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Enantiomeric discrimination in the NMR spectra of underivatized amino acids and α-methyl amino acids using (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid

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Abstract

The compound (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid (**I**) is a useful chiral NMR discriminating agent for underivatized amino acids and α-methyl amino acids. The α-methyl amino acids, when mixed with **I** in methanol, are protonated through a reaction with a carboxylic moiety of **I** and associate with the crown ether. Amino acids such as tryptophan, valine, alanine, and phenylglycine can be solubilized at suitable concentrations in methanol through the addition of deuterium chloride. The NMR spectra of mixtures of these amino acids with **I** show enantiomeric discrimination. Addition of ytterbium(III)nitrate to crown-substrate mixtures causes only small shifts and no discernible enhancements in enantiomeric discrimination in the NMR spectra of the substrates.

Keywords: amino acids and derivatives; asymmetry; crown ethers; enantiomeric purity; NMR.

Chiral crown ethers are widely applied as chromatographic or capillary electrophoretic phases, NMR solvating agents, and extracting agents to separate or distinguish optical isomers of protonated, primary amines.[1–6] The compound 2,3:4,5-bis[1,2-(3-phenylnaphtho)-1,6,9,12,15,18-hexaoxacycloeicosa-2,4-diene (**II**) is often used in chromatographic applications and usually the benchmark to which other chiral crown ethers are compared.[7] Compound **II** is less useful in NMR applications because it is sparingly soluble in solvents such as methanol or acetonitrile, which are especially suited for solubilizing protonated amines.[3,6] The compound 1,2:5,6-di-*O*-isopropylidene-3,4-[(*tert*-butylbenzenediyl)bis(oxyethoxy)ethyl-3-mannitol (**III**) is soluble in methanol and was shown to be a more effective and versatile chiral NMR solvating agent than **II**.[3] More recently, (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid (**I**), which is commercially available and is soluble in methanol and acetonitrile, was shown to be even more effective than **II** or **III** as a chiral NMR solvating agent for protonated salts of esters of amino acids, aromatic amines such as 1-(1-naphthyl)ethyl amine and 1-phenylethyl amine, amino alcohols, and aliphatic amines such as 1-cyclohexylethyl amine.[6] Furthermore,

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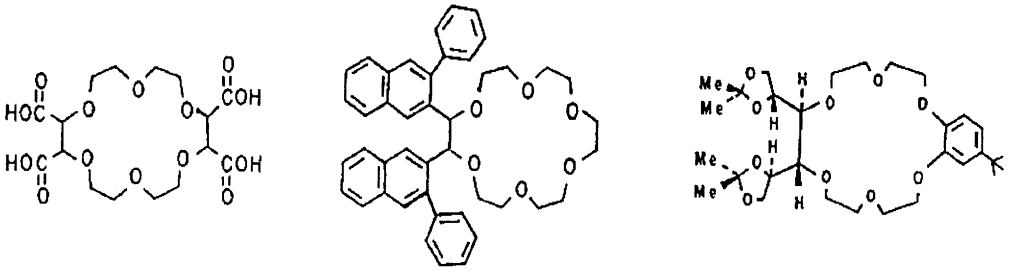
protonation of the amine could be achieved by simply adding **I** to the solution.[6] A neutralization reaction between one of the carboxylic acid moieties of the crown and the primary amine was responsible for protonation of the amine.



I **II** **III**

One of the more important applications of chiral crown ethers is in the determination of enantiomeric purity of amino acids. Analyses using NMR are rarely performed on underivatized amino acids. Instead, ester derivatives are employed to provide solubility in suitable solvents. A chiral crown ether system capable of enantiomerically discriminating underivatized amino acids in NMR spectroscopy would be quite useful. Furthermore, α-methyl amino acids could not be separated using a commercial liquid chromatographic column with **II** as the stationary phase.[7] We wish to report that **I** is an effective chiral NMR discriminating agent for underivatized amino acids and α-methyl amino acids.

Samples for analysis are prepared by weighing the proper amount of **I** and substrate to achieve the desired concentrations (typically 0.025 M of each) and dissolving them in an appropriate volume of methanol-d_4. The α-methyl amino acids examined in this study, α-methylvalinamide and α-methylvaline, are soluble at the desired concentrations, and in mixtures with **I** become protonated through a neutralization reaction with one of the carboxylic acid moieties of the crown. Amino acids such as valine, tryptophan, alanine, and phenylglycine are not soluble in methanol at concentrations of 0.025 M. Adding **I** did not significantly increase the solubility of these amino acids in methanol; however, adding a drop of concentrated deuterium chloride to a 1 ml sample did effect the desired solubility.

Fig. 1 shows the NMR spectrum of α-methylvalinamide in methanol (0.025 M) with and without 1 equivalent of **I**. The substantial downfield shifts of the substrate resonances on addition of **I** indicate that a neutralization reaction between the crown and amino acid has protonated the amine group. Enantiomeric discrimination is observed for the methyl resonances of the valine group, the methine resonance, and the α-methyl resonance of α-methylvalinamide (Table 1). The reversal in the relative shift order for different resonances of the two enantiomers in the presence of **I** (e.g. the methine compared to the α-methyl resonances in Fig. 1b) are consistent with prior reports.[5,6] Such a reversal suggests that the diastereomeric nature of the crown-substrate complexes is more significant at causing enantiomeric discrimination than differences in association constants of the enantiomers with **I**. Enantiomeric discrimination in the spectrum of α-methylvaline (0.025 M) in the presence of **I** is considerably less than that of α-methylvalineamide (Table 1), but is nonetheless significant for the α-methyl resonance. Solubility limitations prevent the use of **II** as an NMR solvating agent with underivatized α-methyl amino acids. No resolution is observed in the NMR spectra of mixtures of α-methylvalinamide and α-methylvaline with **III** in methanol.

Enantiomeric discrimination is observed in the NMR spectra of valine, tryptophan, alanine, and phenylglycine in mixtures with **I** (Table 1). Included in Table 1 are previously reported $\Delta\Delta\delta$ values for the same resonances on the corresponding amino acid methyl esters with **I** in methanol.[6] The $\Delta\Delta\delta$ values are similar, indicating that the methyl ester group is not significant in affecting the extent of enantiodistinction observed with these compounds. In certain instances, mixtures of **I** with

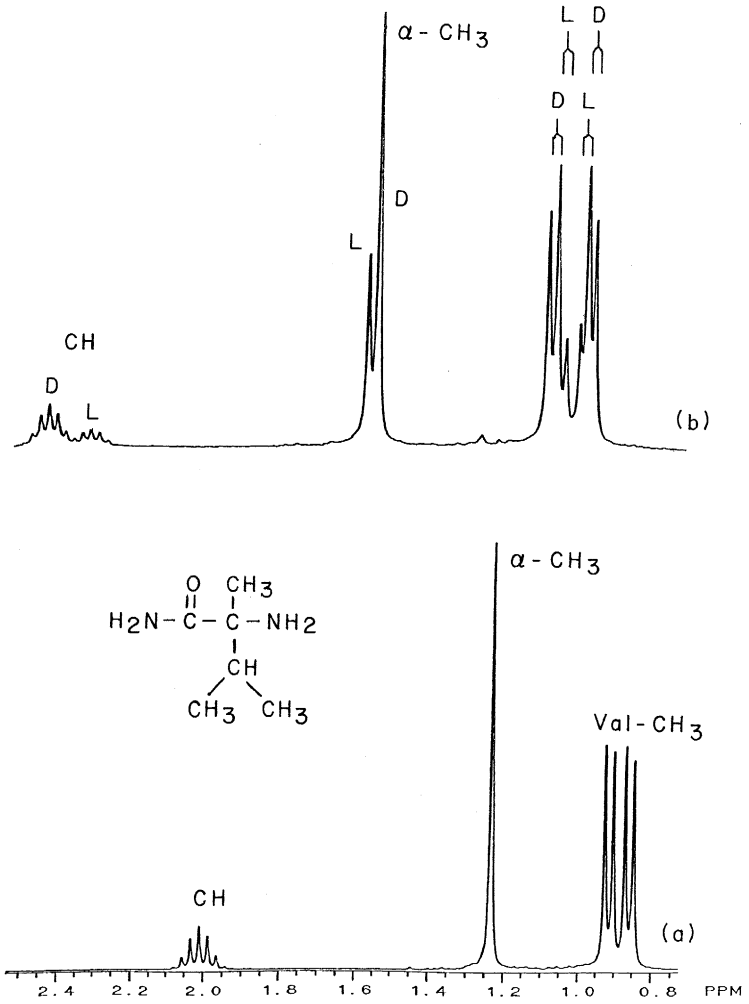


Fig. 1. A portion of the ^{1}H NMR spectrum (300 MHz) of DL-α-methylvalinamide (0.025 M, enriched in D-enantiomer) in methanol-d_4 at 20°C with: (a) no **I**; and (b) 0.0125 M **I**

underivatized amino acids compared to the methyl ester derivatives either eliminates overlapping of the crown and substrate resonances (CH of alanine) or provides enantiomeric discrimination of additional resonances (H_2, H_3, and H_4 of tryptophan). Compound **I** appears to be broadly applicable for the NMR enantiodiscrimination of underivatized amino acids.

In a prior report we showed that Yb(III), when added to mixtures of substrates with **I**, associated with the carboxylic acid moieties of **I** and enhanced the enantiomeric discrimination in the NMR spectra.[6] In contrast, enhancements in enantiomeric discrimination are not observed when Yb(III) is added to mixtures of the underivatized amino acids and α-methyl amino acids with **I** in methanol. A precipitate forms when Yb(III) is mixed with α-methyl amino acids and **I**. Addition of deuterium chloride causes the precipitate to dissolve, but the lanthanide-induced shifts and improvements in enantiomeric discrimination are negligible. The other amino acids do not form a precipitate when mixed with Yb(III) and **I**, but the lanthanide-induced shifts are similarly small. Since the enantiomeric discrimination did not diminish in the presence of Yb(III), it is presumed that the presence of deuterium chloride in the solutions reduces the binding of Yb(III) to the carboxylic acid moieties of **I**.

Table 1
Enantiomeric discrimination ($\Delta\Delta\delta$) in Hz in the ^{1}H NMR spectra (300 MHz) of amino acids (0.025 M) and amino acid methyl esters (0.025 M) with **I** (0.025 M) in methanol-d_4

		Amino acid	Methyl ester
α-methylvaline	CH_3 (val)	4.2	
	CH_3 (α)	11.1	
α-methylvalineamide	CH_3 (val)	5.4	
	CH_3 (val)	8.4	
	CH	35.1	
	CH_3 (α)	8.1	
Alanine	CH_3	9.6	9.3
	CH	30.6	*
Valine	CH_3 (val)	10.5	13.2
	CH_3 (val)	4.5	3.0
Tryptophan	H_1	14.4	14.7
	H_2	2.1	0
	H_3	3.6	0
	H_4	3.6	0
	H_6	34.8	30.0
Phenylglycine	CH	67.8	84.6

*Overlaps with crown resonances

1. Conclusion

Compound **I** is an effective chiral NMR discriminating agent for determining enantiomeric excesses of underivatized amino acids and α-methyl amino acids in methanol. The commercial availability of **I** makes this an especially easy system to use. Amino acids that are not sufficiently soluble in methanol can be dissolved through the addition of deuterium chloride prior to recording the NMR spectrum.

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