Synthesis and Mutagenic Potency of Structural Isomers of 2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine

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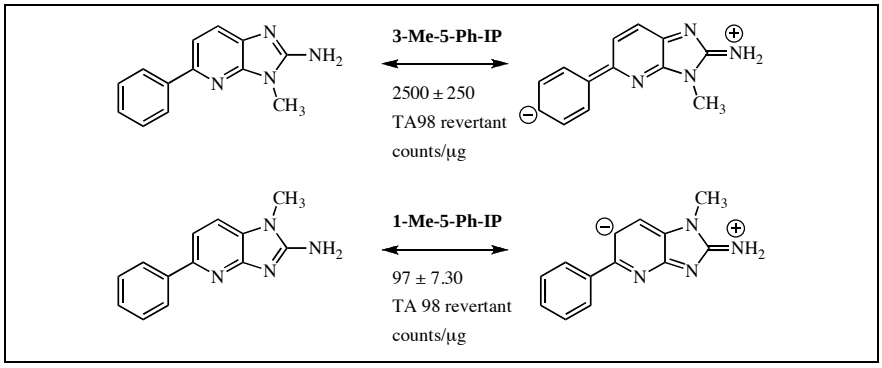

Synthesis of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), three structural isomers, and two desphenyl PhIP congeners has been carried out. Mutagenic potency was evaluated using *S. typhimurium* strain TA98 in the Ames test. Mutagenic potency increased in relation to structural features in these heterocyclic amines that allow extended resonance between the phenyl and imidazo[4,5-*b*]pyridine N^2-amino substituents. By contrast, PhIP isomers, whose substitution disallows involvement of the phenyl group in their aminoimidazo resonance hybrids, and desphenyl congeners were from 86- to 234-fold less mutagenic than PhIP.

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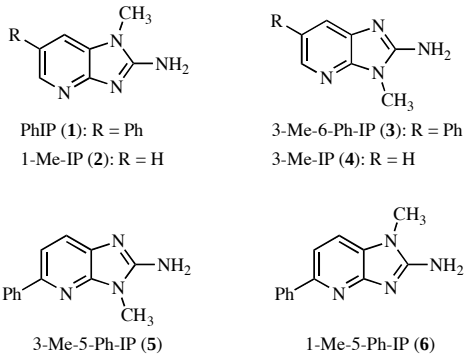
INTRODUCTION

Amino acid pyrolysates, including heterocyclic amines (HAs), are found in fried meat and fish at levels of ng/g; in particular, the imidazopyridine PhIP (**1**) commonly is the most abundant HA in meat and fish [1]. PhIP is a suspected pro-carcinogen [2] that undergoes metabolism to proximate mutagens including predominantly *N*-hydroxy-PhIP [3]. It is well established that PhIP is a substrate for xenobiotic oxidation by cytochrome P450 enzymes en route to either *N*-glucuronide conjugates or heterolytic N-O bond cleavage furnishing the PhIP-nitrenium ion [4]. The impact on human health from PhIP and other HAs [5] and the quantification of HAs obtained from dietary sources [6] are topics of research interest.

Previously we compared PhIP and 3-Me-6-Ph-IP (**3**) following activation by Aroclor induced rat liver, and found that PhIP was 86-fold more mutagenic against *S. typhimurium* TA98 in the Ames test [7]. The difference in potency between these two HAs raised structure-activity questions. Consequently, we have prepared a set of isomeric HAs (Figure 1): PhIP (**1**); PhIP isomers 3-Me-6-Ph-IP (**3**), 3-Me-5-Ph-IP (**5**), and 1-Me-5-Ph-IP (**6**); and the desphenyl PhIP congeners 1-Me-IP (**2**) and 3-Me-IP (**4**). Our studies have resulted in efficient, not previously reported syntheses of PhIP isomers **5** and **6**.



PhIP (**1**) and isomers 3-Me-6-Ph-IP (**3**), 3-Me-5-Ph-IP (**5**), 1-Me-5-Ph-IP (**6**), and desphenyl PhIP congeners 1-Me-IP (**2**), and 3-Me-IP (**4**)

Figure 1

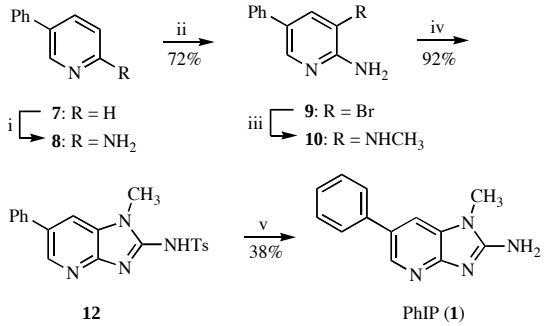
Tests with these six HAs have assessed their relative potency against *S. typhimurium* TA98 and elucidated the possible structure activity relationship (SAR) between the four PhIP isomers and mutagenicity.

RESULTS AND DISCUSSION

I. Synthesis. Synthesis of PhIP (**1**) (Scheme 1) began with 3-phenylpyridine **7** that was aminated in a Chichibabin reaction furnishing 2-aminopyridine **8** in

52% yield. Bromination in acetic acid at 0 °C gave a 72% yield of 3-bromopyridine **9**, which underwent desamino-bromination at 200 °C by the action of aqueous methylamine in the presence of cupric sulfate furnishing unpurified diaminopyridine **10**. Condensing crude diaminopyridine **10** with cyanogens bromide under a

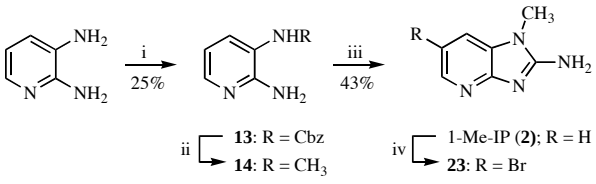
Scheme 1



Reagents: (i) $NaNH_2$, toluene, reflux, 52%; (ii) Br_2, AcOH, 0-20 °C; (iii) CH_3NH_2(aq), $CuSO_4$, 200 °C, 79%; (iv) Cl_2C=NTs (**11**) , K_2CO_3, dioxane, 85 °C; (v) HF, 100 °C

variety of conditions led to low yields of PhIP, plus by-products and recovered starting material. By contrast, when impure **10** and dichloro-*N*-tosylmethanimine (**11**) [8] were condensed in dioxane at 85 °C, the sulfonamido-imidazo[4,5-*b*]pyridine **12** was obtained in 73% yield over two steps from **9**. Attempted detosylation of **12** by standard methods was unsuccessful; *e.g.*, hydrogen bromide in acetic acid resulted in recovered starting material, and hydrolysis in concentrated sulfuric acid at 100 °C or treatment with excess butyl lithium in tetrahydrofuran from –78 °C to 20 °C gave tars [9]. *N*-Toluenesulfonyl removal with anhydrous hydrogen fluoride at 100 °C led to PhIP in 38% yield, although C-18 flash chromatography followed by trituration from ethanol was needed for separation of by-products.

Scheme 2

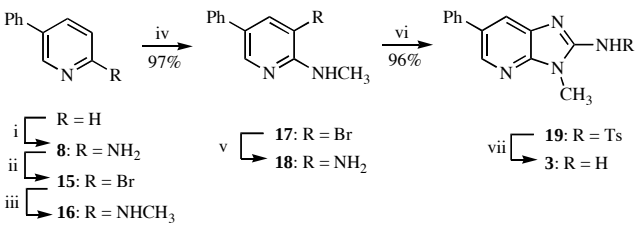


Reagents: (i) $PhCH_2OC(O)Cl$, THF-pyridine, 0-20 °C; (ii) $LiAlH_4$, THF, reflux, 87%; (iii) BrCN, H_2O, 40 °C; (iv) Br, AcOH, 100 °C, 15%

Synthesis of desphenyl PhIP congener 1-Me-IP (**2**) from 2,3-diaminopyridine (Scheme 2) began with a moderately regioselective conversion to N^3-benzylamino-pyridine **13** [10], which was reduced using lithium aluminum hydride in tetrahydrofuran at reflux to provide N^3-methylaminopyridine **14** in 22% yield over two steps. Cyclization with aqueous cyanogen bromide at 40 °C, gave the corresponding imidazo derivative **14** in 43% yield of 1-Me-IP (**2**) after C-18 flash chromatography.

The PhIP isomer 3-Me-6-Ph-IP (**3**) was prepared from 3-phenylpyridine *via* amination with sodium amide (Scheme 3), giving a 52% yield of 2-aminopyridine **8**. Conversion to the corresponding 2-bromopyridine **15** under Sandmeyer reaction conditions gave a 95% yield; it was followed by copper mediated desaminobromination of **15** using aqueous methylamine to furnish a mixture of 2-methylaminopyridine **16** in 63% yield, along with a dehydrobromination by-product. Bromination in acetic acid led to a 97% yield of 3-bromopyridine **17**, which was aminated by the copper-mediated action of aqueous ammonia at 200 °C to give diaminopyridine **18** in 82% yield plus a dehydrobromination by-product. Unpurified diamine **18** and dichloro-*N*-tosylmethanimine (**11**) were condensed in dioxane at 85 °C to give a 96% yield of sulfonamidoimidazo[4,5-*b*]pyridine **19**. Unmasking the amine moiety with anhydrous hydrogen fluoride at 100 °C gave 3-Me-6-Ph-IP in a 43% yield.

Scheme 3



Reagents: (i) $NaNH_2$, toluene, reflux, 52%; (ii) HBr, HCl, Br_2, $NaNO_2$, 0 °C, 95%; (iii) CH_3NH_2(aq), $CuSO_4$, 200 °C, 63%; (iv) Br_2, AcOH, 0-20 °C, 82%; (v) NH_4OH(aq), $CuSO_4$, 200 °C, 82%; (vi) Cl_2C=NTs (**11**), K_2CO_3, dioxane, 85 °C; (vii) HF, 100 °C, 43%

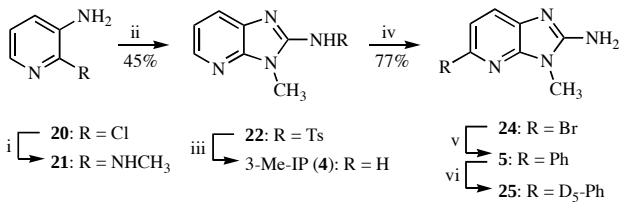
Synthesis of 3-Me-IP (**4**) and 3-Me-5-Ph-IP (**5**) began with 3-amino-2-chloropyridine (**20**). Copper-mediated deaminochlorination with aqueous methylamine at 200 °C led to a mixture of diaminopyridine **21** (Scheme 4) in 53% yield and a dehydrochlorination by-product. Condensation of **21** and dichloro-*N*-tosylmethanimine (**11**) under the standard conditions furnished a 45% yield of sulfonamidoimidazo[4,5-*b*]pyridine **22**. Deprotection using anhydrous hydrogen fluoride at 100 °C left the free amine 3-Me-IP in 68% yield after flash chromatography.

Bromination of 1-Me-IP (**2**) in acetic acid at 100 °C regiospecifically led to 6-bromopyridine **23** (Scheme 2) in 15% yield, without substitution at the pyridine C-5 or C-7 positions; this result is attributable to participation of σ-complex resonance stabilization from the N^2-amino group. By contrast, an imidazomethylamine moiety positioned at

N^3 in 3-Me-IP directs bromination toward C-5. It follows that our synthesis of PhIP isomer **5** involved using a Suzuki cross-coupling (Scheme 4) of 5-bromo-3-Me-IP (**24**) with phenylboronic acid.

Conditions employed for bromination of 1-Me-IP when applied to 3-Me-IP produced exclusively 5-bromopyridine **24**. Under Pd(0)-mediated cross-coupling conditions, **24** was allowed to react with either phenyl- or perdeuterophenylboronic acid, resulting in **5** and **25** in yields of 59% and 71%, respectively.

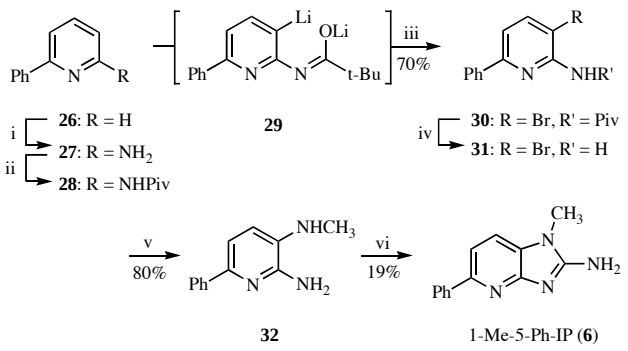
Scheme 4



Reagents: (i) CH_3NH_2(aq), $CuSO_4$, 200 °C, 53%; (ii) Cl_2C=NTs (**11**), K_2CO_3, dioxane, 85 °C; (iii) HF, 100 °C, 68%; (iv) Br_2, AcOH, 100 °C; (v) [Pd]. ethanol-dioxane, 85 °C, $PhB(OH)_2$ 59% for **5**, or D_5-$PhB(OH)_2$ 71% for **25**

Synthesis of PhIP isomer **6** began with amination of 2-phenylpyridine (**26**) employing the Chichibabin reaction conditions used for preparing 2-aminopyridine **8**. Refluxing 2-phenylpyridine, toluene, and sodium amide in xylenes (50 wt% leads unexpectedly to secondary amine bis(6-phenylpyridin-2-yl)amine [11]. By contrast, substituting *N,N*-dimethylaniline for toluene and using dry sodium amide (Scheme 5), the amination led to 2-

Scheme 5



Reagents: (i) $NaNH_2$, *N,N*-dimethylaniline, 170 °C, 81%; (ii) PivCl, NEt_3, THF, 0-20 °C, 56%, (iii) BuLi-TMEDA, Et_2O, -70 °C, then CBr_4; (iv) HCl(aq), 70 °C, 96%; (v) CH_3NH_2(aq), $CuSO_4$, 200 °C, 80%; (vi) BrCN, ethanol, 34 °C

aminopyridine **27** in good yield. When treated with 1.1 equivalent of bromine in acetic acid at 0 °C, 2-aminopyridine **27** was unfortunately converted to a mixture of 2-amino-5-bromo-6-phenylpyridine, 2-amino-3,5-dibromo-6-phenylpyridine, and recovered starting material in nearly equal parts.

Directed lithium-halogen exchange was utilized to bypass this issue in the bromination of aminopyridine **27**. Thus, the amine was converted to pivalamide **28** that is sparingly soluble in diethyl ether, tetrahydrofuran, or 1,2-dimethoxyethane. Initially, *ortho*-directed lithiation of 2-pivaloylaminopyridine **28** using butyllithium, *sec*-BuLi, or *tert*-BuLi in tetrahydrofuran-hexane or lithium diisopropylamide in tetrahydrofuran failed under standard methods [12]. However, the action of butyllithium complexed with tetramethylethylenediamine (TMEDA) in diethyl ether allowed regiospecific metalation of **28**. Dilithiated species **29** as a heterogeneous paste in diethyl ether was treated with tetrabromomethane to give a 70% yield of 2-pivaloylamido-3-bromopyridine **30**. Hydrolysis of the pivaloylamide moiety furnished 3-bromopyridine **31** in 38% yield over three steps from **27**. Deaminobromination with aqueous methylamine under the usual conditions led to 2,3,6-trisubstituted **32** in about 80% yield, along with a dehydrobromination by-product. Unpurified diaminopyridine **32** was allowed to react with cyanogen bromide in ethanol at 34 °C for 20 hours, giving 1-Me-5-Ph-IP (**6**) in 19% yield after flash chromatography and trituration with ethanol.

II. NMR Spectra. ^{1}H and ^{13}C nmr spectra obtained for all synthetic compounds were in accord with their structural assignments. The positions of phenyl and imidazo *N*-Me substituents for each PhIP isomer were confirmed by ^{1}H PFGSE nOe experiments recorded at ambient temperature for dilute samples in degassed dimethyl sulfoxide-d_6 (freeze-pump-thaw). NOe mixing times were on the order of T1 (longitudinal relaxation) for pyridine H-7 that was in the range of 1.5-2.6 seconds.

The ^{1}H nmr spectrum of 3-Me-5-Ph-IP was insufficiently resolved between 7.38 and 7.49 ppm to confirm the phenyl position at pyridine C-5. By contrast, the spectra of $^2H_5$-phenyl congener **25** displayed doublets, J = 8.1 Hz, for vicinal protons H-6 and H-7 in accord with *ortho*-substitution. NOe spectra of **5** showed strong enhancement of the N^2-amino, δ 6.84 (broad singlet), and medium enhancement of the phenyl H-2′ and H-6′ resonances, δ 8.05 (app. d, J = 7.9 Hz), upon irradiation of the N^3-Me, δ 3.60 (singlet). ^{1}H nmr spectra of 1-Me-5-Ph-IP did not clarify the position of the phenyl substituent because H-6, H-7, and phenyl resonances were coincident. The nOe spectra of **6** showed strong enhancement of both the N^2-amino, δ 6.89 (broad singlet), and the doublet arising from H-7, δ 7.43 (app. d, J = 8.0 Hz), when the N^1-Me, δ 3.52 (singlet), was irradiated.

In ^{1}H nmr spectra of 2-amino-5-bromoimidazo[4,5-*b*]-pyridine (**24**), obtained *via* bromination of 3-Me-IP, doublets occurred at δ 7.33 (d, J = 7.9 Hz, H-6) and δ 7.10 (d, J = 8.1 Hz, H-7). Coupling of 8.0 Hz between H-6 and H-7 correlates with vicinal protons in **24**,

whereas the spectrum of 2-amino-1-methyl-6-bromo-imidazo[4,5-*b*]pyridine, showed doublets at δ 7.89 (d, *J* = 1.9 Hz, H-5) and δ 7.56 (d, *J* = 1.9 Hz, H-7) [13].

III. Mutagenic Activity. PhIP and structural isomer **3**, which we have previously compared [7], were synthesized and re-evaluated in this study of the four HA isomers, to ensure that data were self-consistent. While the four isomers **1**, **3**, **5**, and **6** are a complete set for PhIP, two remaining isomers 1-Me-7-Ph-IP and 3-Me-7-Ph-IP have not been reported. PhIP isomers **1**, **3**, **5**, and **6** are linear structures versus tricyclic angular HAs exemplified by 2-amino-3-methyl-imidazo[4,5-*f*]quinoline (IQ) [4b], which would be structurally more closely related to either of the 7-Ph-PhIP isomers.

Mutagenic activity was determined using the standard plate incorporation assay described by Ames *et al*. [14], with *S. typhimurium* strain TA98 (a gift from Professor Bruce Ames, University of California, Berkeley). Two milligrams of Aroclor-induced rat liver S9 protein were added per plate for metabolic activation, and HAs were plated in serial doses to give a linear response covering a range from 0.1 to 100 μg/plate. An HA positive control IQ gave 1200-1500 revertants per 5 ng dose. Dimethylsulfoxide (DMSO) was included in the negative controls (spontaneous revertant counts) and led to 25-60 revertant colonies per plate.

A minimum of four dose points from duplicate platings was used, and from the slope of the linear portion of the curve, the number of revertant colonies per microgram HA was calculated taking the standard error of the linear fit as delta. The Ames test results comparing **1**, **3**, **5**, and **6**, plus desphenyl PhIP congeners **2** and **4**, ranged from 2500 ± 252 to 7.9 ± 0.39 revertant counts per microgram of HA (Table 1).

That the position of the imidazo[4,5-*b*]pyridine *N*-methyl substituent strongly influences the pyridine ring by increasing electron density at C-6 and C-5 of 1-Me-IP (**2**) and 3-Me-IP (**4**), respectively, was demonstrated by the outcome of bromination of **2** and **4**. The electronic effect is preserved in PhIP and 3-Me-5-Ph-IP, extending *via* resonance involvement of the phenyl substituent, and we propose that this effect is implicated in their mutagenicity.

Isomer **5** was two-fold more mutagenic than PhIP, and both HAs favor valence-bond delocalization between the phenyl and 2-aminoimidazo[4,5-*b*]pyridine amine substituents illustrated in resonance hybrids **33** and **35** (Figure 2). By contrast, isomers **3** and **6**, whose substitution pattern disallows involvement of the phenyl group in resonance with the amine substituent, shown in resonance forms **34** and **36**, respectively, were 86-fold and 200-fold less mutagenic than PhIP. Desphenyl *N*-Me-IP congeners **2** and **4**, lacking phenyl contribution to resonance stabilization, were approximately 234-fold less mutagenic than PhIP.

Table 1

Compound	TA98 Revertants/μg [a]
3-Me-5-Ph-IP (**5**)	2500 ± 252
PhIP (**1**)	1800 ± 138
1-Me-5-Ph-IP (**6**)	97 ± 7.30
3-Me-6-PH-IP (**3**)	20 ± 0.78
3-Me-IP (**4**)	8.2 ± 0.22
1-Me-IP (**1**)	7.9 ± 0.39

[a] Five sequential concentrations were used; data points were measured in duplicate; r = 0.93-0.99.

Hydroxylamine metabolites (proximate mutagens) of PhIP give rise to nitrenium ions (primary mutagens) forming covalent bonds with DNA bases or undergoing metabolic decomposition [15]. During these steps an increase in mutagenicity and carcinogenicity frequently accompanies HAs that stabilize positive character on the aminoimidazo nitrogen associated with formation of an HA nitrenium ion, *e.g*., one of the electrophilic metabolites of PhIP [1b,e].

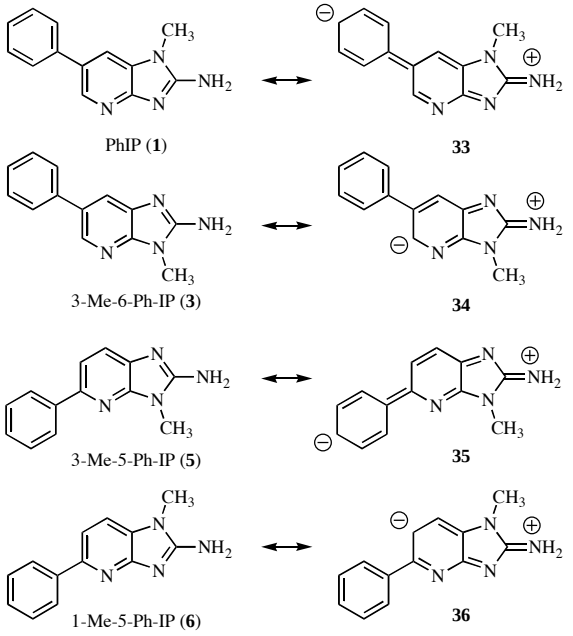


Figure 2. Delocalization between the 2-aminoimidazo[4,5-*b*] amine and phenyl substituents is facilitated by the structure of PhIP and 3-Me-5-Ph-IP.

Despite shortcomings in drawing a SAR using the nonmetabolically activated parent HAs, our data revealed that relative mutagenic activities for these six HAs agree well with an extended resonance effect. Structural isomers that facilitate charge delocalization between the phenyl and 2-aminoimidazo[4,5-*b*]pyridine amine substituents

led to increased mutagenicity. Although the higher mutagenicity for PhIP and in particular 3-Me-5-Ph-IP toward TA98 correlates well with this structural model, the details of the mechanism involved are an open question. It is not clear whether the higher mutagenicity of PhIP and in particular 3-Me-5-Ph-IP results from: (i) facilitated metabolism to HA proximate and ultimate mutagens, or (ii) higher conversion efficiencies of resonance stabilized HA metabolites into DNA-conjugates [16], or (iii) possibly the functional effects of the modified DNA unrelated to electronics of the parent HA [17].

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EXPERIMENTAL

Glassware was dried at 120 °C for 12 hours before use. Procedures employed Schlenck techniques under a nitrogen atmosphere. Solvents were distilled from calcium hydride or sodium/benzophenone, and organic extracts were dried over anhydrous sodium sulfate. All tlc was performed using ANALTECH No. 02521 silica gel uniplates, with tlc and flash chromatography eluents given as a volume ratio. Flash chromatography utilized EM Merck 220–400 mesh, 32–63 μm 60 Å silica gel, or JT Baker octadecyl C-18, 40 μm prep-LC silica gel. Commercially available starting materials were used without further purification. Melting points (uncorrected) were determined on a Mel-Temp II. Nmr spectra were recorded on a Varian MercuryPlus 300VX (300 MHz for ^{1}H and 75 MHz for ^{13}C). Low resolution mass spectra were recorded on a Finnigan LCQ-DUO. All uv spectra were recorded on a Varian Cary-50, and ir spectra (potassium bromide pellets) were obtained on a Perkin-Elmer 1600.

2-Amino-5-phenylpyridine (8). Sodium amide in xylenes (32.3 g, 50 wt%) was combined with toluene (300 mL) and 3-phenylpyridine (15.26 g, 97.1 mmol) was added. The stirred mixture was refluxed for 36 hours, cooled, treated with aqueous potassium hydroxide, and then extracted with ethyl acetate. The extract was dried and filtered, and then concentrated to a dark oil. Flash chromatography on silica gel eluted with methanol:chloroform (1:80-1:20) yielded a tan solid. Crystallization from ethyl acetate provided tan flakes, 8.66 g (52%), tlc R_f = 0.28, methanol:chloroform (1:50), mp = 135-136 °C; ^{1}H nmr (dimethyl sulfoxide-d_6) δ 8.23 (d, J = 2.0 Hz, 1H), 7.68 (dd, J = 2.6, 8.5 Hz, 1H), 7.54 (m, 2H), 7.38 (app. t, J = 7.7 Hz, 2H), 7.25 (app. tt, J = 1.2, 7.3 Hz, 1H), 6.52 (dd, J = 0.6, 8.5 Hz, 1H), 6.02 (br. d, J = 4.1 Hz, NH_2); ^{13}C nmr (chloroform-d) δ 157.9, 146.4, 138.4, 136.6, 129.0, 127.3, 127.0, 126.4, 108.6; ms: *m/z* 172(14), 171(100, M^+ +H), 154(11), 145(3).

2-Amino-3-bromo-5-phenylpyridine (9). Aminopyridine **8** (3.2 g, 18.8 mmol) was combined with acetic acid (30 mL), cooled to 0 °C, stirred, and treated with bromine (1.1 mL) in acetic acid (10 mL) over 5 minutes, then warmed to 20 °C. The slurry was stirred for an additional 30 minutes, concentrated to a solid, and dissolved in water. Sodium hydroxide was added, and the solution was extracted with chloroform. The extract was dried and filtered, and then concentrated to a tan solid, 3.44 g (73%), tlc R_f = 0.34, methanol:chloroform (1:99); ^{1}H nmr (dimethyl sulfoxide-d_6) δ 8.28 (d, J = 2.1 Hz), 8.03 (d, J = 2.1 Hz), 7.58 (m), 7.40 (app. t, J = 8.2 Hz, H-3', H-5'), 7.29 (app. tt, J = 1.8, 7.3 Hz, H-4'), 6.31 (br. s, J = NH_2); ^{13}C nmr (dimethyl sulfoxide-d_6) δ 155.6, 144.9, 137.9, 137.8, 136.4, 135.7, 128.9, 127.7, 126.9, 126.0, 125.7, 124.7, 120.1, 103.4; ms: *m/z* = 252(10), 251(100, M^+ +H, ^{81}Br), 250(11), 249(98, M^+ +H, ^{79}Br).

2-Amino-3-methylamino-5-phenylpyridine (10). Bromopyridine **9** (3.89 g, 15.6 mmol) was combined with cupric sulfate (0.91 g, 3.64 mmol) plus aqueous methylamine (30 mL, 40 wt%) in a Parr vessel and kept at 200 °C for 48 hours, then cooled, poured into water, and extracted with chloroform. The extract was dried and filtered, and then concentrated to an impure blue-green residue that was carried on without purification, 2.89 g (ca. 79%), tlc R_f = 0.34, methanol: chloroform (1:20); ms: *m/z* 201(12), 200(100, M^+ +H).

1-Methyl-6-phenyl-2-*N*-(*p*-toluenesulfonyl)aminoimidazo-[4,5-*b*]pyridine (12). Impure diaminopyridine **10** (2.0 g, *ca*. 8 mmol) was combined with dichloro-*N*-tosylmethanimine (**11**) (2.42 g, 9.60 mmol), potassium carbonate (2.80 g, 20.3 mmol), and dioxane (80 mL). The stirred mixture was heated to 85 °C for 20 hours, cooled, diluted with water, and then extracted with chloroform. The extract was dried and filtered, and then concentrated. Flash chromatograpy on silica gel eluted by methanol:chloroform (1:99) gave a tan solid, 2.75 g (92%), tlc R_f = 0.70, methanol:chloroform (1:20), mp = 315-319 °C (dec); ^{1}H nmr (dimethyl sulfoxide-d_6) δ 12.34 (br. s, NH), 8.46 (d, J = 1.8 Hz), 8.08 (d, J = 1.8 Hz), 7.86 (d, J = 8.1 Hz, 2H), 7.75 (app. d, J = 7.9 Hz, 2H), 7.49 (app. t, J = 7.5 Hz, 1H), 7.39 (m), 7.32 (d, J = 7.8 Hz), 3.49 (s, N-CH_3), 2.34 (s, CH_3); ^{13}C nmr (dimethyl sulfoxide-d_6) δ 149.2, 143.3, 142.2, 141.9, 140.6, 129.3, 125.7, 122.8, 118.5, 118.5, 26.8, 20.8; ir: 3448, 3068, 1591, 1461, 1376, 1272, 1378, 1272, 1138, 1086, 924, 837 cm^{-1}. HRMS: $C_{20}H_{19}N_4O_2S$ (M^+ +H) requires *m/z* 379.1229; found, 379.1237.

1-Methyl-6-phenyl-2-aminoimidazo[4,5-*b*]pyridine; PhIP (1). Sulfonamide **12** (0.43 g, 1.14 mmol) was combined with hydrogen fluoride (8 mL) in a Teflon-lined Parr vessel, heated to 100 °C, and held at that temperature for 2.5 hours. The vessel was cooled and opened, and then concentrated under nitrogen. The residue was washed with benzene, taken up in aqueous sodium hydroxide, and flash chromatographed on C18 silica gel eluted by a methanol:water gradient (1:50-1:5) to yield an impure tan solid. Crystallization from ethanol gave an off a white solid, 96 mg (38%), tlc R_f = 0.26, methanol:chloroform (1:20), mp = 315-317 °C (dec); ^{1}H nmr (methanol-d_4) δ 8.21 (d, J = 1.8 Hz, 1H), 7.70 (d, J = 1.8 Hz, 1H), 7.59 (app. d, J = 8.0 Hz, 2H), 7.40 (app. t, J = 7.6 Hz, 2H), 7.29 (app. tt, J = 1.2, 7.2

Hz, 1H), 3.56 (s, N-CH_3); ^{13}C nmr (dimethyl sulfoxide-d_6) δ 158.0, 156.3, 139.3, 139.1, 128.9, 127.9, 126.6, 126.4, 126.2, 111.7, 28.4; ir: 3294, 3087, 1668, 1593, 1549, 1470, 1440, 1270, 1102, 914, 881, 759 cm^{-1}. HRMS: $C_{13}H_{13}N_4$ (M^+ +H) requires *m/z* 225.1135; found 225.1136.

***N*^{2}-Amino-*N*^{3}-benzyloxyamido-pyridine (13).** 2,3-Diaminopyridine (8.73 g, 79.8 mmol) was combined with pyridine (20 mL) in tetrahydrofuran (200 mL), cooled to 0 °C, and stirred. Benzyl chloroformate (20 mL, 130.0 mmol) in tetrahydrofuran (20 mL) was added over 30 minutes, and the mixture was warmed to 20 °C and then stirred overnight. Water was added, followed by extraction with ethyl acetate. The extract was dried and filtered, then concentrated. Flash chromatography on silica gel eluted by methanol:chloroform (1:20-1:10) yielded an impure tan solid. Trituration from chloroform left a white solid, 4.82 g (25%), tlc R_f = 0.35, methanol:chloroform (1:20), mp = 178-179 °C; ^{1}H nmr (dimethyl sulfoxide-d_6) δ 8.78 (br. s, 1H), 7.73 (dd, *J* = 1.5, 4.8 Hz, 1H), 7.33 (br. d, *J* = 7.6 Hz, 1H), 7.40-7.32 (m, 5H), 6.55 (dd, *J* = 4.8, 7.7 Hz, 1H), 5.76 (br. s, NH_2), 5.13 (s, CH_2-O); ^{13}C nmr (dimethyl sulfoxide-d_6) δ 154.2, 152.7 (br. s), 143.4, 136.6, 130.1 (br. s), 128.4, 128.0, 118.5, 112.3, 65.9; ms: *m/z* 245(15), 244(100, M^+ +H), 228(2), 226(9), 200(2).

2-Amino-3-methylaminopyridine (14). Benzyloxyamidopyridine **13** (0.98 g, 4.03 mmol) in tetrahydrofuran (85 mL) was cooled to 0 °C, combined with lithium aluminum hydride (20 mL, 1.0 *M* tetrahydrofuran), and stirred at 0 °C for 20 minutes. The mixture was refluxed for 3 hours, then cooled and treated with ethyl acetate. Water was added and the solution was acidified with aqueous hydrogen chloride, then washed with diethyl ether. The aqueous phase was cooled and treated with solid potassium hydroxide, and then extracted with diethyl ether. The extract was dried and filtered, then concentrated to yield a tan solid, 0.43 g (87%), tlc R_f = 0.25, methanol:chloroform (1:30), mp = 178-179 °C; ^{1}H nmr (dimethyl sulfoxide-d_6) δ 7.27 (dd, *J* = 4.9, 5.0 Hz, 1H), 6.53 (dd, *J* = 1.8, 7.6 Hz, 1H), 6.47 (dd, *J* = 4.7, 7.6 Hz, 1H), 5.31 (br. s, NH_2), 4.83 (br. app. q, *J* = 4.5 Hz, NH), 2.67 (s, N-CH_3); ^{13}C nmr (chloroform-d) δ 148.9, 136.4, 133.5, 116.4, 116.2, 30.7; ms: *m/z* 125(6), 124(100, M^+ +H), 117(5), 109(2), 97(4), 85(4), 65(1).

1-Methyl-2-aminoimidazo[4,5-*b*]pyridine; 1-Me-IP (2). Diaminopyridine **14** (0.80 g, 6.49 mmol) was combined with cyanogen bromide (0.83 g, 7.83 mmol) in water (20 mL) and stirred at 40 °C for 5 hours before addition of a second quantity of cyanogen bromide (0.25 g, 2.36 mmol). The solution was stirred at 40 °C overnight, then concentrated. Flash chromatography on C18 silica gel eluted by methanol:water (1:3) yielded an off-white solid, 0.41 g (43%), mp = 284-286 °C after crystallization from methanol; ^{1}H nmr (methanol-d_4) δ 7.94 (dd, *J* = 1.4, 5.2 Hz, 1H), 7.43 (dd, *J* = 1.3, 7.7 Hz, 1H), 6.92 (dd, *J* = 5.3, 7.7 Hz, 1H), 3.52 (s, h/2 = 1.1 Hz, N-CH_3); ^{13}C nmr (methanol-d_4) δ 159.2, 156.6, 141.8, 129.5, 115.8, 115.8, 28.8; ir: 3259, 3079, 1680, 1630, 1595, 1546, 1472, 1432, 1267, 1246, 1126, 927, 910, 771 cm^{-1}. HRMS: $C_7H_9N_4$ (M^+ +H) requires *m/z* 149.0821; found 149.0821.

6-Bromo-2-aminoimidazo[4,5-*b*]pyridine (23). 1-Me-IP (2.22 g, 14.98 mmol) in acetic acid (120 mL) at 100 °C was treated with bromine (5.58 g, 34.9 mmol) in acetic acid (10 mL) and the mixture was stirred for 2 hours, then cooled to 50 °C and concentrated. Saturated aqueous potassium carbonate was added, and the solution was extracted with ethyl acetate. The extract was dried and filtered, then concentrated. Flash chromatography on silica gel eluted with methanol:chloroform (1:9) yielded a white solid, 0.51 g (15%), tlc R_f = 0.22, methanol:chloroform (1:9), mp = 178-179 °C; ^{1}H nmr (dimethyl sulfoxide-d_6) δ 7.98 (d, *J* = 2.2 Hz, 1H), 7.68 (d, *J* = 2.2 Hz, 1H), 7.04 (br s, NH_2), 3.49 (s, N-CH_3); ^{13}C nmr (methanol-d_4) δ159.9, 155.7, 142.3, 130.5, 118.4, 110.6, 29.0; ir: 3299, 3134, 1669, 1621, 1588, 1544, 1461, 1428, 1296, 1263, 1103, 938, 861, 773 cm^{-1}. HRMS: $C_7H_7BrN_4$ (M^+ +H, ^{79}Br) requires *m/z* 226.9934; found 225.9926.

2-Bromo-5-phenylpyridine (15). Aminopyridine **8** (3.1 g, 18.21 mmol) was combined with aqueous hydrogen bromide (10.75 mL, 48 wt%) at –5 °C. The stirred solution was treated with bromine (1.0 mL, 19.5 mmol) and aqueous hydrogen chloride (4.5 mL), followed by dropwise addition of sodium nitrite (4.8 g, 69.6 mmol) in water (20 mL) over 15 minutes. After stirring at –5 °C for 1 hour, aqueous sodium hydroxide (11.0 mL, 40 wt%) was added, followed by extraction with chloroform. The extract was dried and filtered, and then concentrated to an impure brown solid, 4.10 g (95%), tlc R_f = 0.65, chloroform; ^{1}H nmr (dimethyl sulfoxide-d_6) δ 8.70 (dd, *J* = 0.7, 2.6 Hz, 1H), 8.03 (dd, *J* = 2.6, 8.2 Hz, 1H), 7.71 (m, 2H), 7.53-7.40 (m, 4H); ^{13}C nmr (dimethyl sulfoxide-d_6) δ 148.3, 140.3, 137.5, 135.7, 135.3, 129.2, 128.6, 128.1, 126.9; ms: *m/z* 237(9), 236(96, M^+ +H, ^{81}Br), 235(10), 234(100, M^+ +H, ^{79}Br), 172(10), 156(12).

2-Methylamino-5-phenylpyridine (16). Bromopyridine **15** (3.83 g, *ca*. 16 mmol) was combined with cupric sulfate (0.82 g, 3.28 mmol) plus aqueous methylamine (23 mL, 40 wt%) in a Parr vessel and kept at 200 °C for 48 hours, then cooled and poured into water, and then extracted with chloroform. The extract was dried and filtered, and then concentrated. Flash chromatography on silica gel eluted by methanol:chloroform (1:50) yielded a tan solid, 1.85 g (63%), tlc R_f = 0.31, methanol:chloroform (1:90), mp = 120-122 °C; ^{1}H nmr (dimethyl sulfoxide-d_6) δ 8.31 (dd, *J* = 0.6, 2.6 Hz, 1H), 7.69 (dd, *J* = 2.6, 8.7 Hz, 1H), 7.55 (m, 2H), 7.39 (app. t, *J* = 8.1 Hz, 2H), 7.25 (app. tt, *J* = 1.8, 7.3 Hz, 1H), 6.58 (br. q, *J* = 4.3 Hz, NH), 6.52 (dd, *J* = 0.7, 8.7 Hz, 1H), 2.79 (br. s, N-CH_3); ^{13}C nmr (dimethyl sulfoxide-d_6) δ 158.9, 158.8, 145.6, 138.2, 134.9, 128.9, 126.2, 125.3, 123.4, 107.7, 28.0, 27.9; ms: *m/z* 186(13), 185(100, M^+ +H), 178(2), 160(6).

2-Methylamino-3-bromo-5-phenylpyridine (17). Methylaminopyridine **16** (1.05 g, 5.69 mmol) in acetic acid (25 mL) was cooled to 0 °C and treated with bromine (0.35 mL, 6.83 mmol) in acetic acid (10 mL), then warmed from 0 °C to 20 °C over 30 minutes, and then concentrated. Cold aqueous sodium hydroxide was added, and the solution was extracted with chloroform. The extract was dried and filtered, then concentrated. Flash chromatography on silica gel eluted with chloroform yielded a tan solid, 1.46 g (97%), tlc R_f = 0.56, chloroform, mp = 68-69 °C; ^{1}H nmr (dimethyl sulfoxide-d_6) δ 8.38 (d, *J* = 2.4 Hz, 1H), 8.03 (d, *J* = 2.1 Hz, 1H), 7.58 (m, 2H), 7.40 (app. t, *J* = 8.2 Hz, 2H), 7.28 (app. tt, *J* = 1.8, 7.3 Hz, 1H), 6.46 (br. q, *J* = 4.6 Hz, NH), 2.88 (d, *J* = 4.7 Hz, N-CH_3); ^{13}C nmr (dimethyl sulfoxide-d_6) δ 154.4, 144.5, 137.4, 136.5, 129.0, 126.8, 125.7, 125.1, 104.9, 28.7. HRMS: $C_{12}H_{12}N_2$ (M^+ +H, ^{79}Br) requires *m/z* 263.0184; found 263.0192.

2-Methylamino-3-amino-5-phenylpyridine (18). Bromopyridine **17** (1.50 g, 5.69 mmol) was combined with cupric sulfate (0.14 g, 0.56 mmol) plus aqueous ammonia (35 mL, 28 wt%) in a stainless steel Parr vessel. The mixture was heated at 200 °C for 48 hours, then cooled and poured into water, and then extracted with chloroform. The extract was dried and filtered,

and then concentrated to yield an impure blue-green solid that was carried on without purification, 1.08 g (*ca*. 82%), tlc R_f = 0.40, methanol:chloroform (1:20); ^{1}H nmr (dimethyl sulfoxide-d_6) δ 7.72 (d, *J* = 1.8 Hz, 1H), 7.48 (app. d, *J* = 7.2 Hz, 2H), 7.37 (app. t, *J* = 7.2, 2H), 7.23 (app. t, *J* = 7.3 Hz, 1H), 6.98 (d, *J* = 1.8 Hz, 1H), 5.76 (br. d, *J* = 4.8 Hz, NH), 4.73 (app. br. d, *J* = 7.4 Hz, NH_2), 2.88 (d, *J* = 4.7 Hz, N-CH_3); ms: *m/z* 201(12), 200(100, M^+ +H).

3-Methyl-6-phenyl-2-*N*-(*p*-toluenesulfonyl)-aminoimidazo-[4,5-*b*]pyridine (19). Impure diaminopyridine **18** (1.08 g) was combined with dichloro-*N*-tosylmethanimine (**11**) (1.76, 6.98 mmol) and potassium carbonate (1.31 g, 13.8 mmol) in dioxane (80 mL), and the solution was stirred at 85 °C for 14 hours. The cooled solution was poured into water and extracted with chloroform. The extract was dried and filtered, and then concentrated. Flash chromatography on silica gel eluted by methanol:chloroform (1:99) yielded a white solid, 1.57 g (ca. 96%), tlc R_f = 0.33, methanol:chloroform (1:99), mp = 228-230 °C (dec); ^{1}H nmr (chloroform-*d*) δ 10.52 (br. s, NH), 8.40 (d, *J* = 1.8 Hz, 1H), 7.89 (app. d, *J* = 8.4 Hz, 2H), 7.66 (d, *J* = 1.8 Hz, 1H), 7.55-7.41 (app. m), 7.29 (app. d, *J* = 8.1 Hz, 2H), 3.60 (s, N-CH_3), 2.40 (s, CH_3); ^{13}C nmr (chloroform-*d*) δ 150.5, 143.8, 143.3, 143.0, 141.9, 140.4, 139.4, 137.9, 133.1, 129.9, 129.7, 129.4, 128.2, 127.5, 126.7, 126.3, 122.8, 116.7, 27.4, 21.7; ir: 3357, 3261, 2924, 1598, 1489, 1340, 1304, 1254, 1159, 1133, 1086, 1004, 837, 814 cm^{-1}. HRMS: $C_{20}H_{19}N_4O_2S$ (M^+ +H) requires *m/z* 379.1229; found 379.1237.

3-Methyl-6-phenyl-2-aminoimidazo[4,5-*b*]pyridine; 3-Me-6-Ph-IP (3). Sulfonamide **19** (1.34 g, 3.54 mmol) was combined with hydrogen fluoride (10 mL) in a Teflon-lined Parr vessel and held at 100 °C for 2.5 hours. The flask was cooled and opened, and then evaporated under nitrogen. The residue was washed with benzene, taken up in aqueous sodium hydroxide, then flash chromatographed on C18 silica gel eluted by methanol:water (1:50-1:5) to yield a tan solid, 0.34 g (43%), mp = 222-223 °C after trituration from methanol; ^{1}H nmr (dimethyl sulfoxide-d_6) δ 8.14 (d, *J* = 1.8 Hz, 1H), 7.66 (app. d, *J* = 1.3 Hz, 1H), 7.63 (app. d, *J* = 7.8 Hz, 2H), 7.44 (app. t, *J* = 7.9 Hz, 2H), 7.33 (app. tt, *J* = 2.4, 7.5 Hz, 1H), 6.83 (br. s, NH_2), 3.53 (s, N-CH_3); ^{13}C nmr (dimethyl sulfoxide-d_6) δ 156.7, 147.8, 139.1, 136.1, 136.0, 129.7, 128.9, 126.8, 118.3, 27.0; ir: 3445, 3029, 1652, 1547, 1494, 1464, 1410, 1361, 760 cm^{-1}; ms: 226(15), 225(100, M^+ +H). HRMS: $C_{13}H_{13}N_4$ (M^+ +H) requires *m/z* 225.1134; found 225.1131.

2-Methylamino-3-aminopyridine (21). Chloropyridine **20** (7.72 g, 60.0 mmol) was combined with cupric sulfate (1.49 g, 6.0 mmol) and aqueous methylamine (45 mL, 40 wt%) in a stainless steel Parr vessel, and the mixture was held at 170 °C for 24 hours. The cooled mixture was treated with aqueous potassium carbonate and continuously extracted for 24 hours using diethyl ether. The extract was dried and filtered, and then concentrated. Flash chromatography on silica gel eluted by ethyl acetate:hexane (2:3) yielded a tan solid, 3.91 g (53%), tlc R_f = 0.34, methanol:chloroform (1:20); ^{1}H nmr (dimethyl sulfoxide-d_6) δ 7.38 (dd, *J* = 5.1, 1.6 Hz, 1H), 6.65 (dd, *J* = 7.26, 1.6 Hz, 1H), 6.32 (dd, *J* = 7.5, 4.8 Hz, 1H), 5.54 (br. app. d, *J* = 4.2 Hz, NH), 4.58 (br. s, NH_2), 2.81 (d, *J* = 4.8 Hz, N-CH_3); ^{13}C nmr (dimethyl sulfoxide-d_6) δ 148.6, 135.0, 130.2, 117.1, 112.0, 28.1; ir: 3318, 3091, 1657, 1606, 1520, 1475, 1442, 1412, 1371, 1302, 1259, 1156, 1126, 1085, 786 cm^{-1}; ms: *m/z* 124 (M^+ +H).

3-Methyl-2-*N*-(*p*-toluenesulfonyl)-aminoimidazo[4,5-*b*]pyridine (22). Diaminopyridine **21** (3.76 g, 30.5 mmol) was combined with dichloro-*N*-tosylmethanimine (**11**) (8.41 g, 33.4 mmol) and potassium carbonate (10.92 g, 79.6 mmol) in dioxane (120 mL) and stirred at 85 °C for 20 hours. The mixture was cooled and diluted with water, and then extracted with chloroform. The extract was dried and filtered, and then concentrated. Flash chromatography on silica gel eluted by methanol:chloroform (1:99) provided a tan solid, 4.16 g (45%), tlc R_f = 0.27, methanol:chloroform (1:99), mp = 208-209 °C; ^{1}H nmr (dimethyl sulfoxide-d_6) δ 11.91 (br. s, NH), 8.14 (dd, *J* = 5.1, 1.3 Hz, 1H), 7.81 (app. d, *J* = 6.8 Hz, 2H), 7.66 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.32 (app. d, *J* = 7.9 Hz, 2H), 7.19 (dd, *J* = 7.8, 5.1 Hz, 1H), 3.41 (s, N-CH_3), 2.33 (s, CH_3); ^{13}C nmr (dimethyl sulfoxide-d_6) δ 149.2, 143.3, 142.2, 141.9, 140.6, 129.3, 125.7, 122.8, 118.5, 118.5, 26.8, 20.8; ir: 3294, 1628, 1594, 1492, 1416, 1300, 1282, 1138, 1087, 1003, 897, 833, 786 cm^{-1}; ms: *m/z* = 325.10 (M^+ +Na).

3-Methyl-2-aminoimidazo[4,5-*b*]pyridine; 3-Me-IP (4). Sulfonamide **22** (0.95 g, 3.14 mmol) was combined with hydrogen fluoride (8 mL) in a Teflon-lined Parr vessel and held at 100 °C for 2.5 hours. The vessel was cooled and opened, and then concentrated under nitrogen. The residue was washed with benzene, taken up in aqueous sodium hydroxide, then flash chromatographed on C18 silica gel eluted by methanol:water (1:50-1:5) to yield a tan solid, 0.32 g (68%), mp = 220-221 °C after trituration with ethanol; ^{1}H nmr (dimethyl sulfoxide-d_6) δ 7.83 (dd, *J* = 1.4, 5.1 Hz, 1H), 7.37 (dd, *J* = 1.2, 7.6 Hz, 1H), 6.93 (dd, *J* = 5.1, 7.7 Hz, 1H), 6.73 (br. s, NH_2), 3.49 (s, N-CH_3); ^{13}C nmr (dimethyl sulfoxide-d_6) δ 155.9, 148.0, 137.4, 135.6, 120.0, 116.6, 26.9; ir: 3317, 3135, 1655, 1609, 1552, 1493, 1407, 1290, 1217, 1112, 930, 766 cm^{-1}; ms: *m/z* 149(100), 135(1), 134(5), 132(2). HRMS: $C_7H_9N_4$ (M^+ +H) requires *m/z* 149.0821; found 149.0816.

5-Bromo-3-methyl-2-aminoimidazo[4,5-*b*]pyridine (24). 3-Me-IP (0.32 g, 2.16 mmol) was combined with acetic acid (35 mL), and the stirred solution was heated at 100 °C. Bromine (0.30 mL, 5.8 mmol) in acetic acid (10 mL) was added. The mixture was stirred for 1 hour, concentrated, taken up in aqueous potassium carbonate, and then extracted with ethyl acetate. The extract was dried, filtered, and then concentrated. Flash chromatography on silica gel eluted by methanol: chloroform (1:20) yielded a tan solid, 0.38 g (77%), tlc R_f = 0.25, methanol:chloroform (1:20), mp = 198-201 °C (dec); ^{1}H nmr (dimethyl sulfoxide-d_6) δ 7.33 (d, *J* = 7.9 Hz, 1H), 7.10 (d, *J* = 8.1 Hz, 1H), 6.91 (br. s, NH_2), 3.47 (s, N-CH_3); ^{13}C nmr (methanol-d_4) δ157.9, 148.9, 135.5, 130.4, 124.2, 121.6, 27.6; ir: 3450, 3308, 3084, 2282, 1659, 1626, 1548, 1496, 1396, 1257, 1115, 1090, 939, 831, 806, 763 cm^{-1}. HRMS: $C_7H_8N_4Br$ (M^+ + H, ^{79}Br) requires *m/z* 226.9932; found 226.9929.

3-Methyl-5-phenyl-2-aminoimidazo[4,5-*b*]pyridine; 3-Me-5-Ph-IP (5). Bromoimidazopyridine **24** (1.11g, 4.89 mmol) was combined with phenylboronic acid (1.37 g, 11.21 mmol), tetrakis(triphenylphosphine)palladium (0.60 g, 5.19 x 10^{-4} mol) and dioxane (70 mL). Potassium carbonate (2.81 g, 20.3 mmol) in water (15 mL) and ethanol (18 mL) was added, and the mixture was heated overnight at 85 °C. The mixture was cooled and diluted with brine, then extracted with ethyl acetate. The extract was dried and filtered, then concentrated. Flash chromatography on silica gel eluted by methanol:chloroform (1:20) yielded a tan solid, 0.65 g (59%), tlc R_f = 0.26, methanol:chloroform (1:20), mp = 209-210 °C after crystallization from methanol; ^{1}H nmr (dimethyl sulfoxide-d_6) δ 8.05 (app. d, *J* = 7.9 Hz, 2H), 7.55 (d, *J* = 8.0 Hz, 1H), 7.44

(app. d, *J* = 8.2 Hz, 1H), 7.45-7.39 (m, 2H), 7.30 (app. tt, *J* = 0.9, 7.3 Hz, 1H), 6.84 (br. s, NH_2) 3.60 (s, N-CH_3); ^{13}C nmr (dimethyl sulfoxide-d_6) δ 156.6, 148.1, 144.7, 139.7, 135.4, 128.5, 127.3, 125.7, 120.6, 113.5, 26.9; ir: 3442, 3312, 3056, 1654, 1542, 1495, 1409, 1300, 1247, 1126, 828, 758 cm^{-1}. HRMS: $C_{13}H_{13}N_4$ (M^+ +H) requires *m/z* 225.1140; found 225.1143.

3-Methyl-5-(phenyl-$^2H_5$)-2-aminoimidazo[4,5-*b*]pyridine (25). The procedure for **5** was employed with bromopyridine **24** (0.33 g, 1.45 mmol), phenyl-($^2H_5$)-boronic acid (0.37 g, 2.95 mmol), tetrakis(triphenylphosphine)palladium (0.14 g, 1.19 x 10-4 mol), and dioxane (35 mL). Flash chromatography on silica gel eluted by methanol:chloroform (1:20) yielded a tan solid, 0.23 g (71%), tlc R_f = 0.24-0.29, methanol:chloroform (1:20), mp = 209-210 °C after crystallization from methanol; ^1H nmr (dimethyl sulfoxide-d_6) δ 7.55 (app. d, *J* = 8.1 Hz, 1H), 7.44 (app. d, *J* = 8.0 Hz, 1H), 6.84 (br. s, NH_2) 3.60 (s, N-CH_3); ^{13}C nmr (dimethyl sulfoxide-d_6) δ 156.6, 148.1, 144.6, 139.6, 135.4, 129.0 (t, J_{CD} = 23.2 Hz), 126.8 (t, J_{CD} = 22.3 Hz), 125.3 (t, J_{CD} = 22.9 Hz), 120.6, 113.5, 26.9; ir: 3442, 3048, 1653, 1539, 1495, 1494, 1414, 1382, 1316, 1286, 1227, 1125, 819, 762 cm^{-1}. HRMS: $C_{13}H_8{}^2H_5N_4$ (M^+ +H) requires *m/z* 230.1454; found 230.1446.

2-Amino-6-phenylpyridine (27). The solvent was decanted off sodium amide in xylenes, leaving a semidry solid (11.7 g, *ca*. 0.30 mol), which was combined with phenylpyridine **26** (9.71 g, 62.5 mmol) in *N,N*-dimethylaniline (30 mL) and stirred at 170 °C for 4 hours under a slow flow of nitrogen. The cooled mixture was treated with ice followed by aqueous potassium hydroxide, and extracted with diethyl ether. The extract was dried and filtered, then concentrated. Flash chromatography on silica gel eluted by a ethyl acetate:hexane:methanol (1:5:0.15) yielded a tan solid, 8.62 g (81%), tlc R_f = 0.33, ethyl acetate:hexane (1:5), mp = 64-65 °C; ^1H nmr (dimethyl sulfoxide-d_6) δ 7.95 (m, *J* = 1.4, 8.0 Hz, 2H), 7.31-7.47 (m, 4H), 7.03 (dd, *J* = 0.8, 7.4 Hz, 1H), 6.43 (dd, *J* = 0.7, 8.0 Hz, 1H), 5.94 (br. s, NH_2); ^{13}C nmr (dimethyl sulfoxide-d_6) δ 159.6, 154.4, 139.4, 138.0, 128.5, 128.4, 126.3, 108.4, 107.1; ir: 3469, 3383, 3330, 3188, 3055, 1955, 1613, 1572, 1466, 1449, 1344, 1274, 984, 805, 762 cm^{-1}; ms: *m/z* 172(12), 171(100, M^+ +H).

2-Pivalylamido-6-phenylpyridine (28). Aminopyridine **27** (0.78 g, 4.58 mmol) was combined with dry tetrahydrofuran (20 mL) and triethylamine (6.5 mL) and cooled to 0 °C. The solution was treated with pivaloyl chloride (2.14 g, 17.7 mmol) at 0 °C, then stirred at 20 °C for 5 hours. Methylene chloride was added, and the solution was diluted with aqueous sodium hydroxide then extracted with chloroform. The extract was dried and filtered, and then concentrated. Flash chromatography on silica gel eluted by ethyl acetate:hexane (1:5) provided a white solid, 0.65 g (56%), tlc R_f = 0.65, ethyl acetate:hexane (1:5); mp = 93-94 °C; ^1H nmr (dimethyl sulfoxide-d_6) δ 9.58 (br. s, NH), 8.11 (app. dd, *J* = 1.6, 8.2 Hz, 2H), 7.99 (dd, *J* = 0.6, 8.2 Hz, 1H), 7.84 (app. t, *J* = 7.9 Hz, 1H), 7.65 (dd, *J* = 0.9, 7.6 Hz, 1H), 7.39-7.55 (br. m, 3H), 1.27 (s, tert-butyl); ^{13}C nmr (dimethyl sulfoxide-d_6) δ 177.1, 154.5, 151.9, 138.9, 138.1, 129.0, 128.5, 126.6, 115.5, 112.9, 39.3, 26.9; ir: 3510, 3285, 2967, 2874, 1952, 1763, 1664, 1573, 1569, 1441, 1382, 1291, 1158, 933, 759 cm^{-1}. HRMS: $C_{16}H_{19}N_2O_2$ (M^+ +H) requires *m/z* 255.1482; found 225.1482.

2-Pivalylamido-3-bromo-6-phenylpyridine (30). Pivaloylamide **28** (0.12 g, 0.47 mmol) was combined with tetramethyl ethylenediamine (0.21 mL) and diethyl ether (5.0 mL), and the stirred solution was cooled to –70 °C, then treated with butyl lithium (0.81 mL, 1.6 *M* in hexanes). The resulting paste was warmed to 20 °C over 1.5 hours, recooled to –70 °C, then treated with tetrabromomethane (0.42 g, 1.25 mmol) in diethyl ether (4.0 mL). After 15 minutes at –70 °C, the mixture was slowly warmed to 20 °C, poured into water, diluted with aqueous sodium hydroxide, and then extracted with ethyl acetate. The extract was dried and filtered, and then concentrated. Flash chromatography on silica gel eluted by ethyl acetate:hexane (1:10) yielded white flakes, 0.11 g (70%), tlc R_f = 0.23, ethyl acetate:hexane (1:10); mp = 110-111 °C; ^1H nmr (dimethyl sulfoxide-d_6) δ 9.78 (br. s, NH), 8.18 (d, *J* = 8.2 Hz, 1H), 8.03 (m, 2H), 7.81 (d, 8.4 Hz, 1H), 7.42-7.53 (app. m, 3H), 1.26 (s, tert-butyl); ^{13}C nmr (dimethyl sulfoxide-d_6) δ 176.5, 154.4, 149.6, 142.7, 137.0, 129.5, 128.8, 126.5, 119.8, 117.4, 38.7, 27.1; ir: 3312, 2966, 2871, 1666, 1573, 1506, 1437, 1174, 1026, 934, 830, 772 cm^{-1}. HRMS: $C_{16}H_{18}N_2OBr$ (M^+ +H, ^{79}Br) requires *m/z* 333.0597; found 333.0587.

2-Amino-3-bromo-6-phenylpyridine (31). Pivalylamide **30** (0.21 g, 0.63 mmol) in aqueous hydrochloric acid (15 mL, 3.0 *M*) was stirred at 75 °C for 12 hours, then cooled and poured over ice. Water was added, and the solution was treated with aqueous ammonium hydroxide then extracted with chloroform. The extract was dried and filtered, and then concentrated. Flash chromatography on silica gel eluted by ethyl acetate:hexane (1:10) yielded a white solid, 0.15 g (96%), tlc R_f = 0.46, ethyl acetate:hexane (1:10); mp = 97-98 °C; ^1H nmr (chloroform-*d*) δ 7.91 (dd, *J* = 1.8, 8.4 Hz, 2H), 7.70 (d, *J* = 7.9 Hz, 1H), 7.28-7.47 (app. m, 3H), 6.99 (d, *J* = 7.9 Hz, 1H), 4.97 (br. s, NH_2); ^{13}C nmr (chloroform-*d*) δ 155.5, 155.3, 141.1, 129.2, 128.9, 126.9, 112.3, 103.2; ir: 3482, 3316, 3176, 3056, 1963, 1778, 1628, 1565, 1457, 1322, 1276, 1151, 1053, 1024, 995, 804, 765 cm^{-1}. HRMS: $C_{11}H_{10}N_2Br$ (M^+ +H, ^{79}Br) requires *m/z* 249.0020; found 249.0020.

2-Amino-3-methylamino-6-phenylpyridine (32). Bromopyridine **31** (0.78 g, 3.13 mmol) was combined with cupric sulfate (0.31 g, 1.24 mmol) plus aqueous methylamine (35 mL, 40 wt%) in a stainless steel Parr vessel. The mixture was held at 200 °C for 60 hours, cooled, poured into water, then extracted with chloroform. The extract was dried and filtered, then concentrated to yield an impure blue-green solid that was carried on without purification, 0.74 g (ca. 80%), tlc R_f = 0.52, methanol:chloroform (1:20); ms: *m/z* 201(12), 200(100, M^+ +H).

1-Methyl-5-phenyl-2-aminoimidazo[4,5-*b*]pyridine; 1-Me-5-Ph-IP (6). Impure diaminopyridine **32** (0.81 g, *ca*. 3.4 mmol) was combined with cyanogen bromide(0.34 g3.2 mmol) in ethanol (40 mL) and stirred at 32 °C for 8 hours. Additional cyanogen bromide (0.46 g, 4.3 mmol) was added, and the mixture was stirred for 12 hours then concentrated. Flash chromatography on silica gel eluted by methanol:chloroform (1:10) yielded a brown solid, 0.15 g (19%), tlc R_f = 0.32, methanol:chloroform (1:10); mp = 272-273 °C (dec) after trituration from ethanol; ^1H nmr (dimethyl sulfoxide-d_6) δ 8.01 (d, *J* = 8.4 Hz, 2H), 7.39-7.50 (app. m, 4H), 7.30 (app. tt, *J* = 0.6, 1.8 Hz, 1H), 6.92 (br. s, NH_2), 3.52 (s, N-CH_3); ^{13}C nmr (dimethyl sulfoxide-d_6) δ 157.9, 156.6, 148.0, 140.4, 128.4, 127.3, 127.1, 126.0, 114.0, 110.4, 28.4; ir: 3443, 3314, 3125, 1664, 1545, 1477, 1422, 1274, 1238, 1147, 1101, 936, 818, 764 cm^{-1}. HRMS: $C_{13}H_{13}N_4$ (M^+ +H) requires *m/z* 225.1140; found 225.1135.

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[11] bis(6-Phenylpyridin-2-yl)amine ($C_{22}H_{17}N_3$); tlc R_f = 0.31, hexane:chloroform (1:10, v/v); mp = 123-124 °C; ^{1}H nmr (dimethyl sulfoxide-d_6) δ 9.77 (br. s, h/2 = 8.2 Hz, NH), 8.07-8.10 (m, 4H), 7.76-7.84 (m, 4H), 7.40-7.53 (m, 8H); ^{13}C nmr (chloroform-*d*) δ 155.96, 153.94, 139.76, 138.68, 129.02, 128.90, 127.08, 113.31, 110.36. ir 3407, 3066, 3030, 1961, 1912, 1850, 1592, 1564, 1513, 1441, 1331, 1283, 1182, 1163, 988, 803 cm^{-1}. *Anal*. Calcd. for $C_{22}H_{18}N_3$ (M^+ +H) m/z 324.1495. Found: hrms; m/z 324.1509.

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