# Electronic Supporting Information (ESI) for

## One-pot analysis of enantiomeric excess of free amino acids by electrospray ionization mass spectrometry

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**Contents of SI**

## 1. General and Materials

**General.** $^1$H-NMR (270 MHz) and $^{13}$C-NMR (67.5 MHz) spectra were measured with a JEOL JNM EX-270. Tetramethyl silane (TMS, δ 0 ppm) was used as the internal standard in CDCl$_3$. High resolution mass spectra (ESI, positive ion mode) were measured with a JEOL AccuTOF LC-plus 4G mass spectrometer and JEOL YOKUDELNA ion peak [M + Na]$^+$ (*m/z* 430.9141952) was used as an internal standard for mass calibration. IR spectra were measured with a HORIBA FT-IR 730 in the range of 650-4000 cm$^{-1}$. Circular dichroism (CD) spectra were measured in the range of 230–400 nm in 1 cm quartz cuvette with a Jasco J-820. Elemental analysis was measured with a CE INSTRUMENTS EA-1110 CHNS-O. Melting point was measured with a SEIKO DSC SSC/5200. Optical rotation was measured on a Jasco P-1020 with a 10 cm quartz cuvette irradiated with sodium D line. TLC was performed by using a Merck TLC Silica gel 60 F$_{254}$ 25 Glass plates detected by iodine as indicator.

**Materials.** Chiral tetradentate *N,N*´-ethylene-bis(*N*-methyl-*S*-alanine methyl amide) (*S,S*-**L**) and *N,N*´-ethylene-bis(*N*-methyl-*R*-alanine methyl ester) (*R,R*-**L**-OMe) were synthesized according to previous reports.[S1–S3] The synthesized compounds were purified by column chromatography using NH silica gel (Fuji silysia chemical, LTD.) as the stationary phase. Methanol for synthesis was distilled over quicklime as a desiccant.

LC/MS grade methanol (Fujifilm Wako pure chem. Co.) was purchased and used for ESI-MS. All other reagents containing metal salts and amino acids were purchased from commercial suppliers and used without further purification.

## 2. Synthesis of *R,R*-**L**-*d$_6$*[S4]
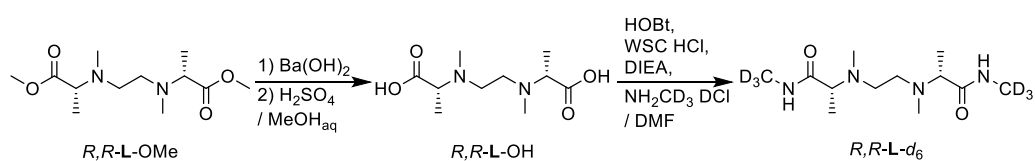


To a solution of *R,R*-**L**-OMe (1.03 g, 3.96 mmol) in 5 mL methanol, 0.1 M Ba(OH)$_2$ aqueous solution (9.6 mL) was added and the mixture was stirred for 30 min at room temperature. After starting material was disappeared on the TLC (silica gel, CH$_3$OH/CHCl$_3$ = 1/9, v/v), 0.1 M H$_2$SO$_4$ aqueous solution (8 mL) was added to the solution. The precipitation was removed by filtration and the solution was evaporated. The residue was dried in vacuo to give crude *R,R*-**L**-OH (0.93 g). *R,R*-**L**-OH was used for next reaction without further purification.

To a solution of *R,R*-**L**-OH (0.093 g, 0.401 mmol), HOBt (0.108 g, 0.802 mmol) and DIEA (0.184 g, 1.60 mmol) in 10 mL DMF, WSC hydrochloride (0.153 g, 0.801 mmol) in 6 mL DMF was added in 3 portions every 5 min and the solution was stirred for 30 min in an ice bath. $NH_2CD_3$ deuteriumchloride (0.054 g, 0.802 mmol) was added to the solution and the mixture was stirred overnight in a water bath. The product was extracted with chloroform and the organic phase was washed 3 times with saturated $NaHCO_3$ aqueous solution, and then the organic phase was dried over anhydrous sodium sulfate. The solution was evaporated, and the residue was purified with column chromatography (NH silica gel, $CH_3OH/CHCl_3$ = 1/5, v/v) to give colorless oil. The liquid was diluted with diethyl ether and the solution was evaporated. This operation repeated several times to give a white solid. The solid was washed with diethyl ether chilled in refrigerator and dried in vacuo to give *R,R*-**L**-$d_6$ (0.036 g, 34.8%). $\delta$H (270 MHz, $CDCl_3$) 7.87 (bs, 2H, N<u>H</u>) 3.29 (q, 2H, $J$ = 7.1, Ala-<u>H</u>), 2.56 (d, 2H, $J$ = 9.1, ethylene), 2.30 (s, 6H, N-C<u>H</u>$_3$), 2.27 (d, 2H, $J$ = 9.1 Hz, ethylene), 1.27 (d, 6H, $J$ = 7.1 Hz, Ala-C<u>H</u>$_3$); $\delta$C (67.5 MHz, $CDCl_3$) 9.67, 39.9, 51.1, 63.9, 76.6, 174.1; $\nu_{max}$(KBr)/cm$^{-1}$ 3300, 2979, 1655, 1522, 1365, 1213, 1115; Found: C, 53.9; H, 10.01; N, 21.0; calculated for $C_{12}H_{20}D_6N_4O_2$ C, 54.5; H, 7.62; D, 4.57; N, 21.2; HRMS (ESI) calculated for $C_{12}H_{20}D_6N_4O_2$ [M + Na]$^+$ for *m/z* 287.2330, found *m/z* 287.2372; [$\alpha$]$^{26.5}_D$ −23.3 (*c* 0.100, MeOH); mp 87.9 ℃.

The presence of deuterium affects the elemental analysis of hydrogen. The hydrogen and deuterium contents were corrected by the following formula from the observed hydrogen value.[S5]

H (%) = observed H value (%) × number of H / (number of H + number of D × 1.028)

$$= 10.01 \times 20 / (20 + 6 \times 1.028)$$

$$= 7.65 \ (\%)$$

D (%) = observed H value (%) × number of D × {1.028 / (number of H + number of D × 1.028)} × 1.944

$$= 10.01 \times 6 \times \{1.028 / (20 + 6 \times 1.028)\} \times 1.944$$

$$= 4.59 \ (\%)$$

### 3. Mass spectrometry

The machine condition of ESI mass spectra (positive ion mode) with a JEOL AccuTOF LC-plus JMS-T 100LP mass spectrometer was optimized to detect such metal complex ions with high sensitivity as follows; voltage of spray needle = 2.5 kV, orifice1 = 40 V, orifice2 = 1 V, ring lens = 15 V, temperature of desolvation chamber = 100 °C, temperature of orifice1 = 50 °C, mass range = m/z 150-1000. The mass spectrum data was collected as the following condition: acquisition time = 0.497 s, wait time = 0.003 s (= single scan time = 0.500 s), measurement time = 2 min for 240 scans.

The accuracy of the 1:1 equivalent of the $Cu^{II}/S,S$-**L** and $Cu^{II}/R,R$-**L**-$d_6$ was calibrated on basis of the relative peak intensity (*IR/IS* values) of the complex ions, [$Cu^{II}(R,R$-**L**-$d_6$)(Gly − H)]$^+$ and [$Cu^{II}(S,S$-

**L**)(Gly – H)]$^+$, obtained by adding glycine (Gly).

## 4. Sample preparation

Host solution is prepared by following procedure; 1.20 mL of *S,S*-**L** and *R,R*-**L**-$d_6$ (1.00 × 10$^{-3}$ M each) in methanol and 1.00 mL of copper(II) chloride (2.00 × 10$^{-3}$ M) in methanol were mixed which was diluted to 20 mL by adding methanol. When the sample solution is prepared at a concentration lower than the follows and the mass spectrum is measured, the contribution of noise to the peak intensity value becomes large not to determine the *ee* of AA. Therefore, the following preparation conditions were adopted.

Single analysis sample solution is prepared by following procedure; 1.00 mL of host solution and 0.01 mL of equimolar mixture of *S*-AA (1.00 × 10$^{-3}$ M each) containing K$_2$CO$_3$ (equimolar for carboxyl group of AA) in water were mixed for the solution of mass measurements (mole ratio: CuCl$_2$/*S,S*-**L**/*R,R*-**L**-$d_6$/*S*-AA = 1.0/0.6/0.6/0.1). In the case adding *S*-Asp, *S*-Glu, *S*-Lys and *S*-Ser, 1.00 mL of host solution and 0.05 mL of equimolar mixture of *S*-AA (1.00 × 10$^{-3}$ M each) containing K$_2$CO$_3$ (equimolar for carboxyl group of AA) in water were mixed for the solution of mass measurements (mole ratio: CuCl$_2$/*S,S*-**L**/*R,R*-**L**-$d_6$/*S*-AA = 1.0/0.6/0.6/0.5).

Simultaneous analysis sample solution is prepared by following procedure; 1.00 mL of host solution and 0.15 mL of 16 AAs mixture (*S*-Ala, *S*-Asn, *S*-Gln, *S*-His, *S*-Hyp, *S*-Ile, *S*-Leu, *S*-Met, *S*-Phe, *S*-Phg, *S*-Pro, *S*-Thr, *S*-Tle, *S*-Trp, *S*-Tyr, *S*-Val, *S*-Arg, *S*-Asp, *S*-Glu, *S*-Lys, and *S*-Ser, 1.00 × 10$^{-3}$ M each) containing K$_2$CO$_3$ (equimolar for carboxyl group of AA) in water were mixed for the solution of mass measurements (mole ratio: CuCl$_2$/*S,S*-**L**/*R,R*-**L**-$d_6$/AA(total) = 1.0/0.6/0.6/1.5).

Under the optimized preparation conditions, equivalents of the AAs to the copper complex are high (1.5) and it does not appear to be a competitive condition. However, when the concentration is small (*ca.* 10$^{-5}$ M), the association constants are small, which may be a competitive condition. As shown in Figure S8, the concentration ratio of the three-component complex ions generated in the three-component complexation equilibrium system changes depending on the association constants ($K_{SS\text{-}S}$ and $K_{RR\text{-}S}$) and the values approach the ratio of the association constants ($K_{SS\text{-}S}/K_{RR\text{-}S}$, here, it is assumed at 1.5) under competitive condition. When the association constants are small ($K_{SS\text{-}S}$ = 150 M$^{-1}$, $K_{RR\text{-}S}$ = 100 M$^{-1}$), it becomes a competitive condition even if the amino acid is excessive (dotted line B) and the concentration ratio of the three-component complex ions is almost maintained. Indeed the *Ie* value was maintained even when equivalent of AAs was actually changed from 0.5 eq. to 1.5 eq. shown in

Figure S9. This suggests that the association constants ($K_{SS-S}$ and $K_{RR-S}$) of this complexation equilibrium system are about 100 $M^{-1}$.

## 5. References

S1   L. N. Schoenberg, D. W. Cooke and C. F. Liu, *Inorg. Chem.*, 1968, **7**, 2386–2393.

S2   H. Miyake, K. Yoshida, H. Sugimoto and H. Tsukube, *J. Am. Chem. Soc.*, 2004, **126**, 6524– 6525.

S3   T. Nakakoji, H. Sato, D. Ono, H. Miyake, S. Shinoda, H. Tsukube, H. Kawasaki, R. Arakawa and M. Shizuma, *Chem. Commun.*, 2020, **56**, 54–57.

S4   H. Miyake, H. Kamon, I. Miyahara, H. Sugimoto and H. Tsukube, *J. Am. Chem. Soc.*, 2008, **130**, 792–793.

S5   M. Okumiya, in the abstract book of *19th The mini-salon of organic micro analysis*. 1998.

## 6. Figures
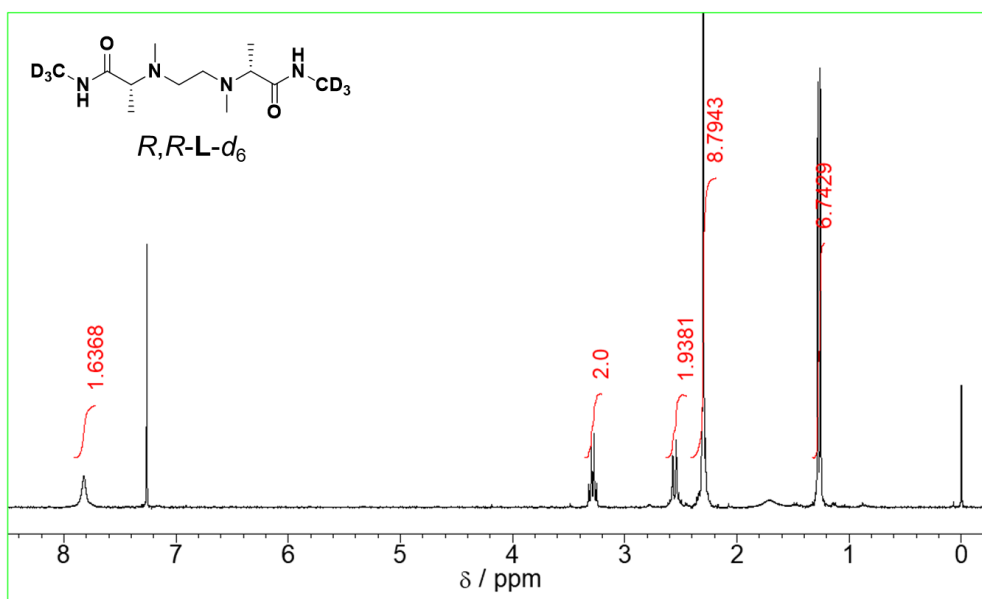


**Figure S1.** ¹H-NMR spectrum of *R,R*-**L**-*d₆*
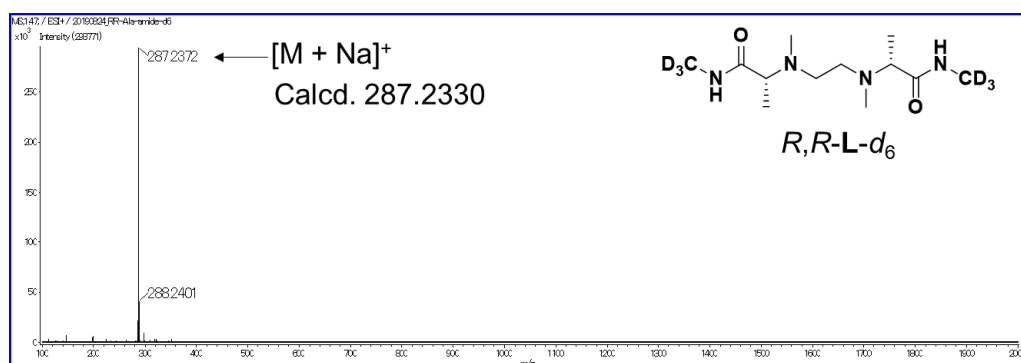


**Figure S2.** HR mass spectrum of *R,R*-**L**-*d₆*
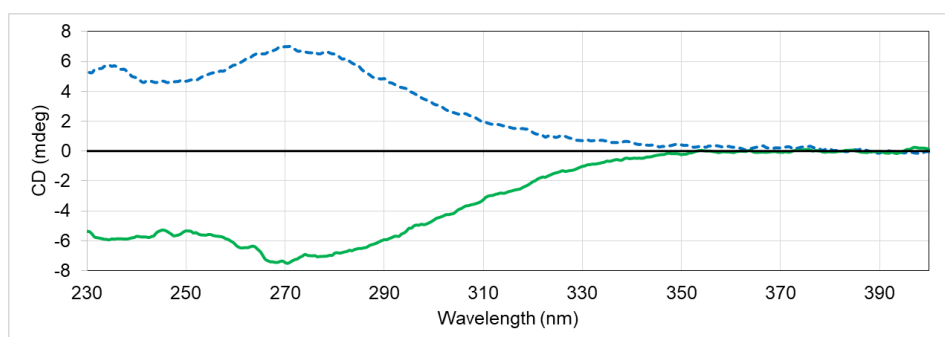


**Figure S3.** CD spectra of a mixed solution of CuCl$_2$ and *S,S*-**L** (blue dashed line) and mixed solution of CuCl$_2$ and *R,R*-**L**-*d₆* (green solid line) in methanol. [CuCl$_2$]$_0$ = [*S,S*-**L**]$_0$ = [*R,R*-**L**-*d₆*]$_0$ = $1.0 \times 10^{-4}$ M.
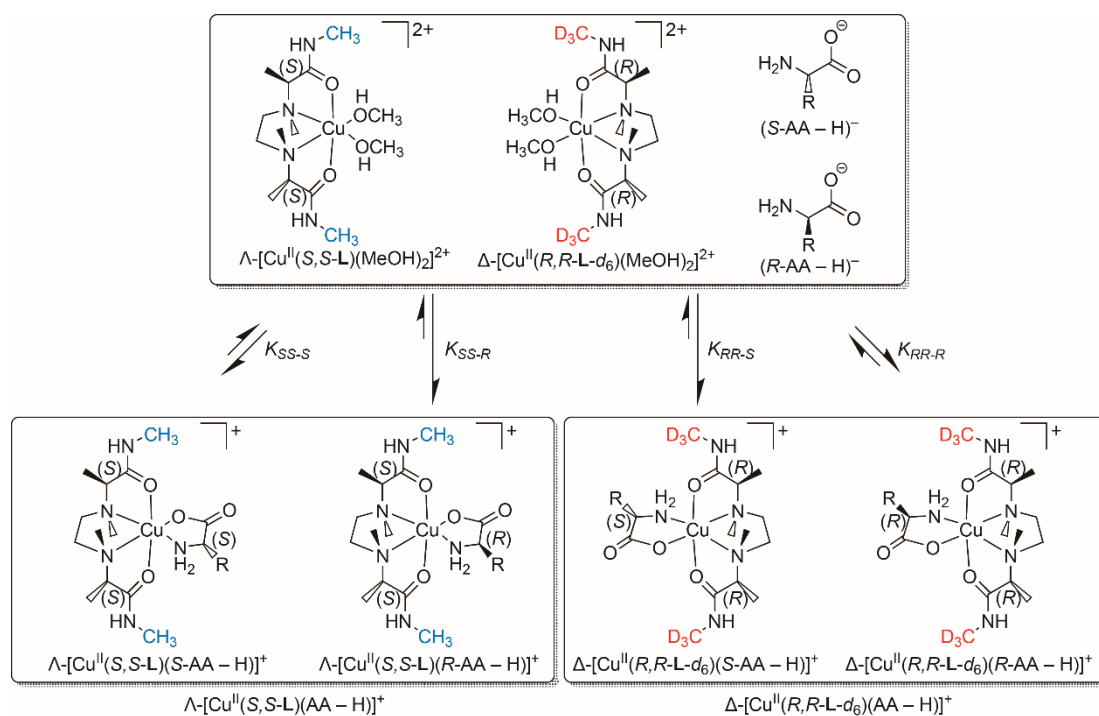
**Figure S4.** Formation of three-component complexes in a system of CuCl$_2$/*S,S*-**L**/*R,R*-**L**-*d$_6$*/*S*-AA/*R*-AA.
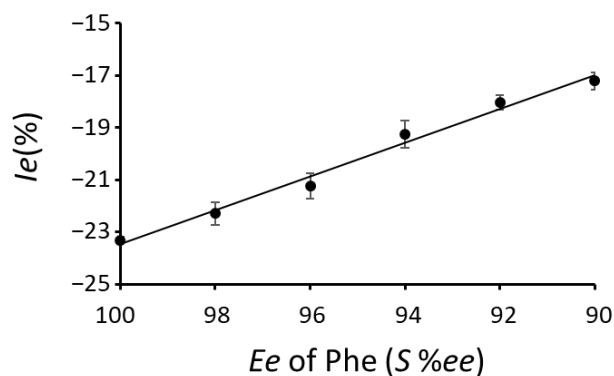


**Figure S5.** Correlation between *Ie* vales of [Cu$^{II}$(*S,S*-**L**)(Phe − H)]$^+$ and [Cu$^{II}$(*R,R*-**L**-*d$_6$*)(Phe − H)]$^+$ in mass spectra using *S*90–100 %*ee* of Phe. [CuCl$_2$]$_0$ = 9.90 × 10$^{-5}$ M, [*S,S*-**L**]$_0$ = [*R,R*-**L**-*d$_6$*]$_0$ = 5.94 × 10$^{-5}$ M, and [Phe]$_0$ = [K$_2$CO$_3$]$_0$ = 9.90 × 10$^{-6}$ M. [CuCl$_2$]$_0$/[*S,S*-**L**]$_0$/[*R,R*-**L**-*d$_6$*]$_0$/[Phe]$_0$ = 1.0/0.6/0.6/0.1.

(a)
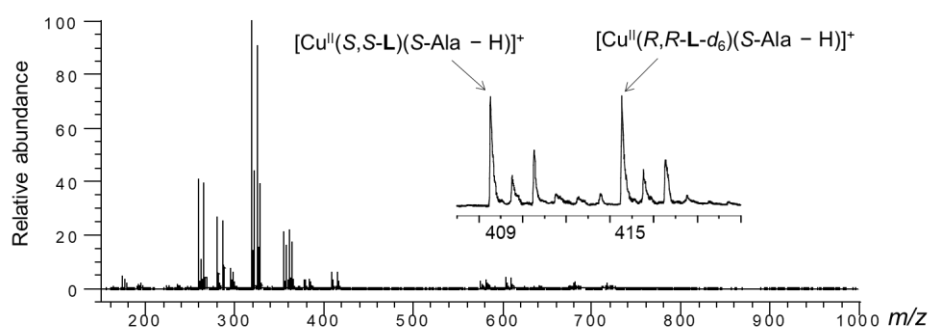


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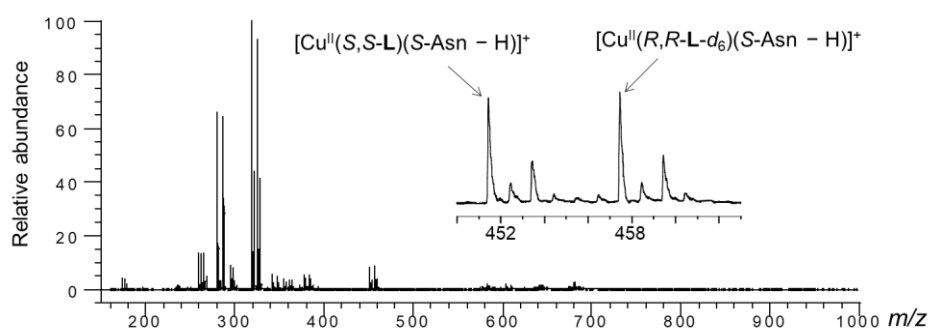


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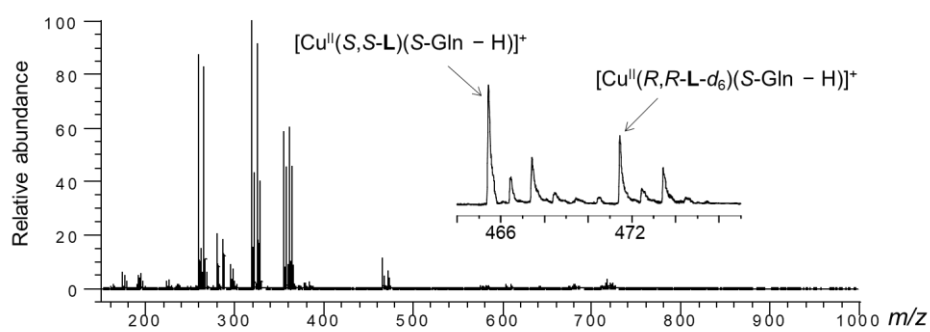


***Figure S6.*** Mass spectra of a mixture of $Cu^{II}$/*S,S*-**L**/*R,R*-**L**-$d_6$/*S*-AA in water/methanol (1/100, v/v). $[CuCl_2]_0 = 9.90 \times 10^{-5}$ M, $[S,S$-$L]_0 = [R,R$-$L$-$d_6]_0 = 5.94 \times 10^{-5}$ M and $[S$-$AA]_0 = [K_2CO_3]_0 = 9.90 \times 10^{-6}$ M. $[CuCl_2]_0/[S,S$-$L]_0/[R,R$-$L$-$d_6]_0/[S$-$AA]_0 = 1.0/0.6/0.6/0.1$. AA = (a) Ala, (b) Asn and (c) Gln.
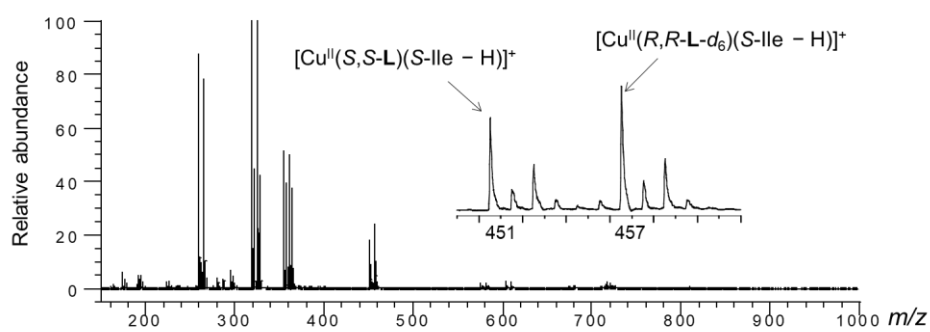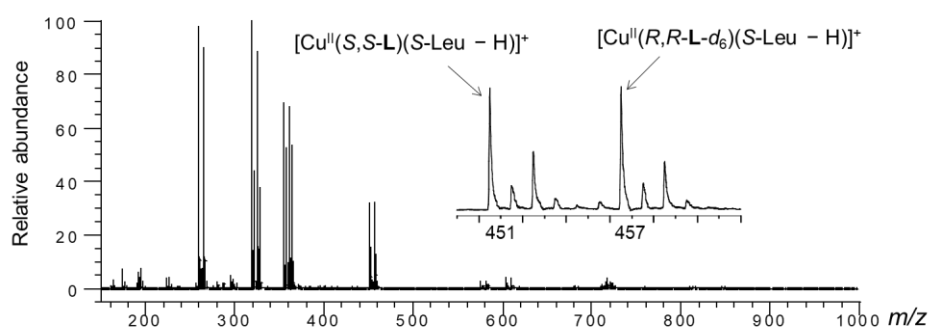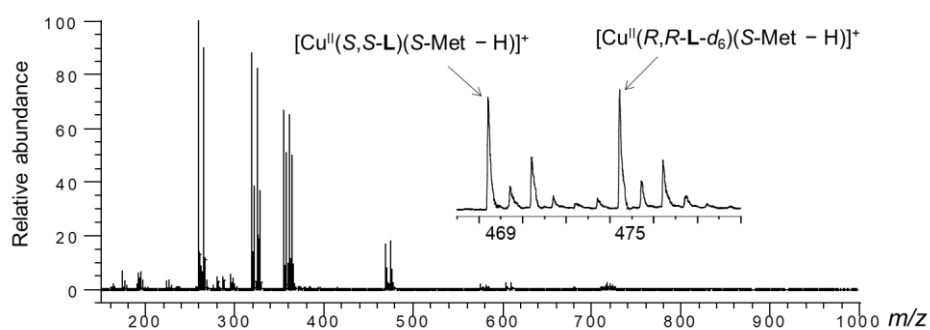
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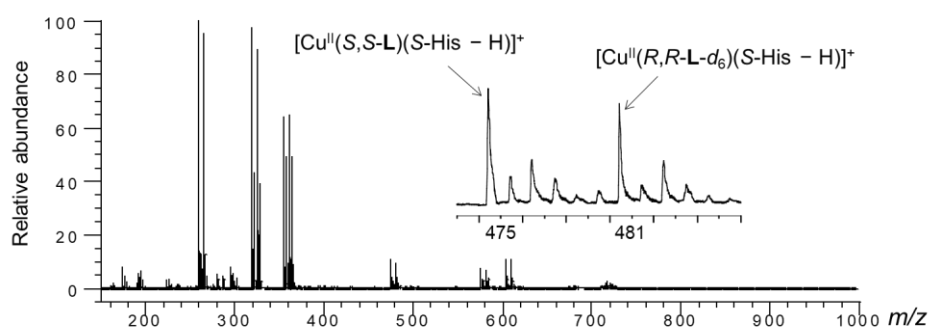


(e)
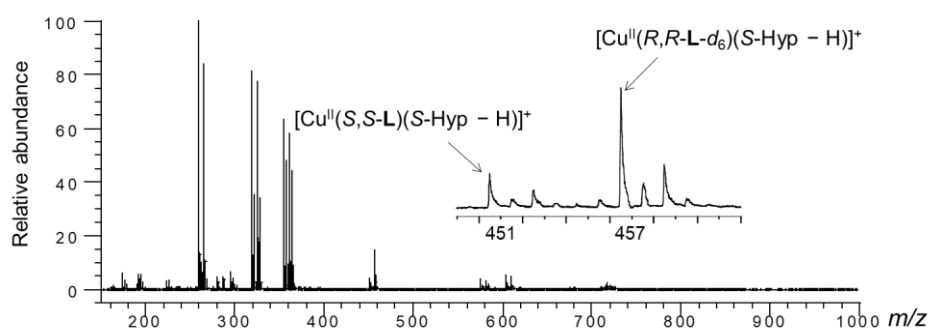


(f)



***Figure S6.*** (continued). Mass spectra of a mixture of $Cu^{II}/S,S$-**L**/$R,R$-**L**-$d_6$/$S$-AA in water/methanol (1/100, v/v). $[CuCl_2]_0 = 9.90 \times 10^{-5}$ M, $[S,S$-**L**$]_0 = [R,R$-**L**-$d_6]_0 = 5.94 \times 10^{-5}$ M and $[S$-AA$]_0 = [K_2CO_3]_0 = 9.90 \times 10^{-6}$ M. $[CuCl_2]_0/[S,S$-**L**$]_0/[R,R$-**L**-$d_6]_0/[S$-AA$]_0 = 1.0/0.6/0.6/0.1$. AA = (d) His, (e) Hyp and (f) Ile.

(g)



(h)



(i)
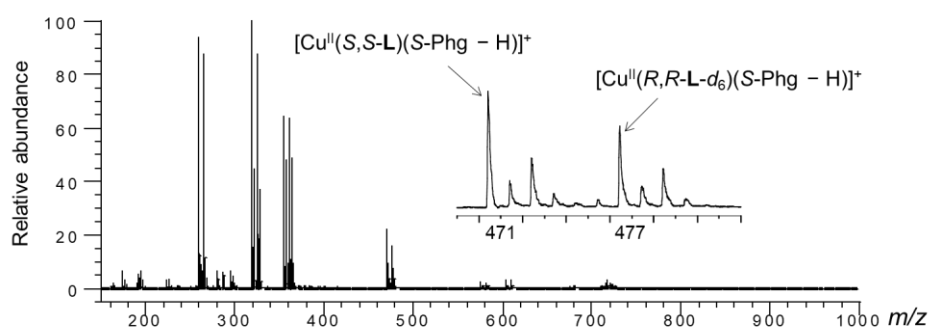


***Figure S6.*** (continued). Mass spectra of a mixture of $Cu^{II}/S,S\text{-}\mathbf{L}/R,R\text{-}\mathbf{L}\text{-}d_6/S\text{-}AA$ in water/methanol (1/100, v/v). $[CuCl_2]_0 = 9.90 \times 10^{-5}$ M, $[S,S\text{-}\mathbf{L}]_0 = [R,R\text{-}\mathbf{L}\text{-}d_6]_0 = 5.94 \times 10^{-5}$ M and $[S\text{-}AA]_0 = [K_2CO_3]_0$ $= 9.90 \times 10^{-6}$ M. $[CuCl_2]_0/[S,S\text{-}\mathbf{L}]_0/[R,R\text{-}\mathbf{L}\text{-}d_6]_0/[S\text{-}AA]_0 = 1.0/0.6/0.6/0.1$. AA = (g) Leu, (h) Met and (i) Phg.

(j)
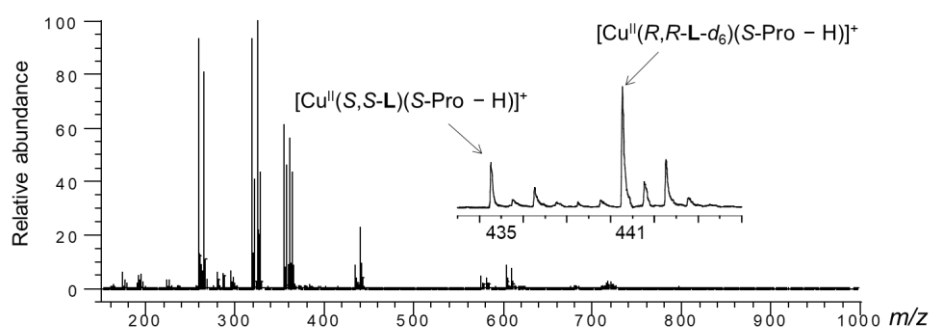


(k)
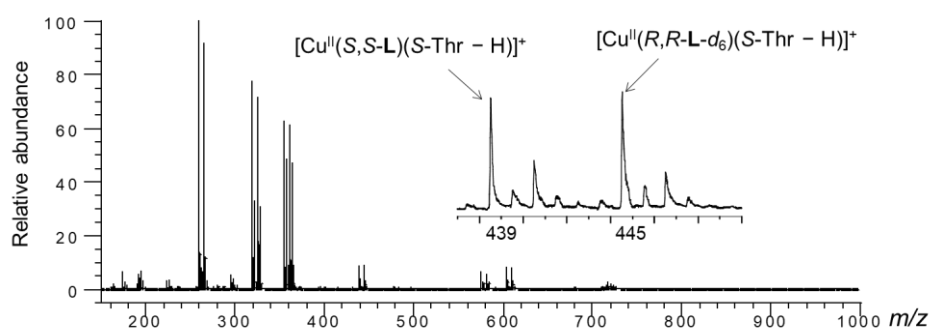


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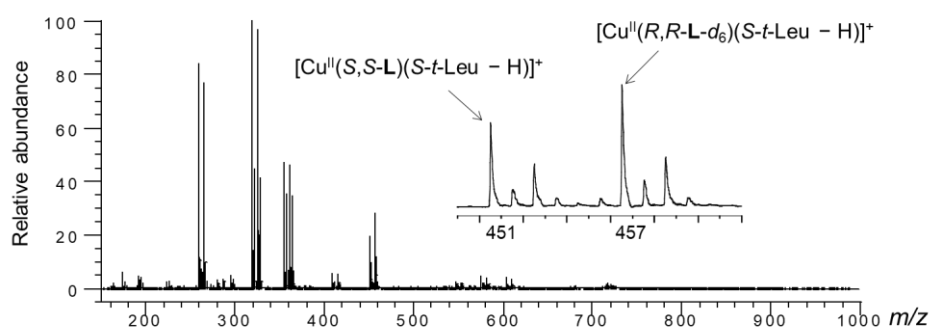


**Figure S6.** (continued). Mass spectra of a mixture of $Cu^{II}/S,S$-**L**/$R,R$-**L**-$d_6$/ $S$-AA in water/methanol (1/100, v/v). $[CuCl_2]_0 = 9.90 \times 10^{-5}$ M, $[S,S$-**L**$]_0 = [R,R$-**L**-$d_6]_0 = 5.94 \times 10^{-5}$ M and $[S$-AA$]_0 = [K_2CO_3]_0 = 9.90 \times 10^{-6}$ M. $[CuCl_2]_0/[S,S$-**L**$]_0/[R,R$-**L**-$d_6]_0/[S$-AA$]_0 = 1.0/0.6/0.6/0.1$. AA = (j) Pro, (k) Thr and (l) $t$-Leu.

(m)
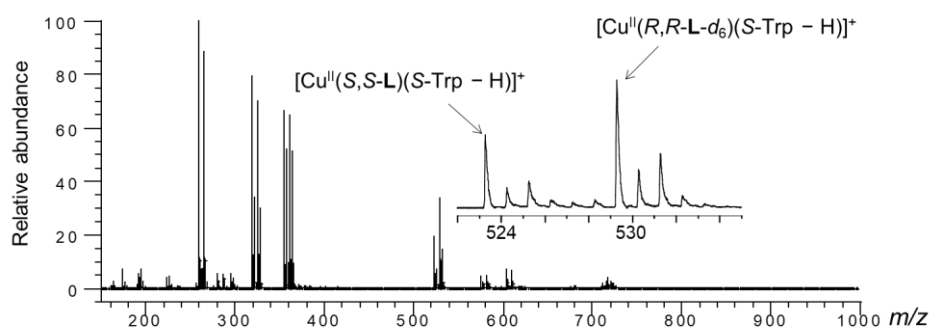


(n)
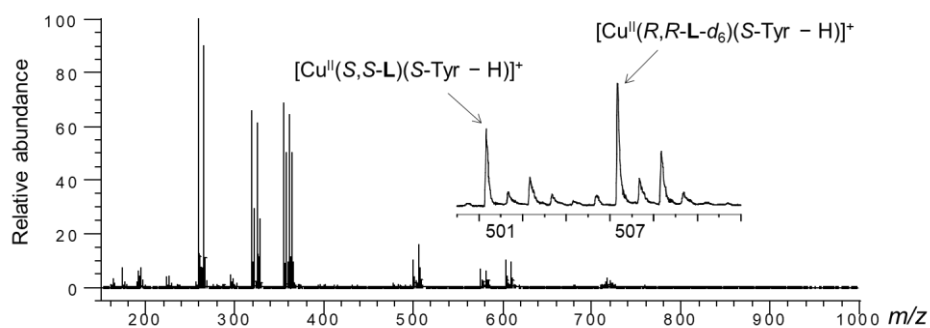


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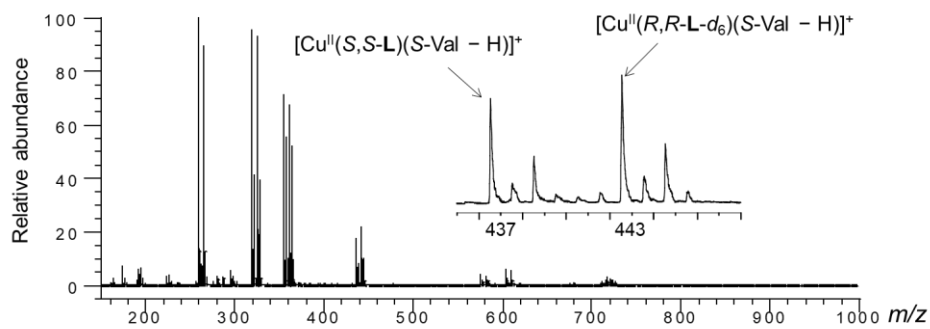


***Figure S6.*** (continued). Mass spectra of a mixture of $Cu^{II}$/*S,S*-**L**/*R,R*-**L**-$d_6$/ *S*-AA in water/methanol (1/100, v/v). $[CuCl_2]_0 = 9.90 \times 10^{-5}$ M, $[S,S\text{-}\mathbf{L}]_0 = [R,R\text{-}\mathbf{L}\text{-}d_6]_0 = 5.94 \times 10^{-5}$ M and $[S\text{-}AA]_0 = [K_2CO_3]_0 = 9.90 \times 10^{-6}$ M. $[CuCl_2]_0/[S,S\text{-}\mathbf{L}]_0/[R,R\text{-}\mathbf{L}\text{-}d_6]_0/[S\text{-}AA]_0 = 1.0/0.6/0.6/0.1$. AA = (m) Trp, (n) Tyr and (p) Val.

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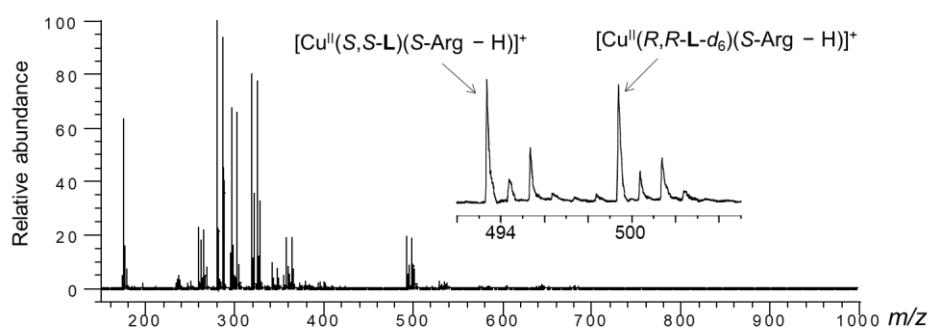


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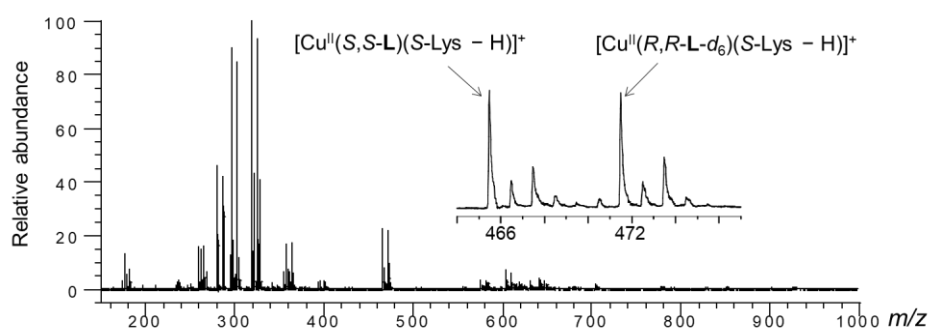


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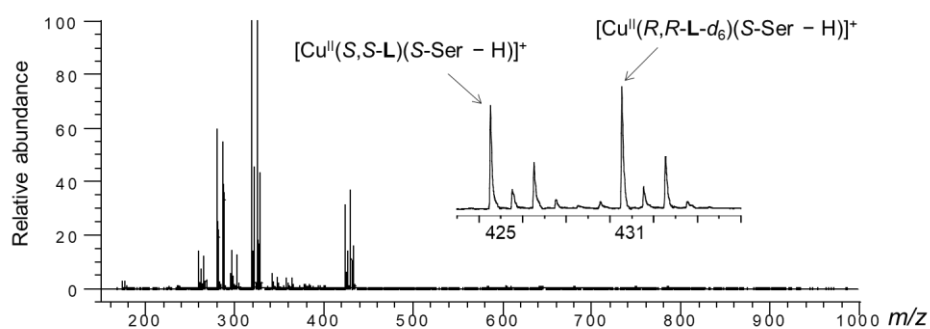


***Figure S7.*** Mass spectra of a mixture of $Cu^{II}/S,S$-**L**/$R,R$-**L**-$d_6$/$S$-AA in water/methanol (1/20, v/v). $[CuCl_2]_0 = 9.52 \times 10^{-5}$ M, $[S,S$-**L**$]_0 = [R,R$-**L**-$d_6]_0 = 5.71 \times 10^{-5}$ M and $[S$-AA$]_0 = [K_2CO_3]_0 = 4.76 \times 10^{-5}$ M. $[CuCl_2]_0/[S,S$-**L**$]_0/[R,R$-**L**-$d_6]_0/[S$-AA$]_0 = 1.0/0.6/0.6/0.5$. AA = (a) Arg, (b) Lys and (c) Ser.

(d)
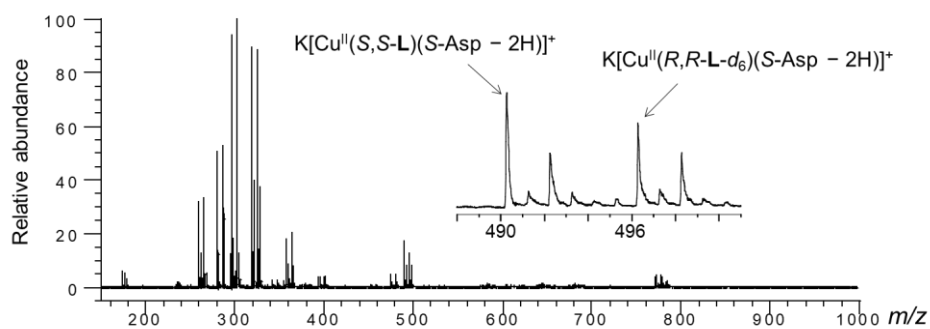


(e)
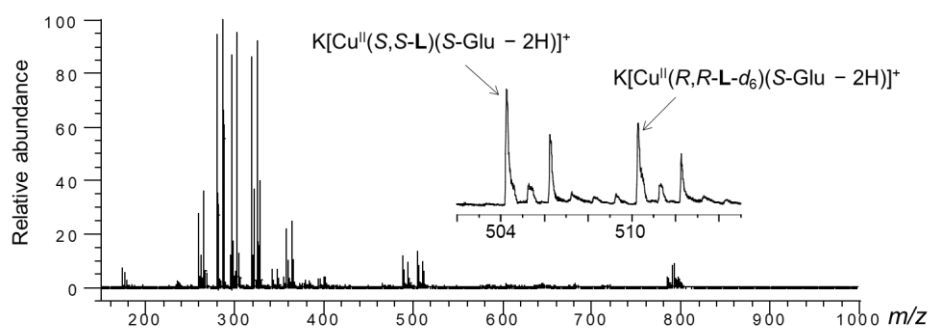


**Figure S7.** (continued). Mass spectra of a mixture of $Cu^{II}/S,S$-**L**/$R,R$-**L**-$d_6$/$S$-AA in water/methanol (1/20, v/v). $[CuCl_2]_0 = 9.52 \times 10^{-5}$ M, $[S,S$-**L**$]_0 = [R,R$-**L**-$d_6]_0 = 5.71 \times 10^{-5}$ M, $[S$-AA$]_0 = [K_2CO_3]_0 = 4.76 \times 10^{-5}$ M. $[CuCl_2]_0/[S,S$-**L**$]_0/[R,R$-**L**-$d_6]_0/[S$-AA$]_0 = 1.0/0.6/0.6/0.5$. AA = (d) Asp and (e) Glu.
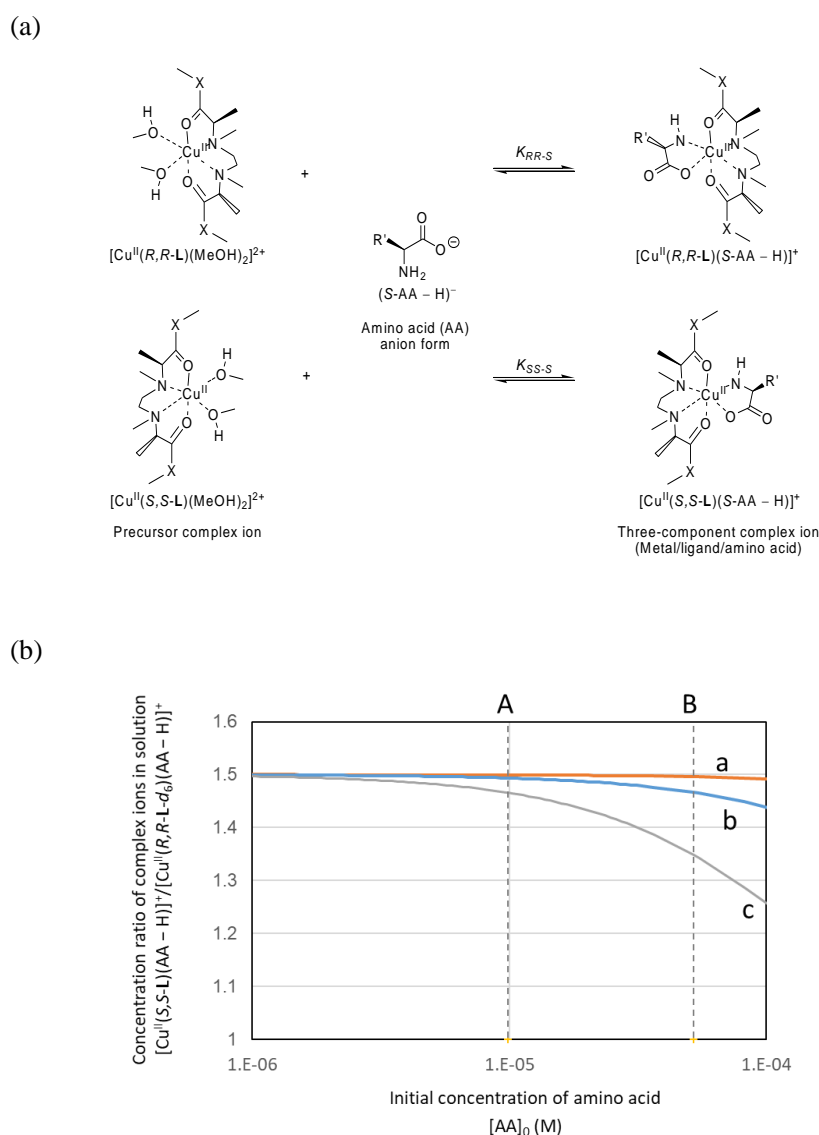
(a)



(b)



***Figure S8.*** Enantioselective three-component complexation equilibrium system and plots of the calculated concentration ratio of the diastereomeric complex ions, $[Cu^{II}(S,S\text{-}\mathbf{L})(S\text{-}AA - H)^+]/[Cu^{II}(R,R\text{-}\mathbf{L1})(S\text{-}AA - H)^+]$, in solution (line) versus the initial concentration of $[AA]_0$ in solution. (a) Enantioselective complexation equilibrium system of enantiomeric pair of the precursor complex ions with AA (anion form), (b) plots of $[Cu^{II}(S,S\text{-}\mathbf{L})(S\text{-}AA - H)^+]/[Cu^{II}(R,R\text{-}\mathbf{L1})(S\text{-}AA - H)^+]$ vs $[AA]_0$ (the initial concentration of the precursor complex ions $[Cu^{II}(S,S\text{-}\mathbf{L1})(CH_3OH)_2{}^{2+}]_0 = [Cu^{II}(S,S\text{-}\mathbf{L1})(CH_3OH)_2{}^{2+}]_0 = 4.95 \times 10^{-5}$ M). In the calculation, the ratio of the association constants was assumed to be constant ($K_{RR\text{-}S}/K_{SS\text{-}S} = 1.50$). Line a, $K_{RR\text{-}S} = 150$ M$^{-1}$, $K_{SS\text{-}S} = 100$ M$^{-1}$; line b, $K_{RR\text{-}S} = 1500$ M$^{-1}$, $K_{SS\text{-}S} = 1000$ M$^{-1}$; line c, $K_{RR\text{-}S} = 15000$ M$^{-1}$, $K_{SS\text{-}S} = 10000$ M$^{-1}$. Dotted line A, *a* condition of single AA analysis shown in Table; dotted line B, condition of simultaneous AA analysis shown in Figs. 5 and 6.
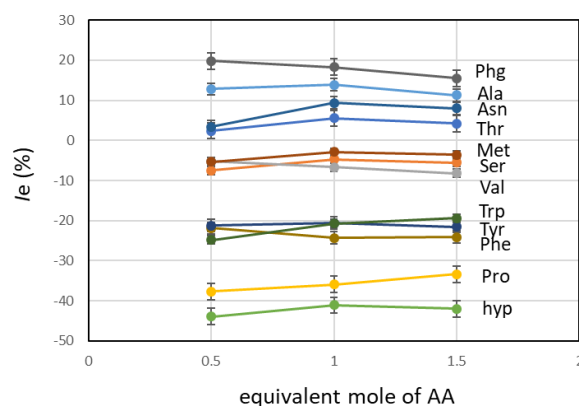
**Figure S9.** *Ie* (%) value of the simultaneous analysis against equivalent mole of AA for $CuCl_2$ and ligands (*S,S*-**L**, *R,R*-**L**-$d_6$). $[CuCl_2]_0 = 8.70 \times 10^{-5}$ M. $[CuCl_2]_0/[S,S\text{-}\mathbf{L}]_0/$ $[R,R\text{-}\mathbf{L}\text{-}d_6]_0/[S\text{-AA (total)}]_0/[K_2CO_3]_0 = 1.0/0.6/0.6/x/4x$ (x = 0.5, 1.0, or 1.5).
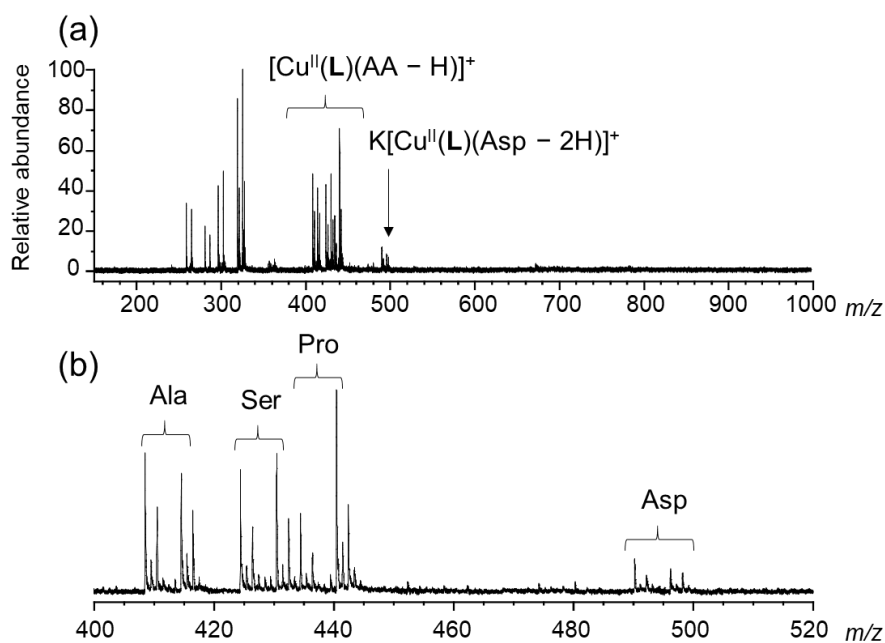


**Figure S10.** Mass spectra of a mixture of $Cu^{II}/S,S$-**L**/$R,R$-**L**-$d_6$/$S$-AA in water/methanol (3/20, v/v). (a) Full mass range. (b) Enlarged view (mass range: *m/z* 400-520) of mass spectra of a mixture of $Cu^{II}/S,S$-**L**/$R,R$-**L**-$d_6$/$S$-AA. *S*-AA is mixture of *S*-Ala, *S*-Asp, *S*-Pro and *S*-Ser. $[CuCl_2]_0 = 8.70 \times 10^{-5}$ M, $[S,S\text{-}\mathbf{L}]_0 = [R,R\text{-}\mathbf{L}\text{-}d_6]_0 = 5.22 \times 10^{-5}$ M, $[S\text{-AA(each)}]_0 = 3.24 \times 10^{-5}$ M and $[K_2CO_3]_0 = 1.30 \times 10^{-4}$ M. $[CuCl_2]_0/[S,S\text{-}\mathbf{L}]_0/$ $[R,R\text{-}\mathbf{L}\text{-}d_6]_0/[S\text{-AA (total)}]_0 = 1.0/0.6/0.6/0.37$.