A Novel ^{99m}Tc Labelling Strategy for the Development of Silica Based Particles for Medical Applications

Supporting Information

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 $4.1 [^{99}TcO(norb)(tacn)]Br \cdot 2H_2O ([\textbf{2}]Br \cdot 2H_2O)$

5 References

1 Experimental Part

1.1 General Information

Caution: ⁹⁹Tc is a weak β ⁻ emitter. All experiments have to be done in appropriate laboratories approved for handling low-level radioactive materials.

The precursor complexes $[^{99(m)}TcO_3(tacn)]^+$ and the MCM-41 particles were prepared according to published procedures.¹⁻³ Bicyclo[2.2.1]hept-2-en (Fluka), 5-(bicycloheptenyl)triethoxysilane 97% (ABCR), (NH_4) ⁹⁹TcO₄] (Oak Ridge) and all other chemicals were of reagent grade and used without further purification. FT-IR spectra were measured as with pellets on a Perkin Elmer Spectrum Two spectrophotometer. ¹H and ¹³C NMR spectra were recorded on a Bruker DRX500 500 MHz and a Bruker AV-400 400 MHz. ¹³C NMR spectra were proton decoupled. For technetium content measurements, pure compounds were dissolved in the appropriate solvents. The measurements were carried out with a scintillation cocktail (Packard Ultimate Gold XR) and a liquid scintillation counter (TRICARB 2200CA, Packard). γ-counting of the ^{99m}Tc solutions was performed on a COBRA 5002 (Packard). HPLC analyses were performed on a Merck Hitachi LaChrom L 7100 pump coupled to a Merck Hitachi LaChrom L7200 tunable UV detector and a radiodetector. Uv/vis detection was performed at 250 nm. The detection of radioactive ⁹⁹Tc/^{99m}Tc complexes was performed with a Berthold LB508 radiodetector equipped with YG/BGO cells, respectively. Separations were achieved on a Macherey-Nagel HILIC column using a isocratic MeCN/NH₄OAc (80 : 20, 200mM, pH 4.5). The grafting of the 5-(bicycloheptenyl)triethoxysilane at the MCM-41 particles was quantified by elemental analysis (LecoCHNS-932) of the modified particles. The degree of occupancy was calculated under the estimation, that only one ethoxy group of the 5-(bicycloheptenyl)triethoxysilane was hydrolyzed, forming the 5-(bicycloheptenyl)triethoxysilane@MCM-41(Sinorb@MCM-41) construct. The overall silane density at the MCM-41 surface was estimated by the BET surface area of 900 m²/g. Nitrogen sorption isotherms were collected at 77 K using a Quantachrome NOVA 3000. Samples were vacuum-degassed at 80 °C for 3 h. The total surface area was calculated by the BET method. Mesopore size distributions were evaluated by the NLDFT method developed for silica exhibiting cylindrical pore geometry (NOVAWin software, Version 11.0, Quantachrome Instruments). The adsorption branch of the respective isotherm was used for the calculations.

1.2 Synthesis of [⁹⁹TcO(norb)(tacn)]Br·2H₂O·2KBr ([2]Br·2H₂O·2KBr)

[⁹⁹TcO₃(tacn)]Cl·2H₂O (9.36 mg, 0.027 mmol) was dissolved in water (3 ml). An excess of norbornene (4.9 mg, 0.052 mmol), dissolved in acetone (1 ml), was added to the reaction solution. After the addition, the colour of the reaction changed continuously from yellow to blue. The reaction solution was stirred for 5 min at room temperature. After removal of the solvent, the excess norbornene was extracted from an aqueous KBr solution with CH_2Cl_2 (3 x 2 ml). The aqueous phase was dried and the residue was dissolved in EtOH (5 ml) and filtered. Compound [2]Br·2H₂O·2KBr was isolated as bright blue crystals after recrystallisation from water. Yield: 8.8 mg (41%).

IR (KBr): 3439 (m), 3366 (m), 3120 (s), 2952 (s), 2868 (s), 2022 (w), 1930 (m), 1636 (s), 1448 (s), 1113 (m), 1065 (m), 981 (s), 948 (s), 850 (m), 802 (m), 632 (s) cm^{-1} .

¹H NMR (400 MHz, D₂O): $\delta = 5.63$ (*s*; 2H, O-C*H*), 4.00 (m, 2H, tacn), 3.69 (m, 2H, tacn), 3.10 (m, 4H, tacn), 2.57 (m, 2H, tacn), 2.45 (*s*, 2H, C*H*), 2.40 (m, 2H, tacn), 1.60 (d, *J* = 8Hz, 2H, C*H*₂), 1.39 (d, *J* = 8Hz, 2H, C*H*₂), 1.24 (d, *J* = 11 Hz, 1H, C*H*_{2bridge}), 0.99 ppm (d, *J* = 11 Hz, 1H, C*H*_{2bridge}). ¹³C

NMR (100 MHz, D₂O): $\delta = 101.05$ (O-*C*H), 54.27 (tacn), 48.81 (tacn), 44.70 (*C*H_{2bridge}), 44.67 (tacn), 31.77 (*C*H), 24.12 ppm (*C*H₂). ⁹⁹Tc analysis: calcd. for C₁₃H₂₉N₃O₅TcK₂Br₃ 13.67%; found 13.62%. Crystals of [**2**]Br·2H₂O, suitable for X-ray diffraction analysis were obtained by slow evaporation of an aqueous reaction solution, containing two drops of a saturated aqueous KBr solution.

1.3 Synthesis of [⁹⁹TcO(norborneneSi(OEt)₃(tacn)]X ([3]X, X = Cl, (BPh₄))

[**3**]Cl:

 $[^{99}$ TcO₃(tacn)]Cl·2H₂O (9.66 mg, 0.028 mmol) was dissolved in H₂O (1 ml). To the resulting yellow solution, 5-(bicycloheptenyl)triethoxysilane (7.95 mg, 0.03 mmol) was added. The colour of the reaction solution changed from yellow to blue. HPLC analysis confirmed a quantitative conversion of the educt.

¹H-NMR (500.25 MHz, D₂O): $\delta = 5.57$ (d, J = 5.0 Hz 1H, O-C*H*); 5.53 (d, J = 5.0 Hz 1H, O-C*H*); 3.90 (m, 2H, tacn); 3.60 (m, 2H, tacn); 3.59 (q, J = 7.3 Hz, 6H, O-C*H*₂CH₃); 3.05 (m, 4H, tacn); 2.48 (m, 3H, C*H* + tacn); 2.34 (m, 3H, C*H* + tacn); 1.54 (m, 2H, C*H*₂); 1.12 (t, J = 7.3 Hz, 9H, CH₃), 1.10 (m, 2H, CH_{2bridge}); 0.83 ppm (m, 1H, Si-C*H*). ¹³C-NMR (125.78 MHz, D₂O): $\delta = 102.59$ (O-CH); 100.39 (O-CH); 57.40 (O-CH₂); 54.25 (tacn); 48.80 (tacn); 45.48 (CH); 44.66 (tacn), 44.60 (CH); 31.40 (*C*H_{2bridge}); 25.99 (*C*H₂); 20.58 (Si-CH); 16.82 ppm (*C*H₃). ²⁹Si-NMR (99.36 MHz, D₂O): $\delta = -40.3$ ppm ($v_{1/2} = 110$ Hz).

[**3**](BPh₄):

[⁹⁹TcO₃(tacn)]Cl·2H₂O (7.17 mg, 0.021 mmol) was dissolved in a H₂O/MeCN mixture (1:1, 1 ml). To the yellow solution, 5-(bicycloheptenyl)triethoxysilane (6.72 mg, 0.026 mmol) was added. The color of the reaction solution changed from yellow to blue within minutes indicating the formation of [**3**]⁺. NaBPh₄ (7.90 mg, 0.023 mmol), dissolved in a H₂O/MeCN mixture (1:1, 1 ml) was added to the reaction solution. After evaporation of the acetonitrile a blue precipitate was formed. The precipitate was filtered, washed with H₂O and dried under vacuum, resulting in [3](BPh₄).

Yield: 10.19 mg (52 %)

IR (KBr): 3417 (s), 3232 (s), 3053 (m), 1633 (w), 1476 (w), 1451 (w), 1423 (w), 1381 (w), 1258 (w), 1149 (m), 1074 (m), 953 (s), 858 (m), 735 (m), 707 (m), 612 (m), 542 (m), 461 (w) cm⁻¹.

NMR data in accordance with compound [3]Cl

⁹⁹Tc analysis: calcd. 11.61%; found 9.99%.

1.4 Synthesis of [⁹⁹TcO(allyltriethoxysilane)(tacn)]Cl

 $[^{99}$ TcO₃(tacn)]Cl·2H₂O (10.5 mg, 0.030 mmol) was dissolved in H₂O (2 ml). To the resulting yellow solution, allyltriethoxysilane (6.54 mg, 0.030 mmol) was added. The colour of the reaction solution changed slowly from yellow to blue. After 12 h, the HPLC analysis confirmed a quantitative conversion of the educt. The product was not isolated, but characterized *in situ* by NMR studies. *syn*-Product:

¹H NMR (500.25 MHz, D₂O): δ = 5.46 (m, 1H, OCH-OCH-CH₂-Si), 5.37 (m, 1H, OCH-OCH-CH₂-Si), 4.05 (m, 1H, O-CH-CHO-CH₂-Si), 3.79 (m, 4H, tacn), 3.49 (q, *J* = 7.2 Hz, 6 H, Si-O-CH₂-CH₃), 2.99 (m, 4H, tacn), 2.44 (m, 2H, tacn), 2.28 (m, 2H, tacn), 1.23 (m, 1H, OCH-OCH-CH₂-Si), 1.06 (m, 1H, OCH-OCH-CH₂-Si), 1.0 ppm (t, *J* = 7.2 Hz, 12H, Si-O-CH₂-CH₃).

¹³C NMR (125.78 MHz, D₂O): δ = 89.88 (OCH-OCH-CH₂-Si), 87.96 (OCH-OCH-CH₂-Si), 58.90 (Si-O-CH₂-CH₃), 56.41 (tacn), 55.10 (tacn), 50.47 (tacn), 50.11 (tacn), 46.40 (tacn), 46.04 (tacn), 20.67 (OCH-OCH-CH₂-Si), 18.27 ppm (Si-O-CH₂-CH₃).

²⁹Si NMR (99.36 MHz, D_2O): $\delta = -41.25$ ppm

anti-Product:

¹H NMR (500 MHz, D₂O): $\delta = 5.39$ (m, 1H, OCH-OCH-CH₂-Si), 4.79 (m, 1H, OCH-OCH-CH₂-Si), 4.46 (m, 1H, OCH-CHO-CH₂-Si), 3.79 (m, 4H, tacn), 3.49 (q, J = 7.2 Hz, 6 H, Si-O-CH₂-CH₃), 2.99 (m, 4H, tacn), 2.44 (m, 2H, tacn), 2.28 (m, 2H, tacn), 1.65 (m, 1H, OCH-OCH-CH₂-Si), 1.46 (m, 1H, OCH-OCH-CH₂-Si), 1.01 ppm (t, J = 7.2 Hz, 12H, Si-O-CH₂-CH₃).

¹³C NMR (125 MHz, D₂O): δ = 89.85 (OCH-OCH-CH₂-Si), 89.61 (OCH-OCH-CH₂-Si), 58.90 (Si-O-CH₂-CH₃), 55.77 (tacn), 55.70 (tacn), 50.72 (tacn), 49.75(tacn), 46.39(tacn), 45.78 (tacn), 19.60 (OCH-OCH-CH₂-Si), 18.27 ppm (Si-O-CH₂-CH₃).

²⁹Si NMR (99.36 MHz, D_2O): $\delta = -41.16$ ppm

1.5 Grafting of 5-(bicycloheptenyl)triethoxysilane at MCM-41 (Sinorb@MCM-41)

Method a:

MCM-41 particles (50 mg) were dried by heating (140 °C) under high vacuum for 4 h.

MCM-41(50 mg), 5-(bicycloheptenyl)triethoxysilane (68.97 mg, 0.269 mmol), and dry toluene (7 ml) were mixed on a filter frit with a valve. Nitrogen gas was used to keep the suspension bubbling for 24 h. The white powder was then washed with toluene (2 x 6 ml) and dried under high vacuum resulting in Sinorb@MCM-41 (43 mg, 86 %).

Method b: MCM-41 particles (35 mg) were dried by heating (140 °C) under high vacuum for 4 h.

MCM-41 (35 mg), 5-(bicycloheptenyl)triethoxysilane (134.10 mg, 0.523 mmol), and dry toluene (3 ml) were placed in a vial, the resulting suspension was then sonicated for 3 h at 60°C. Filtration of the suspension yielded a white powder that was washed with toluene (3 x 1 ml) and dried under high vacuum resulting in Sinorb@MCM-41 (31.4 mg, 90 %).

Pore-size distribution:



Figure SI1: Pore-size distribution of MCM-41 (black line) and Sinorb@MCM-41 (blue line).

Studied Sinorb@MCM-41:

Synthesis	EA (%)	Silane density	Silane density
Method b	C 6.46, H 1.30	$0.54 \ \mu mol/m^2$	$0.33 \text{ molecules/nm}^2$

1.6 Labelling of Sinorb@MCM-41 with [^{99m}TcO₃(tacn)]⁺

To a suspension of Sinorb@ MCM-41 (10 mg) in a physiological NaCl solution (4 ml) was added the [99m TcO₃(tacn)]Cl solution (0.7 ml, 19.10 MBq). The suspension was sonicated for 2 h at 25 °C. After 0, 5, 10, 15, 20, 25, 30, 40, 50, 60, 90 and 120 minutes aliquots (0.1 ml) were collected and centrifuged separately (5000 rpm) for 8 minutes. An aliquot (10 µl) of each centrifuged sample was collected and the remaining [99m TcO₃(tacn)]Cl in solution was determined by the use of a gamma-counter.

Applied Sinorb@MCM-41:

Synthesis	EA (%)	Silane density	Silane density
Method a	C 4.15, H 1.04	$0.35 \ \mu mol/m^2$	$0.21 \text{ molecules/nm}^2$

2 Kinetic measurments

Kinetic measurements were performed on a Cary 50 UV/Vis spectrometer with solution samples in 1 cm quartz cells under pseudo first-order conditions (conc. Tc complex 0.00212 mol/l, conc. alkene 0.04 mol/l).



Figure SI2: Kinetic measurements of the reaction of $[1]^+$ with 5-(bicycloheptenyl)triethoxysilane.

3.1 [99TcO(norb)(tacn)]Br ([2]Br)

3 NMR Data



Figure SI3: ¹H NMR spectrum of complex [2]Br



Figure SI4: ¹³C NMR spectrum of complex [2]Br



Figure SI5: ¹³C NMR-DEPT spectrum of complex [2]Br





Figure SI6: 1D-NOE spectrum of complex [2]Br

3.2 [99TcO(norborneneSi(OEt)₃(tacn)]Cl ([3]Cl)



Figure SI8: ¹³C NMR-DEPT spectrum of complex [**3**]Cl. Due to the fact that the purchased 5-(bicycloheptenyl)triethoxysilane is a diasteromeric mixture (R,S isomers at the Si substituted carbon atom, ratio 2:1) the influenced ¹³C NMR signals are split into two sets.



Figure SI9: ¹³C,¹H-Correlation spectrum of complex [**3**]Cl.



Figure SI10: ¹H COSY spectrum of complex [**3**]Cl.



Figure SI11: ¹H ²⁹Si-Correlation spectrum of complex [**3**]Cl.



Figure SI12: 1D-NOE spectrum of complex [3]Cl.

3.3 [⁹⁹TcO(allyltriethoxysilane)(tacn)]Cl



Figure SI13: ¹H NMR spectrum of complex [⁹⁹TcO(allyltriethoxysilane)(tacn)]Cl.





Figure SI15: ¹³C NMR-DEPT spectrum of complex [⁹⁹TcO(allyltriethoxysilane)(tacn)]Cl.



Figure SI16: ¹³C ¹H-Correlation spectrum of complex [⁹⁹TcO(allyltriethoxysilane)(tacn)]Cl.



Figure SI17: ¹H ²⁹Si-Correlation spectrum of complex [⁹⁹TcO(allyltriethoxysilane)(tacn)]Cl.



Figure SI18: 1D-NOE spectrum of the syn-product of the complex [⁹⁹TcO(allyltriethoxyilane)-(tacn)]Cl.



Figure SI19: 1D-NOE spectrum of the *anti*-product of the complex [⁹⁹TcO(allyltriethoxyilane)-(tacn)]Cl.

4 Structural Details

Crystallographic data were collected at 183(2) K with Mo K α radiation ($\lambda = 0.7107$ Å) using a graphite monochromator on an Oxford Diffraction Xcalibur system with a Ruby detector. 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.⁴ More details on data collection and structure calculations are contained in table SI1. Structures were solved with direct methods using SIR97⁵ and were refined by full-matrix least-squares methods on F² with SHELXL-97. The refinements have been done with anisotropic thermal parameters for all non-hydrogen atoms. The positions of the hydrogen atoms (except hydrogen atoms of water molecules) have been calculated using the 'riding model' option of SHELXL97.⁶ Hydrogen atoms of water molecules have been assigned from the Fourier map and have been refined with fixed bond lengths and angles (1,3-atom restraints) (DFIX).

Table SI1: Data collection	n and structure calculation details.	
	$[^{99}$ TcO(norb)(tacn)]Br·2H ₂ O	
	[2]Br·2H ₂ O	
Formula	$C_{13}H_{29}BrN_3O_5Tc$	
$M_w / g mol^{-1}$	486.30	
Space group	ΡĪ	
Temperature / K	183	
a / Å	7.1578 (2)	
b / Å	11.8422 (4)	
<i>c</i> / Å	21.8142 (5)	
α/°	92.863 (2)	
β/°	91.351 (2)	
γ / \circ	105.704 (3)	
$1 / mm^{-1}$	3.09	
Z	4	
$\frac{2}{V}$ / Å ³	1776.49 (8)	
$\rho_{calc}/g \text{ cm}^{-3}$	1.815	
Crystal description	blue stick	
Θ range [°]	2.8 to 30.9	
0 1 1	-8<=h<=9,	
Index ranges	-15<=k<=15,	
C	-21<=l<=28	
Refl collected	21199	
Indep refl	8466 [R(int) = 0.052]	
Refl obs $I > 2\sigma(I)$	7008	
Compl to theta	99.9 % to 27.9°	
Max. and min. transm	0.949 and 0.318	
Restraints / param	12 / 439	
Goodness-of-fit on F ²	1.04	
Final diff ρ_{max} (e ⁻ /Å ³)	1.27 and -1.86	
R1 ^{a, c}	0.051	
wR2 ^{b, c}	0.134	
CCDC	968395	
$1 = F_0 - F_c / F_0 ; {}^{b}wR2 =$	$[w(F_0^2 - F_c^2)^2 / (wF_0^2)]^{1/2}$; ^c I > 2 sig	

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4.1 [99 TcO(norb)(tacn)]Br·2H₂O ([**2**]Br·2H₂O)

a



Bond lengths [Å] and angles [°] for [99 TcO(norb)(tacn)]Br·2H₂O.

Tc1-O1 1.663(3)	Tc2-O4 1.669(3)
Tc1-O2 1.907(3)	Tc2-O5 1.927(3)
Tc1-O3 1.922(3)	Tc2-O6 1.926(3)
Tc1-N1 2.282(4)	Tc2-N4 2.269(4)
Tc1-N2 2.174(4)	Tc2-N5 2.173(4)
Tc1-N3 2.192(4)	Tc2-N6 2.161(4)
O2-C10 1.439(6)	O5-C31 1.436(6)
O3-C11 1.442(5)	O6-C30 1.434(6)
C10-C11 1.546(7)	C30-C31 1.550(6)
C11-C12 1.550(7)	C31-C32 1.534(7)
C12-C13 1.536(7)	C32-C33 1.544(8)
C13-C14 1.556(8)	C33-C34 1.556(7)
C14-C15 1.541(7)	C34-C35 1.529(7)
C15-C10 1.531(7)	C35-C30 1.547(7)
C15-C16 1.539(7)	C35-C36 1.547(7)
C16-C12 1.530(7)	C36-C32 1.530(7)
O1-Tc1-O2 110.74(16)	O4-Tc2-O5 110.45(16)
O1-Tc1-O3 109.11(16)	O4-Tc2-O6 109.44(17)
O2-Tc1-O3 82.90(14)	O5-Tc2-O6 82.34(14)
N1-Tc1-N2 75.05(14)	N4-Tc2-N5 75.18(14)
N1-Tc1-N3 74.95(14)	N4-Tc2-N6 74.67(15)
N2-Tc1-N3 79.42(15)	N5-Tc2-N6 79.67(15)

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