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Radioiodine-Labeled *N*,*N*-Dimethyl-*N'*-(2-hydroxy-3-alkyl-5-iodobenzyl)-1,3-propanediamines for Brain Perfusion Imaging

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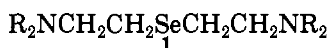
In developing new brain-imaging agents for single photon emission computed tomography (SPECT), we synthesized five radioiodinated *N*,*N*-dimethyl-*N'*-(2-hydroxy-3-alkyl-5-iodobenzyl)-1,3-propanediamines (**12–16**). The appropriate *o*-alkylphenol was formylated via a tin(IV) chloride catalyzed reaction. Iodination with iodine monochloride gave the required 3-alkyl-5-iodosalicylaldehyde, which was reductively aminated with 3-(dimethylamino)propylamine and sodium borohydride. The radioactive labeling (>95% yield) can be achieved by a rapid aqueous exchange reaction in a serum vial at 100 °C. Biodistribution in rats exhibited high initial brain uptake (0.9 to 1.8% dose in brain at 2 min), and the brain activity remained about the same at 1 h for all the compounds. The best brain-imaging agent was *N*,*N*-dimethyl-*N'*-(2-hydroxy-3-methyl-5-iodobenzyl)-1,3-propanediamine (**12**), which showed the highest uptake of 1.80 and 2.02% dose in brain at 2 min and 1 h, respectively. The brain uptake increases when the specific activity of ^{125}I-labeled **12** decreases. An imaging study of [^{123}I]**12** in a monkey showed high initial brain uptake, as well as high uptake in lungs and liver. The brain uptake persists for at least 1 h. Autoradiography of rat brain sections showed a regional distribution that reflects the cerebral perfusion pattern (high activity in gray matter and low activity in white matter). It is apparent that [^{123}I]**12** is readily prepared and is potentially useful for brain perfusion imaging in conjunction with SPECT devices.

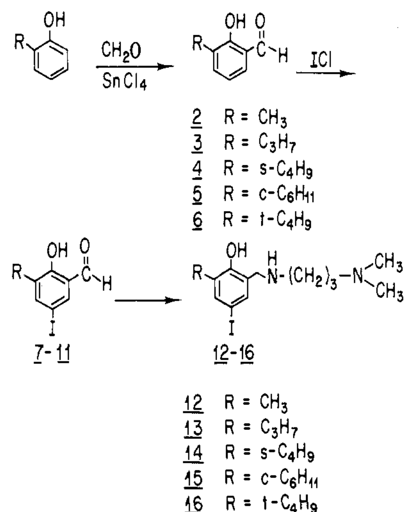
Traditional brain-imaging agents are compounds that cannot pass through the blood–brain barrier (BBB). In areas of brain where the BBB is no longer intact, the imaging agents show a positive uptake. However, many brain diseases in their early stages do not involve defects in the BBB but are characterized by changes in regional perfusion and/or metabolic patterns. Measurement of these physiological changes can provide useful diagnostic information on brain function. This type of brain-imaging agent can be used in conjunction with single photon emission computed tomography (SPECT) to measure or infer regional brain perfusion.[1-3]

Recently a new class of Se-75 labeled diamines, 1, that



can take advantage of the pH gradient that exists between blood (pH ~7.4) and brain (intracellular pH ~7.0) has been developed.[4,5] At high pH these compounds are neutral and lipid soluble and can freely diffuse into cells but at lower pH they become charged and can no longer diffuse out. The minimum concentration gradient is a function of the equilibria established by the local pH shift. Several of these compounds displayed high brain uptake and retention after intravenous injection in rats and monkeys. However, some of the γ rays of Se-75 (260–410 keV) are too high for γ camera imaging, and the long physical half-life ($T_{1/2}$ = 120 days) will give high patient radiation dose, both of which limit the clinical application of Se-75 labeled compounds. A more useful agent might be realized by substituting I-123 for the Se-75. Iodine-123 has superior physical properties; a shorter half-life ($T_{1/2}$ = 13.3 h) will lower the radiation dose to patients, and a better γ energy (159 keV) is more suitable for imaging with single photon imaging devices.

Scheme I



In this report most of the labeling and biodistribution studies were done with a more convenient radionuclide, I-125, because it is cheaper and the half-life is longer ($T_{1/2}$ = 60 days). However, the same procedures are applicable for I-123 labeling.

We report here the synthesis, radioiodide exchange labeling, and biodistribution (in rats and monkeys) of a

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(1) T. F. Budinger, *J. Nucl. Med.*, **22**, 1094 (1981).
(2) R. E. Coleman, B. P. Drayer, and R. J. Jaszczak, *J. Nucl. Med.*, **23**, 266 (1982).
(3) T. C. Hill, *J. Nucl. Med.*, 21, 1197 (1980).
(4) H. F. Kung and M. Blau, *J. Nucl. Med.*, 21, 147 (1980).
(5) H. F. Kung and M. Blau, *J. Med. Chem.*, **23** 1127 (1980).

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Table I. Yield and Melting Points of *N*,*N*-Dimethyl-*N*′-[2-hydroxy-3-alkyl-5-iodobenzyl]-1,3-propanediamine Dihydrochlorides

compd	R	mp, °C	yield, %	anal.
12	CH_3	>161 dec	84	C, H, N
13	n-C_3H_7	>178 dec	76	C, H, N
14	s-C_4H_9	>175 dec	87	C, H, N
15	c-C_6H_{11}	>196 dec	77	C, H, N
16	t-C_4H_9	>198 dec	81	C, H, N

group of *N*,*N*-dimethyl-*N*′-(2-hydroxy-3-alkyl-5-iodobenzyl)-1,3-propanediamines (**12–16**).

Chemistry. Compounds **12–16** were synthesized according to Scheme I. The intermediate salicylaldehydes **2–6** were prepared in moderate yield via a recently reported tin(IV) chloride catalyzed formylation.[6] Iodination of the aldehydes with ICl in glacial acetic acid gave good yields of the 3-alkyl-5-iodosalicylaldehydes (**7–11**). The iodinated salicylaldehydes were reductively aminated with 3-(dimethylamino)propylamine and sodium borohydride to give the diamines **12–16** in excellent yield (Table I).

These compounds are analogues of **1** where one of the *N*-alkyl groups is replaced with an aromatic system to carry the iodine label. Since aromatic iodides are more stable than their aliphatic counterparts, this feature minimizes in vivo deiodination.[7] Moreover, the presence of the phenolic hydroxy markedly enhances radiolabeling (yield >95%). Since the radiolabeling can be achieved by a simple aqueous exchange reaction, the overall procedure is amenable to a kit form for routine use in nuclear medicine clinics. This is especially important for a short-lived radionuclide such as I-123.

This group of compounds differs with respect to the nature of the alkyl substitution on the aromatic ring. In this way the lipid solubility of the compounds can be altered without affecting the pK_a's of the diamines.

Experimental Section

The yields, analyses, and melting points for compounds **12–16** are reported in Table I. NMR spectra were recorded on a Varian T-60A spectrometer, taken in either deuterated chloroform or dimethyl sulfoxide. Infrared spectra were determined on a Perkin-Elmer Model 727B spectrophotometer as KBr pellets. Spectral properties were consistent with the proposed structures. Melting points were determined on a Nalge hot stage and are reported uncorrected. Elemental analyses were performed by Instranal Laboratories, Rensselaer, NY, and all values are within ±0.4% of theoretical numbers. Radioactivity was determined with a Beckman Automatic Gamma Counter (Model 4000). High-performance liquid chromatography was done on a Partisil-10 column (25 cm) eluted with hexane–$CHCl_3$ (3:1).

Synthesis of 3-Alkylsalicylaldehydes (2–6). General Method. To a stirred solution of the appropriate phenol (200 mmol) and tributylamine (14.8 g, 80 mmol) in 100 mL of toluene under a nitrogen atmosphere was added anhydrous $SnCl_4$ (5.2 g, 20 mmol) via glass syringe. The mixture was stirred at room temperature for 30 min. Paraformaldehyde (13.2 g, 440 mmol) was added, and the resulting suspension was heated at 95 °C for 18 h. The reaction mixture was allowed to cool and poured into 2 L of water. The mixture was acidified to pH 2 with 4 N HCl and extracted twice with ether. The organics were washed with saturated NaCl, dried over sodium sulfate, and evaporated under reduced pressure to give the crude salicylaldehydes as liquids. Kugelrohr distillation (0.5–0.8 mm) at 50–100 °C gave the products **2–6**, which were contaminated with 5–15% of the starting phenols (determined by HPLC and NMR). These aldehydes were of sufficient purity to carry through the synthesis to the next step.

Synthesis of 5-Iodo-3-alkylsalicylaldehydes. General Method. A solution of ICl (24.3 g, 150 mmol) in 60 mL of glacial acetic acid was added dropwise to a stirred solution of the appropriate salicylaldehyde (**2–6**) in 60 mL of glacial acetic acid. After the addition was completed, the dark mixture was heated for 4 h and then stirred at room temperature overnight. The reaction mixture was poured into 500 mL of water, and the product was extracted with two portions of methylene chloride. The methylene chloride extracts were combined, washed with saturated sodium thiosulfate, dried over sodium sulfate, and concentrated under reduced pressure to give a dark brown oil. The oil was filtered through silica gel and eluted with petroleum ether (bp 30–60 °C)–chloroform (70:30) to give the iodosalicylaldehydes **7–11** (yield 75–81%). These compounds decomposed on standing and, hence, were used immediately after chromatography in the next reaction.

Synthesis of *N*,*N*-Dimethyl-*N*′-(2-hydroxy-3-alkyl-5-iodobenzyl)-1,3-propanediamines. General Method. A solution of the crude aldehyde **7**, **8**, **9**, **10**, or **11** (3.4 mmol) and 3-(dimethylamino)propylamine (0.4 g, 3.9 mmol) in benzene was refluxed, under a Dean–Stark head to effect the separation of water, for 2 h. The solvent was evaporated under reduced pressure to give a yellow oil. The oil was dissolved in ethanol and $NaBH_4$ (0.39 g, 10.3 mmol) was added by spatula in small portions over 20 min. The reaction mixture was stirred at room temperature for 18 h. The resulting clear solution was concentrated under reduced pressure, and water (100 mL) was added. The product was extracted with methylene chloride. The organic layer was washed with saturated NaCl, dried over sodium sulfate, and evaporated under reduced pressure to give a clear oil. The oil was converted to the dihydrochloride salt by passing dry HCl gas through a methanol solution. Evaporation of the methanol gave the crude dihydrochloride salt of the diamine (**12–16**), which was recrystallized from acetone–MeOH.

Exchange Radiolabeling of the *N*,*N*-Dimethyl-*N*′-(2-hydroxy-3-alkyl-5-iodobenzyl)-1,3-propanediamines (8–12) with I-125. General Method. A solution of the diamine **12**, **13**, **14**, **15**, or **16** (1 mg) and 800–1000 μCi of I-125 (New England Nuclear, 17 Ci/mg) in 1 mL of 0.01 N HCl, in a sealed 10-mL serum vial, was heated in a boiling water bath for 15 min at ambient pressure. The cooled reaction mixture was analyzed for radiochemical incorporation by TLC in two systems: Merck Silica Gel 60, $CHCl_3$–EtOH–NH_4OH (8:1.5:0.5), R_f 0.6–0.8 for labeled amines **12–16** (the radioactivity coincided with the UV-absorbing spot) and 0.0 for free iodide; Gelman ITLC, EtOH–NH_4OH (9.5:0.5), R_f 0.5–0.8 for labeled amines **12–16** and 1.0 for free iodide. In all cases the radiochemical incorporation was greater than 95%. The mixture was diluted with 1 mL of 0.9% saline and passed through a 0.22 μm filter to sterilize the solution. There was no change in radiochemical purity after the filtration. The same procedure was also used for I-123.

Distribution Coefficient. The distribution coefficient was measured by mixing the radioactive diamines **12–16** with 3 g each of 1-octanol and buffer (0.1 M phosphate) at a desired pH in a test tube. This test tube was vortexed (3× 1 min) and then centrifuged for 5 min. Two weighed samples (0.5 g each) from the 1-octanol and buffer layers were counted in a well counter. We determined the distribution coefficient by calculating the ratio of counts per minute per gram of octanol to that of buffer. This procedure was repeated until consistant values were obtained; usually the measurement was repeated three times.

Protein Binding. The ability of compounds **12–16** to bind to human serum proteins was determined by equilibrium dialysis. Human serum (0.4 mL, pooled, GIBCO Labs) and 0.4 mL of phosphate buffer (0.15 M, pH 7.4) were separated by a dialysis membrane (Fisher Scientific Co.). The radioactive diamines **12–16** (0.025 μCi) were mixed with the solution in the buffer side. The dialysis cells were rotated in a water bath at 37 °C for 18 h. At the end of the incubation, aliquots from both sides were weighed and counted. We determined the percent free of protein binding by calculating the radioactivity concentration ratio of buffer to serum multiplied by 100. To determine possible membrane binding, we counted the membrane at the end of the experiment. Usually less than 5% of the original activity was found.

Animal Distribution Study. Rat. Sprague-Dawley male rats (220–300 g) under light ether anesthesia were injected in-

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(7) R. E. Counsell and R. D. Ice, in "Drug Design", Vol. VI, E. J. Ariens, Ed., Academic Press, New York, 1975, Chapter 6.

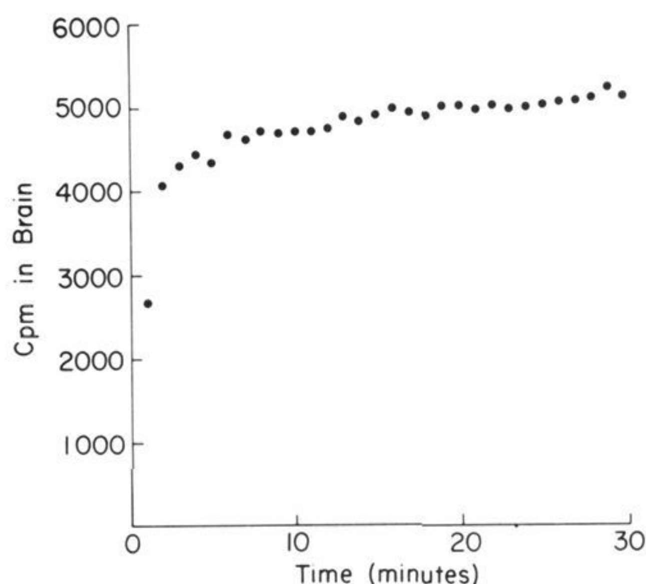

Figure 1. Kinetics of brain uptake in a monkey after an iv injection of [^{123}I]**12**.

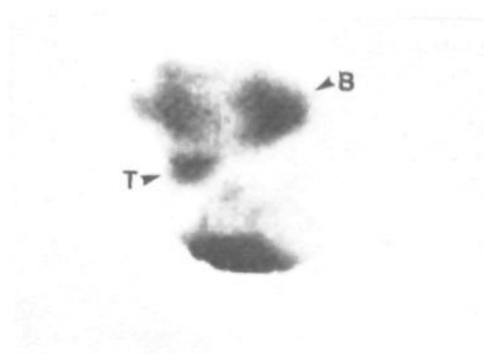

Figure 2. Lateral view of a monkey 45 min after an iv injection of [^{123}I]**12**. B = brain; T = thyroid.

travenously with 0.2 mL of a saline solution containing 0.5–20 μCi of test compound (specific activity 0.5–1.0 mCi/mg). At different time periods after injection the animals were put under ether anesthesia and killed by cardiectomy. The organs of interest were excised, weighed, and counted in a Beckman automatic γ counter (Model 4000).

The percent dose per organ was determined by comparison of tissue radioactivity levels to suitably diluted aliquots of the injected dose. The approximate percent dose per gram of wet tissue or organ can be calculated by dividing the percent dose per organ by the mean organ weight (mean weights: heart, 0.85 g; brain, 1.65 g; blood, 18 g; liver, 9 g; kidneys, 1.9 g; lungs, 1.6 g). The brain to blood concentration ratio was calculated from the percent dose per gram of wet tissue.

Monkeys. Monkeys were sedated with ketamine (0.3 mL, 30 mg/mL) and anesthesized with nembutal. A dose of 3–5 mCi of [^{123}I]**12** (specific activity ~5 mCi/mg) was injected intravenously. Immediately after the injection, images of the head (60 s per frame) were collected by a Searle HP camera and a MDS computer. The brain area was flagged, and the total net counts in this area was plotted vs. time (Figure 1). Static imaging of the head was done 45 min after the injection and a 5–10 min picture was accumulated (Figure 2).

For dissection experiments, the monkey was injected intravenously with a dose of [^{125}I]**12** (~100 μCi, specific activity ~7 mCi/mg). One hour later, the monkey was sacrificed by an overdose of nembutal. Organs of interest were dissected, weighed, and counted. Percent dose per organ was obtained by a method similar to that of the rat distribution studies.

Autoradiography. Male Sprague–Dawley rats (200–300 g) were injected intravenously under light ether anesthesia with 0.2 mL of a solution containing ~100 μCi of [^{125}I]**12** (specific activity ~2 mCi/mg). At 2 min or 1 h after injection, the rats were sacrificed under ether anesthesia. The brain was removed, and the radioactivity was measured. After freezing at −25 °C in embedding medium (Tissue-Tek II, Lab-Tek Products), 20 μm sections were cut with a cryostat microtome maintained at −15 to −20 °C. Each section was mounted on a glass slide and air-dried. Autoradiographs were made with LKB Ultrafilm ^{3}H. The exposure time depended on the amount of activity in each section (10 days for a section containing ~7000 cpm). The exposed films were developed with Kodak D-19 developer (1 to 10 dilution, 5 min) and fixed (Figure 3).

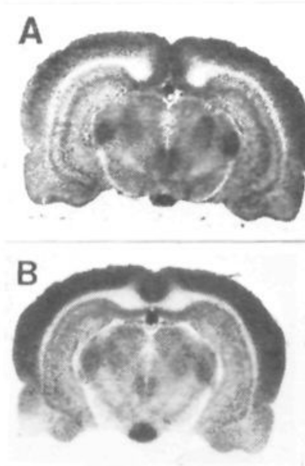

Figure 3. Autoradiographs of brain sections at 2 min (A) and 1 h (B) after an iv injection of [^{125}I]**12** in rats.

Table II. Distribution Coefficient and Protein Binding of Compounds **12–16**

compd	distribution coefficient		protein binding: % free
	pH 7.0	pH 7.4	
12	3.39 ± 0.11	11.6 ± 0.1	48.3 ± 1.6
13	44.8 ± 1.4	176 ± 2	15.9 ± 1.0
14	77.4 ± 2.0	389 ± 31	7.62 ± 0.59
15	260 ± 35	468 ± 69	5.17 ± 0.56
16	522 ± 54	1390 ± 88	6.07 ± 0.54

Results and Discussion

The protein binding and distribution coefficient (DC) of I-125 labeled **12–16** are presented in Table II. The DC (1-octanol/buffer at pH 7.0 and 7.4) of these diamines displayed relatively high lipid solubility (DC > 3) and strong pH dependence (DC at pH 7.4 ≫ DC at pH 7.0). The difference in DC for these diamines between pH 7.4 and 7.0 is the result of shifting of the equilibrium from the neutral to the charged form as pH is lowered. Protein binding generally reflects the lipid-solubility of each compound; when the DC is higher, the percent free of protein binding is lower.

Table III shows the biodistribution of I-125 labeled **12–16** in rats after intravenous injection. The initial brain uptake (2 min after injection) is between 0.9 and 1.8% of the injected dose per whole brain. At 1 h after injection, there is a slight increase in brain activity. Brain to blood ratios at 1 h are higher than those at 2 min. The best brain agent in this group is compound **12**, which displayed brain uptake of 1.80 and 2.02% dose per organ at 2 min and 1 h, respectively. The brain to blood ratio increased from 7.4 at 2 min to 20 at 1 h because of dropping blood levels. Despite the differences in lipid-solubility (DC = 3–1390), every compound in this group exhibits consistent brain uptake at 2 min and 1 h.

Compound **12** displayed the lowest protein binding (48% free) and demonstrated the highest brain uptake in rats. Several of the highly lipid-soluble compounds (**14–16**) showing very low percent free of protein binding (4–7% free) also exhibit significant brain uptake. This is in contrast with the findings of Loberg et al.[8] who reported a group of Tc-99m labeled lipid-soluble compounds that do not pass through blood–brain barrier due to high protein binding. It is apparent that at least for this group of diamines, protein binding is not a significant factor for brain uptake.

It is also apparent from data shown in Table III that the blood level at 2 min is already low for every compound,

(8) M. D. Loberg, *J. Nucl. Med.*, **21**, 183 (1980).

Table III. Biodistribution of I-125 Labeled 12-16 in Rats

	av % dose per organ (range)				
	12, $n = 6$	**13**, $n = 8$	**14**, $n = 3$	**15**, $n = 3$	**16**, $n = 6$
			2 min		
blood	2.63 (2.42-2.84)	4.09 (2.74-5.33)	5.05 (4.84-5.25)	5.30 (3.95-6.09)	4.16 (3.65-4.69)
muscle	9.24 (5.84-12.8)	7.44 (4.59-12.0)	8.38 (7.52-9.77)	6.17 (5.95-6.51)	6.44 (4.49-8.66)
heart	3.23 (3.03-3.55)	2.67 (2.20-3.07)	2.15 (1.95-2.54)	2.51 (2.13-2.96)	3.16 (2.37-4.22)
lungs	16.9 (15.7-18.7)	33.0 (24.6-42.2)	39.8 (36.9-42.9)	33.0 (31.6-34.0)	28.2 (24.3-30.9)
liver	9.51 (7.90-10.6)	9.54 (6.36-14.2)	8.65 (8.10-8.95)	13.8 (12.3-15.0)	11.7 (7.43-15.3)
thyroid	0.22 (0.20-0.23)	0.17 (0.15-0.25)	0.16 (0.13-0.20)	0.16 (0.13-0.19)	0.17 (0.11-0.22)
brain	1.80 (1.71-1.95)	1.71 (1.45-1.89)	1.39 (1.28-1.56)	0.94 (0.85-1.01)	1.67 (1.38-1.86)
brain/blood[a]	7.35	4.49	2.96	1.91	4.31
			1 h		
blood	1.10 (0.92-1.31)	1.15 (0.99-1.37)	1.33 (1.13-1.60)	1.74 (1.64-1.91)	2.79 (2.08-3.71)
muscle	11.9 (8.99-14.4)	14.6 (11.3-17.1)	13.3 (11.9-16.0)	13.4 (11.0-15.8)	14.1 (11.8-19.8)
heart	1.11 (0.97-1.26)	1.49 (1.12-1.86)	1.68 (1.54-1.76)	2.33 (2.29-2.35)	1.33 (0.99-1.80)
lungs	11.7 (10.6-15.1)	16.1 (11.5-26.1)	15.8 (14.0-17.9)	16.3 (15.1-17.1)	15.4 (13.8-17.8)
liver	7.14 (6.22-7.81)	8.01 (7.32-10.1)	9.74 (8.10-10.6)	12.4 (11.0-13.4)	7.38 (6.68-7.93)
thyroid	0.19 (0.17-0.23)	0.21 (0.16-0.26)	0.22 (0.18-0.25)	0.22 (0.19-0.25)	0.20 (0.13-0.26)
brain	2.02 (1.71-2.54)	1.79 (1.51-2.17)	1.43 (1.39-1.47)	0.97 (0.95-1.00)	1.67 (1.41-1.87)
brain/blood[a]	19.7	16.7	11.5	5.99	6.43

[a] Percent dose per gram ratio.

Table IV. Effects of Specific Activity of 12 on Biodistribution in Rats

	av % dose per organ (range of 3 rats) 1 h after injection			
carrier/dose:	6.25 mg	2.5 mg	0.25 mg	0.025 mg
blood	0.61 (0.55-0.64)	0.65 (0.58-0.71)	0.88 (0.83-0.92)	0.91 (0.84-0.99)
muscle	12.3 (11.5-12.9)	16.2 (12.7-18.3)	12.1 (11.0-13.4)	12.2 (11.6-12.9)
heart	0.33 (0.30-0.36)	0.38 (0.34-0.42)	0.72 (0.69-0.74)	1.00 (0.98-1.02)
lungs	4.5 (2.70-7.70)	6.87 (5.89-8.67)	15.2 (12.0-20.3)	12.8 (12.0-14.5)
liver	16.7 (16.5-17.2)	18.9 (17.1-20.7)	8.54 (8.01-9.52)	6.74 (6.23-7.26)
thyroid	0.10 (0.09-0.11)	0.13 (0.12-0.14)	0.17 (0.17-0.19)	0.16 (0.15-0.17)
brain	2.38 (1.95-2.70)	2.21 (2.13-2.36)	1.84 (1.76-1.97)	1.47 (1.39-1.56)
brain/blood[a]	43.0	37.3	22.8	17.6

[a] Percent dose per gram ratio.

indicating free passage of these compounds from blood to cells. The heart exhibited 2-3% uptake at 2 min, but there is rapid clearance from this organ. The lungs, liver, and kidneys showed high initial uptake followed by slow clearance phases.

In Table IV the effect of carrier on the brain uptake in rats is presented. The brain uptake increases slightly when the specific activity of [^{125}I]**12** decreases, indicating there are no saturable specific binding sites in brain. However, there was a shift from lung to liver uptake as the carrier level was increased. It is apparent that at the highest loading dose (6.25 mg/dose), rats survived, indicating toxicity of **12** at such high dose level is not fatal.

Using the same simple exchange technique, we radiolabeled compound **12** with I-123 and injected it intravenously into a monkey. Figure 1 shows the number of counts in a flagged area of the brain at 1-min intervals for 30 min. The radioactivity level in the brain reaches a high level a few minutes after injection and remains stable for the 30-min imaging procedure. Figure 2 is a left lateral image of the monkey head. The radioactivity is localized in the brain, and some areas of soft tissue uptake are observable. Table V shows the results of a biodistribution study carried out on a monkey injected with [^{125}I]**12**. The brain uptake was 3.18% dose/organ, and high lung, liver, and kidney uptakes were observed.

Autoradiographs of normal rat brain at 2 min and 1 h after iv injection of [^{125}I]**12** are presented in Figure 3. All of the sections have a differential distribution with high concentration in gray matter and with little uptake in white matter. This distribution appears to follow regional cerebral perfusion. The pattern of regional distribution

Table V. Biodistribution of [^{123}I]12 in a Monkey 1 h After Intravenous Injection

	% dose/organ	% dose/gram
blood	1.23	0.002
muscle	17.3	0.00491
heart	5.39	0.218
lungs (2)	33.0	1.05
spleen	2.39	0.267
kidneys (2)	16.1	0.602
liver	19.7	0.149
thyroid	0.333	0.0390
bone	1.53	0.00168
brain	3.18	0.0349
brain/blood		17.5

is the same for the 2-min and the 1-h postinjection autoradiographs. In clinical situations, this is highly desirable because it would permit longer imaging time, giving more accumulated counts for better SPECT images.

A number of recent reports have described the brain accumulation of several radioiodine-labeled CNS-active monoamines. The synthesis and biodistribution of 3-(4-[^{131}I]iodo-2,3-dimethoxyphenyl)isopropylamine has been reported and suggested for use in lung and brain imaging.[9] Another monoamine, *N*-isopropyl-*p*-[^{123}I]iodoamphetamine has displayed high brain uptake in animals.[10,11] The

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clinical application of this amine as a cerebral blood flow indicator has recently been demonstrated.[12,13] The mechanism of brain localization for these monoamines may be similar to that of diamines **12–16**. The major advantage of the phenolic diamines **12–16** is the simple labeling procedure that could be used in any nuclear medicine clinic.

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Registry No. **2**, 824-42-0; **3**, 83816-53-9; **4**, 83816-54-0; **5**, 66232-33-5; **6**, 24623-65-2; **7**, 83816-55-1; **8**, 83816-56-2; **9**, 83816-57-3; **10**, 83816-58-4; **11**, 83816-59-5; **12**, 83816-60-8; **12**·2HCl, 83816-67-5; [^{123}I]**12**, 83816-61-9; [^{123}I]**12**, 83816-62-0; **13**, 83816-63-1; **13**·2HCl, 83816-68-6; **14**, 83816-64-2; **14**·2HCl, 83816-69-7; **15**, 83816-65-3; **15**·2HCl, 83816-70-0; **16**, 83816-66-4; **16**·2HCl, 83816-71-1; *o*-methylphenol, 95-48-7; *o*-propylphenol, 644-35-9; *o*-(1-methylpropyl)phenol, 89-72-5; *o*-cyclohexylphenol, 119-42-6; *o*-(1,1-dimethylethyl)phenol, 88-18-6.

Structure–Activity Studies of Highly Potent Cyclic [Cys^4,Cys^{10}]Melanotropin Analogues[1]

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It has been proposed[14] that a β turn or other chain-reversal structure involving the residues His-Phe-Arg-Trp in α-melanocyte stimulating hormone (α-MSH) contributes to the bioactive conformation of this peptide hormone. This proposal is supported by the observation that [Cys^4,Cys^{10}]-α-MSH exhibits superagonist ($\geq$10 000 α-MSH) activity in the frog skin bioassay and is about 30 times more potent in the lizard skin bioassay. Studies on the possible role of a reverse turn in the biological activities of [Cys^4,Cys^{10}]-α-MSH have been extended with the synthesis of Ac-[Cys^4,Cys^{10}]-α-MSH$_{4-10}$-NH$_2$ and Ac-[Cys^4,Cys^{10}]-α-MSH$_{4-13}$-NH$_2$. The cyclic 4–10-heptapeptide was found to be less active than α-MSH in both the frog and lizard skin bioassays, but much more potent (100 times) than its linear congener Ac-α-MSH$_{4-10}$-NH$_2$ in the frog. With the cyclic 4–13-decapeptide, superagonist potency (equipotent to the cyclic tridecapeptide) was observed on the frog skin, and the analogue was equipotent to α-MSH in the lizard skin assay. These results support the suggestion that a cyclic reverse-turn conformation in α-MSH plays a significant role in the hormone–receptor interaction. However, the reduced potency observed with Ac-[Cys^4,Cys^{10}]-α-MSH$_{4-10}$-NH$_2$ and the superagonist activity with Ac-[Cys^4,Cys^{10}]-α-MSH$_{4-13}$-NH$_2$ suggest that the C-terminal tripeptide sequence Lys-Pro-Val-NH$_2$ (or some component thereof) in combination with a reverse turn in the cyclic moiety are both important for the superagonist activity of the latter cyclic analogue and of [Cys^4,Cys^{10}]-α-MSH.

The linear tridecapeptide α-melanocyte stimulating hormone (α-MSH, Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH$_2$) is synthesized and secreted by the pars intermedia of the vertebrate pituitary.[2] This peptide hormone has been implicated in many important physiological functions, including integumental melanogenesis,[3] neural functioning related to facilitated memory and attention and other behavioral paradigms,[4-7] and fetal development.[8] Previous structure–function studies on α-melanotropin analogues and fragments using the in vivo frog skin bioassay system[9-12] have resulted in the proposal that the primary active site of α-MSH consists of the seven residues Met-Glu-His-Phe-Arg-Trp-Gly. Other studies[13] have suggested an additional active sequence containing the carboxamide terminal tripeptide Lys-Pro-Val-NH$_2$.

Recently, Sawyer et al.[14] proposed that a β turn or other peptide chain-reversal conformation within the central active site (His-Phe-Arg-Trp) of α-MSH may be important in the biologically active conformation of the hormone. The cyclic disulfide tridecapeptide analogue [Cys^4,Cys^{10}]-α-MSH (Figure 1) was then synthesized in order to evaluate the effect of covalently stabilizing a reverse turn conformation in α-MSH involving Met-Glu-His-Phe-Arg-Trp-Gly. This compound was found to possess superagonist potency and prolonged in vitro activity in stimulating melanosome dispersion within frog

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(1) Symbols and abbreviations are in accord with the recommendations of the IUPAC–IUB Commission on Biochemical Nomenclature [*J. Biol. Chem.*, **247**, 977 (1972)]. Other abbreviations include: α-MSH, α-melanotropin, α-melanocyte stimulating hormone; Nle, norleucine; 2,4-Cl$_2$-Z, 2,4-dichlorobenzyloxycarbonyl; DCC, dicyclohexylcarbodiimide; 2,6-Cl$_2$-Bzl, 2,6-dichlorobenzyl; *p*-MBHA resin, *p*-methylbenzhydrylamine resin; HOBT, *N*-hydroxybenzotriazole. Taken in part from the Ph.D. Dissertation of T. K. Sawyer, Department of Chemistry, University of Arizona, 1981.
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