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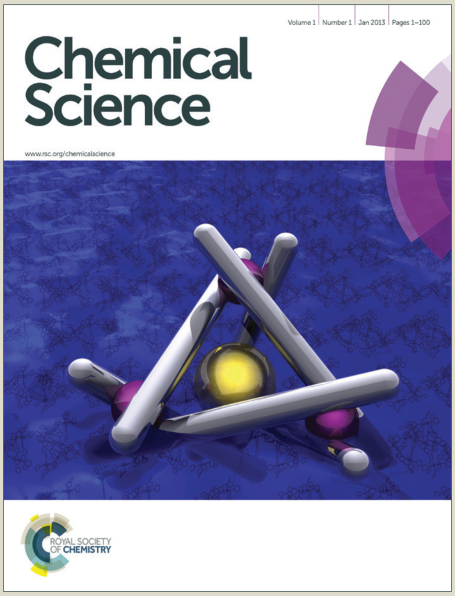

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ARTICLE TYPE

Fluorescent sensing of ^{99}Tc pertechnetate in water

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The selective binding of ^{99}Tc pertechnetate ($^{99}TcO_4^-$) in water is a big challenge. Recently, our group reported on the p-xylyl aza-cryptand, as the first molecular receptor for $^{99}TcO_4^-$ in aqueous solution. Here, we show that the introduction of a fluorescent unit in the azacryptand's framework leads to a new molecular system, able to selectively recognize and sense the pertechnetate anion at μM concentration. In water at pH 2, where the cage is in the hexaprotonated form, pertechnetate recognition is signalled by the quenching of the receptor's emission. Noticeably, environmentally ubiquitous anions, such as chloride, nitrate and sulphate do not interfere in the sensing, as they have no effect on our chemo-sensor's fluorescence. These unprecedented results are promising for the development of simple and smart devices for the recovery of $^{99}TcO_4^-$ from contaminated aqueous solutions.

Introduction.

Technetium is the lightest radioactive element of the periodic table, officially discovered by Carlo Perrier and Emilio Segrè in 1937 at the University of Palermo.[1] The element does not have stable isotopes, therefore technetium has to be produced synthetically.[2] Only very small amounts of this element are found in nature, generally occurring in uranium and molybdenum ores. However, technetium-99 (^{99}Tc) is a radioactive fission product (E_{max}=293 keV, $t_{1/2}$=2.1 × 10^5 years), produced in significant amounts in nuclear reactors. Consequently, ^{99}Tc is element of concern in the nuclear fuel cycle, particularly in waste management, due to its long half-life. The prominent form in which ^{99}Tc is handled at an industrial scale is pertechnetate ($^{99}TcO_4^-$). $^{99}TcO_4^-$ is separated from used nuclear fuel in a reprocessing method known as Plutonium-Uranium Extraction process (PUREX process). $^{99}TcO_4^-$ is a kinetically stable molecule but it has a hazard potential due to its high solubility in water (11.3molL^{-1} for the sodium salt, 20°C).[2]

After an accidental release, $^{99}TcO_4^-$ can spread into the environment and enter the food chain, due to its high solubility in water (high mobility). Because of the very long half-life, environmental contamination by ^{99}Tc may last thousands years.[1, 2] Unfortunately, due to the big size and the low charge density, the selective recognition of pertechnetate is hard to achieve in water.[3-6] Most receptors reported in the literature are based on neutral organic molecules, and work in pure organic solvent.[7-10] Gloe and other authors first considered protonated azacryptands for the perrhenate/pertechnetate extraction from water.[11] More recently, our group have reported on the high affinity of the hexaprotonated p-xylyl azacryptand for $^{99}TcO_4^-$ in water.[12] The azacryptand receptor consists of a macro-bicyclic polyamine, made of two bis-tren units linked by p-xylyl spacers. In aqueous solution, at pH 2.0, the secondary amino groups of the azacryptand are fully protonated. In this situation, the receptor's cavity is expanded and ready to selectively bind the anion. The affinity constant is over 5.5 logarithmic units, i.e. about two and three orders of magnitude higher than for perchlorate and nitrate, respectively. The outstanding affinity for pertechnetate is due to the complementarity between the receptor's cavity and the anion, as documented by the crystal structure of the 1:1 adduct.[12] The inclusion complex is indeed stabilized by multiple H-bonding interactions, involving the protonated amino groups and the oxo-anion within the cavity.

We have now improved the molecular system, by introducing a fluorescent signalling unit in the receptor's skeleton. Fluorescence is indeed particularly attractive in anion recognition, due to the simplicity and high detection limit.[13-16] Of course, the structural modification must not affect the cavity, otherwise the affinity for pertechnetate would be compromised. This goal could be achieved by replacing one of the p-xylyl spacers with a properly chosen fluorescent fragment. The obtained system would be the first fluorescent chemo-sensor for $^{99}TcO_4^-$ in water.

Experimental Section

CAUTION! ^{99}Tc is a weak β-emitter. Although radiation from small amounts of material is completely shielded by glass walls of standard laboratory vessels, to avoid contamination all operations must be carried out in specially equipped laboratories.

Synthesis and characterization of azacryptand **1**, and of the inclusion complexes with perrhenate and pertechnetate are reported in the ESI. Experimental general procedures and details are also included in the ESI. All reagents were purchased form Aldrich/Fluka and used without further purification. $Na^{99}TcO_4$ (Oak Ridge) was purified by recrystallization from an H_2O_2 containing aqueous $Na^{99}TcO_4$ solution. Mass spectra were

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acquired on a Thermo-Finnigan ion-trap LCQ Advantage Max instrument equipped with an ESI source. NMR spectra were taken on a Bruker AVANCE 400 spectrometer (operating at 9.37 T, 400 MHz). UV/Vis spectra were run on a Varian Cary 100 SCAN spectrophotometer with quartz cuvettes of the appropriate path length (0.1-1 cm) at 25.0 ± 0.1 °C under inert conditions. Emission spectra were recorded on a Perkin Elmer LS 50B instrument. Fluorescence spectra at 77 K were measured using quartz sample tubes and a low-temperature luminescence accessory (Perkin- Elmer). ITC studies were performed using a VP-ITC instrument (MicroCal Inc.). FT-IR spectra were obtained with a Nicolet FT-IR iS10 Spectrometer (Nicolet, Madison, WI, USA) equipped with ATR (attenuated total reflectance) sampling accessory (Smart iTR with ZnSe plate) by co-adding 256 scans in the 4000–600 cm^{-1} range at 4 cm^{-1} resolution. X-Ray diffraction data of [$\mathbf{1}H_6(^{99}TcO_4)$] $(CF_3SO_3)_5{\cdot}7H_2O$ were collected at 183(2) K with Cu Kα radiation (λ = 1.54184 Å) on an Agilent SuperNova, Dual source, with an Atlas detector. A suitable crystal (~ 0.29 x 0.22 x 0.19 mm) was covered with the minimal amount of oil (Infineum V8512, formerly known as Paratone N), placed on a nylon loop that is mounted in a CrystalCap Magnetic™ (Hampton Research) and immediately transferred to the diffractometer. X-Ray diffraction data of [$\mathbf{1}H_6(ReO_4)$] $(CF_3SO_3)_5{\cdot}7H_2O$ were collected from a colorless prismatic crystal (~ 0.3 x 0.15 x 0.10 mm), by means of a Bruker-Axs CCD-based three circle diffractometer working at room temperature with graphite-monochromatized Mo-Kα X-radiation (λ = 0.7107 Å). Further information and crystal data are reported in the ESI. (CCDC 961776 and 961777).

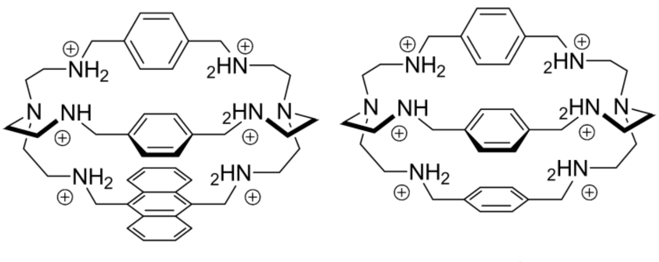
Results and Discussion

Receptor **1** was obtained by replacing one of the spacers of the p-xylyl azacryptand **2** with a 9,10-anthracenyl unit (see Fig. 1). We chose 9,10-anthracenyl as the new spacer, because it displays the same structural features as 1,4-xylyl (i.e. they have the same length). For the synthesis of **1**,† we modified the procedure reported in the literature by Fabbrizzi *et al.*[17] In particular, Fabbrizzi reported on the dizinc complex of **1** as a fluorescent sensor for linear anions in aqueous solution. Among investigated anions, the complex selectively recognizes isostructural N_3^- and NCO^-, because their bites (i.e. the distance between the terminal donor atoms) match the distance between the vacant apical sites of the metal centers in the receptor's cavity.

In this work, we apply the bistren cage **1** in the hexa-protonated form, for the fluorescent sensing of $^{99}TcO_4^-$ in water. First, we have investigated the acid-base properties of the free cage by potentiometric titration, in order to evaluate the distribution of the polyprotonated forms of the receptor, at varying pH values (see Table S1 and Fig. S1).† The obtained protonation constants are close to those reported for **2**,[18] showing that the replacement of one 1,4-xylyl with a 9,10-anthracenyl unit scarcely affects the basicity of the amino groups.

ITC studies.

Anion binding was then studied by ITC measurements[19-20] at pH 2.0 (0.1M CF_3SO_3Na), where the dominant species is $\mathbf{1}H_6^{6+}$, with all secondary amino groups protonated. The titrations were generally performed by adding a standard solution of the receptor (or anion) to the anion (or receptor) solution. In the case of nitrate, a competition-based method was required, according the procedure proposed by Z.-Y. Zhang *et al*.[21] Experimental details and curves are reported in the ESI. With all investigated anions, the ITC curves could be fitted by considering a single equilibrium in solution, leading to the formation of a stable 1:1 adduct.



$\mathbf{1}H_6^{6+}$ $\mathbf{2}H_6^{6+}$

Fig.1 Molecular cages $\mathbf{1}H_6^{6+}$ and $\mathbf{2}H_6^{6+}$.

Table 1. Thermodynamic parameters obtained by ITC measurements on $\mathbf{1}H_6^{6+}$. All titrations have been performed in aqueous solution 0.1M CF_3SO_3Na at pH 2, T =30°C. In parenthesis, standard deviation on the last figure. n.d.: not determined

M-Anion	logK_{11}	$\Delta H°$, Kcal mol^{-1}	$T\Delta S°$, Kcal mol^{-1}
$Na^{99}TcO_4$	5.49(1)	- 13.4(1)	- 5.8(1)
$NaReO_4$	5.20(2)	- 9.15(1)	- 1.9(1)
NH_4ReO_4	5.18(2)	- 9.30(1)	- 2.1(1)
$NaClO_4$	3.61(1)	- 6.50(1)	- 1.5(1)
$NaNO_3$	3.0(1)	- 1.7(1)	n.d.

As expected, the highest affinity is towards the $^{99}TcO_4^-$ anion (see the ITC curve in Fig. 2). The binding constants, as well as the trend of the thermodynamic parameters, are very similar to those of the p-xylyl cage, $\mathbf{2}H_6^{6+}$,[12] and affinity decreases along the series $^{99}TcO_4^- > ReO_4^- > ClO_4^- > NO_3^-$ (see Table 1). Noticeably, the affinity toward anions does not depend on the counter-ion, as demonstrated for ReO_4^-. Actually, the thermodynamic parameters are exactly the same for the sodium and ammonium salts. The interaction with both $^{99}TcO_4^-$ and ReO_4^- is dominated by a strongly exothermic enthalpy contribution, as expected in the formation of strong H-bonding interactions within the cavity. The fit of the anionic guest with the $\mathbf{1}H_6^{6+}$'s cavity can be inferred from the negative variation of entropy, which partially counterbalances the favourable enthalpy term.

X-Ray diffraction studies.

The formation of inclusion complexes with both $^{99}TcO_4^-$ and ReO_4^- has been definitely confirmed by X-ray diffraction studies. Single crystals suitable for X-ray analysis have been obtained by slow evaporation of an aqueous solution containing equimolar amounts of $NaReO_4$ and $\mathbf{1}H_6(CF_3SO_3)_6$.

In a similar way, slow evaporation of an aqueous solution of $\mathbf{1}H_6(CF_3SO_3)_6$, in the presence of $Na^{99}TcO_4$, produced single crystals of the inclusion complex with pertechnetate. Both crystals form as penta-triflate salt heptahydrate:

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$[\mathbf{1}H_6(ReO_4)](CF_3SO_3)_5{\cdot}7H_2O$ and $[\mathbf{1}H_6(^{99}TcO_4)]$ $(CF_3SO_3)_5{\cdot}7H_2O$ (see Table S2). In general, single crystals of both ReO_4^- and $^{99}TcO_4^-$ molecular complexes exhibited a poor X-ray diffraction quality, mainly related to an unresolved positional disorder affecting the triflate counterions.

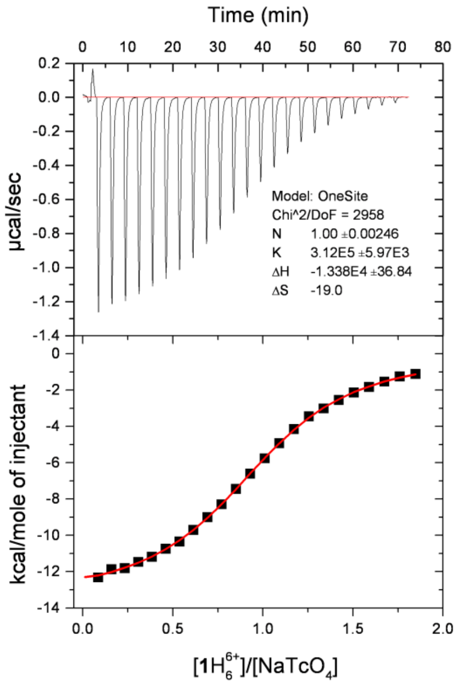

Fig. 2 ITC titration of 35µM $Na^{99}TcO_4$ with $\mathbf{1}H_6^{6+}$ at pH 2.0 (0.1M CF_3SO_3Na). Fitting (red curve, bottom figure) for ligand in the cell, one-site model.

Diffraction data for the ReO_4^- crystal (collected at room temperature with Mo-Kα X-radiation) produced better crystallographic results than those obtained from the $^{99}TcO_4^-$ crystal (collected at 183 K with Cu-Kα X-radiation, see ESI). However, the structural model of both hexaprotonated cages, with the enclosed oxo-anions and water molecules, were considered suitable for the aims of this work, also considering that the two crystal structures are isomorphous and with very similar geometrical features, due to the structural similarity of the two oxo-anions. Only the position of the O(7w) atom site occupied by a water oxygen differs between the two crystal structures (see ESI). In order to emphasize the isomorphism, the crystal structure of $^{99}TcO_4^-$ crystal has been described with a unit cell oriented as in the ReO_4^- crystal, resulting in a non-standard monoclinic cell with a β angle less than 90°.

The molecular structure of the cationic complexes $[\mathbf{1}H_6(^{99}TcO_4)]^{5+}$ and $[\mathbf{1}H_6(ReO_4)]^{5+}$ are shown in Figures 3 and S5, respectively. With respect to the corresponding $^{99}TcO_4^-$ and ReO_4^- complexes of $\mathbf{2}H_6^{6+}$,[12,18] the $\mathbf{1}H_6^{6+}$ cage exhibits C_s molecular symmetry, with a pseudo mirror plane passing through the long axis of the anthracenyl group and the centroid of the cage. Therefore, all protonated amino groups point towards the center of the cavity, and interact with the anion by both direct and water-mediated H-bonds. In particular, two oxygen atoms of the oxo-anions are directly H-bonded to the ammonium groups, whereas the other two involve water molecules to mediate the interactions. The multiple H-bonds are arranged in a very symmetrical fashion (see Table S3 for geometrical details). In the $[\mathbf{1}H_6(^{99}TcO_4)]^{5+}$ complex, the distance between the tertiary amino groups is 10.05(1) Å and the angle formed by the tertiary nitrogen atoms and Tc is 179.4(1)°, which puts the Tc atom on the axis connecting the two tertiary amines. The corresponding values for $[\mathbf{1}H_6(ReO_4)]^{5+}$ are 10.06(1) Å and 179.0(1)°. In both complexes, the distance between the Tc/Re atom and the centroid of the cage is 0.4 Å.

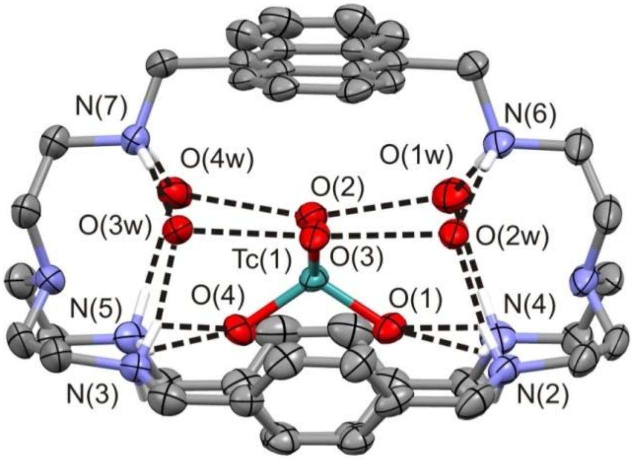

Fig. 3 Plot showing thermal ellipsoids of the $[\mathbf{1}H_6(^{99}TcO_4)]^{5+}$ molecular cation occurring in the $[\mathbf{1}H_6(^{99}TcO_4)](CF_3SO_3)_5{\cdot}7H_2O$ crystal. Ellipsoids are drawn at the 30% probability level, additional water solvent molecules and triflate counter-ions are omitted for clarity. Dashed lines indicate direct and water-mediated H-bonds between the hexa-protonated cage and the pertechnetate oxo-anion.

Noticeably, in the case of $\mathbf{2}H_6^{6+}$ the cavity was more contracted: the distance between the tertiary amines in $[\mathbf{2}H_6(^{99}TcO_4)]^{5+}$ was 9.82(1) Å and the N-Tc-N angle 172.9(1)°.[12] The corresponding values for the $[\mathbf{2}H_6(ReO_4)]^{5+}$ complex were 9.85(1) Å and 174.9(1)°.[18] Moreover, only five ammonium groups pointed towards the included oxo-anion, whereas the sixth group interacted with an external anion. However, the oxo-anions remained at the center of the cage, with distances between ^{99}Tc or Re and the centroid of the cage of 0.3(1) and 0.2(1) Å, respectively.

The higher symmetry observed for $[\mathbf{1}H_6(^{99}TcO_4)]^{5+}$ and $[\mathbf{1}H_6(ReO_4)]^{5+}$ might be attributed to the rigidity imparted by the anthracenyl unit. Interestingly, the O(2)-O(3) edge of the tetrahedral oxo-anion and the long axis of the anthracenyl group are almost parallel (see Fig. 3). Both O(2) and O(3) atoms of $^{99}TcO_4^-$ and ReO_4^- tetrahedra are placed below the middle of the two C-C bonds shared between the fused benzene rings of anthracene, and the resulting C-O distances are in the range 3.19(1)-3.30(1) Å. In particular, several values are below the sum of the van der Waals radii for C and O atom species: 3.22(1) Å.[22] These features suggest the formation of weak anion-π interactions[23] between the anthracenyl moiety of the cage and the O(2) and O(3) atoms of the enclosed oxo-anion (see ESI for details).

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NMR characterization.

The isolated complexes with $^{99}TcO_4^-$ and ReO_4^- have been fully characterized by NMR spectroscopy.† The interaction of $\mathbf{1}H_6^{6+}$ with ReO_4^- has been investigated by ^1H NMR titration in D_2O (0.1M CF_3SO_3Na, at pH 2.0). The main effect of anion binding is the down-field shift of the methylene protons belonging to the bis-tren unit, adjacent to the secondary ammonium groups (see Fig. S6).† This effect is consistent with the polarization of the methylene group exerted by the included oxo-anion. A similar polarization, although to a lesser degree, was observed in the ^1H NMR titrations of $\mathbf{1}H_6^{6+}$ with ClO_4^- and NO_3^-. For these anions, the fitting of the titration profiles (see Figures S7 and S8)† allowed the determination of the binding constants, reported in Table 2.

The $^{99}TcO_4^-$ complex was characterized by both ^1H and ^{99}Tc NMR spectroscopies. It should be noted, that the ^{99}Tc nucleus (I = 9/2) has a notable quadrupole moment, which can lead to line broadening.[24-25] However, line width is sensitive to the symmetry of electron distribution around the nucleus. In the case of the $^{99}TcO_4^-$ anion, the cubic electric field at ^{99}Tc results in a sharp signal, with $\Delta_{1/2}$ =2.7 Hz. The large signal ($\Delta_{1/2} \cong 500$ Hz), observed for a solution of $[\mathbf{1}H_6(^{99}TcO_4)](CF_3SO_3)_5$ in CD_3OD, is consistent with the distortion of the symmetry around the ^{99}Tc nucleus, due to anion inclusion in the cage's cavity (see the Supplementary characterization). †

UV/Vis studies.

Anion binding tendencies of $\mathbf{1}H_6^{6+}$ have been also studied by UV/Vis titrations with standard solutions of the chosen anions, as sodium salts, at pH 2.0 in 0.1M CF_3SO_3Na. The receptor displays the typical UV/Vis spectrum of 9,10-anthracenyl derivatives, with vibrational bands between 330 and 410 nm. Upon the formation of strongly H-bonded complexes (i.e. with $^{99}TcO_4^-$ or ReO_4^-), the vibrational bands of the anthracenyl fragment undergo a significant bathochromic shift. In these cases, the inclusion of the anion in the cavity alters the electronic dipole of the receptor's molecule, thus producing a shift in the spectrum. The presence of isosbestic points is consistent with a single equilibrium in solution, leading to the formation of a 1:1 adduct. Fig. 4 shows the variation of the UV/Vis bands of $\mathbf{1}H_6^{6+}$, between 340 and 480 nm, obtained upon titration with $Na^{99}TcO_4$ (for $NaReO_4$, see Fig. S9) .† For the $^{99}TcO_4^-$ anion, the fitting of the titration curve allowed the calculation of the binding constant: $\log K_{11}$ = 5.5(1) (Table 2). It should be noted that, in the range of concentrations typically used for UV/Vis studies (i.e. $[\mathbf{1}H_6^{6+}]$ = 0.01 – 0.3mM), no significant variation of the receptor's spectrum was observed upon titration with weakly interacting anions (i.e. ClO_4^-, NO_3^- and Cl^-).

Spectrofluorimetric studies.

At pH =2.0, in 0.1M CF_3SO_3Na, the receptor is fully emitting. When excited at 377 nm, the 10^{-5}M solution of $\mathbf{1}H_6^{6+}$ shows the typical anthracene emission with peaks at 406 and 425 nm, and shoulders at 448 and 480 nm.

As already reported by Fabbrizzi, the fluorescence emission of **1** is pH dependent.[17] In particular, the emission intensity is maximum in the 2<pH<4 interval, when the secondary amines are fully protonated. At pH>4, the deprotonation of the ammonium groups leads to the quenching of the anthracenyl unit by photo-induced electron transfer, from the free amine group to the excited anthracene.[26] Thus, a sigmoidal trend of the fluorescence intensity *vs.* pH is obtained. †

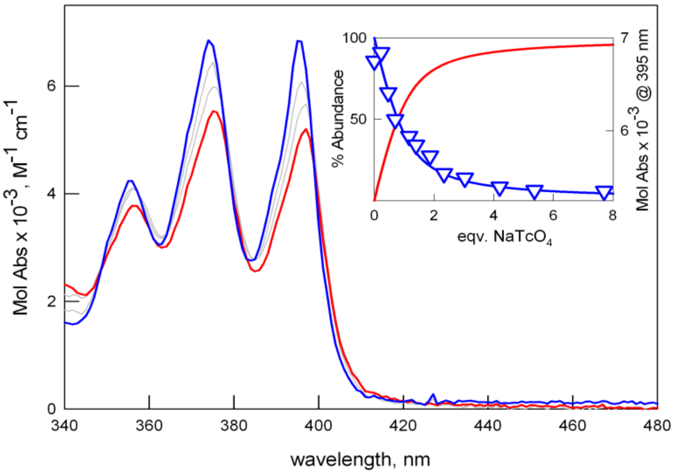

Fig. 4 UV/Vis spectra taken upon addition of $Na^{99}TcO_4$ to a solution of $\mathbf{1}H_6^{6+}$ (0.1 mM in in 0.1M CF_3SO_3Na, pH 2). Initial and final spectra, corresponding to $\mathbf{1}H_6^{6+}$ and $[\mathbf{1}H_6(TcO_4)]^{5+}$ species, are in blue and red, respectively. The inset shows the experimental titration curve (triangles), with the superimposed distribution diagram calculated for $\log K_{11}$ =5.5(1).

In order to verify the activity of $\mathbf{1}H_6^{6+}$ as a fluorescent sensor for $^{99}TcO_4^-$ in water, we performed a spectrofluorimetric titration on $\mathbf{1}H_6^{6+}$ at pH=2, with an aqueous solution of $Na^{99}TcO_4$. Fig. 6 shows the changes in the fluorescence, observed over the course of the titration. Quenching follows the formation of the 1:1 complex (i.e. static mechanism), as schematized in Fig. 5. The calculated equilibrium constant, $\log K_{11}$=5.55(1), is significantly close to that determined by ITC (see Table 1). The inset of Fig. 6 shows the corresponding distribution diagram, with the superimposed profile of I/I_0 at 425 nm vs. equivalents of $^{99}TcO_4^-$. Under these experimental conditions ($[\mathbf{1}H_6^{6+}]$ =10 μM), the receptor's emission is quenched to 75% of the initial intensity by a 5-fold higher concentration of sodium pertechnetate (i.e. 50 μM).

We repeated the titration with $^{99}TcO_4^-$ at a lower concentration of the receptor. The final result is that, upon a 10-fold decrease of $\mathbf{1}H_6^{6+}$'s concentration (i.e. from 10 to 1μM), the fluorophore emission is reduced to 40% in the presence of 10μM $^{99}TcO_4^-$. The fact that a simple molecule such as $\mathbf{1}H_6^{6+}$ can detect $^{99}TcO_4^-$ in low μM concentration demonstrates the potential of this sensor type. In the future, it can be expected that further developments will reduce the detection limit down to the Guidance Level of the World Health Organization for ^{99}Tc in drinking water (100 Bq L^{-1} = 1.61×10^{-3} μmol ^{99}Tc L^{-1}),[27, 28] thus generating an efficient procedure for the detection of low level $^{99}TcO_4^-$ contamination without the need of (expensive) radioanalytical instrumentation.

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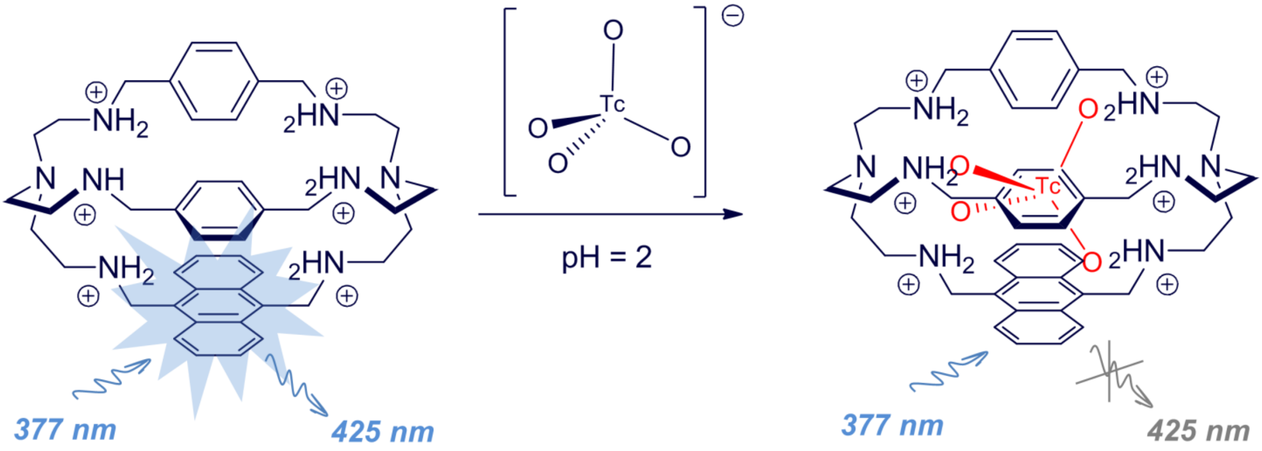

Fig. 5 The formation of an inclusion complex with $^{99}Tc(Re)O_4^-$ is accompanied by the quenching of $\mathbf{1}H_6^{6+}$'s fluorescence.

The corresponding constants (i.e. binding and Stern-Volmer) are reported in Table 2. It should be noted that, with other investigated halides (i.e. bromide and chloride), negligible effects on the receptor's emission have been observed.

Table 2. Binding constants obtained by (a) spectrofluorimetric, (b) ^1H NMR, and/or (c) UV/Vis titrations. All studies have been performed in aqueous solution 0.1M CF_3SO_3Na at pH 2, T =25°C. In parenthesis, standard deviation on the last figure. Further details are available in the ESI.

M-Anion	logK_{11}
$Na^{99}TcO_4$	5.55(1)[a], 5.5(1)[c]
$NaReO_4$	5.2(1)[a], >5[b], 5.3(1)[c]
$NaClO_4$	3.71(1)[b]
$NaNO_3$	3.2(1)[b]
NaCl	< 2[a, b]
NaBr	< 2[a]
NaI	2.57(1)[a] [logK_{SV}=2.0(1)][a]

Anion sensing selectivity.

An important issue for anion sensing is selectivity. Thus, we titrated $\mathbf{1}H_6^{6+}$ with potentially competing anions, under the same experimental conditions as for pertechnetate. Very interestingly, among the competitors studied in this work, only ReO_4^- and I^- quench the receptor's emission.

In the case of iodide, the upward curvature of the Stern-Volmer profile (see Fig. S10)[†] points out the existence of two different pathways: (i) static quenching, corresponding to receptor:anion association, and (ii) dynamic quenching, due to occasional collisions.[29]

In Fig. 7, we compare the experimental profile of I/I_0 at 425 nm vs. equivalents of $^{99}TcO_4^-$, with the curves obtained under the same conditions with several other oxo-anions (i.e. X = ReO_4^-, ClO_4^-, NO_3^-, HSO_4^-) and iodide. As anticipated, in only $^{99}TcO_4^-$ and ReO_4^- significantly quench the anthracene fluorescence; whereas, for ClO_4^-, NO_3^- and HSO_4^-, no decrease of the emission intensity is observed. In the case of ReO_4^-, quenching follows the formation of the 1:1 complex, in a similar but smoother way than observed for $^{99}TcO_4^-$. Very interestingly, environmentally ubiquitous anionic species such as chloride, nitrate and sulphate, even if in large excess, have little effect on the fluorescence of $\mathbf{1}H_6^{6+}$. In particular, a 1000-fold molar excess of chloride switches the fluorescence off by less than 10%. This result is very important, in the view of applying receptor $\mathbf{1}H_6^{6+}$ as a fluorescent sensor for ^{99}Tc pertechnetate in contaminated water.

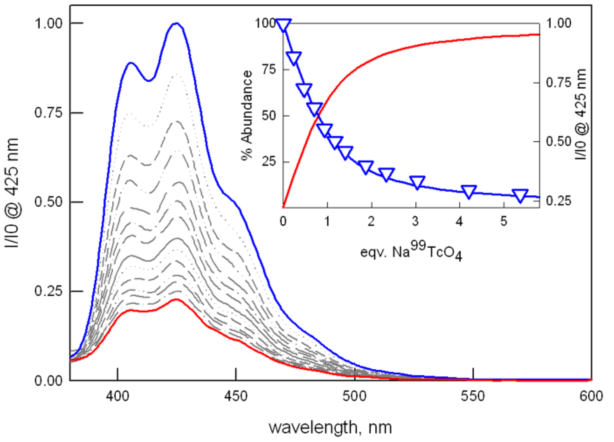

Fig. 6 Spectrofluorimetric titration of $\mathbf{1}H_6^{6+}$ (10µM, 0.1M CF_3SO_3Na, pH = 2.0) with $Na^{99}TcO_4$ at 25°C. $\mathbf{1}H_6^{6+}$ and $[\mathbf{1}H_6(^{99}TcO_4)]^{5+}$ species correspond to blue and red lines, respectively. Inset: distribution diagram with superimposed profile of the normalized emission intensity (*I/I₀*) at 425 nm *vs*. number of equivalents of added anion.

The effect of competing anions was further investigated by performing spectrofluorimetric titrations of $\mathbf{1}H_6^{6+}$ with ReO_4^- (model anion for $^{99}TcO_4^-$), in the presence of a large excess (500 eqv.) of the chosen competitor, X^- (X^-: Cl^-, ClO_4^-, NO_3^-). The obtained titration profiles, as expected, showed a smoother curvature than in the absence of competitors. The curves were fitted with the equation reported in ESI, assuming for ReO_4^- binding, the affinity constant value determined by titration in the absence of X^- (i.e. 5.2(1) logarithmic units, see Table 2). Very interestingly, the binding constants (logK_{11}< 2 for Cl^-; 3.6(1) for

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ClO_4^-; 2.9(1) for NO_3^-) determined in the competition experiments are very close to those determined by ^{1}H-NMR, and shown in Table 2.

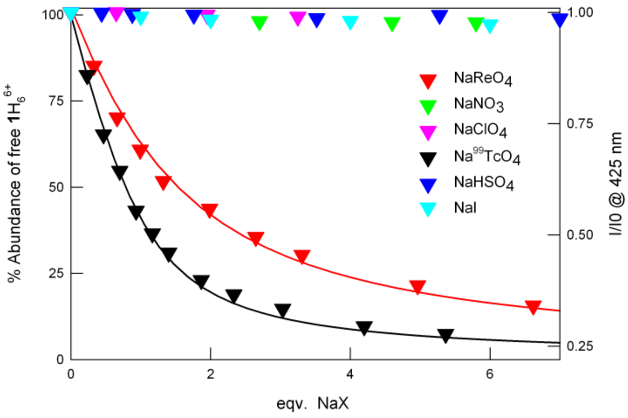

Fig. 7 Plots of I/I_0 at 425 nm *vs.* eqv. of added NaX ([$1H_6^{6+}$] = 10μM). In the case of $^{99}TcO_4^-$ and ReO_4^-, the experimental plots are superimposed to the calculated curves of % Abundance of free receptor vs. eqv. of anion, obtained by considering logK_{11} 5.55 and 5.2, for $^{99}TcO_4^-$ and ReO_4^-, respectively.

Quenching mechanism.

For a better understanding of the quenching mechanism, we performed spectrofluorimetric studies in methanol at the liquid nitrogen temperature, using ReO_4^- as a model (see Fig. S11).† These studies demonstrated the photoinduced electron transfer (eT) nature of quenching. Indeed, the eT process involves charge separation, and, by consequence, the reorganization of solvent molecules, which is of course prevented in vitrified solution. In the presence of excess $NaReO_4$, the vitrified sample of $\mathbf{1}H_6^{6+}$ displays the typical, intense, and well-defined emission spectrum of anthracene, thus proving the occurrence of a photoinduced eT process at room temperature, in liquid solution.

For the eT mechanism, two possible pathways may be hypothesized. First of all, ReO_4^- is known to be a weak oxidant, as well as $^{99}TcO_4^-$.[30] Being kinetically more reactive than ClO_4^-, when bound to our receptor, ReO_4^- (or $^{99}TcO_4^-$) might accept an electron from the nearby excited anthracene, thus deactivating the excited state by intra-complex eT (i.e. oxidative quenching). However, also an intra-molecular deactivation should be considered. In fact, the participation of the ammonium groups to strong H-bonding interactions, such as with ReO_4^- or $^{99}TcO_4^-$, might destabilize the orbital energy level of the protonated amines, enough to promote an intra-molecular eT to the nearby anthracenyl unit. It should be noted that, both ReO_4^- and $^{99}TcO_4^-$ are stronger bases with respect to ClO_4^- and NO_3^-, by consequence they can establish stronger H-bonding interactions with the receptor.

According to the latter mechanism, the Re (or ^{99}Tc) centre plays an auxiliary role, as the oxidative quenching involves, in first instance, the amino groups and anthracene. In the view of applying receptor **1** for the detection/extraction of $^{99}TcO_4^-$ in/from water, we have qualitatively tested the receptor's binding capability, when adsorbed on paper. Paper strips were dipped in a solution of **1** in dichloromethane, dried and treated with an acidic aqueous solution at pH 2 (0.1M CF_3SO_3Na). After drying, they were treated with different anions in water and examined under an UV lamp (λ_{exc} = 250 nm, Fig. 8). Significantly, the pale blue fluorescence of the strips turned dark only in the case of $NaReO_4$, used as non-radioactive surrogate for $^{99}TcO_4^-$. The colour change corresponds to the quenching of $\mathbf{1}H_6^{6+}$'s emission.

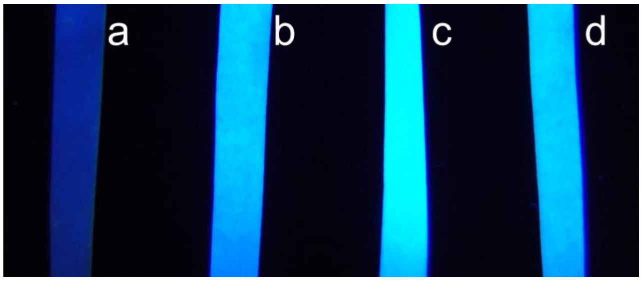

Fig. 8 Picture of paper strips containing the adsorbed receptor $\mathbf{1}H_6^{6+}$ (blank, c), after treatment with aqueous solutions of $NaReO_4$ (0.1mM; a), $NaClO_4$ (10mM; b), and NaCl (10mM; d). The picture was taken in the dark on UV illumination (250 nm).

Conclusions

The aza-cryptand $\mathbf{1}H_6^{6+}$ is the first fluorescent chemo-sensor for ^{99}Tc pertechnetate. In water, at pH = 2, the cage is fully fluorescent, and displays the typical emission spectrum of anthracene. Under these conditions, upon addition of pertechnetate, the fluorescence of $\mathbf{1}H_6^{6+}$ is switched off, following the formation of a stable 1:1 inclusion complex. The high affinity for pertechnetate in water is due to the stabilization of the inclusion complex by multiple strong H-bonding interactions with the protonated amino groups in the cavity. Among the investigated anions, only perrhenate and iodide may compete for the sensing; whereas environmentally ubiquitous anions, such as chloride, sulphate and nitrate, have no effect on the receptor's emission. This result is very important, (i) in the perspective of applying $\mathbf{1}H_6^{6+}$ for pertechnetate sensing in contaminated aqueous samples, and (ii) in the view of developing simple and smart devices for the detection/recovery of ^{99}Tc from acidic aqueous solutions (e.g. the ^{99}Mo/^{99m}Tc generator eluate, in nuclear medicine laboratories, and nuclear waste reprocessing solutions). There are now some future challenges to consider, for instance the development of OFF-ON type chemo-sensors, based on the 'switching on' of the signal upon pertechnetate binding. We are also evaluating new approaches to generate materials and devices for both sensing and extraction of the radioactive pollutant, by immobilizing chemo-sensors on solid supports.[31]

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† Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/b000000x/

‡ Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

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