Synthesis and Preliminary Evaluation of an Iodovinyl-Tetrabenazine Analog as a Marker for the Vesicular Monoamine Transporter

*Daniel J. Canney, Yu-Zhi Guo, Mei-Ping Kung,‡ and Hank F. Kung**

Departments of Radiology and Psychiatry‡, 3700 Market St. Suite 305, University of Pennsylvania, Philadelphia, PA 19104.

SUMMARY

A derivative of tetrabenazine (TBZ, **1**) containing an iodovinyl group (I-TBZ, **3b**) was prepared as a potential radiotracer for the vesicular monoamine transporter. The synthesis of **3a** was achieved by ethynylation of TBZ. The ethynyl derivative **4**, was converted to the corresponding vinylstannane **5**. The vinylstannane intermediate was then treated with I_2 in chloroform at room temperature, to afford the iodovinyl compound (**3a**, I-TBZ). The no-carrier-added [^{125}I]I-TBZ (**3b**) was prepared by treating an ethanolic solution of **5** with sodium[^{125}I]iodide in the presence of H_2O_2. Following i.v. injection, [^{125}I]I-TBZ (**3b**) can readily cross the blood brain barrier and localize in the brain (0.92 and 0.36 % dose/organ, at 2 min and 30 min, respectively). However, no specific regional uptake of the ligand was observed. Subsequent biodistribution studies performed using two well resolved peaks obtained from chiral HPLC separation of **3b**, demonstrated that Fraction **I** displayed higher brain uptake (0.6 % dose/organ, 20 min) than the latter peak (Fraction **II**, 0.3 % dose/organ, 20 min). Fraction **I** also exhibited a modest degree of specificity for the striatum which could be blocked by pretreatment with tetrabenazine. The high lipophilicity of **3a** ($P_{o/b}$ for Fraction **1** and **II** = 1723 and 1395, respectively) may contribute to high nonspecific binding in vivo, and result in the low target/nontarget ratio observed for this compound. Iodovinyl-TBZ analog **3b** is a poor candidate as a SPECT imaging agent for the vesicular monoamine transporter.

Key words: Vesicular monoamine transporter, iodine-125, radioiodinated tetrabenazine analog.

INTRODUCTION

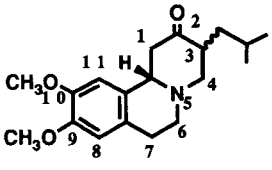
The active transport of monoamine neurotransmitters (dopamine, serotonin, norepinephrine) in the mammalian central nervous system (CNS) plays an important role in synaptic transmission (1). Two distinct sites of active uptake of monoamines are involved in moving the transmitters from the synaptic cleft to the storage vesicles within the neuron. Following the release of the transmitter into the synaptic cleft and interactions at pre- and postsynaptic receptors sites, termination of action often

Portions of this work were presented at the IXth International Symposium on Radiopharmaceutical Chemistry, paper E3, p. 199, 1992.

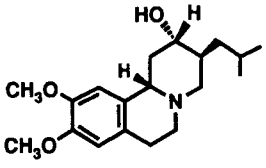
0362–4803/93/040355–14$12.00

Received 4 December, 1992
Revised 17 December, 1992

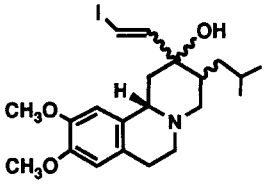
involves Na-coupled reuptake (plasma membrane transport) of the transmitter to either the presynaptic nerve terminal or to glial cells (1). Following uptake of the monoamines into the cytoplasm of the presynaptic neuron, a second type of transporter or reuptake mechanism (vesicular transport) is responsible for storing and packaging the neurotransmitters in monoamine vesicles (or granules). The latter reuptake mechanism is based on common ATP-dependent transporters which are non-selective and are effective for both catecholamines and serotonin. Reserpine is a natural product which inhibits the vesicular monoamine uptake mechanism within the synapse, resulting in a depletion of monoamines in the CNS (2). Tetrabenazine, (3-(2-methylpropyl)-9,10-dimethoxy-1,3,4,6,7,11b-hexahydro-2H-benzo[a]quinolizin-2-one), (TBZ, **1**) is an alkaloid which displays a biological profile similar to reserpine. The depletion of monoamines in the brain by reserpine is long-lasting and the storage mechanism appears to become irreversibly damaged. In contrast, TBZ produces a short lived depletion of monoamines, and does not appear to irreversibly damage the transporter (2,3,4).



Tetrabenazine (1)
(TBZ)



α-Dihydrotetrabenazine (2)
(DH-TBZ)



Iodovinyl-tetrabenazine (3)
(3a = I-TBZ)
(3b = ^{125}I-TBZ)

Recently, tritiated dihydrotetrabenazine (2-hydroxy-3-(2-methylpropyl)-9,10-dimethoxy-1,3,4,6,7,11b-hexahydro-2H-benzo[a]quinolizine; [^{3}H]DH-TBZ; **2**) has been reported as a selective marker for radioligand binding and autoradiographic studies of the monoamine transport system (5, 6, 7). In vitro binding studies have demonstrated that [^{3}H]DH-TBZ has high binding affinity (K_d= 2.9 nM) for the transporter (8, 9, 10, 11). Autoradiography studies have shown that [^{3}H]DH-TBZ labels monoamine rich brain regions (7, 12). Attempts to avoid the long exposure times necessary for the tritiated ligand, resulted in the synthesis and testing of an iodinated analog of ketanserin (known to have moderate affinity for the transporter) (7). The iodinated ligand was shown to be less specific and to exhibit higher nonspecific binding when compared to [^{3}H]DH-TBZ in autoradiography studies. Therefore, the development of specific, high affinity radioiodinated ligands for this uptake site remains an important objective. Such a ligand, when labeled with ^{125}I, could be used for in vitro radioligand binding and autoradiographic studies, while ^{123}I labeled ligands may be used for in vivo

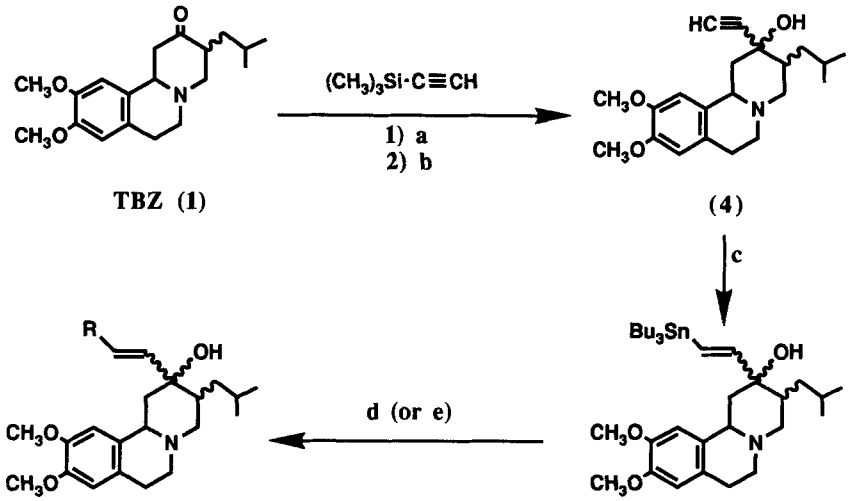
Single Photon Emission Computed Tomography (SPECT) studies involving the vesicular monoamine transporter.

The goal of this work was to synthesize an iodinated analog of TBZ as a potential radioligand for in vitro (radioligand binding and autoradiography) and in vivo (SPECT imaging) studies of the vesicular monoamine transporter. Structure-activity studies involving TBZ suggest that analogs containing considerable steric bulk at the 2 position retain amine depleting activity and /or high affinity for the uptake site (4, 10, 13-21). These data suggest that an iodovinyl group might also be tolerated at the 2-position of the TBZ molecule and retain affinity for the monoamine reuptake site. Consequently, we herein report the synthesis, radiolabeling and initial biodistribution studies of a 2-iodovinyl-analog of TBZ (**3**).

RESULTS and DISCUSSION

The synthetic route utilized for the preparation of the iodovinyl-TBZ analog **3a** is shown in Scheme I. Ethynylation of tetrabenazine (TBZ, **1**) was accomplished by first treating trimethylsilylacetylene with n-butyllithium at -5 to 0 °C in THF, followed by dropwise addition of a solution of TBZ in THF. The trimethylsilyl group was removed under basic conditions (5N NaOH).

Scheme I. Synthesis of Iodovinyl-tetrabenazine (I-TBZ)



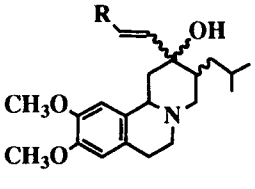
a) n-butylLi, THF, 0 °C; b) KOH, MeOH, 65° C; c) $HSnBu_3$, AIBN, Toluene, 95 °C
d) I_2, $CHCl_3$, 25 °C; e) $Na^{125}I$, 1N HCl/EtOH, H_2O_2

The ethynyl derivative (4) was then treated with tri-n-butyltin hydride in the presence of AIBN and the mixture was heated at 95 °C for 5 h. Treatment of a chloroform solution of the resulting vinylstannane intermediate (3) with a 0.1 M solution of iodine in $CHCl_3$ afforded the cold iodinated compound (3a) in 27.5% overall yield. Elemental analyses and spectroscopic data for each compound were consistent with the structures shown. The coupling constants observed for the vinyl protons of compounds 5 (J = 19.4 Hz) and 3a (J = 11.7 Hz) were consistent with the trans conformations shown for these compounds in Scheme I (22).

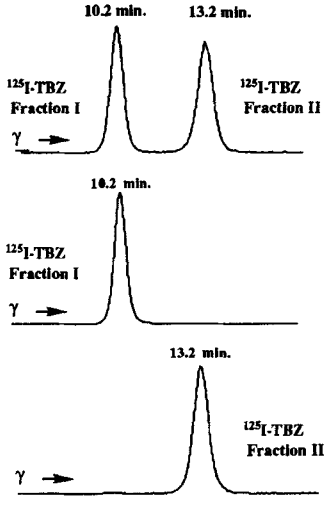
The radiolabeled derivative (3b) was prepared in excellent yield (> 90%) by treating an ethanolic solution of the organotin intermediate with $Na^{125}I$ and 1N HCl in the presence of H_2O_2. Following HPLC purification, the radiochemically pure compound (> 98%) displayed the same retention time as that of the authentic cold compound.

Initial biodistribution studies in rats using racemic [^{125}I]-3b showed that the TBZ derivative can cross the intact blood brain barrier and localize in the brain. However, the regional distribution for 3b in the rat brain showed no selective uptake (data not shown). The apparent lack of specificity of 3b for monoamine containing neurons in the brain may be attributed to the presence of both "active" and "inactive" stereoisomers in the racemic mixture being tested in the in vivo biodistribution studies. Introduction of the iodovinyl group at the 2-carbonyl of TBZ resulted in an additional chiral

Figure 1. Chiral HPLC Separation of ^{125}I-TBZ into Fractions I and II[a]



(3a, R = I)
(3b, R = ^{125}I)



[a] HPLC separations performed on a Rainin System equipped with a Chiracel OD column. The compound was eluted with 5% EtOH/Hexanes at a flow rate of 1.0 mL/min.

center in the molecule. Stereochemical considerations have been shown to be important for the binding of a diasteriomeric pair of dihydro-TBZ analogs to the vesicular monoamine transporter (10). The stereochemistry at carbon 2 of 2α-ethyl-2β-hydoxy-3α-alkyl TBZ derivatives is also known to be important for these analogs to retain amine depleting activity (13). Therefore, **3b** was subjected to chiral HPLC analysis (Chiralcel OD column; n-hexane/ ethanol 95/5; 1mL/min).

Following chiral HPLC separation of racemic no-carrier-added [^{125}I]-**3b**, two well resolved peaks were observed. The HPLC profile of **3b** is shown in Figure 1, suggesting that at least two isomers, or two sets of isomers, can be separated using this method. For convenience, these peaks will be referred to as Fraction **I** (r.t. = 10.2 min) and Fraction **II** (r.t. = 13.2 min). Because the goal of this work was to evaluate **3b** as a potential radiotracer for the monoamine storage site, attempts to determine the absolute configuration of the compound(s) contained in each HPLC fraction were postponed until the potential usefulness of the ligands could be evaluated in biodistribution studies.

TABLE I. Organ Biodistribution of ^{125}I-TBZ (Fractions I and II) in Rats[a,b]

Fraction I

	Time (min) post-injection			
	5	20	60	120
Organ				
Blood	2.15±0.11	1.42±0.56	1.14±0.16	0.95±0.03
Heart	0.91±0.17	0.49±0.04	0.38±0.07	0.26±0.03
Muscle	11.96±1.56	26.79±7.14	21.88±2.74	18.56±2.34
Lung	2.85±0.52	1.65±0.11	1.17±0.06	0.81±0.14
Kidney	4.43±0.56	2.21±0.11	1.52±0.28	1.05±0.1q
Spleen	0.62±0.13	0.44±0.06	0.36±0.04	0.23±0.04
Liver	21.23±2.66	16.16±4.71	10.12±1.26	7.54±0.77
Skin	8.72±1.52	9.00±5.10	14.76±4.35	11.22±3.92
Thyroid	0.08±0.01	0.05±0.01	0.07±0.01	0.07±0.05
Brain	1.12±0.15	0.58±0.01	0.33±0.07	0.18±0.04
Br/Bl	5.51±0.37	4.59±1.10	3.05±0.12	1.96±0.43

Fraction II

	Time (min) post-injection			
	5	20	60	120
Organ				
Blood	4.74±0.36	2.76±0.42	1.89±0.13	1.89±0.34
Heart	0.63±0.03	0.35±0.02	0.16±0.01	0.09±0.02
Muscle	21.12±1.36	19.78±1.90	11.80±2.02	5.72±1.2
Lung	2.02±0.22	1.05±0.04	0.46±0.03	0.27±0.08
Kidney	2.18±0.34	1.37±0.11	0.70±0.06	0.36±0.09
Spleen	0.46±0.03	0.28±0.03	0.14±0.03	0.06±0.01
Liver	22.61±1.85	17.25±1.25	8.36±1.01	4.35±0.50
Skin	7.64±3.09	8.37±1.15	5.45±0.64	4.80±0.46
Thyroid	0.07±0.01	0.05±0.01	0.13±0.03	0.47±0.14
Brain	0.74±0.07	0.27±0.02	0.087±0.009	0.042±0.008
Br/Bl	1.54±0.16	1.06±0.22	0.47±0.05	0.25±0.03

[a] HPLC separations were performed on a Rainin system equipped with a Chiracel OD column (Hex/EtOH, 95/5; 1 mL/min). [b] Values represent the mean ± S D of the % dose/organ for 4 rats.

Biodistribution studies were then performed on the two fractions obtained following chiral HPLC separation (Fractions I and II; Table I). Muscle and liver uptake (% dose/organ) of both isomers remained high relative to other organs for up to 2 h post-injection. The uptake of Fraction I in the brain was consistently higher at all time points as compared to Fraction II. The brain-to-blood ratios for Fraction I ranged from 5.51 at 5 min to 1.96 at 120 min, compared to ratios of 1.54 to 0.25 for Fraction II at the same time points. The amount of activity in the thyroid remained low for Fraction I (< 0.1%) for up to 2 h post-injection. Thyroid uptake for Fraction II remained low at 5 and 20 min.(< 0.1%), but increased to 0.13 % and 0.47 % at 60 and 120 min, respectively.

The results of regional brain uptake studies for Fractions I and II are shown in Table II. As observed in the organ distribution data, identical doses of each HPLC fraction result in larger quantities of Fraction I in the brain at each of the time points investigated. Additionally, at 20 minutes post-injection, the striatum to cerebellum (target to nontarget) ratio for Fraction I is 1.32 vs. 1.17 for Fraction II, suggesting a modest degree of specific uptake into the striatum.

TABLE II. Regional brain uptake of ^{125}I-TBZ, Fractions I and II[a,b]

Fraction I

	Time (min) post-injection			
	5	20	60	120
Region				
Cerebellum	0.708±0.044	0.362±0.017	0.225±0.029	0.116±0.026
Striatum	0.770±0.019	0.479±0.014	0.271±0.046	0.138±0.022
Hippocampus.	0.725±0.066	0.414±0.032	0.230±0.048	0.113±0.041
Cortex	0.792±0.096	0.420±0.031	0.234±0.035	0.129±0.019
Str./Cb.	1.09	1.32	1.20	1.19

Fraction II

	Time (min) post-injection			
	5	20	60	120
Region				
Cerebellum	0.449±0.067	0.162±0.017	0.055±0.005	0.027±0.005
Striatum	0.482±0.066	0.189±0.001	0.062±0.002	0.027±0.003
Hippocampus.	0.458±0.074	0.189±0.012	0.058±0.009	0.027±0.004
Cortex	0.514±0.066	0.183±0.012	0.060±0.007	0.028±0.005
Str./Cb.	1.07	1.17	1.13	1.00

[a] HPLC separations were performed on a Rainin system equipped with a Chiracel OD column (Hex/EtOH, 95/5; 1 mL/min). [b] Values represent the mean ± SD of the % dose/g for 4 rats.

In order to investigate the specificity of the modest uptake observed for **3a**, Fraction I into the striatum, blocking studies were performed. The results of these in vivo competition studies involving cold TBZ and Fractions I and II, are shown in Table III. Tetrabenazine was given i.v. 3 min prior to the test compound. Regional uptake was determined 20 min following injection of Fractions I and II. The pretreatment resulted in a target-to-nontarget ratio for Fraction I of 1.11, compared to 1.31 for un-pretreated controls. Pretreatment with TBZ had no effect on the ratio obtained for Fraction II. The ability of tetrabenazine to block the modest degree of uptake of **3b**, Fraction I into the striatum suggests that the uptake is specific. These observations are consistent with radioligand binding studies which show that **3b**, Fraction I has high affinity (K_d = 0.3 nM) for the monoamine vesicular transporter while no specific binding was observed for Fraction II (23). The details of radioligand binding and autoradiography studies using ^{125}I-labeled **3b** will be published elsewhere.

TABLE III. Regional brain uptake of ^{125}I-TBZ, Fractions I and II[a,b]

	Fraction I		Fraction II	
Region	Control	Pretreated[c]	Control	Pretreated
Cerebellum	0.36±0.02	0.44±0.02	0.19±0.01	0.32±0.05
Striatum	0.47±0.03	0.49±0.02	0.22±0.01	0.35±0.04
Hippocampus	0.39±0.03	0.47±0.01	0.23±0.02	0.33±0.05
Cortex	0.31±0.05	0.37±0.02	0.18±0.01	0.25±0.06
Str./Cb.	1.31	1.11	1.15	1.10
Brain uptake[d]	0.59±0.02	0.69±0.06	0.31±0.02	0.48±0.05

[a] HPLC separations were performed on a Rainin system equipped with a Chiracel OD column (Hex/EtOH, 95/5; 1 mL/min). [b] Values represent the mean ± S D of % Dose/g, 20 min. post injection, n = 4. [c] TBZ (2mg) was given i.v., 3 minutes before the labeled compound. Control values determined without pretreatment with TBZ. [d] % Dose/organ

Recently, Kilbourn and coworkers have demonstrated high uptake of ^{11}C-labeled TBZ into the striatum of mice (24). The data suggest that this agent may be useful as a PET imaging agent for *in vivo* studies involving vesicular monoamine uptake sites. While our preliminary *in vivo* data suggest that I-TBZ-Fraction I exhibits modest specificity for monoamine rich areas in the brain, the low target-to-nontarget ratio is disappointing and precludes the use of this ligand for in vivo SPECT imaging studies. The lipophilicity of **3b** may account for the disappointing target to non-target ratio observed for this compound. High lipophilicity could contribute to significant nonspecific binding, which may make specific binding of the ligand to monoamine rich areas difficult to observe in vivo. The lipophilic nature of ^{125}I-TBZ **3b**, as evidenced by their high partition coefficients in n-octanol/buffer ($P_{o/b}$ = 1723 and 1395, respectively, Table IV), are consistent with this hypothesis.

Relatively high nonspecific binding (30% of total binding) was also observed in radioligand binding studies involving this ligand (23). Less lipophilic derivatives of **3** which retain high affinity for the monoamine storage site may show more promising target to non-target ratios.

TABLE IV. Apparent Partition Coefficients ($P_{o/b}$) of ^{125}I-TBZ and Derivatives[a]

Compound	$P_{o/b}$
I-TBZ Fraction I[b]	1723±644
I-TBZ Fraction II[b]	1395±306
Tetrabenazine (TBZ)[c]	480
Dihydro-TBZ[c,d]	105
Acetyl-dihydro-TBZ[c]	1900

[a] $P_{o/b}$ determined using octanol/K-PO_4 buffer (pH = 7) [b] Values represent the mean ± S D for 3 determinations. [c] Values as reported by Scherman et al.[3]. [d] Unfractionated mixture of isomers

---

Table V. Plasma Protein Binding of ^{125}I-TBZ, Fractions I and II[a, b]

Compd.	Method	
	TCA	Filter
I-TBZ Fraction I	38.1±1.5	81.8±0.5
I-TBZ Fraction II	40.8±0.6	83.7±0.8

[a] HPLC separations were performed on a Rainin system equipped with a Chiracel OD column (Hex/EtOH, 95/5; 1 mL/min). [b] Values represent the mean ± S D for 3 determinations.

Rapid deiodination of the radioligand *in vivo* might also explain the disappointing *in vivo* results obtained for **3b**. However, thyroid uptake in organ biodistribution studies suggest that deiodination is minimal during the first hour post-injection. Preliminary experiments performed with Fraction I and Fraction II showed that a higher percentage of Fraction I (88% ± 3; n = 3) could be extracted from rat plasma (60 min post-injection) as compared to Fraction II (70% ± 3; n = 3). While these results suggest that Fraction I may be more stable than Fraction II *in vivo*, more detailed metabolic experiments are required to assess the metabolic stability of the compounds. Plasma protein binding for Fraction I and Fraction II was also determined in rat plasma and found to be similar for both fractions (38 and 41 %, respectively).

In conclusion, an iodovinyl derivative of TBZ, **3a**, was prepared in modest overall yield (27.5%). The radiolabeled form of the compound was prepared from the tributyltin precursor in high

yield (> 90%) and excellent radiochemical purity (> 98%). When evaluated in biodistribution studies, the parent racemic form of **3b** showed no specific regional uptake in monoamine rich areas of the rat brain. However, when compound **3b** was subjected to chiral HPLC analysis, two well resolved peaks were obtained which were evaluated in biodistribution studies. The more mobile of the two HPLC peaks (Fraction I) was found to show modest uptake in the striatum which could be blocked by TBZ pretreatment. The partition coefficients obtained for the two HPLC fractions suggest that high lipophilicity may contribute to high nonspecific binding *in vivo*, and result in the low target/nontarget ratio observed for this compound. The biodistribution data reported here suggest that the iodovinyl-TBZ analog **3b** is a poor candidate as a potential SPECT imaging agent for the CNS monoamine transporter. However, ^{125}I-TBZ, Fraction I has shown promise as a radioligand for *in vitro* binding and autoradiography studies of the CNS monoamine transporter. The details of these *in vitro* studies will be published elsewhere.

EXPERIMENTAL SECTION

General Methods. Reagents used in syntheses were purchased from Aldrich Chemical Co. (Milwaukee, WI) or Fluka (Ronkonkoma, N.Y.) and were used without further purification unless otherwise indicated. Anhydrous sodium sulfate (Na_2SO_4) was used as a drying agent. Reaction yields are reported without attempts at optimization. Reagent-grade and HPLC-grade solvents were obtained from Emsco Co. (Philadelphia, PA). Tetrahydrofuran was dried by distillation from sodium benzophenone ketyl. Toluene was dried by distillation from sodium metal. Thin layer chromatography (TLC) was performed on EM Science (Gibbstown, N.J.) precoated (0.2 mm) silica gel 60 plates and the spots were detected with I_2 vapor and/or UV light. Silica gel 60 (70-230 mesh) obtained from EM Science (Gibbstown, NJ) was used for column chromatography. ^{1}H and ^{13}C spectra were obtained on a Bruker Model AM 500 or a Varian 360A spectrometer. All samples prepared for NMR analysis were dissolved in $CDCl_3$ purchased from Aldrich Chemical Co. Chemical shifts are reported as δ values with the chloroform or tetramethylsilane resonance used as the internal standard. The multiplicity is defined by s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). The relative peak heights of the resonances are reported as integers after the multiplicity. IR spectra were recorded (KBr pellet or neat as a film) with a Mattson Polaris FT-IR spectrometer. Melting points were determined on a Meltemp apparatus (Cambridge, MA) and are reported uncorrected. Elemental analyses were performed by Atlantic Microlabs Inc. (Norcross, GA). A Rainin liquid chromatograph equipped with a reverse phase cartridge (PRP-1; 25cm x 0.46 cm; Hamilton) or a chiral column (Chiralcel OD; 25 cm x 0.46 cm; Daicel Chemical Industries, Ltd.) was used for high-performance liquid chromatographic (HPLC) separations and analyses. No-carrier-added sodium [^{125}I]iodide (0.1N NaOH solution; 2200 Cu/mmol) was purchased from DuPont NEN Research Products (Boston, MA).

2-Hydroxy-2-ethynyl-3-(2-methylpropyl)-9,10-dimethoxy-1,3,4,6,7,11b-hexahydro-2H-benzo[a]quinolizine (4). Trimethylsilylacetylene (2 eqs.; 20.4 mmol; 2.88 mL) was dissolved in 80 mL of dry THF and stirred under a nitrogen atmosphere at -5 to 0 °C. n-Butyl lithium (20.4 mmol, 12.72 mL) was added dropwise with stirring followed by dropwise addition of a solution of 3-(2-methylpropyl)-9,10-dimethoxy-1,3,4,6,7,11b-hexahydro-2H-benzo[a]quinolizin-2-one (TBZ, **1**) in 30 mL dry THF. The reaction mixture was stirred at -5 to 0 °C for 1 h, allowed to warm to 20 °C, and stirred for 2 h at 20 °C. The reaction was quenched with saturated NH_4Cl. The solvent was evaporated under reduced pressure and the resulting aqueous solution was extracted with ethyl acetate (3 x 100 mL). The organic phase was washed with water (2 x 50 mL) and brine (50 mL) and concentrated. The residue was dissolved in 30 mL MeOH and 5 mL 5N KOH, and heated to 65 °C with stirring for 30 min. The reaction was cooled and quenched with NH_4Cl. The methanol was evaporated and the aqueous phase extracted with ethyl acetate (3 x 75 mL). The organic phase was washed with water (50 mL) and brine (50 mL), dried over Na_2SO_4, and concentrated to yield **4** (53.7 %) as a slightly brown solid: m.p. 53-55 °C; ^1H NMR δ 6.62 (s, 1H), 6.56 (s, 1H), 3.84 (s, 3H), 3.83 (s, 3H), 3.43-3.48 (m, 1H), 2.23-3.18 (m, 8H), 2.12-1.48 (m, 5H), 1.32-1.16 (m, 1H), 0.95 (d, 3H, J = 6.4 Hz), 0.91 (d, 3H, J = 6.4 Hz); IR (neat) λ 3500 (OH), 3290 (≡CH), 2950, 2860, 2250, 1610, 1520; Anal. ($C_{21}H_{29}NO_3$), C, H, N.

2-(2-Tri-n-butylstannylethenyl)-2-hydroxy-3-(2-methylpropyl)-9,10-dimethoxy-1,3,4,6,7,11b-hexahydro-2H-benzo[a]quinolizine (5). The alkyne (1.66 g; 4.9 mmol; **4**), was dissolved in 50 mL dry toluene and treated with tri-n-butyltin hydride (2 eqs., 9.7 mmol; 2.6 mL) while stirring under an argon atmosphere. Azobisisobutyronitrile (AIBN) was added (30 mg) and the mixture was heated to 95 °C for 5 h. The reaction mixture was cooled and the solvent was evaporated under reduced pressure. The residue was chromatographed on a silica gel column and eluted with ethyl acetate/hexane (1:1) to afford 1.89g (60.8%) of **5** as an oil. A second column chromatography was performed to obtain an analytically pure sample: ^1H NMR δ 6.49 (s, 1H), 6.48 (s, 1H), 6.34 (d, 1H, J = 19.4 Hz), 6.33 (d, 1H, J = 19.4 Hz), 3.73 (s, 3H), 3.71 (s, 3H), 3.24 (d, 1H, J = 11.7 Hz), 2.87-3.07 (m, 3H), 2.56-2.60 (m, 1H), 2.43-2.48 (m, 1H), 2.26 (dd, 1H, J = 2.45, 12.4 Hz), 2.16 (t, 1H, J = 11.9 Hz), 1.87-1.92 (m, 1H), 1.67-1.72 (m, 3H), 1.41-1.58 (m, 7H), 1.14-1.26 (m, 7H), 0.78-0.91 (m, 21H); IR (neat) λ 3510 (OH), 3050-2750 (b, n-butyl,C-H), 1610, 1520, 1460; Anal. ($C_{33}H_{57}NO_3Sn$), C, H, N.

2-Hydroxy-2-iodovinyl-3-(2-methylpropyl)-9,10-dimethoxy-1,3,4,6,7,11b-hexahydro-2H-benzo[a]quinolizine (3a). The vinylstannane intermediate (0.24 g; 0.38 mmol) was dissolved in 15 mL $CHCl_3$ and stirred at room temperature. A 0.1 M solution of I_2 in $CHCl_3$ was added dropwise until a brown color persisted. The reaction was stirred at r.t. for 24 h and then quenched with 2 mL of a 1M solution of KF in methanol and 2 mL aqueous sodium bisulfite (5%).

The layers were separated and the aqueous layer extracted with $CHCl_3$ (3 x 20 mL). Organic layers were combined, washed with water (2 x 20 ml) and brine (20 mL), dried over anhydrous sodium sulfate and concentrated to yield (84.3%) **3a** as a slightly yellow oil. ^1H NMR δ 6.92 (d, 1H, J = 14.3 Hz), 6.57 (s, 1H), 6.56 (s, 1H), 6.53 (d, 1H, J = 14.3 Hz), 3.83 (s, 3H), 3.82 (s, 3H), 3.30 (d, 1H, J = 11.7 Hz), 2.92-3.08 (m, 3H), 2.64 (dd, 1H, J= 3.6, 15.5 Hz), 2.52 (tt, 1H, J = 3.8, 11.4 Hz), 2.37 (dd, 1H, J = 2.5, 12.6 Hz), 2.18 (t, 1H, J = 11.8 Hz), 1.86 (m, 1H), 1.52-1.72 (m, 3H), 1.20-1.27 (m, 1H), 0.93-0.96 (m, 1H), 0.91 (d, 3H, J = 6.5 Hz), 0.87 (d, 3H, J = 6.5 Hz); IR (neat) λ 3500 (OH), 2990, 1630, 1530, 1470; Anal. ($C_{21}H_{30}NO_3I$), C, H, N.

Radiolabeling

The I-125 labeled iodovinyl-TBZ derivative ([^{125}I]I-TBZ, **3b**) was prepared by an iododestannylation reaction (25). Aqueous hydrogen peroxide (50 µL, 3% w/v) was added to a mixture of 50 µL tributyl tin precursor (1mg/mL EtOH), 200 µL of 0.1 N HCl and 5 mCi [^{125}I]sodium iodide (2200 Cu/mmol) in a sealed vial. The reaction was allowed to proceed at room temperature for 20 min, after which it was terminated by addition of 0.1 mL sodium bisulfite (300mg/ml). The reaction was then neutralized with $NaHCO_3$ and extracted with ethyl acetate (3 x 1 mL). The combined organic layers were dried under nitrogen to yield (90 %) the desired radioiodinated ligand. The product was purified by HPLC on a reverse phase column (PRP-1, Hamilton) eluting with an isocratic solvent of 90% acetonitrile-10% buffer (5mM 3,3'-dimethylglutaric acid, pH 7.0) and a flow rate of 1 mL/min. The radiochemical purity was analyzed by the above chromatographic system and the purified [^{125}I] product (purity >98%) was obtained for animal experiments. A solution of **3b** dissolved 0.9 % saline was found to be stable at room temperature for up to 15h. The specific activity of **3b** was not determined. However, based on experience with similar iododestannylation reactions where the specific activity was determined to be 2200 Ci/mmol, we feel that it is reasonable to assume that the specific activity of no carrier-added **3b** is approximately 2200 Ci/mmol (25, 26).

Separation of optical isomers was achieved using a Rainin HPLC system in conjunction with a chiral column (Chiracel OD). The racemic compound dissolved in ethanol (10 µL) was injected onto the chiral column and eluted with hexane/ethanol (95/5) at a flow rate of 1 mL/min. The appropriate fractions (Fractions **I** and **II**; retention times of 10.2 and 13.2 min, respectively) containing each isomer (as determined by γ detection) were combined, concentrated and re-extracted with ethyl acetate. The ethyl acetate was evaporated and the residue redissolved in ethanol. Small samples of each of the purified compounds were reinjected and checked for purity using the same chiral HPLC

system. Fractions **I** and **II** showed an optical purity of 99% and 97%, respectively. The overall yield obtained for **3b** following reverse-phase and Chiral HPLC separations was 50 %.

Partition Coefficients

The following procedure was used to determine partition coefficients. The radioligand was mixed with 3 g each of 1-octanol and buffer (pH 7.4, 0.1 M phosphate) in a test tube. The mixture was vortexed for 3 min at room temperature and then centrifuged (4000 rpm) for 5 min. Two aliquots from each layer (0.5 g each) were counted in a well counter. The partition coefficients were determined by calculating the ratio of cpm/g of octanol to that of buffer. Samples from the octanol layer were repartitioned until consistent values were obtained (usually after 3 determinations).

Plasma Protein binding

The following methods were used to determine plasma protein binding of **3b**, Fractions **I** and **II**: Precipitation method. Rat plasma (0.1 mL) was diluted 10-fold with water (1 mL total volume) and mixed with [^{125}I]I-TBZ-**3b** in a test tube. The solution was incubated for 30 minutes at 37 °C. To this mixture, 0.4 mL of 10% trichloroacetic acid (TCA) was added. The test tube was centrifuged for 10 min.(4000 rpm), and the top aqueous layer was removed and transferred to another test tube for counting. The precipitate was washed with water (0.5 mL x 2) and the water was combined with the top aqueous layer (from above) and counted. The residual precipitate was also counted. The protein binding was obtained by dividing counts in the precipitate with counts in the aqueous layer.

Filtration method. Rat plasma (0.1 mL) was diluted 10-fold with water (1 mL final volume) and 0.5 mL of this mixture was combined with [^{125}I]I-TBZ-**3b** in a test tube. After 30 min of incubation at 37 °C, 3 x 50 μL aliqouts of liquid was removed for determination of the total counts. The remaining liquid was passed through an Amicon Micropartition System (W. R. Grace and Co., Danvers, MA) by centrifugation for 20 minutes (2000 rpm) at 0 °C. Four samples (50 μL each) were removed from the filtered solution and counted. The protein binding (% bound) was calculated by using the unit counts in filtrate divided by total counts in liquid prior to filtration.

Biodistribution in rats

Organ biodistribution. While under ether anesthesia, each rat was injected in the femoral vein with 0.2mL saline solution containing either the racemic mixture of [^{125}I]I-TBZ-**3b** (1-5uCi), or Fraction **I** or Fraction **II**. The rats were sacrificed at various time points postinjection by cardiac excision. The tissues were weighed and the radioactivity was measured with a Canberra gamma counter (Model 5000). The percentage dose per organ was calculated by a comparison of the tissue counts to suitably diluted aliquots of the injected material. Total activities of blood and muscle were calculated assuming that they were 7% and 40% of total body weight, respectively.

Brain regional biodistribution. Regional distribution of [^{125}I]I-TBZ-**3b** (with either the racemic mixture or Fractions **I** or **II**) in rat brain was obtained after i.v. injection. By dissecting,

weighing and counting samples from different brain regions (cortex, striatum, hippocampus and cerebellum), % dose/gram of the samples was calculated by comparing the sample counts with the counts of the diluted initial dose. The ratio of uptake in each region was obtained by dividing % dose/gram of each region with that of the cerebellum. The effect of tetrahydrobenazine on the uptake of Fraction I or Fraction II was investigated by pretreating the rats with the TBZ (2 mg/rat) at 3 min before iv injection of [^{125}I]I-TBZ-**3h**. At 20 min. post injection the rats were dissected and the regional brain dissection was carried out as above.

Appendix 1

Elemental analysis data.

Compound **4**.

	Calculated	Found
C	73.44	73.50
H	8.51	8.48
N	4.08	4.09

Compound **5**.

C	62.47	62.42
H	9.05	9.10
N	2.21	2.26

Compound **3**.

C	53.51	53.27
H	6.41	6.56
N	2.97	2.81

Acknowledgements

This work is supported by a grant from Nihon Medi-Physics Inc. The authors wish to thank Karen Loomis for her assistance in preparing this manuscript.

References

1. Kanner BI, Schuldiner S. CRC Critical Reviews in Biochem. 22: 1 (1987).

2. Cooper JR, Bloom FE, Ruth RH. In Biochemical Basis of Neurochemistry 5th ed. Oxford University Press, New York, p. 290 (1986).

3. Weiner N. Drugs that Inhibit Adrenergic Nerves and Block Adrenergic Receptors. In The Pharmacological Basis of Therapeutics, 7th Ed. Gilman AG, Goodman LS, Rall TW, Murad F, Eds., Macmillan Publishing Co., New York, p. 208 (1985).

4. Neumeyer JL. Neuroleptics and axiolytic agents. In Principles of Medicinal Chemistry, Foye WO, Ed. Lea and Febiger, Philadelphia, PA, (1981).

5. Scherman D, Jaudon P, Henry J-P. Proc.Natl. Acad. Sci. 80: 584, 1983; Scherman D, Boschi G, Rips R, Henry J-P. Brain Research 370: 176 (1986); Scherman DJ. Neurochem. 47: 331 (1986); Scherman D, Raisman R, Ploska A, Agid Y-J. Neurochem. 50: 1131 (1988).

6. Darchen F, Masuo Y, Vial M, Rostene W, Scherman D. Neurosci 33: 341 (1989).

7. Henry JP, Sherman D.(Commentary). Biochem Pharmacol 38: 2395 (1989).

8. Meshgin-Azarian S, Chang W, Cugier DL, Vincent MS, Near JA. J Neurochem 50: 824 (1988).

9. Near JA. Mol Pharmacol 30: 252 (1986).

10. Scherman D, Gasnier B, Jaudon P, Henry JP. Mol Pharmacol 33: 72, 1988.

11. Suchi R, Stern-Bach Y, Gabay T, Schuldiner S. Biochem 30: 6490, 1991.

12. Masuo Y, Pelaprat D, Scherman D, Rostene W. Neurosci Lett 114: 45, 1990.

13. Kaiser C, Setler PE. Antipsychotic agents. Burger's Medicinal Chemistry, 4th Ed. Wolf ME, ed. Wiley-Interscience, New York, pp. 860-964 (1981).

14. Clarke FH, Hill RT, Koo J, Lopano RM, Maseda MA, Smith M, Soled S, VonVeh G, Vlattas I. J Med Chem 21: 785 (1978).

15. Saner A, Pletscher A. J Pharmacol Exp Ther 203: 556 (1977).

16. Lednicer D, Mitscher LA. The Organic Chemistry of Drug Synthesis. Wiley-Interscience Inc., New York, pp. 349-361 (1977).

17. Fahrenholtz KE, Capomaggi A, Lurie M, Goldberg MW, Kierstead RW. J Med Chem 9: 304 (1967).

18. Harnden MR, Short JH. J Med Chem 10: 1183 (1967).

19. Tretter JR. U.S. Patent No. 3,053, 845 (1962).

20. Pletscher A, Brossi A, Gey KF. Rev Neurobiol 4: 275 (1962).

21. Brossi A, Lidlar H, Walter M, Schnider O. Helv Chim Acta 41: 119 (1958).

22. Ali H, Rousseau J, Ghaffari MA, van Lier JE. J Med Chem 31: 1946 (1988), Ali H, Rousseau J, Ghaffari MA, van Lier JE. J Med Chem 34: 854 (1991).

23. Kung M-P, Canney DJ, Guo YZ, Billings JJ, Kung HF. Soc Neurosc (Abstract 202.13), 18: 475 (1992).

24. DaSilva JN, Kilbourn MR, Mangner TJ, Toorongian SA. IXth Int Symp Radiopharm Chem Abstract F6, p. 257 (1992).

25. Murphy RA, Kung HF, Kung M-P, Billings JJ. J. Med. Chem. 33: 171 (1990).

26. Kung M-P, Chumpradit S, Billings J, Kung H. Life Sci. 51: 95 (1992).