The Facettes of $[^{99}\text{TcCl}_3(\text{CO})_3]^{2-}$ Chemistry and Its Application to Life Science

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This article gives a brief summary about the current status of radiochemistry and radiopharmaceutical chemistry as related to the fac-$[^{99}\text{mTc(OH}_2)_3(\text{CO})_3]$ and fac-$[^{99}\text{Tc(OH}_2)_3(\text{CO})_3]$ synthon. Since the synthesis and availability of synthons or direct precursors for the labeling of biomolecules (and for the exploration of fundamental inorganic and organometallic chemistry) is a crucial prerequisite for developing new radiopharmaceuticals, some consideration about composition and characteristics of such complexes are discussed at the beginning and their suitability is discussed. Beside widely investigated basic and applied Tc chemistry in the oxidation state (V) with the [Tc=O]$^{3+}$ or [Tc=N]$^{2+}$ moieties, the aforementioned $[^{99}\text{mTc(CO)}_3]$ and $[^{99}\text{Tc(CO)}_3]$ core are new members in this group. Although its pressureless synthesis from organic solvents has been described about eight years ago, it is only recently that exploitation of its radiopharmaceutical potential could reasonably start owing to the availability of a routinely applicable kit. We will summarize basic coordination chemistry of the $[^{99}\text{mTc(CO)}_3]$ and $[^{99}\text{Tc(CO)}_3]$ moieties as relevant for radiopharmaceutical purposes, focusing on some fundamental reactions in the context of low valent Tc chemistry. Due to its special properties, new research directions possible only through its very special physico-chemical properties will be emphasized as well.

**Synthons.** The importance of stable but reactive precursors, so called synthons, is very well recognized in synthetic inorganic chemistry. A synonym for synthon is “building block”, thus, kind of a brick stone for constructing different structures. The situation in chemistry is related to architecture and one of the most important and common building blocks in technetium chemistry is probably $[^{99}\text{TcOCl}_4]$ from which the preparation of most novel complexes started.1,2 The synthon $[^{99}\text{TcOCl}_4]$ is well suited for chemistry in organic solvents, but, switching to aqueous solutions makes it less versatile due to its tendency to hydrolyze and to disproportionate. Real technetium synthons stable in water are rare but highly important since the driving force for research in technetium chemistry is usually the application in radiopharmacy. Suitable properties of such an aqueous synthon are easily defined; i) for well characterized composition, ii) of sufficient stability towards hydrolysis or redox decomposition, iii) exchangeable ligands and optionally iv) forming robust complexes. Typically, aquo-ions of the lanthanides and other triply positive metal-cations fit well in this scheme i) – iii) and are therefore most widely explored in radiopharmaceutical chemistry.3 The situation is different in technetium chemistry, where no real aquo-ions exist. The typical precursors in technetium chemistry comprise the $[^{99}\text{Tc}=\text{O}]^{4+}$ and to a lower extent the $[^{99}\text{Tc}=\text{N}]^{2+}$ core. Despite being very useful, they are not real synthons since they often lack accurate characterization, long term stability or require the presence of additional organic solvents. They can however be stabilized in situ with strong multidentate chelators based on pure or mixed sets of nitrogen, oxygen and sulfur donors or phosphorous donors the number or combination of which is often not very flexible. It is suggested to ask the question at this point what kind of synthon one would ideally like to have for radiopharmaceutical research and development. This is not easily answered and depends strongly on the particular problem. Such compounds probably would have exchangeable water ligands in common, independent of oxidation state or electronic configuration. The synthesis of $[^{99}\text{Tc(OH}_2)_3(\text{CO})_3]$ as shown in Scheme 1 approaches an ideal synthon in that it fulfills, from a chemical point of view, most of the requirements outlined above.6–9 Throughout the following text, the formulation “$[^{99}\text{mTc(OH}_2)_3(\text{CO})_3]$” refers to experiments which have been performed with long-lived $^{99}$Tc and with short-lived $^{99}$mTc. The $[^{99}\text{Tc(OH}_2)_3(\text{CO})_3]$ synthon is synthesized from water, comprises exchangeable water ligands and is stable even on the no carrier added $^{99}$mTc level over extended time periods. Depending on the view point, the three CO ligands might be considered a disadvantage since they provide lipophilicity and are relatively large in comparison to a potential water ligand. However, it is an advantage that they occupy three coordination sites irreversibly and potential ligands must have a maximum of three donors. Ligands with three donors only (or less) might be small and even very small ligands can be designed such as cyclopentadienyl systems (see later in the text).10 The reactivity and coordination chemistry of $[^{99}\text{Tc(OH}_2)_3(\text{CO})_3]$ with many types of mono- bi- and tridentate ligands have been studied in detail but still new surprising features and research directions emerge. Scheme 2 gives an overview on the most important and common ligands for “stand alone chemistry” or suitable for being attached to targeting vectors.11–13

Cyclopentadienyl Chemistry. The example of cyclopenta
dienyl (Cp) ligands shall be discussed in more detail and in front of a general consideration. While reactions with most of the ligands depicted in Scheme 2 are of the “Werner type”, Cp is an organometallic system which chemistry seems not really to be related to water as a solvent. Cyclopentadiene is not stable or soluble in water but the corresponding ligand with an acetyl group (acp) is. Deprotonation occurs easily and at phys-
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![Scheme 2. A selection of divers ligands suitable for strong coordination to the fac-[Tc(CO)₃]⁺ moiety in aqueous solution.](image)

![Figure 1. ORTEP presentation of [(H₃COC₆H₄COC₅H₄)Tc(CO)₃]. Ellipsoids drawn with 50% probability.](image)

Intercalators. A further and rather provocative example in the context of new possibilities is the combination of °⁹⁹Tc with intercalating cell nucleus seeking molecules such as acridine, acridine orange of pyrene. A less known characteristic feature of °⁹⁹Tc is its possibility to emit about 2 Auger and Koster-Cronig electrons per decay (and electrons resulting from interconversion). The principal design of such trifunctional molecules is depicted in Scheme 3.

As could be shown, the °⁹⁹Tc(OH₂)₃(CO)₃⁺ based complexes with a pendant pyrene have in vitro the ability of inducing an activity dependent amount of single and double strand breaks. Combined to the NLS peptides, these radiobioconjugates reduced the cell viability to a significant extent. Cells exposed to equal amounts of °⁹⁹TcO₄⁻ or to the "cold" rhenium surrogates had no effect at all. Although the synthesis of the trifunctional molecule, ligand plus intercalator plus nucleus targeting portion, is relatively demanding and of low flexibility. We have altered the approach by selecting a more flexible [2+1] mixed ligand strategy in which the targeting function is attached to a monodentate ligand and the intercalator to a bidentate chelator or vice versa. This will enable more convenient (fine) tuning of the systems or to select different nucleus seeking agents without the need of repeating complicated multistep organic syntheses every time. The topic of applying °⁹⁹Tc for therapeutic purposes is the subject of controversial discussions for reasons as outlined above. Such reasons might be true or not but it should be emphasized that the same approach or strategy can also be verified with °⁹⁸⁰Re being considered to be among the most important therapeutic radionuclides.

The advantage of using °⁹⁹Tc(OH₂)₃(CO)₃⁺ as a building block is evident in this example. A complicated trifunctional biomolecule simply reacts with this synthon under mild conditions. The concept could also be realized with any of the other °⁹⁹Tc(V) based precursors but would probably be more complicated, leading to byproducts due to the presence of a multicomponent system.

Vitamin B₁₂. So far, ligands coordinated to the fac-[Tc(CO)₃]⁺ moiety were bi- or tridentate for reasons outlined elsewhere. It has been described that even monodentate ligands can replace water and form highly inert complexes. Typical examples are aromatic amines such as imidazole or pyridine but also phosphines and thioethers are appropriate groups. The coordination to monodentate ligands can for instance be used for the direct labeling of biomolecules bearing corresponding coordinating functionalities. A typical example in this respect are the imidazole in the side chains of histidine which have repeatedly
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been described for labeling with \([\text{Tc}^\text{III}(\text{OH}_{2})_3(\text{CO})_3]\).\(^29,30\) Coordinated cyanide is one of the oldest "ligand" known in inorganic chemistry for forming bridges [M]-CN-[M'] between two metal centers. Since cyanides are not common in biological systems, the application of those for labeling purposes is unknown. Vitamin B12 is the exception and comprises in its basic structure a cyanide coordinated to the central Co(III) cation in vitamin B12. We found that this cyanide is very prone to bind to \([\text{Tc}^\text{III}(\text{OH}_{2})_3(\text{CO})_3]\), thereby forming a complex as depicted in Figure 2.\(^31\)

Although the cyanide in this example is a bridging monodentate ligand only, the coordination is very strong. The technetium complex bond to it is only very slowly released even in the presence of serum proteins. Two coordination sites remain in principle occupied by water or chloride and might therefore interact with other competing donors. The stability in serum proves the contrast but these two sites can also be occupied with an anionic bidentate ligand to shield the Tc(III) center from further interactions. Since many bidentate ligands can be considered for this purpose, a (fine) tuning of the biological properties becomes possible without changing the overall topology of the B12 derivative. Clearly, with an asymmetric bidentate ligand two diastereomers are formed which have to be investigated after separation. It is still remarkable that the two diastereomeric forms, once separated from each other, do not interconvert at all proving the high kinetic or thermodynamic stability of the [Co]-CN-[Tc(Re) bond].\(^31\) Since the important transport proteins for B12 in humans bind essentially to the lower hemisphere of B12, the introduction of a metal complex at the upper should not interfere to a large extent with this recognition, an important fact that is confirmed by preliminary biological studies.

Again, it can be emphasized here that the experimental findings with technetium inspired research with other transition metals as well. It is surprising that no studies with B12 and other metals have been presented before. We found that other metal complexes or even building blocks such as different Pt(II) complexes readily bind to cyanide, a fact which is not surprising for an inorganic chemist but still has not been attempted before.\(^35\) The far goal of such a strategy in the context of technetium or other metals is the use of vitamin B12 as a Trojan horse for radio- and/or chemotoxic compounds.

Nucleobases. As a last example, the reactivity towards guanine should briefly be mentioned. As the reaction with vitamin B12 is a good example for a stable monodentate ligand, \([\text{Tc}^\text{III}(\text{OH}_{2})_3(\text{CO})_3]\) and its rhenium analogue are also prone for coordination to the nucleobase guanine. N7 in guanine is the preferred target of cisplatin and its irreversible coordination to two adjacent guanines in DNA is believed to be the reason for its chemotoxic action.\(^33-35\) Octahedral complexes are not generally considered to copy this reactivity since the relatively bulky nucleobases in isolated form or integrated in DNA might sterically interfere with the other ligands. We have studied the reaction of \([\text{Tc}^\text{III}(\text{OH}_{2})_3(\text{CO})_3]\) with 9-methyl guanine (9-MeG) and 7-methyl guanine (7-MeG) and found that two guanines coordinate relatively rapid to Tc(1).\(^36\) An example of the complex \([\text{Tc}^\text{III}(\text{OH}_{2})_3(9-\text{MeG})(7-\text{MeG})]\) is given in Figure 3.

The compound is stable in serum but its formation on the no carrier added level with \([\text{Tc}^\text{III}\text{O}]\) is slow. Still, since \([\text{Tc}^\text{III}(\text{OH}_{2})_3(\text{CO})_3]\) is obviously able to adopt two guanines, one might think about its use for chemotoxic purposes. The numerous problems to overcome are of course comparable to what has been described with the intercalators but the principal physico-chemical authenticity is given. We found that the \([\text{Re}(\text{OH}_{2})_3(\text{CO})_3]\) induces in vitro irreversible conformational changes in plasmide DNA as was previously observed for cisplatin.\(^37,38\) The compound also clearly reduces the viability of cells but on a higher concentration level than cisplatin. Assuming that \([\text{Tc}^\text{III}(\text{OH}_{2})_3(\text{CO})_3]\) or \([\text{Re}(\text{OH}_{2})_3(\text{CO})_3]\) would find their way into the nucleus, an intriguing possibility of combining both radio- and chemotoxicity with one single compound would emerge. The coordination to guanine and small nucleotides includes more realistically the potential of direct labeling of antisense oligonucleotides as requested for gene therapy or for the antisense strategy without the need of conjugating a ligand to these biomolecules.

In conclusion, these three brief examples should underline the importance of new synths in what ever respect and as represented by \([\text{Tc}^\text{III}(\text{OH}_{2})_3(\text{CO})_3]\). It should also encourage inorganic chemists to reactivate the corresponding research with simple compounds and to think about novel building blocks. As shown with the example from cyclopentadienyl chemistry, research directions with other transition metals can be initiated and very interesting results obtained, relevant not only for technetium chemistry but also for very different fields such as catalysis. Although much remains to be done and to be proven, the example with the intercalators stands for new directions within radiopharmacy and exceeding the ever lasting topic of labeling peptides with bifunctional chelators. New synths with properties different from \([\text{Tc}^\text{III}(\text{OH}_{2})_3(\text{CO})_3]\) might lead to other approaches which are in the worst case interesting for fundamental research. Vitamin B12 is a good example for a surprising and unexpected finding, and likely to have a long term impact in basic inorganic and applied radiopharmaceutical chemistry.
References