Synthesis of Mutagenic Heterocyclic Amines PhIP and DMIP

Tominari Choshi, Akiko Tonari, Haruyuki Yoshioka, Kenichi Harada, Eiichi Sugino, and Satoshi Hibino*

Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University, Fukuyama, Hiroshima 729-02, Japan

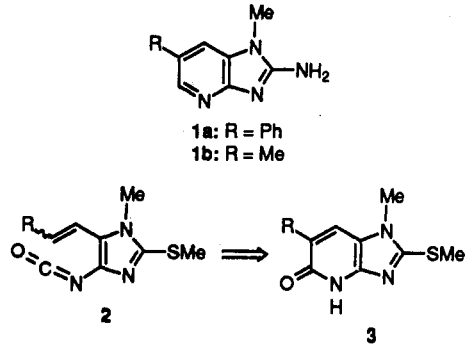
Received July 19, 1993

It has been shown in numerous studies that mutagenic heterocyclic amines are present in the pyrolysates of amino acids, peptides, and proteins and in cooked foods.[1] In 1986, a new mutagen was isolated from cooked beef[2] which was determined to be 2-amino-1-methyl-6-phenylimidazo-[4,5-*b*]pyridine (PhIP, **1a**) by spectral evidence[2] and synthetic study.[3] A few years later, closely related 2-amino-1,6-dimethylimidazo[4,5-*b*]pyridine (DMIP, **1b**) was also isolated from a fried Norwegian sausage along with the other mutagenic amines[4a] and its preliminary synthetic study was similarly reported by the same group.[4b] Recently, it was reported that PhIP (**1a**) is carcinogenic in animal tests.[1f] These compounds are of considerable interest to researchers worldwide who are interested in the role of cooked meat in human cancer. Significant improvements in the methods of synthesis of these compounds are vital to the advancement of this field.

We are currently interested in the synthesis of fused pyridine ring systems by the thermal electrocyclic reaction of monoazahexa-1,3,5-triene systems possessing one double bond of the aromatic or heteroaromatic portion.[5] In seeking a route to the imidazo[4,5-*b*]pyridine ring for the synthesis of PhIP (**1a**) and DMIP (**1b**), we envisaged that 2-azahexa-1,3,5-trienes (**2**) would undergo a thermal electrocyclic reaction, with a tautomeric step to yield the imidazo[4,5-*b*]pyridin-5(4*H*)-ones (**3**), according to the modified Eloy's pyrido-annelation[6] (Chart I). We here report on the use of this reaction to prepare PhIP (**1a**) and DMIP (**1b**).

For the synthesis of the required 5-alkenyl-4-isocyanatoimidazole (**2**), we chose readily available 1-methyl-2,4,5-tribromoimidazole (**4**)[7] as a starting material. Treatment of **4** with BuLi followed by addition of dimethyl disulfide gave the 2-(methylthio)imidazole (**5**) (100%).[7a,8] Subsequent treatment of **5** with BuLi followed by addition of dimethylformamide at −78 °C afforded 5-formylimidazole (**6**) in 81.3% yield. Wittig reaction of **6** with alkyltriphenylphosphorane in turn gave the 5-alkenyl-4-bromoimidazoles **7a** and **7b** (100%). Treatment of **7a** and **7b** with BuLi followed by addition of gaseous carbon dioxide at −78 °C gave the carboxylic acids (**8a** and **8b**) (93.5 and 61.1%) as isocyanate precursors. The carboxylic acids were heated in benzene in the presence of diphenyl phosphorazidate (DPPA) and triethylamine under Curtius conditions[9] to give the relatively stable isocyanates (**2a** and **2b**) (1–2 days at 20–25 °C: 98 and 99.6%), bearing the desired 2-azahexa-1,3,5-triene system (Scheme I).

Chart I



As proposed, the electrocyclic reaction of **2** was carried out by heating in *o*-dichlorobenzene for 5–15 min to obtain the expected imidazo[4,5-*b*]pyridin-5(4*H*)-one derivatives (**3a** and **3b**) (86.4 and 75.7%). Thus, synthesis of the imidazo[4,5-*b*]pyridine ring has been achieved by the pyrido-annelation based on the thermal electrocyclic reaction of the 2-azahexa-1,3,5-triene system (**2**), involving the imidazole 4,5-bond.

For the synthesis of targeted molecules **1**, the pyridones **3a** and **3b** were reduced with sodium bis(2-methoxyethoxy)aluminum hydride (Red-Al) to give the desired imidazo[4,5-*b*]pyridines **9a** (41.5%) and **9b** (43.7%), respectively. Finally, the amination[10] of **9a** and **9b** with sodium amide in dry xylene yielded 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (**1a**: 90.2%) and 2-amino-1,6-dimethylimidazo[4,5-*b*]pyridine (**1b**: 49.9%), respectively. The spectral data of synthetic **1a** and **1b** were identical with those reported for PhIP[2,3] and DMIP.[4] PhIP (**1a**) and DMIP (**1b**) were constructed from 1-methyl-2,4,5-tribromoimidazole (**4**) in 24.3 and 8.2% overall yields, respectively.

As a result, the overall yield of PhIP has been much improved relative to a previous report.[3] However, the total yield of DMIP was slightly lower than that of Becher's earlier route.[4b]

Experimental Section

Melting points were determined with a Yanagimoto micro melting point apparatus and are uncorrected. Infrared spectra were measured with a Shimadzu FTIR-8500 spectrometer. Proton nuclear magnetic resonance spectra were taken with JEOL PMX60Si and JEOL JNM-A500 spectrometers in deuterated $CHCl_3$ with $SiMe_4$ as an internal standard unless otherwise stated. Mass spectra and high resolution mass spectra were recorded on

(1) (a) Sugimura, T.; Sato, S. *Cancer Res. (suppl.)* **1983**, *43*, 2415s–2421s. (b) Hatch, F. T.; Felton, J. S.; Stuermer, D. H.; Bjeldanes, L. F. In *Chemical Mutagens: Principles and Methods for their Detection*; Serres, F. J.; Eds.; Plenum Press: New York, 1984; Vol. 9, pp 111–164. (c) Hashimoto, Y.; Shudo, K.; Okamoto, T. *Acc. Chem. Res.* **1984**, *17*, 403. (d) Sugimura, T. *Science* **1986**, *233*, 312. (e) Kato, R.; Yamazoe, Y. *Jpn. J. Cancer Res. (Gann)* **1987**, *78*, 297. (f) Wakabayashi, K.; Nagao, M.; Esumi, H.; Sugimura, T. *Cancer Res. (suppl.)* **1992**, *52*, 2092s–2098s.

(2) Felton, J. S.; Knize, M. G.; Shen, N. H.; Lewis, P. R.; Andresen, B. D.; Happe, J.; Hatch, F. T. *Carcinogenesis* **1986**, *7*, 1081.

(3) Knize, M. G.; Felton, J. S. *Heterocycles* **1986**, *24*, 1815.

(4) (a) Becher, G.; Knize, M. G.; Nes, J. F.; Felton, J. S. *Carcinogenesis* **1988**, *9*, 247. (b) Becher, G.; Knize, M. G.; Felton, J. S. *Risk Assessment of Cooked Food Mutagens; Reports from the Second Nordic Meeting, Uppsala, Sweden, October 1988; Vår Föda Suppl. 2*, 1989; Vol. 42, pp 85–90.

(5) (a) Hibino, S.; Sugino, E.; Kuwada, T.; Ogura, N.; Sato, K.; Choshi, T. *J. Org. Chem.* **1992**, *57*, 5917. (b) Hibino, S.; Sugino, E.; Kuwada, T.; Ogura, N.; Shintani, Y.; Sato, K. *Chem. Pharm. Bull.* **1991**, *39*, 79 and related refs cited therein.

(6) (a) Eloy, F.; Deryckere, A. *Helv. Chem. Acta* **1969**, *52*, 1755. (b) *ibid.* **1970**, *53*, 645. (c) *Idem. Bull. Soc. Chim. Belg.* **1970**, *79*, 301. (d) *Idem. Bull. Chim. Therap.* **1970**, 121. (e) *Idem. J. Heterocycl. Chem.* **1970**, *7*, 1191.

(7) (a) O'Connel, J. F.; Parquett, J.; Yelle, W. E.; Wang, W.; Rapoport, H. *Synthesis* **1988**, 767. (b) Stencio, K.-E.; Wahlberg, K.; Wahren, R. *Acta Chem. Scand.* **1973**, *27*, 2179.

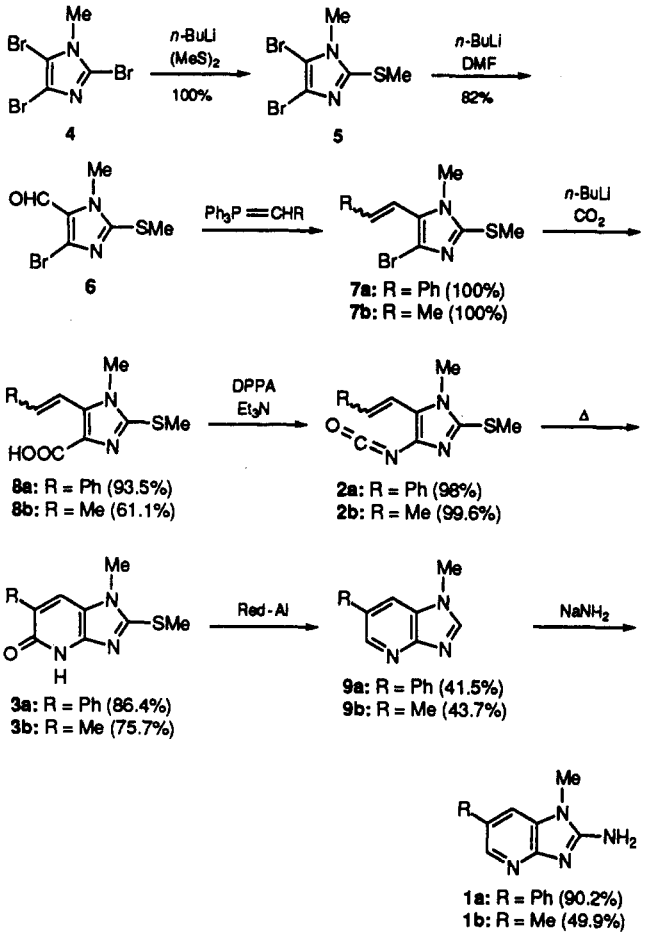
(8) Grimmett, M. R. *Comprehensive Heterocyclic Chemistry*; Katritzky, A. Z., Rees, C. W., Eds.; Pergamon Press: Oxford, 1984; Vol. 5, p 394.

(9) Shioiri, T.; Yamada, S. *Chem. Pharm. Bull.* **1974**, *22*, 849.

(10) Pozharskii, A. F.; Kuz'menko, V. V.; Kolodyazhnyi, Y.-V.; Siminov, A. M. *Chem. Heterocycl. Compd.* **1972**, *8*, 1131.

0022-3263/93/1958-7952$04.00/0

Scheme I



a Shimadzu GC-MS 9020DF spectrometer at 70 eV chamber voltage on a direct inlet system. Silica gel (60–100 mesh, Merck Art 7734) and Iatrobeads (Iatron Chem. Prod.) were used for column chromatography. *Since PhIP is carcinogenic, contact with the reaction mixture should be avoided in the last step.*[1f] *Carcinogenicity of DMIP is unclear, but caution may be necessary.*

4,5-Dibromo-1-methyl-2-(methylthio)imidazole (5). A stirred solution of BuLi (9.3 mL of 1.68 M hexane solution, 15.7 mmol) was added at −78 °C to a solution of 1-methyl-2,4,5-tribromoimidazole (4)[7a] (5.0 g, 15.7 mmol) in anhyd THF (70 mL) under argon atmosphere. After being kept at −78 °C for 30 min, a solution of dimethyl disulfide (1.4 mL, 15.7 mmol) in anhyd THF (10 mL) was added by syringe. Then the solution was stirred at ambient temperature for 12 h, which was quenched with brine, and the mixture was extracted with EtOAc. The EtOAc layer was washed with brine, dried over Na_2SO_4, and concentrated to give the dibromoimidazole (5) (4.48 g, 100%), bp 130–131 °C/1.5 torr: ^{1}H NMR δ 2.57 (3H, s), 3.54 (3H, s); MS *m/z* 288 ($M^+ + 4$), 286 ($M^+ + 2$), 284 (M^+). Anal. Calcd for $C_5H_6Br_2N_2S$: C, 21.00; H, 2.11; N, 9.79. Found: C, 21.15; H, 2.25; N, 9.63.

4-Bromo-1-methyl-2-(methylthio)imidazole-5-carboxaldehyde (6). A stirred solution of BuLi (11.5 mL of 1.68 M hexane solution, 19.3 mmol) was added dropwise at −78 °C to a solution of dibromoimidazole (5) (4.6 g, 16.1 mmol) in anhyd Et_2O (70 mL) under argon atmosphere. After being kept at −78 °C for 30 min, a solution of DMF (1.4 g, 19.3 mmol) in anhyd Et_2O (10 mL) was added by syringe. The solution was stirred at ambient temperature for 12 h, which was quenched with brine, and the mixture was extracted with EtOAc. The EtOAc layer was washed with brine, dried over Na_2SO_4, and concentrated to give the aldehyde (6) (3.1 g, 81.3 %): mp 111–112 °C (Et_2O); IR (KBr) 1662 cm^{-1} (CHO); ^{1}H NMR δ 2.60 (3H, s), 3.77 (3H, s), 9.51 (1H, s); MS *m/z* 236 ($M^+ + 2$), 234 (M^+). Anal. Calcd for $C_6H_7BrN_2OS$: C, 30.65; H, 3.00; N, 11.91. Found: C, 30.68; H, 2.84; N, 12.19.

4-Bromo-1-methyl-2-(methylthio)-5-(2-phenylethenyl)imidazole (7a). A solution of BuLi (2.9 mL of 1.61 M hexane solution, 4.7 mmol) was added dropwise to an ice-cooled mixture of benzyltriphenylphosphonium bromide (2.0 g, 4.7 mmol) in anhyd THF (40 mL) with stirring under argon atmosphere. After stirring at room temperature for 30 min, a solution of the aldehyde (6) (1.0 g, 4.3 mmol) in anhyd THF (40 mL) was added by syringe under cooling with ice. Then the mixture was stirred at ambient temperature for 12 h, which was quenched with brine, and the mixture was extracted with EtOAc. The EtOAc layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography (silica gel, 20 g) with 10% EtOAc/hexane as an eluent to give the oily alkenylimidazole 7a (1.3 g, 100%): ^{1}H NMR δ 2.56 ($^{33}/_{19}$H, s), 2.59 ($^{24}/_{19}$H, s), 3.05 ($^{33}/_{19}$H, s), 3.59 ($^{24}/_{19}$H, s), 5.94 ($^{11}/_{19}$H, d, *J* = 12 Hz), 6.63 ($^{8}/_{19}$H, d, *J* = 18 Hz), 6.72 ($^{11}/_{19}$H, d, *J* = 12 Hz), 7.16 ($^{8}/_{19}$H, d, *J* = 18 Hz), 6.91–7.74 (5 H, m); MS *m/z* 310 ($M^+ + 2$), 308 (M^+); HRMS calcd for $C_{13}H_{13}BrN_2S$ 307.9982, found 308.0012.

4-Bromo-1-methyl-2-(methylthio)-5-(1-propenyl)imidazole (7b). The same procedure as above gave the oily alkenylimidazole 7b (100%) from 6: ^{1}H NMR δ 1.88 (3H, d, *J* = 5 Hz), 2.50 (3H, s), 3.45 (3H, s), 5.74–6.37 (2H, m); MS *m/z* 248 ($M^+ + 2$), 246 (M^+); HRMS calcd for $C_8H_{11}BrN_2S$ 245.9826, found 245.9837.

1-(Methylthio)-5-(2-phenylethenyl)imidazole-4-carboxylic Acid (8a). A solution of BuLi (6.3 mL of 1.56 M hexane solution, 9.9 mmol) was added at −78 °C to a stirred solution of the 4-bromoimidazoles (7a) (1.06 g, 4.5 mmol) in anhyd Et_2O (100 mL) under argon atmosphere. After being kept at −78 °C for 1 h, dry carbon dioxide gas was introduced and the mixture was stirred at ambient temperature for 12 h. The reaction mixture was extracted with an aqueous 1% NaOH solution (30 mL × 3 times). The combined NaOH solution was acidified by AcOH (about pH 4) and then the mixture was reextracted with $CHCl_3$. The $CHCl_3$ layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was recrystallized from $CHCl_3$–hexane to give the carboxylic acid 8a (881 mg, 93.5 %); mp 169–171 °C; IR (KBr) 2929, 1664 cm^{-1} (COOH); ^{1}H NMR δ 2.60 ($^3/_3$H, s), 2.64 ($^6/_3$H, s), 2.89 ($^3/_3$H, s), 3.63 ($^6/_3$H, s), 6.15 (1 H, br s), 6.57–7.60 (7H, m); MS *m/z* 274 (M^+), 229 ($M^+ - 45$). Anal. Calcd for $C_{14}H_{14}N_2O_2S$: C, 61.29; H, 5.14; N, 10.21. Found: C, 61.15; H, 4.93; N, 10.36.

1-(Methylthio)-5-(1-propenyl)imidazole-4-carboxylic Acid (8b). The same procedure as above gave the carboxylic acid 8b (61.1%) from 7b: mp 165–167 °C ($CHCl_3$/hexane); IR (KBr) 3004, 1680 (COOH) cm^{-1}; ^{1}H NMR δ 1.95 (3H, d, *J* = 5 Hz), 2.61 (3H, s), 3.52 (3H, s), 6.38–6.67 (2H, m), 7.16 (1H, br s); MS *m/z* 212 (M^+). Anal. Calcd for $C_9H_{12}N_2O_2S$: C, 50.93; H, 5.70; N, 13.20. Found: C, 51.01; H, 5.88; N, 13.09.

4-Isocyanato-1-methyl-2-(methylthio)-5-(2-phenylethenyl)imidazole (2a). A stirred solution of the carboxylic acid 8a (670 mg, 2.44 mmol), DPPA (1.6 mL, 7.32 mmol), and triethylamine (1.1 mL, 7.32 mmol) in dry benzene (67 mL) was heated at 50 °C for 1 h. After cooling at room temperature, the solvent was removed. The residue was purified by column chromatography (silica gel, 30 g) with 30% EtOAc/hexane to give the isocyanate 2a (649 mg, 98%). The isocyanate was used without further purification because it was unstable for recrystallization (stable for 1–2 days at room temperature): IR (KBr) 2130 (NCO) cm^{-1}; ^{1}H NMR δ 2.74 ($^3/_2$H, s), 2.79 ($^3/_2$H, s), 3.00 ($^3/_2$H, s), 3.75 ($^3/_2$H, s), 6.62 ($^1/_2$H, d, *J* = 12 Hz), 6.98 ($^1/_2$H, d, *J* = 12 Hz), 7.10–7.77 (6H, m); MS *m/z* 271 (M^+); HRMS calcd for $C_{14}H_{13}N_3OS$ 271.0779, found 271.0795.

4-Isocyanato-1-methyl-2-(methylthio)-5-(1-propenyl)imidazole (2b). The same procedure as above gave the isocyanate 2b from 8b (99.6%): IR (KBr) 2132 (NCO) cm^{-1}; ^{1}H NMR δ 2.03 (3H, d, *J* = 6 Hz), 2.70 (3H, s), 3.59 (3H, s), 6.01–6.70 (2H, m); MS *m/z* 209 (M^+); HRMS calcd for $C_9H_{11}N_3OS$ 209.0622, found 209.0641.

1-Methyl-2-(methylthio)-6-phenylimidazo[4,5-*b*]pyridin-5-(4*H*)-one (3a). A stirred solution of the isocyanates 2a (250 mg, 0.92 mmol) in *o*-dichlorobenzene (50 mL) was refluxed at 190 °C for 15 min. After cooling at room temperature, the solvent was removed. The residue was purified by column chromatography (silica gel, 30 g) with 2% MeOH/$CHCl_3$ to give the pyridone 3a (216 mg, 86.4%): mp 226–228 °C ($CHCl_3$/hexane); IR (KBr)

1630 (CO) cm^{-1}; ^1H NMR δ ($CDCl_3$/MeOH-d_4) 2.60 (3H, s), 3.59 (3H, s), 7.01–7.37 (6H, m); MS m/z 271 (M^+), 256 ($M^+ - 15$). Anal. Calcd for $C_{14}H_{13}N_3OS$: C, 61.97; H, 4.83; N, 15.49. Found: C, 62.06; H, 5.04; N, 15.20.

1,6-Dimethyl-2-(methylthio)imidazo[4,5-*b*]pyridin-5(4*H*)-one (3b). The same procedure as above gave the pyridone **3b** from **2a** (75.7%): mp 237–239 °C (MeOH); IR (KBr) 1630 (CO) cm^{-1}; ^1H NMR δ (DMSO-d_6) 2.05 (3H, s), 2.63 (3H, s), 3.61 (3H, s), 7.66 (1H, s); MS m/z 209 (M^+), 194 ($M^+ - 15$). Anal. Calcd for $C_9H_{11}N_3OS$: C, 51.66; H, 5.30; N, 20.08. Found: C, 51.52; H, 5.51; N, 20.26.

1-Methyl-6-phenylimidazo[4,5-*b*]pyridine (9a). A stirred mixture of the pyridone **3a** (100 mg, 0.37 mmol) and $NaAlH_2(OCH_2CH_2OCH_3)_2$ (1.1 mL of 3.46 M toluene solution, 3.69 mmol) in dry toluene (10 mL) was refluxed for 20 h under argon atmosphere. After cooling at room temperature, EtOAc (5 mL) was added. The solvent was removed under reduced pressure and the brine was added. The mixture was extracted with $CHCl_3$. The $CHCl_3$ layer was washed with brine, dried over Na_2SO_4, and evaporated to dryness. The residue was purified by column chromatography (silica gel, 20 g) with 2% MeOH/$CHCl_3$ as an eluent to give the imidazopyridine **9a** (32 mg, 41.5%): mp 131–133 °C ($CHCl_3$/hexane); ^1H NMR δ 3.84 (3H, s), 7.17–7.66 (5H, m), 7.72 (1H, d, J = 2 Hz), 7.94 (1H, s), 8.66 (1H, d, J = 2 Hz); MS m/z 209 (M^+). Anal. Calcd for $C_{13}H_{11}N_3$: C, 74.62; H, 5.30; N, 20.08. Found: C, 74.53; H, 5.04; N, 20.06.

1,6-Dimethylimidazo[4,5-*b*]pyridine (9b). The same procedure as above gave the imidazopyridine (**9b**) from the pyridone **3b** (43.7%): mp 111–113 °C (benzene/hexane); ^1H NMR δ 2.49 (3H, s), 3.81 (3H, s), 7.45 (1H, d, J = 2 Hz), 7.93 (1H, s), 8.34 (1H, d, J = 2 Hz); MS m/z 147 (M^+), 132 ($M^+ - 15$). Anal. Calcd for $C_8H_9N_3$: C, 65.29; H, 6.16; N, 28.55. Found: C, 65.51; H, 6.07; N, 28.82.

2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP: 1a). *Caution: Carcinogenic!* A stirred solution of the imidazopyridine **9a** (30 mg, 0.14 mmol) and $NaNH_2$ (120 mg, 3.08 mmol) in dry xylene (2 mL) was heated at 130 °C for 1.5 h under argon atmosphere. After removal of solvent, the excess $NaNH_2$ in the residue was decomposed with an addition of water, which was evaporated to dryness under reduced pressure. The residue was purified by column chromatography (Iatrobeads, 10 g) with 10% MeOH/$CHCl_3$ as an eluent to give the PhIP (**1a**) (29 mg, 90.2%): mp 329–330.5 °C ($CHCl_3$/hexane) (lit.,[3] mp 327–328 °C); IR (KBr) 3088 (NH_2) cm^{-1}; ^1H NMR δ (500 MHz, DMSO-d_6) 3.56 (3H, s), 7.01 (2H, br s), 7.31 (1H, dd, J = 7.3, 7.3 Hz), 7.45 (2H, dd, J = 7.3, 7.3 Hz), 7.68 (2H, d, J = 7.3 Hz), 7.75 (1H, d, J = 2.1 Hz), 8.28 (1H, d, J = 2.1 Hz); MS m/z 224 (M^+). Anal. Calcd for $C_{13}H_{12}N_4$: C, 69.63; H, 5.39; N, 24.98. Found: C, 69.68; H, 5.57; N, 25.11.

2-Amino-1,6-dimethylimidazo[4,5-*b*]pyridine (DMIP: 1b). *Caution: Potential carcinogen!* The same procedure as above gave the DMIP (**1b**) (49.9%) from the imidazopyridine **9b** (49.9%): mp 273–275 °C ($CHCl_3$/hexane); IR (KBr) 3280 (NH_2) cm^{-1}; ^1H NMR δ (500 MHz, acetone-d_6) 2.35 (3H, dd, J = 0.6 ± 0.15, 0.6 Hz), 3.61 (3H, s), 7.27 (1H, dd, J = 0.6 ± 0.15, 2.0 Hz), 7.91 (1H, dd, J = 0.6, 2.0 Hz); MS m/z 162 (M^+). Anal. Calcd for $C_8H_{10}N_4$: C, 59.24; H, 6.21; N, 32.54. Found: C, 59.47; H, 6.29; N, 32.76.

Acknowledgment. We thank Dr. M. G. Knize, Lawrence Livermore National Laboratory, Biomedical Sciences Division, University of California, for sending an authentic PhIP sample and a proton NMR spectrum (270 MHz) of DMIP. We also thank Miss Y. Murakami and R. Kumanomidoh for their assistance.

Supplementary Material Available: NMR data and peak assignments for PhIP (**1a**) and DMIP (**1b**) (1 page). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.