

## Supporting Information

### Pyrene-modified Cyclic Peptides Detect Cu<sup>2+</sup> Ions by Fluorescence in Water

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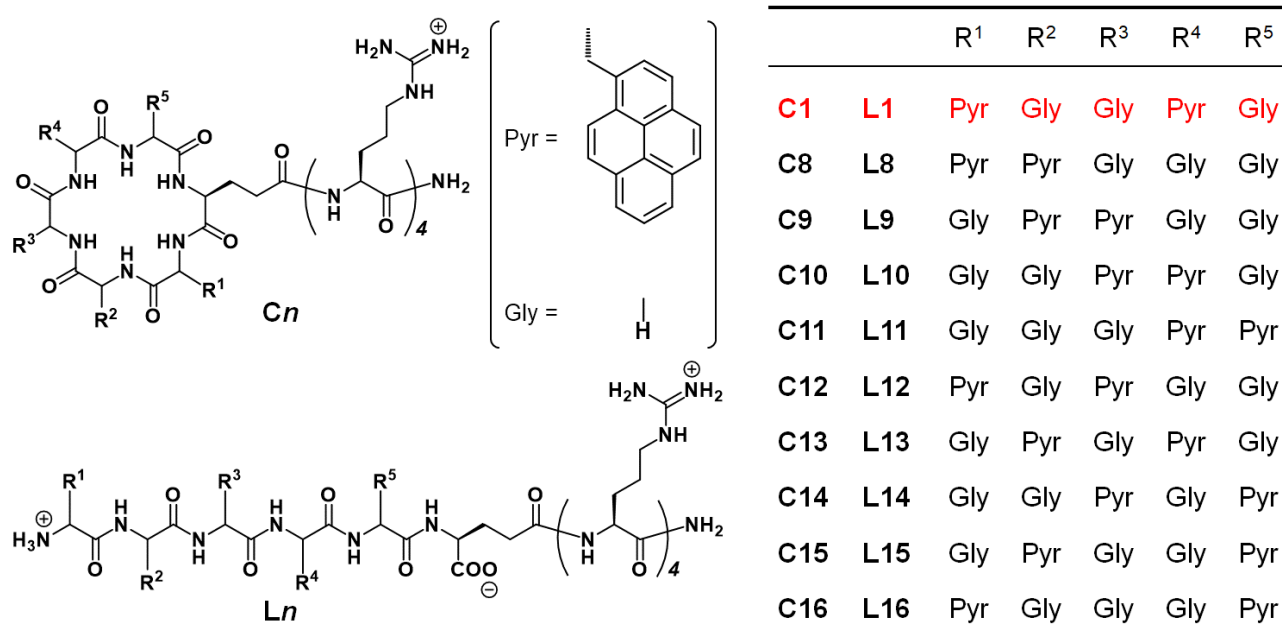


Figure S1. Chemical Structure of **C1** and **C8–C16**, and their linear versions **L1** and **L8–L16**.

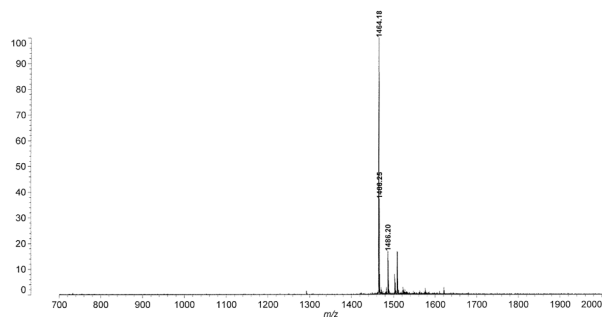
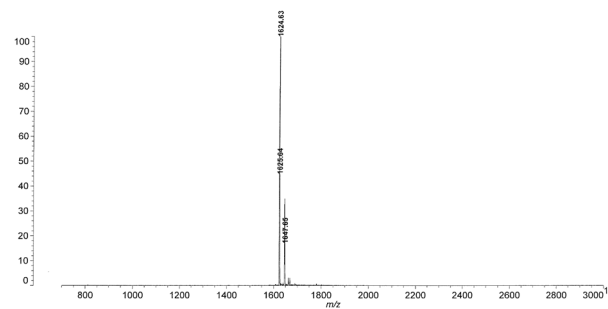
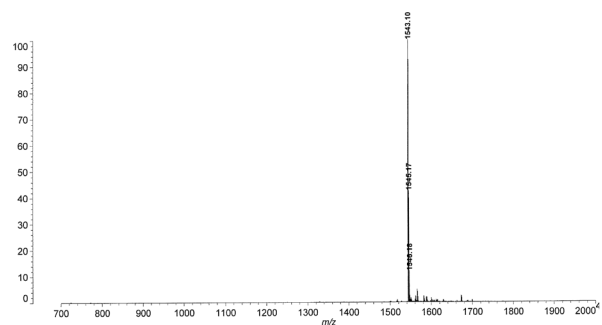
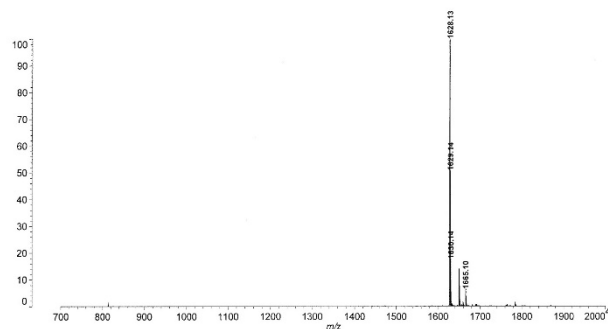
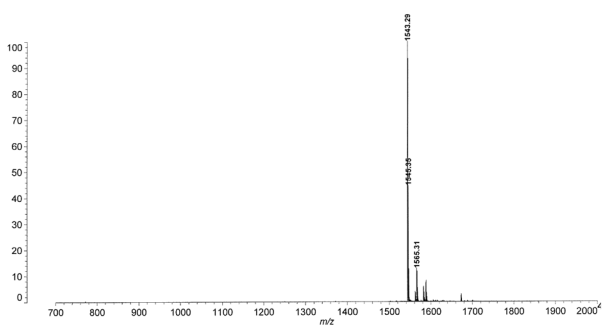
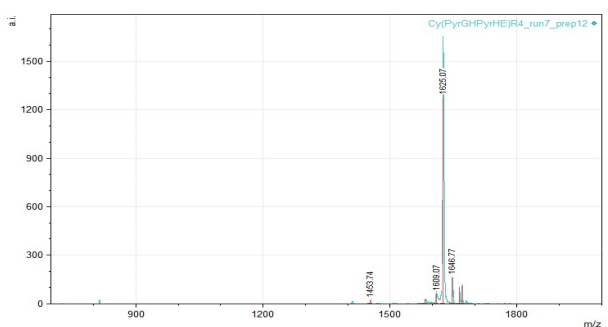
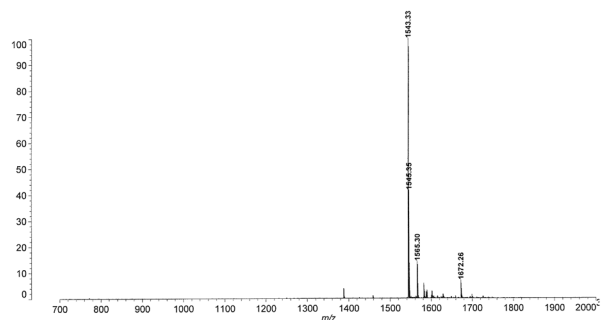
**C1****C5****C2****C6****C3****C7****C4**

Figure S2. MALDI-ToF mass spectra of **C1–C7**. An  $\alpha$ -CHCA ( $\alpha$ -Cyano-4-hydroxycinnamic Acid) was used as a matrix. Calcd.  $[M+H]^+$ ; **C1** = 1466.73, **C2–C4** = 1546.76, and **C5–C7** = 1626.80. Obsd.  $[M+H]^+$ ; **C1** = 1464.18, **C2** = 1543.10, **C3** = 1543.29, **C4** = 1543.33, **C5** = 1624.63, **C6** = 1628.13, and **C7** = 1625.07.

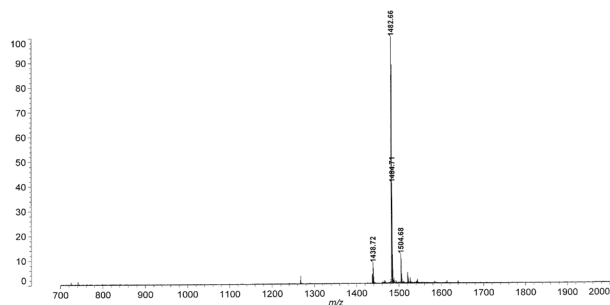
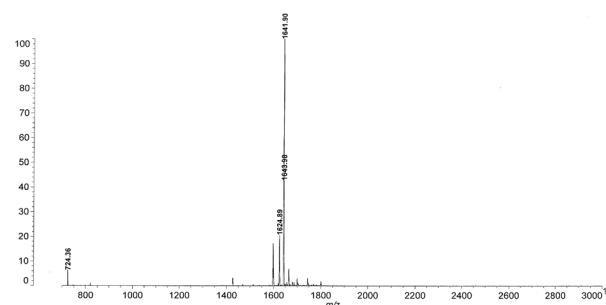
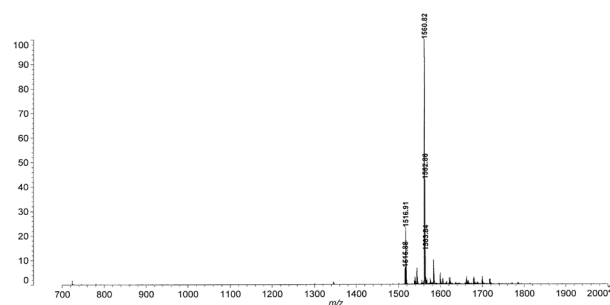
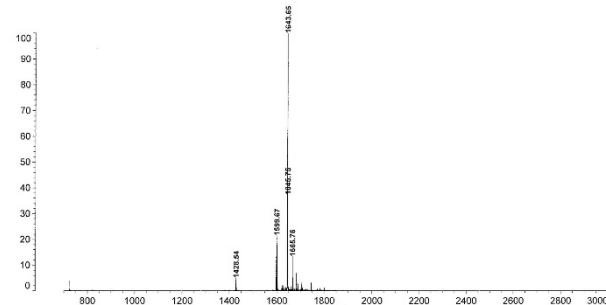
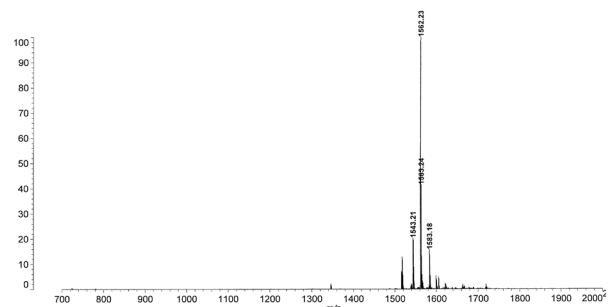
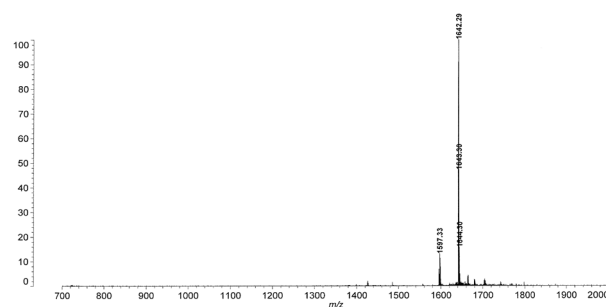
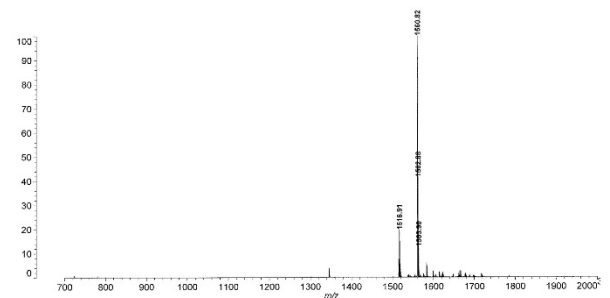
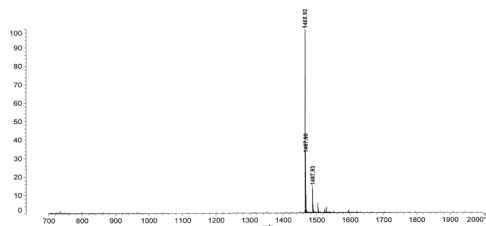
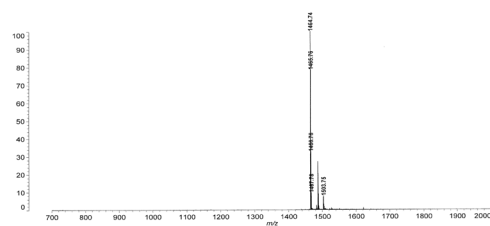
**L1****L5****L2****L6****L3****L7****L4**

Figure S3. MALDI-ToF mass spectra of **L1**–**L7**. An  $\alpha$ -CHCA was used as a matrix. Calcd.  $[M+H]^+$ ; **L1** = 1484.74, **L2**–**L4** = 1564.77, and **L5**–**L7** = 1644.81. Obsd.  $[M+H]^+$ ; **L1** = 1482.66, **L2** = 1560.82, **L3** = 1562.23, **L4** = 1560.82, **L5** = 1641.90, **L6** = 1643.65, and **L7** = 1642.29.

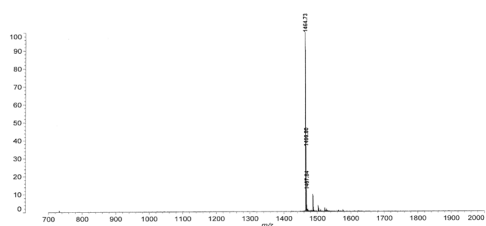
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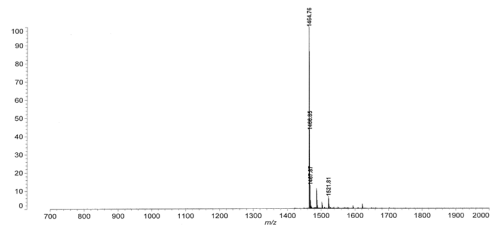
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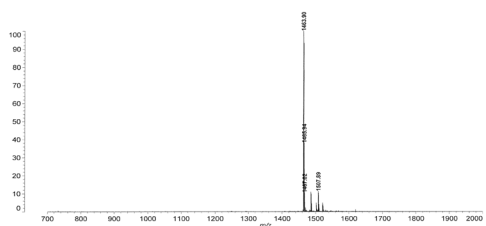
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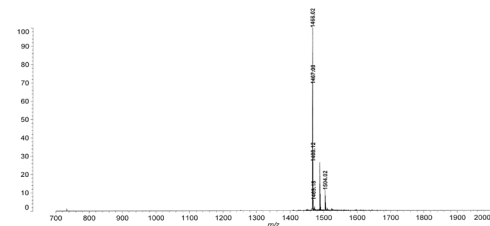
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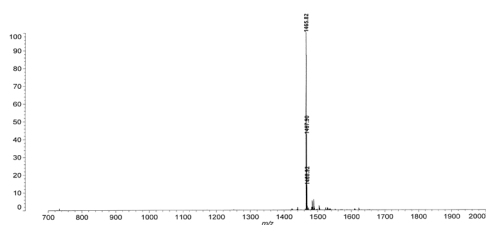
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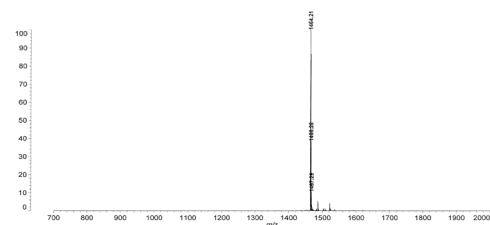
**C15**



**C11**



**C16**



**C12**

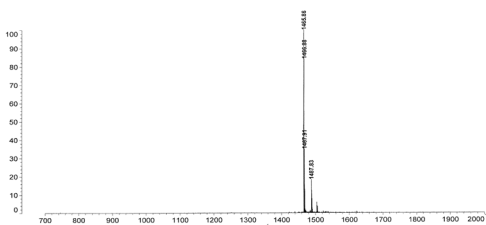


Figure S4. MALDI-Tof mass spectra of **C8–C16**. Calcd.  $[M+H]^+$ ; **C8–C16**=1466.73. Obsd.  $[M+H]^+$  ; **C8** =1465.92, **C9** =1464.73, **C10** =1463.90, **C11** =1465.82, **C12** =1465.86, **C13** =1464.74, **C14** =1464.76, **C15** =1466.02 and **C16** =1464.21.

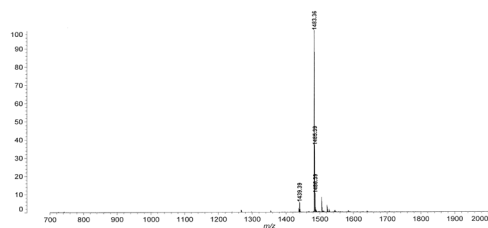
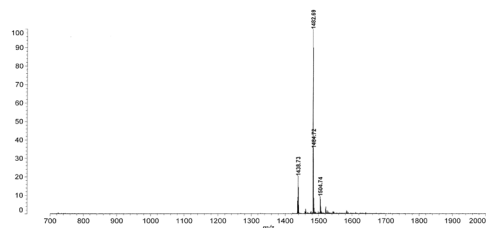
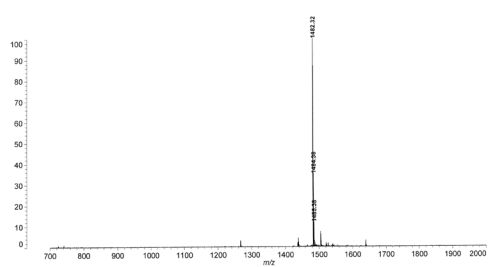
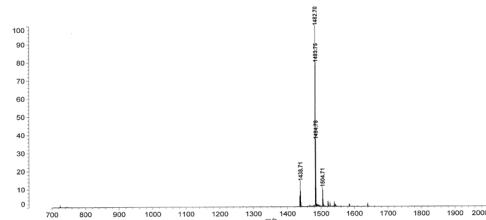
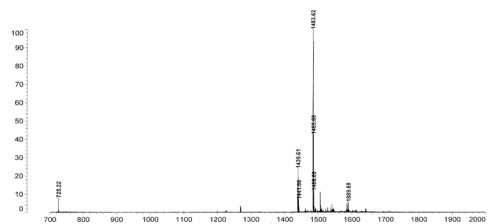
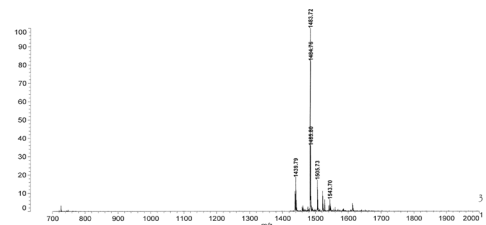
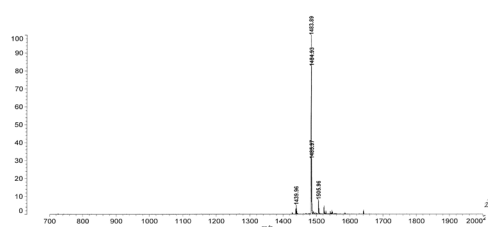
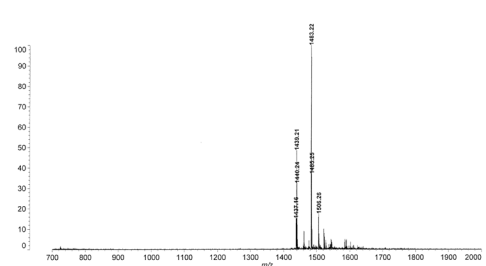
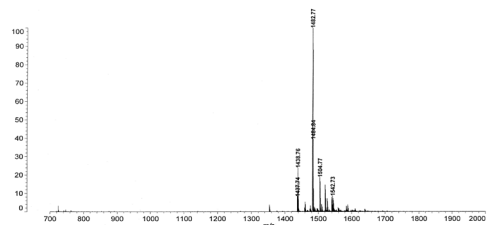
**L8****L13****L9****L14****L10****L15****L11****L16****L12**

Figure S5. MALDI-ToF mass spectra of **L8–L16**. Calcd.  $[M+H]^+$ ; **L8–L16**=1484.74. Obsd.  $[M+H]^+$  ; **L8** =1483.36, **L9** =1482.32, **L10** =1483.62, **L11** =1483.89, **L12** =1482.77, **L13** =1482.69, **L14**=1482.70, **L15**=1483.72 and **L16** =1483.22.

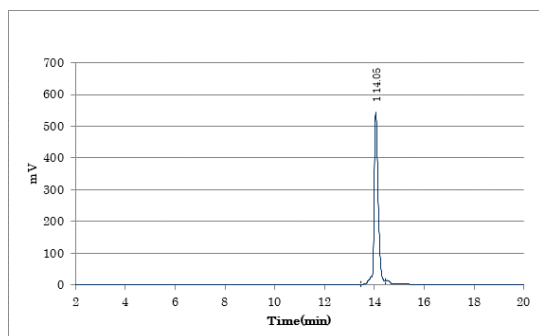
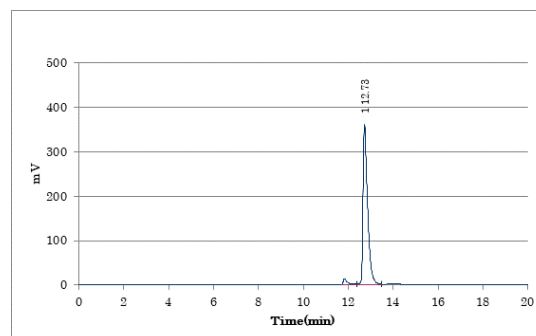
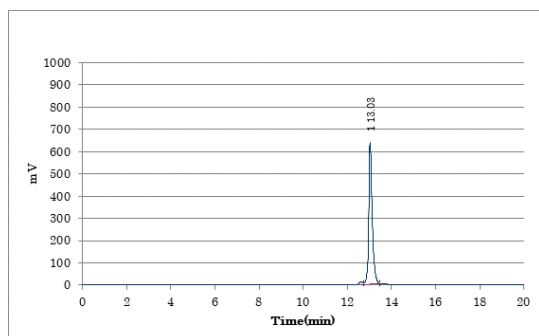
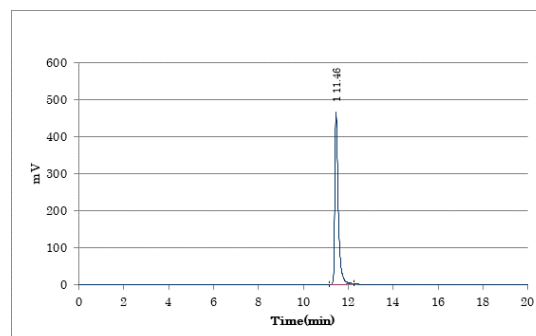
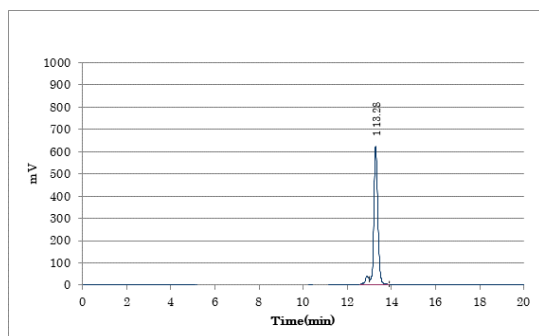
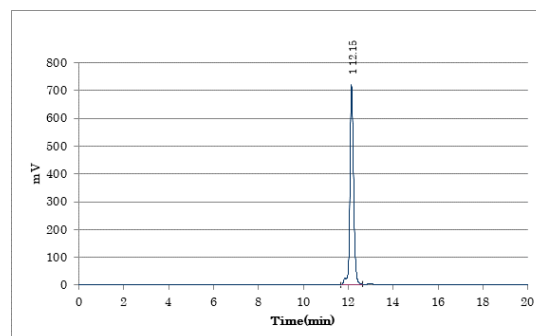
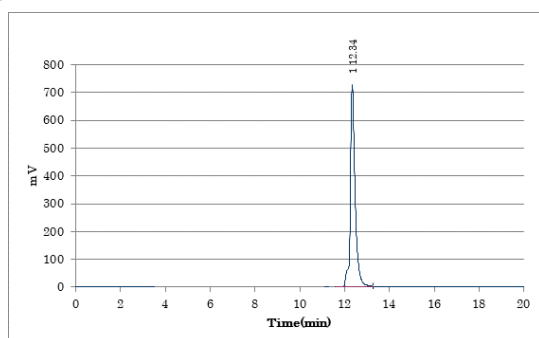
**C1****C5****C2****C6****C3****C7****C4**

Figure S6. RP-HPLC charts of **C1–C7**. Buffer A. 0.1% TFA in water; buffer B, acetonitrile and monitoring at 340 nm with a gradient of 10–80% for 20 min.

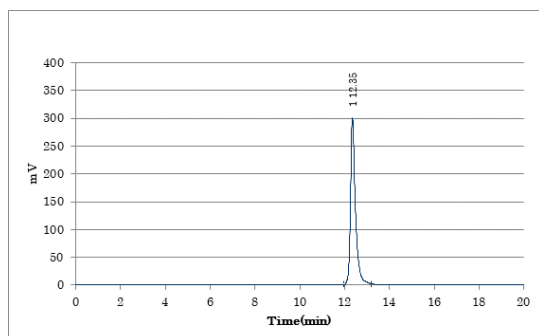
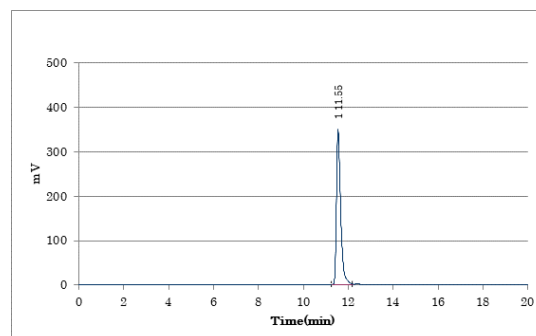
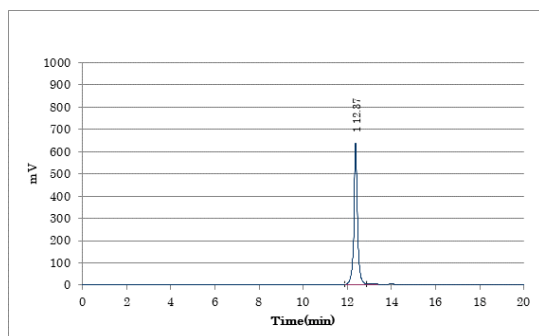
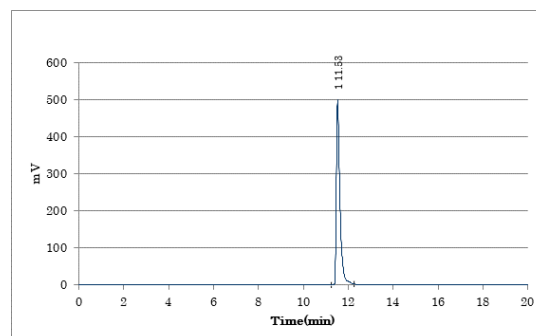
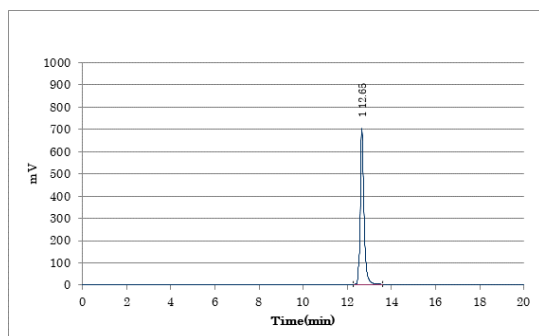
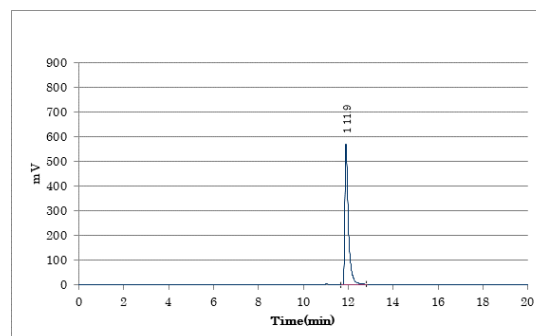
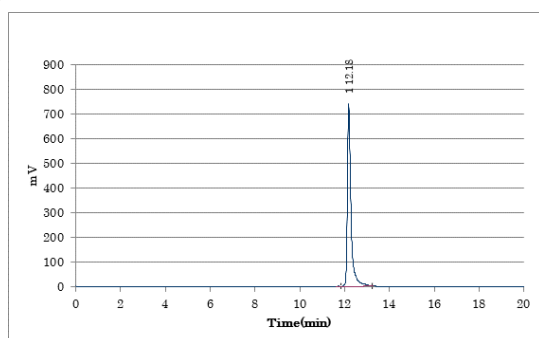
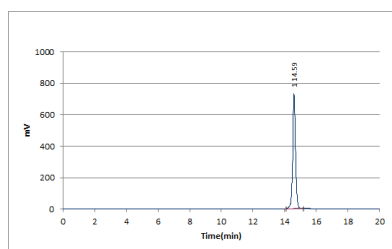
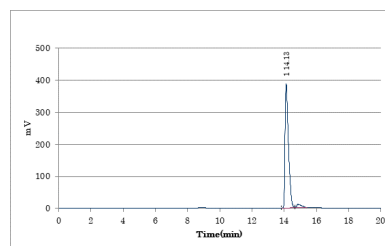
**L1****L5****L2****L6****L3****L7****L4**

Figure S7. RP-HPLC charts of **L1–L7**. Buffer A. 0.1% TFA in water; buffer B, acetonitrile and monitoring at 340 nm with a gradient of 10–80% for 20 min.

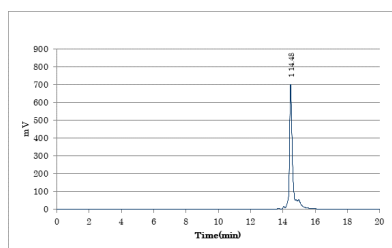
**C8**



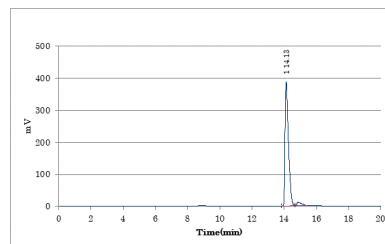
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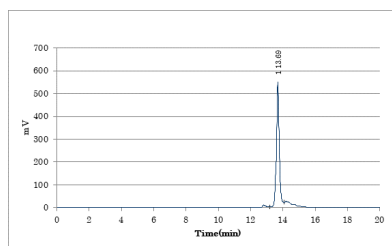
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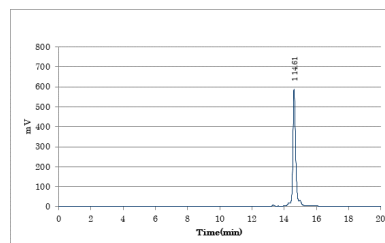
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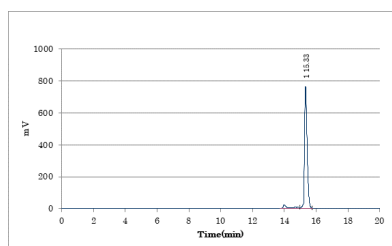
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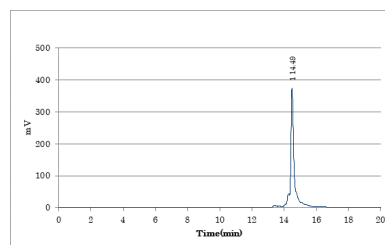
**C15**



**C11**



**C16**



**C12**

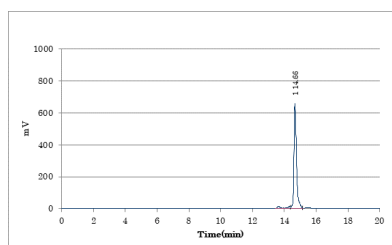
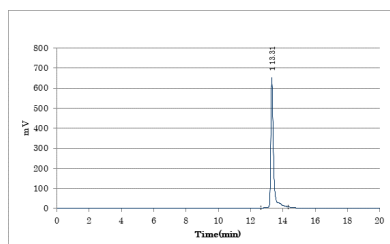
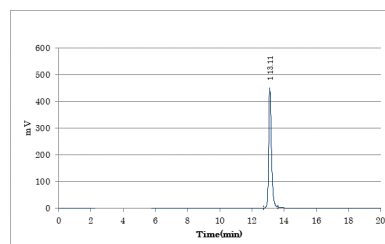


Figure S8. RP-HPLC charts of **C8–C16**. Buffer A. 0.1% TFA in water; buffer B, acetonitrile and monitoring at 340 nm with a gradient of 10–80% for 20 min.

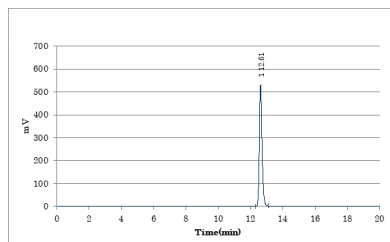
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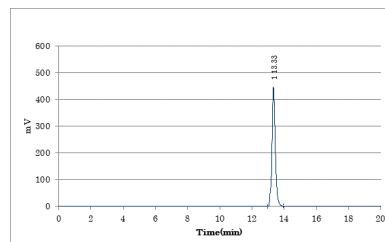
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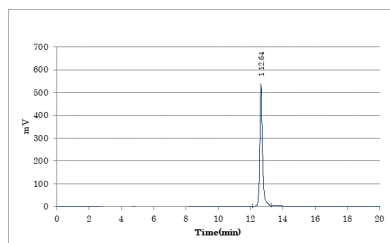
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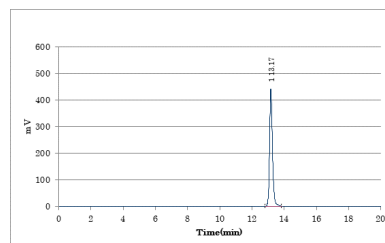
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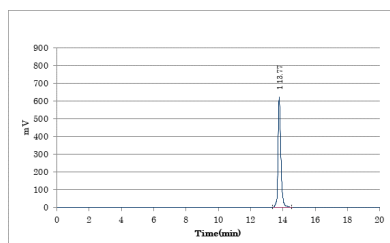
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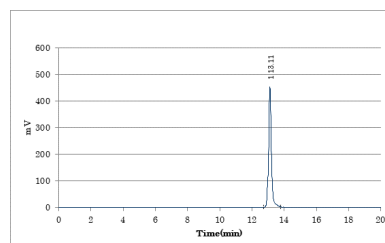
**L15**



**L11**



**L16**



**L12**

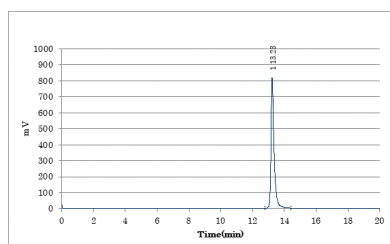


Figure S9. RP-HPLC charts of **L8–L16**. Buffer A. 0.1% TFA in water; buffer B, acetonitrile and monitoring at 340 nm with a gradient of 10–80% for 20 min.

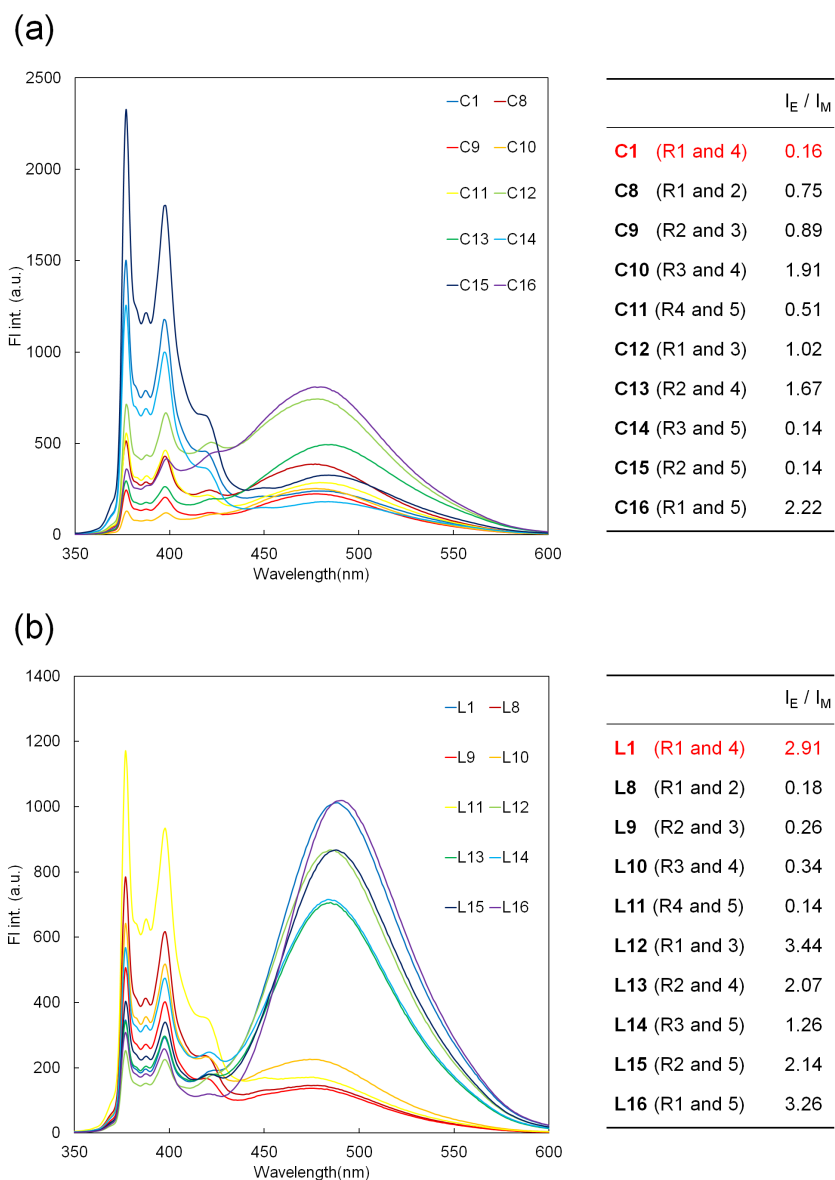


Figure S10. Fluorescence spectra of (a) **C1** and **C8–C16**, and (b) **L1** and **L8–L16** in aqueous solution. Each peptide concentration was 500 nM. Tables to a right of the graphs shows the ratio of the fluorescence intensity at 488 nm to the intensity at 377 nm ( $I_E/I_M$  ratio) for each peptide.

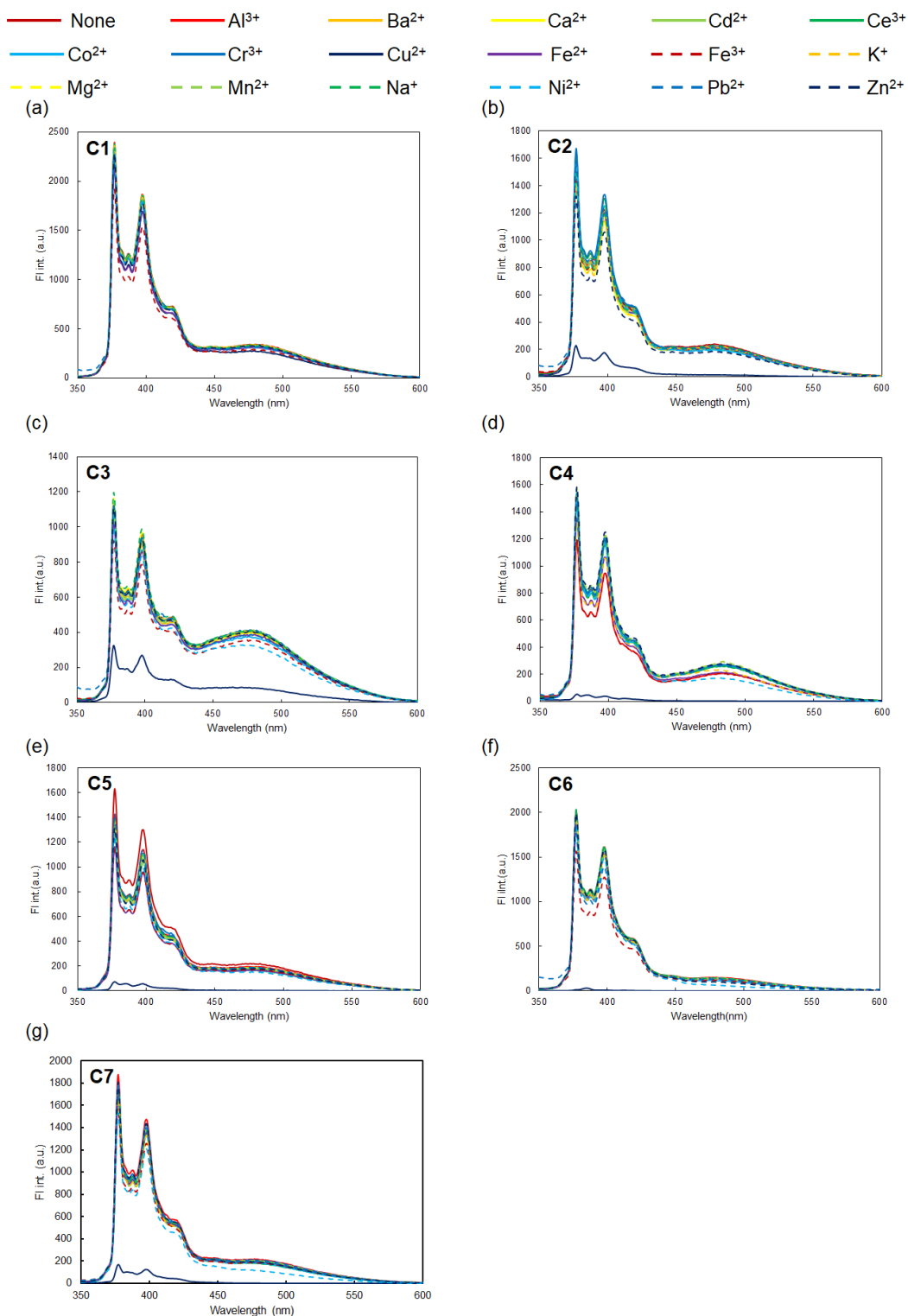


Figure S11. Fluorescence spectra of **C1**–**C7** with 17 metal ions in aqueous solution. The concentration of **C1**–**C7** was 500 nM and the concentration of metal ions was 50  $\mu\text{M}$  (100 eq for the probe).

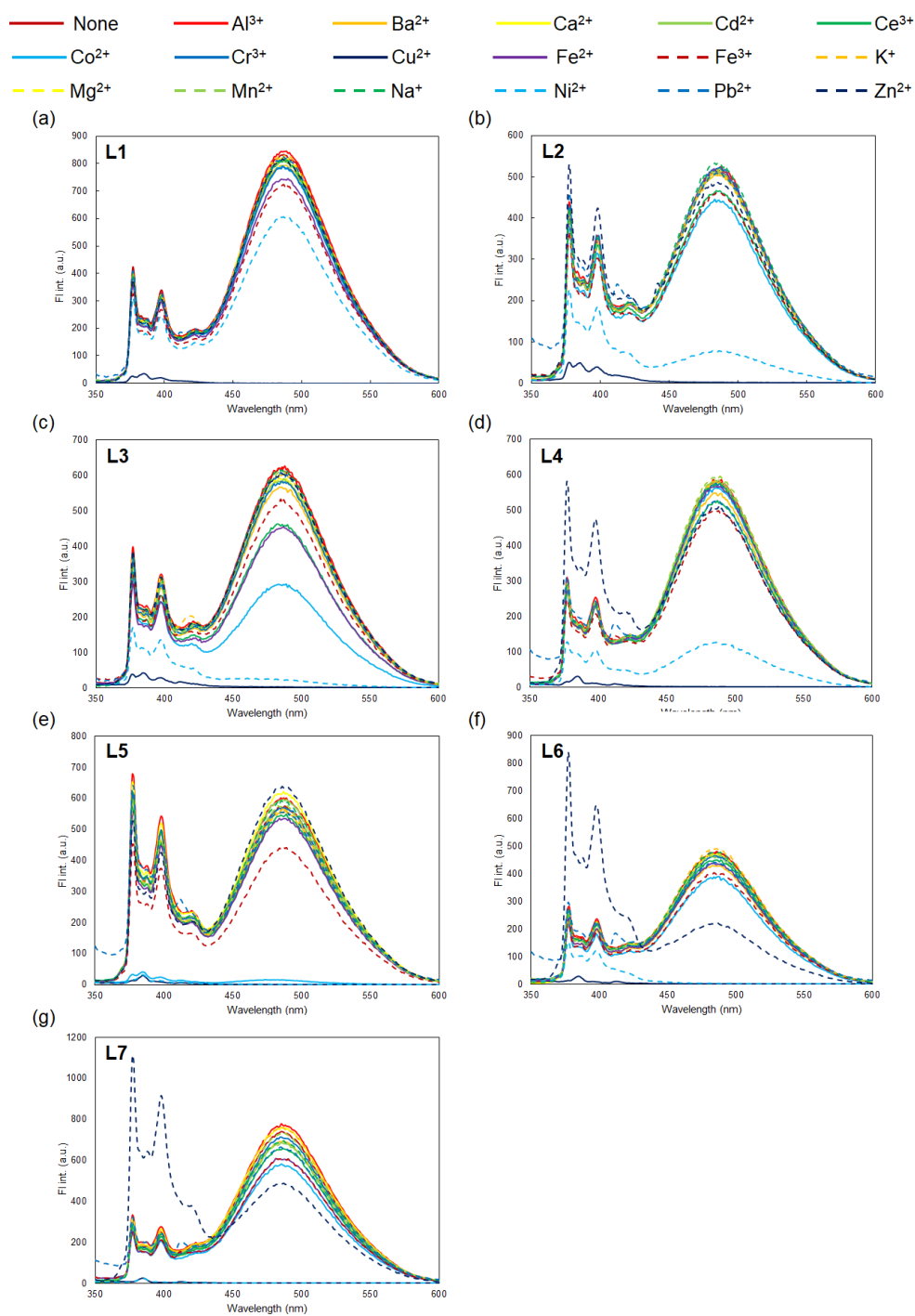


Figure S12. Fluorescence spectra of **L1**–**L7** with 17 metal ions in aqueous solution. The concentration of **L1**–**L7** was 500 nM and the concentration of metal ions was 50  $\mu$ M (100 eq for the probe).

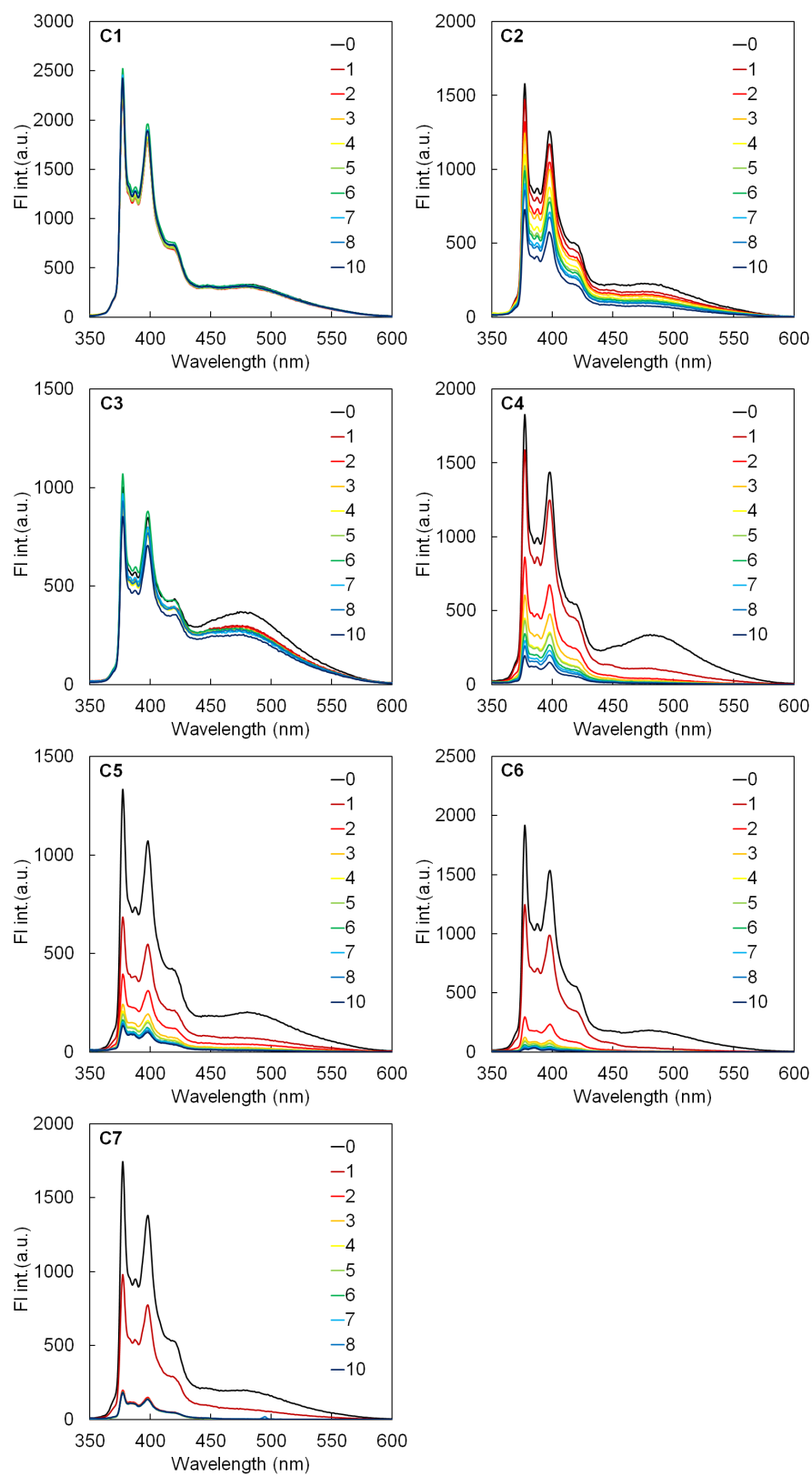


Figure S13. Fluorescence spectra of **C1–C7** used in titration curves of  $\text{Cu}^{2+}$  for the peptides.

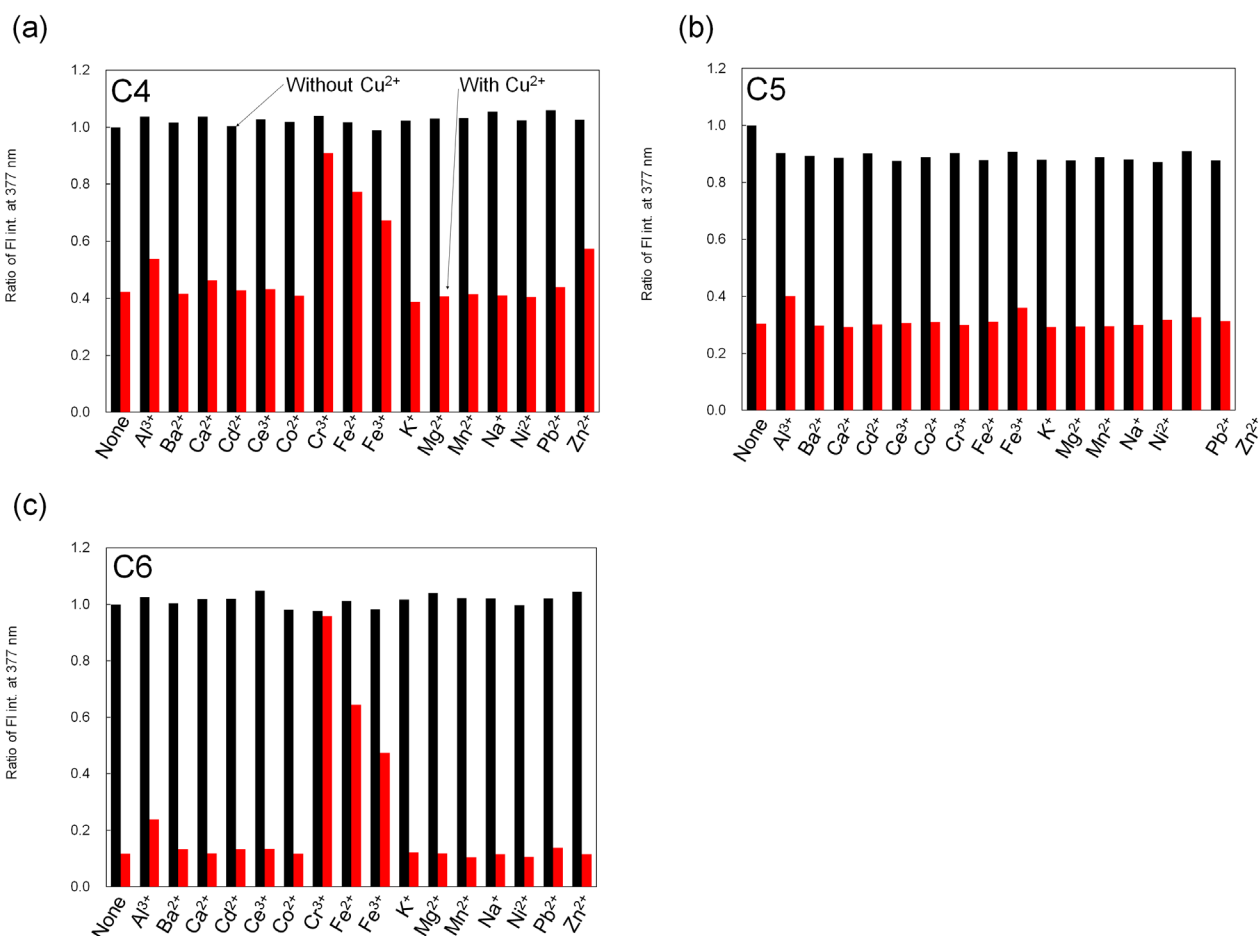


Figure S14. Competition assay of  $\text{Cu}^{2+}$  against other ions by (a) **C4**, (b) **C5** and (c) **C6**. Black bars indicate each peptide solution (500 nM) with 20 equivalents of each metal ion. Red bars indicate each peptide solution (500 nM) in which 20 equivalents of each metal ion and 2 equivalents of  $\text{Cu}^{2+}$ .

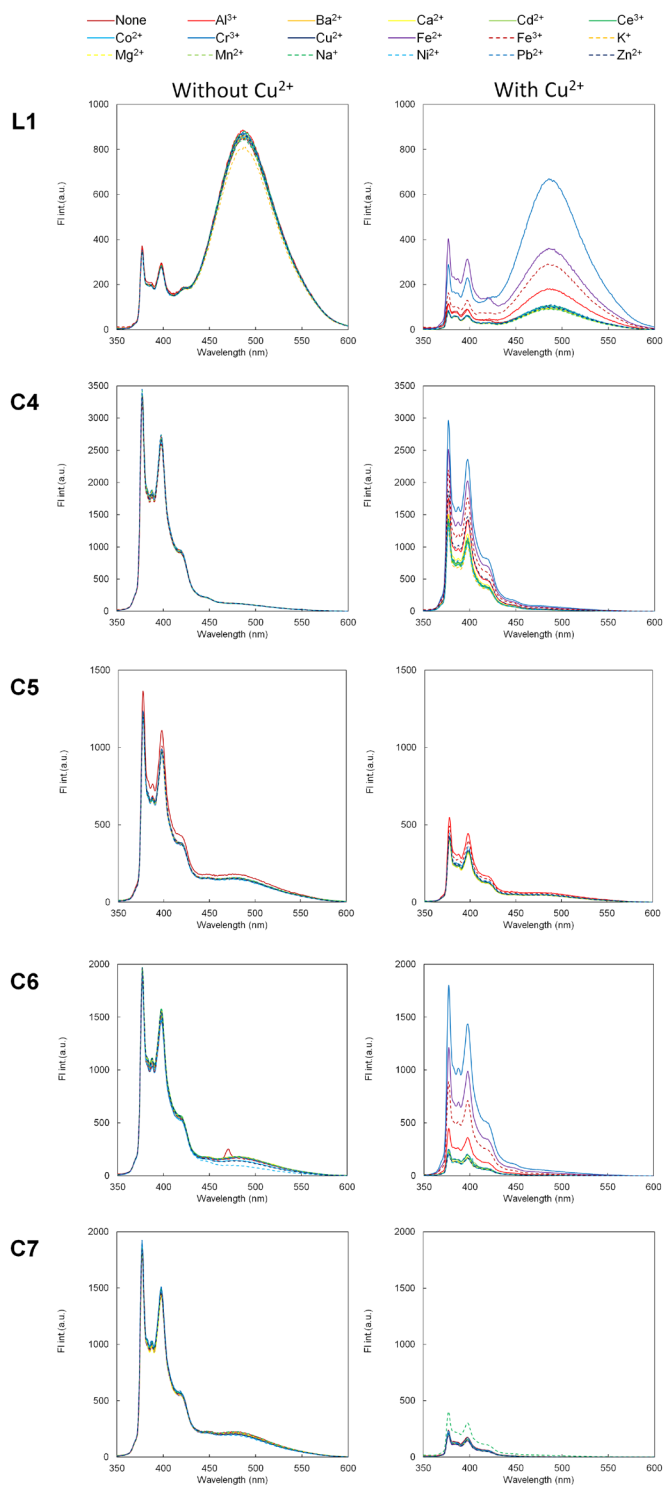


Figure S15. Fluorescence spectra of L1 and C4–C7 used in competition assay of  $\text{Cu}^{2+}$  against other ions.

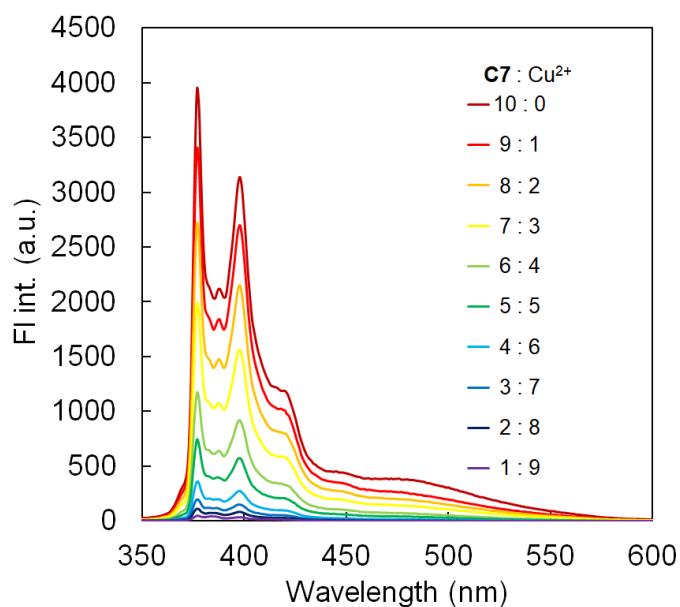


Figure S16. Fluorescence spectra of **C7** with  $\text{Cu}^{2+}$  used in the Job plot in Figure 5a.

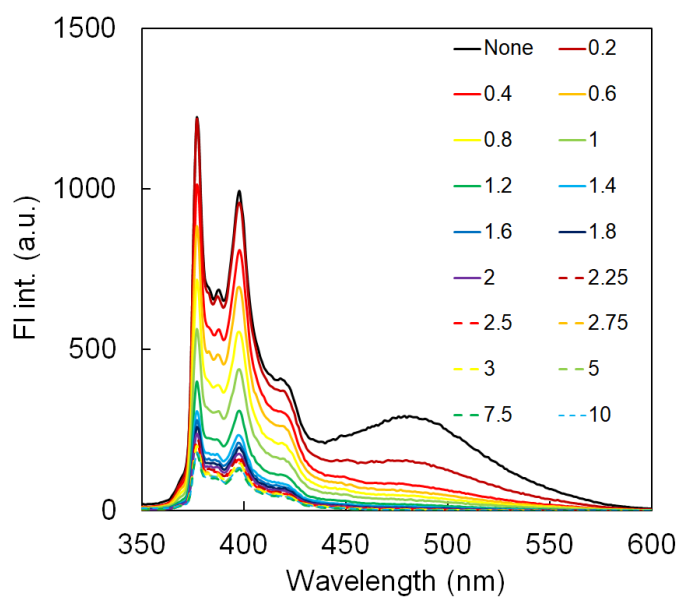


Figure S17. Fluorescence spectra of **C7** (500 nM constant) with various concentrations of  $\text{Cu}^{2+}$  (0–10 eq for the probe) used in the titration curve in Figure 5b.

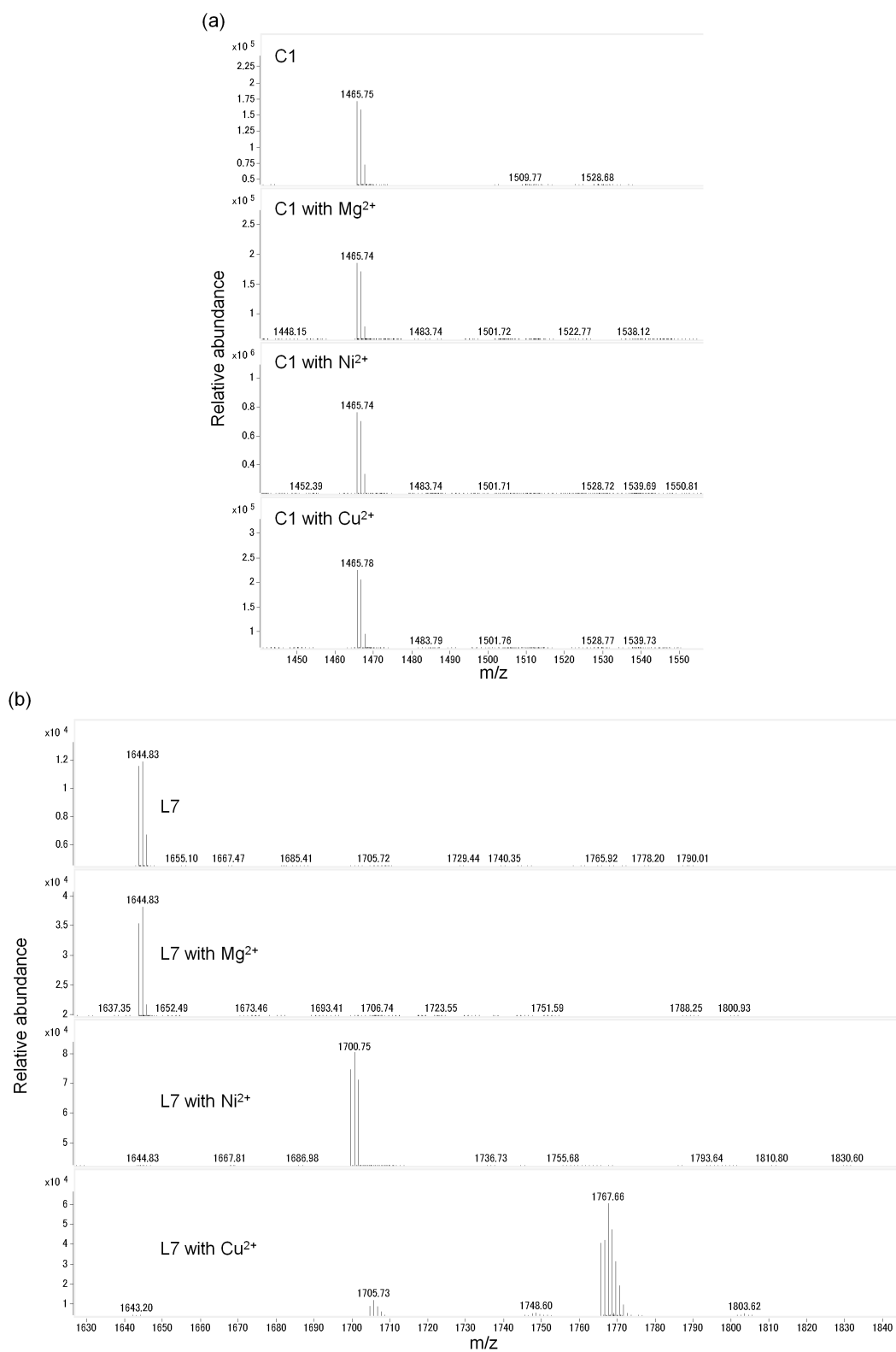


Figure S18. ESI-mass spectra of (a) **C1** and (b) **L7** with and without  $\text{Mg}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Cu}^{2+}$ .