SYNTHESIS AND TRITIUM LABELING OF THE FOOD MUTAGENS IQ AND METHYL-IQ

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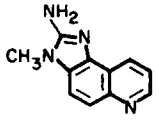
SUMMARY

The mutagens found in cooked meat, 2-amino-3-methylimidazo[4,5-f]-quinoline (IQ) and 2-amino-3,4-dimethylimidazo[4,5-f]quinoline (Methyl-IQ), have been synthesized by unambiguous methods that allow for the preparation of sufficient quantities of material for biological studies. These methods avoid difficult separations of regioisomeric mixtures of products and incorporate specific high level tritium labeling, effected by hydrogenolysis of the appropriately substituted 5-bromo precursors.

Key Words: IQ, Methyl-IQ, synthesis, ^{3}H labeled, food mutagens

INTRODUCTION

In 1980, two highly mutagenic heterocyclic amines were isolated from cooked meats, 2-amino-3-methylimidazo[4,5-f]quinoline (IQ, 1) and 2-amino-3,4-dimethyl-imidazo[4,5-f]quinoline (Methyl-IQ, 2).[1] Considerable investigation has been carried out on these and other highly mutagenic compounds isolated from cooked foods because of the possible connection between exposure to these potent mutagens and diet induced cancer.[2,3] Our objective has been to prepare isomerically pure radiolabeled compounds as well as material in quantity to facilitate these biological investigations.



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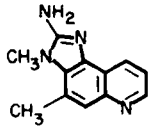

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Our syntheses have been devised to avoid product mixtures that involve closely related regioisomers since efficient purification of such mixtures requires laborious (and in these cases, perhaps dangerous) chromatography.

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Accordingly in the synthesis of methyl-IQ (2) an 8-bromo blocking group was used to prevent the formation of isomeric impurities at two steps. First, it blocks Skraup ring closure to the 5-methyl isomeric products.[4,5] Second, nitration of quinolines 11 and 28 at C-5 would also give substantial amounts of the 8-nitro isomers but for the blocking bromine. Our objective was to prepare radioactive IQ and methyl-IQ of high specific activity for low efficiency biological studies and with the regiochemical specificity desired for metabolic studies. To capitalize on the high specific activity of tritium gas, and the regiospecificity of halogen displacement, hydrogenolysis was clearly the most desirable method for radioactive labeling. For IQ, the most expeditious method was to brominate the final product and then displace that bromine with tritium. In the methyl-IQ case, however, the bromine was already in place, so the final labeling step made a third use of that protecting group.

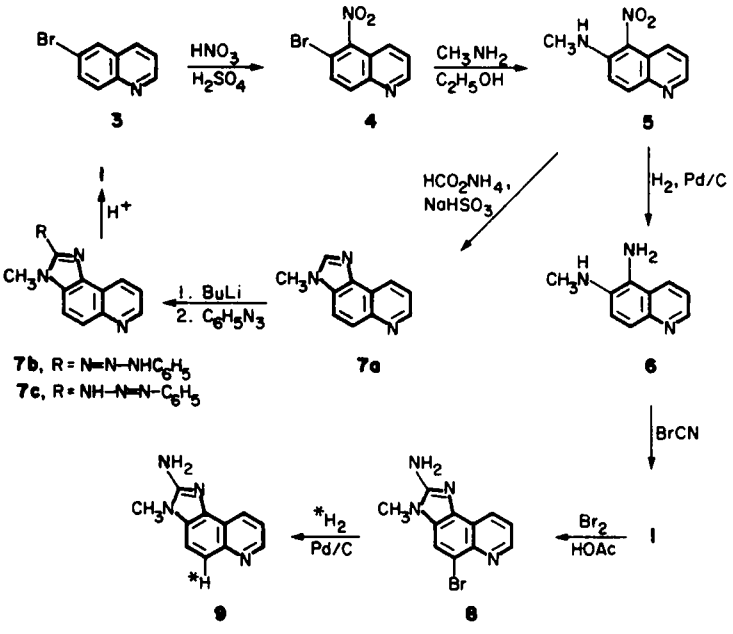
Synthesis of IQ

Our synthesis of IQ (1), which begins from the new educt 3, can be carried out on a 20 g scale in two days with only one purification and involves no chromatography. Following nitration of 6-bromoquinoline (3)[6,7] the crucial methylamine at C-6 is introduced by a simple unambiguous displacement[8] (Scheme I). This avoids the ambiguity of an early synthesis of IQ[9] in which the 3-methyl group was introduced by alkylation using tetramethylammonium hydroxide, a reagent that will alkylate at both the 1- and 3-positions of imidazoquinolines.[10] This material, without purification, is reduced to the diaminoquinoline 6. The latter is air sensitive and is directly added to cyanogen bromide[11] to produce IQ (1).[12]

An alternate route that could be applied in preparing 2-substituted analogues starts with the methylaminonitroquinoline 5 and converts it directly to the imidazoquinoline 7a.[8] Using bisulfite to reduce the nitro group of 5 to an amine, the resulting diamine 6 is cyclized directly with formate to give the imidazole.[13] Deprotonation with kinetic control[14] at low temperature gives the C-2 anion and avoids the equilibration observed at 0° which generates a mixture containing the C-7-anion. The C-2-anion is then treated with phenyldiazonium acide,[15] and the resulting triazene on acid hydrolysis gives IQ (1). The greater electronegativity of the hetero moiety compared to the phenyl group, enhanced by protonation, insures that the required tautomer 7c will predominate. A variety of electrophiles[16] could be used to trap the anion and prepare, directly or indirectly, analogues of 1.

The synthesis of radioactive IQ presented several problems. First, very high level radioactivity was needed so that the labeled material could be useful

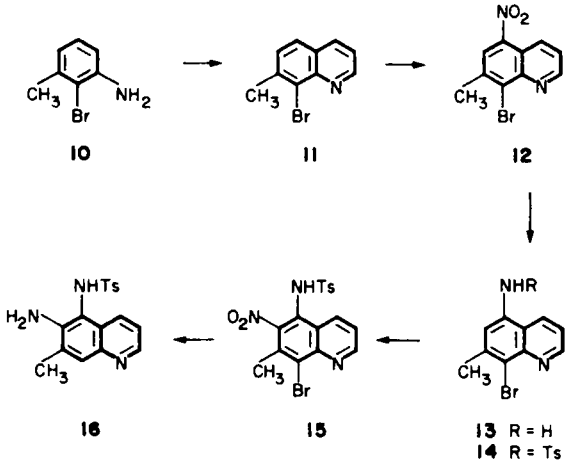
SCHEME I



in low efficiency biological transformations. Secondly, the position of label had to be known in order to avoid ambiguities in metabolic studies. The first requirement precluded exchange methods which are limited to low maximum specific activity. Thus incorporation using pure tritium gas was to be used, and hydrogenolysis could give direct substitution of a halogen with tritium, yielding a site-specific label. Most hydrogenolyses have been carried out in protic (methanol, ethanol) solvents, which in the presence of catalyst quickly exchange their proton with the tritium gas, decreasing its specific activity. We have found that in THF hydrogenolysis takes place in a reasonable period of time using 100 wt % of 10% Pd/C at 1 atm T_2.

To prepare the halogenated precursor, IQ was brominated in the benzo ring to yield 5-bromoIQ (8). The regiochemistry of 8 was established by hydrogenolysis with $^2H_2$ in CH_3OD. An NMR spectrum of the resulting 9 (*H=^{2}H) showed that bromination had occurred at C-5. The same NMR study also showed that the C-4-H and C-5-H chemical shifts are solvent dependent, being reversed in DMSO from that in chloroform. Carrying out the reduction with $^3H_2$ in THF with palladium on carbon afforded 9 (*H=^{3}H) containing 17 Ci/mmol, or about 50% tritium at C-5.

SCHEME II



Synthesis of Methyl-IQ

Our first approach to MeIQ (2) started with Skraup ring closure of bromomethylaniline 10[17] to yield the quinoline 11 (Scheme II). The Skraup reaction on this blocked m-toluidine resulted in an isomerically pure product in contrast to previous approaches[12,18] utilizing 3,4-substituted anilines which give large quantities of the undesired 5,6-substituted quinolines.[4] The Skraup product was then nitrated and the 5-nitroquinoline 12 was the sole isomer. Its structure was established unambiguously by NMR where the typical dramatic deshielding of the 4-proton by a 5-nitro group was clearly observed. Nitroquinoline 12 was then reduced and protected to yield tosylamide 14 which was nitrated to yield 15.[19] After reduction to aminotosylamide 16, however, methylation attempts were thwarted by nucleophilic participation of the 5-tosylamide. Before the methylation of 16 was completed, a new route was devised which circumvented these problems.

The aniline 10 was tosylated to 17 and then nitrated, using nitric acid at 50°C to yield 18 and 19 or, on extended heating, 18 and 20 which were easily separated. Thus the only regioisomeric reaction in the sequence occurs in the first step where the difference between the compounds are maximal and the separation easy. The identities of 18 and 19 were determined by conversion to the known nitro-m-toluidines 21 and 22[20] by one-step debrominative detosylation.[21] The nitration also produced two minor impurities which were characterized after detosylation. These were the dibromo and dinitro anilines noted below.

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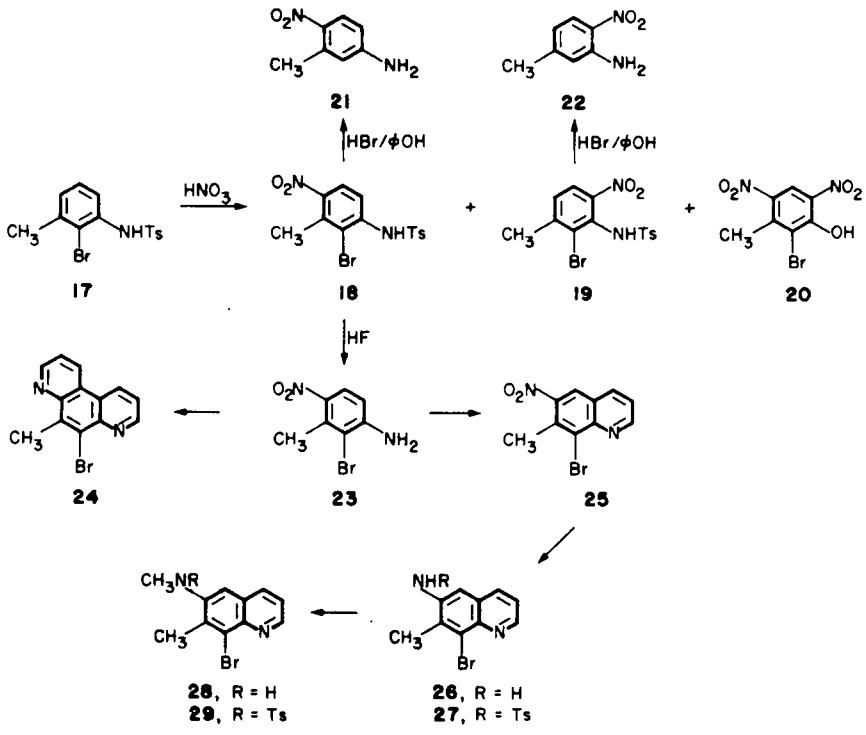
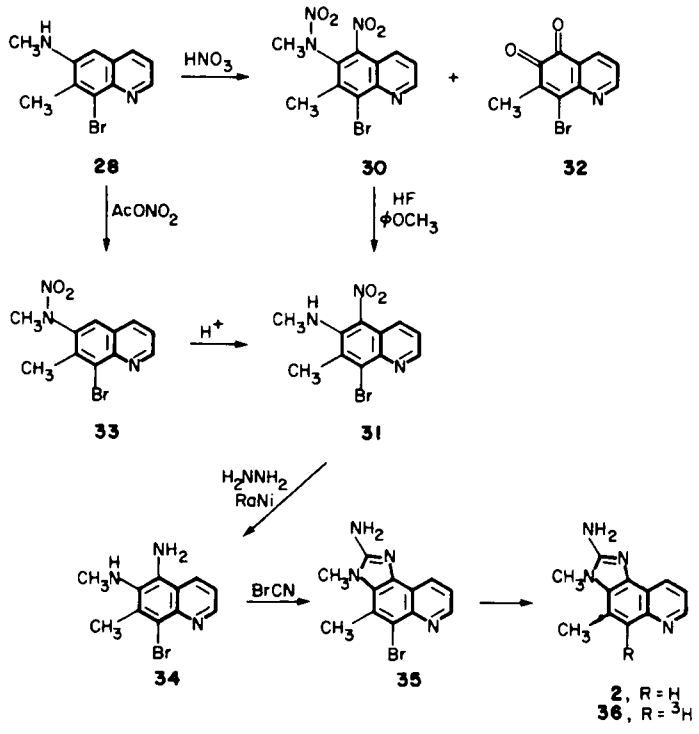


Detosylation of amine 18 without loss of bromine was best done with anhydrous HF. Ring closure of 23 using standard Skraup conditions[6] gave the p-phenanthroline 24 instead of quinoline 25. However, modified Skraup conditions similar to those employed on 2-bromo-4-nitro aniline[22] afforded 25 very cleanly. To avoid tin salts in the isolation, 25 was reduced with hydrazine/Raney nickel[23] rather than with $SnCl_2$. This reduction proceeded with retention of the 8-bromo and gave the amine 26 which was tosylated to give 27 or methylated to 28. Either could be converted to N-tosylmethylamine 29, although the route via 27 is preferable.

Unfortunately, nitration of 29 yielded the dinitro product 30 as the only identifiable material in low yield. Since 30 undoubtedly arose from in situ detosylation, the direct nitration of 28 was tried and both 30 and the o-quinone 32 were formed. No nitric or nitric/sulfuric acid conditions would lead solely to 30 which could be converted cleanly to the desired precursor 31 by N-denitration with anhydrous HF/anisole.[24] However, nitronium acetate nitrated 28 only on the amine group, leading to nitramine 33, which was converted to 31 using

SCHEME IV



aqueous acid. The nitration mixture also contained a significant amount of 30, so the crude product was treated with HF/anisole to convert 30 to the desired 31. TLC evidence indicates that neat trifluoromethanesulfonic acid converts 33 cleanly to 32, an unusually efficient route to this o-quinone.

8-Bromo-7-methyl-6-methylamino-5-nitroquinoline (31) was then reduced to the diamine 34 with hydrazine/Raney Ni leaving the bromine intact, and 34 was cyclized to bromo-MeIQ (35) with cyanogen bromide. Hydrogenolysis with H_2 gave MeIQ (2)[12,18] or with $^3H_2$ gave the radioactive material 36 (*H=^{3}H), again with very high activity and with the label at a known position. Thus, these routes have provided facile and unambiguous preparations of unlabeled and labeled IQ (1) and MeIQ (2).

EXPERIMENTAL

General. Except where noted, all reactions except nitrations were done under nitrogen with magnetic stirring. Tetrahydrofuran (THF) was distilled from sodium/benzophenone, and pyridine from p-toluenesulfonyl chloride. Organic solvent solutions were evaporated on a rotary evaporator after drying over Na_2SO_4. Thin layer chromatography was done on E. Merck silica gel 60F-254, 0.2 mm thick plates; preparative TLC was done on Analtech silica gel GF, 2 mm thick

plates; and column chromatography was performed on E. Merck silica gel 60 (0.063-0.200 mm). ^{1}H-NMR spectra were taken in $CDCl_3$ unless otherwise noted and are reported in ppm downfield relative to internal tetramethylsilane. Elemental analyses were performed by the Analytical Laboratory, Department of Chemistry, University of California, Berkeley, CA.

2-Amino-3-methylimidazo[4,5-f]quinoline (1). 6-Bromoquinoline (3, 50 g, 0.24 mol) was added to an ice cooled mixture of conc. H_2SO_4 (100 mL) and 90% HNO_3 (12.3 mL, d=1.5, 110 mol %) acids. After being stirred for 2 h at 20°C, the mixture was poured onto ice, adjusted to pH 2, and filtered, and the precipitate was dried for 4 h. The resulting 6-bromo-5-nitroquinoline (4) was dissolved in 800 mL of abs. ethanol and methylamine was bubbled into the refluxing solution for 2 h. Cooling and filtering yielded 33.8 g (70%) of 6-methylamino-5-nitroquinoline (5). A portion of 5 (10 g, 49 mmol) dissolved in acetic acid (500 mL) was shaken with hydrogen at 45 psi over 1 g of 10% Pd/C for 3 h. Filtering and evaporating left an oil which was dissolved in water. Basification with aq. NaOH precipitated diamine 6 which was briefly dried then stirred for 72 h in 300 mL of abs. EtOH containing cyanogen bromide (15.6 g, 148 mmol). Addition of 75 mL of ether and filtration gave a precipitate which was dissolved in 50 mL of water containing 2 mL of aq. 48% HBr. To this solution was added excess $K_2CO_3 \cdot 1.5\ H_2O$ to give a solid which was sublimed (210°C/50 μm), affording IQ (1, 4.5 g, 46% yield from 5): mp >300°C; ^{1}H-NMR (DMSO-d_6) δ 8.68 (dd, 1, J=1.5, 4, C-7-H), 8.59 (ddd, J=0.5, 1.5, 8), 7.70 (d, 1, J=9), 7.56 (dd, 1, J=0.5, 9, C-5-H), 7.43 (dd, 1, J=4, 8), 3.63 (s, 3), 3.37 (s, 3); UV, MS, NMR and Ames assay data were identical to those reported for the material isolated from cooked meat.

IQ·2HBr·H_2O was obtained from IQ (1) by dissolution in excess aq. 48% HBr, evaporating, and crystallizing the residue from methanol/ethanol: mp >300°C. Anal. Calcd for $C_{11}H_{14}N_4Br_2O$: C, 34.9; H, 3.7; N, 14.8. Found: C, 35.2; H, 3.6; N, 14.7.

3-Methylimidazo[4,5-f]quinoline (7a). 6-Methylamino-5-nitroquinoline[8] (5, 1.00 g, 5 mmol) in 25 mL formamide was heated to 100°C, ammonium formate (680 mg, 10.8 mmol) was added, then $NaHSO_3 \cdot H_2O$ (415 mg, 3 mmol) in four portions, allowing the vigorous gas evolution to subside between additions. After 3 h the solution was evaporated and the residue was dissolved in 1N HCl. This solution was washed with $CHCl_3$, adjusted to pH 10 with sodium carbonate, diluted with an equal volume of satd aq. NaCl, and extracted four times with $CHCl_3$. The combined dried organic phase was evaporated and the residue sublimed (100°C/50 μm) to yield 540 mg, 59%, of imidazole 7a: mp 185-188°C (lit.[8] mp 189-190°C); ^{1}H-NMR δ 8.94 (d, 1, J=8, C-7-H), 8.93 (d, 1, J=4), 8.01 (d, 1, J=9), 7.99 (s, 1, C-2-H), 7.74 (d, 1, J=9), 7.55 (dd, 1, J=4,8), 3.95 (s, 3).

2-Amino-3-methylimidazo[4,5-f]quinoline (IQ, 1) from 3-Methylimidazo[4,5-f]-quinoline (7a). To imidazole 7a (100 mg, 0.54 mmol) in THF (15 mL) cooled to -70°, was added nBuLi (1.51M, 360 µL, 0.54 mmol) over 15 min. Phenyl azide (70.2 mg, 65 µL, 0.59 mmol) was then added in several portions over 10 min, the solution was allowed to warm to 20°C, methanol (1 mL) was added, and the solution was evaporated. The residue, dissolved in 15 mL methanol, plus 0.2 mL of conc. HCL was heated with stirring at 70° for 2 h. The product which precipitated was pure IQ (1).

2-Amino-5-bromo-3-methylimidazo[4,5-f]quinoline (8). 2-Amino-3-methylimidazo-[4,5-f]quinoline (IQ, 1, 50 mg, 0.25 mmol) was dissolved in 10 mL glacial acetic acid, bromine (40 mg, 13 µL, 0.25 mmol) dissolved in 2 mL acetic acid was added dropwise at 20°C, and after 30 min the solution was evaporated. The residue was dissolved in water and addition of aq. NaOH precipitated 8 as a white solid (50 mg, 72%), mp 310-312°C.

2-Amino-3-methylimidazo[4,5-f]quinoline-5-^{2}H (9, *H=^{2}H). To 5-BrIQ (8, 100 mg, 0.36 mmol) dissolved in 40 mL CH_3OD was added 10% Pd/C (30 mg) and the mixture was shaken under 40 psi D_2 at 40°C for 10 h. The solution was filtered, and the solvent was evaporated to give 9 (*H=^{2}H): ^{1}H NMR δ 8.85 (dd, 1, J=1,4), 8.72 (dd, 1, J=1,8), 7.88 (m, very weak), 7.75 (s, 1), 7.47 (dd, 1, J=4,8). The absorption at δ 7.88 in $CDCl_3$ has been assigned[1] to the C-5-H; in d_6-DMSO, the C-5-H absorbs at δ 7.56 and is not present.

2-Amino-3-methylimidazo[4,5-f]quinoline-5-^{3}H (9, *H=^{3}H). 5-BrIQ (8, 11.0 mg, 40 mmol) was suspended in 2 mL THF and 10% Pd/C (10 mg) was added. The solution was degassed by several freeze-thaw cycles and then exposed to 725 mL $^3H_2$ and stirred magnetically for 5 h. The system was evacuated, the solvent was evaporated the residue was resuspended in THF, centrifuged and decanted, and the catalyst was resuspended in 2 mL THF and 0.5 mL methanol and recentrifuged. The combined supernatants were evaporated three times with re-addition of methanol and the residue was applied to a 250 µ Analtech C-18 reverse-phase silica plate, developing the chromatogram with 70% MeOH/H_2O. The IQ fraction contained 3.13 x 10^{11} DPM or 0.142 Ci, and by UV analysis at 264 nm, 8.46 µmole, for a specific activity of 16.8 Ci/mmol. HPLC analysis (Hamilton PRP-1, 100% H_2O → 100% CH_3OH, 0.1% diethylamine) showed 9 to be >97% radiochemically pure.

8-Bromo-7-methylquinoline (11). 2-Bromo-3-methylaniline[17] (10, 2.0 g, 10.7 mmol), glycerol (3.22 g, 35 mmol) and As_2O_5 (2.89 g, 12.6 mmol) were mixed and stirred mechanically while heating to 130°C, at which point conc. H_2SO_4 (2.95 g) was added dropwise over 10 min. The reaction mixture was heated to 150°C for 4 h, allowing water to distill so that an internal temperature of 150°C

could be maintained. The reaction was allowed to cool, poured into water, and allowed to stand overnight. Filtration was followed by addition of aq. NaOH to pH 11 to the filtrate which was extracted 3x with $CHCl_3$. The combined, dried $CHCl_3$ was evaporated to a residue (1.52 g) which was kugelrohr distilled (110°C/1 mm). The resulting quinoline 11 (1.35 g, 57% yield) remained as an oil at variance with the literature.[25] ^{1}H-NMR δ 8.85 (dd, 1, J=2,4, C-2-H), 7.89 (dd, 1, J=2,8), 7.45 (d, 1, J=8), 7.20 (m, 1), 7.15 (d, 1, J=8), 2.5 (s, 3). Anal. Calcd for $C_{10}H_8BrN$: C, 54.1; H, 3.6; N, 6.3. Found: C, 53.9, H, 3.7; N, 6.2.

8-Bromo-7-methyl-5-nitroquinoline (12). 8-Bromo-7-methylquinoline (11, 213 mg, 0.90 mmol) was added dropwise to an ice chilled mixture of 2 mL of 42% v/v HNO_3 (d=1.6, 22.3 mmoles)/conc. H_2SO_4 acids and stirred at 20°C for 2 h, then poured on ice and filtered to give 12 (215 mg, 89% yield): mp 151-152°C; ^{1}H-NMR δ 9.07 (dd, 1, J=2,5), 8.94 (dd, 1, J=2,8, C-4-H), 8.21 (s, 1, C-6-H), 7.58 (dd, 1, J=5,8), 2.7 (s, 3). Anal. Calcd for $C_{10}H_7BrN_2O_2$: C, 45.0; H, 2.6; N, 10.5. Found: C, 45.0; H, 2.7; N, 10.3.

5-Amino-8-bromo-7-methylquinoline (13). To 8-bromo-7-methyl-5-nitroquinoline (12, 200 mg, 0.75 mmol) dissolved in 2 mL isopropanol and 3 mL THF was added dropwise $SnCl_2 \cdot 2\ H_2O$ (815 mg, 3.6 mmol, dissolved in 2 mL conc HCl) then the solution was chilled on ice. The red precipitate that formed was added to conc KOH and ether and stirred until the precipitate dissolved. The aqueous phase was extracted 3x with chloroform and the combined extracts were dried and evaporated to give 13 (0.14 g, 78% yield): mp 170-172°C from benzene; ^{1}H-NMR δ 8.94 (dd, 1, J=2,5), 8.08 (dd, 1, J=2,8, C-4-H), 7.29 (dd, 1, J=5,8), 6.68 (s, 1, C-6-H), 2.52 (s, 3). Anal. Calcd for $C_{10}H_9BrN_2$: C, 50.7; H, 3.8; N, 11.8. Found: C, 50.6; H, 3.9; N, 11.6.

8-Bromo-7-methyl-5-[N-(4-methylphenyl)sulfonylamino]quinoline (14). Amine 13 (19.22 g, 81.1 mmol) was dissolved in pyridine and p-toluenesulfonyl-chloride (18.55 g, 97.3 mmol) was added. After 18 h the solvent was evaporated and the residue was dissolved in $CHCl_3$ and 1N H_2SO_4 with stirring. The aq. acid phase was extracted with $CHCl_3$ once more and the combined $CHCl_3$ was extracted with 1M NaOH in which the sodium salt precipitated. The salt was dissolved in water which was adjusted to pH 8 with NaH_2PO_4. Cooling to 0°C, filtering, washing, and drying gave 14 (27.7 g, 87% yield); mp 195-197°; ^{1}H-NMR δ 8.98 (dd, 1, J=1.5,4), 8.25 (dd, 1, 8.25 (dd, 1, J=1.5, 8.5), 7.59 (d, 2, J=9), 7.35 (dd, 1, J=4, 8.5), 7.20 (d, 2, J=9), 7.03 (s, 1), 2.59 (s, 3), 2.37 (s, 3).

8-Bromo-7-methyl-6-nitro-5-[N-(4-methylphenyl)sulfonylamino]quinoline (15). 8-Bromo-7-methyl-5-tosylaminoquinoline (14, 330 mg, 0.84 mmol) was dissolved in HNO_3 acid (20 mL, 60%) and heated to 55°. After 1 h the reaction mixture was

poured on ice, made alkaline with sodium bicarbonate, and extracted 3x with EtOAc. The organic phase was dried and evaporated to give 15 (0.29 g, 79% yield): mp 230-231°C; ^{1}H-NMR δ 9.07 (dd, 1, J=2, 5), 8.82 (dd, 1, J=2, 9), 7.50 (dd, 1, J=5, 9), 7.4 (d, 2, J=8), 7.15 (d, 2, J=8), 2.43 (s, 3), 2.36 (s, 3). Anal. Calcd for $C_{17}H_{14}BrN_3O_4S$: C, 46.8; H, 3.2; N, 9.6. Found: C, 46.8; H, 3.3; N, 9.7.

6-Amino-7-methyl-5-[N-(4-methylphenyl)sulfonylamino]quinoline (16). Nitroquinoline 15 (200 mg, 0.46 mmol), dissolved in methanol (30 mL) to which sodium acetate (105 mg, 1.3 mmol) and 10% Pd/C (22 mg) were added, was shaken under 40 psi H_2 for 18 h, then filtered and evaporated. The catalyst was washed with 1N H_2SO_4 which was added to the residue from the filtrate. This acidic aq solution was washed with $CHCl_3$ then isooctane, then made alkaline with Na_2CO_3 to give a white precipitate of 16 (100 mg, 67% yield): mp 258-259°: ^{1}H-NMR δ 8.36 (dd, 1, J=2, 5), 7.5 (m, 3), 7.10 (d, 2, J=8), 6.90 (dd, 1, J=5, 8), 2.37 (s, 6). Anal. Calcd for $C_{17}H_{17}N_3O_2S$: C, 62.4; H, 5.2; N, 12.8. Found: C, 62.0; H, 5.3; N, 12.7.

2-Bromo-3-methyl-4-nitro-[N-(4-methylphenyl)sulfonyl]aniline (18). To aniline 10[17] (5.65 g, 30.4 mmol) dissolved in pyridine (60 mL) and chilled to 5° was added p-toluenesulfonyl chloride (8.67 g, 45.5 mmol) dissolved in 40 mL pyridine, and also chilled to 5°. After stirring this solution for 18 h at 20°C, 12 mL of half satd aq. sodium bicarbonate was added and stirred 45 min. The solution was evaporated and the residue was taken up in 250 mL chloroform and 20 mL 1N H_2SO_4. The aqueous layer (pH 1) was extracted 2x with $CHCl_3$ and the combined extracts were washed with aq. bicarbonate, dried and evaporated to yield 11.0 g of crystalline 17, mp 106-108°C from benzene/pet. ether. The tosylamide 17 (23.0 g, 67.6 mmol) was finely powdered and added over 2 min to nitric acid (770 mL, d=1.4) preheated to 50°. Following 20 min at 50°C (after dissolution of 17), the solution was poured onto 1 L ice and promptly filtered. The orange precipitate obtained was dissolved in 200 mL CH_2Cl_2 and 100 mL water, sodium bicarbonate was added, and the aqueous solution was extracted 2x with CH_2Cl_2. The extracts were filtered, dried, and evaporated to yield 18 (14.8 g, 57% yield): mp 114-116°C; ^{1}H-NMR (acetone-d_6) δ 7.86 (d, 1, J=9), 7.80 (d, 1, J=8), 7.64 (d, 1, J=9), 7.37 (d, 1, J=8), 2.48 (s, 3), 2.37 (s, 3). Anal. Calcd for $C_7H_7BrN_2O_2$: C, 36.4; H, 3.1; N, 12.1. Found: C, 36.5; H, 3.2; N, 12.0.

The initial filtrate was extracted with CH_2Cl_2 which was evaporated, and the residual oil was chromatographed (25% isooctane/$CHCl_3$) to yield phenol 20: ^{1}H-NMR δ 7.61 (s, 1), 2.35 (s, 3); mass spectrum, m/z (relative intensity) 276/278 (M+, 0.17/0.18), 259/261 (0.40, 0.37).

2-Bromo-3-methyl-6-nitro-[N-(4-methylphenyl)sulfonyl]aniline (19). When the above nitration was carried out with 100 mg 17 for 5 min, the organic extracts

yielded 78.3 mg crude product. This was chromatographed (25% isooctane/chloroform) to yield 46 mg 18 and 4 mg 19; ^{1}H-NMR of 19 (acetone-d_6) δ 7.85 (d, 1, J=8), 7.54 (d, 1, J=8), 7.53 (d, 1, J=8), 7.34 (d, 1, J=8), 2.43 (s, 3), 2.42 (s, 3).

3-Methyl-4-nitroaniline (21). Tosylamide 18, treated as described,[21] gave 21 in 42% yield: 133-134°C (lit.[20] mp 133°).

5-Methyl-2-nitroaniline (22). Tosylamide 19, treated as above[21] yielded 22: mp 109-112°C (lit.[20] mp 109°).

2-Bromo-3-methyl-4-nitroaniline (23). Tosylamide 18 (6.55 g, 17.0 mmol) was added to 10 mL liquid HF at 0°C and the solution was allowed to warm to 20°C and stirred for 30 min, after which the HF was evaporated. Aqueous sodium bicarbonate and CH_2Cl_2 were added, and the CH_2Cl_2 solution was separated, dried and evaporated to yield 6.3 g of residue which was dissolved in 75% H_2SO_4 and isooctane and the aq. acid phase washed 2x with isooctane. The acid solution was then diluted with an equal volume of ice water and extracted with CH_2Cl_2. Evaporation yielded 23 (87%): mp 114-116°C; ^{1}H-NMR δ 7.81 (d, 1, J=10), 7.60 (d, 1, J=10), 2.67 (s, 3H); mass spectrum, m/z (relative intensity) 230/232 (M+, 2.7/2.7), 213/215 (2.7/2.6), 200/202 (1.3/1.1), 104 (3.6). Anal. Calcd for $C_7H_7BrN_2O_2$: C, 36.4; H, 3.1; N, 12.1. Found: C, 36.5; H, 3.2; N, 12.0.

Some 20 minute nitrations gave small amounts of impurities which could be isolated by column chromatography (MeOH/isooctane/$CHCl_3$, 5/5/95) in 2-5% yield:
2,4(6)-Dibromo-3-methyl-6(4)-nitroaniline: mp 133-134°C, ^{1}H-NMR δ 8.13 (s, 1), 2.62 (s, 3); mass spectrum, m/z (relative intensity) 308/310/312 (M+, 1.4/2.8/1.4), 291/293/295 (1.9/3.9/1.8). Anal. Calcd. for $C_7H_6Br_2N_2O_2$: C, 27.1; H, 2.0; N, 9.0. Found: C, 27.2; H, 1.8; N, 8.9.
2-Bromo-4,6-dinitro-3-methylaniline: mp 159-160°C; ^{1}H-NMR δ 8.58 (s, 1), 2.75 (s, 3); mass spectrum, m/z (relative intensity) 275/277 (M+, 1.9/1.9), 258/260 (3.0/3.0). Anal. Calcd for $C_7H_6BrN_3O_4$: C, 30.4; H, 2.2; N, 15.2. Found: C, 30.5; H, 2.0; N, 14.9.

9-Bromo-10-methyl-p-phenanthroline (24). Nitroaniline 23 (1.0 g, 4.3 mmol), mixed with As_2O_5 (1.17 g, 5.1 mmol) and glycerol (1.3 g, 14 mmol), was heated to 100° then conc. H_2SO_4 (1.2 mL) was added dropwise, after which the mixture was heated and stirred mechanically at 155°C for 3 h. After cooling, 20 mL water was added and this mixture was stirred overnight at 100°C, filtered, made alkaline with aq. NaOH, and extracted with CH_2Cl_2 which was evaporated. Column chromatography of the residue (25% isooctane/chloroform) gave 87 mg of phenanthroline 24: mp 185-190°C; mass spectrum, m/z (relative intensity) 272/274 (M+, 4.6/4.7), 193 (M+-Br, 5.6); ^{1}H-NMR δ 9.00 (dd, 1, J=2, 5), 8.93 (dd, 1, J=2, 5), 8.68 (d, 2,

J=10), 7.53 (two dd's, 2, J=5,10), 3.05 (s, 3). Anal. Calcd for $C_{13}H_9BrN_2$: C, 57.2; H, 3.3; N, 10.3. Found: C, 57.3; H, 3.0; N, 10.2.

8-Bromo-7-methyl-6-nitroquinoline (25). Nitroaniline 23 (10.0 g, 43.3 mmol) was mixed with water (320 mL), glycerol (80 mL), As_2O_5 (30 g), and conc. H_2SO_4 (200 mL), and stirred mechanically at reflux 5 h. The mixture was then poured on 3000 mL ice, and allowed to sit overnight, after which it was extracted with CH_2Cl_2 (4 x 500 mL). The organic phase was washed with aq. $NaHCO_3$, dried, and evaporated to yield 8.36 g of crude 25. This was sublimed (100°C/50 μm) to return pure nitroquinoline 25 (4.93 g, 43% yield): mp 155-156°C from EtOH; ^{1}H-NMR δ 9.00 (dd, 1, J=2,5), 8.12 (dd, 1, J=2,8, C-4-H), 8.08 (s, 1, C-5-H), 7.43 (dd, 1, J=5,8), 2.65 (s, 3). Anal. Calcd for $C_{10}H_7BrN_2O_2$: C, 45.0; H, 2.6; N, 10.5. Found: C, 44.8; H, 2.6; N, 10.5.

6-Amino-8-bromo-7-methylquinoline (26). Nitroquinoline 25 (115 mg, 0.43 mmol) was dissolved in 10 mL EtOH and added to a solution containing 0.1 g W-2 Raney Nickel (deactivated by a 1 h acetone reflux and washed with MeOH) and 0.3 g $N_2H_4 \cdot H_2O$ in 10 mL MeOH at reflux. Ten minutes after the addition was complete an additional 0.2 g hydrazine hydrate was added, and 15 min later an additional 0.2 g was added. After a final 15 min reflux the RaNI was filtered off with celite, and the solution was evaporated to a yellow residue of aminoquinoline 26 (94 mg, 90% yield): ^{1}H-NMR δ 8.45 (dd, 1, J=2,4), 7.57 (dd, 1, J=2,8), 6.95 (dd, 1, J=4,8), 6.59 (s, 1, C-5-H), 2.25 (s, 3).

8-Bromo-7-methyl-6-methylaminoquinoline (28). To aminoquinoline 26 (1.22 g, 5.1 mmol) dissolved in THF (150 mL) and cooled in liq. N_2/isooctane (-107°C) was added n-BuLi (1.55 M, 3.3 mL, 5.1 mmol) dropwise. The cooling bath was changed to dry ice/isopropanol, and after 15 min methyl iodide (0.33 mL, 5.1 mmol) was added, and the solution was allowed to warm to 20°C for 1 h. Glacial acetic acid was added, the solvent was evaporated, and the residue was dissolved in CH_2Cl_2 which was washed with aq. $NaHCO_3$, dried and evaporated. This residue was chromatographed (Et_2O/isooctane/$CHCl_3$, 3/32/66) to yield three fractions. The first fraction contained 58 mg of 8-bromo-6-dimethylamino-7-methylquinoline as an oil, the second fraction 1.0 g of methylaminoquinoline 28, and the third 150 mg of 28 plus recovered 26. The yield of 28 was 78%: mp 192-193°C; ^{1}H-NMR δ 8.65 (dd, 1, J=1.5, 4), 7.89 (dd, 1, J=1.5, 8, C-4-H), 7.25 (dd, 1, J=4, 8), 6.57 (s, 1, C-5-H), 2.90 (s, 3), 2.48 (s, 3); mass spectrum, m/z (relative intensity) 250/252 (M^+, 5.2/5.0), 235/237 (1.5/1.2). Anal. Calcd for $C_{11}H_{11}BrN_2$: C, 52.6; H, 4.4; N, 11.2. Found: C, 52.6; H, 4.4; N, 11.1.

8-Bromo-7-methyl-6-[(4-methylphenyl)sulfonyl]aminoquinoline (27). The aminoquinoline 26 (167 mg, 0.70 mmol) was dissolved in pyridine (10 mL) with p-

toluenesulfonyl chloride (161 mg, 0.84 mmol). After stirring 2 h, 5 mL water was added, and 1 h later the solvent was evaporated. The residue was dissolved in CH_2Cl_2 and 1N H_2SO_4 and the aqueous phase washed once with CH_2Cl_2. After addition of aq. $NaHCO_3$ to the aqueous phase and extraction 3x with CH_2Cl_2, the CH_2Cl_2 extracts were dried and evaporated to give 27 (252 mg, 91% yield): mp 233-235°C; ^{1}H-NMR ($CDCl_3/CD_3OD$) δ 8.93 (dd, 1, J=2, 5), 8.05 (dd, 1, J=2, 8), 7.71 (s, 1), 7.58 (d, 2, J=8), 7.40 (dd, 1, J=5, 8), 7.17 (d, 2, J=8), 2.39 (s, 6).

8-Bromo-7-methyl-6-(N-methyl)-[N-(4-methylphenyl)sulfonyl]aminoquinoline (29). To tosylamide 27 (226 mg, 0.58 mmol) dissolved in 20 mL THF and cooled to -70°C was added n-BuLi (1.55M, 0.41 mL, 0.63 mmol) dropwise followed by methyl iodide (0.41 µL, 89.8 mg, 0.63 mmol). After 16 h at reflux, 5 drops of glacial acetic acid were added and the solvent evaporated. The residue was dissolved in CH_2Cl_2, washed with aq. $NaHCO_3$, water, and brine, dried and evaporated to give 198 mg, 85% yield, of 29: mp 184-185°C; ^{1}H-NMR δ 9.03 (dd, 1, J=2, 5), 7.88 (dd, 1, J=2, 9), 7.62 (d, 2, J=9), 7.25 (m, 4), 4.21 (s, 3), 2.70 (s, 3), 2.46 (s, 3). Anal. Calcd for $C_{18}H_{17}BrN_2O_2S$: C, 53.3; H, 4.2; N, 6.9. Found: C, 53.3; H, 4.2; N, 6.8.

Nitration of 8-Bromo-7-methyl-6-methylaminoquinoline (28) to Nitramine 30 and Quinone 32. To aminoquinoline 28 (65 mg, 0.26 mmol) dissolved in 2 mL conc. H_2SO_4 and chilled in ice was added dropwise a cold mixture of nitric (90%, d=1.5, 0.5 mL) and conc H_2SO_4 (1 mL) acids. After 1 h at 20°C the solution was poured onto excess ice, made alkaline with aq. $NaHCO_3$, and extracted with CH_2Cl_2 which was dried and evaporated. The residue was separated by preparative TLC (Et_2O/iso-octane/$CHCl_3$, 10/9/81) into two bands. Band A (R_f 0.7) contained 13.8 mg of nitramine 30: ^{1}H-NMR δ 9.21 (dd, 1, J=1.5, 4.2), 8.20 (dd, 1, J=1.5, 8.5), 7.67 (dd, 1, J=4.2, 8.5), 3.73 (s, 3), 2.62 (s, 3); mass spectrum, m/z (relative intensity) 340/342 (0.6/0.8), 294/296 (1.7/2.5), 277/279 (1.6/1.6). Anal. Calcd for $C_{11}H_9N_4O_4Br$: C, 38.7; H, 2.7; N, 16.4. Found: C, 39.1; H, 2.7; N, 16.0. Band B (R_f 0.5) contained 10.9 mg of quinone 32 which was sublimed (110°C/50 µm): mp 130-133°C; ^{1}H-NMR δ 8.95 (dd, 1, J=2,5), 8.33 (dd, 1, J=2,8), 7.48 (dd, 1, J=5,8), 2.38 (s, 3); IR ($CHCl_3$) 1660, 1675 cm^{-1}; mass spectrum, m/z (relative intensity) 253/255 (1.08/0.97), 223/225 (4.6/4.4); calcd for $C_{10}H_6NO_2Br$ (M^+) m/z 250.9581/ 252.9561, found 250.9581/252.9556; calcd for C_9H_6NOBr (M^+-CO) m/z 222.9632/224.9612, found 222.9635/224.9609.

8-Bromo-7-methyl-6-methylamino-5-nitroquinoline (31). A. From 8-Bromo-7-methyl-6-(N-methyl-N-nitro)amino-5-nitroquinoline (30). The dinitroquinoline 30 (19 mg, 55 µmol) was stirred for 1 h in liquid HF containing 1 mL of anisole, the HF was evaporated, and the residue was distributed between CH_2Cl_2 and aq. $NaHCO_3$. The CH_2Cl_2 phase was evaporated and the residue was dissolved in 6N H_2SO_4

which was washed with toluene (3x), adjusted to pH 1, and extracted with $CHCl_3$. After filtration through silica gel, the $CHCl_3$ was evaporated to give 31: 13.3 mg, 80% yield; mp 168-170°C; ^{1}H-NMR δ 8.84 (dd, 1, J=1.5, 4.5), 8.18 (dd, 1, J=1.5, 8, C-4-H), 7.48 (dd, 1, J=4.5, 8.5), 2.99 (d, 3, J=5.5), 2.70 (s, 3); mass spectrum, m/z (relative intensity) 295/297 (M^+, 1.05/0.99), 261/263 (5.6/6.0). Anal. Calcd for $C_{11}H_{10}BrN_3O_2$: C, 44.6; H, 3.4; N, 14.2. Found: C, 44.6; H, 3.5; N, 14.2.

B. From 8-Bromo-7-methyl-6-methylaminoquinoline (28). Acetic anhydride (35 mL) was mixed with nitric acid (3.2 mL, d=1.4) at 25°, and 20 mL of this solution was cooled to 0°. Methylaminoquinoline 28 (349 mg, 1.4 mmol) dissolved in 10 mL glacial acetic acid was added dropwise to this cold solution over 15 min. After another 15 min the nitrating solution was added to excess aq Na_2CO_3. Stirring for 30 min was followed by extraction 3x with CH_2Cl_2. Evaporation yielded 440 mg of crude 33. Chromatography (Et_2O/isooctane/$CHCl_3$, 5/24/71) gave 5% of 8-bromo-7-methyl-6-(N-methyl-N-nitro)amino-5-nitroquinoline 30, 5% of recovered 28, and 70% of 8-bromo-7-methyl-6-(N-methyl-N-nitro)aminoquinoline (33): ^{1}H-NMR δ 9.11 (dd, 1, J=2, 4), 8.18 (dd, 1, J=2, 8, C-4-H), 7.75 (s, 1), 7.53 (dd, 1, J=4, 8), 3.80 (s, 3), 2.05 (s, 3); IR ($CHCl_3$) 1510, 1290 cm^{-1}; mass spectrum, m/z (relative intensity) 249/251 (M^+-NO_2, 8.5/8.3), 169 (10.1), 168 (10.3), 155 (2.7), 141 (7.8).

To the crude 33 was added H_2SO_4 (0°, 10 mL, 85 w/w%) and solution was obtained by swirling and ultrasonic mixing. After standing at 20°C for 30 min, this solution was poured onto ice, chloroform was added, and the acid was neutralized with aq. $NaHCO_3$. The aq. phase was extracted 2x with chloroform and the combined organic phase was dried and evaporated. The residue was treated with HF and anisole (2 mL) and isolated as above. Chromatography (isooctane/Et_2O/CH_2Cl_2, 17/3/80) of the residue gave 0.251 of 31, 60% yield from 28.

2-Amino-5-bromo-3,4-dimethylimidazo[4,5-f]quinoline (35). A solution of nitroquinoline 31 (357 mg, 1.2 mmol) in 50 mL of CH_3OH was added to a refluxing mixture of 100 mg W-2 Raney Ni and hydrazine hydrate (410 mg, 8.2 mmol) in 10 mL of CH_3OH. Additional portions of hydrazine hydrate (130 mg) were added at 15 min intervals, and after 1 h, the solution was filtered and the filtrate evaporated to a yellow residue of 5-amino-8-bromo-7-methyl-6-methylaminoquinoline (34): 309 mg, 96% yield; ^{1}H NMR δ 8.94 (dd, 1, J=1,4), 8.14 (dd, 1, J=1, 8, C-4-H), 7.34 (dd, 1, J=4, 8), 2.74 (s, 3), 2.69 (s, 3).

To crude 34 (202 mg, 0.76 mmol) in 20 mL of ethanol containing diisopropylethylamine (310 mg, 2.4 mmol) was added BrCN (254 mg, 2.4 mmol) in 10 mL of ethanol. After being stirred for 12 h at 25°C, the solvent was evaporated, NaOH (3 mL, 1M) was added to the residue and the water was evaporated. Two

additional 3 mL portions of water were added and evaporated. Chromatography ($CH_3OH/CHCl_3$, 5/20) of the residue gave 147 mg, 66% yield, of 35: mp 275-277°C; ^1H NMR δ 8.86 (dd, 1, J=2,4), 8.65 (dd, 1, J=2, 8, C-9-H), 7.45 (dd, 1, J=4,8), 3.90 (s, 3), 3.02 (s, 3); mass spectrum m/z (relative intensity) 290/292 (M^+, 5.94/5.47), 275/277 (M^+-CH_3, 1.71/1.63), calcd for $C_{12}H_{11}BrN_4$: 290.0167/292.0147, found 290.0155/292.0135.

2-Amino-3,4-dimethylimidazo[4,5-f]quinoline (MeIQ, 2). The bromo-MeIQ 35 (21.2 mg, 73 μmol), dissolved in 20 mL of CH_3OH to which 23 mg of sodium acetate and 4.2 mg of 10% Pd/C were added, was shaken under H_2 at 40 psi for 4 h. Filtration and evaporation of the filtrate left a residue which was chromatographed (15% $CH_3OH/CHCl_3$) giving 12.2 mg of 2, 74% yield: mp >300°C (lit.[12,18] mp >300°C); spectral properties identical to those reported;[12,18] ^1H-NMR δ 8.80 (dd, 1, J=2.4), 8.67 (ddd, 1, J=0.8, 2, 4, C-9-H), 7.54 (t, 1, J=0.8), 7.37 (dd, 1, J=4, 8, C-5-H), 3.91 (s, 3), 2.84 (d, 3, J-1, C-4-CH_3).

2-Amino-3,4-dimethylimidazo[4,5-f]quinoline-5-^3H (36). Bromo-MeIQ (35, 1.5 mg, 5.15 μmol) was dissolved in 3 mL THF containing anhydrous NaOAc (2 mg), freeze-thaw degassed twice, then warmed to 25°C under one atmosphere of $^3H_2$. Catalyst (10% Pd/C, 2.8 mg) was added and the mixture stirred 5 h at the same pressure. The sample was then freeze-thaw degassed twice, the catalyst was removed by centrifugation and washed with methanol twice and the combined supernatants were evaporated, dissolved in methanol, and re-evaporated four times. The residue was chromatographed in two portions on a 4.4 x 250 mm Hamilton PRP-1 column, eluting (1.5 mL/ min) with 65% to 100% CH_3OH/H_2O (25 min), and the 8 min to ~18 min fractions were combined to yield 35 mCi, 2.1 μmol, of 36 with a specific activity of 16.6 Ci/mmol. The final MeIQ-5-^3H (36) was determined (same chromatographic system with 0.5% diethylamine added to the eluting solvent) to be greater than 99% radiochemically pure.

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