Electrospray Ionization Mass Spectrometric Detection of Self-Assembly of a Crown Ether Complex Directed by π-Stacking Interactions

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The self-assembly of ligand-metal-ligand sandwich complexes involving a novel quinoxaline-containing crown ether, **1**, was studied by electrospray ionization mass spectrometry (ESI-MS). Donor-acceptor π-stacking interactions between the electron-poor quinoxaline group of **1** and electron-rich benzene groups from benzo- or dibenzo-18-crown-6 were found to significantly enhance the formation of mixed-ligand sandwich complexes with a free energy gain of up to 17 kJ/mol relative to sandwich formation involving macrocycles without such aromatic functionalities. The relative intensities of the sandwich complexes were greatest with the alkali metals Na^+, K^+, and Rb^+ as well as with the ammonium ion in equimolar concentrations with the macrocycles. The preferential formation of the mixed-ligand sandwich complexes demonstrated that donor-acceptor π-stacking interactions contribute to the assembly of molecular structures and can be monitored by ESI-MS.   (J Am Soc Mass Spectrom 2005, 16, 1162–1171) © 2005 American Society for Mass Spectrometry

In recent years, molecular self-assembly has become a key tool for developing novel chemical structures. Applications as diverse as patterned deposition of metallic monolayers [1], creation of ordered light-harvesting arrays [2], and creation of new biological materials based on self assembled peptide structures [3], have revolutionized the development of materials with nanoscale features. Various types of chemical interactions have been used to direct the assembly of individual subunits into the desired products. In the present report, we present electrospray ionization mass spectrometry (ESI-MS) results on a novel crown ether which demonstrate that donor-acceptor π-stacking interactions can be used to direct the self-assembly of noncovalent macrocycle-metal complexes.

For probing metal complexation of macrocyclic systems, ESI-MS has proven to be an invaluable tool. Since ESI is capable of transferring even very weakly bound noncovalent complexes to the gas phase, mass spectrometry can be used to qualitatively and quantitatively evaluate the binding selectivities of the hosts or the relative binding constants of the resulting host-guest complexes [4–22]. Furthermore, many studies have demonstrated that the results of ESI-MS analysis of these host-guest complexes correlate well with mea-surements of the equilibria in solution [8, 9, 14–17]. Crown ether-metal complexes, in particular, have been the subject of much study, and the binding properties of many types of these macrocyclic complexes have been evaluated through both ESI-MS [4, 5, 7–14, 16, 17, 22–25] and more traditional solution analyses [26–31]. While most such studies have focused on the 1:1 crown ether:metal complexes, 2:1 crown ether:metal "sandwich" complexes are well known [23–25, 28–31] and have been studied successfully by ESI-MS [23–25].

Although binding of the second ligand in a ligand-metal-ligand sandwich complex in solution is typically not as strong as for the first ligand for unsubstituted crown ethers [27], it is expected that the addition of certain functional groups on the macrocycle could enhance the formation of sandwich complexes. An analogous result has been observed for the formation of bis-crown ether-metal complexes studied by ESI-MS [13, 23, 28]. For these macrocycles, the preorganization offered by the covalently linked crown ethers results in very strong metal complexation in which the metal cation is simultaneously coordinated by both crown ethers.

Rather than rely on covalently linked structures, such as those used in bis-crown ethers, other chemical interactions must be leveraged to create sandwich complexes which can self-assemble. π-Stacking interactions, present in many biological systems and having demonstrated utility in strengthening other types of molecular interactions [32–35], represent a novel means of enhanc-
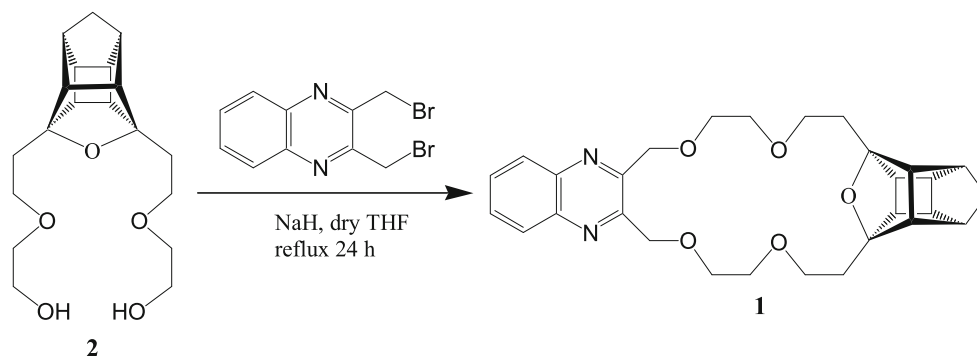
Scheme 1

ing the formation of macrocycle-metal sandwich complexes. In contrast to the well studied interactions between metal ions and π-orbital systems [36–38], the inclusion of aromatic moieties on the macrocycles in this report is expected to result in π-stacking interactions between the π-orbital systems on different macrocycles. These interactions should enhance the formation of sandwich complexes when the π-orbitals have appropriate overlap by allowing the detection of sandwich complexes incorporating larger macrocycles or smaller metal ions, ones not typically observed.

π-Stacking interactions can be further enhanced through the presence of donor-acceptor interactions between the π-orbitals. Including electronegative heteroatoms such as nitrogen or oxygen in an aromatic system creates an electron-deficient π-system in a manner similar to the effect of including an electron-withdrawing substituent on the aromatic ring [32]. The net result is a change in the polarization of the π-orbitals relative to an aromatic moiety without such heteroatoms, causing an additional attractive force as some electron density from the electron-rich π-system is donated to the electron-deficient π-system, strengthening the overall interaction [32, 39]. In the present work, an electron-deficient quinoxaline moiety is incorporated into a crown ether macrocycle, **1** (shown in Scheme **1**), to enhance the sandwich complex formation with benzo-crown ethers as a potential building block for self-assembled structures. The size selectivity of sandwich formation is evaluated based on comparative studies involving different metal ions, and the impact of the donor-acceptor interactions is investigated by use of a variety of reference macrocycles. Additionally, the impact of various solvent systems on sandwich complex formation is studied.

Experimental

Instrumentation

Mass spectra were collected on a LCQ Duo quadrupole ion trap mass spectrometer equipped with an ESI source (ThermoFinnigan, San Jose, CA). Instrumental parameters were optimized for transmission of the 2:1 crown ether:metal complexes, and the heated capillary

was kept at 80 °C to minimize in-source fragmentation. The same parameters were used for all solutions to minimize instrumental deviations. Presented spectra are averages of one hundred scans. High-resolution mass spectral data reported herein for Compound **1** were obtained by personnel at the Mass Spectrometry Facility at the Department of Chemistry and Biochemistry, University of Texas at Austin, by using a ZAB-E double sector high-resolution mass spectrometer (Micromass, Manchester, United Kingdom) that was operated in the chemical ionization mode. Elemental microanalysis of **1** was performed by personnel at M-H-W Laboratories, Inc., Phoenix, AZ.

Synthesis of 1

The quinoxaline-containing caged 19-crown-5, **1**, was prepared by the base-promoted reaction of 2,3-bis(bromomethyl)quinoxaline with **2** as shown in Scheme **1**. A mixture of NaH (obtained as a 60% dispersion in mineral oil, 400 mg, 9.0 mmol) and dry THF (40 mL) was refluxed with stirring under argon. To this refluxing mixture was added dropwise with stirring a solution of **2** [40] (504 mg, 1.5 mmol) and 2,3-bis(bromomethyl)quinoxaline (498 mg, 1.6 mmol) in dry THF (15 mL). After the addition of reagents had been completed, the resulting mixture was refluxed under argon for 24 h. The stirred reaction mixture was allowed to cool gradually to ambient temperature and then was quenched via careful dropwise addition of water (2 mL) until a clear solution resulted. An additional quantity of water (15 mL) was added, and the resulting aqueous solution was extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with brine (20 mL), dried (Na$_2$SO$_4$) and filtered, and the filtrate was concentrated in vacuo. The residue was purified via column chromatography on silica gel by eluting with 40% EtOAc-hexane. Workup of the chromatography fractions thereby obtained afforded crude **1** (335 mg, 46%); fractional recrystallization of this material from EtOAc afforded pure **1** as a colorless microcrystalline solid: mp 140–141 °C; IR (KBr) 2961 (m), 2941 (m), 2894 (m), 2865 (m), 2850 (m), 1295 (m), 1118 (vs), 1102 (vs), 978 (s), 909 (s), 771 cm^{-1} (s); ^{1}H NMR (CDCl$_3$) δ 1.43 (AB, J$_{AB}$ = 10.3

Hz, 1 H), 1.75 (AB, J_{AB} = 10.3 Hz, 1 H), 1.94–2.01 (m, 4 H), 2.32 (br s, 2 H), 2.51–2.60 (m, 6 H), 3.64–3.82 (m, 12 H) , 4.99 (s, 4 H), 7.68–7.73 (upfield half of a centrosymmetric AA'BB' spin pattern, 2 H), 8.04–8.09 (lowfield half of a centrosymmetric AA'BB' spin pattern, 2 H); ^{13}C NMR (CDCl$_3$) δ 32.2 (t), 41.3 (d), 43.4 (t), 43.8 (d), 48.0 (d), 58.9 (d), 68.0 (t), 70.0 (t), 71.1 (t), 72.5 (t), 94.3 (s), 128.9 (d), 129.7 (d), 141.1 (s), 152.2 (s); exact mass (CI HRMS); calculated for $C_{29}H_{34}N_2O_5$: $(M_r + H)^+$ m/z 491.2546; found: $(M_r + H)^+$ m/z 491.2539; calculated for $C_{29}H_{34}N_2O_5$: C, 71.00; H, 7.00; found: C, 71.24; H, 6.80.

Reagents

18-Crown-6, aza-18-crown-6, benzo-18-crown-6, dibenzo-18-crown-6, and dicyclohexano-18-crown-6 and the RbCl, CsCl, SrCl$_2$, and BaCl$_2$ salts were obtained from the Aldrich Chemical Co. (Milwaukee, WI). NaCl, KCl, and NH$_4$Cl salts were obtained from EM Science (Gibbstown, NJ). AgNO$_3$ was obtained from Alfa Aesar (Ward Hill, MA). Aside from the aza-18-crown-6 and dicyclohexano-18-crown-6, which had purities of 95 and 90%, respectively, all reagents were of at least 98% purity. All were used without further purification. Analyzed solutions contained **1**, an additional macrocycle, and a salt in a 1:1:1 ratio with concentrations of 5×10^{-5} M using spectroscopic grade OmniSolv methanol (EM Science) as the solvent. Solvent studies were performed using 1:1:1 solutions as described above using as solvents 1:1 water:methanol (water purified in-house, OmniSolv methanol). Omnisolv methanol, HPLC grade acetonitrile were purchased from Fisher Scientific (Pittsburgh, PA), and Optima grade chloroform, also from Fisher.

Results and Discussion

To evaluate the importance of π-stacking interactions in the self-assembly process, ESI-MS was used to examine solutions containing an equimolar quantity of **1**, a second ligand, and a cation. For each solution, the distribution of complexes containing a single macrocycle and cation (i.e., 1:1 complexes), two of the same macrocycles with one cation (i.e., 2:1 complexes or homo-ligand sandwiches), or two different macrocycles with one cation (i.e., mixed-ligand sandwich complexes) was monitored. The distribution of complexes was then correlated with the structures of the macrocycles, the presence of appropriate donor-acceptor interactions, and the type of cation.

    As a semi-quantitative means of evaluating the contribution of π-stacking towards any observed enhancement in the amount of the mixed-ligand complex, a hypothetical ligand exchange reaction was considered:

$$A_2M^+ + B_2M^+ \Leftrightarrow 2ABM^+ \tag{1}$$

where A and B are two different macrocyclic ligands and M is the cation bound between the ligands. Regardless of whether or not the direct ligand exchange

reaction is kinetically feasible, if the system is at equilibrium, the concentrations of the complexes must adhere to the thermodynamic equilibrium:

$$K_{EX} = \frac{[ABM^+]^2}{[A_2M^+][B_2M^+]} \tag{2}$$

where K_{EX} is the equilibrium constant for Reaction 1. ESI mass spectral intensities, I_X, relate to solution concentrations through the use of an ESI efficiency correction, C_X:

$$[X] = C_X I_X \tag{3}$$

K_{EX} can then be calculated directly from the ESI mass spectral intensities, I_X, of the complex ions:

$$K_{EX} = \left(\frac{C_{ABM^+}{}^2}{C_{A_2M^+}C_{B_2M^+}} \right)\left(\frac{I_{ABM^+}{}^2}{I_{A_2M^+}I_{B_2M^+}} \right) \tag{4}$$
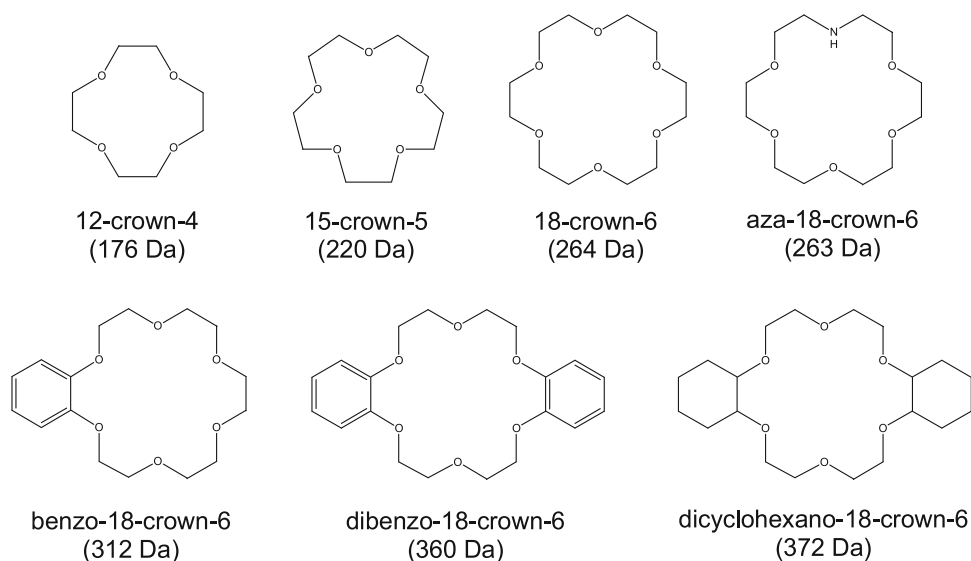
Previous studies suggest that ESI efficiencies for similar host-guest complexes, including ones involving macrocyles or even large biological molecules, are often close enough to ignore [8, 12, 14, 15, 41–46]. When considering this ligand exchange reaction, even if the ESI efficiencies of the different sandwich complexes were very different, they would have only minimal influence on the measured K_{EX} values. Since the mixed-ligand sandwich complex contains one ligand from each of the two homo-ligand complexes, its ESI efficiency should be somewhere midway between the efficiencies of the homo-ligand complexes. As a result, the ratio of ESI efficiencies in eq 4 is near unity, allowing simplification of eq 4 into:

$$K_{EX} = \frac{I_{ABM^+}{}^2}{I_{A_2M^+}I_{B_2M^+}} \tag{5}$$

From K_{EX}, the Gibb's free energy of the ligand exchange reaction can be calculated from:

$$\Delta G_{EX} = -RT \ln K_{EX} \tag{6}$$

where R is the ideal gas constant and T is the temperature. For an electrospray experiment, T is simply the room temperature. This change in free energy represents the extent to which a mixed-ligand complex is energetically favored relative to the two homo-ligand complexes. The ΔG_{EX} values in our study are calculated from ESI mass spectra obtained for solutions containing two different macrocycles, thus allowing direct comparison of the abundances of each homo-ligand sandwich complex to the single mixed-ligand sandwich complex and providing a self-consistent standardization of the spectra. Large positive ΔG_{EX} values signify that the homo-ligand complexes are favored, near-zero values mean that the homo-ligand and mixed-ligand com-

12-crown-4
(176 Da)

15-crown-5
(220 Da)

18-crown-6
(264 Da)

aza-18-crown-6
(263 Da)

benzo-18-crown-6
(312 Da)

dibenzo-18-crown-6
(360 Da)

dicyclohexano-18-crown-6
(372 Da)

Scheme 2

plexes are equally favored, and large negative values reflect a strong preference for formation of mixed-ligand sandwich complexes.

To differentiate the importance of π-stacking interactions from other types of electrostatic interactions, several reference macrocycles were chosen for comparison of their interactions with **1** (see Scheme **2**). 18-Crown-6 and aza-18-crown-6 were selected because these ligands have no functional groups capable of interacting with the quinoxaline moiety of **1**. Benzo-18-crown-6 and dibenzo-18-crown-6 were selected as reference ligands because they have similar metal binding properties, but their aromatic substituents could promote π-stacking interactions with **1**. Dicyclohexano-18-crown-6 has a similar size and shape to that of dibenzo-18-crown-6 but its substituents are not aromatic, thus allowing a way to evaluate the possibility that favorable steric interactions enhance sandwich formation rather than interactions between the aromatic groups. Admittedly, the three-dimensional structure of cyclohexane bears little resemblance to benzene, but as substituents on 18-crown-6 both would add a similar degree of structural rigidity and have approximately the same degree of "bulk" extending from the crown ether.

As nearly ideal singly-charged spheres, the alkali metals Li^+, Na^+, K^+, Rb^+, and Cs^+ were analyzed with all combinations of macrocycles in solution to evaluate the periodic trends in the size-selectivity of sandwich formation. Other metals studied included the alkaline earths Sr^{2+} and Ba^{2+}, chosen because their ionic radii are similar to Na^+ and K^+ yet with much greater charge densities, as well as Ag^+ which is known to have particular affinity for nitrogen-containing compounds [47, 48] and has a size between that of Na^+ and K^+. Finally, formation of sandwich complexes with NH_4^+, a nonmetallic ion with a size similar to that of Rb^+, were examined in order to evaluate the activity of the self-assembly process over a wider range of cations.

As an initial control experiment, **1** was analyzed alone in methanol as shown in Figure 1a. In this spectrum, protonated **1** was the dominant ion, and lesser amounts of $(\mathbf{1} + Na^+)$ and $(\mathbf{1} + K^+)$ complexes were also observed, presumably because of the presence of background solvent contaminants or ionic contaminants leached from the glassware used (Figure 1a). The complete absence of sandwich complexes demonstrated that the levels of ionic contaminants in the solvents used are not sufficient to promote sandwich
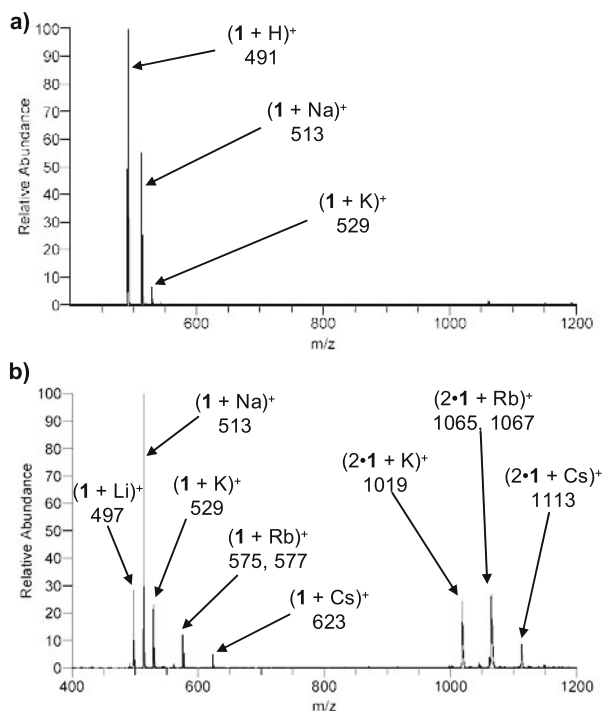
a)

$(\mathbf{1} + H)^+$
491

$(\mathbf{1} + Na)^+$
513

$(\mathbf{1} + K)^+$
529

b)

$(\mathbf{1} + Li)^+$
497

$(\mathbf{1} + Na)^+$
513

$(\mathbf{1} + K)^+$
529

$(\mathbf{1} + Rb)^+$
575, 577

$(\mathbf{1} + Cs)^+$
623

$(2{\cdot}\mathbf{1} + K)^+$
1019

$(2{\cdot}\mathbf{1} + Rb)^+$
1065, 1067

$(2{\cdot}\mathbf{1} + Cs)^+$
1113

Figure 1. ESI-mass spectrum of (**a**) **1** in methanol, and (**b**) **1** with equimolar LiCl, NaCl, KCl, RbCl, and CsCl in methanol.

Table 1. Reported binding constants in methanol for the first and second attachment of either 12-crown-4 (A) or 15-crown-5 (B) to Na^+ or K^+ and free energy change for the ligand exchange reaction for solutions containing both ligands with one of the cations calculated from the ESI-MS measurements. Errors reflect statistical deviation over three replicate measurements

Cation	log K^a				ΔG_{EX} (kJ/mol)
	MA	MA_2	MB	MB_2	
Na^+	1.5	2.2	3.32	2.5	-0.5 ± 0.2
K^+	1.60	1.45^b	3.5	2.5	-1.5 ± 0.1

[a]From reference [27].
[b]From reference [49].

complex formation, eliminating the possibly of contaminant-related errors.

ESI-MS analysis of **1** alone in solution with alkali metal ions added at equimolar concentrations indicates that in the formation of 1:1 macrocycle:metal complexes, **1** has a greater affinity for Na^+ over that of Li^+, K^+, Rb^+, or Cs^+, assuming similar electrospray efficiencies (Figure 1b). $(2 \cdot 1 + K)^+$, $(2 \cdot 1 + Rb)^+$, and $(2 \cdot 1 + Cs)^+$ sandwich complexes are also formed, but sandwiches incorporating Li^+ or Na^+ are not observed because the smaller sizes of Li^+ and Na^+ allow them to be encapsulated within the cavity of **1**, thus preventing the favorable coordination of a second ligand. The larger sizes of K^+, Rb^+, and Cs^+ make them perch partially out of the cavity of **1** and permit the complexation of a second ligand.

Analysis of Model Sandwich Complexes

To confirm the validity of the approach outlined above for evaluating the formation of mixed-ligand sandwich complexes, two model systems were evaluated: sandwich complexes of 12-crown-4, 15-crown-5, and either Na^+ or K^+. Both ligands are known to form homo-ligand sandwich complexes readily [27]. Furthermore, these two ligands are both small and likely have little interaction with the adjacent ligand in a sandwich complex. In an ideal case, if the binding of ligands to a metal were completely independent, the first and second binding constants, K_1 and K_2, would be equal. As seen in Table 1, for each of these ligands with either Na^+ or K^+, K_1 and K_2 values are close to each other. In the absence of any specific interactions between the ligands, the binding in the analogous mixed-ligand sandwich complexes should be nearly independent as well. As a consequence, there should be no significant difference energetically between the homo- and mixed-ligand complexes in a ligand exchange reaction like in eq 1. In other words, with 12-crown-4, 15-crown-5, and either Na^+ or K^+, the value of ΔG_{EX} measured from the ESI mass spectra and calculated with eq 6 should be near zero. As summarized in Table 1, with Na^+, a ΔG_{EX} of -0.5 kJ/mol was measured, and for K^+, ΔG_{EX} was -1.5 kJ/mol, with both values validating our approach.

Sandwich Complexes of Dibenzo-18-Crown-6 with Different Macrocycles

As a first step towards evaluating the propensity of the various crown ethers to form mixed-ligand sandwich complexes, ESI mass spectra were collected for mixtures of dibenzo-18-crown-6, KCl, and various other reference macrocycles, all crown ethers. In these spectra, and all others used for quantitative measurements of the sandwich complexes, the mass ranges were restricted to the region containing the sandwich to avoid space-charge effects in the ion trap from the often abundant 1:1 complexes. From these spectra, the free energy of each ligand exchange reaction between the mixed- and homo-ligand complexes was calculated using eq 6 and listed in Table 2. Of the five other crown ether ligands evaluated, four yielded ΔG_{EX} values near zero, indicating nearly equal preference for formation of both the homo- and mixed-ligand complexes and suggesting the lack of any special ligand interactions that specifically favor formation of mixed-ligand complexes. The smaller size of 15-crown-5 prevents it from fully encapsulating K^+, thus allowing the formation of both mixed-ligand and homo-ligand complexes in the presence of dibenzo-18-crown-6 For experiments involving aza-18-crown-6 and dibenzo-18-crown-6, the amino proton on aza-18-crown-6 introduces a degree of electrostatic repulsion between the crown and the metal, thus preventing complete encapsulation of K^+ and allowing formation of stable mixed-ligand complexes. With either dicyclohexano-18-crown-6 or benzo-18-crown-6, the substituents make both ligands more rigid, resulting in no significant difference in stability of the homo-ligand complexes relative to the mixed-ligand complexes as evidenced by the ΔG_{EX} values near zero (Table 2). None of these four ligands displayed any special enhancement in the formation of mixed-ligand complexes with K^+ and dibenzo-18-crown-6, in striking contrast to the results described below (and also summarized in Table 2) for sandwich formation involving ligand **1**.

The one notable exception in the propensity for formation of mixed-ligand sandwich complexes was for the experiments involving dibenzo-18-crown-6 and 18-crown-6 with K^+, in which the spectra exhibited a

Table 2. Free energy change for the ligand exchange reaction for solutions of either 1 or dibenzo-18-crown-6, K^+ and a second macrocycle. Errors reflect statistical deviation over three replicate measurements

Second macrocycle	ΔG_{EX} (kJ/mol)	
	1	Dibenzo-18-crown-6
15-Crown-5	8.6 ± 0.8	0.0 ± 0.7
18-Crown-6	7.2 ± 0.8	11 ± 1
Aza-18-Crown-6	7.6 ± 0.2	0.0 ± 0.5
Dicyclohexano-18-crown-6	3.74 ± 0.08	1.3 ± 1.3
Benzo-18-crown-6	-8.57 ± 0.03	-0.7 ± 0.3
Dibenzo-18-crown-6	-7.8 ± 0.1	–

marked preference against formation of the mixed-ligand complexes [i.e., (dibenzo-18-crown-6 + 18-crown-6 + K^+)] , with a ΔG_{EX} value of 11 kJ/mol calculated from the mass spectrum. 18-Crown-6 is both large enough and flexible enough to encapsulate the potassium cation, thus prohibiting the coordination of a second ligand and quenching the formation of sandwich complexes.

Sandwich Complexes Involving 1 with Different Macrocycles

To determine the degree of enhancement in the formation of mixed-ligand sandwich complexes attributable to π-stacking interactions, solutions containing KCl, **1** and various other reference macrocycles were analyzed. The ESI mass spectra shown in Figure 2 display the types of product ion distributions observed upon analysis of solutions containing **1**, a second macrocycle, and an alkali metal salt, all at equimolar concentrations. For example, in the spectrum shown in Figure 2a for **1**, 18-crown-6, and KCl, the homo-ligand $(2 \cdot \mathbf{1} + K)^+$ complex is abundant, but the abundances of the sandwich complexes containing 18-crown-6, i.e., $(2 \cdot 18\text{-}crown\text{-}6 + K)^+$ and $(\mathbf{1} + 18\text{-}crown\text{-}6 + K)^+$, are very low. The ΔG_{EX} value calculated from this mass spectrum is 7.2 kJ/mol (Table 2), and the large positive value conveys the preference for formation of the homo-ligand $(2 \cdot \mathbf{1} + K)^+$ complexes over the mixed-ligand $(\mathbf{1} + 18\text{-}crown\text{-}6 + K)^+$ complexes. When the solution containing **1** dibenzo-18-crown-6 and K^+ was analyzed (Figure 2b), the mixed ligand complex, $(\mathbf{1} + $ dibenzo-18-crown-6 + $K)^+$, dominated the spectrum. Its greater abundance relative to the homo-ligand complexes, $(2 \cdot$ dibenzo-18-crown-6 + $K)^+$ and $(2 \cdot \mathbf{1} + K)^+$, results in a ΔG_{EX} of −7.8 kJ/mol that reflects the marked enhancement of the mixed-ligand sandwich complexes attributable to cooperative interactions between the quinoxaline group on **1** and one of the benzene groups on dibenzo-18-crown-6. For the solution containing **1** and dibenzo-18-crown-6 with RbCl instead of KCl (Figure 2c), the mixed-ligand sandwich complex, i.e., $(\mathbf{1} + $ dibenzo-18-crown-6 + $Rb)^+$, again dominated the spectrum, but the preference for formation of the mixed-ligand sandwiches was not as great as observed in Figure 2b, and the calculated ΔG_{EX} value was lower (−4.9 kcal/mol in Table 3). We speculate that the ΔG_{EX} value diminishes on going from K^+ to Rb^+ because the greater size of Rb^+ reduces the overlap of the π-orbitals between **1** and dibenzo-18-crown-6.

The ΔG_{EX} values obtained from this series of ESI-MS experiments are summarized in Table 2. For 15-crown-5, 18-crown-6, and aza-18-crown-6, the ΔG_{EX} values are positive, indicating that the formation of mixed-ligand sandwiches is disfavored relative to the formation of the homo-ligand complexes. With no additional functional groups on these three macrocycles, these were not expected to engage in
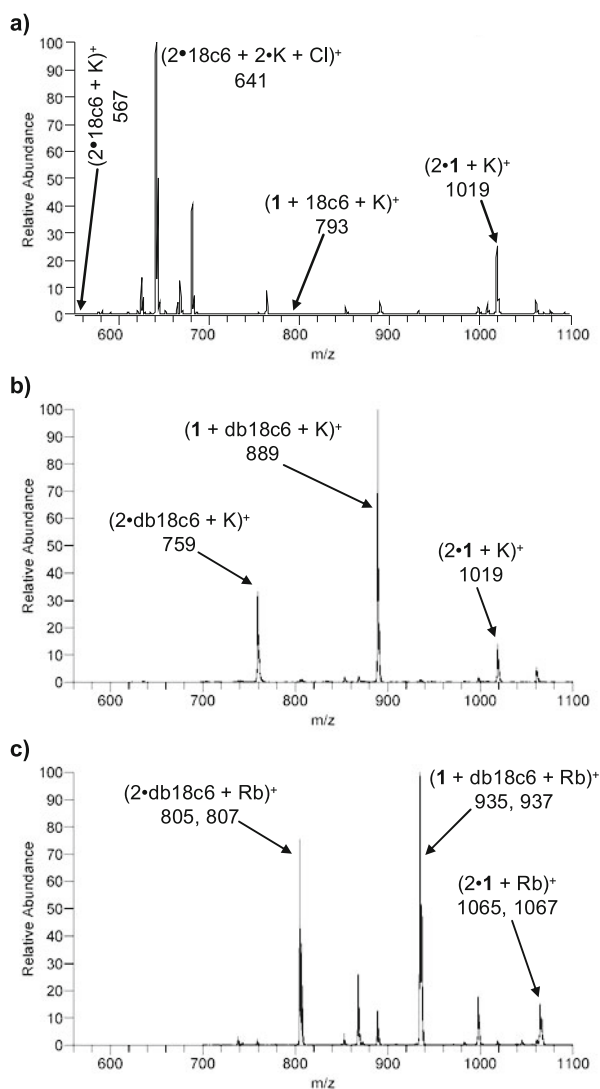

Figure 2. ESI-mass spectra of **1**, a second macrocycle, and a metal ion in methanol. (**a**) **1**, 18-crown-6 (18c6) and K^+, (**b**) **1**, dibenzo-18-crown-6 (db18c6), and K^+, and (**c**) **1**, dibenzo-18-crown-6 and Rb^+. No data was collected below 700 Th in (**c**) to allow the sandwich complexes to be analyzed without peak broadening due to space-charge effects.

any special interactions with **1**, other than the typical electrostatic interactions between the positive metal ion and the heteroatom donor groups that would specifically strengthen the mixed-ligand sandwich complexes. Dicyclohexano-18-crown-6, of similar size as dibenzo-18-crown-6, was evaluated to ensure that a particularly favorable steric situation was not responsible for any observed enhancement. The ΔG_{EX} with dicyclohexano-18-crown-6 was +3.74 kJ/mol, again indicating that the formation of the mixed-ligand sandwich complex is disfavored.

The complexation of **1** and K^+ with two macrocycles possessing aromatic functional groups was also examined: benzo-18-crown-6 and dibenzo-18-crown-6. These two macrocycles have benzene groups capable of donating electron density to the quinoxaline moiety on **1**.

Table 3. Effect on ΔG_{EX} values for mixed-ligand sandwich complexes of 1 and either dibenzo-18-crown-6 or 18-crown-6 with various cations in methanol. Concentrations were 50 μM in all cases. Errors reflect statistical deviation over three replicate measurements

Cation	Ionic Radii [50] (pm)	ΔG_{EX} (kJ/mol)	
		dibenzo-18-crown-6	18-crown-6
Na^+	116	-2.6 ± 0.5	7.9 ± 0.8
K^+	152	-7.8 ± 0.1	7.2 ± 0.8
Rb^+	166	-4.9 ± 0.3	3.6 ± 0.4
Cs^+	181	-0.6 ± 0.8	5.7 ± 0.3
Sr^{2+}	127	0.5 ± 0.7	-10.9 ± 0.7
Ba^{2+}	143	-3.0 ± 0.3	-5.2 ± 0.9
Ag^+	129	2.4 ± 0.5	4 ± 2
NH_4^+	161	-5.4 ± 0.5	3.6 ± 0.9

With benzo-18-crown-6, a ΔG_{EX} of -8.57 kJ/mol was found (Table 2), indicating a significant enhancement in the formation of mixed-ligand sandwiches over that observed for the previous macrocycles. For dibenzo-18-crown-6 with 1 and K^+, a ΔG_{EX} of -7.8 kJ/mol was measured. These values indicate that with either benzo-18-crown-6 or dibenzo-18-crown-6 the mixed-ligand sandwich complexes with 1 are very strongly favored. We speculate that the small increase in ΔG_{EX} for the sandwich formation involving 1 and benzo-18-crown-6 versus 1 and dibenzo-18-crown-6 is due to steric hindrance between the bulky cage group on 1 and the second benzo-group on dibenzo-18-crown-6—a hindrance not present with benzo-18-crown-6. The reactions of 1 with benzo-18-crown-6 and dibenzo-18-crown-6 also suggest that the cage group on 1, included as an anchor point to allow attachment of the macrocycle to a polymer or surface, does not interfere with the π-stacking interactions.

In addition to the positive ΔG_{EX} values obtained upon reaction of 1 and K^+ with the three reference macrocycles that did not possess aromatic groups, all of the observed sandwich complexes with the reference macrocycles had very low abundances in the mass spectra. These abundances were typically one to two orders of magnitude less than that of sandwich complexes formed with macrocycles containing aromatic functional groups, leading to the conclusion that even without any enhancement due to donor-acceptor interactions, π-stacking interactions significantly stabilize the formation of crown ether-metal sandwich complexes.

Sandwich Complexes Involving 1 with Different Cations

The formation of sandwich complexes containing different cations was examined in order to investigate the influence of the size and charge density of the cation on the self-assembly process. The Pauling ionic radii of the cations are listed in Table 3. When solutions containing

1, dibenzo-18-crown-6, and each of the alkali metal chlorides were analyzed, a clear periodic trend emerged in the distribution of mixed-ligand sandwiches relative to the homo-ligand complexes as shown by the data in Figure 3 and Table 3. For the largest ion, Cs^+, the ΔG_{EX} value was near zero, indicating that there was no significant preference for the formation of mixed-ligand sandwiches. The smallest ion, Li^+, resulted in complexes that had very low abundances in the mass spectra, likely attributable to the poor ability of large crown ethers to form sandwich complexes with Li^+ [27], presumably because of a mismatch between the ion and cavity sizes. Because of the low abundances of complexes, ΔG_{EX} values could not be calculated in most cases and were therefore omitted from Table 3. The largest enhancement was seen with K^+, reaching a ΔG_{EX} value of -7.8 kJ/mol, while Na^+ and Rb^+ showed more modest enhancements in the formation of the mixed-ligand sandwich complexes. These results contrast starkly with solutions containing 1 and 18-crown-6 (Table 3, last column). The ΔG_{EX} values obtained upon analysis of solutions containing 1, 18-crown-6, and an alkali metal ion summarized graphically in Figure 3, were significantly positive with all the alkali metals, indicating a consistent preference against formation of the mixed-ligand complexes regardless of the size of the metal ion.

The large enhancement of mixed-ligand sandwich complexes between 1 and dibenzo-18-crown-6 when K^+ in the cation is likely the result of several size-related effects. Periodic trends are well known for 1:1 crown ether:metal complexes [7, 11, 24, 26–28], with binding affinities in solution typically increasing as ions become large enough to interact optimally with more of the potential coordinating sites (oxygen heteroatoms). This size-selective effect is even more pronounced for sandwich complexes because larger ions are not encapsulated by a single macrocycle, thus facilitating coordination by two ligands. As a countering effect seen in the
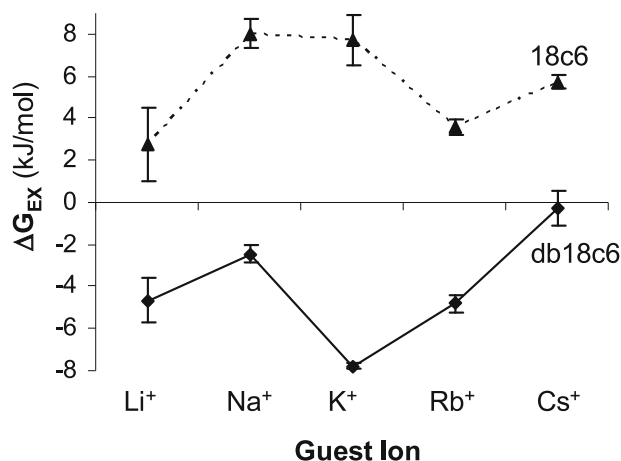

Figure 3. ΔG_{EX} for mixed-ligand sandwich complexes of 1 and either dibenzo-18-crown-6 (solid line) or 18-crown-6 (dotted line) with different alkali metal cations in methanol.

present work, with very large ions such as Cs^+, the spacing between the macrocyclic rings increases, reducing the overlap between the π-orbitals on the aromatic substituents of the benzo-crown ethers and **1** and decreasing the strength of their interactions.

In addition to the alkali metals, sandwich formation involving several other cations was evaluated as well. The alkaline earth cation Sr^{2+}, with an ionic radius comparable to Na^+, demonstrated no significant preference for the formation of mixed-ligand sandwiches involving **1** and dibenzo-18-crown-6, while with Ba^{2+}, similar in size to K^+, only a modestly negative ΔG_{EX} was observed. Both metals, however, resulted in lower ΔG_{EX} values for sandwich complexes incorporating **1** and 18-crown-6 (Table 3). The greater charge of these cations likely results in a shift in the relative contributions of the binding modes. Because of stronger electrostatic attractions between the doubly charged metal ions and the coordinating oxygen atoms, each macrocycle in a sandwich complex would be drawn closer to the metal ion and closer to the second coordinating ligand. The expected steric hindrance between the quinoxaline and cage groups on **1** and the benzene groups of dibenzo-18-crown-6 likely becomes a punitive factor, causing a decrease in the formation of mixed-ligand sandwiches. 18-Crown-6 lacks any bulky substituent, and thus the enhancement in sandwich formation due to the higher charge density of the alkaline earth metal ion is not mitigated by counterproductive steric effects.

To evaluate whether the complexation of **1** was influenced directly by the quinoxaline moiety on **1**, sandwich complex formation with Ag^+ was examined (Table 3). With silver's known affinity for nitrogen heteroatoms [47, 48], an enhancement of sandwich formation might be expected relative to that observed for complexation of K^+, which has a similar ionic radius and no special affinity for nitrogen. No such enhancement is observed, however, and the positive ΔG_{EX} values observed for Ag^+ with **1** and either dibenzo-18-crown-6 (2.4 kJ/mol) or 18-crown-6 (4 kJ/mol) indicate that silver is a poor choice overall for mixed-ligand sandwich complex formation. This result may be related to the general preference of Ag^+ to adopt a linear coordination geometry which creates an orbital alignment problem when forming sandwich complexes [50].

Finally, to determine whether cations other than metals could result in enhancement in mixed-ligand sandwich formation, solutions containing **1**, a second macrocycle, and the ammonium ion were analyzed. With an ionic radius similar to Rb^+, a roughly spherical shape, and a closed valence, NH_4^+ led to a significantly negative ΔG_{EX} value (−5.4 kJ/mol) for the mixed-ligand sandwich complexes containing **1** and dibenzo-18-crown-6, its enhancement second only to K^+ in this study (Table 3). Since NH_4^+ binds to crown ethers though hydrogen bonding interactions rather than metal-ligand coordination, this result suggests that the use of donor-acceptor π-stacking to direct self-assembly
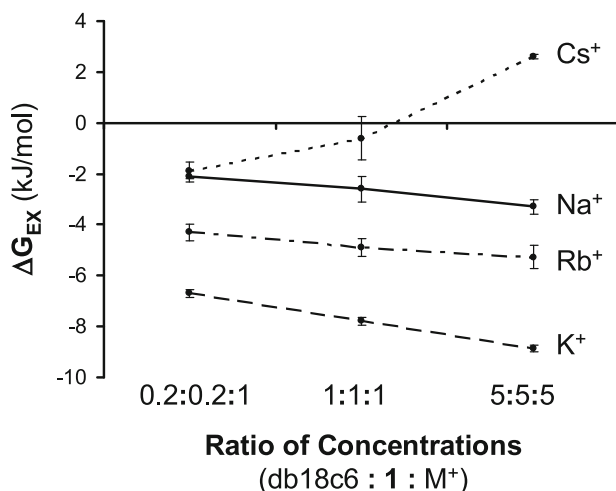

Figure 4. Result of measuring ΔG_{EX} at different relative concentrations of the ligands (dibenzo-18-crown-6 and **1**) and alkali metal cations. Errors reflect statistical deviation over three replicate measurements.

of sandwich complexes might be possible with a wide range of cations.

Concentration Dependence of ΔG_{EX} Measurements

A common endeavor when evaluating the binding of novel ligands is to examine how the binding behavior changes with different relative concentrations of the individual species [7, 8, 10, 14, 16, 17]. One of the advantages of evaluating the binding information thermochemically, however, is that the results should display no dependence on solution concentrations. Accordingly, as a final test of the experimental method, **1** and dibenzo-18-crown-6 were again analyzed with the alkali metals as described above. This time, however, while the metal concentrations remained unchanged, solutions were analyzed in which the ligand concentrations were both increased and decreased fivefold. With Na^+, K^+, and Cs^+, the three metal cations that most readily formed mixed-ligand complexes, increasing ligand concentrations caused very small decreases in the calculated ΔG_{EX} values that were barely significant with respect to variations among the replicate measurements (Figure 4). With Cs^+, however, an increase in ΔG_{EX} was observed with increasing ligand concentrations, possibly the result of in-source fragmentation of the fairly weak Cs^+ sandwich complexes. Changing the relative concentration had virtually no effect on the measured values of ΔG_{EX} in most cases, illustrating the validity of these thermochemical measurements, particularly for the mixed-ligand complexes displaying the greatest enhancement in stability.

Conclusions

Through ESI-MS experiments, the novel crown ether **1** containing an electron-poor quinoxaline moiety was

demonstrated to form 2:1 macrocycle:cation sandwich complexes preferentially with secondary macrocycles containing electron-rich benzene substituents through donor-acceptor π-stacking interactions between the aromatic side groups. The magnitude of the enhancement of these mixed-ligand sandwich complexes was found to be highly dependent on the size of the encapsulated cation, with a preference for cations that maximize the effective interactions of each macrocycle with the cation yet minimize steric effects between the macrocycles. Similar experiments with dibenzo-18-crown-6 and K^+ yielded no additional stabilization of the mixed ligand complexes with any of the other crown ethers examined. That such donor-acceptor π-stacking interactions so readily enhance the formation of crown ether sandwich complexes confirms their utility as building blocks in self-assembled molecular structures. The ESI-MS strategy presented in this work should be applicable to those biological systems in which π-stacking interactions play a role.

Acknowledgments

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