

*Electronic Supporting Information*

**Promising Use of Sm(III)–{1,2-propanediamine-*N,N,N',N'*-tetra( $\alpha,\alpha$ -dideuterioacetate)}  
Complex for Direct Simultaneous NMR Determination of Absolute Configurations of  
Each  $\alpha$ -Amino Acids in Peptide Hydrolysate Mixtures**

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## Materials and methods:

*Preparation of (R)- and (S)-propylenediamine-N,N,N',N'-tetra( $\alpha,\alpha$ -diduterioacetate) [(R)- and (S)-H<sub>4</sub>pdta-d<sub>8</sub>]:* The deuterated ligands were prepared from (R)- and (S)-propylenediamine and bromoacetic acid-d<sub>3</sub> (Aldrich) in the same way as described in the literature for the undeuterated ligands. See R. M. Wing, K. P. Callahan, *Inorg. Chem.* **1969**, *8*, 2303. Deuterium contents of (R)- and (S)-H<sub>4</sub>pdta-d<sub>8</sub> was evaluated to be > 99 % by NMR.

(R)-H<sub>4</sub>pdta-d<sub>8</sub> (recryst. from H<sub>2</sub>O): Mp. 188 °C (decomp.);  $[\alpha]_D^{23}$  -46.4 (*c* 0.45, H<sub>2</sub>O); <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O)  $\delta$  1.10 (3H, d, *J* = 6.6 Hz), 2.96 (1H, dd, *J* = 14.7, 12.2 Hz), 3.14 (1H, dd, *J* = 14.7, 3.3 Hz), 3.59 (1H, m); <sup>13</sup>C NMR (100 MHz, D<sub>2</sub>O)  $\delta$  10.88 (CH<sub>3</sub>), 53 (CD<sub>2</sub>, br), 55.5 (CD<sub>2</sub>, br), 55.78 (CH<sub>2</sub>), 58.06 (CH), 171.84 (CO<sub>2</sub>H), 173.51 (CO<sub>2</sub>H); Anal. Found: C, 38.83; N, 8.02. Calcd for C<sub>11</sub>H<sub>10</sub>D<sub>8</sub>N<sub>2</sub>O<sub>8</sub>·*n*H<sub>2</sub>O (*n* = 1.3): C, 39.02; N 8.27.

(S)-H<sub>4</sub>pdta-d<sub>8</sub> (recryst. from H<sub>2</sub>O):  $[\alpha]_D^{23}$  +47.4 (*c* 0.50, H<sub>2</sub>O): Anal. Found: C, 39.70; N, 8.48. Calcd for C<sub>11</sub>H<sub>10</sub>D<sub>8</sub>N<sub>2</sub>O<sub>8</sub>·*n*H<sub>2</sub>O (*n* = 1): C, 39.75; N, 8.43.

*Preparation of of 'Na[Sm-{(R)-pdta-d<sub>8</sub>}]' and 'Na[Sm-{(S)-pdta-d<sub>8</sub>}]' (Na[(R)-3] and Na[(S)-3]):*

Na[(R)-3] and Na[(S)-3] were prepared from Sm<sub>2</sub>O<sub>3</sub> and the corresponding pdta-d<sub>8</sub> ligands in a similar way for the preparation of undeuterated Eu-pdta. See C. Kabuto, K. Kabuto, Y. Sasaki, T. Nishiyama, K. Umakoshi, *J. Chem. Soc., Chem. Commun.* **1993**, 381.

Na[(R)-3]: Mp. >290,  $[\alpha]_D^{25.5}$  -3.1 (*c* 0.99, H<sub>2</sub>O, pH 8.98),  $[\alpha]_D$  is very dependent on temperature and pH; HRMS(ESI, negative) Calcd for C<sub>11</sub>H<sub>6</sub>D<sub>8</sub>N<sub>2</sub>O<sub>8</sub>Sm (M – Na<sup>+</sup>): 462.0455, Found 462.0460. Anal. Calcd for C<sub>11</sub>H<sub>6</sub>D<sub>8</sub>N<sub>2</sub>NaO<sub>8</sub>Sm·H<sub>2</sub>O: C, 26.34; N, 5.58. Found: C, 26.31; N, 5.52.

Na[(S)-3]:  $[\alpha]_D^{25.5}$  +3.5 (*c* 0.99, H<sub>2</sub>O, pH 9.07); Anal. Calcd for C<sub>11</sub>H<sub>6</sub>D<sub>8</sub>N<sub>2</sub>NaO<sub>8</sub>Sm·H<sub>2</sub>O: C, 26.34; N, 5.58. Found: C, 26.16; H, 3.35; N, 5.378.

*X-ray crystallographic analysis of Na [rac-3]:*

The structure of Na[rac-3] was confirmed by X-ray crystallographic analysis on racemic **3** prepared by mixing equal amounts of Na[(R)-3] and Na[(S)-3]. See C. Kabuto, K. Kabuto, Y. Sasaki, T. Nishiyama, K. Umakoshi, *J. Chem. Soc., Chem. Commun.* **1993**, 381.

Data collected on a Rigaku Saturn CCD diffractometer using MoK $\alpha$  radiation and refined by least-squares method on *F*<sup>2</sup> with the SHELXH-97 program (G. M. Sheldrick, SHELX-97,

Program for the Solution of Crystal Structures, Universität Göttingen, 1997). Crystallographic data have been deposited with Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 651161. The data can be obtained free charge from The Cambridge Crystallographic Data Centre via [www.ccdc.cam.ac.uk/data\\_request/cif](http://www.ccdc.cam.ac.uk/data_request/cif).

*Crystal data:*  $[\text{Sm}(\text{C}_{11}\text{H}_{14}\text{O}_8\text{N}_4)(\text{H}_2\text{O})_2]_6\text{Na}^+_6 \cdot 30\text{H}_2\text{O}$ ;  $M_r=3610.42$ , monoclinic, space group  $P2_1/n$  (No. 14); crystal sizes =  $0.4 \times 0.2 \times 0.15 \text{ mm}^3$ ,  $a = 14.701(1)$   $b = 36.584(17)$ ,  $c = 25.677(1)$  Å,  $\beta = 90.134(3)^\circ$ ,  $V=13809(11)$  Å<sup>3</sup>;  $Z = 4$ ,  $\rho_{\text{calad}} = 1.736 \text{ g/cm}^3$ ,  $\mu(\text{Mo-K}\alpha) = 2.645 \text{ mm}^{-1}$ ,  $\lambda = 0.71073$  Å,  $F(000) = 7224$ ,  $\omega$ -scan mode,  $\theta_{\text{min-max}} = 3.0\text{-}27.0^\circ$ ,  $T = 173\text{K}$ , total data = 158740, unique data = 31008 ( $R_{\text{int}} = 0.075$ ), 1680 parameters,  $R_1(19482 \text{ data with } I > 2\sigma(I)) = 0.0511$  and  $wR_2(\text{all reflections}) = 0.1728$ ,  $\text{GOF} = 1.000$ ,  $\rho_{\text{max}} = 3.40 \text{ e}\text{\AA}^3$ ,  $\rho_{\text{min}} = -2.10 \text{ e}\text{\AA}^3$ . Non-hydrogen atoms in the cationic part and Na atoms were refined anisotropically, and H atoms were fixed. Of which 30 independent guest water molecules, 26 oxygen atoms were refined anisotropically (occupancy = 1.0) and 4 atoms isotropically (occupancy = 0.5), where no H atoms were applied.

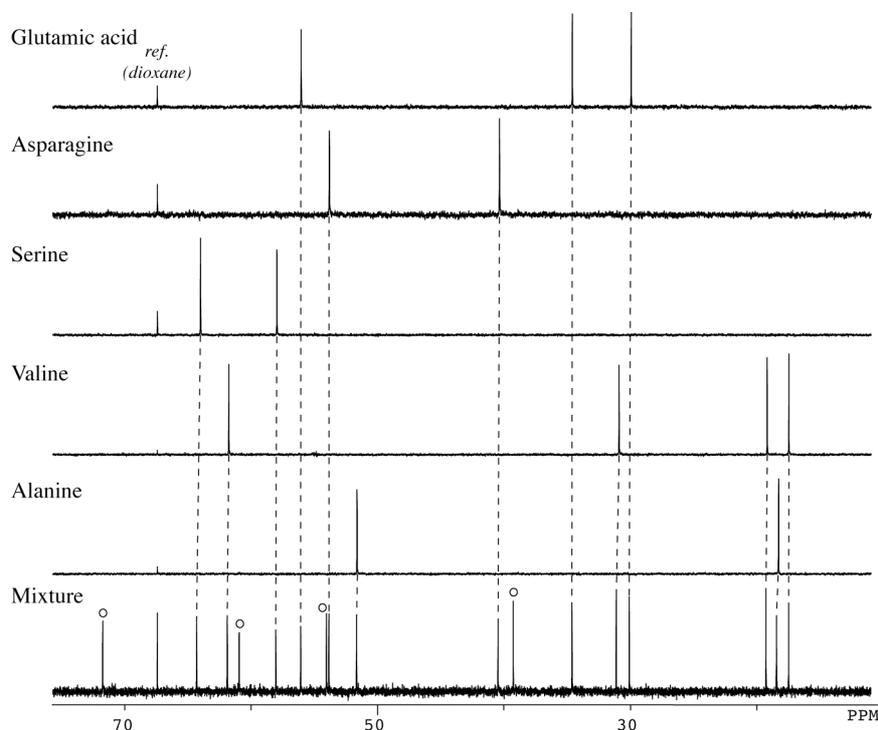
#### *NMR shift study on amino acid mixtures:*

A mixture, consisted of equimolar 2~10 amino acids (0.096 mmol in total), was dissolved in 1.6 ml of D<sub>2</sub>O. After pH of the solution was adjusted to 10.1 using D<sub>2</sub>O solutions of NaOD and DCl (0.1-0.01 M), 0.6 ml portions of the solution were taken in two separated sample tubes. To one of the two tubes, aliquots of 0.36 M D<sub>2</sub>O solution (pH adjusted to 8.0) of (*R*)-**1** were added successively. By the same procedure, D<sub>2</sub>O solution of (*S*)-**1** was added to the other. NMR spectra were determined on each sample solution.

*Hydrolysis of [D-Ala<sup>2</sup>, Met<sup>5</sup>]-enkephalin:* Commercially available [D-Ala<sup>2</sup>, Met<sup>5</sup>]-enkephalin acetic acid salt (Peptide Institute Inc. (Japan), 25 mg, 0.038 mmol) was dissolved in 10 ml of 5.7 M HCl and was heated at 110 °C for 24 h in a sealed tube. After the solution was concentrated to dryness under reduced pressure, the residue was dissolved in 1.6 ml of D<sub>2</sub>O. After adjusting pH to 10 with NaOD-D<sub>2</sub>O solution, the NMR shift study was conducted at 400 MHz.

## A typical procedure for assigning the NMR signals measured for amino acid mixtures.

1. Assignment of each  $^{13}\text{C}$  signal of mixtures by comparison with the NMR spectra of typical amino acids in  $\text{D}_2\text{O}$  pH adjusted around 10.

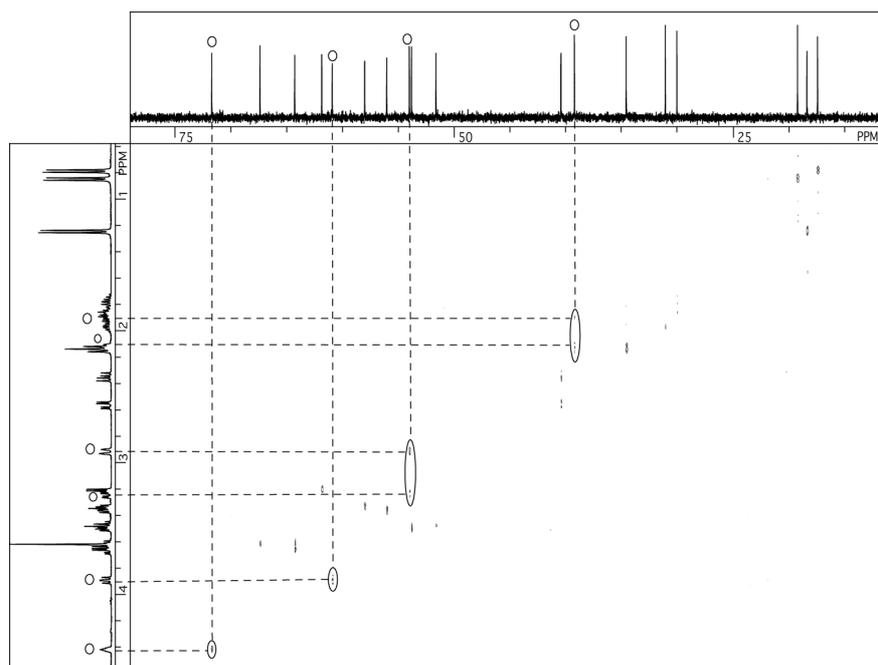


$^{13}\text{C}$  NMR spectra of single amino acids in  $\text{D}_2\text{O}$  (pH ~10).

Dependence of the chemical shifts on the concentration and induced shift on the  $^{13}\text{C}$  signals of substrate amino acids by the Sm reagent were sufficiently small therefore comparison of the  $^{13}\text{C}$  NMR spectrum of the mixture with those of single amino acids is useful to assign the signals observed for mixture.

$^{13}\text{C}$  NMR spectrum of an amino acid mixture containing L-Ala, D-Val, L-Ser, D-Asn, L-Glu, and L-4-hydroxyproline (entry 4 in table 1) in  $\text{D}_2\text{O}$  (pH ~10). Signals marked with circles can be assigned as those of uncommon amino acid (L-4-hydroxyproline).

2. Assignment of  $^1\text{H}$  signal from C-H correlation.

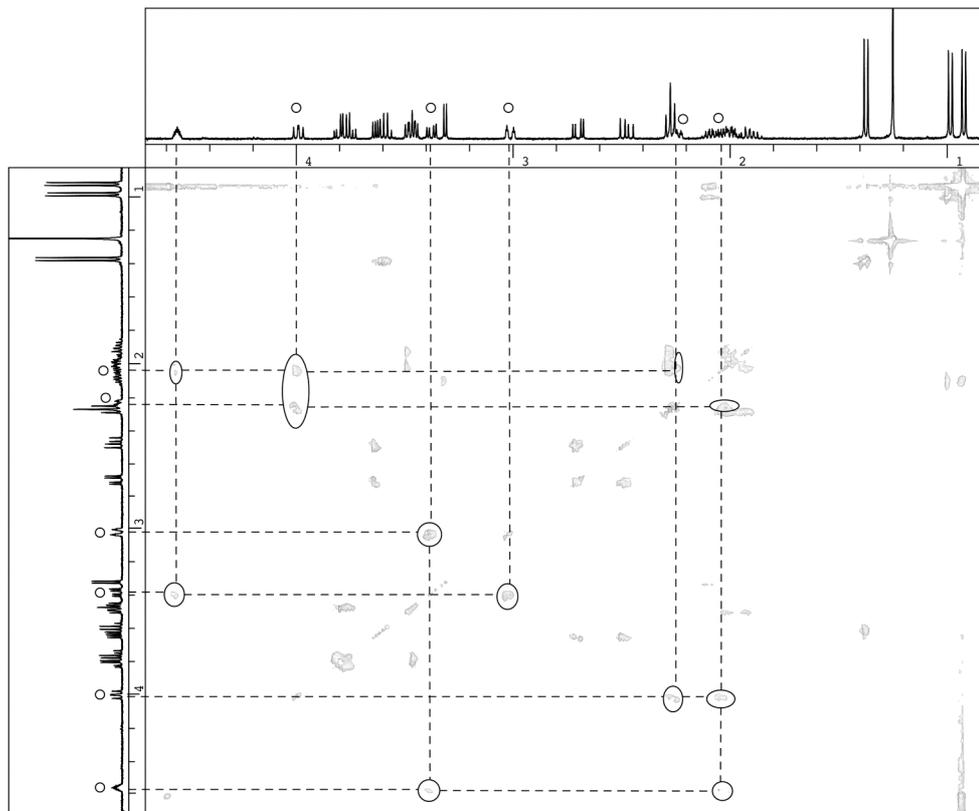


C-H COSY spectrum of an amino acid mixture containing L-Ala, D-Val, L-Ser, D-Asn, L-Glu, and L-4-hydroxyproline (entry 4 in table 1) in  $\text{D}_2\text{O}$  (pH ~10).

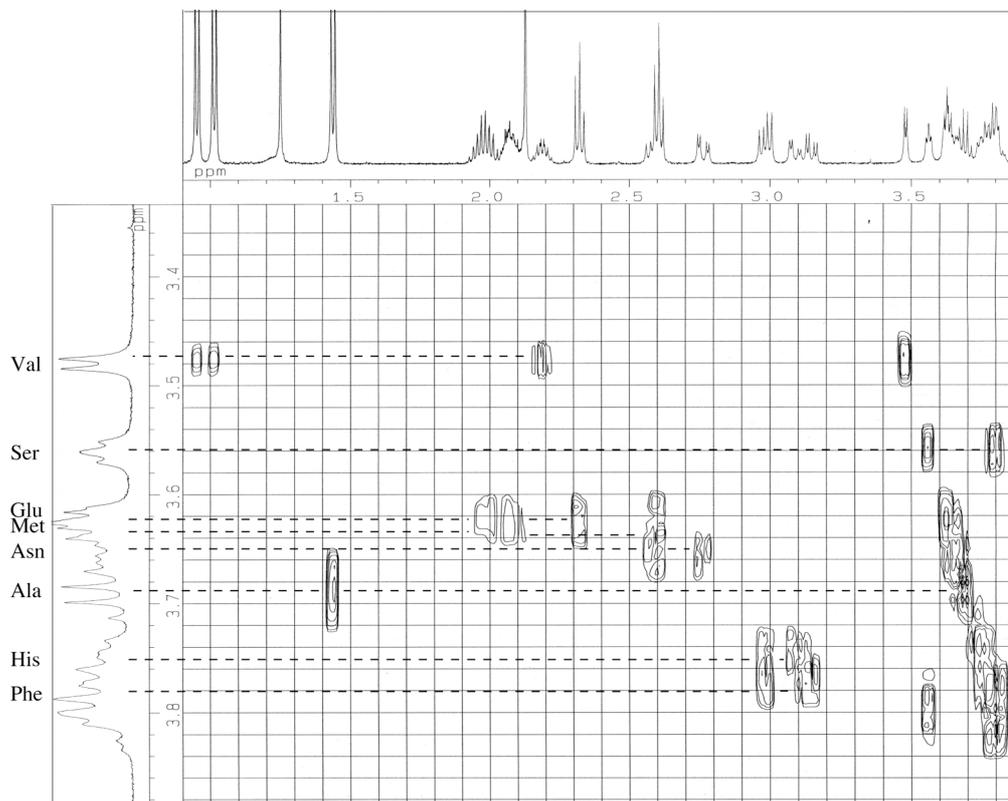
Each  $^1\text{H}$  signal can be assigned on the basis of C-H correlation spectrum.

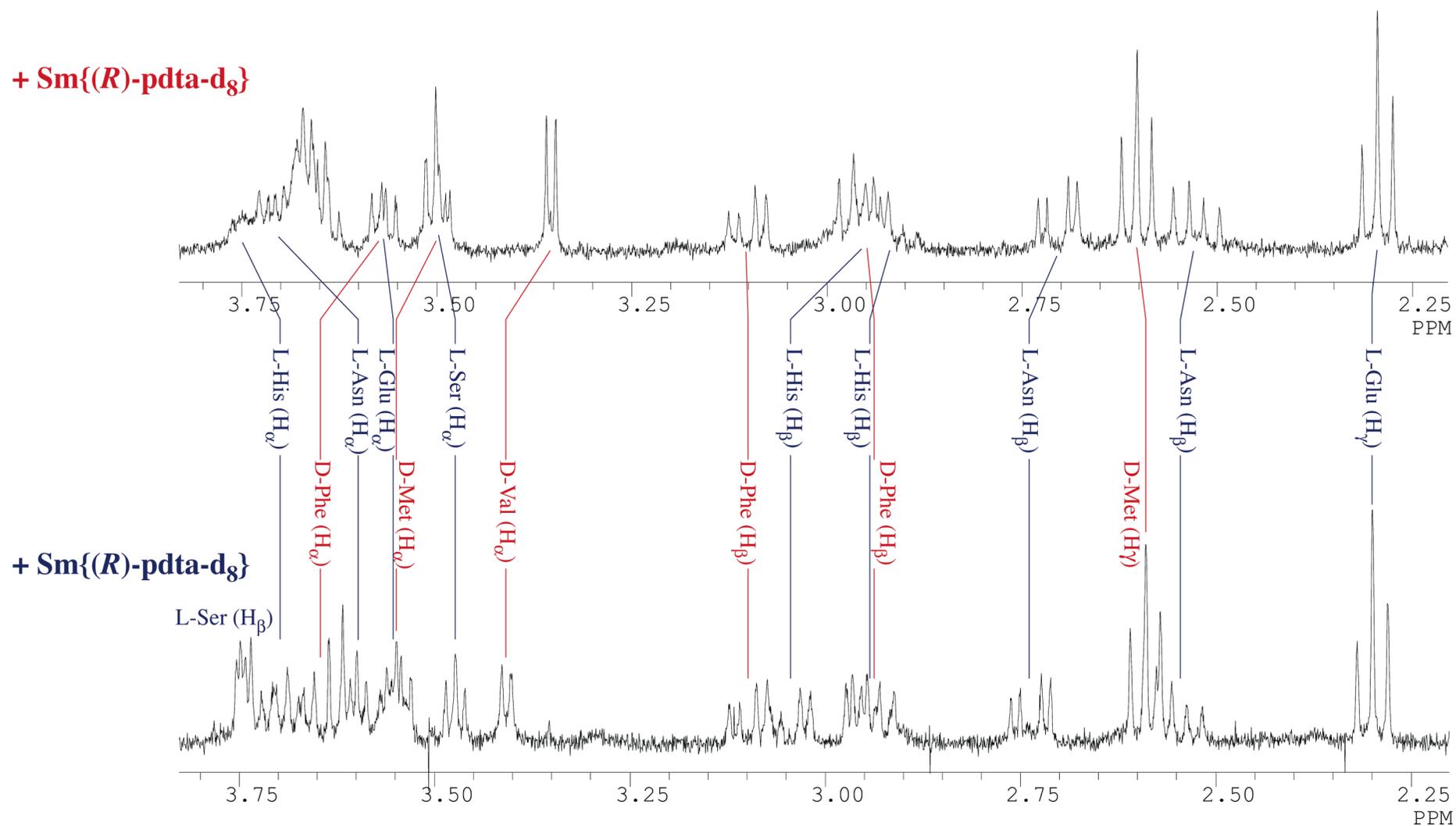
3. Confirmation of the assignment of  $^1\text{H}$  signals by H-H correlation spectrum.

A. H-H COSY of spectrum of a six amino acid mixture (Table 1, entry 4).



B. TOCSY spectrum of a eight amino acid mixture in the presence of  $\text{Sm}\{(\text{S})\text{-pdta-d8}\}$  (Table 1, entry 3).





**Figure S1.** Partial  $^1\text{H}$  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**.  $^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ ), pH 10.1,  $[\text{Sm}] = 6.0$  mM, concentration of each amino acid = 7.5 mM. Upper: in the presence of  $(R)\text{-3}$ , Lower: in the presence of  $(S)\text{-3}$ .