Technetium-99m bis (Aminoethanethiol) Complexes with Amine Sidechains—Potential Brain Perfusion Imaging Agents for SPECT

S.M.N. Efange, H.F. Kung, J. Billings, Y.-Z. Guo, and M. Blau

Department of Nuclear Medicine, State University of New York at Buffalo, Buffalo, New York, and Department of Radiology, Harvard Medical School, Boston, Massachusetts

In an effort to develop new clinically useful technetium-99m bis(aminoethanethiol) ([^{99m}Tc]BAT) complexes for the evaluation of regional cerebral perfusion, two new BAT ligands containing amines in the sidechain were synthesized and subsequently complexed with ^{99m}Tc to yield the target complexes: [^{99m}Tc]DEA and [^{99m}Tc]TMPDA. Each complex was obtained as mixtures of two isomers, *syn* and *anti*, which were separated chromatographically. In biodistribution studies, both isomers of [^{99m}Tc]TMPDA showed little uptake in the brain. In contrast, the brain uptake values at 2 and 15 min for [^{99m}Tc]DEA-*anti* were 0.99 and 0.26, whereas, the corresponding values for DEA-syn were 2.27, 0.64% dose/organ, respectively. Autoradiographic studies (in rats) using both isomers of [^{99m}Tc]DEA show a fixed regional distribution and a higher concentration of radioactivity in the gray matter relative to the white matter. Planar imaging using [^{99m}Tc]DEA-*syn* clearly demonstrates localization of the complex in the brain with a $T_{½}$ of 41 min, suggesting some potential for use with single photon emission computed tomography.

J Nucl Med 28:1012–1019, 1987

The search for suitable agents for use as indicators of regional cerebral perfusion has yielded, in the past few years, the promising agents N,N,N′-trimethyl-N-[2-hydroxy-3-methyl-5-[iodine-123]-iodobenzyl]- 1,3-propanediamine ([^{123}I]HIPDM) and N-isopropyl-4-[^{123}I]iodoamphetamine ([^{123}I]IMP). In conjunction with single photon emission computed tomography (SPECT), both agents have demonstrated utility in the diagnosis of stroke, epilepsy, and various other cerebrovascular disorders, and they are presently in Phase II clinical trials (*1–9*). The limited availability and attendant high cost of the cyclotron-produced pure ^{123}I ($T_{½}$ = 13 hr, gamma energy 159 keV), however, place severe restrictions on the widespread routine use of these agents in nuclear medicine clinics. Considerations such as these are partly responsible for the continuing research on the development of new clinically useful and readily available brain perfusion agents labeled with ^{99m}Tc ($T_{½}$ = 6 hr, gamma energy 140 keV) for SPECT imaging.

Earlier work by Dannals et al. had demonstrated the lipid solubility of the ^{99m}Tc-bis (aminoethanethiol) (BAT) complexes (*10,11*). Based on this information and the fact that lipid solubility is one condition for blood–brain barrier permeability, Kung et al. synthesized and evaluated a series of alkyl-substituted [^{99m}Tc] BAT complexes (*12*). Biologic evaluation in rats revealed that these complexes, indeed, were taken up by the brain in significant quantities. Furthermore, based on autoradiographic studies, the distribution of these compounds in the brain reflected cerebral blood flow, with clear evidence of higher initial uptake in the gray matter (2 min after i.v. injection) relative to white matter. However, redistribution of these compounds in the brain occurred within 15 min, thereby, resulting in a homogenous distribution of radioactivity (*12*). This rapid redistribution was attributed to the absence of a trapping mechanism to prevent facile two-way transport across the cell membrane. Therefore, the high flow area (gray matter) showed higher initial uptake but faster washout; the reverse is true for the low flow area (white matter). Based on these observations, subsequent efforts were aimed at the development of [^{99m}Tc]BAT complexes with similar stability and lipid solubility that would be trapped intracellularly in the brain. Such compounds would presumably exhibit the full comple-

Received Apr. 28, 1986; revision accepted Jan. 20, 1987.

For reprints contact: Hank F. Kung, PhD, Nuclear Medicine, Dept. of Radiology, Hospital of University of Pennsylvania, Philadelphia, PA 19106.

ment of desirable characteristics for SPECT imaging agents, i.e., high initial brain uptake, fixed regional distribution, long brain retention, and a high brain/blood ratio. The present report details the synthesis and biological evaluation of those compounds, which were prepared in the initial stages of this effort.

In the design of [^{123}I]HIPDM, a pH trapping mechanism was utilized (*1*). According to this mechanism, a neutral lipid soluble molecule containing an amine substituent would be trapped intracellularly as a positively charged ammonium species following entry into the brain (*13*). The preceding was based on the reported acidity of the intracellular brain environment (pH 7.0) relative to the extracellular medium (pH 7.4). Although the success of [^{123}I]HIPDM cannot be attributed exclusively to this trapping mechanism, the pH shift hypothesis appears to be a useful working hypothesis for designing new brain imaging agents. In addition, Lever et al. (*16*) recently reported that the ^{99m}Tc complex obtained by N-functionalization of the BAT skeleton (with the N-piperidylethyl group) exhibits high initial uptake and good brain retention in rats and baboons (*16*). The use of alkylamine sidechains, therefore, was adopted to provide a trapping mechanism for the BAT system. In view of the fact that the basicity of any alkylamine substituent would depend partly on its position relative to the BAT ring, the target compound **Ia** (also referred to as DEA) was designed with one amine alpha to the ring, whereas, an additional but distally disposed amine was included in **Ib** (also referred to as TMPDA) (Fig. 1).

Another series of neutral ^{99m}Tc complexes based on propylene diamine dioxines (PnAO), first developed by Volkert et al. and later refined by Amersham, have been reported (*17–19*). Among them, the d,l-isomer of HM-PAO forms a neutral ^{99m}Tc complex showed high brain uptake and retention in brain. The uptake is related to the lipid solubility, whereas, the retention appears to be associated with the in vivo instability of this complex. This agent is presently being evaluated in European countries, and the results seem promising (*19*).

EXPERIMENTAL

General

Melting points were determined on a Nalge hot stage and are reported uncorrected. Elemental analyses were performed commercially and all values are within ± 0.4% of theoretical numbers. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian T60A* (taken in either deuterated chloroform or dimethyl sulfoxide, with tetramethylsilane as the internal standard). Infrared spectra were determined, on a Perkin Elmer 197† or a Nicolet‡ 1180 FT-1R spectrophotometer. Spectral properties were consistent with the proposed structures. High performance liquid chromatography (HPLC) was done on a Hamilton PRP-1 reverse-phase column eluted with acetonitrile/water (85:15), the radioactive eluent was detected by a sodium iodide detector and recorded on a multichannel analyzer.

Chemistry

2,3-bis(t-butoxycarbamoyl)propanoic acid (4). Potassium hydroxide (1.29, 19.90 mmol) was added to a suspension of 6.01 g (18.09 mmol) of ethyl 2,3-bis(t-butoxycarbamoyl) propanoate, 3; prepared by reacting di-t-butyl dicarbonate, following standard methods, with ethyl 2,3-diaminoproprionate dihydrochloride, which was obtained from the Pt-catalyzed hydrogenation of ethyl cyanoglyoxolate-2-oxime (*14*) and the mixture was stirred at room temperature. On completion of the reaction (monitored by TLC, 30% ethyl acetate/methylene chloride on silica gel TLC plates), the reaction was quenched with 2*M* HCl (12 ml), and the resulting solution was extracted with methylene chloride (2 × 100 ml). The organic extract was dried over anhydrous Na_2SO_4 and concentrated in vacuo to a colorless syrup. The latter crystallized from diethyl ether. The white solid product was collected by filtration, washed with hexane, and dried to provide 4.57 g (82.9%) of the product, **4**; mp 118–124°C. ^{1}H NMR (acetone-d_6) δ 1.44 (s, 18, t-butyl), 3.52 (m, 2, -CON-$\underline{CH_2}$-CH-NCO), 4.30 (m, 1, CON-CH_2-$\underline{CH}$-NCO), 5.80–6.24 (m, 3, exchangeable); IR ($CHCl_3$) ν_{max} 3,600–2,500 (broad), 1,710 cm^{-1}.

N-[2,3 - bis(t- butoxycarbamoyl)propanoyloxy]-succinimide (5). To a cooled stirring solution of 2,3-bis(t-butoxycarbamoyl)-propanoic acid, **4**, (prepared from 10.01 g, 48.81 mmol, of ethyl 2,3-diaminopropanoate dihydrochloride) in 75 ml of methylene chloride was added 5.64 g (48.91 mmol) of N-hydroxysuccinimide. N,N′-dicyclohexylcarbodiimide (11.44 g, 55.42 mmol) was subsequently added. The ice bath was removed after 15 min and stirring was continued for a total time of 1 hr. The mixture was subsequently cooled and filtered. The precipitate was washed with a small quantity of cold methylene chloride and discarded. The filtrate was then washed with water (80 ml) and saturated bicarbonate (2 × 75 ml), respectively. The organic extract was subsequently dried over anhydrous Na_2SO_4 and concentrated to a syrup. The latter was treated with diethyl ether and cooled to yield the product as a crystalline material. This material was collected

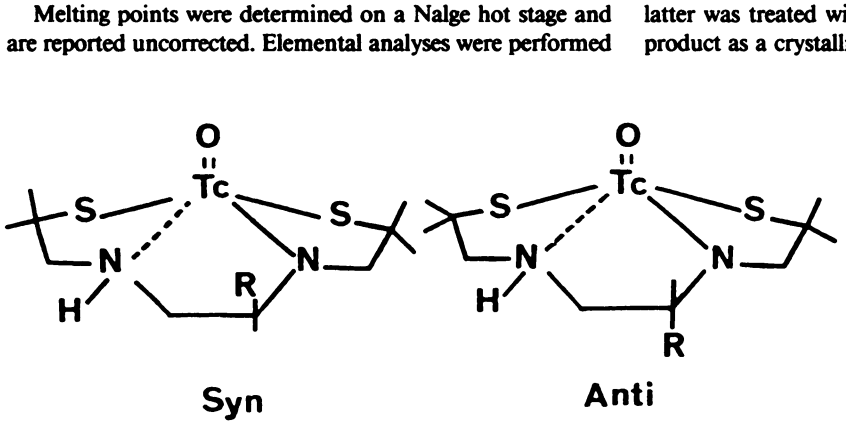


FIGURE 1
Structures of isomeric [^{99m}Tc]BAT complexes. DEA: R = $CH_2N(C_2H_5)_2$; TMPDA: R = $CH_2N(CH_3)(CH_2)_3N(CCH_3)_2$.

by filtration, washed with cold diethyl ether-hexane and dried to give 16.21 g (82.2%) of the product; mp 108°–114°C. ^{1}H NMR ($CDCl_3$) δ 1.47 (s, 18, t-butyl), 2.83 (S, 4, -CO-CH_2-CH_2 CO), 3.67 (m, 2, CON-$\underline{CH_2}$-CH-CON), 4.67 (br s, 1, -CON-CH_2-$\underline{CH}$-CON-), 5.17–6.00 (m, 2, -CONH-), -IR ($CHCl_3$), ν_{max} 3,400, 3,358, 3,280, 3,146, 2,984, 1,812, 1,783, 1,749, 1,713, 1,530, 1,452 cm^{-1}.

6-[N,N-diethylaminocarbonyl] -3,3,10,10-tetramethyl -1,2-dithia-5,8-diazacyclodeca-4,8-diene ***(8)***. 30 ml of diethyl amine was added to a suspension of 21.78 g (54.25 mmol) of the activated ester, **5**, in 100 ml of dry acetonitrile and the mixture was stirred with exclusion of moisture for 5 hr. The mixture was cooled in an ice bath and subsequently filtered; the precipitate was washed with a small amount of cold acetonitrile and discarded. The filtrate was concentrated to a syrup, which was then dissolved in 200 ml of methylene chloride and the resulting solution was washed with saturated bicarbonate (2 × 75 ml). The organic extract was dried over anhydrous Na_2SO_4 and concentrated to give the product, 2,3-bis(t-butoxycarbamoyl)-N,N-diethylpropanamide, **6a**, as a thick syrup: IR (neat) ν_{max} 3,310 (N-H, strech), 2,965, 2,925 (-H, strech), 1,710, 1,690 (carbonyl, carbamate), 1,630 (amide) cm^{-1}.

To a solution of diethyl ether (40 ml) saturated with dry HCl gas (at 25°C) was added a solution of the syrup in diethyl ether (30 ml). HCl gas was then bubbled through the resulting solution for 12–15 min during which time extensive precipitation of the deblocked compound, N,N-diethyl-(2,3-diamino)propanamide dihydrochloride, **7a** occurred. After the introduction of HCl gas was terminated, the mixture was stirred for 15 min more. The precipitate was subsequently collected by filtration, washed with diethyl ether, and added to a mixture of triethylamine (50 ml), anhydrous Na_2SO_4 (15 g) and 11.11 g (53.85 mmol) of bis(2-mercapto-2-methylpropanal) (*12*) in 70 ml of toluene. The mixture was subsequently refluxed, using a Dean–Stark trap, for 3 hr. The reaction mixture was allowed to cool to room temperature and filtered; the precipitate was washed with toluene and discarded. The filtrate was concentrated in vacuo to a minimum volume, treated with diethyl ether, and cooled. The solid obtained was collected by filtration, washed with cold diethyl ether, and dried to give 7.84 g (42.7%) of the diimine **8a**; mp 127°–129°C; ^{1}H NMR ($CDCl_3$) δ 1.13 (t,6, CH_2-$\underline{CH_3}$), 1.42 (d, 12, (S-C[CH_3]$_2$)$_2$, 3.35 (m, 5, $\underline{CH_2}CH_3$ and N-CH_2-$\underline{CH}$-N), 4.18 (m, 2, N-$\underline{CH_2}$-CH-N), 6.93 (s, 1, CH = N), 6.97 (s, 1, CH = N); IR ($CHCl_3$) ν_{max} 3,255, 2,988, 2,962, 2,922, 1,634, (amide), 1,460 cm^{-1}. Anal. calcd. for $C_{15}H_{27}N_3OS_2$: C, 54.68; H, 8.26; N, 12.75; S, 19.46. Found: C, 54.71; H, 8.25; N, 12.64; S, 19.61.

N-methyl-N-(3-N'-N'-dimethylamino)propyl [3,3,10,10-tetramethyl-1, 2 -dithia-5, 8-diazacyclodeca-4, 8-diene]-6-carboxamide ***(8b)***. To a suspension of the activated ester (12.21 g, 30.41 mmol) *5* in 60 ml of dry acetonitrile was added 3.71 g (31.92 mmol) of N,N,N'-trimethylpropane-1,3-diamine. The mixture was stirred for 2 hr, cooled to 0°C, and filtered. The filtrate was concentrated to a syrup, which was dissolved in 200 ml of CH_2Cl_2 and subsequently washed with a saturated solution of sodium bicarbonate (3 × 60 ml). The organic extract was dried over anhydrous $MgSO_4$ and concentrated to give the product 2,3-bis(t-butoxycarbamoyl)-N-methyl-N-[(3-N',N'-dimethylamino)propyl]-propanamide (**6b**), as a syrup; IR (neat) ν_{max} 3,300, 2,975, 1,710, 1,640 (amide) cm^{-1}.

The syrup was then dissolved in 60 ml of ethyl acetate and dry HCl gas was bubbled through the resulting solution for 15 min. The resulting mixture was stirred for 45 min, cooled, and filtered to give the hydrochloride of the deblocked amide, **7b**. The hydrochloride was washed with ethyl acetate and added to a mixture of triethylamine (40 ml), anhydrous Na_2SO_4 (15 g) and 6.3 g (30.5 mmol) of bis(2-mercapto-2-methylpropanal) (*12*) in 100 ml of benzene. The mixture was refluxed for 2.5 hr, allowed to cool to room temperature, and subsequently filtered. The precipitate was washed repeatedly with methylene chloride and discarded. The washes were combined with the mother liquor and the resulting solutions were washed with a saturated solution of sodium bicarbonate (80 ml), dried over anhydrous sodium sulfate, and concentrated to a minimum volume. The product crystallized on standing and was recrystallized from diethyl ether–methylene chloride to give, after drying, 3.43 g (30%) of white material, **8b**; mp 111°–113°C; ^{1}H NMR ($CDCl_3$) δ 1.35 (S, 6, C^3-CH_3 and C^{10}-CH_3), 1.45 (S, 6, C^3-CH_3 and C^{10}-CH_3), 1.73 (m, 2,-$NCH_2\underline{CH_2}CH_2N$-), 2.2 (brs, 8,-$NCH_2CH_2\underline{CH_2}N(CH_3)_2$), 2.95 (s, 3, CON-$CH_3$), 3.37 (m, 3-CON$\underline{CH_2}CH_2CH_2$N- and H_6), 6.97 (d, 2, H_4 and H_9); IR ($CHCl_3$) ν_{max} 3,433, 3,374, 2,975, 2,940, 2,863, 2,815, 2,794, 2,773, 2,718, 1,641 (amide and imine), 1,495, cm^{-1}; Anal. calcd. for $C_{17}H_{32}N_4OS_2$: C, 54.80; H, 8.66; N, 15.04; S, 17.21. Found: C, 54.56; H, 8.43; N, 14.85; S, 17.45.

One step reduction of the heterocycles ***8***. The following is a general procedure used in the reduction of the heterocycles to accomplish simultaneous reduction of the disulfide, the diimine, and the amide.

Reduction of ***8a*** *(to yield* ***9a****)*. A solution of 4.1 g (12.4 mmol) of **8a** in dry THF (50 ml) was added dropwise (under N_2) to a stirring suspension of $LiAlH_4$ in dry THF (50 ml). Subsequent to the addition, the mixture was refluxed for 18 hr and cooled in an ice bath. The reaction mixture was then quenched by careful addition of a solution of Rochelle salt (20 g in 50 ml of H_2O). The resulting mixture was then concentrated to a solid residue, which was extracted with hot ethyl acetate (5 × 150 ml). The combined extracts were dried over anhydrous Na_2SO_4 and concentrated to a syrup: IR (neat) ν_{max} 3,290, 2,950, 2,810, 2,540 (SH) cm^{-1}; ^{1}H NMR ($CDCl_3$) 0.92 (t, 6, NCH_2 $\underline{CH_3}$), 1.27 (S, 12, C-CH_3), 1.90 (S, 4, exchangeable), 2.4 (m, 13, methylene, methine).

This material was then dissolved in cold ethanol and converted to the hydrochloride by passing HCl through this solution. The hydrochloride was obtained as a white precipitate from ethanol and was collected by filtration, washed with ethanol, and dried in vacuo at 50°C to give 3.6 g (67%) of the white product, **9a**; mp 165°–169°C.

Anal. calcd. for $C_{15}H_{38}Cl_3N_3S_2$: C, 41.80; H, 8.89; Cl, 24.68; N, 9.75. Found C, 41.98; H, 8.94; Cl, 24.65; N, 9.56.

Reduction of ***8b*** *(to yield* ***9b****)*. Compound **9b** was prepared by reducing **8b** as described above. IR (neat) (of the free base) ν_{max} 3,290, 2,950, 2,810, 2,540 (SH) cm^{-1}; ^{1}H NMR (of free base, $CDCl_3$) δ 1.22 (br s, 12, C-CH^3), 2.23 (m, 26). Converted to the hydrochloride in diethyl ether. Yield: 56%. mp: 123°–127°C. Anal. calcd. for $C_{17}H_{40}Cl_4N_4S_2$: C, 40.00; H, 8.69; Cl, 27.78; N, 10.97. Found: C, 39.23; H, 8.68; H, 10.53.

Preparation of [^{99}Tc]BAT complexes. A stock solution of stannous glucoheptonate was prepared by dissolving the contents of an NEN® Glucoscan Kit in 2 ml of a 0.09% saline solution. An aliquot of 200 μl of this solution was mixed with

200 μl of a solution of [^{99m}Tc]pertechnetate in saline and to this solution was added 1–3 mg of the ligand. The solution was vortexed and treated with 1 ml of 0.09% saline and extracted with chloroform (2 ml). The organic extract was dried over anhydrous sodium sulfate and concentrated under a stream of dry air. The residue was then dissolved in a minimum amount of absolute ethanol and subjected to further manipulation.

The separation of isomers was carried out on HPLC using a PRP-1 reversed-phase column (with 3,3-dimethylglutaric acid-acetonitrile, 15:85, as solvent). After separation, the isomers were greater than 94% pure. For biodistribution studies, isotonic saline was used as the solvent.

Preparation of [^{99m}Tc]BAT complexes. A solution containing 1.0 mmol of ammonium [^{99}Tc]pertechnetate and 1.2 mmol of the ligand **9a** in 60 μl of ethanol/water (9:1) was stirred and heated at 80°C for 10 min. To this solution was added dropwise a solution of 1.3 mmol of sodium dithionite in 2N sodium hydroxide (3 ml). At the end of this addition, the reaction mixture was stirred at 80°C for 30 min, allowed to cool to room temperature, and extracted with chloroform. The organic extract was dried over anhydrous sodium sulfate and the solution was concentrated in vacuo to a residue. The latter was then subjected to preliminary purification on a short silica gel column (eluted with acetone). The mixture of isomers was crystallized from 50% aqueous acetonitrile and the labeling yield was 28%. The isomers were subsequently separated on a silica gel column eluted with acetone/chloroform (33:67) and recrystallized from acetonitrile/water (2:1).

In order to determine the correspondence between the [^{99m}Tc]BAT and [^{99}Tc]BAT isomeric complexes, a mixture of the two was injected into the HPLC and subjected to simultaneous ultraviolet and gamma detection.

[^{99}Tc]**1a**-anti (DEA-anti)uv: λ_{max} 426 nm (E = 1,856), IR: ν_{max} Tc = 0, 900 cm^{-1}: Anal. calcd. for $C_{15}H_{32}N_3OS_2Tc$: C, 41.5; H, 7.4; N, 9.7; S, 14.8. Found: C, 41.59; H, 7.47; N. 9.7; S, 14.68.

[^{99}Tc] **1a**-syn (DEA-syn) uv: λ_{max} 425 nm (E = 1,297), IR: ν_{max} Tc = 0, 900 cm^{-1}: Anal. calcd. for $C_{15}H_{32}N_3OS_2Tc$: C, 41.5; H, 7.4, N, 9.7; S, 14.8. found: C, 41.64; H, 7.44; N, 9.65; S, 14.69.

Animal Distribution Studies

Male Sprague-Dawley rats (200–300 g) were injected intravenously (under ether anesthesia) with 0.2 ml of a saline solution containing the [^{99m}Tc]BAT complex (0.5–20 μCi). At selected intervals following the injection, blood samples (1 ml each) were collected by cardiac puncture and the animals were killed immediately thereafter by cardiectomy. The organs of interest were subsequently excised, weighed, and counted in a dual-channel automatic gamma counter. The percent dose per organ values were determined by comparison of the tissue radioactivity with suitable dilutions of the injected dose. The percent dose per gram values were computed from the percent dose per organ values and the corresponding mean organ weights (mean organ weights: heart, 0.85 g; brain, 1.65 g; blood, 18 g; liver, 9.0 g; kidneys, 1.9 g; lungs, 1.6 g). Finally, the brain–blood ratio was calculated from the corresponding percent dose per gram values.

The monkey used in the imaging studies was sedated with ketamine (10 mg) and subsequently anesthetized with pentobarbital. A dose of 5 mCi of [^{99m}Tc]DEA-*syn* was then injected intravenously and images were collected, immediately following the injection, at the rate of 1 frame/min and stored in a computer. The brain was flagged and the total net counts in this area was plotted against time to generate the washout curve. Static images were obtained by adding the frames obtained over consecutive 5-min intervals.

Determination of Partition Coefficients

These were determined by mixing the [^{99m}Tc]BAT complex with 3 g each of 1-octanol and buffer (pH 7.0 or 7.4; 0.1*M* phosphate) in a test tube. The tube was vortexed for 3 min at room temperature and subsequently centrifuged for 5 min. Two weighed samples (0.5 g each) from the octanol and aqueous layers were then counted in a well counter. The partition coefficient was calculated as the ratio of the cycles per minute in the octanol layer versus that in the aqueous layer. Samples from the 1-octanol layer were subsequently repartitioned until consistency was achieved. This was usually achieved in three runs.

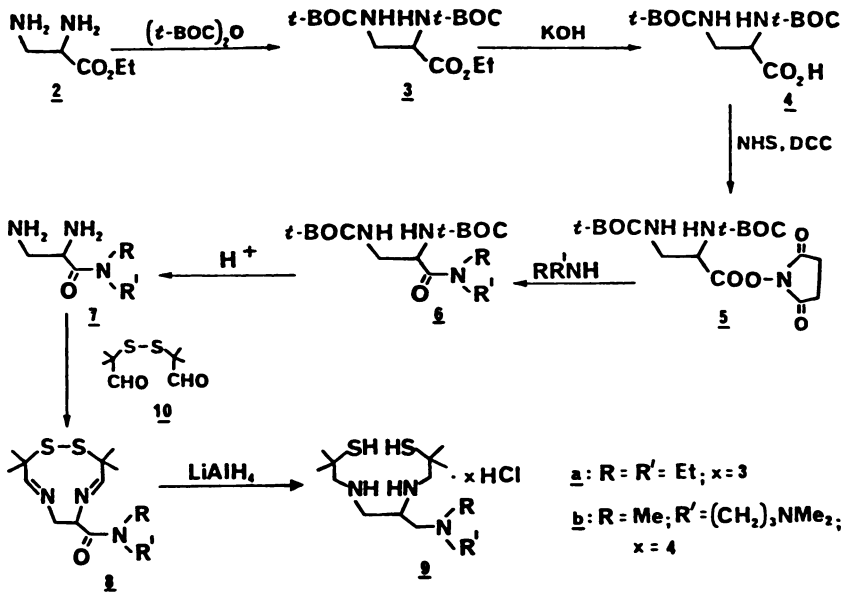
Autoradiography

Male Sprague–Dawley rats (200–300 g) under ether anesthesia were injected intravenously with 0.2 ml of a 20% saline/ethanol solution containing 25–50 mCi of either DEA-syn or DEA-anti. At selected intervals (2 and 15 min) after the injection, the animals were killed while under anesthesia. The brain was quickly but carefully removed and the radioactivity was measured. The brain was then frozen in an embedding medium (OTC compound by Tissue Tek[®]) at −25°C. Following this procedure, 20-micron sections were obtained with a cryostat microtome maintained between −15°C and −20°C. Each section was then mounted on a glass slide and air dried. Autoradiograms were obtained by overnight exposure of these mounted sections to film.[**]

RESULTS

Chemistry

The compounds were synthesized according to Scheme 1. The starting material, ethyl 2,3-diaminopropionate dihydrochloride, **2** was synthesized following a known procedure. Protection of the diamine functionality with the t-butoxycarbonyl group yielded **3**. This was followed by saponification of the latter and subsequent activation of the acid **4** with N,N′-dicyclohexylcarbodiimide and N-hydroxysuccinimide to yield the desired activated ester **5** in 82% yield from **3**. The activated ester **5** was then reacted with the appropriate amine to give **6a** and **6b**, respectively, which were later deblocked with HCl in diethyl ether and/or ethyl acetate. The deprotected compounds **7a** and **7b** were subsequently condensed with 2,2′-dithio-bis(2-methylpropanal) in the presence of triethylamine to yield the substituted diimines **8a** and **8b** in overall yields of 43% and 30%, respectively, from **5**. The diimines were subsequently reduced with excess $LiAlH_4$ to give the target ligands **9a** and **9b**.



SCHEME 1

Radiolabeling

Radiolabeling of the ligands **9a** and **9b** was achieved by simply mixing the ligands with [^{99m}Tc]pertechnetate and stannous glucoheptonate for a few minutes and subsequently extracting with chloroform. The yield of lipid soluble ^{99m}Tc containing material ranged from 42% to 85%. The crude organic extract showed two major peaks on HPLC and these were of essentially equal magnitude. These were separated and checked for purity; the purity of the material used was >94%. The peak exhibiting the higher chromatographic retention is referred to as peak B, whereas, the other is referred to as peak A. These complexes were stable in ethanol and saline/ethanol (20%–80%) mixtures for several hours (monitored by HPLC). Based on the structure proposed by Davidson et al. (*20*), and on x-ray crystallographic data obtained for the analogous [^{99m}Tc]N_2S_2 system, these compounds have been assigned the structures shown in Figure 1. Preliminary x-ray crystallographic data on this series of ^{99m}Tc complexes (data not shown) indicates that the structures shown in Figure 1, indeed, are correct and that the more mobile isomer (peak A) has the *anti* configuration, whereas, the other isomer (peak B) has the *syn* configuration. These isomers are subsequently referred to as DEA-*syn*, DEA-*anti*, TMPDA-*syn*, and TMPDA-*anti*.

The occurrence of isomeric substituted [^{99m}Tc]N_2S_2 complexes was also reported by Schneider et al. (*15*), who suggested that this isomerism was the result of the disposition of the substituent relative to the TcO core.

Biodistribution

At 2 min postinjection, both isomers of [^{99m}Tc] TMPDA (**1b**) showed little or no brain uptake. For TMPDA-*anti* (**1b**-*anti*), the brain uptake at 2 min was 0.05% (of injected dose), whereas, for the corresponding syn isomer, a value of 0.04 was obtained. These values are essentially unchanged at 15 min postinjection. However, there is some redistribution of the radioactivity in other organs such as the lung, liver, and muscle. In contrast, for [^{99m}Tc]DEA-*anti* (**1a**-*anti*) and [^{99m}Tc] DEA-*syn* (**1b**-*syn*), the brain uptake values at 2 min postinjection were 0.99 (brain/blood = 1.98) and 2.27% (brain/blood ratio = 4.41) of the injected dose, respectively. At the end of 15 min, the corresponding value for [^{99m}Tc]DEA-*anti* (**1a**-anti) was 0.26% (brain/blood ratio = 1.18), whereas, that of the syn isomer was 0.64 (brain/blood ratio = 2.53) (Table 1).

TABLE 1
Biodistribution of [^{99m}Tc]BAT Complexes DEA (1*a*) and TMPDA (1*b*) in Rats* %Dose/Organ (Average of three rats)

	DEA-*anti*	DEA-*syn*	TMPDA-*anti*	TMPDA-*syn*
	Distribution at 2 min			
Brain	0.99	2.27	0.05	0.04
Blood	5.01	4.59	3.85	3.45
Muscle	13.30	6.84	16.70	8.76
Heart	0.97	1.54	1.95	2.31
Lungs	13.60	20.00	7.72	23.10
Liver	13.00	9.85	19.20	13.10
Brain/blood ratio†	1.98	4.41	0.12	0.13
	Distribution at 15 min			
Brain	0.26	0.64	0.03	0.04
Blood	2.19	2.16	0.88	0.91
Muscle	18.00	19.80	11.80	14.0
Heart	0.24	0.36	0.81	1.21
Lungs	2.84	4.67	5.07	11.10
Liver	27.30	17.90	15.07	10.7
Brain/blood ratio†	1.18	2.53	0.33	0.46

* Measured as percent dose per organ (average of three rats).
† Blood dose/g.

TABLE 2
Partition Coefficients of [^{99m}TC]BAT Complexes

	Compound	pH = 7.0	pH = 7.4
1*a-anti*	(DEA-*anti*)	203 ± 8	465 ± 25
1*a*-syn	(DEA-*syn*)	309 ± 18	493 ± 6
1*b-anti*	(TMPDA-*anti*)	1.9 ± 0.2	5 ± 1
1*b-syn*	(TMPDA-*syn*)	3.6 ± 0.1	8.9 ± 0.4

Autoradiographic experiments (Fig. 2) using [^{99m}Tc]DEA-*syn* (**1a**-*syn*) clearly reveal, at 2 min postinjection, significant cerebral cortical localization characteristic of flow dependence. At 15 min, a decrease in the radioactivity can be perceived but the regional localization is clearly maintained.

From the static images of a monkey (Fig. 3), which had received an intravenous injection of 5 mCi of [^{99m}Tc]DEA-*syn* (**1a**-*syn*), brain localization is evident. Following a rapid initial influx phase, a plateau is reached; this plateau was taken to represent the initial brain uptake. Using this plateau (maximum) value and the efflux phase of the curve, which is linear, the $T_{½}$ (biologic) was calculated (Fig. 4). The washout curve shows a single component and the $T_{½}$ (biologic) is 42 min (Fig. 4).

The partition coefficient for [^{99m}Tc]TMPDA-*syn* at pH 7.0 was 3.6, but at pH 7.4 the value increased dramatically to 8.9. For [^{99m}Tc]DEA-*syn* (**1a**-*syn*), the partition coefficients at pH 7.0 and 7.4 were 95 and 109, respectively (Table 2).

Experiments using carrier (injected at a level of 0.1 mg/rat, data not shown) indicate that the brain uptake of [^{99m}Tc]DEA-*syn* (**1a**-*syn*) is not influenced by the presence of carrier.

DISCUSSION

Earlier studies by Schneider et al. (*15*) have shown that isomeric [^{99m}Tc]BAT complexes can be obtained from racemic substituted N_{22} ligands. It is not surprising, therefore, to observe that a similar situation is obtained with these new substituted [^{99m}Tc]BAT complexes. Based on these earlier studies, the proposed structures of these isomers are shown in Figure 1. This has been confirmed by preliminary x-ray crystallographic studies on related compounds (data not shown). It also appears that the syn isomer is a more lipophilic and desirable molecule for imaging.

From the data obtained in the biologic evaluation of these [^{99m}Tc]BAT compounds, the inclusion of amines in the sidechain results in significant alterations in the biodistribution and brain uptake profiles relative to the simple alkyl-substituted [^{99m}Tc]BAT complexes evaluated earlier (*12*). Technetium-99m–DEA-*syn* (**1a**-*syn*) not only demonstrates significant initial brain uptake, but also exhibits the desirable feature of fixed regional distribution (Fig. 2). Although the less lipophilic isomer [^{99m}Tc]DEA-*anti* (**1a**-*anti*) exhibits a substantially lower initial brain uptake, the brain uptake profile, nonetheless, is similar. The disparate values of initial brain uptake would suggest that a carrier-mediated process may be responsible for the uptake. However, our experiments with carrier [^{99m}Tc]BAT complexes seem to rule out this possibility. It appears that the brain uptake is a reflection of a simple diffusion mechanism due to high lipid solubility of these agents.

Experiments in a monkey indicate a higher brain retention time for [^{99m}Tc]DEA-*syn* (**1a**-*syn*) ($T_{½}$ = 41 min) relative to the simple alkyl-substituted [^{99m}Tc]BAT-HM (85% with $T_{½}$ = 10 min and 15% with $T_{½}$ = 57 min) (*12*). Although the brain retention determined from the monkey experiment is less than optimum, the washout curve has only one component; similar experiments on the alkyl-substituted [^{99m}Tc]BAT-HM revealed a biophasic washout curve (*12*). In addition, a higher brain/blood ratio is observed for these alkylamine substituted [^{99m}Tc]BAT complexes (Table 1); such a feature is desirable for the reduction of background (improvement of image quality).

In stark contrast to the isomeric [^{99m}Tc]DEA (**1a**), both isomers of [^{99m}Tc]TMPDA (**1b**) exhibit little or no brain uptake. In view of the fact that the partition coefficients obtained for these compounds would suggest blood–brain barrier penetrability, the biologic profile of the isomeric [^{99m}Tc]TMPDA (**1b**) clearly indicates that even in the absence of a carrier, lipophilicity is a necessary but insufficient condition for blood–brain barrier penetrability. In view of the fact that the one major difference, to a first approximation, between [^{99m}Tc]DEA (**1a**) and [^{99m}Tc]TMPDA (**1b**) is the distally

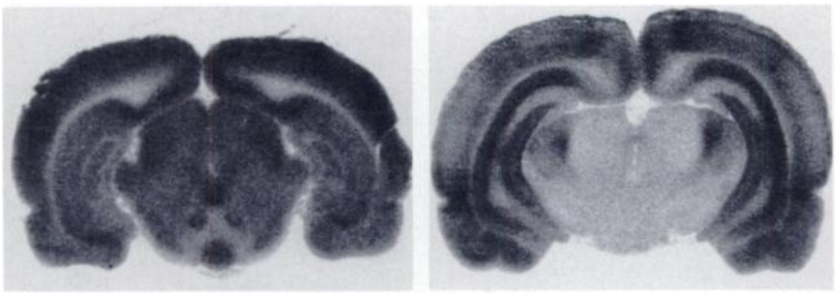


FIGURE 2
Autoradiographs of rat brain sections 2 min (left) and 15 min (right) after i.v. [^{99m}Tc]DEA-*syn*.

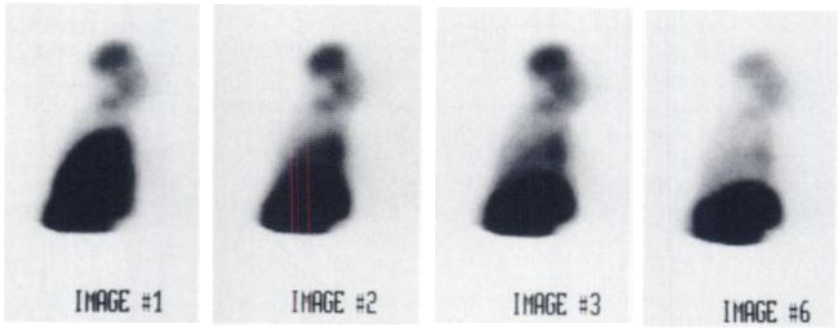


FIGURE 3
Planar images of a monkey injected with [^{99m}Tc]DEA-*syn*. Image #1, 0–5 min; Image #2, 5–10 min; Image #3, 10–15 min; Image #6, 25–30 min.

disposed amine (which is presumably more basic than the others that form a "propylenetriamine fragment"), the aberrant biologic profile of the isomeric [^{99m}Tc] TMPDA (**1b**) may be attributed to the basicity of this amine. One effect of this increased basicity is on the magnitude of change of the partition coefficient as a function of pH. It is evident from the data in Table 2 that the partition coefficient for the isomeric [^{99m}Tc] TMPDA (**1b**) changes by a factor of 2.5 (between pH 7.0 and 7.4), whereas, that of the isomeric [^{99m}Tc]DEA (**1a**) shows only a slight change. These results clearly underscore the relationship between the position of the amine in the sidechain and the biologic profile.

Finally, the autoradiographic data clearly demonstrates both significant initial cortical localization and a higher concentration of radioactivity in the gray matter over the white matter. That this distribution pattern parallels blood flow suggests that these agents could be used as indicators of regional cerebral perfusion. This possibility is further strengthened by the observed fixed regional distribution exhibited by these compounds; the latter also lends additional support to the pH shift hypothesis.

Having thus demonstrated that the incorporation of an amine into the sidechain of substituted [^{99m}Tc]BAT complexes can improve the parameters relevant to imaging, research aimed at the incorporation of design parameters for further optimization of initial brain uptake, brain/blood ratio, fixed regional distribution, and brain retention is presently underway and will be discussed in subsequent reports.

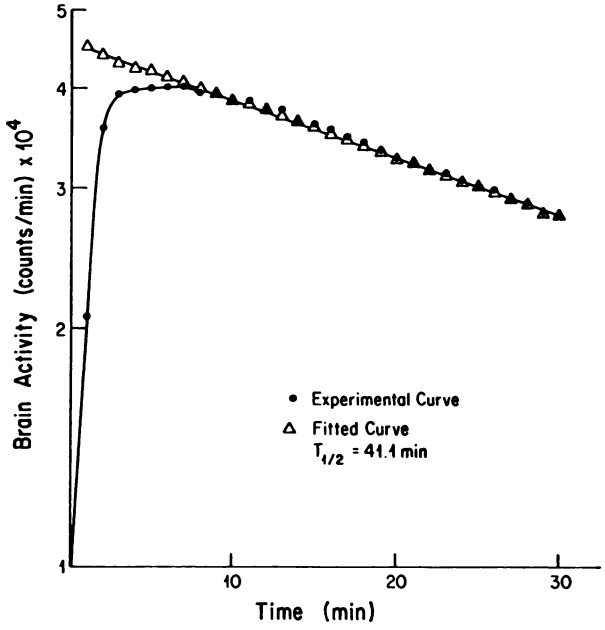


FIGURE 4
Washout curve obtained from planar imaging of a monkey using [^{99m}Tc]DEA-*syn*.

NOTES

* Varian Associates, Palo Alto, CA.
† Perkin-Elmer Corp., Norwalk, CT.
‡ Nicolet Instruments, Madison, WI.
§ DuPont Company, No. Billerica, MA.
¶ Miles Scientific, Elkhart, IN.
** Eastman Kodak Co., Rochester, NY. (Kodak Nuclear Medicine-B Film)

ACKNOWLEDGMENTS

This project is supported by a grant awarded by NIH, NINCDS, NS-18509.

The authors thank Elongia Farrell for her excellent technical assistance and Rebecca Hoffner for preparation of the manuscript. Special thanks for Drs. V. Subramanyan and J. Calabrese (NEN/Dupont) for providing NMR spectra and x-ray crystallography data.

REFERENCES

1. Tramposch KM, Kung HF, Blau M. Radioiodine labeled N,N′-dimethyl-N′(2-hydroxy-3-alkyl-5-iodobenzyl)-1,3-propanediamines for brain perfusion imaging. *J Med Chem* 1983; 26:121–125.
2. Kung HF, Tramposch KM, Blau M. A new brain perfusion imaging agent: [I-123] HIPDM:N,N,N′-trimethyl-N′- (2-hydroxyl-3-methyl-5-iodobenzyl) -1,3-propanediamine. *J Nucl Med* 1983; 24:66–72.
3. Winchell HS, Baldwin RM, Lin TH. Development of I-123-labeled amines for brain studies: localization of I-123 iodophenyl-alkylamines in rat brain. *J Nucl Med* 1980; 21:940–946.
4. Winchell HS, Horst WD, Braun L, et al. N-isopropyl [^{123}I]-p-iodoamphetamine: single pass brain uptake

and washout; binding to brain synaptoosomes; and localization in dog and monkey brain. *J Nucl Med* 1980; 21:947–952.
5. Lassen NA, Henriksen L, Holm S, et al. Cerebral blood flow tomography by SPECT: Xe-133 compared to isopropyl-amphetamine-iodine-123 [Concise communication]. *J Nucl Med* 1983; 24:17–21.
6. Hill RC, Magistretti PL, Holman BL, et al. Assessment of regional cerebral blood flow (rCBF) in stroke using SPECT and N-isopropyl [I-123]-p-iodoamphetamine (IMP). *Stroke* 1984; 15:40–45.
7. Kuhl DE, Barrio JR, Huang S-C, et al. Quantifying local cerebral blood flow by N-isopropyl-p-[I-123]iodoamphetamine (IMP) tomography. *J Nucl Med* 1982; 23:196–203.
8. Fazio F, Lenzi GL, Gerundi P, et al. Tomographic assessment of regional cerebral perfusion using intravenous [I-123] HIPDM and a rotating gamma camera. *J Comp Assist Tomogr* 1984; 8:911–921.
9. Lucignani G, Blasberg R, Patlak CS, et al. Metabolic and kinetic considerations in the use of [I-125] HIPDM as a tracer for quantitative measurement of regional cerebral blood flow. *J Cereb Blood Flow Metab* 1985; 5:86–96.
10. Dannals RF. The preparation and characterization of a nitrogen-sulfur donor ligands and their technecium complexes [PhD Thesis]. Baltimore, MD: Johns Hopkins University, 1984:98–205.
11. Epps LA. The chemistry of neutral, lipid soluble technetium (V) complexes of aminoalcohols and aminothiols [PhD Dissertation]. Baltimore, MD: Johns Hopkins University, 1984:74.
12. Kung HF, Molnar M, Billings J, et al. Synthesis and biodistribution of neutral lipid-soluble Tc-99m complexes that cross the blood brain barrier. *J Nucl Med* 1984; 25:326–332.
13. Kung HF, Blau M. Regional intracellular pH shift: a proposed new mechanism for radiopharmaceutical uptake in brain and other tissues. *J Nucl Med* 1980; 21:147–152.
14. Godefoi EF. 2,3-Diaminopropionic acid esters. *Chem Abstr* 1956; 51:463.
15. Schneider RF, Subramanian G, Feld TA, et al. N,N′-bis(S-benzoylmercaptoacetamido)ethylenediamine and propylenediamine ligands as renal function imaging agents. 1. Alternate synthetic methods. *J Nucl Med* 1984; 25:223–229.
16. Lever SZ, Burns HD, Kervitsky TM, et al. Design, preparation and biodistribution of a Tc-99m triaminedithiol complex to assess regional cerebral blood flow. *J Nucl Med* 1985; 26:1287–1294.
17. Volkert WA, Hoffman TJ, Seger RM, et al. Tc-99m-propyleneamine oxime (Tc-99m-PnAO); A potential brain radiopharmaceutical. *Eur J Nucl Med* 1984; 9:511–516.
18. Holmes RA, Chaplin SB, Royston KG, et al. Cerebral uptake and retention of Tc-99m-hexamethylpropyleneamine oxime (Tc-99m-HM-PAO). *Nucl Med Commun* 1985; 6:443–447.
19. Sharp PF, Smith FW, Gemmell HG, et al. Tc-99m-HM-PAO stereoisomers as potential agents for imaging regional cerebral blood flow in human volunteer studies. *J Nucl Med* 1986; 27:171–177.
20. Davidson A, Jones AG, Orvig C, et al. A new class of oxotechnetium (5+) chelate complexes containing a $TcON_2S_2$ core. *Inorg Chem* 1981; 20:1629–1632.