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An Improved Synthesis of 3,8-Dimethyl-3*H*-imidazo[4,5-*f*]quinoxalin-2-amine ("MeIQx") and Its 2-^{14}C-Labelled Analogue

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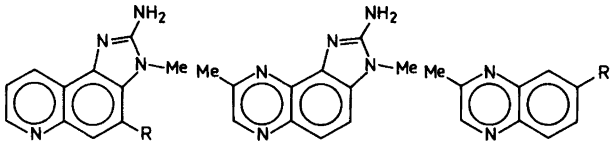
The highly mutagenic title compound (MeIQx) was prepared in 21 % overall yield from 4-fluoro-*o*-phenylenediamine. The 3,7-dimethyl isomer may be obtained as a minor by-product. The ^{14}C-label was introduced in the last step through cyclization with [^{14}C]cyanogen bromide. An alternative synthesis of MeIQx from *p*-fluoroaniline avoided the separation of isomers but gave poorer yield.

In recent years, several highly mutagenic compounds, including *1* (IQ), *2* (MeIQ) and *3a* (MeIQx), have been identified in heated meat or fish. In Ames test,[1] *1*, *2* and *3a* are among the most potent mutagens known for *Salmonella typhimurium* TA98 after activation by liver S9 fraction.[2] Substantial amounts of these so-called IQ compounds are needed as reference samples, for metabolic studies and for the investigation of their possible cancerogenic and other toxic properties. Whereas *1* and *2* may be prepared on a large scale,[3,4] only methods yielding small amounts of *3a* are available. This is because the reported [5] synthesis of *3a* involves the separation of isomers by HPLC at an early stage. In the present synthesis of *3a*, the separation of isomers may be avoided and performed on a larger scale.

By analogy with a synthesis of *1* and *2*,[4] *7-chloro-2-methylquinoxaline (4)*[6] was first considered as starting material. However, attempts to nitrate *4* led to oxidation of its methyl



group, and direct replacement of the chlorine by a methylamino group proceeded very slowly and in poor yield even at 200 °C. The methoxy analogue *5* was readily converted to its 8-nitro derivative,[7] but this did not react with methylamine. The fluoro analogue *10a* was therefore investigated. It was prepared from *p*-fluoroaniline along two routes. The key steps were performed essentially as described for *4*.[6] Thus, pure *10a* was obtained *via* compounds *6*,[8] *7* and *8* (Scheme 1), while a mixture (*10*) of *10a* and its 6-fluoro isomer was prepared *via*

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4-fluoro-*o*-phenylenediamine (*9*).[9] The mixture *10* was preferred to pure *10a* for the further synthesis of *3a*, mainly because of the low yield in the step *8*→*10a*.

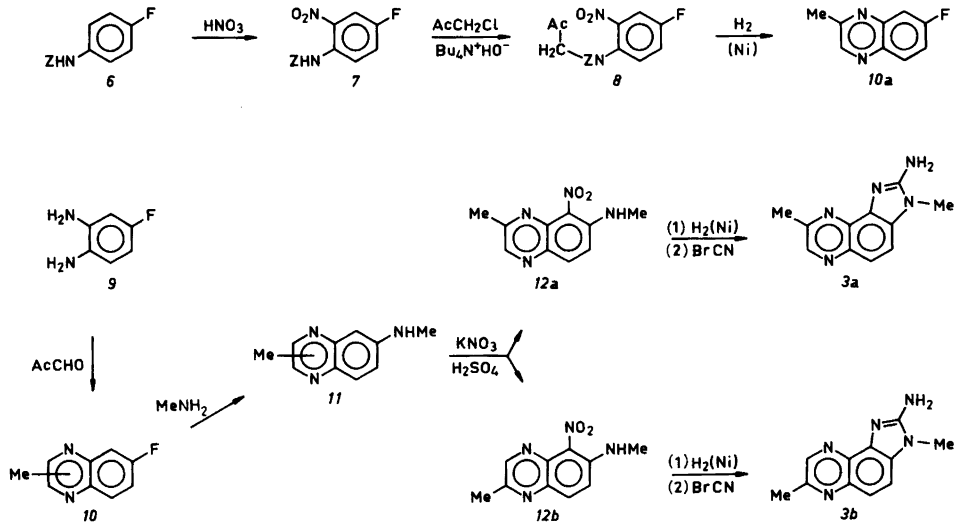
Although the nitration of *10* failed, methylamine converted *10* to the amine mixture *11* in high yield at 175 °C. Nitration of *11* gave the known[5] compounds *12a* and *12b*, which were readily separated by crystallization or LC. In the already[5] described conversions *12a*→*3a* and *12b*→*3b*, catalytic hydrogenation was preferred to reduction with iron, *cf.* the synthesis of *1* and *2*.[4] The mass and ^{1}H NMR spectra of *3a* and *3b* agreed with literature data.[5,10] Since *3a* and notably *3b* are almost insoluble in chloroform-*d*, their ^{1}H NMR spectra in dimethyl sulfoxide-d_6 were also recorded. This solvent change reversed the order of the 4- and 5-H signals. The overall yield of *3a* from *9* was 21 %.

EXPERIMENTAL

All reactions and purifications were monitored by TLC on silica gel (Riedel-de Haën, SIF) or by GC on a 25 m×0.24 mm i.d. capillary column coated with CP Sil 5 and heated from 100 to 250 °C at 10 °C/min. If necessary, the TLC spots were visualized by UV irradiation. Column chromatography (CC) of unlabelled mixtures was performed on silica gel by the "flash" technique.[11] Evaporations were performed at reduced pressure below 40 °C. Melting points are corrected. Mass spectra were recorded at 70 eV, using electron impact and direct insertion. ^{1}H NMR spectra were recorded at 90 MHz and *ca.* 30 °C. The light petroleum boiled at 40–60 °C.

4'-Fluorobenzenesulfonanilide (6). Benzenesulfonyl chloride (22 g, 125 mmol) was added dropwise to a stirred and cooled solution of *p*-fluoroaniline (11.1 g, 100 mmol) in pyridine (50 ml, 620 mmol). The solution was kept at 100 °C for 30 min, then poured on to ice (100 g) and conc. hydrochloric acid (60 ml). The mixture was extracted with chloroform (3×60 ml). The extract was washed with water and evaporated. Crystallization of the residue from 50 % ethanol yielded *6* (22.9 g, 91 %), m.p. 108–109 °C (lit.[8] 108 °C).

4'-Fluoro-2'-nitrobenzenesulfonanilide (7). Nitric acid (≥96 %, 50 ml) was added over 15 min to a stirred mixture of *6* (10.8 g, 43 mmol) and 65 % nitric acid (50 ml), which was kept below 0 °C. After stirring for another 15 min, the solution was poured on to ice. The



Scheme 1. Synthesis of the title compound *3a* and its isomer *3b*. Z=$PhSO_2$.

precipitate was collected and washed with cold water. Crystallization from glacial acetic acid yielded *7* (12.1 g, 95 %), m.p. 98–99 °C. Anal. $C_{12}H_9FN_2O_4S$: C, H, N. MS, *m/z* (rel int.): 77 (100), 141 (31), 51 (23), 78 (8), 50 (6), 82 (5), 296 (5, M), 76 (4), 70 (4), 57 (4). ^{1}H NMR ($CDCl_3$): δ 7.36 (5'-H, ddd), 7.45–7.8 (3'-H and Ph, m), 7.89 (6'-H, ddd), 9.52 (NH, broad s); $|J|$ 0.4 (3',6'), 3.0 (3',5'), 4.9 (4',6'), 7.0 (4'5'), 9.2 (5',6') Hz.

N-*Acetonyl-4'-fluoro-2'-nitrobenzenesulfonanilide* (*8*). Compound *7* (1.45 g, 4.9 mmol) was dissolved in 250 mM sodium hydroxide (100 ml). Tetrabutylammonium hydrogen sulfate (1.8 g, 5.3 mmol) was added in small portions to the stirred solution, which was then extracted with dichloromethane (2×50 ml). The extract was refluxed with chloroacetone (2.0 g, 22 mmol) for 24 h, cooled, washed with water (2×30 ml) and evaporated. CC (CH_2Cl_2–light petroleum, 8:1 v/v) of the residue followed by crystallization from ethyl acetate yielded *8* (1.31 g, 76 %), m.p. 131 °C. Anal. $C_{15}H_{13}FN_2O_5S$: C, H, N. MS, *m/z* (rel. int.): 43 (100), 94 (36), 84 (18), 138 (16), 95 (12), 94 (11), 139 (9), 168 (9), 42 (8), 76 (8). With chemical ionization and ammonia as reaction gas, $M+H^+ + NH_3$ appeared at *m/z* 370. ^{1}H NMR ($CDCl_3$): δ 2.19 (Me, s), 4.70 (CH_2, broad s), 7.23 (5'-H, ddd), 7.4–7.6 (3'-H and Ph, m), 7.69 (6'-H, ddd); $|J|$ 0.3 (3',6'), 3.0 (3',5'), 5.3 (4',6'), 7.3 (4',5'), 8.9 (5',6') Hz.

4-Fluoro-o-phenylenediamine (*9*) was prepared in nearly quantitative yield by Raney nickel-catalysed hydrogenation of 4-fluoro-2-nitroaniline[12] in ethanolic solution under ambient conditions. It was used without purification.

7-Fluoro-2-methylquinoxaline (10a). A strongly stirred solution of *8* (500 mg, 1.42 mmol) in ethyl acetate (70 ml) was hydrogenated under ambient conditions in the presence of Raney nickel (one teaspoon). After 5 min, *ca.* 85 ml of hydrogen had been absorbed. The catalyst was then filtered off quickly and the filtrate evaporated. CC (EtOAc–CH_2Cl_2, 2:1 v/v) of the dark residue, followed by sublimation at 50 °C/1.4 kPa yielded pure *10a* (51 mg, 22 %), m.p. 49.5–50 °C. Found: C 65.9; H 4.3; N 17.1. Calc. for $C_9H_7FN_2$: C 66.7; H 4.3; N 17.2. MS, *m/z* (rel. int.): 162 (100, M), 135 (83), 94 (60), 95 (16), 121 (14), 68 (13), 50 (13), 93 (11), 108 (11), 81 (10). ^{1}H NMR ($CDCl_3$): δ 2.77 (Me, s), 7.48 (6-H, m), 7.63 (8-H, m), 8.06 (5-H, dd), 8.70 (3-H, s); $|J|$ 5.8 (5,7), 8.2 (6,7), 9.1 (5,6), 9.4 (7,8) Hz.

6- and 7-fluoro-2-methylquinoxaline (*10*), *cf.* Ref. 6. Pyruvaldehyde (50 % in water; 2.9 g, 20 mmol) was added to a stirred solution of *9* (*ca.* 2.5 g; from 20 mmol of 4-fluoro-2-nitroaniline) in hot (80 °C) water (50 ml). After 15 min, the solution was cooled and extracted with chloroform. The extract was washed with water and evaporated. CC (CH_2Cl_2–EtOAc, 9:1 v/v) of the residue yielded *10* (2.3 g, 71 %). Its ^{1}H NMR spectrum showed well-separated 3-H singlets at δ 8.71 (*10a*, see above) and 8.74 (isomer of *10a*). Their relative intensity indicated 75–80 % of *10a*.

N,2- and N,3-dimethyl-6-quinoxalinamine (11). A solution of *10* (5.2 g, 32 mmol) in 33 % ethanolic methylamine (125 ml) was kept for 16 h at 170–180 °C in a steel autoclave, cooled and evaporated. The residue was treated with a little water and extracted with dichloromethane. CC (CH_2Cl_2–EtOAc, 1:1 v/v) of the concentrated extract yielded *11* (4.7 g, 85 %). The relative intensity of the ^{1}H NMR signals from the pyrazine protons (δ 8.57 for the *N*,2- and δ 8.41 for the *N*,3-isomer) indicated 75–80 % of *N*,3-dimethyl-6-quinoxalinamine.

N,3-Dimethyl-5-nitro-6-quinoxalinamine (12a), *cf.* Ref. 7. The mixture *11* (3.3 g, 19 mmol) was dissolved in conc. sulfuric acid (90 ml), cooled with ice-water. Powdered potassium nitrate (2.2 g, 22 mmol) was immediately added to the dark solution. This was stirred for 1 h without cooling, poured on to ice, neutralized with conc. ammonia and extracted with dichloromethane. The extract was washed with water, analysed by GC and evaporated. The GC analysis showed *11*, *12a* and *12b*, eluted in that order and in the approximate ratio 1:10:2. CC (CH_2Cl_2–EtOAc, 4:1 v/v) of the residue yielded two fractions. The first one contained only *12a* and was evaporated. Crystallization of the residue (1.9 g, 45 %) from ethyl acetate yielded pure *12a*, m.p. 187.5–188.5 °C. Anal. $C_{10}H_{10}N_4O_2$: C, H, N. MS, *m/z* (rel. int.): 218 (100, M), 157 (60), 143 (48), 171 (45), 103 (39), 75 (35), 116 (31), 51 (31), 144 (29), 145 (28). ^{1}H NMR ($CDCl_3$): δ 2.74 (3-Me, s), 3.15 (NMe, d), 7.30 (7-H, d), ~7.35 (NH, broad s), 8.01 (8-H, d), 8.49 (2-H, s); $|J|$ 5.1 (Me,NH), 9.5 (7,8) Hz.

N,2-Dimethyl-5-nitro-6-quinoxalinamine (12b). The last CC fraction (see preceding paragraph), containing *11* and *12b*, was evaporated. Crystallization of the residue from ethyl acetate yielded pure *12b* (0.3 g, 7 %), m.p. 227–228 °C. Anal. $C_{10}H_{10}N_4O_2$: C, H, N. MS,

m/z (rel. int.): 218 (100, M), 157 (60), 143 (49), 171 (44), 75 (35), 116 (32), 51 (30), 144 (28), 42 (26), 145 (25). ^{1}H NMR ($CDCl_3$): δ 2.70 (2-Me, s), 3.16 (NMe, d), 7.37 (7-H, d), ~7.6 (NH, broad s), 7.99 (8-H, d), 8.75 (3-H); $|J|$ 5.1 (Me,NH), 9.6 (7,8) Hz.

3,8-Dimethyl-3H-imidazo[4,5-f]quinoxalin-2-amine (3a). Two procedures are given: *A* for unlabelled and *B* for labelled *3a, cf.* Ref. 4.

A. A vigorously stirred mixture of *12a* (2.18 g, 10.0 mmol), Raney nickel (two teaspoons) and abs. ethanol (125 ml) was hydrogenated under ambient conditions. After 1–1.5 h, the calculated amount (0.7 l) of hydrogen had been absorbed. The catalyst was then filtered off quickly and the filtrate concentrated to 40 ml. Cyanogen bromide (1.06 g, 10.0 mmol) was dissolved in the filtrate. On the next day, this was treated with conc. ammonia (1.0 ml) and evaporated. CC (CH_2Cl_2–MeOH, 9:1 v/v) of the residue yielded *3a* (1.64 g, 77 %). After crystallization from pyridine, *3a* melted at 295–300 °C (sealed tube) with slight decomposition. ^{1}H NMR (CD_3SOCD_3): δ 2.69 (8-Me, s), 3.66 (3-Me, s), 6.5 (NH_2, broad s), 7.56 (5-H, d), 7.72 (4-H, d), 8.65 (7-H, s); $|J|$ 8.8 (4,5) Hz.

B. 12a (218 mg, 1.00 mmol) was hydrogenated as in *A* and the filtrate evaporated. Potassium cyanide (65–67 mg, 1.00 mmol), labelled with ^{14}C, was dissolved in methanol (2 ml) and added over 20 min to a stirred solution of bromine (160 mg, 1.00 mmol) in methanol (2 ml), cooled in ice-water. Potassium bromide separated gradually. The initially obtained dark evaporation residue was immediately dissolved in the colourless mixture (containing labelled BrCN). On the next day, this was treated with conc. ammonia (0.1 ml) and evaporated. CC ($CHCl_3$–MeOH, 4:1 v/v) of the residue on silica gel at atmospheric pressure yielded *3a* (164 mg, 77 %).

3,7-Dimethyl-3H-imidazo[4,5-f]quinoxalin-2-amine (3b) was prepared from *12b* exactly as described for *12a*→*3a*. After crystallization from pyridine, *3b* decomposed at 330–340 °C (sealed tube) without melting. ^{1}H NMR (CD_3SOCD_3): δ 2.69 (7-Me, s), 3.70 (3-Me, s), 7.3 (NH_2, broad s), 7.67 (5-H, d), 7.88 (4-H, d), 8.81 (8-H, s); $|J|$ 8.7 (4,5) Hz.

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