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Urea derivatives of spirocyclic piperidines endowed with antibacterial activity

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Contents

Experimental procedures and characterization data

General

NMR spectra were acquired with 400 MHz Bruker Avance III spectrometer (400.13 MHz for ^{1}H, 376.49 MHz for ^{19}F and 100.61 MHz for ^{13}C) in CDCl3 or DMSO-*d*$_6$ and were referenced to residual solvent proton signals (δ_H = 7.26 and 2.50, respectively) and solvent carbon signals (δ_C = 77.16 and 39.52, respectively). Multiplicities are abbreviated as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, dd = doublet of doublets, dt = doublet of triplets, ddd = doublet/doublets of doublets; coupling con-stants, *J*, are reported in Hz. Mass spectra were acquired with an HRMS-ESI-qTOF spectrometer Nexera LCMS9030 or MaXis II Bruker Daltonic GmbH (electrospray ion-ization mode, positive ions detection). Flash column chromatography on silica (Merck, 230–400 mesh) was performed with Biotage Isolera Prime instrument. TLC was per-formed on aluminum-backed pre-coated plates (0.25 mm) with silica gel 60 F254 with a suitable solvent system and was visualized using UV fluorescence.

Starting materials

9-Benzyl-1-oxa-9-azaspiro[5.5]undecan-4-yl methanesulfonate **7** and *tert*-butyl 4-hydroxy-1-oxa-9-azaspiro[5.5]undecane-9-carboxylate **4** were synthesized on 5-10 g scale as described previously [A. Lukin *et al*., *Bioorg. Chem.* 2018, **80**, 655-667].

tert-Butyl (9-benzyl-1-oxa-9-azaspiro[5.5]undec-4-yl)carbamate 9

9-Benzyl-1-oxa-9-azaspiro[5.5]undecan-4-yl methanesulfonate **7** (20 g, 58.92 mmol) was dissolved in anhydrous DMF (100 ml). Sodium azide (9.575 g, 147.3 mmol) was added in portions with stirring. The stirring was continued for 12 h while the reaction mixture was heated at 80 ºC on an oil bath. The mixture was cooled to ambient temperature, poured into water (200 ml) and the resulting turbid mixture was extracted with ethyl acetate (2x100 ml). The combined organic extracts were washed with sat. aq. $NaHCO_3$ solution, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was dissolved in methanol (100 ml), and water (3.0 ml) was added. Triphenylphosphine (23.181 g, 88.38 mmol) was added in small portions, and the resulting mixture was heated at reflux for 3 h. The volatiles were removed on a rotatory evaporator, the residue was dissolved in CH_2Cl_2 (100 ml), and the solution was extracted with 30% aqueous citric acid. The acidic aqueous extract was neutralized with K_2CO_3 and extracted with CH_2Cl_2 (2 x 100 ml). The combined organic extracts were dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was triturated with 7:3 hexane-diethyl ether mixture, and the crystalline residue thus formed was separated by filtration and washed with diethyl ether. The solid residue was dried *in vacuo*. Then it was dissolved in CH_2Cl_2 (100 ml) and treated with di-*tert*-butyl dicarbonate (8.883 g, 50.98 mmol) added in small portions. The resulting mixture was stirred at ambient temperature for 5 hours and concentrated *in vacuo*. The residue was crystallized from 1:1 mixture of hexane-diethyl ether. The crystalline product was separated by filtration, washed with hexane and dried in high vacuum to give the title compound as a white crystalline solid (m.p. 134-136 ºC). Yield 14.5 g (69%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36 – 7.21 (m, 5H), 4.46 – 4.24 (m, 1H), 3.85 – 3.70 (m, 2H), 3.68 – 3.55 (m, 1H), 3.53 (s, 2H), 2.67 – 2.51 (m, 2H), 2.50 – 2.35 (m, 1H), 2.35 – 2.22 (m, 1H), 2.11 (d, *J* = 13.7 Hz, 1H), 1.97 – 1.80 (m, 2H), 1.74 – 1.50 (m, 3H), 1.45 (s, 9H), 1.39 – 1.24 (m, 1H), 1.13 (t, *J* = 12.4 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 155.1, 138.1, 129.2, 128.2, 127.1, 79.4, 71.1, 63.1, 59.5, 48.9, 48.7, 43.7, 42.9, 39.1, 33.7, 29.4, 28.4. Anal. Calcd for $C_{21}H_{32}N_2O_3$: C, 69.97; H, 8.95; N, 7.77; found C, 70.02; H, 8.91; N, 7.81.

tert-Butyl (1-oxa-9-azaspiro[5.5]undec-4-yl)carbamate 6

tert-Butyl (9-benzyl-1-oxa-9-azaspiro[5.5]undec-4-yl)carbamate **8** (8.5 g, 24 mmol) was dissolved in methanol (100 ml). Ammonium formate (6.06 g, 96 mmol) was added in portions followed by 5% Pd/C (0.43 g). The mixture was heated at reflux for 8 h, cooled down to room temperature, filtered through a pad of celite. The filtrate was concentrated *in vacuo*, and the residue was extracted with CH_2Cl_2 (3 x 50 ml). The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to give the title compound as clear oil. Yield – 5.1 g (79%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.75 (d, *J* = 7.6 Hz, 1H), 3.65 – 3.47 (m, 4H), 3.01 – 2.55 (m, 3H), 2.02 – 1.85 (m, 1H), 1.71 – 1.55 (m, 2H), 1.43 – 1.24 (m, 14H), 1.07 (t, *J* = 12.1 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 154.8, 79.2, 77.5, 71.1, 58.8, 42.8, 42.2, 41.3, 32.8, 30.3, 28.3. Anal. Calcd. For $C_{14}H_{26}N_2O_3$: C, 62.19; H, 9.69; N, 10.36; found C, 62.22; H, 9.71; N, 10.33.

4.2.4. 9-Benzyl-1-oxa-9-azaspiro[5.5]undecan-4-amine 5

tert-Butyl (9-benzyl-1-oxa-9-azaspiro[5.5]undec-4-yl)carbamate **9** (4.0 g, 11.09 mmol) was dissolved in 4M HCl in 1,4-dioxane (8.3 ml, 33.27 mmol), and the resulting mixture was stirred for 10 h. The mixture was concentrated *in vacuo*, and the residue was neutralized with sat. aq. K_2CO_3 to give the title compound as clear oil. Yield – 2.83 g (95%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.24 – 11.02 (m, 1H), 8.30 – 8.14 (m, 3H), 7.71 – 7.59 (m, 2H), 7.50 – 7.39 (m, 3H), 4.27 (d, J = 4.6 Hz, 2H), 3.73 (dd, J = 12.0, 4.6 Hz, 1H), 3.60 – 3.45 (m, 1H), 3.16 – 3.00 (m, 3H), 2.93 – 2.75 (m, 1H), 2.49 – 2.40 (m, 1H), 2.22 – 1.31 (m, 8H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 131.4, 129.8, 129.3, 128.6, 68.4, 58.6, 46.7, 43.3, 35.0, 29.8, 25.5. Anal. Calcd. for $C_{16}H_{24}N_2O$: C, 73.81; H, 9.29; N, 10.76; found C, 73.86; H, 9.33; N, 10.72.

4-(Pyridin-4-yloxy)-1-oxa-9-azaspiro[5.5]undecane dihydrochloride 10

A solution of 9-*tert*-butoxycarbonyl-1-oxa-9-azaspiro[5.5]undecan-4-ol **4** (4 g, 14.8 mmol) in DMF (20 ml) was added dropwise to a 0 ºC suspension of NaH (1.3 g, 32.6 mmol, 60% dispersion in mineral oil) in dry DMF (100 ml) under argon. The resulting mixture was stirred at 0 ºC for 30 min whereupon a solution of 4-chloropyridine hydrochloride (2.88 g, 19.2 mmol) in DMF (10 ml) was added dropwise. The resulting mixture was allowed to warm up to room temperature and was stirred at this temperature for 18 h. The mixture was poured into water (200 ml), and the aqueous phase was extracted with ethyl acetate (3 x 200 ml). The combined organic extracts were washed with 3% aqueous citric acid, 5% aqueous $NaHCO_3$, brine and water, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was fractionated on silica gel using 0→5% ethyl acetate in hexanes as the eluent, and the fractions containing the desired product (according to LC MS analysis) were combined and concentrated to dryness. Without further purification, the residue was dissolved in CH_2Cl_2 (3 ml mmol^{-1} calculated assuming 100% purity of the material obtained in the previous step), the solution was cooled to 0 ºC, and TFA (1 ml mmol^{-1}) was added. The mixture thus obtained was stirred at 0 ºC for 6 h and then concentrated to dryness to provide, after crystallization from isopropyl alcohol, the target spirocyclic piperidine as a trifluoroacetate salt. The material was converted to hydrochloride salts **10** by treatment of their room temperature suspensions in 1,4-dioxane with 4M HCl in 1,4-dioxane followed by stirring for 3 h, evaporation of the volatiles *in vacuo* and crystallization from isopropyl alcohol.

Yield 2.13 g (6.63 mmol, 45%), white crystalline solid, mp 109-114 ºC. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.38 – 8.33 (m, 2H), 6.99 – 6.96 (m, 2H), 4.91 – 4.80 (m, 1H), 3.77– 3.62 (m, 2H), 2.80 – 2.57 (m, 4H), 2.03 – 1.87 (m, 3H), 1.56 – 1.30 (m, 5H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 163.0, 151.0, 111.1, 71.7, 69.6, 57.7, 41.5, 41.3, 40.6, 32.1, 31.6. MS *m/z* 249.1 (M+H$^+$).

General procedure for the synthesis of compounds 2a-c

Compound **10** (0.58 mmol) thus obtained was dissolved in 1,4-dioxane (10 ml) and treated with the corresponding isocyanate (0.61 mmol) and triethylamine (1 ml). The reaction mixture was stirred overnight, poured into water (25 ml) and extracted with ethyl acetate (2 x 25 ml). The combined organic phase was washed with 3% aqueous citric acid, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel using 0→2% MeOH in CH_2Cl_2 as the eluent. The fractions containing the product were combined, concentrated *in vacuo* to provide the title compound.

***N*-(4-Fluorophenyl)-4-(pyridin-4-yloxy)-1-oxa-9-azaspiro[5.5]undecane-9-carboxamide (2a**)
Yield 100 mg, (44 %), white solid, m.p. 142-143 °C; ^{1}H NMR (300 MHz, DMSO-*d₆*) δ 8.50 (s, 1H), 8.37 (d, *J* = 4.3 Hz, 2H), 7.44 (dd, *J* = 7.6, 5.2 Hz, 2H), 7.14 – 6.93 (m, 4H), 4.97 – 4.83 (m, 1H), 3.91 – 3.63 (m, 4H), 3.11 (dt, *J* = 23.8, 11.5 Hz, 2H), 2.08 – 1.94 (m, 3H), 1.75 – 1.61 (m, 1H), 1.60 – 1.34 (m, 4H); ^{13}C NMR (75 MHz, DMSO-*d₆*) δ 163.1, 157.5 (d, *J* = 238.0 Hz), 155.2, 151.1, 137.1 (d, *J* = 2.5 Hz), 121.7 (d, *J* = 7.6 Hz), 114.9 (d, *J* = 22.0 Hz), 111.3, 71.4, 69.8, 58.2, 40.4, 37.3, 31.5, 31.2; HRMS (ESI), m/z calcd for $C_{21}H_{24}N_3O_3F[M+H]^+$ 386.1802, found 386.1875.

***N*-1-Adamantyl-4-(pyridin-4-yloxy)-1-oxa-9-azaspiro[5.5]undecane-9-carboxamide (2b).**
Yield 143 mg (56%), white solid, m.p. 137-139 °C, ^{1}H NMR (300 MHz, DMSO-*d₆*) δ 8.36 (d, *J* = 5.7 Hz, 2H), 6.97 (d, *J* = 5.8 Hz, 2H), 5.55 (s, 1H), 4.92 – 4.81 (m, 1H), 3.82 – 3.60 (m, 2H), 3.55 – 3.43 (m, 2H), 3.08 – 2.83 (m, 2H), 2.04 – 1.86 (m, 12H), 1.65 – 1.51 (m, 8H), 1.50 – 1.34 (m, 3H); ^{13}C NMR (75 MHz, DMSO-*d₆*) δ 163.0, 156.7, 151.1, 111.2, 71.3, 69.7, 58.0, 50.4, 41.7, 39.6, 37.3, 36.3, 31.5, 31.0, 29.1; HRMS (ESI), m/z calcd for $C_{25}H_{35}N_3O_3$ $[M+H]^+$ 426.2678, found 426.2751.

4-(Pyridin-4-yloxy)-*N*-(4-trifluoromethoxybenzyl)-1-oxa-9-azaspiro[5.5]undecane-9-carboxamide (2c)
Yield 217 mg (78%), clear oil, ^{1}H NMR (300 MHz, DMSO-*d₆*) δ 8.36 (d, *J* = 6.1 Hz, 2H), 7.39 – 7.25 (m, 4H), 7.12 (t, *J* = 5.7 Hz, 1H), 6.98 (d, *J* = 6.2 Hz, 2H), 4.95 – 4.81 (m, 1H), 4.24 (d, *J* = 5.6 Hz, 2H), 3.84 – 3.55 (m, 4H), 3.15 – 2.92 (m, 2H), 2.07 – 1.94 (m, 3H), 1.65 – 1.35 (m, 5H); ^{13}C NMR (75 MHz, DMSO-*d₆*) δ 163.2, 157.6, 151.1, 147.1 (q, *J* = 1.7 Hz), 140.84, 128.9, 120.92, 120.3 (q, *J* = 255.7 Hz), 111.3, 71.4, 69.8, 58.1, 43.0, 40.4, 37.3, 31.5, 31.0; HRMS (ESI), m/z calcd for $C_{23}H_{26}$ $F_3N_3O_4$ $[M+H]^+$ 466.1875 , found 466.1964.

General procedure for the synthesis of compounds 3a-h

9-Benzyl-1-oxa-9-azaspiro[5.5]undecan-4-amine **5** (156 mg, 0.60 mmol) was dissolved in diethyl ether (8 ml) and treated with the corresponding isocyanate (0.63 ml, added dropwise).

The resulting mixture was stirred overnight. The precipitate formed was separated by filtration, washed with diethyl ether and dried *in vacuo* to provide the title compound.

N-(9-Benzyl-1-oxa-9-azaspiro[5.5]undec-4-yl)-N′-(3-fluoro-4-methylphenyl)urea (3a)

Yield 76 mg (31%), white solid, m.p. 228-230 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.84 (s, 0.9H), 7.67 – 7.56 (m, 2H), 7.52 – 7.42 (m, 3H), 7.41 – 7.32 (m, 1H), 7.29 – 7.02 (m, 2H), 6.96 – 6.85 (m, 1H), 6.47 – 6.34 (m, 1H), 4.42 – 4.23 (m, 2H), 3.90 – 3.66 (m, 2H), 3.64 – 3.49 (m, 1H), 3.21 – 3.01 (m, 3H), 3.00 – 2.77 (m, 1H), 2.48 – 2.41 (m, 1H), 2.29 (s, 1H), 2.11 (s, 3H), 2.02 – 1.85 (m, 1H), 1.82 – 1.59 (m, 3H), 1.40 – 1.04 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 160.6 (d, J = 239.9 Hz), 154.6, 140.0 (d, J = 11.3 Hz), 131.4 (d, J = 7.4 Hz), 129.7, 129.0, 116.1 (d, J = 17.5 Hz), 113.4 (d, J = 2.7 Hz), 104.5 (d, J = 27.2 Hz), 78.2, 68.8, 59.7, 47.20, 47.1, 42.1, 32.9, 26.2, 13.6 (d, J = 2.6 Hz); HRMS (ESI) *m/z* calcd for $C_{24}H_{31}FN_3O_2$ [M+H$^+$] 412.2322, found 412.2405.

N-(9-Benzyl-1-oxa-9-azaspiro[5.5]undec-4-yl)-N′-(5-chloro-2-methoxyphenyl)urea (3b)

Yield 128 mg (48%), white solid, m.p. 154-156 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.23 – 8.15 (m, 1H), 7.99 (s, 1H), 7.36 – 7.20 (m, 5H), 7.02 – 6.85 (m, 3H), 3.86 – 3.72 (m, 4H), 3.68 – 3.57 (m, 2H), 3.52 – 3.44 (m, 2H), 2.47 – 2.32 (m, 3H), 2.29 – 2.16 (m, 1H), 2.13 – 2.00 (m, 1H), 1.86 – 1.71 (m, 2H), 1.63 – 1.36 (m, 3H), 1.32 – 1.14 (m, 1H), 1.05 (t, J = 12.1 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 154.7, 146.4, 137.7, 130.9, 129.7, 128.6, 127.6, 124.7, 120.6, 117.4, 112.2, 70.8, 62.3, 59.2, 56.4, 48.6, 48.5, 42.5, 38.5, 33.4, 29.1; HRMS (ESI) *m/z* calcd for $C_{24}H_{31}ClN_3O_3$[M+H$^+$] 444.1976, found 444.2037.

N-(9-Benzyl-1-oxa-9-azaspiro[5.5]undec-4-yl)-N′-(4-fluorophenyl)urea (3c)

Yield 166 mg (70%), white solid, m.p. 166-168 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.32 (s, 1H), 7.44 – 7.18 (m, 7H), 7.11 – 6.98 (m, 2H), 6.00 (d, J = 7.5 Hz, 1H), 3.88 – 3.71 (m, 1H), 3.68 – 3.49 (m, 2H), 3.43 (s, 2H), 2.47 – 2.26 (m, 3H), 2.24 – 2.12 (m, 1H), 2.10 – 1.99 (m, 1H), 1.82 – 1.71 (m, 2H), 1.60 – 1.36 (m, 3H), 1.34 – 1.17 (m, 1H), 1.15 – 1.03 (m, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 157.5 (d, J = 237.4 Hz), 155.2, 138.5, 136.9 (d, J = 2.3 Hz), 129.6, 128.6, 127.4, 120.1 (d, J = 7.6 Hz), 115.6 (d, J = 22.1 Hz), 71.1, 62.7, 59.3, 48.8, 48.7, 42.6, 38.9, 33.6, 29.4; HRMS (ESI) *m/z* calcd for $C_{23}H_{29}FN_3O_2$ [M+H$^+$] 398.2165, found 398.2228.

N-(9-Benzyl-1-oxa-9-azaspiro[5.5]undec-4-yl)-*N′*-cyclohexylurea (3d)

Yield 198 mg (86%), white solid, m.p. 91-93 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 – 7.17 (m, 5H), 5.63 – 5.49 (dd, *J* = 12.32 Hz, *J* =12.11 Hz, 2H), 3.75 – 3.45 (m, 3H), 3.43 (s, 2H), 3.36 – 3.27 (m, 1H), 2.45 – 2.25 (m, 3H), 2.22 – 2.11 (m, 1H), 2.05 – 1.95 (m,1H), 1.78 – 1.56 (m, 6H), 1.55 – 1.34 (m, 4H), 1.30 – 0.94 (m, 7H); ^{13}C NMR (75 MHz, DMSO-*d*$_6$) δ 156.7, 138.6, 128.9, 128.2, 70.6, 62.4, 58.9, 48.6, 48.4, 47.67, 42.8, 42.0, 38.8, 33.8, 33.3, 29.3, 25.3, 24.5; HRMS (ESI) *m/z* calcd for $C_{23}H_{36}N_3O_2$ [M+H$^+$] 386.2729, found 386.2842.

N-(9-Benzyl-1-oxa-9-azaspiro[5.5]undec-4-yl)-*N′*-ethylurea (3e)

Yield 178 mg (90%), white solid, m.p. 159-161 °C. ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 7.35 – 7.18 (m, 5H), 5.68 – 5.59 (m, 2H), 3.77 – 3.45 (m, 3H), 3.43 (s, 2H), 3.05 – 2.92 (m, 2H), 2.45 – 2.25 (m, 3H), 2.23 – 2.10 (m, 1H), 2.07 – 1.94 (m, 1H), 1.76 – 1.63 (m 2H), 1.58 – 1.32 (m, 3H), 1.26 – 1.08 (m,1H), 1.06 – 0.91 (m, 4H); ^{13}C NMR (75 MHz, DMSO-*d*$_6$) δ 157.5, 138.7, 129.0, 128.2, 126.9, 70.7, 62.4, 59.0, 48.2, 48.5, 42.2, 38.9, 34.1, 33.8, 29.3, 15.8; HRMS (ESI) *m/z* calcd for $C_{19}H_{30}N_3O_2$ [M+H$^+$] 332.2260, found 332.2347.

N-(9-Benzyl-1-oxa-9-azaspiro[5.5]undec-4-yl)-*N*'-phenylurea (3f)

Yield 198 mg (87%), white solid, m.p. 161-162 °C. ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 8.28 (s, 1H), 7.45 – 7.11 (m, 9H), 6.96 – 6.79 (m, 1H), 6.01 (d, *J* = 7.5 Hz, 1H), 3.89 – 3.72 (m, 1H), 3.71 – 3.50 (m, 2H), 3.44 (s, 2H), 2.47 – 2.26 (m, 3H), 2.25 – 2.12 (m, 1H), 2.10 – 1.97 (m, 1H), 1.85 – 1.71 (m, 2H), 1.62 – 1.37 (m, 3H), 1.35 – 1.17 (m, 1H), 1.16 – 1.02 (m, 1H); ^{13}C NMR (75 MHz, DMSO-*d*$_6$) δ 154.6, 140.5, 138.7, 129.0, 128.8, 128.2, 126.9, 121.2, 117.8, 70.7, 62.4, 58.9, 48.6, 48.4, 42.6, 42.2, 38.8, 33.5, 29.3; HRMS (ESI) *m/z* calcd for $C_{23}H_{30}N_3O_2$ [M+H$^+$] 380.2260, found 380.2337.

N-(1-Adamantyl)-*N′*-(9-benzyl-1-oxa-9-azaspiro[5.5]undec-4-yl)urea (3g)

Yield 218 mg (83%), white solid, m.p. 190-192 °C. ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 7.37 – 7.17 (m, 5H), 5.52 (d, *J* = 7.6 Hz, 1H), 5.39 (s, 1H), 3.71 – 3.37 (m, 5H), 2.44 – 2.23 (m, 3H), 2.22 – 2.09 (m, 1H), 2.06 – 1.92 (m, 4H), 1.87 – 1.80 (m, 6H), 1.73 – 1.32 (m, 11H), 1.20 – 1.03 (m, 1H), 0.95 (t, *J* = 12.3 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-*d*$_6$) δ 156.3, 138.6, 128.8, 128.1, 126.8, 70.5, 62.3, 58.9, 49.4, 48.5, 48.4, 42.90, 42.1, 41.6, 38.8, 36.1, 33.8, 29.3, 28.9; HRMS (ESI) *m/z* calcd for $C_{27}H_{40}N_3O_2$ [M+H$^+$] 438.3004, found 438.3133.

N-(9-Benzyl-1-oxa-9-azaspiro[5.5]undec-4-yl)-*N'*-(2-fluorophenyl)urea (3h)

Yield 177 mg (74%), white solid, m.p. 167-169 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.22 – 8.03 (m, 2H), 7.36 – 7.22 (m, 5H), 7.20 – 7,02 (m, 2H), 6.96 – 6.85 (m, 1H), 6.54 (d, *J* = 7.4 Hz, 1H), 3.89 – 3.72 (m,1H), 3.70 – 3.50 (m, 2H), 3.45 (s, 2H), 2.47 – 2.27 (m, 3H), 2.26 – 2.13 (m, 1H), 2.10 – 1.99 (m, 3H), 1.86 – 1.73 (m, 2H), 1.62 – 1.37 (m, 3H), 1.32 – 1.16 (m, 1H), 1.14 – 1.01 (m, 1H); 13C NMR (75 MHz, DMSO-d6) δ 151.85 (d, *J* = 240.5 Hz), 128.28 (d, *J* = 10.1 Hz), 124.54 (d, *J* = 3.3 Hz), 121.90 (d, *J* = 7.3 Hz), 120.46 (s), 114.95 (d, *J* = 18.9 Hz); HRMS (ESI) *m/z* calcd for $C_{23}H_{29}$ FN_3O_2 [M+H$^+$] 398.2165, found 398.2254.

General procedure for preparation of compounds 3i-q.

tert-Butyl (1-oxa-9-azaspiro[5.5]undec-4-yl)carbamate **6** (2 g, 74 mmol) was dissolved in CH_2Cl_2 (15 ml) and triethylamine (1.2 ml, 89 mmol) was added dropwise. Within 30 min, the corresponding acylating or sulfonylating agent (81 mmol) was added dropwise. The resulting mixture was stirred at ambient temperature for 10 h, washed with 5% aqueous citric acid, 10% aqueous K_2CO_3, and the organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was dissolved in 4M HCl in 1,4-dioxane (2.65 ml) and stirred at ambient temperature for 10 h. The volatiles were removed *in vacuo*, and the residue was neutralized with saturated aqueous K_2CO_3 to provide clear oil. The requisite portion of the primary amine (0.58 mmol) thus obtained was dissolved in 1,4-dioxane (10 ml) and treated with the corresponding isocyanate (0.61 mmol). The mixture was stirred overnight, poured into water (25 ml) and extracted with ethyl acetate (2 x 25 ml). The combined organic phase was washed with 3% aqueous citric acid, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel using 0→2% MeOH in CH_2Cl_2 as eluent. The fractions containing the product were combined, concentrated *in vacuo* to provide the title compound.

N-(9-Methylsulfonyl-1-oxa-9-azaspiro[5.5]undec-4-yl)-*N'*-(4-trifluoromethylbenzyl)urea (3i)

Yield 91 mg (35%), white solid, m.p. 176-178 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.63 – 7.49 (m, 2H), 7.45 – 7.32 (m, 2H), 4.37 (s, 2H), 4.03 – 3.86 (m, 1H), 3.81 – 3.68 (m, 1H), 3.63 – 3.39 (m, 3H), 3.11 – 2.94 (m, 1H), 2.91 – 2.70 (m, 1H), 2.76 (s, 3H), 2.35 – 2.18 (m, 1H), 1.96 – 1.73 (m, 2H), 1.71 – 1.55 (m, 2H), 1.53 – 1.08 (m, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 157.3, 146.2, 127.6, 127.2 (q, *J* = 31.5 Hz), 125.1 (q, *J* = 3.7 Hz), 124.5 (q, *J* = 271.9 Hz), 69.9, 59.3, 42.6, 42.5, 42.1, 41.3, 41.2, 37.9, 33.7, 33.5, 28.5; HRMS (ESI) m/z calcd for $C_{19}H_{27}F_3N_3O_4S$ [M+H$^+$] 450.1596, found 450.1669.

N-(9-Ethylsulfonyl-1-oxa-9-azaspiro[5.5]undec-4-yl)-*N'*-(4-trifluoromethoxy-benzyl)urea (3j)

Yield 246 mg (86%), clear oil. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 7.40 – 7.25 (m, 4H), 6.30 (t, *J* = 5.9 Hz, 1H), 5.89 (d, *J* = 7.7 Hz, 1H), 4.21 (d, *J* = 5.9 Hz, 2H), 3.80 – 3.62 (m, 2H), 3.59 – 3.49 (m, 2H), 3.41 – 3.25 (m, 3H), 3.19 – 3.10 (m, 1H), 3.02 – 2.93 (m, 1H), 2.22 – 2.13 (m, 1H), 1.77 – 1.69 (m, 2H), 1.60 – 1.46 (m, 2H), 1.41 – 1.04 (m, 3H), 1.20 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-*d6*) δ 158.9, 148.5 (d, *J* = 1.5 Hz), 142.2, 130.4, 126.8, 121.3 (q, *J* = 254.3 Hz), 71.6, 60.8, 44.1, 43.8, 43.6, 42.5, 39.8, 35.1, 30.5, 9.1; HRMS (ESI) *m/z* calcd for $C_{21}H_{31}F_3N_3O_5S$ [M+H$^+$] 494.1858, found 494.1969.

N-Propyl-4-[(4-trifluoromethoxybenzylamino)carbonylamino]-1-oxa-9-azaspiro[5.5]undecane-9-carboxamide (3k)

Yield 198 mg (72.3%), white solid, m.p. 174-176 °C. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 7.40 – 7.25 (m, 4H), 6.40 (t, *J* = 5.3 Hz, 1H), 6.31 (t, *J* = 5.9 Hz, 1H), 5.89 (d, *J* = 7.6 Hz, 1H), 4.21 (d, *J* = 5.9 Hz, 2H), 3.83 – 3.46 (m, 5H), 3.10 – 2.80 (m, 4H), 2.06 – 1.93 (m, 1H), 1.78 – 1.66 (m, 2H), 1.48 – 1.12 (m, 7H), 0.80 (t, J = 7.6 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-*d6*) δ 157.4, 157.3, 147.0, 140.6, 128.8, 120.9, 120.1 (q, *J* = 255.7 Hz), 70.9, 59.2, 42.6, 42.1, 41.9, 39.1, 38.5, 33.6, 29.1, 23.1, 11.4; HRMS (ESI) *m/z* calcd for $C_{22}H_{32}F_3N_4O_4$ [M+H$^+$] 473.2297, found 473.2356.

Methyl 4-oxo-4-{4-[(4-trifluoromethoxybenzylamino)carbonylamino]-1-oxa-9-aza-spiro[5.5]undec-9-yl}butanoate (3l)

Yield 227 mg (78%), white solid, m.p. 101-103 °C. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 7.39 – 7.26 (m, 4H), 6.36 (t, *J* = 5.9 Hz, 1H), 5.94 (d, *J* = 8.0 Hz, 1H), 4.21 (d, *J* = 5.9 Hz, 2H), 4.00 – 3.90 (m, 1H), 3.81 – 3.64 (m, 2H), 3.61 – 3.50 (m, 2H), 3.57 (s, 3H), 3.20 – 2.73 (3m, 2H), 2.59 – 2.53 (m, 2H), 2.49 – 2.43 (m, 2H), 2.17 – 2.03 (m, 1H), 1.79 – 1.67 (m, 2H), 1.58 – 1.04 (m, 5H); ^{13}C NMR (75 MHz, DMSO-*d6*) δ 173.1, 169.0, 157.4, 147.0 (q, *J* = 1.7 Hz), 140.7, 128.8, 120.9, 120.1 (q, *J* = 255.8 Hz), 70.9, 59.4, 59.3, 51.3, 42.7, 42.6, 42.1, 40.5, 38.9, 38.3, 37.0, 36.9, 33.6, 28.8, 27.4; HRMS (ESI) *m/z* calcd for $C_{23}H_{31}F_3N_3O_6$ [M+H$^+$] 502.2087, found 502.2136.

_N_-(9-Methylsulfonyl-1-oxa-9-azaspiro[5.5]undec-4-yl)-_N′_-(4-trifluoromethylbenzyl)urea (3m)

Yield 91 mg (35%), white solid, m.p. 176-178 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.63 – 7.49 (m, 2H), 7.45 – 7.32 (m, 2H), 4.37 (s, 2H), 4.03 – 3.86 (m, 1H), 3.81 – 3.68 (m, 1H), 3.63 – 3.39 (m, 3H), 3.11 – 2.94 (m, 1H), 2.91 – 2.70 (m, 1H), 2.76 (s, 3H), 2.35 – 2.18 (m, 1H), 1.96 – 1.73 (m, 2H), 1.71 – 1.55 (m, 2H), 1.53 – 1.08 (m, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 157.3, 146.2, 127.6, 127.2 (q, *J* = 31.5 Hz), 125.1 (q, *J* = 3.7 Hz), 124.5 (q, *J* = 271.9 Hz), 69.9, 59.3, 42.6, 42.5, 42.1, 41.3, 41.2, 37.9, 33.7, 33.5, 28.5; HRMS (ESI) m/z calcd for $C_{19}H_{27}F_3N_3O_4S$ [M+H$^+$] 450.1596, found 450.1669.

_N_-(4-Bromobenzyl)-_N′_-(9-methylsulfonyl-1-oxa-9-azaspiro[5.5]undec-4-yl)urea (3n)

Yield 83 mg (31.1%), white solid, m.p. 141-143 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.54 – 7.45 (m, 2H), 7.24 – 7.15 (m, 2H), 6.29 (t, *J* = 5.9 Hz, 1H), 5.90 (d, *J* = 7.8 Hz, 1H), 4.15 (d, *J* = 5.9 Hz, 2H), 3.82 – 3.47 (m, 3H), 3.32 – 3.21 (m, 2H), 3.04 – 2.90 (m, 1H), 2.88 – 2.73 (m, 1H), 2.84 (s, 3H), 2.31 – 2.18 (m, 1H), 1.80 – 1.67 (m, 2H), 1.66 – 1.48 (m, 2H), 1.46 – 1.05 (m, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 157.3, 140.6, 131.1, 129.3, 119.5, 69.8, 59.2, 42.5, 42.2, 42.1, 41.2, 41.1, 37.9, 33.7, 33.6, 28.5; HRMS (ESI) *m/z* calcd for $C_{18}H_{26}BrN_3O_4SNa$ [M+Na$^+$] 482.0719, found 482.0720.

_N_-(4-Diethylaminobenzyl)-_N′_-(9-methylsulfonyl-1-oxa-9-azaspiro[5.5]undec-4-yl)urea (3o)

Yield 91 mg (35%), white solid, m.p. 132-133 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.05 – 6.99 (m, 2H), 6.62 – 6.56 (m, 2H), 5.99 (t, *J* = 5.6 Hz, 1H), 5.73 (d, *J* = 7.8 Hz, 1H), 4.03 (d, *J* = 5.6 Hz, 2H), 3.81 – 3.62 (m, 2H), 3.59 – 3.50 (m, 1H), 3.30 – 3.22 (m, 6H), 3.03 – 2.92 (m, 1H), 2.86 – 2.77 (m, 1H), 2.84 (s, 3H), 2.28 – 2.19 (m, 1H), 1.78 – 1.69 (m, 2H), 1.66 – 1.50 (m, 2H), 1.47 – 1.38 (m, 1H), 1.27 – 1.04 (m, 2H), 1.05 (t, *J* = 7.0 Hz, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 158.8, 147.9, 130.0, 128.5, 113.2, 71.4, 60.8, 45.3, 44.2, 44.1, 43.5, 42.8, 42.7, 39.4, 35.3, 35.2, 30.1, 13.9; HRMS (ESI) *m/z* calcd for $C_{22}H_{37}N_4O_4S$ [M+H$^+$] 453.2457, found 453.2540.

_N_-Benzyl-_N′_-(9-methylsulfonyl-1-oxa-9-azaspiro[5.5]undec-4-yl)urea (3p)

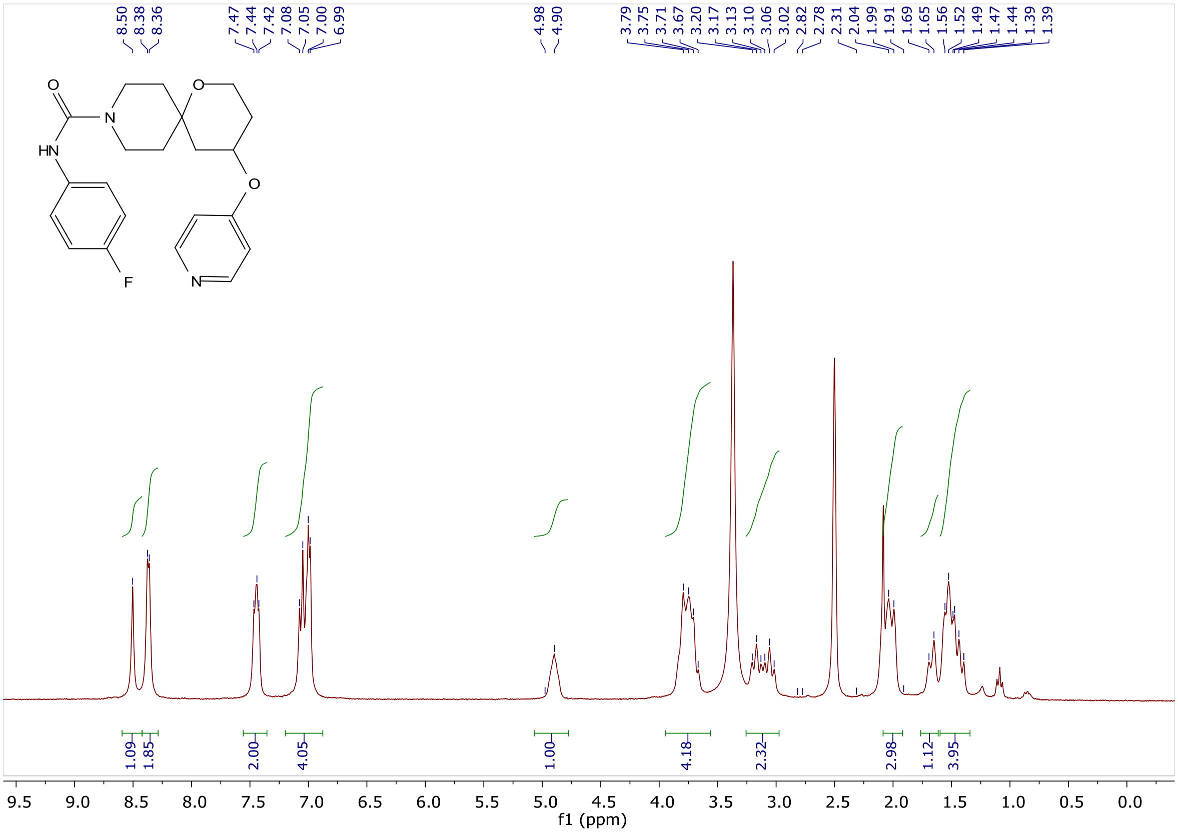
Yield 85 mg (39%), white solid, m.p. 180-182 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.37 – 7.16 (m, 5H), 6.23 (t, *J* = 5.8 Hz, 1H), 5.84 (d, *J* = 7.7 Hz, 1H), 4.19 (d, *J* = 5.8 Hz, 2H), 3.86 – 3.62 (m, 2H), 3.61 – 3.48 (m, 1H), 3.29 – 3.21 (m, 2H), 3.05 – 2.90 (m, 1H), 2.87 – 2.73 (m, 1H), 2.84 (s, 3H), 2.30 – 2.18 (m, 1H), 1.82 – 1.69 (m, 2H), 1.68 – 1.49 (m, 2H), 1.48 – 1.39 (m, 1H), 1.32 – 1.04 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 157.3, 140.9, 128.2, 127.0, 126.6, 69.8, 59.3, 42.8, 42.6, 42.0, 41.2, 41.2, 37.9, 33.7, 33.6, 28.5; HRMS (ESI) *m/z* calcd for $C_{18}H_{28}N_3O_4S$ [M+H$^+$] 382.1722, found 382.1795.

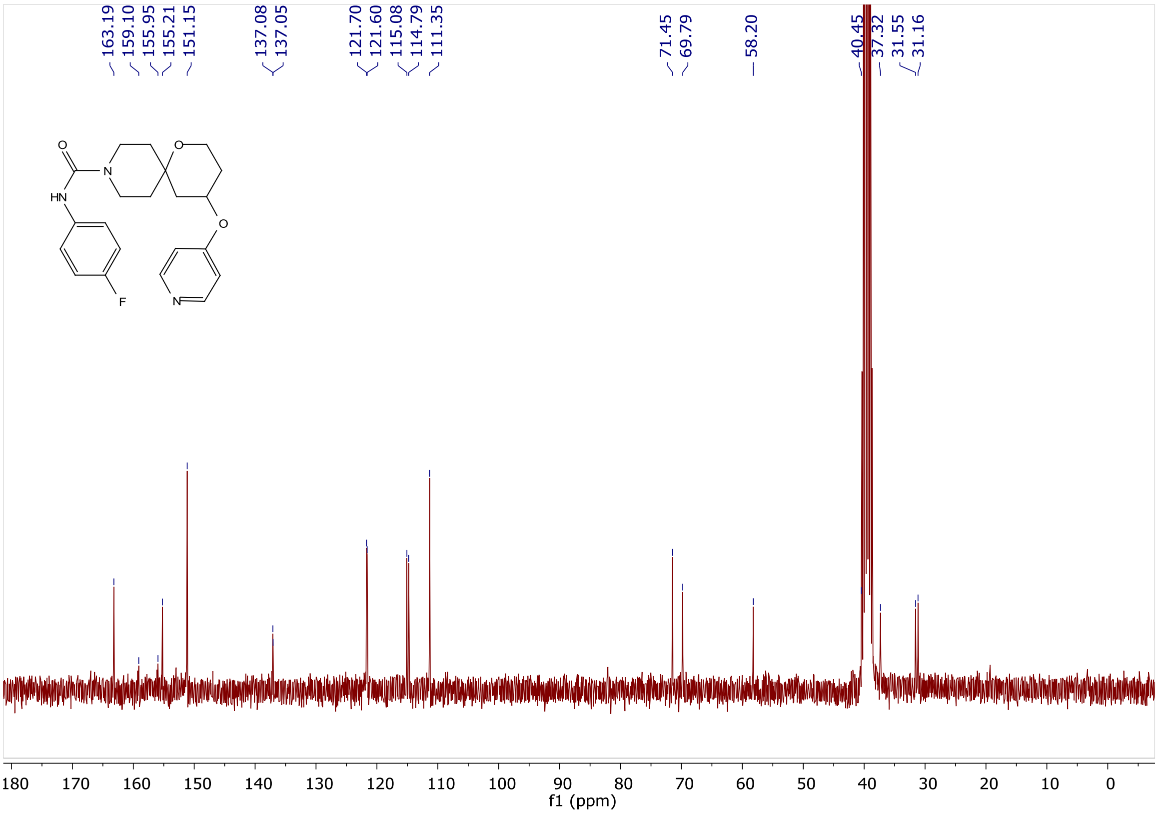
N-(4-Methylbenzyl)-*N'*-(9-methylsulfonyl-1-oxa-9-azaspiro[5.5]undec-4-yl)urea (3q)

Yield 100 mg (43%), white solid, m.p. 141-143 °C. ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 7.15 – 7.06 (m, 4H), 6.17 (t, *J* = 5.9 Hz, 1H), 5.82 (d, *J* = 7.8 Hz, 1H), 4.14 (d, *J* = 5.9 Hz, 2H), 3.83 – 3.61 (m, 2H), 3.60 – 3.47 (m, 1H), 3.31 – 3.20 (m, 2H), 3.04 – 2.91 (m, 1H), 2.86 – 2.74 (m, 1H), 2.84 (s, 3H), 2.29 – 2.19 (m, 1H), 2.26 (s, 3H), 1.80 – 1.69 (m, 2H), 1.67 – 1.34 (m, 3H), 1.30 – 1.02 (m, 2H); ^{13}C NMR (75 MHz, DMSO-*d*$_6$) δ 157.3, 137.8, 135.6, 128.8, 127.0, 69.8, 59.2, 42.6, 42.5, 42.0, 41.2, 41.2, 37.9, 33.8, 33.6, 28.5, 20.7; HRMS (ESI) *m/z* calcd for $C_{19}H_{30}N_3O_4S$ [M+H$^+$] 396.1879, found 396.1952.

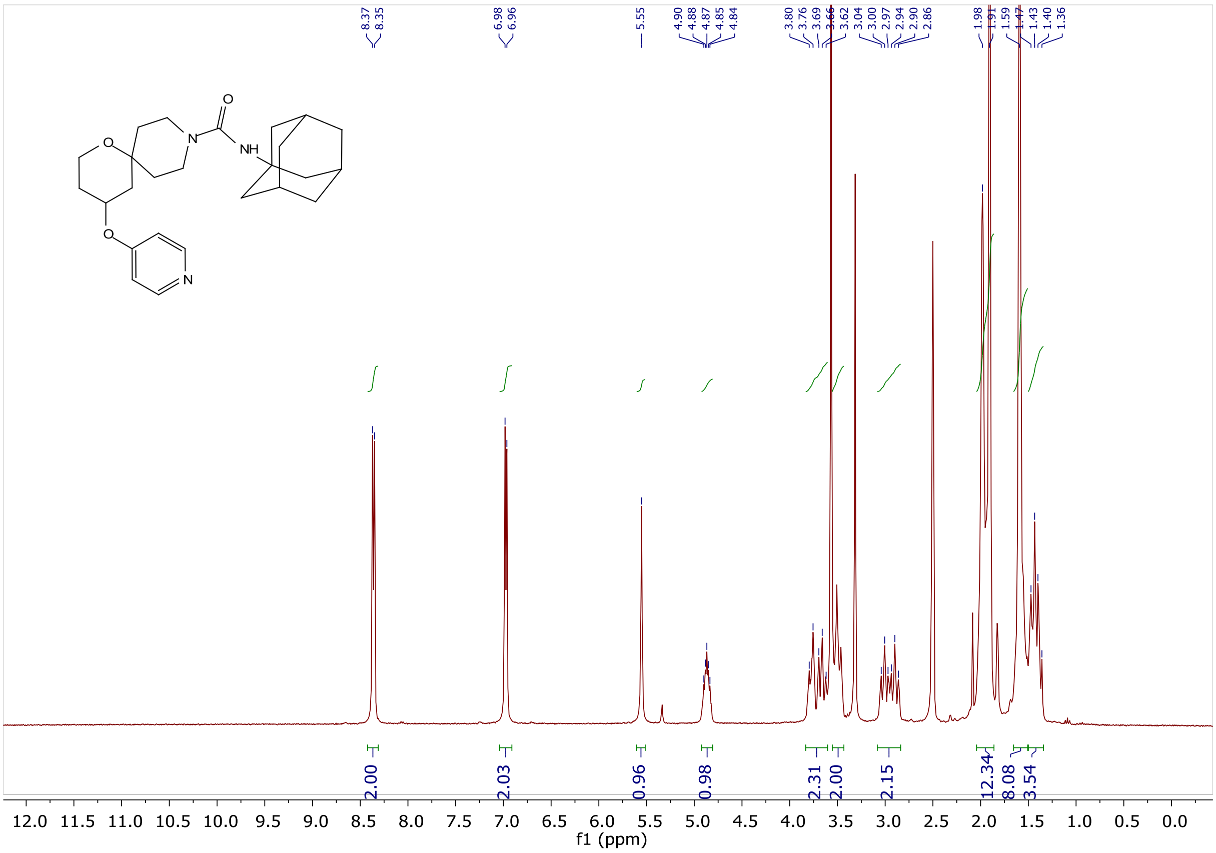
Copies of ^{1}H and ^{13}C NMR spectra

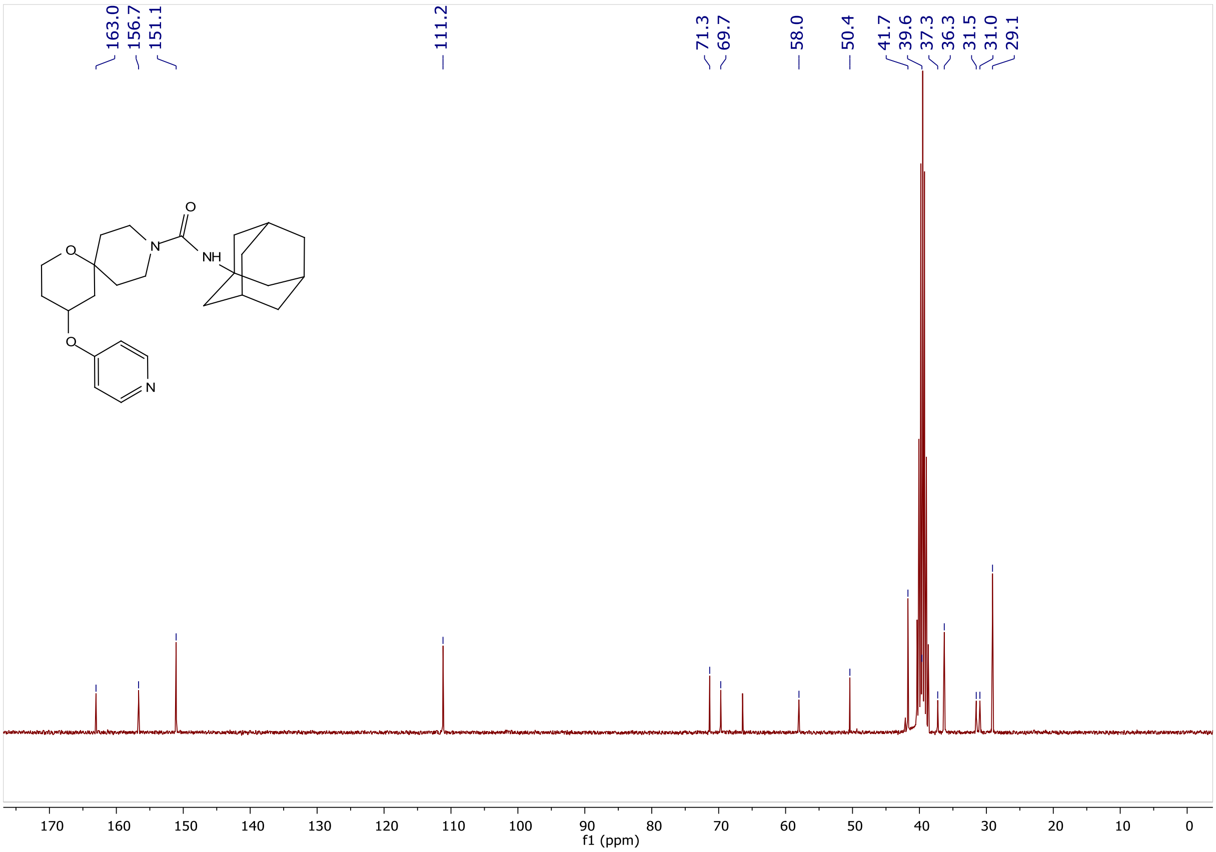
^{1}H and ^{13}C NMR spectrum of compound **2a**



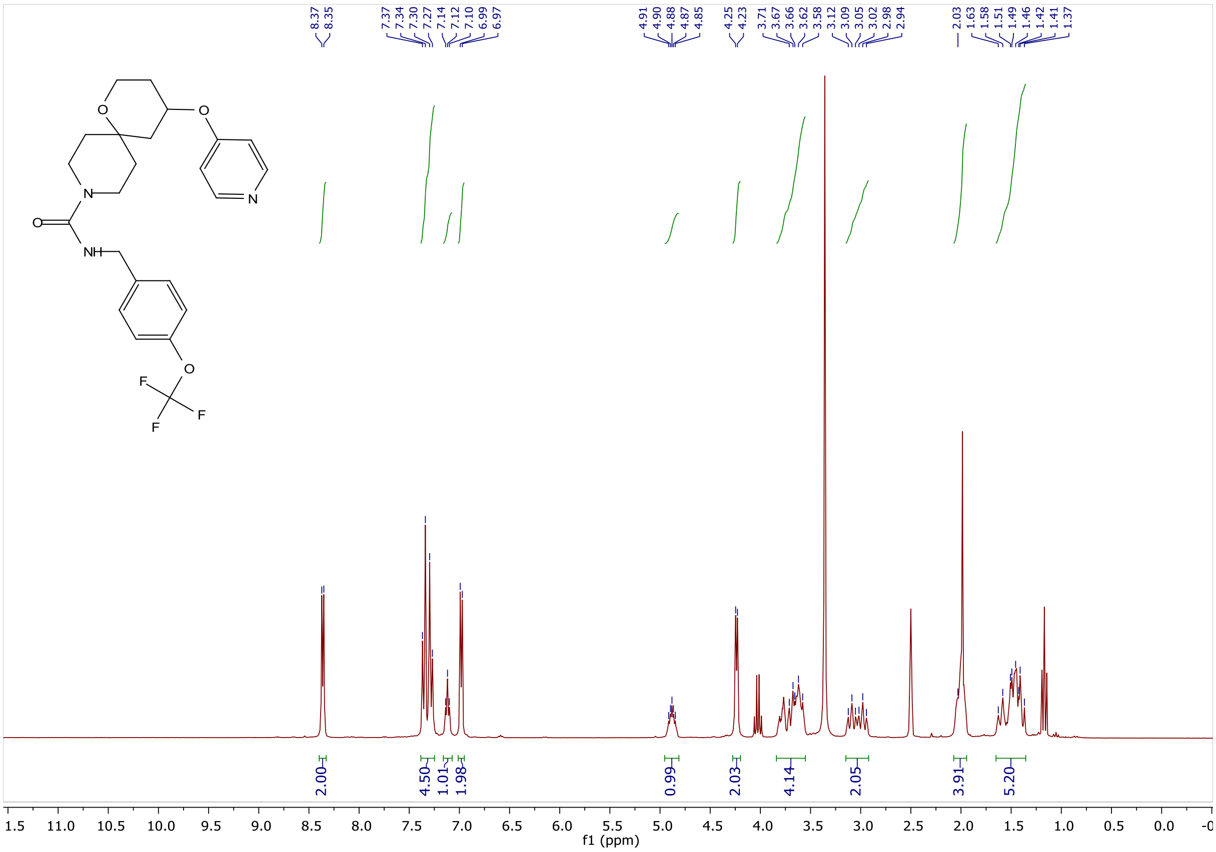


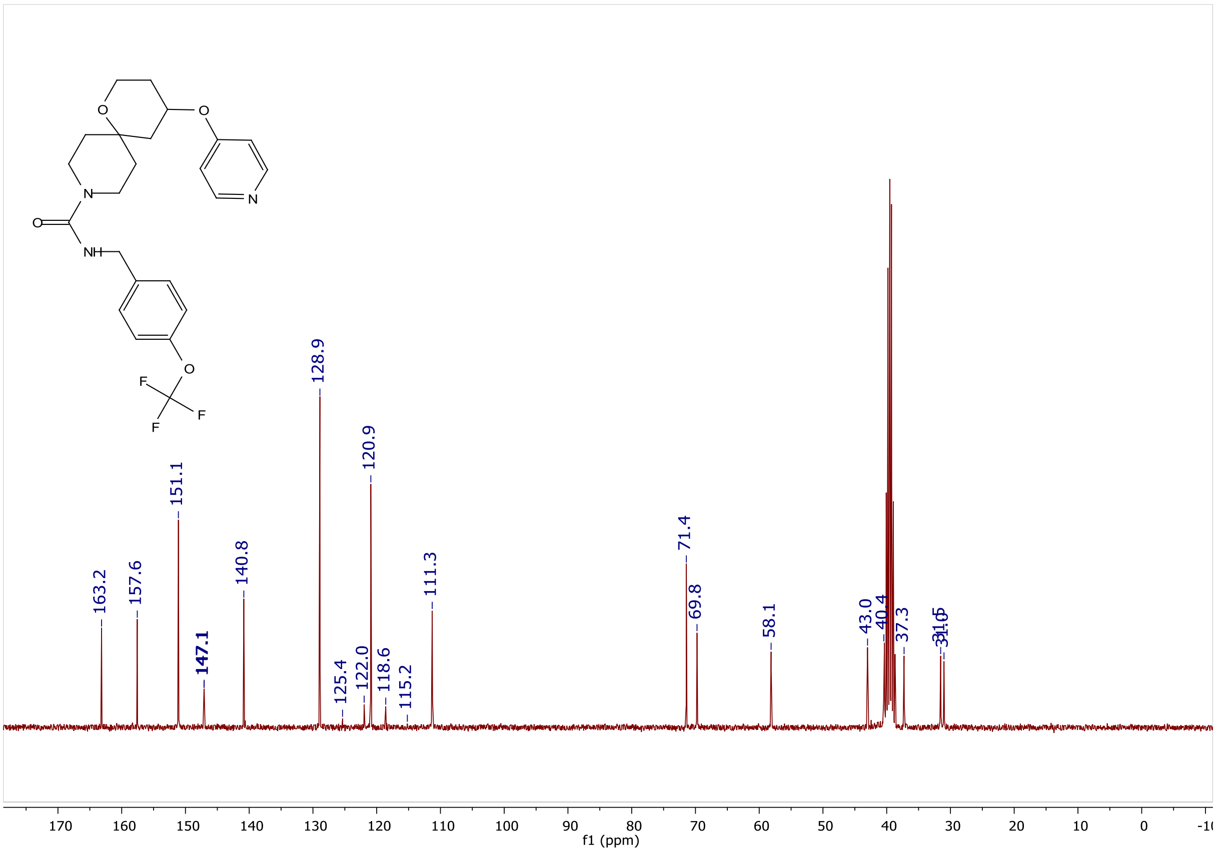
^{1}H and ^{13}C NMR spectrum of compound **2b**



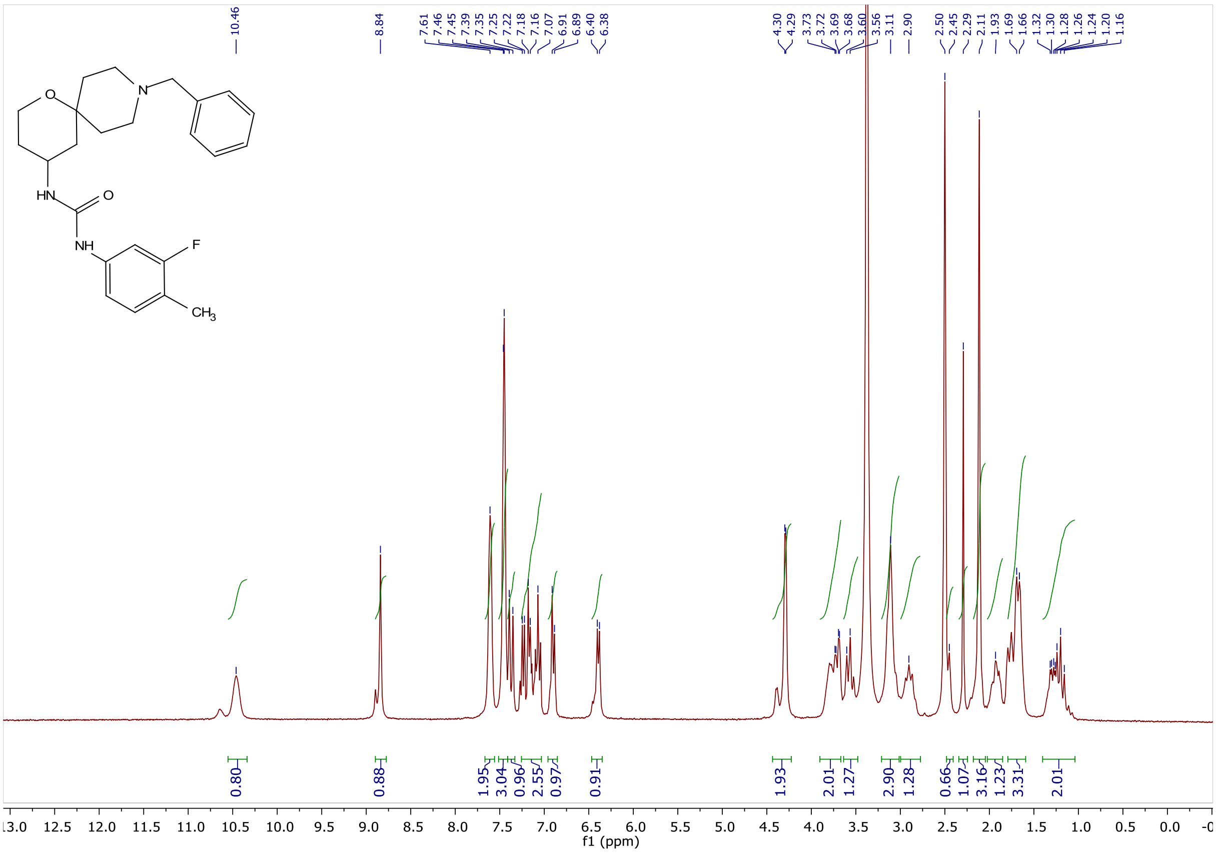


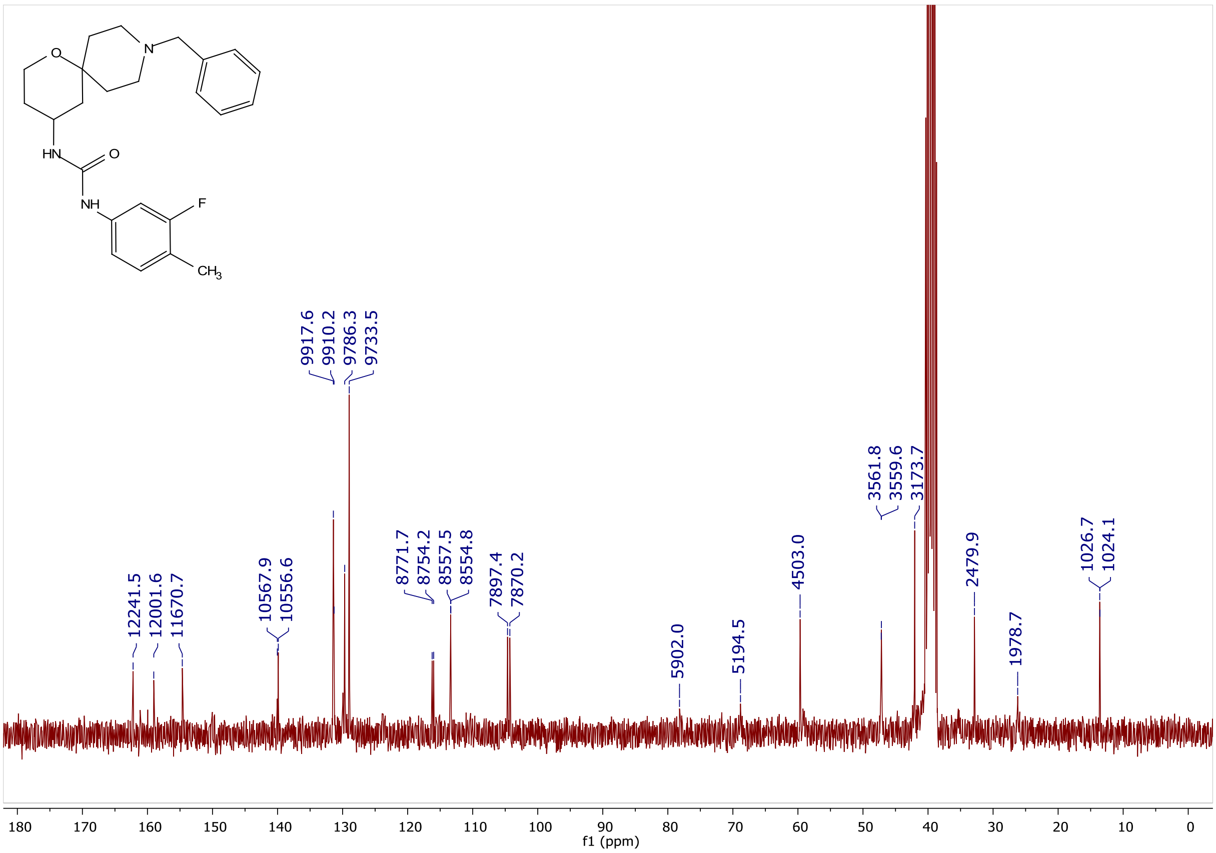
^{1}H and ^{13}C NMR spectrum of compound **2c**



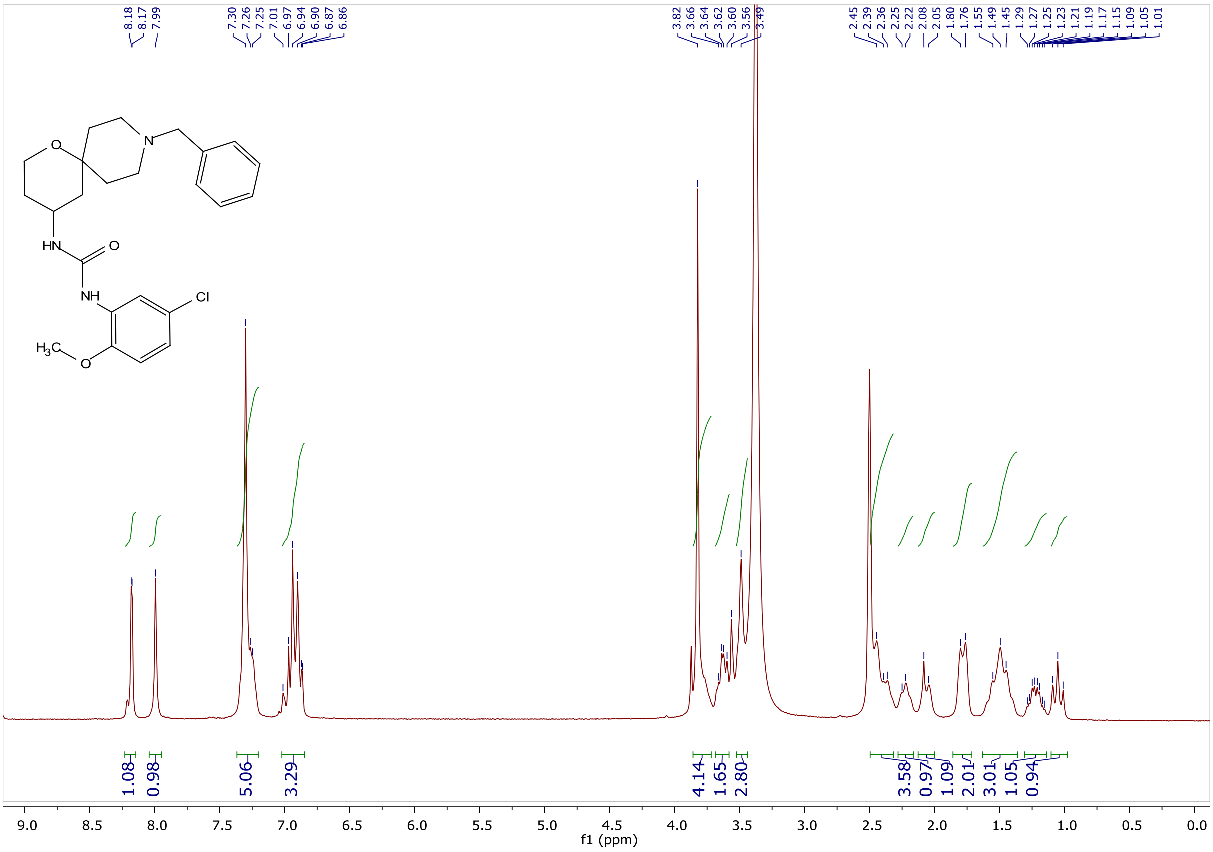


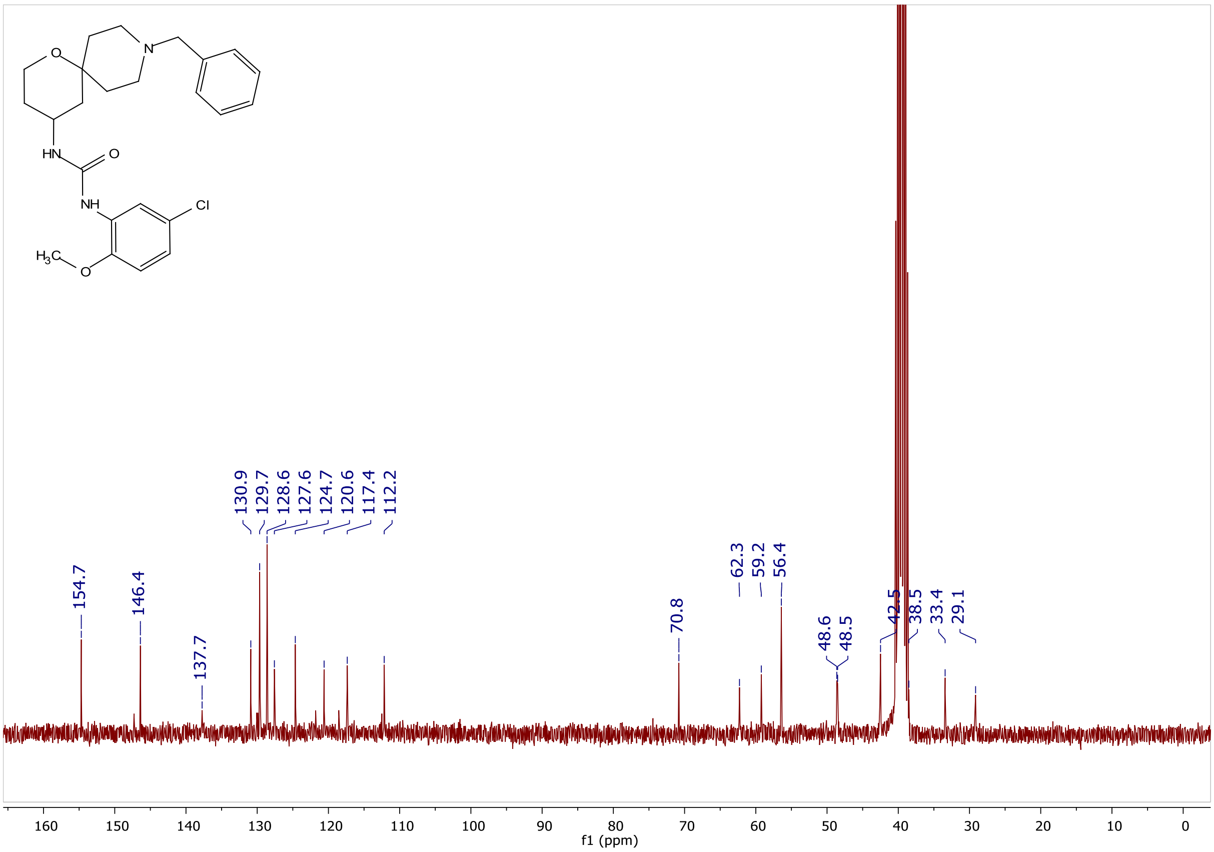
^{1}H and ^{13}C NMR spectrum of compound **3a**



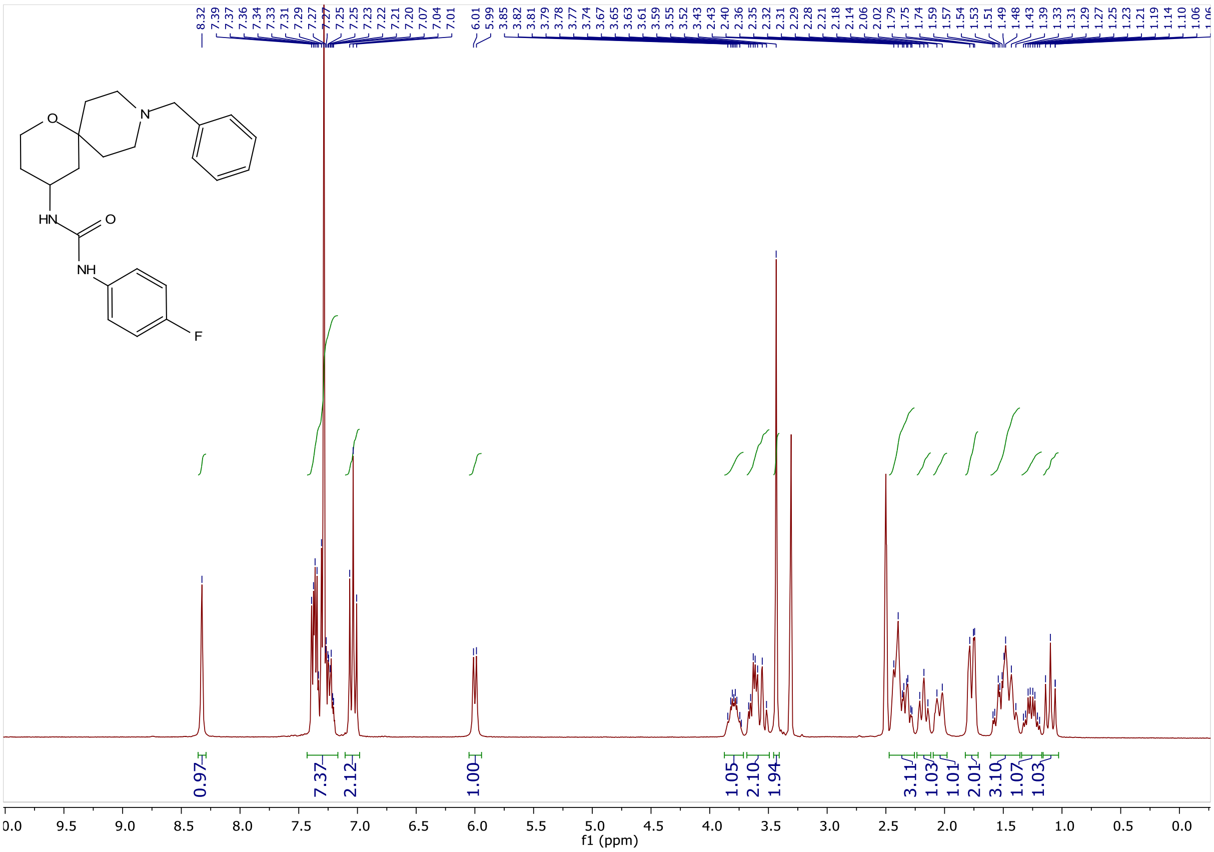


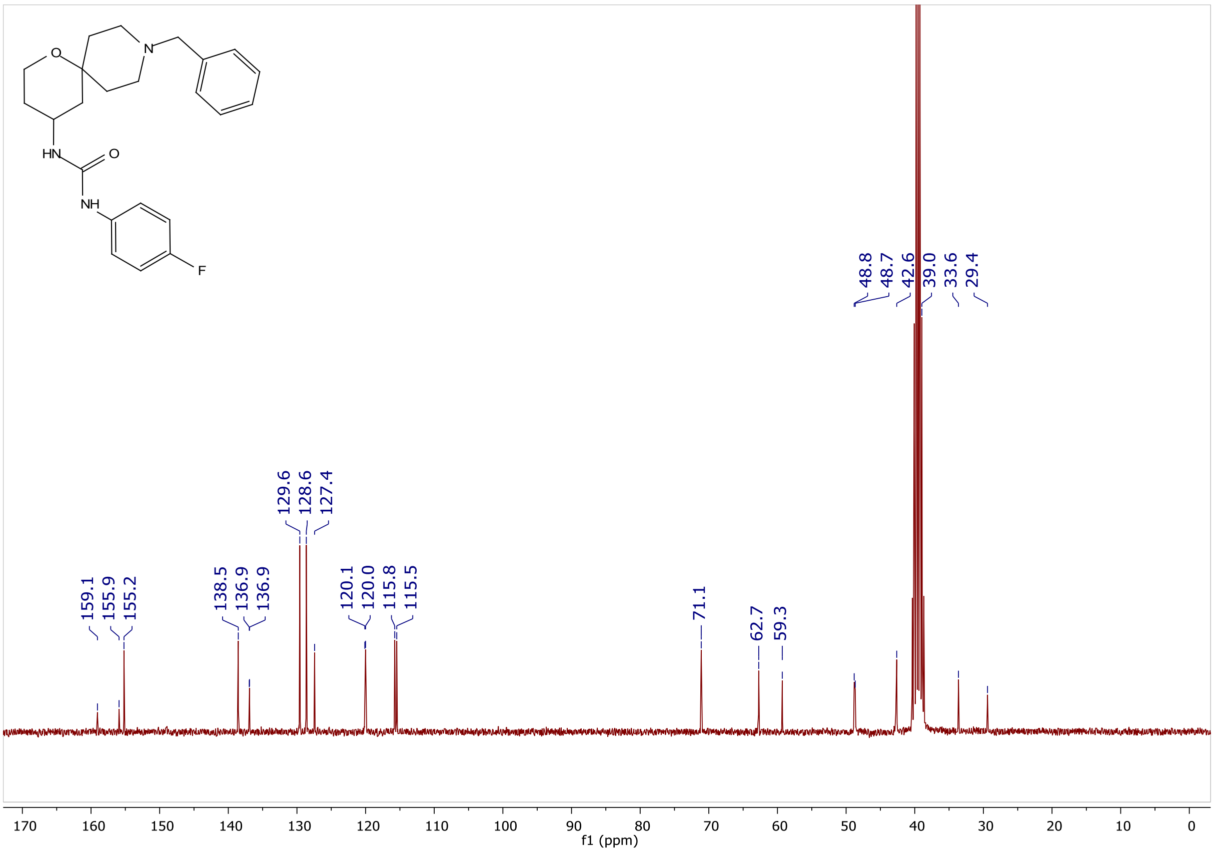
^{1}H and ^{13}C NMR spectrum of compound **3b**



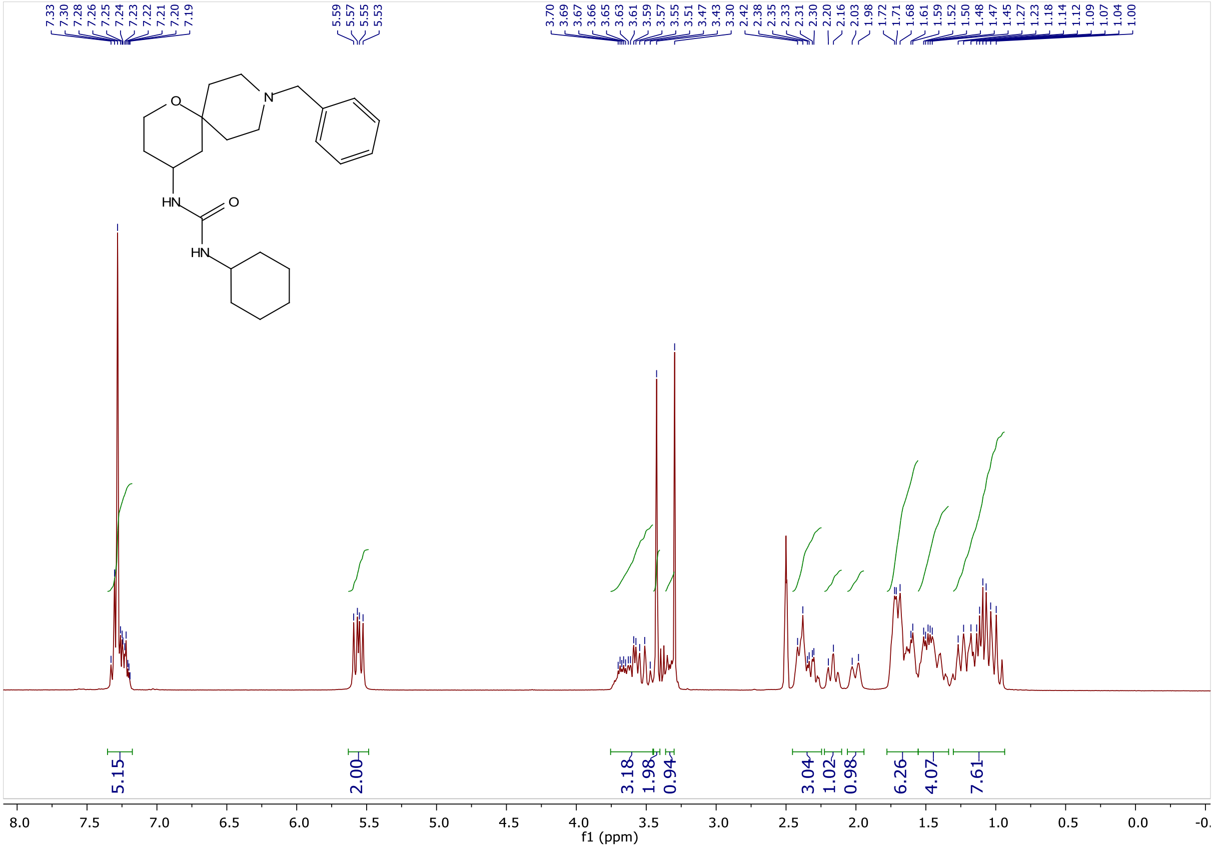


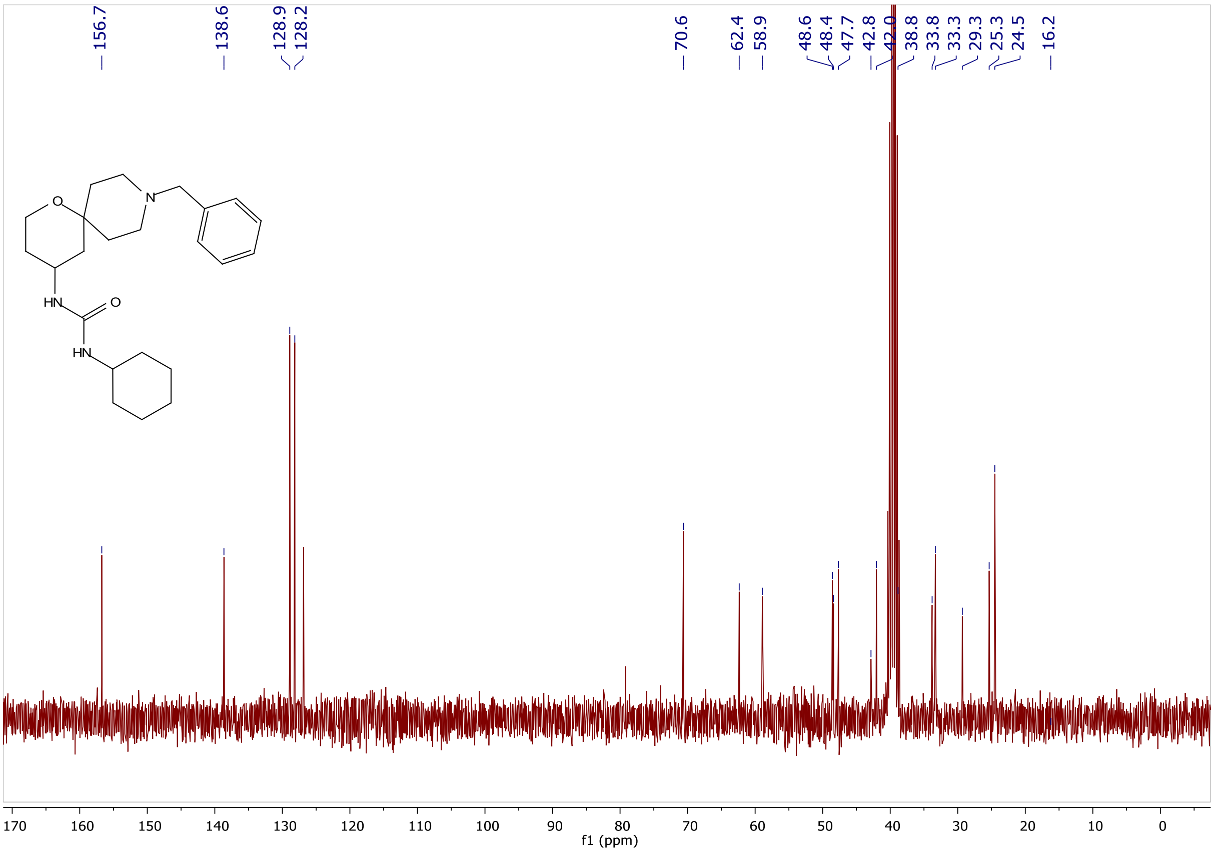
^{1}H and ^{13}C NMR spectrum of compound **3c**



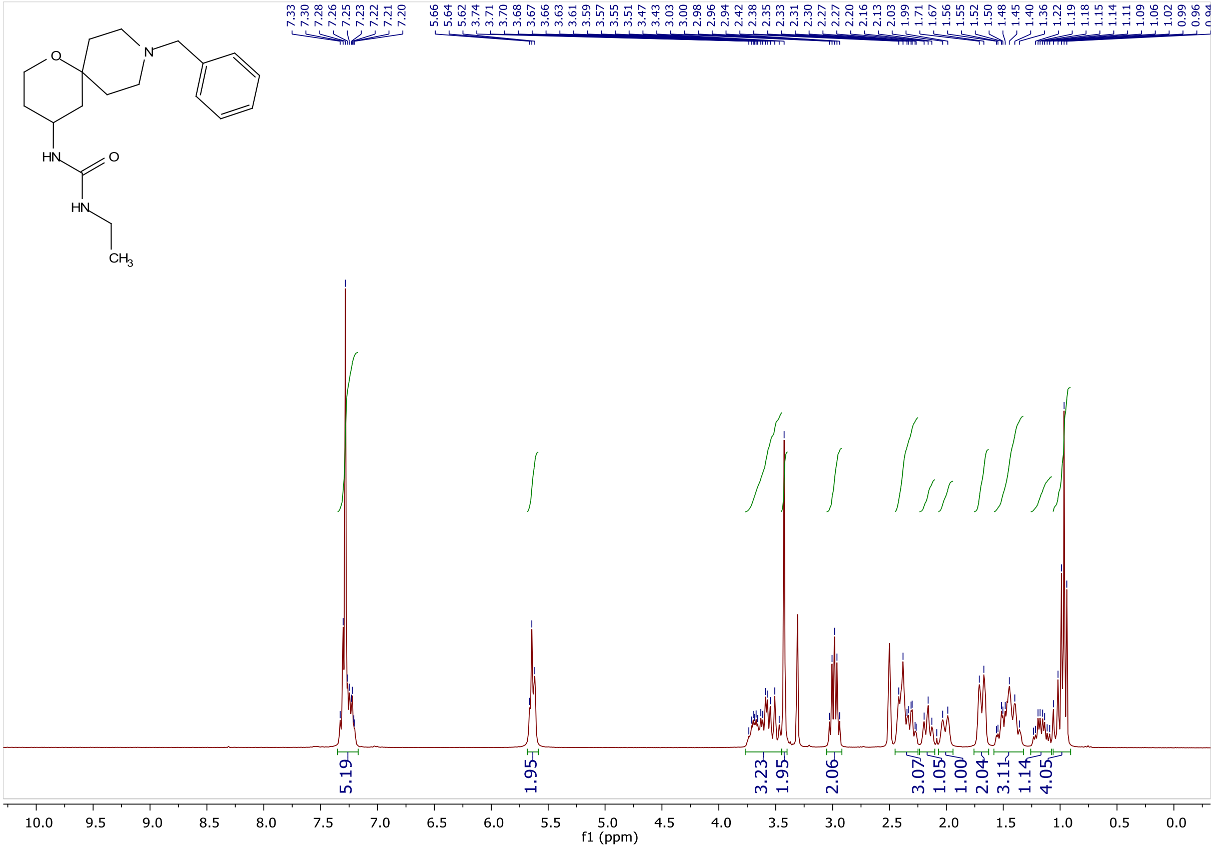


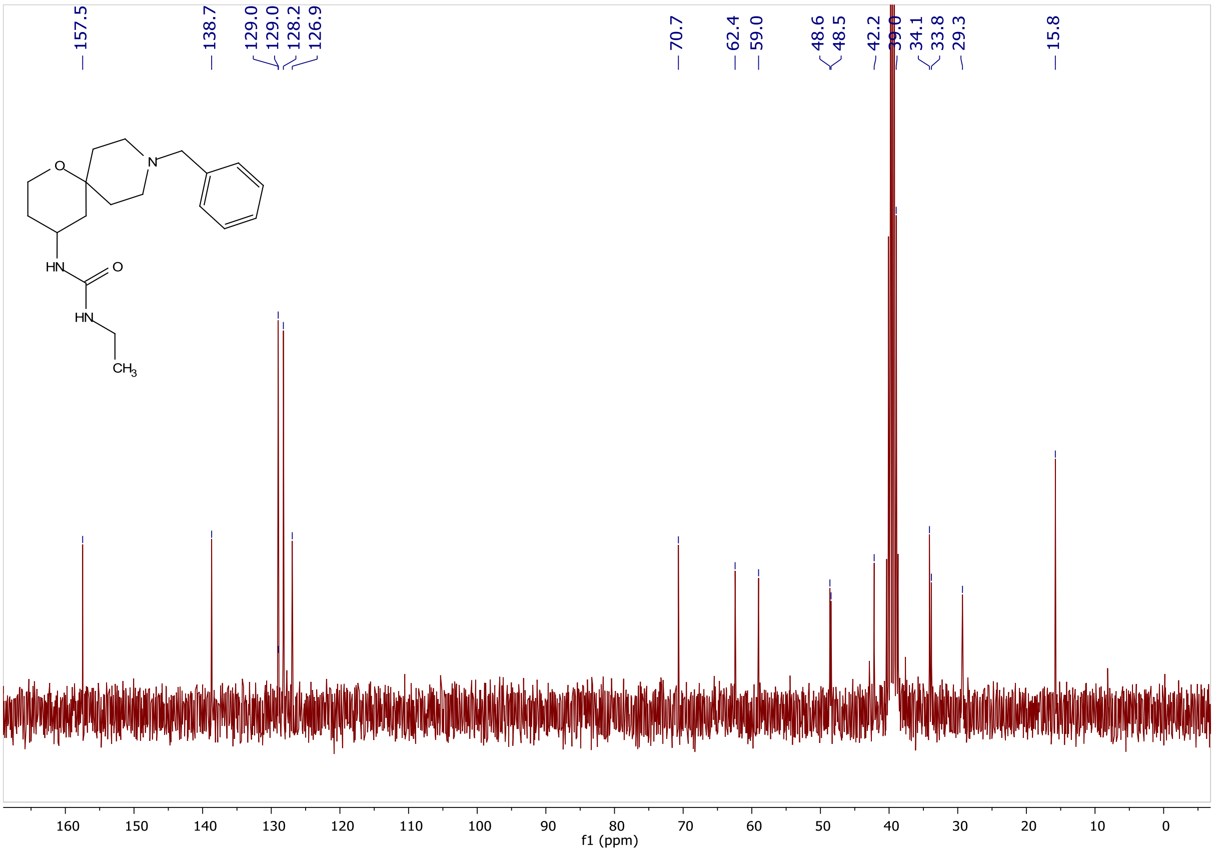
^{1}H and ^{13}C NMR spectrum of compound **3d**



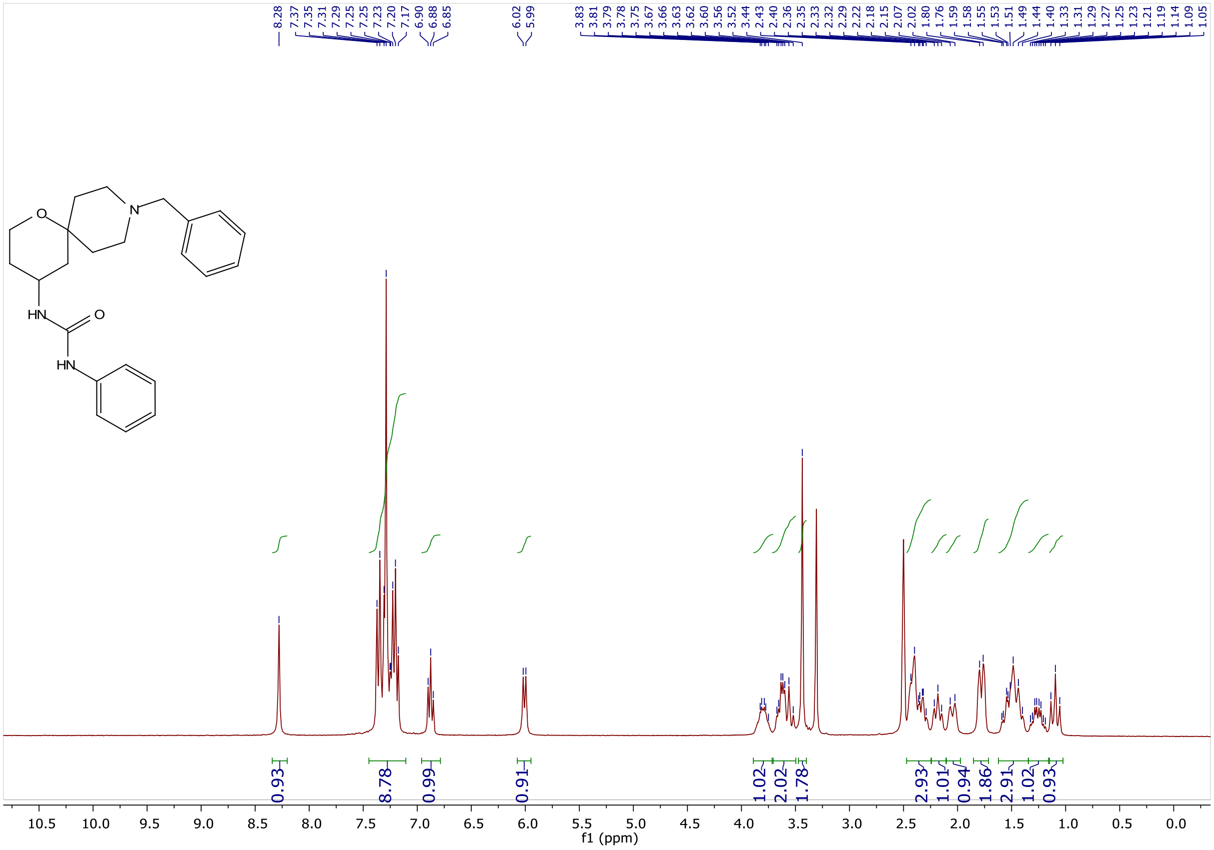


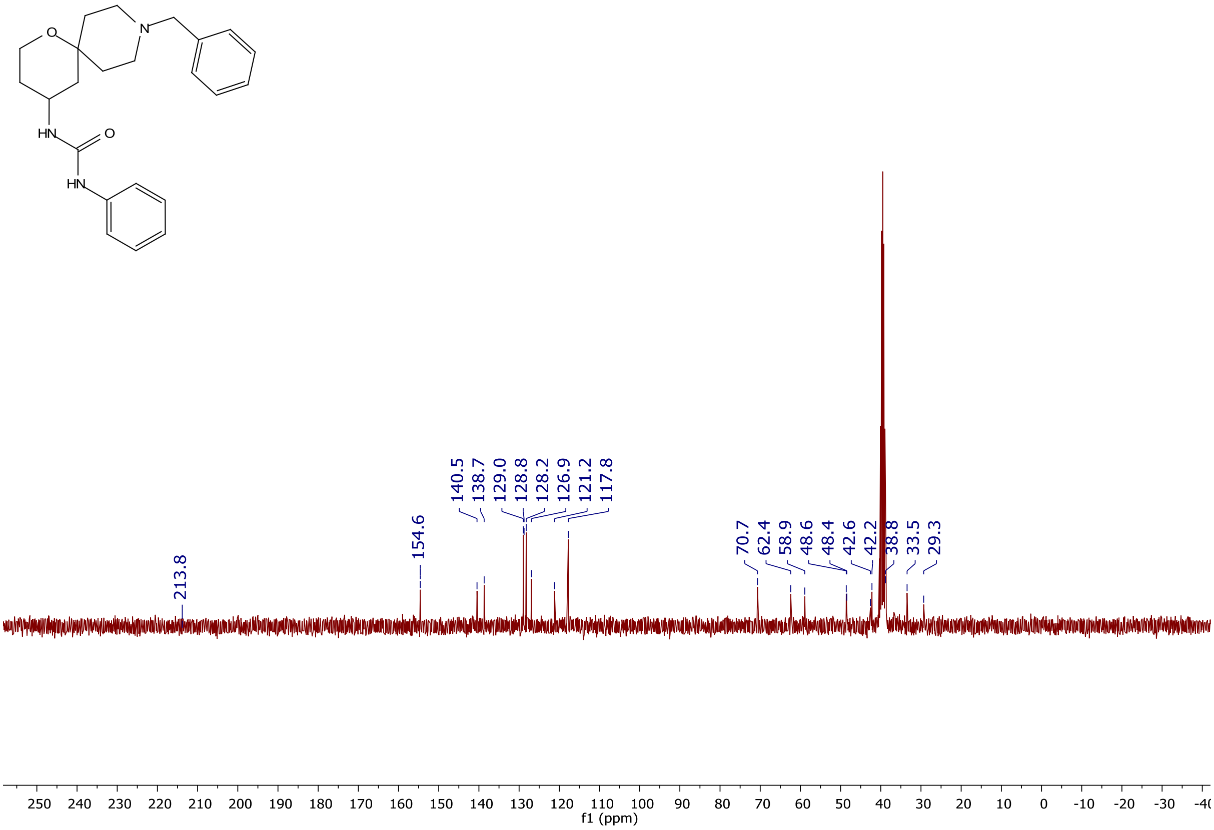
^{1}H and ^{13}C NMR spectrum of compound **3e**



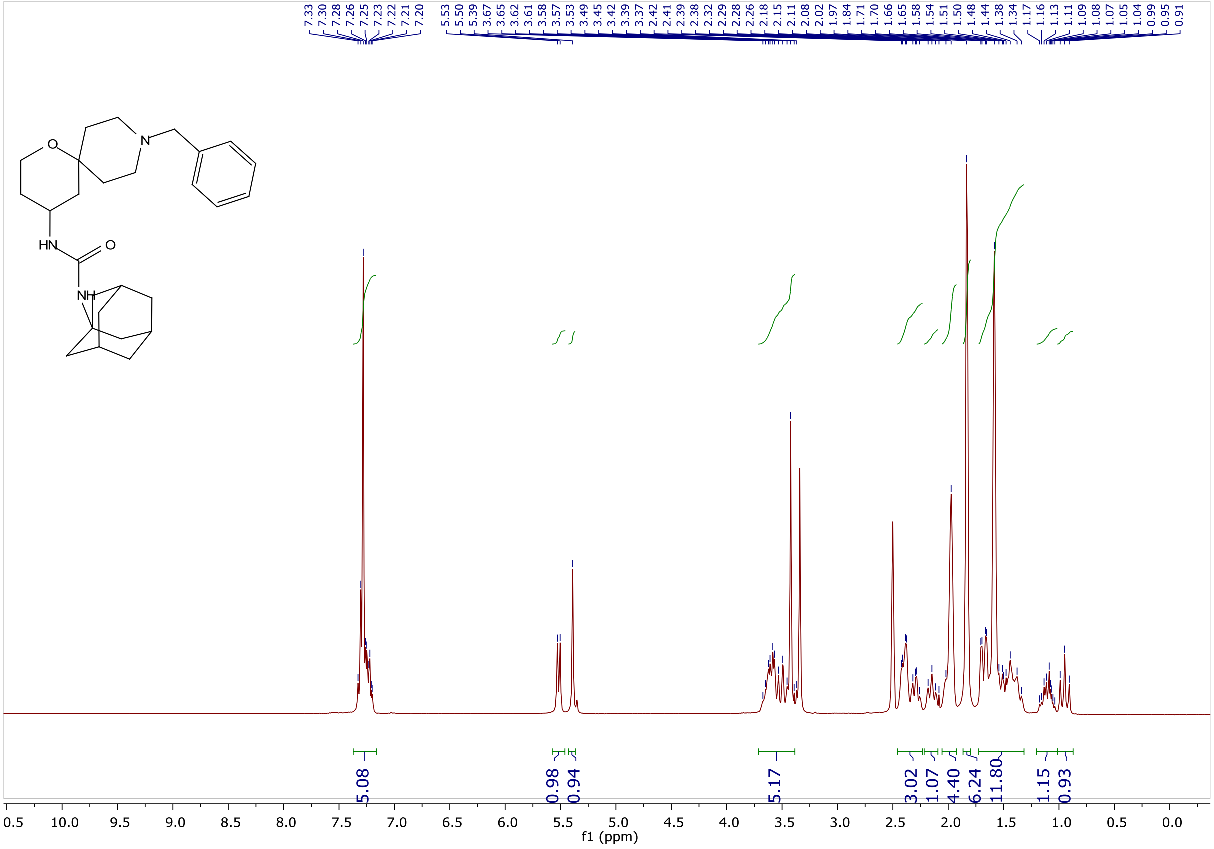


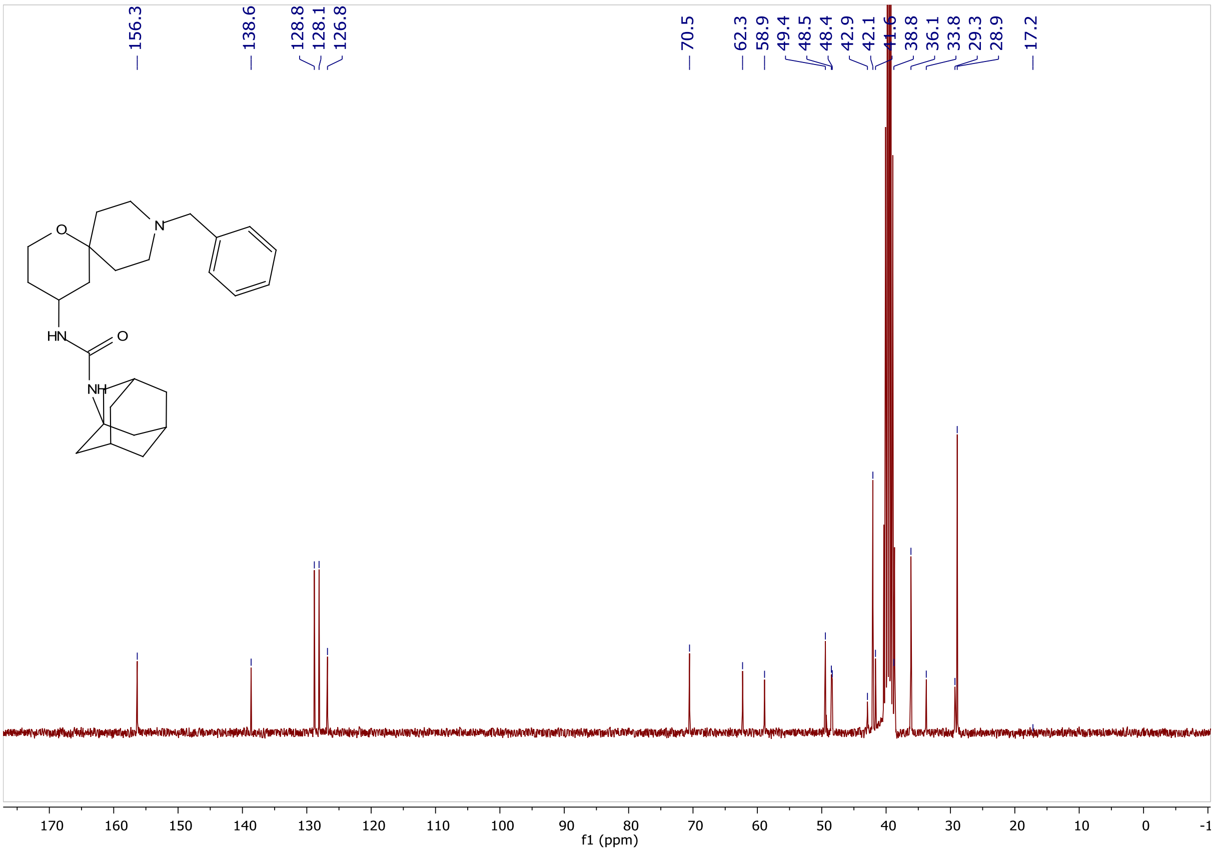
^{1}H and ^{13}C NMR spectrum of compound **3f**



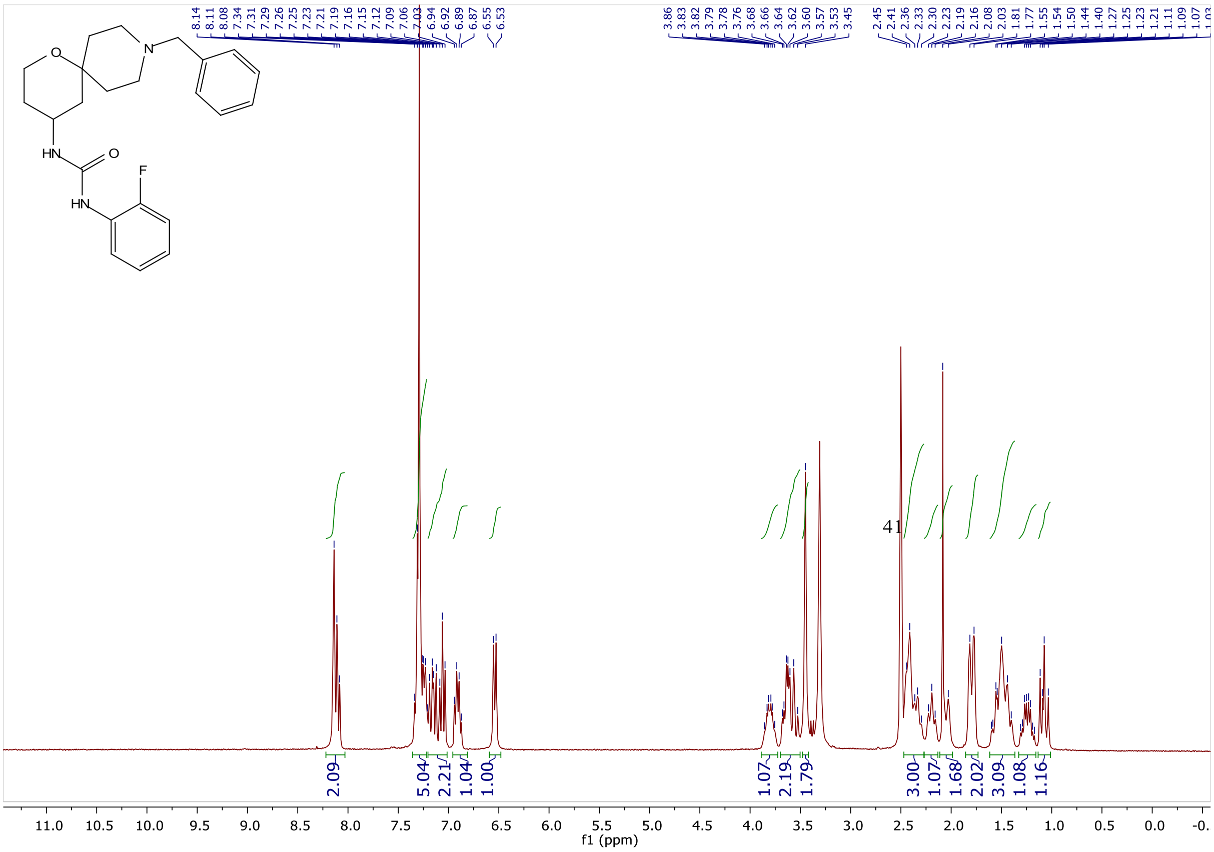


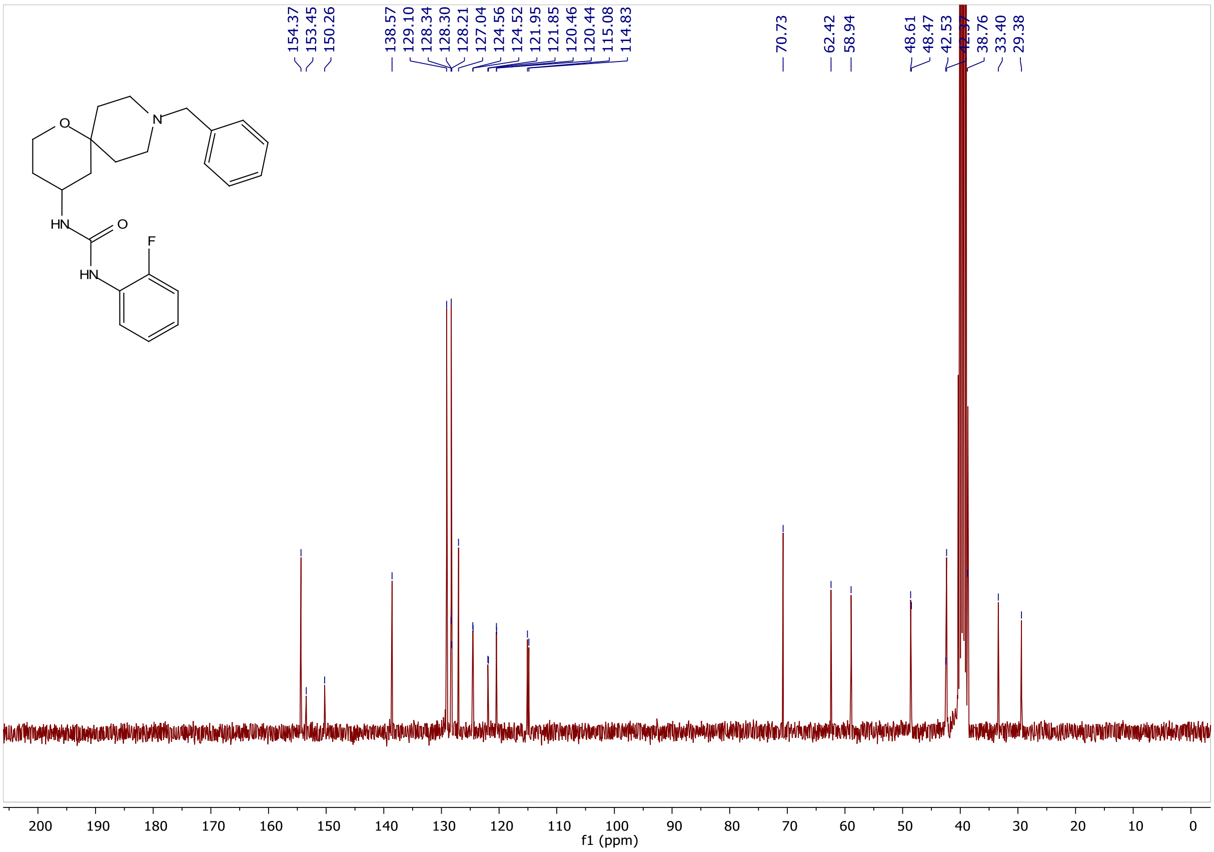
^{1}H and ^{13}C NMR spectrum of compound **3g**



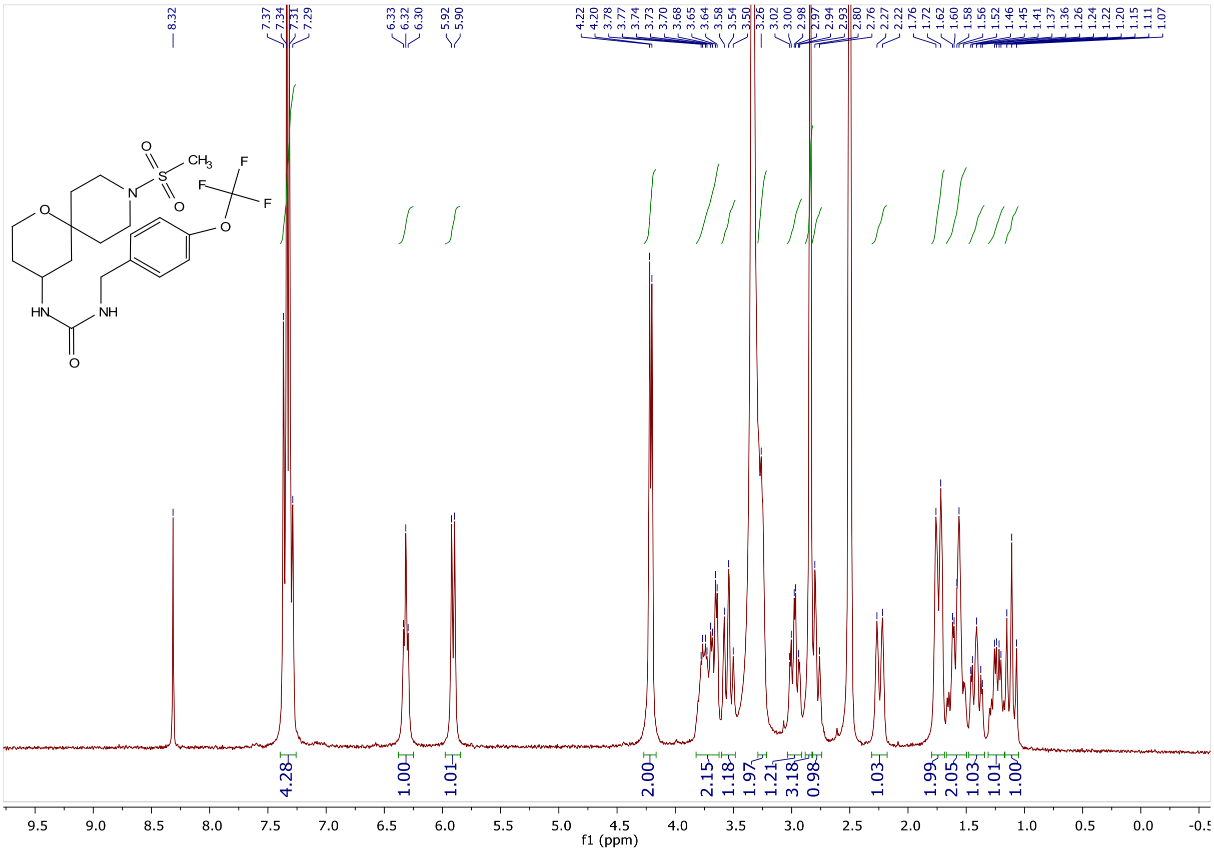


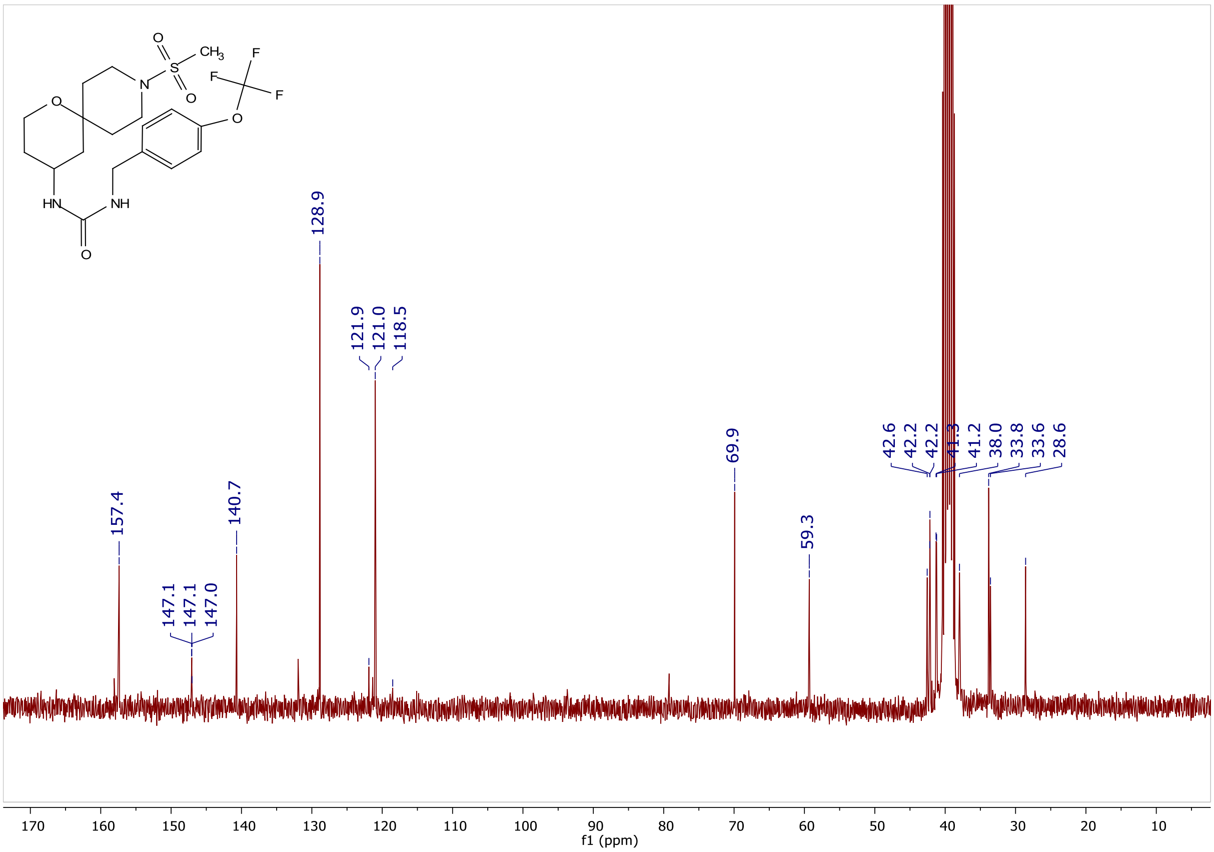
^{1}H and ^{13}C NMR spectrum of compound **3h**



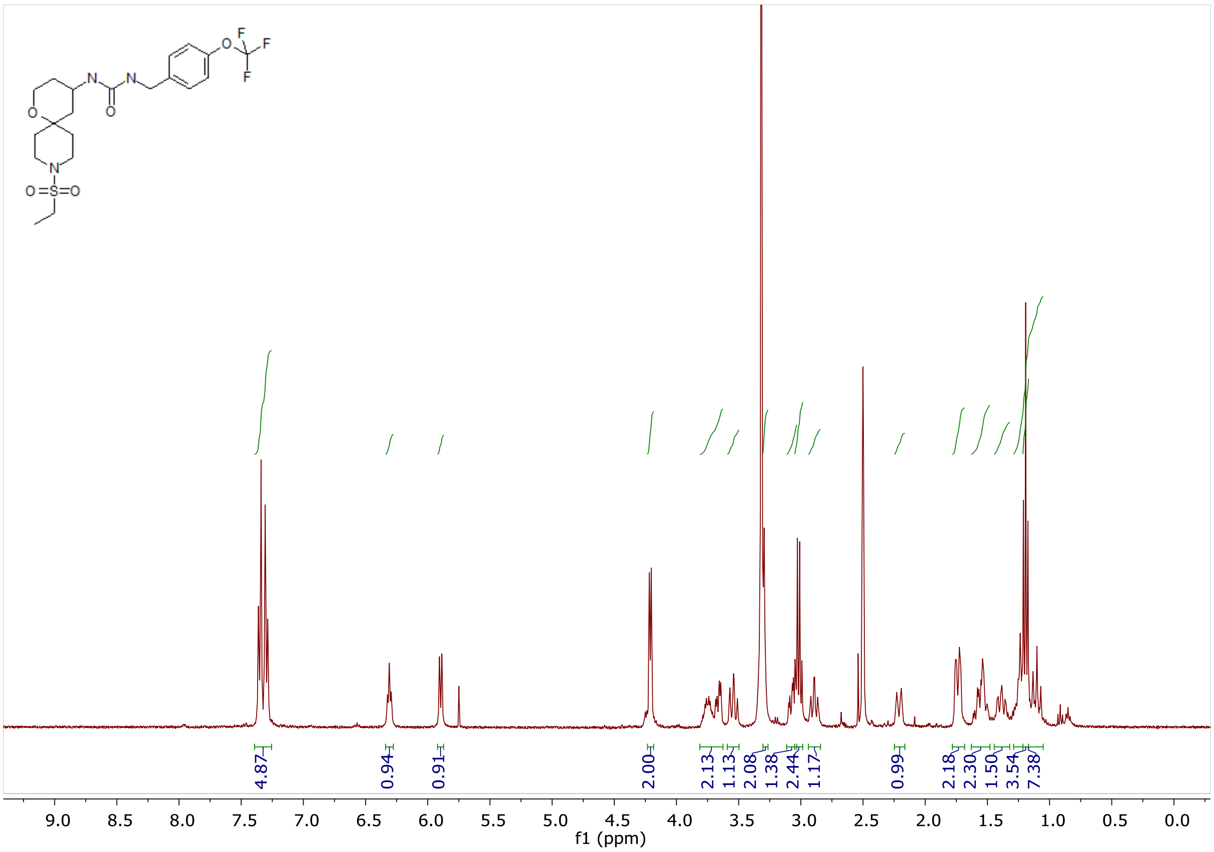


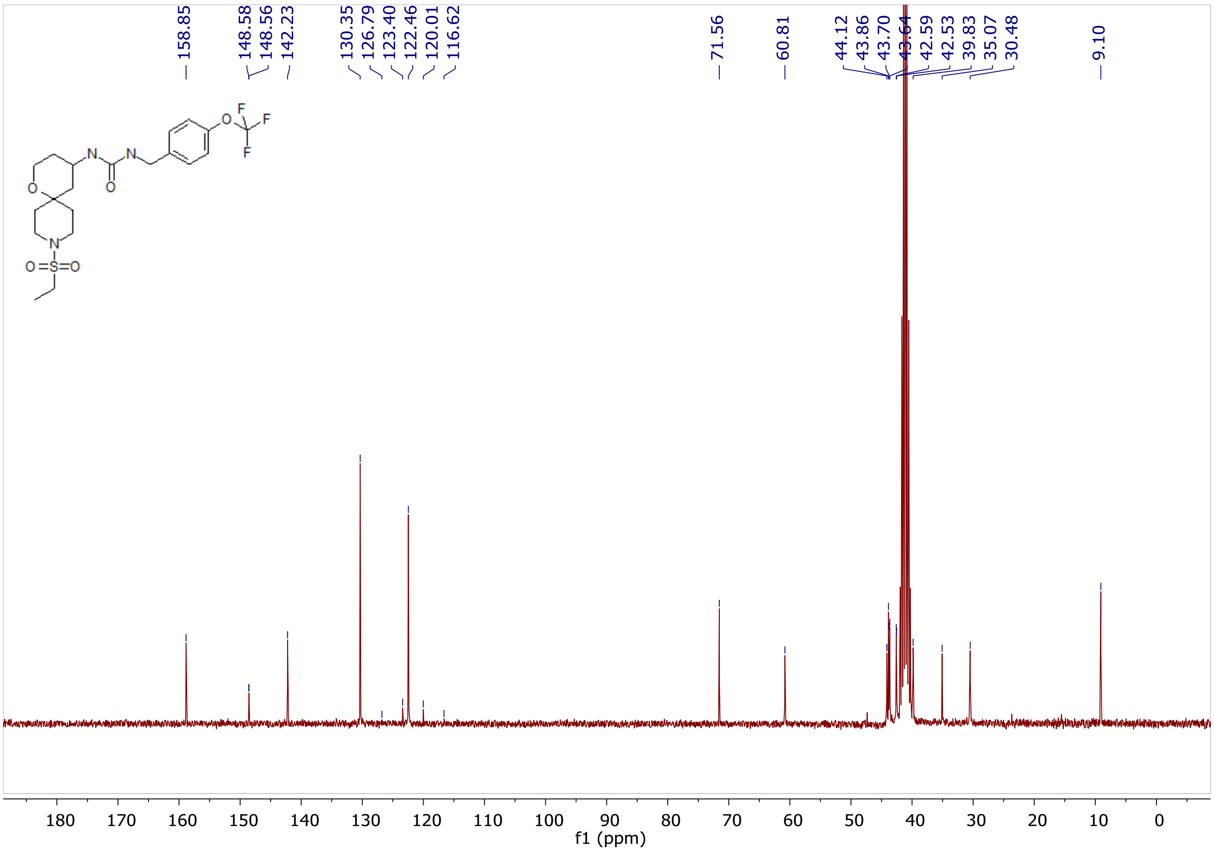
^{1}H and ^{13}C NMR spectrum of compound **3i**



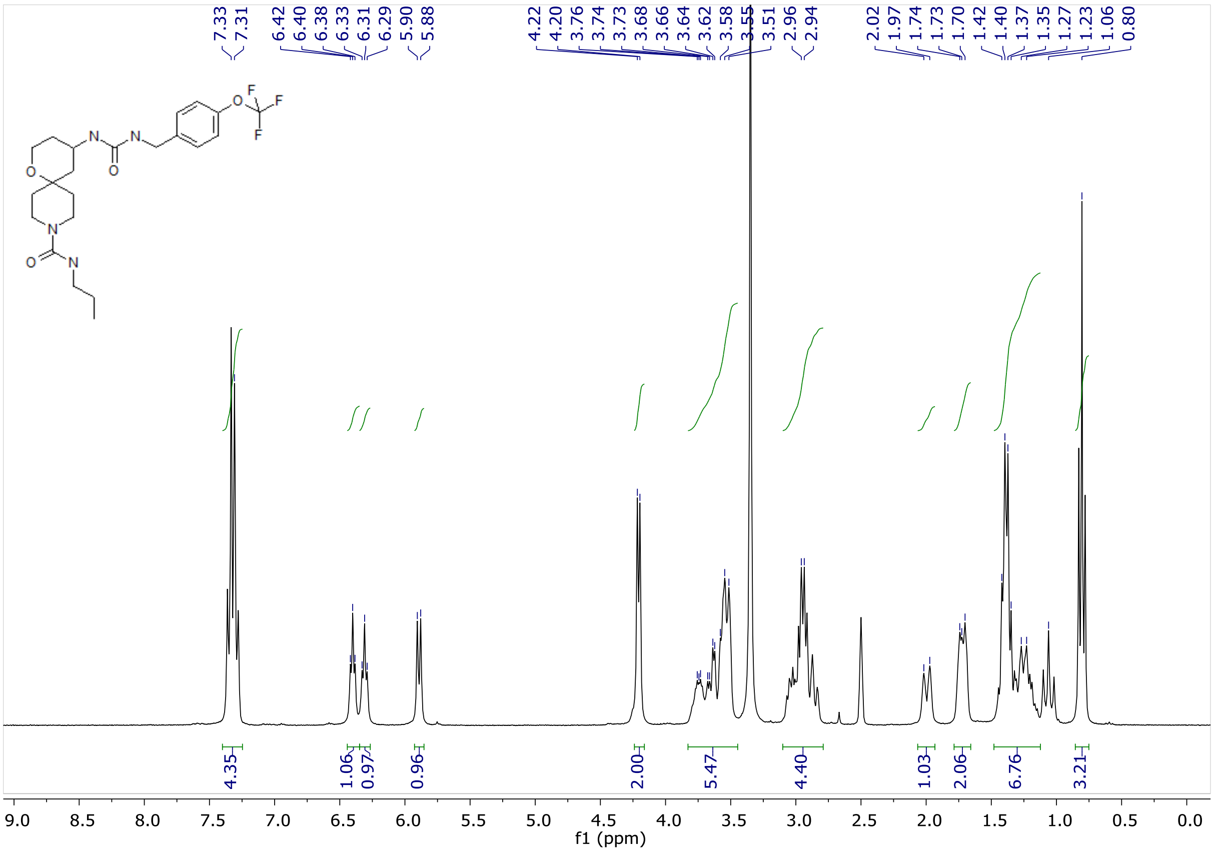


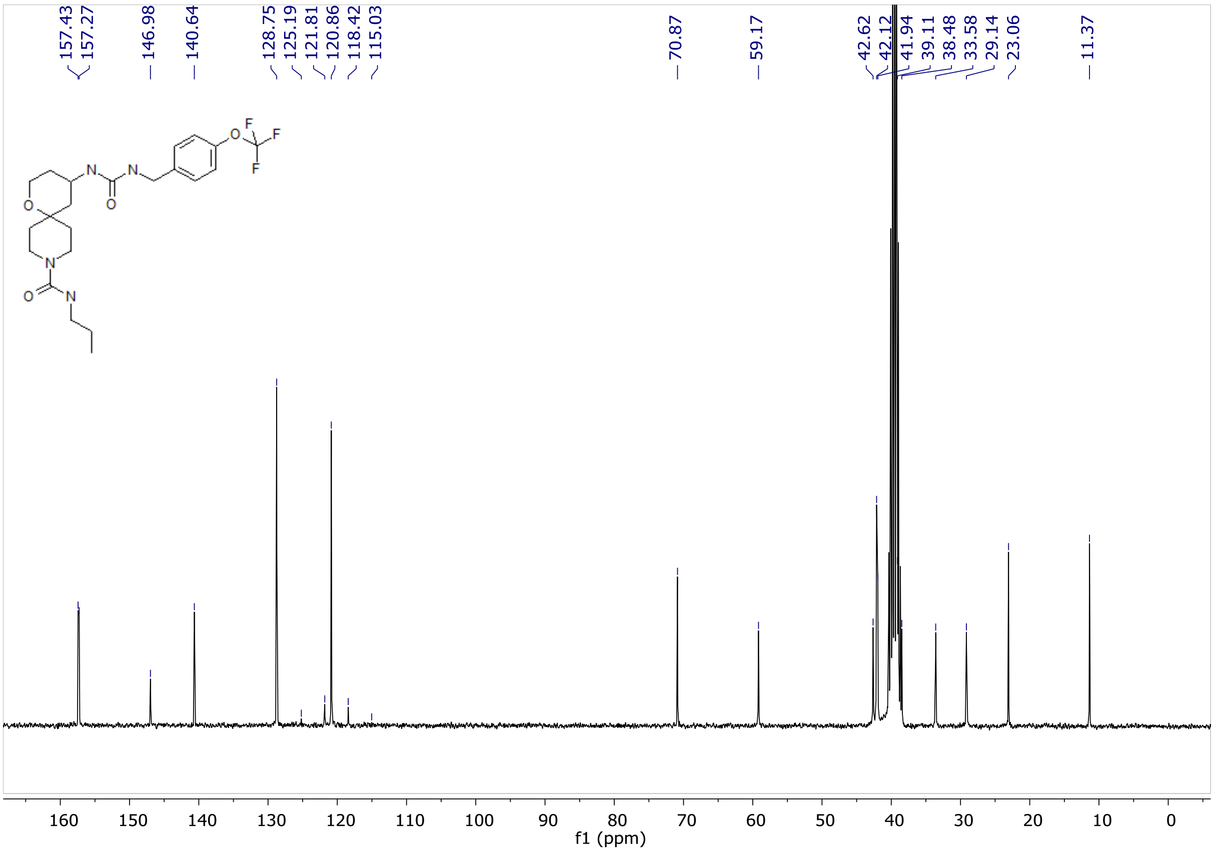
^{1}H and ^{13}C NMR spectrum of compound **3j**



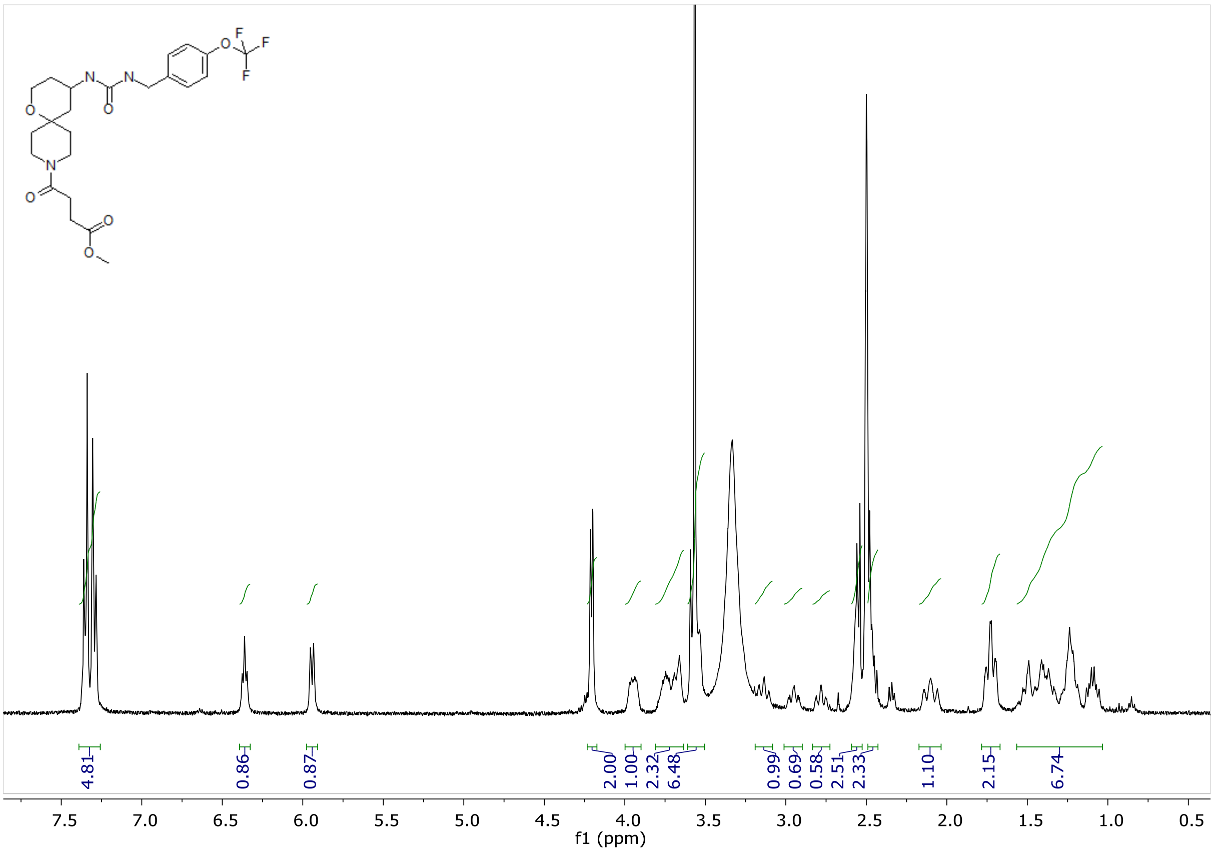


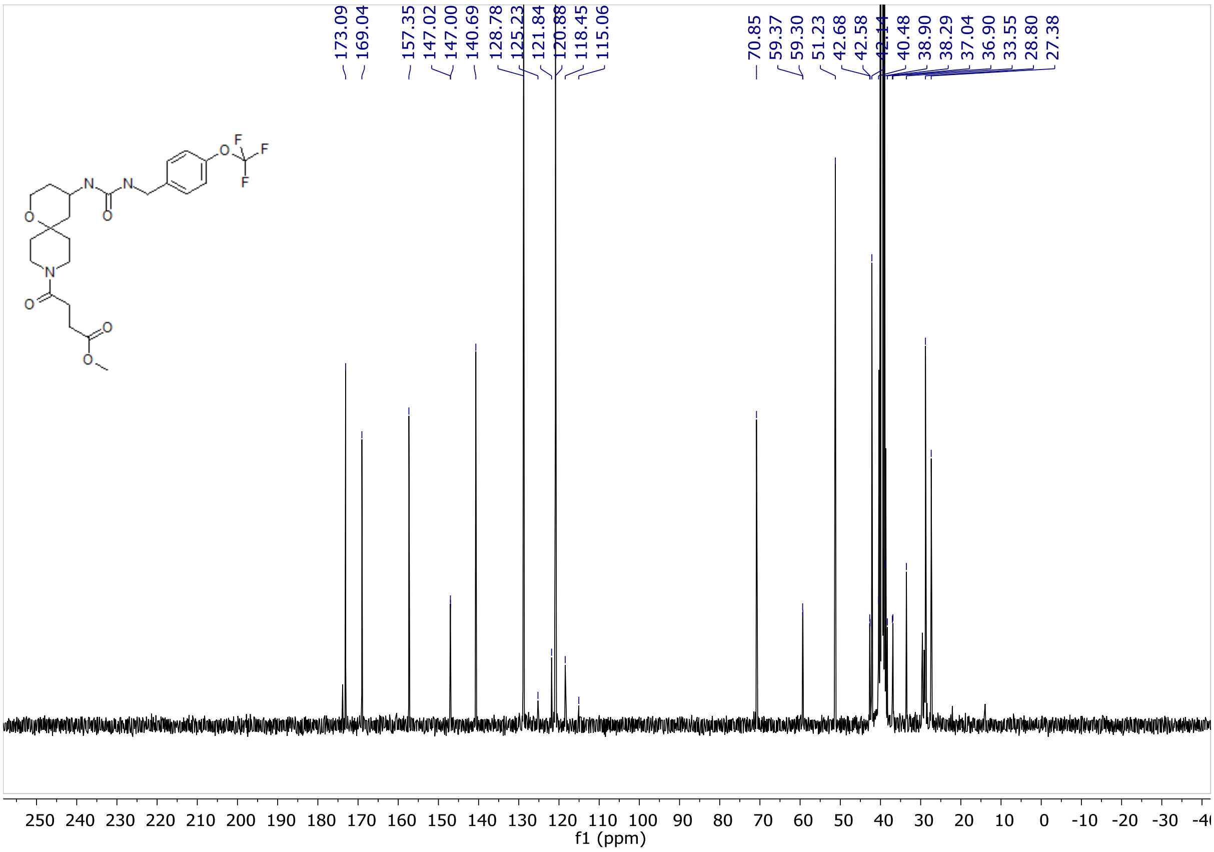
^{1}H and ^{13}C NMR spectrum of compound **3k**



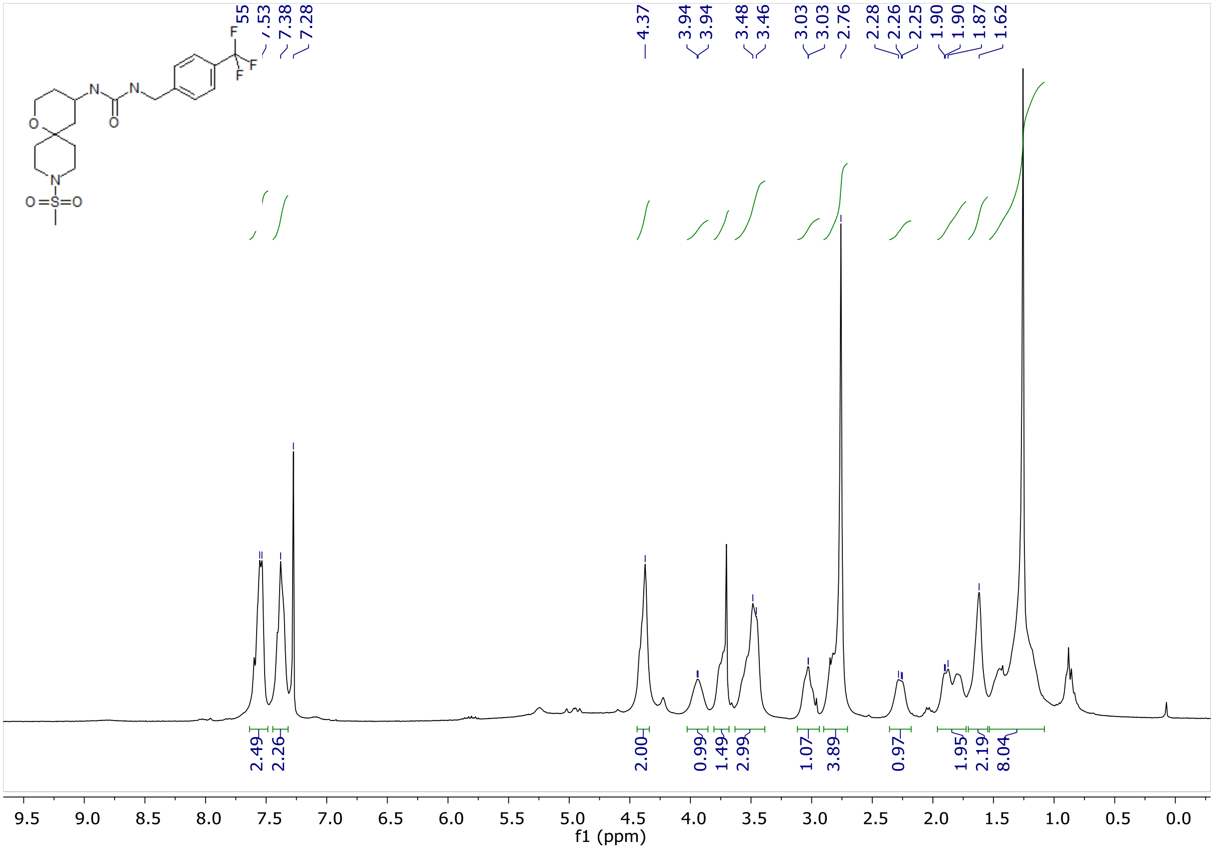


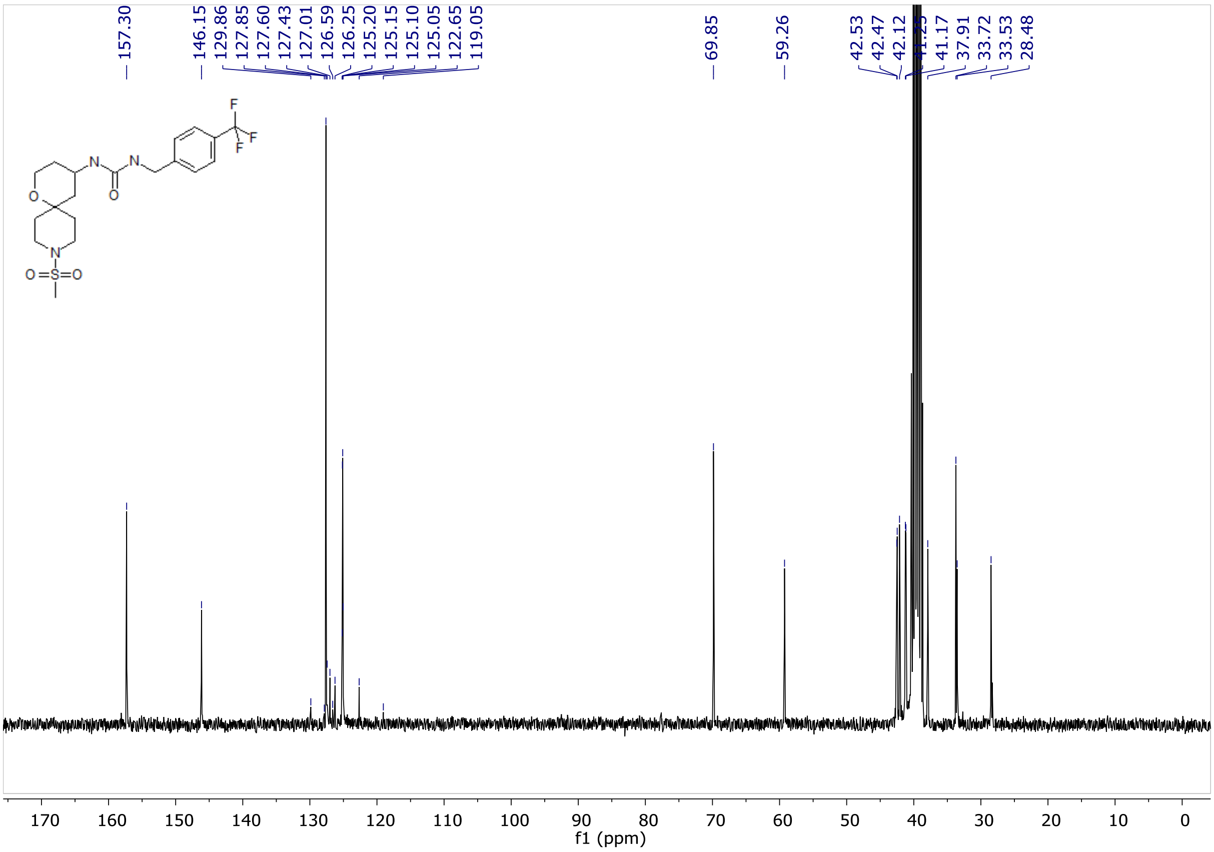
^{1}H and ^{13}C NMR spectrum of compound **3l**



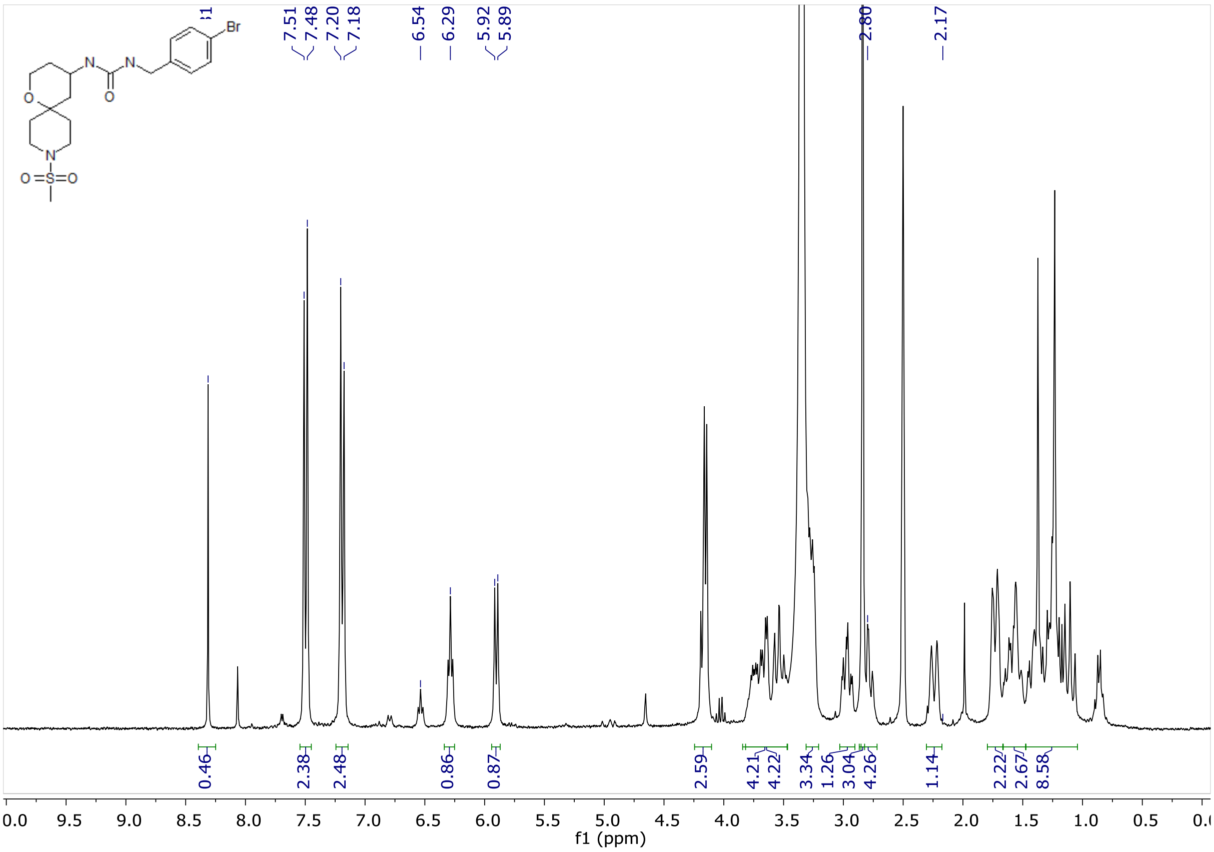


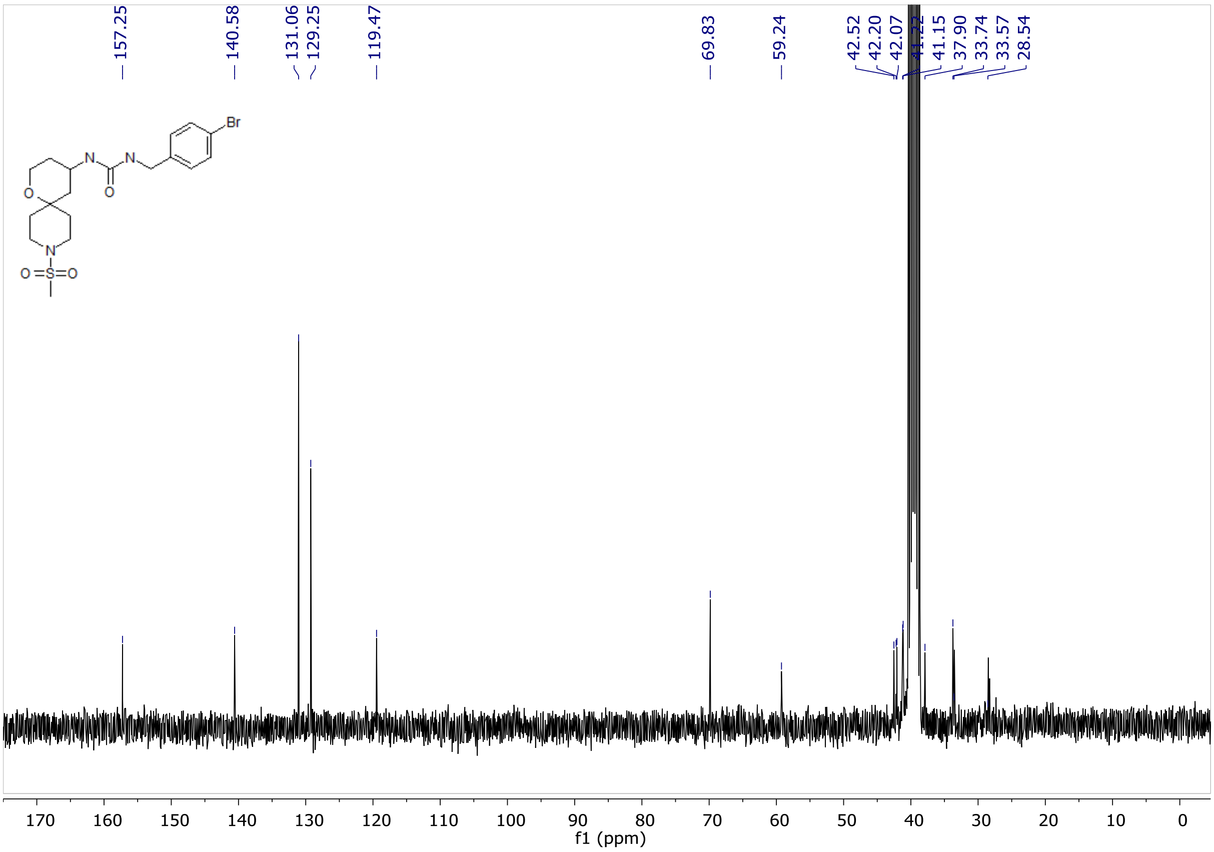
^{1}H and ^{13}C NMR spectrum of compound **3m**



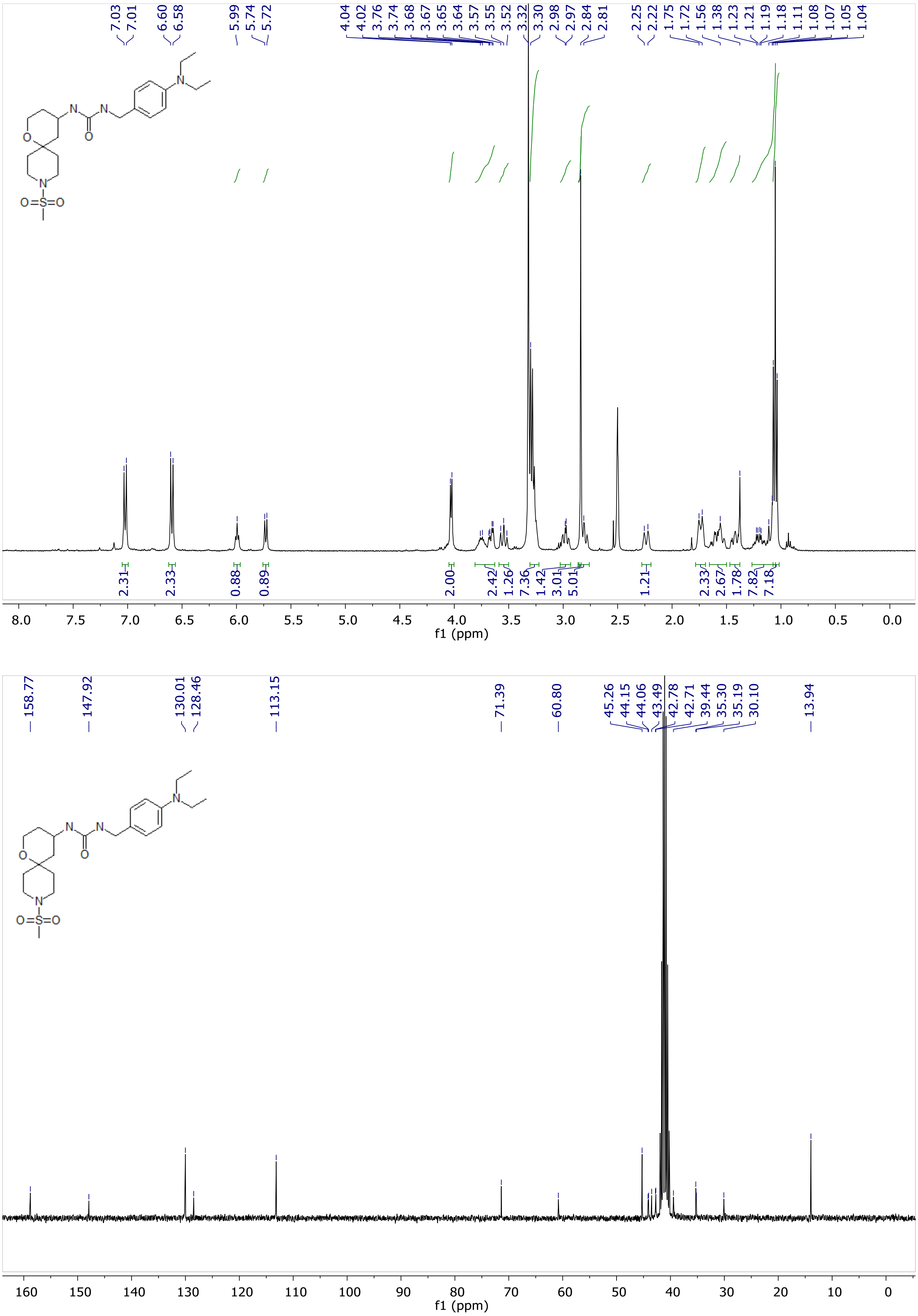


^{1}H and ^{13}C NMR spectrum of compound **3n**

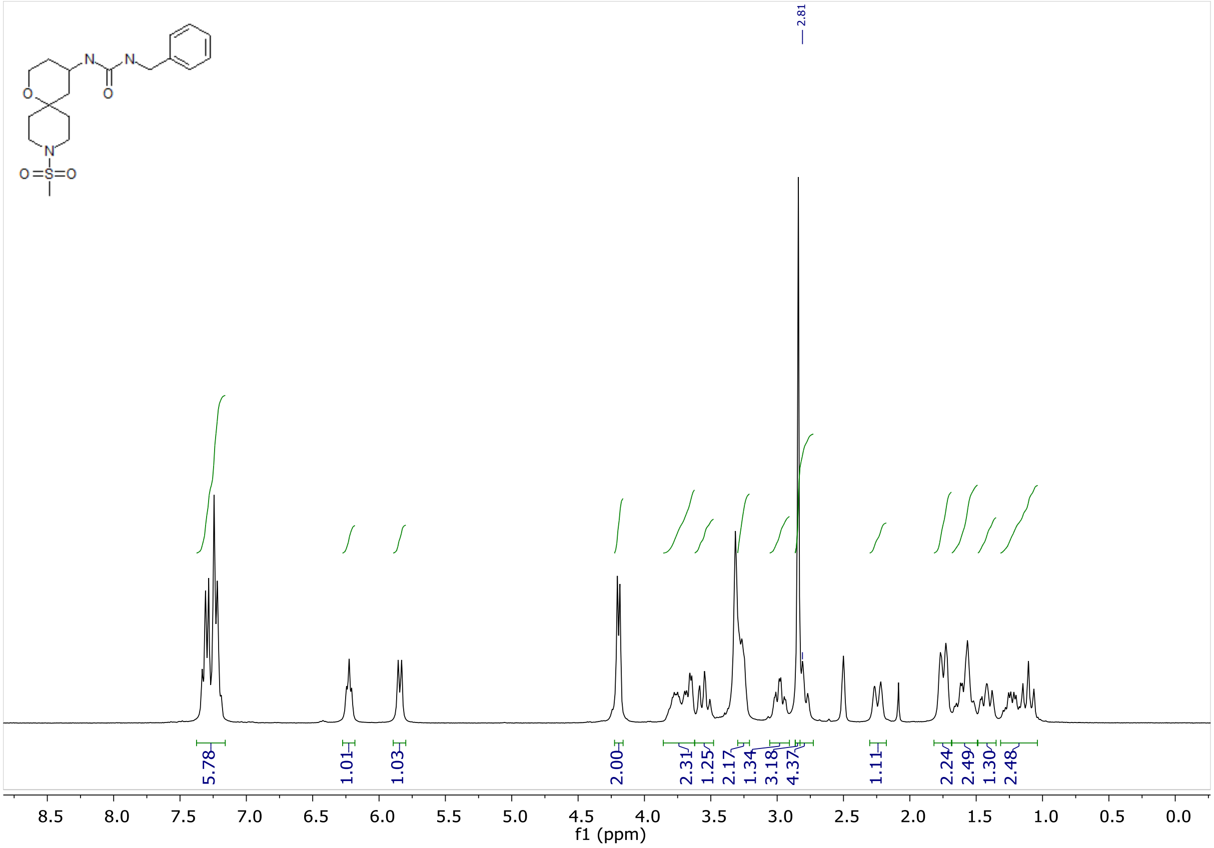


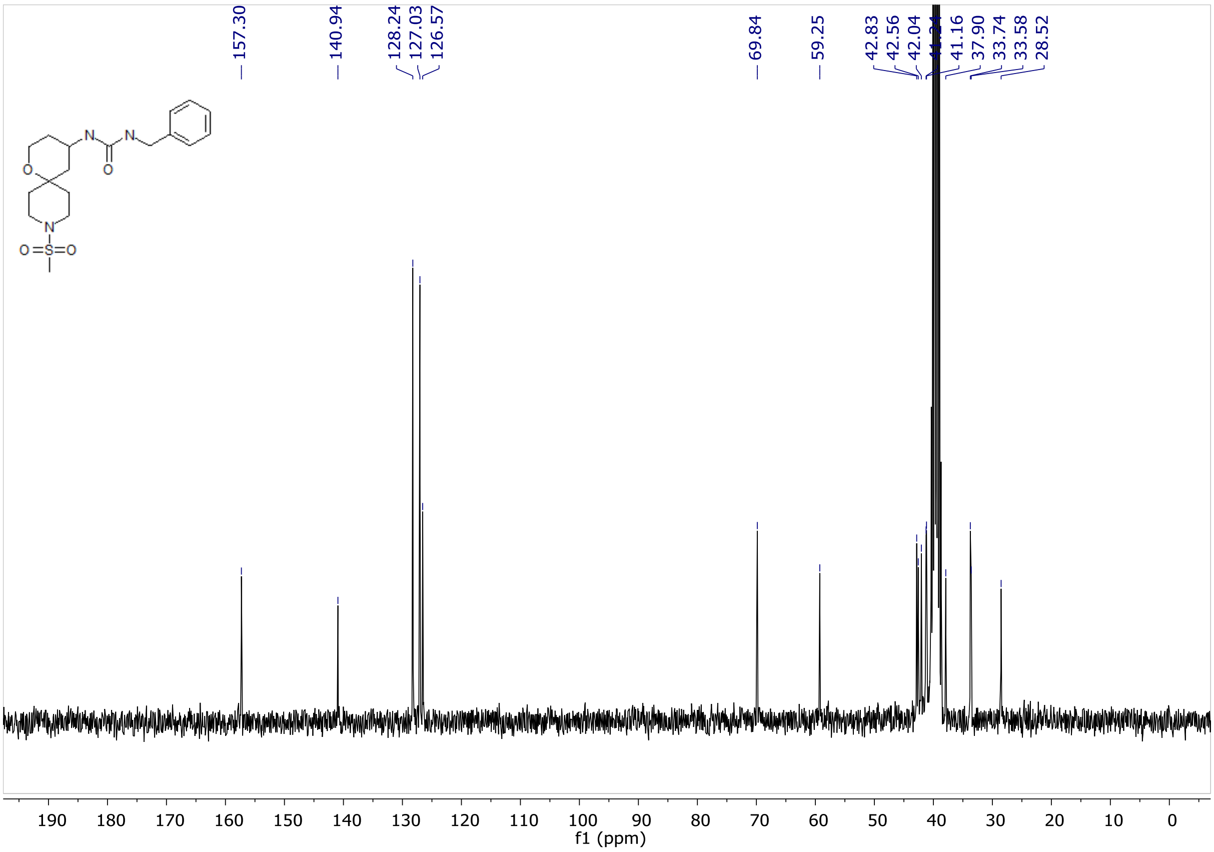


^{1}H and ^{13}C NMR spectrum of compound **3o**



^1H and ^{13}C NMR spectrum of compound **3p**





^{1}H and ^{13}C NMR spectrum of compound **3q**