SUPPORTING INFORMATION

Improvement of Cell Permeability of Human Neuronal Nitric Oxide Synthase Inhibitors Using Potent and Selective 2-Aminopyridine-Based Scaffolds with a Fluorobenzene Linker

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Table S1. Crystallographic data collection and refinement statistics

Data set[a]	nNOS-**7**	nNOS-**8**	nNOS-**10**	nNOS-**12**
Data collection				
PDB code	6AUQ	6AUR	6AUS	6AUT
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions				
a, *b*, *c* (Å)	51.6 111.4 164.4	52.2 110.9 164.2	51.7 110.6 164.2	52.1 111.0 164.4
Resolution (Å)	1.95 (2.02-1.95)	1.75 (1.80-1.75)	1.70 (1.74-1.70)	1.90 (1.96-1.90)
R_{merge}	0.118 (3.279)	0.091 (1.278)	0.071 (2.159)	0.132 (1.766)
R_{pim}	0.088 (2.422)	0.067 (1.035)	0.057 (1.872)	0.098 (1.354)
CC 1/2	0.997 (0.401)	0.997 (0.427)	0.997 (0.455)	0.995 (0.333)
$I / \sigma I$	8.1 (0.6)	8.7 (1.0)	4.7 (0.3)	7.3 (1.0)
No. unique reflections	70015 (4452)	96987 (4699)	104363 (5103)	75792 (4422)
Completeness (%)	99.8 (99.8)	100.0 (99.8)	99.9 (99.8)	99.8 (99.8)
Redundancy	5.0 (5.0)	5.1 (4.4)	5.1 (4.1)	5.0 (4.7)
Refinement				
Resolution (Å)	1.95	1.75	1.70	1.90
No. reflections used	51683 *	96834	104038	75699
R_{work} / R_{free}[b]	0.191/0.256	0.182/0.213	0.192/0.228	0.194/0.235
No. atoms				
Protein	6677	6683	6692	6686
Ligand/ion	171	173	175	175
Water	403	649	506	486
R.m.s. deviations				
Bond lengths (Å)	0.008	0.007	0.007	0.008
Bond angles (deg)	1.213	1.183	1.167	1.187

Data set[a]	nNOS-**13**	nNOS-**16**	nNOS-**17**	nNOS-**18**
Data collection				
PDB code	6AUU	6AUV	6AUW	6AUX
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions				
a, *b*, *c* (Å)	51.7 111.1 164.5	52.1 111.2 164.2	51.9 111.1 164.0	52.0 110.7 164.3
Resolution (Å)	1.85 (1.90-1.85)	1.76 (1.80-1.76)	1.70 (1.74-1.70)	1.90 (1.96-1.90)
R_{merge}	0.102 (2.199)	0.102 (2.807)	0.077 (4.251)	0.130 (2.748)
R_{pim}	0.087 (1.848)	0.047 (1.396)	0.034 (1.924)	0.066 (1.944)
CC 1/2	0.997 (0.349)	0.962 (0.471)	0.999 (0.558)	0.998 (0.318)
$I / \sigma I$	5.3 (0.3)	10.2 (0.5)	11.2 (0.4)	9.5 (0.6)
No. unique reflections	81744 (4365)	95459 (4449)	104763 (4968)	75632 (4246)
Completeness (%)	99.9 (99.8)	99.6 (95.4)	99.3 (99.3)	99.7 (99.7)
Redundancy	4.0 (4.0)	5.8 (4.7)	5.9 (5.5)	10.1 (5.4)
Refinement				
Resolution (Å)	1.85	1.76	1.70	1.90
No. reflections used	81572	92803	101560	75408
R_{work} / R_{free}[b]	0.199/0.240	0.184/0.222	0.194/0.229	0.173/0.210
No. atoms				
Protein	6706	6743	6692	6680
Ligand/ion	193	179	179	181
Water	407	551	395	419
R.m.s. deviations				
Bond lengths (Å)	0.008	0.007	0.007	0.007
Bond angles (deg)	1.168	1.110	1.112	0.914

Data set[a]	hnNOS-**7**	hnNOS-**8**	hnNOS-**10**	hnNOS-**12**
Data collection				
PDB code	6AUY	6AUZ	6AU0	6AU1
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions				
a, *b*, *c* (Å)	52.5 122.0 165.1	52.3 122.0 164.5	52.4 122.1 165.0	52.5 121.2 164.1
Resolution (Å)	1.92 (1.97-1.92)	2.00 (2.07-2.02)	2.00 (2.06-2.00)	2.45 (2.60-2.45)
R_{merge}	0.107 (5.091)	0.128 (2.594)	0.148 (1.761)	0.230 (1.998)
R_{pim}	0.048 (2.276)	0.095 (1.928)	0.108 (1.308)	0.112 (0.956)
CC 1/2	0.998 (0.357)	0.996 (0.395)	0.989 (0.619)	0.990 (0.475)
$I / \sigma I$	8.8 (0.4)	7.5 (0.6)	5.8 (0.9)	4.1 (0.7)
No. unique reflections	81949 (4319)	72141 (4369)	72772 (4199)	39460 (4364)
Completeness (%)	99.8 (96.9)	100.0 (100.0)	99.6 (93.8)	100.0 (100.0)
Redundancy	5.9 (5.8)	4.9 (5.0)	5.3 (5.1)	5.0 (5.2)
Refinement				
Resolution (Å)	1.92	2.00	2.00	2.45
No. reflections used	77143	71989	72635	39227
R_{work} / R_{free}[b]	0.186/0.236	0.200/0.243	0.178/0.218	0.199/0.267
No. atoms				
Protein	6727	6705	6711	6705
Ligand/ion	187	165	167	167
Water	472	390	449	200
R.m.s. deviations				
Bond lengths (Å)	0.007	0.008	0.007	0.008
Bond angles (deg)	1.119	1.133	1.146	1.138

Data set[a]	hnNOS-**13**	hnNOS-**16**	hnNOS-**17**	hnNOS-**18**
Data collection				
PDB code	6AU2	6AU3	6AU4	6AU5
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions□ □				
a, *b*, *c* (Å)	52.2 121.5 165.3	52.5 122.3 164.0	52.6 122.4 164.5	52.5 122.3 164.4
Resolution (Å)	2.10 (2.18-2.10)	1.95 (2.01-1.95)	1.87 (0.92-1.87)	1.90 (1.95-1.90)
R_{merge}	0.148 (1.689)	0.131 (4.078)	0.085 (3.591)	0.114 (1.688)
R_{pim}	0.108 (1.233)	0.058 (1.855)	0.038 (1.605)	0.077 (1.171)
CC 1/2	0.991 (0.416)	0.998 (0.556)	0.999 (0.443)	0.997 (0.462)
$I / \sigma I$	6.5 (0.8)	6.8 (0.4)	10.4 (0.5)	8.8 (0.9)
No. unique reflections	60809 (4365)	77910 (4441)	88965 (4351)	84443 (4412)
Completeness (%)	97.9 (96.9)	99.4 (97.3)	99.7 (96.0)	100.0 (100.0)
Redundancy	5.4 (5.4)	5.9 (5.5)	5.9 (5.7)	6.1 (6.0)
Refinement				
Resolution (Å)	2.10	1.95	1.87	1.90
No. reflections used	60724	74663	87538	84326
R_{work} / R_{free}[b]	0.181/0.225	0.203/0.259	0.188/0.228	0.184/0.219
No. atoms				
Protein	6723	6705	6727	6728
Ligand/ion	172	169	145	173
Water	407	337	407	571
R.m.s. deviations				
Bond lengths (Å)	0.007	0.007	0.007	0.007
Bond angles (deg)	1.161	1.096	1.075	0.977

Data set[a]	heNOS-**8**	heNOS-**12**
Data collection		
PDB code	6AU6	6AU7
Space group	$P2_1$	$P2_1$
Cell dimensions□ □		
a, *b*, *c* (Å)	59.6 152.8 108.9	59.4 152.4 108.7
β (°)	90.8	90.8
Resolution (Å)	2.08 (2.13-2.08)	1.92 (1.96-1.92)
R_{merge}	0.169 (2.916)	0.093 (3.459)
R_{pim}	0.077 (1.313)	0.063 (2.396)
CC 1/2	0.996 (0.623)	0.998 (0.229)
$I / \sigma I$	6.5 (1.0)	8.6 (0.4)
No. unique reflections	116007 (5716)	144907 (6349)
Completeness (%)	99.5 (99.5)	97.7 (86.8)
Redundancy	5.4 (5.5)	5.8 (5.4)
Refinement		
Resolution (Å)	2.08	1.92
No. reflections used	115687	140508
R_{work} / R_{free}[b]	0.194/0.239	0.204/0.254
No. atoms		
Protein	12,857	12,888
Ligand/ion	476	480
Water	326	354
R.m.s. deviations		
Bond lengths (Å)	0.008	0.010
Bond angles (deg)	1.104	1.124

[a] See Figure 2 for nomenclature and chemical formula of inhibitors.

[b] R_{free} was calculated with the 5% of reflections set aside throughout the refinement. The set of reflections for the R_{free} calculation were kept the same for all data sets according to those used in the data of the starting model.

* Reflection file was modified with the Diffraction Anisotropy Server (services.mbi.ucla.edu).

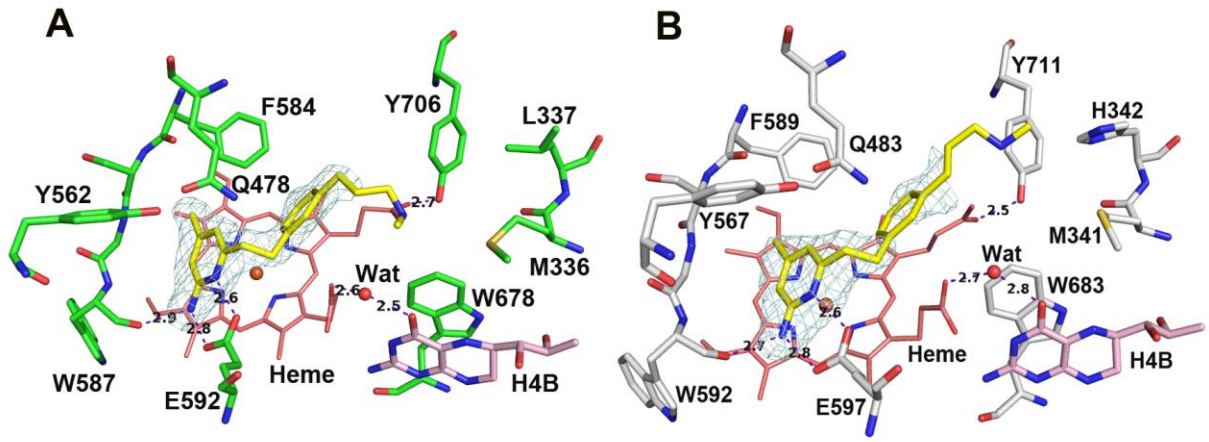


Figure S1. Active site structure of **7** bound to rnNOS (A) and hnNOS (B). The long amine tail showed poor density because of its flexibility.

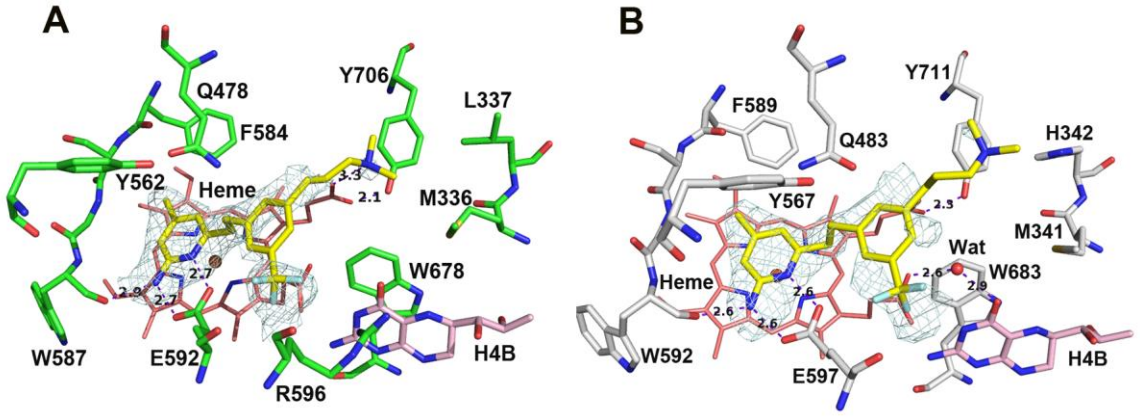


Figure S2. Active site structures of **13** bound to rnNOS (A) and hnNOS (B).

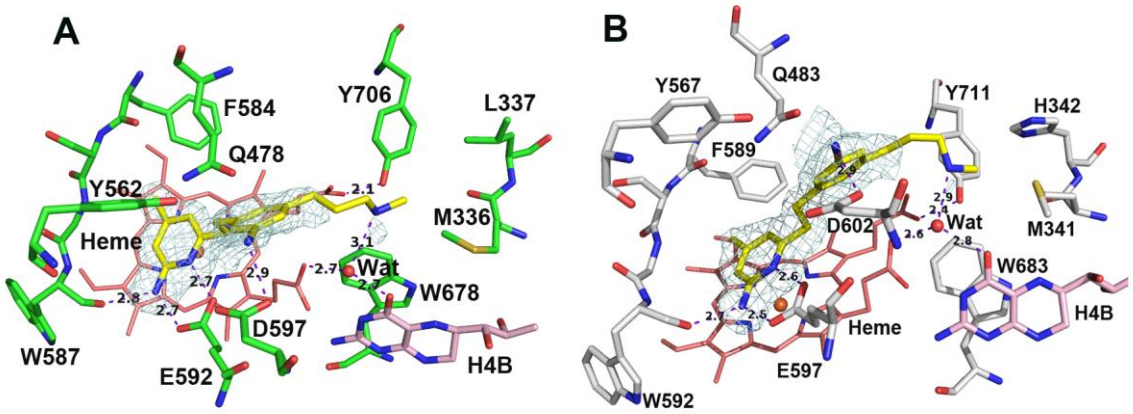


Figure S3. Active site structure of **10** bound to rnNOS (A) and hnNOS (B). The 2.9 Å contact from the cyano group to Asp-597 (or Asp-602) can be a H-bond only if the Asp residue is protonated.

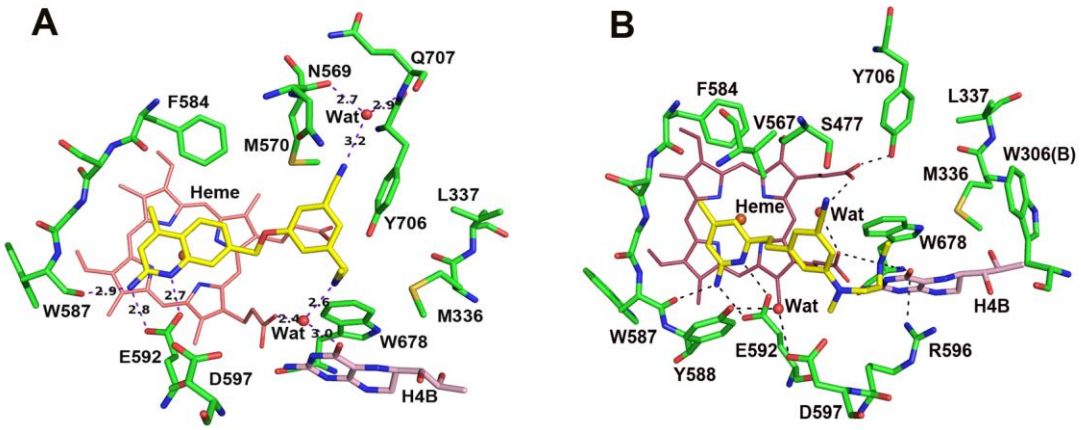


Figure S4. (A) Compound **11** in ref. 22 bound to rnNOS (PDB code 5UNU). (B) Compound **19c** in ref. 24 bound to rnNOS (PDB code 4UH4).

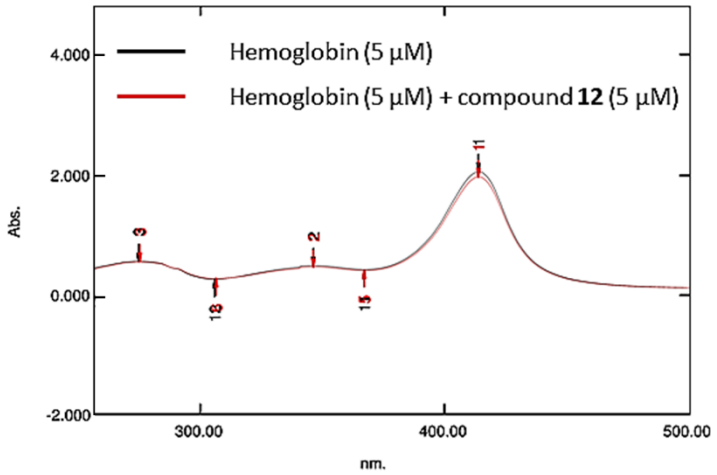
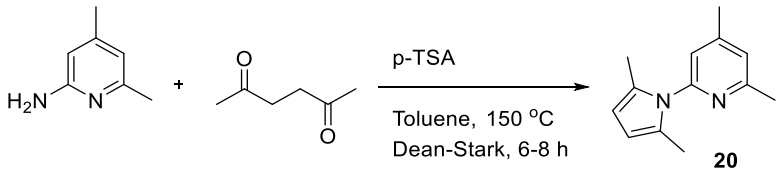
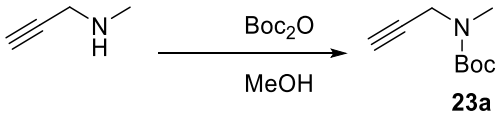


Figure S5. UV-vis Spectra of hemoglobin without (black line) and with (red line) the presence of 2-Aminopyridine **12** indicating that there is little to no interaction between 2-Aminopyridines and the heme in hemoglobin. Therefore, the presence of 2-Aminopyridine-bearing NOS inhibitors do not interfere the hemoglobin NO capture assay of NOS inhibition.

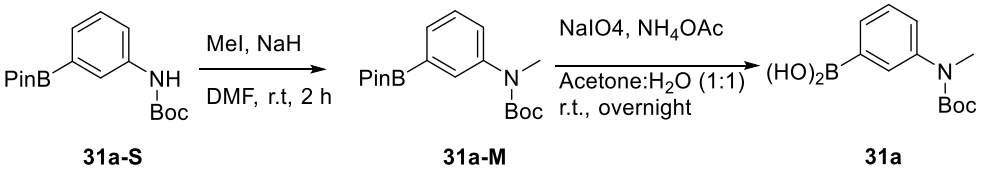
Synthesis of 20, 23a and 31a



2-(2,5-Dimethyl-1H-pyrrol-1-yl)-4,6-dimethylpyridine (20). 20 was synthesized by following a previous report[1] using 2-amino-4,6-dimethylpyridine (1 equiv.) and *p*-TSA (0.1 equiv.), which were dissolved in toluene to make a 0.2 M solution. The reaction mixture was refluxed at 150 °C in a Dean-Stark apparatus. The reaction was monitored by TLC until completion, which takes around 6-8 h. Upon completion, the reaction was allowed to cool, and toluene was removed under reduced pressure. The crude mixture was submitted to flash column chromatography for purification using ethyl acetate and hexanes (1:9) as eluents. **20** was isolated as a light yellow solid (46%).^{1}H NMR (500 MHz, $CDCl_3$): δ 6.99 (s, 1H), 6.84 (s, 1H), 5.87 (s, 2H), 2.54 (s, 3H), 2.38 (s, 3H), 2.12 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 158.1, 151.5, 149.4, 128.4, 122.9, 119.7, 106.6, 24.1, 20.9, 13.1.



***tert*-Butyl-methyl(prop-2-ynyl)carbamate (23a). 23a** was synthesized similarly to a previous report.[1] To a solution of *N*-methylpropargylamine (1 equiv.) in methanol was added slowly di-*tert*-butyl-dicarbonate (1.05 equiv.) at r.t. The reaction was stirred at r.t overnight. Upon completion, the reaction mixture was concentrated under reduced pressure to give **23a** as a light-yellow oil (87%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.00 (brs, 2H), 2.86 (s, 3H), 2.17 (s, 1H), 1.42 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 155.2, 80.1, 79.2, 77.3, 71.6, 33.4, 28.3; MS ESI $[M+H]^+$ = 170.03.



(3-((*tert*-Butoxycarbonyl)(methyl)amino)phenyl)boronic acid (31a). 31a was prepared in two steps from commercially available Boc protected boronic ester **31a-S**. First, **31a-S** (1 equiv.) was dissolved in DMF, forming a 0.5 M solution. NaH (2.37 equiv.) was added slowly to the solution at 0 ^{o}C, and the reaction was stirred at the same temperature for 5 min. MeI (1.5 equiv.) was then added dropwise, and the reaction was run for 2 h at r.t. Upon completion, water was added, and the reaction mixture was diluted with ethyl acetate, which was washed with water (3 times) and brine. The organic extract was concentrated under reduced pressure to afford desired product **31a-M** as a yellow oil (88%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.61 (s, 1H), 7.60 – 7.56 (m, 1H), 7.33 – 7.28 (m, 2H), 3.24 (s, 3H), 1.42 (s, 9H), 1.32 (s, 12H).

Crude **31a-M** (1 equiv.), $NaIO_4$ (1.5 equiv.), and NH_4OAc (1.5 equiv.) were combined in a round bottom flask and diluted with a mixture of acetone and water (1:1) to form a 0.63 M solution.[2] The reaction mixture was stirred vigorously at r.t. for 18 h. The slurry mixture was then filtered, and acetone was removed under reduced pressure. The aqueous solution was then extracted with CH_2Cl_2 (3 times), and the combined organic extracts were washed with brine, dried with Na_2SO_4, and concentrated to give desired product **31a** as a yellow foam (a quantitative yield).

General Procedure SA: Pyrrolyl-lutidine and Aryl Bromide Coupling. A round bottom flask was charged with **20** (1 equiv.), and THF was added to form a 0.2 M solution. *n*-BuLi (1.6 equiv.) was added dropwise to the solution at -78 ºC, and the reaction mixture was stirred for 30 min at 0 ºC. The mixture was then transferred to a 1 M solution of aryl bromide (**21a**, **21b**, **21c** or **21d**) in THF *via* cannula at -78 ºC, which was stirred for an additional 20 min before quenching with H_2O. The crude reaction mixture was partitioned between ethyl acetate and water, and the organic layer was washed with H_2O and brine, dried with Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash column chromatography to give **22a**, **22b**, **22c** or **22d**.

2-(3-Bromophenethyl)-6-(2,5-dimethyl-1*H*-pyrrol-1-yl)-4-methylpyridine (22a). Compound **22a** was synthesized according to general procedure SA using **20** (1.054 g, 5 mmol) and **21a** (1.2398 g, 5 mmol). **22a** was isolated as a light-yellow oil (1.017 g, 55%) after flash column chromatography (ethyl acetate: hexanes 1:19). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.32-7.30 (m, 2H), 7.14-7.10 (m, 2H), 6.89 (s, 1H), 6.87 (s, 1H), 5.90 (s, 2H), 3.06-3.05 (m, 4H), 2.37 (s, 3H), 2.13 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 160.6, 151.8, 149.6, 143.9, 131.6, 130.0, 129.1, 128.5, 127.2, 122.7, 122.4, 120.3, 106.8, 39.4, 35.3, 21.0, 13.3. MS ESI $[M+H]^+$ = 369.05.

2-(3-Bromo-5-fluorophenethyl)-6-(2,5-dimethyl-1*H*-pyrrol-1-yl)-4-methylpyridine (22b). Compound **22b** was synthesized according to general procedure SA using **20** (1.035 g, 5 mmol) and **21b** (1.329 g, 5 mmol). **22b** was isolated as a light-yellow oil (1.0948 g, 57%) after flash column chromatography (ethyl acetate: hexanes 1:19). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.08 (s, 1H), 7.04 (d, J = 8.0 Hz, 1H), 6.88 (s, 1H), 6.85 (s, 1H), 6.80 (d, J = 9.5 Hz, 1H), 5.87 (s, 2H), 3.04-3.02 (m, 4H), 2.35 (s, 3H), 2.10 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 162.6 (d, $J_{C\text{-}F}$ = 248.6 Hz), 159.9, 151.8, 149.7, 145.6 (d, $J_{C\text{-}F}$ = 7.8 Hz), 128.5, 127.6 (d, $J_{C\text{-}F}$ = 2.9 Hz), 122.7, 122.2 (d, $J_{C\text{-}F}$ = 10.0 Hz), 120.4, 116.7 (d, $J_{C\text{-}F}$ = 24.4 Hz), 114.4 (d, $J_{C\text{-}F}$ = 20.8 Hz), 106.8, 39.0, 35.0, 21.0, 13.2. MS ESI $[M+H]^+$ = 389.00.

2-(3-Bromo-5-(trifluoromethyl)phenethyl)-6-(2,5-dimethyl-1*H*-pyrrol-1-yl)-4-methylpyridine (22c). Compound **22c** was synthesized according to general procedure SA using **20** (600 mg, 3 mmol) and **21c** (949. mg, 3 mmol). **22c** was isolated as a light-yellow oil (495 mg, 38%) after flash column chromatography (ethyl acetate: hexanes 1:19). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.56 (s, 1H), 7.48 (s, 1H), 7.28 (s, 1H), 6.87 (s, 1H), 6.85 (s, 1H), 5.88 (s, 2H), 3.13-3.10 (m, 2H), 3.07-3.04 (m, 2H), 2.35 (s, 3H), 2.10 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ

159.6, 151.8, 149.7, 135.0, 132.2 (q, $J_{C\text{-}F}$ = 32.5 Hz), 128.5, 126.1 (d, $J_{C\text{-}F}$ = 3.8 Hz), 124.1 (d, $J_{C\text{-}F}$ = 3.8 Hz), 123.2 (q, $J_{C\text{-}F}$ = 271.3 Hz), 122.8, 122.6, 120.5, 120.0, 106.8, 39.0, 35.0, 20.9, 13.2. MS ESI $[M+H]^+$ = 439.00.

2-(3,5-Dibromophenethyl)-6-(2,5-dimethyl-1*H*-pyrrol-1-yl)-4-methylpyridine (22d). Compound **22d** was synthesized according to general procedure SA using **20** (1.01 g, 5 mmol) and **21d** (1.6629 g, 5 mmol). **22d** was isolated as a light-yellow oil (1.1157 g, 50%) after flash column chromatography (ethyl acetate: hexanes 1:19). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.46 (s, 1H), 7.22 (s, 1H), 7.21 (s, 1H), 6.87 (s, 1H), 6.86 (s, 1H), 5.88 (s, 2H), 3.03-3.01 (m, 4H), 2.36 (s, 3H), 2.11 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 159.8, 151.8, 149.7, 145.5, 131.7, 130.6, 130.5, 128.5, 122.8, 120.4, 106.8, 39.0, 34.9, 21.0, 13.3. MS ESI $[M+H]^+$ = 449.94.

3-Bromo-5-(2-(6-(2,5-dimethyl-1*H*-pyrrol-1-yl)-4-methylpyridin-2-yl)ethyl)benzonitrile (27). A microwave vial was charged with CuCN (1 equiv.), pyridine (40.4 µl), and **22d** (223 mg, 0.5 mmol). The mixture was diluted with DMF to form a 0.2 M solution. The microwave vial was capped, and the reaction mixture was stirred at 150 ºC for 20 h. The cap was removed, and the reaction mixture was diluted with ethyl acetate. The reaction crude product was washed with water and brine, dried with Na_2SO_4, concentrated under reduced pressure, and purified by flash column chromatography (ethyl acetate: hexanes 1:9) to give **27** as a white solid (88 mg, 45%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.58 (s, 1H), 7.53 (s, 1H), 7.37 (s, 1H), 6.88 (s, 1H), 6.87 (s, 1H), 5.88 (s, 2H), 3.12-3.09 (m, 2H), 3.07-3.04 (m, 2H), 2.36 (s, 3H), 2.10 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 159.3, 151.9, 149.9, 145.1, 136.4, 132.3, 130.8, 128.4, 122.8, 122.7, 120.6, 117.4, 114.0, 106.8, 38.7, 34.6, 21.0, 13.3. MS ESI $[M+H]^+$ = 394.04.

Reference

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