

Elucidation of fluorine's impact on pK_a and in vitro Pgp-mediated efflux for a series of PDE9 inhibitors

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General Methods

The LCMS data were acquired using a Waters Acquity UPLC-MS consisting of a Waters Acquity system including column manager, binary solvent manager, sample organizer, PDA detector (operating at 254 nm), ELS detector, and TQ-MS equipped with APPI-source operating in positive ion mode. LC-conditions: The column was Acquity UPLC BEH C18 1.7 μm ; 2.1 \times 50 mm operating at 60 $^{\circ}\text{C}$ with 1.2 mL/min binary gradient consisting of H_2O +0.05% trifluoroacetic acid (TFA) (A) and MeCN+5% H_2O +0.05% TFA (B). Gradient: 0.00 min: 10% B; 1.00 min: 100% B; 1.01 min: 10% B; 1.15 min: 10% B. The retention times provided in the experimental section shall be compared to the total run time of 1.15 min. The HRMS data were acquired with a Bruker Daltonic MicroTOF using internal calibration with ESI in positive mode. NMR data were collected with a Bruker 600-Avance-III spectrometer equipped with a 5 mm TCI cryoprobe operating at 600 and 151 MHz for ^1H and ^{13}C , respectively. ^{19}F NMR spectra were recorded with a Bruker 500-Avance spectrometer equipped with a 5 mm QNP probe operating at 470.6 Hz, using CFCl_3 as reference. The solvents used for NMR were CDCl_3 , with reference signals for CHCl_3 (δ = 7.26 ppm, ^1H) and (δ = 77.16 ppm, ^{13}C), and $\text{DMSO-}d_6$ with the reference signals for residual DMSO (δ = 2.50 ppm, ^1H) and (δ = 39.51 ppm, ^{13}C) using TMS as internal reference. The chemical shifts are provided in ppm and broad proton signals are labeled (br). Commercial reagents and solvents were purchased from Sigma-Aldrich. Wrt. = with regards to. The preparation of enantioenriched pyrrolidinyI carbaldehydes **4** and **5** is described in literature.¹

MDCK-MDR1 assay

The permeability of the test compounds was assessed in MDCK-MDR1 cells that were cultured to confluency (4-6 days) in a 96 transwell plate. Test compounds were diluted with the transport buffer (HBSS + 1% BSA) to a concentration of 0.5 μ M and applied to the apical or basolateral side of the cell monolayer. Permeation of the test compounds from A to B direction or B to A direction was determined in triplicate over a 60-minute incubation time at 37 °C and 5% CO₂ with a relative humidity of 95%. Test compounds were quantified by LC-MS/MS analysis based on the peaks area ratios of analyte/IS in both the receiver and donor wells of the transwell plate. The apparent permeability coefficient Papp (cm/s) was calculated using the equation: $P_{app} = (dCr/dt) \times V_r / (A \times C_0)$ Where dCr/dt is the cumulative concentration of compound in the receiver chamber as a function of time (gM/s); V_r is the solution volume in the receiver chamber (0.05 mL on the apical side; 0.25 mL on the basolateral side); A is the surface area for the transport, i.e. 0.0804 cm² for the area of the monolayer; C₀ is the initial concentration in the donor chamber (μ M). The final MDCK-MDR1 efflux ratio is then calculated $P_{app} BA / P_{app} AB$.

Determination of pK_a values

The pK_a values are determined by a series of three titrations on a dilution of 10 μ L 10 μ M compound stock at 25.0 \pm 0.5 °C and ion strength of 0.16M using methanol as co-solvent. Methanol concentrations in the range 29 – 54% were used in the three titrations on each compound. The titrator is used in the mode with a diode-array-detector. This detector recorded the UV-Vis spectrum of the solution in-line, and thus the titration can be mapped via comparison to the spectra of the protonated and unprotonated species. The real aqueous pK_a –value was determined by extrapolation to zero-methanol content using a Yasuda-Shedlovsky plot.

PDE9 inhibition assay

The assay is performed in 60 μ L samples containing a fixed amount of the PDE9 enzyme (sufficient to convert not more than 20% of the tritiated substrate), a buffer (50 mM HEPES pH 7.6; 10 mM MgCl₂; 0.02% Tween20), 0.1mg/ml BSA, and tritium labeled cGMP to a final concentration of 15 nM and varying amounts of inhibitors. Reactions are initiated by addition of ³H-cGMP, and reactions are allowed to proceed for 1 hour at room temperature before being terminated through mixing with 15 μ L 8 mg/mL yttrium silicate SPA beads (Amersham). The beads are allowed to settle for 1 hour in the dark before the plates are counted in a Wallac 1450 Microbeta counter. The measured signal is converted to activity relative to an uninhibited control (100 %) and IC₅₀ values are calculated using the Xlfit (IDBS) extension to EXCEL.

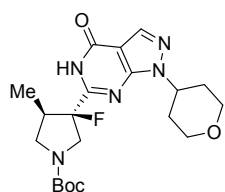
Syntheses

General procedure for the reductive amination

To a stirred solution of **1**, **2**, **6** or **7** (1 equiv.) in MeOH (0.05M wrt. **1**, **2**, **6** or **7**) was slowly added HCl (70 equiv., 4M in dioxane) at rt. The mixture was subsequently stirred 1.5 h followed by a removal of solvent at reduced pressure. To this material was then added 1,2-DCE (0.02M wrt. **1**, **2**, **6** or **7**) and sodium acetate (3 equiv.) followed by the aldehyde (1.5 equiv.) and sodium triacetoxyborohydride (2.5 equiv.) after which the mixture was heated at 40 °C for 1.5 h. Saturated aq. NaHCO₃ was added and the aqueous phase extracted with CH₂Cl₂ after which the combined organic phases were dried over MgSO₄, filtered and concentrated *in vacuo* to provide a crude material that was purified using silica gel chromatography (85:10:5, EtOAc:MeOH:Et₃N) to provide **8-11**.

tert-Butyl

(3R,4R)-3-fluoro-4-methyl-3-(4-oxo-1-(tetrahydro-2H-pyran-4-yl)-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6-yl)pyrrolidine-1-carboxylate (1): To a mixture of **4** (1.00 g, 4.32 mmol) and **3** (1.09 g, 5.19 mmol) in THF (8.6 mL, 0.5M) was added Ti(OEt)₄ (7.89 g, 7.25 mL, 34.6 mmol) and the mixture was stirred for 24 h at 75 °C. Water was added followed by EtOAc and the dense mixture was passed through a plug of Celite. The mixture was extracted with ethyl acetate. The organic phase was washed with brine, dried over MgSO₄ and concentrated *in vacuo* to provide the crude amination intermediate that was dried overnight at reduced pressure. To the dried residue was added CH₂Cl₂ (4 mL) and DMF



(29 mL, 4.32 mmol, 0.15M) and the resulting mixture was heated to 80 °C followed by the addition of I₂ (1.646 g, 6.49 mmol). After 30 min at 80 °C, the heating block was removed and the mixture allowed to cool for 5 min, followed by the addition of sat. aq. NaHCO₃ (15 mL) and 10% aq. Na₂S₂O₃ (35 mL). EtOAc was added and the phases separated, the aqueous phase was extracted with EtOAc (2 x 200 mL) and once with EtOAc/THF (150 mL, 1:1). The combined organic extracts were washed with brine, dried over MgSO₄, filtered and concentrated *in vacuo* to provide a brown residue that was purified using silica gel chromatography to provide **1** (661 mg, 1.568 mmol, 36% yield) as a yellow oil.

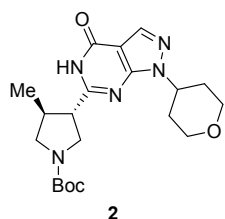
Note: if heating is continued at 80 °C past 30 min in step 2, significant amounts of product will get Boc de-protected. It is then possible to add sat. aq. bicarb, 10% aq. sodium thiosulphate, EtOAc and re-install the Boc *in situ* using cat. 4-DMAP and Boc-anhydride and recover the material by extraction.

¹H NMR (600 MHz, CDCl₃) δ 9.99 - 9.89 (m, 1H), 8.05 (2 x s, 1H), 4.83 - 4.74 (m, 1H), 4.17 - 4.11 (m, 2H), 4.08 - 3.81 (m, 3H), 3.63 - 3.55 (m, 2H), 3.28 - 3.22 (m, 1H), 2.44 - 2.29 (m, 2H), 1.93 - 1.85 (m, 2H), 1.50 - 1.45 (m, 9H), 1.45 - 1.43 (m, 1H), 1.06 - 1.03 (m, 3H). Mixture of conformers.

¹³C NMR (151 MHz, CDCl₃) δ 157.4, 154.9, 154.8, 154.7, 154.6, 154.2, 153.8, 151.1, 151.0, 135.1, 105.3, 101.8 (2 x d, J = 188.4 Hz), 80.3, 67.1, 56.8 (2 x d, J = 23.4 Hz), 54.4, 51.1, 50.6, 42.1 (2 x d, J = 20.9 Hz), 32.4, 32.3, 32.3, 32.2, 28.6. Mixture of conformers.

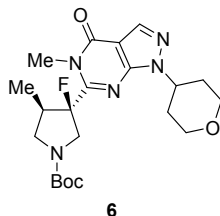
HRMS-ESI: *m/z* C₁₆H₂₁FN₅O₄ [MH⁺-^tBu] calcd. 366.1572; found 366.1578.

***tert*-Butyl (3*S*,4*S*)-3-methyl-4-(4-oxo-1-(tetrahydro-2H-pyran-4-yl)-4,5-dihydro-1H-pyrazolo[3,4-*d*]pyrimidin-6-yl)pyrrolidine-1-carboxylate (**2**):** To a mixture of **5** (1.00 g, 4.32 mmol) and **3** (1.18 g, 5.63 mmol) in THF (9.4 mL, 0.5M)



was added Ti(OEt)₄ (8.56 g, 7.86 mL, 37.5 mmol) and the mixture was stirred for 24 h at 75 °C. The reaction mixture was then poured into water (100 mL) and the resulting suspension was heavily stirred for 10 min after which EtOAc was added and the dense mixture was passed through a plug of Celite. The mixture was extracted with ethyl acetate. The organic phase was washed with brine, dried over MgSO₄ and concentrated *in vacuo* to provide a yellow residue that was taken up in CH₂Cl₂ (15 mL) and transferred to a round-bottom flask. The suspension was boiled and DMF (31 mL, 0.15M) was added while allowing the temperature to reach 80 °C followed by the addition of I₂ (1.785 g, 7.03 mmol). The reaction mixture was stirred for 20 min followed by the addition of sat. aq. NaHCO₃ (30 mL) and 10% sat. aq. Na₂S₂O₃. EtOAc was added and the phases were separated. The aqueous phase was extracted using EtOAc (2 x 150 mL) followed by EtOAc:THF (200 mL, 1:1). The combined organic layers were washed with brine, dried over MgSO₄, filtered and concentrated *in vacuo* to provide a crude residue that was further purified using silica gel chromatography to provide **2** (1.05 g, 2.60 mmol, 56% yield) as a yellow oil. The characterization data are in accordance with literature.²

***tert*-Butyl (3*R*,4*R*)-3-fluoro-4-methyl-3-(5-methyl-4-oxo-1-(tetrahydro-2H-pyran-4-yl)-4,5-dihydro-1H-pyrazolo[3,4-*d*]pyrimidin-6-yl)pyrrolidine-1-carboxylate (**6**):** To a stirred solution of **1** (148 mg, 0.351 mmol)



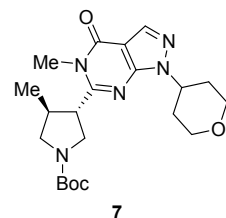
in DMF (540 μL 0.65M) was added NaH (15.5 mg, 0.386 mmol, 60%) and the resulting mixture was stirred for 30 min at 0 °C. MeI (75 mg, 33 μL, 0.53 mmol) was added and stirring was continued for 1.5 h. Water and NH₄Cl were added. The mixture was extracted with EtOAc. The organic phase was washed with brine, dried over MgSO₄ and concentrated *in vacuo*. The crude material was purified using silica gel chromatography to provide **6** (20.0 mg, 0.046 mmol, 13% yield) as a colorless oil.

¹H NMR (600 MHz, CDCl₃) δ 8.08 (s, 1H), 4.77 - 4.70 (m, 1H), 4.27 - 4.08 (m, 3H), 3.88 - 3.74 (m, 2H), 3.74 - 3.71 (m, 3H), 3.63 - 3.54 (m, 2H), 3.48 - 3.35 (m, 1H), 3.24 - 3.18 (m, 1H), 2.46 - 2.31 (m, 2H), 1.96 - 1.90 (m, 2H), 1.51 - 1.44 (m, 9H), 1.18 (d, *J* = 6.6 Hz, 3H).

¹³C NMR (151 MHz, CDCl₃) δ 158.7, 154.0 (d, *J* = 72.7 Hz), 153.7, 153.5, 149.0, 135.0, 105.0, 104.8, 102.9, 102.1 (d, *J* = 126.9 Hz), 80.4 (2 peaks), 67.2 (2 peaks), 55.7 - 55.2 (m), 55.0, 54.8, 50.3, 49.7, 41.6 - 40.4 (m), 32.5, 32.2, 31.9 - 31.5 (m), 28.6, 10.2 (2 peaks). Mixture of conformers.

HRMS-ESI: *m/z* C₁₇H₂₃FN₅O₄ [MH⁺-^tBu] calcd. 380.1729; found 380.1736.

tert-butyl (3*S*,4*S*)-3-methyl-4-(5-methyl-4-oxo-1-(tetrahydro-2*H*-pyran-4-yl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-yl)pyrrolidine-1-carboxylate (7): To a stirred solution of **2** (130 mg, 0.322 mmol) in DMF (496 μL, 0.322 mmol, 0.65*M*) was added NaH (12.9 mg, 0.322 mmol, 60%) and the resulting mixture was stirred for 30 min at 0°C. MeI (69 mg, 30 μL, 0.48 mmol) was added and stirring was continued for 1.5 h. Water and NH₄Cl were added. The mixture was extracted with EtOAc. The organic phase was washed with brine, dried over MgSO₄ and concentrated *in vacuo*. The crude material was using silica gel chromatography to provide **7** (38 mg, 0.091 mmol, 28% yield) as a colorless oil.

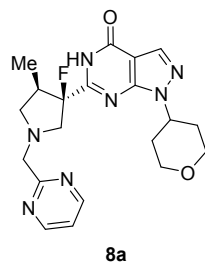


¹H NMR (600 MHz, CDCl₃) δ 8.03 (s, 1H), 4.79 - 4.72 (m, 1H), 4.15 - 4.10 (m, 2H), 3.89 (ddd, *J* = 47.8, 10.7, 7.7 Hz, 1H), 3.75 (ddd, *J* = 33.7, 10.6, 7.4 Hz, 1H), 3.65 - 3.46 (m, 6H), 3.25 - 3.15 (m, 1H), 3.08 (ddd, *J* = 22.7, 10.5, 8.4 Hz, 1H), 2.90 (ddd, *J* = 12.7, 9.2, 6.4 Hz, 1H), 2.43 - 2.29 (m, 2H), 1.95 - 1.88 (m, 2H), 1.47 (2 x s, 9H), 1.14 - 1.11 (m, 3H). Mixture of conformers.

¹³C NMR (151 MHz, CDCl₃) δ 158.9, 158.6, 154.4, 154.2, 149.9, 134.8, 104.3, 104.2, 79.8 (2C), 67.2 (2C), 67.1, 54.4, 54.2, 52.5, 52.0, 50.4, 50.2, 49.9, 49.1, 38.7, 37.6, 32.4, 32.3, 32.2 (2C), 30.3, 28.6, 17.1, 17.0. Mixture of conformers.

HRMS-ESI: *m/z* C₂₁H₃₂N₅O₄ [MH⁺] calcd. 418.2449; found 418.2454.

6-((3*R*,4*R*)-3-fluoro-4-methyl-1-(pyrimidin-2-ylmethyl)pyrrolidin-3-yl)-1-(tetrahydro-2*H*-pyran-4-yl)-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (8a): Synthesized using the general procedure to provide **8a** in 62% yield.



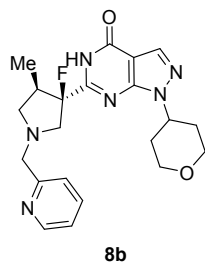
¹H NMR (600 MHz, CDCl₃) δ 10.07 (s, 1H), 8.78 (d, *J* = 4.9 Hz, 2H), 8.09 (s, 1H), 7.23 (t, *J* = 4.9 Hz, 1H), 4.91 - 4.84 (m, 1H), 4.22 (d, *J* = 15.2 Hz, 1H), 4.13 (dd, *J* = 11.8, 4.4 Hz, 2H), 4.08 (d, *J* = 15.2 Hz, 1H), 3.91 (dd, *J* = 28.4, 12.0 Hz, 1H), 3.65 - 3.60 (m, 2H), 3.44 (t, *J* = 8.0 Hz, 1H), 3.18 - 3.08 (m, 1H), 3.02 - 2.92 (m, 1H), 2.76 (t, *J* = 9.7 Hz, 1H), 2.41 - 2.30 (m, 2H), 1.92 - 1.87 (m, 2H), 1.10 - 1.07 (m, 3H).

¹³C NMR (151 MHz, CDCl₃): δ 157.6, 157.5, 151.3, 135.1, 119.7, 105.1, 102.0 (d, *J* = 190.8 Hz), 67.1, 64.1 (d, *J* = 23.8 Hz), 61.7, 60.0, 53.8, 43.5 (d, *J* = 20.7 Hz), 32.4 (d, *J* = 14.2 Hz), 10.3 (d, *J* = 10.0 Hz).

¹⁹F NMR (471 MHz, CDCl₃) δ -170.53.

HR MS-ESI: *m/z* for C₂₀H₂₅FN₇O₂ [MH⁺-^tBu] calcd. 414.2048; found 414.2049.

6-((3*R*,4*R*)-3-fluoro-4-methyl-1-(pyridin-2-ylmethyl)pyrrolidin-3-yl)-1-(tetrahydro-2*H*-pyran-4-yl)-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (8b): Synthesized using the general procedure to provide **8b** in 47% yield.



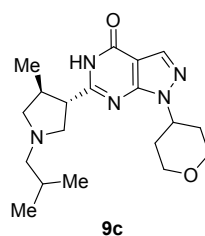
¹H NMR (600 MHz, CDCl₃) δ 9.66 (br, 1H), 8.60 (ddd, J = 4.9, 1.8, 0.9 Hz, 1H), 8.08 (s, 1H), 7.68 (td, J = 7.6, 1.8 Hz, 1H), 7.39 (dt, J = 7.8, 1.0 Hz, 1H), 7.19 (ddd, J = 7.5, 4.8, 1.2 Hz, 1H), 4.84 (tt, J = 11.7, 4.3 Hz, 1H), 4.16 - 4.12 (m, 2H), 4.00 (d, J = 13.7 Hz, 1H), 3.92 (d, J = 13.7 Hz, 1H), 3.71 (dd, J = 30.7, 12.1 Hz, 1H), 3.62 (tt, J = 11.8, 1.9 Hz, 2H), 3.31 - 3.27 (m, 1H), 3.17 - 3.09 (m, 1H), 2.99 - 2.90 (m, 1H), 2.70 (t, J = 9.1 Hz, 1H), 2.41 - 2.31 (m, 2H), 1.93 - 1.87 (m, 2H), 1.06 (dd, J = 7.0, 2.0 Hz, 3H).

¹³C NMR (151 MHz, CDCl₃) δ 158.3, 157.3, 157.1, 151.3, 149.6, 136.8, 135.1, 123.0, 122.5, 105.1, 102.6 (d, J = 189.3 Hz), 67.1, 64.7 (d, J = 23.7 Hz), 61.8, 59.9, 54.1, 43.7 (d, J = 20.6 Hz), 32.3 (d, J = 13.9 Hz), 10.26 (d, J = 10.1 Hz).

¹⁹F NMR (471 MHz, CDCl₃) δ -169.62.

HRMS-ESI: *m/z* C₂₁H₂₆FN₆O₂ [MH⁺] calcd. 413.2096; found 413.2102.

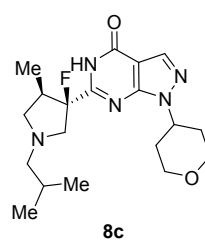
6-((3*S*,4*S*)-1-Isobutyl-4-methylpyrrolidin-3-yl)-1-(tetrahydro-2H-pyran-4-yl)-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (9c): Synthesized using the general procedure to provide **9c** in 33% yield.



¹H NMR (600 MHz, CDCl₃) δ 8.03 (s, 1H), 4.83 (tt, J = 11.7, 4.3 Hz, 1H), 4.17 - 4.11 (m, 2H), 3.61 (tdd, J = 12.0, 7.3, 2.1 Hz, 2H), 3.45 - 3.39 (m, 1H), 3.14 - 3.07 (m, 1H), 2.89 - 2.78 (m, 1H), 2.54 - 2.45 (m, 1H), 2.45 - 2.27 (m, 4H), 1.94 - 1.87 (m, 2H), 1.81 (s, 2H), 1.58 (s, 2H), 1.21 (d, J = 6.9 Hz, 3H), 1.02 (t, J = 6.6 Hz, 6H).

HRMS-ESI: *m/z* C₁₉H₃₀N₅O₂ [MH⁺] calcd. 360.2394; found 360.2403.

6-((3*R*,4*R*)-3-Fluoro-1-isobutyl-4-methylpyrrolidin-3-yl)-1-(tetrahydro-2H-pyran-4-yl)-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (8c): Synthesized using the general procedure to provide **8c** in 33% yield.



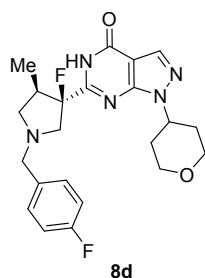
¹H NMR (600 MHz, CDCl₃) δ 9.54 (s, 1H), 8.09 (s, 1H), 4.84 (tt, J = 11.6, 4.3 Hz, 1H), 4.16 - 4.11 (m, 2H), 3.60 (tt, J = 11.9, 2.1 Hz, 3H), 3.20 (t, J = 8.1 Hz, 1H), 2.98 - 2.80 (m, 2H), 2.53 - 2.47 (m, 1H), 2.45 - 2.32 (m, 4H), 1.94 - 1.89 (m, 2H), 1.76 - 1.69 (m, 1H), 1.07 - 1.04 (m, 3H), 0.96 (d, J = 5.2 Hz, 3H), 0.95 (d, J = 5.4 Hz, 3H).

¹³C NMR (151 MHz, CDCl₃) δ 157.7 (d, J = 27.7 Hz), 157.3, 151.4, 135.1, 105.1, 102.2 (d, J = 189.6 Hz), 67.1, 67.1, 65.3, 65.1, 65.0, 60.5, 54.2, 43.4 (d, J = 20.5 Hz), 32.3 (d, J = 18.6 Hz), 27.7, 21.0 (d, J = 12.8 Hz), 10.05 (d, J = 9.8 Hz).

¹⁹F NMR (471 MHz, CDCl₃) δ -170.41

HRMS-ESI: *m/z* C₁₉H₂₉FN₅O₂ [MH⁺] calcd. 378.2300; found 378.2306.

6-((3*R*,4*R*)-3-Fluoro-1-(4-fluorobenzyl)-4-methylpyrrolidin-3-yl)-1-(tetrahydro-2H-pyran-4-yl)-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (8d): Synthesized using the general procedure to provide **8d** in 40% yield.



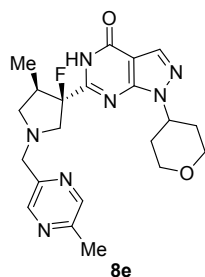
¹H NMR (600 MHz, CDCl₃) δ 9.54 (s, 1H), 8.08 (s, 1H), 7.35 - 7.31 (m, 2H), 7.04 - 7.00 (m, 2H), 4.82 (tt, J = 11.6, 4.2 Hz, 1H), 4.17 - 4.13 (m, 2H), 3.81 - 3.71 (m, 2H), 3.64 - 3.58 (m, 3H), 3.20 - 3.16 (m, 1H), 3.04 (dd, J = 30.1, 12.0 Hz, 1H), 2.96 - 2.85 (m, 1H), 2.59 (t, J = 9.2 Hz, 1H), 2.42 - 2.33 (m, 2H), 1.94 - 1.88 (m, 2H), 1.06 (dd, J = 7.0, 2.1 Hz, 3H).

¹³C NMR (151 MHz, CDCl₃) δ 163.1, 161.4, 157.3, 157.1, 135.1, 134.2, 130.3, 130.3, 115.5, 115.4, 105.1, 102.5 (d, *J* = 189.2 Hz), 67.1, 64.7 (d, *J* = 23.8 Hz), 59.7, 54.3, 43.6 (d, *J* = 20.6 Hz), 32.3 (d, *J* = 10.8 Hz), 10.4 (d, *J* = 10.3 Hz).

¹⁹F NMR (471 MHz, CDCl₃) δ -115.74, -169.38.

HRMS-ESI: *m/z* C₂₂H₂₆F₂N₅O₂ [MH⁺] calcd. 430.2049; found 430.2056.

6-((3*R*,4*R*)-3-fluoro-4-methyl-1-((5-methylpyrazin-2-yl)methyl)pyrrolidin-3-yl)-1-(tetrahydro-2*H*-pyran-4-yl)-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (8e): Synthesized using the general procedure to provide **8e** in 35% yield.



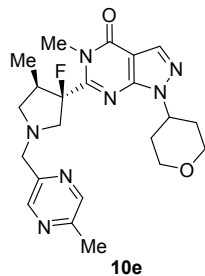
¹H NMR (600 MHz, CDCl₃) δ 9.61 (s, 1H), 8.55 (d, *J* = 1.5 Hz, 1H), 8.44 - 8.43 (m, 1H), 8.08 (s, 1H), 4.87 - 4.80 (m, 1H), 4.16 - 4.11 (m, 2H), 4.01 (d, *J* = 13.8 Hz, 1H), 3.92 (d, *J* = 14.0 Hz, 1H), 3.74 (dd, *J* = 31.7, 12.1 Hz, 1H), 3.62 (tt, *J* = 12.0, 2.5 Hz, 2H), 3.30 - 3.26 (m, 1H), 3.13 (dd, *J* = 30.6, 12.2 Hz, 1H), 3.00 - 2.89 (m, 1H), 2.73 - 2.68 (m, 1H), 2.57 - 2.55 (m, 3H), 2.42 - 2.31 (m, 2H), 1.90 (dddt, *J* = 11.2, 6.7, 4.3, 2.1 Hz, 2H), 1.06 (dd, *J* = 7.0, 1.9 Hz, 3H).

¹³C NMR (151 MHz, CDCl₃) δ 157.3, 156.9 (d, *J* = 27.3 Hz), 152.7, 151.3 (2C), 150.4, 144.0, 143.6, 135.1, 105.1, 102.8 (d, *J* = 188.8 Hz), 67.1, 64.8 (d, *J* = 23.6 Hz), 59.9, 59.1, 54.1, 45.9, 43.7 (d, *J* = 20.7 Hz), 32.3 (d, *J* = 9.0 Hz), 21.4, 10.0 (d, *J* = 9.8 Hz).

¹⁹F NMR (471 MHz, CDCl₃) δ -169.11.

HRMS-ESI: *m/z* C₂₁H₂₇FN₇O₂ [MH⁺] calcd. 428.2205; found 428.2210.

6-((3*R*,4*R*)-3-fluoro-4-methyl-1-((5-methylpyrazin-2-yl)methyl)pyrrolidin-3-yl)-5-methyl-1-(tetrahydro-2*H*-pyran-4-yl)-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (10e): Synthesized using the general procedure to provide **10e** in 48% yield.



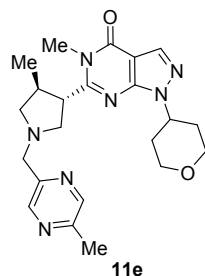
¹H NMR (600 MHz, CDCl₃) δ 8.51 - 8.50 (m, 1H), 8.41 - 8.40 (m, 1H), 8.06 (s, 1H), 4.76 (tt, *J* = 11.7, 4.2 Hz, 1H), 4.14 (dt, *J* = 10.7, 5.0 Hz, 2H), 3.96 (d, *J* = 13.7 Hz, 1H), 3.86 (d, *J* = 13.6 Hz, 1H), 3.69 - 3.64 (m, 1H), 3.62 (d, *J* = 2.8 Hz, 3H), 3.61 - 3.53 (m, 3H), 3.26 - 3.16 (m, 2H), 2.58 - 2.53 (m, 4H), 2.45 - 2.33 (m, 2H), 1.95 - 1.90 (m, 2H), 1.16 (dd, *J* = 6.8, 2.0 Hz, 3H).

¹³C NMR (151 MHz, CDCl₃) δ 158.9, 154.9 (d, *J* = 25.8 Hz), 152.8, 150.3, 149.1, 144.0, 143.7, 134.9, 104.8, 103.7 (d, *J* = 186.5 Hz), 67.2 (2C), 64.7 (d, *J* = 25.0 Hz), 58.8 (d, *J* = 57.2 Hz), 54.8, 41.4, 41.3, 32.4 (d, *J* = 46.3 Hz), 31.7 (d, *J* = 11.2 Hz), 21.4, 10.1 (d, *J* = 10.3 Hz).

¹⁹F NMR (471 MHz, CDCl₃) δ -157.63.

HRMS-ESI: *m/z* C₂₂H₂₉FN₇O₂ [MH⁺] calcd. 442.2361; found 442.2368.

5-methyl-6-((3*S*,4*S*)-4-methyl-1-((5-methylpyrazin-2-yl)methyl)pyrrolidin-3-yl)-1-(tetrahydro-2*H*-pyran-4-yl)-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (11e): Synthesized using the general procedure to provide **11e** in 55% yield.



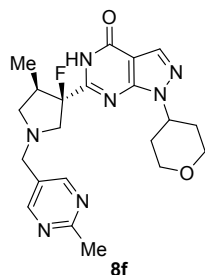
¹H NMR (600 MHz, CDCl₃) δ 8.54 (d, *J* = 1.5 Hz, 1H), 8.40 (dd, *J* = 1.5, 0.6 Hz, 1H), 8.03 (s, 1H), 4.81 (tt, *J* = 11.6, 4.2 Hz, 1H), 4.17 - 4.11 (m, 2H), 3.94 (d, *J* = 13.7 Hz, 1H), 3.80 (d, *J* = 13.8 Hz, 1H), 3.62 (tdd, *J* = 12.0, 3.7, 2.1 Hz, 2H), 3.57 (s, 3H), 3.21 (dd, *J* = 5.9, 3.0 Hz, 2H), 3.13 (p, *J* =

6.9 Hz, 1H), 3.06 (dd, J = 8.8, 7.4 Hz, 1H), 2.93 - 2.85 (m, 1H), 2.57 - 2.52 (m, 4H), 2.44 - 2.33 (m, 2H), 1.93 (ddt, J = 13.0, 5.2, 2.6 Hz, 2H), 1.15 (d, J = 6.6 Hz, 3H).

^{13}C NMR (151 MHz, CDCl_3) δ 160.6, 158.8, 152.5, 150.9, 150.0, 143.9, 143.7, 134.8, 104.1, 67.2 (2C), 61.3, 59.3, 59.0, 54.2, 50.8, 37.8, 32.4, 32.3, 30.5, 21.4, 18.9.

HRMS-ESI: m/z $\text{C}_{22}\text{H}_{30}\text{N}_7\text{O}_2$ [MH^+] calcd. 424.2455; found 424.2460.

6-((3R,4R)-3-fluoro-4-methyl-1-((2-methylpyrimidin-5-yl)methyl)pyrrolidin-3-yl)-1-(tetrahydro-2H-pyran-4-yl)-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (8f): Synthesized using the general procedure to provide **8f** in 75% yield.



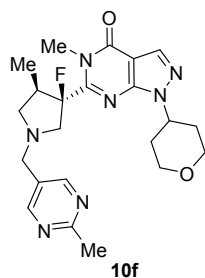
^1H NMR (600 MHz, CDCl_3) δ 8.64 (s, 2H), 8.08 (s, 1H), 4.81 (tt, J = 11.7, 4.2 Hz, 1H), 4.17 - 4.13 (m, 2H), 3.82 (d, J = 13.6 Hz, 1H), 3.74 (d, J = 13.5 Hz, 1H), 3.68 - 3.59 (m, 4H), 3.20 - 3.17 (m, 1H), 3.06 (dd, J = 30.3, 12.0 Hz, 1H), 2.97 - 2.87 (m, 1H), 2.73 (s, 3H), 2.64 - 2.60 (m, 1H), 2.42 - 2.33 (m, 2H), 1.91 (ddt, J = 13.1, 4.6, 1.9 Hz, 2H), 1.06 (dd, J = 7.0, 1.9 Hz, 3H).

^{13}C NMR (151 MHz, CDCl_3) δ 167.6, 157.3, 157.2, 156.7 (d, J = 27.2 Hz), 151.2, 135.1, 128.3, 105.1, 102.8 (d, J = 188.4 Hz), 67.1 (d, J = 2.3 Hz), 64.9 (d, J = 23.7 Hz), 59.6, 55.1, 54.4, 43.9, 43.7, 32.3, 10.11 (d, J = 9.9 Hz).

^{19}F NMR (471 MHz, CDCl_3) δ -168.80.

HRMS-ESI: m/z $\text{C}_{21}\text{H}_{27}\text{FN}_7\text{O}_2$ [MH^+] calcd. 428.2205; found 428.2209.

6-((3R,4R)-3-fluoro-4-methyl-1-((2-methylpyrimidin-5-yl)methyl)pyrrolidin-3-yl)-5-methyl-1-(tetrahydro-2H-pyran-4-yl)-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (10f): Synthesized using the general procedure to provide **10f** in 48% yield.



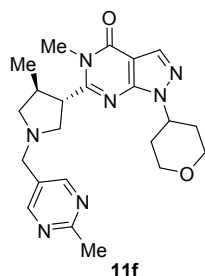
^1H NMR (600 MHz, CDCl_3) δ 8.59 (s, 2H), 8.07 (s, 1H), 4.79 - 4.72 (m, 2H), 4.17 - 4.12 (m, 2H), 3.76 - 3.68 (m, 2H), 3.63 - 3.60 (m, 3H), 3.60 - 3.56 (m, 2H), 3.55 - 3.45 (m, 1H), 3.12 - 3.07 (m, 2H), 2.72 (s, 3H), 2.47 - 2.32 (m, 3H), 1.97 - 1.92 (m, 2H), 1.16 (dd, J = 6.9, 2.1 Hz, 3H).

^{13}C NMR (151 MHz, CDCl_3) δ 167.6, 158.9, 157.3, 154.8 (d, J = 25.6 Hz), 149.1, 134.9, 130.3, 128.2, 104.8, 103.5 (d, J = 186.8 Hz), 67.2 (2C), 64.6 (d, J = 25.0 Hz), 60.6, 58.3, 54.9, 54.8, 41.3 (d, J = 20.8 Hz), 32.4 (d, J = 63.2 Hz), 31.7 (d, J = 11.2 Hz), 25.9, 10.3 (d, J = 10.5 Hz).

^{19}F NMR (471 MHz, CDCl_3) δ -157.49.

HRMS-ESI: m/z $\text{C}_{22}\text{H}_{29}\text{FN}_7\text{O}_2$ [MH^+] calcd. 442.2361; found 302.2368.

5-methyl-6-((3S,4S)-4-methyl-1-((2-methylpyrimidin-5-yl)methyl)pyrrolidin-3-yl)-1-(tetrahydro-2H-pyran-4-yl)-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (11f):



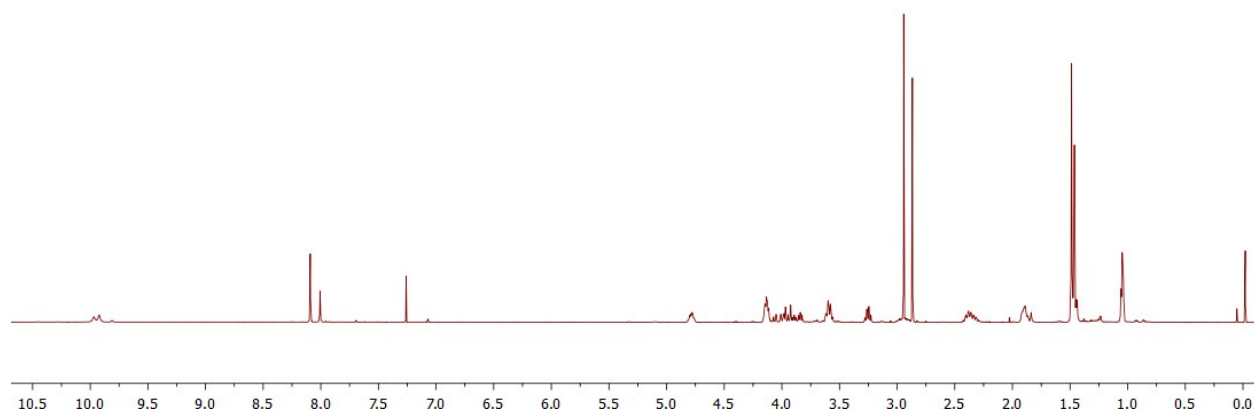
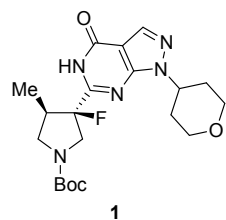
^1H NMR (600 MHz, CDCl_3) δ 8.60 (s, 2H), 8.03 (s, 1H), 4.79 (tt, J = 11.6, 4.2 Hz, 1H), 4.17 - 4.12 (m, 2H), 3.72 (d, J = 13.5 Hz, 1H), 3.64 - 3.59 (m, 3H), 3.57 (s, 3H), 3.21 - 3.16 (m, 1H), 3.14 - 3.04 (m, 2H), 2.99 - 2.95 (m, 1H), 2.82 (dd, J = 9.0, 6.6 Hz, 1H), 2.72 (s, 3H), 2.47 - 2.35 (m, 3H), 1.96 - 1.91 (m, 2H), 1.16 (d, J = 6.8 Hz, 3H).

¹³C NMR (151 MHz, CDCl₃) δ 167.4, 160.6, 158.8, 157.3, 150.0, 134.8, 128.7, 104.2, 67.2 (2C), 61.1, 58.8, 54.8, 54.4, 50.7, 37.8, 32.4, 32.2, 30.5, 25.9, 19.3.

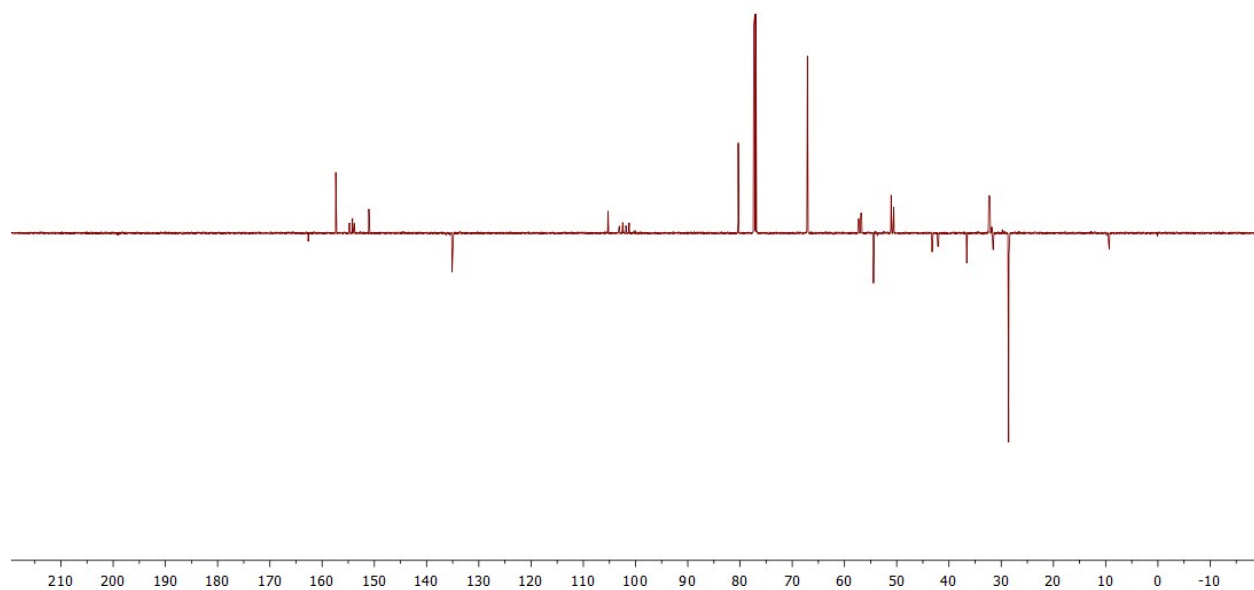
HRMS-ESI: *m/z* C₂₂H₃₀N₇O₂ [MH⁺] calcd. 424.2455; found 424.2452.

Spectra

Compound **1**.

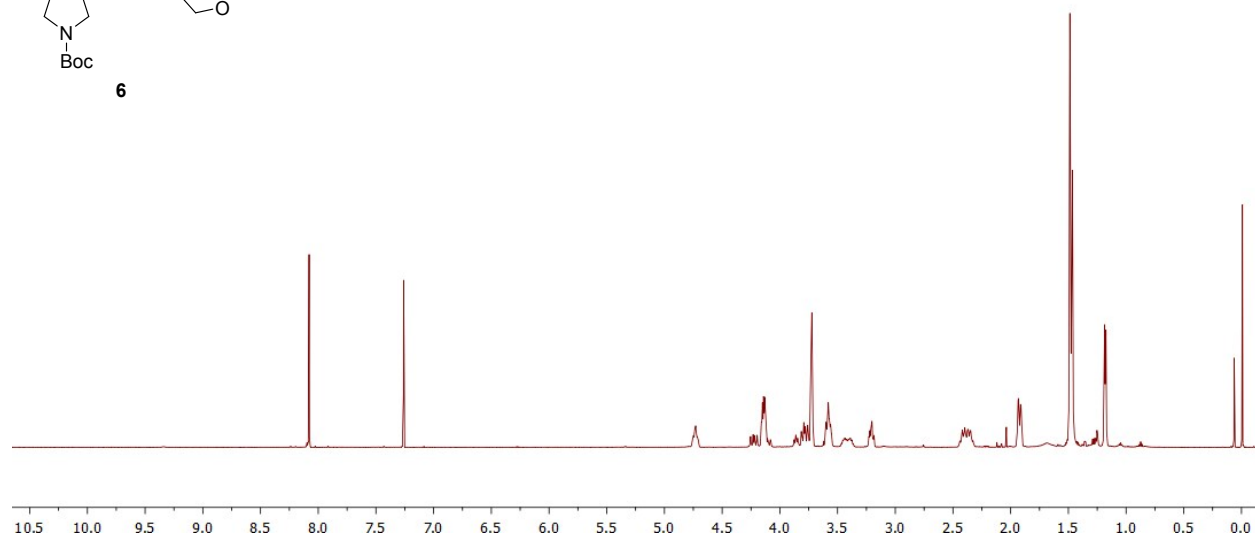
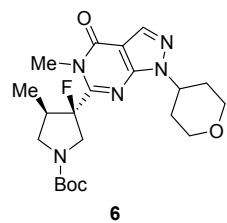


^1H NMR spectrum of **1** acquired in CDCl_3 .

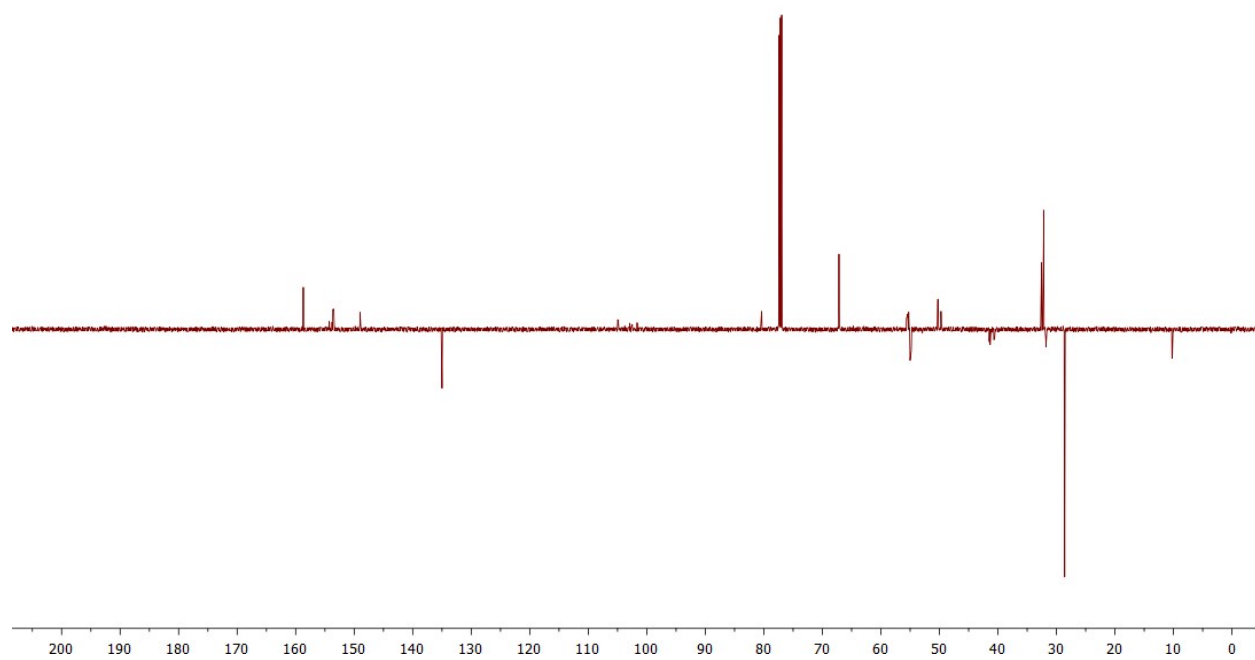


APT spectrum of **1** acquired in CDCl_3 .

Compound **6**.

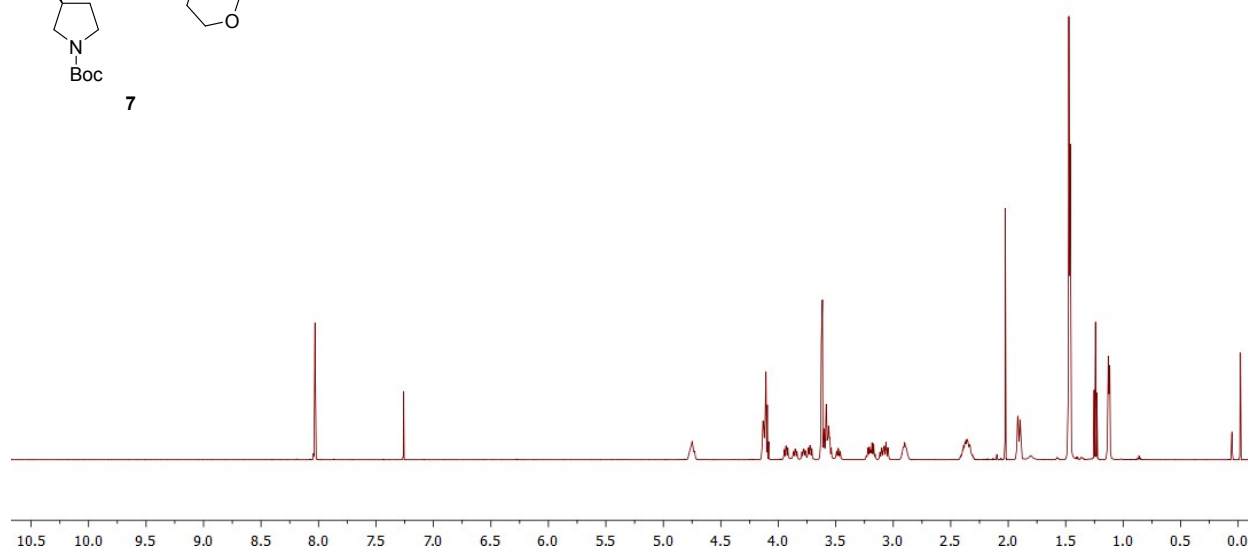
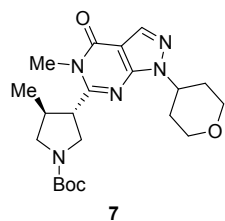


^1H NMR spectrum of **6** acquired in CDCl_3 .

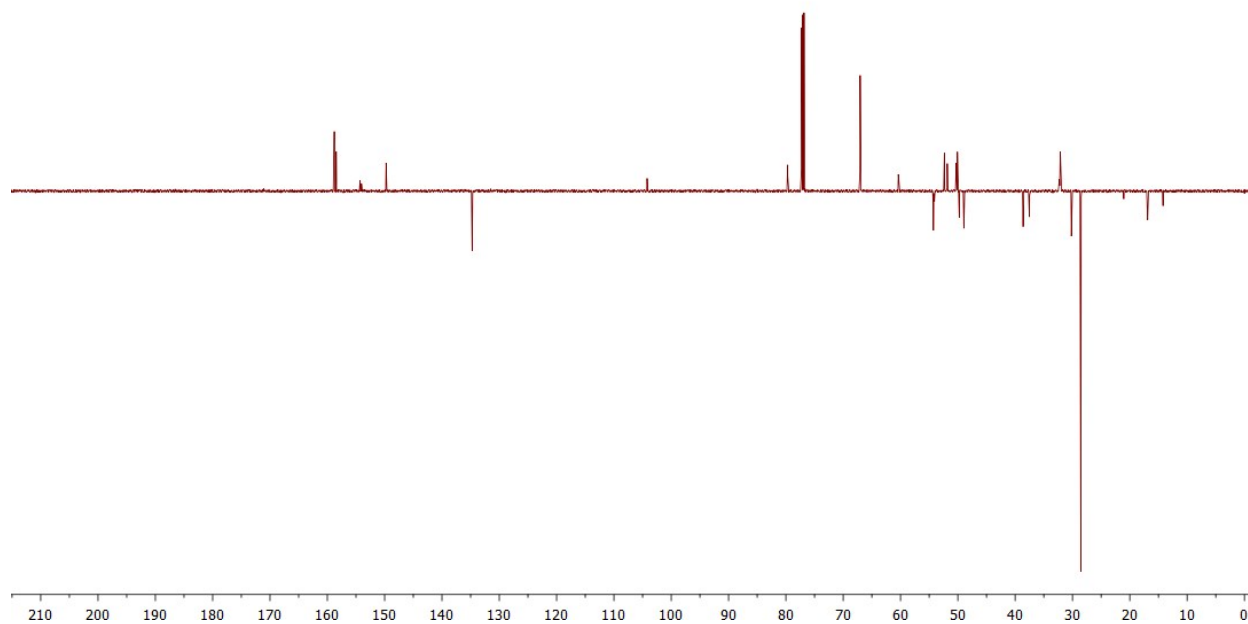


APT spectrum of **6** acquired in CDCl_3 .

Compound 7.

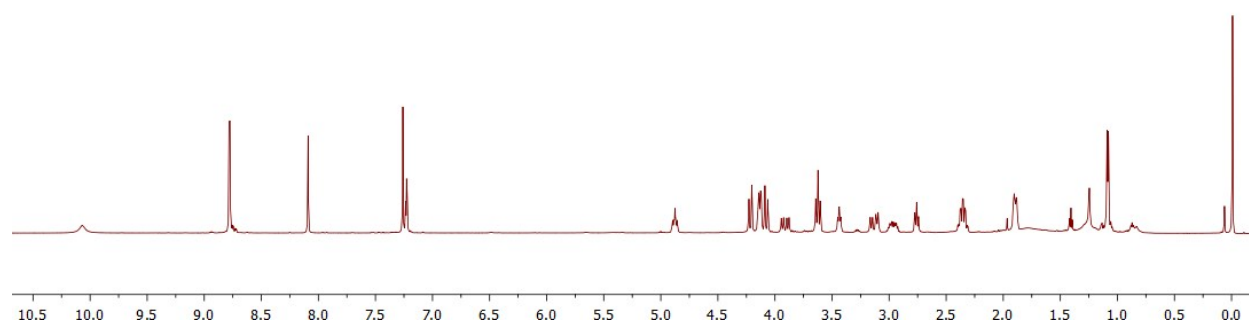
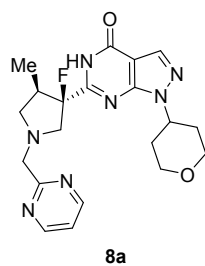


¹H NMR spectrum of 7 acquired in CDCl₃.

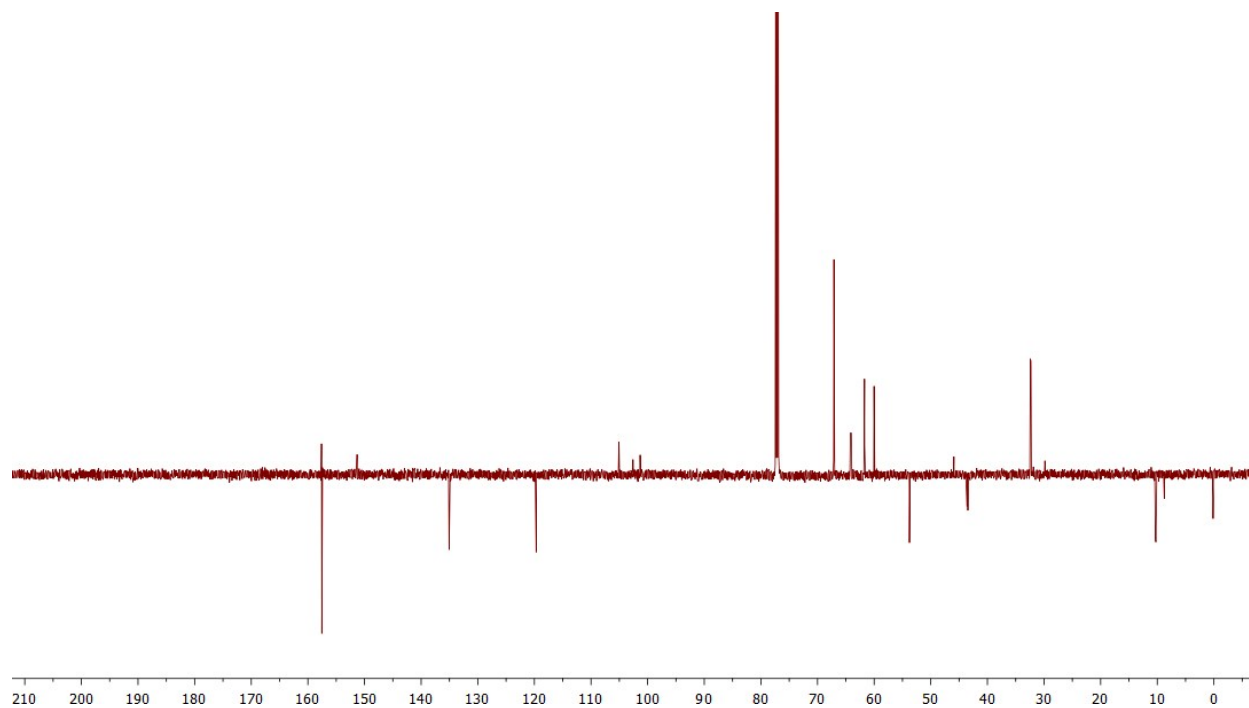


APT spectrum of 7 acquired in CDCl₃.

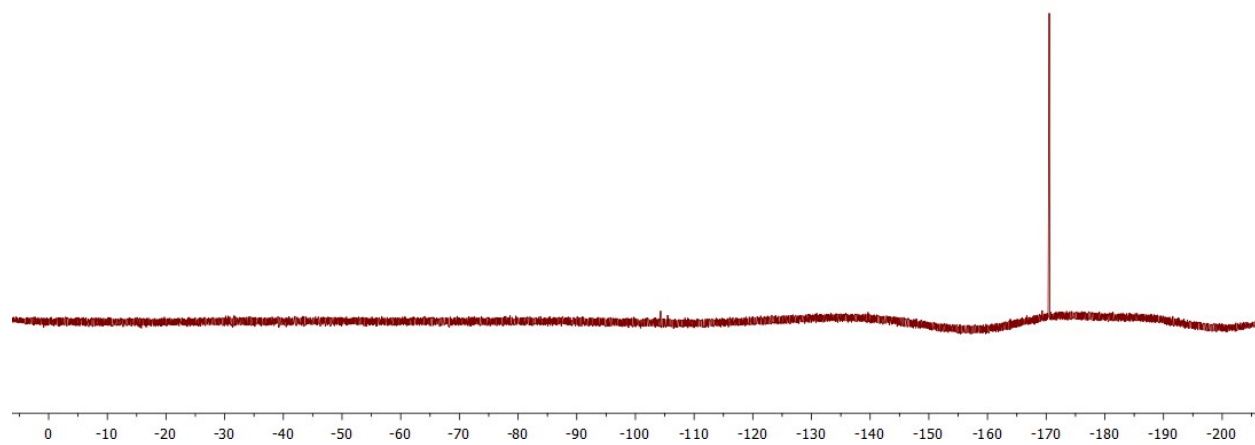
Compound **8a**



¹H NMR spectrum of **8a** acquired in CDCl₃.

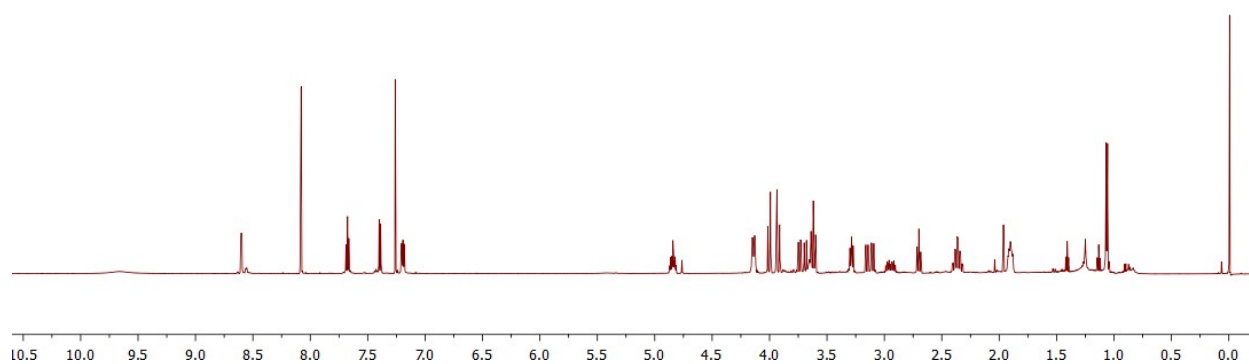
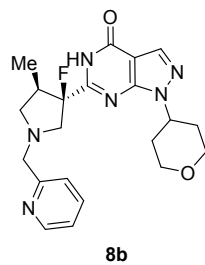


APT spectrum of **8a** acquired in CDCl₃.

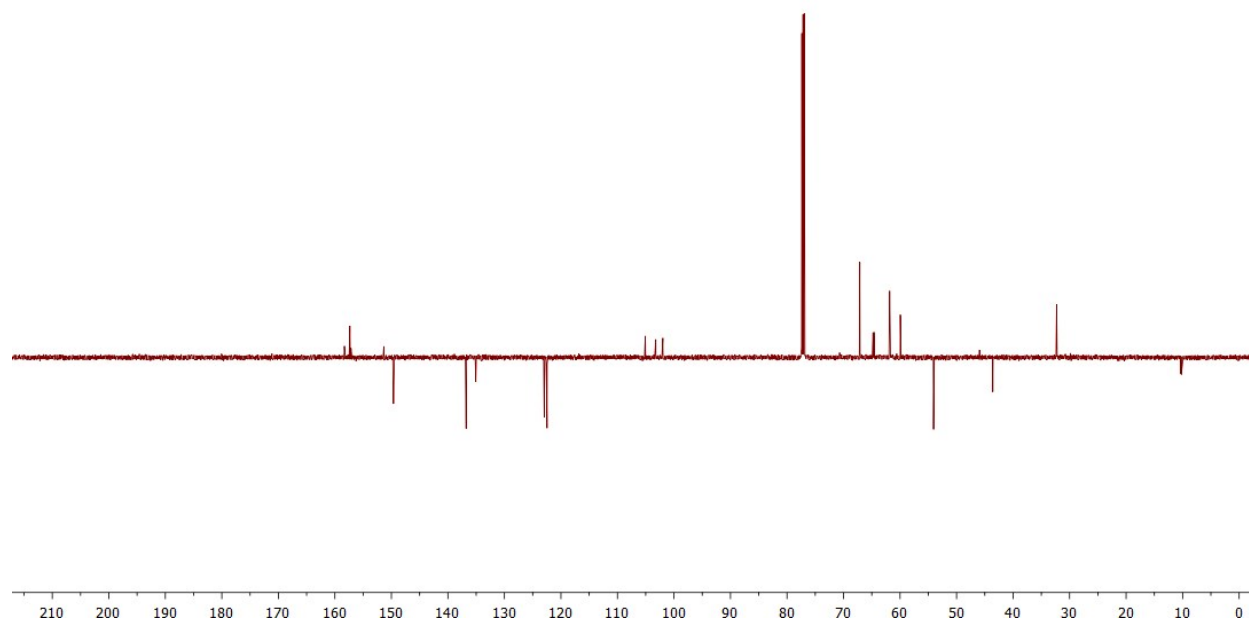


^{19}F NMR spectrum of 8a acquired in CDCl_3 .

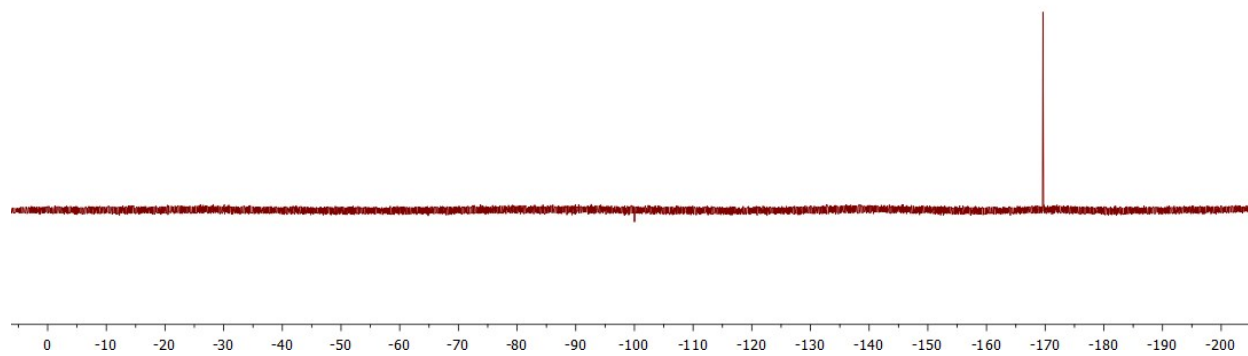
Compound **8b**.



^1H NMR spectrum of **8b** acquired in CDCl_3 .

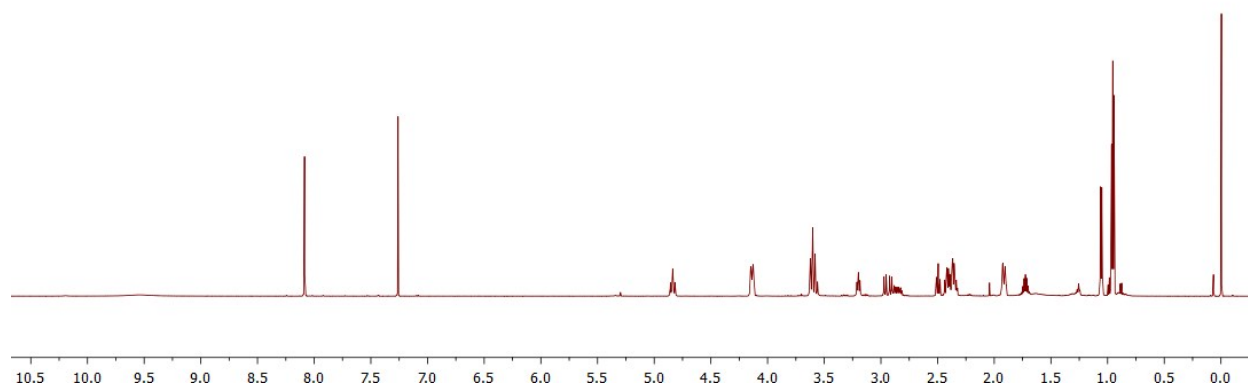
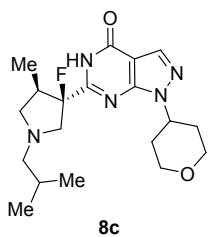


APT spectrum of **8b** acquired in CDCl_3 .

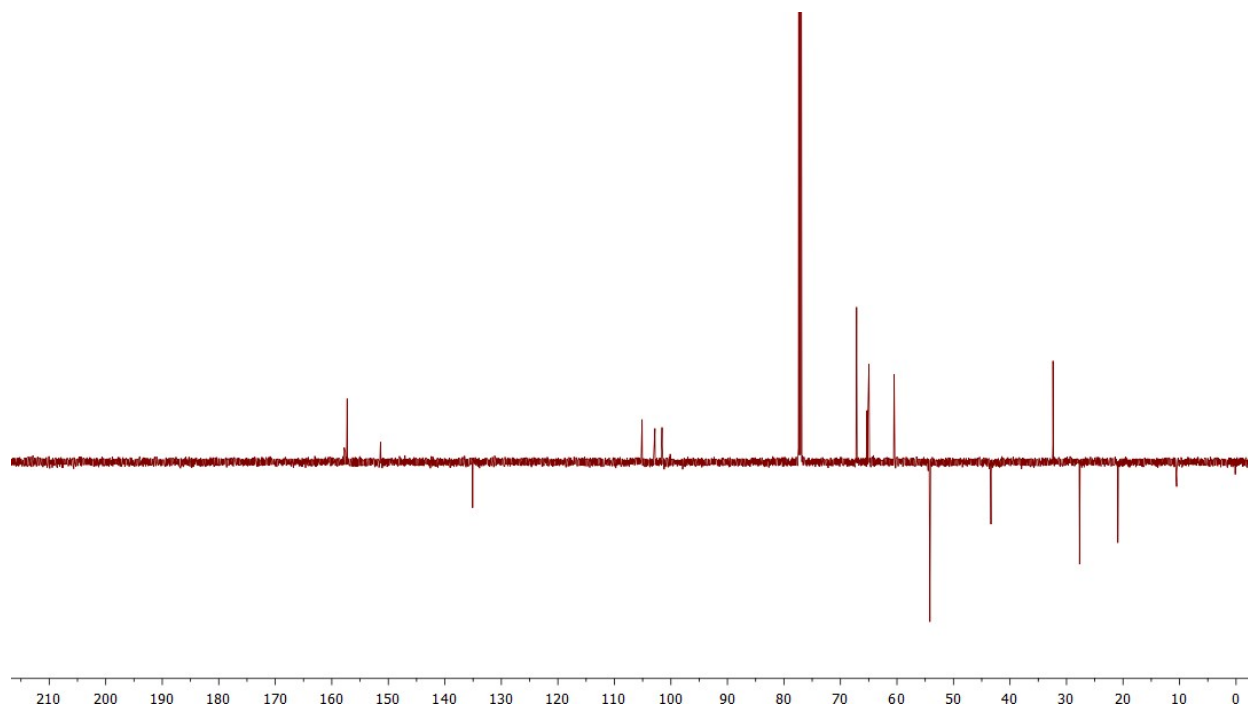


^{19}F NMR spectrum of 8b acquired in CDCl_3 .

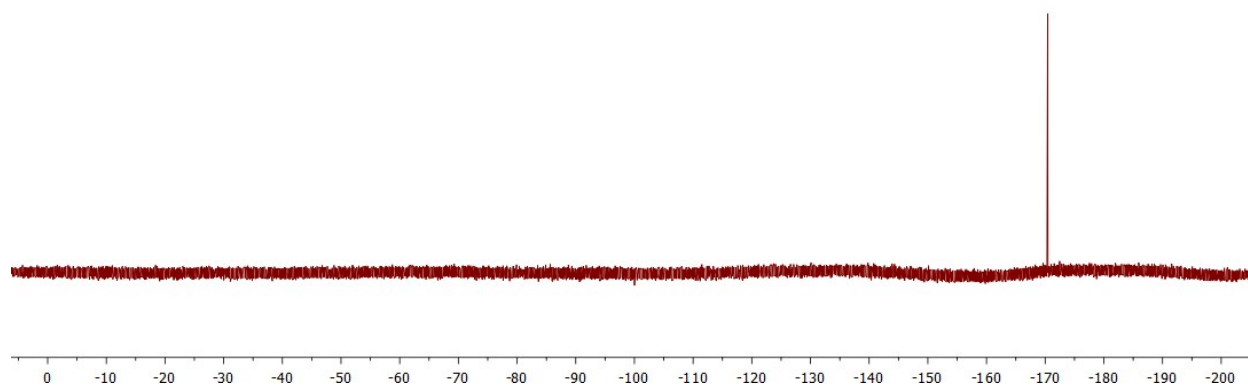
Compound **8c**.



^1H NMR spectrum of **8c** acquired in CDCl_3 .

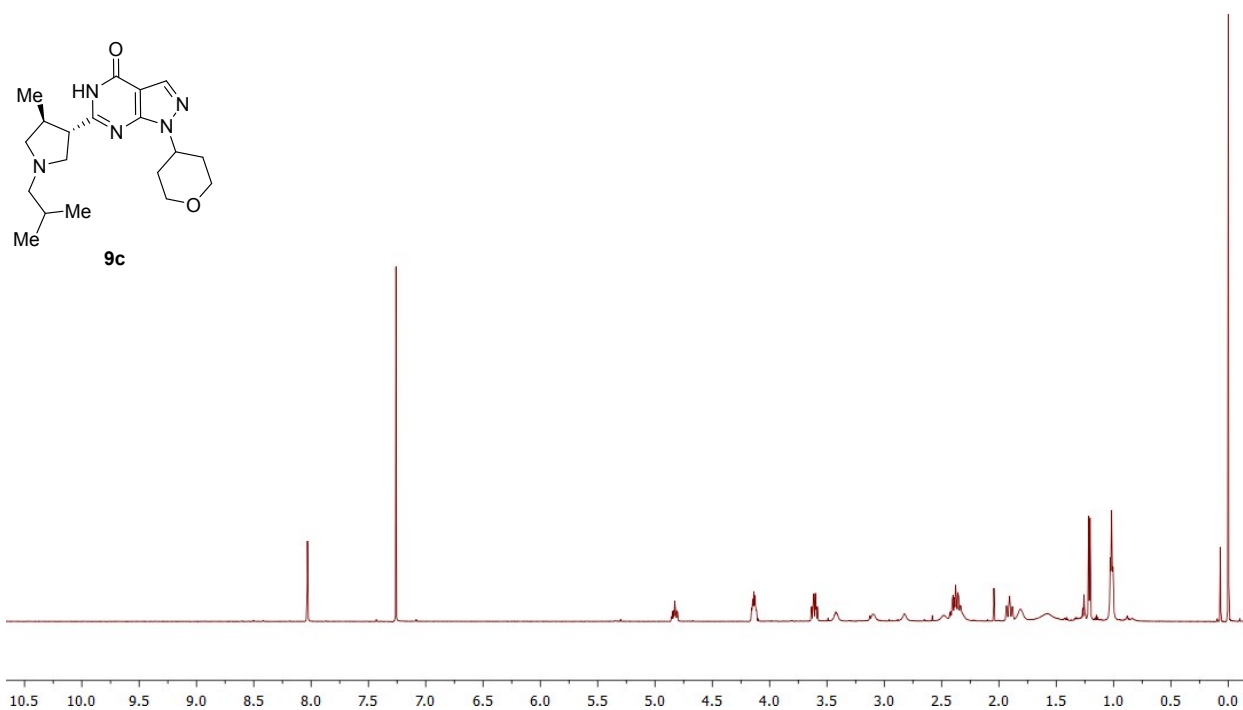


APT spectrum of **8c** acquired in CDCl_3 .



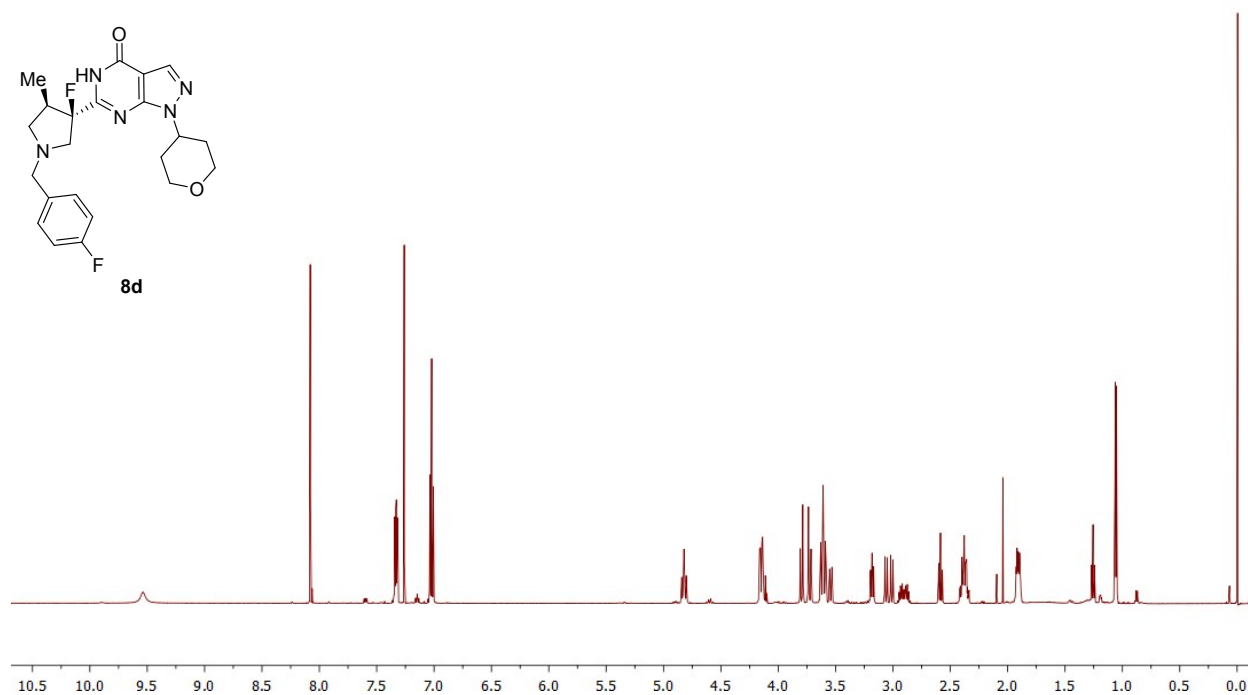
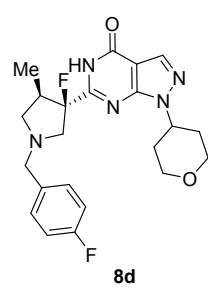
^{19}F NMR spectrum of 8c acquired in CDCl_3 .

Compound **9c**.

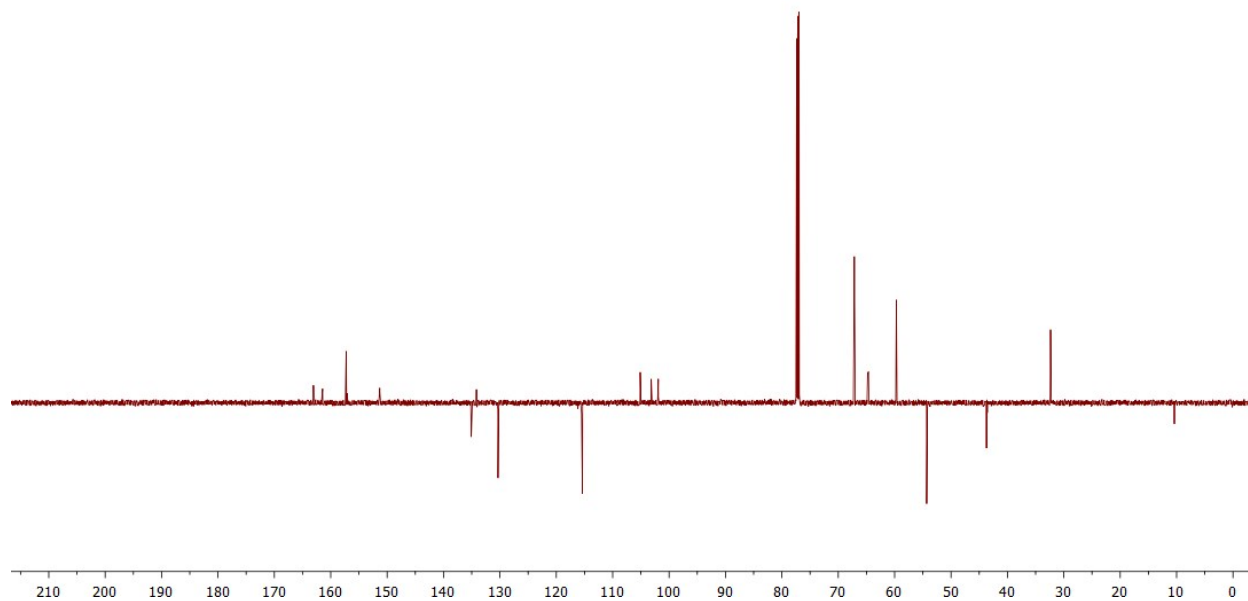


^1H NMR spectrum of **9c** acquired in CDCl_3 .

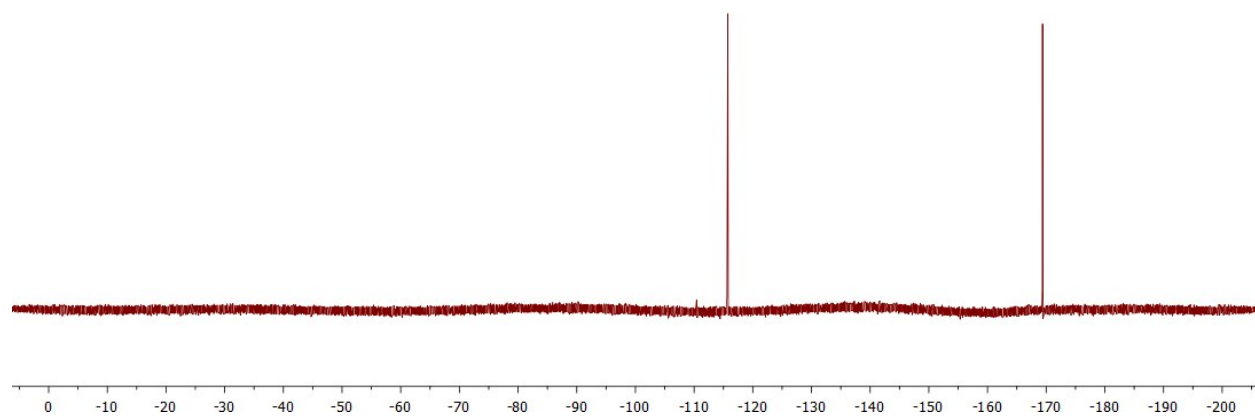
Compound **8d**.



^1H NMR spectrum of **8d** acquired in CDCl_3 .

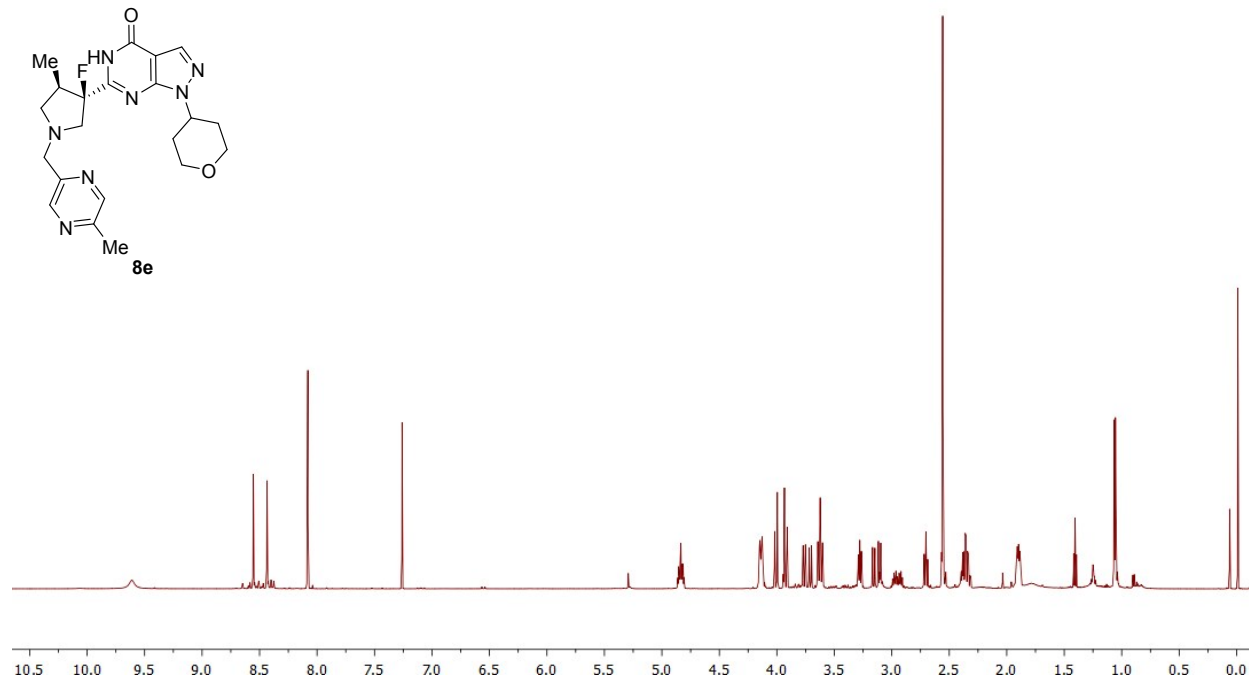
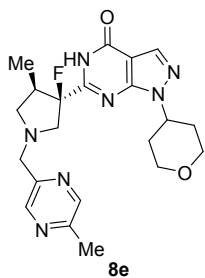


APT spectrum of **8d** acquired in CDCl_3 .

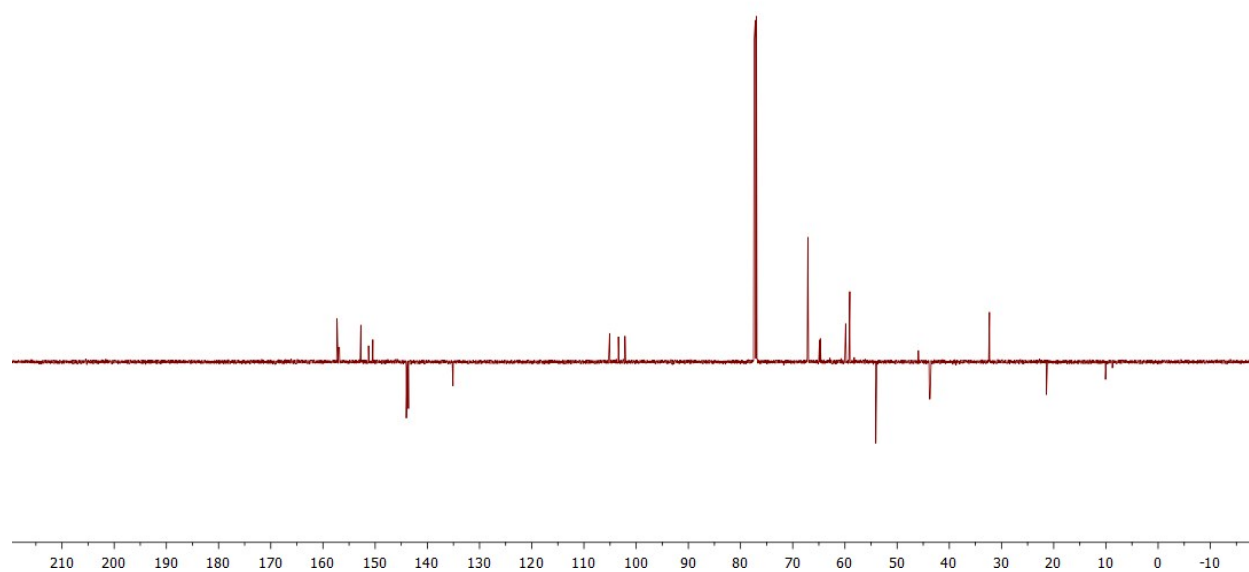


^{19}F NMR spectrum of 8d acquired in CDCl_3 .

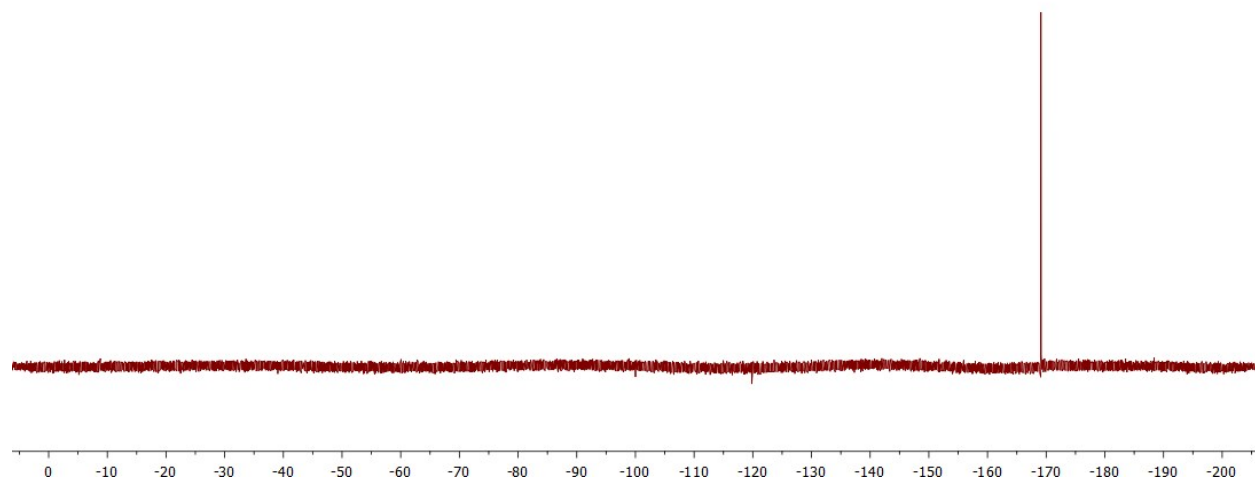
Compound **8e**.



¹H NMR spectrum of **8e** acquired in CDCl₃.

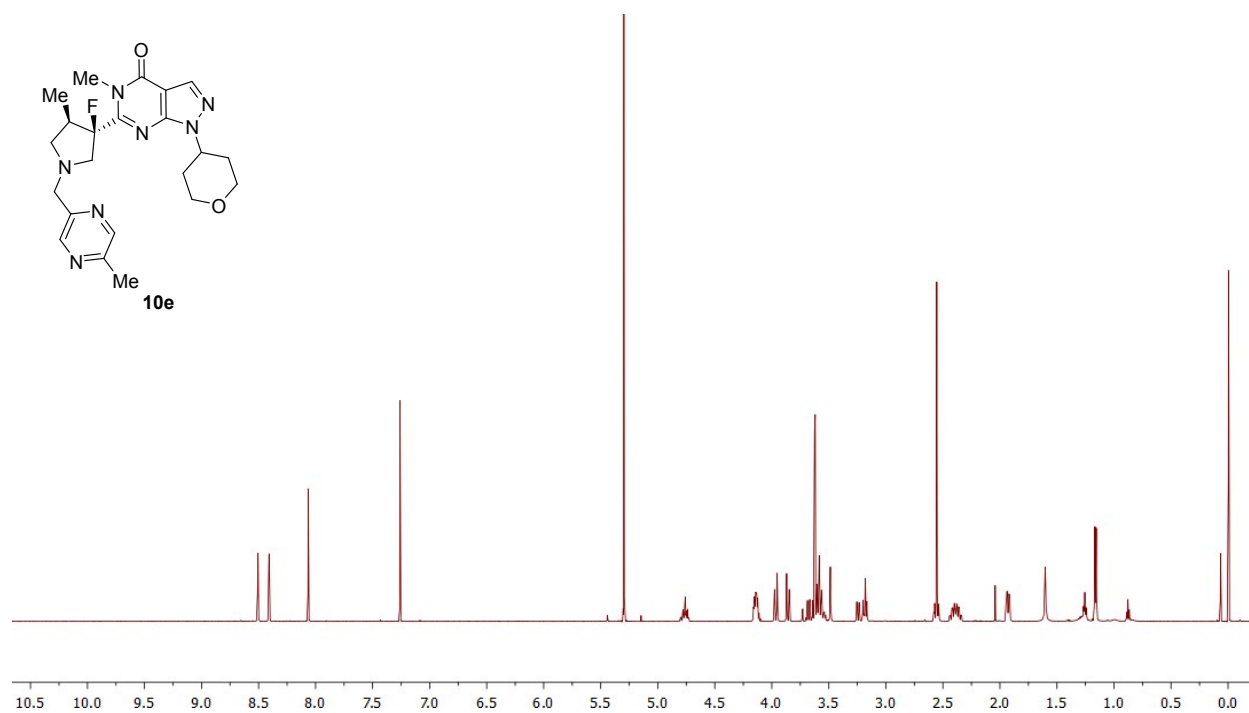


APT spectrum of **8e** acquired in CDCl₃.

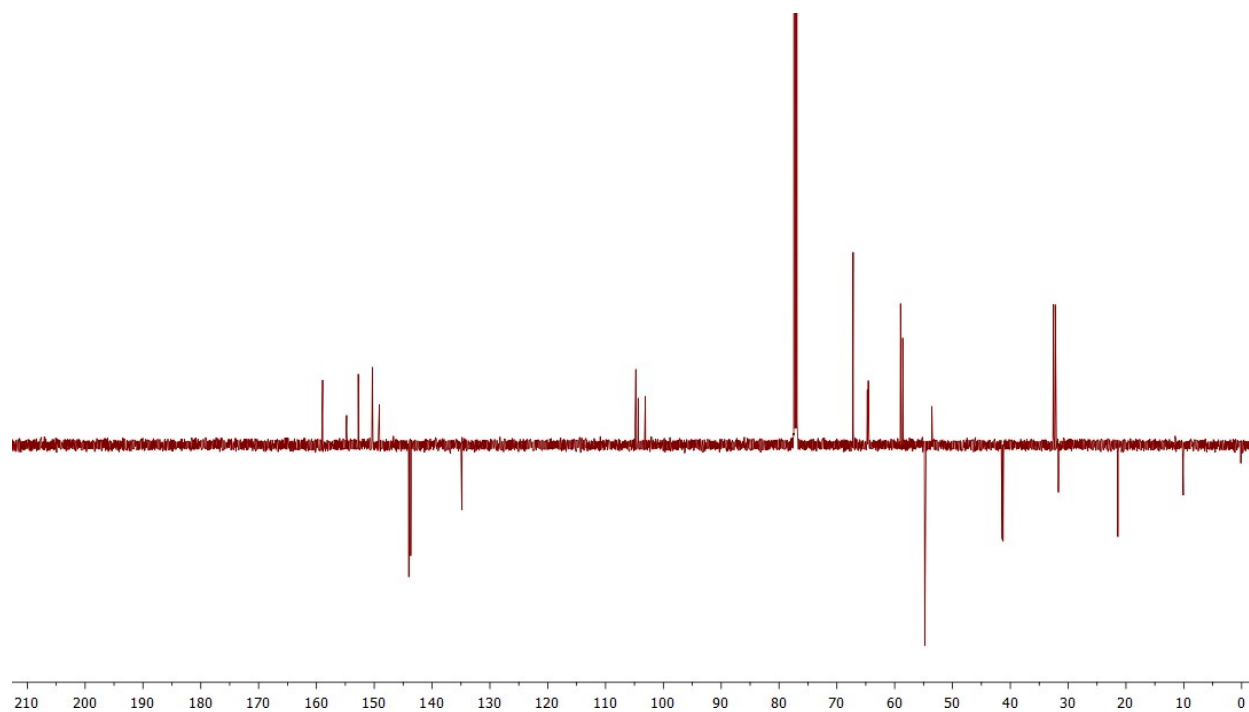


^{19}F NMR spectrum of 8e acquired in CDCl_3 .

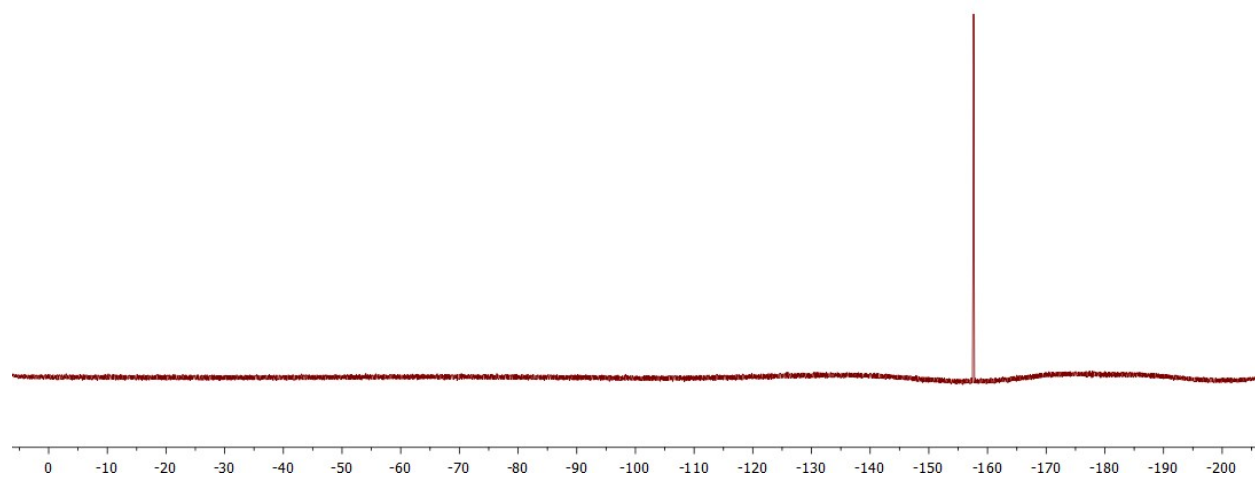
Compound **10e**:



¹H NMR spectrum of **10e** acquired in CDCl₃.

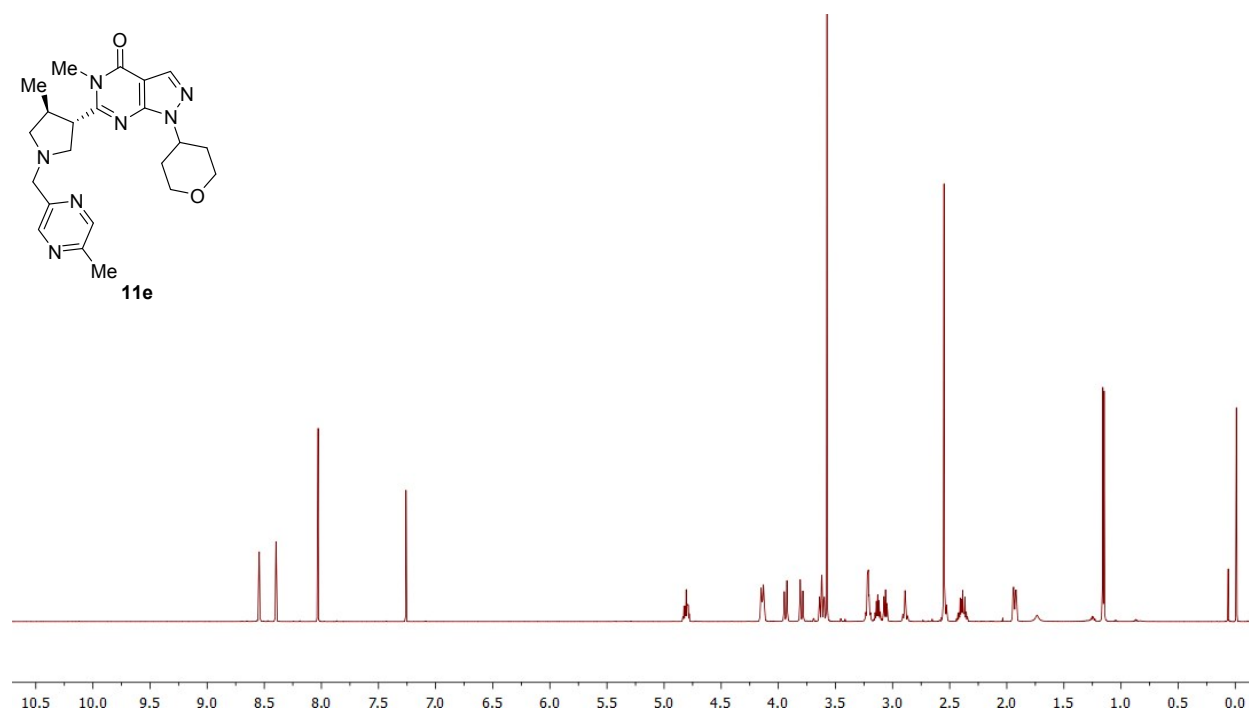
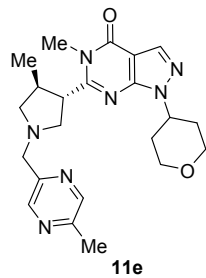


APT spectrum of **10e** acquired in CDCl₃.

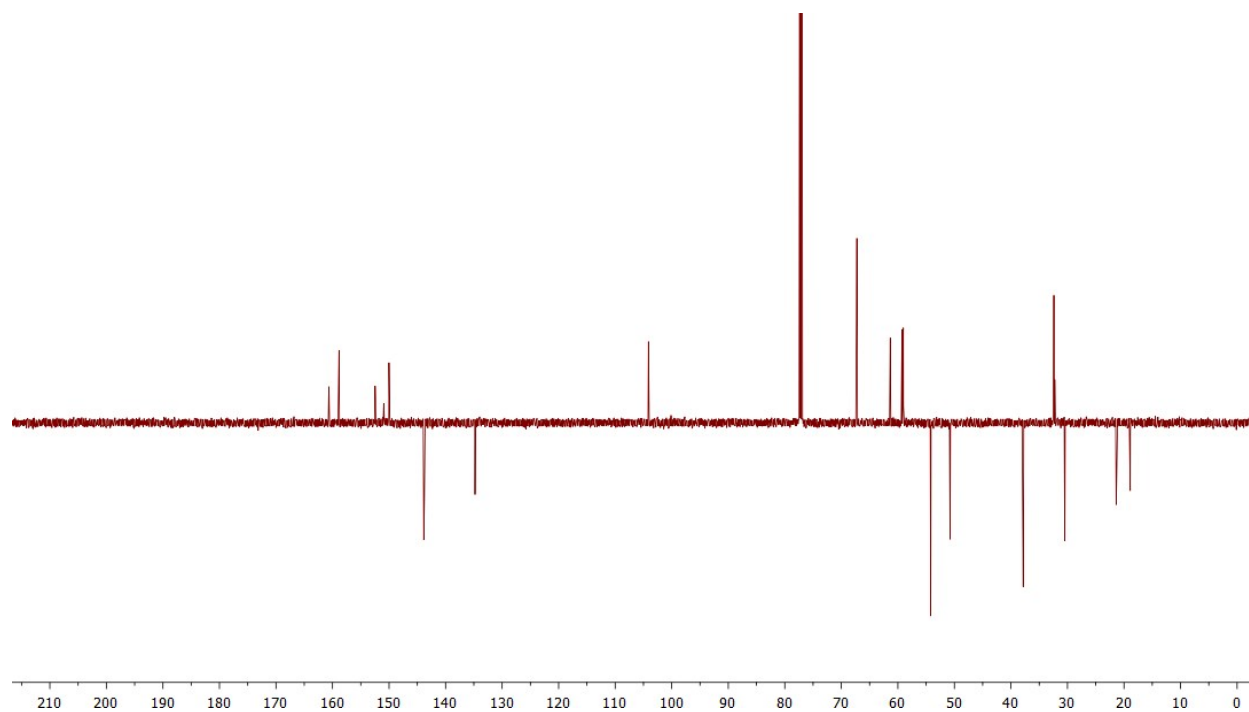


^{19}F NMR spectrum of 10e acquired in CDCl_3 .

Compound **11e**:



¹H NMR spectrum of **11e** acquired in CDCl₃.

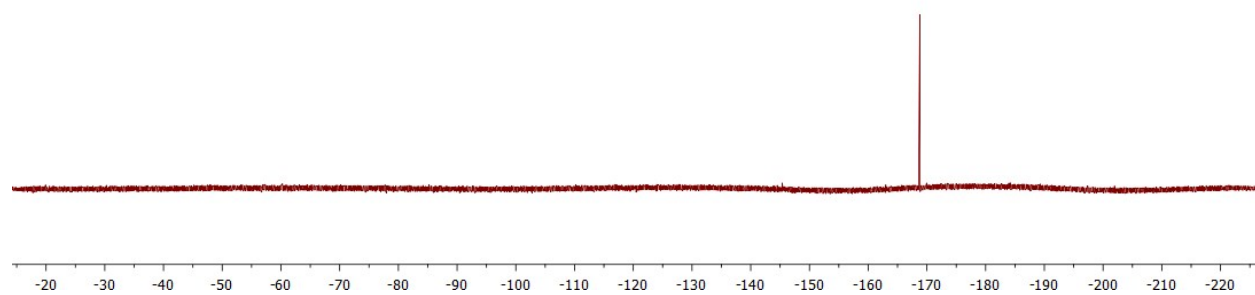


APT spectrum of **11e** acquired in CDCl₃.

8f

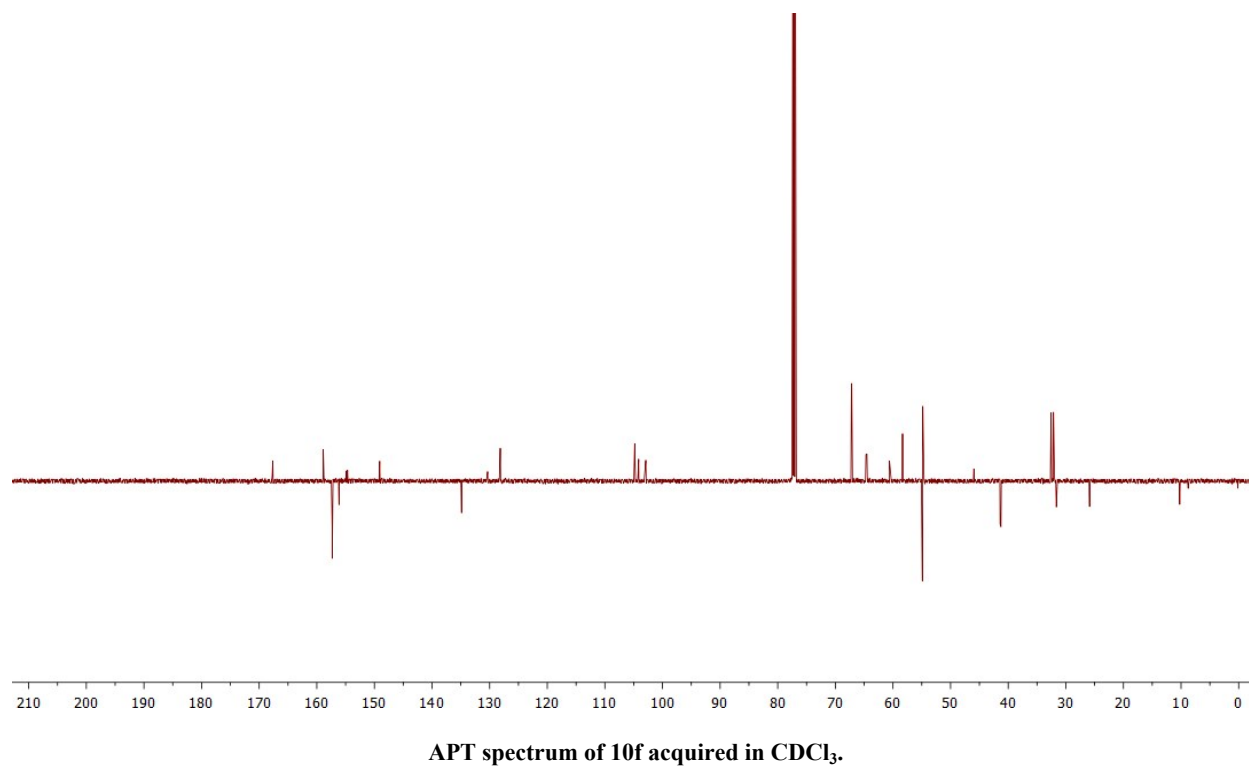
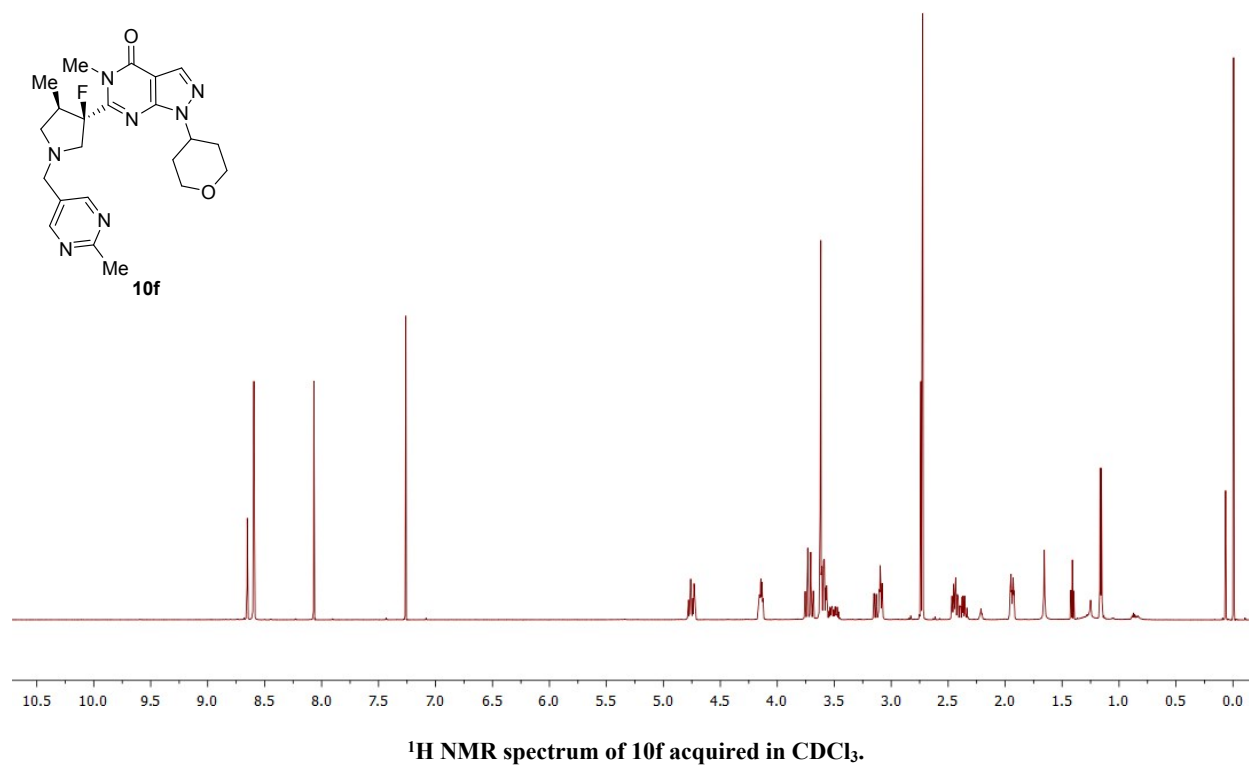
¹H NMR spectrum (CDCl₃) of compound **8f**. The spectrum shows peaks corresponding to the structure, including aromatic protons (7.2, 8.1, 8.6 ppm), a methine proton (5.3 ppm), a methoxy group (3.5 ppm), a methyl group (2.0 ppm), and a methylene group (1.2 ppm). The TMS peak is at 0.0 ppm.

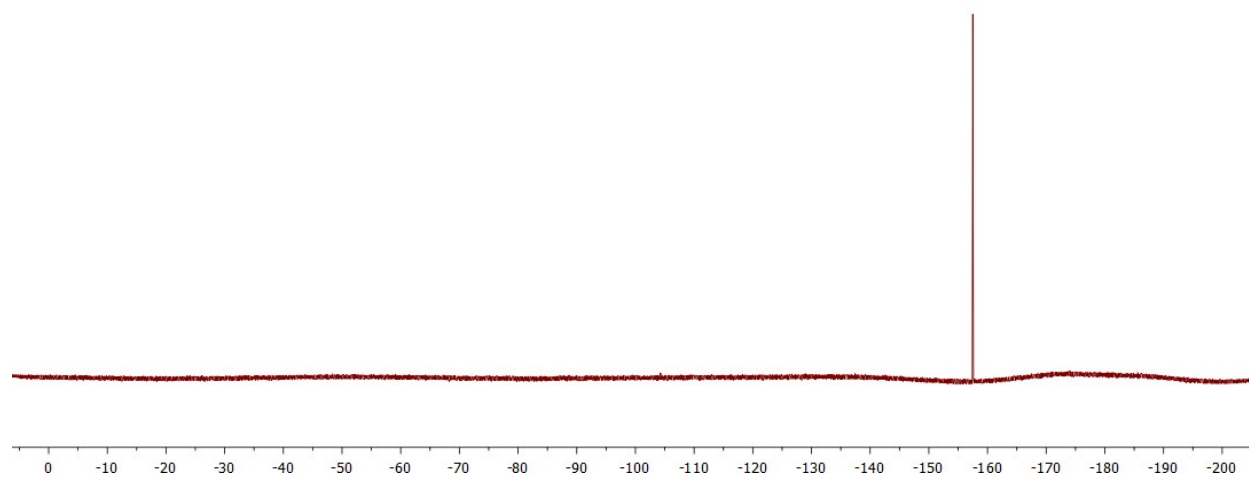
S27



^{19}F NMR spectrum of 8f acquired in CDCl_3 .

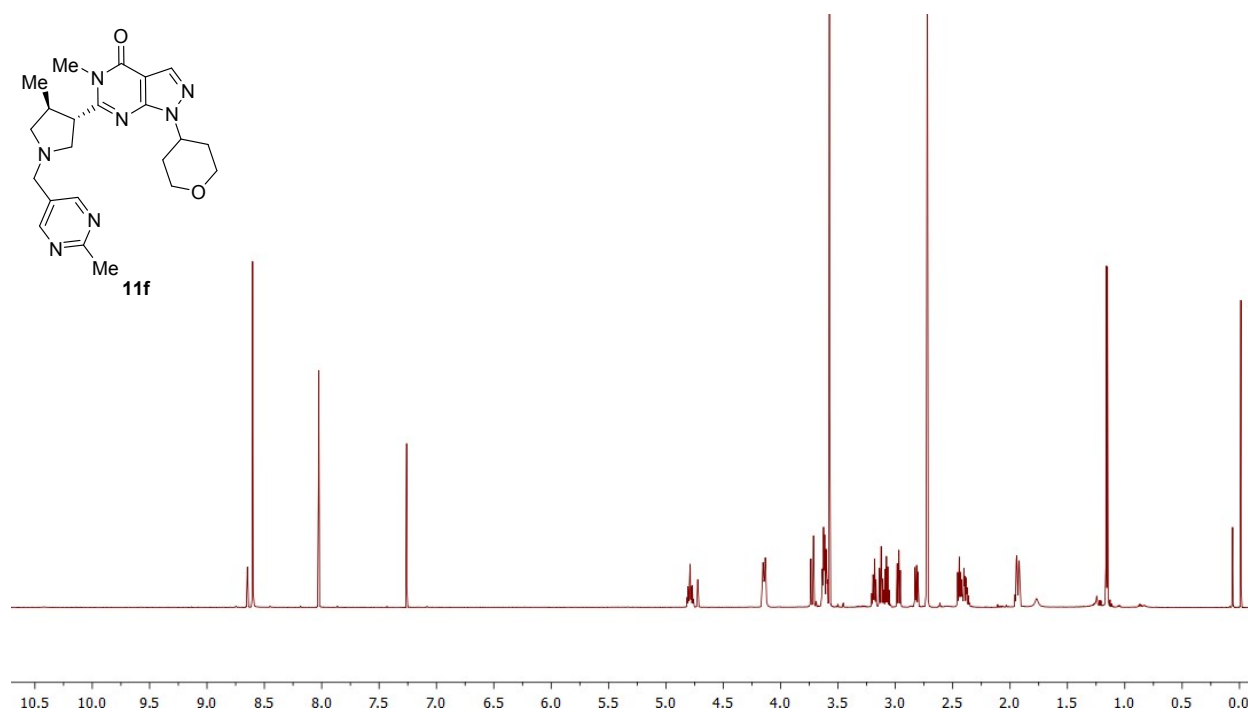
Compound **10f**:



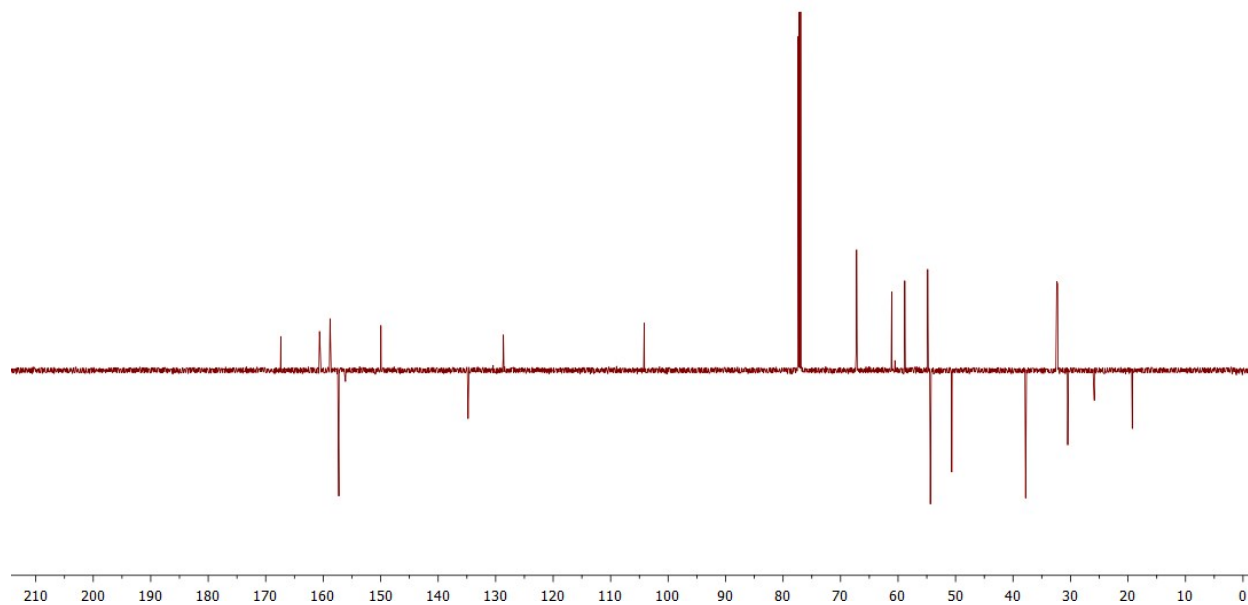


^{19}F NMR spectrum of 10f acquired in CDCl_3 .

Compound **11f**:



^1H NMR spectrum of **11f** acquired in CDCl_3 .



APT spectrum of **11f** acquired in CDCl_3 .

Reference List

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