Dicarboxylate Diamide Dimercaptide (N_2S_2) Technetium-99m Complexes: Synthesis and Biological Evaluation as Potential Renal Radiopharmaceuticals

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Received September 10, 1992

Novel diamide dimercaptide (N_2S_2) ligands **4**, **5**, and **8** have been synthesized and evaluated as potential renal radiopharmaceuticals. The target compounds were prepared in modest overall yields of 22%, 19%, and 20%, respectively, using readily available starting materials. Following in situ deprotection, ^{99m}Tc complexes of high radiochemical purity were obtained in excellent yield and were found to be stable for up to 6 h. The ^{99}Tc complex of ligand **8** was isolated as the $AsPh_4$ salt. The X-ray crystallographic data for [^{99}TcO(**8**)]$AsPh_4$ (space group $P2_1/n$: $Z = 4$, $a = 9.342(3)$ Å; $b = 18.594(5)$ Å; $c = 18.417(7)$ Å; β, deg = 90.61(3); V, Å^3 = 3199.1(20)) show that the Tc is bound to both thiolate sulfur atoms and to two deprotonated amide nitrogen atoms. The coordination geometry about the Tc is square-pyramidal with an -yl oxygen atom in the apical position. The Tc–N bond distances (2.002(12) and 1.984(12) Å), the Tc–S bond distances (2.300(5) and 2.286(5) Å), and the Tc–O bond distance (1.667(11) Å) are in good agreement with bond lengths reported for similar complexes. The carboxylate groups are not bonded to the Tc atom in the solid state, nor in $CDCl_3$ solution, as evidenced by X-ray crystal data and solution NMR data, respectively. In the solid state, [^{99}TcO(**8**)]$AsPh_4$ is monoanionic, therefore, at physiological pH, [^{99m}TcO(**8**)] is presumably trianionic. Biodistribution studies performed in rats with the ^{99m}Tc complexes revealed slow blood clearance and high muscle uptake for these agents. Modest hepatobiliary excretion was observed, and low quantities of the complexes were found in the heart, lungs, and spleen after 1 h. The urinary excretion of the ^{99m}Tc complexes of ligands **4**, **5**, and **8** was found to be slow when compared to the excretion of [^{131}I]OIH in rats (22%, 22%, and 32% vs 85–86%, respectively). Protein binding of ^{99m}Tc complexes of ligands **4**, **5**, and **8** in both rat and monkey plasma was found to be similar to MAG_3. While the synthetic schemes reported here supply facile routes to novel N_2S_2 ligands, biodistribution studies of the ^{99m}Tc complexes performed on rats revealed slow renal excretion rates, accompanied by slow blood clearance and high uptake in muscle tissue. Preliminary planar imaging studies in monkeys also revealed slow renal excretion for these agents. The ^{99m}Tc complexes evaluated here are poor candidates as renal radiopharmaceuticals.

Introduction

[^{131}I]-*o*-Iodohippuric acid (OIH) is used routinely as a renal function and imaging agent. *o*-Iodohippurate is cleared primarily by active tubular secretion with a high renal extraction efficiency (65–80%). However, the inferior physical characteristics of the ^{131}I label limit the spatial resolution of the images and can result in a higher radiation dose to the patient.[1] Consequently, considerable attention during the past decade has focused on the development of technetium-99m (^{99m}Tc) labeled renal imaging agents. The excellent imaging characteristics ($T_{1/2}$ = 6 h, 140 keV), widespread availability, and low cost of ^{99m}Tc make it the radionuclide of choice for renal radiopharmaceuticals.

Numerous tetradentate technetium chelating ligands have been prepared and tested as potential renal imaging agents. Davison and co-workers first reported the rapid renal excretion of the ^{99m}Tc diamide dimercaptide ligand (N_2S_2), 1,2-bis(2-thioacetamido)ethane (DADS, Figure 1), in animals.[2,3] After further biological evaluation, Fritzberg et al. demonstrated that the renal excretion properties of ^{99m}Tc-DADS were inferior to OIH.[4] A monocarboxylate analog of ^{99m}Tc-DADS, 2,3-bis(2-thioacetamido)propanoate (^{99m}Tc-CO_2-DADS, Figure 1), was later reported by Fritzberg et al. to exhibit promising renal excretion properties in mice, dogs, and humans.[1,5,6] The high specificity and efficient renal handling of this dianionic

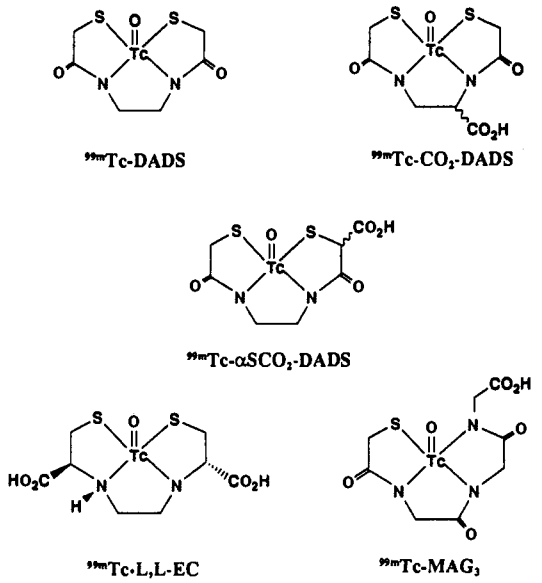

Figure 1. Structures of selected ^{99m}Tc-labeled renal radiopharmaceuticals (^{99m}Tc-DADS, ^{99m}Tc-CO_2-DADS, ^{99m}Tc-αSCO_2-DADS, ^{99m}Tc-L,L-EC, ^{99m}Tc-MAG_3) discussed in text.

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0022-2623/93/1836-1032$04.00/0

^{99m}Tc-CO_2-DADS complex suggested that addition of carboxylate functional groups on the DADS backbone might serve as a means of improving renal handling of these complexes. Consequently, a series of CO_2-DADS analogs in which the carboxylate group(s) was moved to different positions on the chelate ring was synthesized and tested. A single dicarboxylate ^{99m}Tc-DADS derivative was also synthesized and tested. The poor renal excretion observed for this complex was attributed to the second anionic group of the complex. It was suggested that trianionic complexes are poorly handled by tubular transport processes.[1] Further evaluation of this hypothesis is needed using additional dicarboxylate ^{99m}Tc-DADS analogs.

Of the monocarboxylate derivatives of CO_2-DADS evaluated, none exhibited superior excretion characteristics when compared to the parent compound (CO_2-DADS). However, when CO_2-DADS was labeled with ^{99m}Tc, the resulting stereoisomers exhibited very different renal excretion properties in human volunteers. Therefore, the requirement for preparative HPLC purification of CO_2-DADS (component A) precluded its potential clinical usefulness. To obviate the problem of stereoisomers, Fritzberg later changed the core donor atoms from N_2S_2 to N_3S.[7] This work ultimately led to the development of mercaptoacetyltriglycine (MAG_3; Figure 1), a promising new renal radiopharmaceutical which has found widespread clinical utility. The renal clearance of this monocarboxylate, dianionic ^{99m}Tc complex, correlates well with OIH, while its imaging characteristics are superior.[8-10] While MAG_3 is presently the agent of choice for many renal function studies, it is highly bound to plasma proteins, and its renal clearance is significantly lower than that of OIH.[11-13] The superior renal excretion properties of derivatives of MAG_3 also suggest that MAG_3 is not the ideal replacement for [^{131}I]OIH.[14] Therefore, ^{99m}Tc-labeled renal radiopharmaceuticals which more closely approximate the excretion properties of OIH are desirable.

In a more recent study involving ^{99m}Tc-labeled N_2S_2 complexes as renal radiopharmcaceuticals, Verbruggen and co-workers reported the efficient renal excretion properties of the most polar metabolite of a new brain imaging agent, ^{99m}Tc-L,L-ethylenedicysteine diethyl ester (^{99m}Tc-L,L-ECD).[15] The *diamine* dimercaptide metabolite, ^{99m}Tc-ethylenedicysteine (^{99m}Tc-EC; Figure 1), contains two carboxylate moieties, exists as a dianion at physiological pH, and is rapidly and efficiently excreted in the urine of mice, baboons, and human volunteers.[16] Comparisons of ^{99m}Tc-EC with ^{99m}Tc-MAG_3 and [^{131}I]OIH in mice and a baboon demonstrate that the renal excretion characteristics of ^{99m}Tc-EC compare favorably with these commonly used renal radiopharmaceuticals.[17] These data suggest that ^{99m}Tc-EC warrants further study in humans to evaluate its potential as a substitute for ^{99m}Tc-MAG_3 and [^{131}I]OIH in renal function studies.

While numerous, structurally diverse organic molecules are known to undergo efficient tubular secretion, a clear understanding of the structural requirements of this process is lacking.[1] Consistent with Despopoulos's theory, it has been generally accepted that the carbonylglycine moiety (CO-G) of hippuran, CO_2-DADS, and MAG_3 is responsible for the efficient fit of these renal radiopharmaceuticals with tubular transport receptor proteins.[1,17] More recently, this hypothesis has been revised to attribute the efficient renal excretion of ^{99m}Tc-EC to the oxotechnecium-glycine (TcO-G) sequence contained in this compound. It was further suggested that the TcO-G sequence, rather than the CO-G sequence in CO_2-DADS and MAG_3, is the requisite structural moiety for efficient handling by renal tubular proteins. Although the hypothesis holds for many of the efficiently excreted renal agents synthesized and studied to date, interesting exceptions do exist. For example, a monocarboxylate derivative of DADS reported by Fritzberg et al., in which a carboxylate is positioned on the carbon adjacent to the sulfur atom (αSCO_2-DADS; see Figure 1), exhibited renal excretion properties in mice and humans similar to those reported for CO_2-DADS.[1] Similarly, a DADS analog containing a hydroxyl group on an extended chelate ring was also shown to be efficiently excreted into the urine of mice and humans. These data suggest that more can be learned regarding the structural requirements of the renal tubular proteins, and that structural elements other than TcO-G and CO-G may aid in the efficient tubular excretion of ^{99m}Tc-DADS derivatives. In addition, many of the efficiently excreted ^{99m}Tc-N_2S_2 (CO_2-DADS, αSCO_2-DADS, EC) and N_3S (MAG_3) complexes exist as dianions at physiological pH, while a trianionic DADS complex has been reported to be poorly excreted. Therefore, the relationship between the charge being carried by the ^{99m}Tc complex (dianion vs trianion) and efficient renal excretion is of interest.

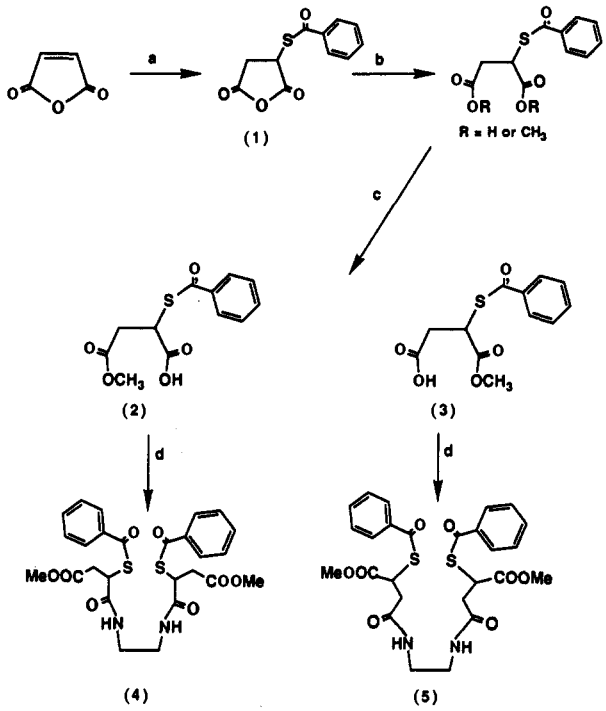
The goal of the present study was to prepare dicarboxylate *diamide* dimercaptide (DADS) derivatives as potential renal radiopharmaceuticals. These compounds are analogs of the monocarboxylate DADS (αSCO_2-DADS) reported by Fritzberg to exhibit promising renal excretion properties in humans. The proposed molecules contain symmetrical carboxylate or acetate groups on the carbons adjacent to the sulfur on the chelate ring. These ^{99m}Tc-dicarboxylate-DADS analogs can also be used to further test the hypothesis that trianionic DADS complexes are poorly excreted by the renal tubular system, since they are expected to exist as trianions at physiological pH. The synthesis, radiolabeling, plasma protein binding, urinary excretion, and biodistribution of the dicarboxylate N_2S_2 technetium chelating ligands are discussed.

Chemistry

Synthesis of Ligands. Scheme I illustrates the synthetic route utilized in the preparation of diamide dimercaptide (N_2S_2) ligands **4** and **5**. The common anhydride precursor **1** was prepared from maleic anhydride and thiobenzoic acid using dibenzoyl peroxide as an initiator. Treatment of the anhydride at −78 °C with NaOMe in methanol resulted in ring opening to form both possible half esters, **2** and **3**. These carboxy esters could be readily separated via column chromatography. Once isolated, each acid was condensed with *N*-hydroxysuccinimide in the presence of dicyclohexylcarbodiimide to afford the respective activated esters. These active esters were then reacted, without purification, with ethylenediamine to supply the desired N_2S_2 ligands, compounds **4** and **5**, in modest overall yield (22% and 19%, respectively).

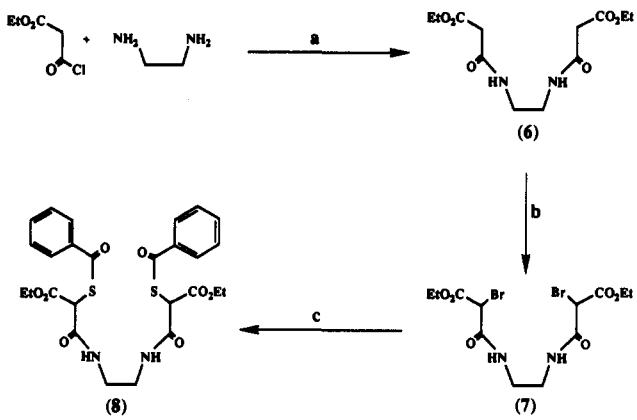
The synthetic route outlined in Scheme II was utilized in the preparation of compound **8**. Commercially available ethyl malonyl chloride was reacted with ethylenediamine (1 equiv) to afford diamide diester **6** in moderate yield (63%). Bromination of **6** was accomplished using *N*-bromosuccinimide and hydrobromic acid at room temperature

Scheme I. Synthesis of Dimethyl 3,10-Bis(benzoylthio)-4,9-dioxo-5,8-diazododecanedioate (4) and Dimethyl 2,11-Bis(benzoylthio)-4,9-dioxo-5,8-diazododecanedioate (5)[a]



[a] (a) Thiobenzoic acid, dibenzoyl peroxide, Et_2O, reflux; (b) $NaOCH_3$, CH_3OH, −78 °C; (c) separation via column chromatography (20% hexanes/Et_2O and 1% AcOH); (d) *N*-hydroxysuccinimide, dicyclohexylcarbodiimide, THF, −5 to 0 °C.

Scheme II. Synthesis of Diethyl 2,9-Bis(benzoylthio)-3,8-dioxo-4,7-diazadecanedioate (8)[a]



[a] (a) CH_2Cl_2, −4 to 0 °C; (b) *N*-bromosuccinimide, hydrobromic acid, CH_2Cl_2, rt; (c) sodium thiobenzoate, EtOH, rt.

to supply 7. Subsequent treatment of 7 with sodium thiobenzoate (prepared from sodium ethoxide and thiobenzoic acid) yielded (20% overall) the desired protected ligand 8.

Radiolabeling with ^{99m}Tc. Thiol deprotection and ester hydrolysis of compounds 4, 5, and 8 was performed in situ at the time of radiolabeling according to a previously published procedure.[9] The chelate–ligand precursor was dissolved in ethanol and 1 N NaOH. Sodium [^{99m}Tc]-pertechnetate as eluent from a commercial ^{99}Mo/^{99m}Tc generator was then added. The mixture was heated for 2 min at 100 °C, the reducing agent was added ($Na_2S_2O_4$), and heating continued for an addtional 3 min. The solution was then cooled to room temperature and neutralized with 1 N HCl. Purification was accomplished by reverse-phase HPLC. Under the HPLC conditions used here, only one component was observed for each of the complexes synthesized.

Characterization of ^{99m}Tc Complexes. The short half-life of ^{99m}Tc (6 h) precludes its use in the structural characterization of technetium compounds. Therefore, the characterization of these complexes has classically been performed using the long-lived isotope ^{99}Tc ($T_{1/2} = 2 \times 10^5$ years). The characterization of similar diamide dimercaptide complexes has been reported previously in the literature.[18,19] Due to the disappointing renal excretion rates of the complexes studied here, we have chosen only compound 8 for structural characterization with ^{99}Tc. It is likely that the other Tc complexes of 4 and 5 possess a similar TcO(V) center core.

Results

Ligands 4, 5, and 8 were synthesized in modest overalls yields of 22%, 19%, and 20%, respectively, with inexpensive, readily available starting materials. The spectroscopic and analytical data obtained for these ligands are consistent with the structures shown. Radiolabeling of 4, 5, and 8 with ^{99m}Tc was performed to evaluate the ability of these ligands to form stable complexes. Thioester and ethyl carboxylate hydrolysis was performed in situ, in alkaline solution, following a previously published procedure. Only one component was observed on reverse-phase HPLC immediately following dithionite labeling with ^{99m}Tc, and up to 6 h afterwards. The syntheses of ligands 4, 5, and 8 are not stereospecific; therefore, the two chiral centers of each compound are likely to be racemic. Following complexation with $[Tc{=}O]^{3+}$, three isomers are expected for each complex. Attempts were made to separate the ^{99m}Tc-5 complex into the expected isomers using reverse-phase (PRP and C-18 columns) and normal-phase (silica gel) HPLC. A variety of mobile phases were evaluated using isocratic and gradient solvent systems which have been reported to be useful for similar ^{99m}Tc-N_2S_2 complexes (see the Experimental Section). Under the various HPLC conditions used here, the expected isomers of the ^{99m}Tc complexes of ligand 5 could not be cleanly separated for further analysis. The single HPLC component for the ^{99m}Tc complexes of 4, 5, and 8 were found to have retention times of 2.4, 2.4, and 2.3 min, respectively, using the conditions described in the Experimental Section. The radiochemical purity of the complexes was determined to be greater than 95% (reverse-phase HPLC). Radiolabeling yield was found to be greater than 90% for each of the ligands evaluated.

Only ligand 8 was chosen for full characterization with ^{99}Tc. Preparation of the carrier added [^{99}TcO(8)]$AsPh_4$ was accomplished by reacting the ligand 8 with the Tc(V) reagent, Na[TcO(eg)$_2$], under basic conditions in water. Purification of the resulting solution involved anion-exchange chromatography to remove benzoic acid and $Na[TcO_4]$. Elution with 5% NaCl resulted in a solution containing the desired [^{99}TcO(8)]Na contaminated with a small amount of $^{99}TcO_4^-$ (determined by IR analysis of the evaporated solid). The residue obtained after concentration of the solution was triturated with acetone to remove traces of $^{99}TcO_4^-$. The resulting solid was redissolved in water and isolated as the $AsPh_4$ salt. Crystallization was accomplished by dissolving the solid in water and allowing the solution to evaporate slowly.

Table I. Comparison of Rat Biodistribution Data for ^{99m}Tc N_2S_2 Dicarboxylate Complexes with [^{131}I]OIH[a]

^{99m}Tc complex	min	kidney	blood	liver	muscle	intestines	heart	lung	spleen
4	10	4.3 ± 0.7	26.7 ± 1.5	5.0 ± 0.4	26.9 ± 1.4	1.7 ± 0.1	0.4 ± 0.03	1.2 ± 0.2	0.3 ± 0.1
	60	2.1 ± 0.1	16.4 ± 0.5	3.1 ± 0.3	21.1 ± 2.1	1.3 ± 0.5	0.2 ± 0.02	0.7 ± 0.02	0.1 ± 0.01
OIH	10	6.9 ± 3.0	5.3 ± 0.7	2.5 ± 0.9	9.9 ± 0.8	1.4 ± 0.9	<0.1%	0.3 ± 0.1	0.1 ± 0.03
	60	0.6 ± 0.2	0.6 ± 0.03	0.3 ± 0.1	1.6 ± 0.3	0.8 ± 0.4	<0.1%	<0.1%	<0.1%
5	10	5.3 ± 1.8	26.2 ± 1.2	5.0 ± 0.1	22.8 ± 2.3	1.4 ± 0.1	0.4 ± 0.01	1.1 ± 0.2	0.2 ± 0.01
	60	1.9 ± 0.1	15.2 ± 0.8	3.0 ± 0.3	20.4 ± 1.8	1.7 ± 0.2	0.3 ± 0.05	0.7 ± 0.03	0.1 ± 0.01
OIH	10	9.9 ± 5.2	4.4 ± 0.2	3.3 ± 1.1	7.9 ± 1.1	1.6 ± 1.3	0.1 ± 0.01	0.2 ± 0.04	<0.1%
	60	0.5 ± 0.1	0.5 ± 0.04	0.5 ± 0.3	1.6 ± 0.4	1.6 ± 1.2	<0.1%	<0.1%	<0.1%
8	10	6.0 ± 0.8	24.0 ± 1.3	4.9 ± 0.9	23.6 ± 3.6	1.6 ± 0.3	0.4 ± 0.02	0.9 ± 0.08	0.2 ± 0.02
	60	4.0 ± 0.7	13.1 ± 1.2	5.0 ± 0.3	21.5 ± 5.4	1.3 ± 0.1	0.2 ± 0.01	0.7 ± 0.1	0.1 ± 0.03
OIH	10	11.4 ± 2.3	4.6 ± 0.5	2.0 ± 1.0	7.6 ± 0.4	1.7 ± 0.3	<0.1%	0.2 ± 0.03	<0.1%
	60	0.9 ± 0.3	0.2 ± 0.09	0.4 ± 0.2	2.0 ± 0.1	1.6 ± 1.1	<0.1%	<0.1%	<0.1%

[a] Values are mean ± SD. Percent injected dose for three to four rats. Corrections were made for ^{131}I spillover into the ^{99m}Tc channel.

The X-ray crystallographic data for [^{99}TcO(8)][$AsPh_4$] demonstrate that technetium is bound to both thiolate sulfur atoms, as well as to two deprotonated amide nitrogen atoms and an -yl oxygen atom, resulting in a −1 charged complex. The carboxylate groups are not complexed to the ^{99}Tc in the solid state (by crystallography and IR data) nor in solution, as a broad resonance at 10.3 ppm is observed in the NMR data ($CDCl_3$), suggestive of the COOH group. The crystallography data show that both carboxylates are syn to the Tc=O bond. This observation does not necessarily suggest that radiolabeling of 8 yields exclusively the syn isomer, but rather, that this isomer was the only one which crystallized under the conditions used. As expected, square-pyramidal coordination geometry about the Tc is observed. The Tc–N1 and Tc–N2 bond distances are 2.002(12) and 1.984(12) Å, respectively. Bond distances for the Tc–S1 and Tc–S2 bonds were found to be 2.300(5) and 2.286(5) Å. The Tc–O7 bond distance is 1.667(11) Å. The Tc–amide nitrogen, Tc–thiolate sulfur, and Tc–O bond distances observed for [^{99}TcO(8)][$AsPh_4$] are consistent with values reported for similar N_2S_2–Tc complexes.[18,19] Bond angles about the nitrogen atoms are close to 120°, consistent with the sp^2 hybridization of these atoms. The N1–Tc–N2 bond angle is 78.1(5)° which is similar to those reported for other structures with ethylenediamine backbones.[19]

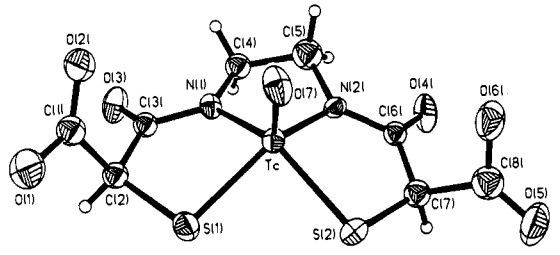


Figure 2. ORTEP drawing and labeling scheme for $[C_8H_6N_2O_7S_2Tc]^-$. Ellipsoids are at 30% probability.

The analytical data (NMR, IR, MS) of the ^{99}Tc-8 complex are also consistent with a complex in which both the nitrogen atoms and sulfur atoms are bound to the metal to form a square-pyramidal coordination environment about the ^{99}Tc, with the oxygen in an apical position. An intense Tc=O absorption at 955 cm^{-1} was observed in the FT-IR spectrum. Also, intense bands at 1745, 1645, and 1550 cm^{-1} were assigned to C=O and carboxylate stretching frequencies, respectively. The NMR data ($CDCl_3$) showed complex multiplets at 3.92 and 4.09 ppm, each integrating for two protons, consistent with the methylene protons of the diamide backbone. A singlet integrating for two protons was observed at 4.87 ppm and was assigned to the protons α to the sulfur atoms. Also, a broad resonance at 10.3 ppm was assigned to the protons of the carboxylic acid groups, suggesting that the carboxylate oxygens are not complexed to the ^{99}Tc in solution. Mass spectral analysis (FAB) showed the expected M^- ion at m/z 407. Also observed in the mass spectrum were peaks at m/z 363 and 319, which correspond to the successive loss of both carboxylate groups. The complex also afforded a reasonable elemental analysis.

Reverse-phase HPLC retention times of co-injected ^{99m}Tc-8 and ^{99}Tc-8 complexes indicate that carrier-added (UV detector) and no-carrier-added (γ detector) complexes exhibit an identical chromatographic profile. While the HPLC coelution suggests that these complexes form similar Tc coordination spheres, the presence of stereoisomers in the HPLC component of the ^{99m}Tc complex cannot be ruled out. The crystal structure of ^{99}Tc-8 shows that the carboxylates are syn to the Tc=O group. It is likely that this isomer is also formed for the ^{99m}Tc-8 complex. However, it is unclear whether other isomers are also formed during labeling which could not be separated using the HPLC techniques described here.

Biodistribution studies were performed in rats with the ^{99m}Tc complexes of ligands 4, 5, and 8 to determine the percent injected dose per organ at 10 and 60 min postinjection. The results of these studies are presented in Table I. Sixty minutes postinjection, the radiolabeled form of ligand 4 showed slow blood clearance and high muscle uptake (16% and 21%, respectively). Only modest hepatobiliary excretion was observed after 1 h. Low quantities of the complex were found in the heart, lungs, and spleen. Compounds 5 and 8 showed similar organ biodistribution characteristics when evaluated in rats. That is, large amounts of activity remained in the blood compartment and in muscle tissue, while only modest hepatobiliary excretion was observed.

The uinary excretion of ^{99m}Tc complexes of ligands 4, 5, and 8 was compared to the excretion of [^{131}I]OIH in rats (Table II). As shown in Table II, 10 min after injection of 4, only 5.8% of the dose was found in the urine, as compared to 50% for OIH at the same time point. Later time points also reflected slow renal excretion of the test compound. After 1 h, only 22% of the injected dose of 4 was found in the urine, compared to 85% for OIH. The urinary excretion rate of compound 5 was also disappointing. One hour following injection of 5, a modest 22% of the dose was excreted in the urine. While the total amount of 8 excreted over 1 h was slightly higher than the

Table II. Comparison of Urinary Excretion of ^{99m}Tc Complexes of Compounds 4, 5, and 8 with OIH in Rats[a]

^{99m}Tc complex	time (min, postinjection)					
	10	20	30	40	50	60
4	5.8 ± 0.7	10.8 ± 0.6	14.8 ± 0.5	17.7 ± 0.4	20.1 ± 0.5	22.0 ± 0.5
OIH	50.3 ± 5.3	68.7 ± 4.5	76.9 ± 3.2	81.0 ± 2.5	83.5 ± 2.0	85.0 ± 1.8
5	6.5 ± 0.5	11.1 ± 0.5	14.4 ± 0.4	17.3 ± 0.4	19.6 ± 0.3	21.5 ± 0.3
OIH	54.8 ± 3.3	69.5 ± 3.2	76.9 ± 2.3	81.5 ± 1.8	84.1 ± 1.5	85.6 ± 1.3
8	7.4 ± 0.9	15.3 ± 1.8	20.8 ± 1.4	25.7 ± 1.3	29.4 ± 1.1	32.1 ± 1.1
OIH	46.6 ± 4.1	67.1 ± 5.5	75.8 ± 3.7	82.0 ± 3.3	85.1 ± 2.7	86.9 ± 2.3

[a] Values are mean ±SD. Percent injected dose for three to four rats. Corrections were made for ^{131}I spillover into ^{99m}Tc channel.

Table III. Comparison of Plasma Protein Binding in Rats and Monkey of N_2S_2 Dicarboxylate ^{99m}Tc Complexes and ^{99m}Tc-MAG_3[a]

^{99m}Tc complex	rat plasma	monkey plasma
MAG_3	30.3 ± 1.7	35.2 ± 9.0
5	22.0 ± 1.0	26.5 ± 0.5
4	31.1 ± 1.2	26.2 ± 1.3
8	21.4 ± 0.4	21.9 ± 1.2

[a] Standard trichloroacetic acid precipitation method. Values are mean ± SD for 3 determinations.

other test agents (32% vs 22% for 4 and 5), this compound also compared very poorly to [^{131}I]OIH in rats.

Because distinct species variability regarding the renal excretion rates of similar complexes has been reported,[20] preliminary planar imaging studies of ^{99m}Tc complexes of 4, 5, and 8 were performed with monkeys. Consistent with the organ distribution data reported in rats, urinary excretion of these ^{99m}Tc complexes in monkey was also slow (data not shown). However, unlike the data obtained in rats, high activity was observed in the liver, heart, and the spleen of the monkey. While differences in biodistribution between rats and monkeys for individual agents were apparent in these preliminary studies, poor renal excretion rates were observed for each of these agents, accompanied by high activity in the liver, heart, and muscle tissue.

Lastly, protein binding of the ^{99m}Tc complexes of ligands 4, 5, and 8 was determined in both rat and monkey plasma. For comparison purposes, the protein binding for MAG_3 is also reported (Table III). MAG_3 is known to be highly protein bound. In each case, the ^{99m}Tc complexes reported here exhibit plasma protein binding values similar to that of MAG_3.

Discussion

The objective of the present work was to synthesize and evaluate dicarboxylate analogs of ^{99m}Tc-CO_2-DADS as potential renal radiopharmaceuticals. Ligands 4, 5, and 8 were designed as dicarboxylate derivatives of ^{99m}Tc-αSCO_2-DADS possessing carboxylate or acetate groups at different positions on the chelate ring. Compound 5 possesses carboxylate moieties on the carbon atom α to the sulfur and also has an extended chelate ring as compared to the ^{99m}Tc-DADS and ^{99m}Tc-EC molecules. Like compound 5, the carboxylate groups of 4 are on the carbon atom α to the sulfur. However, the chelate ring of 4 is two carbons smaller and the carboxylate moieties are extended from the chelate ring as acetates. Complex 8 possesses the same size chelate ring as 4, but like 5, the carboxylate groups are directly attached to the carbon α to the sulfur atom. These agents provide the opportunity to further evaluate the effect that an additional carboxylate (5, 8) or acetate (4), and an extended chelate ring (5), may have on the renal excretion properties of ^{99m}Tc-CO_2-DADS complexes. In addition, the relationship between charge (dianions vs trianions) and the efficient renal handling of such ^{99m}Tc-DADS complexes can be investigated further.

Ligands 4, 5, and 8 were synthesized in modest overall yield and shown to be readily labeled with ^{99m}Tc (>90% radiolabeling yield) following in situ deprotection. The complexes were demonstrated to be of high purity (>95%) and to be stable for up to 6 h following labeling. The short retention times of the ^{99m}Tc complexes of 4, 5, and 8 on reverse-phase HPLC (2.3–2.4 min) were similar to that observed for ^{99m}Tc-MAG_3 (3 min) and suggest that they are polar, hydrophilic compounds. The X-ray crystallography data confirm that the Tc is complexed to ligand 8 through both nitrogen atoms and both sulfur atoms to form a square-pyramidal coordination environment about the ^{99}Tc, with the oxygen in an apical position. The complex is a monoanion and is therefore expected to exist as a trianion at physiological pH. The X-ray crystallographic data for [^{99}TcO(8)][$AsPh_4$] demonstrate that the carboxylate groups are not complexed to the ^{99}Tc in the solid state (by crystallography and IR data) nor in solution, as a broad resonance at 10.3 ppm is observed in the NMR data ($CDCl_3$), suggestive of the COOH group. This observation is consistent with those of Rao et al. concerning the X-ray crystal structure of isomers of ^{99m}Tc-CO_2-DADS. The crystallography data show that both carboxylates are syn to the Tc=O bond. As expected, square-pyramidal coordination geometry about the Tc is observed.

Only one peak was observed in the ^{99m}Tc and ^{99}Tc HPLC data. The synthesis of ligand 8 is not stereospecific; therefore, the two chiral centers are most likely to be racemic. Upon complexation with $[Tc{=}O]^{3+}$, three isomers are expected: one in which both carboxylate groups are syn to the Tc=O bond, one where both COOH groups are anti to the Tc=O bond, and one *d,l* pair in which one COOH is syn and one is anti to the Tc=O bond. It is possible that the isomers could not be separated under the HPLC conditions employed in these experiments and that the isomer obtained for X-ray crystal analysis preferentially crystallized under the conditions used. Although isolation of one peak with our dicarboxylate complexes is difficult to rationalize, our results are similar to those obtained by Brenner.[21] In the latter studies, Tc=O(N_2S_2) dicarboxylate complexes showed one peak in the HPLC data using a mobile phase and column similar to the system used in this work. When monocarboxylate complexes were prepared, two isomers were observed in the HPLC systems; these isomers could be isolated by preparative HPLC and characterized.

The single HPLC component of the ^{99m}Tc complexes of ligands 4, 5, and 8 were likely a mixture of isomers. When evaluated in biodistribution studies, each of the complexes exhibited slow renal excretion and slow blood disappearance. The slow excretion rates observed for these agents suggest that they are poorly handled by glomerular filtration and renal tubular secretory mechanisms. The

biodistribution data (Table I) also suggest that the hepatobiliary excretion of these compounds is slow. The protein binding values obtained for the ^{99m}Tc complexes of 4, 5, and 8 were similar to that found for MAG_3. These results indicate that following iv injection, a significant portion of these tracers are bound to plasma protein. The slow excretion of these agents may be related to their plasma protein binding.

Previous studies involving diamide dimercaptide (N_2S_2) complexes of ^{99m}Tc have shown that the efficient renal handling of these compounds is highly dependent on the position and the electronic and lipophilic nature of substituents on the chelate ring.[22] For example, Fritzberg and co-workers showed that fused cyclohexyl and aromatic N_2S_2 derivatives exhibit very poor renal excretion specificity.[22] In contrast, the monocarboxylate N_2S_2 derivative of 8 (^{99m}Tc-αSCO_2-DADS) has been reported to display promising renal excretion characteristics similar to those of OIH. Therefore, the addition of a carboxylate group in 8 results in a drastic reduction in renal excretion. Extension of the carboxylates of 8 to acetates in compound 4 did not improve renal handling. Increasing the size of the chelate ring in compound 5 also resulted in poor renal excretion of this complex. These results are consistent with previous reports by Fritzberg et al., where acetate analogs and chelate ring extensions had little effect on the renal excretion properties of related ^{99m}Tc-DADS complexes. These data further illustrate that efficient renal handling of radiopharmaceuticals is extremely sensitive to small structural modifications. These results also suggest that the charge of the complexes may be a significant factor in determining the rate of renal excretion.

Monocarboxylate analogs of DADS, which are dianions at physiological pH have been reported to show promise as potential renal radiopharmaceuticals. However, the need for HPLC separation of stereoisomers precluded their practical utility.[22] Therefore, it is desirable to develop complexes which do not have asymmetric centers, or alternatively, to develop agents whose stereoisomeric forms do not exhibit different renal excretion properties and are efficiently excreted.[1] It was also suggested that dicarboxylate derivatives which exist as trianions in vivo were not readily excreted by renal tubular secretion. This suggestion is supported by the renal excretion rates observed here for the ^{99m}Tc complexes of 4, 5, and 8, since they are also expected to exist as trianions in vivo. Consequently, the present data, as well as previously reported studies, support the hypothesis that trianions of this type of ^{99m}Tc complex may be poorly handled by renal excretion mechanisms.

In conclusion, novel N_2S_2 ligands have been synthesized as potential renal imaging radiopharmaceuticals. Following in situ deprotection, ^{99m}Tc complexes of high radiochemical purity were prepared in excellent yield and were found to be stable for up to 6 h. X-ray crystallographic studies of [^{99}TcO(8)][$AsPh_4$] demonstrate that technetium is bound to both thiolate sulfur atoms, as well as to two deprotonated amide nitrogen atoms and an -yl oxygen atom. Biodistribution studies performed on rats and nonhuman primates revealed slow renal excretion rates, accompanied by slow blood clearance and high uptake in muscle tissue. These data support the hypothesis that an additional anionic group (carboxylate) on ^{99m}Tc-CO_2-DADS complexes interferes with the efficient renal excretion of this class of metal complex. The ^{99m}Tc complexes evaluated here are poor candidates as renal radiopharmaceuticals.

Experimental Section

Reagents used in syntheses and deuterated solvents used to prepare NMR samples were purchased from Aldrich Chemical Co. (Milwaukee, WI) and used without further purification unless otherwise indicated. Reagent-grade and HPLC-grade solvents were obtained from Emsco Co. (Philadelphia, PA). Tetrahydrofuran was dried by distillation from sodium benzophenone ketyl. Dichloromethane was dried by distillation from calcium hydride. Thin-layer chromatography (TLC) was performed on EM Science (Gibbstown, NJ) precoated (0.2 mm) silica gel 60 plates, and the spots were detected with I_2 vapor and/or UV light. Silica gel 60 (70–230 mesh) obtained from EM Science (Gibbstown, NJ) was used for column chromatography. Sephadex QAE A25 anion-exchange resin was purchased from Sigma (St. Louis, MO). ^{1}H and ^{13}C spectra were obtained on a Bruker Model AM 500 or a Varian 360A spectrometer. Samples were dissolved in $CDCl_3$, and chemical shifts were reported as δ values with the chloroform or tetramethylsilane resonance as the internal standard. The multiplicity is defined by s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). The relative peak heights of the resonances are reported as integers after the multiplicity. IR spectra (KBr pellet) were recorded on a Mattson Polaris FT-IR spectrometer. Melting points were determined on a Meltemp apparatus (Cambridge, MA) and are reported uncorrected. Elemental analyses were performed by Atlantic Microlabs Inc. (Norcross, GA). A Waters Model 510 liquid chromatograph equipped with a PRP-1 reverse-phase cartridge (25 cm × 0.46 cm; Hamilton; Reno, NE) was used for high-performance liquid chromatographic (HPLC) analysis of ^{99m}Tc complexes. [^{131}I]-*o*-iodohippuran and ^{99m}Tc-MAG_3 were prepared as specified by the manufacturer (Mallinckrodt, St. Louis, MO).

***S*-Benzoylmercaptosuccinic Anhydride (1).** Maleic anhydride (10 g, 102 mmol) and dibenzoyl peroxide (5 mg) were dissolved in anhydrous ether (100 mL) and stirred at room temperature. Thiobenzoic acid (12 mL, 102 mmol) was added dropwise to the solution with stirring. When addition was complete, the reaction was gently refluxed for 5 h. After that time, the reaction mixture was chilled and the precipitate was filtered and washed with ether. The filtrate was concentrated in vacuo and the residue crystallized from $CHCl_3$ to yield (10.5 g, 44%) the product as colorless crystals: mp 112–114 °C; IR (KBr) 1870, 1790 (C=O), 1660 (SC=O) 1220, 920 (COCO) cm^{-1}; ^{1}H NMR 2.95–3.95 (m, 2 H, CH_2), 4.88–5.12 (m, 1 H, CH), 7.40–8.10 (m, 5 H, ArH). Anal. ($C_{11}H_8O_4S$) C, H, S.

S-Benzoylmercaptosuccinic anhydride (4 g, 16.9 mmol) was dissolved in MeOH (125 mL) and chilled to −78 °C. A solution of $NaOCH_3$ [prepared from sodium metal (0.39 g; 16.9 mmol) and dry methanol (10 mL)] was then added dropwise with stirring. The mixture was stirred at −78 °C for 2 h and slowly allowed to warm. When the bath temperature reached −20 °C, the reaction was quenched with 2 N HCl and the methanol evaporated under reduced pressure. The acidic aqueous solution was extracted with EtOAc (3 × 75 mL), and the organic layer was washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. TLC of the white waxy residue (4.1 g, 90.2% yield) indicated that the anhydride had opened to yield both possible half esters in approximately equal proportions. The high and low R_f acids were isolated using silica gel column chromatography (20% hexanes in Et_2O with 1% AcOH) and characterized as described below.

1-Methyl 2-(Benzoylthio)butanedioic Acid Monoester (2, Low R_f Acid). The more polar compound recovered after column chromatography was recrystallized from $CHCl_3$ to yield 2 as a white crystalline solid: mp 83–86 °C; IR (KBr) 3250 (OH), 1730 (C=O), 1710 (C=O), 1660 (SC=O) cm^{-1}; ^{1}H NMR 2.95–3.15 (m, 2 H, CH_2), 3.71 (s, 3 H, OCH_3), 4.76–4.80 (m, 1 H, CH), 7.42–7.96 (m, 5 H, ArH), 8.9–9.3 (br s, 1 H, COOH); ^{13}C NMR 31.85 (-CH_2), 36.39 (-CH-), 47.71 (-OCH_3), 123.06 (meta-C), 124.31 (ortho-C), 129.61 (para-C), 131.34 (C-Ph), 164.52 (S-C=O), 166.32 ($COOCH_3$), 171.94 (COOH). Anal. ($C_{12}H_{12}O_5S$) C, H, S.

4-Methyl 2-(Benzoylthio)butanedioic Acid Monoester (3, High R_f Acid). The less polar compound recovered after column

chromatography was recrystallized from $CHCl_3$ to yield **3** as a white crystalline solid: mp 75–79 °C; IR (KBr) 3280 (OH), 1740 (C=O), 1710 (C=O), 1670 (SC=O) cm^{-1}; ^1H NMR 2.98–3.20 (m, 2 H, CH_2), 3.76 (s, 3 H, OCH_3), 4.74–4.78 (m, 1 H, CH), 7.52–7.98 (m, 5 H, ArH), 9.3–9.6 (br s, 1 H, COOH); ^{13}C NMR 32.20 (-CCH_2), 36.01 (-CH-), 48.68 (-OCH_3), 123.00 (meta-C), 124.3 (ortho-C), 129.52 (para-C), 131.44 (C-Ph), 165.01 (SC=O), 166.26 ($COOCH_3$), 171.68 (COOH). Anal. ($C_{12}H_{12}O_5S$) C, H, S.

Dimethyl 2,11-Bis(benzoylthio)-4,9-dioxo-5,8-diazadodecanedioate (5). The high R_f acid (**3**; 0.2 g, 0.75 mmol) and *N*-hydroxysuccinimide (0.086 g, 0.75 mmol) were dissolved in dry THF (20 mL) and stirred under an inert atmosphere at −5 to 0 °C. A solution of dicyclohexylcarbodiimde (0.185 g, 1.2 equiv) in dry THF (10 mL) was then added dropwise over 30 min. The reaction temperature was maintained at −5 to 0 °C for 2 h, allowed to warm to room temperature, and stirred overnight. The reaction mixture was filtered and concentrated, and the residue was dissolved in $CHCl_3$. Any undissolved materials were filtered, and the filtrate was concentrated. This procedure was repeated three times. Evaporation of solvent and further drying on a vacuum pump for 2 h afforded the activated ester as a slightly yellow solid which was used without purification: IR (KBr) 1820, 1780, 1740 (activated ester), 1740 (C=O), 1675 (SC=O) cm^{-1}; ^1H NMR 2.80 (s, 4 H, CH_2C=O), 3.0–3.22 (m, 2 H, CH_2), 3.72 (s, 3 H, OCH_3), 4.90–5.18 (m, 1 H, CH), 7.10–7.98 (m, 5 H, ArH).

To a stirred solution of the activated ester of **3** in dry THF (25 mL), a solution of ethylenediamine (0.03 mL, 0.35 mmol) in dry THF (10 mL) was added dropwise under an inert atmosphere. The reaction mixture was stirred at room temperature for 18 h, filtered, and concentrated to a white oil. Chloroform (50 mL) and H_2O (20 mL) were added, and the phases were separated. The organic layer was washed with H_2O (20 mL) and brine, dried, and concentrated to a slightly yellow solid. The product was purified by column chromatography on silica gel (40% Et_2O/EtOAc). The recovery of pure **5** was 0.19 g (45%): mp 123–127 °C; IR (KBr) 3320 (NH), 1730 (C=O), 1660 (SC=O) 1630 (NC=O, I), 1550 (NC=O, II) cm^{-1}; ^1H NMR 2.81–3.18 (m, 4 H, CH_2), 3.31–3.51 (m, 4 H, NCH_2CH_2N), 3.69 (s, 6 H, OCH_3), 4.56–4.64 (m, 2 H, S-CH), 6.95 (broad s, 2 H, NH) 7.44–7.98 (m, 10 H, ArH). Anal. ($C_{26}H_{28}N_2O_8S_2$) C, H, N, S.

Dimethyl 3,10-Bis(benzoylthio)-4,9-dioxo-5,8-diazadodecanedioate (4). The procedure described above for the synthesis of **5** was utilized. The activated ester of acid **2** was used without further purification: IR (KBr) 1820, 1785, 1745 (activated ester), 1745 (C=O), 1675 (SC=O) cm^{-1}; ^1H NMR 2.74 (s, 4 H, CH_2C=O), 3.16–3.40 (m, 2 H, CH_2), 3.74 (s, 3 H, OCH_3), 4.62–4.92 (m, 1 H, CH), 7.12–8.10 (m, 5 H, ArH). The title compound was purified by column chromatography on silica gel (40% EtO_2/EtOAc) to afford 0.22 g (55%) of **4**: mp 118–120 °C; IR (KBr) 3320 (NH), 1740 (C=O), 1670 (SC=O) 1650 (NC=O, I), 1550 (NC=O, II) cm^{-1}; ^1H NMR 2.78–3.02 (m, 4 H, CH_2), 3.32–3.46 (m, 4 H, CH_2N), 3.74 (s, 6 H, OCH_3), 4.72–4.78 (m, 2 H, S-CH), 6.45 (broad s, 2 H, NH) 7.38–7.92 (m, 10 H, ArH). Anal. ($C_{26}H_{28}N_2O_8S_2$) C, H, N, S.

Diethyl 3,8-Dioxo-4,7-diazadecanedioate (6). To a chilled (0–4 °C) solution of ethylenediamine (7.10 mL, 106 mmol) in CH_2Cl_2 (150 mL) under an inert atmosphere was added dropwise a solution of methyl malonyl chloride (16 g, 106 mmol) in CH_2Cl_2 (40 mL). The temperature was maintained at 0–4 °C for 3 h, allowed to warm to room temperature, and stirred overnight. The resulting precipitate was filtered and washed with CH_2Cl_2. Water was added to the filtrate, the phases were separated, and the organic phase was washed with brine and dried over Na_2SO_4. Evaporation of the solvent in vacuo and recrystallization of the residue from CH_2Cl_2 afforded 8.7 g (63%) of crystalline **6**: mp 110–112 °C; IR (KBr) 3300 (NH), 1745 (C=O), 1645 (NC=O, I), 1560 (NC=O, II) cm^{-1}; ^1H NMR 1.26 (t, 6 H, J = 7 Hz, OCH_2CH_3), 3.28 (s, 4 H, CO_2CH_2CON), 3.42–3.44 (m, 4 H, NCH_2-CH_2N), 4.18 (q, 4 H, J = 7 Hz, OCH_2CH_3), 7.38 (bs, 2 H, NH). Anal. ($C_{12}H_{20}N_2O_6$) C, H, N.

Diethyl 2,9-Dibromo-3,8-dioxo-4,7-diazadecanedioate (7). To a solution of **6** (2.4 g, 8.3 mmol) and *N*-bromosuccinimide (3.7 g, 10.4 mmol) in dry CH_2Cl_2 (125 mL) was added 3 drops of hydrobromic acid while being stirred under an inert atmosphere. After the mixture was heated at 85 °C for 3 h the precipitate was filtered and the filtrate concentrated. The product was purified by silica gel column chromatography (40% Et_2O/EtOAc). Crystallization from EtOH/ether gave an analytically pure sample of **7** (1.64 g, 44%): mp 120 °C; IR (KBr) 3290 (NH), 1750 (C=O), 1660 (NC=O, I), 1550 (NC=O, II); ^1H NMR 1.32 (t, 6 H, J = 7 Hz, OCH_2CH_3), 3.48–3.54 (m, 4 H, NCH_2CH_2N), 4.28 (q, 4 H, J = 7 Hz, OCH_2CH_3), 4.69 (s, 1 H, CHBr), 4.70 (s, 1 H, CHBr), 7.22 (broad s, 2 H, NH). Anal. ($C_{12}H_{18}Br_2N_2O_6$) C, H, N.

Diethyl 2,9-Bis(benzoylthio)-3,8-dioxo-4,7-diazadecanedioate (8). To a stirred solution of **7** (0.19 g, 0.426 mmol) in absolute ethanol under nitrogen was added dropwise a solution of sodium thiobenzoate (prepared from 0.016 g of sodium in 10 mL of absolute ethanol). The reaction was stirred at room temperature for 2 h and then heated to 80 °C for 30 min. The mixture was cooled and concentrated in vacuo, and the residue was dissolved in EtOAc. The organic phase was washed with H_2O and brine, dried over Na_2SO_4, and concentrated in vacuo. The resulting residue was chromatographed on silica gel (60% EtOAc/hexane) to afford 0.175 g (73%) of **8** which was crystallized from EtOAc: mp 113–115 °C; IR (KBr) 3300 (NH), 1745 (C=O), 1675 (SC=O) 1650 (NC=O, I), 1550 (NC=O, II); ^1H NMR 1.20–1.24 (m, 6 H, OCH_2CH_3), 3.37–3.44 (m, 4 H, NCH_2CH_2N), 4.16–4.23 (m, 4 H, OCH_2CH_3), 4.97 (s, 1 H, CH), 4.98 (s, 1 H, CH), 6.97 (broad s, 2 H, NH), 7.37–7.92 (m, 10 H, ArH). Anal. ($C_{26}H_{28}N_2O_8S_2$) C, H, N, S.

General Procedure for the Synthesis of ^{99m}Tc Complexes. To approximately 1 mg of the ligand was added ethanol (0.3 mL), 1 N NaOH (0.3 mL), and sodium pertechnetate (^{99m}Tc) as eluent from a commercial generator (Du Pont, Billerica, MA). The mixture was heated for 2 min at 100 °C, $Na_2S_2O_4$ was added (0.3 mL, prepared fresh from 100 mg of $Na_2S_2O_4$ in 5 mL of H_2O), and heating continued for an additional 3 min. The solution was then cooled to room temperature and neutralized with 1 N HCl. The synthesis of ligands **4**, **5**, and **8** are not stereospecific; therefore, upon complexation with $[Tc{=}O]^{3+}$, three isomers are expected for each complex. Reverse-phase (PRP; Hamilton; Reno, NE, C-18; Alltech Assoc., Deerfield, IL) and normal-phase (silica gel; J. T. Baker, Phillipsburg, NJ) HPLC were used to attempt to separate the complex of ligand **5** into the expected isomers. For the reverse-phase columns, a variety of mobile phases (MeOH, buffer; CH_3CN, buffer; EtOH, buffer: ratios of 0:100 to 50:50) were evaluated using isocratic and gradient solvent systems which have been reported to be useful for similar ^{99m}Tc-N_2S_2 complexes.[17b,19a,20] Different pH values (6.0–7.8) and flow rates (0.5–1.5 mL/min) were also investigated. In some cases, what appeared to be shoulders on the observed peaks suggested that isomers may be present. However, under the various HPLC conditions used here, the expected isomers of the ^{99m}Tc complexes of ligands **4**, **5**, or **8** could not be cleanly separated for further analysis. Consequently, purification of the respective complexes was accomplished by reverse-phase HPLC (PRP-1) using 85% K_2PO_4 buffer (20 mM; pH 7)–15% ethanol as eluent. Under these HPLC conditions, only one component was observed for each of the complexes synthesized.

Preparation and Characterization of [^{99}TcO(8)]AsPh$_4$. The ^{99}Tc reagent, Na[^{99}TcO(eg)$_2$] was prepared according to previously published procedures.[23] Preparation of [^{99}TcO(8)]-$AsPh_4$ was accomplished as follows. Ligand **8** (62 mg, 0.110 mmol), was dissolved by heating gently under nitrogen in 15 mL of 0.25 N NaOH to give a yellow solution. A solution of Na[^{99}TcO(eg)$_2$] (4.1 mL of a 0.024 M solution in water, 0.1 mmol) was added dropwise to the ligand solution. The color turned brown-gold immediately. The solution was heated under nitrogen for 30 min. A small amount of a brown precipitate was filtered. Drops of 5 N HCl were added to the filtrate to bring the pH to 7–7.2. The solution was loaded onto an anion-exchange column (Sephadex QAE, 25 cm × 1^1/$_2$ cm) and washed with 30 mL of water. The column was washed with 300 mL of 0.9% saline, followed by 300 mL of 3% NaCl to elute benzoic acid and Na[^{99}TcO$_4$]. The column was then washed with 120 mL of 5% NaCl. The first 60 mL of the 5% NaCl eluent was discarded. The last 60 mL were collected. HPLC analysis showed that this fraction coelutes with the complex prepared at the tracer level with ^{99m}Tc (rt = 2.3 min). This solution was evaporated under reduced pressure to a yellow solid. The solid was stirred with 60 mL of acetone for 30 min. The acetone wsa filtered, and the solid was dissolved in 20 mL of water to give a yellow solution.

The pH was adjusted to 1.70. A solution of 41 mg of $AsPh_4Cl$ in 0.5 mL of water was added to the stirring solution, and a yellow precipitate formed over a period of 15 min. The suspension was stirred at room temperature for a total of 30 min. The yellow precipitate was collected by filtration. The solid was redissolved in water by repeating the following procedure three times. The solid was stirred in 40 mL of water for 30 min with gentle heat. The remaining solid was filtered, and the filtrates were combined and allowed to evaporate. After 3 days, yellow rectangular crystals were collected: yield 5–10 mg (5.8–11.5%) $AsPh_4$ [^{99}TcO(8)]; IR (KBr) 680 cm^{-1} s ($AsPh_4$), 730 cm^{-1} s ($AsPh_4$), 955 cm^{-1} s (Tc=O), 1560 cm^{-1} s (C=O), 1720 cm^{-1} s (C=O); ^{1}H NMR 3.92 (m, 2 H, CH_2), 4.09 (m, 2 H, CH_2), 4.87 (s, 2 H, CH), 10.30 (bs, 2 H, COOH), 7.60 (m, 8 H, phenyl), 7.75 (m, 8 H, phenyl), 7.84 (m, 4 H, phenyl); mass spectral data (FAB) m/z 407. Anal. ($C_{32}H_{28}N_2S_2O_7AsTc$) C, H, N; C, calcd, C: 48.61; found, C: 48.06.

Crystal Parameters. $TcC_{32}H_{28}N_2O_7S_2As$, [^{99}TcO(8)]$AsPh_4$, formula weight 787.6, yellow, crystal dimensions (mm) 0.28 × 0.28 × 0.28; monoclinic, $P2_1/n$; V, Å^3 = 3199.1(20); Z = 4; a = 9.342(3) Å; b = 18.594(5) Å, c = 18.417(7) Å; β, deg = 90.61(3); D(calc), g cm^{-3} = 1.635; m(Mo Kα), cm^{-1} = 16.58; temperature = 298 K.

Data Collection. Diffractometer, Siemens P4; reflections collected, 4489; monochromator, graphite; independent reflections, 4170; independent observed reflections ($F_o > n\sigma(F_o)$ (n = 4), 2157; radiation, Mo Kα(λ = 0.710 73 Å); 2θ scan range, deg, 4–45; std rflns, 3 std/197 rflns; data collected (h,k,l), +11,+21,+20; var in stds, %, <1. Unit cell parameters were obtained from the least-squares fit of 25 reflections (20° ≤ 2θ ≤ 25°) on a Siemens P4 diffractometer, graphite monochromated. Preliminary photographic characterization showed 2/m Laue symmetry, and the systematic absences in the diffraction data uniquely established the space group as $P2_1/n$. The semiempirical absorption correction program XABS was applied to the data set.

Solution and Refinement. The structure was solved by direct methods which located the Tc and As atoms. The remaining non-hydrogen atoms were located from the subsequent least-squares and difference Fourier cycles. All non-hydrogen atoms were located from the subsequent least-squares and differences Fourier cycles. All non-hydrogen and non-carbon atoms were refined with anisotropic thermal parameters. All hydrogens were included as idealized isotropic contributions (d(CH) = 0.960 Å; U is fixed at 0.080 Å^2). All computer programs and the sources of the scattering factors are contained in the SHELXTL PLUS program library (4.21) (G. Sheldrick; Siemens, Madison, WI). Refinement: R(F), % = 6.45; R(wF), % = 6.63; Δ/σ(max) = 0.004; $\Delta(\rho)$, e Å^{-3} = 0.824; N_o/N_v = 8.4; GOF = 1.001.

Protein Binding Determinations in Rat and Monkey Plasma. Blood was obtained from male cynomologus monkeys and male Sprague–Dawley rats. Blood samples were first centrifuged at 4000 rpm for 5 min and the plasma recovered. The appropriate ^{99m}Tc chelate ligand dissolved in 50% ethanol/H_2O (0.12 mL; 1 × 10^6 counts) and plasma (1 mL) were incubated at 37 °C for 30 min. Trichloroacetic acid (0.4 mL; 10%) was added and the mixture centrifuged at 4000 rpm for 10 min. Following centrifugation, the pellet was washed with H_2O (2 × 0.5 mL), and the washes and supernatant were combined and counted, as was the pellet. A control containing water rather than plasma which was treated in an identical manner indicated that the ^{99m}Tc complex of ligand 5 was stable under the acidic conditions of the assay. The plasma protein binding is reported as the percentage of counts in the pellet/total number of counts (in both the supernatant and pellet).

Planar Imaging Studies. A male cynomologus monkey (4.0 kg) was sedated with ketamine hydrochloride (10 mg/kg) and anesthetized with a 1–2% isoflurane/oxygen mixture delivered as needed at 0.5–1.0 L/min. Saline (20 mL of 0.45% NaCl) was administered by a slow iv drip over 10–15 min to ensure adequate hydration. Approximately 10–15 min later, the appropriate technetium chelated ligand (5–10 mCi) or MAG_3 was administered as a bolus through the same catheter. A dynamic sequence of 5 min planar images of the monkey in a supine position was obtained on a GE 400T γ camera with a general-purpose medium-energy collimator on line with a GE Star II computer (Milwaukee, WI). Regions of interest were drawn around several anatomical regions (e.g. kidneys, liver, heart, and bladder), and average counts/region vs time were determined.

Animal Biodistribution Studies. The bladders of male Sprague–Dawley rats (200–300 g) under ketamine/xylazine anesthesia (80 mg/kg: 8 mg/kg, respectively) were cannulated with PE 50 tubing for collection of urine. The animals were injected intravenously (femoral vein) with 0.2 mL of a saline solution containing the ^{99m}Tc complexes (20 μCi) and OIH (7 μCi). At selected intervals following the injection, the urine was collected and the bladder was rinsed with saline. Due to the disappointing levels of activity found in the urine of the animals, no attempt was made to determine the chemical form of the ^{99m}Tc. Blood samples were collected by cardiac puncture, and the rats were sacrificed immediately thereafter by cardiectomy. The organs of interest were subsequently excised, weighed, and counted in a Packard (Sterling, VA) Auto-gamma 5000 Series γ counter with a window setting suitable for the separation of counts from each isotope. Corrections were made for ^{131}I spillover into the ^{99m}Tc channel. The percent dose/organ values were determined by comparison of the tissue radioactivity with suitable dilutents of the injected dose. Values represent the mean ±SD of the percent injected dose determined for three to four rats.

Acknowledgment. The authors are grateful to Dr. C. E. Costello and Ms. S. Maleknia of the MIT Mass Spectrometry Facility for providing mass spectral (FAB) data (NIH grant for Mass Spectrometry facilities: RR00317 to K. Biemann). This work was supported by grants from the National Institutes of Health (NS-18509 to H.F.K.) and Nihon Medi-Physics, Inc.

Supplementary Material Available: Crystallography tables of atomic coordinates, bond distances and angles, solution and refinement parameters, anisotropic displacement coefficients, and hydrogen atom coordinates (6 pages); observed and calculated structure factors for [^{99}Tc]-8 (7 pages). Ordering information is given on any current masthead page.

References

(1) Fritzberg, A. R. Advances in Renal Radiopharmaceuticals. In *Radiopharmaceuticals: Progress and Clinical Perspectives*; Fritzberg, A. R., Ed.: CRC Press: Boca Raton, FL, 1986; Vol. 1, pp 61–87.
(2) Davison, A.; Sohn, M.; Orvig, C.; Jones, A. G.; La Tegola, M. R. A tetradendate ligand designed specifically to coordinate technetium. *J. Nucl. Med.* **1979**, *20*, 641 (abstr.)
(3) Davison, A.; Jones, A.; Orvig, C.; et al. A new class of oxotechnetium (+5) chelate complexes containing a $TcON_2S_2$ core. *Inorg. Chem.* **1981**, *20*, 1629–1632.
(4) Fritzberg, A. R.; Klingensmith, W. C.; Whitney, W. P.; Kuni, C. C. Chemical and biological studies of Tc-99m N,N′-bis(mercaptoacetamido)ethylenediamine: A potential replacement for I-131 iodohippurate. *J. Nucl. Med.* **1981**, *22*, 258–263.
(5) Fritzberg, A. R.; Kuni, C. C.; Klingensmith, W. C.; Stevens, J.; Whitney, W. P.; Synthesis and biological evaluation of Tc-99m N,N′-bis(mercaptoacetyl)-2,3-diaminopropanoate: A potential replacement for I-131 iodohippurate. *J. Nucl. Med.* **1982**, *23*, 592–598.
(6) Klingensmith, W. C.; Fritzberg, A. R.; Spitzer, V. M.; Johnson, D. L.; Kuni, C. C.; Williamson, M. R.; Washer, G.; Weil, R. Clinical evaluation of Tc-99m N,N′-bis(mercaptoacetyl)-2,3-diaminopropanoate as a potential replacement for I-131 hippurate. *J. Nucl. Med.* **1984**, *25*, 42–48.
(7) Fritzberg, A. R.; Kasina, S.; Eshima, D.; Johnson, D. L. Synthesis and biological evaluation of technetium-99m MAG_3 as a hippuran replacement. *J. Nucl. Med.* **1986**, *27*, 111–116.
(8) Taylor, A., Jr.; Eshima, D.; Fritzberg, A. R.; et al. Comparison of iodine-131 OIH and technetium-99m MAG3 renal imaging in volunteers. *J. Nucl. Med.* **1986**, *27*, 795–803.
(9) Taylor, A., Jr.; Ziffer, J. A.; Eshima, D. Comparison of Tc-99m MAG3 and Tc-99m DTPA in renal transplant patients with impaired renal function. *Clin. Nucl. Med.* **1990**, *15*, 371–378.
(10) Jafri, R. A.; Britton, K. E.; Nimmon, C. C.; Solanki, K.; Al-Nahhas, A.; Bomanji, J.; Fettich, J.; Hawkins, L. A. Technetium-99m MAG3, a comparison with iodine-123 and iodine-131 orthoiodohippurate, in patients with renal disorders. *J. Nucl. Med.* **1988**, *29*, 147–158.
(11) Taylor, A., Jr.; Eshima, D.; Christian, P. E.; Milton, W. Evaluation of Tc-99m mercaptoacetyltriglycine in patients with impaired renal function. *Radiology* **1987**, *162*, 365–370.
(12) Bubeck, B.; Brandau, W.; Weber, E.; Kalble, T.; Parekh, T.; Georgi, P. Pharmacokinetics of technetium-99m-MAG3 in humans. *J. Nucl. Med.* **1990**, *31*, 1285–1293.

(13) Britton, K. E.; Jafri, R. A.; Nimmon, C. C. Comparison of the clearance of technetium-99m-MAG3 and iodine-131-OIH. *J. Nucl. Med.* **1988**, *29*, 1878–1879.
(14) Bormans, G. M.; Cleynhens, B.; Hoogmartens, M.; DeRoo, M. J.; Verbruggen, A. M. Evaluation of ^{99m}Tc-mercaptoacetyltripeptides in mice and a baboon. *Nucl. Med. Biol.* **1992**, *19*, 375–388.
(15) Verbruggen, A.; Bormans, G.; Van Nerom, C.; Cleynhens, B.; Crombez, D.; DeRoo, M. Isolation of the mono-ester mono-acid derivatives of 99mTc-ECD and their metabolites in mice. In *Technitium and rhenium in chemistry and nuclear medicine 3*; Nicilini, M., Bandoli, G., Mazzi, U., Eds.; Verona: Cortina International, and Raven Press: New York, 1990; pp 445–452.
(16) Verbruggen, A. M.; Bormans, G. M.; Van Nerom, C.; Cleynhens, B.; Osiadacz, D.; DeRoo, M. *J. Labelled Compd. Radiopharm.* **1991**, *30*, 83–85 abstr.
(17) (a) Verbruggen, A. M.; Nosco, D. L.; Van Nerom, C. G.; Bormans, G. M.; Adriaens, P. J.; DeRoo, M. J. Evaluation of Tc-99m-L,L-ethylenedicysteine as a potential alternative to 99mTc MAG3. *Eur. J. Nucl. Med.* **1990**, *16*, 429. (b) Verbruggen, A. M.; Nosco, D. L.; Van Nerom, C. G.; Bormans, G. M.; Adriaens, P. J.; DeRoo, M. J. Techentium-99m-L,L-Ethylenedicysteine: A renal imaging agent. I. Labeling and evaluation in animals. *J. Nucl. Med.* **1992**, *33*, 551–557.
(18) Melnik, M.; Van Lier, J. E. Analyses of structural data of technetium compounds. *Coord. Chem. Rev.* **1987**, *77*, 275–324.
(19) (a) Rao, T. N.; Adhikesavalu, D.; Camerman, A.; Fritzberg, A. R. Technetium(V) and Rhenium(V) Complexes of 2,3-Bis(mercaptoacetamido)propanoate. Chelate Ring Stereochemistry and Influence on Chemical and Biological Properties. *J. Am. Chem. Soc.* **1990**, *112*, 5798–5804. (b) Ohmomo, Y.; Francesconi, L.; Kung, M.-P.; Kung, H. F. New conformationally restricted 99m-Tc N2S2 complexes as myocardial perfusion imaging agents. *J. Med. Chem.* **1992**, *35*, 157–162. (c) John, C. S.; Francesconi, L. C.; Kung, H. F.; Wehrli, S.; Graczyk, G.; Carroll, P. Synthesis and characterization of neutral oxotechnetium(V) bisaminoethanethiol complexes: Potential brain imaging agents. *Polyhedron* **1992**, *11*, 1145–1155.
(20) Bormans, G.; Cleynhens, B.; Jose, D.; Hoogmartens, M.; De Roo, M.; Verbruggen, A. Synthesis and biological characterization of the four stereoisomers of 99mTc-N,N′-bis-(mercaptoacetyl)-2,3-diaminopropanoate. *Nucl. Med. Biol.* **1990**, *17*, 499–506.
(21) Brenner, D. The search for a technetium renal agent: The synthesis, characterization, and in vivo behavior of some oxotechnetium(V^+) bis-amido-bis-thiolato anions. Ph.D. Thesis, MIT, 1984.
(22) Kasina, S.; Fritzberg, A. R.; Johnson, D. L.; Eshima, D. Tissue distribution properties of technetium-99m-diamide-dimercaptide complexes and potential use as renal radiopharmaceuticals. *J. Med. Chem.* **1986**, *29*, 1933–1940.
(23) (a) Brenner, D.; Davison, A.; Lister-James, J.; Jones, A. Synthesis and characterization of a series of isomeric oxotechnetium(V) diamido dithiolates. *Inorg. Chem.* **1984**, *23*, 3793. (b) Linder, K. E. Amino-carboxylate complexes of technetium. Ph.D. Thesis, MIT, 1986.