SYNTHESIS OF ^{123}I- AND ^{125}I-LABELLED 5-IODO-6-NITROQUIPAZINE

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SUMMARY

The syntheses of the potent and selective serotonin reuptake complex radioligands [^{123}I]- and [^{125}I]5-iodo-6-nitroquipazine (5-iodo-6-nitro-2-piperazinylquinoline) are reported. A seven step synthetic sequence provided the BOC-protected 5-tributyltin-6-nitroquipazine precursor for radioiodination. End of synthesis radioiodination yields of ~40% for ^{123}I and ~60% for ^{125}I were achieved resulting in labelled products with high specific activities (>4000 and >2000 Ci/mmol, respectively) and radiochemical purities (>98%).

Key Words: [^{123}I]5-iodo-6-nitroquipazine, [^{125}I]5-iodo-6-nitroquipazine, 5-iodo-6-nitroquipazine, 5-iodo-6-nitro-2-piperazinylquinoline, serotonin reuptake complex

INTRODUCTION

Radioligands which bind to presynaptic biogenic amine reuptake complexes have been the focus of recent in vivo investigations (1-5). Numerous serotonin (5-hydroxytryptamine, 5-HT) reuptake complex ligands have been synthesized, yet few of these agents have proven useful as in vivo markers of the regional concentration of serotonergic neuronal terminals for use in single photon emission computed tomography (SPECT) or positron emission tomography (PET) studies (6,7). Based upon the encouraging in vitro (8,9) and in vivo (10,11) properties of [^{3}H]6-nitroquipazine (6-nitro-2-piperazinylquinoline, Fig. 1), we synthesized 5-[^{125}I]iodo-6-nitroquipazine and reported binding (12) and brain distribution properties in rats (13) and monkeys (14). The syntheses of high specific activity [^{125}I]- and [^{123}I]5-iodo-6-nitroquipazine for studies of the 5-HT reuptake complex are described here.

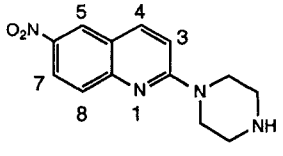

Figure 1. Structure of 6-nitroquipazine showing the ring position numbering.

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RESULTS AND DISCUSSION

A reaction scheme summarizing the syntheses is given in Figure 2. The first step of the synthetic pathway afforded the lowest yield whereas other major transformations were realized in ≥50% yields. Bromination of quinoline to yield 5-bromoquinoline has been reported in ~6% yields using silver sulfate and sulfuric acid (15). A variety of bromination conditions were explored using the commercially available 2-chloroquinoline (**1a**), but the highest yielding bromination reaction utilizing N-bromosuccinimide in concentrated sulfuric acid (16) yielded only 15% **2**. The formation of 8-bromo-2-chloroquinoline in about 17% yield made the isolation of **2** from the reaction mixture difficult and required the use of medium pressure liquid chromatography. An alternative two step synthesis of **2** started with 5-aminoquinoline (**1b**) to afford 5-bromoquinoline (**1c**) via the 5-diazonium salt (17) in good yield (82%). Subsequent reaction of **1c** with peroxyacetic acid followed by treatment of the N-oxide (18) with $POCl_3$ also produced **2**, but the concomitant formation of 5-bromo-4-chloroquinoline reduced the yield of **2** to only 43%. Separation of the 5-bromo-4-chloroquinoline impurity from **2** was easily effected with flash chromatography, and this route was preferred to the one step conversion of **1a** to **2**. Conditions for the nitration of 5-bromoquipazine to form **4** were similar to those described by Hashimoto and Goromaru for the radiosynthesis of [^{3}H]6-nitroquipazine (19). Whereas lithiation reactions in the presence of aromatic nitro groups can be complicated by competing side reactions, an *ortho*-Br substitution pattern is known (20) to facilitate the formation of the lithium anion in the presence of an aromatic nitro moiety at low temperatures and, in this case, afforded the precursor **6** in 50% yield. Macroscopic iodination under conditions similar to radioiodination conditions provided sufficient 5-iodo-6-nitroquipazine (**7**) for structural confirmation and for use as an authentic chromatographic standard.

A variety of radiolabelling conditions were assessed to determine a high yield radioiodination procedure using no-carrier-added [^{125}I]iodide in pilot reactions, and oxidants such as peroxyacetic acid, hydrogen peroxide, chloramine T (CAT), and dichloramine T (DCT) were evaluated. The best conditions led to ^{125}I incorporation yields of about 80% in 30 min utilizing DCT oxidant in acetonitrile. The ^{125}I incorporation yields were measured using radio-HPLC and represent the fractional amount of BOC-protected [^{125}I]5-iodo-6-nitroquipazine compared to the total radioactivity detected in the radio-HPLC analysis. The other oxidizing agents led to considerably lower radioiodination yields ranging from 20-50% in 30 min. Radioiodinations utilizing DCT have been reported (21), and the solubility of this reagent in a variety of organic solvents is an advantage over

CAT. Whereas chlorination side reactions present a potential complication with the use of N-chlorosulfonamide oxidants such as DCT and CAT (21), HPLC purification to provide high specific and effective specific activity products can overcome this complication. Several earlier eluting UV-active peaks (less lipophilic than the title compounds) were observed when DCT was used as oxidant, but these were excluded from the final preparations by careful HPLC fraction collecting. Hydrogen peroxide presents an alternative to the use of chlorine-containing oxidizing agents, but gave lower radioiodination yields (~50% in 30 min) compared to DCT.

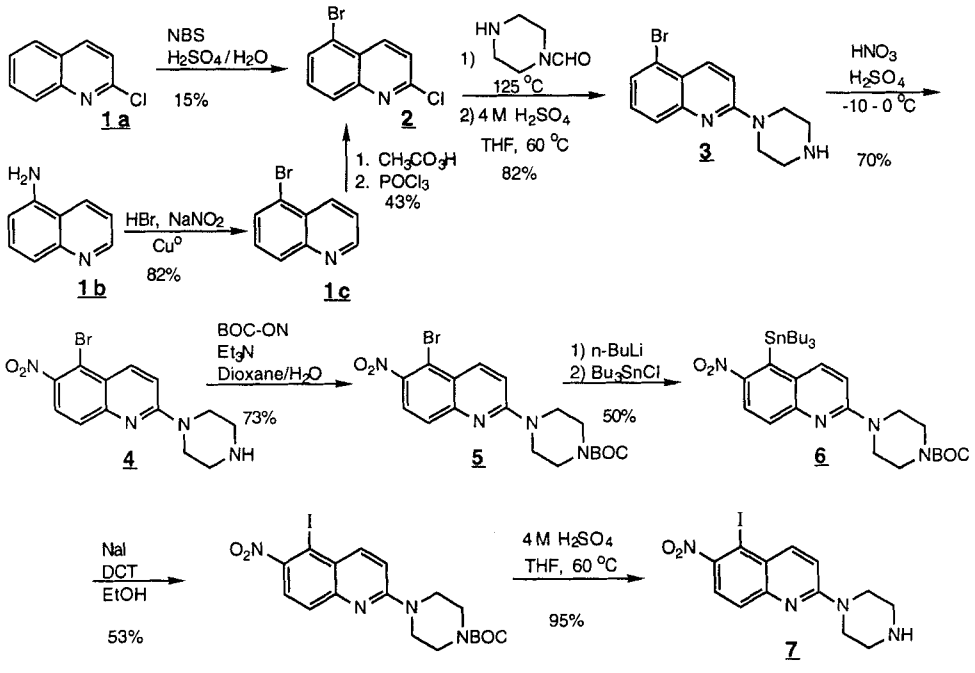

Figure 2. Synthesis of the BOC-protected 5-tributyltin precursor (6) for cold iodination and radioiodination to produce the title compound, 5-iodo-6-nitroquipazine (7).

Using DCT oxidant and acetonitrile as solvent, [^{125}I]5-iodo-6-nitroquipazine (8) was obtained in an overall yield of 63 ± 12% (N = 12) and a radiochemical purity >99% with a specific activity of >2000 Ci/mmol. [^{123}I]5-iodo-6-nitroquipazine (9) was obtained in an overall yield of 40 ± 7% (N = 7) and a radiochemical purity >98% with a specific activity >4000 Ci/mmol. The true specificity of 9 may have been considerably greater than 4000 Ci/mmol, but the mass detection sensitivity of the HPLC UV detection system limited specific activity estimates to this value.

EXPERIMENTAL

Materials and Methods. Reagent chemicals were purchased from Aldrich Chemical Co. and solvents from Burdick and Jackson unless otherwise stated. All nonaqueous reactions were

carried out under an argon or nitrogen atmosphere unless otherwise noted. Preparative high performance liquid chromatography (HPLC) was performed with a Waters Associates 590 pumping system and a Whatman Partisil 10 M9 (silica gel) column. Medium pressure liquid chromatography (MPLC) was performed with a Rainin Rabbit HP preparative pumping system and a silica gel 60 column (230-400 mesh). The ^{1}H NMR spectra were recorded with either a Nicolet 200 or 300 MHz spectrometer with $CDCl_3$ as the internal standard (δ 7.26 ppm). Infrared (IR) spectra were obtained using a Perkin-Elmer Model 1310 spectrometer with the sample as a thin film or in solution (CCl_4). Elemental analyses were performed by the Microanalytical Laboratory, operated by the College of Chemistry, University of California, Berkeley. High (HRMS) and low (LRMS) resolution mass spectrometry data are given for those compounds which repeatedly gave unsatisfactory elemental analyses due to decomposition caused by exposure to air or moisture sensitivity. Mass spectral determinations were made at the Midwest Center for Mass Spectrometry, University of Nebraska-Lincoln. Radioiodinations were conducted in a lead shielded glove box. Analytical HPLC of the reaction products was conducted using a Waters Associates 590 pump, a Waters μBondapak C18 3.9 x 250 mm stainless steel column, an in-line NaI(Tl) radioactivity detector, and a Milton Roy Model 3100 UV detector (@ 254 nm). Purification of high specific activity [^{123}I]- and [^{125}I]-labelled products was performed using a Waters 6000A pump, a Waters μBondapak C18 3.9 x 250 mm stainless steel column, an in-line NaI(Tl) radioactivity detector, and a Waters Model 441 UV detector (@ 254 nm). The eluent used for the HPLC separations consisted of a methanol/H_2O mixture (aqueous buffer made with 0.3% Et_3N/conc. phosphoric acid to pH 7.4).

5-Bromo-2-chloroquinoline (2) via 1a. To a solution of 2-chloroquinoline (**1a**) (1.0 g, 6.1 mmol) in 9 M H_2SO_4 (30 ml) was added N-bromosuccinimide (1.7 g, 9.55 mmol). The mixture was stirred at 35°C for 38 h and then cooled to 0°C. The solution was diluted with 50% NaOH until the pH was between 0 and 1, and the mixture was extracted with CH_2Cl_2. The combined organic layers were washed with sat. $NaHCO_3$ and dried ($MgSO_4$). Evaporation of the solvent afforded a dark brown oil which was sequentially purified by flash chromatography (85:15 hexane:CH_2Cl_2 on silica gel) and MPLC (85:15 hexane:CH_2Cl_2) to afford **2** (0.22 g, 15%) as a white amorphous solid (m.p. 71-72°C; lit. 76-78°C (22)). Anal. calcd. for C_9H_5NBrCl: C, 44.58; H, 2.08; N, 5.78; found: C, 44.65; H, 2.16; N, 5.53. ^{1}H NMR δ (ppm): 7.39 d (CH arom), 7.51 dd (CH arom), 7.74 d (CH arom), 7.91 d (CH arom), 8.37 d (CH arom). IR (cm^{-1}): 3070, 1935.

5-Bromoquinoline (1c). To a solution of 5-aminoquinoline (**1b**) (10 g, 69 mmol) in HBr (48%, 125 ml) cooled to -78°C was added $NaNO_2$ (6 g, 87 mmol) dissolved in H_2O (4 ml). The

mixture was stirred at -78°C for 15 min, warmed to 0°C, and copper dust (0.6 g, 9.4 mmol) was added. The mixture was heated at 100°C for 1 h, poured into ice-water, neutralized with NaOH (2 M), and extracted with EtOAc. The organic layer was dried ($MgSO_4$). The solvent was removed and the residue purified by flash chromatography (1:10 EtOAc:hexanes on silica gel) to yield **1c** (11.9 g, 82%) as a white solid on standing (m.p. 48-49°C, lit. 48-49°C (15)). ^1H NMR δ (ppm): 7.3-7.4 (m, 2 CH arom), 7.41-7.45 (dd, CH arom), 7.67 (d, CH arom), 8.36 (d, CH arom), 8.8 (m, CH arom).

5-Bromo-2-chloroquinoline (2) via 1c. To a solution of 5-bromoquinoline (**1b**) (10 g, 48 mmol) in $CHCl_3$ (100 ml) was added peroxyacetic acid (32%, 40 ml). The mixture was refluxed for 3 h and poured into ice-water followed by the addition of NaOH (4 M) to a final pH of ~10. The N-oxide was extracted with EtOAc and dried ($MgSO_4$). The solvent was removed and the remaining white solid was treated with $POCl_3$ (60 ml) and heated at 100°C for 1 h. The mixture was poured into ice-water, diluted with NaOH (4 M) to a final pH of ~8 and extracted with CH_2Cl_2. Evaporation of the solvent afforded a brown oil that was purified by flash chromatography (EtOAc:hexanes 1:20 on silica gel) to yield **2** (5.0 g, 43%) as a white solid (m.p. and ^1H NMR identical to **2** via **1a** as described above).

5-Bromo-2-piperazinylquinoline (3). A stirred mixture of **2** (342 mg, 1.41 mmol) and 1-piperazinecarboxaldehyde (4 ml) was heated at 125°C for 30 min under argon. The mixture was then cooled and diluted with saturated aqueous $NaHCO_3$. The aqueous phase was extracted with ether (3 x) and the combined extracts were dried ($MgSO_4$). Evaporation of the solvent yielded a solid (400 mg) which was immediately dissolved in THF (10 ml) and 4 M H_2SO_4 (5 ml). The solution was brought to reflux and stirred for 1 h. The solution was cooled and poured into 1 M NaOH. The resulting basic suspension was extracted twice with ether, and the ether extracts were dried ($MgSO_4$). Evaporation of the solvent afforded **3** (300 mg, 82%) as a white solid (m.p. 94-96°C). HRMS: m/z 291.0402 (calcd. for $C_{13}H_{14}N_3Br$, 291.0371). LRMS: m/z 291 (M^+, 20), 263 (22), 249 (51), 235 (65), 223 (100), 208 (30), 149 (28), 127 (40), 69 (19), 56 (32). ^1H NMR δ (ppm): 1.81 s (NH), 2.96 dd (CH_2), 3.67 dd (CH_2), 6.97 d (CH arom), 7.32 dd (CH arom), 7.42 d (CH arom), 7.60 d (CH arom), 8.19 d (CH arom). IR (cm^{-1}): 3345, 3070, 1920.

5-Bromo-6-nitro-2-piperazinylquinoline (4). To a -10°C solution of **3** (300 mg, 1.03 mmol) in conc. H_2SO_4 (10 ml) was added dropwise conc. HNO_3 (0.25 ml). The mixture was stirred 15 min at -10 - 0°C, poured onto ice, and diluted with 1 M NaOH until basic. The mixture was then extracted with CH_2Cl_2 (3 x), and the combined organic layers were dried ($MgSO_4$). Evaporation of

the solvent yielded **4** (240 mg, 70%) as a yellowish solid which was utilized in the next step without further purification. (m.p. > 230°C, dec.). ^{1}H NMR δ (ppm): 1.79 s (NH), 3.00 dd (CH_2), 3.80 dd (CH_2), 7.07 d (CH arom), 7.57 d (CH arom), 7.81 d (CH arom), 8.37 d (CH arom). IR (cm^{-1}): 3300, 1600, 1320. HRMS: m/z 337.1302 (calcd. for $C_{13}H_{13}N_4O_2Br$, 337.1766). LRMS: m/z 337 (M^+, 18), 210 (15), 154 (100), 136 (82), 107 (30).

5-Bromo-6-nitro-2-(1-tert-butylcarboxypiperazinyl)quinoline (5). To a solution of **4** (243 mg, 0.72 mmol) in dioxane (15 ml) and water (10 ml) was added Et_3N (0.19 ml, 1.36 mmol) and BOC-ON (2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile) (208 mg, 0.84 mmol). The mixture was stirred at room temperature for 1.5 h and poured into water. The mixture was extracted with ether, the combined ether extracts washed with water, dried ($MgSO_4$), and the solvent evaporated. The residue was purified by HPLC (90:10 CH_2Cl_2:EtOAc) to afford the carbamate **5** (230 mg, 73%) (m.p. 179-180°C). Anal. calcd. for $C_{18}H_{21}N_4O_4Br$ C, 49.44; H, 4.84; N, 12.81; found: C, 49.38; H, 4.84; N, 12.60. ^{1}H NMR δ (ppm): 1.49 s (3 CH_3), 3.59 dd (CH_2), 3.83 dd (CH_2), 7.09 d (CH arom), 7.61 d (CH arom), 7.93 d (CH arom), 8.42 d (CH arom). IR (cm^{-1}): 3050, 1690, 1350.

5-Tributylstannyl-6-nitro-2-(1-tert-butylcarboxypiperazinyl)quinoline (6). To a -100°C solution of **5** (200 mg, 0.46 mmol) in dry THF (8 ml) was added n-BuLi (1.6 M, 0.50 mmol, 0.31 ml) dropwise. The resulting black-green solution was stirred for 10 min at which time Bu_3SnCl (0.15 ml, 0.54 mmol) was added. The solution was allowed to reach room temperature and stirred 2 h. Water was added, and the mixture was extracted with ether. The combined ether extracts were dried ($MgSO_4$) and concentrated to a brown oil. HPLC purification of the oil (90:10 CH_2Cl_2:EtOAc) afforded the tributylstannyl derivative **6** (150 mg, 50%). ^{1}H NMR δ (ppm): 0.85 t (CH_3), 1.15-1.55 m (3 CH_2), 1.49 s (3 CH_3), 3.58 dd (CH_2), 3.83 dd (CH_2), 7.00 d (CH arom), 7.63 d (CH arom), 8.18 d (CH arom), 8.30 d (CH arom). IR (cm^{-1}): 1695, 1320. HRMS: m/z 647.2778 (calcd. for $C_{30}H_{48}N_4O_4Sn$, 647.4284). LRMS: m/z 647 (M^+, 11), 590 (M-C_4H_9, 100), 535 (38), 375 (12), 235 (11), 177 (52).

5-Iodo-6-nitro-2-piperazinylquinoline (7). To a solution of **6** (70 mg, 0.11 mmol) in 5 ml of ethanol were added 1.0 M NaI (0.22 ml, 0.22 mmol), 1 M H_3PO_4 (0.1 ml), and dichloramine T powder (TCI America, 26 mg, 0.11 mmol). The resulting brown solution was stirred for 20 min at room temperature, and a precipitate formed. The mixture was diluted with 10% $Na_2S_2O_3$ and then extracted with ether. The combined ether extracts were dried ($MgSO_4$) and concentrated to a residue which was immediately dissolved in THF (5 ml) and 4 M H_2SO_4 (5 ml). The solution was refluxed

for 1 h, cooled to room temperature, and made basic with 1 M NaOH. The mixture was extracted with CH_2Cl_2, and the organic phase was dried ($MgSO_4$) and evaporated to afford **7** (21 mg, 50%) as a brown oil. HRMS: m/z 384.0094 (calcd. for $C_{13}H_{13}N_4O_2I$, 384.0083). LRMS: m/z 384 (M^+, 15), 342 (37), 316 (100), 169 (19), 97 (28), 83 (38), 57 (57). ^1H NMR δ (ppm): 1.70 br s (NH), 3.00 dd (CH_2), 3.80 dd (CH_2), 7.02 d (CH arom), 7.60 d (CH arom), 7.86 d (CH arom), 8.32 d (CH arom). IR (cm^{-1}): 1595, 1320.

[^{125}I]5-Iodo-6-nitro-2-piperazinylquinoline (8). Typical preparative radioiodination conditions (utilizing up to 50 mCi of [^{125}I]iodide) involved the sequential addition of 0.5 mg of the BOC-protected 5-tributyltin precursor (**6**), 0.2 ml of acetonitrile, and 10 µl of 2 M H_3PO_4 to a 1 ml Reacti-Vial fitted with a magnetic stirrer. A solution of high specific activity (2175 Ci/mmol) sodium [^{125}I]iodide (New England Nuclear) in pH 9-12 NaOH was then added in volumes varying from 2-100 µl. Dichloramine T (DCT) was added (20 µl of 5 mg DCT in 2 ml acetonitrile), and the vial capped and the contents stirred at room temperature in the dark. Aliquots of the reaction were monitored using radio-HPLC (MeOH:H_20 (pH 7.4) (80:20)), and the reaction was quenched (at 30 min for preparative syntheses) with 50 µl of 2 M $Na_2S_2O_3$. To the reaction mixture were added 200 µl THF and 100 µl 2 M H_2SO_4, and the vial was sealed and heated to 60°C for 15 min to remove the BOC protecting group. The reaction mixture was cooled, made basic with a 1 M $NaHCO_3$ solution, and injected onto a Waters C18 analytical column eluted with methanol:water (pH 7.4) (35:65). The product displayed a retention volume of ~100 ml, and the product fraction was collected in 10 ml of eluant. Unhydrolyzed BOC-protected product and the tributylstannyl precursor (**6**) displayed much longer retention times than **8** using this HPLC purification system and were excluded from the final product (within the detection limits of the system). The product fraction was diluted with 50 ml of 0.1 M $NaHCO_3$ and collected on a Millipore C_{18} "classic" Sep-Pak® cartridge. The Sep-Pak was sequentially washed with 5 ml water, air dryed, and the product eluted with 3 ml ethanol. Analytical radio-HPLC with MeOH:H_20 (pH 7.4) (70:30) eluant (retention volume 19 ml) and TLC with chloroform:methanol:conc. NH_3 (10:10:0.1) eluant (R_f = 0.7) were utilized to determine radiochemical yields and purity. The product was stored in ethanol at -10°C in a sealed vial and diluted with phosphate buffered saline as needed. In ethanol, decomposition of ~20% per month was observed.

[^{123}I]5-Iodo-6-nitro-2-piperazinylquinoline (9). The [^{123}I]-labelled compound was prepared following a procedure similar to that described above for **8** except that high specific activity

sodium [^{123}I]iodide (Nordion) was utilized. The product was stored in ethanol at -10°C in a sealed vial and diluted with phosphate buffered saline as needed. In ethanol, decomposition of <3% per day was observed.

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