Electronic Supplementary Material (ESI) for Chemical Communications.

Electronic Supplementary Information

Rational design and diversity-oriented synthesis of peptoid-based selective HDAC6 inhibitors

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1. Supplemental Figures

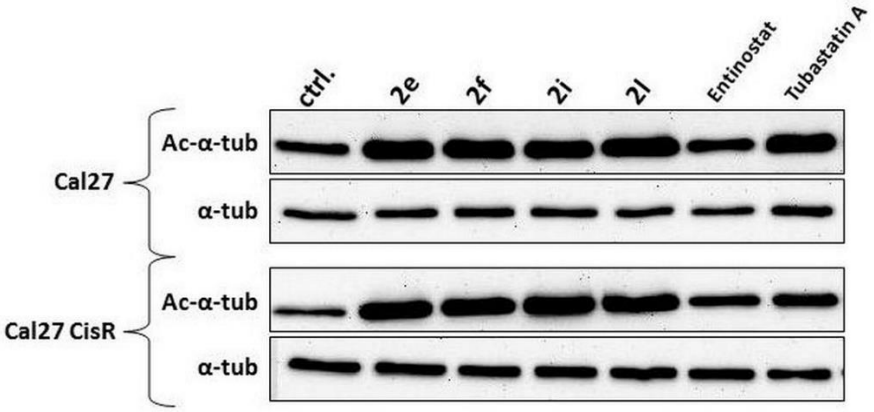


Fig. S1. Hyperacetylation of α-tubulin after 24 h incubation with **2e**, **f**, **i**, **l**, entinostat and tubastatin A (1 µM) in Cal27 and Cal27 CisR.

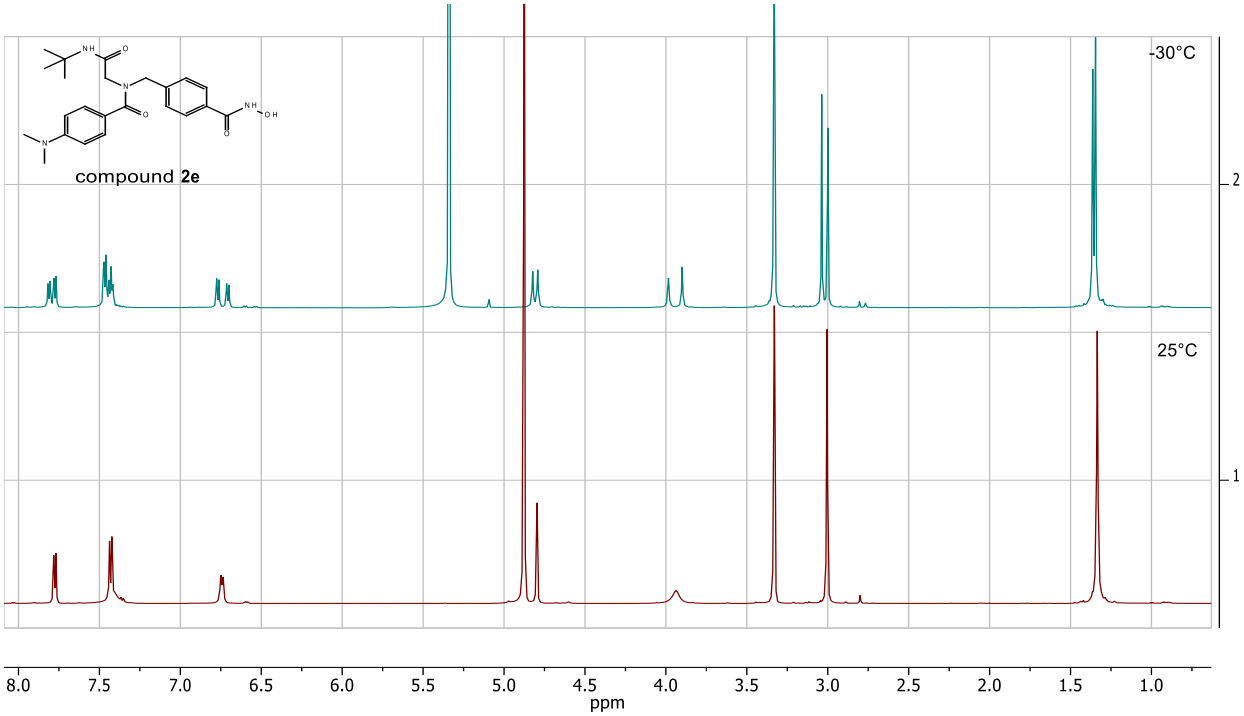


Fig. S2. ^{1}H-NMR spectra of compound **2e** in methanol-d_4 at -30°C and 25°C.

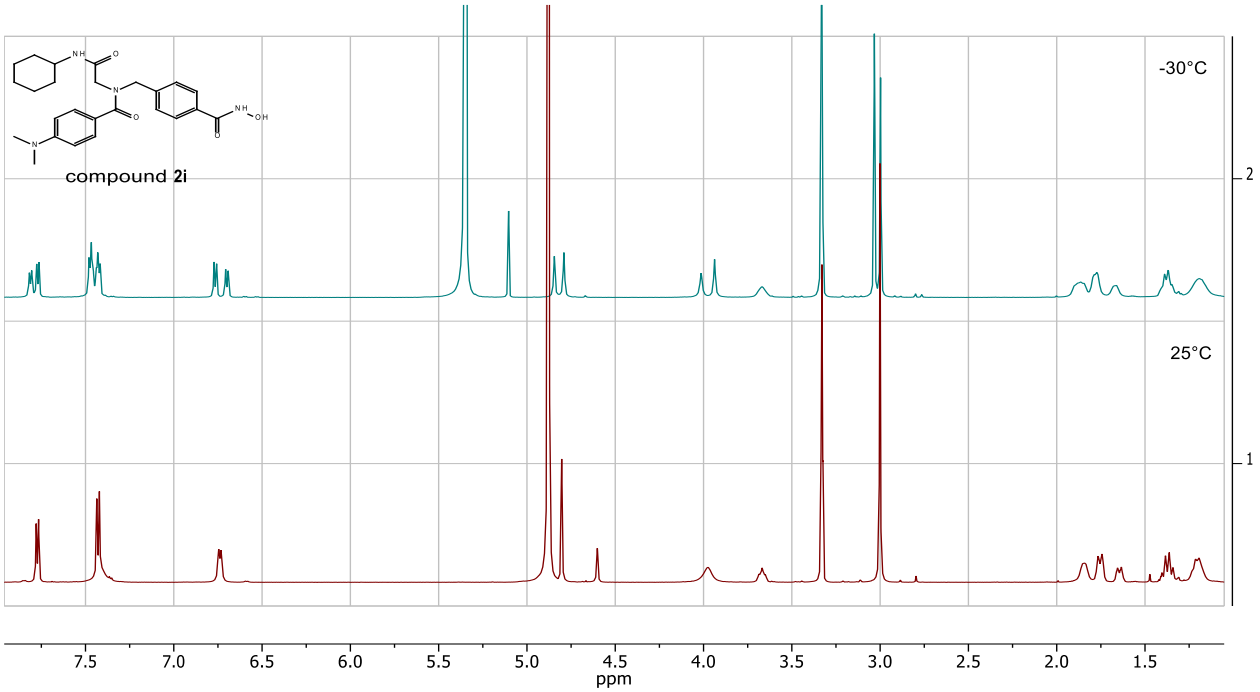


Fig. S3. ^{1}H-NMR spectra of compound **2i** in methanol-d_4 at -30°C and 25°C.

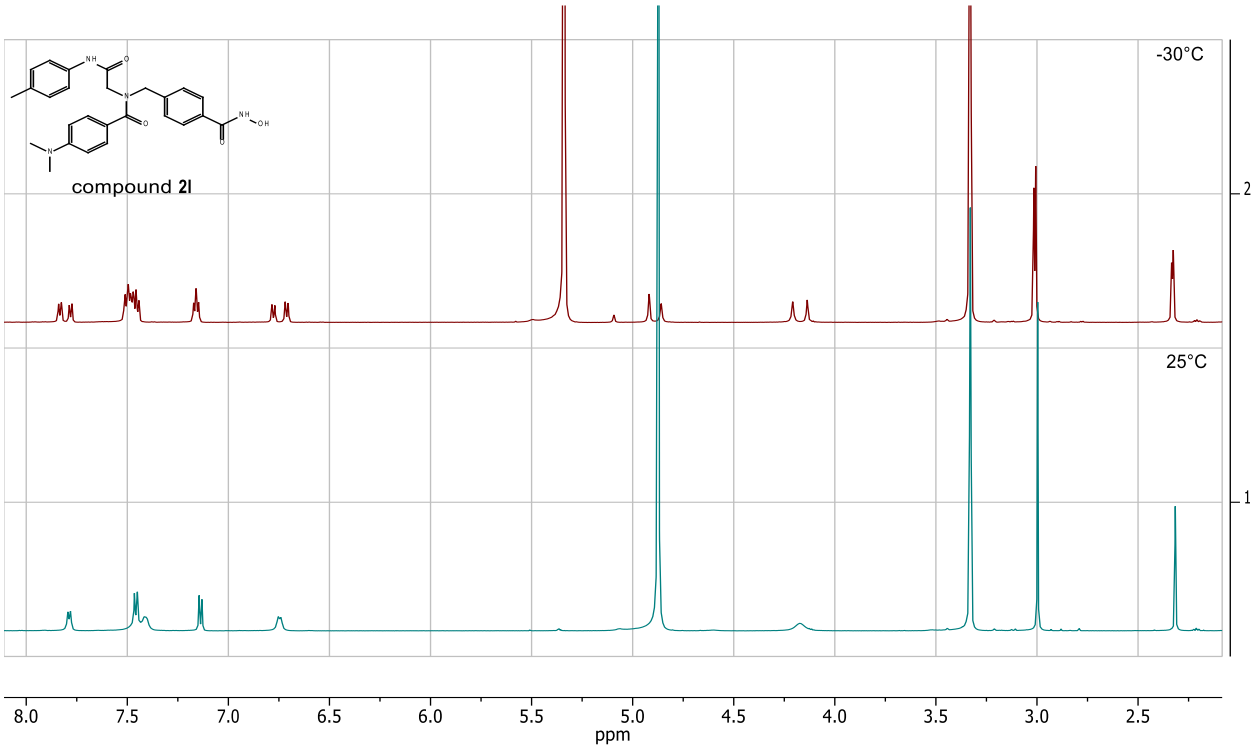


Fig. S4. ^{1}H-NMR spectra of compound **2l** in methanol-d_4 at -30°C and 25°C.

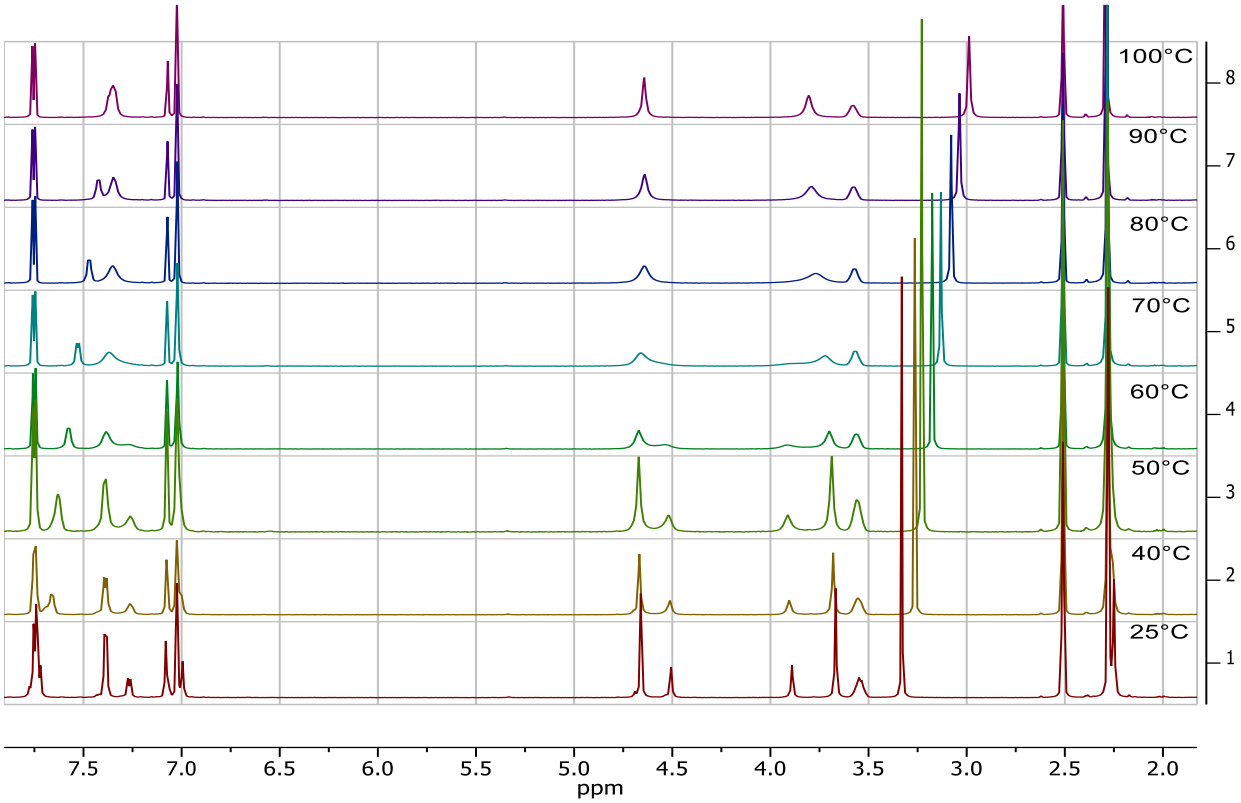


Fig. S5. VT-NMR spectra of compound **2f** in DMSO-d_6.

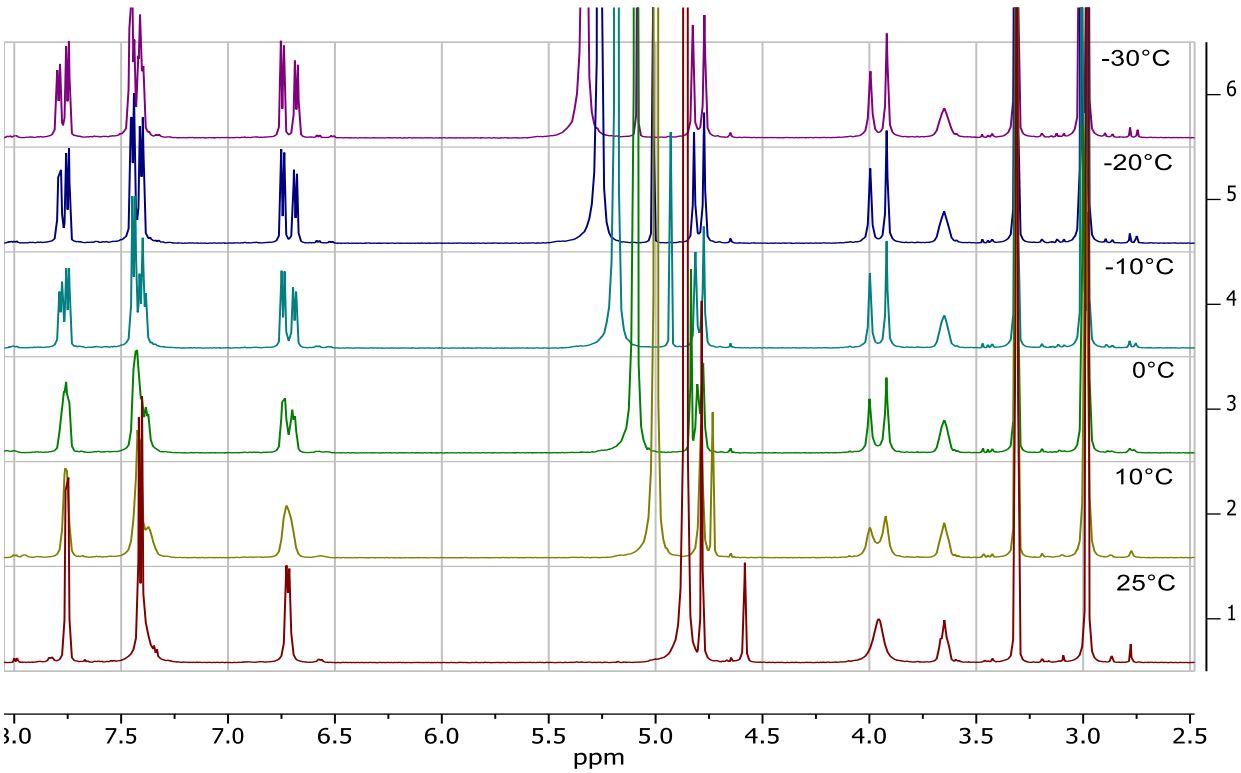


Fig. S6. VT-NMR spectra of compound **2i** in methanol-d_4.

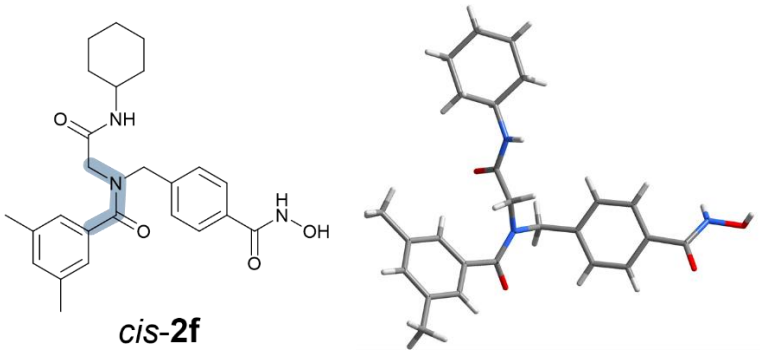


Fig. S7. X-ray structure of compound **2f**.

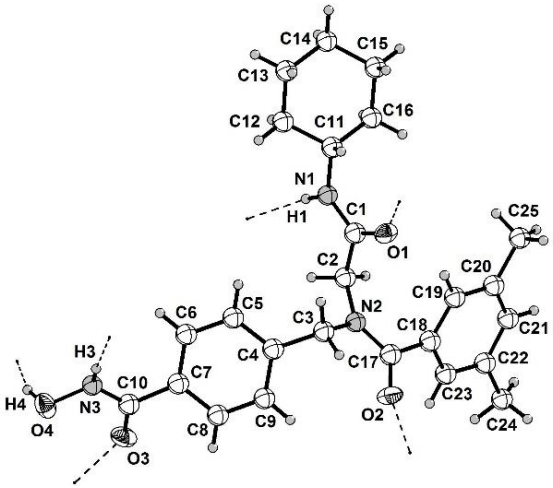


Fig. S8. The asymmetric unit of the title structure **2f** contains one complete molecule. The ellipsoids are drawn at the 45% probability level. Hydrogen bonds are indicated by dashed lines.[1]

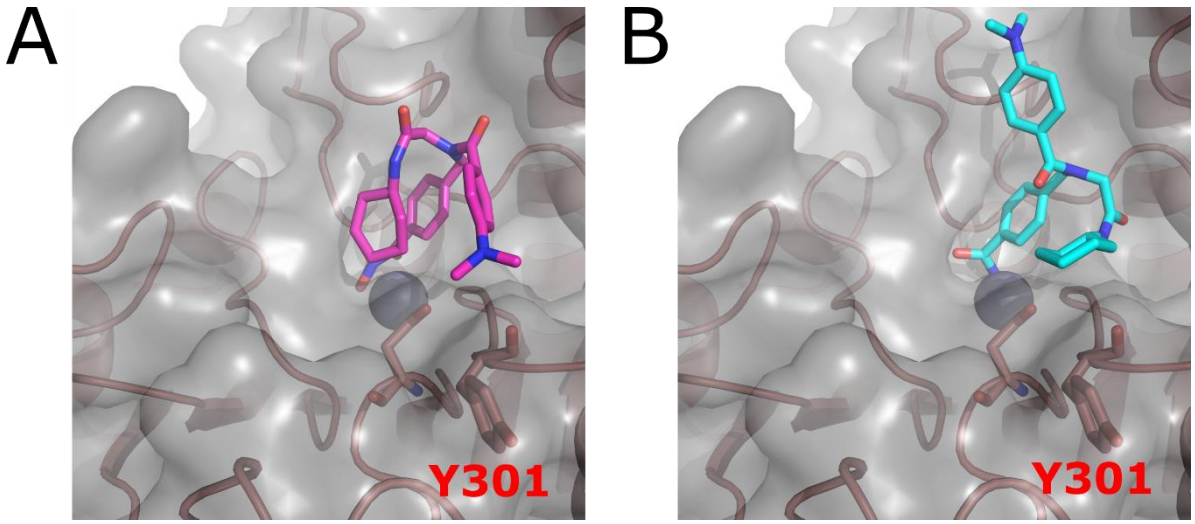


Fig. S9. Docking poses of **2i** as a cis-rotamer (A) and trans-rotamer (B) to HDAC6 with Y301 flipped-out. Zinc is shown as a sphere.

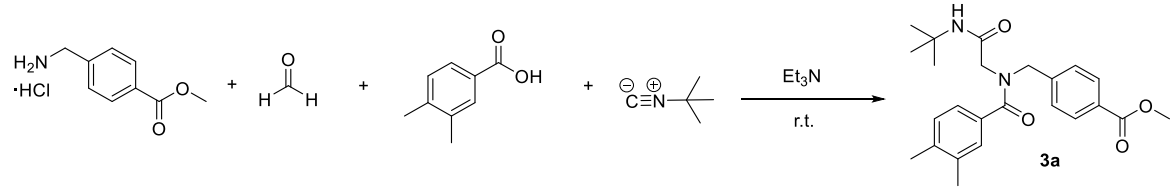
2. Chemistry

General information

All chemicals and solvents were obtained from commercial suppliers (Sigma-Aldrich, Acros Organics, Carbolution Chemicals) and used as purchased without further purification. The progress of all reactions was monitored by thin layer chromatography (TLC) using Merck precoated silica gel plates (with fluorescence indicator UV_{254}). Components were visualized by irradiation with ultraviolet light (254 nm) or staining in potassium permanganate solution following heating. Flash column chromatography was performed using prepacked silica cartridge with the solvent mixtures specified in the corresponding experiment. Melting points (mp) were taken in open capillaries on a Mettler FP 5 melting-point apparatus and are uncorrected. Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on a Bruker Avance 300, 500 or 600 using DMSO-d_6 or $CDCl_3$ as solvents. Chemical shifts are given in parts per million (ppm), relative to residual solvent peak for ^{1}H and ^{13}C. ^{1}H NMR signals marked with an asterisk (*) correspond to peaks assigned to the minor rotamer conformation. Elemental analysis was performed on a Perkin Elmer PE 2400 CHN elemental analyzer. High resolution mass spectra (HRMS) analysis was performed on a UHR-TOF maXis 4G, Bruker Daltonics, Bremen by electrospray ionization (ESI). Analytical HPLC analysis were carried out on a Varian Prostar system equipped with a Prostar 410 (autosampler), 210 (pumps) and 330 (UV-detector) using a Phenomenex Luna 5u C18(2) 1.8 µm particle (250 mm × 4.6 mm) column, supported by Phenomenex Security Guard Cartridge Kit C18 (4.0 mm × 3.0 mm). UV absorption was detected at 254 nm with a linear gradient of 10% B to 100% B in 20 min using HPLC-grade water +0.1% TFA (solvent A) and HPLC-grade acetonitrile +0.1% TFA (solvent B) for elution at a flow rate of 1 mL/min. The purity of all final compounds was 95% or higher.

Optimization of the Ugi four-component reaction (U-4CR) conditions

Table S1: Optimization of the Ugi-4CR.



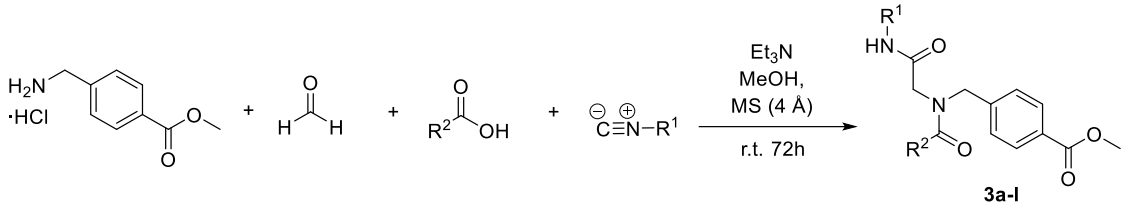
entry	solvent	additive	time	stoichiometries	Concentration[b]	yield (%)[c]
1	Ether	-	72 h	1.2:1.2:1:1	0.5	29
2	THF	-	72 h	1.2:1.2:1:1	0.5	38
3	DCM	-	72 h	1.2:1.2:1:1	0.5	61
4	THF/MeOH 2:1	-	72 h	1.2:1.2:1:1	0.5	68
5	MeOH	-	72 h	1.2:1.2:1:1	0.5	78
6	MeOH[a]	MS (4 Å)	72 h	1.2:1.2:1:1	0.5	86
7	MeOH[a]	MS (4 Å)	24 h	1.2:1.2:1:1	0.5	57
8	MeOH[a]	MS (4 Å)	72 h	1:1:1:1	0.5	82
9	MeOH[a]	MS (4 Å)	72 h	2:2:1:1	0.5	41
10	MeOH[a]	MS (4 Å)	72 h	1.2:1.2:1:1	0.25	74

[a]use of dry methanol
[b]concentration is calculated based on the amount of carboxylic acid
[c]isolated yield after flash column chromatography

A series of experiments were carried out to determine the optimal conditions for the U-4CR using intermediate **3a** as model compound. For all reactions a pre-formation of the imine intermediate was performed, using 4-aminomethyl benzoic acid methyl ester hydrochloride and paraformaldehyde in presence of triethylamine as base. After 30 min 3,4-dimethyl benzoic acid and *tert*-butyl isocyanide were added sequentially and the reaction mixture was stirred at room temperature for the time stated. The results are summarized in Table S1. First, a range of solvents (analytical grade) were screened for the synthesis of **3a** (entry 1-5). Among these solvents, MeOH gave favorable results (entry 5). Addition of crushed molecular

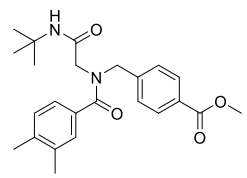
sieves (MS 4 Å) and the use of dry methanol increased the yield from 78% (entry 5) to 86% (entry 6). Hence, further optimization was performed by carrying out the reaction in dry MeOH using molecular sieves as additive. Shortening of the reaction time from 72 h (entry 6) to 24 h (entry 7) led to a lower yield. Finally, investigation of different stoichiometries and concentrations revealed that the best yield was obtained by using 1.2 eq. of 4-aminomethyl benzoic acid methyl ester hydrochloride and paraformaldehyde and a concentration of 0.5 M (entry 6).

General procedure for the synthesis of 3a-l



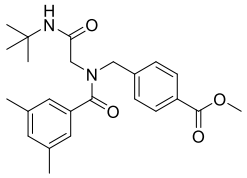
A mixture of 4-aminomethyl benzoic acid methyl ester hydrochloride (484 mg, 2.4 mmol, 1.2 eq), paraformaldehyde (72 mg, 2.4 mmol, 1.2 eq), triethylamine (333 µl, 2.4 mmol, 1.2 eq), and 200 mg of crushed moleculare sieves (MS) 4 Å was stirred in dry methanol (4 mL, 0.5 M) at room temperature for 30 min. Next, the appropriate carboxylic acid (2.0 mmol, 1.0 eq) and isocyanide (2.0 mmol, 1.0 eq) were added sequentially. The reaction mixture was stirred at room temperature for 72 h. After completion of the reaction, the reaction mixture was filtered and the solvent was removed under reduced pressure. Water (15 mL) was added and the pH was adjusted to a pH 4 using 4 M HCl. The mixture was extracted with dichloromethane (3 x 20 mL), the combined organic layers were dried over anhydrous sodium sulfate, filtered, and concentrated in vacuum. The crude products were purified by flash column chromatography (prepacked silica cartridge, hexane-ethylacetate, gradient: 90:10 → 50:50 in 30 min) to yield the desired products **3a-l**.

Methyl 4-((*N*-(2-(*tert*-butylamino)-2-oxoethyl)-3,4-dimethylbenzamido)methyl)benzoate (3a)



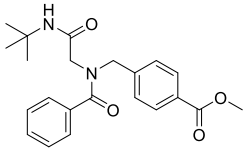
Colorless solid; yield: 700 mg (1.70 mmol, 85%); mp: 195°C; t_R: 17.86 min, purity: 99.1%; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.11-7.96 (m, 2H), 7.51-7.22 (m, 3H), 7.21-7.07 (m, 2H), 6.22/5.32* (2 x s, 1H), 4.84*/4.72 (2 x s, 2H), 3.94/3.73* (2 x s, 2H), 3.91 (s, 3H), 2.26 (s, 3H), 2.23 (s, 3H), 1.32 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.2, 167.9, 166.8, 141.8, 139.35, 137.3, 132.5, 130.3, 129.8, 128.2, 127.2, 124.3, 54.1, 52.3, 51.6, 50.5, 49.8, 28.8, 19.85; Anal. calcd. for $C_{24}H_{30}N_2O_4$: C 70.22, H 7.37, N 6.82, found: C 69.99, H 7.46, N 6.66.

Methyl 4-((*N*-(2-(*tert*-butylamino)-2-oxoethyl)-3,5-dimethylbenzamido)methyl)benzoate (3b)



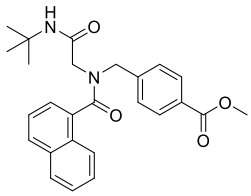
Colorless solid; yield: 665 mg (1.62 mmol, 81%); mp: 157°C; t_R: 18.09 min, purity: 97.0%; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.05-8.00 (m, 2H), 7.45-7.41*/7.28-7.23 (2 x m, 2H), 7.07-7.03 (m, 3H), 6.15/ 5.27* (2 x s, 1H), 4.86*/4.69 (2 x s, 2H), 3.95/3.71* (2 x s, 2H), 3.92 (s, 3H), 2.28 (s, 6H), 1.35/1.30* (2 x s, 9H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 173.4, 167.8, 166.8, 141.7, 138.5, 135.1, 131.9, 130.3, 129.8, 128.6, 127.3, 124.4, 54.1, 52.9, 52.3, 51.5, 50.3, 49.8, 28.8, 21.3; Anal. calcd. for $C_{24}H_{30}N_2O_4$: C 70.22, H 7.37, N 6.82, found: C 70.20, H 7.56, N 6.64.

Methyl 4-((*N*-(2-(*tert*-butylamino)-2-oxoethyl)benzamido)methyl)benzoate (3c)



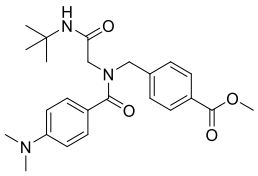
Colorless solid; yield: 650 mg (1.70 mmol, 85%); mp: 144°C; t_R: 16.18 min, purity: 98.6%; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.08-7.96 (m, 2H), 7.46-7.34 (m, 6H), 7.29-7.20 (m, 1H), 6.17/5.26* (2 x s, 1H), 4.86*/4.70 (2 x s, 2H), 3.96/3.68* (2 x s, 2H), 3.91 (s, 3H), 1.34/1.28* (2 x s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 173.0, 167.64 166.8, 142.1, 141.5, 135.5, 135.1, 130.4, 130.3, 129.9, 128.8, 128.5, 127.1, 126.9, 54.0, 52.8, 52.3, 51.5, 50.4, 49.8, 28.8; Anal. calcd. for $C_{22}H_{26}N_2O_4$: C 69.09, H 6.85, N 7.32, found: C 69.27, H 6.80, N 7.29.

Methyl 4-((*N*-(2-(*tert*-butylamino)-2-oxoethyl)-1-naphthamido)methyl)benzoate (3d)



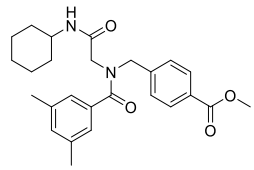
Colorless solid; yield: 700 mg (1.62 mmol, 81%); mp: 143°C; t_R: 18.07 min, purity: 96.9%; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.11-7.82 (m, 5H), 7.63-7.13 (m, 6H), 6.23/4.97* (2 x s, 1H), 5.29*/4.79*/4.51 (3 x s, 2H), 4.44*/3.70*/3.54 (3 x s, 2H), 3.94*/3.89 (2 x s, 3H), 1.42/1.17* (2 x s, 9H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.3, 171.8, 167.6, 166.9, 166.7, 166.7, 142.25, 141.1, 133.7, 133.6, 133.3, 133.2, 130.4, 130.2, 129.9, 129.8, 129.8, 129.6, 128.8, 128.7, 127.6, 127.55, 126.8, 126.8, 125.2, 125.1, 124.8, 124.7, 124.2, 123.9, 77.2, 53.7, 52.35, 52.3, 52.2, 51.7, 51.7, 49.9, 49.4, 28.9, 28.6; Anal. calcd. for $C_{26}H_{28}N_2O_4$: C 72.20, H 6.53, N 6.48, found: C 72.19, H 6.64, N 6.31.

Methyl 4-((*N*-(2-(*tert*-butylamino)-2-oxoethyl)-4-(dimethylamino)benzamido)methyl)benzoate (3e)



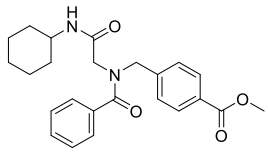
Colorless solid; yield: 683 mg (1.60 mmol, 80%); mp: 187°C; t_R: 13.04 min, purity: 96.2%; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.09-7.96 (m, 2H), 7.47-7.37 (m, 2H), 7.38-7.29 (m, 2H), 6.68-6.55 (m, 2H), 4.81 (s, 2H), 3.91 (s, 5H), 2.97 (s, 6H), 1.32 (s, 9H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 173.4, 168.2, 166.9, 152.0, 142.3, 130.3, 129.7, 129.2, 127.4, 121.3, 111.3, 77.2, 52.3, 51.4, 40.3, 31.7, 28.8, 22.8, 14.2; Anal. calcd. for $C_{24}H_{31}N_3O_4$: C 67.74, H 7.34, N 9.88, found: C 67.75, H 7.52, N 9.60.

Methyl 4-((*N*-(2-(cyclohexylamino)-2-oxoethyl)-3,5-dimethylbenzamido)methyl)benzoate (3f)



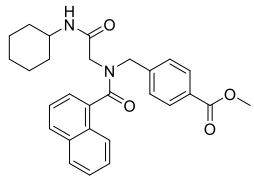
Colorless solid; yield: 698 mg (1.60 mmol, 80%); mp: 196°C; t_R: 18.76 min, purity: 98.8%; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.09-7.96 (m, 2H), 7.46-7.35*/7.31-7.19 (2 x m, 2H), 7.10-6.97 (m, 3H), 6.33/5.62* (2 x s, 1H), 4.83*/4.68 (2 x s, 2H), 4.01/3.76* (2 x s, 2H), 3.91 (s, 3H), 3.84-3.69 (m, 1H), 2.29*/2.27 (2 x s, 6H), 1.95-1.80 (m, 2H), 1.75-1.68 (m, 2H), 1.66-1.55 (m, 1H), 1.43-1.29 (m, 2H), 1.25-1.12 (m, 2H), 1.13-0.98 (m, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 173.6, 167.7, 166.8, 141.6, 138.6, 134.9, 132.0, 130.3, 129.85, 128.5, 127.2, 124.45, 54.1, 52.3, 49.7, 49.5, 48.55, 48.4, 33.1, 33.0, 25.6, 25.5, 24.9, 24.8, 21.35; Anal. calcd. for $C_{26}H_{32}N_2O_4$: C 71.53, H 7.39, N 6.42, found: C 71.26, H 7.46, N 6.22.

Methyl 4-((*N*-(2-(cyclohexylamino)-2-oxoethyl)benzamido)methyl)benzoate (3g)



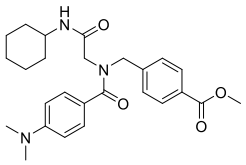
Colorless solid; yield: 637 mg (1.56 mmol, 78%); mp: 137°C; t_R: 16.87 min, purity: 97.3%; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.09-7.98 (m, 2H), 7.59-7.35 (m, 6H), 7.32-7.20 (m, 1H), 6.37/5.54* (2 x s, 2H), 4.87*/4.72 (2 x s, 2H), 4.06/3.77* (2 x s, 2H), 3.94 (s, 3H), 3.87-3.70 (m, 1H), 1.89 (s, 2H), 1.77-1.67 (m, 2H), 1.67-1.57 (m, 1H), 1.45-1.32 (m, 2H), 1.27-1.00 (m, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 173.1, 167.5, 166.8, 141.4, 135.0, 130.5, 130.3, 129.9, 128.8, 128.45, 127.1, 126.9, 54.0, 52.3, 49.8, 49.5, 48.6, 48.3, 33.0, 25.6, 24.8; Anal. calcd. for $C_{24}H_{28}N_2O_4$: C 70.57, H 6.91, N 6.86, found: C 70.47, H 7.12, N 6.60.

Methyl 4-((*N*-(2-(cyclohexylamino)-2-oxoethyl)-1-naphthamido)methyl)benzoate (3h)



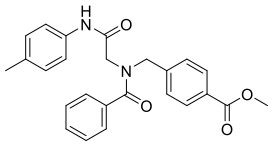
Colorless solid; yield: 580 mg (1.26 mmol, 63%); mp: 153°C; t_R: 18.75 min, purity: 98.7%; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.21-7.77 (m, 5H), 7.70-7.36 (m, 6H), 6.43-6.08/5.26-5.08* (2 x m, 1H), 5.31*/4.75*/4.50 (3 x s, 2H), 4.53*/3.75*/3.59 (3x s, 2H), 3.94*/3.90 (2 x s, 3H), 3.88-3.80/3.69-3.61* (2 x m, 1H), 1.87-0.75 (m, 10H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.4, 171.9, 167.4, 166.9, 166.7, 166.5, 142.1, 141.2, 133.7, 133.6, 133.3, 133.2, 130.4, 130.2, 129.9, 129.8, 129.8, 129.6, 128.75, 128.7, 128.6, 127.6, 127.5, 127.5, 126.85, 126.8, 125.2, 124.9, 124.6, 124.2, 123.9, 77.2, 53.65, 52.34, 52.31, 51.6, 49.2, 48.9, 48.55, 48.5, 33.2, 33.0, 25.6, 25.4, 24.9, 24.85; Anal. calcd. for $C_{28}H_{30}N_2O_4$: C 73.34, H 6.59, N 6.11, found: C 73.17, H 6.55, N 5.91.

Methyl 4-((*N*-(2-(cyclohexylamino)-2-oxoethyl)-4-(dimethylamino)benzamido)methyl)benzoate (3i)



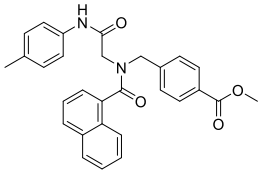
Colorless solid; yield: 409 mg (0.90 mmol, 45%); mp: 189°C; t_R: 13.96 min, purity: 96.5%; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.10-7.96 (m, 2H), 7.47-7.37 (m, 2H), 7.38-7.29 (m, 2H), 6.67-6.54 (m, 2H), 4.80 (s, 2H), 3.98 (s, 2H), 3.91 (s, 3H), 3.82-3.71 (m, 1H), 1.94-1.82 (m, 2H), 1.77-1.64 (m, 2H), 1.63-1.54 (m, 1H), 1.45-1.30 (m, 2H), 1.25-1.06 (m, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 173.5, 168.1, 166.9, 152.0, 142.2, 130.3, 129.7, 129.2, 127.3, 121.1, 111.3, 52.3, 48.2, 40.2, 33.0, 25.6, 24.8; Anal. calcd. for $C_{26}H_{33}N_3O_4$: C 69.16, H 7.37, N 9.31, found: C 69.16, H 7.59, N 9.05.

Methyl 4-((*N*-(2-oxo-2-(*p*-tolylamino)ethyl)benzamido)methyl)benzoate (3j)



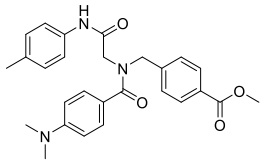
Colorless solid; yield: 593 mg (1.42 mmol, 71%); mp: 153°C; t_R: 17.53 min, purity: 98.8%; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.61 (s, 1H), 8.13-7.92 (m, 2H), 7.59-7.22 (m, 9H), 7.18-7.02 (m, 2H), 4.91*/4.76 (2 x s, 2H), 4.22/3.92* (2 x s, 2H), 3.92 (s, 3H), 2.31 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 173.6, 166.7, 166.6, 141.15, 135.2, 134.6, 134.1, 130.8, 130.4, 130.0, 129.6, 128.9, 128.45, 127.1, 126.9, 120.1, 54.2, 52.35, 50.9, 21.0; HRMS (ESI) Anal. Calcd. for $C_{25}H_{25}N_2O_4$ 417.1804 $[M+H]^+$, Found 417.1809.

Methyl 4-((*N*-(2-oxo-2-(*p*-tolylamino)ethyl)-1-naphthamido)methyl)benzoate (3k)



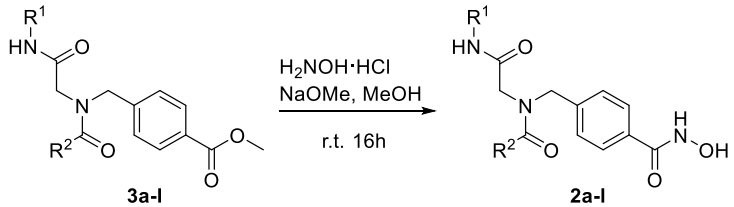
Colorless solid; yield: 710 mg (1.52 mmol, 76%); mp: 147°C; t_R: 19.31 min, purity: 99.4%; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.51/7.20* (2 x s, 1H), 8.10-7.83 (m, 5H), 7.59-7.36 (m, 6H), 7.21-6.99 (m, 4H), 5.41/4.70/4.56/3.96/3.72 (5 x s, 2 x 2H), 3.94*/3.90 (2 x s, 3H), 2.33/2.28* (2 x s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.9, 172.0, 166.7, 166.5, 165.7, 142.0, 140.9, 135.2, 134.65, 134.35, 134.2, 133.7, 133.6, 133.1, 132.9, 130.4, 130.3, 130.0, 130.0, 129.85, 129.8, 129.6, 129.6, 128.8, 128.7, 128.6, 127.8, 127.5, 126.9, 126.8, 125.3, 125.1, 124.9, 124.6, 124.3, 124.0, 120.2, 120.1, 77.2, 53.9, 52.3, 51.95, 49.7, 49.1, 21.03, 20.98; Anal. calcd. for $C_{29}H_{26}N_2O_4$: C 74.66, H 5.62, N 6.00, found: C 74.48, H 5.64, N 5.87.

Methyl 4-((4-(dimethylamino)-*N*-(2-oxo-2-(*p*-tolylamino)ethyl)benzamido)methyl)benzoate (3l)



Colorless solid; yield: 395 mg (0.86 mmol, 43%); mp: 174°C; t_R: 14.84 min, purity: 98.6%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.91 (s, 1H), 8.16-7.97 (m, 2H), 7.54-7.42 (m, 2H), 7.40-7.30 (m, 4H), 7.19-7.03 (m, 2H), 6.81-6.50 (m, 2H), 4.86 (s, 2H), 4.16 (s, 2H), 3.93 (s, 3H), 2.98 (s, 6H), 2.31 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.9, 167.15, 166.8, 151.8, 141.8, 135.4, 134.0, 130.4, 129.8, 129.6, 129.5, 127.1, 121.1, 120.0, 111.7, 54.0, 52.3, 52.2, 40.5, 21.0; Anal. calcd. for $C_{27}H_{29}N_3O_4$: C 70.57, H 6.36, N 9.14, found: C 70.82, H 6.58, N 9.00.

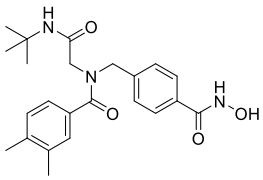
General procedure for the synthesis of 2a-l



3a-l **2a-l**

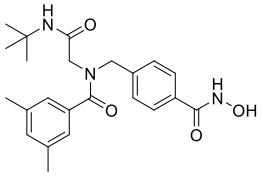
Hydroxylamine hydrochloride (348 mg, 5.0 mmol, 10 eq) was added to a sodium methanolate solution freshly prepared from dry methanol (8 mL) and sodium (175 mg, 7.5 mmol, 7.5 eq). The mixture was stirred for 10 min before the respective ester **3a-l** (0.5 mmol, 1.0 eq) was added. The reaction mixture was stirred at room temperature for 16 h. The solvent was removed under reduced pressure, water (15 mL) was added, and the pH was adjusted to pH 7-8 was using 4 M HCl. The mixture was extracted with ethyl acetate (3 x 20 mL), the combined organic layers were dried over anhydrous sodium sulfate, filtered, and concentrated in vacuum. The crude products were purified by flash column chromatography (prepacked silica cartridge, dichloromethane-dichloromethane/methanol (70:30), gradient: 90:10 → 70:30 in 20 min) to yield the desired hydroxamic acids **2a-l**.

***N*-(2-(*tert*-Butylamino)-2-oxoethyl)-N-(4-(hydroxycarbamoyl)benzyl)-3,4-dimethylbenzamide (2a)**



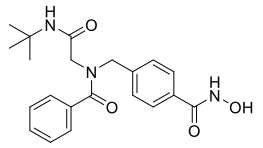
Colorless solid; yield: 116 mg (0.28 mmol, 56%); mp: 127°C; t_R: 12.92 min, purity: 98.2%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.20 (s, 1H), 9.03 (s, 1H), 7.85-7.66 (m, 2H), 7.52-7.08 (m, 6H), 4.64/4.51* (2 x s, 2H), 3.86*/3.65 (2 x s, 2H), 2.24*/2.22 (2 x s, 6H), 1.25*/1.21 (2 x s, 9H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.5, 171.4, 167.1, 166.6, 164.0, 163.8, 140.7, 138.0, 137.9, 136.2, 133.65, 133.4, 131.8, 131.6, 129.3, 127.7, 127.6, 127.25, 127.1, 126.8, 124.15, 123.8, 52.9, 51.3, 50.3, 50.2, 48.85, 47.2, 39.5, 28.6, 28.4, 19.3, 19.25; HRMS (ESI) Anal. Calcd. for $C_{23}H_{30}N_3O_4$ 412.2231 $[M+H]^+$, found 412.2232.

***N*-(2-(*tert*-Butylamino)-2-oxoethyl)-*N*-(4-(hydroxycarbamoyl)benzyl)-3,5-dimethylbenzamide (2b)**



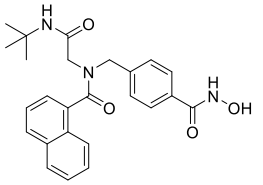
Colorless solid; yield: 142 mg (0.35 mmol, 69%); mp: 164°C; t_R: 13.08 min, purity: 98.0%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.20 (s, 1H), 9.02 (s, 1H), 7.80-7.70 (m, 2H), 7.48*/7.39 (2 x s, 1H), 7.40-7.32/7.30-7.21 (2 x m, 2H), 7.11-7.04 (m, 1H), 7.05-6.94 (m, 2H), 4.64/4.48* (2 x s, 2H), 3.86*/3.62 (2 x s, 2H), 2.27/2.25* (2 x s, 6H), 1.25*/1.21 (2 x s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.5, 167.1, 166.55, 164.0, 140.6, 140.3, 137.65, 137.5, 136.15, 136.0, 131.8, 131.7, 130.8, 127.7, 127.1, 126.9, 124.1, 52.9, 51.2, 50.2, 48.8, 47.1, 28.6, 28.4, 20.8; HRMS (ESI) Anal. calcd. for $C_{23}H_{30}N_3O_4$ 412.2231 $[M+H]^+$, found 412.2229.

***N*-(2-(*tert*-Butylamino)-2-oxoethyl)-*N*-(4-(hydroxycarbamoyl)benzyl)benzamide (2c)**



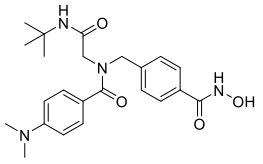
Colorless solid; yield: 117 mg (0.31 mmol, 61%); mp: 116°C; t_R: 11.35 min, purity: 96.1%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.19 (s, 1H), 9.02 (s, 1H), 7.89-7.67 (m, 2H), 7.62-7.15 (m, 8H), 4.64/4.49* (2x s, 2H), 3.88*/3.65 (2 x s, 2H), 1.25*/1.19 (2 x s, 9H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.4, 166.85, 163.8, 140.5, 136.2, 131.6, 129.4, 128.2, 127.55, 127.0, 126.8, 126.5, 52.8, 51.3, 50.2, 48.8, 47.25, 28.5, 28.3; HRMS (ESI) Anal. Calcd. for $C_{21}H_{26}N_3O_4$ 384.1918 $[M+H]^+$, found 384.1916.

***N*-(2-(*tert*-Butylamino)-2-oxoethyl)-*N*-(4-(hydroxycarbamoyl)benzyl)-1-naphthamide (2d)**



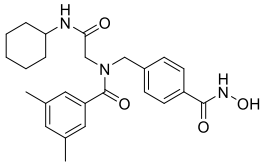
Colorless solid; yield: 92 mg (0.21 mmol, 42%); mp: 149°C; t_R: 13.03 min, purity: 98.2%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.20 (s, 1H), 9.04/9.02* (2 x s, 1H), 8.43-7.75 (m, 4H), 7.72-7.43 (m, 6H), 7.28-7.16 (m, 2H), 5.12/4.68-4.37/4.29/3.73-3.42 (2 x m, 2 x s, 2 x 2H), 1.31/1.10* (2 x s, 9H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.5, 170.4, 166.6, 166.5, 163.85, 163., 140.5, 139.55, 134.2, 133.8, 133.0, 132.8, 131.9, 131.8, 129.2, 129.0, 128.8, 128.8, 128.2, 128.1, 127.8, 127.1, 127.1, 127.0, 126.9, 126.8, 126.5, 126.3, 125.2, 125.2, 125.0, 124.6, 123.7, 123.5, 52.3, 50.8, 50.2, 50.1, 48.7, 46.8, 39.5, 28.5, 28.2; HRMS (ESI) Anal. Calcd. for $C_{25}H_{28}N_3O_4$ 434.2074 $[M+H]^+$, found 434.2070.

***N*-(2-(*tert*-Butylamino)-2-oxoethyl)-4-(dimethylamino)-*N*-(4-(hydroxycarbamoyl)benzyl)benzamide (2e)**



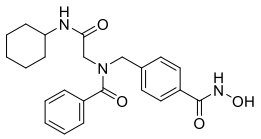
Colorless solid; yield: 120 mg (0.28 mmol, 56%); mp: 162°C; t_R: 8.84 min, purity: 96.6%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.19 (s, 1H), 9.02 (s, 1H), 7.92-7.62 (m, 2H), 7.47 (s, 1H), 7.42-7.14 (m, 4H), 6.85-6.53 (m, 2H), 4.61 (s, 2H), 3.77 (s, 2H), 2.93 (s, 6H), 1.24 (s, 9H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.6, 167.2, 163.9, 151.2, 140.8, 131.6, 128.5, 127.0, 122.2, 110.9, 50.2, 28.4; HRMS (ESI) Anal. Calcd. for $C_{23}H_{31}N_4O_4$ 427.2340 $[M+H]^+$, found 427.2341.

***N*-(2-(Cyclohexylamino)-2-oxoethyl)-*N*-(4-(hydroxycarbamoyl)benzyl)-3,5-dimethylbenzamide (2f)**



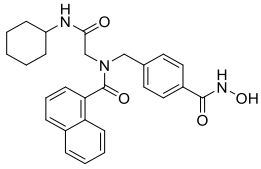
Colorless solid; yield: 127 mg (0.29 mmol, 58%); mp: 179°C; t_R: 13.77 min, purity: 96.0%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.20 (s, 1H), 9.02 (s, 1H), 7.82-7.65 (m, 3H), 7.46-7.34/7.30-7.21* (2 x m, 2H), 7.10-7.04 (m, 1H), 7.04-6.96 (m, 2H), 4.65/4.50* (2 x s, 2H), 3.88*/3.66 (2 x s, 2H), 3.61-3.43 (m, 1H), 2.27/2.24* (2 x s, 6H), 1.79-1.47 (m, 5H), 1.34-0.99 (m, 5H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.4, 166.6, 163.9, 140.45, 137.4, 136.0, 131.6, 130.6, 127.6, 127.0, 124.0, 50.9, 47.0, 48.65, 52.9, 47.55, 32.2, 25.05, 24.3, 20.65; HRMS (ESI) Anal. Calcd. for $C_{25}H_{32}N_3O_4$ 438.2387 $[M+H]^+$, found 438.2392.

***N*-(2-(Cyclohexylamino)-2-oxoethyl)-*N*-(4-(hydroxycarbamoyl)benzyl)benzamide (2g)**



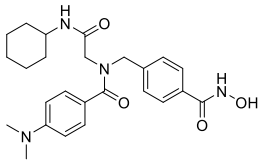
Colorless solid; yield: 87 mg (0.21 mmol, 42%); mp: 148°C; t_R: 12.15 min, purity: 97.8%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.18 (s, 1H), 8.99 (s, 1H), 7.85-7.62 (m, 3H), 7.56-7.13 (m, 7H), 4.66/4.52* (2 x s, 2H), 3.91*/3.69 (2 x s, 2H), 3.62-3.45 (m, 1H), 1.90-1.47 (m, 5H), 1.36-1.00 (m, 5H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.3, 166.4, 163.9, 140.4, 136.1, 131.65, 129.4, 128.2, 127.6, 127.0, 126.7, 126.5, 52.9, 51.0, 48.7, 47.55, 47.1, 32.1, 25.05, 24.3. HRMS (ESI) Anal. Calcd. for $C_{23}H_{28}N_3O_4$ 410.2074 $[M+H]^+$, found 410.2071.

***N*-(2-(Cyclohexylamino)-2-oxoethyl)-*N*-(4-(hydroxycarbamoyl)benzyl)-1-naphthamide (2h)**



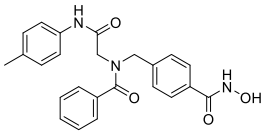
Colorless solid; yield: 98 mg (0.22 mmol, 43%); mp: 165°C; t_R: 13.75 min, purity: 96.5%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.22 (s, 1H), 9.06*/9.03 (2 x s, 1H), 8.54-7.85 (m, 3H), 7.86-7.36 (m, 8H), 7.39-7.06 (m, 1H), 5.24-4.99*/3.80-3.39 (2 x m, 2H + 1H), 4.55*/4.30 (2 x s, 2H), 1.89-1.43 (m, 5H), 1.41-0.84 (m, 5H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.5, 170.4, 166.25, 163.9, 163.7, 140.5, 139.6, 134.2, 133.7, 133.0, 132.8, 131.9, 131.8, 129.2, 129.0, 128.9, 128.8, 128.2, 128.1, 127.9, 127.2, 127.1, 127.0, 126.9, 126.8, 126.5, 126.3, 125.3, 125.2, 125.0, 124.6, 123.8, 123.4, 52.35, 50.5, 48.6, 47.7, 47.4, 46.5, 32.4, 32.0, 25.1, 25.0, 24.4, 24.2; HRMS (ESI) Anal. Calcd. for $C_{27}H_{30}N_3O_4$ 460.2231 $[M+H]^+$, found 460.2230.

***N*-(2-(Cyclohexylamino)-2-oxoethyl)-4-(dimethylamino)-*N*-(4-(hydroxycarbamoyl)benzyl)benzamide (2i)**



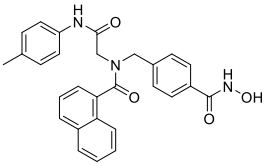
Colorless solid; yield: 153 mg (0.34 mmol, 68%); mp: 151°C; t_R: 9.77 min, purity: 96.9%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.19 (s, 1H), 9.02 (s, 1H), 7.88-7.65 (m, 3H), 7.50-7.19 (m, 4H), 6.77-6.59 (m, 2H), 4.62 (s, 2H), 3.79 (s, 2H), 3.64-3.48 (m, 1H), 2.93 (s, 6H), 1.82-1.49 (m, 5H), 1.35-1.04 (m, 5H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.6, 166.9, 164.0, 151.2, 140.8, 131.6, 128.6, 127.6, 127.2, 122.1, 111.0, 47.6, 39.8, 39.7, 32.3, 25.2, 24.5; HRMS (ESI) Anal. Calcd. for $C_{25}H_{33}N_4O_4$ 453.2496 $[M+H]^+$, found 453.2499.

***N*-(4-(Hydroxycarbamoyl)benzyl)-*N*-(2-oxo-2-(*p*-tolylamino)ethyl)benzamide (2j)**



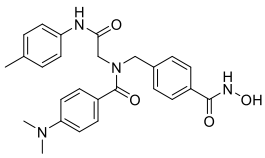
Colorless solid; yield: 134 mg (0.32 mmol, 64%); mp: 158°C; t_R: 13.03 min, purity: 97.3%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.21 (s, 1H), 9.94*/9.80 (2 x s, 1H), 9.03 (s, 1H), 7.86-7.67 (m, 2H), 7.61-7.27 (m, 9H), 7.20-7.01 (m, 2H), 4.72/4.60* (2 x s, 2H), 4.12*/3.95 (2 x s, 2H), 2.24 (s, 3H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.7, 171.5, 166.3, 166.1, 163.9, 136.4, 136.0, 135.95, 135.7, 132.55, 132.2, 131.9, 131.7, 129.8, 129.6, 129.2, 128.6, 128.5, 127.7, 127.3, 127.1, 126.8, 126.6, 126.5, 119.3, 119.05, 53.2, 51.7, 48.7, 48.2, 20.45; HRMS (ESI) Anal. Calcd. for $C_{24}H_{24}N_3O_4$ 418.1761 $[M+H]^+$, found 418.1760.

***N*-(4-(Hydroxycarbamoyl)benzyl)-*N*-(2-oxo-2-(*p*-tolylamino)ethyl)-1-naphthamide (2k)**



Colorless solid; yield: 129 mg (0.28 mmol, 55%); mp: 196°C; t_R: 14.57 min, purity: 99.2%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.21 (s, 1H), 10.05/9.58* (2 x s, 1H) 9.06*/9.02 (2 x s, 1H), 8.47-7.77 (m, 4H), 7.79-7.44 (m, 7H), 7.39-6.96 (m, 4H), 5.17*/4.40 (2 x s, 2H), 4.84-4.51/4.06-3.63 (2 x m, 2H), 2.28/2.23* (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.6, 166.1, 163.7, 140.5, 139.7, 136.3, 135.7, 133.95, 133.6, 133.0, 132.8, 132.4, 132.15, 131.9, 129.2, 129.1, 129.0, 128.9, 128.3, 128.2, 127.9, 127.2, 127.1, 127.0, 126.9, 126.5, 126.4, 125.1, 125.1, 124.5, 123.7, 123.5, 119.2, 119.1, 52.6, 51.1, 48.6, 47.5, 20.35; HRMS (ESI) Anal. Calcd. for $C_{28}H_{26}N_3O_4$ 468.1918 $[M+H]^+$, found 468.1917.

4-(Dimethylamino)-*N*-(4-(hydroxycarbamoyl)benzyl)-*N*-(2-oxo-2-(*p*-tolylamino)ethyl)benzamide (2l)



Pale yellow solid; yield: 125 mg (0.27 mmol, 54%); mp: 167°C; t_R: 10.60 min, purity: 96.6%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.21 (s, 1H), 9.88 (s, 1H), 9.03 (s, 1H), 7.89-7.67 (m, 2H), 7.56-7.25 (m, 6H), 7.22-7.02 (m, 2H), 6.80-6.59 (m, 2H), 4.70 (s, 2H), 4.05 (s, 2H), 2.92 (s, 6H), 2.25 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.8, 166.7, 164.0, 151.3, 140.9, 131.7, 129.1, 128.6, 127.2, 119.1, 111.0, 53.6, 48.9, 39.7, 20.4; HRMS (ESI) Anal. Calcd. for $C_{26}H_{29}N_4O_4$ 461.2183 $[M+H]^+$, found 461.2188.

3. X-ray crystallography

Diffraction quality crystals were obtained by vapor diffusion. 1.0 mg of purified **2f** was added to 0.5 mL of DMSO in a small vial. This vail was then placed within a 20 mL vial containing 3 mL of water. The sample was capped and then left undisturbed for eight weeks to obtain crystals of X-ray quality.

General: All bond lengths are in the expected ranges. Packing: N-H···O and O-H···O hydrogen bonds connect adjacent molecules to form a layered network in the *ab* plane.

Data collection and reduction generally followed the routine procedure[2,3] with an increase of the exposure time. Crystal data of $C_{25}H_{31}N_3O_4$: space group *Pbca*, $Z = 8$, $a = 9.3020\ (4)$ Å, $b = 15.8206\ (5)$ Å, $c = 32.7189\ (11)$ Å, $V = 4815.0\ (3)$ Å^3, $\mu = 0.08$ mm^{-1}, crystal size: 0.22 × 0.20 × <0.01 mm, 55144 measured reflections, 4237 unique reflections, 297 parameters, *wR2*(all reflections) = 0.167, *R1* ($I > 2\sigma I$) = 0.115, $\Delta\rho_{max/min}$: 0.22/-0.21 eÅ^{-3}.

CCDC 1434499 contains the supplementary crystallographic data for this paper. The data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/getstructures.

4. Biological evaluation

Reagents

Cisplatin was purchased from Sigma-Aldrich (Germany), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Serva (Germany). All other reagents were supplied by PAN Biotech (Germany) unless otherwise stated.

Cell lines and cell culture

The human epithelial ovarian cancer cell line A2780 was obtained from the European Collection of Cell Cultures (ECACC, UK). The human tongue cell line Cal27 was obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Germany). The cisplatin resistant CisR sublines were generated by exposing the parental cell lines to weekly cycles of cisplatin in an IC_{50} concentration over a period of 24-30 weeks as described in Gosepath *et al.*[4] and Eckstein *et al.*[5]

All cell lines were grown at 37°C in a humidified atmosphere containing 5% CO_2 in RPMI 1640 (A2780) or DMEM (Cal27) containing 10% fetal calf serum, 120 IU/mL penicillin, and 120 µg/mL streptomycin. The cells were grown to 80% confluency before using them for the appropriate assays.

MTT cell viability assay

Cell viability was evaluated by an improved MTT assay as previously described.[6] In brief, A2780 or Cal27 and their cisplatin resistant sublines were seeded at a density of 5,000 and 2,000 cells/well in 96well plates (Corning, Germany). After 24 h, cells were exposed to test compounds. Incubation was ended after 72 h by addition of MTT solution (5 mg/mL in phosphate buffered saline). The formazan precipitate was dissolved in DMSO (VWR, Germany). Absorbance was measured at 544 nm and 690 nm in a FLUOstar microplate-reader (BMG LabTech, Germany).

Combination experiments

To investigation the effect of **2e**, **2f**, **2i**, and **2l** on cisplatin-induced cytotoxicity, compounds were added 48 h prior to the addition of cisplatin. After 72 h, cell viability was determined by MTT assay.

Whole-cell HDAC inhibition assay

The cellular HDAC assay was based on an assay published by Ciossek *et al.*[7] and Bonfils *et al.*[8] with minor modifications. Briefly, A2780, A2780 CisR, Cal27, and Cal27 CisR were seeded in 96-well tissue culture plates (Corning, Germany) at a density of 1.5 x 10^4 cells/well in a total volume of 90 µL medium. After 24 h, cells were incubated for 18 h with increasing concentrations of test compounds. HDAC assay was started by adding 10 µL of 3 mM Boc-Lys(ε-Ac)-AMC (Bachem, Germany) and incubated for 3 h under cell culture conditions. Then, 100 µl/well stop solution (25 mM Tris-HCl (pH 8), 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, 1% NP40, 2.0 mg/mL trypsin, 10 µM vorinostat) were added, and incubated for 3 h under cell culture conditions. Fluorescence intensity was then measured at an excitation wavelength of 320 nm and emission wavelength of 520 nm in a NOVOstar microplate-reader (BMG LabTech, Offenburg, Germany).

Immunoblotting

Cells were treated with 1 μM of test compounds (**2e**, **2f**, **2i**, **2l**, tubastatin A, entinostat) or vehicle for 24 h. Cell pellets were dissolved with lysis buffer 6 (bio-techne, Germany) and centrifuged at 14.000 g. Protein amount was estimated by bicinchoninic acid assay according to the manufacturer's instructions (Pierce, Thermo Fisher). Equal amounts of total protein (10 μg) were resolved by SDS-PAGE and transferred to polyvinylidene fluoride membranes. Blots were incubated with primary antibodies against acetylated α-tubulin and α-tubulin (Santa Cruz Biotechnology, Germany). Immunoreactive proteins were visualized using luminol reagent (Santa Cruz, Heidelberg, Germany) with an Intas Imager (Intas, Germany).

Data Analysis

Concentration-effect curves were constructed with Prism 4.0 (GraphPad, San Diego, CA) by fitting the pooled data of at least three experiments performed in triplicates to the four parameter logistic equation.

HDAC IC_{50} Profiling

The in vitro inhibitory activity of compounds **2e**, **2f**, **2i**, and **2l** against four human HDAC isoforms (2, 4, 6 and 11) were performed at Reaction Biology Corp. (Malvern, PA) with a fluorescent based assay according to the company's standard operating procedure. The IC_{50} values were determined using 10 different concentrations with 3-fold serial dilution starting at 10 μM. TSA and TMP269 were used as reference compounds.

5. Computational details

Homology modeling

The CD II amino acid sequence of HDAC6 (Gly482-Gly800)[9] was used to search crystal structures of homologues in the Protein Data Bank (PDB) using BLAST.[10] The results showed that HDAC4 is the closest homologue with 48% sequence identity. For the model with Y301 (numbering based on the homology model) in a flipped-out conformation, the protein structure of PDB ID 4CBY[11] was used as a template as it has the best resolution (2.7 Å) among HDAC4 flipped-out crystal structures. For the flipped-in model, the protein structure of PDB ID 2VQW, a H976Y gain of function mutated HDAC4cd crystal structure with Y976 in a flipped-in conformation and a resolution of 3.0 Å,[12] was used as a template for similar reasons described above. The alignment of the sequences of HDAC6 and HDAC4 was calculated using the Align2D routine from the software Modeller9.14.[13] With the alignment, comparative modeling was performed using the standard automodel class from the same software package. In detail, 20 models with a high level of refinement were created and subsequently evaluated depending on their DOPE scores. The respective models with the lowest score were further refined by MD simulations as described next.

Molecular dynamics simulations and clustering

For molecular dynamics simulations of HDAC6 in the Y301 flipped-in and –out conformations, the termini were capped with ACE and NME groups for the N- and C-terminus, respectively. The system was hydrated with TIP3P[14] water in an octahedral box such that the distance between the protein and the box edges is at least 12 Å. Ions were added to neutralize the charge of the system. MD simulations were performed with Amber14[15] using the ff14SB force field and 12-6-4 Lennard-Jones type potentials for divalent ions.[16] The Particle Mesh Ewald[17] method was used to treat long-range electrostatic interactions. Bond lengths involving bonds to hydrogen atoms were constrained using SHAKE.[18] The time step for all MD simulations was 2 fs with a direct-space, non-bonded cutoff of 8 Å. Initially, 12500 steps of steepest descent and conjugate gradient minimization were performed. Harmonic restraints with a force constant of 5 kcal mol^{-1} $Å^{-2}$ were applied to all solute atoms; the force constant was reduced to 1 kcal mol^{-1} $Å^{-2}$ after 2500 steps. Then, NVT-MD (MD simulations with a constant number of particles, volume, and temperature) was carried out for 50 ps during which the system was heated from 100 K to 300 K. Subsequent NPT-MD (MD simulations with a constant number of particles, pressure, and temperature) was used for 100 ps to adjust the solvent density. In both steps, harmonic restraints with a force constant of 1 kcal mol^{-1} $Å^{-2}$ were applied to all solute atoms. A final unrestrained NVT-MD was performed for 200 ps. In the following 1000 ns of unrestrained NVT-MD, conformations were extracted every 40 ps for analysis. Afterwards, the trajectories of the respective HDAC6 in flipped-in and -out conformation were clustered with the protein as a reference. A hierarchical agglomerative clustering algorithm was applied as implemented in cpptraj,[19] with a minimum distance of 1 Å between the clusters in combination with a symmetry-corrected root mean-square deviation (RMSD) between the structures as a distance measure. The cluster means of the biggest cluster for the respective HDAC6 flipped-in and -out structures were used in subsequent docking studies.

Molecular docking

For the molecular docking, the *cis*- and *trans*-rotamers of **2f** and **2i** were drawn with ChemDraw Ultra,[20] converted into a 3D structure, and energy minimized with Moloc using the MAB force field.[21] The HDACi were then docked into HDAC2 (PDB ID: 4LY1[22]) and HDAC4 (PDB ID: 4CBT[11]), and into HDAC6 models with Y301 flipped-in and -out conformations using AutoDock3[23,24] as a docking engine and the DrugScore[25,26] distance-dependent pair-potentials as an objective function as described in ref.[27] Because of the flexibly connected saturated and unsaturated carbon cycles, a clustering RMSD cutoff of 2.0 Å was chosen; for all other docking parameters default values were used. Docking solutions with more than 20% of all configurations in the largest cluster were considered sufficiently converged, and the configuration with the lowest docking

energy of that cluster, binding to the zinc ion in the binding pocket with a distance < 3 Å to the hydroxamic acid oxygen, was used for further evaluation.

Supplemental Table

Table S2. Docking results of HDACi's into HDAC isoforms.

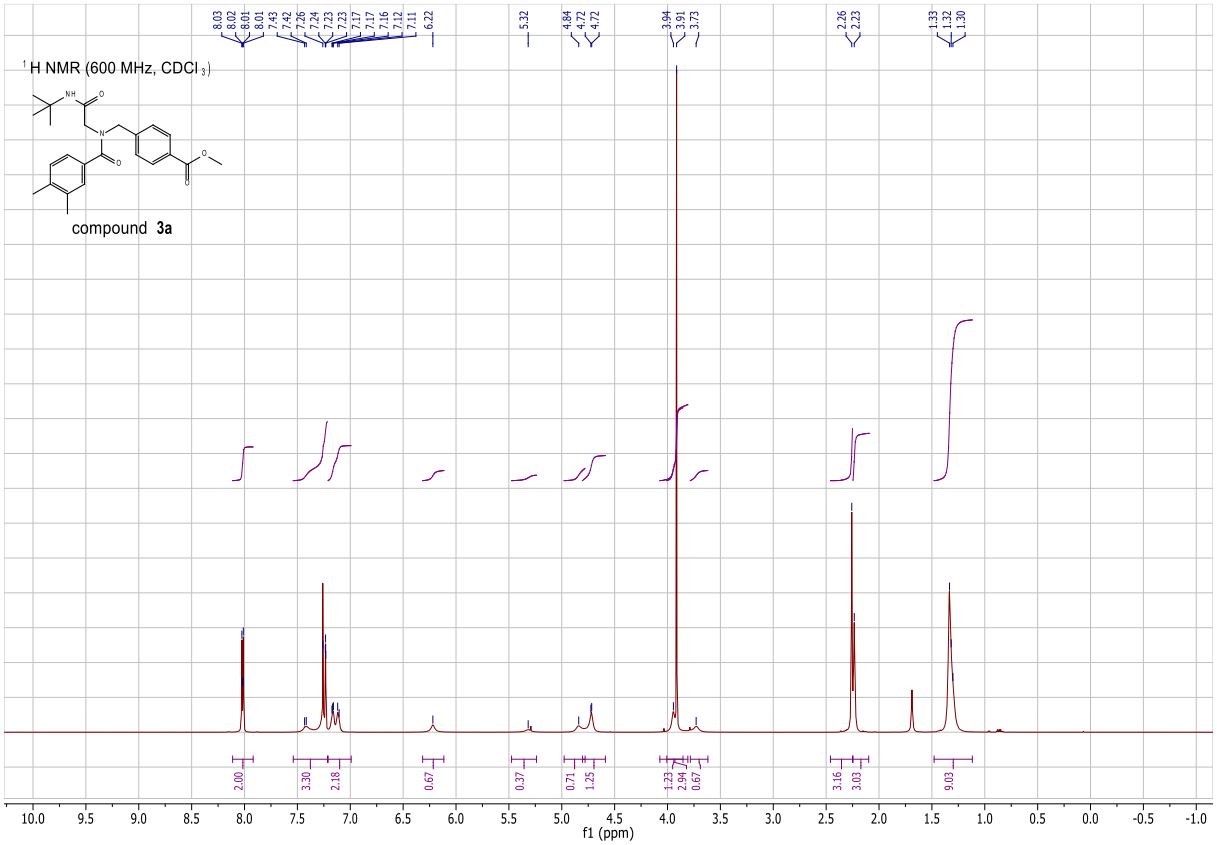
Compound	HDAC2[a]	HDAC4[a]	HDAC6 flipped-out[a]	HDAC6 flipped-in[a]
2f *cis*	-13.61	n/a[b]	-13.91	-15.42
2f *trans*	-13.97	n/a[b]	-13.86	-15.26
2i *cis*	-13.77	n/a[b]	-14.46	-15.58
2i *trans*	-15.5	n/a[b]	-15.53	-16.27

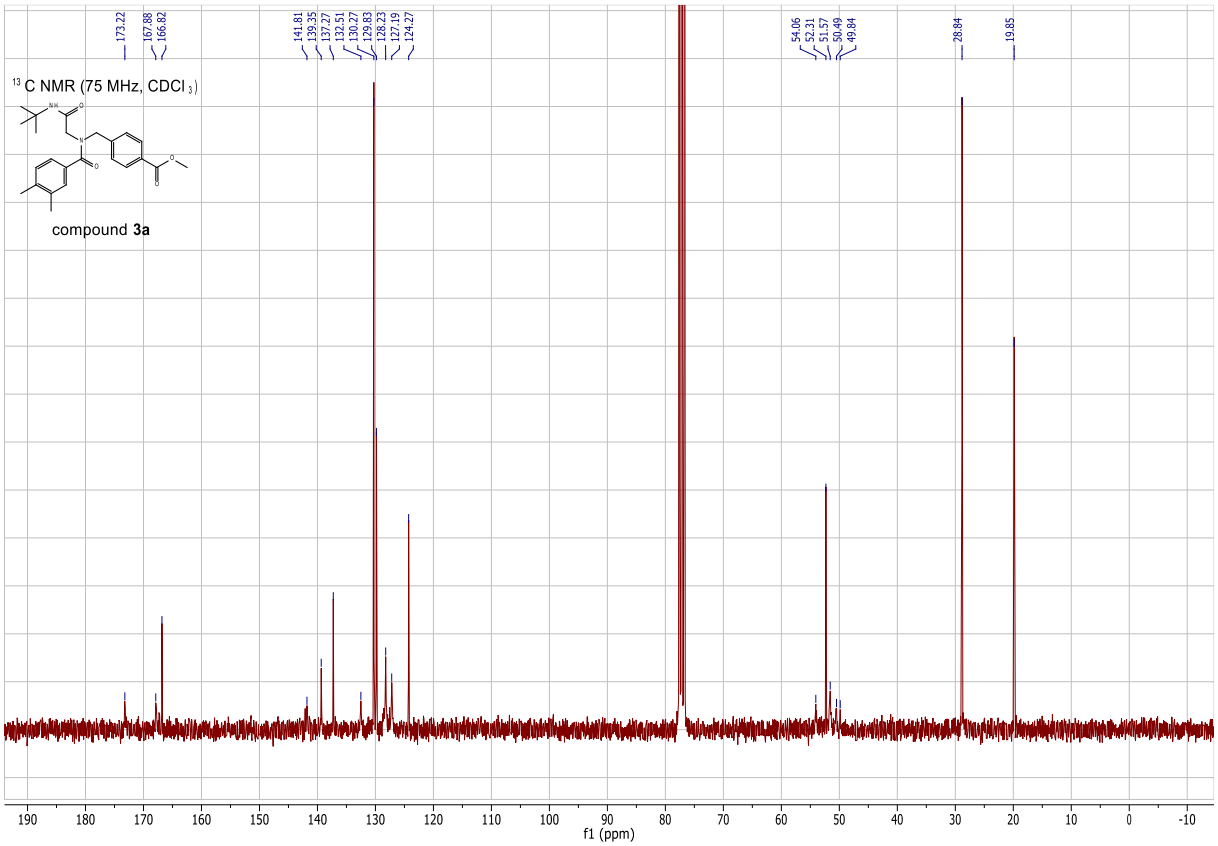
[a] Docking energy of the energetically most favorable configuration in the largest cluster; in kcal mol^{-1}.

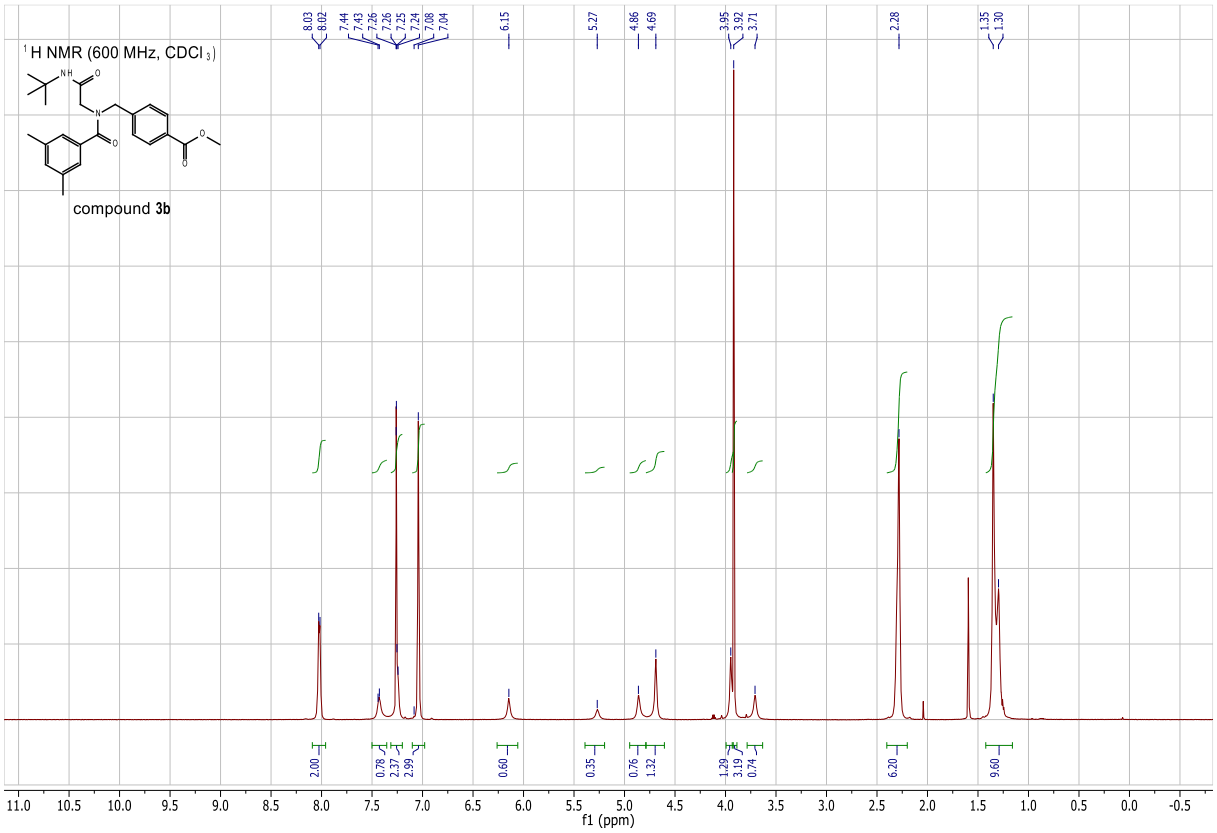
[b] No docking configuration fulfilling the criteria given in the section above could be identified.

6. Spectral data

Copies of ^{1}H-NMR and ^{13}C-NMR spectra of compounds 3a-l and 2a-l



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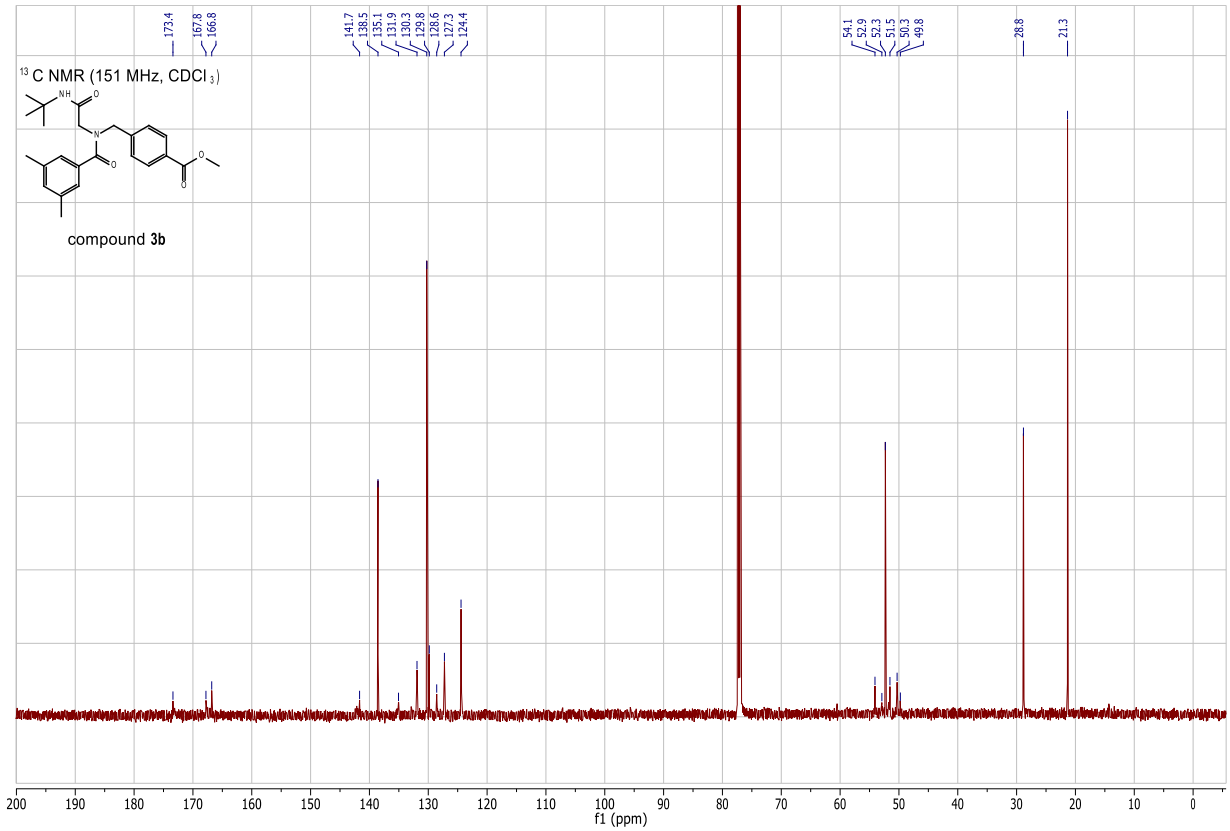
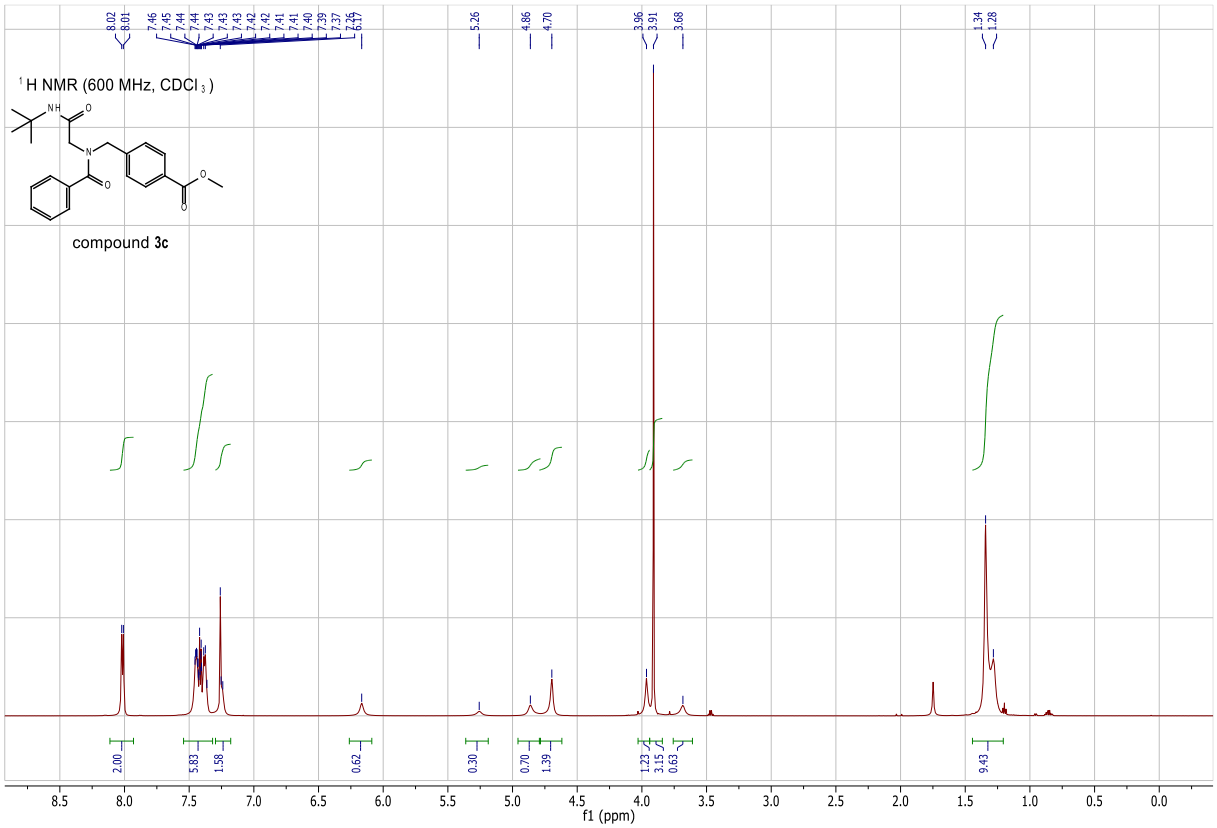
compound 3b
8.03
8.02
7.44
7.43
7.26
7.26
7.25
7.24
7.08
7.04
6.15
5.27
4.86
4.69
3.95
3.92
3.71
2.28
1.35
1.30
2.00
0.78
2.37
2.99
0.60
0.35
0.76
1.32
1.20
3.19
0.74
6.20
9.60
f1 (ppm)


13 C NMR (151 MHz, CDCl 3)
compound 3b
173.4
167.8
166.8
141.7
138.5
135.1
131.9
130.3
129.8
128.6
127.3
124.4
54.1
52.9
52.3
51.5
50.3
49.8
28.8
21.3
f1 (ppm)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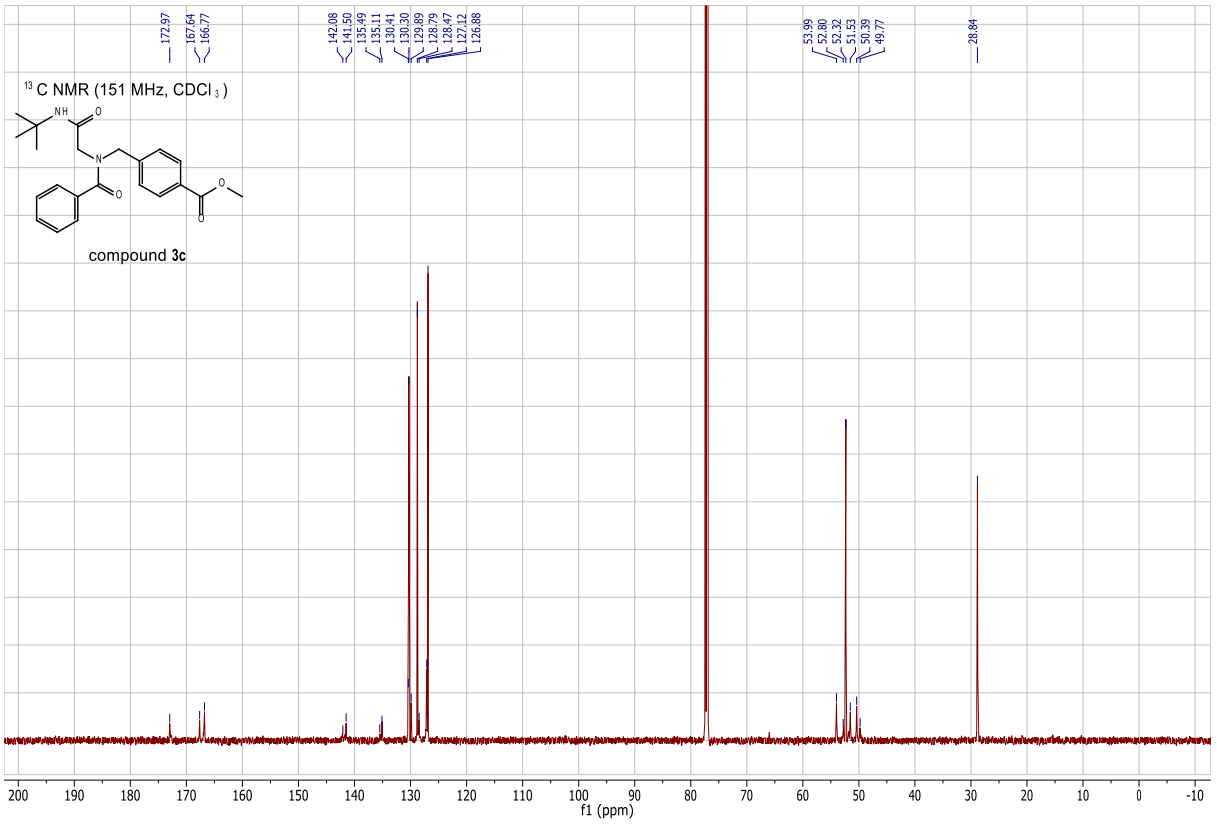
8.01
7.46
7.45
7.44
7.44
7.43
7.43
7.43
7.42
7.42
7.41
7.41
7.40
7.39
7.37
7.36
7.35
6.17
5.26
4.86
4.70
3.96
3.91
3.68
1.34
1.28
^{1}H NMR (600 MHz, CDCl$_3$)
compound 3c
2.00
5.83
1.58
0.62
0.30
0.70
1.39
1.23
3.15
0.63
9.43
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)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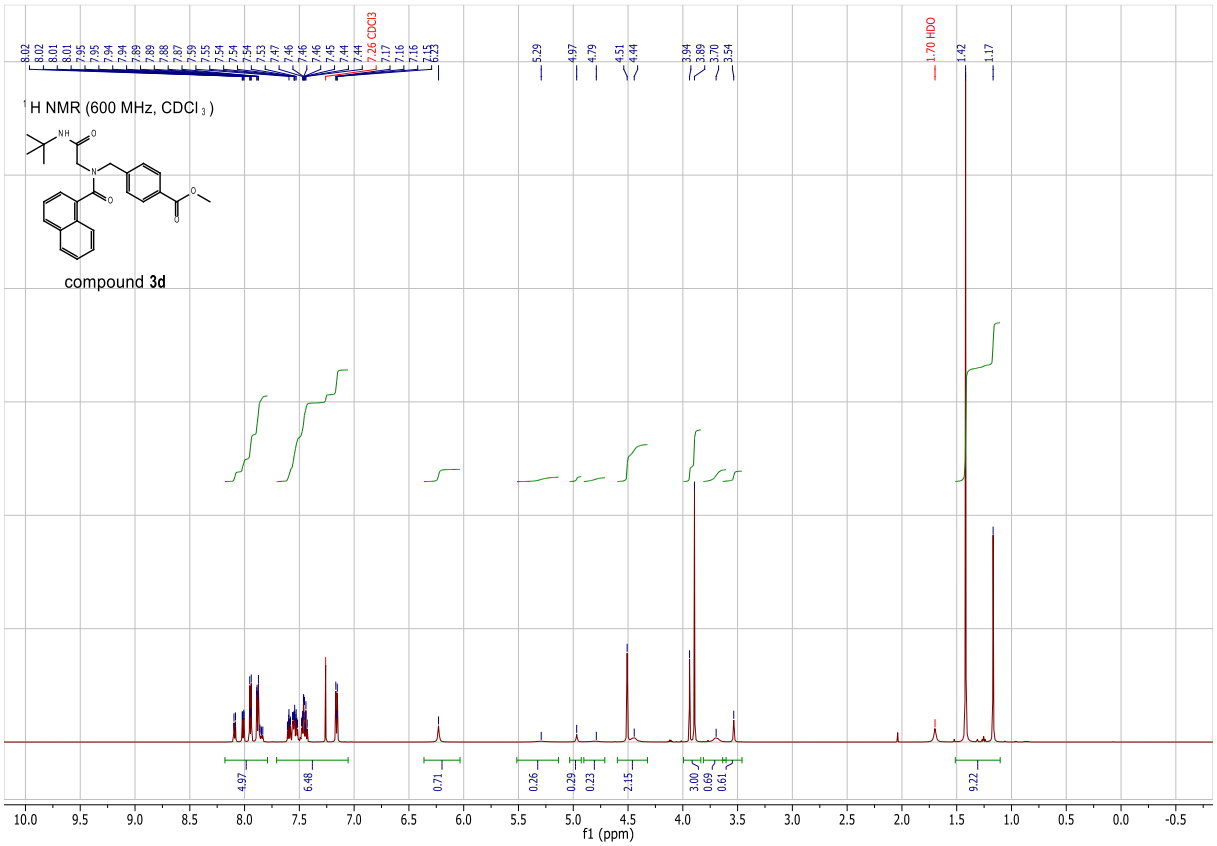
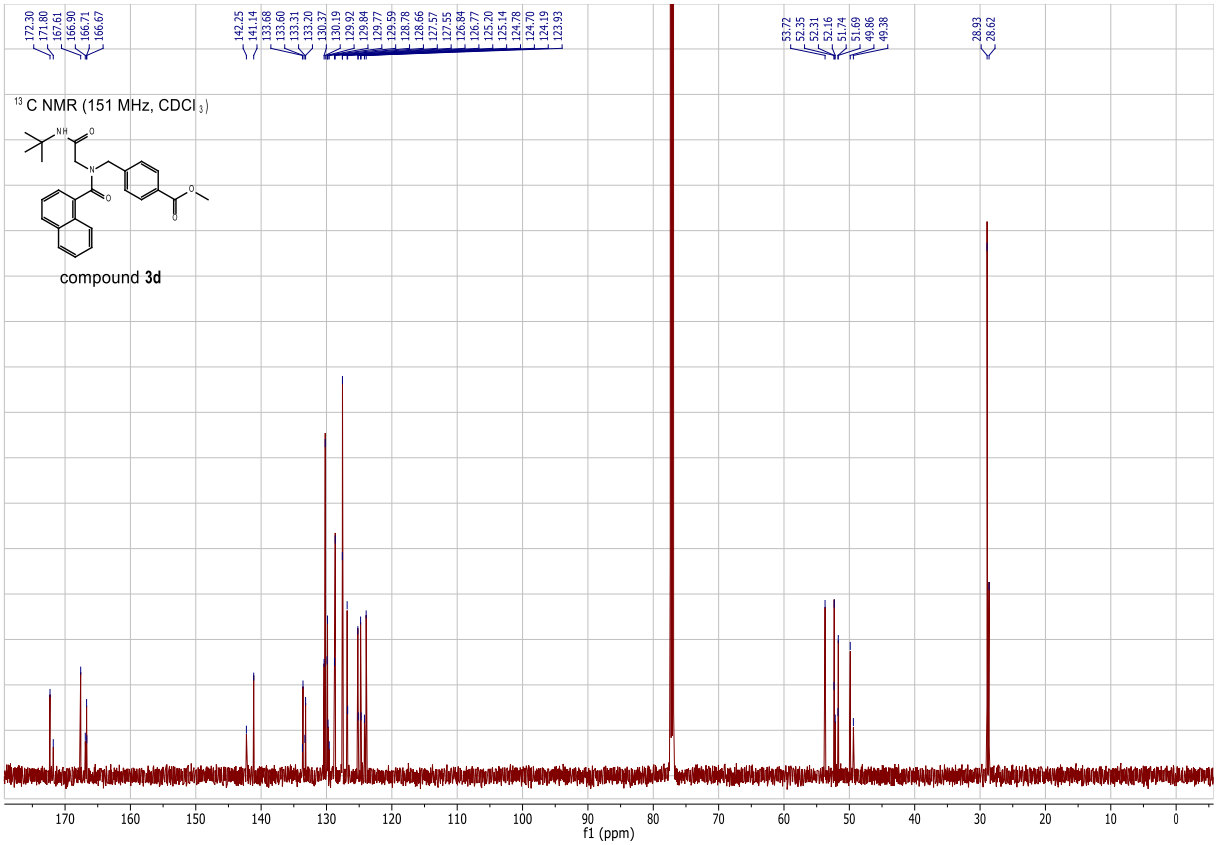
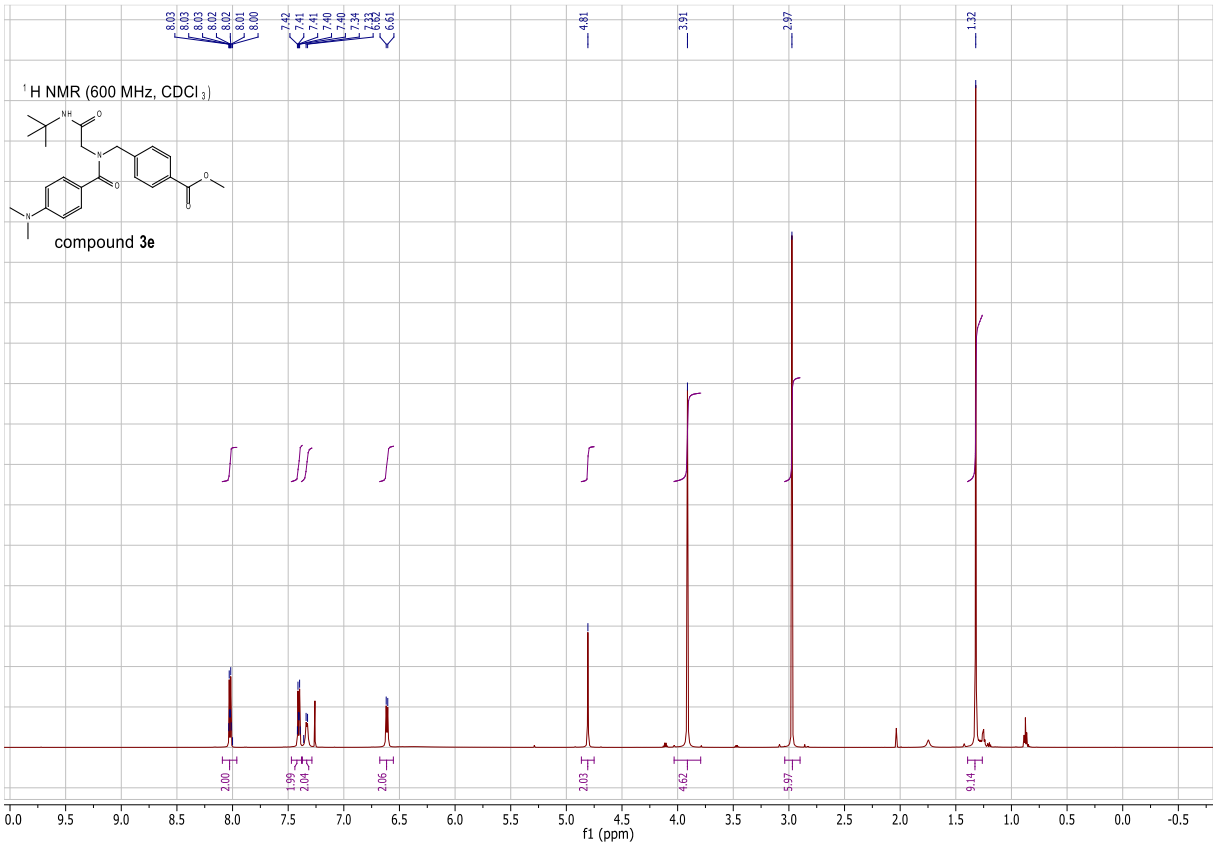
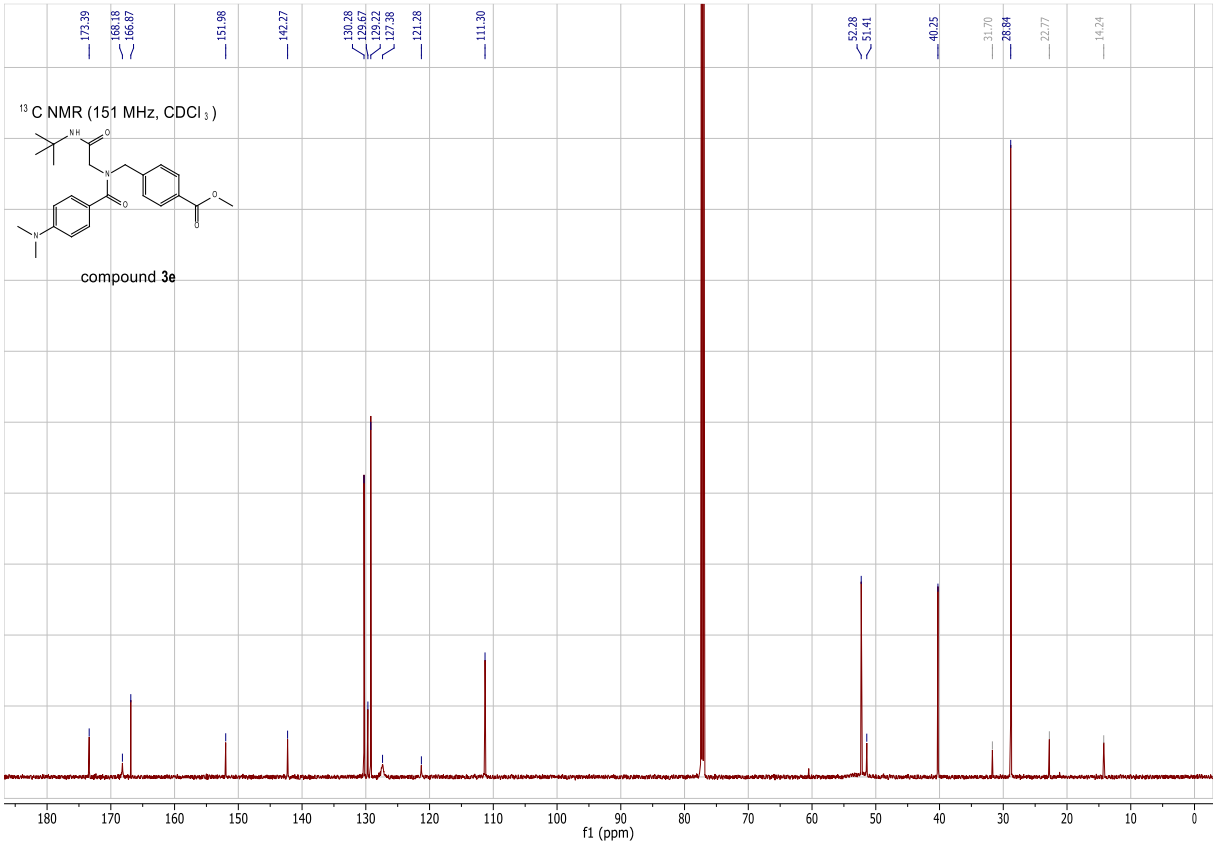
167.64
166.77
142.08
141.50
135.49
135.11
130.41
130.30
129.89
128.79
128.47
127.12
126.88
53.99
52.80
52.32
51.55
50.39
49.77
28.84
^{13}C NMR (151 MHz, CDCl$_3$)
compound 3c
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)


1H NMR (600 MHz, CDCl3)
compound 3d
f1 (ppm)


13C NMR (151 MHz, CDCl3)
compound 3d
f1 (ppm)


8.03
8.03
8.03
8.02
8.02
8.01
8.00
7.42
7.41
7.41
7.40
7.40
7.34
7.33
6.62
6.61
4.81
3.91
2.97
1.32
^{1}H NMR (600 MHz, CDCl$_3$)
compound 3e
2.00
1.99
2.04
2.06
2.03
4.62
5.97
9.14
f1 (ppm)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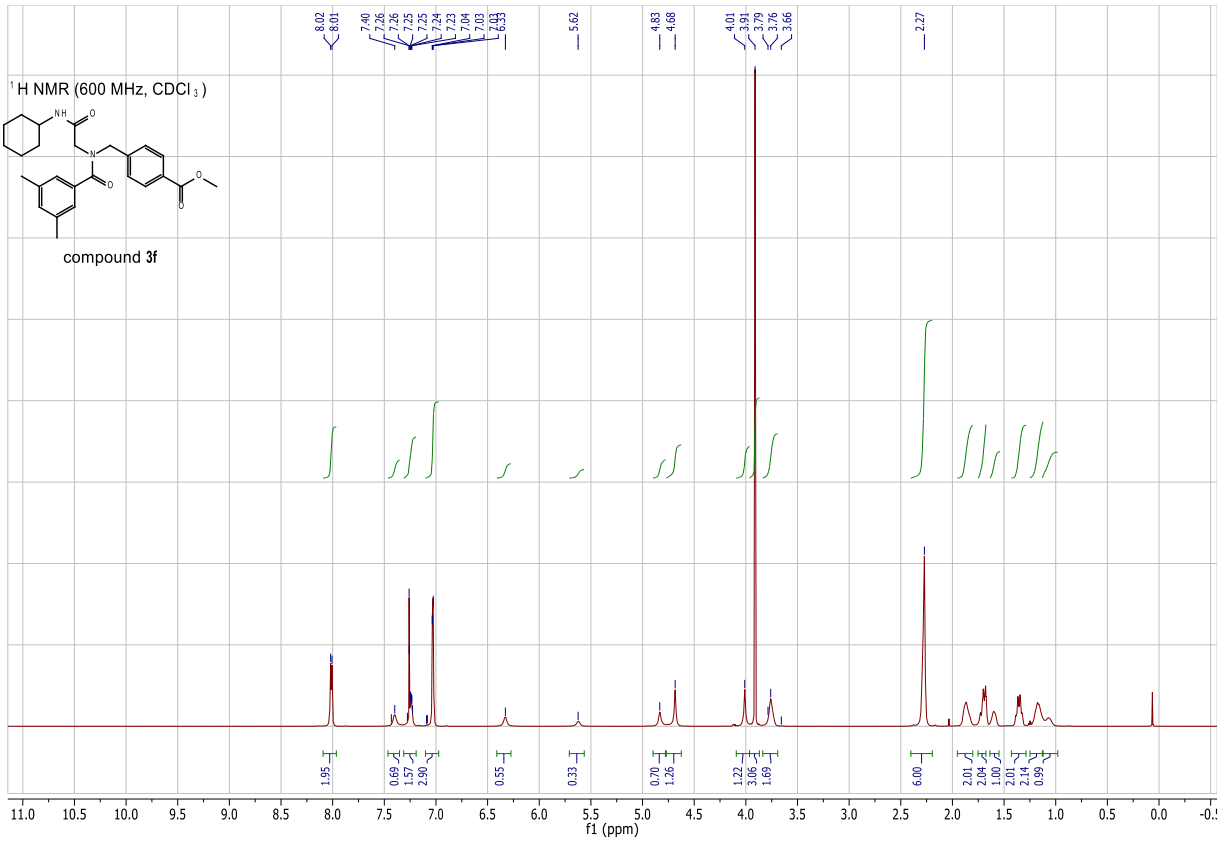
142.27
130.28
129.67
129.22
127.38
121.28
111.30
52.28
51.41
40.25
31.70
28.84
22.77
14.24
^{13}C NMR (151 MHz, CDCl$_3$)
compound 3e
f1 (ppm)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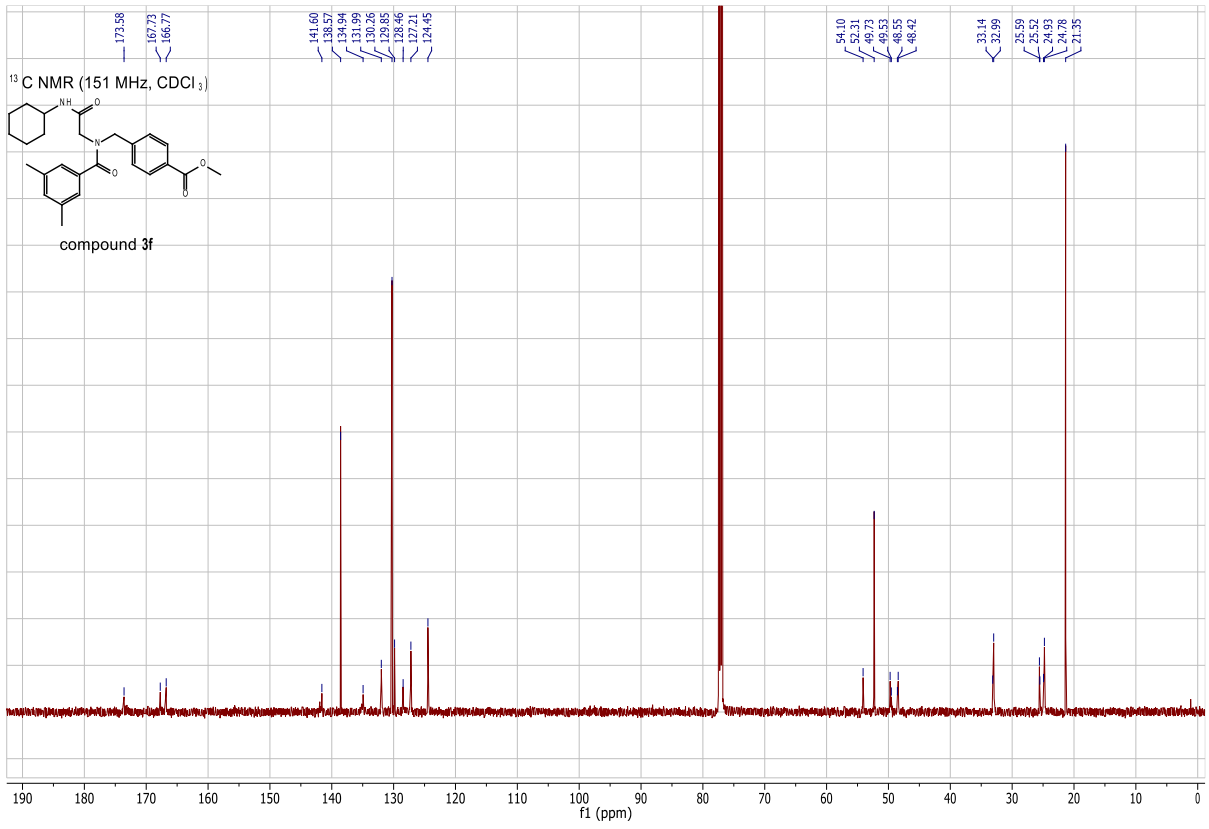
compound 3f
f1 (ppm)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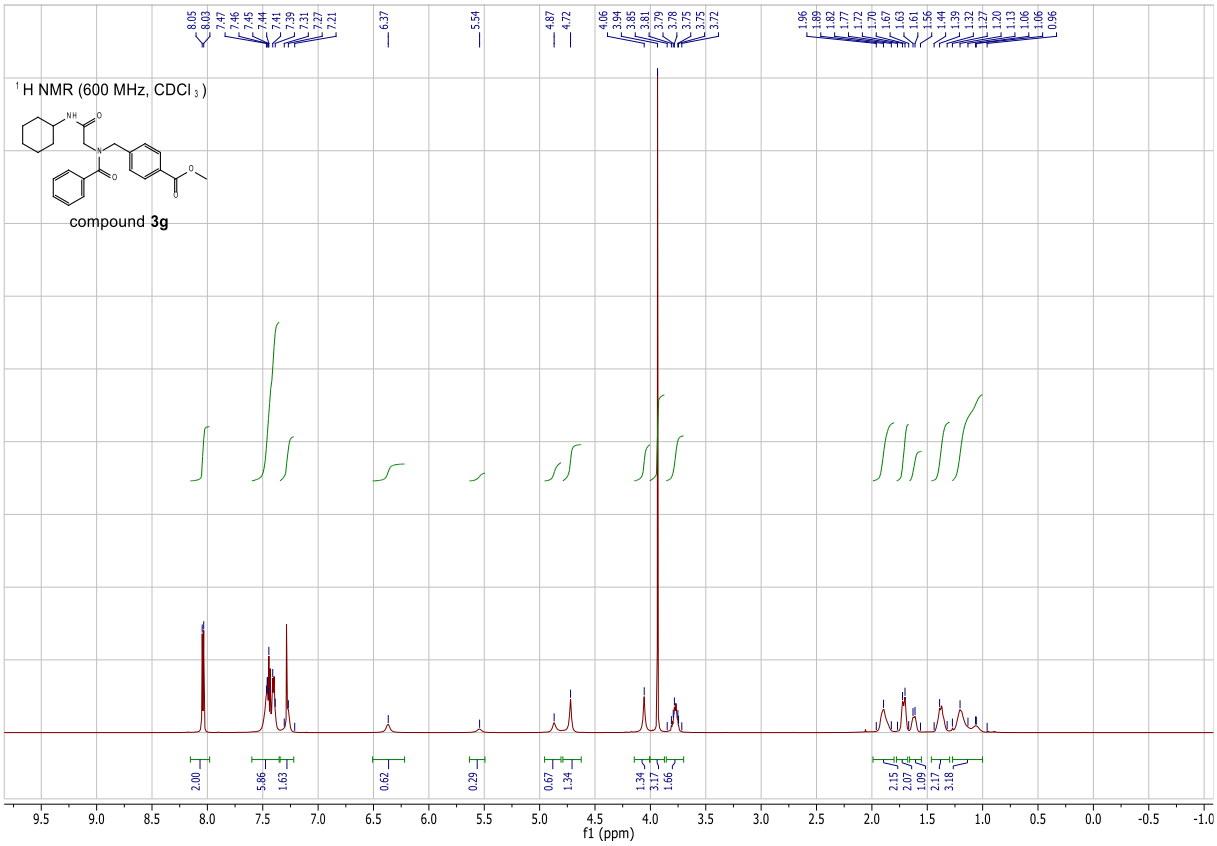
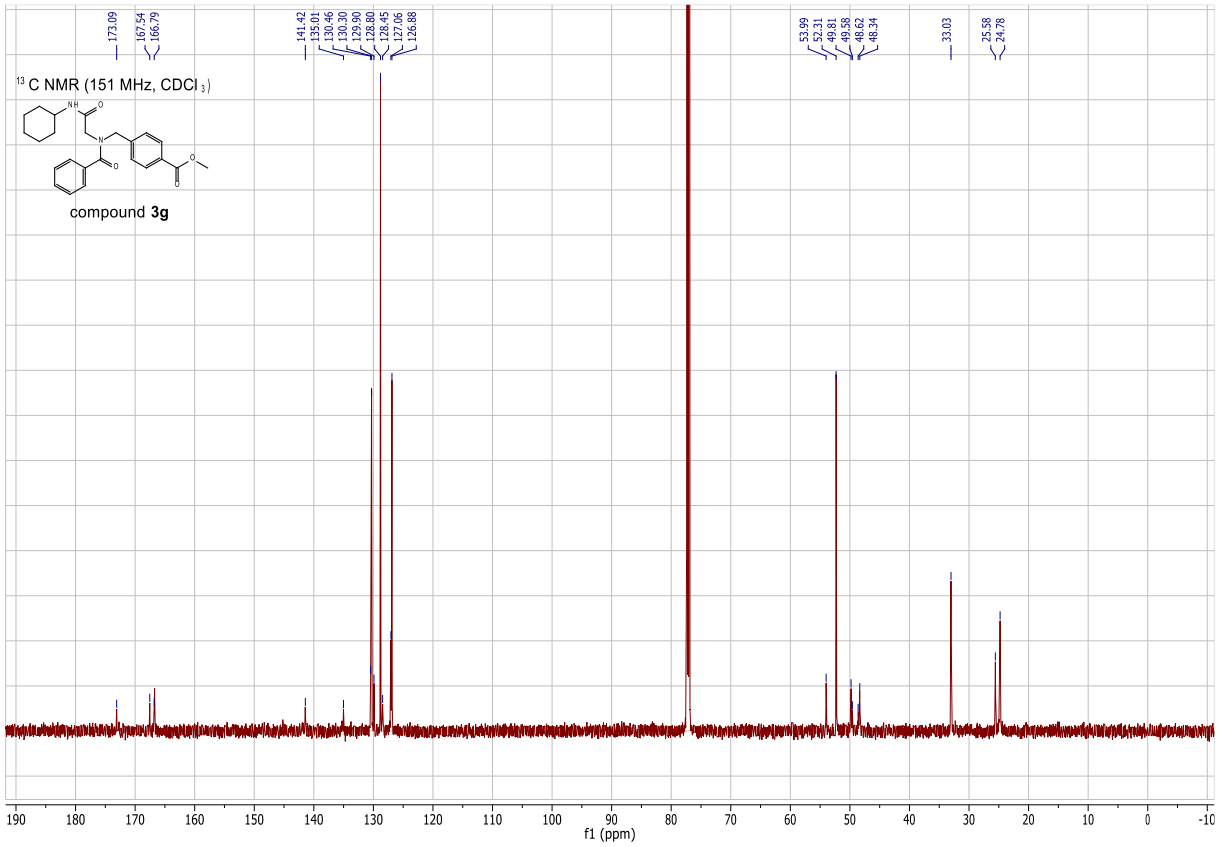
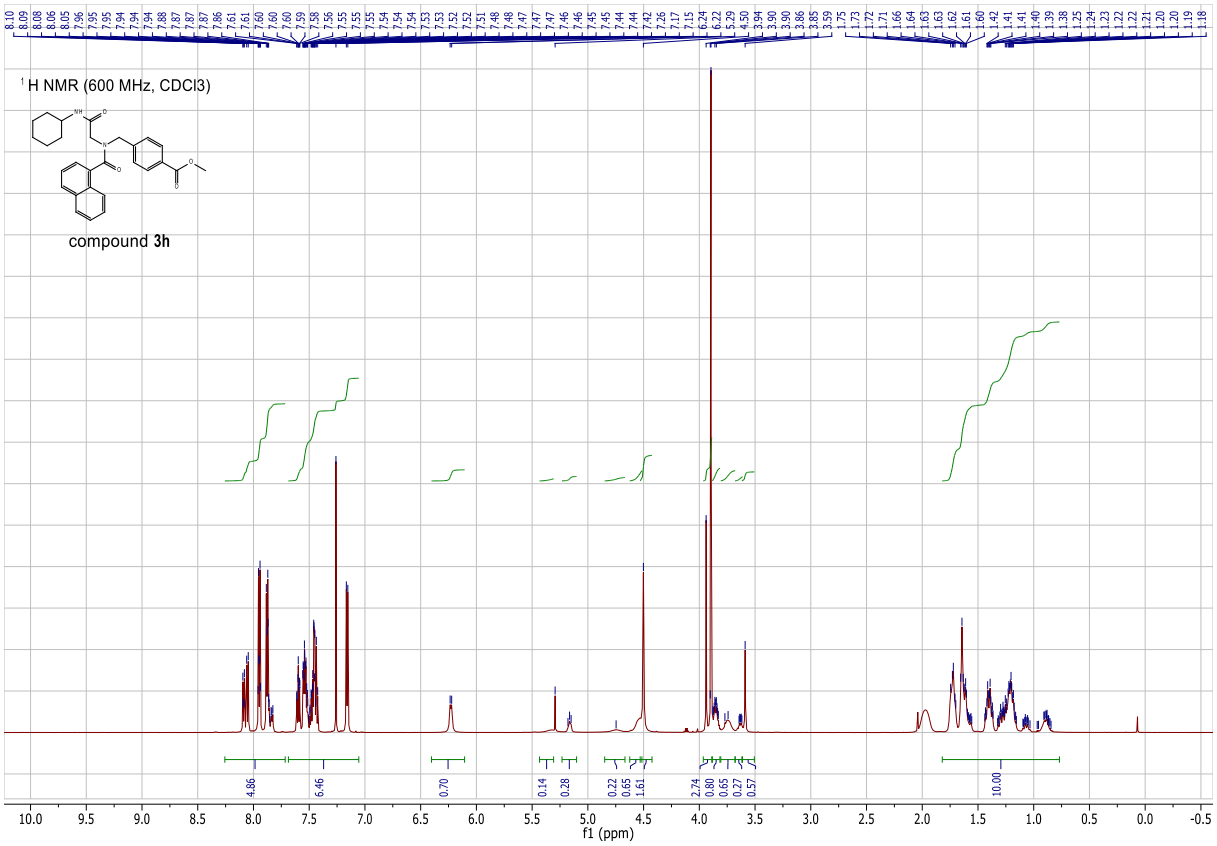
compound 3f
f1 (ppm)


1 H NMR (600 MHz, CDCl 3)
compound 3g
f1 (ppm)


13 C NMR (151 MHz, CDCl 3)
compound 3g
f1 (ppm)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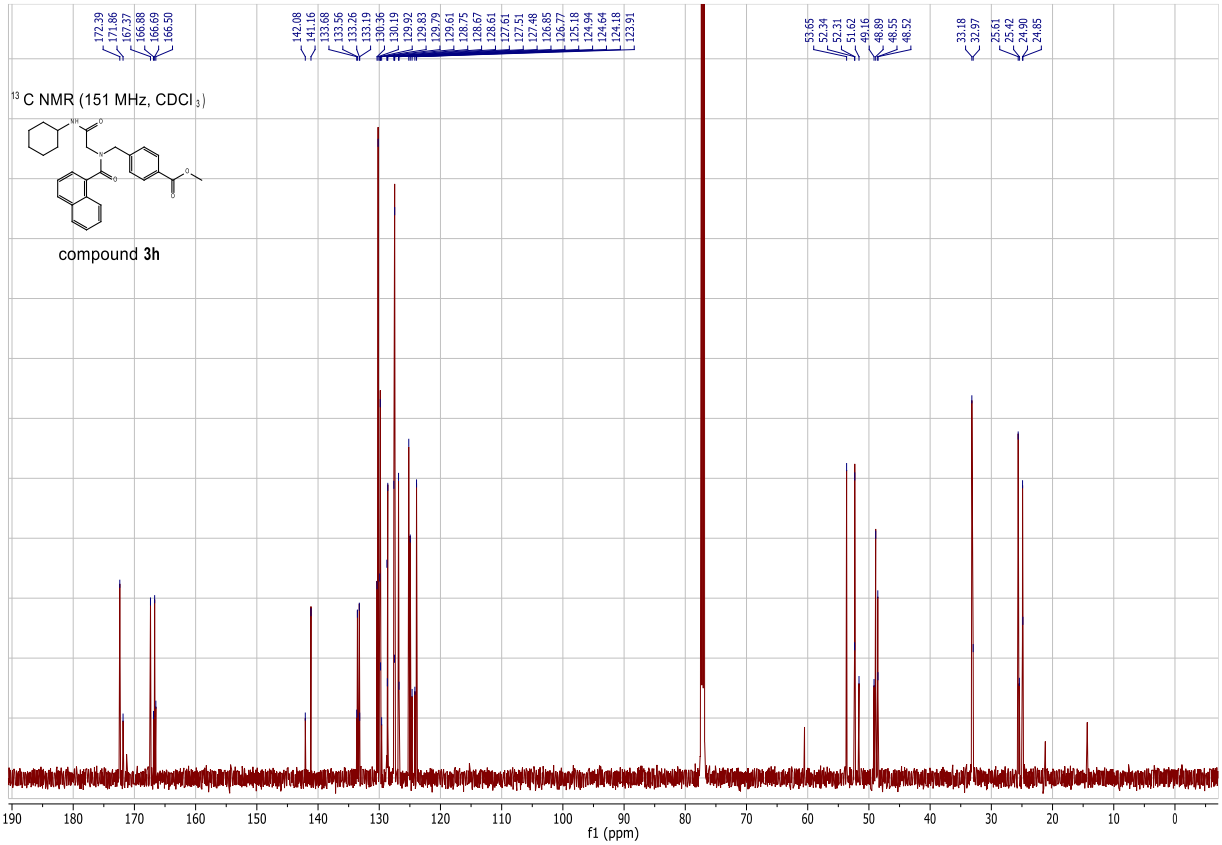
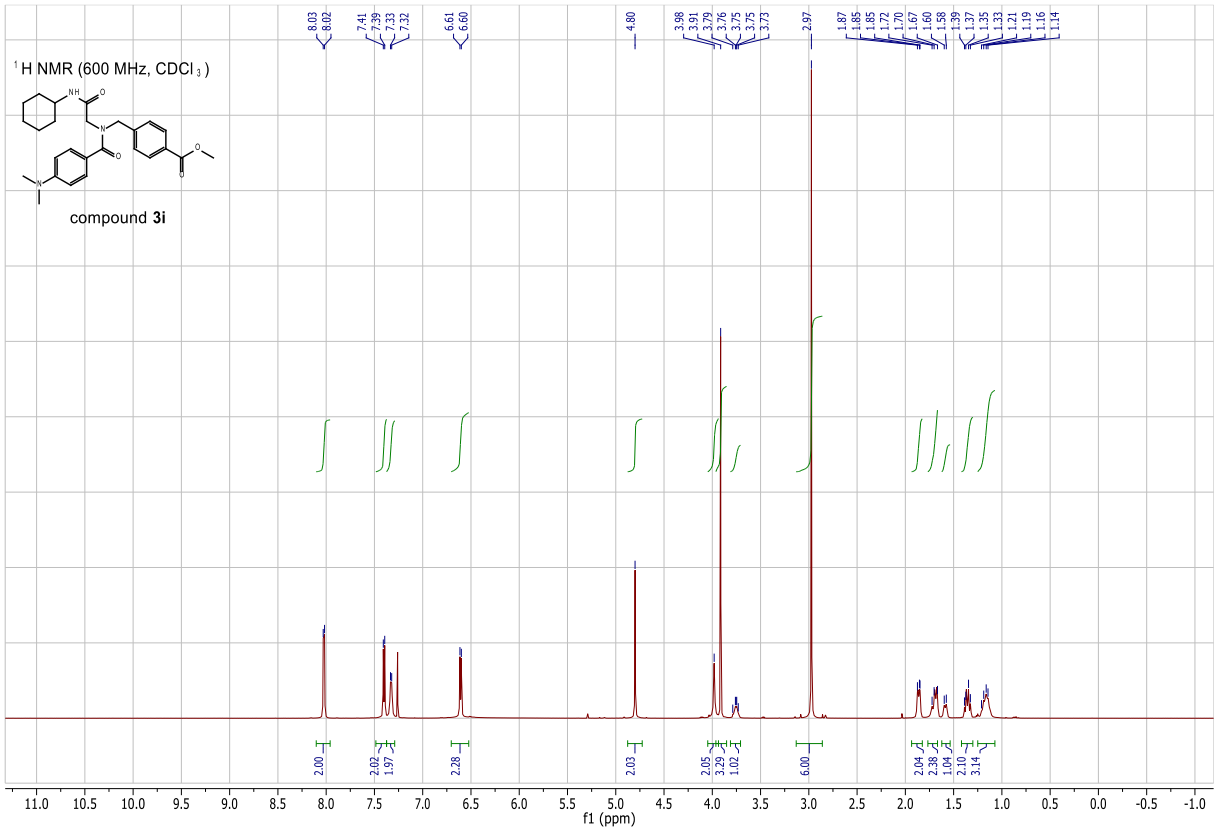
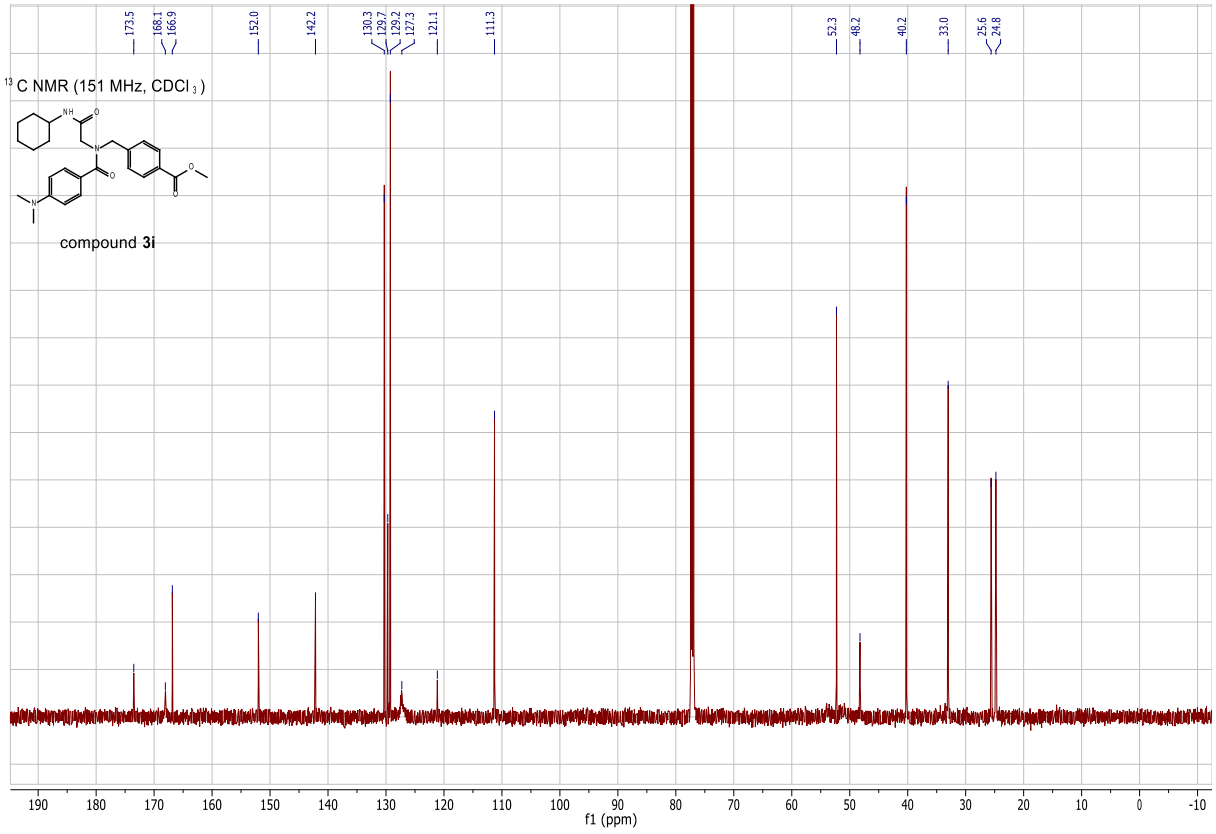
compound 3h
f1 (ppm)


13 C NMR (151 MHz, CDCl3)
compound 3h
f1 (ppm)


1 H NMR (600 MHz, CDCl 3)
compound 3i
f1 (ppm)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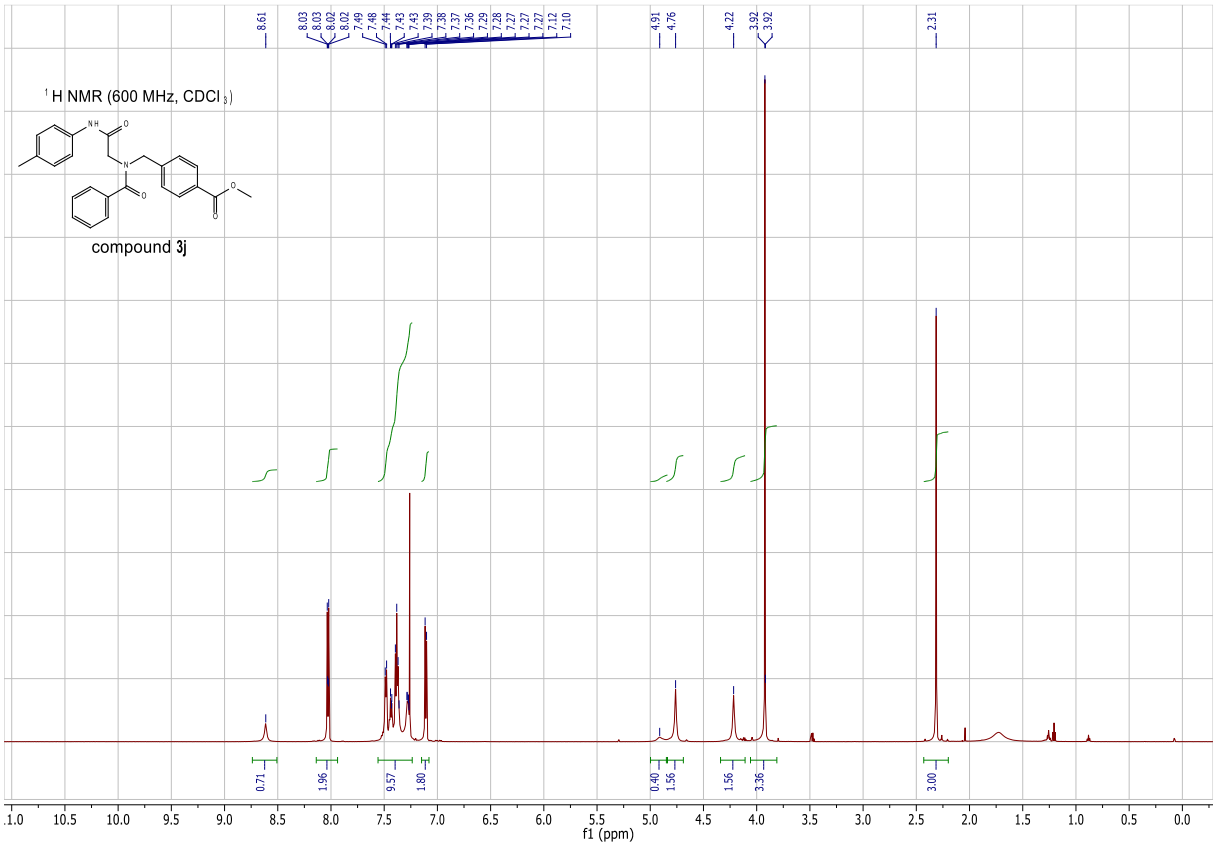
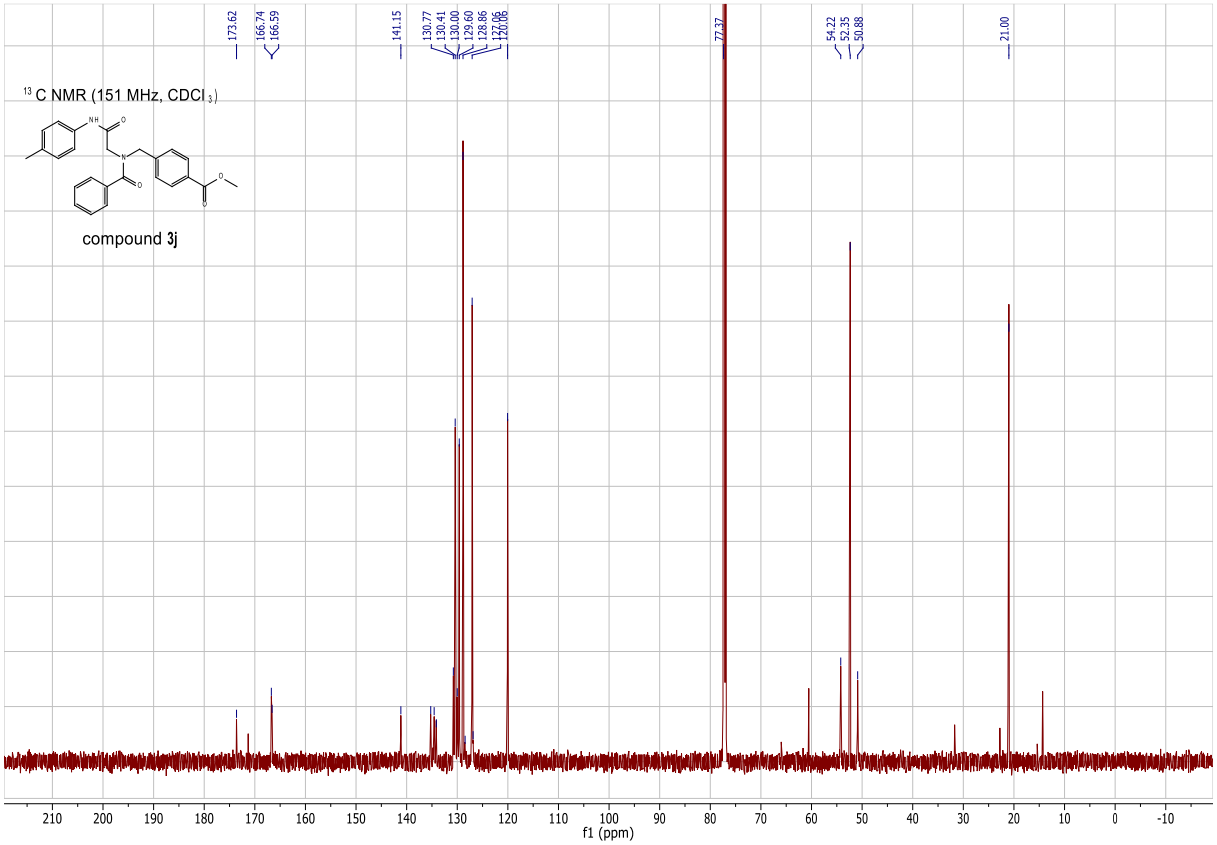
compound 3i
f1 (ppm)



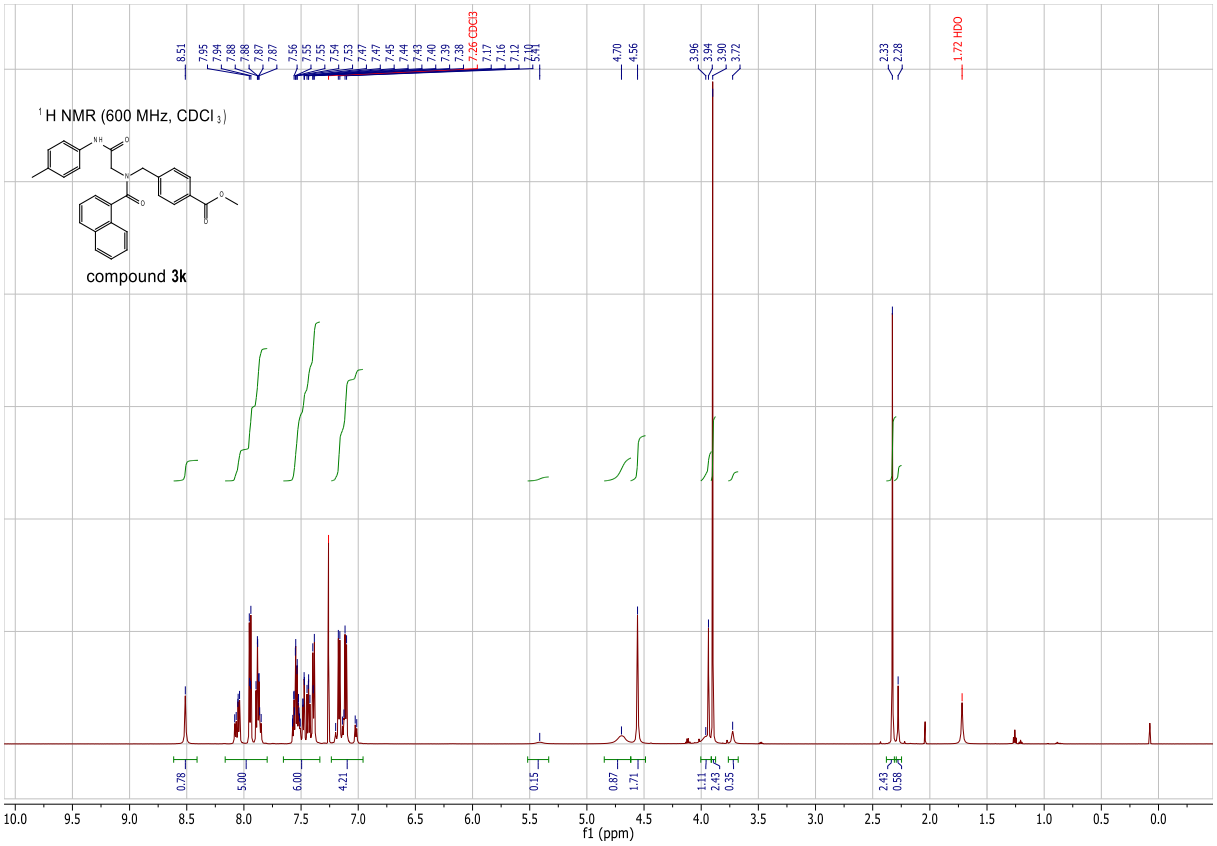
^{1}H NMR (600 MHz, $CDCl_3$)

compound **3j**



^{13}C NMR (151 MHz, $CDCl_3$)

compound **3j**


8.51
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7.94
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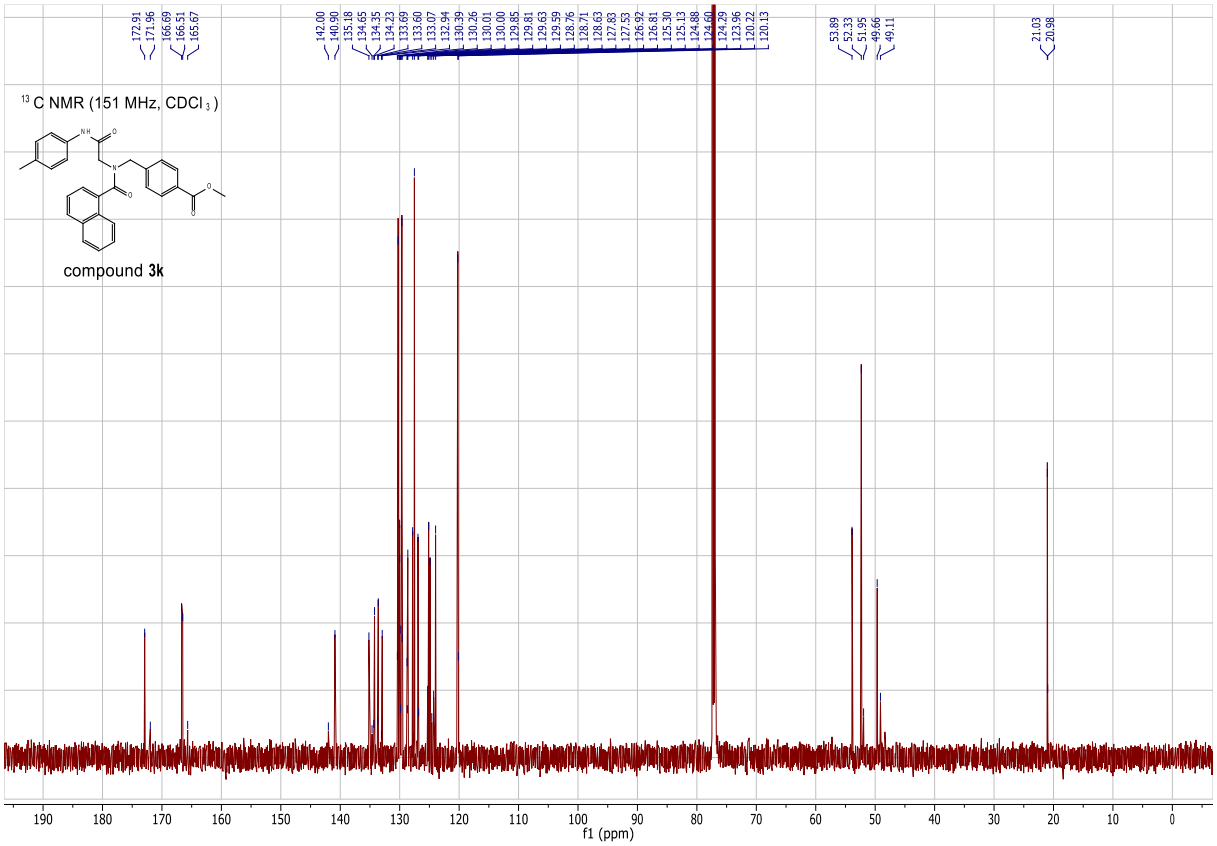
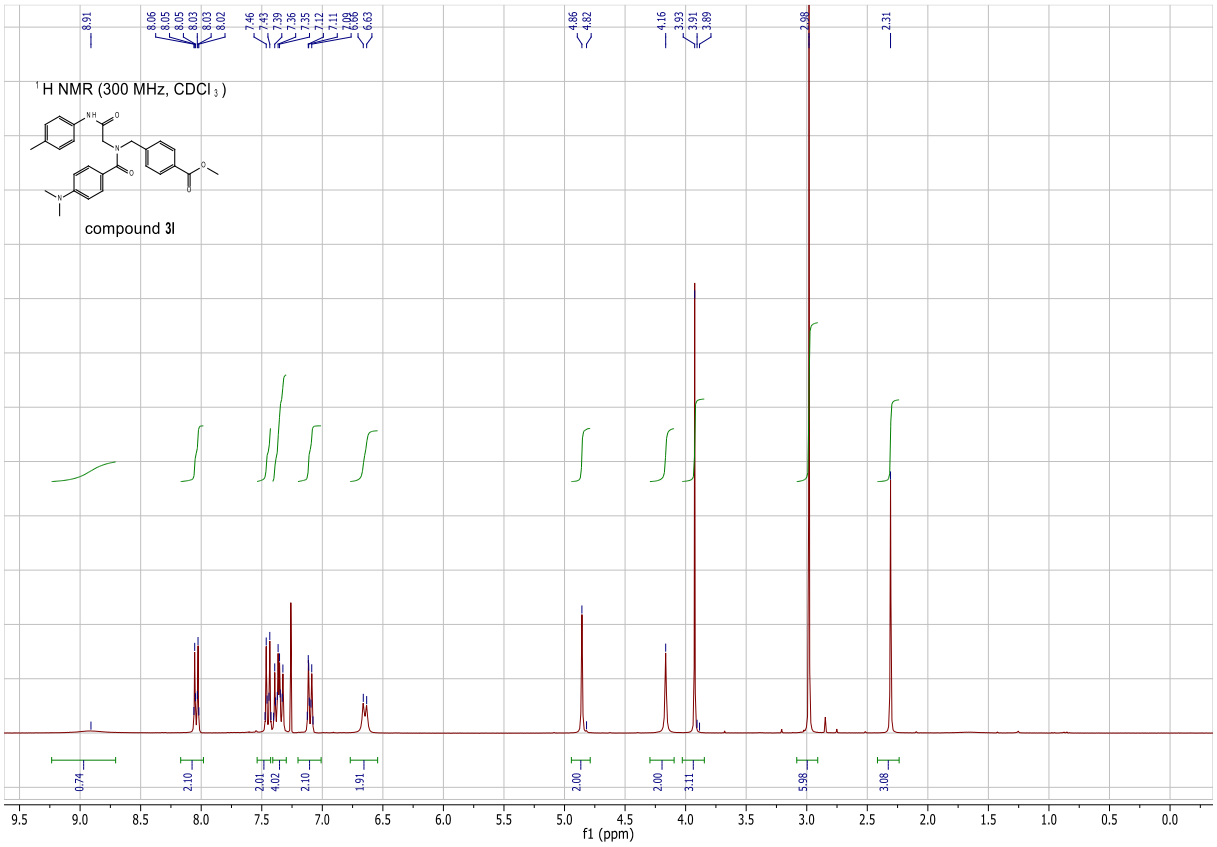
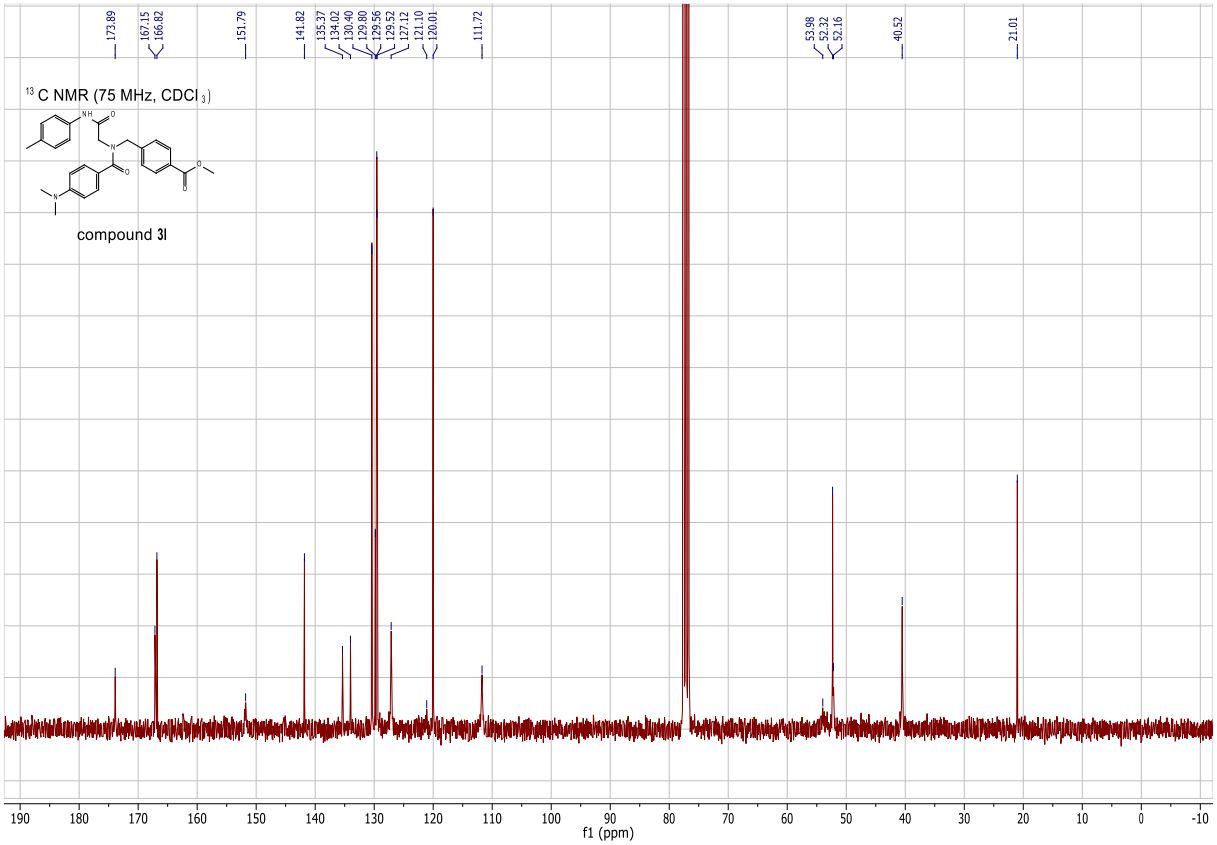
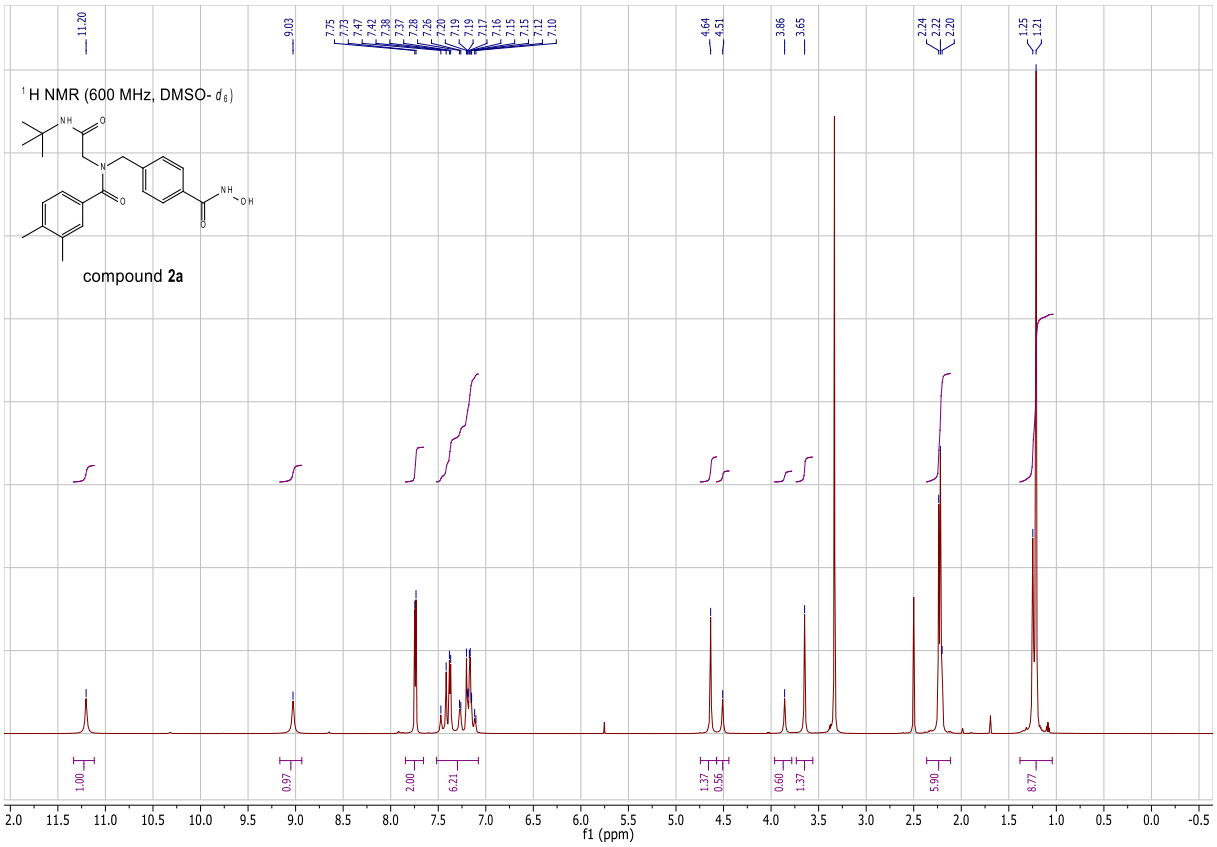
7.88
7.87
7.87
7.56
7.55
7.55
7.54
7.53
7.47
7.47
7.45
7.44
7.43
7.40
7.39
7.38
7.26 CDCl3
7.17
7.16
7.12
5.41
4.70
4.56
3.96
3.94
3.90
3.72
2.33
2.28
1.72 HDO
¹H NMR (600 MHz, CDCl₃)
compound 3k
0.78
5.00
6.00
4.21
0.15
0.87
1.71
1.11
2.43
0.35
2.43
0.58
f1 (ppm)


172.91
171.96
166.69
166.51
165.67
142.00
140.90
135.18
134.65
134.35
134.23
133.69
133.60
133.07
132.94
130.39
130.26
130.01
130.00
129.85
129.81
129.63
129.59
128.76
128.71
128.63
127.83
127.53
126.92
126.81
125.30
125.13
124.88
124.60
124.29
123.96
120.22
120.13
53.89
52.33
51.95
49.66
49.11
21.03
20.98
¹³C NMR (151 MHz, CDCl₃)
compound 3k
f1 (ppm)

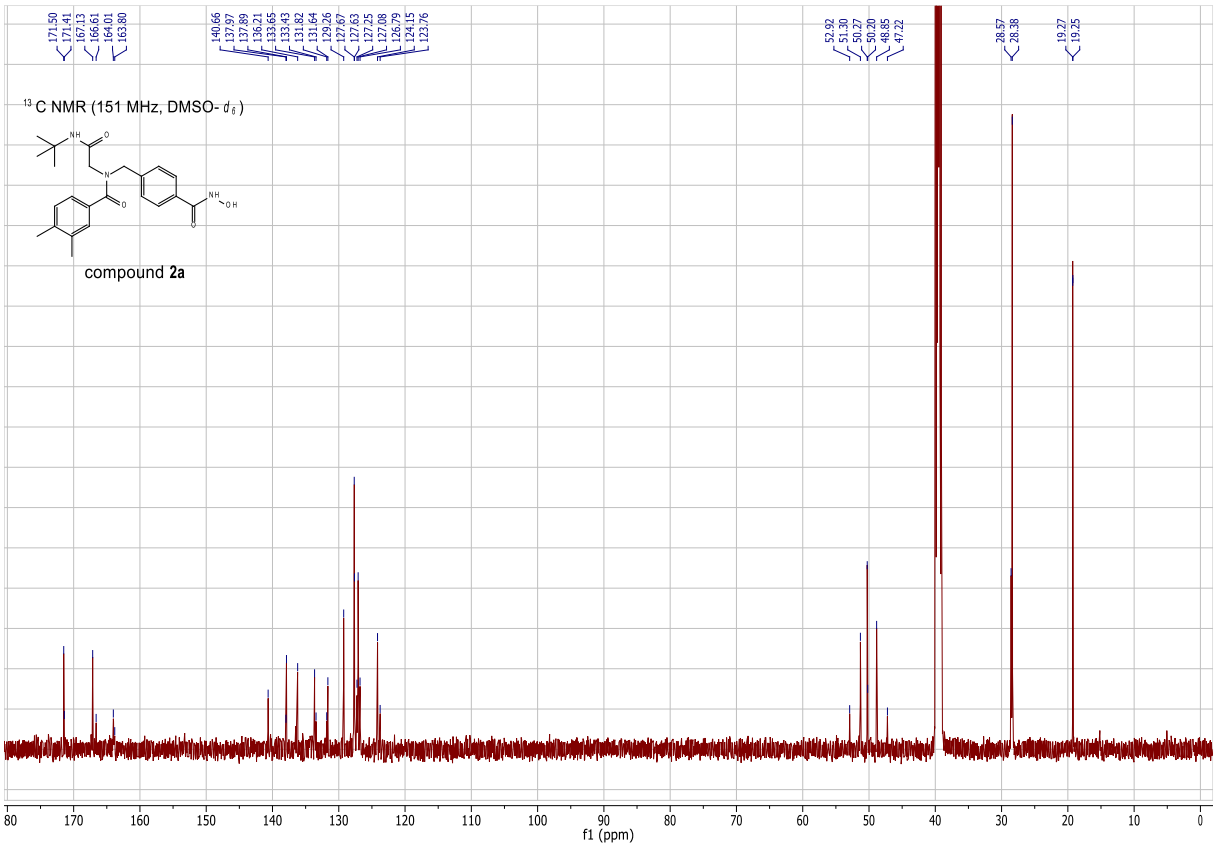

8.91
8.06
8.05
8.05
8.03
8.03
8.02
7.46
7.43
7.39
7.36
7.35
7.12
7.11
6.66
6.63
4.86
4.82
4.16
3.93
3.91
3.89
2.98
2.31
^{1}H NMR (300 MHz, CDCl$_3$)
compound 3l
0.74
2.10
2.01
4.02
2.10
1.91
2.00
2.00
3.11
5.98
3.08
f1 (ppm)


173.89
167.15
166.82
151.79
141.82
135.37
134.02
130.40
129.80
129.56
129.52
127.12
121.10
120.01
111.72
53.98
52.32
52.16
40.52
21.01
^{13}C NMR (75 MHz, CDCl$_3$)
compound 3l
f1 (ppm)



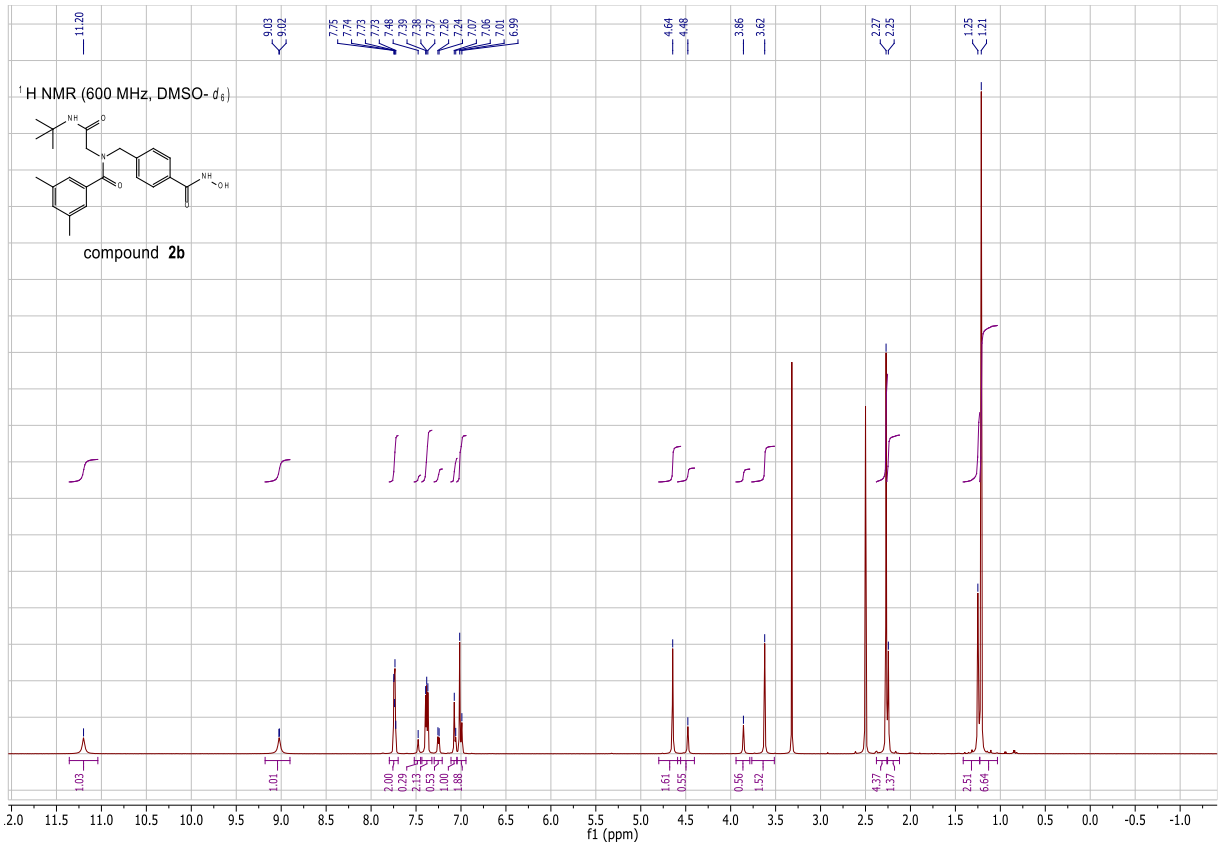
^{1}H NMR (600 MHz, DMSO-d_6)

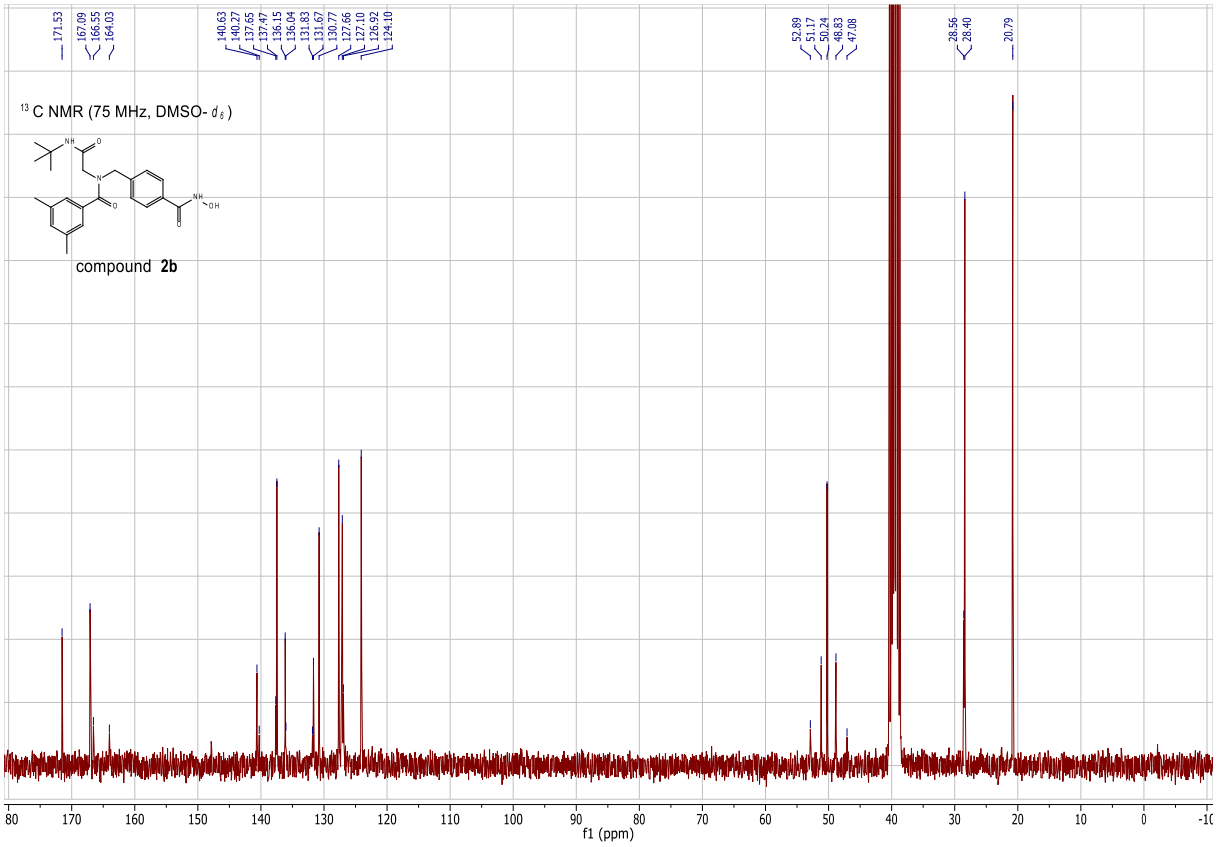
compound **2a**

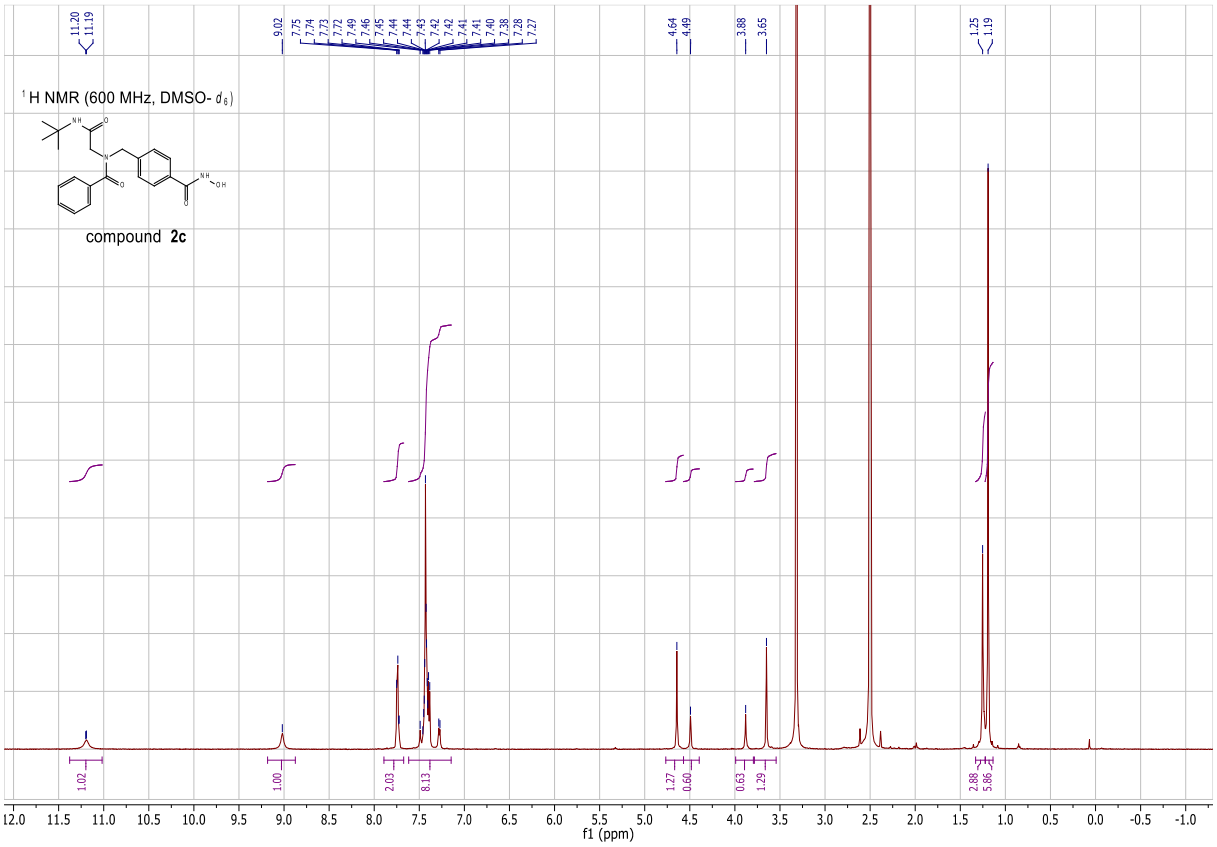


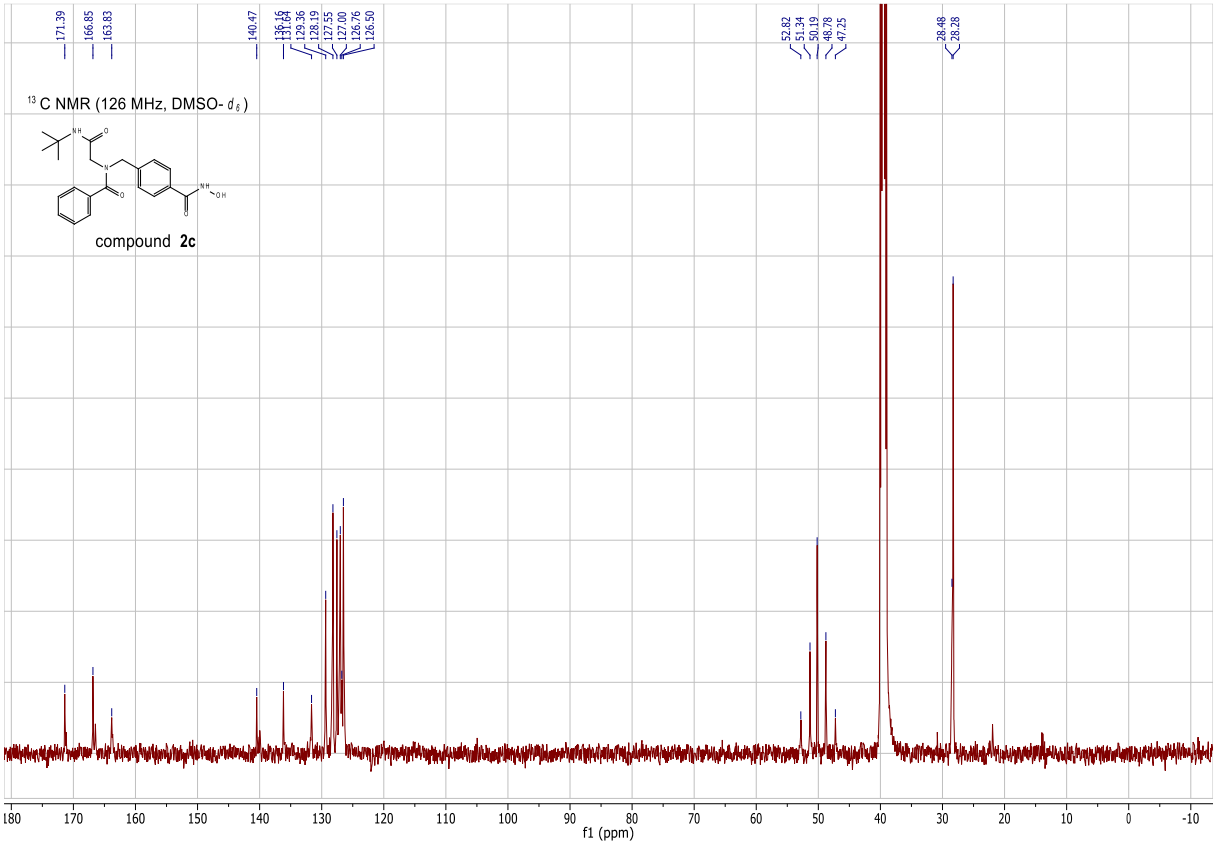
^{13}C NMR (151 MHz, DMSO-d_6)

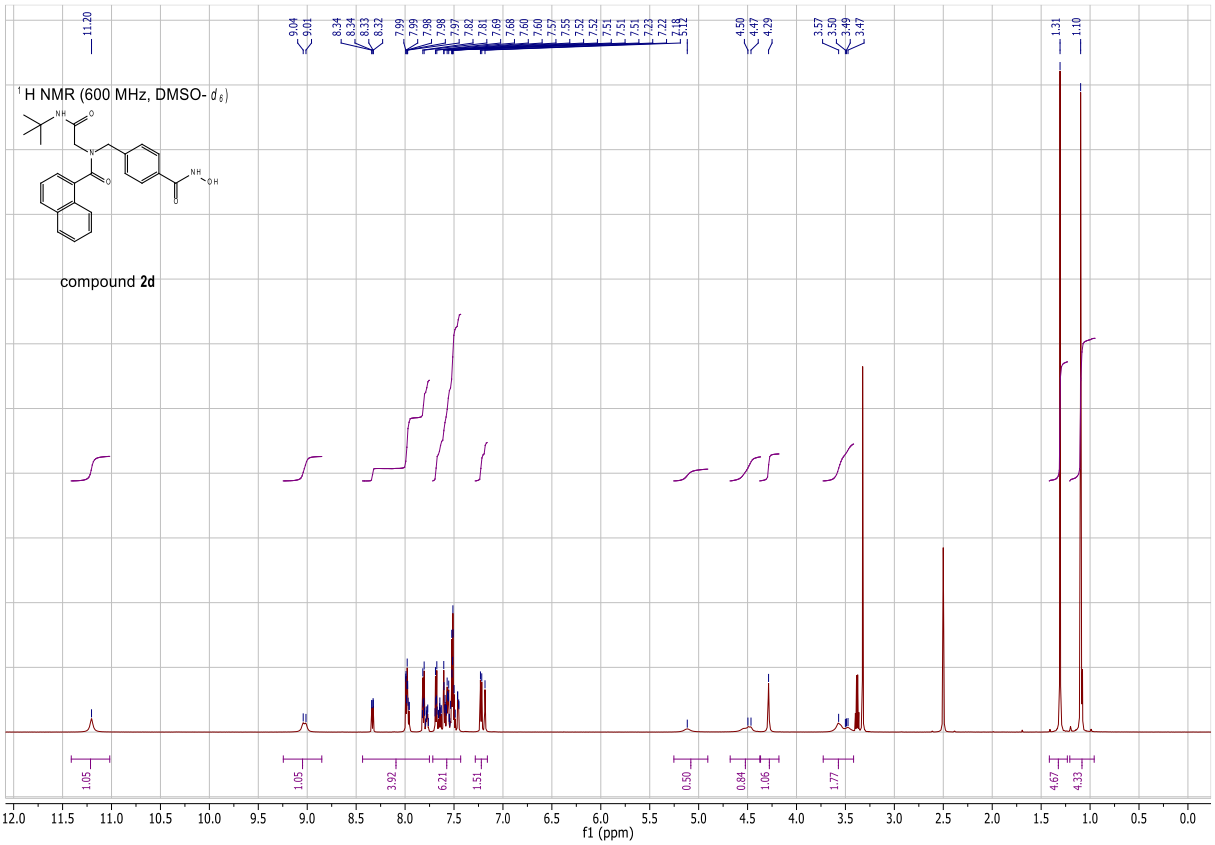
compound **2a**

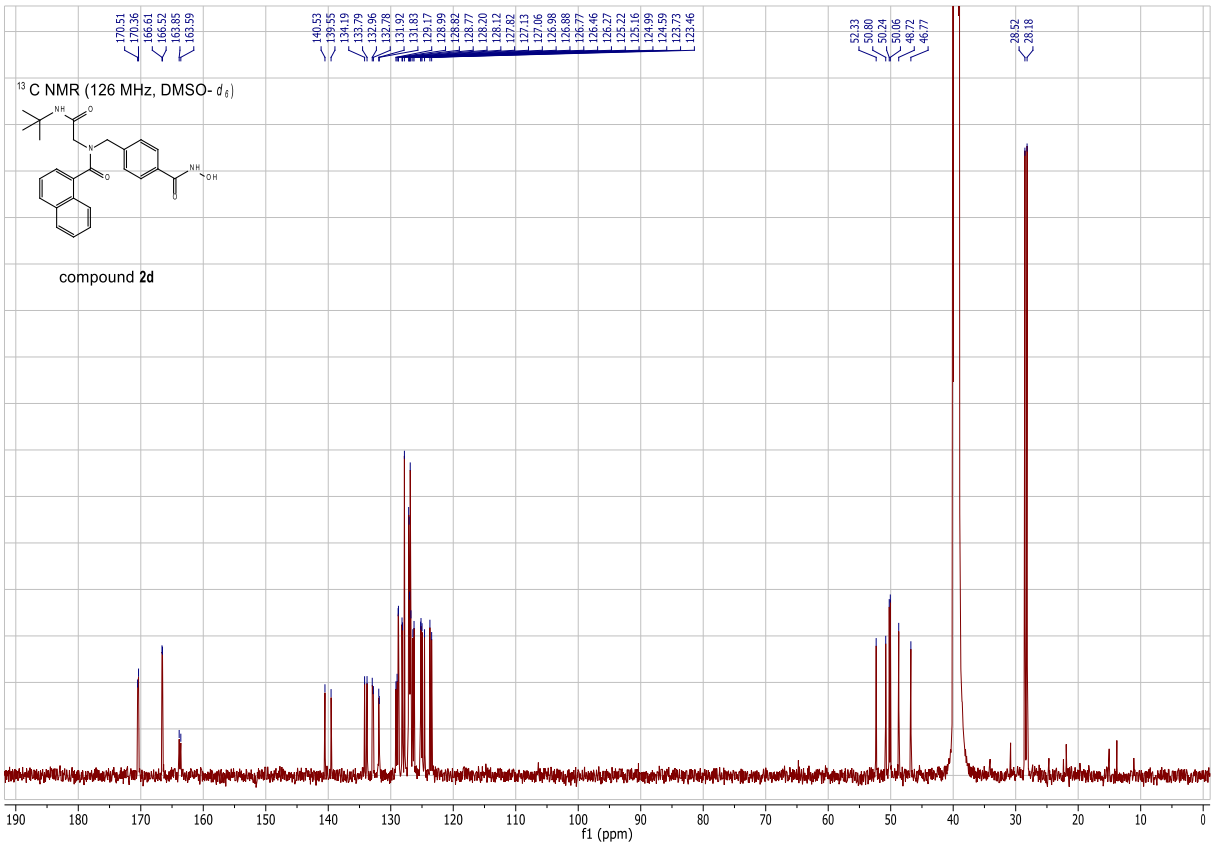

11.20
9.03
9.02
7.75
7.74
7.73
7.73
7.48
7.39
7.38
7.37
7.26
7.24
7.07
7.06
7.01
6.99
4.64
4.48
3.86
3.62
2.27
2.25
1.25
1.21
1H NMR (600 MHz, DMSO- d6)
compound 2b
1.03
1.01
2.00
0.29
2.13
0.53
1.00
1.88
1.61
0.55
0.56
1.52
4.37
1.37
2.51
6.64
f1 (ppm)

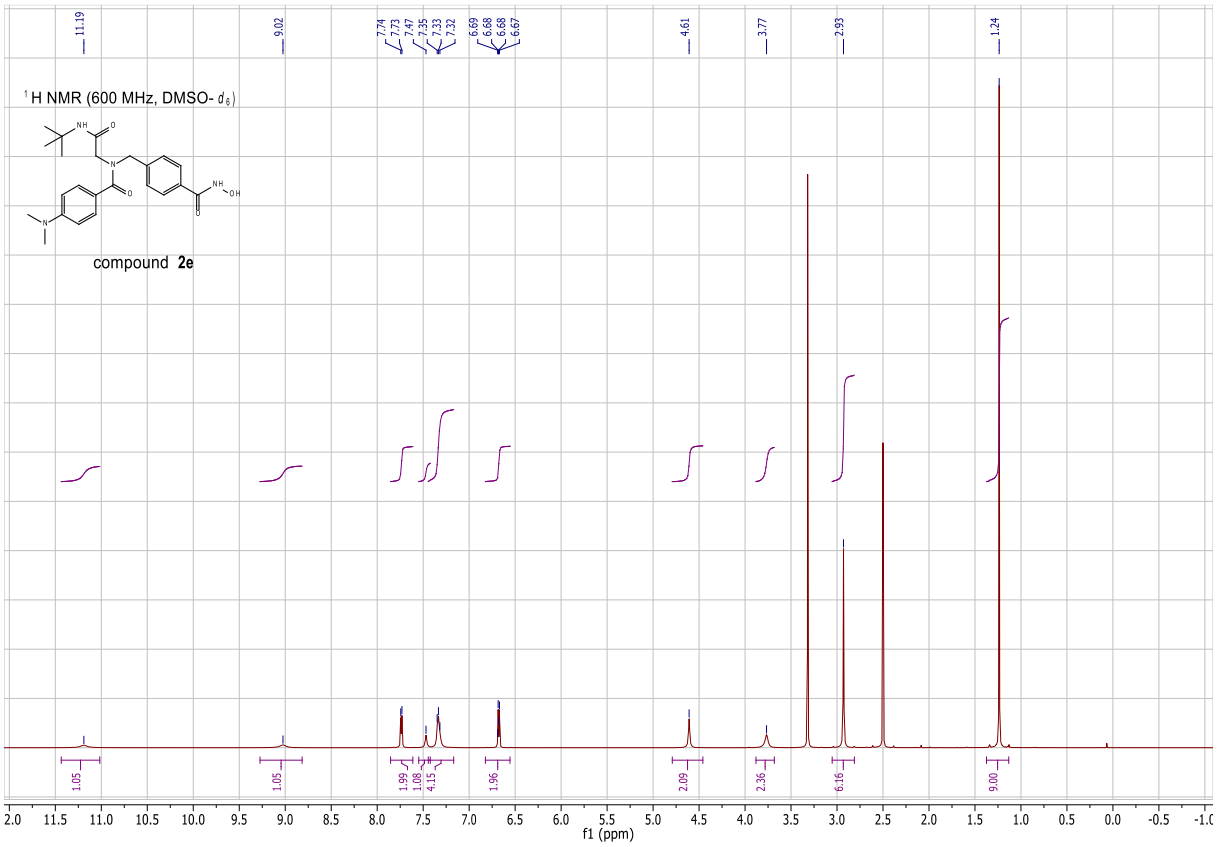

171.53
167.09
166.55
164.03
140.63
140.27
137.65
137.47
136.15
136.04
131.63
131.61
130.77
127.66
127.10
126.92
124.10
52.89
51.17
50.24
48.83
47.08
28.56
28.40
20.79
13C NMR (75 MHz, DMSO- d6)
compound 2b
f1 (ppm)

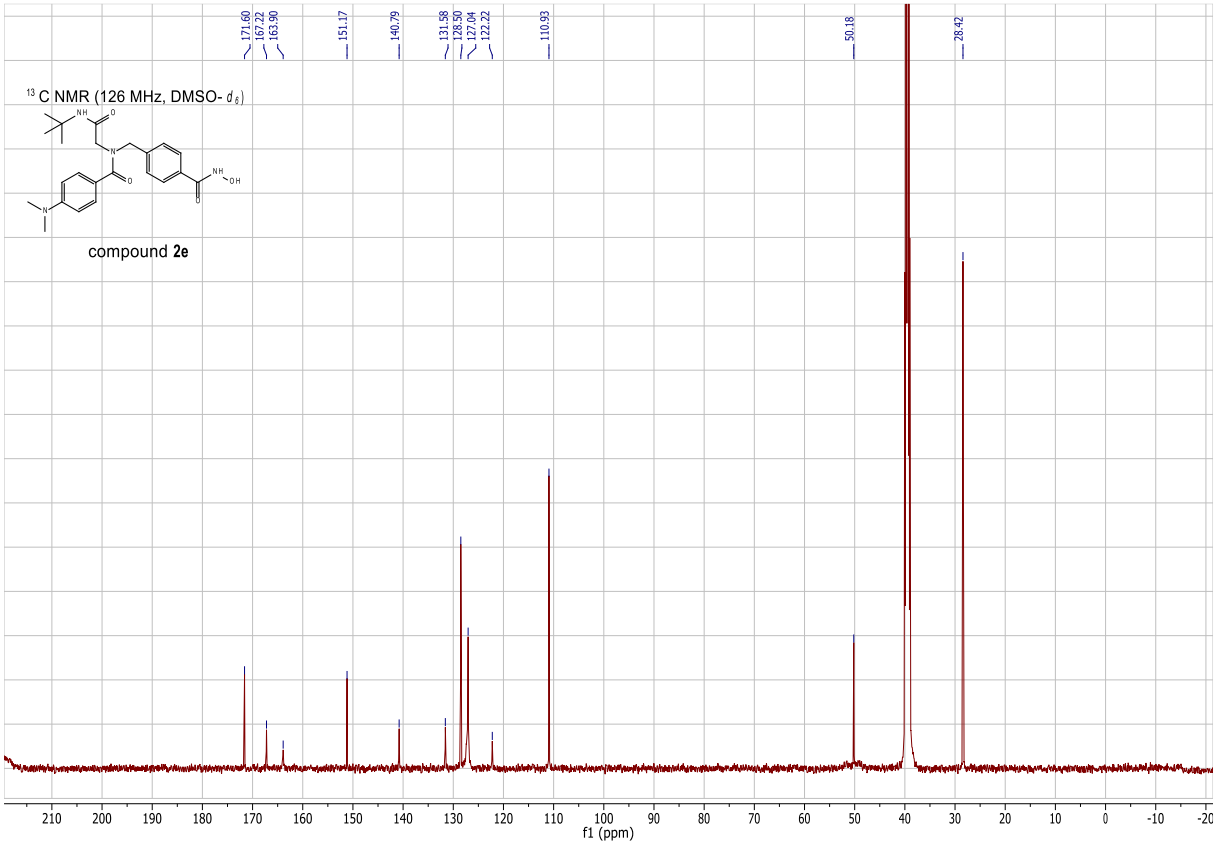

11.20
11.19
9.02
7.75
7.74
7.73
7.72
7.49
7.46
7.45
7.44
7.44
7.43
7.42
7.42
7.41
7.41
7.40
7.38
7.28
7.27
4.64
4.49
3.88
3.65
1.25
1.19
1H NMR (600 MHz, DMSO- d6)
compound 2c
1.02
1.00
2.03
8.13
1.27
0.60
0.63
1.29
2.88
5.86
f1 (ppm)

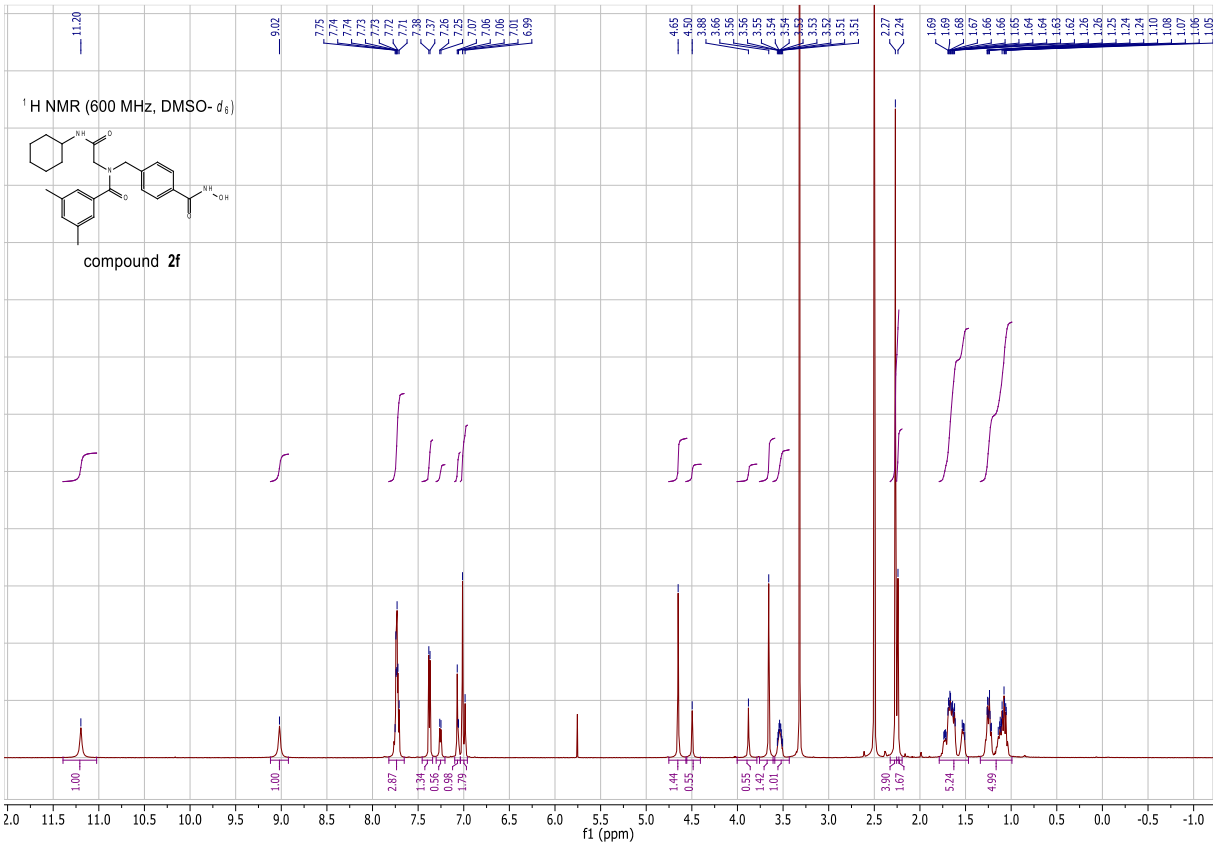

171.39
166.85
163.83
140.47
136.16
135.64
129.36
128.19
127.55
127.00
126.76
126.50
52.82
51.34
50.19
48.78
47.25
28.48
28.28
13C NMR (126 MHz, DMSO- d6)
compound 2c
f1 (ppm)

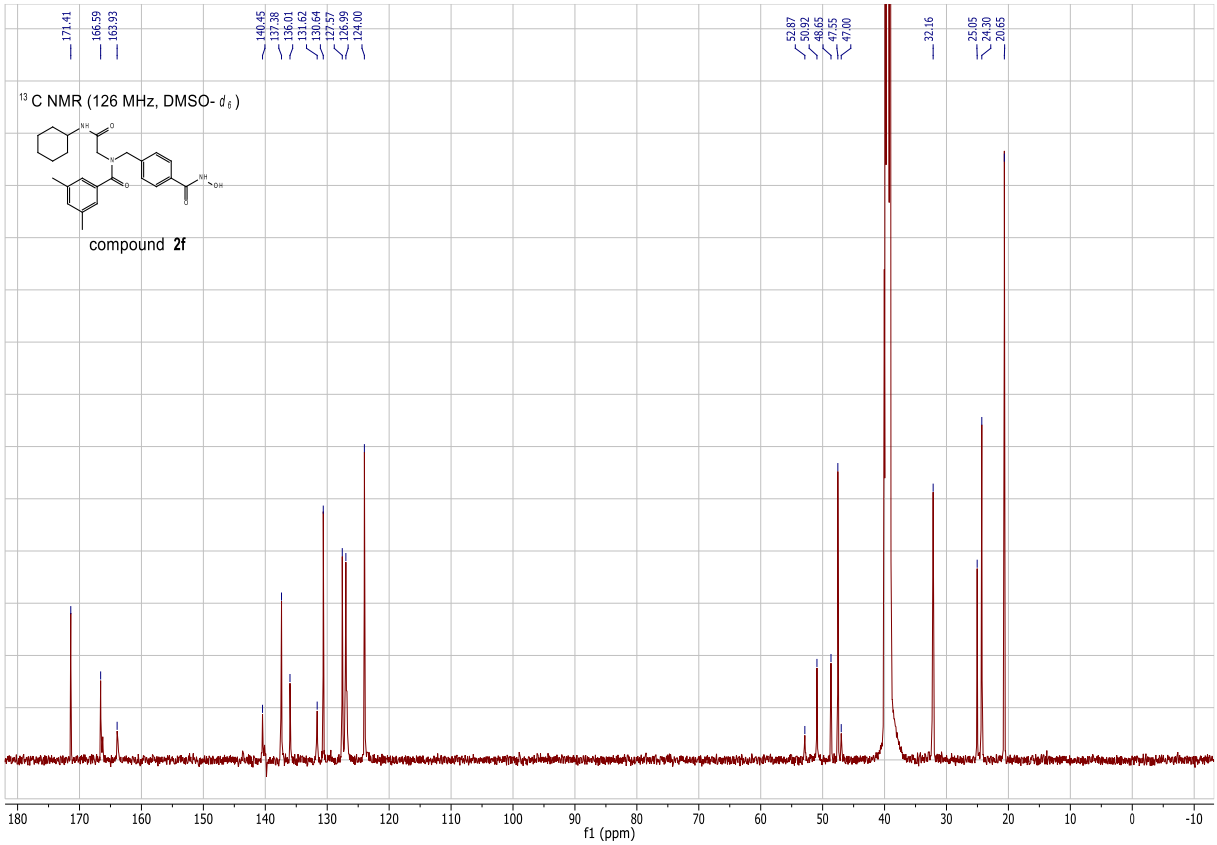

11.20
9.04
9.01
8.34
8.34
8.33
8.32
7.99
7.99
7.98
7.98
7.97
7.82
7.81
7.69
7.68
7.60
7.60
7.57
7.55
7.52
7.52
7.51
7.51
7.51
7.23
7.22
7.18
5.12
4.50
4.47
4.29
3.57
3.50
3.49
3.47
1.31
1.10
1H NMR (600 MHz, DMSO-d6)
compound 2d
1.05
1.05
3.92
6.21
1.51
0.50
0.84
1.06
1.77
4.67
4.33
f1 (ppm)

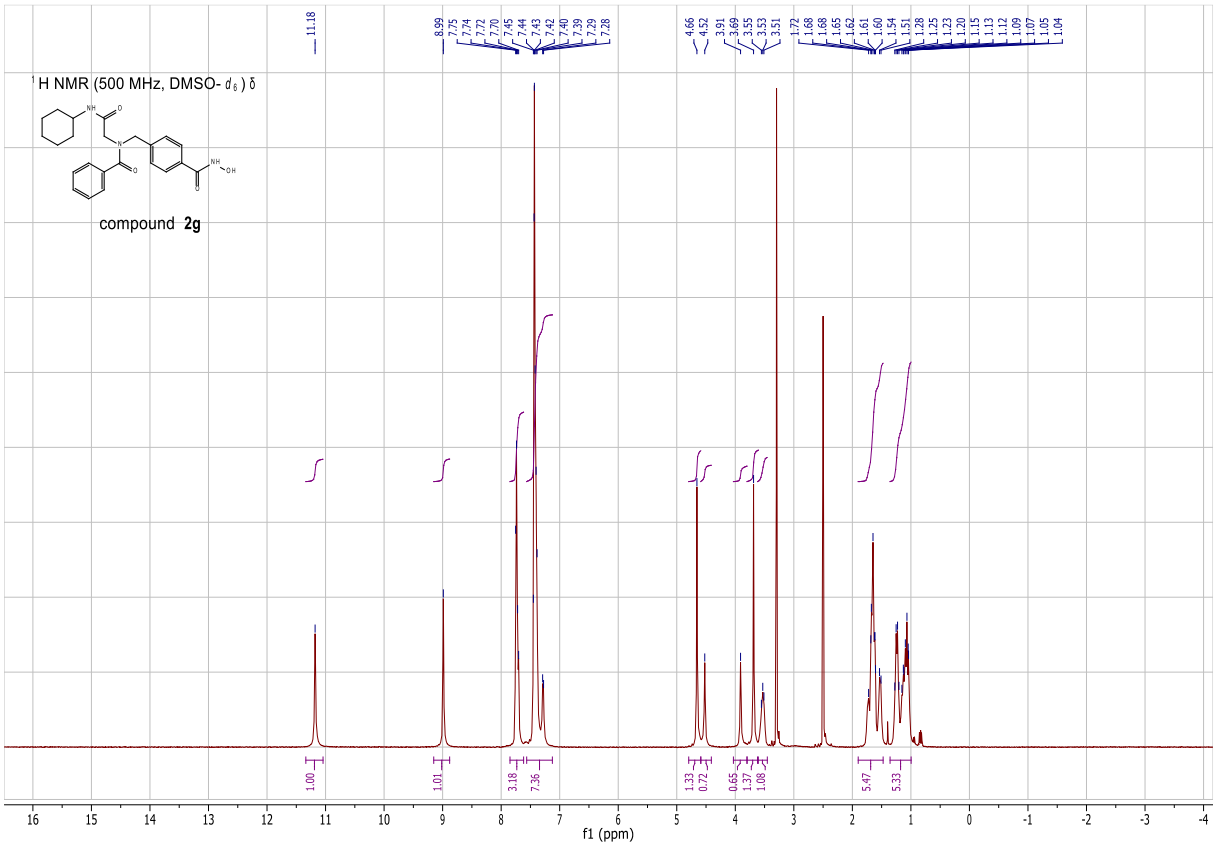

170.51
170.36
166.61
166.52
163.85
163.59
140.53
139.55
134.19
133.79
132.96
132.78
131.92
131.83
129.17
128.99
128.82
128.77
128.20
128.12
127.82
127.13
127.06
126.98
126.88
126.77
126.46
126.27
125.22
125.16
124.99
124.59
123.73
123.46
52.33
50.80
50.24
50.06
48.72
46.77
28.52
28.18
13C NMR (126 MHz, DMSO-d6)
compound 2d
f1 (ppm)

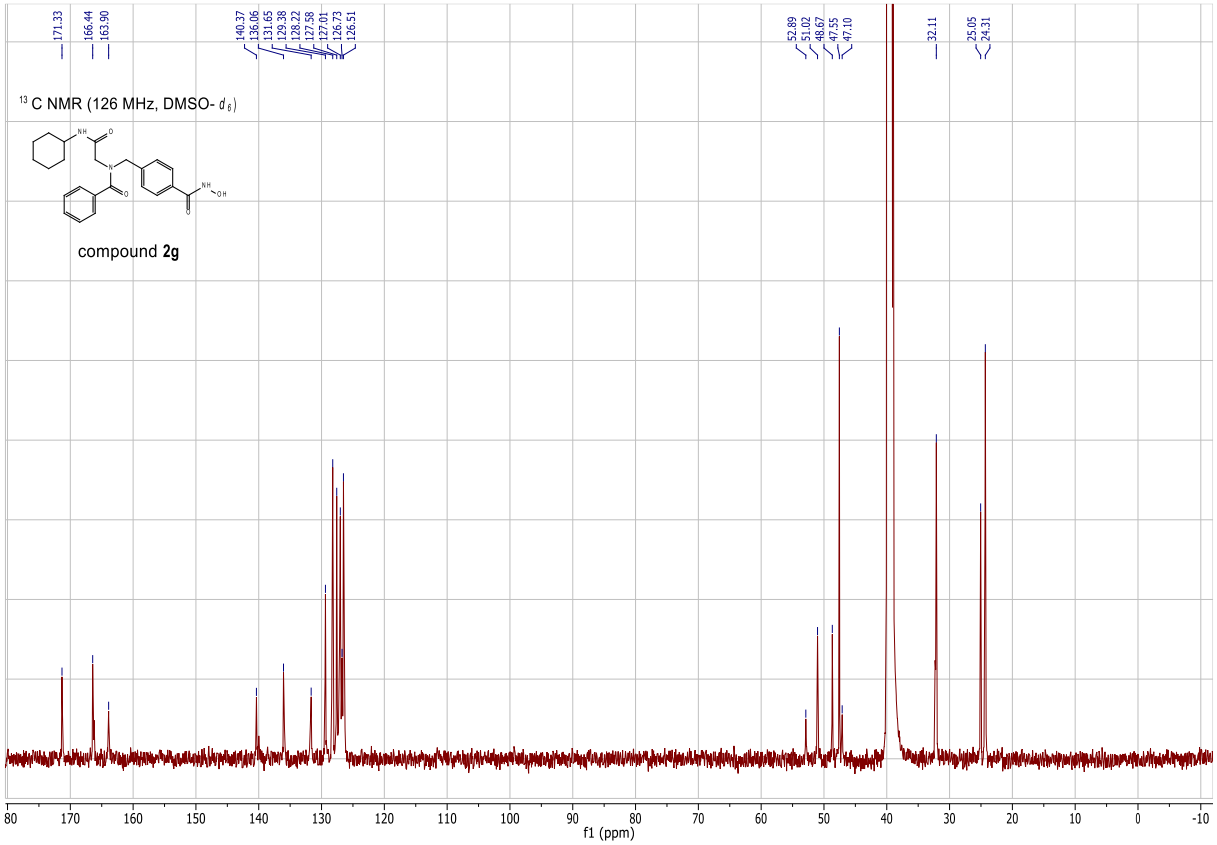

11.19
9.02
7.74
7.73
7.47
7.35
7.33
7.32
6.69
6.68
6.68
6.67
4.61
3.77
2.93
1.24
^{1}H NMR (600 MHz, DMSO- d_6)
compound **2e**
1.05
1.05
1.99
1.08
4.15
1.96
2.09
2.36
6.16
9.00
f1 (ppm)

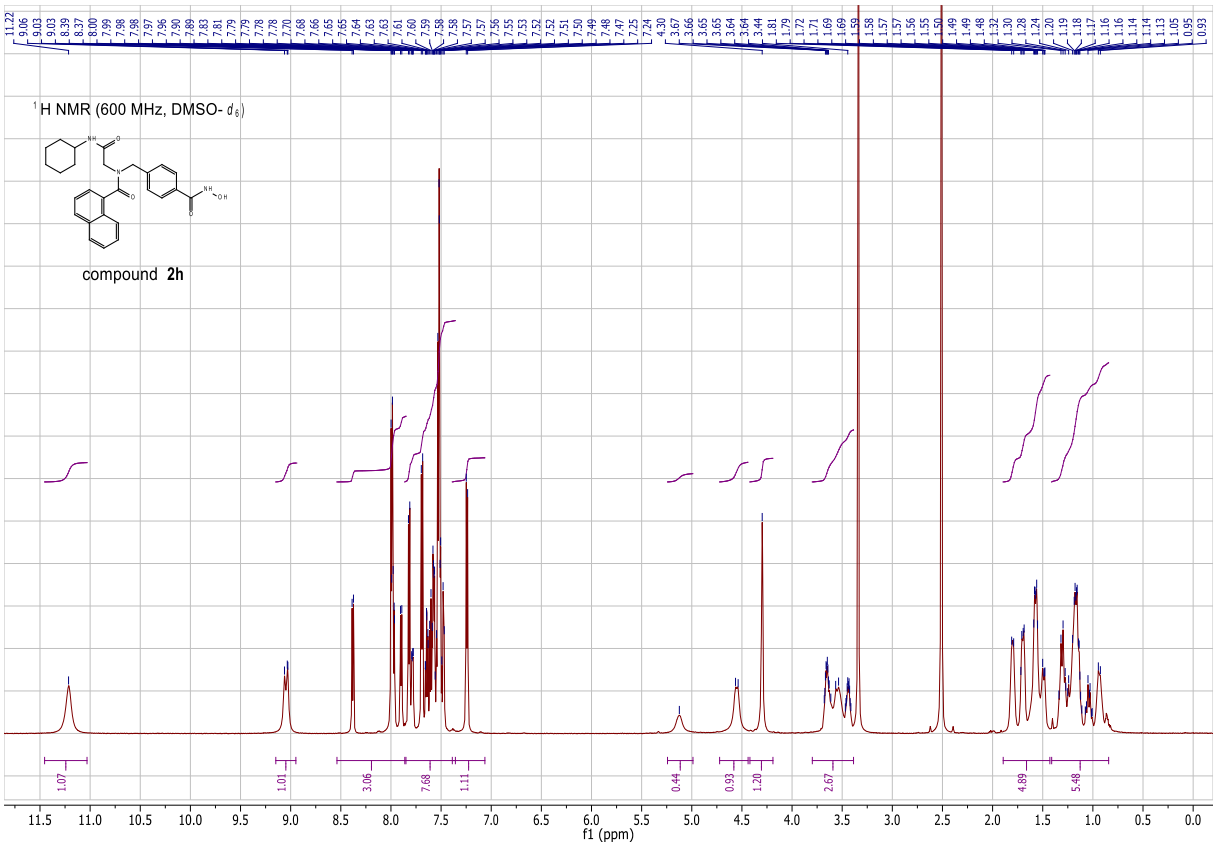

171.60
167.22
163.90
151.17
140.79
131.58
128.50
127.04
122.22
110.93
50.18
28.42
^{13}C NMR (126 MHz, DMSO- d_6)
compound **2e**
f1 (ppm)

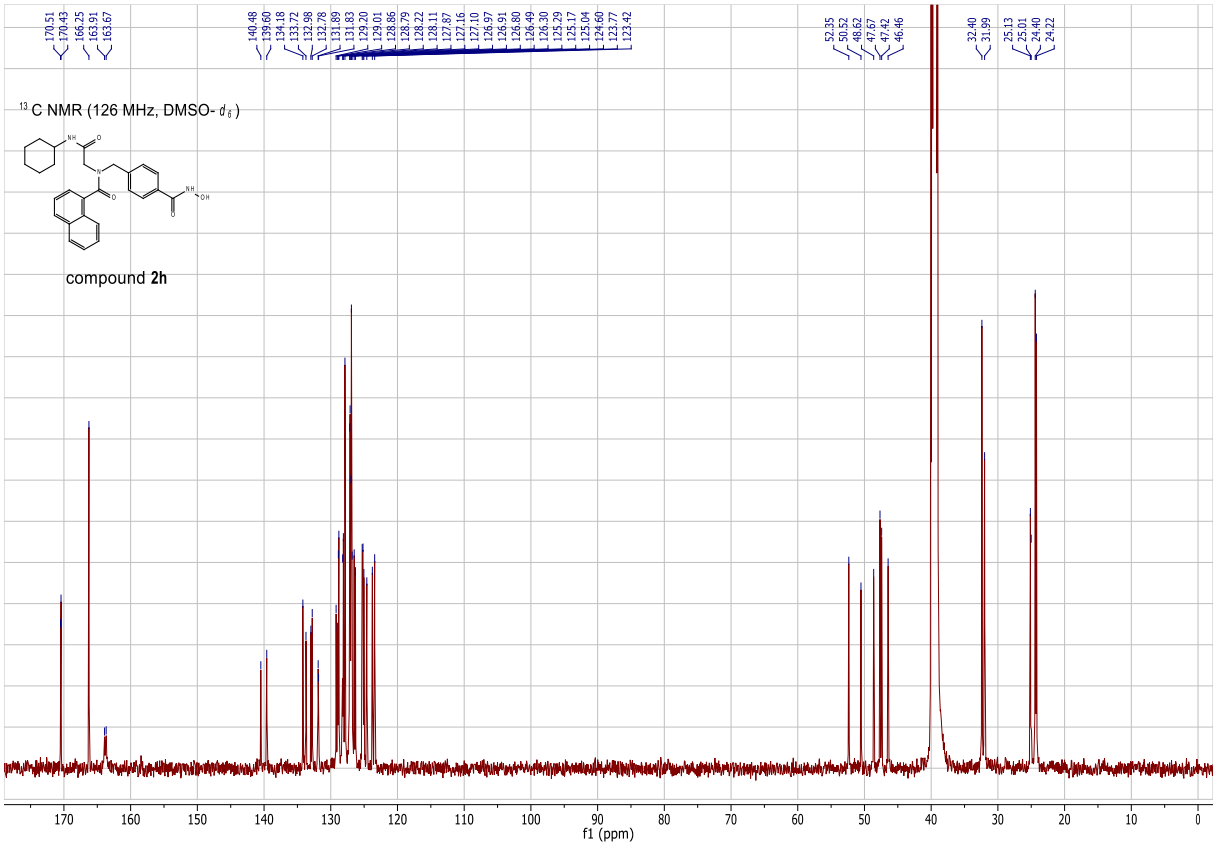

11.20
9.02
7.75
7.74
7.74
7.73
7.73
7.72
7.71
7.38
7.37
7.26
7.25
7.07
7.06
7.01
6.99
4.65
4.50
3.88
3.66
3.56
3.55
3.54
3.53
3.52
3.51
2.27
2.24
1.69
1.69
1.68
1.67
1.66
1.66
1.65
1.64
1.64
1.63
1.62
1.26
1.26
1.25
1.24
1.24
1.10
1.08
1.07
1.06
1.05
1H NMR (600 MHz, DMSO- d6)
compound 2f
1.00
1.00
2.87
1.34
0.56
0.98
1.79
1.44
0.55
0.55
1.42
1.01
3.90
1.67
5.24
4.99
f1 (ppm)

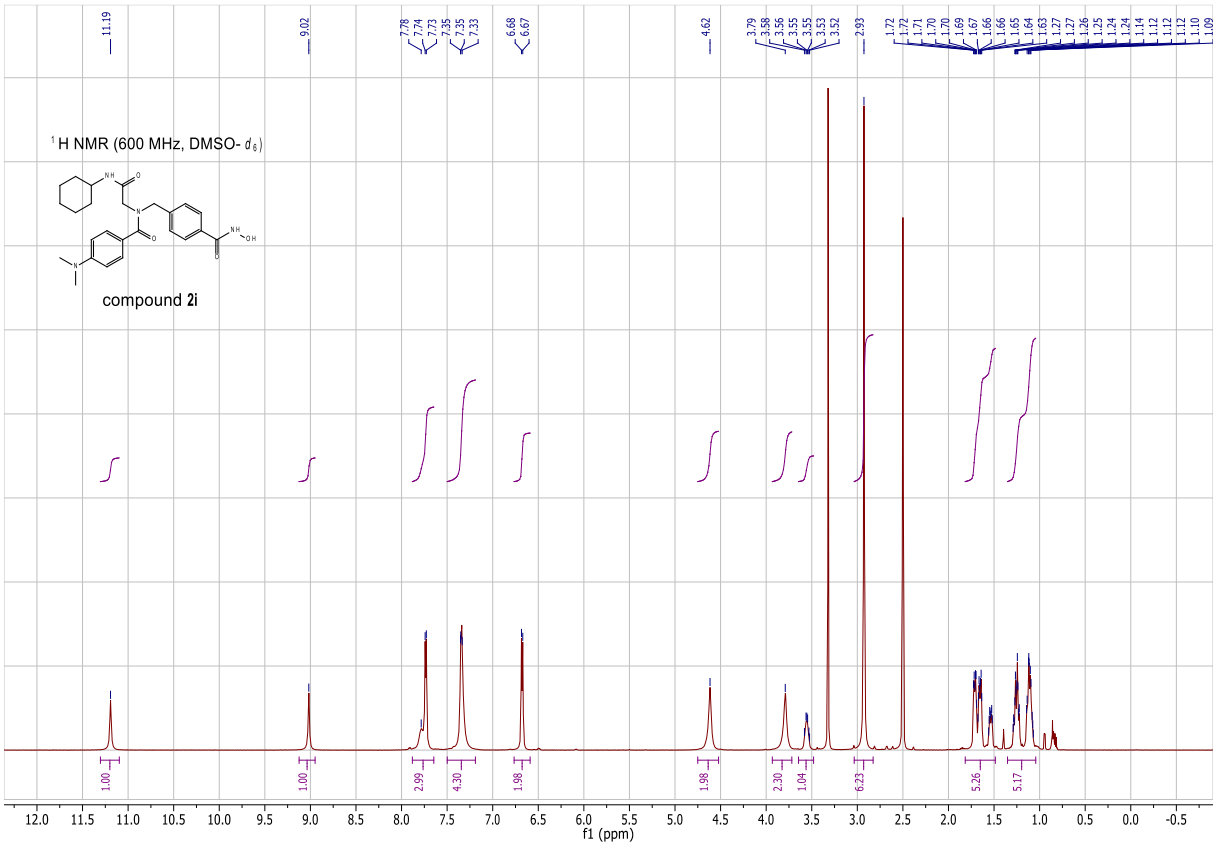

171.41
166.59
163.93
140.45
137.38
136.01
131.62
130.64
127.57
126.99
124.00
52.87
50.92
48.65
47.55
47.00
32.16
25.05
24.30
20.65
13C NMR (126 MHz, DMSO- d6)
compound 2f
f1 (ppm)

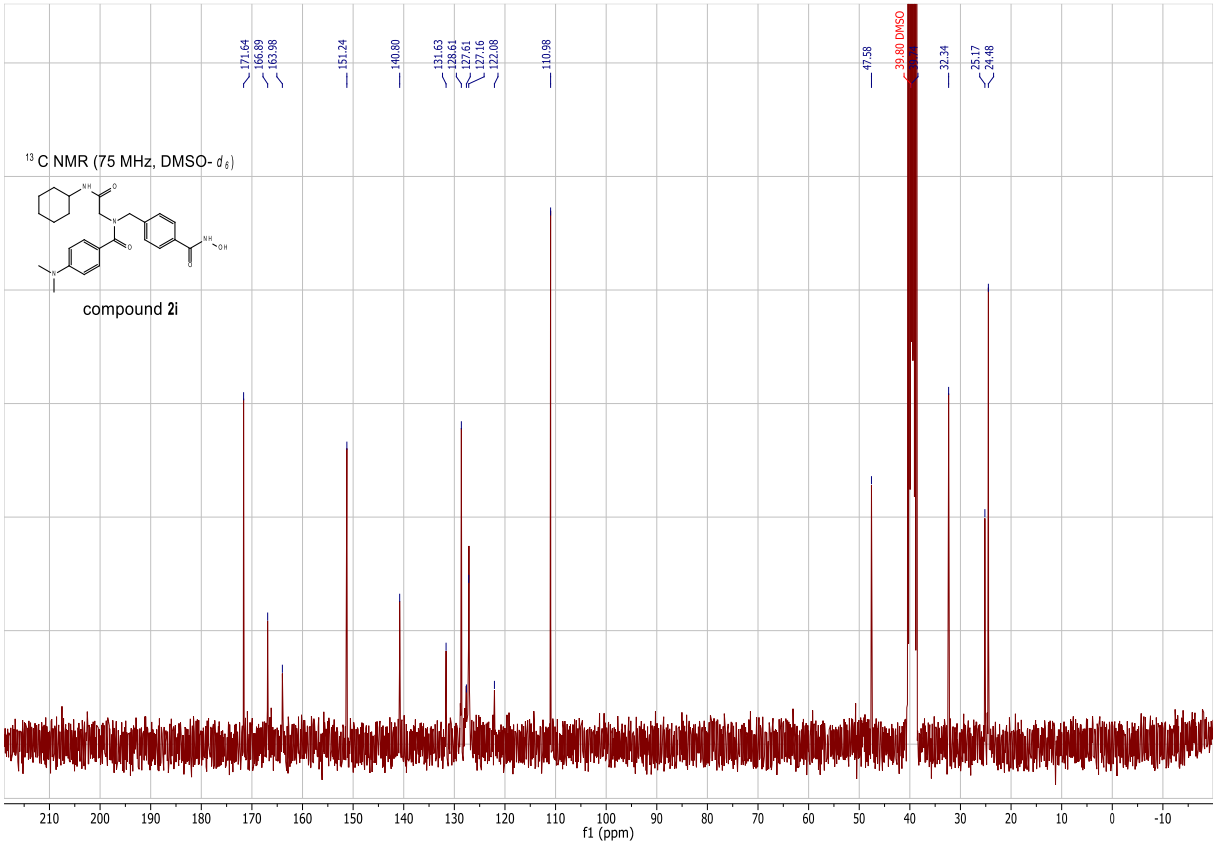

^{1}H NMR (500 MHz, DMSO- d_6) δ
compound **2g**
f1 (ppm)

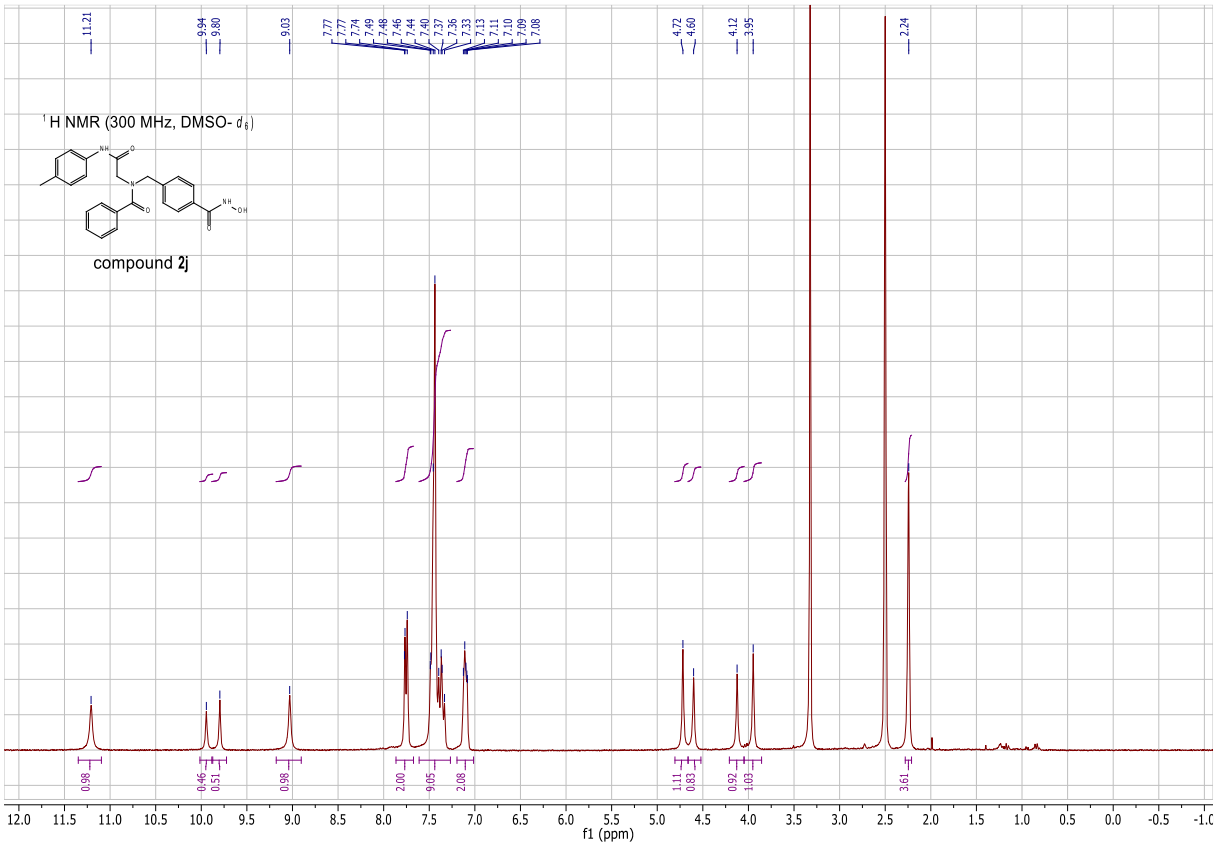

^{13}C NMR (126 MHz, DMSO- d_6)
compound **2g**
f1 (ppm)

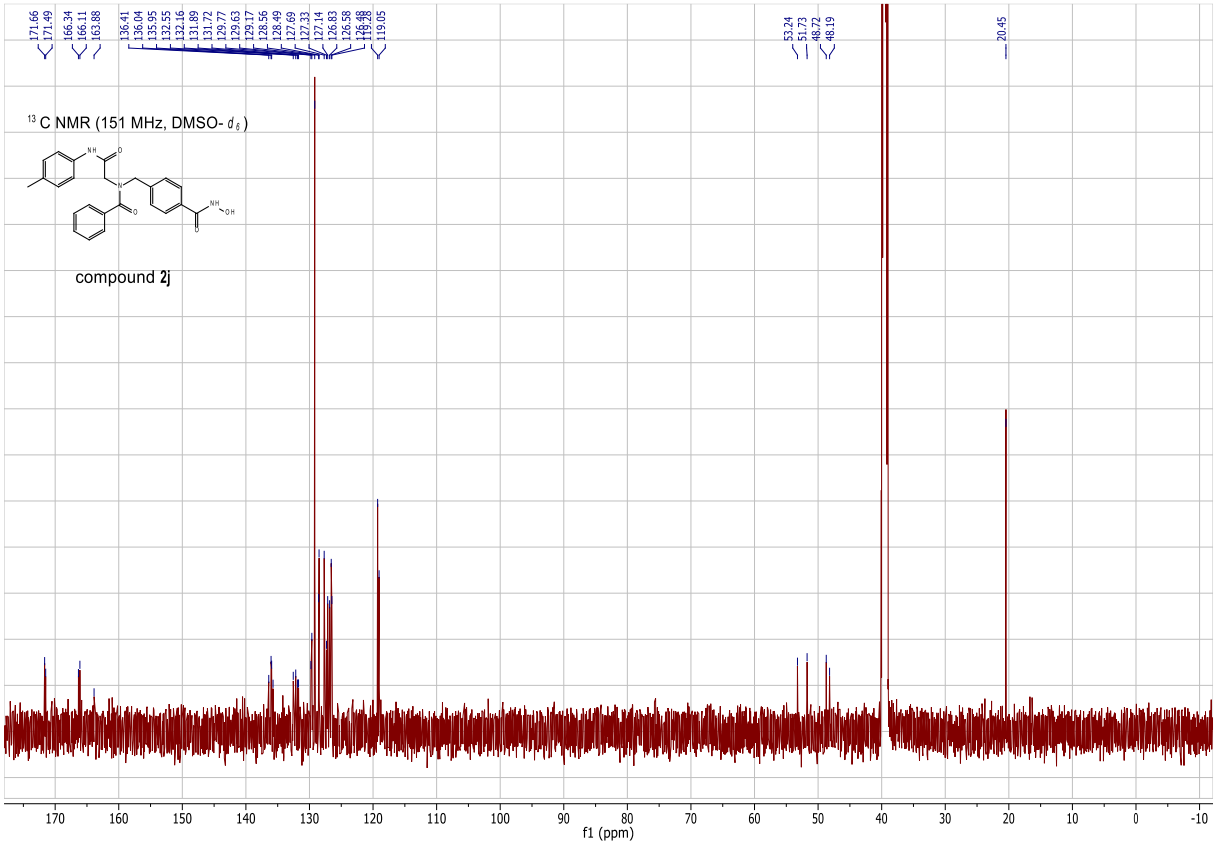

^{1}H NMR (600 MHz, DMSO- d_6)
compound **2h**
f1 (ppm)

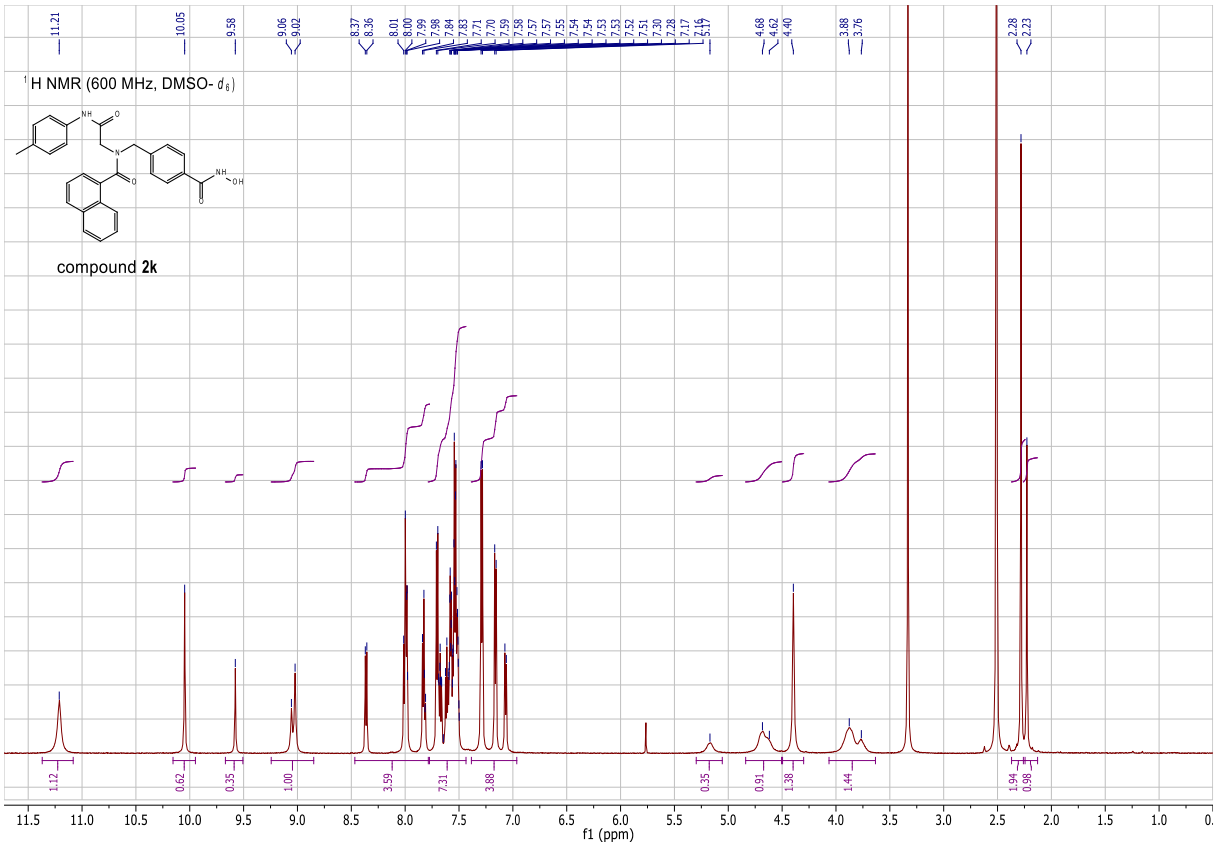

^{13}C NMR (126 MHz, DMSO- d_6)
compound **2h**
f1 (ppm)

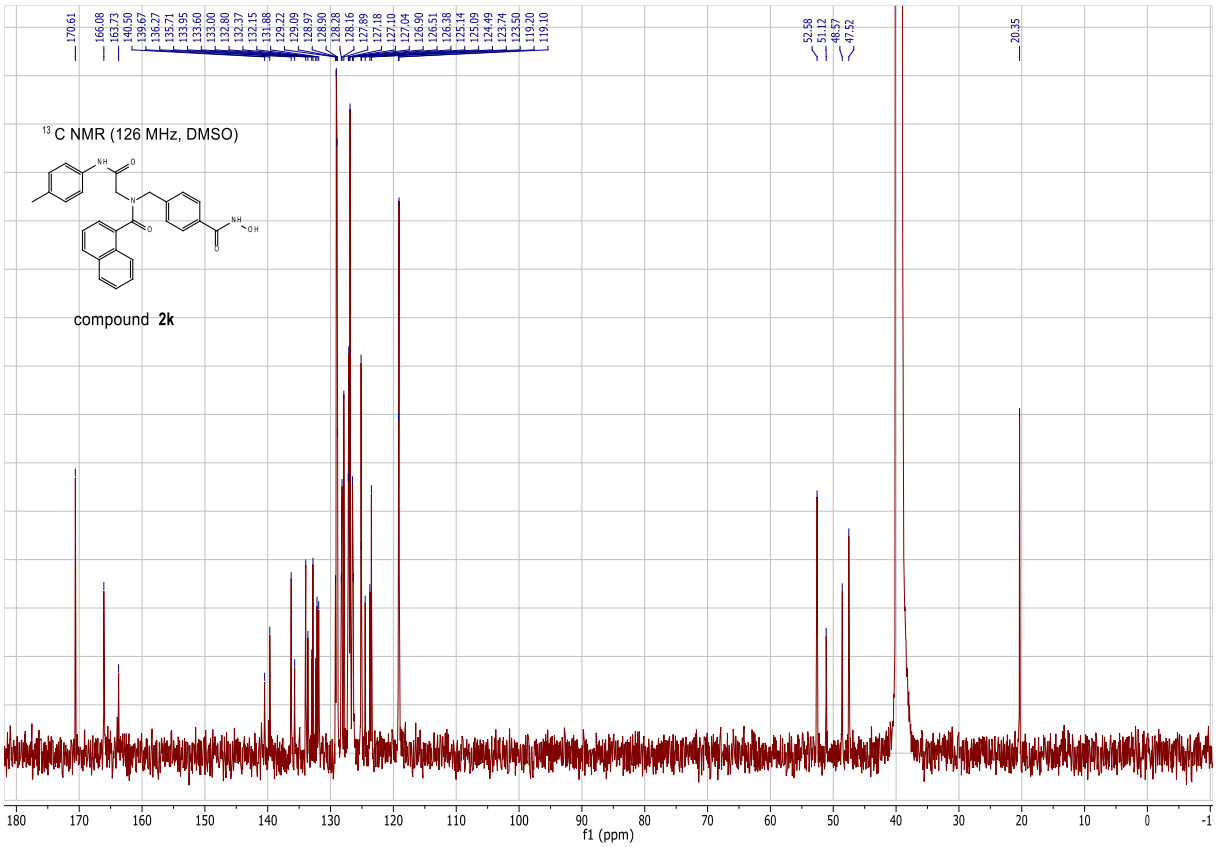

11.19
9.02
7.78
7.74
7.73
7.35
7.35
7.33
6.68
6.67
4.62
3.79
3.58
3.56
3.55
3.55
3.53
3.52
2.93
1.72
1.72
1.71
1.70
1.70
1.69
1.67
1.66
1.66
1.65
1.64
1.63
1.27
1.27
1.26
1.25
1.24
1.24
1.14
1.12
1.12
1.12
1.10
1.09
1H NMR (600 MHz, DMSO- d6)
compound 2i
1.00
1.00
2.99
4.30
1.98
1.98
2.30
1.04
6.23
5.26
5.17
f1 (ppm)

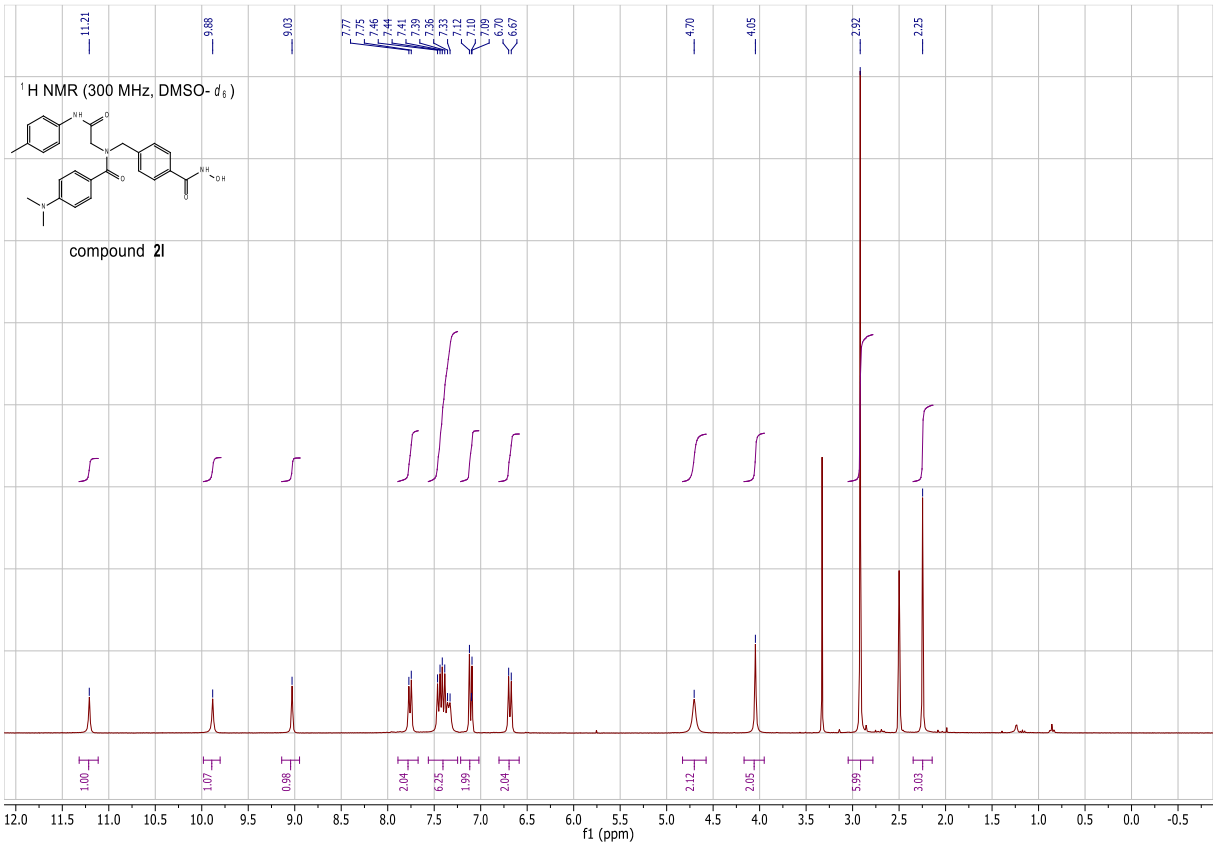

171.64
166.89
163.98
151.24
140.80
131.63
128.61
127.61
127.16
122.08
110.98
47.58
39.80 DMSO
32.34
25.17
24.48
13C NMR (75 MHz, DMSO- d6)
compound 2i
f1 (ppm)

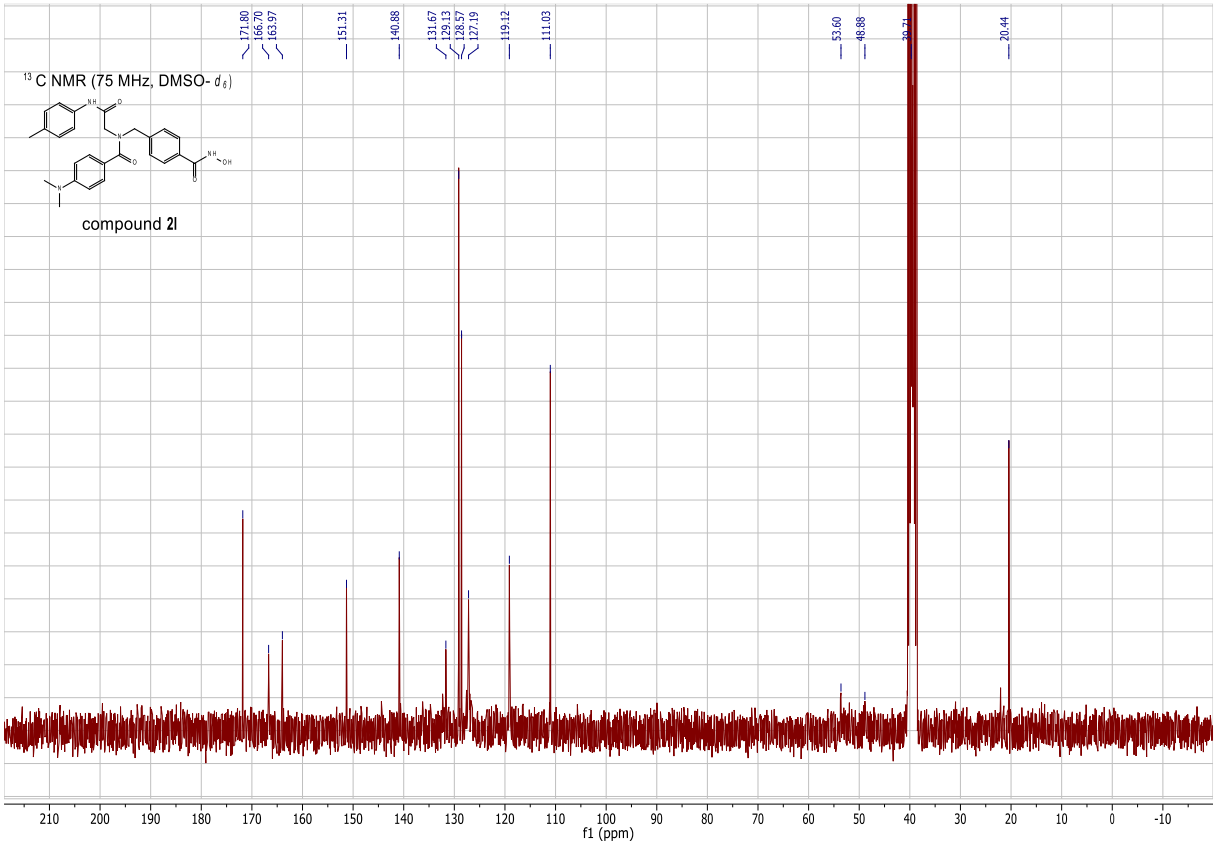

11.21
9.94
9.80
9.03
7.77
7.77
7.74
7.49
7.48
7.46
7.44
7.40
7.37
7.36
7.33
7.13
7.11
7.10
7.09
7.08
4.72
4.60
4.12
3.95
2.24
^{1}H NMR (300 MHz, DMSO- d_6)
compound **2j**
0.98
0.46
0.51
0.98
2.00
9.05
2.08
1.11
0.83
0.92
1.03
3.61
f1 (ppm)


171.66
171.49
166.34
166.11
163.88
136.41
136.04
135.95
132.55
132.16
131.89
131.72
129.77
129.63
129.37
128.56
128.49
127.69
127.34
126.83
126.58
119.28
119.05
53.24
51.73
48.72
48.19
20.45
^{13}C NMR (151 MHz, DMSO- d_6)
compound **2j**
f1 (ppm)


11.21
10.05
9.58
9.06
9.02
8.37
8.36
8.01
8.00
7.99
7.98
7.84
7.83
7.71
7.70
7.59
7.58
7.57
7.57
7.55
7.54
7.54
7.53
7.53
7.52
7.51
7.30
7.28
7.17
5.19
5.17
4.68
4.62
4.40
3.88
3.76
2.28
2.23
^{1}H NMR (600 MHz, DMSO- d_6)
compound **2k**
1.12
0.62
0.35
1.00
3.59
7.31
3.88
0.35
0.91
1.38
1.44
1.94
0.98
f1 (ppm)

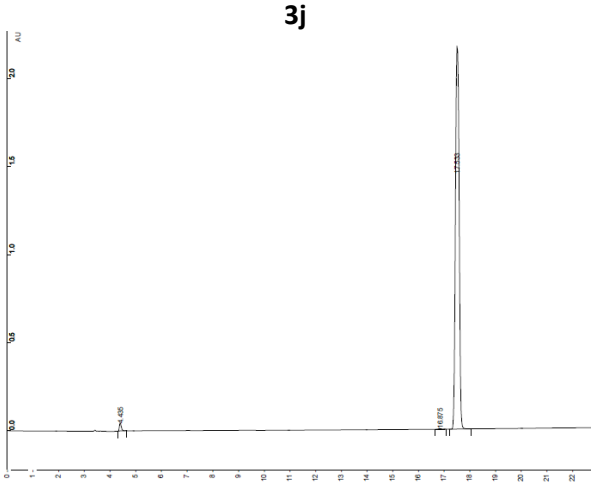

170.61
166.08
163.73
140.50
139.67
136.27
135.71
133.95
133.60
133.00
132.80
132.37
132.15
131.88
129.22
129.09
128.97
128.90
128.28
128.16
127.89
127.18
127.10
127.04
126.90
126.51
126.38
125.14
125.09
124.49
123.74
123.50
119.20
119.10
52.58
51.12
49.57
47.52
20.35
^{13}C NMR (126 MHz, DMSO)
compound **2k**
f1 (ppm)


11.21
9.88
9.03
7.77
7.75
7.46
7.44
7.41
7.39
7.36
7.33
7.12
7.10
7.09
6.70
6.67
4.70
4.05
2.92
2.25
1H NMR (300 MHz, DMSO- d6)
compound 2l
1.00
1.07
0.98
2.04
6.25
1.99
2.04
2.12
2.05
5.99
3.03
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)


171.80
166.70
163.97
151.31
140.88
131.67
129.13
128.57
127.19
119.12
111.03
53.60
48.88
39.71
20.44
13C NMR (75 MHz, DMSO- d6)
compound 2l
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

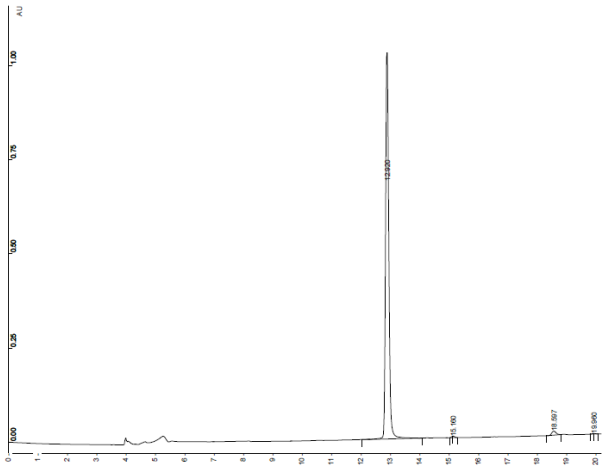
Copies of HPLC chromatograms of 2j and 3a-l

3j



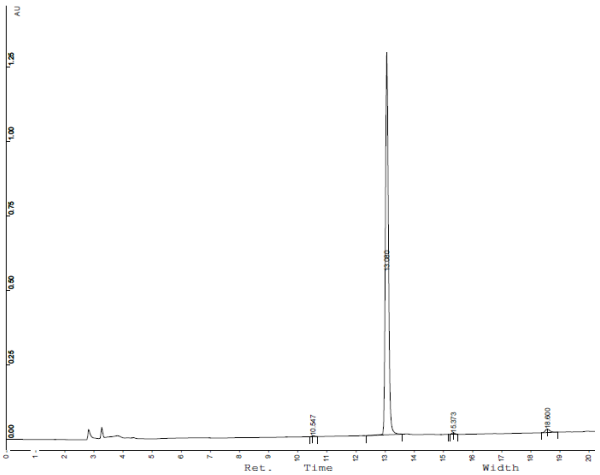
Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		1.0898	4.435	0.000	1202128	BB	4.8	
2		0.1455	16.875	0.000	160500	BB	10.2	
3		98.7647	17.533	0.000	108940416	BB	10.2	
Totals:		100.0000		0.000	110303044			

2a



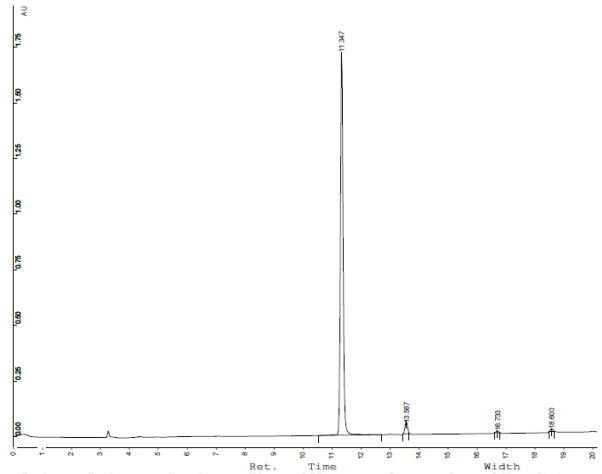
Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		98.1502	12.920	0.000	40729708	BB	7.0	
2		0.3818	15.160	0.000	158456	BB	7.6	
3		1.3493	18.597	0.000	559927	BB	10.9	
4		0.1186	19.960	0.000	49218	BB	8.8	
Totals:		99.9999		0.000	41497309			

2b



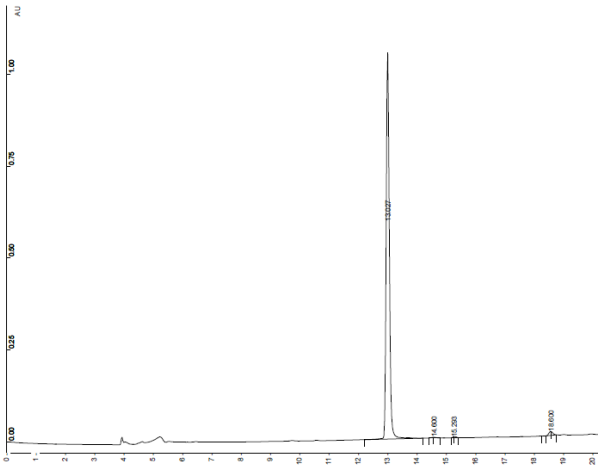
Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		0.2153	10.547	0.000	103942	BB	7.0	
2		98.0159	13.080	0.000	47330468	BB	6.7	
3		0.3754	15.373	0.000	181284	BB	7.3	
4		1.3934	18.600	0.000	672853	BB	10.4	
Totals:		100.0000		0.000	48288547			

2c



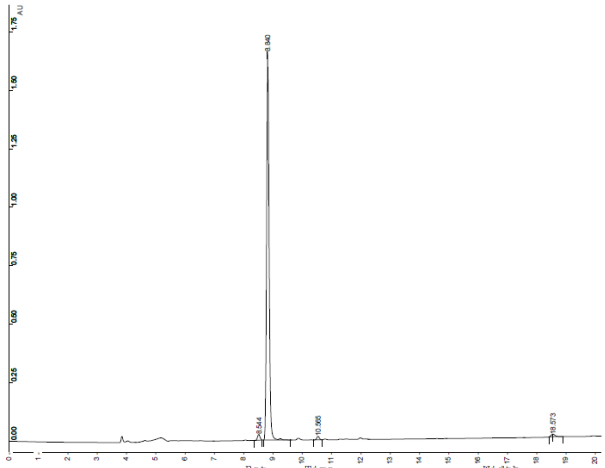
Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		96.0657	11.347	0.000	57804340	BB	5.9	
2		2.9183	13.587	0.000	1755982	BB	6.2	
3		0.3730	16.733	0.000	224417	BB	5.5	
4		0.6431	18.600	0.000	386963	BB	6.2	
Totals:		100.0001		0.000	60171702			

2d



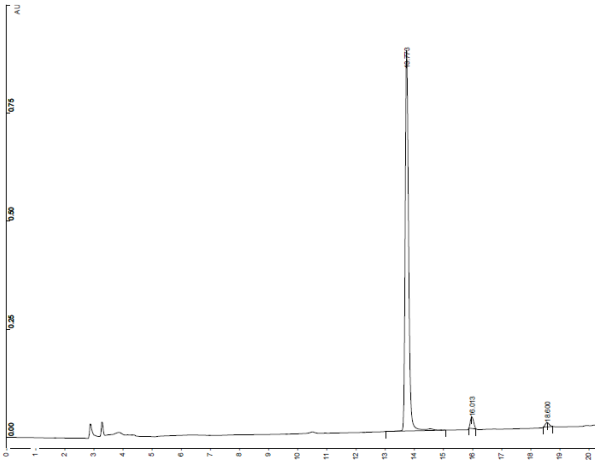
Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		98.1827	13.027	0.000	40740536	BB	6.9	
2		0.2331	14.600	0.000	96704	BB	10.1	
3		0.3325	15.293	0.000	137968	BB	7.3	
4		1.2517	18.600	0.000	519397	BB	10.2	
Totals:		100.0000		0.000	41494605			

2e



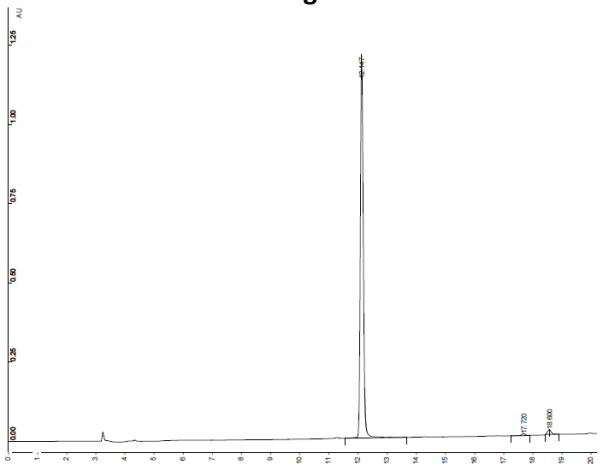
Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		1.5009	8.544	0.000	774520	BB	5.8	
2		96.6321	8.840	0.000	49865236	BB	5.3	
3		0.8841	10.565	0.000	456202	BB	5.9	
4		0.9829	18.573	0.000	507226	BB	8.5	
Totals:		100.0000		0.000	51603184			

2f



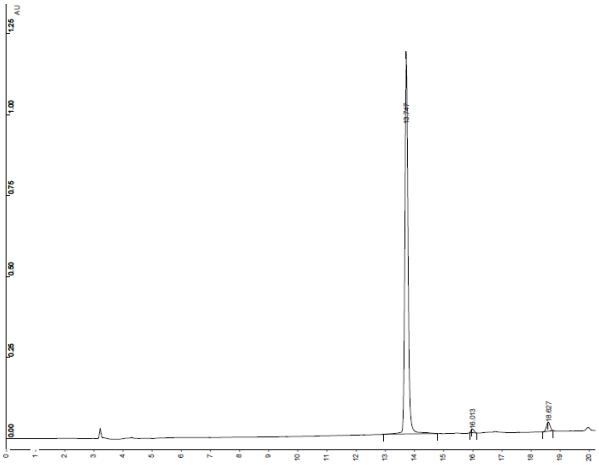
Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		96.0177	13.773	0.000	36967696	BB	7.5	
2		2.7512	16.013	0.000	1059229	BB	7.6	
3		1.2311	18.600	0.000	473992	BB	9.6	
Totals:		100.0000		0.000	38500917			

2g



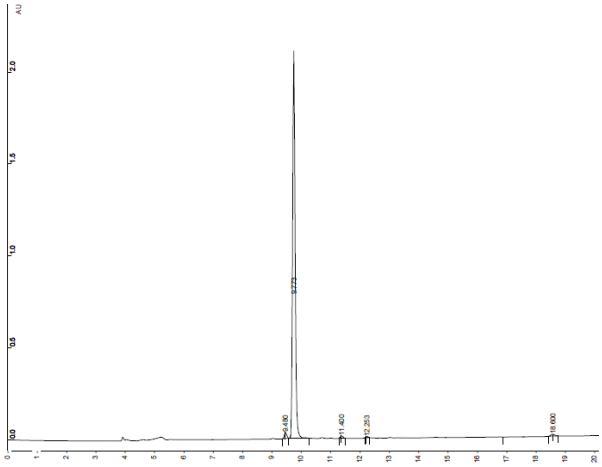
Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		97.7890	12.147	0.000	42870232	BB	6.3	
2		0.5891	17.720	0.000	258256	BB	8.5	
3		1.6219	18.600	0.000	711054	BB	8.5	
Totals:		100.0000		0.000	43839542			

2h



Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		96.5326	13.747	0.000	46073268	BB	6.8	
2		0.7994	16.013	0.000	381520	BB	6.3	
3		2.6680	18.627	0.000	1273387	BB	9.0	
Totals:		100.0000		0.000	47728175			

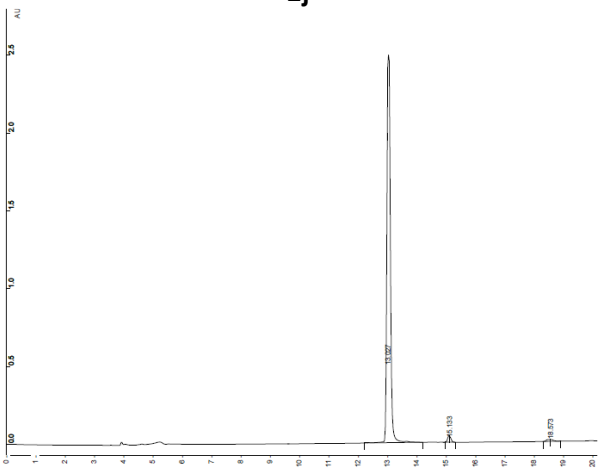
2i



Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		1.4721	9.480	0.000	962822	BB	5.6	
2		96.9483	9.773	0.000	63410260	BB	5.7	
3		0.6161	11.400	0.000	402969	BB	5.7	
4		0.2346	12.253	0.000	153422	BB	5.8	
5		0.7290	18.600	0.000	476796	BB	9.4	
Totals:		100.0001		0.000	65406269			

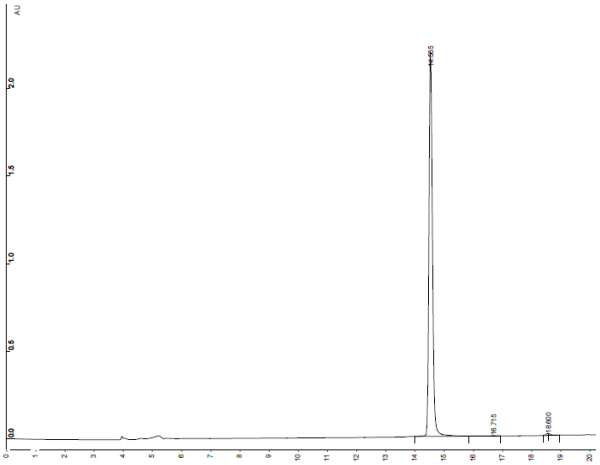
2k

2j

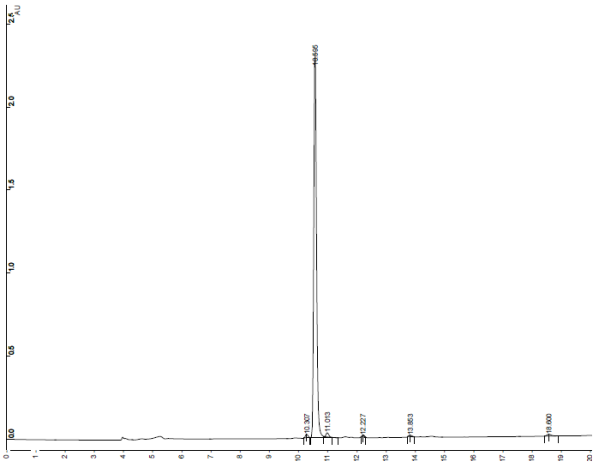


Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		97.2635	13.027	0.000	95985760	BB	8.6	
2		1.6886	15.133	0.000	1666373	BB	7.6	
3		1.0479	18.573	0.000	1034166	BB	16.3	
Totals:		100.0000		0.000	98686299			

2l



Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		99.1524	14.565	0.000	92721424	BB	8.3	
2		0.1784	16.715	0.000	166871	TS	0.0	
3		0.6691	18.600	0.000	625747	BB	10.6	
Totals:		99.9999		0.000	93514042			



Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		0.7888	10.307	0.000	606774	BB	5.7	
2		96.5614	10.595	0.000	74281040	BB	6.7	
3		1.0303	11.013	0.000	792609	TS	0.0	
4		0.4465	12.227	0.000	343466	BB	4.8	
5		0.4409	13.853	0.000	339162	BB	6.8	
6		0.7321	18.600	0.000	563182	BB	10.2	
Totals:		100.0000		0.000	76926233			

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