Cationic $[^{99m}Tc^{III}(DIARS)_2(SR)_2]^+$ Complexes as Potential Myocardial Perfusion Imaging Agents (DIARS = *o*-phenylenebis(dimethylarsine); SR^- = thiolate)

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Reduction−substitution reactions on $[^{99m}TcO_4]^-$ with both *o*-phenylenebis(dimethylarsine) (DIARS) and various thiols produce a series of monocationic $[^{99m}Tc(DIARS)_2(SR)_2]^+$ complexes. Addition of $[^{99g}TcO_4]^-$ to the above reaction mixtures allows the characterization of the "carrier-added" complexes by means of reverse-phase high-performance liquid chromatography with radiometric and optical detection systems. The identity of the $[^{99g}Tc(DIARS)_2(SR)_2]^+$ complexes is confirmed by fast atom bombardment mass spectroscopy; equivalence of the $[^{99g}Tc(DIARS)_2(SR)_2]^+$ and $[^{99m}Tc(DIARS)_2(SR)_2]^+$ species is demonstrated by identical HPLC retention times. All the $[^{99m}Tc(DIARS)_2(SR)_2]^+$ complexes tested accumulate in the myocardium of Sprague−Dawley rats with an average uptake of 1.5−2.0% of injected dose/g at 30 min. Thus, as designed, these nonreducible Tc(III) complexes do not exhibit the rapid myocardial washout observed for reducible Tc(III) complexes. These $[^{99m}Tc(DIARS)_2(SR)_2]^+$ complexes also exhibit an initially high liver uptake, but the presence of ether groups within the thiolate ligands causes this liver uptake to decrease over time without affecting the heart uptake, thereby improving the heart/liver ratio.

Introduction

trans-$[Tc^{III/II}D_2X_2]^{+/0}$ complexes, where D represents a tertiary diphosphine or diarsine and X represents a halide or thiocyanate,[1−3] have received considerable attention in the last decade because (i) they were among the very first technetium complexes to be fully characterized utilizing macroscopic amounts of ^{99g}Tc[2] and (ii) their ^{99m}Tc analogues exhibit important biological properties.[3] In fact, *trans*-$[Tc^{III}(DMPE)_2Cl_2]^+$ (where DMPE = 1,2-bis(dimethylphosphino)ethane) was the first cationic ^{99m}Tc complex to be evaluated as a myocardial perfusion imaging agent in humans.[4] Unfortunately, it suffers *in vivo* reduction to the neutral *trans*-$[^{99m}Tc^{II}(DMPE)_2Cl_2]^0$ form, which washes out of the heart and accumulates in the liver.[5] Even though this initial candidate technetium cation did not succeed as a myocardial imaging agent,[6] the general approach of using cationic technetium complexes has generated several successful examples (Figure 1).

Among these, ^{99m}Tc-MIBI (Cardiolite),[7] hexakis(methoxybutylisonitrile)technetium(I), has been approved by the FDA for myocardial infarct imaging, and at least two other cationic tracers are in clinical trials (^{99m}Tc-Q12, a mixed N_2O_2-donor Schiff base/phosphine Tc(III) cation,[8] and ^{99m}Tc-P53, a *trans*-dioxobis(diphosphine) Tc(V) cation[9]).

Synthetic strategies in the development of new $[Tc^{III/II}D_2X_2]^{+/0}$ complexes include the replacement of the X ligands by thiolato groups (SR^-).[10] In this strategy, the organic R substitutent can be systematically and subtly varied to create Tc complexes which have incorporated carefully controlled properties. Among these properties, the Tc(III/II) redox potential of the $[Tc^{III/II}D_2(SR)_2]^{+/0}$ species is crucial in determining whether the parental Tc(III) complex remains cationic *in vivo* or whether it undergoes *in vivo* reduction to Tc(II) which will lead to myocardial washout.[11] It has been observed that $[Tc^{III}(DMPE)_2(SR)_2]^+$ complexes are much more resistant to reduction to Tc^{II} than their dihalo $[Tc^{III}(DMPE)_2X_2]^+$ analogues;[10,12] in other words, thiolato ligands enhance the redox stability of these Tc(III) complexes. Similarly, while dihalo $[Tc^{III}(DIARS)_2X_2]^+$ (where DIARS = *o*-phenylenebis(dimethylarsine)) complexes are easier to reduce than the corresponding DMPE derivatives because of the increased π-acidity of the As-based backbones, the addition of strong σ-donating alkylthiolato ligands brings $[Tc^{III}(DIARS)_2(SR)_2]^+$ complexes out of the range of biologically accessible reduction.[10d,12] Therefore, *in vivo* reduction of $[Tc^{III}(DIARS)_2(SR)_2]^+$ to its Tc(II) form is not possible, and myocardial washout should be blocked. In addition, variations in the R group of the thiolato ligand allow important properties (*e.g.*, lipophilicity) of the $[^{99m}Tc(DIARS)_2(SR)_2]^+$ complexes to be subtly varied, and in this way the overall biodistribution of the complexes can be controlled.

In this paper we report on the reduction−substitution reactions of "no carrier-added" $[^{99m}TcO_4]^-$ with both DIARS and a series of thiols (*n*-propanethiol, **1**; 2-methoxyethanethiol, **2**; 2,3-dimethoxypropanethiol, **3**; 2,2-dimethyl-4-(mercaptomethyl)-1,3-dioxolane, **4**; 2-(2-mercaptoethyl)-1,3-dioxane, **5**; and 1-methoxy-2-(methoxymethylene)pentane-5-thiol, **6**; see Figure 2) to yield a group of new monocationic $[^{99m}Tc^{III}(DIARS)_2(SR)_2]^+$ complexes. Also reported are the chemical characterizations of these new thiolato Tc(III) complexes, as well as their biodistributions in Sprague−Dawley rats.

Results and Discussion

Ligand Preparation. Thiols **1** and **7** are commercially available. The preparation of thiols **2**−**6** is outlined in Scheme 1. Thiol **2** is made by reacting

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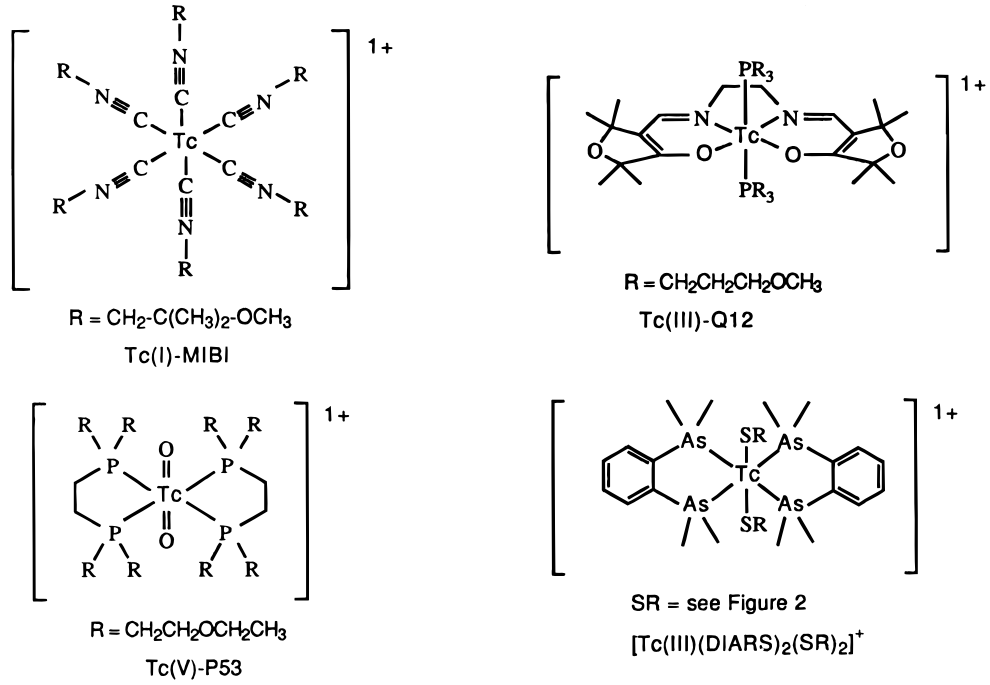


Figure 1. Cationic ^{99m}Tc complexes developed as possible myocardial imaging agents.

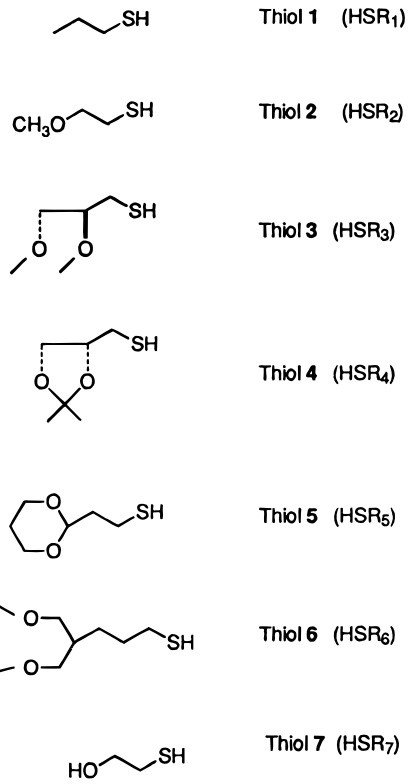


Figure 2. Thiols used in the synthesis of $[Tc^{III}(DIARS)_2(SR)_2]^+$ complexes.

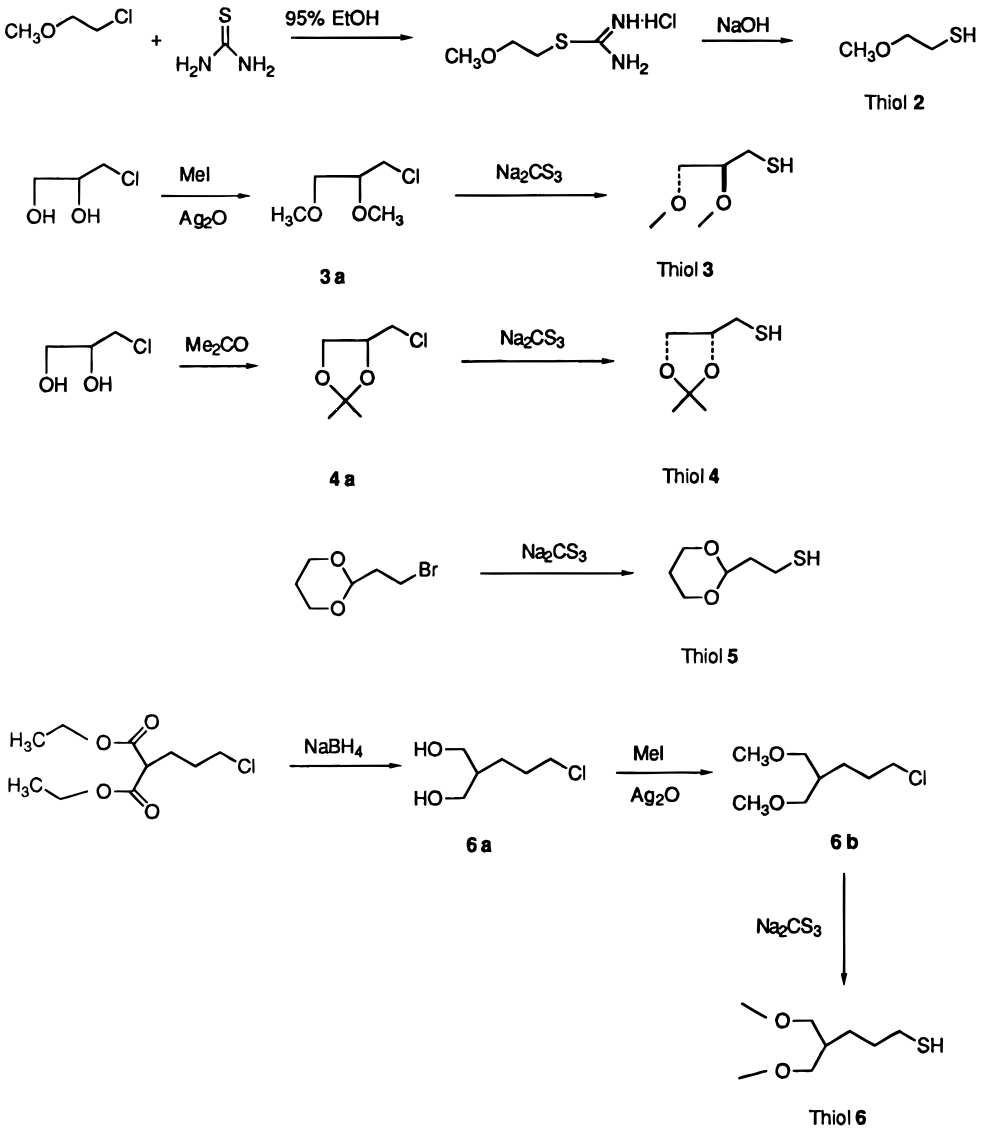
1-chloro-2-methoxyethane with thiourea in refluxing 95% ethanol. The resulting *S*-alkylisothiouronium salt can be hydrolyzed in a sodium hydroxide solution to yield the desired thiol as a separate oil layer. Thiols **3**−**6** are synthesized by the reaction of freshly prepared aqueous sodium thiocarbonate with an alcoholic solution of the appropriate parental halide as detailed in Scheme 1. The resulting mixtures are then acidified with HCl and the target thiols extracted in ether.

Ligand Characterization and pH Stability. Thiols **2**−**6** are characterized by boiling point at reduced pressure, mass spectrometry, and ^{1}H and ^{13}C NMR spectroscopy. Elemental analyses are also reported for thiols **2** and **5**. The physical data are reported in the Experimental Section. In general, the proton NMR spectra of all free thiols show a sharp triplet at ∼1.50 ppm, characteristic of the S-H proton coupled with the vicinal methylene system. The complete assignment of the other proton signals can be made from the relative integrations, spin−spin splittings, homonuclear decoupling, and two-dimensional homonuclear shift correlations (2D-COSY).[13] A typical 2D-COSY contour plot is shown in Figure 3.

The synthesis of $[^{99m}Tc(DIARS)_2(SR)_2]^+$ complexes requires both high temperature and acid conditions (1 M CF_3SO_3H; pH range 1.5−3.5). Consequently, the stability of each free thiol is tested in hot, acidic media by proton NMR to mimic the ethanolic solutions employed during the radiolabeling procedures. Stability tests are conducted in 50/50 D_2O/C_2D_5OD-d_6 mixtures, adjusting the pH with D_3PO_4. Thiols **1**−**3** and **6**, as well as the DIARS ligand, prove stable for 1 h at pH 1.5, 80 °C. Conversely, thiol **4** hydrolyzes completely within a few seconds under these conditions, to yield acetone and 3-mercapto-1,2-propanediol as hydrolysis products. Therefore, thiol **4** was not utilized for labeling. The acetal ring of thiol **5** hydrolizes within 15 min at pH 1.5 and 80 °C, producing a characteristic aldehyde proton signal of the 2-thiopropionaldehyde at 9.50 ppm and two related multiplets of 1,3-propanediol at 3.70 and 1.70 ppm, respectively. Under less stringent conditions (*i.e.*, 80 °C and pH 3.5) the observed hydrolysis is greatly reduced and more than 90% of the free thiol **5** remains intact after 15 min, allowing the radiolabeling reaction to proceed.

Characterization of $[^{99m}Tc^{III}(DIARS)_2(SR)_2]^+$ Agents. The reduction−substitution reaction of $[TcO_4]^-$ with thiols and either diphosphines or diarsines (D) is a well-documented route of synthesis for $[Tc^{III/II}D_2(SR)_2]^{+/0}$ complexes.[10] The thorough characterization of these compounds utilizing macroscopic amounts of the long-

Scheme 1



lived isotope ^{99g}Tc has established their structural, spectroscopic, and electrochemical properties. The redox properties of $[^{99g}Tc^{III}(DIARS)_2(SR)_2]^+$ [10d] complexes confirm that they are incapable of undergoing reduction *in vivo*. Therefore, we have prepared a series of $[^{99m}Tc^{III}(DIARS)_2(SR)_2]^+$ analogues at the "no carrier-added" level in order to investigate both their ability to accumulate in the myocardium and their resistance to myocardial washout which is hypothesized to result from *in vivo* reduction of Tc(III) to Tc(II).

The ^{99m}Tc labeling procedure mimics the reduction–substitution method utilized in the macroscopic ^{99g}Tc syntheses. Commercially available sodium 99m-pertechnetate, metathesized to the organically soluble tetrabutylammonium salt, is brought into reaction with a solution of DIARS and the appropriate neat thiol in acidic ethanol. The reaction produces low-valence cationic species which are monitored by liquid chromatography coupled with radiometric detection. The reduction–substitution reaction is accomplished *via* either a one-step procedure (addition of both DIARS and thiol followed by heating) or a two-step procedure (addition of DIARS first followed by heating/cooling and subsequent addition of the neat thiol with further heating) depending on the thiol employed. The two-step reaction allows the generation of an intermediate species presumed to be a Tc(V) complex such as $[Tc^{V}O_2(DIARS)_2]^+$.[2b] Two representative chromatograms are illustrated in Figure 4. They show the different retention times exhibited by the DIARS-containing Tc(V) intermediate and the mixed DIARS–thiol Tc(III) product under identical chromatographic conditions. The Tc(V) complex elutes first, tracing as a broad peak which characteristically results from interactions of oxo groups with the silica-based stationary phase of the column. The unambiguous identification of the Tc(III) product is obtained by "carrier-added" experiments, wherein both ^{99m}Tc and ^{99g}Tc radionuclides are present in the reaction mixture. The reaction mixture is assayed by HPLC simultaneously monitored by radiometric and optical detection systems. The peaks arising from the radiolabeled species in the radiometric trace show the same retention times as those exhibited by the corresponding species in the UV–vis trace. In addition, the $^{99g}Tc(III)$ species show an intense absorption in the visible region around 600 nm, typical of all the blue $[^{99g}TcD_2(SR)_2]^+$ complexes previously characterized at the macroscopic level.[10] Conclusive characterization of

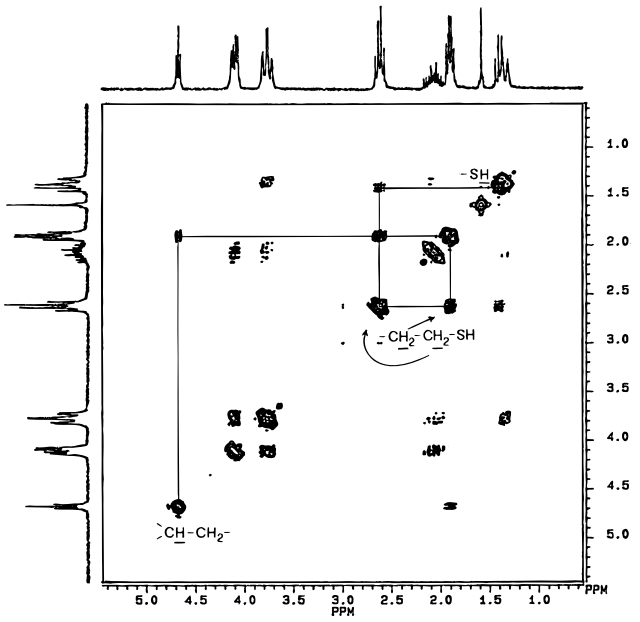


Figure 3. 2D-COSY90 ^{1}H NMR (250-MHz) contour plot of thiol **5**. The cross peaks outside of the diagonal account for the scalar couplings of vicinal protons. The couplings of the alkyl chain protons are connected with a solid line. The other spots on the diagonal and related cross peaks belong to the methylene system of the 1,3-dioxane ring.

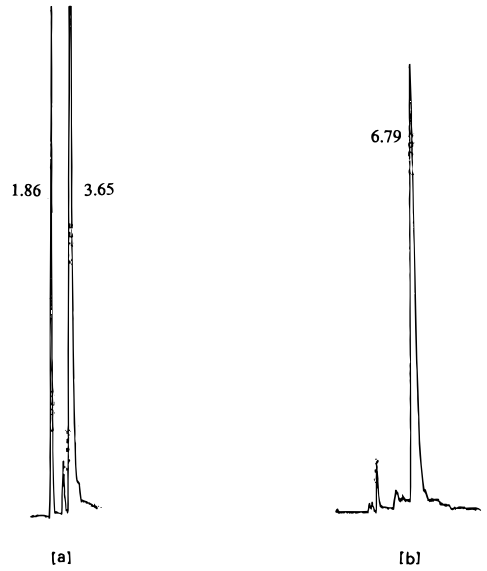


Figure 4. HPLC chromatograms (radiometric traces) under chromatographic conditions MP1 (see Experimental Section) of (a) $[^{99m}Tc^{V}O_2(DIARS)_2]^+$ (t_R = 3.65 min) containing unreacted $[^{99m}TcO_4]^-$ (t_R = 1.86 min) and (b) $[^{99m}Tc^{III}(DIARS)_2(SR_2)_2]^+$ (t_R = 6.79 min). Numbers labeling the peaks represent retention times in minutes. Integrated areas under the peaks represent (a) 25.6% of concentration at 1.86 min, 71.2% at 3.65 min, and (b) 93.0% at 6.79 min.

this colored species comes from positive ion FAB-MS, which shows a parent ion consistent with the $[^{99g}Tc(DIARS)_2(SR)_2]^+$ formulation.

The incorporation of one or more ether functionalities in the thiolato R group (as in thiols **2**−**6)** does not affect the stoichiometry or net charge of the resulting Tc complexes but is expected to influence their biological properties as a function of relative stability and lipophilicity. Relative lipophilicity orderings are derived from the comparison of reverse-phase HPLC retention times under identical chromatographic conditions. The

Table 1. Retention Times in Reverse-Phase HPLC of Related ^{99m}Tc Complexes[a]

complex	t_R (min)
$[Tc^{VII}O_4]^-$	2.5
$[Tc^{V}O_2(DIARS)_2]^+$	7.0
$[Tc^{III}(DIARS)_2(SR_1)_2]^+$	15.0
$[Tc^{III}(DIARS)_2(SR_2)_2]^+$	10.0
$[Tc^{III}(DIARS)_2(SR_3)_2]^+$	8.5
$[Tc^{III}(DIARS)_2(SR_5)_2]^+$	9.7
$[Tc^{III}(DIARS)_2(SR_6)_2]^+$	10.1
$[Tc^{III}(DIARS)_2(SR_7)_2]^+$	5.6

[a] The thiolates represented by SR_1−SR_7 are diagrammed in Figure 2. Chromatographic conditions are described as MP2 in the text. In this chromatographic system, it is usually assumed that lipophilicity increases with increasing retention time. Thus, thiol **1** gives the most lipophilic complex, while thiol **7** gives the least lipophilic complex.

retention times (t_R) are summarized in Table 1. A hydrophilic thiol derivative, 2-mercaptoethanol (thiol **7**), is included in the study for comparison purposes. As expected, $[^{99m}TcO_4]^-$ elutes at the solvent front at t_R = 2.5 min, whereas the Tc(V) species, $[^{99m}TcO_2(DIARS)_2]^+$, follows at 7 min. All the mixed DIARS−thiolato ^{99m}Tc-(III) complexes considered herein for biological studies elute after 8 min with the complexes containing thiols **2**, **5**, and **6** exhibiting practically identical retention times around 10 min. The complex with thiol **1**, which bears no ether functionalities in the thiolato organic R group, elutes much later at 15 min. Interestingly, the complex of thiol **7** shows surprisingly high hydrophilic character for a Tc(III) species, eluting even before the Tc(V) complex.

Biodistribution. The biodistributions of the $[^{99m}Tc^{III}(DIARS)_2(SR)_2]^+$ complexes reported herein are evaluated in female Sprague−Dawley rats. Purification of the radiolabeled product before injection is necessary in order to remove excess ligand as well as any radiochemical impurity formed during the labeling procedure. For this purpose, the radiolabeled reaction mixture is usually diluted with water and loaded onto a Sep-Pak C18 cartridge previously activated with 10% ethanol. The cartridge is then rinsed with water, ethanol/water, and ethanol solutions, and finally, the radiolabled product is eluted with lithium trifluoromethanesulfonate in ethanol. However, one complex, $[^{99m}Tc(DIARS)_2(SR_6)_2]^+$, had to be further purified by HPLC due to heavy loss of activity when the cartridge method was followed. The radiochemical purity and stability of the resulting purified product is verified prior to *in vivo* administration, as well as during the use period, by HPLC, to guarantee >95% purity of the injected radiopharmaceutical. This purified product is administered to the animals through the jugular vein. Groups of at least three rats are sacrificed at time intervals of 5, 15, 30, 60, and 120 min postinjection (pi). Cumulative biodistribution data, calculated as percent injected dose per gram (% ID/g), are shown in Table 2. All complexes are found to be stable with respect to conversion to pertechnetate *in vivo*, as indicated by the low amounts of radioactivity detected in the stomach (these results are not shown). All complexes exhibit low initial activity in the blood followed by a very rapid blood clearance (ca. 0.3% ID/g at 30 min pi). Activity is eliminated from the body through both the hepatobiliary system and the urinary tract. No brain uptake is found, as expected from the overall +1 charge of the complexes. Lung uptake is low, thus offering the possibility of a clear

Table 2. Biodistribution of $[^{99m}Tc^{III}(DIARS)_2(SR)_2]^+$ Complexes in Sprague–Dawley Rats[a]

time (min)	blood	liver	spleen	kidney	heart	lung
		$[^{99m}Tc^{III}(DIARS)_2(SR_1)_2]^+$				
5	0.59(7)	15.43(1.43)		4.35(30)	1.19(10)	
15	0.37(2)	13.72(5)		3.29(51)	0.91(11)	
30	0.29(4)	15.96(1.08)		3.79(43)	0.95(9)	
60	0.17(3)	13.95(2.72)		3.76(23)	1.00(25)	
120	0.13(4)	13.76(81)		3.58(70)	0.88(27)	
		$[^{99m}Tc^{III}(DIARS)_2(SR_2)_2]^+$				
5	1.08(4)	13.04(12)	7.64(1.60)	22.56(1.11)	2.43(38)	2.54(10)
15	0.42(10)	12.66(93)	5.24(34)	16.41(1.77)	1.93(33)	2.22(12)
30	0.31(3)	6.24(72)	5.19(79)	18.71(1.87)	2.17(54)	1.94(9)
60	0.22(3)	2.68(21)	2.57(47)	15.98(37)	1.87(32)	1.75(14)
120	0.24(2)	2.50(27)	2.35(7)	16.92(80)	2.03(8)	1.59(11)
		$[^{99m}Tc^{III}(DIARS)_2(SR_3)_2]^+$				
5	0.34(6)	7.40(1.07)	3.05(21)	10.07(1.02)	1.78(8)	1.42(8)
15	0.34(6)	5.41(29)	1.90(31)	8.73(48)	1.59(12)	1.02(8)
30	0.24(1)	3.85(47)	2.06(22)	6.33(49)	1.45(7)	0.92(5)
60	0.17(1)	2.36(14)	1.71(11)	4.59(39)	1.40(6)	0.79(3)
120	0.20(5)	1.85(12)	1.26(8)	3.20(11)	1.10(4)	0.79(1)
		$[^{99m}Tc^{III}(DIARS)_2(SR_5)_2]^+$				
5	0.68(11)	6.78(1.29)	5.81(1.61)	8.39(57)	1.58(25)	2.74(8)
15	0.35(2)	5.31(82)	3.97(68)	9.82(2.19)	1.78(35)	2.56(19)
30	0.27(2)	3.46(47)	3.63(71)	6.21(1.00)	1.30(13)	2.31(29)
60	0.22(2)	2.49(47)	2.49(34)	7.12(79)	1.28(20)	0.78(8)
120	0.16(1)	1.59(10)	1.31(23)	5.92(61)	1.06(7)	0.54(14)
		$[^{99m}Tc^{III}(DIARS)_2(SR_6)_2]^+$				
5	0.62(8)	3.71(54)	1.21(25)	7.45(1.08)	1.45(20)	4.66(1.52)
15	0.37(0)	2.59(11)	1.11(9)	6.31(1.24)	1.27(13)	3.28(80)
30	0.34(2)	2.53(28)	1.28(11)	5.05(77)	1.30(16)	3.71(96)
60	0.26(2)	2.39(35)	1.37(15)	5.54(49)	1.24(17)	2.38(78)
120	0.16(4)	1.63(26)	1.02(14)	4.36(21)	1.02(5)	1.34(14)

[a] Data are presented as average percent injected dose/gram (% ID/g). Standard deviations of last significant digits(s) are given in parentheses.

visualization of the heart. Rapid heart uptake is observed for all derivatives at 5 min pi (~1.5–2.0% ID/g), and for the prototypical thiols **1** and **2**, this uptake remains relatively constant up to 2 h pi. This rapid heart uptake in combination with the rapid blood clearance results in high heart/blood ratios that increase with time (*i.e.*, for $[^{99m}Tc(DIARS)_2(SR_2)_2]^+$ the initial heart/blood ratio at 5 min is 2.6 and increases to 8.7 at 2 h pi; see Table 3). Interestingly, the more functionalized complexes derived from thiols **2**, **5**, and **6** exhibit a slow myocardial washout.

Of particular interest is the comparison of the liver uptake and washout between the lipophilic $[^{99m}Tc(DIARS)_2(SR_1)_2]^+$ complex ($SR_1 = CH_3CH_2CH_2S^-$) and the rest of the group studied. Whereas $[^{99m}Tc(DIARS)_2(SR_1)_2]^+$ has a relatively high liver uptake (15.4% ID/g at 5 min pi) that remains practically constant up to 2 h pi, the rest of the compounds show lower initial liver uptake and by far more rapid liver clearance (*cf.* $[^{99m}Tc(DIARS)_2(SR_3)_2]^+$ exhibits an initial liver uptake of 7.4% ID/g at 5 min pi which decreases to 1.8% ID/g at 2 h pi). The fast liver washout of these complexes can be attributed to the ether functionalities incorporated into the thiolato ligand. These groups may undergo hydrolysis in the liver, thus giving rise to the corresponding hydroxy derivatives which are very hydrophilic (see Table 1, compound $[^{99m}Tc(DIARS)_2(SR_7)_2]^+$). As a consequence, these agents are then rapidly washed out of the liver, yielding a high heart/liver ratio. The fact that multiple ether functionalities in the thiolate ligand decrease liver uptake and enhance liver clearance further supports this hypothesis (compare liver uptake and washout between $[^{99m}Tc(DIARS)_2(SR_2)_2]^+$ which contains one ether group per thiol at 13.04% at 5 min pi and $[^{99m}Tc(DIARS)_2(SR_6)_2]^+$ which contains two ether groups per thiol at 3.71% at 5 min pi). For thiol **6**, a greater number of hydroxy groups may be "unmasked" in the liver resulting in a higher hydrophilicity and therefore greater washout. The fast liver clearance and constant % ID/g uptake values for the heart for $[^{99m}Tc(DIARS)_2(SR_2)_2]^+$ and $[^{99m}Tc(DIARS)_2(SR_6)_2]^+$ result in increasing heart/liver ratios with time (see Table 3).

Table 3. Heart/Blood and Heart/Liver Ratios for $[^{99m}Tc^{III}(DIARS)_2(SR)_2]^+$ Complexes[a]

complex	time (min)	heart/blood	heart/liver
$[^{99m}Tc^{III}(DIARS)_2(SR_1)_2]^+$	5	2.01(9)	0.08(4)
	15	2.47(15)	0.07(1)
	30	3.32(13)	0.06(8)
	60	5.78(18)	0.07(8)
	120	6.63(21)	0.06(5)
$[^{99m}Tc^{III}(DIARS)_2(SR_2)_2]^+$	5	2.64(96)	0.19(3)
	15	4.82(1.35)	0.16(4)
	30	6.88(1.12)	0.36(14)
	60	8.48(90)	0.69(7)
	120	8.75(1.22)	0.83(12)
$[^{99m}Tc^{III}(DIARS)_2(SR_3)_2]^+$	5	5.29(52)	0.25(4)
	15	4.56(47)	0.29(3)
	30	6.17(71)	0.39(7)
	60	8.09(20)	0.60(5)
	120	5.81(1.38)	0.60(5)
$[^{99m}Tc^{III}(DIARS)_2(SR_5)_2]^+$	5	2.40(77)	0.24(6)
	15	5.08(71)	0.34(10)
	30	4.74(36)	0.38(2)
	60	5.83(58)	0.53(16)
	120	6.66(48)	0.66(3)
$[^{99m}Tc^{III}(DIARS)_2(SR_6)_2]^+$	5	2.36(34)	0.40(6)
	15	3.42(31)	0.47(3)
	30	3.80(30)	0.51(4)
	60	4.77(29)	0.52(4)
	120	6.71(1.46)	0.64(11)

[a] Standard deviations of last significant digits(s) are given in parentheses.

For the complexes of thiols **3**, **5**, and **6**, wherein there are two ether linkages per R group, a slow myocardial washout is observed. Since the complexes of thiols **1** and **2** do not exhibit this washout, it is tempting to presume that it is the multiple ether functionalities of thiols **3**, **5**, and **6** which underlie the myocardial washout phenomenon. If so, this may provide a means of using ^{99m}Tc complexes to monitor a myocardial metabolic function. Further studies may determine if it is feasible to establish bench-mark values for how rapidly these multiple ether complexes are metabolized and then cleared from the myocardium in normal, healthy individuals. This information could be used as a base line against which to measure the clearance rate of these complexes as an early predictor of impaired cardiovascular function.

Nonreducible Tc(III) Myocardial Imaging Agents. The synthetic design strategy pursued herein stems from the results of biodistribution experiments which compared analogous ^{99m}Tc and ^{186}Re complexes. Although the biological milieu cannot distinguish between $[^{99m}Tc^{I}(DMPE)_3]^+$ and $[^{186}Re^{I}(DMPE)_3]^+$ complexes, when $[^{99m}Tc^{III}(DMPE)_2Cl_2]^+$ and $[^{186}Re^{III}(DMPE)_2Cl_2]^+$ are coinjected into rats the chemical processes of the biological milieu readily reduce the $^{99m}Tc(III)$ complex but not the $^{186}Re(III)$ complex.[5] The difference in reduction potential between $[^{99g}Tc^{III}(DMPE)_2Cl_2]^+$ and $[Re^{III}(DMPE)_2Cl_2]^+$ is 190 mV under laboratory conditions. The cationic nature of the $[^{99m}Tc^{III}(DMPE)_2Cl_2]^+$ complex encourages initial myocardial uptake. How-

ever, as the positive charge is lost in the Tc(III) → Tc-(II) reduction, the neutral ^{99m}Tc complex is transported out of the heart and processed through the liver. These observations imply that useful heart imaging agents could be built upon the [^{99m}Tc-"mixed-ligand"]$^+$ template if *in vivo* reduction could be avoided. There are a number of ways to modify [^{99m}Tc^{III}(DMPE)$_2$Cl$_2$]$^+$ so that it becomes more difficult to reduce. One successful approach has been to reduce the number of phosphine ligands, which are characterized by their ability to accept π-density from the central metal, making it more susceptible to chemical reduction. The "Q" series of technetium complexes[8] were designed on this principle and contain an $N_2O_2P_2$ donor set, as opposed to the Cl_2P_4 donor set of [^{99m}Tc^{III}(DMPE)$_2$Cl$_2$]$^+$. Clinical studies have demonstrated the effectiveness of more than one "Q" example as myocardial imaging agents. It is expected that one such complex, "Q12", will be approved for commercial distribution in the United States and Europe (see Figure 1). The success of this strategy to design cationic, mixed-ligand, nonreducible ^{99m}Tc complexes is completely applicable to the [^{99m}Tc(DIARS)$_2$(SR)$_2$]$^+$ complexes evaluated herein. They are sufficiently robust as cations to resist reduction to Tc(II), and they exhibit significant initial myocardial uptake. Yet some interesting biological processing occurs when multiple etheric groups are attached to each thiolato ligand, and this processing may be capitalized on in the future. It is possible that the judicious choice of R group in this series of [^{99m}Tc(DIARS)$_2$(SR)$_2$]$^+$ complexes will produce myocardial imaging agents which produce unique clinical utility.

Experimental Section

Reagents. Unless otherwise noted, all chemicals were of reagent grade. *o*-Phenylenebis(dimethylarsine) (DIARS) was purchased from Strem Chemicals Co., checked before use by proton NMR spectroscopy, and employed as a 1% ethanolic solution. *n*-Propanethiol (thiol **1**) and 2-mercaptoethanol (thiol **7**) were purchased from Aldrich and used without further purification. ^{99m}Tc was eluted as Na[^{99m}TcO$_4$] from a ^{99}Mo/^{99m}Tc commercial generator (Mallinckrodt Medical, Inc.) in 0.9% aqueous NaCl solution. Commercially available K[^{99g}TcO$_4$] (Oak Ridge National Laboratory) was purified from a black contaminant (^{99g}TcO$_2$·*n*H$_2$O) by addition of H_2O_2 and NH_4OH solutions followed by recrystallization as NH_4[^{99g}TcO$_4$] from hot water. The tetraalkylammonium salt was prepared by addition of aqueous *n*-Bu$_4$NCl to a suspension of NH_4[^{99g}TcO$_4$] in water. The white flocculent precipitate of the desired *n*-Bu$_4$N[^{99g}TcO$_4$] salt was removed by filtration and washed with water and a few drops of Et_2O.

Apparatus. Elemental analyses were performed on a Fisons model EA-1108 elemental analyzer. Proton and ^{13}C NMR measurements were performed on a Bruker NR-80 or Bruker WM-250 instrument in $CDCl_3$ using TMS as the internal standard. High-resolution gas−mass spectra were obtained on a Kratos MS-80 mass spectrometer. Positive ion FAB-MS were recorded by using a glycerol matrix on a VG 30-250 spectrometer at ambient temperatures. A Beckman model 114M liquid chromatograph equipped with a Beckman 170 radioactive detector was used to monitor "carrier free" preparations. An ISCO liquid chromatograph equipped with both an HP 1040 photodiode array detector and a Beckman 170 radioactive detector was employed to follow "carrier-added" preparations. Reverse-phase C8 columns (27.5 × 0.5 cm from Hamilton Co.) and guard columns filled with 10 μm size RP-C8 packing material were utilized for all experimental preparations. Reverse-phase RP-C18 Sep-Pak cartridges (Waters Assoc.) were used for both the production of *n*-Bu$_4$N[^{99g}TcO$_4$] and the purification of the radiolabeled species. Biological samples were counted on an LKB Wallace 1282 Compugamma Universal gamma counter.

Syntheses: 2-Methoxyethanethiol (Thiol 2). A mixture of 1-chloro-2-methoxyethane (49 g, 0.52 mol), thiourea (39.45 g, 0.52 mol), and 95% ethanol (250 mL) was refluxed on a steam cone for 24 h. A solution of sodium hydroxide (30 g, 0.75 mol) in water (300 mL) was added, and the mixture was further refluxed for 2 h. During this period the desired thiol separated as an oil. The layers were separated, and the aqueous phase was acidified with dilute sulfuric acid (7 mL of concentrated H_2SO_4 in 50 mL water) and then extracted with one 75 mL portion of diethyl ether. The organic phase was added to the crude thiol layer and dried over anhydrous sodium sulfate (43%): bp 108−110 °C; mass calcd for C_3H_8OS (M^+) *m/z* 92.0296, found 92.03. ^{1}H NMR δ (ppm): 1.56 (t, 1H, S-*H*), 2.68 (dt, 2H, C*H*$_2$-SH), 3.51 (t, 2H, C*H*$_2$-OCH$_3$), 3.36 (s, 3H, O-C*H*$_3$). ^{13}C NMR δ (ppm): 24.2, 58.6, 74.1. Anal. Calcd for (C_3H_8OS): C, 39.1; H, 8.8; S, 34.8. Found: C, 39.8; H, 8.95; S, 34.66.

1-Chloro-2,3-dimethoxypropane (3a). 3-Chloro-1,2-propanediol was dissolved in iodomethane and methylated in the usual manner[14] by addition of silver oxide. The spontaneous reaction was sustained by warming in a water bath; an additional amount of iodomethane was added when the mixture became pasty. The reaction mixture was extracted three times with diethyl ether and the organic solution evaporated to give a clear colorless liquid (54%): bp 156 °C; mass calcd for $C_5H_{11}O_2Cl$ (M^+) *m/z* 138.0447, found 138.04.

2,3-Dimethoxypropanethiol (Thiol 3). 1-Chloro-2,3-dimethoxypropane (**3a**) (14.52 g, 0.105 mol) was added within 25 min to 33% aqueous sodium thiocarbonate[15] (70.88 mL, 0.15 mol) at room temperature. The reaction mixture was then heated at 60 °C and stirred for 5 h (no CS_2 evolution was observed). The basic mixture was treated with diethyl ether and the separated aqueous layer acidified with concentrated HCl, thus promoting the evolution of CS_2. The gas was totally removed by evaporation under reduced pressure. The acidic aqueous layer was treated three times with diethyl ether, and the combined organic extracts were washed with H_2O, dried over anhydrous magnesium sulfate, and evaporated at reduced pressure to give 10 g (70%) of the desired 2,3-dimethoxypropanethiol: bp 88−90 °C at 44 mmHg; mass calcd for $C_5H_{11}O_2S$ (M^+) *m/z* 136.0559, found 136.05. ^{1}H NMR δ (ppm): 1.52 (t, 1H, S-*H*), 2.70 (m, 2H, C*H*$_2$-SH), 3.42 (m, C*H*-OCH$_3$), 3.52 (d, 2H, C*H*$_2$-OCH$_3$), 3.38 (s, 3H, CH$_2$-OC*H*$_3$), 3.44 (s, 3H, CH-OC*H*$_3$). ^{13}C NMR δ (ppm): 25.6, 58.1, 59.7, 72.9, 81.2.

2,2-Dimethyl-4-(chloromethyl)-1,3-dioxolane (4a). Acetone (150 mL, 2.05 mol), 3-chloro-1,2-propanediol (60 g, 0.55 mol), low-boiling petroleum ether (1.30 mL), and *p*-toluenesulfonic acid (1.5 g, 0.009 mol) were placed in a 500 mL three-necked flask equipped with a magnetic stirrer and a fractionating column (2 × 45 cm, packed with glass helices) attached to a reflux phase separating head. The mixture was heated under stirring so that the petroleum ether refluxed as rapidly as the column allowed. The stirring and refluxing were continued for 25 h until no more H_2O was collected in the separating head trap. The mixture was cooled to room temperature, and sodium acetate (1.5 g) was added. The resulting mixture was filtered, and the remaining petroleum ether and acetone were removed from the filtrate by evaporation under reduced pressure. The solution was then distilled and the fraction boiling at 156−158 °C was collected, affording 127.5 g (85%) of the desired product: mass calcd for $C_6H_{11}O_2Cl$ (M^+) *m/z* 150.0448, found 150.05.

2,2-Dimethyl-4-(mercaptomethyl)-1,3-dioxolane (Thiol 4). Compound **4a** (13 g, 0.086 mol) was added dropwise within 25 min to a 33% aqueous sodium thiocarbonate solution (57 mL, 0.12 mol) at room temperature. The mixture was heated under stirring at 60 °C for 5 h, and the resulting alkaline solution was treated once with diethyl ether. The separated layer was acidified with sulfuric acid and then extracted three times with diethyl ether. The combined organic extracts were washed with H_2O, dried over anhydrous magnesium sulfate, and evaporated at reduced pressure to give 7.77 g (61%) of the desired thiol: bp 90−93 °C at 38 mmHg; mass calcd for

$C_6H_{12}O_2S$ (M^+) *m/z* 148.0558, found 148.05. ^1H NMR δ (ppm): 1.47 (t, 1H, S-*H*), 2.68 (m, 2H, C*H*$_2$-SH), 4.21 (quintet, 1H, CH$_2$-C*H*-CH$_2$), 3.77 (dd, 1H, C*H*$_2$(eq)-O), 4.11 (dd, 1H, C*H*$_2$-(ax)-O), 1.36 (s, 3H, C-C*H*$_3$), 1.44 (s, 3H, C-C*H*$_{3'}$). ^{13}C NMR δ (ppm): 25.5, 26.9, 27.6, 68.3, 109.7.

2-(2-Mercaptoethyl)-1,3-dioxane (Thiol 5). 2-(2-Bromoethyl)-1,3-dioxane (25 g, 0.128 mol) in methanol (20 mL) was added dropwise within 1 h to a 33% aqueous sodium thiocarbonate solution (85 mL, 0.182 mol) in methanol (60 mL) under stirring at room temperature. The mixture was allowed to stir for 5 h, during which time essentially no CS_2 formation was observed. The basic reaction mixture was extracted with diethyl ether and the aqueous layer acidified with concentrated HCl, thus promoting the evolution of CS_2 which was removed by evaporation at reduced pressure. The acidic residue was then extracted three times with diethyl ether. The combined ether extract was washed with water, dried over magnesium sulfate, and distilled under reduced pressure to give 10 g (56%) of the desired thiol: bp 60−63 °C at 13 mmHg; mass calcd for $C_6H_{12}O_2S$ (M^+) *m/z* 148.0558, found 148.05. ^1H NMR δ (ppm): 1.39 (t, 1H, S-*H*), 2.62 (dt, 2H, C*H*$_2$-SH), 1.90 (m, 2H, CH$_2$-C*H*$_2$-CH), 4.68 (t, 1H, CH$_2$-C*H*), 3.76 (m, 2H, O-C*H*$_2$), 4.10 (m, 2H, O-C*H*$_{2'}$), 1.33 (m, 1H, CH$_2$-C*H*$_2$(eq)-CH$_2$), 2.07 (m, 1H, CH$_2$-C*H*$_2$(ax)-CH$_2$). ^{13}C NMR δ (ppm): 19.2, 25.7, 39.2, 66.8, 100.4, 187.7. Anal. Calcd for ($C_6H_{12}O_2S$): C, 48.6; H, 8.2; S, 21.6. Found: C, 49.2; H, 8.3; S, 22.0.

2-(3-Chloropropyl)propane-1,3-diol (6a). To a refluxing solution containing diethyl (3-chloropropyl)malonate (42 g, 0.18 mol) and sodium hydroxide (11.09 g, 0.18 mol) in *tert*-butyl alcohol (224 mL) was added methanol (14 mL) in three aliquots over 30 min. The resulting mixture was refluxed for 30 min further and then allowed to cool to room temperature. HCl (5 M) was added with care until the solution became neutral. It was then filtered, and the filtrate was extracted with 2 × 100 mL of ethanol. The ethanolic fractions were combined, and the solvent was removed by rotary evaporation. The residue was extracted again with ethanol (60 mL) and filtered. The solvent was removed to afford 25 g (92%) of the desired product as a colorless liquid.

1-Chloro-4,4-bis(methoxymethyl)butane (6b). Dry silver oxide (153 g, 0.66 mol) was added gradually under mechanical stirring to **6a** (25 g, 0.166 mol) dissolved in iodomethane (188 g, 1.32 mol) and the mixture refluxed for 6 h. Then additional iodomethane (90 g, 0.634 mol) was added and the solution refluxed for another 18 h. After standing at 40 °C overnight, dry diethyl ether was added. The precipitate was filtered off and the filtrate evaporated. The residual oil was distilled to give the desired product (75%): bp 110−115 °C at 12 mmHg.

1-Methoxy-2-(methoxymethylene)pentane-5-thiol (Thiol 6). To a 33% aqueous solution of sodium thiocarbonate (25 mL, 0.053 mol) was added **6b** (6.73 g, 0.037 mol) in methanol (5 mL) dropwise within 1 h with stirring at room temperature. The reaction mixture was heated at 60 °C for 5 h. The cooled solution was acidified with concentrated HCl and extracted three times with diethyl ether. The combined organic fractions were washed with water, dried over anhydrous magnesium sulfate, and distilled to give 4 g (61%) of the desired thiol: bp 105−108 °C at 3 mmHg; mass calcd for $C_8H_{18}O_2S$ (M^+) *m/z* 178.1028, found 178.1031. ^1H NMR δ (ppm): 1.36 (t, 1H, S-*H*), 2.52 (dt, 2H, C*H*$_2$-SH), 1.65 (m, 2H, CH$_2$-C*H*$_2$-CH$_2$), 1.44 (m, 2H, CH$_2$-C*H*$_2$-CH), 1.82 (quintet, 1H, C-*H*), 3.36 (m, 4H, CH-C*H*$_2$-O), 3.33 (s, 6H, O-C*H*$_3$). ^{13}C NMR δ (ppm): 24.8, 27.6, 31.7, 38.9, 58.9, 73.3.

Preparation of "Carrier-Free" ^{99m}Tc Reaction Mixtures. A solution containing a mixture of Na[$^{99m}TcO_4$] (1 mL, 5−20 mCi) and 0.01 M *n*-Bu$_4$NBr (1 mL) was loaded onto an RP-C18 Sep-Pak cartridge previously activated with 95% ethanol (3 mL) followed by 0.01 M *n*-Bu$_4$NBr (3 mL). The cartridge was rinsed with water (20 mL) and the activity collected as *n*-Bu$_4$N[$^{99m}TcO_4$] in ethanol (0.5−1.0 mL) (yield 90−95%). To this ethanolic solution was added 1 M CF_3SO_3H (20−30 μL) (pH = 1−3) in a 5 mL borosilicate vial. The mixture was degassed for 5 min under an Ar stream, and while degassing, a 1% ethanolic DIARS solution (30−50 μL) was added. The vial was quickly capped and placed into an oil bath at 85 °C for 15 min.[16] The reaction mixture was left to cool at ambient temperature, the vial was then opened, and aliquots of the reaction mixture were analyzed by HPLC. The desired thiol (40 μL) was then added to the cooled mixture, and again the capped vial was placed into the oil bath at 85 °C for 10 min. The vial was left to cool at ambient temperature, and aliquots of the reaction mixture were once again tested by HPLC (second step). The two-step reaction procedure was necessary to optimize the yield when thiol **3** was used, whereas in all other cases, both DIARS and thiol were added together in one step to the degassed radioactive solution. The capped vial was placed into the oil bath at 85 °C for 15 min. The reaction mixture was then left to cool at room temperature, and aliquots were again analyzed by HPLC.

Preparation of "Carrier-Added" Reaction Mixtures. A 5 mL borosilicate vial containing an ethanolic solution of *n*-Bu$_4$N[$^{99m}TcO_4$] (0.5 mL, 1−5 mCi) and a 0.02 M ethanolic solution of *n*-Bu$_4$N[$^{99g}TcO_4$] (56 μL) was prepared as described above; then 1 M aqueous CF_3SO_3H (10 μL) was added. The reaction mixture was degassed for 5 min, and while degassing, the appropriate thiol (50 μL) and a 1% DIARS ethanolic solution (120 μL) were added. The vial was quickly capped and placed in an oil bath at 85 °C for 15 min. An intensive blue-violet color always appeared at the end of the reaction. The mixture was left to cool, and aliquots were subjected to HPLC analyses using both radioactive and optical detection modes. The HPLC fraction exhibiting both the correct retention time and the blue absorption was collected, its volume was reduced by rotary evaporation, and finally it was submitted for characterization by positive ion FAB-MS.

Chromatography Conditions. Quality control tests were performed using RP-C8 columns. All HPLC chromatograms were obtained under isocratic conditions using the following mobile phases: (i) 85/15 methanol/water solution containing 0.01 M sodium heptanesulfonate in total volume at a flow rate of 1.5 mL/min (MP1) and (ii) 78/22 ethanol/water solution containing 0.01 M ammonium acetate in total volume at a flow rate of 1.5 mL/min (MP2).

Comparative Lipophilicity Study. All ^{99m}Tc chelates were subjected to HPLC analysis under identical chromatographic conditions. The MP2 conditions outlined above were selected for this experiment.

Purification Procedures: [^{99m}Tc(DIARS)$_2$(SR$_1$)$_2$]$^+$. Three milliliters of water was added to the "carrier free" reaction mixture (*ca.* 1 mL in ethanol) and the content of the vial loaded onto a reverse-phase Sep-Pak C18 cartridge (Waters Assoc.) previously activated with 10% ethanol (10 mL). The cartridge was washed with water (5 mL), 1/2 ethanol/water (20 mL), 1/1 ethanol/water (10 mL), and ethanol (1 mL). The desired cationic complex eluted with 0.13 M lithium trifluoromethanesulfonate in ethanol (1 mL). The filtrate was diluted with physiological saline (9 mL) resulting in a final 10% ethanolic solution suitable for iv injection.

[^{99m}Tc(DIARS)$_2$(SR$_2$)$_2$]$^+$. Nine milliliters of saline was added to the "carrier free" preparation, and the content of the vial was loaded onto a reverse-phase Sep-Pak C18 cartridge (Waters Assoc.) previously activated with 10% ethanol (5 mL). The cartridge was rinsed with water (10 mL) followed by 50% ethanol (10 mL) and ethanol (2 mL). The desired ^{99m}Tc species eluted with 0.13 M lithium trifluoromethanesulfonate in ethanol (1 mL). The activity was diluted with physiological saline (9 mL) to give a 10% ethanolic solution ready for injection.

[^{99m}Tc(DIARS)$_2$(SR$_3$)$_2$]$^+$. A reverse-phase Sep-Pak C18 cartridge (Waters Assoc.) was activated with 10% ethanol (10 mL), and the activity from the "carrier free" preparation previously diluted with physiological saline (9 mL) was loaded onto it. The cartridge was washed with water (10 mL), 50% ethanol (7 mL), and ethanol (2 mL). The ^{99m}Tc complex was collected with 0.13 M lithium trifluoromethanesulfonate in ethanol (1 mL). The filtrate was diluted with physiological saline (9 mL) to obtain a 10% ethanolic solution suitable for injection.

[^{99m}Tc(DIARS)$_2$(SR$_5$)$_2$]$^+$. A reverse-phase Sep-Pak C18 cartridge (Waters Assoc.) was activated by passing 10%

ethanol (5 mL). To the radiolabeled mixture (1 mL) was added physiological saline (9 mL), and the result was loaded onto the cartridge. After rinsing with water (10 mL) and 50% ethanol (5 mL), the activity was collected by eluting 0.13 M lithium trifluoromethanesulfonate in ethanol (1 mL) through the cartridge. The filtrate was then diluted with physiological saline (9 mL) to obtain a 10% ethanolic solution ready for injection.

$[^{99m}Tc(DIARS)_2(SR_6)_2]^+$. This ^{99m}Tc species was purified before injection by HPLC (chromatographic conditions MP2 specified above). The isolated fraction containing $[^{99m}Tc(DIARS)_2(SR_6)_2]^+$ was concentrated by rotary evaporation and the residue brought to the desired volume by adding physiological saline to obtain a final 10% ehanolic solution.

Animal Studies. Female Sprague−Dawley rats (200 ± 20 g), anesthetized under metophan inhalation, were injected intravenously (*via* the jugular vein) with 0.15 mL of a purified ^{99m}Tc complex (30−50 μCi) in 10% ethanol. At 5, 15, and 30 min after injection, groups of at least three rats were sacrificed by cervical dislocation. At 60 and 120 min after injection, additional groups of animals were sacrificed by CO_2 asphixiation. Immediately after death, a blood sample (ca. 1 mL) was withdrawn from the heart. The organs of interest were subsequently excised, weighed, and assayed for ^{99m}Tc content relative to appropriate blank samples and standards. The heart/blood and heart/liver ratios were calculated from the respective percent dose/gram values.

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(16) The temperature must be strictly controlled. In fact, higher temperatures cause formation of a stable chemical impurity having a longer retention time with respect to both Tc(V) and Tc(III) species and is tentatively assigned to be $[Tc^I(DIARS)_3]^+$. See ref 2b.

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