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Molecular Receptors

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$^{99}TcO_4^-$: Selective Recognition and Trapping in Aqueous Solution**

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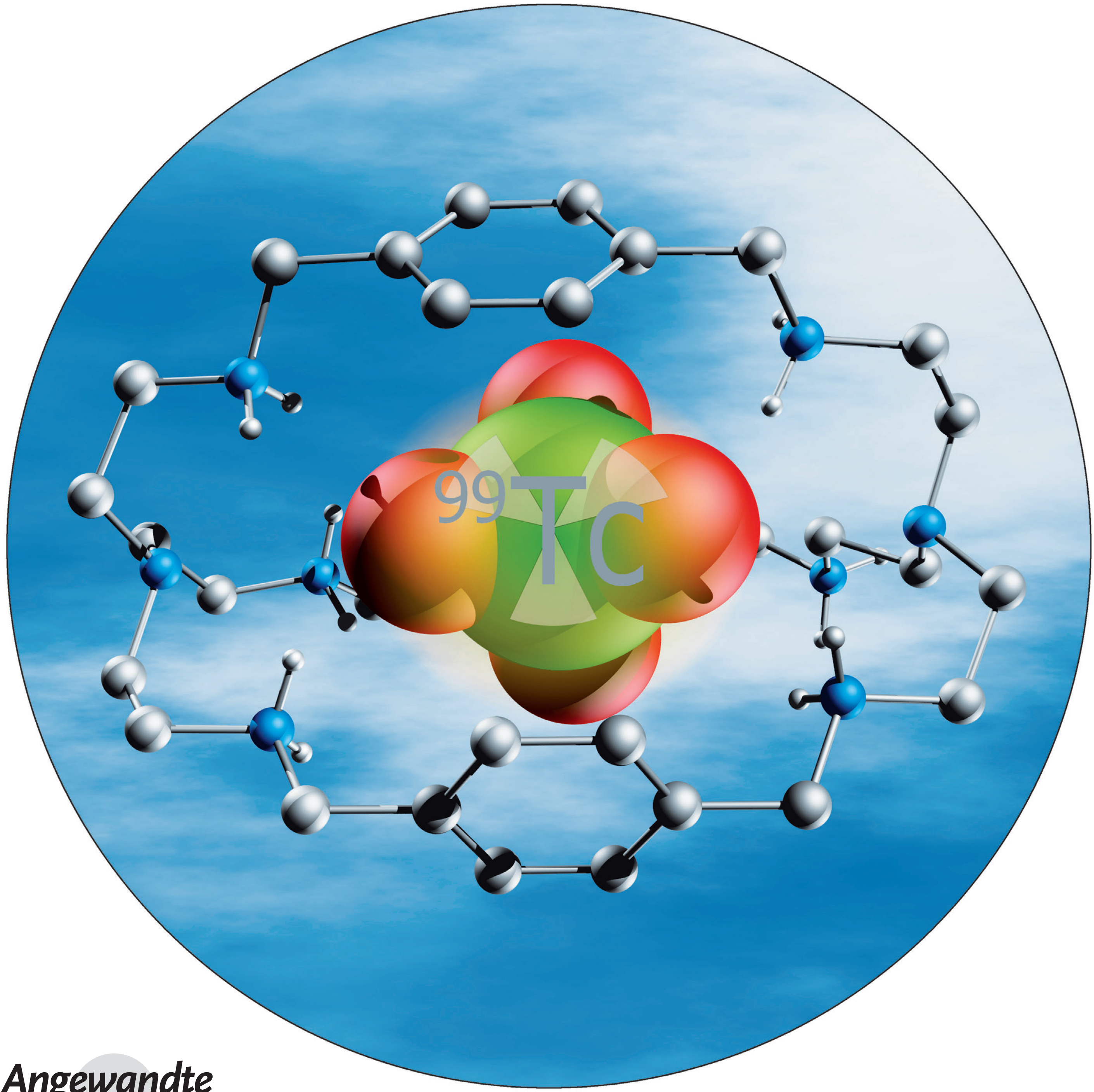
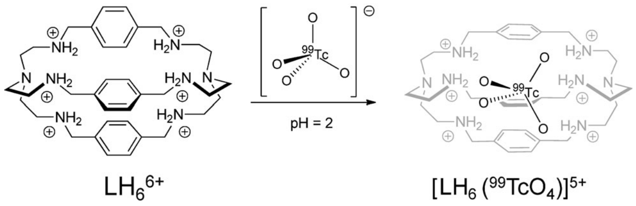


The synthesis of selective molecular receptors[1] and sensors for hazardous and radioactive materials is of great interest. Among potential pollutants, the radioactive anion $^{99}TcO_4^-$ from nuclear waste is especially noteworthy.[2,3] The long-lived isotope ^{99}Tc represents 6 % of the total fission product yield.[4,5] It is of particular importance in nuclear waste management, because the weak β-emitter ($E_{max} = 293$ keV, $t_{1/2} = 2.1 \times 10^5$ years), together with the long-lived isotope ^{129}I ($E_{max} = 194$ keV, $t_{1/2} = 15.7 \times 10^6$ years), dominates the radioactivity of spent fuel for thousands of years. Because of high solubility in water ($11.3\,mol\,L^{-1}$ for the sodium salt, 20 °C), $^{99}TcO_4^-$ easily migrates within the earth's crust and enters the food chain.[6] The large size and low charge density of the oxoanion make selective recognition in aqueous solution a great challenge.[7] Until now, most of the studies on TcO_4^- recognition have been done in pure organic solvents or in water mixtures.[2,3] Because of the governmental restrictions associated with $^{99}TcO_4^-$, ReO_4^- is often used as a structural surrogate,[2,8] even if the chemical analogy is not perfect. As a matter of fact, many of the molecular recognition systems that have been described as potential receptors for $^{99}TcO_4^-$ were actually never tested on this anion. Steps forward on $^{99}TcO_4^-$ recognition were made by Sessler, Katayev, and coworkers, although again not in aqueous solution.[9–11] Recently, our group investigated the binding propensities of poly-protonated azacryptands towards ReO_4^- in water.[12] These studies revealed the receptor LH_6^{6+} to have the most suitable cavity for incorporation of the anion. The selectivity of LH_6^{6+} for ReO_4^- was demonstrated by comparing the affinity constant with those obtained with several other anions (such as chloride and nitrate).[12]

The relevance of $^{99}TcO_4^-$ in the environmental field and the lack of effective receptors for it in aqueous solutions, encouraged us to apply receptor LH_6^{6+} to $^{99}TcO_4^-$ recognition. Receptor L was synthesized as previously described.[13] ITC titrations[14] were performed by addition of L to $NH_4{}^{99}TcO_4$ at pH 2.0 ± 0.1 (0.1 M CF_3SO_3Na, $T = 30$ °C). Under these conditions, the receptor is in the hexaprotonated form, LH_6^{6+}, which is the most suitable for anion binding.[12]

ITC experiments pointed to the formation of a stable 1:1 adduct, according to Scheme 1.[15] The corresponding affinity constant displayed a $\log K_{11} = 5.50(1)$ (see Table 1). To our knowledge, this is the highest value ever reported for $^{99}TcO_4^-$ binding to a receptor in aqueous solution,[2,3] and it is also the highest affinity for LH_6^{6+} with the anions that have been investigated.[12] Table 1 also shows the ITC results from the titration of NH_4ReO_4 with LH_6^{6+} (see ITC curves in Figure S1 of the Supporting Information). Notably, under the same experimental conditions, no interaction was found between ReO_4^- and azacryptands containing different spacers (such as *m*-xylyl).[12] In comparison to $^{99}TcO_4^-$, the affinity constant with ReO_4^- was about 0.3 log units lower. For both anions, binding is mainly driven by the significantly negative enthalpy, as is expected for strong H-bonding interactions. The lower hydration energy of TcO_4^- ($\Delta G_{hydr} = -251\,kJ\,mol^{-1}$ for TcO_4^-; $\Delta G_{hydr} = -330\,kJ\,mol^{-1}$ for ReO_4^-),[16] because of the lower partial negative charge of the O atoms (−0.739 for TcO_4^-; −0.755 for ReO_4^-), made the desolvation of TcO_4^- energetically cheaper, thus favoring complex formation.[7] The enthalpic term was partially counterbalanced by the negative entropy found for both, $^{99}TcO_4^-$ and ReO_4^-. The negative entropic contribution can be attributed to the formation of an anion inclusion complex.[17] It should be noted that the thermodynamic parameters obtained using NH_4ReO_4 were very close to those previously reported for $NaReO_4$.[12] This indicated that the counter ion has little influence on ReO_4^- binding.



Scheme 1. LH_6^{6+} interacts with $^{99}TcO_4^-$ in aqueous solution (pH 2.0). This interaction leads to a 1:1 adduct, with the anion included in the cavity of the receptor.

Table 1: Thermodynamic parameters obtained by ITC titrations.

Anion	$\log K_{11}$	ΔH [kcal mol^{-1}]	$T\Delta S$ [kcal mol^{-1}]	ΔG [kcal mol^{-1}]	*n*
$^{99}TcO_4^-$	5.50(1)	−11.0(1)	−3.35	−7.63(1)	1.0
ReO_4^-	5.22(1)	−10.7(1)	−3.50	−7.24(1)	1.0
NO_3^-[a]	3.41(1)	−3.36(1)	+1.36(1)	−4.72(1)	0.9
Cl^-[a]	2.25(1)	−1.38(1)	+1.76	−3.14(1)	0.9

[a] see ref. [12]. *n* = experimental coefficient. Fitting for a ligand in the cell: one-site model. In parenthesis, is the standard deviation of the last figure.

The binding of $^{99}TcO_4^-$ to LH_6^{6+} was also followed by ^1H NMR spectroscopy (in D_2O, pD 2.0, 0.1 M CF_3SO_3Na). As already measured for other anions,[12] $^{99}TcO_4^-$ promotes the downfield shift of the methylene protons of the bistren units. In particular, for the protons H_b (Figure S2), the variation of the chemical shift upon anion complexation is + 0.16 ppm (as was found for ReO_4^-). The downfield shift may be interpreted as an anion induced polarization effect on the methylene C–H bonds. On the other hand, the moderate upfield shift of the protons belonging to the spacer unit (that is the *p*-xylyl group) may be attributed to the shielding effect exerted by the negative charge of the anion.

Further insights into the interaction between LH_6^{6+} and $^{99}TcO_4^-$ were given by ^{99}Tc NMR spectroscopy. Because of its high sensitivity, the ^{99}Tc nuclide ($I = 9/2$) is extremely convenient for NMR spectroscopy.[19–22] $^{99}TcO_4^-$ is generally

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used as the reference in ^{99}Tc NMR spectroscopy, because of its narrow line width ($\Delta\nu_{1/2} = 2.7$ Hz),[21] resulting from a cubic electric field. Line widths of ^{99}Tc are mainly due to quadrupolar relaxation, and depend on the symmetry of electron distribution around the nucleus. In particular, the more asymmetric the distribution, the shorter the relaxation time (T_1) and the broader the line.[21] Thus, line widths represent a sensitive method to obtain structural information on ^{99}Tc compounds. We recorded the ^{99}Tc NMR spectra of solutions containing both LH_6^{6+} and $NH_4{}^{99}TcO_4$ (in D_2O, pD 2.0, 0.1 M CF_3SO_3Na), at different [$^{99}TcO_4^-$]/[LH_6^{6+}] molar ratios (see Table 2, Figure 1 and Figure S3).

Table 2: ^{99}Tc NMR spectroscopy experiment ($T = 25$ °C).

[TcO_4^-]/[LH_6^{6+}]	% ($^{99}TcO_4^-$)$_{free}$	% [$LH_6(^{99}TcO_4)$]$^{5+}$[a]	$\Delta\nu_{1/2}$ [Hz][b]
[c]	100	[c]	2.7
22.2	95.5	4.5	30
4.4	77.5	22.5	110
2.3	55.0	45.0	225
1.0	4.0	96.0	460
0.52	0.4	99.6	460
0.07	0.2	99.8	460

[a] The percentage of both bound and free $^{99}TcO_4^-$ were determined for $\log K_{11} = 5.50(1)$ by Hyss software (Hyperquad package)[18] [b] ±10 Hz, standard deviation; [c] see ref. [20]. The complete set of spectra are shown in Figure S3.

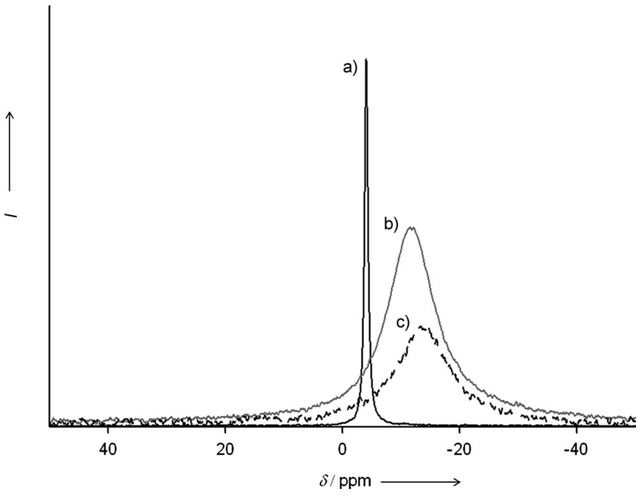

Figure 1. ^{99}Tc-NMR spectra, taken on samples containing both LH_6^{6+} and $NH_4{}^{99}TcO_4$. Spectrum a) [$^{99}TcO_4^-$]/[LH_6^{6+}] = 22.2; b) [$^{99}TcO_4^-$]/[LH_6^{6+}] = 1.0; c) [$^{99}TcO_4^-$]/[LH_6^{6+}] = 0.52.

The $^{99}TcO_4^-$ signal is very sensitive to the interaction with LH_6^{6+}, and the line broadens with the first additions of the receptor. When all the $^{99}TcO_4^-$ is bound to LH_6^{6+} (that is [$^{99}TcO_4^-$]/[LH_6^{6+}] = 1.0), $\Delta\nu_{1/2}$ reaches a limit of 460 ± 10 Hz. In addition, formation of the 1:1 adduct is accompanied by a moderate up-field shift of the ^{99}Tc signal (Figure S3). Both line shift and broadening are due to the H-bonding interactions of $^{99}TcO_4^-$ with the cavity of the receptor. An important contribution to line widths in NMR spectroscopy is given by the exchange equilibrium rate. For the interaction of $^{99}TcO_4^-$ with receptor LH_6^{6+}, the variation of $\Delta\nu_{1/2}$ from the free $^{99}TcO_4^-$ anion (2.7 Hz) to the 1:1 adduct [$LH_6(^{99}TcO_4)$]$^{5+}$ (460 Hz) may depend on both contributions (that is exchange rate and electrical field symmetry).

The formation of an inclusion complex was confirmed by X-ray diffraction studies of the adduct [$LH_6(TcO_4)$](TcO_4)(CF_3SO_3)$_4$·8H_2O. Crystal data are reported in Tables S1–S2. Interestingly, the crystal structure shows two distinct $^{99}TcO_4^-$ anions, one inside the cavity, the other outside (Figure 2).

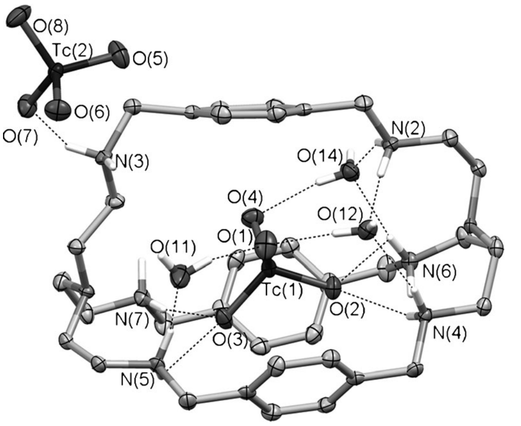

Figure 2. Simplified view of the molecular structure of [$LH_6(TcO_4)$](TcO_4)(CF_3SO_3)$_4$·8H_2O in the crystal. Only $^{99}TcO_4^-$ anions and the water molecules involved in the interaction with the receptor are shown. Atoms are drawn as ORTEP ellipsoids (50% probability level).

Both anions form H-bond interactions with the protonated amino groups of LH_6^{6+} (Table S1). Because of the similarity between $^{99}TcO_4^-$ and ReO_4^- (d(Tc–O) = 1.71 Å; d(Re–O) = 1.72–1.73 Å),[23] the structural features of the inclusion complex [$LH_6(TcO_4)$]$^{5+}$ are close to those previously reported.[12] In particular, the encapsulated anion forms the same number of direct and water-mediated H-bonds as the ReO_4^- complex. Moreover, the distance between the bistren tertiary amines is identical in the two adducts, d(N–N) = 9.8 Å. $^{99}TcO_4^-$ is almost at the center of the cavity, with the Tc atom displaced by only 0.3 Å from the centroid of the cage (0.2 Å for Re).[12]

The X-ray diffraction structures show that both anions fit well into the cavity; therefore, the selectivity of the receptor for $^{99}TcO_4^-$ can be attributed to thermodynamic effects, namely the favorable enthalpic term owing to the lower hydration energy of $^{99}TcO_4^-$.[7] In the ^{1}H NMR spectrum of the crystals dissolved in D_2O (Figure S7), the chemical shifts were close to those of the 1:1 inclusion complex (Figure S2).This indicated that the chemical shifts of the protons on the receptor are mainly affected by the caged anion.

Interestingly, the ^{99}Tc NMR spectrum of the 1:1 adduct (black line in Figure S4) showed two broad signals of different intensities and line widths. This result confirmed the presence of two non-equivalent ^{99}Tc nuclei, as already observed in the crystal structure of the adduct. The broadness of the signals indicated that both $^{99}TcO_4^-$ anions interact with the receptor. The stronger and sharper signal ($\Delta\nu_{1/2} = 252$ Hz) was assigned to the $^{99}TcO_4^-$ anion interacting externally with LH_6^{6+}. The broader and weaker signal (almost hidden behind the first signal) was assigned to the caged $^{99}TcO_4^-$. In fact, both signal positions (approximately −10 ppm versus the free $^{99}TcO_4^-$) and line widths correspond to the ^{99}Tc NMR signal seen for the in situ prepared 1:1 adduct (Table 2, $[^{99}TcO_4^-]/[LH_6^{6+}] \leq 1.0$), as confirmed by the superposition of the corresponding ^{99}Tc NMR spectra (see Figure S4).

In conclusion, receptor LH_6^{6+} is the first molecular receptor to effectively trap $^{99}TcO_4^-$ in aqueous solution. The encapsulation of $^{99}TcO_4^-$ in the cavity of the receptor was demonstrated by the crystal structure of the adduct. The large number of H-bond interactions involving the caged anion are consistent with the large binding constant and the favorable enthalpy measured in solution. These results give new possibilities for developing materials, based on selective receptors, for $^{99}TcO_4^-$ extraction and recovery from acidic aqueous solutions (for example the $^{99}Mo/^{99m}Tc$ generator eluate and nuclear waste streams). Studies on anion solid-phase extraction by polymeric matrices,[24] containing a suitably modified and immobilized azacryptand, will be the subject of further work.

Experimental Section

***Caution**: ^{99}Tc is a weak β-emitter. Although radiation from small amounts of material is completely shielded by the glass walls of standard laboratory vessels, all experiments have been carried out in specially equipped laboratories, to avoid contamination.*

All reagents were purchased form Aldrich/Fluka and used without further purification. $NH_4{}^{99}TcO_4$ (Oak Ridge) was purified by recrystallization from an H_2O_2 containing aqueous $NH_4{}^{99}TcO_4$ solution. ITC studies were performed using a VP-ITC instrument (MicroCal, Inc.). ^1H and ^{99}Tc NMR spectroscopy studies were performed on a Varian Mercury 200 MHz NMR. For the ^{99}Tc chemical shifts, $NH_4{}^{99}TcO_4$ was used as the reference (0 ppm in D_2O). FTIR spectra were recorded on a PerkinElmer, Spectrum Two, as KBr pellets.

Crystallographic data were collected at 183(2) K with Mo K_α radiation ($\lambda = 0.7107$ Å) that was graphite-monochromated on a Stoe IPDS system. Further experimental information and crystal data are reported in Table S2 (CCDC 890213 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.).

Synthesis of $[LH_6(TcO_4)](TcO_4)(CF_3SO_3)_4{\cdot}8H_2O$: Receptor L (21.6 mg, 0.0361 mmol) was dissolved in water (2.0 mL), in the presence of excess CF_3SO_3H (9 equiv., 0.325 mmol). The solution was stirred at room temperature and $NH_4{}^{99}TcO_4$ (1 equiv., 0.0363 mmol) was dissolved. By slow evaporation of the reaction mixture (approximately 5 days), colorless crystals suitable for X-ray diffraction studies were obtained. The residual solvent was evaporated under a slow nitrogen stream, yielding further microcrystalline solid. ^1H NMR (500 MHz, D_2O): $\delta = 7.34$ (s, 12 H, arom.), 4.13 (s, 12 H, CH_2 benzyl.), 3.24 (t, 12 H, tren), 2.69 ppm (t, 12 H, tren); ^{13}C NMR (500 MHz, D_2O): $\delta = 132.0$ (C arom.), 131.3 (CH arom.), 120.1 (q, $CF_3SO_3^-$), 52.3 (CH_2 benzyl), 49.7 (CH_2 tren), 44.8 ppm (CH_2 tren); ^{99}Tc NMR (500 MHz, D_2O): $\delta = -1.73$ ppm ($\Delta\nu_{1/2} = 252$ Hz); ≈ -9 ppm ($\Delta\nu_{1/2}$ not available); IR (KBr): $\tilde{\nu} = 3554$ (m), 3479 (s), 3417 (s), 3079 (m), 2846 (m), 1638 (w), 1617 (m), 1470 (w), 1437 (m), 1283 (s), 1247 (s) 1174 (s), 1030 (s), 898 (m), 816 (w), 762 (w), 639 (s), 600 (w), 576 (w), 518 (w), 487 cm^{-1} (w). See spectra, in Figure S4–S8.

ITC experiments were performed in aqueous solution (0.1 M CF_3SO_3Na), by adding LH_6^{6+} (1.33 mM, syringe) to $NH_4{}^{99}Tc(Re)O_4$ (0.1 mM, cell). Both solutions were adjusted to pH 2.0 with CF_3SO_3H. The association parameters ($\log K_{11}$, ΔH, ΔS, n), were experimentally determined by fitting software (Origin). Blank titrations were performed and subtracted from the corresponding titrations to normalize the effect of the dilution. The ITC apparatus was calibrated using acid–base chemical reaction standards (neutralization of HNO_3 with NaOH, $\Delta H = -13.50 \pm 0.05$ kcal mol^{-1}, in 0.01 M NaCl, 25 °C).[25]

^1H and ^{99}Tc NMR spectroscopy studies were performed in D_2O with 0.1 M CF_3SO_3Na. The spectra were recorded on solutions containing LH_6^{6+} and $NH_4{}^{99}TcO_4$ at different molar ratios. The samples were typically prepared by adding to 1–7 mM solutions of $NH_4{}^{99}TcO_4$ to the desired amount of LH_6^{6+} (or the reverse). The pD value was adjusted to 2.0 by addition of standard CF_3SO_3H in D_2O.

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