Use of Sm(III)–{1,2-propanediamine-*N*,*N*,*N*′,*N*′-tetra(α,α-dideuterioacetate)} complex for NMR determination of absolute configuration of each α-amino acid in peptide hydrolysate mixtures†

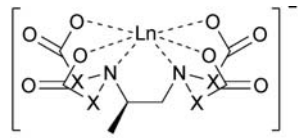
Kenji Omata,*[a] Mika Fujioka,[a] Kuninobu Kabuto[a] and Yoichi Sasaki[b]

Received (in College Park, MD, USA) 5th June 2008, Accepted 23rd July 2008
First published as an Advance Article on the web 12th September 2008
DOI: 10.1039/b809551e

^{1}H NMR analyses of individual α-amino acids in their mixture were simultaneously conducted in the presence of Sm-(pdta–d_8) in water: high regularity, promising for direct simultaneous determination of absolute configurations of each α-amino acids in peptide hydrolysate mixtures, was observed between absolute configuration and the induced shifts.

Simultaneous determination of the absolute configurations of multiple stereogenic centers using spectroscopic method is an intriguing subject. Recently, one of the most successful examples was reported by Kishi *et al*. They demonstrated that absolute configurations of multiple chiral centers in an acyclic polyol can be determined simultaneously by observing ^{13}C NMR spectra in the presence of a shift reagent, Pr(hfc)$_3$.[1] The method is also notable as a rare instance for determining absolute configuration using a chiral shift reagent or a chiral solvating agent that has been usually used for determination of enantiomeric purity. On the other hand, there has been no precedent of application of spectroscopic determination to different molecules in a mixture of chiral compounds.

Previously, we reported that the Eu(III) and Sm(III) complexes of (*R*)- and (*S*)-1,2-propanediamine-*N*,*N*,*N*′,*N*′-tetraacetate (pdta) (Eu-pdta: (*R*)-**1**, (*S*)-**1**; Sm-pdta: (*R*)-**2**, (*S*)-**2**) afford a highly consistent relation between absolute configurations of α-amino acids and their induced shifts.[2,3] The (*R*)- and (*S*)-**2** cause considerably less signal broadening on a high-field NMR instrument than (*R*)- and (*S*)-**1**. Here we examined the application of the Sm(III) complexes of deuterated pdta, 1,2-propanediamine-*N*, *N*, *N*′, *N*′-tetra(α,α-dideuterioacetate) (Sm-{(*R*)-pdta-d_8}: (*R*)-**3** and Sm-{(*S*)-pdta-d_8}: (*S*)-**3**) to mixtures containing up to ten α-amino acids and obtained the same regularity as that observed in single species.‡ This result strongly suggests the possible use of these complexes for simultaneous determination of absolute configurations of component amino acids in a peptide after exhaustive hydrolysis. At present, such determination is possible only by chromatographic methods, for example by Marfey's analysis.[4–6] However, chromatographic methods require standard samples of known absolute configuration. Therefore these methods cannot be applied to novel amino acids or small sample volumes.



Ln = Eu, X = CH_2: (*R*)-**1** [Eu-(*R*)-pdta]
Ln = Sm, X = CH_2: (*R*)-**2** [Sm-(*R*)-pdta]
Ln = Sm, X = CD_2: (*R*)-**3** [Sm-(*R*)-(pdta-d_8)]

Deuterated ligands were newly prepared and used in order to reduce the signals due to the pdta ligand in the range of 2 to 4 ppm which sometimes overlapped with substrate signals to obscure their chemical shifts.

It should not be assumed that the correlation observed for a solution of a single amino acid will be the same for that of a mixture of amino acids, since components may interfere with each other in the chiral discriminating behavior. Six different sample solutions containing a mixture of amino acids were prepared for the examination. The relation between the shift and absolute configuration of each amino acid in the mixture was examined by comparison of the chemical shifts of the corresponding signals due to each amino acid in the two spectra separately measured in the presence of (*R*)- and (*S*)-**3** at the same molar ratio. As a typical example, the ^{1}H NMR spectra of a mixture containing eight amino acids separately measured in the presence of (*R*)- and (*S*)-**3** are shown in Fig. 1.

Table 1 summarizes the chemical shift differences between the corresponding signals in the presence of (*R*)- and (*S*)-**3** at the same molar ratio in terms of ΔΔδ [$\Delta\delta_R - \Delta\delta_S = (\delta_R - \delta_0) - (\delta_S - \delta_0)$; here $\Delta\delta_R$, $\Delta\delta_S$ represent lanthanide induced shifts (LIS) by (*R*)- and (*S*)-**3**, respectively; δ_0, δ_R, and δ_S represent the chemical shifts without shift reagent, in the presence of (*R*)-**3** and (*S*)-**3**, respectively]. The ΔΔδ values for diastereotopic β to γ methylene protons in Table 1 are given only for the signals that showed clear correspondence between signals in the presence and absence of **3** recognizable by the shape of the signals. On the other hand, the signals due to diastereotopic methyl groups of valine and leucine were identified by comparing their shifts with those observed in non-mixture solutions in the presence of Sm-pdta (**2**). Entry 6 is the result of application of this method to the hydrolysate of a synthetic peptide [D-Ala^2, Met^5]-enkephalin (Tyr-D-Ala-Gly-Phe-Met). The peptide was hydrolysed with hydrochloric acid in the

[a] *Department of Chemistry, Graduate School of Science, Tohoku University, 6-3, Aoba, Aramaki, Aoba-ku, Sendai, Japan. E-mail: omata@he.tohoku.ac.jp; Fax: +81 22-795-7721; Tel: +81 22-795-7721*
[b] *Catalysis Research Center, Hokkaido University, Kita-ku, Sapporo, Japan*

† Electronic supplementary information (ESI) available: Preparation, crystallographic data of **3** and procedure for NMR shift study on amino acid mixtures. See DOI: 10.1039/b809551e

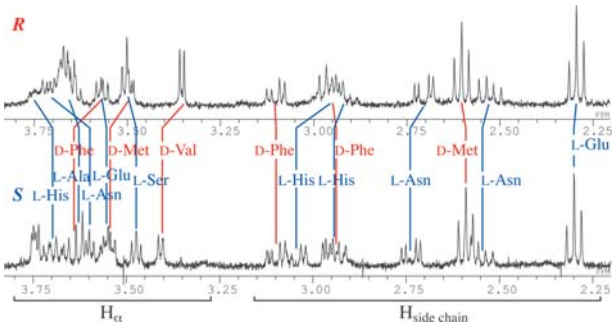


Fig. 1 Portions of the ^{1}H NMR spectra of the mixture of eight amino acids (L-Ala, D-Val, D-Met, D-Phe, L-Ser, L-Glu, L-Asn, L-His) in the presence of enantiomeric **3**. ^{1}H NMR (400 MHz, D_2O), pH 10.1 (adjusted by adding D_2O solutions of NaOD and DCl), [Sm] = 6.0 mM, concentration of each amino acid = 7.5 mM. Upper: in the presence of (*R*)-**3**; lower: in the presence of (*S*)-**3**.

usual way (5.7 M HCl, 110 °C, 24 h in a sealed tube) and the resulting hydrolysate was used for the shift study without further purification.

A consistent correlation was observed between the signs of ΔΔδ and the absolute configuration of each component amino acid. The ΔΔδ values for H_α signals were positive for L- and negative for D-amino acids. It should be noted that we have observed no exceptions for this correlation.

The correlation was also observed for the signals due to the side chain protons with relatively large ($|\Delta\Delta\delta| > 0.005$ ppm) signal separations. In contrast to the ΔΔδ values for H_α signals, the values were negative for L- and positive for D-isomers. These correlations are also the same as those previously observed for the enantiomeric mixtures containing amino acids of a single species (Fig. 2).§ In the case of side chain protons, some inconsistent signs were found. This may be due in part to the experimental errors originated from not exactly identical measurement conditions in two separate measurements (Table 1).¶ These inconsistencies were limited, however, to the signals due to side chain protons with small $|\Delta\Delta\delta|$ (less than 0.004 ppm) except for 2-H of the imidazole ring of histidine, which is one of two exceptions so far observed for amino acids in solutions of single species.[3] Therefore, even with side chain signals, assignment of the absolute

Table 1 Chemical shift differences between α-amino acid signals in the presence of (*R*)- and (*S*)-**3** at the same molar ratio in two separate solutions[a]

		ΔΔδ	
Entry	Amino acid	H_α	$H_{Sidechain}$(site[b])
1	L-Ala	+0.037	−0.017(β)
	D-Leu	−0.052	+0.036(β^{hi}), +0.016(γ), −0.003(δ^{hi}), +0.010(δ^{lo})
2	L-Ala	+0.013	−0.008(β)
	L-Ser	+0.015	−0.040($\beta^{\times 2}$)
	L-Asn	+0.068	−0.014(β^{hi}), −0.022(β^{lo})
3	L-Ala	+0.025	−0.012(β)
	D-Val	−0.054	+0.026(γ^{hi}), +0.001(γ^{lo})
	D-Phe	−0.06	+0.040(β^{hi}), +0.020(β^{lo})
	D-Met	−0.05	+0.012($\gamma^{\times 2}$), +0.005(ε)
	L-Ser	+0.029	−0.088($\beta^{\times 2}$)
	L-Asn	+0.10	−0.033(β^{hi}), −0.021(β^{lo})
	L-His	+0.08	−0.033(β^{hi}), −0.069(β^{lo}), +0.012(2′), −0.049(5′)
	L-Glu	+0.02	−0.005($\gamma^{\times 2}$)
4	L-Ala	+0.030	−0.005(β)
	D-Val	−0.032	+0.013(β^{hi}), +0.003(γ^{lo}), +0.025(γ)
	L-Ser	+0.027	−0.071($\beta^{\times 2}$)
	D-Asn	−0.090	+0.025(β^{hi}), +0.025(β^{lo})
	L-Glu	+0.02	−0.005($\gamma^{\times 2}$), +0.004($\beta^{\times 2}$), −0.025(γ)
	L-(4-OH)-Pro[c]	+0.055	−0.039(δ^{hi}), −0.135(δ^{lo})
5[d]	L-Ala	+0.030	−0.001(β)
	L-Val	+0.054	+0.004(β), −0.018 (γ^{hi}), +0.003 (γ^{lo})
	L-Leu	+0.05	−0.011(β^{hi}), −0.006(γ), +0.003(δ^{hi}), −0.003(δ^{lo})
	L-Phe	+0.06	−0.009(β^{hi}), +0.003(β^{lo})
	L-Met	+0.06	−0.008($\gamma^{\times 2}$), −0.002(ε^{SMe})
	L-Ser	+0.035	−0.020(β^{hi})
	L-Thr	+0.041	−0.014(β), −0.033(γ)
	L-Asn	+0.10	−0.013(β^{hi}), −0.019(β^{lo})
	L-His	+0.08	−0.017(β^{hi}), −0.036(β^{lo}), +0.016(2′), −0.024(5′)
	L-Glu	+0.06	+0.001($\gamma^{\times 2}$)
6	L-Tyr	+0.05	−0.026(β^{hi}), −0.005(β^{lo}), −0.009(2′), −0.017(3′)
	D-Ala	−0.043	+0.022(β)
	L-Phe	+0.092	−0.033(β^{hi}), −0.005(β^{lo})
	L-Met	+0.096	−0.045(β^{hi}), −0.011(β^{lo}), −0.022($\gamma^{\times 2}$), −0.008(ε^{SMe})

[a] ^{1}H NMR (400 MHz) spectra were measured for D_2O solutions of the amino acid mixture under the following conditions: pH 10.1; total concentration of amino acids, 60 mM; concentration of each amino acid: 30, 20, 7.5, 10, 10, 6 mM for entry 1, 2, 3, 4, 5, 6, respectively; concentration of (*R*)-or (*S*)-**3**: 6.00 mM. [b] ΔΔδ values for the signals due to diastereotopic methylene and methyl protons at β–δ positions observed at higher and lower field are designated by ^{hi} and ^{lo}, respectively. Those due to diastereotopic methylene protons not separately observed are denoted by $^{\times 2}$. [c] *cis*-4-Hydroxy-L-proline. [d] Spectra were determined at 600 MHz.

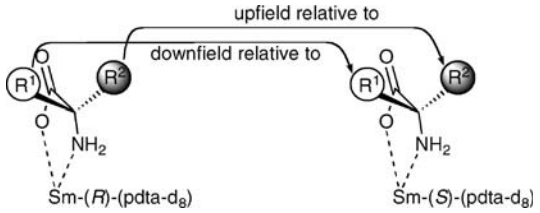


Fig. 2 Relative positions of corresponding α-amino acid signals in the presence of (*R*)- and (*S*)-**2**.[7]

configurations based on the ΔΔδ of sufficiently large separations ($|\Delta\Delta\delta| > 0.01$ ppm) should be reliable. Consequently, the opposite signs of ΔΔδs between H_α and side chain protons are the hallmark for the reliable assignment.

It may be worth commenting on the structural basis for the chiral discrimination in the multicomponent solution. The shift reagents resolve the enantiomer signals through the formation of diastereomeric complexes with enantiomeric substrates in rapid equilibrium. The chemical shift difference between the enantiomer signals is brought by the difference of formation constants (*K*) and the bound shifts (Δ_b).[8] The opposite signs of ΔΔδs observed between H_α and side chain protons indicate that ΔΔδs are determined by Δ_bs for corresponding nuclei in the complexes, and not by the difference in *K*s.‖ If the chemical shift differences are based only on the different formation constants of diastereomeric complexes, a simultaneous determination cannot be attained. In the solution of the mixture of amino acids, the concentration of the shift reagent available for a particular amino acid would be different between the two enantiomer solutions, since other component amino acids form the diastereomeric complex in different extents, depending on the different formation constant. No consistent results of the chemical shift were thus expected if *K* were the important factor.

In conclusion, we observed a highly consistent relationship between the absolute configuration of each of the component amino acids in mixtures of up to ten amino acid and the chemical shift changes induced by the chiral shift reagents (*R*)- and (*S*)-**3**. Increasing interest in bioactive peptides as well as proteins containing D-amino acids propels the need for more simple and reliable methods for the simultaneous determination of the absolute configurations of component amino acids.[9–11] These chiral shift reagents should be promising for the simultaneous determination of absolute configurations of component amino acids of peptides, especially those containing new α-amino acids.

We thank Prof. Shigeki Matsunaga at the University of Tokyo for suggesting the possible application of the samarium complex **3** to amino acid mixtures.

Notes and references

‡ For over 16 of a variety of α-amino acids, the correlations between absolute configurations and the induced shifts upon signals of α-protons and side chain protons were examined. 2,3-diaminopropanoic acid was found as the sole exception for the regularity observes for α-proton. It was confirmed that the β-amino group disturbed the regularity though its coordination with Sm(III). This can be avoided by lowering the pH of the solution used for the measurement; thus, the normal correlation was observed under neutral conditions. All signals of side chain protons conformed to the regularity except for the 2-H signal of imidazole from the side chain of histidine.

§ In ref. 3, we reported the enantiomer signal resolution of a single species of amino acid in the presence of (*R*)-**2**. The relative position of signal due to L-isomers was low for H_α and high for side chain protons. These results correspond to the positive and negative signs of ΔΔδs observed for H_α and other protons, respectively.

¶ The errors in chemical shifts were examined by repeated measurements for the protons of L-valine ([Val] = 0.06 M, pH = 10.0, [(*R*)-**2**]/[Val] = 0.1) to be ±0.005, ±0.003, ±0.002, and ±0.002 ppm for H_α, H_β, H_γ, and H_γ' signals, respectively. Although the error for H_α signal was 1/6 to 1/10 compared to the observed ΔΔδ value in mixtures, those for H_β and H_γ signals were comparable to the observed ΔΔδ values of the corresponding signals of valine with inconsistent signs.

‖ If the signs of ΔΔδs are determined solely by the difference of *K*s, the signs of ΔΔδ for H_α and others must be the same. Although the difference in *K*s is not considered to be the decisive factor in the present case, it might be a source of inconsistent signs together with the experimental errors.

1 I. Ghosh, H. Zeng and Y. Kishi, *Org. Lett*., 2004, **6**, 4715; I. Ghosh, Y. Kishi, H. Tomoda and S. Omura, *Org. Lett*., 2004, **6**, 4719; C. M. Adams, I. Ghosh and Y. Kishi, *Org. Lett*., 2004, **6**, 4723.
2 (*a*) K. Kabuto and Y. Sasaki, *J. Chem. Soc., Chem. Commun*., 1987, 670; (*b*) K. Kabuto and Y. Sasaki, *Tetrahedron Lett*., 1990, **31**, 1031.
3 A. Inamoto, K. Ogasawara, K. Omata, K. Kabuto and Y. Sasaki, *Org. Lett*., 2000, **2**, 3543.
4 P. Marfey, *Carlsberg Res. Commun*., 1984, **49**, 591.
5 T. Iida, T. Santa, A. Toriba and K. Imai, *Biomed. Chromatogr*., 2001, **12**, 319.
6 K. Fujii, Y. Ikai, H. Oka and K. Harada, *Anal. Chem*., 1997, **69**, 5146.
7 The chelate structure of this model is based on the analysis of the LIS for alanine by lanthanide–edta complexes analogous to **1** or **2**: A. D. Sherry, A. Stark, J. R. Ascenso and C. F. C. J. Geraldes, *J. Chem. Soc., Dalton Trans*., 1981, 2078.
8 D. J. Raber, in *Lanthanide Shift Reagents in Stereochemical Analysis*, ed. T. C. Morrill, VCH, New York, 1986, pp. 55–105.
9 For recent papers, see: (*a*) W. Gu, M. Cueto, P. R. Jensen, W. Fenical and R. B. Silverman, *Tetrahedron*, 2007, **63**, 6535; (*b*) D.-C. Oh, P. R. Jensen and W. Fenical, *Tetrahedron Lett*., 2006, **47**, 8625; (*c*) S. Dutertre, N. G. Lumsden, P. F. Alewood and R. J. Lewis, *FEBS Lett*., 2006, **580**, 3860; (*d*) N. Oku, R. Krishnamoorthy, A. G. Benson, R. L. Ferguson, M. A. Lipton, L. R. Phillips, K. R. Gustafson and J. B. McMahon, *J. Org. Chem*., 2005, **70**, 6842.
10 A. Jilekl and G. Kreil, *Monatsh. Chem*., 2008, **139**, 1.
11 (*a*) A. M. S. Mayera and K. R. Gustafson, *Eur. J. Cancer*, 2006, **42**, 2241; (*b*) A. M. S. Mayera and K. R. Gustafson, *Eur. J. Cancer*, 2004, **40**, 2676; (*c*) S. Matsunaga and N. Fusetani, *Curr. Org. Chem*., 2003, **7**, 945; (*d*) D. Faulkner, *Nat. Prod. Rep*., 2002, **19**, 1.