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[^{123}I]IODOMETHANE, A MAIN PRODUCT IN THE SYNTHESIS OF 5-[^{123}I]IODO-6-NITROQUIPAZINE BY IODODESTANNYLATION

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SUMMARY

Radioiodinated 5-iodo-6-nitroquipazine (2-piperazinyl-5-iodo-6-nitroquinoline), a selective serotonin transporter radiotracer, can be prepared by the reaction of its N-t-BOC-5-trimethylstannyl precursor with [^{125}I] or [^{123}I]NaI in the presence of chloramine-T, followed by amine deprotection by heating under acidic conditions. However, the labeling reaction is associated with a large and consistent formation of a volatile radioactive reaction product [37 ± 4% (n = 11) for ^{123}I and 40 ± 4% (n = 3) for ^{125}I].

The purpose of these experiments was to characterize the volatile radioactive product and to examine the effect of reaction temperature on its formation. The volatile radioactive product was identified as [^{125}I] or [^{123}I]iodomethane by its trapping characteristics and by its retention time in two different HPLC systems. Changing the reaction temperature (22-124°C) did not have a significant effect in the amount of labeled iodomethane produced (n = 13).

The conditions chosen for the radioiodination and deprotection were: 50 μg precursor in MeOH, dry Na^{123}I, 50 μl 0.02 M chloramine-T in glacial HOAc/MeOH, heating at 90°C for 15 min, followed by deprotection with CF_3CO_2H for 5-10 min at the same temperature. Purification was achieved by solvent extraction and HPLC.

Radiochemical yields for preparations carried out at temperatures between 86°C and 124°C were 24 ± 5% (n = 11) for [^{123}I]- and 45 ± 13% (n = 3) for 5-[^{125}I]iodo-6-nitroquipazine; radiochemical purity averaged 95 ± 4% and 99 ± 0% respectively.

Key Words: [^{123}I]iodomethane, 5-[^{123}I]iodo-6-nitroquipazine, serotonin transporter radiotracer, SPECT, radioiododestannylation

INTRODUCTION

Several radiotracers have been developed for PET and SPECT studies of the serotonin (5-HT) transporter (1-5). Among these, 5-[^{123}I]iodo-6-nitroquipazine (3, figure 1) has proved to be a

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promising SPECT imaging agent. In vitro, 5-[^{125}I]iodo-6-nitroquipazine has been shown to possess high affinity and selectivity for the 5-HT transporter (5, 6). SPECT brain imaging with 5-[^{123}I]iodo-6-nitroquipazine in nonhuman primates has shown high concentrations of activity in midbrain regions (7), consistent with the presence in such regions of high densities of 5-HT transporters.

Radioiodinated 5-iodo-6-nitroquipazine was first prepared from the N-t-BOC-protected tributylstannyl precursor by Mathis et al (5). Recently, this group reported a radioiodination procedure using the same precursor and dichloramine -T /acetonitrile/2M H_3PO_4 at room temperature, followed by removal of the t-BOC protecting group with 2M H_2SO_4 at 60 °C and purification by solid phase extraction and HPLC (8). The reported overall yields were 40 ± 7% (n = 7) for 5-[^{123}I]iodo-6-nitroquipazine and 63 ± 12% (n = 12) for 5-[^{125}I]iodo-6-nitroquipazine, with radiochemical purity of >98 and >99% respectively. No other major radioiodinated product was indicated.

In this paper we report a radioiodination procedure for 5-iodo-6-nitroquipazine from its N-t-BOC-protected-5-trimethylstannyl precursor (1) using chloramine-T /CH_3OH/CH_3CO_2H followed by deprotection with CF_3CO_2H (figure 1), and the detection and identification of a major radioiodinated volatile reaction product which is consistently formed during the radioiodination reaction: [^{125}I] or [^{123}I]iodomethane.

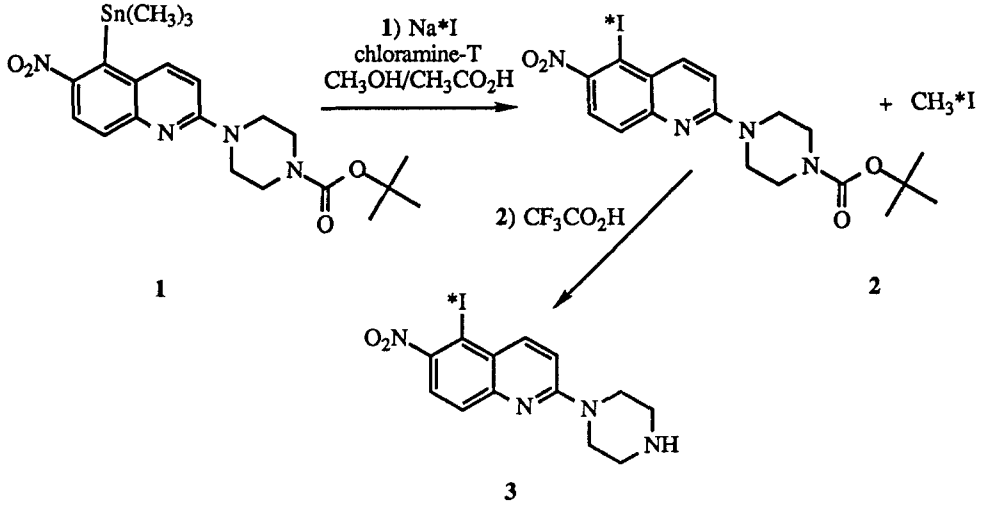

Figure 1. Synthesis of 5-[*I]iodo-6-nitroquipazine by iododestannylation

RESULTS AND DISCUSSION

Nature of Reaction Products. Radiolabeled 5-iodo-6-nitroquipazine (3) was synthesized in two steps from a 5-trimethylstannyl precursor in which the secondary amine was protected as the t-BOC amide (1). In the first step, radioiodine was introduced by iododestannylation with radioactive NaI and chloramine-T. In the second step, the t-BOC group was removed by heating

with trifluoroacetic acid. After the iododestannylation step, the oxidizing agent was reduced with $NaHSO_3$ and the atmosphere was purged into charcoal. Large amounts of volatile radioactivity were consistently released (37 ± 4%, n = 11, for ^{123}I and 40 ± 4%, n = 3, for ^{125}I). After removal of the radioactive volatile product, HPLC radioanalysis (system A, solvents ratio: 85:15:0.2, flow 1 mL/min) of the crude product of the reaction between sodium [^{125}I]iodide and N-t-BOC-5-(trimethylstannyl)-6-nitroquipazine (1) at 22°C, in the presence of chloramine-T, showed: N-t-BOC-5-([^{125}I]iodo)-6-nitroquipazine (2, 78%, retention volume v_R 6.1 mL), possible unreacted [^{125}I]iodide (18%, v_R 2.7 mL), and a secondary byproduct (4%, v_R 4.1 mL). HPLC radioanalysis of the mixture after amine deprotection with trifluoroacetic acid showed: 5-([^{125}I]iodo)-6-nitroquipazine (3, 83%, v_R 5.1 mL), possible unreacted [^{125}I]iodide (12%, v_R 2.6 mL) and a secondary byproduct (4.8%, v_R 4 mL). Under the same HPLC conditions, cold 5-iodo- 6-nitroquipazine had v_R of 5.1 mL. For ^{123}I radioiodinations, the intermediate mixture was not analyzed. HPLC analysis of the mixture (system A) after removal of the t-BOC protecting group yielded 79.9 ± 4.7% (n = 9) of 5-[^{123}I]iodo-6-nitroquipazine (3). These chromatographic results do not reflect the true labeling yield because almost 40% of the starting radioactivity, the released volatile product, is not taken into account.

Radiochemical yield and radiochemical purity. Radioiododestannylation in the presence of chloramine-T, heating the reaction mixture for 15 min at temperatures ranging between 86°C and 124°C, gave reproducible results in 11 preparations on a scale of 26-75 mCi of Na^{123}I. Isolated radiochemical yields were 24 ± 5% for 5-[^{123}I]iodo-6-nitroquipazine with radiochemical purity averaging 95 ± 4% at end of preparation and 92 ± 6 % after 21 ± 3 hours at room temperature. For 5-[^{125}I]iodo-6-nitroquipazine, radiochemical yields were 45 ± 13 (n = 3) and radiochemical purity was 99 ± 0% at both end of preparation and after five days (at 4°C). Of the eleven ^{123}I reactions, those carried out at 90 - 93°C (n = 6) gave radiochemical yield 23 ± 3% and radiochemical purity 94 ± 4%.

The relatively low radiochemical yields obtained with the chloramine-T method reported here are a direct result of the large and consistent generation of [^{123}I]iodomethane during the iododestannylation step; nevertheless this method provided enough 5-[^{123}I]iodo-6-nitroquipazine in a reproducible manner to carry out SPECT studies in monkeys (12 to 17 mCi). 5-[^{123}I]Iodo-6-nitroquipazine ready for injection was obtained in 3 hours total preparation time.

The observed difference in radiochemical yield and radiochemical purity between ^{123}I and ^{125}I radioiodinations is probably due to the different production method of these two radionuclides. It has been suggested for example (9), that trace impurities left over from the target that can inhibit ("poison") labeling reactions, may be insignificant in the case of ^{125}I but become extremely troublesome for ^{123}I labeling.

Nature of the volatile Product. When a series of traps was used, the radioactive volatile material was trapped in MeOH and charcoal but not in aqueous NaOH; a variable amount of radioactivity also adhered to the walls of the two pieces of connecting rubber tubing. The product trapped in methanol was identified by two HPLC systems (B and C) as radioactive iodomethane (Table 1).

Table 1

HPLC of volatile radioiodinated product compared to iodomethane standard

Species	HPLC Retention Volume (mL)	
	System B CH_3CN/NH_4 phosphate	System C $MeOH/H_2O/Et_3N$
Volatile radioiodinated product	5.6	5.0
Standard CH_3I	5.6	5.0
5-[*I]iodo-6-nitroquipazine	13.6	9.9
cold 5-iodo-6-nitroquipazine	13.4	9.9

The characteristics of the radioactive volatile product are similar to those previously reported for 1-[^{123}I]iodobutane (10). It was trapped in organic solvent but not in aqueous NaOH and its HPLC retention time corresponded to that of iodomethane in two different systems, suggesting that it is most likely labeled iodomethane and not an inorganic species such as HOI, I_2, H_2OI^+, HI, ICl or a hydrophilic byproduct.

Effect of reaction temperature in the production of [^{123}I]iodomethane. No significant effect was observed in the production of [^{123}I]iodomethane when the reaction temperature was increased from 22°C to 124°C (Table 2). For the chloramine-T method described here, 90°C was chosen as the reaction temperature to assure rapid radioiodination and t-BOC deprotection, which requires heating, with lower risk of product decomposition at higher temperatures.

Table 2

Effect of reaction temperature

Temperature °C	[^{123}I]CH_3I %	5-[^{123}I]iodo-6-nitroquipazine %	n
22	34-35	24-25	2
86-98	38 ± 3	24 ± 3	8
121-124	34 ± 2	28 ± 2	3

Reaction mechanism. The generation of radioiodinated iodomethane during the reaction between radioactive sodium iodide and 2-(4-t-BOC-piperazin-1-yl)-5-(trimethylstannyl)-6-nitroquinoline (1) is in agreement with our previous observation (10) that when electron-withdrawing groups *ortho* or *para* to the tin atom are present in the aromatic ring, aliphatic substitution becomes a significant competitive reaction pathway. In the case of 2-(4-t-BOC-piperazin-1-yl)-5-(trimethylstannyl)-6-nitroquinoline, the strong electron-withdrawing nitro group *ortho* to the tin atom probably makes the reaction more sensitive to aliphatic substitution than observed for iomazenil, where a

cyclic amide is present in the *ortho* position. In the same study (10), no volatile radioiodinated by-product was detected when the reaction was carried out under the same experimental conditions than those used for the radioiodination of iomazenil, but in absence of the corresponding tributylstannyl precursor.

It is possible that the bulkiness of the alkyl group on the stannyl precursor, or the solvent polarity of the reaction mixture, may have an effect on the amount of radioactive alkyl iodide produced during the iododestannylation. We intend to pursue the study of these and other aspects of the radioiodination of 5-iodo-6-nitroquipazine.

EXPERIMENTAL

Materials and Methods. The t-BOC protected stannyl precursor of 2-(N-piperazin-1-yl)-5-iodo-6-nitroquinoline, [2-(4-t-butoxycarbonylpiperazin-1-yl)-5-(trimethylstannyl)-6-nitroquinoline, **1**] was obtained from Research Biochemicals International (RBI, Natick, MA.) through the NIMH Chemical Synthesis Program; nonradioactive 5-iodo-6-nitroquipazine was obtained from University of Pittsburgh (Pittsburgh, PA). Iodination "kits" for the precursor were prepared by dispensing 50 μL (50 μg) each of a 1 mg/mL chloroform solution of the trimethylstannyl precursor into 1 mL amber blowback reaction vials (The West Co., Lititz, PA), evaporating the solvent to dryness with a stream of N_2, and capping with 13 mm Teflon-lined stoppers. Vials were stored at 4°C protected from light. Dried, no-carrier-added sodium [^{123}I]iodide (nominal radionuclidic purity > 99.8%) was purchased from Nordion International, Ltd (Vancouver, B.C., Canada). Sodium [^{125}I]iodide was obtained from NEN/Dupont (N. Billerica, MA; specific activity 2,200 Ci/mmol).

Trifluoroacetic acid (HPLC spectrograde) was obtained from Pierce Chemical Co. (Rockford, IL). All other chemicals were reagent grade obtained commercially and were used without further purification.

Preparative and analytical high pressure liquid chromatography (HPLC) was carried out with an SP 8800 pump, SP Chrom Jet integrator and SP 200 uv detector (Spectra Physics, San Jose, CA) and a Canberra series 20 multichannel gamma detector (Canberra Industries; Meridien, CT) connected to a NaI scintillation detector. Retention volumes measured on the gamma detector were corrected for the volume of tubing (0.7 mL) between the uv and gamma detector. In this system, the count rate output of the scintillation detector was taken through a ratemeter to an analog-to-digital converter interfaced to a microcomputer for peak detection and integration (Justice Innovations, Palo Alto, CA). HPLC conditions at 254 nm were:

System A: Waters Novapak C_{18} (3.9 x 300 mm) stainless steel column (Waters Associates, Milford, MA) $CH_3OH/H_2O/Et_3N$ (75/25/0.1), 0.7 mL/min.

System B: PRP-1 C_{18}, (4.1 x 250 mm) stainless steel column (Hamilton Co., Reno, NV) CH_3CN/NH_4 phosphate buffer pH 7.0 (82/18), 1 mL/min.

System C: Waters RCM-C_{18}, Radial-Pak™ cartridge (8 x 100 mm), $CH_3OH/H_2O/Et_3N$ (80/20/0.2), 0.5 mL/min.

Radioactivity in charcoal filters, collection tubes and vials was measured in a dose calibrator (Capintec CRC-7, Montvale, NJ) calibrated for each type of vessel used.

5-[^{123}I]iodo-6-nitroquipazine. Methanol (100 μL) was added to a 1 mL amber reaction vial containing 50 μg (0.13 μmol) trimethylstannyl precursor and dissolved by sonication for 2 min. Using 0.5 mL insulin syringes (B-D, Lo-dose, U-100 28G1/2), the precursor solution was added to the dry [^{123}I]NaI shipping vial through the septum, followed by 50 μL of 0.02 M *N*-chloro-*p*-toluenesulfonamide sodium salt trihydrate (chloramine-T hydrate) in glacial acetic acid/methanol (1/1), mixed with an extra volume of glacial acetic acid equal to 0.21 times the volume of 0.1 M NaOH from which the dry [^{123}I]NaI was obtained (indicated by the vendor on the shipping form).

The sealed vial was heated in a block heater at 90-92°C for 15 min, the vial was removed, allowed to cool for 5 min, and then 50 μL (1 mg) aqueous $NaHSO_3$ was added through the septum with an insulin syringe. Volatile radioactivity was removed from the vial by purging with nitrogen gas into a charcoal column (a 10 mL disposable syringe, filled with charcoal and connected to the vial septum through a Vacutainer ™ blood collection set) for 5-10 min; the charcoal column, blood collection set, and reaction vial were assayed in the dose calibrator.

To the unopened reaction vial, using a Hamilton microsyringe/needle, 100 μL CF_3CO_2H was added and the vial was heated at 90-92°C for 5-10 min; after cooling, the vial was opened and the reaction mixture was transferred with a Pasteur pipet to a test tube; H_2O was added (1 mL) followed by 50 μL portions of 10 M NaOH until a yellow color remained permanently (200-300 μL). The mixture was extracted with 3 x 1 mL of ethyl acetate, the organic layer was separated and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure on a rotary evaporator. The residue was dissolved in MeOH/mobile phase (1:1) and was purified by HPLC with system A. The fraction corresponding to ^{123}I product was collected in a 50 mL round bottom flask containing 50 μL (1 mg) of L-ascorbic acid solution and evaporated to dryness under reduced pressure. The residue was dissolved in 400 μL of EtOH followed by 8 mL of 0.9 % NaCl and filtered through a 0.22 μ membrane filter into a sterile 10 mL serum vial. An aliquot of the final product was analyzed by HPLC to determine its radiochemical purity. The stability of the solution was also measured by HPLC after storage at room temperature for 17–25 hours after preparation. Radiochemical yield was calculated by dividing the radioactivity in the final purified product, corrected for decay, by the amount of starting radioactive sodium iodide.

Effect of temperature. To determine if the amount of volatile radioactive product could be reduced by increasing reaction temperature, the reaction was carried out at 22°C, 86°C, 90-93°C, 101-102°C and 121-124°C.

Characterization of the radioactive volatile product. As previously described for the identification of 1-[^{123}I]iodobutane (10), the atmosphere from the reaction mixture was passed into sequential traps containing 0.1 M NaOH, MeOH, and charcoal and assayed in the dose calibrator. The apparatus consisted of two Vacutainer test tubes connected to each other and to the reaction vial through two pieces of latex rubber tubing (4 mm x 20 cm). Each piece of tubing was equipped with a 20 G needle in one end and a spinal needle in the other. The 5 ml charcoal filled syringe and the nitrogen gas cylinder were connected respectively to the MeOH trap and to the reaction vial through Vacutainer blood collection sets.

Methanol was chosen as the organic trapping solvent because it was appropriate for injecting into the HPLC system. The methanolic solution was analyzed by HPLC with system B and C and compared with standards of iodomethane.

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REFERENCES

1. Kung M.P., Chumpradit S., Panyachotipun C., Prapansiri V., Billings J., and Kung H.F.- J. Nucl. Med. 32: 964 (1991)
2. Chumpradit S., Kung M.P., Panyachotipun C., Prapansiri V., Foulon C., Brooks B.P., Szabo S.A., Tegani-Butt S., Frazer A., and Kung H.F.- J. Med. Chem. 35: 4492 (1992)
3. Suehiro M., Scheffel U., Dannals R.F., Hayden T.R., Ricaurte G.A., and Wagner H.N.- J. Nucl. Med. 34: 120 (1993)
4. Biegon A., Mathis C.A., Hanrahan S.M. and Jagust W.J.- Brain Res. 619: 236 (1993)
5. Mathis C.A., Biegon A., Taylor S.E., Enas J.D., and Hanrahan S.M.- Eur. J. Pharmacol. 210: 103 (1992)
6. Mathis C.A., Taylor S.E., Biegon A. and Enas J.D. - Brain Res. 619: 229 (1993)
7. Jagust W., Eberling J.L., Roberts J.A., Brennan K.M., Hanrahan S.M., Van Brocklin H., Enas J.D., Biegon A. and Mathis C.A.- Eur. J. Pharmacol. 242:189 (1993)
8. Mathis C.A., Enas J.D., Hanrahan S.M. and Akgun E. - J. Lab. Comp. Rad. (1994) *In press*
9. Baldwin R.M.- Appl. Radiat. Isot. 37:817 (1986)
10. Zea-Ponce Y., Baldwin R.M., Zoghbi S.S. and Innis R.B. -Appl. Radiat. Isot. 45:63 (1994)