Syntheses of 1,5,6-Trimethyl-2-aminoimidazo[4,5-*b*]pyridine and 3,5,6-Trimethyl-2-aminoimidazo[4,5-*b*]pyridine

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Two potential mutagens found in food, 1,5,6-trimethyl-2-aminoimidazo[4,5-*b*]pyridine and 3,5,6-trimethyl-2-aminoimidazo[4,5-*b*]pyridine, are synthesized.

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Diet is one of the most significant environmental causes of cancer [1]. Recently, a series of mutagenic and carcinogenic compounds has been isolated from cooked meat and fish [2-6], with a common source being protein reaction products formed during the heating of protein-containing foods. These heterocyclic amines are isolated and purified from cooked food by using the *Ames/Salmonella* mutagenicity assay to monitor the fractionation. The compounds are highly potent mutagens that are produced when beef or other muscle foods are cooked by conventional methods at temperatures from 150° to 300° [7,8]. One pathway for the formation of these heterocyclic amines is postulated to result from Maillard reactions involving creatinine, sugars, and amino acids present in raw meat and fish [9-11]. The imidazole portion of the molecule is thought to originate from creatinine, while the pyridine portion is from Strecker degradation products formed in a Maillard reaction between the hexose and the appropriate amino acid [11]. Aldol condensations have been suggested to link the two parts *via* a Strecker aldehyde [12,13].

To assess the risk that consumption of these mutagens pose to humans, it is essential to isolate, identify, and synthesize these compounds. Some of the mutagenic aromatic amines have been identified and synthesized, but one that contributes 10-15% of the total mutagenic activity of the fried meat sample [3-5] has only been identified by mass spectra to have a molecular weight of 176 [4,5]. From the available preliminary data, the mutagenic compound was determined to be one of the twelve isomers of 2-aminotrimethylimidazopyridine (TMIP) [4]. The amino acid isoleucine is thought to give rise to this type of molecule (Figure 1). To investigate the biological risk associated with ingesting this unidentified compound, we synthesized two of the possible isomers, 1,5,6-trimethyl-2-aminoimidazo[4,5-*b*]pyridine (**5**) and 3,5,6-trimethyl-2-aminoimidazo[4,5-*b*]pyridine (**10**), so that they can be compared and tested against the unknown mutagen. The syntheses of these compounds have not previously been reported in the literature.

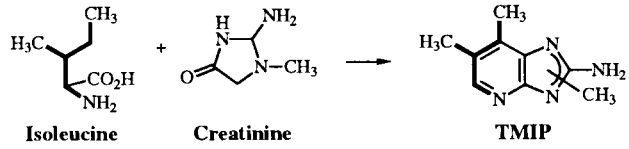
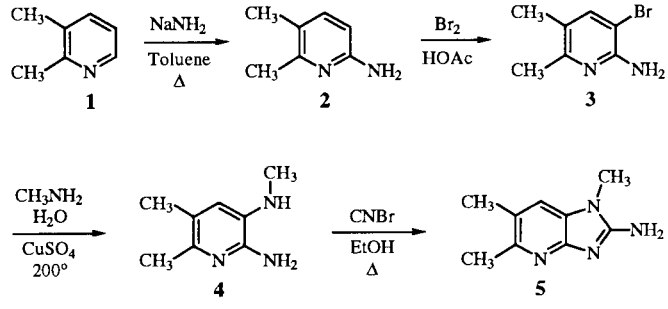


Figure 1. Possible biological source of mutagen.

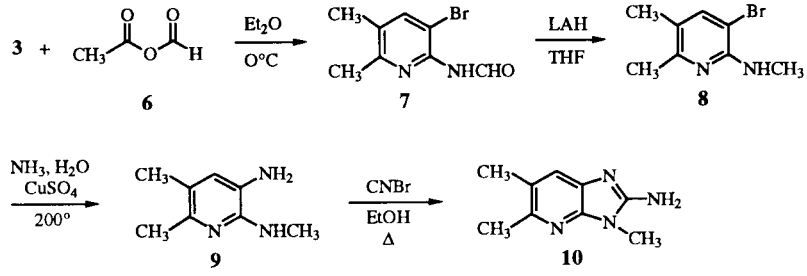
The synthesis of the potential food mutagen 1,5,6-trimethyl-2-aminoimidazo[4,5-*b*]pyridine (**5**) is outlined in Scheme I. Treatment of commercially available 2,3-dimethylpyridine (**1**) with sodium amide suspended in toluene *via* a Chichibabin reaction produces 2-amino-5,6-dimethylpyridine (**2**). Bromination of the amine **2** in acetic acid gives 2-amino-3-bromo-5,6-dimethylpyridine (**3**) in high yield. Treatment of the bromo-compound **3** with 40% aqueous methylamine and copper sulfate in a stainless steel bomb produces 2-amino-5,6-dimethyl-3-(methylamino)pyridine (**4**). The diamine (**4**) in ethanol is reacted with cyanogen bromide in a Teflon-lined bomb at 120° to give the desired final product (**5**). When base was present and a longer reaction time and lower temperature were used, a higher yield of final product (**5**) was obtained.

Scheme I. Synthesis of 1,5,6-trimethyl-2-aminoimidazo[4,5-*b*]pyridine (5)



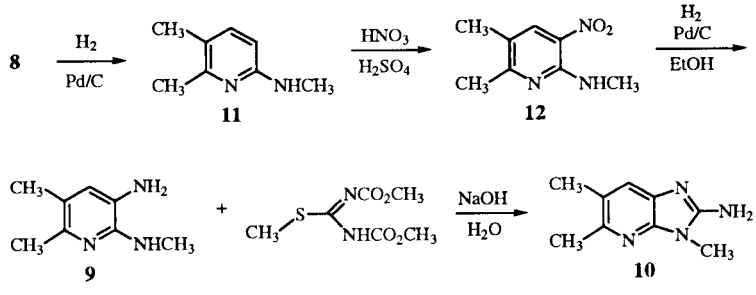
The synthesis of another isomeric food mutagen, 3,5,6-trimethyl-2-aminoimidazo[4,5-*b*]pyridine (**10**), is outlined in Scheme II. The synthesis of the starting material, 2-amino-3-bromo-5,6-dimethylpyridine (**3**), is shown in Scheme I. Formylation of **3** with acetic formic anhydride (**6**) [14] gives 3-bromo-5,6-dimethyl-2-(formylamino)-pyridine (**7**), which is then reduced with lithium aluminum hydride to yield 3-bromo-5,6-dimethyl-2-(methylamino)pyridine (**8**). Treatment of **8** with concentrated ammonium hydroxide and copper sulfate in a stainless steel bomb forms 3-amino-5,6-dimethyl-2-(methylamino)pyridine (**9**). However, it was not possible to completely purify this product. Chromatographies, tritura-

Scheme II. Attempted synthesis of 3,5,6-trimethyl-2-aminoimidazo[4,5-*b*]pyridine (**10**)



tions, and recrystallization were performed without success. The impure material **9** was placed in a bomb with cyanogen bromide, as had been done with the previous synthesis, to make the final product **10**. Repeated efforts varying the conditions of this cyclization failed to yield more than trace amounts of the desired final product, 3,5,6-trimethyl-2-aminoimidazo[4,5-*b*]pyridine (**10**). To overcome these difficulties, we developed the successful alternative approach shown in Scheme III.

Scheme III. Synthesis of 3,5,6-trimethyl-2-aminoimidazo[4,5-*b*]pyridine (**10**)



Dehalogenation of **8** by using hydrogen with palladium on carbon produced a nearly quantitative yield of 5,6-dimethyl-2-(methylamino)pyridine (**11**). Nitration of **11** yielded 5,6-dimethyl-2-methylamino-3-nitropyridine (**12**), which was reduced with hydrogen by using palladium on carbon to give 3-amino-5,6-dimethyl-2-(methylamino)-pyridine (**9**). This material was readily purifiable and identical to the material made by the other route. The thiopseudourea ester **13** was synthesized *in situ* from 2-methyl-2-thiopseudourea sulfate and methyl chloroformate [15]. After the *p*H had been adjusted to 4-5, 3-amino-5,6-dimethyl-2-(methylamino)pyridine (**9**) was added and the reaction mixture was refluxed. The product of the reaction was the desired 3,5,6-trimethyl-2-aminoimidazo[4,5-*b*]pyridine (**10**). The compounds **5** and **10** are undergoing biological testing.

EXPERIMENTAL

Melting points (uncorrected) were obtained using a Thomas-Hoover melting point apparatus. The ir spectra were recorded on a Perkin Elmer 1310 spectrophotometer, the uv spectra on a Varian DMS-90 spectrometer and the nmr spectra were recorded on a Varian Gemini 300-MHz spectrometer. All chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane. The nmr multiplicities are reported using the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; and br, broad. Column chromatography was done using E. Merck silica gel 40 (70-230 mesh, ASTM). All solvents were dried over 3Å molecular sieves, except tetrahydrofuran, which was dried by refluxing over sodium with benzophenone ketyl as an indicator. Microanalyses were performed by Desert Analytics, Tucson, AZ.

Since 1,5,6-trimethyl-2-aminoimidazo[4,5-*b*]pyridine (5) and 3,4,6-trimethyl-2-aminoimidazo[4,5-*b*]pyridine (10) are potential carcinogens and mutagens, direct contact should be avoided.

2-Amino-5,6-dimethylpyridine (**2**).

A mixture of 20.0 g (187 mmoles) of 2,3-dimethylpyridine (**1**) and 14.5 g (187 mmoles) of a 50% suspension of sodium amide in toluene under argon was refluxed for 30 hours. After the reaction mixture was cooled to 0°, 150 ml of 50% aqueous sodium hydroxide solution was added to the black solid. When the reaction had ceased, the mixture was extracted with dichloromethane (3 x 200 ml). The organic fraction was dried (sodium sulfate), filtered, and evaporated *in vacuo* to yield a black oil. The oil was distilled at 80-105° and 0.4 mm of Hg to give 7.79 g (48% based on recovered **1**) of a white solid, mp 41-43°; ^{1}H nmr (deuteriochloroform): δ 2.12 (s, 3H), 2.32 (s, 3H), 4.36 (br s, 2H), 6.27 (d, 1H, J = 8.0 Hz), 7.15 (d, 1H, J = 8.0 Hz); ^{13}C nmr (deuteriochloroform): δ 18.93, 22.93, 106.92, 121.67, 140.56, 155.47, 157.04; ir (potassium bromide): 3352, 1622, 1477, 1406, 1019, 872 cm^{-1}; uv (95% ethanol): λ_{max} 301 nm (ε 4720), 235 (10,253).

Anal. Calcd. for $C_7H_{10}N_2 \cdot 0.1H_2O$: C, 67.82; H, 8.29; N, 22.60. Found: C, 68.19; H, 8.44; N, 22.36.

2-Amino-3-bromo-5,6-dimethylpyridine (**3**).

To 6.0 g (49 mmoles) of 2-amino-5,6-dimethylpyridine (**2**) dissolved in 20 ml of glacial acetic acid under argon was slowly added 8.0 g (50 mmoles) of bromine over 10 minutes. The solution was stirred at room temperature for 1 hour and then the *p*H was adjusted to 11 with cold 50% aqueous sodium hydroxide solution. The mixture was extracted with chloroform. The organic extracts were washed with saturated sodium thiosulfate solution, dried (sodium sulfate), filtered, and evaporated *in vacuo* to give 9.3 g (94%) of a light brown solid, which was used without further purification, mp 114-116°; ^{1}H nmr (deuteriochloroform): δ 2.10 (s, 3H), 2.27 (s, 3H), 4.85 (s, 2H), 7.35 (s, 1H); ^{13}C nmr (deuteriochloroform): δ 18.7, 22.6, 102.0, 123.3, 142.3, 154.2, 155.1; ir (potassium bromide): 3469, 3292, 3154, 1630, 1399, 1464, 966 cm^{-1}; uv (95% ethanol): λ_{max} 271 nm (ε 9760), 238 (8086).

Anal. Calcd. for $C_7H_9N_2Br$: C, 41.82; H, 4.51; N, 13.93. Found: C, 41.95; H, 4.46; N, 13.73.

2-Amino-5,6-dimethyl-3-(methylamino)pyridine (**4**).

To 10.78 g (54.00 mmoles) of 2-amino-3-bromo-5,6-dimethylpyridine (**3**) and 0.81 g (3.24 mmoles) of copper sulfate in a stainless steel bomb was added 100 ml (2.91 moles) of 40% methylamine in water. The bomb was sealed and the reaction mixture heated at 200° for 5 hours and then cooled to 0°. To the mixture was added 3.50 g (63.0 mmoles) of solid sodium hydroxide. The mixture was extracted with chloroform (3 x 200 ml). The combined chloroform extracts were washed with water, dried (sodium sulfate), filtered, and evaporated *in vacuo* to give a black residue, which was distilled. The first faction distilled at 80° and 1 mm Hg to give the starting material, 2-amino-5,6-dimethylpyridine (**2**), while the second fraction distilled at 150° and 1 mm Hg yielded 2.23 g (27%) of product **4** as a yellow solid, mp 110-1.12° dec; ^{1}H nmr (deuteriochloroform): δ 2.17 (s, 3H), 2.29 (s, 3H), 2.81 (s, 3H), 4.04 (br s, 2H), 6.61 (s, 1H); ^{13}C nmr (deuteriochloroform): δ 19.5, 21.8, 31.8, 120.4, 123.0, 132.0, 143.0, 147.2; ir (film): 3328, 1613, 1453, 1339, 1263, 1014 cm^{-1}; uv (95% ethanol): λ_{max} 319 nm (ε 6985), 251 (7426), 201 (19,705).

Anal. Calcd. for $C_8H_{13}N_3 \cdot 0.1H_2O$: C, 62.80; H, 8.70; N, 27.46. Found: C, 63.03; H, 8.74; N, 29.26.

1,5,6-Trimethyl-2-aminoimidazo[4,5-*b*]pyridine (**5**).

A solution of 450 mg (2.98 mmoles) of 2-amino-5,6-dimethyl-3-(methylamino)pyridine (**4**) and 316 mg (2.98 mmoles) of cyanogen bromide in 2 ml of degassed absolute ethanol under argon was sealed in a Teflon bomb and heated at 120° for 4 hours. The cooled reaction mixture was stirred with 200 mg of solid potassium hydroxide for 5 minutes and then evaporated to dryness. The brown residue was dissolved in 200 ml of chloroform, dried (sodium sulfate), filtered, and evaporated. The crude solid was triturated with tetrahydrofuran (5 x 1 ml) and then isopropanol (4 x 1 ml). When the solid was further purified by reverse phase flash column chromatography using Baker octadecylsilyl (C_{18}) packing material and eluting with 40% water/60% methanol, 45 mg (9%) of desired product **5** was obtained as a beige solid, mp 291-293° dec; ^{1}H nmr (deuteriochloroform): δ 2.33 (s, 3H), 2.53 (s, 3H), 3.53 (s, 3H), 5.77 (br s, 2H), 7.06 (s, 1H); ^{13}C nmr (deuteriomethanol): δ 23.0, 25.3, 32.5, 121.3, 127.1, 131.3, 152.6, 158.1, 162.0; ir (potassium bromide): 3327, 3113, 1673, 1549, 1431, 1263 cm^{-1}; uv (95% ethanol): λ_{max} 308 nm (ε 14,014), 246 (2390), 207 (27,782); hrms Calcd. for $C_9H_{12}N_4$: Calcd. 176.1064. Found: 176.1057.

Alternative Synthesis of 1,5,6-Trimethyl-2-aminoimidazo[4,5-*b*]-pyridine (**5**).

To 100 mg (0.66 mmole) of 2-amino-5,6-dimethyl-3(methylamino)pyridine (**4**) and 70 mg (0.66 mmole) of cyanogen bromide dissolved in 2 ml of absolute ethanol was added 371 mg (2.87 mmoles) of *N,N*-diisopropylethylamine. The solution was heated at 80° under argon for 26 hours. To the reaction mixture was slowly added 100 mg (0.72 mmole) of solid potassium carbonate and 50 mg (47 mmoles) of additional cyanogen bromide over a 2-hour period. After 4 additional hours, the reaction mixture was cooled to room temperature and stirred for 4 days. The solution was concentrated *in vacuo*. The residue was dissolved in 10 ml of chloroform and washed with water, dried (sodium sulfate), filtered, and evaporated. The crude material was purified by chromatography on an Analtech tapered uniplate eluting with 5% methanol/95% chloroform. The desired product **5** was further purified by recrystallization from ethyl ether and chloroform to yield 20 mg (17%).

3-Bromo-5,6-dimethyl-2-(formylamino)pyridine (**7**).

To 1.06 g (5.27 mmoles) of 2-amino-3-bromo-5,6-dimethylpyridine (**3**) suspended in 10 ml of anhydrous ethyl ether under argon at 0° was slowly added 0.93 g (10.6 mmoles) of acetic formic anhydride (**6**) [14] dissolved in 5 ml of anhydrous ethyl ether over 10 minutes. The mixture was stirred at room temperature for 3 days. The suspension was centrifuged and the solid rinsed with ethyl ether. The solid was then dissolved in chloroform, washed with 70 ml of 10% aqueous sodium hydroxide solution, dried (sodium sulfate), filtered, and the solvent was evaporated to give 1.05 g (87%) of a light brown solid product **7**, mp 149-151°; ^{1}H nmr (deuteriochloroform): δ 2.21 (s, 3H), 2.37 (s, 3H), 7.53 (s, 1H), 7.97 (br s, 1H), 9.48 (d, 1H, J = 8.6 Hz); ^{13}C nmr (deuteriochloroform): δ 19.2, 23.1, 104.3, 130.0, 142.9, 145.9, 156.3, 163.4; ir (potassium bromide): 3220, 1686, 1578, 1472, 1369, 1290, 1246, 1198, 975 cm^{-1}; uv (95% ethanol): λ_{max} 294 nm (ε 7986), 232 (16,510), 209 (14,765).

Anal. Calcd. for $C_8H_9N_2OBr$: C, 41.95; H, 3.96; N, 12.23. Found: C, 41.73, H, 4.23; N, 12.34.

3-Bromo-5,6-dimethyl-2-(methylamino)pyridine (**8**).

To a stirred suspension of 4.15 g (110 mmoles) of lithium aluminum hydride in 175 ml of anhydrous tetrahydrofuran under argon was added dropwise over 20 minutes 12.5 g (54.6 mmoles) of 3-bromo-5,6-dimethyl-2-(formylamino)pyridine (**7**) dissolved in 50 ml of anhydrous tetrahydrofuran. The mixture was stirred at room temperature for 18 hours. To the reaction mixture was added dropwise 60 ml of 50% water/50% tetrahydrofuran over 20 minutes, followed by 10 ml of 15% aqueous sodium hydroxide solution. After the mixture had stirred for an additional 10 minutes, it was filtered through Celite, evaporated, and then azeotroped with absolute ethanol. The crude material was purified by flash chromatography on silica gel eluting with chloroform to give 7.50 g (63%) of the product **8** as a pale yellow oil; ^{1}H nmr (deuteriochloroform): δ 2.11 (s, 3H), 2.33 (s, 3H), 3.01 (d, 3H, J = 5.0 Hz), 4.72 (br s, 1H), 7.34 (s, 1H); ^{13}C nmr (deuteriochloroform): δ 18.8, 23.2, 29.9, 103.1, 121.3, 141.6, 154.0, 154.8; ir (neat): 3440, 2942, 2861, 1598, 1504, 1404, 1317, 1241, 956 cm^{-1}; uv (95% ethanol): λ_{max} 317 nm (ε 5944), 242 (12,442), 205 (14,147).

Anal. Calcd. for $C_8H_{11}N_2Br$: C, 44.67; H, 5.15; N, 13.03. Found: C, 44.97; H, 5.17; N, 13.14.

3-Amino-5,6-dimethyl-2-(methylamino)pyridine (**9**).

To 640 mg (2.98 mmoles) of 3-bromo-5,6-dimethyl-2-(methylamino)pyridine (**8**) and 45 mg (0.179 mmole) of copper sulfate in a stainless steel bomb was added 20 ml of concentrated ammonium hydroxide. The reaction mixture was heated at 200° for 15 hours and then cooled. The crude mixture was stirred with 200 mg (3.57 mmoles) of solid potassium hydroxide for 5 minutes and then extracted with chloroform (3 x 30 ml). The combined organic extracts were dried (sodium sulfate), filtered, and concentrated to give a purple oil. The oil was purified by reverse phase flash column chromatography using Baker octadecylsilyl (C_{18}) packing material and eluting with 40% water/60% methanol to yield 289 mg (64%) of brown crystals of impure product (**9**); ^{1}H nmr (deuteriochloroform): δ 2.08 (s, 3H), 2.31 (s, 3H), 2.96 (s, 3H), 3.08 (br s, 2H), 6.60 (s, 1H).

5,6-Dimethyl-2-(methylamino)pyridine (**11**).

To 1.51 g (7.02 mmoles) of 3-bromo-5,6-dimethyl-2-(methylamino)pyridine (**8**) in 15 ml of absolute ethanol was added 0.15 g of 10% palladium on carbon. The mixture was hydrogenated at 50 psi of hydrogen for 5 hours, and then 400 mg of potassium hydroxide dissolved in 2 ml of water was added and the mixture was filtered through Celite. After the material was concentrated to near dryness, it was suspended in 10 ml of chloroform, filtered, and concentrated to give 0.91 g (96%) of a red-brown solid, mp 39-41°; ^{1}H nmr (deuteriochloroform): δ 2.14 (s, 3H), 2.33 (s, 3H), 2.86 (d, 3H, J = 5.4 Hz), 4.45 (br s, 1H), 6.18 (d, 1H, J = 8.2 Hz), 7.21 (d, 1H, J = 8.2 Hz); ^{13}C nmr (deuteriochloroform): δ 19.01, 23.31, 30.54, 103.37, 120.51, 140.27, 155.85, 158.94; ir (potassium bromide): 3270, 2918, 1605, 1505, 1368, 1328, 1251, 1161, 1124, 806 cm^{-1}; uv (95% ethanol): λ_{max} 313 nm (ε 5235), 241 (13,260); ms: (70 eV, electron impact) m/z (relative intensity) 136 (100, molecular ion), 107 (92), 94 (15), 83 (18), 53 (15), 42 (11), 27 (10).

Anal. Calcd. for $C_8H_{12}N_2 \cdot 0.1H_2O$: C, 69.63; H, 8.91; N, 20.30. Found: C, 70.00; H, 8.94; N, 20.17.

5,6-Dimethyl-2-(methylamino)-3-nitropyridine (**12**).

To 10 ml of concentrated sulfuric acid and 330 µl (7.78 mmoles) of 90% nitric acid was added 775 mg (5.79 mmoles) of 5,6-dimethyl-2-(methylamino)pyridine (**11**). The solution was stirred at room temperature for 1 hour and then at 60° for another hour. The cooled mixture was poured onto 100 g of ice, brought to a *p*H of 9 with solid sodium carbonate, extracted with chloroform (3 x 150 ml), dried (sodium sulfate), filtered, and concentrated. The material was chromatographed on flash silica gel eluting with chloroform to give 650 mg (63%) of a bright orange solid, mp 137-139°; ^{1}H nmr (deuteriochloroform): δ 2.22 (s, 3H), 2.47 (s, 3H), 3.17 (d, 3H, J = 4.9 Hz), 8.12 (s, 1H), 8.20 (br s, 1H); ^{13}C nmr (deuteriochloroform): δ 18.9, 24.6, 29.1, 120.9 126.9, 135.9, 152.5, 166.9; ir (potassium bromide): 3400, 2944, 1590, 1495, 1399, 1326, 1237, 1159, 1027, 858, 772 cm-^{1}; uv: (95% ethanol): λ_{max} 425 nm (ε 8758), 229 (24,773); ms: (70 eV, electron impact) m/z (relative intensity) 181 (47, molecular ion), 164 (50), 136 (12), 119 (14), 107 (100), 70 (18), 52 (15), 42 (13), 18 (17).

Anal. Calcd. for $C_8H_{11}N_3O_2$: C, 53.03; H, 6.12; N, 23.19. Found: C, 53.05; H, 6.10; N, 22.99.

Alternative Synthesis of 3-Amino-5,6-dimethyl-2-(methylamino)pyridine (**9**).

To 1.74 g (9.62 mmoles) of 5,6-dimethyl-2-(methylamino)-3-nitropyridine (**12**) in 175 ml of ethanol was added 0.17 g of 10% palladium on carbon. The mixture was hydrogenated at 50 psi for 12 hours and then filtered through Celite. Evaporation of the solvent gave 1.44 g (99%) of product **9**, mp 85-86°; ^{1}H nmr (deuteriochloroform): δ 2.10 (s,3H), 2.33 (s,3H), 2.98 (s,3H), 3.02 (br s, 2H), 3.93 (br s, 1H),6.63(s,1H); ^{13}C nmr (deuteriochloroform): δ 19.1, 22.6, 30.0, 120.6, 126.0, 127.0, 146.4, 149.8; ir (potassium bromide): 3436, 3378, 2919, 1602, 1495, 1414, 1243, 1126, 997, 891, 720 cm^{-1}; uv (95% ethanol): λ_{max} 317 nm (ε 7130), 247 (7755), 203 (19,346); ms: (70 eV, electron impact) m/z (relative intensity) 151 (100, molecular ion), 136 (8), 123 (55), 109 (37), 95 (33), 80 (12), 67 (26), 53 (27), 42 (64); hrms Calcd. for $C_8H_{13}N_3$: 151.1111. Found: 151.1109.

3,5,6-Trimethyl-2-aminoimidazo[4,5-b]pyridine (**10**).

To 1.53 g (5.50 mmoles) of 2-methyl-2-thiopseudourea sulfate dissolved in 7.5 ml of warm water and then cooled to 0° was added 0.86 ml (11.0 mmoles) of methyl chloroformate [15]. The *p*H was kept at 8 by the addition of 8 *M* sodium hydroxide solution while the solution stirred at 0° for 30 minutes and room temperature for 1 hour. After the *p*H had stabilized at 8, it was adjusted to 4-5 by the addition of glacial acetic acid. To the reaction mixture was added 0.45 g (1.99 mmoles) of sodium acetate followed by 0.30 g (1.99 mmoles) of 3-amino-5,6-dimethyl-2-(methylamino)pyridine (**9**). After refluxing for 19 hours, the cooled mixture was evaporated to dryness, stirred with 250 ml of ethyl acetate for 15 minutes, and then filtered. The filtrate was dried (sodium sulfate), concentrated, and adsorbed onto 200 mg of Baker octadecylsilyl (C_{18}) reverse phase packing material. This material was added to the top of a prepared C_{18} reverse phase silica gel column and flash chromatographed; it eluted with a gradient of 20% methanol/80% water going to 60% methanol/40% water to give 110 mg (31%) of a white solid, mp 228-230°; ^{1}H nmr (deuteriochloroform): δ 2.33 (s, 3H), 2.52 (s, 3H), 3.63 (s, 3H), 4.80 (br s, 2H), 7.38 (s, 1H); ^{13}C nmr (deuteriomethanol): δ 20.5, 22.6, 28.5, 125.2, 126.9, 135.5, 147.7, 148.0, 158.0; ir (potassium bromide): 3323, 3149, 2944, 1646, 1549, 1405, 1241, 1097, 1000 cm^{-1}; uv (water): λ_{max} 300 nm (ε 14,836), 267 (3267); ms: (70 eV, electron impact) m/z (relative intensity) 176 (100, molecular ion), 161 (25), 148 (17), 134 (9), 92 (9), 66 (14), 51 (12), 42 (23).

Anal. Calcd. for $C_9H_{12}N_4 \cdot 0.4H_2O$: C, 58.93 H, 7.03; N, 30.54. Found: C, 59.30; H, 6.78; N, 30.27.

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