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# Synthesis and *In Vivo* Evaluation of a $^{99m/99}$Tc–DADT–Benzovesamicol: a Potential Marker for Cholinergic Neurons

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The diaminodithiol (DADT) ligand has been conjugated to the neuromuscular blocking agent benzovesamicol (BVM) in the 5-position. DADT–BVM **1** was synthesized by coupling of 5-aminomethylbenzovesamicol with a BCA thiolactone reagent. $^{99m}$Tc radiolabeling of **1** with [$^{99m}$Tc]glucoheptonate gave a 4.7:1 mixture of two $^{99m}$Tc complexes as determined by HPLC. Biodistribution data of the major [$^{99m}$Tc]-**1** complex in CD-1 mice ($n = 4$–5) showed very little uptake and no regional selectivity in the mouse brain. At all time points examined, the lung and liver showed the highest uptake. For whole brain, the % injected dose values were 0.27, 0.12, 0.04 and 0.01% at $t = 1$, 5, 30 and 240 min. The major [$^{99m}$Tc]-**1** product exhibited a $\log P = 3.13 \pm 0.06$ (SD) with an $IC_{50} = 140$–280 nM for the corresponding [$^{99}$Tc]-**1** vs (−)-*N*-[$^3$H]methyl-5-aminobenzovesamicol. The low brain uptake of [$^{99m}$Tc]-**1** vs 5-iodobenzovesamicol is attributed to its higher molecular weight (752) and lower binding affinity.

## Introduction

5-[$^{125/123}$I]Iodobenzovesamicol (Scheme 1, $X = I$) is a stereospecific radiotracer for mapping cholinergic neurons in the brain (Jung *et al.*, 1990, 1993, 1994). (−)-2*R*,3*R*-5-[$^{123}$I]Iodobenzovesamicol is undergoing clinical trials as a potential SPECT clinical agent for the study of Alzheimer's disease (Kuhl *et al.*, 1993). A structural study of benzovesamicol (BVM) derivatives by Rogers *et al.* (1989) showed that substitution of a variety of large functional groups, including biotin, in the 5-position of BVM had little effect on *in vitro* potency. *In vivo* studies of the neuronal mapping potential of a series of iodobenzovesamicols have revealed that considerable bulk tolerance exists in positions 5, 6 and 7 of BVM (Jung *et al.*, 1990, 1993, 1994).

These observations led to the hypothesis that a technetium-99m chelate group might be introduced in the 5-position of BVM without adversely affecting its *in vivo* neuronal mapping characteristics (Scheme 2, [$^{99m}$Tc]-**1**). The chief advantages of a $^{99m}$Tc-radiolabeled BVM are that it: (1) permits the use of the more readily accessible and inexpensive $^{99m}$Tc and (2) allows the possible development of a convenient "kit" formulation. In this paper, we describe the synthesis of the first diaminedithiol (DADT) conjugate of BVM and a preliminary evaluation of its *in vivo* properties.

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## Experimental

### *(±)-trans-5-Cyano-2-hydroxy-3-(4-phenylpiperidino)-tetralin(5-cyanobenzovesamicol)*

To a cooled (≈5°C) heterogeneous solution of (±)-5-aminobenzovesamicol (0.5 g, 1.55 mmol) (Rogers *et al.*, 1989) (Scheme 1, $X = NH_2$) in concentrated HCl (3 mL) and water (10 mL) was added dropwise a cooled solution of sodium nitrite (114 mg, 1.64 mmol) in water (5 mL). The reaction temperature of the resulting clear yellow solution was maintained below 10°C. The diazonium salt was neutralized with sodium carbonate and added to a solution (≈5°C) of nickel chloride (502 mg, 3.88 mmol) and potassium cyanide (808 mg, 12.40 mmol) in 20 mL water. The reaction mixture was stirred for 1 h, allowed to warm to room temperature and stirred for an additional 3 h. The mixture was then gently warmed on a water bath (2 h) and the ensuing brown precipitate was filtered off. The brown precipitate was refluxed for 3 h with ethylene diamine (1 mL) and KCN (1.0 g) in 95% ethanol. The solvent was removed under reduced pressure and the residue was taken up in ethyl

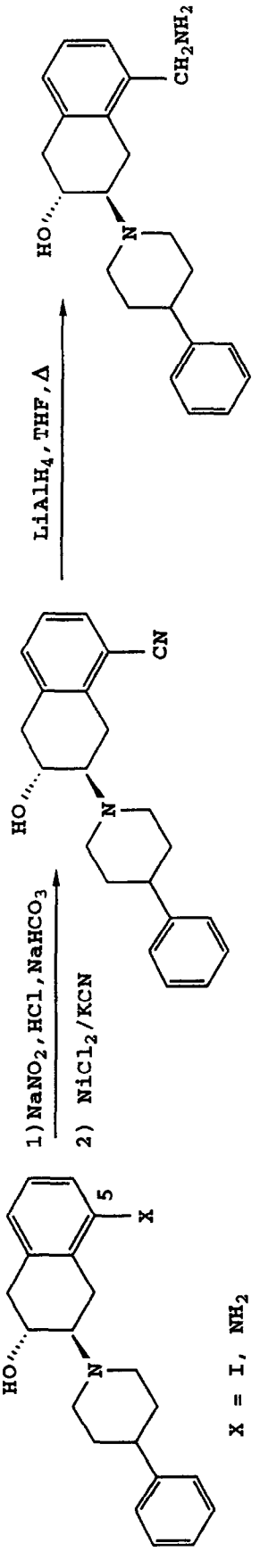
acetate, poured in saturated $NaHCO_3$ and extracted with ethyl acetate. The combined extracts were dried over $Na_2SO_4$ and rotoevaporated to dryness. The residue was purified by flash chromatography (ethyl acetate/hexane, 3:7) on silica V to give 5-cyanobenzovesamicol [(372 mg, 72%); $^1$H-NMR ($CDCl_3$, 360 MHz): $\delta$ 1.72–1.98 (m, 4H), 2.48 (t, d, $J$ = 11.4, 2.1 Hz, 1H), 2.61 (m, 1H), 2.81–2.99 (m, 6H), 3.27 (d, d, $J$ = 16.2, 4.4 Hz, 1H), 3.35 (d, d, $J$ = 16.2, 5.7 Hz, 1H), 3.90 (t, d, $J$ = 10.2, 5.7 Hz, 1H), 4.30 (br. s, OH), 7.18–7.38 (m, 7H), 7.50 (d, $J$ = 7.5 Hz, 1H); MS (EI, 70 eV) *m/e* (relative abundance) 332 (100.0, $M^+$), 315(7.9), 301(2.2), 287(1.0), 254(2.7), 227(1.2), 213(2.7), 202(5.8), 174(77.8), 161(23.2), 155(24.0), 141(10.8); high resolution MS (EI, 70 eV) calcd for $C_{22}H_{24}N_2O$: 332.1889; found 332.1902].

*(±)-trans-5-Aminomethyl-2-hydroxy-3-(4-phenylpiperidino)-tetralin, 5-aminomethylbenzovesamicol*

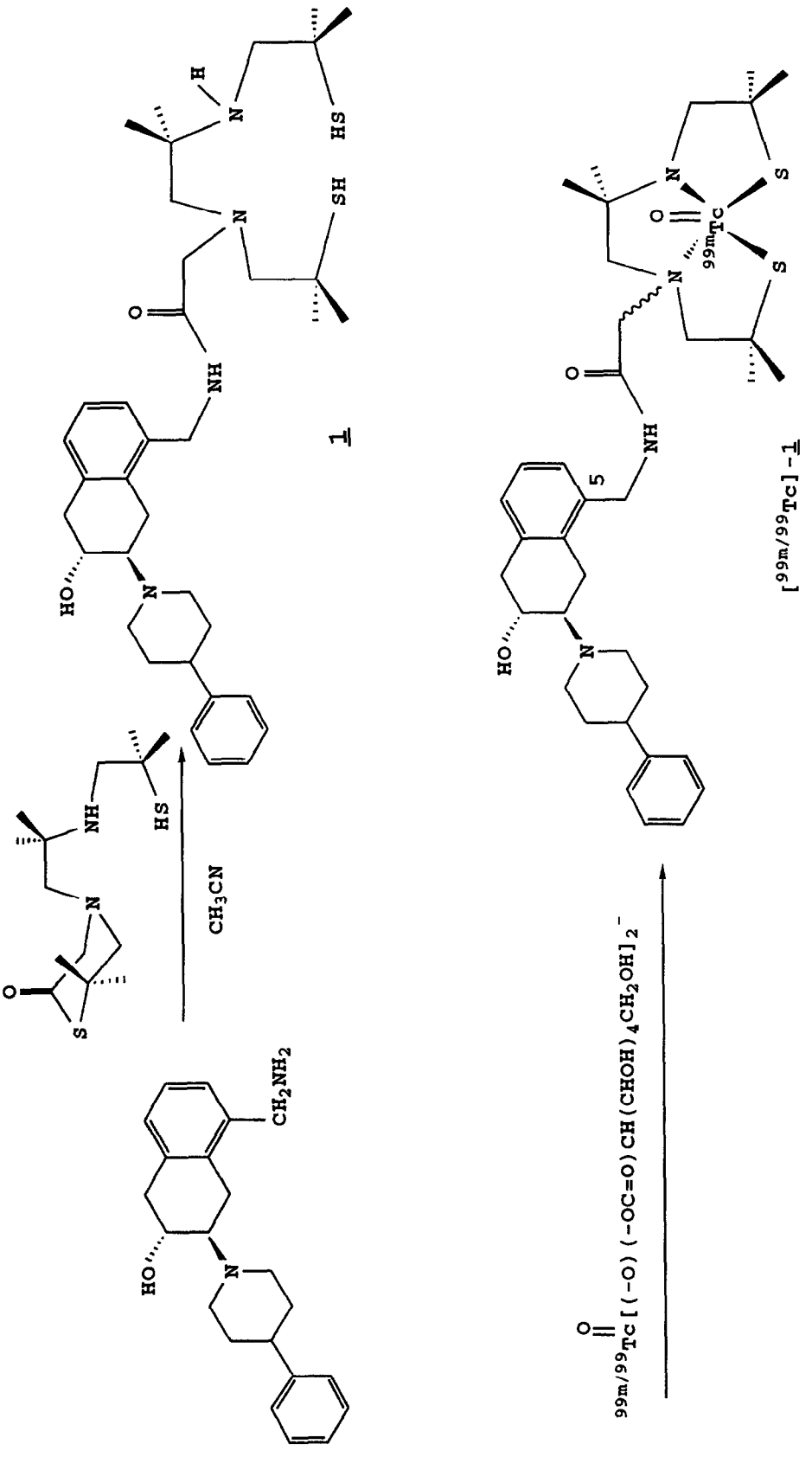
To a solution of 5-cyanobenzovesamicol (620 mg, 1.87 mmol) in dry THF (30 mL) was added dropwise lithium aluminum hydride (10.0 mL of 1.0 M solution in diethyl ether). The mixture was refluxed with stirring for 3 h. The mixture was cooled to room temperature and the unreacted hydride decomposed by addition of ethyl acetate. The mixture was poured into a 2.0 N NaOH solution and extracted with $CH_2Cl_2$. The combined $CH_2Cl_2$ extracts were dried over anhydrous $Na_2SO_4$ and evaporated to dryness under reduced pressure. The residue was flash-chromatographed on alumina with $CHCl_3$/ethanol (97:3) to afford 5-methylaminobenzovesamicol [(489 mg, 78%), $^1$H-NMR ($CDCl_3$, 360 MHz): $\delta$ 1.62–2.05 (m, 4H), 2.46 (t, d, $J$ = 11.3, 1.8 Hz, 1H), 2.68–3.06 (m, 7H), 3.31 (d, d, $J$ = 16.0 Hz, 5.6 Hz, 1H), 3.80–3.93 (m, 3H), 7.05–7.35 (m, 8H)].

*(±)-trans-2-Hydroxy-3-(4-phenylpiperidino)-5-[7-(2,2,5,5,9,9-hexamethyl-4,7-diaza-1,9-dithiadecane)-methylcarbamoyl]methyl-tetralin, DADT–BVM, 1*

To a 5 mL vial equipped with a micro stir bar containing 56.6 mg (0.18 mmol) of 5-methylaminobenzovesamicol was added a solution of the BCA thiolactone compound (Baidoo and Lever, 1990a) (184.4 mg, 0.50 mmol) in 600 $\mu$L acetonitrile. The slurry became a homogeneous light pink–orange clear solution and the mixture was stirred at room temperature. The reaction was monitored by TLC [silica, ethyl acetate/hexane (1:1)]. The desired DADT–BVM conjugate was visualized with bromocresol green and appeared as a streak near the origin. (Excess thiolactone appeared at $R_f > 0.5$.) After 3 h the reaction mixture was purified directly by flash chromatography on silica (13.0 g, K60, 230 mesh, 0.063 mm, EM Sciences) using ethyl acetate/hexane (1:1) as eluent. After elution of the excess thiolactone, the desired DADT–BVM fractions were pooled, rotoevaporated to an oil and dried overnight *in vacuo* to yield **1** as a waxy solid [(64.2 mg, 58%), $^1$H-NMR ($CDCl_3$, 300 MHz): $\delta$ 1.06 (s, 6H) 1.28 (s,




Scheme 1. Synthesis of 5-aminomethylbenzovesamicol.




Scheme 2. Synthesis of DADT–BVM, 1, and [$^{99m}$Tc]-1.

6H), 1.38 (s, 6H), 1.55–2.00 (br, $CH_2$, S–H, N–H and O–H, 8H), 2.74 (s, 2H), 2.63 (s, 2H), 2.48 (s, 2H), 2.36–3.08 (m, overlap, 9H), 3.31 (d, d, $J = 16.4$ Hz, 5.7 Hz, 1H), 3.49 (s, 2H), 3.85 (t, d, $J = 10.5$, 5.7 Hz, 1H), 4.48 (d, $J = 5.7$ Hz, 2H), 6.99–7.41 (m, 8H), 7.97 (t, br, $J \approx 6$ Hz, 1H); MS FAB: 641 ($\{M + 1\}^+$, 100%), 607 ($\{M\text{-}H_2S\}^+$, 11.2%), 553 (11.8%), 363 (19.1%), 337 (12.9%); MS HRFAB calcd for $\{C_{36}H_{56}N_4O_2S_2\}H^+$: 641.3926; found: 641.3928)]. For storage and subsequent radiochemistry, the conjugate was converted to its oxalate salt.

*$^{99m/99}$Tc radiolabeling of DADT–BVM, 1*

Into a 2.0 mL glass MDV containing [$^{99m}$Tc]pertechnetate (30–40 mCi/0.5 mL saline) was added a 0.25 mL aliquot of Glucoscan (NEN, Billerica, Mass.) solution (200 mg sodium glucoheptonate/1 mL). The contents were allowed to stand for 15–20 min at room temperature. Upon addition of 0.50 mL 0.1 M $NH_4OAc$ buffer, a solution of $\leqslant 1$ mg of **1** (oxalate salt) in 1.0 mL absolute ethanol was then added to the [$^{99m}$Tc]glucoheptonate solution. The reaction mixture was allowed to stand at ambient temperature for 1 h with periodic mixing. The mixture was filtered through a Nalgene 4 mm cellulose acetate syringe filter (Waters–Millipore, Milford, Mass.) into a clean 2.0 mL MDV to remove any insoluble material. The filtrate was purified by reverse phase HPLC [Waters Nova Pak C18, 3.9 × 300 mm, 0.4 mL/min, ethanol/0.1 M $NH_4OAc$ (55:45)] to give two radiolabeled products [$R_t = 55$ and 61 min (4.7:1 ratio)] in 20–35% isolated radiochemical yield. The major [$^{99m}$Tc]-**1** peak was collected and utilized for all *in vitro* and *in vivo* studies.

For $IC_{50}$ studies, **1** was radiolabeled with [$^{99}$Tc]glucoheptonate (De Kieviet, 1981). Impure $NH_4TcO_4/TcO_2$ (1.9 mCi, 0.20 g) (NEN, Billerica, Mass.) was first purified according to the method of Deutsch (Stepniak-Biniakiewicz *et al.*, 1992). The resulting white $NH_4TcO_4$ residue (2.0 mmol $^{99}$Tc) was dissolved in an aqueous solution (50.0 mL) of $SnCl_2$ (Aldrich, St Louis, Mo.) (219 mg, 1.16 mmol) and D-glycero-D-gulo-heptonic acid, sodium salt dihydrate (Aldrich, St Louis, Mo.) (8.10 g, 28.5 mmol). For radiolabeling, 1.9 mg (2.7 μmol) of **1** (oxalate salt) in 1.0 mL water/ethanol (1:1) was treated with 70 μL of the [$^{99}$Tc]glucoheptonate solution. Shortly after addition of the deep purple [$^{99}$Tc]glucoheptonate, the solution turned golden-brown. The $^{99}$Tc-radiolabeled products were isolated by HPLC as described above and co-eluted with the corresponding [$^{99m}$Tc]-**1** complexes. After several injections, the combined golden yellow HPLC fractions containing the major [$^{99}$Tc]-**1** product were rotoevaporated to near dryness and the ensuing fine golden yellow precipitate ($\approx 2$ μCi) was carefully collected by gravity filtration through a glass pipet containing a plug of glass wool. The precipitate was dissolved in a minimum amount of ethanol, degassed and stored at −70°C under argon.

*Determination of log P*

The log *P* of the major [$^{99m}$Tc]-**1** product was determined by an analogous procedure used for [$^{99m}$Tc]-*p*-iodophenethyldiaminodithiol (Shiba *et al.*, 1992). Fifty (50) μCi of [$^{99m}$Tc]-**1** was added to 3.5 mL 0.1 M phosphate buffer (pH = 7) and 3.5 mL 1-octanol. The mixture was inverted 60 times and centrifuged for 20 min. A sample of the octanol (10–20 μL) and buffer (100–200 μL) layers was assayed on a Packard Minaxi 5000 γ-counter. Log *P* was calculated from the octanol/buffer cpm ratio. The major portion of the octanol layer (3.0 mL) was diluted with 0.5 mL octanol and mixed with a fresh portion of 3.5 mL phosphate buffer. The equilibration procedure described above was repeated until a constant value of log *P* was obtained. From six equilibrations, the log *P* of the major [$^{99m}$Tc]-**1** product was determined to be $3.13 \pm 0.06$ (SD).

*$IC_{50}$ determination*

The $IC_{50}$ was measured using a modified competitive binding assay (Bennett, 1978) employing (−)-*N*-[$^3$H]methyl-5-aminobenzovesamicol (Jung *et al.*, 1993, 1994) and [$^{99}$Tc]-**1**. The assays were performed by incubating tritiated compound, [$^{99}$Tc]-**1** (>98% pure by HPLC), cortex homogenate extract (2 μg protein/μL) and buffer for 1 h at 37°C (total incubation volume = 500 μL). The protein bound activity was isolated on Whatman GF/B filters (Brandel Inc., Gaithersberg, Md) using a Brandel IP-48LT Cell Harvester. After washing, the filters were cut out and mixed with scintillation fluid. The decrease of $^3$H bound activity and corresponding increase in $^{99}$Tc activity as a function of [$^{99}$Tc]-**1** concentration were measured using a Packard Tri-carb 4530 scintillation counter using the appropriate channels (0–19 for $^3$H and 20–292 MeV for $^{99}$Tc).

*Animal studies*

Radiotracer was formulated for animal injection by a 9-fold dilution of the HPLC purified product with physiologic saline. Female CD-1 mice (20–30 g) were anesthesized (ether), administered 10–20 μCi via i.v. tail vein injection of the above formulation. The mice were sacrificed at 1, 5, 30 and 240 min, respectively and the tissues harvested and counted on a Packard Minaxi 5000 γ-counter. Biodistribution data in the brain and peripheral tissues are given in Tables 1 and 2.

The blood activity contribution to the brain tissues (Table 1) was estimated based on whole body and regional brain blood volume literature data for the rat (Lee and Blaufox, 1985; Cremer and Seville, 1983). The whole body blood volume has been determined to be only ≈4.5–6.3% (Lee and Blaufox, 1985). The regional blood volumes for cortex, cerebellum, hypothalamus and hippocampus ranged from 7.1 to 10.89 μL/1000 mg brain tissue (Cremer and Seville, 1983). Using these values, the blood

Table 1. Brain uptake (% dose/g ± SD) of [$^{99m}$Tc]-**1** in CD-1 mice

| | % Dose/g | | | |
|---|---|---|---|---|
| | 1 min ($n = 4$) | 5 min ($n = 4$) | 30 min ($n = 4$) | 240 min ($n = 5$) |
| Striatum | 0.39 ± 0.07 | 0.14 ± 0.01 | 0.061 ± 0.010 | 0.016 ± 0.006 |
| Cortex | 0.46 ± 0.04 | 0.20 ± 0.04 | 0.069 ± 0.009 | 0.015 ± 0.002 |
| Cerebellum | 0.69 ± 0.03 | 0.26 ± 0.03 | 0.095 ± 0.021 | 0.019 ± 0.005 |
| Hypothalamus | 0.50 ± 0.09 | 0.24 ± 0.04 | 0.13 ± 0.04 | 0.050 ± 0.059 |
| Hippocampus | 0.44 ± 0.02 | 0.22 ± 0.05 | 0.098 ± 0.034 | 0.025 ± 0.006 |

contribution was estimated as follows: with the cerebellum as an example, the amount of blood in 71 mg (mean weight) of cerebellum was calculated to be $\approx 0.773\,\mu$L. From Table 2 ($t = 1$ min), the blood activity contribution in the cerebellum is therefore $0.773\,\mu\text{L} \times 10^3\,\mu\text{g}/\mu\text{L} \times 28.7\%$ ID/$10^6\,\mu$g or ≈0.022% ID or ≈0.31% ID/g cerebellum (0.022% ID/0.071 g cerebellum). The blood activity contribution in the cerebellum is therefore 0.31/0.69 (Table 1) or ≈45% of the total cerebellum activity at 1 min. For other brain sections the blood activity was calculated as: %ID (blood, brain tissue)/g brain tissue ≈ blood volume ($\mu\text{L mg}^{-1}$) × % ID/g blood (at time $t$) assuming a blood density of 1 mg/$\mu$L.

## Results and Discussion

There are only very few published reports of potential $^{99m}$Tc brain receptor mapping reagents. The feasibility of *in vitro* and *in vivo* receptor mediated targeting using small organic molecules labeled with $^{99m}$Tc has been demonstrated unequivocally by Katzenellenbogen and Davison (DiZio *et al.*, 1991, 1992). They published the first detailed study of Re and Tc DADT progestin receptor agents which exhibited high binding affinity to a number of steroid receptors. Lever and co-workers (Lever and Wagner, 1990; Lever *et al.*, 1994) have prepared a [$^{99m}$Tc]-DADT conjugate of quinuclidinyl benzylate (QNB). Receptor binding studies indicated that the affinity of the complex for the muscarinic receptor was in the micromolar range as compared to the nanomolar affinity of QNB itself. A group at Squibb has also prepared a number of QNB–BATO conjugates suitable for $^{99m}$Tc labeling (Nanjappan *et al.*, 1993). Realizing that modification of spiperone at the amide position with large substituents resulted in retention of high affinity for the dopamine D-2 receptor, Ballinger and co-workers prepared a $^{99m}$Tc dithiocarbamate conjugate (Ballinger *et al.*, 1989). However neither the ligand precursor nor the $^{99m}$Tc complex was structurally characterized. The $^{99m}$Tc-radiolabeled product had limited stability and showed negligible brain uptake *in vivo*. In the course of our studies with [$^{125/123}$I]iodobenzovesamicols (Jung *et al.*, 1990, 1993, 1994; Kuhl *et al.*, 1993), it became evident that the precursor, 5-aminobenzovesamicol (Rogers *et al.*, 1989), could easily be functionalized to include a benzylic amine for conjugation to a $^{99m/99}$Tc–DADT chelate.

The $^{99m/99}$Tc ligand DADT–BVM **1** was synthesized by coupling of 5-aminomethylbenzovesamicol (Scheme 1) with the previously reported BCA thiolactone reagent (Baidoo and Lever, 1990a) (Scheme 2). 5-Aminomethylbenzovesamicol was prepared in two steps by cyanation of 5-aminobenzovesamicol (Rogers *et al.*, 1989) followed by reduction with lithium aluminum hydride (Scheme 1). The analytical data for **1** confirms its structure as a 1:1 adduct analogous to that obtained from the reaction of the BCA thiolactone reagent with benzylamine (Baidoo and Lever, 1990b). The 300 $^1$H-NMR spectrum of **1** is characterized by resonances at 7.97 ppm (amide proton), a doublet (two protons) at 4.48 ppm (Ar-C$H_2$-NH-) and a singlet (2 protons) at 3.49 ppm [-CO-C$H_2$-N(C$H_2$)-] in addition to those for the parent BVM and thiolactone precursors. The chemical shift assignments of the two methylene protons are in close agreement with values predicted by Shoolery's rules (Gordon and Ford, 1972) and those of a DADT–benzylamine conjugate (Baidoo and Lever, 1990b). The S–H and N–H resonances appear as a broad overlap at ≈1.8 ppm as has been typically observed for a number of DADT-conjugates (DiZio *et al.*, 1991; Shiba *et al.*, 1991, 1992).

$^{99m/99}$Tc radiolabeling of **1** (Scheme 2) with $^{99m/99}$Tc–glucoheptonate proceeded smoothly to give two [$^{99m/99}$Tc]-**1** complexes (ratio = 4.7/1 for $^{99m}$Tc and 3.3/1 for $^{99}$Tc). As depicted in Scheme 2, the production of two $^{99m/99}$Tc products is consistent with the expected formation of *syn* and *anti* diastereomeric complexes arising from the two possible orientations of the N-substituent with respect to the Tc(=O)$N_2S_2$ plane. In earlier work by Lever and co-workers on a number of N-substituted DADT technetium complexes, the structures of the major and minor product(s) were assigned as the *syn* and *anti* isomers, respectively (Lever *et al.*, 1985, 1990; Lever and Wagner, 1991). In the case of the *N*-ethylpiperidinyl–DADT complexes, it was also found that the major (*syn*) product exhibited better brain uptake (Lever *et al.*, 1985).

In view of these previous findings, its higher yield and easier purification, the predominant [$^{99m/99}$Tc]-**1** peak ($R_t = 55$ min) was chosen for all subsequent preliminary *in vitro* and *in vivo* screening described below. HPLC analysis of the HPLC purified major [$^{99m}$Tc]-**1** complex revealed no degradation throughout the duration of the animal experiments. Cumulative biodistribution data for [$^{99m}$Tc]-**1** in CD-1 mice ($n = 4$–5) after 1, 5, 30 and 240 min post-injection are

Table 2. Uptake (% dose/g ± SD) of [$^{99m}$Tc]-**1** in peripheral tissues

| | % Dose/g | | | |
|---|---|---|---|---|
| | 1 min ($n = 4$) | 5 min ($n = 4$) | 30 min ($n = 4$) | 240 min ($n = 5$) |
| Thyroid | 14.52 ± 5.45 | 8.778 ± 3.04 | 5.80 ± 2.28 | 1.24 ± 0.19 |
| Atria | 16.50 ± 4.75 | 11.326 ± 2.35 | 3.04 ± 0.45 | 0.40 ± 0.12 |
| Ventricles | 19.91 ± 2.62 | 12.019 ± 1.93 | 2.40 ± 0.39 | 0.43 ± 0.04 |
| Lung | 41.10 ± 3.26 | 20.373 ± 3.16 | 13.72 ± 1.43 | 3.57 ± 1.36 |
| Liver | 43.08 ± 4.94 | 46.806 ± 8.22 | 24.42 ± 3.27 | 7.93 ± 0.79 |
| Small intestine | 3.52 ± 0.53 | 7.249 ± 2.62 | 14.08 ± 4.23 | 2.67 ± 0.60 |
| Blood | 28.74 ± 2.66 | 9.628 ± 1.95 | 1.47 ± 0.21 | 0.27 ± 0.02 |

given in Tables 1 and 2. At all time points examined, the lung and liver showed the highest uptake (Table 2). Table 1 shows that [$^{99m}$Tc]-**1** is rapidly cleared from the brain within 4 h. For whole brain, the % ID values were 0.27, 0.12, 0.04 and 0.01% at $t = 1$, 5, 30 and 240 min. In contrast to the biodistribution of 5-iodobenzovesamicol (Jung *et al.*, 1990), no regional selectivity was observed in the striatum, cortex and cerebellum as well as in the heart at all time points examined (Tables 1 and 2). Interpretation of the low brain uptake (Table 1) is further complicated by the corresponding high activities in the blood (Table 2). The blood activity contributions in the brain tissues were calculated to be significantly high, particularly at $t = 1$ and 5 min. The blood activity contribution was estimated using regional brain blood volume data for the rat (Cremer and Seville, 1983) and the observed blood activity data (Table 2). The blood activity contributions in the cortex, cerebellum, hypothalamus and hippocampus were calculated to be 42–57% at 1 min and 29–44% at 5 min of the values reported in Table 1. At later time points, the contributions were significantly lower (8–19 and 4–16% at 30 and 240 min, respectively).

Two principal factors could account for the observed low brain uptake. First, the relatively high molecular weight (752) and larger size of the [$^{99m}$Tc]-**1** complex may have substantially reduced brain permeability. A study by Levin (1980) proposed that an upper limit of 657 exists for crossing the blood–brain barrier. Although other compounds heavier than 657 have shown reasonable brain uptake, these data provided a qualitative limit of ≈600 for most technetium complexes synthesized to date (Nowotnik, 1992).

The measured log *P* of the major [$^{99m}$Tc]-**1** product (3.13) is slightly lower than that of 5-iodobenzovesamicol (3.37) (Jung *et al.*, 1993, 1994). This suggests that the complex is sufficiently lipophilic to cross the **BBB**. The $IC_{50}$ of the corresponding (±)-$^{99}$Tc-**1** (>98% pure by HPLC) was measured using a modified competitive binding assay (Bennett, 1978) employing (−)-*N*-[$^3$H]methyl-5-aminobenzovesamicol (Jung *et al.*, 1993, 1994). Over a concentration range of <1 μM in (±)-$^{99}$Tc-**1**, a decrease in (−)-*N*-[$^3$H]methyl-5-aminobenzovesamicol binding was observed, accompanied by a corresponding increase in (±)-$^{99}$Tc-**1** bound activity. The $IC_{50}$ of (±)-$^{99}$Tc-**1** (140–280 nM) was found to be significantly higher than that of (±)-5-iodobenzovesamicol ($IC_{50} = 2$ nM). The low brain uptake of [$^{99m}$Tc]-**1** is likely due to its large mass; its lack of regional brain selectivity may be attributed to its reduced affinity for the vesamicol binding site.

## Conclusion

We have successfully prepared the first DADT–BVM conjugate **1** and have labeled it with $^{99m/99}$Tc. Although the major $^{99m}$Tc-**1** product showed a lipophilicity similar to that of 5-iodobenzovesamicol, very little uptake and no regional selectivity was observed in the mouse brain. Considered together, the *in vitro* and *in vivo* data for $^{99m/99}$Tc-**1** suggest that its low uptake and retention in the brain are probably due to a combination of both increased size and reduced binding affinity. These initial results not only demonstrate that BVM can be labeled with $^{99m}$Tc, but that labeling can be accomplished without (a) greatly modifying the lipophilicity of the parent molecule or (b) loss of binding ability. Although the $IC_{50}$ of [$^{99m/99}$Tc]-**1** approaches the micromolar range, the binding data is encouraging and suggests that further improvements could be made by reduction of the chelate size (O'Neil *et al.*, 1993). These findings help define the limitations imposed on the design of a $^{99m}$Tc labeled vesamicol analogs for *in vivo* cholinergic nerve mapping. Future efforts will be guided by these constraints.

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