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Technical Report

Title: The Coordination Chemistry of Technetium and Rhenium and Applications to Nuclear Medicine

Principal Investigator: Jon Zubieta, Syracuse University

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Summary

The work focuses on the development of the coordination chemistry of transition metals, with radionuclides of potential use in nuclear medicine, with new chelating reagents and bifunctional ligands. The research has evolved from our preparation of stable ^{99m}Tc -protein conjugates using bifunctional hydrazine ligands to an exploration of analogous Re-containing preparations. The dramatic dependence of Re-binding to functionalized hydrazines on the nature of the coligands associated with the metal site has led to an examination of alkoxy and thiolato ligand types. The observation that multidentate thiolate ligands are highly effective in this latter role suggests the design and synthesis of new chelating polythiolate ligands with high binding affinities for radiometallic nuclides and of functionalized polythiolate ligands for the preparation of radionuclide-conjugated antibodies or peptides and the preparation and characterization of their complexes not only with the Group 7 metals Tc and Re but with the Group 13 metals Ga and In, which also possess radionuclides with useful properties. The long-term goal of this research is to develop methodologies for the synthesis of functionalized polythiolate-protein conjugates which may be labelled with radionuclides with high sulfur affinities, in high yield under mild conditions with significant *in vitro* and *in vivo* stabilities. Since the rational design of these novel bifunctional conjugates requires a fundamental understanding of the coordination chemistry of relevant metals with polythiolate ligands whose syntheses and properties remain unexplored, a significant component of the research effort will be directed toward the preparation of novel polythiolate ligands and the characterization of their complexes with Group 7 and Group 13 metals. The first year of the grant period has focussed on several complementary aspects of the coordination chemistry: (i) the pyridinethiolate derived ligands 2-HSC₅NH₃-3-SiR₃, 2-HSC₅NH₃-6-SiR₃ and 2-HSC₅NH₂-3,6-SiR₃ and the potentially bifunctional polythiolate ligand HOOCCH₂Si(CH₂CH₂SH)₃ have been synthesized; (ii) several aspects of rhenium-hydrazido and rhenium-thiolate chemistry have been developed; (iii) Ga and In complexes of the 2-pyridinethiolate class of ligands have been synthesized and structurally characterized; (iv) the general coordination properties of the 2-pyridinethiolate and related ligand types have been investigated.

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General Considerations

The work evolves from our demonstration that bifunctional hydrazines can be used for the preparation of ^{99m}Tc labelled protein conjugates (Scheme 1). During the course of these studies, we noted that the identity of the coligands coordinated to the Tc site may profoundly influence the structures of complexes with the Tc-hydrazido {TcNNR} core 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 thiolate ligands both as coligands in the preparation of hydrazido-linked protein conjugates and for the development of bifunctional polythiolate ligands.

A. Ligand Synthesis

(i) *Pyridine-2-thiol derivatives.* The synthesis of the 3-(triorganosilyl)pyridine-2-thiol series exploits the utility of the mercapto group in directing *ortho*-lithiation in carbocyclic and heterocyclic aromatic substrates. In this fashion, the reaction of 2-pyridinethiol with trimethylsilyl chloride in thf with lithium diisopropylamide (LDA) yielded 3-(trimethylsilyl)-2-pyridinethiol. Under similar reaction conditions, more hindered chlorosilanes yielded mixtures

of 6- and 3,6-disubstituted 2-pyridinethiols which could be readily separated by column chromatography or by oxidation to the corresponding disulfides (**1** and **2**), followed by fractional crystallization and acidification.

Scheme 1

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(ii) *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 $\text{HOOCCH}_2\text{Si}(\text{CH}_2\text{CH}_2\text{SH})_3$ (**3**) by hydrosilation of a functionalized alkene using trichlorosilane, replacement of the chlorines by vinyl groups, free radical addition of protected thiol equivalents and deprotection.

B. Tc and Re Coordination Chemistry

(i) *Re-catecholate and Re-hydrazido chemistry.* We have observed in a fashion analogous to protein labelling by $^{99\text{m}}\text{Tc}$ that radiochemical yields of greater than 80% of ^{186}Re labelled nicotinyldiazine modified IgG (as determined by HPLC and TLC) may be obtained. However, the yield is highly dependent on the nature of the chelating ligand introduced in the preliminary reduction of $[\text{ReO}_4]^-$ by stannous ion. The most effective ligand has proved to be catechol. This observation led us to examine the Re O-catecholate chemistry and the reactions of these species with organohydrazines.

In view of some rather muddled reports in the literature, it was necessary to reinvestigate the fundamental Re-oxo-catecholate chemistry. We have unambiguously identified the complexes $[\text{ReO}(\text{catecholate})_2]^{1-}$ (**4**) and $[\text{ReO}(\text{catecholate})_2(\text{PPh}_3)]^{1-}$ (**5**). Upon reaction with organosubstituted hydrazines, **4** yields the complexes $[\text{Re}(\text{NNPh}_2)_2(\text{cat})_2]^{1-}$ (**6**) and $[\text{Re}(\text{NNPh})_2(\text{cat})_2]^{1-}$ (**7**), which exhibit the conventional hydrazido(2-) and diazenido(1+) substitution patterns, respectively. The isolation and characterization of **5** and **6** suggest that the

formation of the ^{186}Re containing protein conjugates involves chemistry analogous to that observed for the $^{99\text{m}}\text{Tc}$ conjugates.

Treatment of **7** with $\text{C}_6\text{H}_5\text{Me}/\text{MeOH}$ on silica gel yielded the water soluble Na^+ salt **8**, which exhibits the unusual solid state structure shown in the Figure.

The introduction of other chelates as coligands stabilizes a variety of structural types, as indicated by the tris(pyrazolyl)borate complex $[\text{HB}(\text{pz})_3\text{ReO}(\text{hydralazine})]$ **9**. The tris-(pyrazolyl)borate ligand appears to be particularly effective in stabilizing a variety of Re-hydrazido and Re-oxo-hydrazido species, and further investigations are in progress.

(ii) *Rhenium-thiolate chemistry*. Preliminary investigations of a variety of Re precursors with ligands of the pyridine-2-thiol family reveal an extensive chemistry. The reaction of $[\text{ReOBr}_4(\text{OPBu}_3)]^{1-}$ with pyridine-2-thiol yields the unusual neutral six-coordinate complex $[\text{ReO}(\mu^2\text{-SC}_5\text{NH}_4)_2(\mu^1\text{-SC}_5\text{NH}_4)]$ (**10**). In contrast, reaction of $[\text{ReOBr}_4(\text{OPBu}_3)]^{1-}$ with $\text{HSC}_5\text{NH}_2\text{-3,6-SiR}_3$ yields the oxo-hydroxo core species $[\text{ReO}(\text{OH})(\text{SC}_5\text{NH}_2\text{-3,6-SiR}_3)_2]$ (**11**).

In the presence of the tris(pyrazolyl)borate coligand, species of the type $[\text{ReO}\{\text{HB}(\text{pz})_3\}(\text{SC}_5\text{NH}_3)_2]$ (**12**) are readily isolated. The Re-hydrazido core may also be stabilized in the presence of thiolate ligands as illustrated by the structure of $[\text{ReCl}(\text{N}_2\text{COPh})(\text{SC}_5\text{NH}_4)(\text{PPh}_3)_2]$ (**13**).

Analogous Tc complexes to **10**, **11** and **13** have been isolated and spectroscopically characterized. Further investigation awaits completion of the new laboratory facility (see part E).

C. *Ga and In Coordination Chemistry*. The chemistry of Ga and In with the pyridinethiol class of ligands has been investigated. Two fundamental chemical types are observed, depending on reaction conditions: $[\text{M}\{\text{SC}_5\text{NH}_4\text{-n}(\text{SiR}_3)_n\}_3]$ [$\text{M} = \text{Ga}$, $n = 0$ (**14**); $\text{M} = \text{In}$, $n = 0, 1$ (**15**, **16**)] and $[\text{M}_2(\text{OR})_2(\text{SC}_5\text{NH}_4)_4]$ [$\text{M} = \text{Ga}$ (**17**), In (**18**)]. In collaboration with Dr. Alan Fischman of Massachusetts General Hospital, we have determined that these neutral complexes are, not unexpectedly, lipophilic and that they penetrate cell membranes. Further studies are in progress.

D. *General Coordination Properties of Pyridinethiolate and Related Ligand Types*. We have investigated the coordination chemistry of the pyridinethiolate class of ligands with Group 11 metal cations which should avoid complications due to redox chemistry, such as disulfide formation or C-S bond cleavage. With the coinage metals, we observe wide range of structural types, depending on ligand substituents, reaction conditions and coligands. The binuclear $[(\text{Ph}_2\text{PCH}_2\text{CH}_2\text{PPh}_2)_3\text{Cu}_2(\text{SC}_5\text{NH}_3\text{SiR}_3)_2]$ (**19**), the tetranuclear $[\text{Cu}_4(\text{SC}_5\text{NH}_3\text{SiR}_3)_4]$ (**20**) and the hexanuclear $[\text{Ag}_6(\text{SC}_5\text{NH}_3\text{SiR}_3)_6]$ (**21**) are representative.

The related thiopyrimidine and mercaptoimidazole ligands have also been prepared and some preliminary studies of the coordination chemistry with Group 12 metals undertaken. Quite unexpectedly, the tetranuclear oxo-centered cluster $[\text{Zn}_4\text{O}(\text{SC}_3\text{N}_2\text{H}_2\text{CH}_3)_6]$ (**22**) and the infinite

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linear chain species $[\text{Cd}(\text{SC}_4\text{N}_2\text{CH}_3\text{CF}_3)_2]_\infty$ (23) were isolated. We are currently investigating the chemistry of these novel ligand types with the Group 7 and Group 13 metals.

E. *Construction of Laboratory for Facilities 99-Tc Chemistry.* Due to the latest round of regulatory requirements in New York State, the radiochemistry laboratory in the Chemistry Building was deemed unsuitable for carrying out 99-Tc work. Consequently, a laboratory had to be refurbished to meet the requirements and to guarantee that ductwork and hood motors would not be contaminated by particulate Tc-containing materials. This is a serious concern as both SUNY Albany and MIT have encountered serious contamination problems.

The University generously provided \$30,000 for the laboratory rehabilitation, and the physical plant engineers have provided the expertise for the remodelling. A sketch of the new facility is attached. Work is in progress and should be completed by late January, 1994. While the necessity to refurbish the laboratory has hampered our ability to develop the Tc chemistry, the requirements of worker safety and environmental concerns were deemed to take precedence. The new laboratory will both guarantee safety and provide a state-of-the-art facility which should allow the efficient and timely development of the chemistry.

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