**Project Title: Molecular Engineering of Technetium and Rhenium Based
Radiopharmaceuticals.**

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I. Introduction.

The research is based on the observation that despite the extraordinarily rich coordination chemistry of technetium and rhenium and several notable successes in reagent design, the extensive investigations by numerous research groups on a variety of N_2S_2 and N_3S donor type ligands and on HYNIC have revealed that the chemistries of these ligands with Tc and Re are rather complex, giving rise to considerable difficulties in the development of reliable procedures for the development of radiopharmaceutical reagents. *It is now evident that the MAG_3 technology offers well-defined reagents which are relatively unstable under imaging conditions; in contrast, the HYNIC analogues are chemically robust but less well-defined and poorly characterized at the tracer level. Consequently, the proposed research will focus on the development of an innovative approach to labeling peptides with technetium (^{99m}Tc) and rhenium (^{186}Re and ^{188}Re), by creating single amino acid analogs applicable to current peptide synthetic methods and to bifunctional conjugate strategies that will provide facile and stable complex formation.* The work outlined in the following sections focuses on the development of synthetic strategies and the radiolabeling and testing of ^{99m}Tc -labeled peptides containing single amino acid analogue chelates as novel imaging probes. *The objective is the design of a family of single amino acid chelators (SAAC) based on pyridyl, carboxylate and/or thiolate derivatized lysine, alanine and diamino diacids for conjugation to small peptides by solid phase synthetic techniques.*

The investigations summarized in this section evolve from the demonstration that the technetium-organohydrazino core can be exploited in the development of ^{99m}Tc -peptide conjugates and that the identity of the coligand may dramatically influence the pharmacological preparation, as well as the biodistributions of the compounds. These

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observations drove the elaboration of the coordination chemistry of the Tc-organohydrazino core and the development of novel coligands. However, the extensive investigations on the coordination chemistry of the Tc-hydrazino system led us to conclude that the organohydrazines are *inferior chelates* and that alternative approaches to effective labeling should be explored. The preliminary studies on the development of the **single amino acid analog chelators** exploiting the $\{\text{Tc}(\text{CO})_3\}^{+1}$ core represent a novel strategy for the development of imaging agents.

II. The Technetium(III)-Bishydrazino core $\{\text{Tc}(\text{H}_x\text{NNR})_2\}^{n+}$ in $^{99\text{m}}\text{Tc}$ -Labeled Chemotactic Peptide Derivatives. We have developed chemotactic peptides which incorporated the $^{99\text{m}}\text{Tc}$ binding group HYNIC (hydrazinonicotinamide). This complexing agent when added to the lysine extended C-terminus of the peptide sequence enabled robust labeling at high specific activity. Separation of the unlabeled peptide from the technetium-labeled peptide was easily achievable on HPLC and facilitated by the nature of the starting technetium complex.

A significant structural feature associated with the $[\text{Tc}(\text{HYNIC-peptide})_x(\text{coligand})_y]$ reagents is the necessity of a multidentate coligand to bond to the remaining available coordination sites about the Tc(III)-organohydrazino core. By varying the identity of the coligand, we have demonstrated that the distribution of $^{99\text{m}}\text{Tc}$ -labeled-HYNIC derivatized chemotactic peptides may be modulated. This study employed analogs of the polyhydric ligand, glucoheptonate, which was initially employed to facilitate $^{99\text{m}}\text{Tc}$ radiolabeling of the HYNIC moiety of both proteins and peptides. The compounds used (glucoheptonate, mannitol, glucarate and glucamine) provided a small series of hydroxyl-backbone ligands which differ in the number and type of ionizable functional groups. At imaging times of 3 and 18 h, $[\text{Tc}(\text{HYNIC-f-MLFK})_x(\text{mannitol})_y]$ had the lowest levels of accumulation in bowel and the highest level of accumulation in infected tissue. In addition to influencing biodistribution and excretion, the choice of coligand significantly affects other chemical and biological properties of the conjugates, such as the stoichiometry of the complex, chromatographic separation of labeled and unlabeled peptide and targeting of infection. For infection imaging, peptide radiolabeled with $^{99\text{m}}\text{Tc}$ -mannitol has the most favorable combination of concentration in infected tissue, T/B ratio and biodistribution in infected organs.

However, it is significant that thiolate-based ligands appear to offer considerable advantages in purity of preparations and stability of the complexes.

This latter observation led to the development of a class of tridentate ligands, such as bis(mercaptoethyl)methylamine (NS₂), which may constrain the possible coordination geometries and improve overall stability. To investigate this hypothesis, we synthesized NS₂, converted the [Tc(HYNIC-f-MLFK)_x(mannitol)_y] to the corresponding NS₂ containing complex [Tc(HYNIC-f-MLFK)₂(NS₂)], and

Figure 1. Right: representative whole-body anterior images of rabbits at 3 and 18 h after injection of [99mTc(HYNIC-f-MLFK)_x(mannitol)_y] (left) and [99mTc(HYNIC-f-MLFK)₂(NS₂)] (center and right). Center: Average values of tissue concentration of [99mTc(HYNIC-f-MLFK)_x(mannitol)_y] and [99mTc(HYNIC-f-MLFK)₂(NS₂)] in rabbits at 18 h after intravenous injection. Right: Average values of infected to normal muscle (open bars) and pus to normal muscle (solid bars) ratios at 18 h after injection of [99mTc(HYNIC-f-MLFK)_x(mannitol)_y] and [99mTc(HYNIC-f-MLFK)₂(NS₂)].

compared its infection imaging and biodistribution properties with [Tc(HYNIC-f-MLFK)_x(mannitol)_y]. HPLC, ROI analysis, and tissue radioactivity measurements indicate that the [Tc(HYNIC-f-MLFK)₂(NS₂)] complex is chemically homogeneous and exhibits improved infection localization and biodistribution properties (Figure 1).

In order to provide a structural basis for the interaction of the {Tc-HYNIC} core with NS₂ and related ligand types, the reactions of [MCl₃(NNC₅H₄NH)(NHNC₅H₄N)] [M = Tc (1a), Re (1b)] (see Figure 2) with CH₃N(CH₂CH₂SH)₂ (NS₂), C₅H₃N-2,6-(CH₂SH)₂

Figure 2. The structure of [TcCl₃(NNC₅H₄NH)(NHNC₅H₄N)].

and $\text{O}(\text{CH}_2\text{CH}_2\text{SH})_2$, as well as the bidentate N,S-donor ligands pyridine-2-thiol and 3-(trimethylsilyl)pyridine-2-thiol, were investigated. The resulting thiolate derivatized complexes of the {M-HYNIC} core, $[\text{M}\{\text{CH}_3\text{N}(\text{CH}_2\text{CH}_2\text{S})_2\}(\text{NNC}_5\text{H}_4\text{N})-(\text{NHNC}_5\text{H}_4\text{N})]$ [$\text{M} = \text{Tc}$ (2a), Re (2b)], $[\text{Re}\{\text{C}_5\text{H}_3\text{N}-2,6-(\text{CH}_2\text{S})_2\}(\text{NNC}_5\text{H}_4\text{N})(\text{NHNC}_5\text{H}_4\text{N})]$ (3), $[\text{Re}\{\text{O}(\text{CH}_2\text{CH}_2\text{S})_2\}(\text{NNC}_5\text{H}_4\text{N})(\text{NHNC}_5\text{H}_4\text{N})]$ (4), $[\text{ReCl}(\text{C}_5\text{H}_4\text{N}-2-\text{CH}_2\text{S})(\text{NNC}_5\text{H}_4\text{N})(\text{NHNC}_5\text{H}_4\text{N})]$ (5), and $[\text{Re}(2-\text{SC}_5\text{H}_3\text{N}-3-\text{SiMe}_3)_2(\text{NNC}_5\text{H}_4\text{N})(\text{NHNC}_5\text{H}_4\text{N})]$ (6) were characterized spectroscopically, and in the instances of 2b, 3 and 5 by x-ray crystallography.

The model chemistry invariably shows the presence of the technetium(III)-bisorganohydrazino core, $\{\text{Tc}(\text{H}_x\text{NNR})_2\}^{n+}$. However, in the case of the technetium complex of HYNIC-peptide with tricine and triphenylphosphine as coligands, Liu and Edwards have convincingly demonstrated that the complex formed is formulated as the Tc-monohydrazino species, $[\text{Tc}(\text{HYNIC-peptide})(\eta^4\text{-H}_2\text{tricine})(\text{PPh}_3)]$. In order to reconcile the apparent contradictions, we undertook a detailed investigation of the model chemistry of the metal-organohydrazino core, for $\text{M} = \text{Tc}$ and Re .

Our earlier investigations suggested that chelating organohydrazine ligands in the presence of phosphine coligands yielded monohydrazino species of which $[\text{Re}\{\text{NNC}(\text{O})\text{Ph}\}\text{Cl}_2(\text{PPh}_3)_2]$ (7) is representative. We confirmed this observation using

Figure 3. The structures of $[\text{Re}(\text{HYNIC-lysine})\text{Cl}_2(\text{PPh}_3)_2]$ (7) (left) and $[\text{Re}(\text{HYNIC-lysine})(\text{Me}_2\text{EDDA})(\text{PPh}_3)]$ (8) (right).

HYNIC-lysine as a model for the HYNIC-peptide and dimethylethylenediaminediacetic acid as the N,O-donor coligand in the presence of triphenylphosphine (Figure 3). As anticipated, the ultimate product $[\text{Re}(\text{HYNIC-lys})(\text{Me}_2\text{EDDA})(\text{PPh}_3)]$ (8) exhibits a single coordinated HYNIC unit and, curiously, a monodentate coordination mode. Subsequently, a series of model compounds, incorporating a range of polyhydric and N,S-donor ligands was prepared and structurally characterized, including $[\text{Tc}(\text{NNC}_5\text{H}_4\text{N})(\text{PPh}_3)(\eta^3\text{-H}_2\text{tricine})]$ (9), $[\text{Tc}(\text{HNNC}_5\text{H}_4\text{N})(\text{NNC}_5\text{H}_4\text{NH})(\eta^3\text{-H}_4\text{mannitol})]$ (10), $[\text{TcCl}(\text{HNNC}_5\text{H}_4\text{N})(\text{NNC}_5\text{H}_4\text{N})(\eta^2\text{-SCH}_2\text{C}_5\text{H}_4\text{N})]$ (11) and $[\text{Tc}(\text{HNNC}_5\text{H}_4\text{N})(\text{NNC}_5\text{H}_4\text{N})\{(\text{SCH}_2)_2\text{C}_5\text{H}_3\text{N}\}]$ (12), shown in Figure 4. These results confirm that the Tc-bishydrazino core is persistent, but that in the presence of triphenylphosphine and/or certain polyhydric ligands, the monohydrazino core may be isolated.

Figure 4. Common core structures for technetium and rhenium.

The series of compounds $[\text{Re}(\text{HNNC}_5\text{H}_4\text{N})(\text{NNC}_5\text{H}_4\text{NH})\text{Cl}_3]$ (**1b**), $[\text{Re}(\text{HNNC}_5\text{H}_6\text{N})(\text{NNC}_5\text{H}_4\text{N})\{(\text{SCH}_2)_2\text{C}_5\text{H}_3\text{N}\}]$ (**13**), $[\text{Re}(\text{HYNIC-lys})_2\text{Cl}_3]$ (**14**), $[\text{Re}(\text{HYNIC-lys})_2\{(\text{SCH}_2)_2\text{C}_5\text{H}_3\text{N}\}]$ (**15**), $[\text{Re}(\text{HYNIC-f-MLFK})_2\text{Cl}_3]$ (**16**) and $[\text{M}(\text{HYNIC-f-MLFK})_2\{(\text{SCH}_2)_2\text{C}_5\text{H}_3\text{N}\}]$ (**17**) were prepared and characterized by elemental analysis, ^1H -nmr, ^{13}C -nmr, 2-D-nmr, and mass spectroscopy. The results were consistent with the presence of the $\{\text{M}(\text{HYNIC-R})_2\}^{n+}$ core in all cases. A view of the structure of **17** with $\text{M} = \text{Tc}$, based upon mass spectroscopy and nmr fitting, is shown in Figure 5.

Figure 5. Proposed structure of the radiolabeled conjugate $[\text{Tc}\{(\text{SCH}_2\text{CH}_2)_2\text{NCH}_3\}(\text{HYNIC-f-MLFK})_2]$ (**17**).

These structural results are significant in view of the improved target accumulation and background reduction associated with $[\text{Tc}(\text{HYNIC-f-MLFK})_2\{(\text{SCH}_2)_2\text{C}_5\text{H}_3\text{N}\}]$. First, the structures of the model coordination compounds suggest that the peptides (not the α -nitrogens atoms of the HYNIC moieties) adopt a *trans* orientation. This minimizes the steric interface of the two peptides with each other and may allow either peptide access to the formyl-MLF receptor. If the peptides were *cis* to each other they may interfere with bonding to the receptor by interacting with each other or by sterically blocking the approach of the other to the receptor. This speculation has yet to be proven; however, the fact that the α -nitrogen of HYNIC will not coordinate *trans* to the α -nitrogen of a second HYNIC would dispose the peptides in a mutually *trans* arrangement. Furthermore, the improvement in biological behavior may be derived from the likelihood that *in vivo* ligand substitution (*via* glutathione or cysteine) is unfavorable due to the tridentate nature of NS_2 . Previous work with the bidentate pyridinethiol and pyrimidinethiol showed two pyridinethiol ligands attached to the metal core: one bidentate and one monodentate through the sulfur. The monodentate

pyridinethiol is potentially labile due to the single attachment mode and the *trans* influence of the sulfur from the bidentate pyridinethiol. On the other hand, the NS₂ ligand may enhance the persistence of the complex by adopting a meridional coordination mode for a tridentate chelate.

While these results were significant, several observations made in the course of these investigations were less encouraging. In contrast to the pyridylhydrazino derivative **1b**, the HYNIC analogue [Re(HNNC₅H₄N-4-CO₂H)(NNC₅H₄NH-4-CO₂H)Cl₃] (**18**) can only be isolated in 60% yield from a mixture which also contains a significant amount of a monohydrazino material of composition [Re₂O(HYNIC)₂Cl₄(solvent)] (**19**) from elemental analysis, mass spectroscopy and nmr analysis. The relative amount of **19** formed is dependant on the MeOH/H₂O ratio of the reaction mixture, increasing with H₂O content. Since labeling studies are carried out in aqueous media, purification and yield concerns appear to be issues innate to the chemistry. Furthermore, the coligand chemistry is not without complications. The reactions of **18** with various thiolate coligands, including pyridinethiol, {(SCH₂)₂NC₅H₃}²⁻, and mercatomethylimidazole, invariably produces the desired product, such as **20**, and a binuclear species of which [Re₂O(H_xNNC₅H₄N)₄(SC₃H₂N₂CH₃)₂] (**21**) is characteristic (see Figures 6-8). These observations dramatically illustrate the inherent difficulties associated with the coordination chemistry of the Tc-Re-organohydrazino system which must be overcome in developing an effective imaging agent.

Figure 6. The structure of **18**.

Figure 7. The structure of **20**.

Figure 8. The structure of **21**.

III. Bifunctional chelates for use in activated automated peptide synthesis.

III. 1. The Metal Precursor: The $\{M(CO)_3\}^{+1}$ Core. While the HYNIC approach to infection imaging proved successful, there were a number of disadvantages: the synthesis of the HYNIC-peptide conjugate is quite involved and the coordination/coligand chemistry results in additional synthetic and structural complexities. Consequently, we concluded that it would be expedient to develop approaches exploiting other Tc-core substructures. Recently, the remarkable Tc(I) core $\{Tc(CO)_3\}^{+1}$ has been demonstrated to provide an ideal geometry for the labeling of receptor and biomolecules with high specific activity with retention of the biological activity and specificity.

The Tc(I)-tricarbonyl core offers a numbers of advantages for the design of novel radiopharmaceuticals: (i) Alberto and coworkers developed a facile route to the important intermediate $\{M(H_2O)_3(CO)_3\}^+$ from the permetalate salt MO_4^- under atmospheric pressure of CO and exploiting a reducing agent such as $NaBH_4$; (ii) the complexes are water-soluble and readily undergo ligand exchange; (iii) the $\{Tc(CO)_3\}^{+1}$ core is chemically robust and maintains its integrity under the most forcing conditions; (iv) the organometallic nature of the core renders chelation more covalent in character.

III. 2. Ligand design and coordination chemistry. Ligand design is facilitated by the readily achieved substitution chemistry at the aqua sites of the $\{M(CO)_3(H_2O)_3\}^{+1}$ ($M = Tc, Re$) and $\{Re(CO)_3 X (H_2O)_2\}$ ($X = Cl, Br$) species, and the coordination preferences of Tc(I) and Re(I) for nitrogen and oxygen donors. For example, we have found that $[NEt_4]_2[Re(CO)_3Br_3]$ reacts quantitatively with 2-aminomethylpyridine (amp) to give $[Re(CO)_3Br(amp)]$ (22), shown in Figure 9. Similarly, the reaction of bis-(methyl-2-pyridyl)amine (bamp) with $[NEt_4]_2[Re(CO)_3Br_3]$ yields $[Re(CO)_3\{(C_5H_4NCH_2)_2NH\}]Br$ (23), shown in Figure 10.

This observation suggested the design of bifunctional chelators constructed from amino acids, so as to provide a donor set for effective coordination of Tc(I) and a linker group for attachment to peptide units. The significance of this ligand design is that the bifunctional chelators may be developed as reagents for direct incorporation into conventional solid phase peptide syntheses, thus exploiting the considerable advantages in purity, cost, scale and design afforded by solid phase peptide synthesis (SPPS). In a

preliminary study, the β -alanine derivative $(\text{NC}_5\text{H}_4\text{CH}_2)_2\text{NCH}_2\text{CH}_2\text{CO}_2\text{H}$ (bis-2-pyridylmethyl-

Figure 9. A view of the structure of $[\text{Re}(\text{CO})_3\text{Br}(\text{amp})]$ (22).

Figure 10. A view of the structure of the cationic complex $[\text{Re}(\text{CO})_3(\text{bamp})]^+$ of $[\text{Re}(\text{CO})_3(\text{bamp})]\text{Br}$ (23).

Figure 11. The structure of $[\text{Tc}(\text{CO})_3(\text{L3a})]$.

Figure 12. The structure of $[\text{ReCl}_3(\text{L3a-ethylester})]$.

aminoethylcarboxylic acid, L3a) was prepared by the methods described elsewhere. The Tc(I) complex of L3a $[\text{Tc}(\text{CO})_3(\text{L3a})]$ (24) was prepared in nearly quantitative yield (Figure 11), as well as an unusual material exhibiting the rhenium(III)-trichloride core $[\text{ReCl}_3(\text{L3a-ethylester})]$ (25) (Figure 12). The facile preparations of these model compounds suggested that a family of bifunctional chelators, derived from simple amino acids or bisamino acids could be developed, which through suitable manipulation of the ligand donor groups can provide neutral, cationic or anionic Tc(I) complexes. Neutral and anionic complexes are represented by $[\text{Tc}(\text{CO})_3\{(\text{NC}_5\text{H}_4\text{CH}_2)_2\text{NCH}_2\text{CH}_2\text{S}\}]$ (26) and $[\text{Tc}(\text{CO})_3\{(\text{O}_2\text{CCH}_2)_2\text{NCH}_2\text{C}_5\text{H}_4\text{N}\}]^{-1}$ (27), respectively (Figures 13 and 14).

Figure 13. The structure of 26.

Figure 14. The structure of 27.

III. 3. Bifunctional chelates and peptide conjugates. Our preliminary studies focused on the preparation of model bifunctional chelates derived from β -amino acids and from ϵ -derivatization of lysine. The chelates **M1-3** were prepared by literature methods⁸⁵

while **L1c** was isolated as the Fmoc derivative as illustrated in Scheme 1.

Scheme 1. The synthesis of **L1c**.

Since we had established from our HYNIC-peptide studies that the for-MLF exhibits strong binding to high affinity receptors on the white cell membrane, we chose this peptide as a model for our SAAC peptides and conjugates. The peptide was prepared by solid phase synthetic methods as outlined in Scheme 2.

Scheme 2. The solid phase synthesis and deprotection of the peptide for-MLFKG.

As a test of the versatility of the SAAC approach, two classes of ligand-peptide conjugate were now prepared: the “external chelate” in which the chelating moiety is attached to the $-\text{NH}_2$ side arm of the peptide (analogous to the HYNIC incorporation) and the “internal chelate” in which the derivatized amino acid/donor group is incorporated into the peptide chain itself as a single amino acid with a chelating functional group. Representative solid phase syntheses of these two types of conjugate are illustrated in Schemes 3 and 4, respectively.

Scheme 3. Representative synthesis of an "external chelate" conjugate.

Scheme 4. Representative synthesis of an "internal chelate" conjugate.

III. 4. Preparation of Rhenium and Technetium Complexes: Synthetic Strategies and Stabilities of the Radioconjugates. The $\{M(CO)_3\}^{+1}$ ($M = Tc, Re$) derivatives were prepared in nearly quantitative yield from the free conjugate or by incorporation of the preformed $[M(CO)_3(L1c)]$ complex into the solid phase peptide synthesis, as illustrated in **Scheme 5**. The advantage of this latter approach is the purity of the final product.

Scheme 5. "External chelate" approach to the solid phase synthesis of the $\{Re(CO)_3\}^{+1}$ complex of the conjugate.

The complexes were analyzed by elemental analysis, HPLC and nmr spectroscopy and demonstrated to be monophasic, pure compounds. HPLC showed a single peak, and the nmr spectrum, shown below is consistent with the structure and a single product.

Figure 15. The ^1H nmr spectrum of $[\text{Re}(\text{CO})_3\{\text{f-MLFK}(\text{L1c})\text{G}\}(\text{TFA})]$.

The $^{99\text{m}}\text{Tc}$ analogues were prepared from the reaction of $\{\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3\}^{+1}$, obtained using a previously prepared kit (Mallinckodt, Inc), with f-MLFK(L1c)G and other "external" and "internal chelates" in ethanol/DMSO. The Tc complexes were characterized by radiochromatographic and electrophoretic methods. The radio-HPLC traces gave a single peak associated with the product conjugate and minor peaks associated with ca. 5% of the total Tc content with considerably different retention times.

The stability of the radioconjugates toward oxidation, hydrolysis and transchelation was investigated *in vitro* by challenging the $^{99\text{m}}\text{Tc}$ conjugates with histidine, cysteine and model chelators of the L3 class.^c The radioconjugates were incubated at 37°C for 5 and 24 h in 400 molar excess of the challenging reagent.

Figure 17. C18 radio-HPLC trace of $[\text{Tc}(\text{CO})_3\{\text{f-MLFK}(\text{L1c})\text{G-OH}\}]^{+1}$, after 24 h incubation with cysteine.

Recovery of the ^{99m}Tc complex was of the order of 90-95% after 24 h incubation with the challenging reagents, demonstrating that these radioconjugates are robust chemical species which effectively coordinate and anchor the $\{\text{Tc}(\text{CO})_3\}^{+1}$ subunit for imaging purposes. A representative radio-HPLC plot is shown in **Figure 17**.

IV. Publications.

1. Synthesis and characterization of a '3 + 2' $\{\text{ReO}\}^{+3}$ core complex carrying the ONS/PO donor atom set, Femia, F. J.; Chen, X.; Babich, J. W.; Zubieta, J. *Inorg. Chim. Acta* **2001**, 316, 145.
2. Synthesis and characterization of rhenium(III) organohydrazide compounds. Crystal and Molecular Structures of $[\text{ReCl}(\text{PPh}_3)_2(\text{HNHC}_5\text{H}_4\text{N})(\text{HNNC}_5\text{H}_4\text{N})][\text{Cl}_2]$, $[\text{Re}\{2-(\text{Ph}_2\text{P})\text{C}_6\text{H}_4\text{S}\}\{2-(\text{Ph}_2\text{PO})\text{C}_6\text{H}_4\text{S}\}(\text{NNC}_5\text{H}_4\text{N})(\text{HNNC}_5\text{H}_4\text{N})]$ and $[\text{Re}\{2-(\text{Ph}_2\text{P})\text{C}_6\text{H}_4\text{S}\}_2(\text{NNC}_5\text{H}_4\text{N})]$, Pérez-Lourido, P.; Romero, J.; García-Vázquez, J. A.; Sousa, A.; Maresca, K. P.; Zubieta, J. *Inorg. Chem.* **1999**, 38, 1511.
3. Cationic complexes of the "3 + 1" oxorhenium-thiolate family, Maresca, K. P.; Femia, F. J.; Bonavia, G.H.; Babich, J. W.; Zubieta, J. *Inorg. Chim. Acta* **1999**, 297, 98.
4. Structural systematics of the $\{\text{ReO}\}^{3+}$ core with "3 + 2" ligand donor sets, Femia, F. J.; Babich, J. W.; Zubieta, J. *Inorg. Chim. Acta* **2000**, 300, 462.
5. Oxorhenium (V) complexes containing tridentate Schiff-base and monothiol coligands, Femia, F. J.; Chen, X.; Babich, J. W.; Zubieta, J. *Inorg. Chim. Acta* **2000**, 300, 517.
6. Structural characterizations of a Re(IV) complex $[\text{ReCl}_4(\text{OPPh}_3)_2]$ and of an imino species $[\text{ReOCl}_2(\text{PPh}_3)(\eta^2\text{-OC}_6\text{H}_4\text{-2-CH=NH})]$, prepared from the reaction of $[\text{ReOCl}_3(\text{PPh}_3)_2]$ with salicylaldoxime, Chen, X.; Femia, F. J.; Babich, J. W.; Zubieta, J. *Inorg. Chim. Acta* **2000**, 306, 112.
7. Synthesis and characterization of the $\{\text{ReO}\}^{3+}$ core with SNS and S donor ligands, Femia, F. J.; Chen, X.; Maresca, K. P.; Shoup, T. M.; Babich, J. W.; Zubieta, J. *Inorg. Chim. Acta* **2000**, 306, 30.

8. Investigations of the $\{\text{ReO}\}^{3+}$ core: a "2 + 2" complex from bidentate and potentially tridentate ligands: $[\text{ReO}(\eta^2\text{-HOC}_6\text{H}_4\text{-2-CH}_2\text{NC}_6\text{H}_4\text{S})(\eta^2\text{-SC}_5\text{H}_4\text{N})(\text{PPh}_3)]$, Chen, X.; Femia, F. J.; Babich, J. W.; Zubieta, J. *Inorg. Chim. Acta* **2000**, *306*, 38.
9. Synthesis and crystal and molecular structure of a tetranuclear cluster based on the rhenium(III)-bisorganohydrazino core: $[\text{Re}(\text{HNNC}_4\text{H}_3\text{N}_2)(\text{NNC}_4\text{H}_3\text{N}_2)(\text{OCH}_3)_2]_4$, Femia, F. J.; Chen, X.; Maresca, K. P.; Babich, J. W.; Zubieta, J. *Inorg. Chim. Acta* **2000**, *307*, 160.
10. The syntheses and structures of "3+2" and "2+2+1" oxorhenium mixed-ligand complexes employing 8-hydroxy-5-nitroquinoline as the bidentate N,O donor ligand, Chen, X.; Femia, F. J.; Babich, J. W.; Zubieta, J. *Inorg. Chim. Acta* **2000**, *308*, 80.
11. Schiff base chemistry of the $\{\text{ReO}\}^{3+}$ core: structural characterization of the unusual "3+2" complex $[\text{ReO}(\eta^3\text{-OC}_6\text{H}_4\text{CH}=\text{NC}_6\text{H}_4\text{-2-S})(\eta^2\text{-OC}_6\text{H}_4\text{C}=\text{NC}_6\text{H}_4\text{-2-S})]$, Chen, X.; Femia, F. J.; Babich, J. W.; Zubieta, J. *Inorg. Chim. Acta* **2000**, *307*, 146.
12. Synthesis and characterization of oxorhenium(V)-"3+1" mixed thiolate $[\text{SNS}]/[\text{S}]$ and $[\text{ONS}]/[\text{S}]$ complexes. Crystal and molecular structures of $[\text{ReO}(\eta^3\text{-SCH}_2\text{C}_5\text{H}_3\text{NCH}_2\text{S})(\eta^1\text{-C}_6\text{H}_4\text{Br-4-S})]$, $[\text{ReO}(\eta^3\text{-SCH}_2\text{C}_5\text{H}_3\text{NCH}_2\text{O})(\eta^1\text{-C}_6\text{H}_4\text{X-4-S})]$ (X = Cl, OMe), $[\text{ReO}(\eta^3\text{-SCH}_2\text{C}_5\text{H}_3\text{NCH}_2\text{O})(\eta^1\text{-C}_6\text{H}_4\text{OCH}_3\text{-4-CH}_2\text{S})]$ and $[\text{ReO}(\eta^3\text{-SCH}_2\text{C}_5\text{H}_3\text{NCH}_2\text{S})(\eta^1\text{-C}_5\text{H}_4\text{NH-2-S})][\text{Cl}]$, Chen, X.; Femia, F. J.; Babich, J. W.; Zubieta, J. *Inorg. Chim. Acta* **2000**, *307*, 88.
13. Exploring oxorhenium "3+1" mixed-ligand complexes carrying the S-benzyl 3-[(2-hydroxyphenyl)methylene] dithiocarbamate $[\text{ONS}]/\text{monothiol } [\text{S}]$ donor set: synthesis and characterization, Chen, X.; Femia, F. J.; Babich, J. W.; Zubieta, J. *Inorg. Chim. Acta* **2000**, *307*, 154.
14. $^{99\text{m}}\text{Tc}$ labeled chemotactic peptides: influence of coligand on distribution of molecular species and infection imaging properties. Synthesis and structural characterization of model complexes with the $\{\text{Re}(\eta^2\text{-HNNC}_5\text{H}_4\text{N})(\eta^1\text{-NNC}_5\text{H}_4\text{N})\}$ core, Babich, J. W.; Graham Coco, W.; Barrow, S.; Fischman, A. J.; Femia, F. J.; Zubieta, J. *Inorg. Chim. Acta* **2000**, *309*, 123.

15. An unexpected "4+2" [N₃S]/[NS] rhenium(IV) complex formed upon cleavage of a Re(V) imido bond, Chen, X.; Femia, F. J.; Babich, J. W.; Zubieta, J.A. *Inorg. Chim. Acta* **2000**, *310*, 237.
16. Syntheses and structural characterization of rhenium-bis-hydrazinopyrimidine core complexes with thiolate and Schiff base coligands, Femia, F. J.; Chen, X.; Maresca, K. P.; Babich, J. W.; Zubieta, J. *Inorg. Chim. Acta* **2000**, *310*, 210.
17. Rhenium(I) carbonyl complexes of 2,4,6-tris(2-pyridyl)-2,3,5-triazine(TPT). Rhenium(I) promoted methoxylation of the triazine ring carbon atom in dinuclear rhenium complexes, Chen, X.; Femia, F. J.; Babich, J. W.; Zubieta, J. *Inorg. Chem.* **2001**, *40*, 2769.
18. Synthesis and structural characterization of rhenium(I) tricarbonyl complexes with the bidentate ligands o-(diphenylphosphino)benzaldehyde (P∩O) and o-[diphenylphosphino)benzylidene]aniline (P∩N), Chen, X.; Femia, F. J.; Babich, J. W.; Zubieta, J. *Inorg. Chim. Acta* **2001**, *315*, 147.
19. Spectroscopic and structural studies of complexes of the *fac*-[Re(N∩N)(CO)₃L]ⁿ⁺ type (N∩N = 2-(2-pyridyl)benzothiazole; L = Cl, Br, CF₃SO₃⁻, CH₃CN), Chen, X.; Femia, F. J.; Babich, J. W.; Zubieta, J. *Inorg. Chim. Acta* **2001**, *314*, 91.
20. Synthesis, characterization and crystal structures of mono-, di- and trinuclear rhenium(I) tricarbonyl complexes of 2, 3, 5, 6-tetra(2-pyridyl) pyrazine, Chen, X.; Femia, F. J.; Babich, J. W.; Zubieta, J. *Inorg. Chim. Acta* **2001**, *315*, 66.
21. Schiff base chemistry of the rhenium(V)-oxo core with '3+2' ligand donor sets, Chen, X.; Femia, F. J.; Babich, J. W.; Zubieta, J. *Inorg. Chim. Acta* **316**, 33; (v) 6-Mercaptomethylpyridine-3-carboxylic acid (MEMNIC): A new reagent for peptide labeling with Tc-99m, Babich, J. W.; Graham, W.; Femia, F. J.; Dong, Q.; Barzane, M.; Ferrill, K.; Fischman, A. J.; Zubieta, J. *Inorg. Chim. Acta*, **2001**, *323*, 23.
22. HYNIC-maleimide, a thiol-specific linker for labeling peptides with Tc-99m: conjugation, labeling and *in vivo* evaluation, Dong, Q.; Babich, J. W.; Graham, W.; Barzane, M.; Ferrill, K.; Fischman, A. J.; Ray, S.; Krishna, M. L.; Zubieta, J., in preparation.

23. N,N-bis(2-mercaptoethyl)methylamine(NS₂): a new coligand for Tc-99m labeling of hydrazino-nicotinamide (HYNIC) peptides, Babich, J. W.; Dong, Q.; Graham, W. A.; Barzana, M.; Fischman, A. J.; Maresca, K. P.; Ray, S.; Krishna, M. L.; Zubieta, J., in preparation.
24. Synthesis, characterization, and biodistribution of a Tc-99m fatty acid derivative. The crystal and molecular structures of [ReO{ η^3 -(SCH₂CH₂)₂N(C₁₅H₃₀CO₂H)}(η^1 -C₆H₅CH₂S)], [ReO{ η^3 -(SCH₂CH₂)₂S}(η^1 -SC₁₀H₂₀CO₂H)] and [ReO{ η^3 -(SCH₂CH₂)₂S}(η^1 -SC₄H₈CO₂H)]], Maresca, K. P.; Babich, J. W.; Femia, F. J.; Zubieta, J. *Inorg. Chim. Acta*, **2002**, *338*, 149.
25. Rhenium(I) carbonyl complexes of bis(N,N-diethylamino) carbeniumcarboxylate, a novel inner-salt type sulfur donor ligand. Spectroscopic and structural studies, S. R. Banerjee, A. Nagasawa, J. Zubieta, *Inorg. Chim. Acta*, **2002**, *340*, 155.
26. {Re(III)Cl₃ core complexes with bifunctional chelators constructed from amino acids and analogues, S. R. Banerjee, L. Wei, M. K. Levadala, N. Lazarova, K. A. Stephenson, J. F. Valliant, J. W. Babich, J. Zubieta, *Technetium, Rhenium and Other Metals in Chemistry and Nuclear Medicine*, 6, M. Nicolini, U. Mazzi, eds., Servizi Grafici Editoriali pnc, Padova, Italy, 2002 p. 227.
27. Novel ligands for cerebral monoamine transporters: rhenium and technetium oxo-complexes of 4-arylpiperidines, M. P. Smith, D. Keith, K. P. Maresca, N. S. Kula, R. J. Baldessarini, F. I. Tarazi, J. A. Zubieta, J. W. Babich, *Technetium, Rhenium and Other Metals in Chemistry and Nuclear Medicine*, 6, M. Nicolini, U. Mazzi, eds., Servizi Grafici Editoriali pnc, Padova, Italy, 2002 p. 183.
28. Amino acid-based bifunctional chelates for labeling of biomolecules with the {Te(CO)₃}⁺¹ and {Re(CO)₃}⁺¹ cores, S. R. Banerjee, N. Lazarova, K. M. Levadala, J. Valliant, K. Stephenson, K. P. Maresca, J. Zubieta, J. W. Babich, *Technetium, Rhenium and Other Metals in Chemistry and Nuclear Medicine*, 6, M. Nicolini, U. Mazzi, eds., Servizi Grafici Editoriali pnc, Padova, Italy, 2002 p. 111.
29. Dipyrindinemethylamine tridentate chelates for labeling with the {Te(CO)₃}⁺¹ and {Re(CO)₃}⁺¹ cores, K. P. Maresca, S. R. Banerjee, N. Lazarova, K. M. Levadala, J. Zubieta, C. D. McCusker, A. J. Fischman, J. W. Babich, *Technetium, Rhenium and*

Other Metals in Chemistry and Nuclear Medicine, 6, M. Nicolini, U. Mazzi, eds., Servizi Grafici Editoriali pnc, Padova, Italy, 2002 p. 155.

30. Bifunctional Single Amino Acid Chelates (SAAC) for Labeling of Biomolecules with the $\{\text{Tc}(\text{CO})_3\}^{+1}$ and $\{\text{Re}(\text{CO})_3\}^{+1}$ Cores. The Crystal and Molecular Structures of $[\text{ReBr}(\text{CO})_3(\text{H}_2\text{NCH}_2\text{C}_5\text{H}_4\text{N})]$, $[\text{Re}(\text{CO})_3\{(\text{C}_5\text{H}_4\text{NCH}_2)_2\text{NH}\}]\text{Br}$, $[\text{Re}(\text{CO})_3\{(\text{C}_5\text{H}_4\text{NCH}_2)_2\text{NCH}_2\text{CO}_2\text{H}\}]\text{Br}$, $[\text{Re}(\text{CO})_3\{\text{X}(\text{Y})\text{NCH}_2\text{CO}_2\text{CH}_2\text{CH}_3\}]\text{Br}$, (X = Y = 2-pyridylmethyl; X = 2-pyridylmethyl, Y = 2-(1-methylimidazolyl)methyl; X = Y = 2-(1-methylimidazolyl)methyl, $[\text{ReBr}(\text{CO})_3\{(\text{C}_5\text{H}_4\text{NCH}_2)\text{NH}(\text{CH}_2\text{C}_4\text{H}_3\text{S})\}]$ and $[\text{Re}(\text{CO})_3\{(\text{C}_5\text{H}_4\text{NCH}_2)\text{N}(\text{CH}_2\text{C}_4\text{H}_3\text{S})(\text{CH}_2\text{CO}_2)\}]$, S. R. Banerjee, M. K. Levadala, N. Lazarova, L. Wei, J. F. Valliant, K. A. Stephenson, J. W. Babich, K. P. Maresca, J. Zubieta, *INORG. CHEM.*, **2002**, *41*, 6417.
31. $\{\text{Re}(\text{III})\text{Cl}_3\}$ Core complexes with bifunctional single amino acid chelates, S. R. Banerjee, L. Wei, M. K. Levadala, N. Lazarova, V. O. Golub, C. J. O'Connor, K. A. Stephenson, J. F. Valliant, J. W. Babich, J. Zubieta, *INORG. CHEM.*, **2002**, *41*, 5795 .
32. Rhenium tricarbonyl core complexes with ligands derived from arylpiperazines, L. Wei, S. R. Banerjee, M. K. Levadala, J. W. Babich, J. Zubieta., *INORG. CHEM. COMMUN.*, in press.