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Syntheses of High-Valent *fac*-$[^{99m}TcO_3]^+$ Complexes and [3+2] Cycloadditions with Alkenes in Water as a Direct Labelling Strategy

Henrik Braband, Yuji Tooyama, Thomas Fox, and Roger Alberto*[a]

Abstract: Reported herein is a new concept for the labelling of biomolecules with small $[^{99m}TcO_3]^+$ complexes through a [3+2] cycloaddition with alkenes for radiopharmaceutical applications. We developed convenient reactions for the synthesis of small, water stable *fac*-$[TcO_3(tacn\text{-}R)]^+$ complexes (^{99}Tc and ^{99m}Tc, tacn = 1,4,7-triazacyclononane, R = H, $-CH_2-C_6H_5$, $-CH_2-C_6H_4COOH$). With alkenes, these high valent $[^{99m}TcO_3]^+$ complexes undergo [3+2] cycloaddition with formation of the corresponding Tc^V–glycolato complexes. The $^{99m}Tc^V$ and $^{99m}Tc^{VII}$ complexes are stable at 37 °C in water and in the presence of serum proteins. Therefore, new opportunities in technetium chemistry are enabled with a high potential for medicinal and biological applications. In contrast to classical labelling, the presented strategy is ligand and not metal-centred.

Keywords: alkenes · cycloaddition · labelling · medicinal chemistry · radiopharmacy · technetium

Introduction

Molecular imaging has emerged as a novel and powerful strategy for the visualisation of biological events on a molecular level.[1] Molecular imaging relies on bioactive compounds that are labelled with, for example, fluorescing agents or radionuclides.[2] For radioimaging purposes, the positron emitters ^{18}F and ^{11}C came into focus, in particular since they can be introduced in target molecules without imposing substantial structural and electronic changes. Due to its availability and physical properties, ^{99m}Tc is still the favoured radionuclide, but its site specific and stable conjugation to biomolecules is at least challenging. For labelling receptor ligands, such as peptides, oligonucleotides or small molecules, the bifunctional chelator concept (BFC) is usually applied. A chelator (1st function) is covalently bound to a functional group on the targeting molecule.[3] In the general concept, the chelator (2nd function) coordinates then to a ^{99m}Tc precursor by ligand substitution, a step that governs labelling rates and yields. Typical precursors are complexes with the $\{Tc{=}O\}^{3+}$ core stabilised by glucoheptonate and $[^{99m}Tc(OH_2)_3(CO)_3]^+$.[4,5] Some of these ^{99m}Tc complexes tend to be cleaved from the vector by reoxidation to $[^{99m}TcO_4]^-$. This is not desired since, reminiscent to the behaviour of iodide, $[^{99m}TcO_4]^-$ accumulates in the thyroid.

A small complex is a prerequisite for not affecting the biological activity of the bioconjugate. In comparison to the *fac*-$\{^{99m}Tc(CO)_3\}^+$ core, complexes with the isolobal $\{^{99m}TcO_3\}^+$ core are substantially smaller. Since technetium is in its highest oxidation state +VII, these complexes are evidently not oxidation (though still hydrolysis) sensitive anymore. Complexes of Tc^{VII} are rare and a synthesis from aqueous solution is unknown. Besides the binary oxide $[^{99}Tc_2O_7]$, Davison et al. reported $[^{99}TcO_3(HB(pz)_3]$ (pz = pyrazolyl) from nitric acid oxidation of $[^{99}Tc(HB(pz)_3(CO)_3]$ or from $[HTcO_4]$.[6] The purely organic synthesis of $[^{99}TcO_3\{(CH_3)_3SnO\}]$ and its silyl analogue is known.[7] Due to the readily available $[Re_2O_7]$, chemistry with the $\{ReO_3\}^+$ core is well explored, though again not by syntheses from water.[8–10] Rhenium(VII) complexes exert a substantially reduced (redox) activity and are therefore more stable. Recently, Abram et al. reported the triazacyclononane (tacn) complex $[^{99}TcO_3(tacn)]^+$ and we reported complexes of tris-(1-pyrazolyl)methane derivatives with the *fac*-$[^{99}TcO_3]^+$ core.[11,12] To explore the potential of such small, high-valent, ^{99m}Tc complexes for labelling, stability in and synthesis from $[^{99m}TcO_4]^-$ in water is crucial but difficult to achieve, since $[^{99m}TcO_4]^-$ is thermodynamically and kinetically very stable in an aqueous environment.

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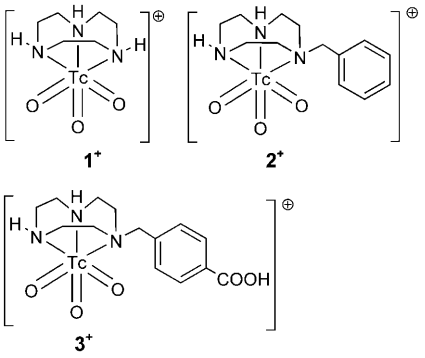
We report here about the syntheses of complexes $[^{99m}TcO_3(N^3\text{-R})]^+$ (N^3-R = 1,4,7-triazacyclononane and derivatives) directly from $[^{99m}TcO_4]^-$. These complexes are stable at 37 °C in water and in the presence of serum proteins. They represent a new kind of ^{99m}Tc complex for labelling purposes, produced from an unusual coupling reaction. When $[^{99m}TcO_3(N^3\text{-R})]^+$ is treated with alkenes in water, a [3+2] cycloaddition to the corresponding $^{99m}Tc^V$–glycolato complexes $[^{99m}TcO(glyc\text{-}R')(N^3\text{-R})]^+$ occurs at reasonable rates. We call this "clack chemistry" due to some similarities with the very well established "click chemistry".[13] "Clack chemistry" represents a novel way of labelling that does not take place at the metal centre as commonly encountered (BFC approach), but on the reactive oxo ligands activated by the metal centre. The Tc^{VII} compounds **1**–**3** discussed are shown here.



Results and Discussion

High-valent technetium complexes are only useful for biological or medicinal application if they are stable under physiological conditions. Hence, a first step was the preparation of model complexes and the assessment of their behaviour in water. The reaction of 1,4,7-triazacyclononane (tacn) and derivatives (tacn-R) with $[TcOCl_4]^-$ in the presence of glycol gave the Tc^V complexes $[^{99}TcO(glyc)(tacn\text{-R})]^+$. Oxidation with hypochlorite $[OCl]^-$ under acidic conditions in water (Cl_2) produced the corresponding complex $[TcO_3(tacn\text{-R})]^+$ (**1**$^+$; R = H) in 97% yield at room temperature. The high yield demonstrated the stability of **1**$^+$ under acidic conditions. No hydrolysis back to $[^{99}TcO_4]^-$ and free ligand was observed even after days. Accordingly, the complexes $[TcO_3(tacn\text{-bz})]^+$ (**2**$^+$) and $[TcO_3(tacn\text{-bz-COOH})]^+$ (**3**$^+$) (bz = benzyl) could be synthesised in 95 and 99% yield, respectively. The oxidation of the $^{99}Tc^V$ compounds with hypochlorite (or Cl_2) in acidic medium seemed to be a general and mild route to complexes with the *fac*-$[^{99}TcO_3]^+$ core and tripodal ligands. Earlier methods in, for example, concentrated nitric acid were difficult to control and gave, if ever, very variable yields. The general synthetic approach is given in Scheme 1.

The structures of **2**-Cl (Supporting Information) and **3**-Cl could be elucidated by single-crystal analyses and a presentation of **3**$^+$ is given in Figure 1.[14] All complexes with the *fac*-$[^{99}TcO_3]^+$ core structurally characterised so far displayed O-Tc-O angles close to 109°, mirroring the spatial demand of the terminal oxo ligands. The complexes **1**$^+$–**3**$^+$ are stable in water over a pH range from 1–10 at 37 °C for at least 24 h, without any significant decomposition. Water stability implied that the corresponding ^{99m}Tc complexes could potentially be useful for biomedical applications.

Scheme 1. Synthetic approach to tacn complexes with the *fac*-$[^{99}TcO_3]^+$ core.

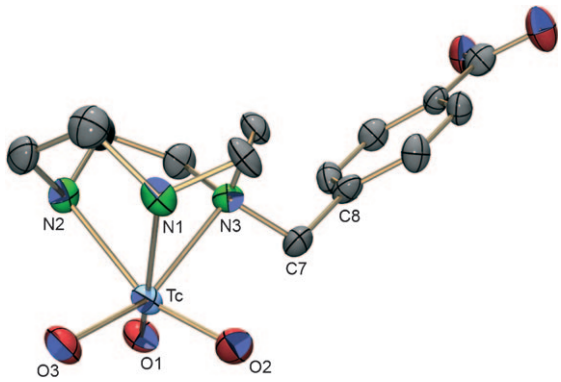


Figure 1. ORTEP representation[15] of the $[TcO_3(tacn\text{-bz-COOH})]^+$ (**3**$^+$) ion. Thermal ellipsoids represent 50% probability. Hydrogen atoms are omitted for clarity. Selected bond lengths [Å] and angles [°]: Tc–O1 1.708(4), Tc–O2 1.722(4), Tc–O3 1.725(5), Tc–N1 2.234(5), Tc–N2 2.216(5), Tc–N3 2.286(5), O1-Tc-O2 106.7(2), O1-Tc-O3 106.9(2), O2-Tc-O3 105.6(2), N1-Tc-N2 74.7(2), N1-Tc-N3 75.6(2), N2-Tc-N3 75.9(2).

The ^{99}Tc NMR signals displayed chemical shifts as expected for $^{99}Tc^{VII}$ complexes.[16] We found $\delta = 375$ ppm for **2**$^+$ and **3**$^+$ (relative to $[^{99}TcO_4]^-$ at 0 ppm). Due to quadrupole line broadening ($I = 9/2$) and the low symmetry of the complexes, the half line widths $\Delta\nu_{1/2}$ of **2**$^+$ (1800 Hz) and **3**$^+$ (4800 Hz) were relatively broad, but the signals still detectable due to the high receptivity of ^{99}Tc in NMR spectroscopy. The ^{17}O signals of **1**$^+$ appeared at $\delta = 958$ ppm in D_2O (Supporting Information). The retention times on reversed phase (RP) HPLC are critical, since they are related to the hydrophilicity and, to a limited extent, to the size of the complexes. Complex **1**$^+$ eluted with the front of any mobile phase on an RP-18 column. Differentiation of **1**$^+$ from $[^{99}TcO_4]^-$ was only possible by delaying the latter (see Figure 2 and Supporting Information). Hence, complex **1**$^+$ is highly hydrophilic in spite of the organic ligand. On the same gradient, $[^{99}Tc(CO)_3(tacn)]^+$ eluted much later at 10.5 min underlining the differences between the $^{99}Tc^{VII}$ complex **1**$^+$ and the principally similar $^{99}Tc^{I}$ complex. This

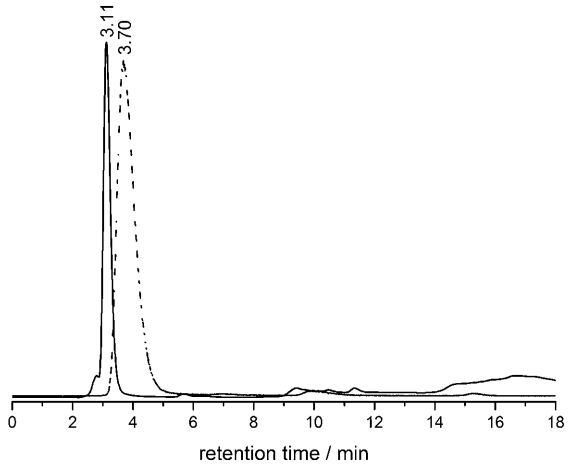
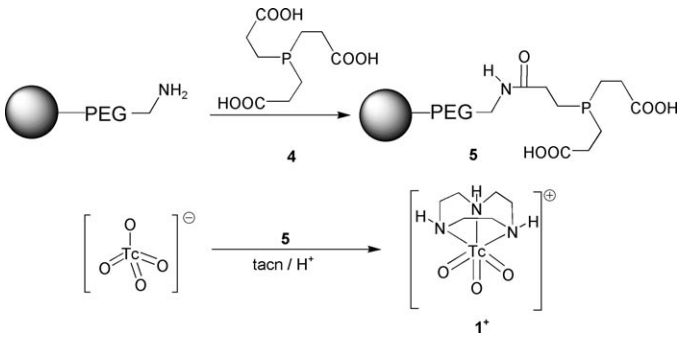


Figure 2. HPLC traces of a co-injection of $[^{99m}TcO_3(tacn)]^+$ and $[^{99}TcO_3(tacn)]^+$: $[^{99}TcO_3(tacn)]^+$ with UV detection (straight) and $[^{99m}TcO_3(tacn)]^+$ with γ detection (dashed). Retention time of $[TcO_4]^- = 6.8$ min. Due to the separation of the two detectors, the γ signal is delayed by 0.6 min relative to the UV signal.



Scheme 2. Synthesis of phosphine-derivatised solid phase support and aqueous heterogeneous synthesis of complexes $\mathbf{1}^+$ (^{99m}Tc).

also demonstrated the distinct ability of the terminal oxo ligands to interact with H_2O by forming H^+ bridges. The retention times of $\mathbf{2}^+$ and $\mathbf{3}^+$ were higher, 15.6 and 13.7 min, respectively, but still much shorter than the corresponding $[^{99}Tc(CO)_3(tacn\text{-}R)]^+$ complexes (20.6 and 19.5 min, respectively). The relatively high hydrophilicity of $\mathbf{1}^+$–$\mathbf{3}^+$ is essential, since correspondingly labelled biomolecules will clear more rapidly from the blood than lipophilic analogues.

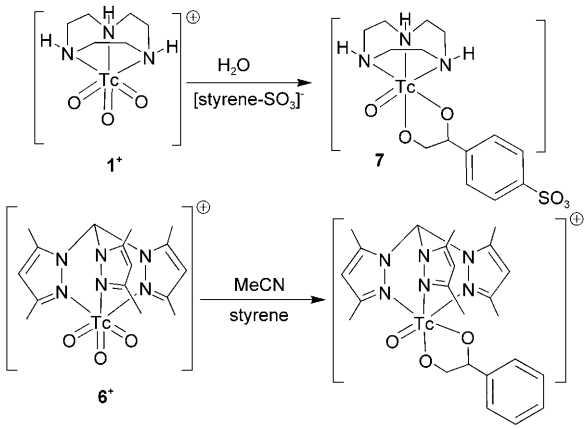
Due to the presence of strong and toxic oxidants such as $[OCl]^-$, the synthetic conditions for ^{99}Tc complexes $\mathbf{1}^+$–$\mathbf{3}^+$ were evidently not transferable to the routine preparation of the corresponding ^{99m}Tc compounds. Applying the synthetic concept for ^{99}Tc to the preparation of the corresponding complexes $\mathbf{1}^+$–$\mathbf{3}^+$ with ^{99m}Tc, the N^3-ligand could be coordinated through a sequential reduction–oxidation (O_2) reaction starting from $[^{99m}TcO_4]^-$. Accordingly, a homogenous reaction of $[^{99m}TcO_4]^-$ with hypophosphoric acid H_3PO_2 in the presence of tacn at pH 1 and 95 °C produced complex $\mathbf{1}^+$ (^{99m}Tc) in 64 % yield, without any detectable intermediates. We propose that H_3PO_2 reduced $[^{99m}TcO_4]^-$ to a lower oxidation state, tacn coordinated and residual O_2 in the solution then reoxidised the intermediate back to the product $[^{99m}TcO_3(tacn)]^+$. This first synthesis was the proof of principle that $^{99m}Tc^{VII}$ complexes with the $[^{99m}TcO_3]^+$ core were accessible directly from an aqueous solution. Since H_3PO_2 was not ideal for routine synthesis either, we used a PPh_3-conjugated polystyrene resin. Alternatively, the phosphine 3,3′,3″-phosphinetripropanoic acid **4** was conjugated to a solid phase support by an amide bond (Scheme 2). Resins have the advantage that they can simply be filtered after a heterogeneous reaction which allows to remove excess reducing agent.

In the presence of these polymers and tacn as ligand, complex $\mathbf{1}^+$ formed in up to 80 % yield after 1 h at 95 °C as the only product from $[^{99m}TcO_4]^-$. Residual $[^{99m}TcO_4]^-$ remained stably bound to the polymer. Since $\mathbf{1}^+$ did not react under these conditions with the polymer or the polymer bound phosphines, it is likely that $[^{99m}TcO_4]^-$ reacted directly with the organic polymeric material, thereby being irreversibly trapped. This reduced the overall yield, but gave complexes $\mathbf{1}^+$ and $\mathbf{2}^+$ in >95 % radiochemical purity. Reaction with tacn-bz-COOH gave two products, one being the desired complex $\mathbf{3}^+$, the other one complex $\mathbf{1}^+$. We concluded that the N3–C7 bond (Figure 1) was polarised due to the strong Lewis acidity of the Tc^{VII} centre and cleaved probably by means of a nucleophilic attack of $[OH]^-$ to the α-carbon C7. Earlier, we found an analogous behaviour with $[^{99m}Tc(CO)_3]^+$ complexes.[17] To assess the authenticity of ^{99m}Tc complexes $\mathbf{1}^+$, $\mathbf{2}^+$ and $\mathbf{3}^+$, HPLC traces were compared to the corresponding ^{99}Tc complexes. For compound $\mathbf{1}^+$, an example is shown in Figure 2. The reaction conditions still need optimisation, but we want to emphasise that they represent the first syntheses in water to give defined complexes with the *fac*-$[^{99m}TcO_3]^+$ core.

Despite the inherent oxidative properties of Tc^{VII} complexes, we found $\mathbf{1}^+$–$\mathbf{3}^+$ (^{99m}Tc) to be stable in water and in the presence of serum proteins at 37 °C, probably rather for kinetic than for thermodynamic reasons. These basic complex structures $[^{99m}TcO_3(tacn\text{-}R)]^+$ could now be applied for the labelling of biomolecules according to the BFC approach. As exemplified by tacn-bz-COOH, the basic tacn ligand is derivatised with a spacer and a functionality (carboxylate group) for conjugation to the biomolecule. Applying the heterogeneous reaction conditions outlined previously will yield a biomolecule labelled with the $[^{99m}TcO_3]^+$ core.

It has been reported that $[^{99}Tc^{VII}O_3Cl(bpy)]$ underwent a metal-mediated vicinal *cis*-dihydroxylation reaction (alkene–glycol interconversion) by [3+2] cycloaddition with alkenes to form the water-unstable Tc^V complex $[^{99}TcOCl(OCH_2CH_2O)(bpy)]$ (**5**).[18] Such a reaction of $\mathbf{1}^+$–$\mathbf{3}^+$ with alkenes is intriguing, since a novel pathway for direct labelling through metal-mediated ligand activation would emerge. We therefore investigated complexes $\mathbf{1}^+$–$\mathbf{3}^+$ together with the pyrazolyl-based complex $[^{99}TcO_3(tpzm^*)]^+$ ($\mathbf{6}^+$)[12] (tpzm* = tris-dimethylpyrazolylmethane) for reactivity towards alkenes such as styrene, potassium 4-styrene-sulfonate (styrene-SO_3^-), allyl alcohol, ethyl vinyl ether, acrylic acid and 2-methyl-3-buten-2-ol in water and acetonitrile. Upon addi-

tion of alkenes to either $[^{99}TcO_3(N^3)]^+$ complex, a colour change from yellow to blue occurred, revealing a rapid [3+2] cycloaddition reaction under quantitative formation of the corresponding $^{99}Tc^{V}$ complexes, for example, $[TcO(O_2styrene\text{-}SO_3)(tacn)]$ (**7**) (Scheme 3). The structure of **7** could be confirmed by X-ray analysis (Figure 3).[14]



Scheme 3. [3+2] cycloaddition of *fac*-$[^{99}TcO_3]^+$-based Tc^{VII} complexes with alkenes.

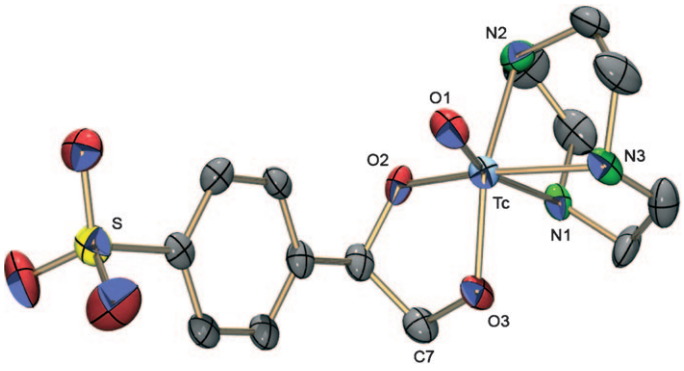


Figure 3. ORTEP representation[15] of $[TcO(O_2styrSO_3)(tacn)]$ (**7**). Thermal ellipsoids represent 50% probability. Hydrogen atoms are omitted for clarity. Selected bond lengths [Å] and angles [°]: Tc–O1 1.658(3), Tc–O2 1.933(3), Tc–O3 1.938(3), Tc–N1 2.274(3), Tc–N2 2.166(3), Tc–N3 2.140(3), O1-Tc-O2 107.8(2), O1-Tc-O3 110.4(2), O2-Tc-O3 82.6(2), N1-Tc-N2 74.8(2), N1-Tc-N3 75.2(2), N2-Tc-N3 80.4(2).

The *cis*-dihydroxylation of alkenes with OsO_4 or $[MnO_4]^-$ have been known for a long time and were originally proposed to occur through a [2+2][19] or [3+2] cycloaddition as the rate-limiting step.[20,21] Since Tc^{VII} is coordinatively saturated, we expected a concerted attack of the alkenes directly to two oxo ligands. Kinetic studies under pseudo-first-order conditions revealed a significant rate dependence of the nature of the alkene, but only a weak one of the solvent (see the Supporting Information). The second-order rate constants at room temperature in water for $\mathbf{1}^+$ gave 0.107 ± 0.001 for styrene-SO_3^-, 4.4 ± 0.1 for allyl alcohol, 22.6 ± 0.5 for acrylic acid and $46.0\pm2.7\,M^{-1}s^{-1}$ for 2-methyl-3-buten-2-ol. Complexes $\mathbf{2}^+$ and $\mathbf{3}^+$ reacted faster with styrene-SO_3^- than $\mathbf{1}^+$ and the corresponding rate constants were 0.42 ± 0.01 and $0.26\pm0.01\,M^{-1}s^{-1}$, respectively. Complex $\mathbf{6}^+$ finally reacted more rapidly with styrene-SO_3^- than any of $\mathbf{1}^+$–$\mathbf{3}^+$ and its rate with styrene in acetonitrile was found to be $3.4\pm0.1\,M^{-1}s^{-1}$. The pseudo-first-order rate constants k_{obs} depended linearly on alkene concentration over a broad range and we could not achieve rate saturation. Activation data as obtained from an Eyring plot gave $\Delta H^{\neq}=44\,kJ\,mol^{-1}$ and $\Delta S^{\neq}=-114$ e.u. for the reaction of $\mathbf{1}^+$ with styrene-SO_3^- (Supporting Inforamtion). The strongly negative activation enthalpy is in support of an associatively driven mechanism.

These kinetic data enabled the prediction of labelling rates on the ^{99m}Tc level. Accurate kinetic analyses with ^{99m}Tc is difficult (no spectroscopic methods possible); however, we compared the reaction rates of $\mathbf{1}^+$ for ^{99}Tc and ^{99m}Tc by HPLC. Although an uncommon method, comparable reaction rates would further support the identity of the complexes $\mathbf{1}^+$–$\mathbf{3}^+$ with ^{99}Tc and ^{99m}Tc on the no carrier added level. Under pseudo-first-order conditions, the reaction of for example, $\mathbf{1}^+$ with styrene-SO_3^- in water and at room temperature occurred with $t_{1/2}=22$ min for ^{99}Tc (spectroscopy) and 43 min for ^{99m}Tc (chromatography, see Figure 4). Thus, the rate constants were comparable and supported the chromatographically established identities of the ^{99}Tc and ^{99m}Tc products.

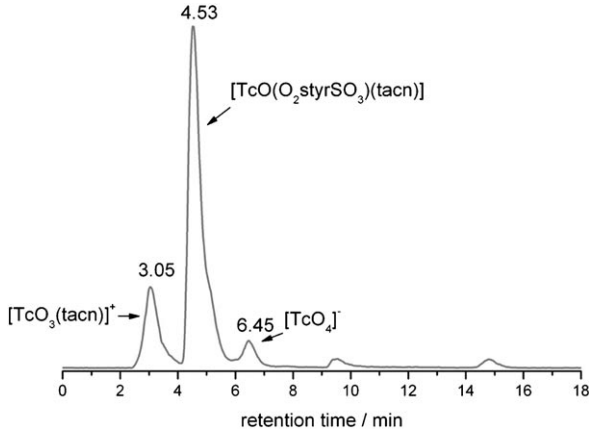


Figure 4. HPLC (γ-trace) chromatogram during the reaction between styrene-SO_3^- and $[^{99m}TcO_3(tacn)]^+$.

A prerequisite for applying ^{99m}Tc complexes in a biological environment is their stability in the presence of serum and serum proteins. It should be emphasised at this point that the Tc^{VII} complexes $\mathbf{1}^+$– $\mathbf{3}^+$ as well as the resulting Tc^{V} complexes formed after reaction with alkenes were stable in the presence of large concentrations of serum proteins or even serum itself.

The [3+2] cycloaddition of alkenes with $^{99m}Tc^{VII}$ complexes represents a novel way of labelling. The labelling procedures presented here are ligand and not metal-centred as in the classical BFC approach. Considering the ease (no protecting groups required) with which alkenes can be conjugated to biomolecules, ligand-centred reactivity paves the

way for smooth and well-defined labelling of biomolecules. The rates of labelling and the physicochemical properties of the radiolabelled bioconjugates can be tuned by selecting an appropriate alkene.

Conclusions

Complexes containing the *fac*-$[^{99}TcO_3]^+$ core stabilised by the triazacyclononane ligand reveal new opportunities in ^{99}Tc chemistry. For their ^{99m}Tc analogues, we have developed a simple and efficient syntheses to yield small and water stable *fac*-$[^{99m}TcO_3(tacn\text{-}R)]^+$ complexes in good yield. These precursor complexes react by a [3+2] cycloaddition with alkenes to give glycolato complexes. If the alkenes are conjugated to biomolecules, a new labelling strategy with high potential for radiopharmaceutical applications is enabled. In addition, biomolecules can also be directly labelled by the classical BFC approach. We want to emphasise that the [3+2] cycloaddition with alkenes is of general interest for catalytic *cis*-dihydroxylation of alkenes, since reductive cycloaddition and oxidative cleavage are reversible reactions.

Experimental Section

General procedure for the synthesis of the $^{99}Tc^{V}$ starting complexes: The synthetic method for the Tc^{V} complexes was adapted from a published method.[11] $[NBu_4][TcOCl_4]$ (100 mg, 0.2 mmol) was dissolved in THF (4 mL) and ethylene glycol (25 mg, 0.4 mmol) was added. After the slow addition of Et_3N (0.5 mL) the colourless precipitate was filtered and tacn·3 HCl, tacn-bz or tacn-bz-COOH·3 HBr (0.2 mmol) was added to the purple solution. The mixture was refluxed for 2 h. A light blue solid precipitated ([TcO(tacn-bz-COO)(glyc)], purple) during this time. The powder was collected by filtration and washed with THF (a few mL). In the case of $[TcO(tacn)(glyc)]^+$ and $[TcO(tacn\text{-}bz)(glyc)]^+$ the solid was suspended in a saturated KBr solution (4 mL) and stirred for 10 min. The blue solid was collected by filtration, and recrystallisation from water yields deep blue prisms. For [TcO(tacn-bz-COO)(glyc)], recrystallisation of the purple powder from MeOH yielded blue purple mirco crystals.

[^{99}TcO(tacn)(glyc)]Br: Yield 53 mg (70 %); other data for [^{99}TcO(tacn)-(glyc)]Br can be found in reference [11].

[^{99}TcO(tacn-bz)(glyc)]Br: Yield: 47 mg (50 %), IR (KBr): $\tilde{\nu}$ = 3434 (m), 3103 (m), 3040 (s), 2907(m), 2857 (m), 2840 (m), 2039 (w), 1921 (w), 1496 (w), 1450 (m), 1118 (w), 1102 (w), 1053 (m), 1014 (s), 970 (w), 949 (vs), 937 (s), 900 (s), 867 (w), 844 (w), 808 (w), 763(w), 732 (w), 708 (m), 654 (m), 648 (m), 619 (s), 573 (w), 537 (m), 475 (w), 447 cm^{-1} (w); ^{1}H NMR (500 MHz, D_2O): δ = 7.40 (m, 5 H; arom), 5.31 (m, 1 H; glycol), 5.01 (m, 1 H; glycol), 4.61 (d, J = 13.5 Hz, 1 H; CH_2 benzyl), 4.48 (m, 1 H; glycol), 4.39 (d, J = 13.5 Hz, 1 H; CH_2 benzyl), 4.07 (m, 2 H; CH_2 tacn), 3.47 (m, 2 H; CH_2 tacn), 3.04 (m, 1 H; CH_2 tacn), 2.87 (m, 2 H; CH_2 tacn), 2.76 (m, 1 H; CH_2 tacn), 2.52 (m, 2 H; CH_2 tacn), 2.27 (m, 1 H; CH_2 tacn), 2.23 ppm (m, 1 H; CH_2 tacn); ^{13}C NMR (500 MHz, D_2O): δ = 134.05 (C arom), 130.95 (C arom), 130.24 (C arom), 84.76 (CH_2 glycol), 84.29 (CH_2 glycol), 70.95 (CH_2 benzyl), 63.10 (CH_2 tacn), 55.95 (CH_2 tacn), 55.00 (CH_2 tacn), 53.57 (CH_2 tacn), 46.67 (CH_2 tacn), 45.46 ppm (CH_2 tacn); Tc analysis: calcd: 20.92 %; found: 20.25 %.

[^{99}TcO(tacn-bz-COO)(glyc)]: Yield: 39 mg (98 %); IR (KBr): $\tilde{\nu}$ = 3394 (br), 2969 (s), 2938 (s), 2733 (m), 2676 (s), 2490 (m), 1594 (m), 1549 (m), 1474 (m) 1432 (m), 1397 (s), 1381 (m), 1362 (m), 1281 (w), 1261 (w), 1170 (m), 1116 (w), 1102 (w), 1079 (w), 1054 (m), 1035 (s), 1012 (m), 941 (s), 898 (m), 867 (w), 847 (w), 802 (w), 777 (w), 651 (m), 614 (m), 537 (m), 464 (w), 453 cm^{-1} (w); ^{1}H NMR (500 MHz, D_2O): δ = 7.77 (d, J = 8 Hz, 2 H; arom), 7.45 (d, J = 8 Hz, 2 H; arom), 5.44 (m, 1 H; glycol), 5.04 (m, 1 H; glycol), 5.03 (m, 1 H; glycol), 4.61 (d, J = 13.5 Hz, 1 H; CH_2 benzyl), 4.49 (m, 1 H; glycol), 4.22 (d, J = 13.5 Hz, 1 H; CH_2 benzyl), 4.08 (m, 2 H; CH_2 tacn), 3.47 (m, 2 H; CH_2 tacn), 3.05 (m, 2 H; CH_2 tacn), 2.86 (m, 1 H; CH_2 tacn), 2.75 (m, 1 H; CH_2 tacn), 2.53 (m, 2 H; CH_2 tacn), 2.28 (m, 1 H; CH_2 tacn), 2.21 ppm (m, 1 H; CH_2 tacn); ^{13}C NMR (500 MHz, D_2O): δ = 175.48 (C carboxyl), 142.94 (C arom), 136.49 (C arom), 133.92 (2 C arom), 130.73 (2 C arom), 84.98 (C glycol), 84.50 (C glycol), 70.47 (C benzyl), 63.24 (C tacn), 56.00 (C tacn), 55.10 (C tacn) 51.95 (C tacn), 46.79 (C tacn), 45.24 ppm (C tacn); Tc analysis: calcd: 19.10 %; found: 17.16 %

General procedure for the synthesis of the $[^{99}TcO_3]^+$ complexes: A $[TcO(N^3\text{-}R)(glyc)]^{+/0}$ complex (0.05 mmol) was dissolved in water (3 mL) and a freshly prepared acidic (HCl, pH 1) solution of NaOCl (14 %; 0.1 mL) was added. Slow evaporation (approx. 3 d) of the solvent gave the corresponding complexes **1** and **2** (yellow crystals) and **3** (green crystals).

[^{99}TcO$_3$(tacn)]Cl (1-Cl): Yield: 15 mg (97 %). Other data for **1** can be found in reference [11].

[^{99}TcO$_3$(tacn-bz)]Cl (2-Cl): Yield: 19 mg (95 %); IR (KBr): $\tilde{\nu}$ = 3419 (m), 3000 (s), 2846 (m), 2784 (m), 1634 (w), 1496 (w), 1453 (m), 1116 (w), 1076 (m), 1065 (m), 1038 (w), 1017 (m), 984 (m), 962 (m), 898 (vs), 867 (m), 848 (m), 817 (m), 767 (m), 731 (m), 709 (m), 667 (w), 647 (m), 615 (w), 573 (w), 525 (w), 489 (w), 475 (w), 451 (w), 433 cm^{-1} (w); ^{1}H NMR (500 MHz, D_2O): δ = 7.54 (m, 2 H; arom), 7.48 (m, 3 H; arom), 4.65 (s, 2 H; CH_2 benzyl), 3.48 (m, 2 H; CH_2 tacn), 3.35 (m, 4 H; CH_2 tacn), 3.15 (m, 2 H; CH_2 tacn), 3.08 (m, 2 H; CH_2 tacn), 2.95 ppm (m, 2 H; CH_2 tacn); ^{13}C NMR (500 MHz, D_2O): δ = 133.58 (2 C arom), 133.57 (C arom), 130.88 (C arom), 130.27 (2 C arom), 66.16 (CH_2 benzyl), 54.60 (2 CH_2 tacn), 49.49 (2 CH_2 tacn), 48.68 ppm (2 CH_2 tacn); ^{99}Tc NMR (D_2O): δ = 375.74 ppm (line width 1800 Hz); Tc analysis: calcd: 24.64 %; found: 22.20 %.

[^{99}TcO$_3$(tacn-bz-COOH)]Cl (3-Cl): Yield: 22 mg (99 %); IR (KBr): $\tilde{\nu}$ = 3566 (m), 3390 (m), 3333 (m), 3282 (m), 3018 (s), 2890 (m), 2845 (m), 1720 (s), 1611 (m), 1452 (m), 1419 (w), 1256 (s), 1183 (m), 1110 (m), 1068 (m), 1019 (m), 958 (m), 897 (vs), 873 (s), 846 (m), 811 (w), 785 (w), 765 (m), 733 (w), 704 (w), 556 cm^{-1}(w); ^{1}H NMR (500 MHz, D_2O): δ = 8.03 (d, J = 8 Hz, 2 H; arom), 7.64 (d, J = 8 Hz, 2 H; arom), 4.70 (s, 2 H; CH_2 benzyl), 3.45 (m, 2 H; CH_2 tacn), 3.34 (m, 4 H; CH_2 tacn), 3.10 (m, 2 H; CH_2 tacn), 3.06 (m, 2 H; CH_2 tacn), 2.97 ppm (m, 2 H; CH_2 tacn); ^{13}C NMR (500 MHz, D_2O): δ = 172.50 (C carboxyl), 138.67 (C arom), 133.89 (C arom), 132.93 (C arom), 131.15 (C arom), 65.50 (CH_2 benzyl), 54.62 (CH_2 tacn), 49.44 (CH_2 tacn), 48.63 ppm (CH_2 tacn); ^{99}Tc NMR (D_2O): δ = 375.20 ppm (line width 4800 Hz); Tc analysis: calcd: 22.21 %; found: 19.58 %.

[^{99}TcO(O$_2$styrSO$_3$)(tacn)] (7): Complex **1** (31 mg, 0.1 mmol) was dissolved in approximately water (4 mL) and Na[4-styrene-SO_3] (21 mg, 0.1 mmol) was added. The yellow colour of the reaction mixture changed quickly to deep blue. The solution was stirred over night at room temperature. Slow evaporation of the solvent gave blue prisms of compound **7**. Yield: 31 mg (60 %); IR (KBr): $\tilde{\nu}$ = 3221 (s), 3127 (s), 2930 (m), 2874 (m), 1635 (m), 1485 (w), 1453 (m), 1382 (w), 1306 (w), 1189 (vs), 1122 (s), 1032 (s), 1004 (s), 956 (s), 913 (m), 835 (w), 798 (m), 769 (w), 712 (m), 674 (m), 610 (s), 567 (m), 546 (w), 523 cm^{-1} (w); ^{1}H NMR (500 MHz, D_2O): δ = 7.65 (d, J = 8 Hz, 2 H; arom), 7.33 (d, J = 8 Hz, 2 H; arom), 6.25 (dd, J = 5 Hz, 1 H; glycol), 5.56 (dd, J = 5 Hz, 1 H; glycol), 4.43 (dd, J = 5 Hz, 1 H; glycol), 3.80 (m, 2 H; tacn), 3.55 (m, 2 H; tacn), 3.08 (m, 1 H; tacn), 2.97 (m, 3 H; tacn), 2.50 (m, 2 H; tacn), 2.33 ppm (m, 2 H; tacn); ^{13}C NMR (500 MHz, D_2O): δ = 145.89 (C arom), 143.52 (C arom), 128.91 (C arom), 127.27 (C arom), 93.42 (C glycol), 88.63 (C glycol), 56.66 (C tacn), 55.16 (C tacn), 50.50 (C tacn), 50.39 (C tacn), 46.53 (C tacn), 46.07 ppm (C tacn); Tc analysis: calcd: 19.28 %; found: 19.81 %.

^{99m}Tc Experiments—[^{99m}TcO$_3$(tacn)]Cl (1-Cl): Polymer (10 mg) [NovaSyn® amino resin (90 μm), coupled to tris(2-carboxy-ethyl)phosphine hydrochloride (≈0.3 mmol g^{-1}resin) by standard SPPS technique] was transferred with 1,4,7-triazacyclononane trihydrochloride (23.5 mg,

10^{-4} mol) to a vial, tightly closed and flushed with N_2 for 10 min. $[^{99m}TcO_4]^-$ (1 mL) elute was added and the reaction mixture heated for 1 h at 95 °C. The reaction solution was cooled, the resin filtered and the solution neutralised by the addition of NaOH (0.1 M). Radiochemical purity was measured by HPLC (SI). Yields: 60–80 %, radiochemical purity > 93 %.

$[^{99m}TcO(O_2styrSO_3)(tacn)]$ (7): Compound **1** (^{99m}Tc) was prepared by the heterogeneous method described above. Sodium [4-styrene-sulfonate] (3.2 mg, 15.5 μmol) was added to the filtered and neutralised solution (0.8 mL). The reaction mixture was heated to 95 °C for 1.5 h. Yield: 78 %.

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[14] The crystal data and experimental details for the structural refinement of **2**-Cl, **3**-Cl, **7** and [TcO(glyc)(tacn-bz)]Br are provieded in the Supporting Information. CCDC 689761 (**2**), 689762 (**3**), 689763 (**7**) and 689760 ([TcO(glyc)(tacn-bz)]Br) contain the supplementary crystallographic data for this paper. 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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