$2[Cu^{I}(16Se4)]^{+} + [16Se4]^{2+}$. The reaction likely proceeds via the radical cation $[16Se4]^{\bullet +}$ (λ_{max} 318 nm). In support of this contention, oxidation of the free ligand with $NOBF_4$[8] leads to the appearance of an absorbance band at 318 nm.

Finally, dissolution of **1** in CH_2Cl_2/CH_3CN (1/1) for 5 min at room temperature followed by cooling gave yellow crystals[4] that were identified crystallographically as the salt $[16Se4]^{2+}$-$([CF_3SO_3]^{-})_2$ (**3**).[9] The structure of **3** contains the $[16Se4]^{2+}$ cation shown in Figure 2. The two transannular Se–Se bonds are nearly collinear. This configuration permits greater charge delocalization than would a single Se–Se transannular bond similar to the S–S bond in the recently published structure of the dithiacyclooctane dication.[10] The bonds to the central Se atom (Se(1)) have a ψ-trigonal-bipyramidal arrangement typical of tetracoordinate Se(IV), with the more electron withdrawing substituents ($Se^{\delta+}$) in the axial positions and the nonbonding electron pair in the equatorial plane. Similar nearly linear Se–Se–Se arrangements, displaying comparable Se–Se bond lengths, have been observed previously in the triselenourea dication[11] (Se–Se, 2.664 (2) Å) and 6a-selenaselenophthene and its derivatives (2.548 (3)–2.583 (3) Å).[12] The Se(1)- - -Se(3) distance, which is significantly less than twice the accepted van der Waals radius of Se (3.8 Å), suggests a weak secondary interaction between these atoms, although this could merely represent a constraint of the ring size. A few Se- - -O distances, which are slightly shorter than the sum of the van der Waals radii, 3.42 Å (the shortest being 3.152 (7) Å to Se(2)), are consistent with reasonable dipolar electrostatic attraction between cation and anion. These interactions are clearly weaker than the S- - -O interactions in the dithiacyclooctane dication[10] (S- - -O, 2.682(6) Å; cf. sum of van der Waals radii, 3.32 Å) where the positive charge is more localized.

While the formation of radical monocations and dications of polythia ethers is documented,[13] and Musker et al.[14] have reported the oxidation of dithiacyclooctane derivatives by Cu(II) complexes, the present study reports the first selenium coronand dication resulting from an electron-transfer reaction of a metal–selenium coronand complex. The structures of other cyclic selena cations have also been reported, e.g., the radical monocation and dication of 1,2,4-triselena-3,5-diazacyclopentane,[15] Se_8^{2+}, and Se_{10}^{2+}.[16,17] We note also that the dication of 1,5-diselenacyclooctane has recently been prepared.[18]

Further studies of the redox chemistry of metal–selenium coronand complexes and of the free ligands[1] themselves are currently in progress, and the results will be reported in due course.

Acknowledgment. We thank the Natural Sciences and Engineering Research Council of Canada for financial support.

Supplementary Material Available: Synthetic details and spectroscopic and microanalytical data for **1–3** and details of the structure determination and tables of atomic coordinates, anisotropic thermal parameters, selected bond lengths, bond angles, and torsion angles for **1** and **3** (21 pages); tables of calculated and observed structure factors for **1** and **3** (36 pages). Ordering information is given on any current masthead page.

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[8] Musker, W. K.; Wolford, T. L.; Roush, P. B. *J. Am. Chem. Soc.* **1978**, *100*, 6416.

[9] $[(Se(CH_2)_3)_4][SO_3CF_3]_2$ 0.88/0.12 $(NCCH_3)/CH_2Cl_2$; triclinic; $P\bar{1}$; $T = 195$ K; $a = 9.015$ (2) Å; $b = 12.850$ (3) Å; $c = 13.835$ (3) Å; $\alpha = 63.98$ (2)°; $\beta = 74.71$ (2)°; $\gamma = 73.59$ (2)°; $Z = 2$; $\lambda = 0.71069$ Å; μ(Mo Kα) = 55.69 cm^{-1}; crystal dimensions 0.23 × 0.22 × 0.08 mm; transmission 0.352–0.659, corrected analytically; $4° \leq 2\theta \leq 42°$; data 2099 ($I \geq 2.5\sigma(I)$); refined parameters 254; restraints 41; $R = \sum||F_o| - |F_c||/\sum|F_o| = 0.042$; maximum |shift/error| = 0.6 (disordered solvent, else 0.06). Bond angles: Se(2)–Se(1)–Se(4), 175.54 (6)°; C–Se(1)–Se, 85.8 (3)–96.8 (3)°; Se(1)–Se–C, 88.9 (3)–96.6 (3)°; C(12)–Se(1)–C(1), 102.0 (4)°; C(3)–Se(2)–C(4), 96.6 (5)°; C(8)–Se(4)–C(9), 95.3 (4)°; C(6)–Se(3)–C(7), 95.2 (5)°; C–C–Se, 108.5 (7)–116.8 (8)°; C–C–C, 111.9 (9)–155.2 (9)°.

[10] Iwasaki, F.; Toyoda, N.; Akaishi, R.; Fujihara, H.; Furukawa, N. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 2563.

[11] Hauge, S. *Acta Chem. Scand. A* **1979**, *33*, 317.

[12] Hordvik, A.; Porten, J. A. *Acta Chem. Scand. A* **1973**, *27*, 485 and references therein.

[13] For example: Asmus, K. D. *Acc. Chem. Res.* **1979**, *12*, 436. Musker, W. K. *Acc. Chem. Res.* **1980**, *13*, 200. Tamaoki, M.; Serita, M.; Shiratori, Y.; Itoh, K. *J. Phys. Chem.* **1989**, *93*, 6052. Drewello, T.; Lebrilla, C. B.; Asmus, K.-D.; Schwarz, H. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 1275.

[14] Musker, W. K.; Wolford, T. L. *J. Am. Chem. Soc.* **1976**, *98*, 3055. Musker, W. K.; Olmstead, M. M.; Kessler, R. M. *Inorg. Chem.* **1984**, *23*, 1764.

[15] Awere, E. G.; Passmore, J.; White, P. S.; Klapotke, T. *J. Chem. Soc., Chem. Commun.* **1989**, 1415.

[16] McMullan, R. K.; Prince, D. J.; Corbett, J. D. *Inorg. Chem.* **1971**, *10*, 1749.

[17] Burns, R. C.; Chan, W.-L.; Gillespie, R. L.; Luk, W.-C.; Sawyer, J. F.; Slim, D. R. *Inorg. Chem.* **1980**, *19*, 1432.

[18] Fujihara, H.; Akaishi, R.; Erata, T.; Furukawa, N. *J. Chem. Soc., Chem. Commun.* **1989**, 1789. Pinto, B. M.; Zhou, X.-M., unpublished results.

Demonstration of a Conformational Equilibrium in Acyl Carrier Protein from Spinach Using Rotating Frame Nuclear Magnetic Resonance Spectroscopy

Yangmee Kim and James H. Prestegard*

Chemistry Department, Yale University
New Haven, Connecticut 06511

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Advances in NMR technology and computational methods have made it possible to determine the three-dimensional structures of small proteins in solution on the basis of distance constraints from interproton nuclear Overhauser effects (NOEs).[1-5] Most of the structures determined have been calculated with the assumption that the structure is static. While in most cases good agreement with X-ray structures supports the validity of this assumption,[2,4,6] there are cases where violation of this assumption can lead to structures of poor quality or even misrepresentation of the state of the system. Errors are most likely to occur when conformers interconvert rapidly enough to show a single set of resonances but slowly enough to average NOEs observed in each state (10^3–10^7 s^{-1}).

Recently we have introduced a structure determination procedure that takes account of averaged NOEs in the limit where two discrete conformers are involved, and we have applied this procedure in a structure determination of *Escherichia coli* acyl carrier protein (ACP, 8847 Da (daltons)).[7] However, other than some improvement in the fit of the structural model to experimental data, and a general improvement in the quality of the structure, there is little direct evidence for the existence of two and only two conformers for this protein. In the rapid exchange limit, only a single set of resonances would be observed, regardless of the number of conformational states. Here we present data on a closely related protein, ACP-I from spinach (9173 Da). While the NMR spectrum has not been completely assigned, it is clear that in this protein there are more resonances than can be assigned to protons from a single structural species.[8] We are now able to demonstrate, using rotating frame NMR experiments, that the extra resonances arise from a second conformer in dynamic equilibrium with the first. This clear demonstration underscores the importance of considering dynamic equilibrium

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[1] Braun, W.; Wider, G.; Lee, K. H.; Wuthrich, K. *J. Mol. Biol.* **1983**, *169*, 921–948.

[2] Clore, G. M.; Gronenborn, A. M.; Brunger, A. T.; Karplus, M. *J. Mol. Biol.* **1985**, *186*, 435–455.

[3] Holak, T. A.; Kearsely, S. K.; Kim, Y.; Prestegard, J. H. *Biochemistry* **1988**, *27*, 6135–6142.

[4] Kline, A. D.; Braun, W.; Wuthrich, K. *J. Mol. Biol.* **1988**, *204*, 675–724.

[5] Clore, G. M.; Gronenborn, A. M. *CRC Rev. Biochem. Mol. Biol.* **1989**, *24*, 479–564.

[6] Wagner, G.; Braun, W.; Havel, T. F.; Schaumann, T.; Go, W.; Wuthrich, K. *J. Mol. Biol.* **1987**, *196*, 611–639.

[7] Kim, Y.; Prestegard, J. H. *Biochemistry* **1989**, *28*, 8792–8797.

[8] Kim, Y.; Prestegard, J. H. *J. Biochem. Pharmacol.*, in press.

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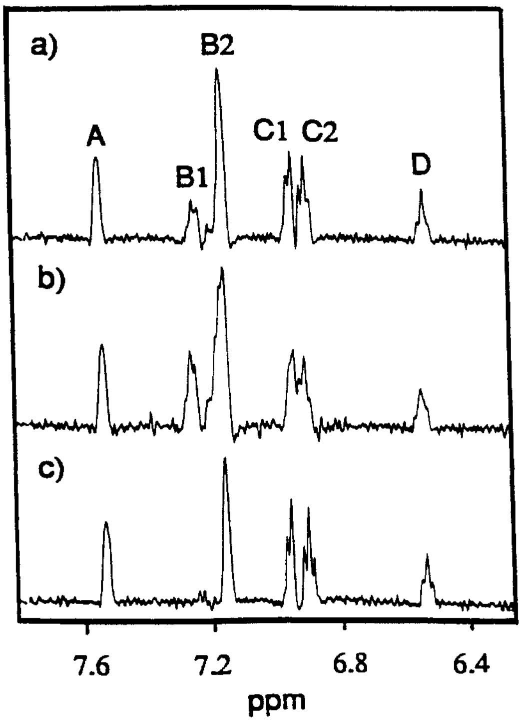

Figure 1. ^1H NMR spectra (500 MHz) of the aromatic region of spinach ACP-I in D_2O showing the effects of varying pH, temperature, and divalent ion content. Spectra were collected under the following conditions: (a) pH 6.0, 303 K; (b) 6.0, 313 K; (c) pH 6.0, 303 K, with 2 equiv of Mg^{2+}. Sample concentration was approximately 1 mM, and acquisitions required approximately 2 min.

effects in the structure determination of other proteins.

The CAMELSPIN[9] or ROESY[10-12] (rotating frame Overhauser enhancement spectroscopy) experiment provides a useful tool for demonstrating exchange between conformational states. The ROESY experiment has most frequently been used as an alternative to the NOESY experiment for the measurement of magnetization transfer by cross relaxation when the product of spectrometer frequency and the rotational correlation time for the molecule is near 1 and leads to very small NOEs.[13-15] However, magnetization can transfer between sites by both cross relaxation (NOEs) and chemical exchange mechanisms. Unlike the NOESY experiment, the ROESY experiment on a macromolecule can distinguish exchange transfers from cross relaxation transfers because, in a phase-sensitive mode, cross peaks due to NOEs have a sign opposite to that of chemical exchange cross peaks.[9-12,14,16]

The upper spectrum in Figure 1 shows a one-dimensional spectrum of the aromatic-ring region of spinach ACP-I in D_2O at pH 6.0, 303 K. The NMR sample was prepared as described in ref 8, and spectra were acquired in the pulsed FT mode on a Bruker 500-MHz spectrometer. Spinach ACP-I has resonances from only two phenylalanines in this region, each with an A_2B_2C five-spin system. Therefore, there should be only 10 protons in this region. Peaks labeled C1, C2, and D correspond to ortho, meta, and para protons of one phenylalanine spin system (Phe 31) as demonstrated by RELAY and NOESY spectra.[8] However, the integral of these peaks, if referenced to what must be the remaining five protons in peaks A, B1, and B2 (Phe 52), is low by more than 10%.

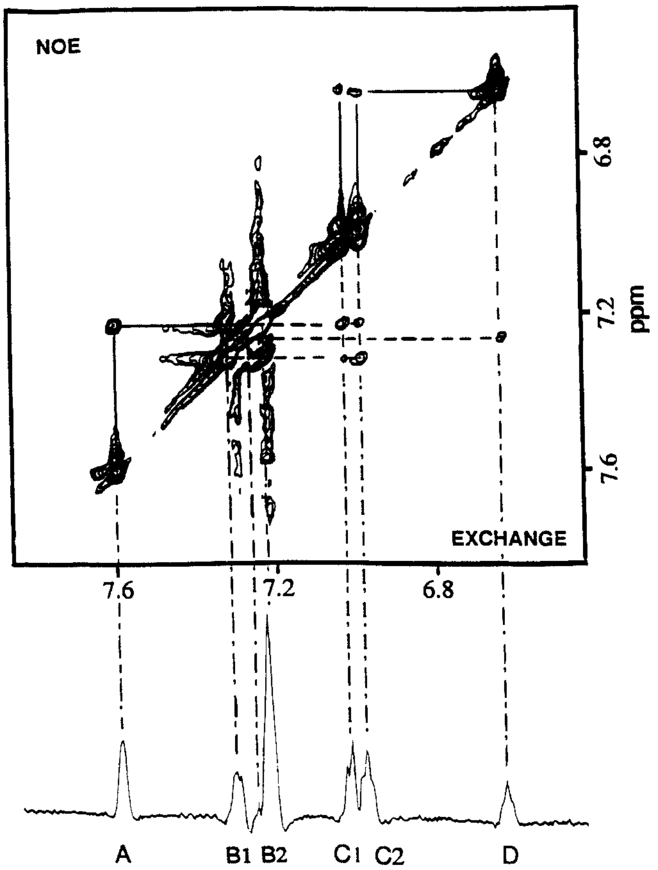

Figure 2. ROESY spectrum (500 MHz) of the aromatic region of spinach ACP-I in D_2O at pH 5.9, 303 K. Exchange cross peaks are indicated by dotted lines to the lower right of the diagonal, and NOE cross peaks are indicated by solid lines to the upper left of the diagonal. The solid–dotted lines correlate cross-peak positions with the positions of each peak in the 1D spectrum.

The remaining spectra show the effect of varying temperature and divalent ion content. Shifts in peak positions are not large, but the peak B1 clearly gains intensity at the expense of peaks C1, C2, and D as divalent metals are removed (a) or temperature is raised (b). These entirely reversible changes are characteristic of seeing discrete resonances for two conformers in slow exchange and assignment of B1 and possibly peaks underlying B2 to the minor conformer.

The two-dimensional spectrum in Figure 2 shows the aromatic region of a ROESY spectrum of a sample at pH 5.9, 303 K in D_2O. It was recorded in a pure absorption mode by the time proportional phase incrementation method,[17,18] and data were processed with the FTNMR software program.[19] The carrier frequency was set 1000 Hz upfield from peak D, and a weak spin-lock field (2200 Hz) was used to suppress Hartmann–Hahn type transfers.[20,21] The data were acquired with a spin-lock time of 160 ms. The cross peaks with negative intensities (those due to NOEs) have been plotted to the upper left of the diagonal, and those with positive intensities (those due to exchange) have been plotted to the lower right of the diagonal. NOESY spectra in this region show cross peaks at exactly the same positions except that the signs of the cross peaks are all positive and no selective plotting of one set is possible.[8] Therefore, in the NOESY spectrum, it is not easy to tell whether extra cross peaks come from remote interresidue connectivities between Phe 31 and Phe 52 or from exchange between two conformers. In the ROESY spectrum,

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(9) Bothner-By, A. A.; Stephens, R. L.; Lee, J.; Warren, C. D.; Jeanloz, R. W. *J. Am. Chem. Soc.* **1984**, *106*, 811–813.

(10) Bax, A.; Davis, D. G. *J. Magn. Reson.* **1985**, *63*, 207–213.

(11) Bax, A. *J. Magn. Reson.* **1988**, *77*, 134–147.

(12) Rance, M. *J. Magn. Reson.* **1987**, *74*, 557–564.

(13) Breg, J.; Romijn, D.; Vliegenthart, J. F. G. *Carbohydr. Res.* **1988**, *183*, 19–34.

(14) Belleney, J.; Delepierre, M. *Magn. Reson. Chem.* **1989**, *27*, 491–495.

(15) Dabrowski, J.; Poppe, L. *J. Am. Chem. Soc.* **1989**, *111*, 1510–1511.

(16) Brown, L. R.; Farmer, B. T., II *Methods Enzymol.* **1989**, *176*, 199–216.

(17) Bodenhausen, G.; Vold, R. L.; Vold, R. R. *J. Magn. Reson.* **1980**, *37*, 93–106.

(18) Redfield, A. G.; Kuntzs, D. *J. Magn. Reson.* **1975**, *19*, 250–254.

(19) FTNMR is a program licensed from Hare Research Inc., Woodinville, WA.

(20) Cavanagh, J.; Keeler, J. *J. Magn. Reson.* **1988**, *80*, 186–194.

(21) Griesinger, C.; Ernst, R. R. *J. Magn. Reson.* **1987**, *75*, 261–271.

the distinction is clear. Cross peaks in the lower part of this ROESY spectrum show exchange of peaks C1 and C2 with a peak under B2 and with the peak B1, respectively, and the peak D with the small shoulder just downfield of B2.

Thus, spinach ACP exists in at least two conformationally discrete forms in slow exchange. It is possible that related proteins that show a single set of resonances, such as *E. coli* ACP, do this more by virtue of a shift in time scales of interconversion than by elimination of one of the discrete conformational forms.[7] This possibility suggests caution in the indiscriminate application of a rigid model in the determination of protein structures from NMR data for ACPs as well as for other proteins.

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Alkali Metal Ion Complexation of Crown Ethers and Related Ligands Studied by Californium-252 Plasma Desorption Mass Spectrometry

Nageshwar Malhotra,[†] Peter Roepstorff,[‡]
Thomas K. Hansen,[†] and Jan Becher*,[†]

Department of Chemistry and
Department of Molecular Biology
Odense University, DK-5230, Odense-M, Denmark

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The potential for ^{252}Cf plasma desorption mass spectrometry (PDMS) to be used in the study of the relative complexation of crown ethers and other ligands with different alkali-metal cations has been investigated. In the PD spectra of different ligands after exposure to a mixture of alkali-metal salts (LiCl, NaOAc, and KOAc), it was found that the ratio of molecular ion abundances reflected the relative complexation trends.

Crown compounds and cryptands have many applications in analytical chemistry. Variable ring sizes as well as type, number, and position of the donor atoms in the ring permit a selective adaptation to specific cations. Monomeric cyclic polyethers are used mainly in the separation and determination of elements of the first and second main groups of the periodic table.[1] Several methods have been employed for the determination of the complexation constants of crown ethers, and these have been discussed in a number of reviews.[2]

^{252}Cf PDMS has previously been employed for the determination of molecular weights of large involatile molecules.[3] It has hitherto found extensive use in studies of peptides, proteins, and related compounds as well as porphyrins and related compounds;[5] its use for the characterization of polyethers has also been described.[6] Recently it has been shown[7] that fast atom bombardment mass spectrometry (FAB-MS) can be used in determination of the crown ether–alkali cation stability constants. Here we report the use of ^{252}Cf PDMS for analysis of crown ethers and their relative complexation with alkali-metal cations. The technique takes advantage of the nonsolubility of the studied compounds in aqueous solutions when these are fixed on a nitrocellulose matrix and thereby the possibility of including the cation by washing a sample with an alkali-metal salt solution.

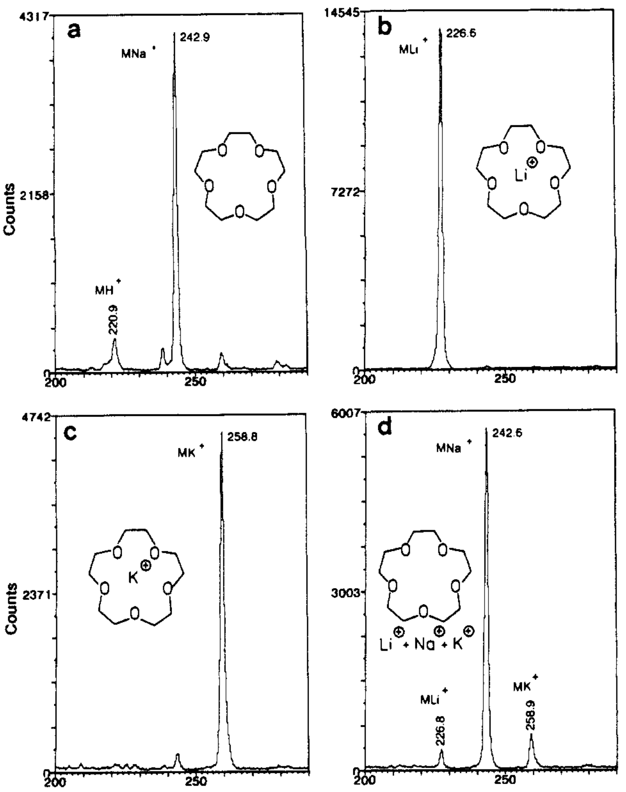
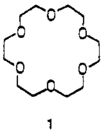


Figure 1. Positive-ion Pd spectra of 15-crown-5 (**2**): (a) The commercial sample dissolved in 100% TFA. (b) After complexation with a 0.1 M solution of LiCl. (c) After complexation with a 0.1 M solution of KOAc. (d) After complexation with a 0.1 M (1:1:1) solution of LiCl–NaOAc–KOAc.

The positive-ion ^{252}Cf Pd spectra of compounds **1–7** are summarized in Table I. All spectra were obtained[8] without attempts to remove the cations already present in the sample. For com-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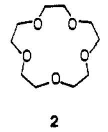
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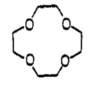
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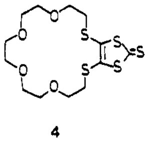
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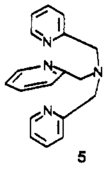
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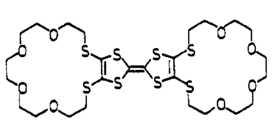
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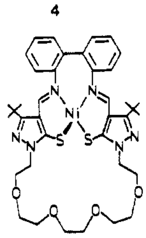
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[†] Department of Chemistry.

[‡] Department of Molecular Biology.

(1) Blasius, E.; Janzen, K. *Top. Curr. Chem.* **1981**, *98*, 163.

(2) (a) Izatt, R. M., Christensen, J. J., Eds. *Synthetic Multidentate Macrocyclic Compounds*; Academic Press: New York, 1978. (b) Izatt, R. M.; Eatough, D. J.; Christensen, J. J. *Struct. Bonding* (*Berlin*) **1973**, *16*, 161. (c) Burgermeister, W.; Winkler-Oswatitsch, R. *Inorganic Biochemistry II. Top. Curr. Chem.* **1977**, *69*, 91.

(3) (a) Cotter, R. J. *Anal. Chem.* **1988**, *60*, 681A. (b) Roepstorff, P. *Acc. Chem. Res.* **1989**, *22*, 421.

(4) Panell, L. K.; Fales, H. M.; Scovill, J. P.; Klayman, D. L.; West, D. X.; Tate, R. L. *Transition Met. Chem.* (*London*) **1985**, *10*, 141.

(5) (a) Chait, B. T.; Field, F. H. *J. Am. Chem. Soc.* **1982**, *104*, 5519. (b) Hunt, J. E.; Macfarlane, R. D.; Kartz, J. J.; Dougherty, R. C. *J. Am. Chem. Soc.* **1981**, *103*, 6775.

(6) Chait, B. T.; Shpungin, J.; Field, F. H. *Int. J. Mass Spectrom.* **1988**, *58*, 121.

(7) Bonas, G.; Bosso, C.; Vignon, M. R. *Rapid Commun. Mass Spectrom.* **1988**, *2*, 88.

(8) The samples of compounds **1–7**[13] were applied on a nitrocellulose matrix prepared by electrospraying 25–50 μL of a 2μg/L solution of nitrocellulose (Bio-Rad Laboratories, Richmond, CA) in acetone onto an aluminized Mylar foil. The samples were dissolved in 100% trifluoroacetic acid (TFA) to a concentration of 1 μg/μL. Between 2 and 3 μL of this solution was slowly deposited on the nitrocellulose matrix with simultaneus evaporation of the solvent. For exchange of the cation or for determination of the relative complexation, 2 μL of a 0.1 M solution of LiCl, NaOAc, or KOAc or an equimolar mixture of all three was applied directly on the nitrocellulose-bound sample. After a few minutes (to allow the ion-exchange reaction), the matrix was dried by spinning of the target. The plasma desorption mass spectra were obtained on a Bio-ion Bin 10k plasma desorption instrument (Bio-ion AB, Uppsala, Sweden). The instrument and data handling procedures have been described earlier.[14] The spectra were accumulated for 500000 fission events. A smooth background has been subtracted from all spectra.

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