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Synthesis of the Food Mutagens MeIQx and 4,8-DiMeIQx by Copper(I) Promoted Quinoxaline Formation

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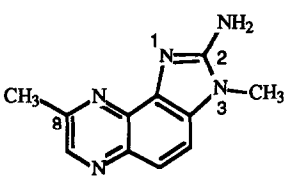
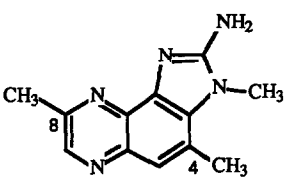
The regiocontrolled syntheses of the title compounds (**1** and **2**) is described. The key step is a new intramolecular alkyne amination and aromatization process (**5** → **6**; **8** → **9**) effected by tetrakis(acetonitrile)copper(I) tetrafluoroborate.

Several highly mutagenic and carcinogenic quinolines and quinoxalines have been identified in heated meat and fish. Among the quinoxalines, 3,8-dimethyl-*3H*-imidazo[4,5-*f*]quinoxalin-2-amine (MeIQx, **1**) has been found in fried beef,[2-5] beef extract,[5,6] and heated fish;[7,8] 3,4,8-trimethyl-*3H*-imidazo[4,5-*f*]quinoxalin-2-amine (4,8-DiMeIQx, **2**) has also been isolated from fried beef,[4] beef extract,[9] and heated fish.[7,8] MeIQx induced liver, skin, Zymbal gland, and clitoral gland tumors in F344 rats.[10] The related quinolines 2-amino-3-methylimidaza[4,5-*f*]quinoline and 2-amino-3,4-dimethylimidaza[4,5-*f*]quinoline induced tumors in several rat and mouse organs.[10] No reports have appeared as yet concerning the carcinogenicity of 4,8-DiMeIQx.



MeIQx (**1**)



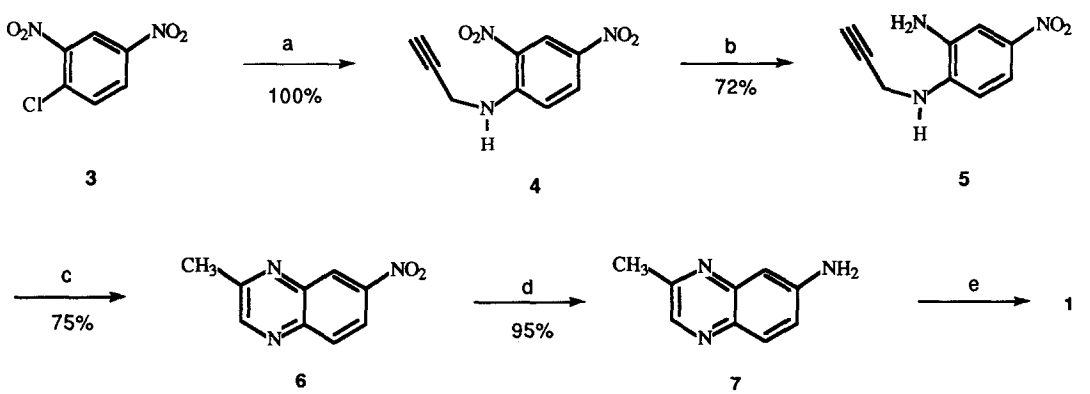
4,8-DiMeIQx (**2**)

In order to assess the human carcinogenic potential of these materials, they must be synthesized in quantities adequate for animal feeding assays, metabolic and pharmacokinetic studies, and analytical methods development. A number of research groups have undertaken synthetic studies on these compounds.[11,12] Non-selective routes to **1**[12ab] and **2**[12c] have been reported, and a preliminary letter[12d] describes the preparation of **1** through a benzoselenenadiazole intermediate. Our own work has focused on the construction of the quinoxaline nucleus by intramolecular amination of an N-propargyl chain, and we are pleased to report the regiocontrolled synthesis of both **1** and **2** by efficient routes that feature a new method for this cyclization.

Results and Discussion

The synthesis and cyclization of the appropriately differentiated phenylenediamine derivative **5** is displayed in Scheme 1. Reaction of commercially available 1-chloro-2,4-dinitrobenzene (**3**) with propargylamine followed by selective reduction of the *ortho* nitro group[13] gave **5** in good overall yield. Several reagents were examined for the cyclization[14] of **5** to **6**, and attempts using iodine in tetrahydrofuran solution, bis(collidine)bromonium perchlorate in refluxing toluene solution, and copper(I) iodide in refluxing toluene solution were unsuccessful. The "monomeric" copper reagent tetrakis(acetonitrile)copper(I) tetrafluoroborate,[15] however, caused smooth cyclization and aromatization to the quinoxaline **6** without the accumulation of any intermediate detectable by TLC analysis. A stoichiometric quantity of the copper(I) reagent was required for the reaction to proceed to completion. Reduction of the remaining nitro group gave amino quinoxaline **7**, whose melting point and UV spectrum agreed with the reported values.[16] Conversion of **7** to MeIQx (**1**) was accomplished by the five step literature procedure (TsCl, pyr; HNO_3, AcOH; H_2SO_4; NaI, CH_3I; Fe, HCl; BrCN),[12a] and the product was identical in all respects with authentic material prepared by the Grivas method.[12b]

Scheme 1. Synthesis of MeIQx (**1**)[a]



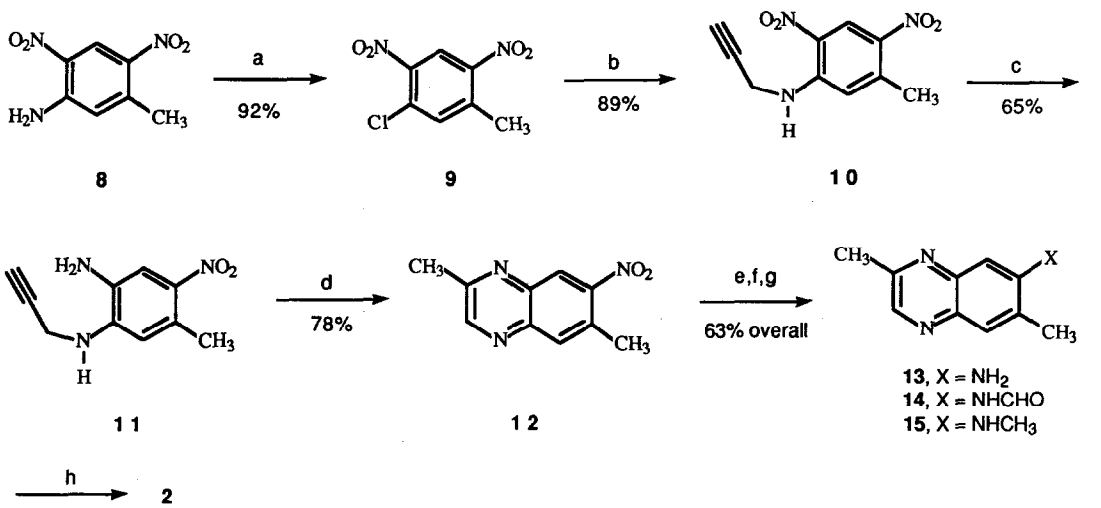
[a]**Reagents:** (a) propargylamine, Et_3N, ethanol, reflux, 4 h; (b) H_2N-NH_2, Raney nickel, ethanol, dichloroethane, 60°, 4 h; (c) $(CH_3CN)_4CuBF_4$, toluene, 90°, 20 h; (d) H_2 (1 atm), Raney nickel, aq ethanol, 25°, 1 h; (e) see reference 12ab.

The N-trideuteriomethyl analogue of **1** ("MeIQx-d_3") was also prepared by substituting CD_3I in the methylation step.[12a] This compound, which may be of use as an internal standard for quantitative mass spectral analysis,[11e] showed the expected molecular ion at m/z 216.

For the synthesis of 4,8-DiMeIQx (**2**, Scheme 2), 5-amino-2,4-dinitrotoluene (**8**)[17] was converted to 5-chloro-2,4-dinitrotoluene (**9**) according to Doyle,[18] and displacement of chloride by propargylamine, cyclization, and aromatization proceeded as before, leading to the nitro quinoxaline **12**. Conversion of **12** to the methylamino quinoxaline **15**[12c] was accomplished by reduction and alkylation,[19] and the final transformation to **2** required three steps ($NaNO_3$, AcOH, H_2SO_4; H_2, Ni; BrCN) following the literature method.[12c]

In summary, both MeIQx (**1**) and 4,8-DiMeIQx (**2**) have been synthesized by efficient routes that feature a new method for intramolecular alkyne amination. This process should serve as a general synthesis for 2-methylquinoxalines, since the position of other substituents relative to the methyl group is predetermined by the location of the propargyl chain.

Scheme 2. Synthesis of 4,8-Me_2IQx (**2**)[a]



[a]**Reagents:** (a) tBuONO, $CuCl_2$, acetonitrile, 65°, 2 h; (b) propargylamine, Et_3N, ethanol, reflux, 4 h; (c) H_2N-NH_2, Raney nickel, ethanol, dichloroethane, 60°, 4 h; (d) $(CH_3CN)_4CuBF_4$, toluene, 90°, 20 h; (e) H_2 (1 atm), Raney nickel, aq ethanol, 25°, 1 h; (f) HCO_2H, Ac_2O, 25°; (g) $LiAlH_4$, THF, 5°, 3 h; (h) see reference 12c.

Experimental Section

Apparatus and Reagents. Melting points were determined on an Electrothermal apparatus and are uncorrected. Fourier transform infrared (FT-IR) spectra were recorded on thin films using a Mattson Cygnus 100 spectrophotometer (selected absorption maxima are reported in cm^{-1}). Proton nuclear magnetic resonance (NMR) spectra were obtained from deuteriochloroform solutions using a Varian Associates XL-400 or VXR-200 spectrometer. Chemical shifts are reported in parts per million using the residual chloroform signal (7.24 ppm) as internal standard, and apparent coupling constants (J) are reported in hertz. Both low and high (10,000) resolution desorption chemical ionization mass spectra (DCI-MS) were obtained on a Finnigan MAT 8230 mass spectrometer with isobutane as the reagent gas. Data processing was done using the Finnigan MAT SS300 data system. The ultraviolet (UV) spectrum was measured using a Shimadzu spectrophotometer model UV-160. Elemental analysis was performed by Robertson Laboratory, Madison, NJ.

Precoated silica gel plates (E. Merck Si250F, 5715-7) were used for analytical thin layer chromatography (TLC). Machery Nagel silica gel 60 (230-400 mesh) was employed for column chromatography. THF was distilled from benzophenone ketyl. Acetonitrile and toluene were distilled from calcium hydride. Bulk grade ether, petroleum ether, and dichloromethane were distilled prior to use. Other solvents and reagents were obtained commercially and used as received. Organic solutions were dried over anhydrous magnesium sulfate.

2,4-Dinitro-*N*-propargylaniline (4). A solution of 2 g of 1-chloro-2,4-dinitrobenzene (**3**), 1 mL of propargylamine, and 2 mL of triethylamine in 40 mL of absolute ethanol was heated at reflux for 4 h. The solution was allowed to cool to room temperature, whereupon the product crystallized. The crystals were collected, washed with a little cold ethanol, and air dried, giving 2.1 g (100%) of **4**, mp 157-158 °C. NMR (200 MHz) 9.16 (d, 1 H, J = 2.8), 8.65 (br s, 1 H), 8.35 (dd, 1 H, J = 9.4, 2.6), 7.06 (d, 1 H, J = 9.4), 4.23 (dd, 2 H, J = 5.6, 2.4), 2.37 (t, 1 H, J = 2.2); IR 3376, 3278, 3099, 2923, 1615, 1313; DCI-MS 221 $(M+1)^+$. Anal. Calcd for $C_9H_7N_3O_4$: C, 48.87; H, 3.19; N, 19.00. Found: C, 48.69; H, 3.23; N, 19.00.

2-(*N*-Propargylamino)-5-nitroaniline (5). Raney nickel (W-2, 50% aq dispersion, 0.6 g) was added in three portions over 40 min to a stirred solution of 1 g of **4** and 0.58 g of hydrazine hydrate in 5 mL of ethanol and 5 mL of 1,2-dichloroethane. The temperature was kept below 60°C during the addition. After 3.5 h of additional stirring at 60°C, the reaction was cooled, filtered, and concentrated. Chromatography using 3 : 2 petroleum ether/ether as the eluant gave 0.62 g (72%) of **5** as a deep red solid, mp 143-145 °C. NMR (200 MHz) 7.84 (dd, 1 H, J = 4.4, 1.4), 7.63 (d, 1 H, J = 1.2), 6.68 (d, 1 H, J = 4.4), 4.03 (d, 2 H, J = 1.0), 2.28 (t, 1 H, J = 1.0); DCI-MS 192 $(M+1)^+$.

3-Methyl-6-nitroquinoxaline (6). A mixture of 0.1 g of **5** and 0.18 g of tetrakis(acetonitrile)copper(I) tetrafluoroborate[15] in 30 mL of toluene was heated at 80-90°C for 20 h, during which time a black precipitate formed, and the solution turned from dark red to light yellow. The solution was cooled and decanted, and the precipitate was ground with a spatula and extracted five times with dichloromethane (total about 100 mL). The combined toluene and dichloromethane extracts were concentrated and chromatographed using ether as the eluant to afford 0.075 g (75%) of **6** as light yellow crystals, mp 154-155 °C. NMR (400 MHz) 8.44 (s, 1 H), 7.81 (d, 1 H, J = 9), 7.09 (dd, 1 H, J = 8.9, 2.6), 7.05 (d, 1 H, J = 2.5), 4.15 (br s, 1 H), 2.67 (s, 3 H); IR 3051, 2926, 1531, 1352; High resolution MS calcd for $C_9H_7N_3O_2$ 189.0538, found 189.0538. Anal. Calcd: C, 57.14; H, 3.73; N, 22.21. Found: C, 57.26; H, 3.68; N, 21.96.

3-Methyl-6-aminoquinoxaline (7). A mixture of 0.1 g of **6** and about 0.1 g of Raney nickel in 20 mL of ethanol was stirred under 1 atm of hydrogen pressure until TLC indicated the disappearance of starting material, about 1 h. The solution was filtered and the ethanol removed to give 0.08 g (95%) of **7** as a tan powder, mp 170-172 °C, lit[16] 177.5-178 °C. NMR (400 MHz) 8.44 (s, 1 H), 7.81 (d, 1 H, J = 9.0), 7.09 (dd, 1 H, J = 9.1, 2.6), 7.05 (d, 1 H, J = 2.5), 2.67 (s, 3 H); IR 3361, 3180, 1619, 1510, 1235; DCI-MS 160

$(M+1)^+$; UV (methanol) λ_{max} 259 (ε = 19100) and 386.5 (6200), lit[16] 259 (19054) and 385 (6166).

5-Chloro-2,4-dinitrotoluene(9). A mixture of 0.47 g of copper(II) chloride and 0.6 mL of *tert*-butylnitrite in 10 mL of acetonitrile was heated at 65°C, and 0.5 g of 2,4-dinitro-5-methylaniline (**8**)[17] was added over 5 min. The mixture was heated at 65°C for 2 h, then cooled using an ice bath, and 50 mL of 20% aq hydrochloric acid was added. The mixture was extracted with ether, and the organic extract was washed with water, dried, and concentrated to give 0.46 g (92%) of **9** as a red-brown solid, mp 72-75 °C. NMR (200 MHz) 8.60 (s, 1 H), 7.60 (s, 1 H), 2.69 (s, 3 H); DCI-MS 217 $(M+1)^+$.

2,4-Dinitro-5-(*N*-propargylamino)toluene (10). Compound **9** (0.35 g) was treated with propargylamine following the procedure for **4**. The crude reaction mixture was chromatographed using 1 : 1 petroleum ether/ether as the eluant to give 0.33 g (89%) of **10** as yellow crystals, mp 122-124 °C. NMR (200 MHz) 9.10 (s, 1 H), 8.48 (br s, 1 H), 6.81 (s, 1 H), 4.18 (m, 2 H), 2.72 (s, 3 H), 2.36 (br s, 1 H); DCI-MS 236 $(M+1)^+$.

4-Methyl-5-nitro-2-(*N*-propargylamino)aniline (11). Following the procedure for **5**, 0.2 g of **10** was selectively reduced to give 0.11 g (65%) of **11** as a deep red powder, mp 140-141 °C. NMR (200 MHz) 7.60 (s, 1 H), 6.47 (s, 1 H), 4.01 (s, 2 H), 2.59 (s, 3 H), 2.28 (s, 1 H); DCI-MS 206 $(M+1)^+$.

3,7-Dimethyl-6-nitroquinoxaline (12). Cyclization of 0.1 g of **11** as described for **6** gave 0.078 g (78%) of **12** as a tan powder, mp 134-136 °C. NMR (400 MHz) 8.81 (s, 1 H), 8.58 (s, 1 H), 8.00 (s, 1 H), 2.79 (s, 3 H), 2.74 (s, 3 H); IR 3068, 2992, 1622, 1529, 1377, 1353; High resolution MS calcd for $C_{10}H_9N_3O_2$ 203.0695, found 203.0601. Anal. Calcd C, 59.11; H, 4.46; N, 20.68. Found: C, 58.98; H, 4.28; N, 20.60.

3,7-Dimethyl-6-aminoquinoxaline (13). Reduction of 0.07 g of **12** as described for **7** gave 0.054 g (90%) of **13** as a tan solid, mp 190-191 °C. NMR (200 MHz) 8.43 (s, 1 H), 7.71 (s, 1 H), 7.12 (s, 1 H), 4.20 (br s, 2 H), 2.69 (s, 3 H), 2.37 (s, 3 H); DCI-MS 174 $(M+1)^+$.

3,7-Dimethyl-6-formamidoquinoxaline (14). Formylation of 0.05 g of **13** following the literature procedure[19] gave 0.049 g (85%) of **14** as a white powder, mp 215-216 °C. NMR (400 MHz) unassigned peaks from amide rotamers at 8.87, 8.84, 8.81, 8.61, 8.59, 7.85; 8.65 (s, 1 H), 7.90 (s, 1 H), 7.74 (s, 1 H), 2.73 (s, 3 H), 2.50 (s, 1 H); IR 3331, 2966, 1659, 1531; DCI-MS 202 $(M+1)^+$.

3,7-Dimethyl-6-(*N*-methylamino)quinoxaline (15). Reduction of 0.49 g of **14** using 5 equiv of lithium aluminum hydride at 0-5 °C for 3 h gave 0.038 g (82%) of **15** as a viscous oil. NMR (400 MHz) 8.38 (s, 1 H), 7.65 (s, 1 H), 6.88 (s, 1 H), 4.15 (br s, 1 H), 3.00 (d, 3 H, J = 3.9), 2.66 (s, 3 H), 2.32 (s, 3 H); lit[12c] NMR (90 MHz) 8.40, 7.68, 6.92, 4.16, 3.02, 2.67, 2.34; IR 3360, 2918, 1626, 1530, 1259; DCI-MS 188 $(M+1)^+$.

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