Fourier Transform Ion Cyclotron Resonance Mass Spectrometry as a Probe of Chiral Recognition

David V. Dearden, Chandin Dejsupa, and Yongjiang Liang

Department of Chemistry
Brigham Young University
Provo, UT 84602-4670

Dr. H. Guard
Office of Naval Research
800 North Quincy Street
Arlington, VA 22217-5000

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David V. Dearden, Chadin Dejsupa, and Yongjiang Liang

Department of Chemistry and Biochemistry
C100 Benson Science Building
Brigham Young University
Provo, UT 84602

Phone: (801)378-2355
Fax: (801)378-5474
Internet: david_dearden@byu.edu

Abstract

We have employed Fourier transform ion cyclotron resonance (FTICR) mass spectrometry to investigate the recognition of chiral amines by chiral crown ether hosts. Our prior studies involved competition between a chiral ligand and an achiral ligand for the enantiomers of a chiral ammonium ion. Conditions were chosen so equilibrium was attained in the exchange of each enantiomeric host between the two guests. Comparison of the equilibrium constants for the two enantiomers yields a measurement of the relative degree of recognition of the chiral host for the two guests. These studies were hampered by the relatively low volatility of the ligands, which led to difficulties in introducing them into the vacuum chamber and in measuring their partial pressures. To circumvent these problems, we have developed a new procedure wherein the relatively involatile chiral ligand is easily ionized using electrospray ionization to produce a protonated host molecule. The protonated host is captured in the FTICR trapping cell. Neutral amines, which are generally fairly volatile, are also readily introduced into the cell, where they react with the protonated host to form crown-ammonium complexes. Measurement of the partial pressures of the two amines is straightforward. Equilibrium constants are determined for exchange of a chiral amine with an achiral amine in the complex. Comparison of the equilibrium constants for the two enantiomeric amines measures the relative degree of recognition by the host. Results are obtained for the recognition by protonated (R,R)- and (S,S)-dimethyl diketopyridino-18-crown-6 of R- and S- α(1-naphthyl)ethylamine, and are in agreement with results obtained using the older ligand transfer method. Comparison of the recognition of chiral sec-butylamine, cyclohexylethylamine, and phenylethylamine reveals the importance of the π-π stacking interaction in this system: the enantiomers of the first two guests, which have no π systems, are not recognized, while chiral recognition of phenylethylamine, which is too small to stack efficiently, is much less than for the larger naphthylethylamine.
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Introduction

One of the most important insights provided by modern chemical science is the realization that molecular shape and size play crucial roles in determining reactivity. The selective reactivity or "recognition" a molecule exhibits for species of a specific shape or size is particularly important in the chemistry of life and in catalysis. In the influential 1985 "Pimentel Report,"\(^1\) which outlines frontier areas and priorities for chemical research, the chemistry of molecular recognition is featured prominently. Receptor-substrate interactions, which "selectively mediate essentially all biological processes," are controlled by the chemistry of recognition. In setting priorities for study of the chemistry of life, with particular reference to receptor-substrate interactions, the report notes that "...we must be able to identify the structures of these substrates, ...analyze their receptor-interactions in physical-chemical as well as biological terms, and modify their structures to suit desired uses."\(^1\)

Likewise, recognition effects are vital to the function and design of highly specific, energy-efficient catalysts. Catalysts which yield products with well-defined stereochemistry are particularly valuable, especially in the synthesis of natural products and biologically active materials such as pharmaceuticals, where correct stereochemistry is often essential for proper function. Fundamental work is still vitally needed. In the words of the Pimentel Report, "...the basic factors that produce stereochemical control [in catalysis] are not completely understood. Mechanistic studies are needed, and the potential rewards are great."\(^1\)

Undoubtedly, recognition also plays a role in the biochemical processes which enable truly "smart" materials such as brain tissue to function. While the concepts of molecular-size switches and molecular-scale circuit elements are still in their infancy, a clear understanding of the chemistry of recognition is certainly a crucial part of the basis for intelligent design of such components.

One of the most challenging areas of this emerging field involves recognition of molecules which possess the property of chirality, or "handedness." When a molecule is chiral, it is structurally distinct from its mirror image, which otherwise possesses the same functional groups. Only the order of arrangement around the stereocenter(s) differs in such a mirror pair. The members of the pair are called enantiomers. Chirality is especially common in biomolecules, since biochemistry in general produces only one of the possible enantiomers. The characterization of
chiral species presents formidable analytical problems, because the enantiomers have identical elemental compositions and atom connectivities--only the arrangement around the stereocenter(s) differs.

**Chiral Recognition in Mass Spectrometry**

Despite its great power as an analytical technique, one of the severest limitations of mass spectrometry lies in its inability to easily yield information on molecular stereochemistry. Likewise, spectroscopic methods capable of providing stereochemical information for gaseous ions are both rare and extremely difficult. 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.²

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.³⁻⁶ However, with FAB it is difficult to determine whether the adducts formed in solution prior to desorption, in the selvedge region as they are desorbed, or in the gas phase, and the relative peak intensities likely do not reflect equilibrium conditions. 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 The equilibrium approach has the advantage that equilibrium constants are easily related to free energy changes, so the degree of chiral recognition can be quantified. Our own studies of chiral recognition in gas-phase ion-molecule reactions use similar equilibrium techniques.

**Ligand Exchange Equilibrium Measurements**

For several years, our group has investigated crown ethers and their molecular recognition abilities in the gas phase, beginning with simple interactions between crowns and alkali metal ions.⁹⁻¹² Chiral crown ethers have been synthesized, and have been demonstrated to enantioselectively form complexes with chiral ammonium cations in solution (via temperature-dependent NMR measurements) and in the solid state (by X-ray crystallographic measurements).¹³⁻¹⁵ We chose to investigate the same system in the gas phase, with the objectives of demonstrating and quantifying chiral recognition in a gas phase ion-molecule reaction, and of clarifying the role of solvent in the recognition process by obtaining solvent-free results.

The host-guest system we initially investigated is 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 the methyl group is attached to the crown rings. Each of the
stereocenters can have either $R$ or $S$ absolute configuration (the designations specify the geometry at the stereocenter). For the molecule to be chiral, the configurations must be the same at both stereocenters. Both the $R,R$ and $S,S$ enantiomers were available to us through a collaboration with Professor Jerald Bradshaw of Brigham Young University.

From the solution studies of 1, one of the best-recognized chiral guest species is $\alpha$-(1-naphthyl)ethylammonium ion (hereafter referred to as NapEtNH$_3^+$).

X-ray structures for the complexes suggest two kinds of interaction are important in complex formation: hydrogen bonding between the ammonium group of the guest and the nitrogen and two oxygens of the crown, and $\pi-\pi$ 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 condensed media, the $R$ ammonium ion is preferentially bound by $S,S$-1. Both enantiomers of the amine are commercially available, so we set out to determine how their binding to $S,S$-1 differs in the gas phase.

In our initial experiments, the chiral host, $S,S$-1, and the achiral host, 18-crown-6, were both admitted into the trapping region 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 re-establishment 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 $\xrightarrow{\text{equilib}}$ 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. 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.

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 direct-exposure solids probe. 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? Fourth, most of the chiral ligand was wasted as it was slowly pumped away by the vacuum system. Finally, measurement of the partial pressures of the ligands, which is crucial to the results, is difficult and introduces a great deal of uncertainty into the results.

An Improved Method: Amine Exchange Equilibrium Measurements

We set out to circumvent these problems by designing new experiments around the capabilities of our APEX 47e Fourier transform ion cyclotron resonance mass spectrometer (Bruker Instruments, Billerica, MA), which is equipped with electrospray ionization. Since this instrument has an external ion source, the ionization and complex formation aspects of the experiment can be separated such that formation of neutral complexes is not possible. Even more importantly, electrospray ionization offers a means of generating protonated host molecules which avoids the problems inherent in the low volatility of the chiral ligands, and enables us to eliminated neutral pressures as variables in these experiments. Further, with electrospray ionization only a few tenths of a milligram of the expensive chiral ligands are required for a whole range of experiments. In fact, sample consumption in our experiments is limited by the minimum mass we can accurately weigh, rather than by the amount actually needed for electrospray detection.

In our new experiments, rather than observing equilibria for exchanging an ammonium guest among neutral hosts, we observe the exchange of neutral amines by a protonated host ion, reaction (2). One enantiomer of the chiral amine, as well as an achiral reference amine, are introduced into the trapping cell as neutrals. One of the host enantiomers is electrosprayed in the external source, and the resulting protonated host ions are guided into the trapping cell and captured. Reaction of the protonated host with the neutral amines results in formation of complexes. Exchange of the chiral and achiral amines on the protonated chiral host is allowed to proceed to equilibrium, which is verified as before by attainment of a constant product ratio and by re-establishment of the same equilibrium ratio after the system is perturbed by ejection of either the reactant complex or the product complex. The experiments are then repeated with the other enantiomer of the host or the guest.

$$ (S,S)^{-}1H^{+} \cdot R\text{-NapEtNH}_{2} + \text{cyclohexylamine} \underset{\text{equilibrium}}{\longrightarrow} (S,S)^{-}1H^{+} \cdot \text{cyclohexylamine} + R\text{-NapEtNH}_{2} $$

(2)
When both host enantiomers are available (as is the case for host 1), switching between the two enantiomers can be done rapidly enough (by simply flushing the solution of one enantiomer out of the electrospray needle, and replacing it with a solution of the other enantiomer) that pressure conditions in the trapping region do not change appreciably between the two measurements. Since the results for the degree of chiral recognition depend on a ratio of the two equilibrium constants for the two enantiomers, if the partial pressures of the amines do not change the pressures cancel in the ratio and pressure is no longer a variable in the measurement. This is a very significant advantage, since the pressure measurements are the largest potential sources of error in the experiments.

The approach of the system to equilibrium is illustrated in Figures 2 and 3. The figures show ion intensities from a series of mass spectra obtained with progressively increasing reaction times. In these experiments, the achiral reference amine was cyclohexylamine, the chiral amine was R-NapEtNH₂, and the chiral ligand was R,R-1H⁺. Ejection of either the complex of R,R-1H⁺ with the chiral amine (at m/z 525) or with the achiral amine (at m/z 453) was followed by re-establishment of the equilibrium ratio of about 1 for these two peaks. Spectra obtained at equilibrium for complexation of both S,S-1 and R,R-1 with R-NapEtNH₂ and cyclohexylamine reference are shown in Figure 4. The R-NapEtNH₂•S,S-1H⁺ complex peak is much more intense relative to the reference than the R-NapEtNH₂•R,R-1H⁺ complex peak. This corroborates our earlier results, obtained with the more difficult experimental method, that indicated stronger complexation between R-NapEtNH₃⁺ and S,S-1.

Quantitatively, the difference in equilibrium constants corresponds to the free energy of binding in the R-NapEtNH₂•S,S-1 complex being 3.1 ± 0.4 kJ mol⁻¹ greater than in S-NapEtNH₂•S,S-1. The results obtained using amine exchange equilibrium are in good agreement with those obtained earlier using ligand exchange, especially when it is noted that the two experiments were done at different temperatures.

The simplicity of the new experiments, along with the ease of electrospraying large, enantiomeric crowns, has now enabled us to examine a number of other chiral guests for recognition by 1. The results, shown in Table 1, reveal some of the structural requirements for recognition in this system. Both enantiomers of sec-butylamine and cyclohexylethylamine reacted identically with respect to the achiral reference; the chirality of both these guests was not recognized. Recognition was observed for phenylethylamine, but the difference in free energy of binding for the two enantiomers was only about 0.7 kJ mol⁻¹. This suggests that the π-π stacking interaction is essential for recognition in this system. No recognition is seen in guests lacking the π system, and recognition is much weaker for phenylethylamine than for naphthylethylamine, because the former is too small to simultaneously allow optimal hydrogen bonding and π system overlap.
The potential of these electrospray ionization, Fourier transform ion cyclotron resonance mass spectrometry methods for the study of chiral recognition is exciting. With sub-milligram samples and a few hours’ work, the degree of recognition in chiral host-guest systems can be quantified under solvent-free conditions.

Acknowledgments

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References


Table 1. Difference in Free Energy of Binding Chiral $R$-amines by $S,S$-1 vs. $R,R$-1 at 300 K.

<table>
<thead>
<tr>
<th>amine</th>
<th>$\Delta \Delta G^\circ$, kJ mol$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>sec-butyl</td>
<td>0</td>
</tr>
<tr>
<td>cyclohexylethyl</td>
<td>0</td>
</tr>
<tr>
<td>phenylethyl</td>
<td>$0.7 \pm 0.4$</td>
</tr>
<tr>
<td>naphthylethyl</td>
<td>$3.1 \pm 0.4$</td>
</tr>
</tbody>
</table>
Figure Captions

Figure 1. A chiral host-guest system. Structures of the host and guest are drawn on the left, and side and top views of a space-filling model of the complex are shown on the right. The guest molecule is outlined in black. Note that the ammonium group of the guest inserts into the central cavity of the host, and that the π system of the guest stacks over the pyridino π system of the host.

Figure 2. Approach to equilibrium in an amine exchange reaction, as documented using Xmass. In this experiment, ejection of the \( R\text{-NapEtNH}_2\cdot R,R\text{-1H}^+ \) complex defined the time origin.

Figure 3. Approach to equilibrium in both the “forward” and “reverse” directions. In the left frame, the cyclohexylamine\( \cdot R,R\text{-1H}^+ \) complex was initially isolated, while in the right frame the \( R\text{-NapEtNH}_2\cdot R,R\text{-1H}^+ \) complex was isolated to define the time origin. In both cases the same equilibrium product/ reactant ratio is reached.

Figure 4. Mass spectra at equilibrium for amine exchanges using \( S,S\text{-1} \) and \( R,R\text{-1} \) hosts. The free energy of binding \( R\text{-NapEtNH}_2 \) by \( S,S\text{-1H}^+ \) is \( 3.1 \pm 0.4 \text{ kJ mol}^{-1} \) greater than that by \( R,R\text{-1H}^+ \).
S,S-dimethyl diketopiperazine-18-crown-6

Host

S,S-1-R-NapEtNH₃⁺

Guest

α-(1-naphthyl)ethylammonium

"NapEtNH₃⁺"
Amine Exchange Equilibrium

cyclohexylamine\(\cdot R,R-1H^+\) + \(R\)-NapEtNH\(_2\) ⇌ cyclohexylamine + \(R\)-NapEtNH\(_2\)\(\cdot R,R-1H^+\)

cyclohexylamine\(\cdot R,R-1H^+\)

\[ \text{ESI of } R,R-1 \text{ And } R\text{-NapEtNH}_2 \]
0.1 mg/ml in H\(_2\)O-MeOH-TFA (18:80:2)
0.8-8 Seconds, SI=16K, NS=16, Eject 354 & 525
February 2, 1996

Reaction Delay, sec

30
20
10
5

0

450 470 490 510 530

m/z
"Forward" and "Reverse" Approaches to Amine Transfer Equilibrium

cyclohexylamine•$R,R$-1H$^+$ + $R$-NapEtNH$_2$ $\rightleftharpoons$ cyclohexylamine + $R$-NapEtNH$_2$•$R,R$-1H$^+$

Graphs showing intensity and 525/453 ratio as a function of reaction delay, sec.
Chiral Recognition by the Amine Transfer Equilibrium Method

\[
\begin{align*}
\text{cyclohexylamine} \cdot S,S-1H^+ & \quad R\text{-NapEtNH}_2 \cdot S,S-1H^+ \\
\frac{R \cdot S,S-1}{\text{achiral}} &= 4.0 \pm 0.4 \\
\text{cyclohexylamine} \cdot R,R-1H^+ & \quad R\text{-NapEtNH}_2 \cdot R,R-1H^+ \\
\frac{R \cdot R,R-1}{\text{achiral}} &= 1.2 \pm 0.4
\end{align*}
\]

\[\Delta \Delta G_{300} = 3.1 \pm 0.4 \text{ kJ mol}^{-1}, \text{amine transfer} \]
\[\Delta \Delta G_{350} = 4.2 \pm 0.4 \text{ kJ mol}^{-1}, \text{ligand transfer}\]