Supporting Information

Design, synthesis, and biological evaluation of imidazopyridines as PD-1/PD-L1 antagonists

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General information

Reagents were available from commercial suppliers (Sigma Aldrich, ABCR, Acros and AK Scientific) and used without any purification unless otherwise noted. Thin layer chromatography was performed on Fluka precoated silica gel plates (0.20 mm thick, particle size 25 µm). Flash chromatography was performed on a Teledyne ISCO Combiflash Rf, using RediSep Rf Normal-phase Silica Flash Columns (Silica Gel 60 Å, 230 – 400 mesh) and on a Reveleris® X2 Flash Chromatography, using Grace® Reveleris Silica flash cartridges (40 grams, 24 grams, 12 grams and 3 grams). All HTRF experiments were performed using a Cisbio Bioassays Human PD1/PD-L1 biochemical binding assay. All microwave irradiation reactions were carried out in a Biotage Initiator+. Nuclear magnetic resonance spectra were recorded on a Bruker Avance 500 spectrometer. Chemical shifts for ^{1}H NMR were reported relative to TMS ($\delta = 0$ ppm) or the corresponding solvent peak ($CDCl_3$ $\delta = 7.26$ ppm, DMSO-d_6 $\delta = 2.50$ ppm, CD_3OD $\delta = 3.31$ ppm) and coupling constants were reported in Hertz (Hz). The following abbreviations were used for spin multiplicity: s = singlet, bs = broad singlet, d = doublet, dd = doublet of doublets, t = triplet, ddt = doublet of doublet of triplets, q = quartet, and m = multiplet. Chemical shifts for ^{13}C NMR were reported in ppm relative to the solvent peak (Chloroform-*d* $\delta = 77.2$ ppm, Methanol-d_4 $\delta = 49.0$ ppm, DMSO-d_6 $\delta = 39.5$ ppm). High resolution mass spectra (HRMS) were recorded using an Orbitrap-Velos Proat a resolution of 60,000. Melting points were obtained on a melting point apparatus and were uncorrected.

Experimental procedures and analytical data

Procedure A: General procedure for the Suzuki-reaction

A mixture of (3-bromo-2-methylphenyl)methanol (1 eq.), Boronic acid (1.5 eq.) in toluene:ethanol:sat. aq. sodium bicarbonate solution (5:1:5, 0.3 M) was placed under nitrogen and degassed for 10 minutes. [1,1'-Bis(diphenylphosphino)ferrocene]palladium(II) chloride (0.5 mol%) was added and the reaction mixture was heated at 85 °C for 12 h. Ethyl acetate and water were added to the reaction mixture. The organic phase was washed with 1M sodium hydroxide solution and brine. The organic extract was dried over magnesium sulfate and concentrated by rotatory evaporation. The residue was purified by silica gel flash chromatography using PE–EA as eluent.

Procedure B: General procedure for the nucleophilic aromatic substitution & reduction

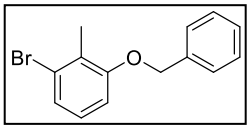
A Solution of the hydroxide component (1 eq.) and para-fluoro-nitropyridine component (1 eq.) in DMSO (1 M) was stirred at 0°C. Potassium hydroxide (2 eq.) was finely ground and added to the reaction mixture. The reaction mixture was stirred at rt until complete conversion (TLC monitoring, 10-60 min.). Ethyl acetate was added to the reaction mixture followed by cold water. The water phase was extracted thrice with ethyl acetate. The combined organic phases were washed with brine and dried over magnesium sulfate. Concentration of the organic phases resulted in orange oil. The crude product was used without further purification for the next reaction.

A suspension of nitropyridine component (1 eq.), ethanol:water (5:1, 0.1 M), iron (10 eq.) and Hydrochloric acid (37%, 3 eq.) was stirred at reflux for 2 h (TLC monitoring). After completion, the reaction mixture was concentrated by rotatory evaporation, diluted with sat. bicarbonate solution and ethyl acetate where added. The water phase has been extracted thrice with ethyl acetate. The combined organic phase was filtrated over magnesium sulfate and silica, concentration resulted in an orange solid. The residue was purified by silica gel flash chromatography using DCM–MeOH as eluent

<u>Procedure C</u>: General procedure for Groebke–Blackburn–Bienaymé reaction & deprotection reaction

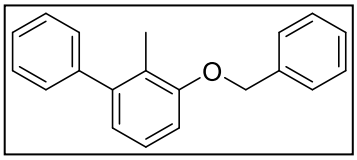
A mixture of Aminopyridine component (1 eq.), aldehyde (1.7 eq.), scandium triflate (0.1 eq.) and isocyanide (1.7 eq.) in DCM/MeOH (2:1; 0.3 M) was placed in a glass microwave vial and sealed. The reaction vial was heated in a microwave for 1 hour at 120°C. Evaporation of the solvents was followed by purification by silica gel flash chromatography using PE–EA as eluent. The still slightly impure product was used directly in the further step. Afterwards the crude product was treated with 50 eq. 7N HCl in 2-Propanol for 20h at rt. Concentration of the reaction mixture under vacuum resulted in an orange oil. Chromatographic purification on silica gel (DCM/MeOH) resulted in the corresponding products.

1-(benzyloxy)-3-bromo-2-methylbenzene (A)



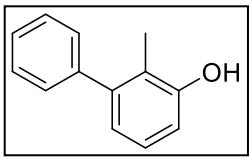
Benzyl alcohol (20 mmol) was added to a suspension of NaH (20 mmol) in N-methylpyrrolidone. The freshly prepared solution was added to 1-bromo-3-fluoro-2-methylbenzene (5.41 mmol) in N-methylpyrrolidone. The reaction was heated at 100 °C until complete conversion of the starting material (TLC monitoring). Ethyl acetate was added to the reaction mixture followed by water. The water phase was extracted thrice with ethyl acetate. The combined organic phases were washed with brine and dried over magnesium sulfate. Concentration and chromathographic purification over silica using PE–EA as eluent afforded **A** (1.38g, 4.98 mmol, 92%) as a colorless oil; ^{1}H NMR (500 MHz, chloroform-*d*) δ = 7.39 – 7.32 (m, 4H), 7.30 – 7.26 (m, 1H), 7.13 (dd, *J* = 8.1, 1.1 Hz, 1H), 6.93 (t, *J* = 8.1 Hz, 1H), 6.76 (dd, *J* = 8.1, 1.1 Hz, 1H), 4.98 (s, 2H), 2.36 (s, 3H); ^{13}C NMR (126 MHz, chloroform-*d*) δ = 157.5, 137.0, 128.6, 128.0, 127.4, 127.3, 127.2, 126.0, 124.9, 110.6, 70.4, 16.0; HRMS (APCI) m/z calculated for $C_{14}H_{14}OBr$ $[M+H]^+$: 277.0223, found $[M+H]^+$: 277.0221.

3-(benzyloxy)-2-methyl-1,1'-biphenyl (B)



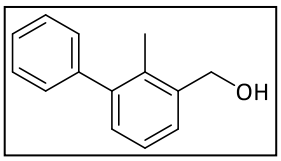
Synthesis according to procedure **A** using compound A (1.00 g, 3.6 mmol) and phenylboronic acid (658 mg, 5.41 mmol) afforded **B** (770 mg, 2,81 mmol, 78%) as an colorless amorphous solid; ^{1}H NMR (500 MHz, chloroform-*d*) δ = 7.47 (dd, *J* = 8.3, 1.2 Hz, 2H), 7.43 – 7.37 (m, 4H), 7.36 – 7.30 (m, 4H), 7.19 (t, *J* = 7.8 Hz, 1H), 6.92 (dd, *J* = 8.3, 1.2 Hz, 1H), 6.89 (dd, *J* = 7.8, 1.2 Hz, 1H), 5.13 (s, 2H), 2.20 (s, 3H); ^{13}C NMR (126 MHz, chloroform-*d*) δ = 157.2, 143.6, 142.0, 137.6, 129.5, 128.7, 128.1, 127.9, 127.3, 126.9, 126.1, 124.9, 122.6, 110.4, 70.3, 13.7; HRMS (APCI) m/z calculated for $C_{20}H_{19}O$ $[M+H]^+$: 275.1430, found $[M+H]^+$: 275.1430.

2-methyl-[1,1'-biphenyl]-3-ol (C)



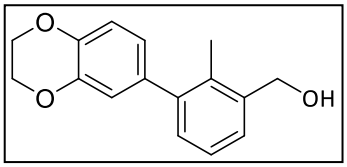
3- (benzyloxy) -2-methyl-1,1'-biphenyl (500 mg, 1.82 mmol) was hydrogenated under atmospheric hydrogen with 10% Pd/C on carbon (containing 50% water, 50 mg) as catalyst in MeOH (5 ml) and THF (2 ml) at room temperature for 72 hours. Filtration of precipitated, wash with ethyl acetate, then concentration gave the afforded **C** (319 mg, 1.73 mmol, 95%) as a colorless oil; ^{1}H NMR (500 MHz, chloroform-*d*) δ = 7.40 – 7.33 (m, 2H), 7.34 – 7.23 (m, 3H), 7.07 (t, *J* = 7.9 Hz, 1H), 6.83 (dd, *J* = 7.5, 1.3 Hz, 1H), 6.74 (dd, *J* = 7.9, 1.3 Hz, 1H), 5.11 (s, 1H), 2.13 (s, 3H); ^{13}C NMR (126 MHz, chloroform-*d*) δ = 154.0, 143.9, 141.8, 129.4, 128.1, 127.0, 126.3, 122.7, 121.9, 114.0, 13.1.; HRMS (APCI) m/z calculated for $C_{13}H_{13}O$ $[M+H]^+$: 185.0961, found $[M+H]^+$: 185.0960.

(2-methyl-[1,1'-biphenyl]-3-yl)methanol (3a)



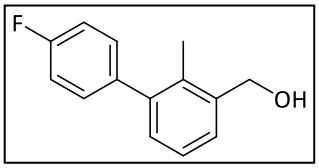
Synthesis according to procedure **A** using (3-bromo-2-methylphenyl)methanol (2.7 g, 13.4 mmol), [1,1'-Bis(diphenylphosphino)ferrocene]palladium(II) chloride (51 mg, 70 µmol), and phenylboronic acid (2.46 g, 20.2 mmol) afforded **3a** (2.52 g, 12.7 mmol, 95%) as a colorless solid; ^{1}H NMR (500 MHz, chloroform-*d*) δ = 7.40 (q, *J* = 8.6, 7.7 Hz, 2H), 7.36 – 7.33 (m, 1H), 7.30 – 7.27 (m, 2H), 7.26 – 7.25 (m, 1H), 7.20 (dd, *J* = 7.7, 1.6 Hz, 1H), 4.77 (d, *J* = 5.6 Hz, 2H), 2.24 (s, 3H), 1.65 (t, *J* = 5.6 Hz, 1H); ^{13}C NMR (126 MHz, chloroform-*d*) δ = 142.8, 142.1, 139.3, 133.6, 129.5, 129.5, 128.1, 126.9, 126.7, 125.6, 63.9, 15.9; HRMS (APCI) m/z calculated for $C_{14}H_{13}$ $[M+H-H_2O]^+$: 181.1012, found $[M+H-H_2O]^+$: 181.1009.

(3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-2-methylphenyl)methanol (3b)



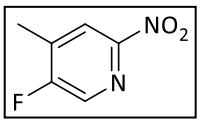
Synthesis according to procedure **A** using (3-bromo-2-methylphenyl)methanol (5.2 g, 26 mmol), [1,1'-Bis(diphenylphosphino)ferrocene]palladium(II) chloride (95 mg, 130 µmol), and (2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)boronic acid (6.5 g, 39 mmol) afforded **3b** (6.00 g, 23.4 mmol, 90%) as a colorless solid; ^{1}H NMR (500 MHz, chloroform-*d*) δ = 7.35 (dd, *J* = 7.6, 1.6 Hz, 1H), 7.21 (t, *J* = 7.6 Hz, 1H), 7.16 (dd, *J* = 7.6, 1.6 Hz, 1H), 6.89 (d, *J* = 8.2 Hz, 1H), 6.80 (d, *J* = 2.1 Hz, 1H), 6.75 (dd, *J* = 8.2, 2.1 Hz, 1H), 4.73 (s, 2H), 4.28 (s, 4H), 2.24 (s, 3H).); ^{13}C NMR (126 MHz, chloroform-*d*) δ = 143.1, 142.6, 142.3, 139.3, 135.5, 133.7, 129.6, 126.6, 125.6, 122.6, 118.3, 116.9, 64.5, 64.5, 64.0, 16.0; HRMS (APCI) m/z calculated for $C_{16}H_{15}O_2$ $[M+H-H_2O]^+$: 239.1067, found $[M+H-H_2O]^+$: 239.1066.

(4'-fluoro-2-methyl-[1,1'-biphenyl]-3-yl)methanol (3c)



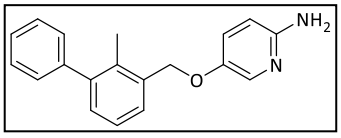
Synthesis according to procedure **A** using (3-bromo-2-methylphenyl)methanol (2.0 g, 10 mmol) [1,1'-Bis(diphenylphosphino)ferrocene]palladium(II) chloride (37 mg, 50 µmol), and (4-fluorophenyl)boronic acid (2.1 mg, 15 mmol) afforded **3c** (2.2 g, 9.7 mmol, 97%) as a colorless solid; ^{1}H NMR (500 MHz, chloroform-*d*) δ = 7.38 (dd, *J* = 7.5, 1.5 Hz, 1H), 7.26 – 7.22 (m, 3H), 7.16 (dd, *J* = 7.5, 1.5 Hz, 1H), 7.11 – 7.07 (m, 2H), 4.74 (s, 2H), 2.21 (s, 3H), 1.84 (s, 1H);^{13}C NMR (126 MHz, chloroform-*d*) δ = 162.0 (d, J_{CF} = 245.6 Hz) 141.9, 139.4, 138.0 (d, J_{CF} = 3.5 Hz), 133.8, 131.0 (d, J_{CF} = 7.9 Hz), 129.7, 127.0, 125.8, 115.1 (d, J_{CF} = 21.3 Hz)., 64.1, 16.0; HRMS (APCI) m/z calculated for $C_{14}H_{12}F$ $[M+H-H_2O]^+$: 199.0918, found $[M+H-H_2O]^+$: 199.0915.

5-fluoro-4-methyl-2-nitropyridine (4b)



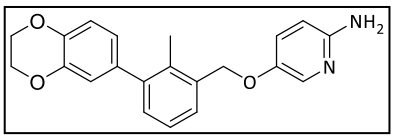
Sulfuric acid (15 ml, 97%) was mixed with 30% hydrogen peroxide solution (13.2 ml) at 0°C. After addition of 5-fluoro-4-methylpyridin-2-amine (2.70 g 21.4 mmol) the reaction mixture was let warm up to rt and stirred for 18h. Neutralization with Sodium bicarbonate at 0°C was followed by extraction of the reaction mixture thrice with Ethyl acetate. The combined organic phase was filtrated over magnesium sulfate, concentration resulted in a yellow solid. The residue was purified by silica gel flash chromatography using PE–EA as eluent afforded **4b** (3.1 g, 19.8 mmol, 93%) as a yellow solid; ^{1}H NMR (500 MHz, DMSO-d_6)δ = 8.59 (s, 1H), 8.43 (d, J = 5.3 Hz, 1H), 2.43 (d, J = 1.8 Hz, 3H); ^{13}C NMR (126 MHz, chloroform-*d*) δ = 161.3 (d, J_{CF} = 263 Hz), 152.2, 138.3 (d, J_{CF} = 17.0 Hz), 136.2 (d, J_{CF} = 28.6 Hz), 121.4 (d, J_{CF} = 4.60 Hz), 14.6 (d, J_{CF} = 2.70 Hz), HRMS (APCI) m/z calculated for $C_6H_6FN_2O_2$ $[M+H]^+$: 157.0408, found $[M+H]^+$: 157,0408.

5-((2-methyl-[1,1'-biphenyl]-3-yl)methoxy)pyridin-2-amine (6a)



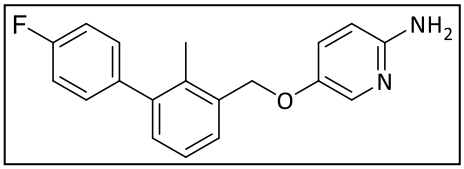
Synthesis according to procedure **B** using 3a (800 g, 4,04 mmol) and 5-Fluoro-2-nitropyridine (574 mg, 4.04 mmol) afforded **6a** (1.02 g 3.51 mmol, 87%) as an orange solid; ^{1}H NMR (500 MHz, chloroform-*d*) δ = 7.89 (dd, J = 3.0, 0.7 Hz, 1H), 7.45 – 7.37 (m, 3H), 7.36 – 7.32 (m, 1H), 7.31 – 7.28 (m, 2H), 7.27 – 7.21 (m, 2H), 7.17 (dd, J = 8.8, 3.0 Hz, 1H), 6.47 (dd, J = 8.8, 0.7 Hz, 1H), 5.03 (s, 2H), 4.25 (s, 2H), 2.24 (s, 3H); ^{13}C NMR (126 MHz, chloroform-*d*) δ = 153.4, 148.9, 143.0, 142.0, 135.3, 134.8, 134.3, 130.3, 129.5, 128.2, 128.3, 126.9, 126.8, 125.7, 109.4, 70.7, 16.2; HRMS (ESI) m/z calculated for $C_{19}H_{19}ON_2$ $[M+H]^+$: 291.1492, found $[M+H]^+$: 291.1490.

5-((3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-2-methylbenzyl)oxy)pyridin-2-amine (6b)



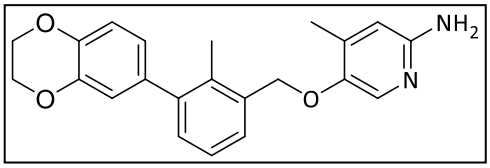
Synthesis according to procedure **B** using 3b (3.56 g, 13.9 mmol) and 5-Fluoro-2-nitropyridine (1.98 g, 13.9 mmol) afforded **6b** (4.31 g, 12.4 mmol, 89%) as an orange solid; ^{1}H NMR (500 MHz, chloroform-*d*) δ = 7.89 (dd, J = 3.0, 0.7 Hz, 1H), 7.37 (dd, J = 6.3, 2.8 Hz, 1H), 7.24 – 7.19 (m, 2H), 7.18 (dd, J = 8.8, 3.0 Hz, 1H), 6.90 (d, J = 8.2 Hz, 1H), 6.83 (d, J = 2.1 Hz, 1H), 6.77 (dd, J = 8.2, 2.1 Hz, 1H), 6.48 (dd, J = 8.8, 0.7 Hz, 1H), 5.02 (s, 2H), 4.30 (s, 4H), 4.22 (s, 2H), 2.26 (s, 3H); ^{13}C NMR (126 MHz, chloroform-*d*) δ = 153.3, 148.9, 143.1, 142.7, 142.5, 135.4, 135.2, 134.5, 134.4, 130.3, 127.9, 127.0, 125.6, 122.7, 118.3, 117.0, 109.6, 70.7, 64.5, 64.5, 16.3; HRMS (ESI) m/z calculated for $C_{21}H_{21}O_3N_2$ $[M+H]^+$: 349.1547, found $[M+H]^+$: 349.1545.

5-((4'-fluoro-2-methyl-[1,1'-biphenyl]-3-yl)methoxy)pyridin-2-amine (6c)



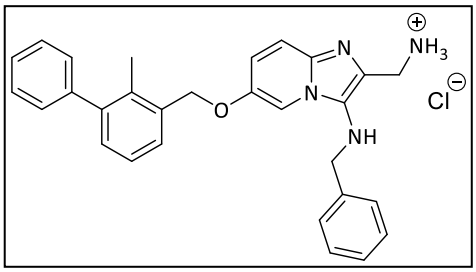
Synthesis according to procedure **B** using 3c (1.08 g, 5.00 mmol) and 5-Fluoro-2-nitropyridine (711 mg, 5.00 mmol) afforded **6c** (1.42 g, 4.6 mmol, 92%) as an orange solid; ^{1}H NMR (500 MHz, chloroform-*d*) δ = 7.89 (d, *J* = 2.9 Hz, 1H), 7.40 (dd, *J* = 7.5, 1.6 Hz, 1H), 7.28 – 7.22 (m, 3H), 7.21 – 7.16 (m, 2H), 7.13 – 7.07 (m, 2H), 6.49 (d, *J* = 8.8 Hz, 1H), 5.03 (s, 2H), 4.24 (bs, 2H), 2.23 (s, 3H); ^{13}C NMR (126 MHz, chloroform-*d*) δ = 162.0 (d, J_{CF} = 245.7 Hz), 153.4, 148.8, 142.0, 137.9 (d, J_{CF} = 3.3 Hz), 135.4, 134.5, 134.4, 131.0 (d, J_{CF} = 7.9 Hz), 130.3, 128.3, 127.0, 125.8, 115.09 (d, J_{CF} = 21.2 Hz), 109.5, 70.6, 16.2; HRMS (ESI) m/z calculated for $C_{19}H_{18}FN_2O$ $[M+H]^+$: 309.1398, found $[M+H]^+$: 309.1397.

5-((3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-2-methylbenzyl)oxy)-4-methylpyridin-2-amine(6d)



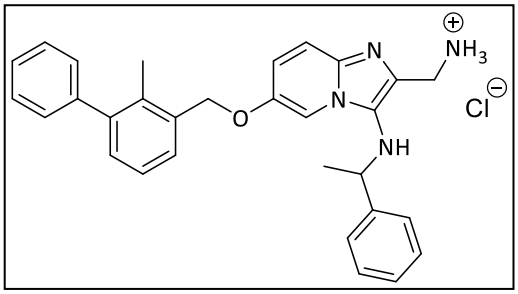
Synthesis according to procedure **B** using 3a (1.15 g, 4.50 mmol) and **4b** (703 mg, 4.50 mmol) afforded **6d** (1.17 g 3.24 mmol, 72%) as a pale white solid; ^{1}H NMR (500 MHz, chloroform-*d*) δ = 7.73 (s, 1H), 7.40 (dd, *J* = 7.0, 2.1 Hz, 1H), 7.26 – 7.20 (m, 2H), 6.91 (d, *J* = 8.2 Hz, 1H), 6.83 (d, *J* = 2.1 Hz, 1H), 6.78 (dd, *J* = 8.2, 2.1 Hz, 1H), 6.38 (s, 1H), 5.03 (s, 2H), 4.30 (s, 4H), 4.16 (bs, 2H), 2.26 (s, 3H), 2.19 (s, 3H); ^{13}C NMR (126 MHz, chloroform-*d*) δ = 153.2, 148.0, 143.1, 142.7, 142.4, 140.0, 135.7, 135.4, 134.2, 131.4, 130.1, 127.5, 125.6, 122.7, 118.4, 117.0, 111.1, 70.6, 64.6, 64.5, 16.3, 16.2; HRMS (ESI) m/z calculated for $C_{22}H_{23}O_3N_2$ $[M+H]^+$: 363.1701, found $[M+H]^+$: 363.1703.

(3-(benzylamino)-6-((2-methyl-[1,1'-biphenyl]-3-yl)methoxy)imidazo[1,2-a]pyridin-2-yl)methanaminium chloride (9a)



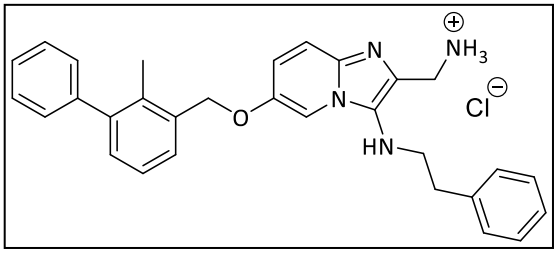
Synthesis according to procedure **C** using 6a (145 mg, 0.5 mmol), paraformaldehyde (25.5 mg, 0.850 mmol), Scandium triflate (25 mg, 50 µmol), and benzyl isocyanide (99.6 mg, 0.850 mmol) afforded **9a** (175 mg, 0.36 mmol, 72%)as a colorless solid; ^{1}H NMR (500 MHz, Methanol-d_4) δ = 7.82 (d, J = 2.3 Hz, 1H), 7.51 (d, J = 9.7 Hz, 1H), 7.46 – 7.38 (m, 3H), 7.37 – 7.33 (m, 1H), 7.33 – 7.22 (m, 9H), 7.20 (dd, J = 7.7, 1.5 Hz, 1H), 5.06 (s, 2H), 4.20 (s, 2H), 3.97 (s, 2H), 2.24 (s, 3H); ^{13}C NMR (126 MHz, Methanol-d_4) δ = 150.4, 144.48, 143.22, 141.00, 138.62, 135.78, 135.65, 131.36, 130.36, 129.81, 129.77, 129.28, 128.77, 128.05, 127.30, 126.67, 124.66, 116.82, 116.68, 107.97, 107.75, 71.44, 53.33, 53.21, 53.08, 35.57, 16.52, 16.48; HRMS (ESI) m/z calculated for $C_{29}H_{29}ON_4$ $[M+H]^+$: 449.2336, found $[M+H]^+$: 44.2335.

(6-((2-methyl-[1,1'-biphenyl]-3-yl)methoxy)-3-((1-phenylethyl)amino)imidazo[1,2-a]pyridin-2-yl)methanaminium chloride (9b)



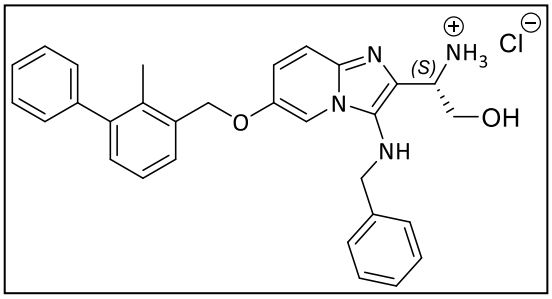
Synthesis according to procedure **C** using 6a (145 mg, 0.5 mmol), paraformaldehyde (25.5 mg, 0.850 mmol), Scandium triflate (25 mg, 50 µmol), and (1-isocyanoethyl)benzene (112 mg, 0.85 mmol) afforded **9b** (120 mg, 0.24 mmol, 48%) as a pale white solid; ^{1}H NMR (500 MHz, Methanol-d_4) δ = 7.90 (d, J = 2.3 Hz, 1H), 7.66 (d, J = 9.7 Hz, 1H), 7.55 (dd, J = 9.7, 2.3 Hz, 1H), 7.43 (tt, J = 8.2, 1.6 Hz, 3H), 7.39 – 7.34 (m, 1H), 7.33 – 7.21 (m, 9H), 5.20 (d, J = 11.6 Hz, 1H), 5.13 (d, J = 11.6 Hz, 1H), 4.27 (q, J = 6.8 Hz, 1H), 4.01 (d, J = 14.6 Hz, 1H), 3.93 (d, J = 14.6 Hz, 1H), 2.27 (s, 3H), 1.67 (d, J = 6.8 Hz, 3H); ^{13}C NMR (126 MHz, Methanol-d_4) δ = 151.20, 145.46, 144.58, 143.14, 136.80, 135.71, 135.48, 131.64, 131.49, 130.36, 129.92, 129.39, 129.25, 128.98, 128.09, 127.90, 126.75, 124.42, 115.26, 108.83, 108.70, 71.69, 59.54, 59.01, 58.49, 34.43, 16.61, 16.49; HRMS (ESI) m/z calculated for $C_{30}H_{31}ON_4$ $[M+H]^+$: 463.2492, found $[M+H]^+$: 463.2495.

(6-((2-methyl-[1,1'-biphenyl]-3-yl)methoxy)-3-(phenethylamino)imidazo[1,2-a]pyridin-2-yl)methanaminium chloride (9c)



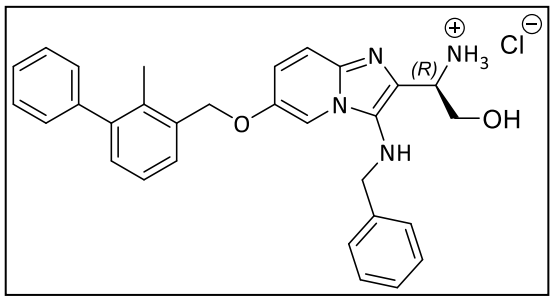
Synthesis according to procedure **C** using 6a (145 mg, 0.5 mmol), paraformaldehyde (25.5 mg, 0.850 mmol), Scandium triflate (25 mg, 50 µmol), and (2-Isocyanoethyl)benzene (112 mg, 0.850 mmol) afforded **9c** (172 mg, 0.255 mmol, 51%) as a pale white solid; ^{1}H NMR (500 MHz, Methanol-d_4) δ = 7.77 (d, *J* = 2.3 Hz, 1H), 7.67 (d, *J* = 9.7 Hz, 1H), 7.56 (dd, *J* = 9.7, 2.3 Hz, 1H), 7.47 – 7.41 (m, 3H), 7.38 – 7.32 (m, 1H), 7.31 – 7.20 (m, 8H), 7.17 – 7.10 (m, 1H), 5.06 (s, 2H), 4.31 (s, 2H), 3.41 (t, *J* = 6.8 Hz, 2H), 2.95 (t, *J* = 6.8 Hz, 2H), 2.25 (s, 3H); ^{13}C NMR (126 MHz, Methanol-d_4) δ = 151.44, 144.59, 143.16, 140.92, 136.40, 135.94, 135.34, 132.86, 131.62, 130.34, 130.15, 129.77, 129.51, 129.30, 128.09, 127.47, 126.75, 122.18, 115.22, 114.99, 108.49, 108.25, 71.97, 50.03, 37.72, 34.52, 16.57, 16.51; HRMS (ESI) m/z calculated for $C_{30}H_{31}ON_4$ $[M+H]^+$: 463.2492, found $[M+H]^+$: 463.2490.

(S)-1-(3-(benzylamino)-6-((2-methyl-[1,1'-biphenyl]-3-yl)methoxy)imidazo[1,2-a]pyridin-2-yl)-2-hydroxyethan-1-aminium chloride (9d)



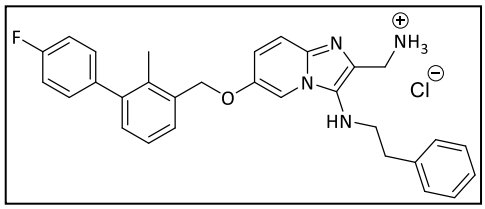
Synthesis according to procedure **C** using 6a (145 mg, 0.5 mmol), (R)-(−)-3-Boc-2,2-dimethyloxazolidine-4-carboxaldehyde (195 mg, 0.85 mmol), Scandium triflate (25 mg, 50 µmol), and benzyl isocyanide (99.6 mg, 0.850 mmol) afforded **9d** (160 mg, 0.31 mmol, 62%) as a pale white solid; ^{1}H NMR (500 MHz, Methanol-d_4) δ = 7.95 (s, 1H), 7.71 (d, *J* = 9.7 Hz, 1H), 7.56 (d, *J* = 9.7 Hz, 1H), 7.48 – 7.39 (m, 3H), 7.38 – 7.19 (m, 10H), 5.12 (s, 2H), 4.50 (dd, *J* = 5.6, 4.2 Hz, 1H), 4.30 (d, *J* = 13.9 Hz, 1H), 4.21 (d, *J* = 13.9 Hz, 1H), 3.79 (dd, *J* = 11.5, 4.2 Hz, 1H), 3.74 (dd, *J* = 11.5, 5.6 Hz, 1H), 2.25 (s, 3H); ^{13}C NMR (126 MHz, Methanol-d_4) δ = 151.1, 144.5, 143.2, 140.8, 137.4, 135.7, 135.5, 131.7, 131.4, 130.3, 129.9, 129.8, 129.5, 129.2, 128.9, 128.1, 126.7, 115.8, 115.6, 108.5, 71.6, 62.2, 53.1, 49.1, 16.6. ; HRMS (ESI) m/z calculated for $C_{30}H_{31}O_2N_4$ $[M+H]^+$: 479.2442, found $[M+H]^+$: 479.2441.

(R)-1-(3-(benzylamino)-6-((2-methyl-[1,1'-biphenyl]-3-yl)methoxy)imidazo[1,2-a]pyridin-2-yl)-2-hydroxyethan-1-aminium chloride (9e)



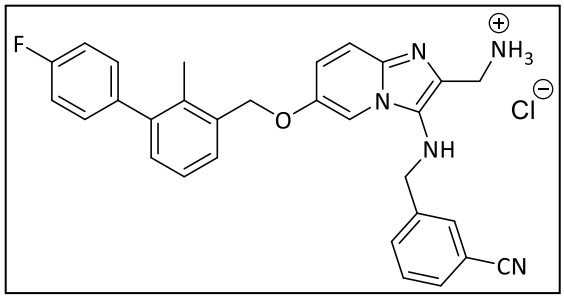
Synthesis according to procedure **C** using 6a (145 mg, 0.5 mmol), (S)-(−)-3-Boc-2,2-dimethyloxazolidine-4-carboxaldehyde (195 mg, 0.85 mmol), Scandium triflate (25 mg, 50 µmol), and benzyl isocyanide (99.6 mg, 0.850 mmol) afforded **9e** (149 mg, 0.29 mmol, 58%) as a pale white solid; ^{1}H NMR (500 MHz, Methanol-d_4) δ = 7.68 (d, *J* = 2.3 Hz, 1H), 7.45 – 7.39 (m, 4H), 7.37 – 7.33 (m, 1H), 7.32 – 7.23 (m, 8H), 7.20 (dd, *J* = 7.7, 1.6 Hz, 1H), 7.15 (dd, *J* = 9.7, 2.3 Hz, 1H), 5.02 (s, 2H), 4.40 (dd, *J* = 8.6, 4.5 Hz, 1H), 4.24 – 4.10 (m, 2H), 3.84 (dd, *J* = 11.5, 8.6 Hz, 1H), 3.61 (dd, *J* = 11.5, 4.5 Hz, 1H), 2.24 (s, 3H); ^{13}C NMR (126 MHz, Methanol-d_4) δ = 149.6, 144.5, 143.3, 141.2, 140.1, 136.0, 135.6, 131.3, 131.1, 130.5, 130.4, 129.8, 129.8, 129.2, 128.7, 128.0, 126.6, 122.6, 118.0, 107.5, 107.3, 71.3, 63.2, 53.5, 50.9, 16.5; HRMS (ESI) m/z calculated for $C_{30}H_{31}O_2N_4$ $[M+H]^+$: 479.2442, found $[M+H]^+$: 479.2444.

(6-((4'-fluoro-2-methyl-[1,1'-biphenyl]-3-yl)methoxy)-3-(phenethylamino)imidazo[1,2-a]pyridin-2-yl)methanaminium chloride (9g)



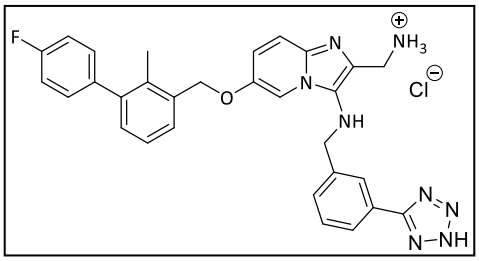
Synthesis according to procedure **C** using 6c (161 mg, 0.5 mmol), paraformaldehyde (25.5 mg, 0.850 mmol), Scandium triflate (25 mg, 50 µmol), and (2-Isocyanoethyl)benzene (112 mg, 0.850 mmol) afforded **9g** (199 mg, 0.385 mmol, 77%) as a colorless solid; ^{1}H NMR (500 MHz, Methanol-d_4) δ = 8.53 (s, 3H), 7.73 (d, *J* = 2.4 Hz, 1H), 7.48 (dd, *J* = 7.7, 1.5 Hz, 1H), 7.44 (d, *J* = 9.7 Hz, 1H), 7.37 – 7.31 (m, 2H), 7.31 – 7.22 (m, 7H), 7.19 (dd, *J* = 7.7, 1.5 Hz, 1H), 7.17 – 7.13 (m, 1H), 7.10 (dd, *J* = 9.7, 2.3 Hz, 1H), 5.38 (t, *J* = 6.6 Hz, 1H), 5.06 (s, 2H), 4.09 (s, 2H), 3.20 (q, *J* = 6.9 Hz, 2H), 2.84 (t, *J* = 7.4 Hz, 2H), 2.20 (s, 3H); ^{13}C NMR (126 MHz, Methanol-d_4) δ = 163.4 (d, J_{CF} = 244.4 Hz), 149.8, 143.4, 141.2, 139.8, 139.3 (d, J_{CF} = 3.3 Hz), 136.0, 135.9, 132.2, 132.2, 131.5, 131.2, 130.1, 129.8, 129.5, 128.9, 127.4, 126.7, 122.7, 117.9, 116.0, 115.8, 107.2, 71.5, 50.6, 37.9, 36.6, 16.6; HRMS (ESI) m/z calculated for $C_{30}H_{30}ON_4F$ $[M+H]^+$: 481.2398, found $[M+H]^+$: 481.2398.

(3-((3-cyanobenzyl)amino)-6-((4'-fluoro-2-methyl-[1,1'-biphenyl]-3-yl)methoxy)imidazo[1,2-a]pyridin-2-yl)methanaminium chloride (9h)



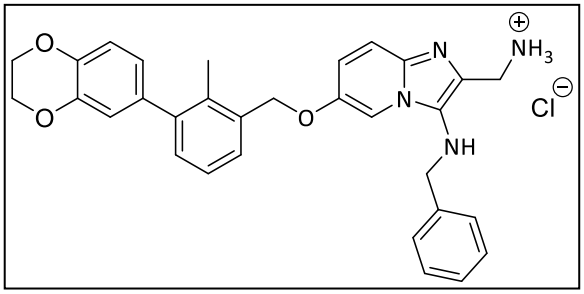
Synthesis according to procedure **C** using 6c (161 mg, 0.5 mmol), paraformaldehyde (25.5 mg, 0.850 mmol), Scandium triflate (25 mg, 50 µmol), and 3-(isocyanomethyl)benzonitrile (121 mg, 0.85 mmol) afforded **9h** (227 mg, 0.43 mmol, 86%) as a colorless solid; ^{1}H NMR (500 MHz, DMSO-d_6) δ = 8.49 (s, 3H), 7.92 – 7.86 (m, 2H), 7.73 (ddt, *J* = 8.3, 6.8, 1.5 Hz, 2H), 7.50 (q, *J* = 7.7 Hz, 2H), 7.42 – 7.33 (m, 3H), 7.29 – 7.22 (m, 3H), 7.21 (dd, *J* = 7.7, 1.5 Hz, 1H), 7.08 (dd, *J* = 9.7, 2.4 Hz, 1H), 5.89 (t, *J* = 6.3 Hz, 1H), 5.11 (s, 2H), 4.21 (d, *J* = 6.3 Hz, 2H), 3.91 (s, 2H), 2.21 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ = 161.4 (d, J_{CF} = 243.7 Hz), 147.3, 141.8, 141.2, 137.6 (d, J_{CF} = 3.2 Hz), 135.0, 134.3, 133.6, 133.5, 132.2, 132.0, 131.2 (d, J_{CF} = 8.0 Hz), 130.9, 130.0, 129.5, 129.5, 128.4, 125.7, 119.5, 118.9, 117.1, 115.1 (d, J_{CF} = 21.2 Hz), 111.1, 69.5, 50.8, 35.1, 16.0, 15.9; HRMS (ESI) m/z calculated for $C_{30}H_{27}ON_5F$ $[M+H]^+$: 492.2194, found $[M+H]^+$: 492.2195.

(3-((3-(2H-tetrazol-5-yl)benzyl)amino)-6-((4'-fluoro-2-methyl-[1,1'-biphenyl]-3-yl)methoxy)imidazo[1,2-a]pyridin-2-yl)methanaminium chloride (9i)



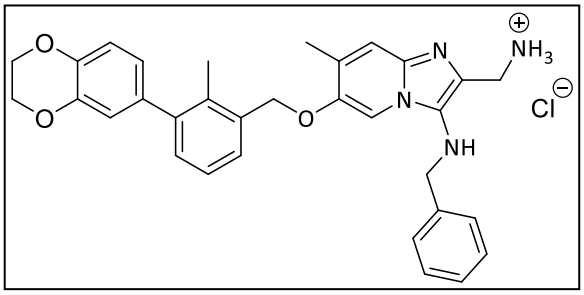
A mixture of **6c** (308 mg, 1 mmol, 1 eq.), *N*-Boc-2-aminoacetaldehyde (271 mg, 1,7 mmol, 1.7 eq.), scandium triflate (49mg, 0,1 mmol, 0.1 eq.) and 3-(isocyanomethyl)benzonitrile (242mg, 1,7 mmol, 1.7 eq.) in DCM/MeOH (2:1; 3ml, 0.3 M) was placed in a glass microwave vial and sealed. The reaction vial was heated in a microwave for 1 hour at 120°C. Evaporation of the solvents was followed by purification by silica gel flash chromatography using PE–EA as eluent. The still slightly impure product was used directly in the further step. Sodium Azide (mg, mmol, 1,2 eq), Zink chloride (mg, mmol, 1 eq.), and 5 ml of n-Propanol where added to the crude Product. The reaction mixture was heated to 95°C for 20h. After removal of the organic solvents the reaction mixture purified via a reverse-phase purification with H_2O/MeOH/NH_3(0,1%) as eluent. Afterwards the purified product was directly treated with 2.8 ml 7N HCl in 2-Propanol (3.9 mmol, 50 eq.) for 20h at rt. Concentration of the reaction mixture under vacuum afforded **9i** (223 mg, 0.39 mmol 39%) as an yellow solid; ^{1}H NMR (500 MHz, MeOD/$CDCl_3$(10%)) δ = 8.08 (s, 1H), 7.95 (d, *J* = 7.7 Hz, 1H), 7.73 (d, *J* = 2.4 Hz, 1H), 7.35 (d, *J* = 9.6 Hz, 1H), 7.32 (dt, *J* = 7.7, 4.0 Hz, 2H), 7.28 – 7.24 (m, 2H), 7.23 – 7.17 (m, 2H), 7.16 – 7.15 (m, 1H), 7.13 (s, 1H), 7.11 (d, *J* = 7.7 Hz, 1H), 7.08 (dd, *J* = 9.6, 2.4 Hz, 2H), 4.96 (s, 3H), 4.22 (s, 2H), 3.94 (s, 2H), 2.14 (s, 2H)); ^{13}C NMR (126 MHz, MeOD/$CDCl_3$(10%))) 163.3 (d, J_{CF} = 244.4 Hz), 149.6, 143.2, 141.9, 140.2 139.3 (d, J_{CF} = 3.5 Hz), 135.96, 135.73, 132.13 (d, J_{CF} = 7.8 Hz), 131.62, 131.25, 130.42, 130.33, 130.04, 129.92, 129.54, 127.74, 127.72, 126.94, 126.81, 126.62, 122.29, 118.03, 115.85 (d, J_{CF} = 21.4 Hz), 107.10, 106.88, 71.18, 53.37, 36.52, 16.33; HRMS (ESI) m/z calculated for $C_{30}H_{28}ON_8F$ $[M+H]^+$: 535.2365, found $[M+H]^+$: 535.2365.

(3-(benzylamino)-6-((3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-2-methylbenzyl)oxy)imidazo[1,2-a]pyridin-2-yl)methanaminium chloride (9j)



Synthesis according to procedure **C** using 6b (174 g, 0.5 mmol), paraformaldehyde (25.5 mg, 0.850 mmol), Scandium triflate (25 mg, 50 µmol), and benzyl isocyanide (99.6 mg, 0.850 mmol) afforded **9j** (174 mg, 0.32 mmol 64%) as a pale white solid; ^{1}H NMR (500 MHz, DMSO-d_6) δ = 8.91 (s, 3H), 8.23 (d, *J* = 2.3 Hz, 1H), 7.90 (d, *J* = 9.7 Hz, 1H), 7.73 (dd, *J* = 9.7, 2.3 Hz, 1H), 7.45 (dd, *J* = 7.6, 1.5 Hz, 1H), 7.42 – 7.36 (m, 2H), 7.36 – 7.22 (m, 4H), 7.20 (dd, *J* = 7.6, 1.5 Hz, 1H), 6.92 (d, *J* = 8.2 Hz, 1H), 6.79 (d, *J* = 2.1 Hz, 1H), 6.75 (dd, *J* = 8.2, 2.1 Hz, 1H), 5.20 (s, 2H), 4.28 (s, 6H), 3.99 (s, 2H), 2.23 (s, 3H); ^{13}C NMR (126 MHz, MeOD) δ = 159.3, 152.5, 152.1, 151.3, 148.7, 143.9, 143.8, 143.7, 142.1, 140.8, 139.7, 138.0, 138.0, 137.7, 137.0, 136.7, 135.1, 131.7, 131.6, 128.5, 127.4, 127.1, 126.4, 126.2, 122.5, 117.4, 79.6, 73.6, 60.1, 41.2, 25.6, 25.5; HRMS (ESI) m/z calculated for $C_{31}H_{31}O_3N_4$ $[M+H]^+$: 507.2391, found $[M+H]^+$: 507.2390.

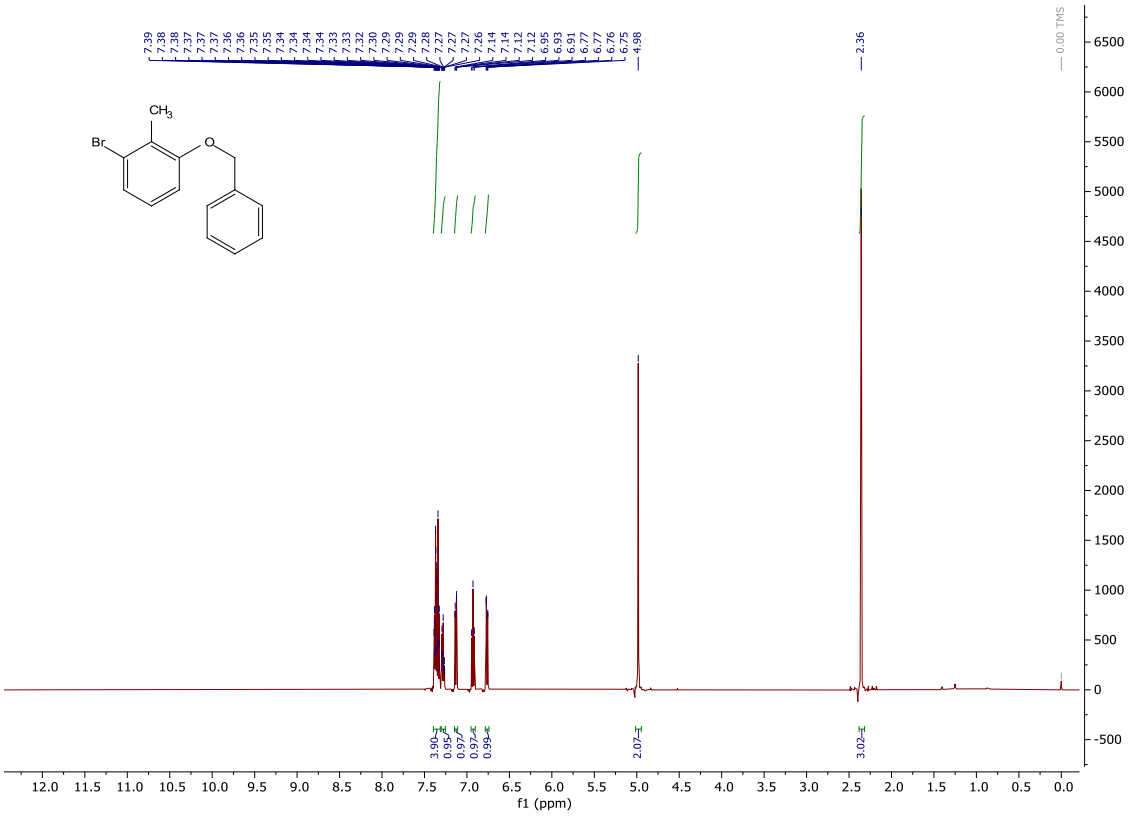
(3-(benzylamino)-6-((3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-2-methylbenzyl)oxy)-7-methylimidazo[1,2-a]pyridin-2-yl)methanaminium chloride (9k)

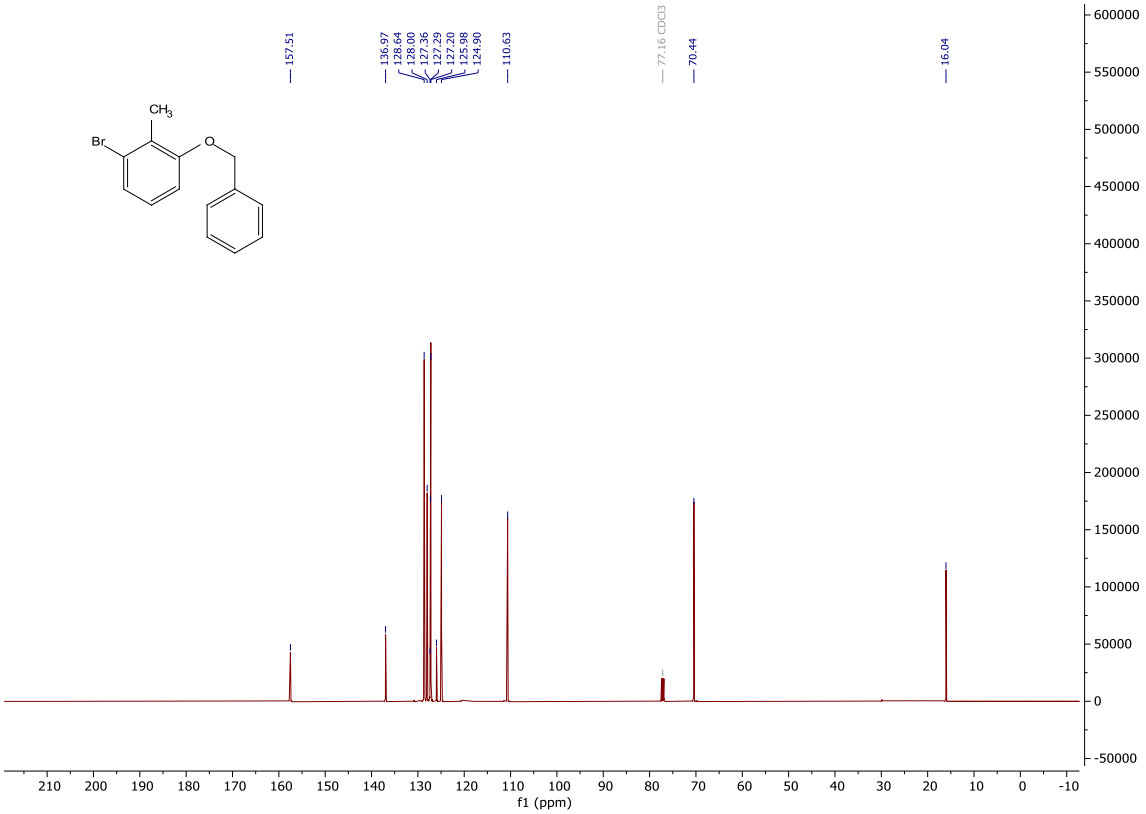


Synthesis according to procedure **C** using 6d (181 mg, 0.5 mmol), paraformaldehyde (25.5 mg, 0.850 mmol), Scandium triflate (25 mg, 50 µmol), and benzyl isocyanide (99.6 mg, 0.850 mmol) afforded **9k** (175 mg, 0.315 mmol 63%) as an orange solid; ^{1}H NMR (500 MHz, Methanol-d_4) δ = 7.60 (s, 1H), 7.40 (dd, *J* = 7.5, 1.6 Hz, 1H), 7.34 – 7.15 (m, 8H), 6.87 (d, *J* = 8.2 Hz, 1H), 6.75 (d, *J* = 2.1 Hz, 1H), 6.72 (dd, *J* = 8.2, 2.1 Hz, 1H), 4.99 (s, 2H), 4.27 (s, 4H), 4.12 (s, 2H), 3.85 (s, 2H), 2.30 (s, 3H), 2.26 (s, 3H); ^{13}C NMR (126 MHz, Methanol-d_4) δ = 149.0, 144.7, 144.2, 144.0, 141.4, 140.3, 136.4, 136.1, 135.5, 133.1, 131.3, 129.9, 129.8, 129.8, 128.8, 128.7, 126.6, 123.3, 119.2, 119.1, 118.0, 116.7, 106.0, 105.8, 71.0, 65.7, 53.7, 36.6, 16.9, 16.9, 16.5, 16.5; HRMS (ESI) m/z calculated for $C_{32}H_{33}O_3N_4$ $[M+H]^+$: 521.2547, found $[M+H]^+$: 521.2546.

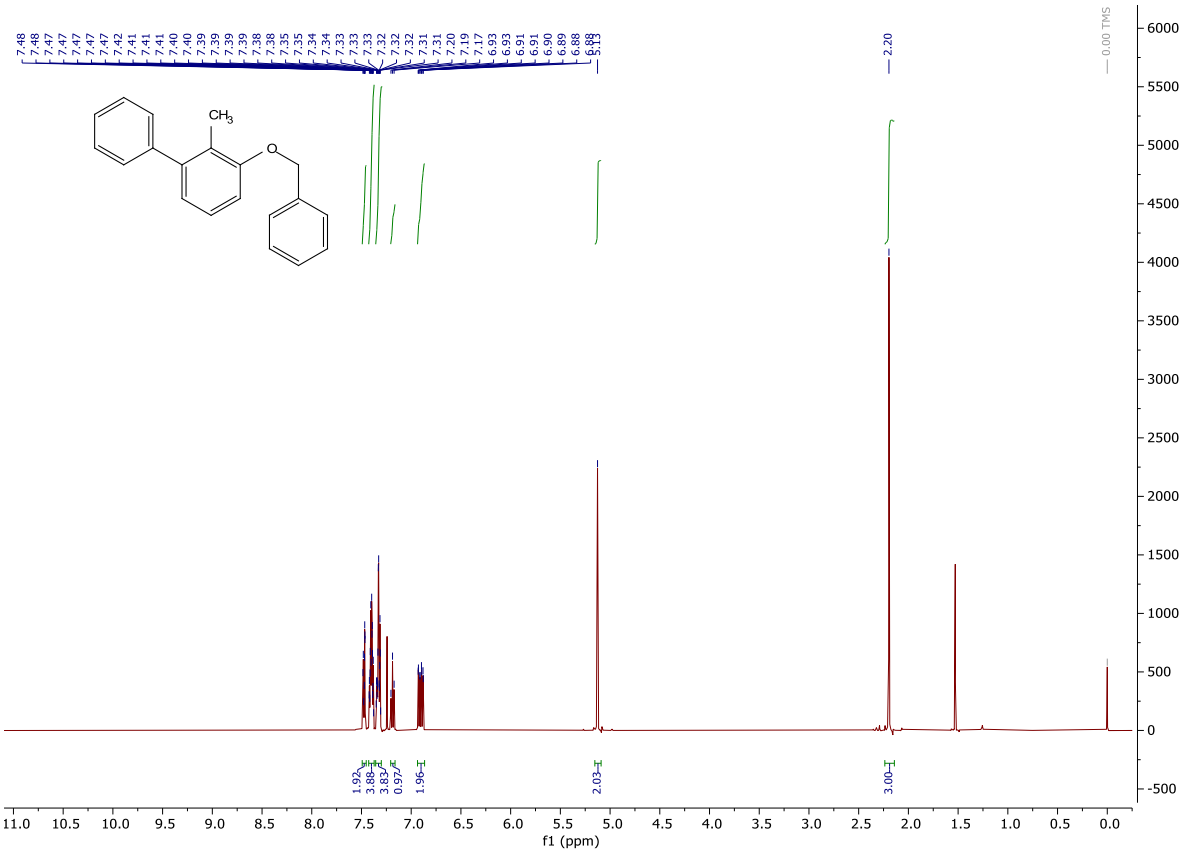
NMR spectra

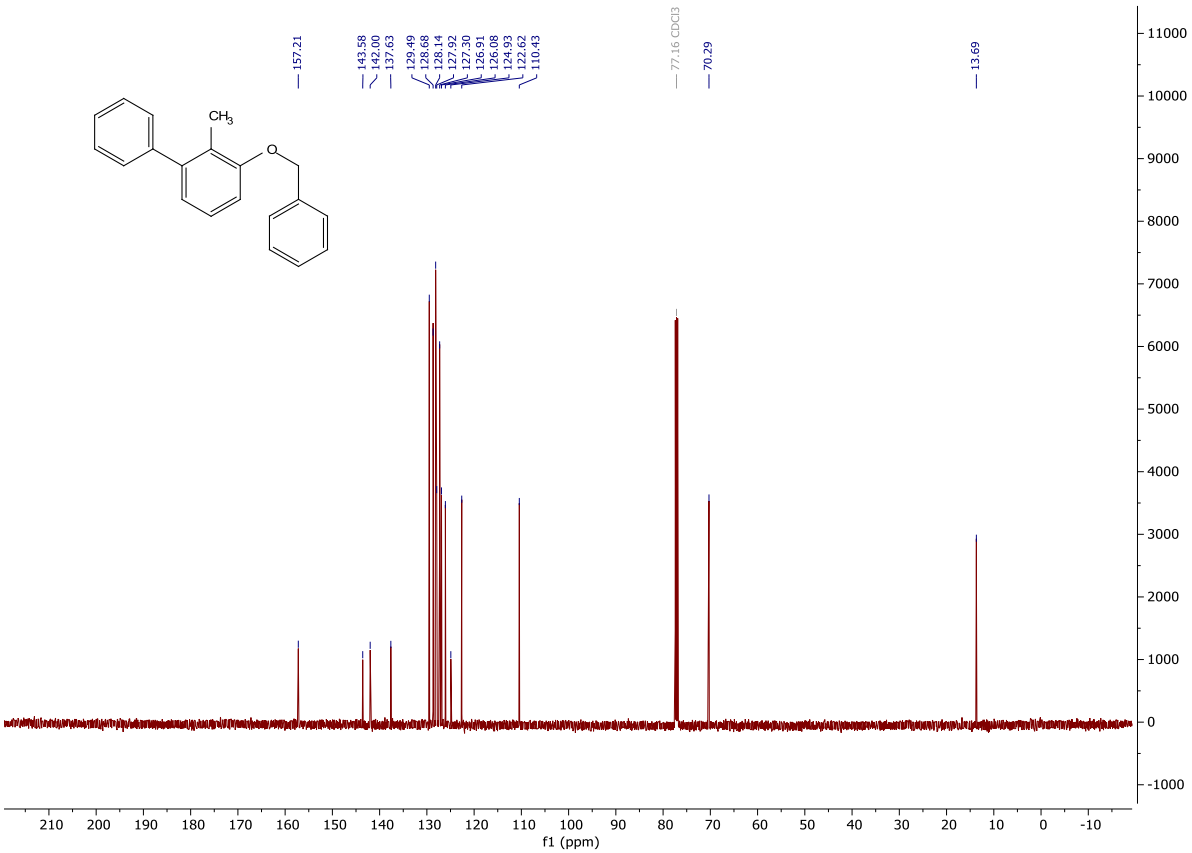
1-(benzyloxy)-3-bromo-2-methylbenzene (A)



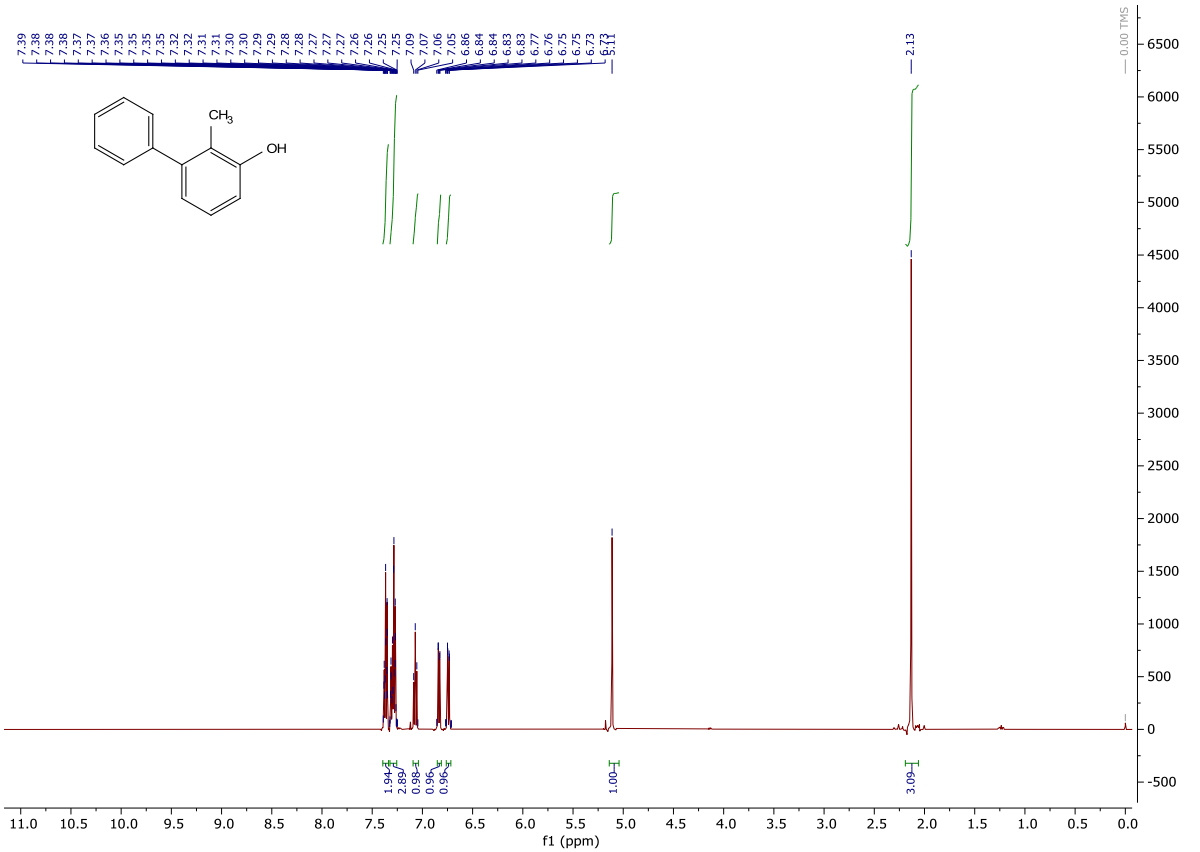


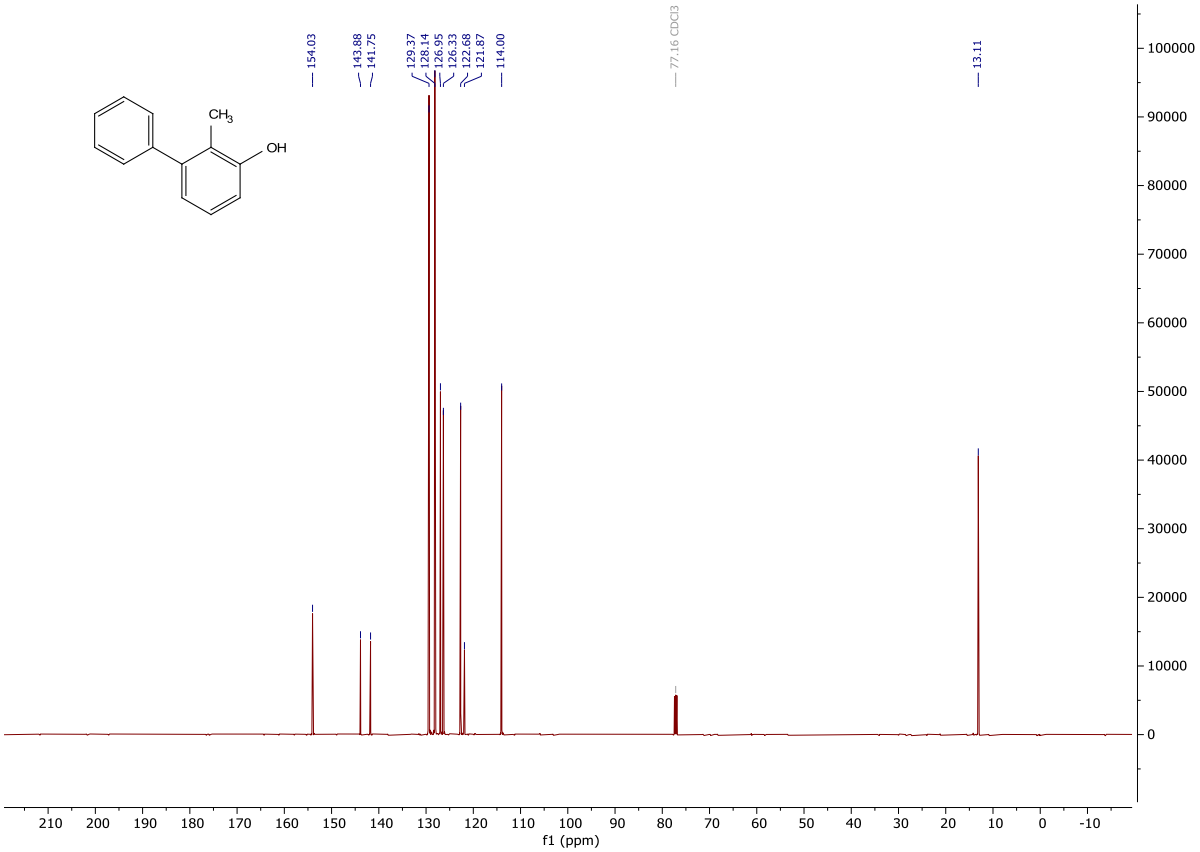
3-(benzyloxy)-2-methyl-1,1'-biphenyl (B)



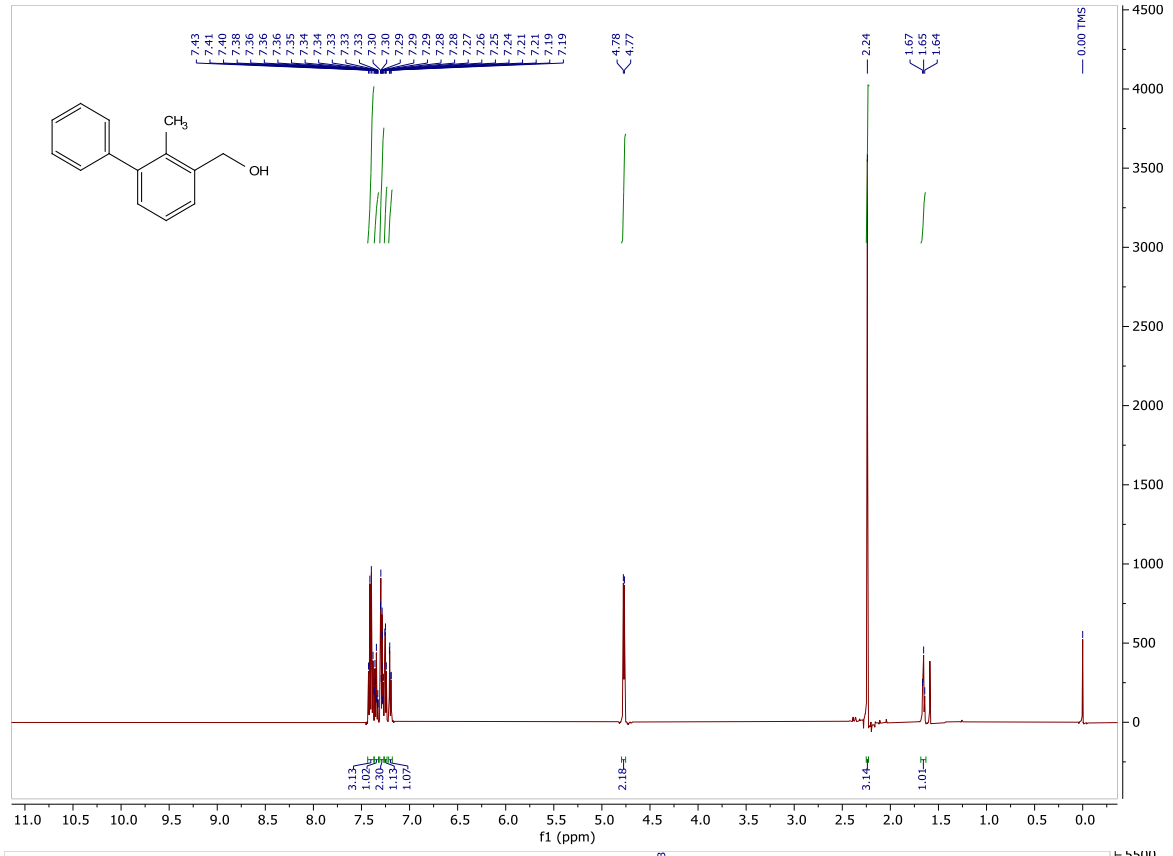


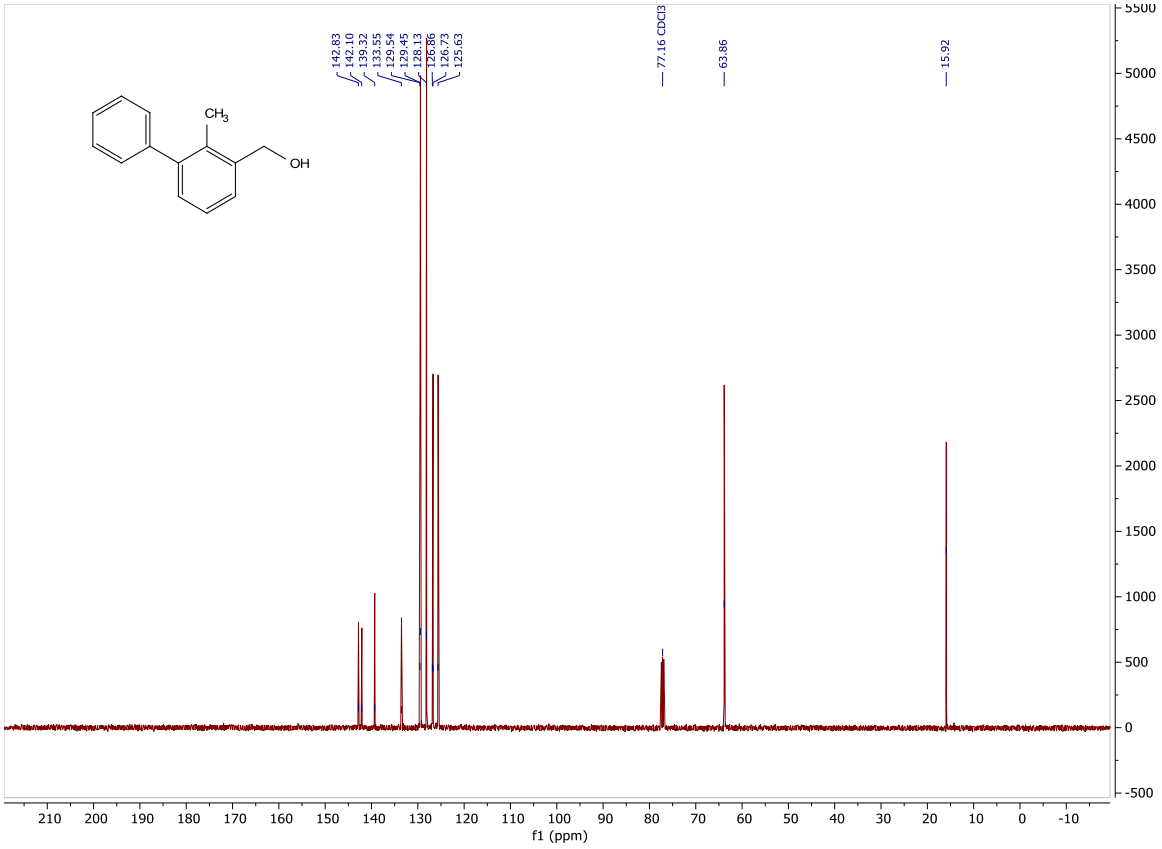
2-methyl-[1,1'-biphenyl]-3-ol (C)



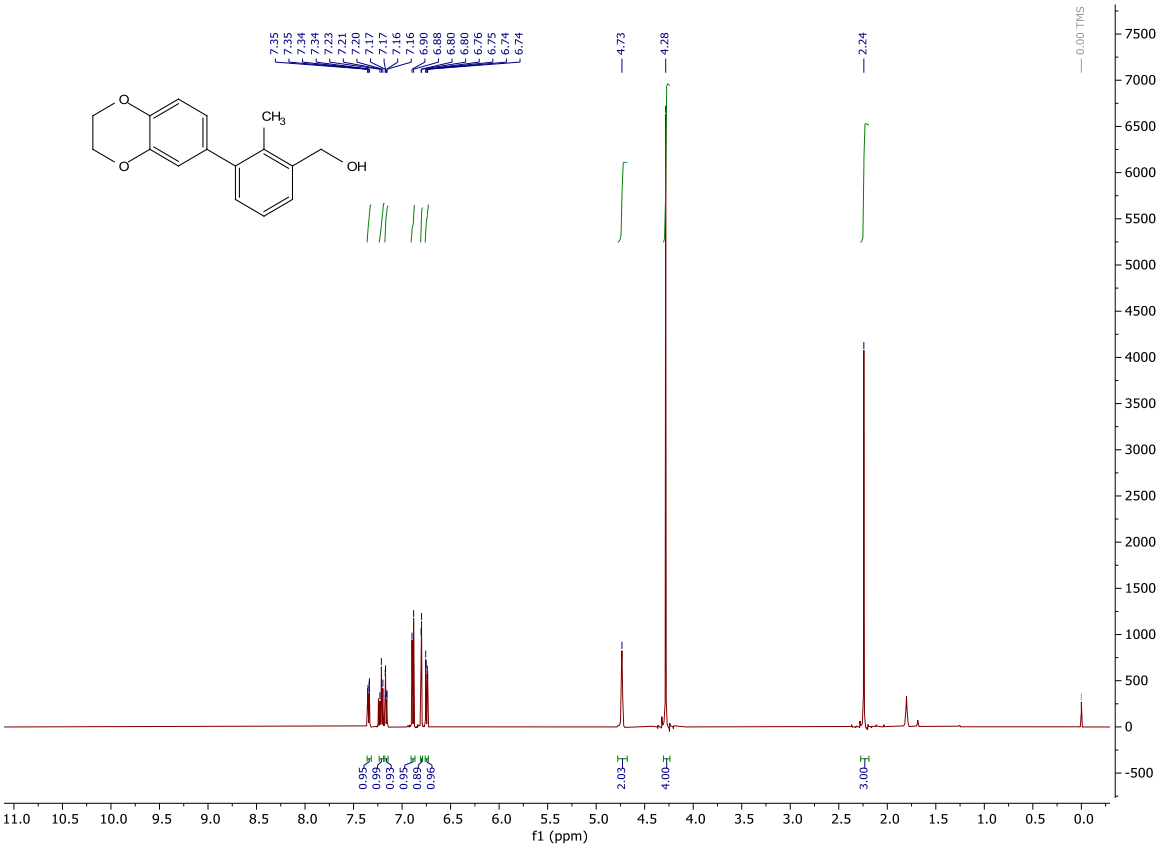


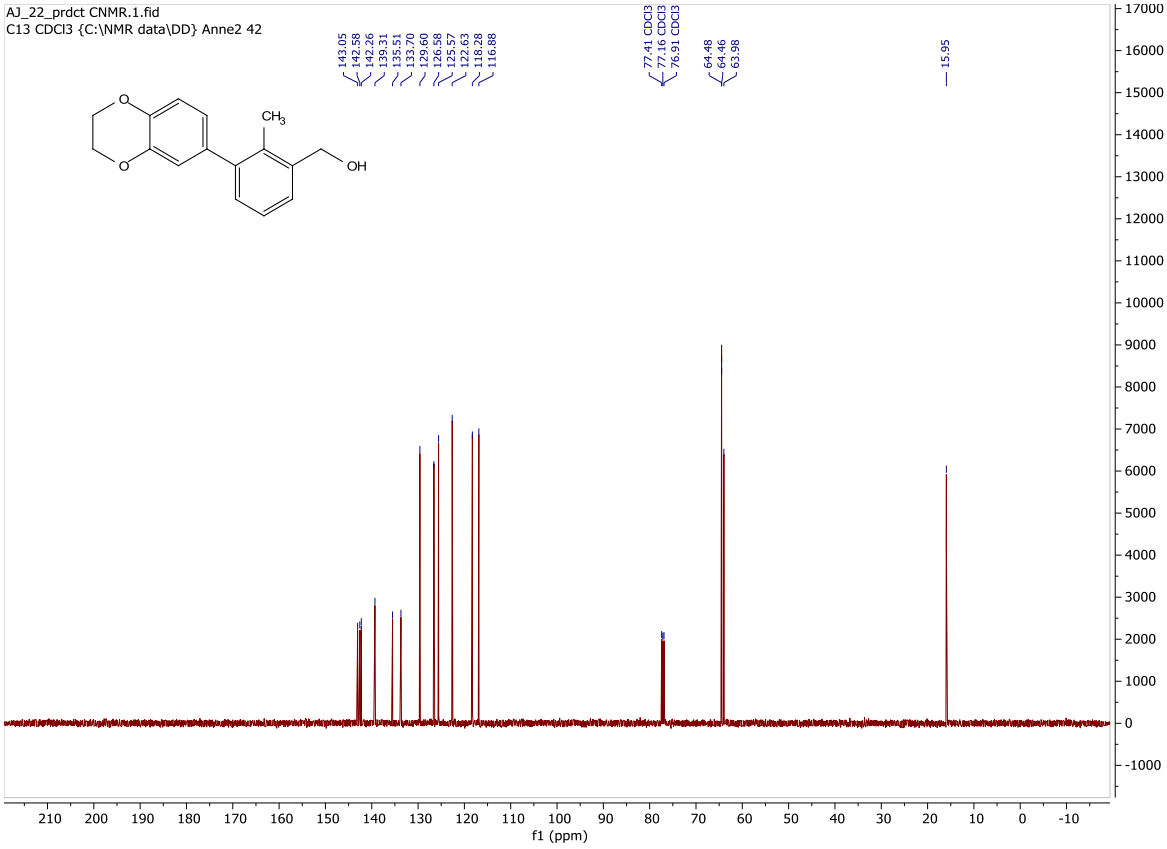
***(2-methyl-[1,1'-biphenyl]-3-yl)methanol* (3a)**



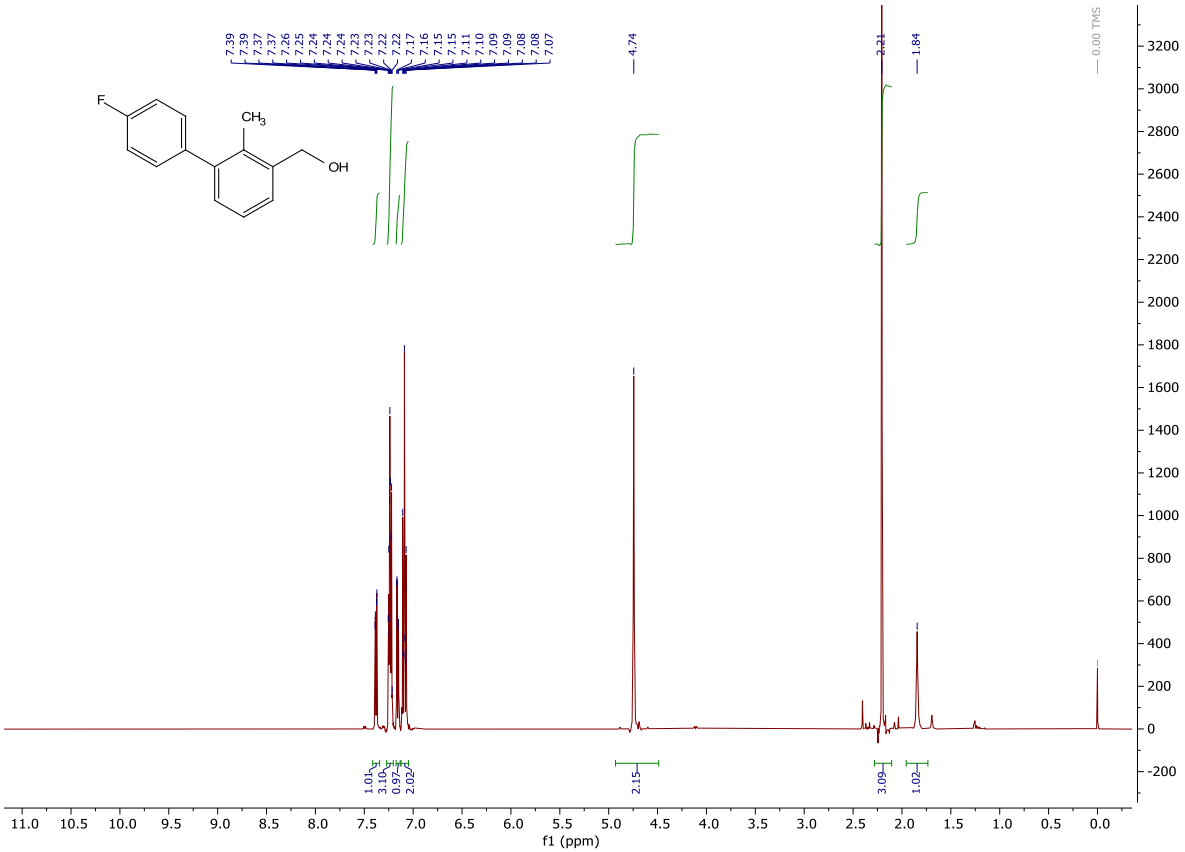


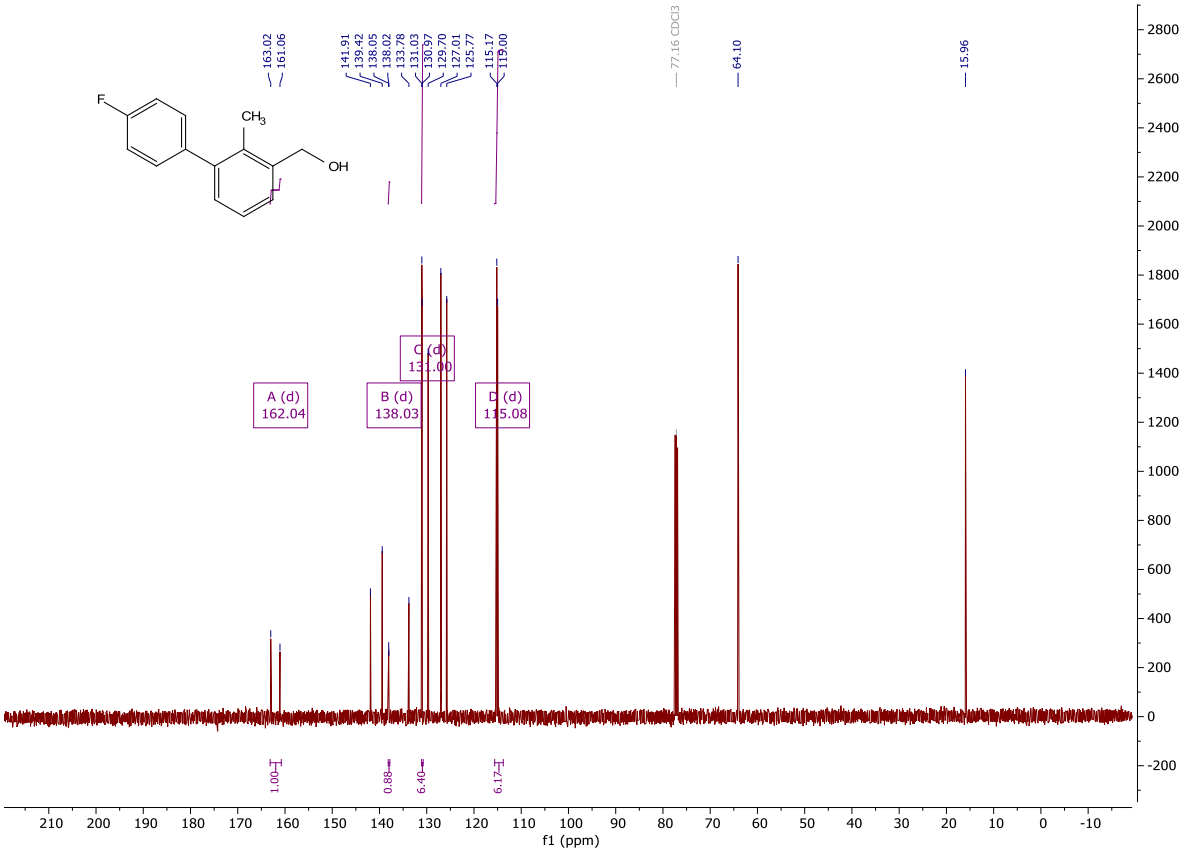
***(3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-2-methylphenyl)methanol* (3b)**



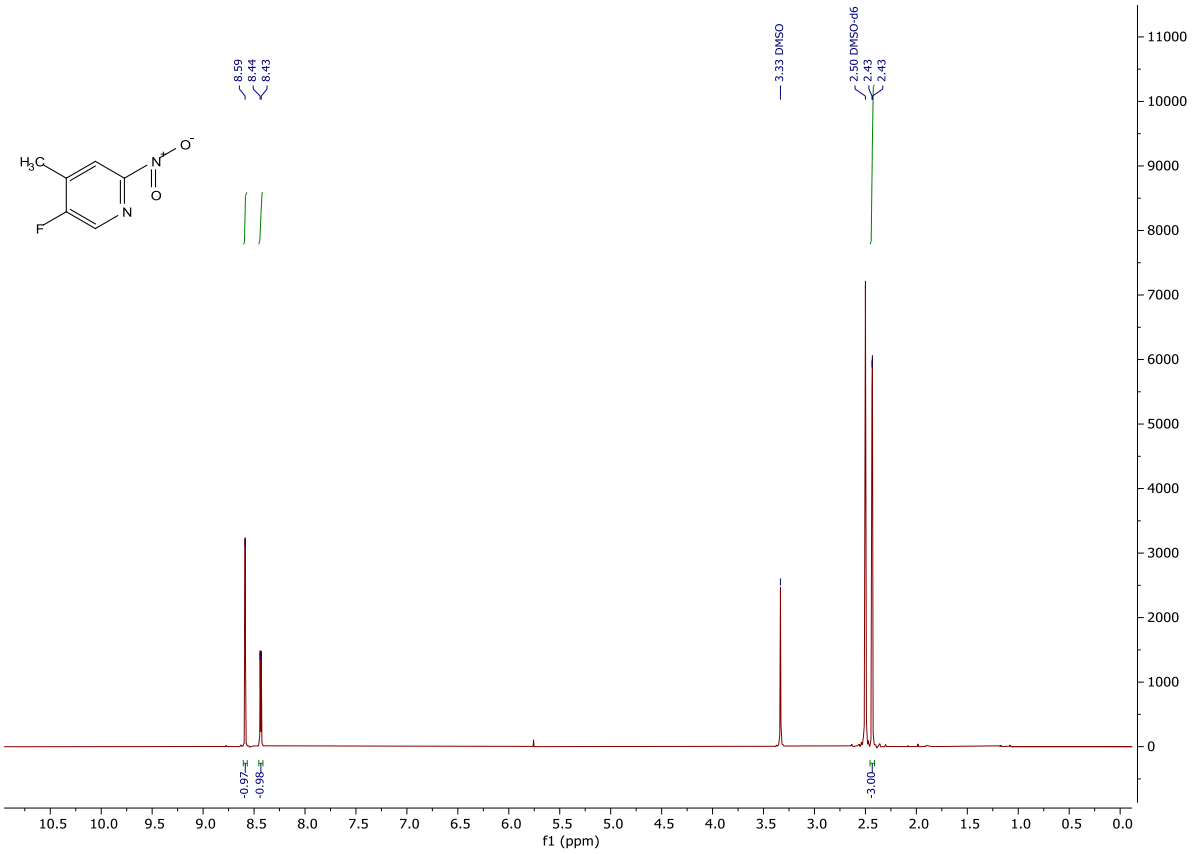


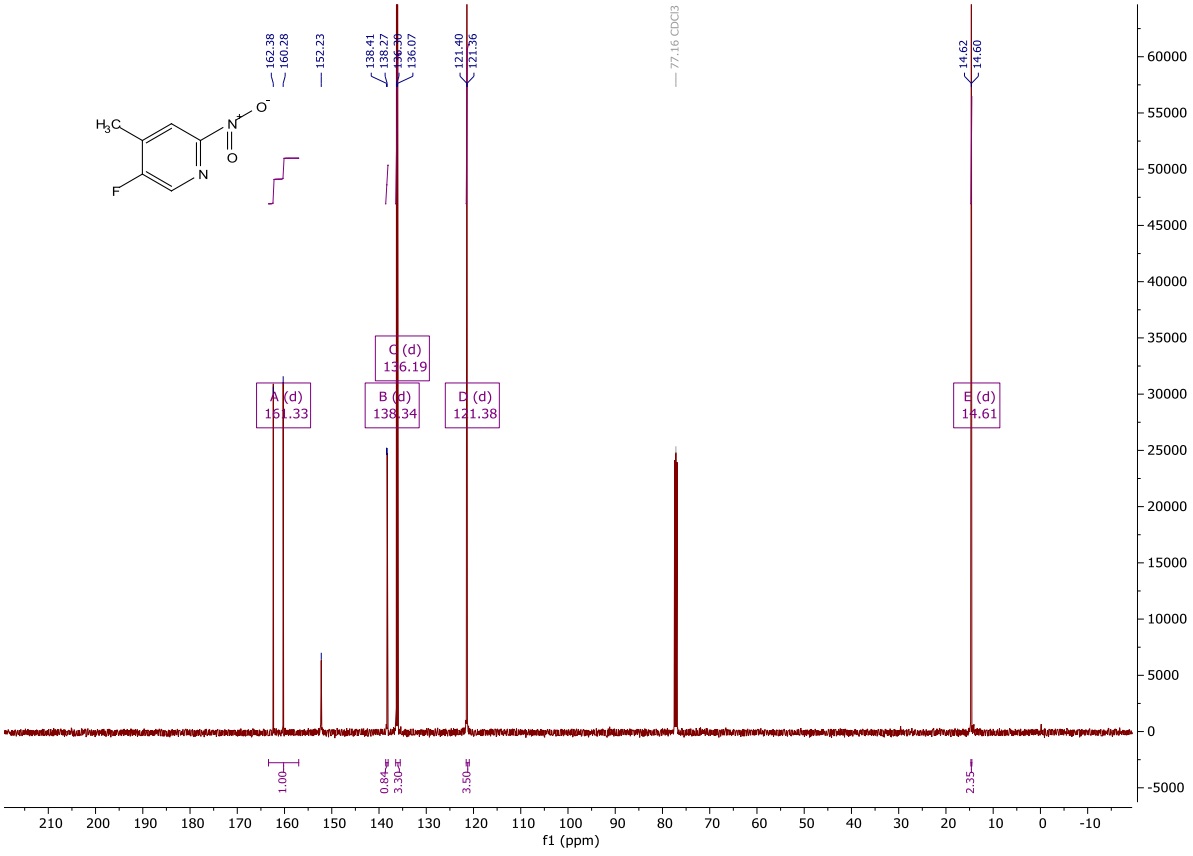
(4’-fluoro-2-methyl-[1,1’-biphenyl]-3-yl)methanol (3c)



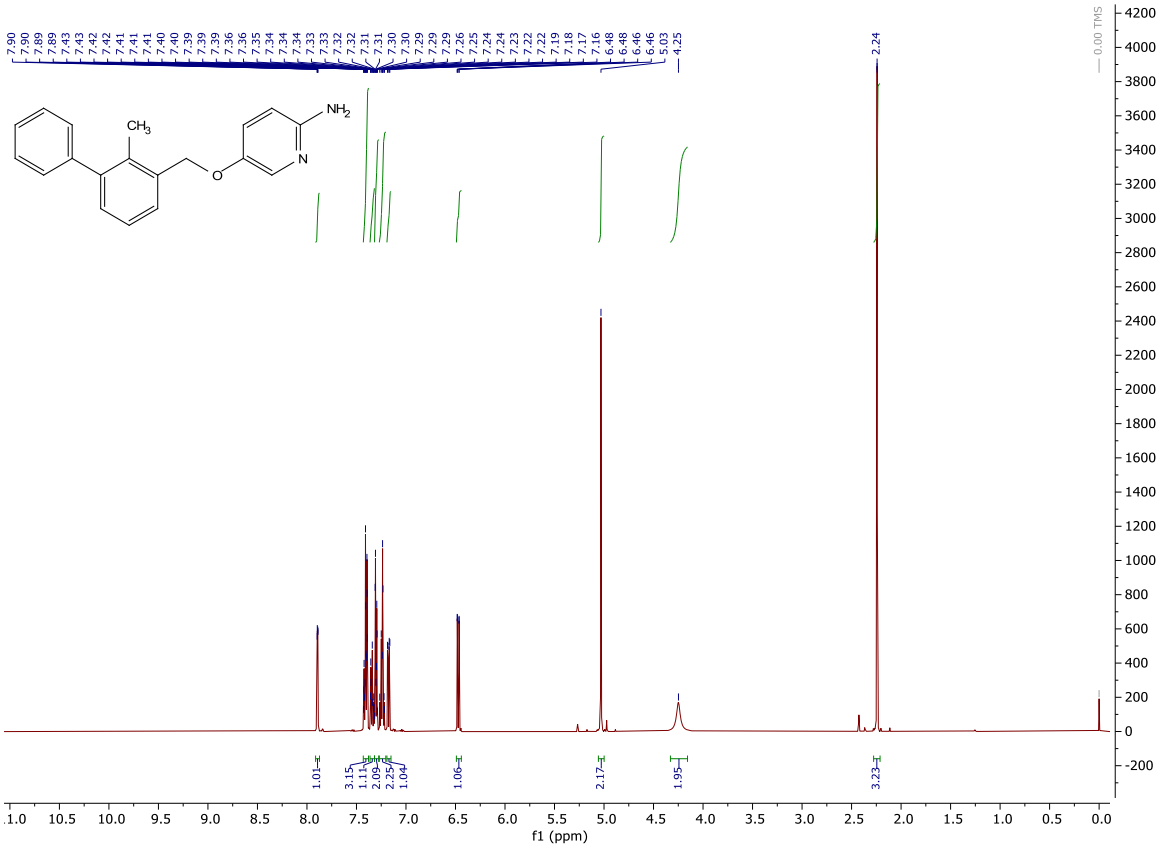


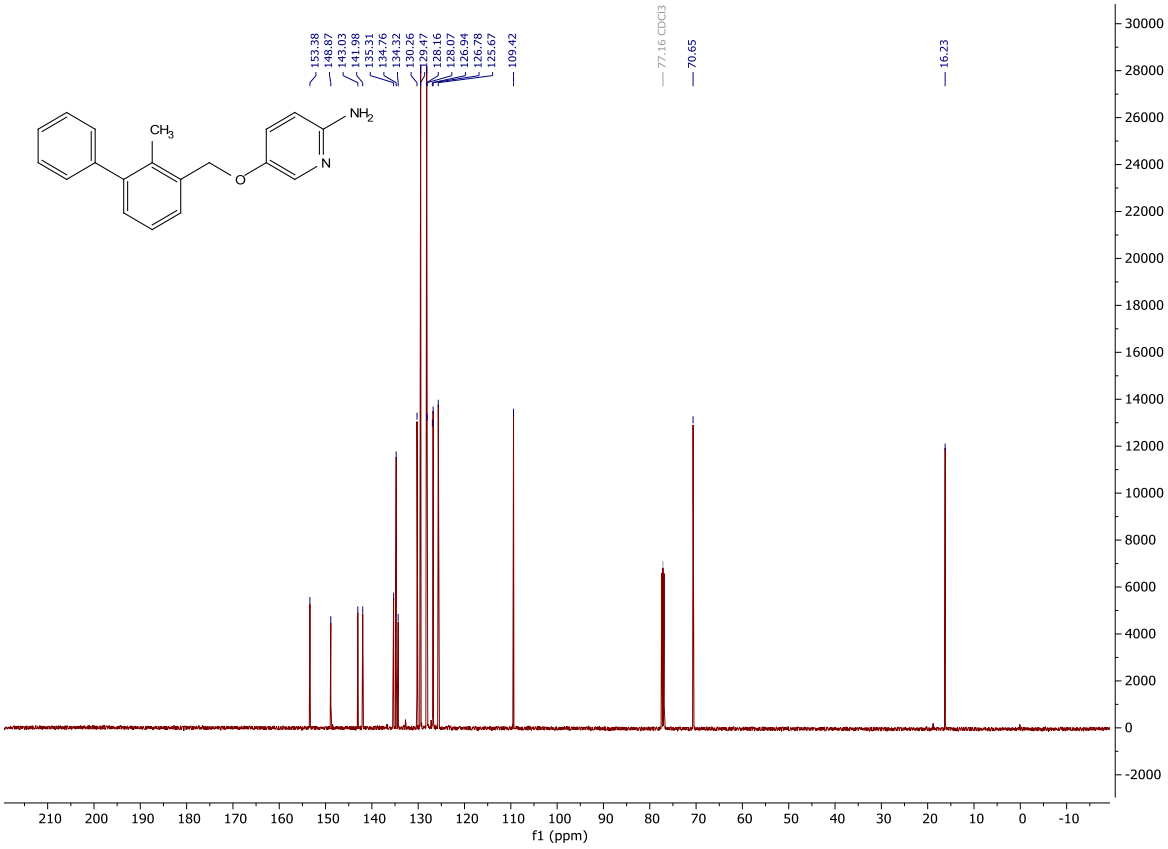
5-fluoro-4-methyl-2-nitropyridine (4b)



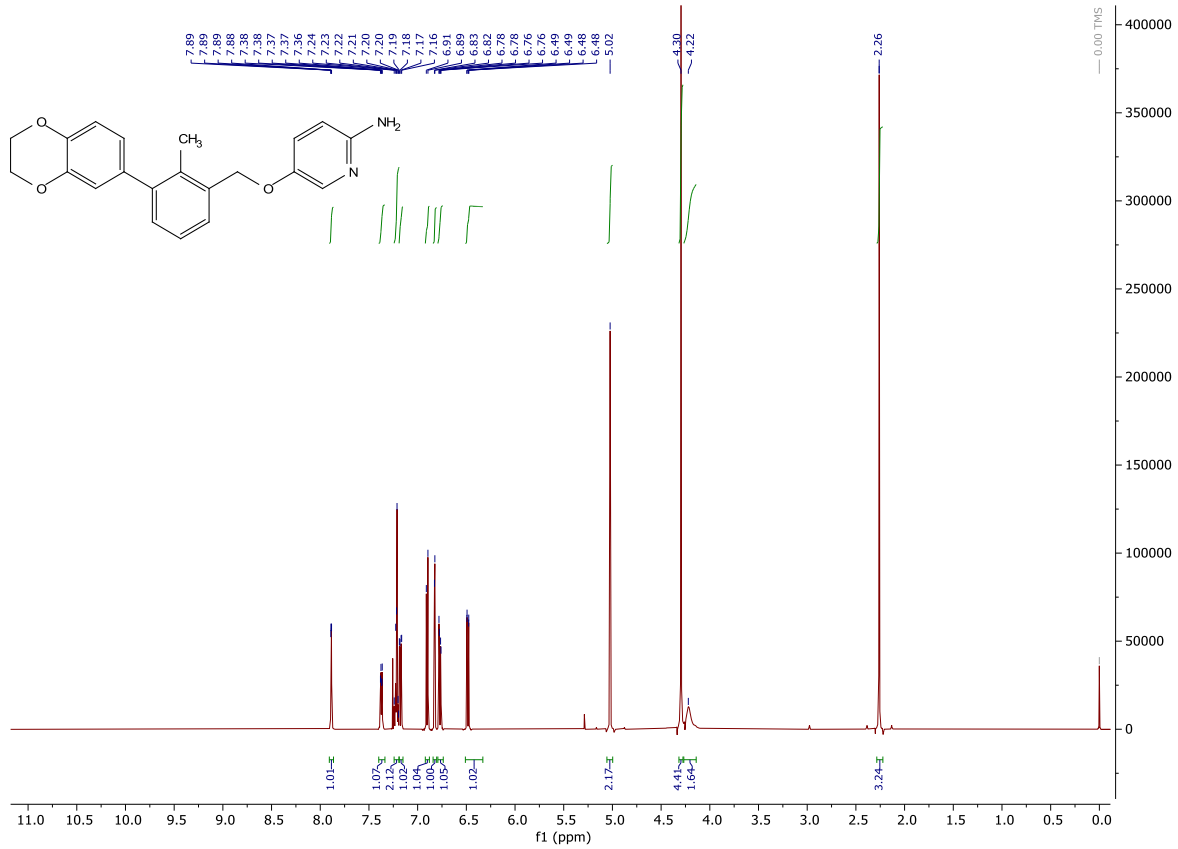


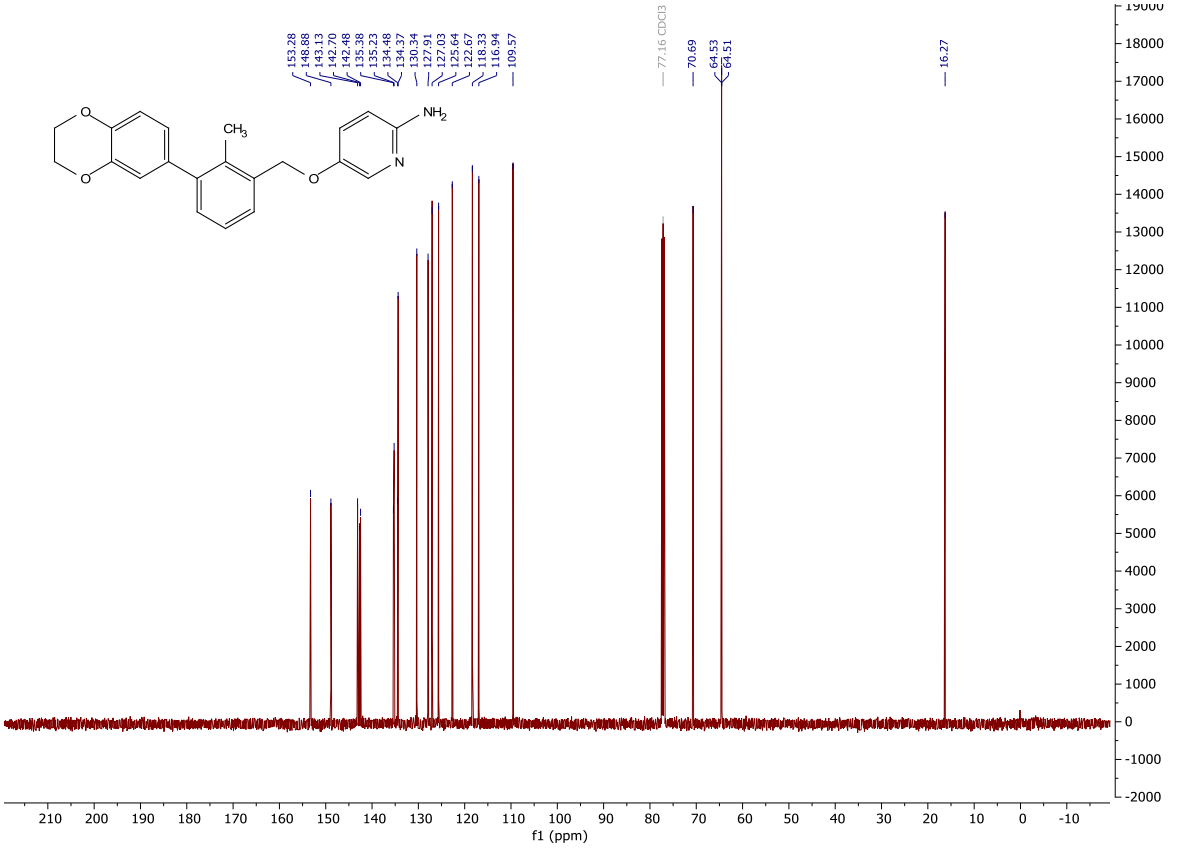
5-((2-methyl-[1,1’-biphenyl]-3-yl)methoxy)pyridin-2-amine (6a)





5-((3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-2-methylbenzyl)oxy)pyridin-2-amine (6b)





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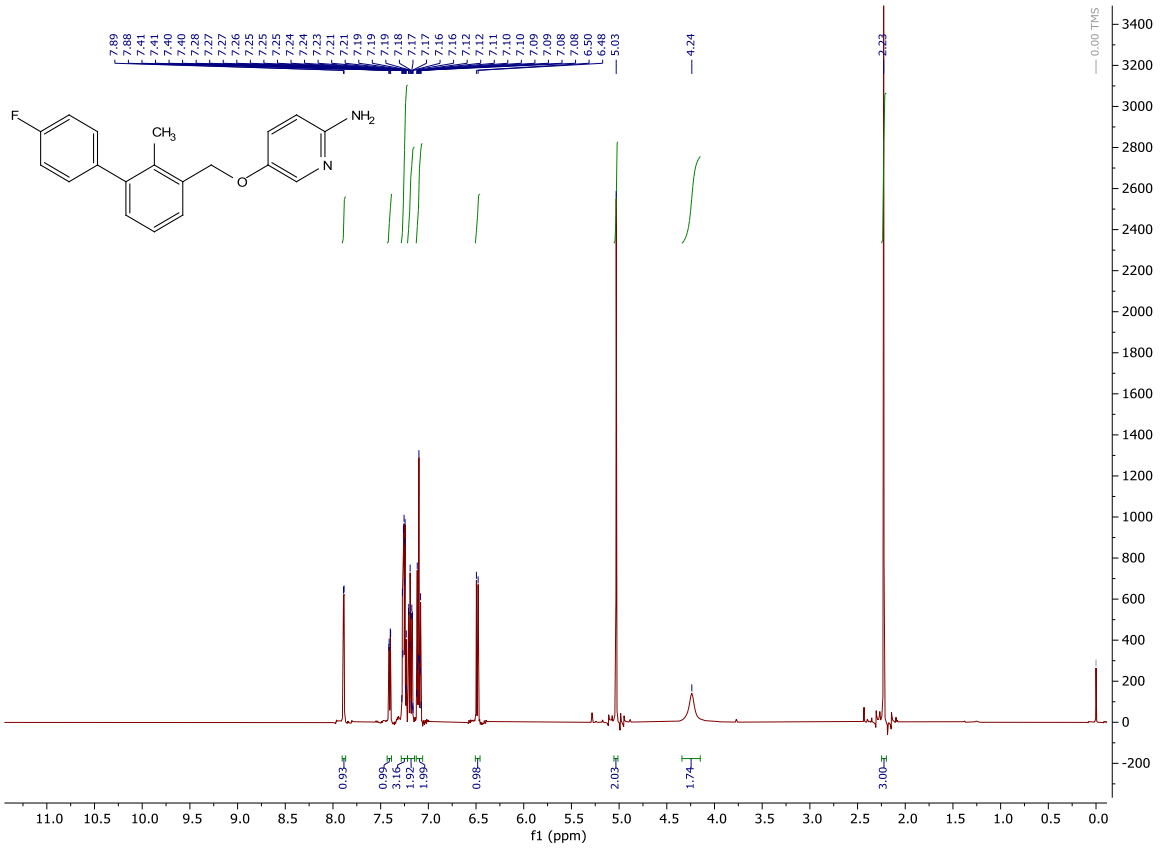
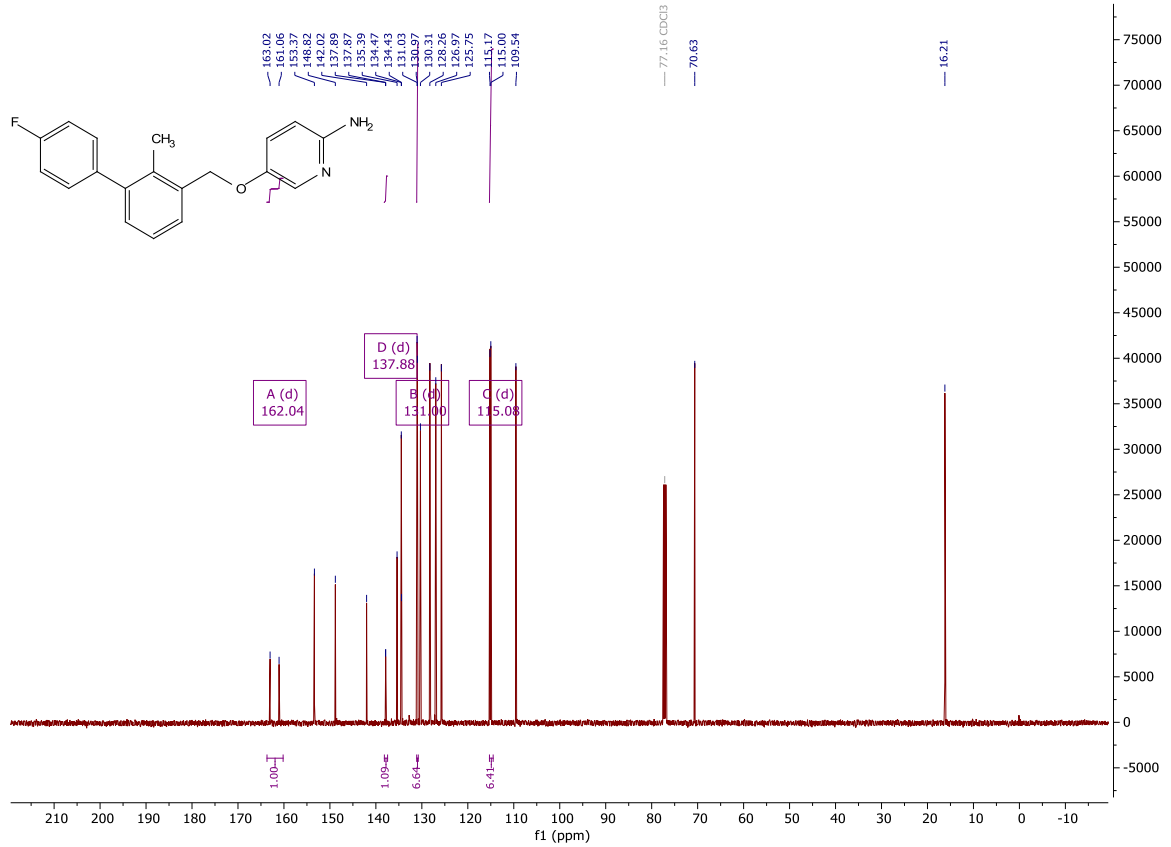
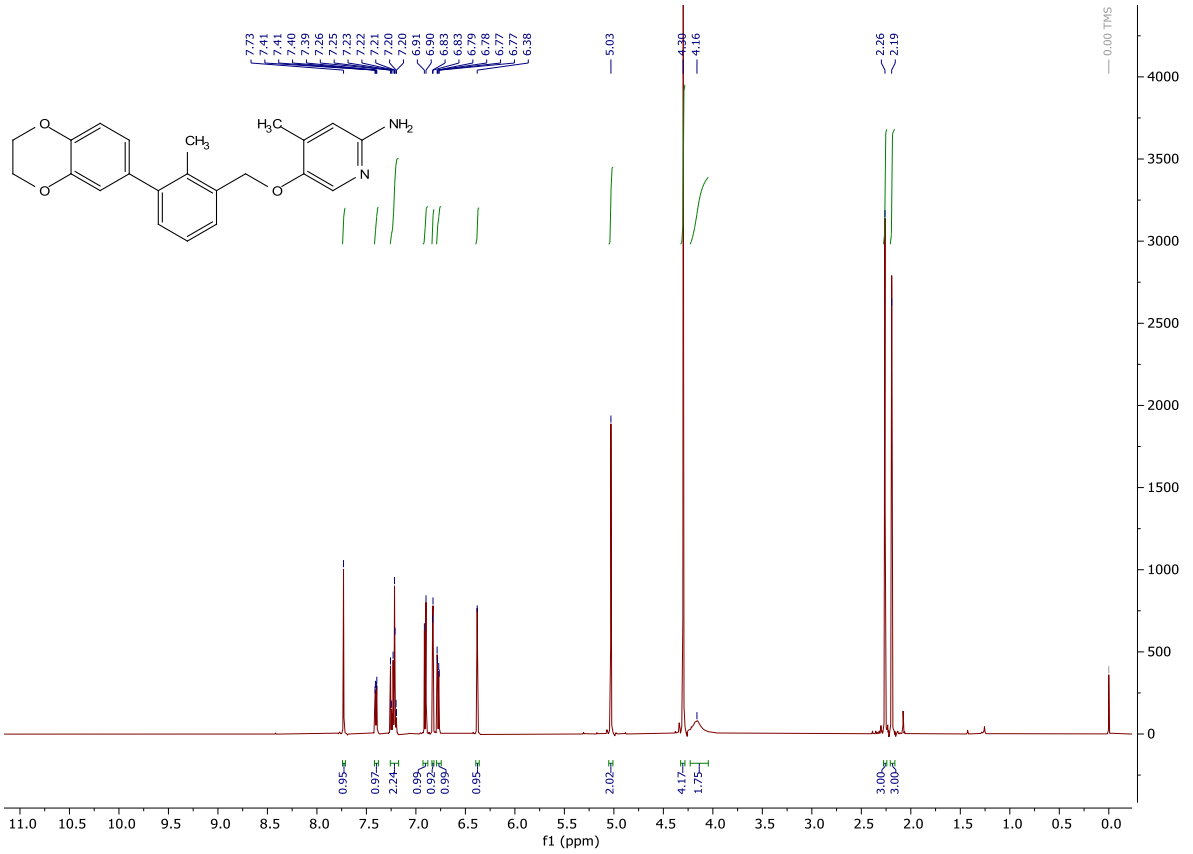
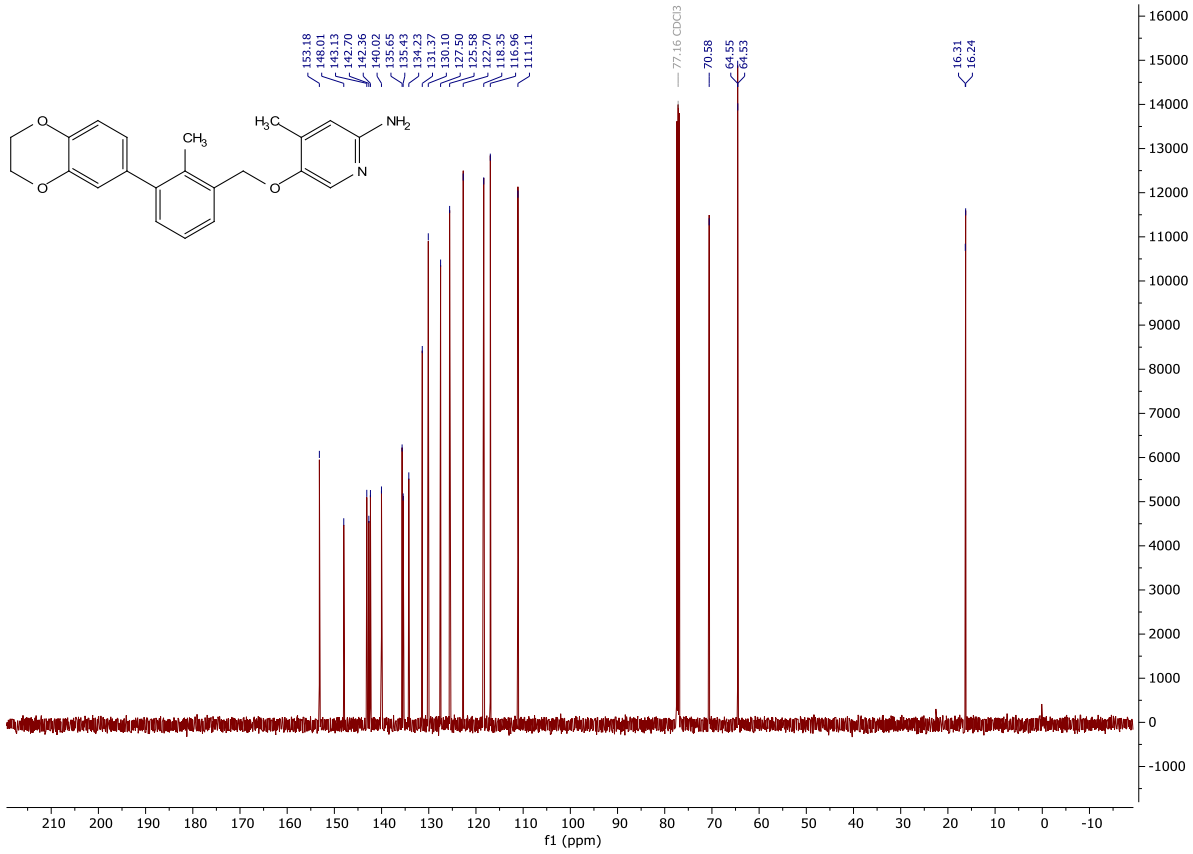


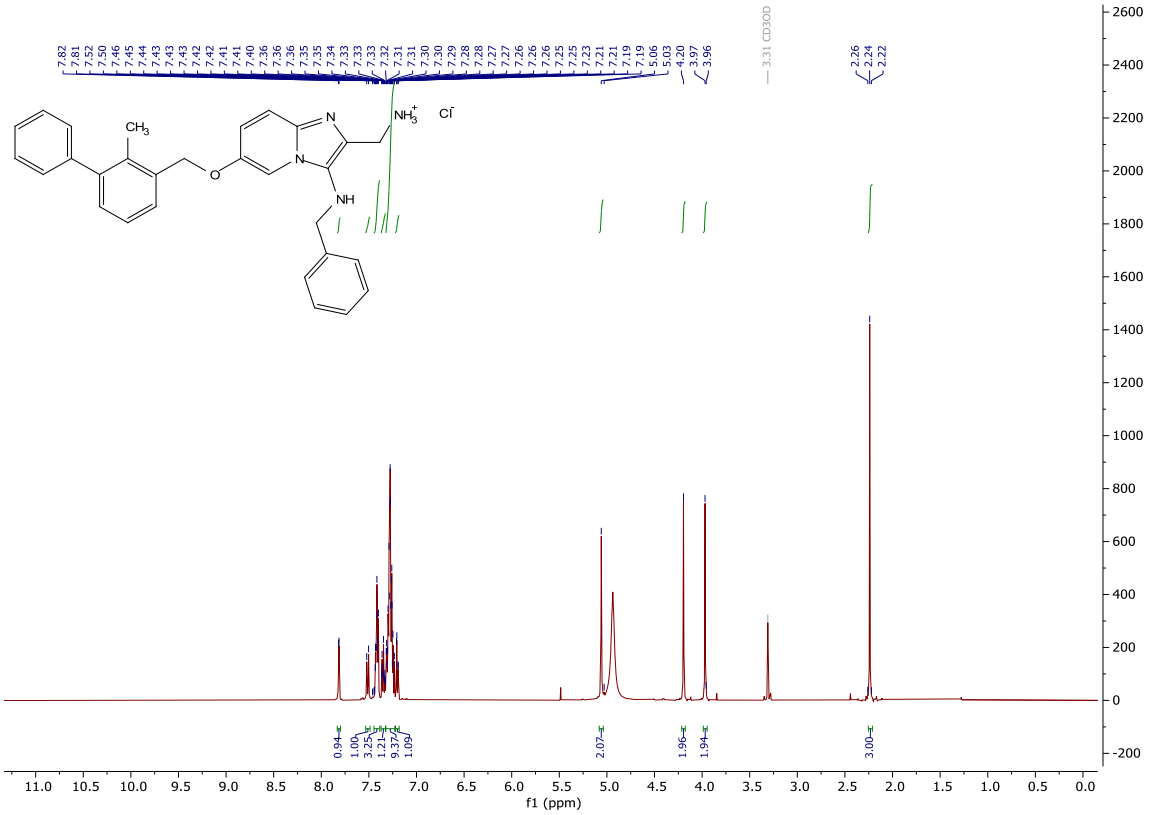


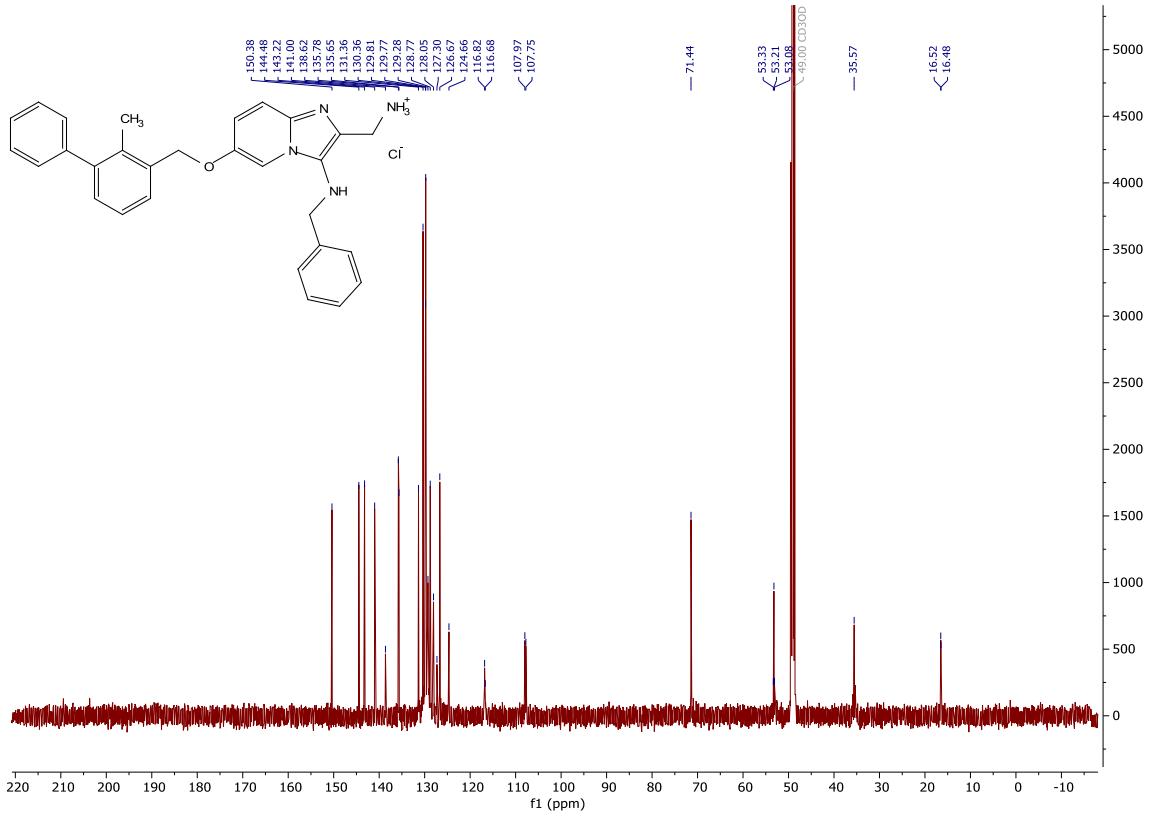
5-((3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-2-methylbenzyl)oxy)-4-methylpyridin-2-amine (6d)



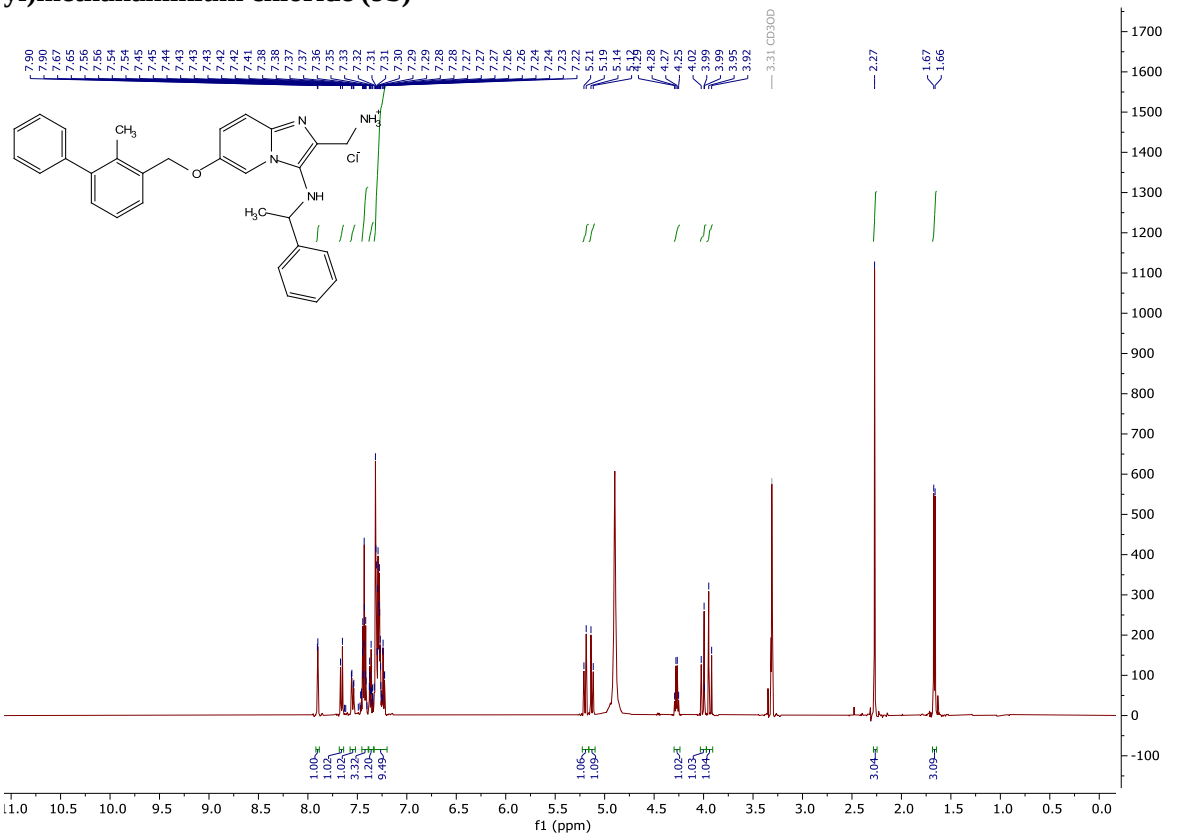


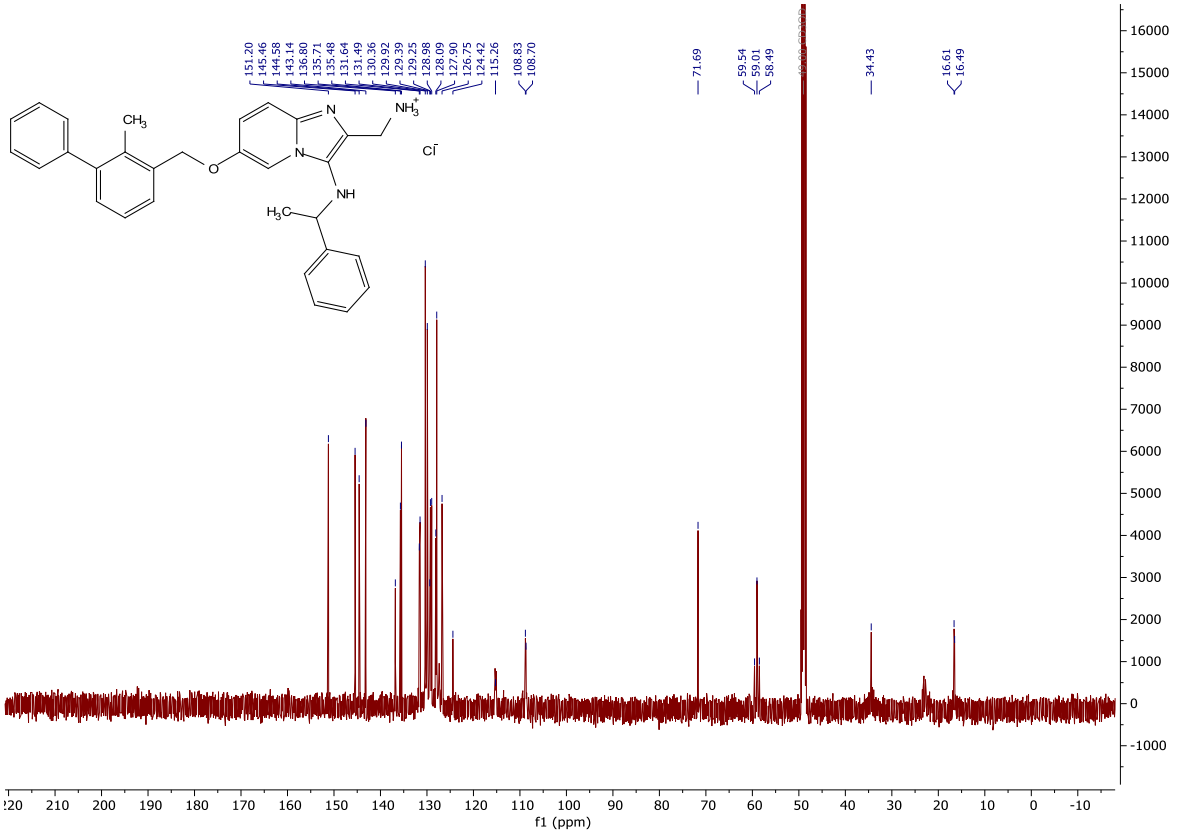
(3-(benzylamino)-6-((2-methyl-[1,1'-biphenyl]-3-yl)methoxy)imidazo[1,2-a]pyridin-2-yl)methanaminium chloride (9a)



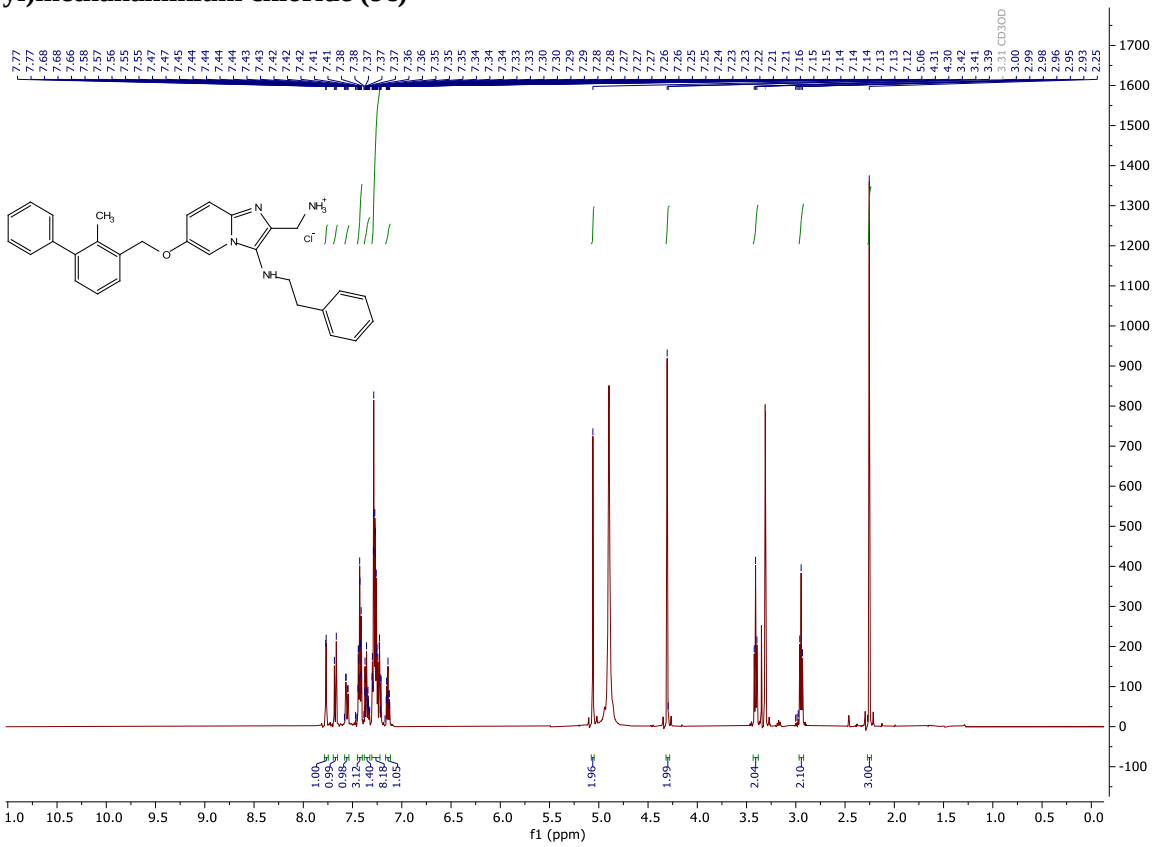


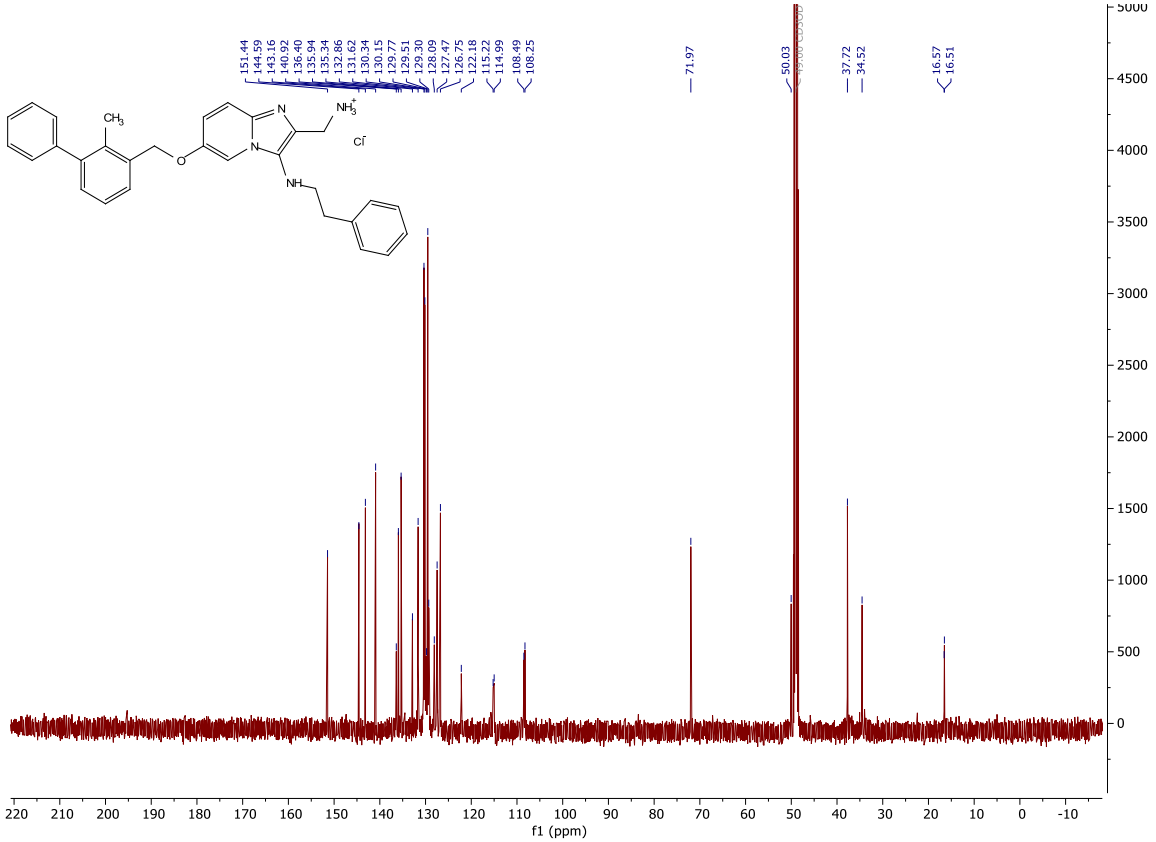
(6-((2-methyl-[1,1'-biphenyl]-3-yl)methoxy)-3-((1-phenylethyl)amino)imidazo[1,2-a]pyridin-2-yl)methanaminium chloride (9b)



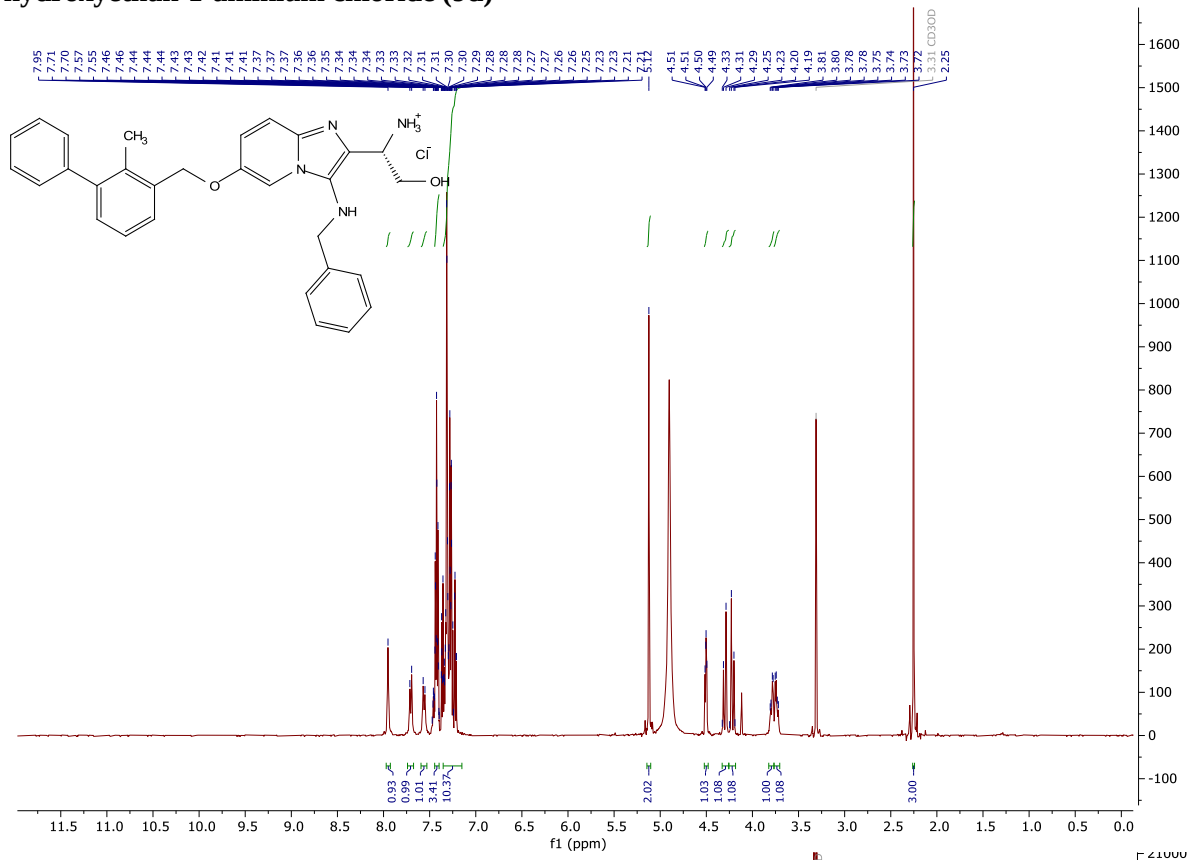


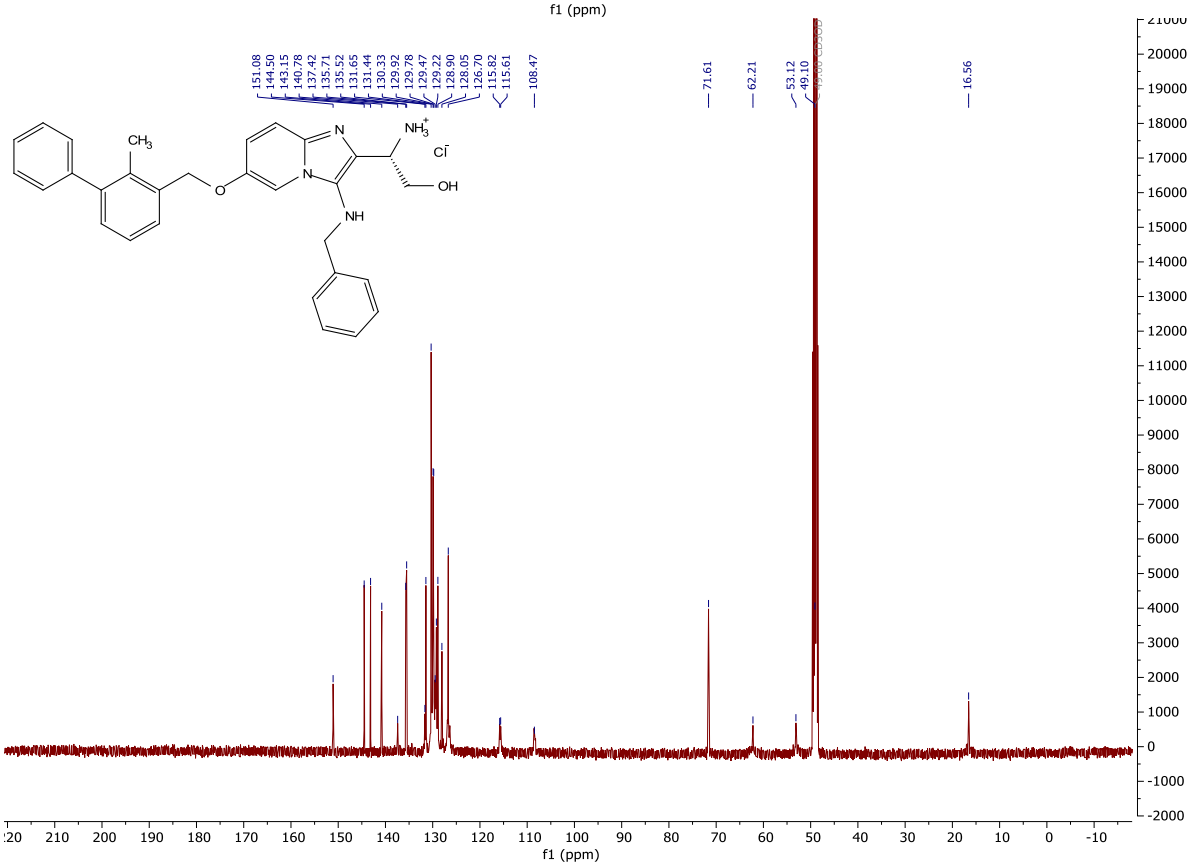
(6-((2-methyl-[1,1'-biphenyl]-3-yl)methoxy)-3-(phenethylamino)imidazo[1,2-a]pyridin-2-yl)methanaminium chloride (9c)



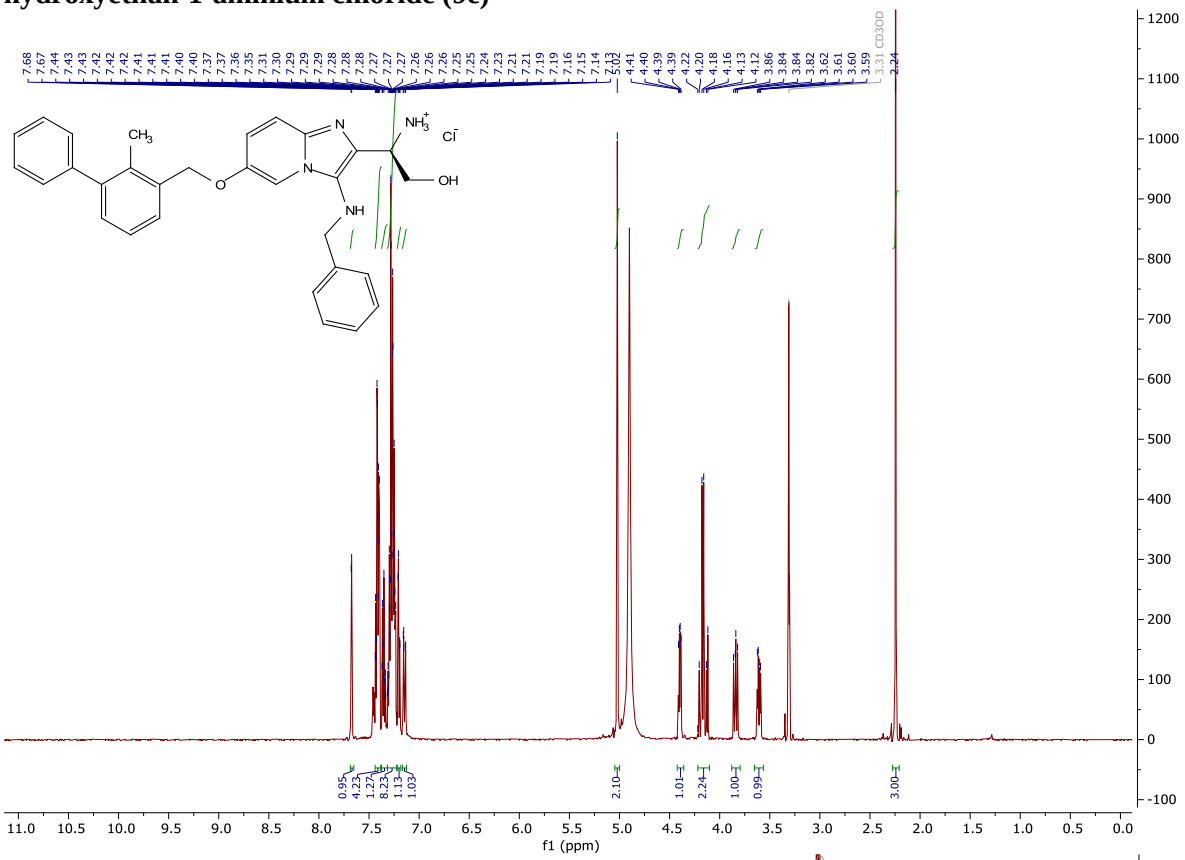


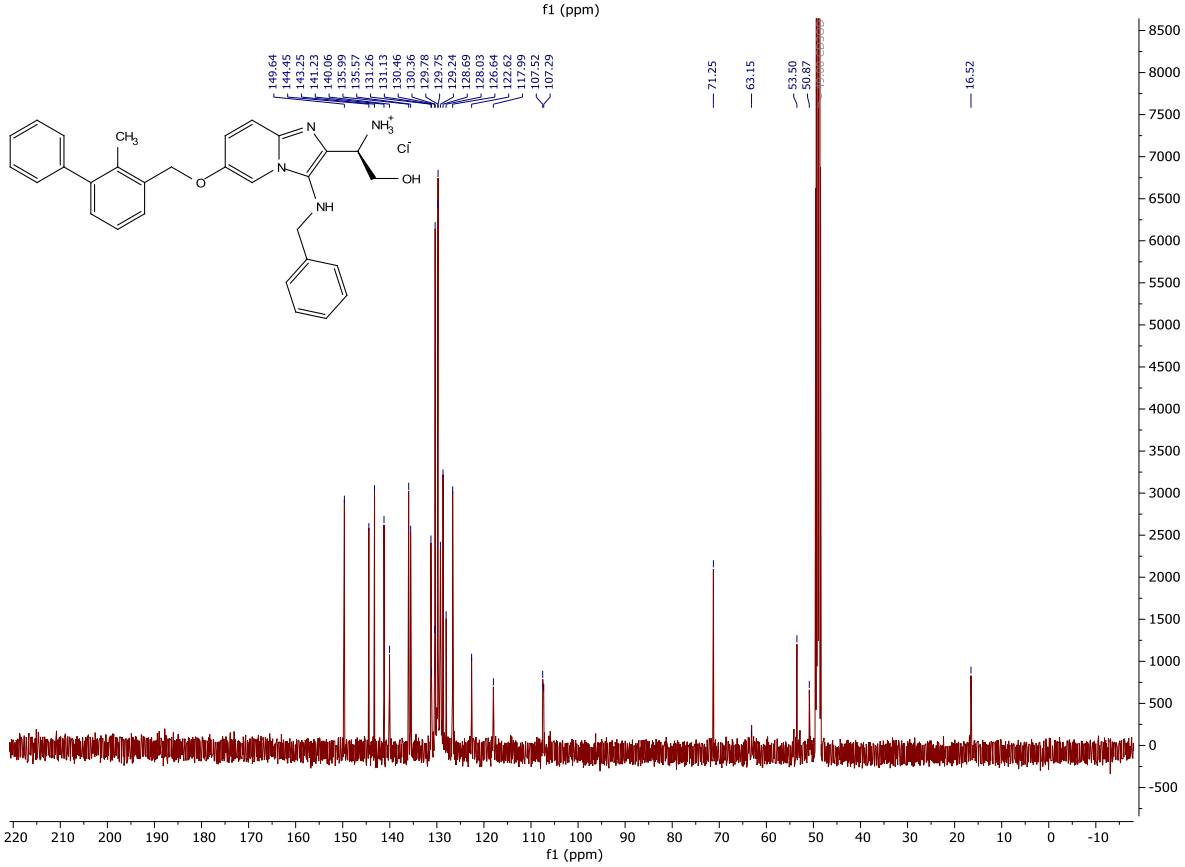
(S)-1-(3-(benzylamino)-6-((2-methyl-[1,1'-biphenyl]-3-yl)methoxy)imidazo[1,2-a]pyridin-2-yl)-2-hydroxyethan-1-aminium chloride (9d)



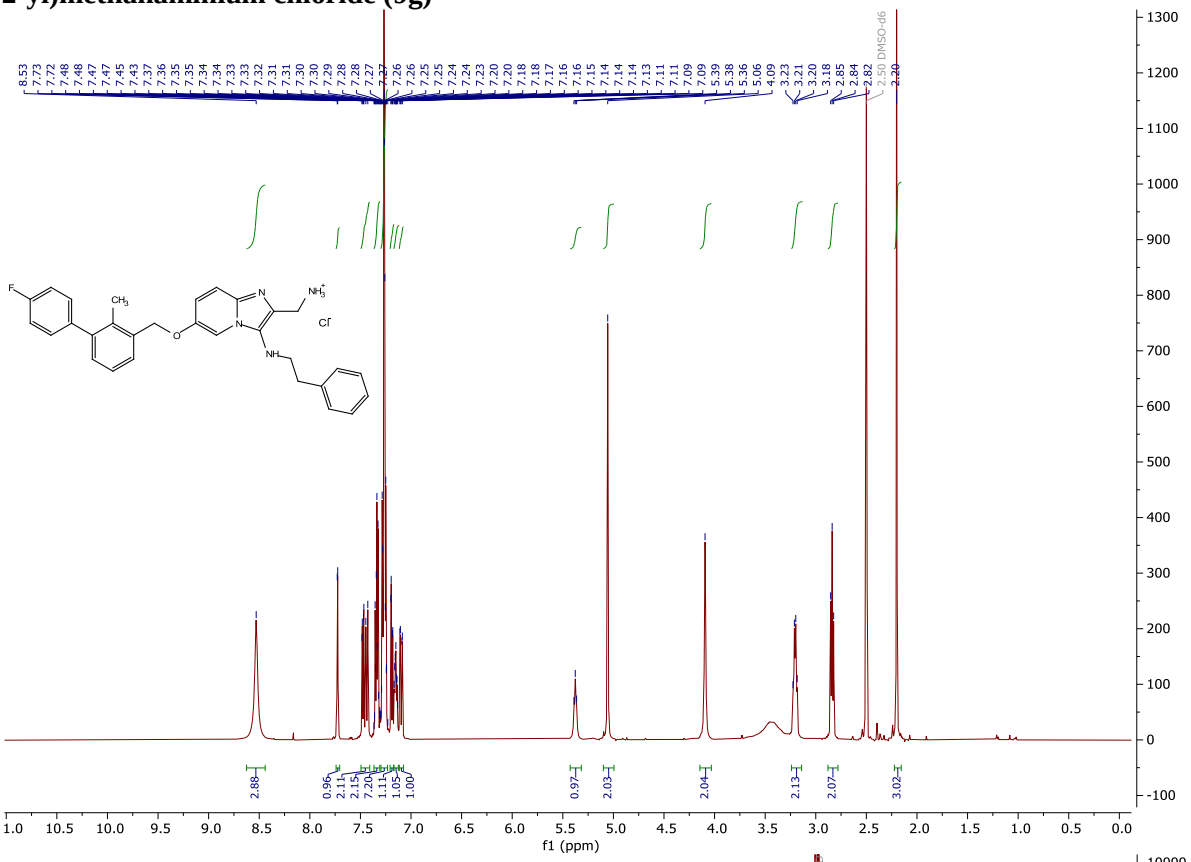


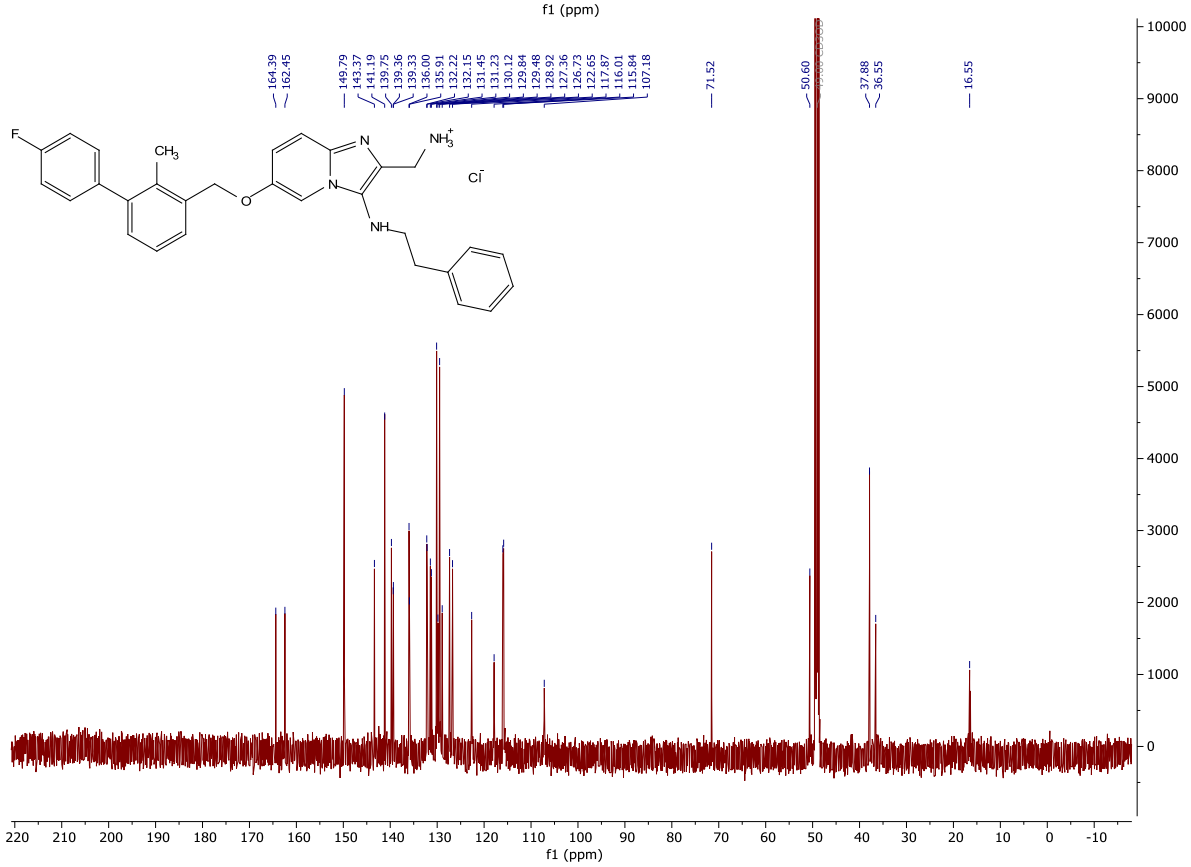
(R)-1-(3-(benzylamino)-6-((2-methyl-[1,1'-biphenyl]-3-yl)methoxy)imidazo[1,2-a]pyridin-2-yl)-2-hydroxyethan-1-aminium chloride (9e)



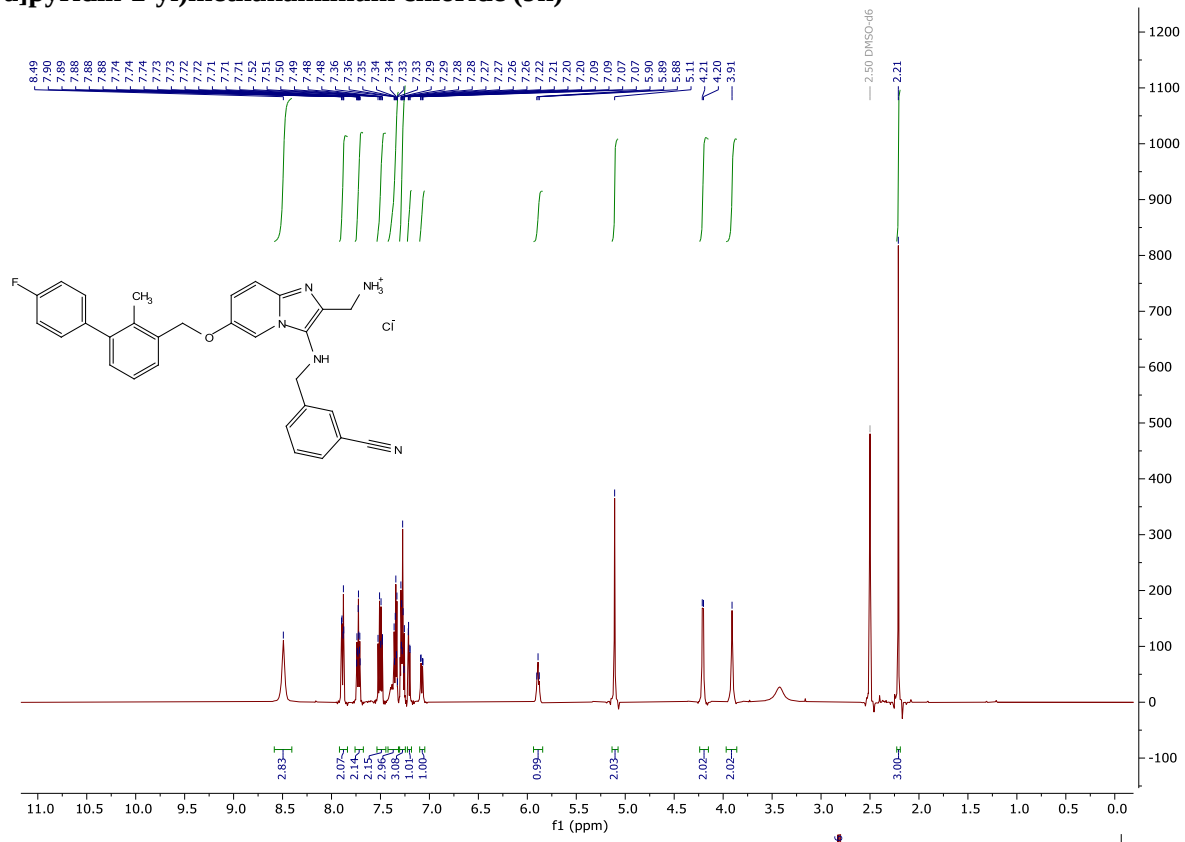


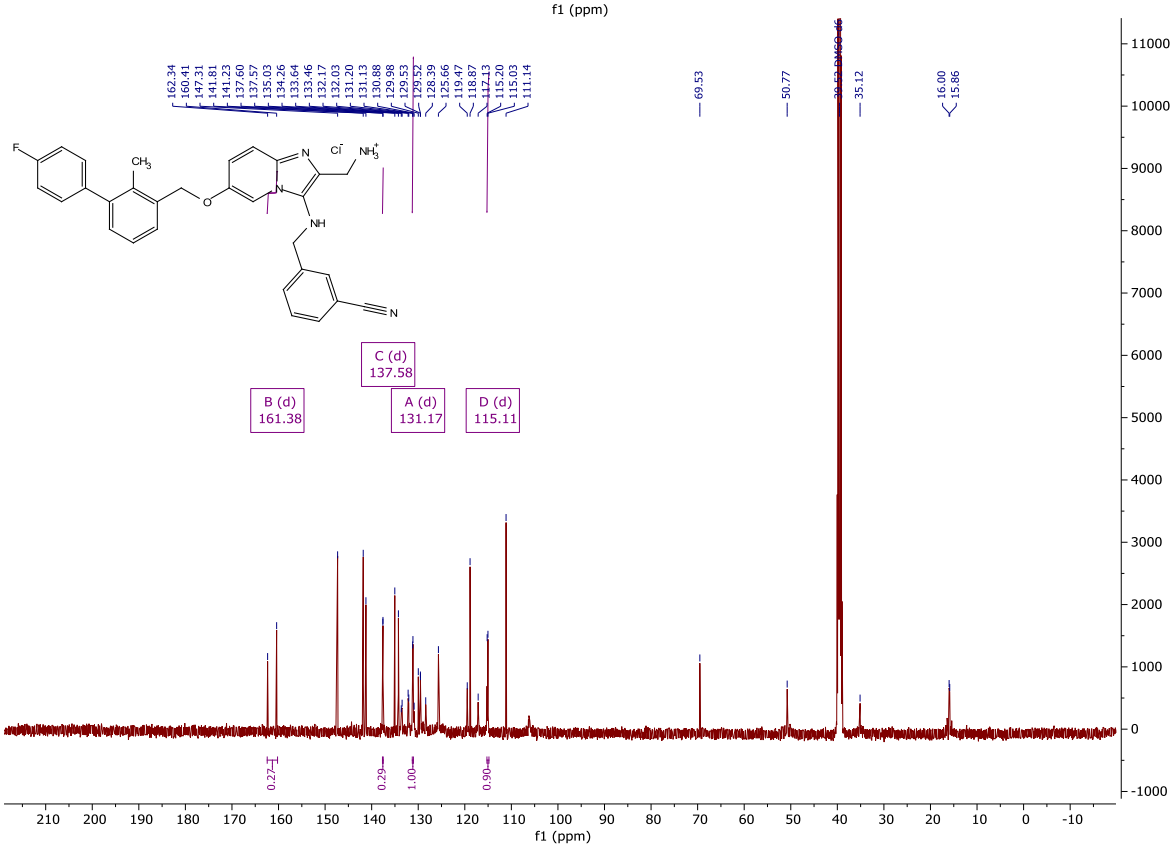
(6-((4'-fluoro-2-methyl-[1,1'-biphenyl]-3-yl)methoxy)-3-(phenethylamino)imidazo[1,2-a]pyridin-2-yl)methanaminium chloride (9g)



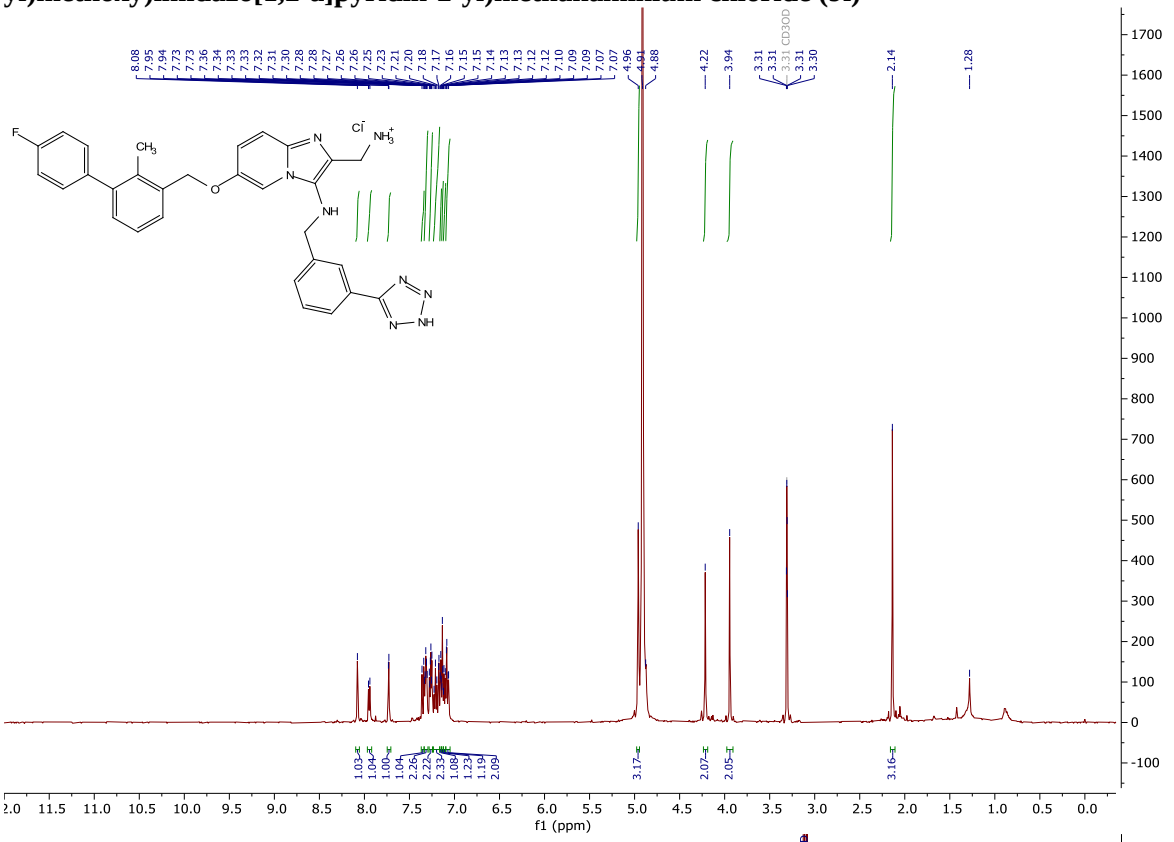


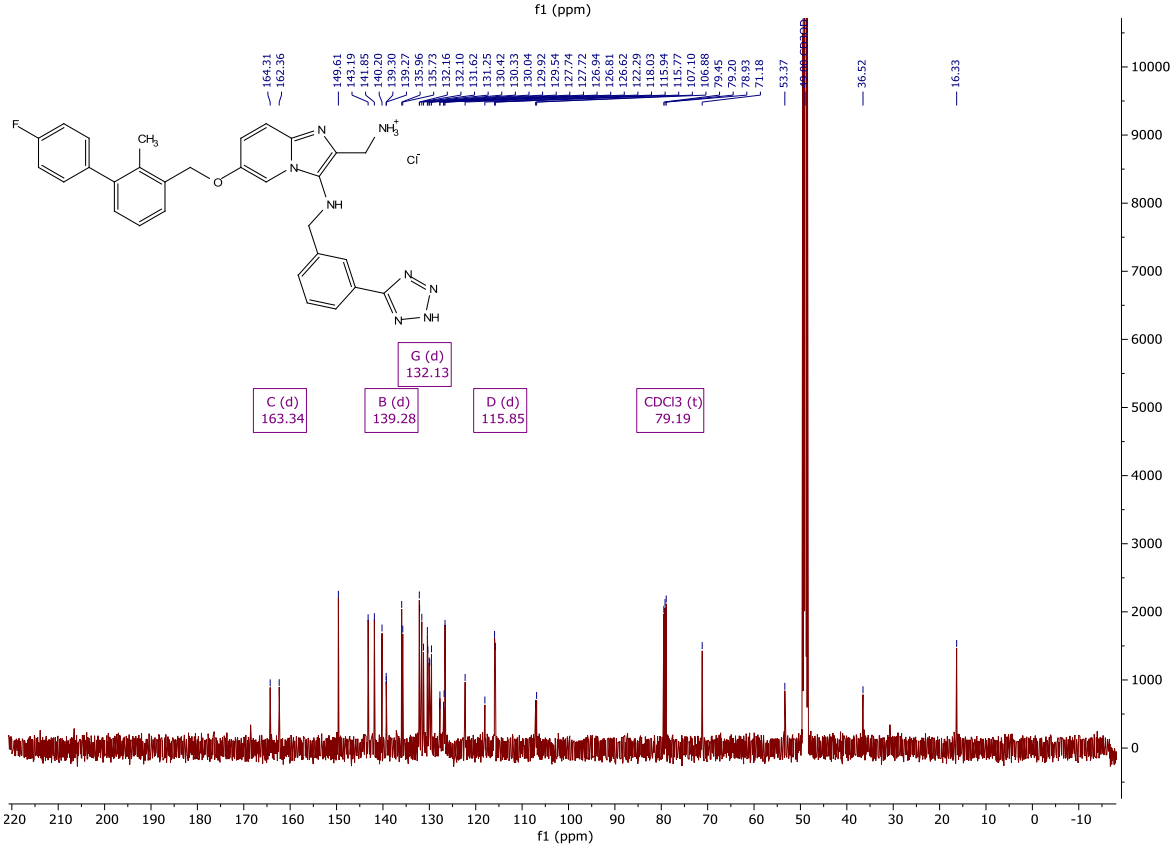
(3-((3-cyanobenzyl)amino)-6-((4'-fluoro-2-methyl-[1,1'-biphenyl]-3-yl)methoxy)imidazo[1,2-a]pyridin-2-yl)methanaminium chloride (9h)



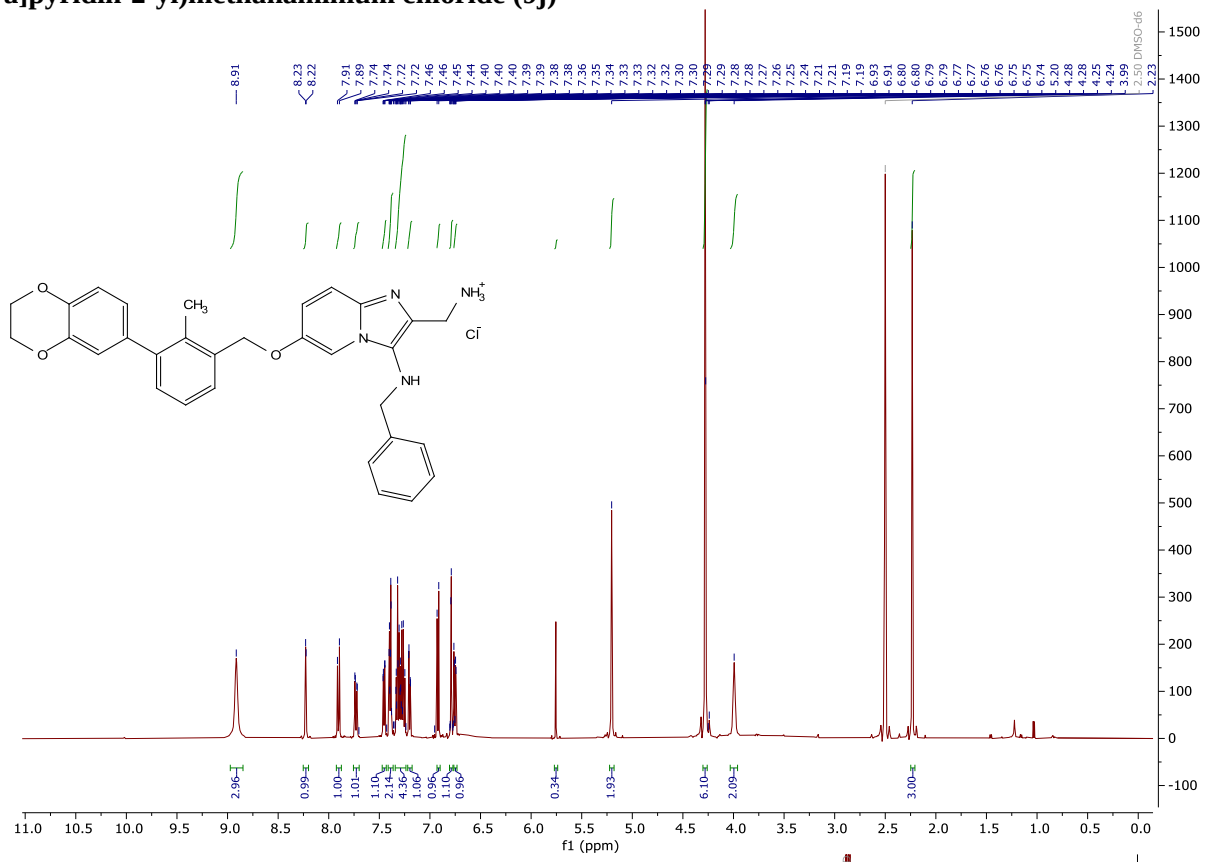


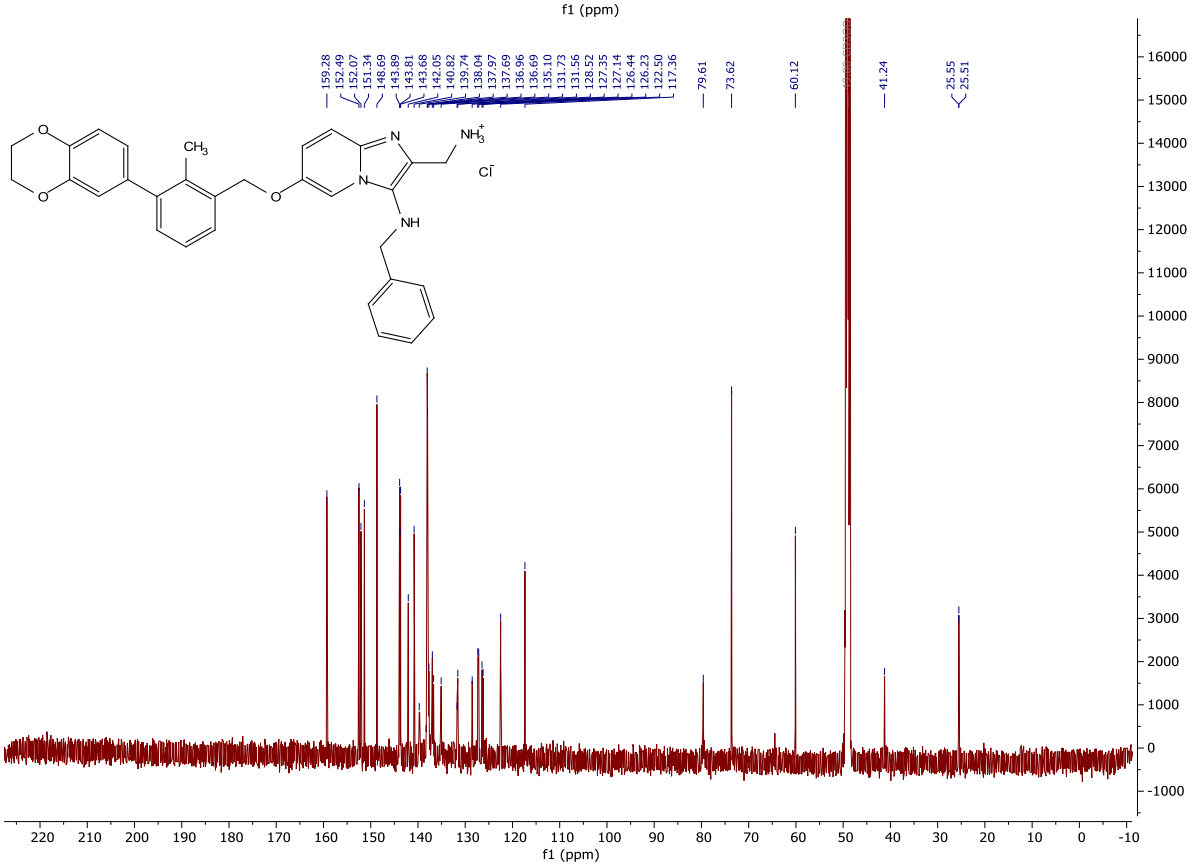
(3-((3-(2H-tetrazol-5-yl)benzyl)amino)-6-((4'-fluoro-2-methyl-[1,1'-biphenyl]-3-yl)methoxy)imidazo[1,2-a]pyridin-2-yl)methanaminium chloride (9i)



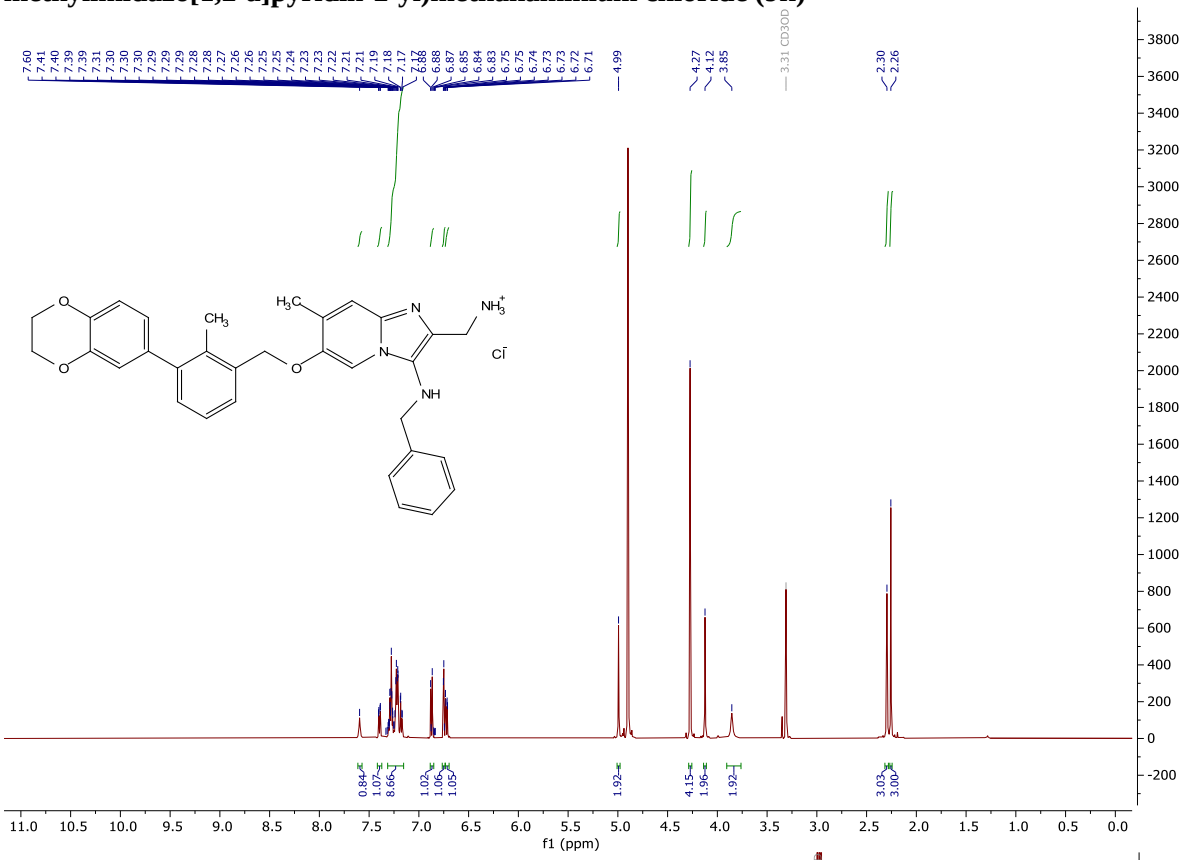


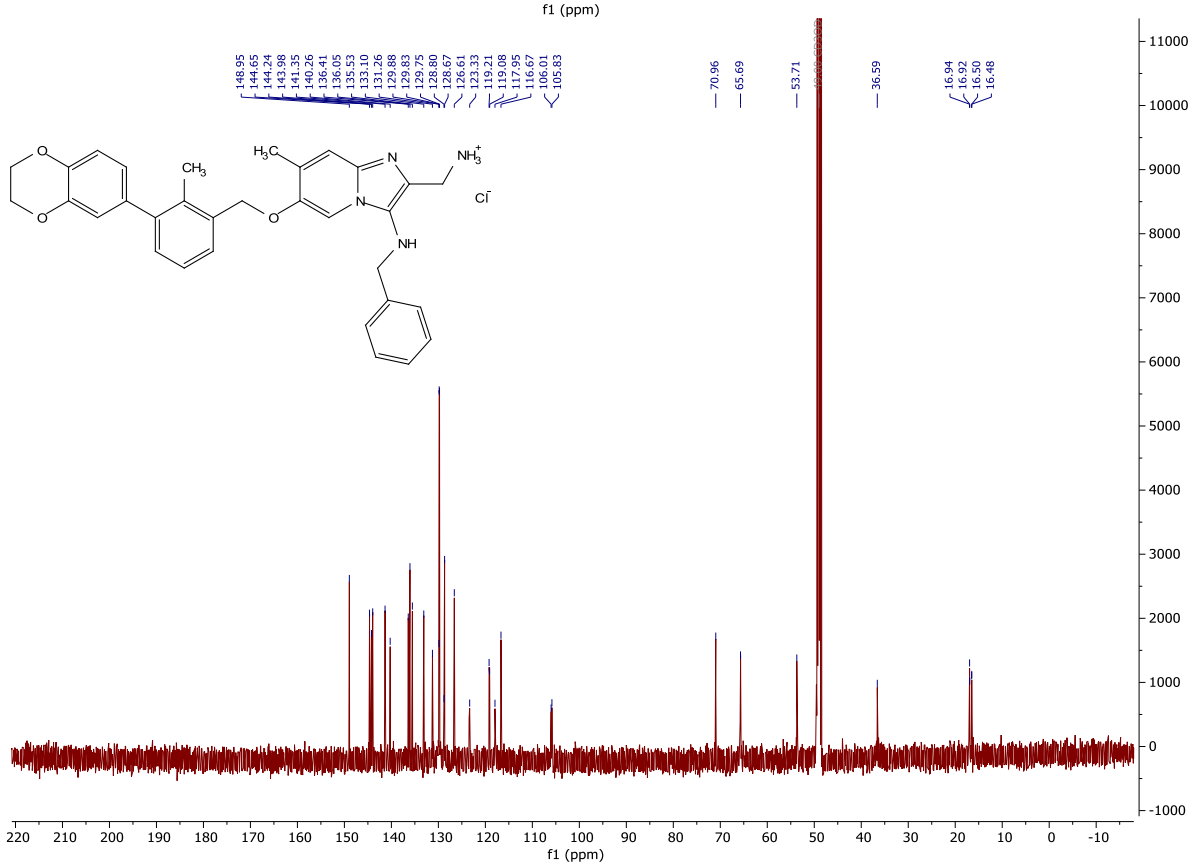
(3-(benzylamino)-6-((3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-2-methylbenzyl)oxy)imidazo[1,2-a]pyridin-2-yl)methanaminium chloride (9j)





(3-(benzylamino)-6-((3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-2-methylbenzyl)oxy)-7-methylimidazo[1,2-a]pyridin-2-yl)methanaminium chloride (9k)





NMR binding assay

For NMR measurements, the buffer was exchanged by gel filtration to PBS pH 7.4. 10% (*v*/*v*) of D_2O was added to the samples to provide the lock signal. All spectra were recorded at 300 K using a Bruker Avance III 600 MHz spectrometer equipped with the nitrogen cryo-probe head.

Determination of binding of compounds to PD-L1 was carried out with the ^1H NMR. The line width broadening in the proton NMR of PD-L1 suggests that the compounds induce protein oligomerization. In all the cases, the well resolved narrow resonance peaks in the aliphatic region of 1H NMR spectrum of apo-PD-L1 exhibited significant broadening upon addition of each compound indicating significant increase in the molecular weight of the complex (Figure 2A). The molecular weight of each complex estimated from relaxation time analysis, which can only be explained by the compound induced PD-L1 dimerization. No significant changes were observed upon addition of the PD-1 with the tested compounds.

PD-L1 cocrystalization

PD-L1 expression and purification

The IgV domains of human PD-L1 protein (hPD-L1 residues: 18-134, C-terminal His-tag) was expressed and purified as described previously (Zak et al., 2016). Briefly, protein was expressed in E. coli BL21 (DE3) strain as inclusion bodies which were collected by centrifugation, washed, and dissolved. Protein was refolded by drop-wise dilution into refolding buffer: 0.1 M Tris pH 8.0, 1 M L-Arg hydrochloride, 2 mM EDTA, 0.25 mM oxidized glutathione and 0.25 mM reduced glutathione. Refolded protein was dialyzed 3 times over 48-72 h against buffer containing 10 mM Tris pH 8.0 and 20 mM NaCl. On the final step, PD-L1 was concentrated and loaded to a size exclusion chromatography column HiLoad 26/600 Superdex 75 (GE Healthcare) pre-equilibrated with buffer containing 10 mM Tris pH 8.0 and 20 mM NaCl for crystallization or PBS pH 7.4 for NMR experiments.

PD-L1 cocrystalization

Purified PD-L1 was concentrated to 5 mg/ml, mixed with the inhibitor in 1:3 molar ratio (protein:compound) and clarified by centrifugation at 15 000 × g for 10 min. Supernatant was used for screening using a sitting-drop vapor diffusion method and commercially available buffer sets. Diffraction-quality crystals were obtained at room temperature from the condition containing: 1.2 M

sodium citrate tribasic dihydrate 0.01 M sodium borate, pH 8.5. The crystal was flash-cooled in liquid nitrogen.

Crystal structure determination and refinement

The X-ray diffraction data were collected at the BL14.1 beamline operated by the Helmholtz-Zentrum Berlin (HZB) at the BESSY II (Berlin Adlershof, Germany).[1] The data were indexed, integrated, and scaled using XDS, XSCALE, and Aimless.[2–4] Initial phases were obtained by molecular replacement calculated in Phaser.[5] The model building was performed in Coot and refinement was performed using Phenix or PDB-REDO server.[6–8] Water molecules were added automatically and inspected manually. Coordinates and structure factors were deposited in the Protein Data Bank under accession code PDB: 7BEA.

Table S1 Data collection and refinement statistics (molecular replacement)

Data collection	
Wavelength (Å)	0.9184
Space group	P 2 21 21
Cell dimensions	
a, *b*, *c* (Å)	32.62 54.62 140.96
α, *β*, *γ* (o)	90.00 90.00 90.00
Resolution range (Å)	46.99 - 2.45 (2.55 - 2.45)
Rmerge	0.138 (1.915)
I/σI	10.9 (0.9)
Completeness (%)	99.8 (98.9)
Redundancy	7.1 (7.4)
Total reflections	69799 (7901)
CC1/2	0.997 (0.474)
Refinement statistics	
No. reflections	9843 (956)
*R*work/*R*free	0.2741/0.2868 (0.3569)/(0.3895)

Wilson B-factor	49.3
No. atoms	1982
Protein	1906
Water	41
Ramachandran favoured (%)	92.65
Ramachandran allowed (%)	6.53
Ramachandran outliers (%)	0.82
B-factors	36.58
Protein	35.91
Water	44.20
R.m.s deviations	
Bond lengths (Å)	0.011
Bond angles (o)	1.555

Homogenous Time-Resolved Fluorescence (HTRF) assay

The HTRF assay was performed using the certified Cis-Bio assay kit at 20 µL final volume using their standard protocol as described by Musielak et al..[9] Measurements were performed on individual dilution series to determine the half maximal inhibitory concentration (IC50) of tested compounds. After mixing all components according to the Cis-Bio protocol, the plate was incubated for 2 h at RT. TR-FRET measurement was performed on the Tecan Spark 20M. Collected data was background subtracted on the negative control, normalized on the positive control, averaged and fitted with normalized Hill's equation to determine the IC50 value using Mathematica 12. For the compounds with IC50 values were too high due to e.g. solubility issues a "dissociation value" at the concentration of 50 µM is presented for the sake of comparability to other inhibitors. The dissociation value represents the percentage of the PD-1/PD-L1 complex that is undissolved.

Molecular docking

Structure of 9j compound was prepared and minimized in VEGA ZZ software[10]. Subsequently, the conformational search using Boltzman jump and 5000 steps was performed. The conformation of 9j with lowest energy was selected and used in molecular docking with AutoDock Vina. The structure of compound and PD-L1 dimer (PDB: 6R3K) were prepared in AutoDock Tools. All water molecules and original ligand (BMS-1166) were removed and polar hydrogens atoms were added to the receptor. A grid box of dimensions 20x20x20 Å and following coordinates x: -8.746, y = 18.049 and z = -21.934 was placed at the interface of PD-L1 homodimer. Docking was carried out with exhaustiveness = 16[11]. The obtained binding poses were carefully visually inspected in PyMol

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