A Novel Organometallic Aqua Complex of Technetium for the Labeling of Biomolecules: Synthesis of $[^{99m}Tc(OH_2)_3(CO)_3]^+$ from $[^{99m}TcO_4]^-$ in Aqueous Solution and Its Reaction with a Bifunctional Ligand

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We present herein the first synthesis of the water and air stable organometallic aqua complex $[^{99m}Tc(OH_2)_3(CO)_3]^+$ directly from $[^{99m}TcO_4]^-$ in saline under 1 atm of CO. Subsequent substitution of the labile water ligands by a bifunctional ligand attachable to biomolecules enables the introduction of carbonyl complexes in life sciences in general and in nuclear medicine in particular.

The application of organometallic complexes in nuclear medicine (i.e. for the labeling of receptor binding biomolecules like steroid hormones, brain tracers, and others) has been proposed in the literature.[1−4] Those approaches, however, were based on classical organometallic syntheses, and thus, the routine use suffered from the lack of practical preparations of precursors. The radionuclide ^{99m}Tc is important in diagnostic nuclear medicine. It is inexpensive and readily available in any hospital. Consequently, it is of high priority to develop efficient labeling methods (in terms of yield and ligand/metal ratio) for biomolecules, derivatized with a chelating function for the ^{99m}Tc center. To date, the published methods mostly rely on the stabilization of Tc(V) with tetradentate N and S ligands.[5−8] Besides nonpredictable retention of biological activity of such conjugates, the methods entailed in most cases purification either from cold, unlabeled material (receptor saturation) or from decomposition products such as $^{99m}TcO_2$.

We recently presented the synthesis of $[^{99}TcCl_3(CO)_3]^{2-}$ (**1**) directly from $[^{99}TcO_4]^-$ under 1 atm of CO in THF.[9] In water, complete substitution of the $[Cl]^-$ by H_2O was observed and the organometallic aqua ion $[^{99}Tc(OH_2)_3(CO)_3]^+$ (**2**) formed. Starting from **2**, highly inert complexes (d^6 electron configuration) of composition $[^{99}TcXL(CO)_3]$ became accessible in water.[10,11] Introduction of organometallics in nuclear medicine, however, must be based on quantitative aqueous synthesis.

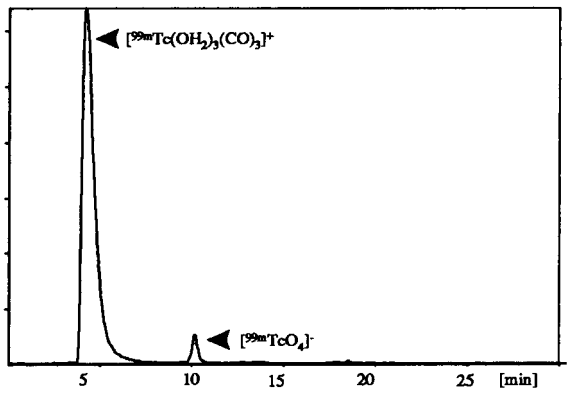
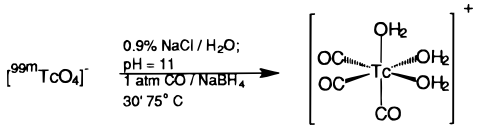


Figure 1. HPLC trace of **2** (^{99m}Tc) on RP-18 column.

Scheme 1



We found that **2** (^{99m}Tc) can be prepared not only in THF, but also directly from saline (0.9% $NaCl/H_2O$) in a closed vial and in yields >95%. Small amounts of $NaBH_4$ were used as reducing agents, and the vial was flushed with CO and heated to 75 °C for 30 min.[12] A HPLC trace is shown in Figure 1 and the reaction in Scheme 1. The therapeutically interesting homologue $[^{188}ReO_4]^-$ reacted similarly but at pH 7.4 within minutes. Considering the low stability of $NaBH_4$ even at high pH and the low solubility of CO in water, quantitative formation of the carbonyl complex **2** is not obvious. Although the ^{99m}Tc concentration is low (μM in a typical generator eluate), one could expect the +I valency to be formed in small steady state concentrations only, before being reoxidized to oxo or hydroxo species. This is not the case, and the Tc(I) center must be captured very efficiently by three CO molecules. This is supported by the observation that, in the absence of CO, exclusively small amounts of $^{99m}TcO_2$ were found which readily reoxidized to $[^{99m}TcO_4]^-$. No other intermediate such as a hypothetical hexaqua complex "$[^{99m}Tc(OH_2)_6]^+$" could be observed. The fact that $NaBH_4$ is an excellent reducing agent to achieve complexes in the valencies +IV and +V implies a synergistic action of CO and $NaBH_4$ to form Tc(I). The presence of large amounts of ligands (tartrate, citrate), known to trap Tc(IV) or Tc(V), did not seriously suppress the formation of **2**. On the other hand, the reducing agent $[S_2O_4]^{2-}$ as used in the preparation of the heart perfusion agent $[^{99m}Tc(CNR)_6]^+$ gave no yield at all.[13]

2 is stable from pH 1 to pH 13 for hours. Serum stability could not be tested, since **2** labeled the proteins very efficiently.

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(12) Na_2CO_3 (0.004 g, 0.038 mmol) and $NaBH_4$ (0.005 g, 0.13 mmol) were added to a penicillin vial (10 mL) which was closed and flushed for 10 min with CO. A 3 mL sample of a generator eluate (containing up to 30 GBq $Na[^{99m}TcO_4]$ in saline) was added by a syringe and the solution heated to 75 °C for 30 min. For safety reasons, the syringe should be kept in the serum stopper during the procedure. After cooling to room temperature 0.3 mL of a 1 M PBS solution was added (pH 7.4). Quality control either by TLC (silica gel, MeOH.HCl (95/5 v/v)), $R_f = 0.35$, or by gradient HPLC. Yield > 95%.

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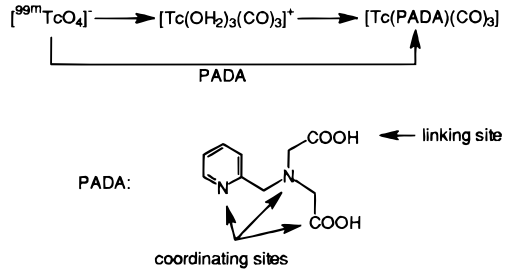
Scheme 2



However, no $[^{99m}TcO_4]^-$ was detected after 48 h, showing the stability of the labeled conjugate.

Owing to this convenient method, we emphasize the unique possibility of introducing a second class of organometallic complexes in routine nuclear medicine. **2** is principally different from $[^{99m}Tc(CN\text{-}R)_6]^+$ or $[^{99m}Tc(\eta^6\text{-}C_6H_6)_2]^+$ [14] due to the substitution labile H_2O ligands. As is obvious from the reaction with serum proteins, they exchange easily with ligand groups from biomolecules.

To exemplify the potential of **2** as an efficient label for derivatized biomolecules, we studied the reaction with the bifunctional ligand picolinamine-*N*,*N*-diacetic acid (PADA). PADA can be considered as a model since it provides three coordinating sites and one group for the covalent attachment to a biomolecule (Scheme 2). In methanol or water, PADA coordinated readily to **2** (^{99}Tc). Neutralization of protons was not required, demonstrating the high stability of $[Tc(PADA)(CO)_3]$ (**3**).[15] The structure of **3** was elucidated (Figure 2).[16] The pyridine nitrogen, the tertiary amine, and one carboxylic acid are facially coordinated to Tc. To our knowledge, **3** is the first example of a structurally characterized Tc(I) or Re(I) complex, where this combination of ligand atoms is encountered. Although Tc(I) is considered to be "soft" and ligands like PADA (aminopolycarboxylic acid) are rather expected to form stable complexes with harder metal centers, **3** exhibited high stability (presumably of kinetic origin) and endured refluxing in 1 M HCl for hours. The second carboxylic acid points outward and is well accessible for covalent linking to a biomolecule. Steric interaction between biomolecule and complex, diminishing complex formation or bioactivity, might not be expected.

Quantitative formation of **3** (^{99m}Tc) was achieved in phosphate buffer after 30−60 min. Only μM solutions of PADA were necessary (ligand/metal ratio about 5/1). Comparing the HPLC trace of the isolated ^{99}Tc complex to that of the solution prepared with ^{99m}Tc proved their identity. **3** is stable in serum for 48 h at 37 °C.

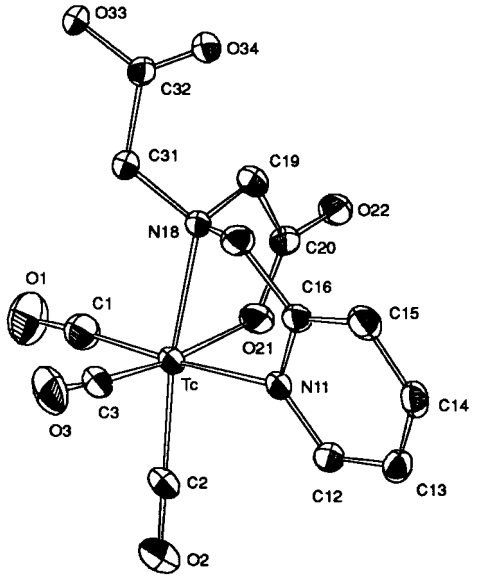


Figure 2. ORTEP presentation of $[Tc(PADA)(CO)_3]$. Ellipsoids are drawn at 30% probability.

Besides the successive synthesis of **3** from **2**, it is highly desirable to prepare **3** in one step, again directly from $[^{99m}TcO_4]^-$. Thus, PADA was added to the reaction vial, containing CO and the reducing agent. Applying the same reaction conditions as for **2**, **3** was formed in one step from $[^{99m}TcO_4]^-$ in saline. Although PADA could stabilize intermediate oxidation states (as found with its fragment imino-N,N-diacetic acid[17] or pyridine[18]), such behavior was not observed and **3** (^{99m}Tc) formed in quantitative yield. The analogues labeling of biomolecules with **2** in the described one-step procedure from $[^{99m}TcO_4]^-$ should be possible.

The major profit for nuclear medical research and practice will originate in the high kinetic stability of d^6 complexes prepared in that way. For instance, $[ReX(L)(CO)_3]$, where L is a DNA intercalating ligand, revealed a potential as probes for nucleic acids.[19] It can be anticipated that **2** as a direct precursor of such intercalating complexes can induce the use of ^{186}Re or ^{99m}Tc complexes in cancer diagnosis or therapy involving DNA−DNA pretargeting.[20] Additionally, the high tendency of complex formation of **2** should allow a much more flexible choice of ligand, adapted to the properties of the biomolecule, than in published procedures.

Supporting Information Available: X-ray data for **3** (10 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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(15) $[NEt_4]_2[TcCl_3(CO)_3]$ (0.055 g, 0.1 mmol) was dissolved in methanol (5 mL). Upon complete dissolution, picolinamine-*N*,*N*-diacetic acid (0.025g, 0.1 mmol) was added and stirring continued at room temperature for 2 h. Reaction can be followed by TLC, $R_f = 0.45$ (silica gel, MeOH/concentrated HCl (95/5 v/v)). **3** precipitated in 80% yield. More product is collected by evaporation of CH_3OH and washing of the residue with CH_2Cl_2 to remove $[NEt_4]Cl$. Yield: 0.036 g, 90%. $IR_{\nu(CO)}$ (KBr): ν(CO) 2013 (s, sh), 1924 (s, br) cm^{-1}. ^1H NMR (DMSO): δ 8.79 (1H, d), 8.18 (1H, t) 7.87 (1H, d), 7.67 (1H, t), 4.93 (1H, d), 4.82 (1H, d), 4.54 (1H, d), 4.33 (1H, d), 4.04 (1H, d), 3.53 (1H, d), all $-CH_2-$ are diastereotopic. Crystals for X-ray analysis from hot methanol.

(16) Crystal data: $C_{13}H_{11}N_2O_7Tc$, MW = 405.2, colorless plates, orthorhombic, *Pbca*, $a = 13.225(1)$ Å, $b = 14.660(1)$ Å, $c = 14.942(2)$ Å, $V = 2897.9(5)$ Å^3, $Z = 8$, $D_{calc} = 1.858$ Mg/m^3, μ(Cu Kα) = 0.8461 cm^{-1}, Enraf Nonius CAD4 diffractometer, Cu Kα radiation (λ = 1.541 74 Å), 2667 reflections, 2341 with $F > 2\sigma(F)$ used for refinement, $R = 0.0386$, $wR2 = 0.1082$, hydrogens fully refined.

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