First Application of *fac*-[^{99m}Tc(OH$_2$)$_3$(CO)$_3$]$^+$ in Bioorganometallic Chemistry: Design, Structure, and in Vitro Affinity of a 5-HT$_{1A}$ Receptor Ligand Labeled with ^{99m}Tc

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Organometallic complexes exhibit unique features, which make them very attractive mainly in catalysis. More recently, their application in life science has become a field of growing interest as well.[1] Beside investigations on the metabolical behavior of pure organometallic compounds,[2] bioorganometallic chemistry, as introduced by Jaouen et al.,[3] stands for the combination of an organometallic moiety and a receptor targeting molecule. For the latter purposes, the metal complex should be "innocent" but provide advantages in metabolical stability. We present herein the first example where the organometallic moiety *fac*-[^{99m}Tc(CO)$_3$]$^+$ was successfully applied for the straightforward labeling of a biomolecule designed for binding to the serotonergic receptors 5-HT$_{1A}$ in the central nervous system.[4] Besides retention of in vitro binding affinity and selectivity, the convenience of the labeling is a significant improvement of this method. The organometallic nature of the precursor permits an adaptable design of the ligand attached to the targeting molecule.

Among the relevant isotopes for imaging purposes, ^{99m}Tc is most widely used.[5] It is inexpensive, readily available in any hospital, easy to image, and has decay energies avoiding high-dose burden to the patients. A number of diagnostics for functional organ imaging are commercially available.[6] The challenge for a new generation of radiopharmaceuticals is essentially the targeting of specific receptors. Whereas the labeling of larger biomolecules such as peptides or proteins with ^{99m}Tc has progressed,[7] the radiolabeling of central nervous system (CNS) receptor ligands with ^{99m}Tc in research and application is challenging. Consequently, efforts have been made in the past few years to label specific CNS receptor ligands with ^{99m}Tc. Kung and co-workers proved that tropane derivatives, acting as dopamine transporter ligands, can be labeled with ^{99m}Tc in principle. The compounds crossed the blood−brain barrier (BBB) and bound with high affinity and selectivity to the corresponding transporter.[8] The relatively high concentration of ligand required for complete labeling, the low first-pass brain uptake, and the fixed ligand type confines this method. Only the choice of another type of Tc-precursor will permit overcoming the latter decisive point.

Such a precursor is represented by [^{99m}Tc(OH$_2$)$_3$(CO)$_3$]$^+$ **1** for which we recently presented a convenient one-step kit procedure from saline in high yield.[9] The closed shell Tc(I) center has a d^6 electronic configuration. Ligand exchange is expected to be based a pure dissociative or interchange dissociative mechanism.[10] The kinetic stability often encountered with such complexes is a basic prerequisite for in vivo application. Potential ^{99m}Tc complexes attached to CNS receptor ligands have to be neutral in charge and lipophilic to cross the BBB. Thus, we have focused our interest on neutral bidentate amine ligands (N∧N′). The occupation of two coordination sites by such a set of donors entails the coordination of Cl$^-$ at the third position, neutralizing the positive charge. To elucidate the rate of complex formation and serum stability, we have chosen different types of N∧N′ ligands (Scheme 1).

Whereas aliphatic diamines were found to be weak (or slow) chelators, all combination of nitrogen donors containing an aromatic amine group were extremly efficient. In particular **3**, **4**, and **6** formed quantiatively complexes at μM concentrations (close to stoichiometry relative to ^{99m}Tc) after 10 min at 90° C. The ^{99m}Tc complexes did not decompose in serum within 24 h at 37° C. The high kinetic stability was shown be challenging these complexes with an about 10^5 fold excess of the cold complex [Re(OH$_2$)$_3$(CO)$_3$]$^+$. Even after 24 h at 50° C no exchange of the ligands from ^{99m}Tc to Re could be detected by HPLC.

In particular the Schiff base ligand **2** can be prepared just by reacting an aliphatic amine, a frequent functionality in biomolecules, with a corresponding aromatic aldehyde of choice. In contrast to the Tc(V) approach, an unpredecented variety of functionalities can be introduced for the study of the metabolic behavior without influencing the coordination sphere. To examplify this procedure, we have derivatized a receptor ligand from the class of arylpiperazines, which belongs to the class of most thoroughly studied molecules for the 5-HT$_{1A}$ subclass of serotonergic receptors.[11] 4-(3-Aminopropyl)-1-(2-methoxyphenyl)-piperazine **7** was reacted with 2-pyridine-carbaldehyde to yield the Schiff base **8** (Scheme 2). According to the conditions worked out with, e.g., **2**, labeling was achieved by incubating the precursor **1** for 10 min at 90 °C with the corresponding amount of **8**. Although Schiff base type ligands are as efficient as the hydrazine derivatives **3**, **4**, or **5**, they suffer at very low concentration from competing hydrolysis. This decreases the effective concentration (and thus the labeling rate) significantly. Nevertheless, specific activities in the range of 30 GBq/μmol (800 mCi/μmol) could be achieved in 90−95% yield without subsequent purification. This is by a factor of ∼10 higher than in a comparable procedure with

(1) Severin, K.; Bergs, R.; Beck, W. *Angew. Chem., Int. Ed.* **1998**, *37*, 1634−1654. Chen, H.; Ogo, S.; Fish, R. *J. Am. Chem. Soc*. **1996**, *118*, 4993−5001.

(2) Smith, D. P.; Baralt, E.; Morales, B.; Olmstead, M. M.; Maestre, M. F.; Fish, R. H. *J. Am. Chem. Soc*. **1992**, *114*, 10647−10649. Krämer, R. *Angew. Chem., Int. Ed. Engl*. **1996**, *35*, 2141−2143. Ryabov, A. D. *Angew. Chem.* **1991**, *103*, 945−955.

(3) Jaouen, G.; Vessières, A.; Top, S.; Ismail, H. H.; Butler, I. S. *J. Am. Chem. Soc.* **1985**, *107*, 4778−480. Vessières, A.; Top, S.; Ismail, A. A.; Butler, I. S.; Louer, M.; Jaouen, G. *Biochemistry* **1988**, *27*, 6659−6666. Top, S.; El Hafa, H.; Vessières, A.; Quivy, J.; Vaissermann, J.; Hughes, D. W.; McGlinchey, M. J.; Mornon, J.-P.; Thoreau, E.; Jaouen, G. *J. Am. Chem. Soc*. **1995**, *117*, 8372−8380. Jaouen, G.; Vessières, A.; Butler, I. S. *Acc. Chem. Res.* **1993**, *26*, 361.

(4) Glennon, R. A. *Drug Dev. Res*. **1992**, *26*, 251−257.

(5) Schwochau, K. *Angew. Chem., Int. Ed.* **1994**, *33*, 2258−2267.

(6) Jurisson, S.; Berning, D.; Jia, W.; Dangshe, M. *Chem. Rev.* **1993**, *93*, 1137−1156.

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

(8) Meegalla, S.; Plössl, K.; Kung, M.-P.; Stevenson, D. A.; Liable-Sands, L. M.; Rheingold, A. L.; Kung, H. F. *J. Am. Chem. Soc.* **1995**, *117*, 11037−11038. Meegalla, S.; Plössl, K.; Kung, M.-P.; Chumpradit, S.; Stevenson, D. A.; Frederick, D.; Kung, H. F. *Bioconjugate Chem.* **1996**, *7*, 421−429. Meegalla, S. K.; Plössl, K.; Kung, M.-P.; Chumpradit S.; Stevenson, D. A.; Kushner, S. A.; McEglin, W. T.; Mozley, P. D.; Kung, H. F. *J. Med. Chem*. **1997**, *40*, 9−17.

(9) Alberto, R.; Schibli, R.; Egli, A.; Schubiger, P. A.; Abram, U.; Kaden, Th. A. *J. Am. Chem. Soc*. **1998**, *120*, 7987−7988. Alberto, R.; Schibli, R.; Egli, A. Patent WO 9848848.

(10) Aebischer, N.; Sidorenkova, E.; Ravera, M.; Laurenczy, G.; Osella, D.; Weber, J.; Merbach, A. *Inorg. Chem*. **1997**, *36*, 6009−6020.

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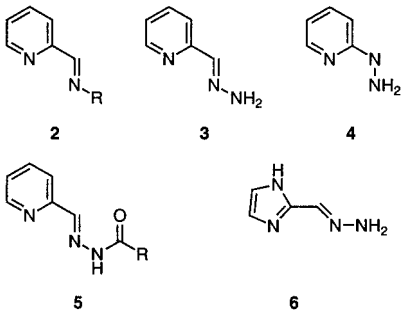
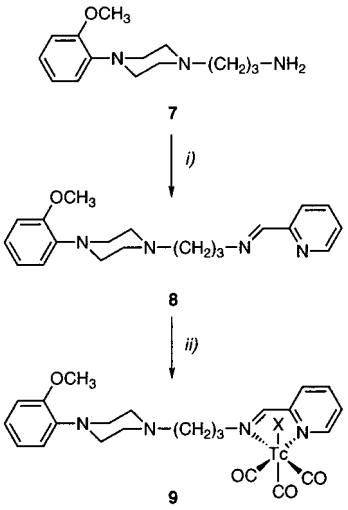
Scheme 1. Bidentate Ligands N∧N′ Which Coordinate $[^{99m}Tc(OH_2)_3(CO)_3]^+$ near Stochiometric Amounts



Scheme 2. Preparation of the Derivatized Aryl-piperazines **8** from **7** and the Corresponding Tc(I) Complex[a]



[a] (*i*) CH_3OH, rt 30 min. (*ii*) CH_3OH, 1 equiv $[NEt_4]_2[TcCl_3(CO)_3]$,[14] rt 60 min.

N2S2 ligands (after purification). The application of ligand **3** instead of the Schiff base **2** would enhance the specific activity by another factor of 10 since derivatives of **3** are much less hydrolyzed. The stability of **9** was tested by means of UV/vis spectroscopy (with cold rhenium) and by TLC at the no carrier added (nca) level. The strong absorption band at 390 nm ($\epsilon = 1100$) remained unchanged even after extended incubation in serum. The TLC did not show decomposition after 24 h at 37° C.

To assess the retention of biological activity and selectivity, the in vitro binding affinities of **9** with cold rhenium instead of Tc were tested.[12] The affinity (IC_{50} values) to the 5-HT_{1A} receptor was 5 ± 2 nM (competitor [^{3}H]8-OH-DPAT). The selectivity (competitor in brackets) was investigated with the following other receptors: 5-HT_{2A} ([^{3}H]ketanserin), dopamine-D2 ([^{3}H]spiperone) 5-HT-transporter ([^{3}H]paroxetine) and D-transporter ([^{3}H]WIN35, 428). In all cases the affinity was found to be >1 μM. The rhenium-labeled compounds revealed, thus, a very good selectivity for the 5-HT_{1A} receptor. Due to the chemical relationship between Re and Tc, the values for the latter are not significantly different.[13] The good selectivity justifies further investigations on the in vivo behavior and in particular on the brain uptake of CNS receptor ligands labeled with the *fac*-$[^{99m}Tc(CO)_3]$ moiety.

To get unambiguous evidence for the composition of **9**, we have synthesized the corresponding complexes with long-lived ^{99m}Tc. Formation of **9** was carried out by the reaction of

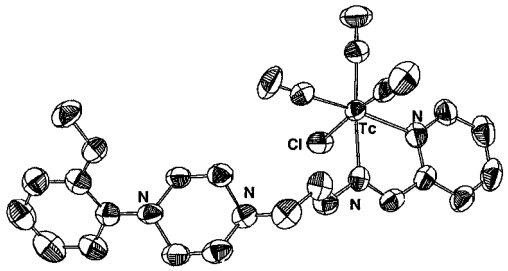


Figure 1. ORTEP presentation of the complex molecule **9**. Ellipsoids are drawn at the 30% probability level.

$[^{99}TcCl_3(CO)_3]^{2-}$ in methanol[14] with 1 equiv. of **8** at rt. The structure was elucidated by X-ray analysis. HPLC comparison of the ^{99m}Tc complex and the material prepared on the nca. level finally established their identity. An ORTEP presentation of **9** is given in Figure 1.[15] The technetium acts as a chirality center. Consequently, two enantiomeric forms of the compound exist with ^{99m}Tc. The complex is far away from the effective binding site of the optically inactive receptor ligand, and it can be anticipated that interference and decrease of affinity should not be initiated by this chiral center.

The versatile protocol presented here is a significant improvement for obtaining ^{99m}Tc-radiolabeled biomolecules. Clinically applied radiopharmaceuticals must be readily available without subsequent purification from cold material or byproducts. In the present case, these improvements clearly result from the organometallic nature of the precursor. The three CO ligands are tightly bound, small, and particularly not subject to protonation which is the major competing reaction in water. The low valency makes the metal center not very attractive for hard donors such as hydroxides or carboxylates, which are present in large amounts. The trans effect of the CO labilizes the H_2O ligands but stabilizes the final metal complex through a push−pull effect with the appropriate ligands. Finally, the strong d-orbital splitting induced by the COs (and d^6 electronic configuration) lead to a very inert system, which is the basis for any biological application.

In conclusion, it was shown for the first time that organometallic complexes such as $[^{99m}Tc(OH_2)_3(CO)_3]^+$ can be applied in aqueous systems for the labeling of bioactive molecules under retention of their receptor affinity. Thus, the concept of bioorganometallic chemistry could be established on a routinely applicable basis. The partially organometallic coordination sphere is responsible for many advantages, i.e., very low biomolecule concentration, high kinetic stabilities, and flexibility in the choice of ligands.

Supporting Information Available: Crystallographic data, atomic coordinates and displacement parameters, bond lengths and angles, anisotropic displacement parameters, and hydrogen coordinates and isotropic displacement parameters (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) Kulkarni, S. K.; Aley, K. O. *Drugs Today* **1988**, *24*, 175−183. Glennon, R. A.; Westkaemper, R. B.; Bartyzer, P. *Serotonin receptor subtypes*; Peroutka, S. J., Ed.; Wiley-Liss: New York, 1991; pp 19−64. Cliffe, I. A.; Fletcher, A. *Drugs Future* **1993**, *18*, 631−642. Wilson, A. A.; Inaba, T.; Fischer, N.; Dixon, L. M.; Nobrega, J.; DaSilva, J. N.; Houle, S. *Nucl. Med. Biol*. **1998**, *25*, 769−776.

(12) 5-HT_{1A} receptor binding assay: Hippocampus rat brain, homogenized in 0.05 M tris-HCl buffer, pH = 7.6. [^{3}H]Ketanserin was used as a radioligand for 5-HT_{2A} receptor binding. The binding assay was carried out in a final volume of 5 mL, containing 0.12 nM [^{3}H]ketanserin, membrane homogenate, and various concentrations of the Re-complex, dissolved in DMSO up to 1 mM and then diluted with buffer. Nonspecific binding was defined as the amount of [^{3}H]ketanserin bound in the presence of 1 μM mianserin. Incubation was accomplished in triplicate at 20 °C for 60 min.

(13) Deutsch, E.; Libson, K.; Vanderheyden, J.-L.; Ketring, A. R.; Maxon, H. R. *Nucl. Med. Biol.* **1986**, *13*, 465−477.

(14) Alberto, R.; Schibli, R.; Egli, A.; Schubiger, P. A.; Herrmann, W. A.; Artus, G. M.; Abram, U. Kaden, Th. A. *J. Organomet. Chem.* **1995**, *493*, 119−127.

(15) Crystal data: $C_{23}H_{26}ClN_4O_4Tc$, MW = 555.93, dark yellow needles, orthorhombic, $P2_12_12_1$, $a = 6.919(1)$ Å, $b = 15.315(3)$ Å, $c = 23.727(2)$ Å, $V = 2514.2(7)$ Å^3, $Z = 4$, $D_{calc} = 1.469$ Mg/m^3, μ(Cu Kα) = 0.5924 cm^{-1}, Enraf Nonius CAD4 diffractometer, Cu Kα radiation ($\lambda = 1.54174$ Å), 4262 reflections, 3732 with $F > 2\sigma(F)$ used for refinement: $R = 0.0333$, $wR2 = 0.0759$, hydrogens calculated, riding model.