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Convenient Cyclopentadiene Modifications for Building Versatile (Radio-)Metal Cyclopentadienyl Frameworks

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The synthesis of a bifunctional cyclopentadiene-based building block, bearing an alkyl linker with a terminal chloride and an ester group is described. This building block was modified by nucleophilic substitution, leading to two new *fac*-$[Re(CO)_3]^+$ containing derivatives of known drug candidates for brain imaging and multi-drug resistance in cancer in as few as two steps. Furthermore, the chloride was replaced by an azide, which was subsequently coupled to phenylacetylene as a model alkyne to demonstrate its versatility for undergoing Click-type reactions. The resulting triazole was labelled with ^{99m}Tc to yield the corresponding piano-stool complex in a radiochemical purity of 86%. The identities were confirmed by coinjection with the Re homologue.

Organometallic complexes of the group 7 transition metals rhenium and technetium have attracted great interest in medicinal chemistry. While the radioactive isotopes ^{186}Re/^{188}Re find application in radionuclide therapy and ^{99m}Tc in nuclear medicinal imaging, cold rhenium compounds could have applications as luminescent probes for cell imaging or as anti-cancer agents.[1]

Complexes of the type $[(\eta^5\text{-}C_5H_5)M^I(CO)_3]$ (M=Re, ^{99m}Tc) are of special interest for radiopharmaceutical applications due to the extremely strong binding of the tridentate cyclopentadienyl (Cp) ligand to the *fac*-$[^{99m}Tc(CO)_3]^+$ moiety. High chemical and physiological stabilities are essential since both increase the target/non-target ratio, i.e. the background, but also prevent the label from undergoing undesired decompositions and metabolisms. Eventual toxic effects are less a concern due to the very low concentrations of ^{99m}Tc.[2]

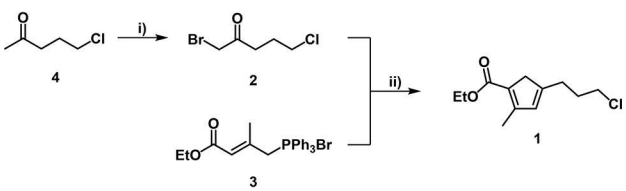
More than 70 complexes of the type $[\eta^5\text{-}(C_5H_5)^{99m}Tc(CO)_3]$, so-called cytectrenes, and substantially more Re compounds are reported in literature.[3] However, none has successfully passed clinical trials thus far, as they did not meet the necessary requirements.

Most ^{99m}Tc cytectrenes are synthesized by the double ligand transfer (DLT) reaction pathway as developed by Wenzel *et al.* in 1992.[4] While straight for making cytectrenes, the synthetic conditions are limiting the nature of pharmaceutically active compounds to be labelled. Systematic screening is difficult and requires extensive post-labelling purifications, inconvenient for clinical settings. Furthermore, the DLT reaction has a rather low functional group tolerance. Alternatives for the direct labelling of cyclopentadienes have been described,[5] however the syntheses of the respective precursors are laborious. Some Cp structures are prone to undergo *Diels-Alder* dimerization. Functionalization of cyclopentadiene depends frequently on the use of different starting materials from multi-step syntheses,[6] rendering them costly and time consuming. All these reasons make the synthesis and biological evaluation of new ^{99m}Tc cytectrenes not very attractive, which hinders the development of new radiopharmaceutical compounds of this class.

For these reasons, new Cp-based building blocks are needed for facilitating the development of new ^{99m}Tc cytectrenes. Such building blocks require a straight synthesis with few steps. The key compounds should be easy to functionalize and stable towards *Diels-Alder* dimerization. Ideally, after functionalization, the pK_a of the cyclopentadiene is sufficiently lowered for allowing its deprotonation in water, a critical point for direct Cp labelling with the $[^{99m}Tc(CO)_3]^+$ core.[7]

We herein report about the synthesis of ethyl 4-(3-chloropropyl)-2-methylcyclopenta-1,3-diene-1-carboxylate (**1**), a doubly functionalized cyclopentadiene framework (Scheme 1). This Cp-based building block is modified in fast and straight ways for matching the chemical properties of pharmaceutically active groups to be introduced. The resulting cyclopentadienyl-based scaffolds are accessible for labelling with the $[^{99m}Tc(CO)_3]^+$ core or for preparing the cold rhenium homologous.

The cyclopentadiene derivative **1** contains both a strongly electrophilic position at the terminus of the alkyl substituent as well as a carbonyl-functionality, adjacent to the cyclopentadiene. While the former allows for modifications of the scaffold via nucleophilic substitution, the latter lowers the pK_a of the Cp unit, which makes it accessible for deprotonation in water. Due to the electron deficient nature of the Cp unit, **1** and its



Scheme 1. Synthesis of **1**; i) Br_2, MeOH, 0 °C to r. t., 24 h, 65%; ii) $NaHCO_3$, CH_2Cl_2/H_2O, r. t., 24 h, 76%.

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derivatives are stable for several weeks at room temperature and for several months at −25 °C.

Based on the work of Hatanaka *et al.* and previous work of our group,[6,8] Cp **1** was synthesized from the α-bromoketone **2** and the Wittig salt **3** under alkaline conditions in 76% yield. Bromoketone **2** was synthesized in 65% yield by treating **4** with Br_2 at low temperatures. Compound **3** was synthesized in three steps from 3,3-dimethylacrylic acid according to a literature procedure.[8c] The carboxilic acid was esterified with EtOH, brominated with *N*-bromosuccinimid (NBS) and azobis (isobutyronitril) (AIBN), and subsequently treated with PPh_3 in toluene to deliver **3**.

Compound **1** was successfully employed in the synthesis of two precursors to derivatives of known drug candidates (Figure 1, compounds [^{99m}**Tc**]**5** and [^{99m}**Tc**]**6**) in one step, or to cold Re derivatives of the corresponding drug candidate in two steps. The first derivative (**7**) was inspired by cytectrene [^{99m}**Tc**]**5**, which was reported by Zenati *et al.* in 2017 for brain imaging.[9] The structure of the second derivative (**8**) had its origin in cytectrene [^{99m}**Tc**]**6**, a compound developed by Li *et al.* in 2018 for the imaging of multi-drug resistance (MDR) in cancer cells.[10] Phosphonium compounds also showed application in targeting mitochondria. A number of ^{99m}Tc compounds have been described for this purpose, albeit not with cytectrene-based complexes.[11] The newly synthesized derivatives **7** and **8** deviate from the imitated lead structures [^{99m}**Tc**]**5** and [^{99m}**Tc**]**6** primarily in additional substituents located on the cyclopentadienyl ligand. Depending on the biological target, these additional substituents may serve different functions.

Compound [^{99m}**Tc**]**5** crossed the blood-brain barrier (BBB), however, it displayed low first-pass uptake into the brain and fast clearance thereof.

Conceptually, the ester group on the cytectrene moiety should allow the compound to accumulate in the brain by passing the blood-brain-barrier (BBB) with the ester group staying intact. In the brain, the ester is hydrolysed to the corresponding carboxylic acid, which traps the compound in the brain. This leads to longer retention times in a similar mechanistic action as is already successfully employed in *Neurolite*, a commercial ^{99m}Tc brain imaging perfusion agent.[12]

Cyclopentadiene **9** was synthesized by reacting **1** with *N*-(*o*-methoxy)phenylpiperazine in the presence of NaI and diisopropylethylamine (DIPEA) in DMF at 120 °C for 4 h in 33% yield. Subsequent reaction with $[Re_2(CO)_{10}]$ in *o*-xylene at 220 °C for 15 min using μ-wave heating gave **7** in 39% yield (Scheme 2). $[Re_2(CO)_{10}]$ was employed as Re-precursor. This precursor works very well for this kind of reaction as we showed in a previous study.[8c] Other Re-precursors have thus not been investigated.

Compound **8** was synthesized by reacting **1** with tris(4-methoxyphenyl)phosphine in the presence of NaI and DIPEA, delivering **10**, with subsequent Re coordination with $[Re_2(CO)_{10}]$ as Re source. The ester functionality in **8**, compared to [^{99m}**Tc**]**6**, is available for conjugation to a second bioactive moiety such as a cytotoxin, or its hydrolysed form simply increases its water solubility for better bioavailability and improved pharmacology.

For expanding the scope and the nature of coupling reactions, the chloride in **1** was replaced by an azide group (**11**, Scheme 3). This azide group can later be employed in Click chemistry with an alkyne-derivatized lead structure of choice. Click reactions are widely used in medicinal chemistry but are not so common in radiopharmaceutical chemistry.[13] Since a plethora of biologically active compounds with alkinyl groups is commercially available, a screening by correspondingly derivatizing **11** becomes easily feasible.

Compound **11** was synthesized along two pathways. Direct reaction of **1** with NaN_3 emphasises the role of **1** as a fast modifiable scaffold, but **11** was obtained in very poor yield only (2% from **4**). A more promising approach is the direct introduction of the azide in a first step by reacting **4** with a slight excess NaN_3. The resulting organo-azide **12** is then treated with Br_2 at low temperatures, yielding the bromoketone **13** in 68% yield. **13** is reacted with **3** along a traditional Wittig-type reaction to give **11** in moderate yields (33% from **4**). The second method to introduce the azide earlier is favourable since it increases the overall yield substantially.

Azide **11** readily undergoes Click reactions with phenylacetylene as model in the presence of Cu^{II} and sodium ascorbate, yielding triazole **14**. Formation of the corresponding Re complex **15** was achieved by reacting **14** with $[Re_2(CO)_{10}]$ in good yield (78%).

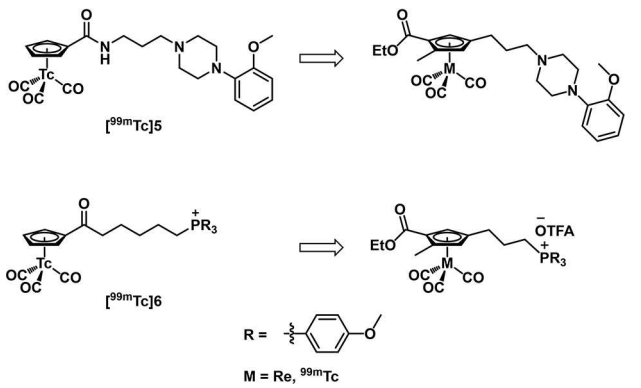
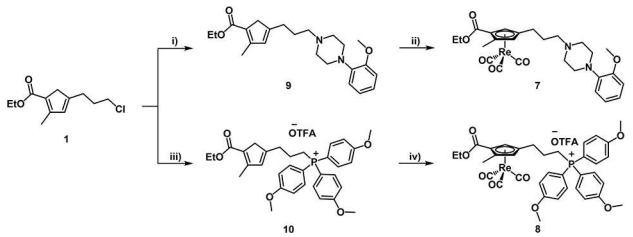
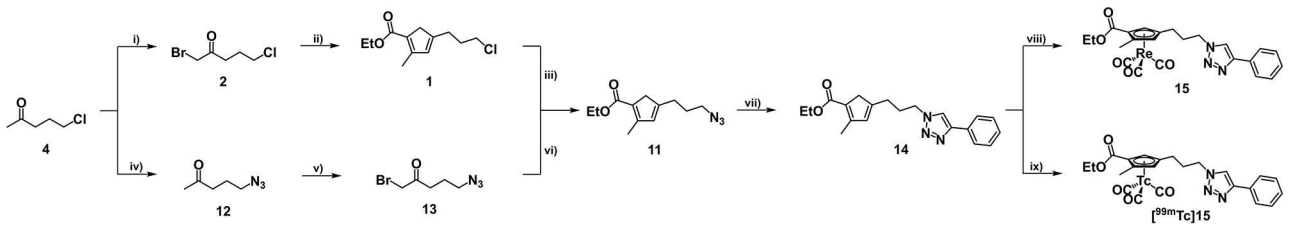


Figure 1. Design of new [η-^{5}(C_5H_5)M^I($CO)_3$] (M=Re, ^{99m}Tc) complexes for medicinal applications based on previously reported drug candidates [^{99m}**Tc**]**5** and [^{99m}**Tc**]**6**.



Scheme 2. Modification of **1** by nucleophilic substitution with subsequent Re complex formation; i) 1-(*o*-methoxy)phenyl piperazine, DIPEA, NaI, DMF, μ-wave, 120 °C, 4 h, 33%; ii) $[Re_2(CO)_{10}]$, *o*-xylene, μ-wave, 220 °C, 15 min, 39%; iii) tris(4-methoxyphenyl)phosphine, NaI, DMF, μ-wave, 120 °C, 90 min, 80%; iv) $[Re_2(CO)_{10}]$, *o*-xylene, μ-wave, 220 °C, 15 min, 70%.



Scheme 3. Synthetic pathway for the introduction of an azide functionality on **1**, click couplings to the products thereof, and subsequent complex formation with Re and labelling with ^{99m}Tc; i) Br_2, MeOH, 0 °C to r. t., 24 h, 65%; ii) **3**, $NaHCO_3$, CH_2Cl_2/H_2O, r. t., 24 h, 76%; iii) NaN_3, NaI, DMF, 60 °C, 23 h, 5%; iv) NaN_3, DMF, 60 °C, 25 h, 82%; v) Br_2, MeOH/cyclohexane, 0 °C to r. t.,16 h, 68%; vi) **3**, $NaHCO_3$, CH_2Cl_2/H_2O, r. t., 24 h, 51%; vii) phenylacetylene, $CuOAc_2$, sodium ascorbate, MeOH, μ-wave, 100 °C, 20 min, 94%; viii) $[Re_2(CO)_{10}]$, *o*-xylene, μ-wave, 220 °C, 15 min, 78%; ix) two-pot: $[^{99m}Tc(H_2O)_3(CO)_3]^+$, EtOH/$H_2O$, μ-wave, 130 °C, 30 min, 86% RCP (hydrolysed ester); one-pot: *Isolink*® kit, EtOH/H_2O, μ-wave, 120 °C, 60 min, 67% RCP.

In order to exemplify labelling of these click-coupled conjugates for ^{99m}Tc, **14** coordinated to $[^{99m}Tc(H_2O)_3(CO)_3]^+$ (**16**) when a freshly synthesized solution of **16** in H_2O (pH = 13) was added to an ethanolic solution of **14**. For confirming the identity of the formed ^{99m}Tc species *via* HPLC analysis, the reaction solution was spiked with a small amount of **15** prior to the formation of [**^{99m}Tc**]**15**. The resulting mixture was heated to 130 °C for 30 min in a μ-wave oven, yielding the hydrolyzed form of [**^{99m}Tc**]**15** in 86% radiochemical purity (RCP). HPLC traces of hydrolysed **15** and [**^{99m}Tc**]**15** are given in Figure 2. Hydrolysis of the ethyl ester functionality occurred due to the strongly basic, aqueous conditions of the labelling reaction.

Since one-pot reactions are preferable to two-pot reactions in a clinical setting, a one-pot labelling reaction directly from $[^{99m}TcO_4]^-$ was carried out. Compound **14** was added directly to the *Isolink*® kit in 50% EtOH and heated to 120 °C for 1 h. The desired complex [**^{99m}Tc**]**15** was thereby obtained in 67% RCP. The one pot reaction is possible, albeit lower yields are obtained than in the two-step procedure. The main impurities in the one-pot reaction were unreacted $[^{99m}TcO_4]^-$ (9%) and residual $[^{99m}Tc(H_2O)_3(CO)_3]^+$ (18%). These conditions can certainly be improved e.g. by additional ethanol to increase the solubilities of highly lipophilic compounds such as **14**, and cyclopentadienes in general. For more hydrophilic alkynes, the ethanol content can be reduced and distinctly hydrophilic compounds can be labelled without any organic solvents, as we recently demonstrated.[8c]

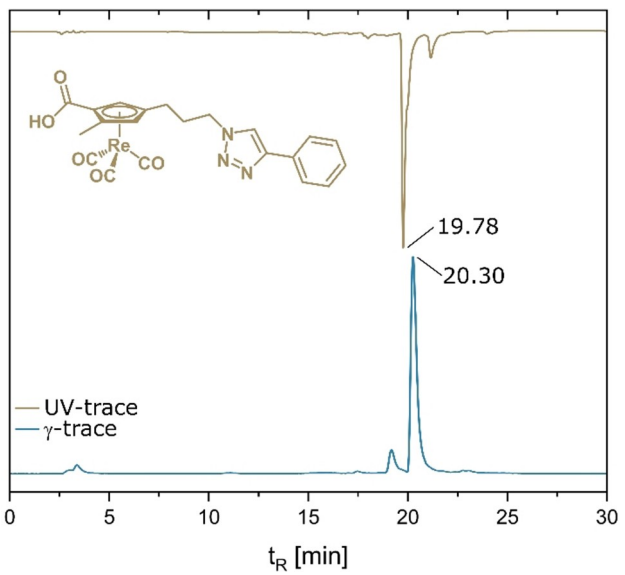


Figure 2. UV-HPLC trace and γ-HPLC trace of hydrolysed **15** and [**^{99m}Tc**]**15**.

Of note, the carboxylic acid function in hydrolysed **15** remains reactive under acidic conditions. It rapidly re-esterified with both EtOH and MeOH if used as eluents on the HPLC. This was evident from the respective comparisons with the rhenium homologous. Interestingly, this behaviour was not observed for the corresponding ^{99m}Tc compound. For avoiding re-esterifications, MeCN should be employed as eluent.

In conclusion, we have demonstrated that **1** presents a viable platform for fast and convenient syntheses of $[\eta^{-5}(C_5H_3RR')M^I(CO)_3]$ (M = Re, ^{99m}Tc) type complexes. The synthesis requests few steps, is straight and provides decent to good yields. Compound **1** carries two functionalities on the cyclopentadiene ring, which can be conjugated to bioactive molecules of choice. Two new drug-candidates were prepared in only two steps. Furthermore, azide can replace the chloride group to obtain **11**, which readily undergoes Click-reactions. The resulting triazole was labelled with *fac*-$[^{99m}Tc(CO)_3]^+$ in both one- or two-step reactions. These new building blocks will facilitate the systematic screening and finding of new ^{99m}Tc cytectrenes for imaging. Furthermore, they expand on the already available methods for metal conjugates of interesting biomolecules such as peptides and antibodies.[14]

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: Bioorganometallic chemistry · Click chemistry · Cyclopentadiene ligands · Rhenium · Technetium

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