Final Report

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A. Particulars

- I. Award No.: DE-FG02-93ER61571
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- III. Institution: Department of Chemistry, Syracuse University
- IV. Title: The Coordination Chemistry of Technetium and Rhenium and Applications to Nuclear Medicine.

B. The Role of Coordination Chemistry of Technetium in the Development of

Radiopharmaceuticals. While ^{99m}Tc -based radiopharmaceutical agents and their targets display a remarkable diversity, an understanding of the underlying coordination chemistry of technetium is pivotal to their future development. The structural characterization of the Tc-ligand complex is fundamental both to the understanding of the chemical and physical properties of the agent and to the design of novel ligands possessing the appropriate donor atoms, complex stability, and three-dimensional structure to allow specificity in targeting. Consequently, investigations on the macroscopic ^{99}Tc level of the structural chemistry of such complexes is crucial in the assessment of those characteristics which influence their relevant chemical and physical properties. The proposed research will focus on the development of broadly interrelated aspects of the coordination chemistry of technetium of relevance to bifunctional linker design, and to the general development of ligand types for specific binding. The analogous chemistry of rhenium will be developed with a view to understanding the structural correlation to technetium.

C. WORK IN PROGRESS.

This work evolves from the demonstration that bifunctional hydrazines can be used for the properties of ^{99m}Tc labeled protein conjugates. Since the elaboration of the coordination chemistry is driven by the studies on the radiolabeled peptide derivatives, a summary of the most recent results of our collaborators on these systems will be reviewed, with an emphasis on those observations which require development of the ligand or core functional group chemistry of Tc and Re.

C.I. ^{99m}Tc -Labeled Chemotactic Peptide Derivatives. The results of our collaborators' investigations cited in Section B.II.3 have addressed several significant areas in the elaboration of these reagents including: (a) synthesis and chemical characterization of chemotactic peptide receptor agonists and antagonists that are suitable for ^{99m}Tc labeling; (b) *in vitro* characterization of the peptides with respect to bioactivity and receptor binding; (c) radiolabeling with ^{99m}Tc ; (d) *in vivo* studies of the effects of chemotactic peptides on leukocyte levels in monkeys; (e) biodistribution and gamma camera imaging studies of focal sites of *E. coli* infection in rats, rabbits and dogs; and (f) the mechanisms by which radiolabeled chemotactic peptide analogues localize at

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sites of infection. Several more recent observations which will serve to drive a significant fraction of the coordination chemistry are presented in more detail.

C.I.1. Effect of Co-Ligand on Infection Imaging and Biodistribution in Rabbits. The ^{99m}Tc -complex (co-ligand) used for radiolabeling can have profound effects on biodistribution. In this study, the distribution of molecular species formed with a variety of "co-ligands" was correlated with infection localization and biodistribution in *E. coli* infected rabbits.

Methods: Five ^{99m}Tc -co-ligand complexes (mannitol, glucamine, glucarate, tricine and glucoheptonate) were used to label f-MLFK-HYNIC and radiolabeled species were characterized by reverse phase HPLC. One mCi of each ^{99m}Tc -co-ligand-peptide complex was injected into rabbits 24h after infection and imaging was performed 3-4 and 16-17 hrs later. After acquiring the final images, the animals were sacrificed and biodistribution was measured.

Results: With all 5 co-ligands, ^{99m}Tc labeled peptide was obtained in > 90% radiochemical purity. Multiple radiolabeled species were obtained with each reagent, however, mannitol yielded the most homogeneous product. Image analysis demonstrated that, ^{99m}Tc -mannitol-f-MLFK-HYNIC produced the highest target to background ratios (T/B). At 16-17 hrs, the T/B's were: 3.91 ± 0.99 , 6.15 ± 1.07 , 2.8 ± 1.07 , 2.57 ± 1.07 , 3.41 ± 1.07 , and 1.8 ± 1.07 at 3-4 h and 11.9 ± 0.99 , 3.94 ± 1.07 , 3.79 ± 1.07 , 4.81 ± 1.17 , 7.0 ± 1.31 and 5.32 ± 1.07 at 16-17, for mannitol, tricine, glucamine, glucarate-a, glucarate-b, and glucoheptonate, respectively. At both imaging times, ^{99m}Tc -mannitol-f-MLFK-HYNIC had the lowest relative levels of accumulation in bowel.

Biodistribution measurements demonstrated that the mannitol preparation had the highest level of accumulation (%ID/g) in infected tissue; mannitol > glucoheptonate = glucarate-b > glucarate-a > glucamine > tricine; $p < 0.01$. The infected to normal muscle ratio for ^{99m}Tc -mannitol-f-MLFK-HYNIC was ~50:1. **Conclusions:** These results support our previous findings in rats that co-ligands can markedly effect the biodistribution and infection localization of ^{99m}Tc labeled HYNIC chemotactic peptides. For infection imaging, the mannitol preparation had the most favorable combination of: accumulation in infected tissue, T/B ratio and biodistribution in uninfected organs.

Figure 1: Radio-HPLC chromatogram of ^{99m}Tc -glucarate-f-MLFK-HYNIC preparation showing both radioactivity and UV traces: a: ^{99m}Tc -glucarate-a-f-MLFK-HYNIC, b: ^{99m}Tc -glucarate-b-f-MLFK-HYNIC, c: ^{99m}Tc -glucarate, d: $^{99m}\text{TcO}_4^-$, e: unlabeled f-MLFK-HYNIC.

Figure 2: Representative anterior images of the infection site in rabbits at 17 hours after injection of f-MLFK-HYNIC radiolabeled with ^{99m}Tc -mannitol (A) and ^{99m}Tc -tricine (B). The animals were infected 24 hours before radiopharmaceutical administration. Intense bowel accumulation is noted with tricine as well as a less intense infection localization as compared with the mannitol prep (A).

Figure 3: Gamma camera images of a rabbit at 5 (left) and 15 (right) hours after injection of ^{99m}Tc -HP2 radiolabeled using mannitol as coligand.

C.I.1.1. Optimization of Sn(II)-Mannitol Formulation to Control Radiolabeled Speciation.

As discussed above, the nature of the starting Tc-species used to label Formyl-M-L-F-K-HYNIC significantly influences the biological fate and infection localizing characteristics of the peptide conjugate. To date, Tc-mannitol appears to offer significant advantages over several other coligands we have studied with regard to the number of radiolabeled species generated, normal tissue distribution and infection localization. As seen with other polyhydric ligands, the reaction of Tc-mannitol with formyl-ML-FK-HYNIC generates several radiolabeled peptide species. This is the likely result of formation of isomers between Tc and the polyhydric ligand. As our objective is to produce a clinically useful tracer for infection imaging, we undertook a study to optimize the Sn(II) Mannitol formulation so as to achieve rapid and quantitative peptide labeling while limiting the number of radiolabeled species generated.

Mannitol concentration, Sn(II) concentration and pH were studied as a function of time to determine the effect on labeling yield and speciation, as determined by reverse phase HPLC (0.1% TFA in a MeCN varying concentrations of mannitol [80-0.10 mg/ml] and a fixed amount of $\text{SnCl}_2/\text{H}_2\text{O}$ [100 ug/ml] at pH 5.0-5.2. The effect of $\text{SnCl}_2/\text{H}_2\text{O}$ concentration was studied at 80 mg/ml and 10 mg/ml mannitol at pH 5.0-5.2. Kits contained 0.5 cc of the Sn-mannitol solution and Tc-mannitol was prepared by addition of 2.0 ml NaTcO_4 . Formation of Tc-mannitol was monitored by radio-TLC using ITLC-sg eluted with acetone ($R_f = 0.0$) and saline ($R_f = 1.0$).

The rapid formation of labeled peptide was favored at low concentrations of mannitol [≤ 2.5 mg/kit] with 2 predominant Tc-peptide peaks. However, at mannitol concentrations ≤ 0.5 mg/kit, greater than 80% of the Tc-peptide was found in the more hydrophobic peak. Sn(II) concentrations did not affect labeling yields or speciation at mannitol concentrations of 0.5 mg/kit but an inverse correlation was found between Sn(II) concentration and labeling yield at mannitol

concentrations of 40 mg/kit. These results indicate the optimal formulation of Sn(II)-mannitol to be 0.5 mg/kit mannitol 50 ug/kit $\text{SnCl}_2/\text{H}_2\text{O}$ at pH 5.0-5.2.

FIGURE 4: RP-HPLC showing several radiolabeled.

FIGURE 5: RP-HPLC showing several radiolabeled species.

C.I.1.2. Pyridinethiols and Pyrimidinethiols (PT) as Co-Ligands for Tc-99m Labeled Hydrazinonicotinamide (HYNIC) Conjugated Peptides. As previous studies have demonstrated, Tc-99m-mannitol gives superior imaging results when used as the starting species (or coligand) to label For-MLFK-HYNIC, as compared with other polyhydric 'coligands'. However, radio-HPLC indicated the presence of several $^{99\text{m}}\text{Tc}$ -peptide species. We are interested in producing $^{99\text{m}}\text{Tc}$ -HYNIC-peptides as homogenous radiochemical species in an effort to simplify formulation and mechanistic studies. Previous work at the 'carrier' level in our laboratory showed these ligands form stable and reproducible complexes with Tc-hydrazinopyridine. To this end, the potential of pyrimidinethiols and pyridinethiols as coligands was investigated.

The Tc-mannitol-HYNIC-peptide was converted to the corresponding pyrimidine-2-thiol, 4-methyl-pyrimidine-2-thiol(P2T), pyridine-2-thiol, and 3-trimethylsilyl-pyridine-2-thiol containing complexes. Formyl-MLFK-HYNIC was labeled by incubating equal volumes of $^{99\text{m}}\text{Tc}$ -mannitol with peptide [20 $\mu\text{g}/\text{ml}$] in 50mM acetate buffer pH 5.2 for 1 hr. Labeling was determined by reverse phase-HPLC eluted with 0.1% TFA and a water - acetonitrile gradient. Conversion to the PT containing complex was accomplished by incubation of equal volumes of PT [1 mg/ml DMSO] and Tc-peptide at room temperature for 1 hr.

For all PTs, HPLC analysis showed the formation of unique hydrophobic species with retention times greater than the Tc-mannitol labeled peptide. In the case of P2T a primary

homogenous peak was obtained which when isolated and re-chromatographed eluted at the same R_f . Initial imaging experiments with this complex were performed in rabbits with *E. coli* infections in the left posterior thigh. 24 hrs after infection, rabbits ($n = 3$) were injected i.v. with 0.5 mCi of Tc-P2T-peptide and imaged at 4 and 18 h p.i. The animals were sacrificed at 18 h and tissue activity determined. ROI analysis gave target/background of 4.26 ± 0.97 and 9.83 ± 1.6 , at 4 and 18 h, respectively. Tissue counting (18 h) resulted in pus:normal muscle $>150:1$ and infected muscle:normal muscle $>50:1$. At 4 and 18h GI accumulation was evident. PTs offer excellent infection localization, however further work is necessary to limit GI accumulation. *In summary, the PT's provide improved radiochemical formulations by limiting the radioactive species present, as compared to polyhydric ligands.*

Figure 6. A to C. Shows that addition of the thiol ligands to the preformed Tc-mannitol-peptide complex results in a Tc-complex with retention times greater than the Tc-mannitol labeled peptide. Radio-HPLC chromatograms of (A) Tc-99m-mannitol-For-MLFK-HYNIC, (B) Tc-99m-pyrimidine-2-thiol and (C) Tc-99m-mannitol-For-MLFK-HYNIC after incubation with pyrimidine-2-thiol. (D) and (E): Gamma camera images of Tc-99m-[pyrimidine-2-thiol]-peptide in infected rabbits at 3h (A) and 18h (B) post injection. The infected thigh muscle can clearly be delineated at 3 h and the activity in the infected thigh is intense at 18h.

C.I.1.3. N,N-Bis(2-mercaptoethyl)methylamine (NS2): A New Co-Ligand for ^{99m}Tc Labeling of Hydrazino-Nicotinamide (HYNIC) Peptides. With the aim of limiting radiochemical speciation of ^{99m}Tc -HYNIC-peptides radiochemical species with more predictable radiochemical and biological properties we previously investigated bidentate aminothiols, pyrimidinethiols and pyridinethiols, as coligands. These ligands resulted in an improved radiochemical formulation by limiting the number of radiochemical species observed with reverse phase HPLC. However, the use of these ligands resulted in undesirable significant GI tract accumulation of radiolabel

Tridentate ligands, such as NS2, may constrain the possible coordination geometries and improve overall stability. To investigate this, we synthesized NS2, converted the Tc-mannitol-For-MLFK-HYNIC to the corresponding NS2 containing complex (Tc-NS2-For-MLFK-HYNIC) and compared its infection imaging and biodistribution properties with Tc-mannitol-For-MLFK-HYNIC. Formyl-MLFK-HYNIC was labeled by incubating equal volumes of Tc-99m-mannitol with peptide [20 $\mu\text{g}/\text{ml}$] in 50mM acetate buffer pH 5.2 for 1 hr. Conversion to the NS2 containing complex was accomplished by addition of NS2 [1 mg/ml DMSO] to the Tc-mannitol-For-MLFK-HYNIC solution at room temperature. HPLC showed a single unique hydrophobic species with retention time greater than the Tc-mannitol-For-MLFK-HYNIC. Imaging experiments with this complex were performed in rabbits with *E. coli* infections in the left thigh. 24 hrs after infection, rabbits ($n = 6$) were injected i.v. with 0.5 mCi of Tc-NS2-peptide or Tc-mannitol-peptide and imaged at 4 and 18 h p.i. The animals were sacrificed at 18 h and tissue activity determined.

ROI analysis gave target/ background ratios of 3.91 ± 0.32 and 11.89 ± 2.21 for ^{99m}Tc -mannitol-For-MLFK(HYNIC) and 3.93 ± 0.69 and 22.40 ± 5.52 for ^{99m}Tc -NS2-For-MLFK(HYNIC) at 3 and 18 h. Tissue radioactivity measurements (18h) demonstrated that compared to Tc-mannitol-peptide, accumulation of Tc-NS2-peptide was lower in blood, heart and normal muscle and higher in spleen, infected muscle and pus ($p < 0.01$). There was no significant difference in accumulation into the GI tract. These results indicate that the Tc-NS2-peptide complex is more chemically homogenous than the Tc-mannitol peptide and exhibits substantially improved infection localization and biodistribution properties.

C.I.2. HYNIC-Maleimide, a Thiol-Specific Linker for Labeling Peptides with ^{99m}Tc : Conjugation, Labeling and In Vivo Evaluation. Currently, the chemistry of attachment to proteins and peptides involves the acylation of primary amino groups via the N-hydroxysuccinimidyl ester of HYNIC (SHNH). Since many peptides of biological interest possess more than one primary amino group, use of SHNH for conjugation can lead to heterogeneous products. In some cases, this lack of site-specificity may lead to derivatization of residues critical for biological activity. To overcome these limitations we have developed a method which allows site directed conjugation of a stable ^{99m}Tc chelating agent. The method makes use of a novel HYNIC-maleimide (HM) bifunctional linker and free thiols, of Cys residues at non-critical sequence positions. The utility of HM was tested by conjugation to a chemotactic peptide analog, For-MLFC, labeling via ^{99m}Tc -mannitol and comparison of its infection imaging and biodistribution properties with For-MLFK-HYNIC, in a rabbit model of infection. Sites of infection were produced in New Zealand white rabbits ($n = 6/\text{peptide}$) by injection of a suspension of *E. coli* in a posterior thigh. 24 hrs after infection, the rabbits were injected i.v. with 0.5 mCi of HPLC purified ^{99m}Tc -peptide and imaged at 3 and 18 h p.i. The animals were sacrificed at 18h and tissue activity determined. At 3 and 18h p.i., the organ distribution of both preparations was qualitatively similar, with high accumulation in liver, spleen and marrow and minimal bowel accumulation. ROI analysis gave target/background ratios of 3.91 ± 0.32 and 11.89 ± 2.21 for For-MLFK(HYNIC) and 3.67 ± 0.47 and 11.18 ± 0.89 for For-MLFC(HM), at 3 and 18 h, respectively. Tissue radioactivity measurements (18h) demonstrated that compared to For-MLFK(HYNIC), the accumulation of For-MLFC(HM) was greater in liver, spleen and marrow and lower in heart, kidney, adrenal, stomach, normal muscle and testes ($p < 0.01$). However, there was no significant difference in accumulation into GI tract, infected muscle or pus. Reverse-phase HPLC indicated that HM increases peptide lipophilicity as compared with SHNH, which may account for minor differences in biodistribution. These results indicate that the HYNIC-maleimide bifunctional chelator can be successfully used to incorporate HYNIC into a Cys containing peptide. This derivative can be readily labeled with Tc-99m and exhibits *in vivo* characteristics similar to peptide conjugated with HYNIC.

C.I.3. Summary and Significance. The results to date demonstrate that structural modifications to the peptide and its radiolabeled analog can be made so that alterations in pharmacological characteristics (i.e. potential unwanted side effects) and localizing characteristics can be improved over the initial peptides and their radiolabeled complexes. This is significant because it demonstrates the feasibility of developing a diagnostic peptide analog with low potential for side effects and improved localizing ability.

The structural modifications to the peptide MLF included both amino-terminal and carboxy-terminal modifications. Amino terminal modifications being critical for receptor binding and activation characteristics and the carboxy terminus being important for chemical modification to produce radiolabeled analogs. The peptide was able to tolerate these modifications and

maintain biological and receptor binding characteristics. This finding is critical for development of a successful diagnostic drug.

Studies of the influence of coligand on the biological fate of the radiolabeled peptide complex is also significant. While it is important to preserve receptor binding affinity (as described above) receptor affinity alone will not predict localizing properties nor will its assure good localization. The demonstration that the coligand can markedly influence the distribution of the peptide complex in various tissues suggests that peptides which have good binding affinities can be prepared with suitable coligands to 'tailor' their distribution, thereby enhancing signal to noise of the tracer. This is a novel finding which we believe is a direct effect of utilizing hydrazinonicotinamide as the radiometal chelating group.

C.II. Coordination Chemistry of Technetium and Rhenium. The work evolves from our demonstration that bifunctional hydrazines can be used for the preparation of ^{99m}Tc labeled protein conjugates. However, despite the manifest potential of bifunctional organohydrazino ligands in protein radiolabeling for nuclear medicinal applications, the nature of the metal coordination to the HYNIC linker has not been elucidated. In order to define some structural possibilities for the coordination chemistry relevant to these reagents, we investigated the chemistry of Tc and Re with hydrazinopyridine, which not only established the formation of the robust $\{M=NNR\}$ core, but also implicated chelate formation through the pyridine nitrogen as a significant structural determinant.⁵⁸

During the course of these studies, we also noted that the identity of the coligands coordinated to the Tc site may profoundly influence the structures of complexes with the Tc-hydrazido core, $\{TcNNR\}$, and the radiochemical yield of the protein conjugate. Sulfur ligands were particularly effective in the stabilization of the Tc-hydrazido core as a consequence of the high affinity of Tc for sulfur donors. These observations suggested a systematic study of Tc and Re coordination chemistry with hydrazine ligand types and with thiolate ligands both as coligands in the preparation of hydrazido-linked protein conjugates and for the development of bifunctional polythiolate ligands.

C.II.1. Technetium and Rhenium Coordination.

C.II.1.1. Models for $\{Tc\text{-(coligand)-HYNIC-peptide}\}$ Radiopharmaceuticals. In an effort to model the interaction of Tc and Re precursors with the hydrazinonicotinamide unit of the most effective linker (HYNIC), the reactions of perrhenate with hydrazinopyridine were investigated. Significantly, hydrazinopyridine acts as **both ligand and reductant** to give the compound $[MCl_3(HNNpy)(NNpyH)]$ ($M = \text{Tc (1a), Re (1b)}$) in quantitative yield. While the participation of the pyridyl nitrogen in chelate formation is significant in complex stabilization, it is also noteworthy that the protonation pattern excludes the hydrazido(2-) unit as a contributor to the overall structure. Furthermore, the preferential protonation of the α -nitrogen and the pyridyl nitrogen establishes the relatively weak basicity of the β -nitrogen site.

These complexes are stable in organic solvents and in mixed organic/aqueous media,⁵⁹ demonstrating the robust nature of the metal-organohydrazine core which extends to a variety of substituents such as the

hydrazinoimidazoles of which $[\text{ReCl}_3(\text{NNC}_3\text{H}_4\text{N}_2\text{H})(\text{HNNHC}_3\text{H}_4\text{N}_2\text{H})]$ (2) is representative. However, detailed synthetic and structural investigations of this class of compounds revealed a complex coordination chemistry, manifested in both monodentate terminal linkage and chelation by organohydrazine ligands and by several distinct patterns for the relative orientations of the ligands within the $\{\text{M}(\eta^1\text{-NNR})(\eta^2\text{-HNNR})\}$ cores, which reflect the metal electronic requirements and the degree of ligand protonation. This latter observation is illustrated by the structure of $[\text{HNEt}_3][\text{ReCl}_3(\text{NNC}_5\text{H}_4\text{N})(\text{HNNC}_5\text{H}_4\text{N})]$ (3). Thus, it is not surprising that the labeled protein conjugates are mixtures of materials as determined by radio-HPLC. While the chemistry of the pyridylhydrazino ligand demonstrates the facility of complex formation and the robust nature of such materials, it also suggests a variety of coordination modes for the radiopharmaceutical materials.

Since formulation and mechanistic studies of $^{99\text{m}}\text{Tc}$ -HYNIC-peptide radiochemical species require homogeneous materials with favorable pharmacokinetics, the synthesis of high purity compositions required some attention. The addition of thiol ligands such as pyrimidine -2-thiol resulted in complexes exhibiting a primary homogeneous peak, which when isolated and rechromatographed eluted at the same t_R .

In order to provide a structural basis for such radiopharmaceutical preparations, the chemistry of the $\{\text{M}(\eta^1\text{-NNR})(\eta^2\text{-HNNR})\}$ core with thiolate coligands was investigated. The prototypical structure is represented by $[\text{Re}(\text{HNNpy})(\text{NNpyH})(\eta^2\text{-SC}_5\text{H}_4\text{N})(\eta^1\text{-SC}_5\text{H}_4\text{N})]$ (4). However, it was subsequently noted that while Tc-chemotactic peptide radiochemical species using pyridinethiol and pyrimidinethiol coligands exhibited excellent infection localization, the GI uptake would hinder the use of such a ligand system.

Further elaboration of the coligand theme demonstrated that tridentate dithiolamine ligand types not only provide more homogeneous radiopharmaceutical preparations with improved stability, but also exhibited rapid localization and retention at the site of infection, constant or decreased concentrations in normal tissues, and no adverse biological effects.⁶⁰ Structural models for the metal coordination geometry were developed and $[\text{M}(\text{NNpyH})(\text{HNNpy})\{\text{SCH}_2\text{CH}_2\text{N}(\text{Me})\text{CH}_2\text{CH}_2\text{S}\}]$ ($\text{M} = \text{Tc}$ (5a), Re (5b)) and $[\text{Re}(\text{NNpyH})(\text{HNNpy})\{(\text{SCH}_2)_2\text{C}_6\text{H}_3\text{N}\}]$ (6) provide representative examples.

C.II.1.2. "Technetium Essential" Complexes. The neutral character, lipophilicity, and small molecular weight of the model complexes suggested an evaluation of their biodistributions with a view toward brain-imaging agents. While Tc-hydrazido core complexes of the types discussed above did not exhibit useful brain uptake properties, thiolate complexes of the Tc-oxo and Tc-imido cores, $\{\text{Tc}=\text{O}\}$ and $\{\text{Tc}=\text{NR}\}$, respectively, traverse the blood-brain barrier and accumulate in the brain. In designing these materials, we have exploited the "3 + 1" concept of using a tridentate dithiolate with a -2 charge in combination with a monothiol of -1 charge to preserve the oxo-metal core and the formal +5 oxidation state of the metal.^{61, 62} Representative structures are provided by $[\text{MO}(\text{SCH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{S})(\text{SCH}_2\text{C}_6\text{H}_4\text{X})]$ ($\text{M} = \text{Tc}$ (7a), Re (7b)) and $[\text{ReO}(\text{SCH}_2\text{CH}_2\text{SCH}_2\text{CH}_2\text{S})\{\text{SCH}_2\text{CH}(\text{OH})\text{CH}(\text{OH})\text{CH}_2\text{SH}\}]$ (8). The pendant arm of such structure types may, of course, be functionalized to link metal centers into binuclear complexes, such as $[\text{Re}_2\text{O}_2(\text{SCH}_2\text{CH}_2\text{SCH}_2\text{CH}_2\text{S})_3]$ (9).

Since the monothiolate ligand may be derivatized at will, the "3 + 1" concept may also be exploited for the synthesis of cationic species, such as $[\text{ReO}(\text{SCH}_2\text{CH}_2\text{SCH}_2\text{CH}_2\text{S})(\text{SCH}_2\text{CH}_2\text{NH}_3)]^+\text{Cl}^-$ (10). This observation suggests a route to potential heart-imaging agents. These themes will be further elaborated in the following sections.

C.II.2. Synthesis of Ligands.

C.II.2.1. *Bifunctional Polythiolates*. We have observed that functionalized thiols add rapidly and in excellent yield under free radical conditions to mono, tris and tetravinyl silanes. Encouraged by these results, we have successfully prepared the polythiolate $\text{HOOCCH}_2\text{Si}(\text{CH}_2\text{CH}_2\text{SH})_3$ (11) by hydrosilation of a functionalized alkene using trichlorosilane, replacement of the chlorines by vinyl groups, free radical additions of protected thiol equivalents and deprotection.

An alternative approach to potential linkers, is provided by the NS_3 tripodal ligands.⁶³

We have also developed a new reagent for peptide labeling with $^{99\text{m}}\text{Tc}$, 6-mercaptomethyl pyridine-3-carboxylic acid (MEMNIC). As shown below (Schematic 3), the N-epsilon MEMNIC derivative of for-MLFK was prepared using the N-hydroxyniccinimidyl ester of MEMNIC. Biodistribution and imaging studies of $^{99\text{m}}\text{Tc}$ labeled for-MLFK-MEMNIC exhibit lower accumulation in most organs and normal muscle compared to for-MLFK-HYNIC and good infection localization. These results indicate that MEMNIC and related thiolate-based linkers can be effective for $^{99\text{m}}\text{Tc}$ labeling of peptides.

Schematic 3. Preparation of the MEMNIC-Chemotactic Peptide Derivative.