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Europium(III)-*N,N′*-Ethylenebis(L-amino acid) Complexes as New Chiral NMR Lanthanide Shift Reagents for Unprotected α-Amino Acids in Neutral Aqueous Solution

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Three *N,N′*-ethylenebis(L-amino acid) ligands have been obtained simultaneously with three α,α′-(1,4-piperazinediyl)bis[(*S*)-alkanoic acid] and four *N,N′*-ethylenedipeptide products, by reacting a mixture of L-histidine methyl ester and L-aspartic acid dimethyl ester with glyoxal in the presence of sodium cyanotrihydroborate in methanol. Europium(III) complexes with *N,N′*-ethylenebis(L-amino acid) ligands were useful as chiral NMR shift reagents for some unprotected natural α-amino acids as substrates in neutral aqueous solution, as characterized by large enantiomeric shift differences and unbroadened signal shapes on high-resolution NMR spectroscopy. In addition, the acid-dissociation constants of six bis(amino acid) ligands and the stability constant of the europium(III) complex with *N,N′*-ethylenedi(L-histidine) were obtained by potentiometric titration.

Most lanthanide-based chiral NMR shift reagents, such as tris[3-(2,2,3,3,4,4,4-heptafluoro-1-hydroxybutylidene)-(+)-camphorato]europium(III) and tris[3-(2,2,2-trifluoro-1-hydroxyethylidene)-(+)-camphorato]europium(III), have been used to determine the optical purities of organic compounds in organic media (e.g., chloroform and dichloromethane).[1] Some water-soluble reagents have also been investigated. Pioneer work was done by Reuben in 1979,[2] in which a self-resolution approach was introduced to resolve the resonance of enantiomeric mixtures and of enantiotopic protons. Thereafter, water-soluble europium complexes were introduced to resolve the enantiomeric nuclei of chiral-unprotected α-amino acid, and were found to be useful in alkaline or acidic aqueous solutions. For example, (*S*)-2-carboxymethoxysuccinatoeuropium(III) complex,[3] (*R*)-*N,N,N′,N′*-propylenediaminetetraacetatoeuropium(III) complex[4] and *N,N′*-ethylenedi(L-asparto)europium(III) complex[5] were used to separate the proton signals on the enantiomeric position of unprotected α-amino acids, like alanine at pH = 3, 11 and 9–11, respectively. Recently, Feringa et al.[6] synthesized some chiral disuccinate ligands and showed that their europium(III) complexes are useful for determination of the enantiomeric excess of several unprotected α-amino acids and α-alkylated amino acids; this study was also done at pH = 9–10. Even more recently, Kabuto et al. first reported a chiral NMR shift reagent, $[EuCl_2\{(R)\text{-tppn}\}]^+$ where (*R*)-tppn = (*R*)-*N,N,N′,N′*-tetrakis(2-pyridylmethyl)propylenediamine, which is very useful in neutral aqueous solution.[7] The advantage of using a neutral pH range for this kind of study is that a possible undesired change of substrates under alkaline conditions employed for other shift reagents is avoided, and that the relatively weak interaction of the substrate with the europium(III) complex results in sharp NMR signals. Signal broadening caused by the europium(III) complex is quite a serious problem. It was just in 1999 that Kabuto et al. discovered that a diamagnetic lanthanum(III) complex with (*R*)-tppn resolves the enantiomer signal of α-amino acid in a weak acidic solution in which the signal separation is smaller, but clearer, than the corresponding europium(III) complex.[8]

We are interested in the chiral-recognition property by europium(III) complex with *N,N′*-ethylenebis(L-amino acid) in which two chiral centers exist in the outside ethylenediamine. It is also expected that several combinations of coordination sites (imidazolyl nitrogen, carboxylic oxygen, amine nitrogen etc.) to europium(III) center brings some new property other than (*R*)-tppn and *N,N′*-ethylenedi(L-aspartic acid). In this paper, we describe the synthesis of various bis(L-amino acid) derivatives as multidentate ligands for europium(III) ions from L-histidine methyl ester and L-aspartic acid dimethyl ester using an extension of a method we reported earlier.[9] The compounds described here are summarized in Chart 1 with their abbreviations. We show the usefulness of europium(III) complexes with *N,N′*-ethylenebis(L-amino acid) as chiral NMR shift reagents for unprotected α-amino acids in neutral aqueous solution by large enantiomeric shift differences and unbroadened signal shapes, even on high-resolution NMR spectroscopy. We also report the acid dissociation constants of six bis(L-amino acid) derivatives among these compounds and the stability constant of an europium(III) complex with *N,N′*-ethylenedi(L-histidine).

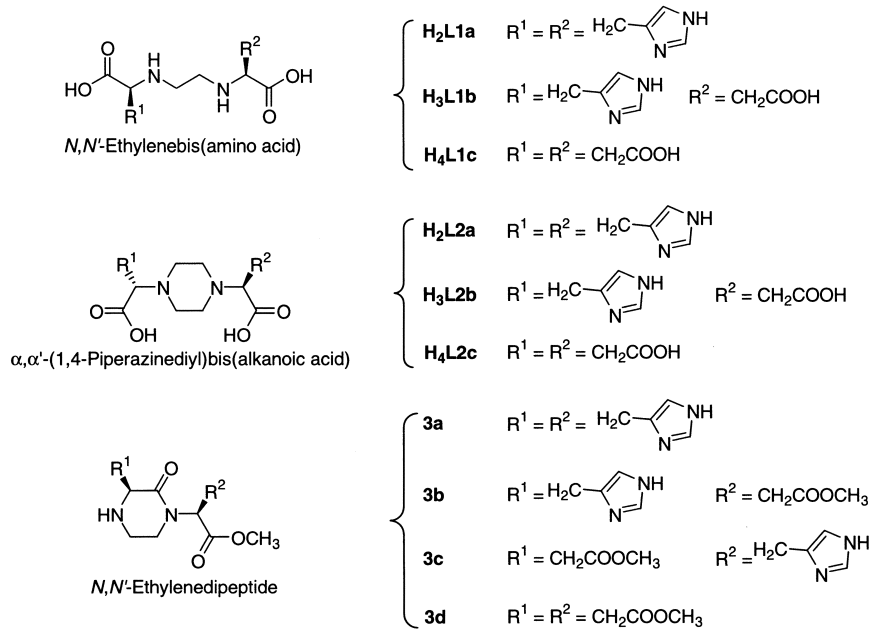
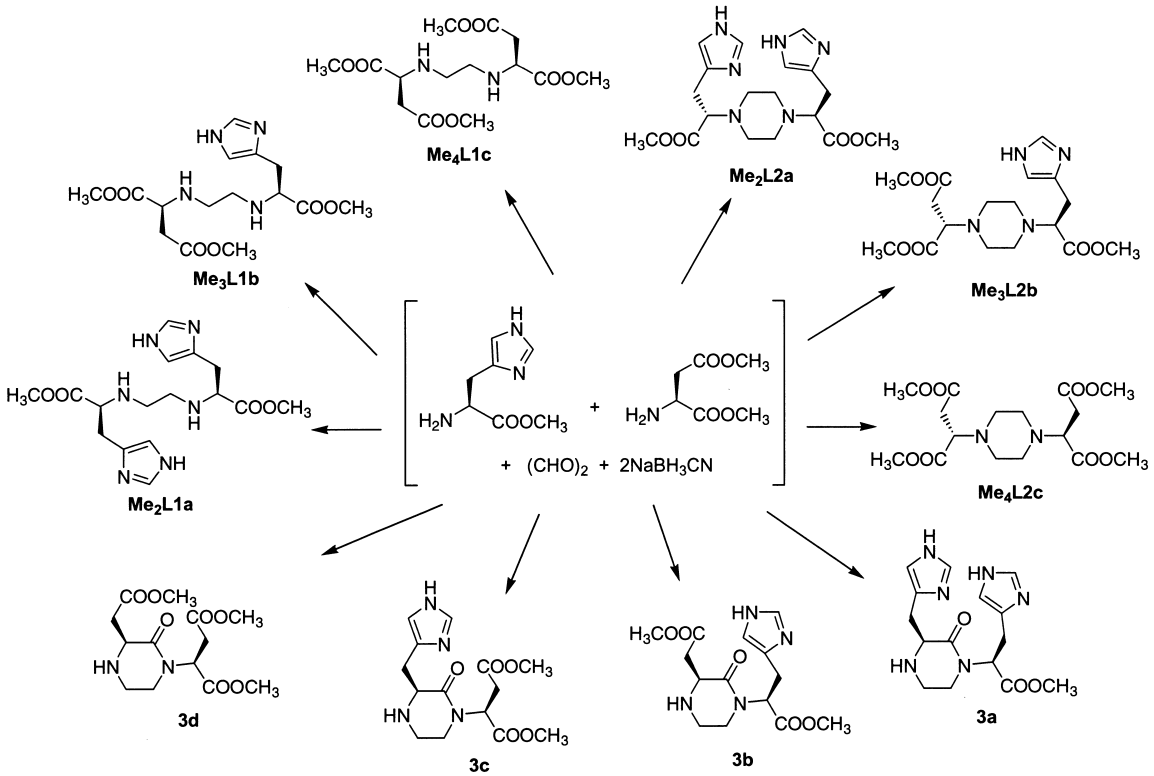


Chart 1.

Experimental

Materials. All starting reagents and solvents were commercially available and used without further purification.

Synthesis and Characterization of Pro-ligands. The reaction scheme and the abbreviations of pro-ligands are summarized in Scheme 1. After a methanol solution of 40% glyoxal aqueous solution (4.35 g, 30 mmol) was added to a methanol solution of L-aspartic acid dimethyl ester hydrochloride (5.93 g, 30 mmol) at 0 °C, the solution was stirred for half an hour. A methanol solution of L-histidine methyl ester dihydrochloride (7.26 g, 30 mmol) and triethylamine (3.04 g, 30 mmol) was added at 0 °C, and the stirring was continued for another half an hour. A methanol solution of sodium cyanotri-



Scheme 1.

hydroborate (3.77 g, 60 mmol) was added to the solution at 0 °C. The resulting solution was stirred for 12 h while the temperature was raised gradually to room temperature. After removal of a white precipitate by filtration, the filtrate was evaporated. The crude oily residue was separated into three parts by basic silica-gel column chromatography (Fuji Silysia Chromatorex NH-DM1020, chloroform/methanol = 30/1–3/1), where the first portion consisted of **Me_4L1c**, **Me_4L2c** and **3d**, the second of **Me_3L1b**, **Me_3L2b**, **3b** and **3c** and the last of **Me_2L1a**, **Me_2L2a** and **3a**. Each portion was further separated into products, except for **3b** and **3c**, by silica gel (Fuji Silysia BW-820MH, chloroform/methanol = 60/1, 20/1 and 5/1–1.5/1, respectively). The dipeptides **3b** and **3c** were separated by silica gel (toluene/ethyl acetate/methanol = 5/4/4). Further purification was achieved by gel filtration (Sephadex LH-20, methanol).

N,N′-ethylenedi(L-histidine methyl ester) **Me_2L1a**[10,11] and *N,N′*-ethylenedipeptide **3a**[9] were identified according to published data. The dipeptides, **3b** and **3c**, were distinguished by the chemical shifts of β-protons located in C-terminal amino acid because of the down-magnetic field shift by anisotropic effect of the amide group. The obtained product yields are given in Table 1.

Table 1. Each Product Yields Obtained

Product	Yield/%
Me_2L1a	18.5
Me_3L1b	17.3
Me_4L1c	24.9
Me_2L2a	8.6
Me_3L2b	10.1
Me_4L2c	4.6
3a	12.7
3b	3.4
3c	2.8
3d	trace
Total yield from Asp–$(OMe)_2$	63.1
Total yield from His–OMe	73.4

N^{α}-(2-L-Aspartoethyl)-L-histidine trimethyl Ester (Me_3-L1b). Found: C, 47.15; H, 7.08; N, 14.63%. Calcd for $C_{15}H_{24}N_4O_6$•1.5H_2O: C, 46.99; H, 7.10; N, 14.61%. ^{1}H NMR ($CDCl_3$) δ 2.58 (m, 2H, en), 2.68 (dd, 1H, J = 16.0, 7.0 Hz, Asp-β-CH_2), 2.79 (dd, 1H, J = 16.1, 5.6 Hz, Asp-β-CH_2), 2.82 (bd, 2H, J = 8.3 Hz, en), 2.87 (dd, 1H, J = 14.6, 7.5 Hz, His-β-CH_2), 3.01 (dd, 1H, J = 14.9, 4.9 Hz, His-β-CH_2), 3.53 (dd, 1H, J = 7.8, 4.9 Hz, His-α-CH), 3.66 (dd, 1H, J = 6.8, 5.6 Hz, Asp-α-CH), 3.68 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 6.79 (s, 1H, His-δ-CH), 7.52 (d, 1H, J = 1.0 Hz, His-ε-CH); IR ($CHCl_3$) 1738 cm^{-1} for $\nu_{C=O}$; $[\alpha]_D$ −18.9° (MeOH); FAB MS *m/z* 357 ($[M + H]^+$).

***N,N′*-Ethylenebis(L-aspartic acid dimethylester) (Me_4-L1c).** Found: C, 46.98; H, 6.96; N, 7.72%. Calcd for $C_{14}H_{24}N_2O_8$•0.5H_2O: C, 47.05; H, 7.05; N, 7.84%. ^{1}H NMR ($CDCl_3$) δ 2.60 (d, 2H, J = 7.7 Hz, en), 2.66 (dd, 2H, J = 15.9, 6.9 Hz, β-CH_2), 2.76 (dd, 2H, J = 15.9, 6.0 Hz, β-CH_2), 2.80 (d, 2H, J = 7.7 Hz, en), 3.67 (dd, 2H, J = 6.7, 6.2 Hz, α-CH), 3.69 (s, 6H, 2OCH_3), 3.74 (s, 6H, 2OCH_3); IR ($CHCl_3$) 1737 cm^{-1} for $\nu_{C=O}$; $[\alpha]_D$ −29.4° (MeOH); FAB MS *m/z* 349 ($[M + H]^+$).

2,2′-(1,4-Piperazinediyl)bis[(*S*)-3-(4-imidazolyl)propionic Acid Methyl Ester] (Me_2L2a). Found: C, 53.60; H, 6.86; N, 20.67%. Calcd for $C_{18}H_{26}N_6O_4$•0.75H_2O: C, 53.52; H, 6.86; N, 20.80%. ^{1}H NMR ($CDCl_3$): δ 2.61 (bd, 4H, J = 7.8 Hz, en), 2.81 (bd, 4H, J = 7.8 Hz, en), 2.96 (dd, 2H, J = 14.9, 6.8 Hz, β-CH_2), 3.08 (dd, 2H, J = 15.1, 7.8 Hz, β-CH_2), 3.53 (dd, 2H, J = 7.3, 7.3 Hz, α-CH), 3.69 (s, 6H, 2OCH_3), 6.83 (s, 1H, δ-CH), 7.54 (d, 1H, J = 1.0 Hz, ε-CH); IR ($CHCl_3$): 1732 cm^{-1} for $\nu_{C=O}$; $[\alpha]_D$ −35.8° (MeOH); FAB MS *m/z* 391 ($[M + H]^+$).

(*S*)-2-{4-[(*S*)-1-Carboxy-2-(4-imidazolyl)ethyl]-1-piperazinyl}butyric Acid (Me_3L2b). Found: C, 52.94; H, 6.91; N, 14.52%. Calcd for $C_{17}H_{26}N_4O_6$•0.2H_2O: C, 52.90; H, 6.89; N, 14.51%. ^{1}H NMR ($CDCl_3$): δ 2.54 (bd, 4H, J = 12.7 Hz, en), 2.63 (dd, 1H, J = 16.0, 6.2 Hz, Asp-β-CH_2), 2.75 (bd, 4H, J = 8.5 Hz, en), 2.86 (dd, 1H, J = 16.1, 8.8 Hz, Asp-β-CH_2), 2.92 (dd, 1H, J = 14.9, 6.6 Hz, His-β-CH_2), 3.06 (dd, 1H, J = 14.9, 8.1 Hz, His-β-CH_2), 3.51 (dd, 1H, J = 7.8, 6.8 Hz, His-α-CH), 3.67 (s, 3H, OCH_3), 3.68 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 3.75 (dd, 1H, J = 8.8, 6.3 Hz, Asp-α-CH), 6.81 (s, 1H, His-δ-CH), 7.53 (d, 1H, J = 1.0 Hz, His-ε-CH); IR ($CHCl_3$): 1733 cm^{-1} for $\nu_{C=O}$; $[\alpha]_D$ −60.3° (MeOH); FAB MS *m/z* 383 ($[M + H]^+$).

2,2′-(1,4-Piperazinediyl)di[(*S*)-succinic acid] Tetramethyl Ester (Me_4L2c). Found: C, 51.21; H, 7.06; N, 7.47%. Calcd for $C_{16}H_{26}N_2O_8$: C, 51.33; H, 7.00; N, 7.48%. ^{1}H NMR ($CDCl_3$): δ 2.47 (bd, 4H, J = 7.4 Hz, en), 2.60 (dd, 2H, J = 16.1, 6.1 Hz, β-CH_2), 2.69 (bd, 4H, J = 7.4 Hz, en), 2.84 (dd, 2H, J = 16.1, 8.8 Hz, β-CH_2), 3.67 (s, 6H, 2OCH_3), 3.71 (dd, 2H, overlapped signals, α-CH), 3.72 (s, 6H); IR ($CHCl_3$): 1734 cm^{-1} for $\nu_{C=O}$; $[\alpha]_D$ −93.6° (MeOH); FAB MS *m/z* 375 ($[M + H]^+$).

Compounds **3b**, **3c**, and **3d** were identified by ^{1}H and 2D (H-H) COSY NMR, IR, and HRFAB MS.

(*S*)-2-[(*S*)-3-(4-Imidazolylmethyl)-2-oxo-1-piperazinyl]succinic Acid Dimethyl Ester (3b). ^{1}H NMR ($CDCl_3$) δ 2.95 (dd, 1H, J = 17.1, 8.5 Hz, Asp-β-CH_2), 3.05 (o.s., 1H, en), 3.10 (o.s., 1H, His-β-CH_2), 3.14 (o.s., 1H, en), 3.17 (o.s., 1H, His-β-CH_2), 3.19 (dd, 1H, J = 17.1, 5.4, Asp-β-CH_2), 3.37 (ddd, 1H, J = 11.2, 3.7, 2.4 Hz, en), 3.46 (ddd, 1H, J = 10.9, 10.9, 4.4 Hz, en), 3.70 (s, 3H, OCH_3), 3.70 (o.s., 1H, His-α-CH), 3.74 (s, 3H, OCH_3), 4.68 (dd, 1H, J = 8.8, 5.4 Hz, Asp-α-CH), 6.87 (s, 1H, His-δ-CH), 7.58 (d, 1H, J = 0.7 Hz, His-ε-CH); IR ($CHCl_3$): 1737 (ester) and 1645 (amide) cm^{-1} for $\nu_{C=O}$; HRFAB MS *m/z* 325.1512. Calcd for $C_{14}H_{21}N_4O_5$ ($[M + H]^+$) 325.1512.

(*S*)-2-[(*S*)-3-Carboxymethyl-2-oxo-1-piperazinyl]-3-(4-imidazolylmethyl)]propionic Acid Dimethyl Ester (3c). ^{1}H NMR ($CDCl_3$) δ 2.64 (dd, 1H, J = 17.1, 8.3 Hz, Asp-β-CH_2), 2.92 (dd, 1H, J = 17.1, 3.6 Hz, Asp-β-CH_2), 2.95 (ddd, 1H, overlapped signals (o.s.) en), 3.10(ddd, 1H, J = 15.2, 3.9, 2.5 Hz, en), 3.15 (o.s., 1H, en), 3.17 (dd, 1H, J = 15.3, 10.5 Hz, His-β-CH_2), 3.34 (dd, 1H, J = 15.4, 5.2 Hz, His-β-CH_2), 3.50 (ddd, 1H, J = 11.1, 11.1, 4.3 Hz, en), 3.67 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 3.78 (dd, 1H, J = 8.3, 3.5 Hz, Asp-α-CH), 5.06 (dd, 1H, J = 10.3, 5.1 Hz, His-α-CH), 6.84 (s, 1H, His-δ-CH), 7.54 (s, 1H, His-ε-CH); IR ($CHCl_3$): 1736 (ester) and 1644 (amide) cm^{-1} for $\nu_{C=O}$; HRFAB MS *m/z* 325.1502. Cal-

cd for $C_{14}H_{21}N_4O_5$ ([M + H]$^+$) 325.1512.

(*S*)-2-[(*S*)-3-Carboxymethyl-2-oxo-1-piperazinyl]succinic Acid Trimethyl Ester (3d). ^{1}H NMR ($CDCl_3$): δ 2.76 (dd, 1H, J = 17.3, 8.3 Hz, β-CH_2 in ring), 2.92 (dd, 1H, J = 16.6, 8.5 Hz, β-CH_2 out of ring), 2.98 (dd, 1H, J = 17.0, 3.3 Hz, β-CH_2 in ring), 3.07 (ddd, 1H, J = 13.0, 10.8, 4.0 Hz, en), 3.16 (dd, 1H, J = 16.8, 5.9 Hz, β-CH_2 out of ring), 3.18 (ddd, 1H, J = 12.7, 4.6, 2.2 Hz, en), 3.37 (ddd, 1H, J = 11.2, 3.8, 2.5 Hz, en), 3.60 (ddd, 1H, J = 10.9, 10.9, 4.5 Hz, en), 3.69(s, 3H, OCH_3), 3.71 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3), 3.77 (dd, 1H, J = 8.3, 3.4 Hz, α-CH in ring), 4.81 (dd, 1H, J = 8.3, 5.9 Hz, α-CH out of ring); IR ($CHCl_3$): 1734 (ester) and 1652 (amide) cm^{-1} for $\nu_{C=O}$; HRFAB MS *m/z* 317.1326. Calcd for $C_{13}H_{21}N_2O_7$ ([M + H]$^+$); 317.1348.

Preparation of Complexes. [Eu(L1a)]Cl. After an aqueous solution of LiOH•H_2O (0.10 g, 0.24 mmol) was added to a methanol solution of **Me$_2$L1a** (0.44 g, 0.12 mmol), the solution was stirred overnight. After hydrolysis was finished (checked by TLC), the solvent was evaporated. The resulting residue was dissolved in a small amount of H_2O and an aqueous solution of $EuCl_3$•$6H_2O$ was added. After 8 h, a white precipitate was removed by filtration and the filtrate was evaporated. The resulting residue was purified by reprecipitation (H_2O–EtOH) and gel filtration (Sephadex LH-20, H_2O). Yield 66%. Found: C, 27.93; H, 4.57; N, 13.89%. Calcd for Eu($C_{14}H_{18}N_6O_4$)Cl•$4.5H_2O$: C, 27.89; H, 4.51; N, 13.94%. Mp > 300 °C. $[\alpha]_D$ −1.3° (H_2O).

Other three complexes, [Eu(**L1b**)], Li[Eu(**L1c**)] and [Eu(**L2a**)]Cl were prepared by the similar method as that of [Eu(**L1a**)]Cl.

[Eu(L1b)]. Yield 24%. Found: C, 27.05; H, 4.28; N, 10.19%. Calcd for Eu($C_{12}H_{15}N_4O_6$)•$4H_2O$: C, 26.93; H, 4.33; N, 10.47%. Mp > 300 °C. $[\alpha]_D$ +30.2° (H_2O).

Li[Eu(L1c)]. Yield 87%. Found: C, 22.49; H, 4.15; N, 5.48%. Calcd for LiEu($C_{10}H_{12}N_2O_8$)•$5H_2O$: C, 22.36; H, 4.13; N, 5.21%. Mp > 300 °C. $[\alpha]_D$ +32.6° (H_2O).

[Eu(L2a)]Cl. Yield 25%. Found: C, 29.02; H, 4.61; N, 12.52%. Calcd for Eu($C_{16}H_{20}N_6O_4$)Cl•$6.5H_2O$: C, 28.90; H, 5.00; N, 12.64%. Mp > 300 °C. $[\alpha]_D$ +23.3° (H_2O).

Potentiometric Measurements. All potentiometric titrations were performed in a double-walled titration cell with a capacity of 100 mL, and all hydrogen ion concentrations were determined by a TOA Electronics Ltd. IM 40S ion meter. The temperature of the solutions was maintained at 25.0±0.05 °C by circulating thermostated water through the outer jacket of the cell. A pH combination electrode was calibrated using commercially available buffer solution standards (4.01, 6.86, and 9.18). The ionic strength (I) of the experimental solutions was maintained at 0.1 mol dm^{-3} by the addition of an appropriate amount of 1.0 mol dm^{-3} potassium nitrate. The ion product of water at 25.0 °C in potassium nitrate media was taken to be the same as that in Ref. 12. The solutions were stirred with a magnetic stirrer and bubbled by nitrogen gas and positive nitrogen pressure was maintained during all experiments in order to prevent contamination of the atmosphere.

In this study, pH is defined as the logarithm of a concentration, (−log [H$^+$]), instead of the generally used hydrogen ion activity function. The concentrations of the reactants in the experimental solutions were of the order of 2.0–5.0×10^{-3} mol dm^{-3} for each component.

The acid dissociation constants of *N,N′*-ethylenebis(amino acid) and α,α'-(1,4-piperazinediyl)bis[(*S*)-alkanoic acid] products and stability constants of Eu^{3+} complexes with *N,N′*-ethylene-bis(L-amino acid) ligands were directly obtained by potentiometric data using BEST.[13]

Spectroscopic Measurements. ^{1}H NMR spectra were recorded on a Jeol GX-400 spectrometer. Me_4Si was used as an internal standard in $CDCl_3$. The pH value of each sample solution was measured with a long-stem combination electrode, which was calibrated with standard aqueous buffers. The measured pH values were converted to pD values by pD = pH_{measd} + 0.44.[14] A minimum quantity of dilute NaOD solution or dilute DCl was used for adjusting pD. Each amino acid for measurements was purified by recrystalization from hot water. The measurements for shift reagent property were performed in D_2O solution at $pH_{measd} \approx 7.0$ and 35 °C containing an Eu^{3+} complex (0.04 mol dm^{-3}), an amino acid ([L-form] : [D-form] = 0.08 : 0.04 mol dm^{-3}) and 1,4-dioxane as an internal standard (δ_H 3.530 and δ_C 67.40). IR spectra were obtained on a Jasco FT/IR-420 spectrometer. Melting points were determined on a Yanaco MP-J3 apparatus. Specific rotations ($[\alpha]_D$) were taken on a Jasco DIP 300 polarometer. Mass spectra were recorded on a JEOL AX-500 mass spectrometer.

Results and Discussion

Syntheses of Pro-ligands (Methyl Ester Forms) and Their Europium(III) Complexes. Pro-ligand syntheses were carried out under the standard reductive *N*-alkylational conditions from a mixture of L-histidine methyl ester and L-aspartic acid dimethyl ester as starting materials according to our previous method (Scheme 1).[9] The product mixture consisted of three types of compounds: 1) *N,N′*-ethylenebis(amino acid), 2) α,α'-(1,4-piperazinediyl)bis(alkanoic acid), and 3) *N,N′*-ethylenedipeptide. These 10 pro-ligands were easily isolated and purified by acidic and basic silicagel column chromatography and gel filtration. Table 1 lists the desired pro-ligand yields. We added histidine methyl ester dihydrochloride and trimethylamine to the solution of aspartic acid dimethyl ester hydrochloride and glyoxal to enhance the yield of methyl ester form of *N,N′*-ethylenebis(amino acid) (**Me$_2$L1a**, **Me$_3$L1b** and **Me$_4$L1c**), which are suitable ligands for metal ion, as described later. Otherwise, the formation of 2,2′-(1,4-piperazinediyl)bis[(*S*)-3-(4-imidazolyl)propionic acid methyl ester] (**Me$_2$L2a**) preceded all other ligands because of the much higher reactivity of histidine with glyoxal compared with that of aspartic acid with glyoxal.[15] In addition, because the ester form of *N,N′*-ethylenebis(amino acid) prepared is easily cyclized to form *N,N′*-ethylenedipeptide ester,[16] the yield of **3a** is also high, and it is noteworthy that evaporation of the reactive solution should be done at as low a temperature as possible and purified immediately. Mayer et al.[17] and Neal et al.[18] reported the preparation of **H$_4$L1c** by the reaction of L-aspartic acid and 1,2-dibromoethane under basic conditions. However, their method is not suitable for the syntheses of *N,N′*-ethylenebis(amino acid) products containing different amino acids because of the difficulty of purification and low product yield.[19] Although Moriguchi et al. synthesized several *N,N′*-ethylenebis(amino acid) products including **H$_2$L1a** and **H$_3$L1b** from L-

histidine, more than six step reactions were required and total yields were low (only 10–15%).[10] Feringa et al.[6] reported the syntheses of **H_4L1c** and **H_4L2c** by asymmetric syntheses, but this also required multistep reactions. Compared with these methods, our one-step reaction is very simple and 10 different compounds were prepared simultaneously and isolated easily.

Syntheses of europium(III) complexes, [Eu(**L1a**)]Cl, [Eu(**L1b**)] and Li[Eu(**L1c**)], were accomplished by hydrolysis of methyl ester moieties of corresponding pro-ligand, and subsequently by complexation with $EuCl_3$ in H_2O. The purification was achieved by reprecipitation and gel filtration (LH-20). However, in the cases of piperazine derivative ligands (**L2a**, **L2b** and **L2c**), insoluble Eu^{3+} complexes (probably polymeric complexes) were precipitated, and only the soluble complex of [Eu(**L2a**)]Cl was obtained in low yield. Since the piperazine ring prefers a chair form,[20] it is thought that monomeric complexation with europium(III) ion was prevented. *N*,*N*′-Ethylenedipeptides (**3a**–**3d**) themselves are not suitable ligands for NMR shift reagents, because *N*- and *C*-terminal sites cannot coordinate to the same metal ion with functional groups on side chains. *N*- and *C*-terminal blocked *N*,*N*′-ethylenedipeptides could be used as ligands for model complexes of the non-heme metalloproteins, hemocyanin[9,21] and galactose oxidase.[22]

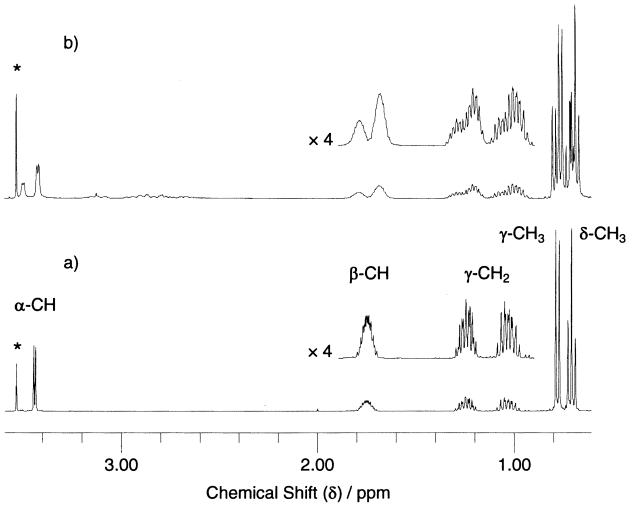


Fig. 1. ^{1}H NMR spectra (ppm) of L- and D-isoleucine a) in the absence and b) in the presence of [Eu(**L1a**)]Cl in aqueous solution at pD = 7.3 and 35 °C. *: Dioxane.

NMR Shift Reagents. As a typical example of NMR shift resolution, ^{1}H and ^{13}C NMR spectra of isoleucine (Ile, L-Ile : D-Ile = 2 : 1) with or without [Eu(**L1a**)]Cl in neutral aqueous solutions are shown in Figs. 1 and 2. Both signals of ^{1}H and ^{13}C NMR spectra, which were less broad, were clearly resolved into pairs of enantiomers with signals of L-enantiomer in the upper-magnetic field. Table 2 gives the chemical shift difference values ($\Delta\Delta\delta$) of Ile using [Eu(**L1a**)]Cl, [Eu(**L1b**)], Li[Eu(**L1c**)] or [Eu(**L2a**)]Cl. Complex [Eu(**L1a**)]Cl obviously shows larger enantiomeric signal resolution than complex [Eu(**L1b**)]. Although Kido et al.[5] and Feringa et al.[6] reported that Li[Eu(**L1c**)] showed the enantiomeric peak resolutions of α-amino acids in basic aqueous solution (pH 9–11), we did not observe the peak resolution of α-amino acids in neutral aqueous solution. The order of enantiomeric resolution effects is [Eu(**L1a**)]Cl > [Eu(**L1b**)] > Li[Eu(**L1c**)] (none), depending on the positive charge of the complex ion ([Eu(**L1a**)]Cl: monopositive complex, [Eu(**L1b**)]: neutral complex, Li[Eu(**L1c**)]: mononegative complex). In neutral aqueous solution, α-amino acid exists mainly in the zwitterion form. Therefore, it is suggested that the carboxylate anion of α-amino acid interacts more strongly with the positive cation of complex [Eu(**L1a**)]Cl than with other complexes, [Eu(**L1b**)] > Li[Eu(**L1c**)]. However, the possibility that another effect, such as a steric hindrance, enhances the peak resolution cannot be excluded. Although complex [Eu(**L2a**)]Cl is also a monopositive ion, no peak resolution was observed because of its weak complexation. As described in the previous section, the piperazine ring of **L2a** prefers a chair form; this fact may weaken the chiral recognition property of [Eu(**L2a**)]Cl compared with [Eu(**L1a**)]Cl.

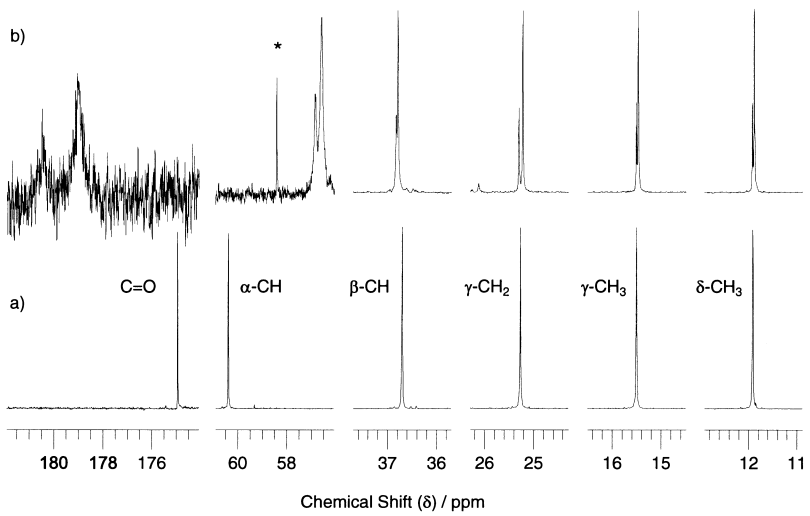


Fig. 2. ^{13}C NMR spectra (ppm) of L- and D-isoleucine a) in the absence and b) in the presence of [Eu(**L1a**)]Cl in aqueous solution at pD = 7.3 and 35 °C. *: Impurity from ligand.

Table 2. Enantiomeric Chemical Shift Differences ($\Delta\Delta\delta$) of the ^{1}H and ^{13}C NMR Spectra of L- and D-Ile in the Presence of Europium(III) Complexes in D_2O at 35 °C[a)]

Complex	pD	Molar ratio of [Ile]/[complex]	Nuclei	$\Delta\Delta\delta$					
				αCH	βCH	γCH_2	γCH_3	δCH_3	COO^-
[Eu(**L1a**)]Cl	7.3	3.1	^{1}H	0.074	0.103[b)]	sh[c)]	0.033	0.027	—
			^{13}C	0.26	0.04	0.08	0.04	0.04	1.40
[Eu(**L1b**)]	7.4	3.0	^{1}H	0.040	0.0	0.0	0.008	0.0	—
			^{13}C	0.0	0.05	sh[c)]	0.0	0.0	0.49
Li[Eu(**L1c**)]	7.4	3.1	^{1}H	br[b)]	br[b)]	br[b)]	0.0	0.0	—
			^{13}C	0.0	0.0	0.0	0.0	0.0	0.0
[Eu(**L2a**)]Cl	7.3	3.3	^{1}H	br[b)]	br[b)]	br[b)]	br[b)]	br[b)]	—
			^{13}C	br[b)]	0.0	0.0	0.0	0.0	sh[c)]

a) All signals of L-isomer were observed at upper-magnetic field. Spectra were recorded using 1,4-dioxane as an internal standard (δ_H: 3.530 and δ_C: 67.40). b) Broad signals. c) Shoulder.

Table 3. Enantiomeric Chemical Shift Differences ($\Delta\Delta\delta$) of the ^{1}H and ^{13}C NMR Spectra of L- and D-Ala, -Val, -Ile and -His in the Presence of [Eu(**L1a**)]Cl in D_2O at 35 °C[a)]

Amino acid (AA)	pD	Molar ratio of [AA]/[complex]	Nuclei	$\Delta\Delta\delta$					
				αCH	βCH_{1-3}	γCH_2	γCH_3	δCH_3	COO^-
Ala	7.5	2.9	^{1}H	0.038	0.031				
			^{13}C	0.09	0.02				0.76
Val	7.4	3.0	^{1}H	0.034	sh[c)]		0.027, 0.009		
			^{13}C	0.44	0.03[d)]		0.02, 0.00		0.35
Ile	7.3	3.1	^{1}H	0.074	0.103[c)]	sh[d)]	0.033	0.027	
			^{13}C	0.26	0.04	0.08	0.04	0.04	1.40
His	7.8	2.9	^{1}H	0.0[b)]	sh[c)]				
			^{13}C	0.52	0.23[d)]				0.38[d)]

a) All signals of L-isomer were observed at upper-magnetic field except for any other comment. Spectra were recorded using 1,4-dioxane as an internal standard (δ_H: 3.530 and δ_C: 67.40). b) Broad signals. c) Shoulder. d) The peak of D-isomer is observed at upper-magnetic field.

On the basis of the above results, the chemical shift difference values on ^{1}H and ^{13}C NMR of alanine (Ala), valine (Val), Ile and histidine (His) in the presence of [Eu(**L1a**)]Cl were also examined in neutral aqueous solutions (Table 3). In the cases of Ala, Val, and Ile, which contain alkyl side chains, almost all of the signals observed in the upper-magnetic field were assigned to L-enantiomers. On the other hand, β- and carbonyl carbon signals of D-isomer of His were observed at the higher field, suggesting that the coordination environment of His is different from other amino acids which have no extra functional group on their side chains. Since unprotected α-amino acid exists in a zwitterion form in neutral aqueous media, it is expected that the carboxyl oxygen of this α-amino acid binds to the europium(III) center and that the hydrogen of ammonium cation may interact with the carbonyl oxygen of ligand via hydrogen bonding.[23] In addition to this two-point interaction, the imidazole nitrogen of His can interact with the europium(III) center or carbonyl oxygen of the ligand. Thus, it is suggested that the coordination mode of His differs from those of other amino acids used in this work, and induced a different tendency in the chemical shift differences between the D- and L-isomers.

The previously reported europium(III) shift reagents, which were effective in aqueous solution, had been studied by low magnetic field NMR spectroscopy (90 MHz).[4,5,7] This was presumably due to signal broadening in a high magnetic field NMR spectroscopy resulting from a chemical exchange phenomenon.[1,24] Although the chemical shift difference values induced by complex [Eu(**L1a**)]Cl (400 MHz) in this work are smaller than those induced by previously reported [Eu{(*R*)-tpdpn}Cl_2]$^+$,[7] the signal broadening is very slight. To our knowledge, complex [Eu(**L1a**)]Cl is the first example of an europium(III) complex to have shown enantiomeric shift resolution without signal broadening, even on high-resolution NMR spectroscopy.

Potentiometric Measurements. The potentiometric titration curves of six ligands (**L1a–L1c** and **L2a–L2c**) and that of europium(III) complex with **L1a** are shown in Figs. 3a and b. In both cases, the experimental data points indicated by a series of symbols in the figures are in fair agreement with the calculated values of the solid lines, which were obtained by the computer programs of PKAS[13] for pK_a and BEST[13] for log K. These acid dissociation constants for the ligands and some of the stability constants studied are listed in Table 4, together with the pK_a values of the parent compounds.

Among the pK_a values obtained for new types of *N,N′*-ethylenebis(amino acid) and α,α'-(1,4-piperazinediyl)bis(alkanoic acid) ligands synthesized in the present study, only the pK_a values for **L1c** can be compared with reported values.[25a] Taking into account that amino acid tends to form a zwitterion in neutral aqueous solution, it can be understood that, for **L1c**, the first and second pK_a values should correspond to the deproto-

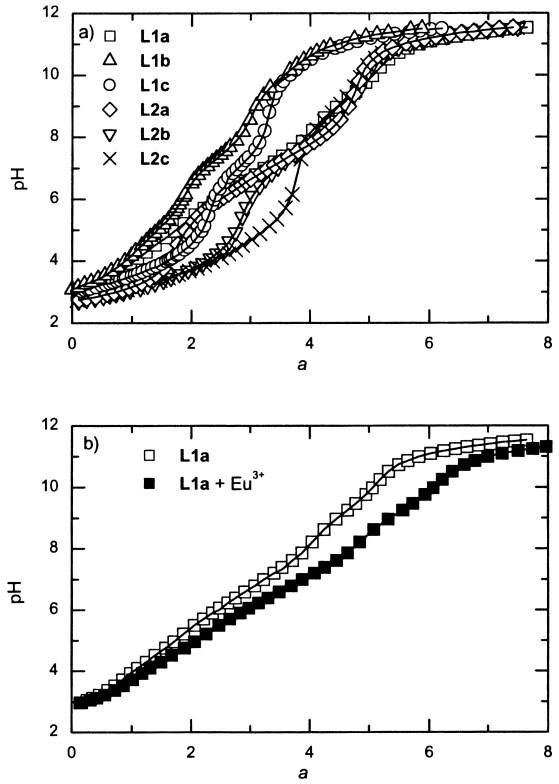


Fig. 3. Potentiometric titration curves for a) bis(amino acid) products **L1a**, **L1b**, **L1c**, **L2a**, **L2b**, and **L2c**, b) **L1a** with or without europium(III) ion, I = 0.1 mol dm^{-3} (KNO_3), at 25 °C. The solid lines represent calculated values. a = [OH^-]/[ligand or complex].

nation of the carboxylate oxygen atoms of aspartic acid side chain (assigned as PH_1 and PH_2 in the Table). Consequently, the 3rd and 4th pK_a values must be those of the amine bound protons. These pK_a values, corresponding to the deprotonation of the amine group of **L1c**, are lower than those[25b] of the parent compound of ethylenediamine. This is thought to be the result of a lowering of the basicity of the nitrogen atom by substitution of the aspartic group.

In the cases of **L1a** and **L1b**, since the difference in chemical structures between these compounds and **L1c** is that one or two aspartic moieties are substituted for the histidine moiety, it is easily deducible that the lowest values (pK_a = 2.74 and 3.23 for **L1a** and **L1b**) and the highest values (pK_a = 9.24 and 10.8) are attributable to the acid dissociation constants of the carboxylic acids and those of one of the nitrogens in the amine groups, respectively. Moreover, based on the reported literature values, it is known that the deprotonations of imidazolyl moiety occur around pH = 6–7 (e.g., L-His (6.02)[25a], cyclo(Gly–L-His) (6.25)[26] and cyclo(L-Met–L-His) (6.31)[26] and an imidazole molecule (7.01)[25c]). Thus, the pK_a values in this region shown in Table 4 (pK_a = 6.04 and 7.21 for **L1a**, and pK_a = 7.43 for **L1b**) are assigned to be the deprotonations originated from the corresponding imidazolyl moieties.

The above conclusions are well substantiated by the independently obtained ^{1}H NMR experimental results. For example, as shown in Fig. 4a, drastic chemical shift changes to the upper-magnetic field were observed in the region of pD ~ 7.8 for the proton peaks belonging to imidazolyl moiety (labeled as His ε-CH and His δ-CH) of **L1b**. In contrast, the split methylene proton signals (labeled as –CH_2–CH_2–) changed greatly at pD ~ 5.5 and again at pD ~ 10.5. Thus, it is reasonable to conclude that the pK_a values of 5.12 and 10.8 correspond to the amine protons. In addition, for **L1a**, because the two imidazole groups attach to the nitrogen atoms of ethylenediamine, the basicity of the amine nitrogen is lowered a bit, and one of the histidine carboxylic acids is determinable.

The assignments of the acid dissociation constants of **L2a**–**L2c** are rationalized in the same way, since their structures are mutually quite analogous to **L1a**–**L1c**. The only structural difference in these, e.g., **L1a**–**L2a**, is that piperazine moiety is substituted for ethylenediamine moiety. As shown in Table 4, the structural difference apparently affects mainly the pK_a values of the amine protons, and the pK_a values of the rest of other functional groups are small. Around pD ~ 4.5 in Fig. 4b, one of the –CH_2–CH_2– signals on the piperazine group resulted in chemical shift to an upper-field, suggesting that the deprotona-

Table 4. Acid Dissociation Constants of *N,N′*-Ethylenebis(amino acid) and α,α'-(1,4-Piperazinediyl)bis(alkanoic acid) Products and Stability Constants of Europium(III) Ion with **L1a** and **L1c** at 25.0 °C and I = 0.1 mol dm^{-3}

Possible Assignment	**L1a**	**L1b**	**L1c**	**L2a**	**L2b**	**L2c**	Ethylene diamine	Piperazine
	Obsd.	Obsd.	Obsd. (Ref.[a])	Obsd.	Obsd.	Obsd.	(Ref.[b])	(Ref.[b])
Acetic (AcH_1)								
Acetic (AcH_2)	2.74			$\ll 2$	2.49	≈ 2		
Propionic (PH_1)		3.23	3.32 (2.40)		2.78	3.16		
Propionic (PH_2)			3.53 (3.86)			4.15		
Imidazolyl (IH_1)	6.04	7.43		6.11	7.06			
Imidazolyl (IH_2)	7.21			7.10				
Amine (AmH_1)	4.81	5.12	6.29 (6.83)	3.51	4.29	5.34	(7.08)	(5.59)
Amine (AmH_2)	9.24	10.8	9.87 (9.82)	≈ 12	10.66	11.01	(9.89)	(9.71)
Standard Deviation (σ)	0.011	0.006	0.041	0.002	0.018	0.021		
Stability Constant / log K	9.40	c)	12.3 (13.5)	c)	c)	c)		

a) Refer to Ref. 25a. b) Refer to Ref. 25b. c) Since precipitation was occurred during the titration, stability constant was not measured.

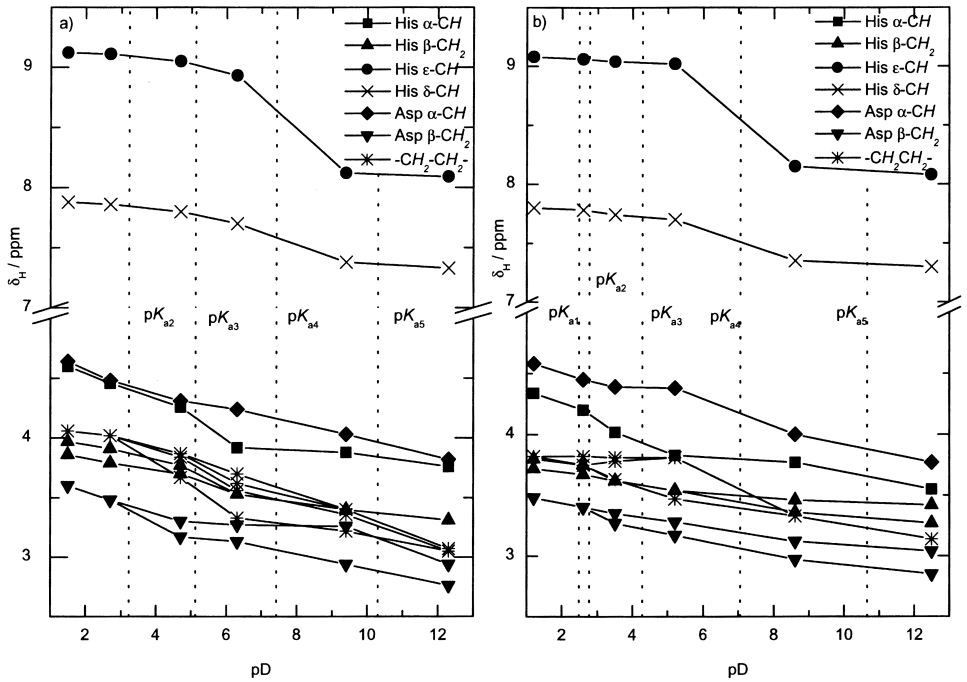


Fig. 4. The pD dependence of the ^{1}H NMR chemical shift of a typical ligand a) **L1b** and b) **L2b** at room temperature in D_2O.

tion occurs on a piperazine nitrogen atom. The corresponding pK_a value for the amine nitrogen is much lower than that of **L1b**. This might reflect a difference in the lowest pK_a values in the parent compound, i.e., pK_a = 7.08 for ethylendiamine[25b] and pK_a = 5.59 for piperazine.[25b] This phenomenon is reasonably explained by the partial contribution of the intramolecular hydrogen bonding between two amine nitrogens in the ethylenediamine residue or between amine nitrogens and carboxy oxygen via a proton.[27] Although the second dissociation constant of **L2a** (pK_a = 3.51) is assigned to be the deprotonation of protonated amine on piperazine moiety and this seems to be rather acidic, the comparison of the difference between pK_1 of ethylendiamine and pK_2 of **L1a** with that between pK_1 of piperazine and pK_2 of **L2a** is roughly equivalent. Thus, the low pK_a value is not too surprising.

In the determination of the formation constants of *N,N′*-ethylenebis(amino acid) and α,α'-(1,4-piperazinediyl)bis(alkanoic acid) ligands with europium(III) ion, only those of **L1a**/Eu^{3+} and **L1c**/Eu^{3+} systems were evaluated by the potentiometric measurements; precipitations occurring in the alkaline region (> 9.0) made such measurements impossible for all other combinations of the ligands with the europium(III) ion.

The formation constant of **L1c** with europium(III) ion is larger that that of **L1a** with the europium(III) ion. The difference may be due to the pK_a difference between the imidazolyl group in **L1a** and the carboxylate group in **L1c**.

Conclusion

We have successfully prepared various *N,N′*-ethylenebis(L-amino acid) ligands and demonstrated that novel europium(III) complexe with *N,N′*-ethylenedi(L-histidine) is an excellent chiral NMR shift reagent for unprotected α-amino acids as substrates in neutral aqueous solution on high magnetic field NMR spectroscopy. The enantiomeric peak separation of isoleucine was increased in proportion to the increase in the positive charge on the complex and the decrease in the stability constant of europium(III) complex, suggesting that control of these factors is very important for enhancing this kind of chiral-recognition phenomenon.

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11 It is also possible to prepare *N,N′*-ethylenedi(L-histidine methyl ester) (**Me_2L1a**) (42% yield) from only L-histidine methyl ester dihydrochloride by reductive *N*-alkylation method described in experimental section.

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