J|A|C|S
COMMUNICATIONS

Published on Web 01/11/2008

Metal-Mediated Retro Diels−Alder of Dicyclopentadiene Derivatives: A Convenient Synthesis of [(Cp-R)M(CO)$_3$] (M = ^{99m}Tc, Re) Complexes

Yu Liu, Bernhard Spingler, Paul Schmutz, and Roger Alberto*

University of Zürich, Institute of Inorganic Chemistry, Winterthurerstrasse 190, 8057 Zürich, Switzerland

Received October 16, 2007; E-mail: ariel@aci.unizh.ch

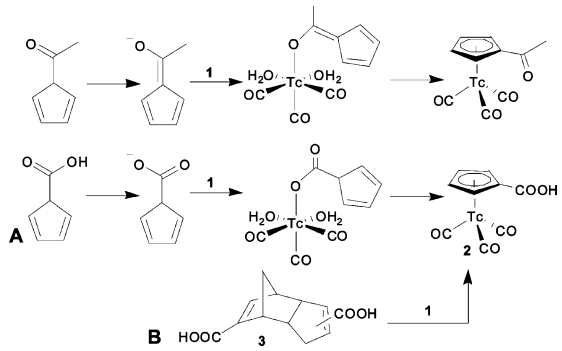
Site-specific targeting of receptors and overexpressed genes as related to cancer or neurodegenerative diseases is the basic concept of molecular imaging with radionuclides or contrast agents.[1] Achieving a high target/nontarget ratio within a short period of time is crucial for a precise diagnosis. In molecular radioimaging, ^{99m}Tc plays still a major role, but the design of a ^{99m}Tc bioconjugate fulfilling the "*conditio sine qua non*" mentioned before is very challenging. The size of the complex and chelator functionalities may govern the biological behavior of the vector, rather than the vector itself.[2] To optimize these parameters, the cyclopentadienyl (Cp) ligand is an obvious choice. Cp is one of the smallest "innocent" ligands and stably binds to the [^{99m}Tc(CO)$_3$]$^+$ core. Notably, it mimics arene rings in pharmaceuticals or biomolecules such as amino acids. This structural analogy was successful in ferrocifen, the ferrocene (Fc) analogue of tamoxifen, as pioneered by Jaouen et al.[3−6] or steroid hormones combined with [(Cp)Re(CO)$_3$].[7,8] Recent results from our group indicated that amino acids derivatized with Fc or [(Cp)Re(CO)$_3$] are surrogates for natural amino acids.[9]

Whereas biomolecules with pendent [(Cp)Re(CO)$_3$] or Fc are accessible via classical organometallic methods, the ^{99m}Tc analogues must be prepared directly from aqueous buffer, apparently incompatible conditions for HCp which rapidly decomposes in water. Efforts by Katzenellenbogen et al. to prepare [(Cp-R)^{99m}Tc(CO)$_3$] by the so-called double ligand transfer reaction gave the desired piano-stool complexes but from organic solvents in autoclaves.[10,11] Alternatively, the [Fe(Cp)]$^+$ moiety in Fc-derivatized biomolecules could be replaced by [Tc(CO)$_3$]$^+$ but still at T > 100 °C and in the presence of DMSO.[12] We reported an aqueous synthesis at T < 100 °C with biomolecules bound to the cyclopentadiene ring via an α-keto group. Still, all these Cp-derivatized compounds are sensitive and limit types of biomolecules and chemistry.[13,14]

It would be highly desirable to find a general approach to [(Cp-R)^{99m}Tc(CO)$_3$]-type complexes from stable precursors, conjugated to biomolecules and generating the active cyclopentadiene *in situ*. We present in this paper the unexpected formation of [(Cp-R)^{99m}Tc(CO)$_3$]-type complexes from their Diels−Alder dimerized precursors (HCp-R)$_2$. Since a thermal *retro* Diels−Alder reaction, (HCp-R)$_2$ → HCp-R, did not occur to a measurable extent under our conditions, monomerization and coordination to [^{99m}Tc(CO)$_3$]$^+$ are concerted; hence, cleavage is metal-mediated.

Originally, we supposed that the mechanism of the aqueous synthesis of [(Cp-COCH$_3$)^{99m}Tc(CO)$_3$] involved deprotonation at the cyclopentadiene ring followed by direct coordination to [^{99m}Tc(OH$_2$)$_3$(CO)$_3$]$^+$, **1**.[13] Initial coordination to the enolate followed by an intramolecular recoordination to η^5-Cp seemed less likely. On a mechanistic base, no complex formation was expected with cyclopentadiene carboxylic acid (HCp-COOH) since acidity of C−H is too low in aqueous solution.[15] We found that **1** still reacted with HCp-COOH in aqueous buffer at almost the same rate as with HCp-COCH$_3$ to yield [(Cp-COOH)^{99m}Tc(CO)$_3$], **2**, in high yield. Since alkenes do not coordinate to **1** in water (i.e., no reaction with HCp at all) and [HCp-COO$^-$] did not ring-deprotonate, the formation of **2** must consist in initial σ-binding to carboxylate with subsequent or concerted coordination to cyclopentadiene (path A in Scheme 1).

Scheme 1. Stepwise Reaction of **1** with HCp-R and Thiele's Acid **3**, Respectively



The formation of Cp complexes is a thermodynamic driving force. After carboxylate coordination, the Lewis acid [^{99m}Tc(CO)$_3$]$^+$ exerts a strong electronic interaction with the adjacent cyclopentadiene. This distinct affinity for the formation of {(η^5-Cp)Tc} implied the use of a "pre-cyclopentadiene" instead of the comparably reactive HCp-R itself. Evidently, the Diels−Alder dimer (HCp-COOH)$_2$, **3** (Thiele's acid), is such a "protected" cyclopentadiene. Although thermal cracking of **3** requires T > 160 °C, reaction of **3** with **1** at 95 °C for 30 min in buffer resulted in the quantitative formation of **2** (path B).

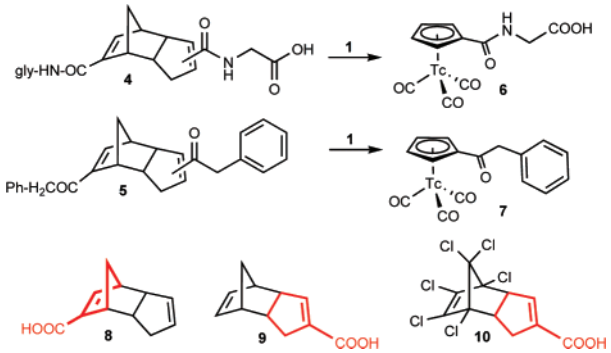
No free HCp-COOH was detected, excluding an *in situ* thermal *retro* Diels−Alder reaction and subsequent entry into path A. Complex **2** was also prepared directly from [^{99m}TcO$_4$]$^-$ in the presence of **3** and under the same conditions as used for the preparation of **1** (Supporting Information [SI]).[16] To our knowledge, this is the first evidence of a metal-mediated *retro* Diels−Alder reaction with concerted formation of the corresponding Cp complex. It can be hypothesized that other metal centers will behave similarly and new mixed Cp-H$_2$O complexes will become accessible.

A metal-mediated *retro* Diels−Alder reaction implies a general approach to [(Cp-R)^{99m}Tc(CO)$_3$] with more complex functionalities, "R", bound to the dicyclopentadiene building block. Synthetically, biomolecules can be conjugated to precursors such as **3** via, for example, amide formation as exemplified with **4** (Scheme 2). Alternatively, NaCp is first reacted with, for example phenyl-acetic acid ethyl ester, and then dimerized to yield **5**.

Compounds **4** and **5** reacted directly with [^{99m}TcO$_4$]$^-$ or **1** to give **6** and **7** after 30−120 min at 95 °C in quantitative yield. We emphasize that no measurable amounts of the respective monomers of **4** and **5** were observed. Further derivatives behaved comparably (SI). For all reactions, one HCp-R per ^{99m}Tc must be released but

10.1021/ja077741l CCC: $40.75

Scheme 2. Reaction of **1** or $[^{99m}TcO_4]^-$ (Isolink Kit) with $(HCp\text{-}R)_2$[a]



[a] $Na[H_3BCO_2H]$ (4 mg), $Na_2B_4O_7{\cdot}10H_2O$ (7 mg), Na_2tartrate$\cdot 2H_2O$ (7 mg) and, e.g., **4** (10^{-3} M), 30−60 min, 95 °C.

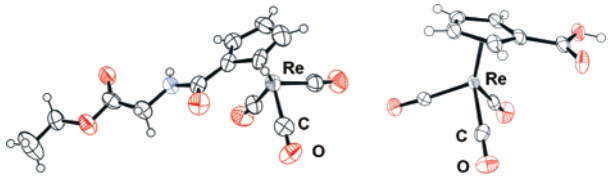


Figure 1. ORTEP presentations of complexes **6**(ethyl ester) and **2**.

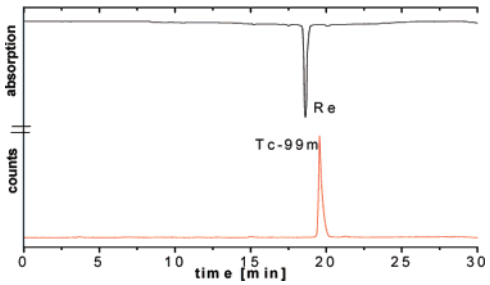


Figure 2. HPLC traces of macroscopic **6** (Re) and the corresponding ^{99m}Tc complex prepared from $[^{99m}TcO_4]^-$. Time difference due to detector separation.

does not play a role since ^{99m}Tc is present at concentrations 10^{-8}−10^{-7} M. To assess the authenticity of the ^{99m}Tc complexes **2**, **6**, and **7**, we applied the reaction to "cold" Re and ^{99}Tc. Since Re is much more robust than Tc, reactions of $[Re(OH_2)_3(CO)_3]^+$ with **3** require longer times or higher temperatures. A hydrothermal reaction at 160 °C gave the Re analogue of **2**. The X-ray structures of the Re surrogates of **2** and **6** (ethyl ester) (Figure 1) and a comparison of the HPLC retention times (Figure 2 and SI) confirmed their authenticity. Other $(HCp\text{-}R)_2$ compounds reacted analogously, indicating that the reaction is general, regardless of the pendent group R.

Diels−Alder reactions with HCp-R produce homodimers such as **3**. It was therefore of interest if the former diene or dienophile was the source for Cp-COOH in **2**. We synthesized and separated the two Diels−Alder heterodimers **8** and **9** (Scheme 2) in a ratio of about 1 to 3.[17] Surprisingly, **8** or **9** reacted only very slowly with $[^{99m}TcO_4]^-$ or **1** with yields of <5% under conditions as above. This implies that two binding or activating groups are required for metal-mediated retro Diels−Alder reactions. Furthermore, a strong leaving group effect has been demonstrated in thermal retro Diels−Alder reactions with HCp.[18] To probe this effect qualitatively, we prepared the heterodimers **10** from C_5Cl_6 and HCp-COOH. Again, the desired product was not received. We conclude that potentially coordinating functionalities such as keto-, amide-, or carboxylate groups should be present on the former diene and the dienophile in the Diels−Alder product. Since concentrations are extremely low, the presence of two biologically active groups is not impairing labeling reactions or applicability.

We have shown that $[^{99m}Tc(OH_2)_3(CO)_3]^+$ mediates the retro Diels−Alder reaction of dicyclopentadiene derivatives with concomitant formation of corresponding piano-stool complexes. A weakly coordinating, Cp pendent group binds to the metal and initiates a retro Diels−Alder reaction and coordinative shift. This opens an avenue to the versatile preparation of $[(Cp\text{-}R)^{99m}Tc(CO)_3]$ complexes with attached targeting molecules "R", avoiding thereby reactive organic precursors. Solid-phase bound Diels−Alder products will yield no carrier-added complexes.

In conclusion, labeling of biomolecules with piano-stool-like ^{99m}Tc and Re complexes from water is now to become an option in the development toward novel radiopharmaceuticals.

Acknowledgment. This research was supported in part by a Eureka project and Mallinckrodt Med. B.V.

Supporting Information Available: Labeling and synthetic procedures, HPLC conditions, and crystallographic data for **2**, **6** and **10** in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Weissleder, R. *Science* **2006**, *312*, 1168−1171.
(2) Liu, S. *Chem. Soc. Rev.* **2004**, *33*, 445−461.
(3) Top, S.; Dauer, B.; Vaissermann, J.; Jaouen, G. *J. Organomet. Chem.* **1997**, *541*, 355−361.
(4) Jaouen, G.; Top, S.; Vessieres, A.; Alberto, R. *J. Organomet. Chem.* **2000**, *600*, 23−36.
(5) Jaouen, G. *Chem. Br.* **2001**, 36−38.
(6) Jaouen, G.; Top, S.; Vessières, A.; Pigeon, P.; Leclercq, G.; Laios, I. *Chem. Commun.* **2001**, 383−384.
(7) Top, S.; Hafa, H. E.; Vessières, A.; Quivy, J.; Vaissermann, J.; Hughes, D. D.; McGlinchey, M. J.; Mornon, J.-P.; Thoreau, E.; Jaouen, G. *J. Am. Chem. Soc.* **1995**, *117*, 8372−8380.
(8) Top, S.; Vessieres, A.; Pigeon, P.; Rager, M. N.; Huche, M.; Salomon, E.; Cabestaing, C.; Vaissermann, J.; Jaouen, G. *ChemBioChem* **2004**, *5*, 1104−1113.
(9) K_d values of Fc-gly and $[(Cp\text{-}R)Re(CO)_3]$ with R = $-(CH_2)_3CH(-NH_2)(-COOH)$ with LAT1 are 33 and 16 μM respectively.
(10) Spradau, T. W.; Katzenellenbogen, J. A. *Organometallics* **1998**, *17*, 2009−2017.
(11) Minutolo, F.; Katzenellenbogen, J. A. *J. Am. Chem. Soc.* **1998**, *120*, 13264−13265.
(12) Masi, S.; Top, S.; Boubekeur, L.; Jaouen, G.; Mundwiler, S.; Spingler, B.; Alberto, R. *Eur. J. Inorg. Chem.* **2004**, 2013−2017.
(13) Wald, J.; Alberto, R.; Ortner, K.; Candreia, L. *Angew. Chem., Int. Ed.* **2001**, *40*, 3062−3066.
(14) Bernard, J.; Ortner, K.; Spingler, B.; Pietzsch, H. J.; Alberto, R. *Inorg. Chem.* **2003**, *42*, 1014−1022.
(15) Top, S.; Lehn, J.-S.; Morel, P.; Jaouen, G. *J. Organomet. Chem.* **1999**, *583*, 63−68.
(16) Alberto, R.; Ortner, K.; Wheatley, N.; Schibli, R.; Schubiger, A. P. *J. Am. Chem. Soc.* **2001**, *123*, 3135−3136.
(17) Peters, D. *J. Chem. Soc.* **1961**, 1037−1042.
(18) Birney, D.; Lim, T. K.; Koh, J. H. P.; Pool, B. R.; White, J. M. *J. Am. Chem. Soc.* **2002**, *124*, 5091−5099.

JA077741L