Electronic Supplementary Information (ESI)

Mass spectrometric detection of enantioselectivity in three component complexation, copper(II)-chiral tetradentate ligand-free amino acid in solution

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1. General and Materials

1-1 General. ^{1}H-NMR (270 MHz) and ^{13}C-NMR (67.5 MHz) spectra were taken with a JEOL JNM EX-270 FT-NMR spectrometer. ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) spectra were taken with a JNM AL300 FT-NMR spectrometer (JEOL). Tetramethyl silane (TMS, δ 0 ppm) was used as the internal standard in [^{2}H]-chloroform. Solvent signal (δ 4.8 ppm) was used as the internal standard in [^{2}H]$_2$-water. 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* 158.9645782) was used as an internal standard for mass calibration. IR spectra were taken with a HORIBA FT-IR 730 in the range of 650-4000 cm^{-1}. UV-vis spectra were measured by a Jasco V-560 equipped with a Peltier type temperature-controlled cell holder (ETC-505) with a 1 cm quartz cuvette in the range of 210-500 nm at 25ºC. Circular dichroism (CD) spectra were measured in the range of 210–500 nm in 1 cm quartz cell using a Jasco J-820. Elemental analysis was measured with a CE INSTRUMENTS EA-1110 CHNS-O or J-Science MICRO CORDER JM10. Melting point was measured with a SEIKO DSC SSC/5200. Optical rotation was measured with a Jasco P-1020 with a 10 cm quartz cell irradiating sodium D line. TLC was performed by using a Merck TLC Silica gel 60 F_{254} 25 Glass plates detected by a UV lamp (254 nm) or iodine as indicator.

1-2 Materials. Chiral tetradentate ligands (*S*,*S*-**L1** and *S*,*S*-**L2**) were synthesized according to previous reports.[S1,S2] The synthesized compounds were purified by column chromatography using silica gel 60 (Merck) and silica gel 60N (Merck) as the stationary phase. Acetonitrile and methanol for synthesis were distilled over calcium hydride and quicklime as a desiccant, respectively.

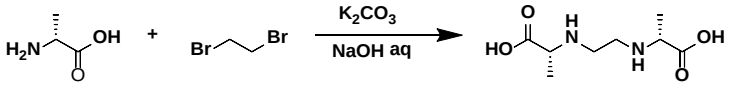
Deuterium-labelled *S*-amino acids (CDN ISOTOPES and ISOTEC inc) were purchased and used for qusaienantiomers (**Table S2**). LC/MS grade methanol (Fujifilm Wako pure chem. Co.) was purchased and used for ESI-MS. Spectral analysis grade methanol (Fujifilm Wako pure chem. Co.) was purchased and used without further purification for UV-visible and CD spectral measurements. All other reagents containing metal salts and amino acids were purchased from commercial suppliers and used without further purification.

2. Synthesis of ligands

R,*R*-**L1** and *R*,*R*-**L2** were similarly prepared to the synthetic procedure of corresponding *S*,*S*-isomers.[S1,S2]

2-1 *N,N´*-Dimethyl-*N,N´*-ethylene-bis(*R*-alanine methyl ester) (*R,R*-L1)

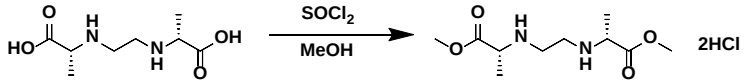
・*N,N'*-Ethylene-bis(*R*-alanine)



R-Ala *N,N'*-Ethylene-bis(*R*-alanine)

To a solution of *R*-alanine (20.0 g, 225 mmol) dissolved in 10 M NaOH aqueous solution (60 mL), 1,2-dibromoethane (23.2 g, 123 mmol) and potassium carbonate (8.56 g, 0.0620 mmol) were added in 10 portions and the mixture was refluxed for 3 h. After cooling to room temperature, saturated HCl aqueous solution was added dropwise to the solution to adjust pH to 5.5. The resulting solution was stored in refrigerator overnight. The precipitated white powder was corrected on vacuum filter and vacuum dried (2.76 g, 12.0%). δH (270 MHz; 1% sodium [^{2}H]-hydroxide in [^{2}H]$_2$-water) 3.16 (q, 2H, *J* = 6.76 Hz, C<u>H</u>), 2.64 (m, 4H,C<u>H</u>$_2$), 1.24 (d, 6H, *J* = 6.76 Hz, C<u>H</u>$_3$); δC (67.5 MHz; 1% sodium [^{2}H]-hydroxide in [^{2}H]$_2$-water) 19.3, 47.4, 59.6, 184.2; ν_{max}(KBr)/cm^{-1} 2976, 2850, 1589, 1469, 1398, 1363, 1284; Found: C, 46.2; H, 7.9; N, 13.4; calculated for $C_8H_{16}N_2O_4$ 0.2H_2O: C, 46.2; H, 8.0; N, 13.5; $[\alpha]^{27}_D$ 27.9 (*c* 0.10 in 0.5 M NaOH aq); mp 252.5 ºC.

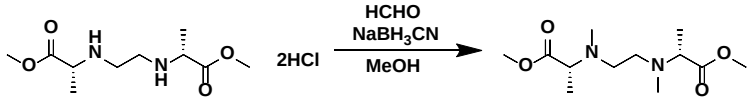
・*N,N'*-Ethylene-bis(*R*-alanine methyl ester) dihydrochloride



N,N'-Ethylene bis(*R*-alanine) *N,N'*-Ethylene bis(*R*-alanine methyl ester) dihydrochloride

To a suspension of *N,N'*-ethylene-bis(*R*-alanine) (2.76 g, 0.014 mmol) in 100 mL methanol, thionyl chloride (4.03 g, 0.035 mmol) was added, and the mixture was stirred at 50 °C overnight. The reaction mixture was cooled to room temperature, and evaporated to obtain the white solid. The collected solid was washed with chloroform to give *N,N'*-ethylene-bis(*R*-alanine methyl ester) dihydrochloride (3.70 g, 92.0 %). δH (300 MHz; [^{2}H]$_2$-water) 4.27 (q, 2H, *J* = 7.34 Hz, C<u>H</u>), 3.90 (s, 6H, OC<u>H</u>$_3$), 3.54 (s, 4H,C<u>H</u>$_2$), 1.64 (d, 6H, *J* = 7.34, C<u>H</u>$_3$); δC (75 MHz; [^{2}H]$_2$-water) 15.2, 43.0, 54.6, 56.7, 172.4; ν_{max}(KBr)/cm^{-1} 3446, 2958, 2740, 1743, 1556, 1240; Found: C, 34.4; H, 7.5; N, 8.2; calculated for $C_{10}H_{20}N_2O_4$ 2HCl 2.5H_2O: C, 34.3; H, 7.8; N, 8.0; HRMS (ESI) calculated for $C_{10}H_{22}N_2O_4$ $[M + H]^+$ 233.1501, found 233.1511; $[\alpha]^{27}_D$ 35.7 (*c* 0.10 in H_2O); mp 51.2 ºC.

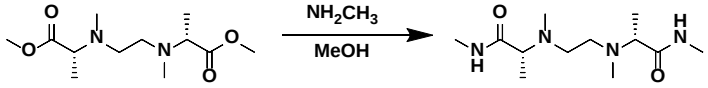
・*N*,*N*´-Dimethyl-*N*,*N*´-ethylene-bis(*R*-alanine methyl ester) (*R*,*R*-**L1**)



N,*N*'-Ethylene-bis(*R*-alanine methyl ester) dihydrochloride *R*,*R*-**L1**

To a solution of *N*,*N*´-ethylene-bis(*R*-alanine methyl ester) dihydrochloride (5.60 g, 18.6 mmol) in 50 mL methanol was added 37% formaldehyde aqueous solution (5.59 g, 74.5 mmol) at ice bath. The solution was adjusted to pH 4.0 by adding 30% of trimethylamine aqueous solution. After stirring for 30 min, $NaBH_3CN$ (2.35 g, 37.2 mmol) was added to the mixture, and the resulting solution was further stirred overnight. The product was extracted with chloroform and the organic phase was washed with saturated $NaHCO_3$ aqueous solution, and then the organic phase was dried over anhydrous sodium sulfate. The solution was evaporated and the residue purified with flash column chromatography (silica gel, ethyl acetate/*n*-hexane = 3/1, v/v) to give *R*,*R*-**L1** (3.87 g, 80.5%). δH (270 MHz, [^{2}H]-chloroform) 3.70 (s, 6H, OC$\underline{H}_3$), 3.44 (dd, 2H, *J* = 7.16 Hz, C$\underline{H}$), 2.63 (m, 4H,C$\underline{H}_2$), 2.35 (s, 6H, NC$\underline{H}_3$), 1.29 (d, 6H, *J* = 7.16 Hz, C$\underline{H}_3$); δC (67.5 MHz, [^{2}H]-chloroform) 14.7, 38.7, 51.3, 52.5, 61.7, 173.6; ν_{max}(neat)/cm^{-1} 2981, 2952, 1734, 1456, 1165; Found: C, 54.3; H, 9.1; N, 10.7; calculated for $C_{12}H_{24}N_2O_4$ 0.29H_2O: C, 54.3; H, 9.3; N, 10.6; HRMS (ESI) calculated for $C_{12}H_{24}N_2O_4$ [M + Na]$^+$ *m/z* 283.1634, found 283.1606; [α]$^{25}_D$ 56.6 (*c* 0.12, CH_3OH).

2-2 *N*,*N*´-Dimethyl-*N*,*N*´-ethylene-bis(*R*-alanine methyl amide) (*R*,*R*-L2)

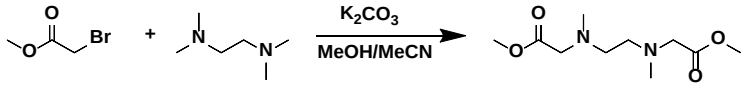


R,*R*-**L1** *R*,*R*-**L2**

R,*R*-**L1** (0.30 g, 1.15 mmol) was dissolved in excess amount of 40% of methylamine solution in methanol, and the solution was stirred at 50 °C overnight. The solution was evaporated and the residue was purified with flash column chromatography (silica gel, chloroform/methanol = 1/5, v/v) to give *R*,*R*-**L2** as white solid (0.18 g, 60.0%). δH (270 MHz, [^{2}H]-chloroform) 7.87 (bs, 2H, N$\underline{H}$), 3.29 (q, 2H, *J* = 7.1 Hz, C$\underline{H}$), 2.81 (d, 6H, *J* = 4.9 Hz, NH-C$\underline{H}_3$), 2.56 (d, 2H, *J* = 9.1 Hz, ethylene), 2.30 (s, 6H, NC$\underline{H}_3$), 2.27 (d, 2H, *J* = 9.1 Hz, ethylene), 1.27 (d, 6H, *J* = 7.1 Hz, Ala-C$\underline{H}_3$); δC (67.5 MHz, [^{2}H]-chloroform) 9.7, 26.2, 40.0, 51.0, 63.9, 174.0; ν_{max}(KBr)/cm^{-1}, 3301, 2937, 1658, 1531, 1365, 1213, 1115; Found: C, 55.3; H, 10.1; N, 21.5; elemental analysis, calculated for $C_{12}H_{26}N_4O_2$ 0.13H_2O: C, 55.3; H, 10.2; N, 21.5; HRMS (ESI), calculated for $C_{12}H_{26}N_4O_2$ [M + Na]$^+$ *m/z* 281.1953, found

281.1924; $[\alpha]^{25}_{D}$ −20.2 (*c* 0.88, CH_3OH); mp 90 ºC.

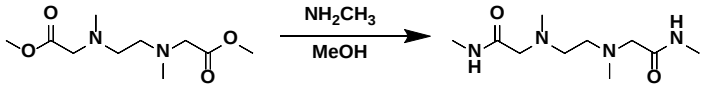
2-3 *N,N′*-Ethylene-bis(sarcosine methyl ester) (L3)



L3

Methyl bromoacetate (2.00 g, 13.1 mmol) and *N,N′*-dimethylethylenediamine (0.58 g, 6.55 mmol) were added to a solution of potassium carbonate in methanol/acetonitrile (25 mL/25 mL, v/v) at ice bath and then, the mixture was stirred for one day at room temperature. The reaction mixture was extracted with chloroform and the organic phase was washed with 10% citric acid aqueous solution and dried over anhydrous sodium sulfate. The solution was evaporated and the residue was purified with flash column chromatography (silica gel, chloroform/methanol = 9/1, v/v) to give **L3** as light yellow oil (0.93 g, 30.5%). δH (270 MHz, [^{2}H]-chloroform) 3.71 (s, 6H, OC$\underline{H}_3$), 3.38 (s, 4H, C$\underline{H}_2$) 2.73 (s, 4H, C$\underline{H}_2$), 2.43 (s, 6H, C$\underline{H}_3$); δC (67.5 MHz, [^{2}H]-chloroform) 42.4, 51.4, 54.1, 58.1, 171.0; ν_{max}(neat)/cm^{-1} 3504, 2952, 1743, 1437, 1204; calculated for $C_{10}H_{20}N_2O_4$ 0.13$CHCl_3$ 0.13H_2O: C, 48.8; H, 8.2; N, 11.2; Found: C, 48.7; H, 8.1; N, 11.2; HRMS (ESI) calculated for $C_{10}H_{20}N_2O_4$ [M + Na]$^+$ *m/z* 255.1321, found 255.1308.

2-4 *N,N'*-Ethylene-bis(sarcosine methyl amide) (L4)



L4

L3 (0.3g, 1.30 mmol) was dissolved in 40% methylamine solution in methanol (1.0 g, 13.0 mmol), and the solution was stirred at 50 °C overnight. The product was extracted with chloroform and the organic phase washed with 10% ammonium chloride aqueous solution, and then the organic phase was dried over anhydrous sodium sulfate. The solution was evaporated and the residue was recrystallized with chloroform/*n*-hexane to give **L4** as needle crystal (0.075 g, 25.1%). δH (300 MHz, [^{2}H]-chloroform) 7.50 (bs, 2H, N$\underline{H}$), 3.10 (s, 4H, C$\underline{H}_2$), 2.85 (d, *J* = 4.9 Hz, 6H, NH-C$\underline{H}_3$), 2.50 (s, 4H, ethylene), 2.32 (s, 6H, NHC$\underline{H}_3$); δC (75 MHz, [^{2}H]-chloroform) 25.8, 43.7, 55.9, 61.7, 171.2; ν_{max}(KBr)/cm^{-1} 3318, 2972, 2800, 1666; Found: C, 51.8; H, 9.71; N, 23.8; calculated for $C_{10}H_{22}N_4O_2$ 0.09H_2O: C, 51.8; H, 9.6; N, 24.2; HRMS (ESI) calculated for $C_{10}H_{22}N_4O_2$ [M + Na]$^+$ *m/z* 253.1640, found 253.1620; mp, 110 ºC.

3. X-ray crystal analysis

The X-ray diffraction data of [Cu(*S*,*S*-**L2**)$(CH_3OH)_2$]$(ClO_4)_2$ $(CH_3OH)_2$ were collected by Rigaku / MSC Mercury CCD diffractometer with graphite monochromated Mo K_α radiation (λ= 0.71070 Å) to $2\theta_{max}$ of 55.0°. The resulting data were processed on a PC using CrystalClear software (Rigaku). The crystal structure was solved by the direct methods using Sir-2004[S3] and refined by full-matrix least squares on F^2 using SHELXL-2014/7[S4] on Yadokari-GX 2009 software.[S5] All non-hydrogen atoms were refined anisotropically with disordered ClO_4^- anion as 60/40 occupancy ratio. All hydrogen atoms were placed on ideally geometrical positions and not refined. Absolute configuration of the complex was determined by the configuration of ligand and Flack parameter. Crystal data for [Cu(*S*,*S*-**L2**)$(CH_3OH)_2$]$(ClO_4)_2(CH_3OH)_2$, $C_{16}H_{42}Cl_2CuN_4O_{14}$, *M*r=648.97, trigonal, space group $P3_221$, *a*=8.729(9), *b*=8.729(9), *c*=33.58(4) Å, *V*=2216(5) Å^3, *Z*=3, *T*=173(2) K, 16969 reflections collected of which 3269 unique (R_{int} = 0.0465). Final *R* values: *R*1=0.0415 [$I>2\sigma(I)$], *wR*2=0.0971 (all data). GOF=1.277. Flack parameter=0.001(8).

4. Geometry optimizations by DFT calculations

The geometry optimizations were performed on windows 10-PC machine using Gaussian 09W (C.01).[S6] Twelve Initial structures of the three component complexes [Cu^{II}(*S*,*S*-**L2**)(Phe − H)]$^+$ were constructed from crystal structure of [Cu^{II}(*S*,*S*-**L2**)$(CH_3OH)_2]^{2+}$ complex (see **Fig. 1**) by replacing two coordinating methanol molecules with bidentate phenylalanine [$NH_2CH(CH_2Ph)COO^-$], followed by changing location of benzyl group as following combinations for both (*S*)- and (*R*)- configuration of phenylalanine; (a) equatorial or axial position in 5-membered ring, -Cu-NH_2-CH(Bzl)-CO-O-, (b) three staggered conformations by rotation of α-β bond of phenylalanine anion. The method used was B3LYP with LANL2DZ as the basis set. The calculation was performed on a doublet electronic state in methanol. Seven optimized conformers were obtained as summarized in **Table S1**, all of which was confirmed that no imaginary frequencies were arisen at calculated structure by frequency calculation.

5. Mass spectrometry

Generally, metal complex coordinated with weak organic ligand is unstable under a default instrumental condition of the mass spectrometer. The machine condition of ESI mass spectra (positive ion mode) with a JEOL AccuTOF LC-plus 4G or a AccuTOF LC-plus JMS-T100LP mass spectrometer was optimized to detect such metal complex ions with high sensitivity as follows; voltage of spray needle = 1 kV, orifice1 = 50 V, orifice2 = 1 V, ring lens = 5 V, temperature of desolvation chamber = 100 ºC, temperature of orifice1 = 50 ºC, mass range = *m/z* 150-1000. The mass spectrum data was

collected under following conditions: acquisition time = 0.397 s (wait time = 0.003 s, recoding time = 0.4s), measurement time = 2 min.

The accuracy of the 1:1 equivalent of a *R*-AA and a deuterium-labelled *S*-AA ($[^2H]_n$-*S*-AA) was calibrated on the basis of I_R/I_S (relative peak intensity ratio of two diastereomeric complex ion peaks $I[Cu^{II}(\mathbf{L})(R\text{-AA} - H)]^+/I[Cu^{II}(\mathbf{L})([^2H]_n\text{-}S\text{-AA} - H)]^+$) values obtained by ESI-MS spectrum of three-component copper complex with achiral ligand (**L3** and **L4**) and pseudo-racemic mixture of amino acid (*R*-AA and $[^2H]_n$-*S*-AA).

1) Screening of metal cation

Metal cation suitable for a chiral host complex with a tetradentate ligand in ESI mass spectrometry was searched using achiral ligand **L3** as shown in **Fig. S7**. The sample solution was prepared as following procedures: (i) 1.20 mL of methanol solution containing metal chloride (2.00×10^{-3} M) and 1.00 mL of methanol solution containing **L3** (2.00×10^{-3} M) were mixed which was diluted to 20 mL in volumetric flask by adding methanol (mole ratio: metal chloride/**L3** = 1.2/1.0).

2) Screening of anion

As copper(II) cation was one of the suitable metal ions, the counter anion (CA) was searched using $Cu(CA)_2$/**L3**/*S*-Ala system (**Fig. S8**). The sample solution was prepared as following procedures: (i) 1.20 mL of methanol solution containing copper(II) cation (2.00×10^{-3} M) and 1.00 mL of methanol solution containing **L3** (2.00×10^{-3} M) were mixed which was diluted to 20 mL in volumetric flask by adding methanol; (ii) 1.00 mL of solution (i) and 0.100 mL of mixture of *S*-Ala (1.00×10^{-3} M) containing equimolar K_2CO_3 in water were mixed (mole ratio: $Cu(CA)_2$/**L3**/*S*-Ala = 1.2/1.0/1.0).

3) Sample preparation for I_R/I_S measurement in ESI-MS

I_R/I_S measurement was carried out under condition optimized from **Fig. S10**. The sample solutions of **Figs. S9** and **S12** were prepared as following procedures: (i) 1.20 mL of methanol solution containing copper(II) chloride (2.00×10^{-3} M) and 1.00 mL of methanol solution containing *S*,*S*-**L1**, *S*,*S*-**L2** or *R*,*R*-**L2** (2.00×10^{-3} M) were mixed which was diluted to 20 mL in volumetric flask by adding methanol; (ii) 1.00 mL of solution (i) and 0.400 mL of equimolar mixture of *R*-enantiomer and deuterium-labelled *S*-enantiomer (5.00×10^{-4} M each) containing K_2CO_3 (1.00×10^{-3}M) in water were mixed (mole ratio: $CuCl_2$/**L**/*R*-AA/$[^2H]_n$-*S*-AA = 1.2/1.0/2.0/2.0).

6. Concentration ratio of complex ions

The complexation equilibrium system including metal ion (Cu^{2+}), a chiral ligand (ex. *S*,*S*-**L1**), amino acid (anion form), solvent (CH_3OH), and counter ion (Cl^-) is so much complicate. Seven possible main equilibria are shown below (eqs. 1–7). Here, the counter ion is omitted for

simplification.

$Cu^{2+} + S,S\text{-}\mathbf{L1} + 2CH_3OH \rightleftharpoons [Cu^{II}(S,S\text{-}\mathbf{L1})(CH_3OH)_2]^{2+}$ (eq. 1)

$Cu^{2+} + (Phe - H)^- + 2CH_3OH \rightleftharpoons [Cu^{II}(Phe - H)(CH_3OH)_2]^+$ (eq. 2)

$[Cu^{II}(Phe - H)(CH_3OH)_2]^+ + (Phe - H)^- \rightleftharpoons [Cu^{II}(Phe - H)_2] + 2CH_3OH$ (eq. 3)

$[Cu^{II}(S,S\text{-}\mathbf{L1})(CH_3OH)_2]^{2+} + (Phe - H)^- \rightleftharpoons [Cu^{II}(S,S\text{-}\mathbf{L1})(Phe - H)]^+ + 2CH_3OH$ (eq. 4)

$2[Cu^{II}(S,S\text{-}\mathbf{L1})(CH_3OH)_2]^{2+} + (Phe - H)^- \rightleftharpoons [\{Cu^{II}(S,S\text{-}\mathbf{L1})(CH_3OH)\}(Phe - H)\{Cu^{II}(S,S\text{-}\mathbf{L1})(CH_3OH)\}]^{3+} + 2CH_3OH$ (eq. 5)

$[Cu^{II}(S,S\text{-}\mathbf{L1})(Phe - H)]^+ + (Phe - H)^- \rightleftharpoons [Cu^{II}(S,S\text{-}\mathbf{L1})(Phe - H)_2]$ (eq. 6)

$[Cu^{II}(S,S\text{-}\mathbf{L1})(Phe - H)]^+ + (Phe - H)^- \rightleftharpoons [Cu^{II}(Phe - H)_2] + S,S\text{-}\mathbf{L1}$ (eq. 7)

However, the complex ion related to $[Cu^{II}(Phe - H)(CH_3OH)_2]^+$, $[Cu^{II}_2(S,S\text{-}\mathbf{L1})(CH_3OH)_2(Phe - H)]^{3+}$ and $[Cu^{II}(S,S\text{-}\mathbf{L1})(Phe - H)_2]$, including the cation, anion or/and solvent attached molecules and the fragment ions, were not detected in the mass spectra. Therefore, the complexation equilibria described by eq. 2, 5 and 6 are considered to contribute hardly to the overall system. As shown in **Fig. S10** (g), release of the ligand (*S*,*S*-**L1**) and generation of $[Cu^{II}(Phe - H)_2]$ were recognized under the condition in the presence of more than 1.0 equivalent of Phe. It suggests that the continuous complexation equilibria described by eq. 1, 4 and 7 mainly dominate this complexation system. Furthermore, the titration profile by MS measurement {**Fig. S10** (g)} showed good agreement with that by UV-vis measurement (**Fig. S5**).

Therefore, it is allowed to discuss the correlation between the enantioselective coordination of amino acid to the precursor complex and the relative peak intensity of the three-component complex ion obtained by mass spectrometry based on the complexation behaviors in solution.

Equilibrium constant (K_R and K_S) of complexation between Cu-*S*,*S*-**L1** and Phe (*R*-Phe and *S*-Phe) in solution is defined as follows,

$[Cu^{II}(S,S\text{-}\mathbf{L1})(CH_3OH)_2]^{2+} + (R\text{-}Phe - H)^- \overset{K_R}{\rightleftharpoons} [Cu^{II}(S,S\text{-}\mathbf{L1})(R\text{-}Phe - H)]^+ + 2CH_3OH$

$[Cu^{II}(S,S\text{-}\mathbf{L1})(CH_3OH)_2]^{2+} + (S\text{-}Phe - H)^- \overset{K_S}{\rightleftharpoons} [Cu^{II}(S,S\text{-}\mathbf{L1})(S\text{-}Phe - H)]^+ + 2CH_3OH$

$$K_R = \frac{[Cu^{II}(S,S\text{-}\mathbf{L1})(R\text{-}Phe - H)]^+}{[Cu^{II}(S,S\text{-}\mathbf{L1})(CH_3OH)_2{}^{2+}]\ \ [(R\text{-}Phe - H)^-]}$$

$$K_S = \frac{[Cu^{II}(S,S\text{-}\mathbf{L1})(S\text{-}Phe - H)]^+}{[Cu^{II}(S,S\text{-}\mathbf{L1})(CH_3OH)_2{}^{2+}]\ \ [(S\text{-}Phe - H)^-]}$$

In the case of K_R/K_S = 1.65, correlation plots of concentration ratio of the diastereomeric complex ions, [Cu^{II}(*S*,*S*-**L1**)(*R*-Phe − H)$^+$]/[Cu^{II}(*S*,*S*-**L1**)(*S*-Phe − H)$^+$], *vs.* concentration of Phe is able to be obtained theoretically (line) under a certain initial concentration of the complex [Cu^{II}(*S*,*S*-**L1**)$(CH_3OH)_2]^{2+}$ (7.14×10^{-5} M). Actually, there is release of the ligand (page S9, eq.7), so the concentration of the complex is not constant. When I_R/I_S values evaluated based on mass spectra (**Fig. S10** (a)-(f)) was plotted (circle in **Fig. S11**), the concentration ratio curve calculated with several equilibrium constants and I_R/I_S values were disagreed (**Fig. S11** (a)). Since the ESI has concentration process of the sample solution via desolvation,[S7,S8] the concentration ratio of the complex ions, [Cu^{II}(*S*,*S*-**L1**)(*R*-Phe − H)$^+$]/[Cu^{II}(*S*,*S*-**L1**)(*S*-Phe − H)$^+$], was calculated under the 100 times higher concentration condition than the experimental concentration of the solution (**Fig. S11** (b)). The I_R/I_S values calculated under conditions 100 times concentrated than the experimental conditions became close to the profile of concentration ratio of the diastereomeric complex ions shown by green line (K_R = 1650 M^{-1}, K_S = 1000 M^{-1}) in **Fig. S11** (b). Although several assumptions were made, it was confirmed that the concentration effect of the solution was reflected in the peak intensity value of MS.

7. References

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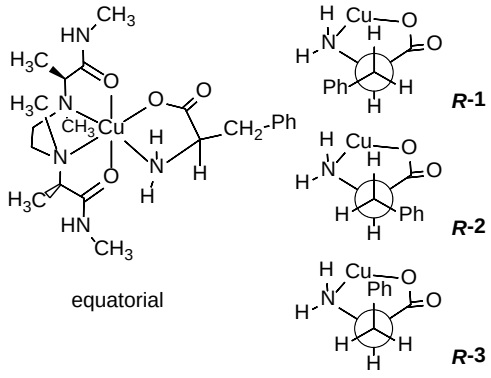
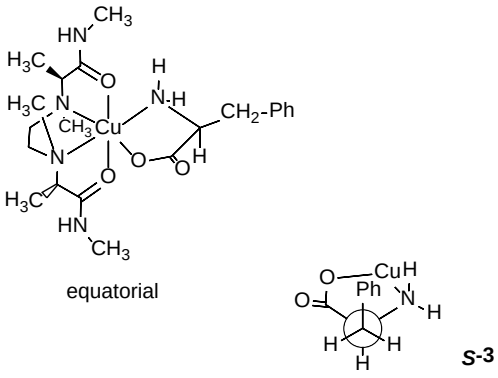
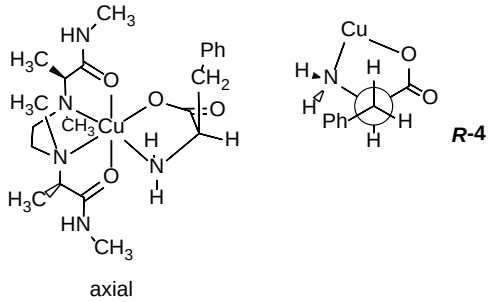
8. Figures and table

Table S1 Summary of the calculated relative energies, and thermal Boltzmann populations for conformers of the chiral Cu(II) complex [Cu(*S*,*S*-**L2**)(Phe − H)]$^{+}$ at 298 K in methanol.

	torsion angle (°) of Cu-N(H_2)-C(H)-C(H_2Ph)	relative energy, kcal mol^{-1}	% population		torsion angle (°) of Cu-N(H_2)-C(H)-C(H_2Ph)	relative energy, kcal mol^{-1}	% population
***R*-1**	155.8	≡0	30.1				
***R*-2**	162.3	2.54	0.4				
***R*-3**	153.3	0.57	11.5	***S*-3**	–154.5	0.70	9.3
***R*-4**	94.4	0.22	20.7	***S*-4**	–99.1	0.07	26.7
				***S*-5**	–90.1	1.85	1.3
total			62.7	total			37.3







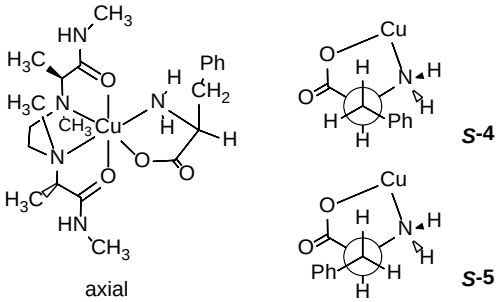
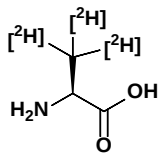
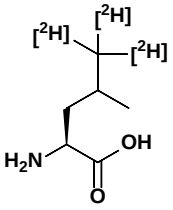
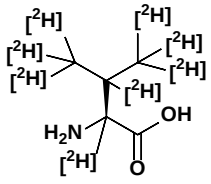
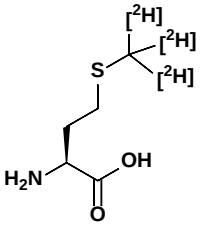
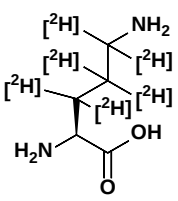
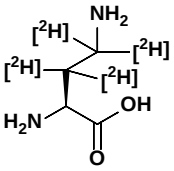
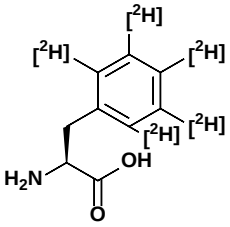
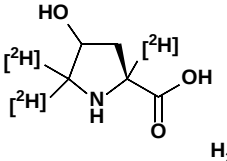
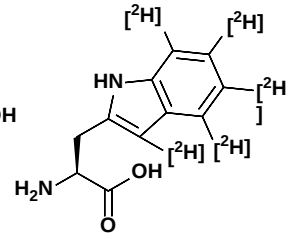


Table S2 Structures of deuterium-labelled *S*-amino acids.

Amino acid	$[^2H]_3$-*S*-Ala	$[^2H]_3$-*S*-Leu	$[^2H]_8$-*S*-Val
$[^2H]$ content (atom %)	99.1	99.0	98.9
Structure			
Amino acid	**$[^2H]_3$-*S*-Met**	**$[^2H]_6$-*S*-Orn**	**$[^2H]_4$-*S*-Lys**
$[^2H]$ content (atom %)	**99.1**	**99.1**	**98.3**
Structure			
Amino acid	**$[^2H]_5$-*S*-Phe**	**$[^2H]_3$-*S*-Hyp**	**$[^2H]_5$-*S*-Trp**
$[^2H]$ content (atom %)	**99.2**	**99.2**	**98.8**
Structure			

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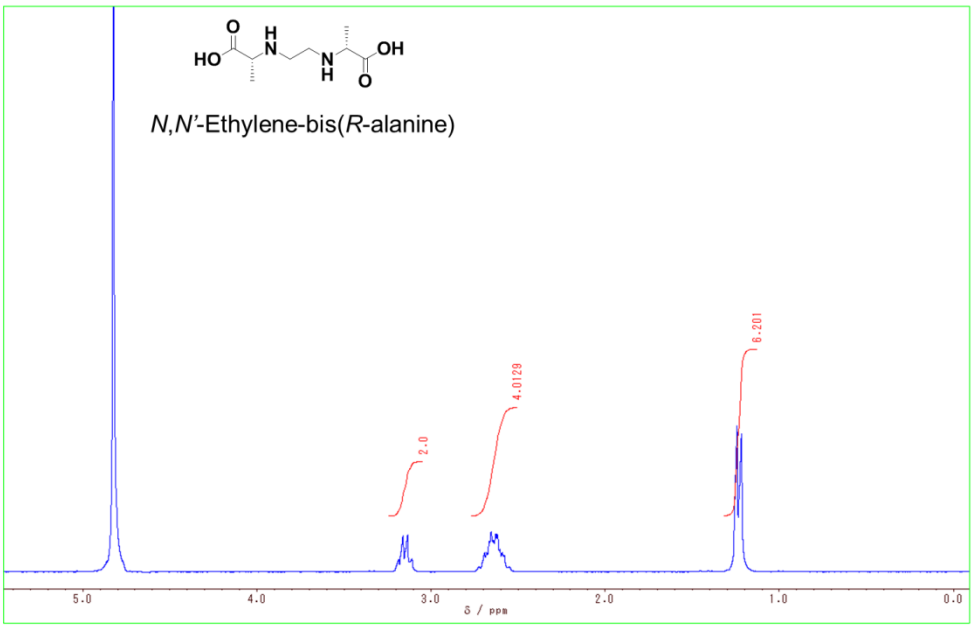

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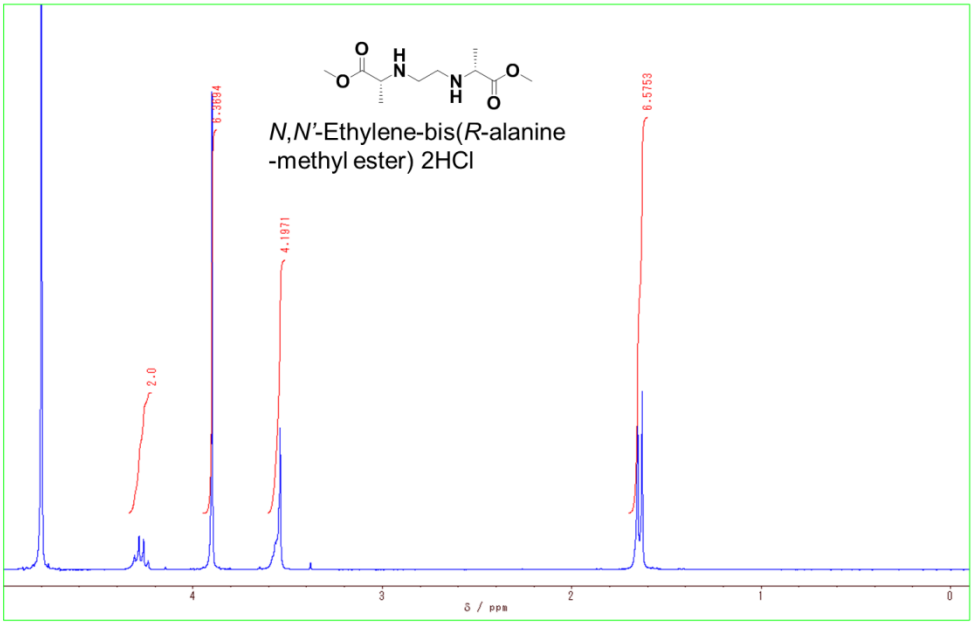
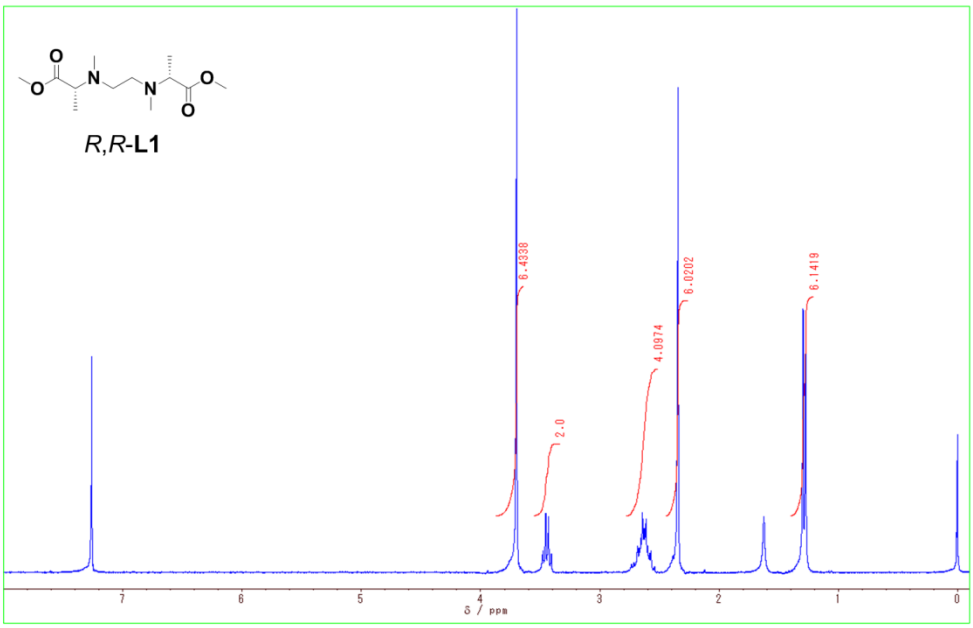


Fig. S1 ^{1}H-NMR spectra of ligands. (a) *N,N'*-Ethylene-bis(*R*-alanine) in 1% sodium [^{2}H]-hydroxide in [^{2}H]$_2$-water at room temperature, (b) *N,N'*-Ethylene-bis(*R*-alanine methyl ester) 2HCl in [^{2}H]$_2$-water at room temperature.

(c)



(d)

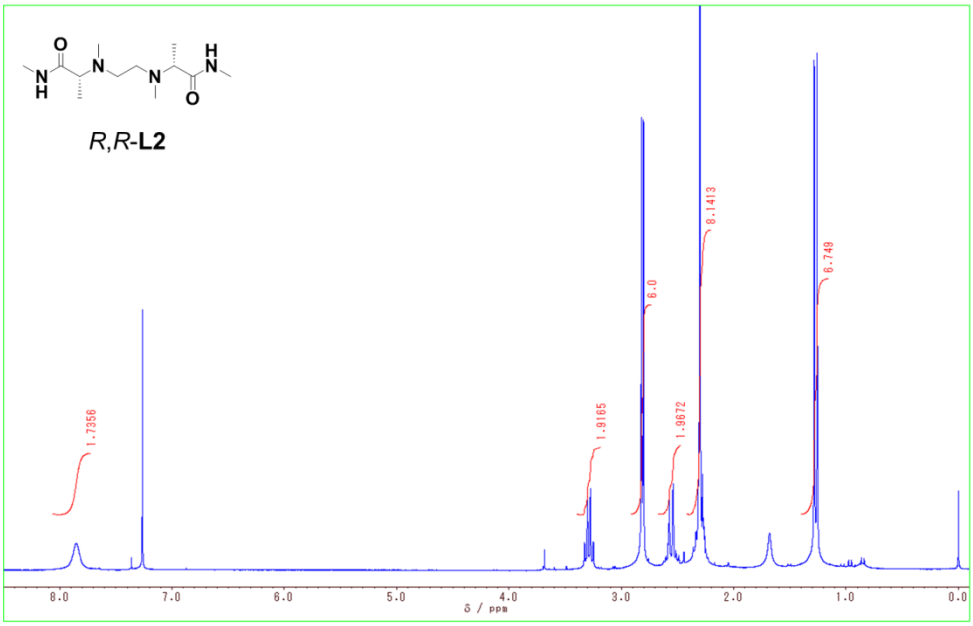
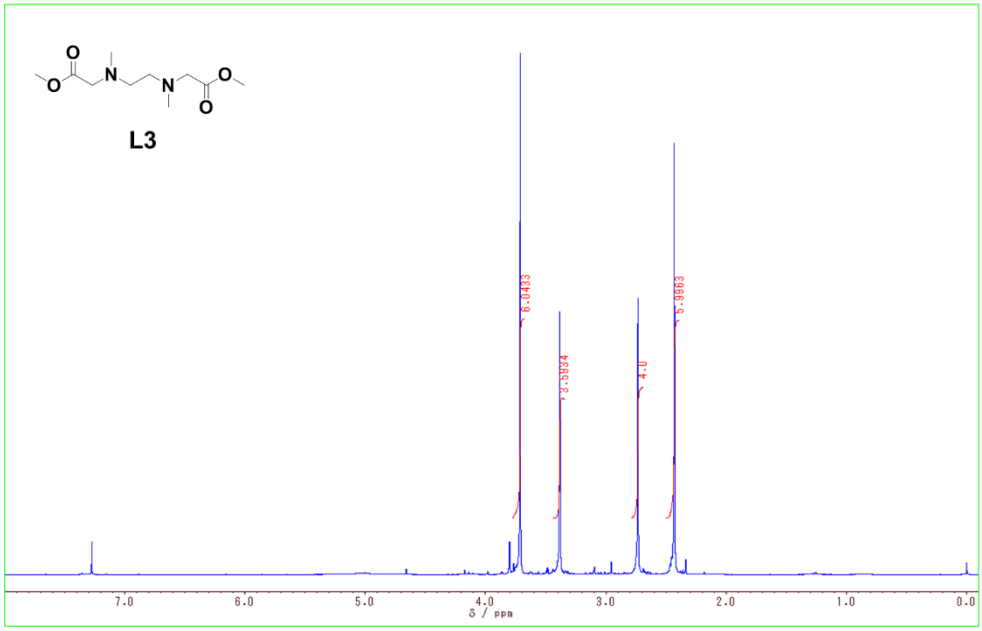


Fig. S1 ^{1}H-NMR spectra of ligands (continued). (c) *R*,*R*-**L1** in [^{2}H]-chloroform at room temperature, (d) *R*,*R*-**L2** in [^{2}H]-chloroform at room temperature.

(e)



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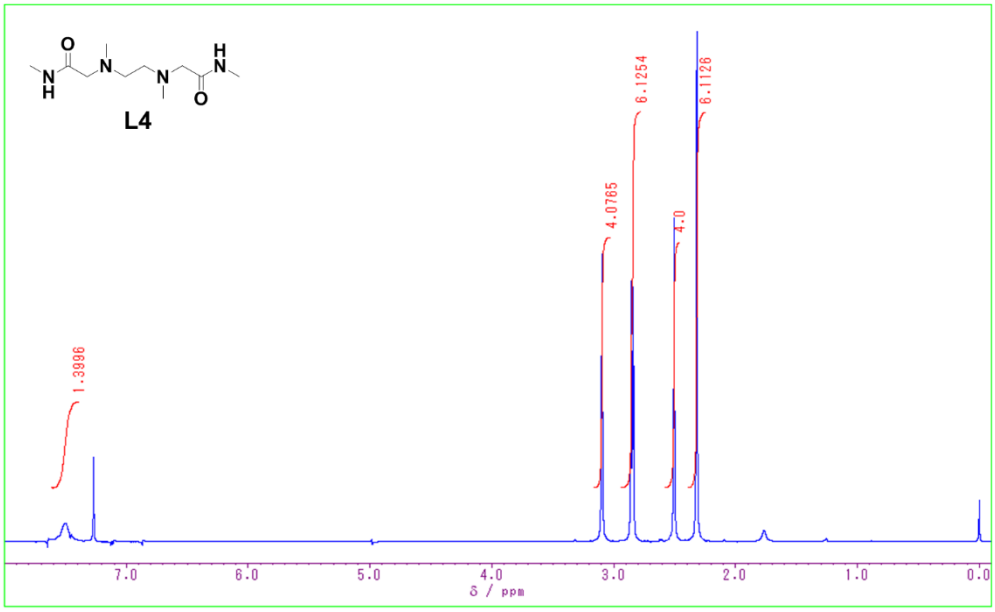
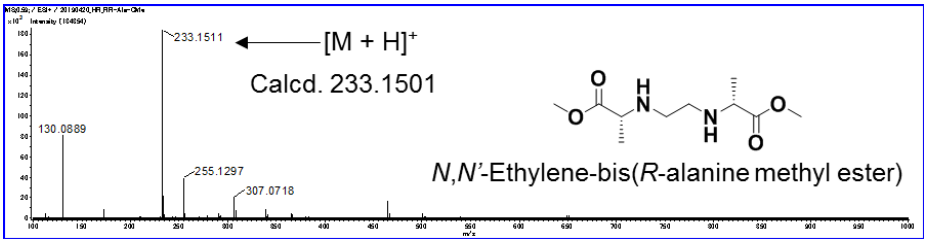


Fig. S1 ^{1}H-NMR spectra of ligands (continued). (e) **L3** in [^{2}H]-chloroform at room temperature, (f) **L4** in [^{2}H]-chloroform at room temperature.

(a)



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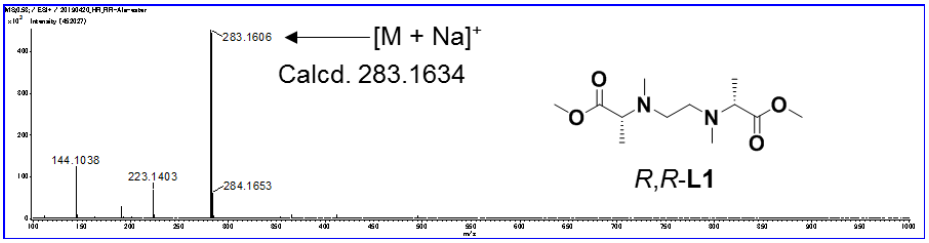

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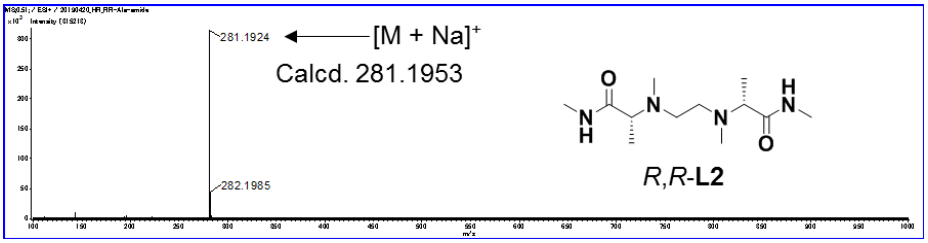

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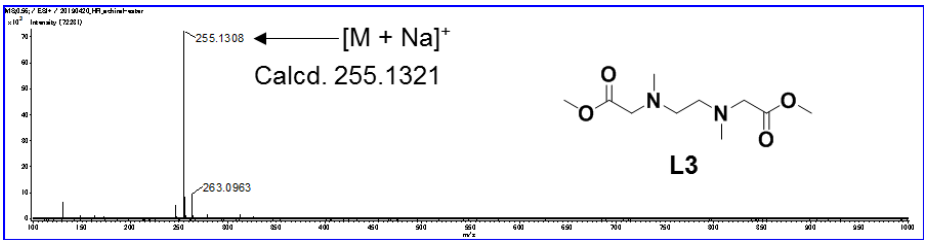

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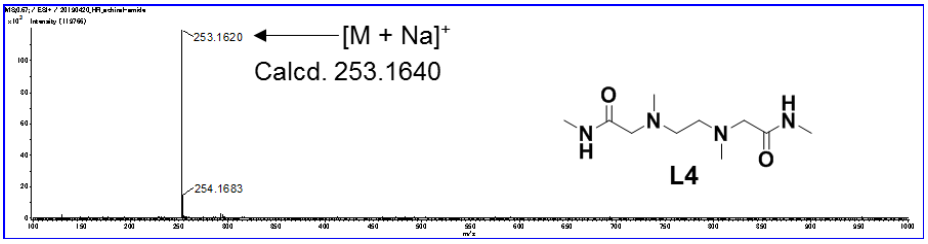


Fig. S2 HR mass spectra of ligands. (a) *N*,*N*'-Ethylene-bis(*R*-alanine methyl ester), (b) *R*,*R*-**L1**, (c) *R*,*R*-**L2**, (d) **L3**, (e) **L4**.

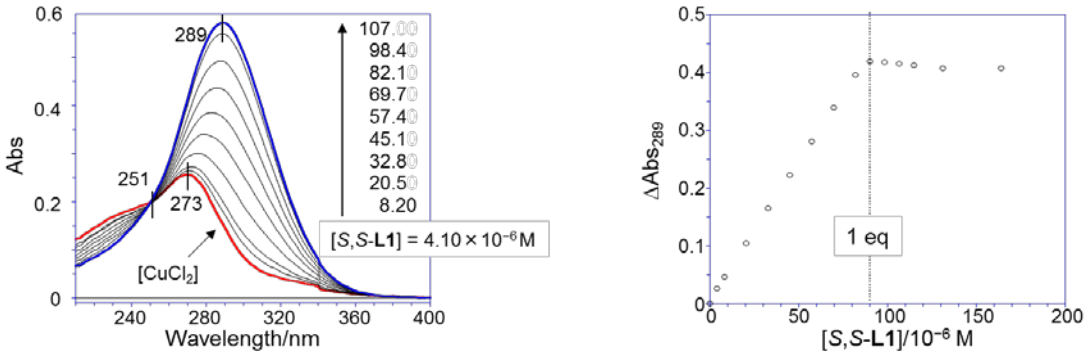


Fig. S3 UV-vis spectral change of $CuCl_2$ upon addition of *S*,*S*-**L1** in methanol (left) and titration profiles (right) at 25°C in 1 cm quartz cell. $[CuCl_2]_0 = 97.8 \times 10^{-6}$ M.

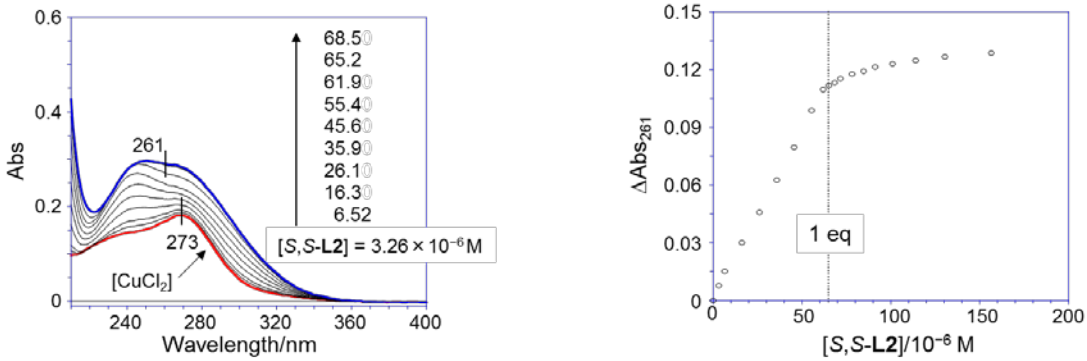


Fig. S4 UV-vis spectral change of $CuCl_2$ upon addition of *S*,*S*-**L2** in methanol (left) and titration profiles (right) at 25°C in 1 cm quartz cell. $[CuCl_2]_0 = 68.8 \times 10^{-6}$ M.

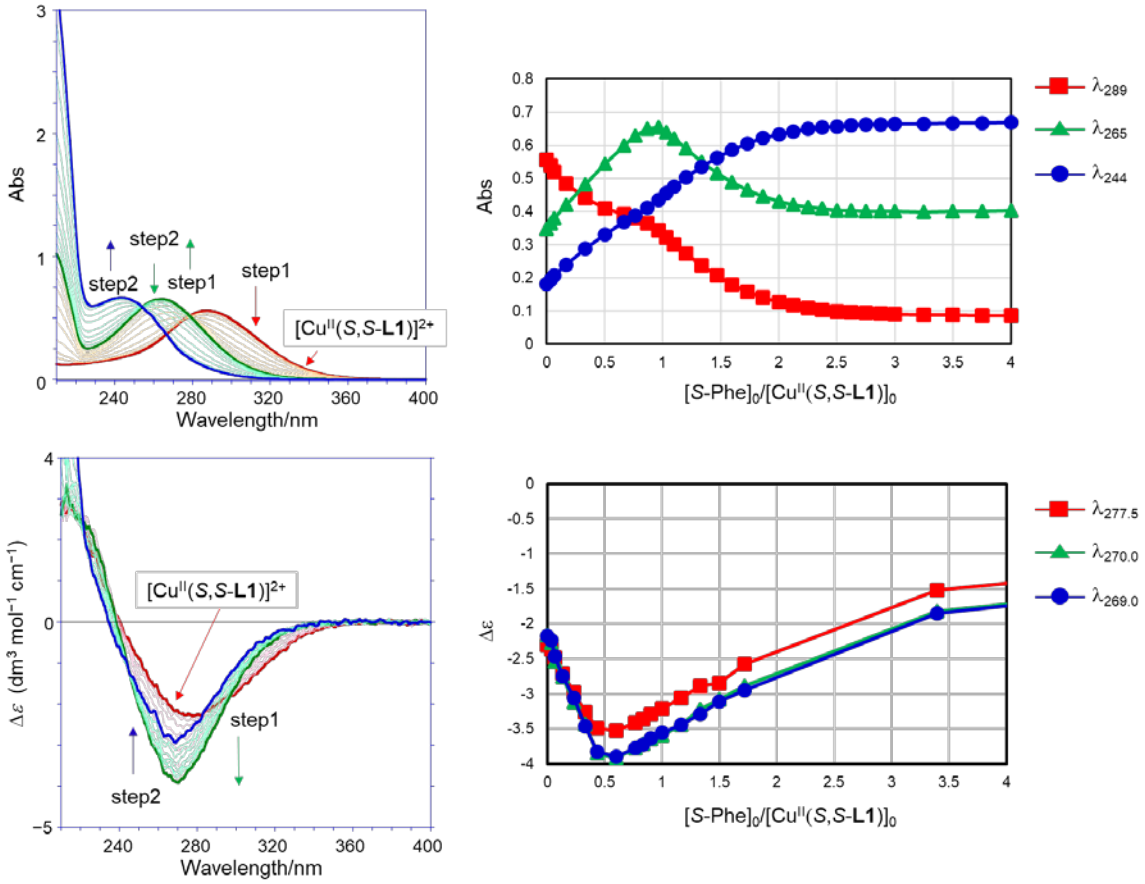


Fig. S5 UV-vis (upper) and CD (bottom) spectral change of *in situ* generated [Cu^{II}(*S*,*S*-**L1**)] complex in methanol upon adding aqueous solution of *S*-Phe and K_2CO_3 at 25°C in 1 cm quartz cell and their titration profiles. $[CuCl_2]_0 = [S,S\text{-}\mathbf{L1}]_0 = 1.00 \times 10^{-4}$ M (red line), step 1 : $[S\text{-Phe}]_0 = [K_2CO_3]_0 = 0 - 1.00 \times 10^{-4}$ M (green line), step 2: $[S\text{-Phe}]_0 = [K_2CO_3]_0 \geq 1.00 \times 10^{-4}$ M (blue line).

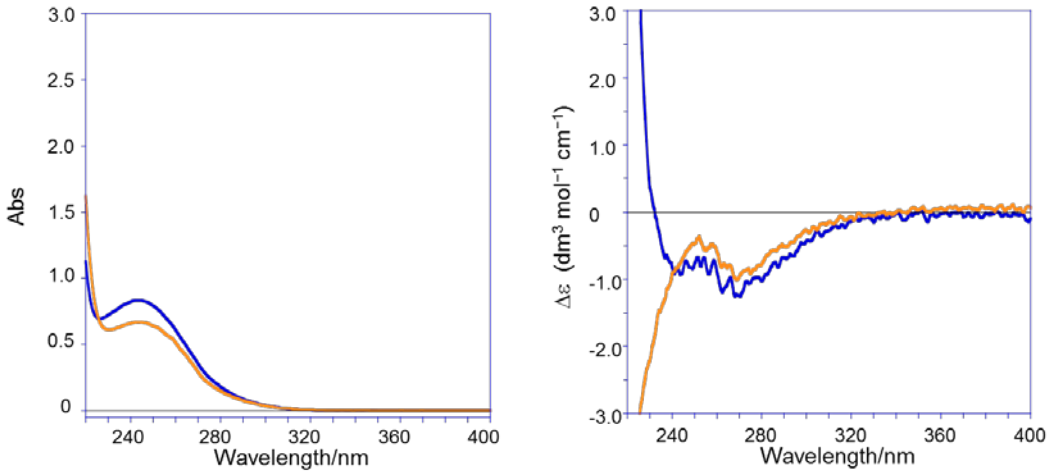
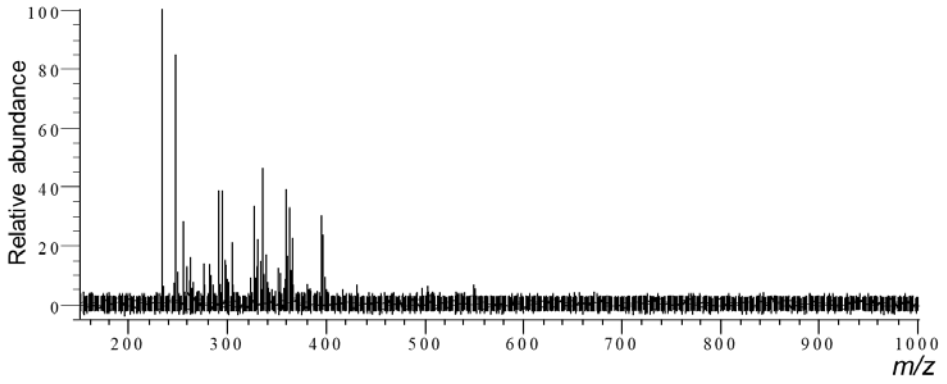


Fig. S6 UV-vis (left) and CD (right) spectra of *in situ* generated copper(II)-*S*-Phe complex, [Cu^{II}(*S*-Phe)$_2$], (orange line) and Cu^{II}-(*S*,*S*-**L1**) complex in the presence of excess amount of *S*-Phe (blue line) in water/methanol. For [Cu^{II}(*S*-Phe)$_2$], $[CuCl_2]_0 = 1.00 \times 10^{-4}$ M and $[S\text{-Phe}]_0 = [K_2CO_3]_0 = 2.00 \times 10^{-4}$ M in water/methanol = 1/50 (v/v). For Cu^{II}-(*S*,*S*-**L1**) complex with excess *S*-Phe, $[CuCl_2]_0 = [S,S\text{-}\mathbf{L1}]_0 = 1.00 \times 10^{-4}$ M and $[S\text{-Phe}]_0 = [K_2CO_3]_0 = 5.00 \times 10^{-4}$ M in water/methanol = 1/20 (v/v).

(a)



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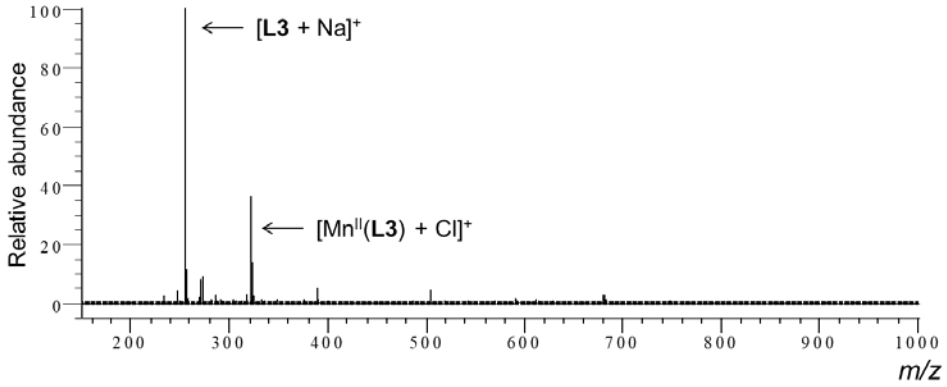

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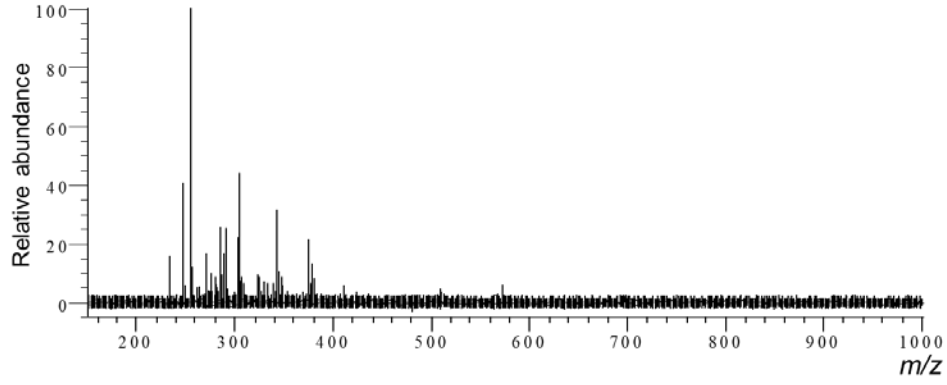
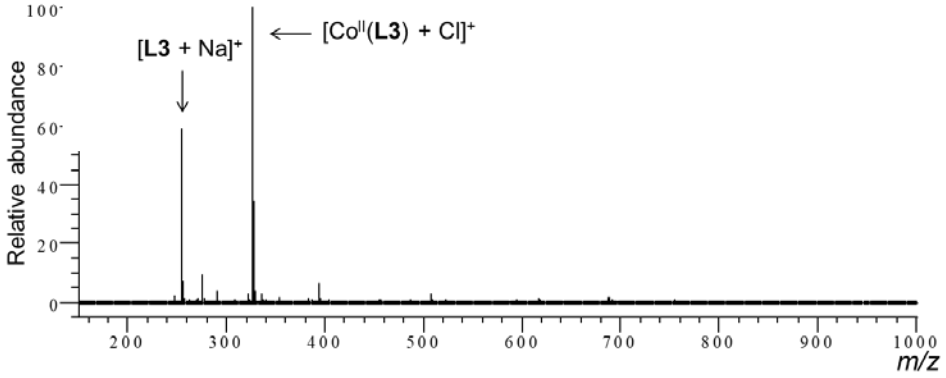


Fig. S7 ESI mass spectra of mixing system of metal chloride/**L3** in methanol. [metal chloride]$_0$ = 1.20×10^{-4} M in methanol and [**L3**]$_0$ = 1.00×10^{-4} M in methanol. [metal]$_0$/[**L3**]$_0$ = 1.2/1. (a) metal chloride = $CrCl_3$, (b) metal chloride = $MnCl_2$, (c) metal chloride = $FeCl_3$.

(d)



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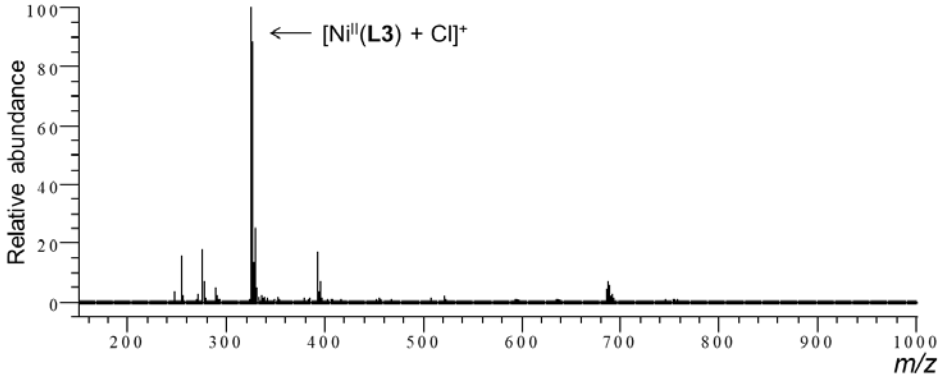


(f)

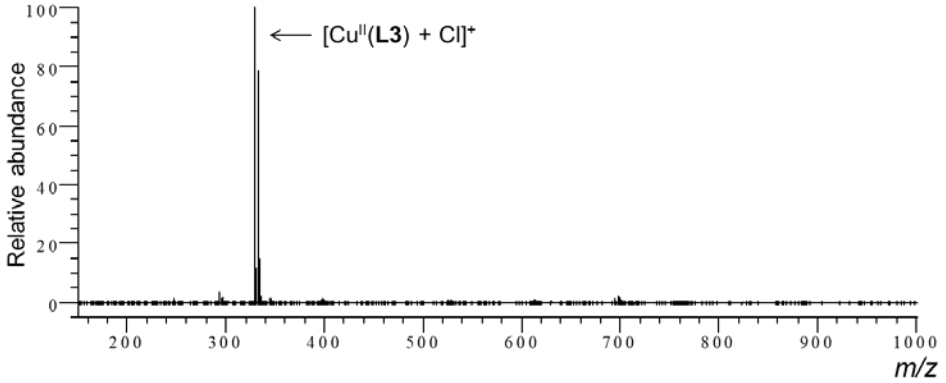
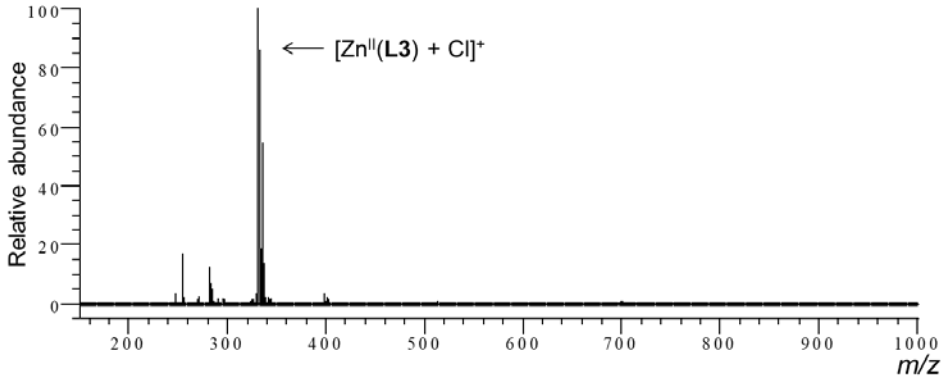


Fig. S7 (continued) (d) metal chloride = $CoCl_2$. (e) metal chloride = $NiCl_2$, (f) metal chloride = $CuCl_2$.

(g)



(h)

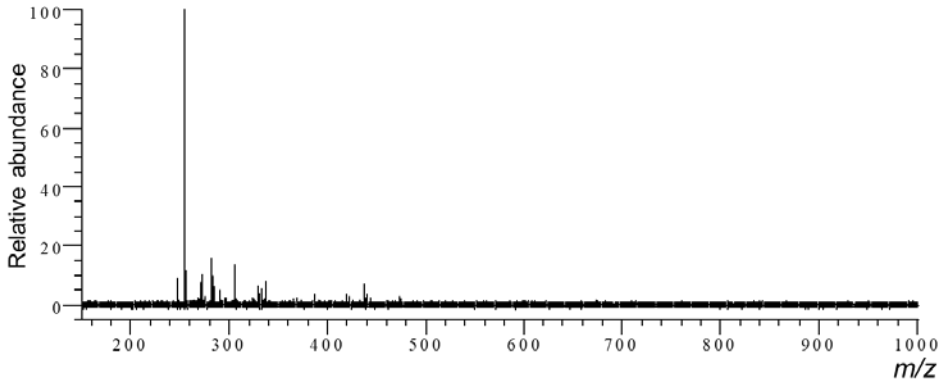
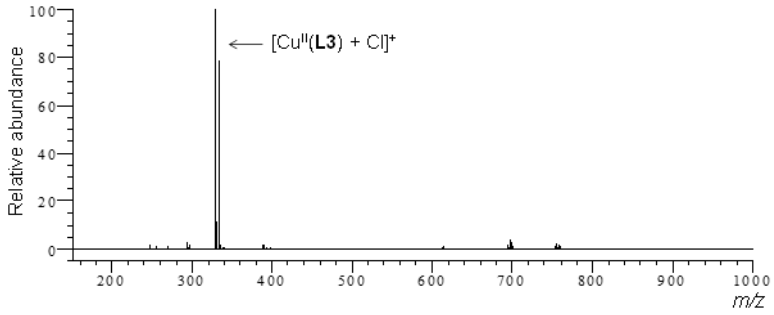
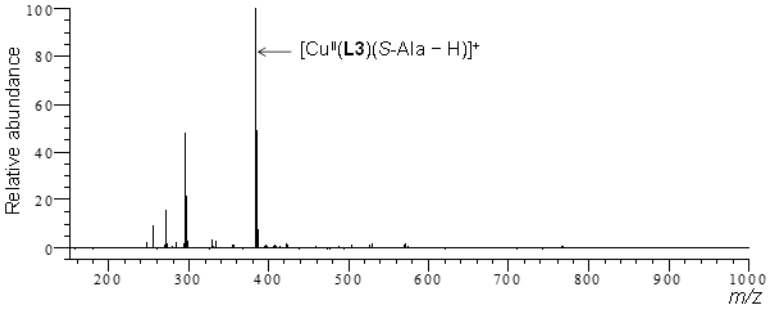


Fig. S7 (continued) (g) metal chloride = $ZnCl_2$, (h) metal chloride = $LaCl_3$.

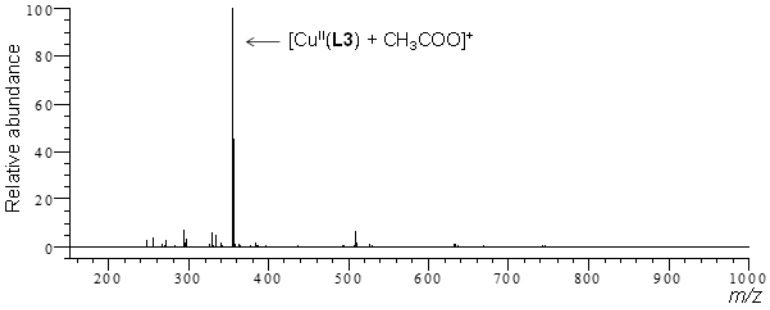
(a)



(b)



(c)



(d)

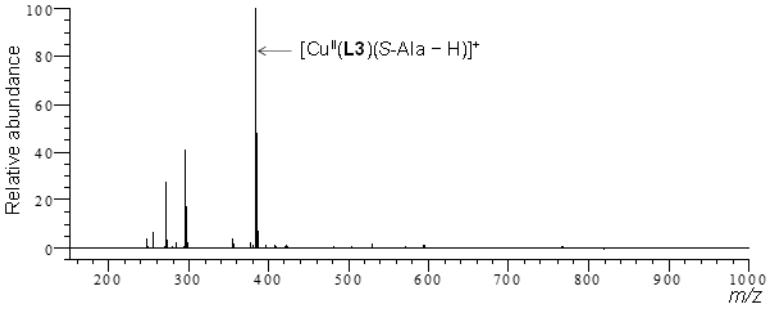
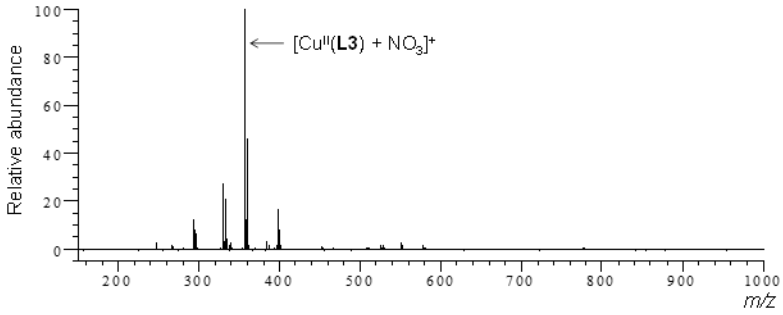
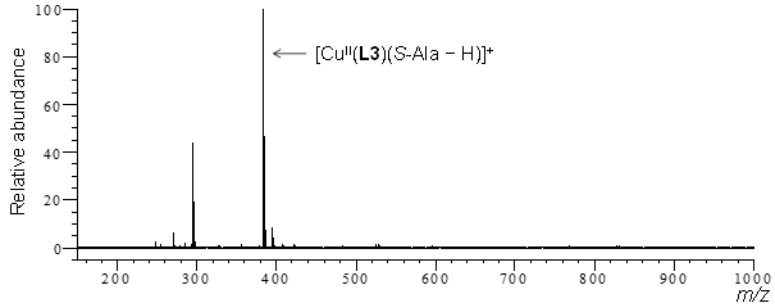


Fig. S8 ESI mass spectra of mixing system of Cu^{II} salt/**L3**, and *S*-Ala in water/methanol (1/100, v/v). [Cu(II) salt]$_0$ = 1.19 × 10^{-4} M, [**L3**]$_0$ = 9.90 × 10^{-5} M, [*S*-Ala]$_0$ = 9.90 × 10^{-5} M and [K_2CO_3]$_0$ = 9.90 × 10^{-5} M. (a) [$CuCl_2$]$_0$/[**L3**]$_0$ = 1.2/1, (b) [$CuCl_2$]$_0$/[**L3**]$_0$/[*S*-Ala]$_0$ = 1.2/1/1, (c) [$Cu(CH_3COO)_2$]$_0$ /[**L3**]$_0$ = 1.2/1, (d) [$Cu(CH_3COO)_2$]$_0$/[**L3**]$_0$/[*S*-Ala]$_0$ = 1.2/1/1.

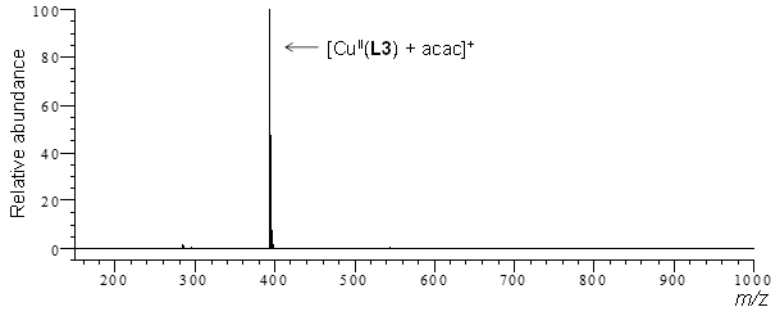
(e)



(f)



(g)



(h)

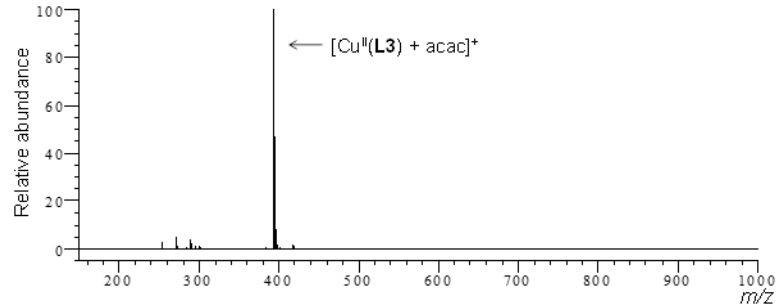
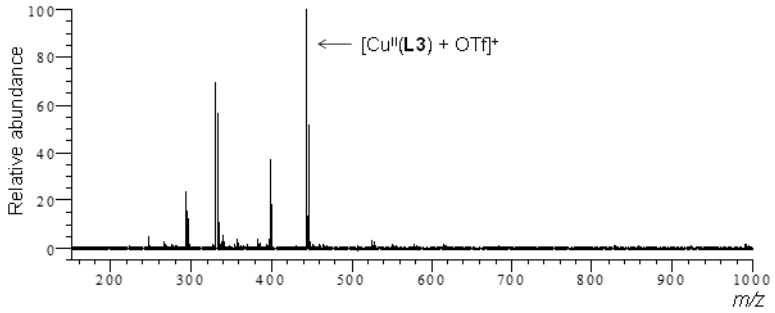


Fig. S8 (continued) (e) $[Cu(NO_3)_2]_0/[\mathbf{L3}]_0$ = 1.2/1, (f) $[Cu(NO_3)_2]_0/[\mathbf{L3}]_0/[S\text{-Ala}]_0$ = 1.2/1/1, (g) $[Cu(acac)_2]_0/[\mathbf{L3}]_0$ = 1.2/1, (h) $[Cu(acac)_2]_0/[\mathbf{L3}]_0/[S\text{-Ala}]_0$ = 1.2/1/1.

(i)



(j)

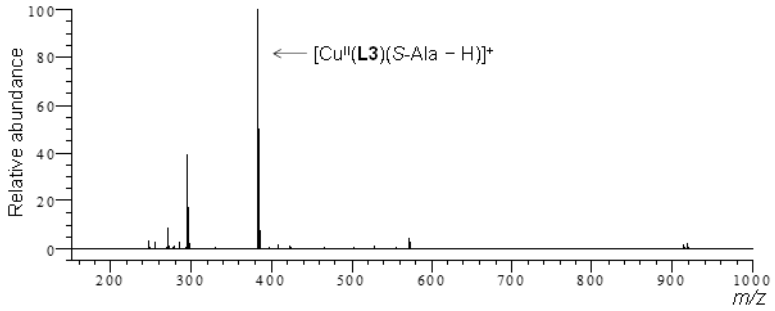
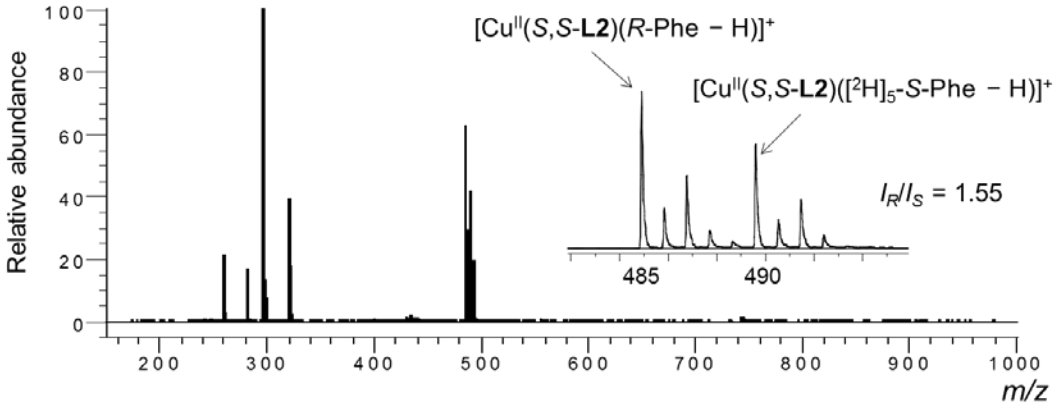


Fig. S8 (continued) (i) $[Cu(OTf)_2]_0/[\mathbf{L3}]_0$ = 1.2/1, (j) $[Cu(OTf)_2]_0/[\mathbf{L3}]_0/[S\text{-Ala}]_0$ = 1.2/1/1.

(a)



(b)

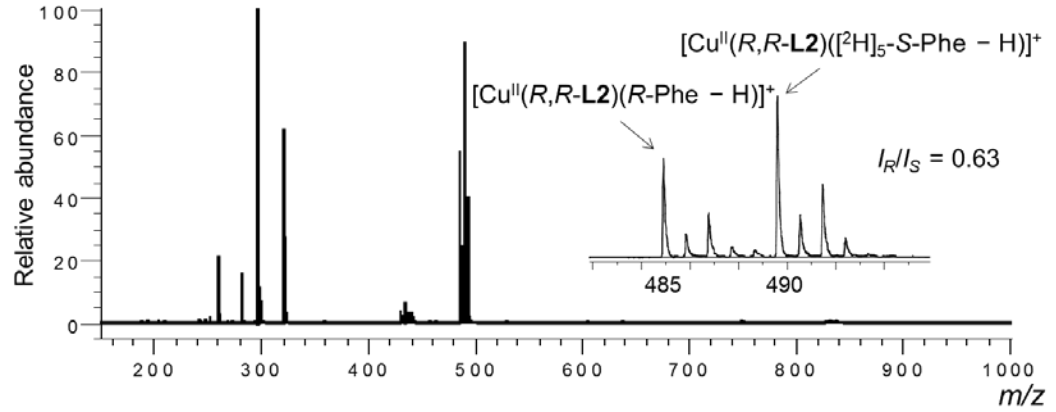
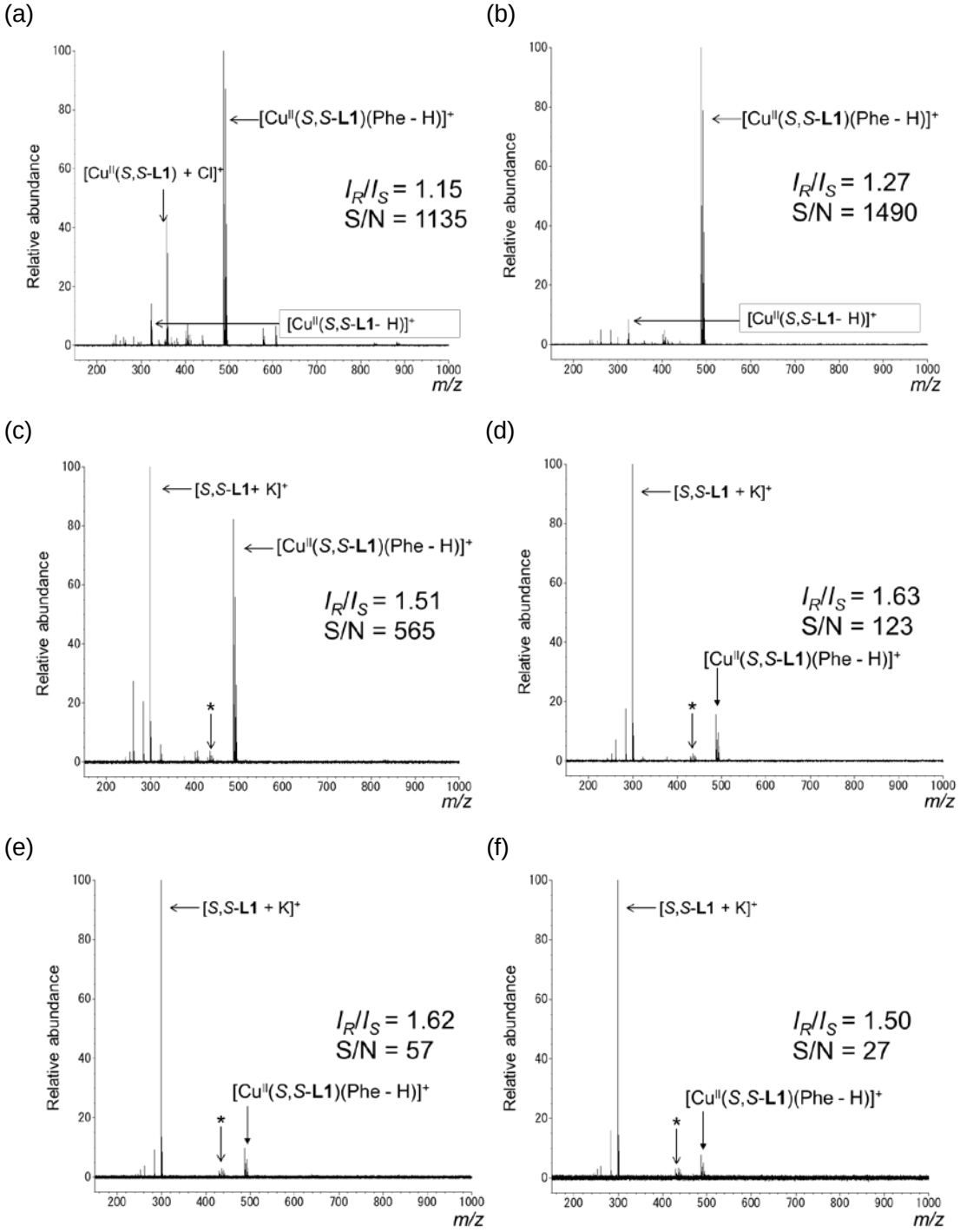


Fig. S9 Comparison of ESI mass spectra of mixing system of (a) $CuCl_2$/*S*,*S*-**L2**/*R*-Phe/[^{2}H]$_5$-*S*-Phe and (b) $CuCl_2$/*R*,*R*-**L2**/*R*-Phe/[^{2}H]$_5$-*S*-Phe in water/methanol (2/5, v/v). $[CuCl_2]_0 = 8.57 \times 10^{-5}$ M, $[\mathbf{L2}]_0 = 7.14 \times 10^{-5}$ M, $[R\text{-Phe}]_0 = 1.43 \times 10^{-4}$ M, $[[^2H]_5\text{-}S\text{-Phe}]_0 = 1.43 \times 10^{-4}$ M and $[K_2CO_3]_0 = 2.83 \times 10^{-4}$ M. $[CuCl_2]_0/[\mathbf{L2}]_0/[R\text{-Phe}]_0/[[^2H]_5\text{-}S\text{-Phe}]_0 = 1.2/1.0/2.0/2.0$.


(a)
[Cu^{II}(*S*,*S*-**L1**)(Phe - H)]$^+$
[Cu^{II}(*S*,*S*-**L1**) + Cl]$^+$
I_R/I_S = 1.15
S/N = 1135
[Cu^{II}(*S*,*S*-**L1**- H)]$^+$
Relative abundance
m/z
(b)
[Cu^{II}(*S*,*S*-**L1**)(Phe - H)]$^+$
I_R/I_S = 1.27
S/N = 1490
[Cu^{II}(*S*,*S*-**L1**- H)]$^+$
Relative abundance
m/z
(c)
[*S*,*S*-**L1**+ K]$^+$
[Cu^{II}(*S*,*S*-**L1**)(Phe - H)]$^+$
I_R/I_S = 1.51
S/N = 565
*
Relative abundance
m/z
(d)
[*S*,*S*-**L1** + K]$^+$
I_R/I_S = 1.63
S/N = 123
[Cu^{II}(*S*,*S*-**L1**)(Phe - H)]$^+$
*
Relative abundance
m/z
(e)
[*S*,*S*-**L1** + K]$^+$
I_R/I_S = 1.62
S/N = 57
[Cu^{II}(*S*,*S*-**L1**)(Phe - H)]$^+$
*
Relative abundance
m/z
(f)
[*S*,*S*-**L1** + K]$^+$
I_R/I_S = 1.50
S/N = 27
[Cu^{II}(*S*,*S*-**L1**)(Phe - H)]$^+$
*
Relative abundance
m/z

(g)

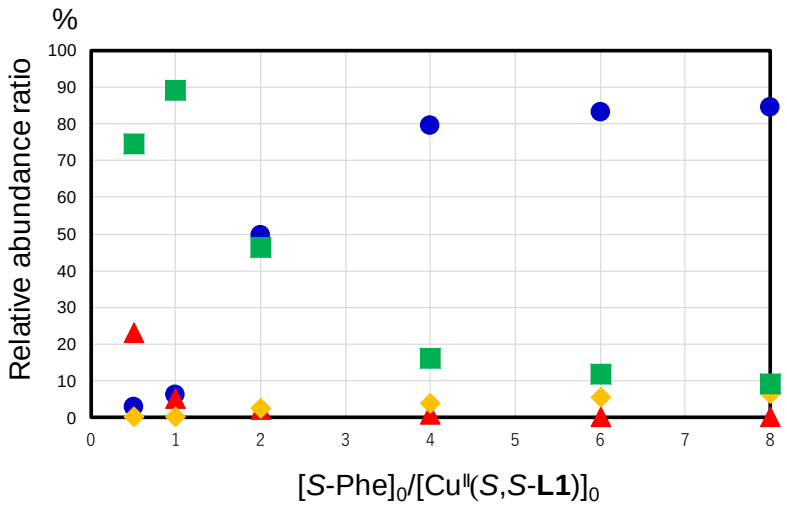
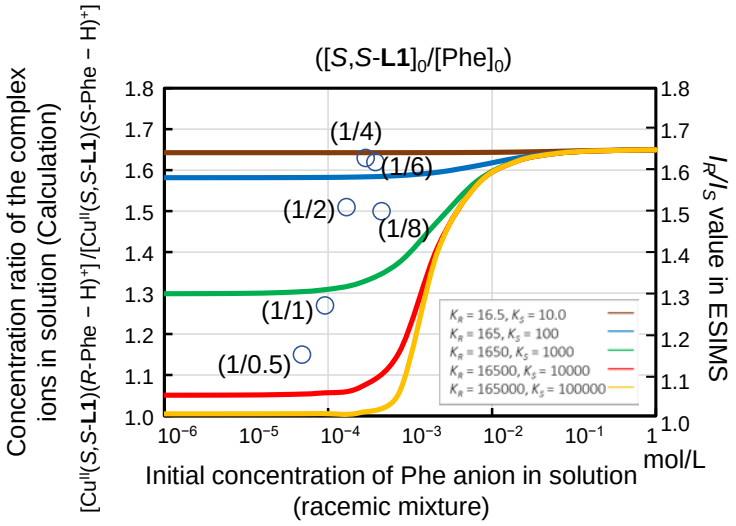


Fig. S10 ESI mass spectra of $CuCl_2$/*S*,*S*-**L1**/*R*-Phe/$[^2H]_5$-*S*-Phe in water/methanol. The sample solution was prepared by mixing 1.00 mL of *in situ* prepared complex solution in methanol ($[CuCl_2]_0 = 1.2 \times 10^{-4}$ M and $[S,S\text{-}\mathbf{L1}]_0 = 1.0 \times 10^{-4}$ M) and a solution of an equimolar mixture of *R*-Phe and $[^2H]_5$-*S*-Phe ($[R\text{-Phe}]_0 = [[^2H]_5\text{-}S\text{-Phe}]_0 = 5.0 \times 10^{-4}$ M and $[K_2CO_3]_0 = 1.0 \times 10^{-3}$ M) in water. The resulting mole ratio of each component $[CuCl_2]_0/[\mathbf{L1}]_0/[R\text{-Phe}]_0/[[^2H]_5\text{-}S\text{-Phe}]_0$ (the amount of adding aqueous solution) : (a) 1.2/1.0/0.25/0.25 (50 μL), (b) 1.2/1.0/0.5/0.5 (100 μL), (c) 1.2/1.0/1.0/1.0 (200 μL), (d) 1.2/1.0/2.0/2.0 (400 μL), (e) 1.2/1.0/3.0/3.0 (600 μL), (f) 1.2/1.0/4.0/4.0 (800 μL). * $[Cu^{II}(Phe - H)_2 + K]^+$. (g) Titration profiles of ions derived from their complex. The sum of the intensity values of the ions derived from each complex was plotted as percentage. The original complex and the derived ions are shown blow. (■) $[Cu^{II}(S,S\text{-}\mathbf{L1})(Phe - H)]^+$ related ions, $[Cu^{II}(S,S\text{-}\mathbf{L1})(R\text{-Phe} - H)]^+$ (*m/z* 485) and $[Cu^{II}(S,S\text{-}\mathbf{L1})([^2H]_5\text{-}S\text{-Phe} - H)]^+$ (*m/z* 490); (▲) $[Cu^{II}(S,S\text{-}\mathbf{L1})(CH_3OH)_2]^{2+}$ related ions, $[Cu^{II}(S,S\text{-}\mathbf{L1}) - H]^+$ (*m/z* 323) and $[Cu^{II}(S,S\text{-}\mathbf{L1}) + Cl]^+$ (*m/z* 358); (●) *S*,*S*-**L1** related ions, $[S,S\text{-}\mathbf{L1} + H]^+$ (*m/z* 259), $[S,S\text{-}\mathbf{L1} + Na]^+$ (*m/z* 281) and $[S,S\text{-}\mathbf{L1} + K]^+$ (*m/z* 297); (◆) $[Cu^{II}(Phe - H)_2]$ related ions, $[Cu^{II}(R\text{-Phe} - H)_2 + K]^+$ (*m/z* 430), $[Cu^{II}(R\text{-Phe}– H)([^2H]_5\text{-}S\text{-Phe} – H) + K]^+$ (*m/z* 435) and $[Cu^{II}([^2H]_5\text{-}S\text{-Phe} - H)_2 + K]^+$ (*m/z* 440).

(a)



(b)

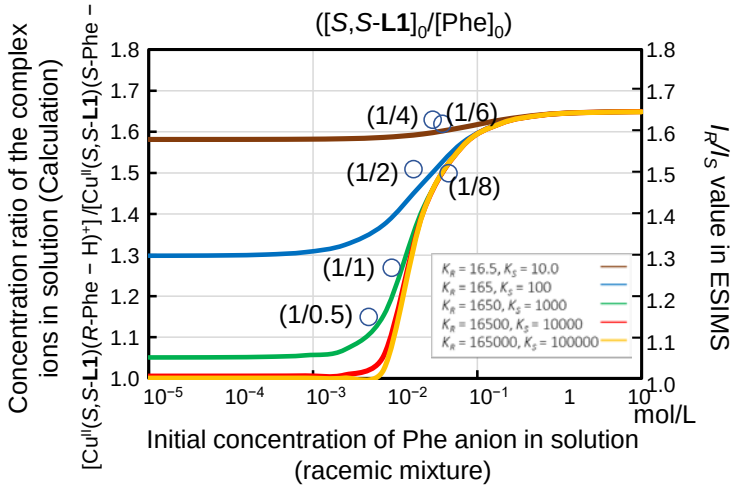


Fig. S11 Plots of the calculated concentration ratio of the diastereomeric complex ions, [Cu^{II}(*S*,*S*-**L1**)(*R*-Phe − H)$^+$]/[Cu^{II}(*S*,*S*-**L1**)(*S*-Phe − H)$^+$], in solution (line) and the I_R/I_S values obtained by the MS/EL method (circle) in **Fig. S10** versus the initial concentration of [Phe]$_0$ = [*R*-Phe]$_0$ + [*S*-Phe]$_0$ ([*R*-Phe]$_0$/[*S*-Phe]$_0$ =1) in solution. (a) Concentration of solution is experimental condition (the initial concentration of the complex [Cu^{II}(*S*,*S*-**L1**)$(CH_3OH)_2]^{2+}$: 7.14×10^{-5} M), (b) concentration of solution is 100 times higher than the experimental condition (the initial concentration of the complex [Cu^{II}(*S*,*S*-**L1**)$(CH_3OH)_2]^{2+}$: 7.14×10^{-3} M),. In the calculation, the ratio of the association constants was assumed to be constant (K_R/K_S = 1.65).

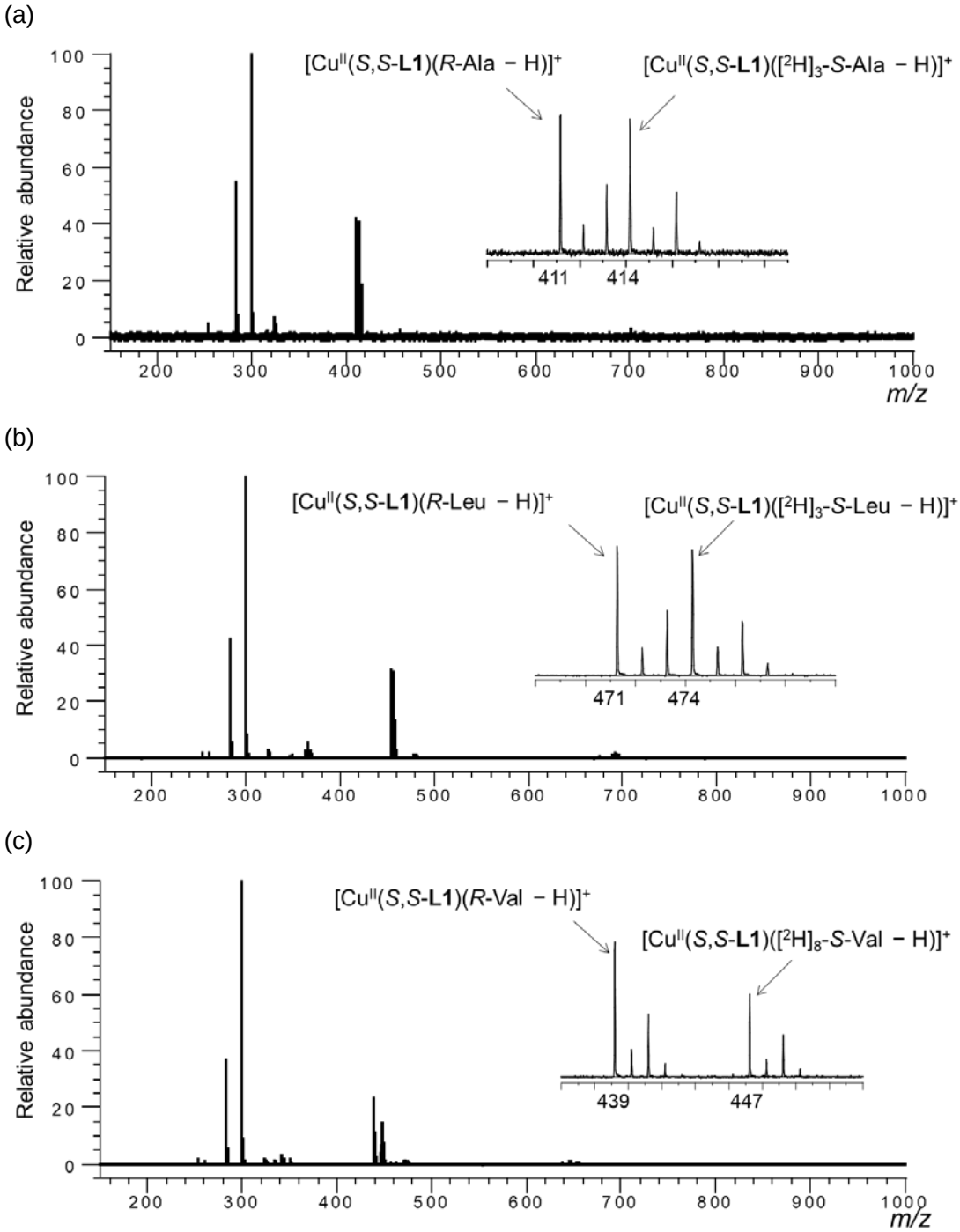
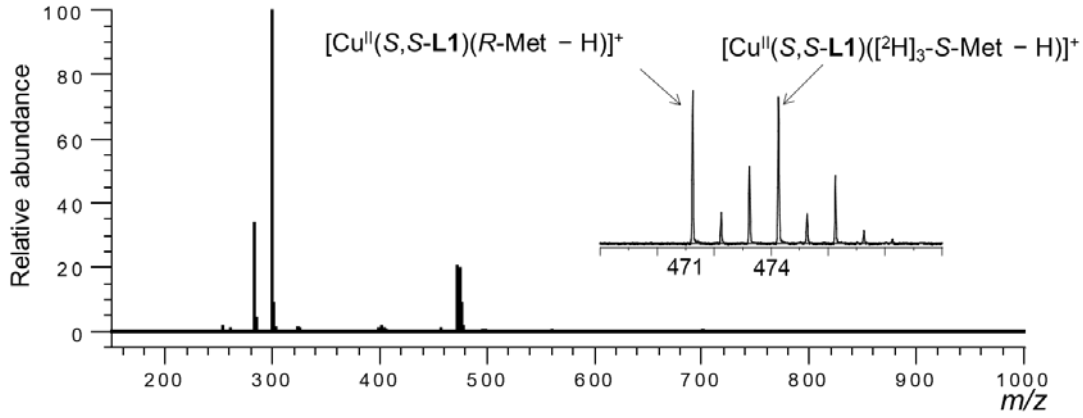
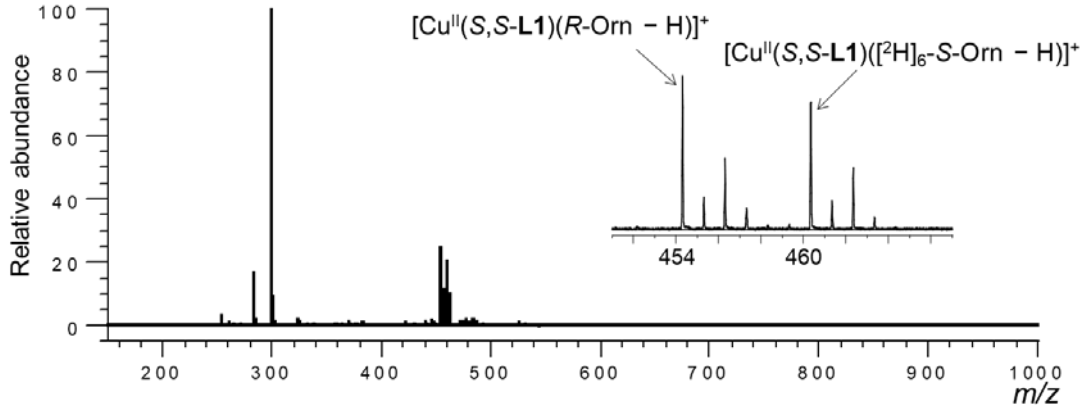


Fig. S12 ESI mass spectra of $CuCl_2$/**L**/*R*-AA/[^{2}H]$_n$-*S*-AA in water/methanol (2/5, v/v). $[CuCl_2]_0$ = 8.57×10^{-5} M, $[\mathbf{L}]_0 = 7.14 \times 10^{-5}$ M, $[R\text{-AA}]_0 = 1.43 \times 10^{-4}$ M, $[[^2H]_n\text{-}S\text{-AA}]_0 = 1.43 \times 10^{-4}$ M and $[K_2CO_3]_0 = 2.83 \times 10^{-4}$ M. $[CuCl_2]_0/[\mathbf{L}]_0/[R\text{-AA}]_0/[[^2H]_n\text{-}S\text{-AA}]_0$ = 1.2/1.0/2.0/2.0. (a) **L** = *S*,*S*-**L1**, AA= *R*-Ala/[^{2}H]$_3$-*S*-Ala, (b) **L** = *S*,*S*-**L1**, AA= *R*-Leu/[^{2}H]$_3$-*S*-Leu, (c) **L** = *S*,*S*-**L1**, AA= *R*-Val/[^{2}H]$_8$-*S*-Val.

(d)



(e)



(f)

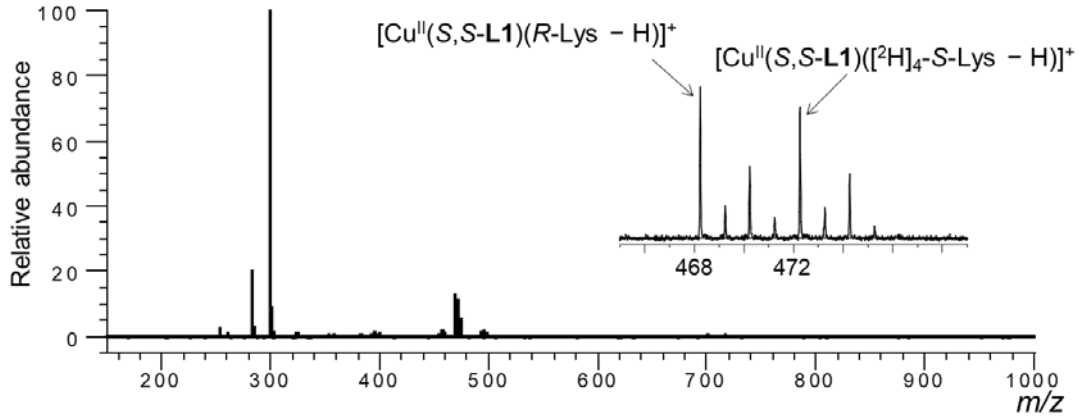
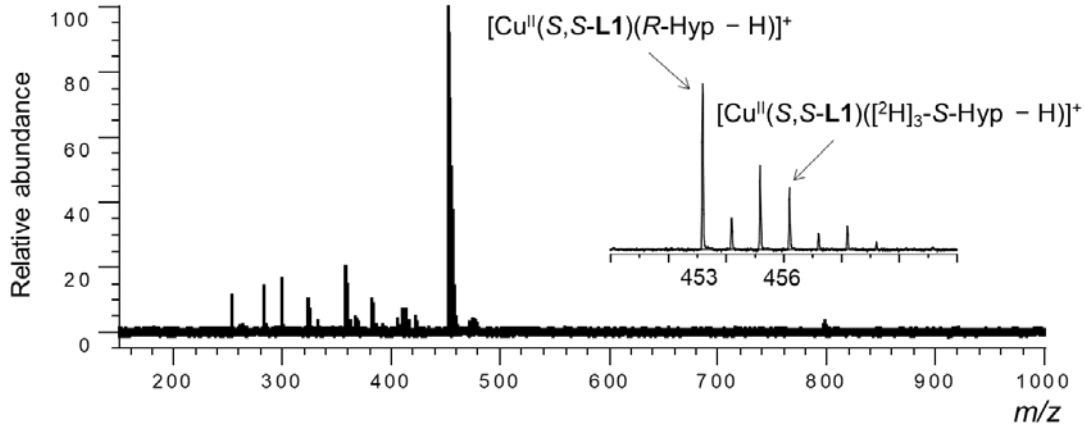
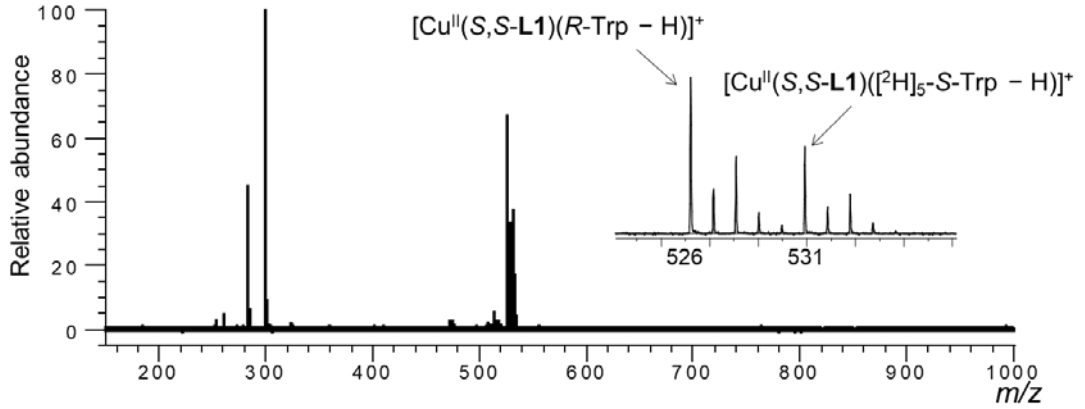


Fig. S12 (continued) (d) **L** = *S*,*S*-**L1**, AA= *R*-Met/[^{2}H]$_3$-*S*-Met, (e) **L** = *S*,*S*-**L1**, AA= *R*-Orn/[^{2}H]$_6$-*S*-Orn, (f) **L** = *S*,*S*-**L1**, AA= *R*-Lys/ [^{2}H]$_4$-*S*-Lys.

(g)



(h)



(i)

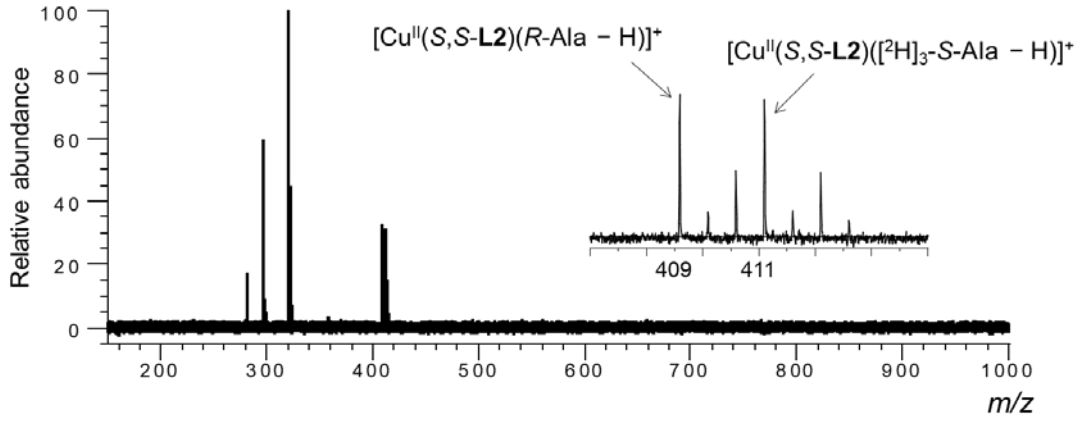
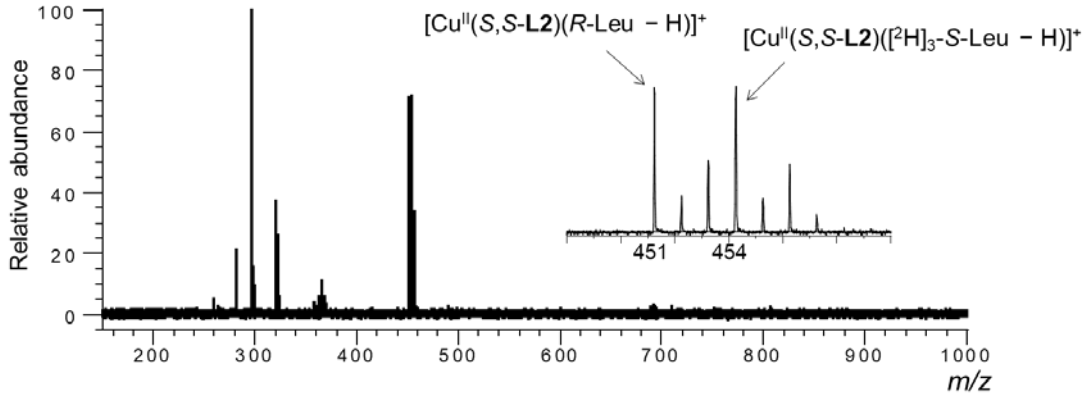
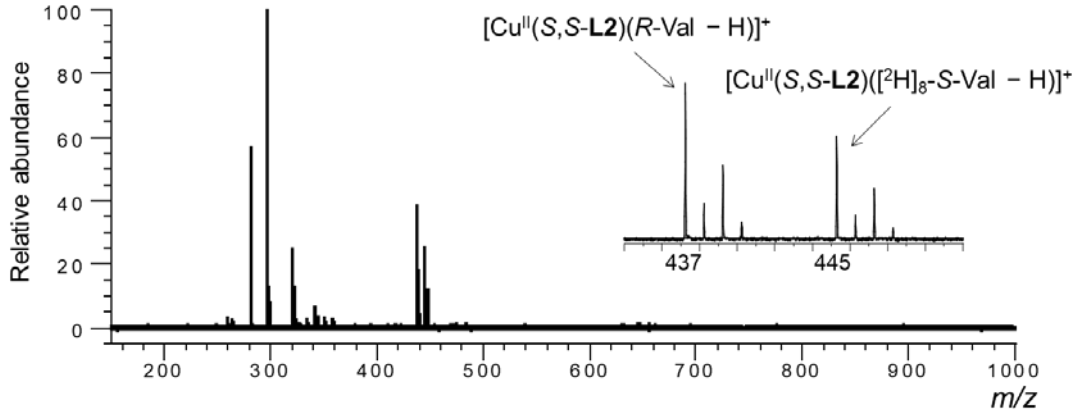


Fig. S12 (continued) (g) **L** = *S*,*S*-**L1**, AA= *R*-Hyp/[^{2}H]$_3$-*S*-Hyp, (h) **L** = *S*,*S*-**L1**, AA= *R*-Trp/[^{2}H]$_5$-*S*-Trp, (i) **L** = *S*,*S*-**L2**, AA= *R*-Ala/[^{2}H]$_3$-*S*-Ala.

(j)



(k)



(l)

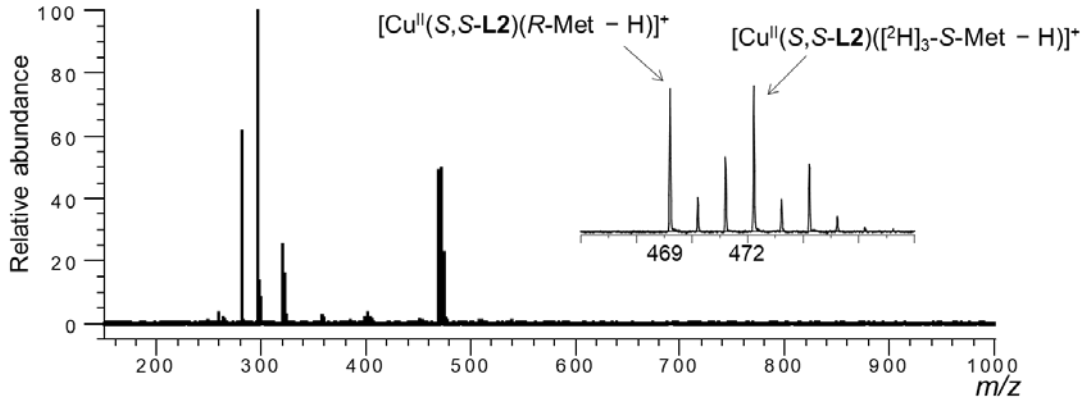
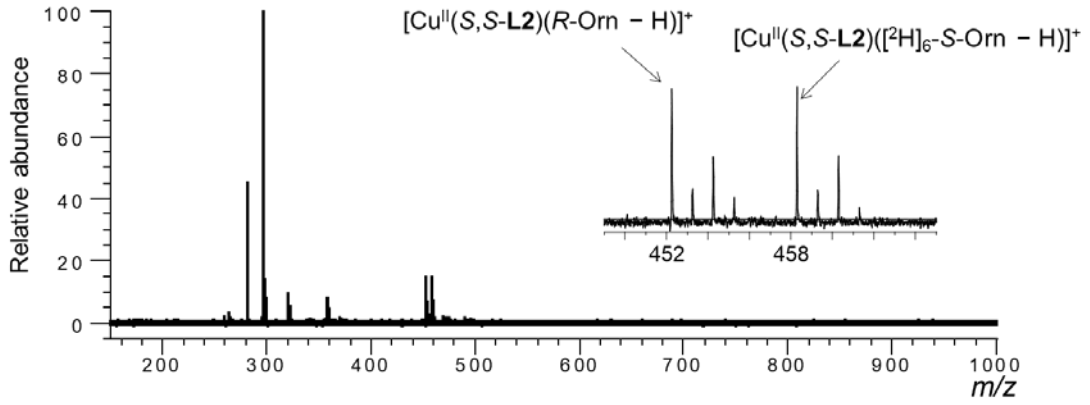
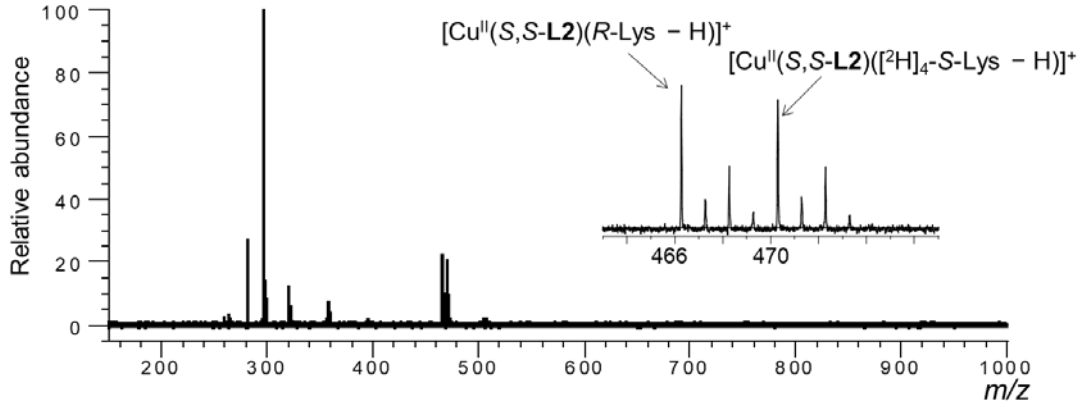


Fig. S12 (continued) (j) **L** = *S*,*S*-**L2**, AA= *R*-Leu/[^{2}H]$_3$-*S*-Leu, (k) **L** = *S*,*S*-**L2**, AA= *R*-Val/[^{2}H]$_8$-*S*-Val, (l) **L** = *S*,*S*-**L2**, AA= *R*-Met/ [^{2}H]$_3$-*S*-Met.

(m)



(n)



(o)

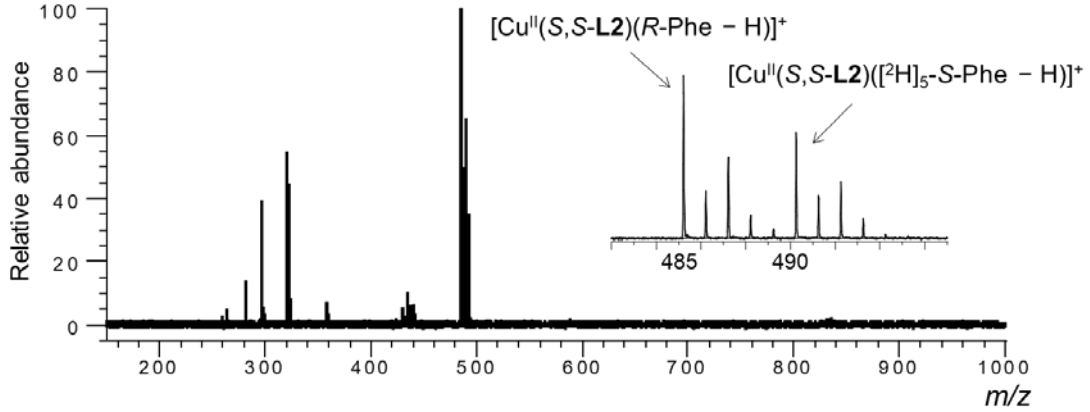
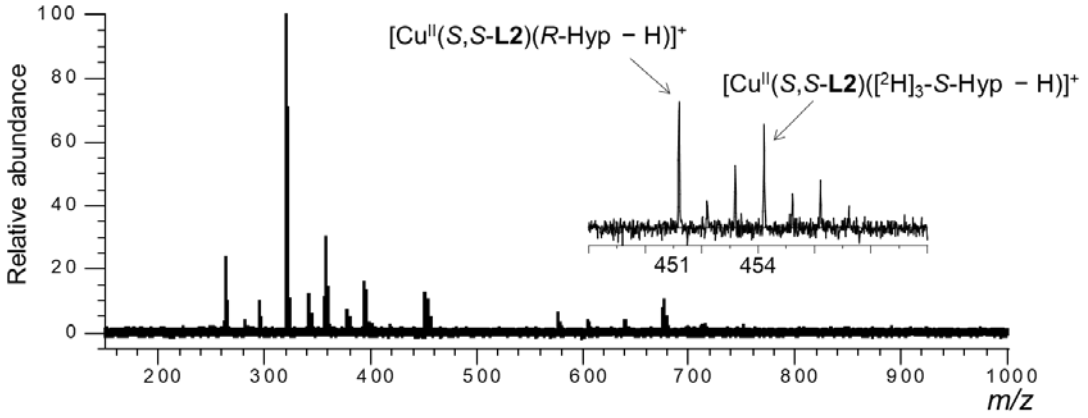


Fig. S12 (continued) (m) **L** = *S*,*S*-**L2**, AA= *R*-Orn/[^{2}H]$_6$-*S*-Orn, (n) **L** = *S*,*S*-**L2**, AA= *R*-Lys/[^{2}H]$_4$-*S*-Lys, (o) **L** = *S*,*S*-**L2**, AA= *R*-Phe/[^{2}H]$_5$-*S*-Phe.

(p)



(q)

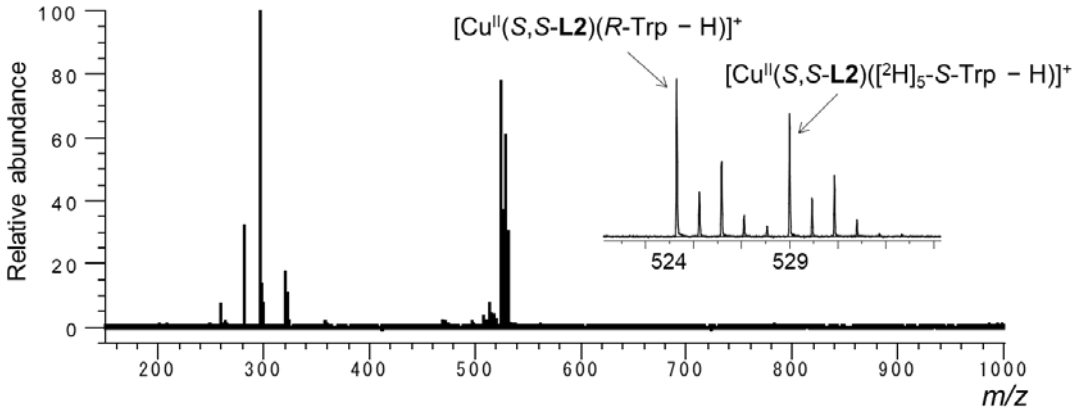


Fig. S12 (continued) (p) **L** = *S*,*S*-**L2**, AA= *R*-Hyp/[^{2}H]$_3$-*S*-Hyp, (q) **L** = *S*,*S*-**L2**, AA= *R*-Trp/[^{2}H]$_5$-*S*-Trp.