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$[Tc(CO)_3]^+$ chemistry: a promising new concept for SPET?

For

The relevance of the "carbonyl approach"—the concise term for the use of $[M(OH_2)_3(CO)_3]^+$ (M=^{99m}Tc, ^{188}Re) for the labeling of biomolecules—is the subject of continuing controversy. This commentary presents arguments in favor of the carbonyl approach, mainly based on chemical considerations. "New approaches are not essential" and "what is available already satisfies our needs" are quite frequent objections to application of the carbonyl approach. It is redundant to comment on this, since any reasonable and worthwhile approach will contribute in extending the scientific horizons of a research field. Ultimately, the innovations offered by researchers and market demand will decide the utility of a novel concept.

With the introduction of the "Isolink" kit (Mallinckrodt Medical B.V.), it has become feasible to complement the radiopharmaceutical research armamentarium with an organometallic moiety as an additional powerful tool. Thus, the complex $[^{99m}Tc(OH_2)_3(CO)_3]^+$ has mutated from an "exotic organometallic moiety with toxic ligands" to a serious, routinely available compound [1]. This progress paves the way for new radiopharmaceuticals both in current research developments and in future clinical applications.

Arguments for and against a proposition are frequently complementary. Often, they are ranked from a subjective standpoint; hence, positive (or negative) arguments regarding any novel approach first require a general (subjective) analysis of the actual situation. This analysis should include future objectives and the rationale for identifying new targets. In the current context, the limitations of ^{99m}Tc-based radiopharmacy need to be considered, with a view to ascertaining (a) whether there are persuasive scientific arguments in favor of the carbonyl approach relative to the established methods and (b) whether arguments supporting the feasibility of the carbonyl approach are sufficient to lead to an impact on market demand. There is in fact no doubt that ^{99m}Tc radiopharmacy needs innovative inputs from chemistry and not only from biology in order to retain its strong position vis-à-vis more competitive non-radiodiagnostic methods. New approaches aiming to improve functional diagnosis for a variety of diseases and a better understanding of physiological processes are inherently attractive.

Despite enormous efforts worldwide, the number of FDA-approved ^{99m}Tc- and ^{188}Re-labeled vectors is rather disappointing. One reason for this failure is that, chemically speaking, a metal complex tagged to a biological vector is not innocent; in addition, the suitability of the radiopharmaceutical for routine application may be insufficient to warrant commercial production. The search for clinically useful and affordable ^{99m}Tc- and ^{188}Re-based compounds relies essentially on the $[Tc=O]^{3+}$ core, wrapped by tetradentate NS or NO ligands and covalently linked to a targeting molecule. These well-established techniques are complemented to a minor extent by the mixed ligand [3+1] or the HYNIC approach [2].

Chemical steps in the conventional development of novel radiopharmaceuticals are outlined in Fig. 1. There are essentially two aspects to be considered, the preparation of the precursor and the selection of the ligand. Precursors such as $^{111}In^{3+}$ or Ln^{3+} form well-defined aquo ions $[M(OH_2)_n]^{3+}$ and do not require cumbersome redox chemistry, as in the case of $[^{99m}TcO_4]^-$. Routine application is therefore very convenient. The multitude of potential ligands that may be coordinated to the same core (and hence the multitude of complexes) allows systematic screening. Since the metal complex will affect the vector in a negative or (rarely) a positive way, the more the physicochemical properties of the metal complex can be altered, the greater is the chance of finding an ideal candidate. Such variation of the metal side in radiopharmaceutical chemistry means drug development. This can be compared to what is carried out with normal pharmaceuticals. The well-established ligands for stabilization of the $[Tc=O]^{3+}$ moiety are problematic for varying the properties of the ^{99m}Tc tag since tetra- and tridentate chelators tend to be synthetically and stereochemically demanding. Other labeling approaches such as HYNIC

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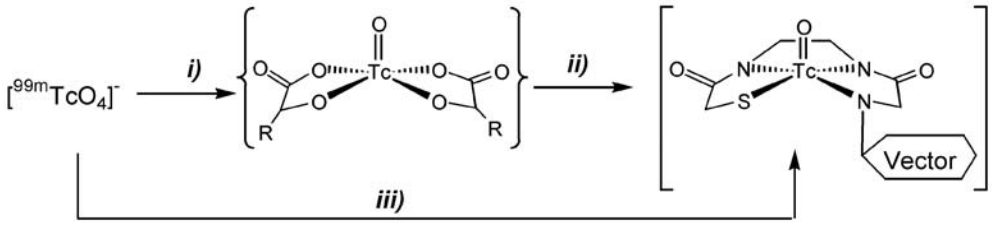

Fig. 1. Steps in the conventional development of novel radiopharmaceuticals: i) reduction, stabilization with coligands; ii) labeling of biomolecule; iii) one-step pathway

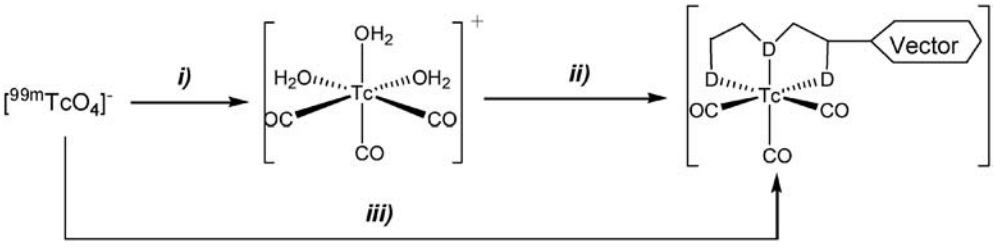

Fig. 2. Steps in the synthesis of labeled vectors in the carbonyl approach

are more restricted with respect to the vector for which they are suitable. The arguments supporting the carbonyl approach discussed later in this commentary refer to these fundamental considerations.

Accordingly, the situation as depicted in Fig. 1 would be significantly improved by the existence of an aquo ion of ^{99m}Tc forming stable complexes with a wide variety of ligands. The more ligand variations are available to coordinate to the metal center along an identical labeling route, the better are the chances of finding a novel radiopharmaceutical. A class of chelators could then be selected matching the physicochemical properties of the vector.

The carbonyl approach (Fig. 2) is in harmony with both of these requirements as it merges the advantages of aquo ions (In^{3+} or Ln^{3+}) with the electronic possibilities of transition metal ions.

Aquo ions of Tc, desirable but not likely to exist, are replaced by $[^{99m}Tc(OH_2)_3(CO)_3]^+$. This precursor is the focus of the carbonyl approach and can be called a "semi aquo ion" with three tightly bound COs and three labile H_2O ligands. This situation compares well with that of $[Ln(aq)]^{3+}$; however, the carbonyl approach is more favorable since only three coordination sites have to be considered for ligand design. $[^{99m}Tc(OH_2)_3(CO)_3]^+$ represents a stable, but substitution-reactive precursor with a d^6 electronic configuration. Importantly, as a precursor it does not necessarily require coligands, as in Tc(V) oxo species, to stabilize the +I oxidation state, and independent of temperature, pH and time, the only product prepared in the kit is $[^{99m}Tc(OH_2)_3(CO)_3]^+$. It can be stored for hours in the kit like a normal 1st row transition metal aquo ion. Sensitivity at room temperature against O_2 is moderate compared with the Tc(V) intermediates. $[^{99m}Tc(OH_2)_3(CO)_3]^+$ is a general precursor for labeling chemistry (step ii), but it can also be prepared in situ (step iii), as discussed later. We should not forget at this point that therapy is the future goal. Radionuclide-based therapy is not in competition with other non-nuclear medicine therapeutic methods and can complement chemotherapy in the treatment of cancer. Synthesis of $[^{188}Re(OH_2)_3(CO)_3]^+$ is also possible, although it entails more difficulties, and in contrast to higher oxidation states, its complexes are more stable than those of $[^{99m}Tc(OH_2)_3(CO)_3]^+$ [3]. The availability of stable therapeutic precursors is a strong argument in favor of the carbonyl approach. The weakly bound H_2O ligands of $[^{99m}Tc(OH_2)_3(CO)_3]^+$ and $[^{188}Re(OH_2)_3(CO)_3]^+$ can be replaced by essentially any ligand, which leads to a second aspect of the carbonyl approach.

The ability to coordinate to multiple ligands is rationalized by the d^6 low spin electron configuration, rendering closed shell complexes in an octahedral environment that are highly robust. It has been argued that kinetic stability is preferential to thermodynamic stability in compounds related to life science. The most prominent example is sestamibi, in which technetium is also in the +I oxidation state. The Tc–C bond energy would not prevent substitution in physiological media but there is simply no reaction pathway of reasonable energy for this purpose. The robust nature of the Tc(I) center in $[^{99m}Tc(OH_2)_3(CO)_3]^+$ provides a unique and very high degree of freedom in selecting the ligands and designing novel radiopharmaceuticals. The shape of the ligand, the combination of donors and even denticity are not restricted. Ligands with structures appropriate for particular biomolecules can be designed "ab initio" based on receptor-ligand interactions from molecular modeling studies. The possibility of designing a complex extends to modifying an existing framework. The "semi aquo ion" as a precursor and the formation of robust complexes introduce a high degree of diversity in radiopharmaceutical research.

This concept has been experimentally verified with model complexes and labeled biomolecules. Monoden-

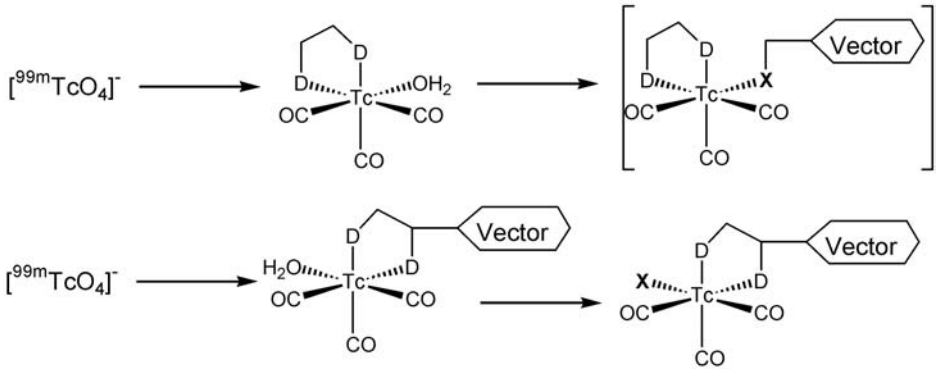

Fig. 3. [2+1] mixed ligand concept for drug development, with X the ligand to be varied

tate ligands such as imidazole or purines, ubiquitous in biology, bidentate ligands such as histamine, amino acids, and picolinic acid and tridentate ligands such as histidine efficiently replace the H_2O ligands and shield the $[^{99m}Tc(CO)_3]^+$ moiety against trans-metallation or reoxidation. The introduction of a linker into the basic ligand framework or an already present functional group allows coupling to a biomolecule. Surprisingly, potent ligands, independently of whether their character is "hard" or "soft", span the full range from polyaminopolycarboxylates such as EDTA to agostic hydrides and cyclopentadiene. Although even tridentate ligands are not expected to have high thermodynamic stability constants, all the model complexes studied so far have shown no decomposition for days at 37°C in physiological solution and have been excreted chemically unchanged [4]. Furthermore, the complexes are essentially stable towards reoxidation under aerobic conditions. Synthesis of the complexes does not demand ligand-dependent modifications, but consists in mixing and, at very low ligand concentration ($<10^{-5}$ *M*), in heating. Thermal stability of the products is a relevant issue for the preparation of radiopharmaceuticals at high vector dilution. For routine use, the concentration of receptor targeting molecules in step ii) or iii) of Fig. 1 or 2 should be low to afford radiopharmaceuticals of very high specific activity. Such second-order reactions are slow at low concentrations. The thermal stability of $[^{99m}Tc(OH_2)_3(CO)_3]^+$ and the products allows reactions to be carried out at up to 100°C, accelerating the reactions without decomposition of the products. It has been shown that strong (fast) ligands give reasonable yields, even close to a ^{99m}Tc/ligand ratio of 1:1. This favorable coordination chemistry allows the selection of ligands from chemical catalogues. In our own research, we have focused, among others, on tridentate histidine for peptides [5] and on cyclopentadiene for CNS receptor ligands [6], demonstrating the possible variations. For peptides, hydrophilic amino acid-derived ligands are suitable, whereas for CNS receptor ligands, lipophilicity and the molecular weight of the tag are important issues. The organometallic nature of $[^{99m}Tc(OH_2)_3(CO)_3]^+$ allows stable occupation of three coordination sites by a ligand with only five atoms of the Cp ring. Considering the core complex only, $[CpTc(CO)_3]$ [7] has a molecular weight of 248 whereas neutral $[TcO(N_2S_2)]$ has a molecular weight of 279. Even smaller ligands can be introduced to further reduce the molecular weight: 248 is not the lower limit.

Substitution reactions in the carbonyl approach normally yield a single product even with ligands of a denticity less than 3. Mixtures are unlikely since the intermolecular rates are too slow compared with intramolecular reactions (a consequence of the d^6 configuration). One single direction of the reaction pathway is essential in (in)organic medicinal chemistry. Anionic bidentate ligands L^2, such as imidazole-carboxylic acid, yield exclusively $[Tc(OH_2)(L^2)(CO)_3]$ and neutral bidentate ligands, $[TcCl(L^2)(CO)_3]$ [8, 9]. Mixtures of two bidentate ligands (one monodentately bound) have not been observed. Water in $[Tc(OH_2)(L^2)(CO)_3]$ exchanges for an appropriate mononuclear ligand to produce $[Tc(L'^1)(L^2)(CO)_3]$, leading to a novel mixed ligand [2+1] concept (Fig. 3). By keeping L^2 constant and modifying L'^1 bound to a vector, structure-activity relationships can be systematically studied. Alternatively, the targeting molecule is bound to L^2 and the coligand L'^1 is varied. It has to be emphasized that the precursor $[Tc(OH_2)(L^2)(CO)_3]$ can be synthesized in one step from $[^{99m}TcO_4]^-$, provided that the ligand is stable against reduction. Polarity, charge and (metabolizable) functional groups can be altered, an essential property for the development of radiopharmaceuticals. Once formed, the [2+1] complexes are extraordinarily stable for hours (i.e. in boiling saline).

Consequently, the carbonyl approach generally results in a single, chemically well defined product, this being essential in order to receive FDA approval. The chemical form of the complex can be predicted and analytically confirmed by synthesizing the Re surrogate. Some complexes even give a clear mass signal with ESI-MS at the ^{99m}Tc level. The products at the carrier and n.c.a. level are always identical.

Finally, one-step synthesis of the labeled vector directly from $[^{99m}TcO_4]^-$, according to pathway iii) in Fig. 1 or 2, would be preferential for future routine preparation, but this requirement is difficult to fulfill. Where-

as the temperature during labeling is often not a critical issue, the reducing agent may be. Analogous to Sn(II), boranocarbonate, as used in the preparation of $[^{99m}Tc(OH_2)_3(CO)_3]^+$, can reduce disulfide bonds. However, under typical reaction conditions amides are not reduced and biomolecules not susceptible to reduction could be labeled in a single step from $[^{99m}TcO_4]^-$. This is surprising since stabilization of intermediate valencies such as Tc(IV) by tridentate ligands, leading to side products, could be expected but has not been found. Thus, a one-step reaction for routine application can be achieved, and kit preparation of a ^{99m}Tc labeled vector is feasible.

From a chemical standpoint, the convenience of preparing $[^{99m}Tc(OH_2)_3(CO)_3]^+$ in a kit, its clean reaction with a multitude of free or biomolecule-coupled ligands at room temperature (10^{-4} *M*) or at 100°C (10^{-6} *M*) to yield chemically and biologically stable complexes, and the possibility of selecting ligands from the bench are the most important arguments in favor of the carbonyl approach. Although discussed in the context of free ligands, these properties also apply to chelator-derivatized biomolecules. The carbonyl approach is feasible and inexpensive and an increasing number of groups have stepped into this new field. Accepting an organometallic moiety as a versatile precursor seems difficult. It is essential to recognize that the organometallic nature of $[^{99m}Tc(OH_2)_3(CO)_3]^+$ is the origin of many of the arguments in favor, and coordination compounds may not exhibit the observed behavior. This fact will hopefully encourage research into other organometallic cores for life science. Admittedly, there is still a lack of biological data to support the value, or even the superiority, of the carbonyl approach in radiopharmacy. Comparative studies are required to prove or disprove its complementarity with the other major labeling techniques. Finally, it should be emphasized that while to consider the carbonyl approach as the only solution would be incorrect, its many advantages, as outlined here, open up novel possibilities.

Regardless of the success of carbonyls in radiopharmaceutical chemistry, the research leading to $[^{99m}Tc(OH_2)_3(CO)_3]^+$ was also recognized by the inorganic and organometallic community. Procedures introduced here are likely to be investigated for other transition metals as well. It is an unusual situation in which research from an applied field inspires fundamental chemistry.

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Against

In this commentary we will discuss some of the reasons why the $[Tc(CO)_3]^+$ approach to technetium radiopharmaceuticals has not obtained a place in the commercial arena. We do not disagree with the chemical achievements that were made in preparing bioactive technetium-labeled molecules utilizing the $[Tc(CO)_3]^+$ or $[Re(CO)_3]^+$ approach. For example, it is almost a decade since this approach was used in the synthesis of rhenium-labeled estrogen derivatives with high affinity for