Threshold Dissociation Energies of Protonated Amine/Polyether Complexes in a Quadrupole Ion Trap

Wendi M. David and Jennifer S. Brodbelt
Department of Chemistry and Biochemistry, University of Texas, Austin, Texas, USA

Electrospray ionization mass spectrometry (ESI-MS) is increasingly used to probe the nature of noncovalent complexes; however, assessing the relevance of gas-phase results to structures of complexes in solution requires knowledge of the types of interactions that are maintained in a solventless environment and how these might compare to key interactions in solution. This study addresses the factors impacting the strength of hydrogen bonding noncovalent interactions in the gas phase. Hydrogen bonded complexes consisting of ammonium ions bound to polydentate ethers are transported to the gas phase with ESI, and energy-variable collisional activated dissociation (CAD) is used to map the relative dissociation energies. The measured relative dissociation energies are correlated with the gas-phase basicities and steric factors of the amine and polyether constituents. To develop correlations between hydrogen bonding strength and *structural features* of the donor and acceptor molecules, a variety of amines with different gas-phase basicities and structures were selected, including primary, secondary, and tertiary amines, as well as those that are bidentate to promote intramolecular hydrogen bonding. The acceptor molecules are polydentate ethers, such as 18-crown-6. Four primary factors influence the observed dissociation energies of the polyether/ammonium ion complexes: the gas-phase basicities of the polyether and amine, steric effects of the amines, conformational flexibility of the polyethers, and the inhibition of intramolecular hydrogen bonds of the guest ammonium ions in the resulting ammonium/polyether noncovalent complexes. (J Am Soc Mass Spectrom 2003, 14, 383–392) © 2003 American Society for Mass Spectrometry

Electrospray ionization mass spectrometry (ESI-MS) is being increasingly used for the analysis of noncovalent complexes, especially those involving biomolecules, because it is a "soft" method for the transfer of solution species into the gas phase [1–5]. A key question revolves around the nature of the noncovalent complexes once they enter the gas phase. The specificity and the type of interactions that are retained in a solventless environment are important considerations when assessing the relevance of gas-phase results to the structures of solution-phase complexes [1–5]. Information on the relative stabilities of noncovalent complexes is often obtained from their gas-phase dissociation behavior [6], and many recent examples of biologically interesting macromolecular complexes demonstrate the importance of electrostatic interactions and hydrogen bonds in maintaining these noncovalent associations in the gas phase [7–14]. In some cases, the stabilities of complexes obtained based on gas-phase measurements do not correlate well with those obtained in solution [14], whereas other reports have shown remarkable agreement between gas-phase and solution results [13].

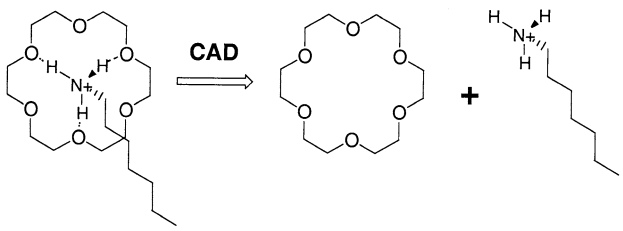
Hydrogen bonds are one of the most important types of noncovalent interactions in the gas phase, both in simple protonated molecules, such as peptides and proteins, and in large biological complexes, such as protein-protein and DNA-drug complexes [15]. The energies of hydrogen bonds are known to range from 0.2–40 kcal/mol [15], with those in solution typically weaker than those in the gas phase due to solvent dispersal and disruption of donor-acceptor interactions. Hydrogen bonds also span a range of bond distances and angles, with near-linear bonds generally being the strongest. Assessing the strength of hydrogen bonding interactions and correlating the interactions with structural factors should lead to a better understanding of the stabilities of noncovalent complexes in the gas phase [1–5]. Is the magnitude of the electrostatic interactions in a particular noncovalent complex the only determinant of gas-phase stability? Although hydrophobic effects are not operative in the absence of solvent, a local environment for a particular complex still exists in the gas phase which can affect the overall binding interactions.

Published online March 14, 2003

Address reprint requests to Dr. J. S. Brodbelt, Department of Chemistry and Biochemistry, University of Texas, 1 University Station, Austin, TX 78712-1167, USA. E-mail: jbrodbelt@mail.utexas.edu

1044-0305/03/$30.00
doi:10.1016/S1044-0305(03)00070-9

Received November 25, 2002
Revised January 31, 2003
Accepted January 31, 2003



Scheme 1. Collisionally activated dissociation (CAD) of a hydrogen bonded complex between 18-crown-6 and protonated heptylamine. The ammonium ion and neutral polyether are produced as the primary fragments during these low energy collisions.

Host-guest complexes between crown ethers and various guests have previously been utilized as gas-phase models to study multiple binding interactions [16–23]. In this study, energy-variable collisional activated dissociation is used to map the relative dissociation energies of a series of hydrogen bonded complexes consisting of ammonium ions bound to polydentate ethers (Scheme **1**). The measured relative dissociation energies are correlated with the gas-phase basicities and steric factors of the amine and polyether constituents. To develop correlations between hydrogen bonding strength and *structural features* of the donor and acceptor molecules, a variety of amines with different gas-phase basicities and structures were selected, including primary, secondary, and tertiary amines, as well as those that are bidentate to promote intramolecular hydrogen bonding (see Table 1). The amines were chosen to span a range of gas-phase basicities yet retain similar sizes to prevent significant bias in the dissociation thresholds due to mass-dependent and degree-of-freedom effects. The acceptor molecules are polydentate ethers, such as 18-crown-6 and other analogs (Table 1).

Experimental

Stock solutions of each amine guest and polyether were prepared in methanol at mM concentrations. All chemicals were obtained commercially and used without further purification. Lysine, threonine, and valine were obtained as D,L-isomers, while glutamine was the L-isomer. The desired amine and polyether were mixed in a 1:1 ratio at concentrations of 20 μM and allowed to interact for at least five min to ensure complexation prior to analysis by ESI-MS.

ESI-MS Experiments

The noncovalent host-guest complexes were admitted by syringe pump at 5–10 μL/min to a ThermoFinnigan LCQ Duo quadrupole ion trap mass spectrometer operated in the positive mode with a source voltage of 4.5 kV. The heated capillary was kept at 120 °C to minimize dissociation of the complexes during desolvation. Instrument response was initially tuned for the protonated (18-crown-6 + 6-amino-1-hexanol) complex and these conditions were used for all subsequent analyses. The desired singly-charged complex was isolated in the ion trap by resonant ejection and subjected to energy-variable collisional activated dissociation in which the applied collision energy was raised incrementally. Ten scans (each consisting of 10 microscans) were averaged for each spectrum.

Plots of relative abundance of the parent ion versus applied collisional energy were generated with Microcal Origin 5.0 (Microcal Software, Inc., Northampton, MA) to determine $E_{1/2}$ values, defined as the point at which half of the isolated parent complex had dissociated, for each polyether-ammonium ion complex. These dissociation curves were determined in triplicate for each complex. In order to correct for instrumental variations over longer periods of time, a "calibration factor" was determined whenever data was collected and used to correct the $E_{1/2}$ values. This calibration procedure was necessary since slight variations in pressure and interface conditions in the mass spectrometer have an effect on the internal energies of the ions and the collisional activation process, leading to slightly different $E_{1/2}$ values over time. The protonated (18-crown-6 + heptylamine) complex was used for calibration purposes. An initially determined average $E_{1/2}$ value (from five replicates) for this complex was compared to the $E_{1/2}$ value obtained for this same complex on any subsequent day that data was collected for other complexes, and a calibration factor was calculated according to the following equation:

$$\text{Factor} = E_{1/2}\,(\text{measured})/E_{1/2}\,(\text{average value}) \quad (1)$$

All other $E_{1/2}$ values determined for other complexes on that particular day were multiplied by the determined calibration factor.

The $E_{1/2}$ value for each complex was also corrected for the degrees-of-freedom in the complex, relative to the protonated (18-crown-6 + *N,N,N′,N′*-tetramethylethylenediamine) complex, chosen as a reference.

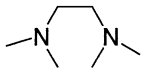
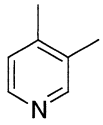
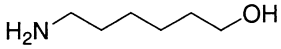
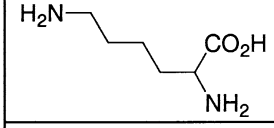
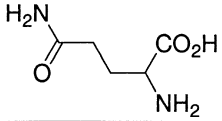
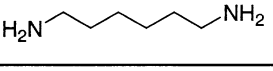
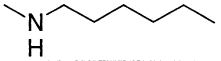
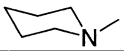
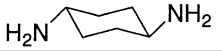
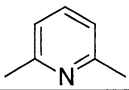
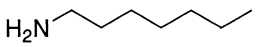
$$E'_{1/2} = [E_{1/2}\,(\text{complex})][\#\text{ of DOF (reference)}]/[\#\text{ of DOF (complex)}] \quad (2)$$

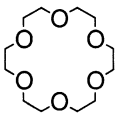
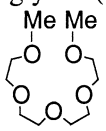
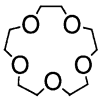
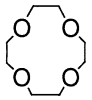
where $E'_{1/2}$ is the DOF-corrected value.

Modeling Experiments

Molecular modeling experiments were undertaken using the commercially available software package PC Spartan Pro (Wavefunction, Inc., Irvine, CA). The lowest energy conformer for each hydrogen bonded complex (with a particular hydrogen bond orientation) was first determined with the molecular mechanics force field MMFF94. Geometry optimization of these structures was undertaken utilizing the semi-empirical PM3 model.

Table 1. Amines and polyethers

Amine Guest	*GPB (PA)*[1]	*MW*	*Amine Guest*	*GPB (PA)*[1]	*MW*
N,N,N', N'-tetramethyl-ethylenediamine (**TMEDA**) 	232.2 (242.3)	116	3,4-Lutidine (**3,4-L**) 	221.4 (229.0)	107
Quinuclidine (**Quin**) 	227.9 (235.2)	111	6-Amino-1-hexanol (**6AH**) 	219.1 (231.8)	117
Lysine (**lys**) 	227.5 (238.3)	146	Glutamine (**glu**) 	215.3 (224.4)	146
1,6-hexanediamine (**HDA**) 	226.4 (239.1)	116	*N*-Methylhexylamine (**MHA**) 	~215[2]	115
1-Methylpiperidine (**1MP**) 	224.9 (232.3)	99	*Trans*-1,4-diamino-cyclohexane (**DCH**) 	~213[2]	114
2,6-Lutidine (**2,6-L**) 	222.8 (230.4)	107	Heptylamine (**HA**) 	212.8 (220.9)	115

Polyether Host	*GPB* (PA)	*MW*	*Polyether Host*	*GPB (PA)*	*MW*
18-crown-6 (**18C6**) 	217.6 (231.3)	264	Tetraglyme (**TG**) 	214.8 (228.2)	222
15-crown-5 (**15C5**) 	215.2 (225.8)	220	12-crown-4 (**12C4**) 	213.0 (221.8)	176

[1] GPB = gas-phase basicity, PA = proton affinity, in kcal/mol, from ref. 24
[2] estimated

Results and Discussion

Dissociation Energies of Ammonium/Polyether Noncovalent Complexes

Singly-protonated ammonium ion/polyether complexes were formed by ESI and isolated in a quadrupole ion trap mass spectrometer. The relative dissociation energies of the resulting noncovalent complexes were subsequently obtained by energy-variable CAD, in which the resonant voltage was varied (Figure 2). In all cases, the lowest energy (threshold) dissociation process involves the loss of the polyether molecule, resulting in the free protonated amine, as illustrated in Scheme **1** and Figure 1. The loss of the neutral polyether is uniformly observed for the complexes listed in Table 2. For amines with gas-phase basicities that are much lower than those of the polyether hosts, such as valine (gas-phase basicity = 208.7 kcal/mol) [24], dissociation

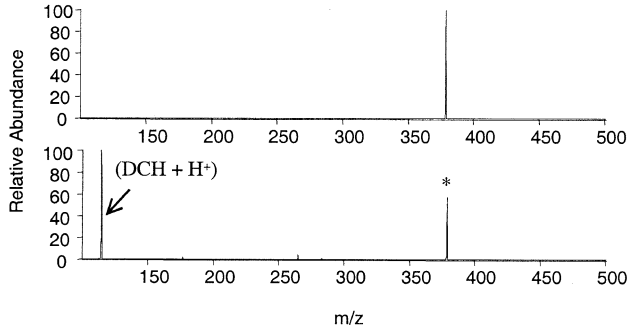


Figure 1. Isolation and CAD of a hydrogen bonded complex between 18-crown-6 and protonated *trans*-1,4-diaminocyclohexane. The parent complex is denoted by an asterisk.

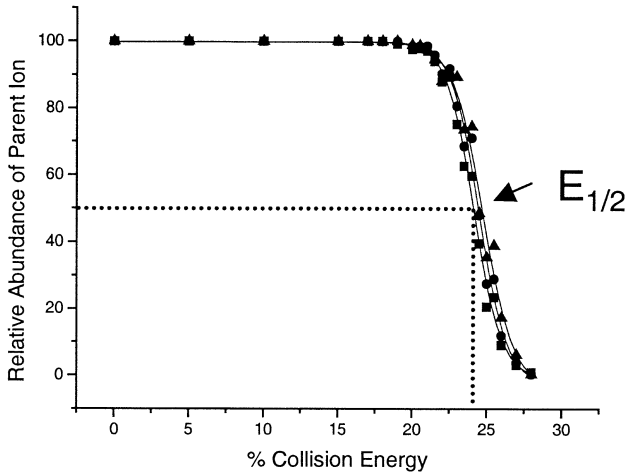


Figure 2. Triplicate series of dissociation curves for the protonated (18-crown-6 + *trans*-1,4-diaminocyclohexane) complex. (Data represented by the triangles was acquired on 2/27/02; circles on 2/22/02, and squares on 2/26/02.)

of the noncovalent complex generates the protonated polyether and neutral amine as the primary fragments. In this situation, such amines and their complexes were discarded from further study. A triplicate series of dissociation curves is shown in Figure 2 to illustrate the reproducibility of the method when using a quadrupole ion trap instrument. Day-to-day variations in instrumental conditions result in standard deviations of the $E_{1/2}$ values that vary by $\pm 0.2\%$, about 1–2% of the net reported values. Over longer periods of time, the $E_{1/2}$ values differed from previous determinations by up to 3–5%. After application of a calibration factor (see Experimental section details), the $E_{1/2}$ values were again within 1–2% of the reported values.

The point at which 50% of the initial protonated polyether/amine complexes dissociated was tabulated as the $E_{1/2}$ value (Table 2). The measured $E_{1/2}$ values range from about 12–25% (of the maximum CAD voltage of 5 $V_{p\text{-}p}$). The numbers given in parenthesis in Table 2 represent the $E_{1/2}$ values corrected for the number of degrees-of-freedom of the complexes. With all other factors being equal, complexes with more degrees of freedom intrinsically are expected to require greater amounts of internal energy to dissociate, giving $E_{1/2}$ values that are correspondingly higher and not directly comparable to the values obtained for the complexes with fewer degrees of freedom. For the data in Table 2, all the $E_{1/2}$ values in parentheses are adjusted for the degrees of freedom relative to one selected standard, the protonated (18-crown-6 + *N,N,N′,N′*-tetramethylethylenediamine) complex, chosen because all the $E_{1/2}$ values for the other 18-crown-6 complexes are higher than this benchmark value. Rhyzhov and coworkers recently showed that the stabilities of noncovalent complexes correlated with the number of degrees of freedom of the complexes, when other factors were equal [23]. Both the experimentally obtained $E_{1/2}$ and the degree-of-freedom adjusted $E_{1/2}$ values are shown in Table 2 because the degree-of-freedom procedure is rudimentary and does not account for possible slight mass-dependent differences in the CAD process or variations in the initial internal and kinetic energies of the complexes, nor does it consider specific active modes. Thus, comparisons of $E_{1/2}$ values for complexes involving the same polyether but different amines or those involving comparisons of complexes containing 15-crown-5 to complexes containing tetraglyme are least dependent on the degree-of-freedom factor.

Table 2. $E_{1/2}$ values[1]

Amine	*12C4*	*TG*	*15C5*	*18C6*
N,N,N′,N′-tetramethylethylene diamine		12.1 (13.1)	12.4 (13.9)	13.7 (13.7)
Quinuclidine	13.1 (17.6)	14.6 (16.6)	14.3 (16.9)	15.8 (16.6)
Lysine		17.3 (18.7)	17.6 (19.7)	20.4 (20.4)
1,6-Hexanediamine	14.1 (17.9)	17.5 (18.9)	18.1 (20.2)	21.6 (21.6)
1-Methylpiperidine	12.7 (17.5)	15.3 (17.7)	15.0 (18.0)	16.6 (17.7)
2,6-Lutidine		14.7 (18.0)	12.7 (16.1)	14.8 (16.6)
3,4-Lutidine		15.4 (18.8)	15.8 (20.1)	17.4 (19.5)
6-Amino-1-hexanol		19.7 (21.7)	19.1 (21.7)	23.4 (23.8)
Glutamine				18.2 (19.4)
N-methylhexylamine	16.6 (21.1)	20.1 (21.7)	19.4 (21.7)	20.7 (20.7)
Trans-1,4-diaminocyclohexane	17.6 (23.2)	21.0 (23.5)	20.9 (24.2)	24.2 (25.0)
Heptylamine	19.7 (24.5)	22.8 (24.3)	22.8 (25.1)	25.5 (25.1)

[1] $E_{1/2}$ values for protonated amine/polyether noncovalent complexes listed as a percentage of 5 $V_{p\text{-}p}$. $E_{1/2}$ values corrected for degrees-of-freedom given in parentheses.

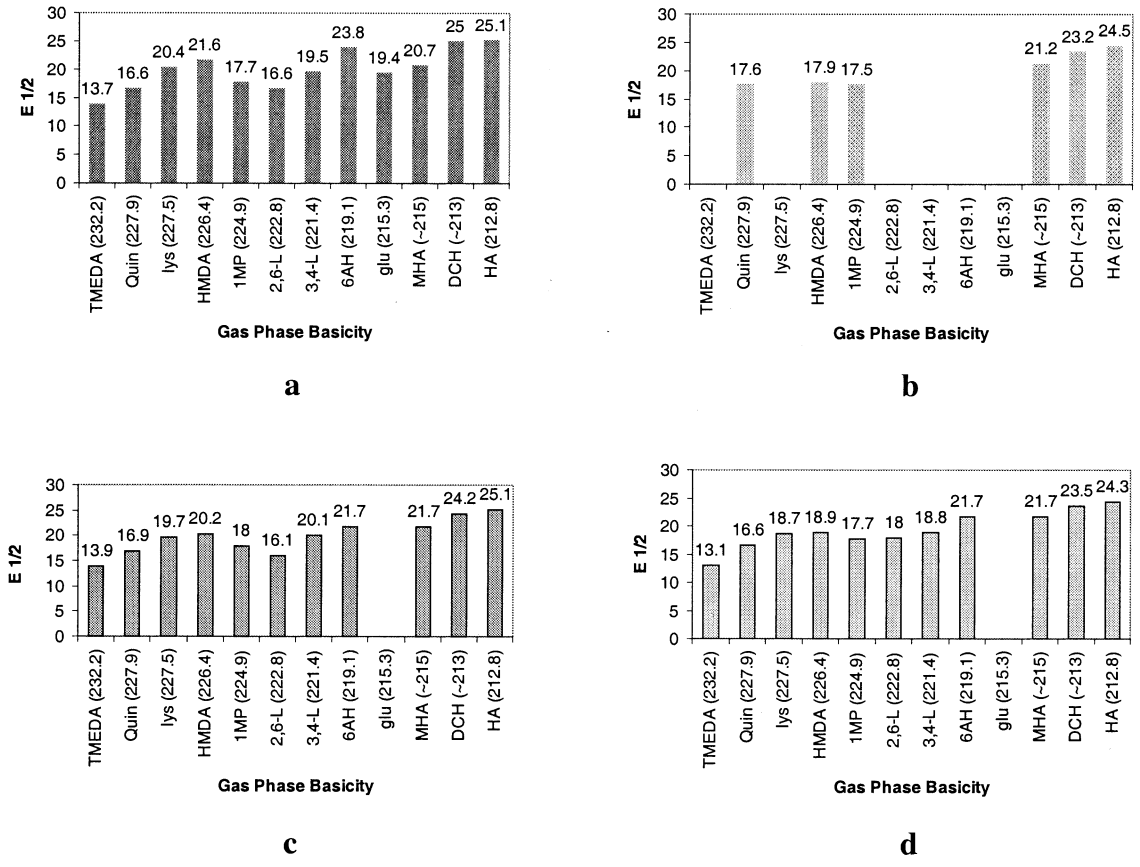


Figure 3. Trend for $E_{1/2}$ values versus gas-phase basicity. Graph (**a**) displays data for protonated amine/18-crown-6 complexes; (**b**) shows data for 15-crown-5 complexes; (**c**) shows data for 12-crown-4 complexes; and (**d**) shows data for tetraglyme complexes.

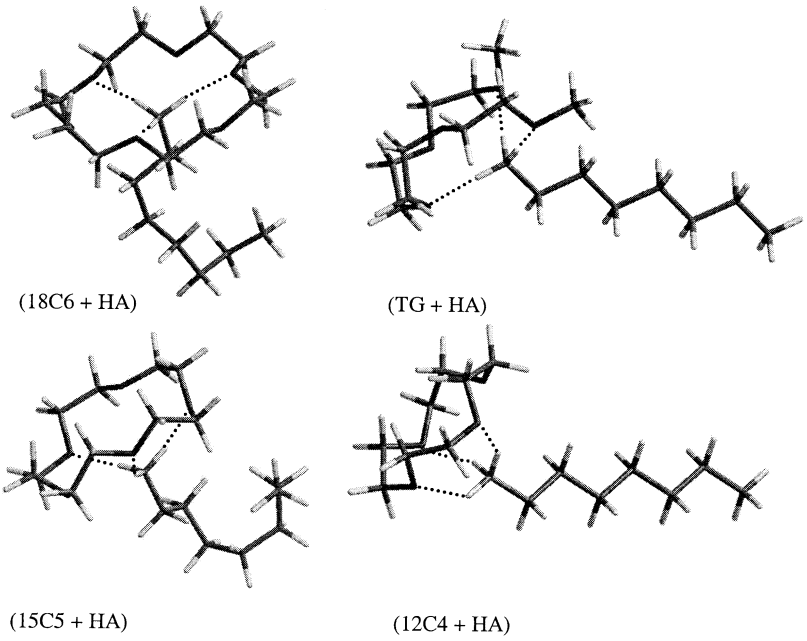


Figure 4. Molecular models of protonated heptylamine (HA)/polyether noncovalent complexes. (18-crown-6 + HA), (15-crown-5 + HA), (TG + HA), and (12-crown-4 + HA) shown left to right. Hydrogen bonds are denoted by dashed lines.

The amine guests shown in Table 1 were chosen to span a range of gas-phase basicities, with *N,N,N′,N′*-tetramethylethylenediamine being the most basic (gas-phase basicity = 232.2 kcal/mol) [24] and heptylamine the least basic (gas-phase basicity = 212.8 kcal/mol) [24]. All of the amines are similar in size, except for lysine and glutamine, which are substantially larger than the others. The comparison between lysine and glutamine is especially interesting since they both have identical nominal molecular weights but quite different gas-phase basicities. Lysine has a basic side chain that is the preferred protonation site, whereas glutamine does not. Other amines, such as 2,6-lutidine and 3,4-lutidine, were selected to allow an assessment of the influence of the steric environment of the ammonium ion on the stabilities of the resulting complexes. The measured gas-phase basicities between these two substituted pyridines only differ by 1.4 kcal/mol, but 2,6-lutidine is much more sterically hindered than the 3,4-isomer. Also, bifunctional amines, such as 1,6-hexanediamine and 6-amino-1-hexanol, which are known to engage in intramolecular hydrogen bonding when protonated, were selected for comparison to amines that can not form intramolecular hydrogen bonds. It was expected that comparison of the complexes containing these amines would support the formation of intermolecular versus intramolecular hydrogen bonds.

Four primary factors influence the observed dissociation energies of the polyether/ammonium ion complexes: the gas-phase basicities of the polyether and amine, steric effects of the amines, conformational flexibility of the polyethers, and the inhibition of intramolecular hydrogen bonds of the guest ammonium ions in the resulting ammonium/polyether noncovalent complexes.

Influence of Gas-Phase Basicity of Amine and Polyether

The relative gas-phase basicities of the amine and polyether affect the formation of stable hydrogen bonds in the complexes. It is well-known that compounds that have similar basicities typically favor the formation of stronger hydrogen bonds [25]. The polyethers in this study have gas-phase basicities that are lower than most of the amines, so it is clear that the amine binds the proton more strongly in the complexes. With a couple of notable exceptions, the magnitude of the $E_{1/2}$ values increases as the gas-phase basicity of the amine decreases, reflecting that the complexes are more stable going down the column in Table 2.

The trend for $E_{1/2}$ values versus gas-phase basicity of the amine is illustrated in Figure 3 for each polyether. A general inverse correlation between $E_{1/2}$ value and gas-phase basicity of the amine is evident for each series of ammonium ion/polyether complexes. In these complexes, the amine guests with higher gas-phase basicities possess a greater proton-binding strength, which correspondingly reduces the strength of the hydrogen bonds to the oxygen atoms of the polyethers. Consequently, complexes involving amine guests with *higher* gas-phase basicities are less stable and *lower* $E_{1/2}$ values are observed. For example, the $E_{1/2}$ value for the protonated (18-crown-6 + *N,N,N′,N′*-tetramethylethylenediamine) complex is lower (13.7) than the $E_{1/2}$ values for any other 18-crown-6 complexes. The very low $E_{1/2}$ value for the protonated (18-crown-6 + *N,N,N′,N′*-tetramethylethylenediamine) complex is attributed to the large gas-phase basicity of *N,N,N′,N′*-tetramethylethylenediamine (232.2 kcal/mol) [24]. The hydrogen proton is less available for hydrogen bonding with the polyether host since it is much more tightly associated with the amine. In addition, the protonated nitrogen atom in this noncovalent complex is extremely sterically hindered, restricting the optimal alignment of the hydrogen proton with the dipoles of the polyether host oxygen atoms. This deviation from linearity of the hydrogen bond stabilizing the noncovalent complex further weakens the association between 18-crown-6 and protonated *N,N,N′,N′*-tetramethylethylenediamine.

For each polyether host, the noncovalent complex with the highest stability is with protonated heptylamine, the amine with the lowest gas-phase basicity of the series (212.8 kcal/mol) [24]. The heptylammonium guest is more likely to "share" its available hydrogen atoms with the polyether host, and the formation of three favorable hydrogen bonds between the polyether oxygens and heptylamine is particularly favorable with this primary amine (Figure 4). Among the protonated heptylamine/polyether complexes shown, the average NH–O bond distance is greatest for the 12-crown-4 complex, as expected due to the more rigid structure of 12-crown-4 in comparison to the other polyethers, which restricts its flexibility in alignment of the oxygen dipoles with the hydrogen atoms of the heptylammonium ion. The hydrogen bonds in this complex are not as linear, and the $E_{1/2}$ value for this complex is correspondingly lower. The other primary amines of the study (lysine, 1,6-hexanediamine, 6-amino-1-hexanol, *trans*-1,4-diaminocyclohexane, glutamine) may also form stable noncovalent complexes involving three favorable hydrogen bonds with the polyether hosts, and these complexes have *higher* $E_{1/2}$ values than those observed for complexes with the secondary and tertiary amine guests (Table 2). The same type of correlation between the number of hydrogen bonds formed in an ammonium ion/polyether complex and its stability has also been demonstrated in solution [26]. In these primary ammonium/polyether complexes an inverse correlation between $E_{1/2}$ values and amine gas-phase basicities is maintained, except in the case of lysine and glutamine, the two amino acids (discussed later). Again, 12-crown-4, with its lower gas-phase basicity, results in complexes that generally have lower $E_{1/2}$ values than the analogous 15-crown-5 or 18-crown-6 complexes.

The molecular models of three noncovalent com-

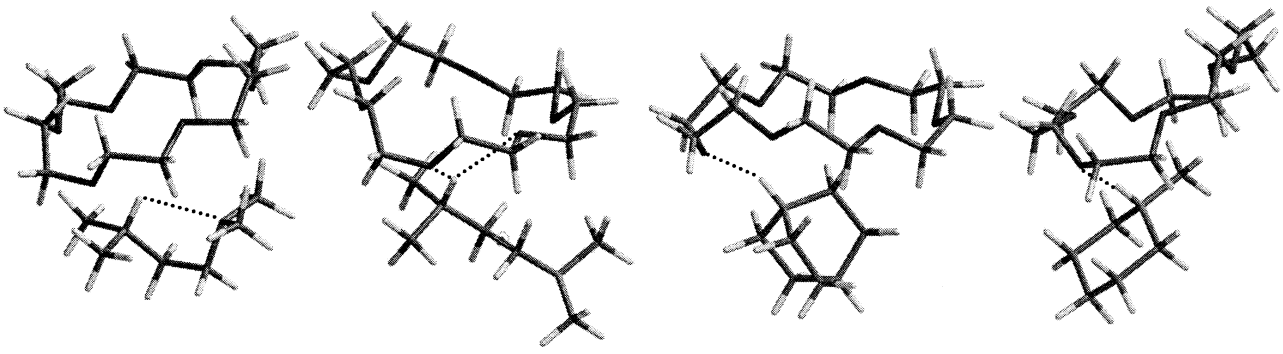


Figure 5. Molecular models for 18-crown-6 noncovalent complexes with protonated *N,N,N′,N′*-tetramethylethylenediamine (TMEDA) in two different hydrogen bonding patterns, protonated quinuclidine (quin), and protonated 1-methylpiperidine (1MP), shown left to right. Dashed lines denote primary hydrogen bonds in the complexes.

plexes involving 18-crown-6 and either *N,N,N′,N′*-tetramethylethylenediamine, quinuclidine, or 1-methylpiperidine nicely illustrate the differences in binding that are related to the different gas-phase basicities of the amines (Figure 5). These three protonated amine guests are all tertiary amines, capable of forming only one hydrogen bond, but their gas-phase basicities and steric environments are quite different. The possible structures for the protonated (18-crown-6 + *N,N,N′,N′*-tetramethylethylenediamine) complex either place the *N,N,N′,N′*-tetramethylethylenediamine with an intramolecular hydrogen bond that is also directed to the center of the crown ether, or with a hydrogen atom bound in an edge orientation to two oxygen atoms of the crown ether. While both protonated quinuclidine and 1-methylpiperidine also only form one hydrogen bond with 18-crown-6, the different orientations of these hydrogen bonds are more favorable, and the hydrogen atom that participates in binding the crown ether oxygens is more equally shared between the amine guest and polyether host as a result of the lower gas-phase basicity of these amines. This effect is reflected in the slightly increased N–H bond length in complexes of increasing stability (1.010 Å for the N–H bond in *N,N,N′,N′*-tetramethylethylenediamine, $E_{1/2}$ = 13.7; 1.028Å for quinuclidine, $E_{1/2}$ = 16.6; and 1.032Å for 1-methylpiperidine, $E_{1/2}$ = 17.7 in the 18-crown-6 complexes).

Influence of the Steric Effects of the Amines

Steric effects impact the formation of stable hydrogen bonds based on the restriction of the most favorable bond angles. This factor is illustrated in the lower $E_{1/2}$ values obtained for the complexes containing 2,6-lutidine versus those containing 3,4-lutidine, both dimethyl-substituted pyridines. The differences in $E_{1/2}$ values are larger than would be predicted based on the difference in the gas-phase basicities of these two pyridines (only 1.4 kcal/mol). Molecular modeling (Figure 6) predicts that protonated 3,4-lutidine forms a much shorter, more favorable hydrogen bond with 18-crown-6 than does 2,6-lutidine. This type of steric effect may also contribute to the unusually low $E_{1/2}$ values obtained for the complexes containing protonated *N,N,N′,N′*-tetramethylethylenediamine (see Figure 5) and protonated *N*-methylhexylamine (Figure 7). A recent examination of the gas-phase dissociation behavior of protonated cyclodextrin/amino acid inclusion complexes found that steric factors exert a large effect on the observed complex stabilities [27]. In this case, 'steric locking' increased the stability of certain complexes. Moreover, no simple correlation between gas-phase basicity of the amino acids and stabilities of the inclusion complexes was exhibited [27]. Although these cyclodextrin inclusion complexes are more complicated than the simple protonated amine/polyether complexes investigated here, it is clear that the contribution of the strength of hydrogen bonding interactions to gas-phase stability can be affected by steric factors.

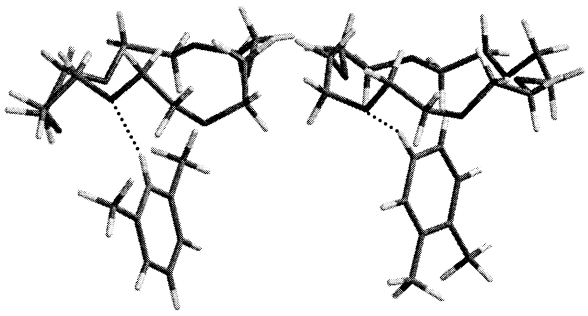


Figure 6. Noncovalent complexes of 18-crown-6 with protonated 2,6-lutidine (2,6-L) and 3,4-lutidine (3,4-L), are shown. Hydrogen bonds are shown by the dashed lines.

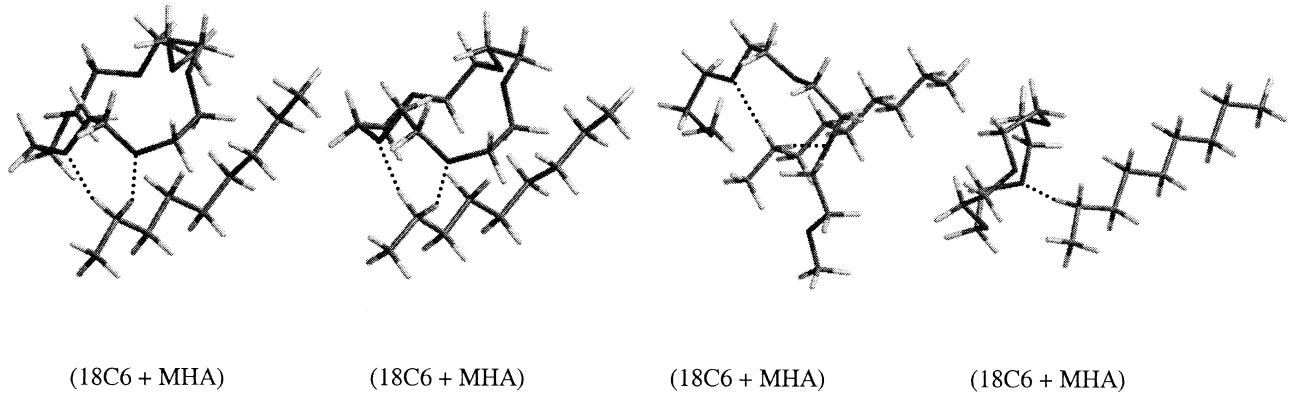


Figure 7. Molecular models of polyether noncovalent complexes with protonated *N*-methylhexylamine (MHA). (18-crown-6 + MHA), (15-crown-5 + MHA), (TG + MHA), and (12-crown-4 + MHA) shown left to right. Dashed lines denote hydrogen bonds.

Conformational Flexibility of the Polyethers

The influence of the conformational flexibility of the polyethers is best illustrated by comparison of the $E_{1/2}$ values obtained for the 15-crown-5 versus tetraglyme complexes. Both of these polyethers have similar sizes and gas-phase basicities, but 15-crown-5 has a more rigid, preorganized structure. For complexes containing unhindered amines, such as 1,6-hexanediamine, 3,4-lutidine, 6-amino-1-hexanol, heptylamine, and *trans*-1,4-diaminocyclohexane, the $E_{1/2}$ values for both the 15-crown-5 and tetraglyme complexes are similar. For one of the most sterically hindered amines, 2,6-lutidine, the $E_{1/2}$ value for the tetraglyme complex is considerably greater than that for the 15-crown-5 complex. This difference likely relates to the greater conformational flexibility of the acyclic ether, allowing better optimization of the alignment of the dipoles associated with the oxygen atoms with the proton bound to the amine, as illustrated in Figure 8.

Inhibition of Intramolecular Hydrogen Bonds

Two of the most striking cases that illustrate the impact of intramolecular versus intermolecular hydrogen bonds involve complexes that incorporate the 1,6-hexanediamine and 6-amino-1-hexanol amines. These two amines have much higher basicities than their monofunctional counterpart, heptylamine, and this enhanced basicity is attributed to the ability of the diamine or aminoalcohol compounds to form intramolecular hydrogen bonds in the gas-phase, thus forming cyclized protonated species. Based on the high basicities of these two amines, relatively low $E_{1/2}$ values would be predicted for the polyether/ammonium complexes because the amine would bind the proton far more strongly than would the polyether. However, the experimental $E_{1/2}$ values are unusually high relative to other amines in the series. In fact, the $E_{1/2}$ values are similar to that obtained for the monofunctional heptylamine/polyether complexes. This deviation from predicted behavior suggests that the intramolecular hydrogen bonds possible in the protonated bifunctional amines are replaced by the intermolecular hydrogen bonds involving the polyether, thus reducing the effective basicity of the bifunctional amines in the complexes.

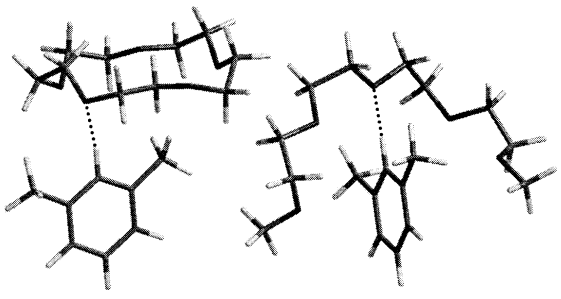


Figure 8. Molecular models of noncovalent complexes between protonated 2,6-lutidine (2,6-L) and 15-crown-5 and tetraglyme, respectively. Dashed lines denote hydrogen bonds.

A similar "reverse ordering" of $E_{1/2}$ values is evident in comparing the protonated lysine/polyether complexes with the analogous protonated glutamine/polyether complexes. These two amino acids have identical nominal molecular weights and similar degrees-of-freedom, but lysine has a significantly higher gas-phase basicity in comparison to glutamine. The $E_{1/2}$ values of the lysine/polyether complexes would be expected to be *lower* than those for the complexes with glutamine based on the inverse correlation between the basicity of the amine and the $E_{1/2}$ values. In fact, the opposite trend is observed (Table 2). Since lysine has two primary amino groups, it could also form a cyclized protonated species similar to 1,6-hexanediamine and 6-amino-1-hexanol with a net basicity that would be effectively higher than the basicity exhibited by the isolated primary side-chain amine when the proton is solvated by the polyether. Molecular models for crown ether binding of each possible singly-protonated lysine ammonium ion were generated (Figure 9). The complex

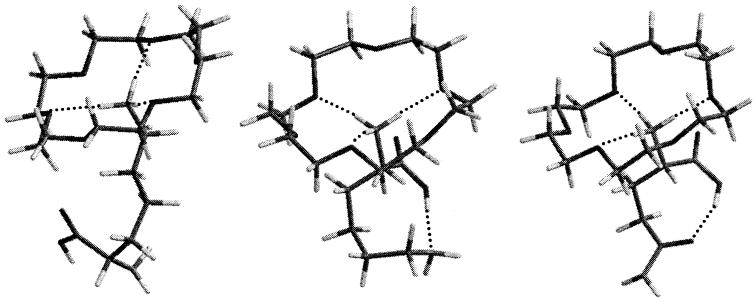


Figure 9. Molecular models of 18-crown-6 complexes with protonated lysine (lys) and glutamine (glu). (18-crown-6 + lys, side-chain ammonium), (18-crown-6 + lys, terminal ammonium), and (18-crown-6 + glu) shown left to right. Hydrogen bonds are shown by the dashed lines.

arising from hydrogen bonding between the side-chain amino functionality of lysine and the crown ether is predicted to be slightly lower in energy. In this complex three favorable, nearly linear hydrogen bonds are predicted. Two strong hydrogen bonds and a third weaker bond are predicted for the structure involving complexation between the protonated terminal amino group of lysine and the crown ether. A possible structure in which lysine is zwitterionic is similarly predicted to bind the crown ether through the protonated terminal amino group and is less favorable than either of the singly-protonated structures. Protonated glutamine can only hydrogen bond to the polyether oxygens through the terminal ammonium ion, and the predicted structure is similar to the structure involving the terminal protonated amino functionality of lysine (Figure 9). In addition, intramolecular hydrogen bonding in this structure still exists and is not abrogated by complexation with the crown ether, further weakening the overall noncovalent complex.

Conclusion

Noncovalent polyether/ammonium ion complexes bound only by hydrogen bonds can be transported to the gas phase by ESI and analyzed by collisional activated dissociation to estimate relative binding energies. A comparison of the relative binding energies for all polyether/ammonium ion complexes reveals a general inverse relationship between the complex stabilities and gas-phase basicities of the amine guests and polyether hosts. The stabilities of the complexes increase as the gas-phase basicity of the amine guest decreases. In addition, an increase in the number of optimal hydrogen bonds imparts greater stability to the noncovalent complexes, as expected. Those complexes between polyethers and primary amines, such as heptylamine and trans-1,4-diaminocyclohexane which possess low gas-phase basicities, thus have the highest stabilities among the series compared in this study.

Several notable exceptions to the general trend between complex stability and gas-phase basicity are evident, however. Molecular models assist in elucidating features that impede optimal hydrogen bonding between donor/acceptor groups, leading to an overall decrease in the gas-phase stability of certain noncovalent complexes. In particular, when bulky steric groups are proximal to the hydrogen bond donor, the magnitude of the hydrogen bonding interactions is decreased and the complex stability is less than expected based on the gas-phase basicities of the amine and polyether alone. This effect is most pronounced in polyether complexes associated with N-methylhexylamine, 2,6-lutidine, and *N,N,N′,N′*-tetramethylethylenediamine. Similarly, a lack of conformational flexibility can be detrimental for the optimal alignment of dipoles associated with the hydrogen bonding interactions, decreasing the complex stability. The greater stability of the protonated 2,6-lutidine complex with tetraglyme compared to 15-crown-5, which is less flexible, illustrates the importance of maintaining strong hydrogen bonding interactions for the stability of noncovalent complexes in the gas phase.

The inhibition of intramolecular hydrogen bonds of bifunctional amines and preferential formation of intermolecular hydrogen bonds in the complexes demonstrated in this study show that it is possible for different hydrogen bonding patterns to occur in multifunctional compounds, depending on the local environment in the complex. This factor is extremely relevant in considering the importance of gas-phase results for most biologically relevant macromolecular complexes since they possess many different points of interactions. A local environment which may shield or impede electrostatic interactions is likely to decrease gas-phase stability, even when the solution stability may be high under these same conditions due to hydrophobic interactions. The interactions maintained in the gas phase may very well be different than the associations initially present in the solution complex, as well.

Acknowledgments

The authors gratefully acknowledge support from the National Science Foundation (CHE-9820755) and Welch Foundation (F-1155).

References

1. Veenstra, T. D. Electrospray Ionization Mass Spectrometry in the Study of Biomolecular Noncovalent Interactions. *Biophys. Chem.* **1999,** *79,* 63–79.
2. Loo, J. A. Electrospray Ionization Mass Spectrometry: A Technology for Studying Noncovalent Macromolecular Complexes. *Int. J. Mass Spectrom.* **2000,** *200,* 175–186.
3. Schalley, C. A. Supramolecular Chemistry Goes Gas Phase: The Mass Spectrometric Examination of Noncovalent Interactions in Host-Guest Chemistry and Molecular Recognition. *Int. J. Mass Spectrom.* **2002,** *194,* 11–39.
4. Daniel, J. M.; Friess, S. D.; Rajagopalan, S.; Wendt, S.; Zenobi, R. Quantitative Determination of Noncovalent Binding Interactions Using Soft Ionization Mass Spectrometry. *Int. J. Mass Spectrom.* **2002,** *216,* 1–27.
5. Brodbelt, J. S. Probing Molecular Recognition by Mass Spectrometry. *Int. J. Mass Spectrom.* **2000,** *200,* 57–69.
6. Rodgers, M. T.; Armentrout, P. B. Noncovalent Metal–Ligand Bond Energies as Studied by Threshold Collision-Induced Dissociation. *Mass Spec. Rev.* **2000,** *19,* 215–247.
7. Gabelica, V.; De Pauw, E. Comparison Between Solution-Phase Stability and Gas-Phase Kinetic Stability of Oligodeoxynucleotide Duplexes. *J. Mass Spectrom.* **2001,** *36,* 397–402.
8. Kitova, E. N.; Bundle, D. R.; Klassen, J. S. Thermal Dissociation of Protein-Oligosaccharide Complexes in the Gas Phase: Mapping the Intrinsic Intermolecular Interactions. *J. Am. Chem. Soc.* **2002,** *124,* 5902–5913.
9. Sannes-Lowery, K. A.; Mei, H.-Y.; Loo, J. A. Studying Aminoglycoside Antibiotic Binding to HIV-1 TAR RNA by Electrospray Ionization Mass Spectrometry. *Int. J. Mass Spectrom.* **1999,** *193,* 115–122.
10. Hunter, C. L.; Mauk, A. G.; Douglas, D. J. Dissociation of Heme from Myoglobin and Cytochrome b_5: Comparison of Behavior in Solution and the Gas Phase. *Biochem.* **1997,** *36,* 1018–1025.
11. Jørgenson, T. J. D.; Roepstorff, P.; Heck, A. J. R. Direct Determination of Solution Binding Constants for Noncovalent Complexes between Bacterial Cell Wall Peptide Analogues and Vancomycin Group Antibiotics by Electrospray Ionization Mass Spectrometry. *Anal. Chem.* **1998,** *70,* 4427–4432.
12. Tito, M. A.; Miller, J.; Walker, N.; Griffin, K. F.; Williamson, E. D.; Despeyroux-Hill, D.; Titball, R. W.; Robinson, C. V. Probing Molecular Interactions in Intact Antibody/Antigen Complexes, an Electrospray Time-of-Flight Mass Spectrometry Approach. *Biophys. J.* **2001,** *81,* 3503–3509.
13. Rosu, F.; Gabelica, V.; Houssier, C.; De Pauw, E. Determination of Affinity, Stoichiometry and Sequence Selectivity of Minor Groove Binder Complexes with Double-Stranded Oligodeoxynucleotides by Electrospray Ionization Mass Spectrometry. *Nucleic Acid Res.* **2002,** *30,* e82.
14. Nesatyy, V. J. Gas-Phase Binding of Noncovalent Protein Complexes between Bovine Pancreatic Trypsin Inhibitor and Its Target Enzymes Studied by Electrospray Ionization Tandem Mass Spectrometry. *J. Mass Spectrom.* **2001,** *36,* 950–959.
15. Jeffrey, G. A.; Saenger, W. *Hydrogen Bonding in Biological Structures.* Springer-Verlag: Berlin, 1991; p. 569–582.
16. Maleknia, S.; Brodbelt, J. Cavity-Size-Dependent Dissociation of Crown Ether/Ammonium Ion Complexes in the Gas Phase. *J. Am. Chem. Soc.* **1993,** *115,* 2837–2843.
17. Liou, C.-C.; Wu, H.-F.; Brodbelt, J. S. Hydrogen Bonding Interactions in Gas-Phase Polyether/Ammonium Ion Complexes. *J. Am. Soc. Mass Spectrom.* **1994,** *5,* 260–273.
18. Dearden, D. V.; Dejsupa, C.; Liang, Y.; Bradshaw, J. S.; Izatt, R. M. Intrinsic Contributions to Chiral Recognition: Discrimination Between Enantionmeric Amines by Dimethyldiketopyridino-18-Crown-6 in the Gas Phase. *J. Am. Chem. Soc.* **1997,** *119,* 353–359.
19. Meot-Ner, M.; Sieck, L. W.; Liebman, J. F.; Scheiner, S. Complexing of the Ammonium Ion by Polyethers. Comparative Complexing Thermochemistry of Ammonium, Hydronium, and Alkali Cations. *J. Phys. Chem.* **1996,** *100,* 6445–6450.
20. Kellersberger, K. A.; Dejsupa, C.; Liang, Y.; Pope, R. M.; Dearden, D. V. Gas Phase Studies of Ammonium-Cyclodextrin Compounds Using Fourier Transform Ion Cyclotron Resonance. *Int. J. Mass Spectrom.* **1999,** *193,* 181–195.
21. Julian, R. R.; Beauchamp, J. L. Site Specific Sequestering and Stabilization of Charge in Peptides by Supramolecular Adduct Formation with 18-Crown-6 Ether by way of Electrospray Ionization. *Int. J. Mass Spectrom.* **2001,** *210/211,* 613–623.
22. Julian, R. R.; Akin, M.; May, J. A.; Stoltz, B. M.; Beauchamp, J. L. Molecular Recognition of Arginine in Small Peptides by Supramolecular Complexation with Dibenzo-30-Crown-10 Ether. *Int. J. Mass Spectrom.* **2002,** *220,* 87–96.
23. Jellen, E. E.; Chappell, A. M.; Ryzhov, V. Effects of Size of Noncovalent Complexes on Their Stability During Collision-Induced Dissociation. *Rapid Commun. Mass Spectrom.* **2002,** *16,* 1799–1804.
24. Hunter, E. P.; Lias, S. G. In *NIST Standard Reference Database Number 69*; Mallard, W. G.; Linstrom, P. J., Eds.; National Institute of Standards and Technology: Gaithersburg, 1997; (http://webbook.nist.gov).
25. Davidson, W. R.; Sunner, J.; Kebarle, P. Hydrogen Bonding of Water to Onium Ions. Hydration of Substituted Pyridinium Ions and Related Systems. *J. Am. Chem. Soc.* **1979,** *101,* 1675–1680.
26. Rüdiger, V.; Schneider, H.-J.; Solov'ev, V. P.; Kazachenko, V. P.; Raevsky, O. A. Crown Ether-Ammonium Complexes: Binding Mechanisms and Solvent Effects. *Eur. J. Org. Chem.* **1999**, 1847–1856.
27. Garcia, B.; Ramirez, J.; Wong, S.; Lebrilla, C. B. Thermal Dissociation of Protonated Cyclodextrin-Amino Acid Complexes in the Gas Phase. *Int. J. Mass Spectrom.* **2001,** *210/211,* 215–222.