CHEMISTRY
A EUROPEAN JOURNAL

Supporting Information

Multistep Continuous-Flow Synthesis in Medicinal Chemistry: Discovery and Preliminary Structure–Activity Relationships of CCR8 Ligands

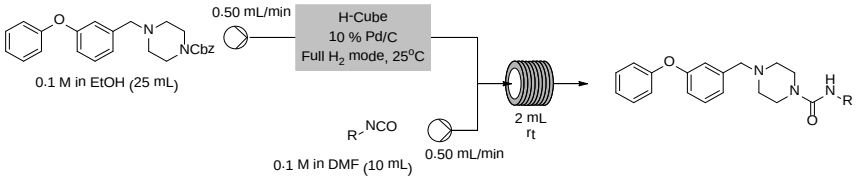
Trine P. Petersen,[a, b] Sahar Mirsharghi,[a] Pia C. Rummel,[c] Stefanie Thiele,[c] Mette M. Rosenkilde,[c] Andreas Ritzén,*[b, d] and Trond Ulven*[a]

chem_201204350_sm_miscellaneous_information.pdf

Contents

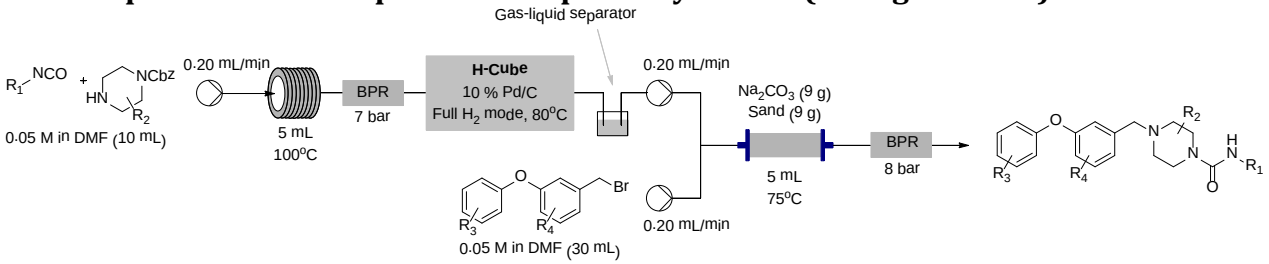
Experimental procedures

General procedure for 2-step flow synthesis



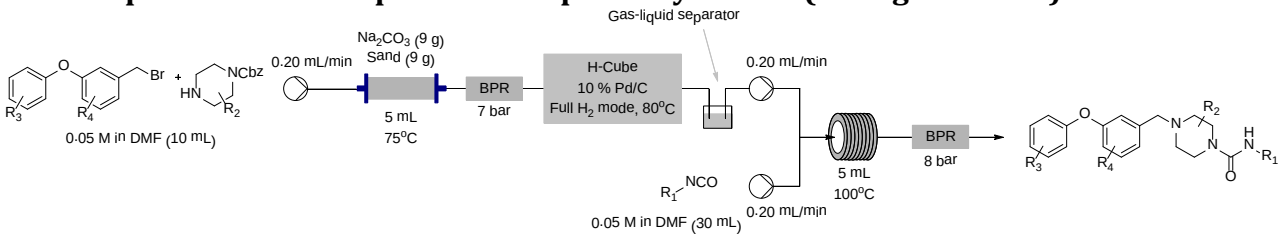
Benzyl 4-(3-phenoxybenzyl)piperazine-1-carboxylate (2.5 mmol) was dissolved in EtOH (25 mL). The solution was pumped at a flow rate of 0.50 mL/min through an H-Cube (catalyst: 10 % Pd/C 30x4 mm cartridge, full H_2 mode, 25°C). Isocyanate (1.0 mmol) was dissolved in DMF (10 mL) and pumped via a second pump (0.50 mL/min) to mix with the intermediate from the H-Cube. The mixture continued through a stainless steel coiled reactor (2 mL, rt). The product was collected, diluted with water (20 mL), extracted with EtOAc (3x15 mL), dried over $MgSO_4$ and filtered. The solvent was evaporated under reduced pressure.

General procedure for improved 3-step flow synthesis (Configuration I)



Cbz-protected diamine (0.50 mmol) and isocyanate (0.50 mmol) were dissolved in DMF (10.0 mL) in a pre-dried flask under argon. The solution was pumped at a flow rate of 0.20 mL/min through a coiled reactor (PFA, 5 mL, 100°C) followed by an H-Cube (catalyst: 10 % Pd/C 30x4 mm cartridge, full H_2 mode, 80°C). The product was collected in a flask (5 mL) to release excess H_2 and from there passed on via second pump (0.20 mL/min). Alkylating agent (1.5 mmol) was dissolved in DMF (30 mL) and pumped via a third pump (0.20 mL/min, started 40 min after the first pump) to mix with the intermediate from the second pump. The mixture continued through a glass column filled with Na_2CO_3/sand (9+9 g, 5 mL, 75°C). The solution was collected, the solvent evaporated under reduced pressure and the product purified by column chromatography using a semi-automatic Combiflash system (24 g silica, eluent gradient: Heptane/EtOAc)

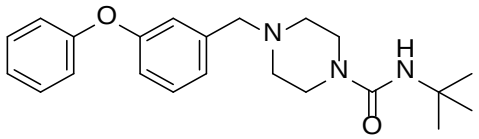
General procedure for improved 3-step flow synthesis (Configuration II)



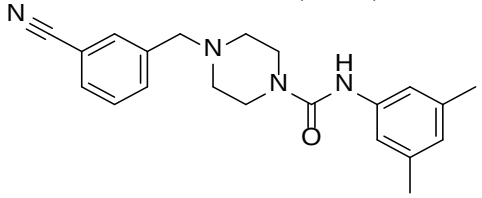
Cbz-protected diamine (0.50 mmol) and alkylating agent 0.50 mmol) were dissolved in DMF (10.0 mL). The solution was pumped at a flow rate of 0.20 mL/min through a glass column filled with Na_2CO_3/sand (9+9 g, 5 mL, 75°C) followed by an H-Cube (catalyst: 10 % Pd/C 30x4 mm cartridge, full H_2 mode, 80°C). The product was collected in a flask (5 mL) to release excess H_2 and from there passed on via second pump (0.20 mL/min). Isocyanate (1.5 mmol) was dissolved in DMF (30 mL) and pumped via a third pump (0.20 mL/min, started 40 min after the first pump) to mix with the intermediate from the second pump. The mixture continued through a coiled reactor (PFA, 5 mL, 100°C). The solution was collected, the solvent evaporated under reduced pressure and the

product purified by column chromatography using a semi-automatic Combiflash system (24 g silica, eluent gradient: Heptane/EtOAc).

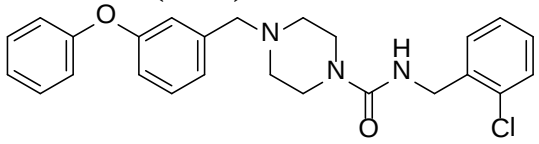
Compound characterization



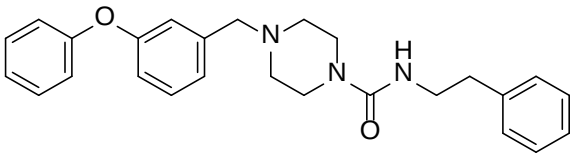
***N*-(*tert*-Butyl)-4-(3-phenoxybenzyl)piperazine-1-carboxamide (5)** was prepared according to general procedure (Configuration I) from benzyl piperazine-1-carboxylate (108.9 mg, 0.494 mmol), *tert*-butyl isocyanate(49.3 mg, 0.497 mmol) and 1-(bromomethyl)-3-phenoxybenzene (397.7 mg, 1.51 mmol) to afford the title compound (116.2 mg, 64%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33 (t, *J* = 7.9 Hz, 2H), 7.27 (t, *J* = 7.9 Hz, 1H), 7.09 (t, *J* = 7.4 Hz, 1H), 7.06 (d, *J* = 7.6 Hz, 1H), 7.04–6.98 (m, 3H), 6.89 (dd, *J* = 8.0, 1.6 Hz, 1H), 4.36 (s, 1H), 3.49 (s, 2H), 3.35–3.28 (m, 4H), 2.46–2.37 (m, 4H), 1.35 (s, 9H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 157.23, 157.20, 157.01, 140.05, 129.71, 129.52, 123.86, 123.18, 119.39, 118.76, 117.54, 62.50, 52.68, 50.68, 43.76, 29.46 ppm; HRMS calcd. for $C_{22}H_{30}N_3O_2$ (M+H): 368.2333, found: 368.2319.



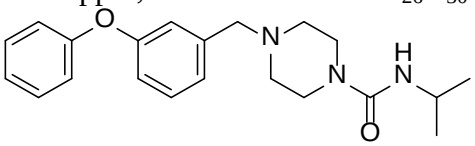
4-(3-Cyanobenzyl)-*N*-(3,5-dimethylphenyl)piperazine-1-carboxamide (6) was prepared according to general procedure (Configuration I) from benzyl piperazine-1-carboxylate (110.4 mg, 0.501 mmol), 1-isocyanato-3,5-dimethylbenzene (73.8 mg, 0.501 mmol) and 3-(bromomethyl)benzonitrile (294.1 mg, 1.50 mmol) to afford the title compound (156.6 mg, 90%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.65 (s, 1H), 7.56 (t, *J* = 7.7 Hz, 2H), 7.43 (t, *J* = 7.7 Hz, 1H), 6.98 (s, 2H), 6.66 (s, 1H), 6.54 (s, 1H), 3.53 (s, 2H), 3.51–3.45 (m, 4H), 2.46–2.40 (m, 4H), 2.25 (s, 6H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.24, 139.71, 138.91, 138.47, 133.40, 132.38, 131.04, 129.25, 124.93, 118.97, 117.99, 112.49, 61.96, 52.77, 44.12, 21.43 ppm; HRMS calcd. for $C_{21}H_{25}N_4O$ (M+H): 349.2023, found: 349.2016.



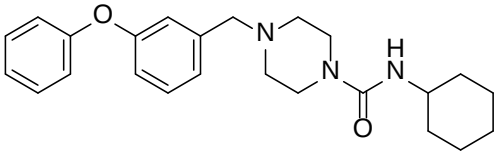
4-(3-Phenoxy-benzyl)-piperazine-1-carboxylic acid 2-chloro-benzylamide (15) was prepared according to the general procedure for the 2-step flow synthesis using 2-chlorobenzylisocyanate (126.1 mg, 1.01 mmol) to afford the title compound: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.37 (d, *J* = 7.4 Hz, 1H), 7.33–7.28 (m, 3H), 7.26 (t, *J* = 7.8 Hz, 1H), 7.21–7.14 (m, 2H), 7.08 (t, *J* = 7.4 Hz, 1H), 7.04 (d, *J* = 7.6 Hz, 1H), 7.01–6.97 (m, *J* = 6.3, 5.3 Hz, 3H), 6.88 (dd, *J* = 8.1, 2.4 Hz, 1H), 5.40 (s, 1H), 4.46 (d, *J* = 5.8 Hz, 2H), 3.47 (s, 2H), 3.39–3.35 (m, 4H), 2.42–2.36 (m, 4H) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 157.56, 157.22, 157.15, 139.93, 136.95, 133.27, 129.80, 129.72, 129.52, 129.27, 128.44, 126.95, 123.87, 123.19, 119.38, 118.75, 117.53, 77.37, 77.16, 76.95, 62.46, 52.60, 43.72, 42.59 ppm; HRMS calcd. For $C_{25}H_{27}ClN_3O_2$ (M+H): 436.1786, found: 436.1788.



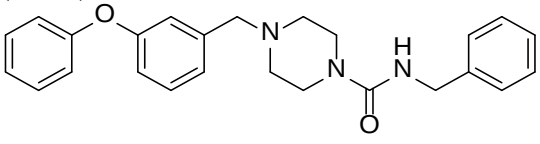
4-(3-Phenoxy-benzyl)-piperazine-1-carboxylic acid phenethyl-amide (16) was prepared according to the general procedure for the 2-step flow synthesis using (2-isocyanatoethyl)-benzene (150.2 mg, 1.02 mmol) to afford the title compound: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.35–7.25 (m, *J* = 30.8, 15.8, 7.7 Hz, 5H), 7.23–7.17 (m, *J* = 18.4, 7.6 Hz, 3H), 7.10 (t, *J* = 7.0 Hz, 1H), 7.05 (d, *J* = 7.6 Hz, 1H), 7.02–6.98 (m, *J* = 8.7 Hz, 3H), 6.89 (dd, *J* = 8.1, 2.4 Hz, 1H), 4.67 (s, 1H), 3.48 (s, 2H), 3.48–3.45 (m, 2H), 3.33–3.29 (m, 4H), 2.81 (t, *J* = 7.0 Hz, 2H), 2.41–2.37 (m, 4H) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 157.66, 157.28, 157.21, 140.00, 139.49, 129.77, 129.58, 128.89, 128.57, 126.36, 123.91, 123.25, 119.45, 118.81, 117.59, 77.37, 77.16, 76.95, 62.55, 52.66, 43.67, 42.11, 36.39 ppm; HRMS calcd. for $C_{26}H_{30}N_3O_2$ (M+H): 416.2333, found: 416.2345.



4-(3-Phenoxy-benzyl)-piperazine-1-carboxylic acid isopropylamide (17) was prepared according to the general procedure for the 2-step flow synthesis using isopropylisocyanate (126.1 mg, 1.01 mmol) to afford the title compound: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.34 (t, *J* = 7.9 Hz, 2H), 7.30–7.26 (m, *J* = 9.1, 6.6 Hz, 1H), 7.11 (t, *J* = 7.2 Hz, 1H), 7.07 (d, *J* = 7.6 Hz, 1H), 7.04–7.00 (m, 3H), 6.90 (dd, *J* = 8.1, 2.2 Hz, 1H), 4.27 (s, 1H), 3.98 (dq, *J* = 13.4, 6.6 Hz, 4H), 3.50 (s, 2H), 3.37–3.32 (m, 4H), 2.45–2.40 (m, 4H), 1.16 (d, *J* = 6.5 Hz, 6H) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 157.34, 157.29, 157.19, 140.07, 129.82, 129.63, 123.98, 123.30, 119.52, 118.87, 117.67, 77.37, 77.16, 76.95, 62.62, 52.74, 43.75, 42.61, 23.56 ppm; HRMS calcd. for $C_{21}H_{28}N_3O_2$ (M+H): 354.2176, found: 354.2183.

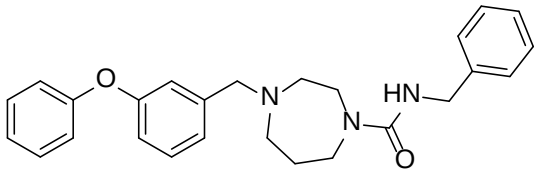


4-(3-Phenoxy-benzyl)-piperazine-1-carboxylic acid cyclohexylamide (18) was prepared according to the general procedure for the 2-step flow synthesis using cyclohexaneisocyanate (126.1 mg, 1.01 mmol) to afford the title compound: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.29–7.23 (m, 2H), 7.19 (t, *J* = 7.8 Hz, 1H), 7.05–7.00 (m, *J* = 7.4 Hz, 1H), 6.98 (d, *J* = 7.5 Hz, 1H), 6.96–6.89 (m, *J* = 7.8, 6.9 Hz, 3H), 6.83–6.78 (m, 1H), 4.54 (d, *J* = 7.3 Hz, 1H), 3.59–3.50 (m, 1H), 3.41 (s, 2H), 3.30–3.24 (m, 4H), 2.37–2.31 (m, 4H), 1.89–1.81 (m, 2H), 1.67–1.58 (m, *J* = 10.1, 3.4 Hz, 2H), 1.57–1.49 (m, 1H), 1.34–1.21 (m, *J* = 12.5 Hz, 2H), 1.10–0.97 (m, 3H) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 157.09, 157.06, 140.00, 129.62, 129.41, 123.78, 123.08, 119.27, 118.63, 117.40, 77.37, 77.16, 76.95, 62.43, 52.59, 49.34, 43.60, 33.79, 25.57, 25.05 ppm; HRMS calcd. for $C_{24}H_{32}N_3O_2$ (M+H): 394.2489, found: 394.2489.

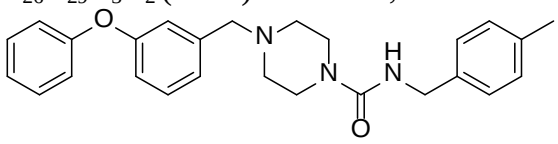


4-(3-Phenoxy-benzyl)-piperazine-1-carboxylic acid benzylamide (19) was prepared according to general procedure (Configuration I) from benzyl piperazine-1-carboxylate (110.1 mg, 0.500 mmol), benzyl isocyanate (68.8 mg, 0.517 mmol) and 1-(bromomethyl)-3-phenoxybenzene (397.5 mg, 1.511 mmol) to afford the title compound (188.3 mg, 94%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.32–7.28 (m, 2H), 7.26 (d, *J* = 6.9 Hz, 2H), 7.22–7.19 (m, 1H), 7.07 (tt, *J* = 7.6, 1.1 Hz, 1H), 7.03 (d, *J* = 7.7 Hz, 1H), 7.01–6.99 (m, 1H), 6.99–6.96 (m, *J* = 4.5, 3.3, 1.8 Hz, 2H), 6.87 (ddd, *J* = 8.1, 2.5, 0.8

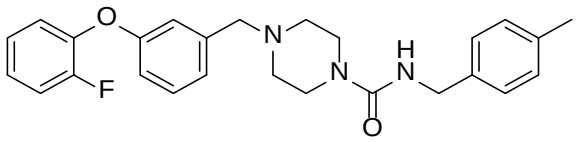
Hz, 1H), 5.16 (t, *J* = 5.6 Hz, 1H), 4.35 (d, *J* = 5.6 Hz, 2H), 3.44 (s, 2H), 3.37–3.29 (m, 4H), 2.39–2.31 (m, 4H) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 157.67, 157.22, 157.15, 139.98, 139.61, 129.72, 129.53, 128.49, 127.55, 127.12, 123.84, 123.20, 119.37, 118.76, 117.53, 62.47, 52.62, 44.75, 43.73 ppm; HRMS calcd. for $C_{25}H_{27}N_3O_2$ (M+H): 402.2176, found: 402.2174.



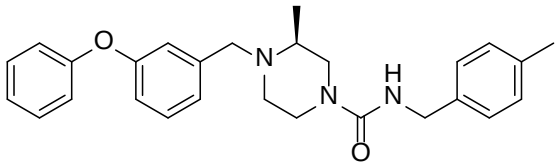
4-(3-Phenoxy-benzyl)-[1,4]diazepane-1-carboxylic acid benzylamide (20) was prepared according to general procedure (Configuration I) from benzyl 1-homopiperazine carboxylate (117.2 mg, 0.500 mmol), benzyl isocyanate (69.1 mg, 0.519 mmol) and 1-(bromomethyl)-3-phenoxybenzene (394.9 mg, 1.501 mmol) to afford the title compound (102.5 mg, 49 %): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.33–7.27 (m, 7H), 7.25–7.22 (m, 2H), 7.09–7.06 (m, *J* = 8.5, 2.1, 1.1 Hz, 1H), 7.05 (d, *J* = 7.6 Hz, 1H), 7.03–7.01 (m, 1H), 7.00–6.97 (m, 2H), 6.89–6.86 (m, 1H), 4.76 (t, *J* = 5.4 Hz, 1H), 4.41 (d, *J* = 5.5 Hz, 2H), 3.59 (s, 2H), 3.52 (s, 2H), 3.44 (t, *J* = 6.0 Hz, 2H), 2.68–2.63 (m, 2H), 2.63–2.58 (m, 2H), 1.88–1.79 (m, 2H) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 157.51, 157.24, 157.18, 140.93, 136.82, 136.56, 129.73, 129.51, 129.23, 127.75, 123.76, 123.17, 119.37, 118.71, 117.38, 57.58, 54.87, 50.36, 44.64, 43.93, 40.90, 21.11, 15.76 ppm; HRMS calcd. for $C_{26}H_{29}N_3O_2$ (M+H): 416.2333, found: 416.2331.



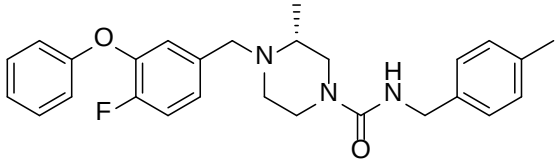
4-(3-Phenoxy-benzyl)-piperazine-1-carboxylic acid 4-methyl-benzylamide (21) was prepared according to general procedure (Configuration I) from benzyl piperazine-1-carboxylate (111.3 mg, 0.505 mmol), 4-methylbenzyl isocyanate (73.9 mg, 0.502 mmol) and 1-(bromomethyl)-3-phenoxybenzene (397.9 mg, 1.512 mmol) to afford the title compound (177.4 mg, 85%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.37–7.32 (m, 2H), 7.29 (t, *J* = 7.8 Hz, 1H), 7.19 (d, *J* = 8.1 Hz, 2H), 7.14–7.10 (m, 3H), 7.07 (d, *J* = 7.6 Hz, 1H), 7.05–7.03 (m, 1H), 7.03–7.00 (m, 2H), 6.93–6.90 (m, 1H), 5.15 (t, *J* = 5.5 Hz, 1H), 4.35 (d, *J* = 5.5 Hz, 2H), 3.48 (s, 2H), 3.40–3.33 (m, 4H), 2.43–2.36 (m, 4H), 2.33 (s, 3H) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 157.64, 157.20, 157.13, 139.95, 136.67, 136.54, 129.70, 129.51, 129.14, 127.62, 123.82, 123.18, 119.34, 118.74, 117.51, 62.45, 52.60, 44.53, 43.68, 21.06 ppm; HRMS calcd. for $C_{26}H_{29}N_3O_2$ (M+H): 416.2333, found: 416.2331.



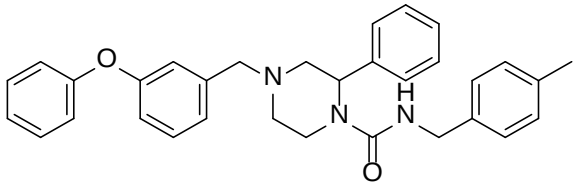
4-(3-(2-Fluorophenoxy)benzyl)-*N*-(4-methylbenzyl)piperazine-1-carboxamide (22) was prepared according to general procedure (Configuration I) from benzyl piperazine-1-carboxylate (109.4 mg, 0.497 mmol), 4-methylbenzyl isocyanate (73.6 mg, 0.500 mmol) and 1-(3-(bromomethyl)phenoxy)-2-fluorobenzene (421.3 mg, 1.50 mmol) to afford the title compound (198.0 mg, 92%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28 (t, *J* = 7.9 Hz, 1H), 7.23–7.17 (m, 3H), 7.17–7.11 (m, *J* = 11.9, 4.9 Hz, 4H), 7.10–7.05 (m, *J* = 13.0, 5.0 Hz, 2H), 7.02 (s, 1H), 6.88 (dd, *J* = 8.1, 2.0 Hz, 1H), 4.99 (t, *J* = 5.3 Hz, 1H), 4.38 (d, *J* = 5.4 Hz, 2H), 3.50 (s, 2H), 3.42–3.36 (m, 4H), 2.47–2.39 (m, 4H), 2.35 (s, 3H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 157.67 (s), 157.47 (s), 154.35 (d, *J* = 248.6 Hz), 143.72 (d, *J* = 11.3 Hz), 140.02 (s,), 136.85 (s), 136.53 (s), 129.54 (s), 129.25 (s), 127.76 (s), 124.83 (d, *J* = 6.9 Hz), 124.72 (d, *J* = 3.6 Hz), 123.76 (s), 121.85 (s), 117.82 (s), 117.09 (d, *J* = 18.2 Hz), 115.96 (s), 62.48 (s), 52.66 (s), 44.69 (s), 43.78 (s), 21.11 (s) ppm; HRMS calcd. for $C_{26}H_{29}FN_3O_2$ (M+H): 434.2238, found: 434.2233.



(*S*)-3-Methyl-4-(3-phenoxy-benzyl)-piperazine-1-carboxylic acid 4-methyl-benzylamide (23) was prepared according to general procedure (Configuration I) from (*S*)-benzyl 2-methylpiperazine-1-carboxylate (115.0 mg, 0.491 mmol), 4-methylbenzyl isocyanate (73.4 mg, 0.499 mmol) and 1-(bromomethyl)-3-phenoxybenzene (395.4 mg, 1.503 mmol) to afford the title compound (131.1 mg, 62%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.36–7.31 (m, 2H), 7.27 (t, *J* = 7.8 Hz, 1H), 7.19 (d, *J* = 8.1 Hz, 2H), 7.14–7.08 (m, 3H), 7.07 (d, *J* = 7.6 Hz, 1H), 7.05–7.03 (m, 1H), 7.02–6.99 (m, 2H), 6.90–6.87 (m, 1H), 4.97 (t, *J* = 5.4 Hz, 1H), 4.35 (dd, *J* = 5.4, 1.3 Hz, 2H), 3.98 (d, *J* = 13.6 Hz, 1H), 3.68–3.61 (m, 1H), 3.55 (d, *J* = 12.5 Hz, 1H), 3.17 (d, *J* = 13.6 Hz, 1H), 3.06 (ddd, *J* = 12.8, 10.0, 3.1 Hz, 1H), 2.83 (dd, *J* = 12.7, 8.9 Hz, 1H), 2.68 (dt, *J* = 7.2, 3.4 Hz, 1H), 2.44 (dqd, *J* = 9.4, 6.2, 3.3 Hz, 1H), 2.33 (s, 3H), 2.11 (ddd, *J* = 11.6, 10.1, 3.3 Hz, 1H), 1.11 (d, *J* = 6.2 Hz, 3H) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 157.51, 157.24, 157.18, 140.93, 136.82, 136.56, 129.73, 129.51, 129.23, 127.75, 123.76, 123.17, 119.37, 118.71, 117.38, 57.58, 54.87, 50.36, 44.64, 43.93, 40.90, 21.11, 15.76 ppm; HRMS calcd. for $C_{27}H_{31}N_3O_2$ (M+H): 430.2489, found: 430.2489.

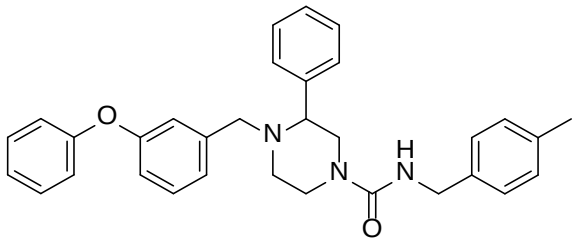


(*R*)-4-(4-Fluoro-3-phenoxy-benzyl)-3-methyl-piperazine-1-carboxylic acid 4-methyl-benzylamide (24) was prepared according to general procedure (Configuration I) from (*R*)-benzyl 2-methylpiperazine-1-carboxylate (116.6 mg, 0.498 mmol), 4-methylbenzyl isocyanate (73.6 mg, 0.500 mmol) and 4-(bromomethyl)-1-fluoro-2-phenoxybenzene (422.9 mg, 1.504 mmol) to afford the title compound (193.6 mg, 87 %): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.34–7.30 (m, 2H), 7.18 (d, *J* = 8.1 Hz, 2H), 7.14–7.06 (m, 6H), 6.98–6.95 (m, 2H), 4.97 (t, *J* = 5.4 Hz, 1H), 4.39–4.31 (m, 2H), 3.92 (d, *J* = 13.6 Hz, 1H), 3.63 (ddd, *J* = 12.6, 2.9, 1.3 Hz, 1H), 3.54 (d, *J* = 12.6 Hz, 1H), 3.11 (d, *J* = 13.6 Hz, 1H), 3.05 (ddd, *J* = 12.8, 9.9, 3.1 Hz, 1H), 2.82 (dd, *J* = 12.7, 8.8 Hz, 1H), 2.63 (dt, *J* = 7.3, 3.3 Hz, 1H), 2.42 (dqd, *J* = 9.4, 6.2, 3.3 Hz, 1H), 2.33 (s, 3H), 2.08 (ddd, *J* = 11.6, 9.9, 3.3 Hz, 1H), 1.08 (d, *J* = 6.3 Hz, 3H) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 157.55 (s), 157.53 (s), 153.40 (d, *J* = 247.9 Hz), 143.22 (d, *J* = 11.7 Hz), 136.88 (s), 136.59 (s), 135.85 (d, *J* = 3.5 Hz), 129.75 (s), 129.28 (s), 127.79 (s), 125.05 (d, *J* = 6.7 Hz), 123.06 (s), 122.34 (s), 117.00 (s), 116.76 (d, *J* = 18.3 Hz), 57.05 (s), 54.90 (s), 50.38 (s), 50.22 (s), 44.70 (s), 43.96 (s), 21.15 (s), 15.69 (s) ppm; HRMS calcd. for $C_{27}H_{30}FN_3O_2$ (M+H): 448.2395, found: 448.2395.

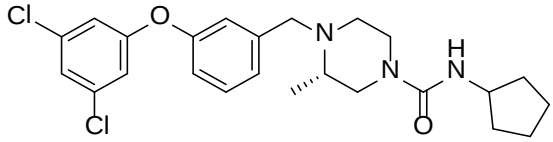


***N*-(4-Methylbenzyl)-4-(3-phenoxybenzyl)-2-phenylpiperazine-1-carboxamide (25)** was prepared according to general procedure (Configuration I) from benzyl 3-phenylpiperazine-1-carboxylate (145.5 mg, 0.491 mmol), 4-methylbenzyl isocyanate (74.3 mg, 0.505 mmol) and 1-(bromomethyl)-3-phenoxybenzene (393.3 mg, 1.50 mmol) to afford the title compound (159.2 mg, 66%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34–7.28 (m, *J* = 8.0 Hz, 4H), 7.26–7.19 (m, *J* = 9.1, 6.2 Hz, 4H), 7.11–7.05 (m, 5H), 6.97 (d, *J* = 8.1 Hz, 3H), 6.93 (s, 1H), 6.87 (dd, *J* = 8.1, 1.5 Hz, 1H), 5.10 (s, 1H), 4.82 (t, *J* = 5.3 Hz, 1H), 4.34 (d, *J* = 5.4 Hz, 2H), 3.73 (d, *J* = 13.0 Hz, 1H), 3.51 (d, *J* =

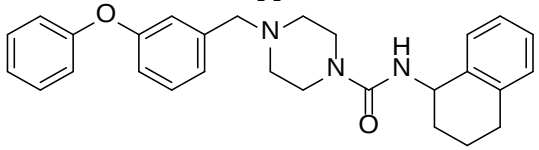
13.3 Hz, 1H), 3.38 (d, *J* = 13.3 Hz, 1H), 3.25–3.14 (m, 2H), 2.76 (d, *J* = 10.9 Hz, 1H), 2.43 (dd, *J* = 11.7, 4.1 Hz, 1H), 2.30 (s, 3H), 2.20 (td, *J* = 11.4, 3.4 Hz, 1H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 158.09, 157.68, 140.53, 140.45, 137.25, 136.85, 130.20, 129.94, 129.66, 128.85, 128.05, 127.85, 127.42, 124.29, 123.64, 119.82, 119.21, 118.16, 63.02, 56.21, 54.23, 53.56, 45.19, 40.96, 21.55 ppm; HRMS calcd. for $C_{32}H_{34}N_3O_2$ (M+H): 492.2646, found: 492.2645.



***N*-(4-Methylbenzyl)-4-(3-phenoxybenzyl)-3-phenylpiperazine-1-carboxamide (26)** was prepared according to general procedure (Configuration I) from benzyl 2-phenylpiperazine-1-carboxylate (148.2 mg, 0.500 mmol), 4-methylbenzyl isocyanate (75.7 mg, 0.514 mmol) and 1-(bromomethyl)-3-phenoxybenzene (398.2 mg, 1.51 mmol) to afford the title compound (140.5 mg, 57%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41 (d, *J* = 7.3 Hz, 2H), 7.32 (dd, *J* = 15.0, 7.1 Hz, 4H), 7.27 (d, *J* = 7.2 Hz, 1H), 7.22 (t, *J* = 7.8 Hz, 1H), 7.18 (d, *J* = 7.9 Hz, 2H), 7.14–7.08 (m, 3H), 6.99 (d, *J* = 8.0 Hz, 3H), 6.95 (s, 1H), 6.84 (dd, *J* = 8.1, 2.0 Hz, 1H), 4.70 (t, *J* = 5.2 Hz, 1H), 4.35 (d, *J* = 5.3 Hz, 1H), 3.86 (dd, *J* = 12.7, 1.7 Hz, 1H), 3.76 (dd, *J* = 13.1, 4.1 Hz, 1H), 3.26 (dd, *J* = 10.5, 3.0 Hz, 1H), 2.99 (td, *J* = 12.5, 2.3 Hz, 1H), 2.92–2.80 (m, 2H), 2.32 (s, 2H), 2.12 (td, *J* = 11.8, 2.9 Hz, 1H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 157.37, 157.27, 140.89, 140.73, 137.13, 136.40, 129.84, 129.54, 129.41, 128.88, 128.03, 127.98, 123.58, 123.35, 119.09, 118.97, 117.33, 66.97, 58.61, 51.80, 51.54, 44.89, 44.11, 21.21 ppm; HRMS calcd. for $C_{32}H_{34}N_3O_2$ (M+H): 492.2646, found: 492.2635.

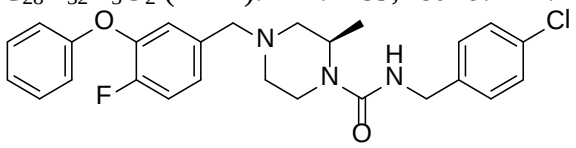


(*S*)-*N*-Cyclopentyl-4-(3-(3,5-dichlorophenoxy)benzyl)-3-methylpiperazine-1-carboxamide (27) was prepared according to the general procedure (Configuration I) from (*S*)-benzyl 2-methyl-piperazine-1-carboxylate (115.9 mg, 0.495 mmol), isocyanatocyclopentane (60.3 mg, 0.542 mmol) and 1-(3-(bromomethyl)phenoxy)-3,5-dichlorobenzene (486.8 mg, 1.47 mmol) to afford the title compound (142.0 mg, 62%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32 (t, *J* = 7.8 Hz, 1H), 7.15 (d, *J* = 7.6 Hz, 1H), 7.06 (dd, *J* = 4.4, 2.7 Hz, 2H), 6.91 (dd, *J* = 8.1, 1.9 Hz, 1H), 6.85 (d, *J* = 1.6 Hz, 2H), 4.34 (d, *J* = 6.7 Hz, 1H), 4.09 (h, *J* = 6.9 Hz, 1H), 4.00 (d, *J* = 13.7 Hz, 1H), 3.61 (dd, *J* = 12.6, 1.4 Hz, 1H), 3.51 (d, *J* = 12.6 Hz, 1H), 3.20 (d, *J* = 13.7 Hz, 1H), 3.09–3.01 (m, 1H), 2.81 (dd, *J* = 12.6, 8.9 Hz, 1H), 2.68 (dt, *J* = 11.5, 3.5 Hz, 1H), 2.50–2.42 (m, 1H), 2.13 (td, *J* = 11.6, 3.2 Hz, 1H), 1.99 (td, *J* = 11.9, 6.4 Hz, 2H), 1.71–1.62 (m, 2H), 1.61–1.54 (m, 2H), 1.33 (td, *J* = 13.4, 6.9 Hz, 2H), 1.12 (d, *J* = 6.2 Hz, 3H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 159.12, 157.48, 155.56, 141.74, 135.66, 130.00, 125.26, 123.07, 120.25, 118.48, 116.78, 57.63, 55.07, 52.64, 50.51, 50.47, 44.01, 33.72, 23.79, 15.93 ppm; HRMS calcd. for $C_{24}H_{30}Cl_2N_3O_2$ (M+H): 462.1710, found: 462.1707.

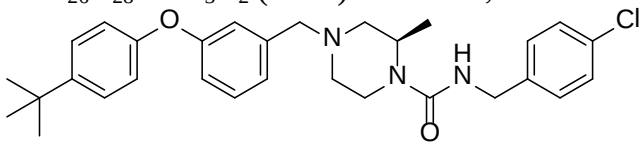


4-(3-Phenoxybenzyl)-*N*-(1,2,3,4-tetrahydronaphthalen-1-yl)piperazine-1-carboxamide (28) was prepared according to general procedure (Configuration I) from benzyl piperazine-1-carboxylate (109.6 mg, 0.497 mmol), 1-isocyanato-1,2,3,4-tetrahydronaphthalene (87.5 mg, 0.505 mmol) and 1-(bromomethyl)-3-phenoxybenzene (393.9 mg, 1.50 mmol) to afford the title

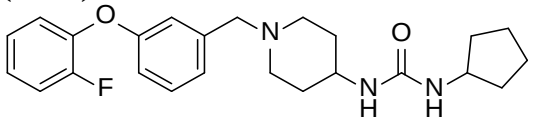
compound (185.6 mg, 84%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36 (t, *J* = 7.2 Hz, 3H), 7.30 (dd, *J* = 13.1, 5.1 Hz, 1H), 7.21–7.16 (m, 2H), 7.12 (dt, *J* = 11.2, 7.3 Hz, 3H), 7.08–7.01 (m, 3H), 6.93 (d, *J* = 8.1 Hz, 1H), 5.15–5.08 (m, *J* = 6.6 Hz, 1H), 4.81 (d, *J* = 8.0 Hz, 1H), 3.53 (s, 2H), 3.43–3.32 (m, 4H), 2.89–2.72 (m, 2H), 2.46 (t, *J* = 4.7 Hz, 4H), 2.08 (dd, *J* = 12.8, 8.3 Hz, 1H), 1.85 (d, *J* = 4.7 Hz, 3H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 157.23, 157.20, 157.01, 140.05, 129.71, 129.52, 123.86, 123.18, 119.39, 118.76, 117.54, 62.50, 52.68, 50.68, 43.76, 29.46 ppm; HRMS calcd. for $C_{28}H_{32}N_3O_2$ (M+H): 442.2489, found: 442.2488.



(*R*)-*N*-(4-Chlorobenzyl)-4-(4-fluoro-3-phenoxybenzyl)-2-methylpiperazine-1-carboxamide (29) was prepared according to general procedure (Configuration II) from (*R*)-benzyl 2-methylpiperazine-1-carboxylate (116.5 mg, 0.497 mmol), 4-(bromomethyl)-1-fluoro-2-phenoxybenzene (149.7 mg, 0.533 mmol) and 4-chlorobenzyl isocyanate (258.9 mg, 1.55 mmol) to afford the title compound (184.0 mg, 79%): ^{1}H NMR (500 MHz, DMSO) δ 7.35–7.29 (m, 5H), 7.22 (t, *J* = 7.7 Hz, 3H), 7.12–7.04 (m, 3H), 6.99 (t, *J* = 5.7 Hz, 1H), 6.95 (d, *J* = 8.3 Hz, 2H), 4.23–4.11 (m, 1H), 4.05 (s, 1H), 3.65 (d, *J* = 12.7 Hz, 1H), 3.47 (d, *J* = 13.7 Hz, 1H), 3.28 (d, *J* = 13.7 Hz, 1H), 2.93–2.84 (m, 1H), 2.70 (d, *J* = 10.3 Hz, 1H), 2.47 (s, 1H), 1.96 (dd, *J* = 11.0, 3.3 Hz, 1H), 1.88 (td, *J* = 11.5, 2.8 Hz, 1H), 1.01 (d, *J* = 6.5 Hz, 1H) ppm; ^{13}C NMR (126 MHz, DMSO) δ 158.90 (s), 157.75 (d, *J* = 46.9 Hz), 153.21 (d, *J* = 245.5 Hz), 143.71 (d, *J* = 11.9 Hz), 141.08 (s), 140.87 (s, *J* = 26.8 Hz), 136.74 (d, *J* = 2.4 Hz), 131.84 (d, *J* = 19.9 Hz), 130.90 (s), 129.69 (s), 128.93 (d, *J* = 14.2 Hz), 125.72 (s, *J* = 6.6 Hz), 124.29 (s), 121.97 (s), 118.07 (s), 117.62 (d, *J* = 18.2 Hz), 61.41 (s), 57.63 (s), 53.72 (s), 46.97 (s), 43.70 (s), 43.17 (s), 16.08 (s) ppm; HRMS calcd. for $C_{26}H_{28}ClFN_3O_2$ (M+H): 468.1849, found: 468.1848.

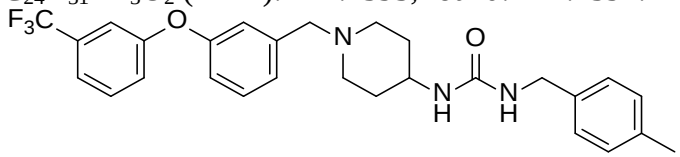


(*R*)-4-(3-(4-(*tert*-Butyl)phenoxy)benzyl)-*N*-(4-chlorobenzyl)-2-methylpiperazine-1-carboxamide (30) was prepared according to general procedure (Configuration II) from (*R*)-benzyl 2-methylpiperazine-1-carboxylate (116.6 mg, 0.498 mmol), 1-(bromomethyl)-3-(4-(*tert*-butyl)phenoxy)benzene (162.3 mg, 0.508 mmol) and 4-chlorobenzyl isocyanate (259.8 mg, 1.55 mmol) to afford the title compound (224.5 mg, 89%): ^{1}H NMR (500 MHz, DMSO) δ 7.39 (d, *J* = 8.6 Hz, 2H), 7.35 (d, *J* = 8.3 Hz, 2H), 7.26 (d, *J* = 8.1 Hz, 2H), 7.03 (d, *J* = 6.7 Hz, 2H), 6.95 (d, *J* = 8.3 Hz, 3H), 6.89 (d, *J* = 7.9 Hz, 1H), 4.22 (qd, *J* = 15.6, 5.7 Hz, 2H), 4.10 (s, 1H), 3.70 (d, *J* = 12.6 Hz, 1H), 3.53 (d, *J* = 13.9 Hz, 1H), 3.34 (d, *J* = 13.7 Hz, 1H), 2.92 (t, *J* = 11.3 Hz, 1H), 2.74 (d, *J* = 10.6 Hz, 1H), 2.55 (d, *J* = 11.0 Hz, 1H), 2.01 (dd, *J* = 10.8, 2.8 Hz, 1H), 1.92 (t, *J* = 10.2 Hz, 1H), 1.28 (s, 9H), 1.05 (d, *J* = 6.5 Hz, 3H) ppm; ^{13}C NMR (126 MHz, DMSO) δ 157.42, 157.04, 153.96, 145.89, 140.64, 140.24, 130.87, 129.66, 128.80, 127.97, 126.63, 122.86, 118.63, 117.28, 116.69, 61.22, 56.91, 52.91, 46.12, 42.80, 38.54, 34.00, 31.21, 15.23 ppm; HRMS calcd. for $C_{30}H_{37}ClN_3O_2$ (M+H): 506.2569, found: 506.2562.

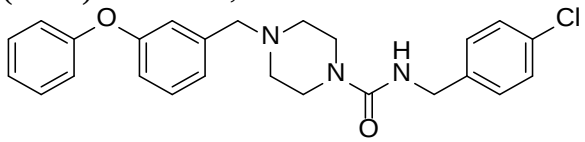


1-Cyclopentyl-3-(1-(3-(2-fluorophenoxy)benzyl)piperidin-4-yl)urea (31) was prepared according to general procedure (Configuration II) from benzyl piperidin-4-ylcarbamate·HCl (135.6 mg, 0.501 mmol), 1-(3-(bromomethyl)phenoxy)-2-fluorobenzene (149.0 mg, 0.530 mmol) and isocyanatocyclopentane (166.9 mg, 1.50 mmol) to afford the title compound (102.6 mg, 50%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.22 (t, *J* = 7.8 Hz, 1H), 7.18–7.11 (m, 1H), 7.11–7.05 (m, 2H), 7.04–

7.00 (m, *J* = 8.7, 5.1 Hz, 2H), 6.98 (s, 1H), 6.81 (dd, *J* = 8.0, 1.3 Hz, 1H), 5.16 (dd, *J* = 42.4, 7.2 Hz, 2H), 4.03–3.92 (m, 1H), 3.58 (d, *J* = 7.6 Hz, 1H), 3.44 (s, 2H), 2.77 (d, *J* = 11.0 Hz, 2H), 2.09 (t, *J* = 10.9 Hz, 2H), 1.97–1.85 (m, 4H), 1.68–1.51 (m, 4H), 1.46–1.30 (m, 4H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 157.96 (s), 157.30 (s), 154.30 (d, *J* = 248.5 Hz), 143.92 (d, *J* = 11.3 Hz), 140.88 (s), 129.41 (s), 124.68 (s), 124.63 (s), 123.82 (s), 121.68 (s), 118.06 (s), 117.05 (d, *J* = 18.1 Hz), 115.81 (s), 62.68 (s), 52.48 (s), 51.86 (s), 46.99 (s), 33.63 (s), 33.13 (s), 23.78 (s) ppm; HRMS calcd. for $C_{24}H_{31}FN_3O_2$ (M+H): 412.2395, found: 412.2397.



1-(4-Methylbenzyl)-3-(1-(3-(3-(trifluoromethyl)phenoxy)benzyl)piperidin-4-yl)urea (32) was prepared according to general procedure (Configuration II) from benzyl piperidin-4-yl-carbamate·HCl (134.9 mg, 0.498 mmol), 1-(bromomethyl)-3-(3-(trifluoromethyl)phenoxy)benzene (167.2 mg, 0.505 mmol) and 4-methylbenzyl isocyanate (219.5 mg, 1.49 mmol) to afford the title compound (202.3 mg, 82%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40 (t, *J* = 8.0 Hz, 1H), 7.33–7.24 (m, 2H), 7.21 (s, 1H), 7.13 (dd, *J* = 8.2, 1.5 Hz, 1H), 7.10–7.06 (m, *J* = 7.8 Hz, 3H), 7.05–7.00 (m, *J* = 13.5, 6.0 Hz, 3H), 6.88 (dd, *J* = 8.0, 2.1 Hz, 1H), 5.40 (s, 1H), 5.01 (d, *J* = 6.8 Hz, 1H), 4.18 (dd, *J* = 13.4, 5.6 Hz, 2H), 3.49 (d, *J* = 7.5 Hz, 1H), 3.41 (s, 2H), 2.68 (d, *J* = 10.5 Hz, 2H), 2.27 (s, *J* = 3.4 Hz, 3H), 2.00 (t, *J* = 10.7 Hz, 2H), 1.79 (d, *J* = 10.5 Hz, 2H), 1.32 (dd, *J* = 20.3, 10.6 Hz, 2H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 158.86 (s), 158.08 (d, *J* = 12.7 Hz), 156.17 (s), 141.37 (s), 136.73 (s), 136.47 (s), 132.21 (d, *J* = 32.6 Hz), 130.36 (s), 129.82 (s), 129.28 (s), 127.40 (s), 124.87 (s), 122.73 (s), 121.54 (s), 119.97 (s), 119.56 (d, *J* = 3.5 Hz), 118.01 (s), 115.15 (d, *J* = 3.5 Hz), 62.65 (s), 52.40 (s), 47.20 (s), 44.09 (s), 32.94 (s), 21.11 (s) ppm; HRMS calcd. for $C_{28}H_{31}F_3N_3O_2$ (M+H): 498.2363, found: 498.2369.



4-(3-Phenoxy-benzyl)-piperazine-1-carboxylic acid 4-chloro-benzylamide (33) was prepared according to general procedure (Configuration II) from benzyl piperazine-1-carboxylate (112.8 mg, 0.512 mmol), 1-(bromomethyl)-3-phenoxybenzene (134.6 mg, 0.512 mmol) and 4-chlorobenzyl isocyanate (251.8 mg, 1.502 mmol). After collecting the title compound it was stirred with *N*-2-(aminoethyl)-1,2-ethanediamine (478.4 mg, 4.637 mmol) for 1 hour at rt, the solvent was evaporated under reduced pressure and the product purified by column chromatography using a semi-automatic Combiflash system (24 g silica, eluent gradient: Heptane/EtOAc) to afford the title compound (164.9 mg, 74%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.30 (tt, *J* = 4.3, 2.1 Hz, 2H), 7.25–7.22 (m, 3H), 7.18 (d, *J* = 8.4 Hz, 2H), 7.09–7.06 (m, 1H), 7.03 (d, *J* = 7.6 Hz, 1H), 7.00–6.96 (m, 3H), 6.87 (dd, *J* = 8.1, 2.5 Hz, 1H), 4.97 (t, *J* = 5.6 Hz, 1H), 4.32 (d, *J* = 5.7 Hz, 2H), 3.46 (s, 2H), 3.38–3.28 (m, 4H), 2.41–2.34 (m, 4H) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 157.60, 157.33, 157.25, 140.03, 138.23, 132.97, 129.82, 129.64, 129.06, 128.72, 123.93, 123.31, 119.47, 118.86, 117.66, 62.59, 52.69, 44.22, 43.87 ppm; HRMS calcd. for $C_{25}H_{26}ClN_3O_2$ (M+H): 436.1786, found: 436.1794.

Biological Assays

Transfections and Tissue Culture

COS-7 cells were grown at 10% CO_2 and 37°C in Dulbecco´s modified Eagle´s medium with glutamax (Invitrogen, UK) adjusted with 10% fetal bovine serum, 180 units/ml penicillin, and 45 µg/ml penicillin/streptomycin (PenStrep). Transfection of the COS-7 cells was performed by the calcium phosphate precipitation method (1). Briefly, 6 x 10^6 cells were transfected with 20 µg of human CCR8 cDNA (purchased from www.cDNA.org) in addition to 30 µg of the promiscuous chimeric G protein, $G\alpha_{qi4myr}$, which is recognized as a $G\alpha_i$-subunit, but transmit a $G\alpha_q$ signal. Thus, the presence of this chimeric G protein turns the $G\alpha_i$ signal, the most common pathway for endogenous chemokine receptors, into a $G\alpha_q$ signaling pathway (phospholipase C activation measured as IP turnover).[1]

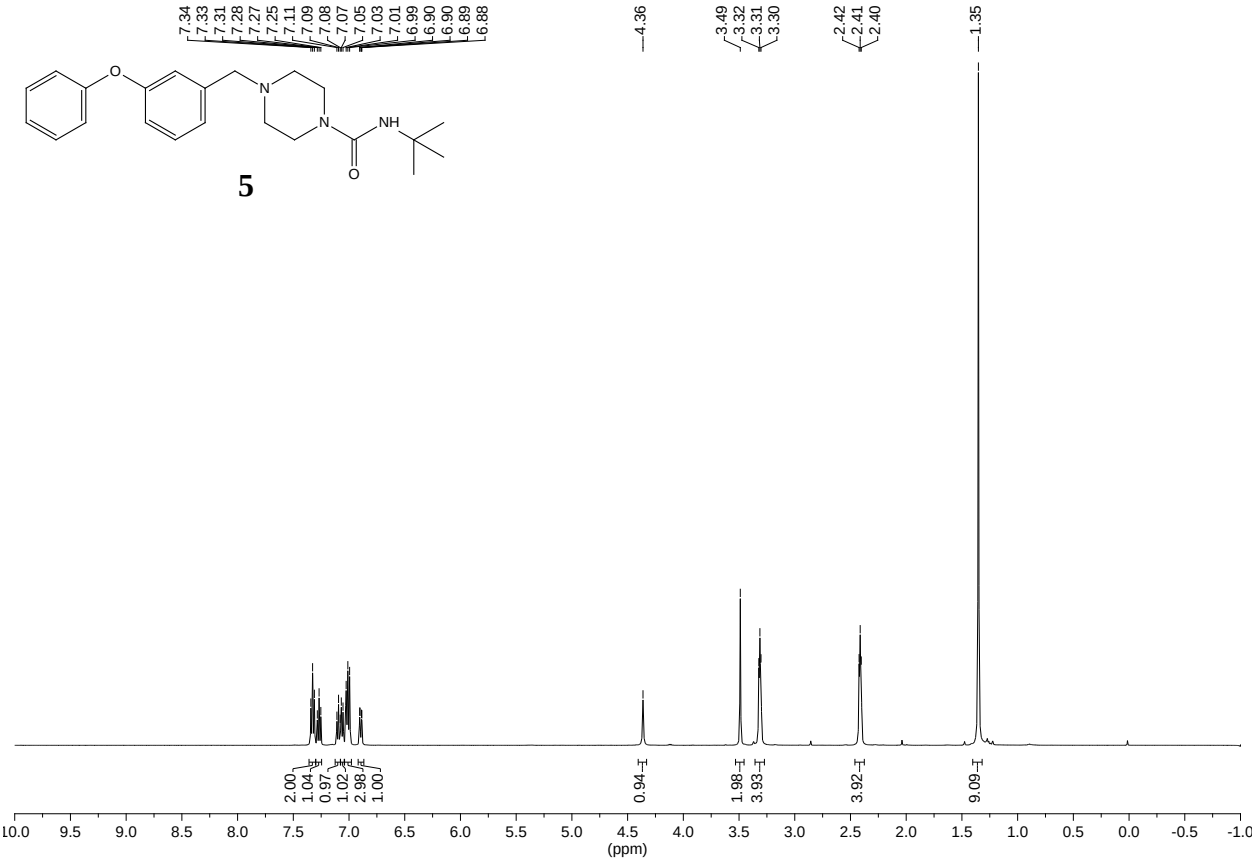
Inositol Phosphate Turnover (IP Turnover)

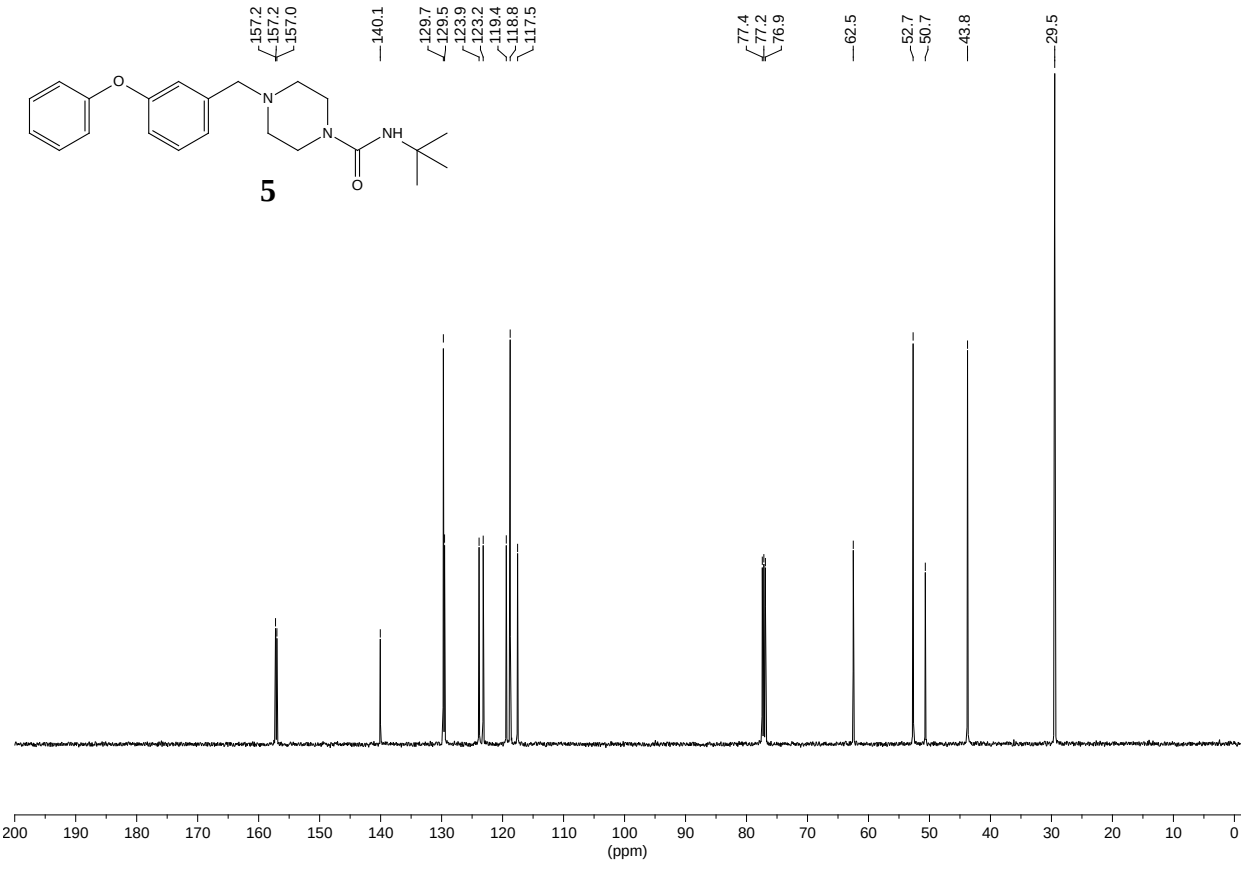
COS-7 cells were transfected according to the procedure mentioned above. One day after transfection, the cells (35.000 cells/well) were incubated for 24h with 0.67 µCi of *myo*[^{3}H]inositol in 0.1 ml of growth medium per well in a 96-well plate. Cells were washed twice in PBS and subsequently incubated for 90min in 0.1 ml Hanks´ Balanced Salt Solution (Invitrogen, UK) supplemented with 10 mM LiCl at 37 °C in the presence of various concentrations of ligands. Cells were extracted by addition of 50 µl of 10 mM formic acid to each well followed by incubation on ice for 30 min. 20µl of the extract were transferred to a white 96-well plate and 80 µl of 1:8 diluted YSi Poly-D-lysine coated beads (Perkin Elmer, MA, USA) were added. The plates were sealed and shaked at maximum speed for at least 30 min, centrifuged for 5 min at 1500 rpm and γ-radiation was counted in a Packard Top Count NXT counter. Determinations were made in duplicates. This general functional read-out has previously been used with success in other chemokine receptors.[2]

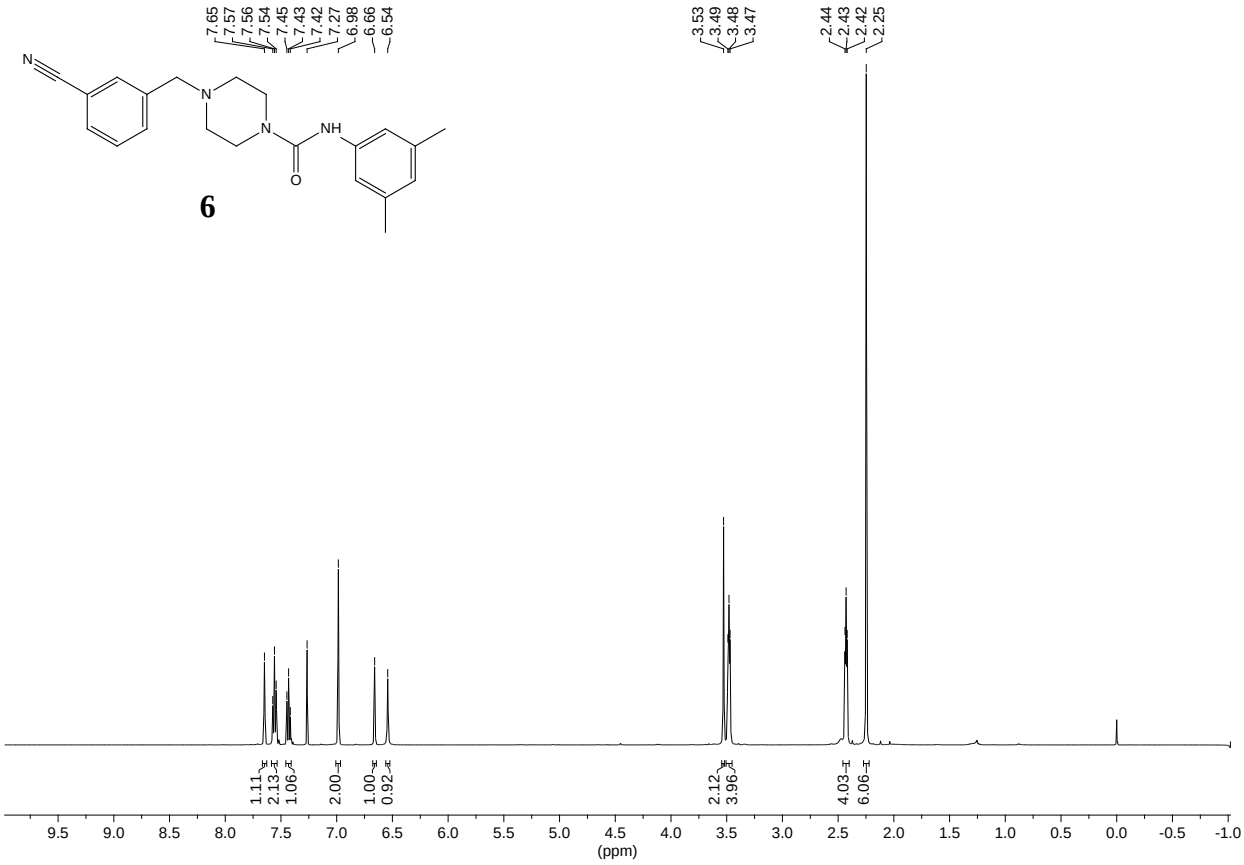
[1] a) Rosenkilde, M. M., Cahir, M., Gether, U., Hjorth, S. A., Schwartz, T. W., *J. Biol. Chem.*, **1994**, *269*, 28160; b) Kostenis, E., Zeng, F. Y., Wess, J., *J. Biol. Chem.* **1998,** *273*, 17886

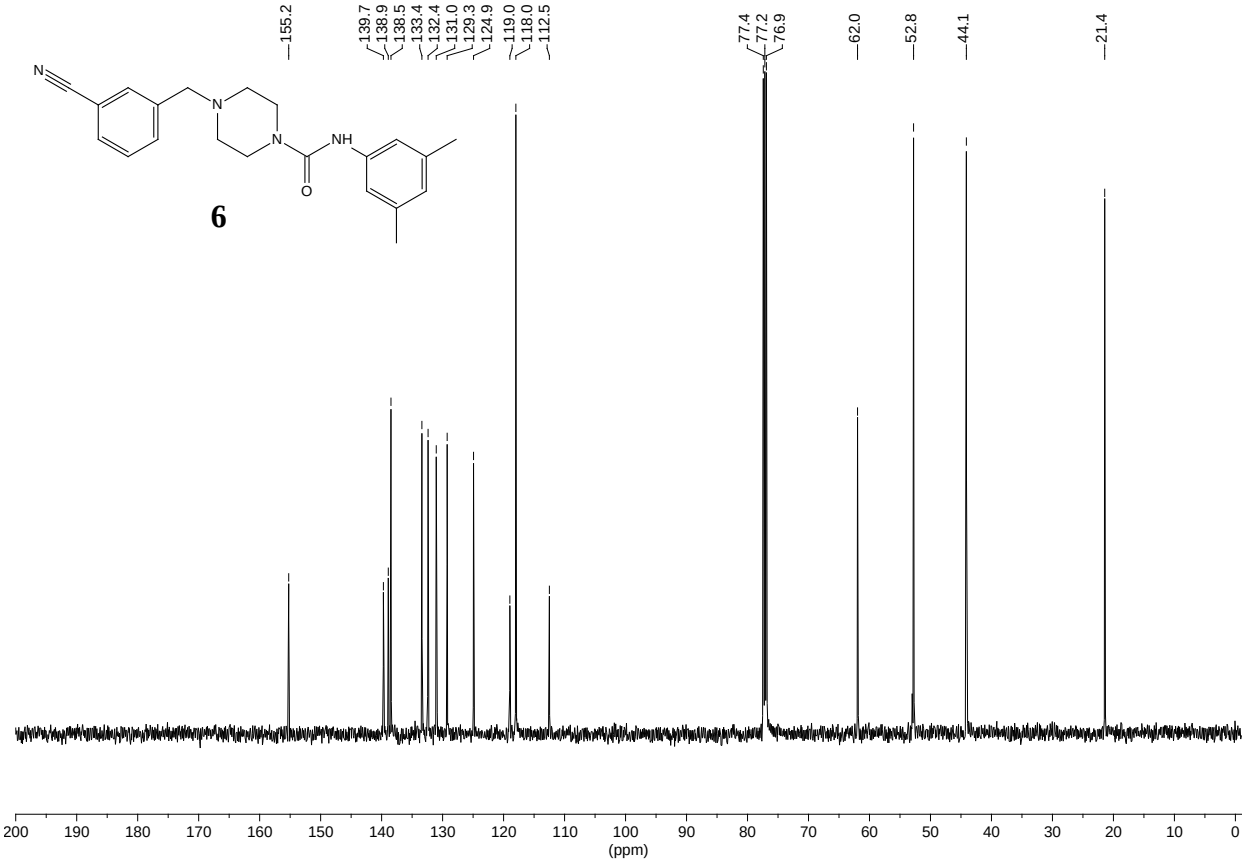
[2] a) Jensen, P. C., Thiele, S., Ulven, T., Schwartz, T. W., Rosenkilde, M. M., *J. Biol. Chem.*, **2008**, *283*, 23121; b) Thiele, S., Steen, A., Jensen, P. C., Mokrosinski, J., Frimurer, T. M., Rosenkilde, M. M., *J. Biol. Chem.*, **2011**, *286*, 37543

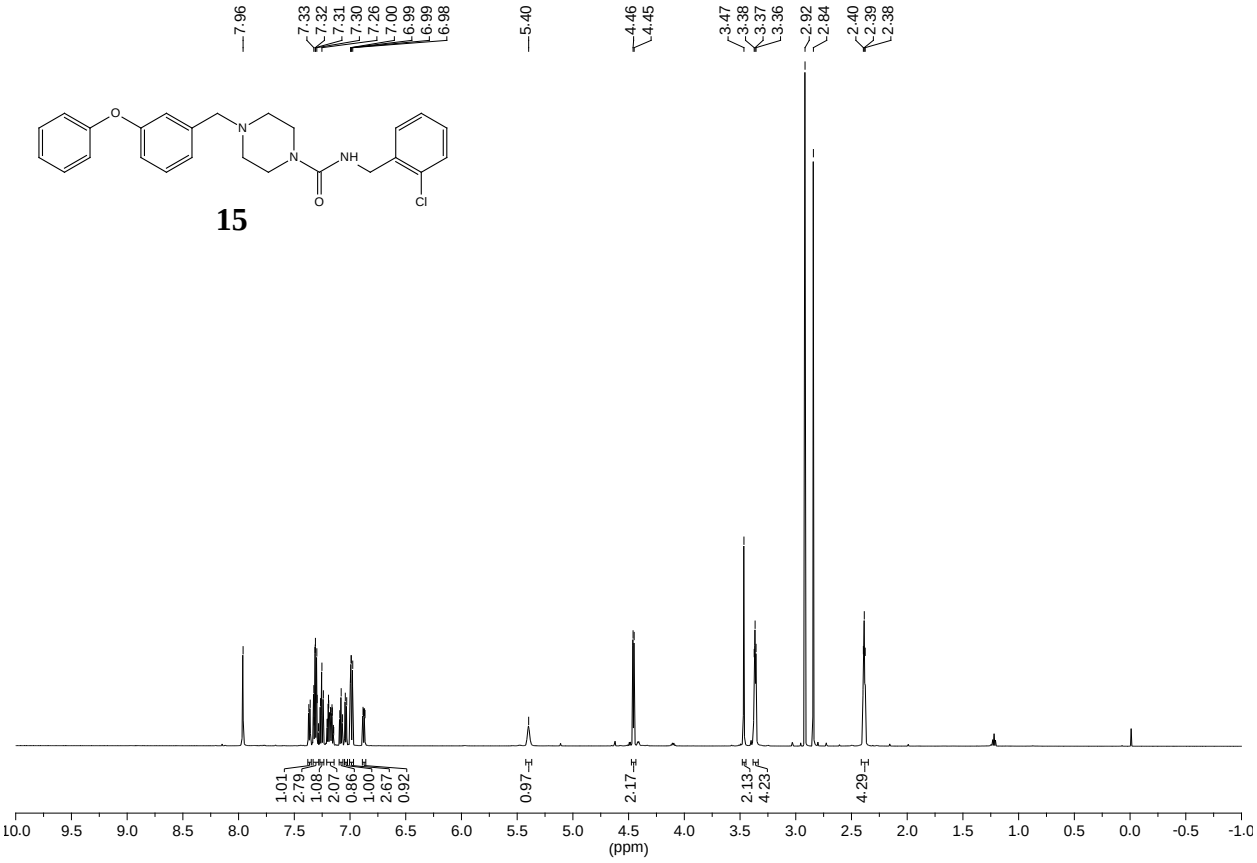
NMR spectra

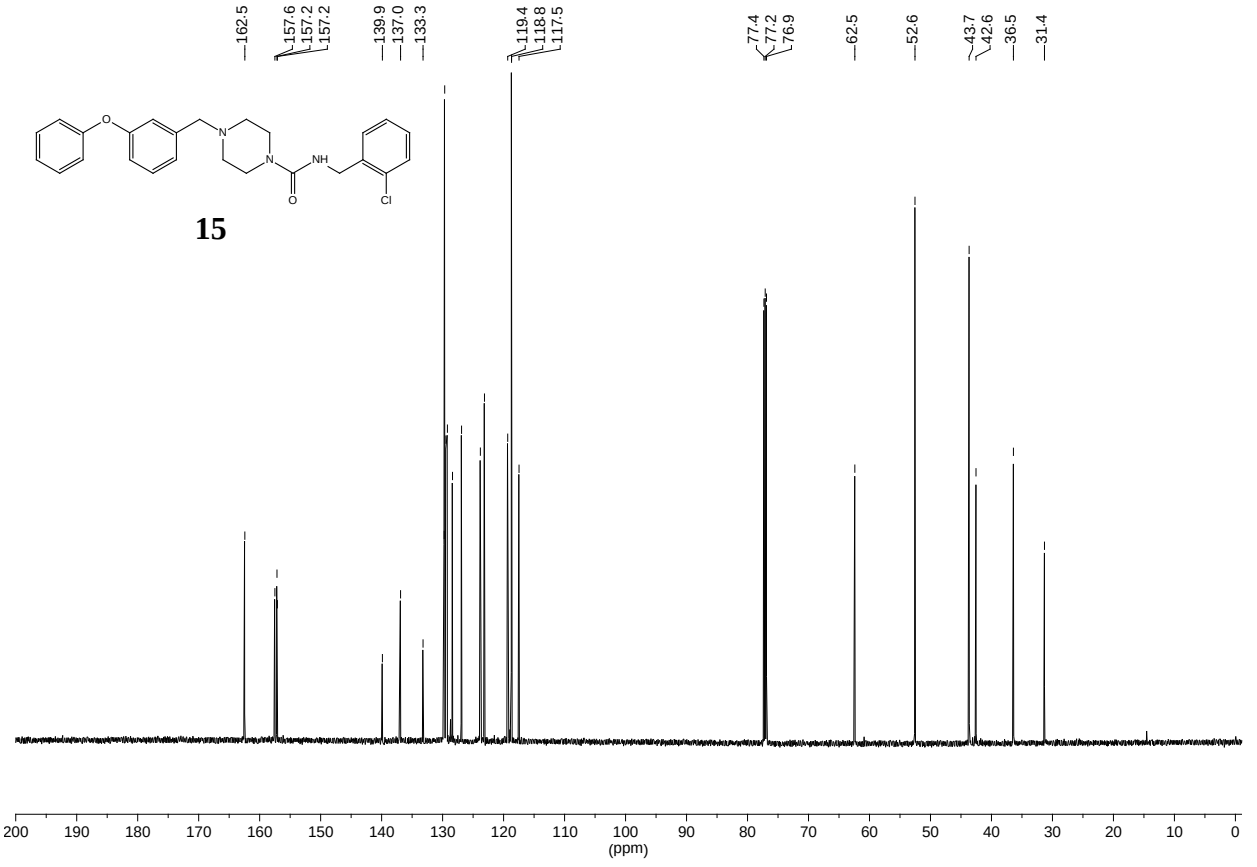

7.34
7.33
7.31
7.28
7.27
7.25
7.11
7.09
7.08
7.07
7.05
7.03
7.01
6.99
6.90
6.90
6.89
6.88
4.36
3.49
3.32
3.31
3.30
2.42
2.41
2.40
1.35
O
N
N
NH
O
5
2.00
1.04
0.97
1.02
2.98
1.00
0.94
1.98
3.93
3.92
9.09
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
(ppm)

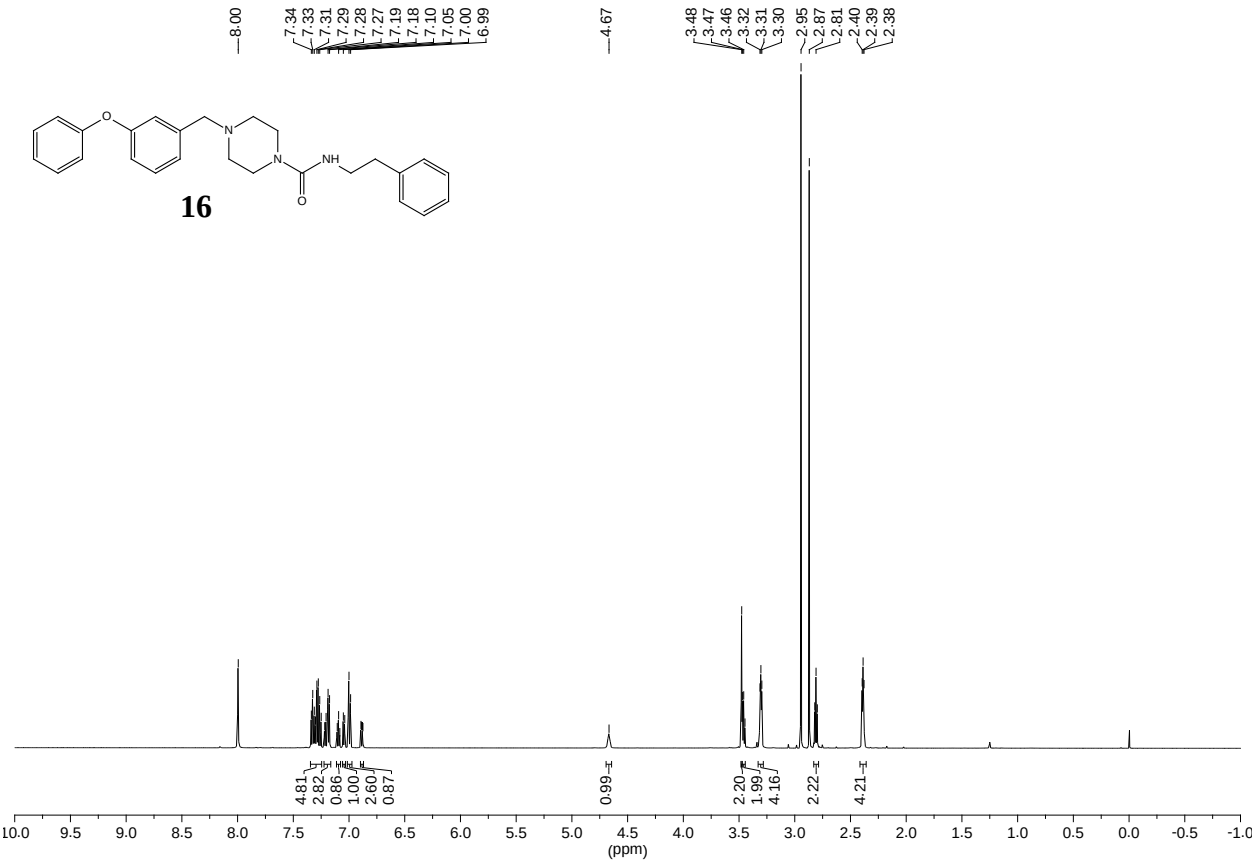

157.2
157.2
157.0
140.1
129.7
129.5
123.9
123.2
119.4
118.8
117.5
77.4
77.2
76.9
62.5
52.7
50.7
43.8
29.5
O
N
N
NH
O
5
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
(ppm)

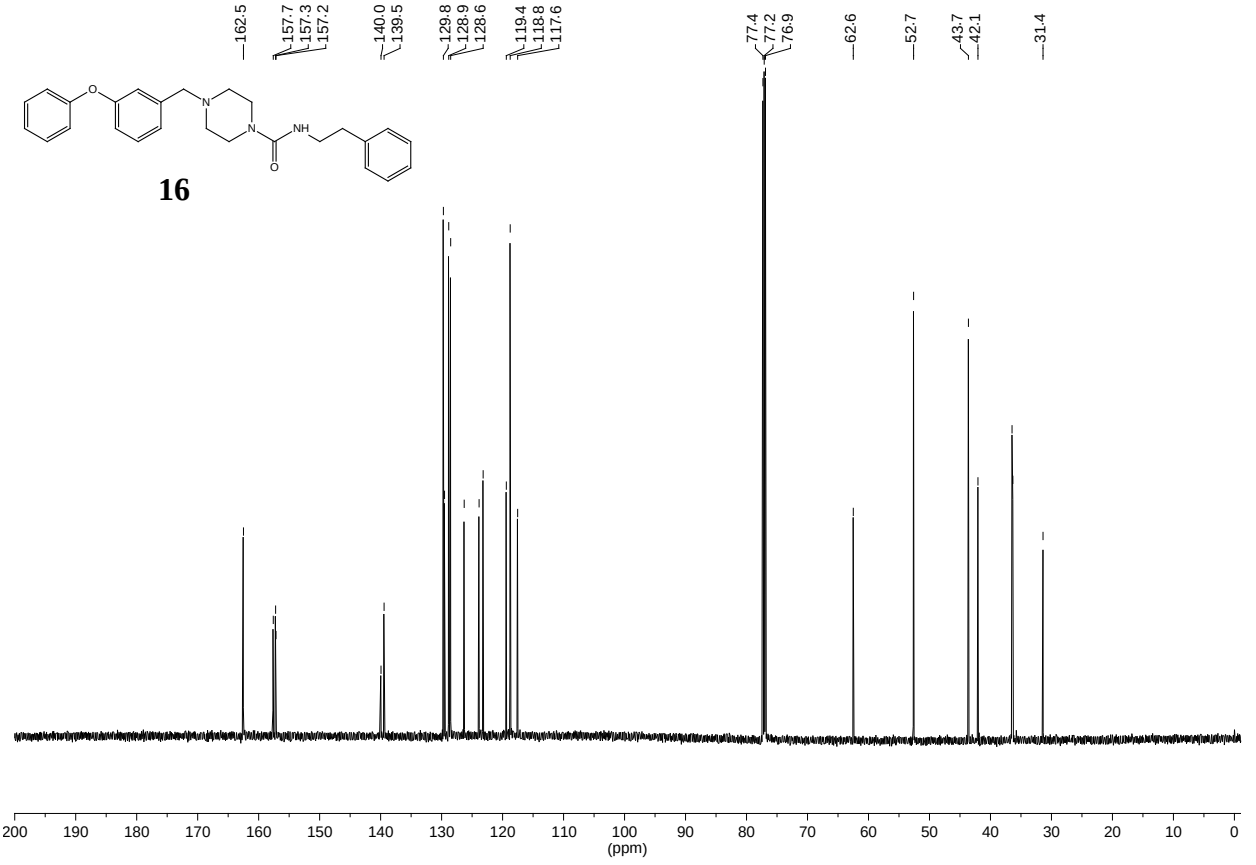

7.65
7.57
7.56
7.54
7.45
7.43
7.42
7.27
6.98
6.66
6.54
3.53
3.49
3.48
3.47
2.44
2.43
2.42
2.25
N
N
N
NH
O
6
1.11
2.13
1.06
2.00
1.00
0.92
2.12
3.96
4.03
6.06
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
(ppm)

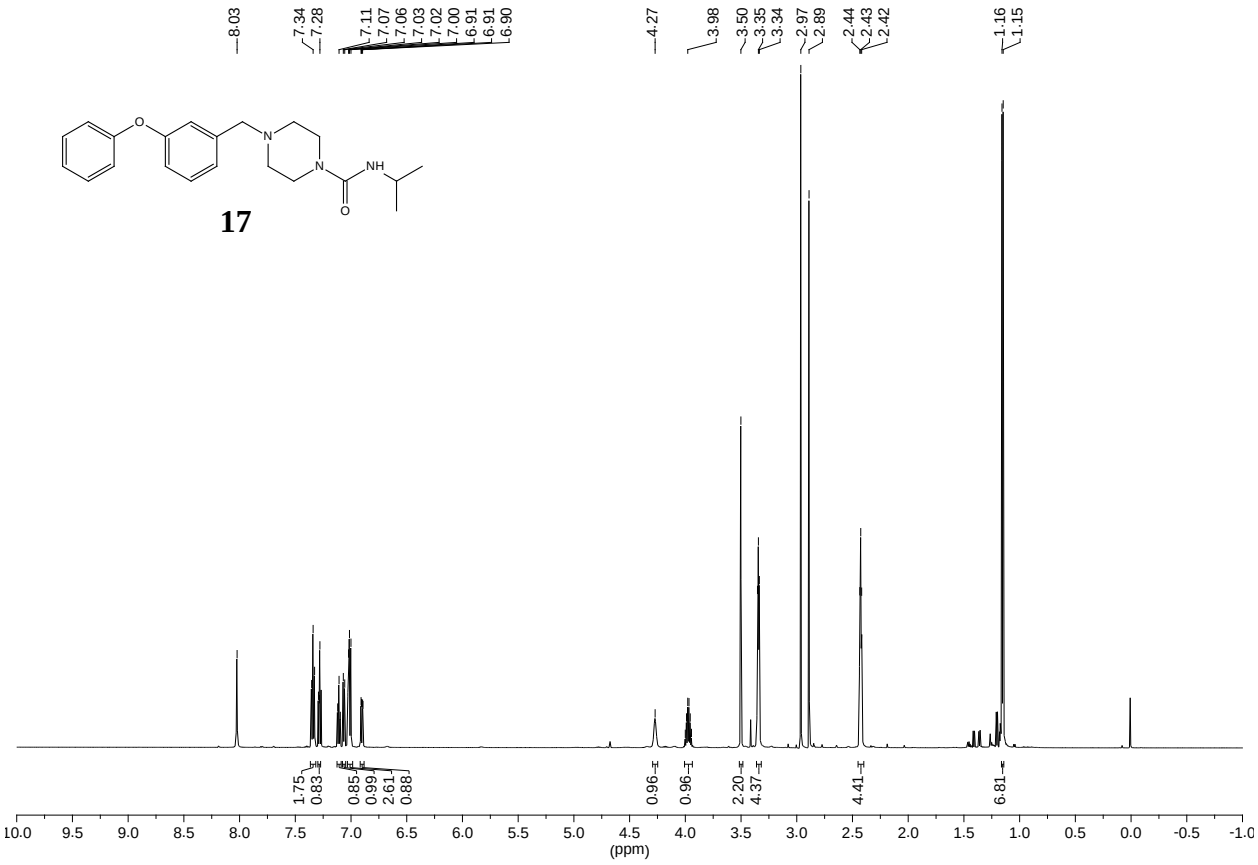

155.2
139.7
138.9
138.5
133.4
132.4
131.0
129.3
124.9
119.0
118.0
112.5
77.4
77.2
76.9
62.0
52.8
44.1
21.4
N
N
N
NH
O
6
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
(ppm)

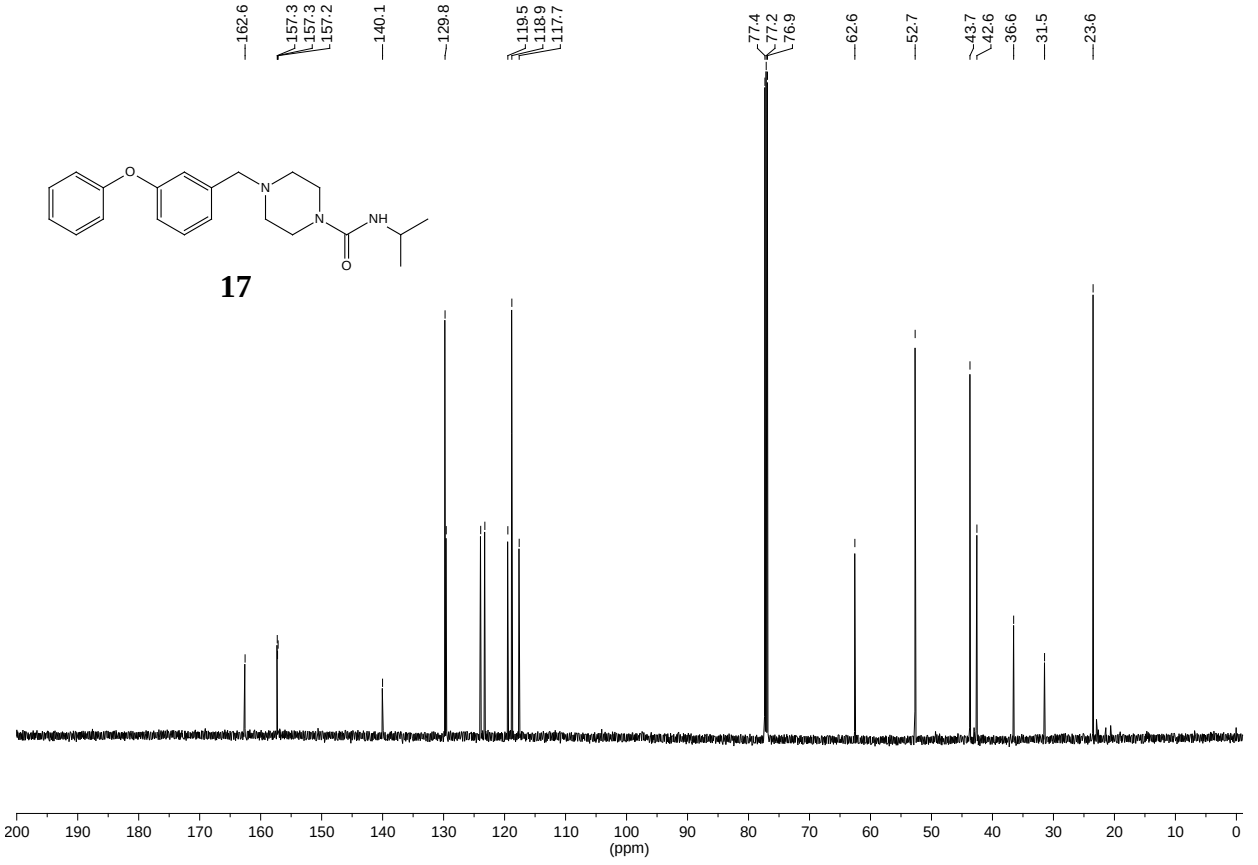

7.96
7.33
7.32
7.31
7.30
7.26
7.00
6.99
6.99
6.98
5.40
4.46
4.45
3.47
3.38
3.37
3.36
2.92
2.84
2.40
2.39
2.38
O
N
N
NH
O
Cl
15
1.01
2.79
1.08
2.07
0.86
1.00
2.67
0.92
0.97
2.17
2.13
4.23
4.29
(ppm)

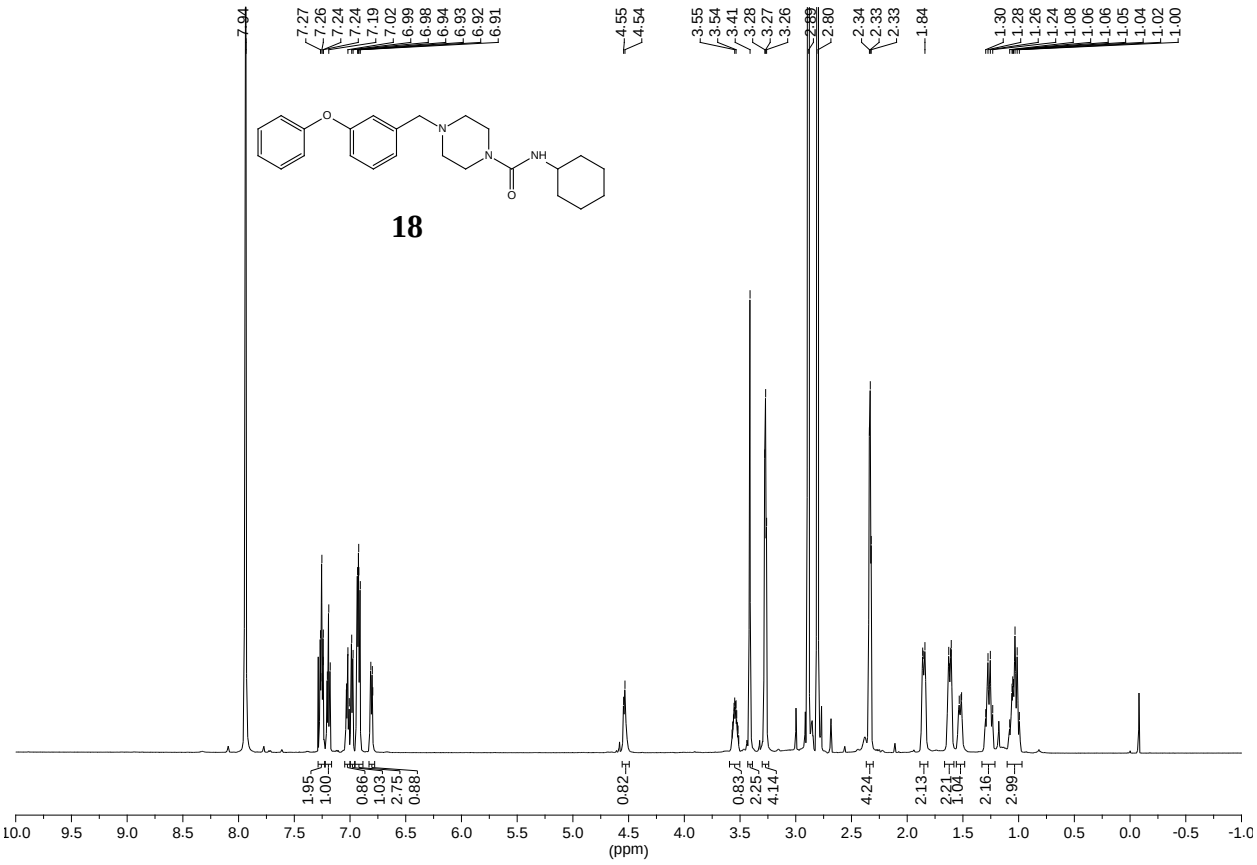

162.5
157.6
157.2
157.2
139.9
137.0
133.3
119.4
118.8
117.5
77.4
77.2
76.9
62.5
52.6
43.7
42.6
36.5
31.4
O
N
N
NH
O
Cl
15
(ppm)


8.00
7.34
7.33
7.31
7.29
7.28
7.27
7.19
7.18
7.10
7.05
7.00
6.99
4.67
3.48
3.47
3.46
3.32
3.31
3.30
2.95
2.87
2.81
2.40
2.39
2.38
O
N
N
NH
O
16
4.81
2.82
0.86
1.00
2.60
0.87
0.99
2.20
1.99
4.16
2.22
4.21
(ppm)

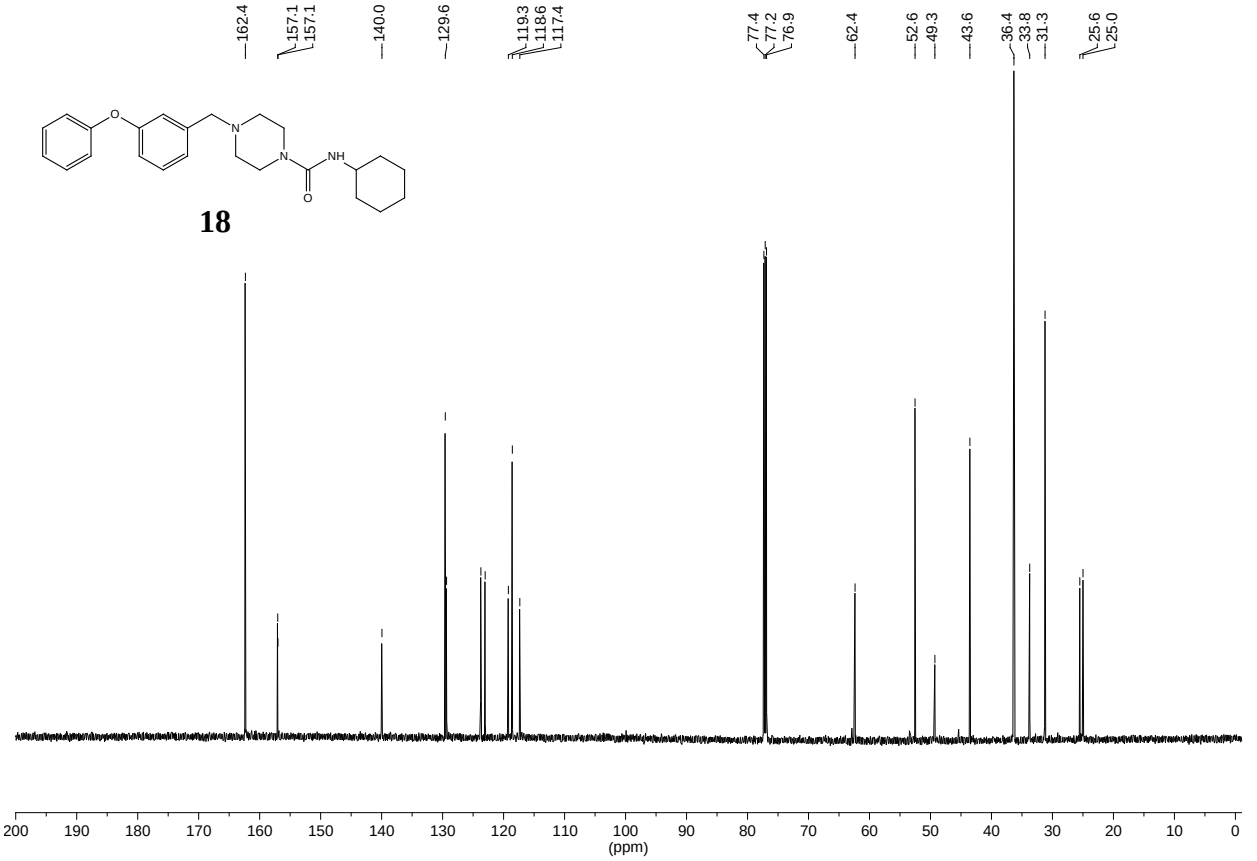

162.5
157.7
157.3
157.2
140.0
139.5
129.8
128.9
128.6
119.4
118.8
117.6
77.4
77.2
76.9
62.6
52.7
43.7
42.1
31.4
O
N
N
NH
O
16
(ppm)


8.03
7.34
7.28
7.11
7.07
7.06
7.03
7.02
7.00
6.91
6.91
6.90
4.27
3.98
3.50
3.35
3.34
2.97
2.89
2.44
2.43
2.42
1.16
1.15
O
N
N
NH
O
17
1.75
0.83
0.85
0.99
2.61
0.88
0.96
0.96
2.20
4.37
4.41
6.81
(ppm)

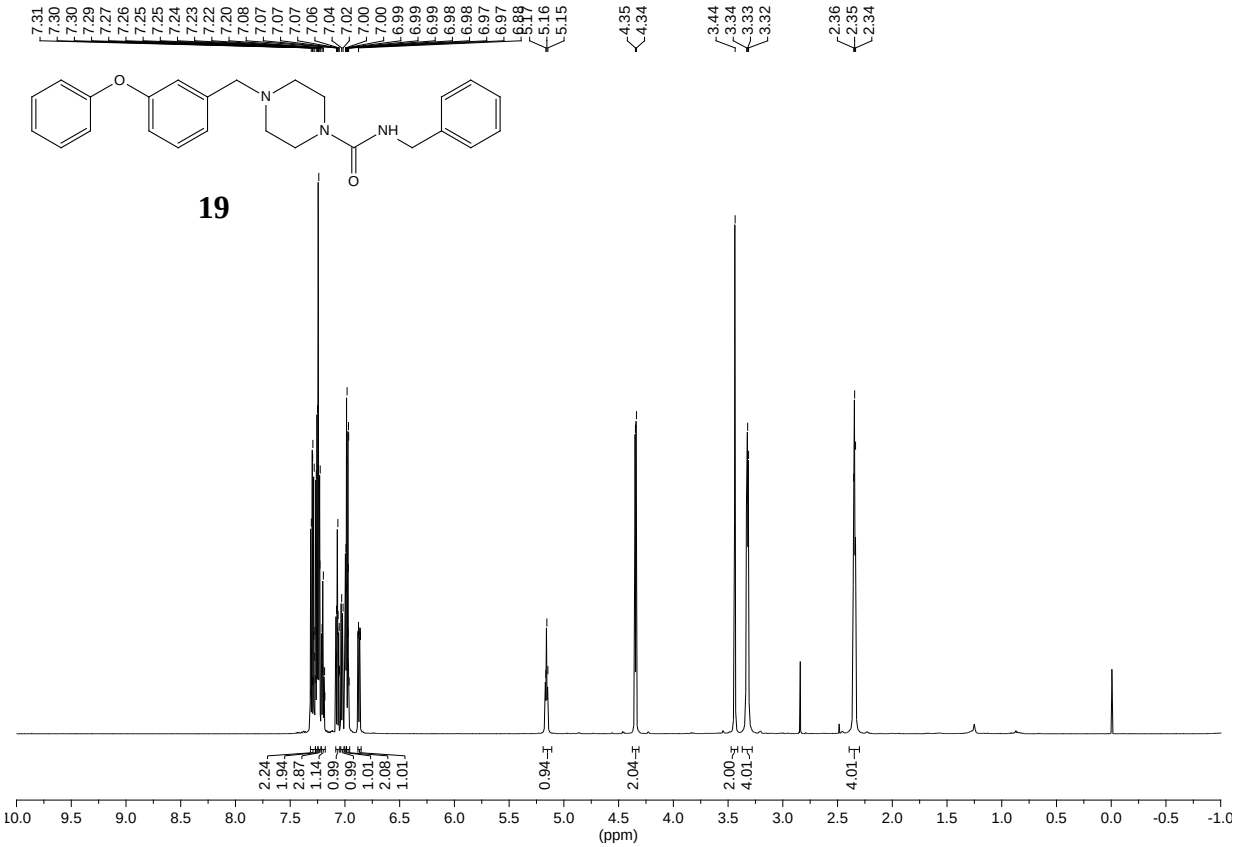

162.6
157.3
157.3
157.2
140.1
129.8
119.5
118.9
117.7
77.4
77.2
76.9
62.6
52.7
43.7
42.6
36.6
31.5
23.6
O
N
N
NH
O
17
(ppm)

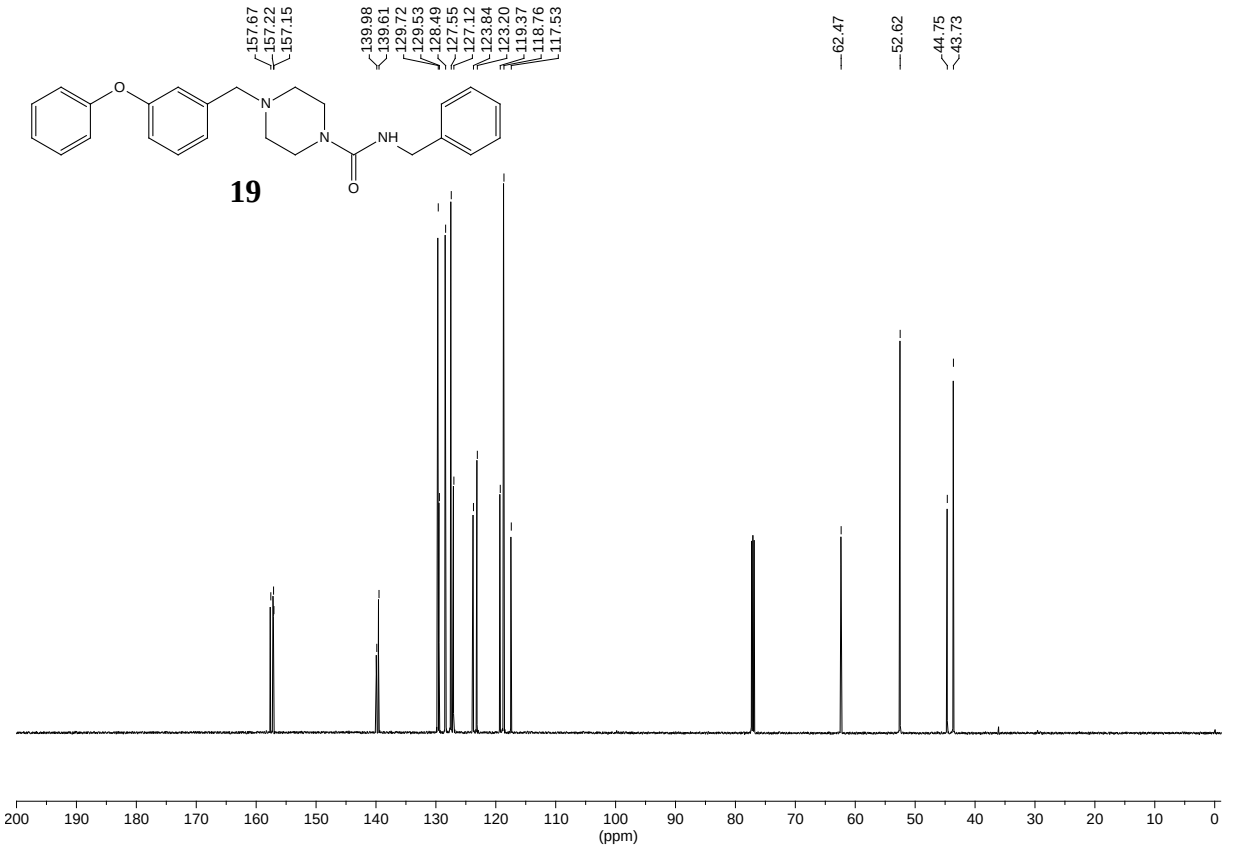

7.94
7.27
7.26
7.24
7.24
7.19
7.02
6.99
6.98
6.94
6.93
6.92
6.91
4.55
4.54
3.55
3.54
3.41
3.28
3.27
3.26
2.80
2.34
2.33
2.33
1.84
1.30
1.28
1.26
1.24
1.08
1.06
1.06
1.05
1.04
1.02
1.00
1.95
1.00
0.86
1.03
2.75
0.88
0.82
0.83
2.25
4.14
4.24
2.13
2.21
1.04
2.16
2.99
(ppm)

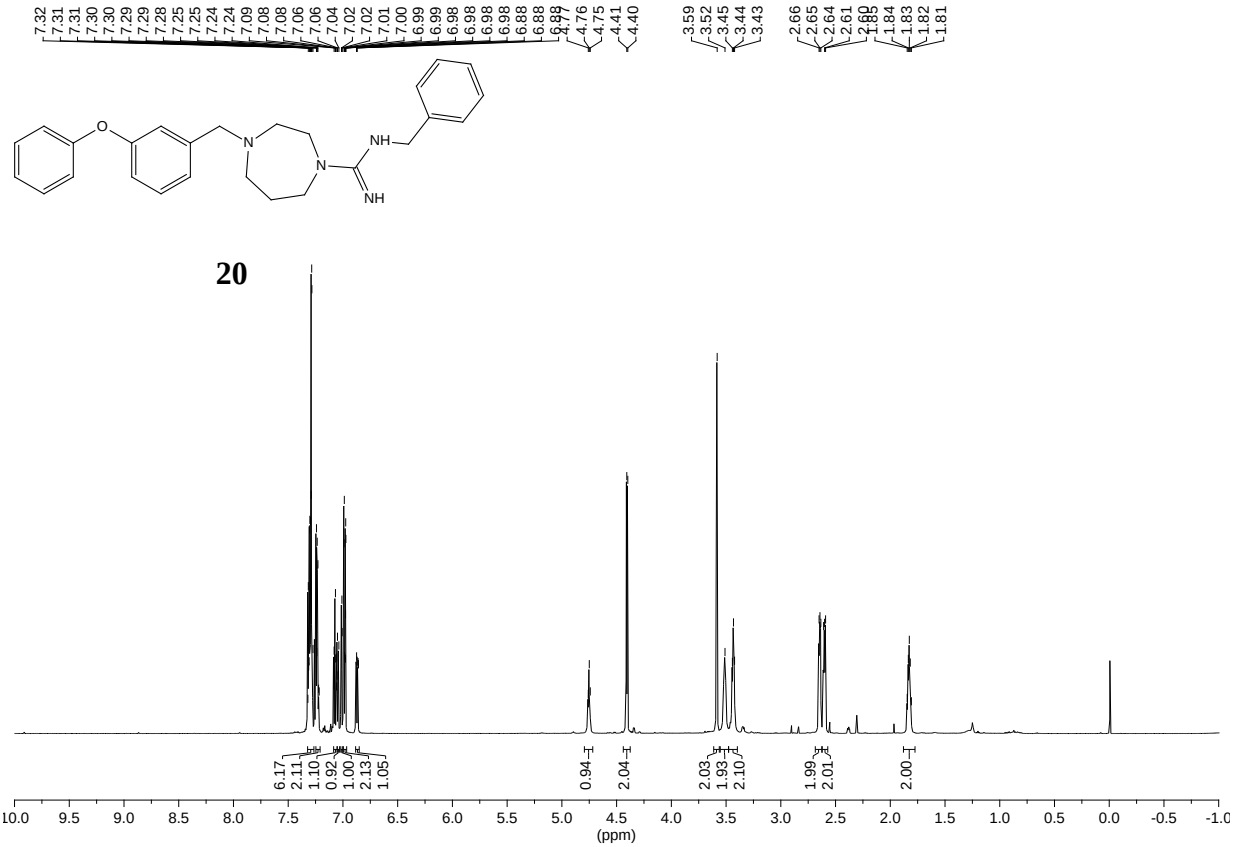
18

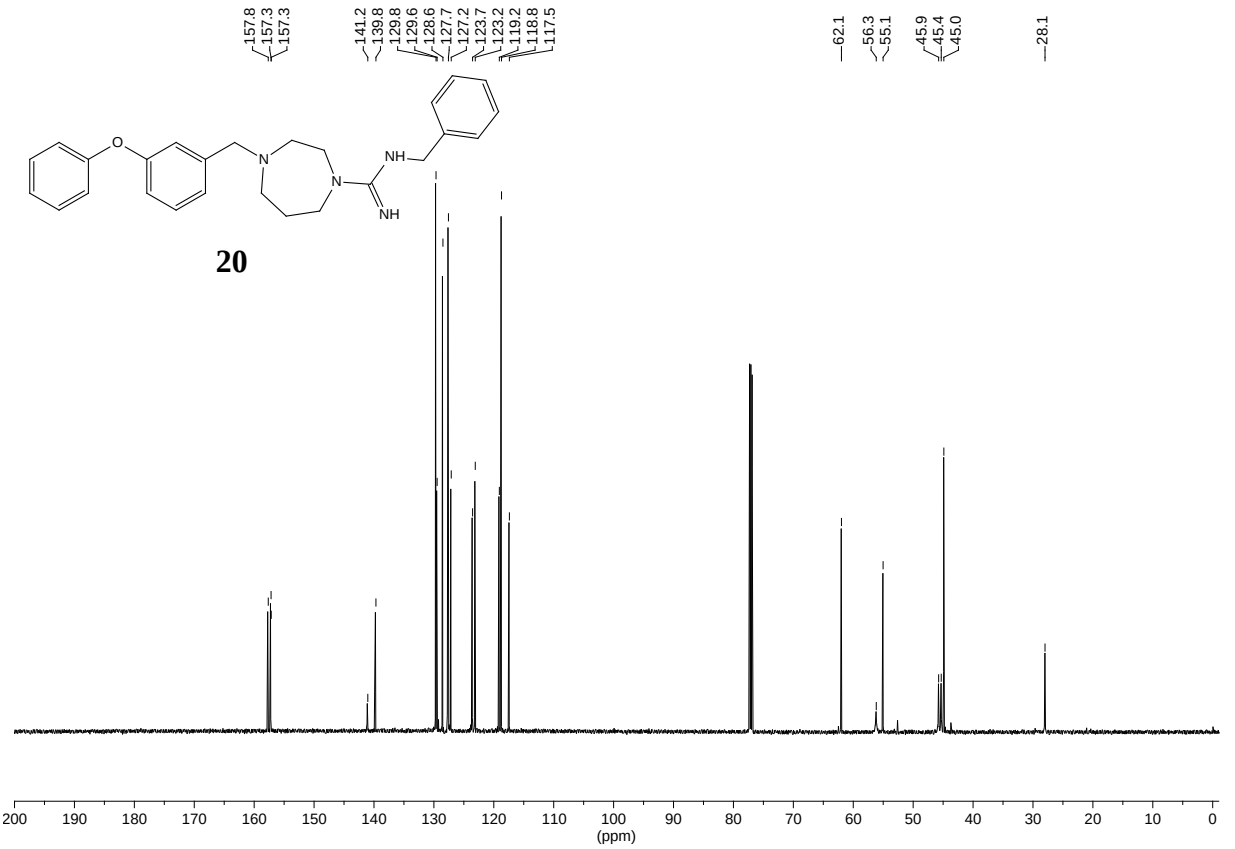

162.4
157.1
157.1
140.0
129.6
119.3
118.6
117.4
77.4
77.2
76.9
62.4
52.6
49.3
43.6
36.4
33.8
31.3
25.6
25.0
(ppm)

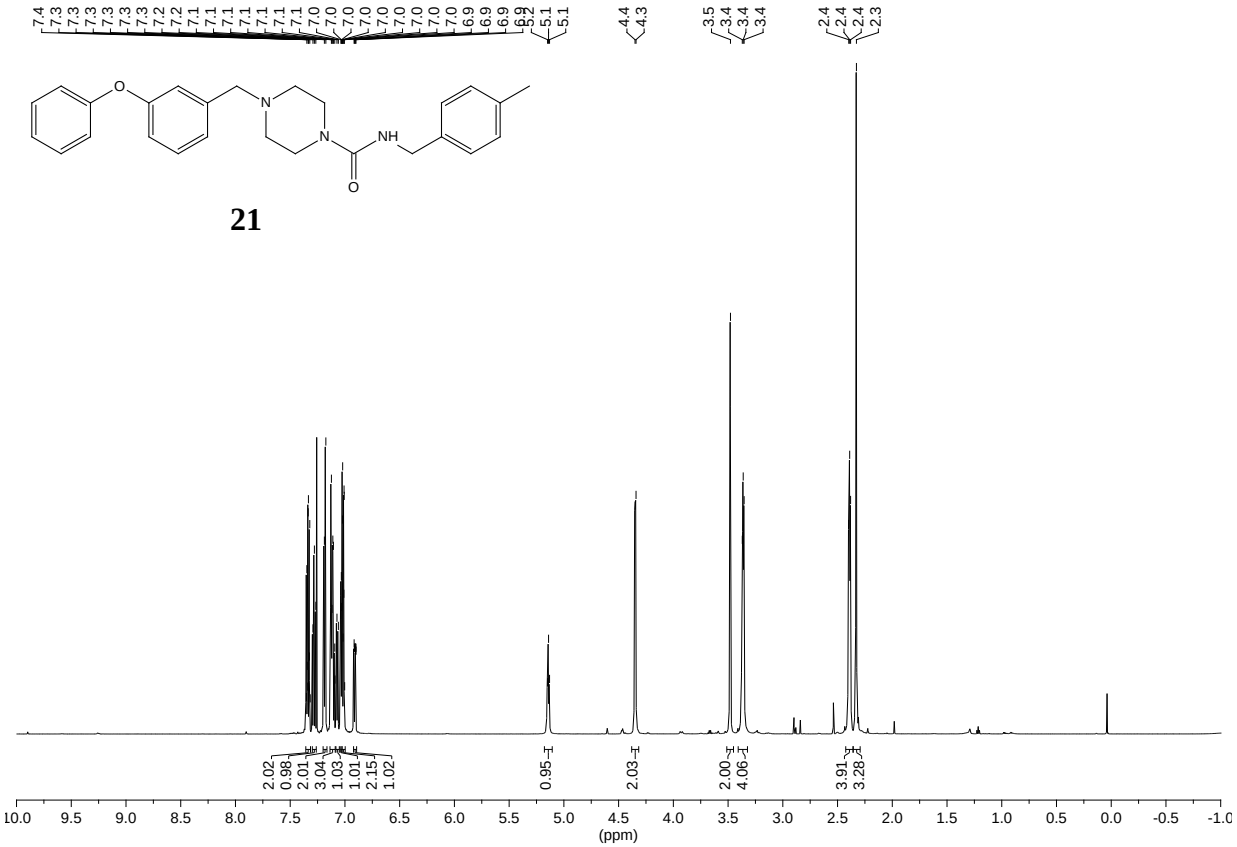
18

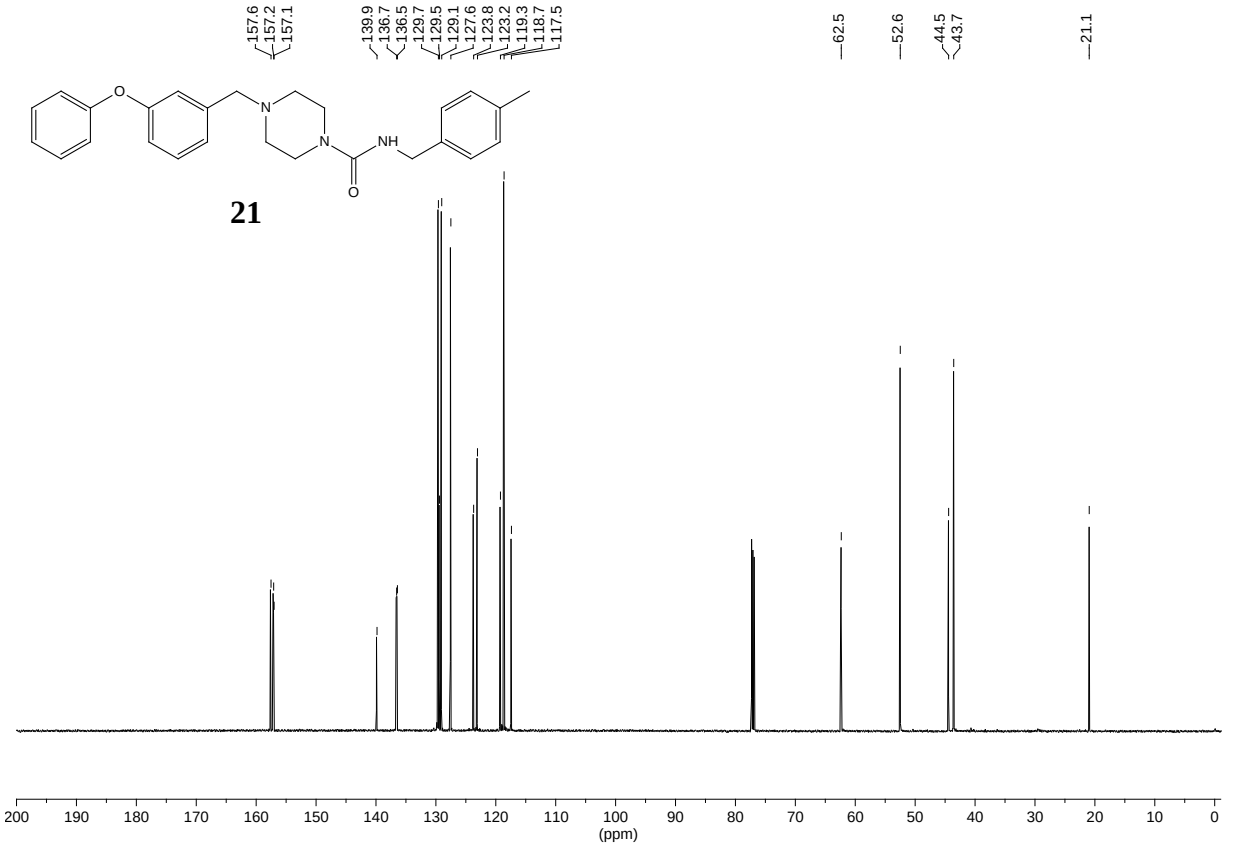

7.31 7.30 7.30 7.29 7.27 7.26 7.25 7.25 7.24 7.23 7.22 7.20 7.08 7.07 7.07 7.07 7.06 7.04 7.02 7.00 7.00 6.99 6.99 6.98 6.98 6.97 6.97 5.17 5.16 5.15 4.35 4.34 3.44 3.34 3.33 3.32 2.36 2.35 2.34
O
N
N
NH
O
19
2.24 1.94 2.87 1.14 0.99 0.99 1.01 2.08 1.01 0.94 2.04 2.00 4.01 4.01
10.0 9.5 9.0 8.5 8.0 7.5 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 -1.0
(ppm)

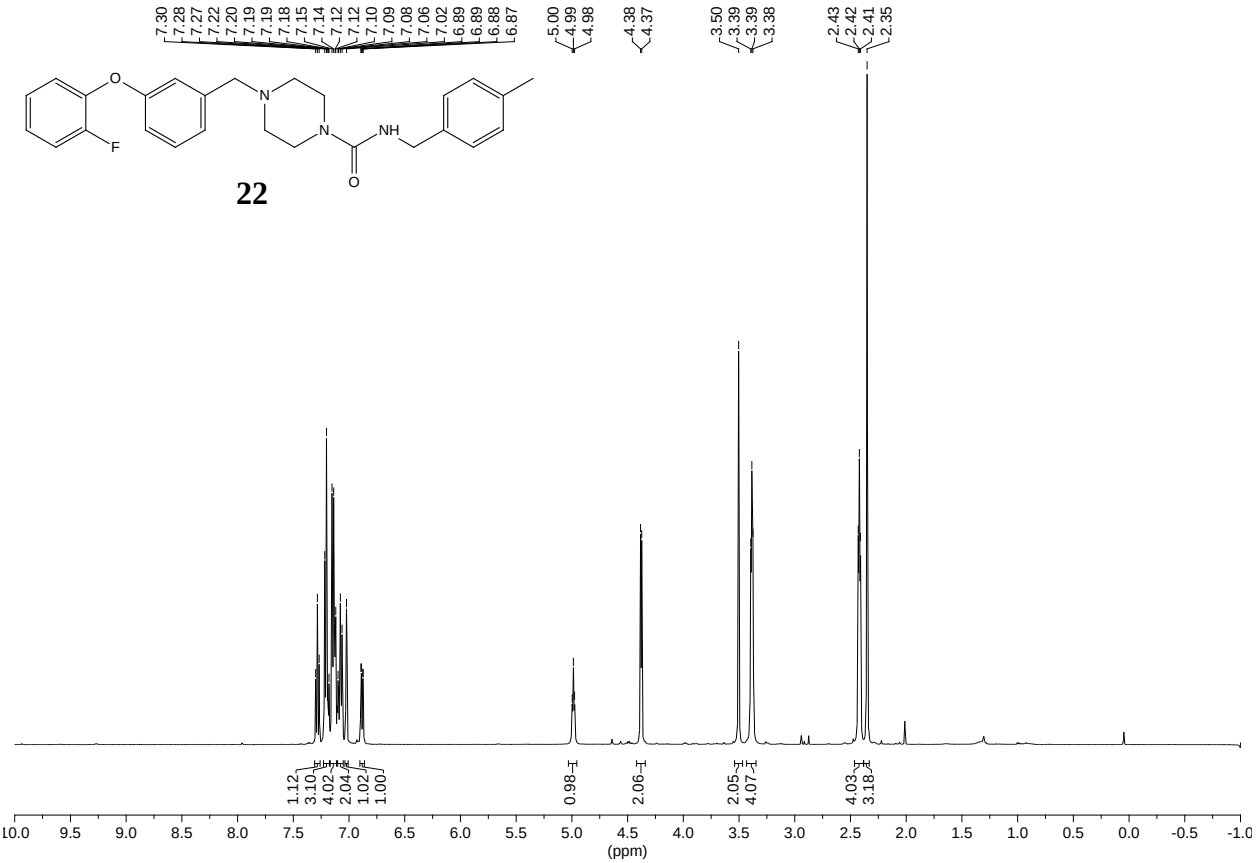

157.67 157.22 157.15 139.98 139.61 129.72 129.53 128.49 127.55 127.12 123.84 123.20 119.37 118.76 117.53 62.47 52.62 44.75 43.73
O
N
N
NH
O
19
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
(ppm)


7.32 7.31 7.31 7.30 7.30 7.29 7.29 7.28 7.25 7.25 7.24 7.24 7.09 7.08 7.08 7.06 7.06 7.04 7.02 7.02 7.01 7.00 6.99 6.99 6.98 6.98 6.98 6.88 6.88 4.77 4.76 4.75 4.41 4.40
3.59 3.52 3.45 3.44 3.43
2.66 2.65 2.64 2.61
1.85 1.84 1.83 1.82 1.81
O
N
N
NH
NH
20
6.17 2.11 1.10 0.92 1.00 2.13 1.05
0.94 2.04
2.03 1.93 2.10
1.99 2.01
2.00
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 -1.0
(ppm)

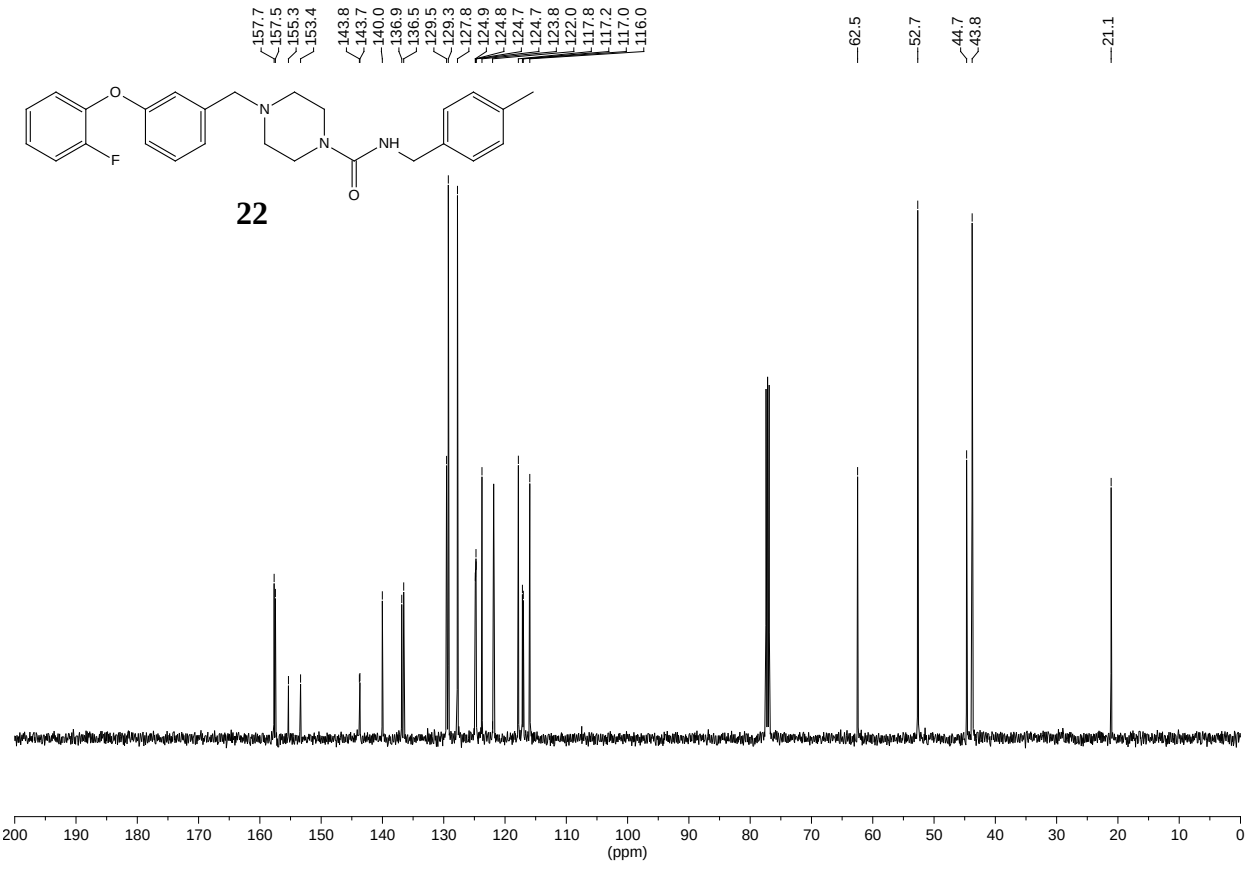

157.8 157.3 157.3
141.2 139.8 129.8 129.6 128.6 127.7 127.2 123.7 123.2 119.2 118.8 117.5
62.1 56.3 55.1 45.9 45.4 45.0 28.1
O
N
N
NH
NH
20
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
(ppm)


O
N
N
NH
O
21
(ppm)

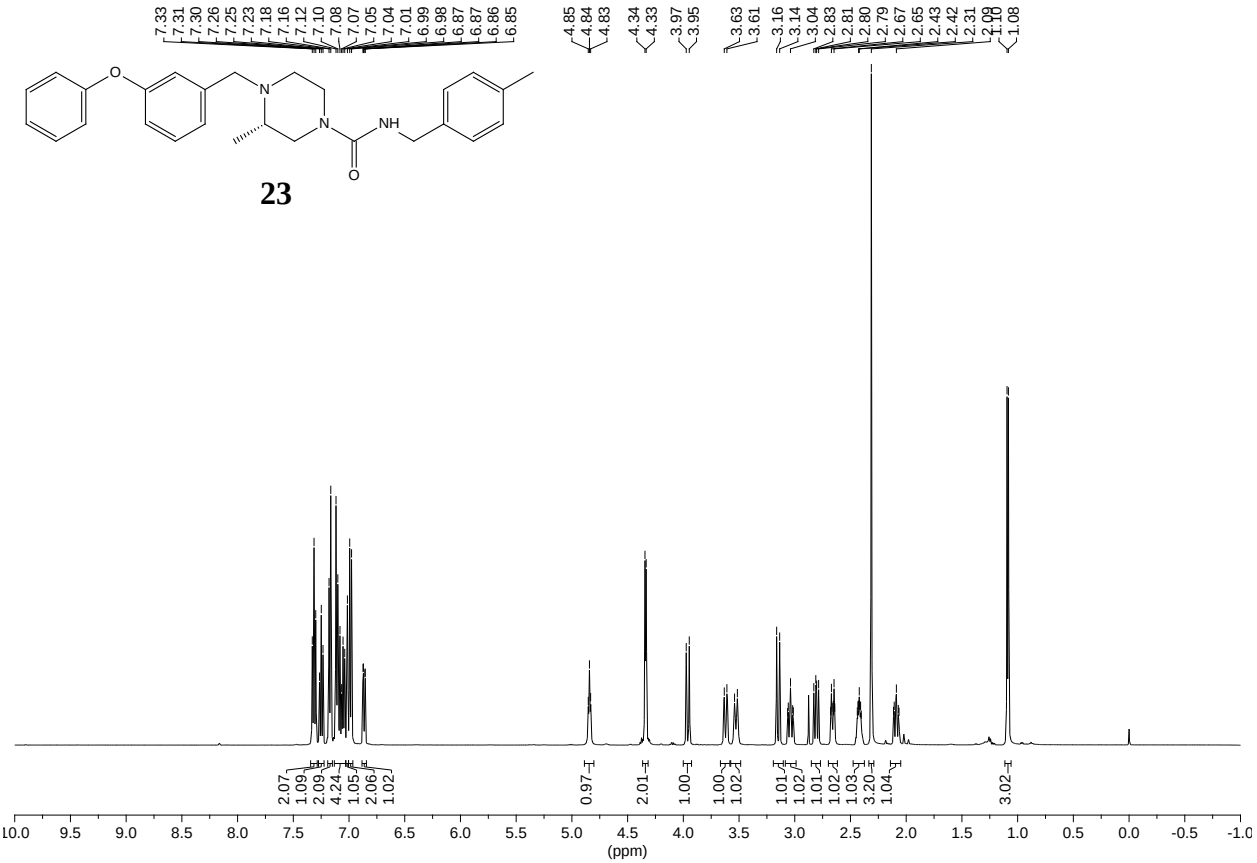

O
N
N
NH
O
21
(ppm)

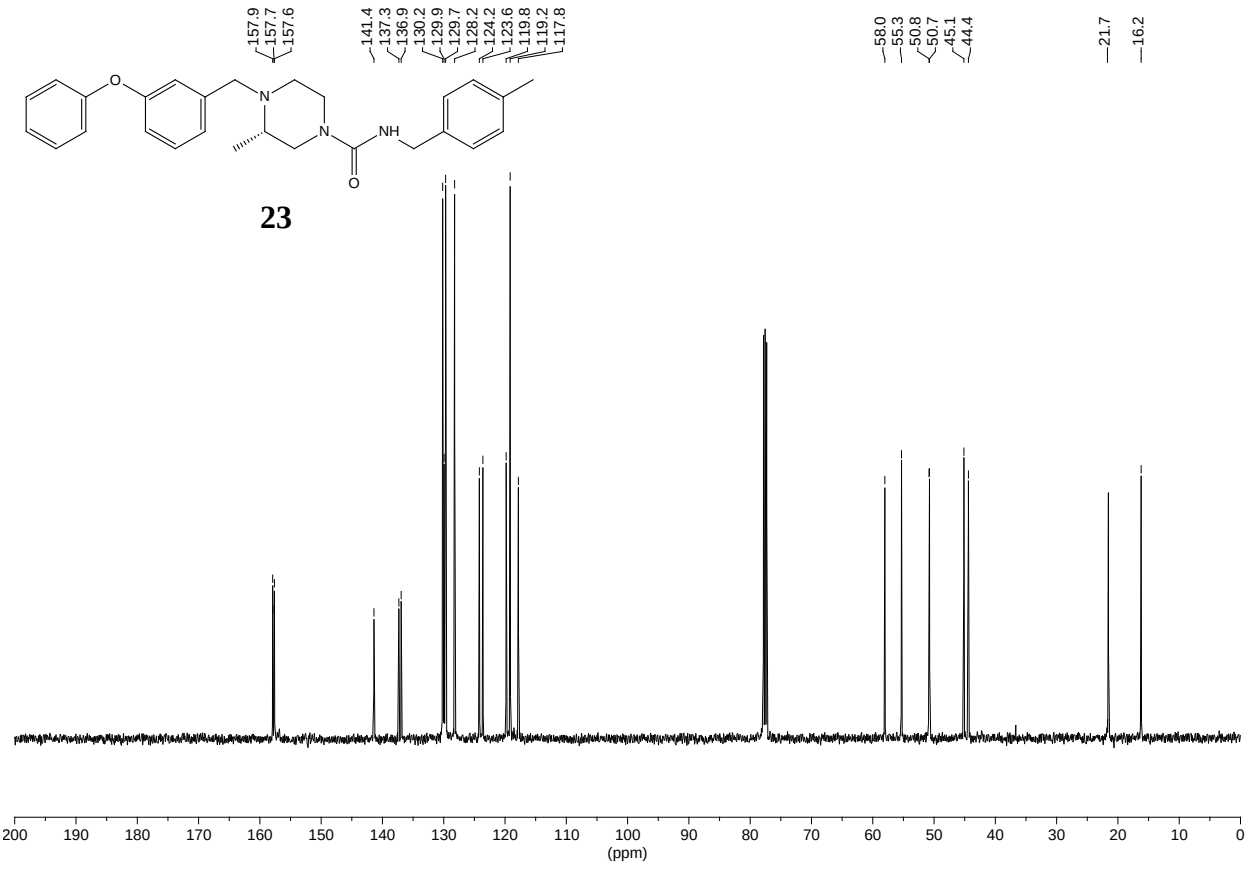

7.30
7.28
7.27
7.22
7.20
7.19
7.19
7.18
7.15
7.14
7.12
7.12
7.10
7.09
7.08
7.06
7.02
6.89
6.89
6.88
6.87
5.00
4.99
4.98
4.38
4.37
3.50
3.39
3.39
3.38
2.43
2.42
2.41
2.35
O
F
N
N
NH
O
1.12
3.10
4.02
2.04
1.02
1.00
0.98
2.06
2.05
4.07
4.03
3.18
(ppm)

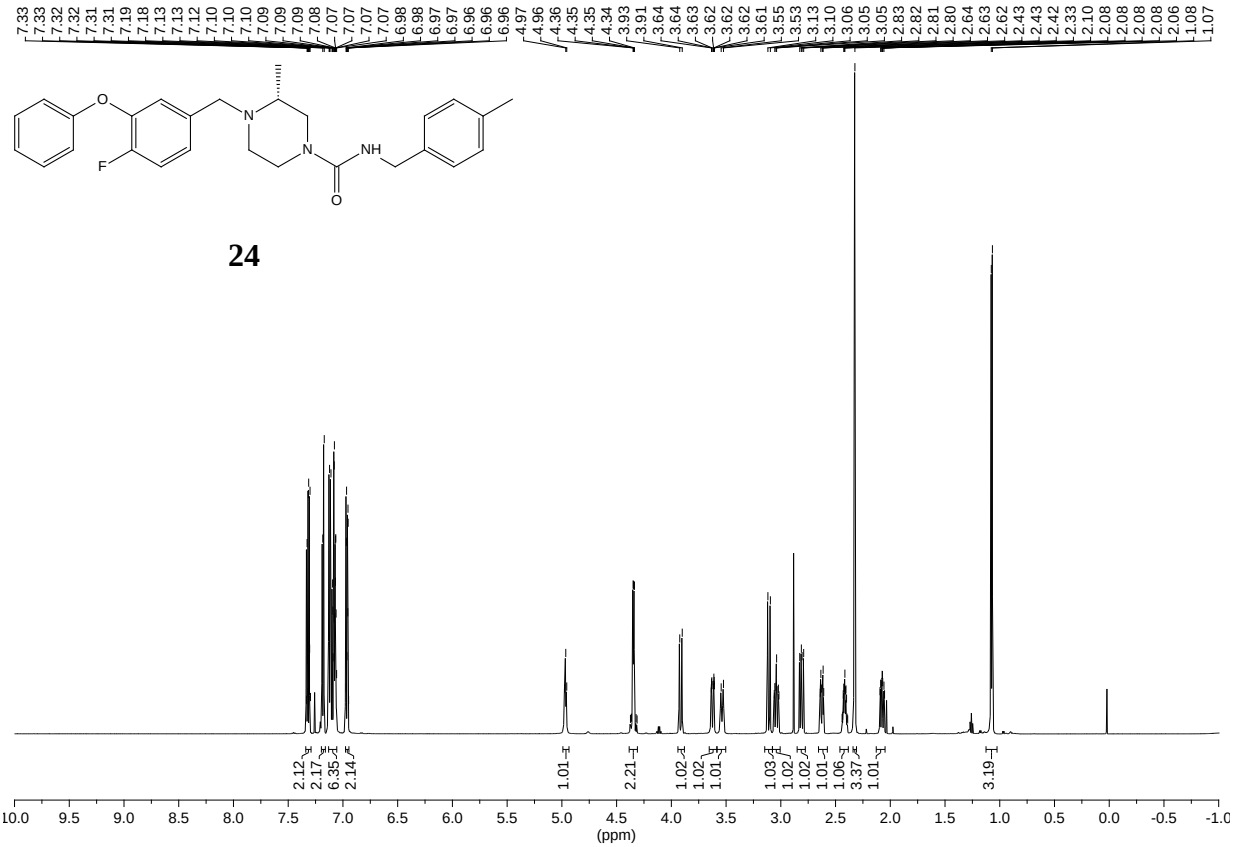
22

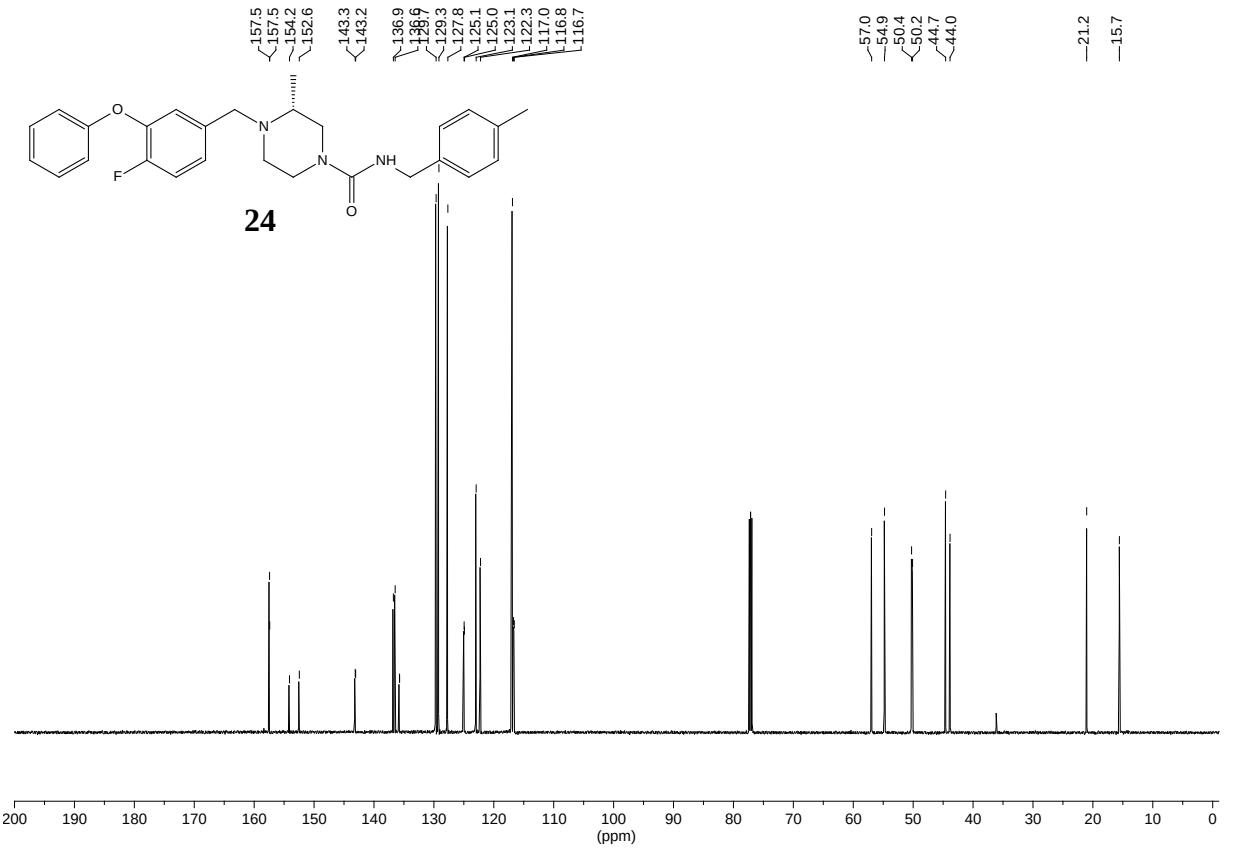

157.7
157.5
155.3
153.4
143.8
143.7
140.0
136.9
136.5
129.5
129.3
127.8
124.9
124.8
124.7
124.7
123.8
122.0
117.8
117.2
117.0
116.0
62.5
52.7
44.7
43.8
21.1
O
F
N
N
NH
O
(ppm)

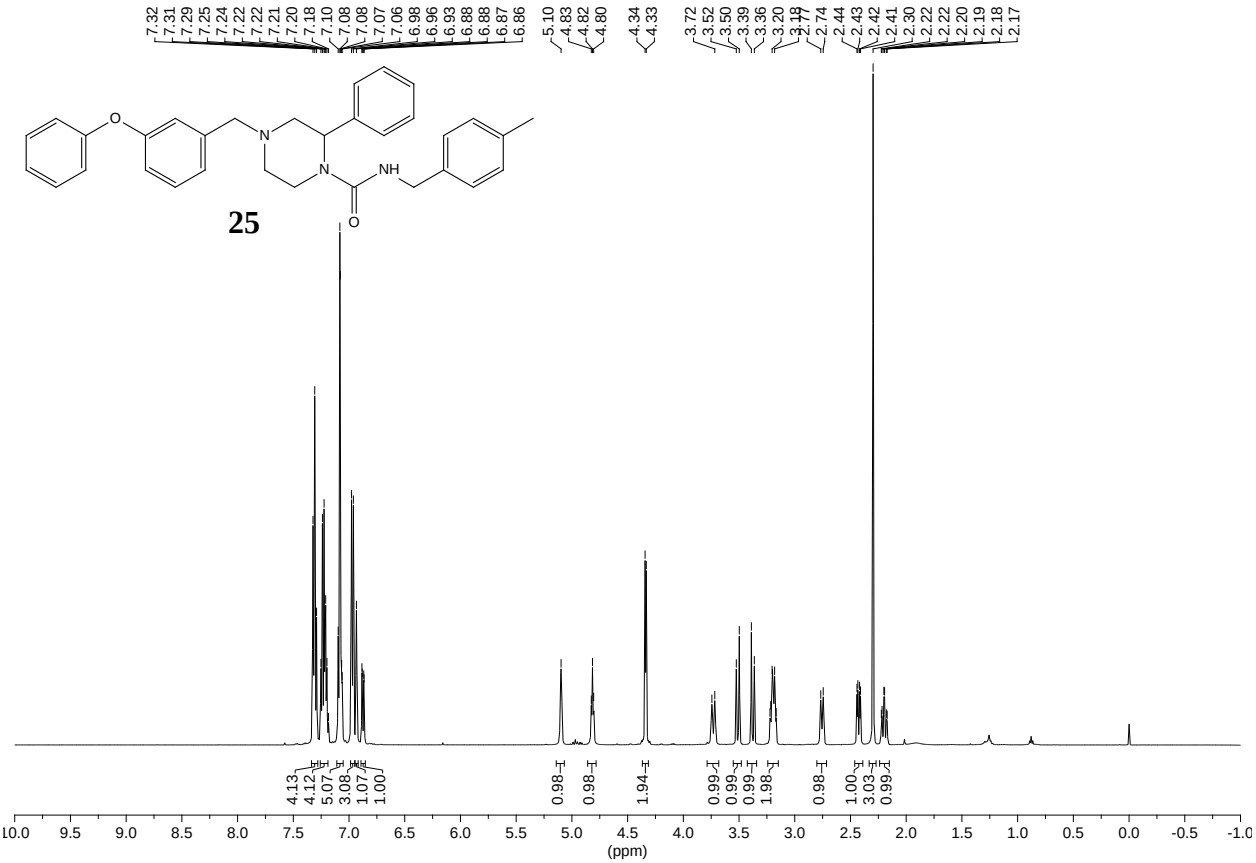
22

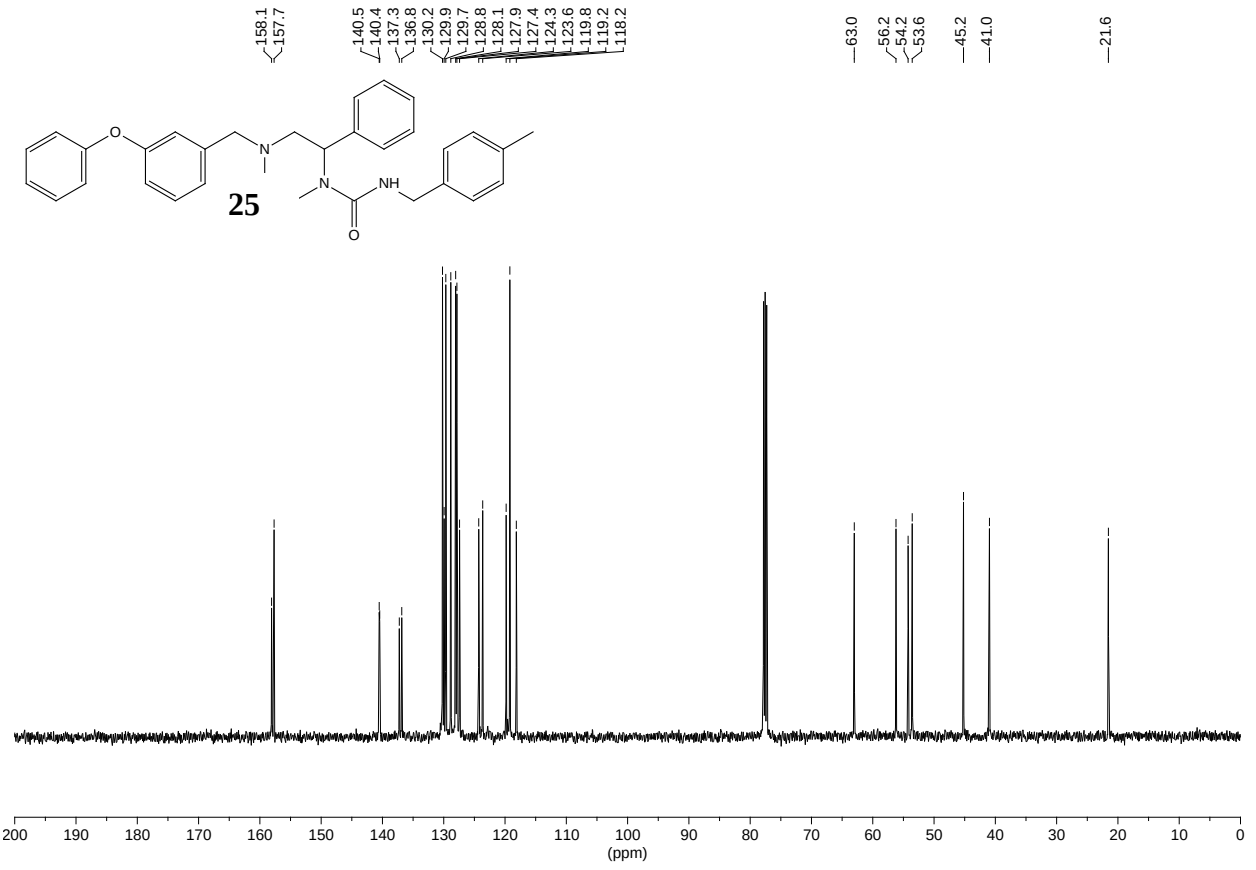

7.33
7.31
7.30
7.26
7.25
7.23
7.18
7.16
7.12
7.10
7.08
7.07
7.05
7.04
7.01
6.99
6.98
6.87
6.87
6.86
6.85
4.85
4.84
4.83
4.34
4.33
3.97
3.95
3.63
3.61
3.16
3.14
3.04
2.83
2.81
2.80
2.79
2.67
2.65
2.43
2.42
2.31
1.09
1.08
O
N
N
NH
O
23
2.07
1.09
2.09
4.24
1.05
2.06
1.02
0.97
2.01
1.00
1.00
1.02
1.01
1.02
1.01
1.02
1.03
3.20
1.04
3.02
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
-1.0
(ppm)

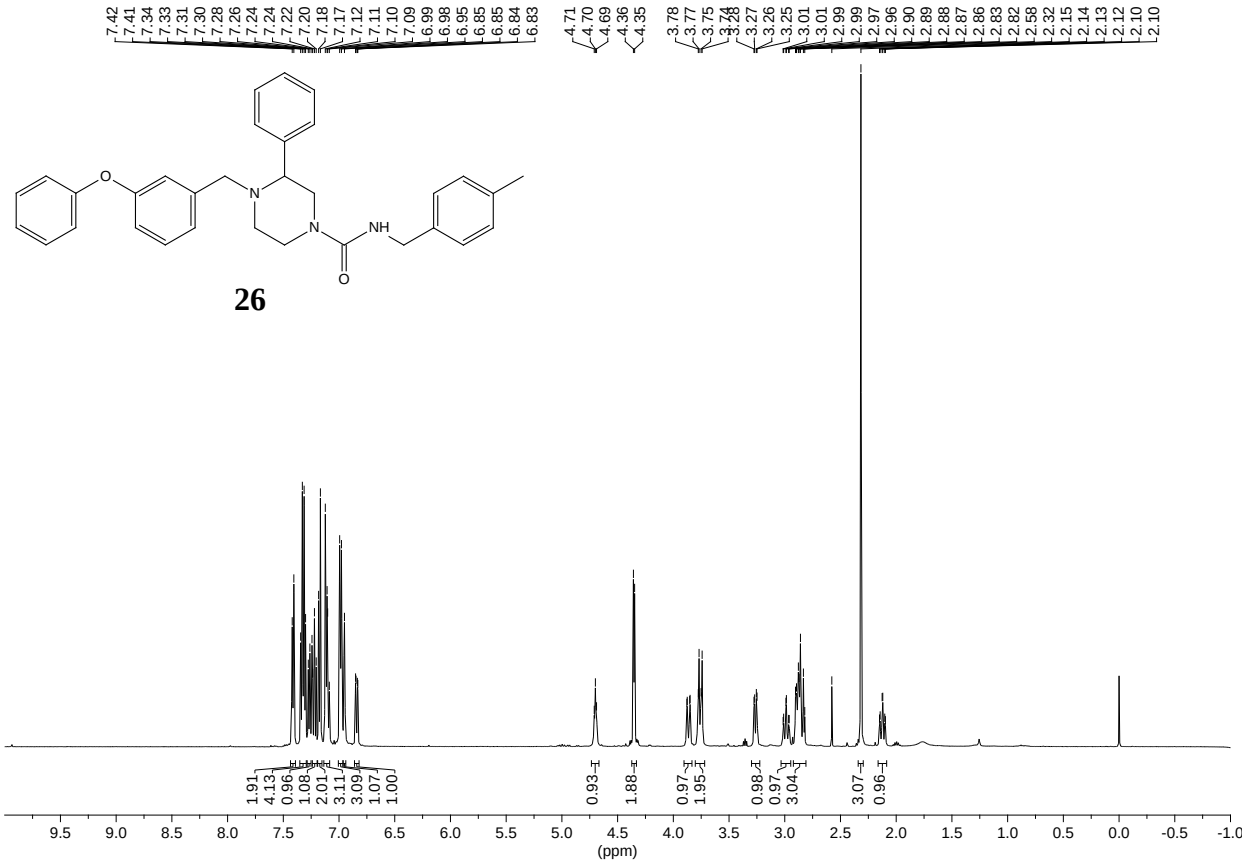

157.9
157.7
157.6
141.4
137.3
136.9
130.2
129.9
129.7
128.2
124.2
123.6
119.8
119.2
117.8
58.0
55.3
50.8
50.7
45.1
44.4
21.7
16.2
O
N
N
NH
O
23
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)


7.33 7.33 7.32 7.32 7.31 7.31 7.19 7.18 7.13 7.13 7.12 7.10 7.10 7.10 7.09 7.09 7.09 7.08 7.07 7.07 7.07 7.07 6.98 6.98 6.97 6.96 6.96 6.96 4.97 4.96 4.36 4.35 4.35 4.34 3.93 3.91 3.64 3.64 3.63 3.62 3.62 3.62 3.61 3.55 3.53 3.13 3.10 3.06 3.05 3.05 2.83 2.82 2.81 2.80 2.64 2.63 2.62 2.43 2.43 2.42 2.33 2.10 2.08 2.08 2.08 2.08 2.06 1.08 1.07
O
N
N
NH
F
O
24
2.12 2.17 6.35 2.14 1.01 2.21 1.02 1.02 1.01 1.03 1.02 1.02 1.01 1.06 3.37 1.01 3.19
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 -1.0
(ppm)

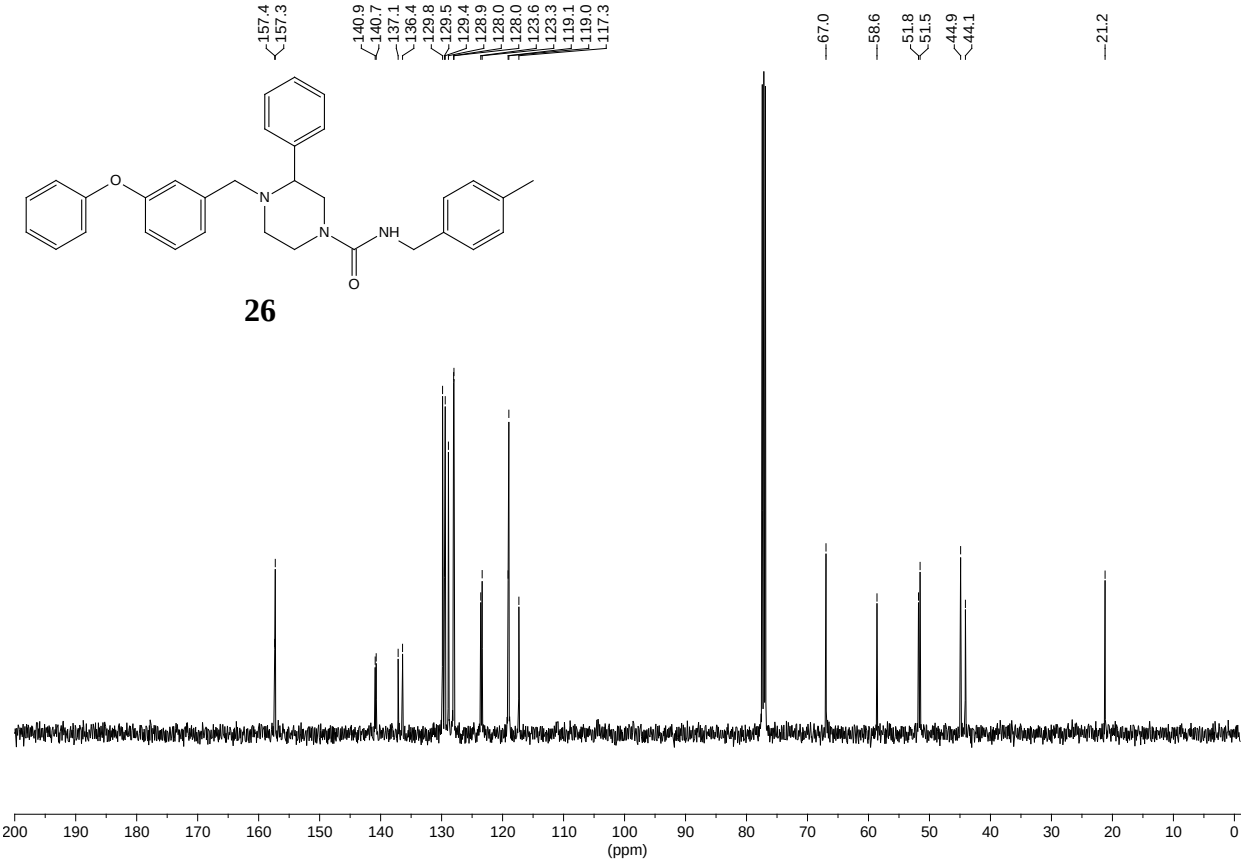

157.5 157.5 154.2 152.6 143.3 143.2 136.9 129.3 127.8 125.1 125.0 123.1 122.3 117.0 116.8 116.7
57.0 54.9 50.4 50.2 44.7 44.0 21.2 15.7
O
N
N
NH
F
O
24
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
(ppm)


7.32 7.31 7.29 7.25 7.24 7.22 7.22 7.21 7.20 7.18 7.10 7.08 7.08 7.07 7.06 6.98 6.96 6.93 6.88 6.88 6.87 6.86
5.10 4.83 4.82 4.80
4.34 4.33
3.72 3.52 3.50 3.39 3.36 3.20 2.74 2.44 2.43 2.42 2.41 2.30 2.22 2.22 2.20 2.19 2.18 2.17
O
N
N
NH
O
25
4.13 4.12 5.07 3.08 1.07 1.00
0.98 0.98 1.94 0.99 0.99 0.99 1.98 0.98 1.00 3.03 0.99
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 -1.0
(ppm)

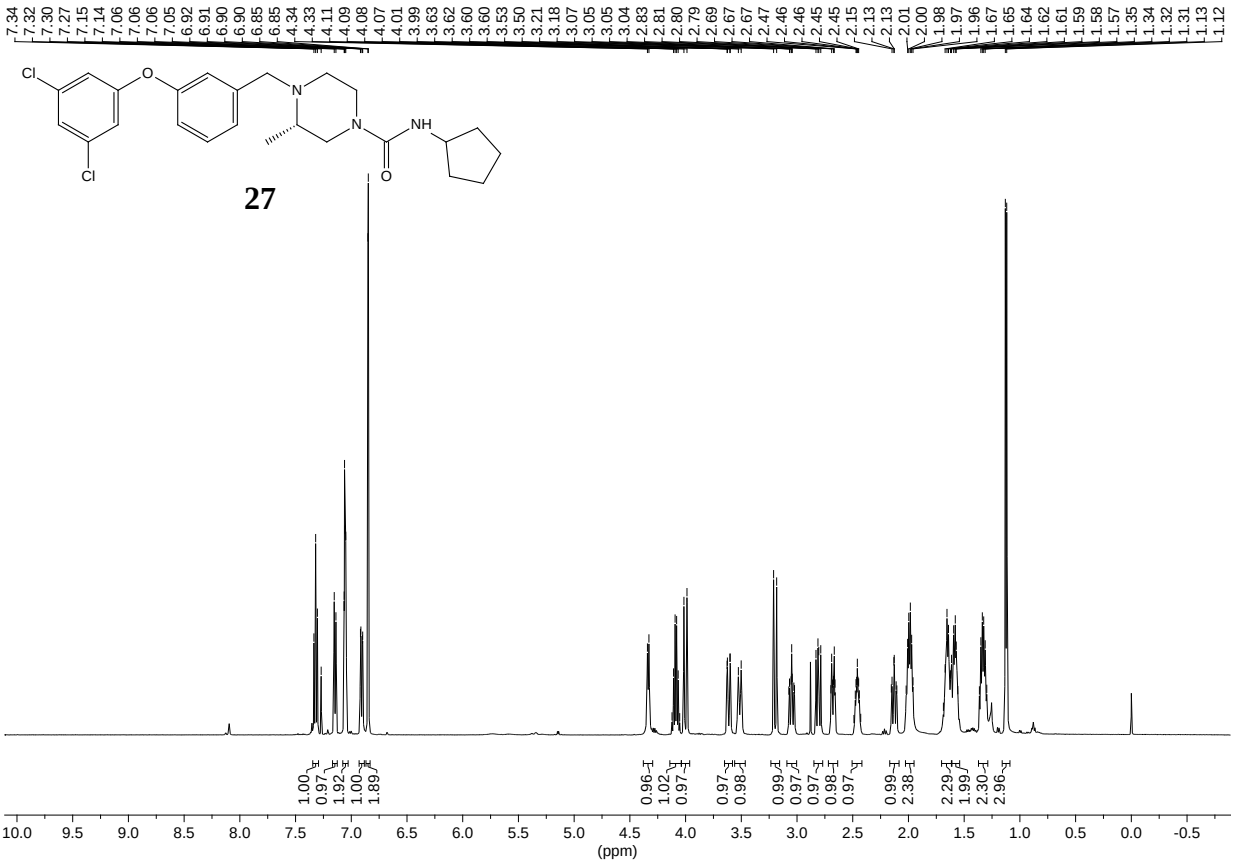

158.1 157.7
140.5 140.4 137.3 136.8 130.2 129.9 129.7 128.8 128.1 127.9 127.4 124.3 123.6 119.8 119.2 118.2
63.0
56.2 54.2 53.6
45.2
41.0
21.6
O
N
N
NH
O
25
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
(ppm)

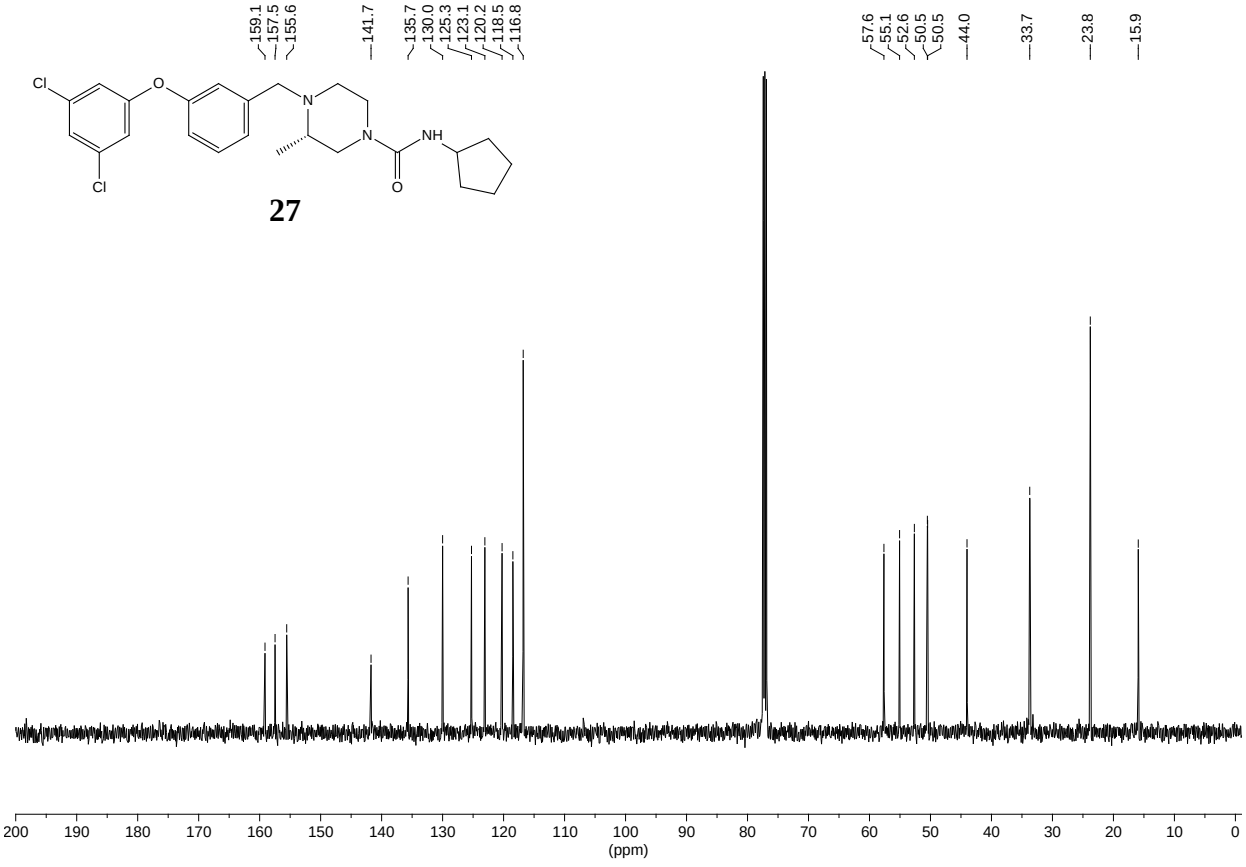

7.42 7.41 7.34 7.33 7.31 7.30 7.28 7.26 7.24 7.24 7.22 7.20 7.18 7.17 7.12 7.11 7.10 7.09 6.99 6.98 6.95 6.85 6.84 6.83
4.71 4.70 4.69 4.36 4.35
3.78 3.77 3.75 3.27 3.26 3.25 3.01 3.01 2.99 2.99 2.97 2.96 2.90 2.89 2.88 2.87 2.86 2.83 2.82 2.58 2.32 2.15 2.14 2.13 2.12 2.10 2.10
O
N
N
NH
O
1.91 4.13 0.96 1.08 2.01 3.11 3.09 1.07 1.00
0.93 1.88 0.97 1.95 0.98 0.97 3.04 3.07 0.96
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 -1.0
(ppm)

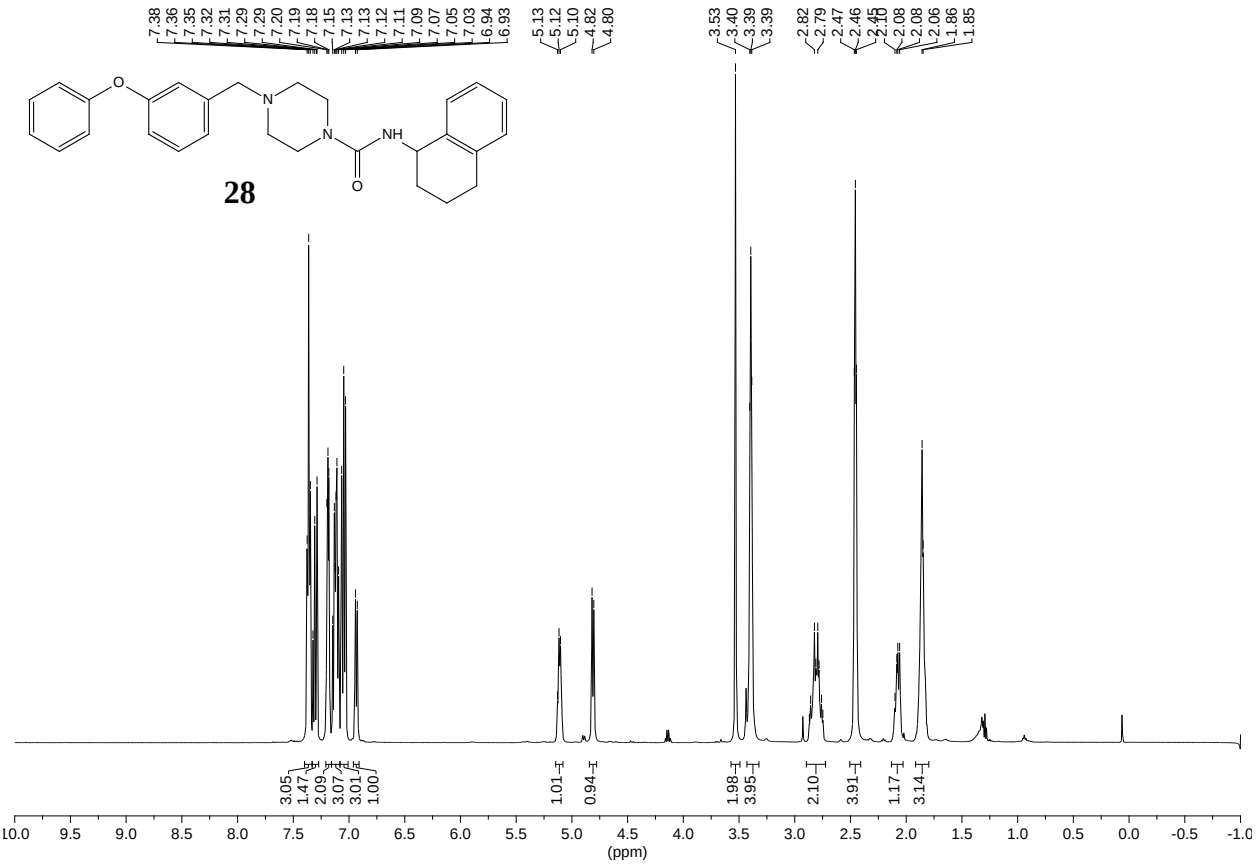
26

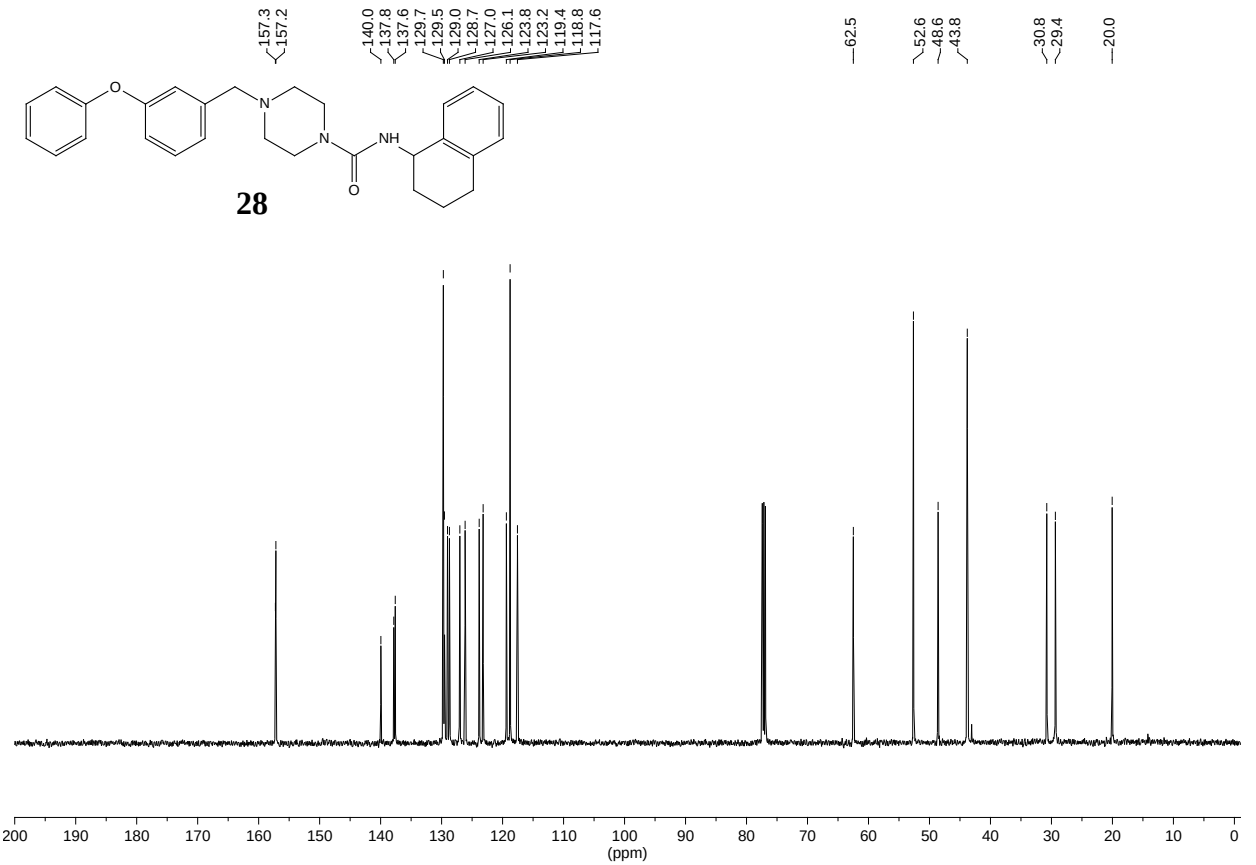

157.4 157.3
140.9 140.7 137.1 136.4 129.8 129.5 129.4 128.9 128.0 128.0 123.6 123.3 119.1 119.0 117.3
67.0
58.6
51.8 51.5
44.9 44.1
21.2
O
N
N
NH
O
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
(ppm)

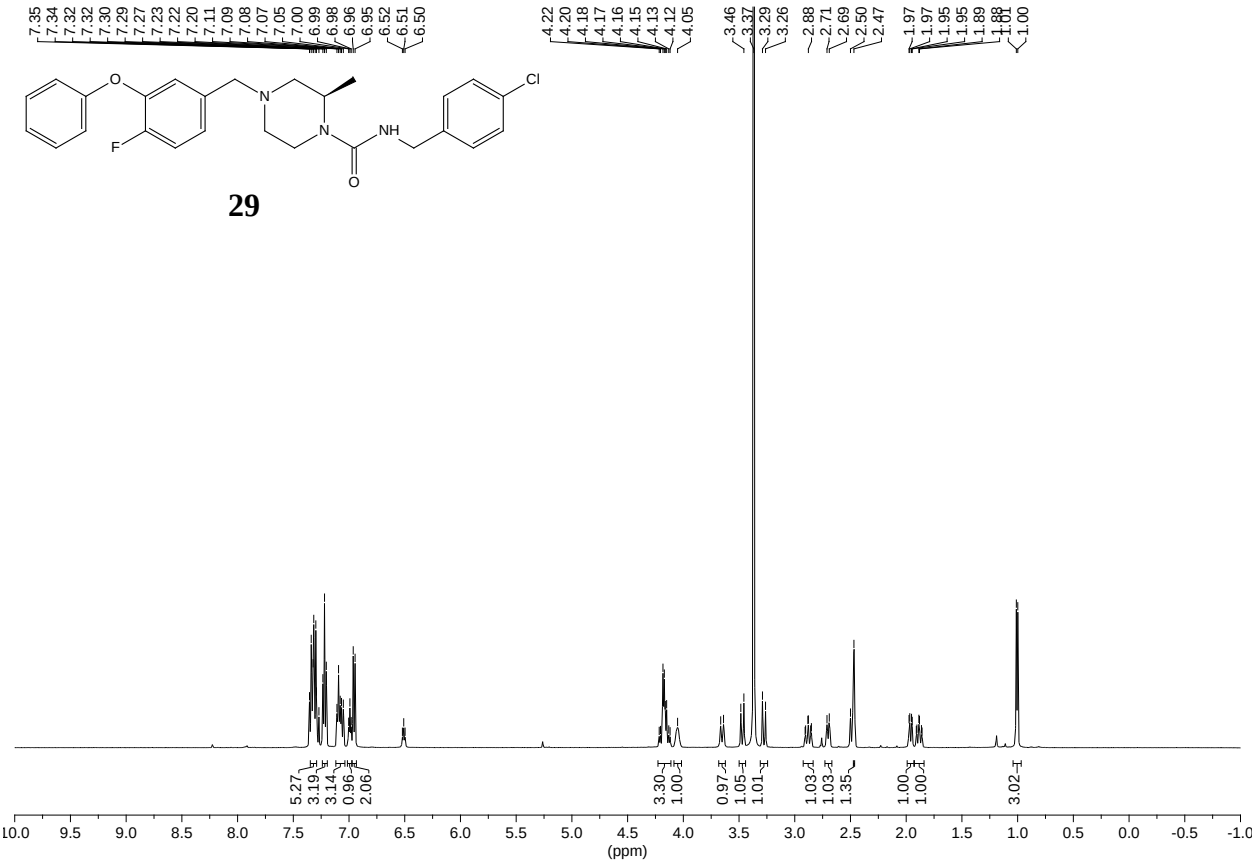
26

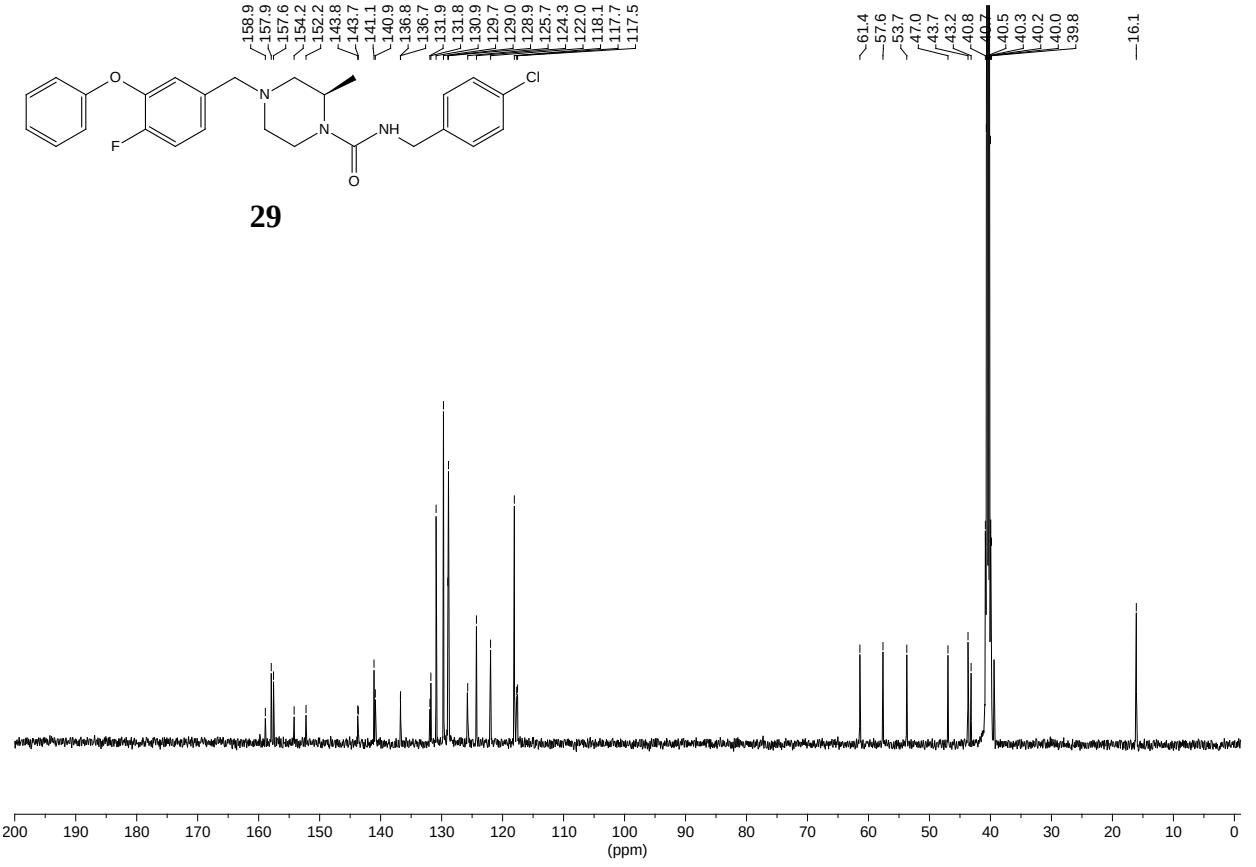

7.34 7.32 7.30 7.27 7.15 7.14 7.06 7.06 7.06 7.05 6.92 6.91 6.90 6.90 6.85 6.85 4.34 4.33 4.11 4.09 4.08 4.07 4.01 3.99 3.63 3.62 3.60 3.60 3.53 3.50 3.21 3.18 3.07 3.05 3.05 3.04 2.83 2.81 2.80 2.79 2.69 2.67 2.67 2.47 2.46 2.46 2.45 2.45 2.15 2.13 2.13 2.01 2.00 1.98 1.97 1.96 1.67 1.65 1.64 1.62 1.61 1.59 1.58 1.57 1.35 1.34 1.32 1.31 1.13 1.12
Cl
O
N
N
NH
O
Cl
27
1.00 0.97 1.92 1.00 1.89 0.96 1.02 0.97 0.97 0.98 0.99 0.97 0.98 0.97 0.99 2.38 2.29 1.99 2.30 2.96
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
(ppm)

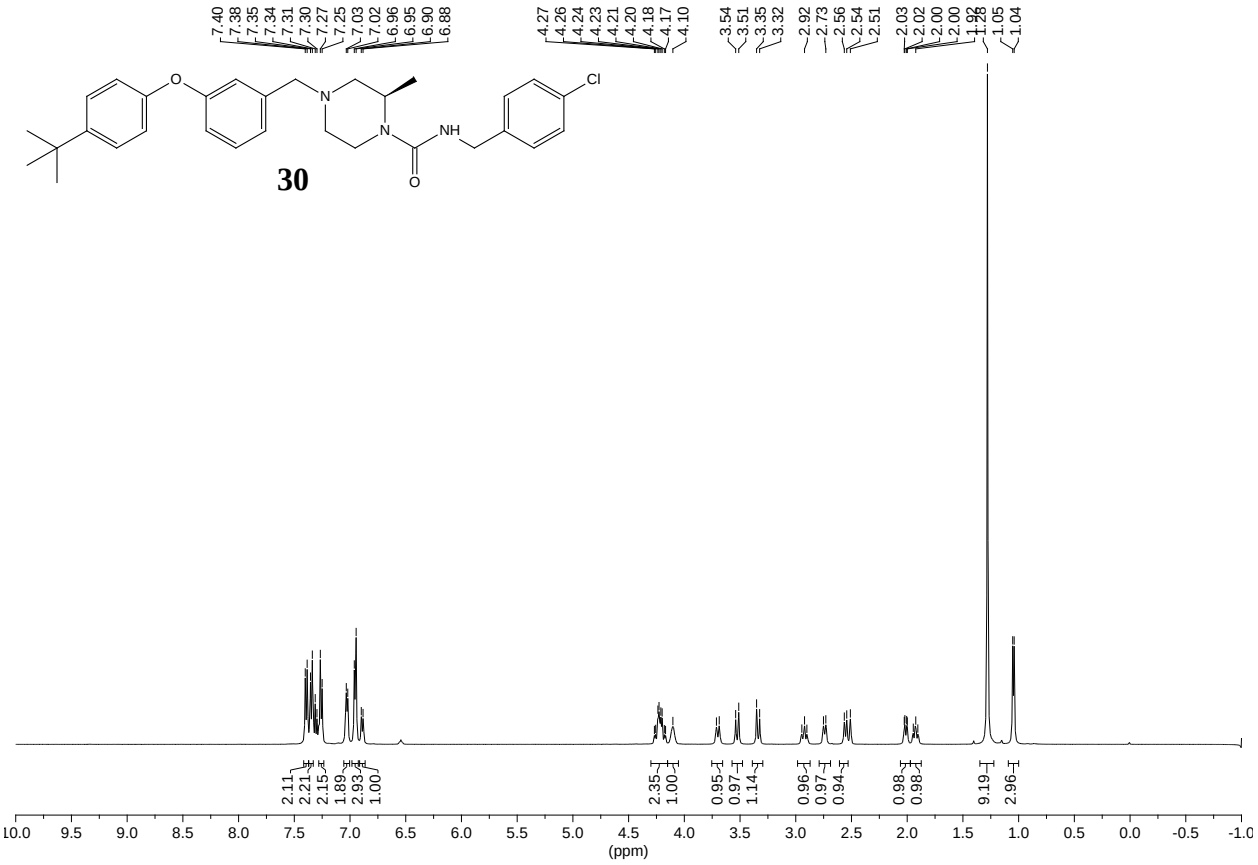

159.1 157.5 155.6 141.7 135.7 130.0 125.3 123.1 120.2 118.5 116.8
57.6 55.1 52.6 50.5 50.5 44.0 33.7 23.8 15.9
Cl
O
N
N
NH
O
Cl
27
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
(ppm)

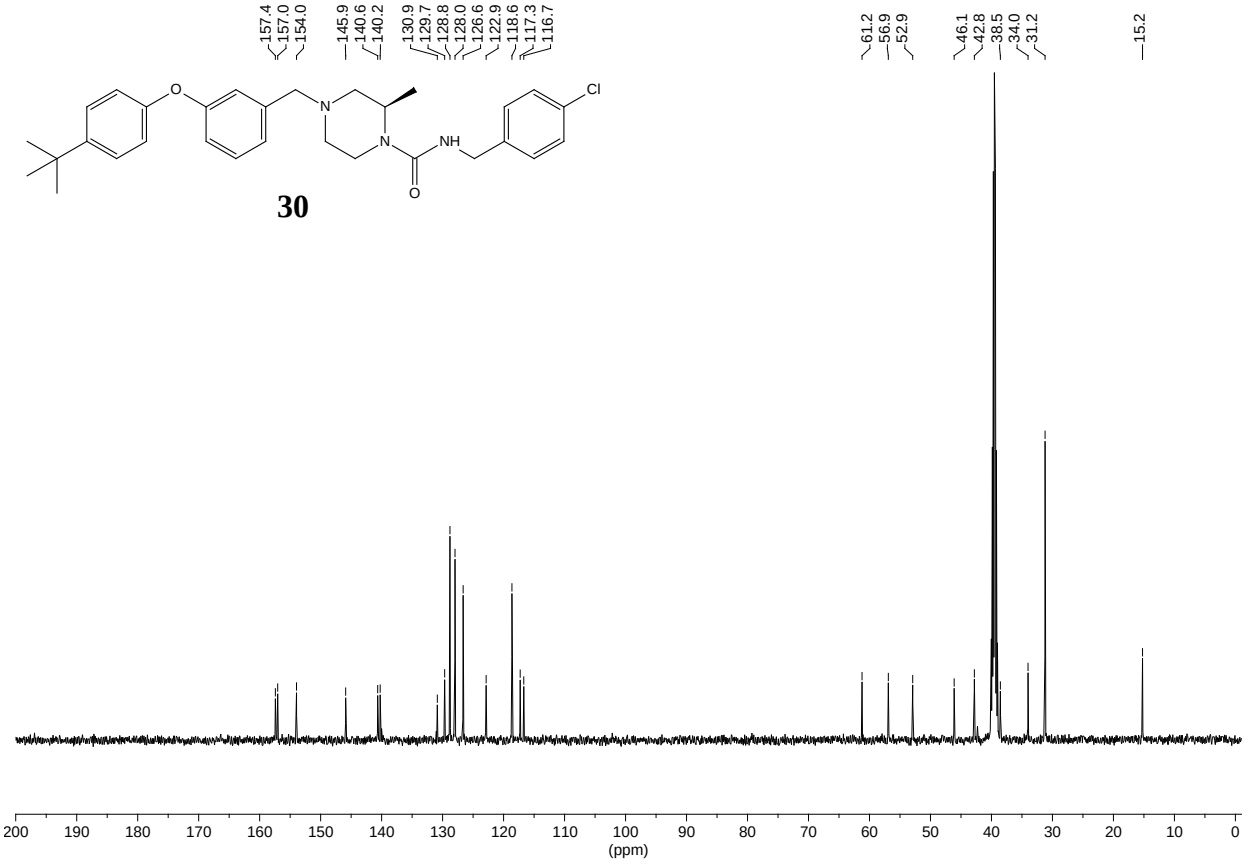

7.38
7.36
7.35
7.32
7.31
7.29
7.29
7.20
7.19
7.18
7.15
7.13
7.13
7.12
7.11
7.09
7.07
7.05
7.03
6.94
6.93
5.13
5.12
5.10
4.82
4.80
3.53
3.40
3.39
3.39
2.82
2.79
2.47
2.46
2.45
2.10
2.08
2.08
2.06
1.86
1.85
O
N
N
NH
O
28
3.05
1.47
2.09
3.07
3.01
1.00
1.01
0.94
1.98
3.95
2.10
3.91
1.17
3.14
10.0 9.5 9.0 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 -1.0
(ppm)

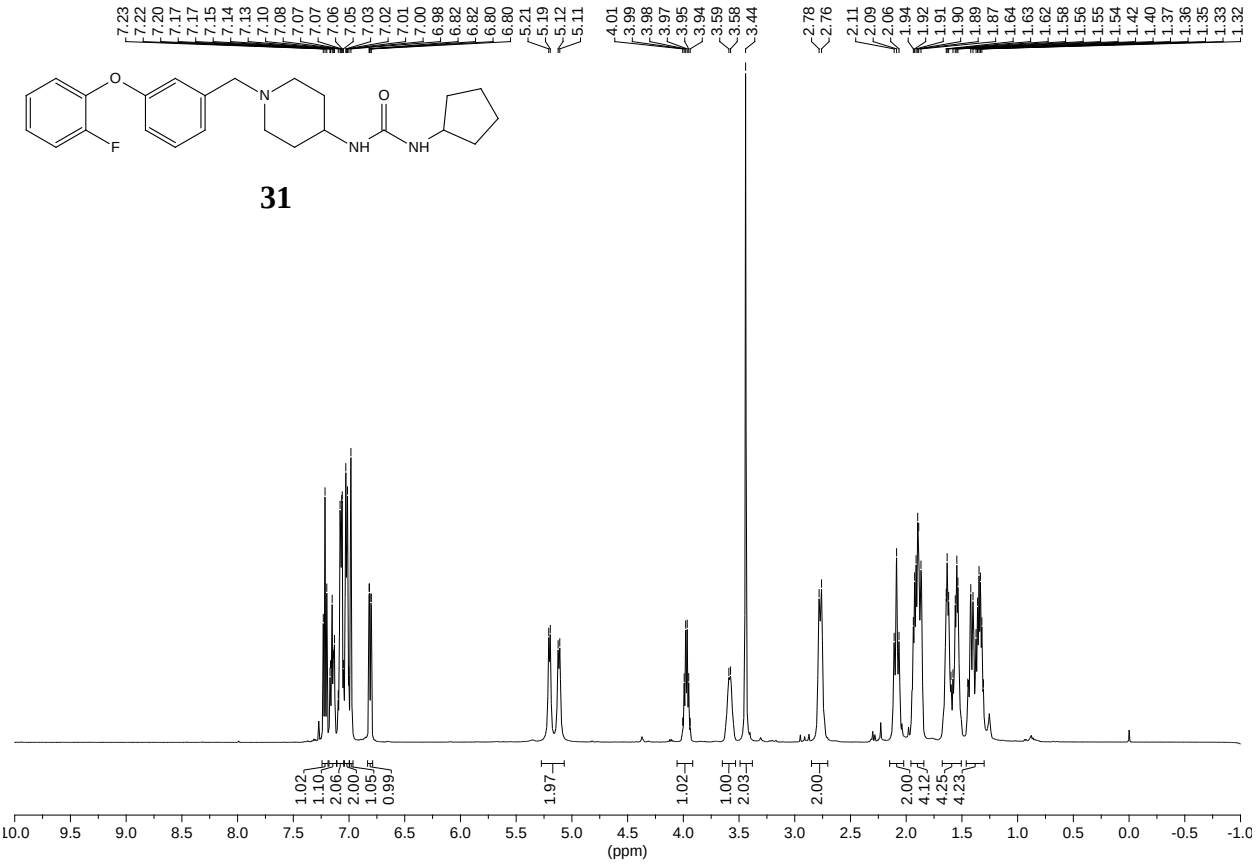

157.3
157.2
140.0
137.8
137.6
129.7
129.5
129.0
128.7
127.0
126.1
123.8
123.2
119.4
118.8
117.6
62.5
52.6
48.6
43.8
30.8
29.4
20.0
O
N
N
NH
O
28
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
(ppm)


7.35 7.34 7.32 7.32 7.30 7.29 7.27 7.23 7.22 7.20 7.11 7.09 7.08 7.07 7.05 7.00 6.99 6.98 6.96 6.95 6.52 6.51 6.50
4.22 4.20 4.18 4.17 4.16 4.15 4.13 4.12 4.05
3.46 3.37 3.29 3.26
2.88 2.71 2.69 2.50 2.47
1.97 1.97 1.95 1.95 1.89 1.88 1.01 1.00
O
F
N
N
NH
O
Cl
29
5.27 3.19 3.14 0.96 2.06
3.30 1.00 0.97 1.05 1.01 1.03 1.03 1.35 1.00 1.00 3.02
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 -1.0
(ppm)

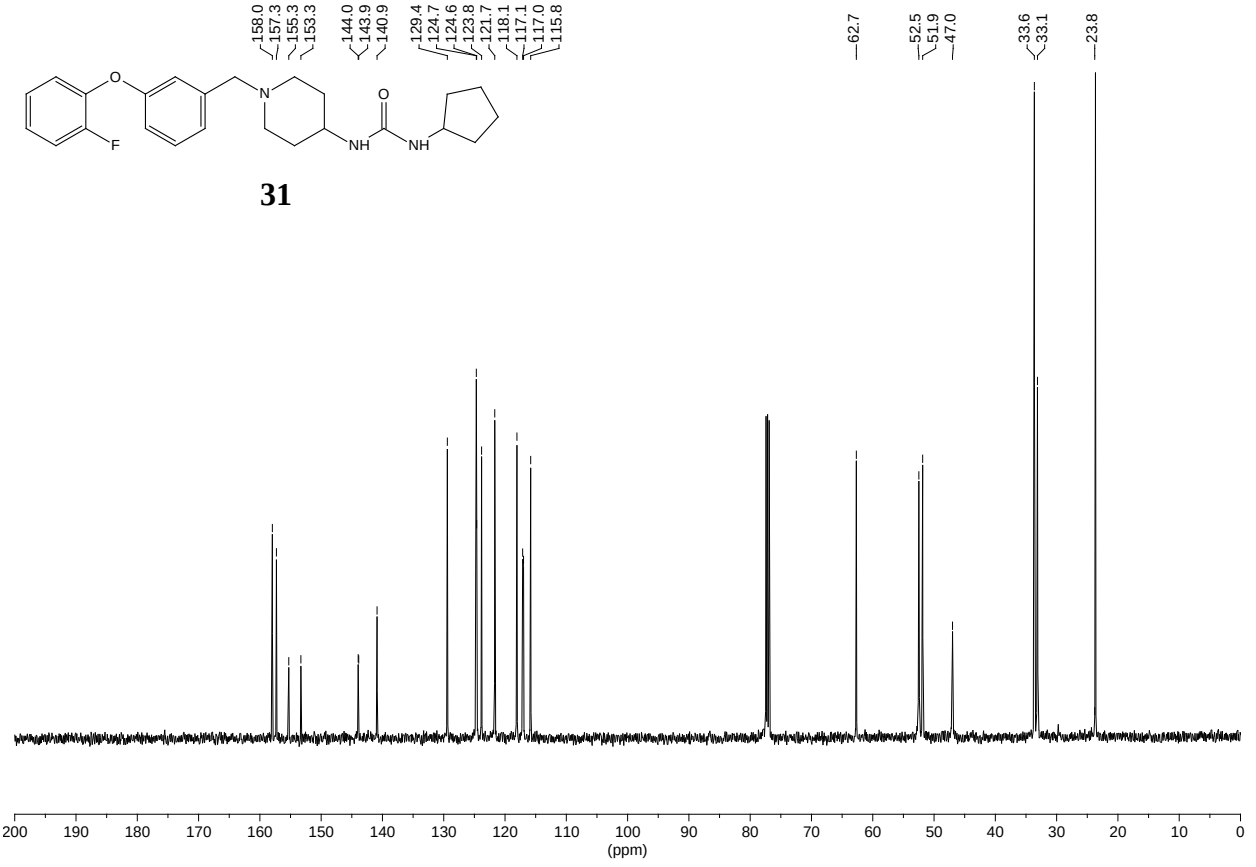

158.9 157.9 157.6 154.2 152.2 143.8 143.7 141.1 140.9 136.8 136.7 131.9 131.8 130.9 129.7 129.0 128.9 125.7 124.3 122.0 118.1 117.7 117.5
61.4 57.6 53.7 47.0 43.7 43.2 40.8 40.5 40.3 40.2 40.0 39.8
16.1
O
F
N
N
NH
O
Cl
29
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
(ppm)


7.40
7.38
7.35
7.34
7.31
7.30
7.27
7.25
7.03
7.02
6.96
6.95
6.90
6.88
4.27
4.26
4.24
4.23
4.21
4.20
4.18
4.17
4.10
3.54
3.51
3.35
3.32
2.92
2.73
2.56
2.54
2.51
2.03
2.02
2.00
2.00
1.05
1.04
O
N
N
NH
O
Cl
30
2.11
2.21
2.15
1.89
2.93
1.00
2.35
1.00
0.95
0.97
1.14
0.96
0.97
0.94
0.98
0.98
9.19
2.96
(ppm)

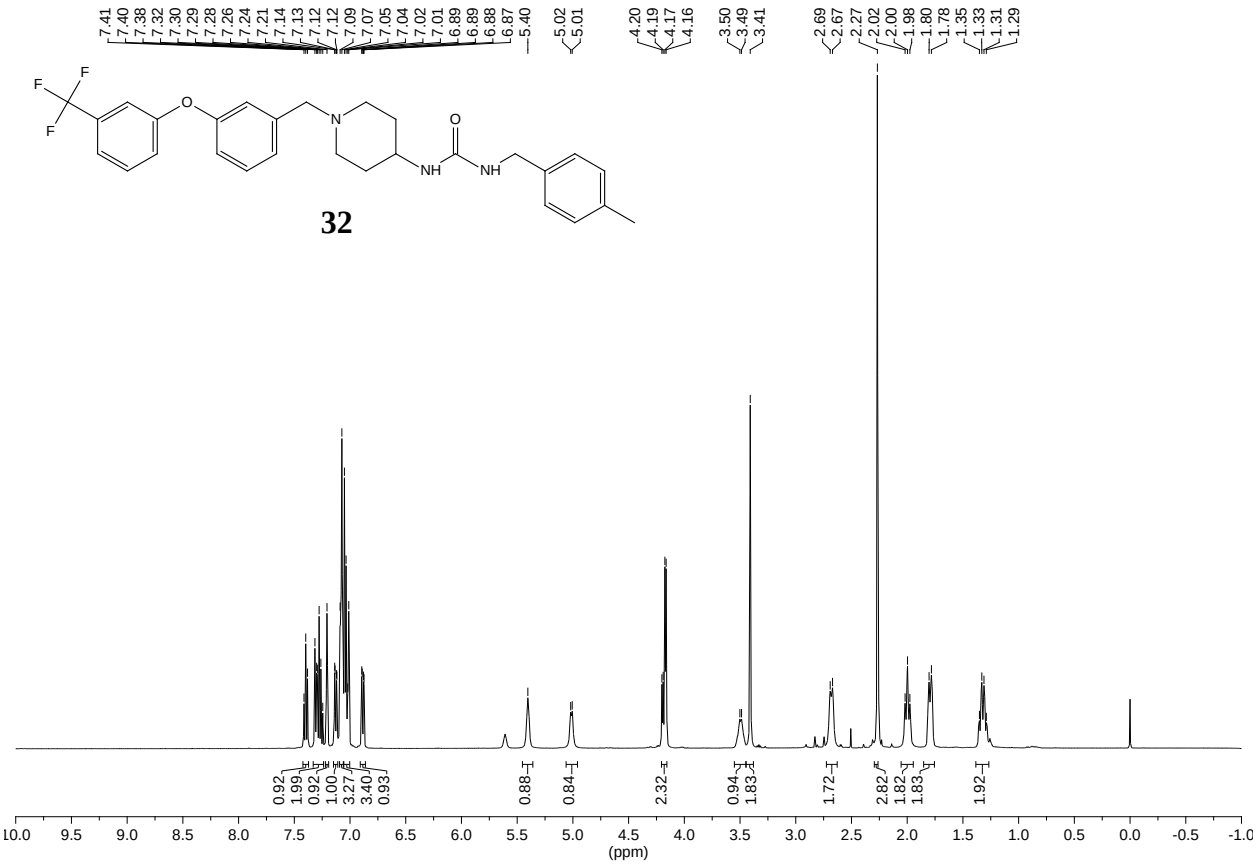

157.4
157.0
154.0
145.9
140.6
140.2
130.9
129.7
128.8
128.0
126.6
122.9
118.6
117.3
116.7
61.2
56.9
52.9
46.1
42.8
38.5
34.0
31.2
15.2
O
N
N
NH
O
Cl
30
(ppm)

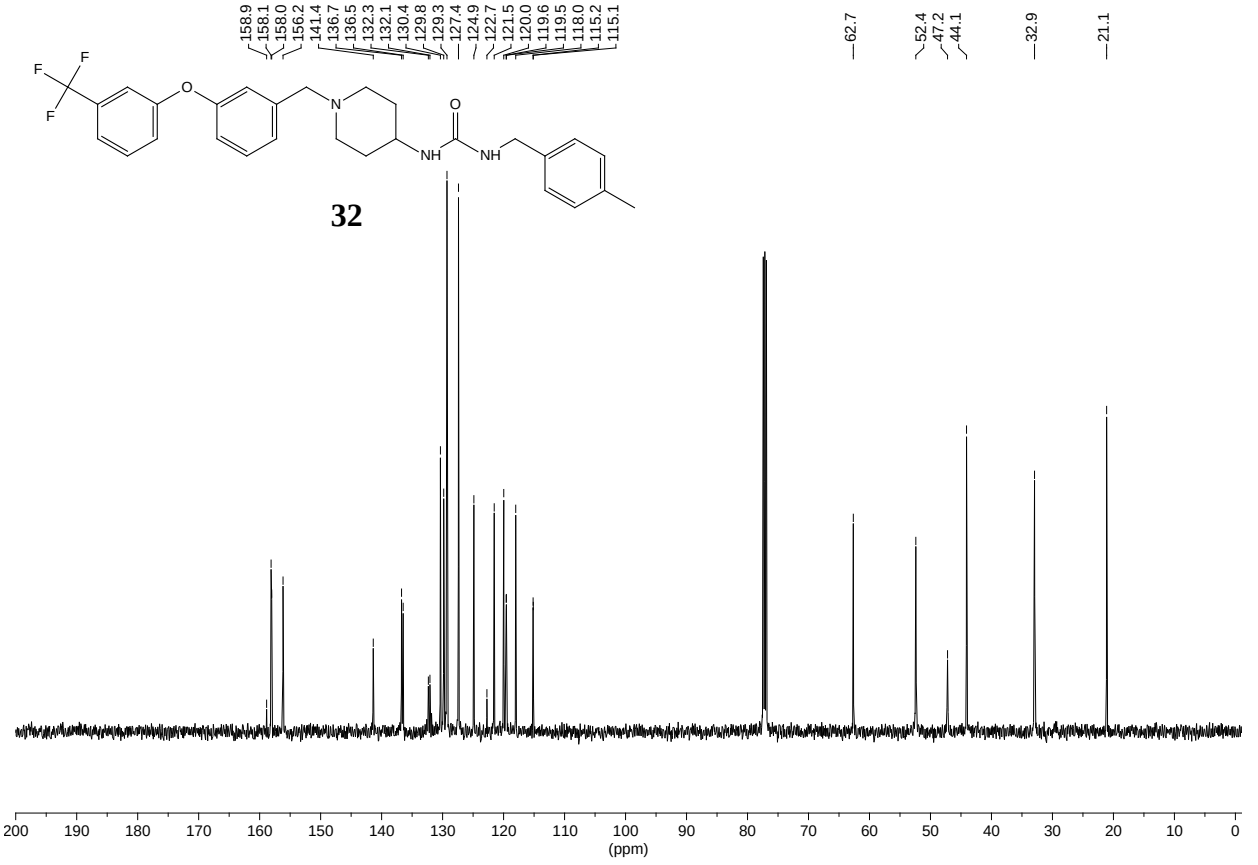

7.23 7.22 7.20 7.17 7.17 7.15 7.14 7.13 7.10 7.08 7.07 7.07 7.06 7.05 7.03 7.02 7.01 7.00 6.98 6.82 6.82 6.80 6.80
5.21 5.19 5.12 5.11
4.01 3.99 3.98 3.97 3.95 3.94 3.59 3.58 3.44
2.78 2.76
2.11 2.09 2.06 1.94 1.92 1.91 1.90 1.89 1.87 1.64 1.63 1.62 1.58 1.56 1.55 1.54 1.42 1.40 1.37 1.36 1.35 1.33 1.32
O
N
O
F
NH
NH
1.02 1.10 2.06 2.00 1.05 0.99
1.97
1.02
1.00 2.03
2.00
2.00 4.12 4.25 4.23
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 -1.0
(ppm)

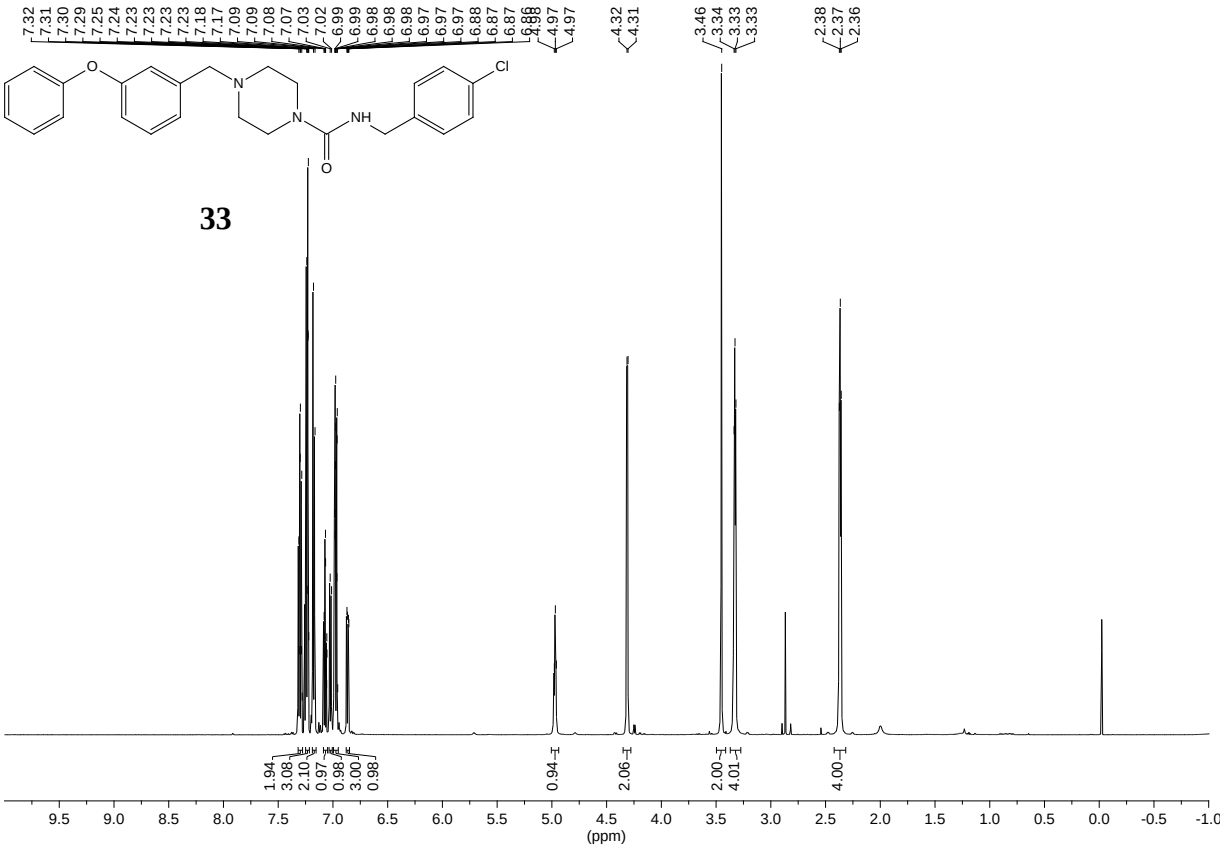
31

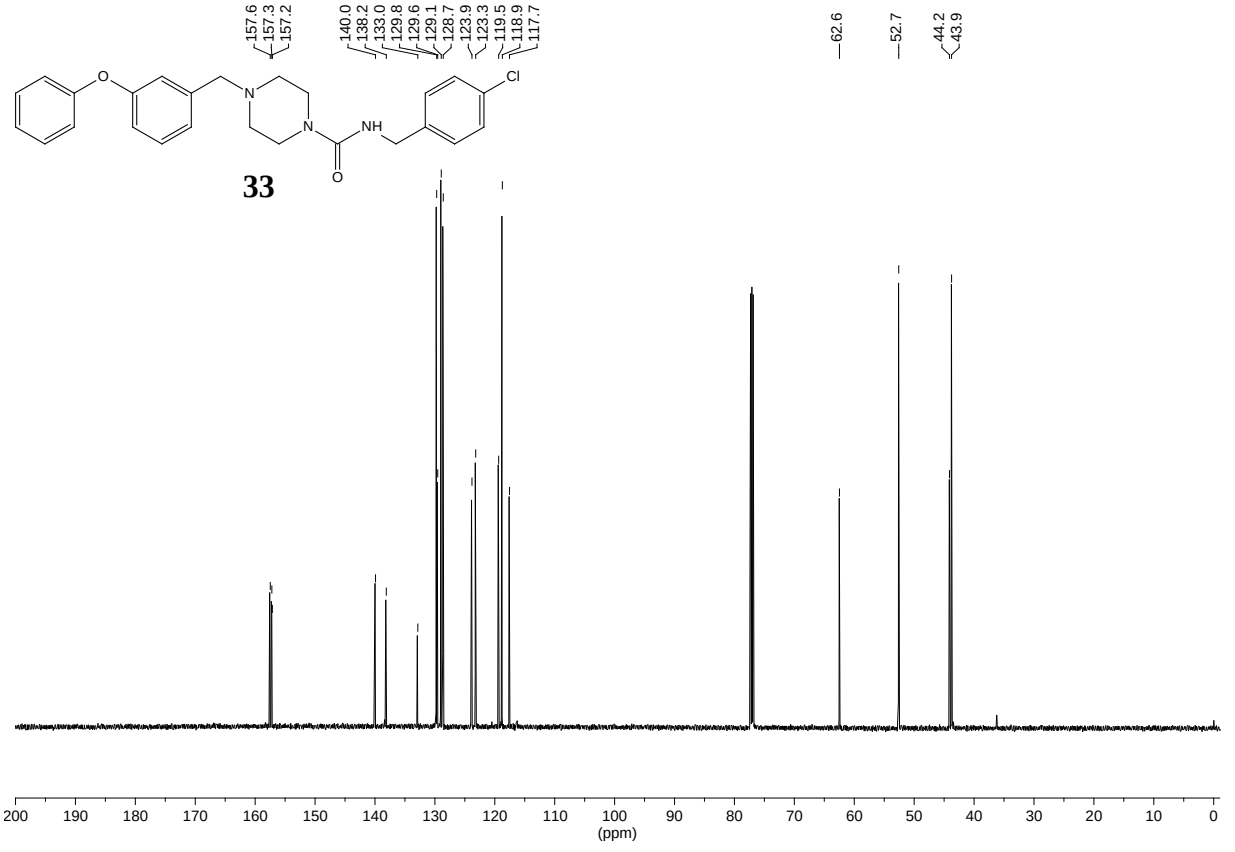

158.0 157.3 155.3 153.3
144.0 143.9 140.9
129.4 124.7 124.6 123.8 121.7 118.1 117.1 117.0 115.8
62.7
52.5 51.9 47.0
33.6 33.1
23.8
O
N
O
F
NH
NH
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
(ppm)

31


7.41
7.40
7.38
7.32
7.30
7.29
7.28
7.26
7.24
7.21
7.14
7.13
7.12
7.12
7.09
7.07
7.05
7.04
7.02
7.01
6.89
6.88
6.87
5.40
5.02
5.01
4.20
4.19
4.17
4.16
3.50
3.49
3.41
2.69
2.67
2.27
2.02
2.00
1.98
1.80
1.78
1.35
1.33
1.31
1.29
F
F
F
O
N
O
NH
NH
32
0.92
1.99
0.92
1.00
3.27
3.40
0.93
0.88
0.84
2.32
0.94
1.83
1.72
2.82
1.82
1.83
1.92
(ppm)


158.9
158.1
158.0
156.2
141.4
136.7
136.5
132.3
132.1
130.4
129.8
129.3
127.4
124.9
122.7
121.5
120.0
119.6
119.5
118.0
115.2
115.1
62.7
52.4
47.2
44.1
32.9
21.1
F
F
F
O
N
O
NH
NH
32
(ppm)


7.32
7.31
7.30
7.29
7.25
7.24
7.23
7.23
7.23
7.23
7.18
7.17
7.09
7.09
7.08
7.07
7.03
7.02
6.99
6.99
6.98
6.98
6.98
6.97
6.97
6.97
6.88
6.87
6.87
6.86
4.98
4.97
4.97
4.32
4.31
3.46
3.34
3.33
3.33
2.38
2.37
2.36
O
N
N
NH
O
Cl
33
1.94
3.08
2.10
0.97
0.98
3.00
0.98
0.94
2.06
2.00
4.01
4.00
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 -1.0
(ppm)


157.6
157.3
157.2
140.0
138.2
133.0
129.8
129.6
129.1
128.7
123.9
123.3
119.5
118.9
117.7
62.6
52.7
44.2
43.9
O
N
N
NH
O
Cl
33
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
(ppm)