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Combining Bifunctional Chelator with (3 + 2)-Cycloaddition Approaches: Synthesis of Dual-Function Technetium Complexes

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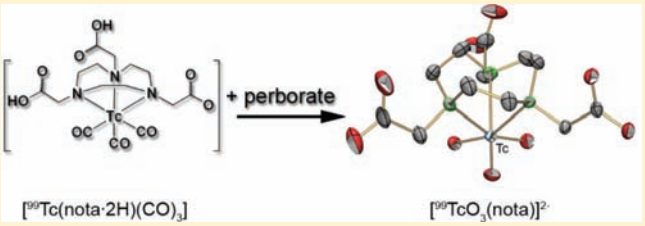
Supporting Information

ABSTRACT: A new concept for the synthesis of dual-functionalized technetium (Tc) compounds is presented, on the basis of the reactivity of *fac*-$\{Tc^{VII}O_3\}^+$ complexes. The concept combines the "classical" bifunctional chelator (BFC) approach with the new ligand centered labeling strategy of *fac*-$\{TcO_3\}^+$ complexes with alkenes ((3 + 2)-cycloaddition approach). To evidence this concept, *fac*-$\{^{99}TcO_3\}^+$ model complexes containing functionalized 1,4,7-triazacyclononane (tacn) derivatives *N*-benzyl-2-(1,4,7-triazonan-1-yl)acetamide (tacn-ba) and 2,2′,2″-(1,4,7-triazonane-1,4,7-triyl)triacetic acid (nota·3H) were synthesized and characterized. Whereas $[^{99}TcO_3(tacn\text{-}ba)]^+$ $[\mathbf{2}]^+$ can be synthesized following a established oxidation procedure starting from the Tc^V complex $[^{99}TcO(glyc)(tacn\text{-}ba)]^+$ $[\mathbf{1}]^+$, a new synthetic pathway for the synthesis of $[^{99}TcO_3(nota)]^{2-}$ $[\mathbf{5}]^{2-}$ had to be developed, starting from $[^{99}Tc(nota{\cdot}3H)(CO)_3]^+$ $[\mathbf{4}]^+$ and using sodium perborate tetrahydrate ($NaBO_3{\cdot}4H_2O$) as oxidizing reagent. While $[^{99}TcO_3(nota)]^{2-}$ $[\mathbf{5}]^{2-}$ is a very attractive candidate for the development of trisubstituted novel multifunctional radioprobes, (3 + 2)-cycloaddition reactions of $[^{99}TcO_3(tacn\text{-}ba)]^+$ $[\mathbf{2}]^+$ with 4-vinylbenzenesulfonate (styrene-SO_3^-) demonstrated the suitability of monosubstituted tacn derivatives for the new mixed "BFC-(3 + 2)-cycloaddition" approach. Kinetic studies of this reaction lead to the conclusion that the alteration of the electronic structure of the nitrogen donors by, e.g., alkylation can be used to tune the rate of the (3 + 2)-cycloaddition.



INTRODUCTION

Molecular imaging has gained enormous importance over the past decade for visualizing biological events in noninvasive diagnostics.[1,2] ^{99m}Tc-compounds are among the most favorable radiotracers due to their physical properties (low energy γ-rays 140.5 keV, half-life time 6 h) and ready availability (generator nuclide). The bifunctional chelator approach (BFC), in which a ligand for a ^{99m}Tc precursor is conjugated to a targeting molecule, is a powerful tool in the development of new radiopharmaceuticals.[2] As an approach complementary to the BFC principle, we have introduced the ligand-centered (3 + 2)-cycloaddition, based on the high-valent, *fac*-$\{Tc^{VII}O_3\}^+$-core. This labeling method has been established as an effective synthetic pathway toward novel $^{99(m)}$Tc complexes.[3,4] Thus, a combination of the BFC and the cycloaddition strategies is an excellent opportunity for the development of site specific dual-functional imaging agents. Conceptually, the synthesis of a site specific, dual-functional technetium probe is based on "functional" groups which can be selectively linked to a biovector for targeting and a second, which can be used to introduce an imaging modality (e.g., fluorescence marker) or a second targeting moiety, e.g., for cell nucleus targeting.

Current labeling strategies are based on ligand substitution reactions at the ^{99m}Tc-center (metal-centered reactivity).[5] Thereby, some weakly bound ligands are replaced by the BFC. In contrast, labeling with the *fac*-$\{^{99m}TcO_3\}^+$ core does not change the ligand atoms in the first coordination sphere but is achieved solely at the ligands (ligand-centered reactivity). Thus, for synthesizing dual-functionalized imaging agents, a first "function" is introduced by modification of the stabilizing tripodal ligand, in this particular case the 1,4,7-triazacyclononane (tacn) ligand, and the second "function" via cycloaddition to the oxo ligands of the *fac*-$\{^{99m}TcO_3\}^+$ core ((3 + 2) approach) (Scheme 1). We showed recently how different bioactive functionalities can be introduced via (3 + 2)-cycloaddition.[4]

In this report, we investigated derivatized 1,4,7-triazacyclononane complexes of the *fac*-$\{^{99}TcO_3\}^+$-core in order to exemplify the suitability for the dual-functionality approach. Complexes of the general form $[^{99}Tc^{VII}O_3(tacn\text{-}R)]^+$ were obtained from oxidation of $[^{99}Tc^{V}O(glyc)(tacn\text{-}R)]^+$ (glyc = ethylene glycol) type complexes by sodium hypochlorite (NaOCl) under acidic conditions.[3,6] We report selected coordinating properties of the 1,4,7-triazacyclononane (tacn) derivatives *N*-benzyl-2-(1,4,7-triazonan-1-yl)acetamide (tacn-ba) and 2,2′,2″-(1,4,7-triazonane-1,4,7-triyl)triacetic acid (nota·3H) and the synthesis of their respective *fac*-$\{^{99}TcO_3\}^+$ complexes (Scheme 2).

Whereas $[^{99}TcO_3(tacn\text{-}ba)]^+$ ($[\mathbf{2}]^+$) could be synthesized by the established procedure, a new synthesis for $[^{99}TcO_3(nota)]^{2-}$ ($[\mathbf{5}]^{2-}$) is described, starting from $[^{99}Tc^{I}(nota)(CO)_3]^{2-}$ and

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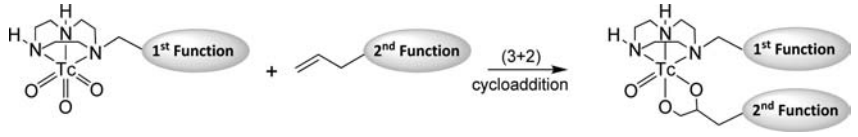
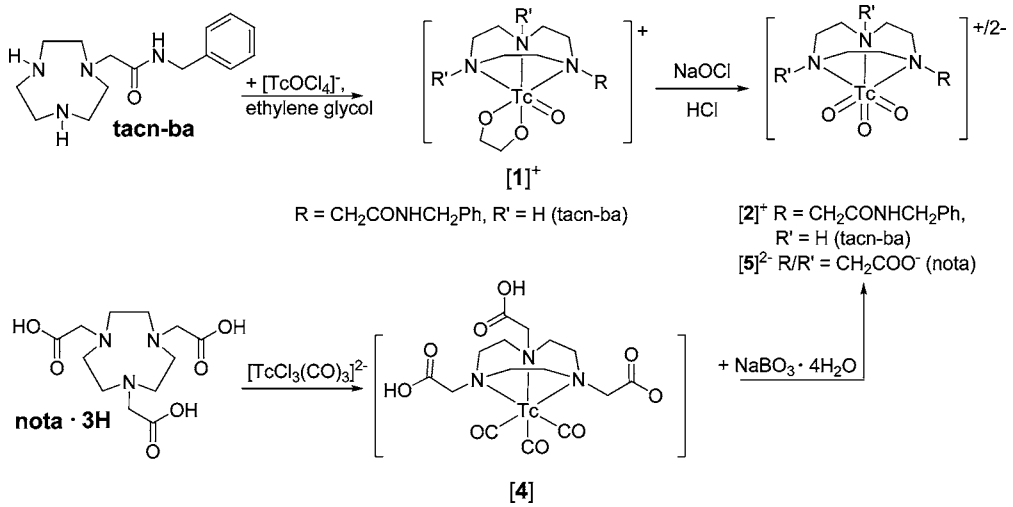
Scheme 1. Concept for the Synthesis of Site Specific Dual-Functionalized *fac*-$\{^{99m}TcO_3\}^+$ Probes Based on the BFC Approach (First Function) and (3 + 2)-Cycloaddition (Second Function)



Scheme 2. Synthesis of $[^{99}TcO_3(tacn\text{-}ba)]^+$ ([2]$^+$) and $[^{99}TcO_3(nota)]^{2-}$ ([5]$^{2-}$)



sodium perborate tetrahydrate ($NaBO_3{\cdot}4H_2O$) as the oxidizing reagent.

EXPERIMENTAL SECTION

Caution: *^{99}Tc is a weak β^- emitter. All experiments have to be done in appropriate laboratories for low-level radioactive materials.* All reactions were carried out under an inert N_2 atmosphere. The ligand 1,4,7-triazacyclononane-*N*,*N*′,*N*″-triacetic acid (nota·3HCl)[7,8] as well as the common precursor complexes $(NEt_4)_2[MX_3(CO)_3]$ (M = Re, ^{99}Tc; X= Cl, Br)[9,10] and $(NBu_4)[TcO(glyc)_2]$[3,11] (glyc = ethyleneglycol) were synthesized according to published procedures. 1,4,7-Triazacyclononane, free base (≥97%), and nota, free base (≥97%), were purchased from CheMatech; $(NH_4)[^{99}TcO_4]$ (Oak Ridge) and all other chemicals were of reagent grade and used without further purification.

Elemental analyses (EA) were performed on a Leco CHNS-932 and a Leco TruSpec Micro elemental analyzer. ^1H, ^{13}C, and ^{99}Tc NMR spectra were recorded on a BrukerDRX500 500 MHz spectrometer. ^{13}C NMR spectra were proton decoupled recorded. ESI-MS were performed on a Bruker esquire/HCT spectrometer. UV−vis spectra and kinetic studies were recorded with an Agilent Cary 50 spectrometer with solution samples in 1 cm quartz cells. During the measurement the temperature was controlled to maintain 293 K. HPLC conditions are described in the Supporting Information. For technetium content measurements, pure compounds were dissolved in water. The measurements were carried out with a scintillation cocktail (Packard Ultimate Gold XR) and a liquid scintillation counter (TRI-CARB 2200CA, Packard).

Synthesis. *$[^{99}TcO(glyc)(tacn\text{-}ba)]Br$ ([1]Br).* To a solution of $(NBu_4)[^{99}TcO(glyc)_2]$ (0.1 mmol) in 4 mL of THF was added tacn-ba·3HCl (38.4 mg, 0.1 mmol). The resulting suspension was refluxed for 2 h. During this time the formation of a light blue precipitate was observed. The blue precipitate was filtered, washed twice with fresh THF, and dried *in vacuo* to yield a blue powder. This product was suspended in a saturated KBr solution and stirred for some minutes. The dark blue powder was filtered off and dried *in vacuo*. X-ray quality crystals (blue needles) were grown by slow evaporation of an aqueous solution of [TcO(glyc)(tacn-ba)]Br. Yield: 44.6 mg (84%). IR ($\nu_{Tc=O}$, KBr): 952 (s) cm^{-1}. ^1H NMR (500 MHz, D_2O): δ = 7.35 (m, 5H, arom), 5.55 (m, 1H, glycol), 5.41 (m, 1H, glycol), 5.08 (m, 1H, glycol), 4.56 (m, 1H, glycol), 4.26 (m, 7H, tacn-CH_2-CONH/CONH-CH_2-Ph/tacn), 3.64 (m, 1H, tacn), 3.36 (m, 1H, tacn), 3.27 (m, 1H, tacn), 2.98 (m, 1H, tacn), 2.91 (m, 1H, tacn), 2.60 (m, 2H, tacn), 2.36 ppm (m, 2H, tacn). ^{13}C NMR (125 MHz, D_2O): δ = 170.25 (CONH), 139.03 (*ipso*-C_{arom}), 130.34 (C_{arom}), 129.13 (C_{arom}), 128.95 (C_{arom}), 84.92 (2C, glycol), 68.01 (tacn-CH_2-CONH), 64.96 (tacn), 57.01 (tacn), 54.67 (tacn), 50.29 (tacn) 46.69 (tacn), 45.10 (tacn), 44.56 ppm (CON-CH_2-Ph). Tc analysis: calcd 18.62%; found 18.04%.

$[^{99}TcO_3(tacn\text{-}ba)]Br$ ([2]Br). This compound was prepared according to a modification of a synthetic procedure reported previously.[3] $[^{99}TcO(glyc)(tacn\text{-}ba)]Br$ (26.56 mg, 0.05 mmol) was dissolved in 1 mL of H_2O, and a freshly prepared acidic solution of NaOCl (14%; 0.1 mL) was added. After about 30 min the dark blue solution turned yellow. Slow evaporation of the solvent yielded yellow crystals that were filtered from the mother liquor and washed with a small amount of saturated KBr solution. Yield: 20.25 mg (80%). IR (ν_{TcO3}, KBr): 896 (s), 889 (s) cm^{-1}. ^1H NMR (500 MHz, MeOH-d_4): δ = 7.28 (m, 5H, arom), 4.39 (s, 2H, CONH−CH_2−Ph), 4.32 (s, 2H, tacn-CH_2-CONH), 3.35 ppm (m, 12H, tacn). ^{13}C NMR (125 MHz, MeOH-d_4): δ = 169.19 (1C, CONH), 139.68 (1C, *ipso*-C_{arom}), 129.81 (C_{arom}), 128.87 (2C, C_{arom}), 128.57 (C_{arom}), 62.63 (tacn-CH_2−CONH), 55.64 (2C, tacn), 49.44 (2C, tacn), 48.75 (2C, tacn), 44.18 ppm (CONH-CH_2−Ph). ^{99}Tc NMR (113 MHz, MeOH-d_4): δ = 392 ppm (s, $\Delta\nu_{1/2}$ = 1200 Hz). Tc analysis: calcd 18.98%, found 19.35%.

$[Re(nota{\cdot}2H)(CO)_3]$ ([3]). $(NEt_4)_2[ReBr_3(CO)_3]$ (65.3 mg, 0.08 mmol) was dissolved in 2 mL of MeOH. To the colorless solution was added a solution of nota (32.8 mg, 0.1 mmol) and sodium acetate (58.0 mg, 0.7 mmol) in 2 mL of MeOH. The mixture was stirred at room temperature overnight. The solvent was then removed under reduced pressure, and the remaining colorless powder was dissolved in 2 mL of water. Upon acidification with 1 M HCl to pH 2−3, the colorless product precipitated from the aqueous solution. Crystals, suitable for X-ray diffraction analysis (colorless sticks), were obtained by slow evaporation of the MeOH reaction solution. Yield: 29.7 mg

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Table 1. Crystallographic Data for [TcO(glyc)(tacn-ba)]Br ([1]Br), [TcO_3(tacn-ba)]Br·H_2O ([2]Br·H_2O), Na_2[Re(nota)$(CO)_3$]·2.5H_2O (Na_2[3]·2.5H_2O), [Tc(nota·3H)$(CO)_3$]Cl ([4]Cl), and $Na_{1.5}$[TcO_3(nota·0.5H)]·4H_2O ($Na_{1.5}$[5]·4H_2O)

	[TcO(glyc)(tacn-ba)]Br [1]Br	[TcO_3(tacn-ba)]Br·H_2O [2]Br·H_2O	Na_2[Re(nota)$(CO)_3$] ·2.5H_2O Na_2[3] ·2.5H_2O	[Tc(nota·3H)$(CO)_3$]Cl [4]Cl	$Na_{1.5}$[TcO_3(nota·0.5H)]·4H_2O $Na_{1.5}$[5]·4H_2O
formula	$C_{17}H_{28}BrN_4O_4Tc$	$C_{15}H_{26}BrN_4O_5Tc$	$C_{15}H_{23}N_3Na_2O_{11.5}Re$	$C_{15}H_{21}ClN_3O_9Tc$	$C_{12}H_{26.5}N_3Na_{1.5}O_3Tc$
M_w	530.34	520.31	661.54	520.80	553.35
space group	$P\bar{1}$	$P2_1/n$	$P\bar{1}$	$P\bar{1}$	$P\bar{1}$
a/Å	7.8503(3)	10.1014(2)	7.7667(5)	8.794(2)	6.4835(2)
b/Å	10.8590(3)	11.4750(8)	11.9159(8)	8.958(2)	14.2176(5)
c/Å	12.3046(4)	17.935(2)	12.1265(6)	13.342(3)	14.3398(5)
α/deg	106.501(3)	90	109.724(5)	94.55(2)	66.044(4)
β/deg	93.887(3)	95.344(4)	95.710(5)	98.59(2)	87.183(3)
γ/deg	96.816(3)	90	90.975(5)	107.66(2)	89.303(3)
μ/mm^{-1}	2.768	2.66	5.897	0.92	0.685
Z	2	4	2	2	2
V/Å^3	992.92(6)	2069.9(4)	1049.7(1)	981.6(4)	1206.47(7)
ρ_{calcd}/g cm^{-3}	1.774	1.670	2.093	1.762	1.523
R1[a,c]	0.0375	0.0480	0.0334	0.0369	0.0450
wR2[b,c]	0.0476	0.0916	0.0640	0.0457	0.0762
CCDC	847663	847664	847665	847661	847662

[a] $R1 = |F_o - F_c|/|F_o|$. [b] $wR2 = [w(F_o^2 - F_c^2)^2/(wF_o^2)]^{1/2}$. [c] $I > 2\sigma(I)$.

(65%). IR ($\nu_{Tc(CO)3}$, KBr): 2030 (s), 1924 (s), 1910 (s) cm^{-1}. ESI-MS (MeOH): m/z = 574.1 [M + H]$^+$. ^{1}H NMR (500 MHz, MeOH-d_4): δ = 4.28 (s, 6H, tacn-CH_2-COO$^-$), 3.76 (m, 6H, tacn), 3.58 ppm (m, 6H, tacn). ^{13}C NMR (125 MHz, MeOH-d_4): δ = 196.23 (3 C, Re-CO), 172.24 (3 C, tacn-CH$_2$-*COO*$^-$), 67.19 (3 C, tacn-*CH*$_2$-COO$^-$), 58.61 ppm (6 C, tacn). Anal. Calcd for $C_{15}H_{20}N_3O_9Re$ (%): C, 31.47; H, 3.52; N, 7.34. Found: C, 31.28; H, 3.73; N, 7.34.

[^{99}Tc(nota·2H)(CO)$_3$] ([4]). $(NEt_4)_2$[^{99}Tc$Cl_3(CO)_3$] (23 mg, 0.05 mmol) was dissolved in 6 mL of MeOH and nota (25.1 mg, 0.08 mmol) added to the solution. After addition of triethylamine (10 drops), the solution was refluxed for 5 h under stirring. The solvent was removed under reduced pressure and the remaining colorless powder taken up in 2 mL of water. Upon acidification to pH 2−3, a colorless product precipitated. Crystals of [^{99}Tc(nota·3H)$(CO)_3$]Cl, suitable for X-ray diffraction analysis, were obtained by slow evaporation of an acidic aqueous solution (pH 1) of [Tc(nota·2H)-$(CO)_3$]. Yield: 15.2 mg (72%). IR ($\nu_{Tc(CO)3}$, KBr): 2034 (s), 1926 (s), 1892 (s) cm^{-1}. ^{1}H NMR (500 MHz, MeOH-d_4): δ = 4.09 (s, 6H, tacn-CH_2-COO$^-$), 3.65 (m, 6H, tacn), 3.50 ppm (m, 6H, tacn). ^{13}C NMR (125 HMz, MeOH-d_4): δ = 169.35 (3 C, tacn-CH$_2$-*COO*$^-$), 68.4 (3 C, tacn-*CH*$_2$-COO$^-$), 57.5 ppm (6 C, tacn). ^{99}Tc NMR (113 MHz, MeOH-d_4): δ = −912 ppm (s, $\Delta\nu_{1/2}$ = 1320 Hz).

Na_2[^{99}TcO_3(nota)] Na_2[5]. Perborate tetrahydrate (46.16 mg, 0.3 mmol) was added to an aqueous solution of [^{99}Tc(nota·2H)$(CO)_3$] (16 mg, 0.03 mmol), and the reaction mixture was heated to 55 °C for 2 h. After this time the HPLC trace of the reaction solution (TEAP buffer) showed a quantitatively conversion of the starting compound to the desired product. The basic reaction mixture was acidified by the addition of four drops of 1 M HCl (destruction of excess perborate) and afterward neutralized with 1 M NaOH. The product was purified by column chromatography (SPE-Cartridge, C_{18} ec (L), Chromafix) and was dried under vacuum. Yield: 12.35 mg (82%). IR (ν_{TcO3}, KBr): 906 (s) cm^{-1}. ^{1}H NMR (500 MHz, D_2O): δ = 4.15 (s, 6H, tacn-CH_2-COO$^-$), 3.55 (m, 6H, tacn), 3.24 ppm (m, 6H, tacn). ^{13}C NMR (125 HMz, D_2O): δ = 175.17 (3 C, tacn-CH$_2$-*COO*$^-$), 66.32 (3 C, tacn-*CH*$_2$-COO$^-$), 56.07 ppm (6 C, tacn). ^{99}Tc NMR (113 MHz, D_2O): δ = 479 ppm (s, $\Delta\nu_{1/2}$ = 6800 Hz).

Crystals of $Na_{1.5}$[^{99}TcO_3(nota·0.5H)] ($Na_{1.5}$[5]) suitable for crystal structural analysis were obtained by fast evaporation of a neutralized reaction mixture.

Kinetic Measurements. The kinetics of reduction were measured under pseudo-first-order conditions in doubly distilled H_2O (N_2 purged). Initial concentration of [^{99}TcO_3(tacn-ba)]$^+$ [**2**]$^+$ was 1.7 × 10^{-4} M and 4.53 × 10^{-3} M for sodium 4-vinylbenzenesulfonate (≈26-fold excess). Changes of absorption were observed at two different wavelengths (200 and 375 nm) for the first 2400 s (collection of 20 data points for each wavelength). The data was linearearized and fitted linearly to yield k_{obs}. The kinetic parameter k_1 was then calculated with the initial concentration listed above.

X-ray Diffraction. Crystallographic data were collected at 183(2) K (compounds [**1**]Br, Na_2[**3**], [**4**]Cl, $Na_{1.5}$[**5**]) or 298(2) K (compound [**2**]Br) with Mo Kα radiation (λ = 0.7107 Å) that was monochromated with help of a graphite on either a Stoe IPDS diffractometer ([Tc(tacn)$(CO)_3$]Cl and [**2**]Br) or an Oxford Diffraction Xcalibur system ([**1**]Br, Na_2[**3**], [**4**]Cl, $Na_{1.5}$[**5**]) with a Ruby detector. Suitable crystals were covered with oil (Infineum V8512, formerly known as Paratone N), mounted on top of a glass fiber and immediately transferred to the diffractometer. In the case of the IPDS, a maximum of 8000 reflections distributed over the whole limiting sphere were selected by the program SELECT and used for unit cell parameter refinement with the program CELL.[12] Data were corrected for Lorentz and polarization effects as well as for absorption (numerical). In case of the Oxford system, the program suite CrysAlis Pro was used for data collection, semiempirical absorption correction, and data reduction.[13] Structures were solved with direct methods using SIR97[14] or SHELXL-97 (Na_2[**3**]) and were refined by full-matrix least-squares methods on F^2 with SHELXL-97.[15] For the structure of $Na_{1.5}$[**5**], the disordered solvent molecules had to be treated with the SQUEEZE procedure within Platon.[16] More details on data collection and structure calculation are contained in Table 1 ([Tc(tacn)$(CO)_3$]-Cl, Table S1).

RESULTS AND DISCUSSION

Syntheses. The oxidation of water stable ^{99}Tc^V complexes by hypochlorite $(OCl)^-$ is a synthetic pathway for *fac*-[^{99}TcO_3(tacn)]$^+$ type complexes.[3] Conjugation of biologically active functions to tacn will enable labeling opportunities according to the BFC approach. Accordingly, 1,4,7-triazacyclononane was derivatized with *N*-benzyl-2-bromoacetamide to yield the bioconjugate *N*-benzyl-2-(1,4,7-triazonan-1-yl)-acetamide (tacn-ba) as a model. The complex [^{99}TcO(glyc)-(tacn-ba)]Br ([**1**]Br) was then synthesized from *in situ* prepared [^{99}$TcO(glyc)_2$]$^-$ with 1 equiv of (tacn-ba)·3HCl.

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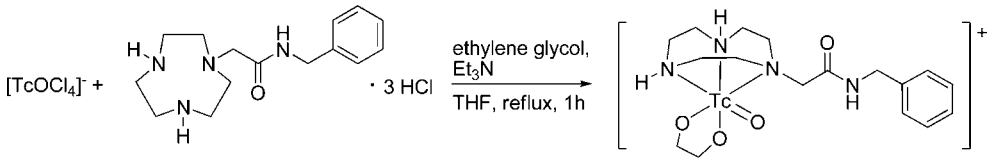
Scheme 3. Synthesis of $[^{99}TcO(glyc)(tacn\text{-}ba)]^+$ ($[\mathbf{1}]^+$)



Compound [**1**]Cl precipitated as blue powder after 2 h. Anion metathesis with a saturated KBr solution gave the corresponding crystalline [**1**]Br in 84% yield (Scheme 3).

[**1**]Br is stable under basic to neutral conditions in aqueous solution and as a solid but slowly decomposes under acidic conditions. Blue, plate-shaped crystals were obtained by slow evaporation of a methanol solution of [**1**]Br. Figure 1 shows the molecular structure of the cation $[\mathbf{1}]^+$.

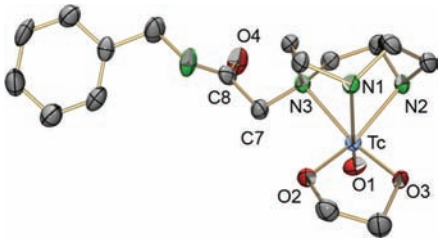


Figure 1. ORTEP[17] representation of the $[^{99}TcO(glyc)(tacn\text{-}ba)]^+$ $[\mathbf{1}]^+$ cation. Thermal ellipsoids represent 50% probability. Hydrogen atoms are omitted for clarity. Selected bond lengths [Å] and angles [deg]: Tc–O1 1.664(2), Tc–O2 1.905(2), Tc–O3 1.922(2), Tc–N1 2.256(2), Tc–N2 2.142(2), Tc–N3 2.229(2), O1–Tc–O2 111.44(9), O1–Tc–O3 107.99(9), O2–Tc–O3 84.06(8), N1–Tc–N2 74.98(9), N1–Tc–N3 76.21(8), N2–Tc–N3 80.39(8).

[**1**]Br crystallizes in the triclinic space group $P\bar{1}$ with one molecule per asymmetric unit. A structural feature of $[\mathbf{1}]^+$ are the two elongated Tc–N bonds (Tc–N1 2.256(2), Tc–N3 2.229(2) Å), in comparison to $[^{99}TcO(glyc)(tacn)]^+$ where only one Tc–N bond (*trans*-oxo) is elongated (2.164(2), 2.174(2), 2.260(2) Å).[6] The extended bond lengths of the Tc–N1 bond can be understood by the *trans*-influence of the terminal oxo ligands and elongation of the Tc–N3 bond in $[\mathbf{1}]^+$ may arise from sterical reasons. The elongation of two Tc–N bonds in $[^{99}TcO(glyc)(tacn\text{-}R)]^+$ type complexes was equally observed in $[^{99}TcO(glyc)(tacn\text{-}bz)]^+$ (bz = benzyl).[3] Bond angles in $[\mathbf{1}]^+$ do not differ significantly from $[^{99}TcO(glyc)(tacn\text{-}bz)]^+$. The O1–Tc–O2 and O1–Tc–O3 angles are 107.99(9)° and 111.44(9)°, respectively, to minimize interligand repulsions between the terminal oxo and the glycolato ligand. An interesting feature of the IR spectrum of compound $[\mathbf{1}]^+$ is the $\nu_{(Tc=O)}$ stretch at 952 cm^{-1}, similar to the comparable complexes $[^{99}TcO(glyc)(tacn)]^+$ (949 cm^{-1}),[6] $[^{99}TcO(glyc)(tacn\text{-}bz)]^+$ (949 cm^{-1}).[3]

The oxidation of [**1**]Br with a small amount of NaOCl in acidic solution rapidly afforded $[^{99}TcO_3(tacn\text{-}ba)]^+$ ($[\mathbf{2}]^+$, Scheme 4).

HPLC monitoring indicated nearly quantitative conversion after one day with a minor amount of $[^{99}TcO_4]^-$ as a side product. $[^{99}TcO_3(tacn\text{-}ba)]$Br ([**2**]Br) formed readily and was isolated as yellow stick-shaped crystals from the aqueous reaction solution in good yield (80%). [**2**]Br is water stable over a wide pH range (1–10) at ambient temperature and partially soluble in methanol. Thus, 2-(1,4,7-triazonan-1-yl) acetic acid represents a good ligand system for stabilizing the *fac*-$\{^{99}TcO_3\}^+$ core even after conjugation to biomolecules via amide bond formation and to act as a bifunctional chelator for the first function. An ORTEP representation of the cation $[\mathbf{2}]^+$ is given in Figure 2.

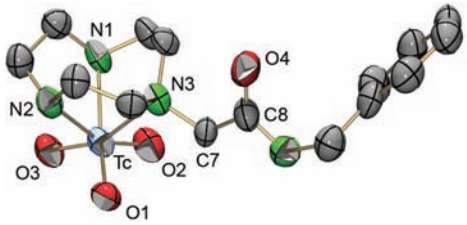
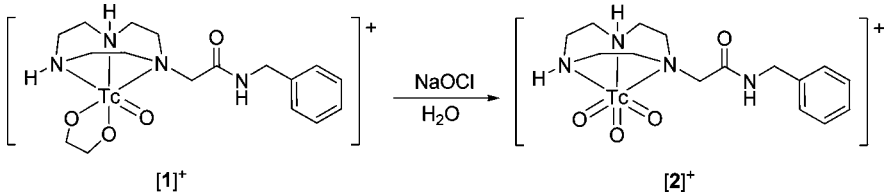


Figure 2. ORTEP[17] representation of the $[^{99}TcO_3(tacn\text{-}ba)]^+$ $[\mathbf{2}]^+$ cation. Thermal ellipsoids represent 50% probability. Hydrogen atoms are omitted for clarity. Selected bond lengths [Å] and angles [deg]: Tc–O1 1.690(4), Tc–O2 1.694(4), Tc–O3 1.693(4), Tc–N1 2.223(5), Tc–N2 2.237(4), Tc–N3 2.360(4), O1–Tc–O2 106.7(2), O1–Tc–O3 107.8(2), O2–Tc–O3 106.9(2), N1–Tc–N2 73.7(2), N1–Tc–N3 75.0(2), N2–Tc–N3 74.5(2).

[**2**]Br crystallizes in the monoclinic space group $P2_1/n$ with one cation and one anion per asymmetric unit. The bromide atom is disordered over two positions with occupancies of 50%. In $[\mathbf{2}]^+$, a distorted octahedral coordination sphere is observed around the Tc center. The N–Tc–N angles are between 73.7(2)° and 75.0(2)° and the O–Tc–O angles between 106.7(2)° and 107.8(2)° wide. This feature is common to all *fac*-$\{MO_3\}^+$ (M = Re, Tc) structures and mirrors the spatial demand of terminal oxo ligands with "M" in its highest

Scheme 4. Synthesis of $[^{99}TcO_3(tacn\text{-}ba)]^+$ ($[\mathbf{2}]^+$)



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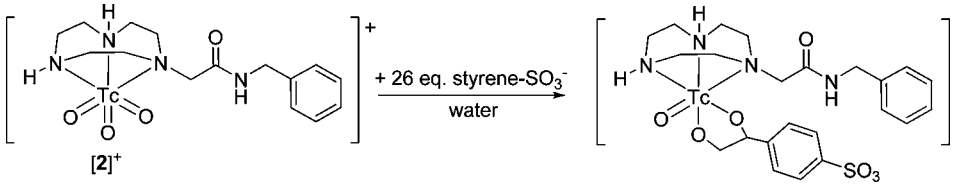
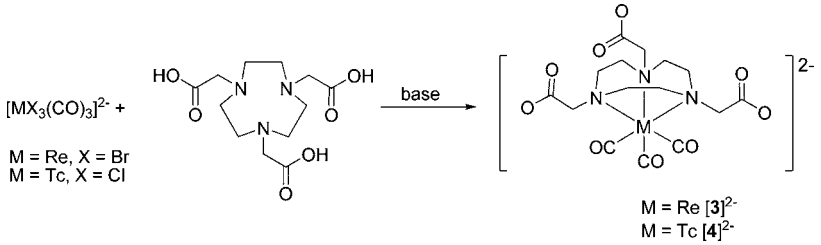
Scheme 5. (3 + 2)-Cycloaddition Reaction of $[2]^+$ with 4-Vinylbenzenesulfonate (Styrene-SO_3^-), Kinetic Measurements



[2]+

Scheme 6. Synthesis of $[M(nota)(CO)_3]^{2-}$ (M = Re, Tc)



oxidation state +VII. Ligand field effects play, if ever, a minor role, and the terminal oxo ligands assume a minimal energy conformation by minimizing electrostatic repulsion. In analogy to the structurally characterized *fac*-$\{^{99}TcO_3\}^+$ complexes $[^{99}TcO_3(tacn\text{-}bz)]^+$ and $[^{99}TcO_3(tacn\text{-}bz\text{-}COOH)]^+$,[3] the Tc−N3 bond (2.360(4) Å) in complex $[2]^+$ is elongated by about 6% relative to the remaining two Tc−N bonds (2.223(5) and 2.237(4) Å, respectively). The ^{99}Tc-NMR signal of $[2]^+$ appeared at 392.39 ppm ($\Delta\nu_{1/2}$= 1200 Hz) relative to $[^{99}TcO_4]^-$ at 0 ppm, thus, in the expected range for *fac*-$\{^{99}TcO_3\}^+$ complexes.[18] IR spectra show additional interesting features. The absorption of the very characteristic symmetric Tc═O stretch is split into two bands at 896 and 889 cm^{-1}, respectively, due to a lowered symmetry (C_{3v} to C_{2v}) as imposed by the insertion of one substituent on the tacn scaffold.

Cycloaddition. According to the dual-functionality concept, the first function is introduced at the tacn ligand and the second by cycloaddition at the *fac*-$\{^{99}TcO_3\}^+$ core. Kinetic (3 + 2)-cycloaddition studies were thus performed with $[2]^+$ to assess convenience for labeling reactions at the tracer level. The kinetic measurements for the reaction of [2]Br with 4-vinylbenzenesulfonate (styrene-SO_3^-) were performed under the same conditions as for $[^{99}TcO_3(tacn\text{-}R)]^+$ (R = H, bz, bz-COOH) (Scheme 5).

The second-order rate constant at 20 °C in water for this reaction was found to be 0.243 ± 0.004 M^{-1} s^{-1} and is in good agreement with the reaction of styrene-SO_3^- with $[^{99}TcO_3(tacn)]^+$ (0.107 ± 0.001 M^{-1} s^{-1}), $[^{99}TcO_3(tacn\text{-}bz)]^+$ (0.42 ± 0.01 M^{-1} s^{-1}), and $[^{99}TcO_3(tacn\text{-}bz\text{-}COOH)]^+$ (0.26 ± 0.01 M^{-1} s^{-1}).[3] This data indicate that $^{99}Tc^{VII}$ complexes with a monosubstituted tacn-ligand react faster in (3 + 2)-cycloadditions. For $[2]^+$, the rate constant for the cycloaddition is about twice as high as for unsubstituted $[^{99}TcO_3(tacn)]^+$. This leads to the conclusion that the alteration of the electronic structure of the nitrogen donors, by, e.g., alkylation, substantially influences the (3 + 2)-cycloaddition rate. This observation is of relevance for tuning the rate of the (3 + 2)-cycloaddition by introducing not only one but two or three additional substituents.

Accordingly, in a next step we studied the multisubstituted tacn-based ligand derivative 2,2′,2″-(1,4,7-triazonane-1,4,7-triyl)triacetic acid (nota·3H). Unexpectedly, reaction of $[^{99}TcO(glyc)_2]^-$ with nota·3H did not lead to the desired $[^{99}TcO(glyc)(nota)]^{2-}$ complex. A similar synthesis published in the literature was not successful as well.[19] Hence, $(OCl)^-$ oxidation of $[^{99}TcO(glyc)(nota)]^{2-}$ was not found to be a successful pathway to higher oxidation states. The corresponding rhenium complex $[ReO_3(tacn)]^+$ was originally prepared by an oxidative decarbonylation of $[Re(tacn)(CO)_3]^+$.[20] To adopt this route, $[M(nota)(CO)_3]^{2-}$ (M = Re, ^{99}Tc) was synthesized by reaction of $[MX_3(CO)_3]^{2-}$ (M = Re, X = Br; M = ^{99}Tc, X = Cl) with 1 equiv of (nota·3H) under alkaline conditions which gave $[M(nota)(CO)_3]^{2-}$ (M = Re $[3]^{2-}$, ^{99}Tc $[4]^{2-}$; Scheme 6) in very good yields.

Complexes [3] and [4] precipitated from an acidic aqueous solution as colorless powders after double protonation. Both compounds [3] and [4] are stable in solution and as solids for weeks. Depending on their protonation grade, they are soluble in water as $[M(nota)(CO)_3]^{2-}$ ($[3]^{2-}$, $[4]^{2-}$) and in methanol as $[M(nota{\cdot}2H)(CO)_3]$ ([3], [4]). The IR spectra of complexes [3] and [4] show strong CO absorption bands at 2030, 1924, 1910 and 2034, 1926, 1892 cm^{-1}, respectively. These frequencies indicate much weaker donation of nota in comparison to the cationic $[Re(tacn)(CO)_3]^+$ (2014 and 1881 cm^{-1}).[21] The ^{99}Tc NMR of compound $[4]^{2-}$ showed a broad signal ($\Delta\nu_{1/2}$= 1320 Hz) at −912.3 ppm, shifted toward lower field as compared to $[^{99}Tc(tacn)(CO)_3]^+$ (−1012 ppm).[22] In contrast to the ^{13}C NMR spectrum of the Re compound [3], which shows a signal for the carbonyl carbon atoms at 196.23 ppm, the ^{13}C NMR signal of the Tc compound [4] cannot be observed in the ^{13}C NMR spectrum, due to the scalar coupling to the ^{99}Tc quadrupole nucleus (spin $^9/_2$). The weaker coordination properties of nota·3H in comparison to the tacn ligand are evident from the X-ray crystal structures of $Na_2[Re(nota)(CO)_3]$ (Na_2[3]) and $[^{99}Tc(nota{\cdot}3H)(CO)_3]Cl$ ([4]Cl), respectively. Since both compounds ($Na_2[Re(nota)(CO)_3]$ and $[^{99}Tc(nota{\bullet}3H)(CO)_3]Cl$) have very similar structural features, only the structure of $[^{99}Tc(nota{\cdot}3H)(CO)_3]Cl$ will be discussed in detail. Crystallographic data for $Na_2[Re(nota)(CO)_3]$ (Na_2[3]) can be found in the Supporting Information (Table SI1). A representation of the triply protonated cation $[^{99}Tc(nota{\cdot}3H)(CO)_3]^+$ $[4]^+$ is given in Figure 3.

Comparing the bond lengths in $Na_2[Re(nota)(CO)_3]$ and $[Tc(nota{\cdot}3H)(CO)_3]Cl$ with the unsubstituted $[M(tacn)$-

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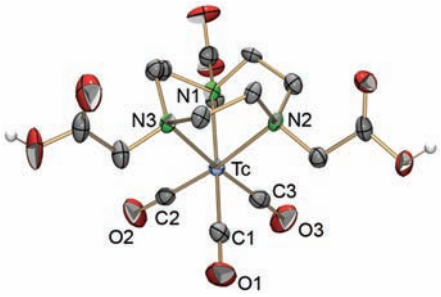

Figure 3. ORTEP[17] representation of the $[^{99}Tc(CO)_3(nota{\cdot}3H)]^+$ cation $[\mathbf{4}]^+$. Thermal ellipsoids represent 50% probability. Noncarboxy hydrogen atoms are omitted for clarity. Selected bond lengths [Å] and angles [deg]: Tc–C1 1.906(3), Tc–C2 1.907(3), Tc–C3 1910(4), Tc–N1 2.237(2), Tc–N2 2.236(2), Tc–N3 2.238(2), C1–O1 1.146(3), C2–O2 1.154(3), C3–O3 1.151(4), C1–Tc–C2 87.8(1), C1–Tc–C3 86.4(1), C2–Tc–C3 87.6(1), N1–Tc–N2 79.35(8), N1–Tc–N3 79.69(9), N2–Tc–N3 79.98(8).

$(CO)_3]^+$ complexes provides an insight into binding properties. The structure of $[Re(tacn)(CO)_3]^+$ is known for over two decades,[21,23,24] but the one of its homologue $[^{99}Tc(tacn)(CO)_3]^+$ was not. To complete this fundamental series, we synthesized and structurally characterized $[^{99}Tc(tacn)(CO)_3]^+$ (SI). Within standard variations, the structures of $[^{99}Tc(tacn)(CO)_3]^+$ and $[Re(tacn)(CO)_3]^+$ are identical.

$[^{99}Tc(nota{\cdot}3H)(CO)_3]Cl$ [**4**]Cl was obtained from an acidified aqueous solution of $[\mathbf{4}]^{2-}$. It crystallizes in the triclinic space group $P\bar{1}$. All acetate groups in nota·3H are protonated, yielding a cationic complex with chloride as counterion. In the crystal structure of $[\mathbf{4}]^+$, the metal center is in a distorted octahedral coordination sphere due to sterical constraints of the aza-macrocycle. Interesting structural features are the elongated Tc–N bonds (2.236(2), 2.237(2), and 2.238(2) Å). These bonds are significantly longer than found in $[M(tacn)(CO)_3]^+$ (M = Re, 2.189(8)–2.222(8),[23] 2.169 – 2.213,[21] 2.195(4) – 2.203(4) Å;[24] M = Tc, 2.195(5)–2.206(5) Å) and confirm nota·3H to be a weaker ligand than tacn.

Starting from [**4**], different oxidizing reagents such as H_2O_2, $(OCl)^-$, and sodium perborate tetrahydrate $(NaBO_3{\cdot}4H_2O)$ were employed. With $NaBO_3{\cdot}4H_2O$, the best results were obtained, and the reaction of a colorless aqueous solution of $[\mathbf{4}]^{2-}$ with this oxidizing reagent gave quantitatively $[^{99}TcO_3(nota)]^{2-}$ ($[\mathbf{5}]^{2-}$) after 2 h at 55 °C (proven by HPLC monitoring). The ^{99}Tc NMR of compound $[\mathbf{5}]^{2-}$ shows a very broad signal ($\Delta\nu_{1/2}$ = 6800 Hz) at 488 ppm. This is remarkable, since the ^{99}Tc NMR shifts of other $[^{99}TcO_3(tacn\text{-}R)]^+$ (R = H, bz, bz-COOH, ba) are in the range 358–392 ppm ($\Delta\nu_{1/2}$ 1200–4800 Hz),[3,6] which suggest a different binding situation in complex $[\mathbf{5}]^{2-}$. The broad signal is a hint for a weakly bound nota ligand, which enables dynamics in solution. In addition, the shift to lower field also indicates a weaker donating property of the nota ligand, which is in agreement with observations made with the *fac*-$\{M(CO)_3\}^+$ (M = Re, ^{99}Tc) core (*vide infra*, compounds $Na_2[\mathbf{3}]$ and [**4**]Cl). The crystal structure analysis of yellow crystals of $Na_{1.5}[^{99}TcO_3(nota{\cdot}0.5H)]$ ($Na_{1.5}[\mathbf{5}]$), which were isolated from a fast evaporated reaction mixture, confirms this hypothesis. A representation of the molecular structure of $[\mathbf{5}]^{1.5-}$ is given in Figure 4.

$[^{99}TcO_3(nota{\cdot}0.5H)]^{1.5-}$ ($[\mathbf{5}]^{1.5-}$) crystallizes with 1.5 Na^+ cations. The residual positive charge is compensated by 0.5 H^+, which could not be localized. All carboxylate oxygen atoms

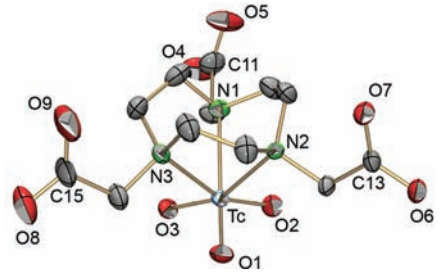

Figure 4. ORTEP[17] representation of the $[^{99}TcO_3(nota{\cdot}0.5H)]^{1.5-}$ anion ($[\mathbf{5}]^{1.5-}$). Thermal ellipsoids represent 50% probability. Hydrogen atoms are omitted for clarity. Selected bond lengths [Å] and angles [deg]: Tc–O1 1.702(3), Tc–O2 1.713(3), Tc–O3 1.706(3), Tc–N1 2.302(4), Tc–N2 2.281(3), Tc–N3 2.296(4), O1–Tc–O2 106.5(2), O1–Tc–O3 106.6(2), O2–Tc–O3 106.2(2), N1–Tc–N2 76.2(1), N1–Tc–N3 76.4(1), N2–Tc–N3 76.6(1).

are acceptors of hydrogen-bridges. There is a residual electron density at O7 in a reasonable geometry, but this would mean that H10d (hydrogen atom of a solvent molecule) would have to be pointing away from O7 in 50% of the cases and this position could not be found. There is also the possibility that the missing half positive charge is located in the disordered solvent. The crystal packing of $Na_{1.5}[\mathbf{5}]$ shows an interesting channel structure with $[\mathbf{5}]^{1.5-}$ and Na^+ forming a two-dimensional network (Figure SI3). These layers are packed in a way that channels are formed along the [100] direction of the crystal. The channels are filled with disordered water molecules (solvent accessible void: 19(2) $Å^3$, 2%$_{vol}$). The Tc–N bond lengths in $Na_{1.5}[\mathbf{5}]$ are all in the same range (2.281(3)–2.302(4) Å) and are significantly longer than in $[^{99}TcO_3(tacn)]$ (2.239(4) Å).[6] The elongation of one Tc–N bond after substitution of one tacn nitrogen atom was observed previously in *fac*-$\{^{99}TcO_3\}^+$ complexes $[^{99}TcO_3(tacn\text{-}bz)]^+$ (2.343(3) Å),[3] $[^{99}TcO_3(tacn\text{-}bz\text{-}COOH)]^+$ (2.286(5) Å),[3] and complex $[\mathbf{2}]^+$ (2.360(4) Å). The substitution of all three nitrogen atoms in nota·3H leads to the elongation and weakening of all three bonds. Substituted nitrogen atoms are usually better σ-donors, and stronger binding to a metal center was expected. Obviously, sterical components overcompensate for strong donation, and overall stability is reduced as it is indicated in the crystal structure of $Na_{1.5}[\mathbf{5}]$. Since the tacn backbone is distorted upon coordination to the metal center and forced to assume a specific conformation, the substituents might keep the tacn ligand from attaining the preferred coordination conformation. However, compound $[\mathbf{5}]^{2-}$ is stable in the solid state and in solution over time (days). As all other *fac*-$\{TcO_3\}^+$ complexes, the yellow compound $[\mathbf{5}]^{2-}$ reacts with 4-vinylbenzenesulfonate (styrene-SO_3^-) to form a blue (3 + 2)-cycloadduct ($[TcO(nota)(styrene\text{-}SO_3)]^{3-}$). On the basis of these observations, the first negatively charged *fac*-$\{^{99}TcO_3\}^+$-complex $[\mathbf{5}]^{2-}$ is a very attractive candidate for the development of novel multifunctional radioprobes. Experiments with the nuclear isomer ^{99m}Tc and kinetic measurements with compound $[\mathbf{5}]^{2-}$ are currently under study.

■ CONCLUSION

The coordination of functionalized tacn-derivatives with the *fac*-$\{^{99}TcO_3\}^+$ core according to the BFC approach leads to new model compounds for radiopharmacy based on high valent $^{99}Tc^{VII}$ chemistry. The coordination properties of the functionalized tacn derivatives *N*-benzyl-2-(1,4,7-triazonan-1-

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yl)acetamide (tacn-ba) and 2,2′,2″-(1,4,7-triazonane-1,4,7-triyl)-triacetic acid (nota·3H) were evaluated in this respect. The former ligand stabilizes the $\{^{99}Tc^{V}{=}O\}^{3+}$ as well as the *fac*-$\{^{99}Tc^{VII}O_3\}^{+}$ core. Corresponding complexes are water stable, and tacn-ba is a model ligand for conjugating targeting functions to 2-(1,4,7-triazonan-1-yl) acetic acid (noma). Noma derivatives therefore enable the BFC approach with *fac*-$\{^{99(m)}TcO_3\}^{+}$ cores. Additional (3 + 2)-cycloadditions are faster than with unsubstituted tacn; thus, a second bioactive function can be introduced enabling the access to dual-functional agents.

The nota ligand does not coordinate as strong as the tacn or monosubstituted derivatives to the metal center. However, *fac*-$\{^{99}TcO_3\}^{+}$ complexes containing the nota ligand are water stable, and corresponding $^{99\,m}$Tc compounds are very attractive candidates for the development of novel multifunctional radioprobes. Beside the new opportunities nota·3H enables for the high oxidation states of technetium, it is also an excellent ligand for the *fac*-$\{M^{I}(CO)_3\}^{+}$ core (M = Re, ^{99m}Tc). Nota containing *fac*-$\{M^{I}(CO)_3\}^{+}$ (M = $^{186/188}$Re, ^{99m}Tc) complexes are hydrophilic complexes with a high potential for radiopharmacetutical imaging or therapy.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional figures, tables, and details. Crystal data in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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