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# Chiral NMR Discrimination of Piperidines and Piperazines Using (18-Crown-6)-2,3,11,12-tetracarboxylic Acid

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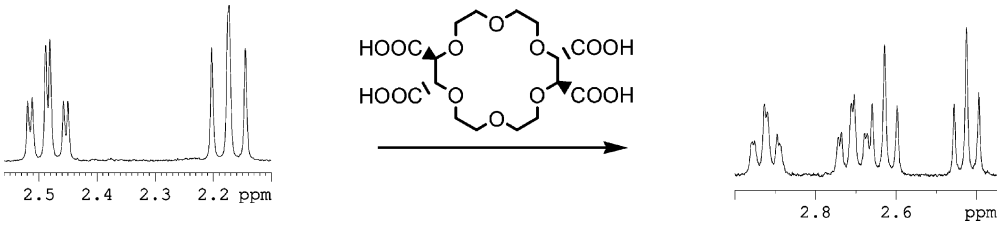
*Received July 31, 2006*

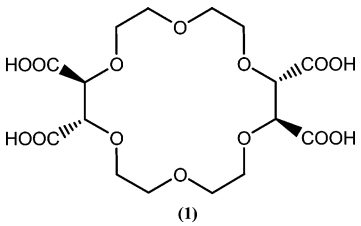



Enantiomeric discrimination is observed in the $^{1}H$ and $^{13}C$ NMR spectra of piperidines and piperazines in the presence of (−)-(18-crown-6)-2,3,11,12-tetracarboxylic acid. The amines are protonated by the carboxylic acid groups of the crown ether to produce the corresponding ammonium and carboxylate ions. Association of the ammonium ion with the crown ether likely involves two hydrogen bonds with the crown ether oxygen atoms and an ion pair with the carboxylate anion. Methyl, hydroxymethyl, phenyl, carboxyl, pyridyl, and cyclohexyl substituent groups α to the nitrogen atom do not inhibit binding of the ammonium ion to the crown ether. The NMR spectra of piperidines with the stereogenic center α or *β* to the nitrogen atom exhibit substantial enantiomeric discrimination. Dibasic substrates such as the piperizines are likely converted to their diprotonated form in the presence of the crown ether, and both nitrogen atoms appear to associate with the crown ether moiety.

## Introduction

NMR spectroscopy represents one of the most common techniques employed for the analysis of chiral compounds because of its ease of use and the availability of many suitable chiral derivatizing or solvating agents.[1] Chiral NMR solvating agents are especially practical because there is no concern associated with kinetic resolution or racemization that accompanies the use of a chiral derivatizing agent. The solvating agent is simply mixed with the compound under study in the NMR tube.

Several important families of chiral NMR solvating agents are cavity compounds that form host−guest complexes with suitable substrates. Cyclodextrins represent one widely known and utilized set of cavity compounds and, depending on the substituent groups on the cyclodextrin, associate with a wide variety of substrate compounds in both organic and aqueous solvents.[2] Crown ethers are a second common family of cavity compounds, and the most common crown ethers with an 18-crown-6 unit are primarily known for their ability to associate with and recognize chiral primary amines.[3] The primary amine is analyzed as its protonated ammonium ion. The $NH_3^+$ unit forms three hydrogen bonds to oxygen atoms of the 18-crown-6 unit as shown in Figure 1a. A substantial number of chiral crown ethers have been studied, although primarily for liquid chromatographic separations rather than as chiral NMR solvating agents. The one exception is (18-crown-6)-2,3,11,12-tetracarboxylic acid (**1**), which is an especially versatile reagent for the analysis of primary amines by NMR spectroscopy.[4] Another




**(1)**

especially attractive feature of **1** is that it is the only com-

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10.1021/jo061586w CCC: $33.50 
Published on Web 11/03/2006

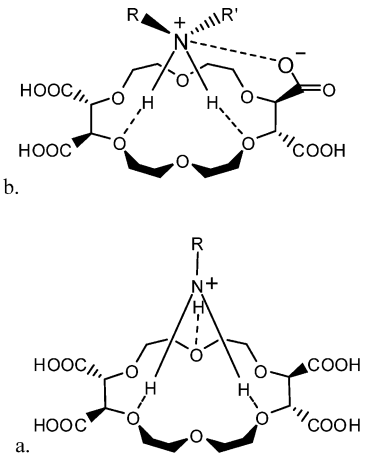


**FIGURE 1.** Association geometries of the ammonium salts of (a) protonated primary amines with neutral **1** and (b) protonated secondary amines with the carboxylate anion of **1**.

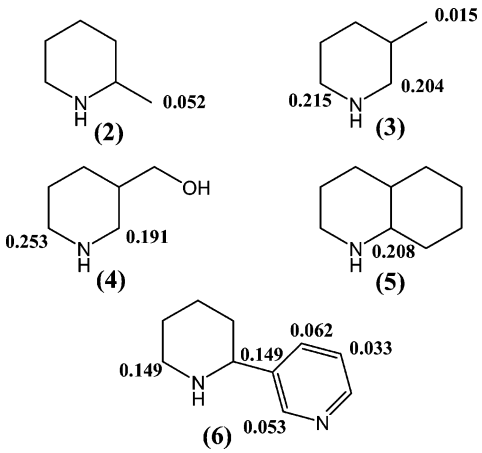


**FIGURE 2.** Enantiomeric discrimination (ppm) in the $^1$H NMR spectra (400 MHz) of piperidines **2**−**6** (10 mM) with **1** (20 mM) in methanol-$d_4$.

mercially available chiral crown ether for use as a chiral NMR solvating agent.

The binding of secondary amines to 18-crown-6 ethers is generally unfavorable because the protonated form of the amine can only form two hydrogen bonds with the crown and steric effects about the secondary amines hinder association with the crown. The use of a much larger pseudo-24-crown-8 ring provided a cavity that was large enough to accommodate secondary amines; however, this reagent is not commercially available, and its synthesis is rather involved.[5] We recently showed that **1** is also an effective chiral NMR solvating agent for secondary amines.[6]

In a mixture of a neutral amine and **1** in methanol-$d_4$, a neutralization reaction between **1** and the amine forms the corresponding ammonium and monocarboxylate ions. The ammonium salt can then associate with **1** through two hydrogen bonds involving the crown oxygen atoms and an ion pair between the ammonium and carboxylate ions, as shown in Figure 1b.[5,7] This interaction provides for strong association between the secondary ammonium ion and **1**, resulting in the observation of substantial enantiomeric discrimination in the $^1$H NMR spectrum of a variety of secondary amines. No discrimination was observed with tartaric acid, the template on which **1** is derived, thereby illustrating the significance of association with the crown in causing the enantiomeric distinction.[6]

In this report, we show that **1** is an especially effective reagent for determining the optical purity of chiral piperidines and piperazines using NMR spectroscopy. The analysis can be performed by recording either the $^1$H or $^{13}$C NMR spectrum. A total of 15 substrates were examined in this study, and all showed enantiomeric discrimination in their $^1$H and/or $^{13}$C NMR spectrum in the presence of **1**. The majority of these was only available as racemates, so we cannot yet determine whether the trends in enantiomeric discrimination correlate with the absolute configuration of the substrate.

## Results and Discussion

Mixing a neutral piperidine with **1** leads to pronounced deshielding of the hydrogens α to the nitrogen atom, indicating that a neutralization reaction between a carboxylic acid group of **1** and the amine has occurred to form the corresponding ammonium ion and carboxylate group. The interaction between the ammonium ion and **1** likely involves two hydrogen bonds and an ion−ion interaction as shown in Figure 1b, although further evidence in the form of a crystal structure will be needed before this is known with certainty.

Substituent groups such as methyl, hydroxymethyl, phenyl, carboxyl, pyridyl, and cyclohexyl at one of the carbons α to the nitrogen in the piperidines and piperazines do not inhibit association of the substrate with **1** because substantial enantiomeric discrimination is observed in the $^1$H and/or $^{13}$C NMR spectra. Furthermore, the spectra of substrates with the stereogenic center $\beta$ to the nitrogen atom also exhibit significant enantiomeric discrimination in the presence of **1**. This strongly suggests that **1** ought to function as an effective chiral NMR solvating agent for the analysis of optical purity of almost any chiral piperidine or piperazine substrate.

**$^1$H NMR Analysis of Piperidines with 1.** Five of the piperidine substrates have one or more resonances that exhibit enantiomeric discrimination ($\Delta\Delta\delta$) in the $^1$H NMR spectrum in the presence of **1**. The $\Delta\Delta\delta$ values for piperidines **2**−**6** are provided in Figure 2. Values are only provided for those resonances that showed separate and isolated peaks for both enantiomers because these would be useful for the analysis of optical purity. In many cases, other resonances were discriminated, but one of them overlapped with other resonances of **1** or the substrate. Enantiomeric discrimination of hydrogen atoms

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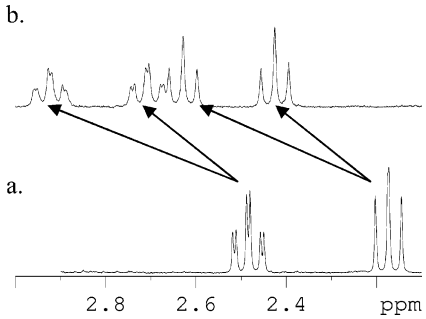


**FIGURE 3.** $^1$H NMR spectra (400 MHz) of two of the (a) α-protons of **3** (10 mM) in methanol-$d_4$ (b) with **1** at 20 mM.

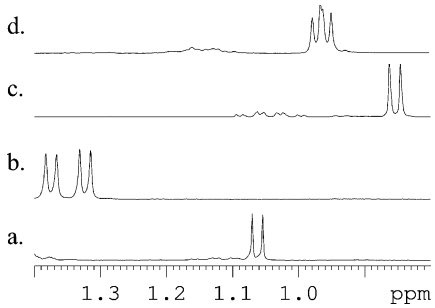


**FIGURE 4.** $^1$H NMR spectra (400 MHz) of the methyl resonance of (a) **2**, (b) **2** with **1** at 20 mM, (c) **3**, and (d) **3** with **1** at 20 mM.

α to the nitrogen atom on the order of about 0.2 ppm was observed for **3**−**6**. An example of the substantial enantiomeric discrimination that is observed for the resonances of hydrogen atoms at the α-position to the nitrogen atom is shown in Figure 3.

The methyl doublets of **2** and **3** are readily monitored, and Figure 4 illustrates the substantially greater enantiomeric discrimination observed for the methyl resonance at the 2-position vs the 3-position. The different proximities to the binding site must account for the larger discrimination of the 2-methyl than the 3-methyl resonance. It has been observed with many chiral solvating agents that nuclei closer to the binding site show greater enantiomeric distinction.[1]

Another common observation with chiral solvating agents is that the closer the substrate's stereogenic center is to the binding site, the larger the enantiomeric discrimination. However, the substantial enantiomeric discrimination of $^1$H resonances of **3** and **4** indicates that piperidines with a stereogenic center at the *β*-position to the nitrogen atom also exhibit significant chiral recognition with **1**.

With **6**, a dibasic substrate with a piperidyl and pyridyl ring, the protons α to the nitrogen of the piperidine ring show large deshielding (shifts of 0.5−0.7 ppm) in the presence of **1**, indicating that the piperidyl nitrogen is protonated. Preferential protonation of the piperidyl nitrogen is expected on the basis of the relative p$K_b$ values of piperidyl and pyridyl nitrogen atoms. At concentrations of **1** equal to or greater than that of **6**, the pyridyl protons are also deshielded (shifts as large as 0.35 ppm), indicating that protonation of the pyridyl nitrogen atom and formation of the diprotonated species likely occur as well.

The series of spectra in Figure 5 shows the pyridyl region of **6** as increasing concentrations of **1** are added. Significant broadening is observed for all the resonances of **1** and **6** at a 2:1 substrate−crown ratio (Figure 5b), whereas the TMS reference peak, which is not shown in Figure 5, is quite sharp.

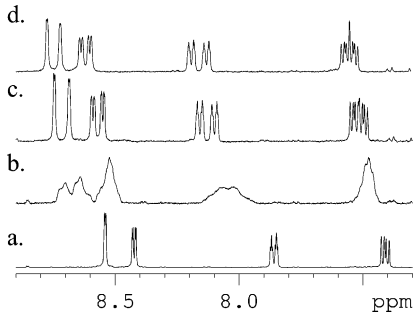


**FIGURE 5.** (a) Aromatic region of the $^1$H NMR spectrum (400 MHz) of **6** (10 mM) in methanol-$d_4$ with **1** at (b) 5 mM, (c) 10 mM, and (d) 20 mM.

This general behavior was observed for many of the substrates. At a crown−substrate ratio of less than 1:1, there tends to be broadening of all of the resonances of the substrate and **1**, suggesting that the system is at an intermediate rate of exchange among the various species. In cases when broadening was observed, raising the concentration of **1** above that of the substrate consistently resulted in a sharpening of all of the resonances, indicating that the exchange rate of crown−substrate complexes was now fast on the NMR time scale. At no time in any of the mixtures were separate resonances observed for bound and unbound forms of any substrate or **1**, such that the splitting of the resonances in the spectrum of **6** is the result of enantiomeric discrimination under fast exchange rather than separate bound forms of the pyridyl and piperidyl nitrogen atoms of **6** with **1**. Raising **1** to 10 mM sharpens all the resonances considerably (Figure 5c), and substantial enantiomeric discrimination is observed for all of the pyridyl resonances. The resonances of two hydrogen atoms α to the nitrogen atom of the piperidine ring also show a significant extent of enantiomeric discrimination in the $^1$H NMR spectrum.

**$^{13}$C NMR Analysis of Piperidines with 1.** For several piperidine substrates, a peak overlap resulting from the complexity of the $^1$H NMR spectra or the shifts that occurred after protonation of the amine prevented the observation of enantiomeric discrimination. There was often an indication that enantiomeric discrimination had occurred (i.e., a resonance with an area that could only correspond to a single enantiomer was observed in the $^1$H NMR spectrum), but a clearly distinct pair of resonances that could be integrated for the purpose of determining optical purity was lacking. COSY spectra could be used to confirm that the $^1$H NMR spectrum had two separate sets of resonances for the two enantiomers. In some cases, homonuclear decoupling to collapse and simplify the $^1$H resonances could also facilitate the analysis. $^{13}$C NMR spectra of piperidines with **1** are conveniently used for the analysis of optical purity. Because the potential exists for different relaxation times and NOEs in the $^{13}$C signals of the two enantiomers, it is important to use an inverted gated acquisition mode when using chiral solvating agents for determining optical purity.

For six of the piperidine substrates (Figure 6), substantial enantiomeric discrimination was observed in the $^{13}$C NMR spectra, and except for resonances that overlapped with the methanol solvent signal and could not be analyzed, almost every $^{13}$C resonance of **7**−**12** showed some degree of enantiomeric discrimination. As seen by the $\Delta\Delta\delta$ values provided in Figure 6, enantiomeric discrimination of the $^{13}$C resonances ranged from a few hundredths to greater than 0.6 ppm. Many of the $^{13}$C nuclei become more shielded in mixtures with **1**, although

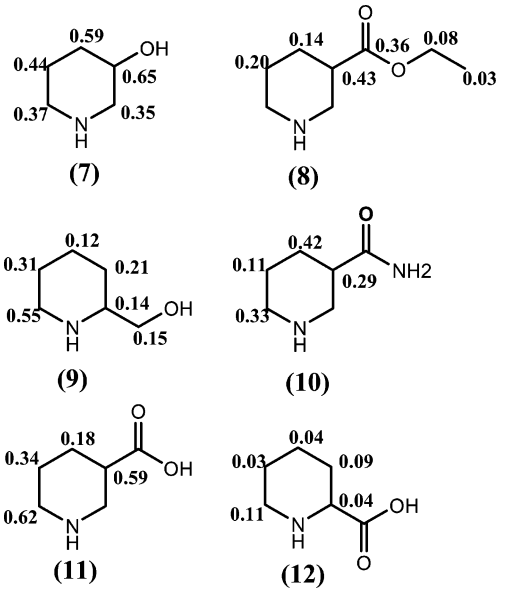


**FIGURE 6.** Enantiomeric discrimination (ppm) in the $^{13}C$ NMR spectra (100 MHz) of piperidines **7**−**12** (10 mM) with **1** (20 mM) in methanol-$d_4$.

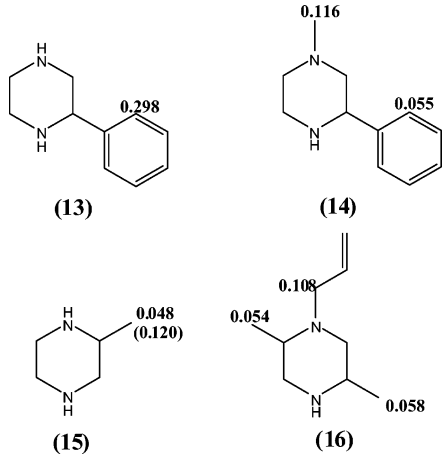


**FIGURE 7.** Enantiomeric discrimination (ppm) in the $^1H$ (400 MHz) and $^{13}C$ (100 MHz) NMR spectra of piperazines **13**−**16** (10 mM) with **1** (20 mM) in methanol-$d_4$.

the changes in the $^{13}C$ spectra that occur on protonation of the nitrogen atom are generally not as significant as those observed in the $^1H$ NMR spectrum. The spectra in Figure 7 illustrate the extent of enantiomeric discrimination that is commonly observed in the $^{13}C$ NMR spectra of piperidines in the presence of **1**.

The spectra of **11** and **12** provide an interesting comparison as substantial enantiomeric discrimination is observed in several resonances in the $^{13}C$ NMR spectrum of **11**, whereas the smallest $^{13}C$ enantiomeric discrimination of any piperidine or piperazine substrate was observed with **12**. Resonances in the $^1H$ NMR spectrum of **12** do not shift that far to higher frequencies on the addition of **1**, indicating that the neutralization does not likely occur to that significant an extent. As such, the ion pair interaction between **12** and **1** is likely absent to a significant extent and association between the two is diminished. A similar observation was reported previously for mixtures of proline with **1**, although high enough concentrations of **1** eventually favored protonation of the proline and association with the crown.[6] For **11**, addition of **1** causes more substantial deshielding in the $^1H$ and $^{13}C$ NMR spectra. Protonation of **11** by **1** likely occurs such that the two associate with each other to a significant extent.

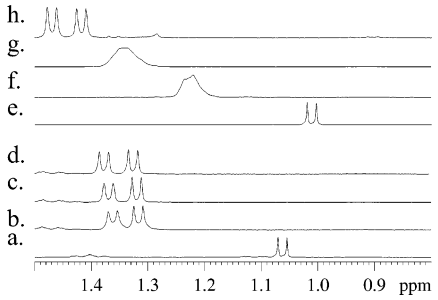


**FIGURE 8.** $^1H$ NMR spectra (400 MHz) of the (a) methyl resonance of **2** (10 mM) with **1** at (b) 5 mM, (c) 10 mM, and (d) 20 mM and (e) of the methyl resonance of **15** (10 mM) with **1** at (f) 5 mM, (g) 10 mM, and (h) 20 mM.

The general trend for most substrates is that the resonances of the carbons α to the nitrogen atom exhibit the largest enantiomeric discrimination, followed by those in the $\beta$- and $\gamma$-positions, respectively. This trend is similar to that observed for hydrogen resonances that exhibit clear enantiomeric discrimination. An exception to the trend in the $^{13}C$ NMR data was observed with **7**. In this case, all of the $^{13}C$ resonances exhibit substantial enantiomeric discrimination and the $\beta$- and $\gamma$-positions were greater than those at the α-position. This suggests that the hydroxyl group at the $\beta$-position of **7** interacts with one or more of the carboxyl groups of **1** and contributes significantly to the chiral recognition.

**Analysis of Piperazines with 1.** All of the ring hydrogen resonances in piperazines **13**−**16** (Figure 7) show substantial deshielding in the $^1H$ NMR spectrum with **1**, indicating that both of the nitrogen atoms are likely protonated by **1** to form the corresponding diammonium salt. The spectra in Figure 8 display an interesting contrast in the behavior of the methyl resonance of **2** and **15**. With **2** (10 mM), addition of **1** (5 mM) causes substantial deshielding (about 0.35 ppm) and immediate enantiomeric discrimination of the methyl resonance. Further addition of **1** up to 20 mM causes only slight increases in the shifts and enantiomeric discrimination. This suggests that the amine (10 mM) is fully converted to its ammonium salt with addition of 5−10 mM of **1** and that the association with **1** is essentially complete by this concentration range. For **15** (10 mM), addition of **1** at 5 and 10 mM causes broadening in the spectra and progressively increasing shifts. By 20 mM of **1**, the spectrum sharpens and significant enantiomeric discrimination (0.048 ppm) is observed that is quite comparable in magnitude to that of the methyl group in **2** (0.052 ppm). Steric hindrance of the methyl group of **15** would seem to favor association with **1** at the N-4 position. If so, the enantiomeric discrimination of the methyl resonance of **15** would likely be more equivalent to that of the 3-methyl resonance in **3** (0.015 ppm). Because substantial deshielding occurs for all of the ring hydrogen atoms of **15** in the presence of **1**, the larger nonequivalence of the methyl resonance of **15** relative to that of **3** suggests that **15** is converted to a diprotonated cation and that each ammonium ion associates to some degree with **1**.

Especially noteworthy in the spectrum of **14** is the pronounced enantiomeric discrimination of the *N*-methyl resonance. One possibility is that the tertiary *N*-methyl nitrogen atom associates to some degree with **1**. A preliminary investigation of two chiral tertiary piperidines, ethyl 1-methylpipecolinate and ethyl 1-methyl-

3-piperidinecarboxylate, indicates that both are protonated in the presence of **1**, that both ammonium ions associate with **1**, and that enantiomeric discrimination is observed in the NMR spectrum. A more thorough investigation of the interaction of tertiary amines with **1** is underway. The *ortho*-hydrogen of the aromatic ring shows substantial enantiomeric discrimination in both **13** and **14**. The chiral recognition in the spectrum of **14** indicates the degree to which potential steric constraints do not seem to inhibit association of this class of substrates with **1**. For **16**, enantiomeric discrimination is clearly apparent for the two methyl and the one α-hydrogen resonances in the $^1$H NMR spectrum.

## Conclusions

Crown ether **1** is a broadly applicable reagent for determining the optical purity of piperidines and piperazines. Either $^1$H or $^{13}$C NMR spectroscopy can potentially be used for the analysis. The neutral amine is mixed with **1** in methanol-$d_4$, and a neutralization reaction between **1** and the amine forms the corresponding carboxylate and ammonium ions. Evidence suggests that dibasic substrates such as the piperizines are likely converted to their diprotonated form in the presence of **1** and that both nitrogen atoms associate with the crown ether moiety. Methyl, hydroxymethyl, phenyl, carboxyl, pyridyl, and cyclohexyl substituent groups α to the nitrogen atom do not inhibit binding of the ammonium ion to **1**. The spectra of piperidines with the stereogenic center α or $\beta$ to the nitrogen atom exhibit substantial enantiomeric discrimination in the NMR spectra in the presence of **1**.

## Experimental

**Reagents.** All substrates, deuterated NMR solvents, and the (−)-isomer of **1** were obtained from commercial suppliers and used as received.

**Instrumentation.** All $^1$H (16 scans) and $^{13}$C spectra (4096 scans) were collected on a 400 MHz NMR spectrometer. Spectra were run in methanol-$d_4$ at ambient probe temperature and calibrated using tetramethylsilane as an internal reference. When necessary, assignments were confirmed using 2D-COSY spectra.

**Procedures for Chiral Discrimination Studies.** Solutions of the chiral substrates (10 mM) in methanol-$d_4$ with 0.05% TMS were prepared and enriched with one of the enantiomers when available. Increments of **1** were added to the sample by appropriately sized aliquots of a 0.3 M stock solution in methanol-$d_4$.

**Acknowledgment.** We thank the National Science Foundation (Research at Undergraduate Institutions Program, Grant CHE-0244742; the Major Instrumentation Program, Grant CHE-0115579) and the Howard Hughes Medical Institute through an institutional award to Bates College for supporting our work.

JO061586W