# **Evaluation of Binding Selectivities of Caged Crown Ligands toward Heavy Metals by Electrospray Ionization/Quadrupole Ion Trap Mass Spectrometry**

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Electrospray ionization (ESI)/quadrupole ion trap mass spectrometry is used to evaluate the heavy metal binding selectivities of five caged crown ethers and two polyether reference compounds in methanol solution. The binding preferences for Hg<sup>2+</sup>, Pb<sup>2+</sup>, Cd<sup>2+</sup>, and Cu<sup>2+</sup> were analyzed by comparison of ESI mass spectral intensities with the aim of developing this method for the rapid screening of binding selectivities of new synthetic ligands. The cage compounds preferentially bind Hg<sup>2+</sup>, except for the cage cryptand derivative, which favors Pb<sup>2+</sup>. The preference for Hg<sup>2+</sup> stems from the favorable positioning of the nitrogen or sulfur atoms for linear coordination of Hg<sup>2+</sup>, whereas the cryptand derivative favors Pb<sup>2+</sup> because of its larger cavity size. The counterions of the metal salts influence the type of complexes observed in the ESI mass spectra because the strengths of the metal-anion bonds affect retention of the anion in the complexes.

The development of new methods to explore structure/binding selectivity relationships in host-guest complexation has become increasingly important due to the wide variety of practical applications of molecular recognition in areas such as drug design and environmental chemistry.<sup>1</sup> The present study focuses on the measurement of heavy metal binding selectivities of novel synthetic host compounds (Figure 1), caged crowns, by a promising method<sup>2</sup> based on electrospray ionization mass spectrometry (ESI-MS).<sup>3</sup> Binding selectivities have traditionally been measured by

potentiometric, NMR, extraction, or UV–vis methods.<sup>4</sup> However, the conventional methods are not appropriate in all solvent systems, do not uniformly give structural information, and can require as much as 100–1000 times more analyte than ESI-MS. ESI-MS offers an alternative way to estimate binding selectivities and provides rapid feedback with minimal sample consumption. The use of electrospray ionization (ESI) mass spectrometry for the evaluation of selectivity in host–guest complexation has been validated previously for polyether compounds, such as crown ethers and cryptands, as well as for the estimation of binding selectivities involving alkali metals and a few transition metals.<sup>2,5</sup> Excellent correlation between the ESI mass spectral data obtained by monitoring relative intensities of host–guest complexes and expected binding selectivities was observed for solutions containing a single host with two metal cations.<sup>4</sup>

In the present study, the binding selectivities of the compounds in Figure 1 for several heavy metals, including  $Hg^{2+}$ ,  $Pb^{2+}$ ,  $Cd^{2+}$ . and  $Cu^{2+}$ , are analyzed in methanolic solutions by comparison of ESI mass spectral intensities. The primary aim is to report for the first time the use of ESI-MS for studies involving screening of novel synthetic ligands for the selective complexation of heavy metals. The caged crowns are designed to complex heavy metals for the selective removal of these toxic metals from waste. Heavy metals accumulate in the environment from many industrial sources,<sup>6</sup> and these heavy metals inhibit metabolism, break down the tissues of many organs, or result in neurological poisoning when accumulated in the body.<sup>6</sup> Much effort is currently

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1,7-Diaza-15-Crown-5

Figure 1. Cage and reference compound structures.

devoted to the development of new synthetic materials for the selective extraction of metals from solution,<sup>7</sup> and thus evaluation of the binding properties of novel ligands is an active area of research.

The analytical strategy presented herein involves mass spectrometric examination of numerous solutions containing one or two host ligands and one or more metals, along with extensive variation of counterions and ionic strengths, to develop a solid framework for using the ESI-MS method in the context of heavy metal binding studies. Experiments with corresponding "reference" compounds including a cryptand and a diaza-crown ether were performed to further understand the factors that influence the metal binding selectivities, such as the preferred coordination geometry and size of the metal, cavity size of the ligand, and variation in the metal counterions. The preferred coordination geometry of mercury is linear, unlike those of the other heavy metals, which prefer octahedral coordination geometries.8 Mercury(II) with linear coordination has a diameter of 0.69 Å, but in its octahedral coordination state, it has a diameter of 1.02 Å.8 Copper, cadmium, and lead have diameters of 0.73, 0.95, and 1.19 Å, respectively, in octahedral binding geometries.<sup>9</sup>

The two reference compounds were chosen because they possess structures that share features to those of the novel caged ligands of interest in this study and because they have been examined previously by conventional methods, thus allowing facile comparison with the present results. Schwing-Weill et al.<sup>10</sup> determined the stability constants for Pb<sup>2+</sup>, Cu<sup>2+</sup>, and Cd<sup>2+</sup> with 1,7-diaza-15-crown-5 (D15C5), 1,4,10-trioxa-7,13-diazacyclopentadecane, and a [2.2.1]-cryptand (221), 4,7,13,16,21-pentaoxa-1,10diazabicyclo[8.8.5]tricosane, in methanolic solutions using conventional potentiometric and spectrophotometric methods. It was found that the host with the smaller binding cavity, D15C5, preferred to bind with the smaller metal cations in the following trend:  $Cu^{2+} > Cd^{2+} > Pb^{2+}$ . Likewise, the larger cavity host, 221, selectively complexed the larger metal cations in the following order:  $Pb^{2+} > Cd^{2+} > Cu^{2+}$ . Binding studies using aqueous

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titrimetric methods were performed by Gramain et al. with the reference compound 1,7-diaza-15-crown-5 (D15C5).<sup>11</sup> Results showed that D15C5 preferentially complexed Cu<sup>2+</sup> and Cd<sup>2+</sup> over other divalent and monovalent metal cations.<sup>12</sup> Anderegg et al. determined the aqueous stability constants for a compound similar to D15C5, 1,10-diaza-18-crown-6, and found that the mercury complex had a significantly higher stability constant than the cadmium complex.<sup>12</sup> Marchand et al. studied the alkali metal picrate extraction behavior of the caged cryptand compound **12** (Figure 1) and found that **12** selectively extracted Na<sup>+</sup> and K<sup>+</sup> picrates in preference to other alkali metals.<sup>13</sup>

#### **EXPERIMENTAL METHODS**

All mass spectrometry experiments were performed on a Finnigan ion trap operating in the mass-selective instability mode with modified electronics to allow axial modulation.<sup>14</sup> The electrospray interface is based on a design developed by Oak Ridge National Laboratory.<sup>15</sup> The Harvard syringe pump system was set at 2.0–3.0  $\mu$ L/min. The needle potential for the methanol solutions ranged from 3.2 to 3.3 kV for all experiments. The vacuum chamber was operated at a pressure of 1 mTorr with He. Each spectrum taken was an average of 25–30 scans. All experimental conditions were held constant throughout each series of experiments to minimize differences arising from inconsistencies in ion formation or transmission.

All solutions were prepared in 100% methanol. The chloride or perchlorate salts were mixed in solution with the host of interest to form the complexes studied. Host–guest solutions containing a single host and a single guest had a concentration ratio of 1:1. All single host—multiple guest solutions had a concentration ratio of 1:1:1:1:1. Typical concentrations of hosts or metal salts were  $1.5 \times 10^{-4}-2 \times 10^{-4}$  M. Molecular modeling of the host compounds was performed using SymApps software, which is based on the MM2 force field. All metal salt compounds were purchased from Aldrich Chemical Co. (Milwaukee, WI) and used without further purification. All caged compounds were synthesized in the Marchand group, and the two main synthetic pathways are outlined in Schemes 1 and 2.

Melting points are uncorrected. Elemental microanalyses were performed by personnel at M-H-W Laboratories, Phoenix, AZ. High-resolution mass spectral data for **11** were obtained at the Mass Spectrometry Facility, Department of Chemistry and Biochemistry, The University of Texas at Austin, by using a ZAB-E double-sector high-resolution mass spectrometer (Micromass, Manchester, England) that was operated in the chemical ionization mode.

3,5-Bis(2-bromoethyl)-4-oxahexacyclo[5.4.1.0<sup>2,6</sup>.0<sup>3,10</sup> .0<sup>5,9</sup>.0<sup>8,11</sup> dodecane (3). Method A. A solution of 3,5-divinyl-4-oxahexacyclo[5.4.1.0<sup>2,6</sup>.0<sup>3,10</sup>.0<sup>5,9</sup>.0<sup>8,11</sup>]dodecane<sup>16</sup> (1, 500 mg, 2.36 mmol) in hexane (10 mL) was cooled to 10 °C via application of an external ice-water bath. To this cooled solution was added, with stirring, benzoyl peroxide (57 mg, 0.23 mmol). Hydrogen bromide gas, generated via reaction of Br<sub>2</sub> (3 mL, 58 mmol, excess) and tetralin (4 mL, 29 mmol, excess), was bubbled through the cooled reaction mixture during 0.5 h. The resulting mixture was poured into a separatory funnel and then was washed sequentially with 10% aqueous NaHCO<sub>3</sub> (5 mL) and water (5 mL). The organic layer was dried (MgSO<sub>4</sub>) and filtered, and the filtrate was concentrated in vacuo. The residue was purified via column chromatography on silica gel by eluting with hexane. Pure 3 (720 mg, 82%) was thereby obtained as a colorless oil: IR (neat) 2980 (s), 1428 (m), 1132 cm<sup>-1</sup> (m); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.51 (AB, J =

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10.2 Hz, 1 H), 1.86 (AB, J = 10.2 Hz, 1 H), 2.28–2.70 (m, 12 H), 3.39 (t, J = 7.0 Hz, 4 H); <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  28.2 (t), 36.2 (t), 43.3 (d), 43.5 (t), 44.0 (d), 47.5 (d), 58.1 (d), 95.0 (s). Anal. Calcd for C<sub>15</sub>H<sub>18</sub>Br<sub>2</sub>O; C, 48.16: H, 4.85. Found: C, 48.53: H, 5.00.

Method B. A solution of 3,5-bis(2-hydroxyethyl)-4-oxahexacyclo-[5.4.1.0.<sup>2,6</sup>0.<sup>3,10</sup>0.<sup>5,9</sup>0.<sup>8,11</sup>]dodecane<sup>16</sup> (2, 300 mg, 1.25 mmol) in benzene (10 mL) was cooled to 0 °C via application of an external ice-water bath. To this cooled solution was added, with stirring, a solution of PBr<sub>3</sub> (146 mg, 0.54 mmol) in benzene (3 mL). After the addition of the brominating agent had been completed, the external ice-water bath was removed, and the stirred reaction mixture was allowed to warm gradually to ambient temperature during 12 h. The reaction was quenched via addition of water (5 mL). The organic layer was separated from the mixture, washed with water (5 mL), dried (MgSO<sub>4</sub>), and filtered, after which the filtrate was concentrated in vacuo. The residue was purified by column chromatography on silica gel by eluting with hexane. Pure 3 (285 mg, 61%) was thereby obtained as a colorless oil. The IR,<sup>1</sup>H NMR, and <sup>13</sup>C NMR spectra of this material were essentially identical to the corresponding spectra obtained for 3 that had been prepared previously via method A (vide supra).

**3**, **5** - **B** is (2 - m er c a p t o e thyl) - 4 - o x a h e x a c y c lo-[5.4.1.0<sup>2,6</sup>.0<sup>3,10</sup>.0<sup>5,9</sup>.0<sup>8,11</sup>]dodecane (4).<sup>17</sup> To a suspension of **3** (830 mg, 2.31 mmol) in water (10 mL) was added thiourea (458 mg, 6.02 mmol), and the resulting mixture was refluxed for 6 h. To the hot reaction mixture was added 10% aqueous NaOH (2 mL, 5 mmol), and the resulting mixture was refluxed for an additional 4 h. The reaction mixture was allowed to cool gradually to ambient temperature and then was acidified by addition of 10% aqueous H<sub>2</sub>SO<sub>4</sub> (2.5 mL). The resulting mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 20 mL). The combined organic extracts were washed with water (10 mL), dried (MgSO<sub>4</sub>), and filtered, and the filtrate was concentrated in vacuo. The residue was purified via column chromatography on silica gel by eluting with 10% EtOAc– CH<sub>2</sub>Cl<sub>2</sub>. Pure **4** (430 mg, 67%) was thereby obtained as a colorless

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Crown Ether 6. To a suspension of NaH (37 mg, 1.56 mmol) in dry THF (10 mL) under argon was added with stirring dithiol 4 (200 mg, 0.71 mmol), and the resulting mixture was stirred at ambient temperature for 0.5 h. To this mixture was then added dropwise with stirring during 0.5 h a solution of triethylene glycol ditosylate (5, 327 mg, 0.71 mmol) in dry THF (20 mL). The resulting mixture was refluxed for 2 days and then was allowed to cool gradually to ambient temperature. The reaction mixture was extracted with  $CH_2Cl_2$  (4 × 25 mL). The combined organic extracts were dried (MgSO<sub>4</sub>) and filtered, and the filtrate was concentrated in vacuo. The residue was purified via column chromatography on neutral alumina by eluting with 10% EtOAc-CH<sub>2</sub>Cl<sub>2</sub>. Pure 6 (260 mg, 43%) was thereby obtained as a colorless oil: IR (film) 2978 (s), 1428 (m), 1220 cm<sup>-1</sup> (m); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.51 (*AB*,  $J_{AB} = 10.1$  Hz, 1 H), 1.84 (*AB*,  $J_{AB} = 10.1$  Hz, 1 H), 1.94-2.10 (m, 6 H), 2.32-2.78 (m, 14 H), 3.60 (s, 4 H), 3.69 (t, J = 7.6 Hz, 4 H); <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  27.4 (t), 30.7 (t), 33.1 (t), 41.3 (d), 43.5 (t), 43.8(d), 47.7(d), 58.5 (d), 70.6 (t), 72.0 (t), 95.0 (s). Anal. Calcd for C<sub>21</sub>H<sub>30</sub>O<sub>3</sub>S<sub>2</sub>: C, 63.92; H, 7.66. Found: C, 63.67; H, 7.39.

**Crown Ether 8 (R = CH<sub>2</sub>Ph).** To a solution of **3** (740 mg, 1.98 mmol) and **7**<sup>18</sup> (542 mg, 1.65 mmol) in CH<sub>3</sub>CN (44 mL) were added sequentially Na<sub>2</sub>CO<sub>3</sub> (1.84 g, 16.5 mmol) and NaI (130 mg, 0.875 mmol), and the resulting mixture was refluxed for 24 h. The reaction mixture was allowed to cool to ambient temperature and then was concentrated in vacuo. The residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (30 mL), and the resulting solution was washed with water (3 × 30 mL). The organic layer was dried (MgSO<sub>4</sub>) and filtered, and the filtrate was concentrated in vacuo. The residue

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was purified via column chromatography on neutral alumina by eluting with 5:1 EtOAc-hexane. Pure **8** (610 mg, 63%) was thereby obtained as a colorless oil: IR (film) 2968 (s), 1630 (m), 1450 (m), 1148 (m), 730 (m), 700 cm<sup>-1</sup> (m); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.50 (*AB*,  $J_{AB} = 10.0$  Hz, 1 H), 1.78–2.04 (m, 5 H), 2.35 (s, 2 H), 2.50–2.70 (m, 10 H), 3.33 (s, 6 H), 3.45 (t, J = 6.6 Hz, 4 H), 3.55–3.62 (m, 8 H), 7.18–7.32 (m, 10 H); <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  30.3 (t), 41.9 (d), 44.2 (t), 44.4 (d), 48.6 (d), 51.0 (t), 53.6 (t), 59.2 (d), 59.4 (t), 95.4 (s), 127.3 (d), 128.7 (d), 129.4 (d), 140.4 (s). Anal. Calcd for C<sub>35</sub>H<sub>44</sub>N<sub>2</sub>O<sub>3</sub>: C, 77.74; H, 8.20. Found: C, 77.53; H, 7.96.

Hydrogenolysis of 8. To a solution of 8 (900 mg, 1.6 mmol) in CH<sub>3</sub>OH (70 mL) was added 10% palladized charcoal catalyst (200 mg, catalytic amount). The resulting mixture was subjected to hydrogenolysis by agitation with excess  $H_2(g)$  at 55 psi in a Parr hydrogenation apparatus at ambient temperature for 12 h. The reaction mixture was filtered through Celite, and the filtrate was concentrated in vacuo. The residue was purified via column chromatography on neutral alumina by eluting with 15% EtOAc-CH<sub>3</sub>OH. Pure 9 (480 mg, 83%) was thereby obtained as a colorless oil: IR (film) 3347 (br, m), 2982 (s), 1468 (m), 1350 (w), 1135 cm<sup>-1</sup> (m); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.52 (AB,  $J_{AB}$  = 9.9 Hz, 1 H), 1.75-2.02 (m, 5 H), 2.32 (s, 2 H), 2.41-2.60 (m, 8 H), 2.70-2.83 (m, 8H), 3.50-3.62 (m, 8 H); <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  31.8 (t), 41.2 (d), 43.4 (t), 43.7 (d), 46.2 (t), 47.6 (d), 49.2 (t), 58.2 (d), 70.0 (t), 71.0 (t), 96.2 (s). Anal. Calcd for C<sub>21</sub>H<sub>32</sub>N<sub>2</sub>O<sub>3</sub>: C, 69.97; H, 8.95. Found: C, 69.79; H, 8.97.

Crown Ether 10. To a solution of 9 (270 mg, 1.98 mmol) and 1-bromo-2-methoxyethane (550 mg, 3.96 mmol) in CH<sub>3</sub>CN (15 mL) under argon were added sequentially Na<sub>2</sub>CO<sub>3</sub> (740 mg, 3.33 mmol) and NaI (110 mg, 2.18 mmol), and the resulting mixture was refluxed for 65 h. The reaction mixture was allowed to cool gradually to ambient temperature. The reaction mixture was then filtered, and the filtrate was concentrated in vacuo. The residue was dissolved in CHCl<sub>3</sub> (20 mL), and the resulting solution was washed with water (3  $\times$  20 mL). The organic layer was dried (MgSO<sub>4</sub>) and filtered, and the filtrate was concentrated in vacuo. The residue was purified via column chromatography on neutral alumina by eluting with 20% EtOAc-hexane followed by continued elution of the chromatography column with 3% CH<sub>3</sub>OH-EtOAc. Compound 10 (190 mg, 73%) was thereby obtained as a colorless oil: IR (film) 2969 (s), 1461 (m), 1351 (w), 1116 cm<sup>-1</sup> (m); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.46 (AB,  $J_{AB} = 9.9$  Hz, 1 H), 1.74–2.02 (m, 5 H), 2.28 (s, 2 H), 2.38-3.79 (m, 17 H), 3.26 (s, 3 H), 3.36 (t, J = 5.6 Hz, 2 H), 3.45–3.60 (m, 8 H); <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  29.6 (t), 31.1 (t), 41.3 (2 C, d), 43.5 (t), 43.78 (d), 43.84 (d), 47.0 (t), 47.5 (d), 47.8 (d), 49.8 (t), 50.2 (t), 53.4 (2 C, t), 58.1 (d), 58.6 (d), 58.8 (d), 70.1 (t), 70.3 (t), 71.2 (t), 71.3 (t), 95.4 (s), 96.6 (s). Anal. Calcd for C<sub>24</sub>H<sub>38</sub>N<sub>2</sub>O<sub>4</sub>: C, 68.87; H, 9.15. Found: C, 68.96; H, 9.09.

**Crown Ether 11.** To a mixture of **9** (210 mg, 0.58 mmol) and  $Na_2CO_3$  (250 mg, 2.33 mmol) in CH<sub>3</sub>CN (10 mL) was added diethyl sulfate (180 mg, 1.17 mmol), and the resulting mixture was refluxed for 34 h. The reaction mixture was allowed to cool gradually to ambient temperature. The reaction mixture was then filtered, and the filtrate was concentrated in vacuo. Dichloromethane (10 mL) was added to the residue; the resulting mixture was filtered, and the filtrate was concentrated in vacuo. The residue was purified via column chromatography on neutral alumina by eluting with 10% EtOAc—hexane. Compound **11** (85

mg, 39%) was thereby obtained as a colorless oil: IR (film) 2969 (s), 1461 (m), 1370 (w), 1122 cm<sup>-1</sup> (m); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.03 (t, J = 6.7 Hz, 4 H), 1.51 (*A*B,  $J_{AB} = 10.5$  Hz, 1 H), 1.75–2.05 (m, 5 H), 2.35 (s, 2 H), 2.38–2.62 (m, 10 H), 2.66 (t, J = 5.4 Hz, 4 H), 2.76 (t, J = 6.2 Hz, 4 H), 3.55 (m, 10 H); <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  12.3 (q), 29.0 (t), 41.4 (t), 43.5 (t), 43.8 (d), 47.97 (t), 47.99 (2 C, d), 49.3 (t), 52.5 (t), 58.6 (d), 70.5 (t), 71.1 (t), 94.8 (s). Exact mass (CI HRMS), amu: calcd for C<sub>25</sub>H<sub>40</sub>N<sub>2</sub>O<sub>3</sub>,  $M_r^+$  416.303 894; found,  $M_r^+$  416.303 048.

# **RESULTS AND DISCUSSION**

Five similar caged aza-crown ether derivatives and two azapolyether reference compounds (Figure 1) were analyzed for their selectivities toward heavy metals. The novel, cage hosts each have a multicyclic cage substituent attached to an aza-polyether ring. The reference compounds, a crown ether and a cryptand, simulate the binding cavities of the cage compounds and are primarily used to undertake detailed studies of the effects of ionic strength, counterions, and other factors on the measurement of heavy metal binding selectivities by ESI-MS. Molecular models indicate that the array of binding sites in the rings of the reference compounds offer slightly more flexibility than those of the cage compounds. This greater degree of flexibility is due to the absence of the cagelike substituent. The cage substituent changes the geometry of the cavity. It provides additional rigidity, with a concomitant increase in preorganization compared to the corresponding noncage-annulated crown ether or cryptand, slightly expands the cavity size compared to the noncaged analogues, and also adds a hydrocarbon residue that increases the lipophilicity of the resulting host system, making the species largely insoluble in water. In addition, the inclusion of the cage substituent provides sites that can be used to attach the host ligand to a soluble polymer and thereby immobilize the host. This approach may be used productively to facilitate handling of the host-guest complexes and eventual recovery of the host ligands after use in the extraction procedures. The guest ions may be stripped from the host-guest complex, and the polymer-attached host system can then be recycled.

This study examines the structure/binding selectivity relationships of the cage compounds, including such structural aspects as host cavity size, and both the numbers and types of binding atoms and ring substituents. Each of the cage and reference compounds were mixed with heavy metal salts, such as  $Cd^{2+}$ ,  $Cu^{2+}$ ,  $Hg^{2+}$ , and  $Pb^{2+}$  chlorides or perchlorates, in methanol solution. The sums of the intensities of the various ligand-metal complexes in the ESI mass spectra were directly compared to determine the binding preferences and trends for each compound with each metal. Because conventional solution methods assume that the counterion is associated in some way with the hostmetal complex, both the free metal and the metal salt complexes in each ESI mass spectrum were considered when the binding selectivities were determined.

**Reference Compounds.** To evaluate the ability to determine binding selectivities for heavy metals and to probe the solution factors that may influence the ESI-MS measurements, a series of experiments involving two reference compounds, [2.2.1]-cryptand and 1,7-diaza-15-crown-5, were performed. The binding trends of these two compounds when studied with metal chloride salts mimic those seen with the cage compounds. The ESI mass

Table 1	. Dominant	Complexes	Observed i	n ESI	Mass	Spectra <sup>A</sup>
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Heavy Metal Salt	1,7-Diaza-15-Crown-5	[2.2.1]-Cryptand
CuCl <sub>2</sub>	$(D + CuCl)^{+}, (D + Cu)^{+}, (D + Cu)^{2+}$	В
CdCl <sub>2</sub>	$(D + CdCl)^+, (D + Cd)^{2+}$	$(C + CdCl)^{+}, (C + Cd)^{2+}$
HgCl <sub>2</sub>	$(D + HgCl)^+$	
PbCl <sub>2</sub>		$(C + PbCl)^{+}, (C + Pb)^{2+}$
Cu(ClO <sub>4</sub> ) <sub>2</sub>	$(D + Cu)^{2+}, (D + Cu)^{+}$	
Cd(ClO <sub>4</sub> ) <sub>2</sub>	$(D + Cd)^{2^+}, (D + CdClO_4)^+$	$(C + Cd)^{2+}$
Hg(ClO <sub>4</sub> ) <sub>2</sub>	$(D + Hg)^{2+}, (D + HgClO_4)^+$	
Pb(ClO <sub>4</sub> ) <sub>2</sub>		$(C + Pb)^{2+}, (C + PbClO_4)^+$
Cu(NO <sub>3</sub> ) <sub>2</sub>	$(D + Cu)^{2+}, (D + Cu)^{+}, (D + CuNO_3)^{+}$	
Cd(NO <sub>3</sub> ) <sub>2</sub>	$(D + Cd)^{2^+}, (D + CdNO_3)^+$	$(C + CdNO_3)^+$
Hg(NO <sub>3</sub> ) <sub>2</sub>	$(D + Hg)^{2^+}, (D + HgNO_3)^+$	$(C + HgNO_3)^+$
Pb(NO <sub>3</sub> ) <sub>2</sub>	$(D + Pb)^{2+}, (D + PbNO_3)^+$	$(C + PbNO_3)^+$

A. In order of decreasing intensity for each set of complexes.

B. Indicates no metal complexes were observed.

spectral trends observed for the perchlorate solutions do not always agree with the trends observed for the chloride salt solutions, as described later.

Metal Chloride Solutions. The examination of the reference compounds began with two sets of experiments. The first set concerned the evaluation of each reference compound mixed in a 1:1 solution with each metal chloride salt. A series of the spectra (one for each metal) were then compared to assess the complexation of the host compound under specific solution conditions and to examine any discrepancies that may have arisen from differences in the relative ESI response factors of the host-guest complexes. The spectra of the 1:1 solutions were used to identify all the ion complexes produced for each combination of host and metal ion, including charge-reduced complexes and ones that retain a counterion. The types of complexes formed from the single host-single metal salt solutions are summarized in Table 1. The second type of experiment involved the analysis of solutions containing one reference compound mixed with all four metal chloride salts in the ratio 1:1:1:1:1. The summations of intensities of both the free metal and the metal chloride complexes, including isotopic peaks, within each spectrum were directly compared to determine the selectivity of the reference compound. Examples of this strategy are shown in Figure 2 for complexation of [2.2.1]cryptand. Because a relatively large mass range is compressed, the individual isotope distributions are not readily discernible in the spectral figures. However, specific regions of the mass spectra are expanded to allow scrutiny of the isotopic distributions, as shown in Figure 2B for the lead-containing complexes of the [2.2.1]-cryptand. The isotopes are not well resolved for the doubly charged Pb2+ complexes (separated by 0.5 amu in the mass spectra) because the quadrupole ion trap only allows nominal unit resolution. The isotope distribution for the singly charged PbClO<sub>4</sub><sup>+</sup> complex that is expanded in Figure 2B shows excellent agreement with that expected for a complex containing one lead and one

chlorine atom. The results for the mixed metal solutions are displayed in Table 2. The reported percentages varied by 10% on a day-to-day basis, with most of the entries in Table 2 representing three to six replicates.

A variety of complexes are seen in the spectra containing the metal chloride salts (Figure 2A). These complexes stem from complexation of the free metal, such as  $Cd^{2+}$ , and of the metal chloride, such as  $CdCl^+$ , by the host ligand. In general, the complexes in which one counterion is retained are the most abundant for the metal chloride experiments.

The mixed-metal 1:1:1:1:1 solutions reveal the nature of the competitive equilibria. The data shown in Figure 2A are used to assess the general trends in binding selectivity (Table 2). For example, Figure 2A shows that the lead-containing complexes are favored over the cadmium complexes and the mercury and copper complexes are obscured in the baseline. The reference compound 1,7-diaza-15-crown-5 (D15C5) is used to imitate the macrocyclic portion of the smaller cage compounds, **9–11**. D15C5 has a cavity diameter of 1.0 Å.<sup>10</sup> With the smaller diameter of its binding cavity, D15C5 tends to favor complexation with smaller metals, such as  $Cu^{2+}$  or  $Cd^{2+}$ , on the basis of the intensities of the complexes formed from the 1:1:1:1 mixed-metal solutions (Table 2). These metals can be fully encapsulated within the ring of the D15C5 molecule unlike a larger metal, such as Pb<sup>2+</sup>. The D15C5 molecule also prefers to bind Hg<sup>2+</sup> over Pb<sup>2+</sup>. Mercury prefers coordination by nitrogen atoms over oxygen atoms,8 thus matching well with D15C5. The complexation of [2.2.1]-cryptand (221) shows a greater degree of selectivity than that observed for D15C5. 221 has a larger binding cavity (1.1 Å<sup>10</sup>) and more donor atoms than D15C5, and therefore it is able to more fully coordinate the larger metals such as lead.

**Metal Perchlorate Solutions.** For the optimal comparison of binding selectivities estimated from ESI-MS to those obtained by conventional solution methods, the experiments were repeated



Figure 2. [2.2.1]-Cryptand with all chlorides vs all perchlorates.

Table 2. Comparison of Metal Selectivity Trends in Reference Compounds (1:1:1:1:1 Solutions)

REFERENCE COMPOUNDS	ESI-MS METAL CHLORIDE TRENDS <sup>A</sup>	ESI-MS METAL PERCHLORATE TRENDS <sup>A</sup>	ESI-MS METAL NITRATE TRENDS <sup>A</sup>	LITERATUR PERCHLORA TRENDS (LOG	RE ATE H K) <sup>B</sup>
Diaza-15-	Cu > Cd > Hg >> Pb	Hg > Cu >> Cd, Pb	Hg >> Cu, Cd, Pb	Cu > Cd > Pb	H⁺
Crown-5	100 45 24 *	100 9 * *	100 * * *	9.5 8.7 7.9	10.1
[2.2.1]-	Pb > Cd >> Hg, Cu	Pb > Cd >> Hg, Cu	Pb > Hg >> Cd, Cu	Pb > Cd > Cu	H⁺
Cryptand	100 41 * *	100 12 * *	100 42 * *	15.1 11.3 10.1	11.5

A. Obtained by summing the intensities of all metal-containing complexes observed in the ESI mass spectra. Percentages listed below trends are scaled relative to the most abundant metal-containing complexes within each set. An asterisk represents no significant amount of complexation seen.

B. Log K trends and values taken from reference 9 for metal perchlorate salts in methanol obtained by potentiometry and from reference 6 (Izatt et al.) for protonation in methanol.

using perchlorate salts because binding constants reported in the literature<sup>10</sup> used these salts. Solutions containing the host and individual metal and mixed metals (following the procedures described earlier in this work) were analyzed for each reference compound with the four metal perchlorate salts mentioned above. The types of complexes formed are summarized in Table 1. For the perchlorate salts, the dominant signals are for the free metal complexes, and the signals for ones that retain a single counterion are less intense or absent. This contrast to the results obtained for the solutions containing the chloride salts is addressed in a later section on counterion effects.

The selectivity trends for the 1:1:1:1:1 perchlorate solutions are summarized in Table 2. D15C5 favors complexation with

mercury, unlike the trend noted for the analogous chloride experiments. This discrepancy stems from a counterion effect that is described in the next section, and it causes the metal selectivities of D15C5 to differ for the chloride versus the perchlorate and nitrate metal salts. For the reference compound [2.2.1]-cryptand, the binding of a larger metal, lead, is still the most favored overall, as seen in Table 2 and Figure 2B. The binding preferences seen with the metal chloride solutions are identical to the orders obtained with the perchlorate solutions for the [2.2.1]-cryptand host. When the concentration of the host, such as [2.2.1]-cryptand, is increased relative to the concentration of the metal guests, metal complexation is suppressed and protonation becomes more favorable, as seen in Figure 2C. In contrast, when the concentration of the host, such as [2.2.1]-cryptand, is decreased relative to the concentration of the metal guests, binding selectivity is increased and complex formation becomes more dominant, as seen in Figure 2D. Comparison of Figure 2B to Figure 2D shows that the preference for complexation of lead is slightly increased, as expected when a solution environment that enhances binding selectivity is created (i.e., greater excess of metals and limited supply of host). The absolute concentrations of the 1:1:1:1:1 solution (i.e., [2.2.1]-cryptand:metals) were also varied to examine the effect of concentration on the observed selectivities in the ESI mass spectra. Neither increasing nor decreasing the total concentration by a factor of 2 had a large impact on the observed distribution of complexes. For example, Figure 2E shows the ESI mass spectrum obtained when the total concentration of the components in the initial solution is halved. Further increases or decreases in the initial concentrations lead to loss of the spray stability due to the high salt content of the solution or reduction in the ion intensities due to the low concentrations of complexes, respectively.

Binding constants obtained using conventional potentiometric methods are available for the two reference compounds, 1,7-diaza-15-crown-5 and [2.2.1]-cryptand, in methanol with three of the perchlorate salts, copper, cadmium, and lead perchlorate,<sup>10</sup> summarized as log *K* values in Table 2. In addition to having very high transition metal binding constants, each of these compounds also has a large proton affinity, thus explaining the presence of great intensities of the protonated molecules in the ESI mass spectra. The orders of the binding constants reported for these compounds qualitatively agree with the selectivities observed in the ESI mass spectra (see Table 2) for the metal perchlorate solutions of 221 and D15C5. The smaller cavity host compound, D15C5, preferentially complexes Cu<sup>2+</sup> over Cd<sup>2+</sup> or Pb<sup>2+</sup>, and likewise the host with the larger cavity, 221, selectively binds the larger metal, Pb<sup>2+</sup>.

Metal Nitrate Solutions. To further explore the effects caused by counterion variation on the binding selectivities observed in the ESI mass spectra, complexation of each reference compound with heavy metal nitrates was also studied. Solutions containing the host and individual-metal nitrates and mixed-metal nitrate salts (following the procedures described earlier) were examined for each reference compound and the four metal nitrate salts. The types of complexes formed are summarized in Table 1. Interestingly, D15C5 favors formation of free metal complexes, whereas all of the 221 complexes incorporate one counterion. For the 1:1: 1:1:1 solutions, D15C5 favors complexation with mercury, similar to the trend observed in the perchlorate studies (see Table 2). For the reference compound [2.2.1]-cryptand, binding of a larger metal, lead, is again the most favorable overall, as seen in Table 2. However, complexation with mercury is also seen in the mixedmetal nitrate ESI mass spectra.

**Counterion Effects.** Although the effects of counterions on host–guest complexation have not been thoroughly scrutinized, several previous reports have noted that the identity of the counterion can affect the conformations of the host–guest complexes due to the degree of ion pairing between the counterion and the metal, even when the metal is complexed to the host.<sup>19</sup> In addition, certain counterions can cause reduction of stability constants for the host–guest complexes due to the competitive

#### A.) D15C5 with HgCl<sub>2</sub> (1:1)



Figure 3. Spectral differences due to counterion variation.

charge delocalization caused by ion pairing.<sup>20</sup> Thus, a closer examination of counterion effects on the ESI mass spectra is warranted. Several differences in the ESI mass spectra are observed among complexes formed with the perchlorate, chloride, and nitrate salts. In general, when chloride and perchlorate solutions of the same concentrations are sprayed on the same day, the chloride mixtures tend to produce spectra that have slightly higher intensities than those of the perchlorate solutions. This general observation suggests that the metal chloride salts increase the formation of charged complexes, perhaps by a desolvation effect or change in the conductivity of the solution. The use of chloride salts promotes formation of complexes that retain one counterion, such as the  $(D15C5 + HgCl)^+$  complex that is seen in Figure 3A or the  $(221 + PbCl)^+$  and  $(221 + CdCl)^+$ ions that are seen in Figure 2A. Small amounts of free metal complexes are also seen occasionally, like the  $(221 + Pb)^{2+}$  and  $(221 + Cd)^{2+}$  ions in Figure 2A. However, when complexation with the perchlorate and nitrate salts, the predominant complexes seen are those formed with the free metal, as illustrated by the  $(221 + Pb)^{2+}$  complex in Figure 2B and the  $(D15C5 + Hg)^{2+}$ complex in Figure 3B. For the perchlorate and nitrate salt studies, small amounts of complexes containing one chlorate or nitrate anion can also be seen, such as the  $(221 + PbClO_4)^+$  complex in Figure 2B, but these complexes are much less abundant. Moreover, in comparison to the complexes formed by the D15C5 crown ether reference, the 221 cryptand shows a greater preference for retention of one counterion with the heavy metal, especially with the nitrate salts. Despite its geometric constraints, the cryptand possesses a slightly larger cavity than D15C5, thus making it more favorable for retention of a loosely bound counterion. Given that the primary ring of the 221 cryptand is an 18-membered one, not

<sup>(19)</sup> Kyba, E. P.; Timko, J. M.; Kaplan, L. J.; de Jong, F.; Gokel, G. W.; Cram, D. J. J. Am. Chem. Soc. **1978**, 100, 4555–4568. Doxee, K. M. J. Org. Chem. **1989**, 54, 4712–4715.

<sup>(20)</sup> Smith, R. M.; Martell, A. E. *Critical Stability Constants*, Plenum Press: New York, 1976; Vol. 4.

a 15-membered one as in the crown ether reference, the counterion may be able to perch above the cavity more favorably than in the crown ether. A previous report by Dearden et al.<sup>21</sup> showed that when the 221 cryptand binds larger metals, the 18-membered face of the cryptand becomes almost planar. This change allows the metal to have optimal contact with six donor atoms on one side of the cryptand and prevents close contact with the seventh donor atom on the opposite side. The counterion is thus able to perch on the side of the 18-membered ring, promoting retention of the counterion with reduced steric hindrance even though the metal is complexed to a bicyclic host.

The differences in the types of complexes formed when various salts are used arise from the strength of the metal-anion bonds and the degree of charge separation. The larger, symmetrical anions, such as the perchlorates and nitrates, allow for the negative charge to be more delocalized, thus creating a weaker ion pair between the counterion and the metal.<sup>22</sup> This weaker bond found in the perchlorate and nitrate salts, along with the bulkier nature of the perchlorate or nitrate anions, enhances the displacement of the counterions by the host because the host is able to more effectively compete with the anion for complexation with the cation. This results in formation of a greater portion of the complexes involving the free metal ion in the ESI process. Nitrates and perchlorates are known to be weaker coordinating anions than the chlorides. For example, the aqueous stability constants<sup>20</sup> reported for the chlorides with heavy metals relative to those of the nitrates or perchlorates with heavy metals are typically 2-4orders of magnitude greater (i.e., log K values averaging 0.1 for the nitrates and perchlorates and 1.2-6.7 for the chlorides), confirming the higher binding energies of the chlorides. With the chloride salts, the dominant complexes observed are those that incorporate one chloride ion because a single anion remains more strongly bound to the metal ion during the desolvation process of ESI.

Differences in the binding selectivity trends for the two reference hosts obtained with metal chlorides versus the nitrates or perchlorates can also be rationalized by the strength of the ion pairs and the sizes of the metals and cavities. The chloride salts form tighter ion pairs, and thus the retention of the chloride ions shifts the observed selectivities of the hosts, making them preferentially bind to smaller metal ions than observed when the counterions are not strongly associated (as for the perchlorates and nitrates). For example, D15C5 prefers to bind mercury over the slightly smaller metals, copper and cadmium, in the perchlorate and nitrate experiments but prefers copper and cadmium over mercury in the chloride experiments. Because the chloride binds to the metal ion in a strong ion pair, thus changing the nominal size and coordination geometry relative to a free metal or weak ion pair that dissociates in the ESI process, the coordination of the smaller metals by D15C5 is more favorable for the metal chloride experiments.

Overall, these results show that the complexation is sensitive to counterion effects in some cases, stemming from the solution chemistry or the ESI process, and this feature may provide a new

A.) Diaza15C5 with HgCl<sub>2</sub> (1:1)



Figure 4. Effect of increased ionic strength with addition of MgCl<sub>2</sub>.

way to probe the influence of counterions on metal binding selectivities. Because counterion effects with respect to heavy metal complexation of the crown ligands have not been evaluated previously by conventional solution methods, such as potentiometry or spectrophotometry, correlations with the present results are not possible.

Ionic Strength Studies. The effect of increasing the ionic strength of the solution on the binding selectivities observed in the ESI mass spectra was also investigated. Previous studies performed by Cole et al. indicated that increasing the ionic strength of an electrosprayed solution caused the ion intensities to decrease without causing a notable change in the charge distribution of the ions.<sup>22</sup> Upon the addition of a second salt to a solution containing a single reference compound and a single heavy metal salt, the ionic strength of the solution was increased in the present experiments. The second salt was chosen to contain a divalent metal that was not expected to bind strongly to the hosts. For example, an equimolar amount of MgCl<sub>2</sub> was added to a host-salt solution, forming a 1:1:1 solution of host-heavy metal salt 1-MgCl<sub>2</sub>. Thus, the total ionic strength of the mixture was increased by a factor of 2. The divalent metal cation, Mg<sup>2+</sup>, should not competitively bind with the host compound in question. For this study, the reference compound 1,7-diaza-15-crown-5 was mixed with MgCl<sub>2</sub> and also CuCl<sub>2</sub>, CdCl<sub>2</sub>, or HgCl<sub>2</sub>. The 1:1 D15C5-HgCl<sub>2</sub> solution produced the spectrum in Figure 4A. The higher ionic strength solution, such as D15C5-MgCl<sub>2</sub>-HgCl<sub>2</sub> (1: 1:1), resulted in the spectrum in Figure 4B under the same instrumental conditions. Comparison of the two spectra shows that increasing the ionic strength of the solution has little effect on the ion intensities and has no effect on the types of metal complexes formed. Examination of numerous other solutions containing the host with pairs or groups of metal salts at a range of ionic strengths and up to 4  $\times$  10^{-4} M total additional salt revealed no changes in the trends in binding selectivities. Thus, no evidence for a significant ionic strength effect has been found.

<sup>(21)</sup> Chen, Q.; Cannell, K.; Nicoll, J.; Deaden, D. V. J. Am. Chem. Soc. 1996, 118, 6336-6344.

<sup>(22)</sup> Wang, G.; Cole, R. B. Anal. Chem. 1994, 66, 3702-3708. Wang, G.; Cole, R. B. In *Electrospray Ionization Mass Spectrometry*, Cole, R. B., Ed.; John Wiley and Sons: New York, 1997; Chapter 4.





**Aza-Crown Ether Derivatives.** The five cage compounds were synthesized and purified, and their respective selectivities toward heavy metal cations were investigated using various mixtures of the host compounds with metal chloride and perchlorate salts. Solutions that contained only a single host and a single metal ion guest (1:1) were investigated, as were solutions that contained a single host and all four metal ion guests (1:1:1:1) (Figure 5). The summations of intensities of both the free metal and the related metal salt complexes within the latter spectra were directly compared to determine the selectivities of the cage compounds.

Single Metal Chloride Solutions. The first set of experiments involved ESI-MS analysis of solutions containing a 1:1 mixture of a single metal chloride salt and a single cage compound. The types of the metal complexes detected in the ESI mass spectra obtained for complexes that involved the various metal salts were then compared with one another. Examples of these spectra are shown in Figure 5 for 9 with CdCl<sub>2</sub>, PbCl<sub>2</sub>, HgCl<sub>2</sub>, and CuCl<sub>2</sub>, respectively. Protonated and sodium-cationized complexes dominate the spectra, highlighting the high basicities and sodium affinities of 9, even in the presence of 10<sup>-4</sup> M concentrations of metal salts. Since the solutions each contained only a single metal, selective complexation could not be monitored directly, but the relative intensities of the complexes provided a preliminary assessment of ESI efficiency for well-defined solution conditions. As shown in Figure 5C, host 9 generates the most intense complex when binding Hg<sup>2+</sup>. In contrast, Pb<sup>2+</sup> complexes are never observed (Figure 5B), presumably due to the low Pb<sup>2+</sup> affinity relative to the preference for proton or Na<sup>+</sup> complexation. The large size of the Pb<sup>2+</sup> ion may prohibit optimal coordination by **9**. The dominant

types of complexes are analogous to those observed for the reference complexes (i.e., retention of one chloride ion), as previously summarized in Table 1.

Mixed Metal Chloride Solutions. The second set of experiments involved ESI analysis of solutions having a 1:1:1:1:1 mixture of the host cage compound and equal amounts of each of each of the four heavy metal chlorides. In previous work aimed at measuring metal binding selectivities, the ESI response factors for different ligand-metal complexes were measured by analyzing solutions containing well-defined concentrations and comparing these results to distributions of complexes calculated for solution equilibria.<sup>2</sup> In fact, few discrepancies between the ESI mass spectral data and the calculated solution equilibrium distributions of complexes were found for systems containing a single ligand with a series of similar metals. The good correlations were attributed to the similar desolvation and spray efficiencies of complexes containing the same host ligand but different metals. Since binding constants are not known for the cage compounds in the present study, calculations of equilibria distributions and detailed measurements of ESI response factors are not possible. Thus, the intensities of all related metal complexes (i.e., all doubly charged complexes plus all those containing a single chloride) in the ESI mass spectra of the 1:1:1:1:1 solutions were compared and presumed to qualitatively reflect the distribution of complexes in the original solutions. An example of one of these spectra can be seen in Figure 5E for 9. A direct comparison of the intensities is made to estimate the binding selectivity of 9 under well-defined solution conditions. Figure 5E shows that 9 prefers to form complexes with copper in the mixed-metal solutions. Interestingly, the spectra in Figures 5A–D indicated that the  $9/Hg^{2+}$  complex

Table 3. Comparison of Metal Selectivity Trends in Cage Compounds (1:1:1:1:1 Solutions)

CAGE COMPOUNDS	METAL CHLORIDE TRENDS <sup>A</sup>	METAL PERCHLORATE TRENDS <sup>A</sup>	
9	Cu > Cd >> Hg, Pb 100 77 * *	Hg >> Cu , Cd, Pb 100 * * *	
10	Cu ≥ Cd >> Hg, Pb 100 83 * *	Hg >> Cu , Cd, Pb 100 * * *	
11	No Complexes	No Complexes	
6	Hg ≥ Cu >> Cd, Pb 100 90 * *	Hg > Cu >> Cd, Pb 100 38 * *	
12	Pb >> Cd, Hg, Cu 100 * * *	Pb >> Cd, Hg, Cu 100 * * *	

A. Obtained by summing the intensities of all metal-containing complexes observed in the ESI mass spectra. Percentages listed below trends are scaled relative to the most abundant metal-containing complexes within each set. An asterisk represents no significant amount of complexation seen.

produced the greatest intensity in the series of ESI mass spectra of the solutions containing a single metal. This complex is not observed at all in Figure 5E for the mixed-metal solution, suggesting that the dominant factor that influences the ESI mass spectra is the competitive equilibria in solution, not the spray efficiencies of the various metal complexes. The results for the other mixed-metal solutions are summarized in Table 3.

Three of the cage compounds differ only by the substituents attached to the nitrogens on the aza-polyether ring, **9**, **10** and **11**, but each of these has a relatively small cavity. **9**, which has no pendant groups and is closely modeled by the compound 1,7-diaza-15-crown-5, tends to be more selective toward the smaller metals such as copper and cadmium, which it can fully encapsulate, rather than lead, which is a larger divalent cation. See Table 3. The smaller metals are preferred because they are better able to fit inside the cavity of the ring to maximize their binding coordination.

Substituents added to the polyether rings greatly affect the binding selectivities of these cage compounds. If an ether substituent is added, as is the case with **10**, the additional oxygen helps stabilize the complexed metal ion by providing another binding site, but the compound still prefers to bind with the smaller copper and cadmium metal cations, which fit better into the cavity. **11**, which has two ethyl substituents added to the binding ring, does not exhibit any metal complexation upon ESI. This result is probably due to steric hindrance caused by the flexible substituents blocking the host's binding cavity. Thus, the pendant arms hinder any metals from being encapsulated by the ring. However, the protonated species of this compound is present in the mass spectrum, indicating that a small charge-dense ion may attach to **11**.

**6** is the sulfur analogue of **9**. This host also has a relatively small binding cavity and tends to selectively bind small metal cations (Table 3). Given that mercury forms strong bonds to sulfur

atoms and also prefers linear coordination,<sup>23</sup> it is no surprise to see that **6** is very selective toward mercury. This preference is similar to that of its nitrogen analogue, **9**. The placement of the sulfur atoms on opposite sides of the ring in **6** may enhance the linear coordination favored by mercury.

12 has a larger binding cavity that can be modeled by the [2.2.1]-cryptand molecule, with a cavity size of 1.1 Å.<sup>10</sup> With a larger cavity and more binding sites, this molecule is able to better coordinate larger metal ions. Therefore, among the metals used in the present study, 12 selectively prefers to bind with lead, which has a diameter of 1.19 Å.<sup>9</sup>

**Mixed-Metal Perchlorate Solutions.** The next set of experiments involved ESI-MS analysis of solutions having a 1:1:1:1:1 mixture of a host cage compound and equimolar amounts of each of the four heavy metal perchlorate salts. The intensities of all the free metal and related metal salt complexes in the observed ESI mass spectrum were compared with one another. The direct comparison of the intensities was then used to evaluate the binding preferences for the metal perchlorate salts. A summary of the binding selectivity trends for the mixed heavy metal perchlorate solutions is found in Table 3.

Some of the binding selectivities of the cage compounds observed for the mixed heavy metal perchlorate solutions are similar to the selectivities noticed for the mixed-metal chloride studies, as seen in Table 3. For example, **6**-A favors complexation with mercury in both the chloride and perchlorate salt solutions, and **12** selectively binds with lead in both cases as well. However, for the compounds **9** and **10**, complexation with mercury is preferred over that with the larger metal cations, unlike the selectivities obtained using metal chloride salts. Since the predominant complexes formed when perchlorate salts are used are

<sup>(23)</sup> Martell, A. E.; Hancock, R. D. Metal Complexes in Aqueous Solutions, Plenum Press: New York, 1996; p 56. Shannon, R. D. Acta Crystallogr., Sect. A 1976, A32, 751.

complexes with the free metal cation, selective complexation with mercury for these two compounds is not surprising because mercury has the smallest ionic diameter in its linear coordination geometry, as stated previously.

Two of the remaining cage compounds, **6** and **12**, have identical metal binding selectivities for the mixed perchlorate solutions as they did for their mixed chloride solutions. These results can be seen in a side-by-side comparison in Table 3. For reasons stated previously, the small sulfur-containing ring of **6** still prefers the small mercury and copper cations and the larger binding cavity of **12** more favorably coordinates the largest metal used in the study, lead. A comparison of the ESI-MS metal selectivity trends to reported values from the literature is not possible because these novel hosts have not been studied previously. **11** again forms only a protonated complex, rather than complexing any of the bulkier metal species, due to steric hindrance.

Quantitation of Binding Selectivities. In previous work,8 it was found that ESI-MS is a useful tool for quantitative evaluation of binding selectivities of host-guest systems. Moreover, ESI mass spectral results correlated well with expected solution equilibrium distributions of complexes, especially when a single host binding a series of similar guest cations was analyzed.9 In the present study, a similar strategy was undertaken to quantitate the binding selectivities of a reference host compound, D15C5, and two heavy metal guests, Cu(ClO<sub>4</sub>)<sub>2</sub> and Cd(ClO<sub>4</sub>)<sub>2</sub>. The known solution binding constants for D15C5 with Cu<sup>2+</sup> and Cd<sup>2+</sup> are log K = 9.45 and log K = 8.72, as reported from potentiometric measurements.<sup>11</sup> In solutions containing 2:1:1 ratios of the host to two metals, there should be no binding selectivity observed due to the large magnitude of the binding constants. Using the method described in our previous work,<sup>10</sup> solutions containing D15C5 and a single heavy metal guest ion (1:1), either  $Cu(ClO_4)_2$ or Cd(ClO<sub>4</sub>)<sub>2</sub>, were sprayed individually to analyze ESI response factors (Figure 6A,B). The ESI response factors reflect differences in the ionization efficiencies between the types of complexes formed for the two metals and are typically related to desolvation effects. Then a multicomponent solution made by mixing the two individual solutions, containing D15C5 with  $Cu(ClO_4)_2$  and Cd- $(ClO_4)_2$  in the ratio 2:1:1, was sprayed (Figure 6C). The ratio of the intensities of the products, (cadmium complexes)/(copper complexes), taken from the latter ESI mass spectrum, was corrected on the basis of the ESI response factors from the analysis of the single-metal solutions. The ESI-MS selectivities were obtained by summing the intensities of all related complexs, singly and doubly charged, because a more optimal way to differentially weight the intensities of the singly versus doubly charged complexes has not been found. The ESI-MS selectivity ratio for the cadmium versus copper complexation could then be compared to the theoretical selectivity ratio calculated from known binding constants. These results are found in Table 4. The corrected ESI ratios (0.97 when considering all of the types of metal complexes formed and 1.1 when considering only the free metal complexes formed) correlate well with the theoretical selectivity ratio of 1.0.

In similar experiments, the concentrations of the heavy metals  $(Cu(ClO_4)_2 \text{ and } Cd(ClO_4)_2)$  were increased relative to the concentration of the host (D15C5), resulting in host:metal ratios of



Figure 6. Complexation of diaza-15-crown-5 with  $Cu(ClO_4)_2$  and  $Cd(ClO_4)_2$ .

SOLUTION	THEORETICAL	UNCORRECTED	CORRECTED	
COMPOSITION	RATIOS	ESI-MS	ESI-MS	
		RATIOS	RATIOS	
D15C5 :	(D15C5 : cadmium	(D15C5 : cadmium	(D15C5 : cadmium	
$Cu(ClO_4)_2$ :	complexes) /	complexes) /	complexes) /	
Cd(ClO <sub>4</sub> ) <sub>2</sub> in	(D15C5 :copper	(D15C5 :copper	(D15C5 :copper	
MeOH 2:1:1	complexes)	complexes)	complexes)	
Considering only	1.0	0.47 ± 0.04	$1.1 \pm 0.1$	
free metal				
complexes				
D15C5 :	(D15C5 : cadmium	(D15C5 : cadmium	(D15C5 : cadmium	
$Cu(ClO_4)_2$ :	complexes) /	complexes) /	complexes) /	
Cd(ClO <sub>4</sub> ) <sub>2</sub> in	(D15C5 :copper	(D15C5 :copper	(D15C5 :copper	
MeOH 2:1:1	complexes)	complexes)	complexes)	
Considering all	1.0	$0.40 \pm 0.04$	$0.97 \pm 0.1$	
metal complexes				
formed				
D15C5 :	(D15C5 : cadmium	(D15C5 : cadmium	(D15C5 : cadmium	
$Cu(ClO_4)_2$ :	complexes) /	complexes) /	complexes) /	
Cd(ClO <sub>4</sub> ) <sub>2</sub> in	(D15C5 :copper	(D15C5 :copper	(D15C5 :copper	
MeOH 1:2.5:2.5	complexes)	complexes)	complexes)	
Considering all	0.25	0.16 ± 0.02	$0.30 \pm 0.03$	
metal complexes				
formed				

Table 4. Binding Selectivity of Diaza-15-Crown-5 for  $Cu(CIO_4)_2$  and  $Cd(CIO_4)_2$ : ESI vs Theoretical Solution Results

1:5 and host:mixed-metal solution ratios of 1:2.5:2.5, to evaluate a more competitive metal binding environment. The results are displayed in Table 4. When one considers all of the metal

complexes formed, the corrected ESI mass spectral ratio correlates well with the theoretical selectivity ratio based on the calculated equilibrium distribution. This excellent agreement shows that the ESI-MS method can be used to estimate heavy metal selectivities in addition to its well-validated capabilities for alkali metal complexation.

Estimation of Relative Binding Constants. Using a method similar to the one described above, the relative heavy metal binding strengths of the two reference compounds, D15C5 and 221, were qualitatively compared to the binding strengths of the two most similar cage compounds, 9 and 12, respectively. For these experiments, a multicomponent mixture containing a reference compound, the corresponding cage compound, and a heavy metal salt in a 1:1:2 ratio was sprayed, and the resulting ESI mass spectrum was analyzed. The intensities of the reference-metal complexes were then compared to the intensities of the cagemetal complexes to provide a qualitative estimation of the binding constant of each cage compound for the given heavy metal relative to the binding constant of each reference compound. For instance, for a mixture of D15C5 and **9** with  $Cu(ClO_4)_2$ , the spectrum in Figure 7A shows only peaks corresponding to 9-copper complexes. Thus, the greater intensities of the 9 complexes indicate that 9 has a substantially greater binding constant for copper than does D15C5. Likewise, in Figure 7B, when the complexation of D15C5 is compared to that of **9** in a mixture with  $Cd(ClO_4)_2$ , the intensities of the 9-cadmium complexes are larger than the intensities of the D15C5-cadmium complexes by a factor of 1.4. Once again, this indicates that 9 has a higher binding constant than D15C5 for cadmium. Finally, the lead binding constants of the reference compound 221 and the cage compound 12 were compared. In this case, 221 formed more intense lead complexes than 12 by a factor of 1.8, which implies that 12 has a lower binding constant than 221 for lead. Although the ESI-MS method does not provide absolute binding constants for these latter series of experiments, further refinements may allow such measurements in the future.

In similar experiments, the concentrations of the hosts (D15C5 and **9**) were increased relative to those of the guests (Cd(ClO<sub>4</sub>)<sub>2</sub>) or Cu(ClO<sub>4</sub>)<sub>2</sub>), resulting in 5:1 host:guest ratios for the single-host solutions and 2.5:2.5:1 ratios for the mixed-host solutions, to create a more competitive environment between the hosts for the metal guest. However, protonation of the hosts proved to be more favorable than complexation with copper or cadmium, as shown in Figure 7C,D.

#### CONCLUSIONS

ESI-MS proves to be a rapid method for determining the heavy metal binding selectivities of a series of caged compounds and thus could be used as an efficient option for screening new synthetic compounds developed for remediation efforts and for evaluating structure/binding selectivity relationships. Sample consumption is minimal, and this method may be applied to many solvent systems that are amenable to ESI but not to any single conventional method for the examination of binding selectivities. Structural modifications of the caged compounds, such as the addition of a diether bridging group across the nitrogen donors, cause significant changes in the binding selectivities. The types of metal complexes formed and the apparent binding preferences of the crown ligands are affected by the counterion A.) Diaza-15-Crown-5 and 9 with Cu(ClO<sub>4</sub>)<sub>2</sub> (1:1:2)



B.) Diaza-15-Crown-5 and 9 with Cd(ClO<sub>4</sub>)<sub>2</sub> (1:1:2)



C.) Diaza-15-Crown-5 and 9 with Cu(ClO<sub>4</sub>)<sub>2</sub> (2.5:2.5:1)







Figure 7. Complexation of diaza-15-crown-5 and 9 with  $Cu(ClO_4)_2$  or  $Cd(ClO_4)_2$ .

of the metal salt. Stronger metal—anion bonds make it more difficult to displace the anion from the complex in the ESI process, resulting in one counterion being incorporated into the complex. Further evaluation by both ESI-MS and conventional solution methods should permit the development of a broader understanding of the influence of counterion effects on binding selectivities.

## ACKNOWLEDGMENT

Funding for this work was provided in part by grants from the National Science Foundation (CHE-9357422), the Welch Foundation (F-1155) and the Texas Advanced Technology Program (003659-0206). A.P.M. thanks the Robert A. Welch Foundation (Grant B-0963) and the Department of Energy (Grant DE-ID-07-98ER14936) for financial support of this study. In addition, we thank Dr. I. N. N. Namboothiri for his kind assistance with the purification and characterization of **4**.

Received for review September 29, 1999. Accepted February 29, 2000.

AC991125T

Analytical Chemistry, Vol. 72, No. 11, June 1, 2000 2445