Supporting Information

Identification of a Fragment-like Small Molecule Ligand for the Methyl-lysine Binding Protein, 53BP1

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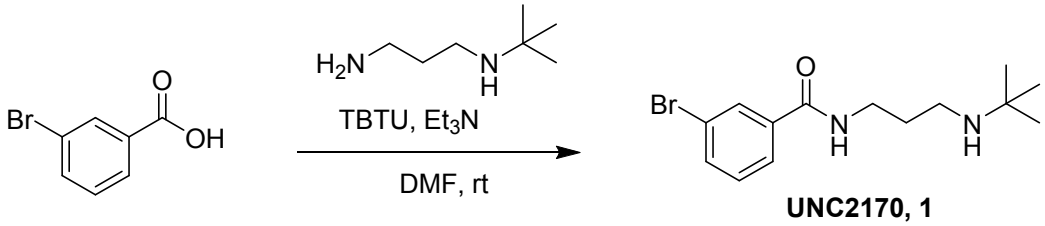
General Procedure for Chemical Synthesis: Analytical LCMS data for all compounds were acquired using an Agilent 6110 series system with the UV detector set to 220 and 254 nm. Samples were injected (<10 µL) onto an Agilent Eclipse Plus 4.6 × 50 mm, 1.8 µm, C18 column at room temperature. A mobile phase of A (H_2O + 0.1% acetic acid) and B (MeOH + 0.1% acetic acid) was used with a linear gradient from 10% to 100% B in 5.0 min, followed by a flush at 100% B for another 2 minutes with a flow rate of 1.0 mL/min. Mass spectra data were acquired in positive ion mode using an Agilent 6110 single quadrupole mass spectrometer with an electrospray ionization source. Nuclear Magnetic Resonance (NMR) spectra were recorded on a Varian Mercury spectrometer at 400 MHz for proton (^{1}H NMR) and 100 MHz for carbon (^{13}C NMR); chemical shifts are reported in ppm (δ). Analytical thin-layer chromatography (TLC) was performed with silica gel 60 F_{254}, 0.25 mm pre-coated TLC plates, generally using a 10% MeOH in DCM solvent system. TLC plates were visualized using UV_{254}, I_2 impregnated silica gel, potassium permanganate with charring, and phosphomolybdic acid with charring. Reverse phase chromatography was used to purify reaction mixtures to obtain final products using a Teledyne Isco CombiFlash Rf 200 chromatography unit equipped with the UV detector set to 220 nm and 254 nm. Samples were injected onto a RediSep Rf 30g C18 high performance Gold column at room temperature and collected at the previously mentioned wavelengths. Mobile phases of A (H_2O + 0.1% TFA) and B (MeOH) were used with a flow rate of 30 mL/min. A general gradient was used consisting of 0-2 minutes at 5% B, 5-15 minutes increasing from 5 to 100% B, and a 100% B flush for another 3 minutes. Small variations in this purification method were made as needed to achieve ideal separation for each compound. All compounds that were evaluated in biochemical and biophysical assays had >95% purity as determined by ^{1}HNMR and LCMS.

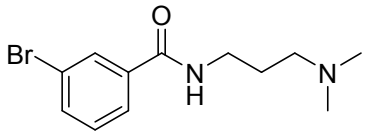
Compound synthesis and characterization:



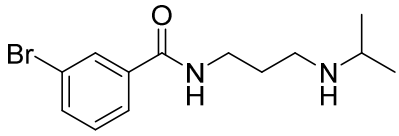
UNC2170, 1

3-Bromo-*N*-(3-(*tert*-butylamino)propyl)benzamide (UNC2170, 1): To a solution of 400 mg (1.98 mmol) of 3-bromobenzoic acid in 2 mL of DMF was added 831 mg (2.6 mmol) of TBTU and the mixture was stirred for 5 min at rt. Upon complete dissolution, 387 mg (2.4 mmol) of *N*-(*tert*-butyl)propane-1,3-diamine and 0.6 mL of Et_3N (6 mmol) was added and the solution was stirred at rt overnight. The

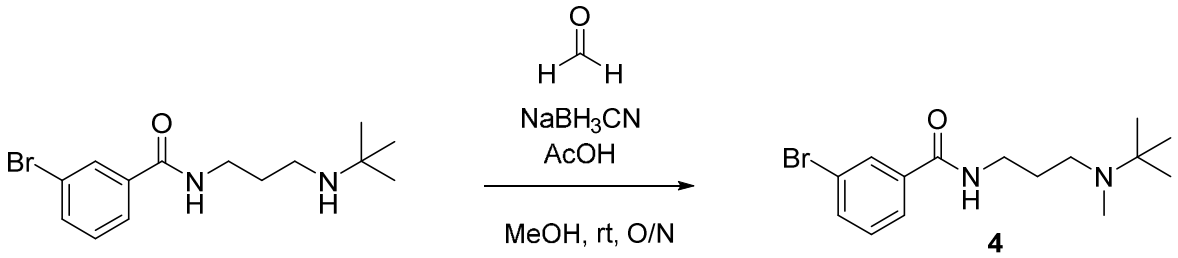
reaction was quenched by addition of 10 mL of sat. aq $NaHCO_3$ and diluted with 15 mL of CH_2Cl_2. The aqueous phase was extracted with CH_2Cl_2 (4 × 15 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4 and filtered. The solvent was evaporated and the crude product was dissolved in 8 mL of CH_2Cl_2 followed by the addition of silica gel. The solvent was again removed by rotary evaporation and the crude material was purified by reverse phase column chromatography using an automated Teledyne Isco chromatography unit to afford 117 mg (69%) of the TFA salt as a yellow, waxy solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.02 (t, *J* = 1.8 Hz, 1H), 7.82 (m, 1H), 7.73 (m, 1H), 7.42 (t, *J* = 7.9 Hz, 1H), 3.51 (t, *J* = 6.5 Hz, 2H), 3.08 – 3.00 (m, 2H), 2.03 – 1.92 (m, 2H), 1.40 (s, 9H). ^{13}C NMR (101 MHz, CD_3OD) δ 169.4, 137.1, 135.9, 131.6, 131.5, 127.1, 123.6, 58.0, 39.9, 37.7, 28.3, 25.9. LC-MS (λ = 254 nm): 100%, t_R = 3.7 min. HRMS calculated for $C_{14}H_{20}N_2OBr$ - H: 311.0759; found: 311.0762 $[M-H]^-$.



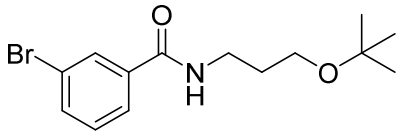
3-Bromo-*N*-(3-(dimethylamino)propyl)benzamide (2): Compound **2** was prepared from 3-bromobenzoic acid (0.15 g, 0.75 mmol) and *N,N*-dimethylpropane-1,3-diamine (0.12 g, 1.1 mmol) by the same procedure as compound **1**. The product was obtained as a clear yellow oil (112 mg, 38%) after purification as the TFA salt. ^{1}H NMR (400 MHz, CD_3OD) δ 8.02 (s, 1H), 7.82 (d, *J* = 7.0 Hz, 1H), 7.69 (d, *J* = 7.2 Hz, 1H), 7.39 (t, *J* = 7.4 Hz, 1H), 3.48 (t, *J* = 6.6 Hz, 2H), 3.19 (t, *J* = 8.1 Hz, 2H), 2.90 (s, 6H), 2.13 – 1.96 (m, 2H). LC-MS (λ = 254 nm): 99%, t_R = 3.1 min. MS (ESI+): 285.1 +287.1 $[M+H]^+$.



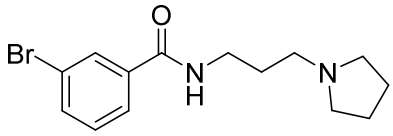
3-Bromo-*N*-(3-(isopropylamino)propyl)benzamide (3): Compound **3** was prepared from 3-bromobenzoic acid (0.15 g, 0.75 mmol) and *N*-isopropylpropane-1,3-diamine (0.10 g, 0.90 mmol) by the same procedure as compound **1**. The product was obtained as a clear yellow oil (113 mg, 37%) after purification as the TFA salt. ^{1}H NMR (400 MHz, CD_3OD) δ 8.01 (t, *J* = 1.8 Hz, 1H), 7.84 – 7.79 (m, 1H), 7.70 (ddd, *J* = 8.0, 1.9, 0.8 Hz, 1H), 7.39 (t, *J* = 7.9 Hz, 1H), 3.50 (t, *J* = 6.5 Hz, 2H), 3.36 (m, 1H), 3.06 (t, *J* = 7.4 Hz, 2H), 2.04 – 1.93 (m, 2H), 1.34 (d, *J* = 6.6 Hz, 6H). LC-MS (λ = 254 nm): 99%, t_R = 3.5 min. MS (ESI+): 299.1 + 301.1 $[M+H]^+$.



3-Bromo-*N*-(3-(*tert*-butyl(methyl)amino)propyl)benzamide (UNC2892, 4): To a solution of **1** (84 mg, 0.27 mmol) in 4 mL of MeOH was added 33 µl (0.44 mmol) formaldehyde solution (37% wt in H_2O), 101 mg (1.6 mmol) sodium cyanoborohydride, and 77 µl (1.3 mmol) acetic acid, and the mixture was stirred overnight at rt. The reaction was monitored by LC-MS until completion and then dried to residue. The crude material was dissolved in 1 mL of DCM on silica and purified by reverse phase column chromatography using the Teledyne Isco automated column system to afford 106 mg (90% yield) as the TFA salt as a cream white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.02 (t, *J* = 1.8 Hz, 1H), 7.82 (m, 1H), 7.71 (m, 1H), 7.40 (t, *J* = 7.9 Hz, 1H), 3.58 – 3.40 (m, 3H), 2.95 (ddd, *J* = 15.2, 13.4, 6.9 Hz, 1H), 2.83 (s, 3H), 2.22 – 2.07 (m, 1H), 2.07 – 1.92 (m, 1H), 1.43 (s, 9H). ^{13}C NMR (101 MHz, CD_3OD) δ 169.0, 137.2, 135.8, 131.5, 131.4, 127.1, 123.6, 65.3, 50.0, 38.0, 35.1, 27.0, 24.6. LC-MS (λ = 254 nm): 99%, t_R = 3.4 min. HRMS calculated for $C_{15}H_{22}N_2OBr$ - H: 325.0916; Found: 325.0921 $[M-H]^-$.

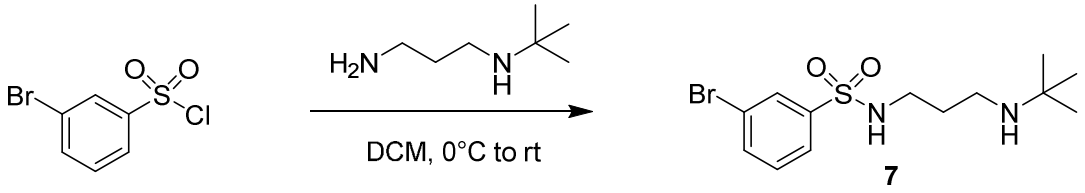


3-Bromo-*N*-(3-(*tert*-butoxy)propyl)benzamide (5): Compound **5** was prepared from 3-bromobenzoic acid (0.10 g, 0.50 mmol) and 3-(*tert*-butoxy)propan-1-amine HCl (0.10 g, 0.60 mmol) by the same procedure as compound **1**. The product was obtained as a clear yellow oil (146 mg, 69%) after purification as the TFA salt. ^{1}H NMR (400 MHz, CD_3OD) δ 7.96 (t, *J* = 1.8 Hz, 1H), 7.77 (m, 1H), 7.67 (m, 1H), 7.37 (t, *J* = 7.9 Hz, 1H), 3.49 (t, *J* = 6.4 Hz, 2H), 3.45 (t, *J* = 7.3 Hz, 2H), 1.87 – 1.78 (m, 2H), 1.19 (s, 9H). LC-MS (λ = 254 nm): 99%, t_R = 5.8 min. MS (ESI+): 336.1 + 338.1 $[M+Na]^+$.



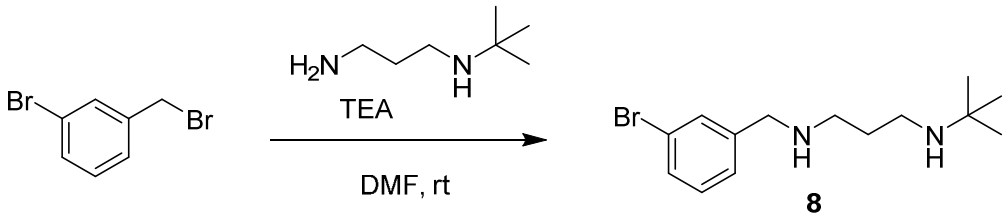
3-Bromo-*N*-(3-(pyrrolidin-1-yl)propyl)benzamide (6): Compound **6** was prepared from 3-bromobenzoic acid (0.5 g, 2.5 mmol) and 3-(pyrrolidin-1-yl)propan-1-amine (0.38 g, 3.0 mmol) by the same procedure

as compound **1**. The product was obtained as a yellow oil (657 mg, 85%) after purification as the TFA salt. ^{1}H NMR (400 MHz, CD_3OD) δ 7.97 (t, *J* = 1.8 Hz, 1H), 7.81 – 7.75 (m, 1H), 7.69 (ddd, *J* = 8.0, 2.0, 1.0 Hz, 1H), 7.39 (t, *J* = 7.9 Hz, 1H), 3.43 (t, *J* = 6.9 Hz, 2H), 2.68 – 2.58 (m, 6H), 1.84 (m, 6H). LC-MS (λ = 254 nm): 99%, t_R = 2.7 min. MS (ESI+): 311.1 + 313.1 $[M+H]^+$.



7

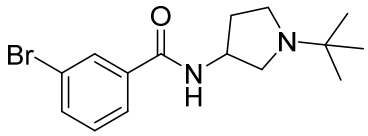
3-Bromo-*N*-(3-(*tert*-butylamino)propyl)benzenesulfonamide (7): To a solution of 3-bromobenzenesulfonyl chloride (0.12 g, 0.47 mmol) in dichloromethane at 0°C was slowly added *N*-(*tert*-butyl)propane-1,3-diamine (0.063 g, 0.48 mmol). The reaction was stirred at 0°C for 30 min and then allowed to warm to room temperature. The reaction was monitored by TLC until it had gone to completion, and then diluted with MeOH, concentrated down, re-suspended in methanol and dried onto silica. The reaction mixture was purified by reverse phase column chromatography using a Teledyne Isco reverse phase chromatography column. The product was obtained as a white solid (147 mg, 68%) after purification as the TFA salt. ^{1}H NMR (400 MHz, CD_3OD) δ 8.00 (s, 1H), 7.81 (m, 2H), 7.51 (t, *J* = 7.9 Hz, 1H), 3.02 (dd, *J* = 10.8, 5.1 Hz, 4H), 1.93 – 1.79 (m, 2H), 1.35 (s, 9H). LC-MS (λ = 254 nm): 99%, t_R = 3.6 min. MS (ESI+): 349.1 + 351.10 $[M+H]^+$.



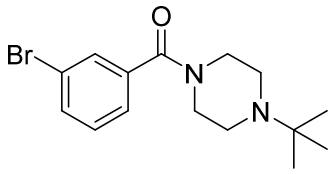
8

***N*^{1}-(3-Bromobenzyl)-*N*^{3}-(*tert*-butyl)propane-1,3-diamine (8):** To a solution of 1-bromo-3-(bromomethyl)benzene (0.23 g, 0.92 mmol) and triethylamine (0.233 g, 2.3 mmol) in 2 mL dimethylformamide at room temperature was slowly added *N*-(*tert*-butyl)propane-1,3-diamine (0.1 g, 0.77 mmol) and the reaction was stirred overnight. Once the reaction had gone to completion via TLC analysis, the reaction mixture was diluted with MeOH, concentrated down to a residue, re-suspended in methanol, and dried onto silica. The reaction mixture was purified by reverse phase column chromatography using a Teledyne Isco reverse phase chromatography column. The product was

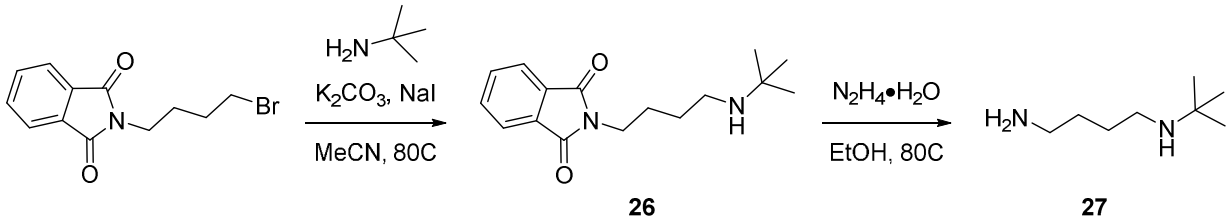
obtained as a clear yellow oil (37 mg, 11%) after purification as the TFA salt. ^{1}H NMR (400 MHz, CD_3OD) δ 7.73 (s, 1H), 7.62 (d, *J* = 8.0 Hz, 1H), 7.49 (d, *J* = 7.7 Hz, 1H), 7.38 (t, *J* = 7.8 Hz, 1H), 4.23 (s, 2H), 3.20 (t, *J* = 7.6 Hz, 2H), 3.10 (dd, J = 9.2, 8.3 Hz 2H), 2.12 (dt, *J* = 15.5, 7.9 Hz, 2H), 1.37 (s, 9H). LC-MS (λ = 254 nm): 99%, t_R = 1.8 min. MS (ESI+): 299.2 + 301.2 $[M+H]^+$.



(3-Bromophenyl)(3-(*tert*-butyl)imidazolidin-1-yl)methanone (9): Compound **9** was prepared from 3-bromobenzoic acid (0.10 g, 0.50 mmol) and 1-(*tert*-butyl)pyrrolidin-3-amine (0.09 g, 0.63 mmol) by the same procedure as compound **1**. The product was obtained as a clear yellow oil (158 mg, 73%) after purification as the TFA salt. ^{1}H NMR (400 MHz, CD_3OD) δ 8.03 (d, J = 1.6 Hz, 1H), 7.86 – 7.80 (m, 1H), 7.70 (d, J = 7.9 Hz, 1H), 7.39 (m, 1H), 4.73 – 4.58 (m, 1H), 3.82 (m, 1H), 3.73 – 3.61 (m, 1H), 3.54 (m, 1H), 3.46 – 3.25 (m, 1H), 2.58 – 2.36 (m, 1H), 2.22 (m, 1H), 1.42 (s, 9H). LC-MS (λ = 254 nm): 97%, t_R = 3.7 min. MS (ESI+): 325.1 + 327.1 $[M+H]^+$.



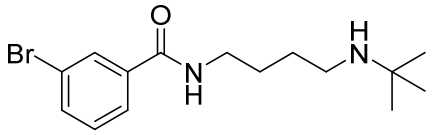
(3-Bromophenyl)(4-(*tert*-butyl)piperazin-1-yl)methanone (10): Compound **10** was prepared from 3-bromobenzoic acid (0.10 g, 0.50 mmol) and 1-(*tert*-butyl)piperazine (0.09 g, 0.63 mmol) by the same procedure as compound **1**. The product was obtained as a clear yellow oil (201 mg, 92%) after purification as the TFA salt. ^{1}H NMR (400 MHz, CD_3OD) δ 7.70 (m, 1H), 7.67 (m, 1H), 7.47 (dt, *J* = 7.7, 1.3 Hz, 1H), 7.40 (m, 1H), 4.71 (m, 1H), 4.23 – 3.25 (m, 5H), 3.17 (m, 2H), 1.43 (s, 9H). LC-MS (λ = 254 nm): 95%, t_R = 3.4 min. MS (ESI+): 325.1 + 327.1 $[M+H]^+$.



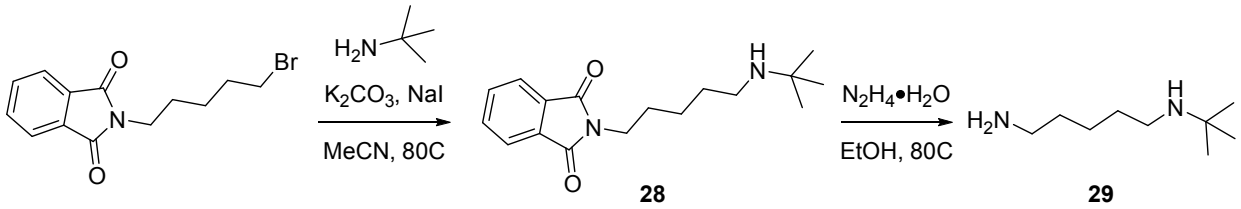
26 **27**

2-(4-(*Tert*-butylamino)butyl)isoindoline-1,3-dione (26): To a solution of 2-(4-bromobutyl)isoindoline-1,3-dione (0.40 g, 1.43 mmol) in acetonitrile (10 ml) was added *N*-*tert*-butylamine (0.14 ml, 1.34 mmol), potassium carbonate (0.39 g, 2.84 mmol), and sodium iodide (0.43 g, 2.83 mmol) and the reaction was stirred overnight at 80°C. After 24 hrs the reaction was cooled to room temperature, dried down to a crude residue, dissolved in water (10 ml), and then extracted with dichloromethane 3 times. The organic layers were combined, dried over Na_2SO_4, and filtered. The crude material was dried onto silica and purified by reverse phase column chromatography using a Teledyne Isco reverse phase chromatography column. The product was obtained as a white solid (117 mg, 22%) after purification as the TFA salt. ^{1}H NMR (400 MHz, CD_3OD)δ 7.85 – 7.78 (m, 4H), 3.74 (t, *J* = 6.7 Hz, 2H), 3.06 - 3.02 (m, 2H), 1.84 - 1.78 (m, 2H), 1.73 - 1.65 (s, 2H), 1.36 (s, 9H). LC-MS (λ = 254 nm): 99%, t_R = 3.6 min. MS (ESI+): 275.2 $[M+H]^+$.

***N*-(*Tert*-butyl)butane-1,4-diamine (27):** To a solution of **26** (0.12 g, 0.31 mmol) in 10 mL of ethanol was added hydrazine monohydrate (0.03 ml, 0.61 mmol) and stirred for 2 hrs at 80°C. The reaction was cooled, dried to a residue, dissolved in chloroform, and filtered. The resulting solution was dried onto silica and purified by reverse phase column chromatography using a Teledyne Isco reverse phase chromatography column and an ELSD detector. The product was obtained as a white solid (34 mg, 29%) after purification as the TFA salt. ^{1}H NMR (400 MHz, CD_3OD) δ 3.03 – 2.97 (m, 4H), 1.81 – 1.71 (m, 4H), 1.37 (s, 9H). LC-MS (λ = 254 nm): 99%, t_R = 0.6 min. MS (ESI+): 145.3 $[M+H]^+$.



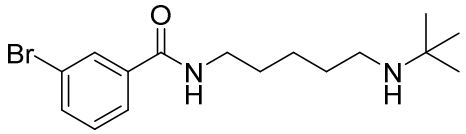
3-Bromo-*N*-(4-(*tert*-butylamino)butyl)benzamide (11): Compound **11** was prepared from 3-bromobenzoic acid (40 mg, 0.19 mmol) and **27** (34 mg, 0.091 mmol) by the same procedure as compound **1**. The product was obtained as a clear yellow oil (23 mg, 57%) after purification as the TFA salt. ^{1}H NMR (400 MHz, CD_3OD) δ 7.99 (t, *J* = 1.7 Hz, 1H), 7.80 (m, 1H), 7.71 (m, 1H), 7.40 (t, *J* = 7.9 Hz, 1H), 3.45 (t, *J* = 6.4 Hz, 2H), 3.07 – 2.99 (m, 2H), 1.79 – 1.67 (m, 4H), 1.37 (s, 9H). LC-MS (λ = 254 nm): 95%, t_R = 4.2 min. MS (ESI+): 327.1 + 329.1 $[M+H]^+$.



28 **29**

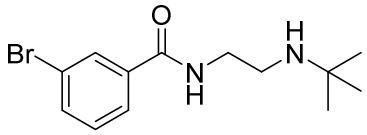
2-(5-(*Tert*-butylamino)pentyl)isoindoline-1,3-dione (28): To a solution of 2-(5-bromopentyl)isoindoline-1,3-dione (0.41 g, 1.37 mmol) in acetonitrile (10 ml) was added *N-tert*-butylamine (0.14 ml, 1.34 mmol), potassium carbonate (0.39 g, 2.70 mmol), and sodium iodide (0.41 g, 2.74 mmol) and the reaction was stirred overnight at 80°C. After 24 hrs the reaction was cooled to room temperature, dried down to a crude residue, dissolved in water (10 ml), and then extracted with dichloromethane 3 times. The organic layers were combined, dried over Na_2SO_4, and filtered. The crude material was dried onto silica and purified by reverse phase column chromatography using a Teledyne Isco reverse phase chromatography column. The product was obtained as a white solid (156 mg, 29%) after purification as the TFA salt. ^{1}H NMR (400 MHz, CD_3OD) δ 7.85 – 7.78 (m, 4H), 3.70 (t, *J* = 8.1 Hz, 2H), 2.98 – 2.94 (m, 2H), 1.78 - 1.68 (m, 4H), 1.52 - 1.42 (m, 2H), 1.36 (s, 9H). LC-MS (λ = 254 nm): 99%, t_R = 4.0 min. MS (ESI+): 289.2 $[M+H]^+$.

***N*-(*Tert*-butyl)pentane-1,5-diamine (29):** To a solution of **28** (0.16 g, 0.40 mmol) in 10 mL of ethanol was added hydrazine monohydrate (0.07 ml, 1.43 mmol) and stirred for 2 hrs at 80°C. The reaction was cooled, dried to a residue, dissolved in chloroform, and filtered. The resulting solution was dried onto silica and purified by reverse phase column chromatography using a Teledyne Isco reverse phase chromatography column and an ELSD detector. The product was obtained as a white solid (71 mg, 47%) after purification as the TFA salt. ^{1}H NMR (400 MHz, CD_3OD) δ 2.99 – 2.92 (m, 4H), 1.75 – 1.67 (m, 4H), 1.53 – 1.46 (m, 2H), 1.37 (s, 9H). LC-MS (λ = 254 nm): 99%, t_R = 0.6 min. MS (ESI+): 159.2 $[M+H]^+$.

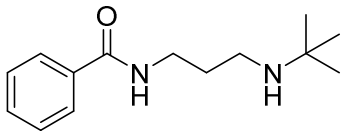


3-Bromo-*N*-(5-(*tert*-butylamino)pentyl)benzamide (12): Compound **12** was prepared from 3-bromobenzoic acid (0.075 g, 0.37 mmol) and **29** (0.071 g, 0.18 mmol) by the same procedure as compound **1**. The product was obtained as a clear yellow oil (47 mg, 57%) after purification as the TFA salt. ^{1}H NMR (400 MHz, CD_3OD) δ 7.99 (t, *J* = 1.8 Hz, 1H), 7.82 – 7.75 (m, 1H), 7.70 (m, 1H), 7.40 (t, *J* = 7.9

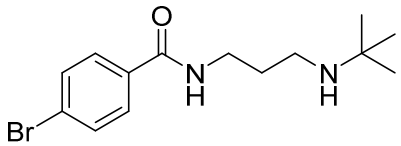
Hz, 1H), 3.41 (t, *J* = 7.0 Hz, 2H), 3.01 – 2.94 (m, 2H), 1.71 (m, 4H), 1.54 – 1.43 (m, 2H), 1.37 (s, 9H). LC-MS (λ = 254 nm): 99%, t_R = 4.3 min. MS (ESI+): 341.3 + 343.1 $[M+H]^+$.



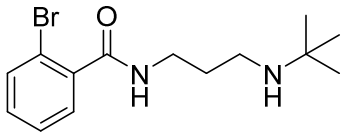
3-Bromo-*N*-(2-(*tert*-butylamino)ethyl)benzamide (13): Compound **13** was prepared from 3-bromobenzoic acid (0.10 g, 0.50 mmol) and *N*-(*tert*-butyl)ethane-1,2-diamine (0.07 g, 0.59 mmol) by the same procedure as compound **1**. The product was obtained as a white solid (119 mg, 58%) after purification as the TFA salt. ^{1}H NMR (400 MHz, CD_3OD) δ 8.05 (m, 1H), 7.85 (m, 1H), 7.74 – 7.68 (m, 1H), 7.40 (t, *J* = 7.9 Hz, 1H), 3.70 (t, *J* = 6.2 Hz, 2H), 3.24 (t, *J* = 6.2 Hz, 2H), 1.39 (s, 9H). LC-MS (λ = 254 nm): 99%, t_R = 3.8 min. MS (ESI+): 299.1 + 301.1 $[M+H]^+$.



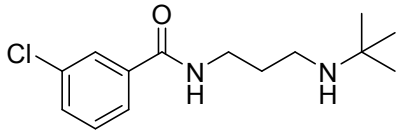
***N*-(3-(*Tert*-butylamino)propyl)benzamide (14):** Compound **14** was prepared from benzoic acid (0.10 g, 0.82 mmol) and *N*-(*tert*-butyl)propane-1,3-diamine (0.13 g, 0.98 mmol) by the same procedure as compound **1**. The product was obtained as a clear oil (192 mg, 67%) after purification as the TFA salt. ^{1}H NMR (400 MHz, CD_3OD) δ 7.85 (m, 2H), 7.59 – 7.54 (m, 1H), 7.51 – 7.45 (m, 2H), 3.53 (t, *J* = 6.5 Hz, 2H), 3.04 (t, *J* = 7.3 Hz, 2H), 2.05 – 1.92 (m, 2H), 1.39 (s, 9H). LC-MS (λ = 254 nm): 96%, t_R = 3.4 min. MS (ESI+): 235.2 $[M+H]^+$.



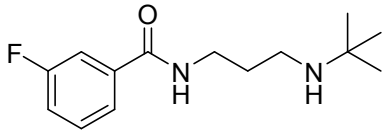
4-Bromo-*N*-(3-(*tert*-butylamino)propyl)benzamide (15): Compound **15** was prepared from 4-bromobenzoic acid (0.10 g, 0.50 mmol) and *N*-(*tert*-butyl)propane-1,3-diamine (0.10 g, 0.74 mmol) by the same procedure as compound **1**. The product was obtained as a clear yellow oil (120 mg, 56%) after purification as the TFA salt. ^{1}H NMR (400 MHz, CD_3OD) δ 7.76 (d, *J* = 8.6 Hz, 2H), 7.63 (d, *J* = 8.6 Hz, 2H), 3.50 (t, *J* = 6.5 Hz, 2H), 3.03 (t, *J* = 7.4 Hz, 2H), 2.04 – 1.93 (m, 2H), 1.37 (s, 9H). LC-MS (λ = 254 nm): 99%, t_R = 3.7 min. MS (ESI+): 313.1 + 315.1 $[M+H]^+$.



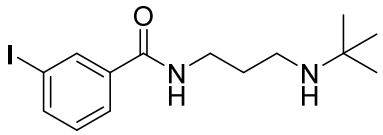
2-Bromo-*N*-(3-(*tert*-butylamino)propyl)benzamide (16): Compound **16** was prepared from 2-bromobenzoic acid (0.10 g, 0.50 mmol) and *N*-(*tert*-butyl)propane-1,3-diamine (0.08 g, 0.60 mmol) by the same procedure as compound **1**. The product was obtained as a clear yellow oil (191 mg, 55%) after purification as the TFA salt. ^{1}H NMR (400 MHz, CD_3OD) δ 7.64 (m, 1H), 7.45 – 7.40 (m, 2H), 7.36 (m, 1H), 3.50 (t, *J* = 6.2 Hz, 2H), 3.17 – 3.07 (m, 2H), 2.06 – 1.95 (m, 2H), 1.38 (s, 9H). LC-MS (λ = 254 nm): 95%, t_R = 3.3 min. MS (ESI+): 313.1 + 315.1 $[M+H]^+$.



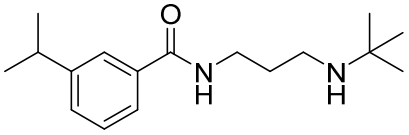
***N*-(3-(*Tert*-butylamino)propyl)-3-chlorobenzamide (17):** Compound **17** was prepared from 3-chlorobenzoic acid (0.10 g, 0.64 mmol) and *N*-(*tert*-butyl)propane-1,3-diamine (0.10 g, 0.76 mmol) by the same procedure as compound **1**. The product was obtained as a clear yellow oil (115 mg, 47%) after purification as the TFA salt. ^{1}H NMR (400 MHz, CD_3OD) δ 7.86 (m, 1H), 7.77 (m, 1H), 7.55 – 7.51 (m, 1H), 7.44 (m, 1H), 3.51 (t, *J* = 6.5 Hz, 2H), 3.03 (t, *J* = 7.4 Hz, 2H), 2.05 – 1.94 (m, 2H), 1.37 (s, 9H). LC-MS (λ = 254 nm): 99%, t_R = 3.4 min. MS (ESI+): 269.2 $[M+H]^+$.



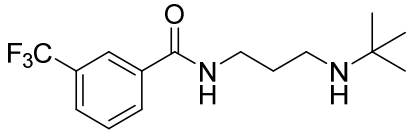
***N*-(3-(*Tert*-butylamino)propyl)-3-fluorobenzamide (18):** Compound **18** was prepared from 3-fluorobenzoic acid (0.10 g, 0.71 mmol) and *N*-(*tert*-butyl)propane-1,3-diamine (0.11 g, 0.85 mmol) by the same procedure as compound **1**. The product was obtained as a clear yellow oil (68 mg, 26%) after purification as the TFA salt. ^{1}H NMR (400 MHz, CD_3OD) δ 7.68 (d, *J* = 7.8 Hz, 1H), 7.61 – 7.56 (m, 1H), 7.49 (td, *J* = 8.0, 5.7 Hz, 1H), 7.29 (td, *J* = 8.4, 2.5 Hz, 1H), 3.52 (t, *J* = 6.5 Hz, 2H), 3.04 (t, *J* = 7.4 Hz, 2H), 2.06 – 1.94 (m, 2H), 1.38 (s, 9H). LC-MS (λ = 254 nm): 99%, t_R = 3.1 min. MS (ESI+): 253.2 $[M+H]^+$.



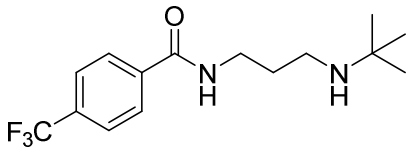
***N*-(3-(*Tert*-butylamino)propyl)-3-iodobenzamide (19):** Compound **19** was prepared from 3-iodobenzoic acid (0.10 g, 0.40 mmol) and *N*-(*tert*-butyl)propane-1,3-diamine (0.06 g, 0.99 mmol) by the same procedure as compound **1**. The product was obtained as a clear yellow oil (90 mg, 47%) after purification as the TFA salt. ^{1}H NMR (400 MHz, CD_3OD) δ 8.20 (t, *J* = 1.6 Hz, 1H), 7.91 – 7.81 (m, 2H), 7.23 (t, *J* = 7.8 Hz, 1H), 3.50 (t, *J* = 6.5 Hz, 2H), 3.03 (t, *J* = 7.4 Hz, 2H), 2.04 – 1.93 (m, 2H), 1.37 (s, 9H). LC-MS (λ = 254 nm): 99%, t_R = 3.8 min. MS (ESI+): 361.1 $[M+H]^+$.



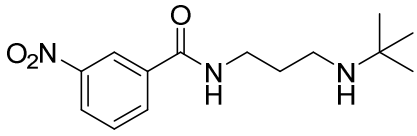
***N*-(3-(*Tert*-butylamino)propyl)-3-isopropylbenzamide (20):** Compound **20** was prepared from 3-isopropylbenzoic acid (95 mg, 0.58 mmol) and *N*-(*tert*-butyl)propane-1,3-diamine (0.1 g, 0.73 mmol) by the same procedure as compound **1**. The product was obtained as a clear oil (171 mg, 76%) after purification as the TFA salt. ^{1}H NMR (400 MHz, CD_3OD) δ 7.75 (t, *J* = 1.8 Hz, 1H), 7.67 (dt, *J* = 7.4, 1.6 Hz, 1H), 7.42 (m, 1H), 7.37 (t, *J* = 7.6 Hz, 1H), 3.53 (t, *J* = 6.5 Hz, 2H), 3.04 (t, *J* = 7.3 Hz, 2H), 2.95 (m, 1H), 2.07 – 1.95 (m, 2H), 1.38 (s, 9H), 1.26 (d, *J* = 6.9 Hz, 6H). LC-MS (λ = 254 nm): 99%, t_R = 4.2 min. MS (ESI+): 277.2 $[M+H]^+$.



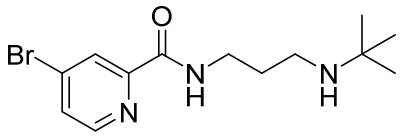
***N*-(3-(*Tert*-butylamino)propyl)-3-(trifluoromethyl)benzamide (21):** Compound **21** was prepared from 3-(trifluoromethyl)benzoic acid (0.10 g, 0.48 mmol) and *N*-(*tert*-butyl)propane-1,3-diamine (0.08 g, 0.63 mmol) by the same procedure as compound **1**. The product was obtained as a clear oil (193 mg, 97%) after purification as the TFA salt. ^{1}H NMR (400 MHz, CD_3OD) δ 8.17 (s, 1H), 8.12 (d, *J* = 7.9 Hz, 1H), 7.85 (d, *J* = 7.5 Hz, 1H), 7.68 (t, *J* = 7.8 Hz, 1H), 3.55 (t, *J* = 6.5 Hz ,2H), 3.06 (t, *J* = 7.5 Hz, 2H), 2.02 (m, 2H), 1.40 (s, 9H). LC-MS (λ = 254 nm): 99%, t_R = 4.1 min. MS (ESI+): 303.2 $[M+H]^+$.



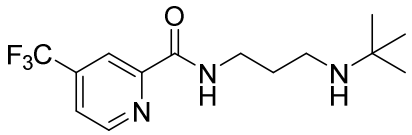
***N*-(3-(*Tert*-butylamino)propyl)-4-(trifluoromethyl)benzamide (22):** Compound **22** was prepared from 4-(trifluoromethyl)benzoic acid (0.10 g, 0.48 mmol) and *N*-(*tert*-butyl)propane-1,3-diamine (0.09 g, 0.72 mmol) by the same procedure as compound **1**. The product was obtained as a clear oil (128 mg, 64%) after purification as the TFA salt. ^{1}H NMR (400 MHz, CD_3OD) δ 8.03 (d, *J* = 8.1 Hz, 2H), 7.78 (d, *J* = 8.2 Hz, 2H), 3.54 (t, *J* = 6.6 Hz, 2H), 3.06 (t, *J* = 7.5 Hz, 2H), 2.02 (m, 2H), 1.39 (s, 9H). LC-MS (λ = 254 nm): 99%, t_R = 4.1 min.MS (ESI+): 303.2 $[M+H]^+$.



***N*-(3-(*Tert*-butylamino)propyl)-3-nitrobenzamide (23):** Compound **23** was prepared from 3-nitrobenzoic acid (0.10 g, 0.6 mmol) and *N*-(*tert*-butyl)propane-1,3-diamine (0.09 g, 0.73 mmol) by the same procedure as compound **1**. The product was obtained as a yellow solid (43 mg, 18%) after purification as the TFA salt. ^{1}H NMR (400 MHz, CD_3OD) δ 8.71 (t, *J* = 2.0 Hz, 1H), 8.41 (m, 1H), 8.25 (m, 1H), 7.75 (t, *J* = 8.0 Hz, 1H), 3.56 (t, *J* = 6.6 Hz, 2H), 3.11 – 3.04 (m, 2H), 2.02 (m, 2H), 1.39 (s, 9H). LC-MS (λ = 254 nm): 99%, t_R = 3.5 min. MS (ESI+): 280.2 $[M+H]^+$.



4-Bromo-*N*-(3-(*tert*-butylamino)propyl)picolinamide (24): Compound **24** was prepared from 4-bromopicolinic acid (0.13 g, 0.65 mmol) and *N*-(*tert*-butyl)propane-1,3-diamine (0.1 g, 0.78 mmol) by the same procedure as compound **1**. The product was obtained as a yellow solid (43 mg, 14%) after purification as the TFA salt. ^{1}H NMR (400 MHz, CD_3OD) δ 8.92 (d, *J* = 5.4 Hz, 1H), 8.34 (s, 1H), 7.89 (d, *J* = 4.9 Hz, 1H), 3.58 (t, *J* = 6.5 Hz, 2H), 3.04 (t, *J* = 7.5 Hz, 2H), 2.02 (m, 2H), 1.38 (s, 9H). LC-MS (λ = 254 nm): 98%, t_R = 3.6 min. MS (ESI+): 314.1 + 316.1 $[M+H]^+$.



***N*-(3-(*Tert*-butylamino)propyl)-4-(trifluoromethyl)picolinamide (25):** Compound **25** was prepared from 4-(trifluoromethyl)picolinic acid (0.10 g, 0.52 mmol) and *N*-(*tert*-butyl)propane-1,3-diamine (0.08 g, 0.63 mmol) by the same procedure as compound **1**. The product was obtained as a yellow solid (40 mg, 18%) after purification as the TFA salt. ^{1}H NMR (400 MHz, CD_3OD) δ 8.91 (d, *J* = 5.0 Hz, 1H), 8.33 (s, 1H), 7.89 (d, *J* = 5.0, 1H), 3.58 (t, *J* = 6.5 Hz, 2H), 3.11 – 3.02 (m, 2H), 2.07 – 1.96 (m, 2H), 1.38 (s, 9H). LC-MS (λ = 254 nm): 99%, t_R = 3.9 min. MS (ESI+): 304.2 $[M+H]^+$.

Protein Expression and Purification: 53BP1, L3MBTL1, L3MBTL3, CBX7, MBTD1, and UHRF1 (tandem tudor-PHD construct) were expressed and purified as previously described.(*1, 2*) The proteins constructs for the aforementioned proteins were provided by the Structural Genomics Consortium. PHF1, PHF19, PHF23, and JARID1A protein constructs were provided by Greg Wang (UNC) and prepared in the same manner. The construct for the 53BP1 D1521A mutant (residues 1484 -1603) was obtained from George Mer (Mayo Clinic) and was expressed and purifed in the same manner as 53BP1.(*1, 3*)

ITC: All ITC measurements were recorded at 25 °C with an AutoITC$_{200}$ microcalorimeter (MicroCal Inc.). All protein and compound stock samples were in the target buffer (25 mM Tris-HCl, pH 8, 150 mM NaCl, and 2 mM β-mercaptoethanol), and then diluted in the same buffer to achieve the desired concentrations: 90 – 200 μM protein and 1 – 2 mM compound depending on the expected dissociation constant. The concentration of protein stock solutions were established using the Edelhoch method, whereas 10 mM compound stock solutions were prepared gravimetrically based on molecular weight. A typical experiment included a single 0.2 μL compound injection into a 200 μL cell filled with protein, followed by 25 subsequent 1.5 μL injections of compound. Injections were performed with a spacing of 180 seconds and a reference power of 8 μcal/sec. If applicable, the heats of dilution generated were then subtracted from the protein-compound binding curves. The titration data was analyzed using Origin Software (MicroCal Inc.) by non-linear least squares, fitting the heats of binding as a function of the compound:protein ratio. The data were fit based on a one set of sites model.

Crystallization:

Protein Expression: The expression construct for 53BP1 tudor domain (residues 1438 – 1603) in pET28-MHL vector was transformed into BL21(DE3)-V2R-pRARE2 cells. The cells were incubated in Terrific Broth medium (TB) in the presence of 50 μg/mL kanamycin and 34 μg/mL chloramphenicol at 37 °C. When the OD_{600} reached 1.5, the overexpression of 53BP1 was induced by addition of isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 0.5 mM, and incubated overnight at 16 °C. Next day, the cells were harvested by centrifugation at 12,227 × g (10 min, 4C) and the cell pellets were flash frozen in liquid N_2 and stored at -80 °C.

Purification: The cell pellet (18.8 g) was thawed and resuspended in 190 mL of lysis buffer (20 mM HEPES, pH 7.5, 500 mM NaCl, 5 mM imidazole, 0.5 mM TCEP, and 5% glycerol). The cell suspension was supplemented with 0.5% (w/v) CHAPS, 5 μl of benzonase (EMD Millipore, cat. no. 70746), protease inhibitor cocktail (Roche) and the cells were sonicated on ice for 5 min total (10 s pulses with 5s interruptions). The lysate was clarified by centrifugation at 20,000 × g, 4 °C, 60 min and the resulting supernatant was filtered through 0.45 μm filter and applied onto 5 mL HisTrap HP column (GE). The column was washed with 10 CV of wash buffer (20 mM HEPES, pH 7.5, 500 mM NaCl, 40 mM imidazole, 0.5 mM TCEP, and 5% glycerol) and the protein was eluted using elution buffer (20 mM HEPES, pH 7.5, 500 mM NaCl, 250 mM imidazole, 0.5 mM TCEP, and 5% glycerol). TEV protease was added to the eluted protein (at ratio of 1 mg of TEV protease per 50 mg protein), and incubated overnight at 4 °C during dialysis against 20 mM HEPES, pH 7.5, 500 mM NaCl, 0.5 mM TCEP, and 5% glycerol. The uncleaved protein (and His-tagged TEV protease) were removed by passing through 1 mL HisTrap HP column (GE). The cleaved protein was collected in the flow-through fraction. As the final purification step, the cleaved protein was applied on 16/600 Superdex 200 (GE) column equilibrated with 20 mM HEPES, pH 7.5, 150 mM NaCl, 0.5 mM TCEP. Final purification yield was 25 mg of protein per 1 L of culture and the purity of the protein was over 95%. The MW (14,103.9 Da) of the purified construct was confirmed by LC/MSD TOF (Agilent).

Crystallization: Purified 53BP1 (42 mg/mL) in 20 mM HEPES, pH 7.5, 150 mM NaCl, 0.5 mM TCEP was pre-incubated with 10 mM UNC2170 (dissolved in water) and the best crystals were obtained by vapor diffusion technique at 20 °C in sitting drops by mixing 1 μL of protein solution with 1 μL of reservoir solution containing 19% PEG3350, 150 mM DL-malic acid pH 7.2. For cryoprotection, the crystals were soaked in the reservoir solution supplemented with 15% ethylene glycol (v/v) for 60 s before flash freezing in liquid N_2.

X-ray Data Collection and Structure Determination: X-ray diffraction data for 53BP1 + UNC2170 was collected at 100K at beam line 19ID of Advanced Photon Source (APS), Argonne National Laboratory. Data sets were processed using the HKL-3000 suite(*4*). The structures of 53BP1 + UNC2170 was solved by molecular replacement using PHASER(*5*) with PDB entry 2G3R as the search template. REFMAC(*6*) and MOLPROBITY(*7*) were used for structure refinement. Geometry constraints for the compound refinement were prepared with GRADE(*8*) developed at Global Phasing Ltd. Graphics program COOT(*9*) was used for model building and visualization.

Protein NMR: All NMR spectra were recorded at 298 K using a Bruker Avance III 700 MHz spectrometer equipped with a cryoprobe. The 53BP1-tudor samples were in 25 mM sodium phosphate buffer at pH 7.5, 90% H_2O/10% D_2O, 0.3 mM 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS), 1.5 mM NaN_3. ^{1}H-^{15}N HSQC-based NMR titrations were conducted by gradual addition of 10 mM UNC2170 to ^{15}N-labeled 53BP1-tudor prepared at a starting concentration of 0.3 mM. The NMR spectra were processed and analyzed using NMRPipe and NMRView.(*10, 11*)

Competitive In-solution Peptide Pulldown Assays: A 5 µL slurry of streptavidin magnetic beads (Pierce) was equilibrated in binding buffer containing 50 mM Tris-HCl (pH 8.0), 300 mM NaCl, and 0.1% NP-40 before being saturated with 500 pmoles of biotinylated peptide for 1 h at room temperature with rotation. Unbound peptide was washed with binding buffer. To pre-complex protein with inhibitor, an 80 pmol solution of His-53BP1 TTD (residues 1485-1611) in binding buffer containing 0.5% BSA was incubated with rotation at 4°C with the indicated concentration of UNC2170 or vehicle (H_2O). The pre-complex was combined with peptide-saturated resin and incubated for 3 h at 4 °C with rotation. Unbound protein was collected and bound protein was washed with binding buffer before being eluted from beads by boiling in 1x SDS loading buffer. Proteins were detected with α-His antibody (Bethyl).

Bi-directional Caco-2 Cell Permeability Assay: This cellular permeability assay was conducted by Absorption Systems (Exton, PA). Assay parameters are available through their website at http://www.absorption.com . The assay results for UNC2170 (**1**) for the bi-directional assay are below, where A is the top aqueous cellular layer and B is lower cellular layer.

Test Article	Direction	Recovery (%)	P_{app} (10^{-6} cm/s)			Efflux Ratio	Permeability Classification	Significant Efflux
			R1	R2	AVG			
UNC2170	A-to-B	93	19.3	17.5	18.4	1.2	High	No
	B-toA	92	20.6	22.0	21.3			

Interpretation and Advancement Potential:

Permeability classification: (P_{app} A-B) < 1.0 x 10^{-6} cm/s: **Low**

(P_{app} B-A) ≥ 1.0 x 10^{-6} cm/s: **High**

Significant Efflux: Efflux ration ≥ 2.0 and (P_{app} B-A) ≥ 1.0 x 1.0 x 10^{-6} cm/s

Overall:

Compound	Permeability Classification	Significant Efflux
UNC2170	High	No

CellTiter-Glo assay: The effect of UNC2170 (**1**) and compound **4** on cell viability was determined using a CellTiter-Glo ATP detection system (Promega #7573). Ten-point, 1:3 dilution curves of compounds starting at 100 µM final concentration were diluted to 5X final concentration in PBS (vehicle control) and then 5 µL were added to 384-well white, clear bottom tissue culture plates (Corning #3707) with a Multimek automated liquid handling device (Nanoscreen, Charleston, SC). Twenty microliters of low passage, subconfluent HEK293T (ATCC CRL-11268) grown in Dulbecco's Modified Eagle's Medium without phenol red (Gibco #31053) and supplemented with 10% Fetal Bovine Serum (GIBCO #26140) were immediately added at a density of 2,500 cells per well using a Multidrop 384 (Thermo-Fisher). Cell plates were incubated for 48 hours at 37oC and 5% C02, and then lysed with 25 microliters of CellTiter-GloTM reagent. Luminescence was measured on an Envision platereader (Perkin Elmer) after 15 minutes at room temperature.

Foci Formation assay: U2OS cells were seeded on a coverslip 24 h prior to the experiment. Cells were pre-treated with 100 µM of the compound for 1h and then exposed to 5 Gy of ionizing radiation (IR) using a RS-2000 Biological Irradiator (Rad Source), or not treated. Cells were rinsed with cold PBS at the indicated times after IR and fixed with 3% formaldehyde (Sigma), followed by permeabilization with 0.5% Triton X-100 (Sigma) and blocking with 10% FCS (Hyclone). The foci staining was performed in 10%

FCS in PBS (anti-γH2AX from Millipore #05-636 and anti-53BP1 from Bethyl Labs #A300-272A at a 1:1000 dilution) for 1h at room temperature followed by 3 washes with PBS. Cells were incubated with secondary antibodies conjugated to AlexaFluor 488 (Invitrogen) for green and to Alexa Fluor 596 (Invitrogen) for red foci staining. Secondary antibodies were diluted 1:500 in 10% FCS in PBS and the coverslips were incubated for 1 h at room temperature. Coverslips were washed 3 times with PBS and air-dried. After drying, the coverslips were mounted on glass slides using ProLong Gold with DAPI (Invitrogen) and pictures were taken using a Zeiss LSM510 META confocal microscope (63x oil objective). Foci quantification was performed using ImageJ software and at least 150 cells per time point were analyzed per experiment for the calculation.

Fluorescence Recovery After Photobleaching (FRAP):

Cell Culture and Plasmid Transfection

Human U2OS were seeded onto 25-mm round coverslips at ~$9x10^5$ cell/mL and cultured overnight at 39.5°C, 5% CO_2 in McCoy's medium with 10% FBS. The cells were then transiently transfected with a GFP-tagged 53BP1 tudor domain (residues 1146 – 1709 which also encompasses the UDR; approximately 1 µg) using Lipofectamine® 2000 (Life Technologies) and incubated overnight with or without treatment of UNC2170 at varying concentrations. After 24 hrs post transfection and compound treatment, the media was changed and then the cells were subjected to FRAP experiments.

Fluorescence Recovery After Polarization assay (FRAP)

FRAP experiments were conducted based on literature procedures(*12-15*), using a Zeiss CLSM 700 confocal laser scanning microscope equipped with a 20x oil objective and Bioptechs open dish live cell imaging system (maintained at 37°C with low CO_2 atmosphere). FRAP experiments were conducted 24hr after transfection with GFP-tagged plasmid. Time-lapse image acquisition was performed on cell nuclei containing GFP-53BP1 tudor domain. Initially 53BP1 foci were scanned for 10 cycles at a 1 sec time interval and then photobleached for 20 iterations using a 405 ʎ laser set at 100% power and then scanned for 200 additional iterations after photobleaching. All confocal and FRAP experimental data and images were acquired and processed using Zess ZEN2011 software package with the recovery time obtained by fitting a single exponential equation. Each data point for recovery measurements was derived from the mean intensities of 3-4 different foci measurements from 3-5 different cells per experimental condition.

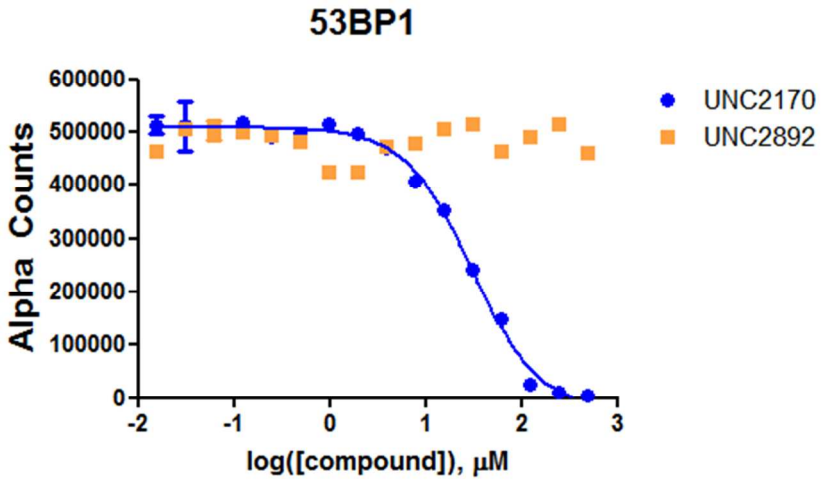
CSR experiments:

Mice and Cells. B cell isolation and culture have been previously described.(*16*) In brief, primary naïve B-lymphocytes from WT C57/BL6 mouse spleens were purified by negative selection with anti-CD43 beads (Miltenyi Biotec) and cultured in RPMI 1640, 1 mM sodium pyruvate, 10% fetal bovine serum (Atlanta Biologicals,), 50 uM 2-mercaptoethanol, 25 µg/ml Lipopolysaccharide(LPS) and 5 ng/ml IL-4 (Sigma-Aldrich). Inhibitors were added at 12 hours culture and cells were analyzed 72 hours later.

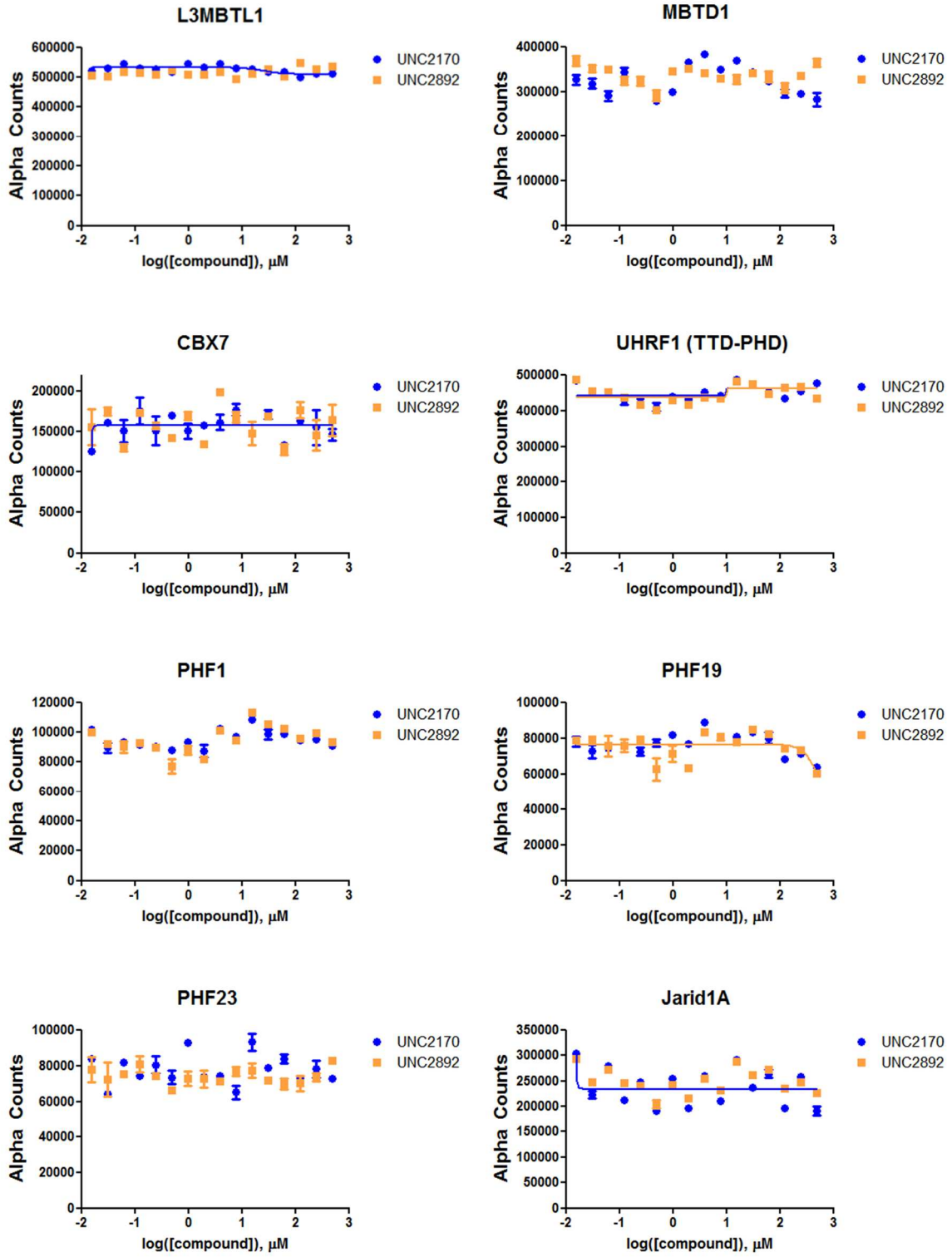
Class switch recombination assay. Cultured splenocytes were stained with anti–mouse IgG1 antibodies (BD). Dead cells were excluded on the basis of forward-side scatter and propidium iodide staining. Cells were analyzed on a LSRFortessa (BD) and data was analyzed with FloJo software. Data is the summary of triplicate culture analysis of 100uM (n=4), 75uM (n=3) and 30uM (n=3) independent experiments. Statistical significance was determined by a two-tailed Student's *t* test assuming unequal variance, p values indicated.

Supplementary Figure 1. AlphaScreen Binding Curves

a) Averaged Alphascreening binding curves for UNC2170 (**1**) and UNC2892 (**4**) against 53BP1. The average IC_{50} for UNC2170 is 28.48 ± 7.44 µM.

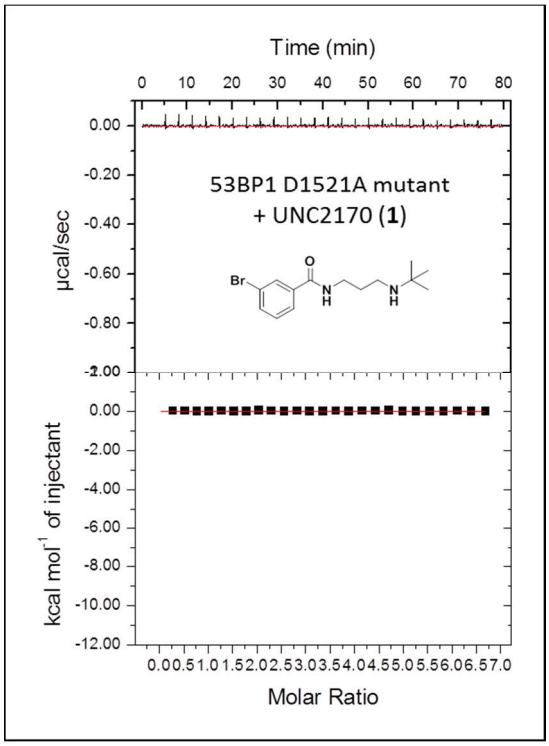


b) UNC2170 (**1**) and UNC2892 (**4**) selectivity data against the Kme panel. Both compounds were inactive against every panel member up to 500 µM.

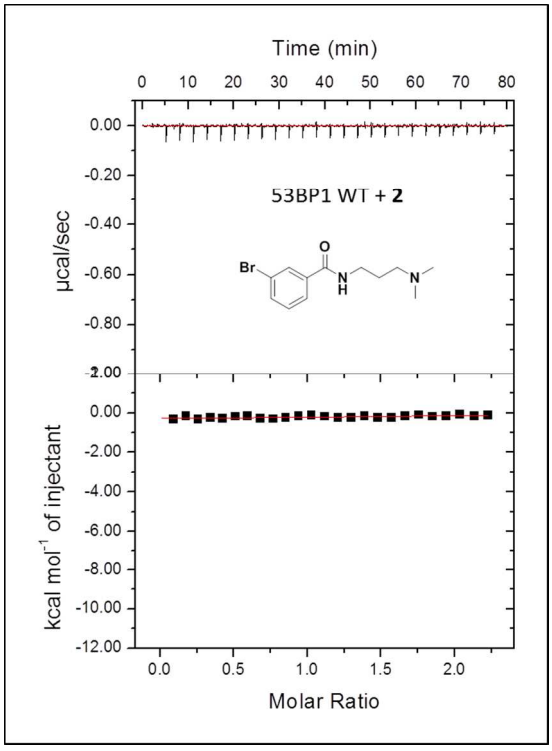


Supplementary Figure 2. Representative ITC binding curves.

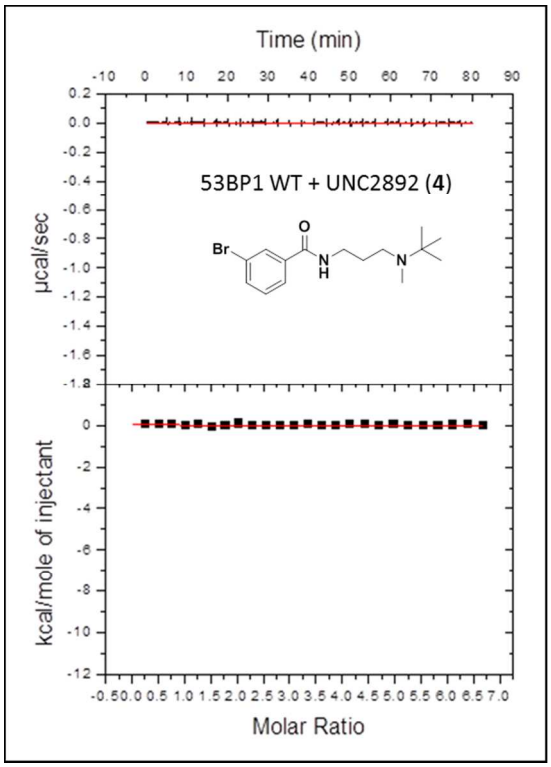
a) UNC2170 (**1**) shows no binding to the 53BP1-TTD D1521A mutant.



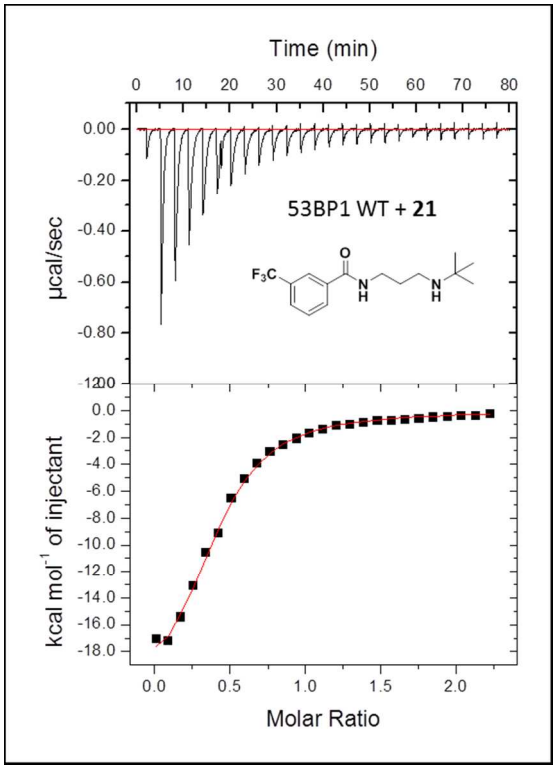
b) Compound (**2**) does not bind 53BP1-TTD.



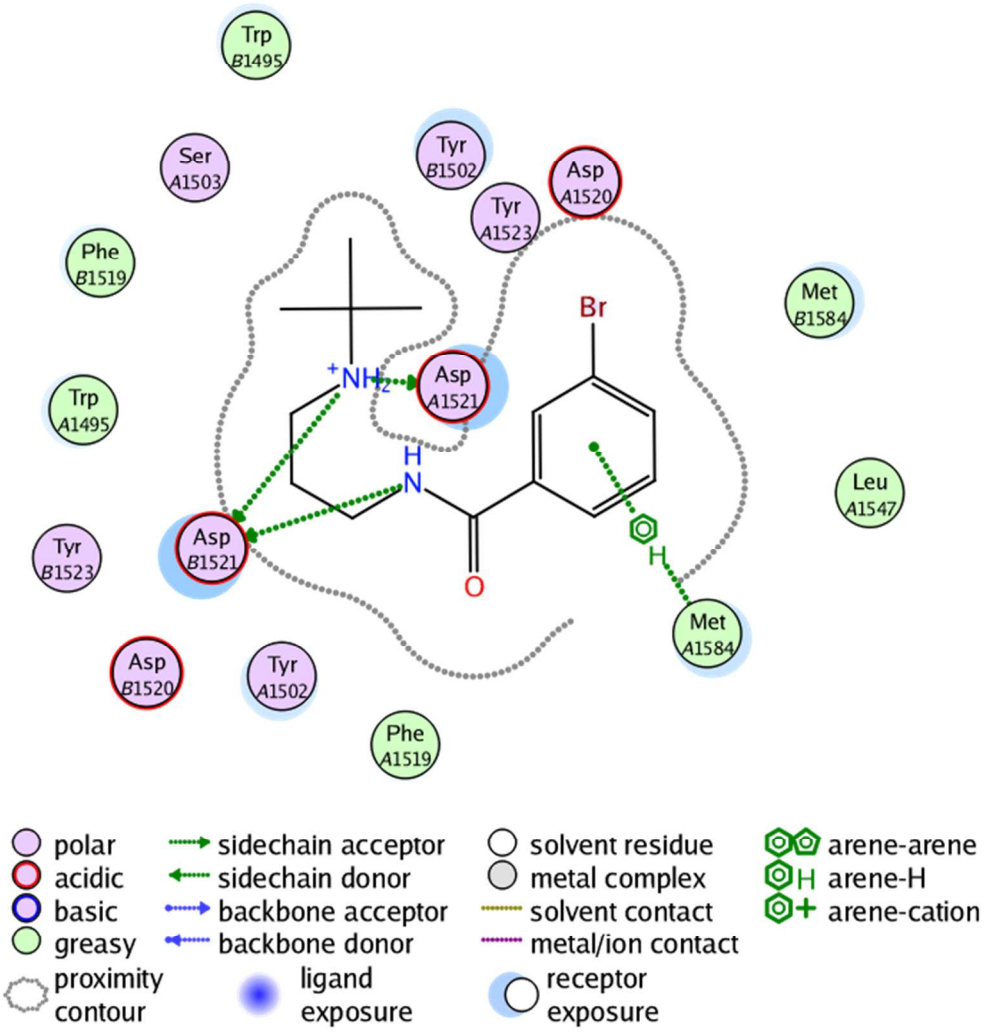
c) UNC2892 (**4**) does not bind 53BP1-TTD.



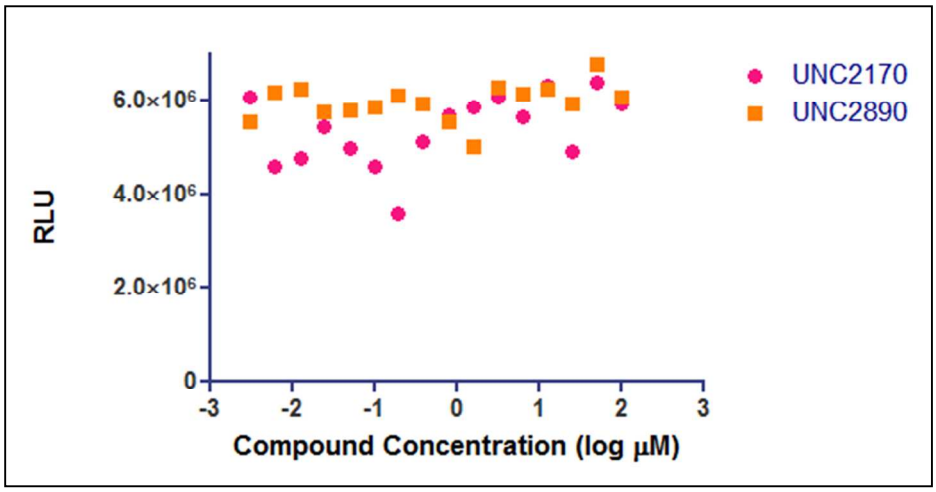
d) Compound **22** binds 53BP1-TTD with a K_d of 10.3 ± 1.0 µM.



Supplementary Figure 3. Key interactions between UNC2170 and the 53BP1 tudor domains. These interactions were determined by MOE based on the UNC2170 co-crystal structure (PDB 4RG2). The residues from one tudor domain are labeled as *A*, and the residues from the second tudor domain are labeled as *B*. The t-butyl group is buried in the Kme pocket of tudor *B*.

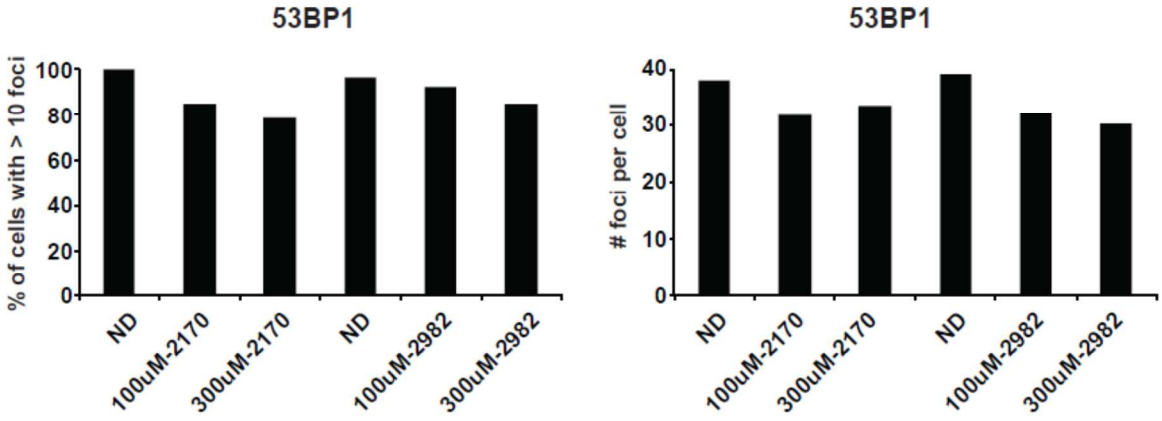


Supplementary Figure 4. CellTiter-Glo luminescent cell viability assay. UNC2170 (**1**) and UNC2892 (**4**) show no toxicity up to 10 µM.

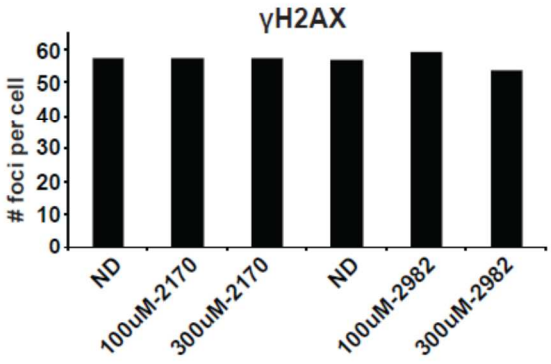


Supplementary Figure 5. Assembly of GFP-53BP1 foci.

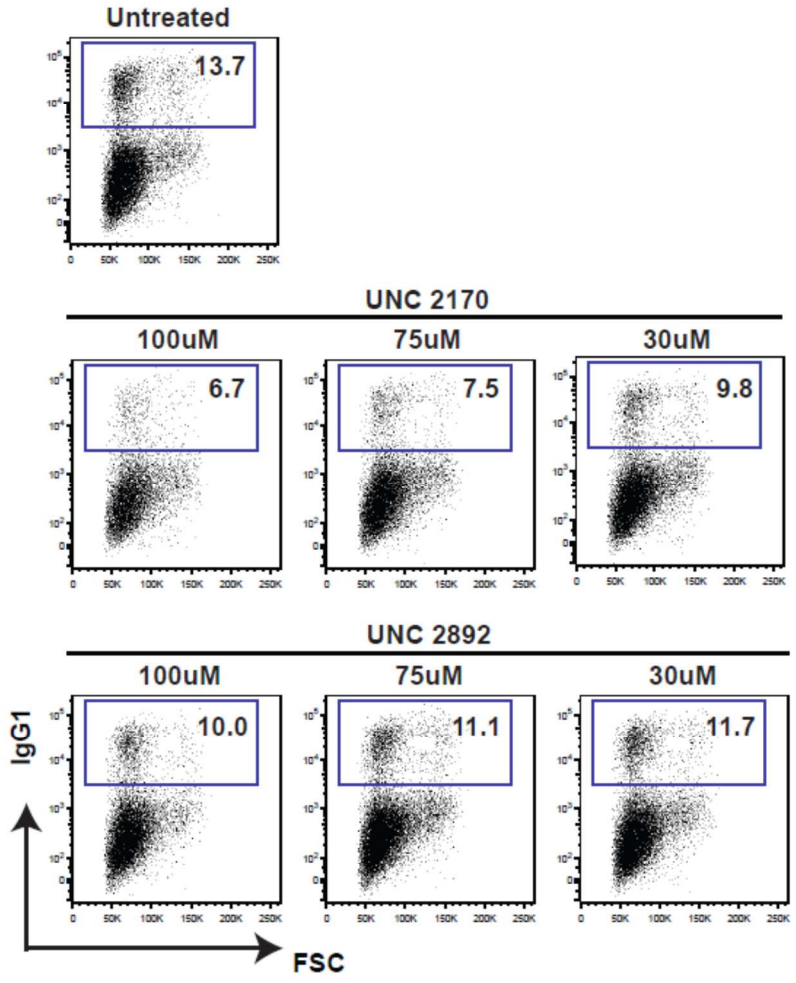
a) U2OS cells subjected to treatment with 5 Gy of ionization radiation causes the accumulation of GFP-53BP1 at DSB sites. Pre-treatment with 100 µM and 300 µM of UNC2170 or UNC2892 results in minimal defects in foci formation, and similar effects are seen with both compounds, indicating that the slight changes observed are not due to the specific inhibition of 53BP1.



b) No changes in γH2AX foci are observed in the same experiments.

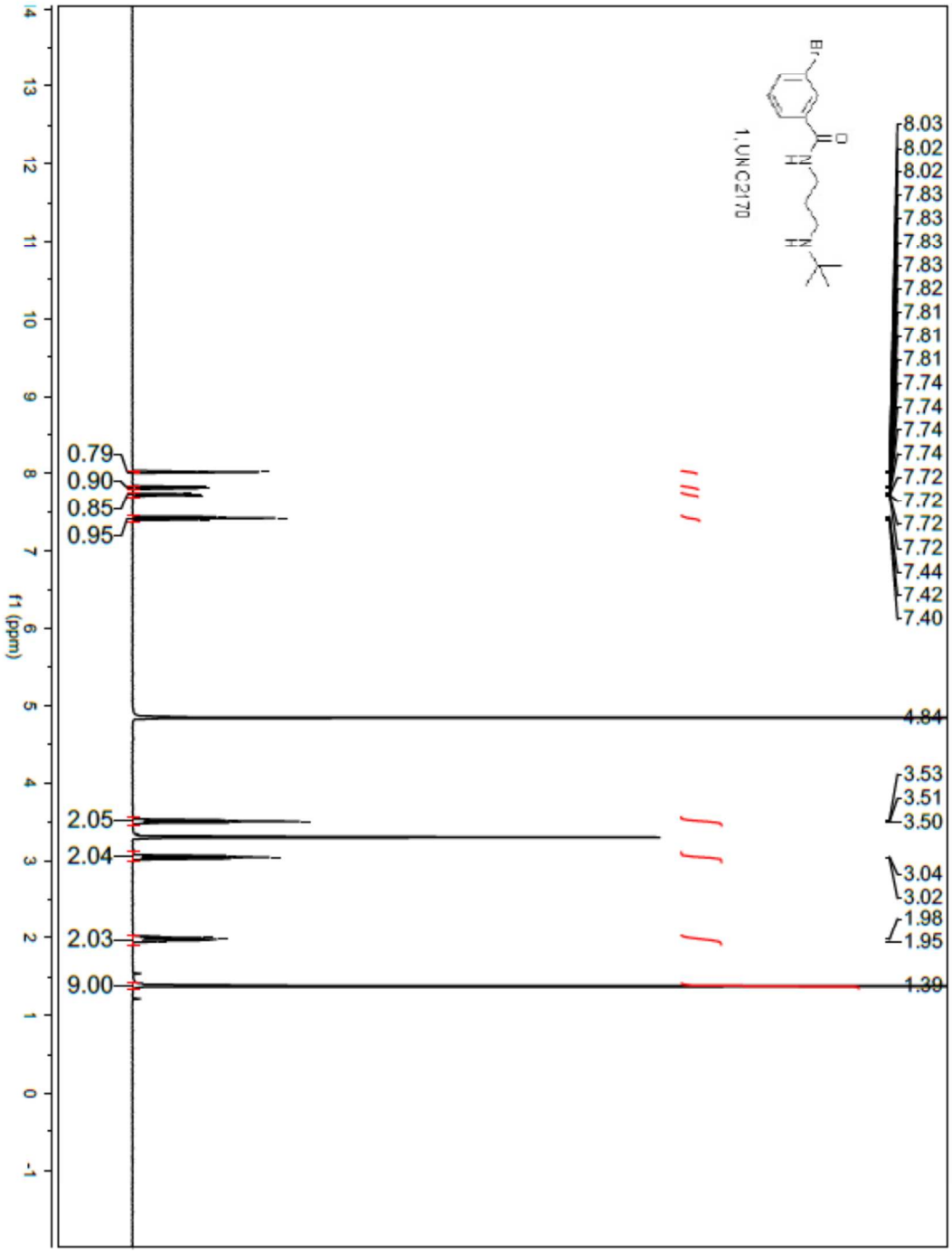


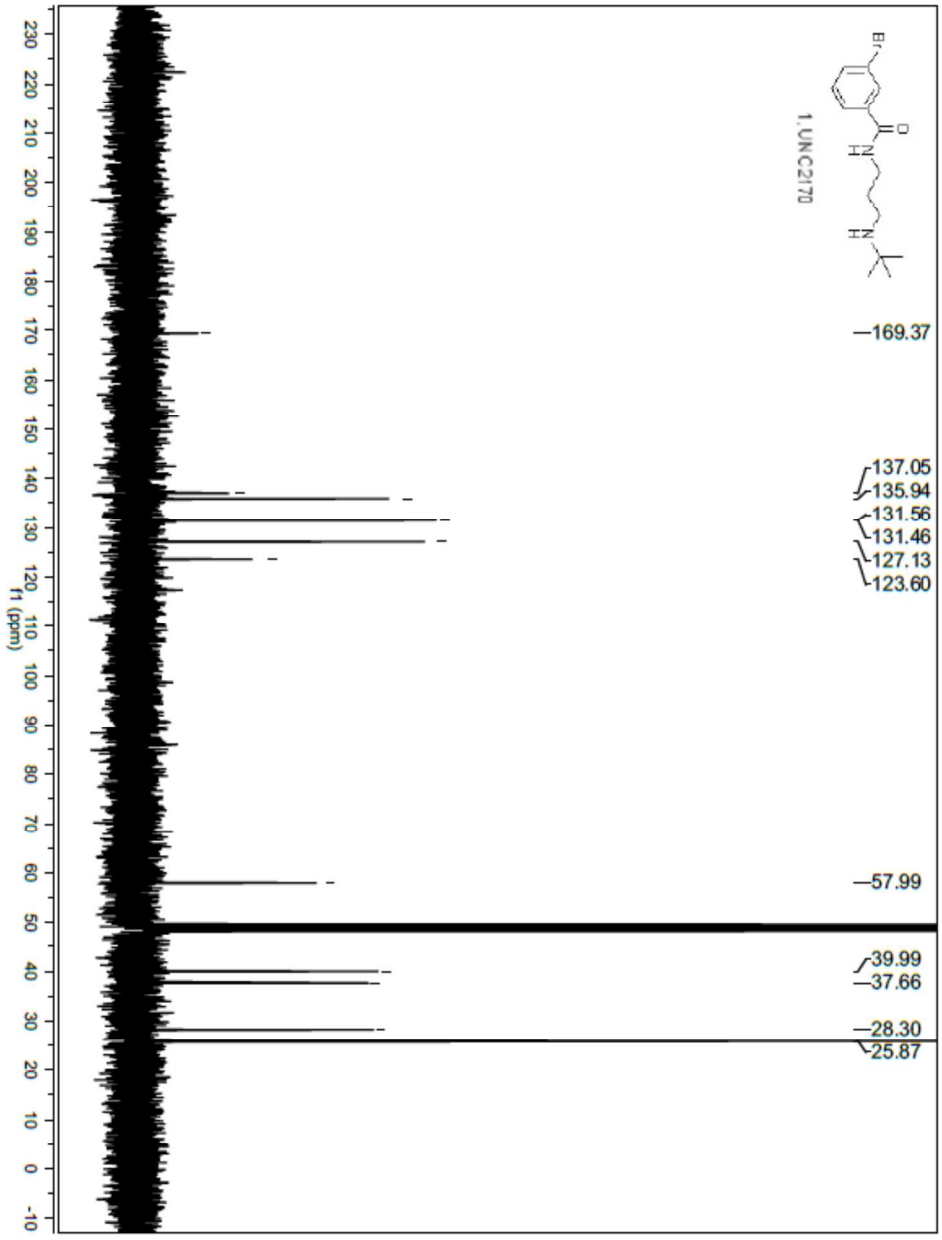
Supplementary Figure 6. Representative flow cytometry plots measuring CSR to IgG1. Analysis of CSR to IgG1 switching in wildtype splenocytes cultured with LPS and IL-4 and incubated with indicated compounds for 3.5 days. Treatment with UNC2170 decreased the number of cells that underwent isotope switching to IgG1, whereas UNC2892 did not have the same effect. CSR was measured by $IgG1^+$ cell surface expression and plotted versus forward size scatter (FSC). The percentage of $IgG1^+$ cells is indicated on the top right of each graph.



Supplementary Figure 7. NMR Spectra.

a) ^{1}H NMR spectra of compounds 1 – 6 and ^{13}C NMR spectra of compound 1 and 4.

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39.99
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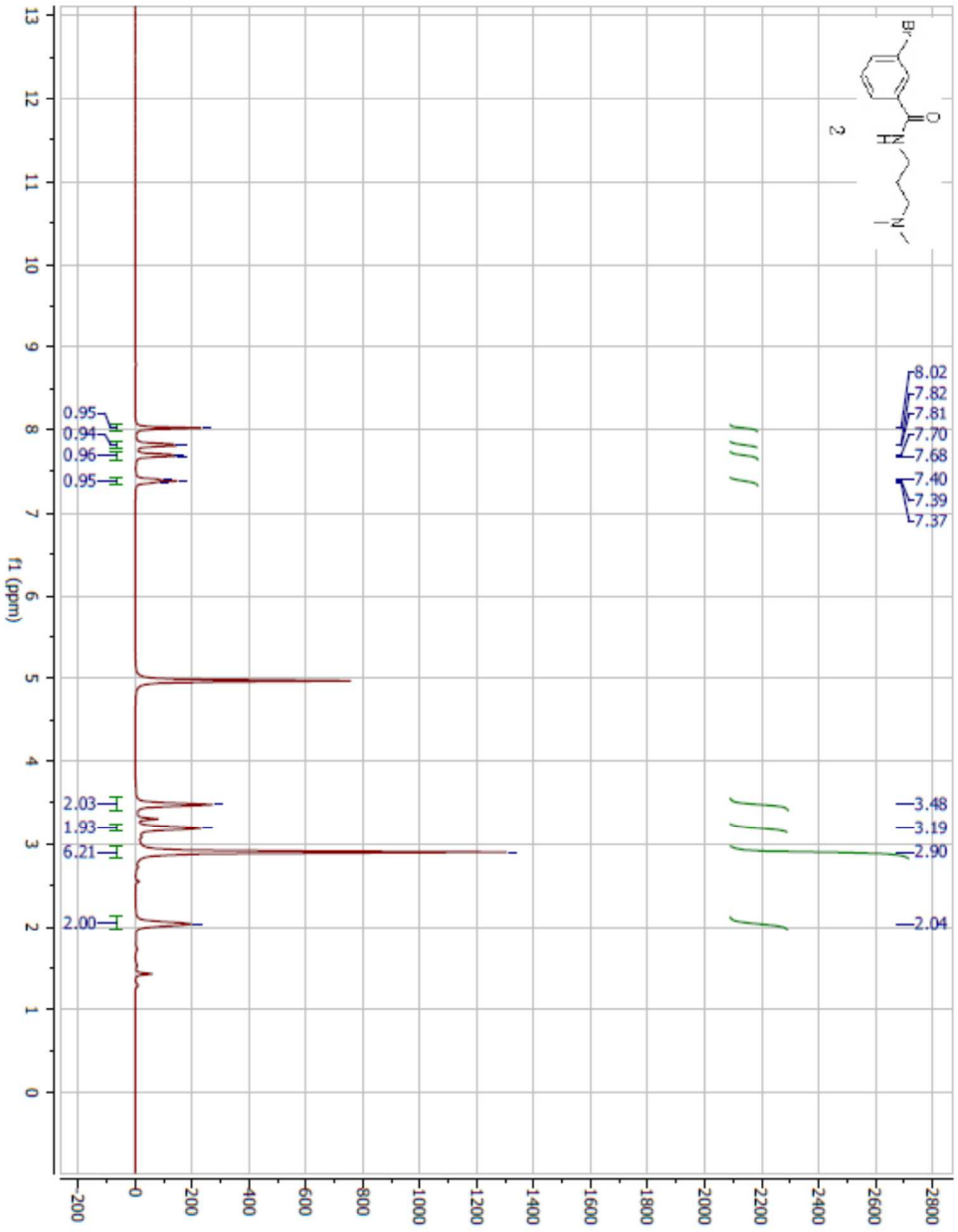
25.87
f1 (ppm)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7.40
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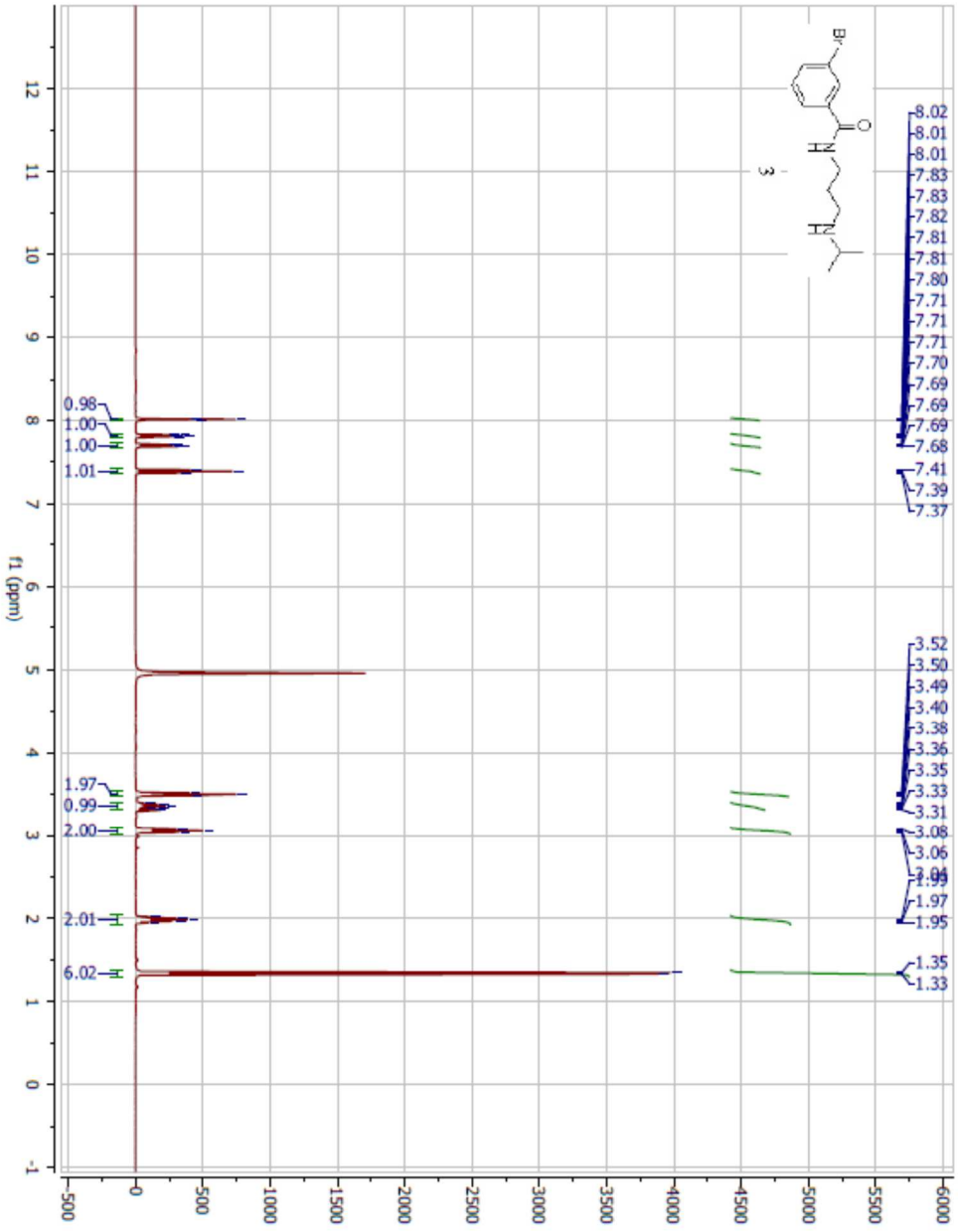
7.37
3.48
3.19
2.90
2.04
2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7.82
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7.37
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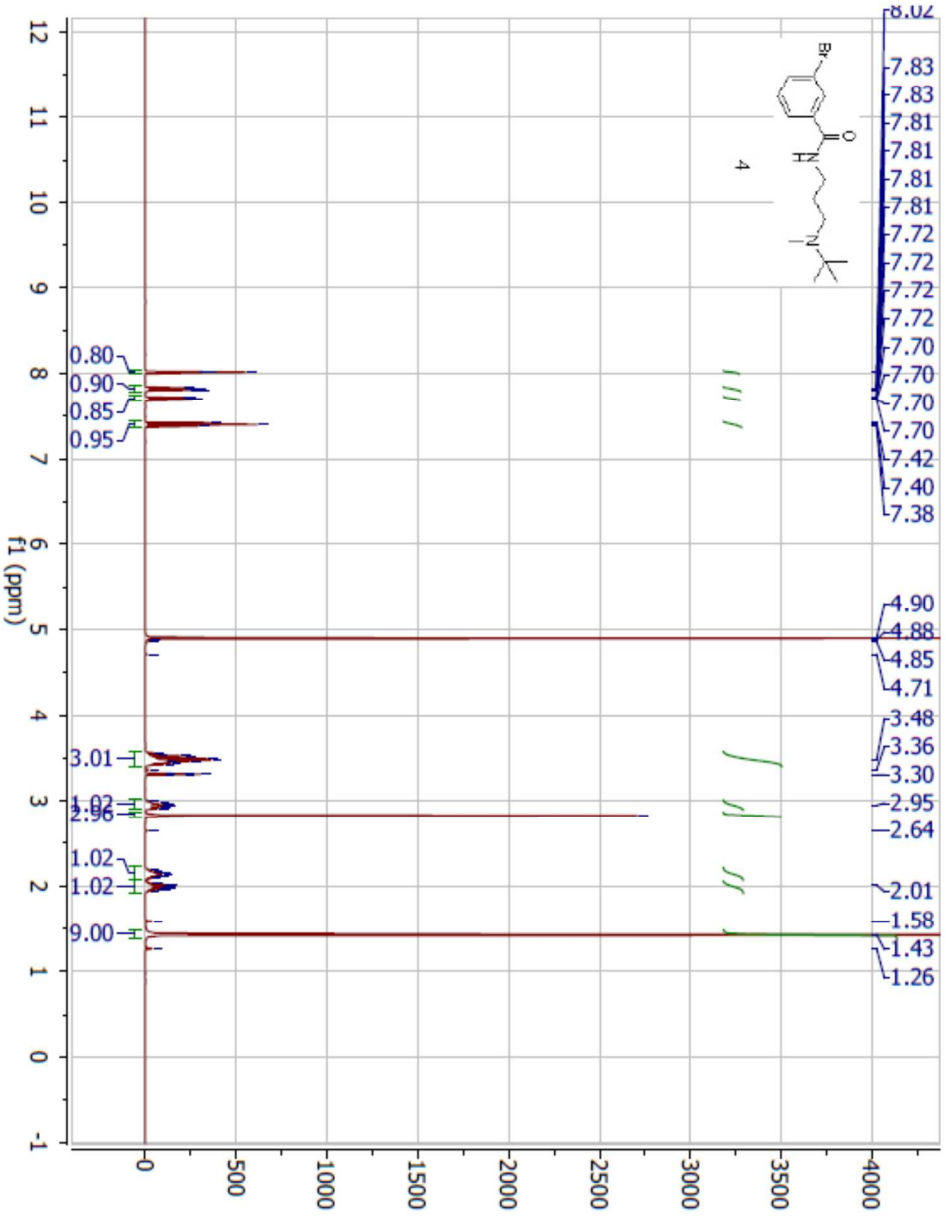
1.33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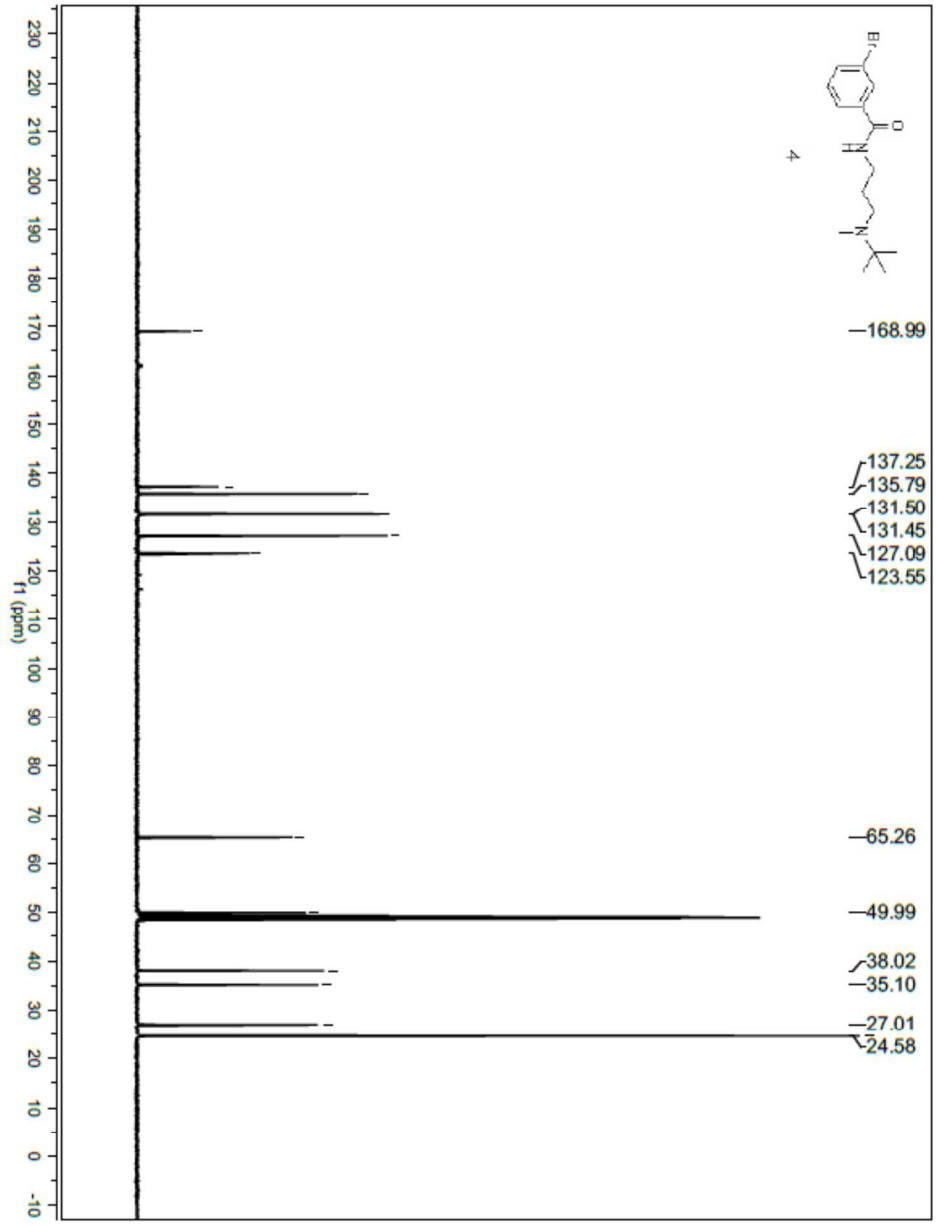
1.26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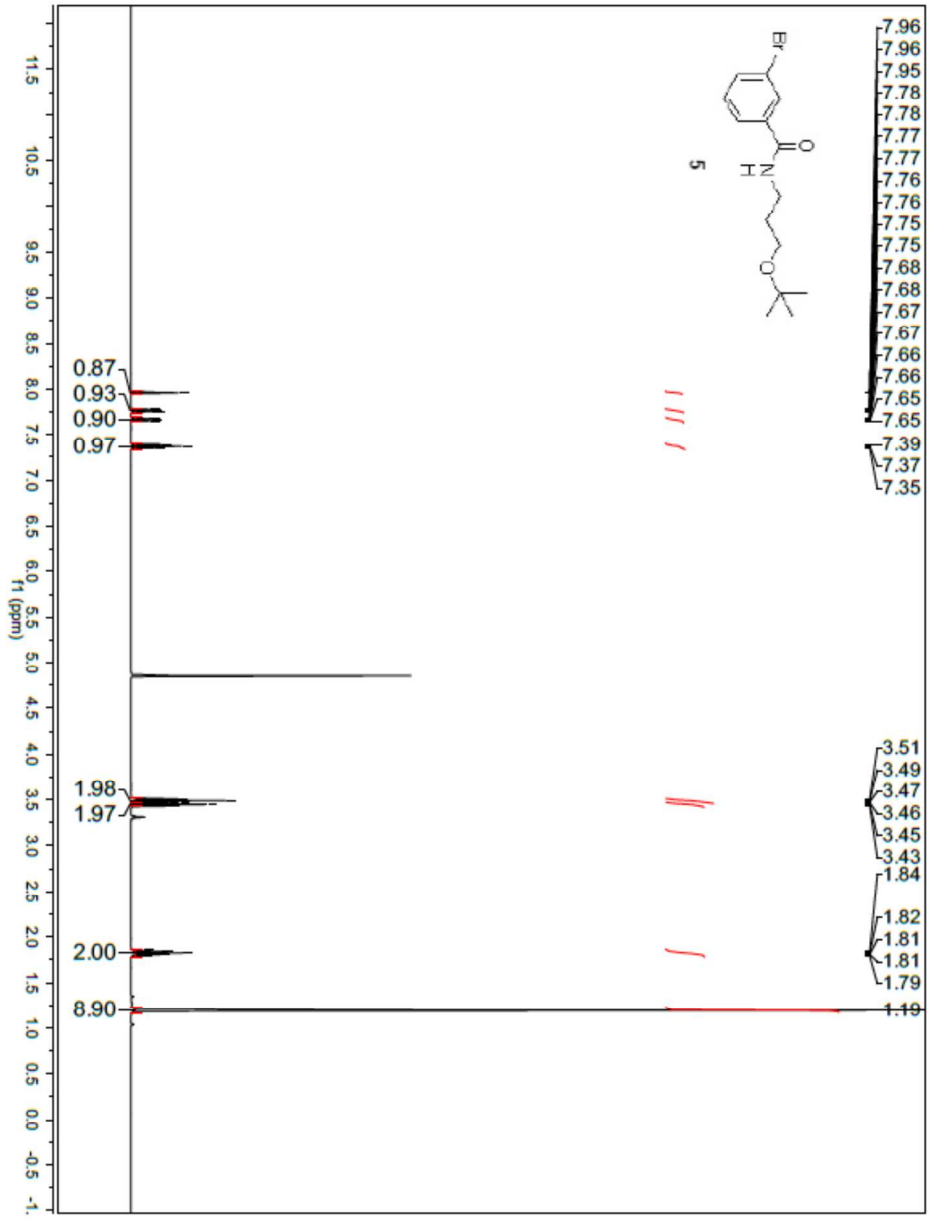
f1 (ppm)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3.43
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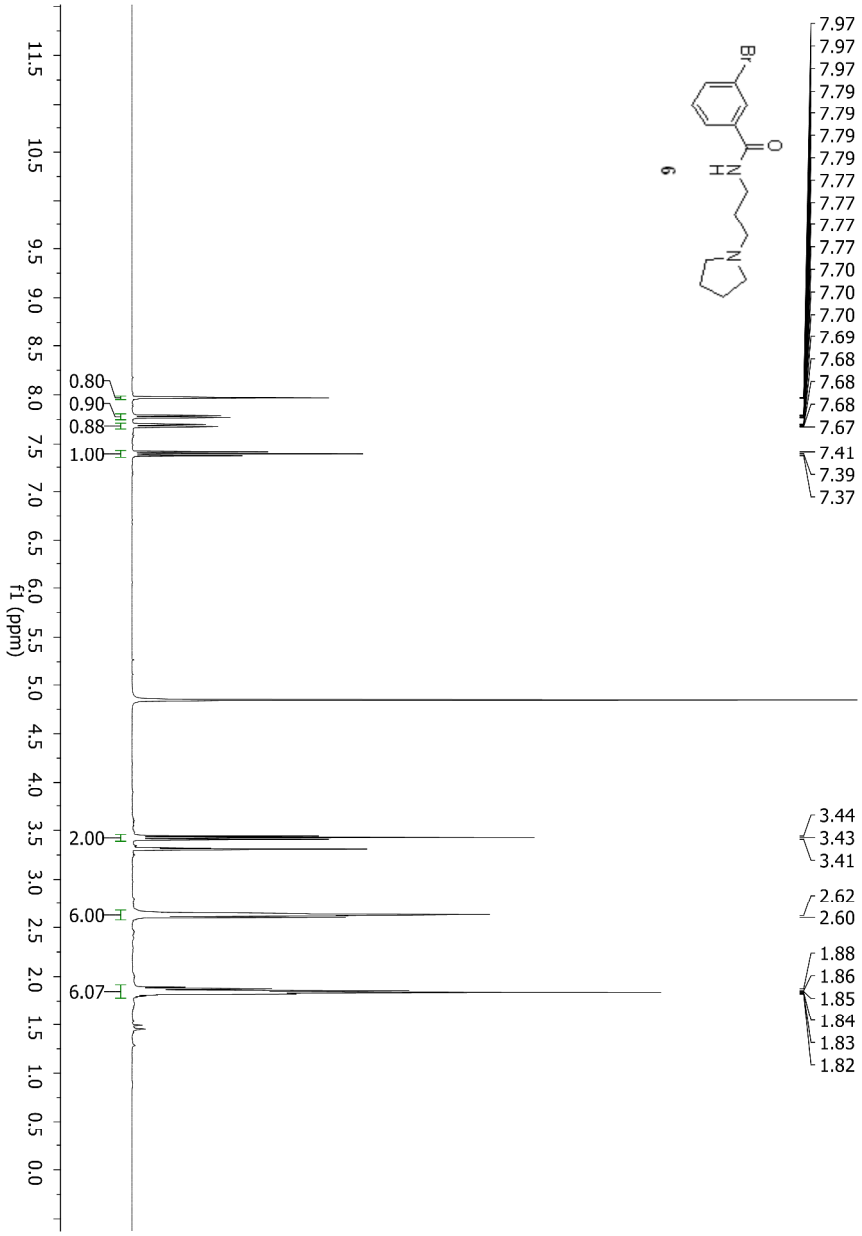
f1 (ppm)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3.5
3.0
2.5
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1.5
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6.07
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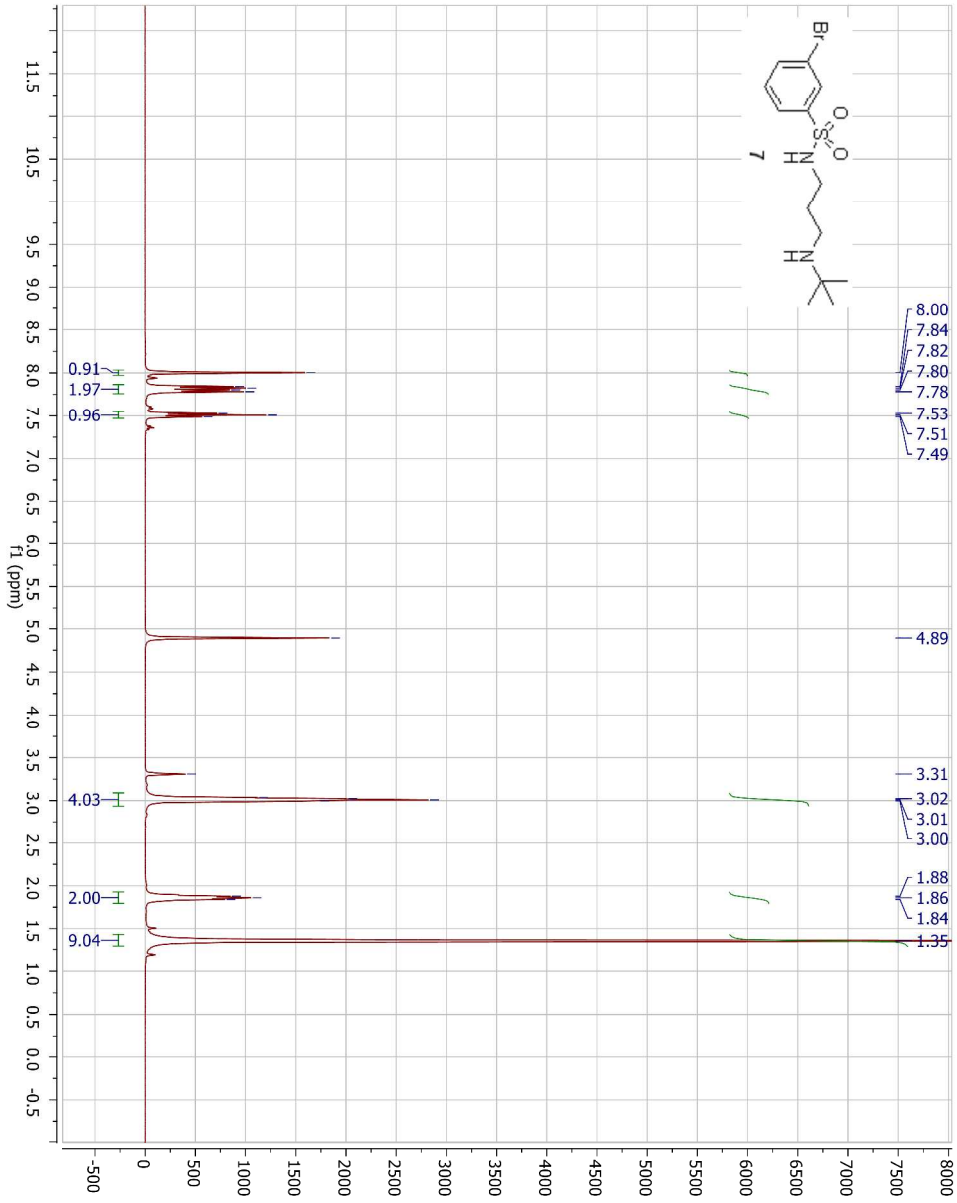
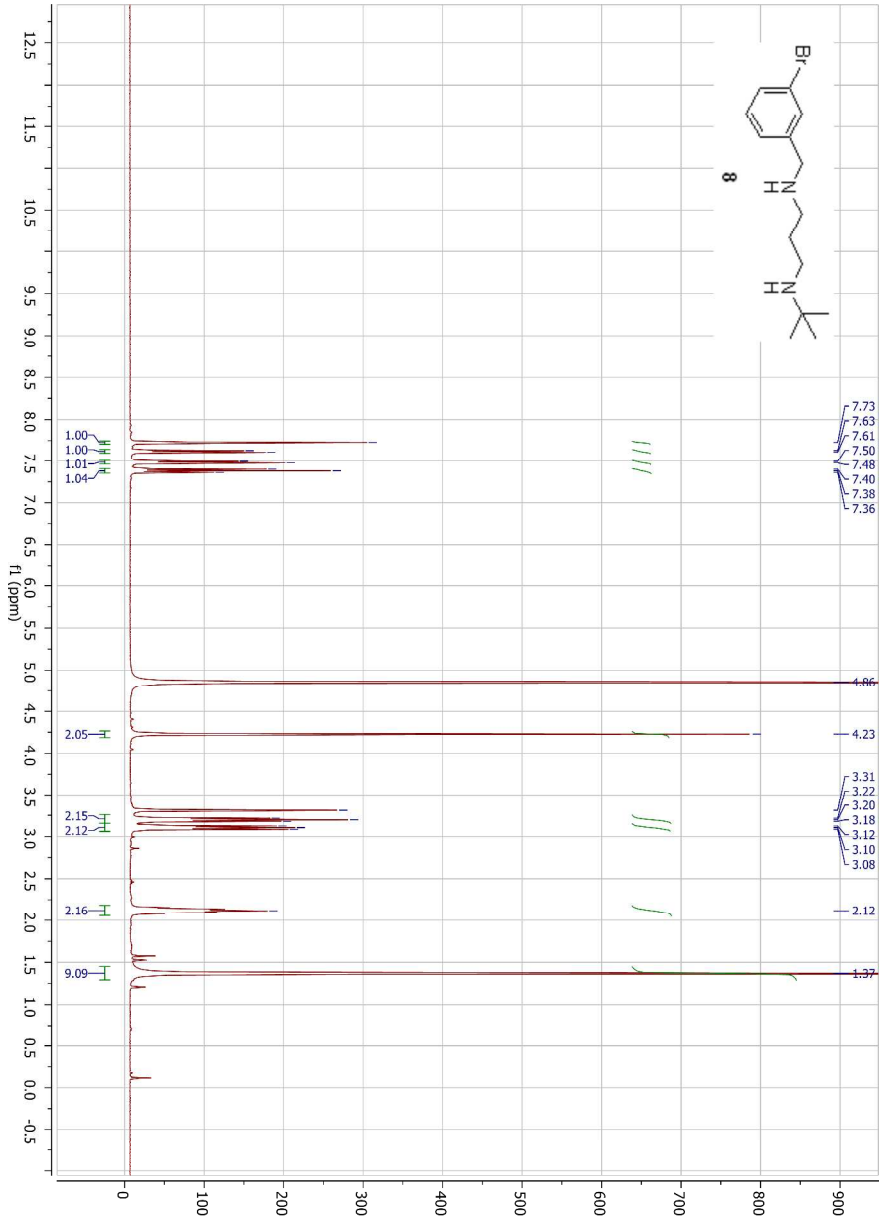
1.82

b) ^{1}H NMR of Compounds 7 – 13.

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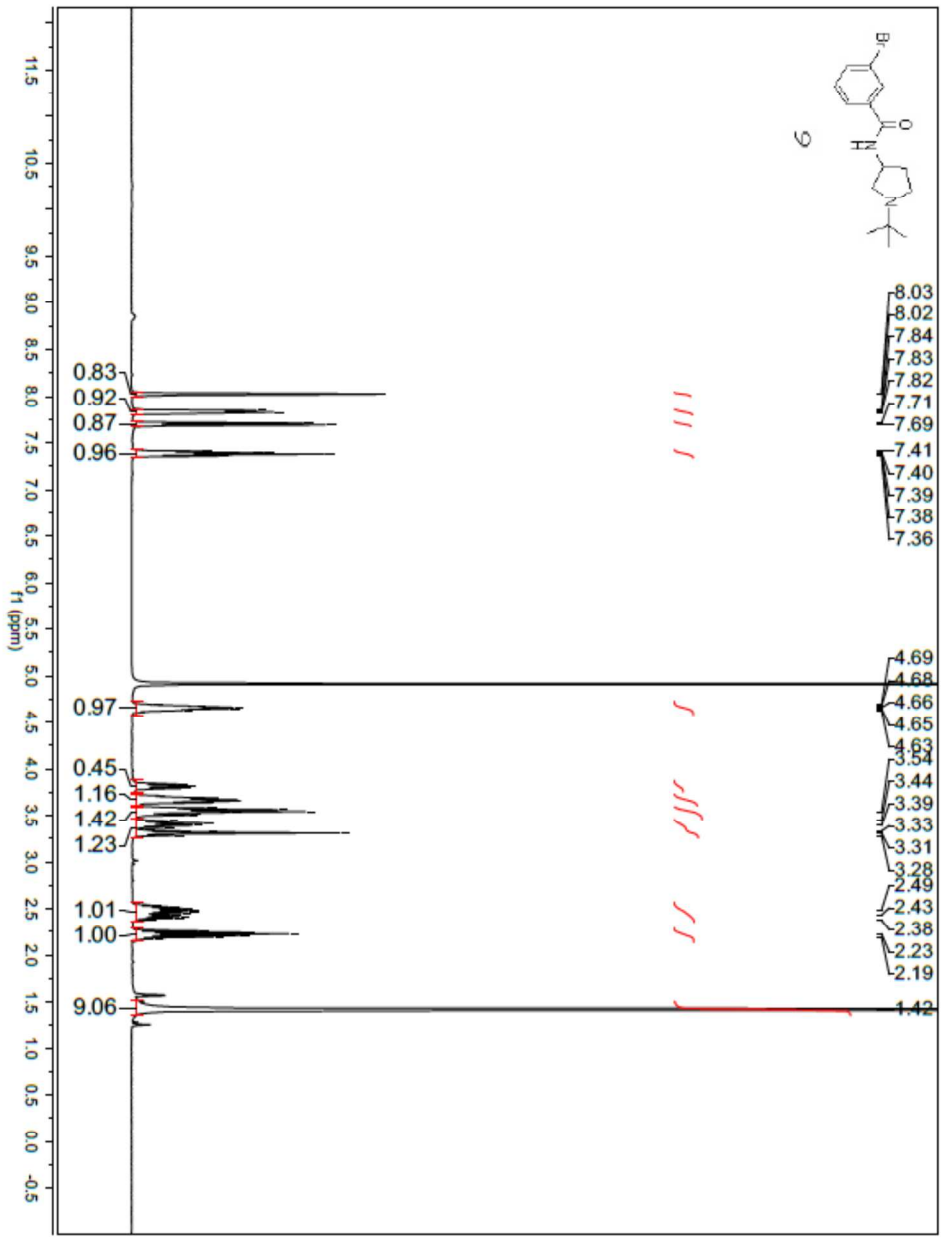
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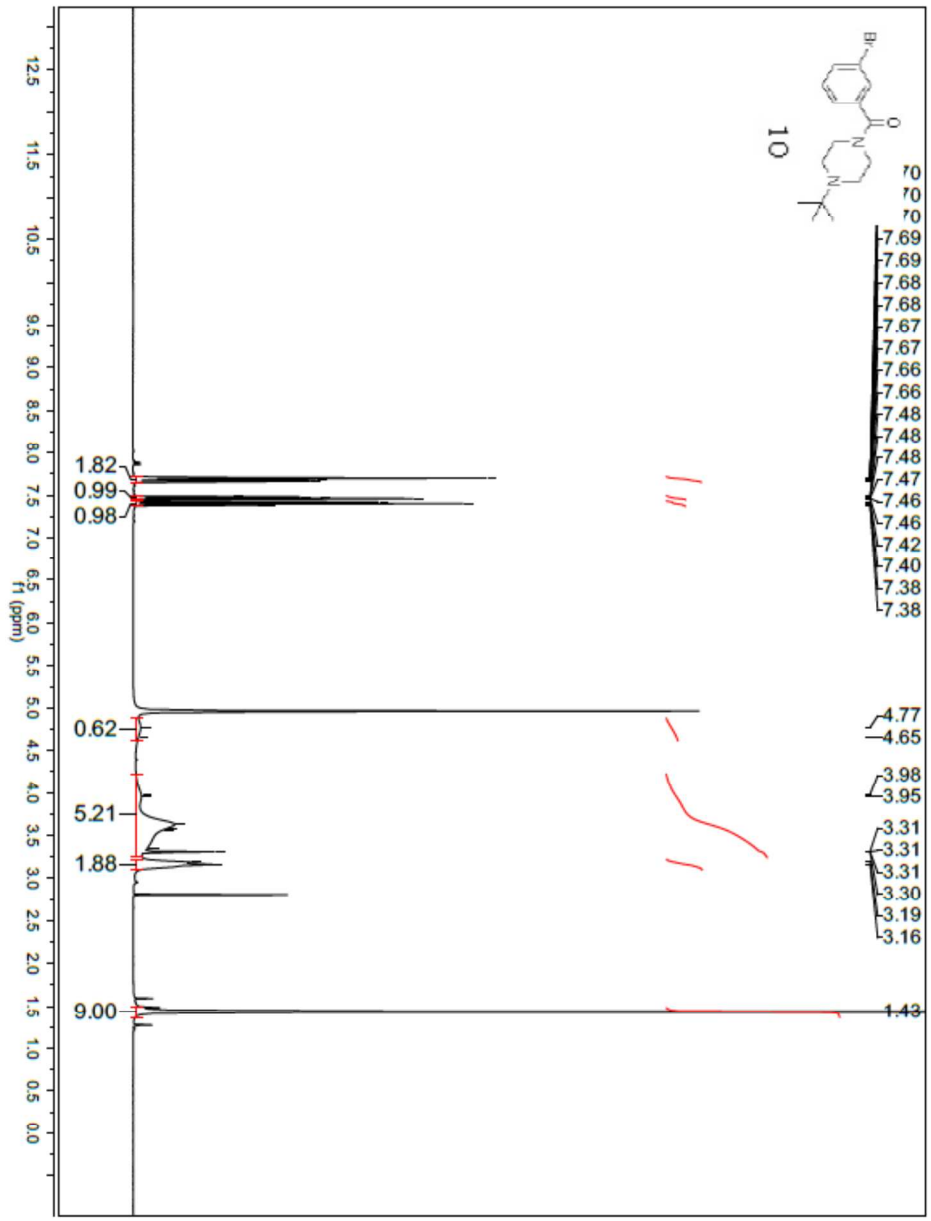
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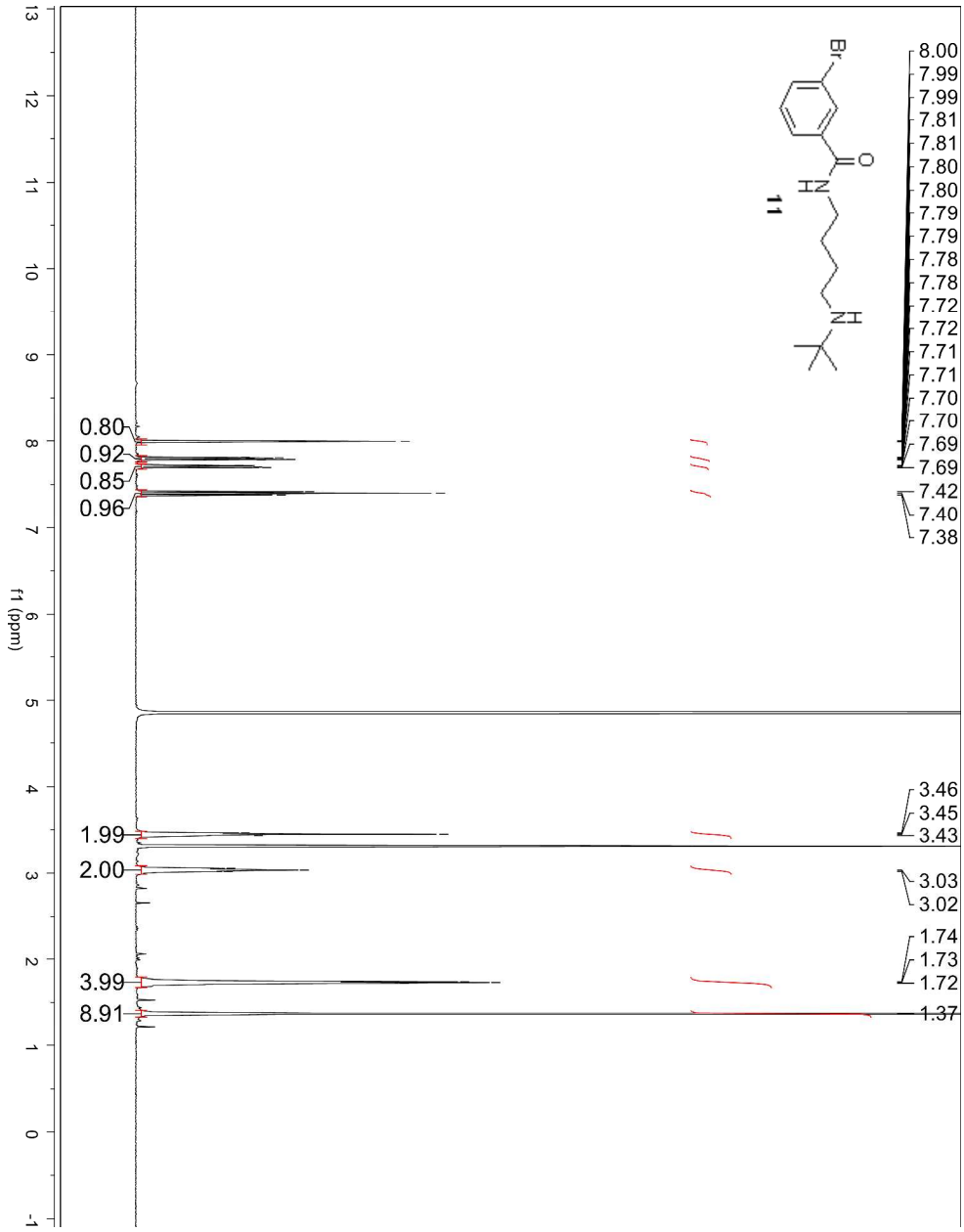
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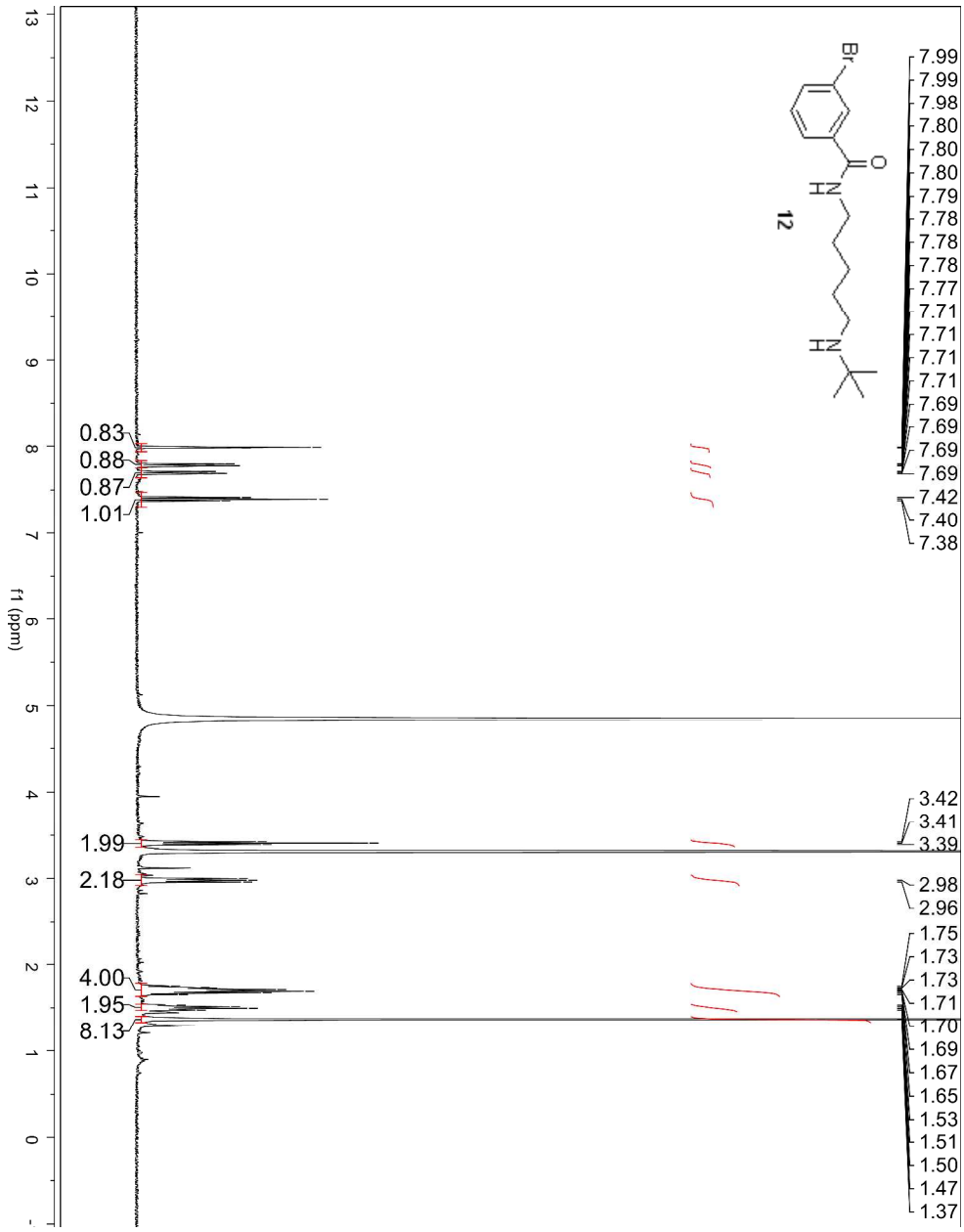
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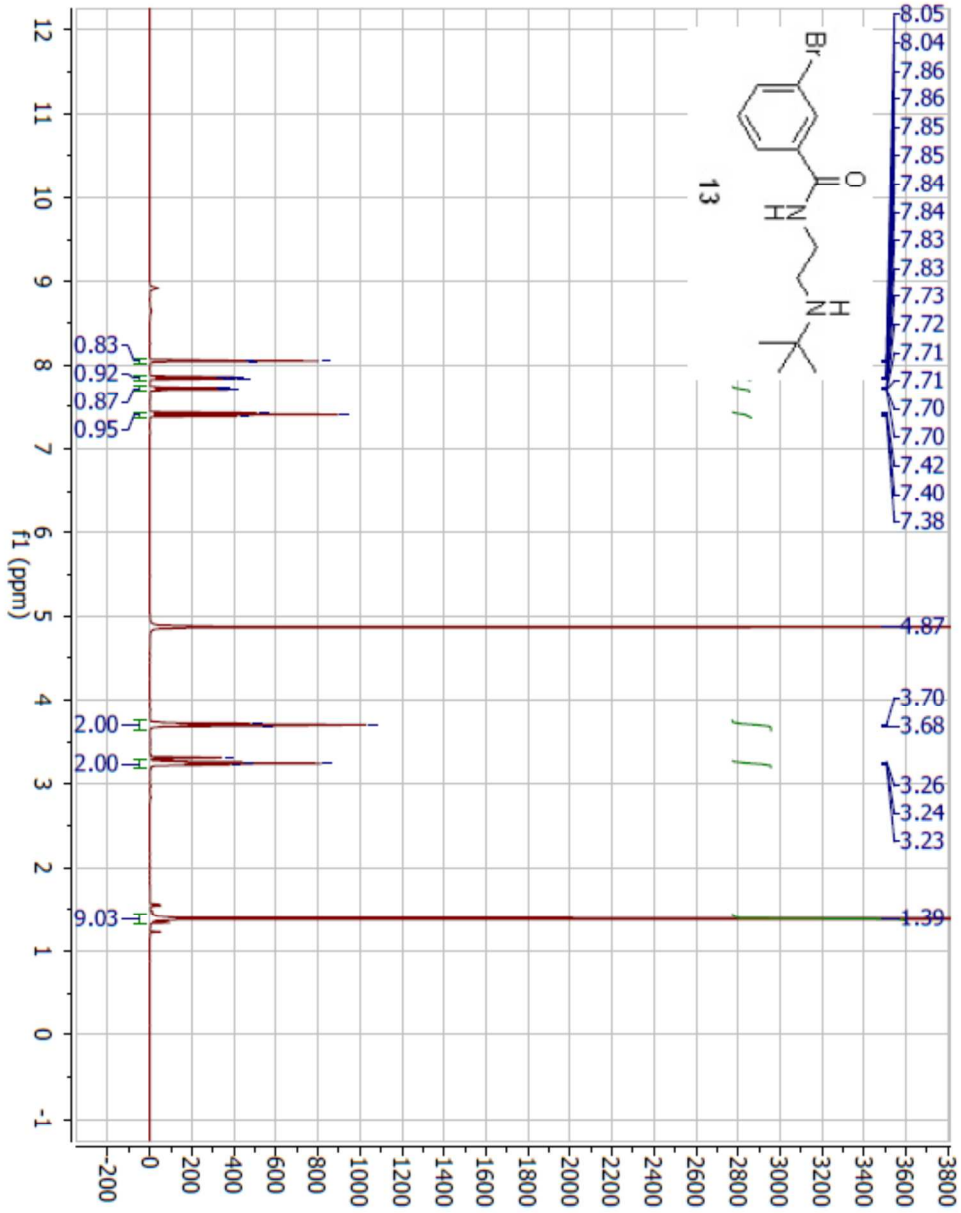
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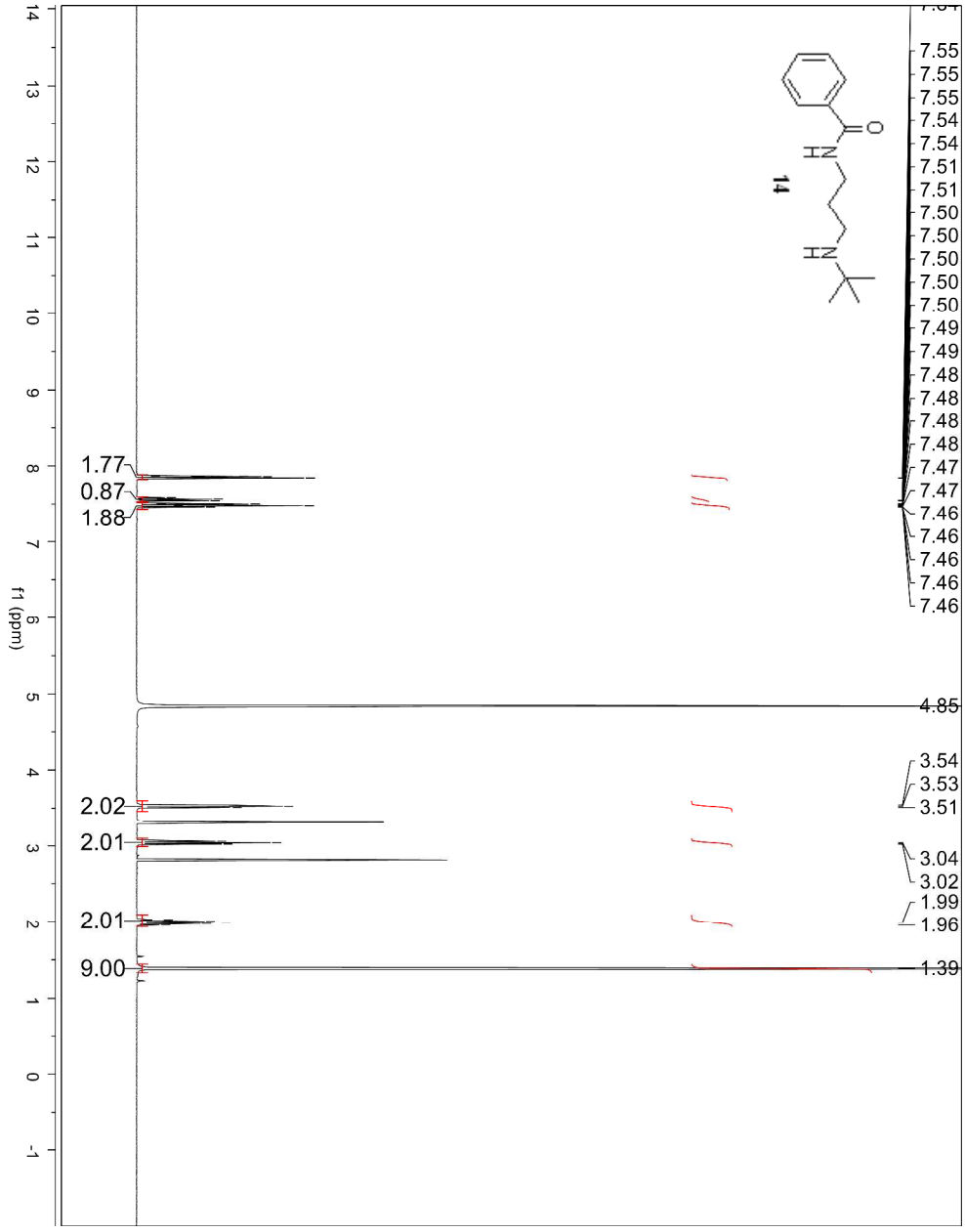
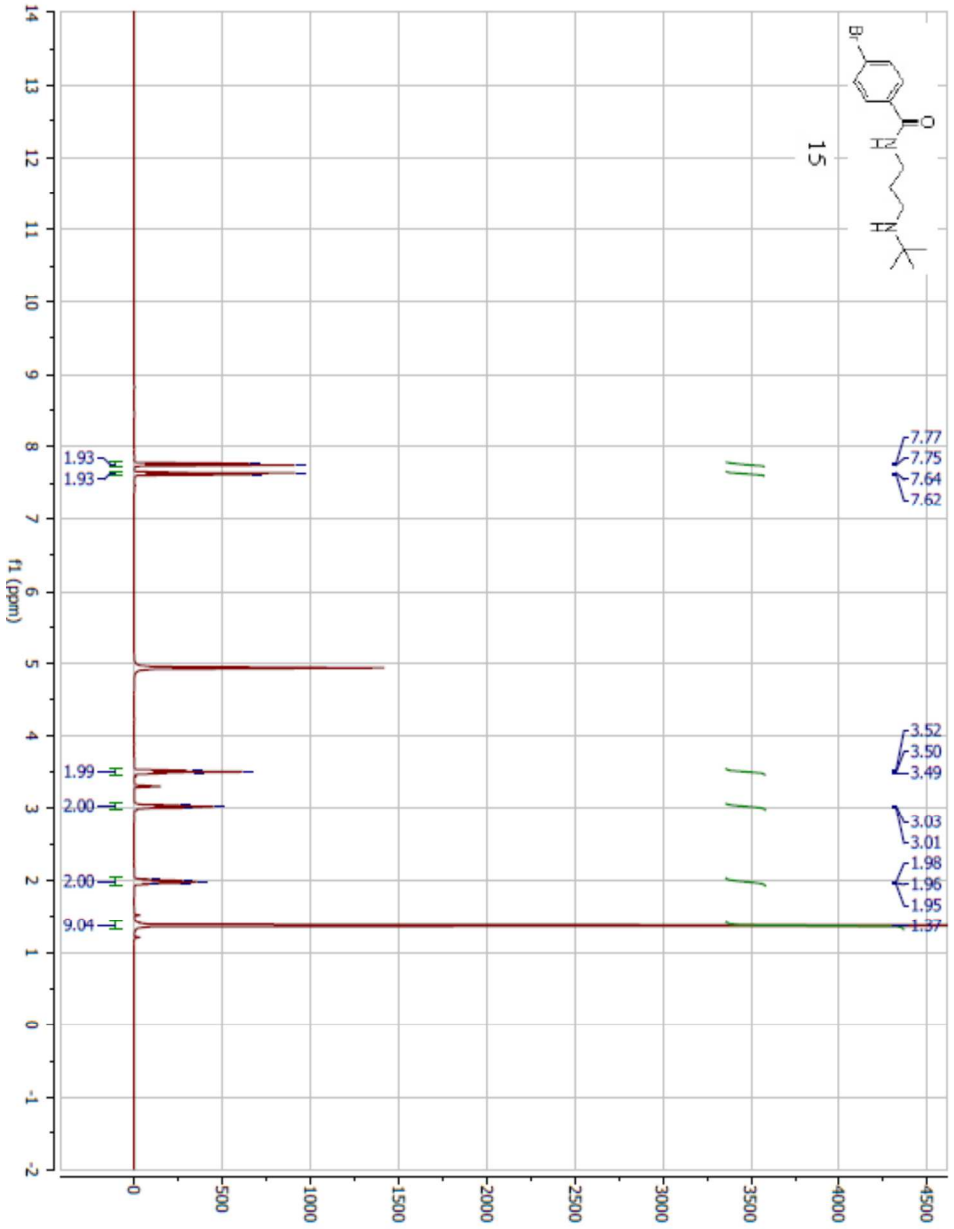
f1 (ppm)

c) ^{1}H NMR of Compounds 14 – 25.

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3.01
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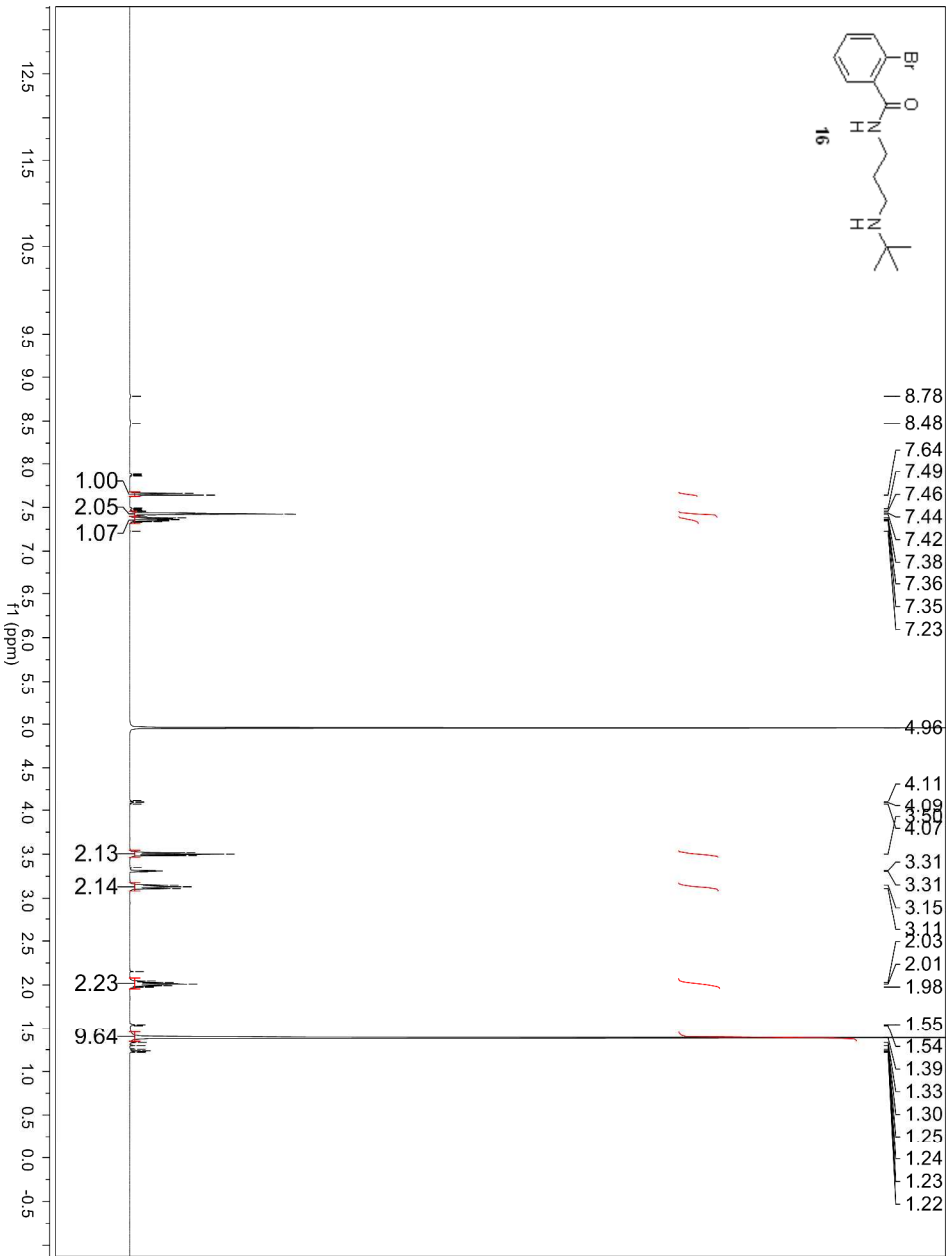
1.95
1.37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7.42
7.38
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7.23
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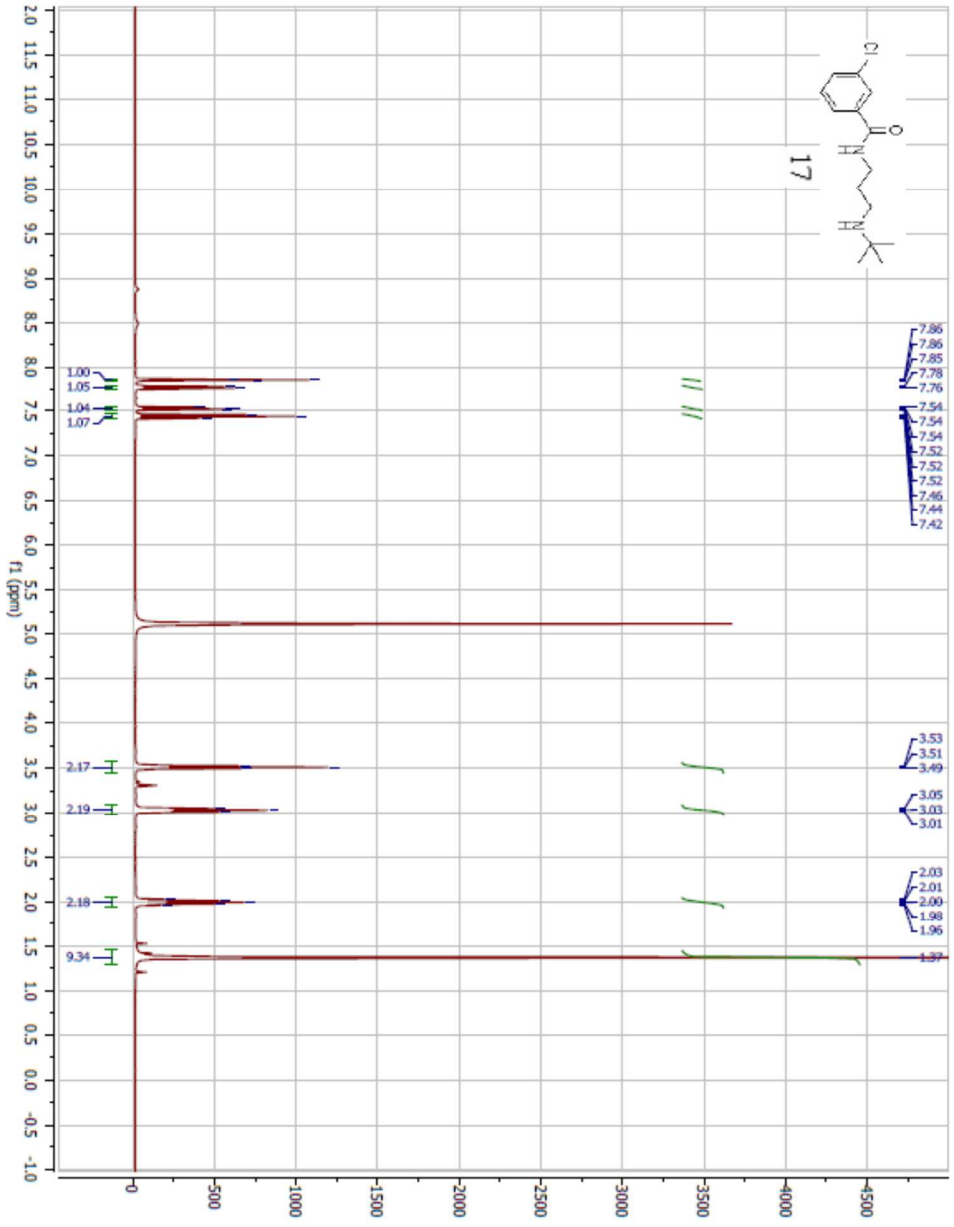
1.23
1.22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f1 (ppm)
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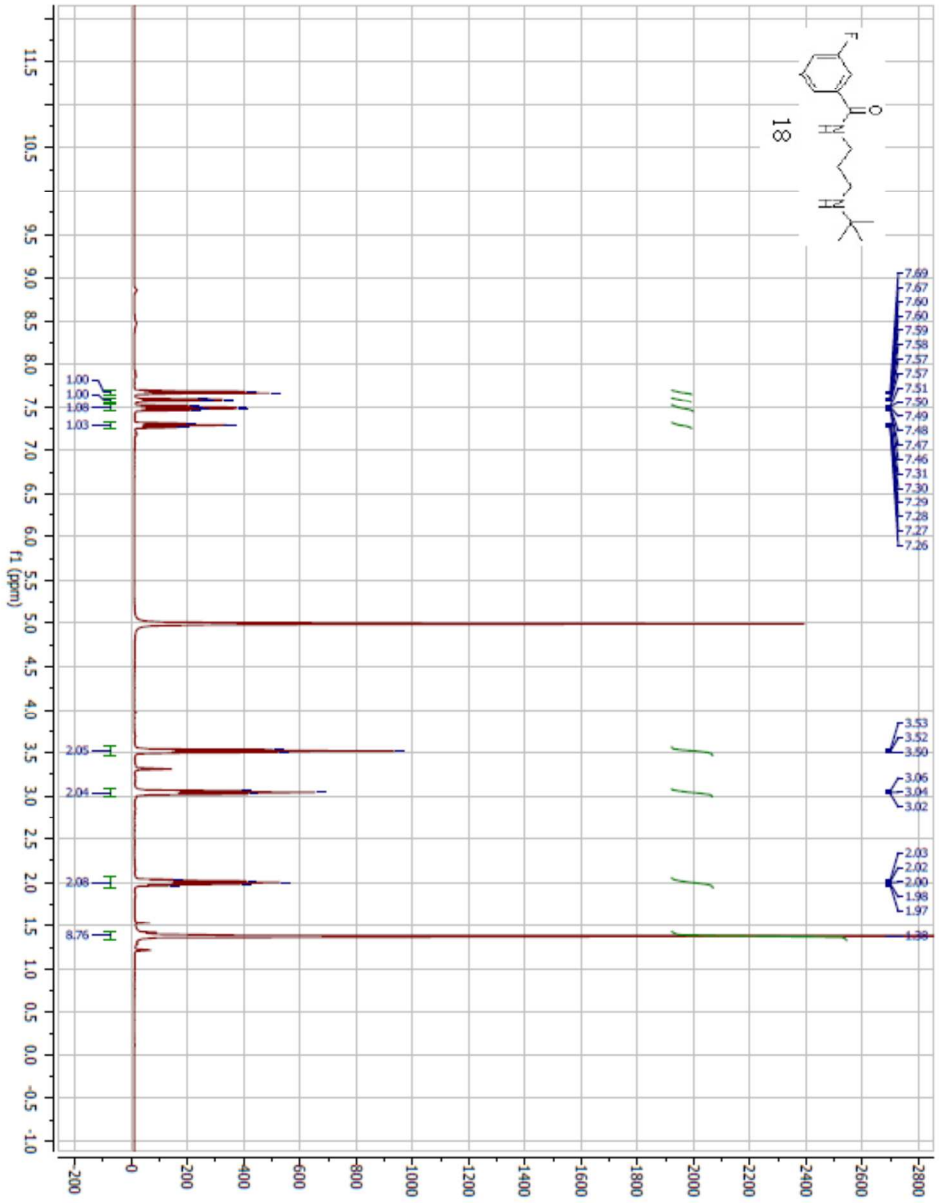
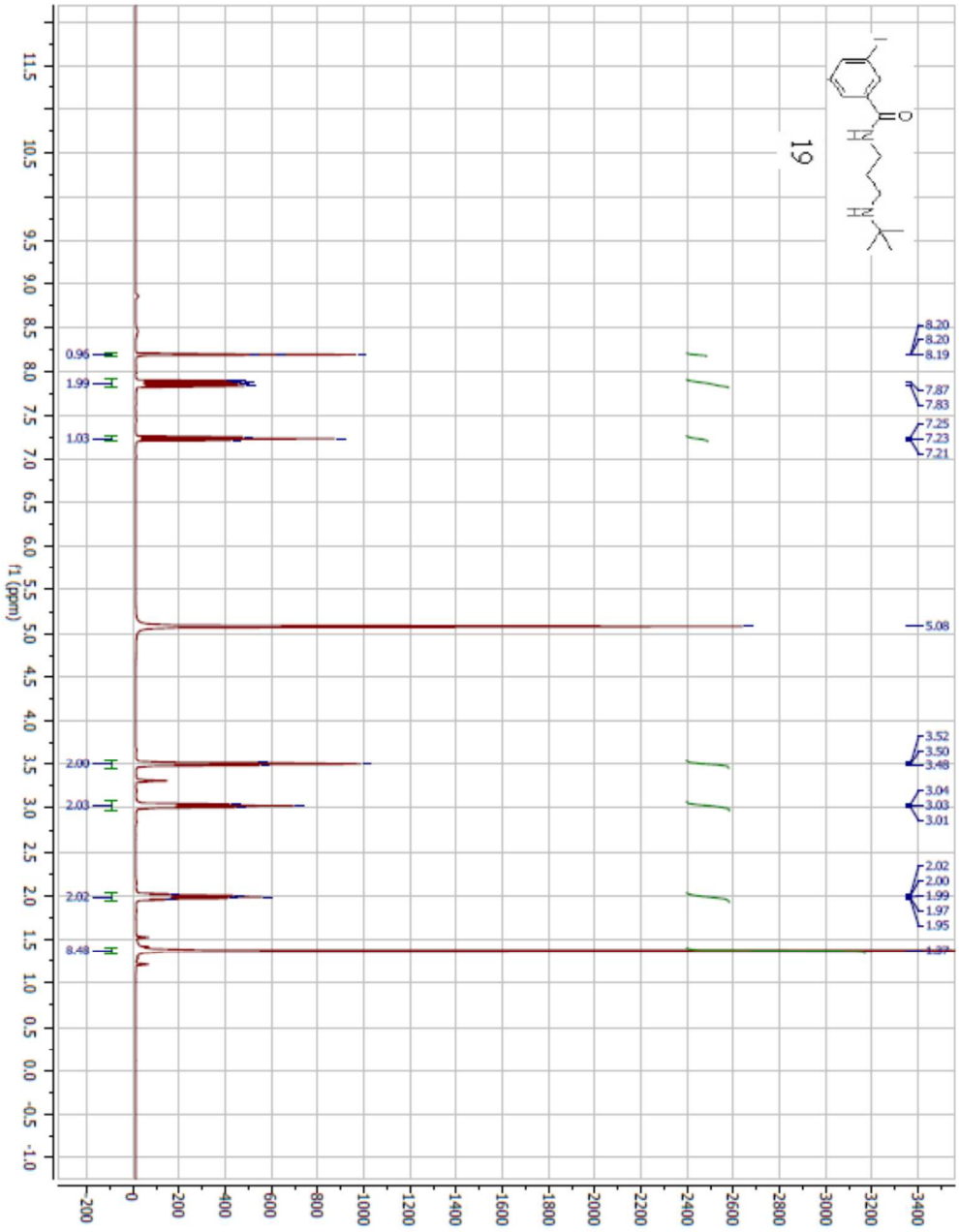
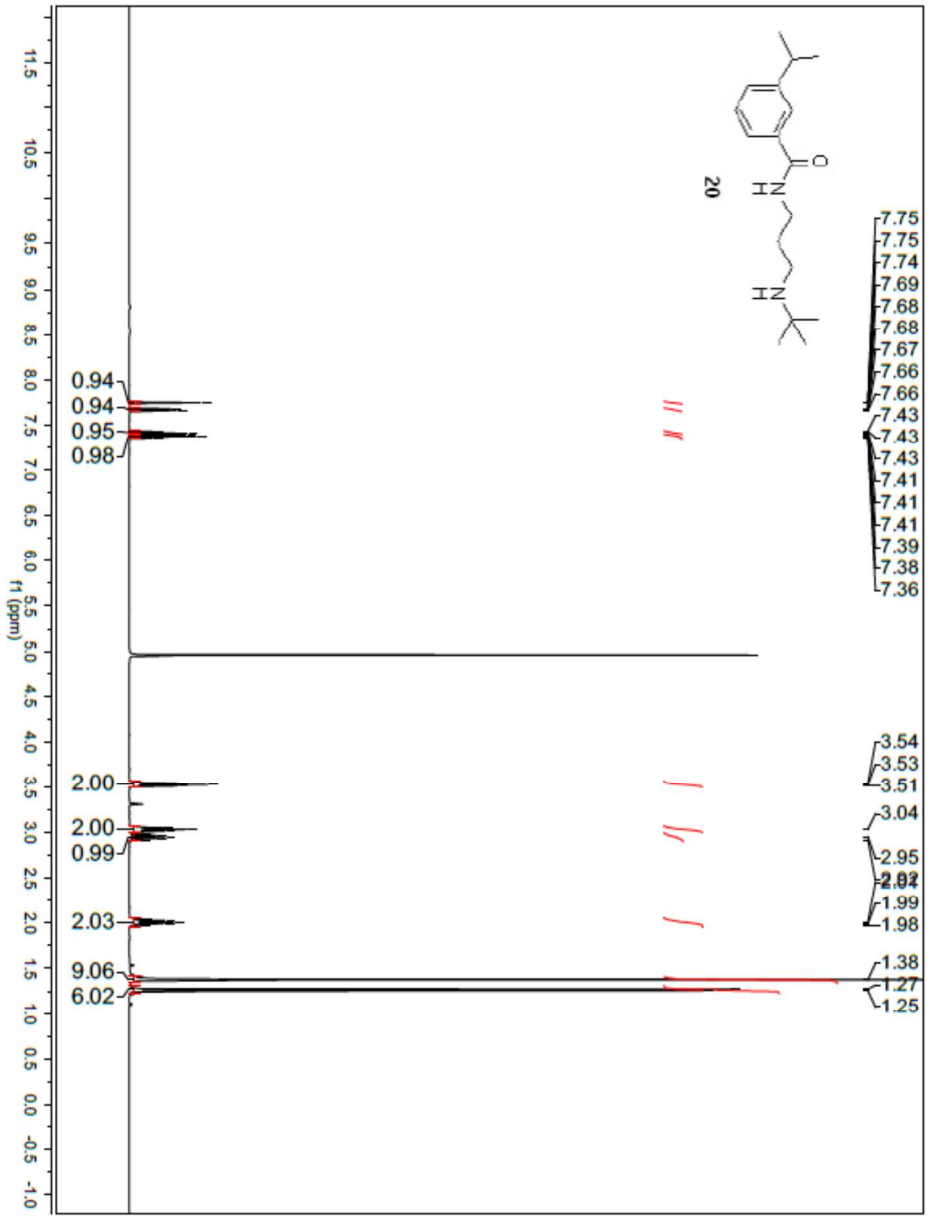
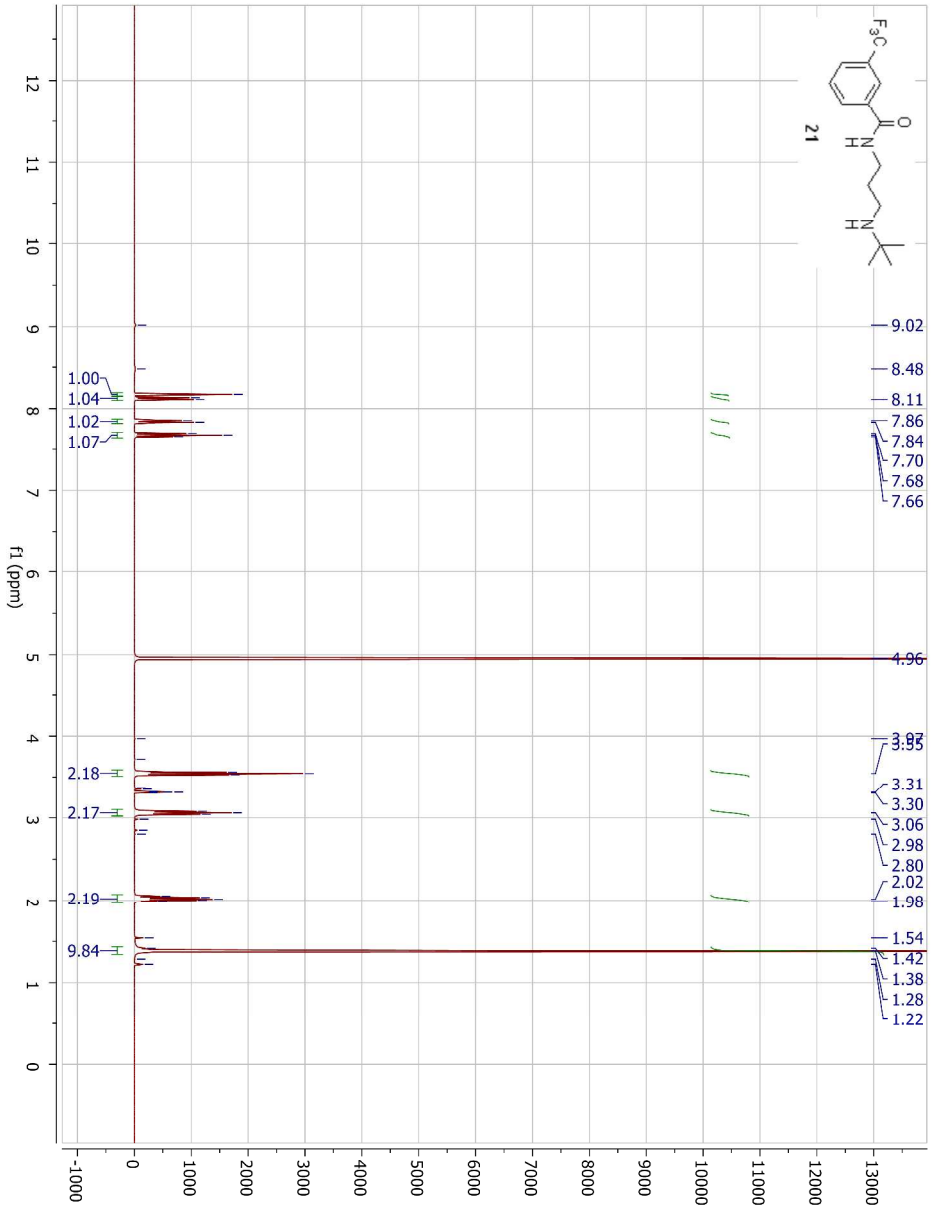
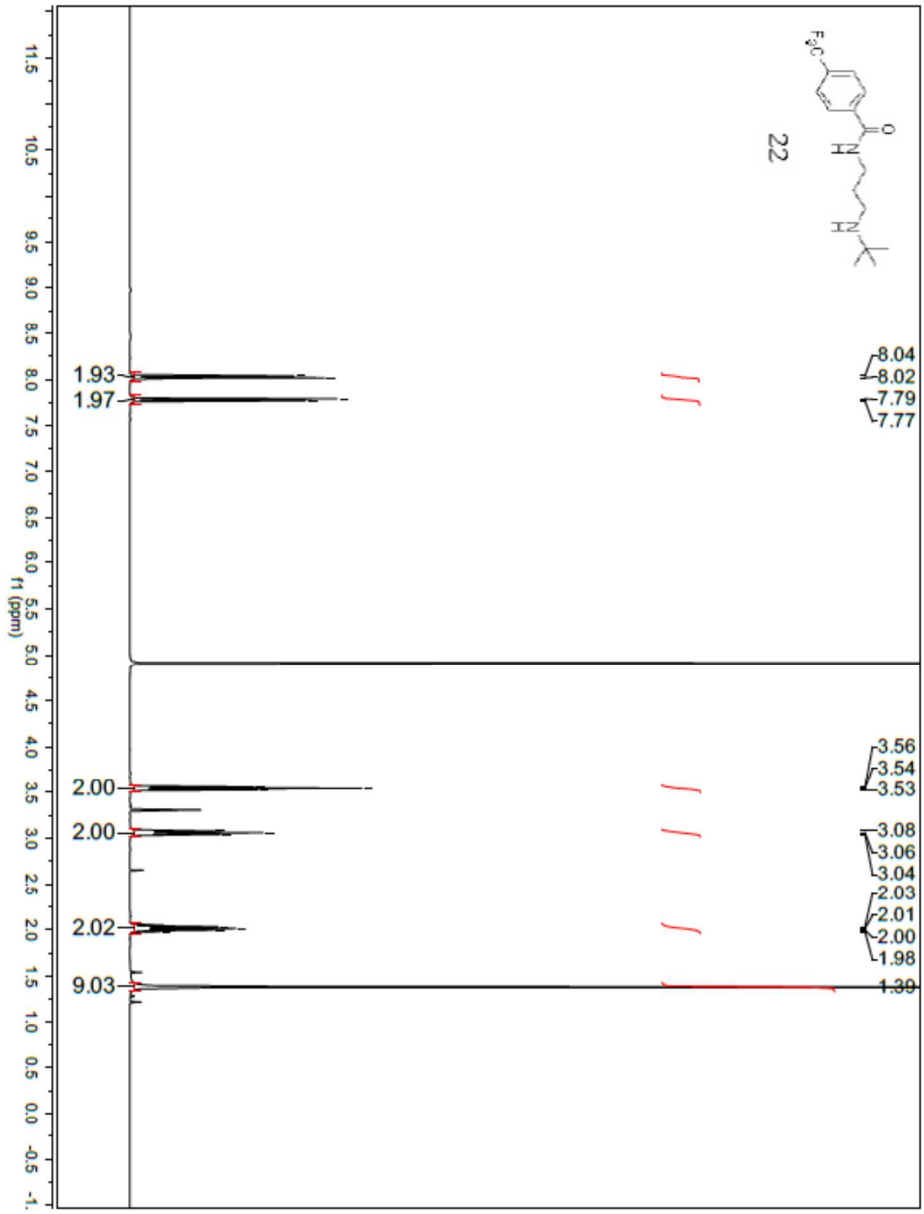
7.86
7.85
7.78
7.76
7.54
7.54
7.54
7.52
7.52
7.52
7.46
7.44
7.42
3.53
3.51
3.49
3.05
3.03
3.01
2.03
2.01
2.00
1.98
1.96
1.00
1.05
1.04
1.07
2.17
2.19
2.18
9.34


18
F
O
H
N
N
H
7.69
7.67
7.60
7.60
7.59
7.58
7.57
7.57
7.51
7.50
7.49
7.48
7.47
7.46
7.31
7.30
7.29
7.28
7.27
7.26
1.00
1.00
1.08
1.03
3.53
3.52
3.50
3.06
3.04
3.02
2.03
2.02
2.00
1.98
1.97
2.05
2.04
2.08
8.76
1.38
f1 (ppm)


19
f1 (ppm)
8.20
8.20
8.19
7.87
7.83
7.25
7.23
7.21
5.08
3.52
3.50
3.48
3.04
3.03
3.01
2.02
2.00
1.99
1.97
1.95
0.96
1.99
1.03
2.00
2.03
2.02
8.48


20
7.75
7.75
7.74
7.69
7.68
7.68
7.67
7.66
7.66
7.43
7.43
7.43
7.41
7.41
7.41
7.39
7.38
7.36
3.54
3.53
3.51
3.04
2.95
1.99
1.98
1.38
1.27
1.25
0.94
0.94
0.95
0.98
2.00
2.00
0.99
2.03
9.06
6.02
f1 (ppm)


21
9.02
8.48
8.11
7.86
7.84
7.70
7.68
7.66
4.96
3.55
3.31
3.30
3.06
2.98
2.80
2.02
1.98
1.54
1.42
1.38
1.28
1.22
1.00
1.04
1.02
1.07
2.18
2.17
2.19
9.84
f1 (ppm)


22
8.04
8.02
7.79
7.77
3.56
3.54
3.53
3.08
3.06
3.04
2.03
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1.98
1.39
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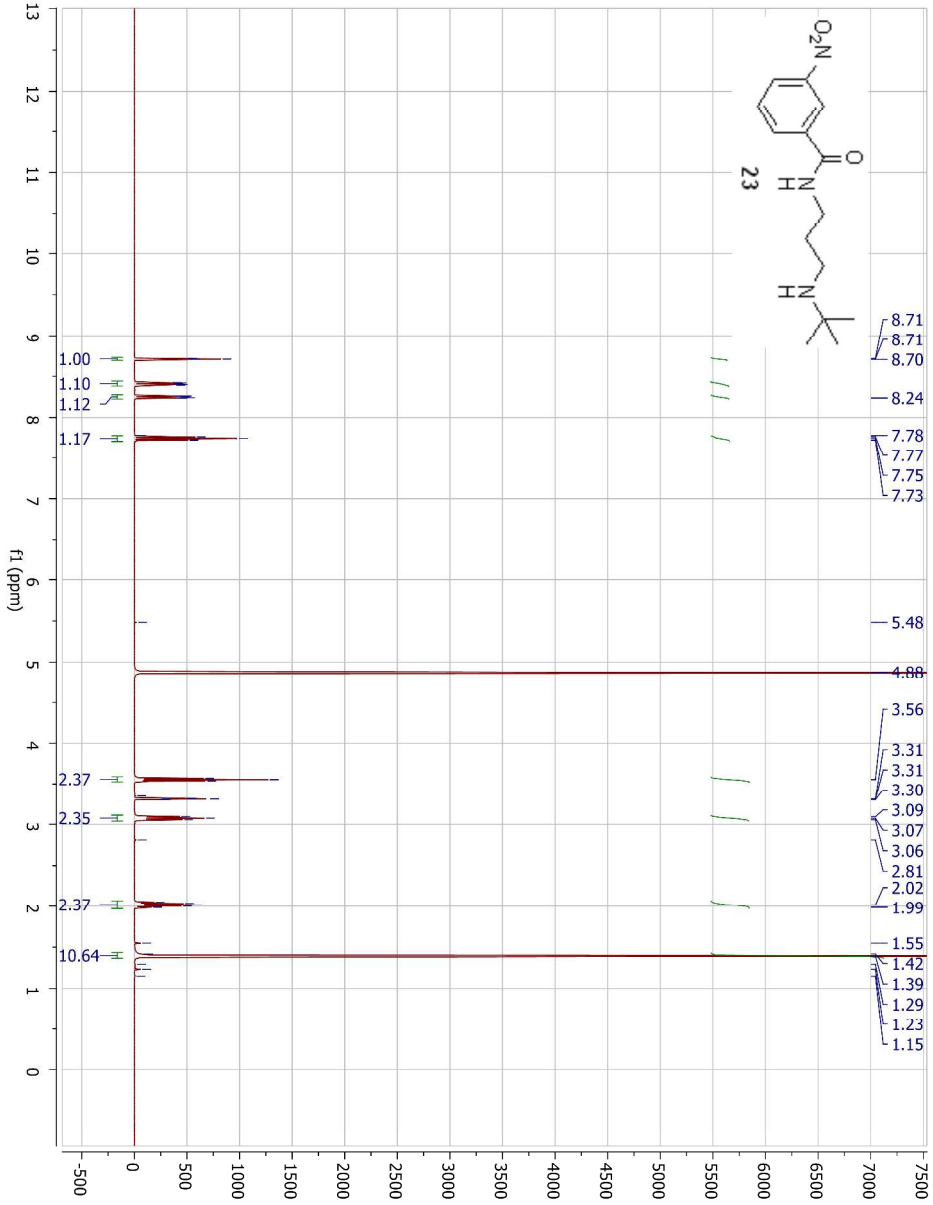
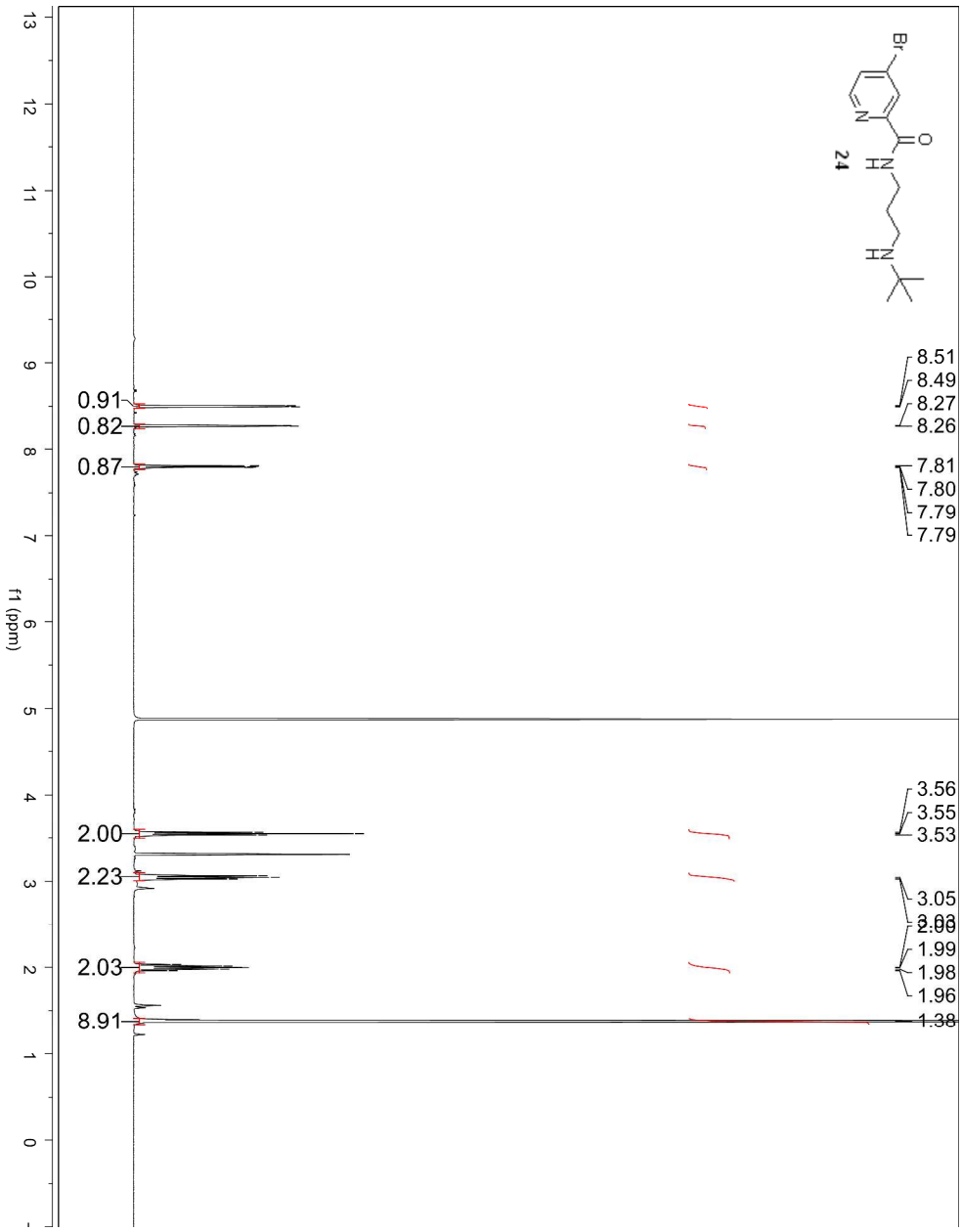
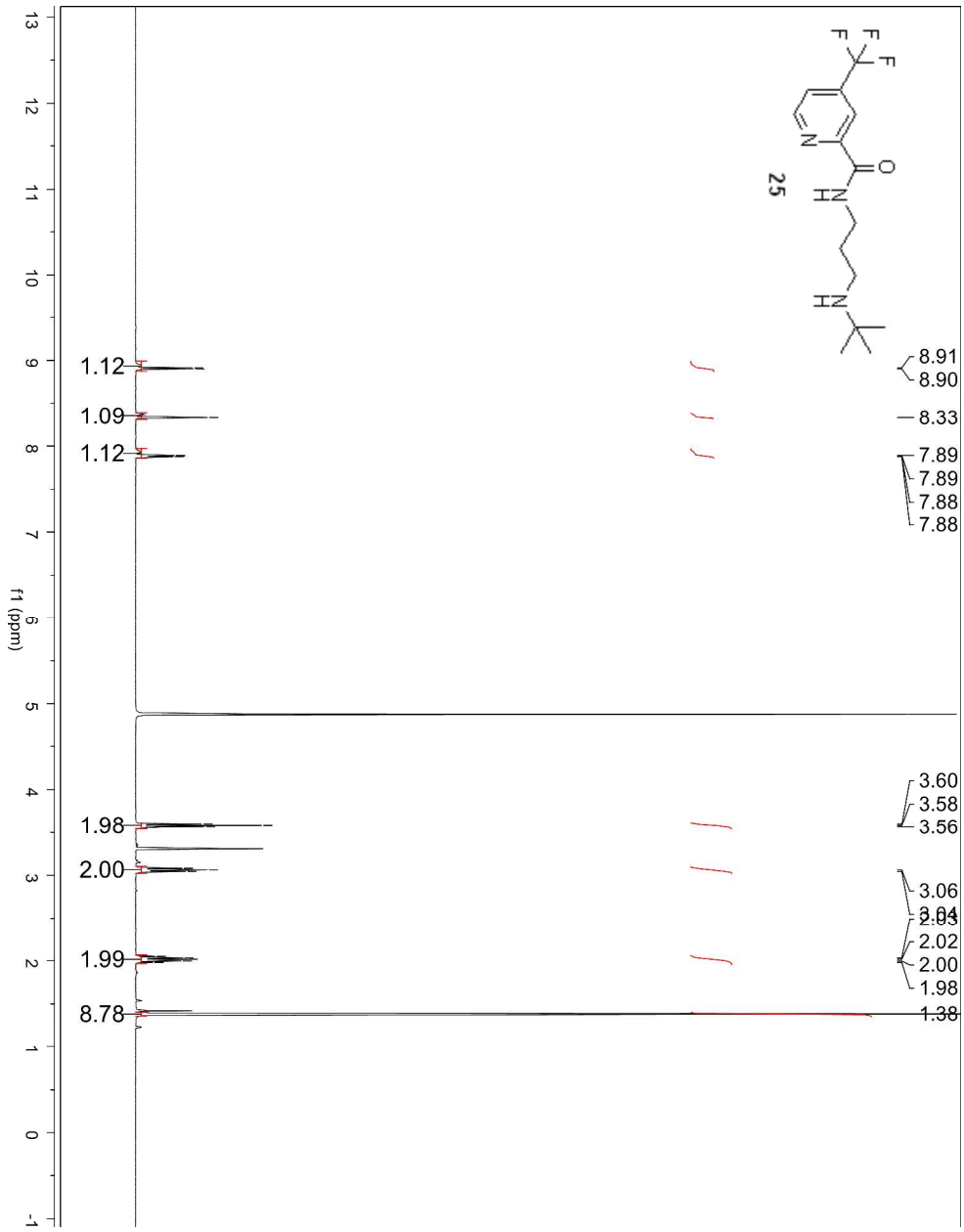
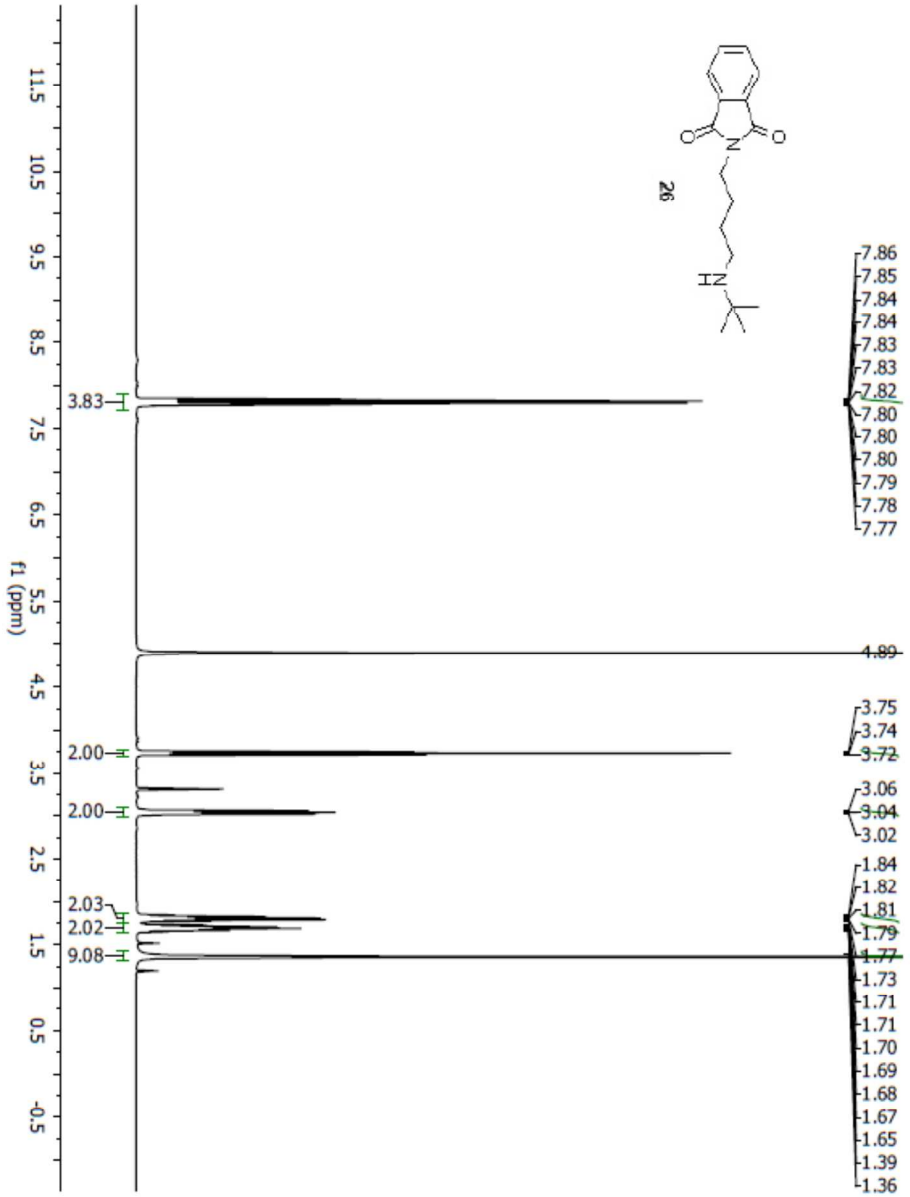
1.97
2.00
2.00
2.02
9.03
f1 (ppm)

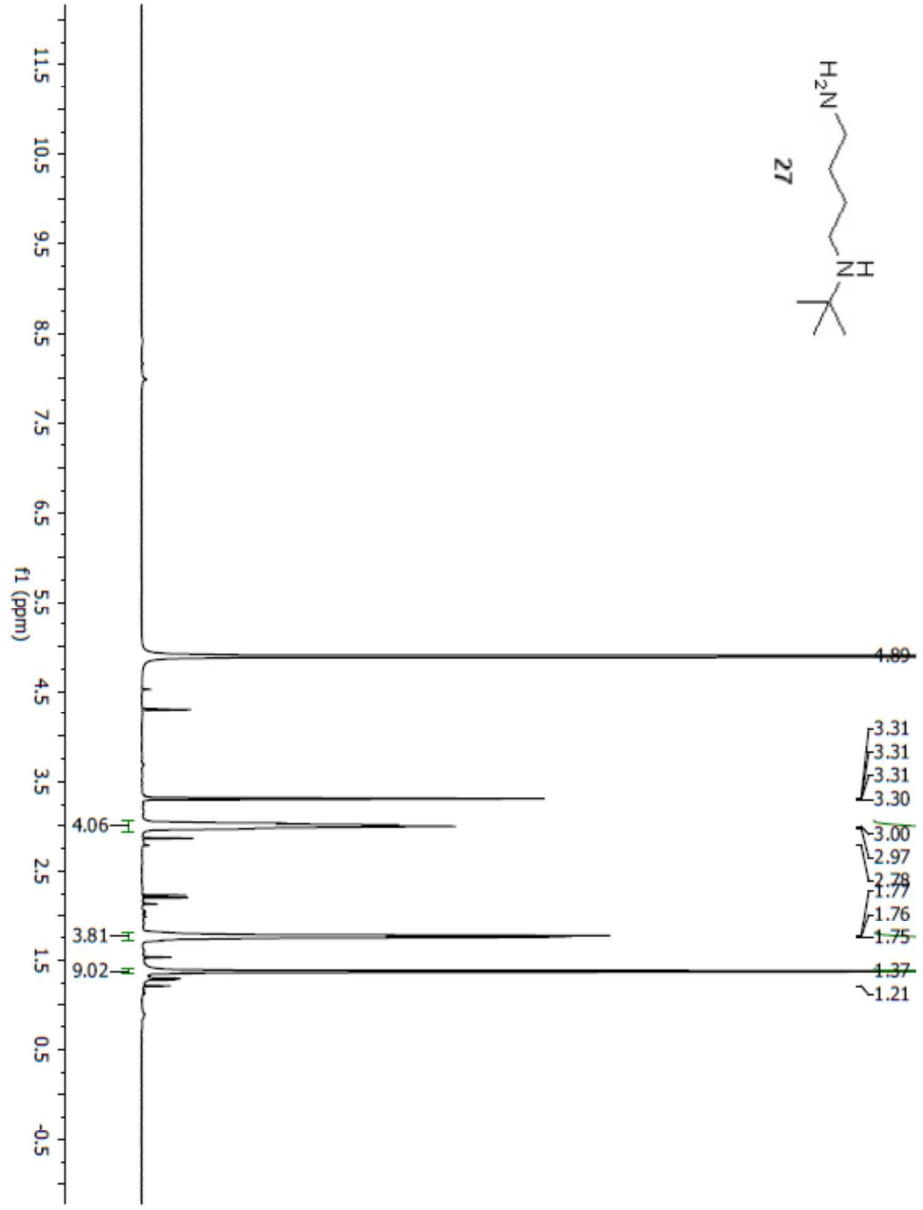

23
f1 (ppm)
1.00
1.10
1.12
1.17
2.37
2.35
2.37
10.64
8.71
8.71
8.70
8.24
7.78
7.77
7.75
7.73
5.48
4.88
3.56
3.31
3.31
3.30
3.09
3.07
3.06
2.81
2.02
1.99
1.55
1.42
1.39
1.29
1.23
1.15

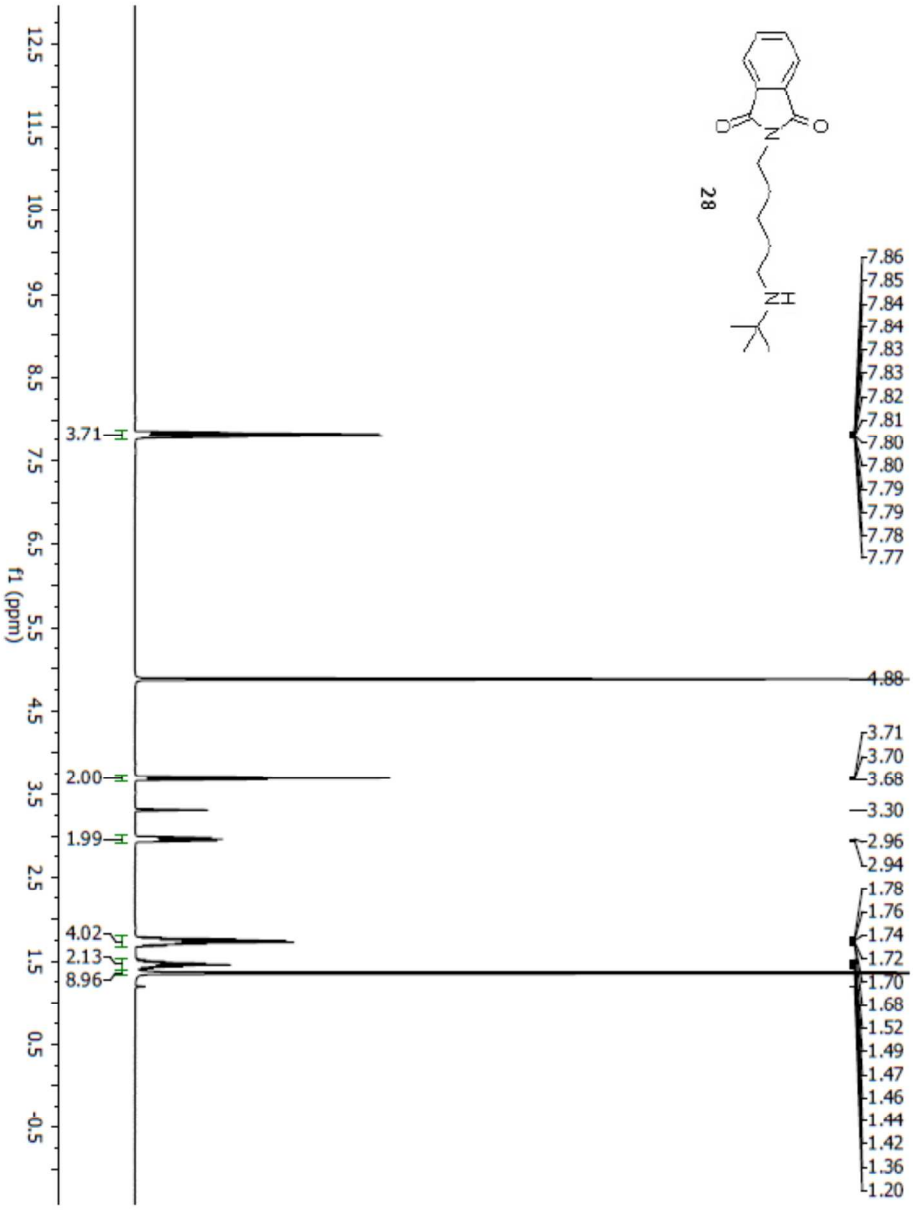

24
f1 (ppm)
0.91
0.82
0.87
2.00
2.23
2.03
8.91
8.51
8.49
8.27
8.26
7.81
7.80
7.79
7.79
3.56
3.55
3.53
3.05
2.00
1.99
1.98
1.96
1.38


25
1.12
1.09
1.12
1.98
2.00
1.99
8.78
8.91
8.90
8.33
7.89
7.89
7.88
7.88
3.60
3.58
3.56
3.06
2.02
2.00
1.98
1.38
f1 (ppm)

d) ^{1}H NMR of intermediates 26 – 29.




27
f1 (ppm)
4.89
3.31
3.31
3.31
3.30
4.06
3.00
2.97
2.78
1.77
1.76
3.81
1.75
9.02
1.37
1.21


28
f1 (ppm)
12.5
11.5
10.5
9.5
8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
0.5
-0.5
3.71
2.00
1.99
4.02
2.13
8.96
7.86
7.85
7.84
7.84
7.83
7.83
7.82
7.81
7.80
7.80
7.79
7.79
7.78
7.77
4.88
3.71
3.70
3.68
3.30
2.96
2.94
1.78
1.76
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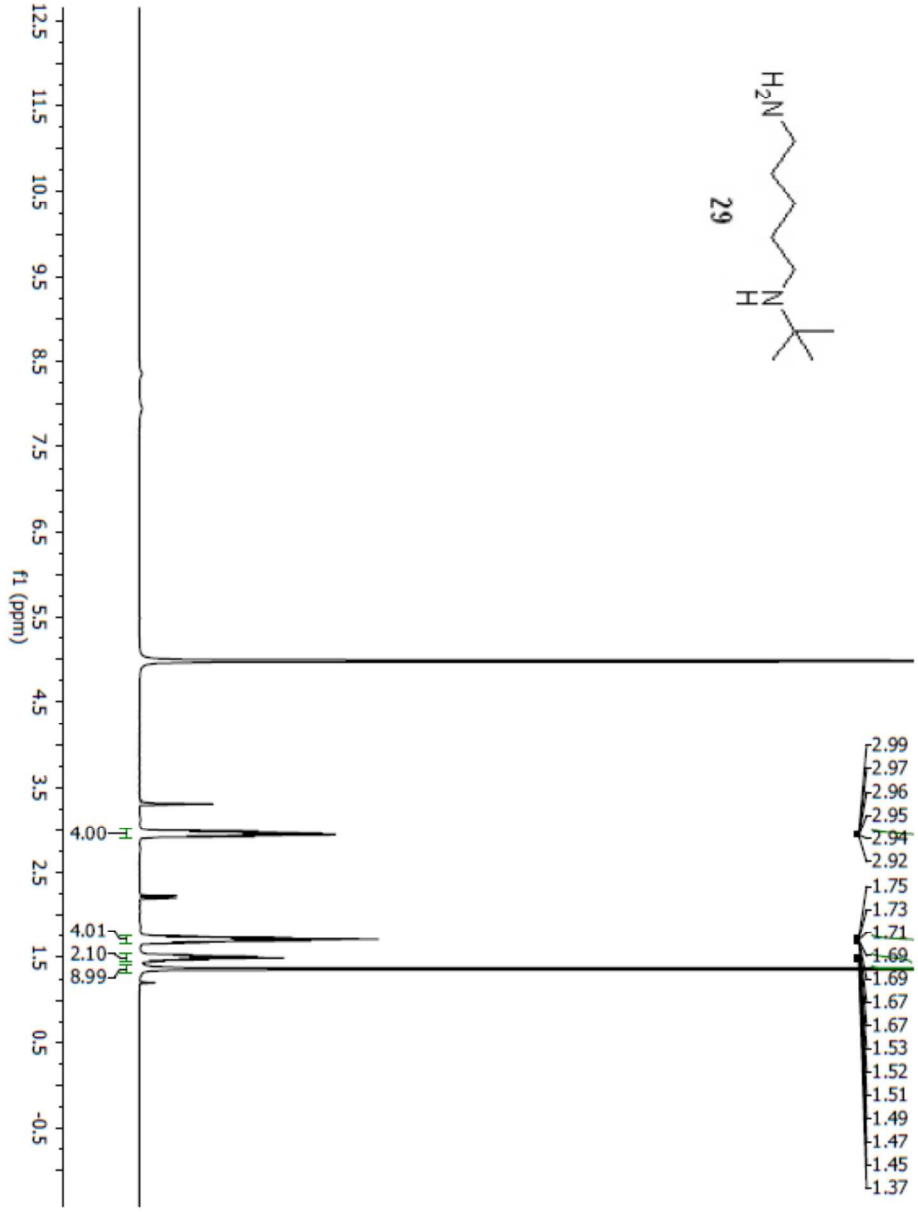
1.47
1.46
1.44
1.42
1.36
1.20


29
H2N
N
H
f1 (ppm)
12.5
11.5
10.5
9.5
8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
0.5
-0.5
4.00
4.01
2.10
8.99
2.99
2.97
2.96
2.95
2.94
2.92
1.75
1.73
1.71
1.69
1.69
1.67
1.67
1.53
1.52
1.51
1.49
1.47
1.45
1.37

Supplementary Table 1. AlphaScreen proteins and their corresponding peptide substrates.

Protein	Peptide	Peptide sequence
53BP1	H4K20Me2	Biotin-AHX-KGGAKRHRK(Me2)VLRDNIQ-OH
L3MBTL1	H4K20Me1	Biotin-AHA-KGGAKRHRK(Me1)VLRDNIQ-OH
L3MBTL3	H4K20Me2	Biotin-AHX-KGGAKRHRK(Me2)VLRDNIQ-OH
MBTD1	H4K20Me1	Biotin-AHA-KGGAKRHRK(Me1)VLRDNIQ-OH
CBX7	H3K9Me3	ARTKQTARK(Me3)STGGKAPRKQL-K(Biotin)-NH2
UHRF1	H3K9Me3	ARTKQTARK(Me3)STGGKAPRKQL-K(Biotin)-NH2
PHF23	H3K4Me3	ARTK(Me3)QTARKSTGGKAPRKQYT-K(Biotin)-NH2
JARID1A	H3K4Me3	ARTK(Me3)QTARKSTGGKAPRKQYT-K(Biotin)-NH2
PHF1	H3K36me3	KSAPSTGGVK(Me3)KPHRYRPGTV-K(biotin)-NH2
PHF19	H3K36me3	KSAPSTGGVK(Me3)KPHRYRPGTV-K(biotin)-NH2

Supplementary Table 2. Crystallography data and refinement statistics.

	53BP1+UNC2170
PDB Code	4RG2
DATA COLLECTION	
Space group	$C222_1$
Cell dimensions	
a, *b*, *c* (Å)	73.5, 100.5, 83.3
α, β, γ (°)	90.0,90.0,90.0
Resolution (Å) (highest resolution shell)	50.00-1.50(1.53-1.50)
Measured reflections	377996
Unique reflections	48740
R_{merge}	4.9(67.0)
$I/\sigma I$	45.0
Completeness(%)	98.1(82.9)
Redundancy	7.8(5.8)
REFINEMENT	
Resolution (Å)	50.0-1.50
No. reflections (test set)	47270(1433)
R_{work}/ R_{free} (%)	22.3/19.8
No. atoms	
Protein	2064
Ligand	36
Water	199
B-factors (Å^2)	
Protein	29.0
Ligand	21.1
Water	35.9
RMSD	
Bond lengths (Å)	0.009
Bond angles (º)	1.350
Ramachandran plot % residues	
Favored	98.2
Additional allowed	1.8
Generously allowed	0
Disallowed	0

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