
Supplementary information

NSD2 targeting reverses plasticity and drug resistance in prostate cancer

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NSD2 targeting reverses plasticity and drug resistance in prostate cancer

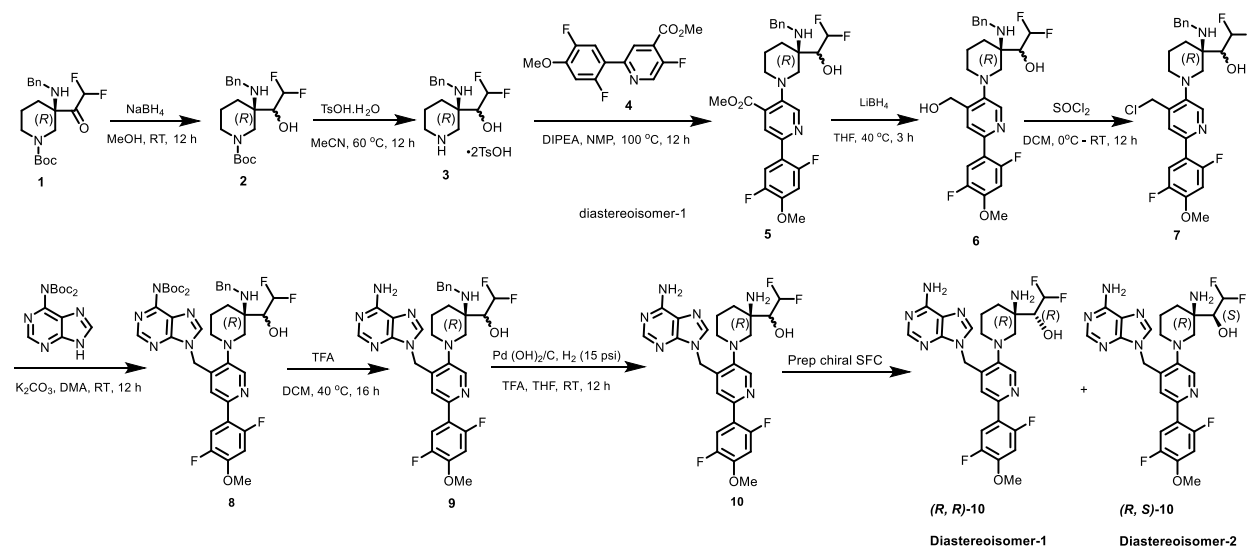
Jia J. Li^{1,2,3,4,9*}, Alessandro Vasciaveo^{2,9,10,11*}, Dimitris Karagiannis^{3,9}, Zhen Sun¹², Kristjan H. Gretarsson^{3,9}, Xiao Chen^{3,9,23}, Ouathek Ouerfelli¹⁴, Fabio Socciarelli¹⁶, Ziv Frankenstein^{7,9}, Hanyang Dong¹⁷, Min Zou^{8,9,24}, Wei Yuan¹⁸, Guangli Yang¹⁴, Gabriel M. Aizenman^{10,11}, Tania Pannellini^{16,25}, Xinjing Xu^{3,9}, Himisha Beltran¹⁹, Yu Chen^{12,13}, Kevin Gardner^{7,9}, Brian D. Robinson¹⁶, Johann de Bono¹⁸, Or Gozani¹⁷, Cory Abate-Shen^{1,2,4,7,8,9}, Mark A. Rubin²⁰, Massimo Loda^{16,21}, Charles L. Sawyers^{12,15}, Andrea Califano^{1,2,5,6,9,22}, Chao Lu^{3,9#}, and Michael M. Shen^{1,2,3,4,9#}

[#]Addresses for correspondence: cl3684@cumc.columbia.edu (C.L.),
mshen@columbia.edu (M.M.S.)

SUPPLEMENTARY INFORMATION

TABLE OF CONTENTS	PAGE
Schema 1: Synthesis of NSD2 inhibitor	S-3
Validation of the two isomers diastereoisomer-1 and diastereoisomer-2	S-4
Schema 2. Synthesis of Cyclic carbamates	S-4
Figure 2. NOESY experiment on cyclic carbamates (<i>R, R</i>)-11 and (<i>R, S</i>)-11	S-5
Experimental Section	S-6
References	S-17
NMR and HPLC Spectra	S-18

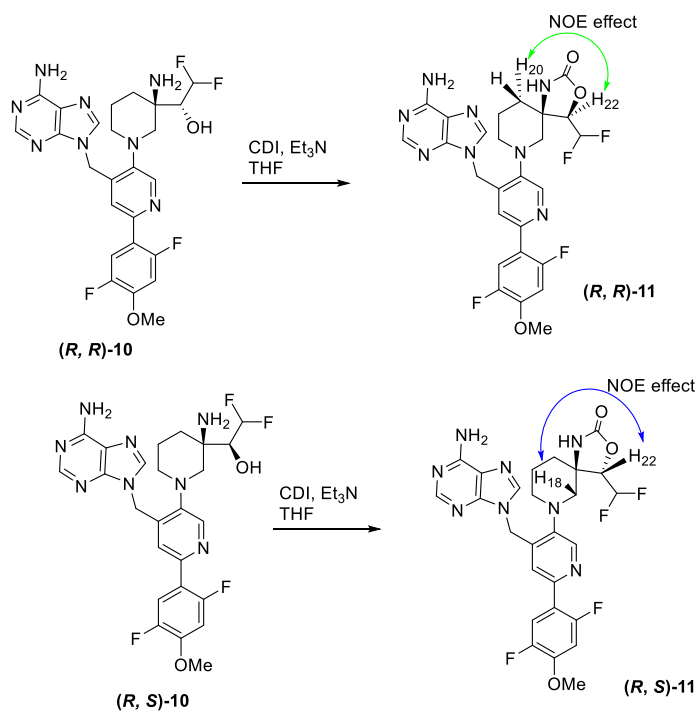
As depicted in Schema 1 below, the synthesis of the NSD2 inhibitor was inspired from the Novartis Patent (Deng et al, 2023) with slight modifications. Briefly, starting from commercially available Boc protected chiral amino ketone **1** which was reduced by sodium borohydride in methanol to provide the diastereomeric mixture of alcohols **2**. The Boc group in **2** was then removed under mild *p*-toluenesulfonic acid (PTSA) conditions at 60 °C to provide piperidine **3** as a bis-PTSA salt. Compound **3** was then condensed with fluoropyridine **4** in *N*-Methyl-2-pyrrolidone (NMP) at 100 °C for 12 hours to give ester **5** in more than 66% yield for the 3 steps. Ester **5** was in turn reduced to the corresponding alcohol **6** before conversion to the respective chloride **7**, which set the stage for reaction with *N,N*-Bis-(Boc) adenine under basic conditions to get to advanced intermediate **8** in 39% yield over 3 steps. Removal of the Boc groups using trifluoroacetic acid (TFA) in dichloromethane (DCM) (1:2, v:v), and hydrogenolysis of the benzyl group in **9** provided compound **10** in 53% yield over 2 steps. Preparative chiral resolution of amino alcohol **10** provided two diastereoisomers, diastereoisomer-1 in 29% yield along with diastereoisomer-2 (Schema 1).



Schema 1: Synthesis of NSD2 inhibitor

Validation of the conformation of the two isomers (*R, R*)-10 (diastereoisomer-1) and (*R, S*)-10 (diastereoisomer-2):

In order to determine and confirm the chiral centers for diastereoisomer-1 and its isomer diastereoisomer-2, we have tested several crystallization conditions. One successful crystallization in water produced white needles. Unfortunately, they didn't diffract X-Rays. Then after running several NMR experiments which were not conclusive, we reckoned that the respective cyclic carbamate of each diastereoisomer should freeze the configuration and would allow Nuclear Overhauser Effects (NOE) to be evaluated based on facial proximities of certain hydrogen atoms. The cyclic carbamates were synthesized by reacting each of the isomers with carbonyldiimidazole (CDI) in the presence of triethylamine in THF. Subsequently NOESY experiments on these cyclic isomers were performed to confirm the stereochemistry (Scheme 2). Predictably, we have registered NOE effect in stereoisomer-1, marked with a green arrow, showing the proximity, and presence on the same face behind the plane of hydrogens H-20 and H-22. On the other hand, for the *R, S*-isomer, another pair of hydrogens; H-18 and H-22 showed NOE effect marked with a blue arrow, and different from the *R, R*-isomer. These NOE effects can only be explained by the configurations *R, R* for **stereoisomer-1** and *R, S* for **stereoisomer-2**.



Scheme 2. Synthesis of Cyclic carbamates

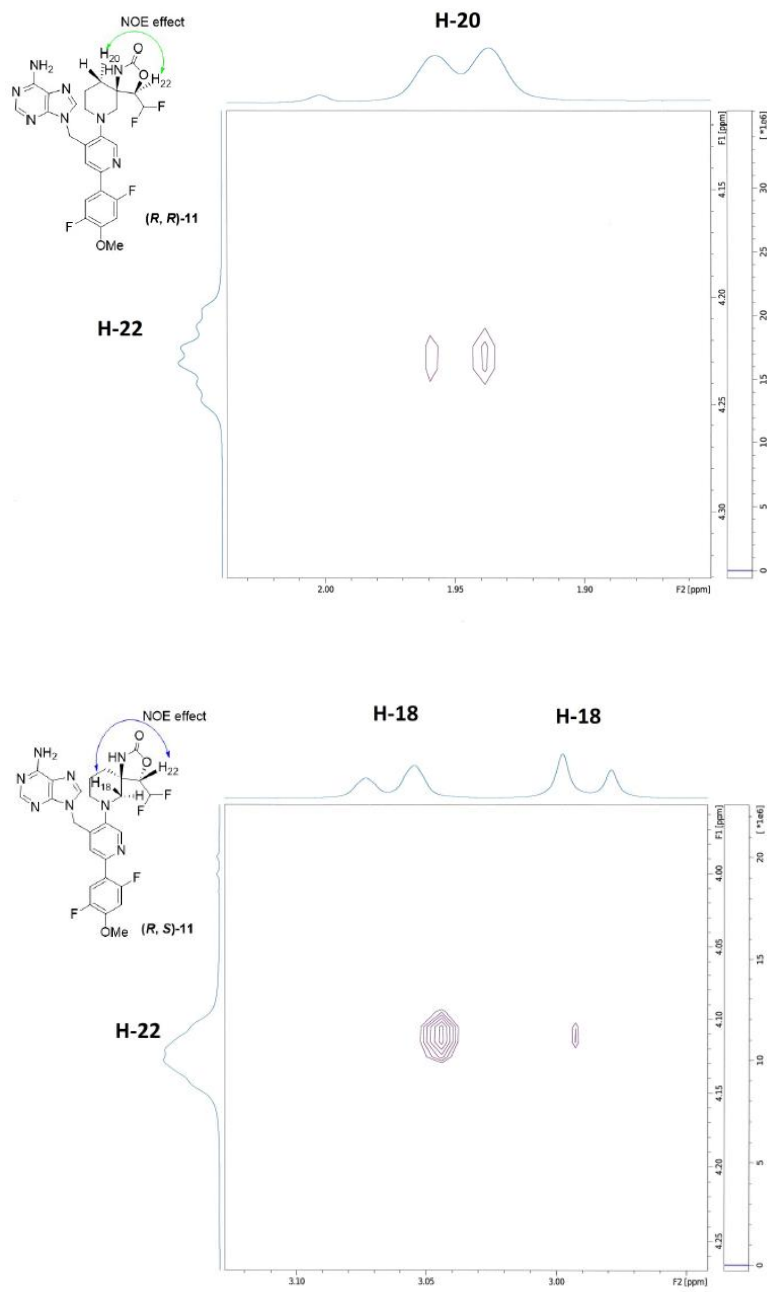


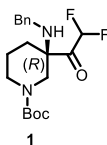
Figure 2. NOESY experiment on cyclic carbamates **(R, R)**-11 and **(R, S)**-11.

Experimental Section

General conditions

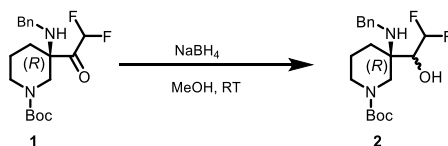
All steps were carefully monitored by the appropriate analytical means. As detailed below, the structures of starting material as well as all intermediates and final compound were carefully analyzed for purity, structural integrity, and chirality wherever applies. Flash column chromatography was performed either manually using Merck-grade silica gel 60 or on a Teledyne ISCO Combiflash NextGen 300+ and prepacked ISCO silica gel columns. NMR spectra were run on a Bruker Avance 600. Specific rotations were measured on a Jasco P-2000 polarimeter, with $[\alpha]_D^{20}$ values reported in degrees with concentrations reported in g/100 mL. LCMS spectra were performed on a Waters Autopure system equipped with 3 detectors in parallel: Evaporative Light Scattering, Diode Array, and Single Quadrupole Mass Spectrometer. NMR values are expressed in parts per million (ppm) and in parentheses peak displays are labeled as follows, bp: broad peak, s: singlet, d: doublet, t: triplet, q: quartet, dd: doublet of doublet, dt: doublet of triplet and m: multiplet. High Resolution Mass Spectra (HRMS) were registered on a Waters LCT Premier XE with electrospray ionization. Melting points were measured on a Fisher Scientific Melting Point apparatus and melting points were not corrected. All starting materials and solvents were purchased from vendors at the highest purity possible and were used without further purification.

Tert-butyl (*R*)-3-(benzylamino)-3-(2,2-difluoroacetyl)piperidine-1-carboxylate (**1**):



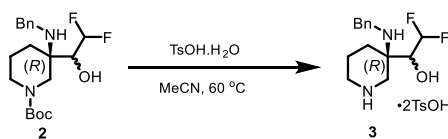
$[\alpha]_D^{20} = +4.87$ ($c = 1.0$, MeOH); ^1H NMR (600 MHz, CDCl_3) δ 7.31 (m, 5H), 6.31 (t, $J = 55.3$ Hz, 1H), 3.86-3.11 (m, 6H), 2.02-1.55 (m, 4H), 1.44 (s, 9H); ^{13}C NMR (151 MHz, CDCl_3) δ 199.8 (t, $J = 21.2$ Hz), 154.8, 139.2, 128.6, 128.1, 127.5, 107.6 (t, $J = 233.1$ Hz), 80.4, 63.4, 47.8, 47.5, 43.2, 30.0, 28.3, 20J 1F); HRMS (ESI) $[\text{M}+\text{H}]^+$ calculated for $\text{C}_{19}\text{H}_{27}\text{F}_2\text{N}_2\text{O}_3^+$: 369.1990, Found: 369.2000.

Tert-butyl (3*R*)-3-(benzylamino)-3-(2,2-difluoro-1-hydroxyethyl)piperidine-1-carboxylate (**2**).



Tert-butyl (*R*)-3-(benzylamino)-3-(2,2-difluoroacetyl)piperidine-1-carboxylate (**1**) (200 g, 542.9 mmol, 1.00 eq) was dissolved in methanol (1.4 L). The solution was then cooled in an ice-water bath before it was treated with sodium borohydride (20.5 g, 542.9 mmol) portion-wise. The resulting reaction mixture was stirred at room temperature for 12 hours. After cooling down in an ice-water bath it was diluted with water (2 L) before extraction with ethyl acetate (600 mL x 3). The combined organic layers were washed with brine (1 L), dried over anhydrous sodium sulfate, filtered over celite, and concentrated under reduced pressure to give a mixture of two diastereomers of *tert*-butyl (3*R*)-3-(benzylamino)-3-(2,2-difluoro-1-hydroxyethyl)piperidine-1-carboxylate (**2**) as a yellow gum, 201 g, 99% yield. This was used as is in the next step: TLC (Ethyl acetate: hexanes, 50: 50 v/v): RF = 0.56; ¹H NMR (600 MHz, CDCl₃) δ 7.31 (m, 5H), 5.94 (dt, *J* = 55.1 and 4.2 Hz, 1H), 4.00-2.80 (m, 7H), 1.91-1.65 (m, 4H), 1.44-1.41 (s, 9H); ¹³C NMR (151 MHz, CDCl₃) δ 155.3, 139.4, 128.7, 128.6, 128.1, 127.4, 127.3, 116.0 (t, *J* = 243.2 Hz), 115.6 (t, *J* = 243.2 Hz), 80.4, 80.3, 71.7 (t, *J* = 22.1 Hz), 56.3, 46.0, 45.4, 28.4, 28.3, 21.1; ¹⁹F NMR (565 MHz, CDCl₃) δ -124.2 (m, 2F); HRMS (ESI) [M+H]⁺ calculated for C₁₉H₂₉F₂N₂O₃⁺: 371.2146, Found: 371.2132.

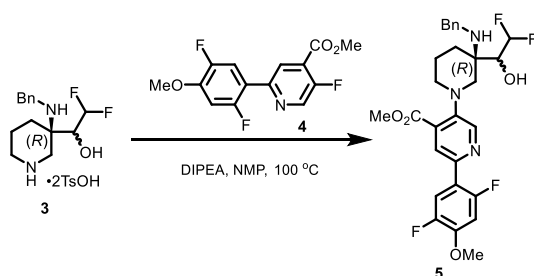
1-((*R*)-3-(Benzylamino)piperidin-3-yl)-2,2-difluoroethan-1-ol (**3**)



Tert-butyl (3*R*)-3-(benzylamino)-3-(2,2-difluoro-1-hydroxyethyl)piperidine-1-carboxylate (**2**) (200 g, 539.9 mmol) was dissolved in acetonitrile (1.4 L). The solution was then treated with *p*-toluenesulfonic acid monohydrate (205.4 g, 1.08 mol) slowly. The resulting reaction mixture was stirred and heated in an oil bath at 60 °C for 12 hours. The reaction mixture was then concentrated to dryness under reduced pressure to give the desired product as a pale white solid 1-((*R*)-3-(benzylamino)piperidin-3-yl)-2,2-difluoroethan-1-ol bis-tosylate salt (**3**) as a mixture of two diastereomers, 331.9 g, 99% yield: ¹H NMR (600 MHz, CD₃OD) δ 7.69 (d, *J* = 8.0 Hz, 4H, 2TsOH), 7.52 (d, *J* = 7.0 Hz, 2H), 7.40 (m, 3H), 7.24 (d, *J* = 8.0 Hz, 4H, 2TsOH),

6.32 (dt, $J = 53.7$ and 4.2 Hz, 1H), 4.50-4.34 (m, 3H), 3.93-3.80 (m, 1H), 3.60-3.57 (m, 1H), 3.33-3.21 (m, 2H), 2.34 (s, 6H, 2TsOH), 2.30-1.97 (m, 4H); ^{13}C NMR (151 MHz, CD_3OD) δ 143.1, 142.0 (TsOH), 132.1, 131.6 (TsOH), 130.9, 130.8, 130.3, 130.2, 129.9 (TsOH), 126.9 (TsOH), 115.8 (t, $J = 243.3$ Hz), 115.7 (t, $J = 243.3$ Hz), 70.2 (m), 61.1, 48.4, 48.0, 46.3, 45.4, 28.7, 22.9, 21.3 (TsOH); ^{19}F NMR (565 MHz, CD_3OD) δ -125.8 (m, 1F), -128.4 (m, 1F); HRMS (ESI) $[\text{M}+\text{H}]^+$ calculated for $\text{C}_{14}\text{H}_{21}\text{F}_2\text{N}_2\text{O}^+$: 271.1622, Found: 271.1620.

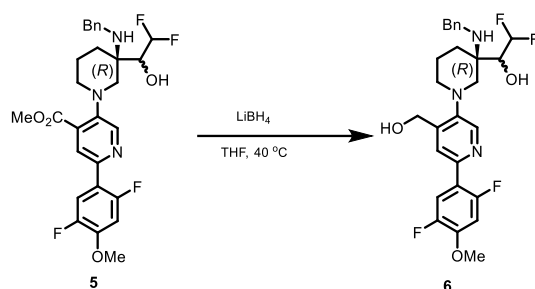
Methyl 5-((3*R*)-3-(benzylamino)-3-(2,2-difluoro-1-hydroxyethyl)piperidin-1-yl)-2-(2,5-difluoro-4-methoxyphenyl)isonicotinate (**5**)



1-((*R*)-3-(Benzylamino)piperidin-3-yl)-2,2-difluoroethan-1-ol (**3**) (2TsOH salts form, 331 g, 538.4 mmol) was dissolved in *N*-Methyl-2-pyrrolidone (NMP) (2.5 L). The solution was added methyl 2-(2,5-difluoro-4-methoxyphenyl)-5-fluoroisonicotinate (**4**) (160 g, 538.4 mmol) and *N,N*-diisopropylethylamine (750 mL, 4.3 mol). The resulting mixture was stirred at 100 °C for 12 hours. The reaction was cooled to room temperature and was poured into water (4 L). The mixture was extracted with ethyl acetate (3 L x 3). The combined organic layers were washed with brine (1 L x 3), dried over anhydrous sodium sulfate for 2 hours, and then filtered over celite, concentrated under vacuum, and the residue was purified by flash column chromatography, silica gel, using the gradient hexanes/ethyl acetate: 100/1 to 1/1, giving methyl 5-((3*R*)-3-(benzylamino)-3-(2,2-difluoro-1-hydroxyethyl)piperidin-1-yl)-2-(2,5-difluoro-4-methoxyphenyl)isonicotinate (**5**) as a mixture of two diastereomers, 200.5 g, 68% yield, as yellow gum: TLC (Ethyl acetate: hexanes, 50:50 v/v): $R_F = 0.47$; ^1H NMR (600 MHz, CDCl_3) δ 8.48-8.46 (s, 1H), 7.95-7.94 (s, 1H), 7.77 (m, 1H), 7.40-7.28 (m, 5H), 6.77-6.73 (m, 1H), 6.11-5.79 (m, 1H), 3.92 (s, 3H), 3.79-3.57 (m, 5H), 3.28-3.22 (m, 2H), 3.08-2.87 (m, 2H), 2.12-1.70 (m, 4H); ^{13}C NMR (151 MHz, CDCl_3) δ 166.7, 166.6, 156.3 (d, $J = 246.8$ Hz), 148.9 (d, $J = 242.2$ Hz), 148.7, 146.8, 146.5, 144.9, 144.8, 142.9, 139.9, 139.4, 132.7, 132.3, 128.6, 128.4, 128.2, 127.4, 127.3, 123.7, 123.6, 118.2-114.0 (m), 101.9, 101.7, 72.5 (t, $J = 21.3$ Hz), 71.4 (t, $J = 20.3$

Hz), 58.8, 56.8, 56.6, 56.5, 54.4, 54.1, 52.6, 52.5, 46.1, 44.9, 28.8, 21.5; ^{19}F NMR (565 MHz, CDCl_3) δ -118.8 (m, 1F), -122.1 to -125.4 (m, 2F), -140.1 (m, 1F); HRMS (ESI) $[\text{M}+\text{H}]^+$ calculated for $\text{C}_{28}\text{H}_{30}\text{F}_4\text{N}_3\text{O}_4^+$: 548.2172, Found: 548.2162.

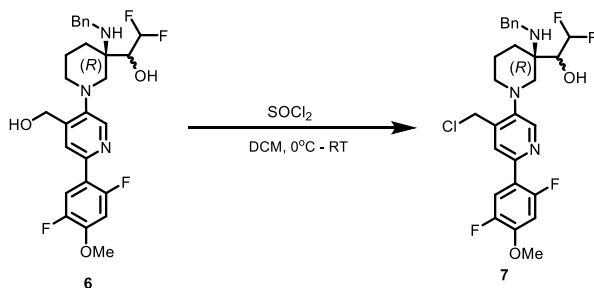
1-((*R*)-3-(Benzylamino)-1-(6-(2,5-difluoro-4-methoxyphenyl)-4-(hydroxymethyl)pyridin-3-yl)piperidin-3-yl)-2,2-difluoroethan-1-ol (**6**)



Methyl 5-((3*R*)-3-(benzylamino)-3-(2,2-difluoro-1-hydroxyethyl)piperidin-1-yl)-2-(2,5-difluoro-4-methoxyphenyl)isonicotinate (**5**) (200 g, 365 mmol) was dissolved in tetrahydrofuran (2.5 L) and methanol (250 mL). The resulting solution was treated with a solution of 2.0 Molar lithium borohydride in tetrahydrofuran (365 mL) dropwise at room temperature. The mixture was heated up to 40 °C and was stirred at that temperature for 3 hours. The reaction mixture was then cooled in an ice-water bath and was quenched with saturated aqueous ammonium chloride solution (3 L). The product was extracted with ethyl acetate (1 L x 3). The combined organic extracts were washed with brine (1 L x 3), dried over anhydrous sodium sulfate, concentrated under vacuum, to give 1-((*R*)-3-(benzylamino)-1-(6-(2,5-difluoro-4-methoxyphenyl)-4-(hydroxymethyl)pyridin-3-yl)piperidin-3-yl)-2,2-difluoroethan-1-ol (**6**) as a mixture of two diastereomers, 180.2 g, 95% yield, as a yellow semi-solid which was used as is in the next step: TLC (Ethyl acetate:hexanes, 50:50 v/v): R_F = 0.29; ^1H NMR (600 MHz, CDCl_3) δ 8.43-8.39 (s, 1H), 7.81-7.80 (s, 1H), 7.73 (m, 1H), 7.36-7.33 (m, 4H), 7.30-7.7 (m, 1H), 6.76-6.72 (m, 1H), 6.13-5.81 (m, 1H), 4.76-4.68 (m, 2H), 3.91 (m, 3H), 3.78-3.64 (m, 3H), 3.15-2.79 (m, 4H), 2.10-1.80 (m, 4H); ^{13}C NMR (151 MHz, CDCl_3) δ 156.3 (d, J = 246.4 Hz), 148.9 (d, J = 242.1 Hz), 148.7, 148.6, 148.5, 145.0, 144.6, 144.3, 144.2, 142.5, 142.2, 139.9, 139.5, 128.8, 128.7, 128.1, 128.0, 127.5, 127.5, 122.8 (d, J = 10.6 Hz), 122.4 (d, J = 10.6 Hz), 118.9 (dd, J = 13.7 and 6.1 Hz), 116.7 (dd, J = 21.7 and 4.9 Hz), 115.8 (t, J = 244.4 Hz), 115.7 (t, J = 243.2 Hz), 101.7 (d, J = 29.5 Hz), 71.7 (t, J = 22.2 Hz), 61.2, 60.9, 59.0, 57.2, 56.9, 56.8, 56.5, 54.0, 53.7, 49.5, 46.1,

45.1, 28.8, 27.9, 27.2, 22.1, 22.0; ^{19}F NMR (565 MHz, CDCl_3) δ -118.9 (m, 1F), -123.3 (d, J = 50.8 Hz, 1F), -124.8 (m, 1F), -140.4 (m, 1F); HRMS (ESI) $[\text{M}+\text{H}]^+$ calculated for $\text{C}_{27}\text{H}_{30}\text{F}_4\text{N}_3\text{O}_3^+$: 520.2223, Found: 520.2213.

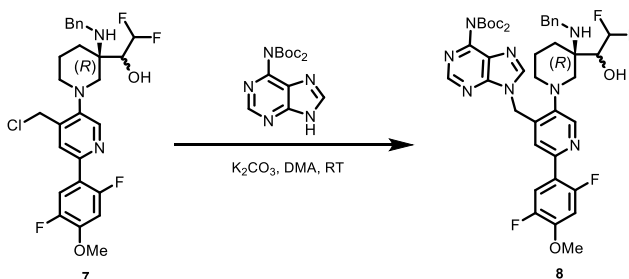
1-((*R*)-3-(Benzylamino)-1-(4-(chloromethyl)-6-(2,5-difluoro-4-methoxyphenyl)pyridin-3-yl)piperidin-3-yl)-2,2-difluoroethan-1-ol (**7**)



1-((*R*)-3-(Benzylamino)-1-(6-(2,5-difluoro-4-methoxyphenyl)-4-(hydroxymethyl)pyridin-3-yl)piperidin-3-yl)-2,2-difluoroethan-1-ol (**6**) (180 g, 346.5 mmol) was dissolved in anhydrous dichloromethane (2.5 L). The solution was cooled down to 4 °C using an ice-water bath and treated with thionyl chloride (63 mL, 866 mmol) under positive argon flow. The mixture was stirred at 0 -5 °C for 2 hours. Water (250 mL) was carefully added, and the mixture was stirred at room temperature for 10 hours. The reaction mixture was carefully neutralized with 10% aqueous sodium carbonate solution (2 L) and extracted with dichloromethane (2 L x 3). The combined organic layers were washed with brine (1 L x 3), briefly dried over anhydrous sodium sulfate, and concentrated under vacuum to provide 1-((*R*)-3-(benzylamino)-1-(4-(chloromethyl)-6-(2,5-difluoro-4-methoxyphenyl)pyridin-3-yl)piperidin-3-yl)-2,2-difluoroethan-1-ol (**7**) (mixture of two diastereomers, 167.8 g, 90% yield) as a yellow semi-solid. This was treated in the next step without purification: TLC (Ethyl acetate: hexanes, 50: 50 v/v): R_F = 0.51; ^1H NMR (600 MHz, CDCl_3) δ 8.45 (s, 1H), 7.82-7.76 (m, 2H), 7.37-7.36 (m, 4H), 7.30-7.29 (m, 1H), 6.77-6.74 (m, 1H), 6.14-5.81 (m, 1H), 4.64-4.54 (m, 2H), 3.92 (s, 3H), 3.84-3.66 (m, 3H), 3.19-2.84 (m, 4H), 2.14-1.74 (m, 4H); ^{13}C NMR (151 MHz, CDCl_3) δ 156.4 (d, J = 246.8 Hz), 148.9 (d, J = 242.2 Hz), 148.8, 148.6, 145.3, 145.2, 143.4, 141.0, 140.8, 128.8, 128.7, 128.1, 128.0, 127.5, 127.4, 125.0 (d, J = 11.3 Hz), 124.9 (d, J = 11.2 Hz), 118.4 (dd, J = 13.7 and 6.1 Hz), 116.7 (dd, J = 21.7 and 4.2 Hz), 115.8 (t, J = 243.4 Hz), 115.7 (t, J = 243.2 Hz), 101.7 (d, J = 30.2 Hz), 72.2 (t, J = 22.0 Hz), 71.5 (t, J = 22.8 Hz), 59.0, 57.3, 57.1, 56.5, 54.6, 54.5, 46.1, 45.0,

40.9, 40.8, 28.8, 22.1, 22.0; ^{19}F NMR (565 MHz, CDCl_3) δ -118.8 (m, 1F), -123.2 (m, 1F), -125.0 (m, 1F), -140.2 (m, 1F); HRMS (ESI) $[\text{M}+\text{H}]^+$ calculated for $\text{C}_{27}\text{H}_{29}\text{ClF}_4\text{N}_3\text{O}_2^+$: 538.1884, Found: 538.1887.

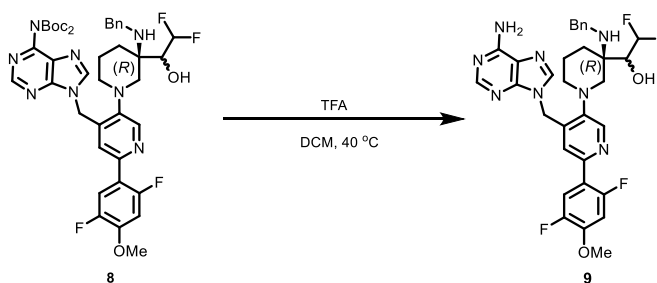
Tert-butyl (9-((5-((3*R*)-3-(benzylamino)-3-(2,2-difluoro-1-hydroxyethyl)piperidin-1-yl)-2-(2,5-difluoro-4-methoxyphenyl)pyridin-4-yl)methyl)-9H-purin-6-yl)(*tert*-butoxycarbonyl)carbamate (**8**)



1-((*R*)-3-(Benzylamino)-1-(4-(chloromethyl)-6-(2,5-difluoro-4-methoxyphenyl)pyridin-3-yl)piperidin-3-yl)-2,2-difluoroethan-1-ol (**7**) (167 g, 310.4 mmol) and *N,N*-bis(9H-purin-6-yl)carbamate (125 g, 372.5 mmol) was dissolved in *N,N*-dimethylacetamide (1.5 L) and the solution was treated with potassium carbonate (86 g, 620.8 mmol) portion-wise. The resulting reaction mixture was stirred at room temperature for 12 hours. It was then cooled in an ice-water bath dilution with water (2 L), and extraction with ethyl acetate (1.5 L x 3). The combined organic layers were washed with brine (1 L x 3), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography, silica gel, with the gradient hexanes/ ethyl acetate: 100/1 to 1/1. *Tert*-butyl (9-((5-((3*R*)-3-(benzylamino)-3-(2,2-difluoro-1-hydroxyethyl)piperidin-1-yl)-2-(2,5-difluoro-4-methoxyphenyl)pyridin-4-yl)methyl)-9H-purin-6-yl)(*tert*-butoxycarbonyl)carbamate (**8**) was isolated as a mixture of two diastereomers, 119.5 g, 46% yield, as white solid: TLC (Ethyl acetate: hexanes, 50: 50 v/v): R_F = 0.19; ^1H NMR (600 MHz, $\text{DMSO}-d_6$) δ 8.77 (s, 1H), 8.67-8.65 (s, 1H), 8.55-8.54 (s, 1H), 7.66-7.63 (m, 1H) 7.40-7.38 (m, 2H), 7.22-7.10 (m, 3H), 7.05-7.01 (m, 1H), 6.90-6.89 (s, 1H), 6.38-6.13 (m, 1H), 5.86- 5.65 (m, 3H), 3.99- 3.73 (m, 6H), 3.34-2.75 (m, 4H), 2.43 (s, 1H), 2.00-1.69 (m, 4H), 1.32 (s, 18H); ^{13}C NMR (151 MHz, $\text{DMSO}-d_6$) δ 155.6 (d, J =245.0 Hz), 153.0, 152.9, 151.7, 149.7, 149.6, 149.2, 147.7, 148.3 (t, J = 12.5 Hz), 147.3, 147.2, 147.1, 146.4, 145.5, 145.4, 142.9, 142.8, 141.6, 141.5, 140.2, 140.1, 128.0, 127.9,

127.8, 127.7, 127.6 (d, $J = 8.3$ Hz), 126.3 (d, $J = 12.3$ Hz), 120.0 (d, $J = 11.1$ Hz), 117.7 (d, $J = 6.2$ Hz), 117.6 (d, $J = 6.2$ Hz), 116.6 (t, $J = 240.0$ Hz), 116.0 (t, $J = 241.1$ Hz), 115.8 (d, $J = 4.9$ Hz), 115.6 (d, $J = 4.9$ Hz), 102.5 (d, $J = 28.8$ Hz), 83.2, 72.5 (t, $J = 22.7$ Hz), 71.4 (t, $J = 22.0$ Hz), 56.6, 56.1, 53.7, 53.0, 45.6, 45.1, 42.3, 42.2, 27.2, 21.4, 21.0; ^{19}F NMR (565 MHz, DMSO- d_6) δ -118.9 (m, 1F), -121.4 (dd, $J = 282.1$ and 52.8 Hz, 1F), -126.6 (ddd, $J = 283.9$, 56.0 and 15.4 Hz, 1F), -140.0 (m, 1F); HRMS (ESI) $[\text{M}+\text{H}]^+$ calculated for $\text{C}_{42}\text{H}_{49}\text{F}_4\text{N}_8\text{O}_6^+$: 837.3711, Found: 837.3719.

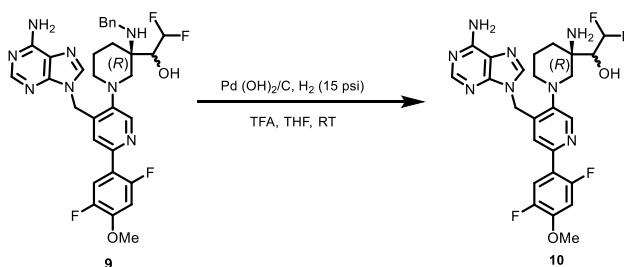
1-((*R*)-1-(4-(((6-Amino-9H-purin-9-yl)methyl)-6-(2,5-difluoro-4-methoxyphenyl)pyridin-3-yl)-3-(benzylamino)piperidin-3-yl)-2,2-difluoroethan-1-ol (**9**)



Tert-butyl (9-(((5-((3*R*)-3-(benzylamino)-3-(2,2-difluoro-1-hydroxyethyl)piperidin-1-yl)-2-(2,5-difluoro-4-methoxyphenyl)pyridin-4-yl)methyl)-9H-purin-6-yl)(*tert*-butoxycarbonyl) carbamate (**8**) (119 g, 142.2 mmol) was dissolved in dichloromethane (1 L). The solution was carefully treated with trifluoroacetic acid (500 mL). The solution was then stirred at 40 °C for 16 hours. The solvents were removed under reduced pressure. The residue was re-dissolved in methanol (200 mL) and then slowly poured into 10% aqueous sodium carbonate solution (2 L). The resulting precipitate was filtered over Buchner, washed with water, and dried under vacuum, to get 1-((*R*)-1-(4-(((6-amino-9H-purin-9-yl)methyl)-6-(2,5-difluoro-4-methoxyphenyl)pyridin-3-yl)-3-(benzylamino)piperidin-3-yl)-2,2-difluoroethan-1-ol (**9**) as a mixture of two diastereomers, 83.3 g, 92% yield, as white solid. This was used as is in the next step: TLC (MeOH: methylene chloride, 10: 90 v/v): $R_F = 0.56$; ^1H NMR (600 MHz, CD_3OD) δ 8.59-8.56 (s, 1H), 8.24 (s, 1H), 8.07-8.03 (s, 1H), 7.63- 7.55 (m, 3H), 7.39-7.36 (m, 3H), 7.22-7.20 (s, 1H), 6.94-6.90 (m, 1H), 6.33 (t, $J = 54.4$ Hz, 1H), 5.70-5.60 (m, 2H), 4.50-4.40 (m, 3H), 3.87 (s, 3H), 3.73-3.39 (m, 2H), 3.06-2.95 (m, 2H), 2.22-1.85 (m, 4H); ^{13}C NMR (151 MHz, CD_3OD) δ 156.3 (d, $J = 245.8$ Hz), 156.1, 156.0, 152.7, 152.6, 149.4, 149.3 (d, $J = 16.4$ Hz), 149.1, 149.0, 148.7 (d, $J = 242.0$ Hz), 144.5, 144.4, 143.5, 143.2, 141.6, 141.5, 141.4, 141.3, 129.7, 129.6, 128.8, 128.7, 121.9, 121.8,

118.7, 117.8, 117.5 (dd, $J = 13.8$ and 5.7 Hz), 115.8, 115.7, 114.9 (t, $J = 241.0$ Hz), 114.6 (t, $J = 243.1$ Hz), 101.7 (d, $J = 30.2$ Hz), 69.5, 67.5, 55.7, 54.3, 54.0, 48.2, 46.6, 45.6, 42.2, 41.9, 26.8, 25.7, 25.1, 20.7; ^{19}F NMR (565 MHz, CD_3OD) δ -120.8 (m, 1F), -124.8 (m, 1F), -127.9 (m, 1F), -142.0 (m, 1F); HRMS (ESI) $[\text{M}+\text{H}]^+$ calculated for $\text{C}_{32}\text{H}_{33}\text{F}_4\text{N}_8\text{O}_2^+$: 637.2663, Found: 637.2667.

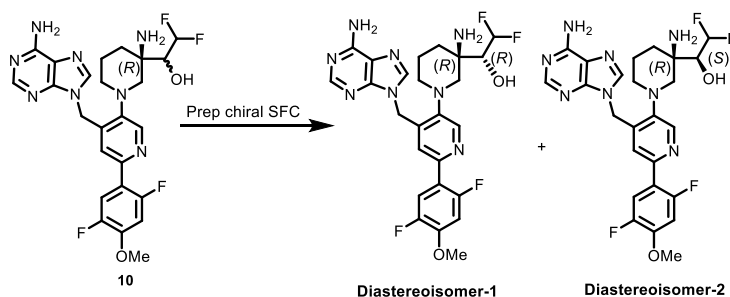
1-((*R*)-3-Amino-1-(4-((6-amino-9H-purin-9-yl)methyl)-6-(2,5-difluoro-4-methoxyphenyl)pyridin-3-yl)piperidin-3-yl)-2,2-difluoroethan-1-ol (**10**)



1-((*R*)-1-(4-((6-Amino-9H-purin-9-yl)methyl)-6-(2,5-difluoro-4-methoxyphenyl)pyridin-3-yl)-3-(benzylamino)piperidin-3-yl)-2,2-difluoroethan-1-ol (**9**) (83 g, 130.4 mmol) was dissolved in tetrahydrofuran (1 L). The solution was treated with trifluoroacetic acid (30 mL, 391 mmol) and palladium hydroxide on carbon (10%, 30 g). The resulting black suspension was degassed and purged with hydrogen for 3 times. The reaction was then stirred under hydrogen atmosphere (15 psi) at 40 °C for 12 hours. After purging with nitrogen, the catalyst was filtered over a thick packed bed of celite and the filtrate was concentrated under reduced pressure to give the crude 1-((*R*)-3-amino-1-(4-((6-amino-9H-purin-9-yl)methyl)-6-(2,5-difluoro-4-methoxyphenyl)pyridin-3-yl)piperidin-3-yl)-2,2-difluoroethan-1-ol (**10**) as a mixture of two diastereoisomers, 41.5 g, 58% yield, as an off-white solid: m.p.: 192–198 °C; ^1H NMR (600 MHz, $\text{DMSO}-d_6$) δ 8.49–8.47 (s, 1H), 8.26 (s, 1H), 8.11 (s, 1H), 7.69 (dd, $J = 12.3$ and 7.4 Hz, 1H), 7.30 (s, 2H), 7.11 (dd, $J = 12.3$ and 7.4 Hz, 1H), 6.97–6.91 (s, 1H), 6.28–6.08 (m, 1H), 5.63–5.54 (m, 3H), 3.83 (s, 3H), 3.54 (s, 1H), 3.11–2.78 (m, 4H), 1.98–1.46 (m, 6H); ^{13}C NMR (151 MHz, $\text{DMSO}-d_6$) δ 156.5, 155.6 (d, $J = 245.1$ Hz), 152.8, 149.8, 149.7, 148.3 (t, $J = 12.4$ Hz), 148.0 (d, $J = 238.1$ Hz), 146.4, 145.7, 145.6, 142.8, 142.7, 141.4, 141.3, 141.0, 140.9, 120.9 (d, $J = 11.9$ Hz), 120.8 (d, $J = 11.9$ Hz), 118.6, 118.5, 117.7 (dd, $J = 13.6$ and 6.1 Hz), 116.0 (t, $J = 240.6$ Hz), 115.9 (t, $J = 240.6$ Hz), 115.6 (dd, $J = 21.4$ and 4.9 Hz), 102.8 (d, $J = 31.3$ Hz), 74.1, 60.6, 60.1, 56.6, 53.2, 53.1, 53.0, 52.9, 41.8, 41.7, 31.1, 31.0, 21.4, 21.0; ^{19}F NMR (565 MHz,

DMSO- d_6) δ -118.9 (m, 1F), -120.9 to -122.5 (m, 1F), -126.8 to -127.9 (m, 1F), -140.1 (m, 1F); HRMS (ESI) $[M+H]^+$ calculated for $C_{25}H_{27}F_4N_8O_2^+$: 547.2193, Found: 547.2205.

(*R*)-1-((*R*)-3-Amino-1-(4-((6-amino-9H-purin-9-yl)methyl)-6-(2,5-difluoro-4-methoxyphenyl)pyridin-3-yl)piperidin-3-yl)-2,2-difluoroethan-1-ol, **Diastereoisomer-1**



The crude 1-((*R*)-3-amino-1-(4-((6-amino-9H-purin-9-yl)methyl)-6-(2,5-difluoro-4-methoxyphenyl)pyridin-3-yl)piperidin-3-yl)-2,2-difluoroethan-1-ol (**10**) mixture of two diastereoisomers, 41 g, was isolated by supercritical fluid chromatography (column: DAICEL CHIRALPAK IC (250 mm x 50 mm, 10 μ m), mobile phase: CO_2 / MeOH (40% / 60%, with 0.1% $NH_3 \cdot H_2O$), to provide (*R*)-1-((*R*)-3-amino-1-(4-((6-amino-9H-purin-9-yl)methyl)-6-(2,5-difluoro-4-methoxyphenyl)pyridin-3-yl)piperidin-3-yl)-2,2-difluoroethan-1-ol (**Diastereoisomer-1**), 11.9 g, 29% yield as a white solid: m.p.: 202–203 $^{\circ}C$; LC-MS: 10-50% Acetonitrile/Water (both containing 0.05% TFA), retention time $t = 5.82$ min; $[\alpha]_D^{20} = +5.32$ ($c = 1.0$, MeOH); 1H NMR (600 MHz, DMSO- d_6) δ 8.49 (s, 1H), 8.27 (s, 1H), 8.12 (s, 1H), 7.69 (dd, $J = 12.3$ and 7.4 Hz, 1H), 7.30 (s, 2H), 7.13 (dd, $J = 12.3$ and 7.4 Hz, 1H), 6.97 (s, 1H), 6.20 (t, $J = 55$ Hz, 1H), 5.63 (s, 1H), 5.58 (s, 2H), 3.84 (s, 3H), 3.56 (s, 1H), 3.07 (d, $J = 11.3$ Hz, 2H), 2.88 (d, $J = 11.3$ Hz, 1H), 2.81 (t, $J = 10.4$ Hz, 1H), 1.98 (m, 3H), 1.69 (m, 2H), 1.54 (d, $J = 12.2$ Hz, 1H); ^{13}C NMR (151 MHz, DMSO- d_6) δ 156.4, 155.6 (d, $J = 245.1$ Hz), 152.8, 149.7, 148.2 (t, $J = 12.4$ Hz), 148.0 (d, $J = 238.1$ Hz), 146.3, 145.6, 142.7, 141.2, 140.9, 120.9 (d, $J = 11.9$ Hz), 118.6, 117.7 (dd, $J = 13.6$ and 6.1 Hz), 115.9 (t, $J = 240.6$ Hz), 115.6 (dd, $J = 21.4$ and 4.9 Hz), 102.7 (d, $J = 31.3$ Hz), 74.1, 60.1, 56.6, 53.1, 53.0, 41.7, 31.1, 21.0; ^{19}F NMR (565 MHz, DMSO- d_6) δ -118.9 (m, 1F), -122.5 (dd, $J = 285.0$ and 54.9 Hz, 1F), -127.5 (ddd, $J = 285.1$, 55.7 and 16.8 Hz, 1F), -140.1 (m, 1F); HRMS (ESI) $[M+H]^+$ calculated for $C_{25}H_{27}F_4N_8O_2^+$: 547.2193, Found: 547.2190.

Further Validation of Diastereoisomers 1 and 2:

LCMS: XBridge C-18 column 3.5 μm , 4.6 x 150 mm, flow rate 1.4 ml/min (Waters), 10 to 50% gradient Acetonitrile in water (both containing 0.05% TFA) on a 12 min run, single peak detected in UV detection at 5.82 minutes for **Diastereoisomers -1**, 5.53 minutes for **Diastereoisomers -2** corresponding to a mass peak in positive mode of 547.4 which matches $[\text{M}+1]^+$ peak with an area providing a 98.7 % purity. The calculated value for a formula consisting of $\text{C}_{25}\text{H}_{26}\text{F}_4\text{N}_8\text{O}_2$ is 546.21.

Chiral Resolution Assessment for **Diastereoisomers -1**:

Method: Supercritical Fluid Chromatography (SFC).

Column: Chiralpack IC-3, 50 x 4.5 mm, 3 μm .

Mobile Phase: A: CO_2 , B: MeOH (containing 0.2% NH_3 (7.0 M in MeOH), v/v)

Gradient: Isocratic A : B = 40 : 60

Flow Rate: 4 mL/min

Column Temperature: 35 $^\circ\text{C}$

Result: single enantiomer.

Product RT = 0.952 min, 100% *e.e* under 220 nm.

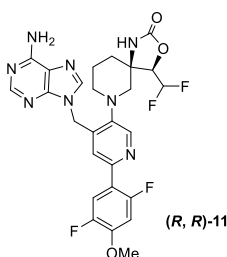
(*S*)-1-((*R*)-3-Amino-1-(4-((6-amino-9H-purin-9-yl)methyl)-6-(2,5-difluoro-4-methoxyphenyl)pyridin-3-yl)piperidin-3-yl)-2,2-difluoroethan-1-ol (**Diastereoisomer-2**)

m.p.: 177–178 $^\circ\text{C}$; LC-MS: 10-50% Acetonitrile/Water (both containing 0.05% TFA), retention time $t = 5.53$ min; $[\alpha]_{\text{D}}^{20} = +4.45$ ($c = 1.0$, MeOH); ^1H NMR (600 MHz, DMSO-d_6) δ 8.49 (s, 1H), 8.26 (s, 1H), 8.12 (s, 1H), 7.68 (dd, $J = 12.4$ and 7.4 Hz, 1H), 7.30 (s, 2H), 7.12 (dd, $J = 12.8$ and 7.3 Hz, 1H), 6.92 (s, 1H), 6.18 (dt, $J = 55.9$ and 3.4 Hz, 1H), 5.73 (s, 1H), 5.55 (dd, $J = 22.0$ and 17.0 Hz, 2H), 3.83 (s, 3H), 3.78 (s, 1H), 3.11-2.75 (m, 4H), 1.88-1.45 (m, 4H), 1.69 (m, 2H), 1.54 (d, $J = 12.2$ Hz, 1H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 156.0, 155.6 (d, $J = 244.8$ Hz), 152.7, 149.7, 148.2 (t, $J = 12.4$ Hz), 148.0 (d, $J = 240.3$ Hz), 146.3, 145.5, 142.6, 141.3, 141.0, 120.8 (d, $J = 11.9$ Hz), 118.5, 117.7 (dd, $J = 13.6$ and 6.1 Hz), 116.1 (t, $J = 241.3$ Hz), 115.6 (dd, $J = 21.6$ and 5.0 Hz), 102.8 (d, $J = 29.9$ Hz), 72.3, 60.6, 56.6, 52.9, 52.8, 41.8, 32.3, 21.3; ^{19}F NMR (565 MHz, DMSO-d_6) δ -118.9 (m, 1F), -121.2 (d, $J = 309.3$ Hz, 1F), -127.4 (d, J

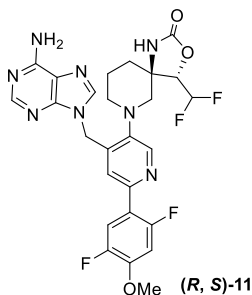
= 283.9 Hz, 1F), -140.1 (m, 1F); HRMS (ESI) $[M+H]^+$ calculated for $C_{25}H_{27}F_4N_8O_2^+$: 547.2193, Found: 547.2200.

General Procedure to synthesize the cyclic carbamates:

Diastereoisomers -1 (23.0 mg, 42 μ mol) was dissolved in anhydrous THF (1 mL), and Et_3N (8 μ L, 57 μ mol) was added followed by 1,1-carbonyldiimidazole (8 mg, 5.1 μ mol) at 0 °C. The reaction mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure. The crude product was purified by column chromatography (5% to 10% MeOH/DCM) providing cyclic carbamate (**(R, R)-11**) as a white solid.



(R, R)-11 1H NMR (600 MHz, CH_2Cl_2 - d_2) δ 9.39 (brs, 1 H, NH), 8.44 (s, 1H), 8.41 (s, 1H), 7.78 (s, 1H), 7.74 (s, 1H), 7.71 (m, 1H), 6.72 (dd, J = 7.0, 12.5 Hz, 1H), 5.91 (complex, 3H including NH_2), 5.57 (d, J = 14.5 Hz, 1H), 5.06 (d, J = 14.5 Hz, 1H), 4.23 (m, 1H), 3.83 (s, 3H, OMe), 3.21 (d, J = 11.0 Hz, 1H), 3.09 (d, J = 11.1 Hz, 1H), 2.81 (t, J = 11.3 Hz, 1H), 2.62 (d, J = 11.1 Hz, 1H), 1.95 (d, J = 12.2 Hz, 1H), 1.62 (m, 3H); LCMS (ESI) $[M+H]^+$ calculated for $C_{26}H_{25}F_4N_8O_3^+$: 573.20, Found: 573.32.



(R, S)-11 1H NMR (600 MHz, CH_2Cl_2 - d_2) δ 9.35 (brs, 1 H, NH), 8.40 (s, 1H), 8.36 (s, 1H), 7.71 (s, 1H), 7.70 (m, 1H), 7.63 (s, 1H), 6.69 (dd, J = 7.0, 12.5 Hz, 1H), 6.10 (brs, 2H, NH_2), 5.95 (dt, J = 5.3, 54.1 Hz, 1H), 5.55 (d, J = 14.8 Hz, 1H), 5.04 (d, J = 14.8 Hz, 1H), 4.12 (m, 1H), 3.81 (s, 3H, OMe), 3.07 (d, J = 11.3 Hz, 1H), 2.99 (d, J = 11.3 Hz, 1H), 2.80 (t, J = 11.3 Hz, 1H), 2.67

(d, $J = 10.8$ Hz, 1H), 1.95 (d, $J = 11.6$ Hz, 1H), 1.67-1.56 (m, 3H); LCMS (ESI) $[M+H]^+$ calculated for $C_{26}H_{25}F_4N_8O_3^+$: 573.20, Found: 573.52.

Reference: Deng H. et al. US Patent No.: US 2023/0002388, Pub. Date: Jan. 5, 2023

Chemical structure of compound **1** is shown as an inset. The structure is a piperidine ring with a BOC group at the nitrogen, a benzyl group at the 2-position, and a 2,2-difluoroacetyl group at the 3-position. The stereochemistry at the 3-position is (R).

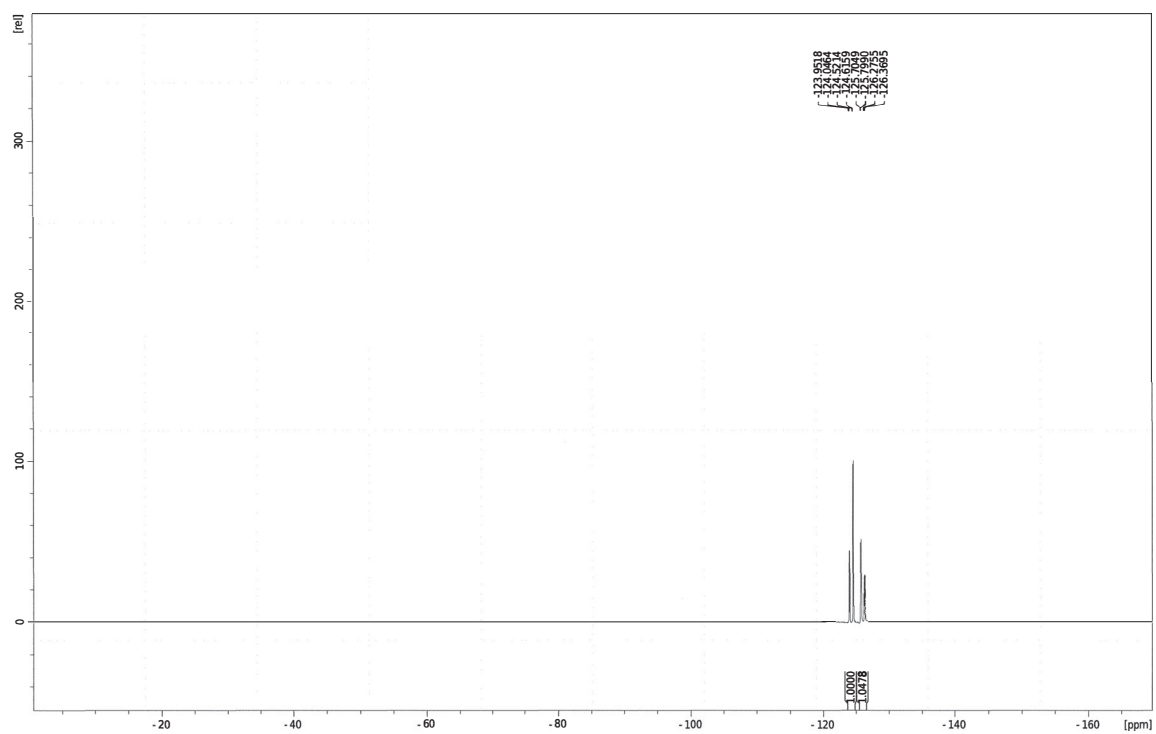
¹H NMR spectrum (CDCl₃) of compound **1**. The x-axis represents the chemical shift in ppm, ranging from 0 to 10. The y-axis represents the intensity in arbitrary units. The spectrum shows several peaks, with integration values provided below the baseline: 4.8306, 1.0000, 6.2723, 6.2838, and 9.1263. A list of chemical shifts (δ) is provided above the spectrum, grouped by brackets.

Chemical shifts (δ) listed above the spectrum:

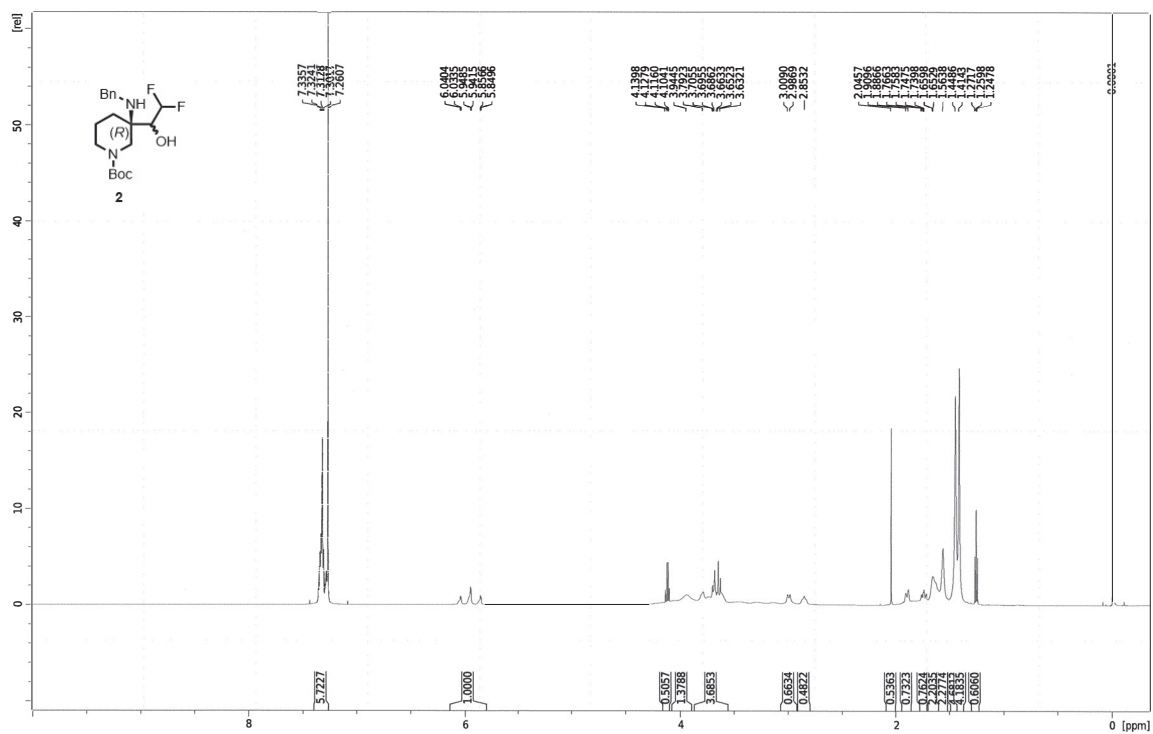
- 7.3389, 7.3346, 7.3303, 7.3260, 7.3217, 7.3174, 7.3131, 7.3088, 7.3045, 7.2999, 7.2956, 7.2913, 7.2870, 7.2827, 7.2784, 7.2741, 7.2698, 7.2655, 7.2612, 7.2569, 7.2526, 7.2483, 7.2440, 7.2397, 7.2354, 7.2311, 7.2268, 7.2225, 7.2182, 7.2139, 7.2096, 7.2053, 7.2010, 7.1967, 7.1924, 7.1881, 7.1838, 7.1795, 7.1752, 7.1709, 7.1666, 7.1623, 7.1580, 7.1537, 7.1494, 7.1451, 7.1408, 7.1365, 7.1322, 7.1279, 7.1236, 7.1193, 7.1150, 7.1107, 7.1064, 7.1021, 7.0978, 7.0935, 7.0892, 7.0849, 7.0806, 7.0763, 7.0720, 7.0677, 7.0634, 7.0591, 7.0548, 7.0505, 7.0462, 7.0419, 7.0376, 7.0333, 7.0290, 7.0247, 7.0204, 7.0161, 7.0118, 7.0075, 7.0032, 6.9989, 6.9946, 6.9903, 6.9860, 6.9817, 6.9774, 6.9731, 6.9688, 6.9645, 6.9602, 6.9559, 6.9516, 6.9473, 6.9430, 6.9387, 6.9344, 6.9301, 6.9258, 6.9215, 6.9172, 6.9129, 6.9086, 6.9043, 6.8999, 6.8956, 6.8913, 6.8870, 6.8827, 6.8784, 6.8741, 6.8698, 6.8655, 6.8612, 6.8569, 6.8526, 6.8483, 6.8440, 6.8397, 6.8354, 6.8311, 6.8268, 6.8225, 6.8182, 6.8139, 6.8096, 6.8053, 6.8010, 6.7967, 6.7924, 6.7881, 6.7838, 6.7795, 6.7752, 6.7709, 6.7666, 6.7623, 6.7580, 6.7537, 6.7494, 6.7451, 6.7408, 6.7365, 6.7322, 6.7279, 6.7236, 6.7193, 6.7150, 6.7107, 6.7064, 6.7021, 6.6978, 6.6935, 6.6892, 6.6849, 6.6806, 6.6763, 6.6720, 6.6677, 6.6634, 6.6591, 6.6548, 6.6505, 6.6462, 6.6419, 6.6376, 6.6333, 6.6290, 6.6247, 6.6204, 6.6161, 6.6118, 6.6075, 6.6032, 6.5989, 6.5946, 6.5903, 6.5860, 6.5817, 6.5774, 6.5731, 6.5688, 6.5645, 6.5602, 6.5559, 6.5516, 6.5473, 6.5430, 6.5387, 6.5344, 6.5301, 6.5258, 6.5215, 6.5172, 6.5129, 6.5086, 6.5043, 6.5000, 6.4957, 6.4914, 6.4871, 6.4828, 6.4785, 6.4742, 6.4699, 6.4656, 6.4613, 6.4570, 6.4527, 6.4484, 6.4441, 6.4398, 6.4355, 6.4312, 6.4269, 6.4226, 6.4183, 6.4140, 6.4097, 6.4054, 6.4011, 6.3968, 6.3925, 6.3882, 6.3839, 6.3796, 6.3753, 6.3710, 6.3667, 6.3624, 6.3581, 6.3538, 6.3495, 6.3452, 6.3409, 6.3366, 6.3323, 6.3280, 6.3237, 6.3194, 6.3151, 6.3108, 6.3065, 6.3022, 6.2979, 6.2936, 6.2893, 6.2850, 6.2807, 6.2764, 6.2721, 6.2678, 6.2635, 6.2592, 6.2549, 6.2506, 6.2463, 6.2420, 6.2377, 6.2334, 6.2291, 6.2248, 6.2205, 6.2162, 6.2119, 6.2076, 6.2033, 6.1990, 6.1947, 6.1904, 6.1861, 6.1818, 6.1775, 6.1732, 6.1689, 6.1646, 6.1603, 6.1560, 6.1517, 6.1474, 6.1431, 6.1388, 6.1345, 6.1302, 6.1259, 6.1216, 6.1173, 6.1130, 6.1087, 6.1044, 6.1001, 6.0958, 6.0915, 6.0872, 6.0829, 6.0786, 6.0743, 6.0699, 6.0656, 6.0613, 6.0570, 6.0527, 6.0484, 6.0441, 6.0398, 6.0355, 6.0312, 6.0269, 6.0226, 6.0183, 6.0140, 6.0097, 6.0054, 6.0011, 5.9968, 5.9925, 5.9882, 5.9839, 5.9796, 5.9753, 5.9710, 5.9667, 5.9624, 5.9581, 5.9538, 5.9495, 5.9452, 5.9409, 5.9366, 5.9323, 5.9280, 5.9237, 5.9194, 5.9151, 5.9108, 5.9065, 5.9022, 5.8979, 5.8936, 5.8893, 5.8850, 5.8807, 5.8764, 5.8721, 5.8678, 5.8635, 5.8592, 5.8549, 5.8506, 5.8463, 5.8420, 5.8377, 5.8334, 5.8291, 5.8248, 5.8205, 5.8162, 5.8119, 5.8076, 5.8033, 5.7990, 5.7947, 5.7904, 5.7861, 5.7818, 5.7775, 5.7732, 5.7689, 5.7646, 5.7603, 5.7560, 5.7517, 5.7474, 5.7431, 5.7388, 5.7345, 5.7302, 5.7259, 5.7216, 5.7173, 5.7130, 5.7087, 5.7044, 5.7001, 5.6958, 5.6915, 5.6872, 5.6829, 5.6786, 5.6743, 5.6699, 5.6656, 5.6613, 5.6570, 5.6527, 5.6484, 5.6441, 5.6398, 5.6355, 5.6312, 5.6269, 5.6226, 5.6183, 5.6140, 5.6097, 5.6054, 5.6011, 5.5968, 5.5925, 5.5882, 5.5839, 5.5796, 5.5753, 5.5710, 5.5667, 5.5624, 5.5581, 5.5538, 5.5495, 5.5452, 5.5409, 5.5366, 5.5323, 5.5280, 5.5237, 5.5194, 5.5151, 5.5108, 5.5065, 5.5022, 5.4979, 5.4936, 5.4893, 5.4850, 5.4807, 5.4764, 5.4721, 5.4678, 5.4635, 5.4592, 5.4549, 5.4506, 5.4463, 5.4420, 5.4377, 5.4334, 5.4291, 5.4248, 5.4205, 5.4162, 5.4119, 5.4076, 5.4033, 5.3990, 5.3947, 5.3904, 5.3861, 5.3818, 5.3775, 5.3732, 5.3689, 5.3646, 5.3603, 5.3560, 5.3517, 5.3474

¹³C NMR spectrum of compound 10a in CDCl₃. The x-axis represents the chemical shift in ppm (0 to 200), and the y-axis represents the intensity in arbitrary units (0 to 10). The spectrum shows several sharp peaks. Key peaks are labeled with their chemical shifts: 199.8989, 199.7585, 199.6179, 154.8110, 139.2029, 128.9943, 127.7807, 127.4826, 109.1676, 107.6238, 80.4113, 77.7807, 76.8055, 63.3993, 47.7706, 43.2084, 39.9652, 38.9500, 20.5708, and -0.0003. A small peak is also visible at approximately 200 ppm.

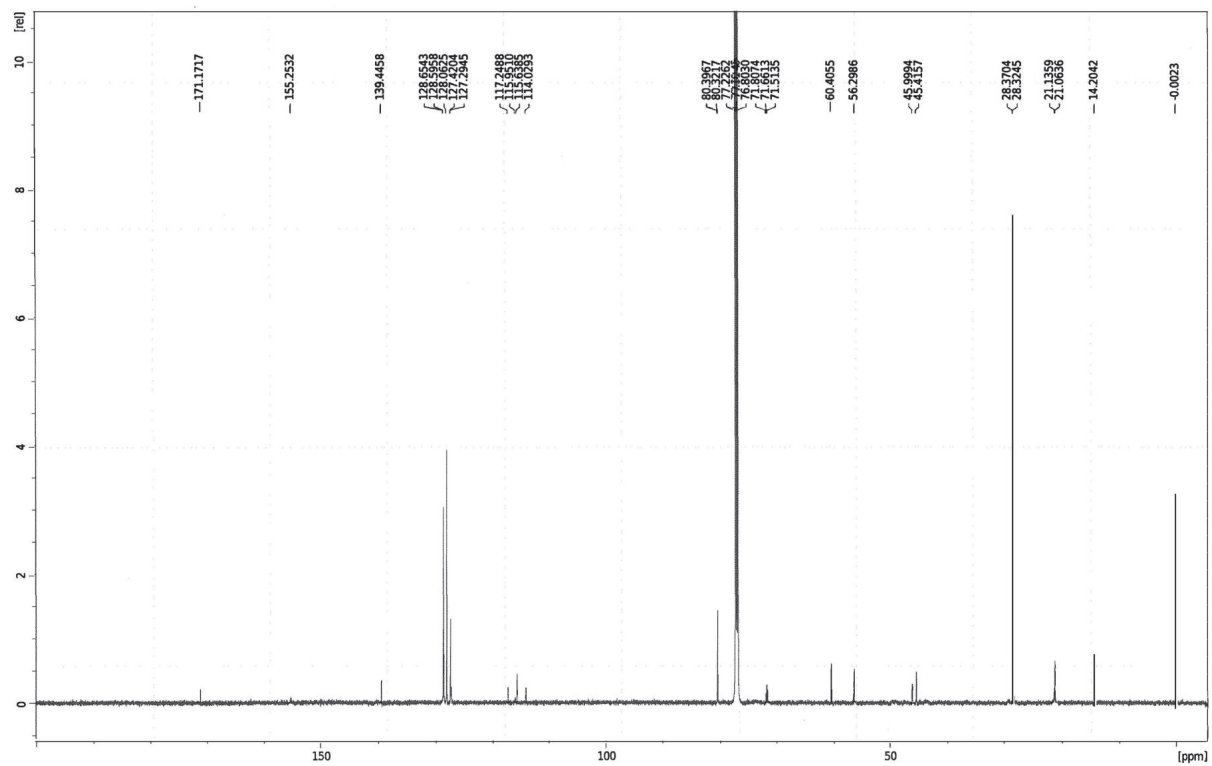
¹⁹F-NMR compound 1



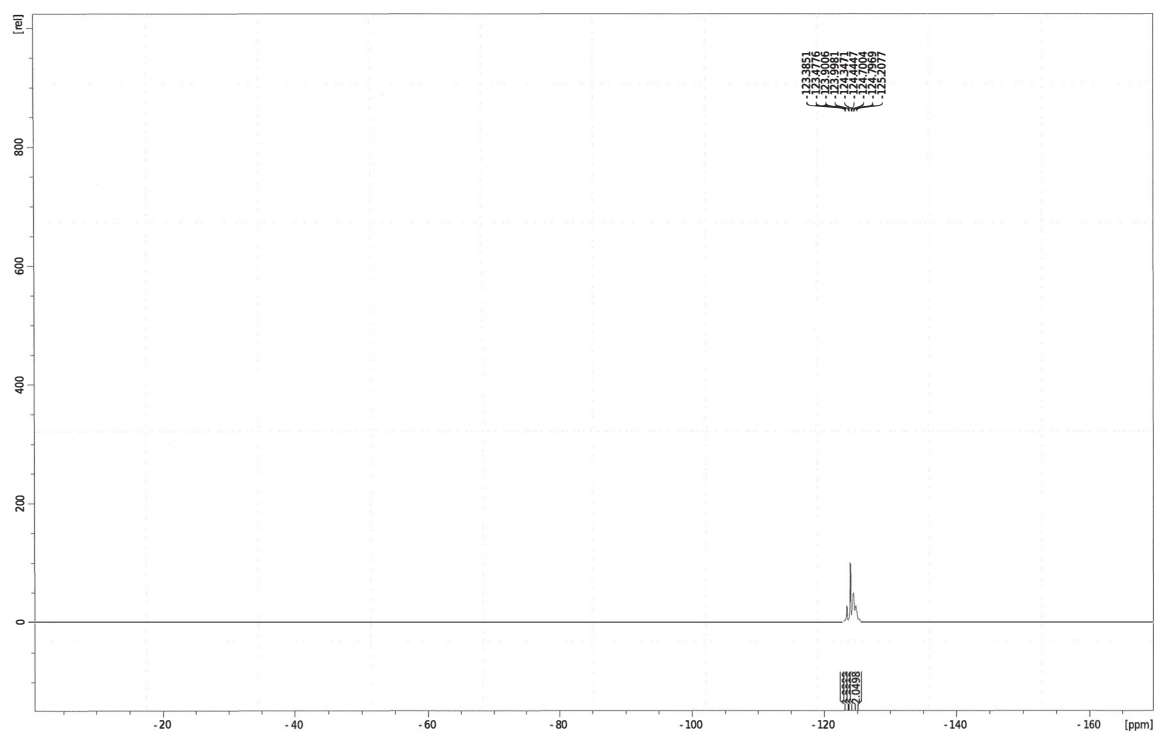
¹H NMR (600 MHz, CDCl₃) Compound 2



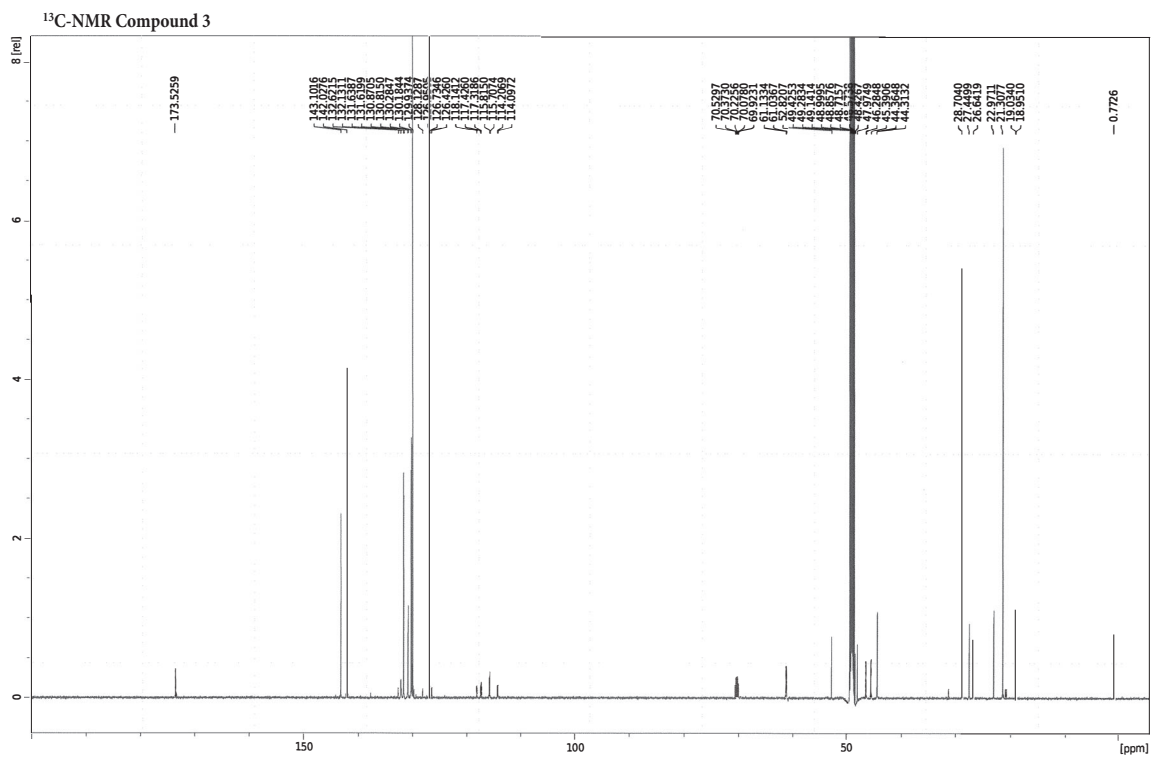
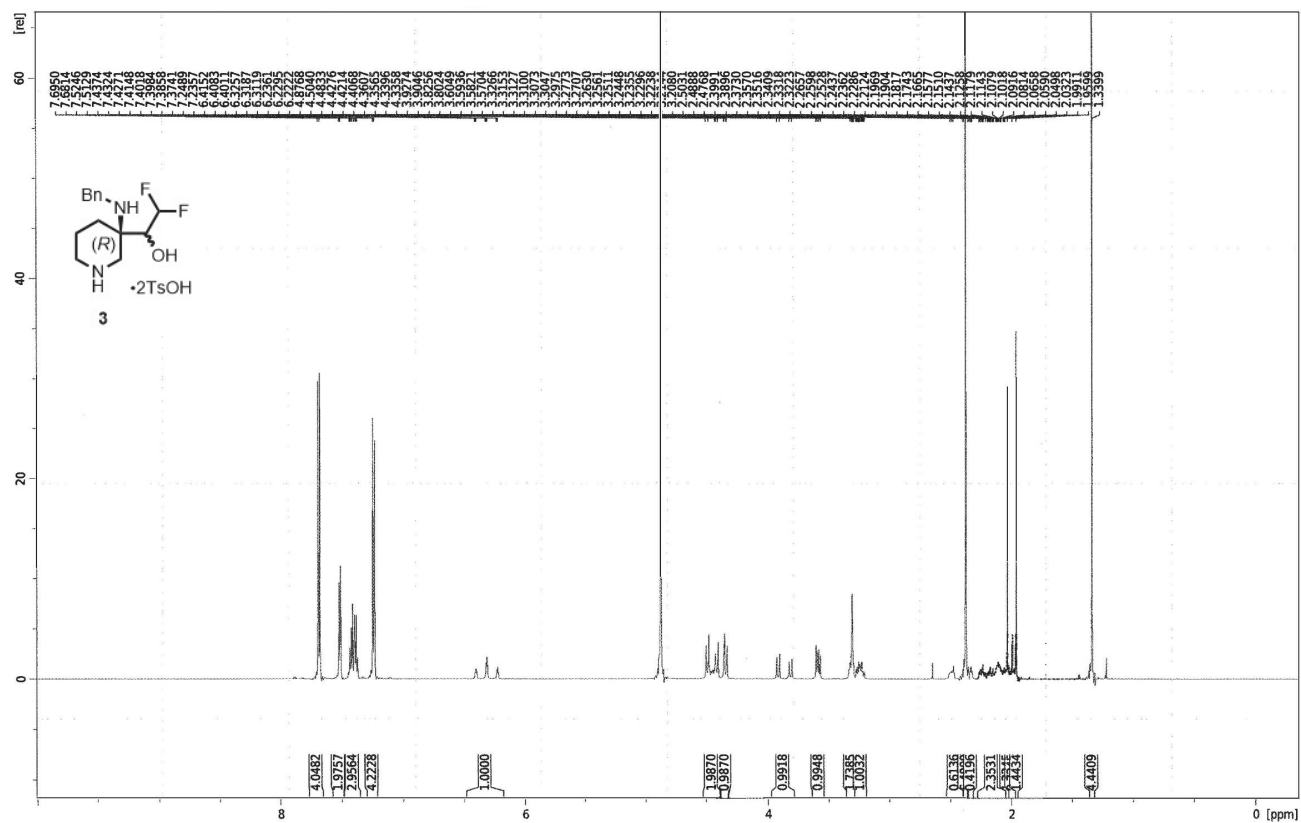
¹³C-NMR Compound 2



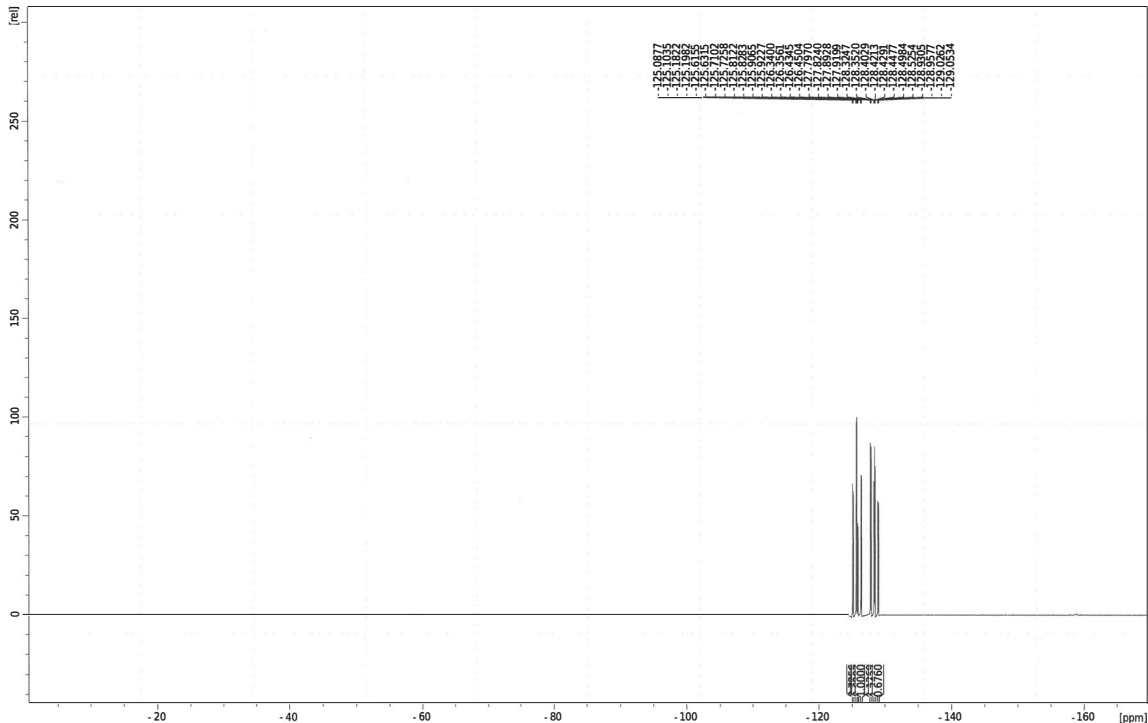
¹⁹F NMR Compound 2



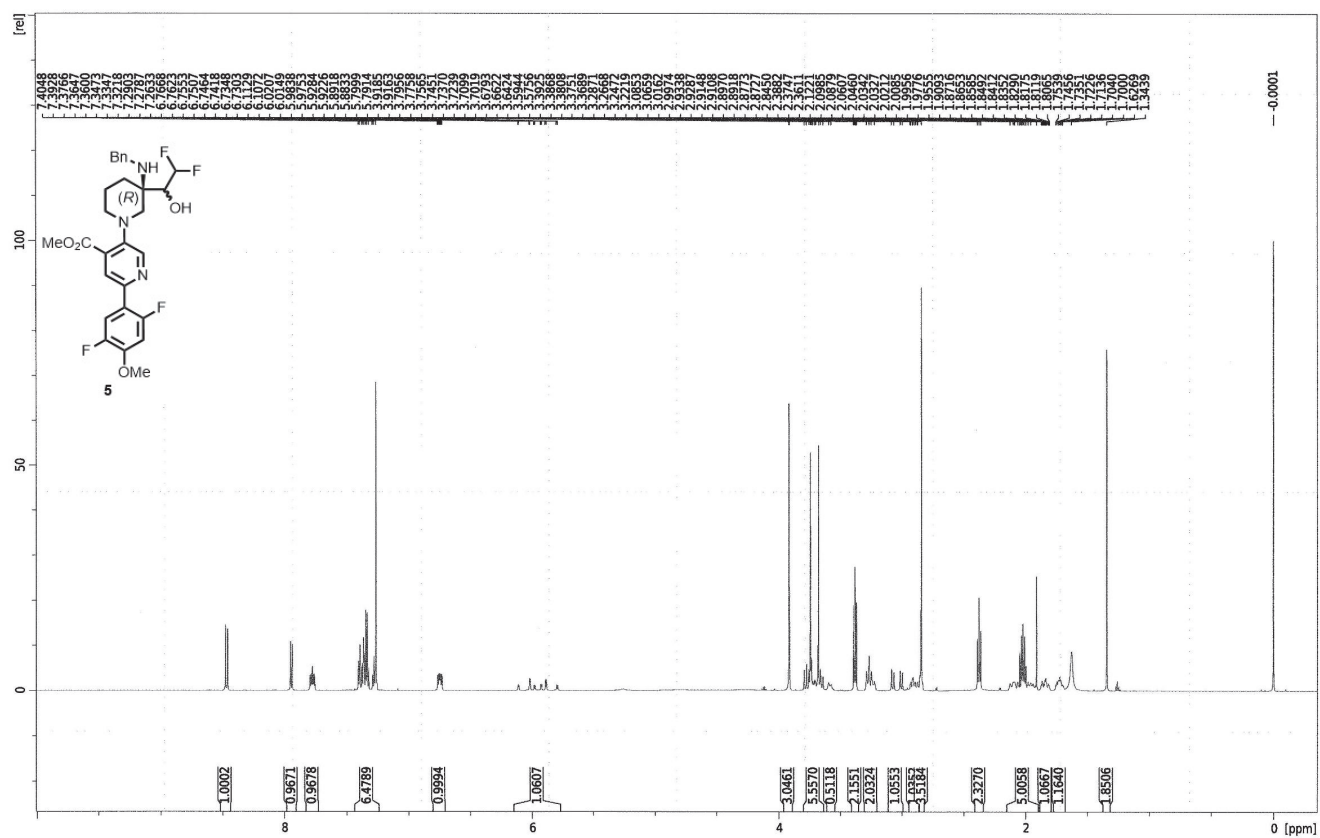
¹H NMR (600 MHz, CD₃OD) Compound 3



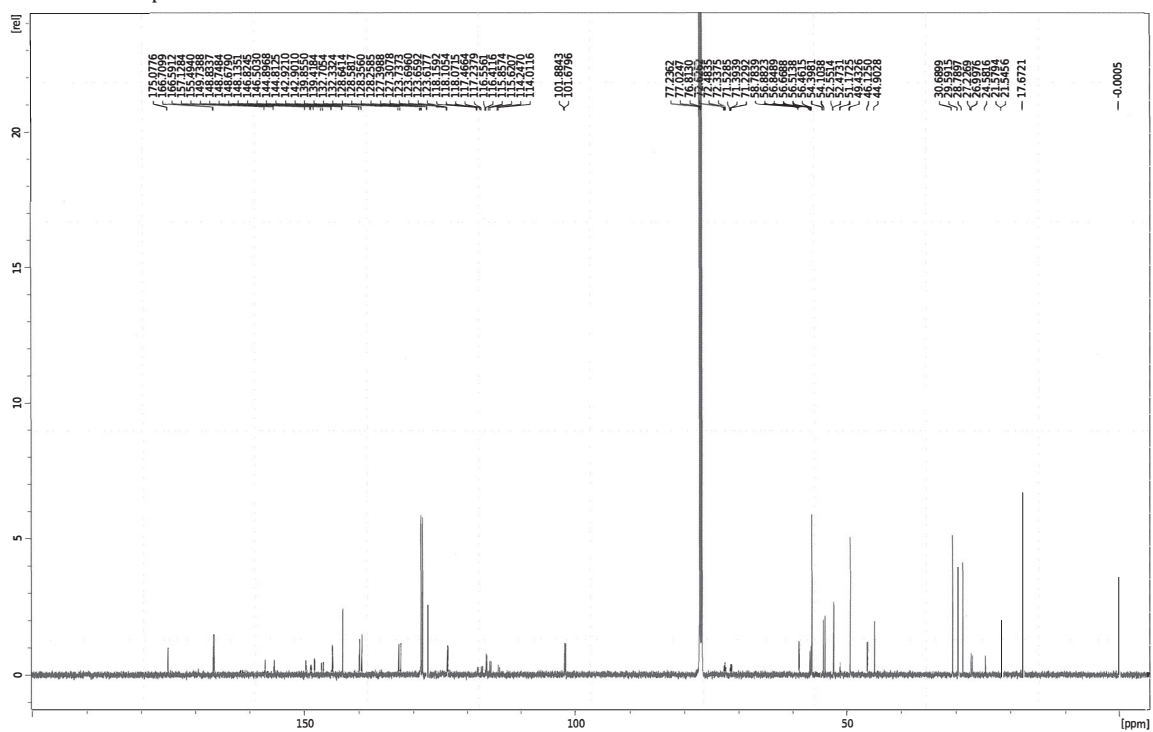
¹⁹F-NMR Compound 3



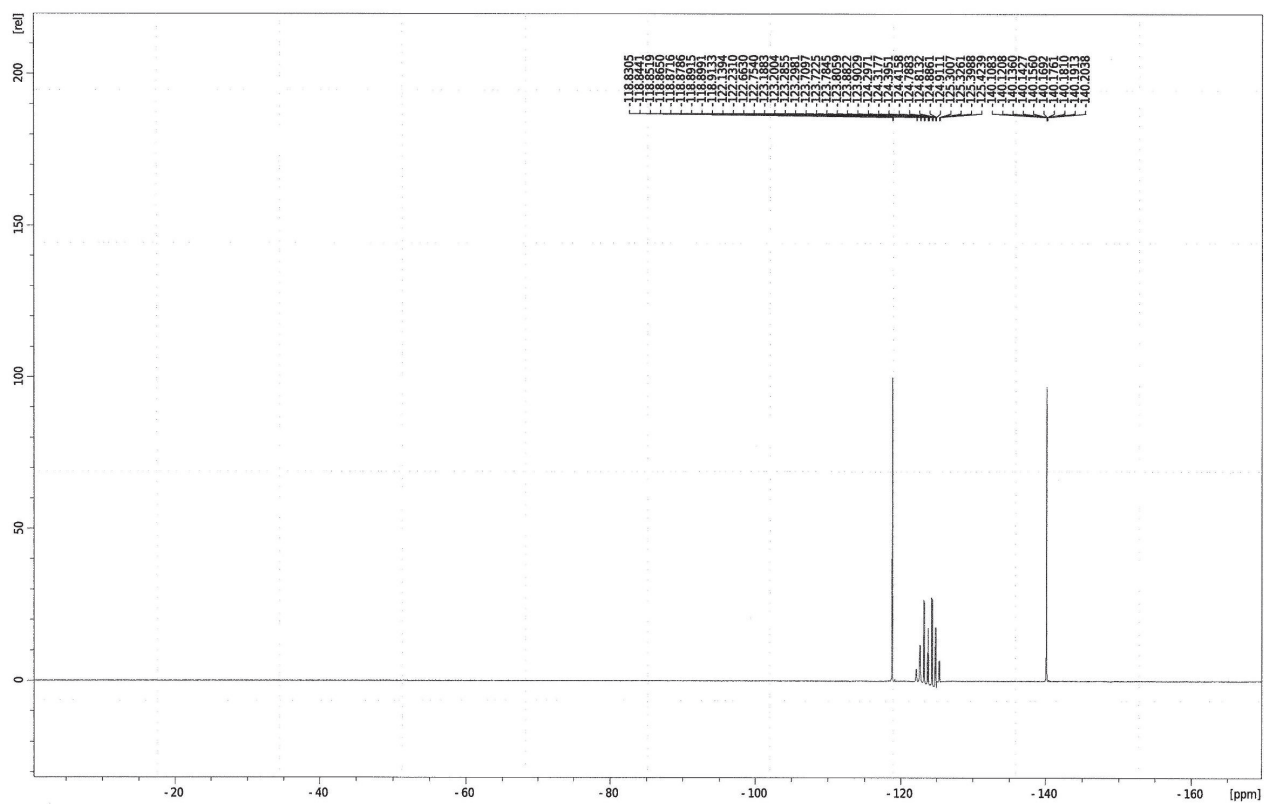
¹H-NMR (600 MHz, CDCl₃) Compound 5



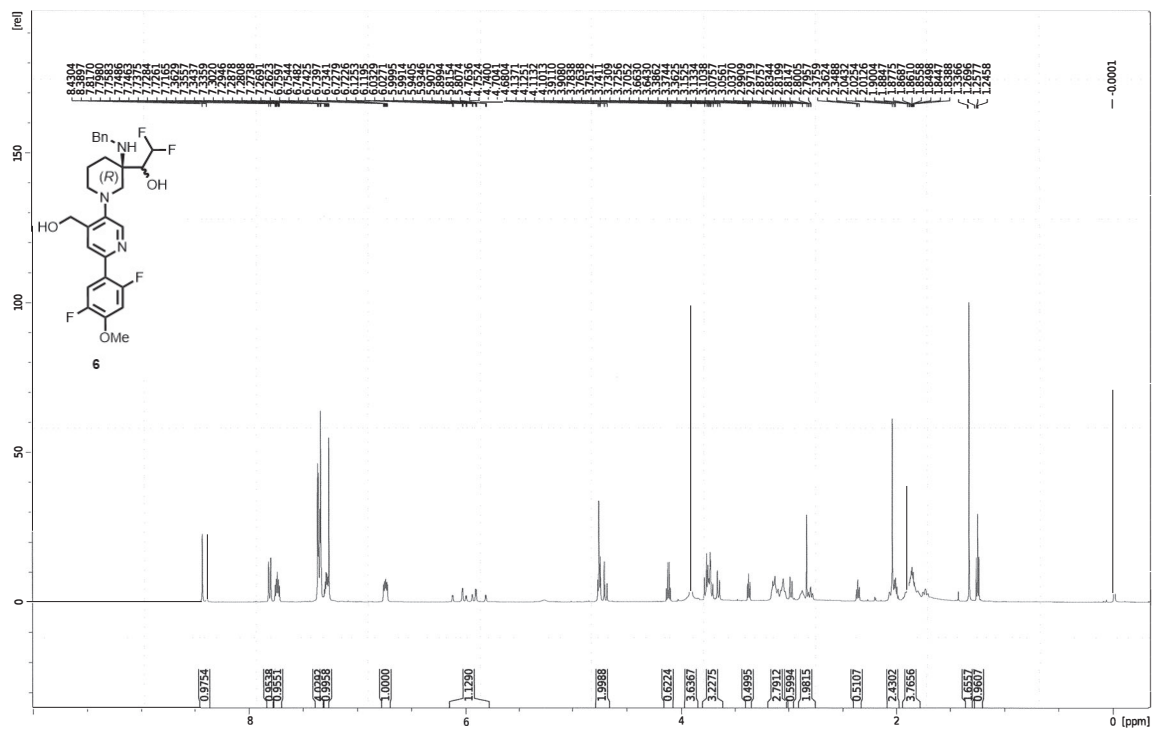
¹³C-NMR Compound 5



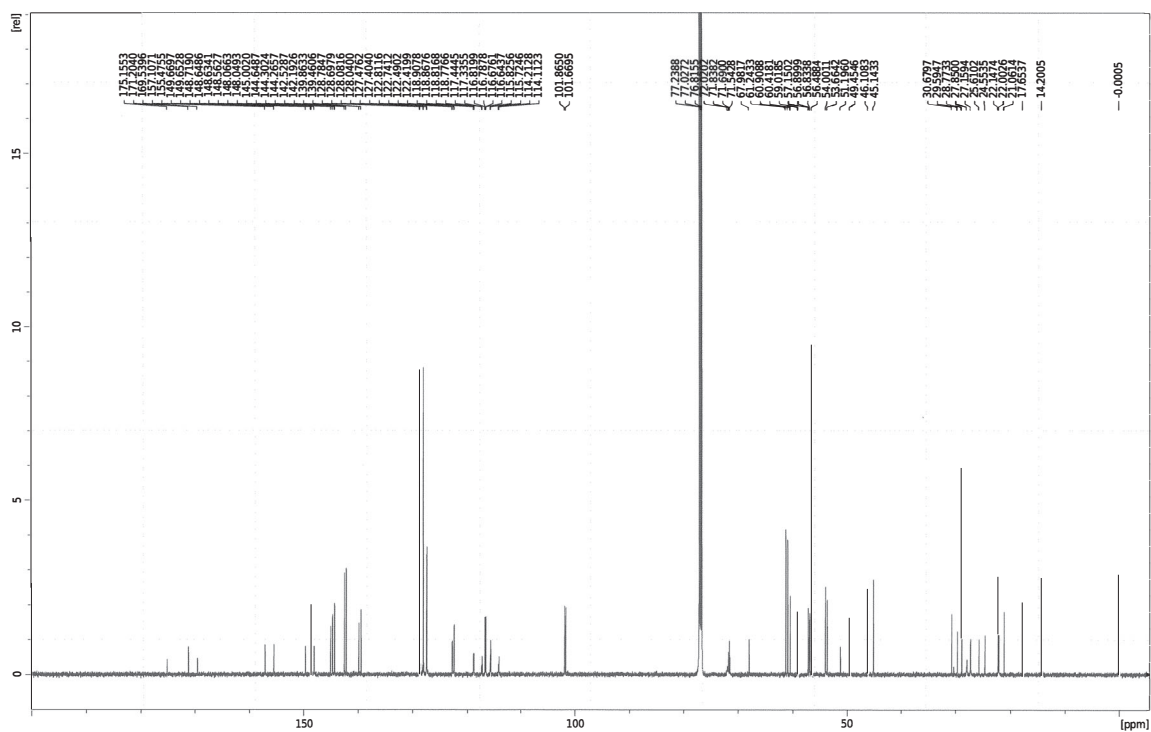
¹⁹F-NMR Compound 5



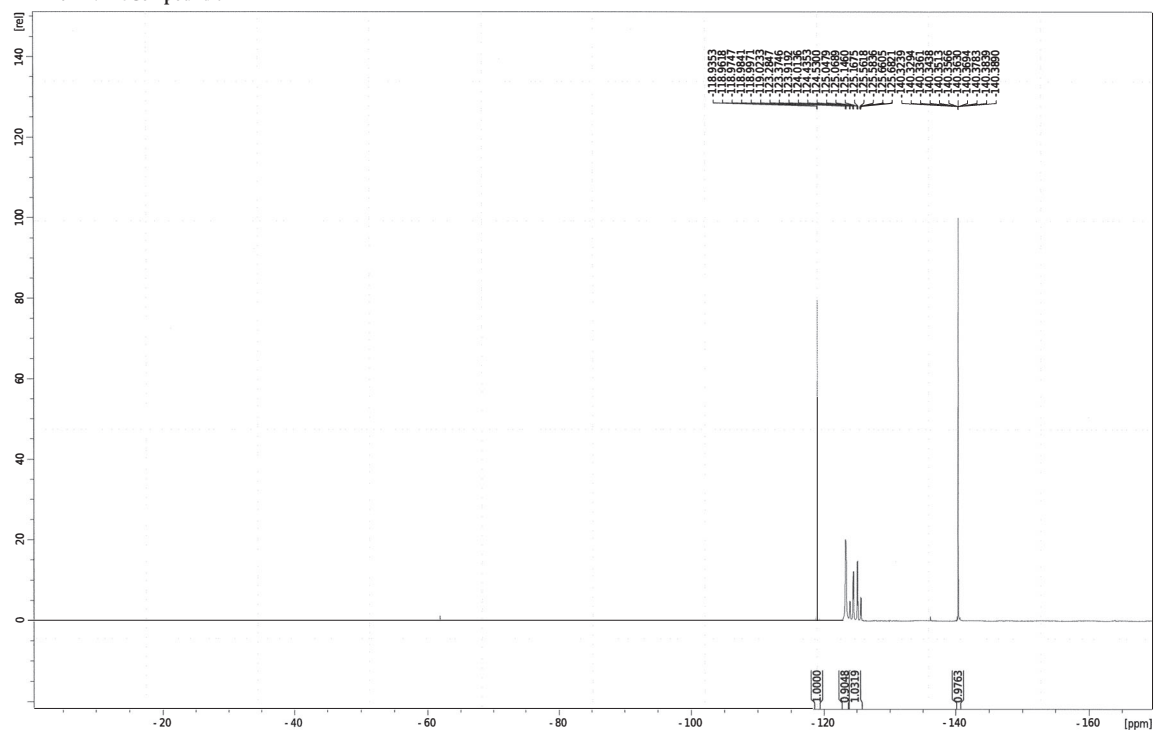
¹H-NMR (600 MHz, CDCl₃) Compound 6



¹³C-NMR Compound 6



¹⁹F-NMR Compound 6



COc1cc(F)ccc1-c2ccc(Cl)cn2C3CCN(C3)C(O)C(F)F

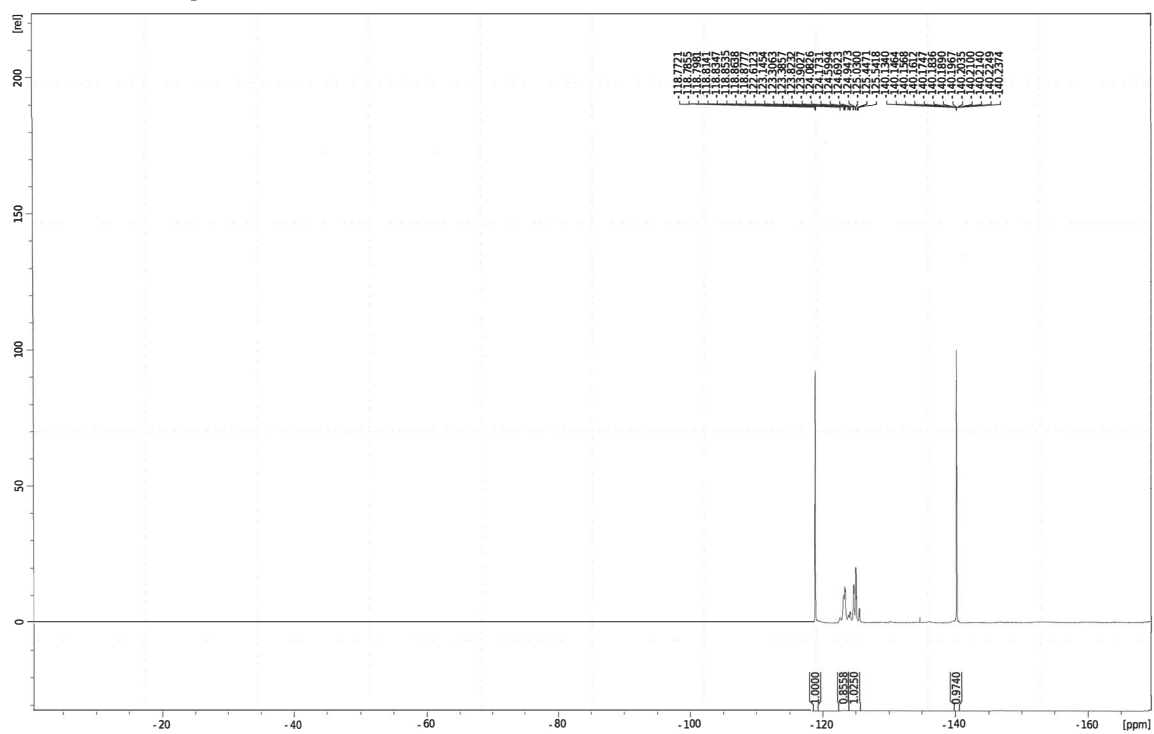
7

1H NMR spectrum (CDCl₃) of compound **7**. The x-axis represents the chemical shift in ppm, ranging from 0 to 10. The spectrum shows several peaks corresponding to the structure of **7**. The integration values are provided below the baseline for each major peak group.

Chemical Shift (ppm)	Integration
~7.3	0.9399
~7.2	1.8846
~7.1	1.4388
~6.8	1.0000
~6.2	1.0462
~4.3	0.1597
~4.2	2.0710
~4.1	0.5564
~4.0	2.2296
~3.9	3.5331
~3.3	0.3612
~3.2	1.4838
~3.1	0.5462
~2.8	1.5788
~2.4	0.3617
~2.3	1.0854
~2.2	2.7440
~2.1	1.5153
~2.0	1.5677
~1.9	0.7752



¹⁹F-NMR Compound 7

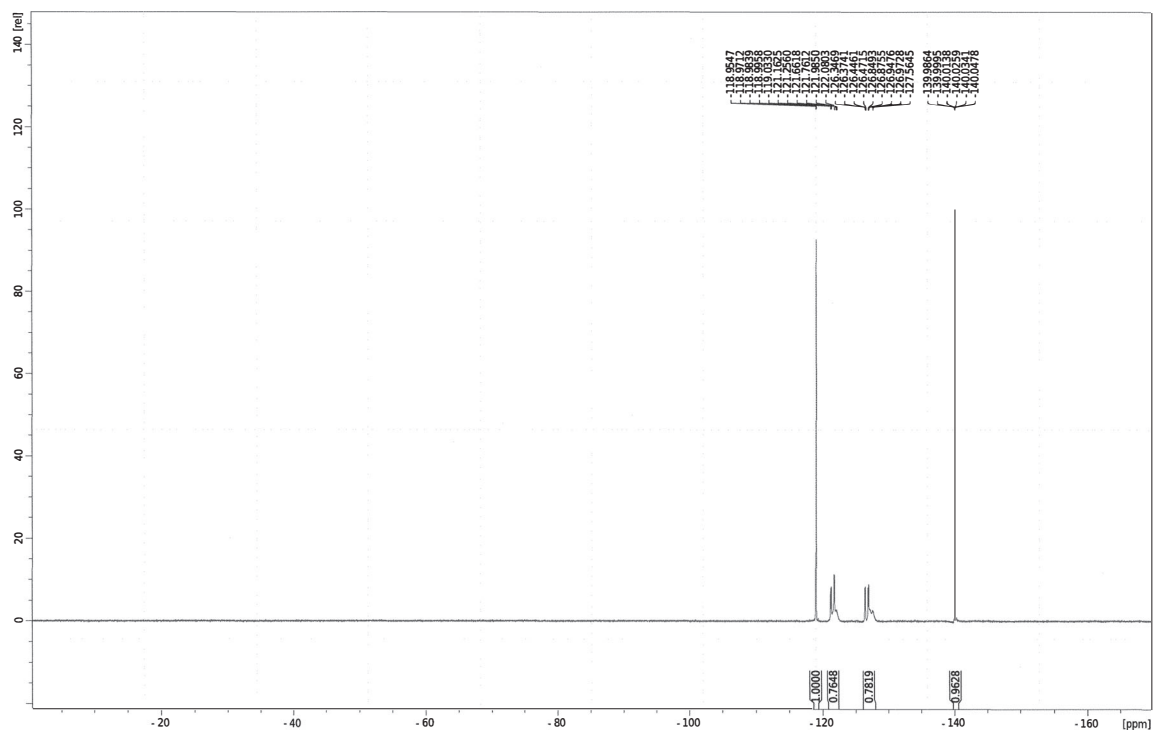


Chemical structure of compound **8** is shown as an inset. The structure is a 4-fluoro-3-methoxyphenyl ring attached to a pyridine ring, which is further attached to a 1,2,4-triazole ring. The triazole ring is substituted with a tert-butyl carbamate group (NBoc₂) and a (R)-1-benzyl-2-fluoro-2-hydroxyethyl group.

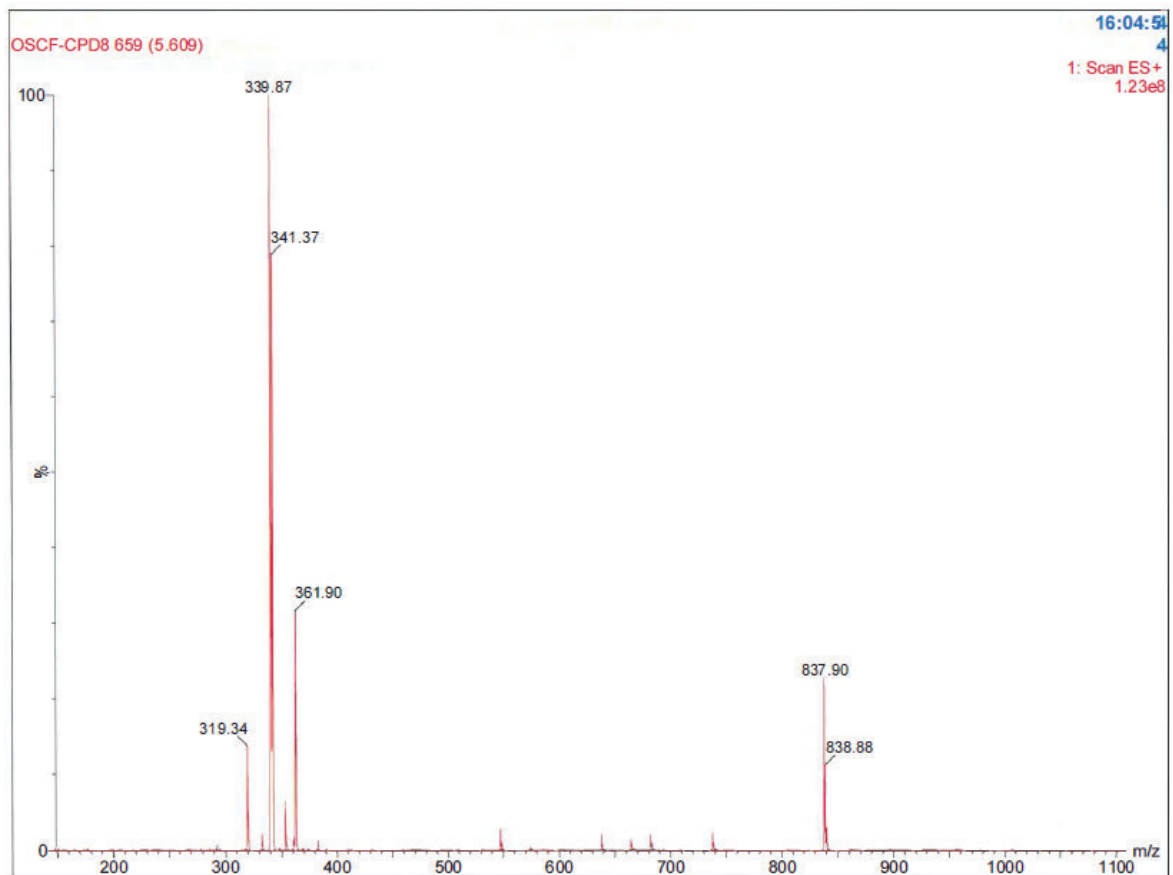
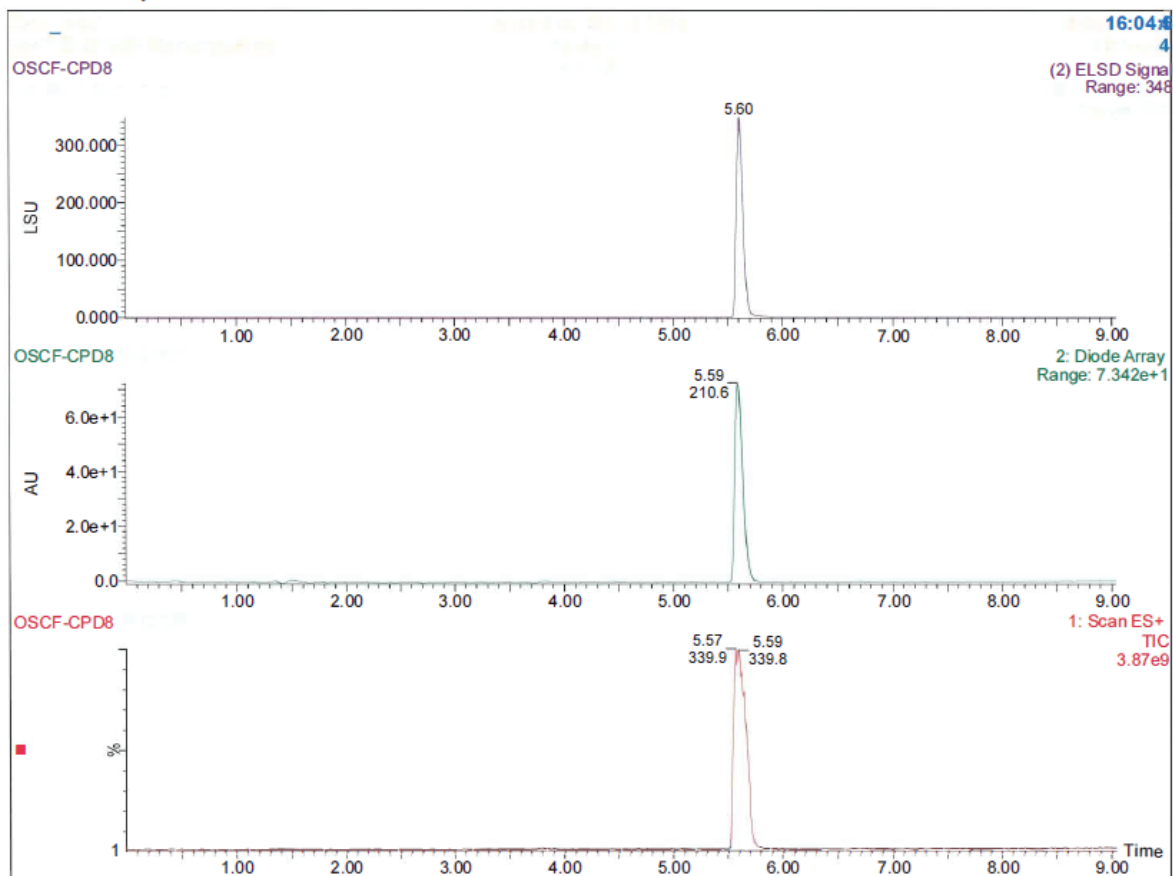
¹H NMR spectrum (CDCl₃) of compound **8** is shown. The x-axis represents the chemical shift in ppm (0 to 8), and the y-axis represents the intensity in arbitrary units (0 to 15). The spectrum displays several peaks corresponding to the protons in the molecule. Integration values are provided below the baseline, and chemical shift values are listed above the peaks.

Chemical Shift (ppm)	Integration
7.833	1.0000
7.818	2.0141
7.803	3.0092
7.788	3.0517
7.773	1.0191
6.1819	1.0002
6.1611	3.0934
6.1403	6.1819
6.1195	20.0611
6.0987	0.9223
6.0779	1.7188
6.0571	1.0317
6.0363	8.9106
6.0155	0.9833
4.1689	4.1689
1.83390	1.83390

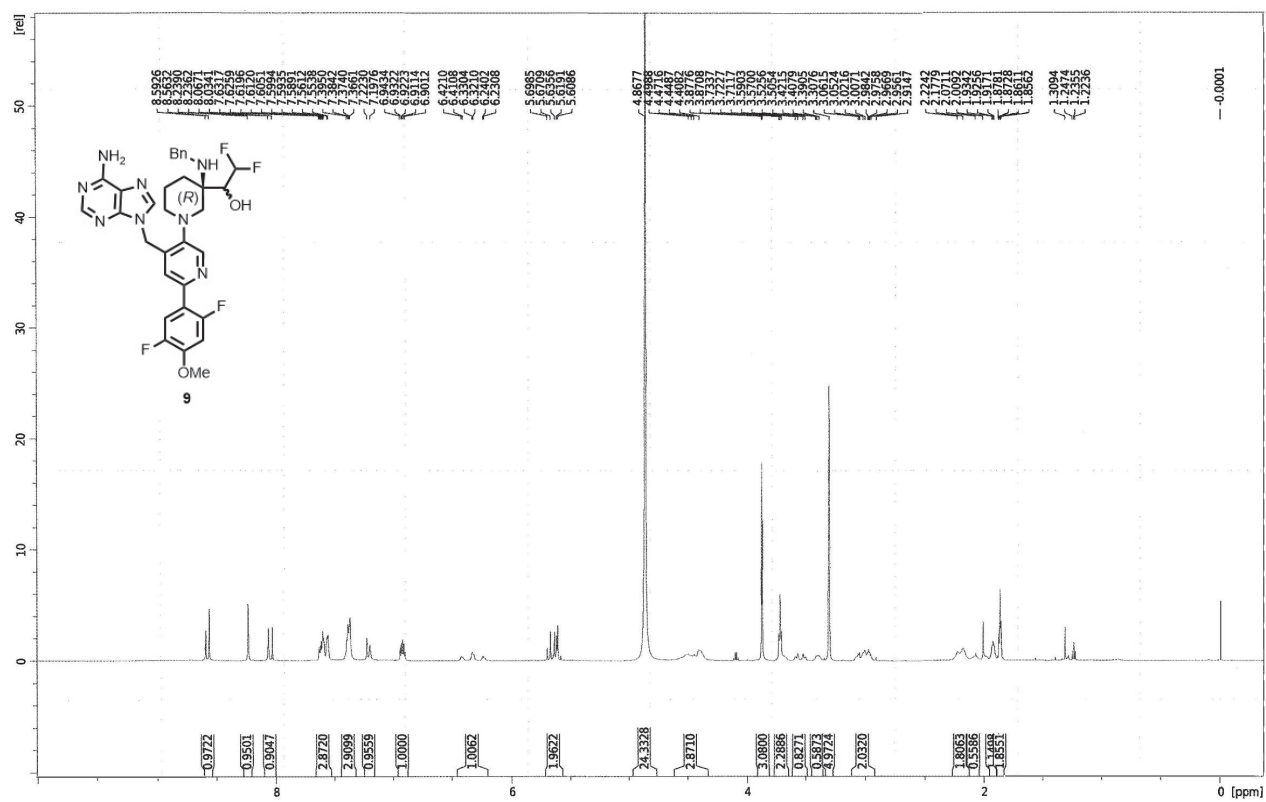
¹⁹F NMR Compound 8



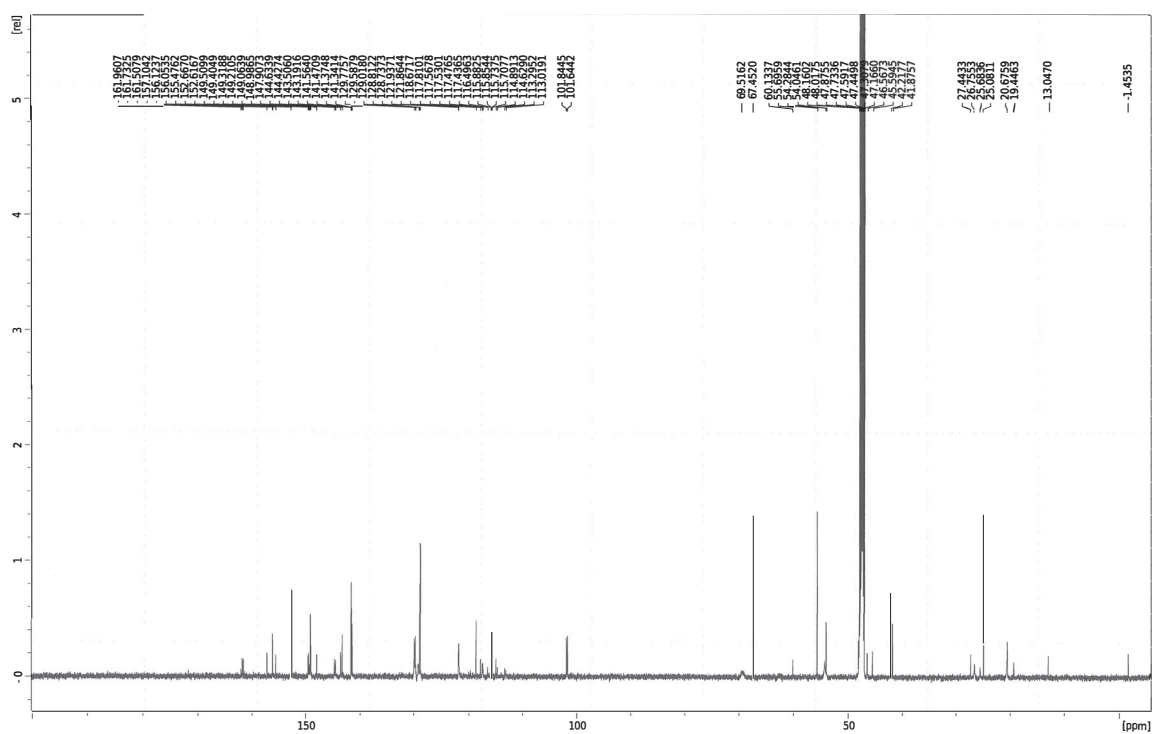
LCMS of compound 8



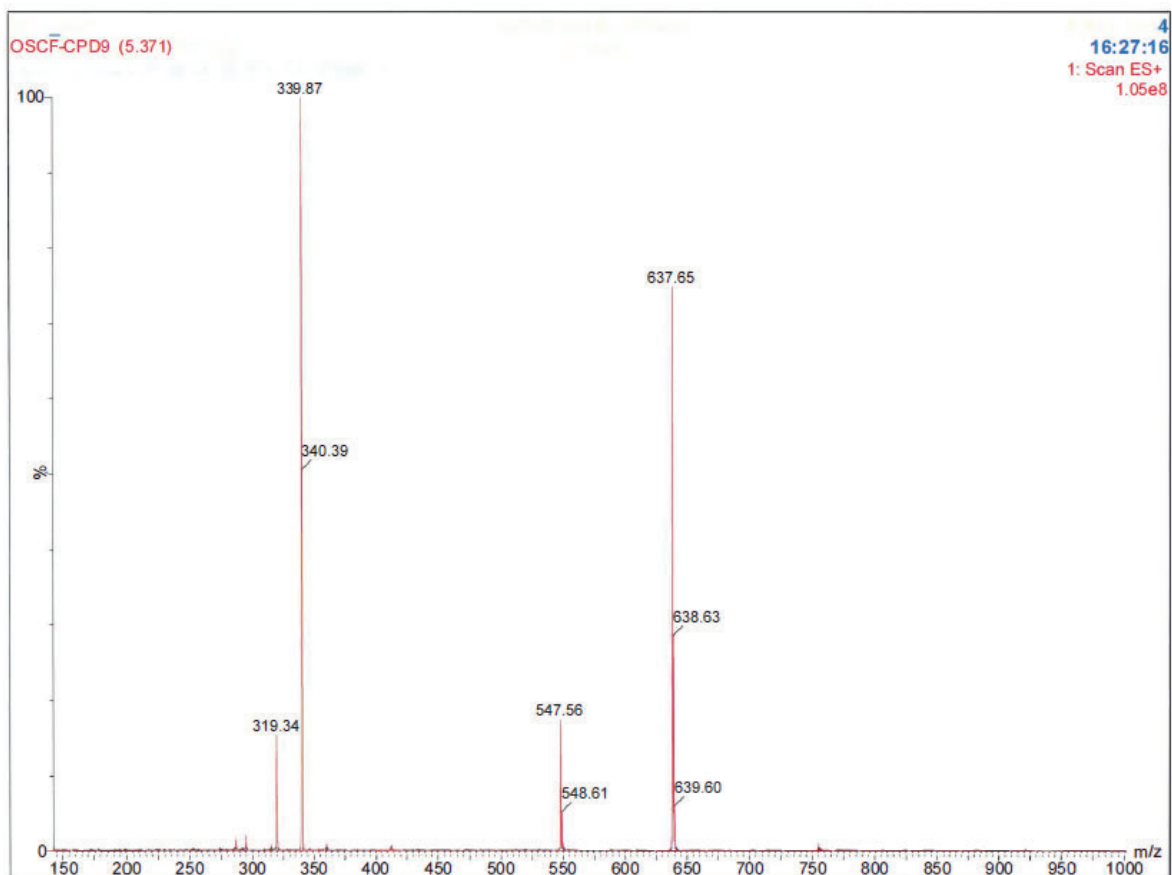
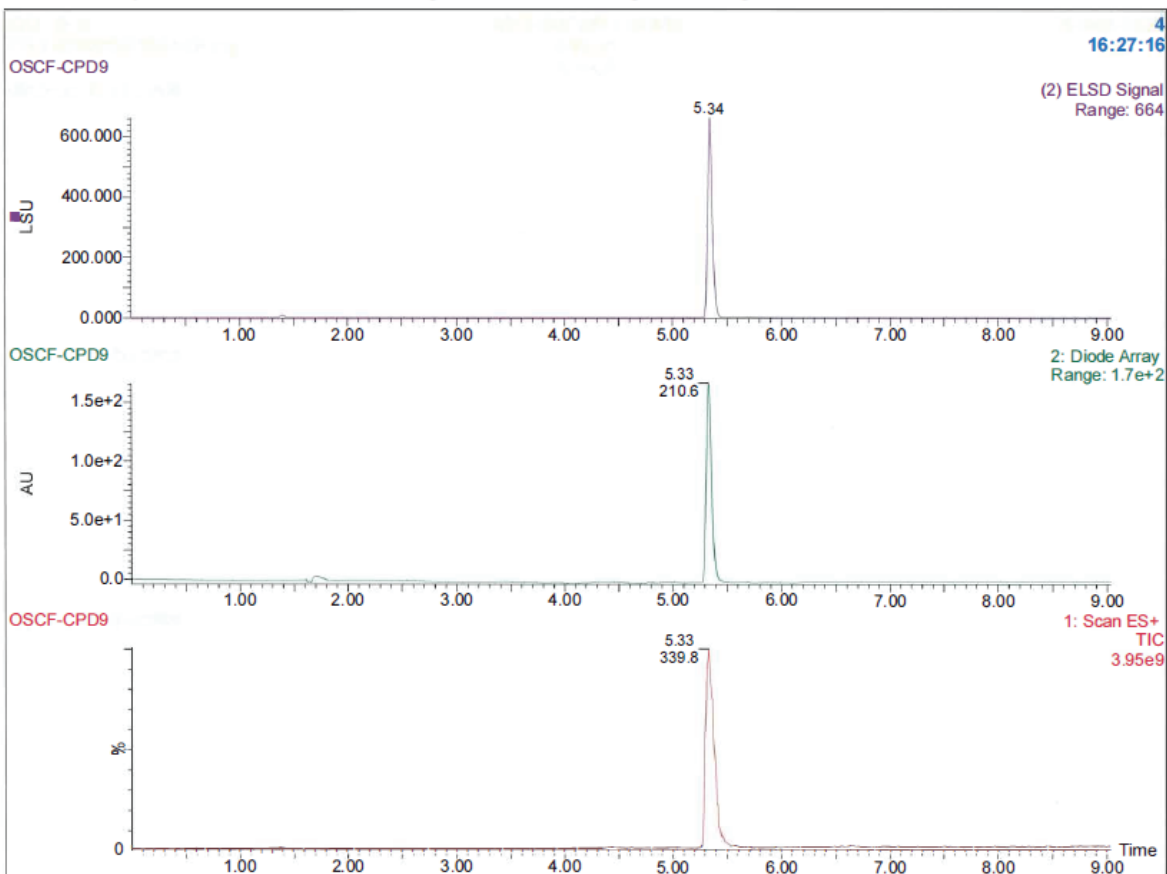
¹H NMR (600 MHz, MeOH-d₄) Compound 9



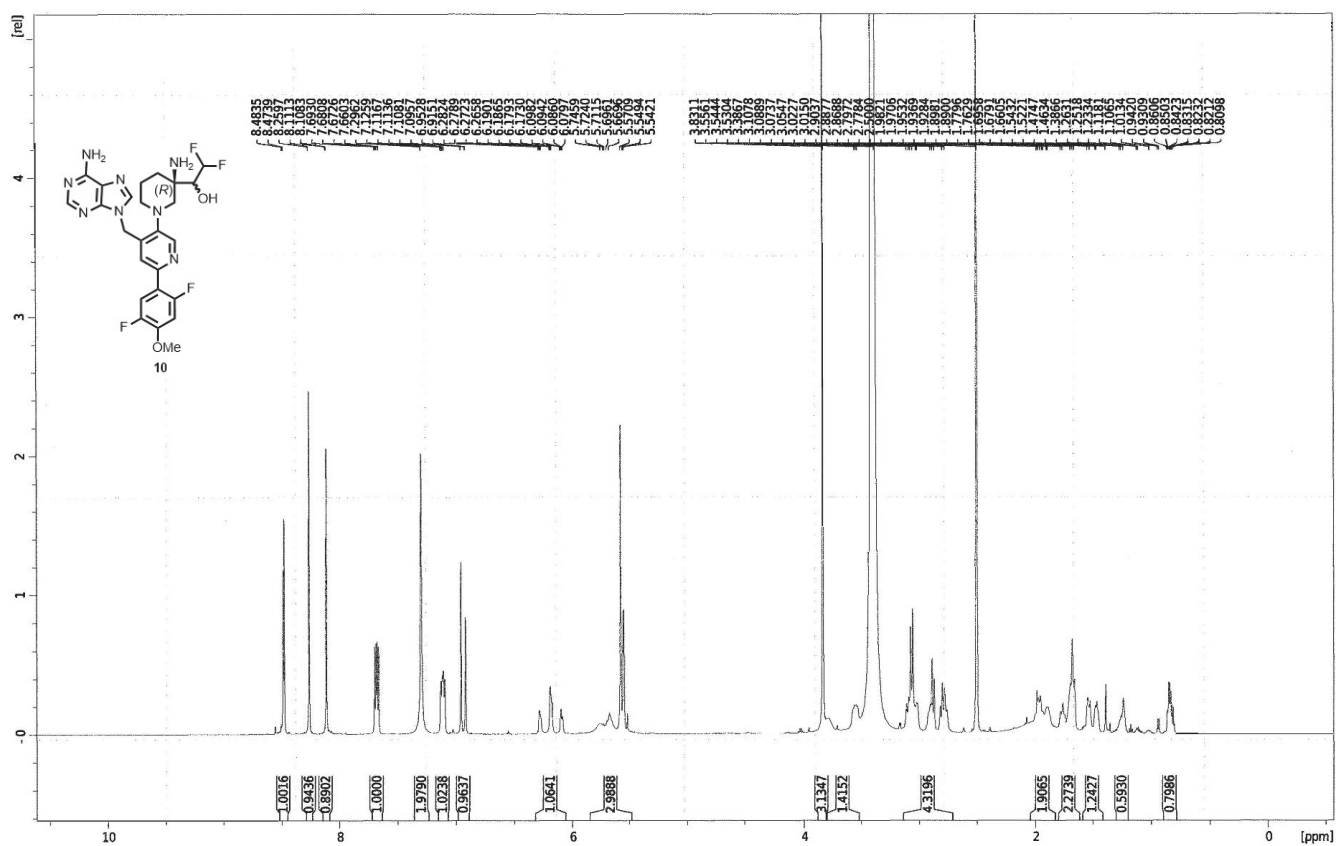
¹³C-NMR Compound 9



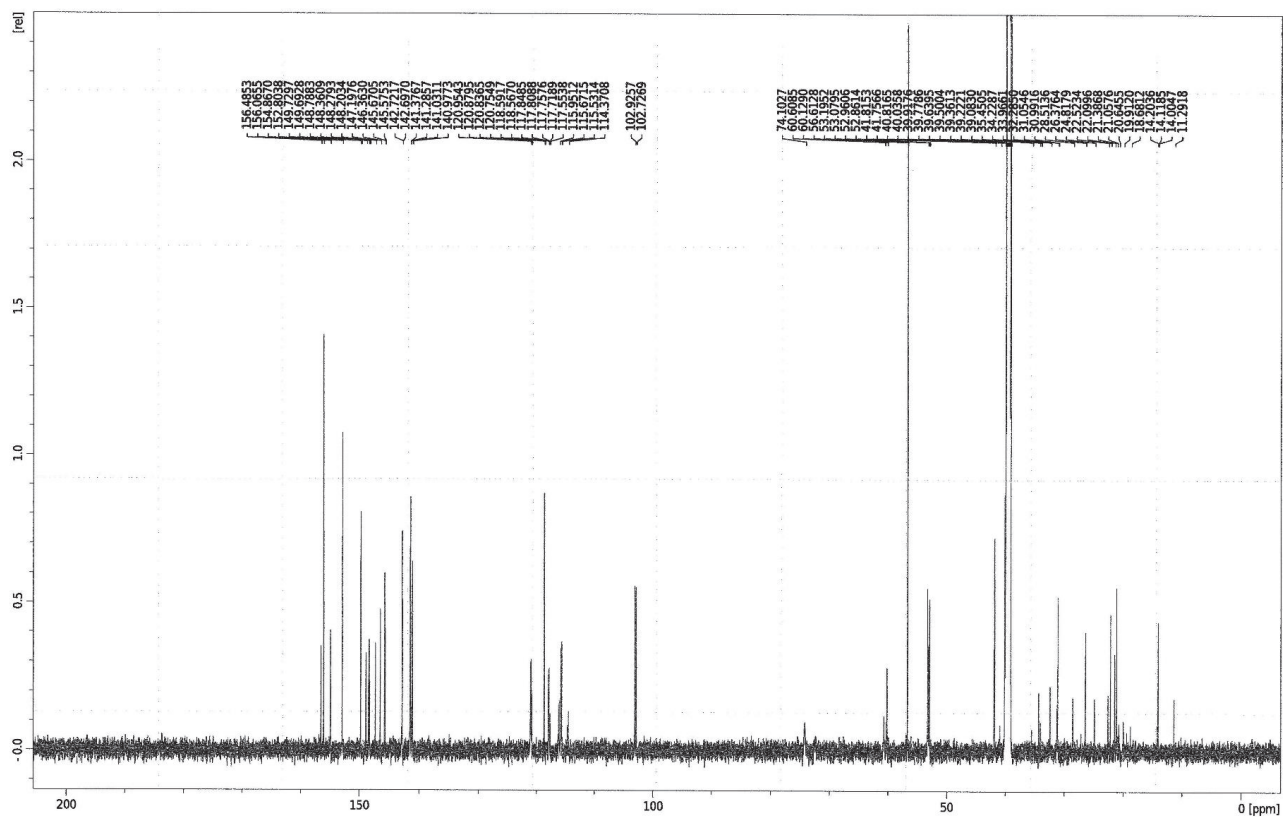
LCMS method: Autopure Waters LCMS, Xbridge C18 3.5 μ m 4.6 x 150 mm column, flow rate 1.4ml/min 8 min run with gradient 5-95% ACN/water containing 0.05% TFA, rest is washing and calibrating.

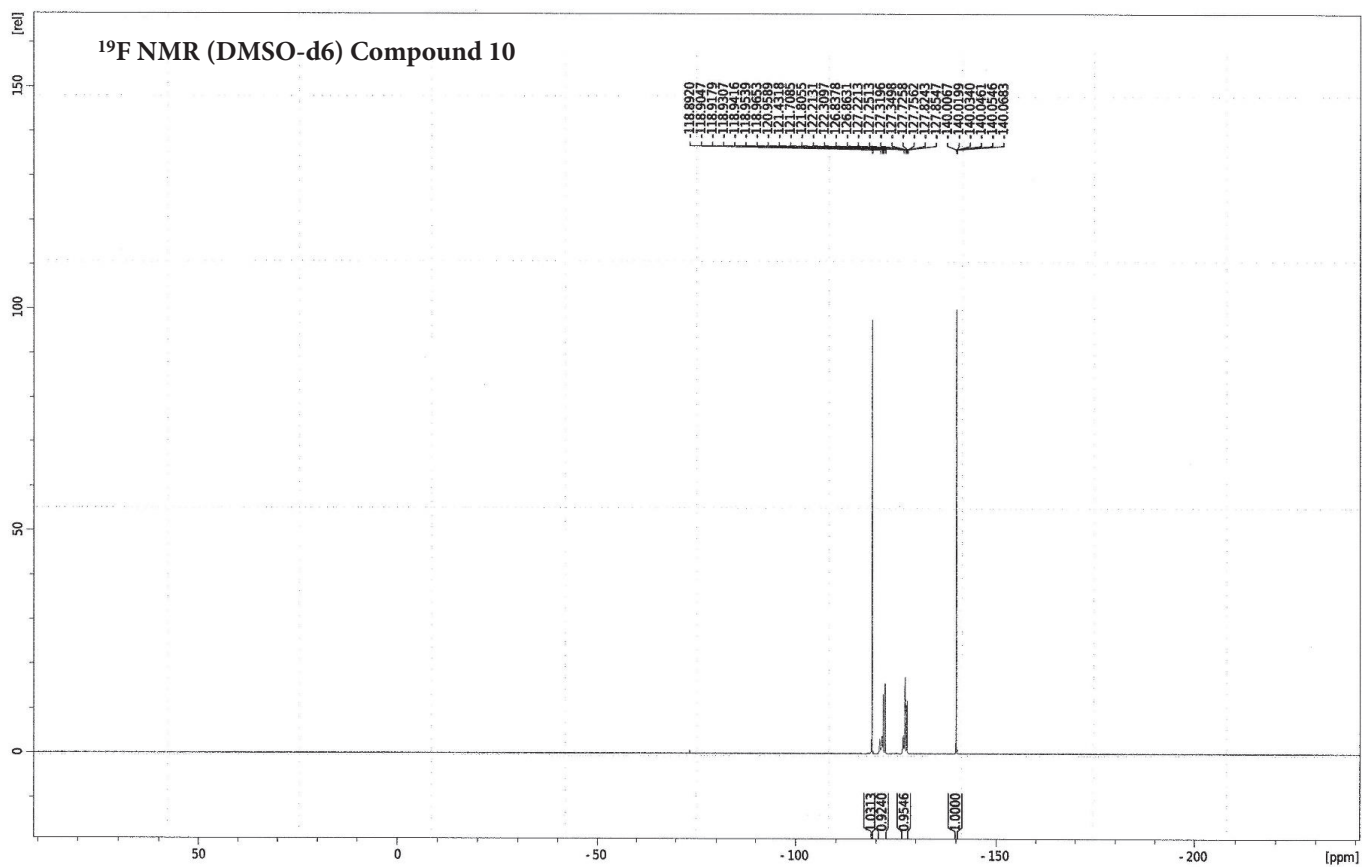


¹H NMR (DMSO-d₆) Compound 10



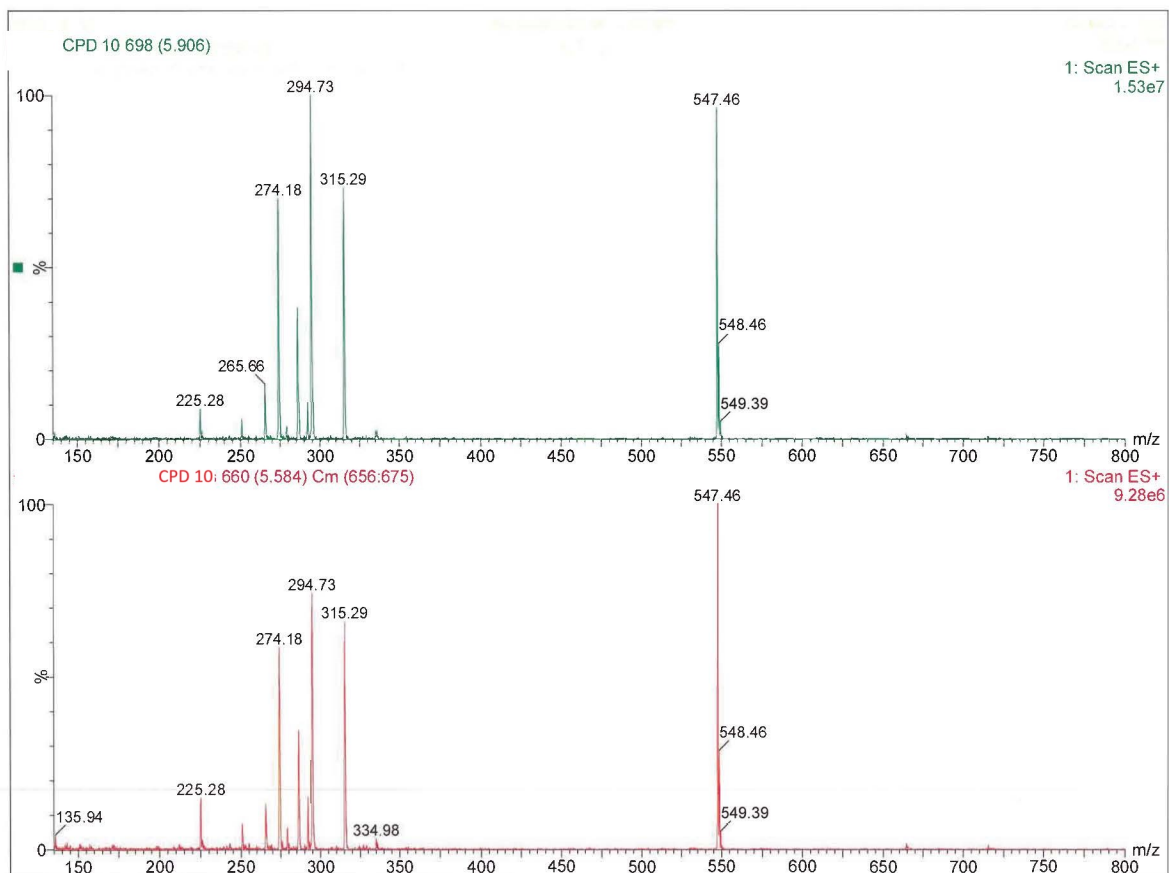
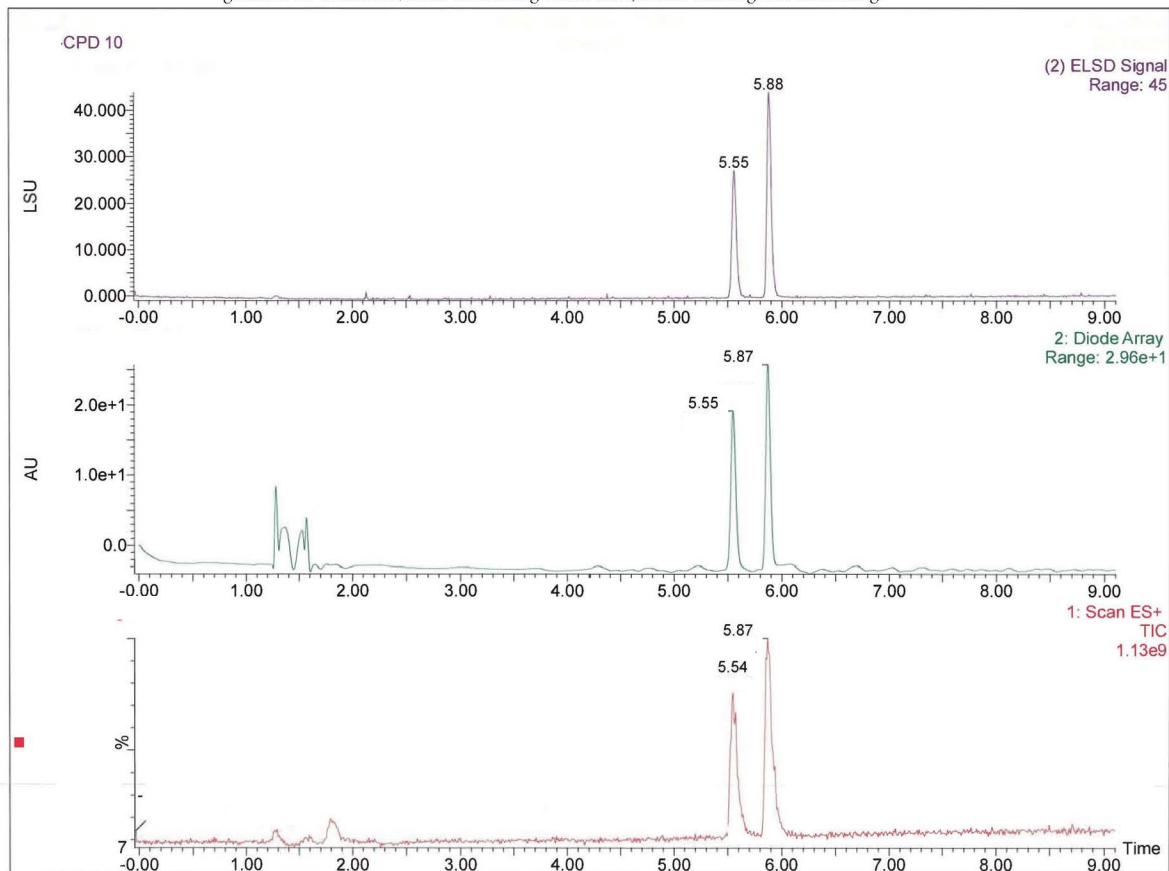
¹³C NMR (DMSO-d₆) Compound 10



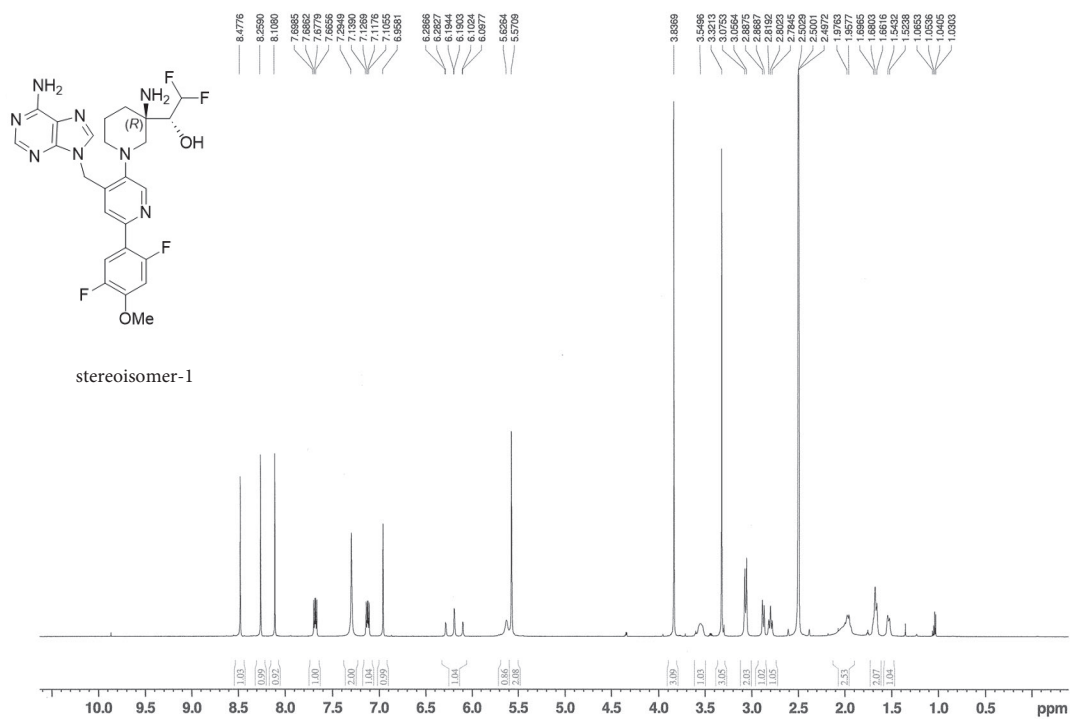


LCMS of Compound 10

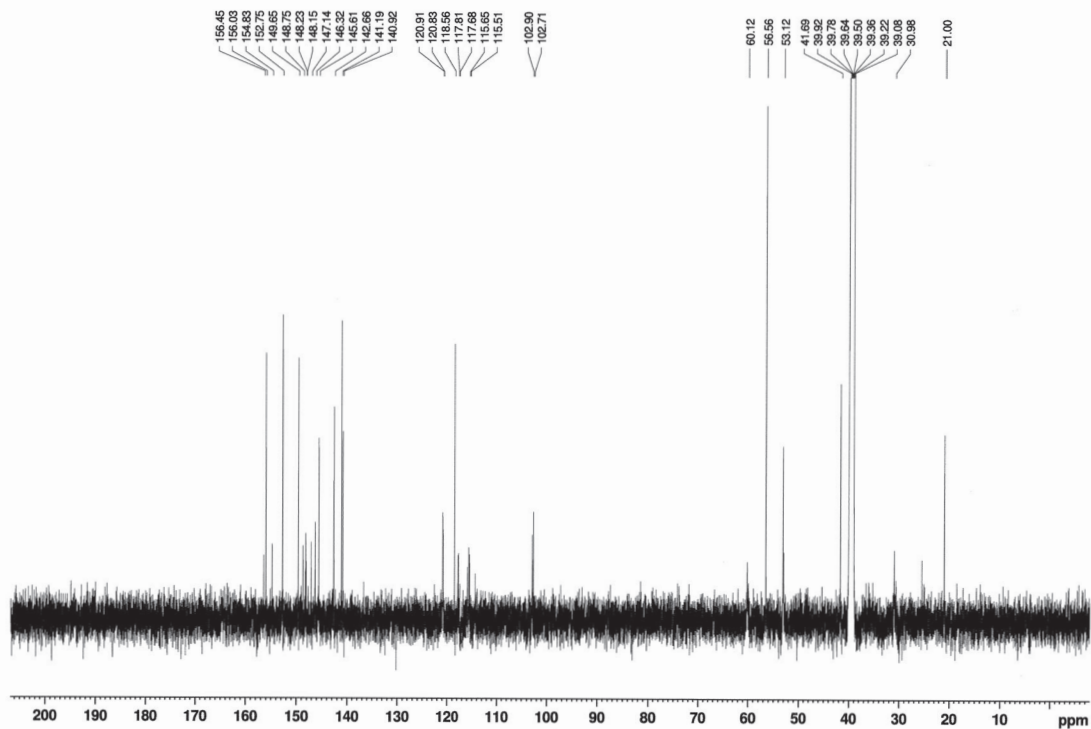
LCMS method: Autopure Waters LCMS, Xbridge C18 3.5 μ m 4.6 x 150 mm column, flow rate 1.4ml/min 8 min run with gradient 10-50% ACN/water containing 0.05% TFA, rest is washing and calibrating.



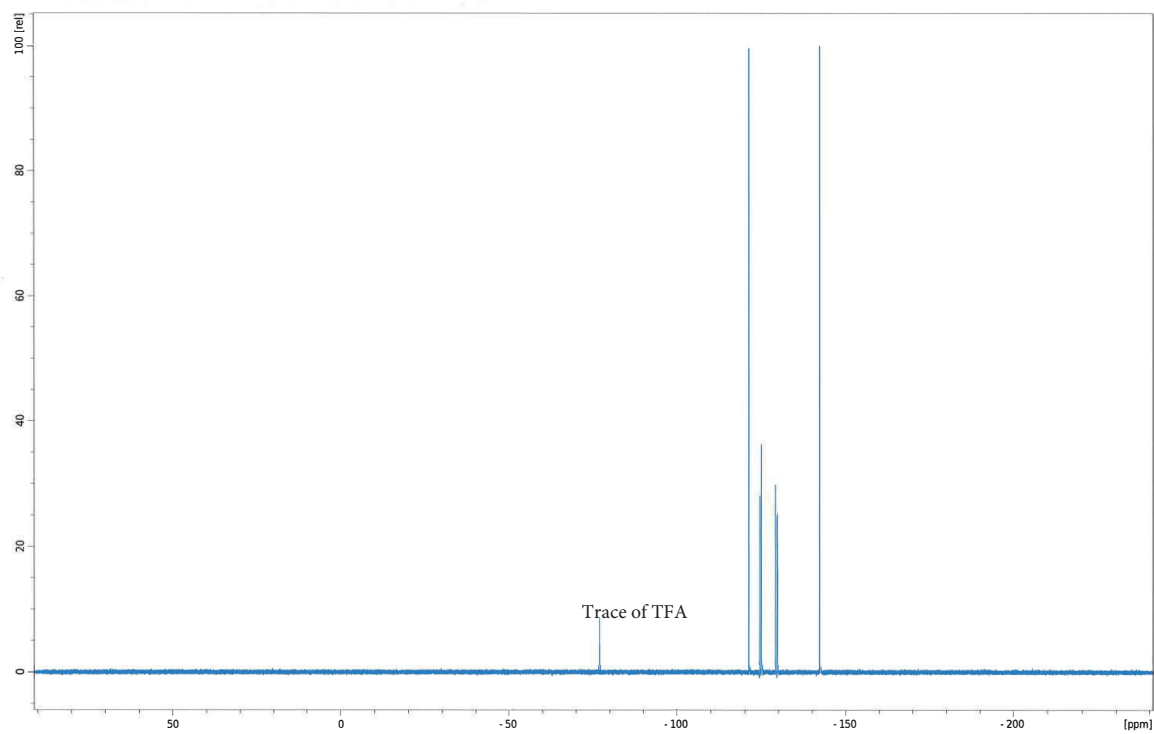
¹H NMR (600 MHz, DMSO-d₆) Stereoisomer-1



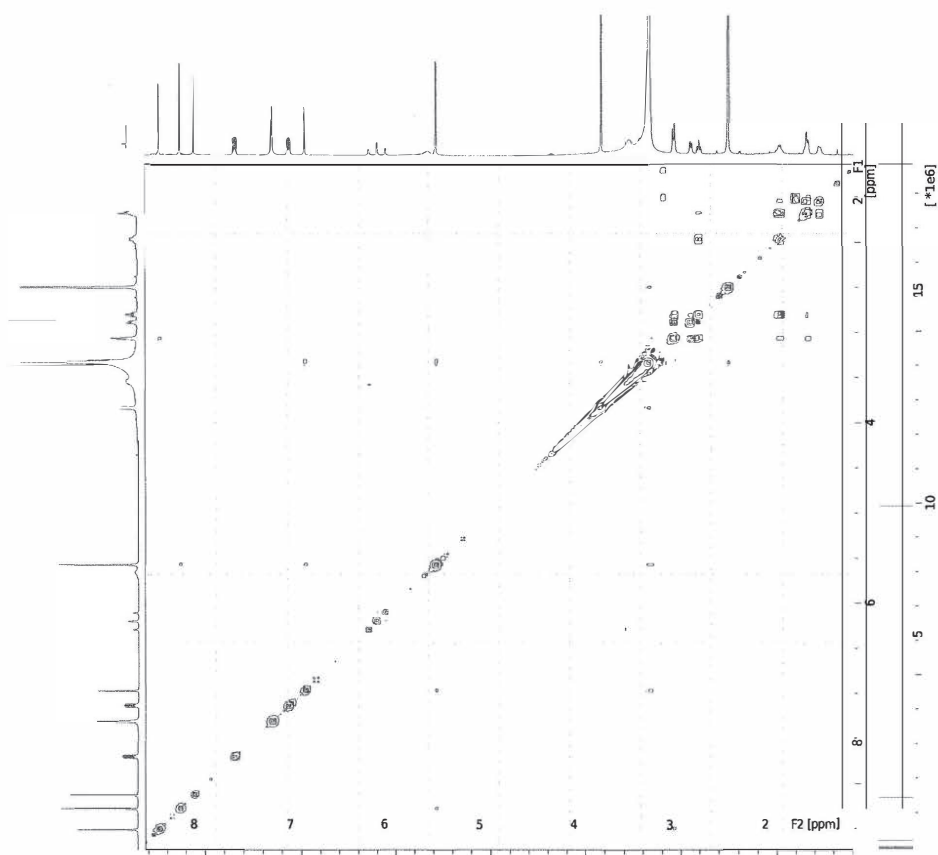
¹³C NMR (151 MHz, DMSO-d₆) Stereoisomer-1

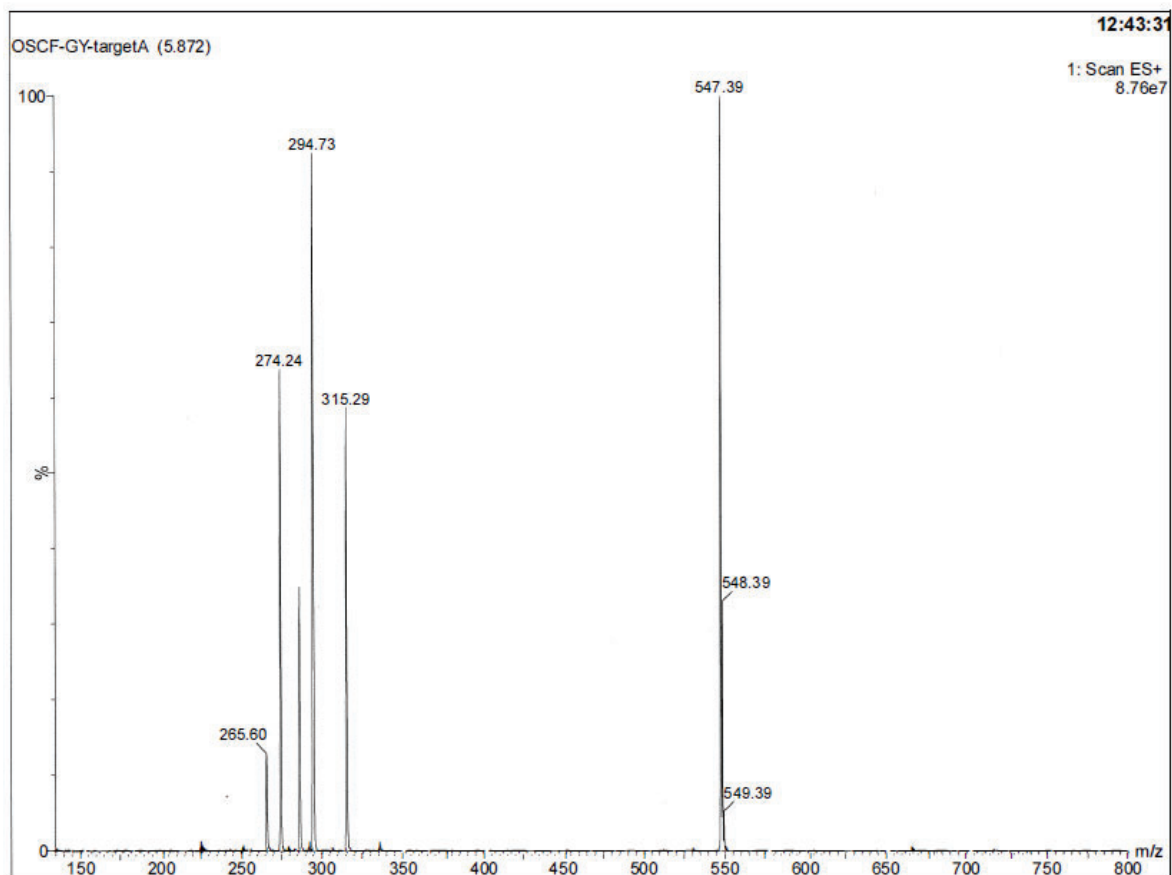
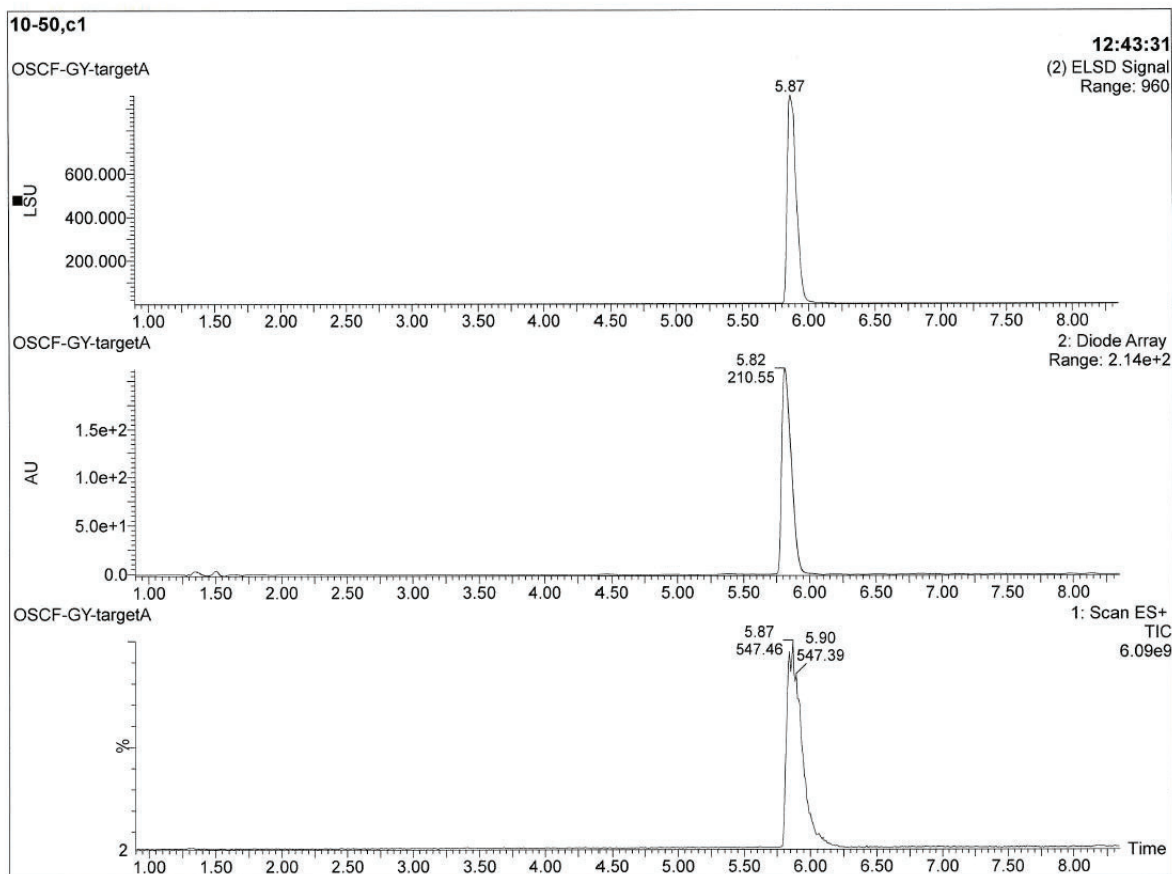


^{19}F NMR (565 MHz, DMSO- d_6) Stereoisomer-1



2D COSY Stereoisomer-1



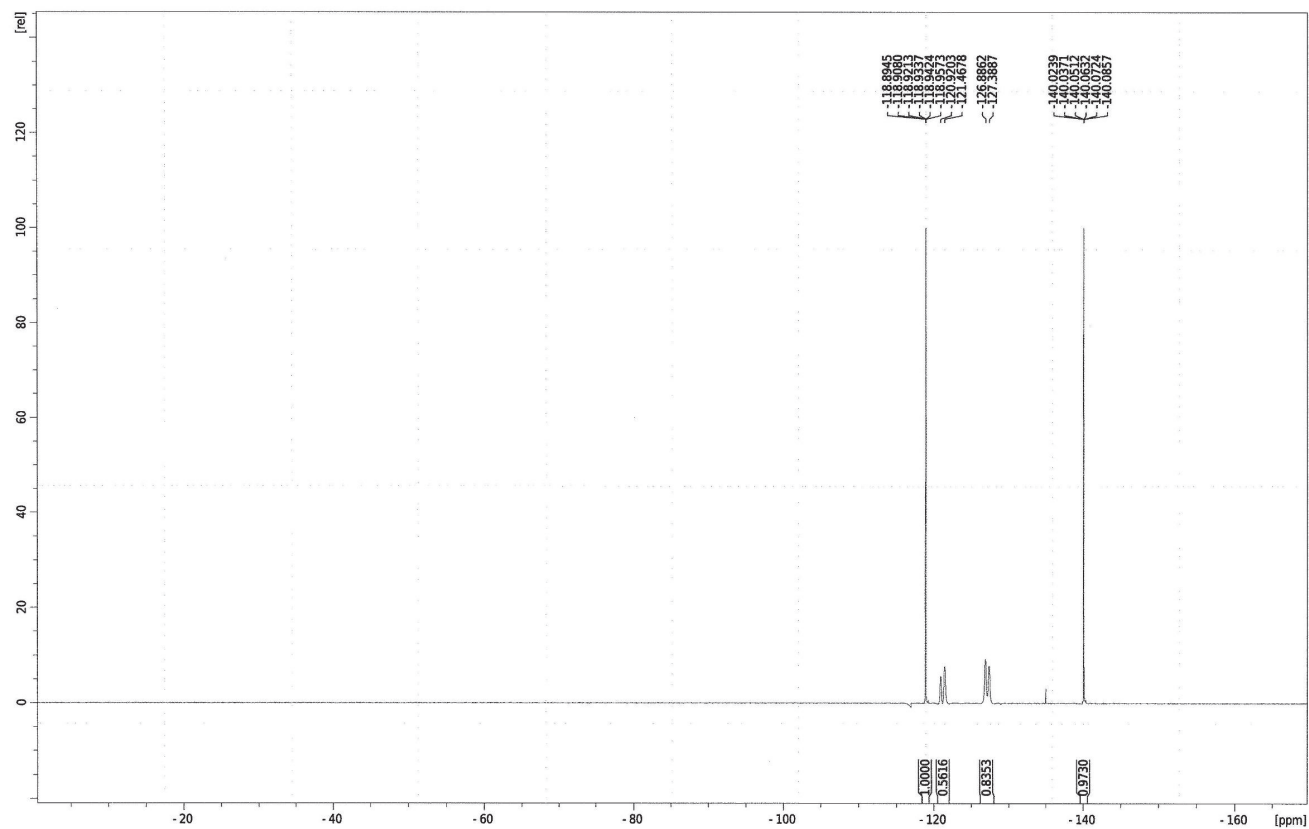


Chemical structure of stereoisomer-2 is shown, which is a pyrazoloquinoline derivative with a 2-fluoro-4-methoxyphenyl group and a (R)-2-amino-2-fluoro-1-hydroxyethyl group.

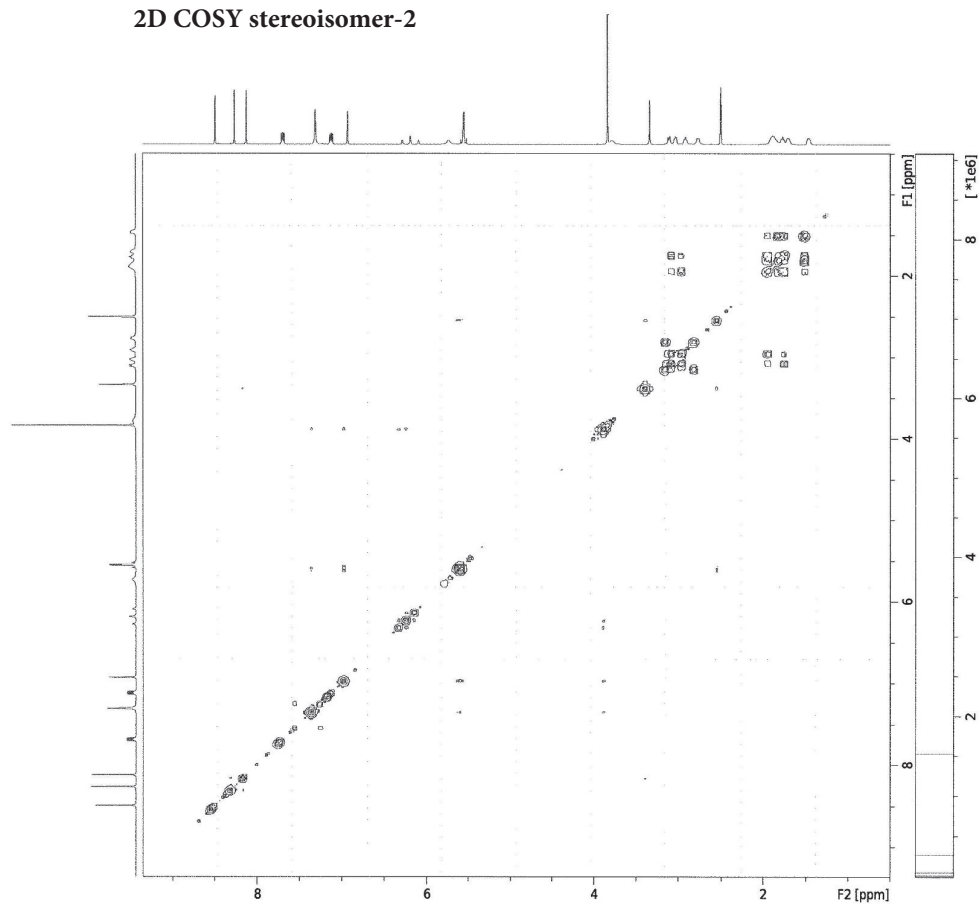
Chemical shifts (ppm) labeled on the left side of the spectrum:

- 156.4504
- 156.0437
- 154.2829
- 152.7485
- 152.0219
- 148.7406
- 148.3017
- 148.1441
- 147.1640
- 146.1172
- 145.2884
- 145.2625
- 141.2979
- 140.9838
- 120.8901
- 120.7214
- 118.4440
- 117.7359
- 117.7234
- 117.6501
- 116.6500
- 115.8184
- 115.7064
- 114.4667
- 102.8933
- 102.8951
- 72.3421
- 60.6195
- 56.5554
- 52.8846
- 52.7576
- 41.7587
- 39.9170
- 39.6391
- 36.5600
- 35.2218
- 35.0827
- 32.2726
- 21.3467

¹⁹F-NMR stereoisomer-2

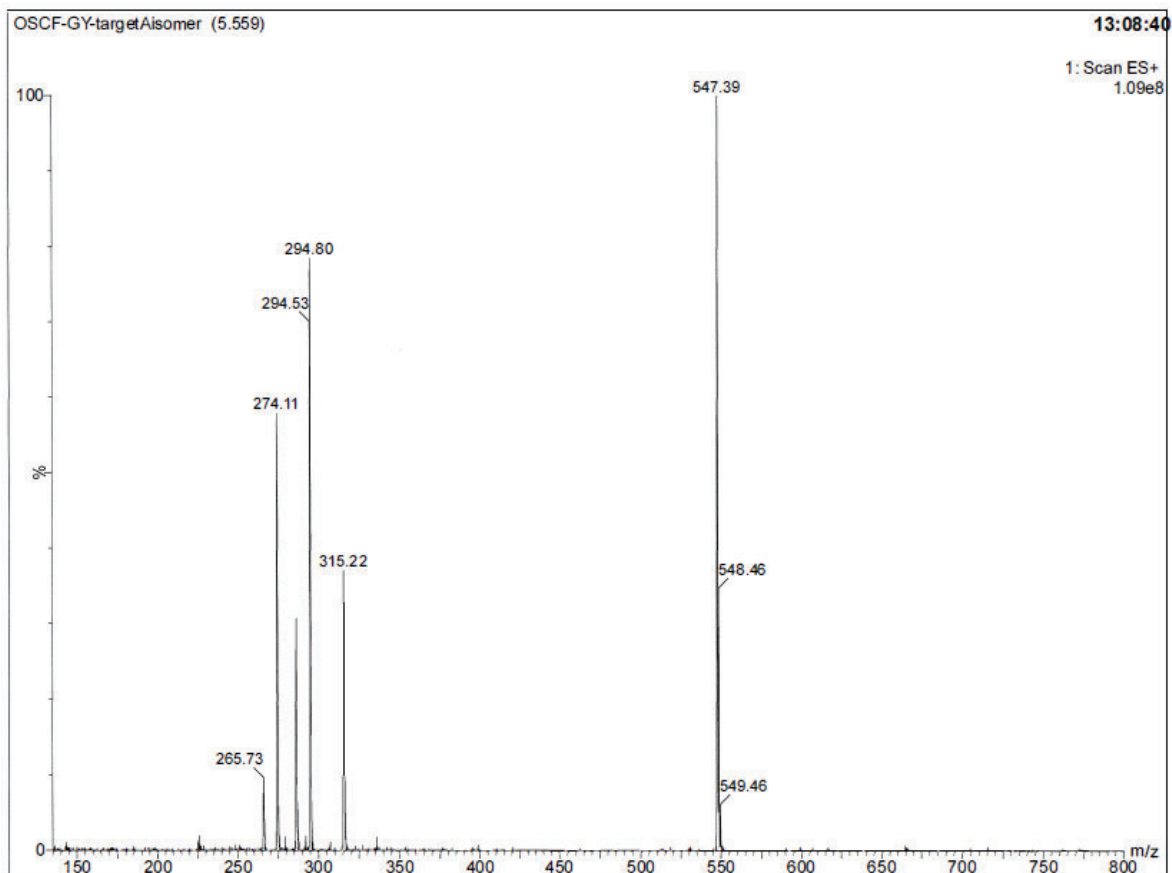
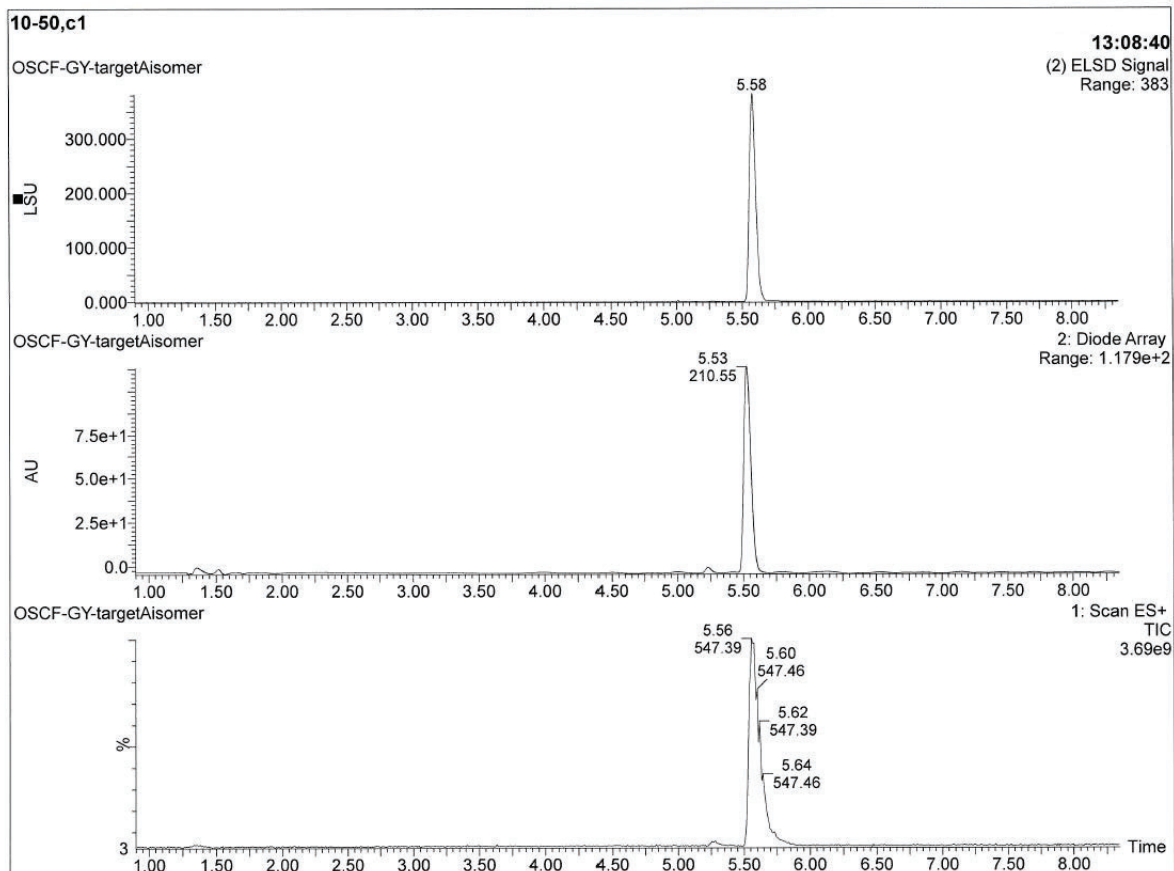


2D COSY stereoisomer-2



LCMS of stereoisomer-2

LCMS method: Autopure Waters LCMS, Xbridge C18 3.5 μ m 4.6 x 150 mm column, flow rate 1.4ml/min 8 min run with gradient 10-50% ACN/water containing 0.05% TFA, rest is washing and calibrating.



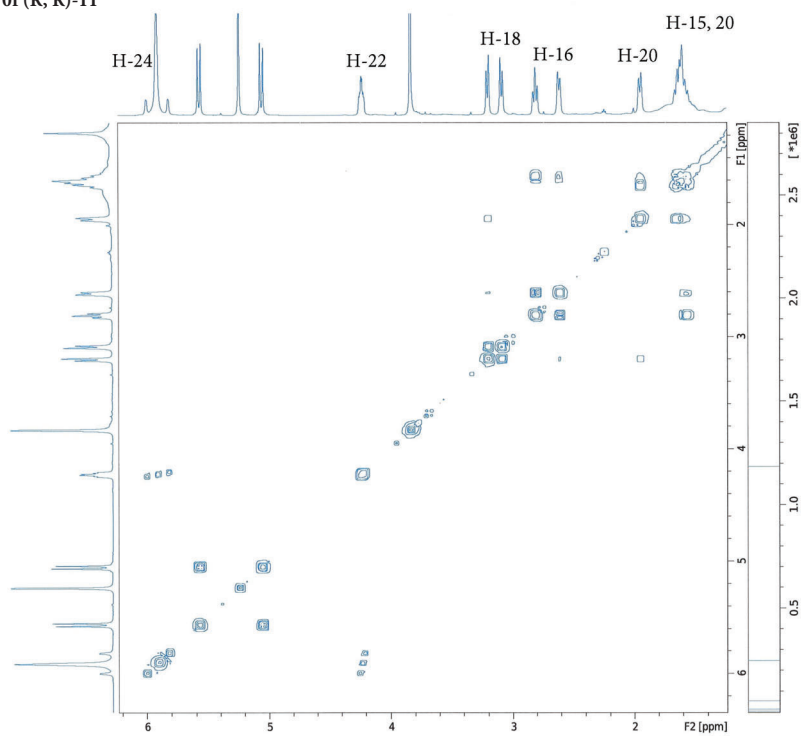
Chemical structure of (R,R)-11 is shown in the top left. The structure is a 1,2,3,4,5,6-hexahydro-1H-benzotriazin-4-ylmethyl derivative. It features a benzotriazine ring system with an amino group (NH₂) at position 6 and a methyl group at position 4. The methyl group is attached to a 1,2,3,4,5,6-hexahydro-1H-benzotriazin-4-ylmethyl group. The stereochemistry is (R,R).

¹H NMR spectrum (CD₂Cl₂) of (R,R)-11. The spectrum shows peaks corresponding to the structure, with integration values provided below the baseline. Key peaks are labeled: NH (~9.3 ppm), H-24 NH₂ (~5.9 ppm), CD₂Cl₂ solvent (~5.3 ppm), OMe (~3.8 ppm), H-22 (~4.2 ppm), H-18 (~2.2 ppm), H-16 (~2.0 ppm), H-20 (~1.7 ppm), H-20, 15 (~1.5 ppm), and two peaks labeled X (~1.2 ppm).

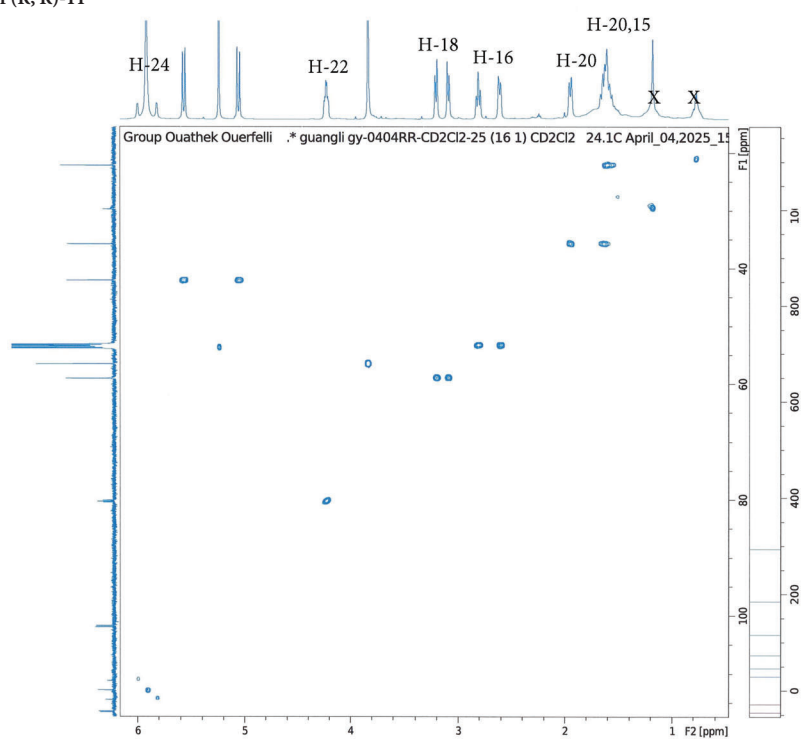
Chemical Shift (ppm)	Integration
9.3446	0.8742
8.4117	2.0352
7.7968	3.0027
7.7498	
7.7468	
7.7438	
7.6894	
6.7363	1.0000
6.7195	
6.7059	
5.9138	3.0474
5.5376	1.0246
5.5324	
5.2391	1.8275
5.0658	1.0414
5.0418	
4.2395	0.9939
4.2355	
3.8306	3.1419
3.2116	1.0268
3.1982	1.0543
3.0781	
3.0756	
2.8101	1.0962
2.7905	1.0755
2.6020	
1.9570	1.1764
1.9444	0.7747
1.6247	4.3183
1.6249	
1.2649	3.9742
1.2649	
1.2633	1.0335

[illegible]

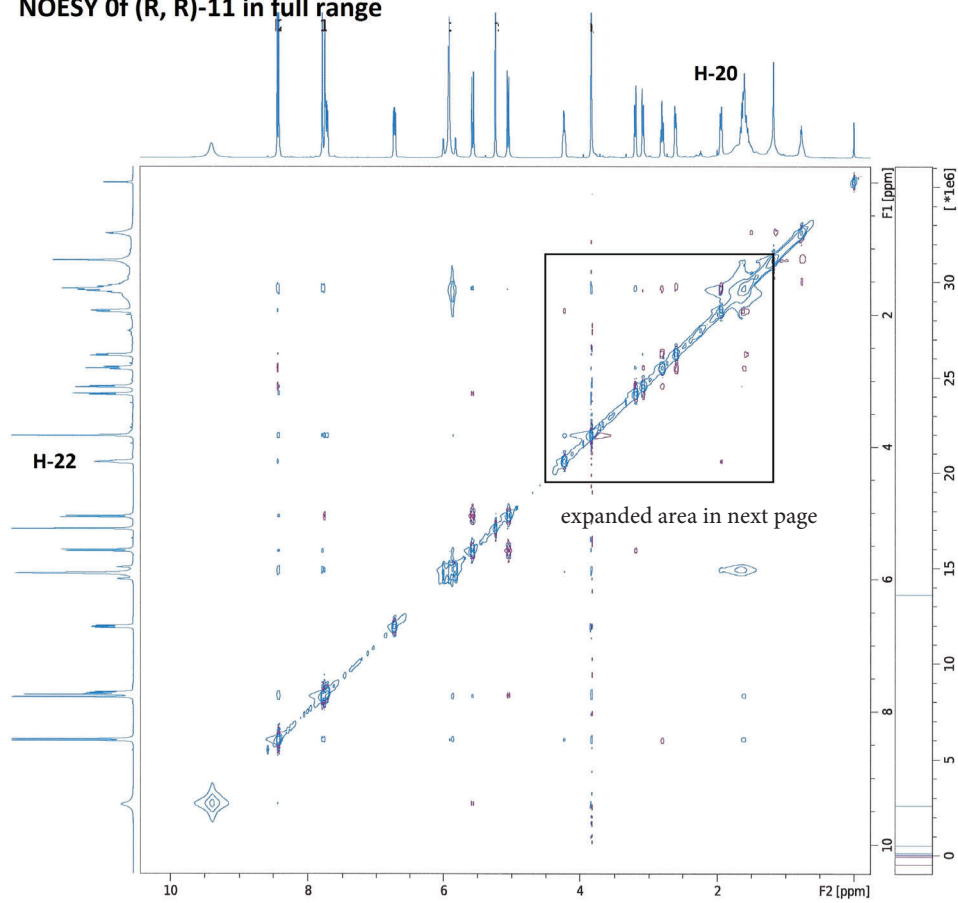
2D-COSY of (R, R)-11



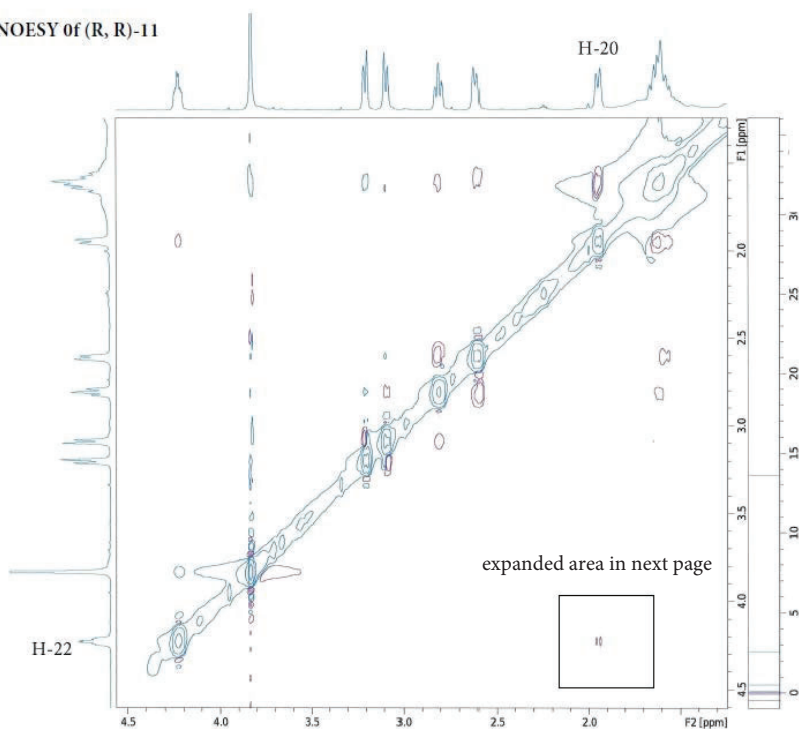
HSQC of (R, R)-11



NOESY of (R, R)-11 in full range

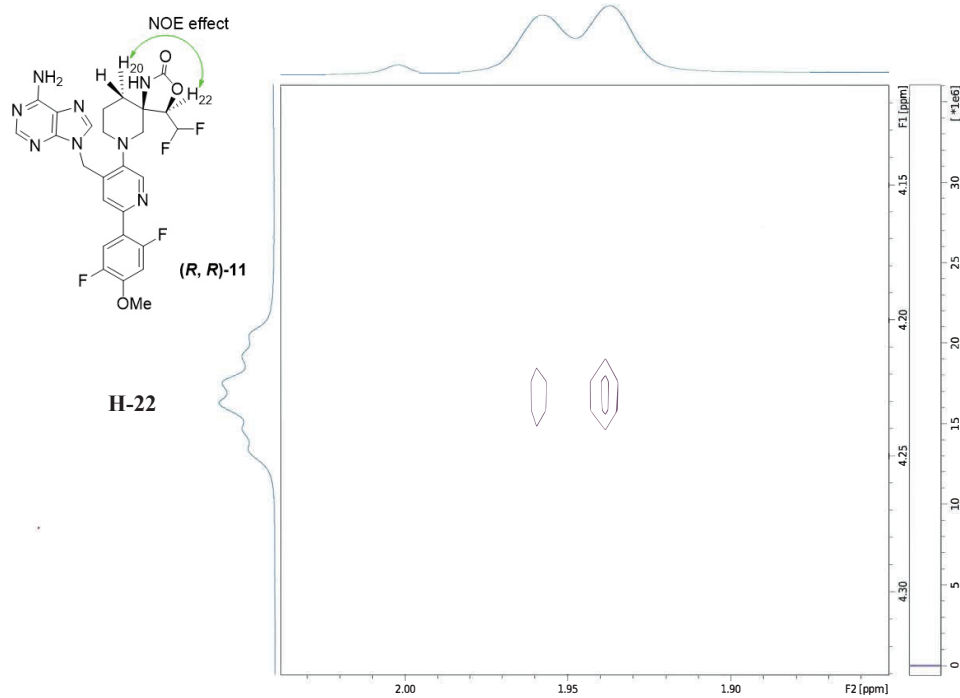


NOESY of (R, R)-11



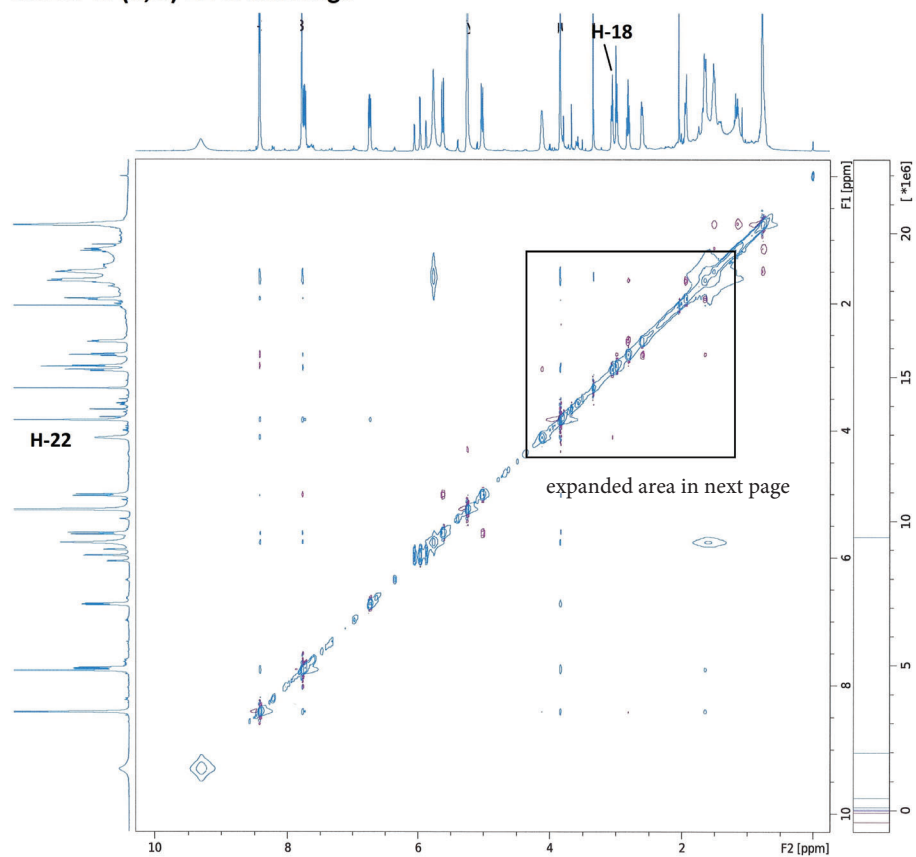
NOSEY of (R, R)-11

H-20

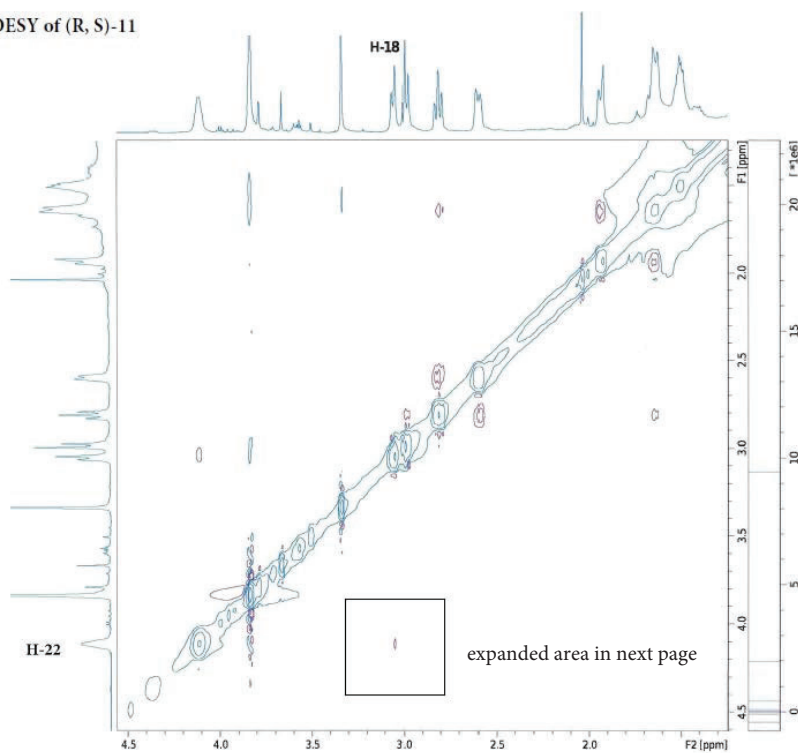


[illegible]

NOESY of (R, S)-11 in full range



NOESY of (R, S)-11



NOSEY of (R, S)-11

