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Aqueous Synthesis of Derivatized Cyclopentadienyl Complexes of Technetium and Rhenium Directed toward Radiopharmaceutical Application

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Half-sandwich complexes of the type $[(RCOCp)M(CO)_3]$ with M = Re and $^{99(m)}$Tc were synthesized from $[M(OH_2)_3(CO)_3]^+$ in water. The R group can be an organic residue or a receptor binding biomolecule with a spacer to cyclopentadienyl (Cp). This provides a general route to Cp complexes of technetium without the need for starting from $[TcBr(CO)_5]$. The X-ray structure of $[\{C_6H_5CH_2COC_5H_4\}Tc(CO)_3]$ has been elucidated. The compound crystallizes in the monoclinic space group $P2_1/c$ with $a = 16.1454(9)$, $b = 7.6300(6)$, and $c = 12.3922(7)$ Å and $\beta = 107.792(6)°$. We have chosen a serotonergic receptor ligand (WAY) as an example for the derivatization of Cp with a bioactive molecule. WAY is linked to Cp by an aliphatic chain of variable length. The half-sandwich complexes were prepared from water and organic solvents. The structure of $[(WAY4–Cp)Re(CO)_3]$ could be elucidated. The compound crystallizes in the monoclinic space group $P2_1/c$ with $a = 15.7112(6)$, $b = 6.8775(3)$, and $c = 25.5217(12)$ Å and $\beta = 103.778(5)°$. Quantification of inhibition constants gave a clear structure–activity relationship. A single methylene group between the receptor binding site and the half-sandwich complex gave an IC_{50} of 217 nM for HT_{1A}, whereas a butylene linker resulted in retention of the inhibition constant with an IC_{50} of 6 nM with respect to underivatized WAY. For use as radiopharmaceuticals, the compounds have also been prepared with ^{99m}Tc in quantitative yield.

Introduction

Technetium and rhenium are the subject of great interest as radiopharmaceuticals for diagnostic (^{99m}Tc) and therapeutic (^{188}Re, ^{186}Re) purposes. ^{99m}Tc is still the most widely used isotope in diagnostic nuclear medicine. It is readily available and inexpensive, has good decay characteristics, and typically results in a low dose burden to the patient. Most complexes investigated in research and applied in clinical practice to this date are of the "Werner type", comprising tetradentate N_xS_y ligands or polyaminocarboxylates with an intermediate oxidation state (i.e. +5). Some recent reviews cover these aspects of technetium chemistry.[1] Davison and co-workers have demonstrated for the first time that low oxidation state metal complexes, in particular those with a d^6 electronic configuration, can be very useful due to their robust nature. They introduced organometallic complexes to radiopharmacy with the synthesis of $[^{99m}Tc(CNR)_6]^+$ which became an important class of imaging agents.[1,2] While most radiopharmaceutical complexes in use today are perfusion agents, the challenge for the future lies in preparing ^{99m}Tc radiopharmaceuticals with specific receptor binding properties. This requires a ^{99m}Tc fragment that can coordinate easily to ligands attached to targeting molecules. Following this idea we introduced the low-valent mixed aquo−carbonyl complex $[^{99m}Tc(OH_2)_3(CO)_3]^+$ as a versatile precursor for the labeling of biomolecules.[3,4] Whereas Davison's compound is inherently stable under reasonable chemical and biological condi-

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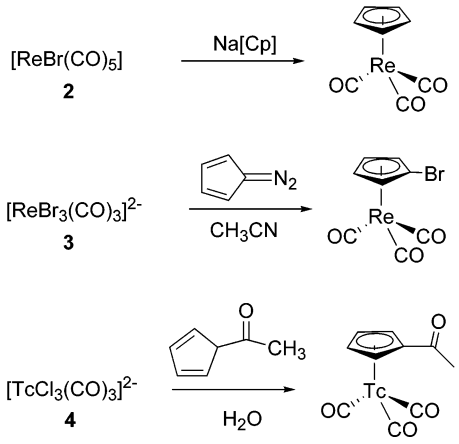
tions, the aquo ligands in $[^{99m}Tc(OH_2)_3(CO)_3]^+$ are easily exchanged for a variety of mono-, bi-, and tridentate ligands, affording complexes of very high kinetic stability.[5] Until now these ligands were essentially histidine, histamine, and related ones. Typical organometallic ligands such cyclopentadienyl (Cp) are normally excluded from this list due to their instability and insolubility in water even though the utility of half-sandwich complexes with rhenium combined with biomolecules has been demonstrated by several groups and has pioneered the field of bioorganometallic chemistry.[6−9] The tridentate Cp ligand offers many advantages for application in nuclear medicine because of its small size and low molecular weight. With these properties in mind, robust complexes of the $[RCOCpRe(CO)_3]$ (R = steroid hormone,[9] antibody[8]) have been prepared by classical organometallic methods and it could be shown that the bioactivity was reasonably maintained, whereas larger complexes lead to loss of affinity for the corresponding receptor. For the first time, Wenzel[10] has shown that it is possible to prepare half-sandwich complexes $[(Cp)^{99m}Tc(CO)_3]$ by a so-called double ligand transfer approach starting with radiopharmaceutically useful $[^{99m}TcO_4]^-$.[11] Though there have been further improvements toward a general synthesis of $[(RCOCp)Re(CO)_3]$, each approach still suffers from unacceptable reaction conditions for routing use.[7,12]

We have recently demonstrated that the cyclopentadienyl ligand can be coordinated to $[^{99m}Tc(OH_2)_3(CO)_3]^+$ in water by introducing the electron-withdrawing acetyl group in cyclopentadiene to give acetyl−Cp. This has a significantly lowered the pK_a value and allowed a one-step preparation of model complexes $[(RCOCp)Tc(CO)_3]$ directly from $[^{99m}TcO_4]^-$. In the way of classical coordination chemistry, cyclopentadienyl is introduced by substituting the three water ligands.[13] In this study we will present a general synthesis of $[(RCOCp)^{99(m)}Tc(CO)_3]$ type half-sandwich complexes from water and $[^{99(m)}TcO_4]^-$ in which R can be different organic functionalities or CNS receptor ligands. This study opens a synthetic approach to labeling biomolecules with half-sandwich complexes that is radiopharmaceutically reasonable and can also be adapted to more basic aqueous chemistry related to the water-soluble acetyl−Cp ligand.

Scheme 1



Results and Discussion

Synthetic Aspects. Various methods are used in organometallic Re and Tc chemistry to prepare "piano stool" complexes, as depicted in Scheme 1. Rhenium carbonyl complexes $[Re_2(CO)_{10}]$ (**1**) are prepared at high temperature and pressure in autoclaves. From $[ReBr(CO)_5]$ (**2**), $[NEt_4]_2[ReBr_3(CO)_3]$ (**3**) may be produced in organic solvents by the substitution of two CO's with two bromides.[14] From **1** half-sandwich complexes are prepared by oxidative addition or from **2** by reaction with M[Cp]. More recently it was shown that the reaction of $[Re(NCCH_3)_3(CO)_3]^+$, prepared from **3**, reacts with diazocyclopentadiene or with stannylated cyclopentadienes to form $[(Cp)Re(CO)_3]$.[7,15] Due to the difficulty of preparing the $[^{99}TcBr(CO)_5]$ analogue under high-pressure and high-temperature conditions, a different method has been devised. Addition of BH_3·THF and CO to $[^{99}TcO_4]^-$ results in reduction to Tc(I) and carbonyl coordination. $[NEt_4]_2[^{99}TcCl_3(CO)_3]$ (**4**) may then be isolated after the addition of excess $[NEt_4]Cl$.[3] When **4** is dissolved in water, the three chloride ligands are replaced by H_2O and $[^{99}Tc(OH_2)_3(CO)_3]^+$ (**5**) is formed.

While convenient for the preparation of macroscopic quantities, these procedures are not feasible for radiopharmaceutical application which requires rapid reaction under aqueous conditions. To meet these requirements, BH_4^- was used as an aqueous reductant in the presence of CO and NaCl affording *fac*-$[^{99m}Tc(OH_2)_3(CO)_3]^+$ (**6**) from $[^{99m}TcO_4]^-$ eluted from a generator at low concentrations (10^{-6}−10^{-8} M). As a further improvement, we have used potassium boranocarbonate ($K_2H_3BCO_2$), the properties of which were reported by us, as an in-situ reducing agent and CO source. This approach allows for the rapid formation of **6** in >95%

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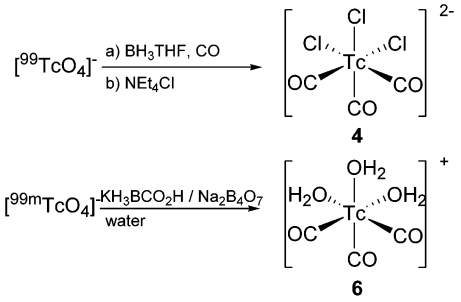
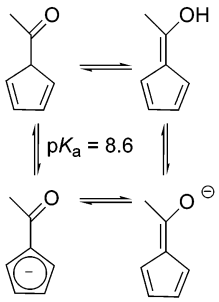
Scheme 2



Scheme 3

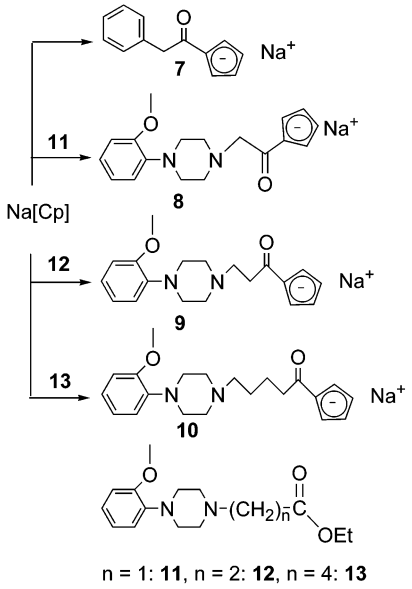


yield and fulfills the requirements for routine application (Scheme 2).[13]

Initial attempts to coordinate Cp or Cp derivatives to the *fac*-$[M(CO)_3]^+$ moiety directly in water failed or resulted in very low yields. Cyclopentadienyl is readily protonated due to its very high pK_a of about 14 and quickly decomposes by polymerization. Alkene coordination as an initial step is unlikely since this type of ligand is uncharged and does not compete for water. Considering coordination chemistry with other ligands, the key step that allows metal coordination to Cp is the introduction of an electron-withdrawing carbonyl group to the Cp ring to yield the Cp derivative of general formula $[RCOC_5H_5]$, referred to as carbonyl−Cp. The resulting keto−enol tautomerism stabilizes the deprotonated form and increases the acidity by about 5 orders of magnitude. For example, the pK_a value for acetyl cyclopentadiene was found to be 8.62(1) in 0.1 M NaCl (Scheme 3). The methyl group in the acetyl functionality can be taken as a linking group for model groups or directly to receptor binding biomolecules.[13]

The introduction of an acetyl group in $[Cp]^-$ results in so-called pentafulvenes. Such derivatization of $[Cp]^-$ with double-bonded functional groups have been performed among many pathways and with a multitude of functionalities. The reaction of Na[Cp] with an ester functionality attached to a biomolecule or an organic model residue in THF allows for the straightforward formation of various differently substituted carbonylcyclopentadienyl compounds Na[RCOCp].[16] Cyclopentadienyl attacks the carbonyl carbon with concomitant cleavage of the corresponding $[R-O]^-$ group. The alcoholato group is sufficiently alkaline to deprotonate the cyclopentadiene. Loss of this ester functionality and the introduction of Cp can be conveniently monitored by ^{1}H NMR spectroscopy. This flexible approach allows for the introduction of a wide variety of (bio)-molecules, linked by an alkyl chain of variable length, to the carbonyl−Cp residue. The synthetic method is shown in Scheme 4.

Scheme 4



To explore this synthetic route to substituted cyclopentadienyls and to investigate the subsequent aqueous coordination chemistry with **5** or **6**, we have chosen the phenyl group as a future model for steroids and 1-(2-methoxyphenyl)-piperazine (WAY) as a serotonergic receptor binding ligand which would allow the assessment of relative binding affinities (RBA). The Cp derivative of phenylacetyl, Na-$[PhCH_2COCp]$ (**7**), was prepared in 70% yield by the reaction of Na[Cp] in THF with the ethyl ester of phenylacetic acid. The crude product from this step was of sufficient purity for the reaction with the Re^- or the ^{99,99m}Tc precursors. For the affinity studies with receptor binding ligands, further purification by preparative HPLC was performed. As an example of a more complicated and receptor binding molecule, a lead structure, 1-(2-methoxyphenyl)piperazine, was chosen. Derivatives of this bioligand are among the most thoroughly studied ligands for the 5-HT_{1A} subclass of serotonergic receptors.[17] After alkylation of the piperazine ring with ethyl bromoacetate to yield **11**, the Cp derivative was prepared by the same method as above to give Na[WAY1−Cp] (**8**). Since it has been reported that small changes in the length of the linker between the metal center and (bio)molecule can have a large effect on the relative binding affinity, we decided to prepare a similar Cp-derivatized biomolecule differing only in the length of the alkyl chain between the (bio)molecule and Cp ligand. The

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synthetic procedure for an ethyl linker Na[WAY2−Cp] (**9**) was again the same as above. However, NMR analysis of the product from the reaction of Na[Cp] with **12** indicated the formation of a neutral fulvene rather than the anionic compound **9** (Scheme 4). This implies that the fulvene form is stabilized by the formation of a six-membered ring with the proton chelated by the piperazine nitrogen and the enol oxygen atom. In principle, this does not prevent the Cp ligand from coordinating. Still, this assumption is in agreement with the observation that all reaction attempts with **4** or **5** respectively with **6** failed or produced unacceptably low yields of [η^5-(WAY2−Cp)Tc(CO)$_3$] (**18**). To avoid fulvene stabilization by intramolecular hydrogen bridge formation, we alkylated 1-(2-methoxyphenyl)piperazine with ethyl 5-bromovalerate to get **13** to generate a butylene linker and a terminal ester function. Reaction of **13** with Na[Cp] produced the substituted cyclopentadienyl compound Na-[WAY4−Cp] (**10**) in about 60% yield after 2−3 days at 70 °C. The reaction was terminated when a ^{1}H NMR sample did not show any remaining resonance of the ester functionality. Product formation was directly indicated by the growth of two doublets from carbonyl-bound Cp centered near 6.1 and 6.6 ppm.

Half-sandwich complexes of the general form [(RCOCp-)M(CO)$_3$] with M = Re or ^{99}Tc can be prepared along two pathways. The classical reaction of **7** with **2** in benzene proceeded smoothly overnight. ^{1}H NMR was used to monitor the shift in the resonance from free to coordinated **7**. Due to the water solubility of all Cp derivatives presented herein, any excess ligand can easily be washed away while the high lipophilicity allows the isolation of the product [(PhCH$_2$-COCp)Re(CO)$_3$] (**14**) into hexane by repeated extraction. Purification by column chromatography gives a clean product from which X-ray-quality crystals could be grown by crystallization from CH_2Cl_2/hexane. Since these organic conditions are not appropriate for the requirements in future application, we assessed the possibility of using Cp derivatives directly in water, comparable to "normal" ligands. Routine application in a hospital setting must rely on the preparation of the complexes [(RCOCp)Tc(CO)$_3$] directly from water. The reaction of **7** with **4**, which readily produces [^{99}Tc(OH$_2$)$_3$(CO)$_3$]$^+$ (**5**) when dissolved in water, proceeds within a few hours at 75 °C or overnight at rt (room temperature), generating [(PhCH$_2$COCp)^{99}Tc(CO)$_3$] (**15**) in modest yield. Since the chlorides do not compete in complexation, it was not necessary to remove them by precipitation with a silver salt. The modest yield is not due to uncontrolled side reactions or decomposition of the ligand (which is nicely stable in water or buffer over this time period) or precursor but by the competing hydrolytic reaction of **5** to form the stable cluster compound [M(μ-OH)(CO)$_3$]$_4$. The complex **15** could be purified by extraction, water washing, and a small silica gel column. From the pale yellow product, crystals suitable for X-ray analysis were obtained, and an ORTEP presentation of the molecule is given in Figure 1. The crystal structures of the rhenium and technetium compounds are isomorphous and reveal the typical piano-stool arrangement with average M−Cp carbon bond

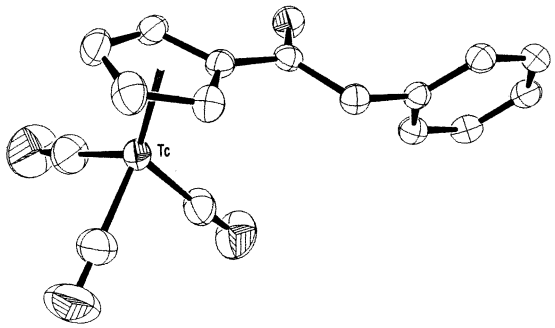

Figure 1. ORTEP presentation of **15**. Ellipsoids are drawn at the 50% level.

lengths of 2.30 Å and M−CO carbon bond lengths of 1.14 Å. The X-ray structures of **14** and **15** are essentially identical. All their corresponding cell constants differ by less than 0.7%, and the equivalent metal carbon bond lengths are not significantly different. The ^{99}Tc NMR spectra confirms nicely the Tc(I) nature of the complex since the chemical shift fits well in the region usually found for Tc(I) compounds.[18] A single and relatively broad signal at −2502 ppm relative to [^{99}TcO$_4$]$^-$ was observed. In comparison to other half-sandwich complexes of ^{99}Tc such as [CpTc(CO)$_3$] at −1716 or [(Cp*)Tc(CO)$_3$] at −1874 ppm, respectively,[19] the resonance is shifted toward stronger shielding which might be a consequence of the additional carbonyl group. The relatively large $\Delta\nu_{1/2}$ of 720 Hz is typical for asymmetric coordination around this spin 9/2 nucleus.

Reactions with both **2** in benzene or **4** in water with **8** proceeded at a similar rate to give [(WAY1−Cp)M(CO)$_3$] in reasonable yield (15−25% for M = Re (**16**) and 40% for M = Tc (**17**), respectively). The structure of **16** has already been described.[13] As with ligand **7**, the modest yield is due to cubic cluster formation as confirmed by HPLC analysis. For the analogue reactions with ^{99m}Tc, this side reaction is not expected to play an important role since the ^{99m}Tc concentration is too low to afford di- or even tetramerization. Preparing Cp complexes with the ligands **7** and **8** in water leads to the possibility of applying this chemistry to other metal centers in a comparable synthetic strategy. The in vitro binding affinity has here been determined for complex **16**. It was found that the inhibition constant (IC_{50}) against the 5-HT$_{1A}$ receptor was 217 nM and that against the 5-HT$_{2A}$ receptor 8753 nM. The former value is decisive but unacceptably low. This number is not surprising since it has been shown by structure−activity relationships (SAR's) that, independent of the nature of the complex attached to the lead structure, one methylene group imposes significant sterical interactions with the binding pocket, resulting in a low inhibition constant as observed in this case.[20] This inhibition constant is still among the smallest known so far

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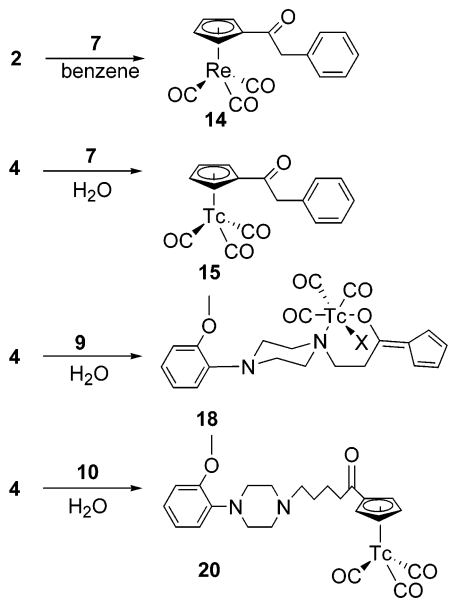
Scheme 5



for this type of a (too) short linker. We emphasize that the small number encountered with a system far from optimal supports the hypothesis of designing a complex as small and lipophilic as possible. To extend the spacer length, we attempted to apply the fulvene compound **9** which is also expected to provide a η^5-coordination to the metal center. Surprisingly, only minimal amounts of complex could be detected analytically under any of the reaction conditions successfully applied to **8** or **7**. Although the compound was not isolated in analytically pure form, it was evident from spectroscopic data that the major product is a complex in which the tertiary piperazine amine and the deprotonated enolate form a six-membered chelate to the metal center (**18** in Scheme 5), thus preventing the ring slippage to η^5 coordination.

This mechanistic result clearly implies that the major pathway of coordination is by initial binding to the enolate and subsequent intramolecular rearrangement, affording the desired half-sandwich complex. Obviously, the carbonyl group in carbonyl−Cp does not only acidify the cyclopentadiene ring but acts the same time as an initial coordinating site. Chelation to nitrogen and oxygen hampers this route for **9**. A comparable failure in Cp coordination was also found for other biomolecules providing an acac donor set including the −CO− group from carbonyl−Cp. We did not observe this behavior with **8** which would have a five-membered chelate. Obviously, the relatively large Tc(I) center prefers six-membered over five-membered rings. To further support this hypothesis, we reacted **10** with **2** in benzene or $[Re(H_2O)_3(CO)_3]^+$ in water generating [(WAY4−Cp)Re(CO)$_3$] (**19**) in reasonable yield. The progress of the reaction is conveniently monitored by ^{1}H NMR spectroscopy or by HPLC. Product purification by silica gel chromatography generated **19** in 25−40% yield. Crystals of X-ray quality were prepared from acidified MeOH/hexane. An ORTEP presentation of **19** is given in Figure 2. The Cp ring, rhenium center, and the ketone moiety possess an almost

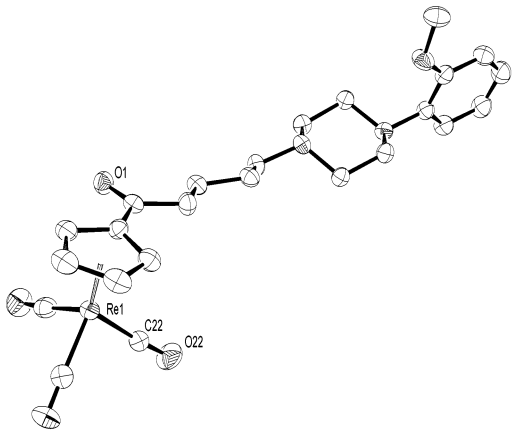


Figure 2. ORTEP presentation of **19**. Ellipsoids are drawn at the 50% level.

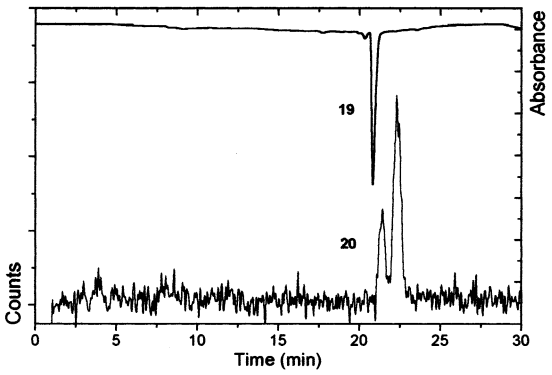


Figure 3. HPLC trace after the synthesis of **20** with ^{99}Tc. The lower trace is monitored with a β^- detector, and the upper trace, with a UV detector at 250 nm for **19** for comparison (delay due to detector separation).

identical geometry in the complexes **14** and **19**. The tris(carbonyl) moiety, however, is twisted by about 57° compared between the two structures. The charge of the diprotonated piperazine is compensated by a chloride and a nitrate anion, both of which form a hydrogen bond to the nitrogen atoms: the distance from the nitrogen atoms to the chloride/oxygen O30 of the nitrate is 3.02/2.80 Å, respectively.

To confirm that [WAY4−Cp^{99}Tc(CO)$_3$] (**20**) can be prepared directly from water as well, **5** was reacted with **10** in an analogous way to the reaction with **8** at rt or at 70 °C. The reaction progress was monitored by means of HPLC with parallel UV and β^- detection. After 2 h at 70 °C, two new product peaks were found in the β^- trace at 21.3 and 22.33 min along with a larger peak of unreacted **5**. After continued reaction overnight, only the two product peaks remained. The HPLC β^- trace of **20** and comparison to **19** are depicted in Figure 3.

The first peak was identified as **20** (30% yield) by comparison of the HPLC retention time with **19**. Furthermore, ESI LC-MS gave the correct mass of this product for the molecule cation (*m*/*e* 522.73; theoretical for $C_{24}H_{27}O_5N_2{}^{99}Tc$, *m*/*e* 522.44). The second β^- active peak was identified as the expected tetrameric $[^{99}Tc(\mu\text{-OH})(CO)_3]_4$ cluster in about 70% yield. For the rhenium reaction in benzene and water and the Tc reaction in water, no evidence was found for the formation of a stable intermediate as detected with

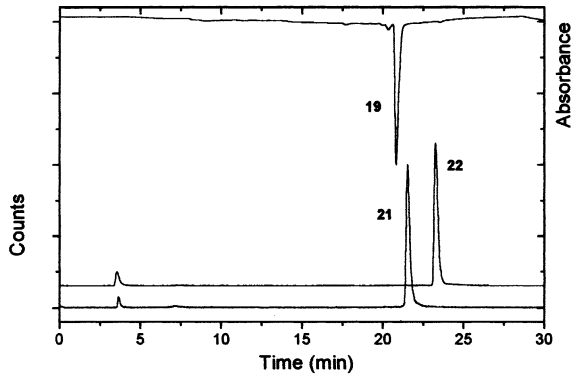


Figure 4. Formation of **21** and **22** with ^{99m}Tc in water from **6**. The lower HPLC traces show the products with γ detection, and the upper trace shows the product with UV detection for **19** with Re for comparison.

9. The reaction proceeds clearly from the educt to the two described products without the formation of any undesirable side products. The successful reaction with **10** supports the hypothesis that the formation of six-membered chelates interferes with the desired formation of the half-sandwich complex. In vitro binding studies confirmed the SAR from other studies. Complex **19** displays remarkable affinity toward the 5-HT_{1A} receptor (IC_{50}: 6 nM) and a lower affinity for the 5-HT_{2A} receptor (IC_{50}: 300 nM). Animal studies with this compound and ^{99m}Tc are currently underway.

Labeling Studies. To further examine the novel reactions of Cp compounds in water and to demonstrate their versatility for radiopharmaceutical purposes, we carried out reactions at the no-carrier-added (nca) level with $[^{99m}TcO_4]^-$ (10^{-6}–10^{-8} M ^{99m}Tc). These are conditions typically encountered in routine application and will demonstrate the practical convenience of this approach. At low concentration, $[M(\mu\text{-}OH)(CO)_3]_4$ cluster is not formed for kinetic reasons. In addition and in contrast to the synthetic methods on the macroscopic level, there is normally several orders of magnitude ligand (or biomolecule) excess though the concentration should still be as low as possible. Thus, the reaction rate is expected to be much lower assuming a second- or pseudo-first-order kinetic substitution reaction. The synthesis of $[(RCOCp)^{99m}Tc(CO)_3]$ can be performed along two pathways. As previously reported, the precursor **6** was prepared from $[^{99m}TcO_4]^-$ eluted in saline from a generator and using potassium boranocarbonate $K_2[H_3BCO_2]$ as an air-stable, in-situ reducing agent and CO source. Addition of 1−4 mL of $[^{99m}TcO_4]^-$ to a vial containing $K_2[H_3BCO_2]$ and borate generates **6** in greater than >98% yield after 15 min at 95 °C. After phosphate buffering of this solution to pH 7.4−8 aqueous solutions of **8** or **10,** respectively, were added to 1 mL of **6** to produce a final concentration of RCOCp between 0.1 and 1 mM. Heating to 90 °C for 15−30 min affords $[(RCOCp)^{99m}Tc(CO)_3]$, i.e., $[WAY4{-}Cp^{99m}Tc(CO)_3]$ (**21**), in high yield. Traces of HPLC γ-detection are given in Figure 4. The identity of the product was confirmed by co-injection of the corresponding Re complex and comparison of the retention time. In Figure 4 the lower trace represents the γ-detection and the upper trace the UV absorption at 250 nm for the rhenium compound **19**. The retention times are identical, and the slight delay of radioactive over optical detection is due to separation of the respective detectors. The steroid model **7** was labeled by the same method to yield $[(PhCH_2COCp)^{99m}Tc(CO)_3]$ (**22**) in 95% yield. Preliminary animal studies in mice showed a relatively high first pass brain uptake of 1.2% ID/g with the expected washout from the brain due to the lack of a trapping mechanism. The compound showed very high lung uptake (>20% ID/g) which even increased over time. The study of the biological mechanism responsible for this behavior in lung is currently underway.

Although the straightforward synthesis of derivatized half-sandwich complexes has been demonstrated, the concentration of the biomolecule still remains a concern. It is known from other approaches that relatively high concentration of unlabeled brain receptor binding molecules may not interfere with potential application since the unlabeled material might be too polar to pass the blood brain barrier.[21] This is probably also the case with **10** since it is zwitterionic to a large extent at physiological pH. We observed in our case that excess RCOCp precipitated under the conditions applied during the reaction with ^{99m}Tc, probably as dimers or oligomers. This precipitate can easily be removed by filtration through a syringe, and the UV trace of the corresponding solution did not show the presence of any Cp derivatives. For in vivo application, this would act to increase the specific activity of the complex by preventing saturation of the target receptor with "cold" substrate.

To further improve the system, a true one-pot synthesis was finally performed, producing the half-sandwich complexes directly from $[^{99m}TcO_4]^-$ in saline and in the presence $K_2[H_3BCO_2]$ as a reducing agent and CO source. The combination of conditions described above in one single vial and 40 min at 90 °C was sufficient to form the corresponding half-sandwich complex in >95% yield. This method can generally be applied to other biomolecules derivatized with a carbonyl−Cp unit, provided that the biomolecule is not susceptible for reduction. We have found in some cases that the carbonyl functionality was reduced to a hydroxy group but optimized conditions should prevent this undesirable side reaction.

Conclusions

We have demonstrated that cyclopentadienes carrying a carbonyl functionality acidify the ring protons sufficiently to be used as aqueous ligands. This allows for the general preparation of half-sandwich complexes $[(RCOCp)Tc(CO)_3]$ directly from $[^{99}Tc(OH_2)_3(CO)_3]^+$ and the corresponding Na[RCOCp]. Presumably, initial coordination is by the deprotonated enolate with subsequent ring slippage to η^5-coordination. We want to emphasize that this pathway can

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probably be applied to other transition metal cations, opening pathways to new mixed Cp and water complexes. Attachment of a biomolecule to carbonyl−Cp, followed by reaction with $[^{99m}Tc(OH_2)_3(CO)_3]^+$ at the nca level, proceeds rapidly and in good yield to generate $[(RCOCp)^{99m}Tc(CO)_3]$ complexes. This procedure has been successfully demonstrated with a serotonergic receptor binding ligand. In addition, this route represents a general synthetic method for the production of radiopharmaceutically relevant technetium and also rhenium complexes, suitable for application in, e.g., CNS receptor imaging. Considering basic SAR's, this method underlines the possibility of introducing the very small and highly lipophilic $[(RCOCp)^{99m}Tc(CO)_3]$ moiety into a variety of receptor binding molecules.

Experimental Section

Chemicals and solvents of reagent grade were purchased from Fluka AG Buchs and used without further purification. All manipulations were carried out using standard Schlenk techniques unless otherwise noted. NaCp was freshly prepared in situ from Na and the corresponding amount of freshly distilled dicyclopentadiene, or purchased as a 2 M solution in THF and stored in a glovebox. NMR spectra were recorded on a Varian Gemini 300 MHz or Varian Mercury 200 MHz instrument. Chemical shifts (ppm) are reported relative to residual solvent. HPLC were measured on two different Merck Hitachi LaChrom D-7000 instruments. One of these was interfaced with an EG&G Berthold LB 508 radioflow detector while the other was interfaced with a Merck Hitachi M-8000 LCMS. A Varian Prostar was used for preparative HPLC. $[^{99m}TcO_4]^-$ was eluted from a Mallinckrodt Med. Inc. generator. The amount of radioactivity varied from 370 MBq to 3.7 GBq, and no dependence of the total ^{99m}Tc concentration on yield was noted. The complexes **3**, **4**, and **6** and $K_2[H_3BCO_2]$ were prepared as described elsewhere.[22] HPLC system: RP-18 column (A = 0.1% CF_3COOH in H_2O, B = MeOH): 0−3 min 100% A; 3−9 min 75% A; 9.1 min 66% A; 9.1−20 min 66% → 0% A; 20−25 min 0% A; 25.1−30 min 100% A.

Caution! *^{99}Tc is a weak β^- emitter ($t_{1/2} = 2.13 \times 10^5$ yrs, $\beta^- = 294$ keV). Therefore, all manipulations were carried out in specially equipped (C-type) laboratories to avoid contamination or ingestion.*

Synthesis of 7. Phenylacetic acid ethyl ester (0.15 mol) was added dropwise to a solution of Na[Cp] (0.10 mol) in dry THF (50 mL) over 45 min. The wine red solution was then heated for 5 h at 75 °C, during which time the solution color changed to orange/brown. After solvent removal, the light tan colored residue was rinsed with hexane and ether and dried under vacuum (yield 90%). The product was used as is for further reactions or purified by preparative HPLC in small quantities taking with care to exclude oxygen. HPLC retention time = 20.34 min. ^1H NMR (DMSO): 7.05−7.30 (aromatic, 5H), 6.22 (d, 2H), 5.63 (d, 2H), 3.68 (s, 2H). ^{13}C NMR (DMSO): 185.5, 140.2, 129.4, 127.8, 125.3, 123.0, 114.8, 113.3, 111.2, 45.2.

Synthesis of 13. 1-(2-Methoxyphenyl)piperazine (15.6 mmol), ethyl 5-bromovalerate (23.4 mmol), and potassium carbonate (31.2 mmol) were added to 150 mL of acetonitrile under an ambient atmosphere, and the mixture was refluxed for 3 days. After filtration on a fritted glass filter, the yellow filtrate was concentrated to an oil on a rotary evaporator. The product was purifed be silica gel chromatography (19:1 ethyl acetate−methanol), giving a pure (TLC) yellow oil (yield >95%). HPLC retention time = 17.09 min. ^1H NMR (DMSO): 6.83−6.93 (aromatic, 4H), 4.02 (quartet, 2H), 3.75 (s, 3H), 2.93 (br, 4H), 2.47 (br, 4H), 2.31 (t, 4H), 1.35−1.65 (m, 4H)), 1.17 (t, 3H). ^{13}C NMR (DMSO): 172.8, 151.9, 141.2, 122.2, 120.7, 117.7, 111.8, 59.6, 57.4, 55.2, 52.9, 50.0, 33.3, 25.6, 22.4, 14.1.

Synthesis of 10. NaCp (12.48 mmol from a 2 M solution in THF) was added dropwise in a glovebox to **13** (3.12 mmol) in THF (10 mL). The reaction mixture was then transferred to a fume hood and heated to 70 °C for 2 days or until a sample removed for ^1H NMR showed no trace or ester remaining (yield 60−80%). Samples were used as is or further purified by preperative HPLC, taking care to exclude oxygen as much as possible. HPLC retention time = 18.8 min. ^1H NMR (D_2O): 6.85−7.11 (aromatic, 4H), 6.53 (d, 2H), 6.06 (d, 2H), 3.74 (s, 3H), 2.79−3.02 (br, 4H), 2.57 (t, 4H), 2.36 (t, 4H), 1.39−1.66 (m, 4H). ^{13}C NMR (D_2O): 152.3, 140.0, 124.5, 121.0, 119.2, 118.8, 113.4, 111.6, 57.6, 55.6, 52.0, 50.0, 48.8, 26.0, 20.6. HPLC−MS, $(M + 2H)^+$: *m*/*e* 341.07 (calcd for $C_{21}H_{27}O_2N_2$, *m*/*e* 339.21).

Synthesis of 14: Method a. $[ReBr(CO)_5]$ (0.739 mmol) and **7** (2.18 mmol) were placed in a round-bottom flask containing 10 mL of benzene and gently refluxed overnight. Evaporation of the benzene left a cream colored residue which was washed with water. The crude product was then extracted with hexane and purified by silica gel chromatography (3:1 CH_2Cl_2−hexane). The yield was 42%. Crystals suitable for X-ray analysis were obtained by slow evaporation of a solution of **14** in hexane. HPLC retention time = 22.37 min. ^1H NMR (MeOH): 7.20−7.36 (aromatic, 5H), 6.27 (t, 2H), 5.62 (t, 2H), 3.96 (s, 2H). ^{13}C NMR (MeOH): 195.5, 193.5, 135.9, 130.4, 129.7, 128.1, 95.4, 90.0, 87.4, 46.5. HPLC−MS, $(M + H)^+$: *m*/*e* 453.46 (calcd for $C_{16}H_{11}O_4Re$, *m*/*e* 454.60).

Synthesis of 15: Method b. Compound **7** (0.2 mmol) in 3 mL of H_2O was added to 1 mL of an aqueous solution of **4** (0.13 mmol) and warmed to 65 °C overnight although HPLC analysis indicated the reaction was mostly complete within a few hours. The crude product was extracted with hexane (3 × 5−10 mL) and dried under vacuum or with N_2 flow. The yellow residue was washed several times with water, filtered on a fritted glass filter, and washed with acetonitrile. After dissolution in a minimal volume of CH_2Cl_2/hexane, the product was transferred to a silica gel column and eluted with CH_2Cl_2. Thin layer chromatograpyh (4:1 CH_2Cl_2−hexane) gave one spot with an $R_f = 0.5$ (yield = 17%). Crystals suitable for X-ray analysis were obtained by slowly cooling a solution of **15** in hexane from room temperature to −27 °C. HPLC retention time = 23.08 min. Analytical data for **15** are as follows. ^{99}Tc NMR (D_3COD): −2502 (740 Hz). ^1H NMR (MeOH): 7.34−7.21 (aromatic, 5H), 6.19 (t, 2H), 5.51 (t, 2H), 3.95 (t, 2H). HPLC−MS, $(M + H)^+$: *m*/*e* 366.60 (calcd for $C_{16}H_{11}O_4{}^{99}Tc$, *m*/*e* 365.16).

Synthesis of 19: Method a. **2** (0.155 mmol) and **10** (0.221 mmol) were placed in a round-bottom flask containing 5 mL of benzene and gently refluxed overnight. Evaporation of the solvent left a cream colored residue which was dissolved in a minimal amount of CH_2Cl_2−MeOH and transferred to a silica gel column. Elution with 20:1 CH_2Cl_2−MeOH gave one spot by TLC (R_f (7:1 CH_2Cl_2−MeOH) = 0.7). The yield varied between 25 and 40%. Crystals suitable for X-ray analysis were obtained by diffusion of hexane into acidified methanol. HPLC retention time = 21.15 min. ^1H NMR (DMSO): 6.86−7.05 (aromatic, 4H), 6.25 (t, 2H), 5.62 (t, 2H), 3.83 (s, 3H), 2.97−3.14 (br, 4H), 2.59−2.74 (br, 4H), 2.50 (t, 4H),1.51−1.74 (m, 4H). ^{13}C NMR (DMSO): 195.5. 193.7, 152.0, 139.5, 123.7, 121.0, 118.4, 112.1, 96.5, 89.3, 87.3, 55.4, 55.2, 51.1, 46.8, 37.5, 22.5, 21.0. MS, $(M + H)^+$: *m*/*e* 609.74 (calcd for $C_{24}H_{27}O_5N_2Re$, *m*/*e* 608.74).

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Table 1. Summary of X-ray Crystallographic Data

	14	**15**	**19**
formula	$C_{16}H_{11}O_4Re$	$C_{16}H_{11}O_4Tc$	$C_{24}H_{29}ClN_3O_8Re$
molecular mass	453.45	365.25	709.15
T, K	183(2)	183(2)	183(2)
space group	$P2_1/c$	$P2_1/c$	$P2_1/c$
a, Å	16.0665(9)	16.1454(9)	15.7112(6)
b, Å	7.6797(4)	7.6300(6)	6.8775(3)
β, deg	107.172(8)	107.792(6)	103.778(5)
c, Å	12.3656(11)	12.3922(7)	25.5217(12)
V, Å^3	1457.73(17)	1453.57(16)	2678.4(2)
Z	4	4	4
ρ_{calcd}, g/cm^3	2.066	1.669	1.759
μ(Mo Kα), mm^{-1}	8.347	1.002	4.688
F(000)	856	728	1400
cryst size, mm^3	0.15 × 0.14 × 0.04	0.20 × 0.17 × 0.16	0.45 × 0.07 × 0.06
cryst description	colorless plate	colorless block	colorless needle
θ range, deg	2.65−28.17	2.65−28.12	2.35−28.0
tot. no. of data	19 852	20 643	25 279
no. of unique data, R_{int}	3529, 0.0510	3390, 0.0369	6444, 0.0780
obsd data[a]	2632	2634	4893
max/min transm	0.7165/0.3104	0.8561/0.8248	0.7657/0.3054
data/params	3529/190	3390/234	6444/343
goodness of-fit on F^2	0.980	0.892	0.891
R[a,b]	0.0367	0.0271	0.0361
wR^2 [a,c]	0.0889	0.0663	0.0848
max, min peaks, e/Å^3	0.977, −1.504	0.519, −0.331	1.745, −1.347

[a] Observation criterion: $I > 2\sigma(I)$. [b] $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$. [c] $wR^2 = \{\Sigma[w(F_o^2 - F_c^2)^2]/\Sigma[w(F_o^2)^2]\}^{1/2}$.

Synthesis of 20: Method b. Compound **10** (0.2 mmol) in 3 mL of H_2O was added to 1 mL of an aqueous solution of **4** (0.13 mmol), and the solution was warmed to 65 °C overnight although HPLC analysis indicated the reaction was mostly complete within a few hours. The product was identified by HPLC with β^- detection and HPLC−MS (30% yield by β^- detection for **20**). Due to the low amount of starting material used, confirmation of **20** was done only by HPLC comparison and by LC−MS (*m*/*e*): 522.73 (calcd for $C_{24}H_{27}O_5N_2{}^{99}Tc$, 522.44). HPLC retention time = 20.97 min (UV detection) and 21.42 min (β^- detection). The rhenium compound **19** can be produced accordingly with **3** as a starting material. The fulvene complex **18** was prepared the same way but not isolated and purified. ^{1}H NMR gave evidence for the presence of complex **18** as depicted in Scheme 5.

Labeling Procedure. A vial was charged with 4 mg of boranocarbonate ($K_2H_3BCO_2$), 7 mg of $Na_2B_4O_7$, and 7 mg of sodium tartrate, sealed, and flushed with N_2. Generator eluate (1−4 mL) containing $[^{99m}TcO_4]^-$ was added and the vial heated to 90 °C for 15−30 min to generate **6** in >95% yield (HPLC). The pH of this solution was then set to 7.4 with phosphate buffer. An aliquot of this solution was then added to an aqueous solution of RCOCp. Further heating at 90 °C for 30 min generates the labeled product of the form $[(RCOCp)^{99m}Tc(CO)_3]$, i.e., **21** or **22**.

In Vitro Binding Studies with 5-HT$_{1A}$ and 5-HT$_{2A}$ Receptors. (a) 5-HT$_{1A}$ Receptor Binding Assay. The hippocampus of rat brain was homogenized in 10 volumes of ice-cold buffer (50 mM Tris-HCl, pH 7.6), centrifuged at 20000*g* for 10 min. The resulting pellet was resuspended and centrifuged again at 20000*g* for 10 min. After the same procedure was repeated, the pellet was resuspended in 10 volumes of buffer and stored at −20 °C until used in binding studies.

[^{3}H]8-OH-DPAT (5494.5 GBq/mmol from NEN) was used as radioligand for the 5-HT$_{1A}$ receptor binding. The binding assay was carried out in a final volume of 2.5 mL Tris-HCl buffer (50 mM, pH 7.4, 0.1% ascorbic acid, 2 mM $CaCl_2$) containing 0.10 nM [^{3}H]8-OH-DPAT, membrane homogenate (about 20 μg/mL protein), and various concentrations of the Re complexes. The complexes were dissolved in DMSO up to 1 mM and then diluted with buffer.

Nonspecific binding was defined as the amount of [^{3}H]8-OH-DPAT bound in the presence of 10 μM serotonin (Sigma) and ranged from 5% to 9% of total binding. The samples were incubated in triplicates at 20 °C for 60 min. The incubation was terminated by rapid filtration through GF/B glass fiber filters (Whatman) using a 30-port Brandel Cell Harvester. The filters were rapidly washed with four 4 mL portions of ice-cold buffer, transferred into 10 mL scintillation fluid (Ultima-Gold, Packard), and analyzed for radioactivity.

(b) 5-HT$_{2A}$ Receptor Binding Assay. The cortex of rat brain was prepared as described above and stored at −20 °C until used in binding studies. [^{3}H]ketanserin (3148.7 GBq/mmol from NEN) was used as a radioligand for 5-HT$_{2A}$ receptor binding. The binding assay was carried out in a final volume of 5 mL of Tris-HCl buffer, pH 7.6, containing 0.12 nM [^{3}H]ketanserin, membrane homogenate (about 20 μg/mL protein), and various concentrations of the Re complexes dissolved and diluted as described above. Nonspecific binding was defined as the amount of [^{3}H]ketanserin bound in the presence of 1 μM mianserin (Sigma) and ranged from 17% to 24% of total binding. The samples were incubated in triplicates at 20 °C for 120 min. Filtration and counting of the samples were the same as described above.

X-ray Crystallography. Crystal data and experimental details are listed in Table 1. Suitable crystals were covered with Paratone N oil, mounted on top of a glass fiber, and immediately transferred to a Stoe IPDS diffractometer. Data were collected at 183(2) K using graphite-monochromated Mo Kα radiation (λ = 0.710 73 Å). A total of 8000 reflections distributed over the whole limiting sphere were selected by the program SELECT and used for unit cell parameter refinement with the program CELL.[23] Data were corrected for Lorentz and polarization effects as well as for absorption (numerical). Structures were solved with direct methods using

(23) *CELL*, *2.87 5*, 1998 ed.; STOE & Cie, GmbH: Darmstadt, Germany, 1998.

SHELXS-97[24] or SIR97[25] and were refined by full-matrix least-squares methods on F^2 with SHELXL-97.[26] Further experimental details are available as Supporting Information.

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Supporting Information Available: Tables of X-ray data, ORTEP diagrams, and complete X-ray data in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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