Notes

Synthesis of Selenium-75 Labeled Tertiary Diamines: New Brain Imaging Agents

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Selenium-75 labeled tertiary diamines, bis[β-(*N*,*N*-dimethylamino)ethyl] selenide (1), bis[β-(*N*,*N*-diisopropylamino)ethyl] selenide (2), bis(β-piperidinoethyl) selenide (3), and bis(β-morpholinoethyl) selenide (4), were prepared by reducing [^{75}Se]selenious acid with sodium borohydride and reacting the intermediate with the N,N-disubstituted aminoethyl chlorides. The effect of pH on lipid solubility (1-octanol/buffer distribution coefficient) was measured for each labeled compound. Biodistribution studies in rats showed high brain uptake for the tertiary diamines, especially for 3 and 4 (1.53 and 1.49% dose/organ, respectively, at 30 min after iv injection). The permanently charged bisquaternary amine, bis[β-(*N*,*N*,*N*-trimethylammonio)ethyl] selenide (5), showed negligible brain uptake (0.06% dose/organ at 30 min).

Recently, a new mechanism for radiopharmaceutical localization based on regional intracellular pH shift was proposed.[1] Many tissues and organs have low intracellular pH either normally or as a result of various metabolic disturbances.[2] Tertiary amines which are neutral and lipid soluble at blood pH (~7.4) can diffuse freely into cells. In those regions where intracellular pH is low (~7.0) they pick up a hydrogen ion and become positively charged. In this form they are no longer lipid soluble and are temporarily "trapped" because they cannot diffuse out of the cell. The influence of regional pH on drug entry and retention in the central nervous system is well known.[3] This paper describes the synthesis and biodistribution of a series of Se-75 labeled tertiary and quaternary diamines designed to test this principle as the basis for new brain imaging agents: bis[β-(*N*,*N*-dimethylamino)ethyl] selenide (1), bis[β-(*N*,*N*-diisopropylamino)ethyl] selenide (2), bis(β-piperidinoethyl) selenide (3), bis(β-morpholinoethyl) selenide (4), and bis[β-(*N*,*N*,*N*-trimethylammonio)ethyl] selenide (5) (Scheme I).

The selenium atom can form two stable Se-C covalent bonds and, therefore, such Se-75 labeled agents are more stable than radiopharmaceuticals based on metal chelates. Selenium metal (Se_x) has often been used as the starting material for the synthesis of organic selenium compounds. It can be reduced to sodium hydrogen selenide (NaHSe) or sodium diselenide (Na_2Se_2) by various reducing agents, including sodium metal in liquid ammonia,[4] magnesium metal in methanol,[5] sodium borohydride,[6] or lithium triethylborohydride.[7] The reduced intermediates can then be reacted with alkyl halides to form various organic compounds.

Similar procedures, starting from $^{75}Se_x$ are employed for the synthesis of Se-75 labeled radiopharmaceuticals.[8] However, the Se-75 isotope is available as selenious acid (H_2SeO_3) at a higher specific activity and lower cost (50 mCi/mg against 1 mCi/mg at a quarter of the cost). Selenious acid is, therefore, the logical choice as the starting material for the synthesis of Se-75 labeled compounds. The reduction of selenious acid to NaHSe or Na_2Se_2 by sodium borohydride has been reported.[9] Using this reducing agent, we have developed a simple method for preparing Se-75 labeled selenides.

Scheme I

$$H_2SeO_3 \xrightarrow[\text{(excess)}]{NaBH_4} [NaHSe] \xrightarrow{2R\text{-}X} R\text{-}Se\text{-}R$$

1, R = $-CH_2CH_2N(CH_3)_2$
2, R = $-CH_2CH_2N[CH(CH_3)_2]_2$
3, R = $-CH_2CH_2$-c-NC_5H_{10}
4, R = $-CH_2CH_2$-c-$N(CH_2CH_2)_2O$
5, R = $-CH_2CH_2N^+(CH_3)_3$

Chemistry. Selenious acid (H_2SeO_3) was successfully reduced by sodium borohydride (in excess) to form NaHSe. After reacting with N,N-disubstituted aminoethyl chlorides, the dialkyl selenides were the only products and no diselenides were isolated. The unreacted NaHSe was reoxidized during the workup to insoluble Se_x which was easily separated by filtration. The product was separated from unreduced H_2SeO_3 by chloroform extraction. This new procedure for the preparation of Se-75 labeled symmetrical selenides is simple and efficient. With slight modification (i.e., reduction of selenite to sodium diselenide, formation of dialkyl diselenides followed by reduction and reaction with another alkyl halide) it should be possible to prepare unsymmetrical alkyl selenides.

Effect of pH on Lipid Solubility. The distribution coefficients for the Se-75 labeled tertiary diamines were determined at pHs from 6.0 to 8.5 at 37 °C (Figure 1).

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Table I. Physical Data on Tertiary and Quaternary Diamines

compd	mp, °C	method[a]	yield, %	R_f values[b] A	B	C	D	formula	anal.
1	>300	A	30						
		B	50	0.13	0.93	1.0	0.81	$C_8H_{20}N_2Se \cdot 2HCl$	C, H, N, Cl
2	212-215	B	50	0.0-0.5	0.93	0.91	0.93	$C_{16}H_{36}N_2Se \cdot 2HCl \cdot 0.5H_2O$	C, H, N
3	238-240	B	59	0.0-0.3	0.91	0.91	0.83	$C_{14}H_{28}N_2Se \cdot 2HCl \cdot 0.25H_2O$	C, H, N
4	203-206	B	77	0.06	0.91	0.91	0.93	$C_{12}H_{24}N_2O_2Se \cdot 2HCl$	C, H, N, Cl
								$C_{12}H_{24}N_2O_2Se \cdot 2HCl \cdot 0.5H_2O$	C, H, N
5	254-256	B	86	0.0	0.4-0.9	0.91	0.93	$C_{10}H_{26}I_2N_2Se$	C, H, N

[a] Method A, reduction of Se_x; method B, reduction of H_2SeO_3. [b] Solvent systems: (A) chloroform; (B) 50% MeOH; (C) 1-butanol-acetic acid-H_2O (4:1:2); (D) ethylene glycol monomethyl ether-acetic acid-H_2O saturated with NaCl (7:1.5:1.5).

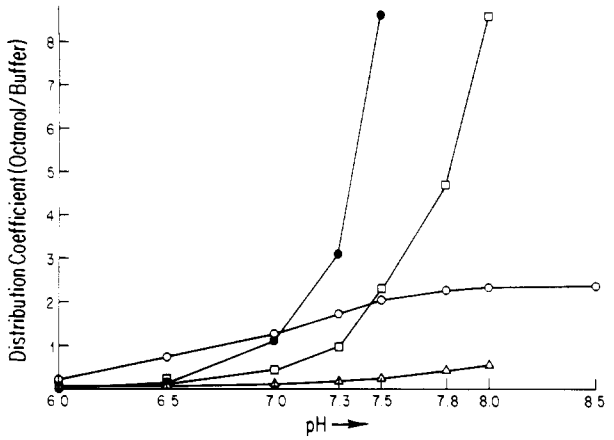

Figure 1. Distribution coefficients of Se-75 labeled tertiary diamines at different pHs. Average of four to six measurements: (Δ–Δ) compound 1; (●–●) compound 2; (□–□) compound 3; (○–○) compound 4.

The distribution coefficients for 2 and 3 are quite sensitive to pH; compounds 1 and 4, on the other hand, change more gradually.

Oldendorf[10,11] had determined that compounds with 1-octanol/water partition coefficients above about 0.5 can pass freely into the brain, but compounds with lower partition coefficients cannot. The distribution coefficient of 3 is strongly dependent on pH and passes thru 0.5 at pH 7.3 (Figure 1). The pH of blood is normally about 7.4 and that of brain cells about 7.0–7.1.[2] Compound 3 most closely follows the desired behavior of changing from a neutral, lipid-soluble molecule at blood pH to a charged water-soluble form at intracellular pH.

Biological Distribution. The organ distributions in rats at various times after intravenous injection of the Se-75 compounds are presented in Table II. Blood levels are very low for the tertiary amines at 2 min after injection, suggesting free passage out of blood and out of extracellular fluid into cells. Movement into cells is confirmed by the high muscle and brain uptake. Liver and kidney uptakes are high at 30 min and remain high at 2 h. The bisquaternary amine, 5, on the other hand, exhibited a different distribution pattern with lower muscle uptake and negligible brain uptake. The molecule is permanently charged and probably not able to cross cell walls or the blood–brain barrier.

Brain uptakes for 3 and 4 were significantly higher than for the other compounds and these were studied further. The detailed time course of the brain uptake and wash-out (Figures 2 and 3) corresponds to the predictions based on the distribution coefficient measurements. Compound 4, which is more lipid soluble at blood pH, is taken up in brain more readily; however, because the lipid solubility changes only moderately with pH (Figure 1), it is not as effectively trapped. Compound 3 is somewhat less lipid soluble at blood pH and, therefore, is taken up in brain

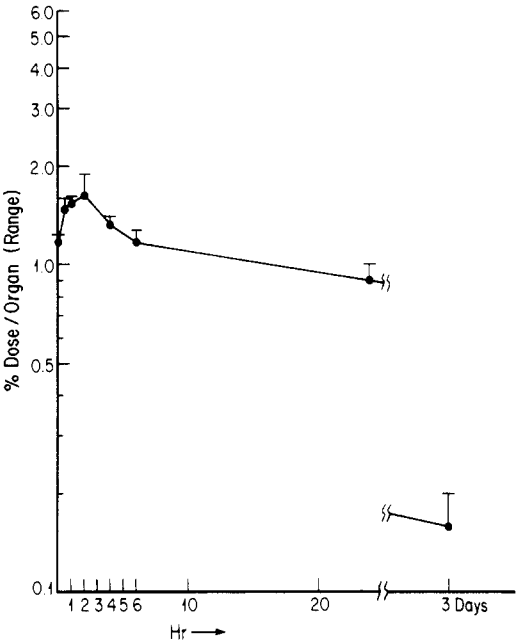

Figure 2. Brain uptake of compound 3 in rats. Average of three to six rats.

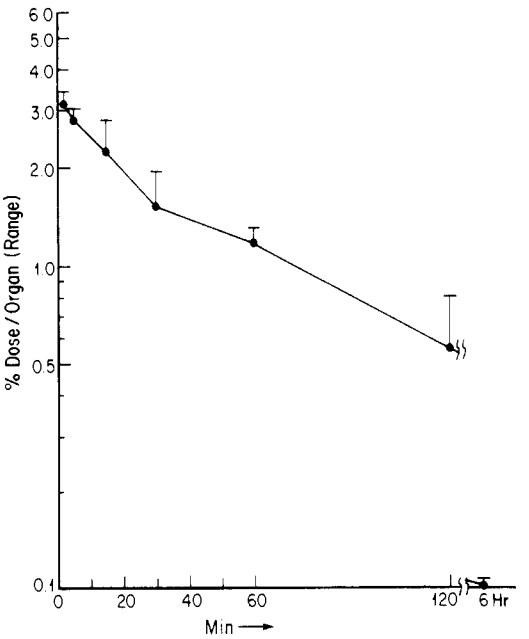

Figure 3. Brain uptake of compound 4 in rats. Average of three to six rats.

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Table II. Organ Distribution of Se-75 Labeled Tertiary and Quaternary Diamines

	% dose/organ (range)				
	1[a]	2[a]	3[a]	4[a]	5[b]
			2 min		
blood	2.82 (2.27-3.79)	2.96 (2.20-4.48)	4.58 (3.75-5.48)	3.13 (2.76-3.36)	
muscle	14.1 (6.02-20.3)	9.88 (5.75-14.0)	7.39 (6.33-8.44)	10.5 (9.64-11.0)	
heart	2.21 (1.76-2.50)	2.35 (2.03-2.68)	3.04 (2.36-4.13)	0.45 (0.36-0.52)	
lung (2)	11.36 (8.11-13.1)	11.2 (7.84-16.9)	20.0 (17.5-22.9)	2.77 (2.17-3.37)	
liver	12.82 (11.7-14.4)	11.9 (8.41-16.8)	12.0 (9.44-13.1)	18.3 (16.4-21.1)	
kidney (2)	9.46 (5.88-12.5)	11.4 (7.3-14.4)	7.14 (6.18-8.49)	9.89 (9.57-10.4)	
brain	0.49 (0.42-0.54)	0.25 (0.18-0.30)	1.09 (0.96-1.28)	3.19 (2.41-3.88)	
			30 min		
blood	0.74 (0.60-0.88)	1.17 (1.12-1.26)	0.87 (0.81-0.98)	2.82 (2.63-3.02)	5.16 (4.42-5.90)
muscle	11.8 (9.70-13.9)	14.2 (13.5-14.7)	13.4 (11.2-17.8)	14.4 (10.7-17.9)	9.52 (7.63-11.4)
heart	0.81 (0.71-0.91)	0.55 (0.44-0.71)	0.89 (0.78-0.98)	0.22 (0.20-0.23)	0.15 (0.14-0.16)
lung (2)	5.77 (4.43-7.11)	3.44 (2.65-5.01)	2.83 (2.42-3.43)	1.79 (1.07-3.04)	0.63 (0.49-0.78)
liver	15.1 (14.6-15.6)	16.0 (13.1-20.7)	14.3 (12.5-15.7)	23.2 (22.0-24.3)	5.74 (5.73-5.75)
kidney (2)	5.60 (5.50-5.60)	6.19 (5.13-7.70)	9.21 (7.90-10.6)	4.03 (3.77-4.52)	6.01 (5.30-6.72)
brain	0.49 (0.44-0.54)	0.33 (0.32-0.35)	1.49 (1.41-1.60)	1.53 (1.32-1.94)	0.06 (0.05-0.07)
			2 h		
blood	0.53 (0.51-0.57)	1.04 (0.94-1.13)	0.65 (0.59-0.71)	2.46 (2.24-2.66)	0.36 (0.29-0.43)
muscle	15.2 (14.7-16.0)	15.0 (13.9-15.8)	12.0 (9.88-14.4)	17.7 (17.5-17.8)	5.48 (2.23-8.73)
heart	0.54 (0.46-0.66)	0.30 (0.24-0.37)	0.19 (0.17-0.21)	0.18 (0.14-0.22)	0.05 (0.04-0.06)
lung (2)	4.74 (4.00-5.89)	1.11 (1.05-1.16)	0.85 (0.80-0.89)	0.94 (0.75-1.30)	0.20 (0.20-0.21)
liver	12.2 (10.4-14.2)	13.1 (12.1-14.0)	15.9 (11.8-18.3)	21.7 (20.2-23.5)	3.04 (2.59-3.51)
kidney (2)	3.92 (3.40-4.28)	2.28 (1.74-2.49)	2.89 (2.68-3.00)	3.42 (3.05-3.78)	2.75 (2.08-3.42)
brain	0.71 (0.65-0.74)	0.35 (0.29-0.42)	1.63 (1.40-2.00)	0.56 (0.40-0.82)	0.06 (0.04-0.08)

[a] Average of three rats. [b] Average of two rats.

more slowly. However, because of the sharp decrease in lipid solubility with small pH changes, it is very effectively trapped in the brain. The brain curve for these agents is a reflection of the equilibria established on both sides of the blood–brain barrier. The concentrations of the various charged and uncharged molecular species on both sides depend on the p*K*s of the compound, the local pH, and the transmembrane diffusion rates of the various molecular species. To a first approximation, the concentration gradient and diffusion characteristics of the uncharged form determine the brain uptake and retention curve. Generally speaking, high initial uptake is associated with high lipid solubility at blood pH, and the wash-out rate from brain is determined by the slope of the curve plotting distribution coefficient against pH. The influence of transport kinetics on the wash-out curve, and of possible nonspecific binding to intracellular components, remains to be investigated. These factors may play a significant role in the establishment of the very high brain to blood concentration ratios observed at later times.

Selenium-75 has been used in clinical studies for many years as a label for methionine.[12,13] Although the physical half-life is long (120 days), the rapid biological excretion of these tertiary diamines (>95% excreted in 2 days) would prevent high patient radiation doses. We estimate that the total body dose in humans would be approximately 100 mrem per millicurie administered. A Tc-99m ($T_{1/2}$ = 6 h) pH shift agent would be even more suitable for clinical applications. Burns et al.[14] and Loberg et al.[15] have reported lipid-soluble Tc-99m labeled compounds. Suitable tertiary amine derivatives might be useful as pH shift radiopharmaceuticals. Compounds labeled with I-123 ($T_{1/2}$ = 13 h) might also be advantageous in certain clinical uses.

High brain uptake of a radioactive iodine labeled *N,N,N,N*-tetramethyl-*p*-xylylenediamine prepared as a possible putrescine analogue has been reported.[16] It is likely that the mechanism of localization for this compound is the same as that proposed for the Se-75 labeled tertiary diamines.

Experimental Section

Except for Se-75 labeled compounds, all of the synthesized compounds were characterized by standard spectral analyses. IR spectra, in KBr pellets, were recorded on a Perkin-Elmer Model 700 spectrophotometer. NMR spectra were obtained with a Varian T-60 spectrometer. All of the spectra were consistent with the proposed structures. Melting points (Table I) for all of the cold compounds were obtained on a Nalge apparatus and are uncorrected. Elemental analyses were performed by Instranal Laboratory, Rensselaer, NY. All results were within ±0.4% of the theoretical values. Thin-layer chromatography on Gelman ITLC-SG paper was carried out with four solvent systems: (A) chloroform, (B) 50% methanol, (C) 1-butanol–acetic acid–H_2O (4:1:2), and (D) ethylene glycol monomethyl ether–acetic acid–H_2O saturated with NaCl (7:1:5:1.5). The developed chromatograms were sprayed with 0.4% dipicrylamine in 50% acetone solution to give yellowish-pink spots which corresponded to the tertiary or quaternary amines. After spraying, the radioactive chromatograms were cut into 20 pieces and the radioactivity was counted in a well counter. In all cases, the radioactivity was coincident with the R_f value for the cold compound (Table I). Radiochemical purity was above 95% for all compounds.

Bis[β-(*N,N*-dimethylamino)ethyl] Selenide Dihydrochloride (1). Method A. Reduction of Selenium Metal. Selenium metal (0.23 g, 2.9 mmol) was suspended in 10 mL of water, and sodium borohydride ($NaBH_4$, 0.25 g) was added in small portions until a colorless solution was obtained. *N,N*-Dimethylaminoethyl chloride hydrochloride (0.9 g, 6.2 mmol) was added, and the resulting solution was refluxed for 3 h and then treated with 0.4 g of NaOH and extracted with chloroform (30 mL). The chloroform extracts were separated, dried over anhydrous sodium sulfate, and condensed. The residue was mixed with 1 mL of concentrated HCl and 15 mL of absolute ethanol. After overnight refrigeration, the white precipitate was collected

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to give an analytically pure product (0.26 g, yield 30%). Melting point and chromatography results are shown in Table I.

Method B. Reduction of Selenious Acid. Selenious acid (0.4 g, 3.1 mmol) was dissolved in 25 mL of water and $NaBH_4$ (~0.5 g) was added in small portions. After heating to reflux, a clear solution was obtained. *N,N*-Dimethylaminoethyl chloride hydrochloride (0.95 g, 6.6 mmol) was added and the mixture was heated to reflux for 1 h and then cooled to room temperature. Following treatment with 0.4 g of NaOH and extraction with 30 mL of chloroform, the chloroform layer was separated, dried over anhydrous sodium sulfate, and filtered. The condensed residue was treated with 1 mL of concentrated HCl and 20 mL of absolute ethanol. After refrigeration overnight, the precipitate was filtered and the product was washed with 10 mL of petroleum ether. After drying, 0.52 g of product was obtained (yield 50%). The IR and NMR spectra for this product were the same as those for the product prepared by method A.

Other Tertiary Diamines, 2-4. Method B using the appropriate substituted aminoethyl chlorides was employed for the syntheses of all of the tertiary diamines. The yields and melting points are shown in Table I.

Bis[β-(*N,N,N*-trimethylammonio)ethyl] Selenide Diiodide (5). Selenious acid (1.29 g, 10 mmol) was dissolved in 50 mL of water and $NaBH_4$ (~1.5 g) was added in small portions. Upon heating to reflux, a clear solution was obtained. *N,N*-Dimethylaminoethyl chloride hydrochloride (2.9 g, 21 mmol) was added, and the resulting solution was refluxed for 1 h. The reaction mixture was cooled to room temperature, treated with 0.4 g of NaOH, and extracted with chloroform (2 × 30 mL). The combined extracts were condensed and the residue was dissolved in 5 mL of methyl iodide and 20 mL of ethanol. Upon standing at room temperature for 5 min, white crystals precipitated. The mixture was refrigerated for 16 h, and the white crystals were collected by filtration and washed with petroleum ether (10 mL) to give 4.4 g of compound 5 (yield 86%).

Se-75 Labeled Tertiary and Quaternary Diamines, 1-5. The desired amount of [^{75}Se]selenious acid was mixed with cold selenious acid and the sequence of method B was carried out until the chloroform extract condensation. The condensed residue was dissovled in saline and the solution was filtered thru a 0.22-μm filter to sterilize the solution. The specific activities ranged from 10 to 1000 μCi/mg, and the yields ranged from 30 to 70% based on radioactivity. For compound 5 the chloroform extract was treated with 1 drop of methyl iodide and 1 mL of absolute ethanol, and the solution was condensed to dryness, dissolved in saline, and filtered thru a 0.22-μm filter. The radiochemical yield was 42%.

Distribution Coefficients. The radioactive compound (0.05–0.2 μCi) was mixed with 1 mL of 1-octanol and 1 mL of phosphate buffer (0.1 M) at the desired pH. This mixture was counted, vortexed for 3 min, and then placed in a water bath shaker at 37 °C for 2 h. After centrifugation (3000 rpm × 5 min), the 1-octanol layer was separated, aliquoted, and counted. The distribution coefficient was calculated by the following equation:

$$\text{distribution coefficient} = \frac{\text{total counts in 1-octanol}}{\text{initial counts} - \text{total counts in 1-octanol}}$$

Organ Distribution Studies. Sprague–Dawley male rats (220–300 g) were injected intravenously (femoral vein) with 0.2 mL of solution (0.5–2.0 μCi) under light ether anesthesia. At different time periods after injection, animals were killed by removing the heart under ether anesthesia, and organs of interest were excised and counted in a well counter. Percent dose was determined by comparison of tissue counts to suitably diluted aliquots of the injected material prepared by injecting a dose into a volumetric flask at the time of administration to the rats using the same syringe. Total activities in blood and muscle were calculated by assuming that they are 7 and 40% of the body weight, respectively. All of the biodistribution data are presented as percent dose per organ (Table II). Percent dose per gram of each organ or tissue can be obtained by dividing percent dose per organ by organ weight (heart, 0.7–1.0 g; lungs, 1.4–1.8 g; liver, 8–10 g; kidneys, 1.8–2.0 g; brain, 1.5–1.8 g).

Synthesis, Absorption, and Toxicity of N^1,N^8-Bis(2,3-dihydroxybenzoyl)spermidine, a Potent Iron Chelator

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A synthesis of N^1,N^8-bis(2,3-dihydroxybenzoyl)spermidine, a potent iron chelator, is developed employing N^4-benzylspermidine as the starting material. Both the toxicity and the absorption properties of this compound are evaluated, further supporting its potential as a clinical iron chelator.

Phlebotomy therapy for the management of hemochromatosis or other conditions characterized by iron overload with normal red blood cell production has been very successful.[1] However, for patients who are anemic, with limited bone marrow reserve, iron chelation therapy is the only option.

The drug most widely used in iron chelation therapy, desferrioxamine B (Desferal), a hydroxamate siderophore isolated from *Streptomyces pilosus*,[2] has had limited success. Unfortunately, it cannot be taken orally and unless patients are subjected to extended infusion therapy they cannot be kept in negative iron balance. For these reasons, workers have been searching for new, clinically useful iron chelators. To date, most of these efforts have focused on siderophores, natural iron chelators, or synthetic models thereof.[3-6]

In a recent paper[7] focused on the evaluation of isoniazid pyridoxal hydrazone as a therapeutic iron chelator, Jacobs et al. also pointed out the potential of N^1,N^8-bis(2,3-di-

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