First dinuclear Re/Tc complex as a potential bimodal Optical/SPECT molecular imaging agent

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Experimental section

*Materials and equipment:* All purchased chemicals were of the highest purity commercially available and used without further purification. Analytical grade solvents were used and not further purified unless specified. Reactions were monitored by TLC on *Kieselgel* 60 F254 (Merck) on aluminium support under UV light (254 nm). Chromatographic purifications were conducted using "gravity" silica gel or neutral alumina obtained from Merck. Compound [Re(CO)3Cl(bipy)] was prepared according to literature protocols. 1 Di-tert-butyl-iminodiacetate (IDA-tBu) and Re(CO)5Cl were purchased from Aldrich Chem. Co. Na[99mTcO4] was eluted from a 99Mo/99mTc generator (Mallinckrodt Inc.) using a 0.9% saline solution.

Infrared spectra were recorded on a Perkin-Elmer FTIR 1725x spectrophotometer. Samples were prepared as KBr pellets (solid sample) or applied to NaCl plates (liquid sample). Selected characteristic absorption frequencies are reported in cm⁻¹. ¹H and ¹³C spectra were recorded on a Bruker Avance 300 MHz and on a Bruker Avance 500 MHz spectrometers; chemical shifts are indicated in δ values (ppm) downfield from internal TMS, and coupling constants (J) are given in Hertz (Hz). Multiplicities were recorded as s (singlet), d (doublet), t (triplet), q (quadruplet) and m (multiplet). Electrospray (ES) mass spectra were obtained on a Q TRAP Applied Biosystems spectrometer and High-Resolution Mass Spectra (HRMS) on a LCT Premier Waters spectrometer. DCl, NH₃ mass spectra were obtained on a DSQ II Thermo Fisher. The rhenium complexes were analyzed by RP-HPLC using a Waters Alliance 2695 system with a PDA 2996 detector and using a reverse-phase (RP) C₈ column: Phenomenex Luna C8(2), 5 µM, 100 Å, 150 x 4.6 mm with a flow rate of 1 mL/min. The analytical procedure was as follows: solvents were 10 mM pH 4 ammonium formate buffer (solvent A) and acetonitrile (solvent B); the compounds were analyzed using the HPLC gradient system beginning with a solvent composition of 90 % A:10 % B and following linear gradient up to 10 % A:90 % B from 0 to 18 min. HPLC purification step was performed using an autopurification system equipped of a Waters 2545 pump, a Waters 2767 injector-collector and a Waters 2998 PDA detector. Preparative HPLC was performed using a Phenomenex Luna C8(2), 5 µM, 100 Å, 150 x 10 mm with a flow rate of 5 mL/min and a gradient system beginning with a solvent composition of 90 % A:10 % B and following linear gradient up to 37 % A:63 % B from 0 to 11.5 min. RP-HPLC analysis and purification of ⁹⁹mTc-complex 6

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was carried out on a Nucleodur C18 endcapped RP column (Macherey Nagel analytical column 125 x 4 mm, 5 µm). The RP-HPLC conditions were as follows: flow rate was 1mL/min, eluents were 0.1% TFA in H₂O/MeOH 90/10 (Solvent A) and 0.1% TFA in H₂O/MeOH 10/90 (Solvent B) and gradient system was 0-15 min, 30% A to 62% B; 15-20 min, 62% B; 20-21 min, 62% B to 30% A. In all analytical and semi-preparative separation, the wavelength used for UV detection was 300 nm. Absorption measurements were done with a Hewlett Packard 8453 temperature-controlled spectrometer. Fluorescence spectra were obtained with a Cary Eclipse spectrofluorimeter with a Xenon flash lamp source and a Hamamatsu R928 photomultiplier tube. The measurements were carried out at pH 7.4 in Tris buffer (50 mM). Spectra were corrected for both the excitation light source variation and the emission spectral response. The fluorescence quantum yields for the rhenium complexes were determined by the method described by Haas and Stein, using as standard [Ru(bipy)₃]²⁺ in aerated water (Ø = 0.028). Unfortunately, with this spectrofluorimeter, we are not able to calculate the emission lifetimes of our rhenium(I) complexes which are too short (< 30 µs) to be estimated with that equipment.

**Synthesis of compounds 1-5.**

[Re(CO)₃(bipy)(ACN)][OTf], (1). According to a slight modification of the literature,¹ a solution of silver trifluoromethanesulphonate, AgOTf (256 mg, 1 mmol) in 5 mL of THF was added to a solution of [Re(CO)₃Cl(bipy)] (462 mg ; 1 mmol) in 50 mL of fresh distilled acetonitrile in the dark, under N₂, and the mixture was allowed to reflux overnight. The reaction mixture was filtered through a pad of Celite to remove any residual AgCl. All volatiles were removed under reduced pressure and the recrystallization was performed by layering Et₂O on a CHCl₃ solution of the crude product at -18°C yielding to the desired product 1 as a triflate salt. Yellow needles (465 mg; 75%). ¹H NMR δH (300 MHz, CDCl₃) 8.93 (ddd, J = 5.5 Hz, J = 1.6 Hz, J = 0.7 Hz, 2H, CH Ar), 8.67 (m, 2H, CH Ar), 8.28 (m, 2H, CHAr), 7.65 (ddd, J = 7.7 Hz, J = 5.5 Hz, J = 1.3 Hz, 2H Ar), 2.25 (s, 3H, CH₃CN). ¹³C NMR δC (75 MHz, CDCl₃) 195.7 (CO), 193.4 (CO), 189.9 (CO), 156.0 (C₃(bipy)), 153.0 (CHbipy), 141.2 (CHbipy), 128.1 (CHbipy), 125.6 (CHbipy), 123.7 (CF₃SO₃), 122.2 (CH₃-CN), 3.6 (CH₃-CN). MS DCI/NH₃ m/z 594.0 [M – CH₃-CN + NH₄]⁺. MS Calcd for C₁₄H₁₂F₃N₃O₆ReS 594.00.

4-PyrIDA-tBu, (2). To a solution of 4-(bromomethyl)pyridine hydrobromide (517 mg, 2.04 mmol) in 40 mL of fresh distilled acetonitrile were added potassium iodide (43 mg, 0.26 mmol) and potassium carbonate (1.41 g, 10.2 mmol). After stirring for 20 min at room temp., di-tertbutyl iminodiacetate (IDA-tBu) (510 mg, 2.08 mmol) was added and the reaction mixture was allowed to reflux overnight. The solvent was removed under reduce pressure and the residue was purified by column chromatography on silica gel (eluant: CH2Cl2/MeOH 98/2) to give the desired product as a light brown oil (305 mg; 45\%).

\[^1H\ \text{NMR}\ \delta_H (300\ \text{MHz, CDCl}_3) 8.56 (m, 2H, CHAr), 7.39 (m, 2H, CHAr), 3.94 (s, 2H, CH2), 3.43 (s, 4H, 2xCH2), 1.50 (s, 18H, CH3 tBu).\]

\[^{13}C\ \text{NMR}\ \delta_C (75\ \text{MHz, CDCl}_3) 170.3 (C=O), 149.6 (C_{pyr}), 148.5 (C_{q\ pyr}), 123.8 (C_{pyr}), 81.2 (C_{q\ tBu}), 56.6 (CH2), 55.3 (CH3), 28.2 (CH3).\]

MS ESI\+ m/z 337.2 [M+H]\+. MS Calcd for C18H28O4N2 336.2.

[Re(CO)\textsubscript{3}(bipy)(4-PyrIDA-tBu)][OTf], (3). To a solution of 2 (150 mg, 0.24 mmol) in 5 mL of distilled acetonitrile were added 1 (82 mg, 0.24 mmol) and NaHCO\textsubscript{3} (30 mg, 0.36 mmol). The reaction mixture was then allowed to reflux overnight. The solvent was removed under reduced pressure and the crude product was extracted with CH2Cl\textsubscript{2}, concentrated in vacuo, loaded on a silica column (eluant: CH2Cl2/MeOH 85/15) to obtain the desired product 3 as an orange oil (106 mg; 48\%).

\[^1H\ \text{NMR}\ \delta_H (300\ \text{MHz, CDCl}_3) 9.04 (dd, J = 5.4 Hz, J = 1 Hz, 2H, CHAr), 8.87 (d, J = 8.4 Hz, 2H, CHAr), 8.32 (t, J = 7.4 Hz, 2H, CHAr), 8.01 (d, J = 6.3 Hz, 2H, CHAr), 7.71 (dd, J = 7.4 Hz, J = 5.7 Hz, 2H, CHAr), 7.46 (d, J = 6.3 Hz, 2H, CHAr), 3.89 (s, 2H, CH2), 3.34 (s, 4H, 2xCH2), 1.41 (s, 18H, CH3 tBu).\]

\[^{19}F\ \text{NMR}\ \delta_F (300\ \text{MHz, CDCl}_3) -78.5.\]

\[^{13}C\ \text{NMR}\ \delta_C (75\ \text{MHz, CDCl}_3) 195.5-193.4 (CO), 170.1 (O=C-OR), 155.8 (C_{q\ Ar}), 154.4 (C_{q\ Ar}), 152.7 (C_{Ar}), 151.1 (C_{Ar}), 141.8 (C_{Ar}), 128.9 (C_{Ar}), 126.4 (C_{Ar}), 126.0 (C_{Ar}), 123.5 (CF3SO3), 81.6 (C_{q\ tBu}), 56.2 (CH2), 55.7 (CH2), 28.1 (CH3).\]

HRMS-DCI-CH4\+ m/z 763.2153 [M]+. Calcd for C31H36N4O7Re 763.2142.

[Re(CO)\textsubscript{3}(bipy)(4-PyrIDA)][OTf], (4). To a solution of 3 (89 mg ; 97.6 \mu mol) in CH2Cl\textsubscript{2} was added at 0 °C, trifluoroacetic acid (0.25 mL, 3.37 mmol) and the reaction mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure and the crude product redissolved in acetone to be evaporated again. This procedure was repeated three times. The residue was then washed with water (5x1 mL), sonicated, centrifugated and the supernatant was finally evaporated at reduce pressure to yield to a thick orange oil 4 (68 mg; 87\%).

\[^1H\ \text{NMR}\ \delta_H (300\ \text{MHz, D}_2O) 9.31 (d, J = 4.7 Hz, 2H, CHAr), 8.41 (d, J = 6.8 Hz,
$^{19}$F NMR $\delta_F$ (300 MHz, CDCl$_3$) - 78.8. $^{13}$C NMR $\delta_C$ (125 MHz, D$_2$O) 196.0-192.0 (CO), 169.5 (O=C-OH), 155.7 (C$_q$ Ar), 153.5 (C$_{Ar}$), 152.6 (C$_{Ar}$), 142.2 (C$_q$ Ar), 140.9 (C$_{Ar}$), 128.3 (C$_{Ar}$), 127.9 (C$_{Ar}$), 124.4 (C$_{Ar}$), 119.0 (CF$_3$SO$_3$), 55.7 (CH$_2$), 55.5 (CH$_2$). IR (NaCl/cm$^{-1}$): $\nu_{O-H}$ = 3439; $\nu_{C=O}$ (COOH) = 1693, $\nu$(CO) = 1893, 1916, 2027.

HRMS DCl CH$_4$ + m/z 649.0880 [M]. Calcd for C$_{23}$H$_{20}$N$_4$O$_7$Re m/z = 649.0862.

HPLC Rt = 7.14 min.

UV/Vis (Tris buffer, 50 mM, pH = 7.4) : $\lambda_{max}$ (ε, L.mol$^{-1}$.cm$^{-1}$) = 249 (12800), 263 (12400), 306 (8200), 319 (8500), 352 (2500).

Fluorescence (Tris buffer, 50 mM, pH = 7.4) $\lambda_{exc}$= 352 nm, $\lambda_{em}$ = 570 nm.

$^{[\text{Re(CO)}_3(bipy)]\{4-\text{PyrIDA}\text{Re(CO)}_3\}}$, (5). A methanolic solution of 4 (24 mg, 30 µmol), Re(CO)$_5$Cl (12 mg, 33 µmol) and Et$_3$N (13 µL, 180 µmol) was allowed to reflux 8 hours. The solvents were evaporated under reduce pressure and the crude product was purified by preparative HPLC following general procedure given in “Materials and equipment” part, to give 5 as a yellow oil (5 mg; 18%). $^1$H NMR $\delta_H$ (500 MHz, CD$_3$OD) 9.33 (d, J = 5.3 Hz, 2H, CH$_{Ar}$), 8.56 (d, J = 8.1 Hz, 2H, CH$_{Ar}$), 8.48 (d, J = 6.1 Hz, 2H, CH$_{Ar}$), 8.33 (m, 2H, CH$_{Ar}$), 7.86 (m, 2H), 7.59 (d, J = 6.3 Hz, 2H, CH$_{Ar}$), 4.35 (s, 2H, CH$_2$), 3.77-3.80 (ABsys, J = 15.5 Hz, 2H, CH$_2$), 3.15-3.18 (ABsys, J = 15.5 Hz, 2H, CH$_2$). $^{13}$C NMR $\delta_C$ (125 MHz, CD$_3$OD) 197.0 (CO), 195.1 (CO), 191.1 (CO), 180.3 (COOH), 156.5 (C$_q$ Ar), 153.0 (C$_{Ar}$), 152.2 (C$_{Ar}$), 145.6 (C$_q$ Ar), 141.1 (C$_{Ar}$), 129.5 (C$_{Ar}$), 128.7 (C$_{Ar}$), 124.6 (C$_{Ar}$), 68.8 (CH$_2$), 62.8 (CH$_2$). IR (NaCl/cm$^{-1}$): $\nu_{C=O}$ (COORe) = 1642, $\nu$(CO) = 1884-2021.

HRMS-ESI$^+$ m/z 938.9993 [M + Na]. Calcd for C$_{26}$H$_{18}$N$_4$O$_{10}$NaRe$_2$ m/z = 938.9980.

HPLC Rt = 10.49 min.

UV/Vis (Tris buffer, 50 mM, pH = 7.4) : $\lambda_{max}$ (ε, L. mol$^{-1}$.cm$^{-1}$) = 243 (9600), 262 (9200), 319 (5500), 305 (5900), 352 (1500).

Fluorescence (Tris buffer, 50 mM, pH = 7.4) $\lambda_{exc}$= 352 nm, $\lambda_{em}$ = 569 nm.

$^{99m}$Tc-labelling procedure.

$^{[\text{Re(CO)}_3(bipy)]\{4-\text{PyrIDA}\text{Tc(CO)}_3\}}$, (6). In a vial, 50 µL of the freshly prepared $^{[99m}$Tc(CO)$_3$(OH$_2$)$_3$$^+$ precursor$^3$ were added to 100 µL of 5 (2.22 mM) in MeOH/AcOH solution. The mixture was heated at 70°C for 30 min. After cooling down, the resulting radiocomplex was analyzed and purified by HPLC leading to one component 6 with good

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radiochemical purity (> 91%) with $R_t = 19.22$ min. In the same analytical conditions the di-
henium complex 5 was eluted at $R_t = 19.06$ min.

**Stability versus histidine.**

Histidine challenge experiment was carried out on the purified complex 6 using a large molar excess of histidine (1:250 molar ratio). In a borosilicated vial containing phosphate buffer (200 μL, 0.2 M, pH 7.2), water (100 μL) and purified technetium complex 6 (50 μL), an aliquot (50 μL) of a freshly prepared aqueous solution of histidine (10 mM) was added. The solution was stirred and incubated at 37°C for various time intervals (1, 2 and 6 h). Periodically incubate aliquots were removed and analysed by RP-HPLC.
$^1$H and $^{13}$C NMR spectra of compound 4
HPLC-UV and HRMS-DCI-CH$_4^+$ spectra of compound 4 (HPLC purity 94%)
$^1$H and $^{13}$C NMR spectra of compound 5
HPLC-UV and HRMS-ESI⁺ spectra of compound 5 (HPLC purity 97%)