Discovery of a Novel Class of Imidazo[1,2-a]Pyridines with Potent PDGFR Activity and Oral Bioavailability

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General Methods and Materials

In the examples described below, unless otherwise indicated all temperatures are set forth in degrees Celsius. Reagents were purchased from commercial suppliers such as Aldrich Chemical Company, Lancaster, TCI or Maybridge, and were used without further purification unless otherwise indicated. Tetrahydrofuran (THF), dichloromethane (DCM, methylene chloride), toluene, and dioxane were purchased from Aldrich in Sure seal bottles and used as received. The reactions set forth below were done generally under a positive pressure of nitrogen or argon or with a drying tube (unless otherwise stated) in anhydrous solvents, and the reaction flasks were typically fitted with rubber septa for the

introduction of substrates and reagents via syringe. Air and water sensitive reactions were performed in glassware that was oven dried or flame-dried. ^{1}HNMR spectra were obtained as $CDCl_3$, CD_3OD, D_2O or d_6-DMSO solutions (reported in ppm), using tetramethylsilane (0.00 ppm) or residual solvent ($CDCl_3$: 7.25 ppm; CD_3OD: 3.31 ppm; D_2O: 4.79 ppm; d_6-DMSO: 2.50 ppm) as the reference standard. When peak multiplicities are reported, the following abbreviations are used: s (singlet), d (doublet), t (triplet), m (multiplet), br (broadened), dd (doublet of doublets), dt (doublet of triplets), obs (obscured). Coupling constants, when given, are reported in Hertz (Hz).

Abbreviations

Abl, Ableson leukemia oncogene cellular homolog; AUC, total area under the plasma drug concentration-time curve; DBA(dba), dibenzylideneacetone; DCE, 1,1-dichloroethane; ER, extraction ratio; FLT3, fms-like tyrosine kinase receptor-3; cFMS, colony-stimulating factor; HMDS, 1,1,1,3,3,3-hexamethyldisilazane; KDR, kinase insert domain receptor; cKIT, stem cell factor receptor; MDR1, multi-drug resistance gene; PDGFR, platlet-derived growth factor receptor; Pgp, P-glycoprotein; PK, pharmacokinetic; pKa, logarithmic acid dissociation constant; PKPD, pharmacokinetic-pharmacodynamic; SAR, structure-activity relationship; TFA, trifluoroacetic acid, X-Phos, 2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl.

Cellular PDGFR Assay

The ability of the compounds described to inhibit PDGF-induced PDGFR phosphorylation was assessed by using mouse NIH3T3 cells. 25,000 cells in DMEM supplemented with 10% fetal bovine serum were added to each well of a black 96-well cell culture plate. Plates were incubated in a 37°C/5% CO_2 incubator for 6-8 hours. Plates were then washed and incubated with serum-free DMEM, and the cells were returned to the 37 °C/5% CO_2 incubator for 16-20 hours.

Compound test solutions were added at a final concentration of 0.5% DMSO, and the cells were incubated in a 37 °C/5% CO_2 incubator for 1 hour. PDGF-BB ligand was then added (75 ng/mL) and incubated for 15 minutes. Cells were washed with PBS and fixed

in 3.7% formaldehyde in PBS for 10 minutes. This was followed by washing in PBS/0.2% Triton X-100 and permeabilizing in 100% MeOH for 10 minutes. Cells were blocked in Odyssey blocking buffer (LI-COR Biosciences) for 1 hour. Antibodies to phosphorylated PDGFRβ and total PDGFRβ were added to the cells and incubated for 3 hours. After washing with PBS/0.2% TritonX-100, the cells were incubated with fluorescently-labeled secondary antibodies (goat anti-rabbit IgG-IRDye800 and goat anti-mouse IgG-Alexa Fluor 680) for an additional hour. Cells were then washed with PBS and analyzed for fluorescence at both wavelengths using the Odyssey Infrared Imaging System (LI-COR Biosciences). Phosphorylated PDGFR signal was normalized to total PDGFR signal.

pPDGFR PKPD Protocol

Title: PK/PD of PDGFR inhibitors in nude mice bearing C6 tumor xenografts
Purpose: Evaluate the effect of PDGFR inhibitors in C6 PK/PD model

Cell Line: C6

Number cells, injection volume, and route of implantation:

6 x 10^6 cells per mouse, SC in 100 ul PBS. Cells at passage P+4, grown in DMEM/F12 with 10% FBS

Taconic-NCI stock female nude mice

Study groups:

Group	Compound	Dose / schedule	# of Animals
1	**20% solutol in water vehicle, 2 HOUR**	**10 ml/kg, PO**	**4**
2	**28, 2 HOUR**	**10 mg/kg**	**4**
3	**28, 2 HOUR**	**30 mg/kg**	**4**
4	**28, 2 HOUR**	**100 mg/kg**	**4**
5	**20% solutol in water vehicle, 6 HOUR**	**10 ml/kg, PO**	**4**
6	**28, 6 HOUR**	**10 mg/kg**	**4**
7	**28, 6 HOUR**	**30 mg/kg**	**4**
8	**28, 6 HOUR**	**100 mg/kg**	**4**
9	**20% solutol in water vehicle, 12 HOUR**	**10 ml/kg, PO**	**4**
10	**28, 12 HOUR**	**10 mg/kg**	**4**
11	**28, 12 HOUR**	**30 mg/kg**	**4**
12	**28, 12 HOUR**	**100 mg/kg**	**4**

Vehicle: 20% solutol in water

Dose volume: 10 ml/kg

Dose Schedule: single dose

Route of Administration: PO

Dose solution Preparation and Characteristics:

28 (85.68% Active) 10mg/kg:
4.66mg + 3.96mL solutol
Clear, in solution

28 (85.68% Active) 30mg/kg:
16.21mg + 4.6mL solutol
clear, in solution

28 (85.68% Active) 100mg/kg:
28.1mg + 2.4mL solutol
Clear, in solution

Test Animal Species: Nu/nu female nude mice.

Deviations: The experiment will be run in 3 rounds.

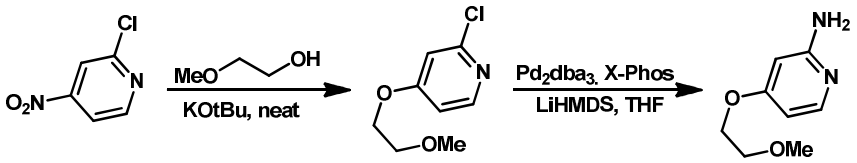
	ANIMAL WEIGHT, g				TUMOR WEIGHT, mg			
GROUP	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
1	24	20	23	22	134/83	132/87	178/178	202/128
2	24	26	25	25	236/215	434/333	120/87	206/
3	25	25	25	23	147/116	151/157	173/162	68/58
4	27	25	24	23	67/155	106/125	183/144	136/147
5	23	22	24	27	129/60	206/178	428/326	129/143
6	24	26	24	24	199/180	72/51	150/113	249/154
7	23	25	23	23	188/167	260/238	394/185	125/162
8	24	24	23	26	130/126	233/257	81/142	354/276
9	22	23	21	21	225/142	150/143	154/93	492/386
10	22	22	24	22	203/39	521/143	159/158	170/175
11	20	23	22	23	384/302	182/101	184/175	67/80
12	21	25	23	25	271/90	90/57	278/234	127/94

Comparison of Protein Sequence Variations in the ATP Binding Pocket

Kinase	Gate Keeper	Inner Floor	Outer Floor	Kinase	Gate Keeper	Inner Floor	Outer Floor
PDGFR*a*	Thr	Cys	Asp - Asn	FLT3	**Phe**	Cys	Asp - Asn
PDGFR*b*	Thr	Cys	Asp - Asn	KDR (VEGFR2)	**Val**	Cys	**Asn - Asn**

KIT	Thr	Cys	Asp - Asn	FLT1 (VEGFR1)	**Val**	Cys	**Asn - Asn**
cFMS (CSF1R)	Thr	**Gly**	Asp - Asn	FLT4 (VEGFR3)	**Val**	Cys	**Asn - Asn**

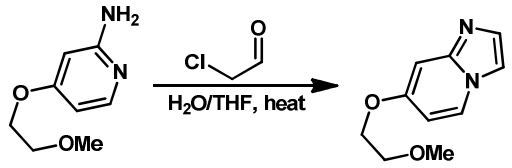
Experimental Details and Data for the Synthesis of Compounds 3-11, **27** and **28**



2-Chloro-4-(2-methoxyethoxy)pyridine (3). 2-chloro-4-nitropyridine (120 g, 757 mmol) was treated with 2-methoxyethanol (731 g, 9,612 mmol) and the mixture was cooled to 0 °C. Then solid KOtBu (93.43 g, 832.58 mmol) was added portion wise so that the internal temperature did not rise above 10 °C. The mixture was stirred at 0 °C for 30 minutes and then warmed to ambient temperature where the mixture stirred for 16 hours. TLC (Hexanes/Ethyl Acetate) showed clear consumption of the starting material and formation of a single new spot. The mixture was concentrated *in vacuo* and then diluted with water (500 mL). The mixture was extracted twice with methylene chloride (500 mL each) and the combined organic layers were dried over Na_2SO_4 filtered and concentrated *in vacuo* to an amber oil, (143 g, quant) that was used directly in the subsequent step. Data are: ^{1}H NMR ($CDCl_3$, 400 MHz) 8.19 (d, J = 5.9 Hz, 1H), 6.86 (d, J = 2.4 Hz, 1H), 6.79 (dd, J = 5.9 Hz, 2.4 Hz, 1H), 4.17 (m, 2H), 3.76 (m, 2H), 3.45 (s, 3H); MS APCI (+) *m/z* 188.1 (M+1) detected.

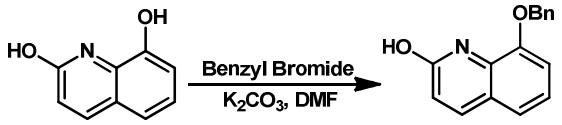
4-(2-methoxyethoxy)pyridin-2-amine (4). A solution of 2-chloro-4-(2-methoxyethoxy)pyridine (142 g, 756.8 mmol) in THF (260 mL) was added to a suspension of Pd_2dba_3 (13.86 g, 15.14 mmol) and XPHOS (14.43 g, 30.27 mmol) in THF (1000 mL) at ambient temperature. The reaction mixture was degassed (vacuum cycling with nitrogen refills, 5 cycles) and then LiHMDS (1,597 mL, 1,597 mmol, 1.0M THF) was added. The mixture was degassed again, (vacuum cycling with nitrogen refills, 5 cycles) and then heated to an internal temperature of 60 °C where the mixture stirred for 18 hours. The reaction was cooled to 5 °C in an ice bath and then treated with cold 1N HCl (700 mL). Then 3N HCl was added in 100 mL portions while maintaining an

internal temperature of less than 20 °C until the mixture had reached a pH = ~1 (700 mL). The mixture was then diluted with MTBE (1 L). The layers were mixed and separated and the organic layer was removed and then washed once with 1N aqueous HCl (500 mL). The combined acidic aqueous layers were extracted with a fresh portion of MTBE (500 mL) and the aqueous layer was then filtered through celite to remove a dark insoluble material. The filtered aqueous layer was cooled to 0 °C and adjusted to pH >10 with solid NaOH. The resulting basic aqueous layer was washed with methylene chloride (2 x 400 mL, 2 x 200 mL) and the combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The resulting tan slurry was diluted with heptane (400 mL) and then concentrated again *in vacuo*. The resultant brown thick slurry was again suspended in heptane (300 mL), stirred to homogenize the solids and then filtered. The solids were washed with a small amount of fresh heptane and the solids thus isolated were then dried, providing the desired compound (110.5 g, 86%) as a tan solid. Data are: ^{1}H NMR ($CDCl_3$, 400 MHz) 7.90 (d, *J* = 5.9 Hz, 1H), 6.29 (dd, *J* = 5.9 Hz, 2.0 Hz, 1H), 6.00 (d, *J* = 2.0 Hz, 1H), 4.36 (brs, 2H), 4.10 (m, 2H), 3.73 (m, 2H), 3.44 (s, 3H); MS APCI (+) *m/z* 169.1 (M+1) detected.

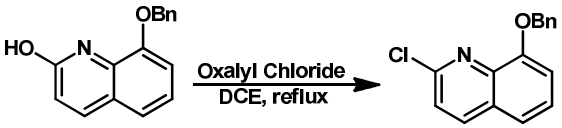


7-(2-Methoxyethoxy)imidazo[1,2-a]pyridine (5). 4-(2-methoxyethoxy)pyridin-2-amine (22.0 g, 130.8 mmol) was dissolved in THF (220 mL). The solution was degassed and back filled with Nitrogen 3x. Then 2-chloroacetaldehyde (49.84 mL, 392.4 mmol, ~50% water) was added and the mixture was heated to 50 °C where it stirred overnight. The mixture was then diluted with saturated aqueous Na_2CO_3 (13 mL). The mixture was then allowed to cool to ambient temperature. The mixture was then diluted with H_2O (200 mL) and the mixture was extracted with MTBE. The combined organic layers were then washed with 200 mL of 1M aqueous HCl. The aqueous layer was then treated with 160 mL of a saturated aqueous Na_2CO_3 solution to achieve a pH = ~9. The basic mixture was then extracted with Ethyl Acetate (EtOAc) (2 x 200 mL). The basic aqueous layer was found to contain additional product so it was treated further with saturated aqueous

Na_2CO_3 (50 mL) and extracted with EtOAc again. The combined organic layers were then washed with brine and dried over Na_2SO_4, filtered and concentrated to dryness *in vacuo*. This provided 24.6 g (97%) of the title compound as a brown semisolid. Data are: ^{1}H NMR ($CDCl_3$, 400 MHz) 7.94 (d, J = 7.4 Hz, 1H), 7.48 (s, 1H), 7.42 (s, 1H), 6.91 (d, J = 2.7 Hz, 1H), 6.59 (dd, J = 7.4 Hz, 2.7 Hz, 1H), 4.17 (m, 2H), 3.79 (m, 2H), 3.46 (s, 3H); MS APCI (+) *m/z* 193.0 (M+1) detected.



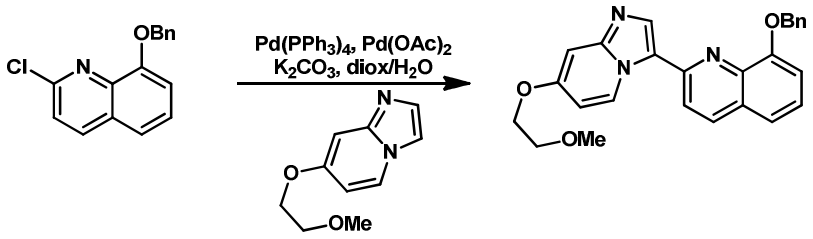
8-(Benzyloxy)quinolin-2-ol (7). Quinoline-2,8-diol (66.0 g, 410 mmol) was combined with K_2CO_3 (56.6 g, 410 mmol) and then slurried in DMF (430 mL). The slurry was treated with (bromomethyl)benzene (48.6 mL, 410 mmol) and warmed to 65 °C for 15 hours. HPLC showed an incomplete reaction therefore an additional aliquot of (bromomethyl)benzene (4.86 mL, 41.0 mmol) was added to the hot mixture and heating continued for an additional 4 hours. The warm mixture was poured into water (3.3 L) and the mixture was stirred for 15 hours. The solids were collected by filtration and the solids were washed with Et_2O (two portions of 1 L each). The resultant solid was dried to a tan solid, (91.9 g, 89%). Data are: ^{1}H NMR (DMSO, 400 MHz) 10.76 (brs, 1H), 7.88 (d, J = 9.4 Hz, 1H), 7.58 (m, 2H), 7.38 (m, 2H), 7.31 (m, 1H), 7.22 (m, 2H), 7.08 (t, J = 7.8 Hz, 1H), 6.53 (d, J = 9.8 Hz, 1H), 5.30 (s, 2H); MS APCI (+) *m/z* 252.1 (M+1) detected.



8-(Benzyloxy)-2-chloroquinoline (8). 8-(Benzyloxy)quinolin-2-ol (91.9 g, 366 mmol) was slurried in 1,2-dichloroethane (360 mL). Then DMF (0.5 mL) was added followed by the dropwise addition of oxalyl chloride (63.8 mL, 731 mmol). After the addition was complete, the mixture was heated to 85 °C, where it stirred overnight. The mixture was cooled to ambient temperature and concentrated *in vacuo*. The residue was dissolved in dichloromethane and washed with 50% saturated aqueous $NaHCO_3$. The aqueous was washed twice with methylene chloride and the combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo* to a tan solid. The residue was suspended in

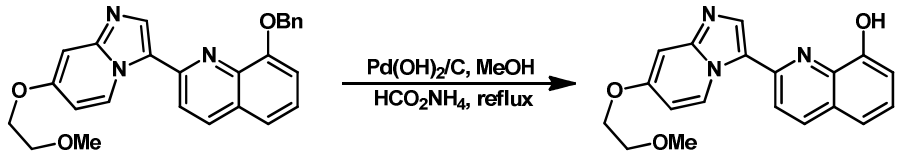
toluene and stirred overnight. The product was collected by filtration, washed with cold toluene, followed by hexanes and dried, providing the desired product as a tan solid (75.3 g).

Additional product was detected in the filtrate which was concentrated *in vacuo* then dissolved in hot toluene (50 mL) and treated with activated carbon. After heating for 20 minutes, the carbon was removed by filtration and concentrated to 25-35 mL. This mixture was allowed to stand in the refrigerator. The solid was then recovered by filtration and the solid was washed with cold toluene and dried to a tan solid, providing an additional 14.6 g (total recovery was 89.9 g, 91%) of the title compound. Data are: ^{1}H NMR ($CDCl_3$, 400 MHz) 8.06 (d, J = 8.6 Hz, 1H), 7.51 (m, 2H), 7.42-7.35 (m, 5H), 7.32-7.28 (m, 1H), 7.06 (m, 1H), 5.45 (s, 2H); MS APCI (+) *m/z* 270.0 (M+1) detected.

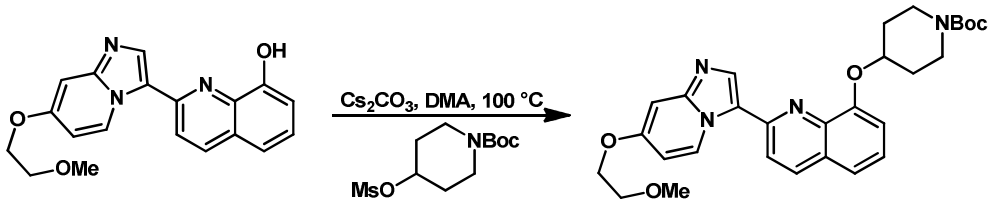


8-(Benzyloxy)-2-(7-(2-methoxyethoxy)imidazo[1,2-a]pyridin-3-yl)quinolone. 8-(Benzyloxy)-2-chloroquinoline (60.17 g, 223.1 mmol), 7-(2-methoxyethoxy)imidazo[1,2-a]pyridine (42.88 g, 223.1 mmol), $Pd(PPh_3)_4$ (12.89 g, 11.15 mmol), $Pd(OAc)_2$ (2.50 g, 11.15 mmol) and K_2CO_3 (92.49 g, 669.2 mmol) were combined and slurried in dioxane (880 mL) and water (8.8 mL). The reaction mixture was purged with argon (vacuum cycling five times) and heated to 100 °C for 60 hours. The mixture was cooled to ambient temperature. The mixture was treated with methylene chloride (750 mL) and with activated charcoal (EM Science, (Darco G-60), 30 g) and stirred for 30 minutes. The mixture was then filtered through celite. The charcoal solids were then resuspended in methylene chloride (1000 mL) and stirred for 30 minutes then filtered, and the combined filtrate was then concentrated *in vacuo*. The resulting material was triturated in 1:1 Ethyl Acetate/hexanes (500 mL) with stirring for 30 minutes then filtered and the remaining tan solids were washed with a small amount of 1:1 Ethyl Acetate/hexanes (two portions of 100 mL each) then dried, providing the title compound as a yellow solid (81.4g, 85%). Data are: ^{1}H NMR ($CDCl_3$, 400 MHz) 10.48 (d, J = 7.4 Hz, 1H), 8.21 (s, 1H), 8.10 (d, J

= 9.0 Hz, 1H), 7.84 (d, *J* = 8.6 Hz, 1H), 7.67 (m, 2H), 7.53-7.37 (m, 5H), 7.19 (dd, *J* = 2.0 Hz, 7.0 Hz, 1H) 6.98 (d, *J* = 2.7 Hz, 1H), 6.49 (dd, *J* = 2.7 Hz, 7.8 Hz, 1H), 5.32 (s, 2H), 4.21 (m, 2H), 3.84 (m, 2H), 3.50 (s, 3H); MS APCI (+) *m/z* 426.2 (M+1) detected.

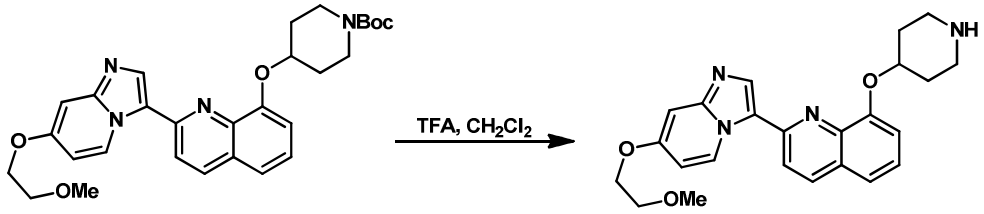


2-(7-(2-Methoxyethoxy)imidazo[1,2-a]pyridin-3-yl)quinolin-8-ol (9). 8-(Benzyloxy)-2-(7-(2-methoxyethoxy)imidazo[1,2-a]pyridin-3-yl)quinolone (56.3 g, 132.3 mmol) was slurried in MeOH (1.3 L). Then ammonium formate (83.44 g, 1,323 mmol) and $Pd(OH)_2/C$ (9.29 g, 6.616 mmol, 10% by wt) were added. The mixture was then heated to reflux where it stirred for 2 hours. The reaction mixture was cooled to ambient temperature and formic acid was added to the slurry until the solids went into solution. The reaction mixture was then filtered and the solids were washed with 100 mL of 10% formic acid in methanol. The golden filtrate was then concentrated to an oil. To the oil was added NH_3 (2.0M methanol) and resulting solids dried *in vacuo*. Water was then added to the solids and the mixture was allowed to stir for 1 hour (pH was 6.5-7.0). The solids were isolated by vacuum filtration and slurried in toluene and concentrated to dryness, providing 43.7g (98%) of the desired product. Data are: ^{1}H NMR (DMSO, 400 MHz) 10.35 (d, *J* = 7.8 Hz, 1H), 9.70 (s, 1H), 8.49 (s, 1H), 8.27 (d, *J* = 9.0 Hz, 1H), 8.09 (d, *J* = 8.6 Hz, 1H), 7.36 (m, 2H), 7.17 (m, 2H), 6.90 (dd, *J* = 2.7 Hz, 7.8 Hz, 1H), 4.27 (m, 2H), 3.74 (m, 2H), 3.35 (s, 3H); MS APCI (+) *m/z* 336.1 (M+1) detected.

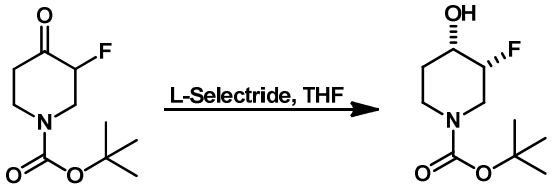


tert-Butyl 4-(2-(7-(2-methoxyethoxy)imidazo[1,2-a]pyridin-3-yl)quinolin-8-yloxy)piperidine-1-carboxylate. To a solution of 2-(7-(2-Methoxyethoxy)imidazo[1,2-a]pyridin-3-yl)quinolin-8-ol (100 mg, 0.30 mmol) in DMA (2 mL) was added cesium

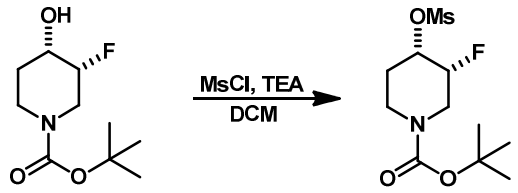
carbonate (291 mg, 0.89 mmol) followed by tert-butyl 4-(methylsulfonyloxy)piperidine-1-carboxylate (125 mg, 0.45 mmol). The heterogeneous mixture was warmed to 100 °C, where it stirred overnight. The mixture was then cooled to ambient temperature and the mixture was treated with water (20 mL) and extracted with EtOAc (3 x 10 mL). The combined organic phases were washed with water (2 x 10 mL) and brine (10 mL) then dried over Na_2SO_4, filtered and concentrated to afford a brown gum. The crude material was then purified via column chromatography (2% MeOH/CH_2Cl_2) to afford the product as a yellow foam (0.099g, 64%).



2-(7-(2-methoxyethoxy)imidazo[1,2-a]pyridin-3-yl)-8-(piperidin-4-yloxy)quinoline bis(2,2,2-trifluoroacetate) (11). To a solution of tert-butyl 4-(2-(7-(2-methoxyethoxy)imidazo[1,2-a]pyridin-3-yl)quinolin-8-yloxy)piperidine-1-carboxylate (126 mg, 0.24 mmol) in CH_2Cl_2 (4 mL) was added TFA (1 mL). The mixture was stirred at ambient temperature for 2 hours after which time an HPLC analysis indicated consumption of starting material. The mixture was then concentrated and dried *in vacuo* overnight. The resulting solid was triturated with ether, filtered and dried *in vacuo* to afford the product salt as a white powder (0.145g 92%). Data are: ^{1}H NMR (CD_3OD, 400 MHz) 10.82 (d, *J* = 7.8 Hz, 1H), 8.75 (s, 1H), 8.41 (d, *J* = 8.6 Hz, 1H), 8.08 (d, *J* = 8.6 Hz, 1H), 7.62-7.56 (m, 2H), 7.42 (m, 2H), 7.35 (dd, *J* = 2.3 Hz, 7.8 Hz, 1H), 5.06 (m, 1H), 4.43 (m, 2H), 3.86 (m, 2H), 3.54 (m, 2H), 3.45 (s, 3H), 3.35 (obs m, 2H), 2.40-2.22 (m, 4H); ^{13}C NMR of freebase material ($CDCl_3$, 125 MHz) δ 32.3, 43.6, 59.3, 67.5, 70.6, 73.8, 95.7, 107.8, 112.2, 118.5, 119.7, 123.4, 125.7, 127.5, 130.1, 135.8, 136.1, 140.1, 148.8, 149.6, 152.9, 157.9; MS APCI (+) *m/z* 419.2 (M+1) detected, HRMS (EI$^+$) found 418.20100 M$^+$, calcd 418.20049 for $C_{24}H_{26}N_4O_3$.

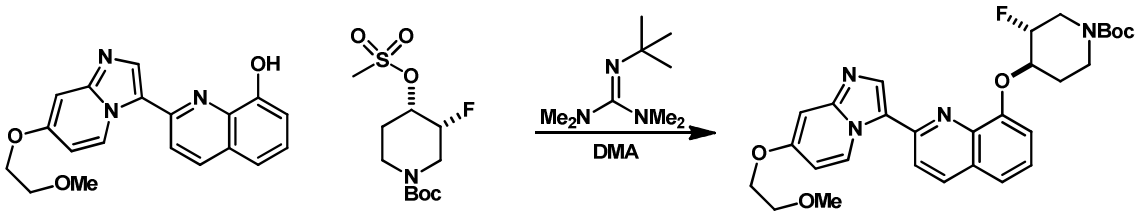


***cis-tert*-butyl 3-fluoro-4-hydroxypiperidine-1-carboxylate.** To a solution of *tert*-butyl 3-fluoro-4-oxopiperidine-1-carboxylate[1] (45.0 g, 207.1 mmol) in THF (600 mL) at 0 ºC was added L-Selectride (270 mL, 1.0 M THF, 270 mmol) via cannula. The mixture was allowed to warm slowly to ambient temperature overnight after which time TLC (hexanes:EtOAc, 1:1, with KMnO4 stain and charring) indicated complete consumption of the starting material. The mixture was transferred to an erlenmeyer flask and treated with methanol (250 mL) followed by 2N NaOH (500 mL). The solution was then cooled to 0 ºC and treated with 30% H_2O_2 (250 mL) from a dropping funnel over ~30 min. The mixture was stirred for an additional 2 hours and then extracted with EtOAc (3 x 400 mL). The combined organic phases were washed with brine (200 mL), dried over Na_2SO_4, filtered and concentrated to afford 37.1g (81%) of a thick yellow syrup. Data are: ^{1}H NMR ($CDCl_3$, 400 MHz) 4.61 (br dt, J = 3.1 Hz, 47.7 Hz, 1H), 4.00-3.83 (m, 2H), 3.81-3.57 (m, 1H), 3.55-3.30 (m, 1H), 3.28-3.08 (m, 1H), 2.01 (br d, J = 5.5 Hz, 1H), 1.88-1.70 (m, 2H), 1.46 (s, 9H).



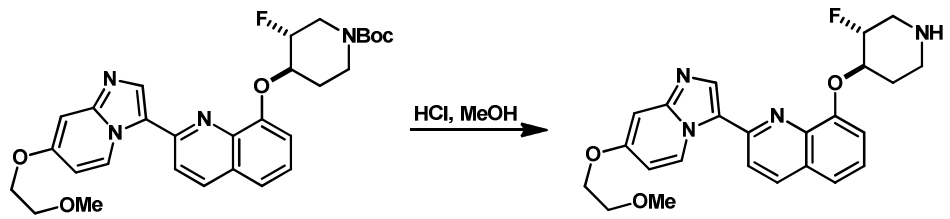
***cis-tert*-butyl 3-fluoro-4-(methylsulfonyloxy)piperidine-1-carboxylate.** *cis-tert*-butyl 3-fluoro-4-hydroxypiperidine-1-carboxylate (5.50 g, 25.09 mmol) was dissolved in dry CH_2Cl_2 (400 mL), then NEt_3 (4.54 mL, 32.6 mmol) was added and the mixture was chilled to 0 ºC. MsCl (2.13 mL, 27.6 mmol) was then added dropwise over a 5 minute period, and then the mixture was removed from the cooling bath. After 3 hours at ambient temperature, TLC (50% EtOAc/hex, with KMnO4 stain and charring) indicated consumption of starting material. The mixture was then diluted with CH_2Cl_2, washed with a saturated aqueous $NaHCO_3$ solution (2 x), dried over Na_2SO_4, filtered and concentrated. The crude product was then purified via column chromatography (1:1

EtOAc/hex) to afford 4.3 g (57%) of an oil that solidified upon standing. Data are: ^{1}H NMR ($CDCl_3$, 400 MHz) 5.00-4.88 (m, 1H), 4.80-4.65 (m, 1H), 3.93-3.27 (m, 4H), 3.09 (s, 3H), 2.19-2.10 (m, 1H), 1.93-1.82 (m, 1H), 1.47 (s, 9H).

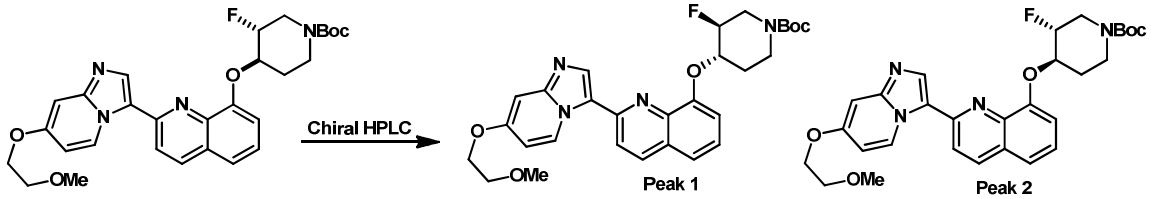


(*trans*)-*tert*-butyl 3-fluoro-4-(2-(7-(2-methoxyethoxy)imidazo[1,2-a]pyridin-3-yl)quinolin-8-yloxy)piperidine-1-carboxylate. 2-(7-(2-methoxyethoxy)imidazo[1,2-a]pyridin-3-yl)quinolin-8-ol (12.7 g, 37.9 mmol) and (*cis*)-*tert*-butyl 3-fluoro-4-(methylsulfonyloxy)piperidine-1-carboxylate (16.9 g, 56.8 mmol) were slurried in DMA (189 ml, 37.9 mmol). To this mixture was added 2-tert-butyl-1,1,3,3-tetramethylguanidine (16.0 mL, 79.5 mmol). The mixture was purged with N2, capped and then warmed to 70 ºC in an oil bath where it stirred for 3 days. The mixture was cooled to ambient temperature and then additional *tert*-butyl 3-fluoro-4-(methylsulfonyloxy)piperidine-1-carboxylate (4.5 g, 15 mmol, 0.4 eq) was added followed by tetramethylguanidine (4 mL, 19.8 mmol, 0.5 eq) and the reaction was again heated to 70 ºC where it stirred for 4 days. The mixture was then concentrated *in vacuo* with heat (65 ºC) and then diluted with $CHCl_3$ (200 mL) and washed with a saturated aqueous NH_4Cl solution (100 mL). The aqueous phase was extracted with 5% *i*-PrOH/$CHCl_3$ (2 x 100 mL) and the combined organic layers were then dried over Na_2SO_4, filtered and concentrated. The crude product was then purified via column chromatography (EtOAc followed by 1 to 5% MeOH/CH_2Cl_2 gradient) to afford the product as an orange foam. The product contained unreacted phenol (5%), the material was then dissolved in EtOAc (200 mL) and was washed with 1N NaOH (2 x 50 mL). The EtOAc layer was then washed with brine and dried over Na_2SO_4, filtered and concentrated. The product was again purified via column chromatography (1 to 5% MeOH/CH_2Cl_2 gradient) to afford the desired product (15.8g, 77%). Data are: ^{1}H NMR ($CDCl_3$, 400 MHz) 10.51 (d, J = 7.8 Hz, 1H), 8.22 (s, 1H), 8.10 (d, J = 8.6 Hz, 1H), 7.84 (d, J = 8.6 Hz, 1H), 7.45-7.37 (m, 2H), 7.21 (d, J = 7.0 Hz, 1H), 7.01 (d, J = 3.1 Hz, 1H), 6.78 (dd, J = 2.3 Hz, 7.8 Hz, 1H), 4.97-4.77 (m, 2H), 4.23 (m, 2H), 3.95 (m, 1H), 3.90

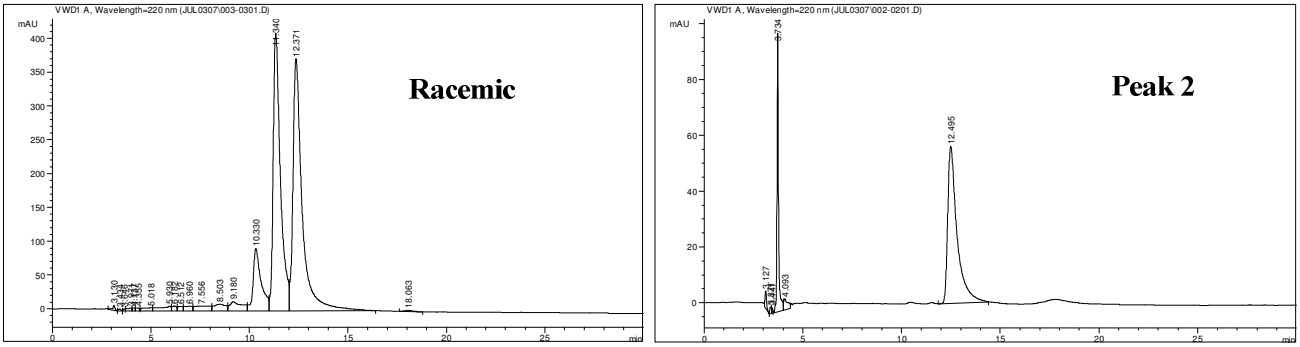
(m, 1H), 3.83 (m, 2H), 3.72-3.61 (m, 2H), 3.50 (s, 3H), 2.27-2.17 (m, 1H), 2.03-1.94 (m, 1H), 1.51 (s, 9H); MS APCI (+) *m/z* 537.1 (M+1) detected.

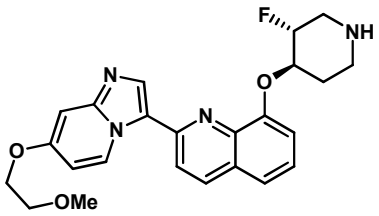


8-(*trans*)-3-fluoropiperidin-4-yloxy)-2-(7-(2-methoxyethoxy)imidazo[1,2-a]pyridin-3-yl)quinolone (*trans*-27). To a mixture of (*trans*)-*tert*-butyl 3-fluoro-4-(2-(7-(2-methoxyethoxy)imidazo[1,2-a]pyridin-3-yl)quinolin-8-yloxy)piperidine-1-carboxylate (15.8 g, 29.4 mmol) and MeOH (147 mL) was added HCl (147 mL, 589 mmol, 4.0M Dioxane) and the solution was stirred at RT for 3 hours. The resulting mixture was then concentrated *in vacuo* and dried to an off-white solid. The solid was made the freebase by dissolving in water and then adding saturated aqueous $NaHCO_3$ until basic, forming a sticky orange precipitate. The sticky solid was broken up using minimal MeOH and water. The mixture was then extracted with CHCl3 (3 x 200 mL) and the combined organic layers were then dried over Na_2SO_4, filtered and concentrated. The product was then purified via column chromatography (2 to 10% MeOH/CH_2Cl_2 gradient) providing the product as a pale yellow foam (8.95g, 69%). Data are: ^{1}H NMR ($CDCl_3$, 400 MHz) 10.60 (d, *J* = 8.2 Hz, 1H), 8.22 (s, 1H), 8.09 (d, *J* = 8.6 Hz, 1H), 7.83 (d, *J* = 9.0 Hz, 1H), 7.42-7.36 (m, 2H), 7.21 (dd, *J* = 2.0 Hz, 6.7 Hz, 1H), 7.01 (d, *J* = 2.7 Hz, 1H), 6.82 (dd, *J* = 2.7 Hz, 7.8 Hz, 1H), 4.90-4.71 (m, 2H), 4.24 (m, 2H), 3.83 (m, 2H), 3.56-3.46 (obs m, 1H), 3.49 (s, 3H), 3.23-3.16 (m, 1H), 3.08-3.00 (m, 1H), 2.85-2.79 (m, 1H), 2.25-2.18 (m, 1H), 1.97-1.89 (m, 1H), 1.61 (br s, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 29.1, 42.1, 47.43, 59.27, 67.4, 70.5, 75.00, 88.5, 95.5, 107.9, 112.9, 118.6, 120.7, 123.2, 125.6, 127.4, 129.8, 135.9, 136.1, 139.8, 148.9, 149.6, 152.5, 157.9; ^{19}F ($CDCl_3$, 376 MHz) -58.3; HRMS (EI$^+$) found 436.19111 M^+, calcd 436.19107 for $C_{24}H_{25}FN_4O_3$.



(3R,4R)-tert-butyl 3-fluoro-4-(2-(7-(2-methoxyethoxy)imidazo[1,2-a]pyridin-3-yl)quinolin-8-yloxy)piperidine-1-carboxylate (*Absolute Stereochemistry Arbitrarily Assigned*). (*trans*)-*tert*-butyl 3-fluoro-4-(2-(7-(2-methoxyethoxy)imidazo[1,2-a]pyridin-3-yl)quinolin-8-yloxy)piperidine-1-carboxylate (0.050g, 0.093 mmol) was separated by chiral HPLC [Chiral Tech. IA, 5% MeOH/15% EtOH/80% hexanes, 5 mL/min, 23 ºC, λ= 220 nm, retention times: (Peak 1) 11.3 min, (Peak 2) 12.3 min] to afford the title compound (Peak 2, 0.019g, 38%, 99% ee) as a colorless film.





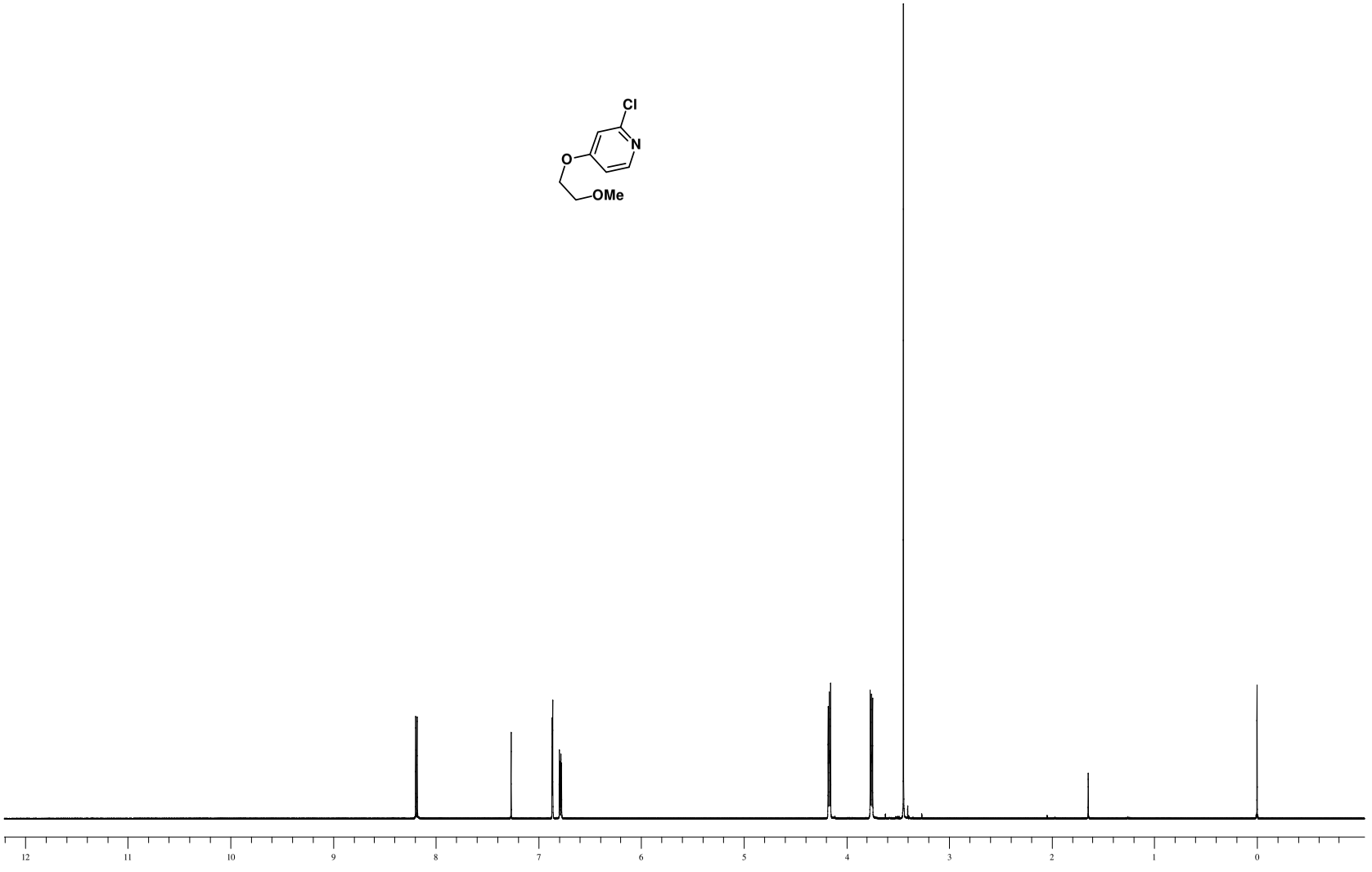
8-(((3R,4R)-3-fluoropiperidin-4-yl)oxy)-2-(7-(2-methoxyethoxy)imidazo[1,2-a]pyridin-3-yl)quinolone bis(2,2,2-trifluoroacetate) (36, *Absolute Stereochemistry Arbitrarily Assigned*). Prepared from (3R,4R)-tert-butyl 3-fluoro-4-(2-(7-(2-methoxyethoxy)imidazo[1,2-a]pyridin-3-yl)quinolin-8-yloxy)piperidine-1-carboxylate as described above for Compound **11**. NMR data is the same as that seen for ***trans*-27**; HRMS (EI^+) found 436.19127 M^+, calcd 436.19107 for $C_{24}H_{25}FN_4O_3$.

References

1. van Niel, M. B.; Collins, I.; Beer, M. S.; Broughton, H. B.; Cheng, S. K. F.; Goodacre, S. C.; Heald, A.; Locker, K. L.; MacLeod, A. M.; Morrison, D.; Moyes, C. R.; O'Connor, D.; Pike, A.; Rowley, M.; Russell, M. G. N.; Sohal, B.;

Stanton, J. A.; Thomas, S.; Verrier, H.; Watt, A. P.; Castro, J. L. *J. Med. Chem.* **1999**, *42*, 2087-2104.

NMR Data: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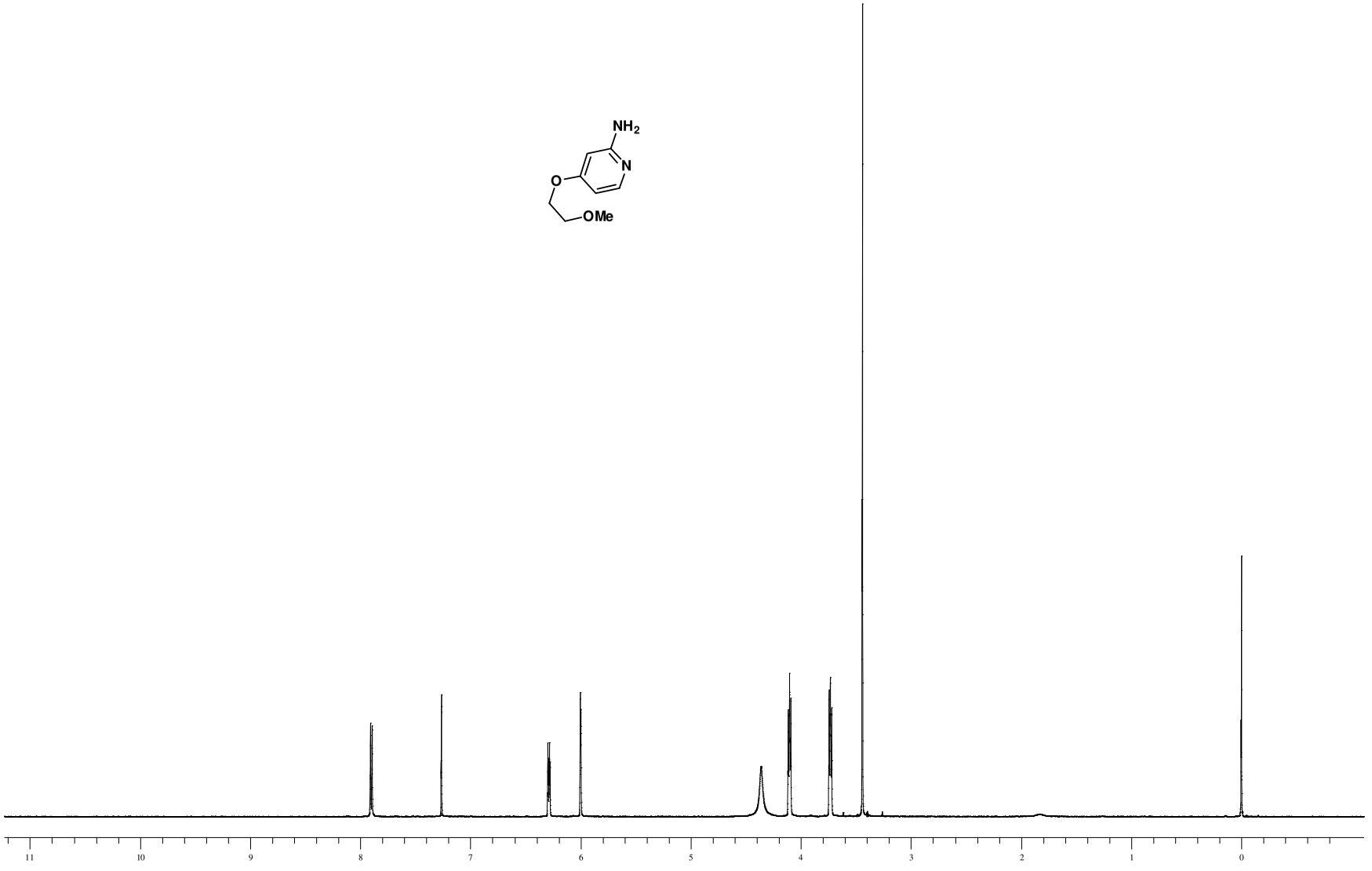
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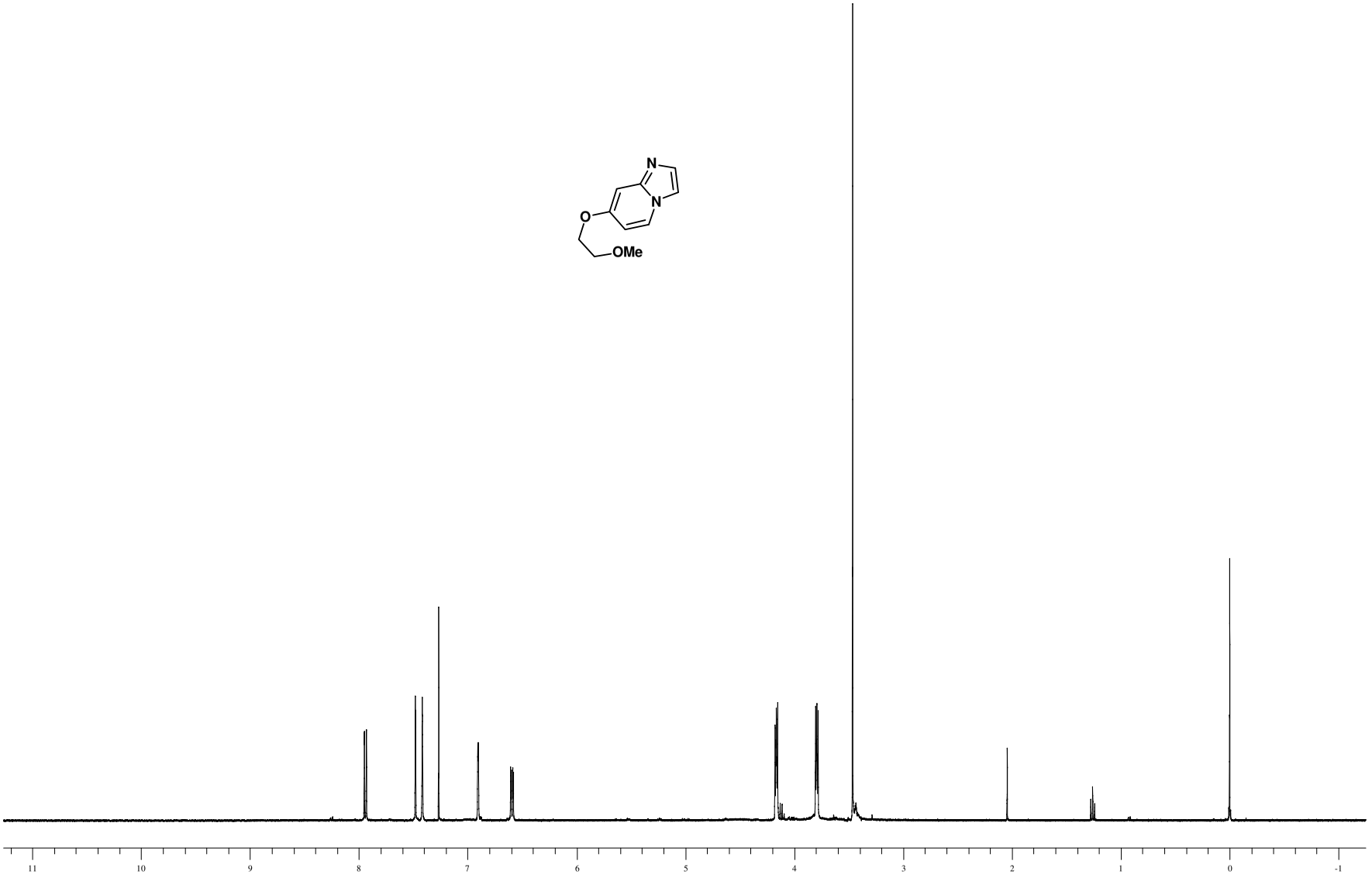
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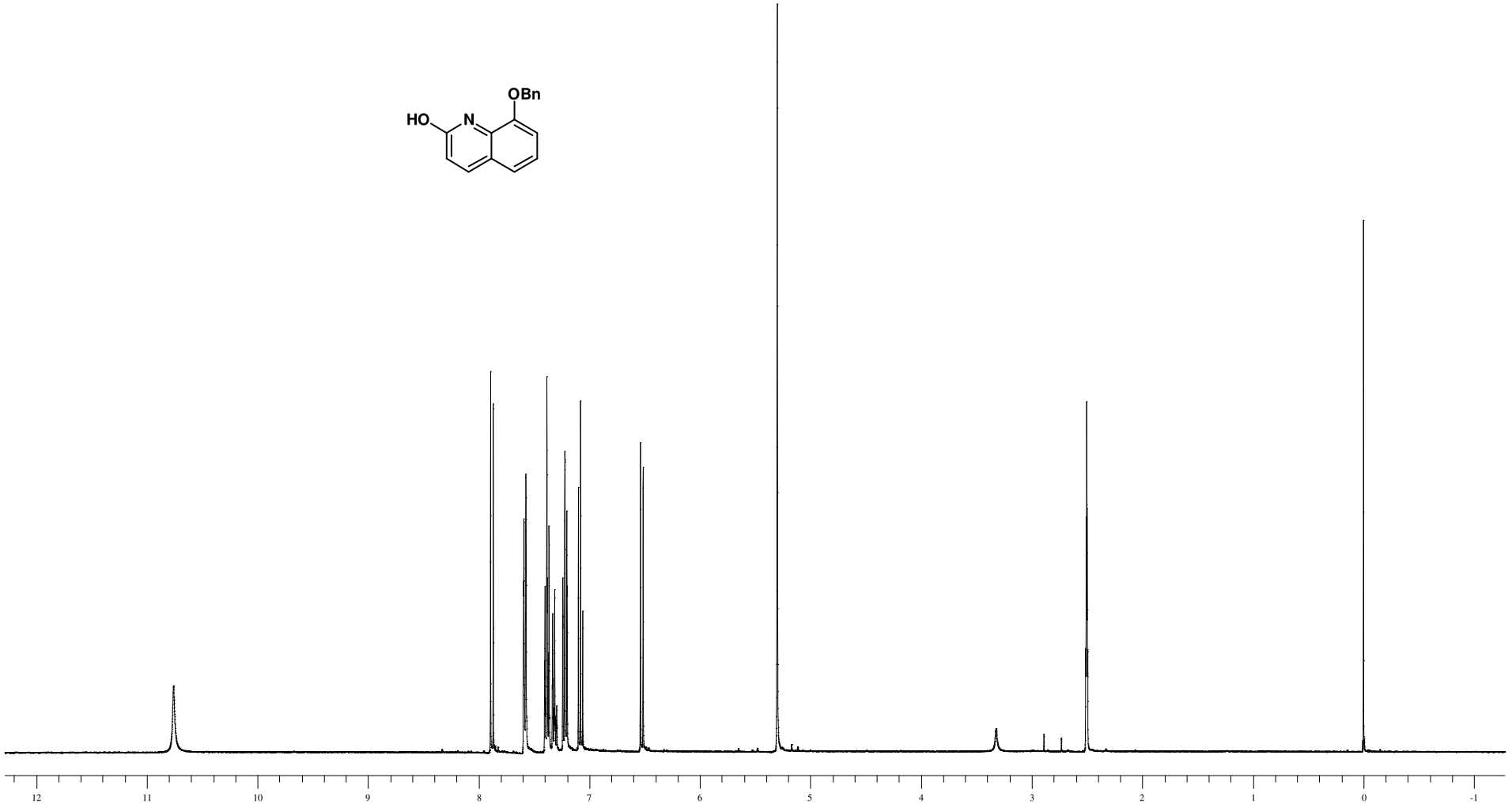
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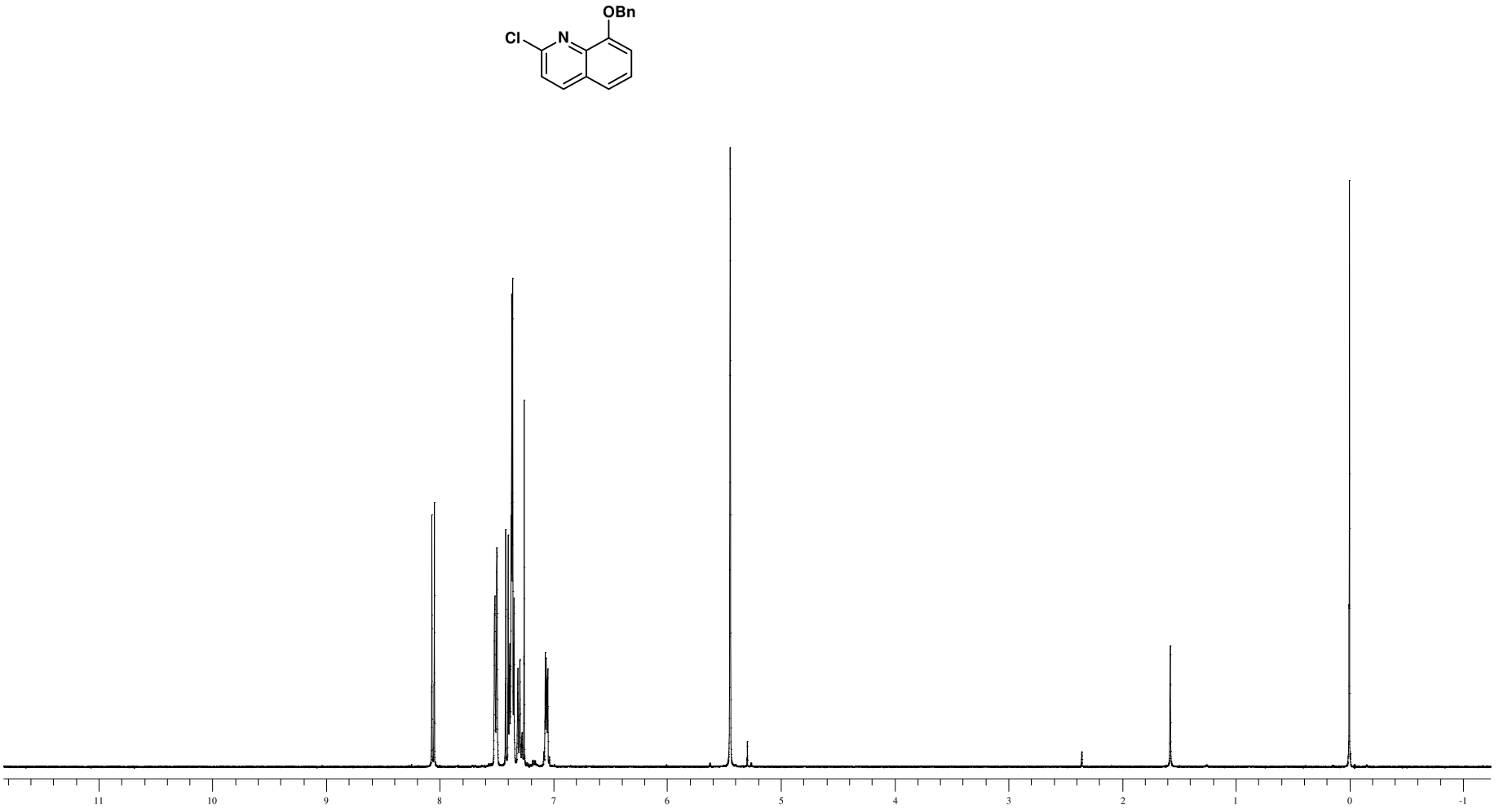
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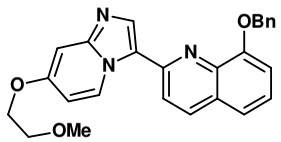
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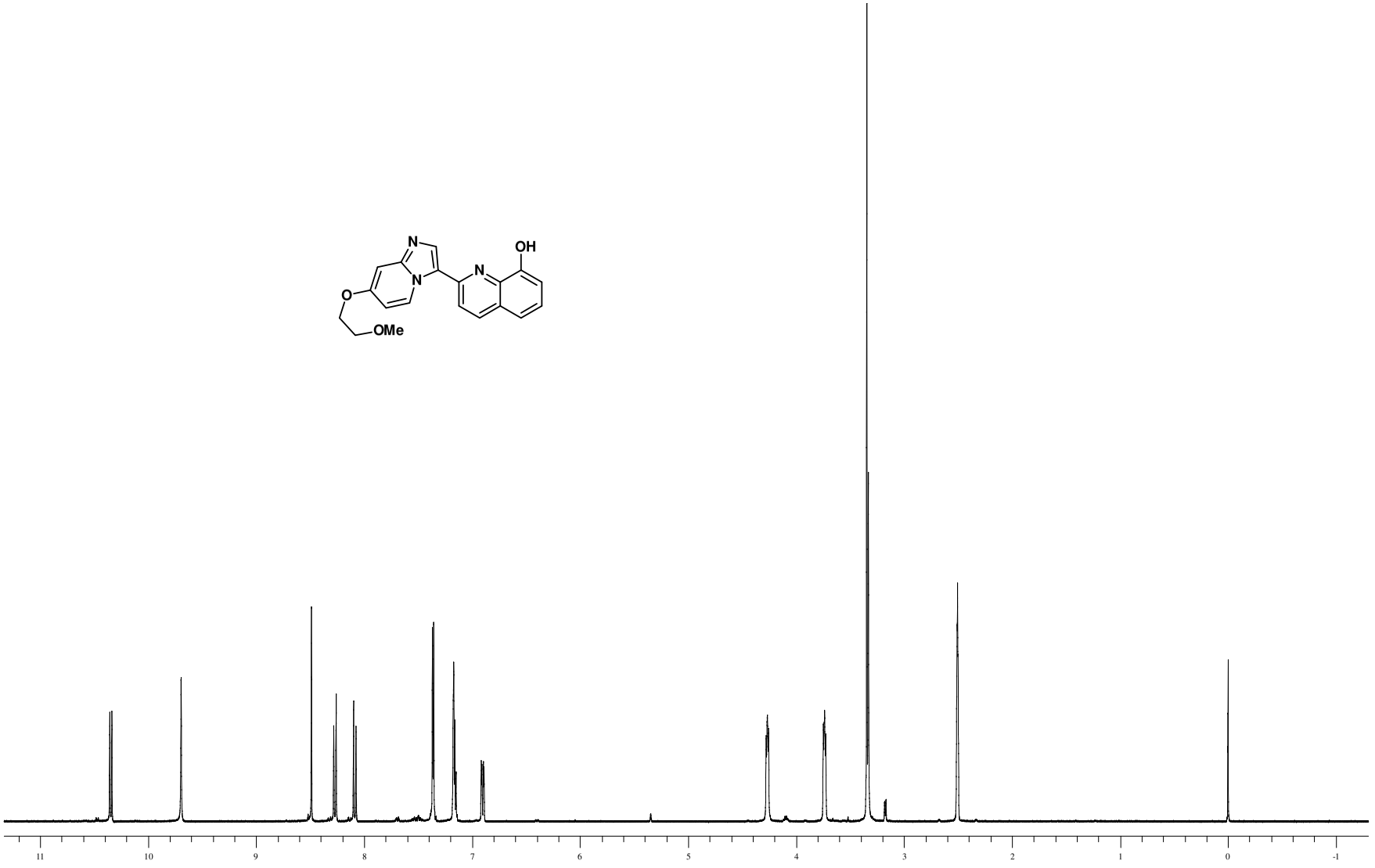
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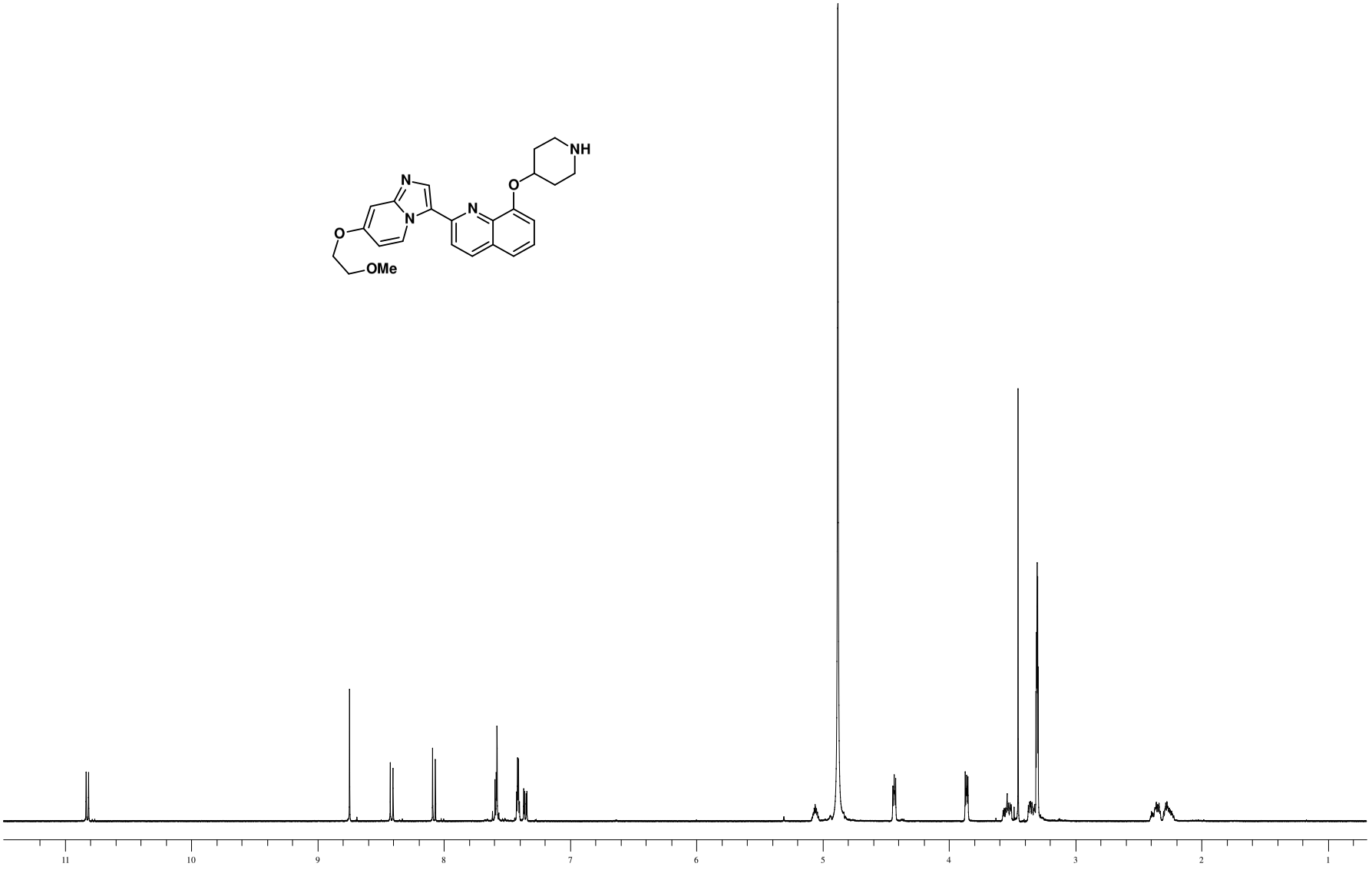
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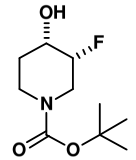
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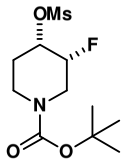
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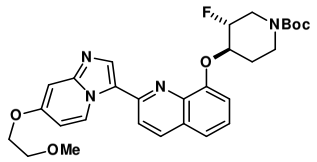
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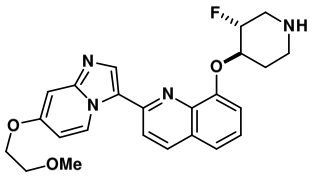
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