Supporting information for

Discovery of 2,6-Dimethylpiperazines as Allosteric Inhibitors of CPS1

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KEYWORDS: Carbamoyl-phosphate synthetase 1, CPS1, allosteric inhibitors, high-throughput screening, cancer.

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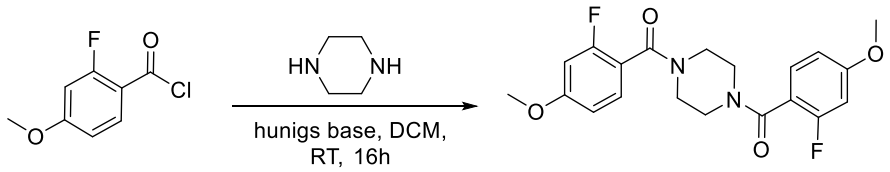
1.1 General Information

Unless otherwise noted, all materials were obtained from commercial suppliers and were used without further purification. Solvent removal was carried out using either a Büchi rotary evaporator or a Genevac centrifugal evaporator. Preparative LC/MS was conducted using a Waters mass directed autopurification system and a Waters 19 x 100mm XBridge 5 micron C18 column under basic mobile phase conditions or an equivalent Waters CSH C18 column under acidic conditions. NMR spectra were recorded using a Bruker Ascend 400MHz spectrometer. Chemical shifts (δ) are reported in ppm relative to the residual solvent signal (measurement range – 6.4 kHz). ^{1}H NMR data are reported as follows: chemical shift (multiplicity, coupling constants and number of hydrogens). Multiplicity is abbreviated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad). Compounds demonstrated the existence of rotamers in solution and hence ^{1}H NMR were run at both 298K and 350K when required.

When the term "inerted" is used to describe a reactor (e.g., a reaction vessel, flask, glass reactor) it is meant that the air in the reactor has been replaced with an essentially moisture-free or dry, inert gas (such as nitrogen, argon).

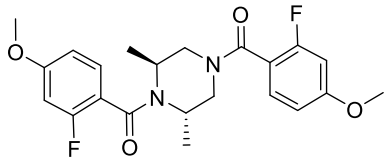
1.2 General procedures for 2, 11-13, 18, 24, 27, 30 and 31

Piperazine-1,4-diylbis((2-fluoro-4-methoxyphenyl)methanone) 2



To a 1 dram vial was added piperazine (9.14 mg, 0.087 mmol), DCM (2.6 mL) and hunig's base (46.3 µl, 0.265 mmol). After stirring for 10 mins at RT, 2-fluoro-4-methoxybenzoyl chloride (50 mg, 0.265 mmol) was added to the crude reaction mixture and the reaction stirred at RT for 16 h. After such time the reaction was concentrated and purified by reverse phase HPLC to afford the title compound (42 mg, 0.107 mmol, 41%) as a white solid. ^{1}H-NMR (400 MHz, DMSO-d6, 298K) δ 7.38-7.34 (m, 2H), 6.94-6.86 (m, 2H), 3.81 (m, 6H), 3.73-3.68 (br m, 2H), 3.65-3.59 (br m, 2H), 3.29-3.24 (br m, 2H). ^{1}H-NMR (400 MHz, DMSO-d6, 350K) δ 7.34 (dd, 2H, J = 8.22 Hz), 6.86 (d, 4H, J = 9.79 Hz), 3.83 (m, 6H), 3.58-3.45 (m, 8H). ^{19}F-NMR (377 MHz, DMSO-d6, 350K) δ -113.33. UPLC-MS: calculated for $C_{20}H_{21}F_2N_2O_4$ $[M+H]^+$ is 391.493, found 391.493 $[M+H]^+$.

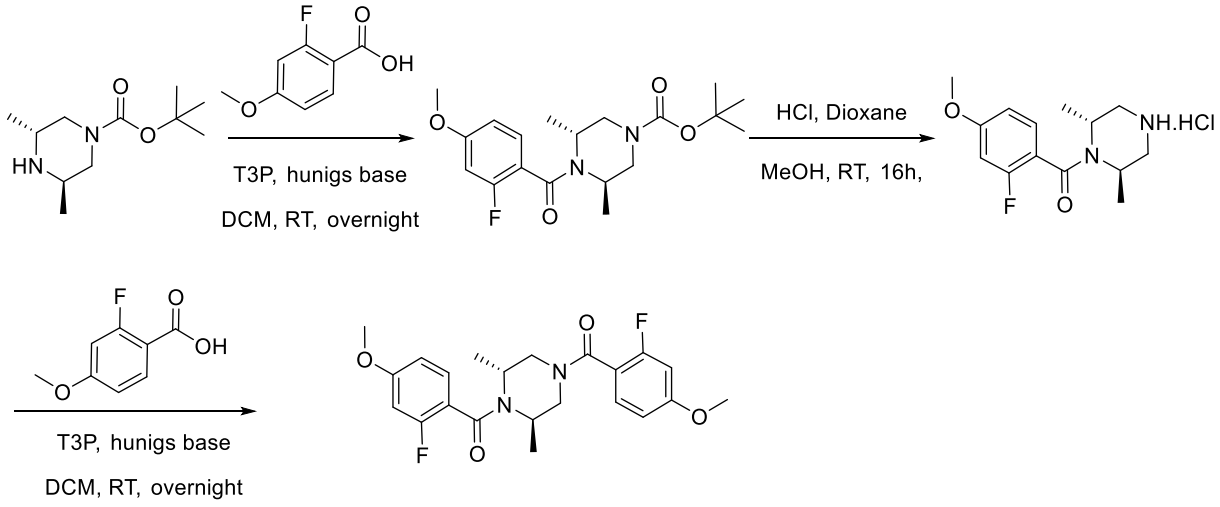
((2*S*,6*S*)-2,6-Dimethylpiperazine-1,4-diyl)bis((2-fluoro-4-methoxyphenyl)methanone) 11



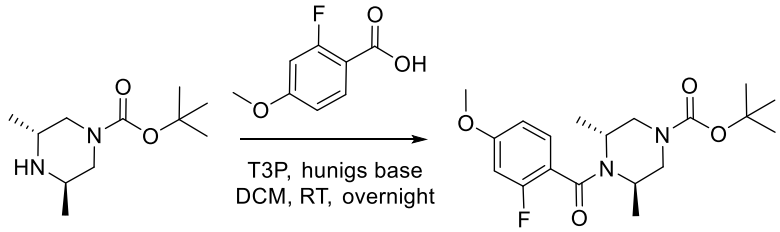
The title compound was synthesized following the approach outlined in procedure **2** substituting (2*S*,6*S*)-2,6-dimethylpiperazine for piperazine to afford the title compound (80.4 mg, 0.576 mmol, 69 %) as a white solid. ^{1}H-NMR (400 MHz, DMSO-d6, 298K) δ 7.40-7.31 (m, 2H), 6.93 (ddd, 2H, *J* = 11.89, 5.99, 2.2 Hz), 6.89-6.84 (m, 2H), 4.28-4.05 (br m, 2H), 3.87 (br d, 1H, *J* = 13.3 Hz), 3.82 (s, 3H), 3.80 (s, 3H), 3.80-3.77 (m, 1H), 3.63-3.58 (m, 1H), 3.27-3.22 (m, 1H), 1.24 (br s, 3H), 1.07 (br s, 3H). ^{1}H-NMR (400 MHz, DMSO-d6, 350K) δ 7.37-7.29 (m, 2H), 6.90-6.84 (m, 4H), 4.25-4.10 (br m, 2H), 3.92-3.81 (br m, 1H), 3.84 (s, 3H), 3.83 (s, 3H), 3.77-3.62 (br m, 2H),

3.32-3.18 (br m, 1H), 1.30-1.10 (br m, 6H). ^{19}F-NMR (377 MHz, DMSO-d6, 298K) δ -111.69, -114.01. UPLC-MS: calculated for $C_{22}H_{25}F_2N_2O_4$ $[M+H]^+$ is 419.178, found 419.173 $[M+H]^+$.

((2*R*,6*R*)-2,6-dimethylpiperazine-1,4-diyl)bis((2-fluoro-4-methoxyphenyl)methanone) H3B-374 (12)



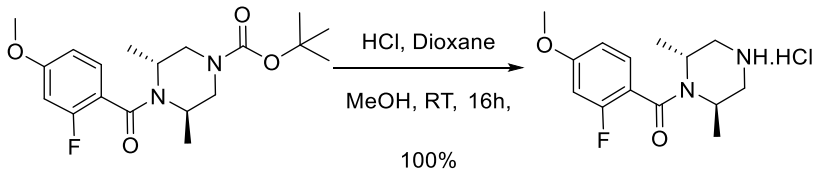
***a. tert-Butyl (3R,5R)-4-(2-fluoro-4-methoxybenzoyl)-3,5-dimethylpiperazine-1-carboxylate*.**



To a r.b flask was added 2-fluoro-4-methoxybenzoic acid (1.19 g, 6.99 mmol) followed by DCM (46.7 mL, 4.67 mmol), hunig'sbase (2.4 mL, 13.99 mmol) and 1-propanephosphonic acid cyclic [50% in EtOAc (16.3 mmol)]. After stirring for 5 mins at RT, tert-butyl (*2R,6R*)-2,6-dimethylpiperazine-1-carboxylate (1.0 g, 4.66 mmol) was added and the reaction was stirred overnight at RT. The reaction was quenched with water (20 mL), the organic layer separated and washed with water (20 mL), dried over $MgSO_4$, filtered and concentrated to give a crude yellow oil. The crude material was purified by flash chromatography on silica eluting with hexane:EtOAc (0-100% gradient) to obtain the title compound (0.90 g, 2.64 mmol, 53 % yield) as a white solid. ^{1}H-NMR (400 MHz, DMSO-d6) δ7.32 (dd, 1H), 6.92 (dd, 1H), 6.85 (dd, 1H), 3.81 (s, 3H), 3.63-

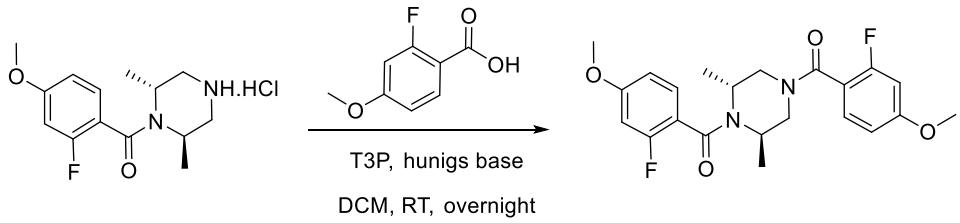
3.52 (m, 3H), 3.48-3.32 (m, 3H), 1.42 (s, 9H), 1.19-1.08 (m, 6H). UPLC-MS: calculated for $C_{19}H_{28}FN_2O_4$ is 367.203, found 367.189 $[M+H]^+$.

b. ((2R,6R)-2,6-Dimethylpiperazin-1-yl)(2-fluoro-4-methoxyphenyl)methanone hydrochloride.



To a r.b flask containing tert-butyl (3*R*,5*R*)-4-(2-fluoro-4-methoxybenzoyl)-3,5-dimethylpiperazine-1-carboxylate (903 mg, 2.46 mmol) was added MeOH (4.5 mL) and hydrochloric acid solution [(4.0M in dioxane), 3.1 mL, 12.32 mmol] and the reaction stirred at RT for 2h. After such time, the reaction was concentrated to afford the title compound (745 mg, 2.46 mmol, 100 % yield) as a white solid. ^{1}H-NMR (400 MHz, DMSO-d6) δ 9.73 (br s, 2H), 7.38 (d, 1H), 6.94 (dd, 1H), 6.87 (dd, 1H), 4.11 (br s, 2H), 3.81 (s, 3H), 3.44-3.34 (m, 2H), 3.19-3.13 (m, 2H), 1.28 (s, 3H), 1.26 (s, 3H).

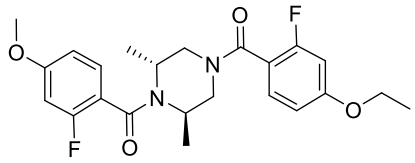
***c.* ((2*R*,6*R*)-2,6-dimethylpiperazine-1,4-diyl)bis((2-fluoro-4-methoxyphenyl)methanone) H3B-374 (12)**



To a reaction vessel was added a solution of ((2*R*,6*R*)-2,6-dimethylpiperazin-1-yl)(2-fluoro-4-methoxyphenyl)methanone, HCl (17 mg, 0.056 mmol), DCM (0.561 mL, 0.056 mmol) and 1-Propanephosphonic acid cyclic anhydride (66.8 mL, 0.112 mmol). After stirring for 10 mins, 2-fluoro-4-methoxybenzoic acid (14 mg, 0.082 mmol) and hunig's base (0.029 mL, 0.168 mmol) were added and the reaction was stirred at RT for another 16h. After such time, the reaction was concentrated and purified by reverse phase HPLC to afford the desired compound (14.2 mg, 0.034

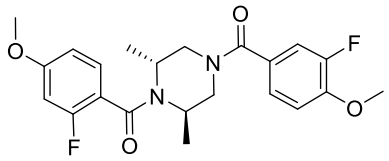
mmol, 61%) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$, 298K) δ 7.39-7.31 (m, 2H), 6.93 (td, 2H, *J* = 11.92, 6.02, 2.26 Hz), 6.86 (td, 2H, *J* = 7.87, 2.32 Hz), 4.35-4.01 (br m, 2H), 3.98-3.75 (m, 2H), 3.82 (s, 3H), 3.81 (s, 3H), 3.63-3.58 (m, 1H), 3.26-3.18 (m, 1H), 1.23 (br s, 3H), 1.08 (br s, 3H). ^{1}H-NMR (400 MHz, $CDCl_3$, 350K) δ 7.37-7.29 (m, 2H), 6.90-6.83 (m, 4H), 4.28-1.06 (br m, 2H), 3.90-3.80 (m, 1H), 3.84 (s, 3H), 3.83 (s, 3H), 3.75-3.63 (m, 2H), 3.30-3.22 (m, 1H), 1.28-1.12 (br s, 6H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 167.9, 166.3, 162.5, 161.9, 160.6, 160.0, 158.1, 157.6, 130.4, 129.4, 117.0, 116.0, 110.8, 110.7, 101.9, 101.7, 101.5, 77.2, 55.76, 48.6, 45.9. ^{13}C-DEPT NMR (101 MHz, $CDCl_3$) δ 130.4, 129.4, 110.8, 110.7, 101.9, 101.7, 101.5, 77.2, 55.7, 48.6, 45.9. ^{19}F-NMR (377 MHz, DMSO-d6, 298K) δ -113.48, -114.77. UPLC-MS: calculated for $C_{22}H_{25}F_2N_2O_4$ $[M+H]^+$ is 419.178, found 419.218 $[M+H]^+$.

((2*R*,6*R*)-4-(4-Ethoxy-2-fluorobenzoyl)-2,6-dimethylpiperazin-1-yl)(2-fluoro-4-methoxyphenyl)methanone 13



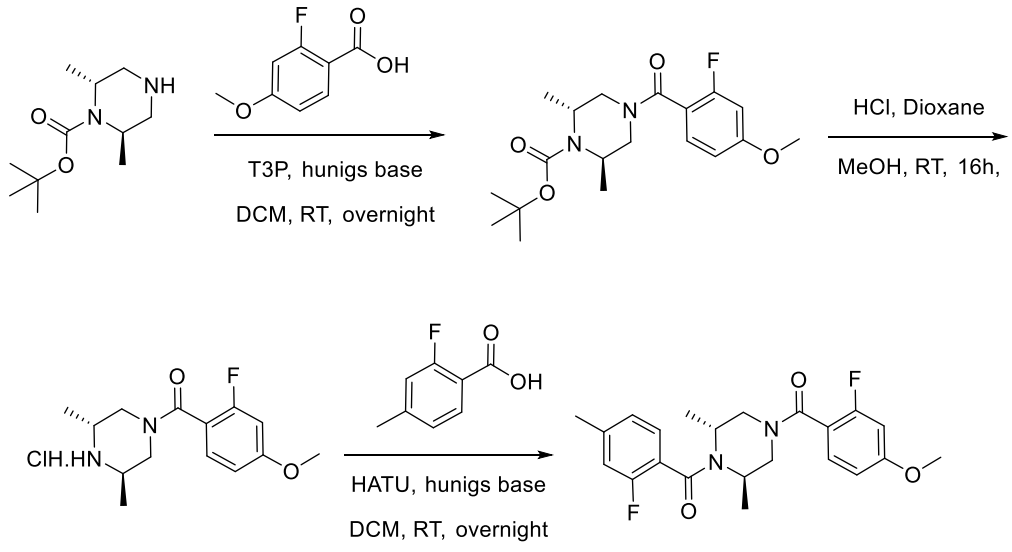
The title compound was synthesized following the approach outlined in procedure **12** substituting 4-ethoxy-2-fluorobenzoic acid for 2-fluoro-4-methoxybenzoic acid in step (c) to afford the title compound (80.4 mg, 0.576 mmol, 69 %) as a white solid. ^{1}H-NMR (400 MHz, DMSO-d6, 298K) δ 7.38-7.30 (m, 2H), 6.94-6.85 (m, 2H), 6.85 (br d, 2H, *J* = 8.66 Hz), 4.41-4.17 (br m, 1H), 4.08 (q, 2H, *J* = 6.96 Hz), 3.88-3.77 (m, 2H), 3.80 (s, 3H), 3.63-3.58 (m, 1H), 3.27-3.21 (m, 2H), 1.34 (t, 3H, *J* = 6.96 Hz), 1.24 (br s, 3H), 1.08 (br s, 3H). ^{1}H-NMR (400 MHz, DMSO-d6, 350K) δ 7.35-7.29 (m, 2H), 6.88-6.83 (m, 4H), 4.29-4.06 (br m, 2H), 4.12 (q, 2H, *J* = 7.03 Hz), 3.83 (s, 3H), 3.77-3.62 (m, 2H), 3.32-3.18 (m, 2H), 1.35 (t, 3H, *J* = 6.96 Hz), 1.27-1.11 (m, 6H). ^{19}F-NMR (377 MHz, DMSO-d6, 350K) δ -113.51, -114.78. UPLC-MS: calculated for $C_{23}H_{27}F_2N_2O_4$ $[M+H]^+$ is 433.194, found 433.306 $[M+H]^+$.

((2*R*,6*R*)-4-(3-Fluoro-4-methoxybenzoyl)-2,6-dimethylpiperazin-1-yl)(2-fluoro-4-methoxyphenyl)methanone 18

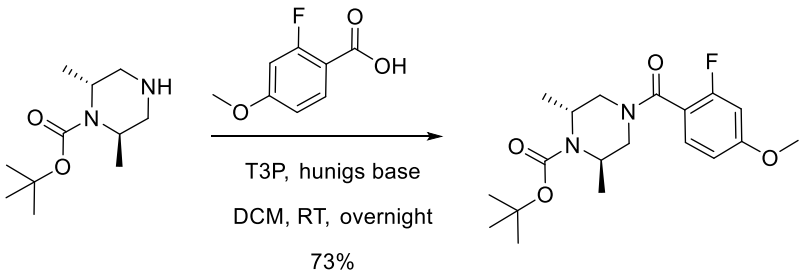


The title compound was synthesized following the approach outlined in procedure **12** substituting 3-fluoro-4-methoxybenzoic acid for 2-fluoro-4-(trifluoromethoxy)benzoic acid in step (c) to afford the title compound (12 mg, 0.028 mmol, 51 %) as a white solid. ^{1}H-NMR (400 MHz, DMF-d7, 350K) δ 7.05-6.97 (m, 3H), 6.92-6.88 (m, 1H), 6.57-7.27 (m, 2H), 3.85 (br , 2H), 3.59 (s, 3H), 3.51 (s, 3H), 3.52-3.43 (br m, 2H), 3.33-3.21 (br s, 2H), 0.89 (br d, *J* = 4.89 Hz, 6H). ^{19}F-NMR (377 MHz, DMF-d7, 350K) δ -114.34, -134.24. UPLC-MS: calculated for $C_{22}H_{25}F_2N_2O_4$ $[M+H]^+$ is 419.194 $[M+H]^+$, found 419.208.

((2*R*,6*R*)-4-(2-Fluoro-4-methoxybenzoyl)-2,6-dimethylpiperazin-1-yl)(2-fluoro-4-methylphenyl)methanone. 21

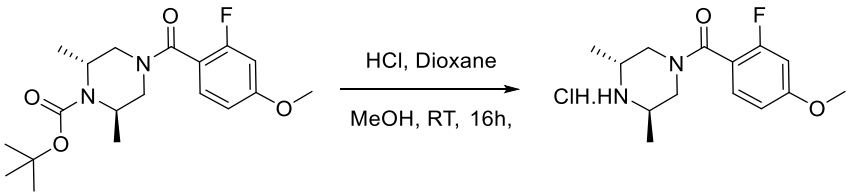


***a. tert-Butyl (2R,6R)-4-(2-fluoro-4-methoxybenzoyl)-2,6-dimethylpiperazine-1-carboxylate*.**



To a r.b flask was added 2-fluoro-4-methoxybenzoic acid (1.19 g, 6.99 mmol) followed by DCM (46.7 mL, 4.67 mmol), hunig's base (2.45 mL, 13.99 mmol) and 1-propanephosphonic acid cyclic anhydride [50% in EtOAc (9.7 mL, 16.3 mmol)]. After stirring for 5 mins at RT, tert-butyl (*2R,6R*)-2,6-dimethylpiperazine-1-carboxylate (1.0 g, 4.66 mmol) was added and the reaction was stirred overnight at RT. After such time the reaction was quenched with water (20 mL), the organic layer separated and washed with water (20 mL), dried over $MgSO_4$, filtered and concentrated to give a crude yellow oil. The crude material was purified by flash chromatography on silica eluting with hexane:EtOAc (0-100% gradient) to afford the title compound (1.25 g, 3.40 mmol, 72.9 % yield) as a white solid. ^{1}H-NMR (400 MHz, DMSO-d6) δ 7.35 (dd, 1H), 6.93 (dd, 1H), 6.87 (dd, 1H), 4.15-4.12 (m, 1H), 3.95-3.92 (m, 1H), 3.82 (s, 3H), 3.81-3.78 (m, 1H), 3.72 (dd, 1H), 3.54 (dd, 1H), 3.18 (dd, 1H), 1.42 (s, 9H), 1.25 (d, 3H), 1.08 (d, 3H). UPLC-MS: calculated for $C_{19}H_{28}FN_2O_4$ is 367.203, found 367.135 $[M+H]^+$.

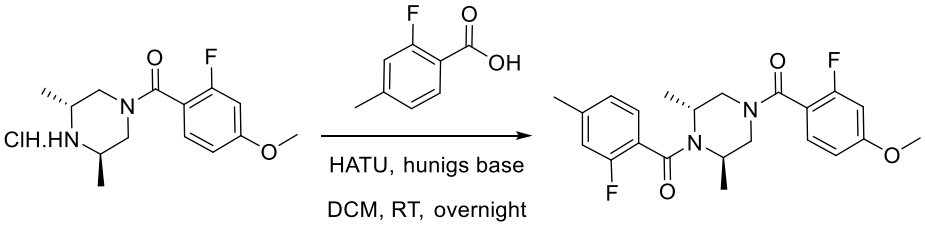
b. ((3R,5R)-3,5-Dimethylpiperazin-1-yl)(2-fluoro-4-methoxyphenyl)methanone hydrochloride.



To a r.b flask containing tert-butyl (*2R,6R*)-4-(2-fluoro-4-methoxybenzoyl)-2,6-dimethylpiperazine-1-carboxylate (1.35 g, 3.67 mmol) was added MeOH (6.7 mL), hydrochloric acid solution [(4.0M in dioxane), 4.6 mL, 18.37 mmol] and the reaction stirred at RT for 2h. After such time, the reaction was concentrated to afford the title compound (1.11 g, 4.00 mmol, 100 % yield) as a white solid. ^{1}H-NMR (400 MHz, DMSO) δ 9.52 (br s, 1H), 7.38 (dd, 1H), 6.95 (dd,

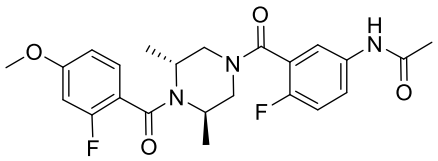
1H), 6.88 (dd, 1H), 3.93 (br s, 1H), 3.82 (s, 3H), 3.65-3.58 (m, 1H), 3.50-3.45 (m, 1H), 3.34-3.32 (m, 2H), 3.28-3.22 (m, 1H), 1.30 (br s, 3H), 1.16 (br s, 3H).

c. ((2*R*,6*R*)-4-(2-Fluoro-4-methoxybenzoyl)-2,6-dimethylpiperazin-1-yl)(2-fluoro-4-methylphenyl)methanone 21



To a reaction vessel was added a solution of ((3*R*,5*R*)-3,5-dimethylpiperazin-1-yl)(2-fluoro-4-methoxyphenyl)methanone, HCl (20 mg, 0.066 mmol) in DCM (0.66 mL, 0.066 mmol) followed by HATU (0.132 mmol), 2-fluoro-4-methylbenzoic acid (0.020 g, 0.130 mmol) and hunig'sbase (0.029 ml, 0.165 mmol). The reaction was stirred at RT for 16 h, after which time the reaction was concentrated and purified by reverse phase HPLC to afford the title compound (8.5 mg, 0.021 mmol, 32%) as a white solid. ^{1}H-NMR (400 MHz, DMSO-d6, 350K) δ 7.35 (dd, 1H, *J* = 8.47 Hz), 7.29-7.25 (m, 1H), 7.11-7.08 (m, 2H), 6.90-6.86 (m, 2H), 4.28-4.06 (br m, 2H), 3.92-3.86 (m, 1H), 3.81 (s, 3H), 3.78-3.61 (m, 2H), 3.32-3.19 (m, 1H), 2.37 (s, 3H), 1.30-1.08 (m, 6H). ^{19}F-NMR (377 MHz, DMSO-d6, 350K) δ -113.48, -118.00. UPLC-MS: calculated for $C_{22}H_{25}F_2N_2O_3$ $[M+H]^+$ is 403.183, found 403.210 $[M+H]^+$.

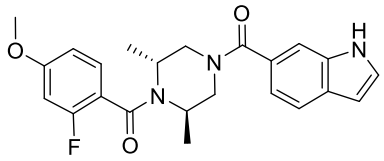
***N*-(4-Fluoro-3-((3*R*,5*R*)-4-(2-fluoro-4-methoxybenzoyl)-3,5-dimethylpiperazine-1-carbonyl)phenyl)acetamide 24**



The title compound was synthesized following the approach outlined in procedure **12** substituting 5-acetamido-2-fluorobenzoic acid for 2-fluoro-4-methoxybenzoic acid in step (c) to afford the title compound (41.1 mg, 0.088 mmol, 49%) as a white solid. ^{1}H-NMR (400 MHz, DMSO-d6, 350K)

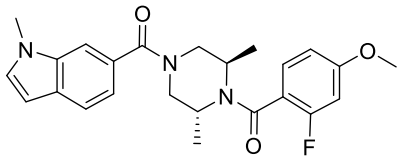
δ 9.86 (s, 1H), 7.66-7.61 (m, 2H), 7.32 (dd, 1H, *J* = 8.53 Hz), 7.24-7.19 (m, 1H), 6.88-6.84 (m, 2H), 4.25 (br s, 1H), 4.11 (br s, 1H), 3.95-3.87 (m, 1H), 3.83 (s, 3H), 3.75-3.62 (m, 2H), 3.27-3.23 (m, 1H), 2.05 (s, 3H), 1.25 (br s, 3H), 1.15 (br s, 3H). ^{1}H-NMR (400 MHz, DMSO-d6, 298K) δ 10.12 (s, 1H), 7.66-7.61 (m, 2H), 7.34 (dd, 1H, *J* = 8.34 Hz), 7.27 (dd, 1H, *J* = 8.97 Hz), 6.92 (dd, 1H, *J* = 11.73, 2.2 Hz), 6.85 (dd, 1H, *J* = 8.53, 2.26 Hz), 4.35-4.02 (br s, 1.5H), 3.91 (br d, 1.5H, *J* = 13.18 Hz), 3.80 (s, 3H), 3.77-3.69 (m, 2H), 3.24 (br d, 1H, *J* = 12.3 Hz), 2.04 (s, 3H), 1.22 (br s, 3H), 1.10 (br s, 3H). UPLC-MS: calculated for $C_{23}H_{26}F_2N_3O_4$ $[M+H]^+$ is 446.189, found 446.313 $[M+H]^+$.

((2R,6R)-4-(1H-indole-6-carbonyl)-2,6-dimethylpiperazin-1-yl)(2-fluoro-4-methoxyphenyl)methanone H3B-616 (25)



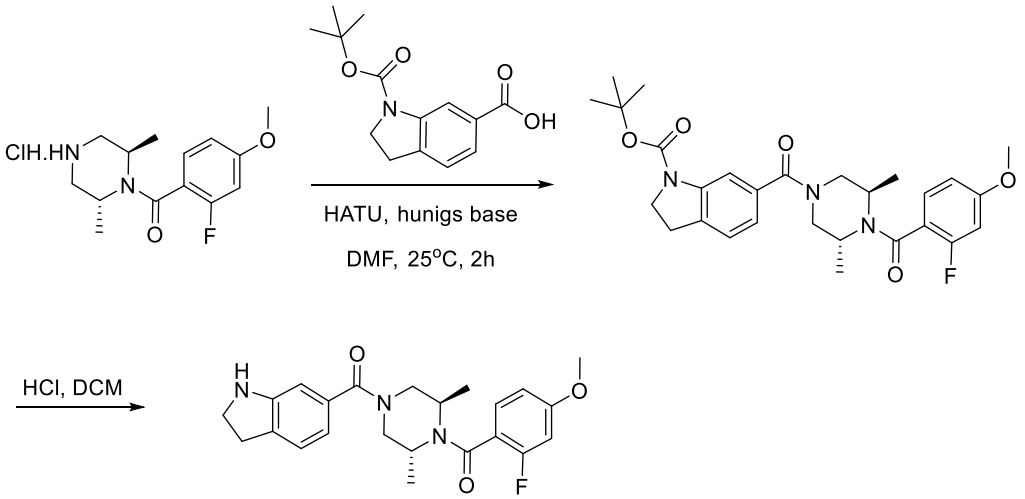
The title compound was synthesized following the approach outlined in procedure **12** substituting 1H-indole-6-carboxylic acid for 2-fluoro-4-methoxybenzoic acid in step (c) to afford the title compound (242 mg, 0.59 mmol, 89 %) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$, 298K) δ 8.67 (s, 1H), 7.58 (d, 1H), 7.53 (s, 1H), 7.23-7.22 (m, 1H), 7.19-7.16 (m, 1H), 7.13-7.09 (m, 1H), 6.68 (dd, 1H), 6.56 (dd, 1H), 6.50 (br s, 1H), 4.45-4.02 (br s, 1H), 3.96-3.85 (m, 2H), 3.83-3.74 (m, 2H), 3.75 (s, 3H), 3.55-3.43 (m, 1H), 1.33 (br s, 3H), 1.10 (br s, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 174.1, 166.4, 161.9, 160.0, 157.6, 135.4, 129.5, 128.1, 126.8, 125.3, 120.3, 118.6, 116.9, 111.4, 110.7, 55.72, 50.3, 46.3, 21.5. ^{13}C DEPT-NMR (101 MHz, $CDCl_3$) δ 129.3, 128.2, 126.8, 125.3, 111.5, 110.8, 102.3, 101.9, 101.7, 77.29, 50.3, 46.3, 21.5. UPLC-MS: calculated for $C_{23}H_{25}FN_3O_3$ $[M+H]^+$ is 410.180, found 410.190 $[M+H]^+$.

((2*R*,6*R*)-2,6-Dimethyl-4-(1-methyl-1H-indole-6-carbonyl)piperazin-1-yl)(2-fluoro-4-methoxyphenyl)methanone 27

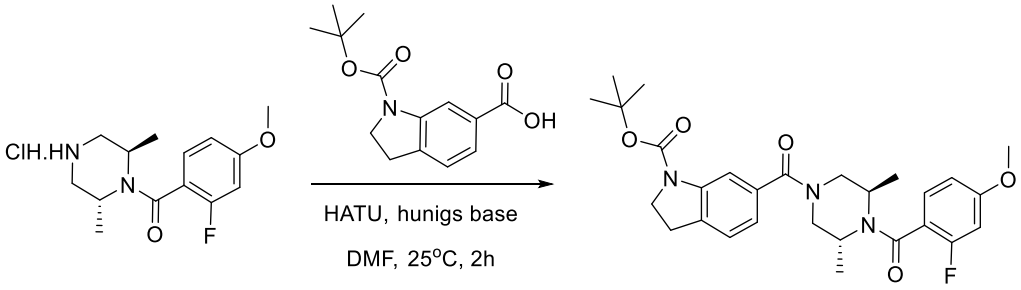


The title compound was synthesized following the approach outlined in procedure **12** substituting 1-methyl-1H-indole-6-carboxylic acid for 2-fluoro-4-methoxybenzoic acid in step (c) to afford the title compound (22.7 mg, 0.053 mmol, 81%) as a white solid. ^{1}H-NMR (400 MHz, DMSO-d6, 298K) δ 7.63 (s, 1H), 7.59 (d, 1H, *J* = 8.16 Hz), 7.46 (d, 1H, *J* = 3.14 Hz), 7.36-7.30 (m, 1H), 7.17 (dd, 1H, *J* = 8.09, 1.19 Hz), 6.95-6.90 (m, 1H), 6.87-6.85 (m, 1H), 6.48 (d, 1H, *J* = 2.89 Hz), 4.50-4.15 (br m, 1H), 4.08 (br d, 1H, *J* = 12.55 Hz), 3.91 (s, 1H), 3.84 (s, 3H), 3.80 (s, 3H), 3.82-3.78 (m, 1H), 3.73-3.65 (br m, 1H), 3.48-3.42 (m, 1H), 1.35 (br s, 3H), 1.04 (br s, 3H). ^{1}H-NMR (400 MHz, DMSO-d6, 350K) δ 7.61-7.57 (m, 2H), 7.41 (d, 1H, *J* = 3.01 Hz), 7.31 (dd, 1H, *J* = 8.47, 8.47 Hz), 7.14 (d, 1H, *J* = 8.16 Hz), 6.89-6.84 (m, 2H), 6.48 (d, 1H, *J* = 3.01 Hz), 4.22-4.14 (br s, 2H), 3.89 (s, 1H), 3.85-3.81 (m, 1H), 3.84 (s, 3H), 3.82 (s, 3H), 3.68-3.59 (m, 2H), 1.23 (br d, 6H). ^{19}F-NMR (377 MHz, DMSO-d6, 350K) δ -114.74. UPLC-MS: calculated for $C_{24}H_{27}FN_3O_3$ $[M+H]^+$ is 424.189, found 424.263 $[M+H]^+$.

((3*R*,5*R*)-4-(2-Fluoro-4-methoxybenzoyl)-3,5-dimethylpiperazin-1-yl)(indolin-6-yl)methanone 30

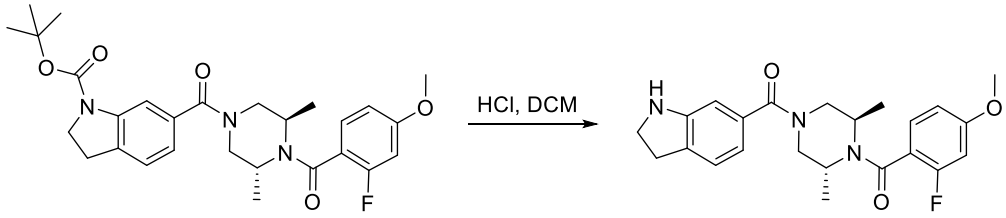


a. tert-butyl 6-[(3*R*,5*R*)-4-(2-fluoro-4-methoxybenzoyl)-3,5-dimethylpiperazine-1-carbonyl]-2,3-dihydroindole-1-carboxylate



Into a 8-mL vial, was placed (2*R*,6*R*)-1-(2-fluoro-4-methoxybenzoyl)-2,6-dimethylpiperazine (80 mg, 0.30 mmol), DMF (2.00 mL), DIEA (0.901 mmol) and 1-(tert-butoxycarbonyl)-2,3-dihydroindole-6-carboxylic acid (79.1 mg, 0.30 mmol,). After stirring at 25 °C for 5 mins, HATU (137 mg, 0.36 mmol) was added and the resulting solution stirred for 2 h at 25 °C. The reaction was then quenched with water (20 mL) and the resulting solution extracted with ethyl acetate (3 x 20 mL). The combined organic was concentrated and purified by column chromatographjy [Silica gel, ethyl acetate/petroleum ether (48%)] to afford the title compound (160 mg, 0.28 mmol, 95%) as a white solid. UPLC-MS: calculated for $C_{28}H_{35}FN_3O_5$ $[M+H]^+$ is 512.256, found 512.27 $[M+H]^+$.

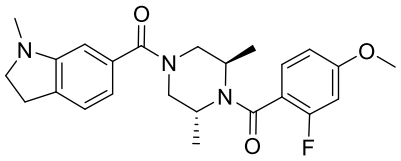
b. (3*R*,5*R*)-4-(2-Fluoro-4-methoxybenzoyl)-3,5-dimethylpiperazin-1-yl)(indolin-6-yl)methanone 30



Into a 50-mL round-bottom flask, was placed tert-butyl 6-[(3*R*,5*R*)-4-(2-fluoro-4-methoxybenzoyl)-3,5-dimethylpiperazine-1-carbonyl]-2,3-dihydroindole-1-carboxylate (130 mg, 0.254 mmol), DCM (1.00 mL), HCl (gas) in 1,4-dioxane (4.00 mL). The resulting solution was stirred for 1 h at 25 °C after which time the reaction mixture was concentrated. The crude material was purified by Prep-HPLC to afford the desired product (28.3 mg, 0.053 mmol, 26%) as a white solid. ^{1}H-NMR (400 MHz, DMSO-d6, 298K) δ 7.33 (dd, 1H, *J* = 8.34 Hz), 7.11 (d, 1H, *J* = 7.4 Hz), 6.92 (dd, 1H, *J* = 11.73, 1.95 Hz), 6.85 (dd, 1H, *J* = 8.53, 2.13 Hz), 6.69 (d, 1H, *J* = 7.65 Hz), 6.62 (s, 1H), 4.25-4.05 (m, 2H), 3.93-3.85 (m, 1H), 3.81 (s, 3H), 3.78-3.73 (m, 1H), 3.64-3.56 (m,

1H), 3.47 (t, 2H, *J* = 8.53 Hz), 3.41-3.35 (m, 2H), 2.96 (t, 2H, *J* = 8.41 Hz), 1.26 (br s, 3H), 1.04 (br s, 3H). ^{1}H-NMR (400 MHz, DMSO-d6, 298K) δ 7.3 (dd, 1H, *J* = 8.41 Hz), 7.09 (d, 1H, *J* = 7.4 Hz), 6.88-6.84 (m, 2H), 6.63 (d, 1H, *J* = 7.4 Hz), 6.58 (d, 1H, *J* = 1.0 Hz), 4.21-4.13 (m, 2H), 3.83 (s, 3H), 3.75-3.68 (m, 2H), 3.64-3.55 (m, 2H), 3.49 (t, 3H, *J* = 8.53 Hz), 2.97 (t, 2H, *J* = 8.34 Hz), 1.19 (br d, 6H, *J* = 6.27 Hz). ^{19}F-NMR (377 MHz, DMSO-d6, 350K) δ -114.77. UPLC-MS: calculated for $C_{23}H_{27}FN_3O_3$ $[M+H]^+$ is 412.204, found 412.212 $[M+H]^+$.

((2*R*,6*R*)-2,6-Dimethyl-4-(1-methylindoline-6-carbonyl)piperazin-1-yl)(2-fluoro-4-methoxyphenyl)methanone 31

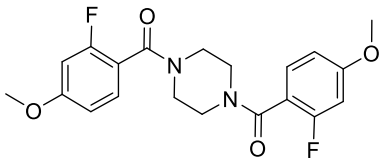


The title compound was synthesized following the approach outlined in procedure **12** substituting 1-methylindoline-6-carboxylic acid for 2-fluoro-4-methoxybenzoic acid in step (c) to afford the title compound (23.9 mg, 0.056 mmol, 85%) as a white solid. ^{1}H-NMR (400 MHz, DMSO-d6, 350K) δ 7.31 (dd, 1H, *J* = 8.47 Hz), 7.07 (d, 1H, *J* = 7.03 Hz), 6.88-6.84 (m, 2H), 6.64 (dd, 1H, *J* = 7.34, 1.44 Hz), 6.47 (d, 1H, *J* = 1.25 Hz), 4.18-4.12 (br s, 2H), 3.83 (s, 3H), 3.79-3.72 (m, 2H), 3.64-3.32 (br m, 2H), 3.35-3.31 (m, 2H), 2.94-2.90 (m, 2H), 2.74 (s, 3H), 1.25-1.15 (br m, 6H). ^{19}F-NMR (377 MHz, DMSO-d6, 350K) δ -114.74. UPLC-MS: calculated for $C_{24}H_{29}FN_3O_3$ $[M+H]^+$ is 426.219, found 426.374M+H]$^+$.

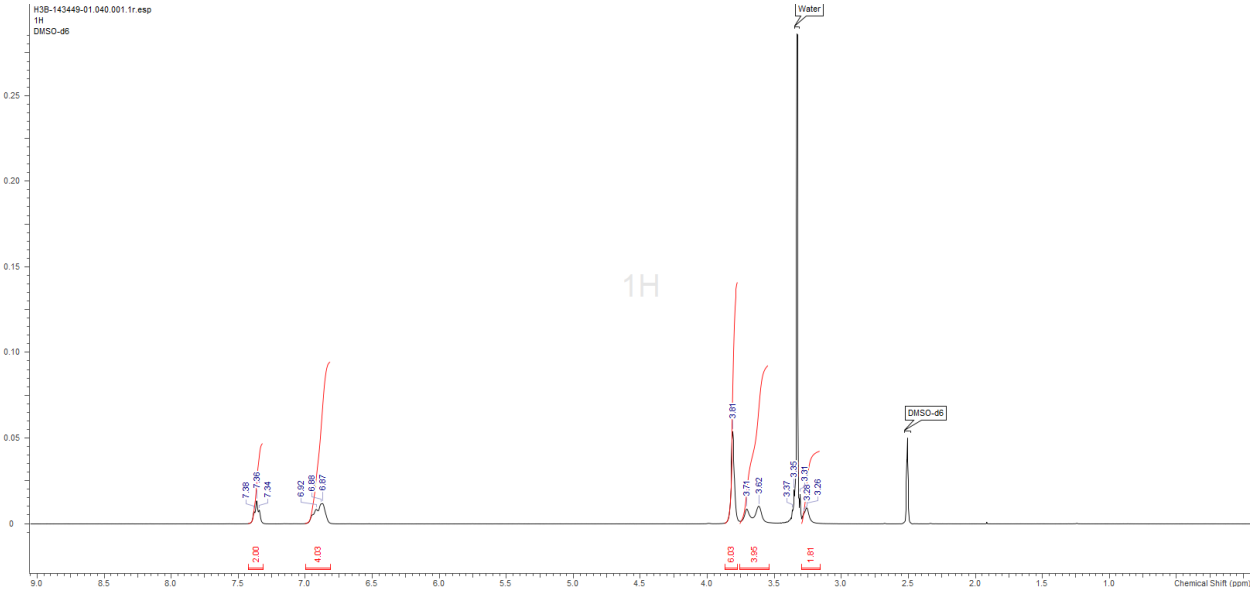
Compounds **4**-**10** were synthesized using the procedure outlined for the synthesis of **2.** Compounds **14**-**17**, **26**, **28**, **29** were synthesized using the procedure outlined for the synthesis of **12**. Compounds **19**-**20**, **22**-**23**, were synthesized using the procedure outlined for the synthesis of **21**. Compound **3** was synthesized starting from commercial (2-Fluoro-4-methoxyphenyl)-4-piperidinylmethanone [1154221-31-4] followed by amide coupling as described in the synthesis of **12,** step (c).

2. NMR spectra

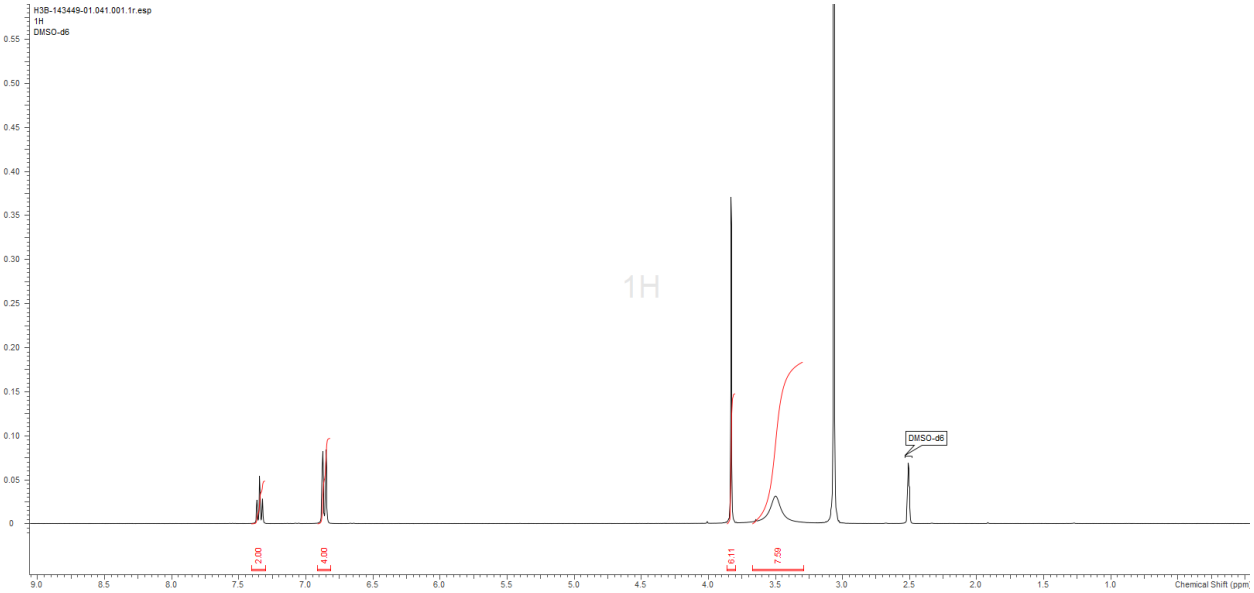
Piperazine-1,4-diylbis((2-fluoro-4-methoxyphenyl)methanone) **2**



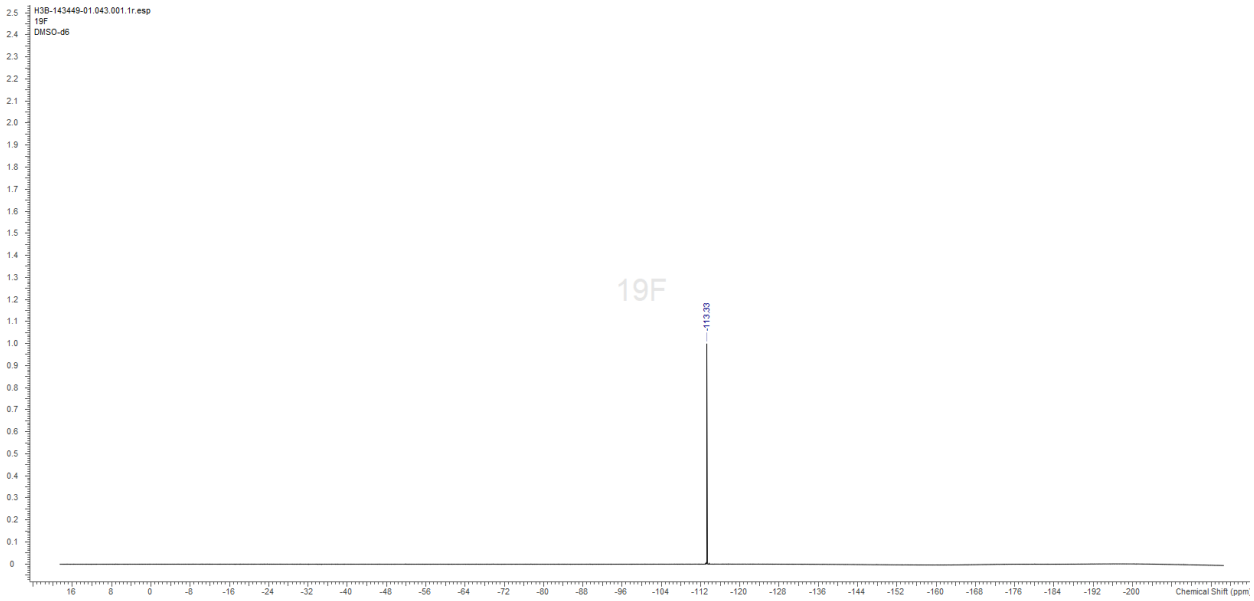
^{1}H NMR 298K



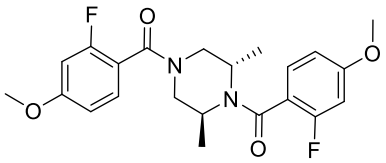
^{1}H NMR 350K



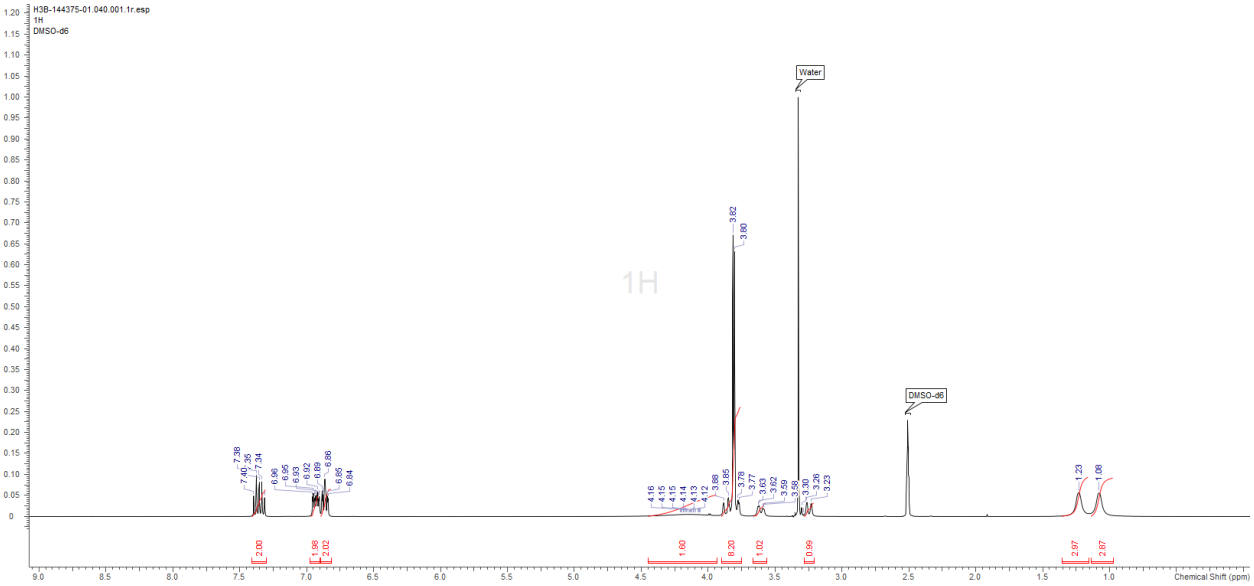
^{19}F NMR 350K



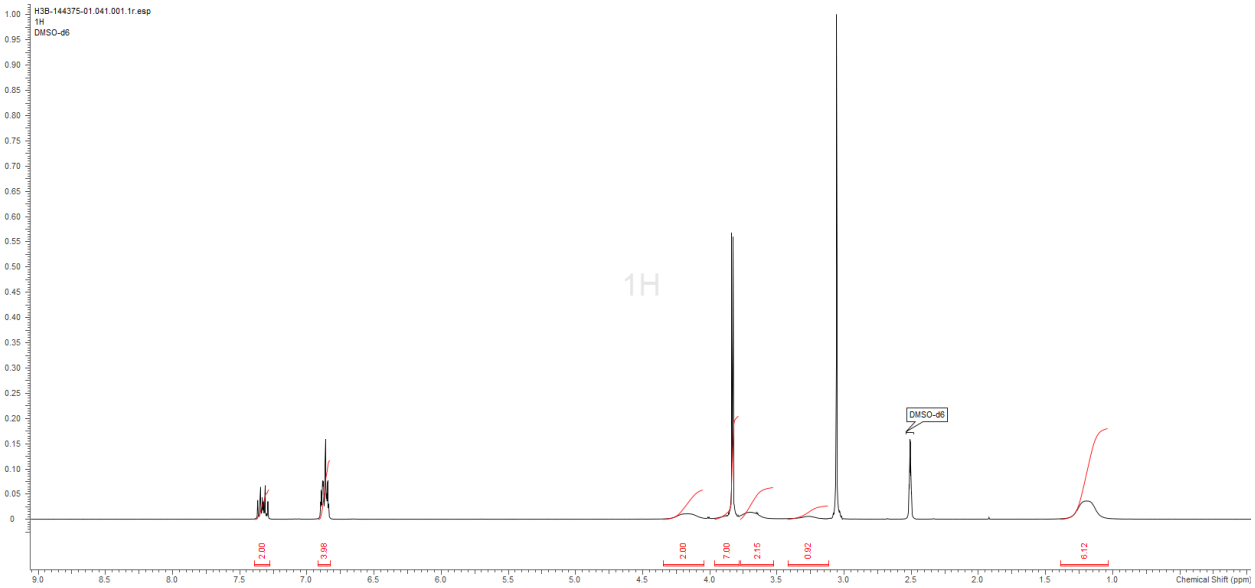
((2*S*,6*S*)-2,6-Dimethylpiperazine-1,4-diyl)bis((2-fluoro-4-methoxyphenyl)methanone) **11**



^{1}H NMR 298K



^{1}H NMR 350K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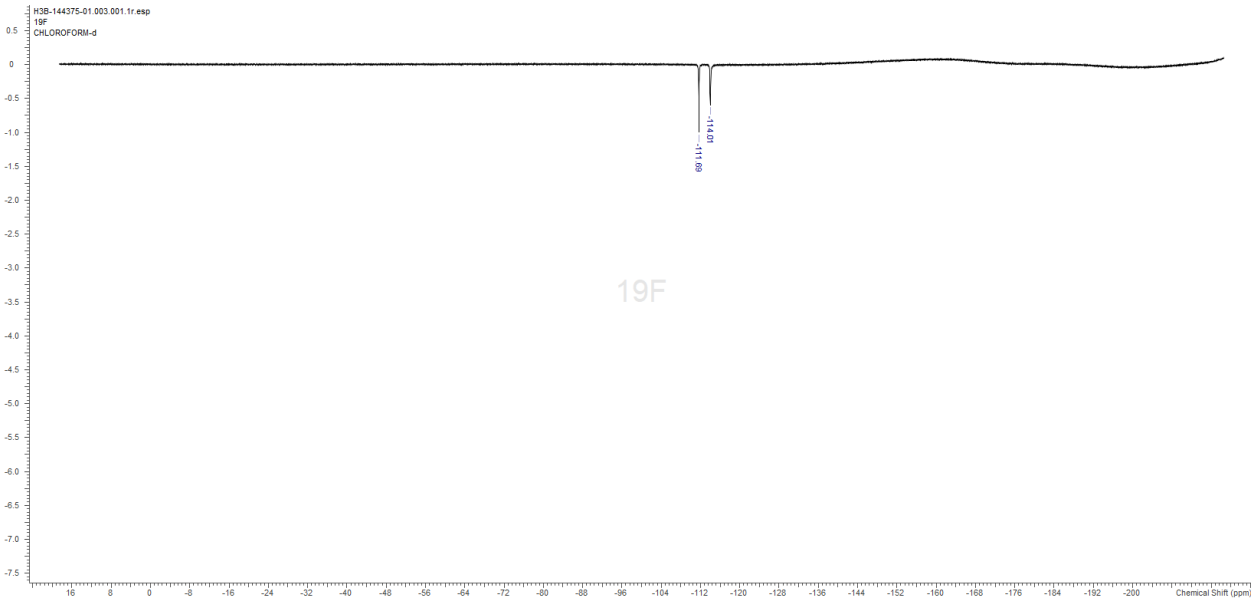
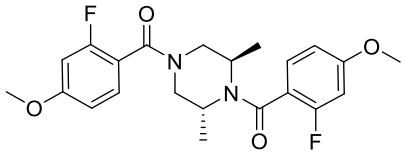
1H
DMSO-d6
1H
DMSO-d6
2.00
3.98
2.00
7.00
2.15
0.92
6.12
Chemical Shift (ppm)

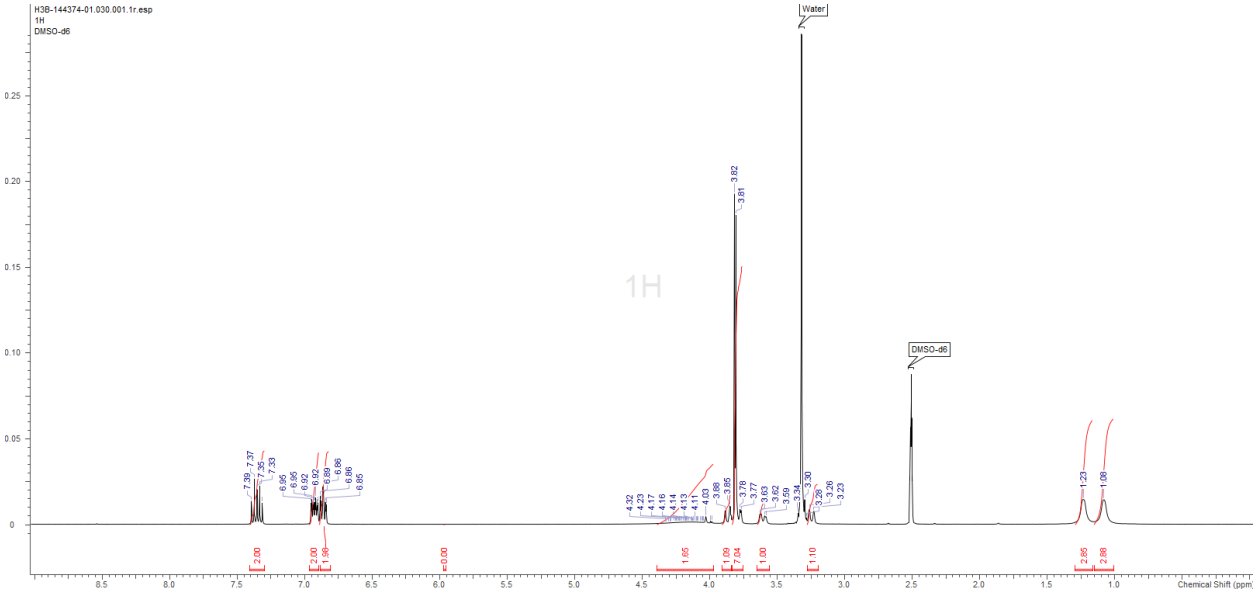
^{19}F NMR 298k


H3B-144375-01.003.001.1r.esp
19F
CHLOROFORM-d
-114.01
-111.69
19F
Chemical Shift (ppm)

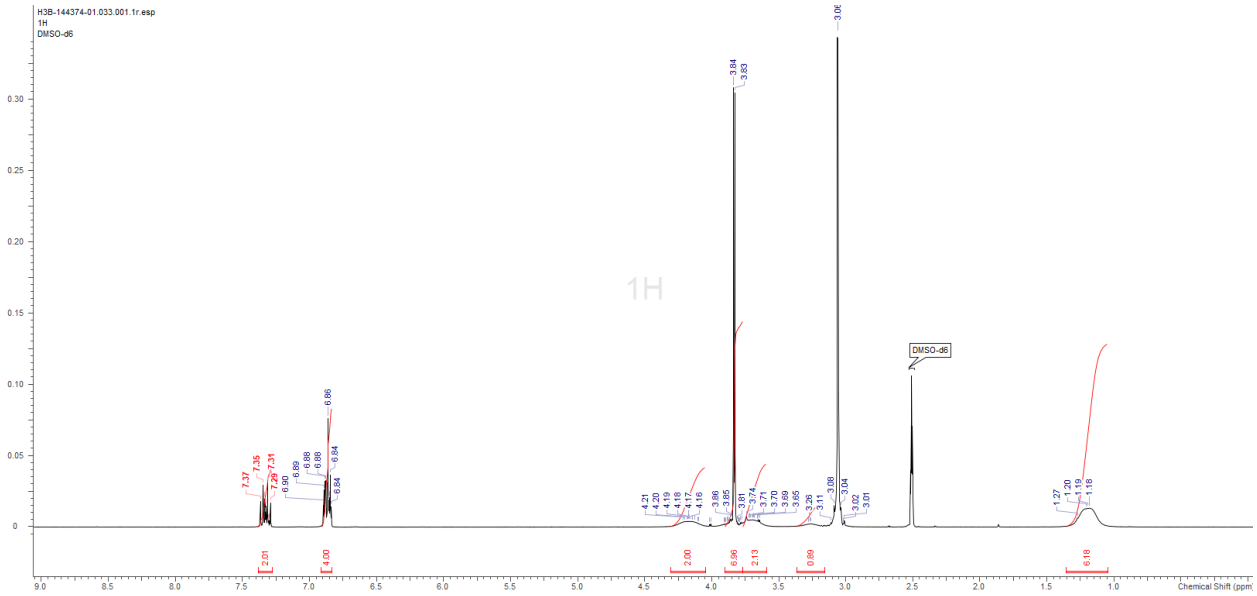
((2*R*,6*R*)-2,6-dimethylpiperazine-1,4-diyl)bis((2-fluoro-4-methoxyphenyl)methanone) **12**



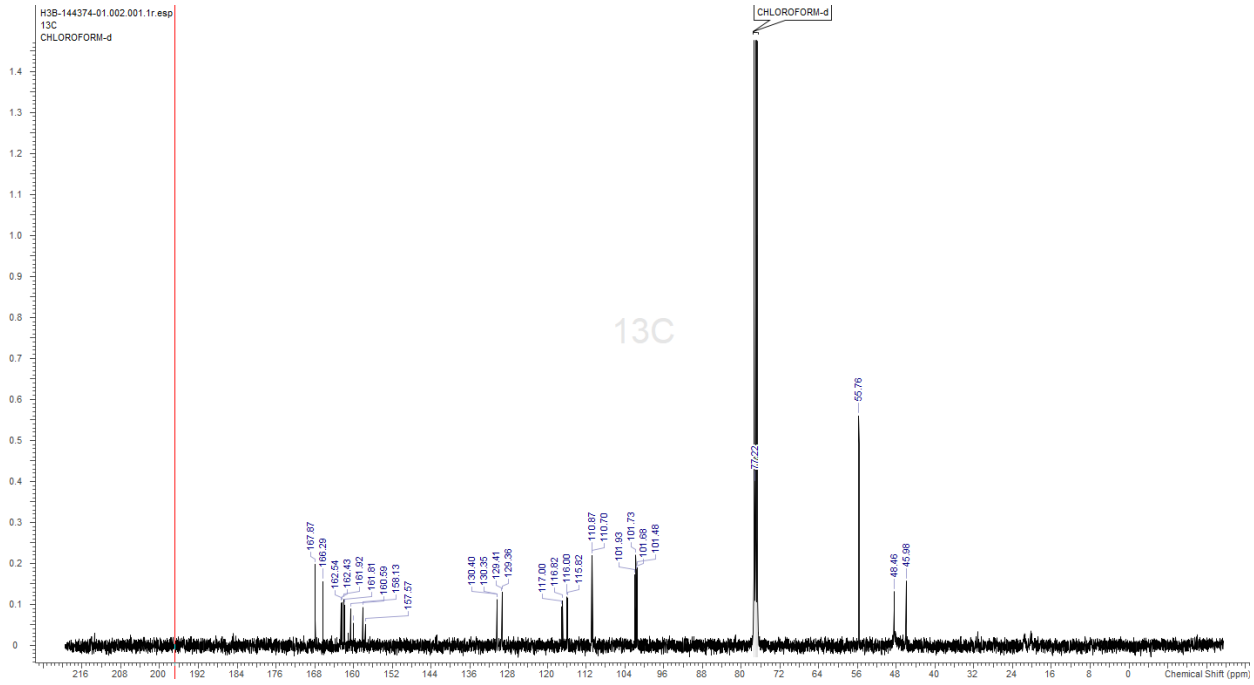
^{1}H NMR 298K



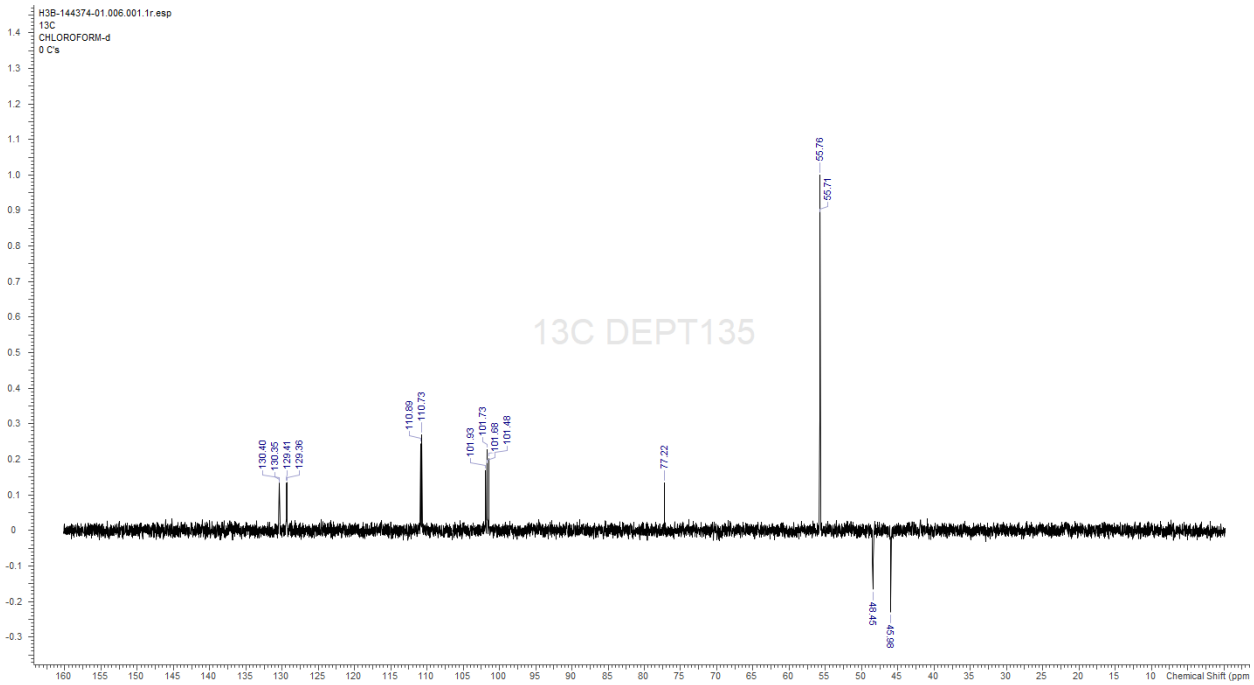
^{1}H NMR 350K



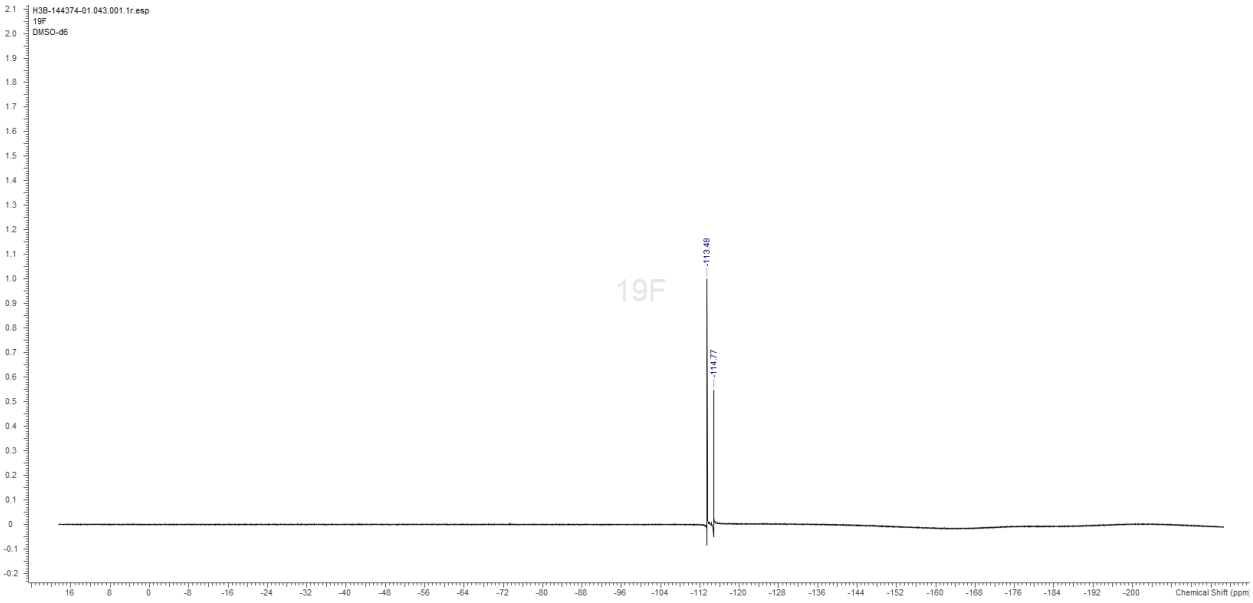
^{13}C NMR 298K



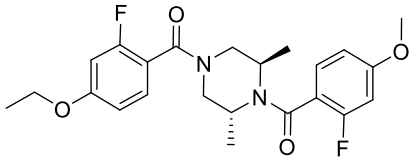
^{13}C DEPT NMR 298K



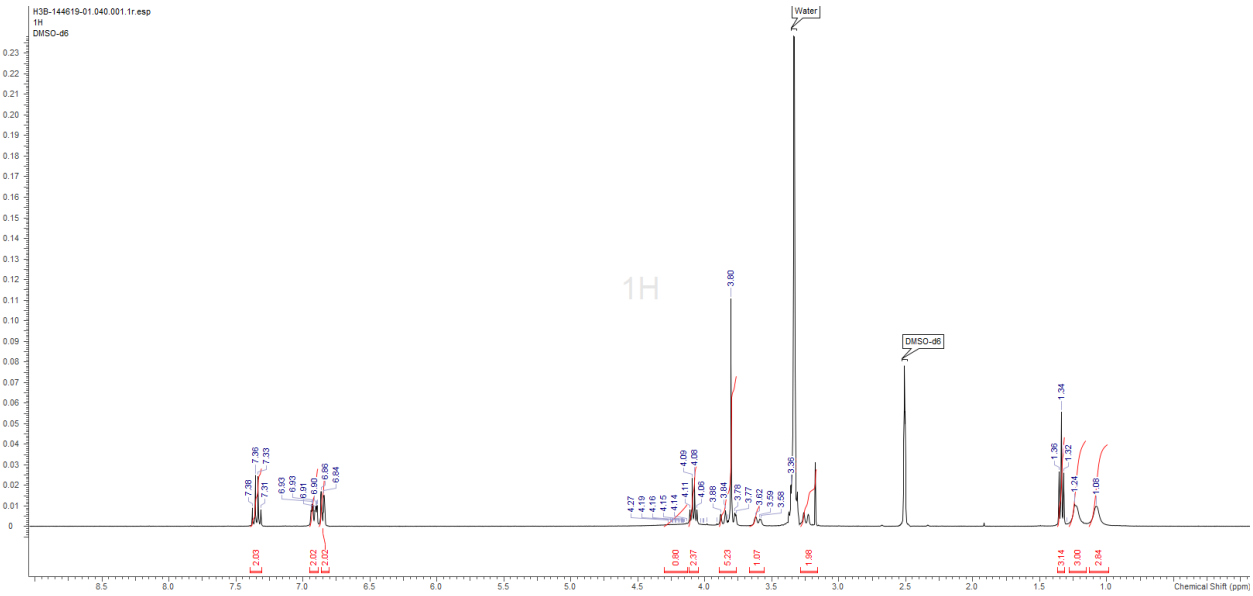
^{19}F NMR 350K



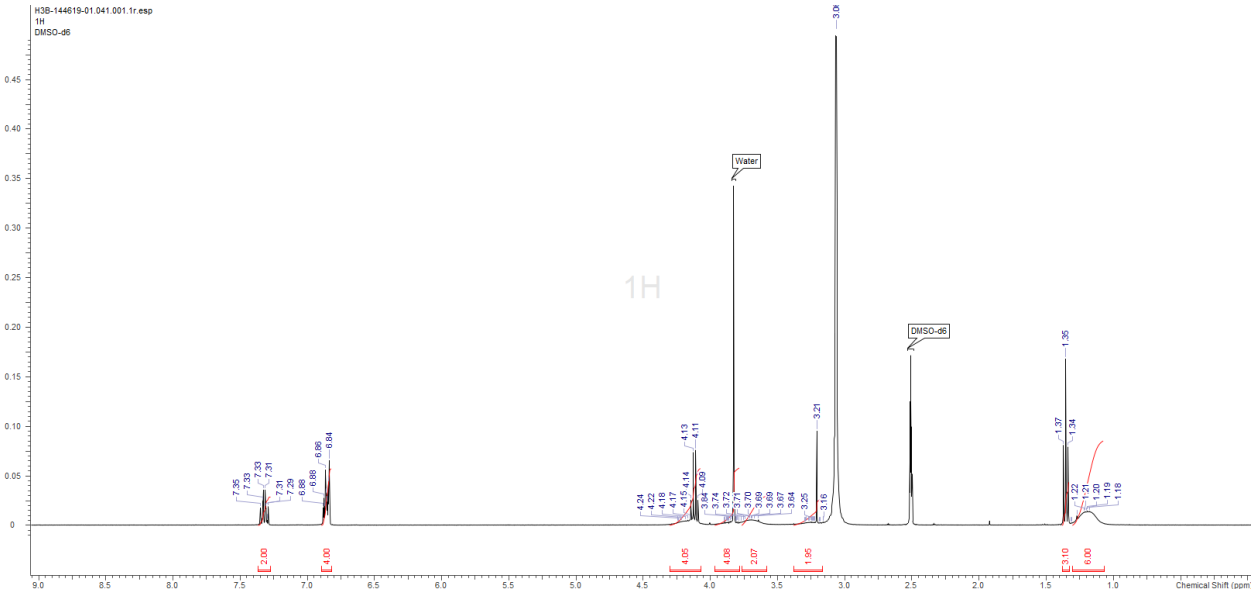
((2*R*,6*R*)-4-(4-Ethoxy-2-fluorobenzoyl)-2,6-dimethylpiperazin-1-yl)(2-fluoro-4-methoxyphenyl)methanone **13**



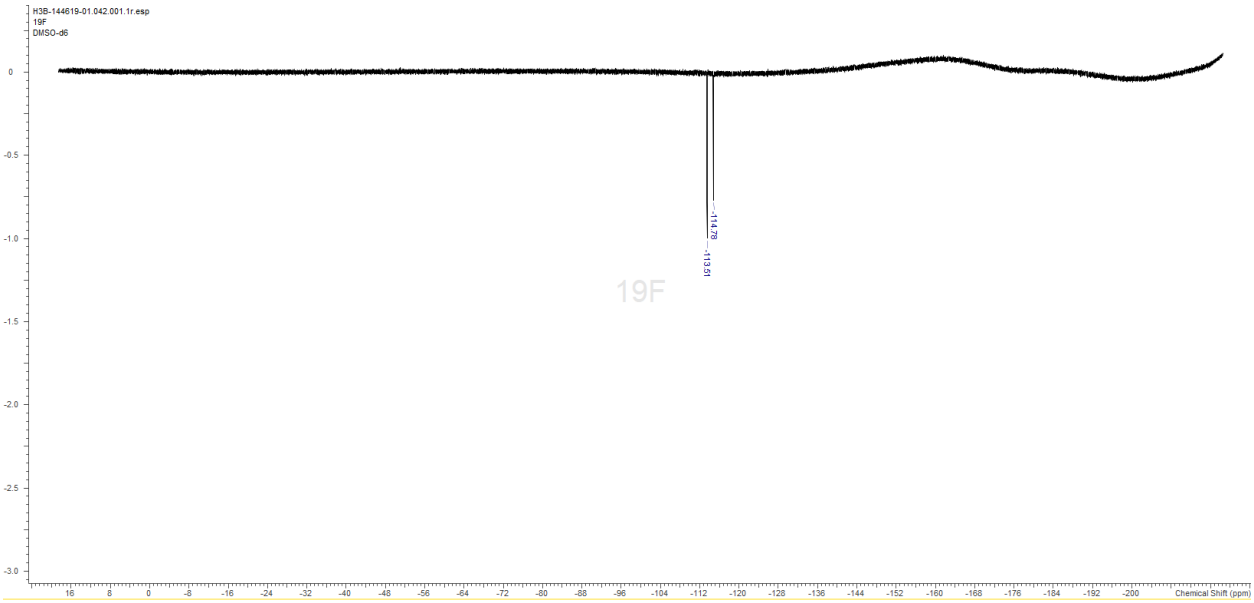
^{1}H NMR 298K



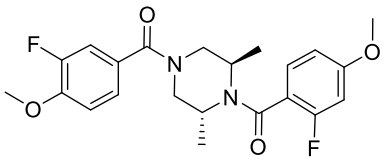
^{1}H NMR 350K



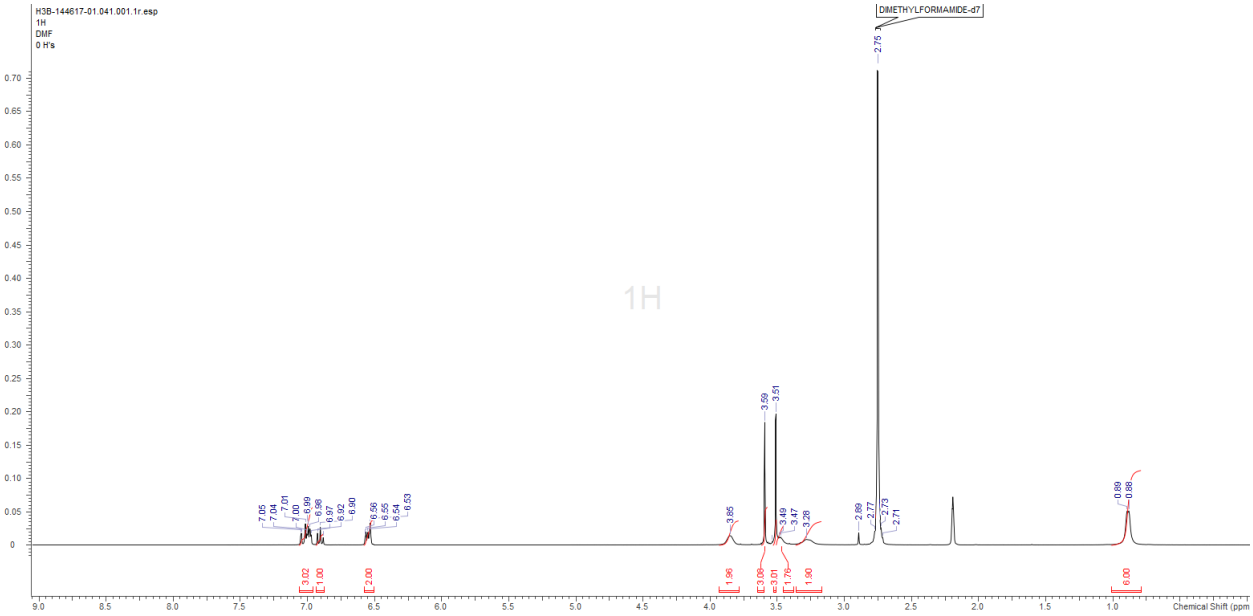
^{19}F NMR 350K



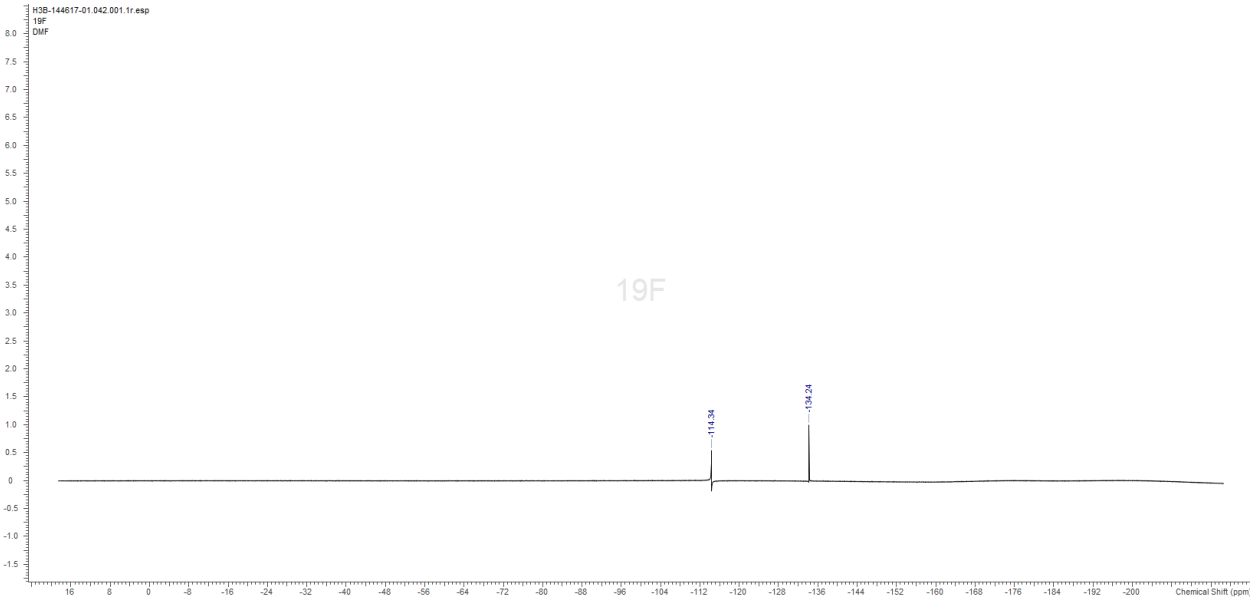
((2*R*,6*R*)-4-(3-Fluoro-4-methoxybenzoyl)-2,6-dimethylpiperazin-1-yl)(2-fluoro-4-methoxyphenyl)methanone **18**



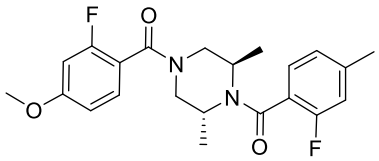
^{1}H NMR 350K



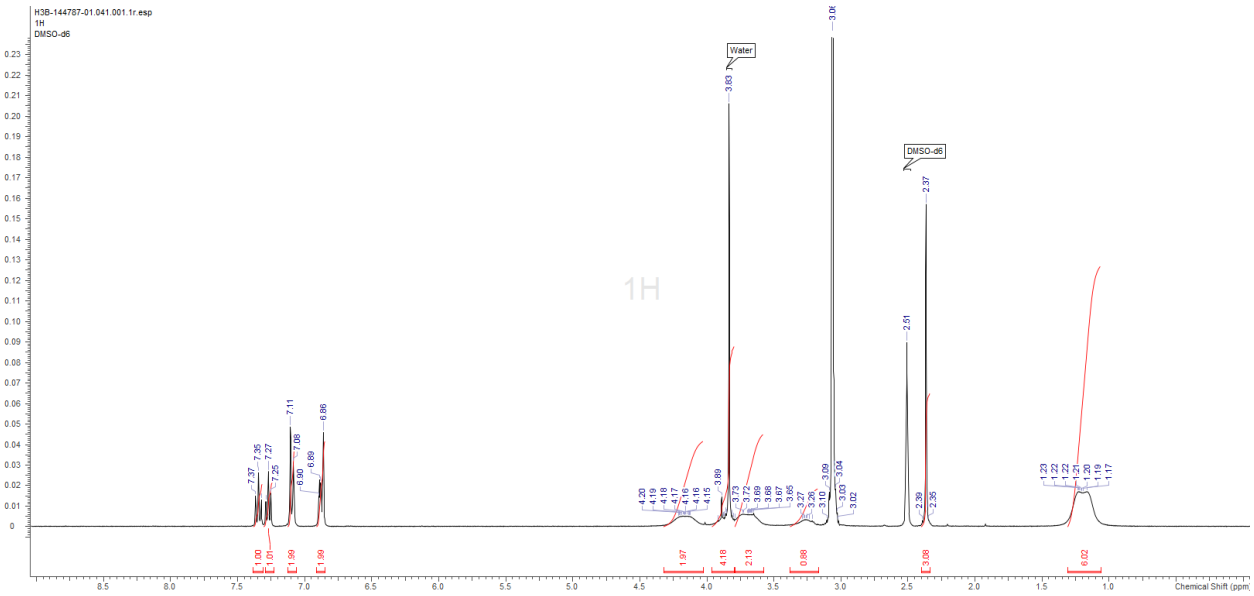
^{19}F NMR 350K



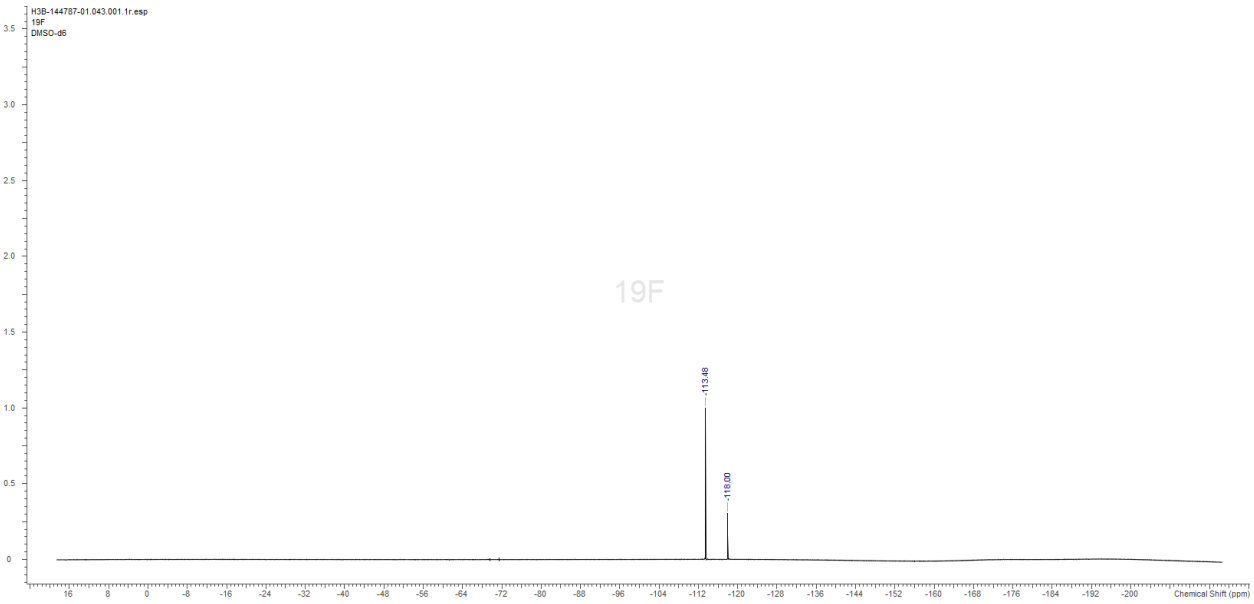
((2*R*,6*R*)-4-(2-Fluoro-4-methoxybenzoyl)-2,6-dimethylpiperazin-1-yl)(2-fluoro-4-methylphenyl)methanone **21**



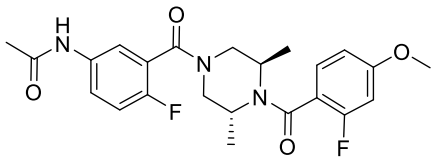
^{1}H NMR 350K



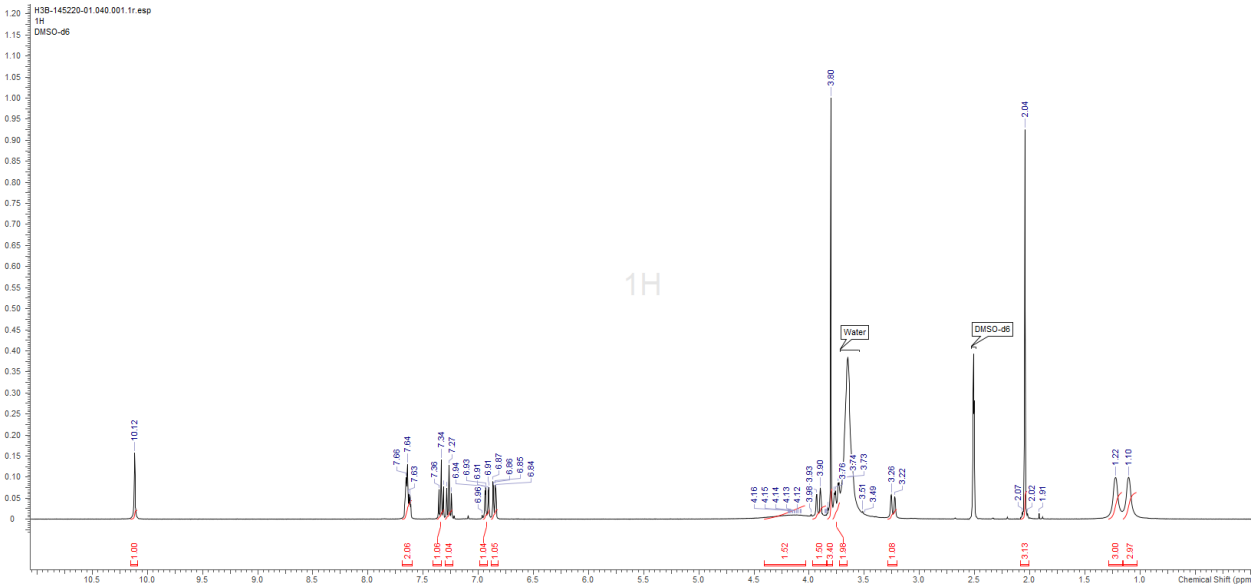
^{19}F NMR 350K



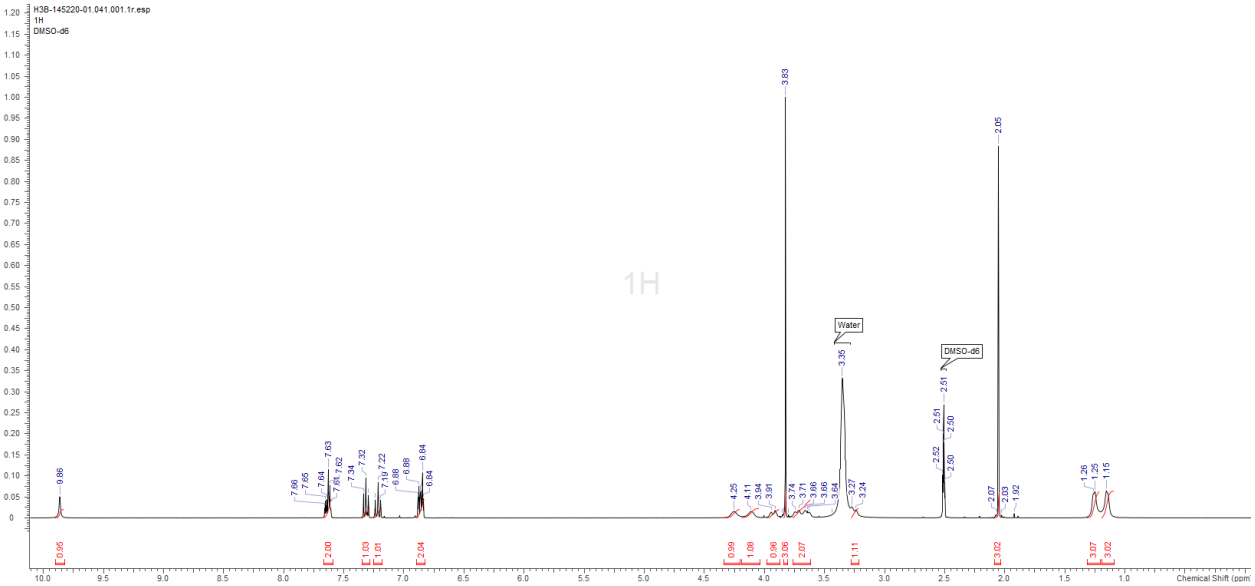
N-(4-Fluoro-3-((3*R*,5*R*)-4-(2-fluoro-4-methoxybenzoyl)-3,5-dimethylpiperazine-1-carbonyl)phenyl)acetamide **24**



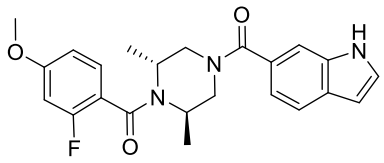
^{1}H NMR 298K



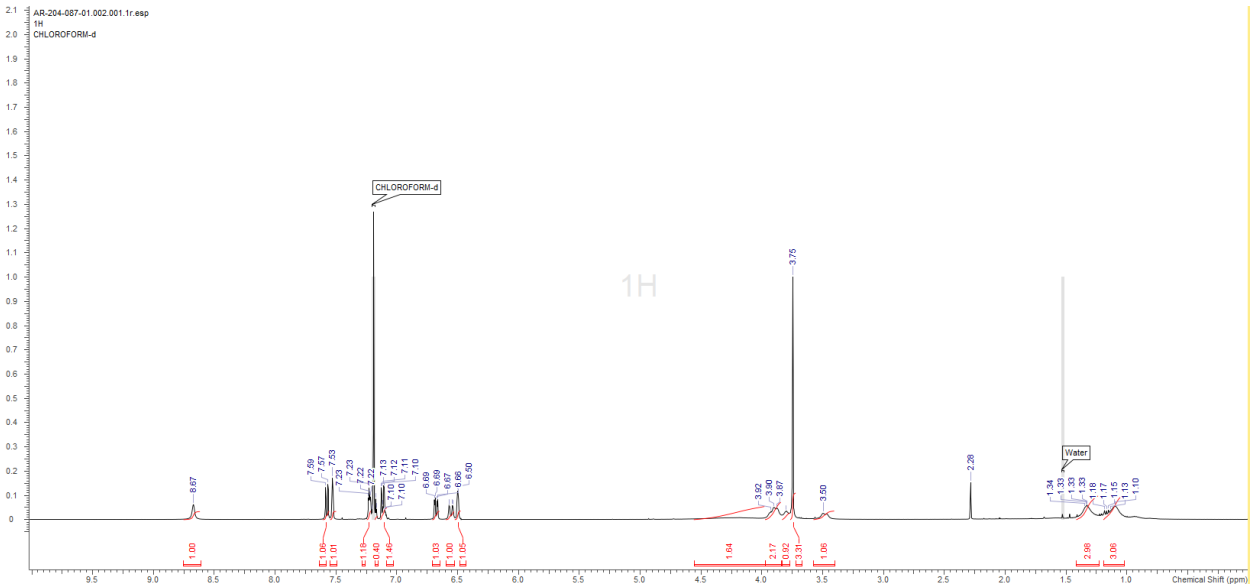
^{1}H NMR 350K



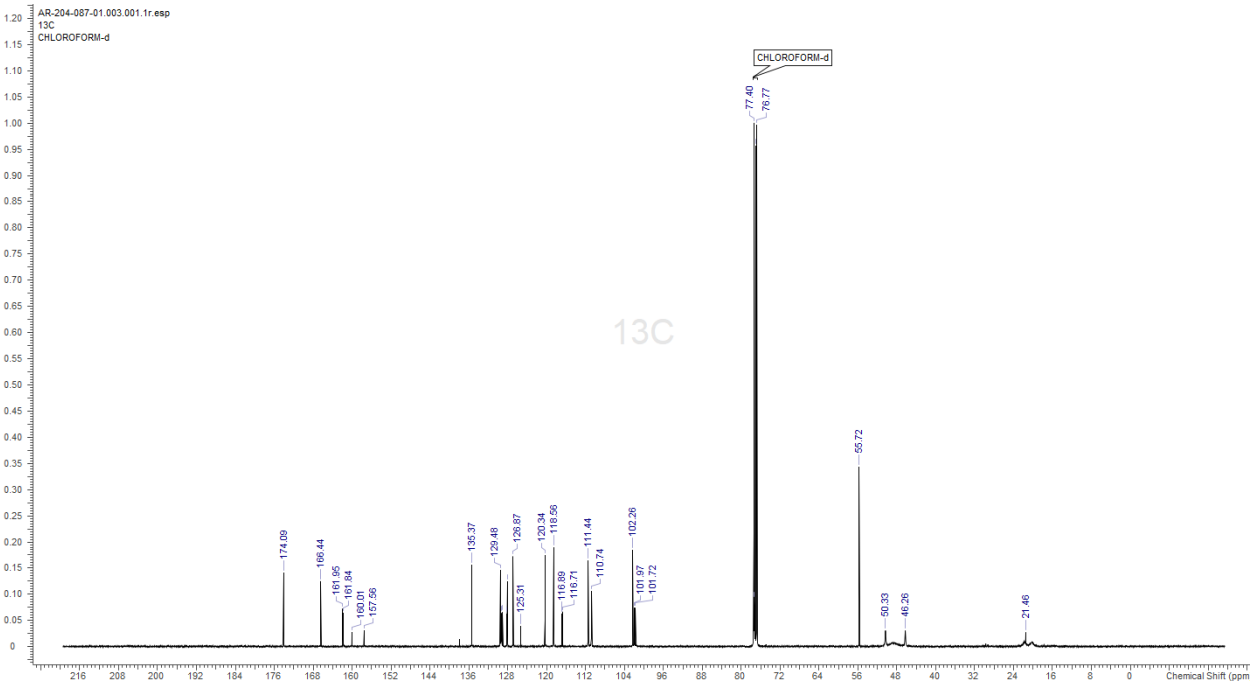
((2*R*,6*R*)-4-(1H-indole-6-carbonyl)-2,6-dimethylpiperazin-1-yl)(2-fluoro-4-methoxyphenyl)methanone **25**



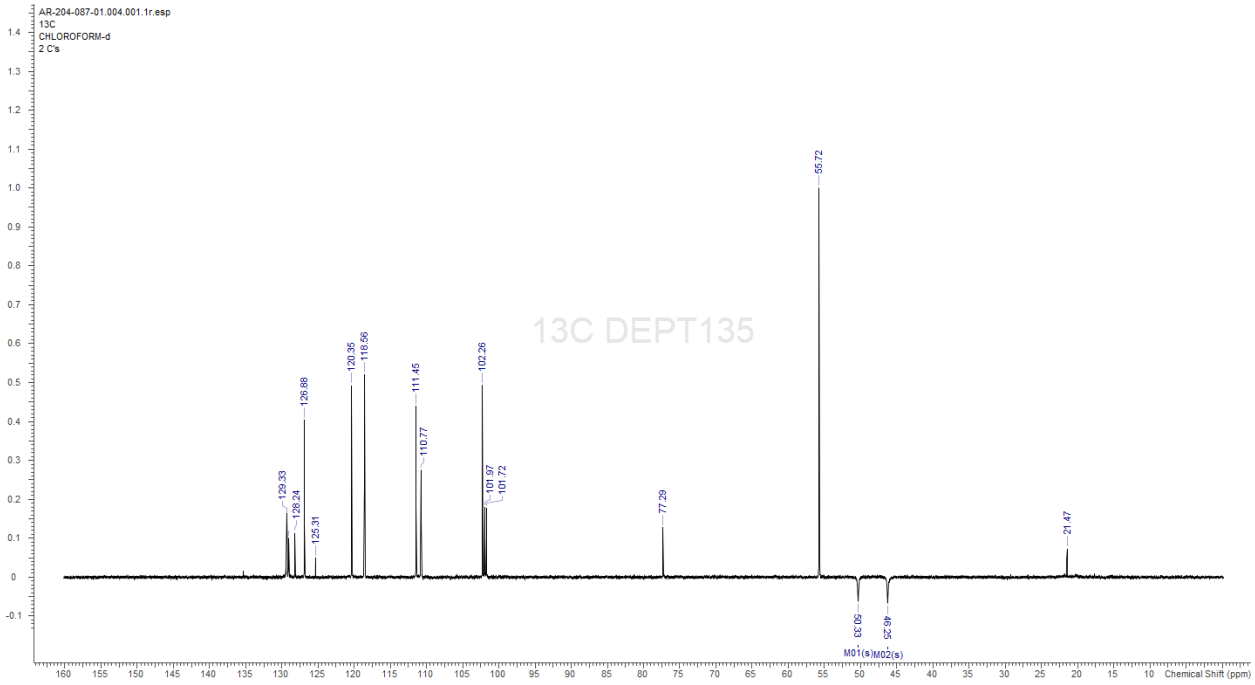
^{1}H NMR 298K



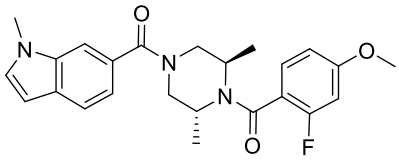
^{13}C NMR 298K



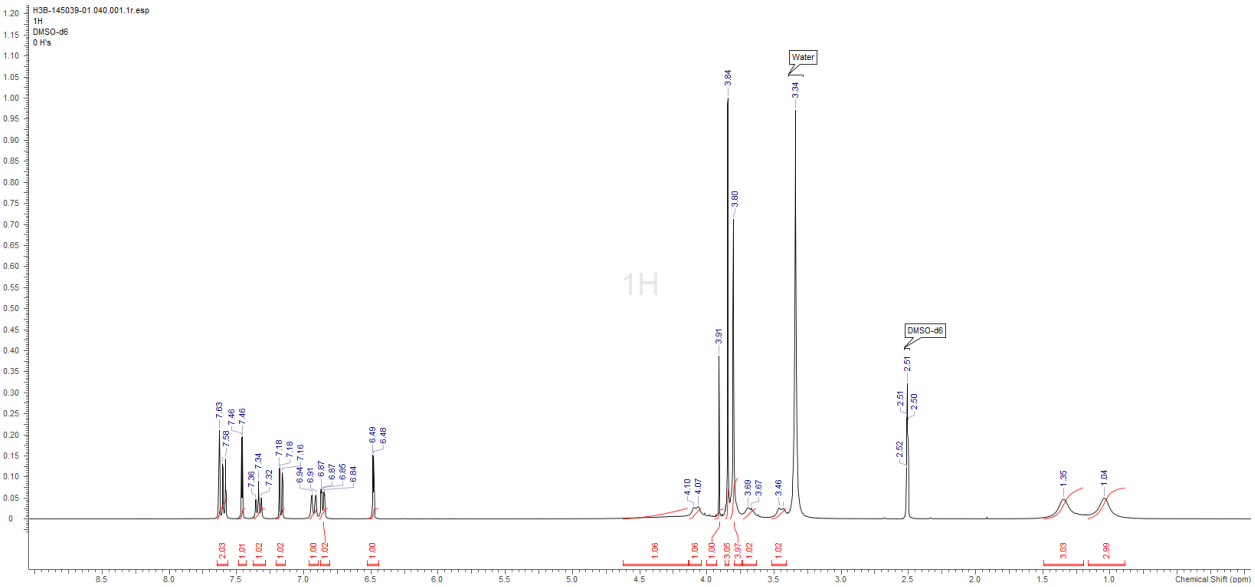
^{13}C DEPT NMR 298K



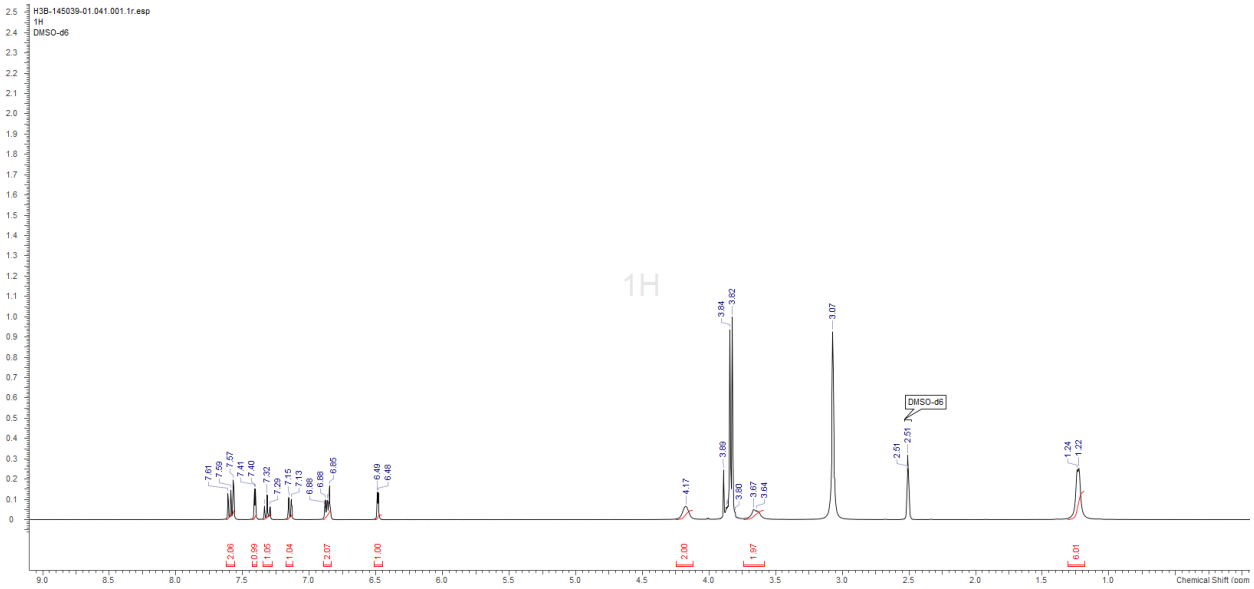
((2*R*,6*R*)-2,6-Dimethyl-4-(1-methyl-1H-indole-6-carbonyl)piperazin-1-yl)(2-fluoro-4-methoxyphenyl)methanone **27**



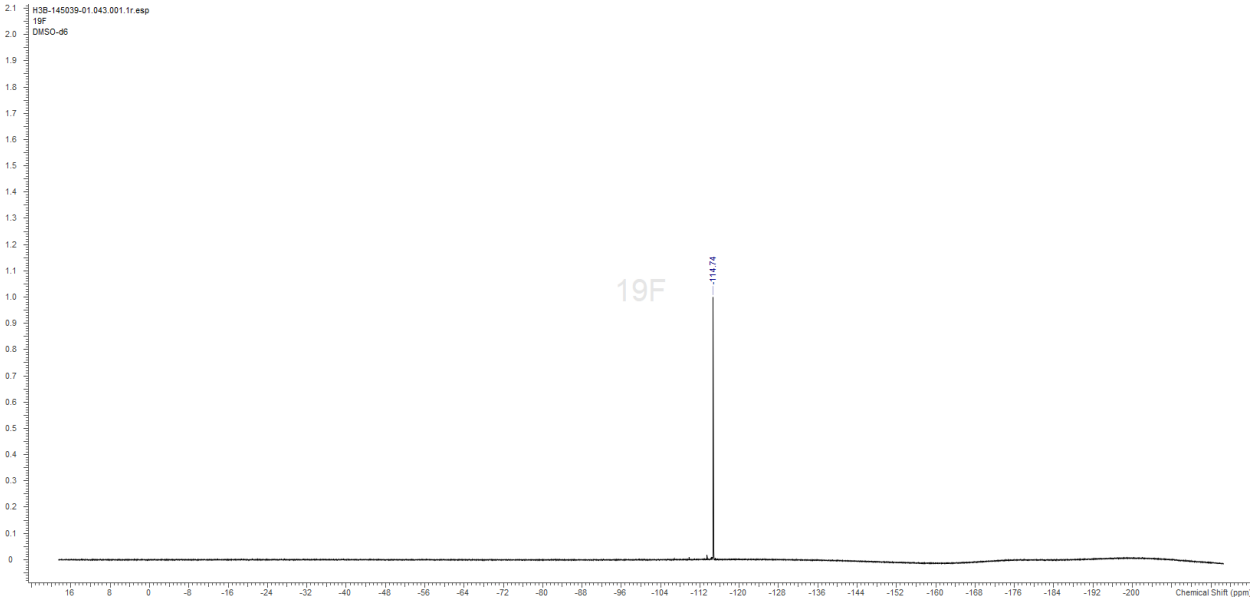
^{1}H NMR 298K



^{1}H NMR 350K

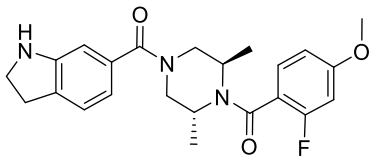


^{19}F NMR 350K

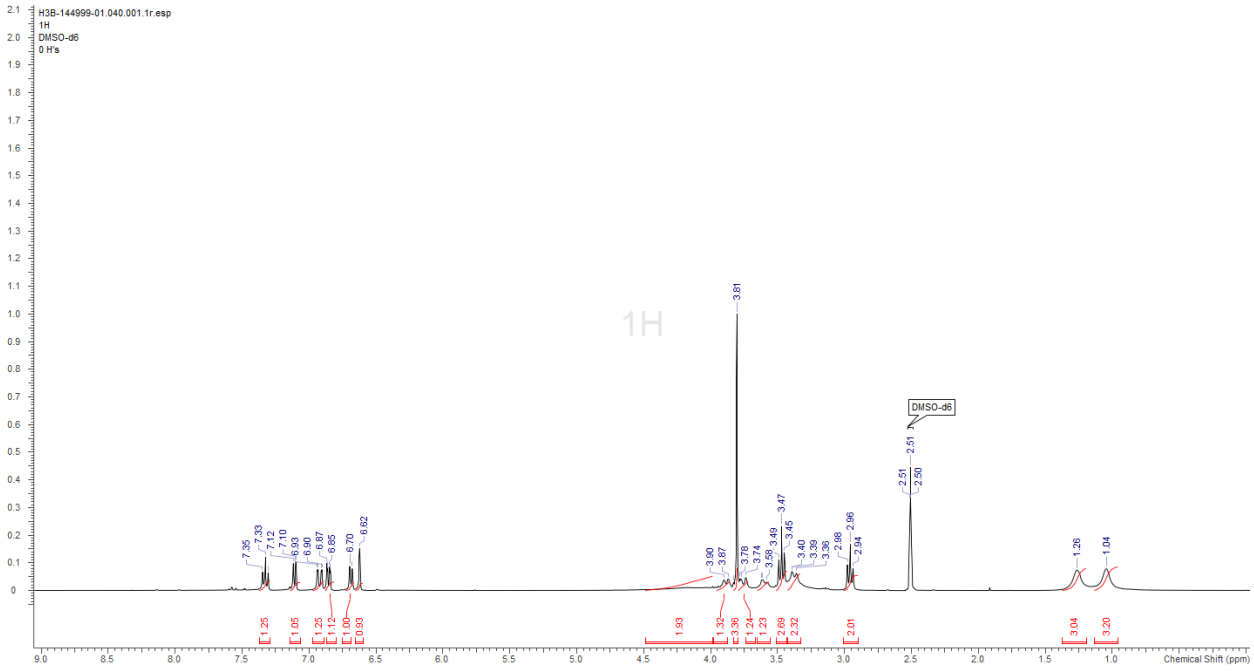


((3*R*,5*R*)-4-(2-Fluoro-4-methoxybenzoyl)-3,5-dimethylpiperazin-1-yl)(indolin-6-yl)methanone

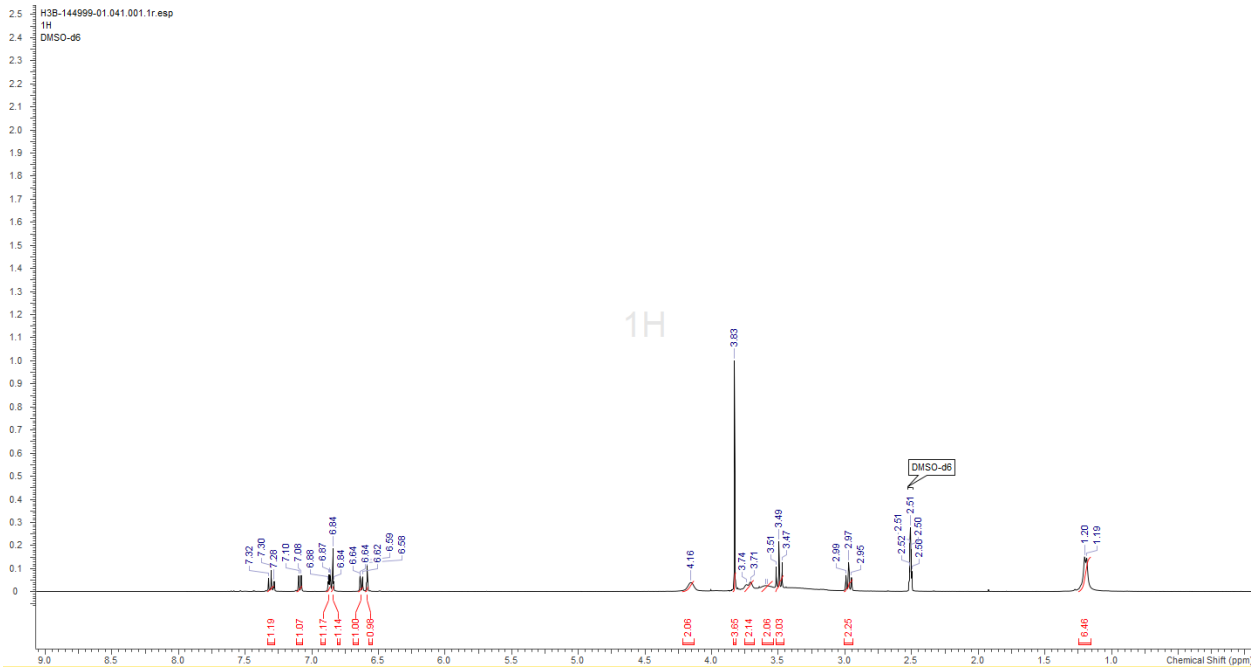
30



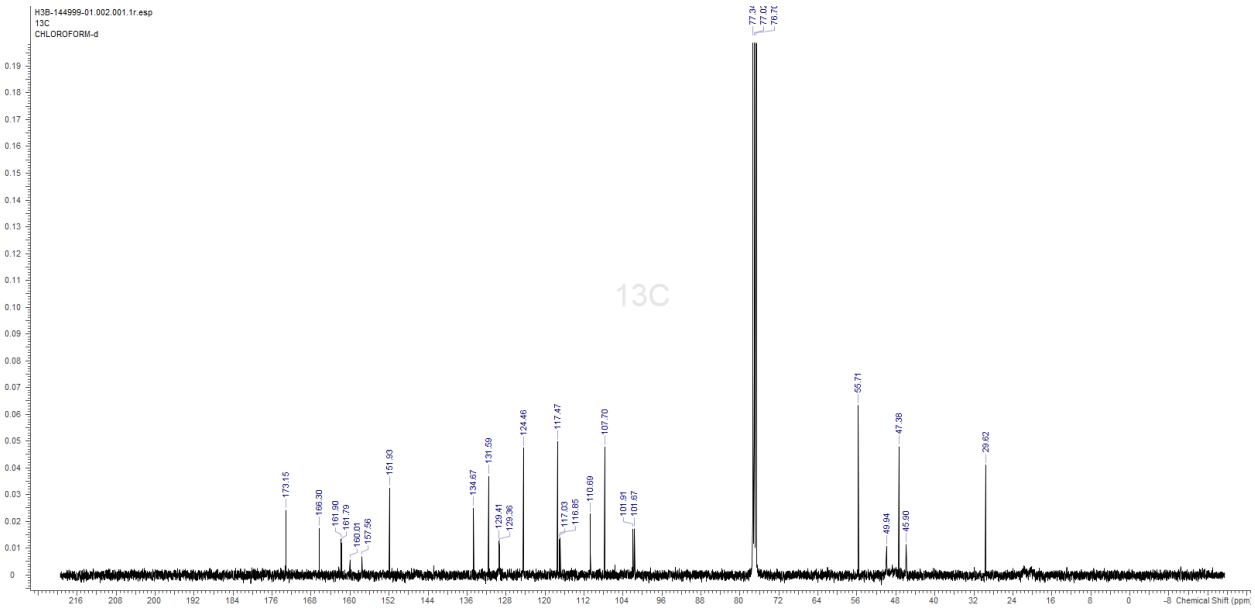
^{1}H NMR (298K)



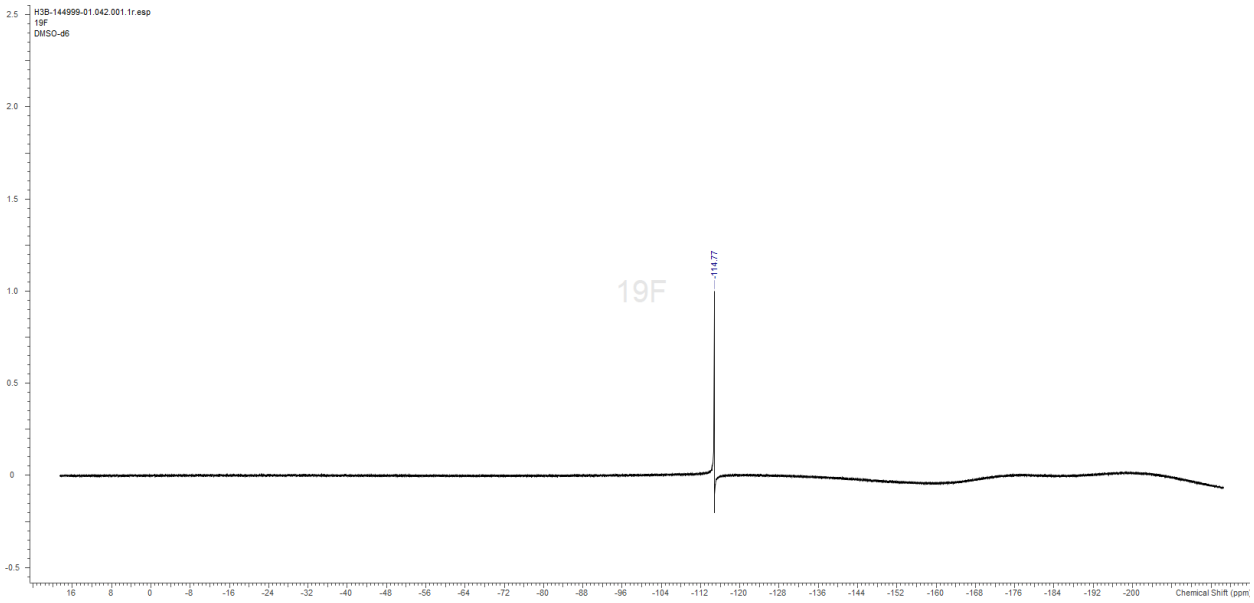
^{1}H NMR (350 K)



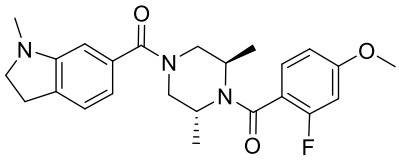
^{13}C NMR (298K)



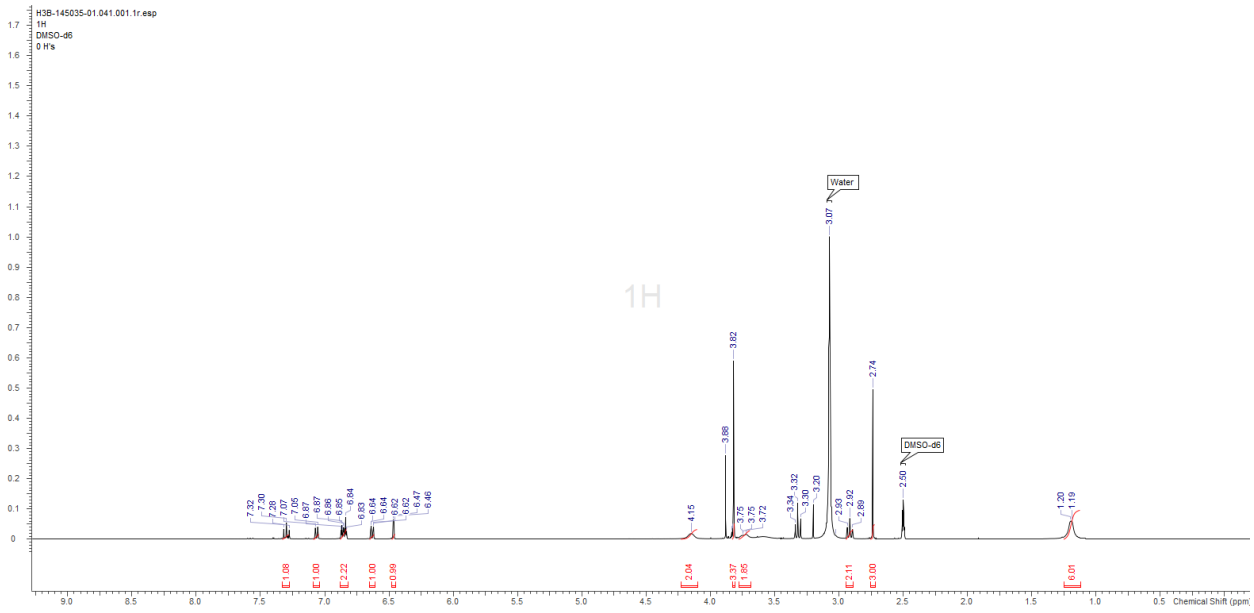
^{19}F NMR (350K)



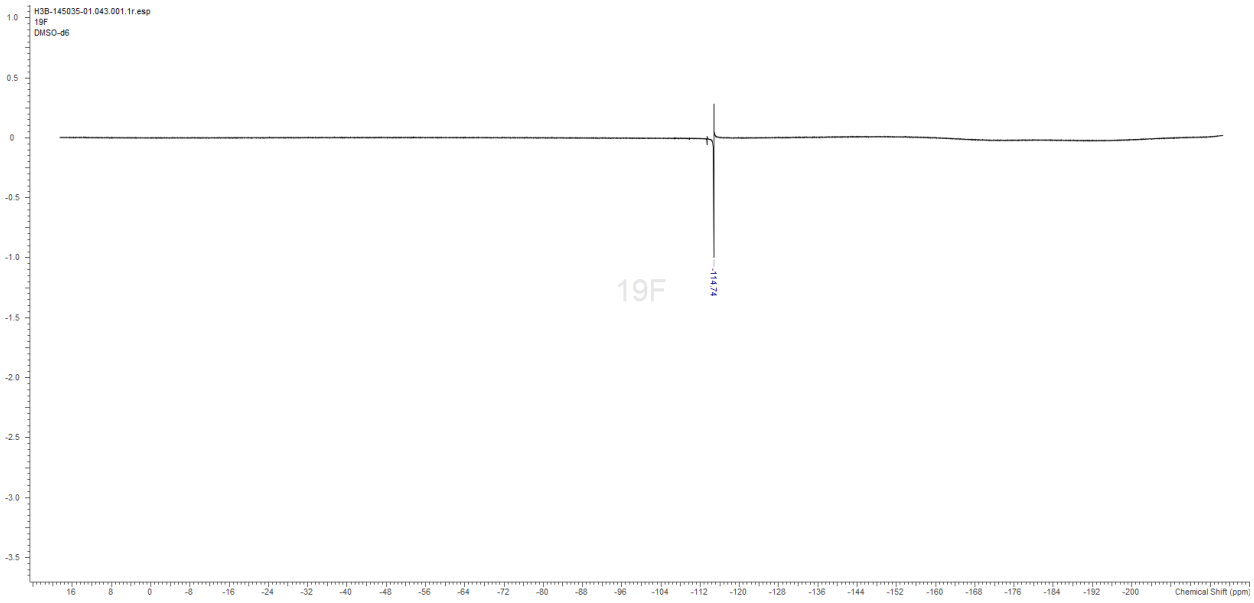
((2*R*,6*R*)-2,6-Dimethyl-4-(1-methylindoline-6-carbonyl)piperazin-1-yl)(2-fluoro-4-methoxyphenyl)methanone **31**



^{1}H 350K



^{19}F 350K


H3B-145035-01.043.001.1r.esp
19F
DMSO-d6
-114.74
19F
Chemical Shift (ppm)

3. X-ray structure-determination statistics

Supplementary table 1 Data collection and refinement statistics

	CPS1 + H3B-374
Data collection	
Space group	P1
Cell dimension	
a, b, c (Å)	71.7, 98.5, 142.5
α, β, γ (°)	102.1, 97.9, 106.1
Wavelength (Å)	0.9201
Resolution (Å)	59.0-2.62 (2.69-2.62)
R_{means} (%)	7.2 (52.3)
$I/\sigma(I)$	9.4 (1.7)
Completeness (%)	96.6 (96.3)
Redundancy	2.1 (2.1)
Refinement	
Resolution (Å)	59.0-2.62 (2.69-2.62)
No. reflections	98,950 (7,326)
R_{work}/R_{free} (%)	19.0/25.0
No. mol / AU	2
No. atoms	
Protein	21555
Ligand	59
Water	136
Zn^{2+}	2
B factors	
Protein	61.8
Ligand	44.0
Water	45.0
R.M.S. deviations	
Bond lengths (Å)	0.01
Bond angles (°)	1.56
Ramachandran	
Preferred	93.9%
Allowed	4.8%
Outliers	1.3%

4. Experimental Details

4.1 Assay Protocols

Complete protocols describing the biochemical activity assays measuring CPS1 and the CPS2 activity of CAD, the co-crystallization procedure of full length CPS1 with inhibitor as well as the hepatocyte urea production assay can be found in Yao et al.

4.2 Compound Data

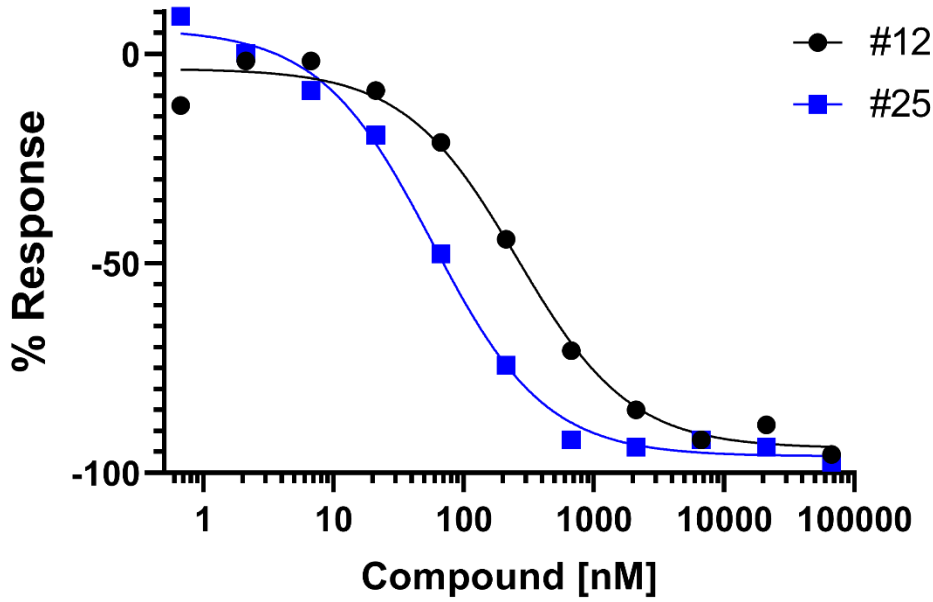
4.2.2 Biochemical Assay and Analytical Data

Cpd	ADPGlo IC_{50} (μM)	Experimental Replicates (n=)	UPLC m/z $[M+H]^+$	Purity (%)
2	7.8	2	391.493	99.2
3	21.5	2	390.503	100
4	18.9	2	405.446	100
5	5.2	2	405.446	97.5
6	6.3	2	419.323	100
7	36	2	419.233	100
8	11.4	2	419.278	100
9	>66	2	403.420	100
10	6.9	2	419.353	100
11	6.5	2	419.173	100
12	0.36	11	419.218	100
13	0.13	6	433.306	100
14	2.2	2	403.600	100
15	2.6	2	407.561	100
16	>66	2	419.578	98.0
17	>66	2	419.448	100

18	0.54	2	419.208	100
19	0.38	2	433.441	100
20	1.4	3	447.349	100
21	0.36	2	403.210	100
22	1.3	2	407.651	100
23	5.1	2	419.308	100
24	>66	2	446.313	100
25	0.066	2	410.190	98.7
26	0.17	2	424.394	100
27	31.0	2	424.263	100
28	>66	2	410.397	99.5
29	4.9	2	411.387	100
30	6.4	2	412.467	96.5
31	7.7	2	426.374	92.0

* Purity assesed using the conditions in section 4.4 Preparative HPLC Conditions

Biochemical inhibitions curves of #12 and #25

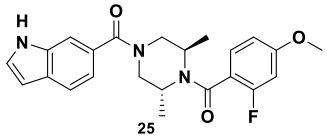


4.2.3 Cellular inhibition data.

Cpd #	IC_{50} (µM)	Experimental replicates (n =)
25	0.24	2
26	1.0	2

4.3 DMPK Data and Experimental

DMPK data 25



25

CPS1 IC_{50} = 66 nM

Solubility pH7 (µM)	148.0
Caco-2 A-B (10^{-6} cm/s)	12.3
MLM CLH (mL/min/kg)	77.1
HLM CLH (mL/min/kg)	7.5
Human hepatocyte stability (µL/min/10-6 cells)	8.7
Human blood stability $t_{1/2}$ (min)	>360

4.3.1 Solubility assay

Instruments:
Instrument: Waters Acquity UPLC I Class:
Column: Acquity HSS T3 column, 2.1 x 50 mm, 1.8 µm

Equipment:
96-well plates (shallow one for filtrate, VWR catalog no. HP5042-1385)
Pre Slit Plate covers (Nalgene catalog no. 276011)
Adhesive Plate covers (VWR catalog no. 60941-090)
Vacuum apparatus
Shaker (800 rpm):Eppindorf Thermomixer R (serial no. 535522603)
Multi-channel pipettes capable of dispersing 200 µL and 795 µL
1200 µL multi-channel pipette: eppendorf 1200 log # 2395404
300 µL multi-channel pipette: Eppendorf 300 log # 4006944

Reagents:

Water: in-house Milli-Q filtered
Acetonitrile: HPLC grade
DMSO: Pure
0.1N HCl: Fluka catalog no. 318965-500mL
Dulbecco's phosphate buffer saline pH7.4:

4.3.2 Caco-2 cell culture and permeability assay

Caco2 cells from American Type Culture Collection (Rockville, MD) were routinely maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco Life Technologies, Carlsbad, CA) containing 10% fetal bovine serum, with high glucose and L-glutamine supplemented with: 10% FBS, 1X penicillin-streptomycin mixture and 1X non-essential amino acids (NEAA). Cells were grown in T-75 flask in an incubator in an atmosphere of 5% CO2 and 95% relative humidity at 37 °C. Allow cells to reach 80-90% confluence before detaching and splitting. Cells are then trypsinized, centrifuged at 120g for 10 min and resuspended in seeding medium at a density of $6.86x10^5$ cells/mL. This cell concentration can be used to seed $2.40x10^5$ cells/cm^2. 50 µL of this cell suspension is then seeded in 96 well Transwell plate (96-well Transwell plate, Corning Costar Co., Corning, NY). The cells on the inserts were cultured for 21 days at 37 °C in a humidified incubator containing 5% CO2 in air. The differentiation status of the formed monolayer was evaluated by measuring the transepithelial electrical resistance (TEER). Permeability studies were conducted with the monolayers that developed TEER values >300 Ω cm2 following 21 days in cell culture.

On the day of the experiment, DMEM was removed, and the monolayers were rinsed two times using pre-warmed HBSS containing 10 mM HEPES, pH 7.4(Transport buffer). Then incubated with the same media for 30 minutes at 37 °C. To assess A-B permeability, the blank transport buffer was removed from the apical compartment and replaced with 75 µL of transport buffer containing 5 µM of test compound or controls (Digoxin, propranolol and Prazosin). The final concentration of DMSO in the incubation system is less than 0.1%. On the basolateral compartment, 235 µL of transport buffer was added. To determine the rate of drug transport from the basolateral to apical direction. Add 235 µL of transport buffer containing 5 µM of test

compound or controls (as stated above). After 2 hrs of incubation, remove 50 µL directly from the apical and basolateral wells (using the basolateral access holes) and transfer to a new pate. Then add 4 volume of cold methanol containing appropriate internal standards (100 nM alprazolam, 200 nM labetalol, 200nM caffeine and 200 nM diclofenac) to terminate the reaction. Vortex for 5 minutes. Samples are centrifuged at 3,220 g for 30 minutes. An aliquot of 100 µL of the supernatant was then mixed with 100 µL of ultra-pure water. The samples were analyzed by LC-MS/MS.

For data analysis, all calculations were carried out using Microsoft Excel. Peak areas were determined from extracted ion chromatograms. The apparent permeability coefficient (Papp), in units of centimeter per second, can be calculated for Caco-2 drug transport assays using the following equation:

$$P_{app} = \frac{V_A}{Area \times time} \times \frac{[drug]_{acceptor}}{[drug]_{initial,donor}}$$

Where VA is the volume (in mL) in the receiver well (0.235 mL for Ap→Bl flux and 0.075 mL for Bl→Ap flux), Area is the surface area of the membrane (0.143 cm2 for HTS Transwell-96 Well Permeable Supports), and time is the total transport time in seconds.
The efflux ratio can be determined using the following equation:

$$Efflux\ Ratio = \frac{P_{app(B-A)}}{P_{app(A-B)}}$$

Where Papp (B-A) indicates the apparent permeability coefficient in basolateral to apical direction, and Papp (A-B) indicates the apparent permeability coefficient in apical to basolateral direction.

4.3.3 Liver microsome metabolic stability assay

Pooled Human liver microsomes (mixed gender) were purchased from Corning Life Sciences (Amsterdam, the Netherlands). CD-1 Mouse liver microsomes were purchased from Xenotech. Liver microsomes (0.5mg/mL) were pre-incubated 5 minutes at 37 °C in the following mixture, 0.1 M phosphate buffer pH 7.4 containing 5 mM $MgCL_2$ and 1mM NADPH. The reaction is initiated by adding the test compound at a final concentration of 2 µM (final DMSO concentration

0.5%). Negative control incubations were included where phosphate buffer was added instead of NADPH. Positive control used was verapamil for both Human and Mouse liver microsomes. Each compound was incubated for 0, 15, 30, 45 and 60 min. The negative and positive controls were also incubated for the same period of time. The reactions were stopped by transferring 50 µL from the reaction solution at 0, 15, 30, 45 and 60 minutes with the addition of 4 volumes of cold acetonitrile 0.1% formic acid- with internal standard(IS) (100 nM alprazolam, 200 nM imipramine, 200 nM labetalol and 2 µM ketoprofen). Samples where then centrifuged at 3,220 g for 40 minutes at 4 °C to precipitate the proteins. Then 90 µL of the supernatant was transferred to each well of a new 96-well plate containing an appropriate volume of ultra-pure water (depends on the LC-MS/MS signal response and peak shape) for LC-MS/MS analysis. *In vitro* $t_{½}$ values were determined by plotting the natural logarithm of the analyte/IS peak area ratios as a function of time, with the slope of the linear regression ($-k$) converted to *in vitro* $t_{½}$ value, where $t_{½} = -0.693/k$. Subsequently, intrinsic CL (CL_{int}) was calculated as: (incubation volume/microsomal protein) × $0.693/t_{½}$ and scaled CL values were obtained using the well-stirred venous equilibration model

4.3.4 Hepatocyte metabolic stability assay

Pooled cryopreserved Human hepatocytes (10-Donor pooled) were purchased from BioIVT. Cells where quickly thawed and re-suspended in Williams E media supplemented with glutamax at a final density of $0.5x10^6$ viable cells/mL. Cells were transferred in a 96 well plate and pre-incubated for 10 minutes at 37°C. The reaction is initiated by adding the test compound at a final concentration of 1 µM (final DMSO concentration 0.5%). Negative control incubations consists of boiled hepatocytes ($0.5x10^6$ viable cells/mL) also incubated with 1 µM test compound. Positive control used was verapamil at a 1 µM final concentration. Each compound was incubated for 0, 15, 30, 60, 90 and 120 min. The negative and positive controls were also incubated for the same period of time. The reactions were stopped by transferring 25 µL from the respective time points with the addition of 6 volumes of cold acetonitrile containing internal standards (IS: 200 nM Caffeine, 200 nM labetalol, 200 nM diclofenac and 100nM alprazolam) to terminate the reactions. The 96 well plate was then centrifuged for 25 minutes at 3,220 g. Aliquots of 100 µL of the supernatants are then mixed with 100 µL ultrapure water and used for LC/MS/MS analysis. *In*

vitro $t_{½}$ values were determined by plotting the natural logarithm of the analyte/IS peak area ratios as a function of time, with the slope of the linear regression ($-k$) converted to *in vitro* $t_{½}$ value, where $t_{½} = -0.693/k$. Subsequently, CL_{int} were calculated as: (incubation volume/number of cells) x $0.693/t_{½}$. Subsequently, scaled CL values were calculated using the well-stirred venous equation model.

4.3.5 Blood stability assay

Human blood was obtained from Healthy Asian volunteers from local vendor, collected in domestic hospitals with Ethical approval. Blood stability was assessed by incubating test compound or positive control (Propantheline) at a final concentration of 2 µM in whole blood (500 µL, 0.5%final solvent concentration). The assay are performed in duplicate. Incubate the reaction samples at 37°C in a water bath with shaking at approximately 60 rpm. Take aliquots of 50 µL from the reaction samples at 0, 15, 30, 45, 60 and 120 minutes. Stop the reaction by adding 350 µL of room temperature quench solution (Acetonitrile containing internal standards (IS, 100 nM alprazolam, 200 nM labetalol, 200 nM Imipramine and 2 µM ketoplofen)). Vortex for 5 minutes. Centrifuge samples in plate at 3,220 g for 30 minutes at room temperature to precipitate protein. Transfer 100 µL of the supernatant to a new plate. The supernatant may be diluted with 100 µL or 200 µL water according to the LC/MS signal response and peak shape. Mix well and analyze samples using LC/MS/MS. For data analysis, all calculations are carried out using Microsoft Excel. Percent parent compounds remaining at each time point will be estimated by determining the peak area ratios from extracted ion chromatograms. *In vitro* $t_{½}$ values were determined by plotting the natural logarithm of the analyte/IS peak area ratios as a function of time, with the slope of the linear regression ($-k$) converted to *in vitro* $t_{½}$ value, where $t_{½} = -0.693/k$.

4.4 Preparative HPLC Conditions

Preparative HPLC Conditions for the Purification of Target Compounds

Chromatography Conditions:

Prep HPLC Instrument: Waters 2545 pump with 2767 fraction collector
Column：For mobile phase (2)Waters Xbridge C18 100mmx19mm,5µm particle size
For mobile phase (1)Waters CSH C18 100mmx19mm, 5µm particle size

MS Detector: Waters 3100 mass detector
UV detector: Waters 2489 dual wavelength UV detector
Flow Rate：30 mL/min
Example Gradient Time:

Time(min)	B%
0	20
1.5	20
6.5	40
6.55	95
8.5	95

Representative Mobile Phase:
(1)
Mobile Phase: A: 0.1%formic acid in water
Mobile Phase: B: 0.1% formic acid in ACN

(2)
Mobile Phase: A: 0.1%NH_4OH in water
Mobile Phase: B: 0.1% NH_4OH in CAN

Other preparative HPLC conditions for the Purification of Target Compounds

Chromatography conditions:
Prep HPLC Instrument: Shimadzu
Column：Ascentis Express C18 or Shim-Pack XR-ODS C18
Detector: SPD-M20A
Flow Rate：1.2 mL/min

Representative Mobile Phase:
(1)
Mobile Phase: A: 0.05%formic acid in water
Mobile Phase: B: 0.05% formic acid in ACN

Preparative SFC Conditions for the Purification of Target Compounds

Chromatography Conditions:
SFC Instrument: Thar SFC Prep Investigator (Waters)
Column：Chiral Technologies chiralpak IA 250mm x10mm,5μm particle size
ELS Detector: Waters 2424 detector
UV detector: Waters 2998 photodiode array detector, 254 nm
Flow Rate：10 mL/min
Isocratic run: 40% isopropanol as a cosolvent

UPLC, HPLC and MS data provided in the examples described below are registered on:

UPLC Waters Acquity SD
- Method name: lc-ms1-2-ba

Equipment**:**

- UPLC Waters Acquity SQZ
- column: Waters Acquity UPLC CSH C18, 50 mm x 2.1 mm x 1.8 μm

Eluents:

- (A) 0.1% formic acid in ACN
- (B) 0.1% formic acid in water

Analytical method:

- Autosampler: Waters2707
- injection volume: 1μL
- Pump:

Time [min]	Flow [ml/min]	%B
0.00	0.8	95
0.20	0.8	95
1.5	0.8	10
1.75	0.8	10
1.85	0.8	0
2.00	0.8	0
2.05	0.8	95

- Column compartment:

- column temperature: ambient
- time of analysis: 2.5 min

- Detector:

- SQZ
-ELSD
-PDA