Organic & Biomolecular Chemistry

Dynamic Article Links

Cite this: *Org. Biomol. Chem.*, 2011, **9**, 7358

www.rsc.org/obc

PAPER

Macrocyclic receptor for pertechnetate and perrhenate anions†

Grigory V. Kolesnikov,[a,b] Konstantin E. German,[c] Gayane Kirakosyan,[c] Ivan G. Tananaev,[c] Yuri A. Ustynyuk,[a] Victor N. Khrustalev[b] and Evgeny A. Katayev*[d]

Received 1st June 2011, Accepted 5th August 2011
DOI: 10.1039/c1ob05873h

The design and synthesis of a neutral macrocyclic host that is capable of perrhenate and pertechnetate recognition is described. The anion affinities and underlying coordination modes were estimated by several experimental and theoretical methods including a new technique—reverse ^{99}Tc NMR titration.

Introduction

The design of anion receptors of analytical and biochemical utility is a recognized challenge within the supramolecular community.[1–4] Perrhenate (ReO_4^-) and pertechnetate (TcO_4^-) are among the anions that are the most "difficult to catch" due to their relatively large size and low charge density.[5] However, these anions are readily available from ^{99}Mo/^{99m}Tc and ^{188}W/^{188}Re generators and are of great interest in nuclear medicine for both diagnostic and therapeutic applications.[6] The most significant source of ^{99}Tc by far is from the nuclear fuel cycle, and it is considered as one of the most hazardous pollutants due to its long half-life and high mobility in the environment. The design and synthesis of receptors capable of achieving the selective recognition of $Re(Tc)O_4^-$ is of great interest because the resulting species could lead to the development of sensors[7,8] and materials[9–11] allowing for the detection and capture of these anions. Appropriate receptors might also permit new approaches for the radiolabeling of organic compounds without the need for the classic reduction steps involving conversion of Re(VII) and Tc(VII) to easier-to-manipulate oxidations states.[12,13] To date, positively charged receptors have proved to be the most efficient for perrhenate and pertechnetate recognition.[7,14–19] However, neutral receptors are promising in the sense that they may display higher binding selectivities for the targeted anions.[15,20]

In this work we describe the synthesis of a neutral macrocyclic host that is capable of $Re(Tc)O_4^-$ recognition. The anion affinities of this system have been estimated on the basis of several known analytical methods. We also introduce here a new technique, namely a ^{99}Tc NMR[21] reverse titration protocol, that facilitates estimation of the pertechnetate anion affinities.

Results and discussion

Synthesis and structure of receptors

In our previous work[22,23] we showed that an anion-induced combinatorial selection of macrocyclic hosts from dialdehyde and diamine building blocks *via* reversible acid-catalyzed imine condensation is a convenient method for host design.[24] On the basis of the O–O distance (2.8 Å) in perrhenate we suggested that compounds **1** and **2** are promising building blocks with which to construct receptors for $Re(Tc)O_4^-$ (Fig. 1). The dialdehyde **1**, with an $N_{pyrrole}$–$N_{pyrrole}$ distance known to vary from 2.5 to 4.3 Å, is particularly attractive since it can provide two different binding sites.[25] Once incorporated in a macrocycle, we propose that a third binding site, located at least 5.8–7.2 Å from other binding sites, is advantageous.[20]

We used $HClO_4$, $HReO_4$, H_2SO_4, HNO_3, HCl and H_3PO_4, as templates in the synthesis of the receptor. $HReO_4$, $HClO_4$ and HCl gave only the [1 + 1] product of the condensation (according to MALDI-TOF spectra) and highest isolated yields of $\boldsymbol{L^1}\cdot(acid)_2$ (45%). The free-base ligand was prepared by neutralization of the salt using triethylamine. An understanding as to why different anions (Cl^- *vs.* ReO_4^- or ClO_4^-) gave rise to different products came from an analysis of the X-ray structures of the corresponding ligand salts (Fig. 2a,b)

According to the X-ray analysis, one perrhenate anion in $\boldsymbol{L^1}\cdot 2HReO_4$ interacts *via* H-bonds with both dipyrromethane and diamidopyridine binding units, while in $\boldsymbol{L^1}\cdot 2HCl$ two chloride anions are found in completely isolated binding sites. The two perrhenate anions are coordinated to the receptor from both sides in different modes (Fig. 2), forming strong H-bonds with pyrrole *NH*'s, and weak H-bonds with amide *NH*'s and benzene *CH*'s. Interestingly, the fact that only three oxygen atoms of the perrhenate anion are involved in H-bonding is in agreement with the H-bond network found by one of us in the structure of guanidinium perrhenate.[26]

[a]*Department of Chemistry, M.V. Lomonosov Moscow State University, Leninskie gory 1/3, 119992, Moscow, Russian Federation*
[b]*A.N. Nesmeyanov Institute of Organoelement Compounds Russian Academy of Sciences, Vavilov st., 28, 119991, Moscow, Russian Federation*
[c]*A.N. Frumkin Institute of Physical Chemistry and Electrochemistry Russian Academy of Sciences, Leninskiy pr. 31, 119991, Moscow, Russian Federation*
[d]*Institut für Organische Chemie, Universität Regensburg, Universitätsstr. 31, 93040 Regensburg, Germany. E-mail: evgeny.katayev@chemie.uni-regensburg.de; Tel: +49 941 9434628*

† Electronic supplementary information (ESI) available. CCDC reference numbers 818214. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c1ob05873h

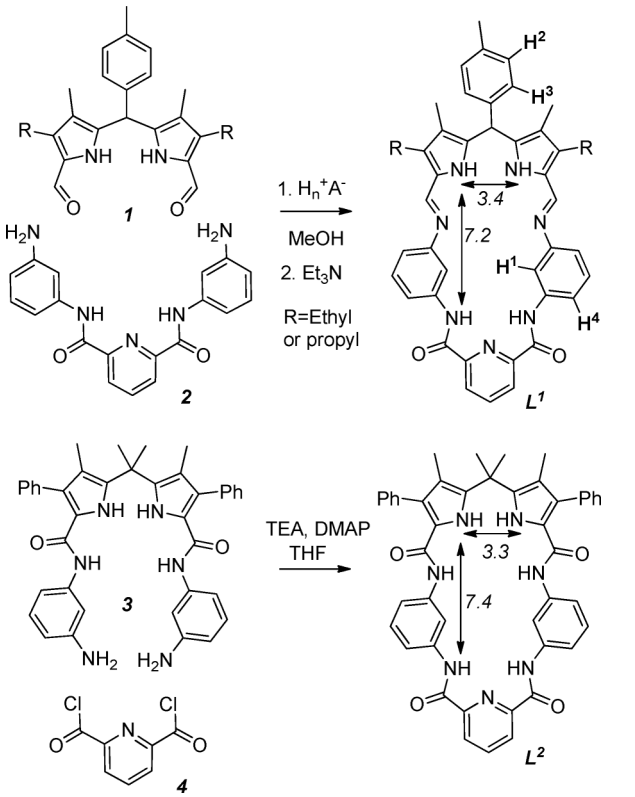

Fig. 1 Synthesis and structures of receptors considered in this study. The distances in Å between binding sites shown in this figure were estimated from DFT calculations.

The slower kinetics of $\boldsymbol{L^1}\cdot 2HCl$ complex formation in comparison with the corresponding perrhenate complex allowed us to determine the half-life of the template-mediated reaction using ^1H NMR spectroscopy; this value was found to be *ca.* 800 s. Addition of one equivalent of HCl to a mixture of **1** + **2** led to monoprotonation of the diamine (green signals, Fig. 3). During the reaction, the diamine loses the proton and the signal disappears but not completely. This is rationalized in terms of the fact that for full transformation to the macrocycle, two equivalents of the acid are needed. Fig. 3 shows the course of the spectral changes as a function of time as the reaction is allowed to run its course. Signals corresponding to the product (red signals) are already visible after 1 min. In order to assign all the signals we conducted ^1H NMR titrations of diamine **2** with HCl and $HReO_4$ (*cf.* ESI†).

Anion binding studies

Binding affinities of the free-base receptor were determined using UV-vis titrations. Both anions ReO_4^- and TcO_4^- have low charge densities and thus produce small changes in the electronic spectrum of the receptor upon interaction. The maximal changes were observed in 1,2-dichloroethane (Table 1, *cf.* ESI†). Interestingly, the first binding event for all anions is 2 : 1 ligand-to-anion. This stoichiometry was additionally supported by Job's plot and ESI(–) mass spectrometry, where the peak with m/z 1625.6 was detected, which corresponds to $2\boldsymbol{L^1}\cdot ReO_4^-$ (*cf.* ESI†).

Because the anion in complex $\boldsymbol{L^1}\cdot 2HReO_4$ is coordinated to the protonated receptor, we cannot directly compare this binding mode with that of the free-base receptor. In order to

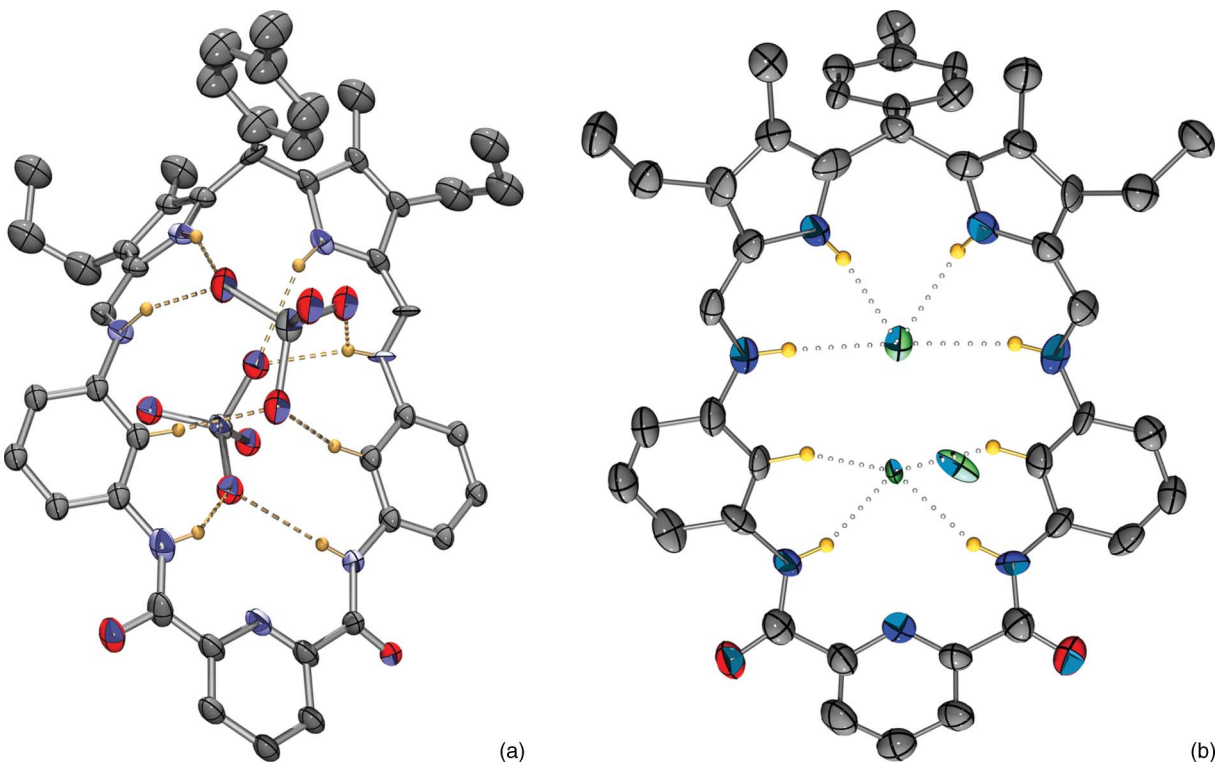

Fig. 2 Structures of $\boldsymbol{L^1}\cdot 2HReO_4$ (a) and $\boldsymbol{L^1}\cdot 2HCl$ (b) according to X-ray analysis. In (b) one of the chloride atoms is disordered. Most of the hydrogen atoms and solvent molecules are omitted for clarity.

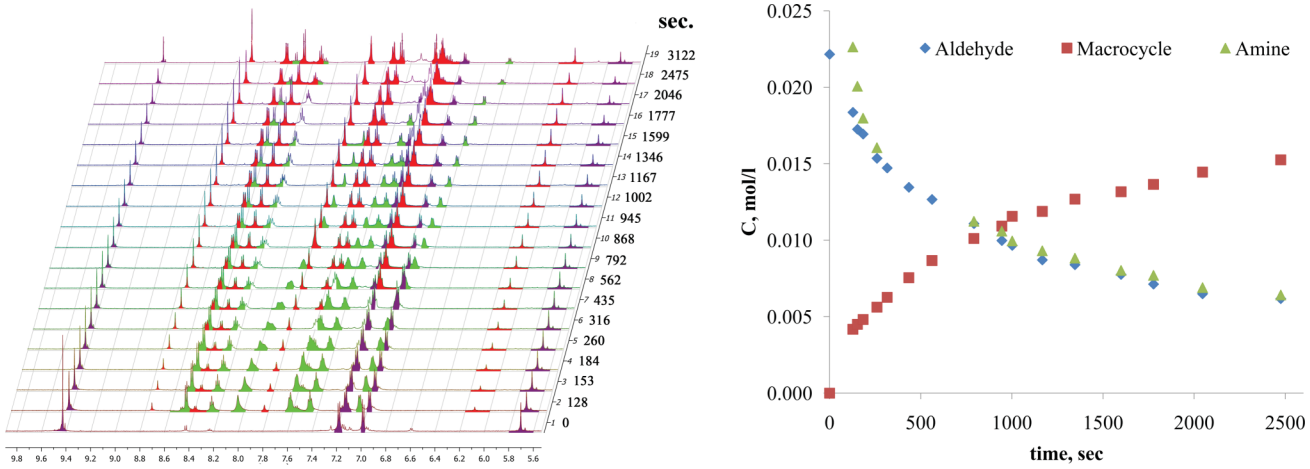

Fig. 3 Changes in the ^{1}H NMR spectrum of an equimolar mixture **1** + **2** in CD_3OD after addition of one equiv. of HCl (26% aqueous solution). The first spectrum represents the spectrum of dialdehyde **1**. Green signals belong to diamine **2**, violet signals belong to dialdehyde **1** and red signals belong to complex $\boldsymbol{L^1}\cdot 2HCl$.

Table 1 Affinity constants ($\log\beta/M^{-1}$) of receptors for anions as determined from UV-vis titrations carried out in two solvents at 25 °C (DCE—1,2-dichloroethane, DMSO—dimethylsulfoxide). Binding stoichiometries other than 1 : 1 are indicated

	$\boldsymbol{L^1}$ DCE	$\boldsymbol{L^1}$ DMSO	$\boldsymbol{L^2}$ DCE	$\boldsymbol{L^2}$ DMSO
$H_2PO_4^-$	10.54(5) *2 : 1* 5.21(1) *1 : 1*	8.73(8) *2 : 1* 4.38(6) *1 : 1*	4.85(1)	4.32(1)
HSO_4^-	11.34(8) *2 : 1* 5.00(10) *1 : 1*	4.42(1)	4.91(2)	5.16(5)[a]
OAc^-	12.72(14) *2 : 1* 5.35(9) *1 : 1*	3.52(2)	6.30(15)[a]	4.64(4)
Cl^-	4.65(9)[a]	3.60(2)	5.50(2)[a]	4.28(2)
NO_3^-	10.83(12) *2 : 1* 5.49(9) *1 : 1*	2.99(14)	4.72(2)	4.71(4)
I^-	9.72(12) *2 : 1*[b] 3.18(10) *1 : 1*	5.26(15)[b]	4.40(2)[b]	5.55(4)[b]
ClO_4^-	8.73(12) *2 : 1* 3.70(8) *1 : 1*	4.60(10)	5.01(1)	4.10(3)
ReO_4^-	9.65(8) *2 : 1* 4.46(5) *1 : 1*	4.61 (5)	4.55(10)[b]	4.95(6)[b]
TcO_4^-	8.60(8) *2 : 1* 4.29(6) *1 : 1*	5.00(30)[b]	4.22(30)[b]	4.60(6)[b]

[a] Average binding constant for all events was calculated. [b] Values were calculated taking into consideration the anion absorption.

estimate the geometry of the host–guest complexes formed in 1,2-dichloroethane, we carried out DFT calculations of 1 : 1 and 2 : 1 receptor–perrhenate complexes (Fig. 4a, b). To establish the reliability of these calculations, we compared the optimized structures and those obtained from the X-ray analysis. On the basis of a bond length comparison, the average difference between the experimental (X-ray, solid state) and optimized (DFT, gas phase) value was found to be 0.13 ± 0.06 Å, which is 10% of the length of the C–C bond with a confidence interval of 95% (*cf.* ESI†). According to the calculations carried out for complex $\boldsymbol{L^1}\cdot ReO_4^-$, the coordination of the anion takes place due to two pairs of hydrogen bonds provided by the dipyrromethane *NH*'s, the benzene *CH*'s and the amide *NH*'s. A conformational and geometrical search for the global minimum of the 2 : 1 structure resulted in the structure shown in Fig. 4b. In this case, all oxygen atoms of the anion are involved in an H-bonding network provided by two molecules of the receptor, which are oriented almost perpendicular to each other. According to the calculations the formation of a 2 : 1 complex (ΔE = –46 kcal mol^{-1}) is more energetically favorable than the formation of a 1 : 1 complex (ΔE = –26 kcal mol^{-1}).

Support for the conclusion that the benzene *CH*'s of $\boldsymbol{L^1}$ are involved in hydrogen bonding interactions with the perrhenate anion was obtained from ^{1}H NMR titrations carried out in $CDCl_3$.

Maximal proton shifts were observed for pyrrole *NH*- (+0.92 ppm) and benzene *CH*-protons H^1 (+0.04 ppm, Fig. 5). Other aromatic *CH*- and amide *NH*-protons move to lower field but with smaller shifts. Fitting the titration data allowed us to estimate the stepwise binding constants as $\log K_{21}$ = 5.15(10) and $\log K_{11}$ = 3.12(10), respectively.

As for UV-vis titrations, addition of $(Bu_4N^+)(ReO_4^-)$ to the receptor induces only small changes in the spectrum of the ligand.

Analysis of the data included in Table 1 reveals that $\boldsymbol{L^1}$ has almost the same affinity for most anions included in this study, with a small preference for the two hydrophilic anions CH_3COO^- and HSO_4^-. However, the solvation energies of the more hydrophobic anions, *e.g.*, ReO_4^-, TcO_4^-, ClO_4^-, are much less than those of these two more hydrophilic anions.[27] Hence, increasing the polarity of the solvent should reverse the selectivity of the host and favor its interaction with the more hydrophobic anions, simply because hydrophilic anions require more energy to be desolvated in polar environments.[5] Indeed, measurement of binding constants in a mixture of DCE–MeOH and DMSO revealed that the selectivity is reversed and that the greatest effect is observed in DMSO (Table 1). Interestingly, in the more polar solvents little evidence of 2 : 1 binding was seen, presumably due to the competition between the solvent and the ligand for coordination of the second anion.

To establish the reliability of UV-Vis titrations we conducted the competition experiment, namely the titration of complex $\boldsymbol{L^1}\cdot HSO_4^-$ with ReO_4^- in both solvents. Changes in the absorbance upon dilution of $\boldsymbol{L^1}\cdot HSO_4^-$ with more than 5–10 equiv. of $\boldsymbol{L^1}$ should follow Lambert–Beer behavior, because the receptor will be in a large excess. However, if ReO_4^- can replace HSO_4^- (the receptor has large affinity for the perrhenate anion), then the changes upon dilution of $\boldsymbol{L^1}\cdot HSO_4^-$ with a mixture of $\boldsymbol{L^1}$ and ReO_4^- should

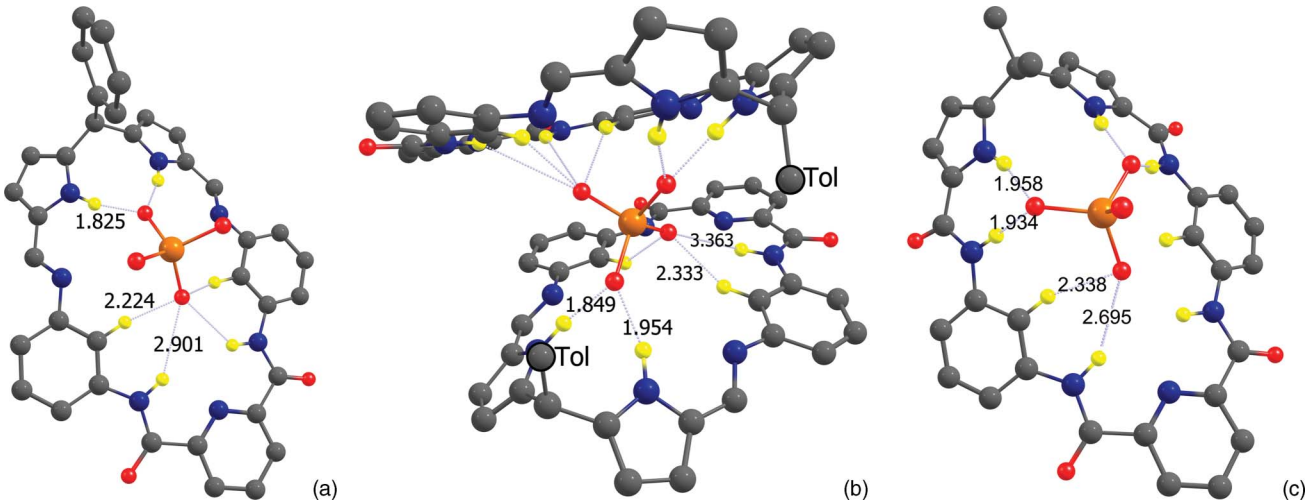

Fig. 4 The structures of complexes $\boldsymbol{L^1}\cdot ReO_4^-$ (a), $2\boldsymbol{L^1}\cdot ReO_4^-$ (b) and $\boldsymbol{L^2}\cdot ReO_4^-$ (c) are optimized using DFT calculations. In (b) "Tol" stands for the tolyl group which is partially removed for clarity; however, the resting carbon atom shows the direction of the tolyl group in space. Most of the hydrogen atoms were removed for clarity as well.

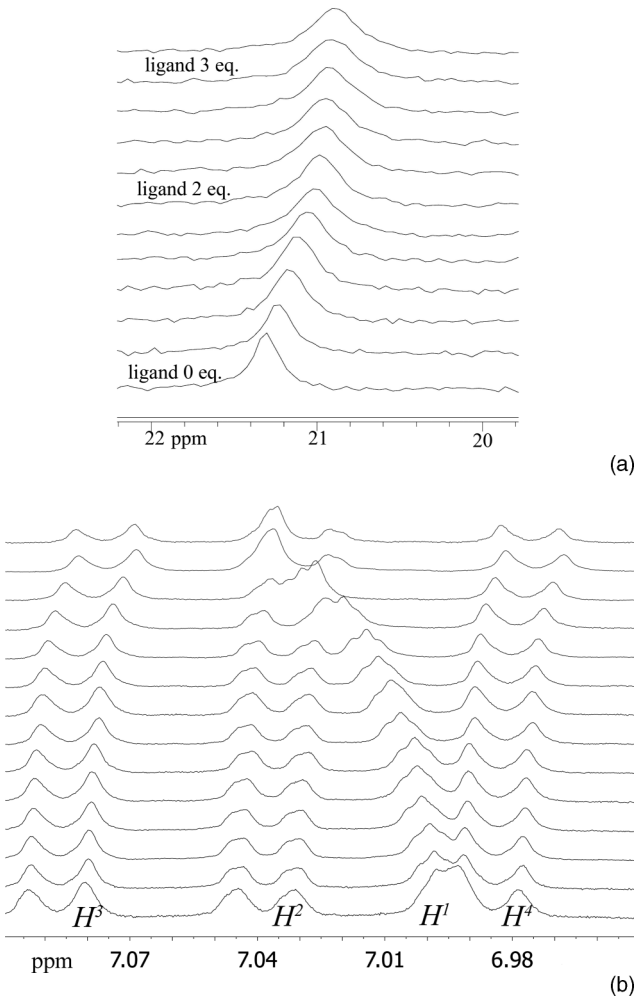

Fig. 5 ^{99}Tc NMR titration of $(Bu_4N^+)(TcO_4^-)$ with $\boldsymbol{L^1}$ (a) and ^{1}H NMR titration of $\boldsymbol{L^1}$ with $(Bu_4N^+)(ReO_4^-)$ (b) in $CDCl_3$ at 25 °C.

not be linear. In this experiment, we observed non-linear changes only in DMSO solution (*cf*. ESI, Fig. S27 and S28†). This fact is in agreement with the observed selectivities (Table 1). Fitting of the data allowed us to obtain a competition binding constant $\log K(HSO_4^-/ReO_4^-) = 3.60(2)$.

We thought that increasing the number of hydrogen bonding donor sites could increase the selectivity for ReO_4^-. Accordingly, a new polyamide receptor, $\boldsymbol{L^2}$, was synthesized. The H-bond network of $\boldsymbol{L^2}$ is similar to that of $[\boldsymbol{L^1}H_2]^{2+}$ (compare Fig. 2a and Fig. 4c). However, the results of screening the anion binding affinities in different solvents did not agree with our expectations (Table 1). Thus, we concluded that by increasing the number of *NH* sites on the receptor in both polar and non-polar media the effect of anion solvation becomes negligible in defining the selectivity of the host. In point of fact, receptor $\boldsymbol{L^1}$ appears to possess a better number and arrangement of H-bond donor sites; certainly, it has allowed us to achieve almost one order of magnitude selectivity for the ReO_4^- anion over HSO_4^-, Cl^- and CH_3COO^-.

Reverse ^{99}Tc NMR titrations

Additional evidence of binding of pertechnetate to the receptors was provided by reverse ^{99}Tc NMR titrations carried out in $CDCl_3$ solution. This method appears to be more reliable in comparison with ^{1}H NMR titration, because the shift of the ^{99}Tc signal is close to 1 ppm when the binding is present. In this experiment the stock solution of tetrabutylammonium pertechnetate was titrated with the ligand and the technetium signal was followed until a 1 : 5 anion–receptor ratio was reached. The technetium peak shifted upfield from 21.30 to 20.88 ppm in the case of $\boldsymbol{L^1}$ ($\log K$ = 3.24(10)) and from 21.30 to 20.54 ppm in the case of $\boldsymbol{L^2}$ ($\log K$ = 1.96(10)). The binding affinities are almost two orders of magnitude lower than those obtained from UV-vis titrations; however, this is in agreement with general correlation between NMR and UV-vis methods observed for similar host–guest complexes.[28,29] The difference of binding affinities arises from the difference in the concentrations used in NMR (*ca.* 10^{-3} mol L^{-1}) and UV-vis (*ca.* 10^{-5} mol L^{-1}) methods. At a concentration of host higher than 10^{-4} mol L^{-1}, the absorbance did not follow Lambert–Beer behavior, indicating that aggregations of the host

are present. Carrying out the reverse titration, a receptor can self-associate and this will compete with the anion binding process. Because in the reverse ^{99}Tc NMR method the chemical shifts are not large, the obtained binding affinities represent a presumably average value which include both 2 : 1 and 1 : 1 events.

Conclusions

We have prepared for the first time what is, to our knowledge, a viable neutral receptor for the perrhenate and pertechnetate anions. The structural and binding data collected in the context of this study provide support for this conclusion. They are also expected to increase our understanding of how these important anions interact with organic hosts. Currently, we are working to generalize these findings by extending the present studies to include receptors that can bind the perrhenate anion in water.

Experimental

All solvents were purchased commercially and were of reagent grade quality. Starting materials were purchased from Aldrich Chemical Co. or Acros Organics and used without further purification. NMR spectra used in the characterization of products were recorded on a Bruker Avance 600 spectrometer. The NMR spectra were referenced to the solvent and the spectroscopic solvents were purchased from Cambridge Isotope Laboratories. All MALDI-TOF mass spectra were recorded on a Reflex 3 Bruker instrument. Elemental analyses were performed by INEOS Analytical Lab and are reported as percentages. TLC analyses were carried out using Baker-flex Silica gel IB-F sheets. Column chromatography was performed on Acros silica gel 60 Å (230–400 mesh). 2,2′-(5-Formyl-3-methyl-4-propyl-pyrrolyl)(*p*-tolyl)methane (**1**) was prepared according to a literature procedure.[1]

Synthesis

Bis(3-aminophenyl)pyridine-2,6-dicarboxamide (2). *N*-Boc-*m*-phenylenediamine 0.50 g (2.40 mmol), TEA 0.37 ml (2.64 mmol) and DMAP 0.03 g (0.24 mmol) were dissolved in 25 ml of DCM and stirred for 30 min. The solution of 0.29 g (1.44 mmol) of pyridine-2,6-diacid dichloride in 10 ml of DCM was added dropwise and the reaction mixture was left stirring for the next 10 h. The reaction mixture was evaporated to dryness and dissolved in MeOH. The precipitate was filtered off, dried under vacuum, redissolved in a dichloromethane–trifluoroacetic acid (15 : 5 ml) mixture and stirred at rt. After 3 h an aqueous 10% NaOH solution was added until a white slurry precipitate was formed. The precipitate was filtered off, washed with water twice, and dried under high vacuum for 4 h at 50 °C. Overall yield – 0.28 g (68%). M.p. 247–248° C. ^{1}H NMR ([D6]DMSO): δ (ppm) 5.18 (4H, bs, NH_2), 6.41 (2H, d, J = 7.8 Hz, CH-benzene), 7.00 (2H, d, J = 7.9 Hz, CH-benzene), 7.06 (2H, t, J = 7.9 Hz, CH-benzene), 7.21 (2H, s, CH-benzene), 8.28 (1H, t, J = 7.6 Hz, CH-pyridine), 8.36 (2H, d, J = 7.7 Hz, CH-pyridine), 10.79 (2H, s, C(O)NH). ^{13}C NMR ([D6]DMSO): δ (ppm) 164.85, 148.86, 140.19, 135.68, 130.20, 128.71, 128.22, 126.88, 109.65, 108.52, 106.21. ESI(+) m/z: calcd [M + H] 348.4, found 348.4. Anal. calcd. for $C_{19}H_{17}N_5O_2$: C, 65.69; H, 4.93; N, 20.16. Found: C, 65.47; H, 5.12; N, 20.25.

Macrocycle L^1 (R = Pr). Diamine **2** 43.0 mg (0.12 mmol) and dialdehyde **1** 50.0 mg (0.12 mmol) were dissolved in 1.0 ml of MeOH and stirred for 10 min. Then 2.5 eq. of a template acid was added to the solution (HCl_{conc} (33%), $HClO_{4\,conc}$ (70%) or $HReO_{4\,conc}$ (70%)). The mixture was allowed to react for the next 10 h at r.t. The red colored precipitate formed was filtered off yielding the macrocyclic product as a salt of the corresponding acid. The solid was dissolved in 95 : 5 (v/v) DCM–MeOH solution and layered with hexane. The resulting crystals were dissolved in 95 : 5 (v/v) DCM–MeOH and treated with 3.3 ml (2.4 mmol) of triethylamine and evaporated. The product was passed through an alumina plug (eluent 95 : 5 (v/v) DCM-MeOH) yielding the free base as a yellow-red powder. Overall yield - 38 mg (45%). ^{1}H NMR ([D6]DMSO): δ (ppm) 1.12 (6H, t, CH_3), 1.60 (4H, m, CH_2),2.24(3H, s, CH_3), 2.67 (4H, m, CH_2), 5.69 (1H, s, CH), 6.90 (2H, d, CH-benzene), 7.07 (2H, d, CH-benzene), 7.16 (2H, d, CH-benzene), 7.43 (4H, m, CH-benzene), 8.27 (2H, m, CH-pyridine), 8.32 (1H, m, CH-pyridine), 8.42 (2H, s, CH═N), 11.15 (2H, bs, NH-amide), 11.71 (2H, bs, NH-pyrrole). ^{13}C NMR ([D6]DMSO): δ (ppm) 161.5, 154.12, 147.22, 139.86, 136.04, 133.32, 131.61, 131.50, 129.72, 129.55, 128.82, 128.13, 127.14, 126.43, 116.86, 116.54, 116.33, 116.20, 113.64, 47.95, 26.16, 25.02, 21.06, 14.00, 9.13. MALDI-TOF (matrix – DHB), m/z: calcd [M + H] 716.4, found 716.4. Anal. calcd for $C_{45}H_{45}N_7O_2$: C, 75.50; H, 6.34; N, 13.70. Found: C, 75.31; H, 6.54; N, 13.87. UV-vis (1,2-dichloroethane): λ_{max} = 342(42630)

5,5′-(Propane-2,2-diyl)bis(*N*-(3-aminophenyl)-4-methyl-3-phenyl-1*H*-pyrrole-2-carboxamide) (3). 5,5′-(Propane-2,2-diyl)bis(4-methyl-3-phenyl-1*H*-pyrrole-2-carboxylic acid) 440 mg (1.0 mmol) was suspended in 25 ml DCM, oxalyl chloride 2.8 ml (32 mmol) in 25 ml DCM was added followed by addition of 20 µl of dry DMF. The reaction mixture was allowed to stir for 4 h at room temperature then evaporated to dryness under vacuum on a water bath at 40–45 °C, and dried in high vacuum for 1 h. The green colored residue was dissolved in 50 ml THF and was added slowly to a mixture of *N*-Boc-*m*-phenylenediamine 416 mg (2.0 mmol), pyridine 1.6 ml (20.0 mmol) and DMAP 30 mg (0.24 mmol) in 50 ml of THF. The reaction mixture was left stirring for the next 12 h. The reaction mixture was then evaporated to dryness, dissolved in MeOH : DCM = 1 : 20 and filtered through a plug of silica gel. The resulting solution was evaporated to dryness, dissolved in 15 ml DCM, and 5 ml of trifluoroacetic acid was added. The reaction mixture was stirred at room temperature for the next 3 h and poured into cold 10% NaOH in water, and extracted with DCM 50 ml twice. The organic fractions were combined and washed with brine 100 ml, and dried with Na_2SO_4. The crude product was recrystallized from EtOAc : Hex. Overall yield was 441 mg (67%). M.p. 359–260° C. ^{1}H NMR ([D6]DMSO): δ (ppm) 1.43 (6H, s, CH_3), 1.82 (6H, s, CH_3), 5.02 (4H, s, NH_2), 6.20 (2H, d, J = 8.0 Hz, CH-benzene), 6.31 (2H, d, J = 8.0 Hz, CH-benzene), 6.70 (2H, s, CH-benzene), 6.84 (2H, t, J = 8.0 Hz, CH-benzene), 7.25–7.44 (10H, m, CH-benzene), 8.15 (2H, s, NH-amide), 10.62 (2H, s, NH-pyrrole). ^{13}C NMR ([D6]DMSO): δ (ppm) 158.3, 149.0, 139.32, 137.0, 135.4, 130.4, 128.9, 128.8, 128.2, 126.9, 119.5, 114.8, 109.1, 106.8, 104.7, 36.2, 27.8, 9.5. ESI(+) m/z: calcd [M + H] 623.7, found. 623.8. Anal. calcd for $C_{39}H_{38}N_6O_2$: C, 75.22; H, 6.15; N, 13.49. Found: C, 75.47; H, 6.30; N, 13.55.

Macrocycle L^2. 62 mg (0.1 mmol) of **3** was dissolved in 150 ml of THF, 140 µl (1 mmol) of Et_3N and DMAP 3 mg (0.02 mmol) was added. The solution of 31 mg (0.15 mmol) pyridine-2,6-diacid dichloride (**4**) in 50.0 ml of THF was added slowly to the former solution. The reaction mixture was left stirring for the next 12 h. The reaction mixture was then evaporated to dryness, dissolved in MeOH : DCM = 1 : 20 and filtered through a plug of silica gel. The product was purified by flash chromatography on silica gel, and eluted with EtOAc–Hex 1 : 2 mixture. Additional purification steps were carried out by suspending the product in Et_2O and filtering through a glass filter. Overall yield was 35 mg (46%). ^{1}H NMR ([D6]DMSO): δ (ppm) 1.93 (6H, s, CH_3), 2.09 (6H, s, CH_3), 6.20 (2H, d, CH-benzene) 7.14–7.24 (4H, CH-benzene), 7.42–7.60 (10H, CH-benzene), 7.72 (1H, s, CH-benzene), 8.07 (1H, t, J = 7.8 Hz, CH-pyridine), 8.30 (2H, d, J = 8.2 Hz, CH-benzene), 8.49 (2H, d, J = 7.8 Hz, CH-pyridine), 9.05 (2H, s, NH-amide), 9.44 (2H, s, NH-amide). ^{13}C NMR ([D6]DMSO): δ (ppm) 160.1, 158.2, 148.0, 138.1, 136.9, 136.5, 135.9, 133.4, 129.6, 128.5, 128.2, 127.4, 125.0, 118.6, 115.8, 115.4, 115.3, 112.6, 28.7, 27.2, 10.4. ESI(+), m/z: calcd [M + H] 754.8, found 754.8. Anal. calcd for $C_{46}H_{39}N_7O_4$: C, 73.29; H, 5.21; N, 13.01. Found: C, 73.17; H, 5.33; N, 12.95. UV-Vis (1,2-dichloroethane) λ_{max} = 300(32000)

UV-Vis titration technique. Stock solutions of the host molecule being studied were made up in DMSO with the final concentrations being between 1.295×10^{-5} M and 2.200×10^{-5} M. For instance, 2.33 mg of receptor L^1 were dissolved in 25.0 mL of DMSO (spectrophotometric grade) yielding a 1.303×10^{-4} M stock solution. This first stock solution was then diluted 10 times to give the titration stock solution with a concentration of 1.303×10^{-5} M. Stock solutions of the guest were prepared by dissolving 10–100 equivalents of tetrabutylammonium salts of the anions used in this study in 1.5–2.5 ml of stock solution of the host, prepared as described above. Making up the anion source solutions in this way allowed the binding studies to be carried out without having to make mathematical corrections to account for changes in host concentration as the result of dilution effects. The general procedure for the UV-Vis binding studies involved making sequential additions of titrant (anionic guest), using Hamilton® syringes, to a 2 mL aliquot of the host stock solution in the spectrometric cell. The data were then collated and combined to produce plots that showed the changes in host spectral features as a function of changes in the concentration of the guest. The experimental data were fitted by HYPERQUAD 2006 computer program.[30]

Reverse ^{99}Tc NMR titrations technique. Caution! Titrations were performed with ^{99}Tc isotope, a low energy (0.292 MeV) β2-particle emitter with a half-life of 2.12×10^5 years. When handled in milligram quantities (the concentration range 10^{-3}–10^{-5} M), ^{99}Tc does not present a serious health hazard since common laboratory materials provide adequate shielding. Bremsstrahlung is not a significant problem due to the low energy of the β2-particle emission, but normal radiation safety procedures must be used at all times to prevent contamination.

Stock solutions of the tetrabutylammonium pertechnetate were prepared in $CDCl_3$ with the final concentration of 1.016×10^{-3} M. Stock solution of the titrant was prepared by dissolving 10 equivalents of the receptor in 1.0 mL of titration stock solution of the anion, prepared as described above. The general procedure for the ^{99}Tc NMR binding studies involved making sequential additions of titrant (*e.g.* receptor **3**) using Hamilton syringes to a 0.5 mL aliquot of the host (anion) stock solution in the NMR tube. The data were combined to produce plots that showed the changes in anion chemical shift (ppm) as a function of changes in the concentration of receptor **3**. The experimental data were fitted by HYPERNMR 2006 computer program.[30]

Combinatorial experiments for imine macrocycle L^1. Diamine **2** 4.6 mg (0.08 mmol) and dialdehyde **1** 5.0 mg (0.08 mmol) were dissolved in 0.6 ml of methanol and stirred for 10 min. Then 2.5 eq. of template acid were added ($H_3PO_{4\,conc}$ (98%), $H_2SO_{4\,conc}$ (98%), HCl_{conc} (26%), $HNO_{3\,conc}$ (73%), $HClO_{4\,conc}$ (70%), $HReO_{4\,conc}$ (76%)). The mixture was allowed to react for the next 12 h under ambient conditions. The yellow-green colored precipitate formed was filtered off then washed with Et_2O, yielding the macrocyclic product as a salt of the corresponding acid. The resulting solid was analyzed by MALDI-TOF spectra (matrix DHB-2,5-dihydroxybenzoic acid).

NMR kinetics measurements of macrocyclization. Diamine **2** 4.6 mg (0.08 mmol) and dialdehyde **1** 5.0 mg (0.08 mmol) were placed in a 5 mm NMR tube and 0.6 ml of CD_3OD was added. Then, 1.0 equiv. of the template acid were added (HCl_{conc} (26%), $HReO_{4conc}$ (76%)). The NMR tube was immediately placed into the spectrometer and the spectra were recorded.

Crystal structure of L^1·2HCl. Crystal data for $C_{44}H_{45}Cl_4N_7O_2$, CCDC 818214 M = 845.67 g mol^{-1}, monoclinic, $P2_1/c$, a = 16.89(4) Å, b = 15.29(4) Å, c = 16.64(4) Å, α = 90°, β = 110.63(3)°°, γ = 90°, V = 4023(16) Å^3, Z = 4, 33694 reflections measured, 7016 independent (R_{int} = 0.1104), which were used in all calculations. The final wR_2 was 0.2213 (all data). Data were collected on a Bruker SMART APEX II CCD diffractometer λ(Mo-Kα)-radiation (0.71073 Å), graphite monochromator, ω and φ scan mode) and corrected for absorption using the *SADABS* program.[31] One of the two chloride anions is disordered over two sites with the occupancies of 0.6 : 0.4. The independent part of the unit cell of L^1·2(HCl) contains a dichloromethane solvate molecule. The hydrogen atoms were placed in calculated positions and refined within the riding model with fixed isotropic displacement parameters (U_{iso}(H) = 1.5U_{eq}(C) for the CH_3 groups and U_{iso}(H) = 1.2U_{eq}(N or C) for the other groups). All calculations were carried out using the *SHELXTL* program.[32]

Acknowledgements

We gratefully acknowledge Prof. Burkhard König for support, Dr D. Laikov for the program "PRIRODA" for structure optimization and Russian Academy of Sciences program "Chemistry and Physico-Chemistry of Supramolecular Systems and Atomic Clusters".

Notes and references

1 J. L. Sessler, P. A. Gale and W.-S. Cho, *Anion Receptor Chemistry (Monographs in Supramolecular Chemistry)*, ed. J. F. Stoddart, Royal Society of Chemistry, 2006.
2 P. A. Gale, *Chem. Soc. Rev.*, 2010, **39**, 3746–3771.
3 S. Kubik, *Chem. Soc. Rev.*, 2010, **39**, 3648–3663.
4 T. H. Rehm and C. Schmuck, *Chem. Soc. Rev.*, 2010, **39**, 3597–3611.

5 E. A. Katayev, G. V. Kolesnikov and J. L. Sessler, *Chem. Soc. Rev.*, 2009, **38**, 1572–1586.
6 W. A. Volkert and T. J. Hoffman, *Chem. Rev.*, 1999, **99**, 2269–2292.
7 A. E. V. Gorden, J. Davis, J. L. Sessler, V. Král, W. Keogh and N. L. Schroeder, *Supramol. Chem.*, 2004, **16**, 91–100.
8 D. P. Cormode, A. J. Evans, J. J. Davis and P. D. Beer, *Dalton Trans.*, 2010, **39**, 6532–6541.
9 N. M. Hassan, W. D. King, D. J. McCabe, L. L. Hamm and M. E. Johnson, *Solvent Extr. Ion Exch.*, 2002, **20**, 211–226.
10 H. H. Fei, D. L. Rogow and S. R. J. Oliver, *J. Am. Chem. Soc.*, 2010, **132**, 7202–7209.
11 S. A. Wang, E. V. Alekseev, D. W. Juan, W. H. Casey, B. L. Phillips, W. Depmeier and T. E. Albrecht-Schmitt, *Angew. Chem., Int. Ed.*, 2010, **49**, 1057–1060.
12 O. K. Hjelstuen, *Analyst*, 1995, **120**, 863–866.
13 H. Stephan, R. Berger, H. Spies, B. Johannsen and F. P. Schmidtchen, *J. Radioanal. Nucl. Chem.*, 1999, **242**, 399–403.
14 S. Nieto, J. Pérez, L. Riera, V. Riera, D. Miguel, J. A. Golen and A. L. Rheingold, *Inorg. Chem.*, 2007, **46**, 3407–3418.
15 P. D. Beer, P. K. Hopkins and J. D. McKinney, *Chem. Commun.*, 1999, 1253–1254.
16 B. Antonioli, K. Gloe, K. Gloe, G. Goretzki, M. Grotjahn, H. Heßke, M. Langer, L. F. Lindoy, A. M. Mills and T. Söhnel, *Z. Anorg. Allg. Chem.*, 2004, **630**, 998–1006.
17 K. T. Holman, M. M. Halihan, S. S. Jurisson, J. L. Atwood, R. S. Burkhalter, A. R. Mitchell and J. W. Steed, *J. Am. Chem. Soc.*, 1996, **118**, 9567–9576.
18 D. Farrell, K. Gloe, K. Gloe, G. Goretzki, V. McKee, J. Nelson, M. Nieuwenhuyzen, Ibolya Pál, H. Stephan, R. M. Town and K. Wichmann, *Dalton Trans.*, 2003, 1961–1968.
19 I. S. Antipin, S. E. Solovieva, I. I. Stoikov, I. S. Vershinina, G. A. Pribylova, I. G. Tananaev and B. F. Myasoedov, *Russ. Chem. Bull.*, 2004, **53**, 127–132.
20 E. A. Katayev, N. V. Boev, V. N. Khrustalev, Y. A. Ustynyuk, I. G. Tananaev and J. L. Sessler, *J. Org. Chem.*, 2007, **72**, 2886–2896.
21 F. Poineau, P. F. Weck, K. German, A. Maruk, G. Kirakosyan, W. Lukens, D. B. Rego, A. P. Sattelberger and K. R. Czerwinski, *Dalton Trans.*, 2010, **39**, 8616–8619.
22 E. A. Katayev, J. L. Sessler, V. N. Khrustalev and Y. A. Ustynyuk, *J. Org. Chem.*, 2007, **72**, 7244–7252.
23 E. A. Katayev, G. V. Kolesnikov, V. N. Khrustalev, M. Y. Antipin, R. K. Askerov, A. M. Maharramov, K. E. German, G. A. Kirakosyan, I. G. Tananaev and T. V. Timofeeva, *J. Radioanal. Nucl. Chem.*, 2009, **282**, 385–389.
24 N. Gimeno and R. Vilar, *Coord. Chem. Rev.*, 2006, **250**, 3161–3189.
25 J. L. Sessler, E. Katayev, G. D. Pantos, P. Scherbakov, M. D. Reshetova, V. N. Khrustalev, V. M. Lynch and Y. A. Ustynyuk, *J. Am. Chem. Soc.*, 2005, **127**, 11442–11446.
26 M. S. Grigoriev, K. E. German and A. Y. Maruk, *Acta Crystallogr., Sect. E: Struct. Rep. Online*, 2007, **63**, m2061.
27 Y. Marcus, *J. Chem. Soc., Faraday Trans.*, 1991, **87**, 2995–2999.
28 E. A. Katayev, N. V. Boev, V. N. Khrustalev, Y. A. Ustynyuk, I. G. Tananaev and J. L. Sessler, *J. Org. Chem.*, 2007, **72**, 2886–2896.
29 C. Schmuck and M. Schwegmann, *Org. Lett.*, 2005, **7**, 3517–3520.
30 P. Gans, A. Sabatini and A. Vacca, *Talanta*, 1996, **43**, 1739–1753.
31 G. M. Sheldrick, *SADABS Bruker/Siemens Area Detector Absorption Correction Program*, (2003) Bruker AXS, Wisconsin.
32 G. M. Sheldrick, *SHELXTL, v. 6.12, Structure Determination Software Suite*, (2001) Bruker AXS, Madison, Wisconsin, USA.

