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Synthesis of deuterated 2-amino-1-methyl-6-phenyl-1H-imidazo[4,5-b]pyridine (PhIP) and its *N*-hydroxy derivative

Mary J. Tanga*, James E. Bupp and Wallace W. Bradford
Pharmaceutical Discovery Division, SRI International, 333 Ravenswood Avenue, Menlo Park, CA 94025, USA

Summary

The syntheses of the deuterium-labeled food mutagen 2-amino-1-methyl-6-phenyl-1H-imidazo[4,5-b]pyridine (PhIP) and its *N*-hydroxy metabolite are described. Unlabeled PhIP is deuterated using a boron trifluoride phosphoric acid complex in one step. Labeled PhIP–$^2H_5$ is nitrosated to give nitro-PhIP, which is then reduced to *N*-hydroxy–PhIP–$^2H_5$. Copyright © 2001 John Wiley & Sons, Ltd.

Key Words: heterocyclic amines; food mutagens; PhIP; nitro-PhIP; *N*-hydroxy–PhIP; boron trifluoride phosphoric–$^2H_3$ acid complex

Introduction

Heterocyclic amines are mutagenic and carcinogenic compounds formed during the cooking of beef, pork, lamb, poultry, and fish at high temperature. The consumption of well-done meats, and thus exposure to heterocyclic amines formed during cooking, may play a significant role in the risk of breast cancer, colon cancer, and other cancers in humans.[1–4] Among the known food mutagens, 2-amino-1-methyl-6-phenyl-1H-imidazo[4,5-B]pyridine (PhIP) is considered very important because of its high content in various foods.[1,4–7] Exposure to PhIP is widespread; PhIP has been found in home-cooked and

*Correspondence to: M. J. Tanga, Pharmaceutical Discovery Division, SRI International, 333 Ravenswood Avenue, Menlo Park, CA 94025, USA. E-mail: mary.tanga@sri.com
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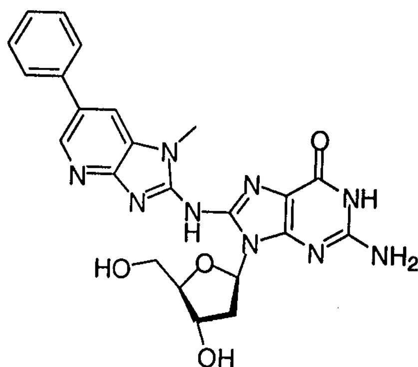

Figure 1. Structure of guanosine–C8–PhIP nucleoside adduct

restaurant foods at levels up to hundreds of parts per billion. It has recently been identified in human urine[2] and as DNA and protein adducts in the colon and blood.[1] Metabolic activation of PhIP leads to *N*-hydroxy–PhIP, which can attack DNA to form adducts (Figure 1).[8,9] An accurate assessment of the risk posed by consumption of heterocyclic amines formed during cooking needs to be determined. Thus, it is essential to have analytically pure standards and labeled analogues of PhIP to assess the risk it poses to humans. A versatile synthesis of PhIP and its *N*-hydroxy metabolite labeled with deuterium is presented.

Results and discussion

Convenient syntheses of deuterium-labeled PhIP (**2**) and its metabolite, *N*-hydroxy–PhIP (**4**) have been developed (Scheme 1). Unlabeled PhIP (**1**) is deuterated using a boron trifluoride phosphoric–$^{2}H_3$ acid complex. The reagent, $BF_3 \cdot {}^{2}H_3PO_4$, is made in quantitative yield at 0°C from deuterium oxide and phosphorus pentoxide, into which is bubbled boron trifluoride gas.[10] The reagent is then added to PhIP (**1**) in anhydrous liquid SO_2 cooled to -78°C. The reaction mixture is heated in a sealed flask at 90°C for 4 days. Subsequent workup and purification of the reaction mixture give a 40% yield of labeled PhIP–$^{2}H_5$ (**2**). Mass spectroscopy showed the deuterium incorporation was 96.6% ^{2}H isotopically pure material labeled exclusively in the phenyl group. Product **2** contains no $^{2}H_0$, $^{2}H_1$, or $^{2}H_2$ species by mass spectroscopy. NMR studies confirmed this result showing no phenyl ring protons.

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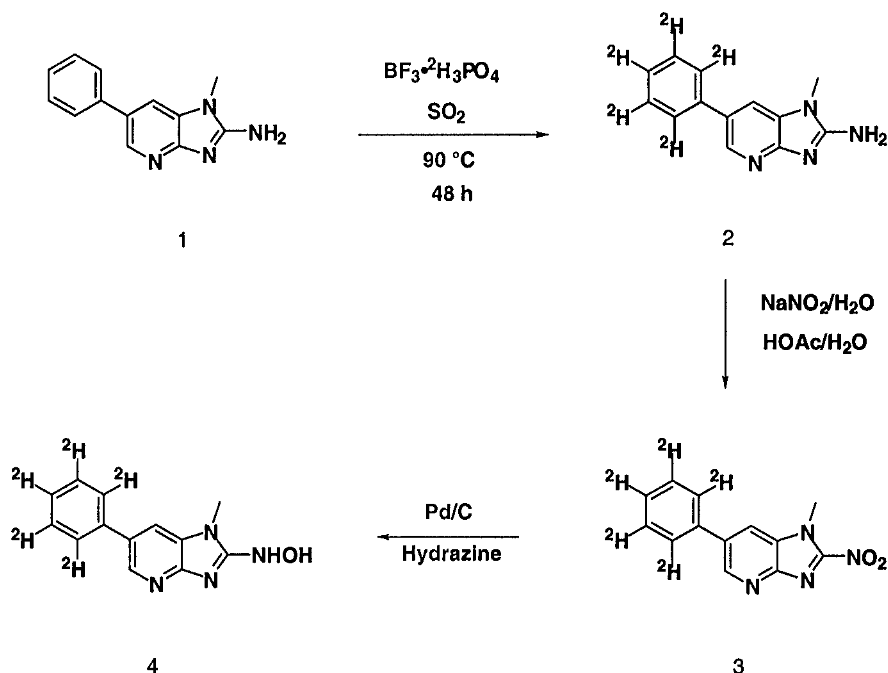

Scheme 1

The synthesis of deuterated **4** is achieved as follows: Labeled PhIP–$^{2}H_5$ (**2**) in HOAc and H_2O is treated with an aqueous solution of sodium nitrite to give 2-nitro–PhIP–$^{2}H_5$ (**3**). This intermediate is reduced to the *N*-hydroxy derivative **4** with hydrazine in the presence of Pd/C catalyst. Compound **4** has shown good stability when stored as a solid at −80°C under Ar but it is extremely sensitive to oxygen, especially when dissolved in organic solvents. All solvents used must be thoroughly degassed with argon or nitrogen before coming in contact with *N*-hydroxy–PhIP–$^{2}H_5$ (**4**). Dilute solutions (0.0001 M) in degassed methanol have been observed to slowly turn pink due to the formation of the azoxy derivative (Figure 2). The reactivity of *N*-hydroxy–PhIP–$^{2}H_5$ (**4**) is consistent with the fact that PhIP (**1**) is metabolically activated to form *N*-hydroxy–PhIP, which on solvolysis gives a nitrenium ion that is attacked by DNA (Figure 2).[11]

Experimental

Infrared spectra were recorded on a Perkin Elmer 1600 FTIR spectrophotomer. Ultraviolet spectra were recorded on a Varian

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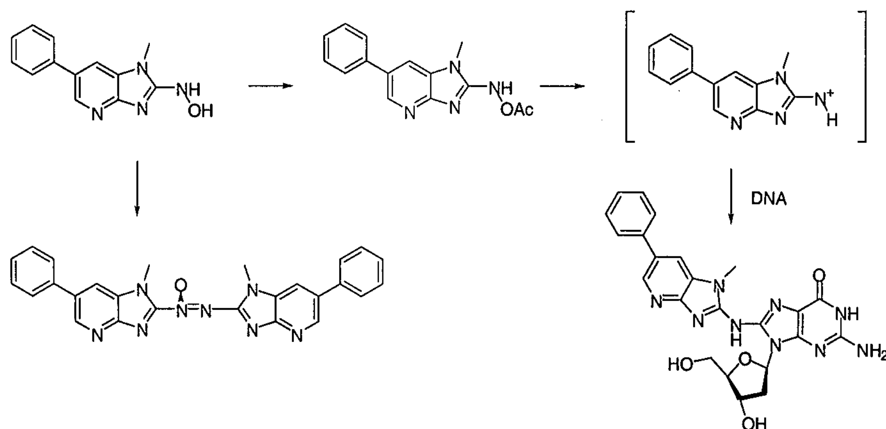

Figure 2. Proposed active-2-nitrenium ion and azoxy derivative

DMS-90. NMR spectra were recorded on a Varian Gemini 300 spectrophotometer using tetramethylsilane as the internal standard. The following abbreviations are used in reporting the NMR multiplicities: apt, apparent; s, singlet; d, doublet; t, triplet, q, quartet; m, multiplet; and br, broad. The mass spectra were obtained on a Ribermag R 10-10 GC/MS. Column chromatography was performed using E. Merck silica gel 40 (70–230 mesh ASTM). All solvents were dried over molecular sieves.

Caution should be taken when manipulating these corrosive liquids. The reactions should be done in a well-ventilated hood with proper shielding.

Boron trifluoride phosphoric–$^{2}H_3$ acid complex

Under Ar in a dry 100 ml round-bottomed flask, a sample of 25.0 g (1.25 mol) of deuterium oxide (99.9 at % D) at 0°C was slowly treated with phosphorus pentoxide 58.0 g, 0.409 mol) over 30 min, using a Teflon®-screw solid feeder. The mixture was stirred overnight at RT. To the reaction solution, a stream trifluoride gas was introduced below the surface at 0°C slowly over 1 h. The resulting product was 138 g (100%) of $BF_3 \cdot {}^{2}H_3PO_4$.[10]

2-amino-1-methyl-6-phenyl-(2,3,4,5,6-[^{2}H]$_5$)-1H-imidazo-[4,5-b]pyridine-d_5[PhIP-$^{2}H_5$(2)]

Under Ar in a dry Pyrex pressure reaction flask, a sample of 2-amino-1-methyl-6-phenyl-1H-imidazo[4,5-b]pyridine hydrochloride [PhIP (**1**)]

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(440 mg, 1.69 mmol) **1** was cooled to $-78^{\circ}C$ and liquid anhydrous SO_2 (16 ml) added. The resulting solution was treated with $BF_3 \cdot {}^2H_3PO_4$ (20 ml, 40.0 g, 237 mmol) and the flask sealed with a Teflon® stopper. The reaction mixture D/H ratio was 60/1. The solution was heated at 90°C for 4 days. After cooling to $-78^{\circ}C$, the ampoule was opened and the SO_2 removed with a stream of Ar at RT. The mixture was cooled and treated with D_2O (7 ml) and then poured over ice and treated with 20% NaOH to pH 10. The mixture was extracted with $CHCl_3$ and evaporated to give 182 mg of crude material, which was triturated with Et_2O and then MeOH to give 155 mg (40%) of pure product **2**. The deuterium incorporation in the phenyl group was found to be 128.14, 137.51, 137.76, 152.20, 157.06; MS m/z (rel. intensity) 230 (M + H, d_5, 100), 229 (d_4, 17), 228 (d_3, 1); FTIR (KBr) 3340, 3163, 1636, 1545, 1472, 1433, 1383, 1274, 1095 cm^{-1}; UV $(CH_3OH)\lambda_{max}$ 222 nm (ε 21,735), 269 (8,598), 315 (16,779), 373 (3,821); TLC System 1: (Bakerflex silica gel IB2-F plates) ($CHCl_3/CH_3OH/Et_3N$, 89.9/10/0.1), $R_f = 0.35$; TLC System 2: (Bakerflex silica gel IB2-F plates) ($CH_2Cl_2/CH_3OH/Et_3N$, 79.9/20/0.1), $R_f = 0.45$; HPLC System 1: Brownlee Spheri-5 RP 8 Column, flow rate 1.00 ml/min, detection 320 nm, solvent CH_3OH/0.5% Et_3N in H_2O (60/40), retention time 2.60 min, purity 99%; HPLC System 2: Brownlee Spheri-5 Phenyl Column, flow rate 1.00 ml/min, detection 320 nm, solvent CH_3OH/0.5% Et_3N in H_2O (50/50), retention time 2.81 min, purity 99%; HRMS (DCI, NH_3 in CH_4) calculated for $C_{13}H_8D_5N_4$ [M + H]: 230.1449; Found: 230.1472.

1-methy-2-nitro-6-phenyl-(2,3,4,5,6-[^{2}H]$_5$)-1H-imidazo[4,5-b] pyridine [2-nitro-PhIP-$^2H_5$ (3)]

To a solution of PhIP-$^2H_5$ **2** (90.0 mg, 0.393 mmol) in $HOAc/H_2O$ (50/50) (4 ml) under Ar at 0°C was added a solution of $NaNO_2$ (1.20 g, 17.4 mmol) in H_2O (2 ml) slowly over a 6 min period with intermittent stirring. A precipitate formed, and the resulting mixture was allowed to stand at 0°C for 15 min and then at RT for 1 h. The mixture was cooled to 0°C, filtered, rinsed with cold H_2O (2×1 ml), and dried under high vacuum to give 53 mg of crude material, which was chromatographed on Et_3N deactivated silica gel (12 g) equilibrated with CH_3OH/CH_2Cl_2 (1/100) (40 ml) and eluted with the same solvent. The first 12 ml of solvent was eluted, and then the next 18 ml was collected and evaporated to give 27.5 mg (28%) of product **3**.

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2-hydroxylamino-1-methyl-6-phenyl-(2,3,4,5,6-[$^2H]_5$)-1H-imidazo [4,5-b]pyridine (N-hydroxy-PhIP-$^2H_5$) (4)

A sample of 10% Pd/C (27.5 mg) was placed in a 100 ml round bottom flask and the flask was evacuated and treated with Ar repeatedly to remove O_2. To the flask was added CH_3OH (10 ml) degassed by purging with Ar, and the mixture was purged with Ar for 5 min. After cooling the mixture with an ice bath, 64% hydrazine hydrate (104 μl) was added and the mixture stirred at 0°C for 30 min. A solution of nitro-PhIP-$^2H_5$ **3** (27.5 mg, 0.108 mmol) in degassed CH_3OH (20 ml) was added over 2 min. The mixture was stirred for 10 min CH_3OH/H_2O (80/20) (3×1 ml) and evaporated to give 5.5 mg (21%) of pure product **4**. MS m/z (rel. intensity) (DCI) 246 (M + H, 55), 230 (100), 215 (47). The mass spectrum indicates that the compound contains 95.6% ^2H in the phenyl ring. UV (CH_3OH) λ_{max} 230 nm (ε 20,495), 318 (13,627): HPLC: Brownlee Spheri-5 RP-18 Column, flow rate 1.50 ml/min, detection 240 nm, solvent CH_3OH/2% ammonium acetate buffer, pH 5.85 (45/55), retention time 5.38 min, purity 97%; HRMS (DCI, NH_3 in CH_4) calculated for $C_{13}H_6D_5N_4O$; 245.1320; found: 245.1466.

Conclusions

This method allows convenient syntheses of deuterium-labeled PhIP and its metabolite *N*-hydroxy–PhIP. Labeled PhIP–$^2H_5$ and *N*-hydroxy–PhIP–$^2H_5$ are available as reference materials from Dr Harold Seifried, National Cancer Institute, 6130 Executive Boulevard, Room 212F, Bethesda, MD 20892.

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