Synthesis of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), a heterocyclic food mutagen

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Abstract

The synthesis of the heterocyclic food mutagen, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) **7**, is reported. PhIP **7** was synthesized in six steps from commercially available 2,3-diaminopyridine **1** in 6% overall yield. This route features a new ring closure reagent, *N*-dichloromethylene-*p*-toluenesulfonamide, to assemble the imidazo[4,5-*b*]pyridine ring system and uses a Suzuki coupling to introduce an aryl ring onto the heterocyclic core as the last synthetic step.

Keywords: 2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine, PhIP, heterocyclic amine, food mutagen

Introduction

Diet is one of the most significant environmental causes of cancer in humans.[1] A series of mutagenic and carcinogenic heterocyclic amines has been identified from cooked meat and fish.[2] These heterocyclic amines are believed to arise from the condensation amino acids, glucose and creatinine via the Maillard reaction during cooking at high temperatures.[3] The most mass abundant of the heterocyclic amines is 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) **7**.[1] PhIP **7** forms covalent DNA adducts resulting in genetic mutations in animal and human models.[4] This compound has also been demonstrated to cause cancer in animal models and is suspected to be responsible for various types of cancer such as prostate, breast and colon in humans.[4-6] Chemical standards of these mutagens need to be made for biological assays to assess the risk associated with their consumption. In our continued effort to develop new methods for the syntheses of biologically important mutagens and carcinogens, we now report a new method for the synthesis of **7**.

We were interested in developing a expedient and flexible synthesis of **7** that would be also amenable to making isotope-labeled and metabolites of **7**. This synthesis features new ring-closure reagent, *N*-dichloromethylene-*p*-toluenesulfonamide,[7] that reacts with an aromatic vicinal

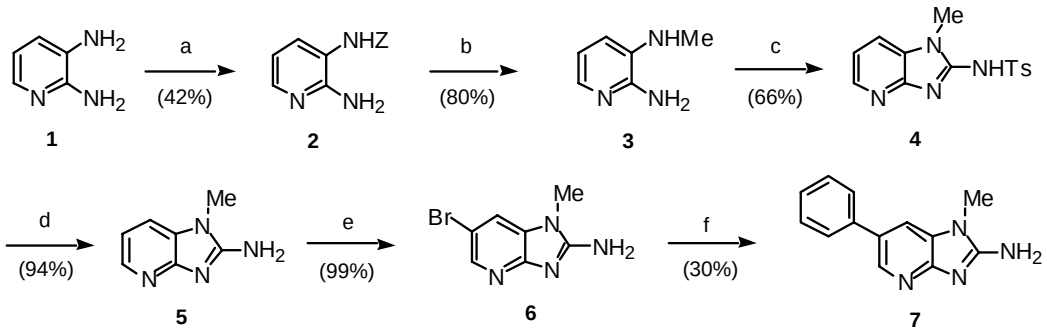
diamine **3** that contains both primary and secondary amine groups to give the tosyl-protected *N*-alkyl imidazo[4,5-*b*]pyridine **4**. This ring-closure reagent is more reactive than the analogous *N*-(bis-methylthio)methylene-*p*-toluenesulfonamide that reacts most readily with primary aliphatic diamines.[8] The *N*-tosyl cyclic amine **4** can be deprotected in high yield by anhydrous HF[8] to give the 2-amino-1-methyl-imidazo[4,5-*b*]pyridine heterocyclic core **5**.

Previous published routes for synthesis of **7**[9-12] were not amenable to facile derivatization of the aryl ring that will be important to make isotope-labeled[13] and metabolites of **7**. The Suzuki coupling[14] stood out as an ideal method to introduce the aryl ring onto a brominated heterocycle in the last step of this synthesis. This flexible coupling reaction allows for the synthesis of **7** and a potentially large variety of aryl-substituted PhIP derivatives from commercially available arylboronic acids.

Results and Discussion

In the first step of this synthesis (**Figure 1**), 2,3-diaminopyridine **1** was allowed to react with benzyl chloroformate in THF using pyridine as an acid scavenger to give N^3-benzoyloxycarbonyl-2,3-diaminopyridine **2** in 42% yield.[15] The carbamate **2** was reduced with $LiAlH_4$ in refluxing THF to give 2-amino-3-methylaminopyridine **3** in 80% yield.[15]

Condensation of the diamine **3** with *N*-dichloromethylene-*p*-toluenesulfonamide in dioxane gave 66% yield of 2-amino-*p*-*N*-toluenesulfonamide-1-methyl-imidazo[4,5-*b*]-pyridine **4**. Cleavage of the toluenesulfonamide group of **4** with anhydrous HF[8] gave a 94% yield of 2-amino-1-methyl-6-imidazo[4,5-*b*]pyridine **5**. Bromination of **5** with 1.5 equivalents of bromine in refluxing acetic acid[16] provided an essentially quantitative yield of 2-amino-1-methyl-6-bromoimidazolo[4,5-*b*]pyridine **6**. The Suzuki coupling of **6** with phenylboronic acid and 10 mol% $Pd(PPh_3)_4$ in refluxing dioxane[14c] gave a 30% isolated yield of PhIP **7**. Spectral data of synthetic **7** was in good agreement with authentic samples.[2,9] **Since compound 7 is a known mutagen and suspected carcinogen, direct contact should be avoided.**



Scheme 1. Synthesis of PhIP **7**.

[a.] CBzCl, Pyr, THF, 0 °C. [b.] $LiAlH_4$, THF, 65 °C. [c.] $TsNCCl_2$, dioxane, 80 °C. [d.] HF, 100 °C. [e.] Br_2, AcOH, 100 °C. [f.] $PhB(OH)_2$, $Pd(PPh_3)_4$, dioxane/EtOH, aqueous K_2CO_3, 110 °C.

Conclusions

We have reported a new route for the synthesis compound **7**, which was prepared in six synthetic steps in 6% overall yield starting from a commercially available 2,3-diaminopyridine **1**. This route features a new ring closure reagent, *N*-dichloromethylene-*p*-toluenesulfonamide, to assemble the imidazo[4,5-*b*]pyridine ring system and uses a Suzuki coupling to introduce an aryl ring onto the heterocyclic core as the last synthetic step.

Experimental Section

General Procedures. NMR spectra were recorded on a Varian Gemini 300 MHz NMR with chemical shifts reported downfield from TMS. IR spectra were recorded on a Perkin-Elmer FTIR Spectrometer Model 3100. UV-VIS spectra were recorded on a Varian DMS 90 UV-VIS Spectrometer. Melting points (uncorrected) were recorded on a Laboratory Devices Mel-Temp Apparatus. Low resolution mass spectra were obtained on a Finnigan LCQ Duo Mass Spectrometer set in Positive Electrospray Ionization (ESI) Mode. High resolution mass spectra were obtained on a Finnigan MAT 95 Mass Spectrometer set in Positive Chemical Ionization (CI) mode. Elemental analysis was performed by Galbraith Laboratories (Knoxville, TN). All chemicals and solvents were purchased from Aldrich Chemical Company (Milwaukee, WI) and used without further purification.

***N*^{3}-Benzyloxycarbonyl-2,3-diaminopyridine (2).** A dry 1000-mL round bottom flask was charged with 200 mL anhydrous THF, 20 mL pyridine and 8.73 g (80 mmol) of finely divided 2,3-diaminopyridine to give a dark brown suspension. At 0 °C, benzyl chloroformate (23.9 g, 140 mmol) was added dropwise over 20 min. The reaction mixture was stirred for 12 h at 25 °C. The reaction mixture was diluted with 400 mL deionized water and the organic layer was extracted with 2 x 200 mL portions of EtOAc. The organic layer was dried over anhydrous Na_2SO_4, filtered and dried under vacuum (25 °C, 0.1 torr) to give a viscous brown oil. The oil was stirred in 200 mL $CHCl_3$ and filtered to give 6.12 g of beige solid. The mother liquor was eluted through a short silica gel column using 10% MeOH/$CHCl_3$ and the solvent was removed under vacuum (25 °C, 0.1 Torr) to give a brown oil. The brown oil was stirred in 100 mL $CHCl_3$ and filtered to give an additional 1.98 g of beige solid. Beige solid (8.11 g, 42%); mp = 175-176 °C (lit.[15] mp =182-183 °C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.47 (s, 2H), 7.09-7.14 (dd, 1H, *J* = 5.7 Hz, *J* = 7.5 Hz), 6.59-6.63 (q, 1H, *J* = 4.8 Hz, J = 7.8 Hz), 7.39-7.48 (m, 3H), 7.54-7.56 (d, 2H, *J* = 7.2 Hz), 7.89-7.92 (dd, 1H, *J* = 1.5 Hz, *J* = 8.1 Hz), 8.06-8.08 (dd, 1H, *J* = 1.5 Hz, *J* = 5.4 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 66.66, 112.99, 119.25, 128.71, 129.11, 130.02, 137.29, 144.13, 154.20, 154.95; LRMS (ESI$^+$) $[M+H]^+$ = 244 m/z; IR (KBr) 3456, 3311, 3065, 1775, 1723, 1703, 1655, 1618, 1543, 1524, 1509, 1494, 1458, 1387, 1339, 1294, 1266,

1212, 1167, 1128, 1052, 1003, 825, 788, 758, 700, 680, 645, 581, 525, 489 cm^{-1}; UV-VIS (95% EtOH) λ_{max} 285 nm (ε = 5,674), 233 (ε = 8,255).

2-Amino-3-methylaminopyridine (3). A dry 250-mL round bottom flask was charged with 50 mL anhydrous THF and N^3-benzoyloxycarbonyl-2,3-diaminopyridine (4.81 g, 20 mmol). At 0 °C, 50 mL of 1 M $LiAlH_4$ in THF was added to the flask over 20 minutes. The reaction mixture was heated to reflux (65 °C) for 4 h. At 0 °C, the reaction was quenched with 10 mL deionized water followed by 100 mL 3 M HCl. The aqueous layer was extracted with 4 x 50 mL portions of diethyl ether. At 0 °C, the aqueous layer was made basic (pH = 12) with solid KOH. The aqueous layer was extracted with 4 x 50 mL portions of THF/diethyl ether. The organic layer was dried over anhydrous $MgSO_4$, filtered and the solvent was removed under vacuum (25 °C, 0.1 torr) to give 3.60 g (80%) of a brown solid; mp = 115-118 °C (lit.[13] 120-121 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.81 (s, 3H), 3.44 (br s, 1 H), 4.17 (br s, 2H), 6.72-6.77 (m, 2H, *J* = 6.3 Hz), 7.57 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 30.72, 116.10, 116.34, 133.53, 136.37, 148.98; LRMS (ESI^+) $[M+H]^+$ = 244 m/z; IR (KBr) 3319, 2919, 2682, 2365, 1967, 1635, 1581, 1458, 1364, 1302, 1278,1223, 1072, 910, 779, 760, 656 cm^{-1}; UV-VIS (95% EtOH) λ_{max} 308 nm (ε = 6,396), 251 (ε = 8,400).

***N*-Dichloromethylene-*p*-toluenesulfonamide.** A 1000-mL round bottom flask was charged with 170 mL glacial acetic acid and *N*-(bis-methylthio)methylene-*p*-toluenesulfonamide (24.82 g, 90.1 mmol). At 15 °C, a gentle stream of chlorine gas was added to the suspension over 1 h. The resulting solution was stirred an additional 1 h at 15 °C. The solvent was removed under vacuum (25 °C, 0.1 torr) and the solid residue was sublimed (75 °C, 0.2 torr) to give 19.1 g (83%) of a white solid; mp = 80-83 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.46 (s, 3H), 7.38 (d, 2H, *J* = 8.5 Hz), 7.86 (d, 2H, *J* = 8.5 Hz).

2-Amino-*p*-*N*-toluenesulfonamide-1-methyl-imidazo[4,5-*b*]pyridine (4). A dry 250 mL round bottom flask was charged with 80 mL anhydrous dioxane, 2-amino-3-methylaminopyridine (1.23 g, 10 mmol), *N*-dichloromethylene-*p*-toluenesulfonamide (2.51 g, 10 mmol) and anhydrous K_2CO_3 (3.45 g, 2.5 mmol). The reaction mixture was heated to 80 °C for 12 h. The reaction mixture was diluted with 50 mL deionized water and the aqueous layer was extracted with 4 x 100 mL portions of $CHCl_3$. The organic layer was dried over anhydrous Na_2SO_4, filtered and dried under vacuum (25 °C, 0.1 torr) to give 3.72 g of crude product as a brown solid. The solid was purified via column chromatography on silica gel with 1% MeOH/$CHCl_3$ as the eluting solvent. The solvent was removed under vacuum (25 °C, 0.1 torr) to give 2.55 g (85%) of the product as a beige solid, mp = 225-227 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.38 (s, 3H), 3.49 (s, 3H), 7.13-7.17 (m, *J* = 5.4 Hz, *J* = 7.8 Hz), 7.23-7.26 (d, 1H, *J* = 7.8 Hz), 7.35-7.38 (dd, 1H, *J* = 1.5 Hz, *J* = 7.8 Hz), 7.85-7.88 (dd, 2H, *J* = 1.8 Hz, J = 8.1 Hz), 8.28-8.30 (dd, 1H, *J* = 1.2 Hz, *J* = 5.1 Hz), 10.92 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.70, 28.55, 115.89, 118.67, 128.41, 137.59, 142.83, 143.21, 143.35, 147.43, 149.87; LRMS (ESI^+) $[M+H]^+$ = 303 m/z; IR (KBr) 3680, 3369, 3018, 2400, 1596, 1484, 1460, 1437, 1409, 1389, 1339, 1301, 1272, 1215, 1162, 1137, 1085, 1038, 1020, 996, 928, 902, 872, 829, 812, 742, 669, 620, 582, 549 cm^{-1}; UV-VIS (95% EtOH) λ_{max} 299 nm (ε = 14,736), 243 (ε = 6,894).

2-Amino-1-methyl-imidazo[4,5-*b*]pyridine (5). A 30-mL Teflon-lined bomb was chilled to 0 °C and charged with 2-amino-*p*-*N*-toluenesulfonamide-1-methyl-imidazo[*4,5-b*]pyridine (1.00 g, 3.32 mmol) and 10 mL liquid anhydrous HF. The bomb was assembled and heated to 100 °C for 4 h. At 4 h, the bomb was chilled in dry ice and carefully disassembled. The HF was evaporated with a gentle stream of argon gas in the fume hood. The residue was rinsed with 4 x 5 mL portions of benzene to remove the *p*-toluenesulfonyl fluoride by-product. The residue was made basic (pH = 12) with 5 mL 3 M NaOH. The residue was chromatographed on Baker Flash C_{18} reverse phase silica gel eluting first with deionized water followed by MeOH. The MeOH fractions were collected, combined and concentrated under vacuum (25 °C, 0.1 Torr) to give 0.450 g (94%) of the product as a brown solid; mp >250 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 3.56 (s, 3H), 6.94-6.98 (dd, 1H, *J* = 5.4 Hz, *J* = 7.5 Hz), 7.46-7.49 (dd, 1H, *J* = 1.5 Hz, *J* = 7.8 Hz), 7.98-8.00 (dd, 1H, *J* = 1.2 Hz, *J* = 5.1 Hz); ^{13}C NMR (75 MHz, CD_3OD) δ 27.59, 114.58, 114.60, 128.26, 140.58, 155.41, 157.99; LRMS (ESI^+) $[M+H]^+$ = 149 m/z; IR (KBr) 1679, 1629, 1593, 1544, 1470, 1430, 1266, 1245, 1125, 926, 908, 769, 750, 707 cm^{-1}; UV-VIS (95% EtOH) λ_{max} 296 (ε = 11,296), 241 (ε = 2,618).

2-Amino-1-methyl-6-bromoimidazolo[4,5-*b*]pyridine (6). A 100-mL round bottom flask was charged with 40 mL of glacial AcOH and 2-amino-1-methyl-imidazo[4,5-*b*]pyridine (265 mg, 1.79 mmol) and was heated at 100 °C to give a clear solution. The heat source was removed, bromine (430 mg, 2.69 mmol) in 1 mL glacial AcOH was added dropwise and heating was continued for 45 min. During the course of the reaction, the product precipitates out of solution as a brown solid. After 45 min at 100 °C, the solvent was removed from the reaction mixture under vacuum (50 °C, 30 torr). Saturated aqueous K_2CO_3 was added to the residue until basic (pH =10). The aqueous layer was extracted with 4 x 50 mL portions of EtOAc. The organic layer was dried over anhydrous Na_2SO_4, filtered and dried under vacuum (25 °C, 0.1 torr) to give 405 mg (99%) of a brown solid, mp = 249-251 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 3.34 (s, 2H, NH_2), 3.55 (s, 3H), 7.66 (d, 1H, *J* = 1.8 Hz), 8.04 (d, 1H, *J* = 1.8 Hz); ^{13}C NMR (75 MHz, CD_3OD) δ 27.81, 109.37, 117.19, 129.34, 141.15, 154.60, 158.74; LRMS (ESI^+) $[M+H]^+$ = 227 m/z; IR (KBr) 3279, 3103, 3020, 1679, 1618, 1546, 1463, 1436, 1266, 1237, 1215, 1108, 1070, 941, 862, 851, 757, 668 cm^{-1}; UV-VIS (95% EtOH) λ_{max} 310 nm (ε = 12,896), 248 (ε = 3,655), 210 (ε = 38,137).

PhIP (2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine) (7). A 100-mL round bottom flask was charged with 35 mL dioxane, 10 mL EtOH and 5 mL saturated K_2CO_3. Argon gas was bubbled through the solvent for 5 min. The flask was charged with 2-amino-1-methyl-6-bromoimidazolo[4,5-*b*]pyridine (243 mg, 1.07 mmol), phenylboronic acid (250 mg, 2.05 mmol) and $Pd(PPh_3)_4$ (120 mg, 10 mol %). The reaction mixture was heated to reflux (100 °C) for 12 h. At 12 h, the reaction mixture was cooled to 25 °C under argon gas. The reaction mixture was diluted with 20 mL saturated aqueous NaCl and extracted with 4 x 50 mL portions of EtOAc. The organic layers were dried over anhydrous Na_2SO_4, filtered and dried under vacuum (25 °C, 0.1 torr) to give a yellow solid. The solid was purified via column chromatography on silica gel using a solvent gradient of 100% $CHCl_3$ to 20% MeOH/$CHCl_3$. The product-containing fractions

were combined and concentrated under vacuum (25 °C, 0.1 torr) to give 150 mg of an off-white solid. The solid was recrystallized from MeOH (20 mL), filtered and dried under vacuum (25 °C, 0.1 torr) to give 47 mg of product. The mother liquor was concentrated under vacuum (30 °C, 30 torr). The resulting solid was recrystallized from MeOH (10 mL), filtered and dried under vacuum (25 °C, 0.1 torr) to give an additional 23 mg of product. White solid (70 mg, 30%); mp = 322-324 °C (lit.[9] 327-328 °C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.52 (s, 3H), 6.92 (s, 2H, NH_2), 7.27-7.30 (t, 1 H, J = 6.9 Hz), 7.38-7.41 (t, 2H, J = 7.2 Hz), 7.63-7.66 (d, 2H, J = 8.1 Hz), 7.70 (s, 1H), 8.24 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 29.09, 112.44, 126.95, 127.15, 127.27, 128.60, 129.57, 139.77, 140.01, 157.00, 158.71; LRMS (ESI$^+$) $[M+H]^+$ = 225 m/z; HRMS (CI$^+$) $[M+H]^+$ = calculated 225.1062, found 225.1145 m/z; IR (KBr) 3289, 3081, 1668, 1630, 1593, 1544, 1470, 1438, 1423, 1269, 1100, 1041, 944, 914, 880, 868, 784, 760, 695, 594, 512 cm^{-1}; UV-VIS (MeOH) λ_{max} 315 nm (ε = 16,392), 271 (ε = 7,341), 225 (ε = 31,645); RP HPLC (Beta Basic-18, 3: 7 MeOH/H_2O with 0.05% Et_3N) RT = 11.95 min, flow rate = 0.2 mL/min, 99.9% purity; elemental analysis for $C_{13}H_{12}N_4$·0.7 MeOH calculated C 66.70 H 6.05 N 22.71, found C 66.69 H 5.74 N 22.54.

Acknowledgements

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