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Basic aqueous chemistry of $[M(OH_2)_3(CO)_3]^+$ (M = Re, Tc) directed towards radiopharmaceutical application

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Contents

Abstract

A review on the synthesis and properties of the organometallic aqua-ion $[M(OH_2)_3(CO)_3]^+$ (M = Re, ^{99}Tc, ^{99m}Tc), as relevant for radiopharmaceutical application, is presented. These important starting compounds can be prepared quantitatively, (a) on the no carrier added (n.c.a.) level (^{99m}Tc) in water, or (b) in organic solvents (Re, ^{99}Tc) at

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atmospheric pressure in a short time and from $[MO_4]^-$. The main characteristics of these carbonyl complexes are the high substitution stability of the three CO ligands and the substitution lability of the coordinated water molecules. $[M(OH_2)_3(CO)_3]^+$ can be considered as a 'semi aquo–ion'. On the macroscopic level, upon titration with OH^-, hydroxo-bridged oligomers have been isolated and characterized. The formation of hydroxo-bridged complexes is a consequence of the considerable Brönstedt acidity of $[M(OH_2)_3(CO)_3]^+$, whereas on the n.c.a. level no such behavior was observed. Conditions and products of the water exchange by imidazole (im) and derivatives thereof (histamine, histidine) will be presented. The different mononuclear complexes with these ligands are of extraordinary inertness, which is the basis for potential applications in biology and nuclear medicine. Finally, as a basis for bioorganometallic chemistry, the adoption of the results from basic coordination chemistry to the labeling of biomolecules with an organometallic moiety will be exemplified with a selected penta-peptide and a recombinant single chain fragment.

Keywords: Tc–carbonyls; Tc–peptide complexes; scFv; Cancer diagnosis; Radiopharmacy

1. Introduction

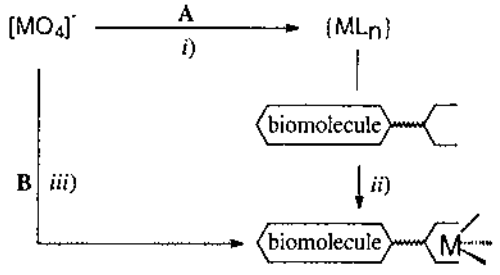
The ultimate goal of radiopharmacy is the application of radiolabeled compounds in nuclear medicine for diagnostic and therapeutic purposes. For several reasons, the radionuclides $^{188/186}Re$ and ^{99m}Tc are of primary interest [1,2]. In nuclear medicine, these transition metal centers are basically applied in two different forms: (a) a highly stable metal complex, that does not contain a specific biomolecule at its backbone and is completely resistant to metabolical degradation, is applied to make biological functions visible, and (b) metal complexes attached to a specific biomolecule, thus, following essentially its biological pathway. Brain and heart perfusing agents such as $[^{99m}Tc(CN–R)_6]^+$ are typical and widely applied examples for the former type of radiopharmaceutical compounds [3,4]. Compounds of this class are predominantly complexes of technetium. In contrast, most compounds of the second class of radiopharmaceuticals are represented by iodinated biomolecules and rarely with transition metals such as ^{99m}Tc or ^{188}Re. Due to availability and convenience in respect of routine application, iodinated biomolecules should be substituted by ^{99m}Tc (diagnosis) or ^{188}Re (therapy) labeled compounds. Thus, a new generation of radiopharmaceutical compounds was born, consisting of a transition metal complex attached to a biological vector as for example a chemotactic peptide [5]. However, the label will become much larger than a single atom like iodine. The interference with the bioactivity and physico–chemical properties of the biomolecule will increase significantly with the size of the label. The metal complex should therefore be kept small in size and its physico–chemical properties (such as hydrophilicity) should adopt those of the biomolecules.

Apart from these chemical requirements, a number of further limitations have to be considered when designing practical procedures to use the radiopharmaceuticals for routine clinical application. These limitations mainly concern their preparation

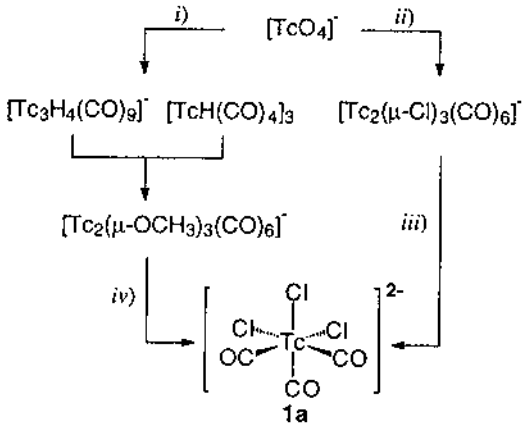
(in one step), final purity (98% yield), biomolecule concentration (preferably 1:1 in respect to the radionuclide) and time consumption (depending on the half-life of the radionuclide). The principal limitations in that respect are the following: (i) any preparation has to be performed in saline solution (0.9% NaCl in water or buffer); (ii) no purification should be necessary, thus, receptor targeting biomolecules are present in extremely low concentrations during the preparation; and (iii) preparation should not exceed a certain time (about 60 min in the case of ^{99m}Tc). Obviously, these conditions and in particular point (i), made the introduction of organometallic compounds rather unattractive until now. In this article we will demonstrate that organometallic precursors are readily prepared even with the above mentioned restrictions. Furthermore, they have significant advantages compared with other precursors applied in the field as a result of the unique properties of typical organometallic ligands such as CO. Finally, they fulfill the limitations mentioned above more easily than high valent precursors especially due to the high kinetic stability of the d^6 metal core. Scheme 1 shows synoptically the development of Tc or Re compounds until their application in nuclear medicine.

2. Synthesis of $[MX_3(CO)_3]^{2-}$ (M = Re, ^{99}Tc) from $[MO_4]^-$ in organic solvents

The syntheses of carbonyl complexes were traditionally performed in autoclaves. This was particularly the case for $[Re_2(CO)_{10}]$ and $[Tc_2(CO)_{10}]$ [6]. $[NEt_4]_2[ReBr_3(CO)_3]$ was prepared from $[Re_2(CO)_{10}]$, by bromination to $[ReBr(CO)_5]$ and substitution of two COs with $[NEt_4]Br$ [7]. The main problem to overcome was the direct reduction of $[MO_4]^-$ from the oxidation state + VII down to + I. For carbonylation at normal pressure, a strong reducing agent has to be found that is soluble in the corresponding solvent but may not coordinate to the reduced metal center. Thus, a lot of the common reductants fail in one or more of these conditions. BH_3, hardly applied as a reductant in organometallic synthesis, is convenient in several respects. The B(III) is very oxophilic, but the resulting borates are not expected to coordinate strongly to low valent metal centers. Accordingly,



Scheme 1. Availability of radiopharmaceuticals: path A: two step, and path B: one step approach. (i) Precursor preparation in saline; (ii) quantitative exchange; and (iii) in saline, conditions dictated by the biomolecule.



Scheme 2. Low pressure carbonylation of $[^{99}TcO_4]^-$, in general: r.t., 1 atm CO, $THF{\cdot}BH_3$: (i) no Cl^-; (ii) with Cl^-; (iii) excess Cl^-; and (iv) NaOH in CH_3OH then HCl.

the reduction of Tc(VII) and/or Re(VII) to Tc(I) or Re(I) under concomitant coordination of three COs could be achieved. Bubbling of CO through a THF solution of the permetallates in the presence of BH_3THF and Cl^- resulted in the reduction of the metal center and coordination of at least three CO ligands [8].

Nevertheless, the mechanism of the reaction remains unclear. No intermediate complex in an oxidation state higher than +I could be isolated or detected in reasonable amounts. However, different complexes in the +I valency were found as products when varying the reaction conditions. When X^- was present in the reaction mixture, $[Tc_2(\mu\text{-}X)_3(CO)_6]^-$ formed quantitatively, addition of excess $[NEt_4]Cl$ lead to cleavage and formation of the final product $[TcX_3(CO)_3]^{2-}$ (**1a**) or $[ReX_3(CO)_3]^{2-}$ (**1b**) (M = Cl, Br), respectively. In the complete absence of X^-, the mixed hydrido–carbonyl complexes $[TcH(CO)_4]_3$ and $[Tc_3H_4(CO)_9]^-$ were formed together in almost 100% yield. Addition of NaOH in methanol and subsequent acidification lead to **1a** (or **1b**). A flow chart of the reaction conditions and the isolated and fully characterized carbonyl compounds is given in Scheme 2. Fig. 1 depicts an ORTEP presentation of $[TcH(CO)_4]_3$ [9].

In can be assumed that the described procedure can also be applied to other permetallates, since the driving force is probably the formation of the borates rather than the hydride transformation to the metal center. This could open an important approach towards other mixed carbonyl–hydride or carbonyl–halide complexes.

The number of CO ligands seemed not to be limited to three. In the absence of halides, a complex with four COs was isolated in good yield (Scheme 2). Obviously, the M(I) center tries to achieve a closed shell. This is most readily, and probably most quickly, achieved either with coordination of Cl^- or, in its absence, by accepting one (or more) additional CO ligands.

3. Synthesis of $[M(OH_2)_3(CO)_3]^+$ from $[MO_4]^-$ in water

The preparation described in Section 2 is very convenient for the starting material **1a** or **1b** on the macroscopic level. The precursor **1a** in particular forms the scaffold for the low valent organometallic technetium chemistry. Obviously, the procedure is not feasible for nuclear medical purposes. As mentioned previously, exclusive syntheses in saline are required. The synthesis of carbonyl complexes in water is practically unknown, since there was no need to develop such an approach. From a scientific point of view such a reaction is hard to develop since low valent organometallic complexes tend to decompose unspecifically in water, or at least to form hydrides rather than resulting in well defined species. BH_3 was not a versatile reductant in this medium since it decomposed almost instantaneously. On the other hand, different BH_3 adducts are reasonably stable at appropriate pH values. The most simple adduct, $[BH_4]^-$, proved to be a versatile reagent for these purposes [10]. At $pH > 10$ it is stable at r.t. for an extended time period, but at elevated temperature it hydrolyzes quite rapidly. However, for the preparation of **1a** with ^{99m}Tc, **1a′**, $[BH_4]^-$ was found to be sufficiently stable for the required reaction time. Thus, **1a′** could be prepared in a single step reaction in saline solution within 30 min. No limitation due to radiolysis at high radioactivity was found. Fig. 2 shows the reaction conditions and a typical HPLC trace of the crude reaction mixture. Compound **1a′** is stable for hours at neutral or acidic pH but is quite sensitive to oxygen at alkaline pH.

Although the concentration of $[^{99m}TcO_4]^-$ is < 10 μM, the straightforward approach is astonishing for several reasons. The solubility of CO in water is low and three of these ligands have to stabilize the reduced metal center by rapid coordination. Since we found that only $[BH_4]^-$ was able to act as a reductant, we conclude that some synergistic effects must be responsible, i.e. CO coordinated to BH_3 or a partially hydrolyzed borohydride. Other reductants, such as $[S_2O_4]^{2-}$,

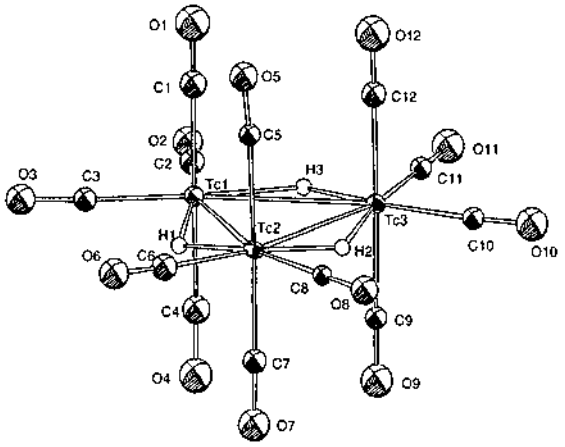
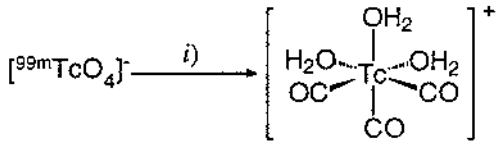


Fig. 1. ORTEP presentation of the mixed hydrido–carbonyl complex $[^{99}Tc(\mu\text{-}H)_3(CO)_4]_3$.



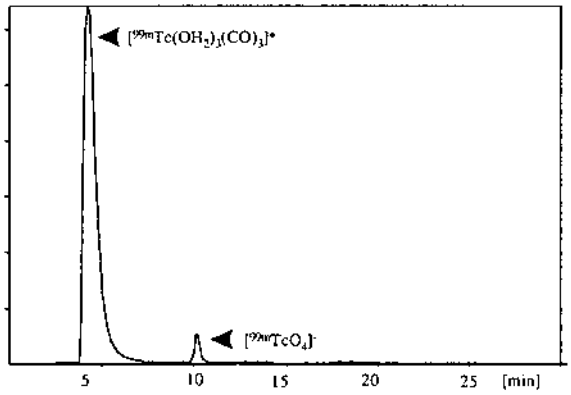


Fig. 2. Reaction conditions for the preparation of **1a′**: (i) generator eluate up to 37 GBq $[^{99m}TcO_4]^-$, 0.9% $NaCl/H_2O$, $NaBH_4$, vial filled with CO, 25 min 75°C (above): HPLC trace of the crude reaction mixture (below).

which is known to reduce Tc(VII) to Tc(I) showed no effect at all, and no formation of **1a′** could be observed. On the other hand, reduction in the absence of CO did not result in the formation of defined species apart from minor amounts of TcO_2.

This approach now fulfills the conditions for routine application. The ingredients can be prepared in a closed vial and flushed with CO. Subsequently, only the generator eluate has to be injected and the solution heated to 75°C for about 20–30 min. We want to emphasize that this entirely new procedure allows the introduction of a stable organometallic Tc(I) or Re(I) moiety, containing substitution labile ligands, in nuclear medical application.

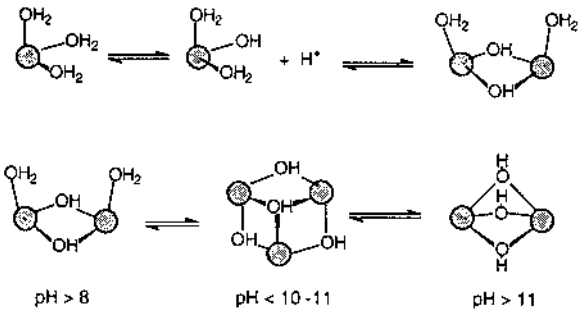
4. Hydrolytic behavior of $[MX_3(CO)_3]^{2-}$ (M = Re, ^{99}Tc) in water

When compounds **1a** and **1b** are dissolved in water, the halides were readily substituted by H_2O and very high halide concentration was required to get small amounts of mono-halide substituted species. Thus, **1a** and **1b** transform completely to the corresponding aqua-ions $[M(OH_2)_3(CO)_3]^+$ (M = Re, **2a**; M = ^{99}Tc, **2b**). Compounds **2a** and **2b** can, like **1a′**, be considered as 'semi aqua-ions'. Three coordination sites are occupied by very tightly bound CO ligands, whereas the remaining sites are occupied by three labilized water ligands. Like most aqua-ions,

2a/**b** act as Brönstedt acids, and can also be expected to exhibit this characteristic. However, mono-cationic species are in general not very acidic and the pK_a values are usually higher than 8 [11]. Deprotonation of coordinated water ligands might lead temporarily to terminal hydroxo complexes which subsequently tend to oligo- or polymerize by formation of μ-OH or μ-O species. If **1a**′ should be applied for labeling purposes, the characteristics of the species present in solution should be known at distinct pH values. Depending on the pK_a values of the coordinated water ligands, hydrolysis can be expected at lower or higher pH values. Macroscopic amounts of **2a** and **2b** have been titrated with $[OH]^-$ and the nature of the corresponding intermediates were deduced from stability constant calculations. Furthermore, most of the postulated species have been isolated and fully characterized [12]. We found, that below physiological pH 7.4, the aqua-ions **2a** or **2b** were indeed the predominant species. Upon addition of base, the expected deprotonation and a well defined oligomerization was observed. Essentially, two species could be rationalized at higher pH values, the trinuclear complex $[Re_3(\mu\text{-}OH)_3(\mu_3\text{-}OH)(CO)_9]^-$ and the dinuclear complex $[Re_2(\mu\text{-}OH)_3(CO)_6]^-$ (Scheme 3).

The pK_a of **2a** is about 7.5. The corresponding Tc complex is about one order of magnitude less acidic. This significant difference mirrors the contrasting electronic properties of the two elements. For both elements however, the hydrolytic oligomerization was slow (but fully reversible) and at least 30 min were required to establish equilibrium (typically at r.t. and with $[M] = 10^{-3}$M). The different pK_a values were not unexpected. Similar observations were also described for other homologue complexes of Tc and Re, in particular for the M(V) system $[MO(OH_2)(CN)_4]^-$ [13]. In that case, the Re compound is even more acidic, by 1.6 pK_a units, than the corresponding Tc complex.

However, formation of oligomers could not be detected at the no carrier added (n.c.a.) level with **1a**′. Even at high pH, only $[^{99m}Tc(OH_2)_3(CO)_3]^+$ was present as evident from HPLC studies. This can be explained by the very low concentrations of complex **1a**′ in solution. Additionally, the kinetic order of the reaction must be second or third order in Tc which reduces the rate dramatically when working in very diluted solution.



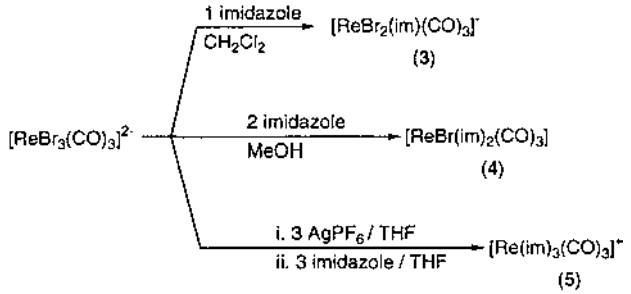
Scheme 3. Hydrolysis of **1a** or **1b** as a function of the pH.

5. Complex formation in aqueous solution

The major advantage of $[M(OH_2)_3(CO)_3]^+$ emerges from the high stability of the *fac*-$M(CO)_3]^+$ moiety in water and the potential of exchanging specifically the labile solvent ligands (i.e. H_2O). For the vast majority of Tc(I) or Re(I) compounds, the low valency is stabilized by pH independent ligands (i.e. phosphanes) which can hardly be substituted by other incoming chelators. Apart from thermodynamic exchange consideration, the M(I) center possesses a d^6 electronic configuration in an octahedral field. In general, such complexes are very well known to be kinetically inert. Combining these two features, one can expect even monodentate ligands, providing low thermodynamic stability, to form very inert complexes. Obviously, such an attempt depends, from case to case, on the electronic characteristics of the incoming ligand. However, even if the final complexes are very stable, the limiting factor for an application is essentially the rate of substitution. Although the water ligands are labilized by the *trans* COs, they are also stabilized by the *cis* COs [14]. If the latter effect governs the substitution process, the exchange might be too slow and will not occur on a reasonable time scale. In such systems, ligand exchange is purely based on a dissociative mechanism since no orbitals of convenient energy are present for the coordination of a seventh ligand (required for associative mechanism). It is well known that the rate of ligand exchange in the first coordination sphere of a metal center is influenced by the type of ligand in the second coordination sphere. Therefore, kinetic inertness of the product might be of higher relevance than the thermodynamic stability of the educt. If **1a′** should be used in the labeling of biomolecules, the mentioned substitution has to be fast and the resulting complexes must be inert.

Since the aqueous chemistry of the '*fac*-$[M(CO)_3]^+$' moiety is almost uncharted, we had to investigate its fundamental properties such as preferred ligand atoms, reaction rate and stability of complexes. It basically turned out that there was a large gap between formation rate and stability. Aliphatic amines as isolated ligand groups coordinate only weakly to **1a′** or **2a**/**b**. Carboxylate groups and other anions showed similar features. Thus, as potential anchor groups in biomolecules, they are not considered to be of high importance. Thioether groups coordinate very slowly, but their complexes were found to be of extremely high (kinetic) stability. On the other hand, aromatic amines combine both characteristics, such as reasonably fast complexation and very high thermodynamic or kinetic stability. From the classical HSAB concept and the comparison of pK_a values of these groups with that of **1a′**, this result could have been predicted in terms of thermodynamic but not of kinetic stability. Consequently, an aromatic amine should be present in the anchor group between the '*fac*-$[M(CO)_3]^+$' moiety and a biomolecule.

If one of the above mentioned groups was combined with an aromatic amine, the stability of the complex, as well as the rate of reaction, could be improved. The combination of an aromatic and an aliphatic amine and even more with an additional carboxylic acid, were found to be most advantageous in aqueous solutions. The fundamental coordination chemistry of the *fac*-$[M(CO)_3]^+$ moiety with such combinations will be exemplified in the next sections with the ligands imidazole, histamine and histidine.



Scheme 4. Reaction conditions for the formation of imidazole complexes from **1a** or **1b**.

6. Formation of imidazole complexes

The imidazole (im) moiety is very often encountered in biological molecules. In particular, this function is present in the side chain of the amino acid histidine (his). The relatively small ligand offers an aromatic sp^2 amine function for the effective coordination to metal centers. The systematic synthesis of the mono-, di- and trisubstituted complexes $[ReBr_2(im)(CO)_3]^-$ (**3**), $[ReBr(im)_2(CO)_3]$ (**4**) and $[Re(im)_3(CO)_3]^+$ (**5**) starting from **1b**, was achieved by varying the reaction conditions as outlined in Scheme 4. ORTEP presentations of the three complexes are given in Fig. 3.

^{1}H NMR investigations, performed in coordinating organic solvents (methanol or DMSO) at room temperature (r.t.), revealed the stepwise formation of the mono- and disubstituted intermediates $[Re(sol)_2(im)(CO)_3]^+$ (**3a**) and $[Re(sol)(im)_2(CO)_3]^+$ (**3b**), respectively. Evidence for the composition of **3a** and **3b** was obtained by

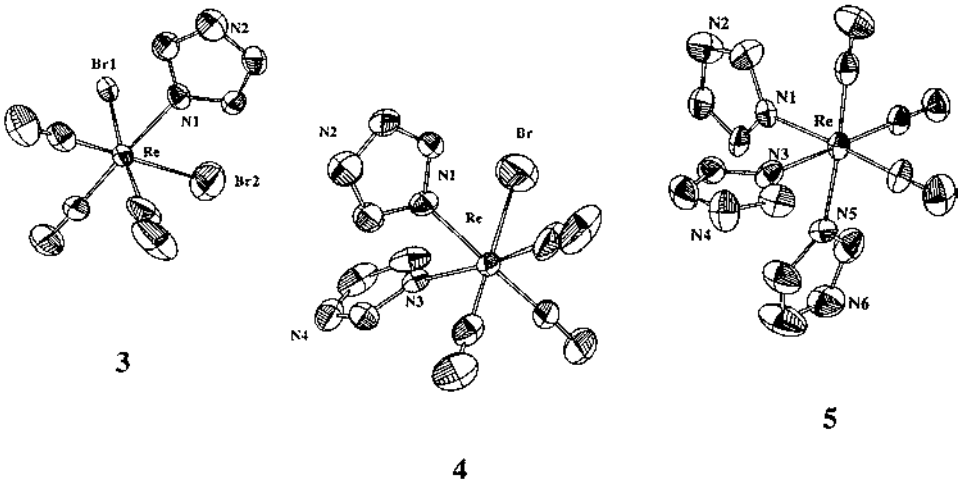


Fig. 3. ORTEP presentations of the three different imidazole complexes $[Re(Br)_2(im)(CO)_3]^-$ (**3**), $[ReBr(im)_2(CO)_3]$ (**4**) and $[Re(im)_3(CO)_3]^+$ (**5**).

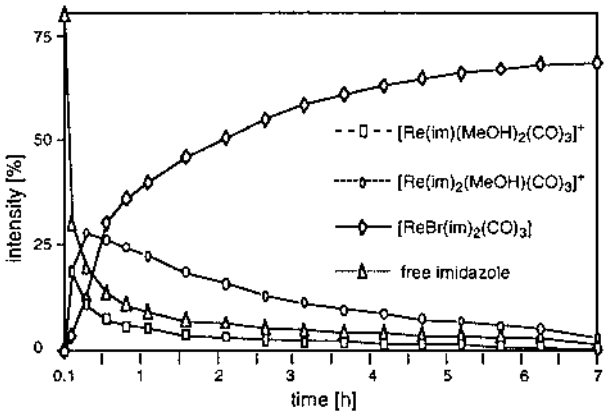


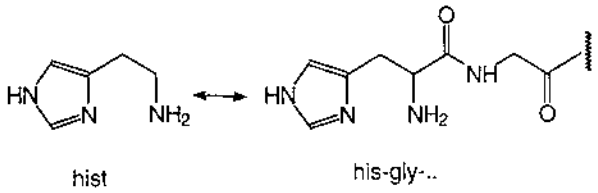
Fig. 4. Product distribution of the formation of imidazole complexes **3a**, **4a** and **4** as a function of time in d^4-methanol at r.t., ([**1b**] = 30 mM) followed by ^1H NMR spectroscopy.

comparison of the ^1H NMR spectrum of the reaction mixture with those of the pure compounds **3** and **4** (before and after precipitation of Br^-). The final step, however, was not the coordination of a third imidazole even when present in excess, but the re-coordination of a Br^- to form the neutral complex **4**. Fig. 4 displays the product distribution and the resulting substitution mechanism of the reaction between **1b** and two equivalents of imidazole in d^4-methanol. The fast coordination of one and two imidazoles, is followed by the slow re-coordination of the bromide.

The extraordinary high kinetic inertness of the coordinated imidazole was proven in a challenge experiment versus a 25-fold excess of fully deuterated d^4-imidazole in d^4-CH_3OH. Even heating of the complexes **3**, **4** or **5** in the presence of d^4-imidazole for several days did not significantly alter the shape and intensity of the original ^1H NMR spectrum. None of the originally coordinated h^4-imidazole was exchanged, but finally, only **5** with one, two or three h^4-imidazole of the original compound was formed. Therefore, the off-rate of coordinated imidazole is negligible.

Reactions on the n.c.a. level with **1a′** and imidazole in aqueous solutions (pH 6–8, phosphate buffer, 70°C) revealed a similar substitution behavior in terms of observed products (evidence for the formation of $[^{99m}Tc(OH_2)_2(im)(CO)_3]^+$ and $[^{99m}Tc(OH_2)(im)_2(CO)_3]^+$ by HPLC comparison with macroscopic amounts of the corresponding Re complexes). Coordination of a halide at the third position could not be detected unambiguously, but the formation of a tri-substituted complex $[^{99m}Tc(im)_3(CO)_3]^+$ could definitely be excluded. The rate of the reaction should be pseudo first order since the ligand concentration can be considered as constant. Complete complex formation with **1a′** was observed only after extended reaction time at elevated temperature (2 h at 75°C) and relatively high (in respect of **1a′** $< 10^{-6}$ M) ligand concentration (10^{-4} M).

Direct comparison of inertness as with macroscopic amounts of **1b** was obviously not possible due to the highly diluted solutions. In phosphate buffer (pH 7.4), a 10^5-fold excess of **2a** was added and the solution kept at about 50°C for several



Scheme 5. Histamine mimics an N-terminal histidine in a peptide sequence.

hours. Imidazole exchange to cold Re should result in an intensity decrease of the radioactive peak of $[^{99m}Tc(OH_2)(im)_2(CO)_3]^+$ detected by HPLC. This was essentially not the case, demonstrating that the off-rate of imidazole is the limiting factor concerning stability of the once formed complexes.

7. Formation of histamine complexes

The bidentate chelate histamine (hist) is a logical extension of the imidazole system and has been chosen as a model for an N-terminal histidine in proteins or peptides. This motive would provide the same coordinating groups to **1a**′ from a biomolecule. The coordination of the *fac*-$[M(CO)_3]^+$ would be realized by the imidazole and the primary amine functionalities. Hist mimics this chelate moiety perfectly (Scheme 5).

The reaction with macroscopic amounts of **1b** yielded the neutral complex $[ReBr(hist)(CO)_3]$ (**6**) almost quantitatively. The substitution rate was similar to that observed with imidazole, thus, coordination of hist was relatively fast ($T_{1/2}$ = 30 min) followed by the slow re-coordination of a bromide. Fig. 5 shows the time dependent ^1H NMR spectrum of the formation of **6** in d^6-DMSO. As expected, the

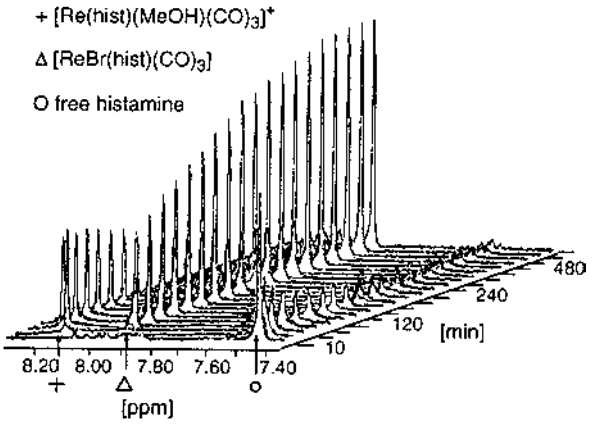


Fig. 5. Formation of $[ReBr(hist)(CO)_3]$ ([**1b**], [hist] = 27 mM) in d^4-methanol at r.t. observed by ^1H NMR spectroscopy as a function of time.

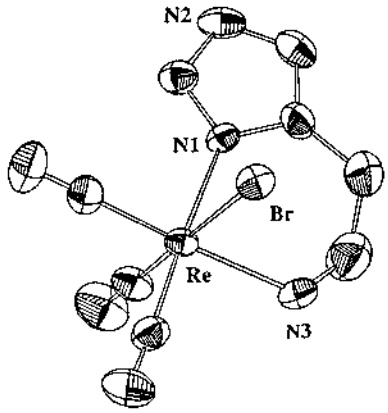


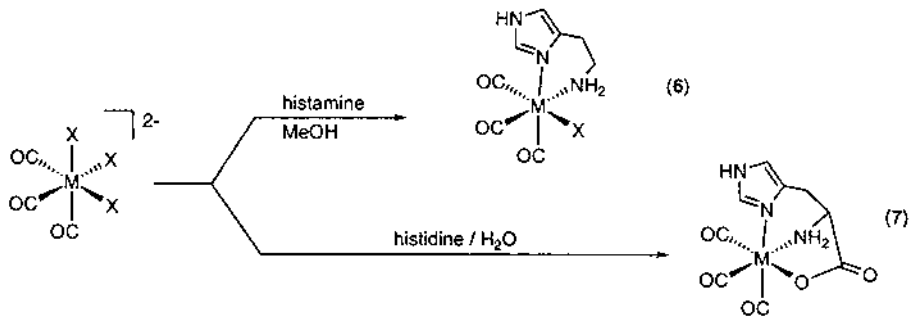
Fig. 6. ORTEP presentation of the neutral complex $[ReBr(hist)(CO)_3]$.

ligand coordinates bidentate with both amine functionalities, forming a flexible six membered ring (Fig. 6).

Complex formation with **1a**′ and hist in phosphate buffer (pH 7.4) was quantitative at ligand concentration as low as 10^{-5} M (30 min at 75°C). Thus, the combination of imidazole with the primary amine function in histamine resulted in an improved complex formation with **1a**′ at a ten times lower ligand concentration than with imidazole only.

8. Formation of histidine complexes

The amino acid histidine offers three potentially coordinating functionalities (Scheme 6). Although it is not obvious from its size and conformation, histidine is able to coordinate tridentately and facially to a metal center with only minor distortion of the complex geometry. The same holds true for the neutral complexes $[M(his)(CO)_3]$ (**7a** and **7b**). The $-NH_2$ group and sp^2-nitrogen form a six membered



Scheme 6. Formation and similarities in the structures of $[ReBr(hist)(CO)_3]$ and $[Re(his)(CO)_3]$.

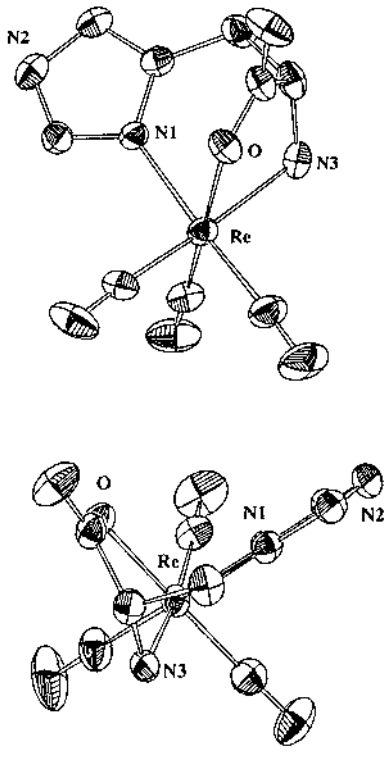


Fig. 7. ORTEP presentations of [Re(his)$(CO)_3$]along two different axes.

ring with the metal center, comparable to the one found as a motive in the structure of **6**, whereas the primary amine and carboxylic acid form a five membered chelate. An ORTEP presentation of **7b** is given in Fig. 7. The ligand has a bent conformation due to the tridentate coordination. The neutral complex precipitated almost quantitatively from water after several hours at r.t. and at a final pH value of close to 1 (H^+ from coordinated histidine).

Although this tridentate coordination cannot be provided by a histidine attached to or integrated into a protein sequence, easy derivatization of histidine at the primary amine with, for example, an acetic acid group could in principle allow the introduction of the same ligand into biomolecules (see Section 10).

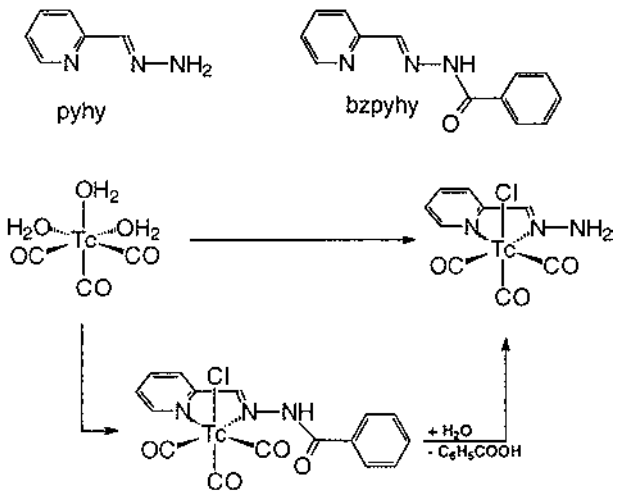
Applying histidine on the n.c.a. level with **1a′** fully confirmed the results found with **2a**/**b**. Quantitative complex formation of **7a′** on the n.c.a. level at ligand concentrations lower than 10^{-6} M and after 60 min at 75°C were realized. This, again, is by a factor of 10 lower in concentration than in the case of histamine. At this concentration level, the ratio between ^{99m}Tc and histidine is almost stoichiometric. Therefore, labeling of biomolecules with the organometallic *fac*-$[M(CO)_3]^+$ moiety and a histidine like ligand would result in preparation of radioconjugates of very high specific activity, which could be applied without separation and/or purification from unlabeled material.

The preparation of **7a′** was not only achieved in a stepwise synthesis ($[^{99m}TcO_4]^- \rightarrow$ **1a′** $\rightarrow$ **7a′**) but also in one step and in situ preparation of **1a′** only. Addition of generator eluate to a vial, containing the ingredients for the preparation of **1a′** and tiny amounts of histidine, yielded complex **7a′** exclusively and quantitatively in one step. The potential of this approach with respect to practical application in nuclear medicine should be emphasized again.

9. Formation of a pyridine–hydrazone complex

A very powerful alternative to the above mentioned chelators is pyridine–hydrazone (pyhy), the condensation product between pyridine-2-aldehyde and hydrazine. This bidentate ligand is known to coordinate to a variety of b-type metal centers. The reaction with **2a**/**b** depicted in Scheme 7 was very fast. As evident from ^1H NMR spectroscopy and elemental analysis, the composition of the corresponding complexes was $[MX(pyhy)(CO)_3]$. The coordination occurred by the two nitrogens and resulted in a five membered chelate. Thus, the $-NH_2$ group remains free for covalent binding to a biomolecule.

Although pyhy coordinates only in a bidentate manner, it reacted rapidly with **1a′** and at almost stoichiometric ratios. The complex $[^{99m}TcX(pyhy)(CO)_3]$ was found to be stable in serum over a 24 h period of time at 37°C without any decomposition. One of the potentials of the $-NH_2$ in the pyhy group could be the formation of hydrazides with carboxylic acids present in a variety of biomolecules. As a model complex, pyhy was linked to benzoic acid to yield bzpyhy. Complex formation of bzpyhy with **1a** occurred readily and with a comparable rate as with pyhy itself. The X-ray structure of the complex $[TcCl(bzpyhy)(CO)_3]$ (**8**) (ORTEP) is presented in two different views in Fig. 8.



Scheme 7. Structure of pyhy and bzhypy, synthesis of $[TcX(pyhy)(CO)_3]$, synthesis of $[TcX(bzpyhy)(CO)_3]$ and subsequent hydrolysis at mild conditions.

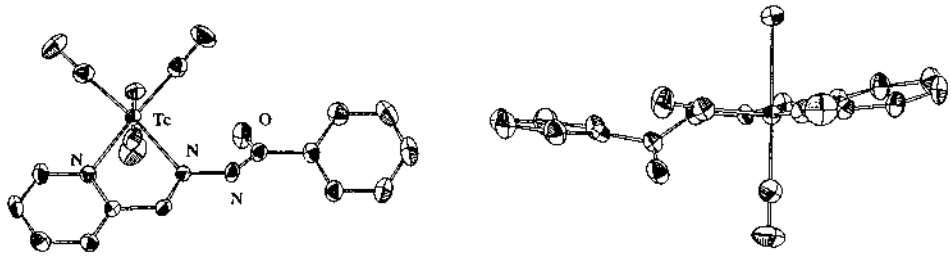


Fig. 8. ORTEP presentations of [TcCl(bzpyhy)$(CO)_3$] along two different axes.

The reaction with **1a**′ and bzbyhy occurred at comparable rates and yields as in the case of pyhy. However, instead of one product, two products were detected by HPLC. One peak represented the intended complex **8**′, whereas the second one was the pyhy complex **7**′. The latter one could only be formed by hydrolysis of the hydrazide bond, which represents an unusual reaction. The ligand itself resisted the applied reaction conditions very well and the hydrazide bond is known to be very stable. The metal coordination must be responsible for the weakening of the –C(O)–NH– bond. This behavior however, became clear when analyzing the structure depicted in Fig. 8. The planar ligand is strongly twisted (the C=O group by an angle of about 30° out of plane) upon coordination. The strong distortion leads to a significant weakening of the hydrazide bond and hydrolytic cleavage becomes possible. It is nevertheless remarkable that complex formation took place at all, despite the fact, that steric interactions between the CO ligand and the carbonyl group of the hydrazide must be very strong.

In respect of application and coordination chemistry, two important conclusions could be drawn from these experiments: (i) the pyridine–hydrazone, although a strong ligand, is not convenient in combination with a potential biomolecule when linked over a carboxylic acid; and (ii) steric interaction between CO and the ligand or the biomolecule must seriously be considered in advance with relevant model ligands.

10. Combination of organometallic chemistry and radiopharmacy: bioorganometallic chemistry

The basic coordination chemistry of the organometallic '*fac*-$[M(CO)_3]^+$' moiety in water with model ligands discussed in the previous sections, must be applied to biomolecules bearing the respective chelators. Only this will lead to bioorganometallic chemistry and, finally, to the application of organometallic precursor complexes in biology or medicine and taking advantage of the unique properties of this type of compounds [15]. In an optimal case, the conditions for complex formation elaborated with the model ligands should be adaptable to biomolecules derivatized with the respective chelators.

The imidazole group is a very versatile ligand for the formation of complexes with **1a**′. Imidazole occurs naturally in the side chain of histidine and is thus, present in most of the proteins. If histidine is integrated into the protein sequence (at the N-terminus), complexation with **1a**′ must be comparable to the results found for histamine. Nowadays, small peptides (penta- to deca-peptides) and recombinant single chain fragments Fv (scFv) are of particular interest in cancer diagnosis and therapy [16]. Two examples from these large groups of biomolecules will be presented in this section to show that the elaborated coordination chemistry can be applied to these biomolecules.

The receptor binding motive of naturally occurring peptides has found growing interest in the last few years. In Particular, octreotide, a cyclic peptide, has been studied extensively in that respect [17]. Our group is interested in the poly-peptide neurotensin [18], a 13-amino-acid poly-peptide, which has the binding sequence H_2N–arg–arg–pro–tyr–ile–COOH (NT8-13). Although NT8-13 contains no histidine, the sequence can be easily extended at the N-terminus by one additional histidine through an amide bond. This derivatization will provide an histamine system for the complexation of the '*fac*-$[M(CO)_3]^+$' moiety since the carboxylic acid group is required for amide bond formation. No other competing sites are present in the peptide. Interference with the binding sequence should therefore be retained at a minimum. For size comparison, a calculated model of the labeled peptide is shown in Fig. 9.

Applying the same conditions for the peptide labeling as described for the complex formation with histamine, resulted in essentially the same yields and in the formation of one single species [19]. Although no direct proof for the anticipated coordination can be given, experiments have shown, that NT8-13 without an N-terminal histidine was not labeled by **1a**′ at all. Therefore, it is likely that **1a**′ is coordinated by the imidazole and by the aliphatic amine group.

Conditions resulting from model studies could directly be transferred to the corresponding biomolecules and the latter one's site specifically labeled with the

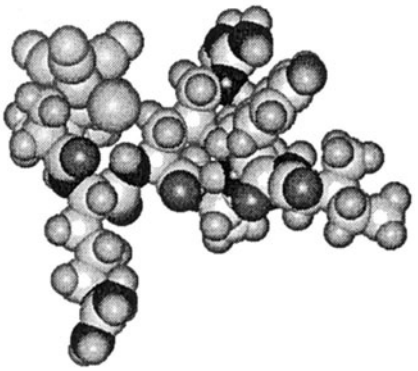


Fig. 9. Calculated structure of $[TcCl(CO)_3$(His–Arg–Arg–Pro–Tyr–Ile)] for relative size comparison and orientation. The *fac*-$Tc(CO)_3$ moiety is in the upper left corner of the peptide.

organometallic complex. The attached complex is relatively small and its hydrophilicity comparable to the whole NT8-13 peptide. The labeled peptide was stable in serum over a 24 h period of time without decomposition. In vitro affinity constants with the receptor were found to be in the same range as for the native peptide confirming no or only weak interaction with the binding site. The advantages compared to other procedures are obvious. The approach is straightforward, neither synthesis of special ligands nor inconvenient derivatization techniques are required. The three CO ligands stabilize the low valency, and additional bidentate chelators are able to protect the metal center in competing environments such as serum against decomposition or transmetallation. The stability, mainly based on inertness of the Tc(I) center, allows application of small and naturally occurring ligand systems.

The second example concerns recombinant single chain F_v antibody fragments. These proteins with a molecular weight of about 25 kD bear at the C-terminus an additional sequence of 5–6 histidines. This sequence is not required for antibody binding but was introduced for ease of purification on Ni-affinity columns. Obviously, the imidazole side chains of these histidines can be used as multidentate anchor groups for the '*fac*-$[M(CO)_3]^+$' moiety. Different modes of coordination could be expected. Monodentate binding to a single imidazole, bidentate complexation by two neighbored, or by two separated imidazoles, are possible, as well as coordination including the carboxylic group at the end of the sequence (C-terminus). In contrast to the aforementioned small peptides, these scFv proteins do not generally resist temperatures above 37°C, otherwise denaturation of the structure with concomitant loss of bioactivity would result. However, the versatility of the '*fac*-$[M(CO)_3]^+$' moiety could also be shown in this case. Mixing the organometallic aqua-ion **1a**′ with the scFv at concentrations of about 10^{-5} M resulted in a rapid and quantitative labeling of the protein [20]. Based on the discussion above, it is not yet clear which of the different modes occurred predominantly. The labeled proteins are stable in serum and, what is of basic importance, no transmetallation to serum proteins could be observed. The carbonyl complex remained tightly bound to the scFv. In vivo studies finally showed that the proteins localized the tumor comparable to their iodinated analogues.

These two examples clearly exhibit the potential of the organometallic aqua-ion $[Tc(OH_2)_3(CO)_3]^+$ as a site specific and easy to handle label of biomolecules. It furthermore shows, that basic studies on the coordination chemistry with model complexes can be transformed to the same coordination chemistry but in the presence of a biomolecule. These two experiments clearly show that **1a**′ can act as a general label for a wide variety of vectors, since no complicated and inconvenient derivatization of the latter ones is demanded.

11. Summary

The complexes $[MX_3(CO)_3]^{2-}$ (M = Tc, Re; X = Cl^-, Br^-) can be prepared by normal pressure low temperature approaches directly from $[MO_4]^-$ in quantitative

yield. In particular, the ^{99}Tc complex is a versatile synthon for the development of low valent organometallic technetium chemistry. In water, the three halides are substituted by H_2O forming the tris-aqua complex $[M(OH_2)_3(CO)_3]^+$, which is perfectly stable in this medium. For trace amounts of ^{99m}Tc, the same complex can be prepared directly from water in a single step procedure, resulting directly in quantitative amounts of $[^{99m}Tc(OH_2)_3(CO)_3]^+$. The coordination chemistry of the latter one in water can best be described as that of a typical first row transition element aqua-ion, but with only three water ligands available for exchange reactions. The water ligands are exchanged by a wide variety of ligands. As a consequence of the d^6 electronic configuration, the resulting complexes are of extraordinary inertness and do not exchange the ligands even in strongly competing media. In the context of radiopharmaceutical application, ligands derived from the imidazole function were shown to offer a major advantage. Finally, $[^{99m}Tc(OH_2)_3(CO)_3]^+$ reacts easily with derivatized biomolecules to form in vitro and in vivo stable radioconjugates for application in nuclear medicine.

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