Complete Carbonylation of *fac*-[Tc(H_2O)$_3$(CO)$_3$]$^+$ under CO Pressure in Aqueous Media: A Single Sample Story!**

Nicolas Aebischer, Roger Schibli, Roger Alberto, and André E. Merbach*

Diagnostic nuclear medicine is one of the prominent fields where radio nuclides of transition metals (e.g. $^{99\mathrm{m}}$Tc) have played an important role in functional organ imaging for two decades.[1, 2] However, until recently most of the ligand systems produced classical Werner-type compounds, which are thermodynamically very stable but suffer frequently from hydrolysis under physiological conditions. The "second generation" of radiopharmaceuticals, which combine receptor-specific, bioactive molecules with a radioactive isotope, require metal complexes that provide the highest possible stability, and avoid interference of the metal center with the binding site of the biomolecule. Thus, organometallic carbonyl compounds attract considerable attention due to their unique features in terms of kinetic inertness, small size, and in vivo stability.[3] It has recently been shown that the novel Tc$^{\mathrm{I}}$ organometallic precursor *fac*-[$^{99\mathrm{m}}$Tc(H_2O)$_3$(CO)$_3$]$^+$ (**1**) represents a powerful alternative to Tc$^{\mathrm{V}}$ compounds for the successful labeling of even the smallest molecules under retention of their bioaffinity and selectivity.[4] The precursor produces very kinetically inert complexes with a variety of mono-, bis-, and tridentate ligand systems.[5] However, very little is known about the water exchange reaction of the *fac*-[Tc(H_2O)$_3$(CO)$_3$]$^+$ complex, the reactivity of the coordinated CO ligands, or the influence of the functionalities of an entering ligand system on the rate of the substitution reactions. To develop appropriate ligand systems and optimize the labeling conditions of functionalized biomolecules, we believe that it is of critical importance to understand the fundamental behavior of the corresponding metal precursor in aqueous media.

This study of the water and CO exchange rate was performed by using multinuclear NMR (^{13}C, ^{17}O, ^{99}Tc) techniques under normal and moderately pressurized conditions. All the experiments described in this paper were carried out over a period of several weeks using only one single sample of *fac*-[Tc(H_2O)$_3$(CO)$_3$]$^+$. During this period no decomposition of this species was observed (as verified by NMR spectroscopy) although the sample was kept in a highly acidic medium (2 M $HClO_4$) and heated to 100 °C.

First, complex **1** was dissolved at 277 K in ^{17}O enriched water (oxygen isotopic composition: ^{16}O 57.9 %, ^{17}O 5 %, ^{18}O 37.1 %). The first ^{17}O NMR spectrum was recorded three minutes after the preparation of the sample and exhibited a single broad peak at $\delta = -52$, which was attributed to the coordinated water molecules in **1**. The signal of the bulk water was suppressed by addition of Mn$^{\mathrm{II}}$ (see Experimental Section). No variation of the signal intensity was observed, demonstrating that the water exchange was completed after three minutes at 277 K. When the sample was heated to 363 K, no significant broadening of the signal at $\delta = -52$ (resulting from the exchange between the coordinated and bulk water molecules) was observed. Combining these observations, the estimated half-life of the water exchange rate at 277 K is between 1 s and 1 min. Comparable results were reported for the water *trans* to the CO ligand in [Ru(H_2O)$_5$CO]$^{2+}$ ($k_{\mathrm{ax}} = 3.8 \times 10^{-3}\,\mathrm{s}^{-1}$, $t_{1/2} \approx 3$ min).[7] For this Ru$^{\mathrm{II}}$ complex the CO exchange rate was dependent on the rupture of the Ru–H_2O_{ax} bond.[8] The half-life for the observed CO exchange was about three days, much longer than the half-life of the water exchange reaction due to the very low concentration of CO in water. As Tc$^{\mathrm{I}}$ and Ru$^{\mathrm{II}}$ have an identical electronic configuration (d^6 low spin), the same mechanism for the CO exchange on *fac*-[Tc(H_2O)$_3$(CO)$_3$]$^+$ may be expected. If this is true, and based on the proposed range of the water exchange rate on **1**, a half life of the CO exchange between 1 and 24 h is expected.

To verify this prediction, the Tc solution was transferred into a 10 mm NMR sapphire tube[9] and pressurized with 4.4 MPa of ^{13}CO. The CO exchange was then monitored by ^{99}Tc NMR spectroscopy (Figure 1 a–d). The substitution of the coordinated ^{12}CO molecules by one, two, and finally three ^{13}CO led to the appearance of a doublet, a triplet, and finally a quadruplet due to the coupling between the Tc center with the coordinated ^{13}CO molecules. The $^1J_{99\mathrm{Tc},13\mathrm{C}}$ coupling constant is 354 Hz and is, to our knowledge, the first ^{99}Tc–^{13}C coupling constant reported. Moreover, each substitution of one ^{12}CO by one ^{13}CO causes an isotopic shift of $\Delta\delta = -1.05$ per carbon mass unit (-95 Hz at 9.4 T, Figure 1 a–d).

The chemical scheme of the CO exchange on *fac*-[Tc(H_2O)$_3$(CO)$_3$]$^+$ is also shown in Figure 1. The mathematical model describing this type of exchange has already been developed for the ligand exchange on [Ru(H_2O)$_5$L]$^{2+}$.[8] The second-order rate constants, k_{CO}, were determined at 277 K and 310 K by fitting the differential equations to the concentrations as a function of time of the different isotopomers of **1** (Figures 1 e and 1 f). The curves in Figure 1 e,f were fitted by using a single rate constant as the adjustable parameter. It is also worth mentioning that the CO exchange on **1** can be monitored at many different temperatures using the same Tc solution by alternatively pressurizing it with ^{13}CO (Figure 1 e) and ^{12}CO (Figure 1 f).

To test for complete carbonylation, the Tc solution was kept for more than two weeks at ambient temperature under 50 bar of ^{13}CO. The ^{99}Tc spectrum recorded after this time revealed three new signals at $\delta = -1050$, -1418, and -1961 as well as the quartet at $\delta = -869.7$ from **1** (Figure 2 a). The signal at $\delta = -1961$ is a septet, with a coupling constant of 261 Hz and must be the result of the coupling between a Tc center and six

---

[*] Prof. Dr. A. E. Merbach, Dr. N. Aebischer
Institut de Chimie Minérale et Analytique
Université de Lausanne, BCH
1015 Lausanne (Switzerland)
Fax: (+41) 21-692-38-75
E-mail: Andre.Merbach@icma.unil.ch

Dr. R. Schibli
Paul Scherrer Institut
Villigen (Switzerland)

Prof. R. Alberto
University of Zürich
Zürich (Switzerland)

[**] We thank the Swiss National Foundation for Scientific Research (A.E.M.) and Mallinckrodt Medical Inc. for the support to this work (R.S. and R.A.)

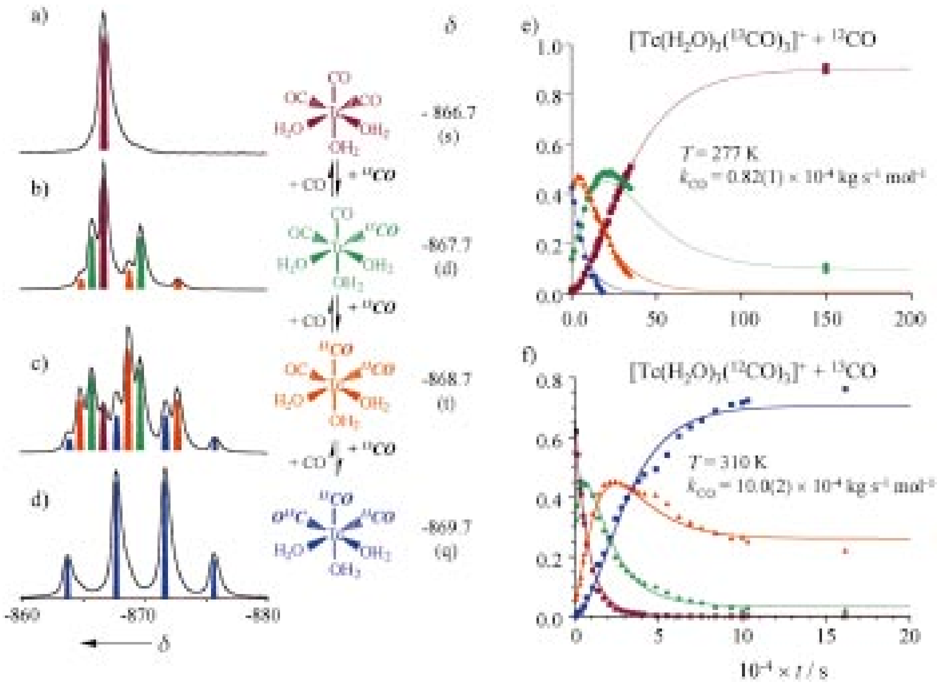

Figure 1. CO exchange on *fac*-$[Tc(H_2O)_3(CO)_3]^+$: time dependancy of ^{99}Tc NMR spectra of a solution containing complex **1** under 44 bar of ^{13}CO at 277 K. The spectra were recorded 2 min (a), 4 h (b) and 35 h (c) after applying the ^{13}CO pressure; spectrum (d) was recorded after removing all ^{12}CO present; evolution of the mole fraction of the different isotopomers of complex **1** under 44 bar of ^{12}CO at 277 K (e) and 44 bar of ^{13}CO at 310 K (f).

equivalent carbon atoms as in $[Tc({}^{13}CO)_6]^+$. To confirm the presence of $[Tc({}^{13}CO)_6]^+$, the Tc solution was slowly degassed and a ^{13}C NMR spectrum was recorded (Figure 2b). Two decets can be seen in the carbonyl region, one at $\delta = 209.7$ (${}^1J_{99Tc,13C} = 354$ Hz) belonging to $[Tc({}^{13}CO)_3(H_2O)_3]$, and a smaller one at $\delta = 190.2$ whose coupling constant, ${}^1J_{99Tc,13C} = 261$ Hz, is identical to the coupling constant of the septet in the ^{99}Tc spectrum. This confirms unambiguously the existence of six equivalent CO ligands coupling with a Tc center, and therefore, the formation of the homoleptic $[Tc(CO)_6]^+$ (**2**) complex in aqueous solution.

The synthesis of **2** was already reported in 1965[10], from solid $Tc(CO)_5Cl$ under 300 bar of CO at 90 °C in presence of $AlCl_3$. The reaction was performed under anhydrous conditions and the product was characterized only by elemental analysis. In contrast to these rather harsh reaction conditions, we have shown that $[Tc(CO)_6]^+$ can be synthesized under relatively mild and even aqueous conditions. We could characterize **2** unambiguously by ^{99}Tc and ^{13}C NMR techniques. Among the large quantity of data reported for $[M(CO)_6]^{n+}$-type complexes ($n = 1-2$; M = 3 d or 4 d transition metal in Groups 6–8), the value of the ^{13}C NMR chemical shift of **2** ($\delta = 190.2$) was missing. A comparison of this value with that calculated in a density functional study

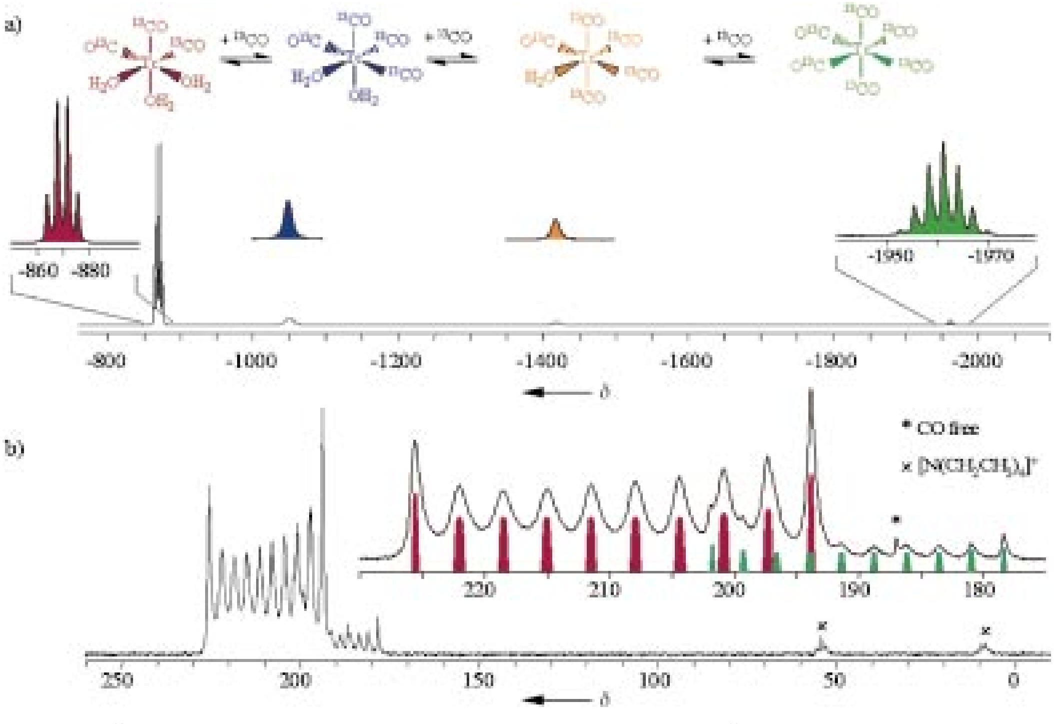

Figure 2. a) ^{99}Tc NMR spectrum, recorded at 310 K, of a solution of *fac*-$[Tc(H_2O)_3({}^{13}CO)_3]^+$ kept three weeks under 44 bar of ^{13}CO at room temperature; b) ^{13}C NMR spectrum recorded at 335 K of the same solution as in (a) after releasing the CO pressure.

($\delta = 193.1$),[11] confirms the quality of the theoretical studies for these systems.

Since the additional two peaks at $\delta = -1050$ and -1418 in the ^{99}Tc spectra (Figure 2a) are much broader and exhibit no splitting, the assignment of these signals is not readily apparent. It seems reasonable to assume that each substitution of a water molecule by one CO will increase the shielding of ^{99}Tc. Therefore, these two signals can logically be attributed to $[Tc(H_2O)_2(CO)_4]^+$ (**3**) and $[Tc(H_2O)(CO)_5]^+$ (**4**). The reduced local symmetry of complexes **3** and **4** and the inequivalence of the CO ligands (complicated coupling pattern) explain the broader signals and the impossibility to resolve the coupling pattern.

When the CO pressure was released, the ^{99}Tc NMR peaks of **3** and **4** disappeared within one hour, and the signal of **2** at $\delta = -1961$ within two days at 25° C; this left only the signal of **1** at $\delta = -867$. The same phenomenon was observed in the ^{13}C spectra. The decrease of the signal at $\delta = 190.2$ was concomitant with an increase of the signals at $\delta = 209.7$ (**1**) and at 187 (free CO). These observations emphasize the high stability of the homoleptic $[Tc(CO)_6]^+$ complex compared to the tetra- or pentacarbonyl complexes and support our kinetic model to describe the CO exchange on **1**. For the CO exchange for $[Ru(H_2O)_5CO]^{2+}$, the *trans*-$[Ru(H_2O)_4(CO)_2]^{2+}$ intermediate was not observed but its existence indirectly demonstrated. In the present study, the signal of the $[Tc(H_2O)_2(CO)_4]^+$ intermediate was observed in the ^{99}Tc spectra, confirming the validity of the model.

In this study, many aspects of the reactivity of *fac*-$[Tc(H_2O)_3(CO)_3]^+$ were observed by using one single sample. A range for the water exchange rate was determined. A kinetic model was developed to describe the CO exchange and the rate could be monitored by ^{99}Tc NMR spectroscopy at 4 °C ($k_{CO} = (0.82 \pm 0.01) \times 10^{-4}\,\mathrm{kg\,s^{-1}\,mol^{-1}}$) and 37 °C ($k_{CO} = (10.0 \pm 0.02) \times 10^{-4}\,\mathrm{kg\,s^{-1}\,mol^{-1}}$). Moreover, the full aqueous carbonylation of *fac*-$[Tc(H_2O)_3(CO)_3]^+$ was unambiguously demonstrated by ^{99}Tc and ^{13}C NMR spectroscopy and the first ^{99}Tc–^{13}C coupling constant was determined.

Experimental Section

$[NEt_4]_2[TcCl_3(CO)_3]$ was synthesized according to the literature.[6] A solution of *fac*-$[Tc(H_2O)_3(CO)_3]^+$ (**1**) was prepared as follows: To a suspension of $[NEt_4]_2[TcCl_3(CO)_3]$ (0.183 g, 0.33 mmol) in dry THF (5 mL) three equivalents of $AgPF_6$ were added and stirred for 5 h. After filtration of AgCl and NEt_4PF_6, THF was evaporated under N_2, and aqueous $HClO_4$ (2 mL, 2 M) was added. To suppress the ^{17}O NMR signal of the bulk water, $Mn(ClO_4)_2$ (0.153 g, 0.69 mmol) was added. This solution was mixed at 277 K with ^{17}O-enriched water (1 mL). The final composition of the solution was: **1** 0.11 mol kg^{-1}, [H$^+$] 1.33 mol kg^{-1}, [ClO_4^-] 1.78 mol kg^{-1}). To monitor the CO exchange reaction of **1**, the solution was transferred into a sapphire tube[9] and a pressure of 44 bar of ^{13}CO was applied. After complete exchange, the solution was degassed and shaken for 10 min under 6 bar of N_2. Then, a pressure of 44 bar of ^{12}CO were applied to monitor the CO exchange at another temperature. This procedure was repeated several times. The CO concentration at 44 bar is 0.044 mol kg^{-1}. The absence of sideproducts of the Tc complexes was checked by ^{99}Tc, ^{17}O, and ^{1}H NMR after each reaction. NMR spectra (^{99}Tc and ^{13}C) were obtained on a Bruker ARX-400 spectrometer. The ^{13}C and ^{99}Tc NMR chemical shifts are referenced to TMS and TcO_4^-, respectively, and measured with respect to dissolved CO ($\delta = 187$),[12] and $[Tc(H_2O)_3(^{12}CO)_3]^+$ ($\delta = -867$), respectively. The CO exchange was monitored by ^{99}Tc NMR spectroscopy. The spectra were obtained at 90.04 MHz in 500–5000 scans with a spectral width of 100 kHz, 32 k data points (acquisition time 0.262 s), and a pulse width of 15 µs. New products formed under CO pressure were characterized by ^{99}Tc and ^{13}C NMR spectroscopy. The ^{13}C NMR spectra were recorded at 100.63 MHz in 200–20000 scans, with a spectral width of 41.7 kHz, 64 k data points (acquisition time 5.8 s) and a pulse width of 15 µs. Rate constants were obtained by fitting the kinetic data to appropriate differential equations by a nonlinear optimization procedure with the program SCIENTIST (version 2.0, MicroMath Inc.).

Received: July 23, 1999
Revised: September 21, 1999 [Z13775]

---

[1] a) J. Steigman, W. Eckelman, *The Chemistry of Technetium in Medicine, Vol. 1*, National Academy Press, Washington D.C., **1992**, pp. 1–180, and references therein; b) S. Jurisson, D. Berning, W. Jia, M. Dangshe, *Chem. Rev.* **1993**, *93*, 1137–1156, and references therein.

[2] a) S. Liu, D. S. Edwards, J. A. Barrett, *Bioconjugate Chem.* **1997**, *8*, 621–636; b) S. Vallbhajosula, B. R. Moyer, J. Lister-James, B. J. McBride, H. Lipszyc, H. Lee, D. Bastidas, R. T. Dean, *J. Nucl. Med.* **1996**, *37*, 1016–1022; c) A. J. T. George, F. Jamar, M.-S. Tai, B. T. Heelan, G. P. Adams, J. E. McCartney, L. L. Houston, L. M. Weiner, H. Oppermann, A. M. Peters, J. S. Huston, *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 8358–8362; d) M. J. Verhaar, P. A. Keep, R. E. Hawkins, L. Robson, J. L. Casey, B. Pedley, J. A. Boden, R. H. Begent, K. A. Chester, *J. Nucl. Med.* **1996**, *37*, 868–872.

[3] a) R. Alberto, R. Schibli, A. Egli, P. A. Schubiger, U. Abram, T. A. Kaden, *J. Am. Chem. Soc.* **1998**, *120*, 7987–7988; b) F. Minutolo, J. A. Katzenellenbogen, *J. Am. Chem. Soc.* **1998**, *120*, 4514–4515.

[4] a) R. Alberto, R. Schibli, P. A. Schubiger, U. Abram, H.-J. Pietzsch, B. Johannsen, *J. Am. Chem. Soc.* **1999**, *121*, 6076–6077; b) A. Egli, R. Alberto, L. Tannahill, R. Schibli, U. Abram, A. Schaffland, R. Waibel, D. Tourwé, L. Jeannin, K. Iterbeke, P. A. Schubiger, *J. Nucl. Med.* **1999**, in press.

[5] a) R. Schibli, R. Alberto, U. Abram, S. Abram, A. Egli, P. A. Schubiger, T. A. Kaden, *Inorg. Chem.* **1998**, *37*, 3509–3516; b) R. Alberto, R. Schibli, P. A. Schubiger, U. Abram, T. A. Kaden, *Polyhedron* **1996**, *15*, 1079–1089.

[6] R. Alberto, R. Schibli, A. Egli, P. A. Schubiger, W. A. Herrmann, G. M. Artus, U. Abram, T. A. Kaden, *J. Organomet. Chem.* **1995**, *493*, 119–127.

[7] N. Aebischer, E. Sidorenkova, M. Ravera, G. Laurenczy, D. Osella, J. Weber, A. E. Merbach, *Inorg. Chem.* **1997**, *36*, 6009–6020.

[8] N. Aebischer, R. Churlaud, L. Dolci, U. Frey, A. E. Merbach, *Inorg. Chem.* **1998**, *37*, 5915–5924.

[9] T. Cusanelli, U. Frey, D. T. Richens, A. E. Merbach, *J. Am. Chem. Soc.* **1996**, *118*, 5265–5271.

[10] W. Hieber, F. Lux, C. Herget, *Z. Naturforsch. B* **1965**, *20*, 1159–1165.

[11] G. Laurenczy, L. Helm, A. Ludi, A. E. Merbach, *Helv. Chim. Acta* **1991**, *74*, 1236–1238.

[12] A. W. Ehlers, Y. Ruiz-Morales, E. J. Baerends, T. Ziegler, *Inorg. Chem.* **1997**, *36*, 5031–5036.