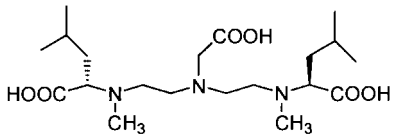
New Chiral NMR Shift Reagent for α-Amino Acids and *N*-Protected Oligopeptides in Aqueous Solution

Masaaki Watanabe, Takayuki Hasegawa, Hiroyuki Miyake, and Yoshitane Kojima*
Department of Chemistry, Graduate School of Science, Osaka City University, Sugimoto, Sumiyoshi-ku, Osaka 558-8585

(Received October 5, 2000; CL-000909)

A europium(III) complex with a new chiral ligand, *N*,*N*-bis[2-{*N*-methyl((1*S*)-1-carboxy-3-methyl)butylamino}ethyl]-glycine, is described as a new chiral shift reagent for α-amino acids and *N*-acyl-oligopeptides in aqueous solutions without signal broadening on high resolution NMR spectroscopy. In addition, the molecular structure of lanthanum(III) complex with this ligand is revealed by X-ray crystallography.

Chiral shift reagents are used to resolve enantiomer signals on NMR spectroscopy. Major studies have been performed with organic solvents, and a few water-soluble chiral shift reagents have also been studied.[1–5] The europium(III) based shift reagent is difficult to use on high resolution NMR spectroscopy, because the strong paramagnetic effect of the Eu(III) ion brings a serial signal broadening problem.[6] In this paper, we report a new water-soluble europium(III) chiral shift reagent with *N*,*N*-bis[2-{*N*-methyl((1*S*)-1-carboxy-3-methyl}butylamino)ethyl]glycine (H_3L)[7] which shows enantiomer shifts without signal broadening at neutral pH regions on high resolution NMR spectroscopy.



H_3L

A ^{1}H-NMR spectrum of (*R*,*S*)-alanine((*R*):(*S*) = 1:2) with 1/3 amount of EuL[8] in D_2O solution is shown in Figure 1. Although the signals of the α-proton are scarcely resolved, those of β-protons are clearly separated into a pair of enantiomers with the signal of (*S*)-enantiomer at the lower magnetic field without signal broadening. The chemical shift difference between the enantiomer signals (ΔΔδ) is small but high resolution NMR spectroscopy could distinguish them. Table 1 shows the ΔΔδ values of several amino acids and their derivatives in the presence of EuL. The ΔΔδ values showed pH dependence. The optimum pH for NMR measurement is between 7 and 8. The enantiomer signals were not resolved under lower pH and were broadened under a higher pH in which paramagnetic Eu(III) complex strongly interacts with the amino acids. Several one-negative-charge Eu(III) chiral shift reagents with four carboxylates, such as (*R*)-propylenediaminetetraacetic acid,[2] (*S*,*S'*)-ethylenediamine-*N*,*N'*-disuccinate (EDDS),[3] and (*R*,*R'*) or (*S*,*S'*)-1,2-diaminobisethylene-*N*,*N*,*N'*,*N'*-disuccinate[4] have been studied in aqueous solutions. Their NMR measurements needed in order to observe a pair of enantiomer signals required a pH = 9–11 on low resolution NMR spectroscopy. The substrate should coordinate to the Eu(III) center in a chelate form (amine and carboxylate) under this condition,[9] inducing signal broadening by strong paramagnetic interactions. Since the Eu(III) complex reported here has no charge, the interaction between the shift reagent and α-amino acid is considered to be weak at a neutral pH because of monodentate coordination (carboxylate oxygen). Therefore the enantiomer signals are observed without signal broadening on high resolution NMR.

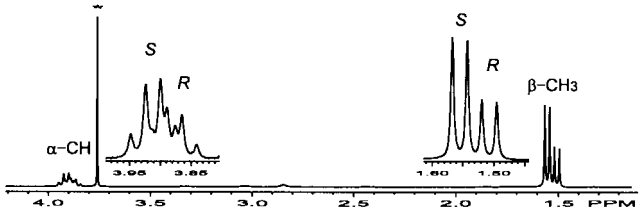


Figure 1. ^{1}H-NMR (300 MHz) spectra of (*R*,*S*)-alanine (0.05 M, (*R*):(*S*) = 1:2) in the presence of EuL (0.017 M) at pH=7.54. *The data were referenced to dioxane (3.76 ppm).

Table 1. The ΔΔδ values ($\Delta\Delta\delta = \Delta\delta_S - \Delta\delta_R$) of α-amino acids(0.05 M) and *N*-acyl-oligopeptides(0.05 M) in the presence of EuL(0.017 M) at room temperature[a]

Run	Compound	pH	α	β	γ	Other signal
1	Ala	7.54	0.035	0.048		
2	Met	7.61	0.000	0.000	0.000	S-CH_3 0.026
3	Val	7.82	0.000	0.000	0.014	
4	Z-Ala	7.61	0.080	0.077		
5	Boc-Gly-Ala	7.51	0.077	0.000		

[a] ^{1}H-NMR spectra were recorded with *t*-BuOH or dioxane as an internal standard on 300 MHz or 400 MHz NMR spectroscopy.

The molecular structure of a lanthanum(III) complex with the ligand described here was determined by X-ray analysis[10] at 297 K (Figure 2), revealing that the complex exists in a trimeric form. The coordination geometry of each La(III) ion is a distorted tricapped trigonal prism. Each La(III) ion is coordinated by three amine nitrogens and three carboxylate oxygens from the ligand, a water oxygen and chelated oxygens from one carboxylate moiety of the neighboring ligand, making a nine coordination structure. One of the chelating oxygens bridges between the La(III) ions to make a trimer to the neighboring La(III) center. Disordered crystal water molecules occupy the central cavity of the trimer. Each La–La nonbonding distance is 4.98 Å. The bond distances from both the terminal amines to the metal ion are longer than that from the central amine. Among the terminal amines, the bond lengths of La–N(1), N(4) and N(7) are longer than that of La–N(3), N(6) and N(9), respectively. This discrepancy is attributed to the bridging carboxylate arm being stretched

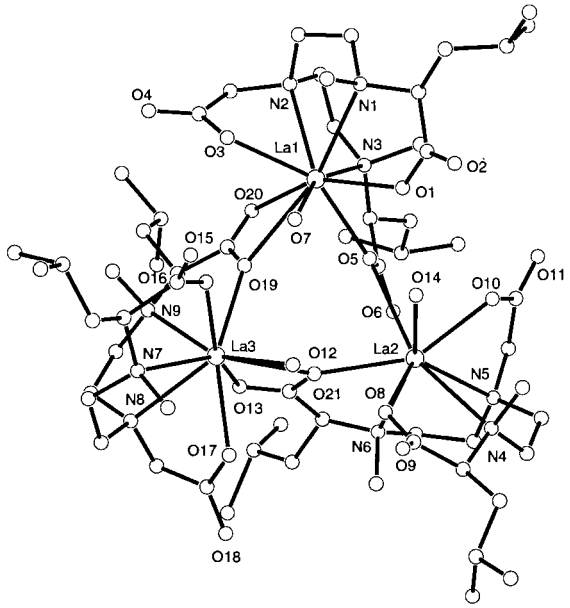


Figure 2. The molecular structure of $[La_3(L)_3(H_2O)_3]\cdot 9H_2O$. Nine crystal water and hydrogen atoms are omitted. Selected bond length (Å) and angles (°): La(1)-O(1) 2.466(5), La(1)-O(3) 2.440(5), La(1)-O(5) 2.509(4), La(1)-O(7) 2.532(4), La(1)-O(19) 2.735(4), La(1)-O(20) 2.629(5), La(1)-N(1) 2.808(5), La(1)-N(2) 2.703(6), La(1)-N(3) 2.739(5), La(2)-O(5) 2.741(4), La(2)-O(6) 2.623(5), La(2)-O(8) 2.469(4), La(2)-O(10) 2.433(5), La(2)-O(12) 2.498(4), La(2)-O(14) 2.527(4), La(2)-N(4) 2.823(5), La(2)-N(5) 2.728(6), La(2)-N(6) 2.732(5), La(3)-O(12) 2.743(4), La(3)-O(13) 2.629(5), La(3)-O(15) 2.463(4), La(3)-O(17) 2.431(5), La(3)-O(19) 2.511(4), La(3)-O(21) 2.534(5), La(3)-N(7) 2.810(5), La(3)-N(8) 2.719(6), La(3)-N(9) 2.725(5), O(1)-La(1)-N(1) 61.7(2), O(3)-La(1)-N(2) 62.5(2), O(5)-La(1)-N(3) 62.4(1), O(19)-La(1)-O(20) 47.9(1), N(1)-La(1)-N(2) 65.6(2), N(2)-La(1)-N(3) 65.8(2), O(8)-La(2)-N(4) 61.6(2), O(10)-La(2)-N(5) 62.7(2), O(12)-La(2)-N(6) 62.1(1), O(5)-La(2)-O(6) 48.1(1), N(4)-La(2)-N(5) 65.3(2), N(5)-La(2)-N(6) 66.3(2), O(15)-La(3)-N(7) 61.6(2), O(17)-La(3)-N(8) 62.4(2), O(19)-La(3)-N(9) 62.5(1), O(12)-La(3)-O(13) 48.4(1), N(7)-La(3)-N(8) 65.8(2), N(8)-La(3)-N(9) 65.9(2).

away from the chelated metal in order to coordinate with the neighboring metal. In addition, the bond lengths of La–O(5), O(12) and O(19) are longer than those of La–O(1), O(8) and O(15), respectively, because of the steric hindrance of the side chain. The average bond lengths of La–O(2.461 Å) and La–N(2.754 Å) are similar to those observed in $[La(HTTHA)]^{2-}$ (average bond length: La–O, 2.492 Å; La–N, 2.812 Å),[11] $[La_2(EDTA)_2(H_2O)_4]^{2-}$ (La–O, 2.545 Å; La–N, 2.812 Å)[12] and $[La_2(HEDTA)_2(H_2O)_4]$ (La–O, 2.520 Å; La–N, 2.785 Å).[13]

The ESI-MS showed that the Eu(III) complex exists in a mixture of monomeric and polymeric structures (*m/z* 597, 599 $[EuL+MeOH]^-$, 1149 $[Eu_2L_2+OH]^-$, 1732 $[Eu_3L_3+Cl]^-$ etc.) in methanol. The addition of alanine to this solution reduced these complicated equilibria to a simple monomeric ternary complex. (*m/z* 653, 655 $[EuL+alanine]^-$). A ^{1}H-NMR spectrum of LaL in D_2O showed broad signals, which were changed to sharp signals in the presence of an equimolar amount of alanine, suggesting that the polymerization of La(III) complex was inhibited by a substrate as indicated by ESI-MS. The X-ray analyses of polyaminocarboxylate–lanthanide(III) complexes sometimes showed polymeric structures.[11–14] Furthermore, it has been reported concerning the $[Tb(EDDS)]^-$ complex that polynuclear species were formed at a low pH in aqueous solutions by luminescence study.[15] So, it is first interesting to observe that polymeric lanthanide(III) complex changes its structure to a monomeric one in the presence of the third substrate to show chiral recognition on a ^{1}H-NMR study.

M.W. thanks the support of the Graduate School of Science, Osaka City University (Special Fund for the Student).

References and Notes

1 T. J. Wenzel, "NMR Shift Reagent", CRC Press, Boca Ratton, FL(1987).
2 K. Kabuto and Y. Sasaki, *J. Chem. Soc., Chem. Commun*., **1984,** 316; K. Kabuto and Y. Sasaki, *J. Chem. Soc., Chem. Commun*., **1987**, 670.
3 J. Kido, Y. Okamoto, and H. G. Brittain, *J. Org. Chem.*, **56**, 1412 (1991).
4 R. Hulst, N. K. de Vries, and B. L. Feringa, *J. Org. Chem.*, **59**, 745 (1994).
5 R. Hazama, K. Umakoshi, C. Kabuto, K. Kabuto, and Y. Sasaki, *Chem. Commun*., **1996,** 15.
6 J. Sato, H. Y. Jin, K. Omata, K. Kabuto, and Y. Sasaki, *Enatiomer*, **4**, 147 (1999).
7 Treatment of L-leucine methylester hydrochloride (Leu-OMe·HCl) (20.0 g, 0.110 mol) and glyoxal dimethylacetal 45% *t*-butyl methyl ether solution (28.0 g, 0.121 mol) with $NaBH_3CN$ (7.90 g, 0.111 mol) in MeOH gave *N*-(2,2-dimethoxyethyl)-Leu-OMe (12.0 g, 0.0571 mol; 47.1%), followed by *N*-methylation with excess HCHO aq (12.9 g, 0.155 mol) and $NaBH_3CN$ (3.30 g, 0.0524 mol) in MeOH. *N*-(2,2-dimethoxyethyl)-*N*-methyl-Leu-OMe (8.3 g, 0.0336 mol; 65.2%) was treated with 28% HBr/AcOH to give corresponding aldehyde. The aldehyde was condensed with glycine methylester hydrochloride (1.45 g, 0.0155 mol) in the presence of $NaBH_3CN$ (0.73 g, 0.0156 mol) in MeOH/H_2O to give L trimethyl ester (1.70 g, 0.00372 mol; 32.3%), which was hydrolyzed by LiOH·H_2O (0.703 g, 0.0167 mol) in MeOH/H_2O to give lithium salt (0.829 g, 0.00170 mol; 45.7%) of title ligand. Anal. Calcd for $C_{20}H_{36}N_3Li_3O_6\cdot 3H_2O$ (hygroscopic): C, 49.09; H, 8.65; N, 8.59%. Found: C, 48.95; H, 8.58; N, 8.81%. $[\alpha]_D$ = 4.39 ° (MeOH). IR (KBr) 1624 cm^{-1} ($\nu_{C=O}$). FAB-MS *m/z* 424 $[M+Li]^+$. ^{1}H-NMR (300 MHz, D_2O, TSP): δ 0.93(d, *J* = 6.6 Hz, 12H), 1.36 (m, 2H), 1.55(m, 2H), 1.73 (m, 2H), 2.42 (s, 6H), 2.62–2.95 (m, 8H), 3.21 (s, 2H), 3.25 (dd, *J* = 4.0 Hz, 10.3 Hz, 2H).
8 The stock solution of EuL complex was prepared from $EuCl_3\cdot 6H_2O$ and equivalent amount of Li_3L in D_2O. Samples for measurements were prepared by mixing a substrate (0.05 mmol, (*R*):(*S*) = 1:2)), stock solution of EuL(1 ml, 0.017 M) and small amount of NaOD or DCl solution for pH adjustment. Pure EuL complex was obtained as white precipitate from Li_3L and $EuCl_3\cdot 6H_2O$ in small amount of water. This complex is solved in both water and organic solvents ($CHCl_3$, CH_3OH etc.). Anal. Calcd for $C_{20}H_{36}N_3EuO_6\cdot 2.5H_2O$: C, 39.22; H, 6.92; N, 6.83%. Found: C, 39.28; H, 6.87; N, 6.75 %. FAB-MS *m/z* 565, 567 $[M]^-$.
9 H. G. Brittain, *J. Am. Chem. Soc*., **102**, 3693 (1980); A. D. Sherry, C. A. Stark, J. R. Ascenso, and C. F. G. C. Geraldes, *J. Chem. Soc. Dalton Trans*., **1981**, 2078; L. Spaulding and H. G. Brittain, *Inorg. Chem*., **24**, 3692 (1985).
10 Crystal data for $[La_3(L)_3(H_2O)_3]\cdot 9H_2O$: $C_{60}H_{132}N_9La_3O_{30}$ (fw = 1876.46), orthorhombic, space group *I*222(#23), *a* = 25.6915(3) Å, *b* = 25.7038(3) Å, *c* = 25.6975(3) Å, *V* = 16969.8(4) $Å^3$, D_{calcd} = 1.469 g/cm^3, D_{obs} = 1.48 g/cm^3, μ(Mo Kα) = 15.55 cm^{-1}, *R* = 0.033 (R_w = 0.043) on 7917 reflections ($I > 2.00\ \sigma(I)$). Anal. Calcd for $C_{60}H_{108}N_9La_3O_{18}\cdot 13H_2O$: C, 38.04; H, 7.13; N, 6.65%. Found: C, 38.06; H, 7.16; N, 6.68%, indicating a position of one crystal water was not determined by X-ray analysis.
11 R. Ruloff, P. Prokop, J. Sieler, E. Hoyer, and L. Beyer, *Z. Naturforsch*., **51b**, 963 (1996); R. Y. Wang, J. R. Li, T. Z. Jin, G. X. Xu, Z. Y. Zhou, and X. G. Zhou, *Polyhedron*, **16**, 1361 (1997).
12 S. Y. Chen, C. H. Wang, D. Li, and X. Wang, *Sci. China, Ser B*, **32**, 918 (1989).
13 C. C. Fuller, D. K. Molzahn, and R. A. Jacobson, *Inorg. Chem*., **17**, 2138 (1978).
14 M. B. Inoue, M. Inoue, and Q. Fernando, *Acta Crystallogr*., *Sect. C*, **50**, 1037 (1994); S. J. Franklin and K. N. Raymond, *Inorg. Chem*., **33**, 5794 (1994); S. Aime, A. Barge, F. Benetollo G. Bombieri, M. Botta, and F. Uggeri, *Inorg. Chem*., **36**, 4287 (1997).
15 L. Spaulding and H. G. Brittain, *Inorg. Chim. Acta*, **110**, 197 (1985).