Synthetic Methods

Abbreviations:

AcOH	acetic acid
ACN	acetonitrile
app	apparent
ATP	adenosine 5'-triphosphate
BI-DIME	3-(tert-butyl)-4-(2,6-dimethoxyphenyl)-2,3-dihydrobenzo[d][1,3]oxaphosphole
BISPIN	4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane)
BOC	tert-butyloxycarbonyl
br	broad
Bu	butyl
CDI	carbonyldiimidazole
d	doublet
dd	doublet of doublets
DCE	dichloroethane
DCM	dichloromethane
DIPEA	diisopropylethylamine
DMA	dimethylacetamide
DMAP	4-dimethylaminopyridine
DME	1,4-dimethoxyethane
DMF	N,N-dimethylformamide
DMSO	dimethylsulfoxide
ESI	electrospray ionization
Et	ethyl
EtOAc	ethyl acetate
h	hour(s)
HATU	1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate
HBTU	1-[bis(dimethylamino)methylene]-1H-benzotriazoliumhexafluorophosphate(1-) 3-oxide
HOBt	1-hydroxy-7-azabenzotriazole
HPLC	high pressure liquid chromatography
LCMS	liquid chromatography and mass spectrometry
MeCN	acetonitrile
MeOH	methanol
MHz	mega hertz
MS	mass spectrometry

MW	microwave
m	multiplet
mg	milligram
min	minutes
ml	milliliter
mmol	millimol
m/z	mass to charge ratio
NBS	N-bromosuccinimide
NMR	nuclear magnetic resonance
o/n	overnight
$PdCl_2$(dppf)	1,1'-Bis(diphenylphosphino)ferrocene-palladium(II)dichloride
$Pd(OAc)_2$	palladium(II) acetate
Pd/C	palladium on carbon
Ph	phenyl
ppm	parts per million
rac	racemic
RBF	round bottom flask
Rt	retention time
RT	room temperature
s	singlet
sat.	saturated
SCX	strong cation exchange sorbent column
SM	starting material
t	triplet
TBME	methyl tert-butyl ether
tBu	tertiary butyl
TEA	triethylamine
tert	tertiary
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography

Purification

Purification of intermediates and final products was carried out via either normal or reverse phase chromatography. Normal phase chromatography was carried out using prepacked SiO_2 cartridges (e.g., RediSep® Rf columns from Teledyne Isco, Inc.) eluting with gradients of appropriate solvent systems (heptane and ethyl acetate, or DCM and MeOH, unless otherwise indicated). Reverse phase preparative HPLC was carried out using the methods described below:

(1) Basic method: XBridge 5μm column, 5 mM NH_4OH in acetonitrile and water.

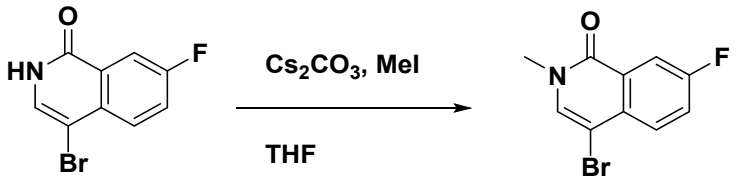
(2) TFA method: Sunfire 5μm column, 0.1% TFA in acetonitrile and water.

(3) Formic acid method: XBridge 5μm column; 0.1% formic acid in acetonitrile and water.

All of the above three HPLC methods run a focused gradient from the starting % acetonitrile to the final % acetonitrile. The initial and final conditions for each gradient are as follows: Method 0: 2-12% acetonitrile; Method 1: 7.5-20% acetonitrile; Method 2: 10-30% acetonitrile; Method 3: 15-40% acetonitrile; Method 4: 25-50% acetonitrile; Method 5: 35-60% acetonitrile; Method 6: 45-70% acetonitrile; Method 7: 55-80% acetonitrile; Method 8: 65-95% acetonitrile; and Method 9: 5-95% acetonitrile.

General Synthetic Schemes

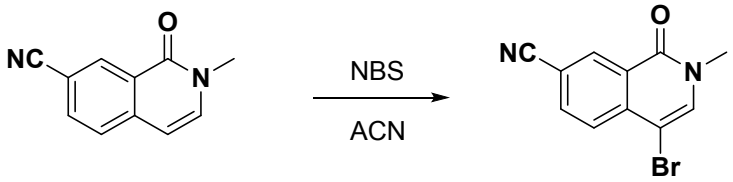
General Procedure for Methylation



To a stirring solution of cesium carbonate (4.04 g, 12.39 mmol) in THF (Volume: 41.3 ml) was added 4-bromo-7-fluoroisoquinolinone (1 g, 4.13 mmol). The reaction was sonicated vigorously, after which time iodomethane (0.310 ml, 4.96 mmol) was added dropwise and the reaction was allowed to stir at RT o/n.

The following morning, the reaction was concentrated by rotary evaporation to remove THF. The crude material was diluted with water and extracted with EtOAc. The organics were washed 3x with water, then brine, then filtered over a bed of magnesium sulfate and concentrated to afford the product as an off-yellow solid (950 mg, 3.71 mmol, 90% yield).

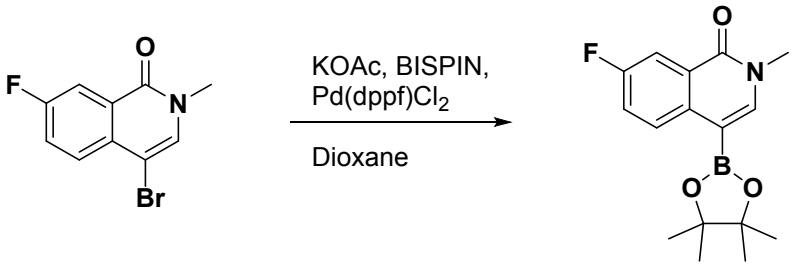
General Procedure for Bromination



NBS (276 mg, 1.553 mmol) and isoquinolinone (260 mg, 1.412 mmol) were suspended in acetonitrile (0.1 M) in a 50mL RBF equipped with a stir bar and stirred at RT o/n.

The following morning, the reaction was concentrated to a solid, re-suspended in EtOAc and filtered to remove the succinimide byproduct. The organic layer was concentrated to afford the desired product as a cream solid (268 mg, 1.019 mmol, 72.2% yield).

General Procedure for Borylation

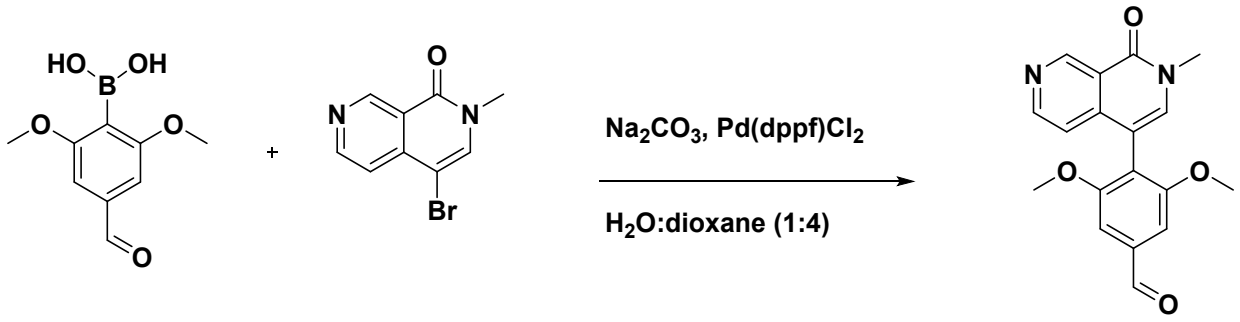


Isoquinolinone (500 mg, 1.93 mmol), BISPIN (744 mg, 2.93 mmol), potassium acetate (479 mg, 4.88 mmol) and $Pd(dppf)Cl_2$ (286 mg, 0.391 mmol) were suspended in dioxane (0.1 M) in a 20 mL pressure relief vial under N_2. The mixture (a brown-orange suspension) was stirred at 90°C (suspension became

darker in color with heat) o/n.

The following morning, LC reveals conversion to the desired borane species. The reaction was diluted with DCM and washed 3x with sat. sodium bicarbonate. The organic layers were combined, passed through a bed of sodium sulfate, and concentrated to a brown oil. Assume quantitative yield, this material was used directly without further manipulation.

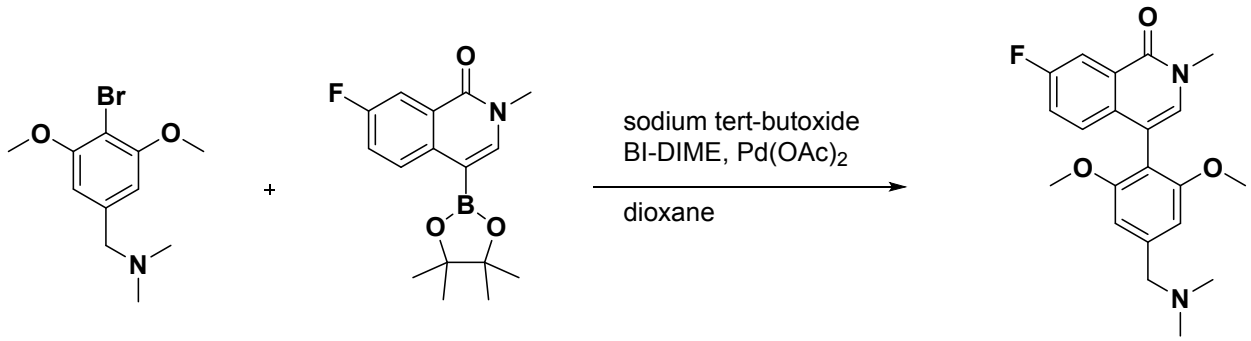
General Procedure for Cross-Coupling – ROUTE A



$Pd(dppf)Cl_2$ (30.6 mg, 0.042 mmol), sodium carbonate (66.5 mg, 0.627 mmol), napthyridinone (50 mg, 0.209 mmol) and 4-formyl-2,6-dimethoxy phenylboronic acid (43.9 mg, 0.209 mmol) were suspended in 4:1 dioxane/water (0.1 M) in a 2 mL MW vial. The reaction was sealed and heated in a MW at 160 °C for 5 min.

The reaction was concentrated to a solid, suspended in a small volume (1 mL) of 1:1 ACN/water and filtered. The crude material was purified by HPLC (basic, method 2, Rt 4.45) to afford the desired product as a brown solid (18 mg, 0.055 mmol, 26.5% yield).

General Procedure for Cross-Coupling – ROUTE B

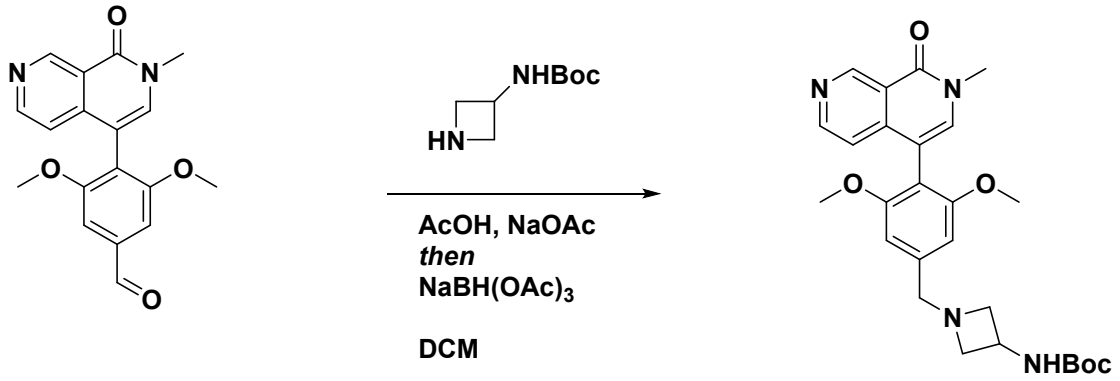


$Pd(OAc)_2$ (2.457 mg, 10.94 µmol), sodium tert-butoxide (31.5 mg, 0.328 mmol), BI-DIME ligand (7.23 mg, 0.022 mmol), isoquinolinone (33.2 mg, 0.109 mmol) and bromo dimethylamine (30 mg, 0.109 mmol) were suspended in dioxane (0.1 M) in a 2 mL MW vial. The reaction was sealed and heated in a MW at 160 °C for 1 hr.

The reaction was quenched by the addition of sodium bicarbonate (2 x 5 mL) and the crude material was extracted 3 times with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and

concentrated to a brown oil. The crude material was purified by HPLC (acidic, method 2, Rt 2.55) to afford the desired product as a white solid (6.7 mg, 0.017 mmol, 15.7% yield)

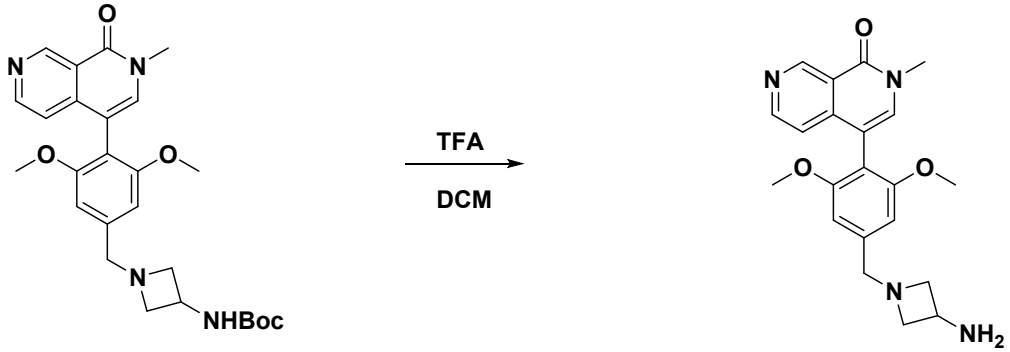
General Procedure for Reductive Aminations



In a 4 mL pressure relief vial, a mixture of sodium acetate (6.37 mg, 0.078 mmol), acetic acid (3.18 µl, 0.055 mmol), 3-N-Boc-amino azetidine (13.38 mg, 0.078 mmol), and aldehyde (18 mg, 0.055 mmol) in DCM (0.1 M) were stirred at 0° C for 30 minutes under a stream of nitrogen gas. Then, sodium triacetoxyborohydride (23.52 mg, 0.111 mmol) was added in one portion and the reaction mixture was stirred at RT o/n.

The following morning, saturated $NaHCO_3$ solution was added and the layers were separated. The aqueous layer was extracted 3x with DCM. The combined organic layers were dried with Na_2SO_4 and concentrated onto a bed of celite. The material was purified by ISCO (0-10% MeOH in DCM) to afford the target compound as a light brown solid (14 mg, 0.029 mmol, 52.5% yield).

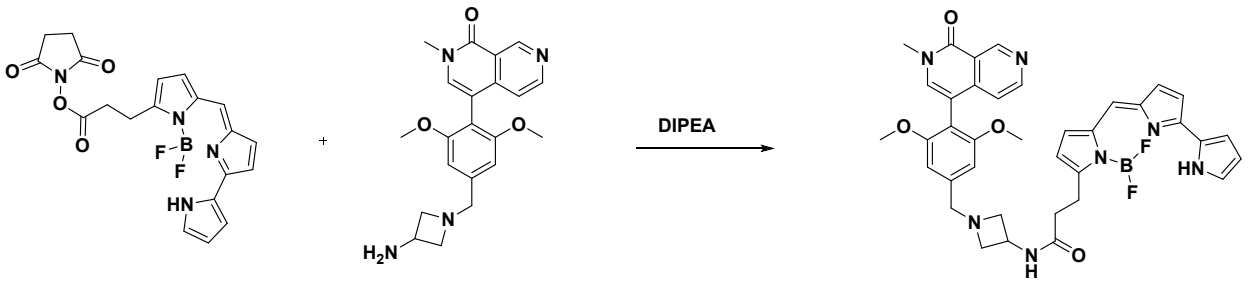
General Procedure for BOC-deprotection



To a stirring solution of BOC-protected amine (14 mg, 0.029 mmol) in DCM (0.1 M) at RT was added TFA (33.7 µl, 0.437 mmol). The reaction was allowed to stir at RT o/n.

The following morning, the crude reaction was concentrated to an oil, re-suspended in 1 mL of 1:1 ACN:water and was purified by HPLC (basic, method 2, Rt 3.12). The product fractions were combined and concentrated to afford the target as a white solid (7.3 mg, 0.019 mmol, 65.2% yield).

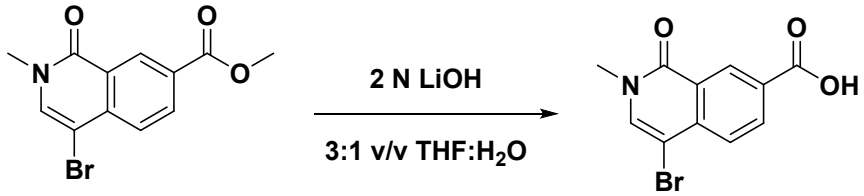
General Procedure for Amide Couplings



To a solution of azetidine amine (10 mg, 0.026 mmol) in DCM (0.1 M) at RT was added DIPEA (8.17 µl, 0.047 mmol) and stirred for five minutes. Then BODIPY dye (5 mg, 0.012 mmol) was added and the reaction stirred for 2.5 hr.

Upon complete consumption of SM by LCMS, the crude material was concentrated to a solid, resuspended in ACN, and purified by HPLC (basic, method 4, Rt 3.80). The product fractions were combined and concentrated to afford the target compound as a purple solid (1.9 mg, 0.00261 mmol, 22.3% yield).

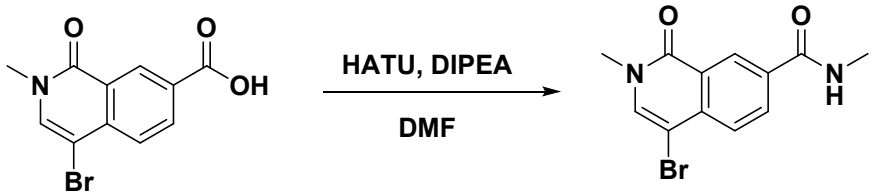
General Procedure for Saponification



To the methyl ester (150 mg, 0.507 mmol) in 3:1 solution of THF/water (0.1 M) vortexing at RT was added LiOH dropwise (507 uL, 1.013 mmol). The reaction stirred overnight.

The following morning, LC revealed consumption of SM to the desired product. The crude material was concentrated to a solid and used directly on the subsequent steps, assuming quantitative yield of the lithium salt.

General Procedure for HATU-mediated Amidation

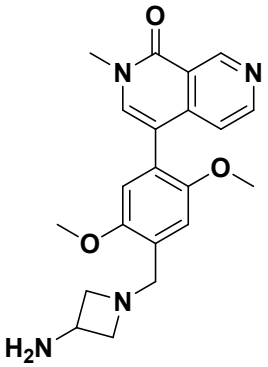


To a solution of carboxylic acid (143 mg, 0.507 mmol) in DMF (0.25 M) at RT was added DIPEA (328 mg, 2.54 mmol), and amine (68 mg, 1.014 mmol) and the reaction stirred for five minutes. Then HATU (231 mg, 0.608 mmol) was added and the reaction stirred for an additional 2.5 hr.

When LCMS revealed complete consumption of the acid, the crude material was concentrated onto a bed

of celite and purified by ISCO (0-10% MeOH in DCM). The desired fractions were combined and concentrated to afford the target compound as a brown solid (27.6 mg, 0.093 mmol, 18.3% yield).

Compound Synthesis and Characterization



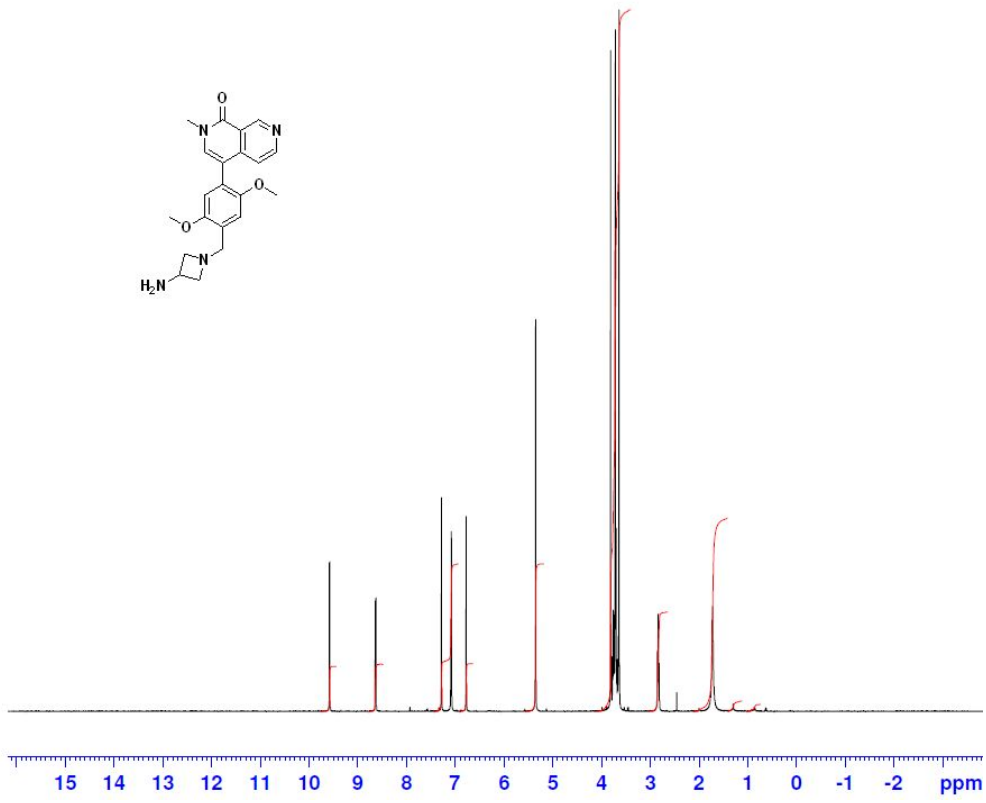
4-(4-((3-aminoazetidin-1-yl)methyl)-2,5-dimethoxyphenyl)-2-methyl-2,7-naphthyridin-1(2H)-one

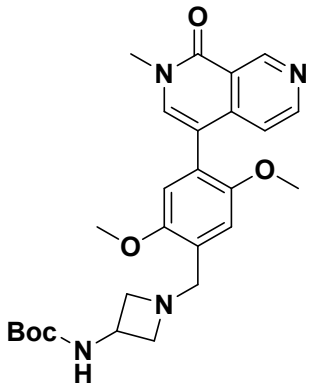
59.9%, 0.071 mmol, 27.3 mg

Compound 1

1H NMR (400 MHz, Methylene Chloride-d2) δ 9.59 (d, J = 0.9 Hz, 1H), 8.64 (d, J = 5.6 Hz, 1H), 7.29 (s, 1H), 7.09 (dd, J = 4.5, 1.0 Hz, 2H), 6.78 (s, 1H), 3.82 (s, 3H), 3.72 (s, 3H), 3.65 (s, 4H), 2.84 (t, J = 6.9 Hz, 2H), 1.73 (s, 4H).

[M+H] = 381.0, Rt = 0.64 min



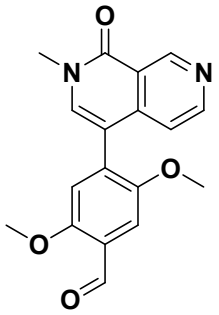


tert-butyl (1-(2,5-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzyl)azetidin-3-yl)carbamate

Intermediate 1D

1H NMR (400 MHz, CHLOROFORM-d) δ ppm 1.46 (s, 9 H) 2.76 - 4.03 (m, 17 H) 6.74 (s, 1 H) 6.99 - 7.08 (m, 2 H) 7.24 (s, 1 H) 8.65 (d, J=5.81 Hz, 1 H) 9.68 (d, J=0.76 Hz, 1 H)

[M+H]: 481.4, Rt = 0.90 min

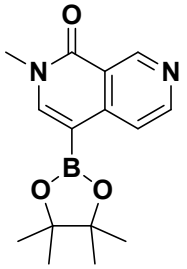


2,5-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzaldehyde

Intermediate 1C

1H NMR (400 MHz, CHLOROFORM-d) δ ppm 3.69 (s, 3 H) 3.76 (s, 3 H) 3.94 (s, 3 H) 6.93 (s, 1 H) 7.04 (d, J=5.56 Hz, 1 H) 7.29 (s, 1 H) 7.48 (s, 1 H) 8.67 (d, J=5.56 Hz, 1 H) 9.70 (s, 1 H) 10.52 (s, 1 H)

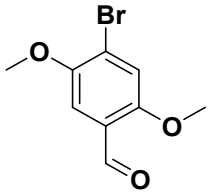
[M+H]: 325.1, Rt = 0.73 min



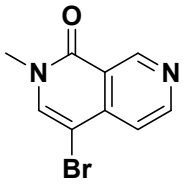
2-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2,7-naphthyridin-1(2H)-one

Intermediate 1B

[M+H]: 287.0, Rt = 0.73 min



Commercially available (CAS 31558-40-4); 4-Bromo-3,5-dimethoxybenzaldehyde

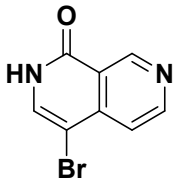


4-bromo-2-methyl-2,7-naphthyridin-1(2H)-one

Intermediate 1A

1H NMR (400 MHz, CHLOROFORM-d) δ ppm 3.64 (s, 3 H) 7.57 (s, 1 H) 7.62 (d, J=5.56 Hz, 1 H) 8.87 (d, J=5.56 Hz, 1 H) 9.62 (s, 1 H)

[M+H]: 239.1, Rt = 0.54 min



Commercially available (CAS 3951-95-9); 4-Bromo-1(2H)-isoquinoline

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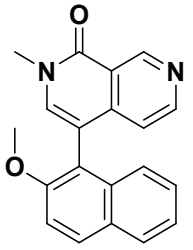


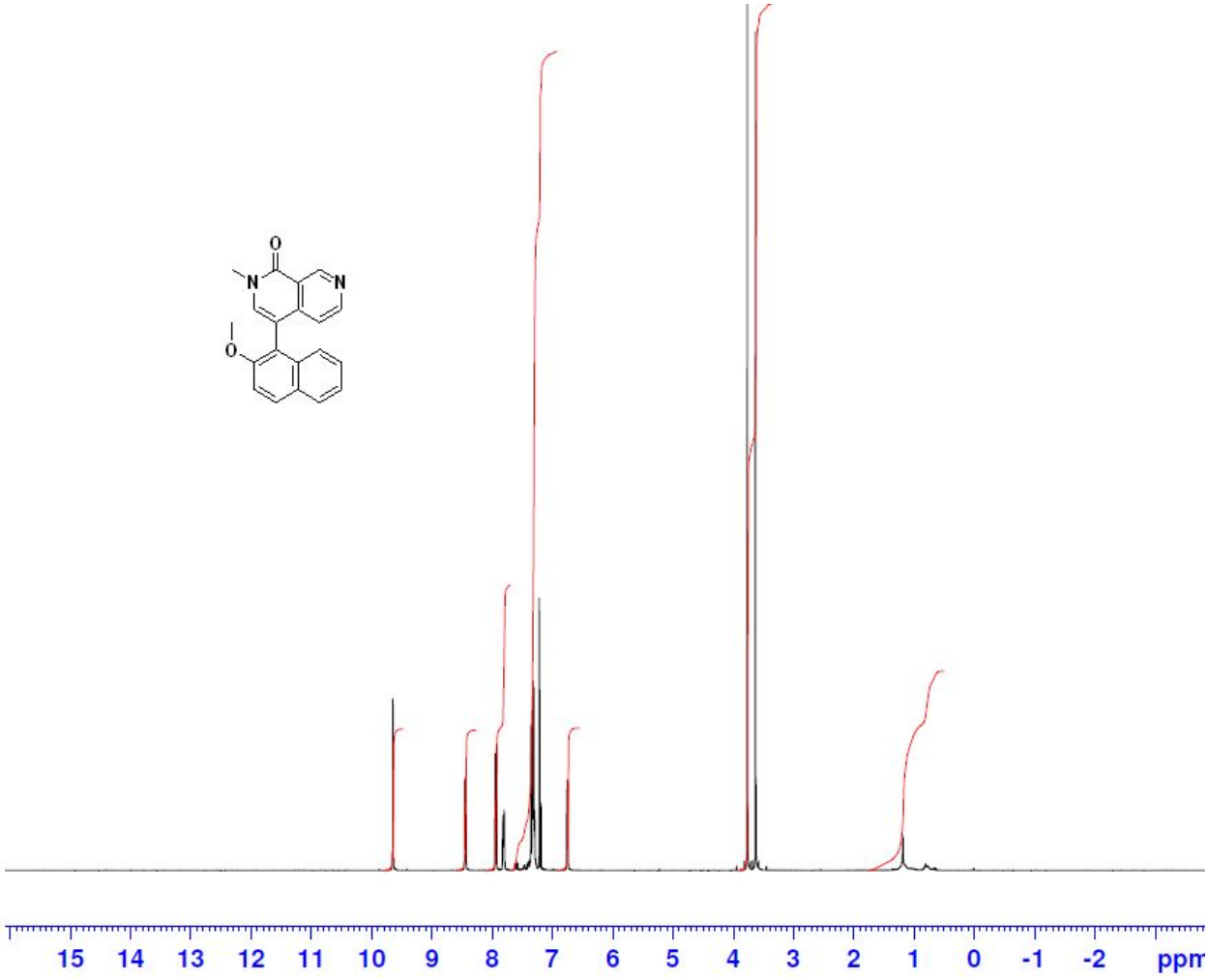
4-(2-methoxynaphthalen-1-yl)-2-methyl-2,7-naphthyridin-1(2H)-one

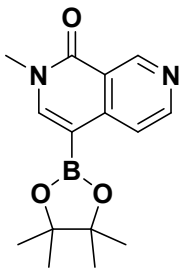
43.0%, 0.043 mmol, 13.6 mg

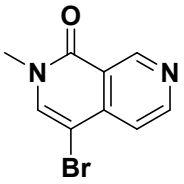
Compound 2

^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.64 (s, 1H), 8.44 (d, *J* = 5.7 Hz, 1H), 7.93 (d, *J* = 9.0 Hz, 1H), 7.86 – 7.77 (m, 1H), 7.39 – 7.27 (m, 4H), 7.21 (s, 1H), 6.75 (d, *J* = 5.6 Hz, 1H), 3.76 (s, 3H), 3.62 (s, 3H).

[M+H] = 317.2, Rt = 0.90 min

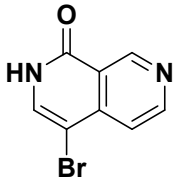




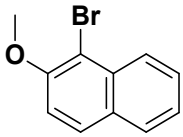


Intermediates 1A + 1B

See above



Commercially available (CAS 3951-95-9); 4-Bromo-1(2H)-isoquinoline



Commercially available (CAS 3401-47-6); 1-Bromo-2-methoxynapthalene

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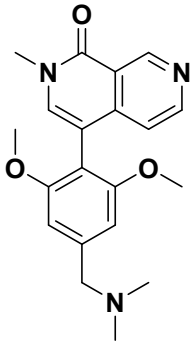


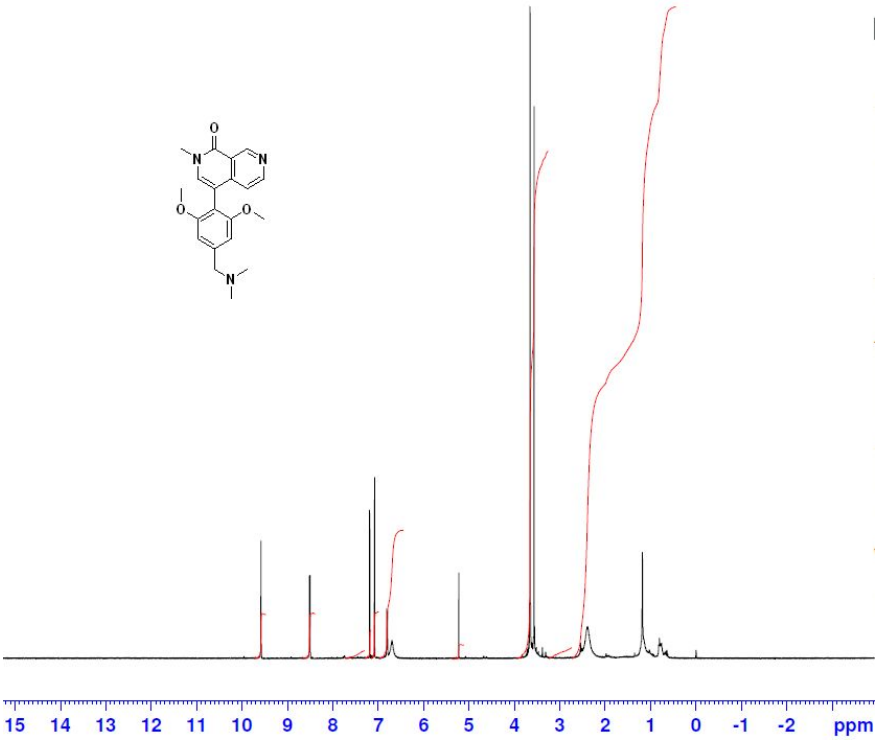
4-(4-((dimethylamino)methyl)-2,6-dimethoxyphenyl)-2-methyl-2,7-naphthyridin-1(2H)-one

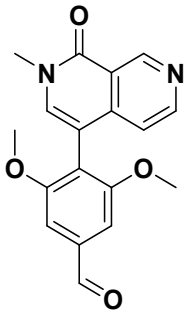
43.0%, 0.043 mmol, 13.6 mg

Compound 3

^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.59 (d, *J* = 0.9 Hz, 1H), 8.51 (d, *J* = 5.6 Hz, 1H), 7.09 (s, 1H), 6.81 (d, *J* = 5.6 Hz, 1H), 6.70 (s, 2H), 3.75 – 3.47 (m, 11H), 2.47 (d, *J* = 60.0 Hz, 6H).

[M+H] = 354.2, Rt = 0.75 min

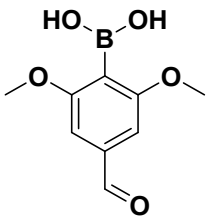




Intermediate 3A

3,5-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzaldehyde

[M+H]: 325.0, Rt = 0.58 min



Commercially available (CAS 1256355-34-6); 2,6-Dimethoxy-4-forymlphenylboronic acid

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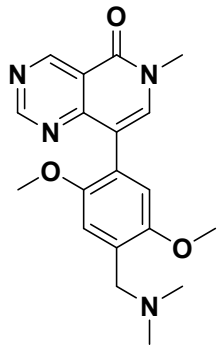


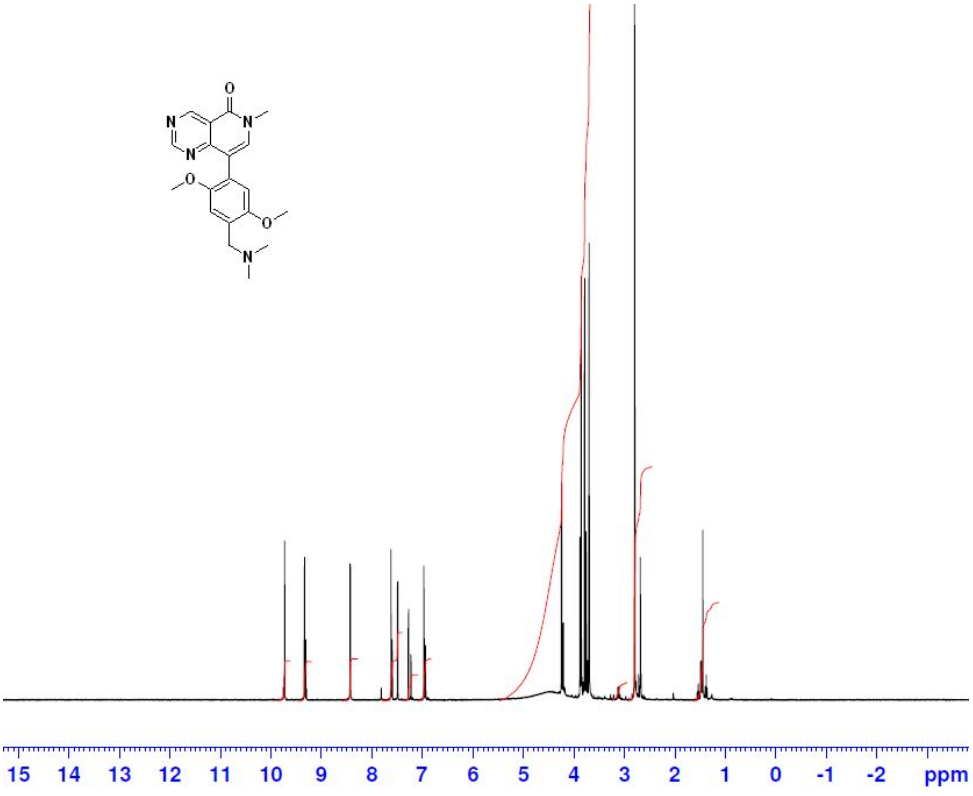
8-(4-((dimethylamino)methyl)-2,5-dimethoxyphenyl)-6-methylpyrido[4,3-d]pyrimidin-5(6H)-one

11.5%, 0.011 mmol, 3.3 mg

Compound 4

^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.74 (d, *J* = 1.2 Hz, 1H), 9.33 (d, *J* = 9.9 Hz, 1H), 8.44 (s, 1H), 7.62 (d, *J* = 10.4 Hz, 1H), 6.96 (d, *J* = 13.0 Hz, 1H), 4.25 (s, 2H), 3.87 (d, *J* = 9.8 Hz, 3H), 3.78 (d, *J* = 12.5 Hz, 3H), 3.70 (d, *J* = 2.6 Hz, 3H), 2.74 (d, *J* = 45.3 Hz, 6H).

[M+H] = 355.2, Rt = 0.63 min



See patent (WO2016/139361 A1; EP3265453 A1; US2018/44335 A1; JP2018/507238 A) for intermediate analysis.

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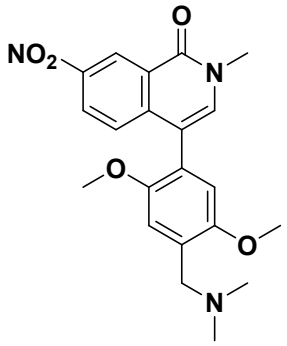


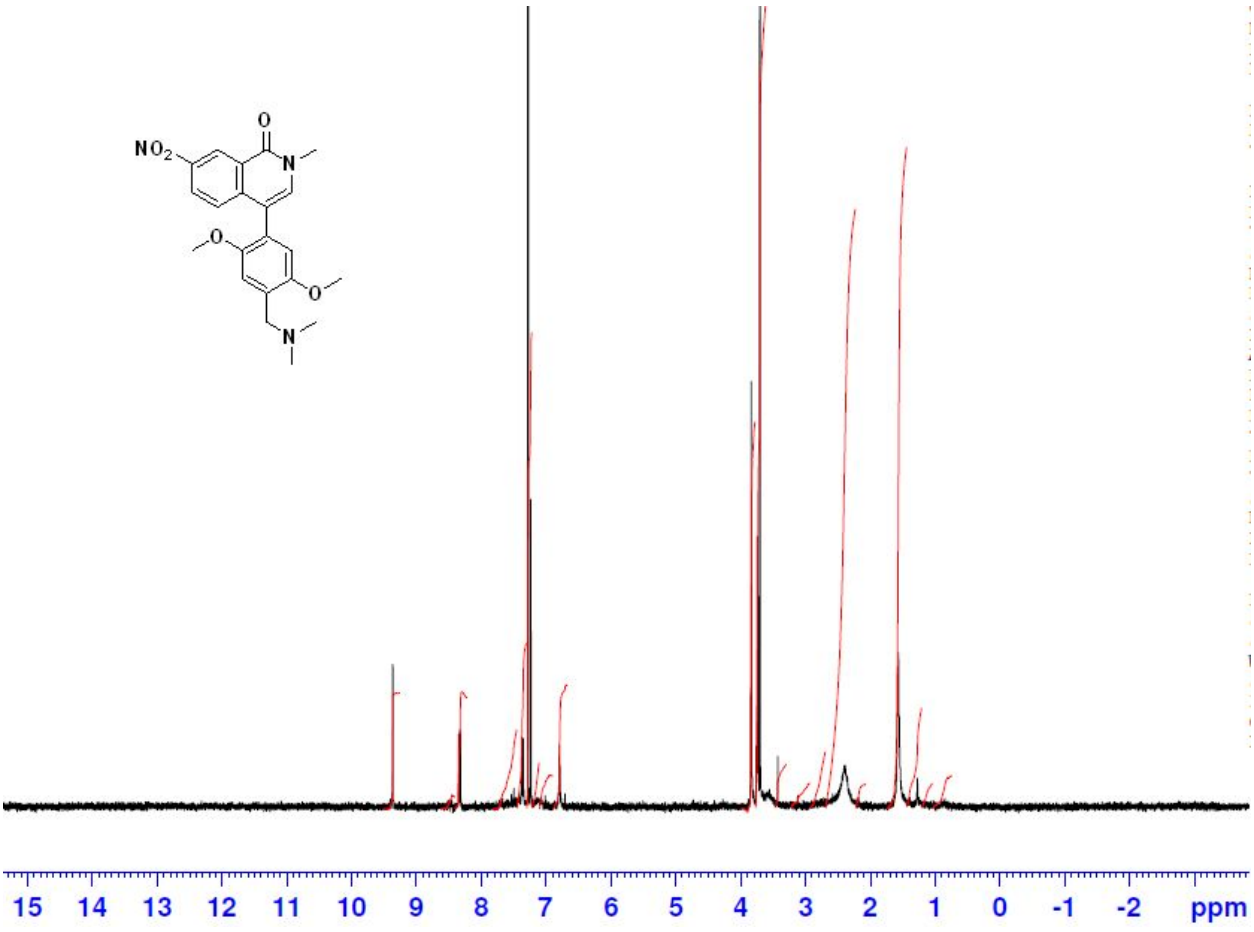
4-(4-((dimethylamino)methyl)-2,5-dimethoxyphenyl)-2-methyl-7-nitroisoquinolin-1(2H)-one

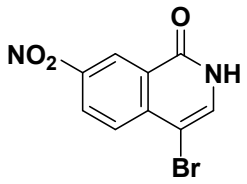
11.5%, 0.011 mmol, 3.3 mg

Compound 5

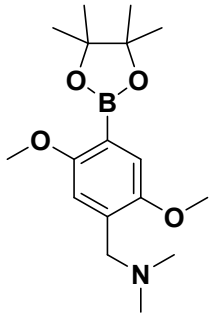
^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.74 (d, *J* = 1.2 Hz, 1H), 9.33 (d, *J* = 9.9 Hz, 1H), 8.44 (s, 1H), 7.62 (d, *J* = 10.4 Hz, 1H), 6.96 (d, *J* = 13.0 Hz, 1H), 4.25 (s, 2H), 3.87 (d, *J* = 9.8 Hz, 3H), 3.78 (d, *J* = 12.5 Hz, 3H), 3.70 (d, *J* = 2.6 Hz, 3H), 2.74 (d, *J* = 45.3 Hz, 6H).

[M+H] = 398.2, Rt = 0.98 min





Commercially available (CAS 1036390-36-9); 4-Bromo-7-nitroisoquinolin-1(2H)-one



Intermediate 5A

1-(2,5-dimethoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-N,N-dimethylmethanamine

[M+H]: 322.3, Rt = 0.69 min

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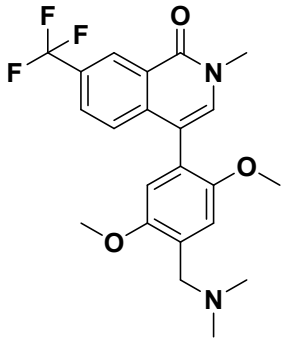


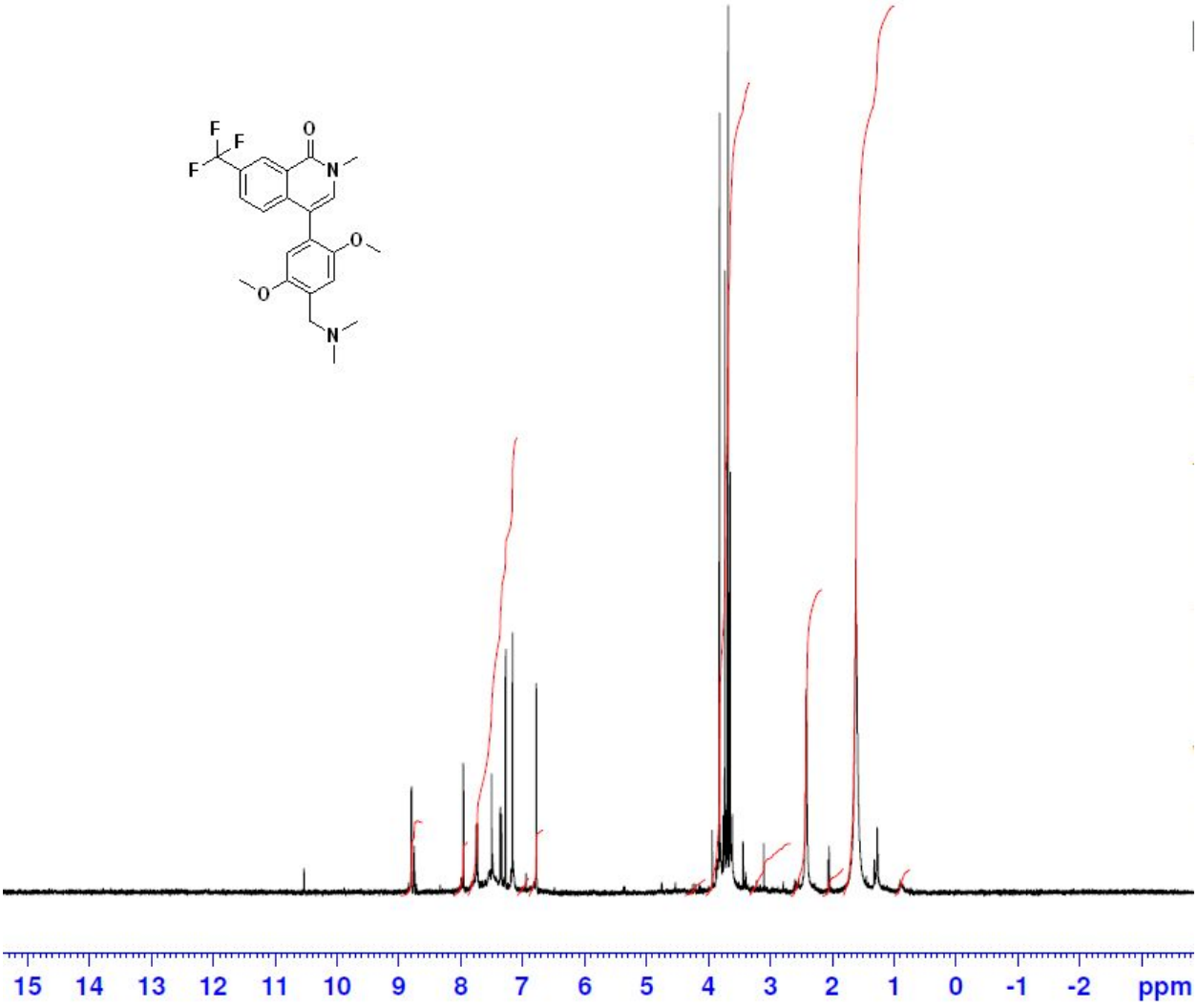
4-(4-((dimethylamino)methyl)-2,5-dimethoxyphenyl)-2-methyl-7-(trifluoromethyl)isoquinolin-1(2H)-one

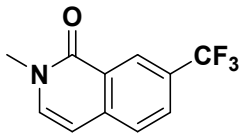
3.03%, 0.00346 mmol, 1.6 mg

Compound 6

1H NMR (400 MHz, Chloroform-d) δ 8.82 - 8.75 (m, 1H), 7.96 (d, J = 1.5 Hz, 1H), 7.75 (dd, J = 8.7, 2.0 Hz, 1H), 7.51 (s, 1H), 7.36 (d, J = 8.5 Hz, 1H), 7.17 (s, 1H), 6.79 (s, 1H), 3.83 (s, 3H), 3.74 (s, 3H), 3.69 (s, 3H), 3.66 (s, 1H), 2.42 (s, 5H).

[M+H] = 421.4, Rt = 0.77 min



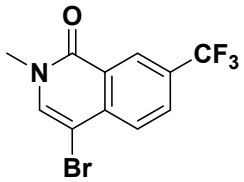


Intermediate 6B

2-methyl-7-(trifluoromethyl)isoquinolin-1(2H)-one

1H NMR (400 MHz, Chloroform-d) δ 8.75 (s, 1H), 7.84 (dd, J = 8.3, 2.0 Hz, 1H), 7.64 (d, J = 8.3 Hz, 1H), 7.21 (d, J = 7.3 Hz, 1H), 6.55 (d, J = 7.4 Hz, 1H), 3.66 (s, 3H).

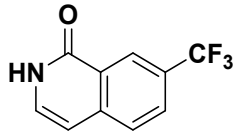
[M+H] = 229.1, Rt 0.88 min



Intermediate 6A

4-bromo-2-methyl-7-(trifluoromethyl)isoquinolin-1(2H)-one

[M+H] = 308.0, Rt = 1.02 min



Commercially available (CAS 410086-28-1); 7-(trifluoromethyl)-1(2H)-isoquinolinone

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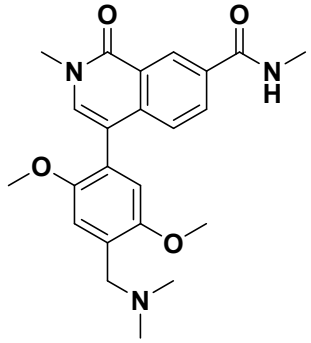


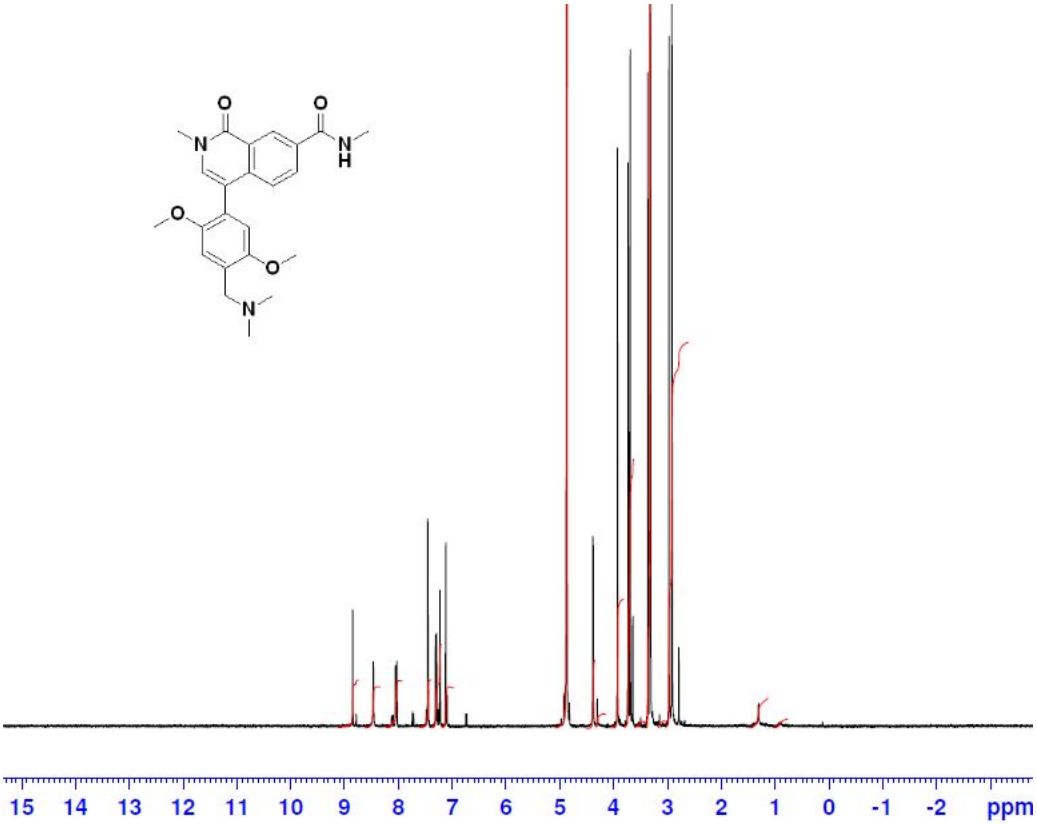
4-(4-((dimethylamino)methyl)-2,5-dimethoxyphenyl)-N,2-dimethyl-1-oxo-1,2-dihydroisoquinoline-7-carboxamide

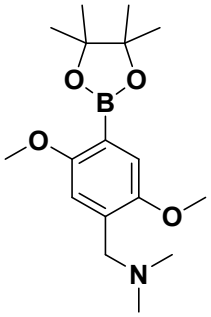
8.6%, 0.00766 mmol, 3.3 mg

Compound 7

1H NMR (400 MHz, Methanol-d4) δ 8.86 (d, J = 1.9 Hz, 1H), 8.47 (s, 1H), 8.04 (dt, J = 8.4, 2.5 Hz, 1H), 7.49 - 7.43 (m, 1H), 7.31 (d, J = 8.6 Hz, 1H), 7.23 (d, J = 4.6 Hz, 1H), 7.13 (s, 1H), 4.39 (s, 2H), 3.93 (d, J = 1.4 Hz, 3H), 3.73 (d, J = 2.9 Hz, 3H), 3.70 (s, 3H), 2.98 (s, 3H), 2.92 (s, 5H)

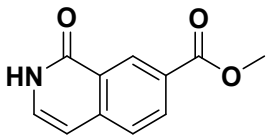
[M+H] = 410.4, Rt = 0.86 min



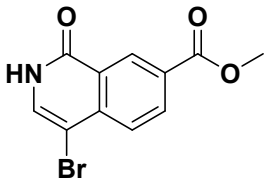


Intermediate 5A

See above



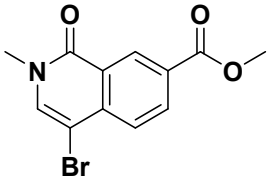
Commercially available, (CAS 658082-39-4); methyl 1-oxo-12-dihydro-7-isoquinolinecarboxylate



Intermediate 7A

methyl 4-bromo-1-oxo-1,2-dihydroisoquinoline-7-carboxylate

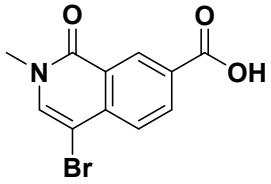
[M+H] = 282.0, Rt = 0.80 min



Intermediate 7B

methyl 4-bromo-2-methyl-1-oxo-1,2-dihydroisoquinoline-7-carboxylate

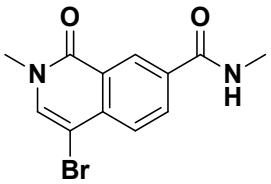
[M+H] = 298.0, Rt = 0.90 min



Intermediate 7C

4-bromo-2-methyl-1-oxo-1,2-dihydroisoquinoline-7-carboxylic acid

[M+H] = 283.9, Rt = 0.72 min



Intermediate 7D

4-bromo-N,2-dimethyl-1-oxo-1,2-dihydroisoquinoline-7-carboxamide

[M+H] = 295.0, Rt = 0.66 min

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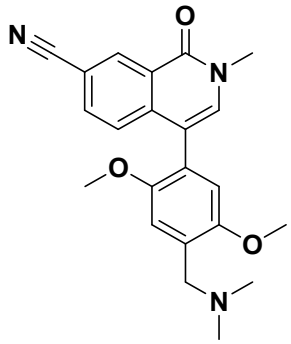


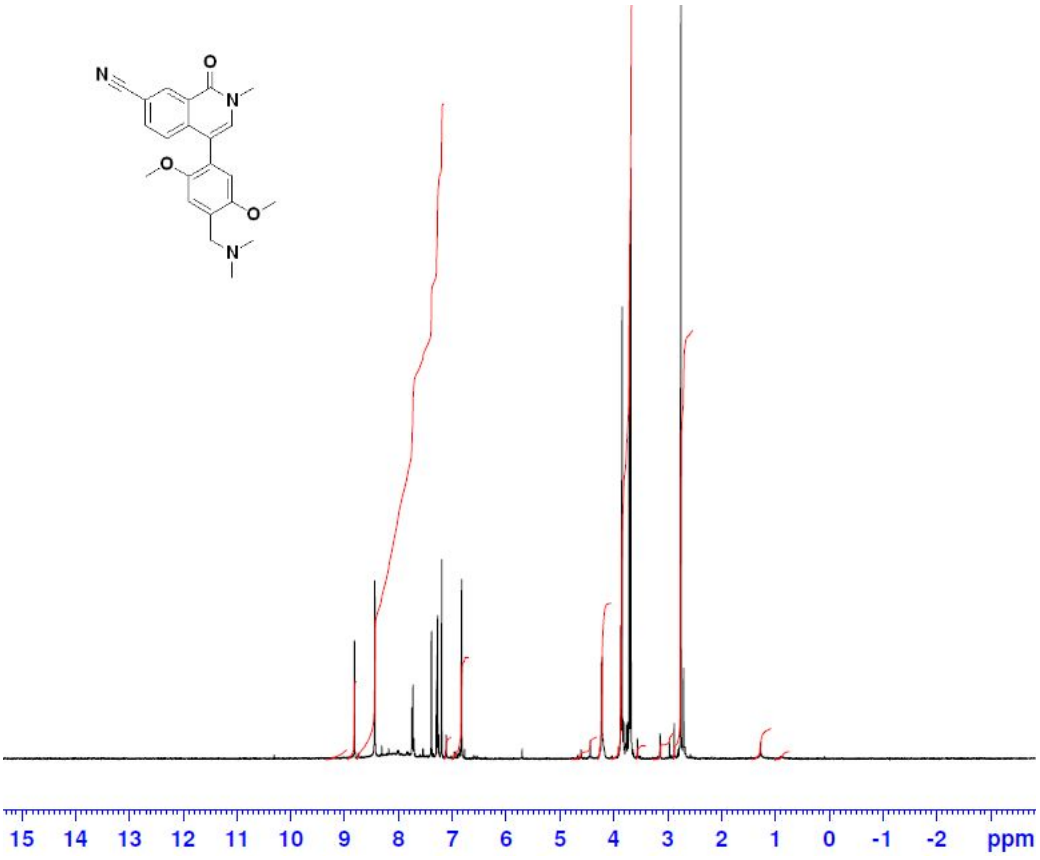
4-(4-((dimethylamino)methyl)-2,5-dimethoxyphenyl)-2-methyl-1-oxo-1,2-dihydroisoquinoline-7-carbonitrile

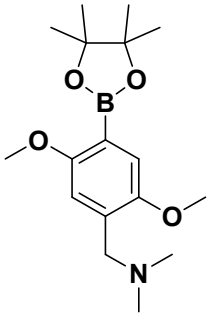
9.9%, 0.011 mmol, 4.5 mg

Compound 8

1H NMR (400 MHz, Chloroform-d) δ 8.82 (d, J = 1.8 Hz, 1H), 8.45 (s, 2H), 7.74 (td, J = 7.9, 7.2, 1.8 Hz, 1H), 7.39 (s, 1H), 7.28 (d, J = 2.0 Hz, 1H), 7.20 (s, 1H), 6.83 (s, 1H), 4.23 (d, J = 4.4 Hz, 2H), 3.86 (d, J = 9.3 Hz, 3H), 3.71 (d, J = 2.3 Hz, 3H), 3.69 (s, 3H), 2.76 (s, 6H).

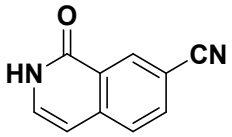
[M+H] = 378.3, Rt = 0.97 min



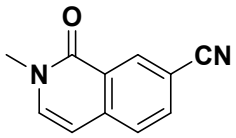


Intermediate 5A

See above



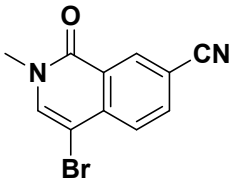
Commercially available, (CAS 1184913-64-1); 1-oxo-1,2-dihydroisoquinoline-7-carbonitrile



Intermediate 8A

2-methyl-1-oxo-1,2-dihydroisoquinoline-7-carbonitrile

[M+H] = 185.1, Rt = 0.64 min



Intermediate 8B

4-bromo-2-methyl-1-oxo-1,2-dihydroisoquinoline-7-carbonitrile

[M+H] = 264.9, Rt = 0.81 min

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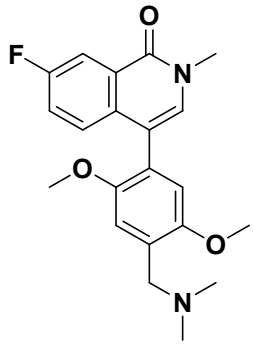


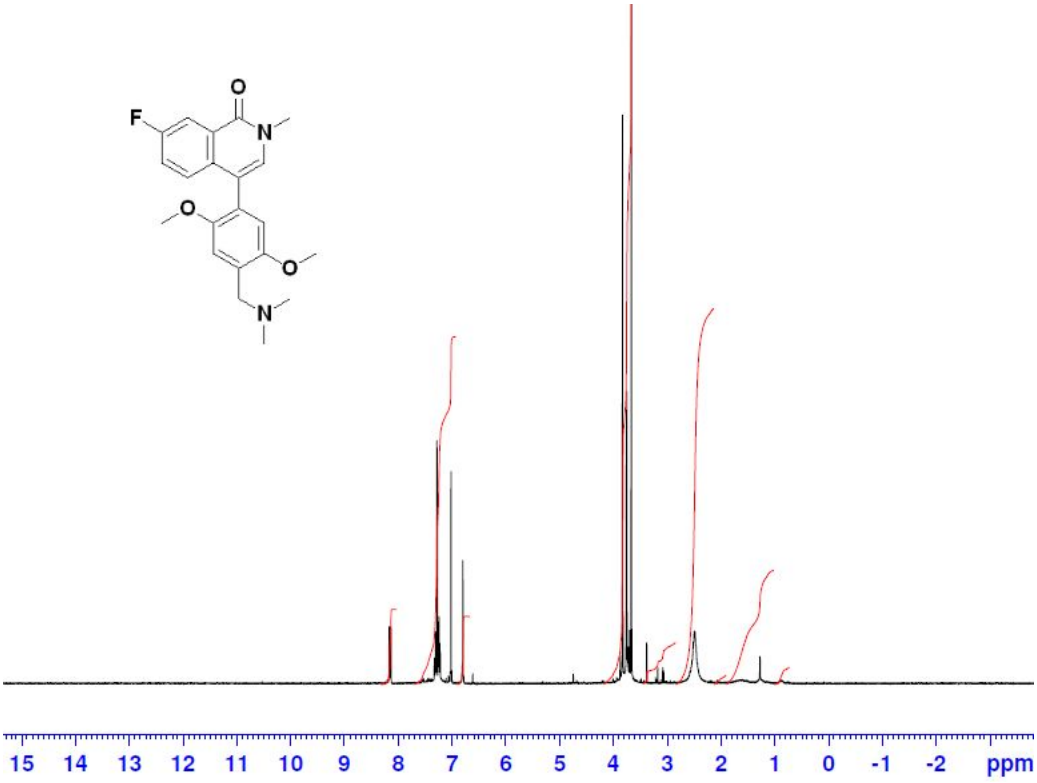
4-(4-((dimethylamino)methyl)-2,5-dimethoxyphenyl)-7-fluoro-2-methylisoquinolin-1(2H)-one

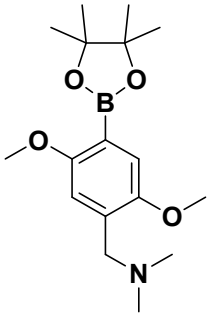
15.2%, 0.024 mmol, 9.5 mg

Compound 9

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.16 (dd, *J* = 9.4, 2.8 Hz, 1H), 7.35 – 7.19 (m, 3H), 7.03 (s, 1H), 6.80 (s, 1H), 3.83 (s, 3H), 3.76 (s, 5H), 3.67 (s, 3H), 2.50 (s, 6H).

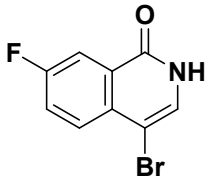
[M+H] = 371.3, Rt = 0.95 min



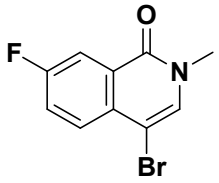


Intermediate 5A

See above



Commercially available, (CAS 1227607-99-9); 4-bromo-7-fluoroisoquinolin-1(2H)-one



Intermediate 9A

4-bromo-7-fluoro-2-methylisoquinolin-1(2H)-one

[M+H] = 255.9, Rt = 0.84 min

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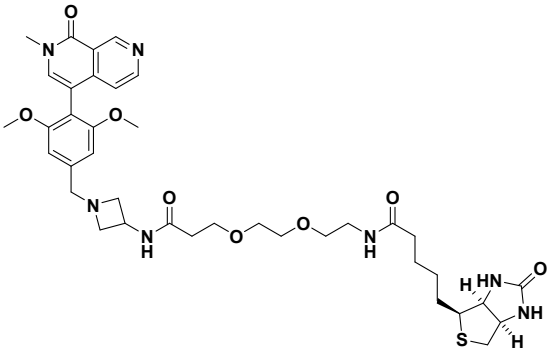


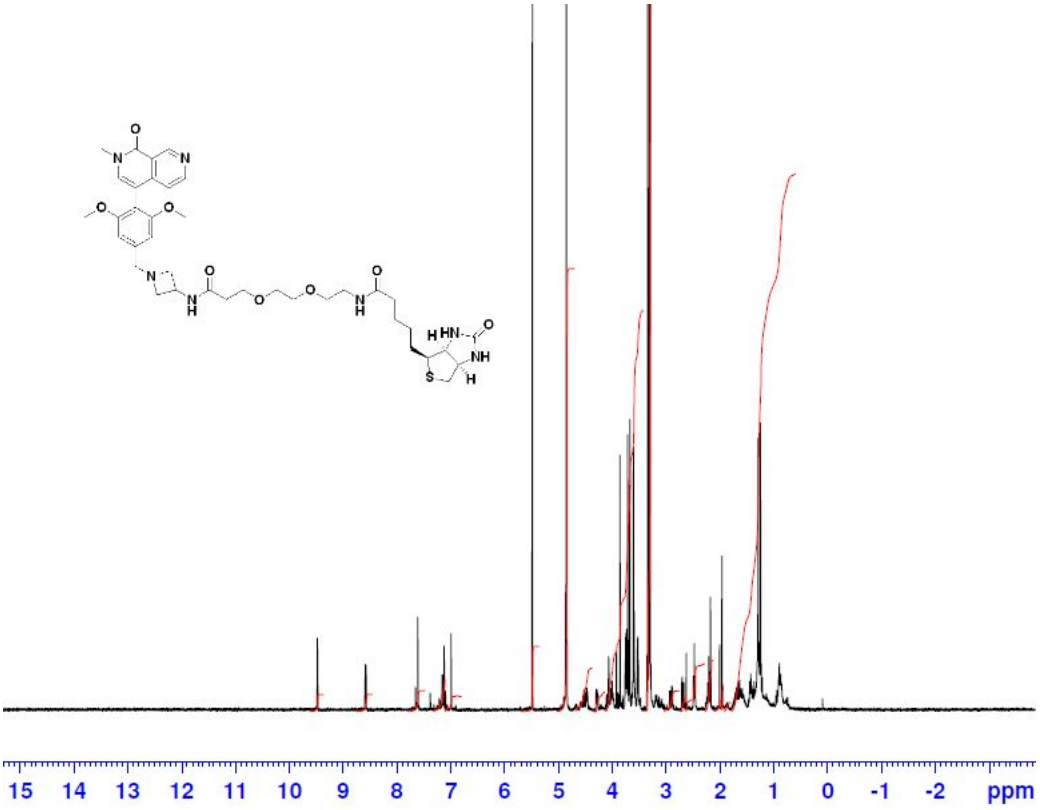
N-(2-(2-(3-((1-(2,5-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzyl)azetidin-3-yl)amino)-3-oxopropoxy)ethoxy)ethyl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide

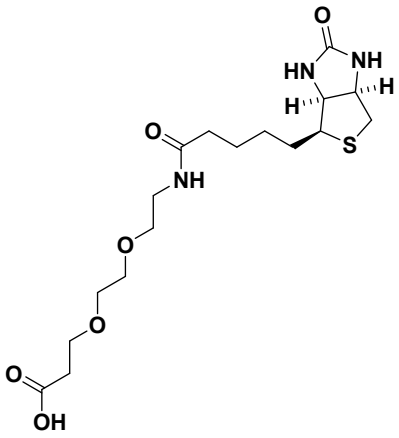
24.5%, 0.00979 mmol, 7.5 mg

Compound 1 - BIOTIN

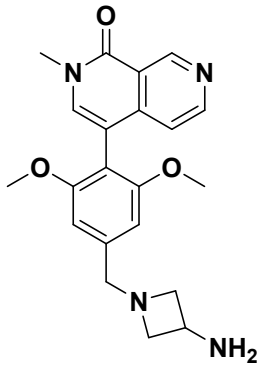
^{1}H NMR (400 MHz, Methanol-d_4) δ 9.48 (d, *J* = 1.0 Hz, 1H), 8.58 (dd, *J* = 5.8, 4.0 Hz, 1H), 7.61 (s, 1H), 7.17 – 7.08 (m, 2H), 6.99 (s, 1H), 5.48 (s, 4H), 4.59 – 4.42 (m, 2H), 4.28 (dd, *J* = 7.9, 4.6 Hz, 1H), 4.13 – 3.96 (m, 4H), 3.85 (s, 2H), 3.81 – 3.70 (m, 5H), 3.67 (d, *J* = 3.7 Hz, 4H), 3.60 (d, *J* = 3.7 Hz, 6H), 3.52 (t, *J* = 5.5 Hz, 3H), 2.47 (t, *J* = 6.1 Hz, 2H), 1.65 (tdt, *J* = 20.8, 15.6, 7.0 Hz, 6H).

[M+H] = 766.4, Rt = 0.50 min





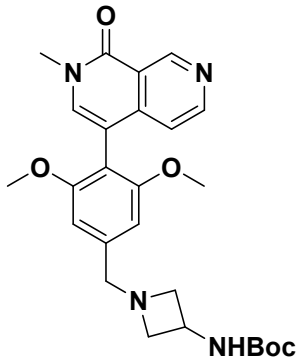
Commercially available, (CAS 1365655-89-5); 3-{2-[2-({5-[(3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl]pentanoyl}amino)ethoxy]ethoxy}propanoic acid



Intermediate 1-BIOTIN-A

4-(4-((3-aminoazetidin-1-yl)methyl)-2,6-dimethoxyphenyl)-2-methyl-2,7-naphthyridin-1(2H)-one

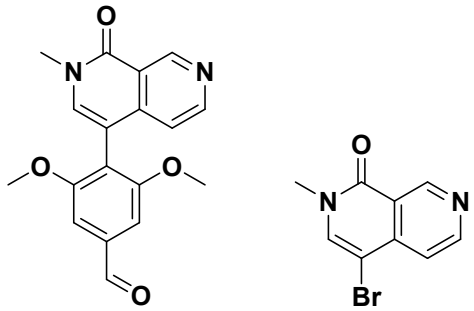
[M+H] = 381.2, Rt = 1.10 min



Intermediate 1-BIOTIN-B

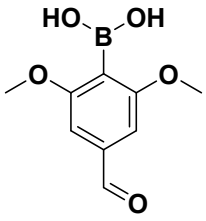
tert-butyl (1-(3,5-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzyl)azetidin-3-yl)carbamate

[M+H] = 480.9, Rt = 0.59 min



Intermediates 3A + 1A

See above



Commercially available (CAS 1256355-34-6); 2,6-Dimethoxy-4-forymlphenylboronic acid

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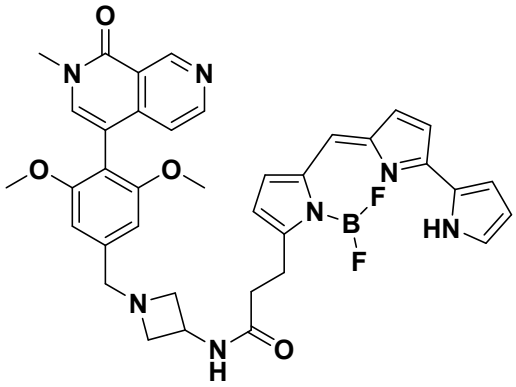


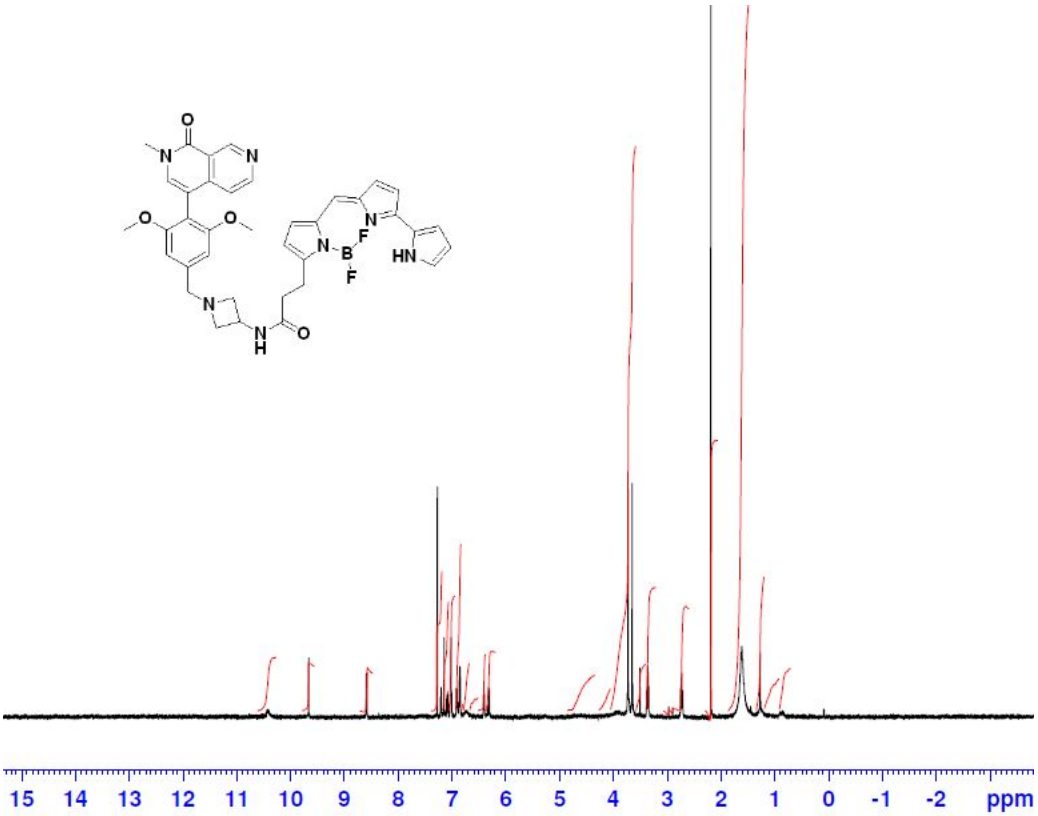
(Z)-3-(5-(1H,5'H-[2,2'-bipyrrol]-5'-ylidenemethyl)-1-(difluoroboraneyl)-1H-pyrrol-2-yl)-N-(1-(3,5-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzyl)azetidin-3-yl)propanamide

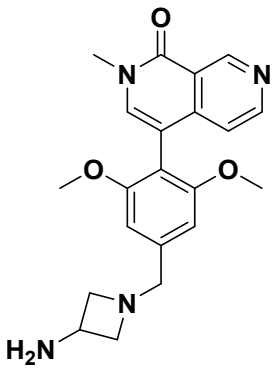
22.3%, 0.00261 mmol, 1.9 mg

Compound 3-TRACER

1H NMR (400 MHz, Chloroform-d) δ 9.67 (s, 1H), 8.59 (d, J = 5.6 Hz, 1H), 7.28 (s, 2H), 7.21 (s, 1H), 7.15 (s, 1H), 7.11 - 7.07 (m, 1H), 7.02 (d, J = 2.4 Hz, 2H), 6.92 (d, J = 4.8 Hz, 1H), 6.86 (d, J = 4.9 Hz, 2H), 6.41 (s, 1H), 6.32 (d, J = 3.9 Hz, 1H), 3.73 (s, 6H), 3.65 (s, 4H), 3.37 (t, J = 7.5 Hz, 3H), 2.74 (t, J = 7.6 Hz, 2H), 1.62 (s, 6H).

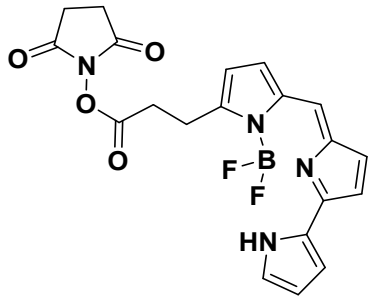
[M+H] = 692.3, Rt = 0.95 min





Intermediate 1-BIOTIN-B

See above



Commercially available, 4,4-difluoro-5-(2-pyrrolyl)-4-bora-3a,4a-diaza-s-indacene-3-propionic acid, succinimidyl ester (BODIPY® 576/589, SE)

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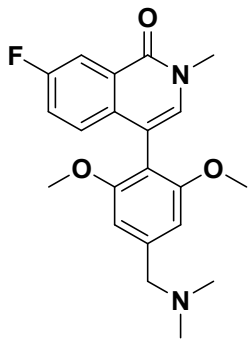


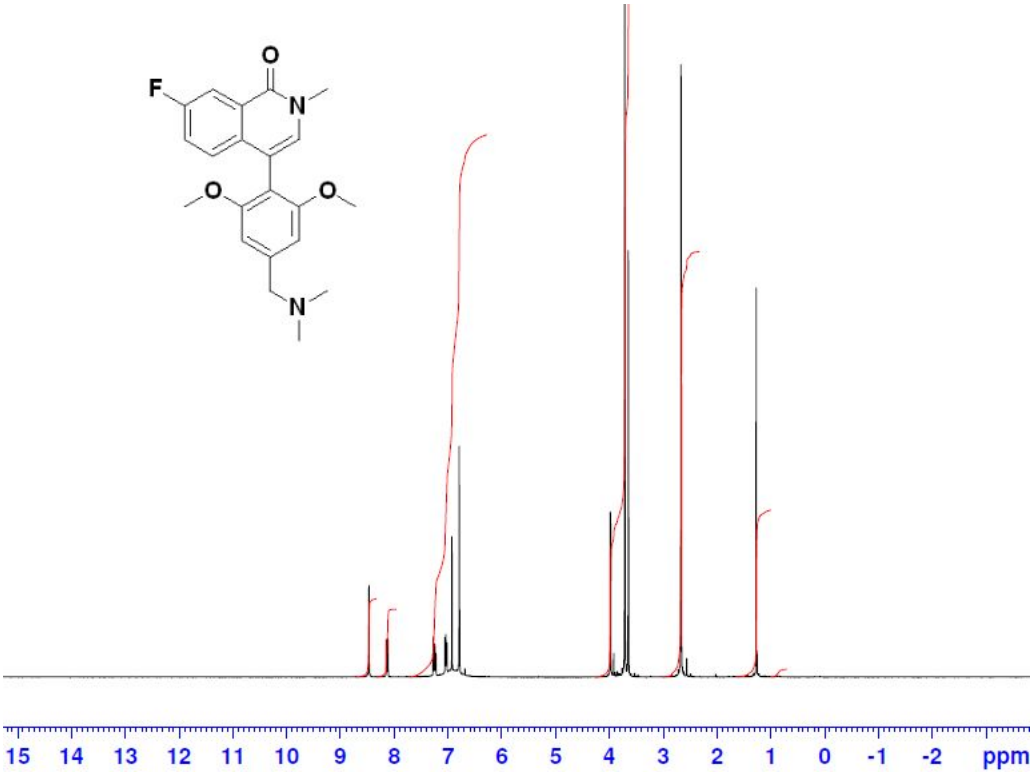
4-(4-((dimethylamino)methyl)-2,6-dimethoxyphenyl)-7-fluoro-2-methylisoquinolin-1(2H)-one

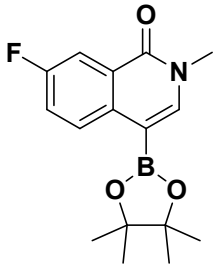
15.7%, 0.017 mmol, 6.7 mg

Compound DN01

1H NMR (400 MHz, Chloroform-d) δ 8.48 (s, 1H), 8.14 (dd, J = 9.5, 2.8 Hz, 1H), 7.30 - 7.22 (m, 1H), 7.04 (dd, J = 8.9, 5.2 Hz, 1H), 6.93 (s, 1H), 6.80 (s, 2H), 3.98 (s, 2H), 3.72 (s, 6H), 3.65 (s, 3H), 2.67 (s, 6H)

[M+H] = 371.2, Rt = 0.63 min

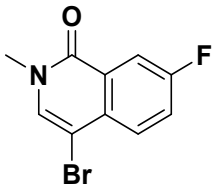




Intermediate DN01-A

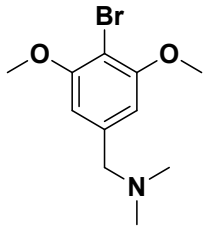
7-fluoro-2-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoquinolin-1(2H)-one

[M+H] = 305.2, Rt = 1.14 min



Intermediate 9A

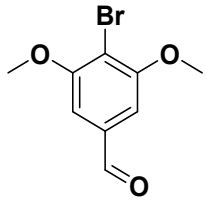
See above



Intermediate DN01-B

1-(4-bromo-3,5-dimethoxyphenyl)-N,N-dimethylmethanamine

[M+H] = 275.1, Rt = 0.54 min



Commercially available, (CAS 31558-40-4); 4-bromo-3,5-dimethoxybenzaldehyde

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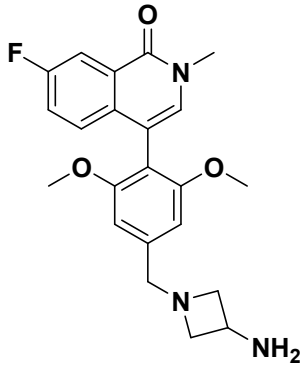


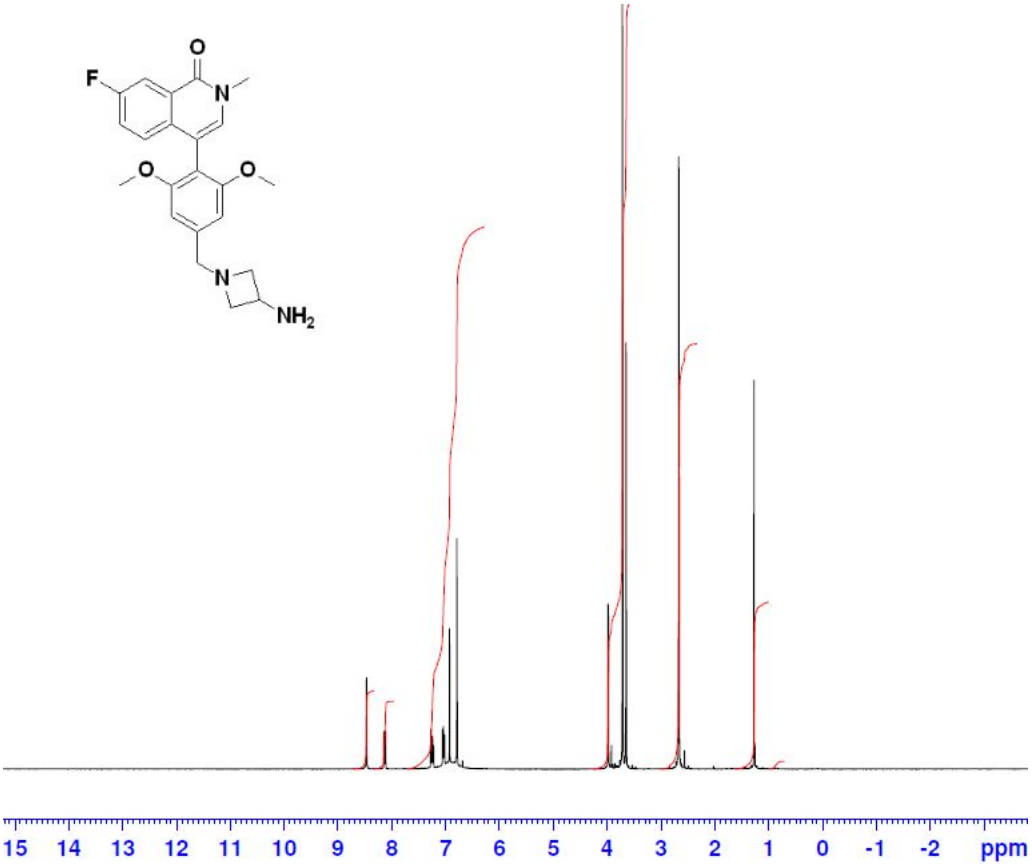
4-(4-((3-aminoazetidin-1-yl)methyl)-2,6-dimethoxyphenyl)-7-fluoro-2-methylisoquinolin-1(2H)-one

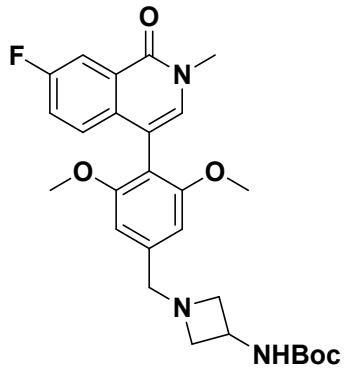
34.9%, 0.0021 mmol, 0.9 mg

Compound DN02

1H NMR (400 MHz, Chloroform-d) δ 8.14 (dd, J = 9.5, 2.7 Hz, 1H), 7.24 (td, J = 8.5, 2.8 Hz, 1H), 7.07 (dd, J = 8.9, 5.2 Hz, 1H), 6.93 (s, 1H), 6.69 (s, 2H), 3.80 (s, 4H), 3.73 (s, 7H), 3.65 (s, 3H).

[M+H] = 398.2, Rt = 0.70 min

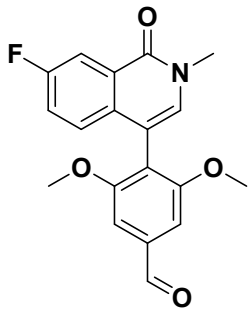




Intermediate DN02-B

tert-butyl (1-(4-(7-fluoro-2-methyl-1-oxo-1,2-dihydroisoquinolin-4-yl)-3,5-dimethoxybenzyl)azetidin-3-yl)carbamate

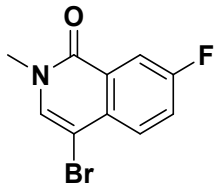
[M+H] = 498.5, Rt = 0.78 min



Intermediate DN02-A

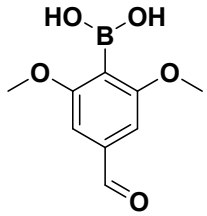
4-(7-fluoro-2-methyl-1-oxo-1,2-dihydroisoquinolin-4-yl)-3,5-dimethoxybenzaldehyde

[M+H] = 342.1, Rt = 0.88 min



Intermediate 9A

See above



Commercially available (CAS 1256355-34-6); 2,6-Dimethoxy-4-forymlphenylboronic acid