634, 1477, 1410, 1369; ESI-MS calcd. for $C_{12}H_{15}N_2O_2$ $[M+H]^+$ *m/z*: 219.1128, found, 219.1140.

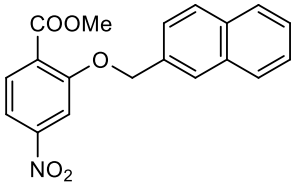
Methyl-4-nitrosalicylate (4)[4]

Ester **4** was obtained from 4-nitrosalicylic acid with modification in the reported procedure.



Acid (8 g, 43.68 mmol, 1 equiv.), $SOCl_2$ (6.40 mL, 87.38 mmol, 2 equiv.), CH_3OH (30 mL), pale yellow solid (8.20 g, 95%), R_f = 0.42 (eluent: EtOAc/ hexane, 30:70 v/v); ^{1}HNMR (500 MHz, $CDCl_3$) δ: 10.98 (s, 1H), 8.02–8.01 (d, *J* = 8.69 Hz, 1H), 7.81 (s, 1H), 7.71–7.69 (d, *J* = 8.72 Hz, 1H), 4.02 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 169.2, 161.9, 152.1, 131.2, 117.1, 113.5, 113.0, 53.1.

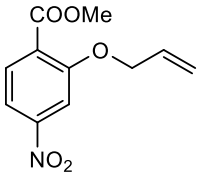
Methyl 2-(naphthalen-2-ylmethoxy)-4-nitrobenzoate (5)[4]



General procedure A, compound **4** (2.68 g, 12.58 mmol, 1 equiv.), K_2CO_3 (2.82 g, 21.82 mmol, 1.5 equiv.), DMF (20 mL), 2-(bromomethyl)naphthalene (3.6 g, 16.32 mmol, 1.2 equiv.), White solid (4.44 g, 97%), m.p: 163–165 °C, R_f = 0.50 (eluent: EtOAc/hexane, 20:80, v/v); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.96 (s, 1H), 7.96–7.84 (m, 7H), 7.60–7.58 (dd, *J* = 8.46 Hz, 1.64 Hz, 1H), 7.53–7.49 (m, 2H), 4.53 (s, 2H), 3.97 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 165.3, 158.1, 150.6, 133.2, 133.1, 132.7, 132.3, 128.6, 128.0, 127.8, 126.6, 126.4, 126.3, 126.2, 124.6, 115.3, 108.5, 71.2, 52.6; IR (KBr) ν (cm^{-1}): 3122, 2951, 2871, 1732, 1536, 1424, 1397; ESI-MS calcd. for $C_{19}H_{15}NO_5$, $[M+Na]^+$ *m/z*: 360.0842, found, 360.0833.

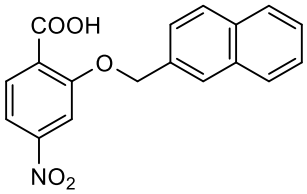
Methyl 2-(allyloxy)-4-nitrobenzoate (6)[5]

General procedure A, compound **4** (3.5 g, 17.75 mmol, 1 equiv.), K_2CO_3 (3.68 g, 26.63 mmol, 1.5 equiv.), DMF (20 mL), allyl bromide (1.84 mL, 21.30 mmol, 1.2 equiv.),



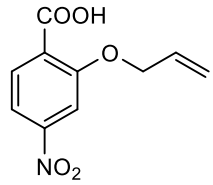
puffy white material (3.9 g, 93%), m.p: 67–69 °C, R_f = 0.46 (eluent: EtOAc/hexane, 20:80, v/v); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.91–7.89 (d, *J* = 8.46 Hz, 1H), 7.83–7.81 (dd, *J* = 8.46 Hz, 2.07 Hz, 1H), 7.791–7.787 (d, *J* = 2.02 Hz, 1H), 6.09–6.02 (ddt, *J* = 17.24 Hz, 10.60 Hz, 4.88 Hz, 1H), 5.55–5.51 (dq, *J* = 17.25, 1.61 Hz, 1H), 5.37–5.34 (dq, *J* = 10.64, 1.46 Hz, 1H), 4.73–4.71 (dt, *J* = 4.85, 1.62 Hz, 2H), 3.93 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 165.2, 157.1, 150.2, 132.6, 131.6, 126.6, 117.5, 115.4, 108.6, 69.4, 52.7; IR (KBr) ν (cm^{-1}): 3122, 2998, 2952, 2863, 1713, 1531, 1442, 1396.

2-(Naphthalen-2-ylmethoxy)-4-nitrobenzoic acid (5b)[4]



General procedure B, compound **5** (3.6 g, 10.67 mmol, 1 equiv.), $LiOH.H_2O$ (0.9 g, 21.34 mmol, 2 equiv.), THF-water (20 mL), yellow solid (3.34 g, 97%), m.p: 141–143 °C, R_f = 0.33 (eluent: EtOAc/hexane, 70:30, v/v); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.38–8.36 (d, *J* = 8.6 Hz, 1H), 8.08–8.07 (d, *J* = 2.03 Hz, 1H), 8.00–7.98 (dd, *J* = 8.59, 2.04 Hz, 1H), 7.96–7.94 (m, 2H), 7.90–7.88 (m, 2H), 7.57–7.55 (m, 3H), 5.57 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 166.4, 156.9, 149.7, 133.8, 132.7, 132.6, 131.1, 128.6, 128.2, 127.8, 127.7, 126.5, 126.2, 126.0, 125.3, 115.5, 108.6, 70.5; IR (KBr) ν (cm^{-1}): 2931, 2862, 1671, 1610, 1531, 1449, 1349.

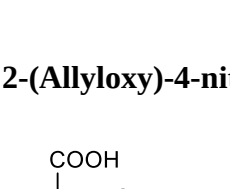
2-(Allyloxy)-4-nitrobenzoic acid (6b)



General procedure B, compound **6** (2 g, 8.43 mmol, 1 equiv.), $LiOH.H_2O$ (0.71 g, 16.86 mmol, 2 equiv.), THF-water (10 mL), white solid (1.86 g, 99%), m.p: 147–149 °C, R_f = 0.27 (eluent: EtOAc/hexane, 70:30, v/v); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.30–8.28 (d, *J* = 8.55 Hz, 1H), 7.95–7.93 (d, *J* = 8.59 Hz, 1H), 7.90 (s, 1H), 6.14–6.07 (ddt, *J* = 16.17, 10.65, 5.44 Hz, 1H), 5.59–5.56 (d, *J* = 17.21 Hz, 1H), 5.50–5.47 (d, *J* = 10.50 Hz, 1H), 4.88–4.87 (d, *J* = 5.27 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 166.5, 156.8, 149.8, 132.7, 131.1, 128.4, 117.6, 115.3, 108.4, 69.3; IR (KBr) ν (cm^{-1}): 2998, 2857, 1689, 1589, 1524, 1452, 1351.

Methyl 2-(allyloxy)-4-aminobenzoate (6a)

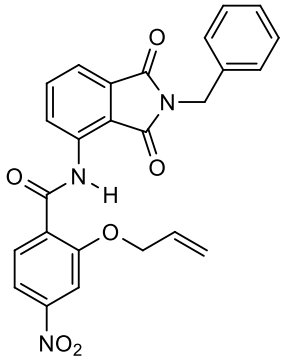
Following the reported procedure[6] compound **6** (1.5 g, 6.32 mmol, 1 equiv.) was dissolved in EtOAc (15 mL) and $SnCl_2.2H_2O$ (7.44 g, 31.62 mmol, 5 equiv.) was added to the substrate. The reaction mixture was stirred for 12 h at room temperature. After completion of the reaction the product was isolated and used for the next step reaction without purification. Light yellow liquid (1.27 g, 97%), R_f = 0.50 (eluent: EtOAc/hexane, 1:1, v/v); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.74 – 7.73 (d, *J* = 8.48 Hz, 1H), 6.23 – 6.21 (dd, *J* = 8.49 Hz, 2.12 Hz, 1H), 6.163 – 6.158 (d, *J* = 2.09 Hz, 1H), 6.09 – 6.01 (ddt, *J* = 17.20 Hz, 10.59 Hz, 4.72 Hz, 1H), 5.56 – 5.51 (dq, *J* = 17.23 Hz, 1.73 Hz, 1H), 5.30–5.27 (dq, *J* = 10.60 Hz, 1.55 Hz, 1H), 4.55–4.53 (dt, *J* = 4.64 Hz, 1.66 Hz, 2H), 4.08 (bs, 2H), 3.82 (s, 3H), ^{13}C NMR (125 MHz, $CDCl_3$) δ: 166.2, 160.6, 151.9, 134.21, 132.8, 117.1, 109.4, 106.7, 99.2, 69.2, 51.4; IR (KBr) ν (cm^{-1}): 3472, 3343, 3222, 3016, 2948, 1696, 1604, 1436, 1334,1256; MALDI-TOF: (matrix: DHB) calcd. for $C_{11}H_{13}NO_3Na$: 230.0788 $(M+Na)^+$, found: 230.5760; calcd. for: $C_{11}H_{13}NO_3K$: 246.0527$(M+K)^+$, found: 245.8064.



***N*-(2-Benzyl-1,3-dioxoisoindolin-4-yl)-2-(naphthalen-2-ylmethoxy)-4-nitrobenzamide (7)**

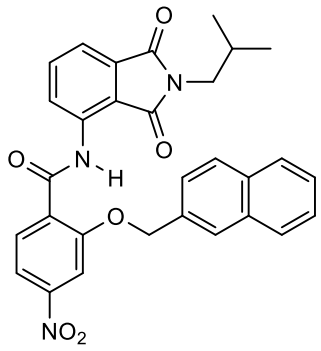
General procedure E; acid **5b** (2.0 g, 6.19 mmol, 1 equiv.), $SOCl_2$ (0.90 mL, 12.38 mmol, 2 equiv.), dry DMF (cat. amount), dry DCM (5 mL). After the removal of solvent and volatile impurities, the acid chloride was taken in dry DCM (10 mL). The solution was added dropwise to a stirred solution of amine **2a** (1.72 g, 6.80 mmol, 1.1 equiv.) and TEA (1.72 mL, 12.38 mmol, 2 equiv.) in dry DCM (20 mL) at 0 °C under nitrogen atmosphere and allowed to stir at rt for 12 h. Work up of the reaction as given in general procedure and purification by precipitation in EtOAc yielded a white solid (2.83 g, 82%), m.p: 206–208 °C, R_f = 0.49 (eluent: DCM/hexane, 50:50, v/v); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 11.75 (s, 1H), 9.08–9.06 (d, *J* = 8.42 Hz, 1H), 8.40–8.38 (d, *J* = 8.61 Hz, 1H), 8.01–7.99 (d, *J* = 9.44 Hz, 2H), 7.91–7.89 (d, *J* = 8.72 Hz, 1H), 7.83–7.78 (m, 3H), 7.76–7.72 (t, *J* = 7.89 Hz, 1H), 7.62–7.58 (m, 2H), 7.52–7.48 (m, 2H), 7.32–7.32 (m, 2H), 7.234–7.227 (m, 3H), 5.86 (s, 2H), 4.60 (s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 169.0, 167.4, 162.7, 156.7, 150.7, 136.8, 136.0, 135.9, 133.9, 133.2, 133.1, 132.3, 131.9, 128.9, 128.7, 128.5, 128.0, 127.9, 127.8, 127.1, 126.7, 126.6, 125.9, 125.0, 118.6, 116.9, 116.0, 108.8, 71.8, 41.3; IR (KBr) ν (cm^{-1}): 3274, 3114, 1761, 1701, 1613, 1533, 1475, 1400, 1345, 1111; MALDI-TOF calcd for $C_{33}H_{23}N_3O_6Na$: 580.1479 $(M+Na)^+$, found 580.0825.

2-(Allyloxy)-*N*-(2-benzyl-1,3-dioxoisoindolin-4-yl)-4-nitrobenzamide (8)



General procedure E; acid **6b** (3 g, 13.44 mmol, 1 equiv.), $SOCl_2$ (1.95 mL, 26.88 mmol, 2 equiv.), dry DMF (cat. amount), dry DCM (7 mL). After the removal of solvent and volatile impurities, the acid chloride was taken in dry DCM (15 mL). The solution was added dropwise to a stirred solution of amine **2a** (3.73 g, 14.78 mmol, 1.1 equiv.) and TEA (3.75 mL, 26.88 mmol, 2 equiv.) in dry DCM (30 mL) at 0 °C under nitrogen atmosphere. Allowed to stir at rt for 12 h. Work up of the reaction as given in general procedure and purification by precipitation from a solvent mixture Et_2O/EtOAc (1:9) yielded a white solid (5.3 g, 86%), m.p: 205–207 °C, R_f = 0.51 (eluent: DCM/hexane, 50:50, v/v);^{1}H NMR (500 MHz, $CDCl_3$) δ: 11.58 (s, 1H), 9.05–9.03 (d, *J* = 8.49 Hz, 1H), 8.40–8.38 (d, *J* = 8.40 Hz, 1H), 7.94–7.92 (m, 2H), 7.74–7.71 (t, *J* = 7.86 Hz, 1H), 7.59–7.58 (d, *J* = 7.30 Hz, 1H), 7.43–7.41 (m, 2H), 7.34–7.31 (t, *J* = 7.24 Hz, 2H), 7.30–7.28 (d, *J* = 7.12 Hz, 1H), 6.18–6.09 (m, 1H), 5.50–5.47 (d, *J* = 17.20 Hz, 1H), 5.38–5.36 (d, *J* = 10.45 Hz, 1H), 5.15–5.14 (d, *J* = 5.32 Hz, 2H), 4.84 (s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 169.1, 167.5, 162.7, 156.7, 150.8, 136.9, 136.1, 135.9, 133.8, 131.9, 131.4, 128.7, 128.5, 127.9, 127.0, 125.9, 120.2, 118.6, 116.9, 115.8, 108.4, 70.6, 41.5; IR (KBr) ν (cm^{-1}): 3321, 3094, 2927, 1760, 1692, 1617, 1545, 1475, 1399, 1346, 1108; ESI-MS calcd. for $C_{25}H_{20}N_3O_6$ m/z: 458.1347$[M+H]^+$, found: 458.1355.

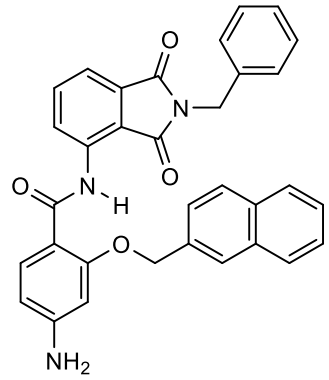
***N*-(2-isobutyl-1,3-dioxoisoindolin-4-yl)-2-(naphthalen-2-ylmethoxy)-4-nitrobenzamide (9)**



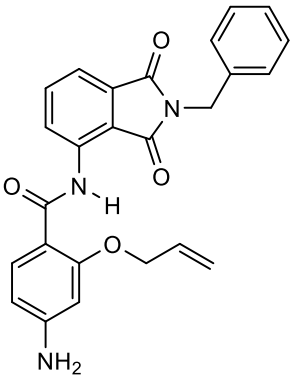
General procedure E; acid **5b** (0.83 g, 2.57 mmol, 1 equiv.), $SOCl_2$ (0.37 mL, 5.13 mmol, 2 equiv.), dry DMF (cat. amount), dry DCM (3 mL). After the removal of solvent and volatile impurities, the acid chloride was taken in dry DCM (5 mL). The solution was added dropwise to a stirred solution of amine **3a** (0.62 g, 2.83 mmol, 1.1 equiv.) and TEA (0.72 mL, 5.14 mmol, 2 equiv.) in dry DCM (10 mL) at 0 °C under nitrogen atmosphere. Allowed to stir at rt for 12 h. Work up of the reaction as given in general procedure and purification by precipitation from a solvent mixture Et_2O/EtOAc (1:1) afforded a white solid (1.2 g, 89%), m.p: 168–171 °C, R_f = 0.5 (eluent: DCM/ hexane, 50:50, v/v); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 11.76 (s, 1H), 9.09–9.07 (d, *J* = 8.5 Hz, 1H), 8.41–8.39 (d, *J* = 8.6 Hz, 1H), 8.011–8.007 (d, *J* = 2.0 Hz, 1H), 7.98 (s, 1H), 7.92–7.89 (dd, *J* = 8.6 Hz, 2.0 Hz, 1H), 7.82–7.72 (m, 4H), 7.61–7.58 (m, 2H), 7.51–7.47 (m, 2H), 5.84 (s, 2H), 3.28–3.27 (d, *J* = 7.4 Hz, 2H), 2.00–1.92 (tt, *J* = 13.8 Hz, 7.0 Hz, 1H), 0.85–0.84 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 169.6, 168.1, 162.7, 156.7, 150.7, 136.7, 135.7, 133.8, 133.25, 133.16, 132.3, 131.9, 128.9, 127.9, 127.8, 127.3, 127.1, 126.6, 125.8, 124.9, 118.5, 116.9, 116.0, 108.8, 71.8, 45.0, 27.8, 20.0; IR (KBr) ν (cm^{-1}): 3246, 3054, 2957, 1765, 1709, 1613, 1533, 1471, 1399, 1349; MALDI-TOF calcd. for $C_{30}H_{25}N_3O_6$: 523.1743 $(M)^+$; found: 523.5655; calcd. for $C_{30}H_{25}N_3O_6Na$: 546.1636 $(M+Na)^+$, found: 546.5092; calcd. for $C_{30}H_{25}N_3O_6K$: 562.1375 $(M+K)^+$, found: 562.6138.

4-Amino-*N*-(2-benzyl-1,3-dioxoisoindolin-4-yl)-2-(naphthalen-2-ylmethoxy)benzamide (7a)

General procedure C, nitro compound **7** (1.5 g, 2.69 mmol, 1 equiv.), $SnCl_2.2H_2O$ (3.03 g, 13.45 mmol, 5 equiv.), EtOAc (15 mL); off-white solid (1.32 g, 93%), m.p: 185–188 °C, R_f = 0.48 (eluent: methanol/DCM, 1:99, v/v); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 11.69 (s, 1H), 9.12–9.11 (d, *J* = 8.47 Hz, 1H), 8.07–8.05 (d, *J* = 8.53 Hz, 1H), 7.90 (s, 1H), 7.84–7.76 (m, 3H), 7.68–7.65 (t, *J* = 7.87 Hz, 1H), 7.58–7.56 (d, *J* = 8.30 Hz, 1H), 7.49–7.47 (m, 3H), 7.28 (m, 2H), 7.19 (m, 3H), 6.34–6.32 (d, *J* = 8.15 Hz, 1H), 6.23 (s, 1H), 5.73 (s, 2H), 4.56 (s, 2H), 3.99 (s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 168.8, 167.8, 164.8, 158.7, 151.9, 138.1, 136.3, 135.5, 134.4, 134.3, 133.2, 133.0, 131.9, 128.54, 128.47, 127.8, 127.72, 127.67, 126.3, 126.2, 125.9, 125.8, 124.6, 117.4, 116.1, 111.6, 108.1, 98.8, 70.7, 41.1; IR (KBr) ν (cm^{-1}): 3481, 3349, 3272, 3218, 3053, 1767, 1707, 1598, 1507, 1395, 1347; MALDI-TOF calcd. for $C_{33}H_{25}N_3O_4Na$: 550.1737 $(M+Na)^+$, found: 550.5296; calcd. for $C_{33}H_{25}N_3O_4K$: 566.1477 $(M+K)^+$, found: 566.5027.



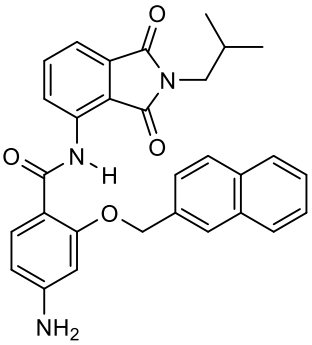
2-(Allyloxy)-4-amino-*N*-(2-benzyl-1,3-dioxoisoindolin-4-yl)benzamide (8a)



General procedure C; nitro compound **8** (2.0 g, 4.37 mmol, 1 equiv.), $SnCl_2.2H_2O$ (4.93 g, 21.86 mmol, 5 equiv.), EtOAc (20 mL); off-white solid (1.78 g, 95%), m.p: 187–189 °C, R_f = 0.46 (eluent: methanol/DCM, 1:99, v/v); ^{1}H NMR (500 MHz, $CDCl_3$) δ:11.49 (s, 1H), 9.08–9.06 (d, *J* = 8.50 Hz, 1H), 8.05–8.03 (d, *J* = 8.52 Hz, 1H), 7.66–7.63 (t, *J* = 7.86 Hz, 1H), 7.49–7.48 (d, *J* = 7.10 Hz, 1H), 7.43–7.41 (d, *J* = 7.37 Hz, 2H), 7.33–7.28 (m, 3H), 6.36–6.35 (d, *J* = 8.50 Hz, 1H), 6.24 (s, 1H), 6.16–6.08 (ddt, *J* = 16.17 Hz, 10.71 Hz, 5.47 Hz, 1H), 5.40–5.36 (d, *J* = 17.2 Hz, 1H), 5.27–5.25 (d, *J* = 10.56 Hz, 1H), 4.99–4.98 (d, *J* = 5.07 Hz, 2H), 4.82 (s, 2H), 4.10 (br, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 168.9, 167.8, 164.8, 158.5, 151.9, 138.0, 136.4, 135.4, 134.4, 132.9, 131.8, 128.6, 128.4, 127.7, 126.0, 118.3, 117.3, 116.1, 111.3, 107.8, 98.2, 69.5, 41.3; IR (KBr) ν (cm^{-1}): 3447, 3339, 3269, 1760, 1705, 1602, 1530, 1399, 1332; MALDI-TOF calcd. for $C_{25}H_{21}N_3O_4Na$: 450.1424 $(M+Na)^+$, found: 450.5577; calcd. for: $C_{25}H_{21}N_3O_4K$: 466.1164 $(M+K)^+$; found: 466.4931.

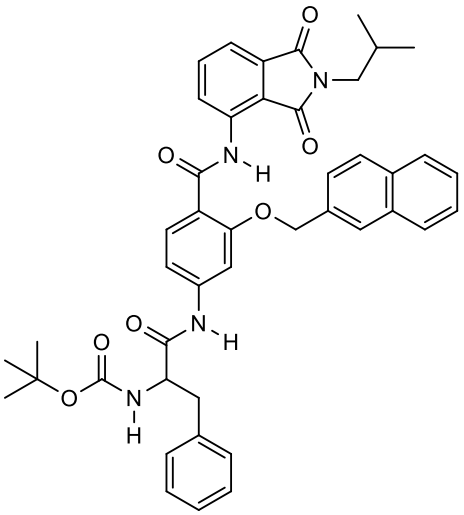
4-Amino-*N*-(2-isobutyl-1,3-dioxoisoindolin-4-yl)-2-(naphthalen-2-ylmethoxy)benzamide (9a)

General procedure C, compound **9** (1.1 g, 2.10 mmol, 1 equiv.), $SnCl_2.2H_2O$ (2.37 g, 10.50 mmol, 5 equiv.), EtOAc (10 mL); off-white solid (0.98 g, 95%), m.p: 116–118 °C, R_f = 0.51 (eluent: methanol/DCM, 1:99, v/v); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 11.67 (s, 1H), 9.13–9.12 (d, *J* = 8.45 Hz, 1H), 8.08–8.06 (d, *J* = 8.55 Hz, 1H), 7.89 (s, 1H), 7.82–7.75 (m, 4H), 7.69–7.66 (m, 1H), 7.57–7.55 (dd, *J* = 8.48, 1.67 Hz, 1H), 7.49 – 7.45 (m, 3H), 6.35–6.33 (dd, *J* =2.10 Hz, 8.56 Hz, 1H), 6.24–6.23 (d, *J* = 2.07 Hz, 1H), 5.71 (s, 2H), 3.98 (s, 2H), 3.24–3.22 (d, *J* = 7.39 Hz, 2H), 1.97–1.89 (m, 1H), 0.82–0.81 (d, *J* = 6.71 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 169.4, 168.4, 164.9, 158.7, 151.8, 137.9, 135.3, 134.4, 134.3, 133.2, 133.0, 131.8, 128.5, 127.8, 127.7, 126.3, 126.1, 125.81, 125.79, 124.6, 117.2, 116.1, 111.7, 108.1, 98.9, 70.7, 44.8, 27.7, 20.0; IR (KBr) ν (cm^{-1}): 3590, 3463, 3356, 3266, 2985, 1762, 1705,



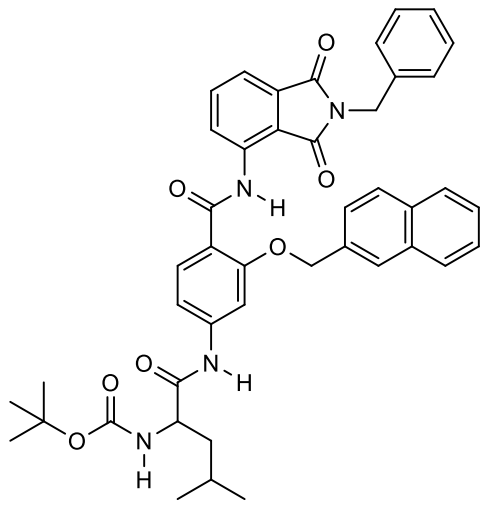
1601, 1530, 1401, 1345; MALDI-TOF calcd. for $C_{30}H_{27}N_3O_4Na$: 516.1894 $(M+Na)^+$, found 516.9026; calcd. for $C_{30}H_{27}N_3O_4K$: 532.1633 $(M+K)^+$, found: 532.8954.

***Tert*-butyl(1-((4-((2-isobutyl-1,3-dioxoisoindolin-4-yl)carbamoyl)-3-(naphthalen-2-ylmethoxy)phenyl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (10)**



General procedure F, amine **9a** (0.50 g, 1.01 mmol, 1 equiv.), Boc-Phe-OH (0.32 g, 1.21 mmol, 1.2 equiv.), PyBOP (0.79 g, 1.51 mmol, 1.5 equiv.), DCM (5 mL), DIPEA (0.26 mL, 1.51 mmol, 1.5 equiv.); white solid (0.61 g, 81%), m.p: 200–203 °C, R_f = 0.36 (eluent: methanol/DCM, 3:97, v/v); ^{1}H NMR (500 MHz, $CDCl_3$) δ:11.73 (s, 1H), 9.10–9.08 (d, *J* = 8.36 Hz, 1H), 8.20 (br, 1H), 8.13–8.11 (d, *J* = 7.92 Hz, 1H), 8.00 (s, 1H), 7.83–7.78 (m, 4H), 7.68–7.65 (m, 1H), 7.63–7.61 (d, *J* = 8.16 Hz, 1H), 7.50–7.46 (m, 3H), 7.19–7.16 (m, 5H), 6.68–6.66 (d, *J* = 8.67 Hz, 1H), 5.79–5.70 (m, 2H), 5.08 (br, 1H), 4.44 (br, 1H), 3.24–3.23 (d, *J* = 6.25 Hz, 2H), 3.12–3.11 (d, *J* = 4.55 Hz, 2H), 1.94 (m, 1H), 1.39 (s, 9H), 0.83–0.82 (d, *J* = 6.08 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 117.0, 169.4, 168.3, 164.2, 157.47, 157.46, 142.4, 137.5, 136.2, 135.4, 133.6, 133.21, 133.18, 133.1, 131.9, 129.2, 128.8, 128.3, 128.0, 127.7, 127.2, 126.24, 126.20, 125.8, 125.4, 117.6, 117.2, 116.4, 111.8, 104.3, 81.0, 70.9, 44.9, 37.9, 29.7, 28.2, 27.7, 20.0; IR (KBr) ν (cm^{-1}): 3264, 3120, 3052, 2965, 1768, 1709, 1666, 1596, 1532, 1431, 1347; MALDI-TOF calcd. for $C_{44}H_{44}N_4O_7Na$: 763.3102 $(M+Na)^+$, found: 763.5829; calcd for $C_{44}H_{44}N_4O_7K$: 779.2842 $(M+K)^+$, found: 779.6258.

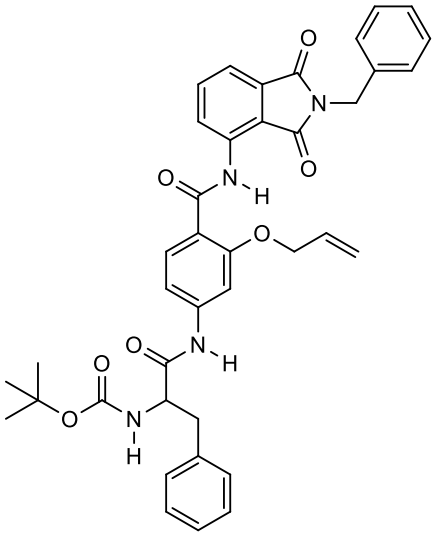
***Tert*-butyl(1-((4-((2-benzyl-1,3-dioxoisoindolin-4-yl)carbamoyl)-3-(naphthalen-2-ylmethoxy)phenyl)amino)-4-methyl-1-oxopentan-2-yl)carbamate (11)**



General procedure F, amine **7a** (0.50 g, 0.95 mmol, 1 equiv.), Boc-Leu-OH.H_2O (0.28 g, 1.14 mmol, 1.2 equiv.), PyBOP (0.74 g, 1.42 mmol, 1.5 equiv.), DCM (5 mL), DIPEA (0.25 mL, 1.42 mmol, 1.5 equiv.); white solid (0.60 g, 85%), m.p: 216–218 °C, R_f = 0.45 (eluent: methanol/DCM, 3:97, v/v); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 11.72 (s, 1H), 9.15 (s, 1H), 9.00–8.99 (d, *J* = 6.25 Hz, 1H), 8.08–8.06 (d, *J* = 8.27 Hz, 1H), 8.02 (s, 1H), 7.95 (s, 1H), 7.78–7.72 (m, 3H), 7.64–7.63 (d, *J* = 8.44 Hz, 1H), 7.46–7.41 (m, 2H), 7.32 (m, 4H), 7.20 (m, 3H), 6.62–6.60 (d, *J* = 7.38 Hz, 1H), 5.87–5.84 (d, *J* = 13.26 Hz, 1H), 5.64–5.62 (d, *J* = 12.93 Hz, 1H), 5.08–5.07 (d, *J* = 7.16 Hz, 1H), 4.56–4.53 (d, *J* = 14.46 Hz, 1H), 4.50–4.47 (d, *J* = 14.53 Hz, 1H), 4.34 (br, 1H), 1.78–1.72 (m, 1H), 1.65–1.59 (m, 2H), 1.43 (s, 9H), 0.95–0.94 (d, *J* = 6.38 Hz, 3H), 0.92–0.90 (d, *J* = 6.25 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 171.7, 168.4, 167.4, 164.1, 157.2, 156.7, 143.1, 137.6, 136.3, 135.1, 133.8, 133.2, 133.0, 132.9, 131.7, 128.8, 128.6, 128.2, 128.1, 127.8, 127.6, 127.5, 126.02, 125.99, 125.8, 125.7, 117.3, 116.1, 111.5, 104.0, 81.0, 70.5, 54.0, 41.1, 40.4, 28.3, 24.7, 23.1, 21.4; IR (KBr) ν (cm^{-1}): 3299, 3113, 3054, 2957, 1762, 1704, 1667, 1602, 1505, 1427, 1396;

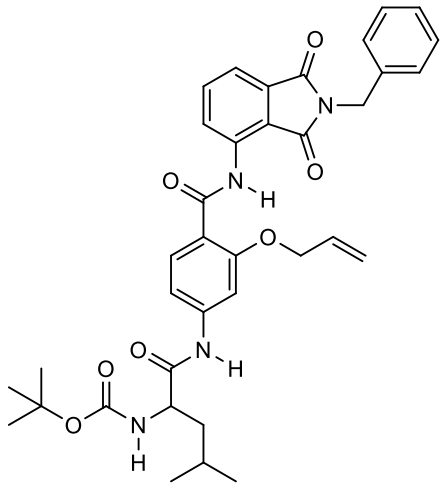
MALDI-TOF calcd. for $C_{44}H_{44}N_4O_7Na$: 763.3102 $(M+Na)^+$, found: 763.4630; calcd. for $C_{44}H_{44}N_4O_7K$: 779.2842 $(M+K)^+$, found: 779.5673.

Tert-butyl(1-((3-(allyloxy)-4-((2-benzyl-1,3-dioxoisoindolin-4-yl) carbamoyl) phenyl) amino)-1-oxo-3-phenylpropan-2-yl) carbamate (12)



This product was prepared either through general procedure F, amine **8a** (0.50 g, 1.17 mmol, 1 equiv.), Boc-Phe-OH (0.37 g, 1.40 mmol, 1.2 equiv.), PyBOP (0.91 g, 1.75 mmol, 1.5 equiv.), DIPEA (0.31 mL, 1.75 mmol, 1.5 equiv.), DCM (5 mL) white solid (0.65 g, 82%); or through general procedure H, acid **15b** (0.50 g, 1.14 mmol, 1 equiv.), Ghosez's reagent (0.23 mL, 1.71 mmol, 1.5 equiv.), amine (0.34 g, 1.37 mmol, 1.2 equiv.), DIPEA (0.30 mL, 1.71 mmol, 1.5 equiv.), DCM (5 mL); white solid (0.61 g, 80%), m.p: 184–186 °C, R_f = 0.51 (eluent: methanol/DCM, 3:97, v/v); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 11.55 (s, 1H), 9.02–9.00 (dd, *J* = 8.39 Hz, 3.78 Hz, 1H), 8.38 (br, 1H), 8.11–8.09 (dd, *J* = 8.52 Hz, 1.63 Hz, 1H), 7.73 (s, 1H), 7.53 (br, 1H), 7.46–7.43 (m, 3H), 7.35–7.30 (m, 4H), 7.29–7.27 (m, 2H), 7.25–7.23 (m, 2H), 6.68–6.66 (d, *J* = 8.20 Hz, 1H), 6.18–6.11 (ddd, *J* = 22.90 Hz, 10.95 Hz, 5.65 Hz, 1H), 5.48–5.44 (dd, *J* = 17.29 Hz, 1.37 Hz, 1H), 5.32–5.30 (dd, *J* = 10.49 Hz, 1.21 Hz, 1H), 5.14 (br, 1H), 5.08–4.99 (m, 2H), 4.81 (s, 2H), 4.54 (s, 1H), 3.21–3.09 (ddd, *J* = 22.67 Hz, 14.10 Hz, 7.19 Hz, 2H), 1.44 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.2 (d, *J* = 3.8 Hz), 168.8 (d, *J* = 2.7 Hz), 167.7 (d, *J* = 1.2 Hz), 164.1, 157.4, 156.1, 142.4, 137.6, 136.35, 136.25, 135.4 (d, *J* = 2.0 Hz), 133.1, 132.4, 131.9, 129.2, 128.9, 128.7, 127.9, 127.2, 125.9, 119.3, 117.7, 116.9, 116.4 (d, *J* = 2.0 Hz), 111.7 (d, *J* = 2.5 Hz), 104.0, 81.1, 69.8, 56.7, 41.4, 37.9, 28.3; IR (KBr) ν (cm^{-1}): 3274, 3119, 3054, 2978, 2927, 1759, 1710, 1664, 1598, 1535, 1430, 1399; MALDI-TOF: (matrix: DHB) calcd. For $C_{39}H_{38}N_4O_7Na$: 697.2633 $(M+Na)^+$; found: 697.4235; calcd. for $C_{39}H_{38}N_4O_7K$: 713.2372 $(M+K)^+$; found: 713.4119.

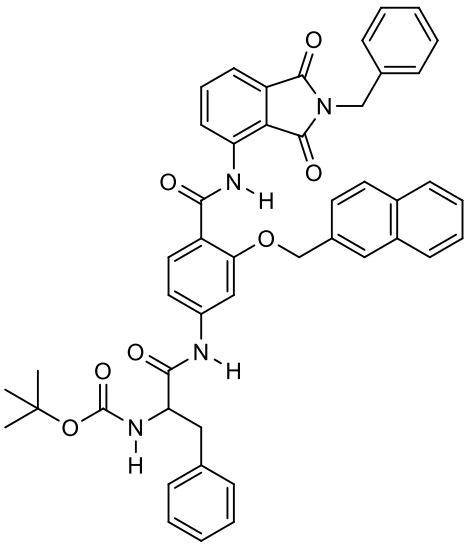
Tert-butyl(1-((3-(allyloxy)-4-((2-benzyl-1,3-dioxoisoindolin-4-yl)carbamoyl)phenyl) amino)-4-methyl-1-oxopentan-2-yl)carbamate (13)



General procedure F, amine **8a** (0.50 g, 1.17 mmol, 1 equiv.), Boc-Leu-OH.H_2O (0.35 g, 1.40 mmol, 1.2 equiv.), PyBOP (0.91 g, 1.75 mmol, 1.5 equiv.), DCM (5 mL), DIPEA (0.31 mL, 1.75 mmol, 1.5 equiv.); white solid (0.66 g, 88%), m.p: 151–154 °C, R_f = 0.46 (eluent: methanol/DCM, 3:97, v/v); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 11.49 (s, 1H), 10.34$_{rotamer}$ (0.9 H), 10.24 $_{rotamer}$ (0.1 H), 8.96–8.95 (d, *J* = 8.63 Hz, 1H), 8.04–8.02 (dd, *J* = 8.67 Hz, 1.32 Hz, 1H), 7.84–7.81 (t, *J* = 7.94 Hz, 1H), 7.68 (s, 1H), 7.58 (dd, *J* = 7.27 Hz, 0.58 Hz, 1H), 7.344–7.336 (m, 5H), 7.29–7.27 (m, 1H), 7.15–7.13 (d, *J* = 6.56 Hz, 1H), 6.15–6.08 (qd, *J* = 10.21 Hz, 5.03 Hz, 1H), 5.41–5.38 (d, *J* = 17.28 Hz, 1H), 5.27–5.25 (d, *J* = 10.47 Hz, 1H), 5.00–4.99 (d, *J* = 4.82 Hz, 2H), 4.77 (s, 2H), 4.17–4.13 (t, *J* = 10.78 Hz, 1H), 1.69 – 1.52 (m, 2H), 1.44 – 1.38

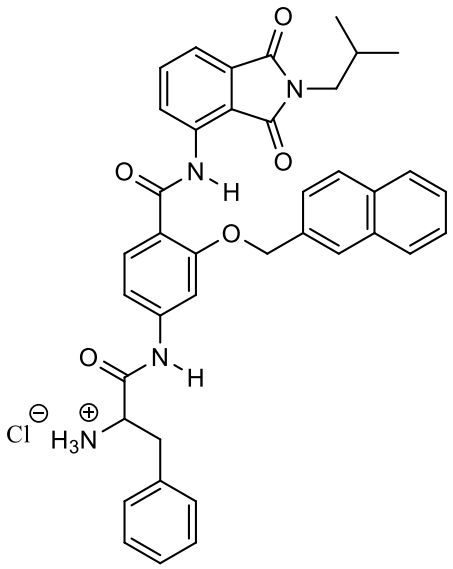
(m, 1H), 1.38$_{rotamer}$ (8H), 1.30 $_{rotamer}$ (1H), 0.91–0.89 (d, *J* = 6.26 Hz, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ:172.6, 168.7, 167.3, 163.4, 156.8, 155.5, 144.4, 136.8, 136.5, 136.0, 132.7, 132.5, 131.8, 128.6, 127.4, 125.1, 119.1, 117.7, 116.3, 115.1, 111.6, 103.5, 78.1, 69.3, 53.7, 40.8, 40.4, 28.18, 24.3, 22.9, 21.4; IR (KBr) ν (cm^{-1}): 3285, 2959, 1765, 1707, 1671, 1601, 1532, 1399, 1349; MALDI-TOF calcd. for $C_{36}H_{40}N_4O_7Na$: 663.2789 $(M+Na)^+$, found 663.0743; calcd for $C_{36}H_{40}N_4O_7K$: 679.2529 $(M+K)^+$, found: 679.1300.

Tert-butyl(1-((4-((2-benzyl-1,3-dioxoisoindolin-4-yl)carbamoyl)-3-(naphthalen-2-ylmethoxy)phenyl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (14)



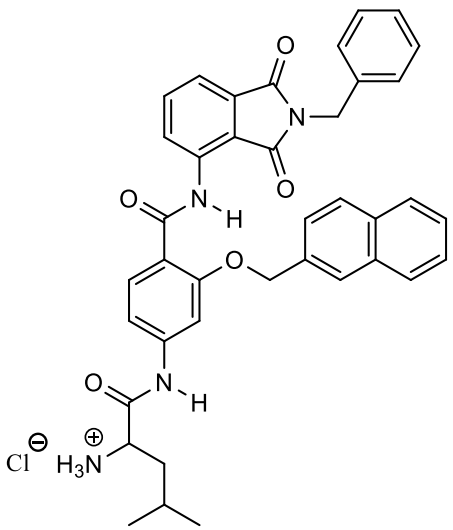
General procedure F, amine **7a** (0.50 g, 0.95 mmol, 1 equiv.), Boc-Phe-OH (0.30 g, 1.14 mmol, 1.2 equiv.), PyBOP (0.74 g, 1.42 mmol, 1.5 equiv.), DCM (5 mL), DIPEA (0.25 mL, 1.42 mmol, 1.5 equiv.); off-white solid (0.60 g, 82%), m.p: 231–233 °C, R_f = 0.32 (eluent: methanol/DCM, 3:97, v/v); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 11.74 (s, 1H), 9.08–9.07 (d, *J* = 8.50 Hz, 1H), 8.16 (br, 1H), 8.12–8.11 (d, *J* = 8.55 Hz, 1H), 8.02 (s, 1H), 7.84–7.76 (m, 4H), 7.64–7.62 (m, 2H), 7.49–7.44 (m, 3H), 7.33–7.31 (m, 2H), 7.22–7.16 (m, 7H), 6.67–6.65 (d, *J* = 8.17 Hz, 1H), 5.82–5.73 (dd, *J* = 32.22 Hz, 13.32 Hz, 2H), 5.07 (br, 1H), 4.57 (s, 2H), 4.44 (br, 1H), 3.12–3.10 (d, *J* = 6.65 Hz, 2H), 1.39 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.1, 168.7, 167.6, 164.2, 157.4, 142.4, 137.6, 136.2, 136.2, 135.5, 135.4, 133.6, 133.2, 133.1, 131.9, 129.1, 128.8, 128.6, 128.3, 128.0, 127.8, 127.7, 127.3, 127.1, 126.1, 125.9, 125.5, 117.7, 117.0, 116.4, 111.8, 104.3, 81.0, 70.9, 59.5, 41.2, 37.9, 28.2; IR (KBr) ν (cm^{-1}): 3272, 3058, 2921, 2852, 1765, 1710, 1667, 1600, 1532, 1398; MALDI-TOF calcd. for $C_{47}H_{42}N_4O_7Na$: 797.2946 $(M+Na)^+$, found: 797.0976; calcd. for $C_{47}H_{42}N_4O_7K$: 813.2685 $(M+K)^+$, found: 813.0001.

1-((4-((2-isobutyl-1,3-dioxoisoindolin-4-yl)carbamoyl)-3-(naphthalen-2-ylmethoxy)phenyl)amino)-1-oxo-3-phenylpropan-2-aminium chloride (SK17)



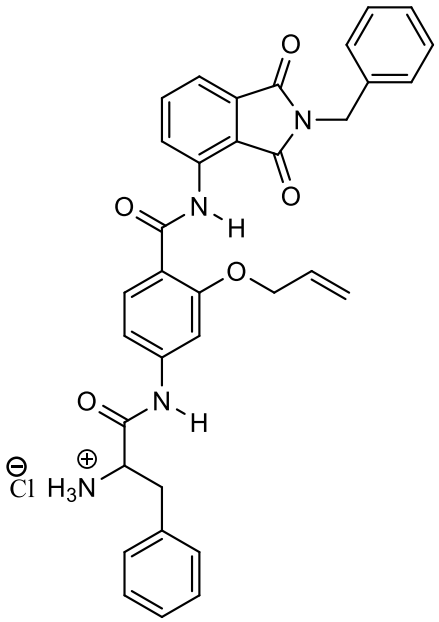
General procedure G, compound **10** (50 mg, 0.067 mmol, 1equiv.), saturated HCl in ACN (4 ml), purified through RP-HPLC chromatography, white solid (27 mg, 60%); ^{1}H NMR (500 MHz, Methanol-d_4) δ: 9.05–9.03 (d, *J* = 8.51 Hz, 1H), 8.91 (bs, 3H), 8.37 (bs, 3H), 8.09–8.08 (d, *J* = 8.63 Hz, 1H), 8.03 (s, 1H), 7.81–7.78 (m, 5H), 7.64–7.62 (dd, *J* = 8.43 Hz, 1.29 Hz, 1H), 7.54–7.53 (d, *J* = 7.28 Hz, 1H), 7.46–7.44 (m, 2H), 7.20–7.15 (m, 5H), 7.11–7.09 (dd, *J* = 8.66 Hz, 1.73 Hz, 1H), 5.83–5.76 (q, *J* = 13.34 Hz, 2H), 4.23–4.20 (t, *J* = 7.33 Hz, 1H), 3.26–3.22 (q, *J* = 6.86 Hz, 3H), 3.16–3.12 (dd, *J* = 13.75 Hz, 7.61 Hz, 1H), 1.92–1.85 (m, 1H), 0.81–0.80 (d, *J* = 6.70 Hz, 6H). MALDI-TOF calcd. for $C_{39}H_{36}N_4O_5Na$: 663.2578 $(M+Na)^+$, found: 663.0743; calcd. for $C_{39}H_{36}N_4O_5K$: 679.2317 $(M+K)^+$, found: 679.8084.

1-((4-((2-benzyl-1,3-dioxoisoindolin-4-yl)carbamoyl)-3-(naphthalen-2-ylmethoxy)phenyl)amino)-4-methyl-1-oxopentan-2-aminium chloride (SK18)



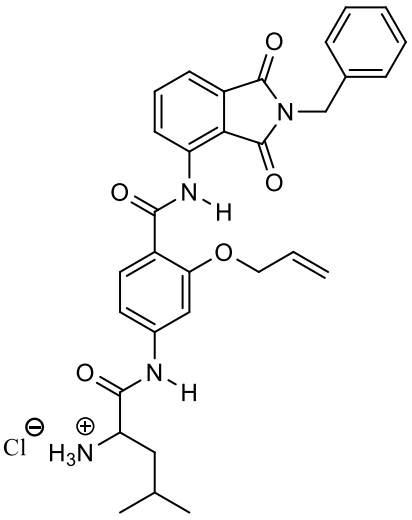
General procedure G, compound **11 (**50 mg, 0.067 mmol, 1equiv.), saturated HCl in ACN (4 ml), purified through RP-HPLC chromatography, off white solid (43 mg, 95%); ^{1}H NMR (500 MHz, Methanol-d_4) δ: 9.05–9.04 (d, *J* = 8.45 Hz, 1H), 8.13–8.12 (d, *J* = 8.62 Hz, 1H), 8.01 (s, 1H), 7.93–7.92 (d, *J* = 1.80 Hz, 1H), 7.82–7.74 (m, 4H), 7.63–7.61 (d, *J* =8.52 Hz, 1H), 7.56–7.54 (d, *J* = 7.23 Hz, 1H), 7.49–7.42 (m, 2H), 7.25–7.17 (m, 6H), 5.82 (s, 2H), 4.57 (s, 2H), 3.99–3.97 (t, *J* = 7.55 Hz, 1H), 1.77–1.74 (m, 2H), 0.98–0.97 (d, *J* = 6.38 Hz, 6H). MALDI-TOF calcd. for $C_{39}H_{36}N_4O_5Na$: 663.2578 $(M+Na)^+$, found: 663.5347; calcd. for $C_{39}H_{36}N_4O_5K$: 679.2317 $(M+K)^+$, found: 679.7514.

1-((3-(allyloxy)-4-((2-benzyl-1,3-dioxoisoindolin-4-yl)carbamoyl)phenyl)amino)-1-oxo-3-phenylpropan-2-aminium chloride (SK19)



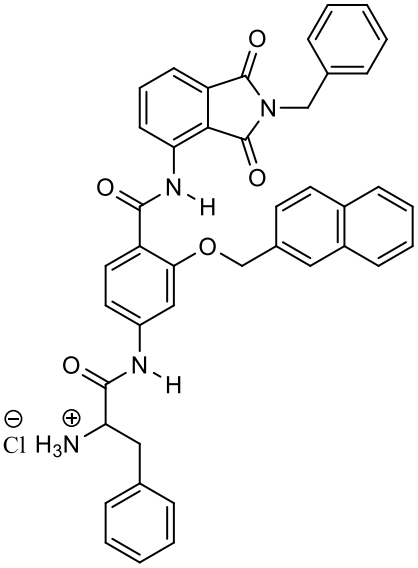
General procedure G, compound **12 (**50 mg, 0.074 mmol, 1equiv.), saturated HCl in ACN (4 ml), purified through precipitation using DCM solvent, white solid (44 mg, 98%); ^{1}H NMR (500 MHz, Methanol-d_4) δ: 8.99–8.97 (d, *J* = 8.47 Hz, 1H), 8.06–8.04 (d, *J* = 8.59 Hz, 1H), 7.72–7.69 (m, 1H), 7.66 (s, 1H), 7.52–7.50 (d, *J* = 7.21 Hz, 1H), 7.38–7.24 (m, 10H), 7.19–7.17 (d, *J* = 8.54 Hz, 1H), 6.16–6.08 (ddd, *J* = 22.55 Hz, 10.69 Hz, 5.47 Hz, 1H), 5.46–5.42 (d, *J* = 17.33 Hz, 1H), 5.29–5.27 (d, *J* = 10.36 Hz, 1H), 5.06–5.05 (d, *J* = 5.28 Hz, 2H), 4.81 (s, 2H), 4.32–4.30 (t, *J* = 6.95 Hz, 1H), 3.21–3.27 (dd, *J* = 13.86, 7.87 Hz, 2H). MALDI-TOF calcd. for $C_{34}H_{30}N_4O_5Na$: 597.2108 $(M+Na)^+$, found: 597.6642; calcd. for $C_{34}H_{30}N_4O_5K$: 613.1848 $(M+K)^+$, found: 613.6924.

1-((3-(allyloxy)-4-((2-benzyl-1,3-dioxoisoindolin-4yl)carbamoyl)phenyl)amino)-4-methyl-1-oxopentan-2-aminium chloride (SK20)



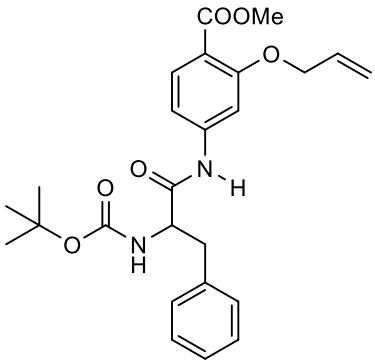
General procedure G, compound **13 (**50 mg, 0.078 mmol, 1equiv.), saturated HCl in ACN (4 ml), purified through precipitation using DCM solvent, white solid (45 mg, 100%); ^{1}H NMR (500 MHz, Methanol-d$_4$) δ: 9.01–9.00 (d, *J* = 8.54 Hz, 1H), 8.11–8.10 (d, *J* = 8.64 Hz, 1H), 7.79–7.74 (m, 2H), 7.56–7.54 (d, *J* = 7.30 Hz, 1H), 7.39–7.37 (d, *J* = 7.14 Hz, 2H), 7.34–7.25 (m, 4H), 6.18–6.10 (ddd, *J* = 22.66 Hz, 10.74 Hz, 5.53 Hz, 1H), 5.47–5.43 (d, *J* = 17.35 Hz, 1H), 5.28–5.26 (d, *J* = 10.51 Hz, 1H), 5.08–5.07 (d, *J* = 5.59 Hz, 2H), 4.82 (s, 2H), 4.11–4.08 (m, 1H), 1.84–1.81 (m, 2H), 1.31–1.28 (d, *J* = 5.67 Hz, 1H), 1.06–1.04 (dd, *J* = 6.25 Hz, 2.82 Hz, 6H). MALDI-TOF calcd. for $C_{31}H_{32}N_4O_5Na$: 563.2265 $(M+Na)^+$, found: 563.7005; calcd. for $C_{31}H_{32}N_4O_5K$: 579.2004 $(M+K)^+$, found: 579.7956.

1-((4-((2-benzyl-1,3-dioxoisoindolin-4-yl)carbamoyl)-3-(naphthalen-2-ylmethoxy)phenyl)amino)-1-oxo-3-phenylpropan-2-aminium chloride (SK21)



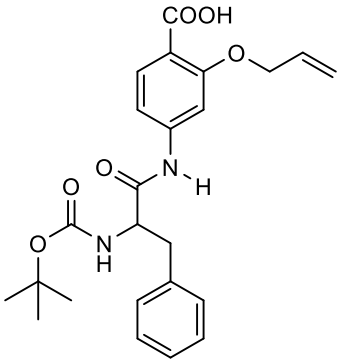
General procedure G, compound **14 (**50 mg, 0.064 mmol, 1equiv.), saturated HCl in ACN (4 ml), purified through RP-HPLC chromatography, yellowish white solid (32 mg, 70%); ^{1}H NMR (500 MHz, Methanol-d$_4$) δ: 9.04–9.0 (d, *J* = 8.49 Hz, 1H), 8. 09–8.08 (d, *J* = 8.61 Hz, 1H), 8.00 (s, 1H), 7.84–7.74 (m, 5H), 7.63–7.61 (d, *J* = 8.42 Hz, 1H), 7.55–7.53 (d, *J* = 7.27 Hz, 1H), 7.49–7.44 (m, 2H), 7.33–7.25 (m, 3H), 7.18–7.16 (m, 7H), 7.01–7.00 (d, *J* = 8.66 Hz, 1H), 5.85–5.78 (q, *J* = 13.49 Hz, 2H), 4.59 (s, 2H), 4.11–4.08 (t, *J* = 7.25 Hz, 1H), 3.23–3.18 (dd, 13.84 Hz, 7.33 Hz, 1H), 3.12–3.07 (dd, 13.73 Hz, 7.44 Hz, 1H). MALDI-TOF calcd. for $C_{42}H_{34}N_4O_5Na$: 697.2421 $(M+Na)^+$, found: 697.4235; calcd. for $C_{31}H_{32}N_4O_5K$: 713.2161 $(M+K)^+$, found 713.5711.

Methyl-2-(allyloxy)-4-(2-((*tert*-butoxycarbonyl)amino)-3-phenylpropanamido)benzoate (15)



Compound **6a** (0.8 g, 3.86 mmol, 1 equiv.) was added to a solution containing Boc-Phe-OH (1.23 g, 4.63 mmol, 1.2 equiv.), PyBOP (3.01 g, 5.79 mmol, 1.5 equiv.) and *N*,*N*-diisopropylethylamine (DIPEA) (1 mL, 5.79 mmol, 1.5 equiv.) in dry DCM (10 mL) at 0 °C under nitrogen atmosphere. After 30 min reaction, the mixture was allowed to stir at room temperature for 12 h. After completion, DCM (20 mL) was added, and the reaction mixture was washed with 2N $KHSO_4$ (50 mL×2) solution followed by saturated $NaHCO_3$ (50 mL×2) solution and brine (50 mL). The organic layer was dried over anhydrous Na_2SO_4, concentrated to obtain desired yield and the product obtained in this step was further purified through column chromatography. Puffy white solid (1.65 g, 94%), m.p: 75–77 °C; R_f = 0.40 (eluent: EtOAc/hexane, 2:3, v/v); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.61 (s, 1H), 7.73–7.71 (d, *J* = 8.42 Hz, 1H), 7.38 (s, 1H), 7.29–7.17 (m, 5H), 6.73–6.71 (d, *J* = 8.31 Hz, 1H), 6.07–5.99 (ddd, *J* = 15.40 Hz, 10.12 Hz, 4.84 Hz, 1H), 5.54–5.51 (dd, *J* = 17.22 Hz, 1.56 Hz, 1H), 5.31–5.27 (m, 2H), 4.54 (s, 3H), 3.85 (s, 3H), 3.18–3.03 (m, 2H), 1.39 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.3, 166.0, 159.2, 156.1, 142.4, 136.3, 132.8, 132.4, 129.3, 129.1, 128.8, 128.5, 127.1, 117.4, 115.3, 110.8, 104.4, 80.8, 69.3, 56.7, 51.7, 38.2, 28.2; IR (KBr) ν (cm^{-1}): 3534, 3311, 2980, 1701, 1674, 1597, 1538, 1415, 1251; MALDI-TOF calcd. for $C_{25}H_{30}N_2O_6Na$: 477.1996 $(M+Na)^+$, found: 477.2279; calcd. for $C_{25}H_{30}N_2O_6K$: 493.1735 $(M+K)^+$, found: 493.2500.

4-(2-((*tert*-butoxycarbonyl)amino)-3-phenylpropanamido)-2-(naphthalen-2-ylmethoxy)benzoic acid (15b)



General procedure B, compound **15** (1 g, 2.20 mmol, 1 equiv.), $LiOH.H_2O$ (184.9 mg, 4.40 mmol, 2 equiv.), THF-water (8 mL), white solid (1.86 g, 99%), m.p: 171–173 °C, R_f = 0.27 (eluent: EtOAc/hexane, 70:30, v/v); ^{1}H NMR (500 MHz, DMSO-d_6) δ: $10.31_{rotamer}$ (0.8H), $10.24_{rotamer}$ (0.2H), 7.70–7.69 (d, *J* = 8.50 Hz, 1H), 7.43 (s, 1H), 7.33–7.18 (m, 7H), 6.09–6.01 (ddd, *J* = 15.25 Hz, 10.03 Hz, 4.58 Hz, 1H), 5.54–5.50 (d, *J* = 17.23 Hz, 1H), 5.28 –5.26 (d, *J* = 11.63 Hz, 1H), 4.59–4.58 (d, *J* = 4.05 Hz, 2H), 4.34–4.30 (td, *J* = 9.85 Hz, 4.63 Hz, 1H), 3.00–2.80 (m, 2H), 1.31 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 171.5, 166.6, 158.3, 155.5, 143.5, 138.1, 137.9, 133.2, 132.4, 129.3, 128.2, 128.1, 126.4, 117.2, 115.3, 110.7, 104.0, 78.2, 68.6, 56.7, 37.3, 28.19; IR (KBr) ν (cm^{-1}): 3304, 3196, 2982, 1279, 1665, 1602, 1537, 1401, 1235; MALDI-TOF calcd. for $C_{24}H_{28}N_2O_6Na$: 463.1840 $(M+Na)^+$; found: 463.2943; calcd. for: $C_{24}H_{28}N_2O_6K$: 479.1579 $(M+K)^+$, found: 479.3070

3. Fluorescence spectrophotometric data

a) Fluorescence data

Fluorescence data were obtained using SHIMADZU RF-6000 spectrofluorophotometer. Stock solution (1 mM) was prepared by dissolving the required amount of compound in MeOH or DMSO. The dilutions were done with the solvents. Measurements were done with 2 mL standard quartz cuvette, stepping rate = 1 nm, continuous scanning, excitation and emission band width = 5.0 nm.

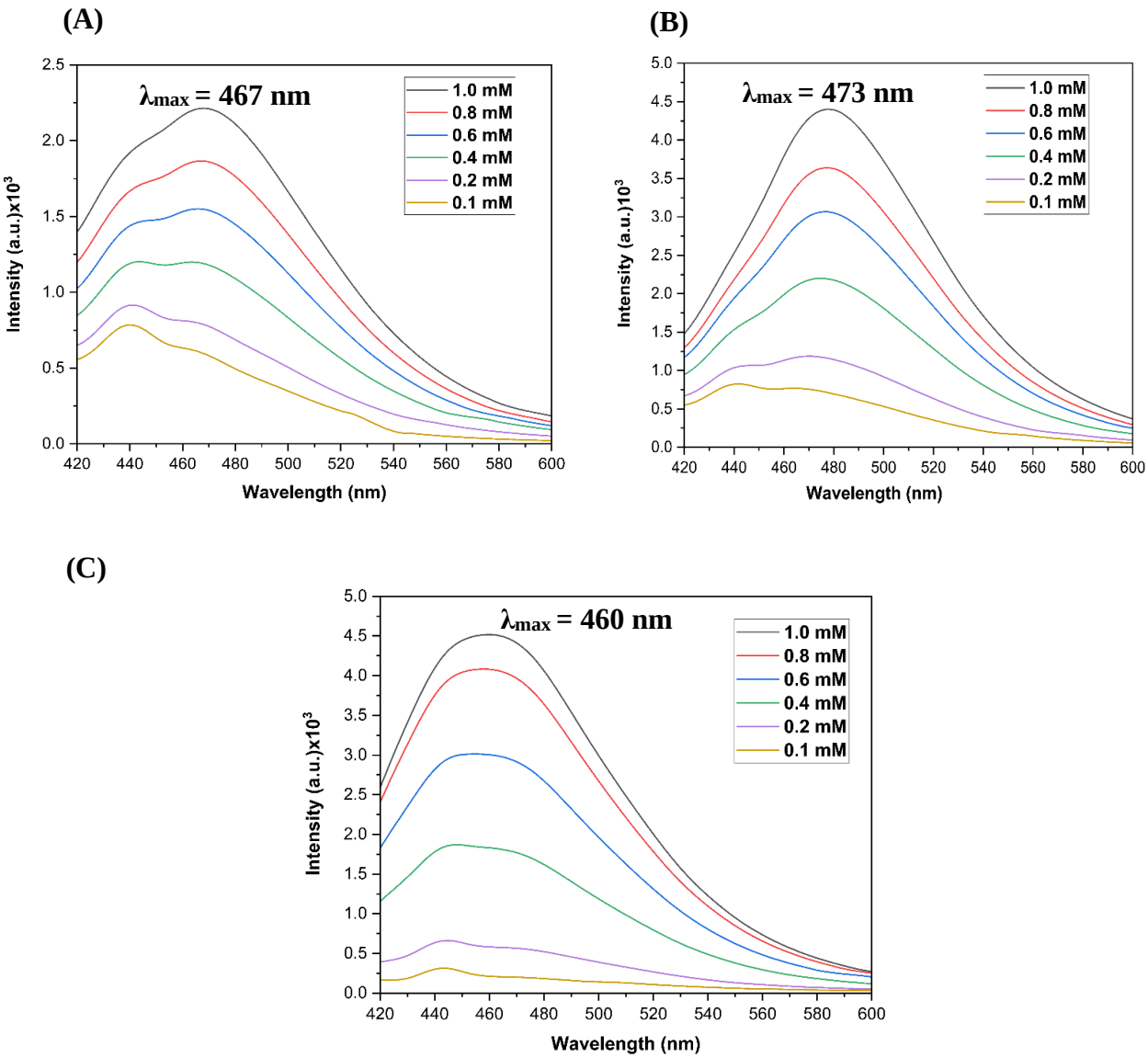


Figure S2. Fluorescence data. (A) **SK18**, (B) **SK20**, and (C) **SK21**

4. Conformational analysis

4.1 Solid-state structure based on SC-XRD

SC-XRD data for all the compounds were collected on a Bruker D8 QUEST instrument with an IμS Mo micro source (λ = 0.71073 Å) and a PHOTON-100 detector. The raw data frames were reduced and corrected for absorption effects using the Bruker Apex 3 software suite programs.[7] The structure was solved using intrinsic phasing method and further refined with the SHELXL[8] program and expanded using Fourier techniques. Anisotropic displacement parameters were included for all non-hydrogen atoms. All H atoms were positioned geometrically and treated as riding on their parent C atoms (C-H = 0.93-0.98 Å, and U_{iso}(H) = 1.5U_{eq}(C) for methyl H or 1.2U_{eq}(C) for other H atoms). The molecular graphics were generated using Mercury 3.10.3 software.

Table S1. Crystallographic data of compounds **7**, **8**, and **12**.

	7	**8**	**12**
CCDC	**2322664**	**2322663**	**2322665**
Chemical Formula	$C_{33}H_{23}N_3O_6$	$C_{25}H_{19}N_3O_6$	$C_{39}H_{38}N_4O_7$
Fw[g/mol]	557.54	457.43	674.73
Crystal system	Monoclinic	Monoclinic	Monoclinic
Space group	$P2_1/n$	$P2_1/n$	$C2/c$
a (Å)	7.8194(2)	9.5530(2)	16.707(3)
b (Å)	22.3939(7)	5.43510(10)	32.088(7)
c (Å)	15.3651(5)	41.8137(8)	16.045 (3)
β	93.848(3)	93.365(2)	117.296(7)
V (Å^3)	2684.47(14)	2167.29(7)	7644(3)
Z	4	4	8
Radiation (Mo K_α)	0.71073	0.71073	0.71073
Temperature (K)	297(1)	230(30)	100 (2)
F(000)	1160	952	2848
μ (mm^{-1})	0.097	0.850	0.081
Measure reflns.	32374	11117	23040
Independent reflns.	5611	4300	8771
Observed reflns.	3603	3851	4295
R1	0.0473	0.0436	0.0824
*w*R2	0.1202	0.1081	0.1984
GOF	1.056	0.846	1.025
$\Delta\rho_{max}$, $\Delta\rho_{min}$(e/Å^3)	0.113, -0.205	0.161, -0.206	0.210, -0.258

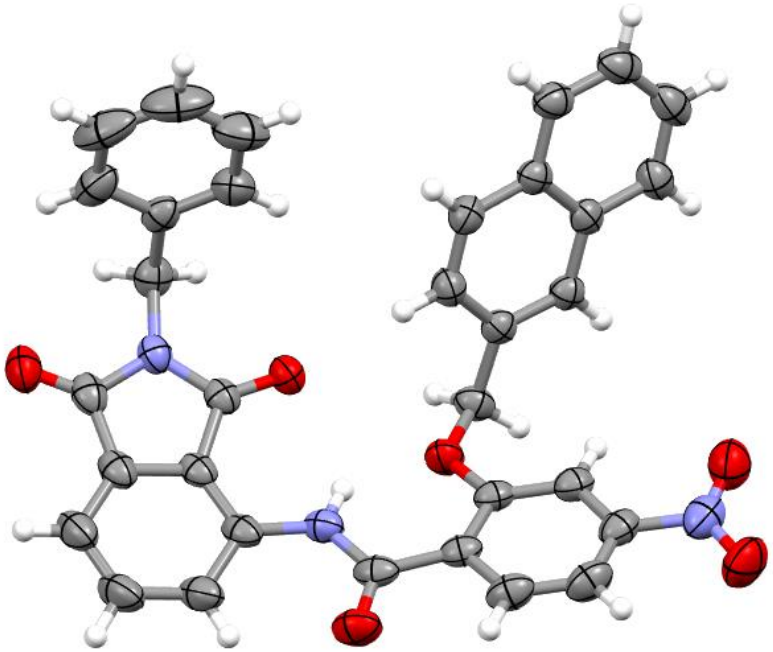


Figure S3. A view of **7** with displacement ellipsoids drawn at the 40% probability level and H atoms are represented by circles of arbitrary radii. For clarity, only one molecule in the asymmetric is shown.

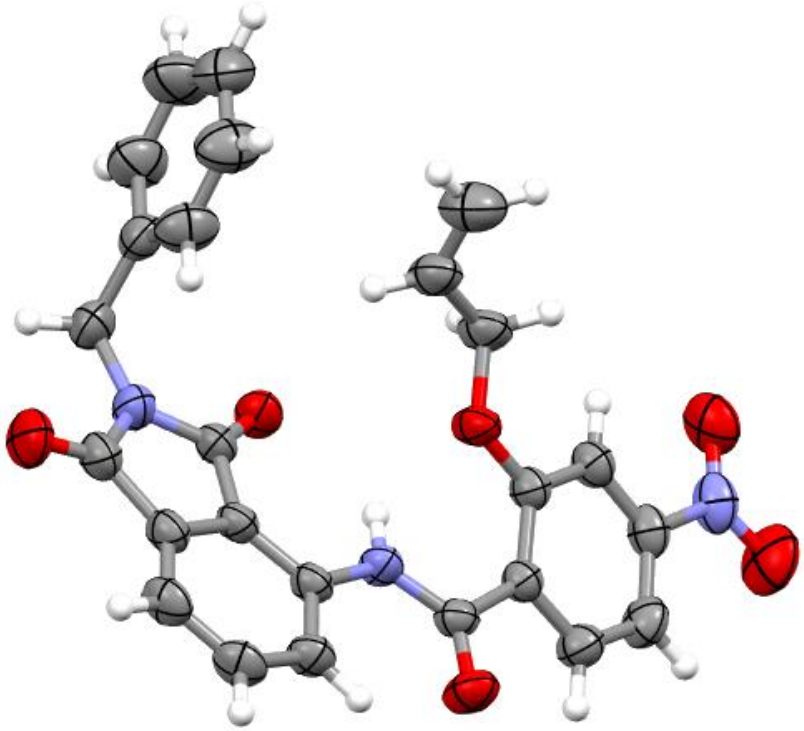


Figure S4. A view of **8** with displacement ellipsoids drawn at the 40% probability level and H atoms are represented by circles of arbitrary radii. For clarity, only one molecule in the asymmetric is shown.

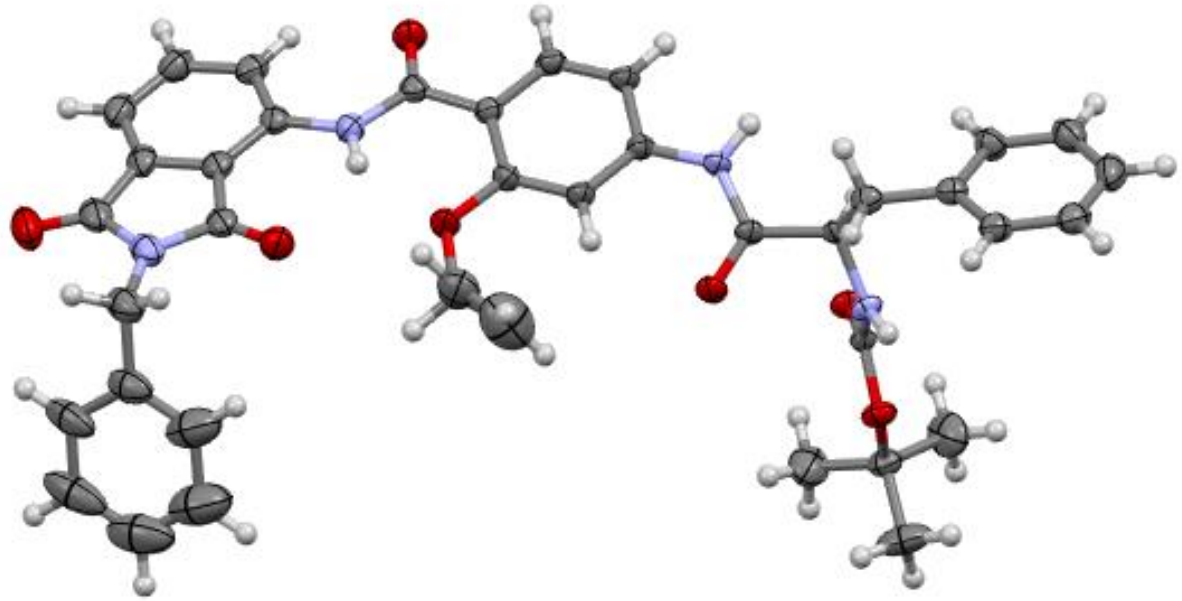


Figure S5. A view of **12** with displacement ellipsoids drawn at the 20% probability level and H atoms are represented by circles of arbitrary radii. Disorder in the five membered rings has been removed for clarity.

4.2 2D-NMR

Table S2. ^{1}H-^{1}H COSY correlations of **11**.

^{1}H (δ ppm)	**^{1}H (δ ppm)**
11.72 (3-NH)	
9.15 (2-NH)	
5.08–5.07 (1-NH)	
9.00–8.99 (27-CH)	7.32
8.08–8.06 (10-CH)	6.61
8.02 (24-CH)	
7.95 (13-CH)	
7.78–7.72 (22, 21 & 19-CH)	7.63, 7.46–7.40
7.64–7.63 (16-CH)	7.46–7.40
7.46–7.41 (20 & 17-CH)	7.75
7.32 (38, 37, 36 & 28 -CH)	8.99
7.20 (40, 39 & 29-CH)	7.32
6.62-6.60 (9-CH)	8.07
5.87–5.84 & 5.64–5.62 (14-CH)	
4.56–4.53 & 4.50–4.47 (34-CH)	
4.34 (2-CH)	5.08, 1.68–1.54
1.78–1.72(4-CH)	0.93, 1.68–1.54
1.65–1.59 (3-CH)	4.34, 1.79–1.71
1.43 (44, 43 & 42-CH)	
0.95–0.91 (6 & 5-CH)	1.79–1.71

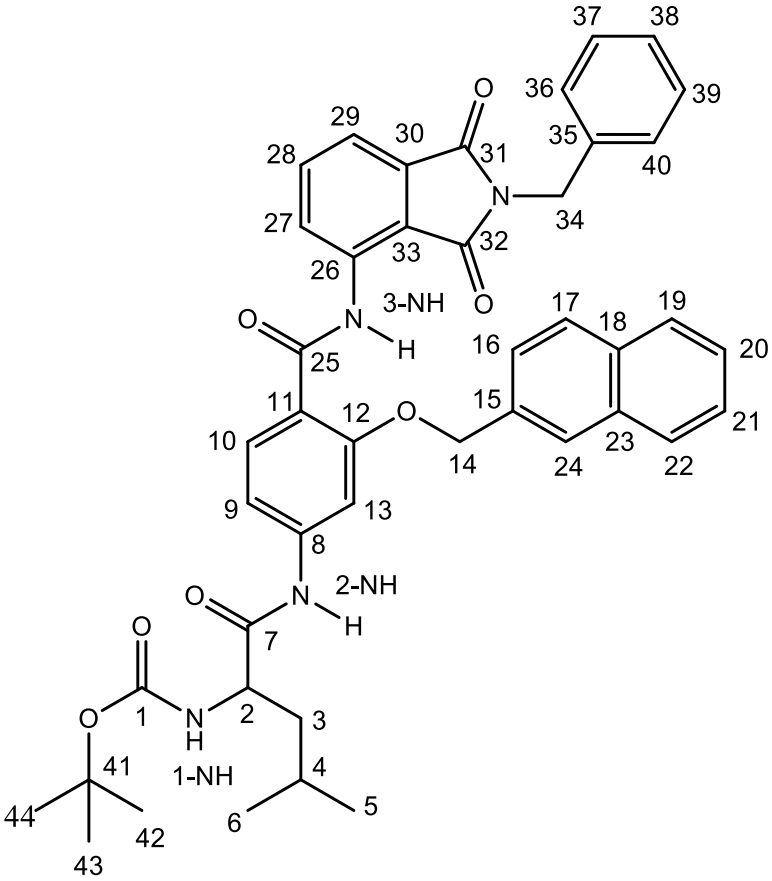


Table S3. ^{1}H-^{1}H TOCSY correlations of **11**.

^{1}H (δ ppm)	**^{1}H (δ ppm)**	
11.72 (3-NH)		
9.15 (2-NH)		
5.08–5.07 (1-NH)	4.34, 1.79–1.71, 1.68–1.54	
9.00–8.99 (27-CH)	7.32	
8.08–8.06 (10-CH)	7.95, 6.61	
8.02 (24-CH)	7.75, 7.63	
7.95 (13-CH)	8.07, 6.61	
7.78–7.72 (22, 21 & 19-CH)	8.02, 7.63, 7.46–7.40	
7.64–7.63 (16-CH)	7.75, 8.02	
7.46–7.41 (20 & 17-CH)	7.75	
7.32 (38, 37, 36 & 28 -CH)	8.99, 7.20	
7.20 (40, 39 & 29-CH)	7.32	
6.62-6.60 (9-CH)	8.07, 7.95	
5.87–5.84 & 5.64–5.62 (14-CH)		
4.56–4.53 & 4.50–4.47 (34-CH)		
4.34 (2-CH)	5.08, 1.79–1.71, 1.68–1.54	
1.78–1.72(4-CH)	5.08. 4.34, 1.68–1.54, 0.93	
1.65–1.59 (3-CH)	5.08. 4.34, 1.79–1.71, 0.93	
1.43 (44, 43 & 42-CH)		
0.95–0.91 (6 & 5-CH)	5.08, 4.34, 1.79–1.71, 1.68–1.54	

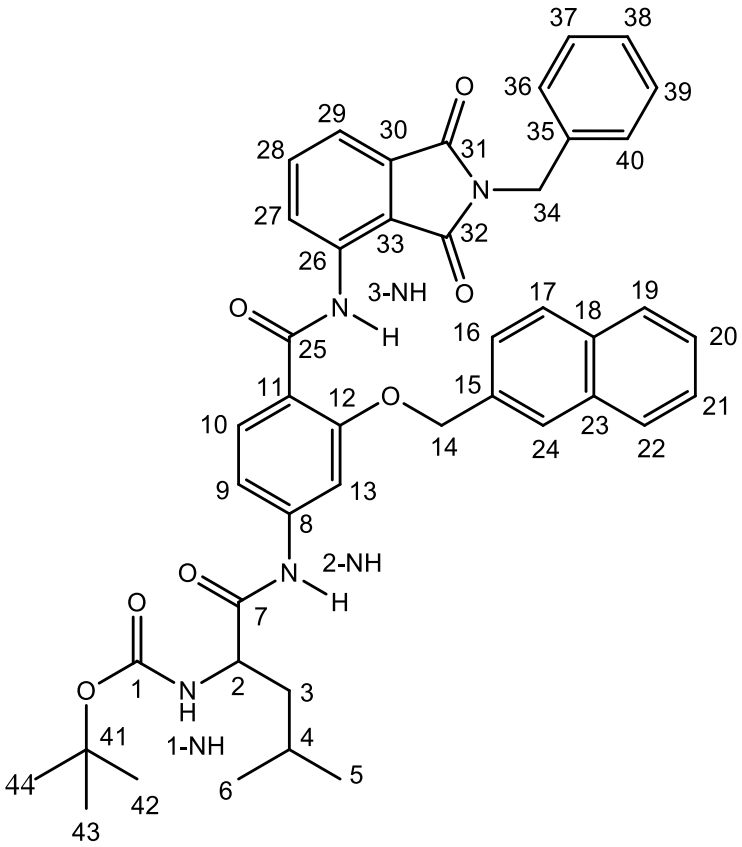


Table S4. ^{1}H-^{13}C HSQC correlations of **11**.

^{1}H (δ ppm)	**^{13}C (δ ppm)**	
11.72 (3-NH)		
9.15 (2-NH)		
5.08–5.07 (1-NH)		
9.00–8.99 (27-CH)	125.7	
8.08–8.06 (10-CH)	133.0	
8.02 (24-CH)	127.5	
7.95 (13-CH)	104.0	
7.78–7.72 (22, 21 & 19-CH)	128.2, 128.1, 126.02	
7.64–7.63 (16-CH)	125.99, 128.1	
7.46–7.41 (20 & 17-CH)	127.5, 126.02	
7.32 (38, 37, 36 & 28 -CH)	135.1, 128.8, 127.8, 117.3	
7.20 (40, 39 & 29-CH)	128.8, 128.6, 127.8	
6.62-6.60 (9-CH)	111.5	
5.87–5.84 & 5.64–5.62 (14-CH)	70.5	
4.56–4.53 & 4.50–4.47 (34-CH)	41.1	
4.34 (2-CH)	54.0	
1.78–1.72(4-CH)	24.7	
1.65–1.59 (3-CH)	40.4	
1.43 (44, 43 & 42-CH)	28.3	
0.95–0.91 (6 & 5-CH)	23.1, 21.4	

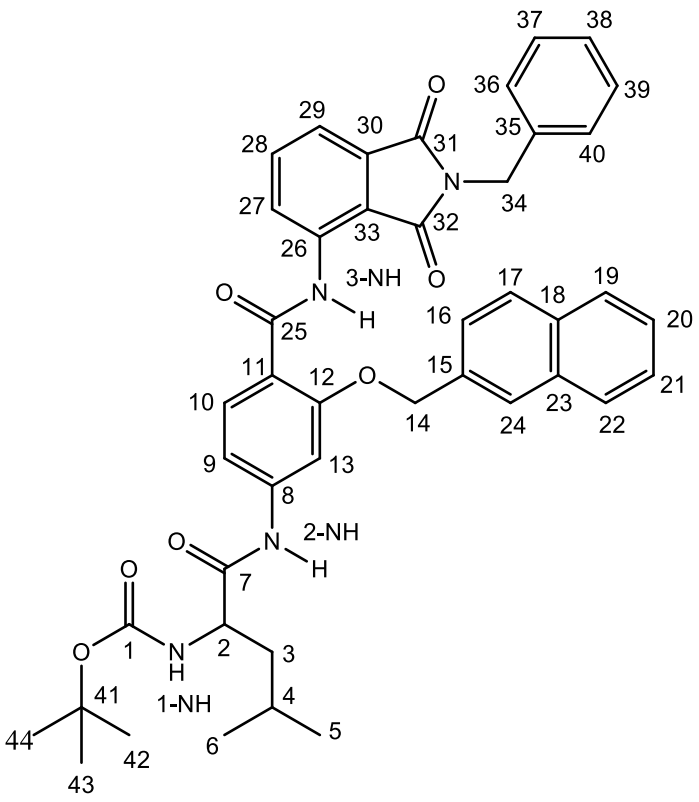


Table S5. ^{1}H-^{13}C HMBC correlations of **11**.

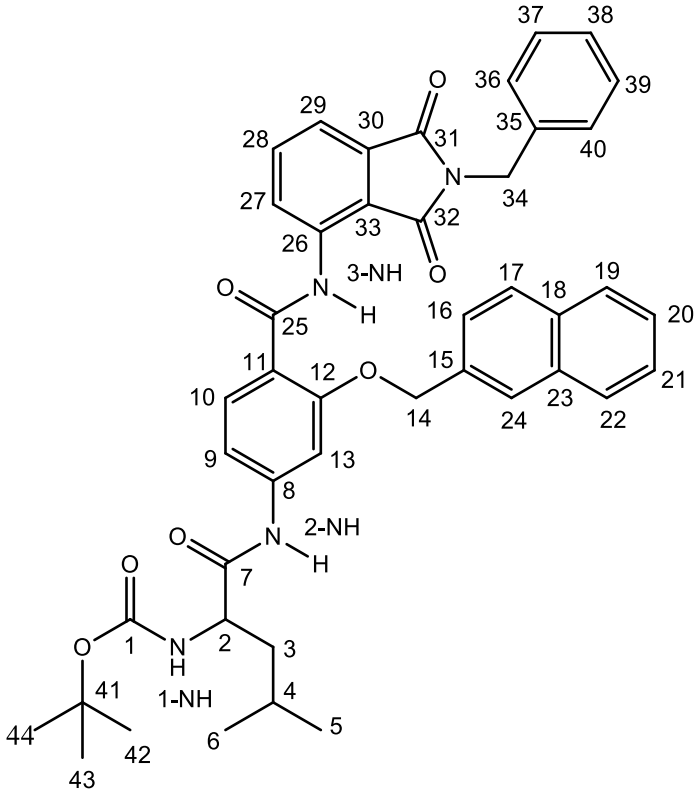
^{1}H (δ ppm)	^{13}C (δ ppm)	
11.72 (3-NH)	164.1, 137.6, 125.7, 116.1	
9.15 (2-NH)	171.7, 111.5, 104.1	
5.08–5.07 (1-NH)	54.0, 40.4	
9.00–8.99 (27-CH)	168.4, 137.6, 117.3, 116.1	
8.08–8.06 (10-CH)	164.1, 157.2, 143.1	
8.02 (24-CH)	133.0, 128.2, 128.1, 126.02, 125.99, 70.5	
7.95 (13-CH)	157.2, 143.1, 116.1, 111.5	
7.78–7.72 (22, 21 & 19-CH)	133.8, 133.2, 133.0,128.2, 128.1, 127.8, 127.6, 127.5	
7.64–7.63 (16-CH)	133.2, 133.0, 127.6, 127.5, 70.5	
7.46–7.41 (20 & 17-CH)	133.2, 133.0, 128.2, 128.1, 127.8, 126.02, 125.8	
7.32 (38, 37, 36 & 28 -CH)	167.4, 136.3, 128.8, 127.8, 125.8, 116.1, 41.1	
7.20 (40, 39 & 29-CH)	137.6, 136.3, 131.7, 128.8, 128.6	
6.62-6.60 (9-CH)	104.0, 116.1	
5.87–5.84 & 5.64–5.62 (14-CH)	157.2, 133.8, 127.6, 127.5, 125.8	
4.56–4.53 & 4.50–4.47 (34-CH)	168.4, 167.4, 136.3, 128.8	
4.34 (2-CH)	40.4	
1.78–1.72(4-CH)	54.0, 40.4, 23.1, 21.4	
1.65–1.59 (3-CH)	171.7, 54.0, 23.1, 21.4	
1.43 (44, 43 & 42-CH)	81.0	
0.95–0.91 (6 & 5-CH)	40.4, 24.7, 23.1, 21.4	

Table S6. ^{1}H peak assignments of **11**.

^{1}H (δ ppm)	**Proton Number**
11.72	3-NH
9.15	2-NH
5.08–5.07	1-NH
9.00–8.99	27-CH
8.08–8.06	10-CH
8.02	24-CH
7.95	13-CH
7.78–7.72	22-CH, 21-CH, 19-CH
7.64–7.63	16-CH
7.46–7.41	20-CH, 17-CH
7.32	38-CH, 37-CH, 36-CH, 28-CH,
7.20	40-CH, 39-CH, 29-CH
6.62-6.60	9-CH
5.87–5.84 & 5.64–5.62	14-CH
4.56–4.53 & 4.50–4.47	34-CH
4.34	2-CH
1.78–1.72	4-CH
1.65–1.59	3-CH
1.43	44-CH, 43-CH, 42-CH
0.95–0.91	6-CH, 5-CH

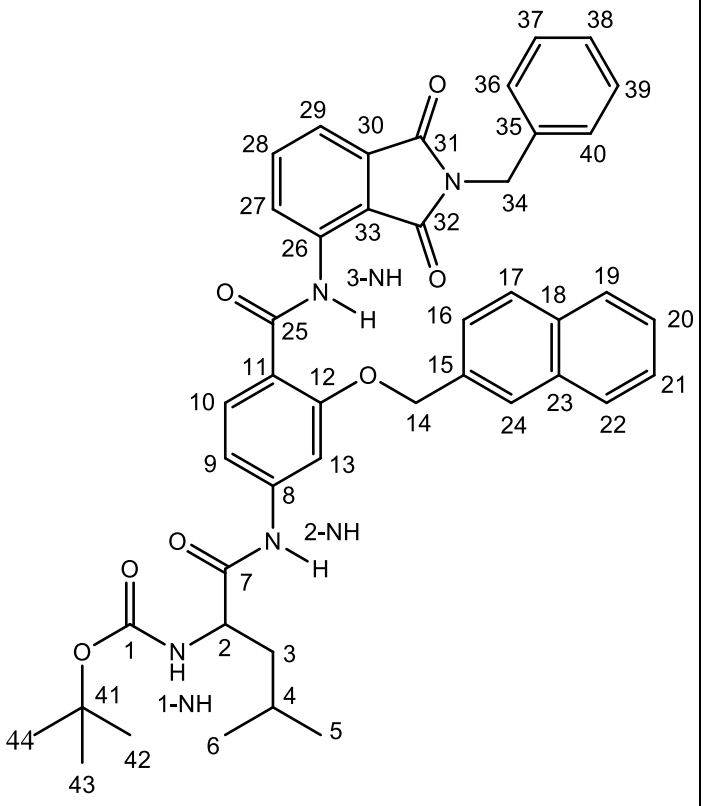
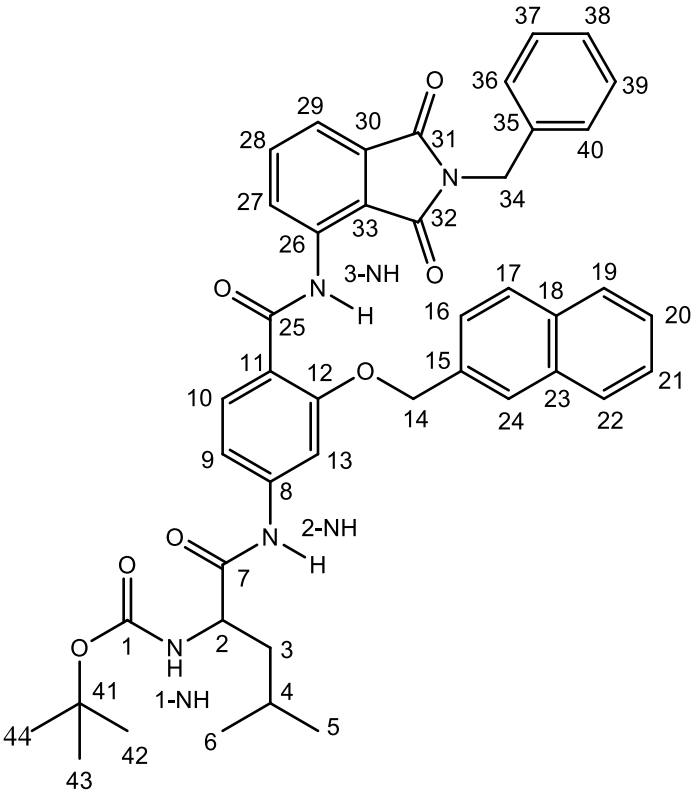


Table S7. ^{13}C peak assignments of **11**.

^{13}C (δ ppm)	Carbon Number
171.7	7-C
168.4	32-C
167.4	31-C
164.1	25-C
157.2	12-C
156.7	1-C
143.1	11-C
137.6	33-C
136.3	35-C
135.1	37-C
133.8	15-C
133.2	18-C
133.0	23-C
132.9	10-C
131.7	30-C
128.8	36-C, 40-C
128.6	39-C
128.2	21-C
128.1	19-C
127.8	38- C
127.6	24-C
127.5	17-C
126.02	22 -C
125.99	16-C
125.8	20 -C,
125.7	27- C
117.3	28-C
116.1	8, 26-C
111.5	9-C
104.0	13-C
81.0	41-C
70.5	14-C



^{13}C (δ ppm)	Carbon Number
54.0	2-C
41.1	34-C
40.4	3-C
28.3	44, 43 & 42-C
24.7	4-C
23.1, 21.4	6 & 5-C

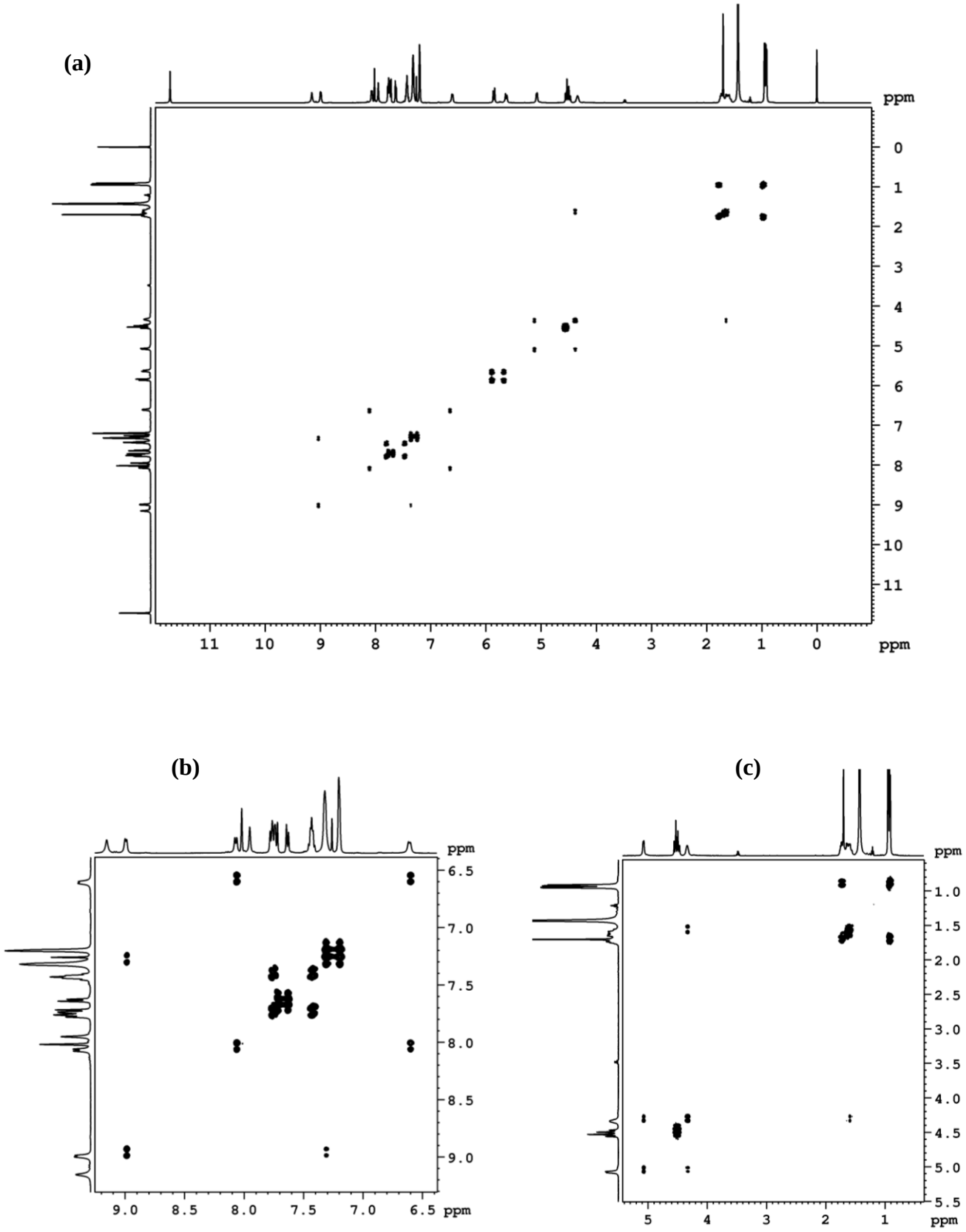


Figure S6. COSY spectrum of **11**. **(a)** full spectrum, **(b)** aromatic region, and **(c)** aliphatic region.

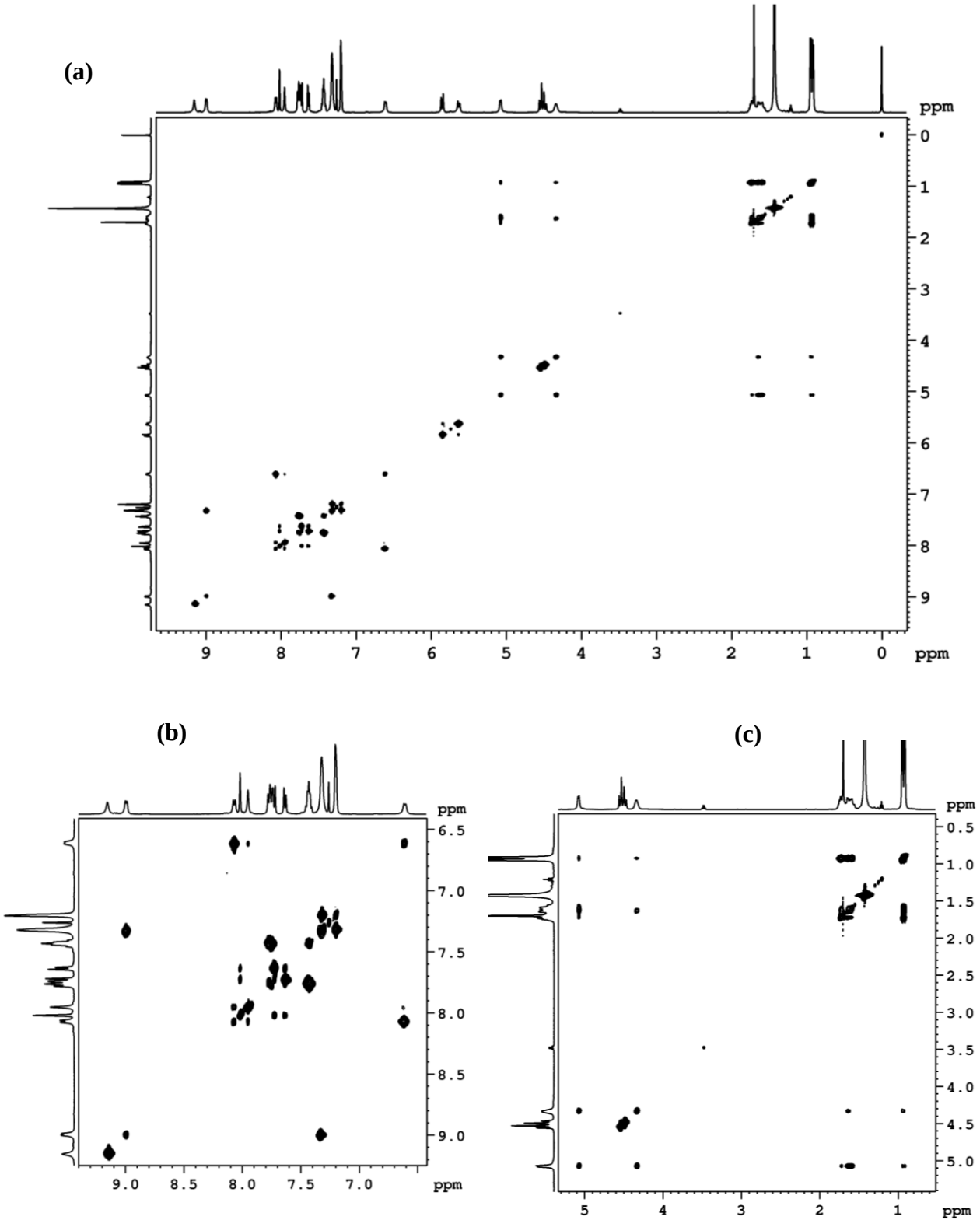


Figure S7. TOCSY spectrum of **11**. **(a)** full spectrum, **(b)** aromatic region, and **(c)** aliphatic region.

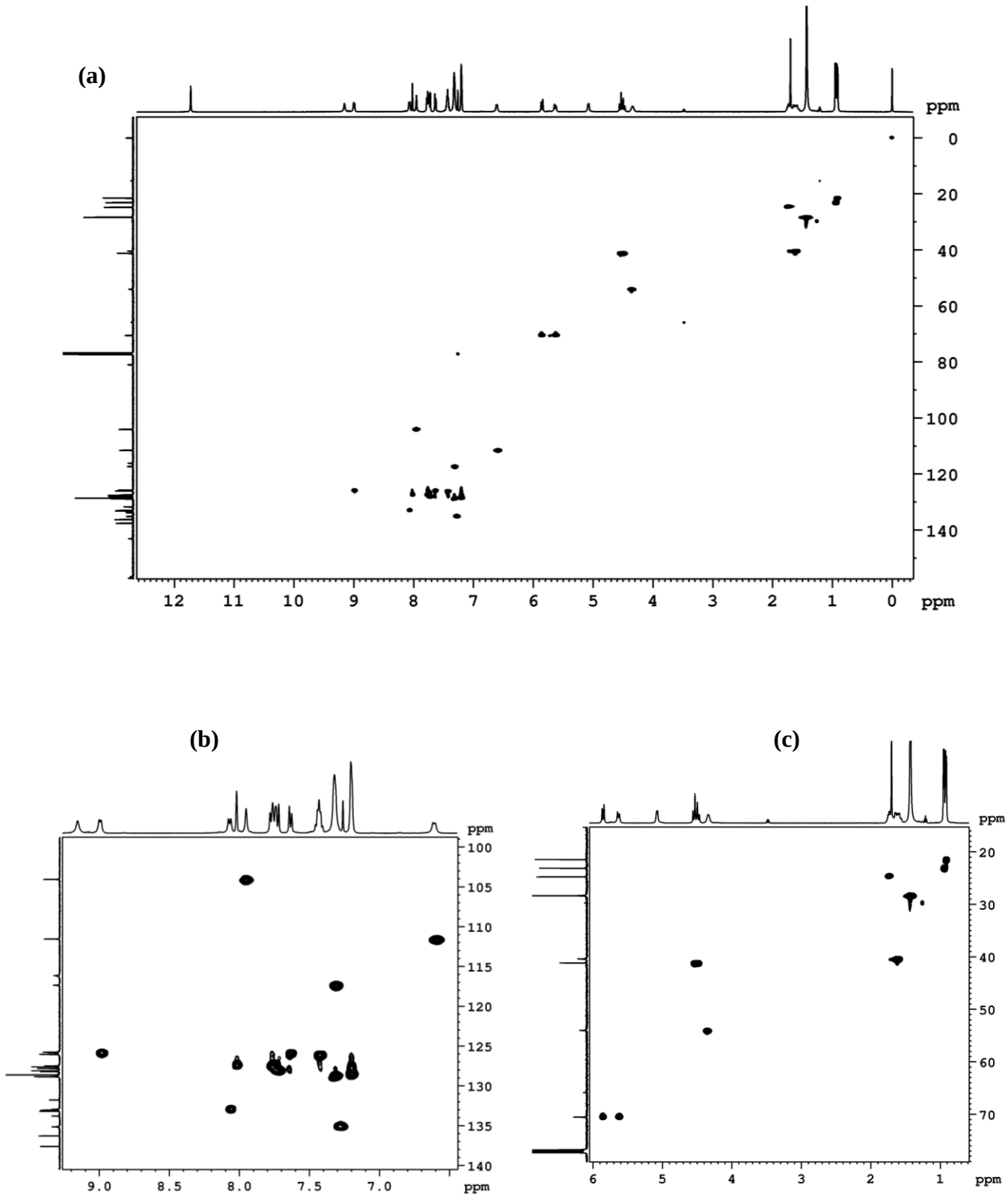


Figure S8. HSQC spectrum of **11**. **(a)** full spectrum, **(b)** aromatic region, and **(c)** aliphatic region.

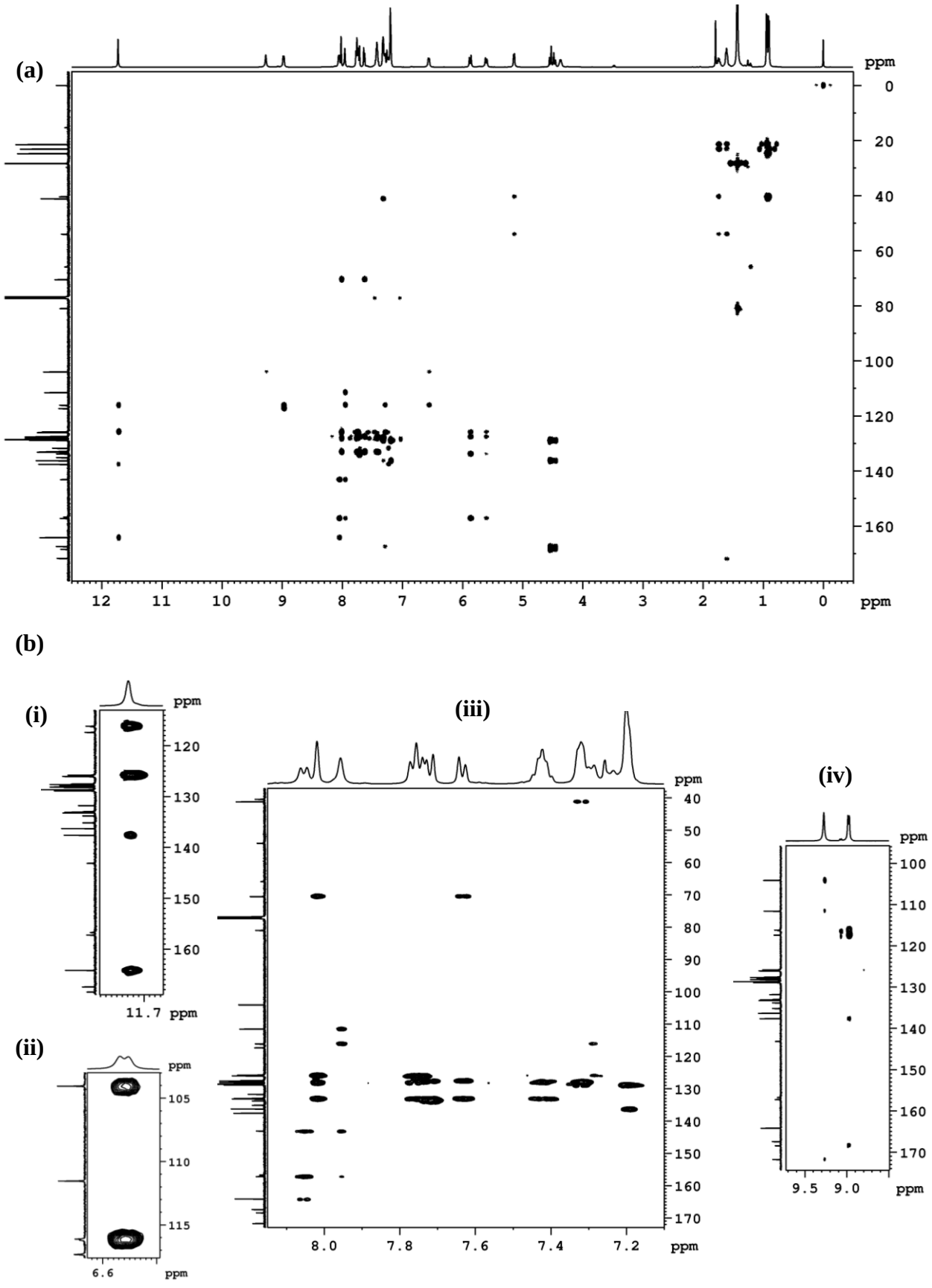


Figure S9. HMBC spectrum of **11**. **(a)** full spectrum, **(b)** partial aromatic region **(i)-(iv)**

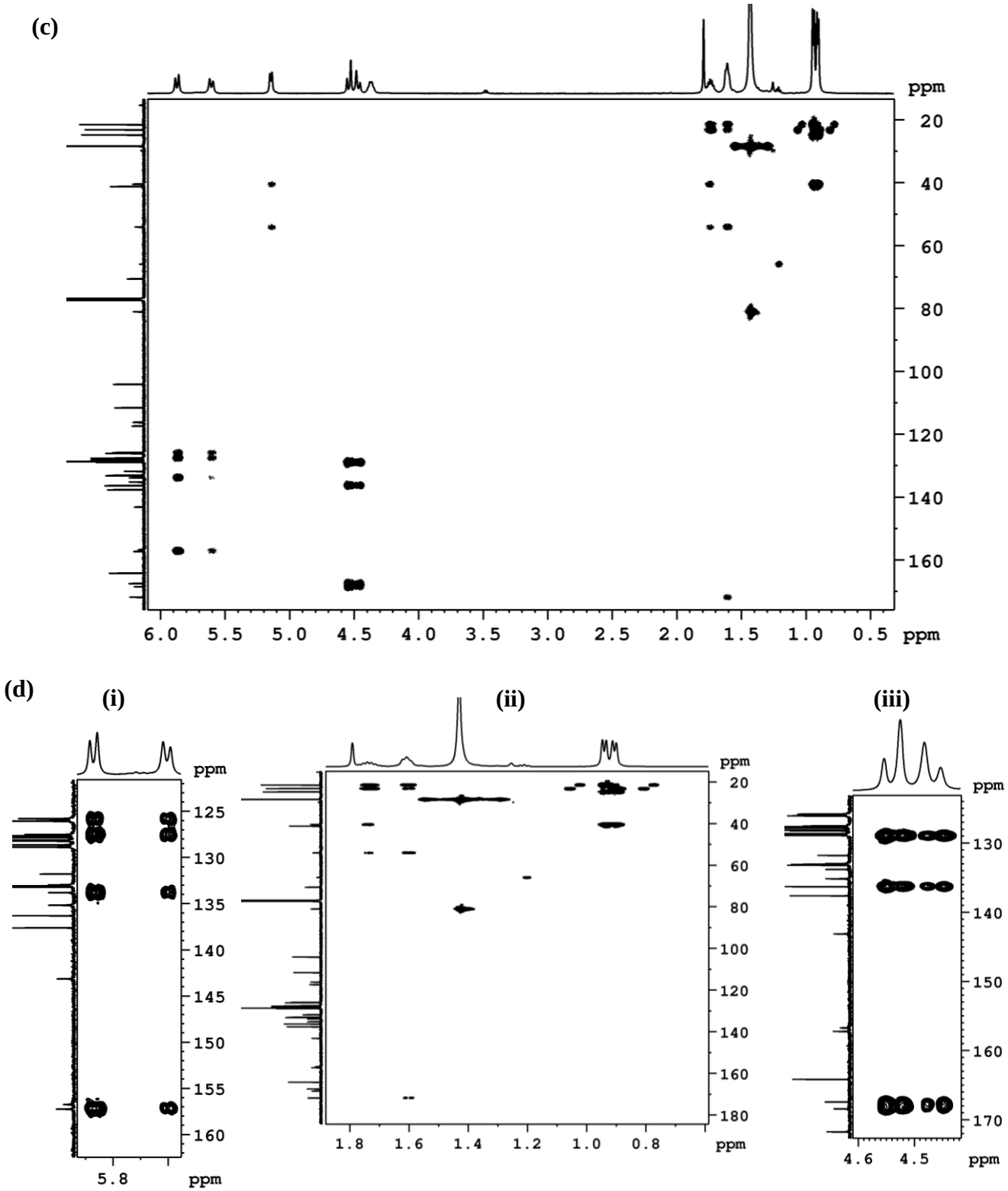


Figure S10. HMBC spectrum of **11**. **(c)** aliphatic region, **(d)** partial aliphatic region **(i)**-**(iii)**

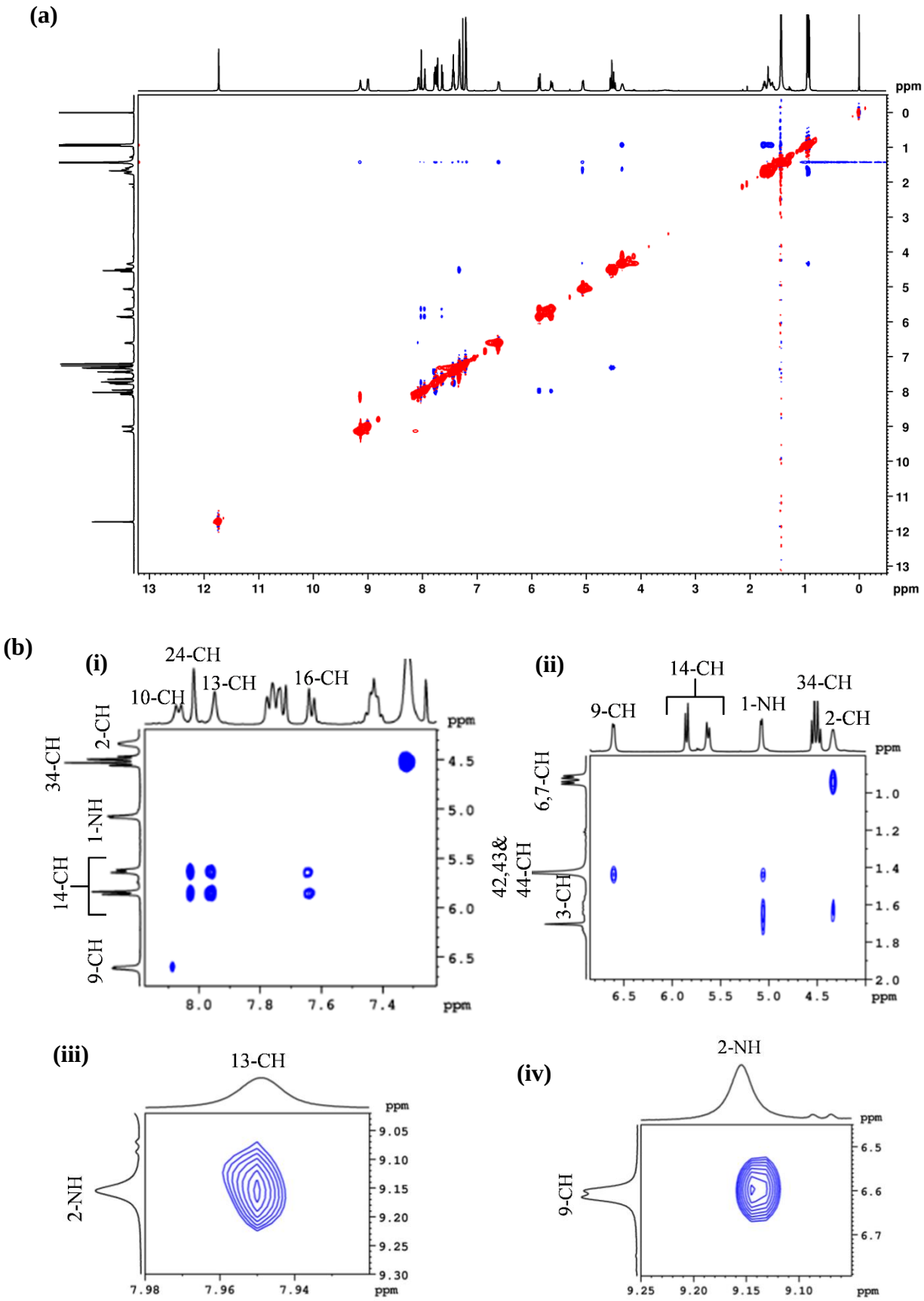


Figure S11. **NOESY** spectrum of **11**. **(a)** full spectrum **(b)** partial spectrum **(i)-(iv)**

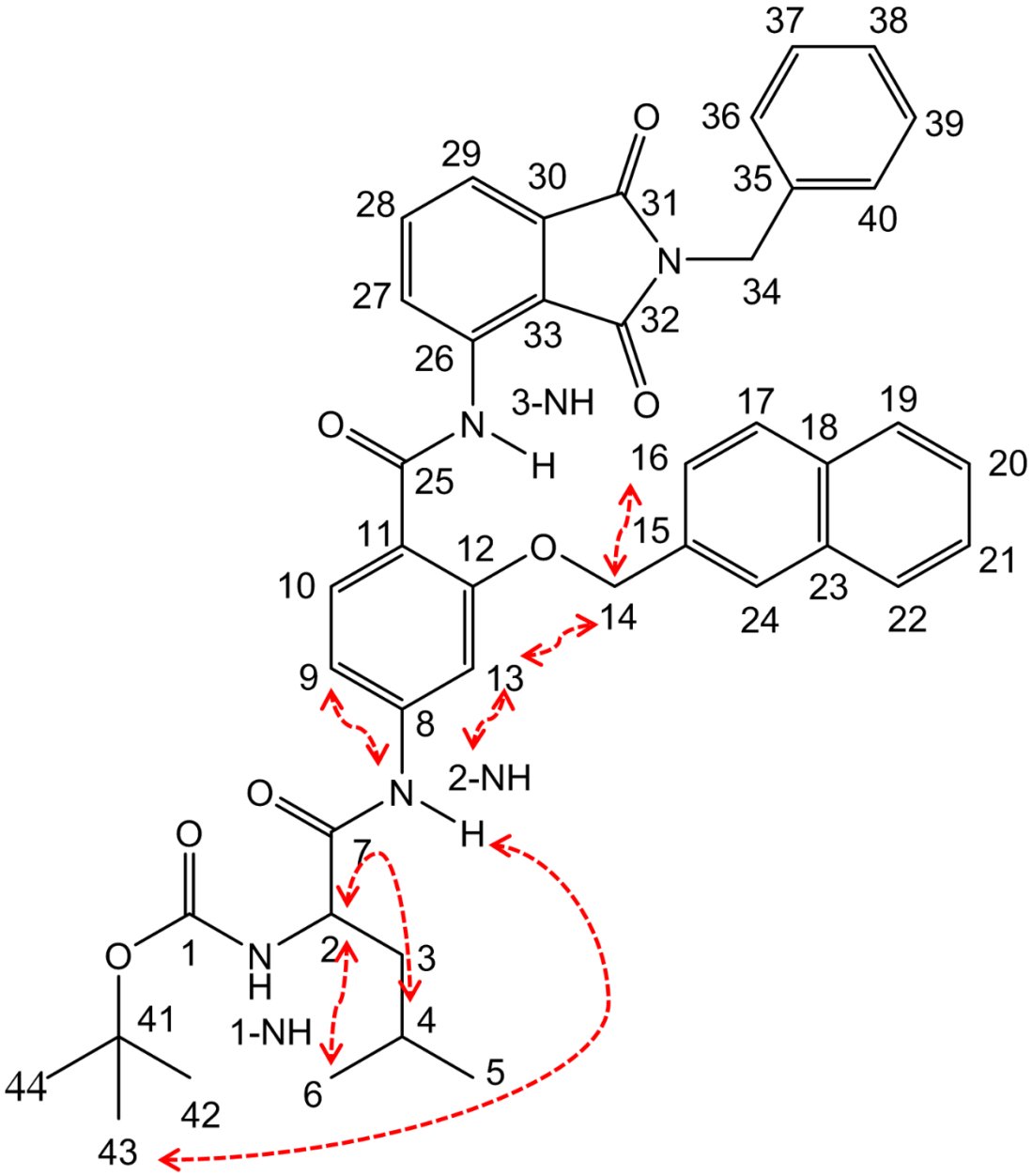


Figure S12. Selected NOE interactions of compound **11.**

4.3 Computational studies

All calculations were carried out using the Gaussian09[9] program Revision D01, we have chosen the following important classes of DFT functionals for our study, M06L functional, geometry optimizations and energy evaluations were performed using same level of theory. The electronic structure calculations for all these structures were modelled using 6-31G(d,p) basis set. Following geometry optimisation, frequency calculations were also carried out on the optimised geometries at the same level of theory to assess the nature of stationary points. All these structures are minima on the potential energy surface (Nimag=0).

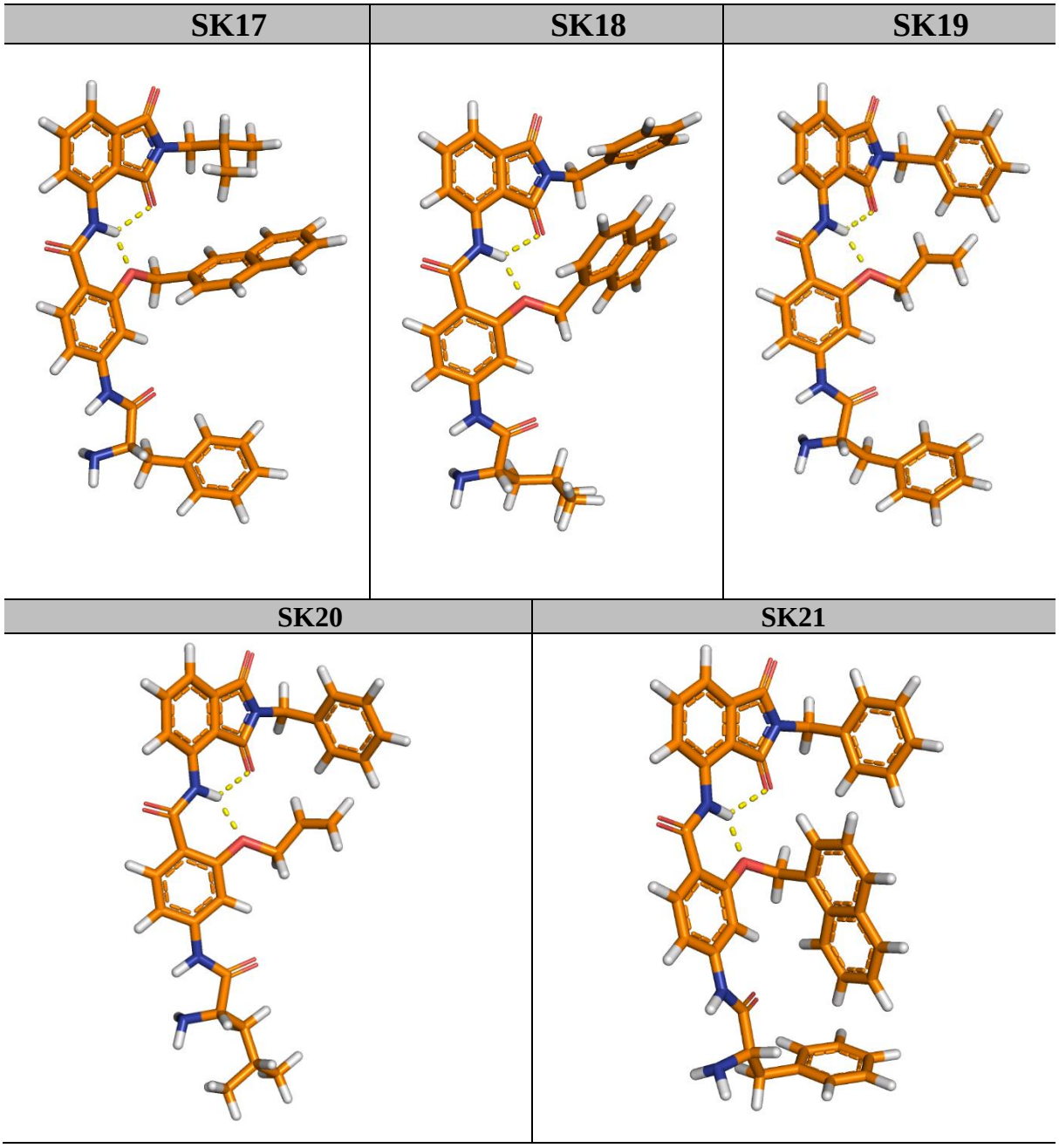


Figure S13. Energy minimized structures of free amine **SK17-SK21**. Molecular modelling was done using Gaussian Software. All these structures were optimized at M06L/6-31G (d,p) level of theory.

5. Molecular docking studies

To check the binding affinity of these helix mimetics, two different protein crystal structures were obtained from the protein data bank (PDB) and used for the docking studies: PDB ID: 1YCR chain A (MDM2 protein bound to p53 peptide) and PDB ID: 1T4F chain M (HDM2 protein bound to a high affinity p53 peptide).

All the water molecules and the p53 helix bound to the protein were removed. The protonation states were assigned manually. For the docking with MDM2 protein a grid box with x, y and z coordinates 50, 42 and 42, spacing of 0.508 Å and centered on 27.859, -23.859, -4.765 was generated. To perform the docking, we used 2.5 million function evaluations, 27,000 generations and 150 population size to produce 50 docked conformations.

For the docking with HDM2 protein a grid box with x, y and z coordinates 52, 58 and 48, spacing of 0.375 Å and centered on 13.119, 18.969, 10.941 was generated. Docking was performed with both the proteins based on Lamarckian Genetic Algorithm. After docking all the conformations were analyzed and only the lowest energy conformation structures given below.

A) Docking study with MDM2 protein.

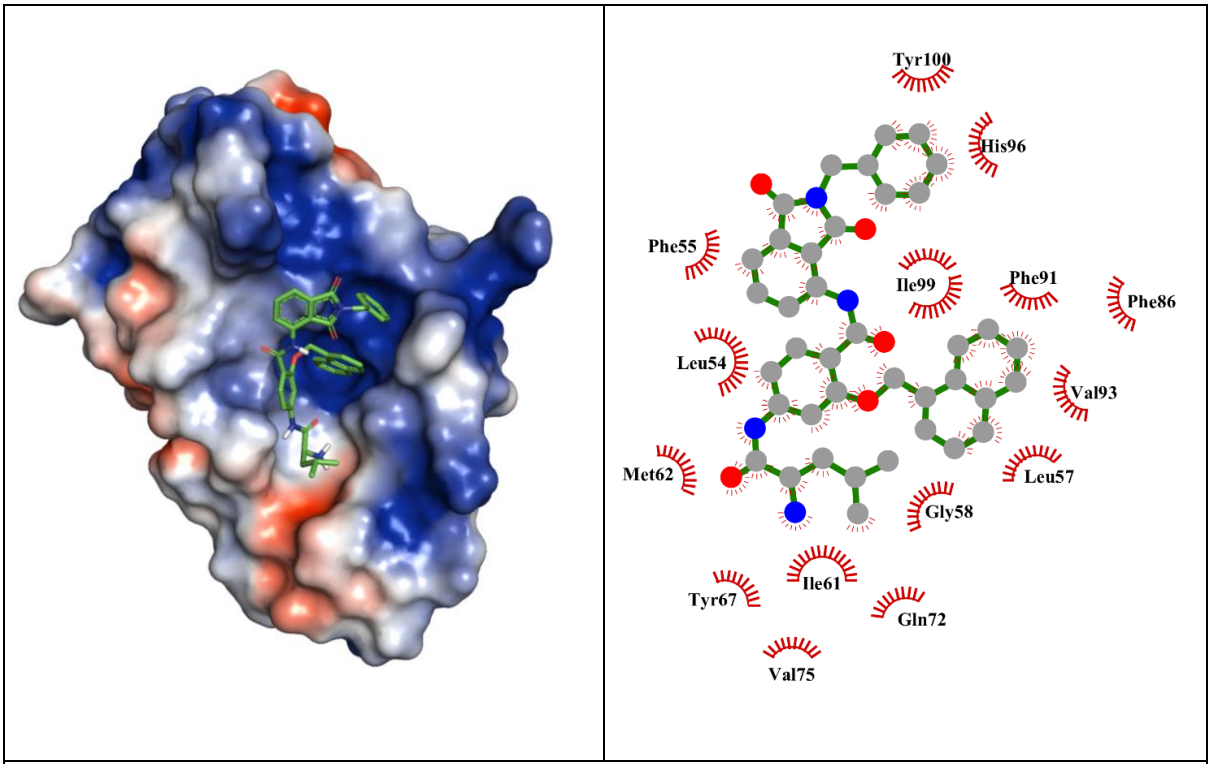


Figure S14. A) Docked conformation of **SK18** (as free amine) with MDM2 protein (PDB: 1YCR). The figure was made by PyMOL software. B) 2D-plot showing possible non-covalent interactions between helix mimetic and MDM2. The figure was made by LigPlot+.

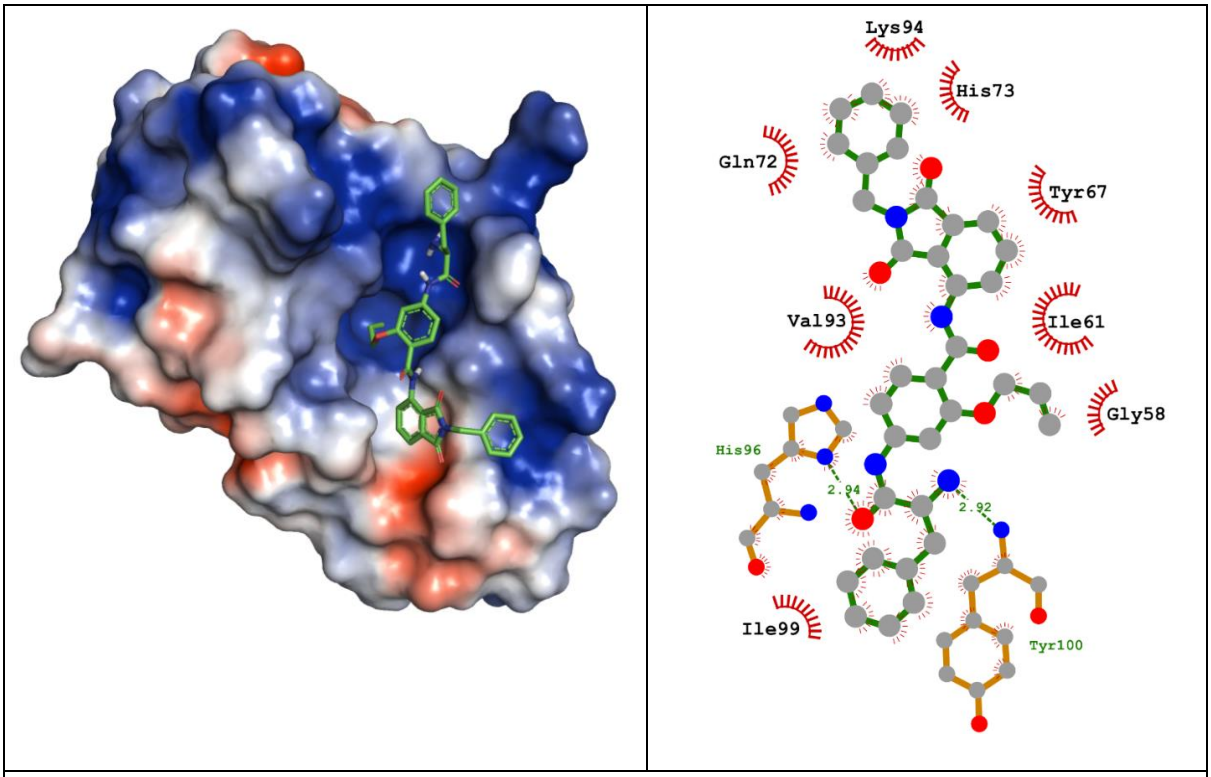


Figure S15. A) Docked conformation of **SK19** (as free amine) with MDM2 protein. The figure was made by PyMOL software. B) 2D-plot with possible non-covalent interactions between helix mimetic and MDM2. The figure was made by LigPlot+.

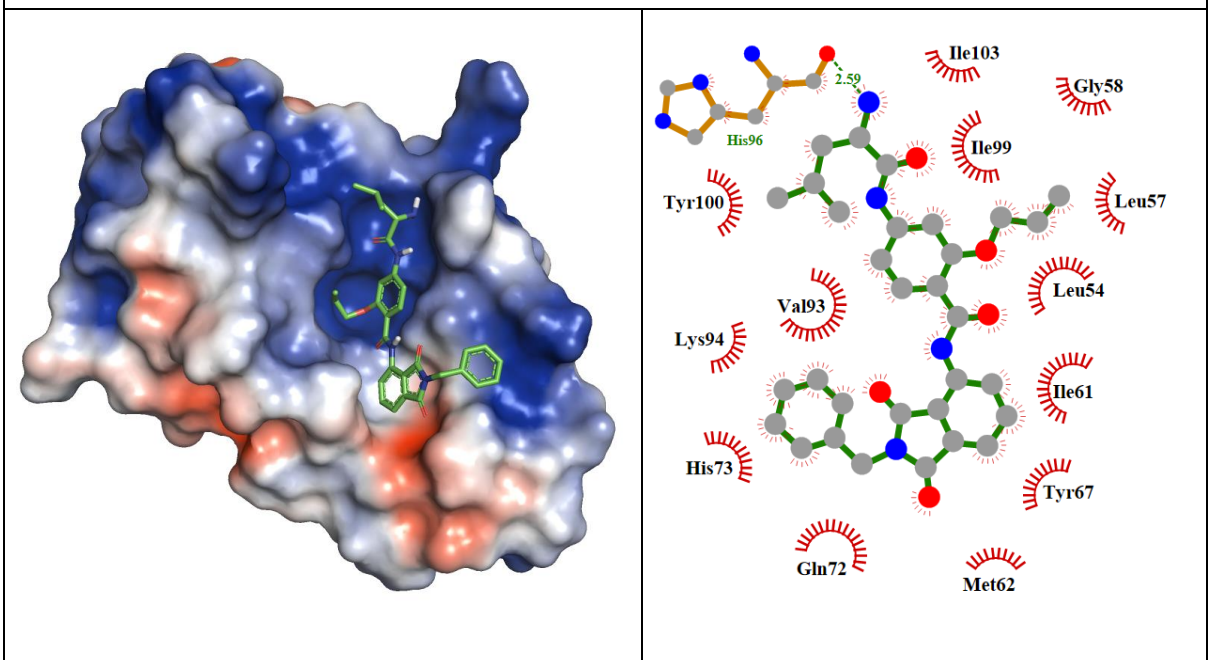


Figure S16. A) Docked conformation of **SK20** (as free amine) with MDM2 protein. The figure was made by PyMOL. B) 2D-plot with possible non-covalent interactions between helix mimetic and MDM2. The figure was made by LigPlot+.

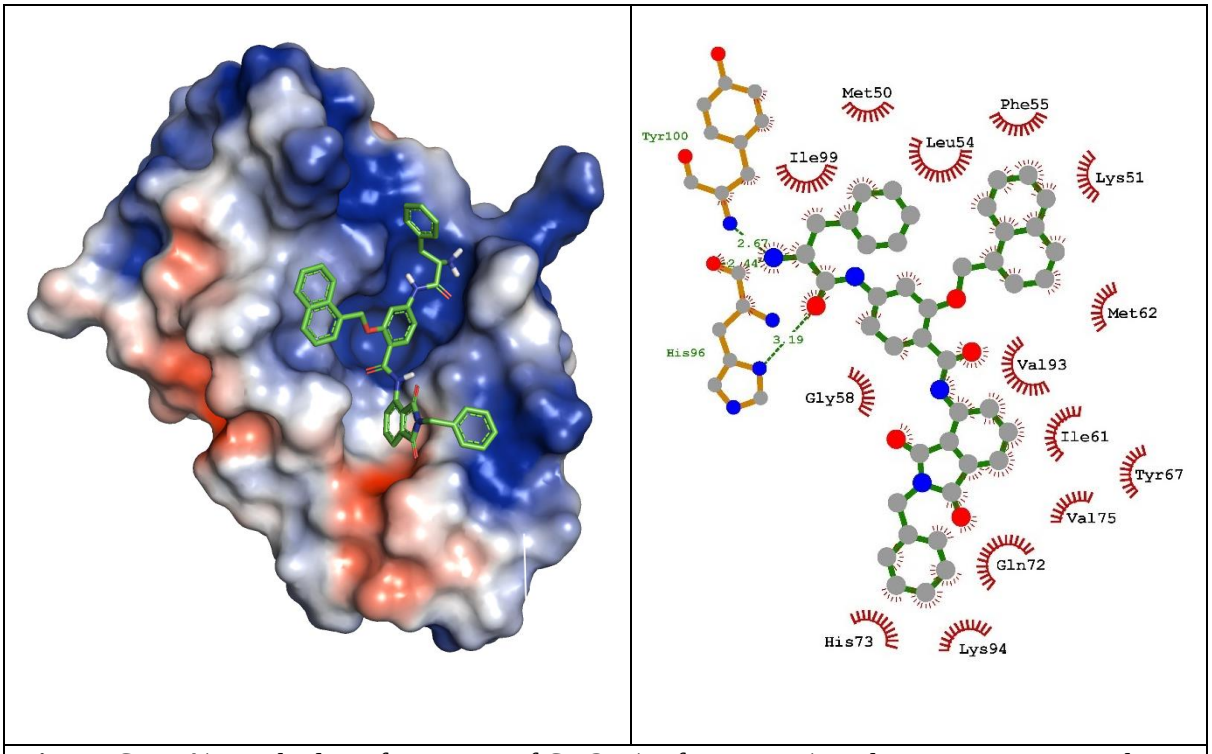


Figure S17. A) Docked conformation of **SK21** (as free amine) with MDM2 protein. The figure was made by PyMOL. B) 2D-plot with possible non-covalent interactions between helix mimetic and MDM2. The figure was made by LigPlot+.

Table S8. Analysis of Docking study with helix mimetic with MDM2 protein (PDB:1YCR).

Helix Mimetics	Binding Energy (Kcal/mol)	Inhibition Constant (K_i) at 298.15 K	Non-covalent Interactions	H-Bonding
SK17	-8.58	515.72 nM	Met50, Leu54, Leu57, Gly58, Ile61, Met62, Tyr67, Gln72, His73, Val75, Phe86, Phe91, Val93, His96, Ile99, Tyr100, Ile103.	Between Gln72 and ligand terminal amine
SK18	-7.94	1.52 µM	Leu54, Phe55, Leu57, Gly58, Ile61, Met62, Tyr67, Gln72, Val75, Phe86, Phe91, Val93, His96, Ile99, Tyr100.	No H-bonding detected
SK19	-7.78	1.98 µM	Gly58, Ile61, Tyr67, Gln72 His73, Val93, Lys94, Ile99.	Between His96 and terminal C=O of ligand. another one between ligand amine N-H and Tyr100 of the receptor.
SK20	-6.77	10.92 µM	Leu54, Leu57, Gly58, Ile61, Met62, Tyr67, Gln72, His73, Val93, Lys94, Ile99, Tyr100, Ile103.	Between His96 and ligand terminal amine
SK21	-9.56	98.26 nM	Met50, Lys51, Leu54, Phe55, Gly58, Ile61, Met62, Tyr67, Gln72, His73, Val75, Val93, Lys94, Ile99.	Terminal amine and amide C=O of the ligand form two H-bonds with His96. Another one between Tyr100 and terminal amine of the ligand.

B) Docking conformation with HDM2 protein (PDB: IT4F).

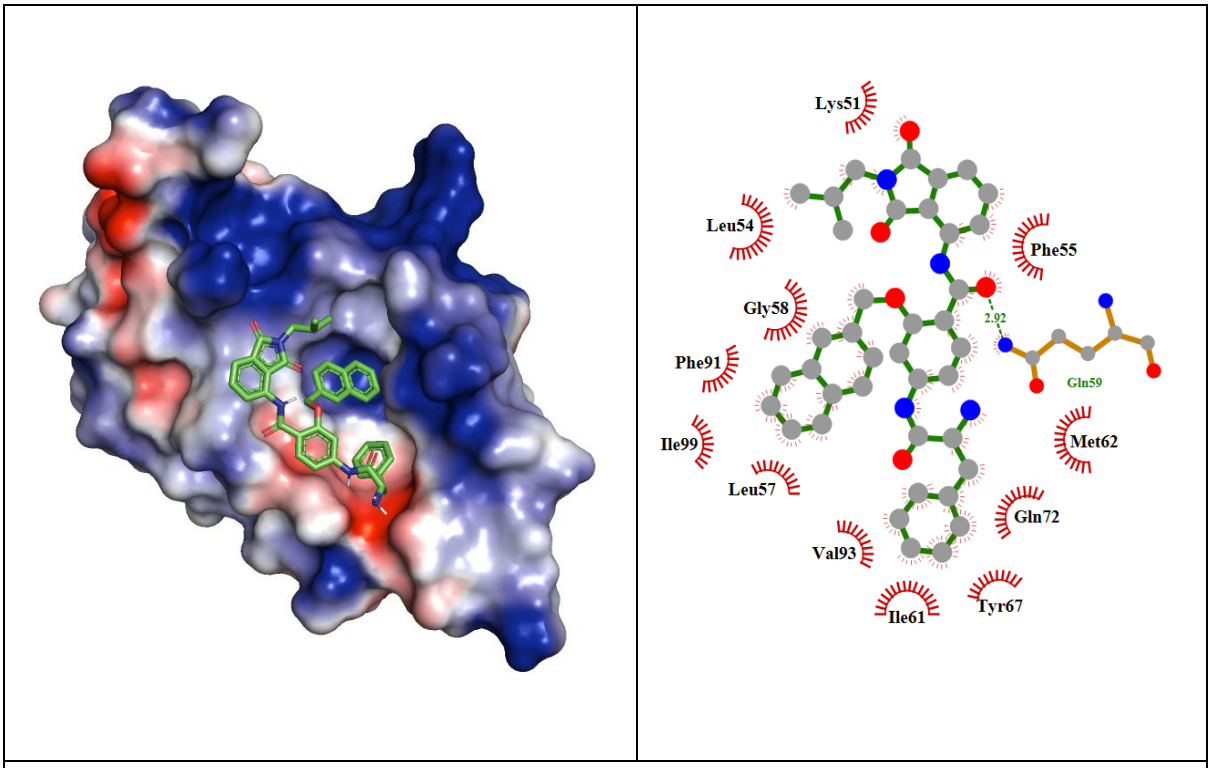


Figure S18. A) Docked conformation of **SK17** (as free amine) with HDM2 protein. The figure was made by PyMOL software. B) 2D-plot showing possible non-covalent interactions between helix mimetic and HDM2. The figure was made by LigPlot+.

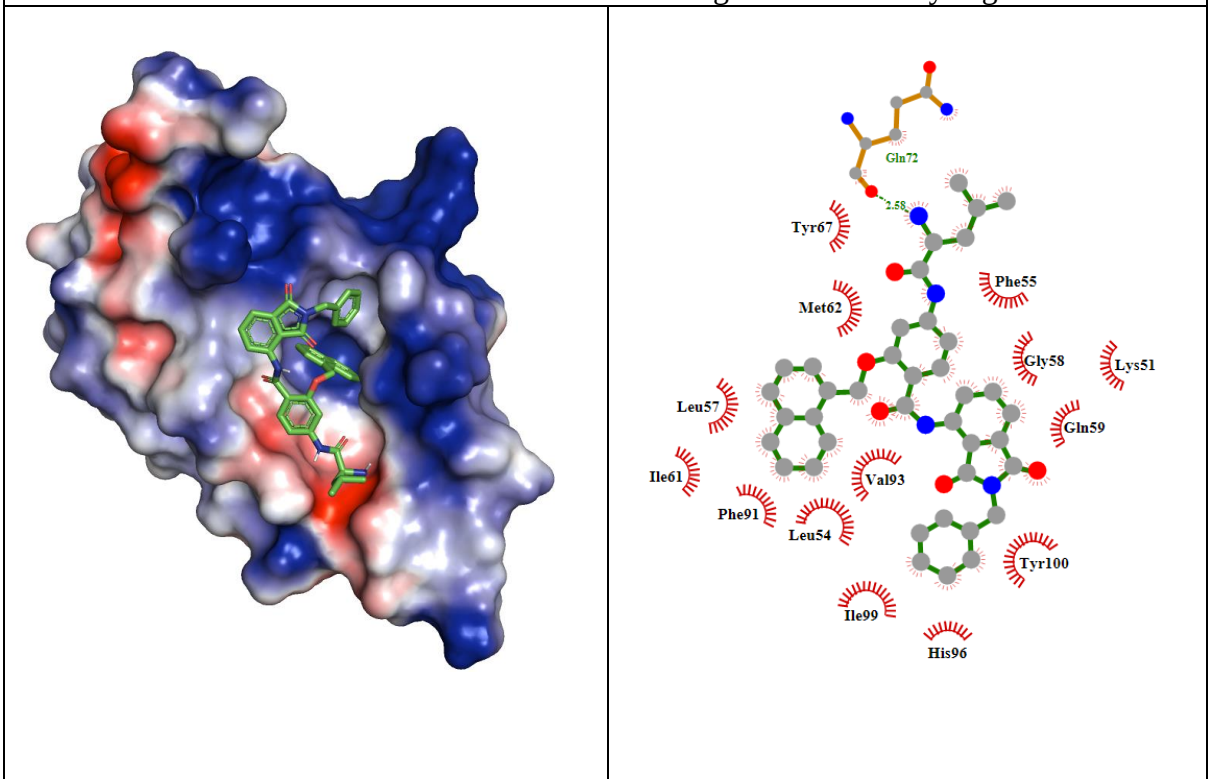


Figure S19. A) Docked conformation of **SK18** (as free amine) with HDM2 protein. The figure was made by PyMOL software. B) 2D-plot with possible non-covalent interactions between helix mimetic and HDM2. The figure was made by LigPlot+.

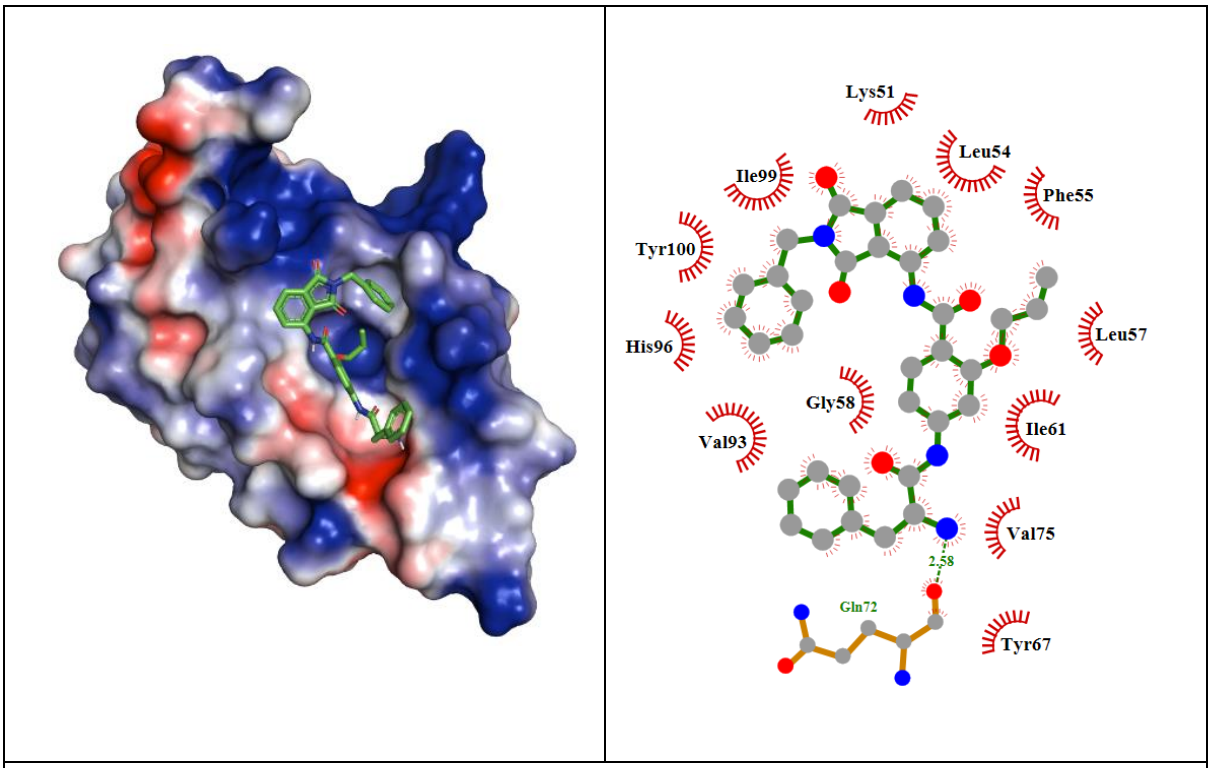


Figure S20. A) Docked conformation of **SK19** (as free amine) with HDM2 protein. The figure was made by PyMOL. B) 2D-plot with possible non-covalent interactions between helix mimetic and HDM2. The figure was made by LigPlot+.

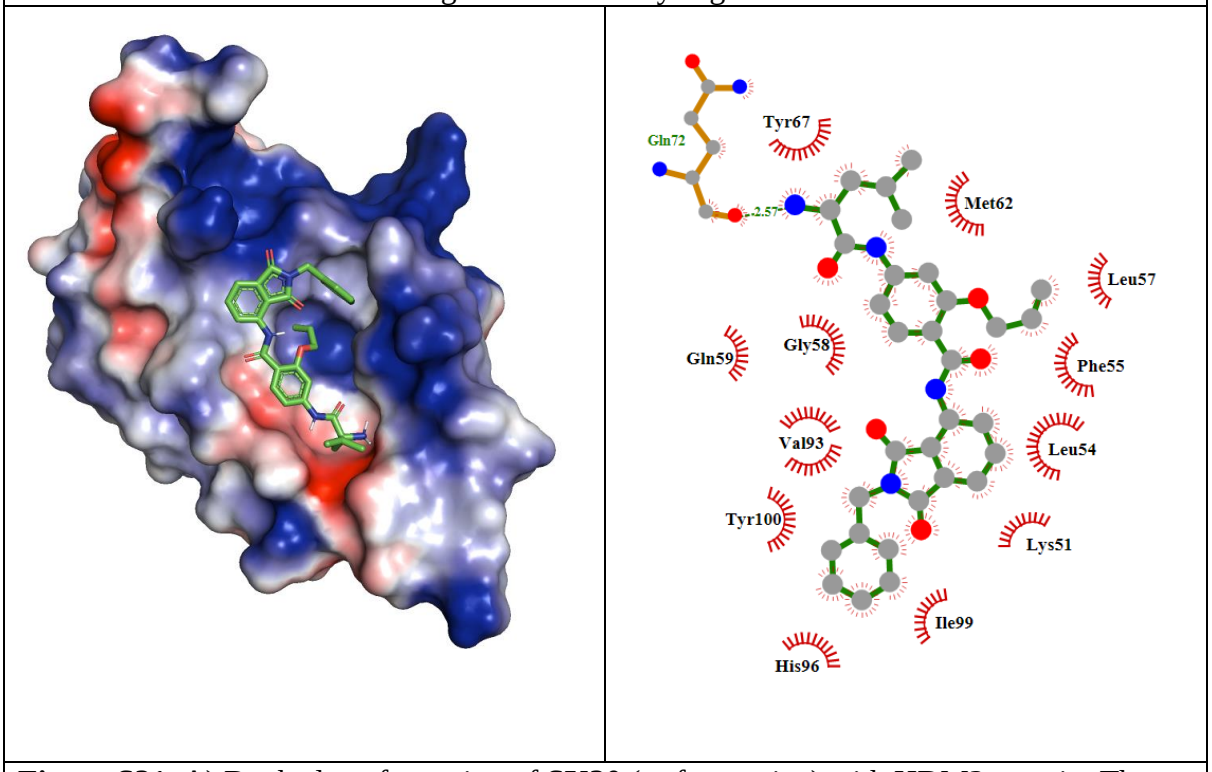


Figure S21. A) Docked conformation of **SK20** (as free amine) with HDM2 protein. The figure was made by PyMOL. B) 2D-plot with possible non-covalent interactions between helix mimetic and HDM2. The figure was made by LigPlot+.

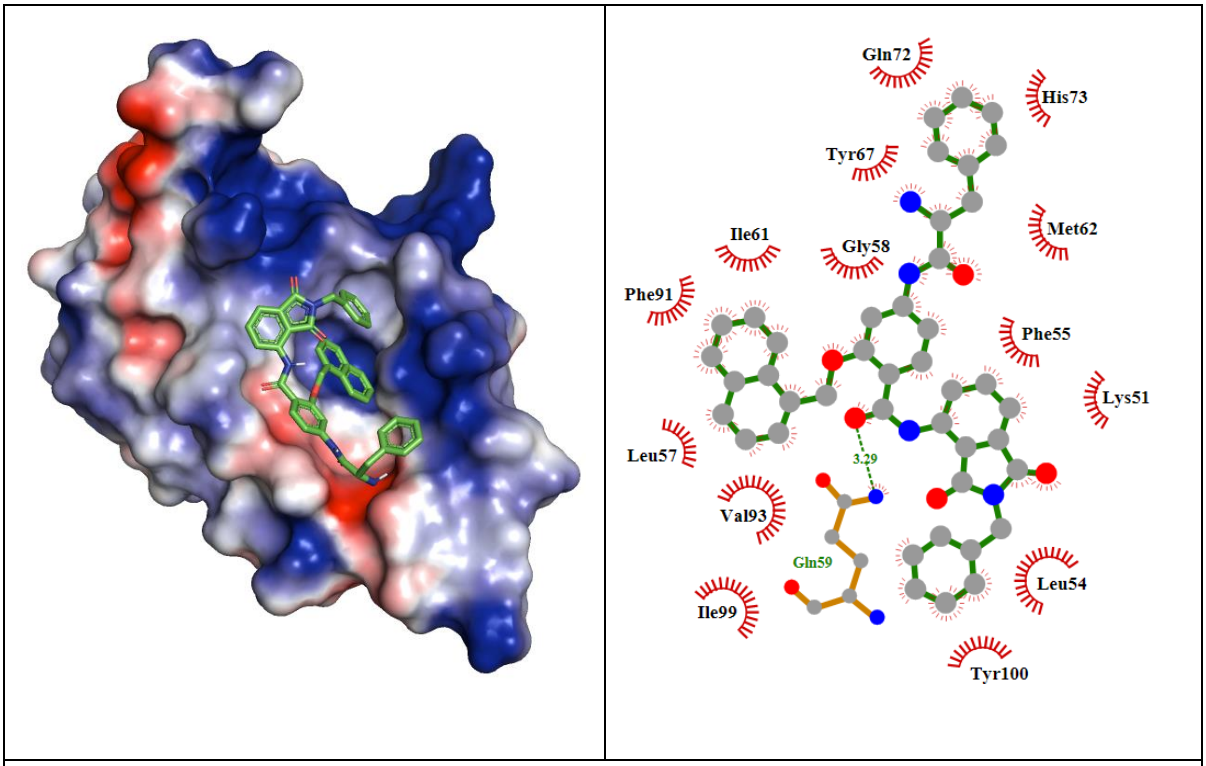


Figure S22. A) Docked conformation of **SK21** (as free amine) with HDM2 protein. The figure was made by PyMOL. B) 2D-plot with possible non-covalent interactions between helix mimetic and HDM2. The figure was made by LigPlot+.

Table S9. Analysis of docking study with helix mimetic and HDM2 protein (PDB:1T4F).

Helix Mimetics	Binding Energy (Kcal/mol)	Inhibition Constant (K_i) at 298.15 K	Non-covalent Interactions	H-Bonding
SK17	-8.64	467.96 nM	Lys51, Leu54, Phe55, Leu57, Gly58, Ile61, Met62, Tyr67, Gln72, Phe91, Val93, Ile99.	Between Gln59 and ligand amide carbonyl.
SK18	-9.29	155.03 nM	Lys51, Leu54, Phe55, Leu57, Gly58, Gln59, Ile61, Met62, Tyr67, Phe91, Val93, His96, Ile99, Tyr100.	Between Gln72 and ligand terminal amine.
SK19	-8.77	369.90 nM	Lys51, Leu54, Phe55, Leu57, Gly58, Ile61, Tyr67, Val75, Val93, His96, Ile99, Tyr100.	Between Gln72 and ligand terminal amine.
SK20	-7.77	2.02 µM	Lys51, Leu54, Phe55, Leu57, Gly58, Gln59, Met62, Tyr67, Val93, His96, Ile99, Tyr100.	Between Gln72 and ligand terminal amine.
SK21	-9.18	187.42 nM	Lys51, Leu54, Phe55, Leu57, Gly58, Ile61, Met62, Tyr67, Gln72, His73, Phe91, Val93, Ile99. Tyr100.	Between Gln59 and ligand amide carbonyl.

6. Biological Studies and SEM data

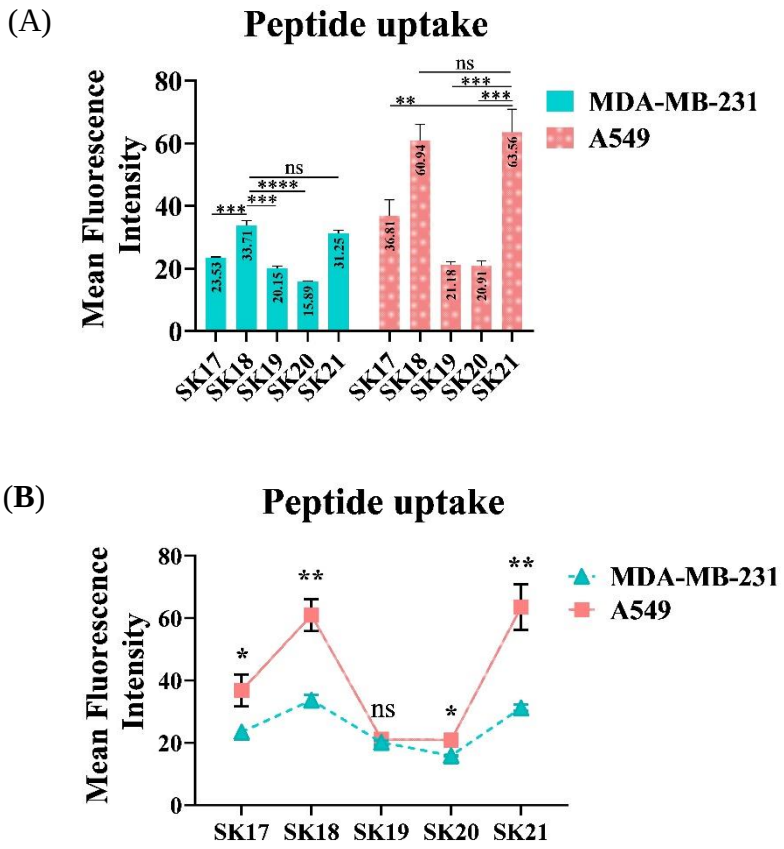


Figure S23. (A)Graphs representing the statistical analysis of mean fluorescence intensity derived from the fluorescence microscopic images of cells treated with **SK17**, **SK18**, **SK19**, **SK20**, and **SK21**. MDA-MB-231 cells (green) and A549 cells (red). (B) The mean fluorescence intensity difference of the same compound treated within two different cell lines are being statistically analysed.

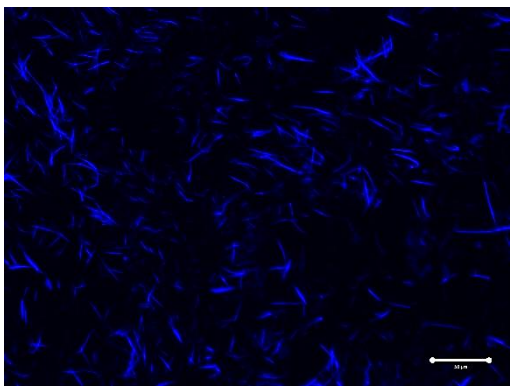
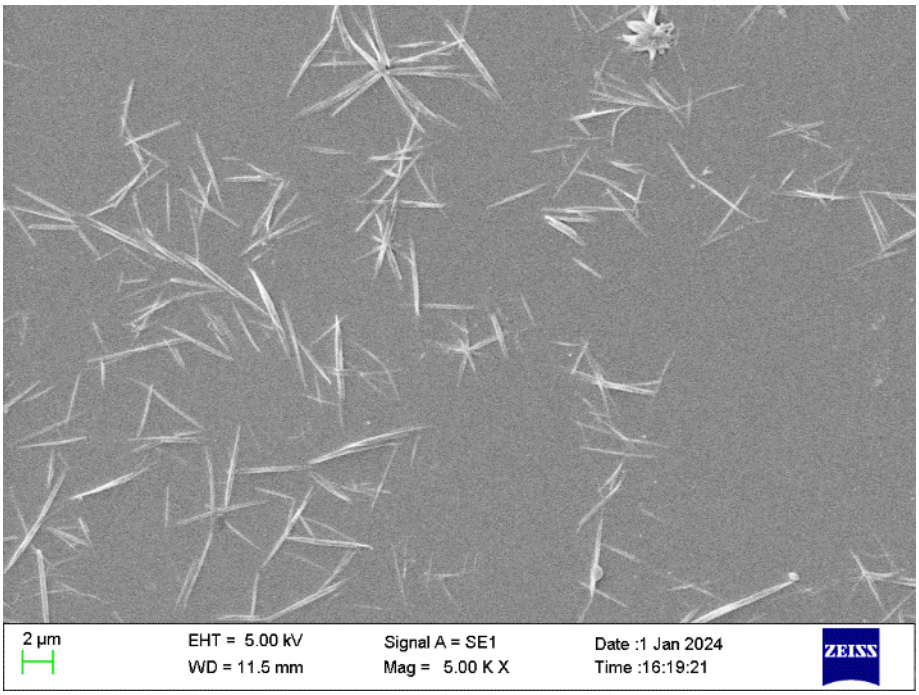


Figure S24. Needle morphology of **SK20** captured through Fluorescence microscope with 20X objective lens.

SEM data

10 mM stock solution of the helix mimetic **SK20** was prepared in DMSO and sonicated for 20 min. After sonication, 25 μM solution was prepared by diluting the stock solution with water. Again, sonicated for 20 min and 5μL of **SK20** aliquot was drop casted on a silicon wafer and dried in a vacuum desiccator for 12 h. After coating with gold, SEM images were taken at 5 kV using EV018 Zeiss instrument.

(**A**)



(**B**)

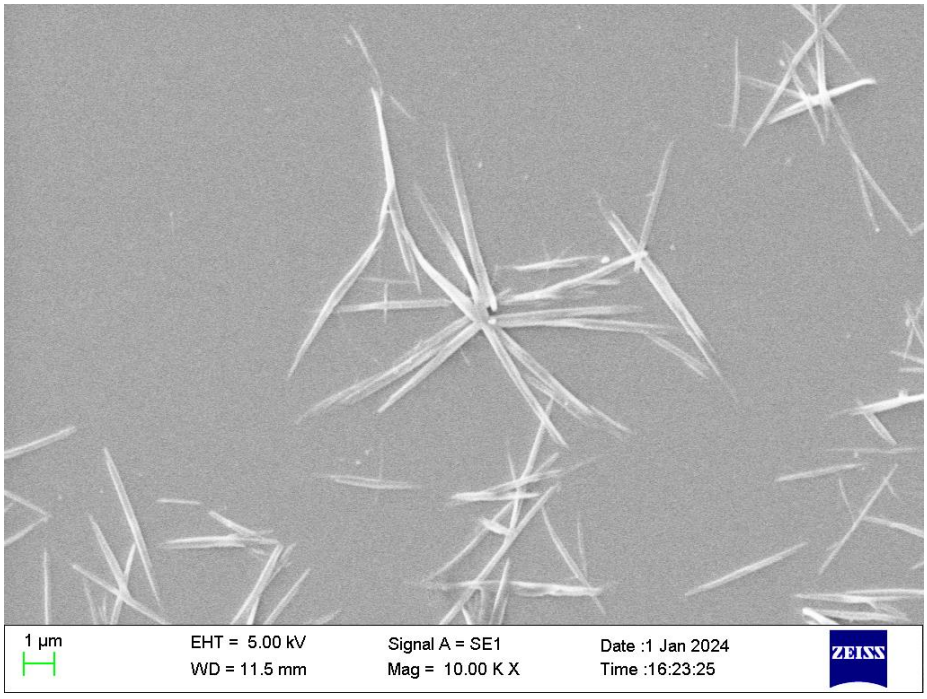
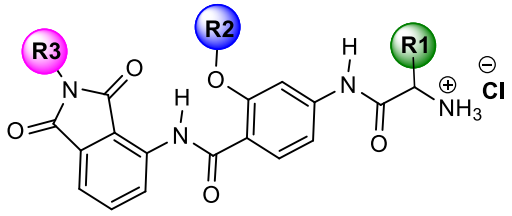
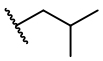
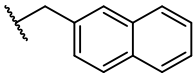
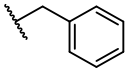
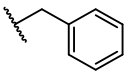
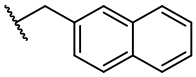
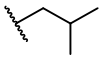
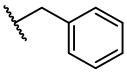
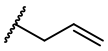
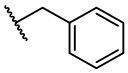
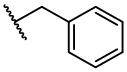
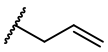
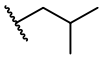
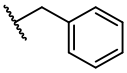
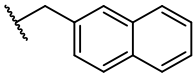
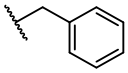


Figure S25. SEM images (**A** & **B**) **SK20** helix mimetic in 0.1% DMSO in water at 25 μM concentration showing the morphology of assembled structures.

Table S10. Cell viability results with helix mimetics in three different cancer cell lines.



Compound	R3	R2	R1	IC50 (μM) A549[a]	IC50 (μM) MDA-MB-231[a]	IC50 (μM) U87MG[b]
SK17				21.08	32.57	84.18
SK18				16.29	**16.64**	**23.89**
SK19				114.00	67.12	67.06
SK20				36.57	28.63	50.49
SK21				**6.61**	27.31	>150

[a] IC_{50} values calculated based on MTT cell viability assay. [b]The values determined by MTS cell viability assay.

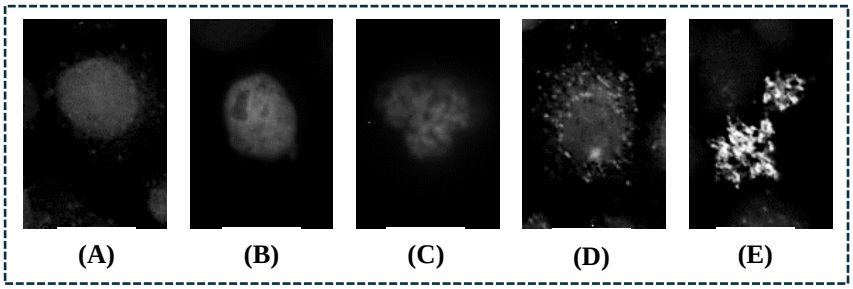


Figure S26. Nucleus stained with 7-AAD converted to grey scale images using ImageJ from the fluorescence images captured at 63X magnification. Images show varied stages of chromatin condensation as a part of apoptosis observed in groups treated with different helix mimetics: (A) uncondensed, (B) early condensation, (C) advanced condensation, (D) necklace condensation and (E) Nuclear disassembly.

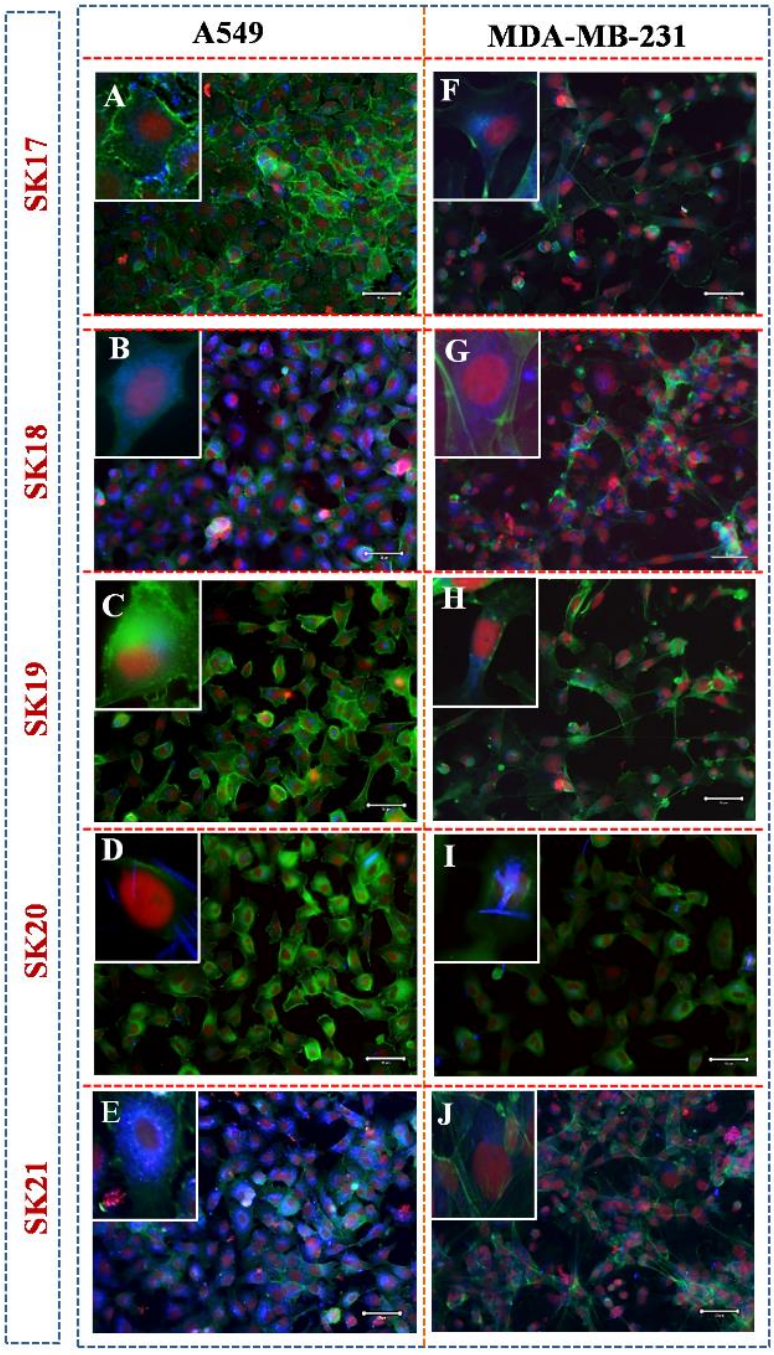
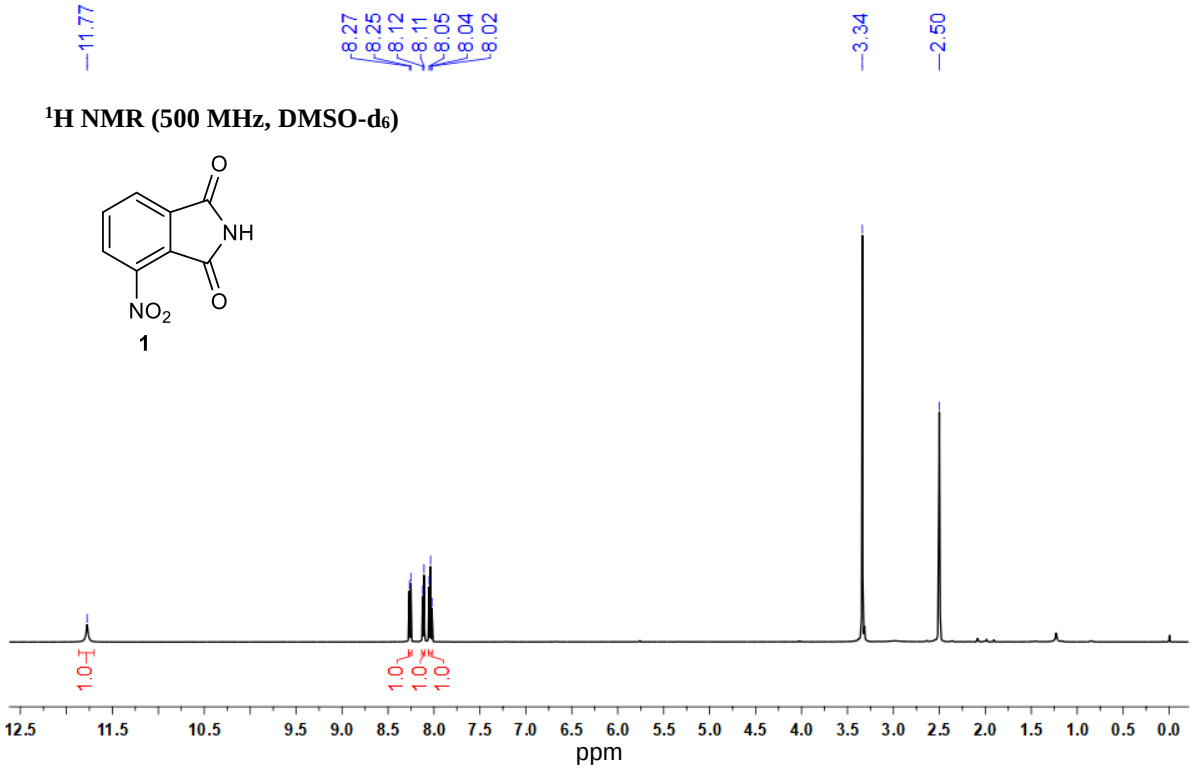


Figure S27. Fluorescence microscopic images showing helix mimetics uptake post 2 hours of incubation with 25 µM of helix mimetics individually with MDA-MB-231 and A549 cells captured at 20X objective lens, and the inset picture is the zoomed view. Helix mimetics (blue–self-fluorescent), actin (green), nucleus (red) and the merged images. Scale bar = 50µm.

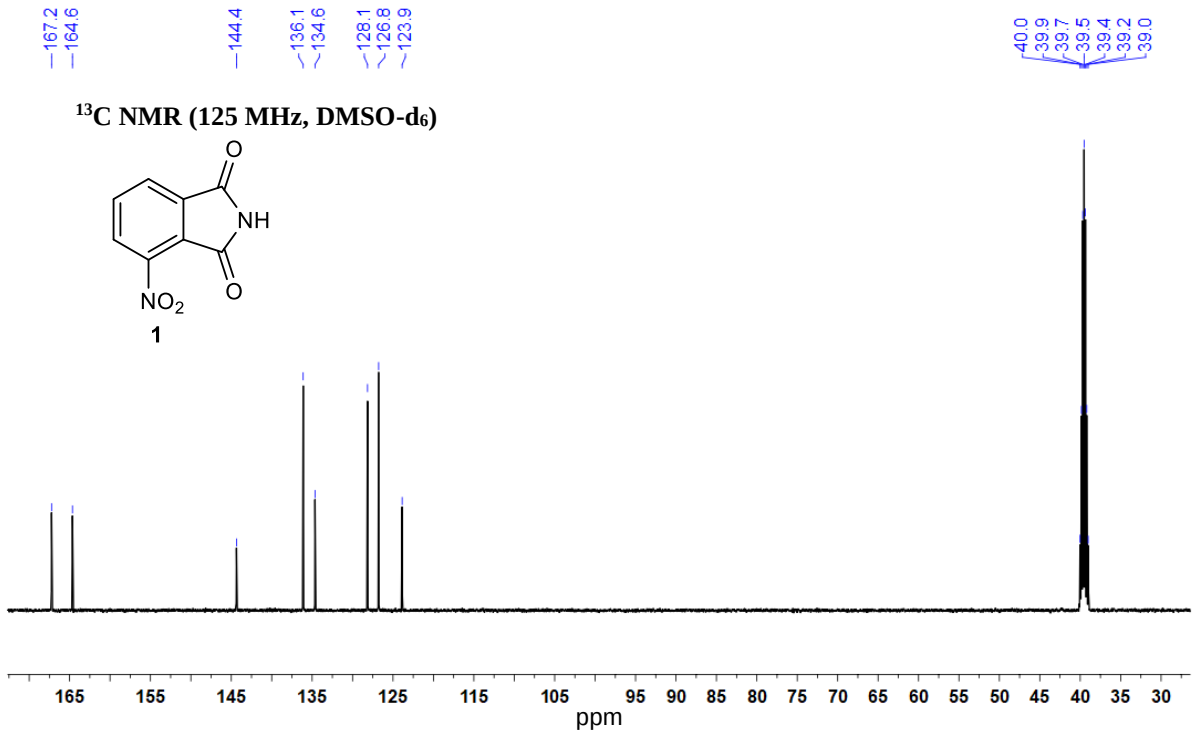
7. Characterization

7.1 ^{1}H and ^{13}C NMR spectra



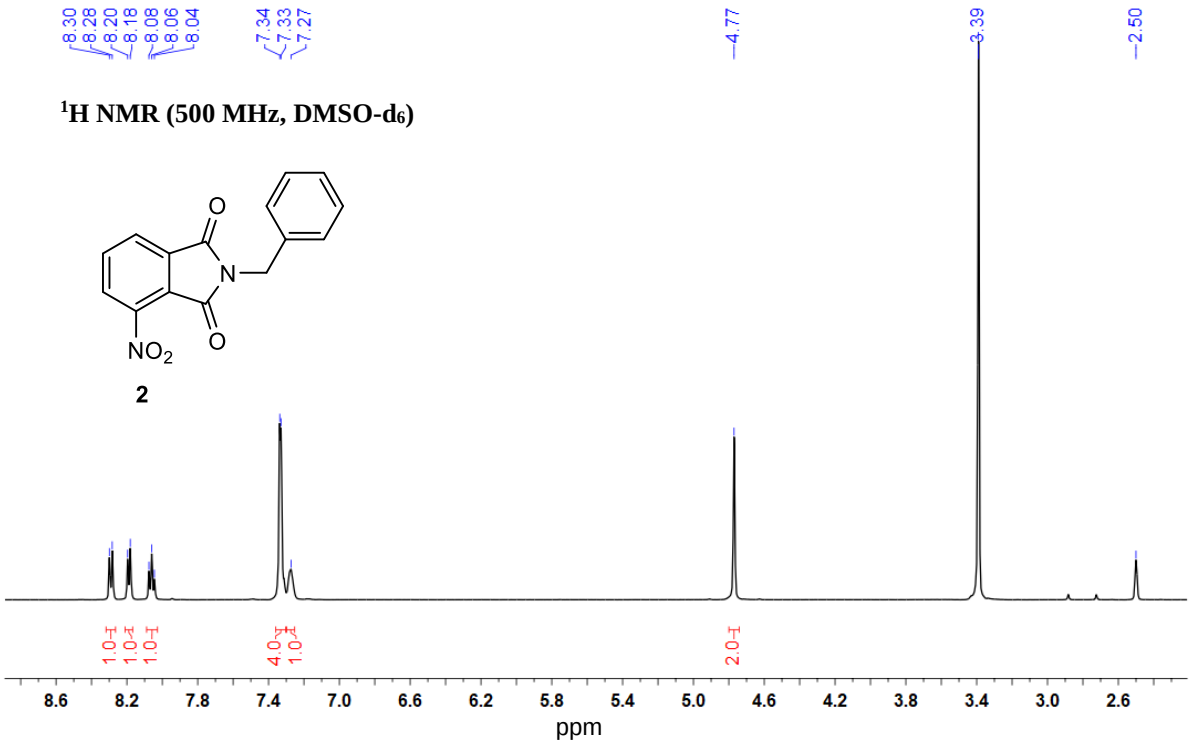
^{1}H NMR (500 MHz, DMSO-d$_6$)

1



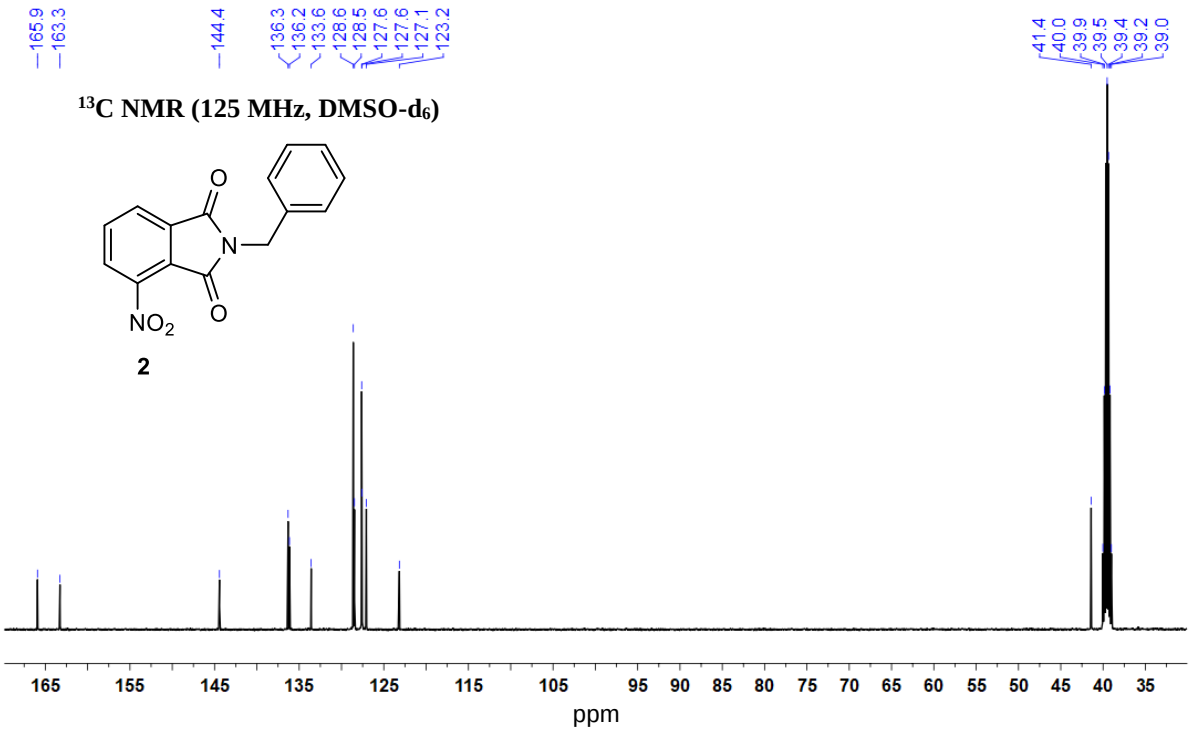
^{13}C NMR (125 MHz, DMSO-d$_6$)

1



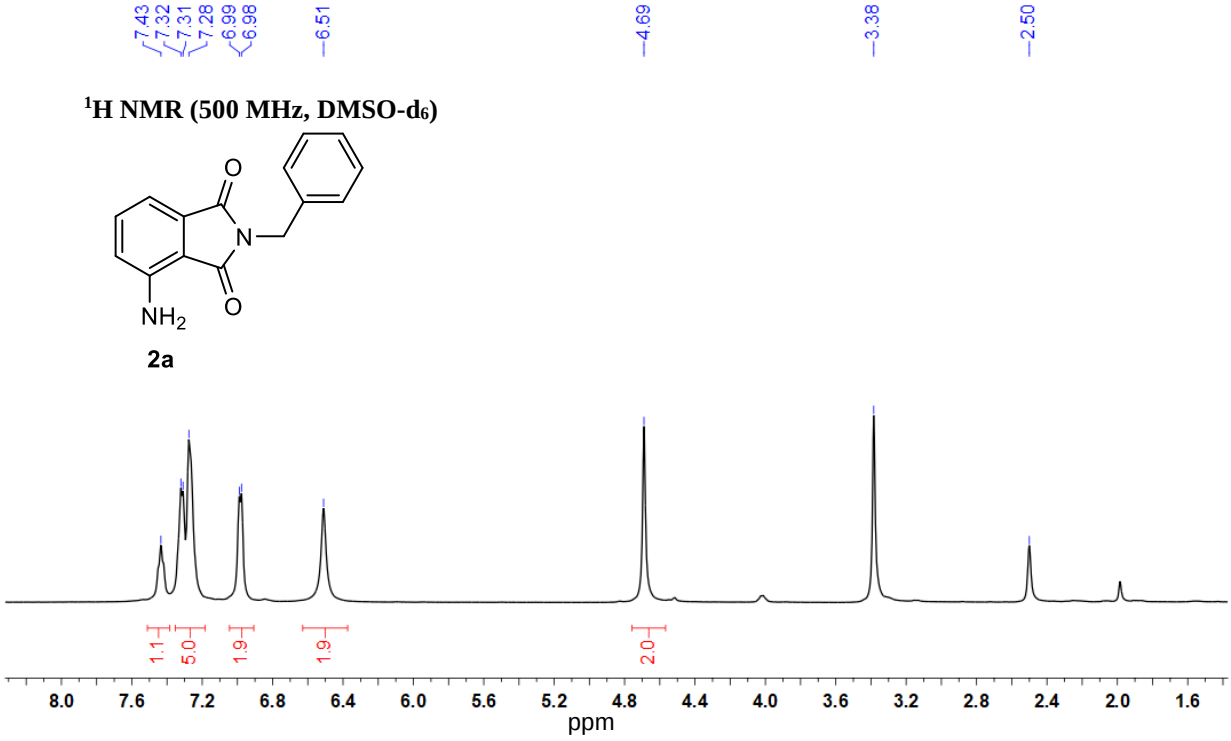
^{1}H NMR (500 MHz, DMSO-d$_6$)

2



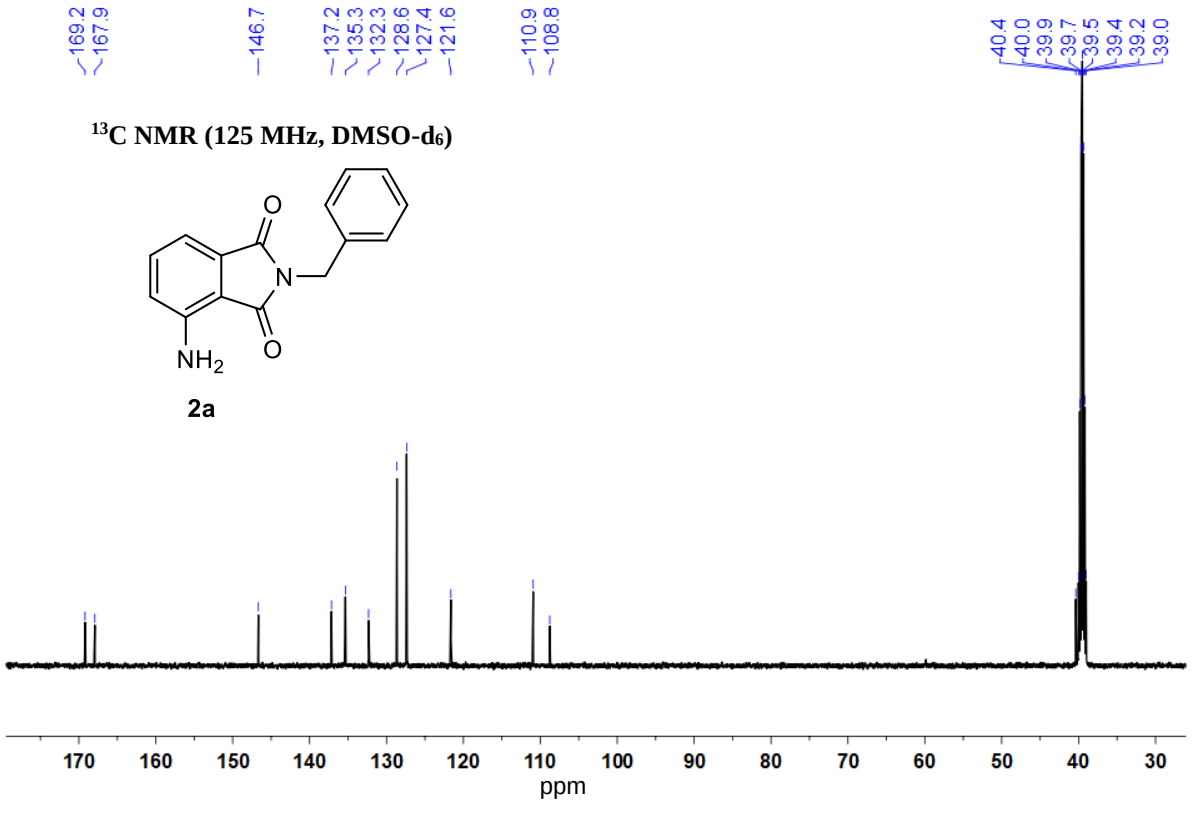
^{13}C NMR (125 MHz, DMSO-d$_6$)

2



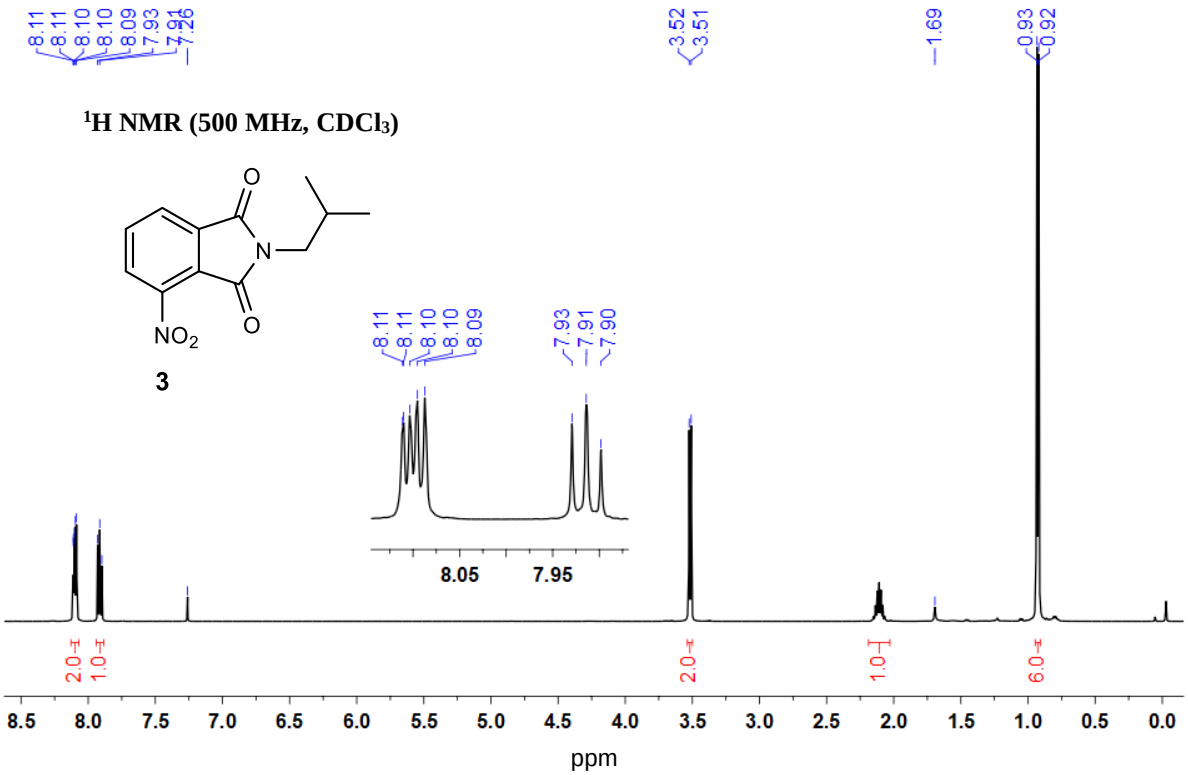
^{1}H NMR (500 MHz, DMSO-d$_6$)

2a



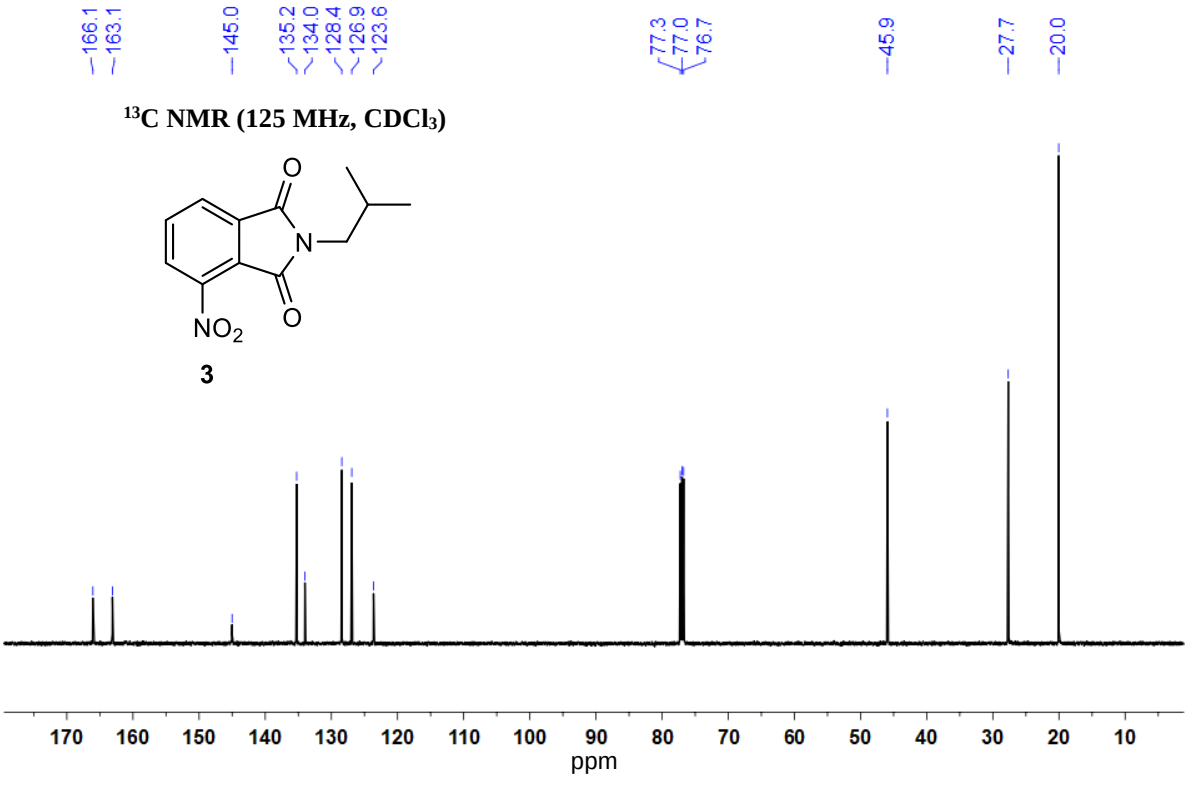
^{13}C NMR (125 MHz, DMSO-d$_6$)

2a



^{1}H NMR (500 MHz, $CDCl_3$)

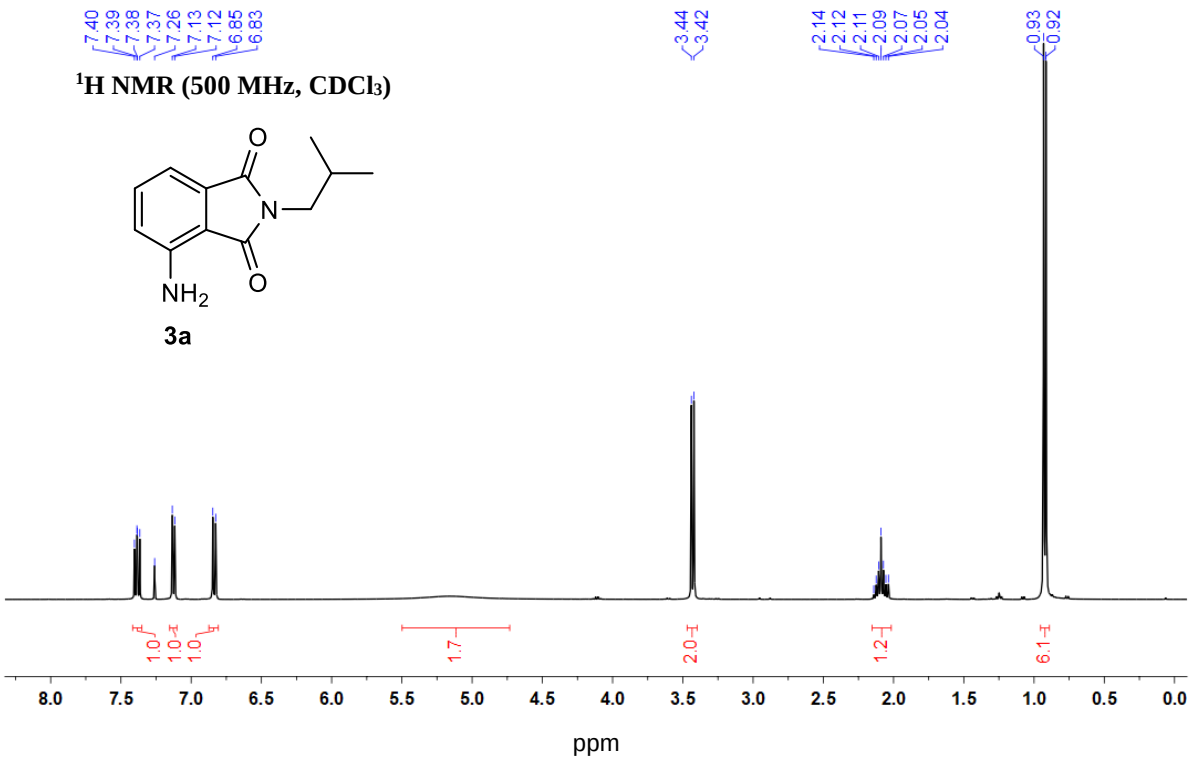
3



^{13}C NMR (125 MHz, $CDCl_3$)

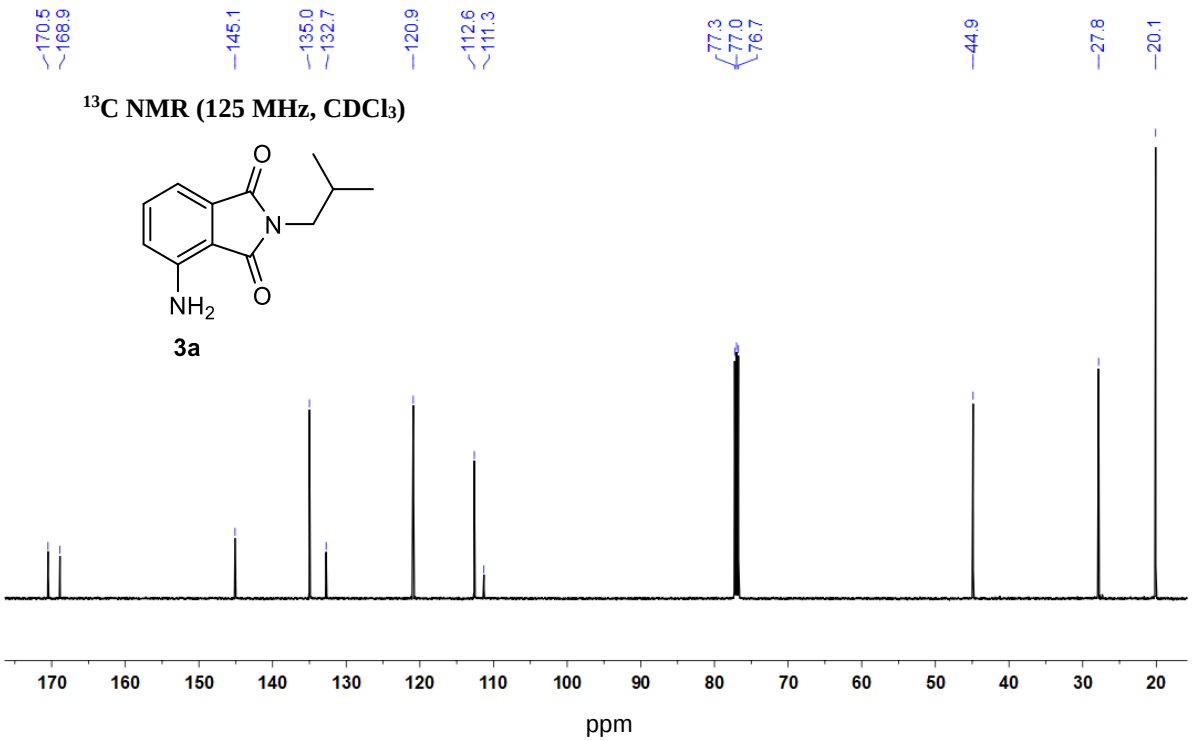
3

^{1}H NMR (500 MHz, $CDCl_3$)



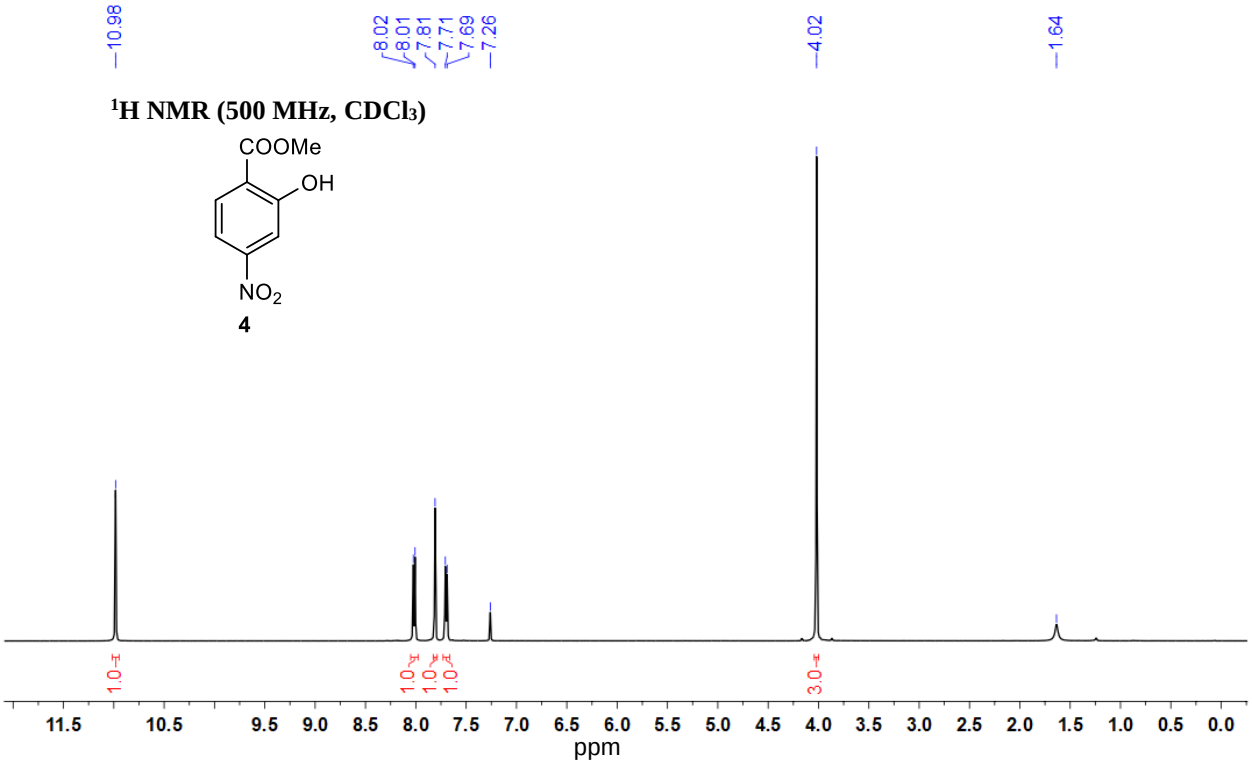
3a

^{13}C NMR (125 MHz, $CDCl_3$)

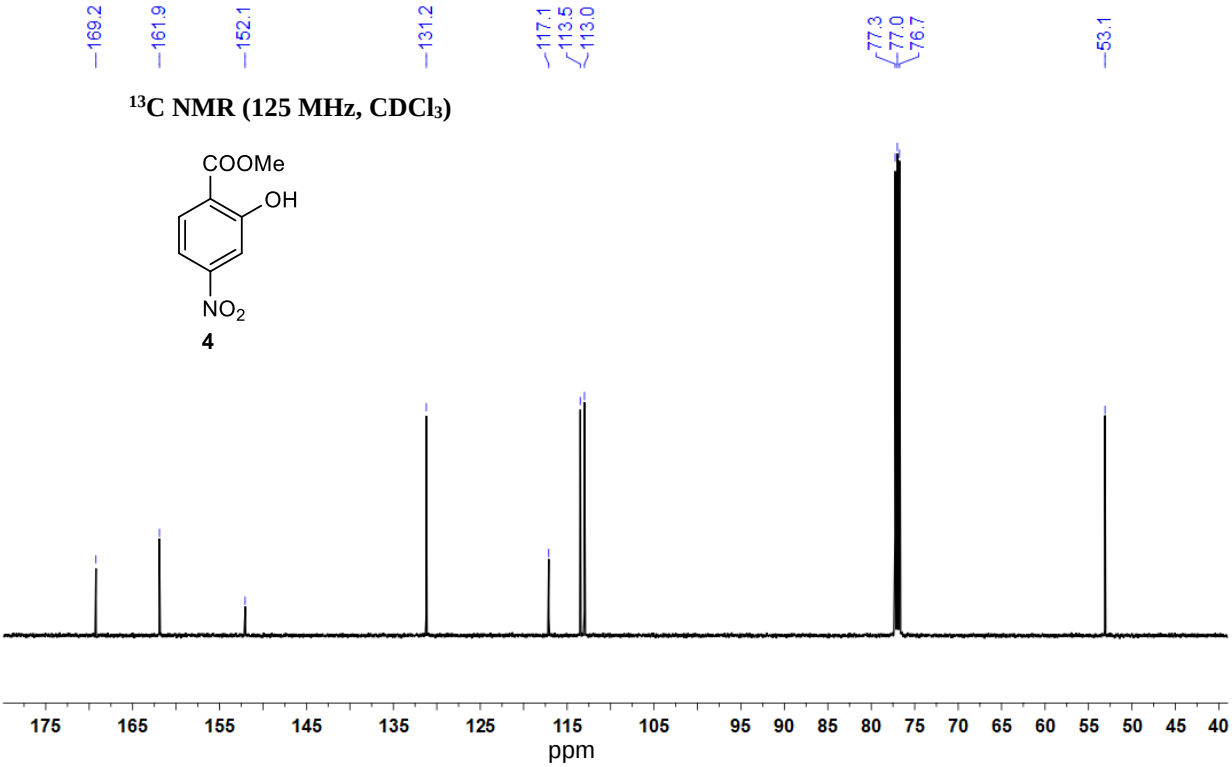


3a

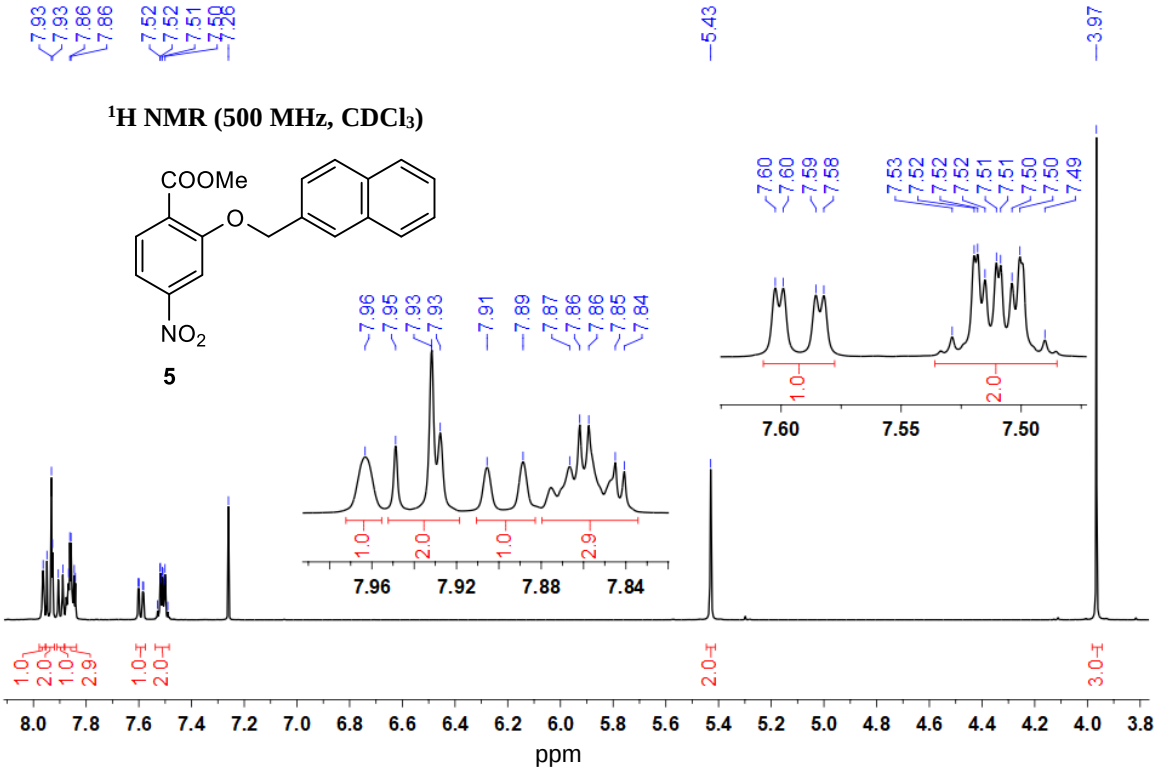
^{1}H NMR (500 MHz, $CDCl_3$)



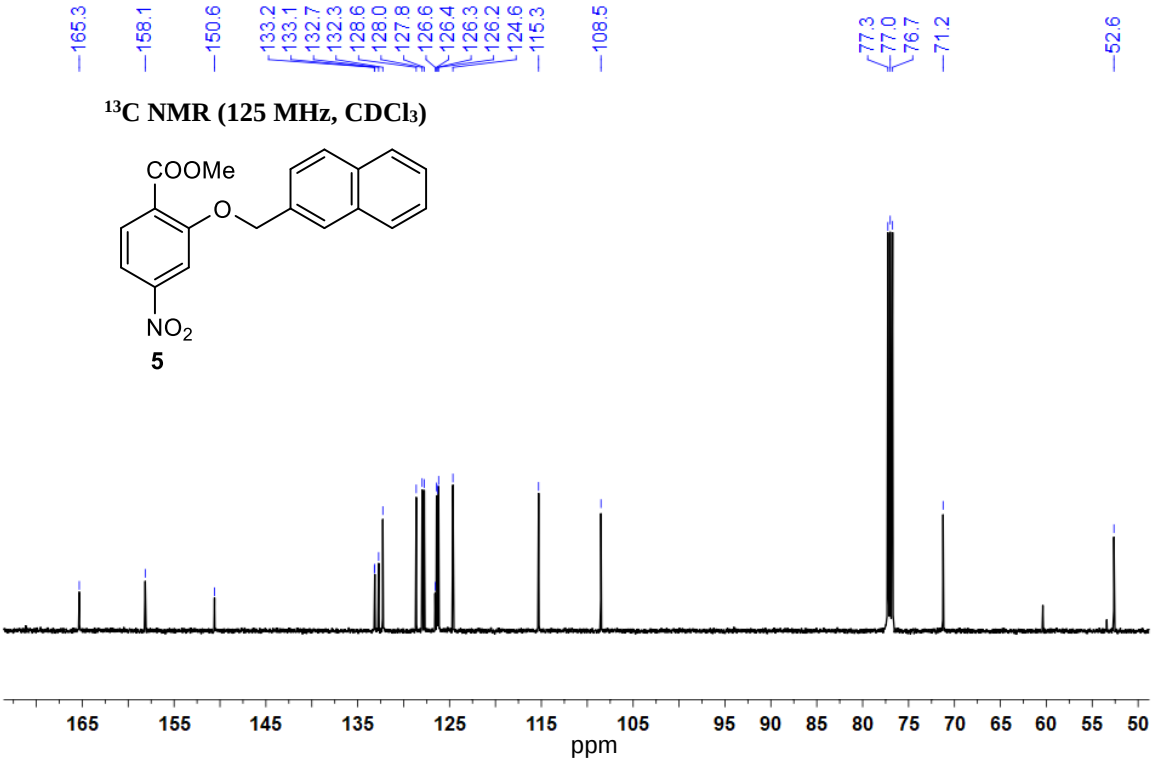
^{13}C NMR (125 MHz, $CDCl_3$)



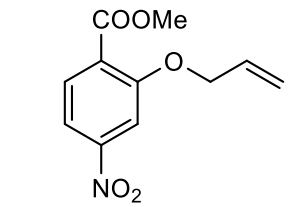
^{1}H NMR (500 MHz, $CDCl_3$)



^{13}C NMR (125 MHz, $CDCl_3$)

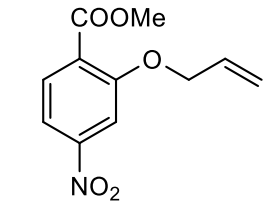


^{1}H NMR (500 MHz, $CDCl_3$)

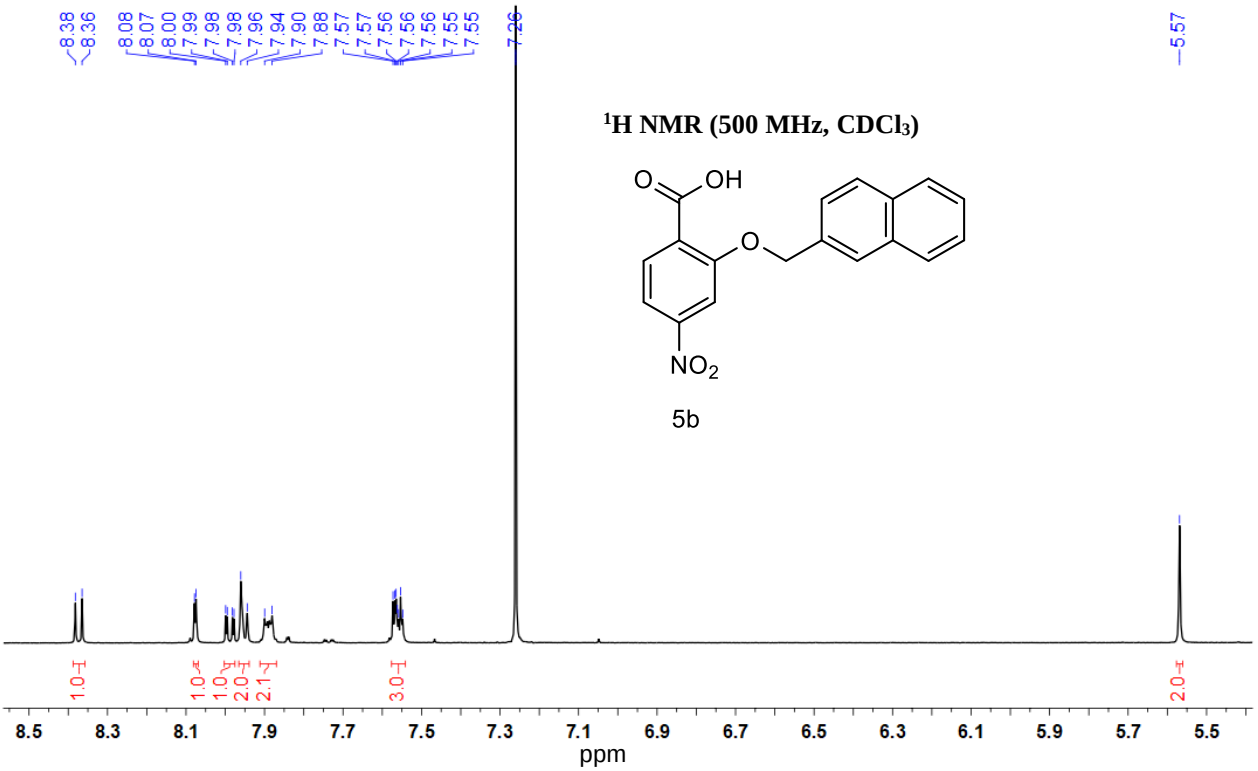


6

^{13}C NMR (125 MHz, DMSO-d_6)

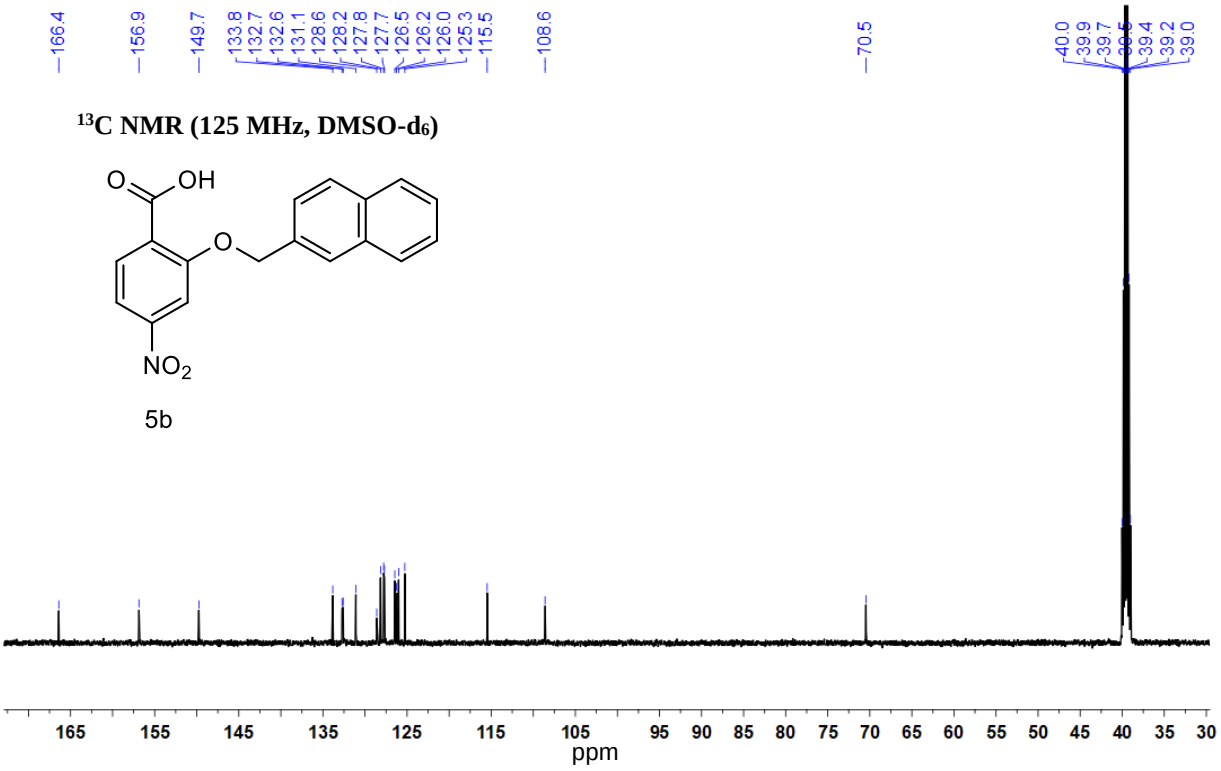


6



^{1}H NMR (500 MHz, $CDCl_3$)

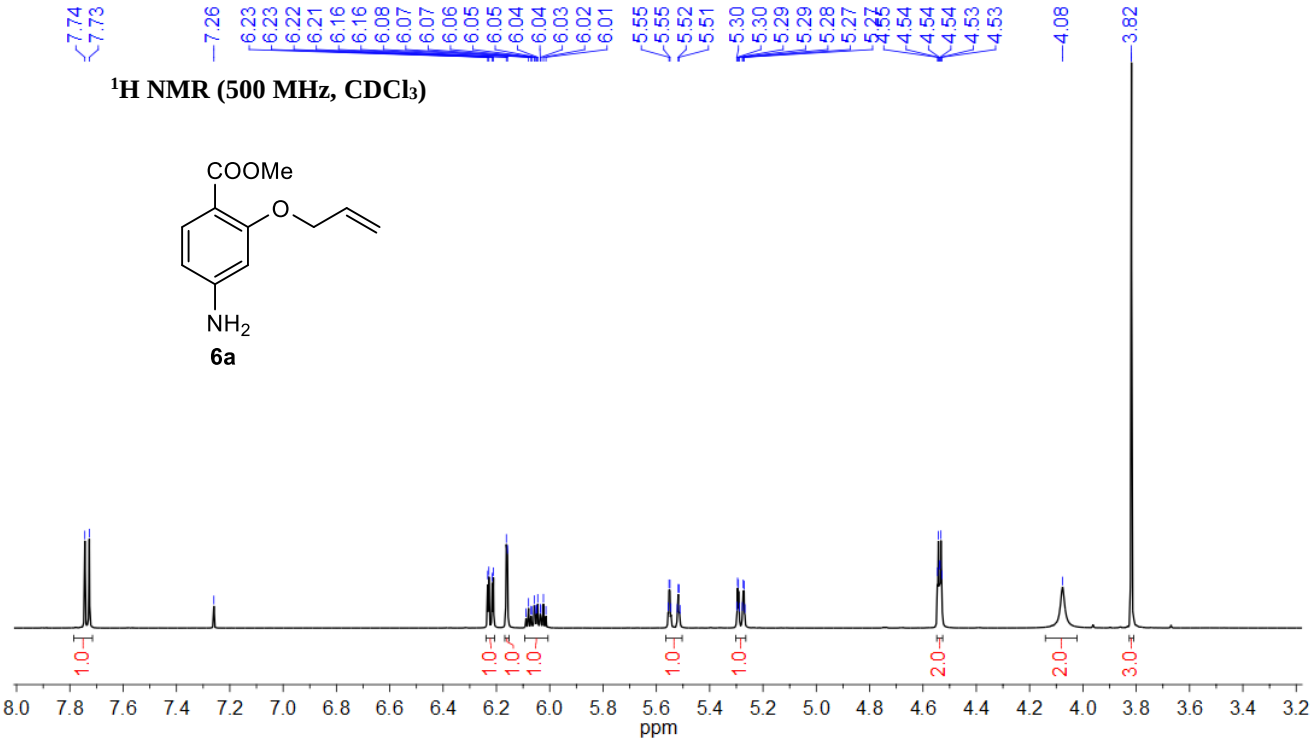
5b



^{13}C NMR (125 MHz, DMSO-d_6)

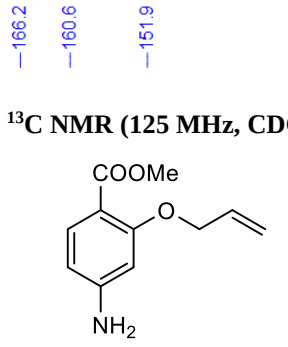
5b

^{1}H NMR (500 MHz, $CDCl_3$)



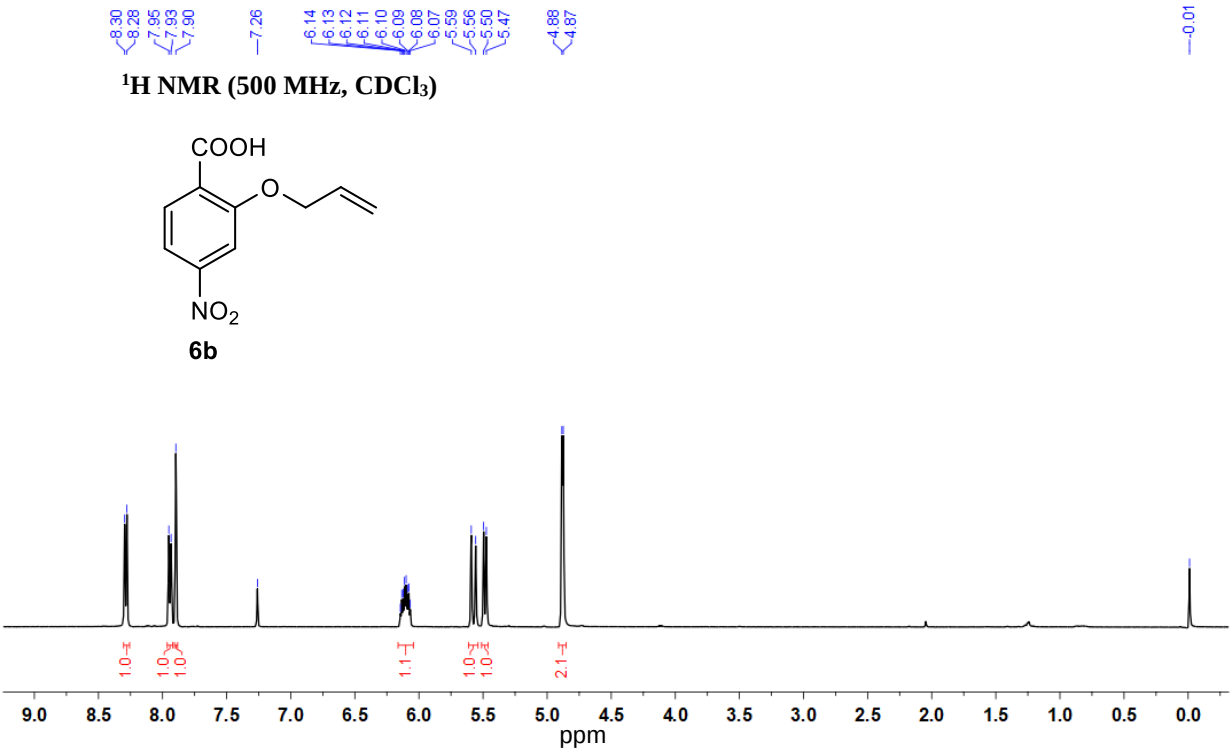
6a

^{13}C NMR (125 MHz, $CDCl_3$)



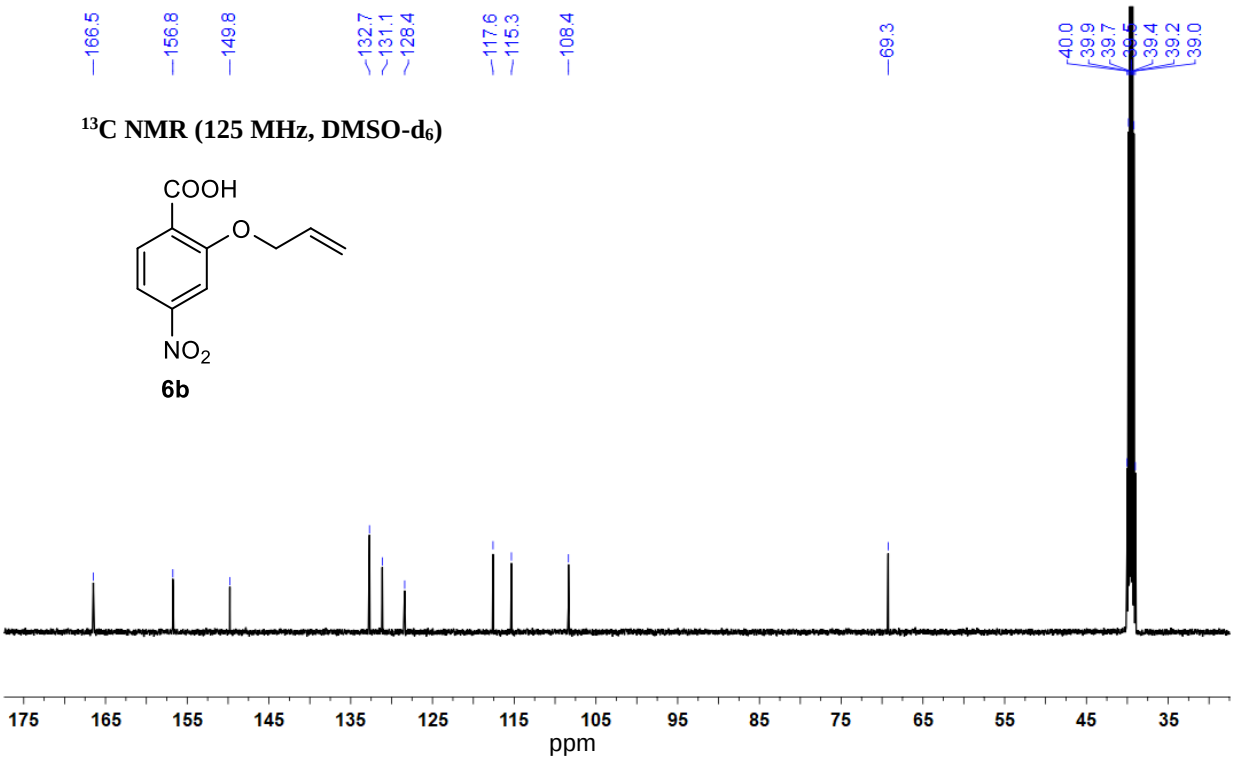
6a

^{1}H NMR (500 MHz, $CDCl_3$)



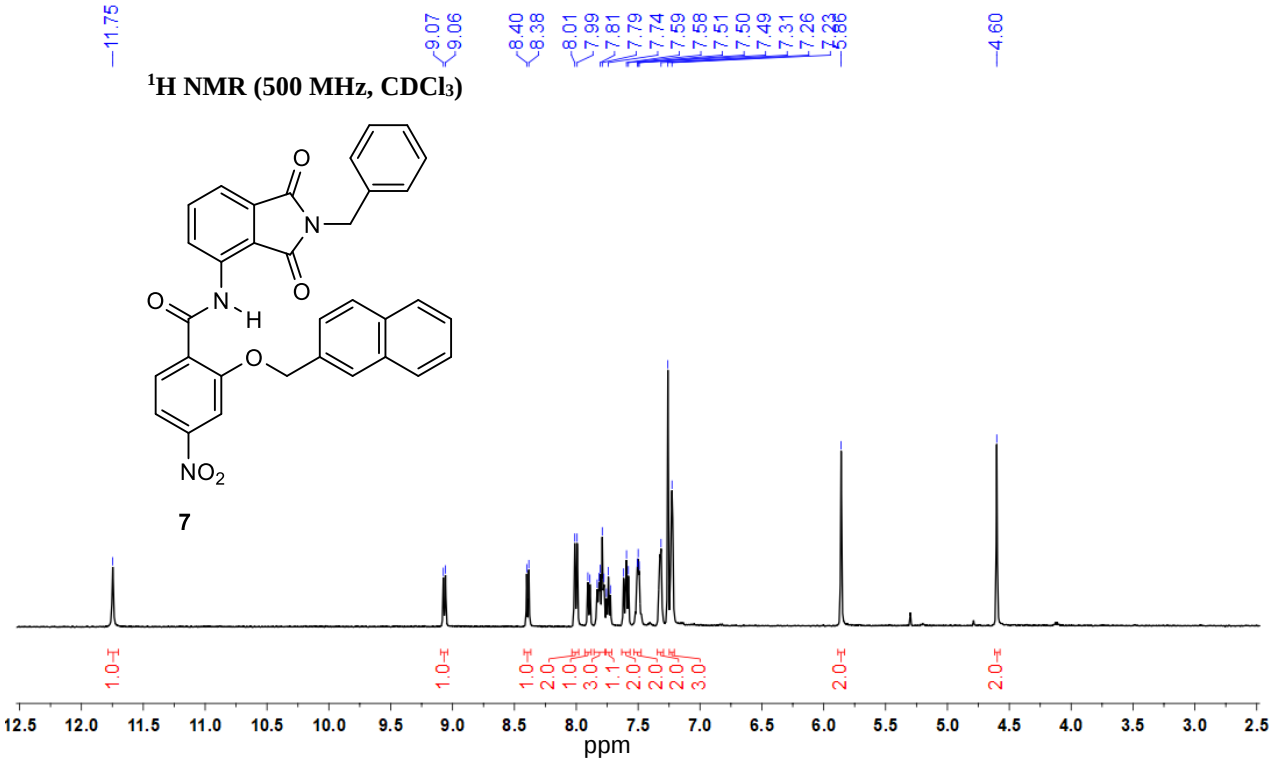
6b

^{13}C NMR (125 MHz, DMSO-d_6)

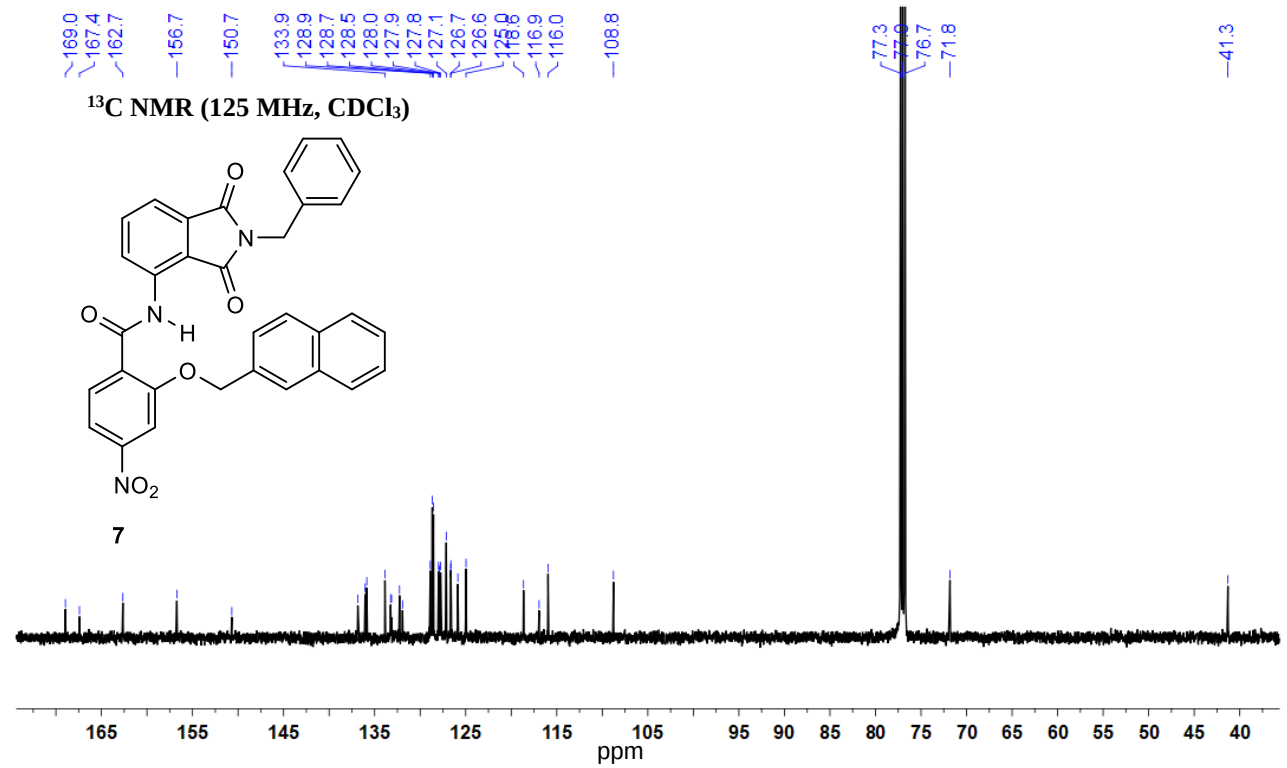


6b

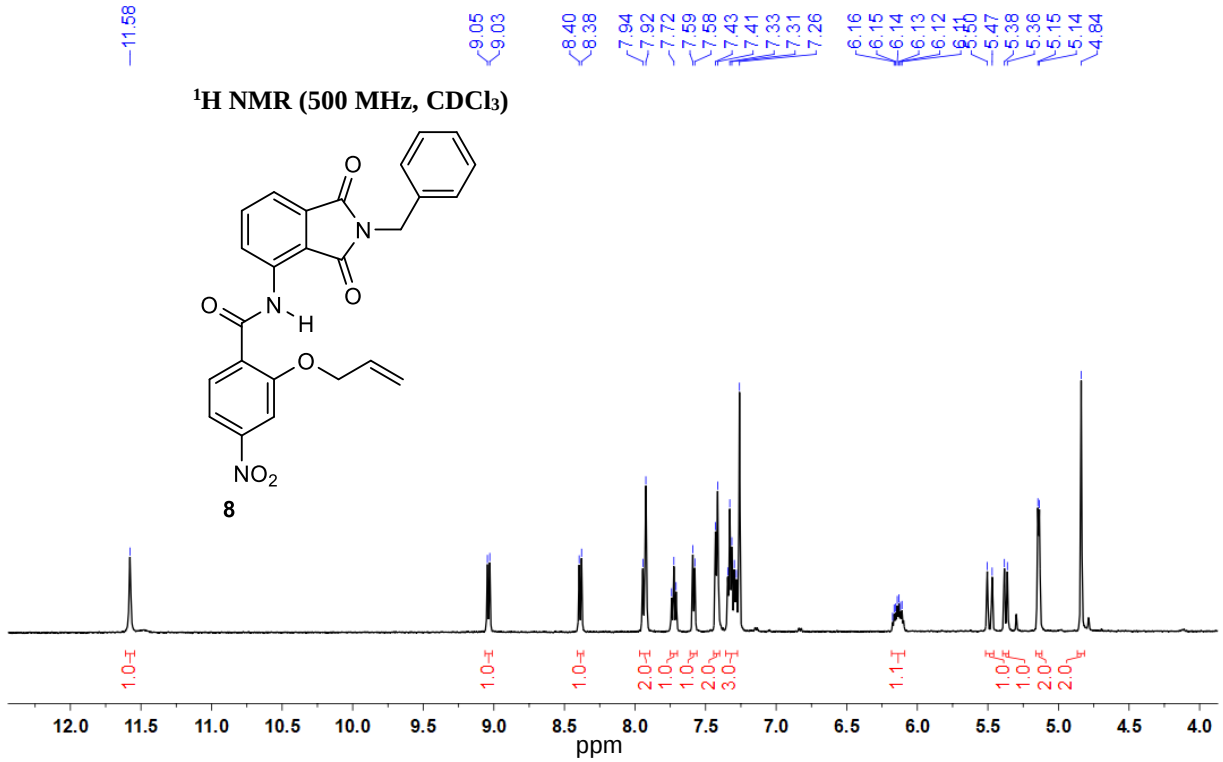
^{1}H NMR (500 MHz, $CDCl_3$)

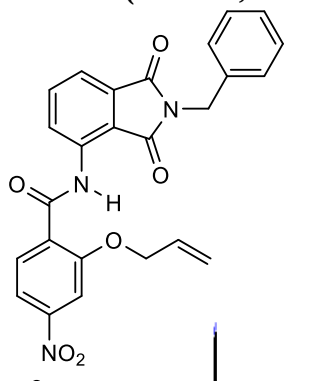


^{13}C NMR (125 MHz, $CDCl_3$)



^{1}H NMR (500 MHz, $CDCl_3$)



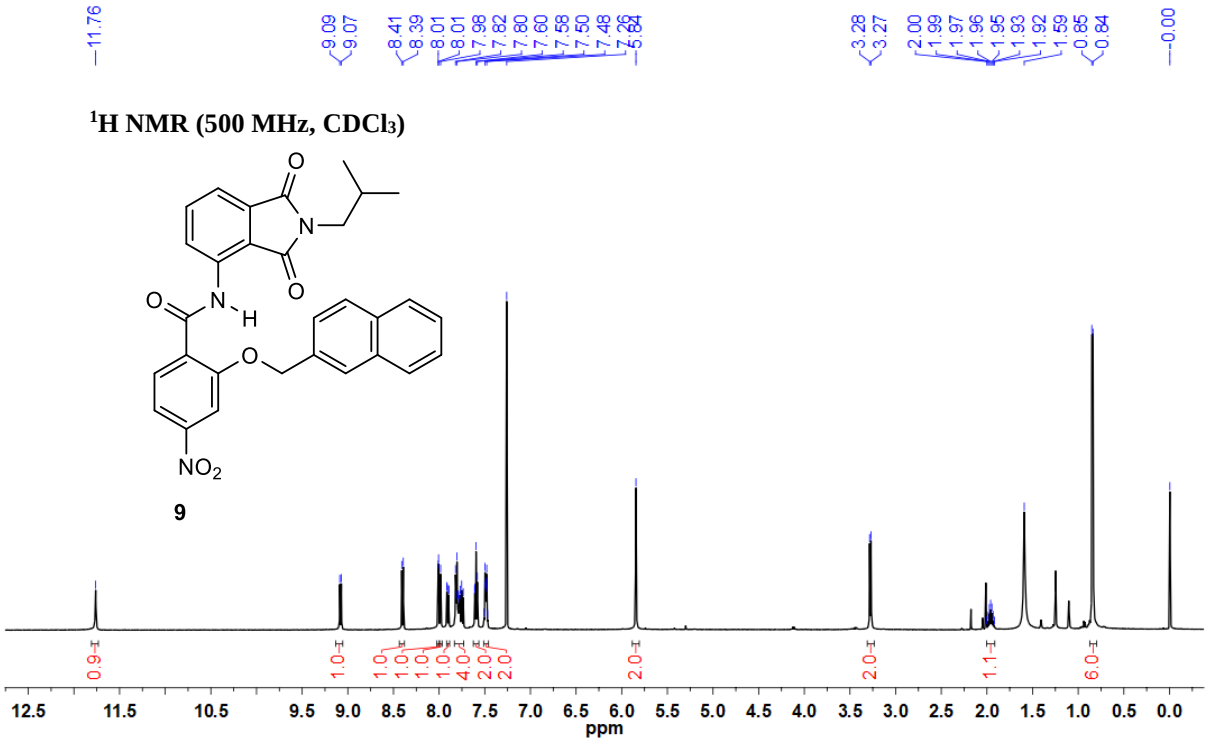


8

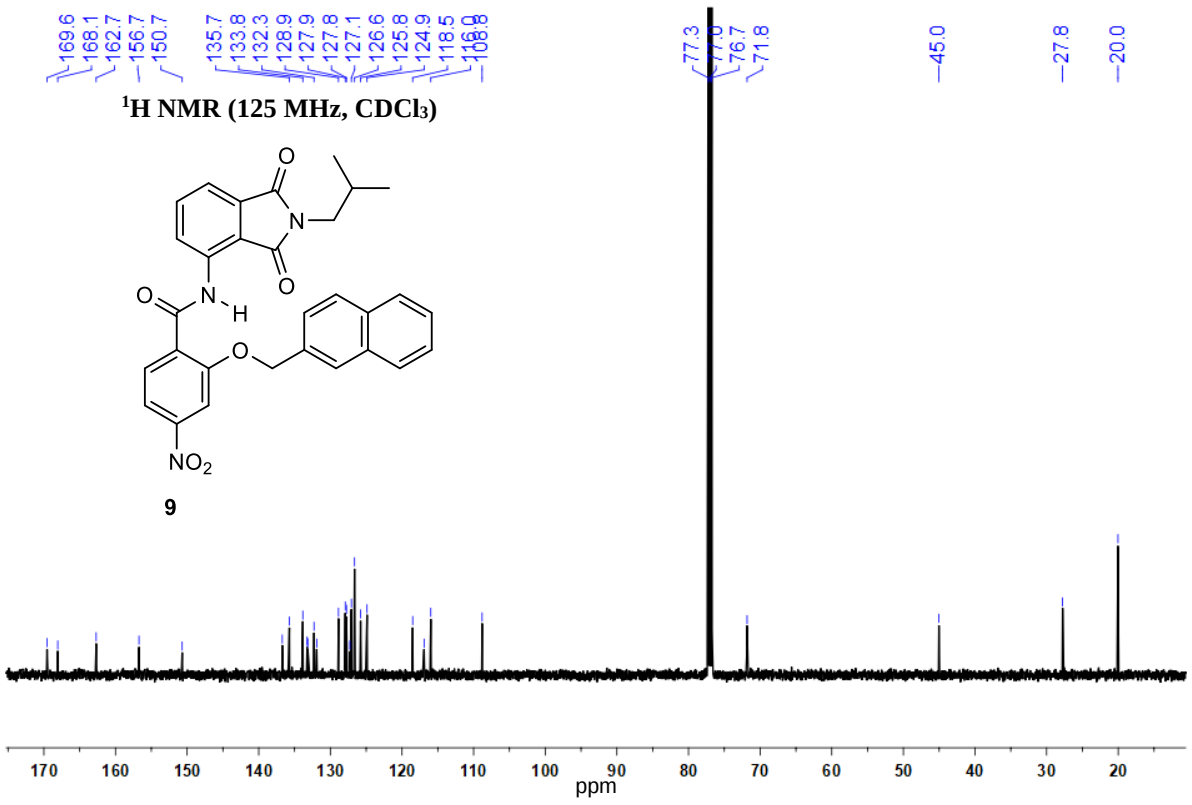
^{13}C NMR (125 MHz, $CDCl_3$)

8

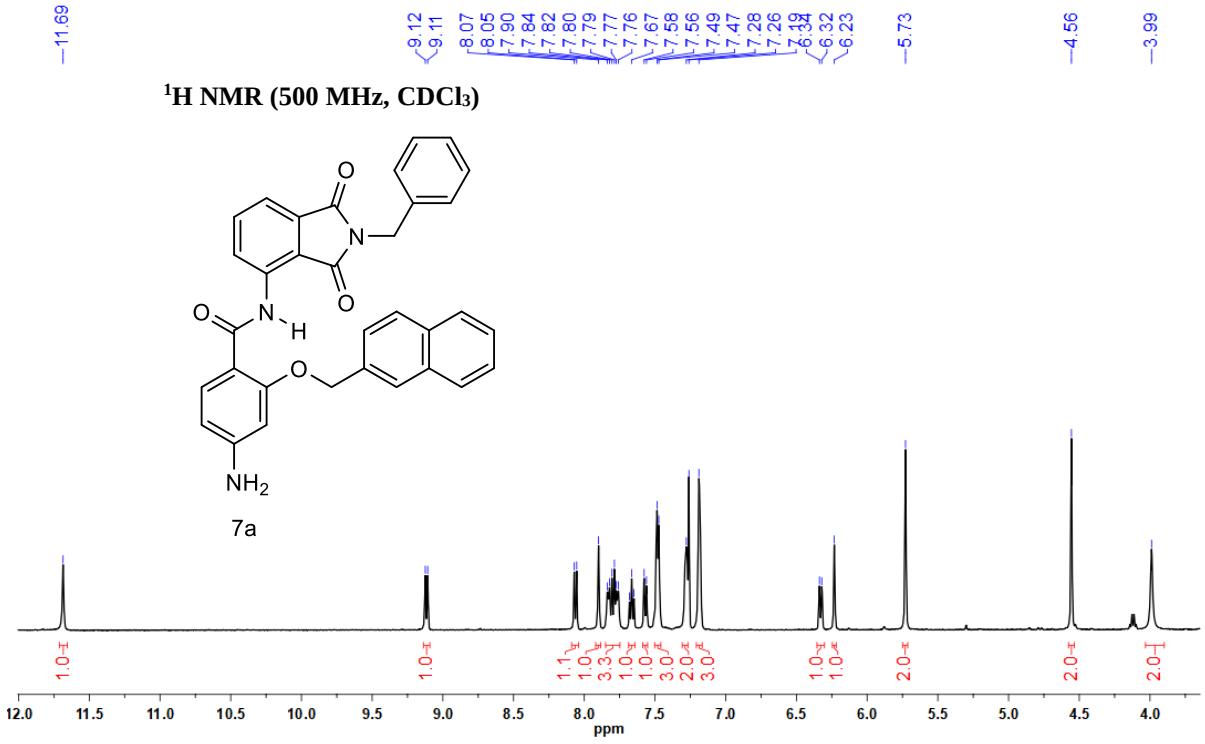
^{1}H NMR (500 MHz, $CDCl_3$)



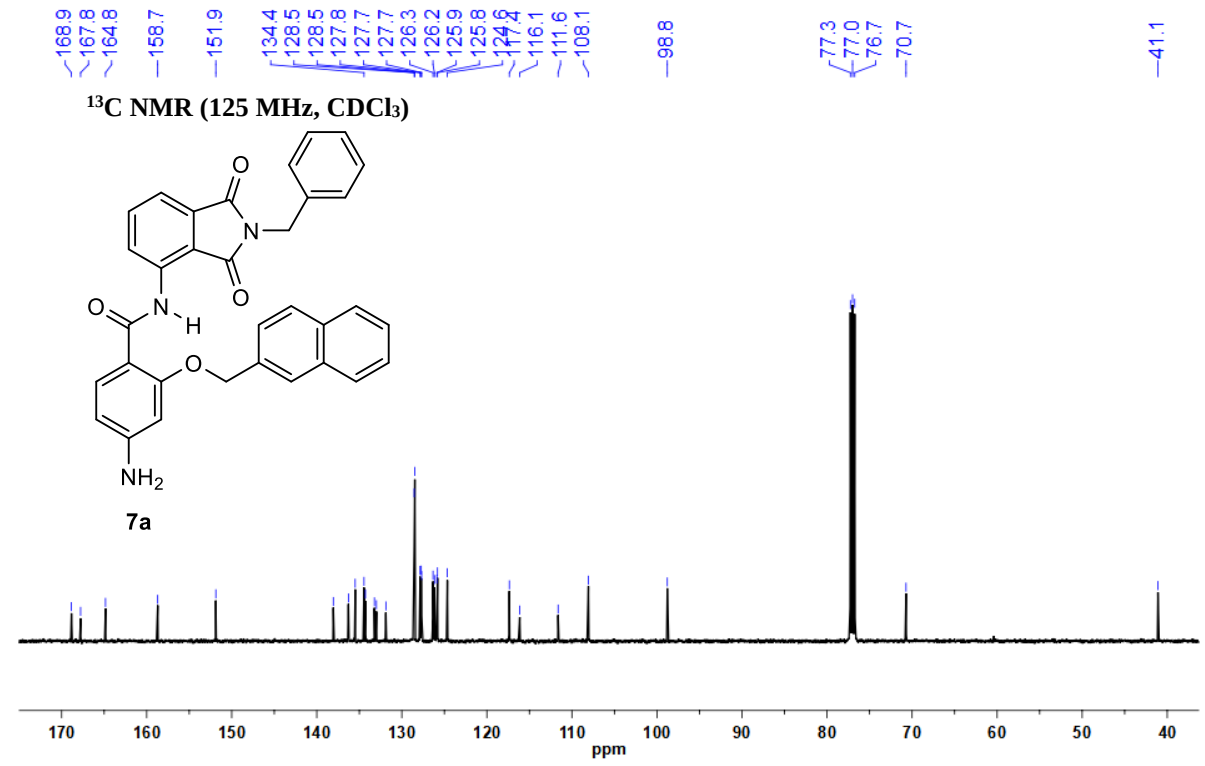
^{1}H NMR (125 MHz, $CDCl_3$)



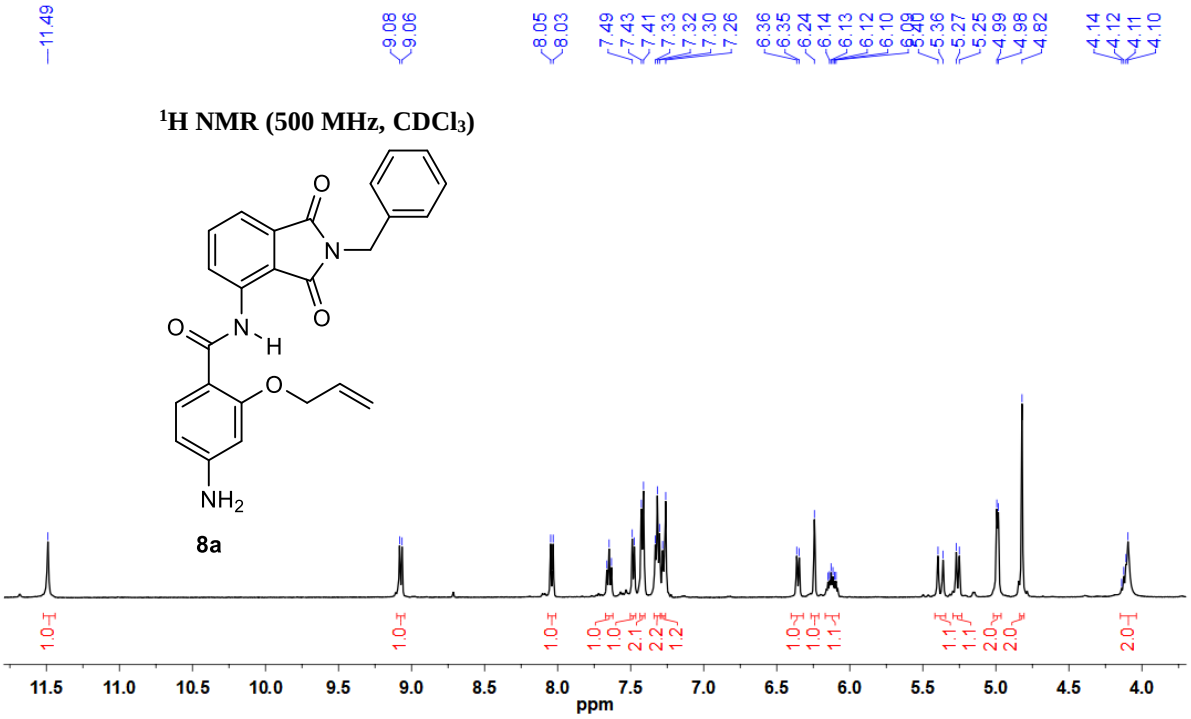
^{1}H NMR (500 MHz, $CDCl_3$)



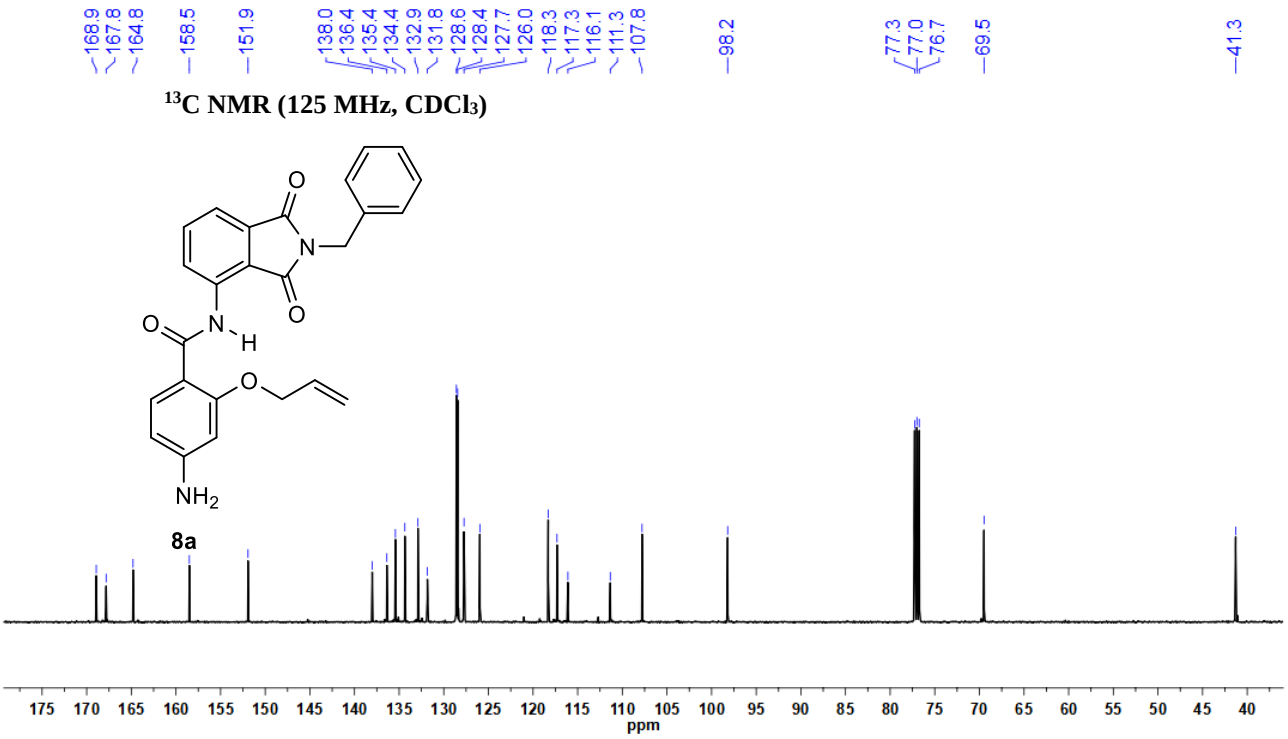
^{13}C NMR (125 MHz, $CDCl_3$)

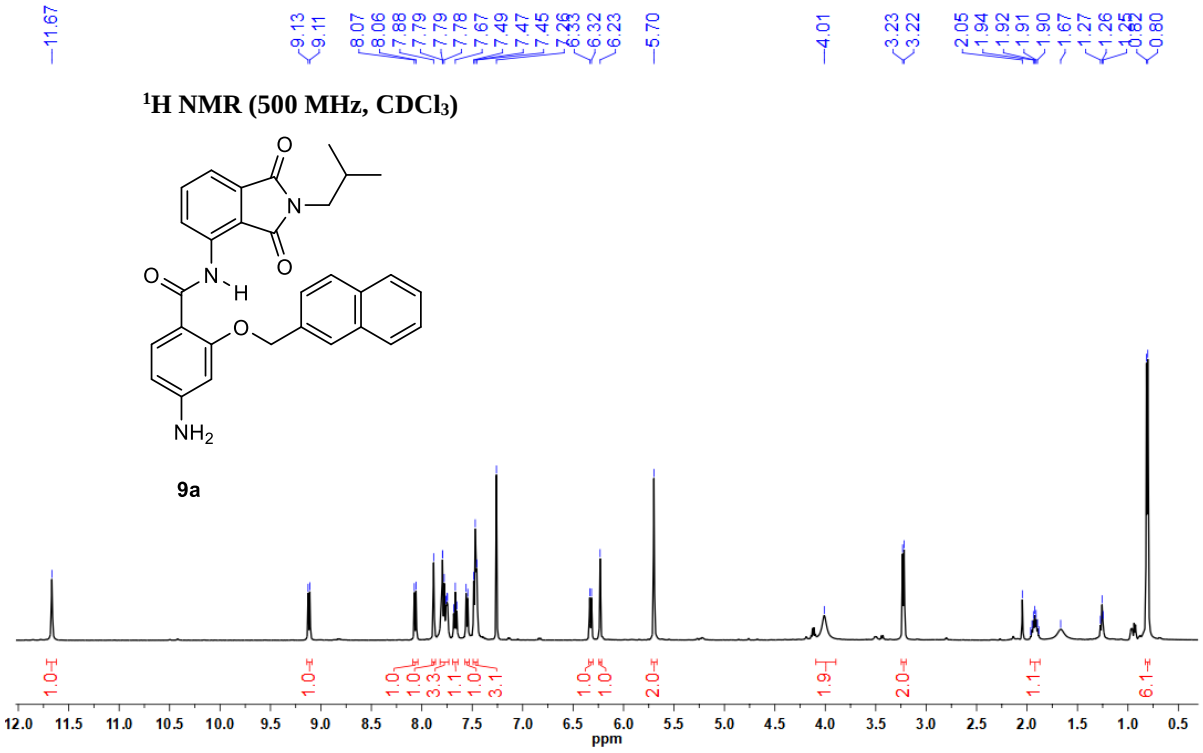


^{1}H NMR (500 MHz, $CDCl_3$)



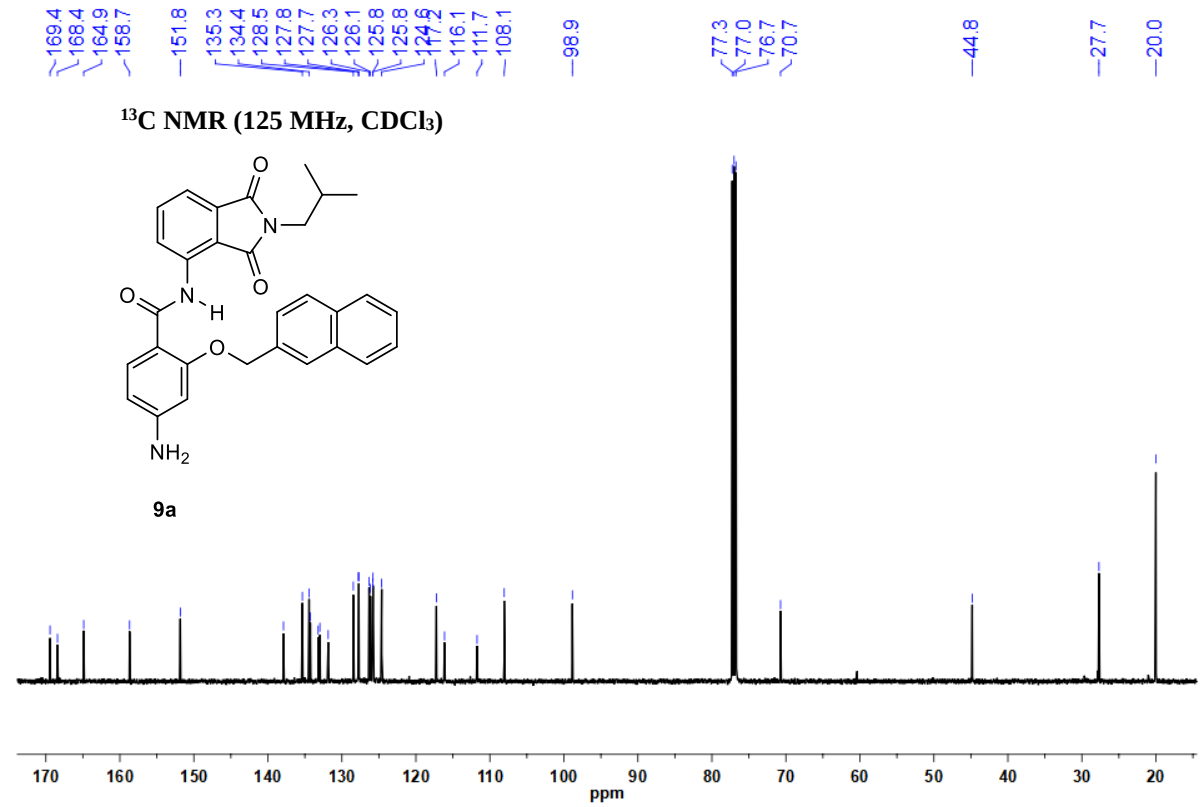
^{13}C NMR (125 MHz, $CDCl_3$)





^{1}H NMR (500 MHz, $CDCl_3$)

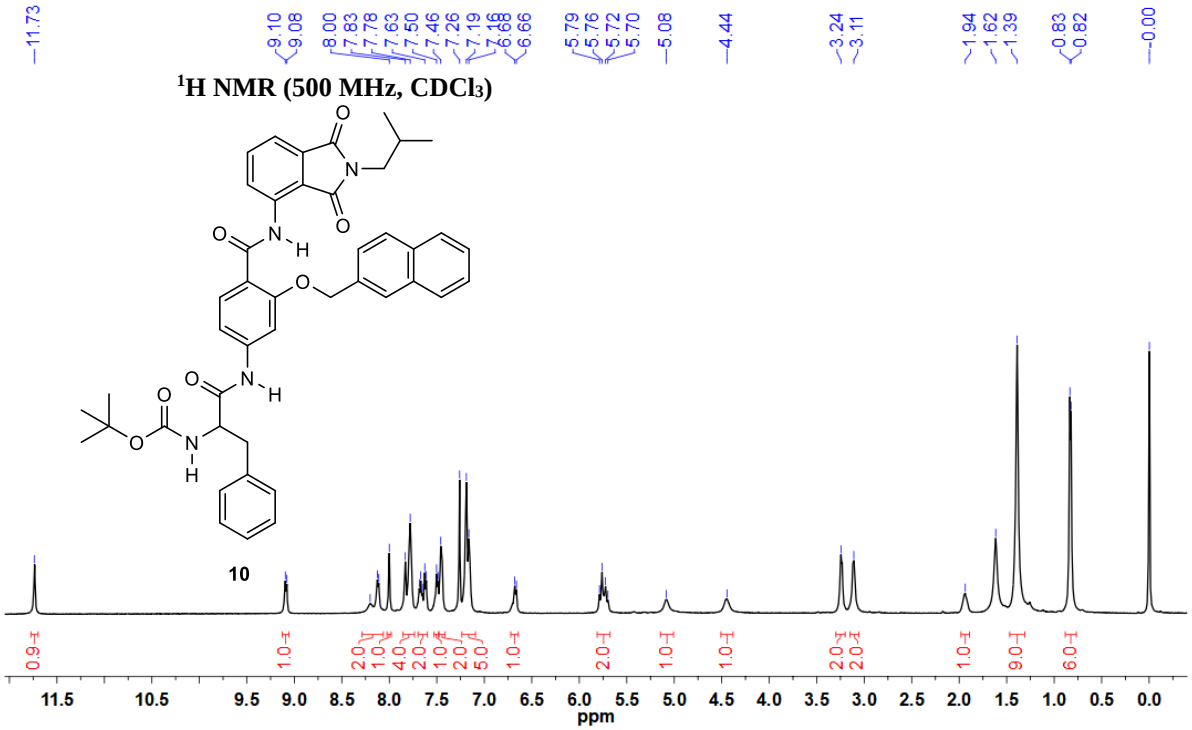
9a



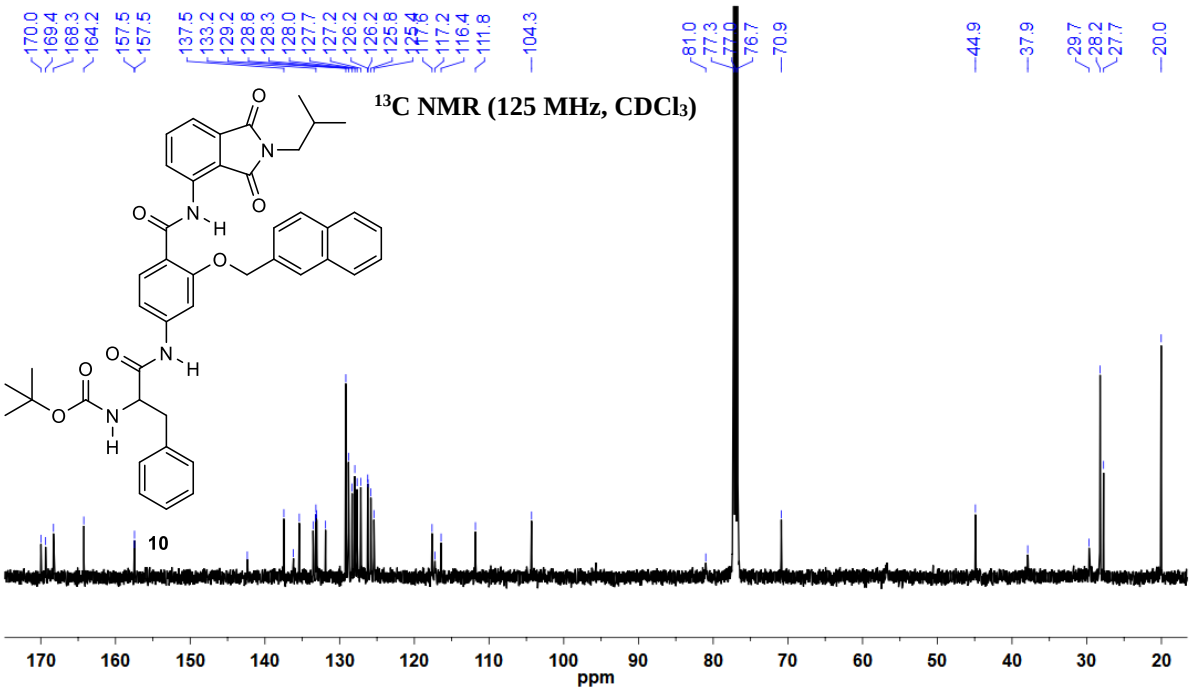
^{13}C NMR (125 MHz, $CDCl_3$)

9a

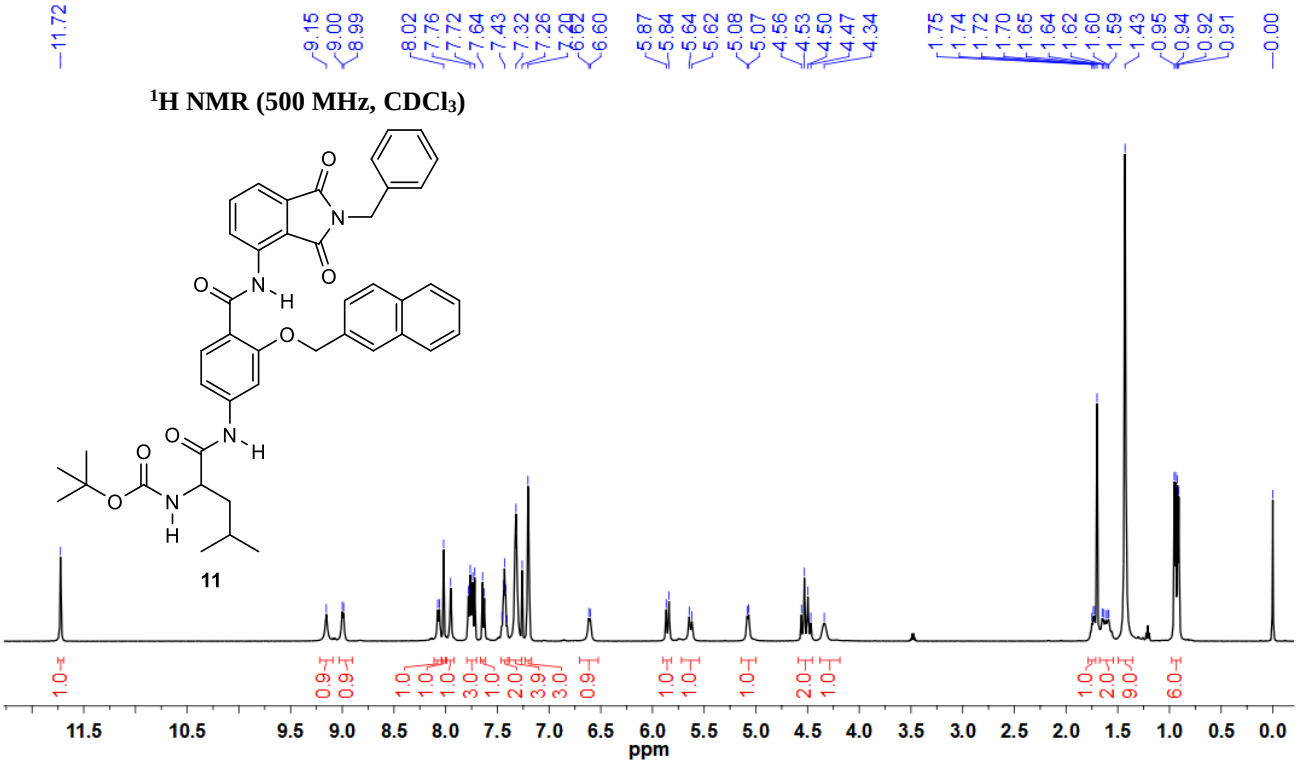
^{1}H NMR (500 MHz, $CDCl_3$)



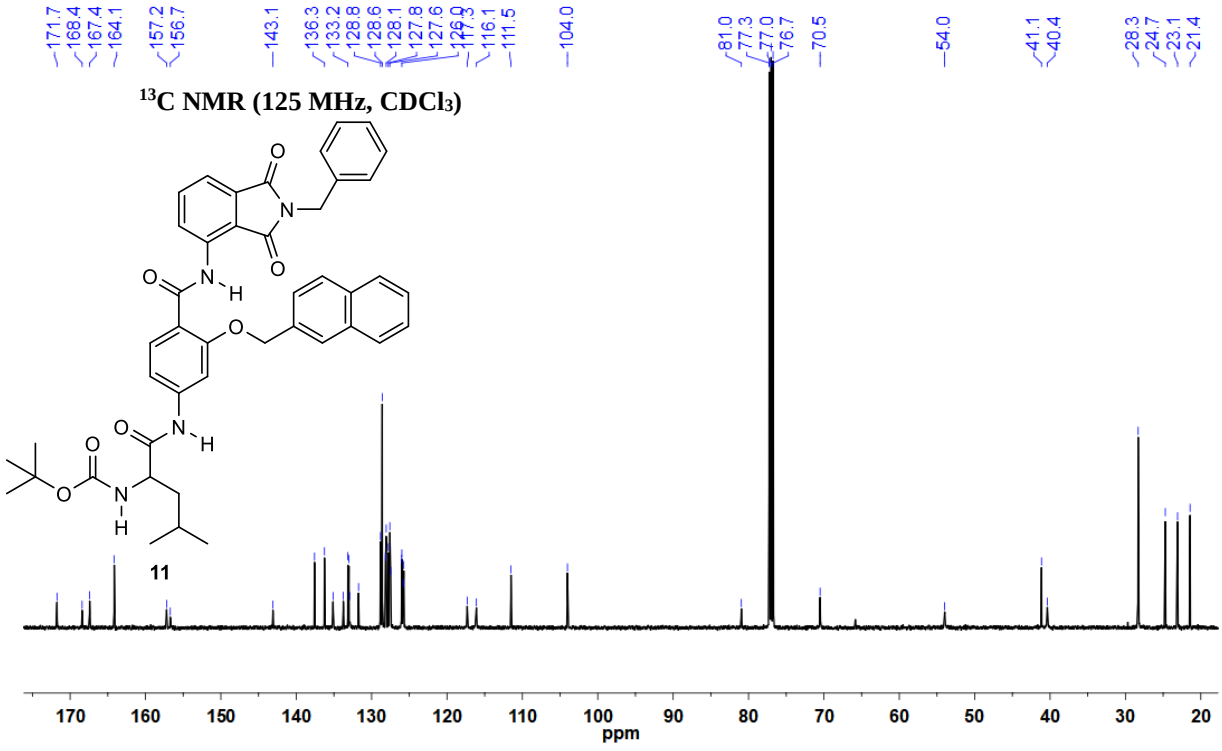
^{13}C NMR (125 MHz, $CDCl_3$)

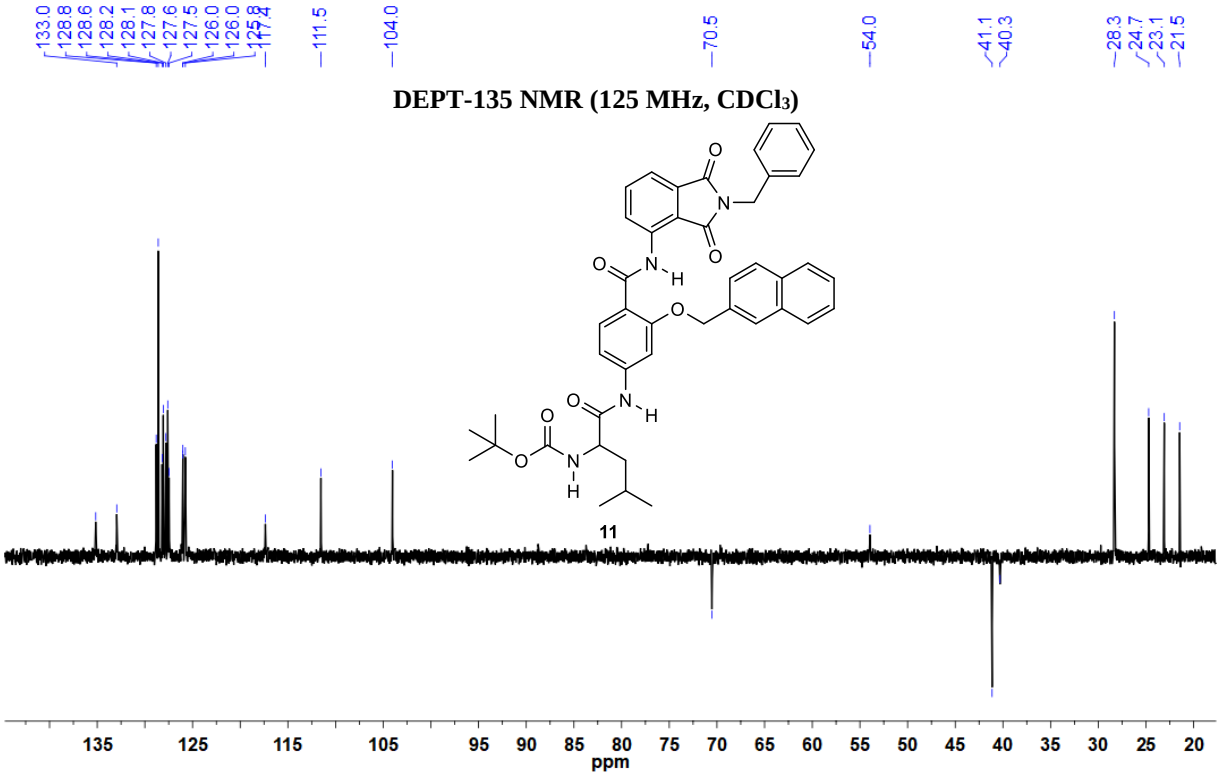


^{1}H NMR (500 MHz, $CDCl_3$)

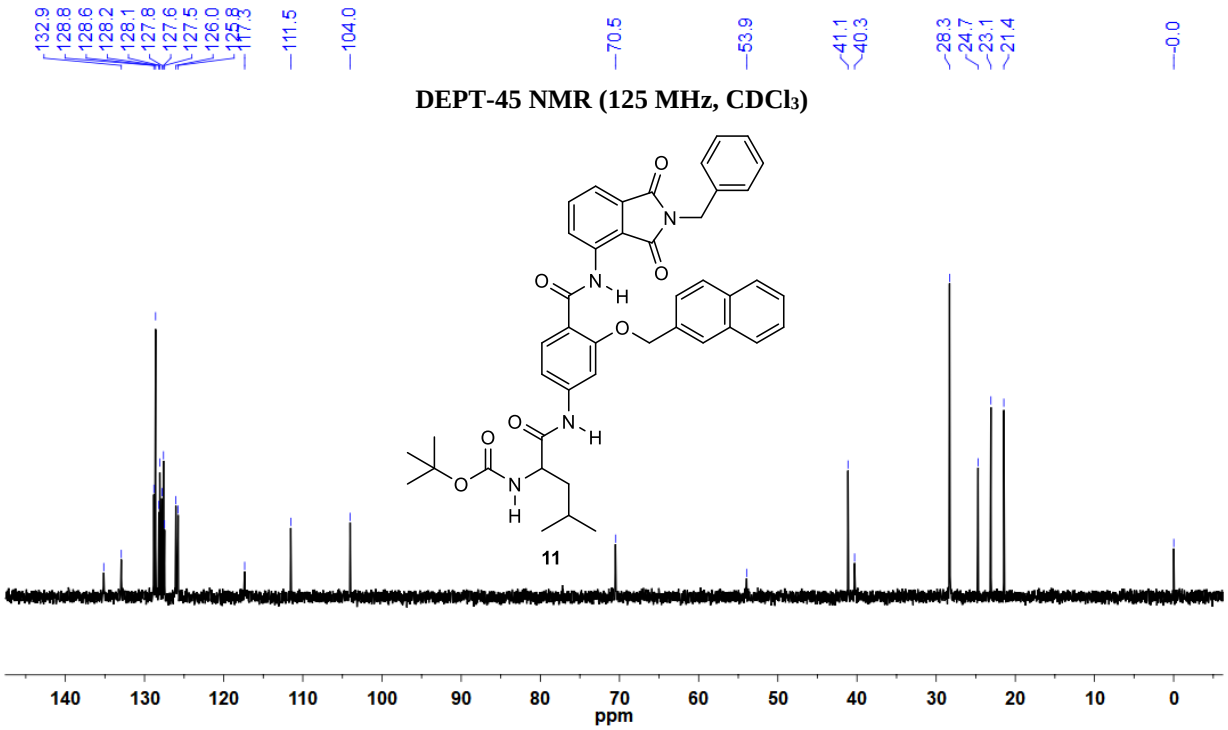


^{13}C NMR (125 MHz, $CDCl_3$)

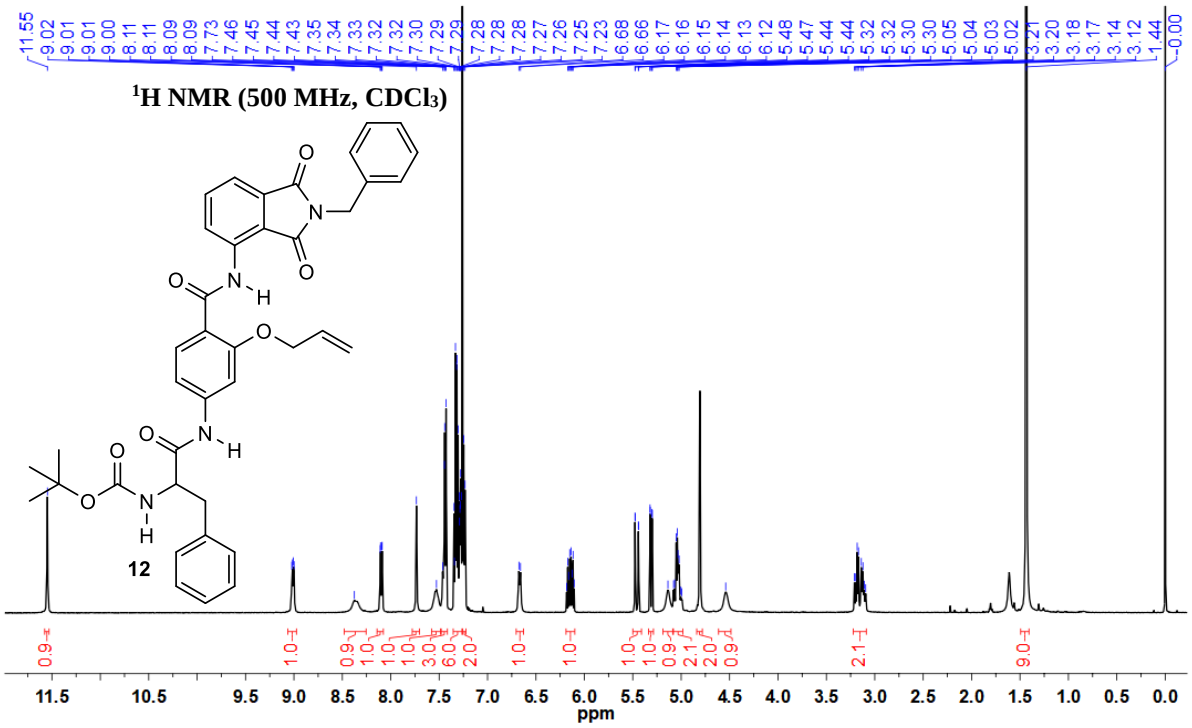


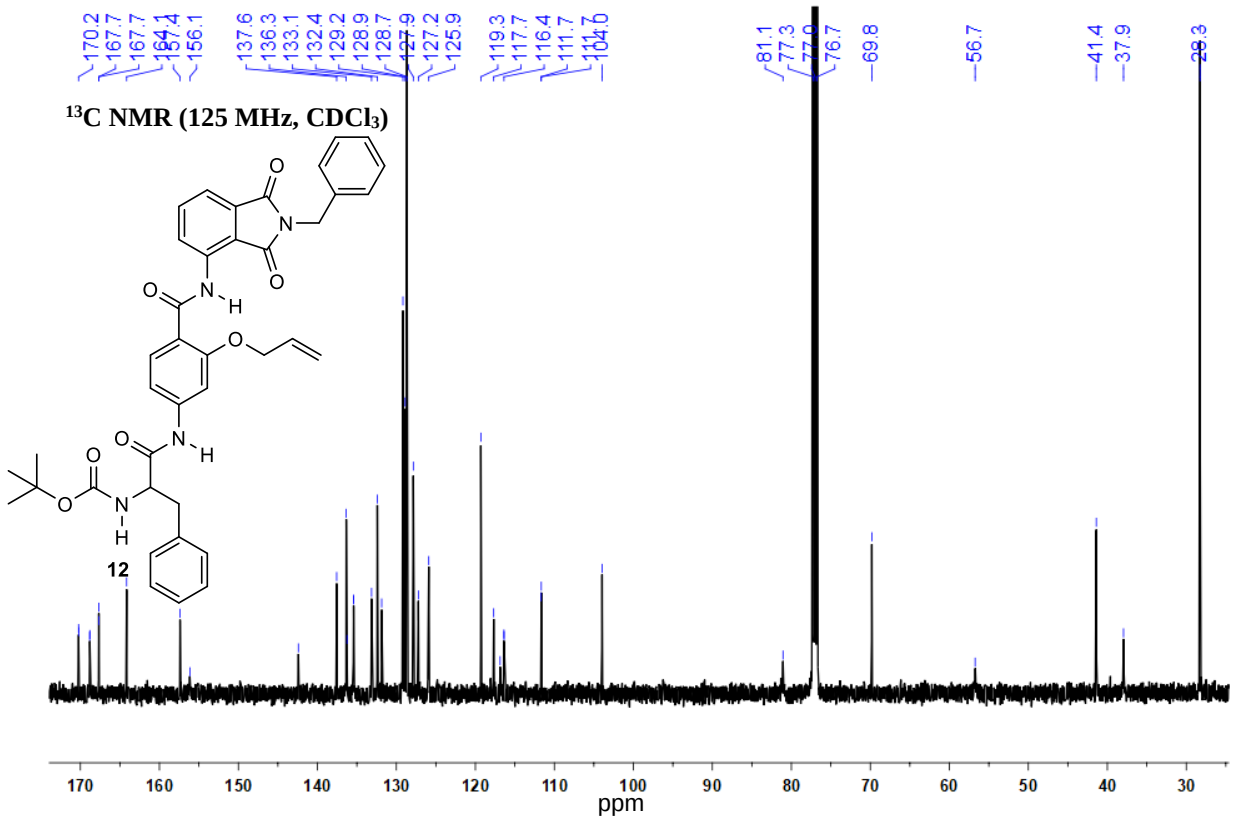


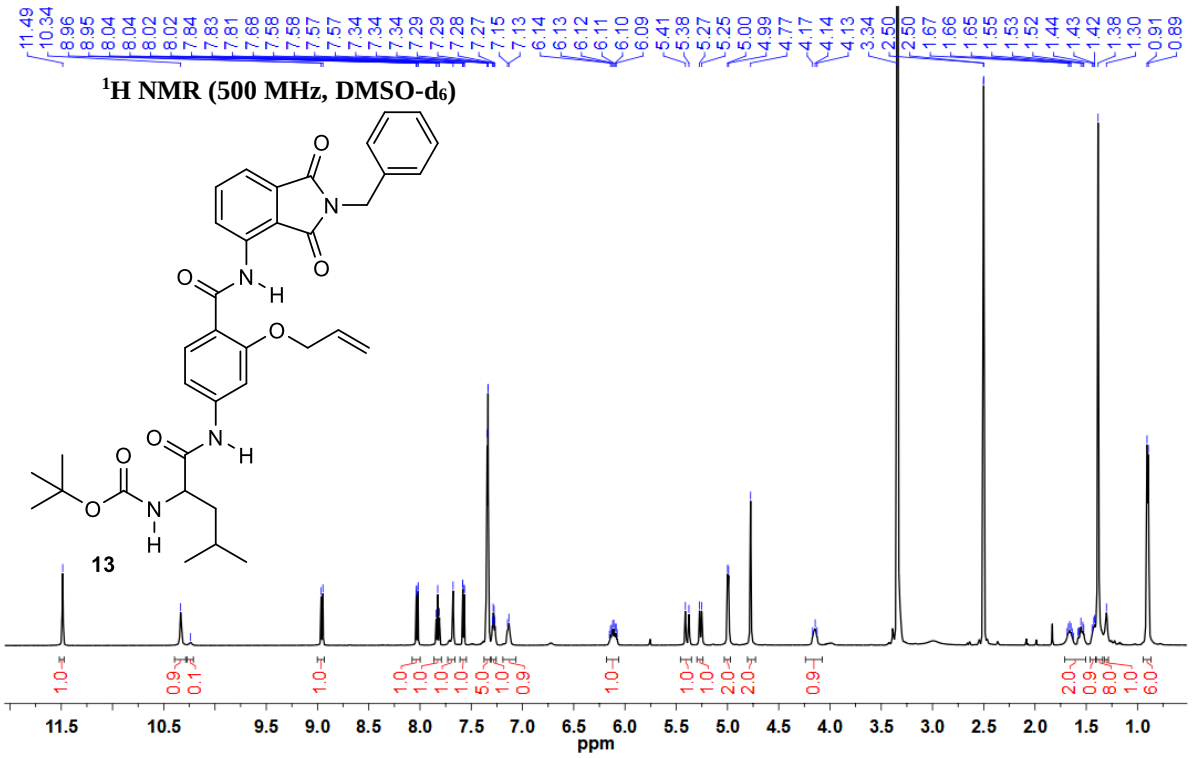
DEPT-135 NMR (125 MHz, $CDCl_3$)

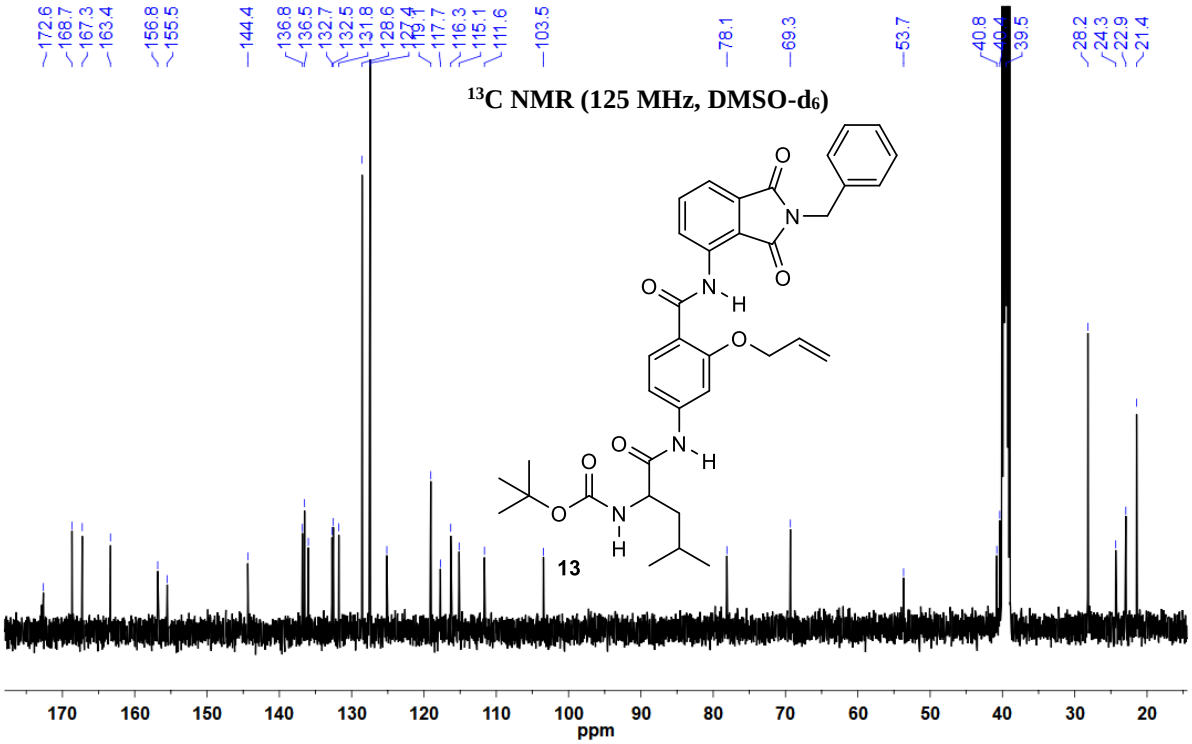


DEPT-45 NMR (125 MHz, $CDCl_3$)

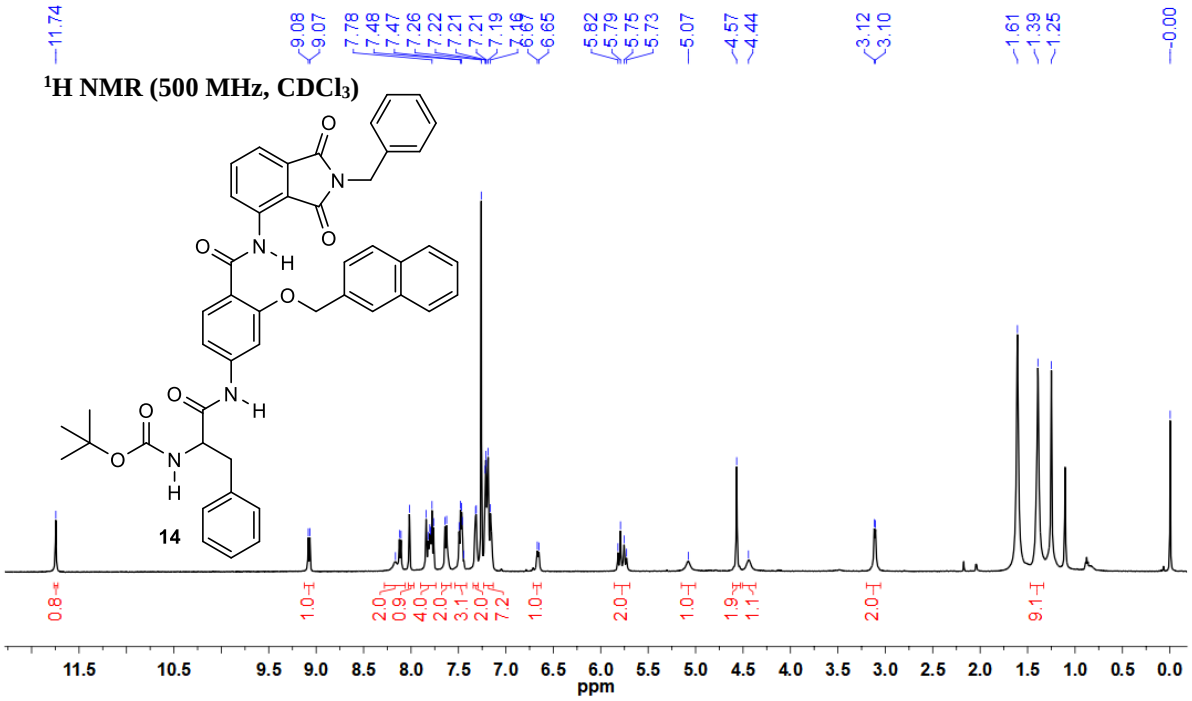

^{1}H NMR (500 MHz, CDCl$_3$)
12
ppm


^{13}C NMR (125 MHz, CDCl$_3$)
12
ppm

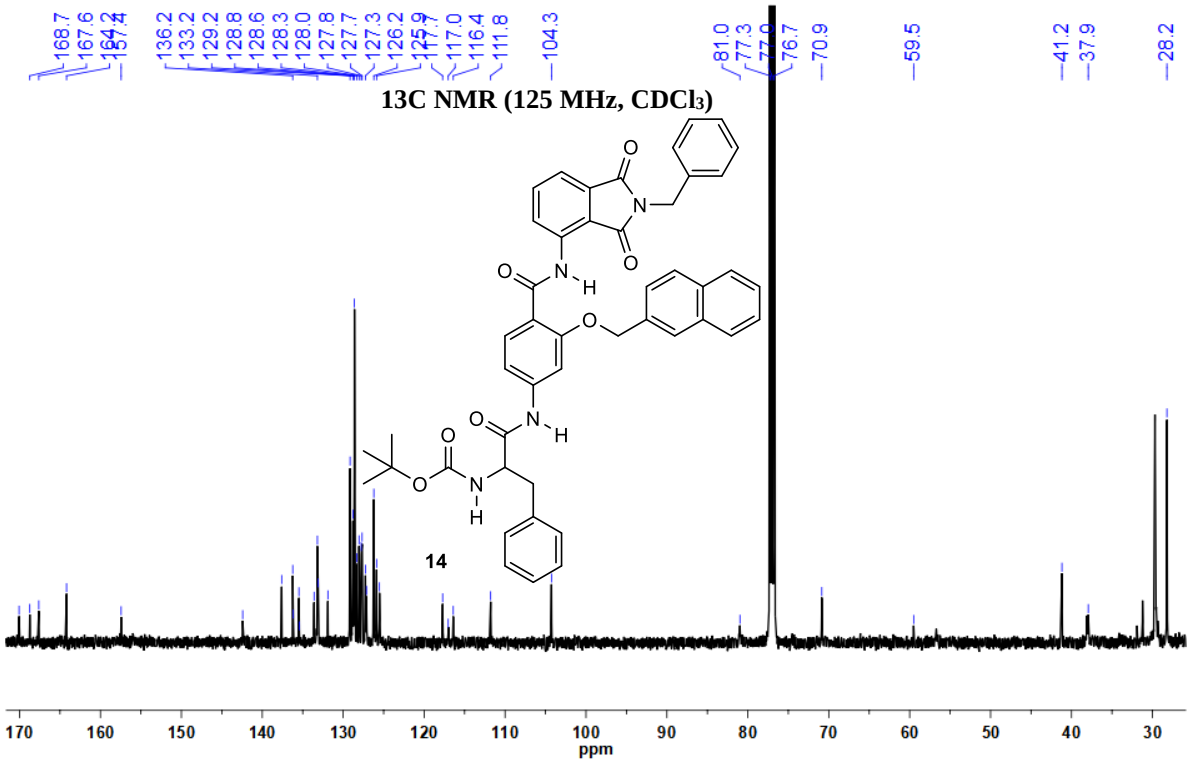

11.49
10.34
8.96
8.95
8.04
8.04
8.02
8.02
7.84
7.83
7.81
7.68
7.58
7.58
7.57
7.57
7.34
7.34
7.34
7.29
7.29
7.28
7.27
7.15
7.13
6.14
6.13
6.12
6.11
6.10
6.09
5.41
5.38
5.27
5.25
5.00
4.99
4.77
4.17
4.14
4.13
3.34
2.50
2.50
1.67
1.66
1.65
1.55
1.53
1.52
1.44
1.43
1.42
1.38
1.30
0.91
0.89
1H NMR (500 MHz, DMSO-d6)
13
ppm

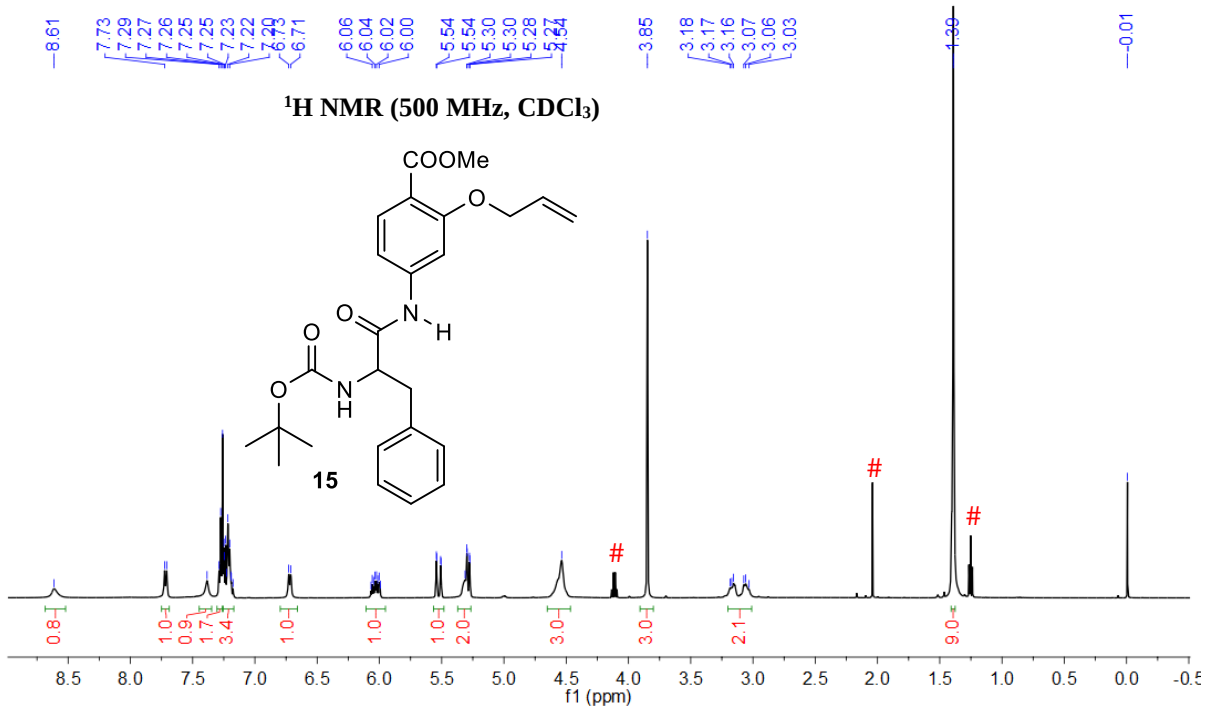

172.6
168.7
167.3
163.4
156.8
155.5
144.4
136.8
136.5
132.7
132.5
131.8
128.6
123.4
119.1
117.7
116.3
115.1
111.6
103.5
78.1
69.3
53.7
40.8
40.4
39.5
28.2
24.3
22.9
21.4
13C NMR (125 MHz, DMSO-d6)
13
ppm

^{1}H NMR (500 MHz, $CDCl_3$)



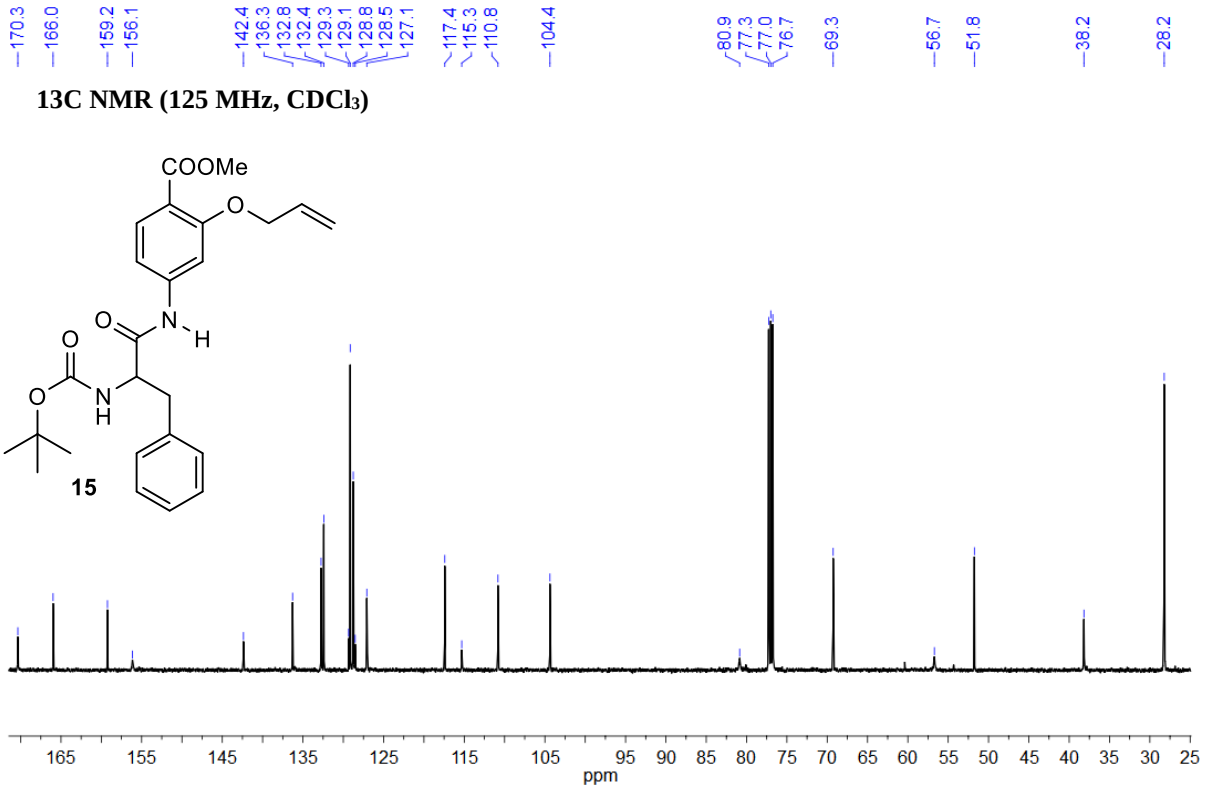
13C NMR (125 MHz, $CDCl_3$)



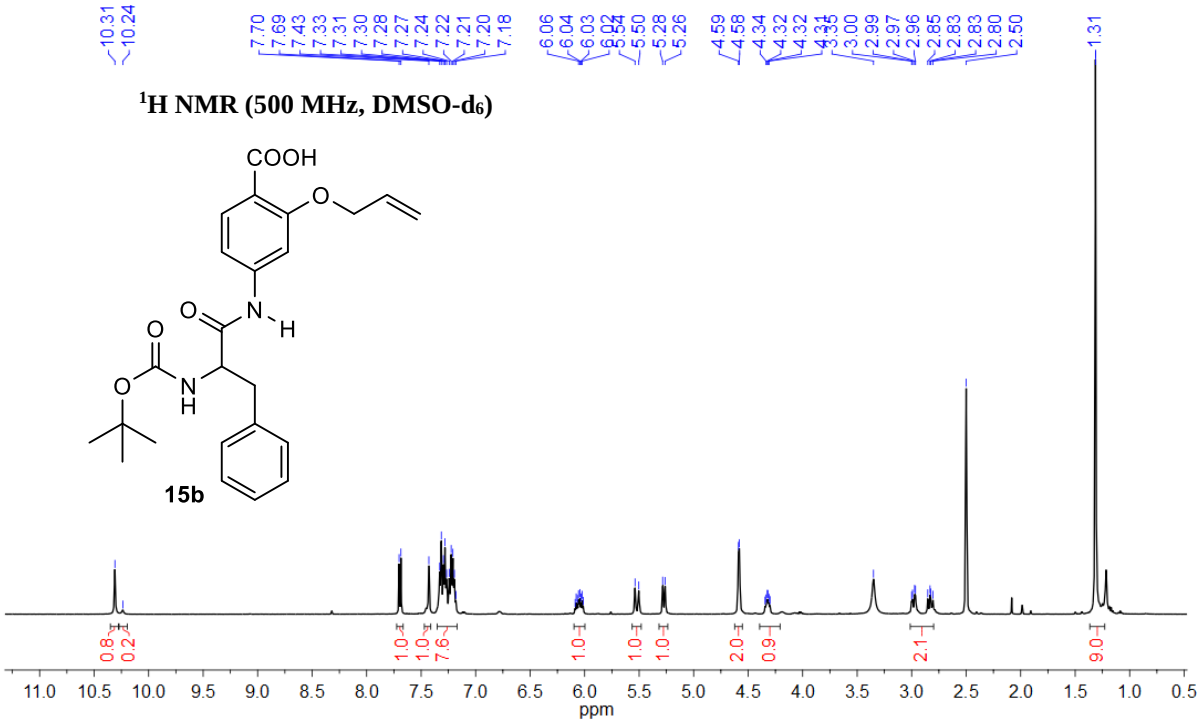


^{1}H NMR (500 MHz, $CDCl_3$)

= Solvent peak (Ethyl acetate)

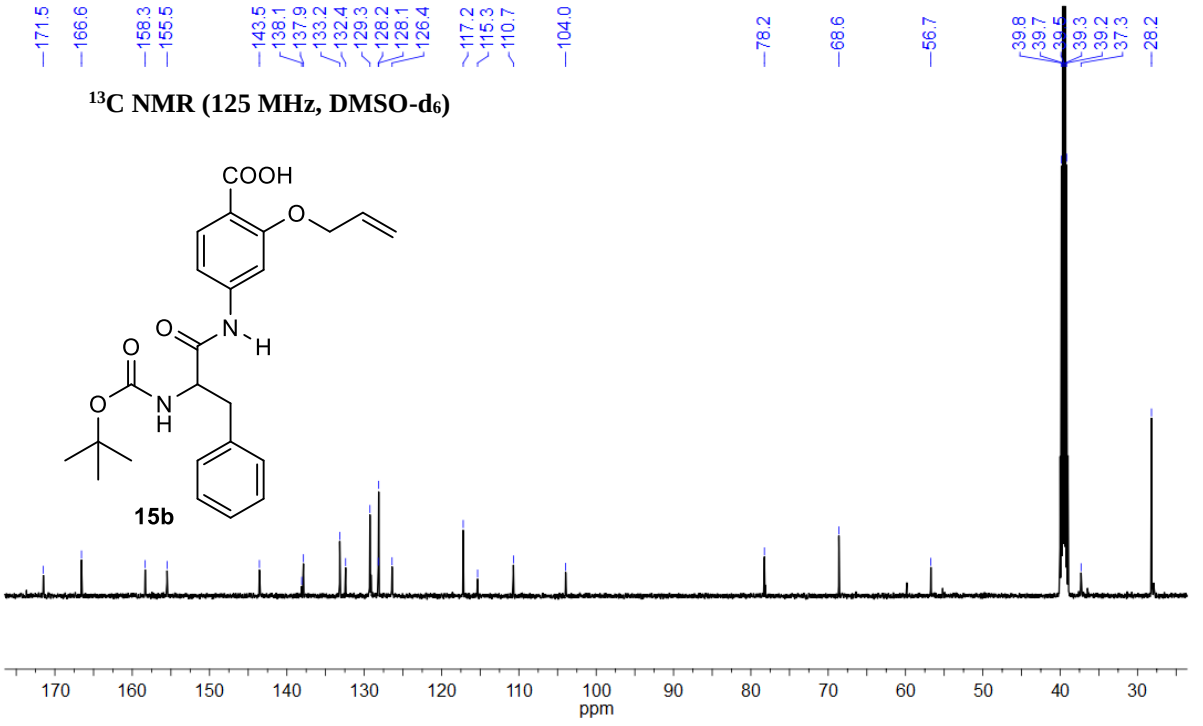


13C NMR (125 MHz, $CDCl_3$)



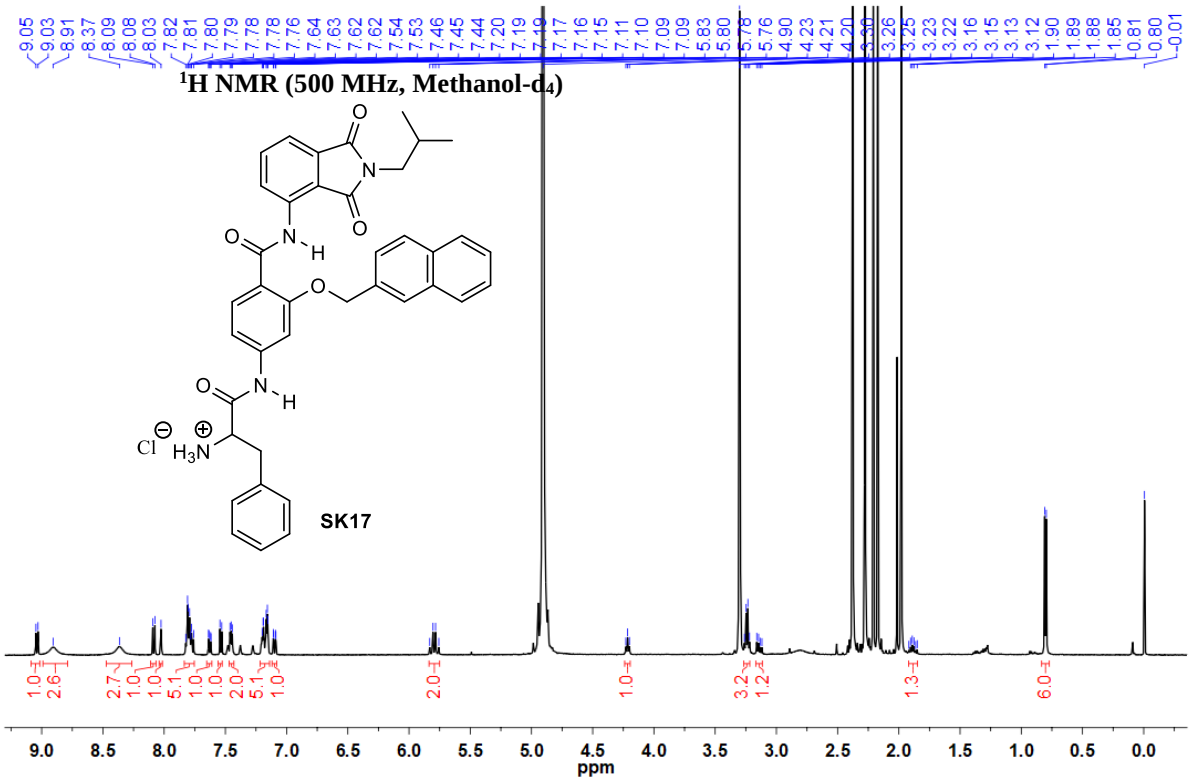
^{1}H NMR (500 MHz, DMSO-d$_6$)

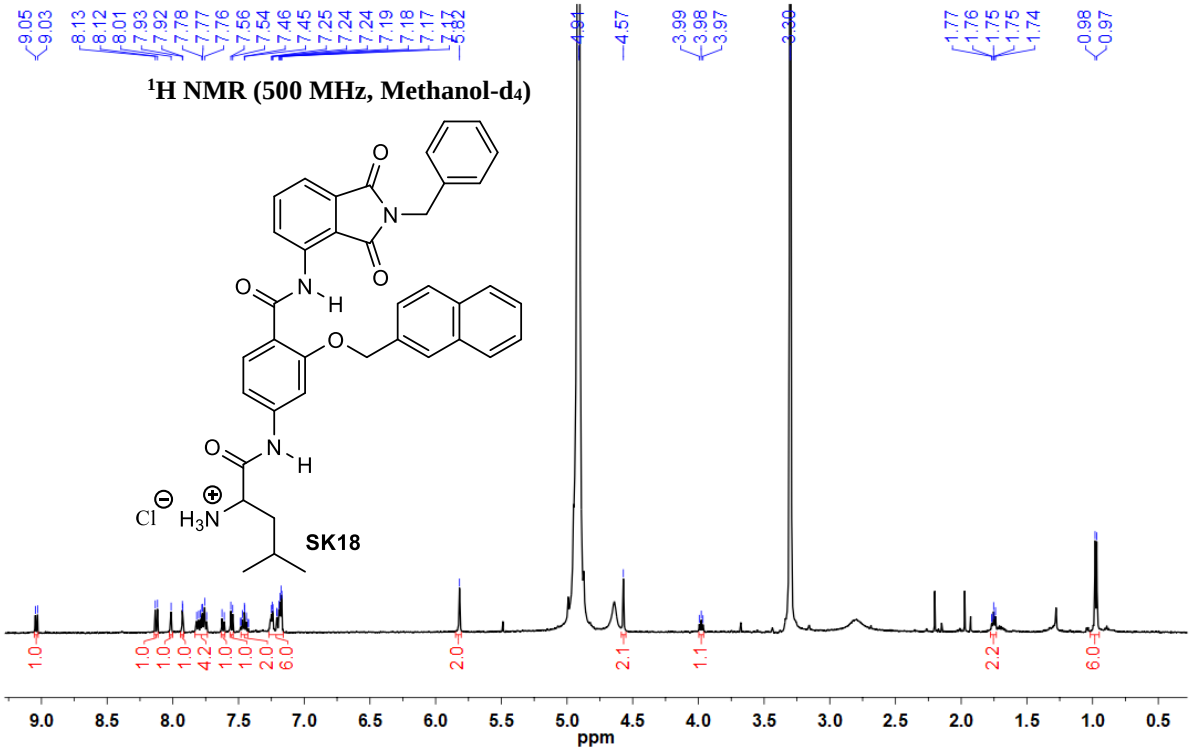
15b

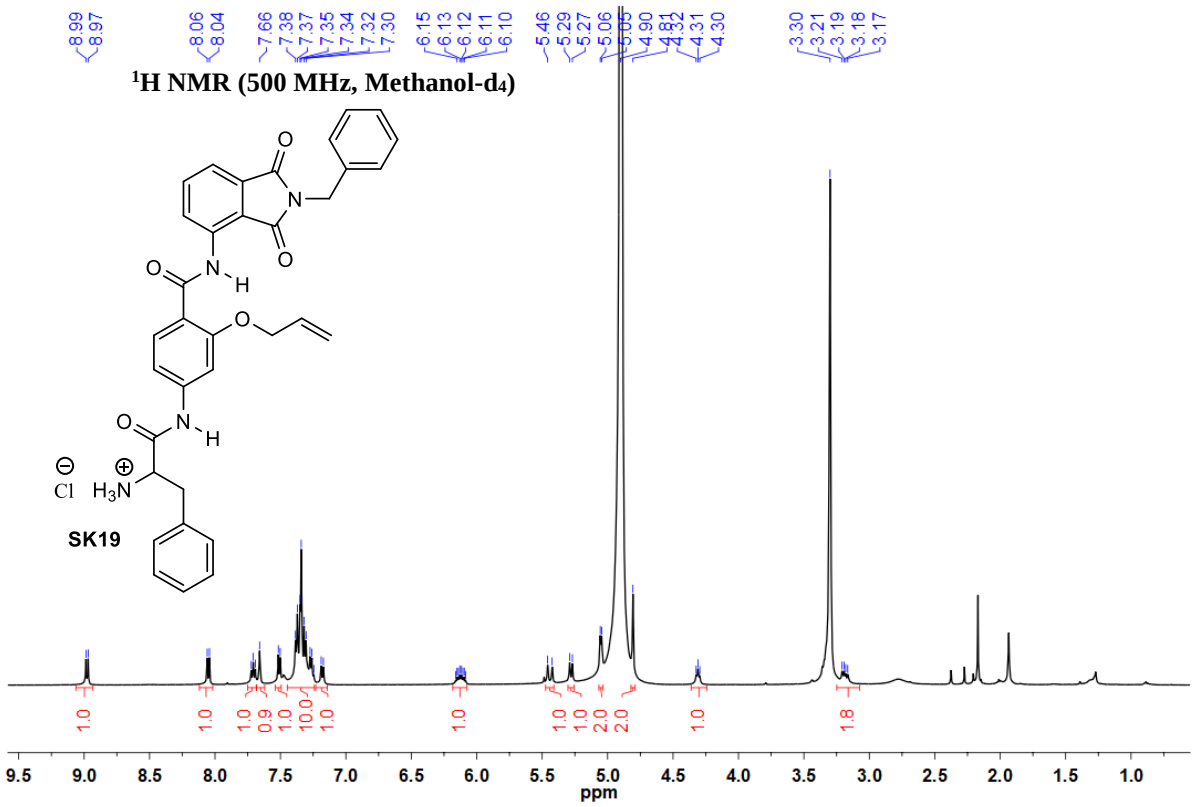


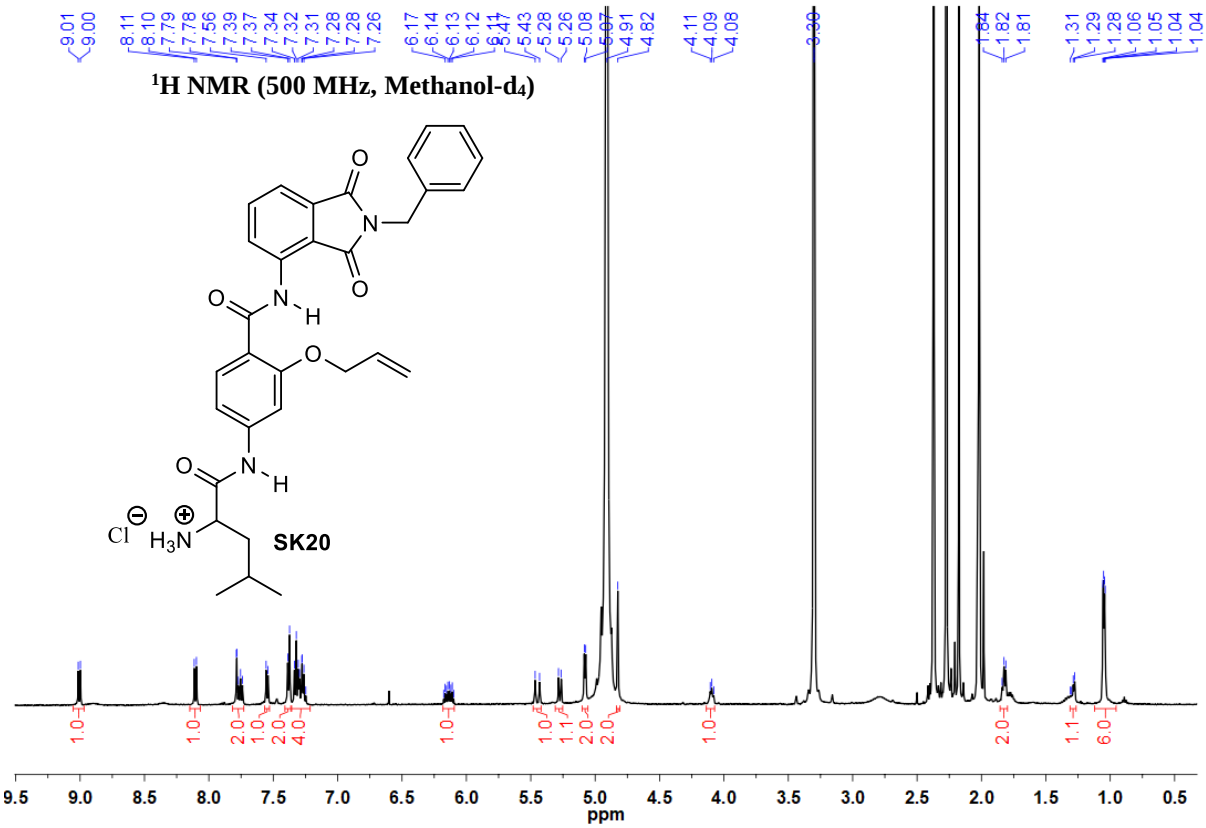
^{13}C NMR (125 MHz, DMSO-d$_6$)

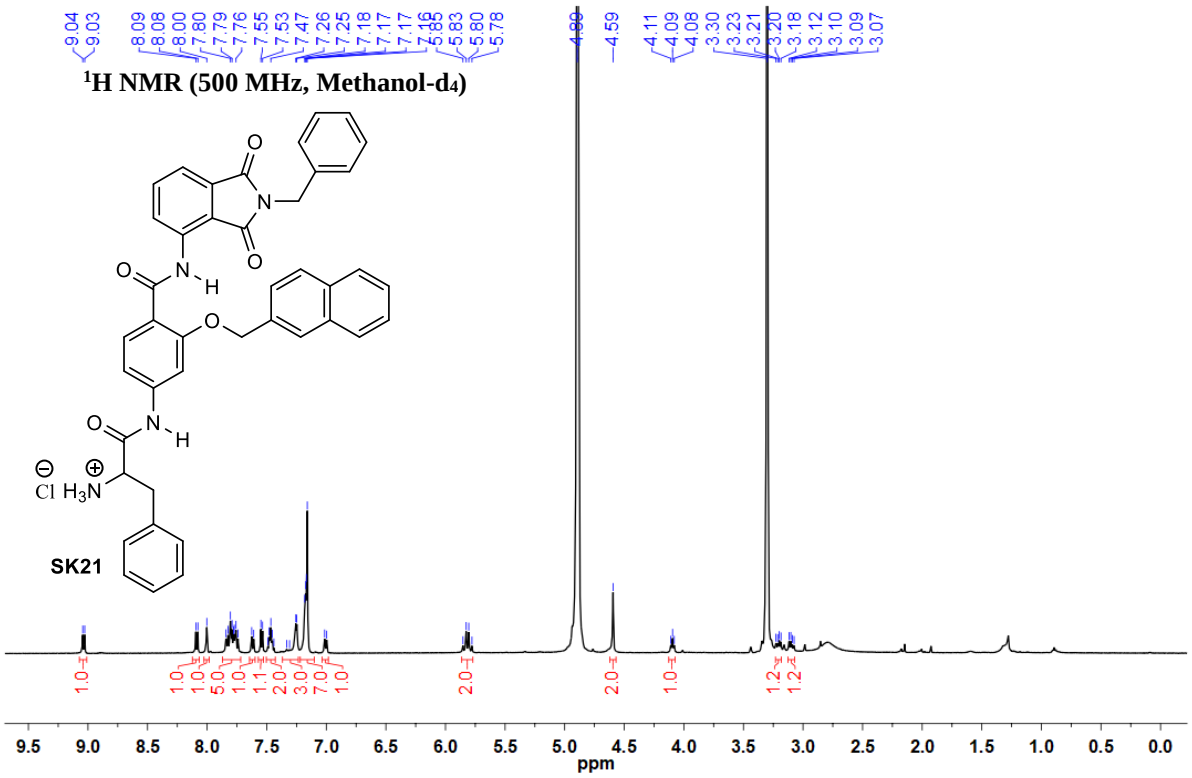
15b


^{1}H NMR (500 MHz, Methanol-d$_4$)
SK17
ppm

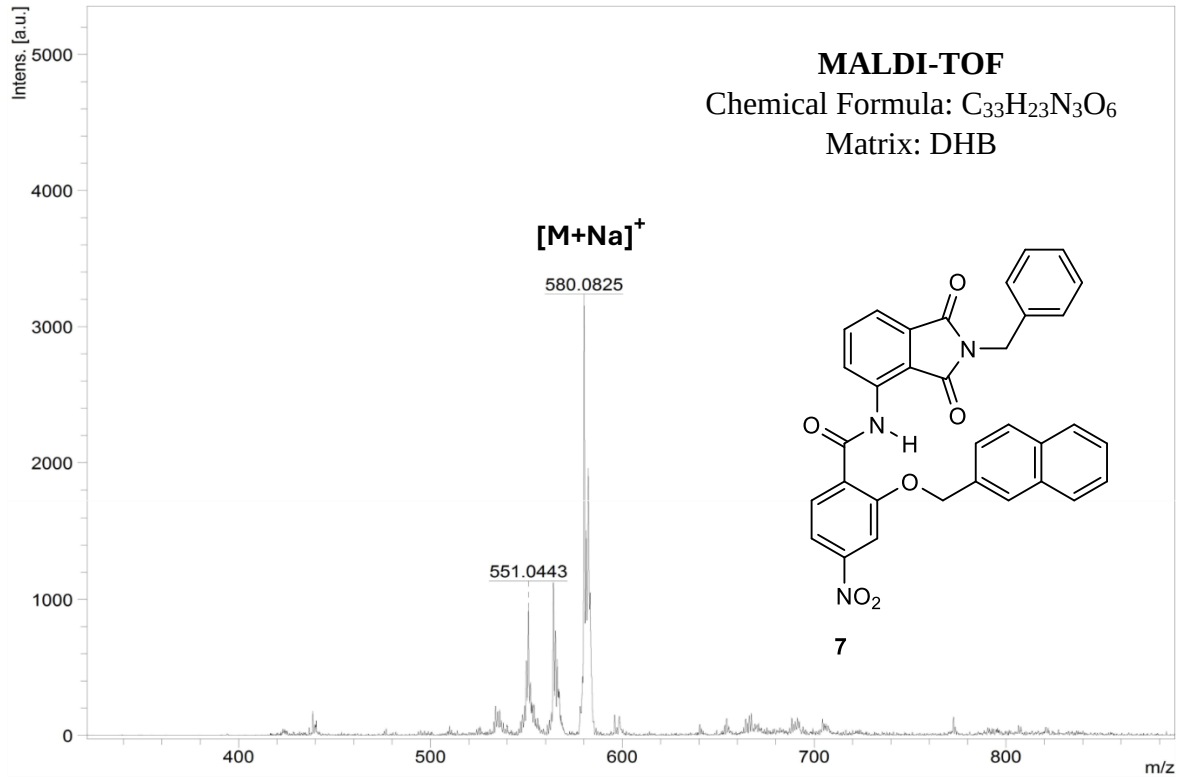

^{1}H NMR (500 MHz, Methanol-d$_4$)
SK18
ppm

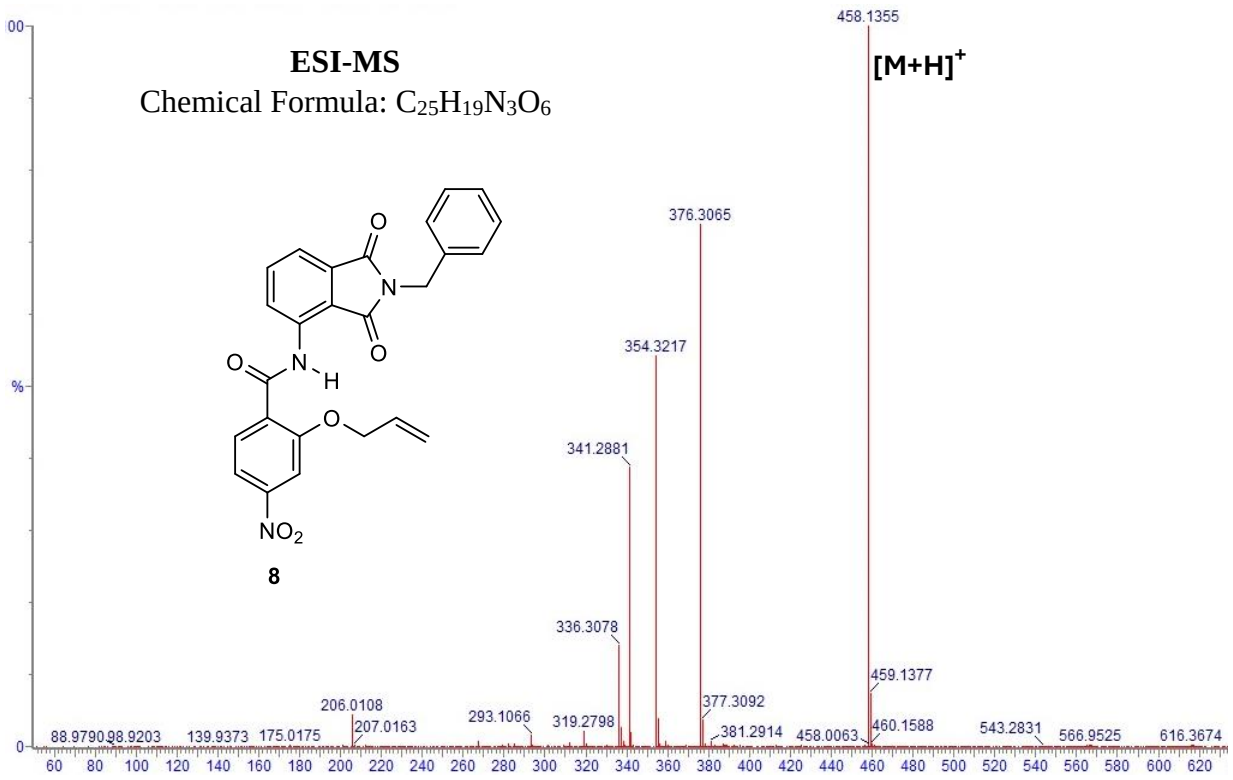

^{1}H NMR (500 MHz, Methanol-d$_4$)
SK19
ppm

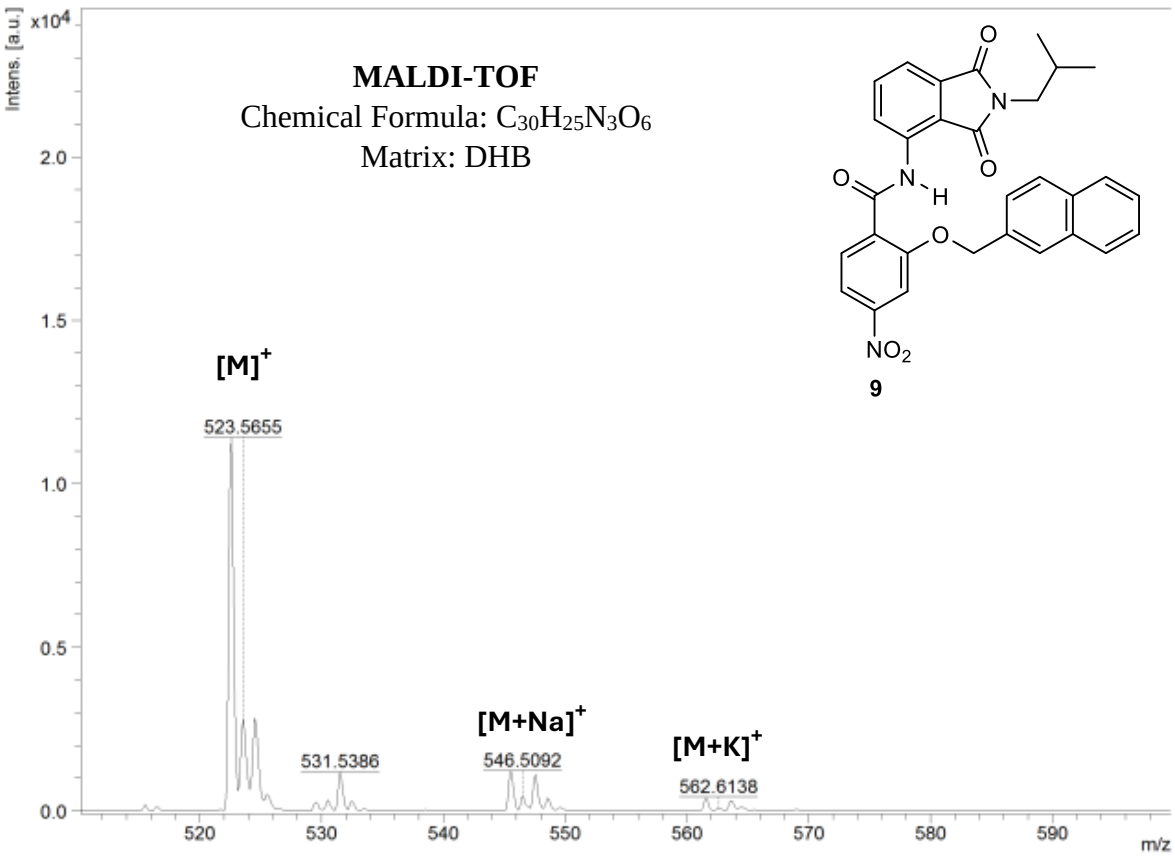

^{1}H NMR (500 MHz, Methanol-d$_4$)
SK20
ppm

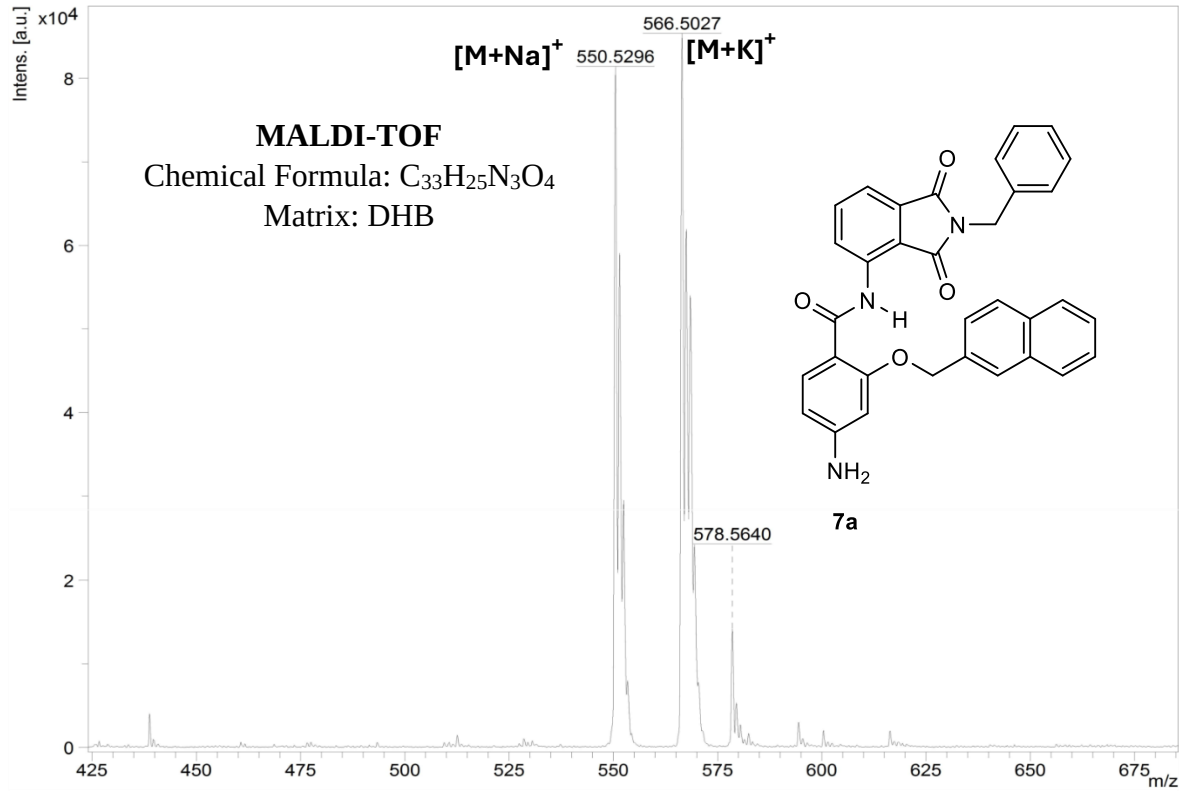

9.04
9.03
8.09
8.08
8.00
7.80
7.79
7.76
7.55
7.53
7.47
7.26
7.25
7.18
7.17
7.17
5.83
5.80
5.78
4.59
4.11
4.09
4.08
3.30
3.23
3.21
3.20
3.18
3.12
3.10
3.09
3.07
1H NMR (500 MHz, Methanol-d4)
SK21
ppm

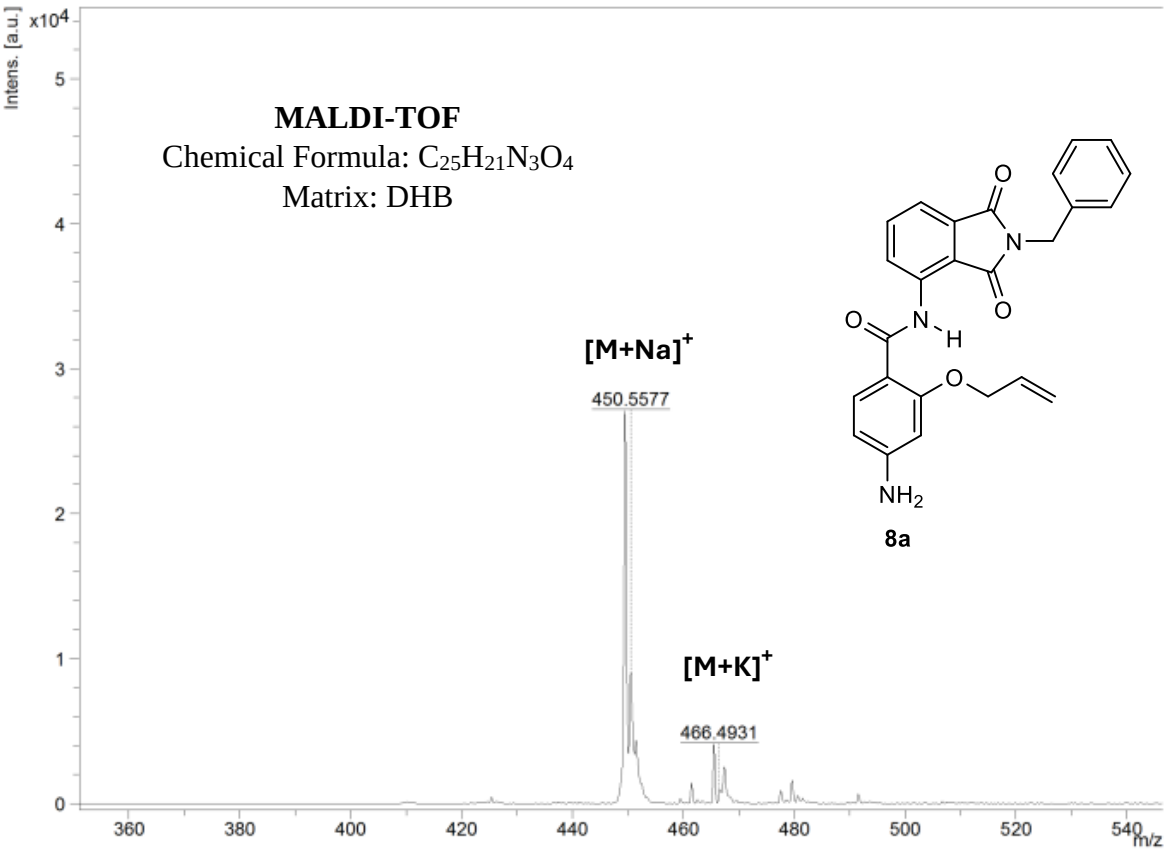
7.2 Mass Spectra






MALDI-TOF
Chemical Formula: C30H25N3O6
Matrix: DHB
9
[M]+
523.5655
531.5386
[M+Na]+
546.5092
[M+K]+
562.6138
Intens. [a.u.]
x10^4
m/z


MALDI-TOF
Chemical Formula: C33H25N3O4
Matrix: DHB
7a
[M+Na]+
550.5296
566.5027
[M+K]+
578.5640
Intens. [a.u.]
x10^4
m/z

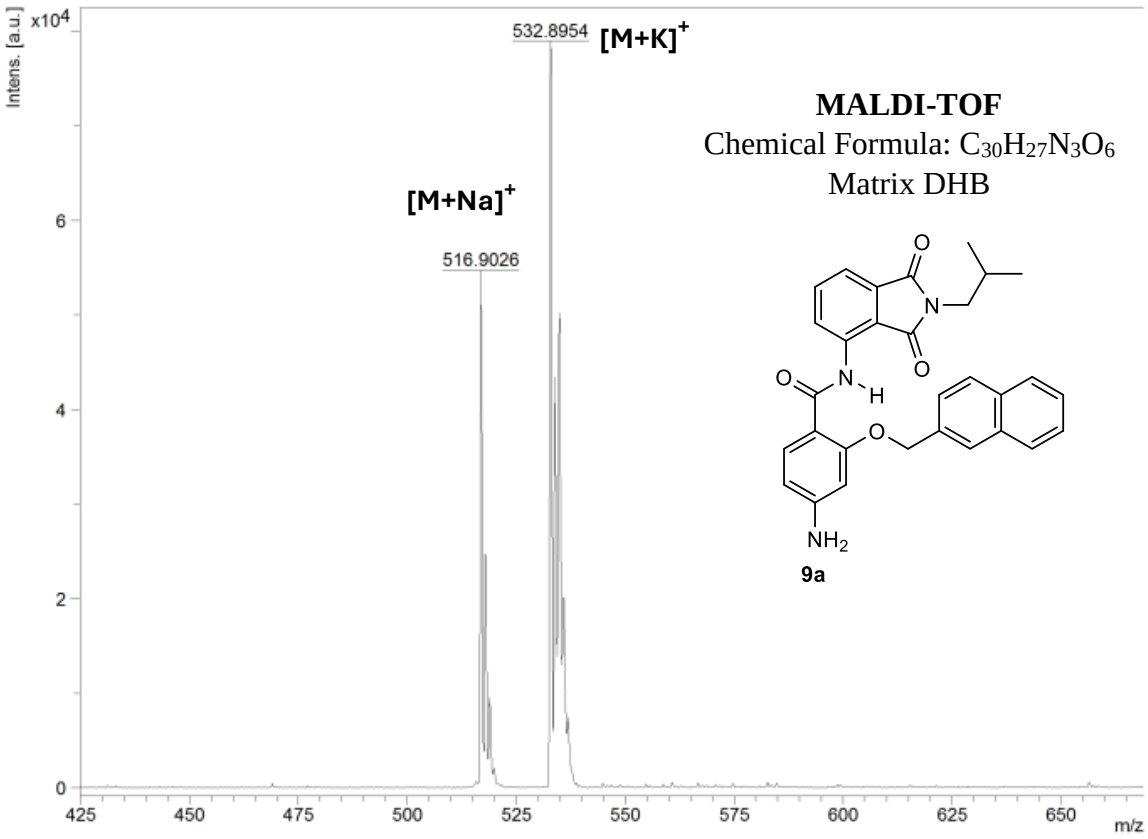


MALDI-TOF

Chemical Formula: $C_{25}H_{21}N_3O_4$

Matrix: DHB

8a

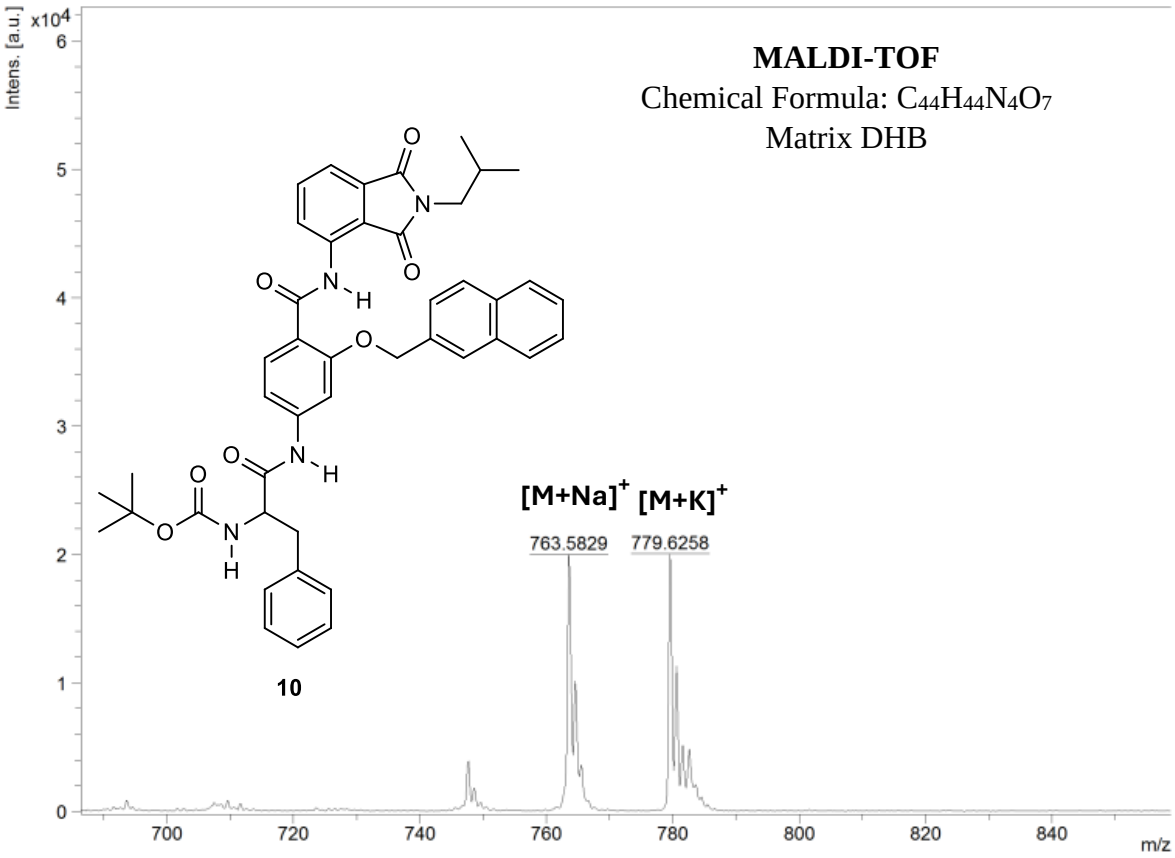


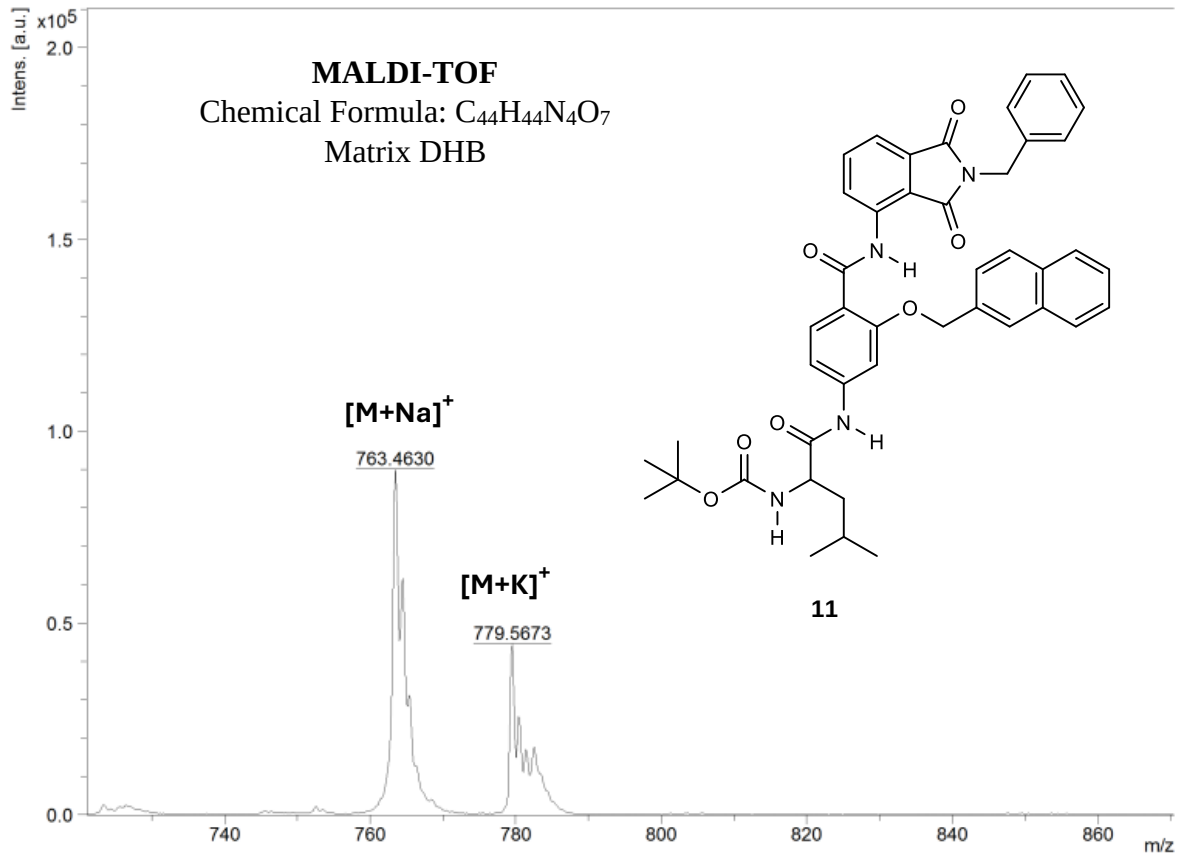
MALDI-TOF

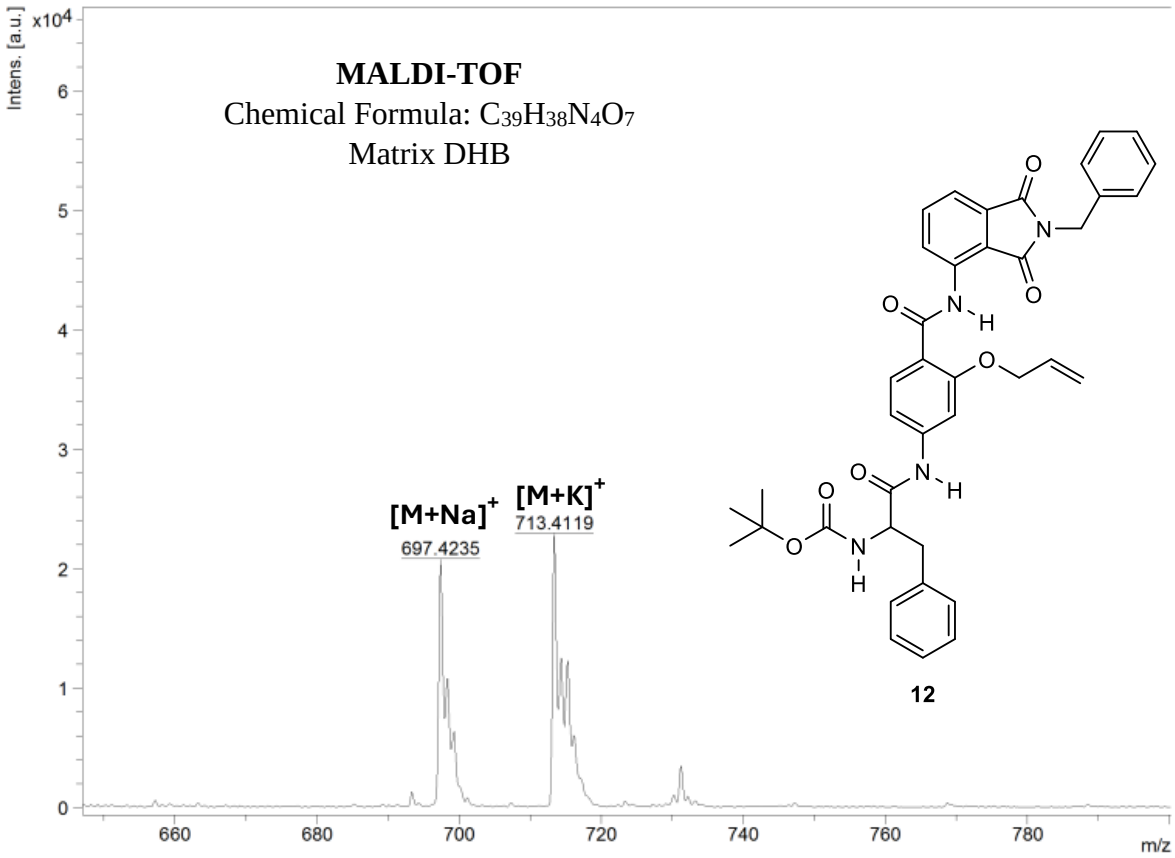
Chemical Formula: $C_{30}H_{27}N_3O_6$

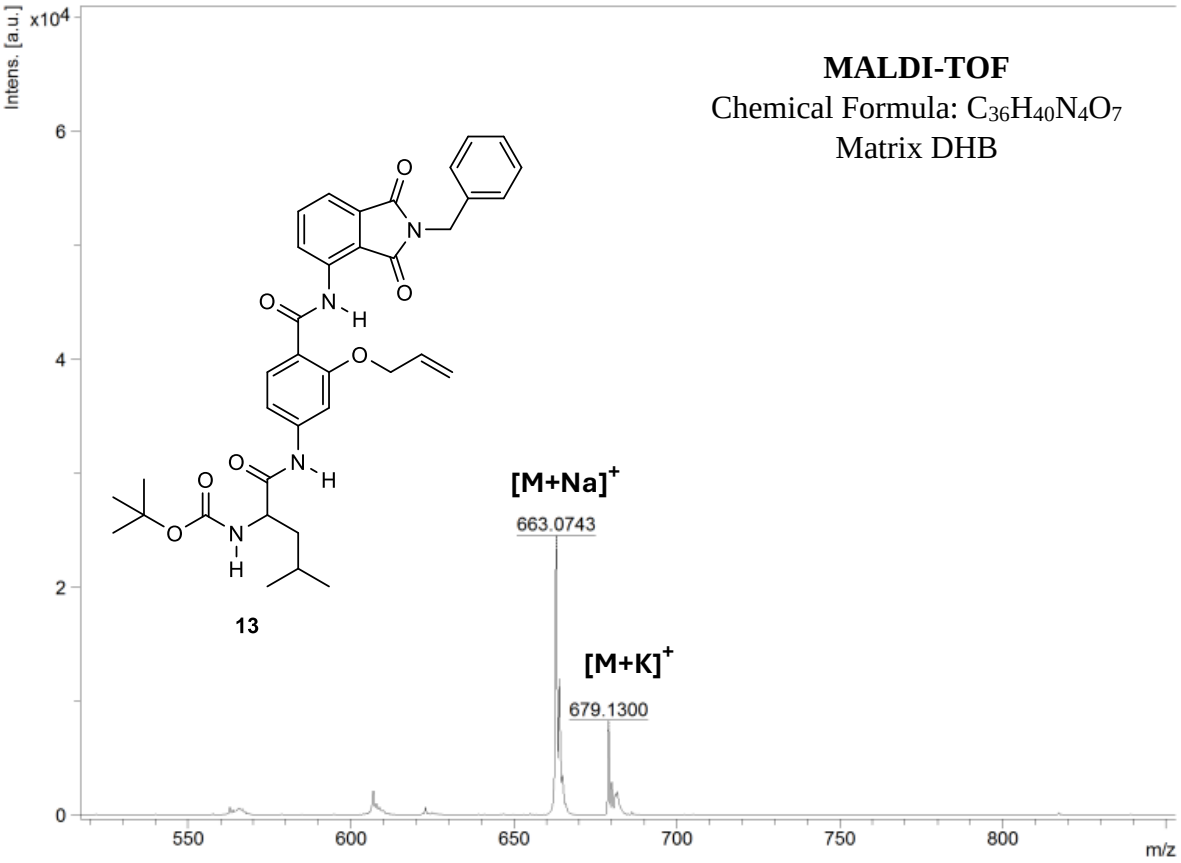
Matrix DHB

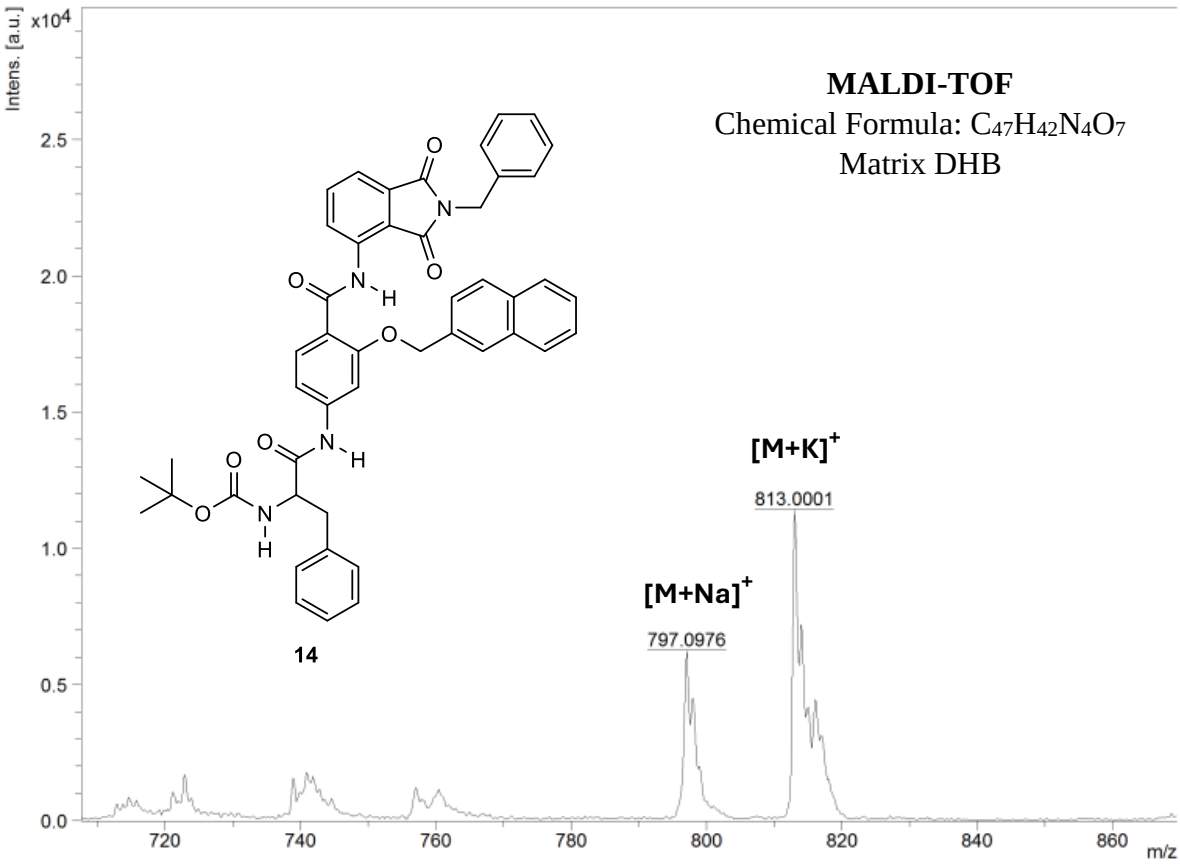
9a


MALDI-TOF
Chemical Formula: C44H44N4O7
Matrix DHB
[M+Na]+ [M+K]+
763.5829 779.6258
10
Intens. [a.u.]
x10^4
m/z

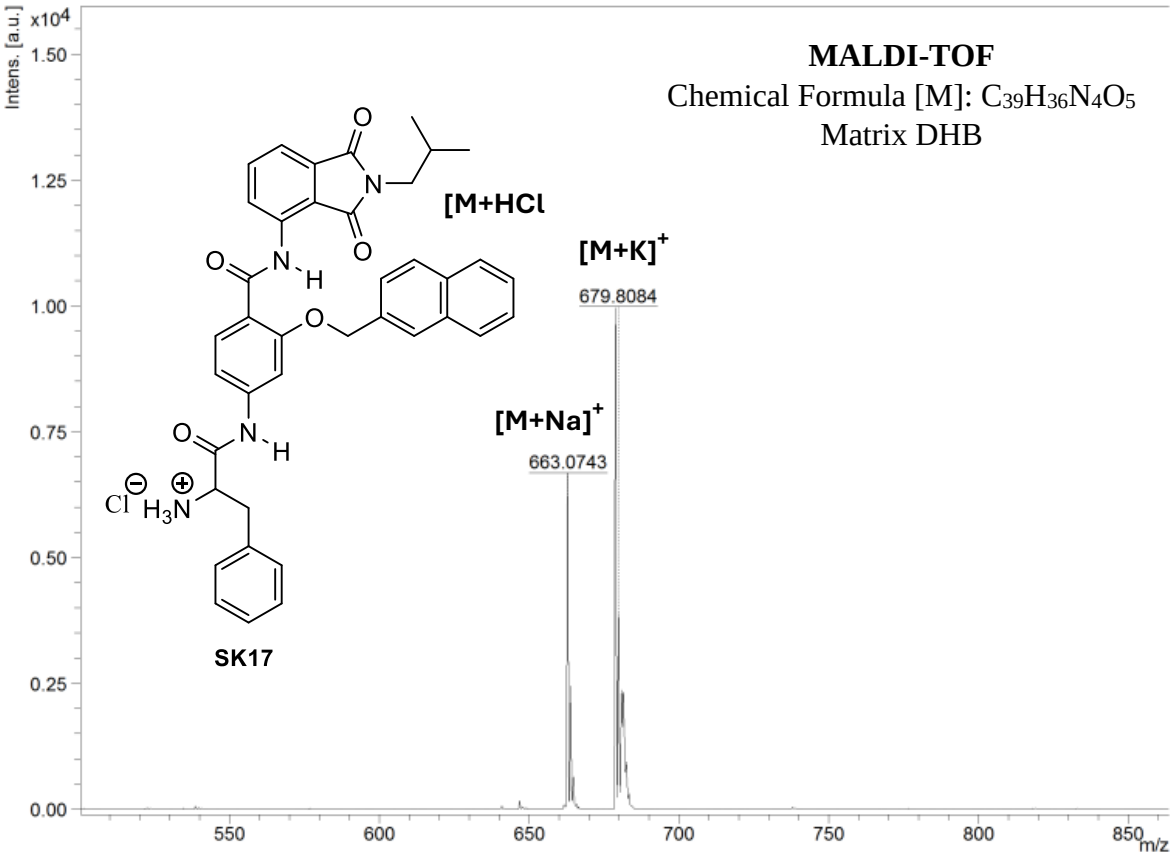

MALDI-TOF
Chemical Formula: C44H44N4O7
Matrix DHB
[M+Na]+
763.4630
[M+K]+
779.5673
11
Intens. [a.u.]
x10^5
m/z


MALDI-TOF
Chemical Formula: C39H38N4O7
Matrix DHB
[M+Na]+
697.4235
[M+K]+
713.4119
12
Intens. [a.u.]
x10^4
m/z

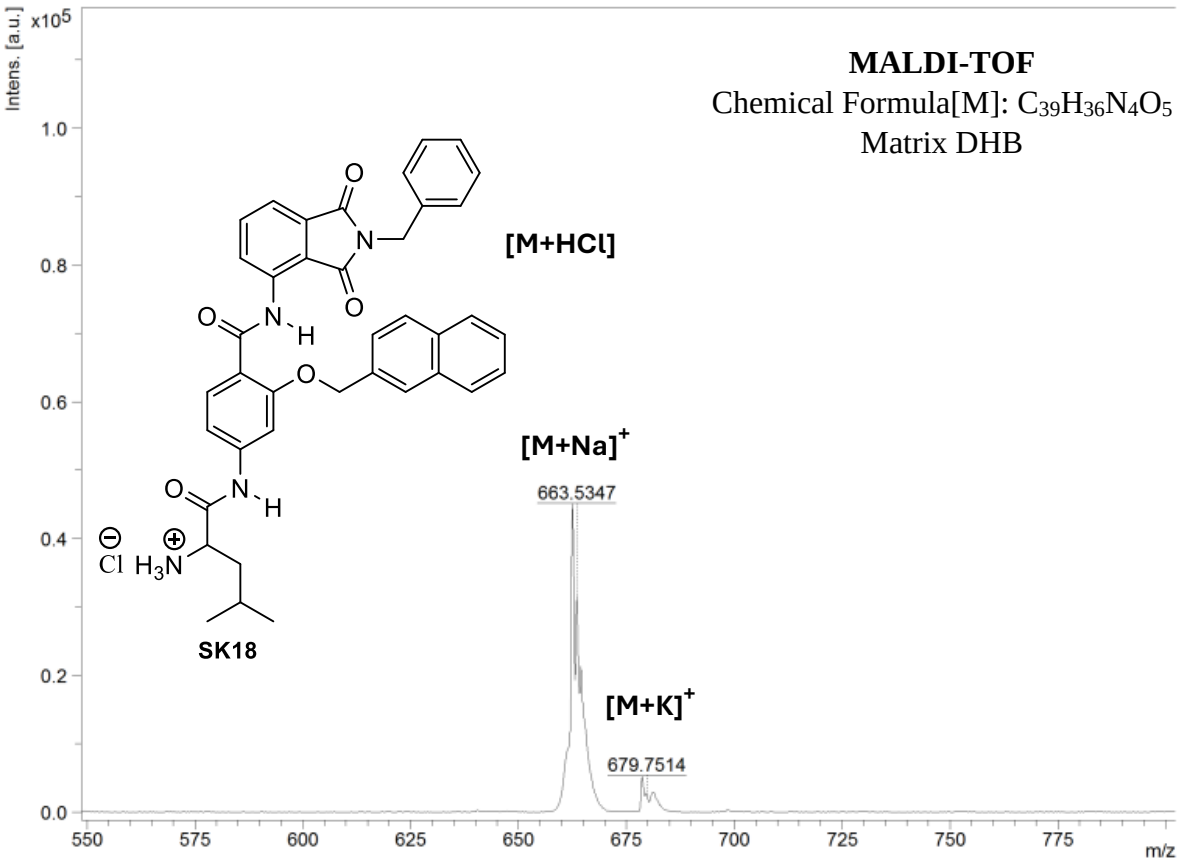

MALDI-TOF
Chemical Formula: C36H40N4O7
Matrix DHB
[M+Na]+
663.0743
[M+K]+
679.1300
13
Intens. [a.u.]
x10^4
m/z

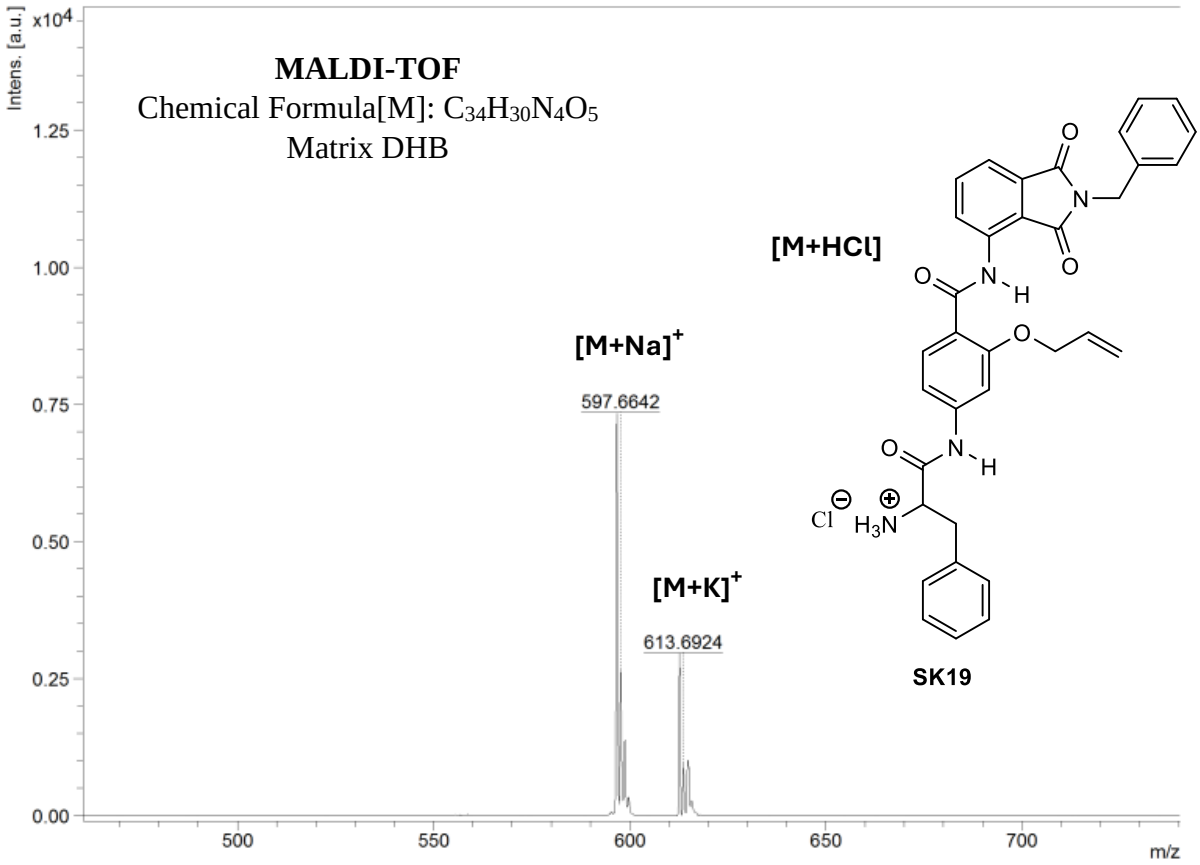


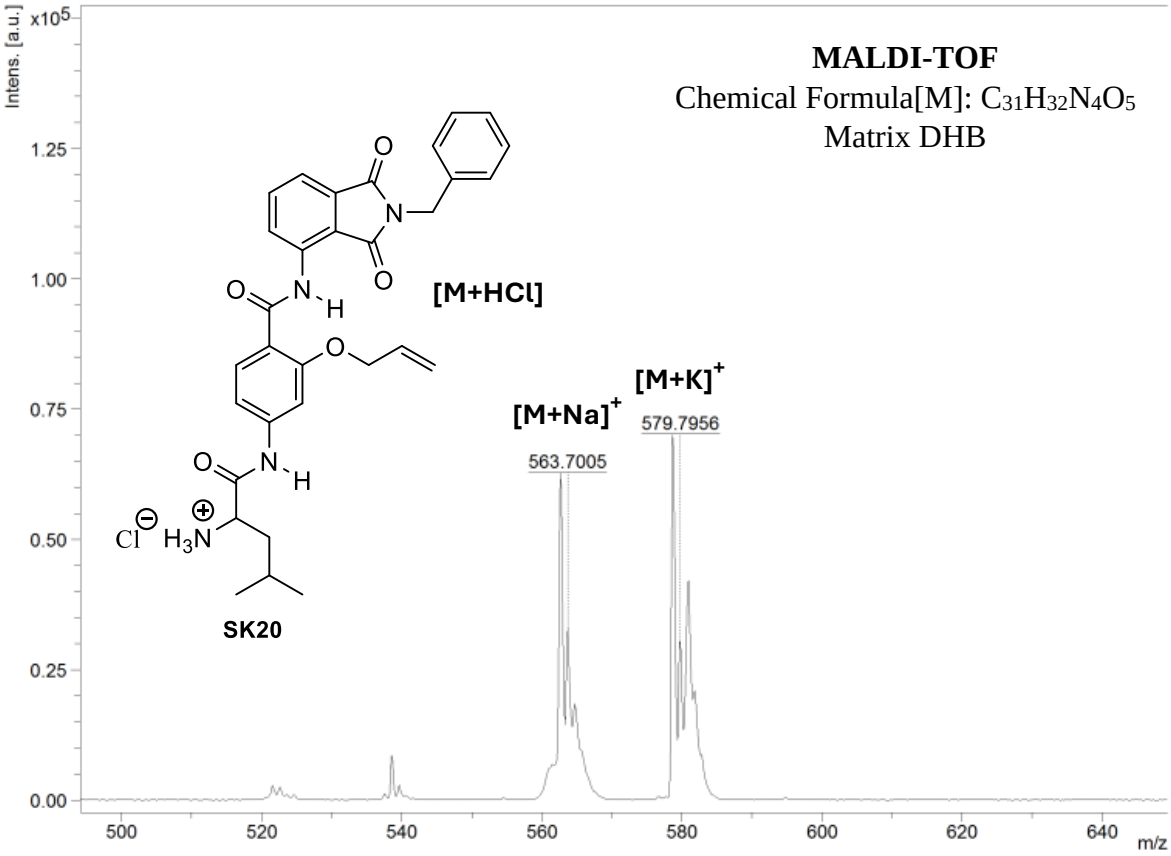
MALDI-TOF
Chemical Formula: $C_{47}H_{42}N_4O_7$
Matrix DHB

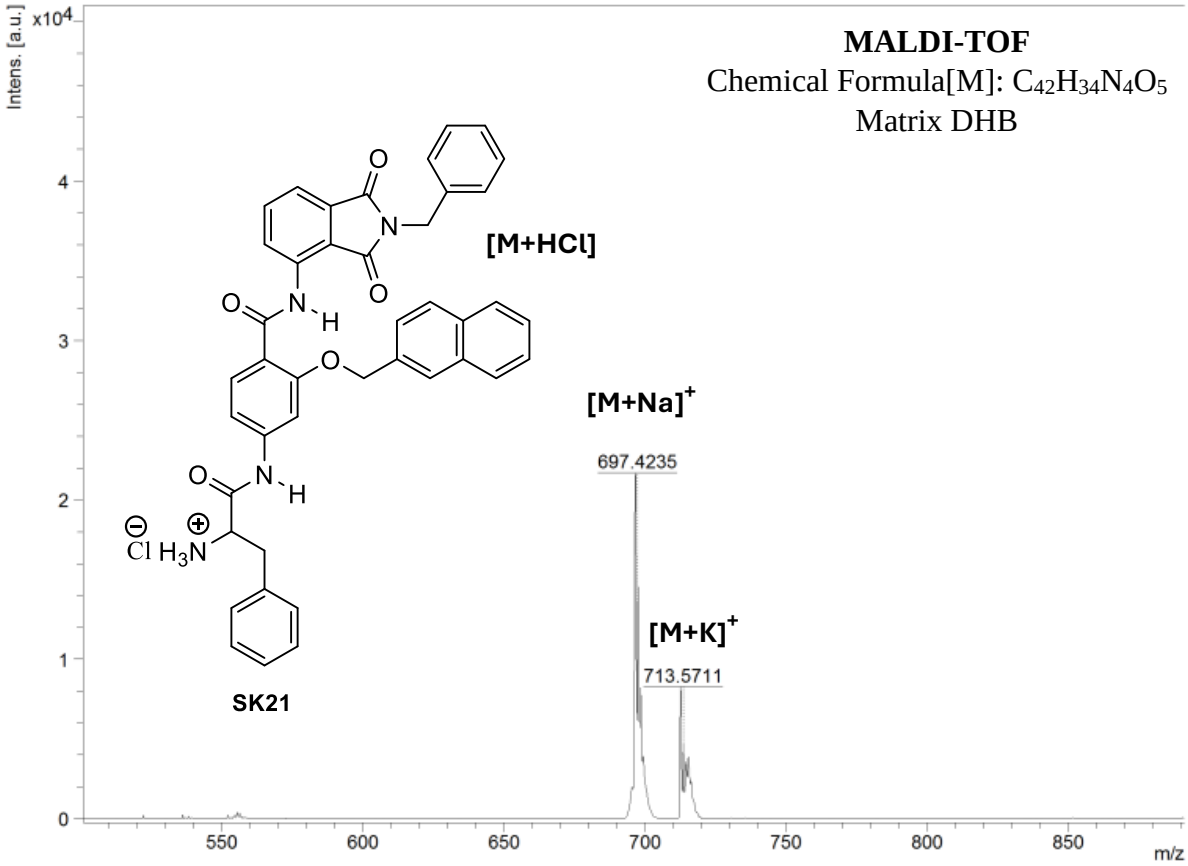


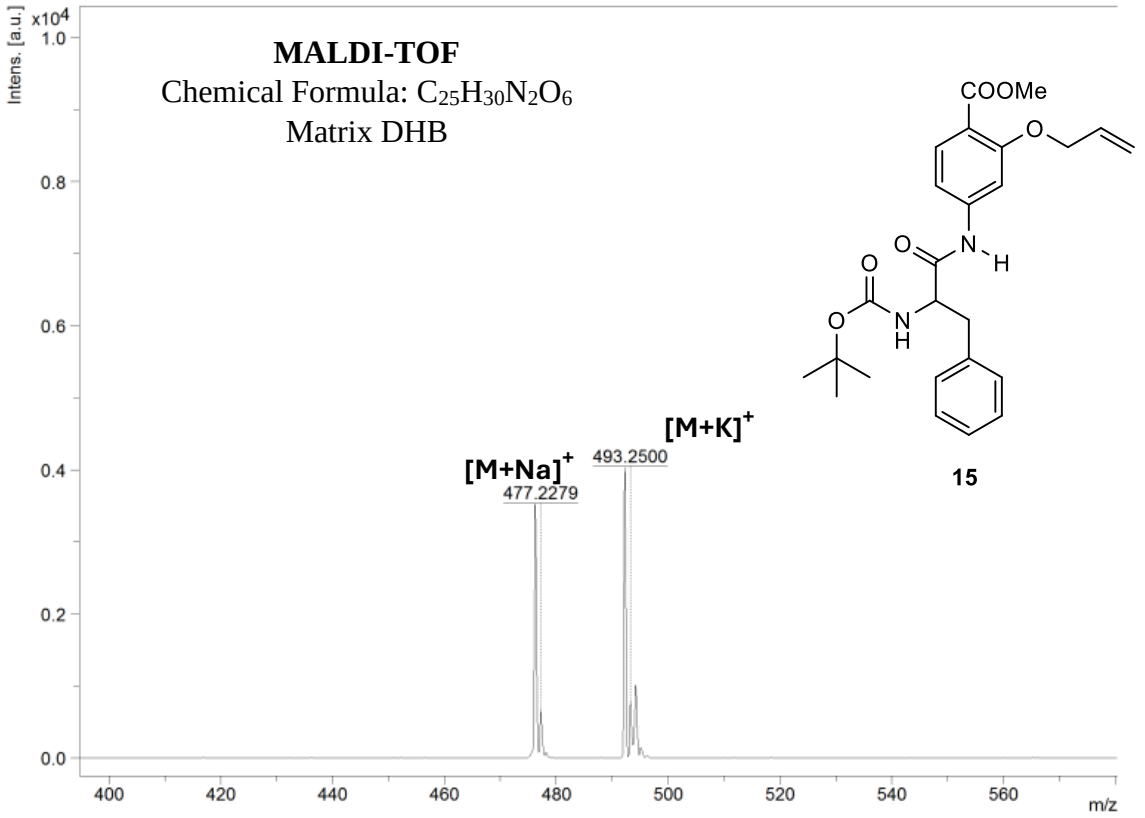
MALDI-TOF
Chemical Formula [M]: $C_{39}H_{36}N_4O_5$
Matrix DHB

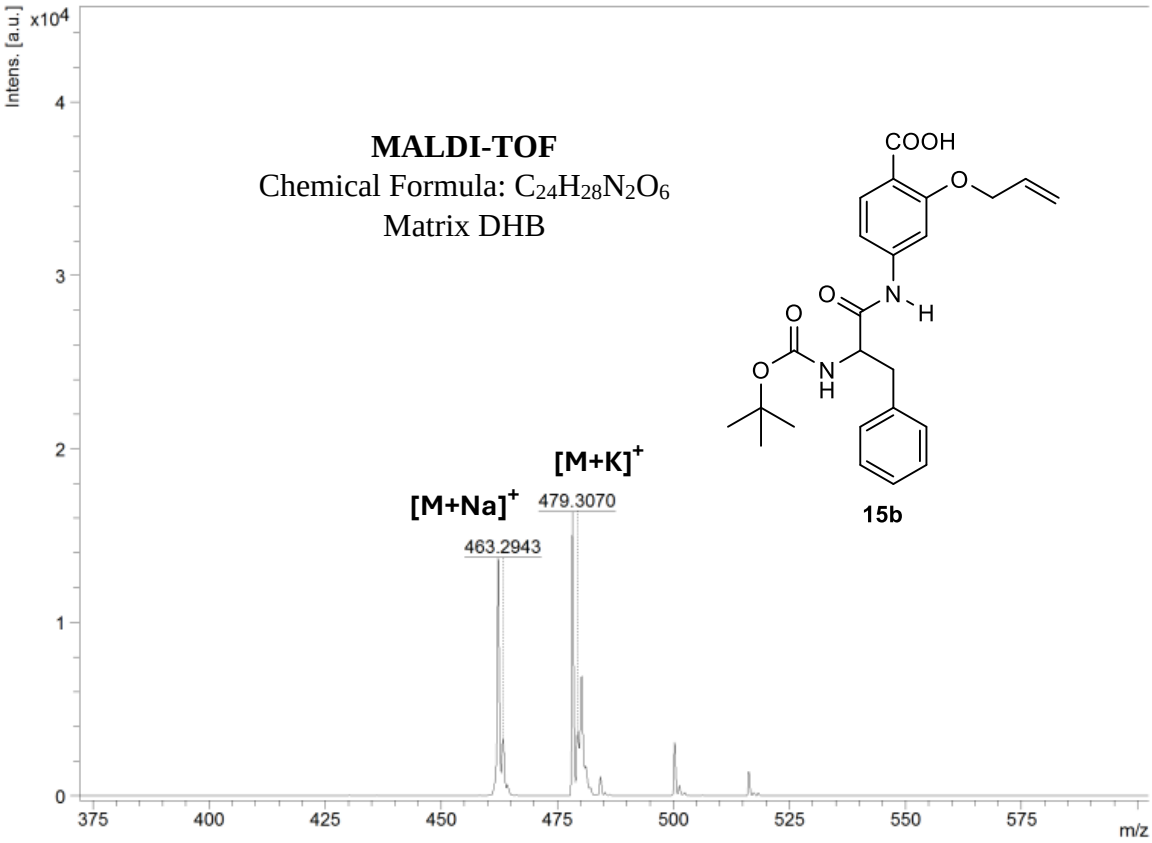

MALDI-TOF
Chemical Formula[M]: C39H36N4O5
Matrix DHB
[M+HCl]
[M+Na]+
663.5347
[M+K]+
679.7514
SK18
Intens. [a.u.]
x10^5
m/z


MALDI-TOF
Chemical Formula[M]: C34H30N4O5
Matrix DHB
[M+HCl]
[M+Na]+
597.6642
[M+K]+
613.6924
SK19
Intens. [a.u.]
x10^4
m/z


MALDI-TOF
Chemical Formula[M]: C31H32N4O5
Matrix DHB
Intens. [a.u.]
x10^5
1.25
1.00
0.75
0.50
0.25
0.00
[M+HCl]
[M+Na]+
563.7005
[M+K]+
579.7956
SK20
500
520
540
560
580
600
620
640
m/z


MALDI-TOF
Chemical Formula[M]: C42H34N4O5
Matrix DHB
Intens. [a.u.]
x10^4
4
3
2
1
0
[M+HCl]
[M+Na]+
697.4235
[M+K]+
713.5711
SK21
550
600
650
700
750
800
850
m/z


MALDI-TOF
Chemical Formula: C25H30N2O6
Matrix DHB
COOMe
O
O
N
H
O
O
N
H
O
15
[M+K]+
493.2500
[M+Na]+
477.2279
Intens. [a.u.]
x10^4
1.0
0.8
0.6
0.4
0.2
0.0
400
420
440
460
480
500
520
540
560
m/z


MALDI-TOF
Chemical Formula: C24H28N2O6
Matrix DHB
COOH
O
O
N
H
O
O
N
H
O
15b
[M+K]+
479.3070
[M+Na]+
463.2943
Intens. [a.u.]
x10^4
4
3
2
1
0
375
400
425
450
475
500
525
550
575
m/z

7.3 RP-HPLC data

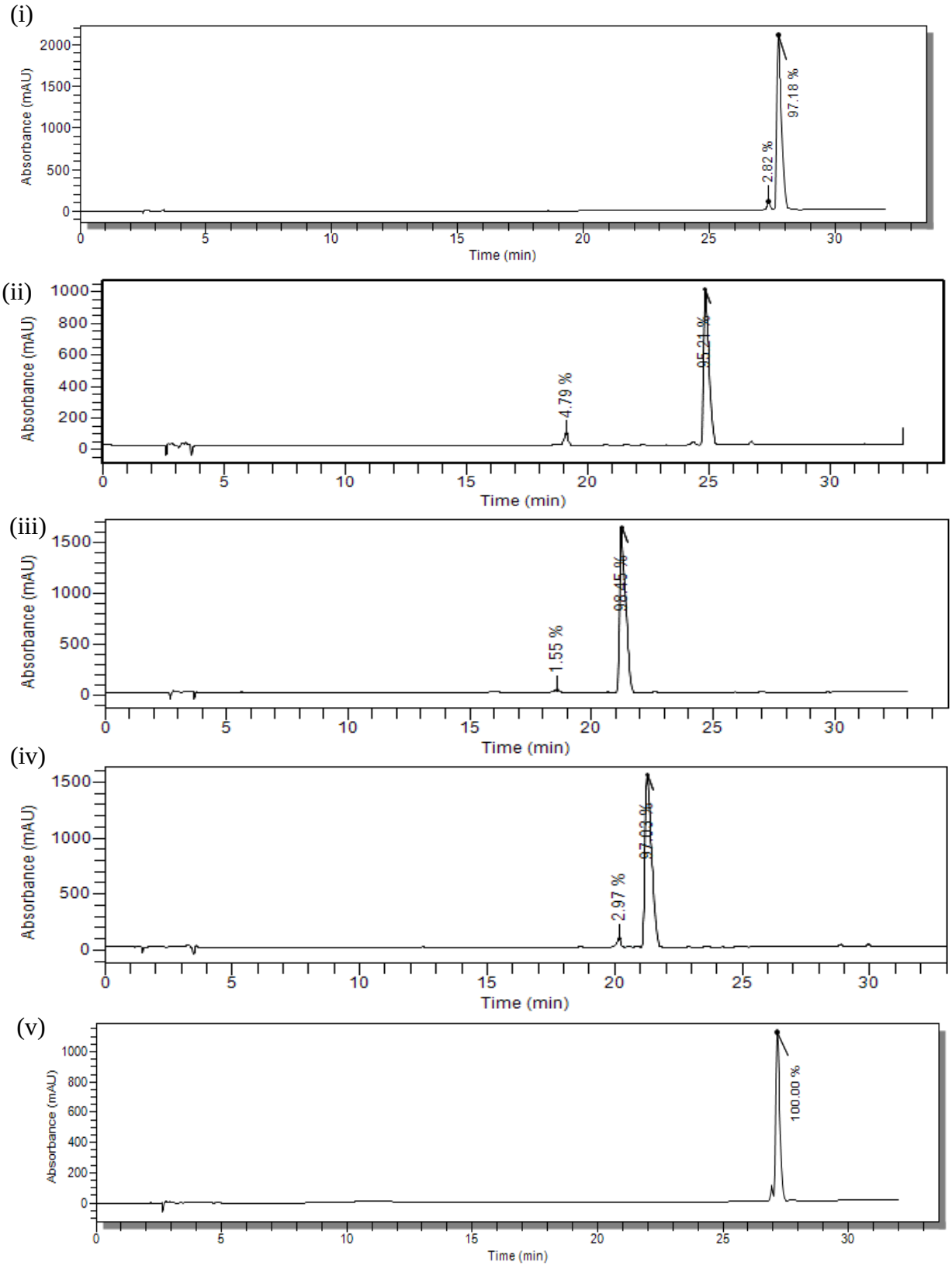


Figure S28. RP-HPLC chromatograms of HCl salts of helix mimetics i) **SK17**, (ii) **SK18**, (iii) **SK19**, (iv) **SK20** and (v) **SK21** Gradient: 5-95 % ACN:water containing 0.1% TFA.

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