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Synthesis of the ^{123}I- and ^{125}I-labeled Cholinergic Nerve Marker (−)-5-Iodobenzovesamicol*

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The highly toxic curaremimetic and cholinergic neuron marker (−)-5-iodobenzovesamicol (IBVM) has been labeled with iodine-125 and iodine-123. [^{125}I]IBVM, suitable for animal distribution and *ex vivo* autoradiographic studies, was synthesized by solid-state exchange; isolated yields were 65–89% with specific activities in the range of 130–200 Ci/mmol. The synthesis of no-carrier-added (−)-5-[^{125}I]IBVM from the corresponding chiral (−)-5-(tri-*n*-butyltin) derivative using Na^{125}I was evaluated using the oxidants H_2O_2, peracetic acid and chloramine-T. Both peracetic acid and chloramine-T gave good yields (70–95%). However, when Na^{123}I was utilized, acceptable yields of [^{123}I]IBVM were obtained only with chloramine-T. Use of the latter oxidant did produce 5-chlorobenzovesamicol which was eliminated during HPLC purification. After optimization of the reaction parameters, [^{123}I]IBVM in batch sizes of 10–27 mCi, is routinely obtained with a specific activity of 30–70,000 Ci/mmol, radiochemical purity (>97%) and chiral purity (>98%). Isolated radiochemical yields have averaged 71% ($N = 40$). Distribution analyses of [^{125}I]IBVM and [^{123}I]IBVM in mice 4 h following intravenous administration show essentially equivalent concentrations of the two tracers in the four brain regions sampled. The exceptionally high specific activity of [^{123}I]IBVM has made possible the evaluation of this radiotracer in humans.

Introduction

We have demonstrated that 5-[^{125}I]iodobenzovesamicol (IBVM, **2**) is a highly specific *in vivo* marker for cholinergic neurons of the brain (Jung *et al.*, 1990, 1993). The promising results obtained in these studies has prompted the clinical evaluation of [^{123}I]IBVM as a SPECT agent for the non-invasive mapping of cholinergic nerve loss in dementing disorders such as Alzheimer's disease and Parkinson's disease. Vesamicol (**1**), and likely the benzovesamicols as well (see Fig. 1 for structures), are thought to produce their curare-like action by non-competitive inhibition of the acetylcholine transporter located on the intraneuronal storage vesicle (Rogers and Parsons, 1990). IBVM, like the parent compound vesamicol, is a potent neuromuscular blocking agent, having a LD_{50} of 218 μg/kg, i.v., in rats and 120–180 μg/kg, i.v., in rabbits (Kostyniak *et al.*, 1991). IBVM, however, is slightly less toxic than *d*-tubocurarine which has a LD_{50} in mice of 130 μg/kg, i.v. (Sweet, 1987), and in rabbits of 20 μg/kg, i.v. (von Szendey and Munnes, 1965). To avoid pharmacological effects following i.v. injection of an imaging dose of IBVM to a patient, the development of a no-carrier-added synthesis of [^{123}I]IBVM became essential. The radiolabeling method was first developed and optimized using iodine-125 and then subsequently modified for the synthesis of [^{123}I]IBVM suitable for human SPECT studies of brain cholinergic neurons (Kuhl *et al.*, 1993).

Materials and Methods

Melting points were obtained using a Thomas–Hoover melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin–Elmer 727B spectrometer. ^{1}H-NMR spectra were obtained

*Part of this study has been reported in abstract form: Gildersleeve D. L., Jung Y.-W., Van Dort M. E., Hagen C. A., Kuhl D. E., Wieland D. M. (1992) Purification and quality control procedures for the production of (−)-5-[^{123}I]Iodobenzovesamicol: a presynaptic SPECT imaging agent for brain cholinergic neurons. In *Abstracts of the Third International Symposium on Radiohalogens*, 20–23 September 1992, Banff, Alberta, Canada.

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on a Bruker WM 300 MHz spectrometer with tetramethylsilane (TMS) as internal standard. Mass spectra were obtained on a Finnigan 4021 GCMS/DS (low resolution) or a UG70-250-S (high resolution) instrument.

Racemic, (+)-, and (−)-5-iodobenzovesamicol **2** were synthesized as previously reported (Jung *et al.*, 1990). Benzovesamicol **6**, used as a HPLC standard, was synthesized by the method of Rogers *et al.* (1989). Na[^{125}I]iodide and Na[^{123}I]iodide were obtained from Nordion Ltd, Ontario, Canada as no-carrier-added solutions in 0.1 M NaOH (pH = 10–12). Hydrogen peroxide (30% by weight in H_2O), peracetic acid (32% by weight in dilute acetic acid), chloramine-T hydrate (98%), sodium metabisulfite and all other chemicals were of reagent grade and purchased from Aldrich Chemical Company, Milwaukee, Wis. Hydrogen peroxide and peracetic acid were diluted to 3 and 0.32%, respectively, with deionized water prior to use.

Concentrated hydrochloric acid and concentrated sulfuric acid were purchased from J. T. Baker Inc., Phillipsburg, N.J. and Mallinckrodt Specialty Chemicals Company, Paris, Ky, respectively. Absolute ethanol was purchased from Midwest Grain Products of Illinois, Pekin, Ill. Ethyl acetate and other solvents were of HPLC grade and purchased from J. T. Baker Inc. Flash chromatography was performed by the method of Still *et al.* (1978). Thin-layer chromatography of unlabeled compounds was performed on Analtech silica gel GF glass-backed plates (10 cm, 250 μm). Thin-layer chromatography of the radioactive products was performed on Whatman K6F silica gel glass-backed plates (20 cm, 250 μm) using $CHCl_3$:EtOH:NH_4OH (97:3:0.1) as solvent. The R_f of 5-IBVM was 0.68; Na[^{125}I]iodide remained at the origin in this TLC system. TLC chromatograms were scanned for radioactivity using a Berthold Model LB 2832 TLC-linear analyzer equipped with a Model LB 500 data acquisition system. Radioactivity was assayed with a Capintec radioisotope calibrator model CRC-12R.

Purification of (−)-5-[^{123}I]IBVM was performed with a Beckman Model 110B pump equipped with a Rheodyne Model 7010 injection valve and a Phenomenex Ultracarb ODS (30) column (2 × 30 mm; 5 μm particle). Ultraviolet absorption was monitored with an Applied Biosystems 757 u.v./vis. detector at 210 nm and radioactivity was monitored with a Beckman 170 radioactivity detector. Output from the detectors was directed to two separate Spectra-Physics SP 4400 Integrators for data analysis. The HPLC elution solvent was 0.1 M NH_4OAc:95% EtOH (1:1) at a flow rate of 0.5 mL/min. Conditions for radio-HPLC analysis of the final product were similar to the above except that a longer Ultracarb ODS (30) column (2 × 100 mm; 5 μm particle) was used with 0.1 M NH_4OAc:CH_3CN:95% EtOH (30:45:25) as eluant at a flow rate of 0.3 mL/min. Analysis of the optical purity of the labeled product was performed using a Chiracel OD column (4.6 × 250 mm; 10 μm particle, Daicel Chemical Industries Ltd) with hexane:isopropanol:diethylamine (90:10:0.1) as eluant at a flow rate of 1 mL/min. Ultraviolet absorption was monitored at 254 nm. Under these conditions, the retention times for (−) and (+)5-IBVM were 6.8 and 9.9 min, respectively.

Specific activity determination of (−)-5-[^{123}I]IBVM

An ethanolic stock solution of (−)-5-IBVM at a concentration of 1 ng/μL was prepared by serial dilution. Aliquots of this stock solution (75–200 μL) were diluted with a mixture of 100 μL of 0.1 N NaOH, 50 μL of 0.2 N ethanolic H_2SO_4, and 50 μL of 0.2 N NH_4OH, respectively (to simulate actual conditions present in the reaction mixture), and subjected to HPLC analysis. The u.v. absorbance peak area corresponding to 5-IBVM was determined by comparison with a standard curve, relating injected mass of 5-IBVM to the u.v. absorbance peak area. The fraction containing HPLC purified (−)-5-[^{123}I]IBVM was assayed for radioactivity in a Capintec dose calibrator and the mass of IBVM present determined from the standard curve using its u.v. absorbance peak area in the preparative run. The specific activity (at time of purification) was then calculated as follows:

$$\text{specific activity (Ci/mmol)} = \frac{(\text{activity in curies}) \times 433.34}{(\text{mass of IBVM in milligrams})}$$

No 5-chlorobenzovesamicol **5** could be detected by HPLC analysis (λ = 210 nm) in the HPLC-purified

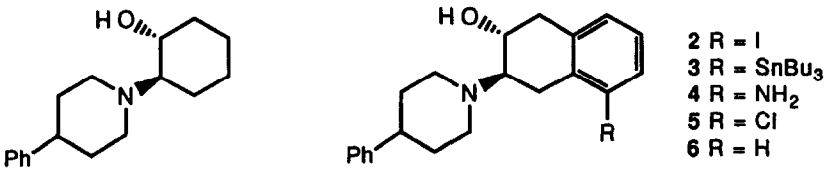

Fig. 1. Chemical structures of vesamicol and benzovesamicols.

batches of (−)-5-[^{123}I]IBVM. Compound **5** elutes 7–8 min prior to elution of the tracer. Taking into account the limit of sensitivity for detection for **5** in this HPLC system, a typical 20 mCi batch of (−)-5-[^{123}I]IBVM, after HPLC purification, would contain no more than 35 ng of compound **5**, a value that is approx. 8-fold lower than the usual mass of IBVM present.

(−)-5-(Tri-n-butyltin)benzovesamicol (3)

To a solution of (−)-5-IBVM **2** (151 mg, 34.8 μmol) and hexabutylditin (808 mg, 1.39 mmol) in dry toluene (5 mL) was added tetrakis-(triphenylphosphine)palladium(0)(80 mg, 70.0 μmol). The mixture was degassed by bubbling argon through the reaction mixture for 5 min and then refluxed for 20 h under an argon atmosphere. As the reaction proceeded, the reaction mixture changed in color from yellow to black. The black precipitate was filtered and the filtrate was concentrated under reduced pressure. The residue was flash chromatographed on silica with EtOAc:hexanes (15:85) to afford the title compound (137 mg, 66%). $[\alpha]^{23}D = -24.4°$ ($c = 11.5$, $CHCl_3$). IR (KBr): 3640–3210 (br), 3020, 2950, 2920, 2862, 2840, 1600, 1490, 1450, 1440, 1373, 1070 cm^{-1}; ^{1}H–NMR ($CDCl_3$): δ 0.91 (t, $J = 7.3$ Hz, 9H), 1.09 (t, $J = 8.3$ Hz, 6H), 1.36 (pentet, $J = 7.3$ Hz, 6H), 1.50–1.60 (m, 6H), 1.62–1.96 (m, 4H), 2.33 (t, $J = 10.6$ Hz, 1H), 2.51–2.60 (m, 1H), 2.77–3.02 (m, 7H), 3.31 (d, d, $J = 16.0$, 5.8 Hz, 1H), 3.89 (m, 1H), 4.3 (br s, OH), 7.06–7.35 (m, 8H); MS (EI, 70 eV) *m/z* (relative intensity) 597 (8.8, M^+), 540 (50.0), 379 (31.5), 361 (56.2), 307 (100), 277 (45.2), 247 (23.0), 174 (98.7), 160 (28.5), 146 (23.9), 129 (38.7), 115 (37.5), 91 (39.7); high-resolution MS (EI, 70 eV). Calcd for $C_{33}H_{51}NO^{120}Sn$: 597.2992. Found: 597.2980.

(±)-5-Chlorobenzovesamicol (5)

This compound was synthesized for use as a standard in determining the purity and effective specific activity of (−)-5-[^{123}I]IBVM. A solution of $NaNO_2$ (23 mg, 0.33 mmol) in water (2 mL) was added dropwise to a cooled (5°C) solution of (±)-5-aminobenzovesamicol (100 mg, 0.31 mmol) in a mixture of acetic acid (2 mL) and concentrated HCl (2 mL). The reaction was stirred and maintained at a temperature below 10°C for 30 min and then added in portions to a stirred, cooled (5°C) solution of Cu_2Cl_2 (67.5 mg, 0.68 mmol) in concentrated HCl (2 mL). The mixture was allowed to warm to ambient temperature, stirred for 30 min, and then heated at 80°C for an additional 30 min. The solution was basified with aqueous 2 N NaOH, extracted three times with $CHCl_3$ and the combined organic layers dried over Na_2SO_4. The crude product was flash chromatographed on silica with hexane:EtOAc (7:3) to give 95 mg (90%) of the title compound. ^{1}H-NMR ($CDCl_3$) δ 1.69–1.96 (m, 4H), 2.48 (t, d, $J = 11.5$, 2.2 Hz, 1H), 2.54–2.99 (m, 7H), 3.14 (d, d, $J = 16.1$, 4.5 Hz, 1H), 3.30 (d, d, $J = 16.1$, 5.6 Hz, 1H), 3.85 (t, d, $J = 10.2$, 5.6 Hz, 1H), 4.35 (br.s, OH), 7.01–7.12 (m, 2H), 7.18–7.35 (m, 6H) ppm. Anal. calcd for $C_{21}H_{24}ClNO$: C, 73.78; H, 7.08; N, 4.10. Found: C, 73.88 H, 6.97, N, 4.19

Synthesis of (+), (−) and racemic 5-[^{125}I]iodobenzovesamicol by solid-state isotopic exchange

In a typical procedure, a solution of $(NH_4)_2SO_4$ (5.0 mg in 15 μL of deionized H_2O), the respective stereoisomer or racemic **2** (20 μg, 46 nmol, in 20 μL of EtOH), Na^{125}I (7.8 mCi in 16 μL of 0.1 N NaOH) and three layers of 5 mm borosilicate glass beads were combined in a 3 mL glass multi-dose vial. The inside of the reaction vial was rinsed with acetone (2 × 50 μL) followed by absolute EtOH (2 × 50 μL) using the same syringe that was used for the dispensing of the Na^{125}I. The vial was sealed with a Teflon-coated rubber septum and an aluminum cap; a 5 cm^3 disposable syringe fitted with a bent 18 gauge syringe needle was inserted through the rubber septum to serve as a condenser for the distillate. A disposable charcoal trap (5 cm^3 syringe) was attached to the top of the condenser via a rubber septum, which was in turn connected to a thiosulfate trap. The vial was heated in an oil bath to 140°C and air was then slowly pushed (3 × 10 cm^3 over a 3 min period) through the reaction vial using a 10 cm^3 syringe fitted with a butterfly needle assembly. Heating was continued at 140–150°C for 25 min and the vial was then allowed to cool to room temperature. Radio-TLC analysis of the crude product (silica; $CHCl_3$:EtOH:NH_4OH; 97:3:0.1) showed 76% radiochemical purity. Purification was achieved using a solvent-activated silica gel Sep-Pak cartridge. The crude product was transferred in CH_2Cl_2 (3 × 1 mL) to the Sep-Pak and eluted using slight suction. The Sep-Pak cartridge was then eluted sequentially with hexane (3 × 2 mL), EtOAc:hexane (1:9) (4 × 2 mL), and EtOAc:hexane (1:1) (4 × 2 mL). A yield of 5.12 mCi (66%) of 5[^{125}I]IBVM eluted in the last solvent system; radiochemical purity as shown by radio-TLC analysis was 99% and the specific activity was 133 Ci/mmol. The solvent was removed under an argon stream; the product was redissolved in absolute EtOH to a concentration of 1 mCi/mL and stored at 4°C until further use. Under these conditions, the labeled product showed less than 5% radiolytic decomposition in 7 days by radio-TLC analysis. The radiotracer was formulated in 0.005 M NaOAc buffer (pH = 4.2):EtOH (95:5) for animal evaluation.

General procedure for labeling with H_2O_2

In a typical procedure, 50 μg (84 nmol) of racemic **3** in EtOH (50–150 μL) in a Sarstedt polypropylene V-vial was treated with either aqueous HCl (0.1–0.3 N) or ethanolic HCl (0.5 N) followed by the appropriate amount of Na^{125}I in 0.1 N NaOH (see Table 1). The reaction was initiated with 50 μL of

Table 1. Hydrogen peroxide catalyzed labeling of IBVM with Na[^{125}I]iodide

Entry	Volume of EtOH (μL)*	Acid/solvent/vol (μL)	mCi $Na^{125}I$/ vol (μL)	Radiochemical yield (purity)
1	50	0.1 N HCl/H_2O/50	2.4/3	92% (98%)
2	100	0.1 N HCl/H_2O/50	1.4/31	82% (98%)
3	100	0.3 N HCl/H_2O/50	1.7/102	67% (96%)
4	150	0.5 N HCl/EtOH/25	2.4/102	46% (94%)

*50 μg (84 nmol) of (±)-5-(tri-*n*-butyltin)benzovesamicol in 50 μL of EtOH was used for each reaction.

aqueous H_2O_2 (3% w/v). The vial was capped and shaken occasionally for 15 min at ambient temperature. The reaction mixture was quenched by the addition of aqueous sodium metabisulfite (100 μL, 1 mg/mL) followed by the addition of saturated aqueous $NaHCO_3$ (100 μL). After extraction with EtOAc (3 × 1 mL), the combined organic layers were dried over anhydrous Na_2SO_4; the radiochemical purity of the solution was determined by radio-TLC on silica. The radiochemical yield of [^{125}I]IBVM was determined by dividing the radioactive counts assigned to [^{125}I]IBVM in the radiochromatogram of the EtOAc layer by the total counts present in the organic and aqueous layers.

General procedure for labeling with peracetic acid

Racemic **3** (40 μg, 67 nmol) in EtOH (40 μL) in a Sarstedt polypropylene V-vial was treated with ethanolic 0.2 N H_2SO_4 (50 μL) followed by 0.20–2.90 mCi of $Na^{125}I$ (Table 2) or 5.66–32.2 mCi of $Na^{123}I$ (Table 3) in 0.1 N NaOH. The pH of the reaction was determined (pH = 4.5) and the reaction initiated with 12 μL of peracetic acid (0.32% w/w). The effect of temperature and reaction time on the product yield and composition was studied. Workup and radio-TLC analysis were performed as described above for the radiosynthesis with H_2O_2.

General procedure for labeling with chloramine-T

To a Sarstedt polypropylene V-vial was added in the following order: racemic **3** (30 μg, 51 nmol), ethanolic 0.2 N H_2SO_4 (50 μL) and the appropriate amount of $Na^{125}I$ in 0.1 N NaOH (Table 4). The pH of the reaction mixture, which was generally between 4 and 5, was monitored and the reaction was initiated by adding 5 μL of freshly prepared aqueous chloramine-T solution (5 μg/μL). The vial was capped and shaken occasionally for 2 min at ambient temperature. Workup and radio-TLC analysis were performed as described for the radiosynthesis using H_2O_2.

Synthesis of (−)-5-[^{123}I]iodobenzovesamicol for clinical studies

To the shipping vial containing 20–30 mCi of $Na^{123}I$ in 65–135 μL of 0.1 N NaOH was added 100–150 μL of 0.2 N ethanolic H_2SO_4 followed by (−)-5-(tri-*n*-butyltin)benzovesamicol **3**, (30 μg, 51 nmol) in 30 μL of EtOH. The pH of the reaction mixture was monitored (pH = 4–5) and the reaction was initiated by the addition of 5 μL of freshly prepared aqueous chloramine-T (5 μg/μL), followed by vigorous shaking for a few seconds. The reaction was allowed to proceed for 2 min and then quenched by the addition of 50 μL of 0.2 N NH_4OH. The contents of the reaction were remotely loaded onto a 500 μL injection loop for HPLC purification. Both u.v. and radioactivity traces were monitored during the collection of the labeled product which eluted at 17 min. Under these conditions, IBVM is well resolved from the 5-(tri-*n*-butyltin) precursor **3**, (retention time > 60 min) as well as other potential side products such as 5-chlorobenzovesamicol **5** (retention time 9.2 min) and benzovesamicol **6** (retention time 2.4 min). The eluant was filtered via a sterile 0.2 μm alumina filter (Anotop 10, Whatman) connected directly to the HPLC outlet line. Typically, the (−)-5-[^{123}I]IBVM elutes in a 4.0 mL volume with an ethanol content of 47.5%; this eluant fraction is then diluted to 19 mL with 0.9% normal saline to afford a directly injectable solution containing 10% or less ethanol. The radiochemical purity of the formulated product is routinely monitored by TLC analysis. Between syntheses the HPLC column is washed with 95% ethanol to remove any residual (−)-5-(tri-*n*-butyltin)benzovesamicol precursor. The average radiochemical and chemical purity was determined to be greater than 97%; the average specific concentration of the formulated product was 1.12 mCi/mL.

Table 2. Peracetic acid catalyzed labeling of IBVM with Na[^{125}I]iodide

Entry*†‡	mCi $Na^{125}I$/vol (μL)	Temperature (°C)	Time (min)	Radiochemical yield (purity)
1	2.95/83	23	5	95% (98%)
2	0.20/78	23	10	70% (78%)
3	0.20/78	40	10	86% (91%)

*A constant mass of 40 μg (67 nmol) of (±)-5-(tri-*n*-butyltin)benzovesamicol in 40 μL of EtOH was used for each reaction.
†Reactions were acidified with 50 μL of 0.2 N H_2SO_4 in absolute EtOH.
‡Reaction was initiated with 12 μL of aqueous peracetic acid (0.32% w/w).

Table 3. Peracetic acid catalyzed labeling of IBVM with Na[^{123}I]iodide

Entry*‡	0.2 N Ethanolic H_2SO_4 (μL)	mCi Na^{123}I/vol (μL)	Temperature (°C)	Time (min)	Radiochemical yield (purity)
1	80	32.2/90	23	10	38%§
2	18	5.81/15	23	10	77% (95%)
3	18	5.71/15	23	20†	81% (91%)
4	18	5.66/15	60	10	88% (97%)
5	55	9.87/77	60	20†	62%§
6	55	12.4/80	80	20†	0%§

*A constant mass of 40 μg (67 nmol) of (±)-5-(tri-*n*-butyltin)benzovesamicol in 40 μL of EtOH was used for each reaction.
†Reactions were magnetically stirred.
‡Reaction was initiated with 12 μL of aqueous peracetic acid (0.32% w/w).
§Radiochemical yield determined by radio-TLC analysis of the crude reaction mixture.

The average specific activity ($N = 14$) was 43,600 Ci/mmol with an optical purity greater than 98%.

Distribution comparison between [^{125}I]IBVM and [^{123}I]IBVM in mice

CD-1 female mice (Charles River, Wilmington, Mass.) weighing 23–30 g were allowed free access to food and water. Under light ether anesthesia, 5 animals were given tail vein injections of 35–46 μCi of [^{123}I]IBVM in 0.05–0.10 mL of 0.15 M sodium acetate buffer (pH 4.5)/ethanol (95/5); a second set of 5 mice were given tail vein injections of 8–9 μCi of [^{125}I]IBVM in equal volumes of the same vehicle. The mice were sacrificed by decapitation 4 h after tracer injection. The brain was quickly isolated and samples (20–150 mg) of striatum, hippocampus, cortex, cerebellum and blood were excised, weighed and counted on a Packard 5780 autogamma counter. Tissue radioactivity concentrations were expressed as % injected dose/g of tissue (% dose/g) normalized to a standard 25 g mouse. All animal procedures had the prior approval of the University of Michigan Committee on Use and Care of Animals.

Results and Discussion

Racemic 5-IBVM was synthesized from (±)-5-aminobenzovesamicol **4** by the Sandmeyer diazotization reaction as previously reported (Jung *et al.*, 1990). The enantiomer (−)-5-IBVM was resolved by preparative-TLC separation of the corresponding diastereoisomeric MTPA esters of racemic 5-IBVM followed by hydrolysis (Jung *et al.*, 1990). The synthesis of racemic and (−)-5-(tri-*n*-butyltin)benzovesamicol **3** (Fig. 2) was carried out by treating their respective iodinated isomers with hexabutylditin and tetrakis (triphenylphosphine) palladium (0) in refluxing dioxane (Mais *et al.*, 1991). Flash chromatography with hexane:EtOAc provided pure product in 66% yield.

The $(NH_4)_2SO_4$ catalyzed solid-state isotopic exchange method (Fig. 3) provided (−)-5-[^{125}I]IBVM in specific activities (130–200 Ci/mmol) suitable for small animal biodistribution and *ex vivo* autoradiographic studies. This radiosynthetic method routinely provided high radiochemical yields (65–89%; $N = 18$). In all cases radioiodide appeared to be the only radiochemical impurity thus allowing a relatively straightforward purification by solid phase extraction. Chiral HPLC analysis of the labeled product confirmed that no racemization had occurred during the labeling process.

The synthesis of no-carrier-added radioiodinated (−)-5-IBVM was carried out by iododestannylation of the corresponding (−)-5-(tri-*n*-butyltin)benzovesamicol precursor as shown in Fig. 2. This approach is the current method of choice for the regiospecific incorporation of radioiodine in high specific activity in unactivated molecules. The tin precursors are relatively shelf-stable, easily prepared from the corresponding iodo or bromo analogs, and undergo radioiododestannylation in excellent radiochemical yields under mild conditions (Hanson *et al.*, 1981; Tonneson *et al.*, 1981). An added advantage is the increased lipophilicity of the tin precursor compared to the iodinated product; a feature which allows easy purification of the radiotracer by reversed-phase HPLC.

To optimize the synthesis of 5[^{123}I]IBVM, we compared the effect of the three oxidizing agents—hydrogen peroxide, peracetic acid and chloramine-T—on the radiochemical yield and purity of the labeled product. In certain cases, additional variables such as

Table 4. Chloramine-T catalyzed labeling of IBVM with ^{125}I/^{123}I-iodide

Entry	Acid/solvent/vol (μL)	mCi ^{125}I/[^{123}I]iodide/vol (μL)	Radiochemical yield (purity)
1*	0.2 N H_2SO_4/EtOH/50	0.18 mCi Na^{125}I/80	80% (98%)
2	0.2 N H_2SO_4/EtOH/50	20.5 mCi Na^{123}I/65	84% (97%)
3†	0.2 N H_2SO_4/EtOH/50	20.3 mCi Na^{123}I/68	82% (98%)

*30 μg (50 nmol) of (±)-5-(tri-*n*-butyltin)benzovesamicol was used in each reaction.
†Reaction conducted directly in the Na[^{123}I]iodide shipping vial. The 0.2 N ethanolic H_2SO_4 was initially added to the vial followed by the tin precursor and chloramine-T solution.

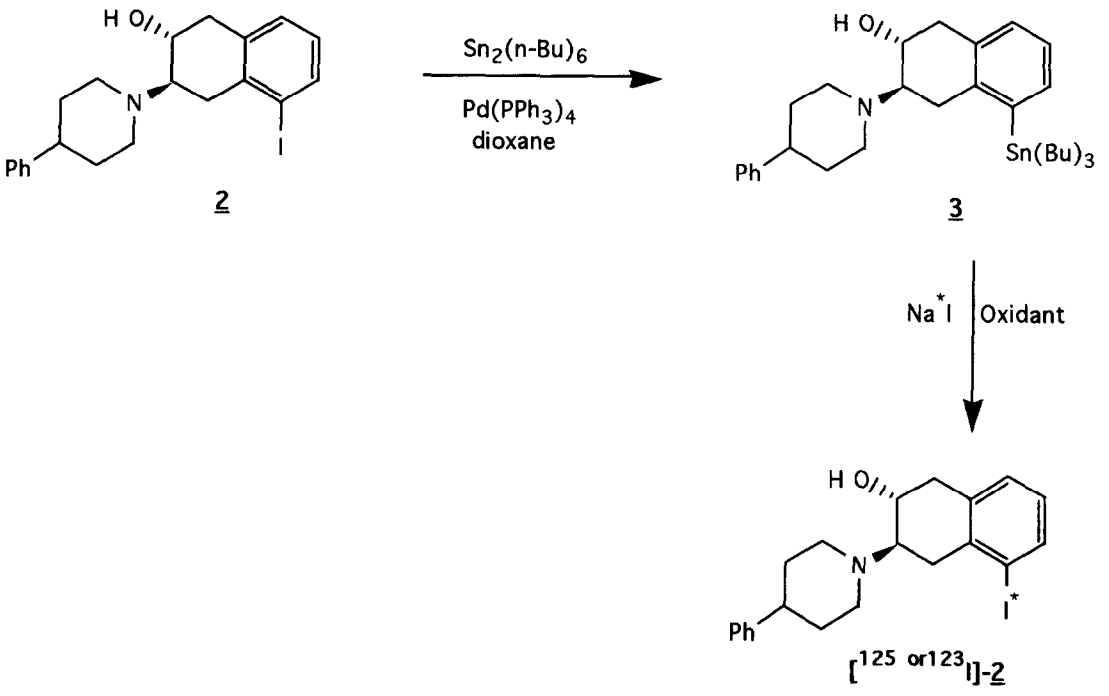

Fig. 2. Radiosynthesis of n.c.a. (−)-5-[^{123,125}I]IBVM by iododestannylation.

solvent composition, reaction times, temperature and stirring were also evaluated. For convenience and economy, preliminary evaluations were conducted on racemic **3** with $Na^{125}I$. Commercially available $Na^{125}I$ is frequently supplied in 0.1 N NaOH at higher concentrations than $Na^{123}I$—approx. 1 mCi/μL for $Na^{125}I$ and 0.23 mCi/μL for $Na^{123}I$. In order to obtain valid comparisons between the two radioisotopes, reactions employing $Na^{125}I$ were diluted with 0.1 N NaOH to match the radioiodide concentrations in the $Na^{123}I$ reactions.

Dilute aqueous HCl or ethanolic HCl solutions were employed in the H_2O_2 catalyzed radioiodinations to neutralize the base accompanying the radioiodine and thus assure an acidic pH for the radioiodination. Due to our concern that the vast excess of chloride ion might lead to formation of the corresponding 5-chloro derivative of BVM (**5**), subsequent acidifications were conducted with dilute H_2SO_4 rather than HCl. Studies reported by Kung and Kung (1989) and others (Petzold and Coenen, 1981; Wilson *et al.*, 1989) have demonstrated that optimum radiochemical yields in electrophillic radioiodinations occur at acidic pH. The pH of the reaction was therefore routinely monitored prior to addition of the oxidant; additional acid was added, as required, to maintain the pH between 4 and 5.

Hydrogen peroxide catalyzed reactions

As noted in Table 1, at low total reaction volumes (entry 1), radioiodinations conducted with $Na^{125}I$ using aqueous H_2O_2 as oxidizing agent gave high radiochemical yields and high radiochemical purities. A moderate increase in reaction volume could be tolerated without sacrificing radiochemical yields provided that equal volume ratios of EtOH to H_2O were present in the reaction medium (entries 1 and 2). Higher reaction volumes and disproportionate EtOH-to-H_2O ratios, however, resulted in lower yields (entries 3 and 4).

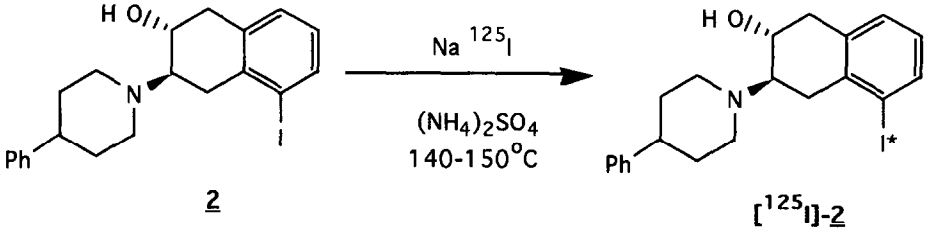

Fig. 3. Solid-state synthesis of (−)-5-[^{125}I]iodobenzovesamicol.

Peracetic acid catalyzed reactions

As in the case of H_2O_2, high radiochemical yields were obtained with peracetic acid as oxidizing agent providing low total reaction volumes were maintained (compare entries 1 and 2 in Table 2). Moderate increases of temperature (40°C) resulted in both higher radiochemical yields and purity (entry 3).

When Na^{123}I was substituted for Na^{125}I, however, a 50% decrease in radiochemical yield was observed (compare entry 1 in Table 3 versus entry 2 in Table 2). This result was obtained even though similar stoichiometric ratios of iodinating species-to-substrate were used in the two reactions: 0.2 mCi of [^{125}I]iodide is stoichiometrically equivalent to 22 mCi of [^{123}I]iodide. However, if the reaction was performed with smaller volumes of aqueous [^{123}I]iodide, radiochemical yields improved dramatically (entry 2 versus entry 1, Table 3). Since a fixed mass of 5-(tri-*n*-butyltin)benzovesamicol was used in all the reactions, the lower radiochemical yields observed when [^{123}I]iodide was used may be due to the limited solubility of **3** in aqueous/organic solvent mixtures. Stirring the reaction mixture gave no added advantage (entry 3). Moderate increase of temperature (60°C) was advantageous; however, at higher temperatures (80°C), no radiolabeled product was observed.

Chloramine-T catalyzed reactions

As the data in Table 4 shows, chloramine-T catalyzed reactions afforded excellent radiochemical yields and radiochemical purities with both Na^{125}I and Na^{123}I. These reactions were conducted at room temperature with typical reaction times of 2 min. In addition, there was no significant variability in radiochemical yields at higher reaction volumes as was observed in the case of the H_2O_2 or peracetic acid catalyzed reactions. Although analysis of the u.v. trace of the preparative HPLC chromatogram does suggest that small amounts of 5-chlorobenzovesamicol **5** are formed (< 400 ng), this product, fortunately, is well separated from 5-IBVM under the preparative HPLC conditions employed, and therefore is not a detectable contaminant in the final product. Another major advantage of the method described here is that the radiosynthesis can be conducted directly in the vial in which it is shipped and the crude product then transferred to the injection loop of the HPLC for immediate purification. This feature shortens synthesis time and minimizes radiation exposure during the synthesis.

Distribution studies in mice

The distributions of radioactivity in mouse brain 4 h after i.v. injection of [^{125}I]IBVM and [^{123}I]IBVM are compared in Fig. 4. Brain concentration values for the two tracers in the four brain regions sampled were within one standard deviation of each other. Taking into account differences in radioactive dose

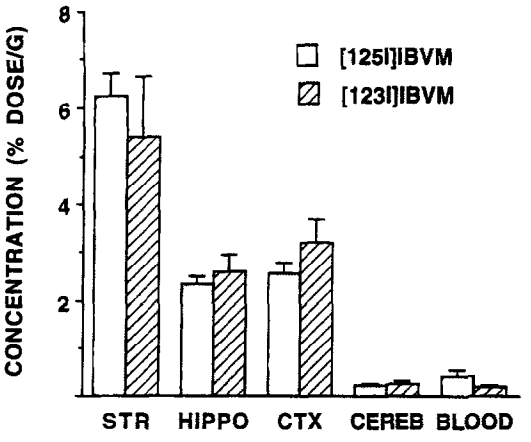

Fig. 4. Regional brain distribution of [^{125}I]IBVM and [^{123}I]IBVM in mice 4 h after i.v. tracer injection: STR, striatum; HIPPO, hippocampus; CTX, cortex; CEREB, cerebellum.

administered and tracer specific activity, the mass of IBVM administered was ≥20-fold higher with [^{125}I]IBVM than with [^{123}I]IBVM. As reported earlier (Jung *et al.*, 1990), the regional distribution pattern of [^{125}I]IBVM in mouse brain 4 h after i.v. injection correlates well with other markers of cholinergic innervation such as choline acetyltransferase. In particular, the low cerebellar retention and high striatal uptake of these tracers are consistent with the known cholinergic nerve density in these two brain areas. The major purpose of the distribution comparison between [^{125}I]IBVM and [^{123}I]IBVM was to ascertain whether the two tracers behave similarly *in vivo*; the results presented here, though preliminary in nature, support the equivalence of the two tracers.

Specific activity considerations

The major goal of this work was to develop a dependable synthesis of [^{123}I]IBVM that could be safely injected into humans for image analysis of cholinergic nerve defects. Specific activity was our chief concern in view of the potent neuromuscular blocking action of vesamicol and its benzo derivatives (Rogers *et al.* 1989). Although use of shorter half-life radioisotopes such as the positron emitters carbon-11 and fluorine-18 can theoretically provide tracers with specific activities an order of magnitude higher than iodine-123, in practice this has not been the case. Environmental carbon is likely responsible for causing the specific activities of carbon-11 radiotracers to be generally less than 5000 Ci/mmol (Fowler and Wolf, 1986). Similar reasoning has been given for the relatively low specific activities (5–10% of theoretical) that are generally reported for fluorine-18 labeled tracers (Kilbourn, 1990).

The 30–70,000 Ci/mmol specific activity range ($N = 14$) obtained for [^{123}I]IBVM equates to a maximum of 2.9 ng/kg of IBVM per patient; this calculation is based on a worst-case situation of a specific

activity of 30,000 Ci/mmol for a 10 mCi dose administered to a 50 kg adult. A dose of 2.9 ng/kg is 75,000 times lower than the i.v. LD_{50} of IBVM in male Sprague–Dawley rats which is 218 μg/kg (Kostyniak *et al.*, 1991). Perhaps more important is the pharmacological safety factor for IBVM, which to date has not been investigated in detail. Slowing of respiratory rate is generally the first symptom of neuromuscular blockade by curares and curaremimetics (Taylor, 1990). IBVM first produces lethargy in rats at doses of 75–150 μg/kg (Kostyniak *et al.*, 1991); a dose level that is still 25,000 times higher than the anticipated maximum patient dose of IBVM.

Summary

Carrier-added and no-carrier-added syntheses of the radioiodinated cholinergic neuronal marker (−)-5-IBVM are described. The synthesis of no-carrier-added 5-[^{125}I]IBVM from the corresponding chiral 5-(tri-*n*-butyltin) precursor was evaluated using the oxidants hydrogen peroxide, peracetic acid and chloramine-T. Both peracetic acid and chloramine-T gave good yields of [^{125}I]IBVM; however, analogous studies with Na^{123}I revealed that chloramine-T oxidation is the method of choice for the synthesis of [^{123}I]IBVM. The advantages of this method include rapid reaction times at room temperature, high radiochemical yield and excellent product purity and specific activity. The use of a mini-bore C-18 chromatography column provides a rapid and efficient HPLC purification of the final product, which is well resolved from potential side products such as benzovesamicol, 5-chlorobenzovesamicol and the 5-(tri-*n*-butyltin) precursor. The radiochemical yield of (−)-5-[^{123}I]IBVM was optimized by: (1) conducting the iodination directly in the shipment vial; (2) using ethanol as a co-solvent to enhance the solubility of the tri-*n*-butyltin precursor; (3) maintaining a small reaction volume; and (4) conducting the reaction at pH 4–5. The high specific activities of [^{123}I]IBVM achieved by this method, i.e. 30–70,000 Ci/mmol, make possible the safe i.v. administration of imaging doses of 10 mCi to human subjects. The regional distribution patterns of [^{125}I]IBVM and [^{123}I]IBVM in mouse brain 4 h after i.v. injection are nearly identical.

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