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(18-Crown-6)-2,3,11,12-tetracarboxylic acid as a chiral NMR solvating agent for determining the enantiomeric purity and absolute configuration of β-amino acids

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ABSTRACT

(18-Crown-6)-2,3,11,12-tetracarboxylic acid is an excellent chiral NMR solvating agent for cyclic β-amino acids and acyclic derivatives with aliphatic, aromatic, and heterocyclic aromatic moieties. The β-amino acids are mixed with the crown ether in methanol-d_4 in either their neutral or protonated form. Substantial enantiomeric discrimination typically occurs for the resonances of the α-methylene and β-methine hydrogen atoms. Resonances of the substituent group of the β-amino acid often exhibit enantiomeric discrimination. The enantiomeric discrimination of the α-methylene and β-methine resonances of specific groups of compounds shows consistent patterns that correlate with the absolute configuration.

1. Introduction

Optically active β-amino acids are important in a variety of natural products including β-lactam antibiotics, alkaloids, peptides, and compounds with anti-tumor properties.[1–5] β-Amino acids that have pharmacological and neurological properties, act as enzyme inhibitors, and function as receptor antagonists have been discovered.[6] They have been used in pharmaceutical development and a variety of strategies have been developed for their synthesis.[5,7] Given the importance of β-amino acids, easily applicable methods for their enantiomeric analysis are necessary.

The most common methods for enantiomeric analysis involve chromatographic separations or NMR spectroscopic discrimination. Chromatographic methods afford the possibility of isolating the two enantiomers, whereas NMR spectroscopic methods using chiral solvating agents are easy to perform. Chiral NMR reagents that are broadly suitable for the analysis of chiral β-amino acids are limited in scope.[8] The Eu(III) complex of propylenediaminetetraacetate causes enantiomeric discrimination in the NMR spectra of β-amino acids in water and the differential changes in chemical shifts of the enantiomers correlate with absolute configuration.[9] However, the complex must be synthesized using a commercially available ligand. Furthermore, lanthanide shift reagents are often limited in utility because of paramagnetic broadening that occurs in the NMR spectrum.

A palladium dimer with *N*,*N*-dimethyl-(1-phenyl)ethylamine and bridging chloro ligands causes enantiomeric discrimination of β-amino acids in methanol-d_4. The β-amino acid displaces the bridging chloro ligands in the dimer to form a monopalladium complex. Differences in the *cis*- and *trans*-complexes that form with the β-amino acids correlate with the absolute configuration. A limitation of this reagent is that the palladium complex must first be prepared from a commercially available ligand.[10]

Liquid chromatographic separations of β-amino acids have been achieved using bonded phases that incorporate an (18-crown-6)-2,3,11,12-tetracarboxylic acid **1** unit.[11–13] The 18-crown-6 moiety exhibits favorable association with protonated primary amines by forming three hydrogen bonds (Fig. 1). Compound **1** is commercially available and we and others have demonstrated its effectiveness as a chiral NMR solvating agent for primary amines.[14–18] The analysis is performed directly in an NMR tube by mixing either the ammonium salt or neutral amine to a solution of **1**. In the latter case, a neutralization reaction between the amine and a carboxylic acid group of **1** produces the protonated primary amine needed for association. Earlier studies have shown that enantiomeric discrimination

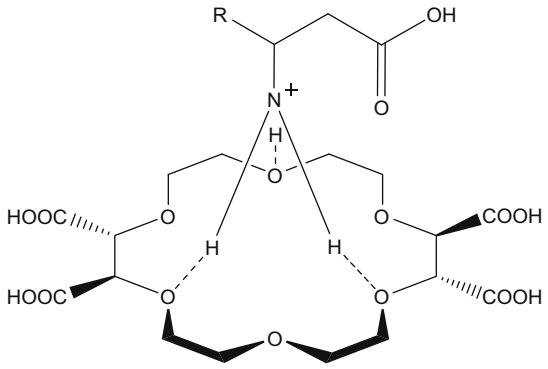

Figure 1. Association of a protonated β-amino acid with **1**.

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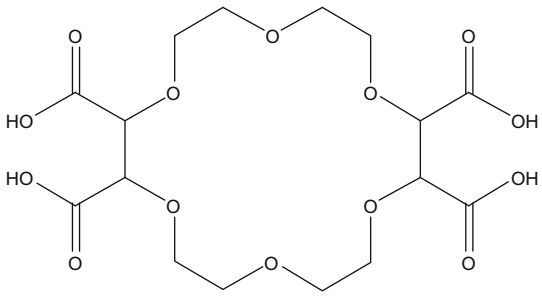
in the ^{1}H NMR spectra of primary amines is essentially the same regardless of whether the ammonium salt or neutral amine is mixed with **1**.[14,17] Compound **1** has also been shown to be an effective chiral NMR solvating agent for secondary amines, provided the neutral amine is mixed with it instead of the ammonium salt.[19–22]

Herein we show that **1** is an exceptional chiral NMR solvating agent for the analysis of β-amino acids. Several resonances of the β-amino acids can be used for the determination of enantiomeric purity. Empirical trends indicate that the pattern of changes in chemical shifts for certain hydrogen atoms correlates with the absolute configuration of the β-amino acid.



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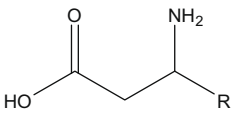
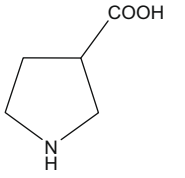


	R
2	CH_2CH_3
3	$CH_2CH_2CH_3$
4	$CH_2CH_2CH_2CH_3$
5	$CH(CH_2CH_3)_2$
6	CH_2CH_2Ph
7	Cyclohexyl
8	Ph
9	2-Cl-Ph
10	2-Br-Ph
11	2-Me-Ph
12	2-HO-Ph
13	2-MeO-Ph
14	3-Cl-Ph
15	3-MeO-Ph
16	4-F-Ph
17	4-Cl-Ph
18	4-Br-Ph
19	4-Me-Ph
20	4-CH_3-Ph
21	4-MeO-Ph
22	3,4-DiCl-Ph
23	1-naphthyl
24	2-furyl
25	2-thienyl
26	3-pyridyl



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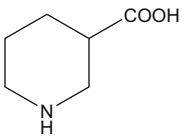

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2. Results and discussion

The effectiveness of **1** as a chiral NMR solvating agent for β-amino acids was tested with **2–28**. These compounds have a variety of substituent groups that include aliphatic (**2–7**), phenyl **8–22**, naphthyl **23**, and heterocyclic aromatic **24–26** moieties. Two cyclic β-amino acids **27** and **28** were examined as well. Sufficient solubility of the substrates and excellent enantiomeric discrimination have been observed in prior studies of amines with **1** in methanol-d_4.[14–22] At least one resonance of every β-amino acid examined herein exhibits an enantiomeric discrimination that is suitable for the determination of enantiomeric purity in the ^{1}H NMR spectrum with **1** in methanol-d_4. The resonances of the α-methylene and methine hydrogen atoms are especially useful for the enantiomeric analysis of β-amino acids. Furthermore, the discrimination of the α-methylene and β-methine hydrogen atoms shows trends that correlate with the absolute configuration.

2.1. α-Methylene hydrogen atoms

The diastereotopic methylene hydrogen atoms, which are designated H_A and H'_A herein, are doublets of doublets because of geminal coupling to each other and vicinal coupling to the adjacent methine hydrogen. At 400 MHz, H_A and H'_A generally appear as separate resonances for **2–26**. The degree of distortion of the doublets of doublets (e.g., Figs. 2a and 3a) depends on the proximity of chemical shifts of the two resonances. The series of spectra in Figure 2 show the general pattern that occurs for the H_A and H'_A resonances of every substrate examined herein in the presence of increasing concentrations of **1**.

The resonance designated H_A initially occurs at a lower frequency than that of H'_A. The H_A atom of one enantiomer is strongly deshielded in the presence of **1**, (Fig. 2a–i) such that its resonance is eventually at the highest frequency of any of the methylene signals. The resonance of the H_A atom of the other enantiomer of **7** is only slightly perturbed from its initial position in the presence of **1** such that the enantiomeric discrimination of H_A is quite large (e.g., 0.351 ppm for **7**). The H'_A resonances of the two enantiomers of **7** show only small perturbations from their original position. For **7**, the H'_A atom of one enantiomer is slightly shielded, whereas the other is slightly deshielded in the presence of **1** (Fig. 2a–i). For **7**, the differences in shielding of the two enantiomeric H'_A atoms are sufficient enough to cause enantiodifferentiation of 0.042 ppm.

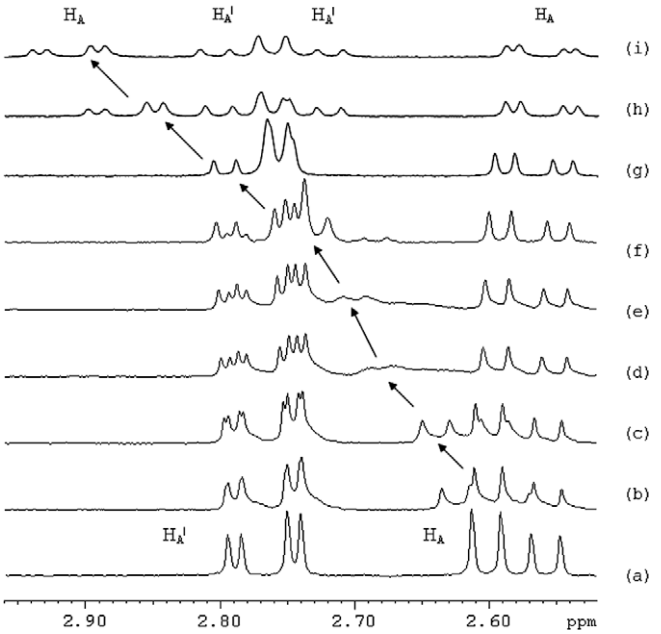

Figure 2. ^{1}H NMR spectra (400 MHz, methanol-d_4, 23 °C) of the (a) α-methylene hydrogen atoms of **7** (10 mM) with **1** at (b) 1 mM, (c) 2 mM, (d) 3 mM, (e) 4 mM, (f) 5 mM, (g) 10 mM, (h) 15 mM, and (i) 20 mM.

The spectra in Figure 3 show the methylene resonances of **8**. Unlike **7**, the H_A and H'_A atoms of both enantiomers are deshielded in the presence of **1**. Nevertheless, the overall pattern of changes in chemical shifts is consistent with that of **7**. The H_A atom of one of the enantiomers experiences the largest deshielding and substantial enantiomeric discrimination occurs (0.400 ppm). The H'_A atoms of the two enantiomers experience different degrees of deshielding

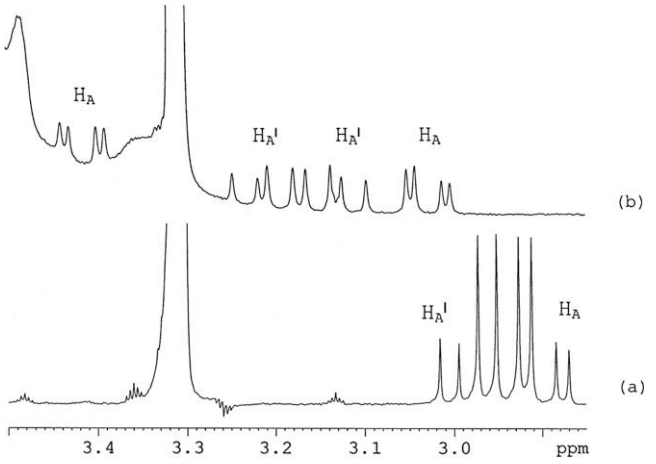

Figure 3. ^{1}H NMR spectra (400 MHz, methanol-d_4, 23 °C) of the (a) α-methylene hydrogen atoms of **8** (10 mM) with **1** at (b) 10 mM.

such that substantial enantiomeric discrimination occurs as well (0.076 ppm).

The initial chemical shifts of the H_A and H'_A resonances of **8** (Fig. 3a) are at a higher frequency than those of **7** (Fig. 2a). For **7** (10 mM), the chemical shifts of the H_A and H'_A resonances with **1** (20 mM) are still at a lower frequency than the methanol peak at 3.32 ppm (Fig. 2b–i). For **8** (10 mM), the change in chemical shift of one of the H_A resonances with **1** (20 mM) moves it to a higher frequency than the methanol peak (Fig. 3b). For most substrates, the H_A and H'_A resonances are discernible from the methanol resonance and resonances of **1**. The only exceptions are the H_A resonance of **10**, **12**, **13**, **23**, and **24** and the H'_A resonance of **10**, **12**, and **23**, which overlap with either resonances of **1** or the methanol resonance in the NMR spectra. In most cases with these substrates, the H_A or H'_A resonance for only one enantiomer overlaps with others. For mixtures enriched in one of the enantiomers, it was possible to identify the configuration of the unobstructed resonance by comparing its area to other resonances in the spectrum.

Compounds **10** and **12** are unusual in that extra peaks begin to appear in the NMR spectra upon the addition of **1**. This suggests that two favorable rotational conformations occur on complexation with **1**. Presumably the large bromine substituent group in **10** and hydrogen bonding hydroxyl group in **12** that are *ortho* to the position of the β-amino moiety are important in causing the presence of two preferred rotamers. This behavior does not occur when a chlorine atom, methyl group, or methoxy group is at the *ortho*-position. The magnitudes of the enantiomeric discrimination of the H_A and H'_A resonances for **2–26** (10 mM) with **1** (20 mM) are provided in Table 1. As seen in Figure 2a–i, using a higher concentration of **1** relative to substrate enhances the magnitude of the enantiomeric discrimination. A more important reason for recording data at a 2:1 crown-substrate ratio is that the ^{1}H NMR spectra of several substrates in methanol-d_4 at 10 mM show appreciable broadening when **1** is at 5, 10, and 15 mM. For example, the H_A resonance of **7** that shows the largest perturbation in chemical shift is broadened at intermediate concentrations of **1** shown in Figure 2d–g.

Similar broadening has been observed in previous studies with **1** at comparable concentration ratios.[22] The broadening likely occurs from exchange effects. Studies with chiral NMR solvating agents are best carried out under conditions of fast exchange such that the spectrum of the substrate is a time-average of its bound and unbound forms.[8] Intermediate exchange rates lead to broadening. In this and prior studies using **1** as a chiral NMR solvating agent, the rate of exchange exhibits a concentration dependence for many substrates which implies that fast exchange occurs when either the substrate or crown ether is present at a much higher concentration than the other. When the two concentrations are more equal, the exchange appears slower and broadening some-

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

	CH_2		CH	Other hydrogen atoms
	H_A	H'_A		
2	0.168(*S*)	0.074(*R*)		CH_3: 0.023
3	0.159	0.082		CH_3: 0.018
4	0.159	0.084		CH_3: 0.004
5	0.244	0.093		CH_3: 0.006
6	0.201	0.108		
7	0.351	0.042		
8	0.400	0.076	0.158	H*o*: 0.019; H*m*,[a] H*p*[a]
9	0.271(*S*)	0.040(*R*)	0.189(*R*)	H3′[a]; H4′[a]; H5′[a]; H6′: 0.182
10	—[b] (*S*)	—[b]	0.275(*R*)	H4′: 0.070; H5′: 0.020; H6′: 0.222
11	0.165(*S*)	0.096(*R*)	0.149(*R*)	H6′: 0.208
12	—[b]	0.128(*R*)	—[a]	H3′: 0.020; H6′: 0.150
13	—[a] (*R*)	—[a]	—[a] (*S*)	H3′: 0.155; H4′: 0.021; H5′: 0.057; H6′: 0.040
14	0.406(*R*)	0.040(*S*)	0.197(*S*)	H2′: 0.010; H4′: 0.015; H5′: 0.019; H6′: 0.041
15	0.436	0.051	—[a]	H4′: 0.026; H5′[a]; H6′[a]
16	0.372	0.061	0.163	H*o*: 0.036; H*m*, 0.041
17	0.369(*R*)	0.040(*S*)	0.158(*S*)	H*o*: 0.021; H*m*, 0.041
18	0.365	0.039	0.123	H*o*: 0.020; H*m*, 0.041
19	0.367	0.043	0.124	H*o*: 0.032; H*m*, 0.036; CH_3: 0.008
20	0.365	0.040	0.151	H*o*: 0.020; H*m*, 0.040
21	0.375	0.029	0.155	H*o*: 0.030; H*m*, 0.037; CH_3: 0.007
22	0.350	0.040	0.201	H2′: 0.013; H5′: 0.044; H6′: 0.039
23	—[a]	—[a]	0.146	H2′: 0.223; H8′: 0.249
24	—[a]	0.060	0.141	H3′: 0.104; H4′: 0.018; H5′: 0.013
25	0.397(*R*)	0.024(*S*)	0.176(*S*)	H3′: 0.081; H4′: 0.023
26	0.397(*R*)	0.024(*S*)	0.202(*S*)	H3′: 0.111; H4′: 0.020; H5′: 0.060; H6′: 0.047
27				NCH_2 (H2): 0.284
28				NCH_2 (H2): 0.055[c], NCH_2 (H6): 0.068[c]

Enantiomer shifted to higher frequency 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] Resonances show enantiomeric discrimination but are too complicated because of the presence of two preferred rotamers.
[c] Concentration of **1** is 5 mM.

times occurs in the ^{1}H NMR spectrum at 400 MHz. Therefore, studies with β-amino acids are best carried out with a concentration of **1** twice that of the substrate.

For β-amino acids with a phenyl, cyclohexyl, or heterocyclic aromatic substituent group on the β-carbon, the enantiomeric discrimination of the H_A resonance generally ranges from 0.350 to 0.436 ppm. β-Amino acids with a straight-chain aliphatic substituent group exhibit smaller enantiomeric discrimination of the H_A resonance which ranges from 0.159 to 0.244 ppm. The exception to these general trends occurs with substrates that have a substituent group on the phenyl ring *ortho* to the carbon with the β-amino acid moieties **9–13**. This may account for the much smaller enantiodifferentiation of the H_A resonance relative to other aromatic-substituted substrates. The enantiomeric discrimination of the H'_A resonances for substrates with a phenyl, cyclohexyl, or heterocyclic aromatic group ranges from 0.029 to 0.076 ppm, with most values at about 0.040 ppm. Enantiomeric discrimination of the H'_A resonance of substrates with a straight-chain aliphatic substituent group is larger and ranges from 0.082 to 0.108 ppm. The *ortho*-substituted β-amino acids exhibit smaller enantiomeric discrimination of the H_A resonance and larger enantiomeric discrimination of the H'_A resonance than the other phenyl-containing substrates.

Another observation apparent in the spectra in Figures 2 and 3 is a change in the vicinal coupling constants between the methylene and methine hydrogen atoms in the presence of **1**. The changes in vicinal coupling constants indicate that the time-averaged dihedral angle between the diastereotopic methylene and methine hydrogen atoms is altered upon association of β-amino acids with **1**. The geminal coupling constant between H_A and H'_A remains constant over the series (17.5 Hz for **7**). The vicinal coupling constant between H_A and the adjacent methine hydrogen atom (J_{CH-HA}) of **7** (10 mM) starts at 8.5 Hz and diminishes to 4.3 Hz with **1** at 20 mM. The vicinal coupling constant between H'_A and the adjacent methine hydrogen ($J_{CH-HA'}$) atom of **7** (10 mM) starts at 4.0 Hz and increases to 8.3 Hz with **1** at 20 mM. The same pattern of changes in the vicinal coupling constants between H_A and H'_A and the adjacent methine hydrogen atom in the presence of **1** occurs for all of the substrates with an aliphatic substituent group **2–7**.

The pattern of the initial vicinal coupling constants between the methine hydrogen atom and H_A and H'_A for β-amino acids with an aromatic or heterocyclic aromatic substituent group is different from those with an aliphatic substituent group. Whereas J_{CH-HA} is consistently greater than $J_{CH-HA'}$ for **2–7** (e.g., Fig. 2a), J_{CH-HA} is less than or about equal to $J_{CH-HA'}$ for **8–26** (e.g., Fig. 3a). The addition of **1** to solutions of **8** and **14–26** causes a pattern of change in the coupling constants which is consistent with the observations for **2–7**; J_{CH-HA} becomes smaller while $J_{CH-HA'}$ becomes larger. An exception occurs with **9–12**. In these cases, J_{CH-HA} becomes larger whereas $J_{CH-HA'}$ becomes smaller in the presence of **1**. Compounds **9–12** also have anomalous trends in the relative magnitude of the enantiomeric discrimination of H_A and H'_A. Interestingly, compound **13** with a methoxy group at the *ortho*-position exhibits changes in J_{CH-HA} and $J_{CH-HA'}$ analogous to those phenyl-containing substrates with a substituent at the *meta*- or *para*-position. As will be discussed later, **13** is also anomalous to **9–12** in the trends that occur related to absolute configuration.

Since the general pattern of changes in chemical shift and enantiomeric discrimination of H_A and H'_A in the presence of **1** is consistent for all of the β-amino acids, the changes in coupling constants do not compromise the use of these resonances for determining enantiomeric purity.

2.2. Methine hydrogen atom

For substrates in which the substituent group at the β-carbon is an aromatic or heterocyclic aromatic moiety, the methine hydrogen is a doublet of doublets from the vicinal coupling to the diastereotopic hydrogen atoms of the methylene group (Fig. 4a). For some substrates, the vicinal coupling constants are nearly identical and the resonance appears as a 1:2:1 triplet (Fig. 5a). For many substrates, the methine resonance occurs at a lower frequency than the HOD resonance (Fig. 4a). The addition of **1** deshields the methine hydrogen such that its resonance moves to a higher frequency than the HOD resonance (Fig. 4b). The methine resonance exhibits substantial enantiomeric discrimination in the presence of **1**. As discussed earlier, altering the time-averaged dihedral angle on complexation with **1** alters the vicinal coupling constants and the methine resonances of both enantiomers appear as doublets of doublets (Figs. 4b and 5d).

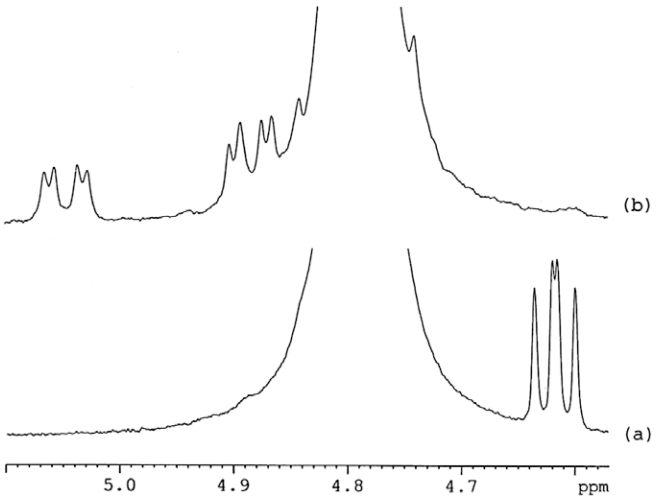

Figure 4. ^{1}H NMR spectra (400 MHz, methanol-d_4, 23 °C) of the (a) methine hydrogen atom of **16** (10 mM) with **1** at (b) 20 mM.

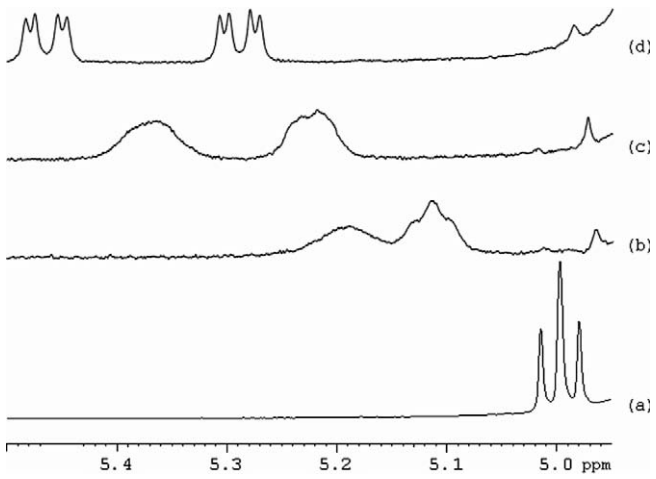

Figure 5. ^{1}H NMR spectra (400 MHz, methanol-d_4, 23 °C) of the (a) methine hydrogen atom of **25** (10 mM) with **1** at (b) 5 mM, (c) 10 mM, and (d) 20 mM.

The methine resonance of **25** occurs at a higher frequency than the HOD resonance. The series of spectra in Figure 5 show the progression of the methine resonance of **25** (10 mM) with increasing concentrations of **1**. Broadening is observed with **1** at 5 and 10 mM (Fig. 5b and c). As discussed earlier, an intermediate rate of exchange of bound and unbound forms of the substrate likely causes the broadening. Mixtures with the substrate at 10 mM and **1** at 20 mM are the best conditions to use for measuring the enantiomeric discrimination of the methine resonance.

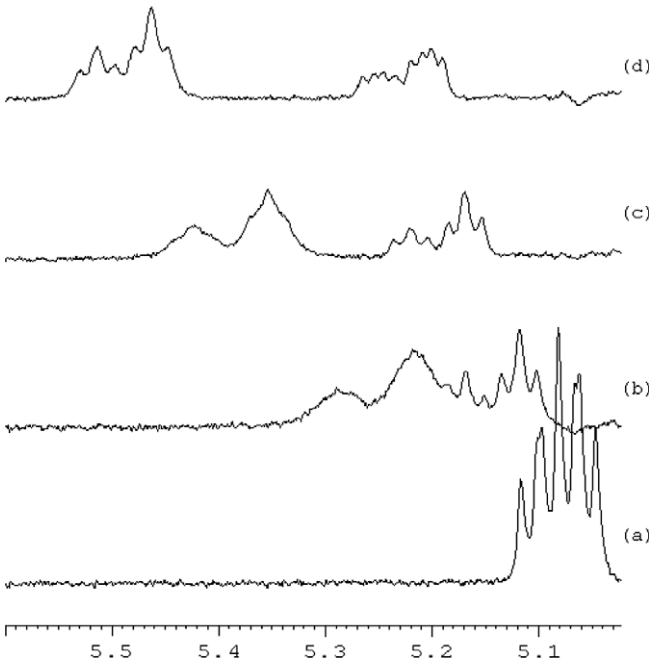

Figure 6. ^{1}H NMR spectra (400 MHz, methanol-d_4, 23 °C) of the (a) methine hydrogen atom of **10** (10 mM) with **1** at (b) 5 mM, (c) 10 mM, and (d) 20 mM.

The spectra in Figure 6 show the methine resonance of **10** in the presence of **1**. The set of two resonances at higher frequency (5.5 ppm) in the shifted spectra (Fig. 6d) are those of the (*R*)-enantiomer, whereas those at the lower frequency (5.25 ppm) belong to the (*S*)-enantiomer. The further splitting of these resonances is evidence of the presence of two rotamers, one of which has a higher preference than the other. This observation was unique to only **10** and **12**.

The enantiomeric discrimination of the methine resonances is reported in Table 1 and ranges from 0.123 to 0.275 ppm. Enantiodifferentiation is sufficiently large enough for accurate determination of enantiomeric purity. There are only three substrates **12**, **13**, and **15** where the methine resonance of one of the enantiomers overlaps with the HOD resonance in the spectrum with **1**. For β-amino acids with an aliphatic substituent group **2–7** or cyclic structure **27** and **28**, the methine hydrogen has additional vicinal coupling and a chemical shift that overlaps with resonances of the crown ether. The complexity of the spectrum where the methine resonance is observed with β-amino acids **2–7**, **27**, and **28** makes it impractical or impossible to use this resonance for the determination of enantiomeric purity with these substrates.

2.3. Other hydrogen atoms

2.3.1. Aliphatic substituent groups

Compounds **2–7** have aliphatic substituent groups. Four of these compounds have terminal methyl groups that are 2-, 3- or 4-carbon atoms removed from the amine-substituted carbon. The magnitude of the enantiomeric discrimination of the methyl resonance varies in the order **2** (0.023 ppm) > **3** (0.018 ppm) > **4** (0.004 ppm). This trend is not surprising given the relative proximity of the methyl group to the chiral carbon in **2–4**. The methyl groups in **5** are diastereotopic and, at 400 MHz, the addition of **1** leads to diastereotopic resolution and a small degree of enantiomeric discrimination for one of the methyl groups (Table 1). The enantiomeric discrimination of the methyl resonances of **2–5** is too small for a quantitative analysis of enantiomeric purity, but sufficient enough to qualitatively determine whether one or two enantiomers are present. The resonances of the cyclohexyl hydrogen atoms of **7** are too complex with too many overlapping peaks to determine whether enantiomeric discrimination occurs. Peak overlap and the complexity of resonances of the substituent group of **6** preclude an assessment of whether enantiomeric discrimination occurs. The phenyl resonances of **6** do not show enantiomeric discrimination, presumably because of their distance from the stereogenic center. Fortunately, the H_A and H'_A resonances of **2–7** exhibit enantiomeric discrimination of a suitable magnitude for the quantitative analysis of enantiomeric purity.

The spectra of **27** and **28** are also quite complex with several resonances that overlap with those of **1** or the solvent. Nevertheless, at least one resonance showed clear enantiomeric discrimination that could be used to determine the enantiomeric purity for each of these substrates.

2.3.2. Aromatic and heterocyclic aromatic substituent groups

All of the β-amino acids with an aromatic or heterocyclic aromatic substituent group attached at the stereogenic center exhibit substantial enantiomeric discrimination of one or more of the aromatic resonances (Table 1). The series of spectra in Figure 7 show the unusually pronounced enantiomeric discrimination of the H6′ resonance of **9** on addition of **1**. The hydrogen atom of **9** *ortho* to the carbon substituted with the β-amino acid moiety is deshielded in the presence of **1**, whereas the hydrogen atoms at the *meta*- and *para*-positions are shielded. A comparable pattern of deshielding (*ortho*-position) and shielding (*meta*- and *para*-positions) in the presence of **1** occurs with the other phenyl-containing substrates as well.

Figure 8 shows a series of spectra for **10** in the presence of **1**. In contrast to the spectrum of **9** (Fig. 7a), the bromine atom at the 2-position of the aromatic ring causes unique resonances for all four aromatic hydrogen atoms (Fig. 8a). Substantial enantiomeric discrimination occurs for the H4′ and H6′ resonances in the presence of **1**. A further splitting of the H6′ resonance occurs as the concentration of **1** is increased from 10 mM (Fig. 8c) to 20 mM (Fig. 8e). This splitting is evidence that **10** exhibits two preferred conformations on binding to **1**.

The spectra in Figure 9 show the progression of the aromatic resonances of **17** in the presence of **1**. Again, the hydrogen atoms *ortho* to the β-amino acid moiety are deshielded, whereas those at the *meta*-position are shielded. Both signals split into two doublets, one for each enantiomer. An interesting observation is the larger enantiomeric discrimination for H*m* than for H*o*, even though H*m* is further removed from the stereogenic center. Every other substrate shows the same trend with larger enantiomeric discrimination of H*m* than of H*o*. A subtlety of the geometry of the associated complex must position H*m* in such a way that leads to larger enantiomeric discrimination than that occurring for H*o*.

Figure 10 shows the series of spectra for **22** in the presence of increasing concentrations of **1**. The broadening at the intermediate concentrations of the crown ether is apparent. All of the aromatic resonances exhibit substantial enantiomeric discrimination, the largest of which occurs for H5′, when **1** is at 20 mM. The spectra in Figure 11 show the significant deshielding and enantiomeric discrimination that occur for the H2′ and H8′ resonances of **23** in the presence of **1**. The other hydrogen atoms of **23** experience modest shielding and no readily apparent enantiomeric discrimination with **1**.

The resonances of the heterocyclic ring hydrogen atoms of **24–26** also show pronounced enantiomeric discrimination in mixtures with **1**. Figure 12 shows the effect of **1** on the furyl ring resonances of **24**. Protons H4′ and H5′ are slightly shielded in the presence of **1**, whereas H3′ is deshielded. The enantiomeric discrimination of the H3′ and H5′ resonances is of suitable magnitude for the accurate determination of enantiomeric purity.

The series of spectra of the pyridyl resonances of **26** in the presence of **1** are shown in Figure 13. All of the pyridyl resonances are

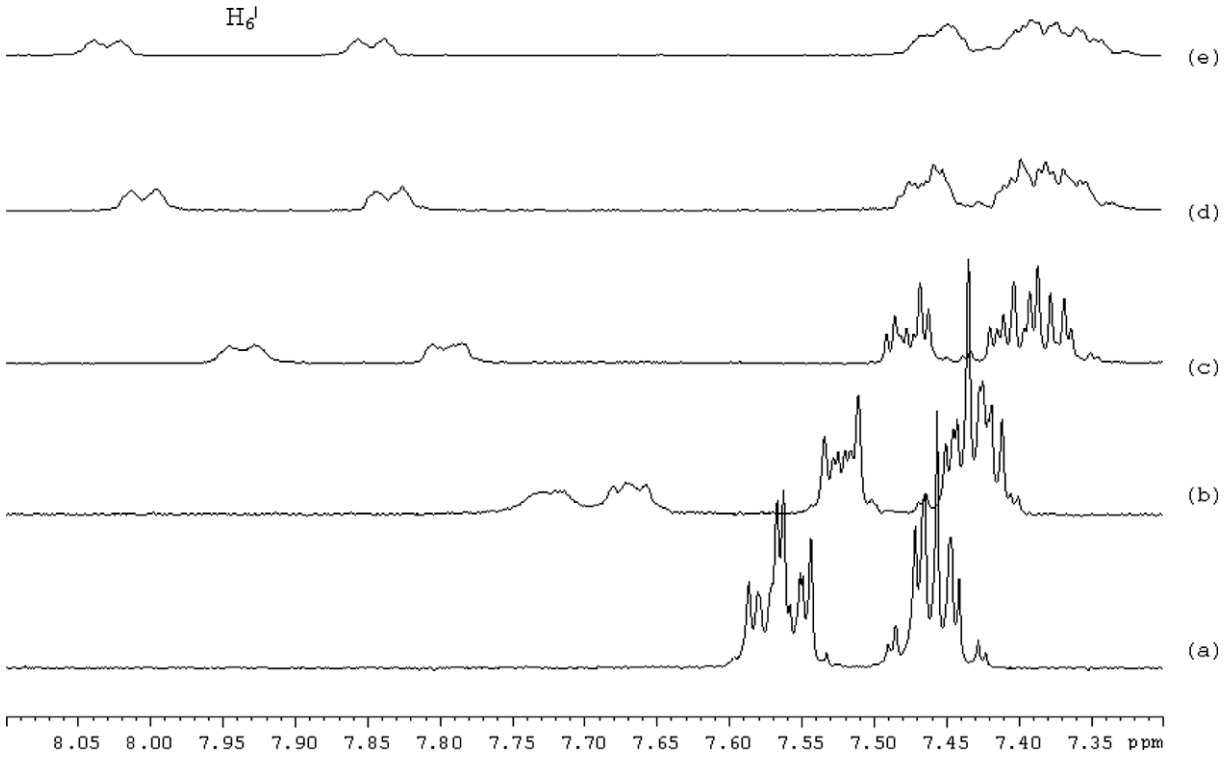

Figure 7. [1]H NMR spectra (400 MHz, methanol-d_4, 23 °C) of the (a) aromatic region of **9** (10 mM) with **1** at (b) 5 mM, (c) 10 mM, (d) 15 mM, and (e) 20 mM.

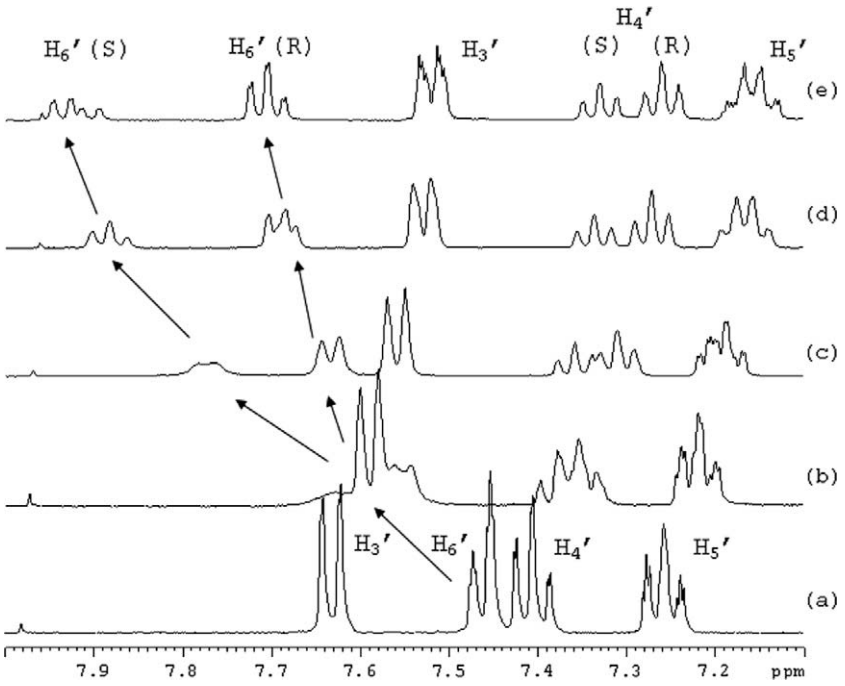

Figure 8. [1]H NMR spectra (400 MHz, methanol-d_4, 23 °C) of the (a) aromatic region of **10** (10 mM) with **1** at (b) 5 mM, (c) 10 mM, (d) 15 mM, and (e) 20 mM.

deshielded in mixtures with **1**, although H2′ and H4′ show the largest changes in chemical shifts. The H2′, H5′, and H6′ resonances exhibit enantiodifferentiation of sufficient magnitude to use for the quantitative determination of enantiomeric purity. The enantiomeric discrimination of H2′ is especially pronounced. The H4′ resonance is unusual in that a small degree of enantiomeric discrimination is observed when the concentration of **1** is 15 mM (Fig. 13c), but raising the concentration of **1** to 20 mM causes the two H4′ resonances to re-coalesce. This contrasts with the behavior for the H2′, H5′, and H6′ resonances, which show the largest enantiomeric discrimination at the highest concentration of **1**.

2.4. Assignment of absolute configuration

Several of the substrates were examined in enantiomerically enriched mixtures to determine whether any empirical trends occur

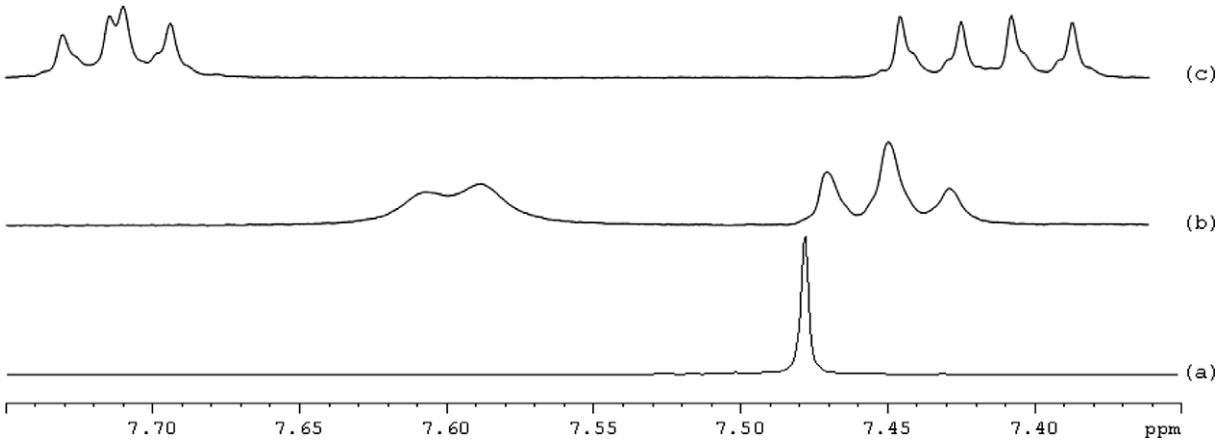

Figure 9. ^{1}H NMR spectra (400 MHz, methanol-d_4, 23 °C) of the (a) aromatic region of **17** (10 mM) with **1** at (b) 5 mM and (c) 10 mM.

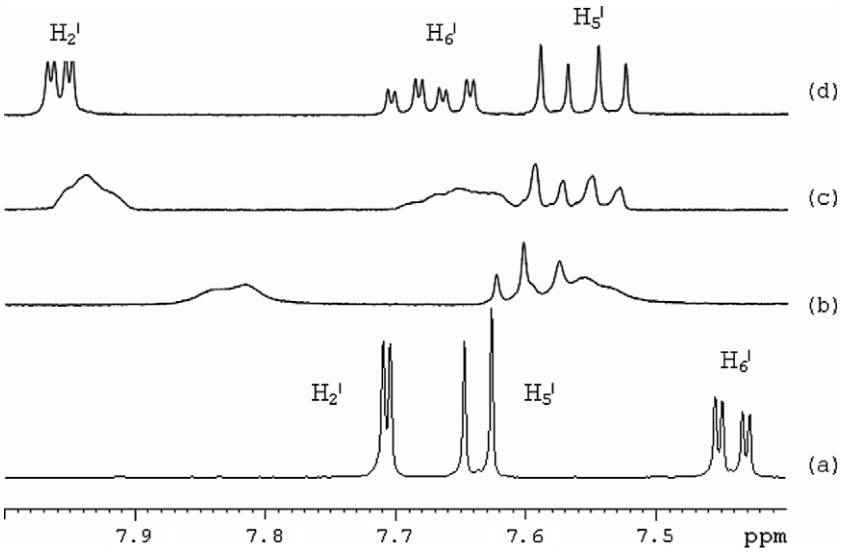

Figure 10. ^{1}H NMR spectra (400 MHz, methanol-d_4, 23 °C) of the (a) aromatic region of **22** (10 mM) with **1** at (b) 5 mM, (c) 15 mM, and (d) 20 mM.

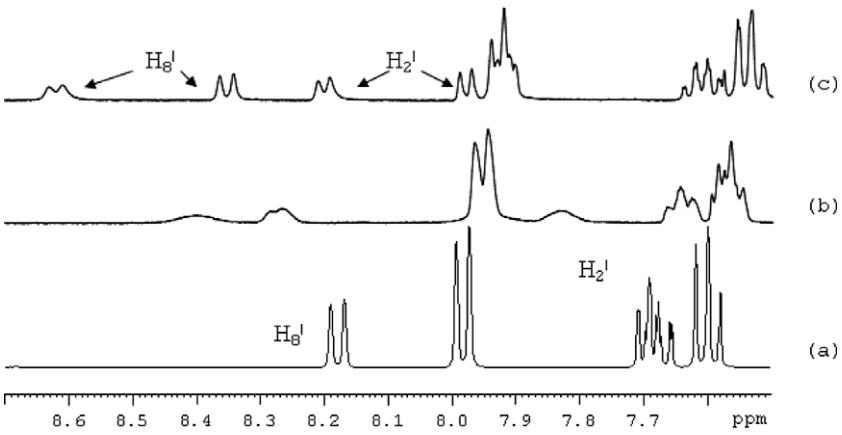

Figure 11. ^{1}H NMR spectra (400 MHz, methanol-d_4, 23 °C) of the (a) aromatic region of **23** (10 mM) with **1** at (b) 5 mM and (c) 15 mM.

that could be used to the assign absolute configuration. Table 1 includes information on which enantiomeric resonance shifted to a higher frequency in the presence of (+)-**1**. Particular groups of substrates exhibit consistent patterns that agree with the absolute configuration.

For β-amino acids with a *meta*- or *para*-substituted aromatic ring or with a heterocyclic aromatic group, the (*R*)-resonance of H_A and the (*S*)-resonances of H'_A and the methine hydrogen atom consistently shift to higher frequency in the presence of (+)-**1**. With one exception (compound **13**), substrates with an *ortho*-substi-

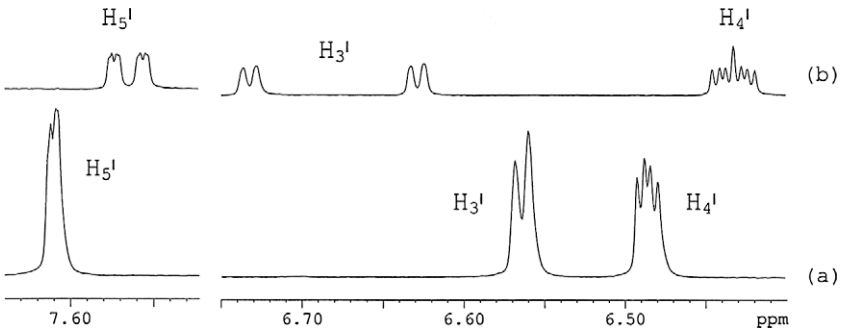

Figure 12. [1]H NMR spectra (400 MHz, methanol-d_4, 23 °C) of the (a) aromatic region of **24** (10 mM) with **1** at (b) 15 mM.

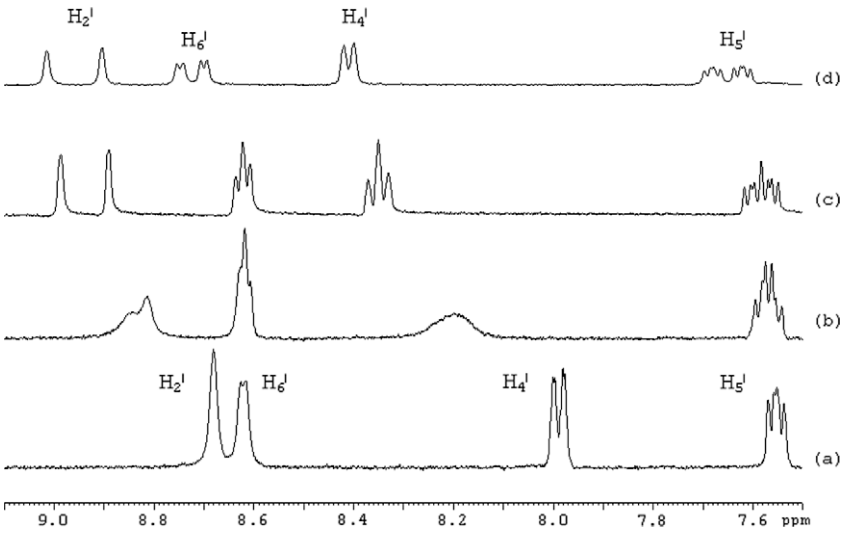

Figure 13. [1]H NMR spectra (400 MHz, methanol-d_4, 23 °C) of the (a) aromatic region of **26** (10 mM) with **1** at (b) 5 mM, (c) 10 mM, and (d) 20 mM.

tuted aromatic ring showed exactly the opposite trend in the shift order of the (*R*)- and (*S*)-resonances for H_A, H'_A and the methine hydrogen atom. We had noted previously in a discussion of changes of the vicinal coupling constants between the methine and α-methylene protons how **13** showed anomalous behavior relative to the other β-amino acids with *ortho*-substituted phenyl rings. For every substrate, the H'_A and methine hydrogen atoms exhibit an order for the (*R*)- and (*S*)-resonances that is the opposite of the order for the H_A resonance. For compound **2**, which has an aliphatic substituent group, the resonance of the (*S*)-enantiomer of H_A is at a higher frequency, whereas the reverse order occurs for the H'_A resonance in the presence of (+)-**1**.

The utilization of empirical trends in the assignment of absolute configuration must always be applied with caution. However, the consistency of the various trends, including observations of the relative magnitudes of enantiomeric discrimination and changes in coupling constants, suggests that there are patterns among the different groups of compounds that can be used to reliably assign the absolute configurations of β-amino acids with unknown configurations. The analysis of suitable model compounds with known configurations is always recommended when assigning absolute configurations using such empirical trends.

3. Conclusions

Compound **1** is an excellent chiral NMR solvating agent for the analysis of β-amino acids. Twenty-seven β-amino acids with a variety of substituent groups that include aliphatic, aromatic, and heterocyclic aromatic moieties all have one resonance or more resonances that exhibit enantiomeric discrimination of a sufficient magnitude to reliably determine the enantiomeric purity. The α-methylene and β-methine hydrogen resonances were often free of interferences and exhibited large enantiomeric discrimination in the presence of **1**. In cases where these resonances were obstructed by solvent or crown ether resonances, other resonances of the β-amino acid are suitable for the analysis of enantiomeric purity. The α-methylene and β-methine resonances of specific groups of compounds also show patterns in the presence of **1** that correlate with the absolute configuration. With proper caution, compound **1** can also be used to assign the absolute configuration of β-amino acids.

4. Experimental

4.1. Reagents

All substrates, methanol-d_4, and the (+)- and (−)-isomer of **1** were obtained from commercial suppliers (BioBlocks, San Diego, CA; Peptech, Burlington, MA, 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 run in methanol-d_4 at ambient probe temperature and calibrated using tetramethylsilane (0.05%) as an internal reference. When necessary, assignments were confirmed using 2D-COSY spectra.

4.3. Procedures for chiral discrimination studies

Solutions of the chiral substrates were prepared using two procedures. If the β-amino acid was sufficiently soluble, a 10 mM solution of the neutral compound was prepared directly in methanol-d_4. If the β-amino acid was not sufficiently soluble in methanol-d_4 to prepare a 10 mM solution, a stoichiometric equivalent of hydrochloric acid was added to form the more soluble ammonium chloride salt. The mixture was enriched with one of the enantiomers when available. An amount of **1** necessary to prepare the desired concentration was then added by weight to the sample in an NMR tube.

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