

M. J. Tanga,* W. W. Bradford, J. E. Bupp, and J. A. Kozocas

SRI International, 333 Ravenswood Avenue, Menlo Park, California 94025

Received January 23, 2003

The syntheses of the potential heterocyclic amine food mutagens 1,4,6-trimethyl-2-aminoimidazo[4,5-*c*]-pyridine and 1,5,7-trimethyl-2-aminoimidazo[4,5-*b*]pyridine are described.

J. Heterocyclic Chem., **40**, 569(2003).

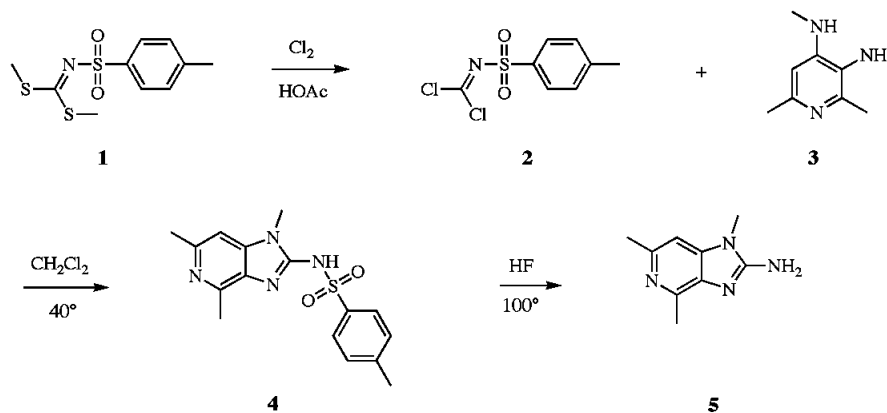
Our diet is a complex mixture that provides nutritional sustenance but may also be important in the causation, modulation, and prevention of human disease. Cooking, heat processing, and pyrolysis of protein-rich foods induce formation of mutagenic and carcinogenic heterocyclic amines through chemical reactions involving amino acids, creatine, and proteins [1,2]. The formation of these compounds requires high temperatures, such as are found in radiative or conductive cooking (*e.g.*, grilling or frying) but usually not in indirect convective cooking (*e.g.*, roasting) or boiling methods (*e.g.*, poaching or steaming) [3,4]. The dietary concentration of heterocyclic amines can be reduced by making changes to cooking methods, for example marinating meat before grilling [5], and eating foods containing catechins, flavonoids, and caffeic acid [6,7].

Heterocyclic amines are potent mutagens in the Ames/*Salmonella* assay and are also carcinogens in laboratory animals [8,9] and suspected carcinogens in humans [10]. Recent epidemiological studies have shown that these substances are ingested by humans in cooked meats and fish products [1,4,11]. A positive association has been reported between the preference for well-done red meat and the risk of postmenopausal breast cancer [11,12]. A recent study demonstrated that heterocyclic amines are bioavailable to the human colon following defined dietary-relevant doses, and DNA and protein adducts are formed

[13]. These data are consistent with increased risk of cancer from ingestion of heterocyclic amines [1,4,11-13].

To understand the risk that consuming heterocyclic amines formed during cooking meat and fish poses to humans, it is essential to isolate, identify, and synthesize these compounds. Although some of these compounds have been identified and synthesized, one that contributes 10-15% of the total mutagenic activity of a fried meat sample [14-17] has only been identified by mass spectra to have a molecular weight of 176 [14,16,17]. From the available preliminary data, the mutagenic compound has been determined to be one of the twelve isomers of 2-amino-trimethylimidazopyridine (TMIP) [14,17]. To investigate the biological risk associated with ingesting this unidentified compound, we have previously synthesized six of the possible isomers: 1,5,6-trimethyl-2-aminoimidazo[4,5-*b*]pyridine; 3,5,6-trimethyl-2-aminoimidazo[4,5-*b*]pyridine [11]; 3,5,7-trimethyl-2-aminoimidazo[4,5-*b*]pyridine; 1,4,7-trimethyl-2-aminoimidazo[4,5-*c*]pyridine; 1,6,7-trimethyl-2-aminoimidazo[4,5-*c*]pyridine; and 3,4,6-trimethyl-2-aminoimidazo[4,5-*c*]pyridine [18,19]. We now report the syntheses of two other possible isomers-1,4,6-trimethyl-2-aminoimidazo[4,5-*c*]pyridine (**5**) and 1,5,7-trimethyl-2-aminoimidazo[4,5-*b*]pyridine (**14**)-so that they can be compared and tested against the unknown mutagen [20]. The syntheses of the two compounds have not previously been reported in the literature.

Scheme I

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

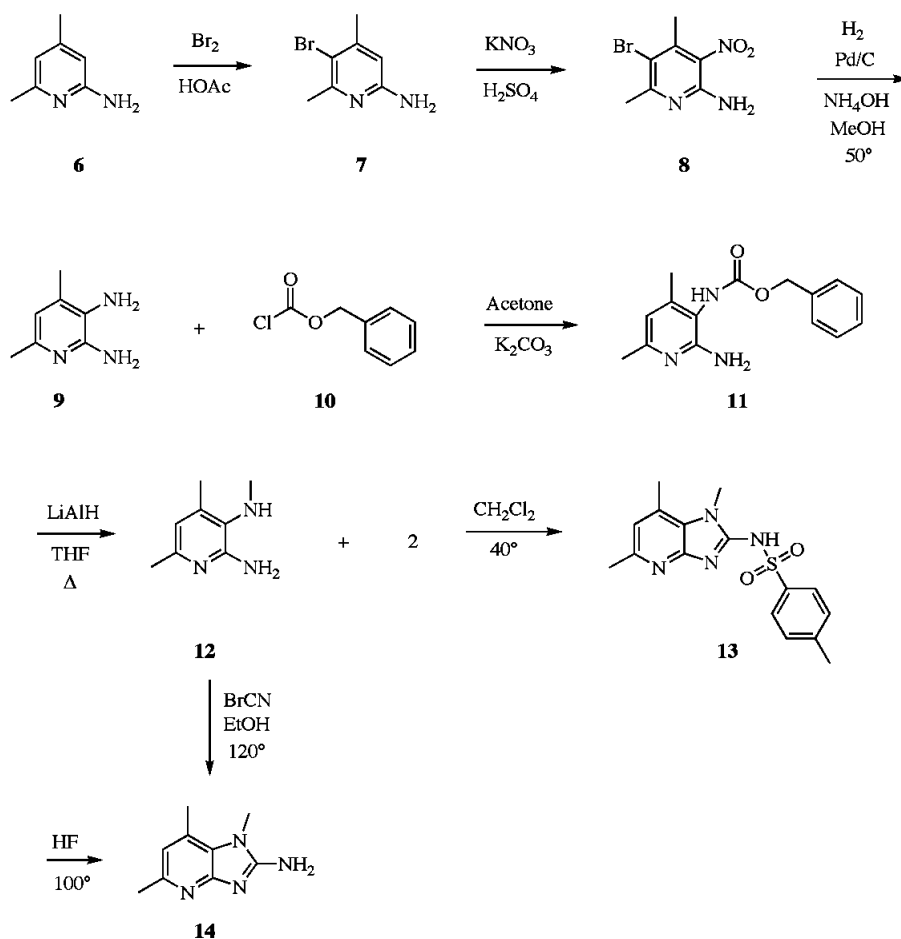
The successful synthesis of 1,4,6-trimethyl-2-aminoimidazo[4,5-*c*]pyridine (**5**) is outlined in Scheme I. Previous attempts to synthesize **5** resulted in the synthesis of 1,4,6-trimethyl-7-aminoimidazo[4,5-*c*]pyridine or in the inability to ring close 3-amino-2,6-dimethyl-4-(methylamino)pyridine (**3**) by methods such as cyanogen bromide [19]. The success of this synthesis is based on the use of the reagent *N*-dichloromethylene-*p*-toluenesulfonamide (**2**) [21] to accomplish the ring closure and introduce the desired amino function at the 2-position. It is a facile way to form the desired heterocycle **5**, and purification is simplified.

The reagent *N*-dichloromethylene-*p*-toluenesulfonamide (**2**) was formed in good yield (83%) from commercially available *N*-[bis(methylthio)methylene]-*p*-toluenesulfonamide (**1**) by treatment with excess chlorine gas in glacial acetic acid [21]. One equivalent each of the diamine **3** and the reagent **2** in dichloromethane forms a solution at 40°, and after 5 minutes the product **4** begins to precipitate as the hydrochloride salt. Cleavage of the sulfonamide **4** to give the desired product, 1,4,6-trimethyl-2-

aminoimidazo[4,5-*c*]pyridine (**5**), was accomplished in good yield (75%) by using anhydrous hydrofluoric acid with heat. We explored alternative methods for the cleavage of the sulfonamide **4**, such as using potassium carbonate in aqueous methanol with heat, concentrated sodium hydroxide in aqueous methanol with heat, concentrated sulfuric acid with heat, concentrated sulfuric acid in aqueous methanol with heat, and finally hydrofluoric acid in pyridine with heat. None of these methods gave the desired product **5**, and usually unreacted starting material **4** was recovered.

The synthesis of the potential food mutagen 1,5,7-trimethyl-2-imidazo[4,5-*b*]pyridine (**14**) is shown in Scheme II. The product **14** has been prepared by the cyanogen bromide method as well as by the sulfonamide method. Bromination of commercially available 2-amino-4,6-dimethylpyridine (**6**) in glacial acetic acid gave the desired 2-amino-5-bromo-4,6-dimethylpyridine (**7**) plus a small amount of 2-amino-3,5-dibromo-4,6-dimethylpyridine. Trituration in hexane separated the two compounds, which were formed in a 12:5 ratio.

Scheme II

Synthesis of 1,5,7-Trimethyl-2-aminoimidazo[4,5-*b*]pyridine (**14**)

The amine **6** was brominated to direct the nitration to the 3-position. Nitration of the amine **7** with potassium nitrate in sulfuric acid gave the nitro compound **8** in 64% yield. Reduction and dehydrohalogenation of **8** in one step, using hydrogen with 10% palladium on carbon as the catalyst in ammonium hydroxide and methanol at 50°, yielded 2,3-diamino-4,6-dimethylpyridine (**9**) in 98% yield. Treatment of the diamine **9** with benzyl chloroformate (**10**) yielded N³-benzyloxycarbonyl-2,3-diamino-4,6-dimethylpyridine (**11**) in 49% yield with a small amount of the bis-benzylated material (9%), which was separated by column chromatography. Reduction of **11** with lithium aluminum hydride gave 2-amino-4,6-dimethyl-3-(methylamino)pyridine (**12**), which was immediately used in the ring cyclization reaction with cyanogen bromide in ethanol in a bomb at 120° to give the desired final product **14** in 10% yield. In an effort to synthesize greater quantities of product **14**, which could be more readily purified, the sulfonamide **13** was prepared from the diamine **12** and *N*-dichloromethylene-*p*-toluenesulfonamide (**2**) in 51% yield. The sulfonamide **13** was cleaved using anhydrous hydrofluoric acid to give the product **14** in 40% yield, which was identical to **14** synthesized using the cyanogen bromide method. The final products of these syntheses are undergoing biological testing [17].

In conclusion, two potential food mutagens were synthesized for biological evaluation using an approach that is applicable to the preparation of similar heterocyclic compounds. In all, ten of the twelve isomers of 2-aminotrimethylimidazopyridine (TMIP) are presently available as reference materials from the National Cancer Institute [20].

EXPERIMENTAL

Melting points (uncorrected) were obtained using a Thomas-Hoover melting point apparatus. The ir spectra were recorded on a Perkin Elmer 1600 FTIR spectrophotometer, the uv spectra on a Varian DMS-90 spectrometer, and the nmr spectra on a Varian Mercury VX-300 MHz spectrometer. All chemical shifts are reported in parts per million () downfield from tetramethylsilane. The nmr multiplicities are reported using the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; and b, broad. Column chromatography was carried out using E. Merck silica gel 40 (70-230 mesh, ASTM). All solvents were dried over 3 Å molecular sieves, except tetrahydrofuran, which was dried by refluxing over sodium with benzophenone ketyl as an indicator. Microanalyses were performed by Atlantic Microlab, Inc., Norcross, GA.

After extensive effort we were unable to obtain reasonable elemental analysis for compounds **5** and **14**. It is our experience that these compounds do not give reasonable elemental analysis. Analysis by HPLC shows the compounds to be greater than 95% pure.

Since 1,4,6-trimethyl-2-aminoimidazo[4,5-*c*]pyridine (**5**) and 1,5,7-trimethyl-2-aminoimidazo[4,5-*b*]pyridine (**14**) are potential carcinogens and mutagens, direct contact should be avoided.

N-Dichloromethylene-*p*-toluenesulfonamide (**2**) [21].

A suspension of 25.0 g (90.8 mmoles) of *N*-[bis(methylthio)methylene]-*p*-toluenesulfonamide (**1**) in 170 mL of glacial acetic acid was cooled to 15°. A stream of chlorine gas was slowly introduced over 1 hour, keeping the reaction temperature at 15°. The resulting solution was stirred at 10° for 1 hour. The sample was evaporated under vacuum and the solid residue sublimed at 70-75° (5 μm of mercury) to give 19.1 g (83%) of pure product (**2**), mp 80-83°; ¹H nmr (deuteriochloroform): 2.46 (s, 3H), 7.38 (d, 2H, J = 8.45 Hz).

2-Amino-*N*-*p*-toluenesulfonamide-1,4,6-trimethylimidazo[4,5-*c*]pyridine (**4**).

A sample (392 mg, 2.59 mmoles) of 3-amino-2,6-dimethyl-4-(methylamino)pyridine (**3**) in 10 mL of dichloromethane was treated with 653 mg (2.59 mmoles) of *N*-dichloromethylene-*p*-toluenesulfonamide (**2**). The reaction solution was heated to 40° for 18 hours, then treated with 5 g of anhydrous sodium sulfate and stirred overnight to form the free base. The sample was filtered through a Millipore filter and evaporated to give 1.15 g of crude product (**4**). The filtrate was filtered through 5 g of silica gel eluting with 125 mL of chloroform/methanol/triethylamine (94.5/5/0.5). Evaporation of the eluent gave 653 mg (76%) of desired product (**4**); mp 239-241°; ¹H nmr (deuteriochloroform):

2.40 (s, 3H), 2.58 (s, 3H), 2.60 (s, 3H), 3.45 (s, 3H), 6.80 (s, 1H), 7.27 (d, 2H, J = 8.28 Hz), 7.86 (d, 2H, J = 8.28 Hz); ¹³C nmr (deuteriochloroform): 12.70, 14.46, 17.61, 21.57, 94.36, 115.22, 119.04, 122.34, 130.04, 132.50, 133.18, 135.64, 143.17, 145.49; ir (potassium bromide) 3258, 2920, 2365, 1578, 1542, 1522, 1458, 1402, 1375, 1331, 1277, 1200, 1132, 1088, 1040, 986, 918, 831, 806, 788, 696, 666, 570, 540 cm⁻¹; uv (methanol) _{max} 311 nm (2,347), 286 (7,410), 242 (27,072), 220 (19,809).

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

In a Teflon lined bomb, 327 mg (0.990 mmole) of 2-amino-*N*-*p*-toluenesulfonamide-1,4,6-trimethylimidazo[4,5-*c*]pyridine (**4**) in 10 mL of anhydrous hydrofluoric acid was heated to 100° for 1 hour. After cooling to 0°, the bomb was carefully opened and the hydrofluoric acid evaporated with a gentle stream of argon. The solid residue was rinsed into a continuous extraction apparatus with 1 mL of 4 *N* sodium hydroxide solution and 100 mL of water, extracted with chloroform overnight, and then dried (sodium sulfate), filtered, and evaporated to give 280 mg of crude product (**5**). This material (**5**) was triturated with 3 x 5 mL of warm hexanes and then with 3 x 2 mL of ethyl ether to give 130 mg (75%) of pure product (**5**); mp 244-248°; ¹H nmr (deuterioethanol): 2.41 (s, 3H), 2.50 (s, 3H), 3.46 (s, 3H), 6.55 (br s, 2H), 6.89 (s, 1H); ¹³C nmr (deuterioethanol): 16.86, 17.36, 34.18, 128.97, 132.07, 132.89, 139.84, 140.09, 148.24; ir (potassium bromide) 3342, 3246, 3068, 1665, 1615, 1582, 1454, 1413, 1259, 1125 cm⁻¹; uv (95% ethanol) _{max} 281 nm (4,745), 253 (5,468), 215 (30,725); ms (DCI-NH₃) m/z (relative intensity) 177 (100, molecular ion + H), 123 (10), 78 (10); hrms Calcd. for C₉H₁₂N₄: 176.1062. Found: 176.1050.

2-Amino-5-bromo-4,6-dimethylpyridine (**7**).

A solution of 25.6 g (0.21 mole) of 2-amino-4,6-dimethylpyridine (**6**) in 50 mL of glacial acetic acid under argon was treated with a solution of 33.5 g (0.21 mole) of bromine in 25 mL of glacial acetic acid over 20 minutes with water bath cooling

keeping the temperature below 20°. The solution became a solid mass and was allowed to stand at room temperature for 1 hour. After cooling in an ice bath, the material was treated with 500 mL of cold 20% sodium hydroxide solution. The mixture was extracted with 3 x 250 mL of dichloromethane, dried (sodium sulfate), filtered, and evaporated to provide 44.3 g of crude product (**7**). Trituration of the material (**7**) with 3 x 250 mL of hot hexane gave 24.7 g (59%) of pure product (**7**); mp 134-137°; ¹H nmr (deuteriochloroform): 2.27 (s, 3H), 2.50 (s, 3H), 4.40 (br s, 2H), 6.24 (s, 1H); ¹³C nmr (deuteriochloroform): 24.3, 26.2, 109.0, 113.4, 149.6, 156.5, 157.4; ir (potassium bromide) 3431, 3310, 3174, 2367, 2343, 1637, 1592, 1543, 1506, 1458, 1419, 1260, 1210, 1020, 960, 942, 740, 671, 621, 578, 525 cm⁻¹.

Anal. Calcd. for C₇H₉N₂Br: C, 41.82; H, 4.51; N, 13.93. Found: C, 42.10; H, 4.51; N, 13.90.

2-Amino-5-bromo-4,6-dimethyl-3-nitropyridine (**8**).

A sample of 7.04 g (69.66 mmoles) of potassium nitrate was carefully added over 30 minutes to a stirring solution of 10.00 g (49.76 mmoles) of 2-amino-5-bromo-4,6-dimethylpyridine (**7**) in 30 mL of concentrated sulfuric acid. The mixture was stirred for 1 hour at room temperature and then poured onto 200 g of ice. To the solution was added 300 mL of chloroform, and the mixture was neutralized with sodium carbonate, filtered through Celite, extracted into chloroform (2 x 300 mL), dried (sodium sulfate), filtered, and concentrated to afford 7.89 g (64%) of pure product (**8**); mp 148-151°; ¹H nmr (deuteriochloroform): 2.56 (s, 3H), 2.57 (s, 3H), 4.51 (br s, 2H); ¹³C nmr (deuteriochloroform): 21.26, 26.55, 112.72, 118.25, 144.37, 150.45, 161.22; ir (potassium bromide) 3446, 3296, 3171, 1635, 1573, 1543, 1496, 1434, 1378, 1324, 1243, 1122, 1022, 1002, 949, 849, 777, 672, 540, 496 cm⁻¹.

2,3-Diamino-4,5-dimethylpyridine (**9**).

To 3.00 g (12.20 mmoles) of 2-amino-5-bromo-4,5-dimethyl-3-nitropyridine (**8**) in 200 mL of methanol with 6 mL of ammonium hydroxide was added 300 mg of 10% palladium on carbon. The reaction mixture was hydrogenated for 18 hours at 50 psi of hydrogen and 50°. The mixture was filtered through Celite, concentrated, suspended in 30 mL of water, and treated with sodium carbonate until basic. The material was extracted into chloroform (3 x 200 mL), dried (sodium carbonate), filtered, and evaporated to afford 1.64 g (98%) of a yellow solid (**9**); mp 157-160°; ¹H nmr (deuteriochloroform): 2.14 (s, 3H), 2.30 (s, 3H), 3.11 (br s, 2H), 4.12 (br s, 2H), 6.40 (s, 1H); ¹³C nmr (deuteriochloroform): 16.69, 23.05, 117.78, 124.56, 131.69, 146.20, 148.15; ir (potassium bromide) 3319, 3162, 2914, 2374, 1652, 1636, 1559, 1476, 1244, 1152, 1116, 1030, 932, 830, 756, 613, 528 cm⁻¹; ms (electrospray ionization) m/z (relative intensity) 138 (100, molecular ion + H), 121 (26), 105 (16).

N³-Benzyloxycarbonyl-2,3-diamino-4,6-dimethylpyridine (**11**).

A suspension of 381 mg (2.77 mmoles) of 2,3-diamino-4,6-dimethylpyridine (**9**) in 10 mL of acetone at 0° under argon was treated with 1.53 g (11.1 mmoles) of potassium carbonate, and 473 mg (2.77 mmoles) of benzyl chloroformate (**10**) was added dropwise. The reaction mixture was stirred at room temperature for 18 hours. The solvent was removed under vacuum and the residue extracted with 3 x 200 mL of chloroform. Evaporation left approximately 1 g of crude material, which was chromatographed on silica gel eluting with 5% methanol/95% chloro-

form to give 100 mg (9%) of biscarbamate and 370 mg (49%) of the desired product (**11**); mp 155-157°; ¹H nmr (deuteriochloroform): 2.12 (br s, 3H), 2.31 (s, 3H), 4.62 (br s, 2H), 5.18 (s, 2H), 6.11 (br s, 1H), 6.42 (s, 1H), 7.30-7.45 (m, 5H); ¹³C nmr (deuteriochloroform): 18.5, 24.7, 68.5, 114.6, 117.1, 129.1, 129.2, 129.4, 129.5, 129.6, 137.1, 156.2, 156.4; ir (potassium bromide) 3411, 3276, 3156, 2964, 2371, 1686, 1638, 1605, 1567, 1523, 1474, 1458, 1371, 1254, 1235, 1061, 848, 812, 774, 747, 693, 576, 483 cm⁻¹; ms (70 eV, electron impact) m/z (relative intensity) 271 (25, molecular ion), 136 (51), 91 (100).

2-Amino-4,6-dimethyl-3-(methylamino)pyridine (**12**).

A solution of 349 mg (1.29 mmoles) of N³-benzyloxycarbonyl-2,3-diamino-4,5-dimethylpyridine (**11**) in 10 mL of dry tetrahydrofuran under argon was treated with 340 mg (8.96 mmoles) of lithium aluminum hydride. The reaction mixture was refluxed for 1 hour. The mixture was cooled to 0° and a solution of 5 mL of 50% tetrahydrofuran/50% water was very slowly added, followed by the addition of 1 mL of 15% sodium hydroxide solution. The resulting mixture was filtered and the residue washed with water and tetrahydrofuran. The tetrahydrofuran was removed under vacuum and the aqueous suspension continuously extracted with chloroform overnight. The extract was dried (sodium sulfate), filtered, and evaporated to give 255 mg of crude material, which was triturated into hot hexane (3 x 200 mL) and evaporated to give 140 mg (99%) of crude product (**12**); ¹H nmr (deuteriochloroform): 2.19 (s, 3H), 2.31 (s, 3H), 2.67 (s, 3H), 6.35 (s, 1H); ir (potassium bromide) 3362, 3028, 2922, 2858, 2360, 1513, 1476, 1452, 1410, 1381, 1238, 1206, 1136, 1022, 735, 698 cm⁻¹; uv (methanol) _{max} 299 nm (2,604), 230 (2,453), 203 (10,697).

1,5,7-Trimethyl-2-aminoimidazo[4,5-*b*]pyridine (**14**).

To 650 mg (4.30 mmoles) of 2-amino-4,6-dimethyl-3-(methylamino)pyridine (**12**) was added 1.37 g (12.91 mmoles) of cyanogen bromide in 6 mL of methanol. The mixture was sealed in a Teflon lined bomb under argon and heated at 120° for 6 hours with stirring. The mixture was cooled, evaporated to dryness, redissolved in 2 mL of methanol, and purified by preparative reverse phase C₁₈ hplc column eluting with 25% methanol/75% water with 0.5% triethylamine. From the chromatography was obtained 75 mg (10%) of a white solid product (**14**); mp 237°; ¹H nmr (deuteriodimethylsulfoxide): 2.33 (s, 3H), 2.49 (s, 3H), 3.65 (s, 3H), 6.44 (s, 1H), 6.62 (br s, 2H); ¹³C nmr (deuteriodimethylsulfoxide): 16.7, 23.6, 30.7, 115.9, 123.6, 126.0, 148.6, 155.8, 157.1; ir (potassium bromide) 3323, 3171, 1647, 1620, 1544, 1473, 1419, 1392, 1121 cm⁻¹; uv (95% ethanol) _{max} 299 nm (12,178), 209 (28,307); ms (70 eV, electron impact) m/z (relative intensity) 176 (53, molecular ion), 161 (14), 148 (7), 133 (9), 106 (7), 92 (8), 77 (18), 65 (60), 51 (43), 42 (100); hmrs Calcd. for C₉H₁₂N₄: 176.1062. Found: 176.1062.

2-Amino-*N-p*-toluenesulfonamide-1,5,7-trimethylimidazo[4,5-*b*]pyridine (**13**).

A solution of 430 mg (2.85 mmoles) of 2-amino-4,6-dimethyl-3-(methylamino)pyridine (**12**) in 8 mL of dichloromethane was treated with 720 mg (2.85 mmoles) of *N*-dichloromethylene-*p*-toluenesulfonamide (**2**). The reaction solution was heated at 40° for 1 hour under argon. After the mixture was cooled to 0°, 0.57 mL (5.7 mmoles) of 10.0 *M* sodium hydroxide solution was added. The

mixture was evaporated to dryness to give a brown gum, which was triturated with 20 mL of dichloromethane, placed on a 2 cm x 30 cm silica gel column, and eluted with 97% dichloromethane/3% methanol and a trace of triethylamine. The product fractions were combined and evaporated to yield 477 mg (51%) of desired product (**13**); ¹H nmr (deuteriochloroform): 2.39 (s, 3H), 2.50 (s, 3H), 2.56 (s, 3H), 3.69 (s, 3H), 6.74 (s, 1H), 7.23-7.33 (m, 2H), 7.83 (m, 2H); ir (potassium bromide) 2922, 2847, 2360, 2339, 1587, 1450, 1263, 1141, 1085, 840, 812 cm⁻¹; uv (methanol) λ_{\max} 338 nm (ε); ms (atmospheric pressure chemical ionization) m/z (relative intensity) 331 (100, molecular ion + H), 176 (11).

Alternative Synthesis of 1,5,7-Trimethyl-2-aminoimidazo[4,5-*b*]pyridine (**14**).

In a Teflon lined bomb, a solution of 477 mg (1.44 mmoles) of 2-amino-*N-p*-toluenesulfonamide-1,5,7-trimethylimidazo[4,5-*b*]pyridine (**13**) in 10 mL of anhydrous hydrofluoric acid under argon was heated at 100° for 1 hour. After cooling to 0°, the bomb was carefully opened and the hydrofluoric acid evaporated under a gentle stream of argon. The solid residue was treated with 4 mL of 15% sodium hydroxide solution. The mixture was evaporated to dryness and then refluxed in 400 mL of chloroform overnight to dissolve the product away from any salts. The organic extract was dried (sodium sulfate), filtered, and evaporated to give 230 mg of material. This residue was triturated with 3 x 5 mL of ethyl ether and the insoluble residue chromatographed on a 1 cm x 27 cm silica gel column eluting with 80% chloroform/19% methanol/1% triethylamine. The product fractions were combined and evaporated to give 102 mg (40%) of desired product (**14**); mp 237°; ¹H nmr (deuteriochloroform): 2.33 (s, 3H), 2.49 (s, 3H), 3.65 (s, 3H), 6.46 (s, 1H), 6.65 (br s, 2H); ¹³C nmr (deuteriochloroform): 16.6, 23.4, 30.7, 115.9, 123.7, 126.3, 148.4, 155.6, 157.0; ir (potassium bromide) 3342, 3167, 1653, 1623, 1547, 1481, 1426, 1397, 1310, 1282, 1225, 1121, 1028 cm⁻¹; uv (methanol) λ_{\max} 299 nm (ε 12,968), 208 (30,182); ms (direct chemical ionization-ammonia) m/z (relative intensity) 177 (100, molecular ion); hrms Calcd. for C₉H₁₂N₄: 176.1062. Found: 176.1062.

Acknowledgments.

This work was supported by the National Cancer Institute under Contract No. N01-CB-67257 and Grant No. PO1-CA55861. We would also like to thank Dr. M. G. Knize, Lawrence Livermore National Laboratory, Biomedical Sciences Division, University of California, for his assistance.

REFERENCES AND NOTES

- [1] D. W. Layton, K. T. Bogen, M. G. Knize, F. T. Hatch, V. M. Johnson, and J. S. Felton, *Carcinogenesis*, **16**, 39 (1995).
- [2] F. T. Hatch, M. G. Knize, S. K. Healy, T. Slezak, and J. S. Felton, *Environ. Mol. Mutagen.*, **12**, 1 (1988).
- [3] S. Robbana-Barrat, M. Rabache, E. Rialland, and J. Fradin, *Environ. Health Perspect.*, **104**, 280 (1996).
- [4] K. Skog, G. Steineck, K. Augustsson, and M. Jägerstad, *Carcinogenesis*, **16**, 861 (1995).
- [5] C. P. Salmon, M. G. Knize, and J. S. Felton, *Food Chem. Toxicol.* **35**, 433 (1997).
- [6] A. Oguri, M. Suda, Y. Totsuka, T. Sugimura, and K. Wakabayashi, *Mutat. Res.* **402**, 237 (1998).
- [7] R. H. Dashwood, M. Xu, J. F. Hernaez, N. Hasaniya, K. Youn, and A. Razzuk, *Proc. Soc. Exp. Biol. Med.* **220**, 239 (1999).
- [8] J. D. Felton, M. G. Knize, C. Wood, B. J. Wuebbles, S. K. Healy, D. H. Struermer, L. F. Bjeldfanes, B. J. Kimble, and F. T. Hatch, *Carcinogenesis*, **5**, 95 (1984).
- [9] T. Sugimura, S. Sato, and K. Wakabayashi, Chemical Induction of Cancer, Structural Bases and Biological Mechanisms, Y. T. Woo, D. Y. Lai, J. C. Arcos, and M. F. Argus, eds., Academic Press, New York, 1988, p. 681.
- [10] IRAC Monographs on the Evaluation of Carcinogenic Risks to Humans. No. 56, Some Naturally Occurring Substances: Food Items and Constituents. Heterocyclic Aromatic Amines and Mycotoxins, International Agency for Research on Cancer, Lyon, France, 1993, p. 165.
- [11] G. A. Keating, D. W. Layton, and J. S. Felton, *Mutation Res.*, **443**, 149 (1999).
- [12] W. Zheng, D. R. Gustafson, R. Sinha, J. R. Cerhan, D. Moore, C. P. Hong, K. E. Anderson, L. H. Kushi, T. A. Sellers, and A. R. Folsom, *J. Natl. Cancer Inst.* **90**, 1687 (1998).
- [13] K. H. Dingley, K. D. Curtis, S. Nowell, J. S. Felton, N. P. Land, and K. W. Turteltaub, *Cancer Epidemiol. Biomarkers Prevention* **8**, 507 (1999).
- [14] J. S. Felton, M. G. Knize, N. H. Shen, B. D. Andersen, L. F. Bjeldanes, and F. T. Hatch, *Environ. Health Perspect.*, **67**, 17 (1986).
- [15] J. S. Felton, M. G. Knize, M. Roper, E. Fultz, N. H. Shen, and K. W. Turteltaub, *Cancer Res.*, **52** (Suppl.), 2103 (1992).
- [16] G. Becher, M. G. Knize, I. F. Nes, and J. S. Felton, *Carcinogenesis*, **9**, 247 (1988).
- [17] J. S. Felton, M. G. Knize, F. T. Hatch, M. J. Tanga, and M. E. Colvin, *Cancer Lett.*, **143**, 127 (1999).
- [18] M. J. Tanga, J. E. Bupp, and T. K. Tochimoto, *J. Heterocyclic Chem.*, **31**, 1641 (1994).
- [19] M. J. Tanga, J. E. Bupp, and T. K. Tochimoto, *J. Heterocyclic Chem.*, **34**, 717 (1997).
- [20] The ten isomers 1,5,6-trimethyl-2-aminoimidazo[4,5-*b*]pyridine; 3,5,6-trimethyl-2-aminoimidazo[4,5-*b*]pyridine; 3,5,7-trimethyl-2-aminoimidazo[4,5-*b*]pyridine; 1,4,7-trimethyl-2-aminoimidazo[4,5-*c*]pyridine; 1,6,7-trimethyl-2-aminoimidazo[4,5-*c*]pyridine; 3,4,6-trimethyl-2-aminoimidazo[4,5-*c*]pyridine; 1,4,6-trimethyl-2-aminoimidazo[4,5-*c*]pyridine; 1,5,7-trimethyl-2-aminoimidazo[4,5-*b*]pyridine; 3,6,7-trimethyl-2-aminoimidazo[4,5-*b*]pyridine; and 3,6,7-trimethyl-2-aminoimidazo[4,5-*c*]pyridine are presently available as reference materials from Dr. Harold Seifried, National Cancer Institute, 6130 Executive Boulevard, EPN 3158, Bethesda, MD 20852.
- [21] R. Gompper and R. Kunz, *Chem. Ber.*, **99**, 2900 (1966).