Europium (*S,S*)-Ethylenediamine-*N,N'*-disuccinate as a Chiral Lanthanide Shift Reagent for Aqueous Solutions

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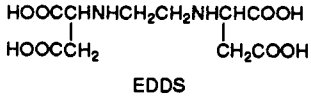
The title compound has been utilized for ^{1}H NMR spectral resolution of enantiomeric amino acids in aqueous solutions. For the α-protons of D,L-phenylglycine, an enantiomeric shift difference, $\Delta\Delta\delta$ = 0.4 ppm, was observed at an Eu(EDDS):substrate ratio of 0.3. Association constants between Eu(EDDS) and D,L-phenylglycine were found to be different for each isomer, K_L = 3.7 and K_D = 6.1. Spectral resolution of other amino acids including alanine, serine, threonine, and phenylalanine was also achieved.

Nuclear magnetic resonance (NMR) spectroscopy is one of the most valuable techniques for the study of molecular structures. The use of NMR spectroscopy is sometimes restricted due to insufficient sensitivity of proton chemical shifts to changes in the chemical and stereochemical environment. Lanthanide shift reagents (LSRs) are effective in reducing such equivalence of nuclei. Alteration of the chemical shifts is caused by (1) transfer of electron spin density through covalent-bond formation from the metal ion to the associated nuclei (contact shift) or (2) magnetic effects of the unpaired electron magnetic moment (pseudocontact shift).

Chiral LSRs were separately introduced by Whitesides[1] and Goering[2] and have been widely used to resolve enantiomers in ^{1}H NMR spectra. The majority of studies involving chiral shift reagents are performed in organic media owing to solubility concerns, and very few aqueous chiral shift reagents are known.

Reuben[3] used a "self-resolution" approach to resolve the enantiomeric nuclei of α-hydroxy carboxylic acids by paramagnetic lanthanide ions. The first genuine aqueous chiral shift reagents were proposed by Peters, who used lanthanide derivatives of (*S*)-[(carboxymethyl)oxy]succinic acid (CMOS) to resolve the enantiomeric nuclei of amino acids and hydroxy carboxylic acids.[4] However, it has been shown that this particular ligand system is not a good choice for shift reagent work, due to the self-association tendency associated with lanthanide–CMOS complexes.[5] Kabuto and Sasaki demonstrated the utility of Eu^{3+}–(*R*)-propylenediaminetetraacetate (PDTA) as a chiral shift reagent and suggested that this type of ligand is promising.[6]

Recently we reported the suitability of the Eu^{3+} derivative of (*S,S*)-ethylenediamine-*N,N'*-disuccinic acid (EDDS) as an aqueous chiral reagent.[7] In this paper, we will provide a full account of the utility of this reagent.



EDDS

Experimental Section

(*S,S*)-Ethylenediamine-*N,N'*-disuccinic acid (EDDS) was prepared by the condensation of two molecules of L-aspartic acid with dibromoethane, using the method of Majer et al.,[8] as modified by Neal and Rose.[9] It was verified by these workers that, under the synthesis condition used, the EDDS ligand forms with retention of configuration at each asymmetric atom of the aspartic acid precursors. EDDS was purified three times by slow acidification of the alkaline solution of EDDS with concentrated hydrochloric acid to pH 3.5. The IR and NMR spectra of the EDDS were identical with those in the literature.[9]

L-Aspartic acid, D- and L-phenylglycine, D- and L-alanine, D- and L-serine, D- and L-threonine, D- and L-phenylalanine, deuterium oxide (99.8 atom % D), and sodium deuterioxide (40 wt %) were obtained from Aldrich and used without further purification. Hexahydrated europium chloride ($EuCl_3 \cdot 6H_2O$) (99.9%) was purchased from Research Chemicals and used as received.

NMR spectra were taken with a Varian EM 390 NMR spectrometer or Jeol JNM-FX 90Q FT NMR spectrometer at room temperature.

A Corning pH meter Model 240 with an automatic temperature compensation (fixed at 25 °C) and a Corning calomel combination electrode were used for pH measurements. Buffer solutions (pH 4.0, pH 7.0, and pH 10.0) were used to calibrate the pH meter before each series of measurements.

For the NMR studies, the Eu(EDDS) and amino acid solutions were separately prepared and combined in appropriate ratios. Eu(EDDS) solutions were prepared by dissolving EDDS in D_2O with appropriate amount of NaOD, which was added to the D_2O solution of $EuCl_3 \cdot 6H_2O$. Each amino acid was dissolved in D_2O with an equivalent amount of NaOD and the appropriate amount of NaCl to keep the final NaCl concentration at 2 M after combining with the Eu(EDDS) solution.

Results and Discussion

The formation constant of Eu(EDDS) is quite high, with log *K* equal to 13.54 having been reported.[10] It has been shown that, above pH 8, the 1:1 lanthanide–EDDS complexes are monomeric and exhibit no oligomerization tendencies.[11] The three water molecules bound at the

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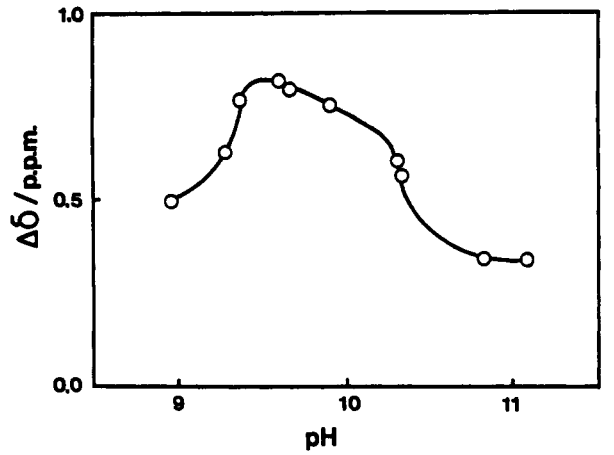

Figure 1. pH dependence of induced chemical shift (Δδ): 0.1 M L-phenylglycine, 0.03 M Eu(EDDS), 2 M NaCl. The data were referenced to *tert*-butyl alcohol.

inner coordination sphere of the lanthanide ion are replaceable, and the existence of ternary complexes has been demonstrated.[12] These trends are exactly what would be required for a well-behaved chiral shift reagent.

The pH dependence of the induced chemical shift by the Eu(EDDS) was first investigated. Because of the simplicity of the NMR spectra, L-phenylglycine was chosen as a substrate to be studied. The resonance of the α-proton of L-phenylglycine was shifted upfield by Eu(EDDS) and was found to be dependent on the solution pH (Figure 1). The induced chemical shift, Δδ, was observed in the pH range 9–11 and has a bell-shaped profile exhibiting a maximum at pH 9.5–10. The sharp decreases in Δδ on the acidic side (pH ~9.4) and the basic side (pH ~10.5) probably correspond to the oligomerization[11] and the hydroxo complex formation,[11] respectively, of the Eu(EDDS) complex. Because the solutions were turbid in the pH range below 9 and over 11, all the work using Eu(EDDS) could only be conducted in the pH range 9–11.

The ^{1}H NMR spectra of 0.1 M D_2O solutions of D-, L-, and D,L-phenylglycine in the presence and absence of 0.03 M Eu(EDDS) are shown in Figure 2. A larger upfield shift of the α-proton resonances was observed for the the D isomer relative to the L isomer. The data in Figure 2 signify total resolution of the enantiomeric proton resonances in racemic phenylglycine, for which ΔΔδ equaled 0.4 ppm at an Eu(EDDS):substrate ratio of 0.3. The magnitude of the lanthanide-induced shift was found to increase in a regular fashion with the Eu(EDDS):substrate ratio. This well-behaved nature of the Eu(EDDS)-induced shifts implies the formation of a single type of Eu(EDDS)(adduct) species, which is in agreement with other spectroscopic work, where it was noted that the chirality of Eu(EDDS) adducts was not dependent on the concentration of substrate used.[12] At the same time, the degree of line broadening for the α-proton of D-phenylglycine was found to be greater upon association with Eu(EDDS) than that of the L isomer. For D-phenylglycine, the addition of 1 equiv of Eu(EDDS) led to such line broadening that the position of the α-proton could not be determined.

There are two mechanisms that can lead to enantiomeric resolution in the NMR spectra of chiral substrates in the presence of chiral shift reagents. The first is a difference in the equilibrium constant for association between the enantiomers and the shift reagent (stereoselectivity). Secondly, the geometry of the complex between each enantiomer and the shift reagent may be different. Different geometries then can bring different distance and angle terms into the pseudocontact shift equation. Either one or both mechanisms have been shown to be important for nitrogen- and oxygen-containing substrates in the presence of the tris-chelates.[13]

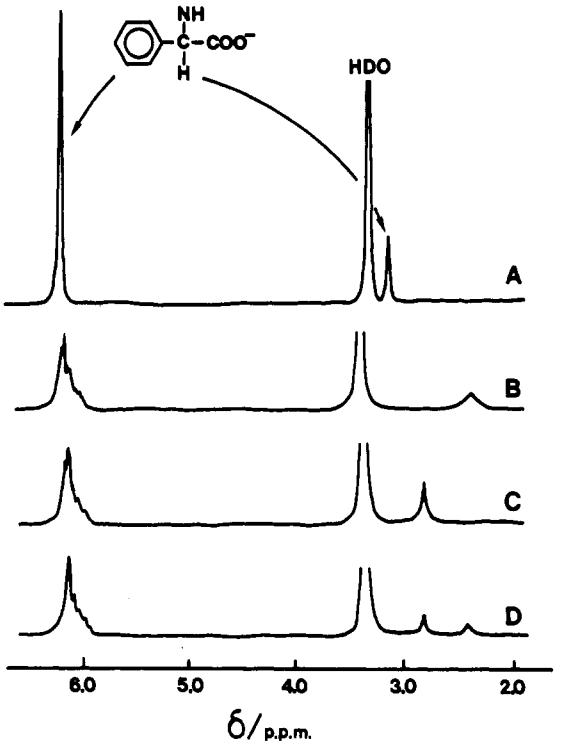

Figure 2. ^{1}H NMR (90 MHz) spectra of 0.1 M phenylglycine in the presence (traces B–D) and absence (trace A) of 0.03 M Eu(EDDS) at pH 9.5 in D_2O solution. Spectra are shown for D-phenylglycine (trace B), L-phenylglycine (trace C), and D,L-phenylglycine (trace D). *tert*-Butyl alcohol (at 0.0 ppm) was used as a reference.

The NMR shift reagent data permitted determination of the formation constants for the ternary complexes. Assuming that only 1:1 Eu(EDDS)–phenylglycine complexes are formed with the equilibrium constant of K, the following equation is described:[14]

$$S_0 = \left(L_0 + \frac{[LS]^2}{S_0}\right)\frac{\Delta_b}{\Delta\delta} - \left(L_0 + \frac{1}{K}\right) \qquad (1)$$

where Δ_b is the bound shift of the complex and L_0 and S_0 represent the total concentration of lanthanide shift reagent and substrate, respectively; δ_{obsd} and δ_0 are the observed chemical shifts of a nucleus in the presence and absence of shift reagent, respectively. At small K values, the term $[LS]^2/S_0$ must be small in comparison to $[L_0]$, and this results in simplification of eq 1 to the following relationship:

$$S_0 = \frac{L_0\Delta_b}{\Delta\delta} - \left(L_0 + \frac{1}{K}\right) \qquad (2)$$

or

$$\frac{1}{\Delta\delta} = \frac{S_0 + \frac{1}{K}}{\Delta_b}\frac{1}{L_0} + \frac{1}{\Delta_b} \qquad (3)$$

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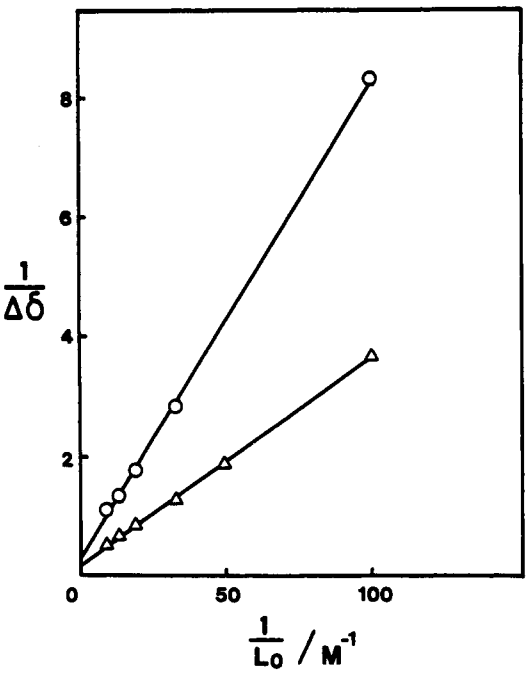

Figure 3. Plots of $1/\Delta\delta$ vs $1/L_0$ for L-phenylglycine (O) and D-phenylglycine (Δ).

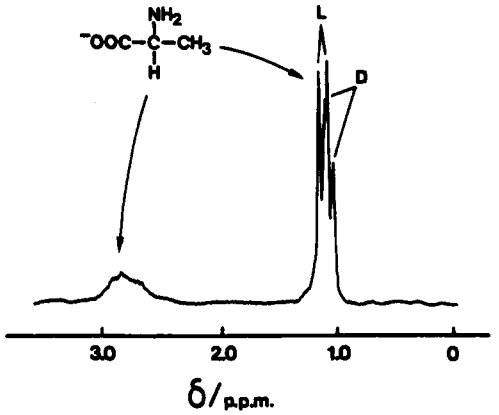

Figure 4. ^{1}H NMR (90 MHz) spectra of 0.1 M D,L-alanine in the presence of 0.03 M Eu(EDDS) at pH 11.0: 2.0 NaCl. The data were referenced to HDO (4.65 ppm).

This equation indicates that if the reciprocal of the induced shift $\Delta\delta$ is plotted against the reciprocal of the shift reagent concentration L_0 (at constant substrate concentration), the experimental points should give a straight line. The intercept of the line affords the bound shift, and the slope yields the association constant.

By plotting $1/\Delta\delta$ vs $1/L_0$ for our system (Figure 3), we calculated the association constants and the bound shifts for the Eu(EDDS)(phenylglycine) system. It was found that the association constant for the D isomer (K_D = 6.1) was greater than that for the L isomer (K_L = 3.7). The bound shift for the D isomer (Δ_{bD} = 7.5) was also greater than that for the L isomer (Δ_{bL} = 4.6). These results indicate that the ternary complex formed between the D isomer and Eu(EDDS) is probably geometrically more favorable than that formed with the L isomer, which results in more stability for the D-isomer complexes.

The enantiomeric separation of the ^{1}H NMR signals of some other chiral amino acids was also demonstrated, and the results are summarized in Table I.

The ^{1}H NMR spectrum of alanine, which is the most structurally similar to phenylglycine, was resolved with Eu(EDDS), as seen in Figure 4. The best separation was observed for the β-protons and showed a $\Delta\Delta\delta$ value of 0.08 ppm at 0.5 equiv of Eu(EDDS). Unlike phenylglycine, the signal of the α-proton of alanine was a quartet, and due to line broadening it was impossible to locate its exact position in the presence of Eu(EDDS).

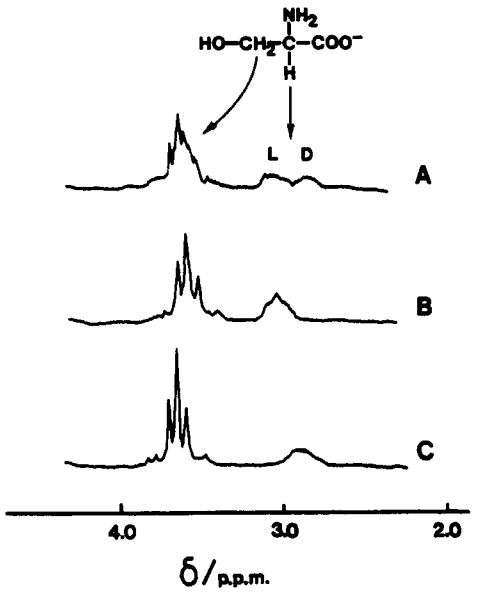

Figure 5. ^{1}H NMR (90 MHz) spectra of 0.1 M serine in the presence of 0.1 M Eu(EDDS) at pH 10.9: 2.0 NaCl M. The data were referenced to HDO (4.65 ppm). Spectra are shown for D,L-serine (trace A), L-serine (trace B), and D-serine (trace C).

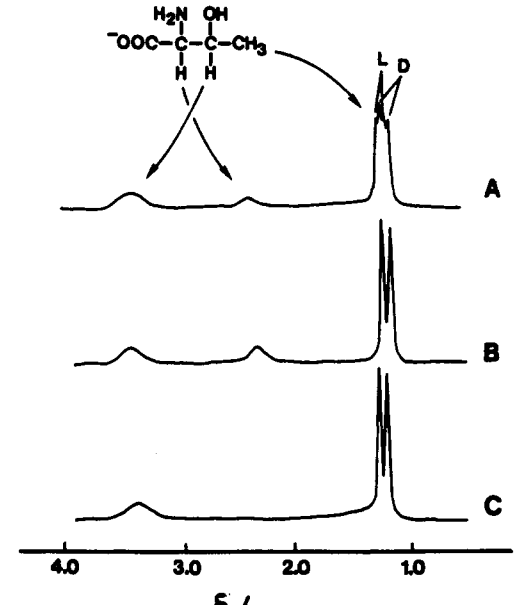

Figure 6. ^{1}H NMR (90 MHz) spectra of 0.1 M threonine in the presence of 0.03 M Eu(EDDS) at pH 10.1: 2.0 M NaCl. The data were referenced to HDO (4.65 ppm). Spectra are shown for D,L-threonine (trace A), D-threonine (trace B), and L-threonine (trace C).

The enantiomeric proton resonance lines of serine, which was a hydroxy group attached to the methyl group of the alanine structure, were separated in the NMR spectrum (shown in Figure 5). In the presence of 0.1 equiv of Eu(EDDS), the α-proton signal showed a $\Delta\Delta\delta$ of 0.19 ppm, with the D-isomer resonance being located at higher field. The β-proton signal showed a $\Delta\Delta\delta$ of 0.033 ppm, with the L-isomer resonance being located at higher field.

In the case of threonine, which has a methyl group attached to serine, a large separation ($\Delta\Delta\delta$ = 0.27 ppm) was observed for the α-protons after addition of 0.1 equiv of

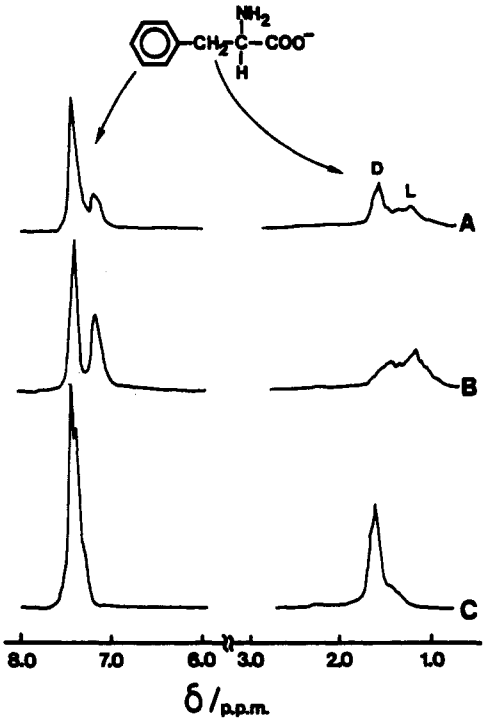

Figure 7. ^{1}H NMR (90 MHz) spectra of 0.1 M phenylalanine in the presence of 0.05 M Eu(EDDS) at pH 10.1: 2.0 M NaCl. The data were referenced to HDO (4.65 ppm). Spectra are shown for D,L-phenylalanine (trace A), L-phenylalanine (trace B), and D-phenylalanine (trace C).

Eu(EDDS), as seen in Figure 6. The resonance of the D isomer was shifted more than that of the L isomer. No enantiomeric resolution of the β-proton was observed, although the $\Delta\delta$ value was found to be 0.56 ppm. A clear separation was achieved for the γ-proton with 0.3 equiv of Eu(EDDS), yielding $\Delta\Delta\delta$ = 0.032 ppm.

In the presence of 0.5 equiv of Eu(EDDS), the signals for the α-protons of phenylalanine could not be located due to line broadening and overlapping. The β-proton was resolved, however, having a $\Delta\Delta\delta$ of 0.36 ppm (Figure 7). A distinct difference was found in the phenyl proton signals. For the D isomer, these consisted of two closely located signals at 2.77 and 2.71 ppm. For the L isomer, the two signals were further split and were located at 2.71 and 2.48 ppm.

Table I. Spectral Resolution of the 90-MHz ^{1}H NMR Signals of Enantiomers in the Presence of Eu(EDDS)

substrate[a]	mole ratio[b]	nucleus	$\Delta\Delta\delta$,[c] ppm	larger $\Delta\delta$[d]
alanine	0.3	β	0.05	D
	0.5	β	0.08	D
serine	0.1	α	0.19	D
		β	0.03	L
threonine	0.1	α	0.27	D
		β	0	-
	0.3	γ	0.03	D
phenylalanine	0.5	β	0.36	L

[a] Substrate concentration = 0.1 M, in the presence of an equivalent amount of NaOD; pH 10–11; [NaCl] = 2 M. [b] Eu(EDDS): substrate. [c] HDO signal used as an internal standard. Measurements at room temperature. [d] Enantiomer in the higher field.

As shown above, spectral resolution of enantiomeric amino acids was achieved, but was somewhat limited by line broadening in those systems where the spectra were taken at 90 MHz. This can be attributed to the insoluble europium oxides that are present as colloids in the sample. Since the K values of Eu(EDDS) to amino acids are rather small compared to usual lanthanide shift reagents for organic solution, the higher concentration of Eu(EDDS) is required to obtain reasonable spectral resolution, which consequently causes more line broadening. However, it should be possible to achieve complete resolution of the enantiomers at a higher frequency such as 500 MHz with much lower concentrations of the shift reagent and, hence, with less line broadening.

Conclusion

The Eu(EDDS) system was shown to be a useful chiral shift reagent for aqueous work, as long as the solution pH was maintained between 9 and 11. In the presence of Eu(EDDS), the ^{1}H NMR spectra of several chiral amino acids were successfully obtained. It was also shown that the induced chemical shift differences were due to differences not only in the adduct geometry but also in the association constants of the ternary complexes formed between Eu(EDDS) and substrates.

Registry No. EDDS, 20846-91-7; Eu(EDDS), 130856-12-1; (*R*)-H_2NCHPhCOOH, 875-74-1; (*S*)-H_2NCHPhCOOH, 2935-35-5; (±)-H_2NCHPhCOOH, 2835-06-5; H-DL-Ala-OH, 302-72-7; H-DL-Ser-OH, 302-84-1; H-Ser-OH, 56-45-1; H-D-Ser-OH, 312-84-5; H-DL-Thr-OH, 80-68-2; H-D-Thr-OH, 632-20-2; H-Thr-OH, 72-19-5; H-DL-Phe-OH, 150-30-1; H-Phe-OH, 63-91-2; H-D-Phe-OH, 673-06-3.