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7,10-BIS(2-MERCAPTO-2-METHYL)PROPYL-7,10-DIAZAPALMITIC ACID: A NOVEL, N_2S_2 LIGAND FOR TECHNETIUM-99m

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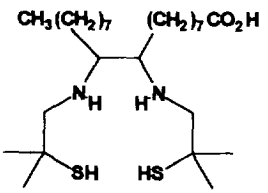
Abstract: 7,10-Bis(2-mercapto-2-methyl)propyl-7,10-diazapalmitic acid (**7**) was synthesized and evaluated as a new ligand for technetium-99m (^{99m}Tc). The title fatty acid analog, an achiral, bis(aminoethanethiol) derivative in which the amines are tertiary, gave a stable complex with ^{99m}Tc. The biodistribution of ^{99m}Tc-labeled **7** in rats is reported.

The development of technetium-99m (^{99m}Tc) labeled radiopharmaceuticals based on a diamin(id)odithiol (N_2S_2) core continues to attract considerable interest.[1-7] A specific goal has been the realization of a ^{99m}Tc-labeled fatty acid derivative.[8-10] We recently reported on the synthesis and biodistribution of a ^{99m}Tc-labeled stearic acid derivative (**1**).[10] Although the identity of the ultimate N_2S_2 ligand was ascertained by positive-ion fast atom bombardment mass spectrometry (FABMS) and elemental analysis of its nickel (II) complex, the ligand was undoubtedly a diastereomeric mixture as a consequence of the synthetic scheme employed. Herein is described the synthesis of a new, achiral N_2S_2 ligand (**7**) that incorporates a free carboxylate group, and in which the amines are tertiary. The synthetic pathway obviates the stereochemical concerns inherent in our previous study. The biodistribution of the ^{99m}Tc-labeled ligand was studied in rats.



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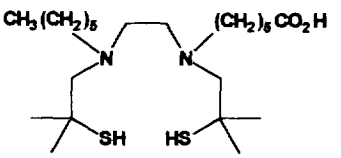

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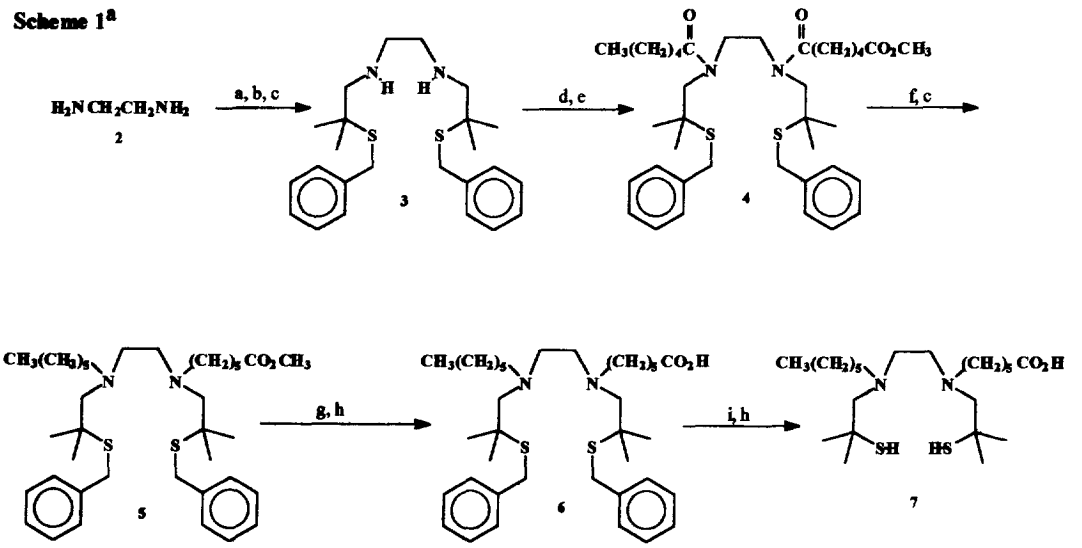
The synthesis of the title compound is outlined in Scheme 1. Ethylenediamine (**2**) was condensed with two equivalents of 2-methyl-2-(benzylthio)propanal that had been prepared in a one-step reaction from benzyl mercaptan, isobutyraldehyde and N-chlorosuccinimide.[11] The resultant diimine was not isolated, but reduced with $NaBH_4$ to give the corresponding diamine (**3**) isolated as the dihydrochloride salt in 73% yield based on **2**.[12] Compound **3** was acylated with one equivalent of hexanoyl chloride to give after flash chromatography[13] the desired monoamide in 70% yield.[14] Subsequent acylation with methyl adipyl chloride afforded diamido ester **4** in 77% yield.[15] The amide groups were selectively reduced with 1 M BH_3 THF to give after flash chromatography the corresponding diamine (**5**) as a syrup in 45% yield.[16] Saponification of **5** with KOH in 50% aqueous ethanol gave, after neutralization with HCl and flash chromatography, the free acid (**6**) in 60% yield.[17] Debenzylation of **6** with Na in liquid NH_3 gave the ultimate ligand (**7**) as a clear syrup in 25% yield after flash chromatography.[18,19]

Scheme 1[a]



[a] (a) 2-methyl-2-(benzylthio)propanal; (b) $NaBH_4$/EtOH; (c) HCl (Et_2O); (d) hexanoyl chloride/CH_2Cl_2/TEA; (e) methyl adipyl chloride/CCl_4/TEA; (f) 1 M BH_3·THF; (g) KOH; (h) dil HCl; (i) Na/NH_3

Based on our previous success of characterizing ligand **1** as its Ni(II) complex,[10] we prepared a Ni(II) complex of **7** by heating it with an ethanolic solution of $Ni(OAc)_2$. The brick red solid was flash chromatographed to give an amorphous golden powder with a broad melting range (120-140 °C). The complex FABMS of the powder did not confirm the identity of the Ni(II) complex, but gave a base peak at *m/e* 433, which is consistent with the formation of the protonated disulfide corresponding to ligand **7**.[9,20]

Ligand 7 was radiolabeled by reacting it with sodium [^{99m}Tc]pertechnetate in the presence of a reconstituted Glucoscan^{R} kit and the radiochemical yield ranged from 60-80%.[21] **^{99m}Tc-7** (≈30 μCi) was coinjected with 15-[^{125}I]-(p-iodophenyl)pentadecanoic acid (IPPA; ≈50 μCi),[22] an accepted internal standard for estimating myocardial fatty acid uptake and metabolism, through a tail vein into Fischer CD rats (255-300 g). The rats were sacrificed by cervical dislocation at 1, 5, and 15 min postinjection. The appropriate organs were excised and the radioactivity measured in an automatic dual-channel counter. (Table 1)

Table 1. Distribution of radioactivity in fasted rats following concomitant intravenous administration of **^{99m}Tc-7** and IPPA.[a]

		Blood	Heart	Lung	Liver	Kidney	Spleen	Stomach
% Injected	**^{99m}Tc-7**	17.16 ± 3.01	0.22 ± 0.05	1.19 ± 0.11	37.64 ± 4.47	3.42 ± 0.25	0.70 ± 0.16	0.27 ± 0.17
Dose/Organ	IPPA	13.01 ± 3.27	1.86 ± 0.42	1.25 ± 0.14	16.50 ± 1.91	2.07 ± 0.42	0.54 ± 0.08	0.42 ± 0.07
% Injected	**^{99m}Tc-7**	0.79 ± 0.19	0.56 ± 0.09	0.73 ± 0.24	2.65 ± 0.59	1.18 ± 0.14	0.78 ± 0.39	0.18 ± 0.13
Dose/Gram	IPPA	0.60 ± 0.19	2.94 ± 0.85	0.77 ± 0.27	1.14 ± 0.11	0.71 ± 0.17	0.57 ± 0.14	0.27 ± 0.19

[a] Mean values ± standard deviation for three rats 15 min postinjection.

At 15 min, radioactivity in the heart associated with **^{99m}Tc-7** was approximately 0.2% of the injected dose compared with 1.86% for IPPA. These values are consistent with data obtained from our previous study where ratios of 0.45/2.28 and 0.19/1.87 were obtained for radioactivity in the heart as a percentage of the injected dose for ^{99m}Tc-labeled **1** vs IPPA at 5 and 30 min, respectively. The level of radioactivity in the liver provided by **^{99m}Tc-7** was: (a) lower than that provided by ^{99m}Tc-labeled **1** at 5 and 30 min (44.81 and 45.29 % injected dose, respectively); (b) more than twofold higher than that achieved with IPPA (ratios of 3/1 and 5/1 were obtained for radioactivity in the liver as a percentage of the injected dose for ^{99m}Tc-labeled **1** vs IPPA at 5 and 30 min, respectively). Low levels of radioactivity in stomach and kidneys suggest little, if any, dissociation of **^{99m}Tc-7** in vivo.

The incorporation of a technetium chelating moiety into a fatty acid chain profoundly and invariably alters the behavior of the fatty acid in regard to its myocardial profile.[10] Our approach has been to build an N_2S_2 moiety into approximately the middle of the fatty acid backbone, anticipating that this modification would be least disruptive in terms of the physicochemical properties of the parent fatty acid. This approach has met with only limited success, which suggests that the site of incorporation of the chelating moiety into the fatty acid backbone may not be of major importance. It is likely that **^{99m}Tc-7** is a neutral complex with a net charge of plus one for the complex core compensated by a net charge of minus one for the carboxylate anion. It is perhaps the inability of the neutral complex to be recognized as a fatty acid which results in its disapppointing myocardial profile. Alternately, myocardial uptake may be influenced by the increased polarity of the complex contributed by the chelating moiety itself.[9] Further investigations into this class of ligands are warranted.

In summary, we report a new synthetic route to functionalized N_2S_2 ligands that would seem to be of general applicability. The title compound, a heretofore unreported, achiral bis(tertiary aminoethanethiol), effectively chelated technetium-99m. The biodistribution of **^{99m}Tc-7** in rats was similar to that observed for our prototype technetium-99m - labeled fatty acid, and was inferior to IPPA with regard to its myocardial profile.

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12. N,N'-Bis[2'-methyl-2'(benzylthio)propyl]ethylenediamine Dihydrochloride (**3**). (a) A mixture of 2-methyl-2-(benzylthio)propanal[8] (1.94 g, 10 mmol) and ethylenediamine (**2**) (0.33 mL, d 0.899, 5 mmol) was stirred under Ar at 85 ± 5° C for 1.5 h. After cooling the reaction mixture was partitioned between dichloromethane and water. The organic phase was washed with brine, dried (Na_2SO_4) and concentrated in vacuo to a golden-yellow liquid; (b) The liquid was diluted with 20 mL ethanol to which was added sodium borohydride (0.58g, 15 mmol). The mixture was stirred under Ar at 45±5° for 24 h, then concentrated to near dryness. The residue was partitioned between ether-petroleum ether (2:1) and water. The organic layer was washed with brine, dried ($MgSO_4$) and concentrated to a syrup. The syrup was dissolved in ethanol:ether (1:1) and HCl gas was bubbled through the solution producing a voluminous precipitate. The mixture was filtered and the solid was washed with ethanol, then with ether, then dried in vacuo. Yield: 1.77 g as the dihydrochloride (73%; based on ethylenediamine); mp 215-216° C. ^{1}H NMR (CCl_4; free base) δ 1.31 (s, 12, C$\underline{H}_3$), 2,53 (s, 4, C$\underline{H}_3$), 2.53 (s, 4, C$\underline{H}_2$N), 2.58 (s, 4, C$\underline{H}_2$N), 3.7 (s, 4, C$\underline{H}_2$C$_6$H$_5$), 7.25 (s, broad, 12, C$_6\underline{H}_5$ and N$\underline{H}$, exchangeable); Anal. calcd. for $C_{24}H_{38}Cl_2N_2S_2$ (489.6): C, 58.87; H, 7.82; N, 5.72. Found: C, 58.80; H, 7.99; N, 5.67.
13. Flash chromatography was performed according to: Still, W. C.; Kahn, M; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

14. N-Hexanoyl-N,N'-bis[2'-methyl-2'-(benzylthio)propyl]ethylenediamine (**3a**). To a solution of dihydrochloride **3** (1.10 g, 2.25 mmol) in 50 mL dichloromethane containing 1 mL triethylamine stirred at 0 ± 5° C under Ar, was added hexanoyl chloride (0.31 mL, d 0.963, 2.22 mmol) in 50 mL cold dichloromethane, dropwise over 1.5 h. Stirring was continued in the cold for an additional 1.5 h. Removal of the solvent in vacuo gave a semi-solid residue, which was triturated with ether and filtered. The filtrate was concentrated to a clear syrup which was flash chromatographed (4% MeOH-dichloromethane) to give **3a** as a clear syrup. Yield: 800 mg (70%); ^1H NMR (CCl_4) δ 0.97 (t, broad, 3, C*H*$_3$), 1.37 (s, broad, 18, C*H*$_2$ and C*H*$_3$), 2.20-2.80 (m, broad, 6, C*H*$_2$ and C*H*$_2$C(O)N), 3.7 (s, 2, C*H*$_2$NC(O)), 3.87 (s, 2, C*H*$_2$NC(O)), 4.0 (s, 4, C*H*$_2$,C_6H_5), 7.25 (s, broad, 12, C*H*$_2C_6H_5$ and N*H*, exchangeable).

15. N-Hexanoyl-N'-(6-methyloxycarbonylhexanoyl)-N,N'-bis[2'-methyl-2'-(benzylthio)propyl]ethylenediamine (**4**). To a solution of **3a** (810 mg, 1.6 mmol) in 15 mL carbon tetrachloride containing 0.25 mL triethylamine stirred at 0° C under Ar was added methyl adipyl chloride (316 mg, 1.77 mmol) in 15 mL carbon tetrachloride, dropwise. After stirring for 1 h in the cold, the mixture was filtered and the filtrate was concentrated to a syrup. The syrup was covered with petroleum ether and refrigerated, whereupon solidification occurred. The solvents were decanted and the solid was triturated with petroleum ether, then dried under N_2 to give a white powder, mp 48-52° C. Crystallization from hot petroleum ether gave mp 50-52° C. Yield: 800 mg (77%); ^1H NMR (CCl_4) δ 0.95 (m, broad, 3, C*H*$_3$), 1.37 (m, broad, 18, C*H*$_2$ and C*H*$_3$), 1.73 (m, broad, 4, C*H*$_2$), 2.40 (m, broad, 6, C*H*$_2$C(O)N and C*H*$_2$C(O)O), 5.3-3.9 (m, broad, 8, C*H*$_2$NC(O)), 3.83 (s, 3, C*H*$_3$O), 4.00 (s, 4, C*H*$_2C_6H_5$), 7.80 (s, 10, C_6*H*$_5$); Anal. calcd for $C_{37}N_{56}N_2O_4S_2$ (657): C, 67.64; H, 8.59; N, 4.26. Found: C, 67.61; H, 8.62; N, 4.29.

16. N-hexyl-N'-(6-methyloxycarbonylhexyl)-N,N'-bis[2'-methyl-2'-(benzylthio)propyl]ethylenediamine (**5**). To a solution of **4** (1.97 g, 3 mmol) in 12 mL dry THF stirred at -12 ± 3° C under Ar was added dropwise over 2 h, BH_3-THF complex (10 mL, 1.0 M solution in THF). After stirring in the cold for an additional 10 min, the reaction mixture was allowed to warm slowly to room temperature where it stirred for 45 min. Ten mL MeOH was added, followed by 20 mL 1 N HCl (Et_2O). The clear solution stood overnight at room temperature, then was heated at reflux for 1 h. The solvents were removed in vacuo and replaced with 10 mL MeOH and 10 mL 1 N HCl (Et_2O). After 1 h at reflux, the solvents were removed to give a golden syrup which was flash chromatographed (4% MeOH-$CHCl_3$) to give **5** (850 mg; 45%) as a clear syrup. ^1H NMR ($CDCl_3$) δ (t, 3, C*H*$_3$), 1.37 (broad, 26, C*H*$_2$ and C*H*$_3$), 2.40-2.80 (broad, 14, C*H*$_2$N and C*H*$_2CO_2$), 3.87 (s, 3, CO_2C*H*$_3$), 4.00 (s, 4, C*H*$_2C_6H_5$), 7.25 (s, 10, C_6*H*$_5$). The syrup was dissolved in MeOH and treated with 1 N HCl (Et_2O) to give the hydrochloride salt: mp 144-147° C. Anal. calcd for $C_{37}H_{60}N_2O_2S_2$·2 HCl·H_2O(719.94): C, 61.72; H, 8.96; N, 3.89. Found: C, 61.83; H, *8.54*; N, 3.94

17. N,N'-Bis[2'-methyl-2'-(benzylthio)propyl]-7,10-diazahexadecanoic Acid (**6**). A mixture of **5** (850 mg, 1.35 mmol) and KOH (1.0 g, 15.5 mmol) in 40 mL 50% EtOH was stirred at reflux for 4 h. The reaction mixture was reduced to a small volume, then diluted with water (pH >14). pH was adjusted to 3-4 with varying concentrations of HCl providing a gummy precipitate. The cloudy aqueous phase was decanted and extracted with $CHCl_3$. The gummy precipitate was washed with water. The $CHCl_3$ extract and the precipitate were combined and washed with water. The organic phase was washed with brine, dried (Na_2SO_4), and concentrated. The residue was flash chromatographed (5% MeOH-$CHCl_3$) to give **6** (550 mg; 66%) as a clear syrup. ^1H NMR (CCl_4) δ 0.93 (t, 3, C*H*$_3$), 1.30 (broad, 26, C*H*$_2$ and C*H*$_3$), 2.30-2.77 (broad, 14, C*H*$_2$N and C*H*$_2CO_2$), 3.93 (s, 4, C*H*$_2CH_6CH_5$), 7.25 (s, 10, C_6*H*$_5$), 11.43 (s, CO_2*H*). Anal. calcd for $C_{36}H_{58}N_2O_2S_2$·0.25 CH_3OH (623): C, 69.88; H, 9.55; N, 4.50. Found: C, 69.76; H, 9.57; N, 4.42.

18. N,N'-Bis(2'-methyl-2'-mercapto)propyl-7,10-diazahexadecanoic Acid (**7**). To a clear solution of **6** (102 mg, 0.17 mmol) in 1 mL dry THF was added ≈3 mL liquid NH_3. To the resultant suspension, Na metal (94 mg, 0.004 g-atom) was added in 3 approximately equal portions. The characteristic blue color persisted for 10 min after the first portion was added. The second portion was added and after 15 min, the last portion was added. The blue solution was maintained for 1 h under refluxing NH_3, then excess NH_3 and solvents were removed under a stream of Ar. The solid residue was dissolved in water and filtered (pH >14). pH was adjusted to ≈5.5 with 1 N HCl producing a gummy precipitate. The mixture was extracted with EtOAc; the extract was washed with brine, dried (Na_2SO_4), and concentrated to a pale-yellow syrup which was flash chromatographed (5% MeOH-CH_2Cl_2). Compound **7** (18 mg; 25%) was obtained as a clear syrup that gave an immediate and intense reaction in the Ellman assay (Ellman, G. L. *Arch. Biochem. Biophys.* **1958**, *74*, 443). ^{1}H NMR ($CDCl_3$) δ 0.85 (broad, 3, C*H*$_3$), 1.30 - 2.00 (broad, 26, C*H*$_2$ and C*H*$_3$), 2.33-3.20 (broad, 14, C*H*$_2$N and C*H*$_2$CO$_2$), 6.8 (broad, 2, S*H*).

19. All new compounds were characterized by ^{1}H NMR (Varian T 60 NMR Spectrometer) and spectra were consistent with the proposed structures. Elemental analyses were performed by Schwarzkopf Microanalytical Laboratory on compounds **3, 4, 5,** and **6** and were within 0.4% of theoretical values. The Ni(II) complex of **7** failed to give a satisfactory analysis; the identity of ligand **7** was supported by FABMS.

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21. The ligand (**7**, 3.5-4.0 mg, ≈9 μmol) was dissolved in 300 μL EtOH and added to a solution of $Na^{99m}TcO_4$ (11 mCi in 200 μL saline) containing 150 μL of a reconstituted Glucoscan^R kit (New England Nuclear). The resultant cloudy solution was stirred under Ar at 85 ± 5° C. Solvents were removed by increasing the Ar flow while heating (total heating time ≈30 min). The residue was partitioned between saline (1 mL) and CH_2Cl_2 (2 mL x 3). The extracts were combined, washed with water, dried (Na_2SO_4), and concentrated by purging with Ar while heating. The residue was partitioned between water (1 mL) and CH_2Cl_2 (1 mL x 3). The extracts were combined, dried (Na_2SO_4), and concentrated as before. The residue was dissolved in 1 mL EtOH which was diluted with 4 mL propylene glycol. The solution was filtered through a 0.22 μm filter. The radiochemical purity of the complex (≈90%) was ascertained by TLC (10% MeOH-CH_2Cl_2)

22. [^{125}I]IPPA was prepared by adaptation of a literature method: Mangner, T. J.; Win, J.; Weiland, D. M. *J. Org. Chem.* **1982**, *47*, 7481. Thus, 100 μCi of $Na^{125}I$ was added to a 5 mL minivial, and the aqueous solution was evaporated to dryness under Ar. 15-(p-Iodophenyl)pentadecanoic acid (4-5 mg) and ammonium sulfate (4-5 mg) were added to the vial, which was then heated at 140-145° C for 1 h. After the vial had cooled to room temperature, 5% $NaHSO_3$ (0.5 mL) and hexane (2 mL) were added and the mixture was stirred thoroughly. The hexane layer was separated, washed with water (0.5 mL), partially evaporated to a final volume of 0.5 mL, then charged on a silica gel column (1 g, 60-230 mesh). The column was eluted with 10 mL portions of: petroleum ether:Et_2O (9:1); petroleum ether:Et_2O:HOAc (29:1:0.1); petroleum ether:Et_2O:HOAc (14:1:0.1); petroleum ether:Et_2O:HOAc (9:1:0.1). The last fraction, which contained pure [^{125}I]IPPA, was evaporated to dryness under N_2. The residue was dissolved in EtOH (1 mL), then diluted with 5% human serum albumin (8 mL). The final solution was filtered through a 0.22 μm filter. Yield: 30-60%. The radiochemical purity of the complex (>95%) was ascertained by TLC (hexane:ether:HOAc::50:50:1).

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