Tetrahedron: *Asymmetry* 21 (2010) 2289–2294

Contents lists available at ScienceDirect

Tetrahedron: *Asymmetry*

journal homepage: www.elsevier.com/locate/tetasy

Enantiomeric discrimination of cyclic β-amino acids using (18-crown-6)-2,3,11,12-tetracarboxylic acid as a chiral NMR solvating agent

Cora D. Chisholm [a], Ferenc Fülöp [b], Eniko Forró [b], Thomas J. Wenzel [a,*]

[a] *Department of Chemistry, Bates College, Lewiston, ME 04240, USA*
[b] *Institute of Pharmaceutical Chemistry, University of Szeged, Eotvos, Hungary*

ARTICLE INFO

Article history:
Received 7 July 2010
Accepted 23 July 2010
Available online 9 September 2010

ABSTRACT

(18-Crown-6)-2,3,11,12-tetracarboxylic acid is an excellent chiral NMR solvating agent for cyclic β-amino acids with cyclopentane, cyclohexane, cycloheptane, cyclopentene, cyclohexene, bicyclo[2.2.1]heptane, and bicyclo[2.2.1]heptene rings. The crown ether was added to the neutral β-amino acids in methanol-d_4. A neutralization reaction between the crown ether and β-amino acid forms the ammonium ion needed for favorable association. Enantiomeric discrimination of the two hydrogen atoms α to the amine and carboxylic acid moieties of the β-amino acid was observed with every substrate studied. Trends in the order of the enantiomeric discrimination of certain hydrogen atoms for substrates of similar structures correlate with the absolute configuration.

1. Introduction

Optically active β-amino acids are an important class of compounds that act as enzyme inhibitors, have pharmacological and neurological properties, and function as receptor antagonists.[1] They are found in a variety of natural products including β-lactam antibiotics, alkaloids, peptides, and compounds with anti-tumor properties.[2–7]

In the past few years, a large number of syntheses (enantioselective synthesis and enzymatic resolution) have been developed for enantiopure carbocyclic β-amino acid derivatives.[4,6,8] One good possibility for the preparation of enantiopure β-amino acids is the enantioselective ring-opening of the corresponding β-lactams[9] or the enantioselective hydrolysis of β-amino esters.[10]

As the assignment of the absolute configuration of enantiopure β-amino acids is always a requirement, therefore, easily applicable methods for their enantiomeric analysis are necessary.

18-Crown-6 ethers form complexes with protonated primary amines via the formation of three hydrogen bonds (Fig. 1). (18-Crown-6)-2,3,11,12-tetracarboxylic acid **1**, both enantiomers of which are commercially available, is an especially effective chiral NMR solvating agent for primary amines.[11–15] Recently it was shown that **1** is an excellent chiral NMR solvating agent for β-amino acids. The majority of substrates examined in the report were acyclic. The Hα and Hβ resonances of acyclic β-amino acids showed substantial enantiomeric discrimination in the presence of **1** that could be used to determine the enantiomeric purity. Resonances of the substituent group in many of the acyclic β-amino acids exhibited enantiomeric discrimination in the presence of **1** as well. Finally, the relative perturbations of the chemical shifts of the Hα and Hβ resonances of acyclic β-amino acids correlated with the absolute configuration.[16]

Herein we report the utility of **1** as a chiral NMR solvating agent for a series of cyclic β-amino acids. Substrates with cyclopentane, cyclopentene, cyclohexane, cyclohexene, cycloheptane, bicyclo[2.2.1]heptane, and bicyclo[2.2.1]heptene rings are examined.

2. Results and discussion

Prior reports have shown that methanol-d_4 is a particularly useful solvent for the analysis of amines with **1**.[11–15] All of the cyclic substrates examined in this report are soluble in their neutral form in methanol-d_4 at the concentration employed for NMR studies (10 mM). The addition of **1** to a solution of the β-amino acid results in a neutralization reaction that generates the ammonium ion of the substrate and carboxylate ion of **1**. Prior work has shown that substrates that are insoluble in methanol-d_4 can be solubilized by adding an equivalent of DCl to the sample.[16] The ammonium species is then analyzed using the neutral form of **1**. Prior work has demonstrated that ammonium ions associate with either the neutral or anionic forms of **1**.[11,14,16]

The hydrogen atoms near the amine moiety of the cyclic β-amino acids (H1 and H2 in Figs. 2–6) experience significant deshielding in mixtures with **1**, providing evidence that the substrates undergo a neutralization reaction with **1** that protonates the amine. Deshielding of the methine resonances of the β-amino acid that are α to the amine (H2) and carboxylic acid (H1) moieties of

* Corresponding author. Tel.: +1 207 786 6296; fax: +1 207 786 8336.
E-mail address: twenzel@bates.edu (T.J. Wenzel).

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doi:10.1016/j.tetasy.2010.07.031

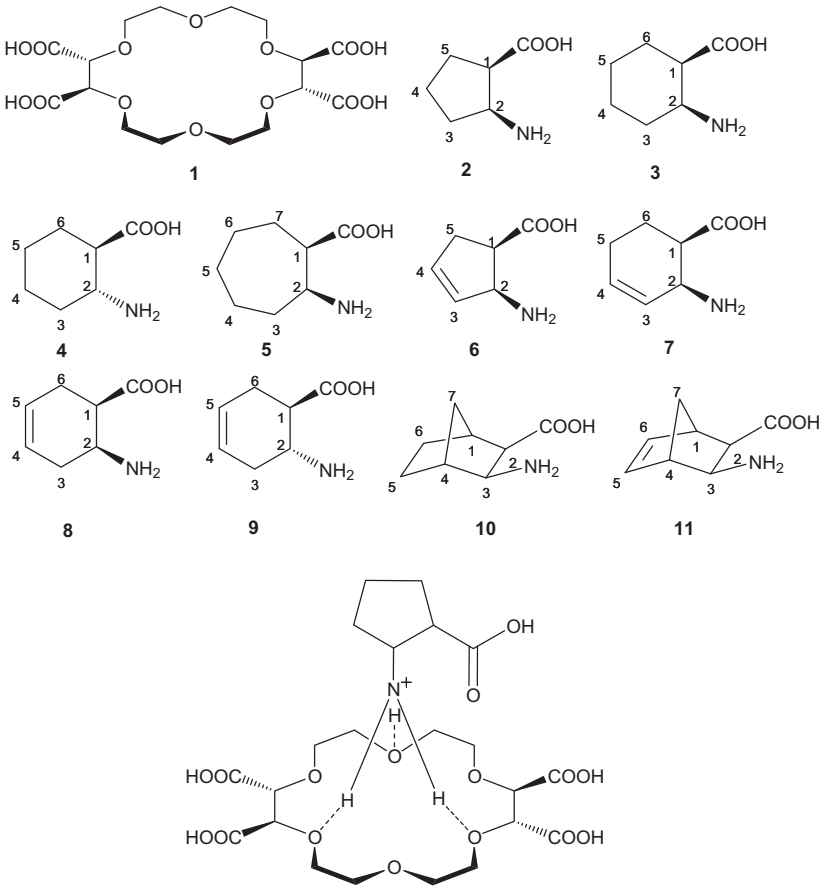

Figure 1. Association of a protonated β-amino acid with **1**.

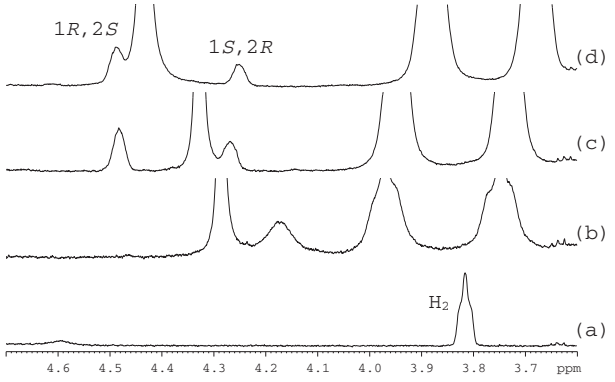

Figure 2. ^{1}H NMR spectra (400 MHz, methanol-d_4, 23 °C) of (a) the H2 resonance of **7** [10 mM—enriched in (1*R*,2*S*)] with **1** at (b) 5 mM, (c) 10 mM and (d) 20 mM.

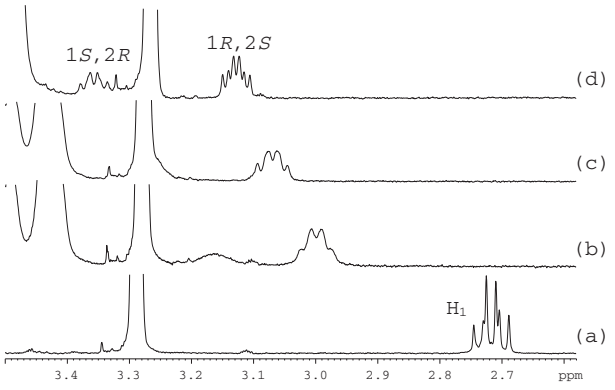

Figure 3. ^{1}H NMR spectra (400 MHz, methanol-d_4, 23 °C) of (a) the H1 resonance of **2** [10 mM—enriched in (1*R*,2*S*)] with **1** at (b) 5 mM, (c) 10 mM and (d) 20 mM.

the monocyclic substrates often moves them into the region of the spectrum where the resonances of **1** and methanol occur. However, the changes in chemical shifts of the substrate resonances vary with the concentration of **1**. For this reason, recording a series of spectra with increasing concentrations of **1** (5, 10 and 20 mM) relative to the β-amino acid (10 mM) is recommended. By recording a series of spectra, it is likely that at least one set of conditions will provide an NMR spectrum with enantiomerically discriminated H1 and H2 substrate resonances free of overlap with resonances of **1** and methanol. The nature of **1** does change over such a series. When a neutral amine (10 mM) is mixed with **1** at a concentration of 5 mM, the primary form of **1** is expected to be a dianion. At a 1:1 crown–substrate ratio, the primary form of **1** is a monoanion.

The substrates examined herein, (1*R*,2*S*)- and (1*S*,2*R*)-*cis*-2-aminocyclopentanecarboxylic acid **2**, (1*R*,2*S*)- and (1*S*,2*R*)-*cis*-2-aminocyclohexanecarboxylic acid **3**, (1*R*,2*S*)- and (1*S*,2*R*)-*trans*-2-aminocyclohexanecarboxylic acid **4**, (1*R*,2*S*)- and (1*S*,2*R*)-*cis*-2-aminocycloheptanecarboxylic acid **5**, (1*R*,2*S*)- and (1*S*,2*R*)-*cis*-2-aminocyclopent-3-enecarboxylic acid **6**, (1*R*,2*S*)- and (1*S*,2*R*)-*cis*-2-aminocyclohex-3-enecarboxylic acid **7**, (1*R*,2*S*)- and (1*S*,2*R*)-*cis*-2-aminocyclohex-4-enecarboxylic acid **8**, (1*R*,2*S*)- and (1*S*,2*R*)-*trans*-2-aminocyclohex-4-enecarboxylic acid **9**, (1*S*,2*R*,3*S*,4*R*)- and (1*R*,2*S*,3*R*,4*S*)-3-*exo*-aminobicyclo[2.2.1]heptane-2-*exo*-carboxylic acid **10**, and (1*S*,2*S*,3*R*,4*R*)- and (1*R*,2*R*,3*S*,4*S*)-3-*exo*-aminobicyclo[2.2.1]hept-5-ene-2-*exo*-carboxylic acid **11** encompass a range

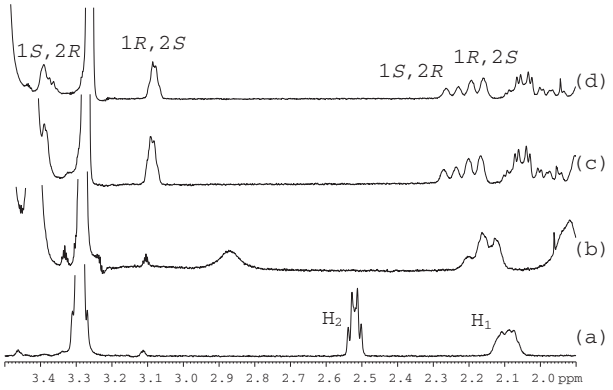

Figure 4. ^{1}H NMR spectra (400 MHz, methanol-d_4, 23 °C) of (a) the H1 and H2 resonances of **3** [10 mM—enriched in (1*R*,2*S*)] with **1** at (b) 5 mM, (c) 10 mM and (d) 20 mM.

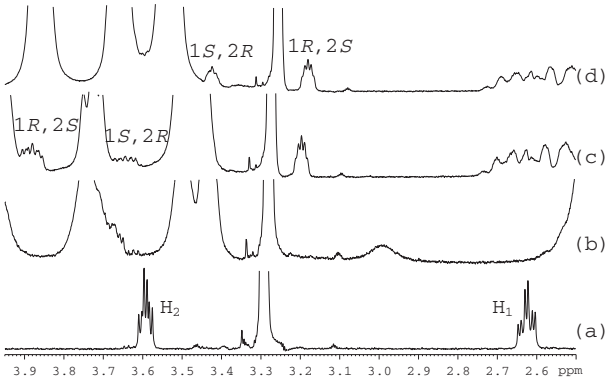

Figure 5. ^{1}H NMR spectra (400 MHz, methanol-d_4, 23 °C) of (a) the H1 and H2 resonances of **8** [10 mM—enriched in (1*R*,2*S*)] with **1** at (b) 5 mM, (c) 10 mM and (d) 20 mM.

of ring sizes, include aliphatic and olefinic rings, and include mono- and bicyclic compounds. All the substrates were available in enantiomerically pure form so the study of enriched mixtures enabled the assignment of the configuration of the enantiomerically discriminated resonances.

Table 1 summarizes the enantiomeric discrimination observed in the NMR spectra of **2**–**11** in mixtures with **1**. Values are reported for hydrogen atoms where the resonances of both enantiomers do not overlap with other signals. In almost every case, the largest enantiomeric discrimination was observed at the highest concentration of **1** (20 mM). In a few instances that will be described later, enantiomeric discrimination was largest at one of the lower concentrations of **1**.

Enantiomeric discrimination was observed for the methine hydrogen atoms α to the carboxylic acid and amine moieties for all 10 substrates. At least one of these signals has enantiomeric discrimination of sufficient magnitude and is free of overlap with other resonances such that the enantiomeric purity can be determined. The signals for H1 and H2 in the monocyclic substrates are especially useful because they are deshielded relative to the other ring hydrogen atoms. Furthermore, H1 and H2 have less coupling than the other hydrogen atoms in the ring and appear as less complex multiplets. The resonances for the other ring hydrogen atoms of the monocyclic substrates often overlap in the initial NMR spectra and show smaller perturbations in chemical shifts upon protonation and complexation with **1**. As mentioned earlier, perturbations in the chemical shifts of the methine resonances frequently move them into the region of the spectrum where the methanol and crown ether resonances occur. In cases where the resonance of one enantiomer overlaps with a resonance of **1** or methanol throughout the entire series of spectra, it is still often possible to use 2D COSY measurements to find the location of the resonance of the other enantiomer.

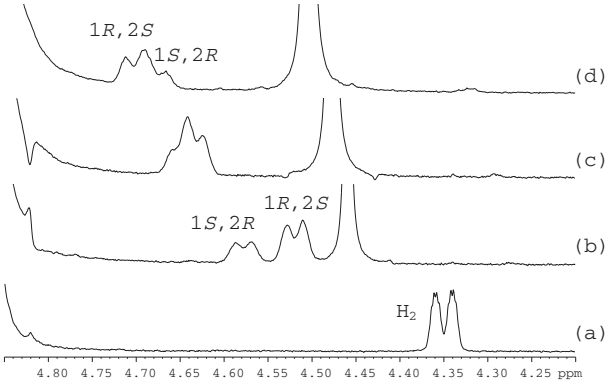

Figure 6. ^{1}H NMR spectra (400 MHz, methanol-d_4, 23 °C) of (a) the H2 resonance of **6** [10 mM—enriched in (1*R*,2*S*)] with **1** at (b) 5 mM, (c) 10 mM and (d) 20 mM.

Figures 2–6 show examples of the enantiomeric discrimination observed for the H1 and/or H2 resonances of several monocyclic substrates in the presence of **1**. The spectra in these figures also illustrate other important aspects with regards to the use of **1** with β-amino acids. Figure 2 shows the progression of the H1 resonance of **7** with increasing concentrations of **1**. The large resonances in the spectra belong to **1**. Of particular interest are the changes in the chemical shifts of the resonances of **1** that occur over the series. As the concentration of **1** is increased, the resonance of one hydrogen atom of **1** shows significant deshielding, whereas the others experience modest shielding. Since **1** and the β-amino acid undergo a neutralization reaction, the primary species for **1** in Figure 2b (crown–substrate ratio of 0.5:1) is a dianion. It is also possible that a 1:2 crown–substrate complex, in which two substrate molecules bind at the two faces of **1**, occurs at this ratio. For the conditions in Figure 2c (crown–substrate ratio of 1:1), the primary species for **1** is a monoanion and a 1:1 crown–substrate complex is expected. Unfortunately, as noted in the previous study of acyclic β-amino acids with **1**, it was not possible to measure the stoichiometry of these complexes using accepted methods such as a Job plot, since they provide the stoichiometry of the neutralization reaction rather than the complexation reaction.[16] The transition from a dianion to monoanion and the expected changes in the stoichiometry of the complex are likely responsible for the changes in the chemical shifts of the resonances of **1** over the series of spectra. However, as can be seen in Figures 3–6, changes in the chemical shifts of the resonances of **1** are not identical in the series of spectra for each of the substrates. The variability in the extent of shielding and deshielding of the hydrogen atoms of **1** with different substrates is another reason to record a series of spectra with increasing concentrations of **1** relative to the substrate.

Figure 3 shows the progression of the H1 resonance of **2** in the presence of increasing concentrations of **1**. Significant broadening is observed in the spectrum with a crown–substrate ratio of 0.5:1 (Fig. 2b). This occurs with other substrates as seen in Figures 4b and 5b. Broadened spectra at this concentration ratio have been noted in prior studies of acyclic β-amino acids and other amines with **1** when the two are mixed together as a neutral species and an acid–base neutralization reaction occurs.[16–19] Broadened NMR spectra with chiral solvating agents usually implies that the substrate is undergoing an intermediate rate of exchange between its bound and unbound form.[20] The broadening and intermediate

Table 1
Enantiomeric discrimination (ppm) in the ^{1}H NMR spectrum of β-amino acids (10 mM) in the presence of **1** (20 mM)

	α to COOH	α to NH_2	Alkene	Other hydrogen atoms
2	0.23 (1*S*,2*R*)	0.17 (1*R*,2*S*)		
3	0.07 (1*S*,2*R*)	0.30 (1*S*,2*R*)		
4	0.03 (1*R*,2*R*)	—[a]		
5	0.26 (1*S*,2*R*)	—[a]		
6	0.14 (1*S*,2*R*)	0.06[b,c]	0.05 (1*S*,2*R*)	
7	0.19 (1*S*,2*R*)[b]	0.24 (1*R*,2*S*)		
8	0.24 (1*S*,2*R*)	0.24[d] (1*R*,2*S*)		
9	0.07[d]	0.17 (1*R*,2*R*)		
10	0.10 (1*R*,2*S*)	0.05[e] (1*R*,2*S*)		H_1: 0.03[e] (1*R*,2*S*), H_4: 0.02[e] (1*R*,2*S*) H_7: 0.05 (1*R*,2*S*), $H_{7'}$: 0.14 (1*R*,2*S*)
11	0.06 (1*S*,2*S*)	0.06[e] (1*S*,2*S*)	0.06 (1*S*,2*S*)	H_7: 0.06 (1*S*,2*S*), $H_{7'}$: 0.24 (1*S*,2*S*)

Enantiomer that shows the largest perturbations in chemical shifts in the presence of (+)-**1** is indicated in parentheses.
[a] Resonances show enantiomeric discrimination but one or both overlap with other resonances in the spectrum.
[b] Concentration of **1** is 5 mM.
[c] Assignment of stereochemistry with largest perturbation in chemical shift is not clear because of the reversal of the resonances with increasing concentration of **1**.
[d] The assignment of stereochemistry is unclear because of partially overlapped resonances.
[e] Concentration of **1** is 10 mM.

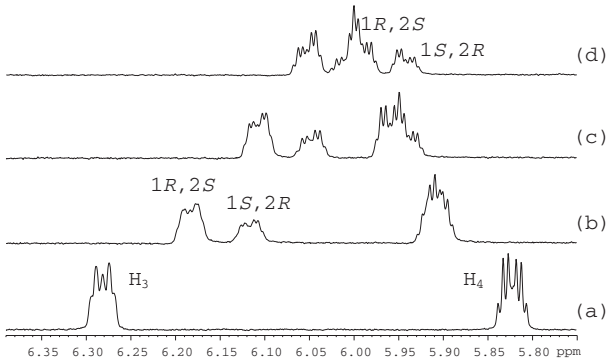

Figure 7. ^{1}H NMR spectra (400 MHz, methanol-d_4, 23 °C) of (a) the alkene resonances of **6** [10 mM—enriched in (1*R*,2*S*)] with **1** at (b) 5 mM, (c) 10 mM and (d) 20 mM.

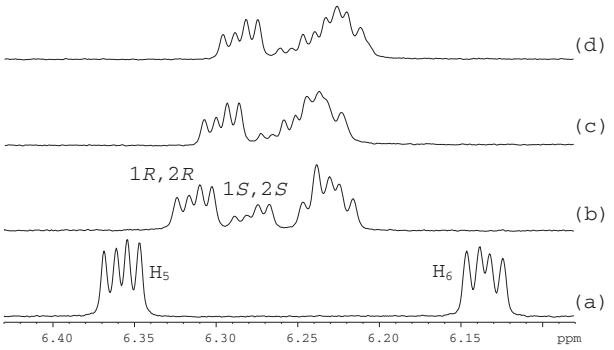

Figure 8. ^{1}H NMR spectra (400 MHz, methanol-d_4, 23 °C) of (a) the alkene resonances of **11** [10 mM—enriched in (1*R*,2*R*,3*S*,4*S*)] with **1** at (b) 5 mM, (c) 10 mM and (d) 20 mM.

exchange rate only occurs at a crown–substrate ratio of 0.5:1, where **1** is primarily in its dianionic form. Increasing the amount of **1** favors a 1:1 crown–substrate complex and speeds up the exchange, thereby reducing the broadening (Fig. 3c and d). Significant enantiomeric discrimination of the H1 resonance of **2** with **1** is observed in the spectra in Figure 3b–d, although the broadening in Figure 3b and overlap of the signal for the (1*R*,2*R*)-isomer with the methanol peak in Figure 3c make these less useful than the spectrum in Figure 3d.

Figures 4 and 5 provide other examples that show the value of recording a series of spectra with increasing concentrations of **1**. In Figure 4, a crown resonance at about 3.4 ppm is deshielded in the series of spectra. The resonance for H2 of **3** exhibits substantial deshielding in the spectra in Figure 4b and c that primarily occurs because of protonation of the amine by the crown ether. However, increasing **1** from 10 to 20 mM led to a slight shielding of H2 of **3** that, combined with the deshielding of the resonance of **1**, removes the overlap of the H2 resonances of both enantiomers (Fig. 4d). The resonance of H1 of **3** shows a small but consistently larger deshielding in the series of spectra (Fig. 4a–d), which is in contrast with that of H2.

The H1 resonance of **8** shown in Figure 5 behaves different to that of H1 in **3**, but rather analogously to H2 in **3** (Fig. 4). A substantial deshielding of H1 of **8** (10 mM) occurs when **1** is at concentrations of 5 and 10 mM (Fig. 5b and c). Raising the concentration of **1** to 20 mM causes a slight shielding of H1 of **8** that eliminates the overlap with the resonance of **1** (Fig. 5d). The enantiomeric discrimination for H2 of **8** is also apparent in Figure 5c, whereas the resonance of one or both enantiomers of H2 overlaps with the resonances of **1** under other conditions.

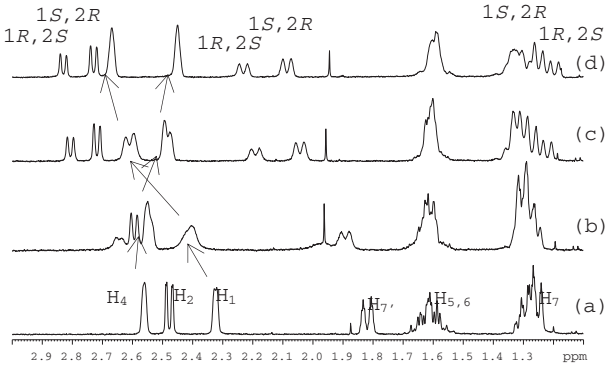

Figure 9. ^{1}H NMR spectra (400 MHz, methanol-d_4, 23 °C) of (a) the aliphatic region of **10** [10 mM—enriched in (1*S*,2*R*,3*S*,4*R*)] with **1** at (b) 5 mM, (c) 10 mM and (d) 20 mM.

Figure 6 shows the unusual behavior for the H2 resonance of **6** in the presence of increasing concentrations of **1**. In the spectrum with a crown–substrate ratio of 0.5:1, (Fig. 6b), the H2 resonance shows significant enantiomeric discrimination while the signal of (1*S*,2*R*)-**6** is deshielded relative to that of (1*R*,2*S*)-**6**. Increasing

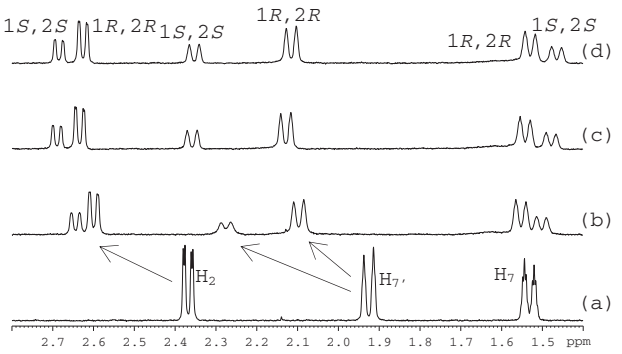

Figure 10. ^{1}H NMR spectra (400 MHz, methanol-d_4, 23 °C) of (a) the aliphatic region of **11** [10 mM—enriched in (1*R*,2*R*,3*S*,4*S*)] with **1** at (b) 5 mM, (c) 10 mM and (d) 20 mM.

the concentration of **1** above 5 mM causes the H2 resonances of the two enantiomers of **6** to recoalesce and then change their relative positions (Fig. 6c and d). The transition from a 1:2 crown–substrate complex under the conditions in Figure 6b to a 1:1 complex under the conditions in Figure 6c and d may account for this unusual behavior, as the relative deshielding of H2 of the two enantiomers may be different in the 1:2 and 1:1 crown–substrate complexes. However, an alternative explanation for the unusual behavior of H2 is possible.

Enantiomeric discrimination with chiral NMR solvating agents can occur because of two mechanisms. The first is that the complexes of the two enantiomers with the chiral solvating agent are diastereomers. The second is that the association constants of the two enantiomers with the chiral solvating agent are often different. In the latter situation, under the conditions of fast exchange, the time-averaged solvation environment of the two enantiomers is different. It is difficult to distinguish between these two mechanisms and both likely contribute to some extent to the enantiomeric discrimination with many chiral solvating agents. If the differences in the association constants represent a significant contribution to the enantiomeric discrimination in the NMR spectrum, unusual behavior can occur in a series of spectra with increasing concentration of the chiral solvating agent relative to the substrate. This situation has already been examined in detail.[21] Given the right conditions, it is possible for a resonance to exhibit enantiomeric discrimination at a low concentration of the chiral solvating agent, recoalesce and reverse the order of the two enantiomers at higher concentrations of the chiral solvating agent.[21] Irrespective of the exact rationale for the behavior of H2 in Figure 6, it further demonstrates the desirability of recording a series of spectra with increasing concentrations of **1** relative to the substrate.

A final observation apparent in Figures 3–5 is that the vicinal coupling constants of H1 and H2 often change over the series of spectra. This same behavior was observed in the prior study of acyclic β-amino acids.[16] Presumably, and perhaps not surprisingly, the association of the β-amino acid with **1** alters the conformational preference of the substrate, which causes changes in the average dihedral angles and vicinal coupling constants.

Five of the ten substrates have alkene moieties and the two alkene resonances are in a region of the spectrum that is free of other resonances. Figures 7 and 8 illustrate the general behavior that is observed for the alkene resonances of **6**–**9** and **11**. Using **6** as an example, the resonance of the alkene hydrogen β to the amine group (H3) is deshielded relative to that of the hydrogen γ to the carboxylic acid (H4) in the spectrum of the substrate (Fig. 7a). The same relative deshielding occurs for substrates where one alkene hydrogen atom is γ to the amine and the other is γ to the carboxylic acid moiety (Fig. 8a). The addition of **1**–**6** led to substantial shielding of H3 and deshielding of H4, which eventually causes the two alkene resonances to overlap as the concentration of **1** is increased (Fig. 7b–d). In **7**–**9**, the overlap of the two alkene resonances that occurs with increasing concentrations of **1** obscures any enantiomeric discrimination that might be present in the NMR spectra. For **6** (Fig. 7), the substantial enantiomeric discrimination of H3 is apparent and can be used to accurately determine the enantiomeric purity. The same is true for H5 of **11** (Fig. 8).

Figures 9 and 10 show the series of spectra obtained for portions of the aliphatic region of the bicyclic substrates **10** and **11**. The H2 and H7′ resonances of **10** and **11** are deshielded upon the addition of **1** and show large enantiomeric discrimination. The H7 resonances of **10** and **11** are only minimally perturbed from their original chemical shifts when **1** is added, but the small shielding for the hydrogen atom of one enantiomer and small deshielding for the other results in enantiomeric discrimination in the NMR spectrum. Compound **1** is an especially effective chiral solvating agent for β-amino acids with bicyclo[2.2.1]heptane and heptene rings. Figure 9 includes the H1 and H4 resonances of **10** as well. Shielding of H4 and deshielding of H1 in the presence of **1** cause these resonances to reverse their positions over the series of spectra. The H1 and H4 resonances show a small degree of enantiomeric discrimination at a 1:1 crown–substrate ratio (Fig. 9c) that no longer occurs at a 2:1 crown–substrate ratio (Fig. 9d).

2.1. Absolute configuration

When **1** was used with acyclic β-amino acids, perturbations of the resonances of the Hα-methine and Hβ-methylene hydrogen atoms exhibited trends that correlated with the absolute configuration.[16] Perturbations of the chemical shifts of the two methine hydrogen atoms of **2**–**11** were examined to see if the changes correlated with the absolute configuration. The enantiomer resonance that exhibits the larger perturbation in the chemical shift in the presence of (+)-**1** is indicated in Table 1. Six of the monocyclic substrates have the amine and carboxylic acid moieties in a *cis*-configuration, and in every case, the resonance for the (1*S*,2*R*)-isomer of the methine hydrogen atom α to the carboxylic acid moiety exhibits the larger perturbation in chemical shift in the presence of **1**. The relative order of the resonances for the methine hydrogen α to the amine moiety exhibited more variability among the different substrates. For the two bicyclic substrates, the resonances of the (2*S*,3*R*)-isomer for the hydrogen atoms α to the amine and carboxylic acid moieties exhibit the larger perturbations in chemical shifts in the presence of **1**. These data suggest that the assignment of the absolute configuration of cyclic β-amino acids can be achieved using **1** as a chiral NMR solvating agent, provided compounds with similar structures, ring sizes, and known configurations are examined for comparison.

3. Conclusions

Compound **1** is an effective chiral NMR solvating agent for cyclic β-amino acids with cyclopentane, cyclohexane, cycloheptane, cyclopentene, cyclohexene, bicyclo[2.2.1]heptane and bicyclo[2.2.1]heptene rings. Spectra are recorded in methanol-d_4 and it is best to record a series of spectra with increasing amounts of **1** relative to the β-amino acid. The enantiomeric discrimination of the methine hydrogen atoms α to the amine and carboxylic acid moieties is observed for all 10 substrates and is large enough to determine enantiomeric purity. The bicyclic substrates have a number of resonances that exhibit enantiomeric discrimination in the presence of **1**. Certain hydrogen atoms of β-amino acids with similar structures show consistent patterns in the enantiomeric discrimination that correlates with the absolute configuration. Pro-

vided compounds of similar structures with known configurations are analyzed, **1** can be used to assign the absolute configuration of cyclic β-amino acids.

4. Experimental

4.1. Reagents

The racemic substrates were prepared and purified using established methods.[4] The (1*R*,2*S*)-β-amino acids **2**, **3**, **5**–**8**, **10**, and **11** were prepared through CAL-B-catalyzed highly enantioselective ring cleavage of the corresponding β-lactams,[9,22,23] while (1*R*,2*R*)-**4** and **9** were prepared via CAL-B-catalyzed enantioselective hydrolysis of corresponding β-amino esters.[10] Methanol-d_4 and the (+)- and (−)-isomer of **1** were obtained from commercial suppliers (Sigma–Aldrich, Milwaukee, WI) and used as received.

4.2. Instrumentation

All ^{1}H NMR spectra (16 scans) were collected on a 400 MHz NMR spectrometer. Spectra were recorded in methanol-d_4 at ambient probe temperature and calibrated using tetramethylsilane (0.05%) as an internal reference.

4.3. Procedures for chiral discrimination studies

Solutions of the chiral substrates, either as racemates or enriched in one isomer, were prepared by dissolving a sufficient amount of the β-amino acid to prepare a 10 mM solution in methanol-d_4. Some amount of **1** necessary to prepare the desired concentration was then added by weight to the sample in an NMR tube.

Acknowledgments

We thank the National Science Foundation (Research at Undergraduate Institutions Program Grant CHE-0653711; Major Instrumentation Program, Grant CHE-0115579) for supporting this work.

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