Intrinsic Contributions to Chiral Recognition: Discrimination Between Enantiomeric Amines by Dimethyldiketopyridino-18-crown-6 in the Gas Phase

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Received July 12, 1996[⊗]

Abstract: We have employed Fourier transform ion cyclotron resonance (FTICR) mass spectrometry to investigate and quantify the recognition of chiral amines in the gas phase by the chiral crown ethers (*R,R*)- and (*S,S*)-dimethyldiketopyridino-18-crown-6, using a new procedure wherein the relatively involatile chiral ligand is easily ionized via electrospray to produce a protonated host molecule. A neutral chiral amine and an achiral reference amine, which are generally fairly volatile, were introduced into the ion trapping cell, where they reacted with the protonated host to form crown−ammonium complexes. Equilibrium constants were determined for exchange of the chiral and achiral amine guests. Electrospray of the other enantiomeric host, followed by guest exchange equilibrium constant determination, enabled characterization of the effects of chirality on complexation equilibria. Comparison of the equilibrium constants for the two enantiomeric hosts measures the relative degree of recognition for a given guest. In all cases, binding of the guest with absolute configuration opposite those of the host stereocenters is preferred. The free energy of binding the preferred enantiomer of α(1-naphthyl)ethylamine is 3.5 ± 0.6 kJ mol^{-1} greater than for the nonpreferred enantiomer, in agreement with results obtained using an older ligand transfer method. Enantiomeric preferences (all in kJ mol^{-1}) for *sec*-butylamine (0.3 ± 0.4), cyclohexylethylamine (0.9 ± 0.2), and methylbenzylamine (2.4 ± 0.5) illustrate intrinsic factors contributing to chiral recognition, including steric bulk and the importance of $\pi-\pi$ stacking interactions to anchor the guest. The interactions of *sec*-butylamine and cyclohexylethylamine can be described using a three-point binding model, while the aromatic amines are more consistent with the four-point binding model described by Cram. The data suggest that recognition in this system arises largely from differing degrees of methyl rotor locking for the two enantiomers, with accompanying differences in the entropy of complexation.

Introduction

Despite the great power of mass spectrometry as an analytical technique, among its greatest limitations is its inability to easily yield information on molecular stereochemistry. One of the most challenging areas of this emerging field involves recognition of molecules that possess the property of chirality. The characterization of chiral species presents formidable analytical problems because the mirror image enantiomers have identical elemental compositions and atom connectivities—only the arrangement around the stereocenter(s) differs.

Mass spectrometry is an attractive method for these studies because of its high speed and very small sample requirements. Successful mass spectrometric methods, like other analytical techniques for chiral species, rely on differences in the reactivity of the enantiomers with a chiral reagent. For example, chemical ionization using a chiral reagent ion may yield different results for enantiomeric analytes.[1]

Two experimental approaches typify work in this field. Recently, fast atom bombardment (FAB) mass spectrometry has been used to examine the relative intensities of adduct peaks arising from interactions of chiral species.[2−6] Often, one of the enantiomers is isotopically labeled so that the chiral adducts can be distinguished on the basis of mass. These experiments can be performed quickly with very small samples and appear to be an excellent means of rapidly screening compounds for chiral selectivity. However, with FAB there is ambiguity about the environment in which recognition occurs, since it is difficult to determine whether the adducts form in solution prior to desorption, in the selvedge region as they are desorbed, or in the gas phase. Further, relative peak intensities from FAB probably do not reflect equilibrium conditions, so interpretation of the results to yield quantitative thermochemical information is not straightforward.

Fourier transform ion cyclotron resonance mass spectrometry was used several years ago to measure equilibrium populations of clusters of chiral molecules such as L- and D-dimethyl tartrate[7,8] and continues to yield interesting results.[9−11] The equilibrium approach has the advantage that equilibrium constants are easily related to free energy changes, so the degree

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[⊗] Abstract published in *Advance ACS Abstracts,* December 15, 1996.

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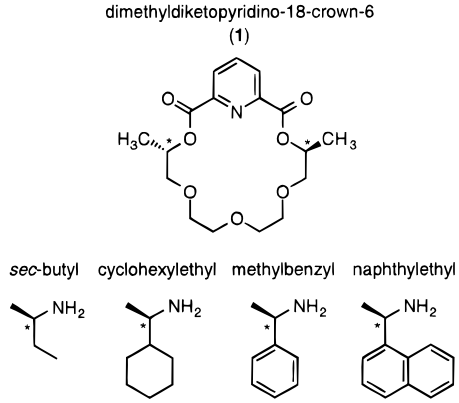

Figure 1. Structures and abbreviations for chiral host and chiral amine guests.

of chiral recognition can be quantified. Further, these are unambiguously gas-phase experiments, carried out under conditions much simpler than those which prevail in solution. On the other hand, these experiments are probably more difficult to perform and require more time than FAB analyses.

Our own studies of chiral recognition are part of an ongoing investigation of molecular recognition under gas phase conditions,[12−17] where solvent and counterion effects are eliminated and recognition arises from the intrinsic interactions between host and guest molecules. The chiral host−guest system we initially investigated involved the chiral ligand shown in Figure 1. The host molecule, dimethyldiketopyridino-18-crown-6 (hereafter designated **1**, for brevity), has two stereocenters, one at each of the carbon atoms where methyl groups are attached to the crown ring. From solution studies of **1**, one of the best-recognized chiral guest species is [α-(1-naphthyl)ethyl]ammonium ion (hereafter referred to as $NapEtNH_3^+$).[18] In condensed media, the *(R)*-form of the ammonium ion is bound in preference to the *(S)*-enantiomer by *(S,S)*-**1**. X-ray structures for the complexes[18−21] suggest two kinds of interaction are important in complex formation: hydrogen bonding between the ammonium group of the guest and the nitrogen and two alternate oxygens of the crown and face-to-face $\pi-\pi$ stacking between the pyridino moiety of the crown and the naphthyl group of the guest. Molecular mechanics models of the complexes indicate the same two types of interactions are dominant in the gas phase.

In our initial experiments,[22] the chiral host, *(S,S)*-**1**, and an achiral host, 18-crown-6, were both admitted (as neutrals) into the trapping cell of a Fourier transform ion cyclotron resonance mass spectrometer. The partial pressures of these two neutral ligands were carefully measured. Either *(R)*- or *(S)*-$NapEtNH_2$ was also admitted into the trapping region, and $NapEtNH_3^+$ was formed by self-chemical ionization. Reaction of $NapEtNH_3^+$ with the neutral ligands afforded the host−guest complexes. The exchange of the guest between the chiral and achiral hosts, reaction 1, was allowed to proceed to equilibrium. The attainment of equilibrium was verified by monitoring ion intensities as a function of reaction time until the ratio of the 18-crown-6·$NapEtNH_3^+$ and *(S,S)*-**1**·$NapEtNH_3^+$ ion intensities became constant. Perturbation of the system away from equilibrium by ejection of either of the complexes always resulted in reestablishment of the same equilibrium ratios after an appropriate delay, attesting to the fact that true equilibrium was reached.

(S,S)-**1**·(*R or S*)-$NapEtNH_3^+$ + 18C6 ⇌
(S,S)-**1** + 18C6·(*R or S*)-$NapEtNH_3^+$ (1)

These experiments found that the equilibrium constant for exchange of *(R)*-$NapEtNH_3^+$ was only about one quarter that for exchange of *(S)*-$NapEtNH_3^+$, corresponding to about 4 kJ mol^{-1} greater free energy of interaction between *(R)*-$NapEtNH_3^+$ and *(S,S)*-**1** than between the *(S)*-guest and the same ligand. Thus, *(S,S)*-**1** preferentially binds *(R)*-$NapEtNH_3^+$ in the gas phase, just as it does in solution.[18] The degree of recognition in the gas phase is about the same as is observed in a weakly-solvating solvent such as dichloromethane and is about twice as great as is seen in methanol, a better solvent.[18]

These were difficult experiments. First, *(S,S)*-**1** is fairly involatile, so that usable vapor pressures were barely attainable when the ligand was inserted into the high vacuum region of the instrument on a heated direct-exposure solids probe. The use of a heated probe also introduces ambiguity about the temperature of the system, making the thermochemical measurements less useful. Second, achiral 18-crown-6 binds $NapEtNH_3^+$ much more strongly than the chiral ligand, making equilibrium difficult to observe unless there is a large excess pressure of the less volatile, chiral *(S,S)*-**1**. Third, the lack of an external ion source on the instrument we used originally made some of the chemistry ambiguous. Could we have simply been transferring protons between neutral complexes? Finally, measurement of the partial pressures of the ligands, which is crucial to the results, is difficult and introduces a great deal of uncertainty.

Herein we again examine interactions between $NapEtNH_2$ and chiral crown ether **1**. Our new investigation takes advantage of recent advances in electrospray ionization mass spectrometry[23,24] to avoid the volatility problems that plagued our earlier experiments and is much faster than the older techniques. No heating of sample probes or of the vacuum chamber is required, so that the entire chamber is maintained at uniform, ambient temperature. The new method allows experiments to be designed so that the results do not depend on the pressures of the neutrals, thus eliminating a large potential source of error. Finally, we use the new technique to examine the interactions of a number of additional chiral amine guests with host **1**. The new results shed important new light on the intrinsic, solvent-

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free interactions that are essential to chiral recognition by this host and illustrate the superior recognition achieved when $\pi-\pi$ interactions help to orient the guest as it binds with the host.

Experimental Section

All experiments were performed using a commercial Fourier transform ion cyclotron resonance mass spectrometer (Model APEX 47e, Bruker Instruments, Billerica, MA), featuring a 4.7 T superconducting magnet and an ion source external to the high-field region of the magnet. Vacuum is maintained by means of two stages of mechanical pumping followed by three regions of differential cryopumping (Edwards) such that with the electrospray source in operation at atmospheric pressure the trapping cell region remains at a typical base pressure of 1×10^{-9} mbar. Ion injection into the magnetic field is accomplished via electrostatic focusing of the ion beam along the field lines into the high-field region, where the ions are captured inside a cylindrical trapping cell designed to approximate a cell of infinite length ("infinity" design).[25] Trapping is facilitated by application of a small voltage "kick" perpendicular to the magnetic field axis as the ions pass through the front trapping plate of the instrument. During all experiments, the trapping cell remained at ambient temperature (about 300 K). The instrument is interfaced to an electrospray ionization source (Analytica, Branford, MA) of the Whitehouse type,[26,27] with pneumatically-assisted nebulization and a Pt-coated glass capillary "drying tube".

Ligand **1** was synthesized using published procedures.[28] Cyclohexylamine (97.9%) was purchased from Fisher. *(R)*-NapEtNH$_2$ (>99%) was purchased from Aldrich, while *(S)*-NapEtNH$_2$ (>99%), D-methylbenzylamine (>98%), *(R)*-1-cyclohexylethylamine (>98%), and *(R)*-*sec*-butylamine (>99%) came from Fluka. All compounds were used as supplied, with the exception that they were degassed through several freeze−pump−thaw cycles prior to introduction into the vacuum system.

In a typical experiment, one enantiomer of the chiral amine of interest was introduced into the ion-trapping region of the vacuum chamber, along with an achiral reference amine. Volatile samples were introduced using precision variable leak valves (Varian, Palo Alto, CA), while less volatile amines were inserted via a direct-exposure solid sample vacuum lock. In most experiments, cyclohexylamine served as the achiral reference, although in experiments with the enantiomers of *sec*-butylamine, isopropylamine served as a better reference because its affinity for the ligand is more similar to that of *sec*-butylamine than is that of cyclohexylamine. The partial pressures of each amine were allowed to stabilize and were determined using a cold cathode ionization gauge (Balzers), which is mounted above the cryopump in the high vacuum region of the instrument and is shielded from the fringing magnetic field. Rough calibration of the gauge was accomplished by measuring proton transfer kinetics, but careful compound-specific calibration was not performed since only relative pressures need be measured in these experiments. We do assume that the gauge has similar response to the chiral and achiral amines, but even this assumption has no bearing on the results, as will be shown.

One enantiomer of the chiral host molecule [*(S,S)*-**1**, for example] was electrosprayed, and the resulting ions were guided into the trapping cell and captured. Typically, concentrations of 0.1 mg mL^{-1} in 80:18:2 methanol:water:acetic acid were used. The countercurrent drying gas was N_2, at approximately 470 K. All source voltages were adjusted to optimize the signal. Under these conditions, the protonated chiral crown was the dominant ion detected. Smaller peaks, arising from alkali metal ion adducts, were also generated, presumably from trace contamination in the source. Ion accumulation times of 200 ms were typically used, after which time the ion beam from the source was electrostatically deflected to stop any further accumulation in the trap.

The trapped protonated host was allowed to react with the neutral amines in the trapping cell, resulting in the formation of complexes. The reaction delay to allow complex formation was typically a few hundred milliseconds. Following this delay, either the complex of the protonated chiral host with the chiral amine, or with the achiral reference amine, was isolated in the trapping cell using standard RF shot ejections. The reaction of the isolated complex with the neutral amines resulted in reestablishment of an equilibrium distribution of complexes with the chiral and achiral amines (reaction 2);this return to equilibrium was

$$(R,R)\text{-}\mathbf{1}H^+\cdot(R\ or\ S)\text{-NapEtNH}_2 + \text{cyclohexylamine} \rightleftharpoons (R,R)\text{-}\mathbf{1}H^+\cdot\text{cyclohexylamine} + (R\ or\ S)\text{-NapEtNH}_2 \quad (2)$$

monitored as a function of time. All such reactions were carried out in both the "forward" (chiral amine complex reacting with achiral amine to yield achiral amine complex) and "reverse" (achiral amine complex reacting with chiral amine to yield chiral amine complex) directions. This procedure served to verify that true equilibrium was attained, since if the system is at equilibrium the same ratio of complex ions should be reached regardless of the direction of approach. The equilibrium constant for reaction 2 is determined from the ratio of the peak intensities of the complexes and the measured partial pressures of the two amines, as has been discussed.[22]

The degree of chiral recognition, $\Delta\Delta G^\circ_S$, is defined as the difference between the free energy obtained for reaction 2, $\Delta G^\circ_{R,R\cdot S}$, and that for the corresponding reaction involving the *(S,S)*-enantiomer of the host, $\Delta G^\circ_{S,S\cdot S}$, eq 3. (An analogous expression can be written for $\Delta\Delta G^\circ_R$,

$$\Delta\Delta G^\circ_S = \Delta G^\circ_{R,R\cdot S} - \Delta G^\circ_{S,S\cdot S} = -RT \ln \frac{K_{R,R\cdot S}}{K_{S,S\cdot S}} = -RT \ln \frac{I_{R,R\cdot S}\, I_{S,S\cdot ref}}{I_{R,R\cdot ref}\, I_{S,S\cdot S}} \quad (3)$$

expressing the degree of preference of the system toward *(R)*-NapEtNH$_2$.) R and T are the ideal gas constant and absolute temperature, respectively. As eq 3 shows, this quantity is obtained from the ratio of the equilibrium constants for the two reactions, $K_{R,R\cdot S}$ and $K_{S,S\cdot S}$, respectively. The pressures of the neutral amines are part of each equilibrium constant, but assuming the pressures do not change during the measurements, these neutral pressures cancel in the determination of the degree of recognition, such that $\Delta\Delta G^\circ_S$ depends only on the mass spectral intensities of the chiral and achiral reference complexes, $I_{R,R\cdot S}$, $I_{R,R\cdot ref}$, $I_{S,S\cdot S}$, and $I_{S,S\cdot ref}$. Operationally, $\Delta\Delta G^\circ_S$ is determined by performing back-to-back experiments with the two enantiomers of either the host or the guest. If both host enantiomers are available (as is the case for **1**), this is most easily accomplished by flushing the electrospray source to remove the original enantiomer and then spraying a solution of the other. This can be done rapidly enough (requiring perhaps 15 min) that the partial pressures of the reference and chiral amines do remain constant, so that the pressures cancel as in eq 3.

Results

Adduct Formation and Proton Transfer. Prior to working with the chiral ligands, a number of model protonated hosts were surveyed for their ability to form complexes with neutral amines in the gas phase. The hosts examined included unsubstituted 18-crown-6 (18C6), dicyclohexano-18-crown-6 (DC18C6, mixture of isomers), and dibenzo-18-crown-6 (DB18C6). The amines included ammonia, *sec*-butylamine, *tert*-butylamine, cyclohexylamine, ethylenediamine, 1,3-diaminopropane, and 1,4-diaminobutane. Protonated 18C6 readily formed adducts with all the amines examined, but neither protonated DC18C6 nor protonated DB18C6 formed adducts with any of the amines. A small amount of proton transfer from each of the crowns to *tert*-butylamine was observed, but no other proton transfer reactions were seen. These may be cases where proton transfer is sterically hindered and entropic barriers make

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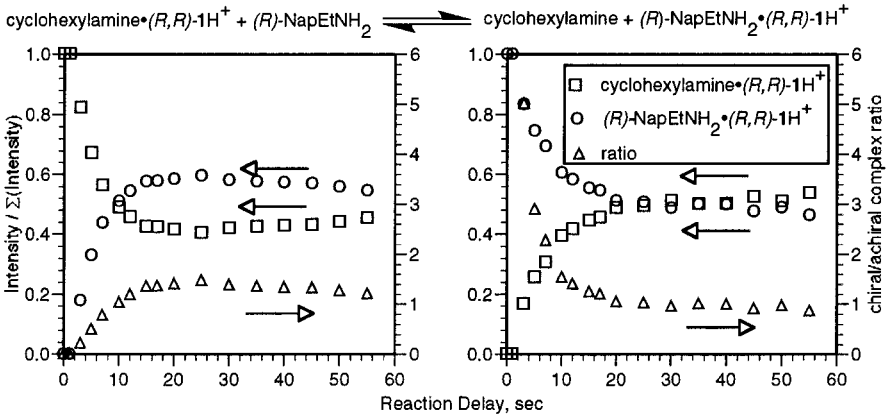

Figure 2. Approach to equilibrium in the forward (left frame) and reverse (right frame) directions, for exchange of cyclohexylamine and *(R)*-$NapEtNH_2$ on *(R,R)*-**1**H^+ in the gas phase.

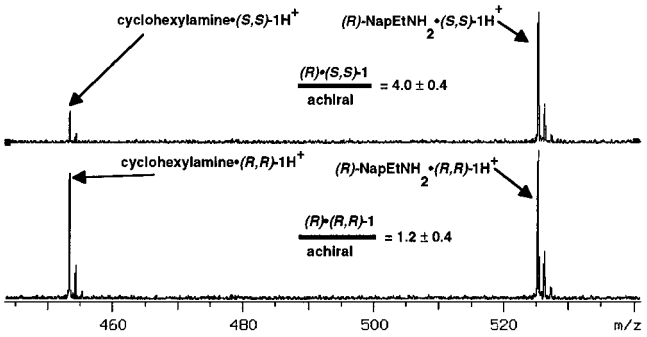

Figure 3. Mass spectra obtained under equilibrium conditions for exchange of cyclohexylamine and *(R)*-$NapEtNH_2$ on *(S,S)*-**1**H^+ (upper spectrum) and on *(R,R)*-**1**H^+ (lower spectrum).

the reactions very slow.[29] Fortunately, protonated **1** readily formed adducts with all of the amines examined.

Chiral Recognition in the 1H$^+$·NapEtNH$_2$ System. We stress the fact that all the results shown herein arise from recognition as a purely *gas-phase* process, since all complexation and guest exchange took place in the gas phase, not in solution prior to electrospray. For example, Figure 2 shows the approach to equilibrium in the gas phase for reaction 2. In the left-hand frame, the complex of achiral cyclohexylamine with *(R,R)*-**1**H^+ was initially isolated and allowed to react with *(R)*-$NapEtNH_2$. In the frame on the right, equilibrium was approached from the opposite direction: the *(R)*-$NapEtNH_2$ complex with *(R,R)*-**1**H^+ was isolated and allowed to react with cyclohexylamine. After about 20 s, the ratio of chiral guest complex:achiral guest complex attains a roughly constant value of about 1:1.

Figure 3 displays two mass spectra obtained under identical equilibrium conditions, except that the upper frame was obtained while electrospraying *(S,S)*-**1**H^+, while the lower frame was obtained a few minutes later while electrospraying *(R,R)*-**1**H^+. Neutral *(R)*-$NapEtNH_2$ and cyclohexylamine were present in the cell during both experiments. The equilibrium ratios of chiral complex:achiral complex are clearly quite different in the two cases, about 4.0 ± 0.4:1 for the *(S,S)*-host and about 1.2 ± 0.4:1 for the *(R,R)*-host. Using eq 3, this corresponds to a $\Delta\Delta G°_R$ value of 3.1 ± 0.4 kJ mol^{-1}.

To further verify the observation of chiral recognition in this system, the recognition of both *(R)*- and *(S)*-$NapEtNH_2$ was examined. The results are shown in Table 1. The *(R)*-enantiomer is bound, on average, 3.9 ± 0.5 kJ mol^{-1} more strongly by *(S,S)*-**1** than by *(R,R)*-**1**. The results for the *(S)*-enantiomer of the amine are in excellent agreement: it is preferentially bound by *(R,R)*-**1**, by essentially the same amount. We take the degree of recognition in this system to be the average of the absolute value of $\Delta\Delta G°_X$ obtained for the system, 3.5 ± 0.6 kJ mol^{-1}. This value should be valid at the temperature of the trapping cell at the time of the measurement, 300 K. The results are in close agreement with the $\Delta\Delta G°_R$ value of 4.2 ± 0.4 kJ mol^{-1} obtained earlier for the **1**·$NapEtNH_3^+$ system using ligand-exchange methods,[22] with an estimate of 3.6 kJ mol^{-1} from MM2 molecular mechanics calculations[22] and with a calculated difference of 2.9 kJ mol^{-1} between free energies of activation for dissociating complexes of the two enantiomeric guests.[20]

Table 1. Degree of Recognition of Protonated Dimethyldiketo-pyridino-18-crown-6 (**1**) for α-(1-Naphthyl)ethylamine, at 300 K

X	no. of trials	$\lvert\Delta\Delta G°_X\rvert$,[a] kJ mol^{-1}
R	4	3.9 ± 0.5
S	6	3.2 ± 0.6
mean	10	3.5 ± 0.6

[a] $\Delta\Delta G°_X$ as defined in eq 3, reported as the mean ± standard deviation for replicate experiments.

Chiral Recognition in Other 1H$^+$·Amine Systems. The procedures described above were used to characterize the recognition of other chiral amines, with the results shown in Table 2. In every case, the guest with the absolute configuration opposite to that of the two host stereocenters was preferentially bound. This is expressed in Table 2 as the hetero/homo ratio, which is the ratio of observed equilibrium constants involving opposite absolute configurations to that involving the same absolute configurations. This corresponds to the equilibrium ratio of hetero to homochiral complexes which would be present when the chiral host reacts with an excess of racemic guest. Table 2 also describes recognition using $\Delta\Delta G°_X$, as defined above. The least-recognized guest was *sec*-butylamine, which has a $\Delta\Delta G°_X$ value within experimental error of zero, while $NapEtNH_2$ was the best-recognized.

Discussion

Comparison with Results from Ligand Exchange. The excellent agreement between the ligand-exchange results[22] and those of the present experiments for the **1**H$^+$·$NapEtNH_2$ system gives us great confidence in these methods for measuring chiral recognition. In addition, we note that in the ligand-exchange experiments complexes were formed by reaction of ammonium cations with *neutral* crowns, while in the amine exchange experiments the complexes were generated by reaction of *protonated* crowns with neutral amines. It is interesting that the degree of recognition is the same whether the proton is

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Table 2. Degree of Recognition of Dimethyldiketopyridino-18-crown-6 (**1**) for Chiral Amines[a]

	gas phase		FAB[b]	methanol solution[c]	
amine	hetero/homo preference	$\Delta\Delta G°_R$, kJ mol^{-1}	hetero/homo preference	hetero/homo preference	$\Delta\Delta G°_R$, kJ mol^{-1}
sec-butyl	1.1 ± 0.2	0.3 ± 0.4			
cyclohexylethyl	1.5 ± 0.1	0.9 ± 0.2			
methylbenzyl	2.6 ± 0.5	2.4 ± 0.5	1.10 ± 0.02	1.7	1.3
naphthylethyl	4.0 ± 1.0	3.5 ± 0.6	1.17 ± 0.02	2.6	2.4

[a] $\Delta\Delta G°_R = \Delta G°$(*S,S*-**1**H$^+$ + *(R)*-amine) − $\Delta G°$(*R,R*-**1**H$^+$ + *(R)*-amine). All results at 300 K, reported as mean ± standard deviation for replicate analyses. [b] Reference 6. [c] Reference 30.

initially located on the amine or on the chiral crown. This strongly suggests that both pathways lead to similar, if not structurally identical, complexes. In retrospect, this is probably not surprising since one would expect the best hydrogen bond donor−acceptor sites in the complex would not depend on the initial location of the proton.

The new method is clearly superior to the old one. First, it eliminates experimental difficulties in achieving adequate vapor pressures of low-volatility host ligands, since the ligand bears the charge and a stable vapor pressure of the involatile material is not required. Instead, only the relatively volatile amines need be present as neutrals. As a result, no heating of the vacuum chamber or of sample probes is required, removing ambiguities in the temperature. Further, the electrospray method uses only a very small quantity of chiral ligand. We have made no attempts to optimize sensitivity, but conservatively, less than 0.1 mg of ligand is required for an entire series of experiments with several different guests. This method is also very rapid, especially for screening many hosts for recognition of a given guest. The main time limitation arises from the time required to flush the electrospray source as enantiomers and/or host species are changed. The biggest advantage of the new procedure is the elimination of a major potential source of systematic error, inaccurate pressure measurements. For this last advantage to be fully realized, however, both enantiomers of the host must be available, increasing the amount of effort which must be devoted to synthesis. If pressure measurements are made carefully, the degree of recognition can be quantified using only one host enantiomer, and all of the other benefits still apply.

Comparison with Results from FAB. Chiral recognition in the complexes of protonated *(S,S)*-**1** with methylbenzylamine and naphthylethylamine has been investigated using fast atom bombardment (FAB) mass spectrometry.[6] That study found the same preference for heterochiral complex formation measured in this work, but the observed degree of recognition was much smaller than we observe and is also significantly less than is observed in solution (Table 2). As the authors of the FAB study noted, their results probably do not reflect true equilibrium conditions. In addition, energy deposited during the FAB desorption process renders the internal temperatures of the ions difficult, if not impossible, to determine, and if the degree of recognition has a strong temperature dependence (as seems likely, *vide infra*), this might account for the differences.

Comparison of the methods suggests that the amine exchange equilibrium methods presented here are a more sensitive measure of chiral discrimination than the FAB methods and are a better source of quantitative equilibrium information.

Comparison with Results in Solution. Solution results are given in Table 2 for comparison with the current gas phase work. Comparison with observations in methanol[30] shows that the degree of recognition is greater in the gas phase than in solution for both methylbenzylamine and naphthylethylamine. This corroborates and extends our earlier report for naphthylethylamine using ligand-exchange methods,[22] where we noted that solvation by an achiral solvent leads to decreased recognition.

Limitations to Adduct Formation. The adduct formation behavior of these systems is unusual. For example, while adducts readily formed for unsubstituted 18C6 and for the chiral ligand **1**, none were observed for DC18C6 or DB18C6. It is somewhat surprising that substitution on the alkyl skeleton of the crown makes such a difference in adduct formation. Steric hindrance might account for the differences, since both of the unreactive crowns include bulky substituents. However, **1** also has somewhat bulky substituents, and examination of molecular models does not support the idea that approach of an amine to the binding region of DB18C6 would be more hindered than in **1** (although the pyridine nitrogen of **1** may also furnish a better hydrogen bonding site than the ether oxygens of DC18C6 or DB18C6).

A better rationalization may originate in the relative flexibilities of the various ligands. The cyclohexyl- or benzo-substituted crowns are certainly less flexible than either 18C6 or **1**; 18C6 is quite flexible, with many energetically similar low-lying conformations,[31] and while the diketopyridino portion of **1** is fairly rigid, the remainder of the molecule should be reasonably flexible and therefore able to adopt conformations appropriate for binding the guests.[20] Although flexibility seems to have little effect on binding affinities for alkali metal cations,[17] it is reasonable to expect that orientational requirements would be much greater for the more directional hydrogen bonds[32,33] involved in these complexes than for electrostatically-bound, nondirectional alkali cation complexes. Crystal structures for complexes of 18C6 with ammonium,[34] methylammonium,[35] and benzylammonium[36] find the crown in a high-symmetry D_{3d} conformation, suggesting that the ability of the crown to adopt such a conformation may be important in forming three strong, linear hydrogen bonds to ammonium ions. Previous gas phase studies of the binding of oxonium[37] and ammonium[38] ions to crown ethers observed enthalpies and entropies of complexation consistent with the formation of three hydrogen bonds in the complexes. In addition, our earlier work[22] noted that the affinity of flexible 18C6 for $NapEtNH_3^+$ is much greater than that of more rigid **1**.

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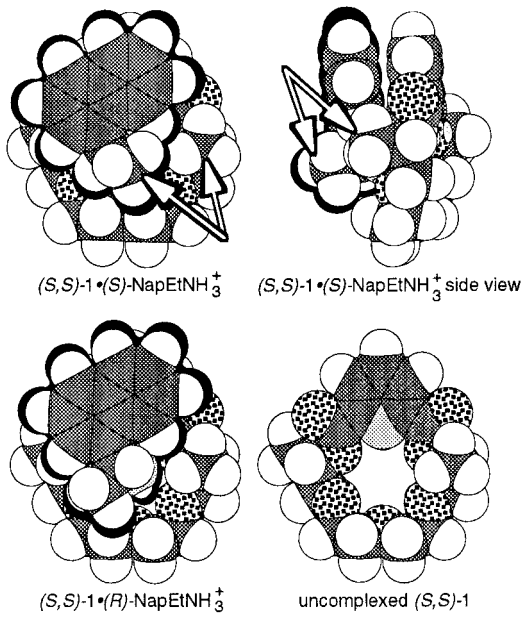

Figure 4. Space-filling models of uncomplexed *(S,S)*-**1** and its complexes with *(R)*- and *(S)*-$NapEtNH_3^+$, determined using MM2 calculations. The guest molecule is denoted by a bold outline. Arrows mark the close methyl−methyl contact in the models of the homochiral complex. The side view of the homochiral complex involves rotation of the top view by 90° clockwise about a vertical axis.

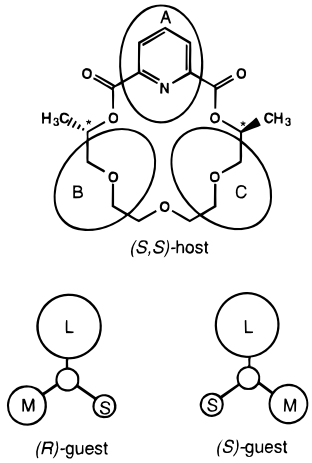

Figure 5. *(S,S)*-$\mathbf{1}H^+$ drawn with steric regions A, B, and C outlined (referring to placement of the guest on top of the host), with schematic depictions of *(R)*- and *(S)*-guests viewed with the C−N bond axis pointing to the rear. Large-, medium-, and small-sized substituents are symbolized by L, M, and S, respectively.

Intrinsic Factors Influencing Chiral Recognition. The data of Table 2 provide insight into the effects of host substituents on the degree of chiral recognition, in the absence of any ambiguities introduced by solvation. The gas-phase results presented here arise *only* from interactions between the host and the guest; hence, we describe these as *intrinsic* interactions.

The gas phase complexation between crown ethers and molecular cation guests, including oxonium[37] and ammonium[38] cations, has been examined. Although the guest ions in those studies were not chiral, many of the fundamental host−guest interactions are similar, so the results are pertinent to the present work. For both oxonium and ammonium guests, the enthalpies of complexation were large enough to suggest the formation of multiple hydrogen bonds involving the oxygen donor groups of the crowns. For the 18-crown-6 complex with cyclohexylammonium, the multiple bonding contribution has been estimated to be as great as 88 kJ mol^{-1}.[38] Not surprisingly, the entropies of complexation were found to be very large and unfavorable: for the reaction between 18-crown-6 and H_3O^+,[37] this was measured to be −230 J mol^{-1} K^{-1}, while for the reaction between cyclohexylammonium and neutral 18-crown-6,[38] this was estimated to be −160 J mol^{-1} K^{-1}. We expect similar effects in the chiral systems examined here.

Prior work with sterically-hindered proton-bound dimers[39] found that the ethalpies of complexation did not vary appreciably with substituent steric bulk, but that the entropies became increasingly unfavorable as substituents became larger and more and more conformational space was sacrificed to allow favorable complexation geometries. It is reasonable that similar factors may account for chiral discrimination in the current systems. Molecular models (Figure 4) suggest that it is not particularly difficult for any of the host−guest enantiomeric complexes to achieve favorable binding geometries, but it is likely that this occurs at the expense of entropically unfavorable partial locking of methyl rotors, and it is probable that the degree to which this occurs differs for enantiomers. It should be noted that locking effects were not observed in proton-bound dimers of hindered, methyl-substituted pyridines,[39] but it is likely that the current chiral complexes require better-defined binding conformations than the pyridines. Thus, chiral discrimination may be largely entropic in the chiral systems. This idea can be tested through variable-temperature experiments, and we are currently carrying out instrument modifications to allow such studies.

As was noted above, in every case the guest with absolute configuration *opposite* that of the two host stereocenters is bound in preference to the guest with the same absolute configuration as the host stereocenters. Referring to Figure 5, there are three regions in which an ammonium ion perched above the cavity of *(S,S)*-**1** by three hydrogen bonds can orient its substituent groups: region A, over the pyridino group, region B, over the downward-pointing methyl group, and region C, toward the upward-pointing methyl group. Region C, with the interfering methyl group, is clearly the most sterically constrained. The relative degree of crowding in regions A and B is harder to evaluate. A quick examination of line drawings suggests A might be somewhat more crowded than B due to proximity to the upward-pointing methyl group, but examination of three-dimensional models indicates that upward-pointing hydrogen atoms make B more crowded than A, where the host is planar and there are no interfering hydrogens.

The experimental results also suggest region A of *(S,S)*-**1** is less hindered than B. For all of the guests in this study, the perched *(R)*-enantiomer can orient its largest substituent toward region A and its smallest toward region C, while for the *(S)*-enantiomer only the less favorable orientation, with the largest substituent in region A and the medium-sized substituent in C, is possible. Thus, if A is the least-hindered region, sterics favor binding the *(R)*-guest, as observed. On the other hand, if B is less hindered than A, then the *(R)*-enantiomer could not orient its largest substituent toward B and its smallest toward C, while the *(S)*-enantiomer could, and would be favored. The latter does not agree with observation.

Consistent with these steric arguments, the smallest degree of recognition (essentially none) is observed for *sec*-butylamine, which also has the smallest and most conformationally mobile "large" substituent (ethyl). The ethyl group can easily rotate out of the way of the chiral barriers on the host and so is ineffective at promoting recognition. Change of the ethyl group to the considerably bulkier and less flexible cyclohexyl leads

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to a significant enantiomeric preference (about 1.5:1) for the guest with absolute configuration opposite to that of the stereocenters of the host. These complexes therefore correspond to the three-point binding model described by Cram,[40] wherein three hydrogen bonds hold the perching guest in the host, and the relative sizes of the various regions above the host determine which enantiomeric guest is preferred.

For guests that include π-systems, additional bonding is possible that may influence recognition. The magnitude of resonance stabilization in gas-phase complexes of radical cations with aromatic neutrals can be substantial: 25 kJ mol^{-1} for the dimer of benzene radical cation with benzene and 15 kJ mol^{-1} for the dimer of naphthalene radical cation with naphthalene.[41] It is reasonable that the π-system should play a role in chiral recognition by $\mathbf{1}H^+$. The electron-withdrawing keto groups make the pyridino moiety a weak π-acid, while both methylbenzylamine and naphthylethylamine are weak π-bases. Thus, in guests where the π-system is present, there is a marked preference for orienting the aromatic substituent toward region A to allow face-to-face π-interactions. Since the aromatic group is also the bulkiest substitutent, preferring orientation toward region A, the steric and π-effects reinforce each other, and again the binding of the *(R)*-guest by *(S,S)*-**1** is more strongly preferred than when crowding alone promotes recognition.

Comparison of the observed degree of recognition by $\mathbf{1}H^+$ for cyclohexylethylamine and methylbenzylamine suggests that the presence of the π-system in the latter greatly enhances chiral recognition. Models of these two amines indicate that the steric bulk of the cyclohexyl and phenyl substituents is roughly the same, or perhaps slightly greater for the aliphatic amine, yet $\Delta\Delta G^\circ_X$ more than doubles when the cyclohexane ring is dehydrogenated to form methylbenzylamine, corresponding with a hetero/homo preference change from 1.5:1 to 2.6:1. The degree of recognition increases further when the π-system is more extensive, as in $NapEtNH_2^+$. $NapEtNH_2^+$ is also the guest with the bulkiest substituent, but molecular models (Figure 4) suggest that the size of the naphthyl group contributes more toward allowing simultaneous hydrogen bonding and π-overlap than it does toward steric bulk.

All of these ideas are consistent with an entropic origin for chiral recognition, as described above. Space-filling models of the complexes (Figure 4) suggest that partial locking of the methyl rotors of the host and guest may occur upon complexation, particularly in the homochiral complex. The difference in the degree of locking for the two guest enantiomers, and thus in entropy, is likely greater for the π-bonded systems, accounting for the increase in recognition. Again, testing this idea awaits variable-temperature experiments.

The aromatic amine guests comprise interesting examples of four-point binding[40] (three hydrogen bonds plus the $\pi-\pi$ interaction) for comparison with the simpler three-point systems: recognition, as quantified through $\Delta\Delta G^\circ_X$, more than doubles when the fourth interaction anchors the guest in the host and supplements the sterics. It will be interesting to examine additional systems to verify these observations. For example, *(S,S)*-**1** may preferentially bind the *(S)*-enantiomer of amines with aromatic groups that are not the bulkiest substituent, as π-interactions work against, rather than reinforcing, sterics.

Conclusions

Measurement of amine exchange equilibria offers a powerful new method for evaluating chiral recognition in the absence of solvation effects and using only very small amounts of material. Herein, using protonated crowns and neutral amines, we found the same results as were observed earlier when protonated amines reacted with neutral crowns, suggesting that both sets of reactants lead to the same host−guest complexes. On the basis of prior work with hindered complexes involving ammonium ions, including complexes with crown ethers, we postulate that chiral recognition in these systems may be largely entropic in origin. We have also demonstrated that the change from three-point to four-point binding in the systems studied leads to enhanced chiral discrimination.

Results such as those described here present a new challenge for molecular modelers, since gas-phase methods can now accurately measure binding preferences on the order of 1 kJ mol^{-1}. Gas-phase results such as these may be useful for fine-tuning force fields to reproduce subtle conformational and/or binding preferences such as those involved in chiral recognition.

An important extension of this work will be its application to assaying the enantiomeric makeup of mixtures of enantiomers. This extension should be straightforward, and we are now investigating the analytical utility of these techniques. In addition, it may be possible to use these methods to examine asymmetric induction in ion-molecule reactions, a possibility we are pursuing.

Acknowledgment. We are grateful to acknowledge support of this work by the National Science Foundation (Grant Nos. CHE-9496141 and CHE-9496150), by the donors of the Petroleum Research Fund, administered by the American Chemical Society (Grant No. 26836-AC6,4), and by the Office of Naval Research.

JA962400L

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