Pergamon

Tetrahedron 55 (1999) 5733–5740

TETRAHEDRON

# New Multidentate Ligands Containing Mercaptobenzyl Functional Groups, and Biodistribution of Gallium-67-TACN-HSB

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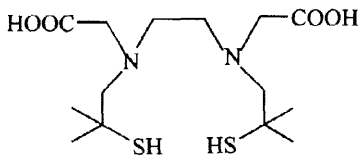
Received 4 February 1999; revised 8 March 1999; accepted 11 March 1999

**Abstract.** The synthesis of two multidentate ligands containing mercaptobenzyl functional groups: N,N'-bis(2-mercaptobenzyl)ethylenediamine-N,N'-diacetic acid and N,N',N''-tris(2-mercaptobenzyl)-1,4,7-triazacyclononane is described. Labeling of N,N',N''-tris(2-mercaptobenzyl)-1,4,7-triazacyclononane with $^{67}$Ga was carried out, and the results are compared with that obtained with tris(2-mercaptobenzyl)amine. 

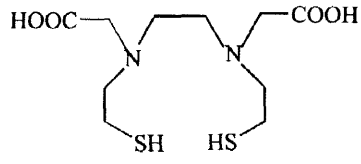
**Keywords.** Chelation, , thiols, gallium (III) compounds, complexes.

## INTRODUCTION

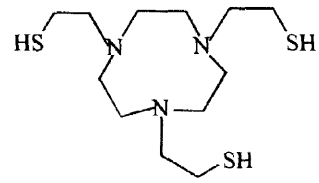
We have recently been successful in investigating several thiol-containing chelates with very high affinity for the coordination of In(III) and Ga(III): N,N'-bis(2,2-dimethyl-2-mercaptoethyl)ethylenediamine-N,N'-diacetic acid (6SS, **1**), $K_{InL} = 10^{39.8}$,[1] $K_{GaL} = 10^{41}$;[1] N,N'-bis(2-mercaptoethyl)ethylenediamine-N,N'-diacetic acid (EDDASS, **2**), $K_{InL} = 10^{37.0}$,[2] $K_{GaL} = 10^{35.6}$;[2] N,N'N''-tris(2-mercaptoethyl)-1,4,7-triazacyclononane (TACN-TM, **3**), $K_{InL} = 10^{36.1}$,[3] $K_{GaL} = 10^{34.2}$.[3] The common feature of these three ligands is that they contain two or three 2-thioethyl groups which form five-membered chelate rings with In(III) and Ga(III). Aromatic thiol groups have lower $pK_a$'s than the aliphatic 2-thioethyl groups. There is interest to prepare similar ligands with lower $pK_a$'s.




6SS, **1**




EDDASS, **2**




TACN-TM, **3**

K. Wieghardt and coworkers[4] synthesized a tripodal ligand containing aromatic thiol groups: N,N',N''-tris(2-thio-4-*t*-butylbenzyl)-1,4,7-triazacyclononane (compound **4**). Govindaswamy, *et al.*[5a] prepared the tripodal mercaptobenzyl containing ligand: tris(2-mercaptobenzyl)amine ($S_3N$, **5**). Cutler, *et al.*[5b] reported that the Ga(III) complexes of $S_3N$ (**5**) cross the blood-brain barrier and also localized in the heart. For further exploration of the relation between ligand structure and biodistribution behavior of their In(III)

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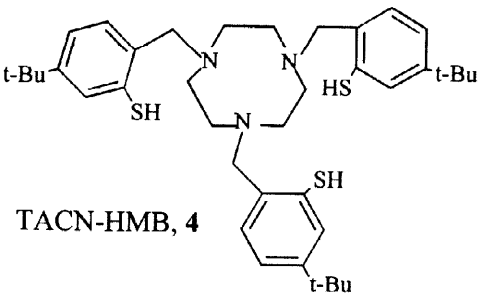
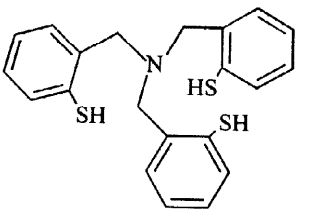
0040-4020/99/$ - see front matter 
*PII:* S0040-4020(99)00243-4

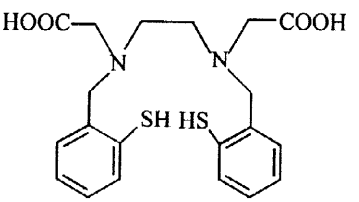
and Ga(III) complexes, the analogues of 6SS (**1**) and TACN-TM (**3**), where the mercaptoethyl groups are replaced by the mercaptophenolate groups: N,N'-bis(2-mercaptobenzyl)ethylenediamine-N,N'-diacetic acid (6SSAr, **6**) and N,N'N''-tris(2-mercaptobenzyl)-1,4,7-triazacyclononane (TACN-HSB, **7**) were prepared, and the biodistribution of the TACN-HSB-$^{67}$Ga complex was studied.



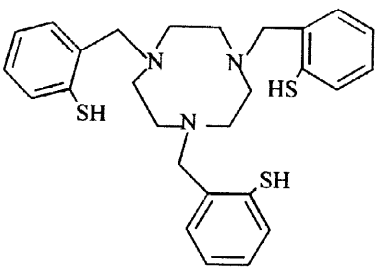

TACN-HMB, **4**




$S_3N$, **5**




6SSAr, **6**



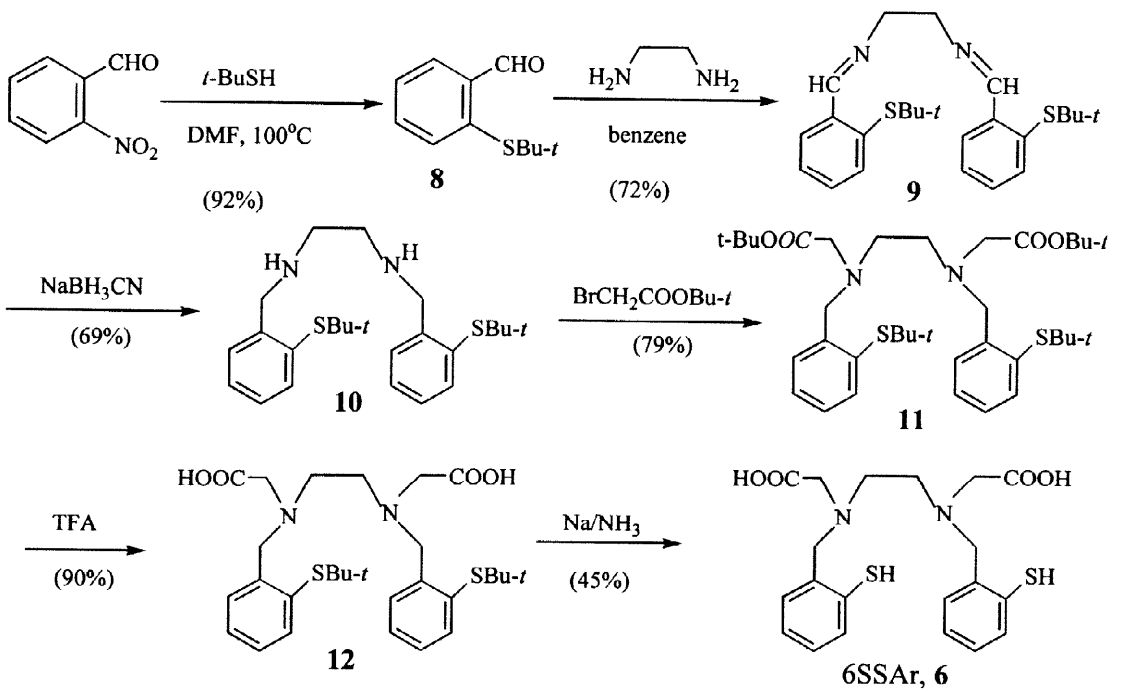

TACN-HSB, **7**

## Results and Discussion

*Synthesis*

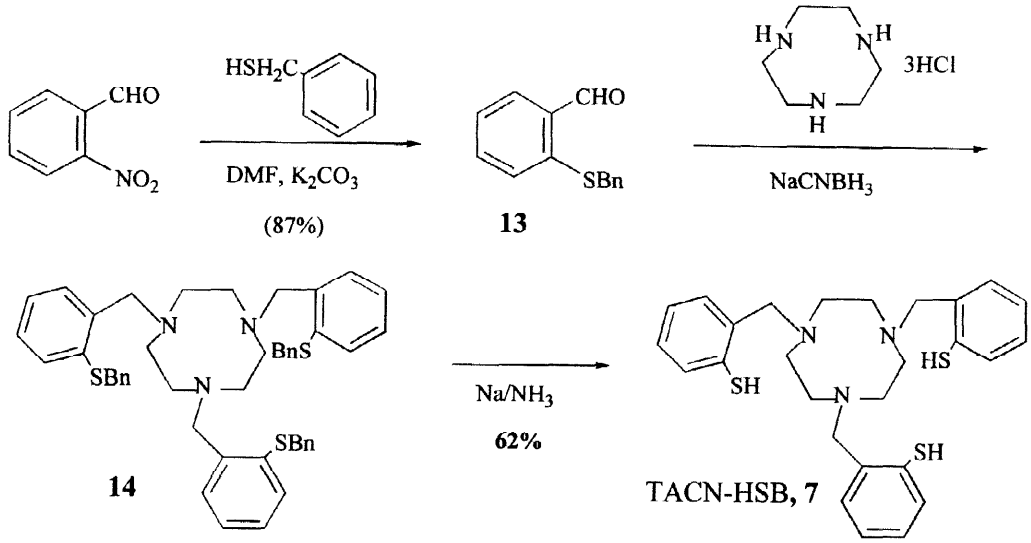
The route used for the synthesis of 6SSAr (**6**) involving intermediates **10, 11, 12,** is shown in Scheme I.

*Scheme I*

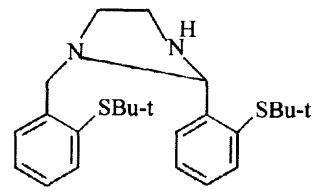


The route used for the synthesis of TACN-HSB (**7**) involving intermediates **13, 14** is shown in Scheme II.

*Scheme II*




Although $NaBH_4$ is a good reagent to reduce Schiff bases to amines, it cannot be used to prepare the diamino compound **10**. The basic condition of this reducing reagent leads to the formation of an impurity **15** which is difficult to remove. This problem can be solved by maintaining the pH of the reaction solution at about 4 and $NaBH_3CN$ is used as reducing agent.

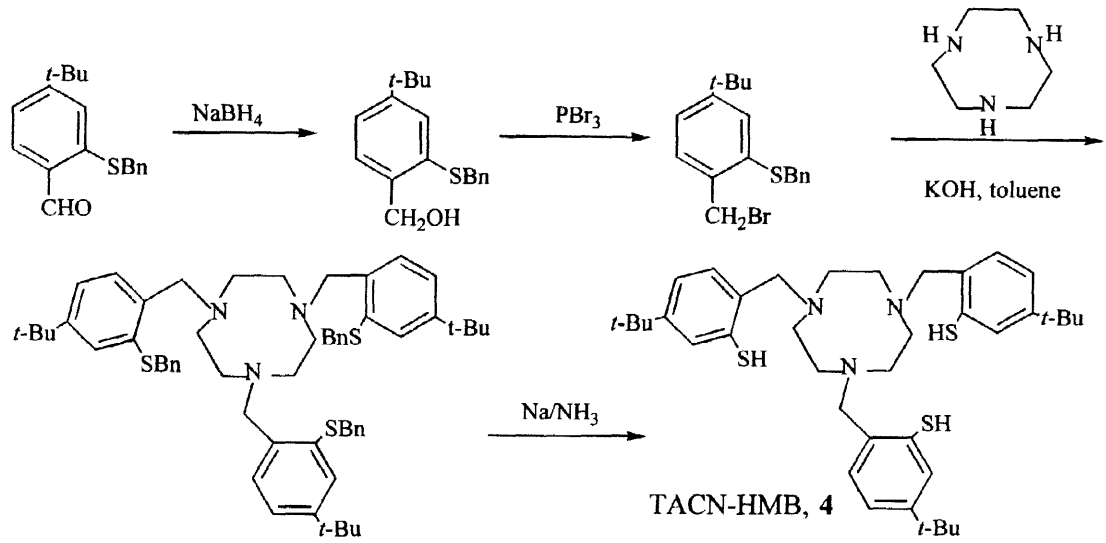



**15**

There are two publications on the synthesis of 2-benzylmercaptobenzaldehyde (**13**). Stacy *et al.*[6] methods start from benzyl-α-chloro-2-tolyl sulfide by the Sommelet reaction or from 2-benzylthiobenzoic acid by the McFadyen and Steven's method. Both need additional steps from commercially available reagents and have relatively low yields. Murata and Yoshida's Patent[7] uses a one-pot reaction; the starting material is 2-chlorobenzaldehyde. Since in aromatic nucleophilic substitution, the nitro group is a much better leaving group than the chloro group, we adapted Meth-Cohn and Tarnowski's work,[8] starting from 2-nitrobenzaldyde with mild reaction conditions and a simple work-up procedure; a 87% yield was obtained.

Wieghardt and coworker[4] synthesized a similar TACN-tris-mercaptobenzyl ligand (**4**) through a four step route (see Scheme III). The first two steps are unnecessary if the starting aldehyde can be directly reacted with triazacyclononane by reductive amination, and this method was successful.

*Scheme III*




*Biodistribution Studies*

Previous attempts at labeling TACN-HSB (**7**) with $^{68}$Ga were unsuccessful due to the instability of the ligand to air and the short half-life of Ga-68 ($t_{1/2}$ = 68 min) prevented monitoring the reaction over long periods of time. The experiment was started with freshly prepared ligand and a longer lived $^{67}$Ga ($t_{1/2}$ = 3.3 days). Sonicating the reaction mixture overnight resulted in yields of about 80%. Two complex peaks were observed for $^{67}$Ga-TACN-HSB that were not completely resolved and migrated with $R_f$'s = 0.5-0.6 and 0.7-0.8 on reversed phase plates and $R_f$'s = 0.4-0.5 and 0.6-0.7 on silica plates. In both systems $^{67}GaCl_3$ remained at the origin. *In vitro* serum stability of $^{67}$Ga-TACN-HSB analyzed by both reversed phase and normal phase TLC in rat serum incubated at 37°C, showed the complex to be > 89% intact out to 1 hr.

As indicted in Table 1, $^{67}$Ga-TACN-HSB exhibits high blood (38 ± 1% ID/organ at 2 min) and liver uptake (33 ± 1% ID/organ at 2 min). The liver uptake increased to 64 ± 10% ID/organ at 15 min post injection remaining fairly constant out to 60 min (59 ± 3% ID/organ). Significant uptake was also seen in the bone (5.4 ± 0.4% ID/organ at 2 min) which slowly decreased over time (2.5 ± 0.2% ID/organ at 60 min). High uptake was also seen in the lungs (3 ± 1% ID/organ at 2 min) which decreased over time (0.9 ± 0.1% ID/organ at 60 min) and the spleen (2.3 ± 0.4% ID/organ at 2 min) which increased slightly over time (2.7 ± 0.1% ID/organ at 60 min). Significant uptake was observed in the brain at 2 min (0.17 ± 0.04% ID/organ) which decreased over time (0.08 ± 0.02% ID/organ at 60 min). Uptake was also seen in the heart (0.682 ± 0.006% ID/organ at 2 min) which decreased by 60 min to 0.15 ± 0.02% ID/organ. The data show that the complex clears primarily through the liver and intestines with only a small amount clearing through the kidneys and bladder. As indicated in Table 2, although $^{67}$Ga-TACN-HSB exhibits uptake in the heart and brain, the concomitant high blood uptake makes this compound useless as a diagnostic imaging agent. However, the differences in biodistribution between $^{67}$Ga-TACN-HSB and the gallium labeled tris(2-mercaptobenzyl)amine compound ($S_3N$, **5**) are of interest.[5] For instance $^{68}$Ga-$S_3N$ exhibits high lung uptake (3 ± 0.9% ID/organ at 2 min) which was accumulated over time (6 ± 0.7% ID/organ at 60 min). The lung accumulation was not observed for $^{67}$Ga-TACN-HSB. The liver uptake of $^{68}$Ga-$S_3N$ was also initially quite high (72 ± 7% ID/organ at 2 min), however, unlike $^{67}$Ga-TACN-HSB which exhibited increased liver uptake, the liver uptake of $^{68}$Ga-$S_3N$ actually decreased over time (21 ± 1% ID/organ at 60 min). The brain uptake of $^{68}$Ga-$S_3N$ differed in that it increased over time instead of decreasing as observed for $^{67}$Ga-TACN-HSB. These differences are of note as these compounds share some of the same chemical similarities.

Table 1. Selected Biodistribution Data for $^{67}$Ga-TACN-HSB, values expresses as % ID/organ.

| Time | 2 min | 15 min | 60 min |
|---|---|---|---|
| Blood | 38 ± 1 | 12 ± 3 | 3.2 ± 2 |
| Liver | 33 ± 1 | 64 ± 10 | 59 ± 3 |
| Bone | 5.4 ± 0.4 | 3.8 ± 0.5 | 2.5 ± 0.2 |
| Lung | 3 ± 1 | 1.7 ± 0.6 | 0.9 ± 0.1 |
| Spleen | 2.3 ± 0.4 | 4.1 ± 0.6 | 2.7 ± 0.1 |
| Brain | 0.17 ± 0.04 | 0.12 ± 0.04 | 0.08 ± 0.02 |
| Heart | 0.68 ± 0.01 | 0.49 ± 0.06 | 0.15 ± 0.02 |

Table 2 Comparison of the biodistribution data of $^{67}$Ga-TACN-HSB and $^{68}$Ga-$S_3N$

| | $^{67}$Ga-TACN-HSB | | $^{68}$Ga-$S_3N$ | |
|---|---|---|---|---|
| Lung | 0.9 ± 0.1 | ID/organ at 60 min | 6 ± 0.7 | ID/organ at 60 min |
| Liver | 33 ± 1 | ID/organ at 2 min | 72 ± 7 | ID/organ at 2 min |
| | 59 ± 3 | ID/organ at 60 min | 21 ± 1 | ID/organ at 60 min |
| Brain | 0.17 ± 0.04 | ID/organ at 2 min | 0.07 ± 0.03 | ID/organ at 2 min |
| | 0.08 ± 0.02 | ID/organ at 60 min | 0.26 ± 0.04 | ID/organ at 60 min |

Biodistributions are not available for $^{111}$In-6SSAr, $^{68,67}$Ga-6SSAr and $^{111}$In-TACN-HSB because labeling efforts failed due to the instabilites of the ligands. These complexes were found (by proton NMR) to decompose during the labeling process, as mentioned above.

## Experimental Section

*Materials and Methods*

Benzyl mercaptan, 2-nitrobenzaldehyde, *t*-butyl bromoacetate, sodium cyanoborohydride, 1,4,7-triazacyclononane trihydrochloride were obtained from Aldrich Chemical Co. and were used as supplied. 2-*t*-Butylthiobenzaldehyde (**8**),[6] N,N'-bis(2-*t*-butylthiobenzylidine)ethylenediamine (**9**)[9] were prepared by previously reported procedures.

The proton and carbon-13 NMR were recorded on a Varian XL-200 spectrometer operating at 200 MHz, and the chemical shifts are reported in ppm relative to tetramethylsilane. The mass spectra were obtained with the Departmental VG analytical 70S high resolution double focusing magnetic sector spectrometer with an attached VG analytical 11/250J data system. Measurements were made by the Departmental mass spectrometry specialist, Dr. Lloyd W. Sumner. The C, H, N analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN. The melting point was determined with a Fisher-Johns melting point apparatus and was corrected.

*Synthetic Procedures*

*N,N'-Bis(o-t-butylthiobenzyl)ethylenediamine (**10**).* N,N'-Bis(o-*t*-butylthiobenzylidine)ethylenediamine (**9**),[9] 4.03 g (9.9 mmol), was dissolved in 50 mL of absolute ethanol with a few drops of bromocresol blue indicator (pH 3.8 - 5.4, yellow to blue). At ice-water temperature, 1.3 g of $NaBH_3CN$ was added portionwise and a 2M HCl-ethanol solution (1 mL of conc. HCl in 5 mL of absolute ethanol) was added to maintain a yellow colored solution (pH is about 3.8). The stirring was continued at room temperature for 2

hr. After filtration, washing with ethanol and ethyl ether and drying, 2.8 g of pure product (dihydrochloride salt) was obtained. The filtrate and washings were combined and made alkaline with 50% NaOH and were extracted with ethyl ether; after purification by flash chromatography another 0.49 g of di-HCl salt was obtained; total yield is 69%. $^1$H NMR (in $CDCl_3$): 7.5-7.40 & 7.35-7.10 (m, 8H, arom.); 4.02 (s, 4H, -$CH_2$- of benzyl); 2.72 (s, 4H, ethylene); 1.79 (b, 2H, -NH-); 1.28 (s, 18H, *t*-butyl).$^{13}$C NMR (in $CDCl_3$): 145.4, 138.9, 131.9, 129.6, 129.0, 126.8 (arom.); 52.3 (-$CH_2$- of benzyl); 48.5 (ethylene); 47.3 (-C-$(CH_3)_3$); 31.1 (-$CH_3$). Anal. Calcd. for $C_{24}H_{36}N_2S_2$: C, 69.23; H, 8.65; N, 6.73. Found: C, 68.81; H, 8.62; N, 6.50.

*N,N'-Bis(2-t-butylthiobenzyl)ethylenediamine-N,N'-diacetic Acid, di-t-butyl ester* (**11**). A mixture of 0.6 g (1.4 mmol) of compound **10**, 0.55g (2.8 mmol) of *t*-butyl bromoacetate, 0.37 g (2.8 mmol) of potassium carbonate, 0.16g (0.93 mmol) of potassium iodide and 28 mL of acetonitrile was stirred at room temperature for 24 hr. After the inorganic salts were removed by filtration and the solvents were removed by vacuum distillation, 1 g of yellow oil was obtained. It was purified by flash chromatography with Silica Gel 60 and the column was eluted with benzene and benzene-ethyl ether mixed solvent. The pure product, consisting of 0.71 g colorless oil was obtained; yield 79%. $^1$H NMR (in $CDCl_3$): 7.7-7.1(m, 8H, arom.); 4.07 (s, 4H, -$CH_2$- of benzyl); 3.26 (s, 4H, ethylene); 2.83 (s, 4H, -$CH_2$COO-*t*-Bu); 1.45 (s, 18H, -COO-t-Bu); 1.25 (s, 18H, -S-*t*-Bu). $^{13}$C NMR (in $CDCl_3$): 171.2 (-COO-t-Bu); 144.8, 138.6, 132.1, 129.8, 128.8, 126.4 (arom.); 80.5 (-$CH_2$COO-*t*-Bu); 56.5 (-$CH_2$- of benzyl); 55.5 (-COO-C$(CH_3)_3$); 52.2 (ethylene); 47.0 (-SC$(CH_3)_3$); 31.1 (-SC$(CH_3)_3$); 28.2 (COO-C$(CH_3)_3$. Anal. Calcd. for $C_{36}H_{56}N_2O_4S_2 \cdot H_2O$: C, 65.26; H, 8.76; N, 4.23. Found: C, 65.39; H, 8.33; N, 4.20.

*N,N'-Bis(2-t-butylthiobenzyl)ethylenediamine-N,N'-diacetic Acid* (**12**). Compound **11**, 0.71g (1.1 mmol), was dissolved in 30 mL of trifluoroacetic acid (TFA, corrosive, toxic) and was allowed to stand at room temperature covered with dry Ar gas for 2 days. The excess TFA was removed by distillation at 60 mm Hg and 25°C. Benzene (about 25 mL) was added and removed under reduced pressure three times to complete the removal of TFA and moisture. The product was vacuum dried at 1 mm Hg and 40°C for 2 hr, and 0.8 g of brown oil was obtained. It was purified with Silica Gel 60 and the column was eluted with benzene, $CH_2Cl_2$, and $CH_2Cl_2$-$CH_3OH$. The product was collected by elution with mixed solvent: $CH_2Cl_2$:$CH_3OH$ = 9:1(v/v); 0.55 g of pure product was obtained (yield 90%). $^1$H NMR (in $CDCl_3$):7.57-7.52 & 7.4-7.2 (m, 8H, arom.); 6.6-6.2 (b, 4H, -COOH); 4.26 (s, 4H, -$CH_2$- of benzyl); 3.71 (s, 4H, -$CH_2$COOH); 3.14 (s, 4H, ethylene); 1.20 (s, 18H, -$CH_3$). $^{13}$C NMR(in $CDCl_3$): 170.7 (-COOH); 139.4, 136.7, 133.7, 131.5, 130.1 & 129.3 (arom.); 56.6 (-$CH_2$- of benzyl); 54.7 (-$CH_2$COOH); 53.5 (ethylene); 48.4 (-SC$(CH_3)_3$); 30.7 (-SC$(CH_3)_3$). FAB. M.S.: [M+H] = 533. Anal: Calcd. for $C_{28}H_{40}N_2O_4S_2 \cdot 0.9$ $CHCl_3$: C, 54.23; H, 6.40; N, 4.38. Found: C, 54.13; H, 6.65; N, 4.07.

*N,N'-Bis(2-mercaptobenzyl)ethylenediamine-N,N'-diacetic Acid* (6SSAr, **6**). Compound **12,** 0.42 g (0.8 mmol), was dissolved in 2 mL of dry THF and added portionwise to a solution of 0.1 g of Na (flammable solid, moisture sensitive) metal in 20 mL of liquid $NH_3$ in a dry ice-i-PrOH bath (about -65°C). The blue colored solution became colorless within 5 min. More Na metal was added (about 0.05 g); the blue solution was maintained at this temperature for 30 min; then the reaction was quenched by the addition of powdered $NH_4Cl$. The liquid $NH_3$ was removed by evaporation and then by vacuum evaporation at 1 mm Hg and room temperature. Degassed water, 4 mL, were added and the insoluble material was removed by filtration under Ar. Dilute hydrochloric acid (1 M ) was added until pH was about 3. The green sticky product was taken out by decantation and washed several times with water. After vacuum drying over $P_2O_5$ for 16 hr. 0.2 g pure product was obtained; yield 45%. $^1$H NMR(in $D_2O$-NaOD pD > 12, *t*-BuOH as internal standard: 1.29 ppm): 7.46 (m, 2H, arom.); 7.24 (m, 2H, arom.); 6.99 (m, 4H, arom.); 3.82 (s, 4H, -$CH_2$- of benzyl); 3.16 (s, 4H, -$CH_2COO^-$); 2.79 (s, 4H, ethylene). $^{13}$C NMR (in $D_2O$-NaOD pD > 12, *t*-BuOH as internal standard: 70.8 ppm & 30.8 ppm): 180.9 (-$COO^-$); 147.7, 139.9, 135.8, 130.2, 127.4, 122.8 (arom.);

59.9 (-$CH_2$-benzyl); 59.1 (-$CH_2$ $COO^-$); 52.8 (ethylene). FAB. M.S.: [M+H] = 421. Anal. for $C_{20}H_{24}N_2O_4S_2 \cdot 1/2\ H_2O$, Calcd: C, 55.94; H, 5.83; N, 6.53; Found: C, 55.87; H, 6.01; N, 6.20.

*2-Benzylthiobenzaldehyde* (**13**). Benzyl mercaptan, 12.3 mL (13g, 0.105 mole) was added to a suspension of 15 g of powdered $K_2CO_3$ in 50 mL of dry (with 4A molecular sieves) DMF. After it was heated in a 70-80°C oil bath for 1.5 hr, the reaction mixture was cooled to room temperature. A solution of 15 g of 2-nitrobenzaldehyde in 40 mL of DMF was added to this suspension in 20 min. It was stirred in 70-80°C bath for 1 hr; and at room temperature for another 1 hr.; then poured into 500 mL of water and was extracted with 2/200 mL of benzene. The benzene phase was washed with 4/500 mL of water; 5/200 mL of 2% NaOH; 2/200 mL of NaCl saturated solution and dried with anhydrous $MgSO_4$ for 2.5 hr. The dry benzene solution was concentrated to about 50 mL. After the addition of 100 mL of hexane, a large amount of pale yellow product was separated. It was filtered and washed with a 1:4 solution of benzene/hexane and air dried; 18.3 g of pure product was obtained. From the filtrate another 1.6 g pure product was obtained; total yield 19.9 g (87%). $^1H$ NMR (in $CDCl_3$): 10.25 (s, 1H, aldehyde); 7.81 (dd, 1H, arom. of thiobenzaldehyde); 7.5-7.35 (m, 3H, arom. of thiobenzaldehyde); 7.3-7.2 (m, 5H, arom. of benzyl); 4.13 (s, 2H, -$CH_2$-) m.p. 74 -76 °C (Lit.[6] 75 - 76 °C).

*N,N,N''-Tris-2-benzylthiobenzyl-1,4,7-triazacyclononane* (**14**). A mixture of 0.95 g (4 mmol) of 1,4,7-triazacyclononane trihydrochloride, 0.7 g (11 mmol) of KOH, 0.8 g $H_2O$ ,4 g of methanol and 2 drops of bromothymol blue indicator (pH 6.0-7.6; yellow to blue) was prepared and stirred at room temperature for 20 min. A solution of 3.4 g (15 mmol) of 2-benzylthiobenzaldehyde in 12 mL of methanol was added; and 0.6 g (9.6 mmol) of $NaBH_3CN$ was added portionwise within 15 min. After stirring at room temperature for 2.5 hr, the pH of the reaction solution became 10.5. A solution of 1:9 of conc. HCl/ MeOH was used to adjust the pH of the solution to 8.3 (just blue color). More aldehyde (1.6g) and $NaBH_3CN$ (0.3g) was added, and the pH of the solution was maintained at about 8.3. This reaction mixture was stirred at room temperature for 2 days. After the solvents were removed, the reaction mixture was allowed to distribute between methylene chloride and water. The organic phase was washed with 2% $Na_2CO_3$ solution and saturated NaCl solution, and dried over anhydrous $MgSO_4$ for 24 hr. The crude product was purified chromatographically twice. The product was collected by elution with a solution of $CH_2Cl_2$:MeOH:conc. $NH_3$-$H_2O$ = 100:15:2; 1.75 g of pure product was obtained, yield 57%. $^1H$ NMR (in $CDCl_3$): 7.4-7.05 (m, 27H, arom.); 4.04 (s, 6H, -$CH_2$- of benzyl); 3.59 (s, 6H, -$CH_2$- of 2-thiobenzyl); 2.73 (s, 12H, ethylene of triazacyclononane). $^{13}C$ NMR (in $CDCl_3$): 139.8 (1-C of thiobenzyl); 137.5 (2-C of thiobenzyl); 136.3 (1-C of benzyl); 130.0-125.8 (3,4,5,6-C of thiobenzyl and 2,3,4-C of benzyl); 60.1 (-$CH_2$- of thiobenzyl); 55.0 (ethylene of triazacyclononane); 38.9 (-$CH_2$- of benzyl). FAB. M.S.: [M+H] = 766. Anal. Calcd. for $C_{48}H_{51}N_3S_3 \cdot H_2O$: C, 73.56; H, 6.77; N, 5.36. Found: C, 73.64; H, 6.87; N, 5.16.

*N,N'N''-Tris(2-thiobenzyl)-1,4,7-triazacyclononane* (TACN-HSB, **7**). Compound **14**, 0.35 g (0.46 mmol) was dissolved in 2 mL of dry THF and added portionwise to a solution of 0.1 g of Na metal in 15 mL of liquid $NH_3$ in a dry ice-*i*-PrOH bath (about -65°C). The blue colored solution became colorless within 2 min. More Na metal was added (about 0.05 g); the blue solution was maintained at this temperature for 40 min; then the reaction was quenched by the addition of powdered $NH_4Cl$. The liquid $NH_3$ was removed by evaporation and then by vacuum evaporation at 1 mm Hg and room temperature. Degassed water, 8 mL was added and the insoluble impurities were removed by filtration under Ar. Dilute hydrochloric acid (2.5 M) was added until the pH was about 4.5-5.0. The white precipitate was separated by filtration and washed several times with water. After it was vacuum dried over $P_2O_5$ for 16 hr, 0.14 g pure product was obtained, yield 62%. $^1H$ NMR (in $CDCl_3$): 7.4 - 7.1(m, 12H, arom.); 3.72 (s, 6H, -$CH_2$- of benzyl); 3.04 (s, 12H, ethylene of the triazacyclononane). $^{13}C$ NMR (in $CDCl_3$): 133.4, 131.9, 129.1, 126.0 (arom.); 60.2 (-$CH_2$- of benzyl); 52.1 (ethylene of the triazacyclononane). FAB. M.S.: [M+H] = 495. Anal. Calcd. for $C_{27}H_{33}N_3S_3 \cdot 1.5H_2O$: C, 62.07; H, 6.90; N, 8.11. Found: C, 62.35; H, 6.46; N, 8.11.

*Radiolabeling*

The ligand (TACN-HSB) solution was prepared by placing 1-2 mg of ligand in 1 mL of ethanol that had been degassed for 15 min with argon. The resultant solution was sonicated until all the ligand was dissolved.

$^{67}GaCl_3$ was obtained from the Cyclotron Consulting Group (Miami, FL) in 0.15 M HCl. The $^{67}GaCl_3$ (2mCi) was first diluted with 1 M HCl and then evaporated to dryness with a heat gun under a stream of nitrogen, redissolved in 400 mL of ethanol and degassed for 10 min with argon. The entire ligand solution was then added to the $^{67}GaCl_3$ which was then capped, vortexed and left at room temperature for 10 min. Quality control was determined by radio-TLC on silica and C18 plates developed in pure methanol. Initially little to no complexation was observed. The reaction mixture was left sonicating overnight and checked the next morning.

The product was purified prior to biodistribution by addition of 3 mL of saline and subsequent loading onto a preconditioned C18Sep-Pak Light (prepared by washing with 3-5 mL of ethanol followed by 3-5 mL of normal saline) to remove excess ligand and uncomplexed $^{67}Ga$. The Sep-Pak was then washed with 3 mL of saline. The complex was eluted with 400 mL of ethanol. Saline was then added to the eluate to give a solution of 85% saline/15% ethanol and the % complex was determined by TLC.

*Serum Stability*

*In vitro* serum stability studies were conducted by placing 100 μL (135 μCi) of $^{67}Ga$-TACN-HSB in 1 mL of freshly drawn rat serum incubating at 37° C. Samples were removed at various times and analyzed by the radio-TLC procedures described above.

*Biodistribution Studies*

Mature male Sprague-Dawley rats (n = 4), average weight 320 g, were anesthetized with Metofane, injected with 5 μCi of $^{67}Gd$-TACN-HSB in 80 μL via the tail vein. The rats were anesthetized prior to sacrifice by decapitation at each time point. The blood, lung, liver, spleen, kidney, bladder, muscle, fat, heart, brain, bone and intestines were removed from each animal, placed on absorbent paper and weighed. Blanks and standards were prepared and counted along with the blood samples in order to calculate the percent injected dose per gram of tissue (% ID/g), percent injected dose per organ (% ID/organ) and to correct for physical decay.

*Acknowledgment.* This research was supported by U.S. Public Health Service, National Cancer Institute, CA-42925. National Science Foundation Grant CHE-8705697 supported the purchase of the mass spectrometers.

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