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Aqueous syntheses of $[(Cp\text{-}R)M(CO)_3]$ type complexes (Cp = cyclopentadienyl, M = Mn, ^{99m}Tc, Re) with bioactive functionalities

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ABSTRACT

We describe reactions of $[^{99m}Tc(H_2O)_3(CO)_3]^+$ (**1**) with Diels–Alder products of cyclopentadiene such as "Thiele's acid" $(HCp\text{-}COOH)_2$ (**2**) and derivatives thereof in which the corresponding $[(Cp\text{-}COOH)^{99m}Tc(CO)_3]$ (**3**) complex did form in water. We propose a metal mediated Diels–Alder reaction mechanism. To show that this reaction was not limited to carboxylate groups, we synthesized conjugates of **2** $(HCp\text{-}CONHR)_2$ (**4a–c**) (**4a**, R = benzyl amine; **4b**, R = N_α-Boc-L-2,3-diaminopropionic acid and **4c**, R = glycine). The corresponding ^{99m}Tc complexes $[(\mathbf{4a})^{99m}Tc(CO)_3]$ **6a**, $[(\mathbf{4b})^{99m}Tc(CO)_3]$ **6b** and $[(\mathbf{4c})^{99m}Tc(CO)_3]$ **6c** have been prepared along the same route as for Thiele's acid in aqueous media demonstrating the general applicability of this synthetic strategy. The authenticity of the ^{99m}Tc complexes on the no carrier added level have been confirmed by chromatographic comparison with the structurally characterized manganese or rhenium complexes.

Studies of the reaction of **1** with Thiele's acid bound to a solid phase resin demonstrated the formation of $[(Cp\text{-}COOH)^{99m}Tc(CO)_3]$ **3** in a heterogeneous reaction. This is the first evidence for the formation of no carrier added ^{99m}Tc radiopharmaceuticals containing cyclopentadienyl ligands via solid phase syntheses. Macroscopically, the manganese analogue **5a** and the rhenium complexes **5b–c** have been prepared and characterized by IR, NMR, ESI-MS and X-ray crystallography for **5a** (monoclinic, $P2_1/c$, a = 9.8696(2) Å, b = 25.8533(4) Å, c = 11.8414(2) Å, β = 98.7322(17)°) in order to unambiguously assign the authenticity of the corresponding ^{99m}Tc complexes.

1. Introduction

The group 7 transition metals Tc and Re are in the main focus for novel radiopharmaceuticals in diagnostic imaging (^{99m}Tc) or for therapeutic (^{186}Re, ^{188}Re) purposes [1–4]. Organometallic compounds are generally considered to require water and air free synthetic conditions and to be therefore not compatible with biomedical applications. However, the preparation of the complex $[^{99m}Tc(CN\text{-}R)_6]^+$ directly from $[^{99m}TcO_4]^-$ in water clearly eliminated this prejudice [5]. This complex contained monodentate ligands only but is still perfectly stable under in vivo conditions due to the kinetic stability of the d^6 electronic configuration. This isocyanide ^{99m}Tc complex is the most widely used radiopharmaceutical in myocardial imaging [6]. Chemically, its high stability towards substitution reactions makes it not suitable for the so called third generation radiopharmaceuticals, labeled targeting biomolecules. On the other hand, the need for innovative and efficient synthetic methods of organometallic compounds from water is crucial for application in the bio-organometallic field [7,8].

Cyclopentadienyl (Cp^-) is one of the most basic ligand in organometallic chemistry. Cyclopentadienyl is relevant since it has a low molecular weight, it blocks three coordination sites and includes the possibility of conjugating targeting vectors. Keeping the principle of using inert complexes for radiopharmaceutical in vivo applications in mind, Cp^- forms robust organometallic $[(Cp\text{-}R)M(CO)_3]$ (M = Re, Tc) complexes with Tc and Re being in the oxidations state +1. In fact, complexes $[(Cp\text{-}R)M(CO)_3]$ have been attached both to antibodies [9,10] and to steroidal hormones [11,12] without substantial loss of receptor recognition and affinity. A more prominent and well explored example is in the context of tamoxifen/ferrocifen where $[CpRe(CO)_3]$ was shown to surrogate ferrocene under retention of biological activity [13,14]. Cyclopentadienyl on other hand has severe disadvantages for applications in water. It is essentially insoluble and unstable in water, tends to di- or polymerize and can hardly be deprotonated ($pK_a \approx 15$). For radiopharmaceutical applications on a routine base, it is desirable to prepare e.g. $[(Cp\text{-}R)^{99m}Tc(CO)_3]$ directly in water. Some approaches to $[Cp\text{-}R^{99m}Tc(CO)_3]$ have been reported but most of them require harsh condition and organic solvents [15–18]. We reported a fully aqueous synthesis of $[(Cp\text{-}R)^{99m}Tc(CO)_3]$ at <100 °C [19]. Still, the drawback of this approach was the sensitivity of Cp group pendent to the biomolecule. To circumvent this issue, protection of

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the Cp^- ligand would be helpful. The Diels–Alder product of dimerized [HCp-R] would be an ideal precursor provided that it could be concertedly cleaved and coordinated. We recently showed that the reaction of $[^{99m}Tc(H_2O)_3(CO)_3]^+$ (**1**) with Diels–Alder products such as Thiele's acid $(HCp\text{-}COOH)_2$ (**2**) gave the corresponding $[(Cp\text{-}COOH)\ ^{99m}Tc(CO)_3]$ (**3**) complex. The labelling conditions were such that **2** did not thermally cleave [20]. Hence, we proposed that the retro Diels–Alder reaction with concerted coordination to the $[^{99m}Tc(CO)_3]^+$ core was metal mediated. A first step might involve the coordination of the carboxylate followed by cleavage and coordination. Dimerized cyclopentadiene compounds can therefore be considered as "pre-cyclopentadiene" able to form the $\{(\eta^5\text{-}Cp)Tc\}$ core.

As an important extension to the previous synthetic approaches, we describe inhere a more general approach to $[(Cp\text{-}R)^{99m}Tc(CO)_3]$ complexes which also allows the introduction of additional functionalities R on the Cp ring. Thiele's acid was coupled with amines to give amides. We show that amides can also act as anchoring group and the corresponding ^{99m}Tc complexes be synthesized along a retro Diels–Alder reaction. The scope of this finding is the principal possibility of coupling different families of biomolecules, including peptides or other small amine containing vectors to the cyclopentadiene ring. Furthermore, we will show that the retro Diels–Alder can also be performed directly on a solid phase support. Thereby, the synthesis of no carrier added $[(Cp\text{-}R)^{99m}Tc(CO)_3]$ type complexes is enabled.

2. Results and discussion

2.1. Synthesis of ligands

Thiele's acid is a very convenient precursor for the preparation of cyclopentadiene derivatives. Starting from [HCp-COOH], Diels–Alder dimerisation gave different stereoisomers [21]. One of the isomers could be separated by fractioned crystallization and its structure being elucidated. An ORTEP together with important bond lengths and angles is given in Fig. 1. All subsequent functionalisations were then performed with this single isomer (Scheme 1). The preparation of ligand **4a** $(HCp\text{-}ba)_2$ and **4b** $(HCp\text{-}dap)_2$ was performed in a two step reaction. Thiele's acid was easily activated with pentafluorophenyl-trifluoroacetate in an *N,N*-dimethylformamide (DMF)/pyridine mixture at room temperature to yield **4** in quantitative yield in solution [22]. Precursor **4** was then allowed to react with benzyl amine to yield **4a** in 43% yield as white solid. Using the same protocol, N_α-Boc-L-2,3-diaminopropionic acid (Boc = *tert*-butoxycarbonyl) was reacted with **4** and the reaction was monitored by HPLC. Deprotection with a trifluoroacetic acid (TFA)/CH_2Cl_2 mixture 1:1 gave **4b** in 95% yield as an off-white solid. The analytical data (see Section 4) confirmed the authenticity of the compounds. The synthesis of **4c** $(HCp\text{-}gly)_2$ was previously reported by us [20]. We would like to emphasize at this point, that this strategy can be followed by principally any biomolecule. Along this route, targeting vectors such as peptides or CNS receptor ligands can be introduced. Taking the clean labelling with ^{99m}Tc into account (vide infra) it became clear that new labelled biomolecules can be introduced in which the amide functionality acts as an anchoring and activation group for the formation of $[(Cp\text{-}R)^{99m}Tc(CO)_3]$ complexes.

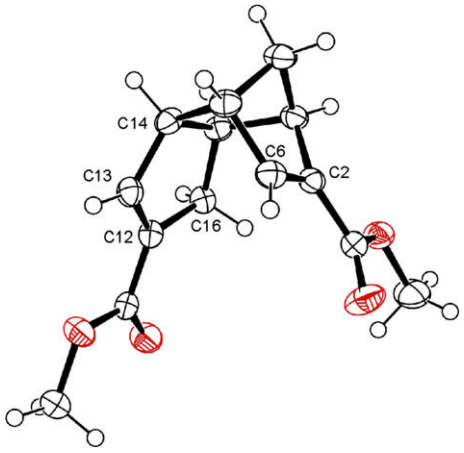
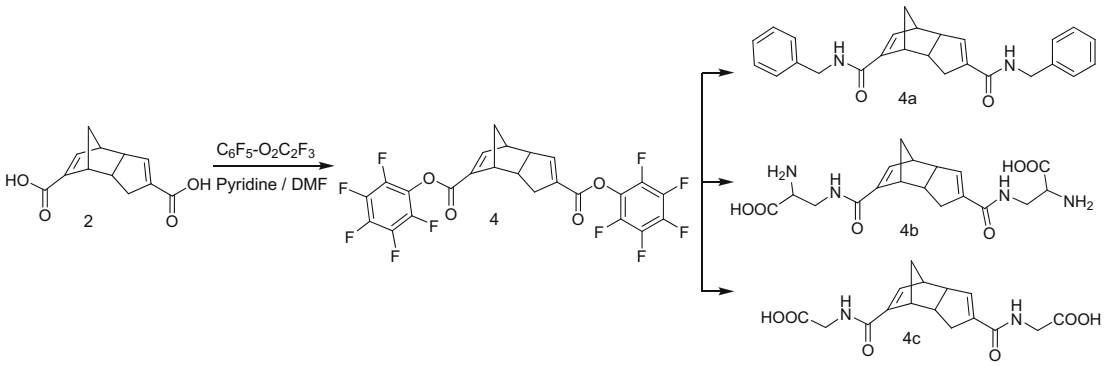


Fig. 1. ORTEP presentation of Thiele's acid dimethyl ester. Important bond lengths (Å) and angles (°) are: C(2)–C(6) = 1.3374(19); C(12)–C(16) = 1.5009(18); C(12)–C(13) = 1.3343(17); C(13)–C(14) = 1.4947(17); C(6)–C(2)–C(1) = 124.76(12); C(6)–C(2)–C(3) = 107.74(11); C(13)–C(12)–C(11) = 126.00(12); C(13)–C(12)–C(16) = 113.05(11); C(12)–C(13)–C(14) = 112.40(11).

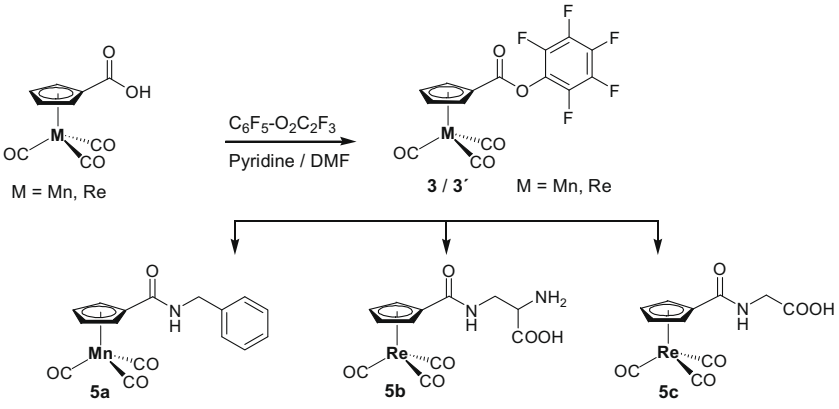
2.2. Synthesis of manganese and rhenium complexes

A comparison of the HPLC retention times of the fully characterized manganese, rhenium or technetium complexes with the corresponding ^{99m}Tc compounds is an accepted method to assess the authenticity of the ^{99m}Tc complexes. Accordingly, the manganese complex $[(Cp\text{-}ba)Mn(CO)_3]$ (**5a**) and the rhenium complexes $[(Cp\text{-}dap)Re(CO)_3]$ (**5b**) and $[(Cp\text{-}gly)Re(CO)_3]$ (**5c**) were synthesized by different methods and fully characterized with spectroscopic methods including X-ray crystallography for **5a** (Scheme 2). As described above for the free ligands, the complexes **5a–c** were synthesized by activation of cymantrene-carboxylic acid $[(Cp\text{-}COOH)Mn(CO)_3]$ or its rhenium analogue with pentafluorophenyl-trifluoroacetate [22] in the presence of pyridine. These activated complexes were then allowed to react with benzylamine to give **5a** as a yellow powder in 65% yield. Compound **5a** could be recrystallised from CH_2Cl_2/hexane to afford X-ray quality crystals. The proton NMR of **5a** in $CDCl_3$ showed the aromatic protons at 7.25 ppm as multiplet and the protons of the cyclopentadienyl ring were observed at 5.23 and 4.70 ppm, respectively, as two distinct multiplets. The NH amide proton and the methylene proton are observed at 5.91 and 4.31 ppm as two broad signals. The infrared spectrum in KBr showed the two characteristic strong bands for ν_{CO} at 2028 and 1927 cm^{-1}, respectively, which confirmed the presence of the *fac*-$[Mn(CO)_3]^+$ moiety. The amide C=O bond stretch showed a strong absorption at 1638 cm^{-1}. Furthermore, the ESI-MS in both, positive and negative mode showed single peaks at 360 and 336 corresponding to M+Na, respectively, M−H. **5b** was prepared using the same protocol with $[(Cp\text{-}COOH)Re(CO)_3]$ and N_α-Boc-L-2,3-diaminopropionic acid followed by TFA deprotection. After workup **5b** was isolated as TFA salt giving a yield of 70% with respect to the starting material $[(Cp\text{-}COOH)Re(CO)_3]$. The ESI-MS positive mode measurements realized in methanol, indicated a peak at 467 as a main peak corresponding to M+H. As expected, the IR spectrum displays two strong vibration ν_{CO} at 2026 and 1926 cm^{-1} associated to *fac*-$[Re(CO)_3]^+$ fragment and one amide C=O bond stretch at 1639 cm^{-1}. The proton NMR spectrum in CD_3OD confirmed the proposed structure with the presence of two triplets associated to Cp ring at 6.16 and 5.59 ppm while the α proton to amino acid function and the CH_2 appear as two multiplets at 3.75 and 3.68 ppm.

Crystals suitable for an X-ray structure analysis were obtained for cymantrene functionalized with benzyl amine **5a** by recrystallization from CH_2Cl_2/hexane. An ORTEP is given in Fig. 2. The compound crystallizes in the monoclinic crystal system (Table 1). The manganese central atom is η^5-coordinated to the C_5H_4–CO–NH–CH_2–C_6H_5 ligand and the three CO groups complete the coordination sphere. The geometry around manganese is pseudo octahedral and, accordingly, C–M–C between CO are close to 90°. The amide group O4–C9–N1 has the usual geometrical parameters with a



Scheme 1. Syntheses of the activated form of Thiele's acid **4**, and amide derivatives **4a**–**4c** containing a benzyl group, an amino acid and glycine.



Scheme 2. Syntheses of the manganese and rhenium complexes **5a**–**c**.

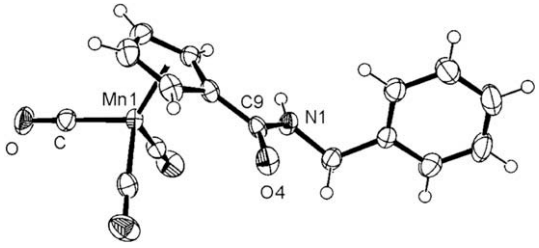


Fig. 2. ORTEP presentation of [(Cp-ba)Mn(CO)$_3$] (**5a**). Important bond lengths (Å) and angles (°) are: Mn(1)–C(1) = 1.7905(16); Mn(1)–C(3) = 1.796(2); Mn(1)–C(2) = 1.7996(17); Mn(1)–C(4) = 2.1210(15); Mn(1)–C(5) = 2.1363(16); C(9)–N(1) = 1.339(2); C(10)–N(1) = 1.452(2); C(1)–O(1) = 1.1414(19); C(2)–O(2) = 1.1423(19); C(4)–C(8) = 1.428(2); C(4)–C(5) = 1.430(2); C(1)–Mn(1)–C(3) = 92.11(8); C(1)–Mn(1)–C(2) = 92.47(7); C(2)–Mn(1)–C(3) = 92.26(8); C(1)–Mn(1)–C(4) = 99.81(7); O(1)–C(1)–Mn(1) = 178.07(15); O(2)–C(2)–Mn(1) = 178.72(15).

C–N bond length of 1.339(2) Å and is essentially in the same plane with the neighboring cyclopentadienyl ring (1.2° torsion angle between the amide group and the Cp plane). This lead to orthogonal orientation between the Cp and the phenyl ring. All the geometrical parameters agreed well with the data reported for $(C_5H_4COCH_3)Mn(CO)_3$ in the literature [23].

2.3. Syntheses of ^{99m}Tc complexes

We recently reported the metal mediated retro Diels–Alder reaction of $(HCp\text{-}COOH)_2$ with $[^{99m}Tc(OH_2)_3(CO)_3]^+$ or $[^{99m}TcO_4]^-$ under formation of the corresponding cyclopentadienyl complex $[(Cp\text{-}COOH)^{99m}Tc(CO)_3]$ (**3**) [20,24]. On the basis of these results, we investigated the possibility of extending this method to retro Diels–Alder reactions with building blocks different from **2**. We were especially interested in other functionalities, particularly amide groups, bound to the cyclopentadiene ring. Although this has principally been shown with ligand **4c**, the presence of the carboxylate group in this ligand might still be the anchoring group required for cyclopentadienyl complex formation. In ligand **4a**, this was not the case anymore. Thus, if **4a** could be labelled, a clear hint was given that a carboxylate group is not an absolute requirement for retro Diels–Alder reaction. This should lead to a more general approach towards $[(Cp\text{-}R)^{99m}Tc(CO)_3]$ complexes conjugated to

Table 1
Crystallography parameters for complex **5a** and Thiele's dimethyl ester

Formula	$C_{16}H_{12}MnNO_4$	$C_{14}H_{16}O_4$
M (g mol^{-1})	337.21	248.27
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1/c$	$P2_1/n$
a (Å)	9.8696(2)	8.0642(2)
b (Å)	25.8533(4)	6.0850(1)
c (Å)	11.8414(2)	24.9771(5)
β (°)	98.7322(17)	94.453(2)
V (Å^3)	2986.45(9)	1221.94(4)
Z	8	4
$D_{calc.}$ (g cm^{-3})	1.500	1.350
Linear absorption coefficient (mm^{-1})	0.900	0.099
Absorption correction	Multi-scan	Multi-scan
Relative trans$_{min}$/trans$_{max}$	0.88553/1.00000	0.91246/1.00000
Measured reflections	38865	13181
Unique reflections [$R_{(int)}$]	9105/0.0387	3734/0.0242
Refined parameters	397	165
$R_1(F)/wR_2$ (F^2) ($I > 2\sigma(I)$)[a]	0.0347/0.0835	0.0480/0.1230
GOF	0.953	1.057

[a] $R_1 = |F_o - F_c|/|F_o|$; $wR_2 = [w(F_o^2 - F_c^2)^2/(wF_o^2)]^{-1/2}$.

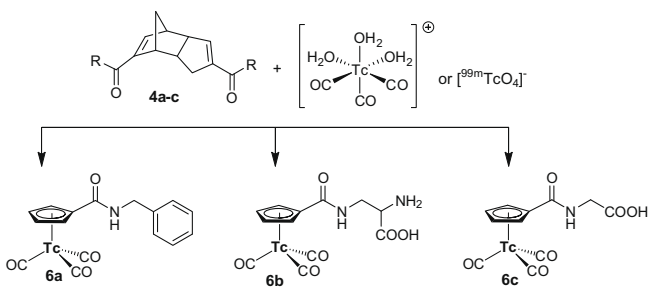
targeting molecules. Complexes **5a–c** are the models based on which the identity of the corresponding ^{99m}Tc complexes can be confirmed.

We used three different procedures to synthesize the ^{99m}Tc complexes **6a–6c**. In particular, pH, reaction time and temperature were varied in order to find optimal conditions for the metal mediated retro Diels–Alder reaction. Method A involved the classical "one pot" reaction which was the direct synthesis from $[^{99m}TcO_4]^-$ in an Isolink® Kit and in the presence of ligands **4a–c**. Method B was a two step procedure under alkaline conditions, thereby $[^{99m}Tc(OH_2)_3(CO)_3]^+$ was synthesized first and then reacted with the corresponding ligand. Method C finally consisted also in a two step reaction, initial synthesis of **1**, neutralizing the solution with phosphate buffer to pH 7.4 and subsequent addition and reaction with **4a–c**. The reactions and the corresponding products are depicted in Scheme 3.

The compounds $(HCp\text{-}CONHR)_2$ (**4a-b**) were reacted directly with $[^{99m}TcO_4]^-$ in the presence of boranocarbonate $[H_3BCOOH]^-$ to give **6a–b** after 30–180 min at 95 °C in quantitative yield. In the reaction solutions, we could not detect measurable amounts of the respective monomers of **4a** or **4b** by HPLC. Still, for all reactions, one HCp-R per ^{99m}Tc must be released. Since the concentration of $[^{99m}TcO_4]^-$ is very low (10^{-8}–10^{-7} M) we did not expect to find the monomer provided that no thermal retro Diels–Alder reaction occurred, which was obviously not [25] the case. Decreasing the temperature to 70 °C reduced the rate of the reaction and we started to observe the formation of side products especially for **4b**. Since no corresponding model compounds could be used to identify the composition of these side products, we assume that under more moderate conditions coordination might also take place at the amino acid part in **4b**. Though not a very favorable bidentate ligand, we showed previously that coordination takes place under formation of well defined amino acid complexes [20]. Unlike of basic direct synthesis of **6a** and **6c**, the preparation of **6b** is best realized with the buffered method which gave quantitative conversion. The pH of the reaction seemed of to be crucial for the metal mediated retro Diels–Alder reaction especially for the zwitterions of amino acid derivatives. The authenticity of the ^{99m}Tc compounds could be assessed by comparison with the cold manganese and rhenium analogues synthesized before. An example for an HPLC comparison is given in Fig. 3. The inverted trace on top shows the radiochromatogram of **6a** whereas the lower one gives the UV–Vis absorption trace of the manganese surrogate **5a**.

2.4. Retro Diels–Alder reaction on a solid phase

The Diels–Alder product of Thiele's acid $(HCp\text{-}COOH)_2$ (**2**) was chosen as a model substrate to investigate if the corresponding complexes $[(Cp\text{-}COOH)^{99m}Tc(CO)_3]$ could be synthesized directly on solid phase support. This would give access to no carrier added ^{99m}Tc complexes. A low concentration of radiopharmaceutical is



Scheme 3. Preparation of the ^{99m}Tc complexes **6a–c** under aqueous conditions.

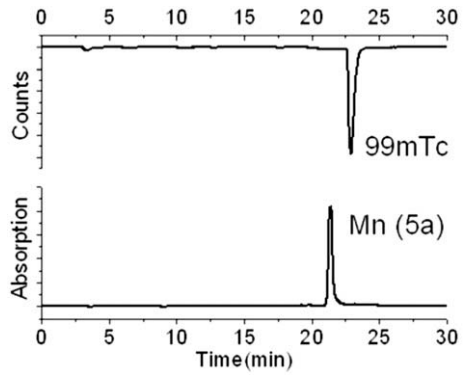
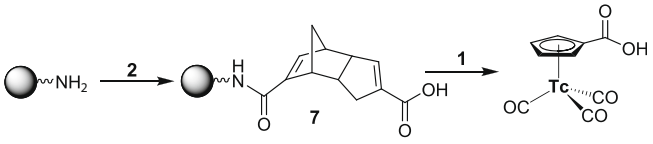


Fig. 3. HPLC trace of the cold manganese complex **5a** and the corresponding ^{99m}Tc complex **6a** complex as prepared directly from $[^{99m}TcO_4]^-$. The time difference is due to detector separation.

crucial in application when working with receptor targeting agents. A too high concentration of e.g. unlabelled peptide would avoid good images since the cold peptides preferentially bind to the receptors. Thus, reasonable images could not be received due to insufficient target to background ratio. The solid phase polymer was a polystyrene matrix functionalized with polyethyleneglycol and bearing terminal NH_2 groups. These polymers had good swelling properties in water. The polymer was then linked via an amide bond to **2** previously activated with the *O*-(7-Azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU)/*N*,*N*-diisopropylethylamine procedure. The efficiency of the coupling reaction was performed according to literature procedure with a 2,4,6-trinitrobenzylsulfonic acid (TNBS) test. This allowed a semi-quantitative analysis of the uncoupled terminal amino groups [26]. Essentially no free NH_2 groups were detected by this method, thus, confirming the complete derivatization of the resin by Thiele's acid. The procedure and the products are shown in Scheme 4. Additional IR spectroscopy analysis in KBr pellets showed the presence of two news peaks at 1535 and 1712 cm^{-1}, respectively. Since these bands were not present in the unmodified resin, we attributed them to the amide bond and the terminal carboxylic group of coupled Thiele's acid.

The resulting polymer **7**, Thiele's acid PEG-NH-CO-$(HCp)_2$COOH derivative was first swelled in water at 4 °C for 3 h and then allowed to react in one pot reaction in presence of $[^{99m}TcO_4]^-$ and $[H_3BCOOH]^-$ at 95 °C for 3 h (Scheme 4). The reaction mixture was then cooled and the solid phase removed by filtration. The HPLC analysis of the remaining solution revealed one single peak at 21.35 min and residual $[^{99m}TcO_4]^-$. The formation of $[(Cp\text{-}COOH)^{99m}Tc(CO)_3]$ (**3**) at 21.35 min in 50% yield in the reaction mixture. This is to our knowledge, the first evidence of aqua metal mediated *retro* Diels–Alder on solid support. Even though the reaction occurred in basic condition with $[^{99m}Tc(H_2O)_3(CO)_3]$ (**1**), the modulation of other parameters such reaction time and temperature did not improved the yield but we observed the rising of additional peaks. It seems that $[^{99m}TcO4]^-$ or **1** could not reach efficiently the polymer bound Thiele's acid in heterogeneous conditions since it is reasonable to assume that the distribution of polymer is not homogeneous in the vial. However, **3** could be easily



Scheme 4. Solid phase synthesis and labeling of Thiele's acid.

assigned by direct synthesis from Thiele's acid and **1** in water buffer with almost quantitative conversion as previously described [20].

3. Conclusion

Thiele's acid has been conjugated to different functional groups. These dimers ligands were found to be compatible to generated piano stool-like ^{99m}Tc complexes in water. Metal mediated *retro* Diels–Alder seems to be a new general approach to produce biomolecules [(Cp-R)^{99m}Tc(CO)$_3$] with more complex functionalities bound to cyclopentadienyl. Furthermore, promising preliminary studies shown that the scope of this *retro* Diels–Alder reaction can be extend to the solid phase synthesis in aqueous media. This water solid phase synthesis of ^{99m}Tc complexes is to our knowledge, the first evidence of metal mediated *retro* Diels–Alder on solid support.

4. Experimental

4.1. General remarks

Reactions were carried out in oven-dried Schlenk glassware under an atmosphere of pure nitrogen when necessary. Solvents were dried over molecular sieves and degassed prior to use. All chemicals were obtained from commercial sources and used without further purification. The acid [(Cp-COOH)M(CO)$_3$] (M = Mn, Re) and **5c** were prepared according to literature procedure [20,21]. NMR spectra were recorded on Bruker Advance 500, 400 and Varian 200 spectrometers. Chemical shifts δ in ppm relative to tetramethylsilane (TMS) and coupling constants *J* are given in Hz. Mass spectra were measured on Bruker Esquire HCT (ESI) instrument, only characteristic fragments are given. The solvent flow rate for ESI measurements was 5 μl min^{-1} with a nebulizer pressure of 15 psi and a dry gas flow rate of 5 l min^{-1} at a dry gas temperature of 300 °C. IR spectra were recorded as KBr pellets on a Perkin Elmer BX II IR spectrometer.

HPLC solvents consisted of 0.1% CF_3COOH in H_2O (solvent A) and methanol (solvent B) with variable gradient (0–3 min, 100% A; 3–3.1 min, 0–25% B; 3.1–9 min, 25% B; 9–9.1 min, 25% B to 34% B; 9.1–20 min, 34% B to 100% B; 20–25 min, 100% B; 25–25.1 min 100% B to 100% A; 25.1–30 min 100% A).

4.2. Bis(pentafluorophenyl) 3a,4,7,7a-tetrahydro-1H-4,7-methanoindene-2,6-dicarboxylate (**4**)

3a,4,7,7a-Tetrahydro-1*H*-4,7-methanoindene-2,6-dicarboxylic acid (Thiele's acid) **2** (881 mg, 4.0 mmol) was dissolved in 9 ml of DMF. Pyridine (708 μl, 8.8 mmol) and pentafluorophenyl-trifluoroacetate (1.6 ml, 9.3 mmol) were added, and the solution stirred at r.t. under N_2 for 5 h. 600 ml ethyl acetate were added, and the solution washed three times with 0.1 N HCl and once with 5% (wt./vol.) aqueous $NaHCO_3$. The organic phase was dried over Na_2SO_4 and the solvent removed *in vacuo* to produce 2.346 g of crude product which contained approximately 20% of mono-activated product. The solid was then dissolved in 200 ml of CH_2Cl_2 and extracted with 400 ml of 5% Na_2HCO_3. The organic phase was dried under reduced pressure and 1.639 g (74% yield) of **4** was isolated as a brownish oil. The product was used for the next synthesis step without further purification.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.28 (d, 1H, *J* = 3.2 Hz, CH), 6.88 (d, 1H, *J* = 2.1 Hz, CH), 3.71 (m, 1H, CH), 3.53 (m, 1H, CH), 3.35 (m, 1H), 3.12 (m, 1H, CH), 2.68 (m, 1H, CH), 2.25 (m, 1H, CH), 1.86 (m, 1H, CH), 1.55 (m, 1H, CH). ^{19}F{^{1}H} NMR ($CDCl_3$): δ −154.6, −159.8, −164.0.

4.3. N,N′-Dibenzyl-3a,4,7,7a-tetrahydro-1H-4,7-methanoindene-2,6-dicarboxamide (**4a**)

Compound **4** (811 mg, 1.47 mmol) was dissolved in 15 ml (DMF) and stirred under N_2 at 0 °C. A solution of benzyl amine (376 mg, 3.52 mmol) and $NaHCO_3$ (296 mg, 3.52 mmol) in 15 ml water was added drop-wise via syringe through a rubber seal cap. The reaction mixture was stirred under N_2at 0 °C for 10 h. The solvent was then removed under reduced pressure and the residue dissolved in 150 ml H_2O and acidified with 1 N HCl to pH 3. The solution was extracted three times with 150 ml of CH_2Cl_2. After each extraction step the pH of the aqueous phase was re-adjusted to 2–3. The combined organic phases were dried over Na_2SO_4. The solvent was removed *in vacuo* and the crude product purified by flash chromatography with a gradient from pure CH_2Cl_2 to CH_2Cl_2/MeOH 1:1. Compound **4a** was isolated as white powder (266 mg, 43% yield). Calcd. for M_r ($C_{26}H_{26}N_2O_2$) 398.5; ESI-MS(CH_3OH, pos. Mode): 421.3 $[M+Na]^+$. Anal. Calc. for $C_{26}H_{26}N_2O_2$: C, 78.36; H, 6.58; N, 7.03. Found: C, 78.30; H, 6.74; N, 6.92%. ^{1}H NMR (200 MHz, $CDCl_3$), δ 7.29–7.32 (m, 10H, Ph), 6.43 (d, 1H, *J* = 3.2 Hz, CH), 6.15 (d, 1H, *J* = 2.8 Hz, CH), 5.75–5.86 (m, 2H, NH), 4.35–4.52 (m, 4H, CH_2), 3.65 (m, 1H, CH), 3.43 (m, 1H, CH), 3.06 (m, 1H, CH), 2.96 (m, 1H, CH), 2.01–2.43 (m, 2H, CH_2), 1.45–1.67 (2H, CH_2).

4.4. 3,3′-[Tricyclo[5.2.1.0^{2,6}]deca-3,8-diene-4,8-diylbis(carbonylimino)]bis(2-aminopropanoic acid) (**4b**)

Compound **4** (1.620 g, 2.93 mmol) was dissolved in 33 ml DMF and stirred under N_2 at 0 °C. A solution of N_α-Boc-L-2,3-diaminopropionic acid (Boc-Dap-OH) (1.438 g, 7.04 mmol) and $NaHCO_3$ (591 mg, 7.04 mmol) in 33 ml H_2O was added drop-wise via syringe. The reaction mixture was stirred under N_2 at 0 °C during 10 h. The solvent was removed and the residue dissolved in 300 ml H_2O and acidified with 1 N HCl to pH 3. The solution was extracted 3× with 300 ml CH_2Cl_2. After each extraction step the pH of the aqueous phase was re-adjusted to 2–3. The combined organic phases were dried over Na_2SO_4. The crude product was purified by flash chromatography as for **4a**. The product (1.020 g, 59% yield) was obtained as an off-white powder. For deprotection, 103 mg (0.17 mmol) was dissolved in 2 ml of a 1:1 (v/v) mixture of F_3CCOOH and CH_2Cl_2. After stirring for 4 h at r.t. under N_2, the solution was cooled to 0 °C and 2 ml of H_3CCN were added, followed by solvent removal in vacuo. This procedure was repeated twice. The dry residue was dissolved in 8 ml 1 N HCl and lyophilized, resulting in 100 mg (95% yield) **4b** as a slightly brown solid. Calcd. for M_r ($C_{18}H_{24}N_4O_6$) 392.41; ESI-MS(CH_3OH, neg. Mode): 391.1 $[M-H]^-$; ^{1}H NMR (500 MHz, D_2O): δ 6.84 (m, 1H, CH), 6.48 (m, 1H, CH), 4.28–4.16 (m, 2H, CH_2), 3.90–3.72 (m, 4H, CH_2), 3.68–3.59 (m, 1H, CH), 3.38–3.31 (m, 1H, CH), 3.29–3.22 (m, 1H, CH), 3.12–3.02 (m, 1H, CH), 2.58–2.47 (m, 1H, CH), 2.01–1.83 (m, 1H, CH), 1.73–1.66 (m, 1H, CH), 1.53–1.46 (m, 1H, CH).

4.5. Synthesis of [(CpCONHCH$_2$C$_6$H$_5$)Mn(CO)$_3$] (**5a**)

Cymantrene-carboxylic acid (248 mg, 1 mmol) was dissolved in 1 ml of dry DMF. Pyridine (81 μl, 0.853 mmol) and pentafluorophenyl-trifluoroacetate (PFT, 174 μl, 1.0 mmol) were added and the solution was stirred for 3 h under N_2 at r.t. The reaction mixture was diluted with 30 ml ethyl acetate and washed 3× with 30 ml of 0.1 M HCl and once with 30 ml of 5% $NaHCO_3$. The organic phase was dried over Na_2SO_4 the solvent removed under reduced pressure and the yellow solid **3** (350 mg, 85% yield) was used without further purification.

Activated cymantrene-carboxylic acid (**3**) (207 mg, 0.5 mmol) was dissolved in 8.5 ml of DMF and stirred under N_2 at 0 °C. A solution of benzyl amine (53 mg, 0.5 mmol) and $NaHCO_3$ (66 mg, 0.78 mmol) in 8.5 ml of H_2O was added via syringe. The reaction mixture was stirred under N_2 at 0 °C for 4 h. After solvent removal, the residue was dissolved in 50 ml of CH_2Cl_2 and washed with 50 ml of 1 mM HCl. A small amount of 0.1 M HCl was added during extraction to ensure a final pH of 3 in the aqueous phase which was then extracted 3× with 150 ml of CH_2Cl_2. The combined organic phases were dried over Na_2SO_4. The crude product was purified by flash chromatography, using a gradient from CH_2Cl_2 to CH_2Cl_2/MeOH 5:1. The product (396 mg, 65% yield) was obtained as a slightly yellow powder which could be recrystallised from CH_2Cl_2/hexane mixture. Calcd. for M_r ($C_{16}H_{12}MnNO_4$) 337.21; ESI-MS (CH_3OH, pos. Mode): 360.0 $[M+Na]^+$, ESI-MS (CH_3OH, neg. Mode): 336.0 $[M-H]^-$. Anal. Calc. for $C_{16}H_{12}MnNO_4$: C, 56.99; H, 3.59; N, 4.15. Found: C, 56.81; H, 3.63; N, 4.16%. ^1H NMR (500 MHz, $CDCl_3$): δ 7.25 (m, 5H, Ph), 5.91 (m, 1H, NH), 5.23 (m, 2H, Cp), 4.70 (m, 2H; Cp), 4.31 (m, 2H, CH_2); $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$): δ 223.90, 138.25 (1C, Ph), 129.34 (1C, Ph), 128.38 (1C, Ph), 128.26 (1C, Ph), 91.13 (1C, Cp), 85.12 (1C, Cp), 83.41 (1C, Cp), 44.36 (1C, CH_2); IR (KBr): 3300, 2028, 1927, 1638, 1560 cm^{-1}.

4.6. Synthesis of [(CpCOOC$_6$F$_5$)Re(CO)$_3$] (**3′**)

[(Cp-COOH)Re(CO)$_3$] (274 mg, 0.775 mmol) was dissolved in 1 ml of dry DMF. Pyridine (69 μl, 0.853 mmol) and PFT (155 μl, 0.900 mmol) were added and the solution was stirred for 3 h under N_2 at r.t. The reaction mixture was diluted with 75 ml ethyl acetate and washed three times with 75 ml of 0.1 M HCl and then with 75 ml of 5% $NaHCO_3$. The organic phase was dried with Na_2SO_4 and the solvent was removed *in vacuo* to yield 344 mg (81%) of **3′**.

^1H NMR (200 MHz; acetone-d^6): δ 6.55 (t, 2H, J = 2.4 Hz, Cp), 5.89 (t, 2H, J = 2.4 Hz, Cp); $^{19}F\{^1H\}$ NMR (acetone-d^6): δ −155.0, −159, 164.5.

4.7. Synthesis of [Re(CO)$_3$(CpCONHCH$_2$CH(NH$_3$)COOH)](CF$_3$COO) (5b)

Compound **3′** (711 mg, 1.30 mmol) was dissolved in 8.5 ml of DMF and stirred under N_2 at 0 °C. A solution of N_α-Boc-L-2,3-diaminopropionic acid (Boc-Dap-OH) (319 mg, 1.56 mmol) and $NaHCO_3$ (132 mg, 1.56 mmol) in 8.5 ml of water was added drop-wise via syringe. The reaction mixture was stirred under N_2 at 0 °C for 4 h, the solvent removed *in vacuo* and the residue dissolved in 150 ml of CH_2Cl_2 and washed with 150 ml of 1 mM HCl. A small amount of 0.1 M HCl was added during extraction to ensure a final pH of 3 in the aqueous phase which was extracted 3× with 150 ml of CH_2Cl_2. The combined organic phases were dried with Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography, using a gradient from CH_2Cl_2 to CH_2Cl_2/MeOH 5:1. The product (396 mg, 65% yield) was obtained as a slightly brownish powder which was used for the next step by dissolving 104 mg (0.175 mmol) in mixture of 1 ml CH_2Cl_2 and 1 ml TFA. The removal of Boc group reaction was complete after stirring at r.t. for 2 h under N_2 as indicated by HPLC. Twenty five milliliters of CH_3CN were then added, followed by solvent removal. This procedure was repeated four times to produce 436 mg (72%) of **5b** as a brownish solid. Calcd. for M_r ($C_{12}H_{11}N_2O_6Re$) 466.43; ESI-MS(CH_3OH, pos. Mode): 467.0 $[M+H]^+$. ^1H NMR (500 MHz, CD_3OD): δ 6.16 (m, 2H, Cp), 5.59 (t, 2H, J = 2.9 Hz, Cp), 3.75 (m, 1H, CH), 3.68 (m, 2H, CH_2); $^{13}C\{^1H\}$ NMR (125 MHz, CD_3OD): δ 194.0, 169.9 166.9, 94.5, 88.7, 41.0. IR (KBr) 3420, 2026, 1926, 1639 cm^{-1}.

5. Technetium complexes synthesis

5.1. Solid phase synthesis

NovaSyn® TG amino resin (400 mg, 0.116 mmol –NH_2 groups) was left to swell in DMF during 1 h and washed several times with DMF. The supernatant was removed. Thiele's acid (64 mg, 0.29 mmol) and *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) (220 mg, 0.58 mmol) were dissolved in 1 ml of DMF and added to the resin. *N*,*N*-diisopropylethylamine (200 μl, 1.16 mmol) was added, and the suspension was stirred very gently for 18 h under N_2 at r.t. The resin was filtered and washed 5× each with 5 ml of DMF, CH_2Cl_2, and finally 2-propanol (to de-swell the resin). The resin was dried *in vacuo* overnight and stored at 4 °C prior to use.

To check the completeness of the coupling a TNBS test was performed according to literature procedure [26]. No free –NH_2 groups were detected, thus confirming the complete derivatization of the resin by Thiele's acid. IR (KBr pellet) showed peaks at 1535 and 1712 cm^{-1} not present in the unmodified resin, which were attributed to the amide bond and the terminal carboxylic group of Thiele's acid, respectively.

5.2. Labelling method A (one-pot labeling)

A vial was charged with $[H_3BCOOH]$– (4 mg) and Na_2[tartrate]$2H_2O$ (7 mg), $Na_2B_4O_7 \cdot 10H_2O$ (7 mg) and the ligands **4a–c**. The vial was then sealed and flushed with N_2. The $[^{99m}TcO_4]^-$ elute (1 mL) was injected into the vial and the resulting mixture was heated at 95 °C for 30 min, after which time the reaction products were analyzed with HPLC coupled with gamma detector.

5.3. Method B: ^{99m}Tc-complexes in alkaline conditions

To a solution of *fac*-$[^{99m}Tc(H_2O)_3(CO)_3]^+$ (**1**) (900 μl) 100 μl of 10^{-2} or 10^{-3} M stock solution of the ligands **4a–c** in MeOH under N_2, were added. The vials was then brought to 95 °C, and the final solution analyzed by HPLC coupled with gamma detector after cooling to r.t.

5.4. Method C: ^{99m}Tc-complexes in buffered conditions

The pH of a solution containing **1** was adjusted (pH 7.4) by a solution of 1 N HCl (respectively 1 N NaOH) and phosphate buffer. Nine hundred microliters of **1** was then added to 100 μl of 10^{-2} or 10^{-3} M stock solution of ligands **4a–c** were mixed together and protected with N_2. The mixture was allowed to heat at 95 °C, and the labeling products were analyzed with HPLC coupled with gamma detector.

5.5. Solid phase labeling of Thiele's acid

Polymer **7** (4 mg) was previously swelled in water at 4 °C for 3 h and then the solid was filtrated off. The vial was charged with $Na[H_3BCO_2H]$ (4 mg), $Na_2B_4O_7 \cdot 10H_2O$ (7 mg) and Na_2[tartrate]$2H_2O$ (7 mg). The vial was then sealed and flushed with N_2. The $[^{99m}TcO_4]^-$ elute (1 mL) was injected into the vial and the resulting mixture was heated at 95 °C for 3 h, after which time the reaction products were analyzed with HPLC coupled with gamma detector.

5.6. X-ray crystallographic data collection and refinement of the structures of dimethyl ester of Thiele's acid and **5a**

Crystallographic data were collected at 183(2) K on an Oxford Diffraction Xcalibur system with a Ruby detector using Mo Kα radi-

ation (λ = 0.7107 Å) that was graphite-monochromated. Suitable crystals were covered with oil (Infineum V8512, formerly known as Paratone N), mounted on top of a glass fibre and immediately transferred to the diffractometer. The program suite CRYSALIS^{Pro} was used for data collection, semi-empirical absorption correction and data reduction [27]. Structures were solved with direct methods using SIR97 [28] and were refined by full-matrix least-squares methods on F^2 with SHELXL-97 [29]. The structures were checked for higher symmetry with help of the program PLATON [30].

Supplementary material

CCDC 704728 contains the supplementary crystallographic data for **5a**. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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