Supporting Information

Utilizing Copper-mediated deprotection of selenazolidine for cyclic peptides synthesis

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The synthesis of peptide thioesters

Analytical Data:
1) 1: Sez-LKFAG-MMP
90 mg crude 1 were obtained, and purified (30 mg, yield 15%). ESI-MS (m/z): obs. 800.17, calc. for [M + H]^+ 800.29.
2) **3a**: Sez-LKFAG-MPAA  
80 mg crude 3a were obtained, and purified (35 mg, yield 16.5%). ESI-MS (m/z): obs.: 848.17, calc. for [M + H]^+ 848.29.

3) **3b**: Sez-LKFAT-MPAA  
75 mg crude 3b were obtained, and purified (28 mg, yield 12.5%). ESI-MS (m/z): obs. 891.92, calc. for [M + H]^+ 892.32.
4) **3c**: Sez-LKFAA-MPAA
81 mg crude 3c were obtained, and purified (31 mg, yield 14.4%). ESI-MS (m/z): obs. 861.83, calc. for [M + H]^+ 862.31.

5) **3d**: Sez-LKFAF-MPAA
85 mg crude 3d were obtained, and purified (36 mg, yield 15.3%). ESI-MS (m/z): obs. 937.92, calc. for [M + H]^+ 938.34.
6) 3e: Sez-LKFAK-MPAA
65 mg crude 3e were obtained, and purified (20 mg, yield 8.7%). ESI-MS (m/z): obs. 919.00, calc. for [M + H]^+ 919.36.

7) 3f: Sez-LKFAV-MPAA
70 mg crude 3f were obtained, and purified (18 mg, yield 8.1%). ESI-MS (m/z): obs. 890.08, calc. for [M + H]^+ 890.34.
MeONH$_2$ deprotects Sez and reacts with the thioester to give 2

Scheme S1. Deprotection of Sez-LKFAG-MMP by MeONH$_2$

Figure S1. MeONH$_2$ deprotects Sez and reacts with the thioester to give 2.
Cu(II)-catalyzed deprotection of Sez in different buffer

Sez-LKFAG-OH (M1) was prepared by standard Fmoc-SPPS (Figure S10) as a model peptide for exploring the deprotection of Sez in different buffers with CuCl₂. Five different conditions was investigated: (I) 1.4 mg Sez-LKFAG-OH (M1, 2 μmol) was dissolved in 500 μL phosphate buffer (0.2 M sodium phosphate, 6 M Gdn·HCl, 1.5 equiv CuCl₂, pH 6) and shaken for 30 min at room temperature; (II) 1.4 mg Sez-LKFAG-OH (M1, 2 μmol) was dissolved in 500 μL phosphate buffer (0.2 M sodium phosphate, 1.5 equiv CuCl₂, pH 6) and shaken for 5 min at room temperature; (III) 1.4 mg Sez-LKFAG-OH (M1, 2 μmol) was dissolved in 500 μL phosphate buffer (0.2 M sodium phosphate, 0.3 equiv CuCl₂, pH 6) and shaken for 30 min at room temperature; (IV) 1.4 mg Sez-LKFAG-OH (M1, 2 μmol) was dissolved in 500 μL HEPES buffer (0.2 M sodium HEPES, 0.3 equiv CuCl₂, pH 6) and shaken for 30 min at room temperature; (V) 1.4 mg Sez-LKFAG-OH (M1, 2 μmol) was dissolved in 500 μL Tris buffer (0.2 M Tris hydrochloride, 0.3 equiv CuCl₂, pH 6) and shaken for 30 min at room temperature.

Figure S2. The deprotection of Sez in different buffers with CuCl₂
One-pot Cu(II)-mediated deprotection of Sez, cyclization and deselenization for the synthesis of cyclic peptides

We wanted to use our recently developed Cu(II)-mediated deprotection of Sez as a general strategy for cyclic peptide synthesis. Sez-LKFAG-MPAA (3a) and Sez-LKFAT-MPAA (3b) were used as two model peptides for optimization of cyclization conditions, as one bearing C-terminal Gly residue, with no steric hindrance and the other with more steric β-branched amino acid, Thr.¹
Figure S3. The synthesis of cyclic peptides c(ALKFAG), 6a, from Sez-LKFAG-MPAA (3a) and c(ALKFAT), 6b, from Sez-LKFAT-MPAA (3b) using different strategies. (a): the cyclization conditions of 3a was in the phosphate buffer (0.2 M NaH₂PO₄, 6 M Gn·HCl, pH 7) but with 0.05 M TCEP, 0.1 M sodium ascorbate, at room temperature. (b): the cyclization conditions of 3a was in the same phosphate buffer but with 5 equiv TCEP, 1 equiv MPAA, at pH 7, at room temperature. (c): the cyclization conditions of 3b was under the same conditions as in b. (d): the cyclization conditions of 3b was under the same conditions as in b but with additional 5 equiv MeONH₂ to quench the release formaldehyde.
The synthesis of different cyclic peptides

1) 6a c(ALKFAG), was prepared in 68% yield (0.8 mg). ESI-MS (m/z): obs. 588.17, calc. for [M + H]^+ 588.35.

2) 6b c(ALKFAT), was prepared in 47% yield (0.6 mg). ESI-MS (m/z): obs. 632.08, calc. for [M + H]^+ 632.38.
3) **6c** c(ALKFAA) was isolated in 67% yield (0.8 mg). ESI-MS (m/z): obs. 602.17, calc. for [M + H]^+ 602.37.

4) **6d** c(ALKFAF) was isolated in 59% yield (0.8 mg). ESI-MS (m/z): obs. 678.08, calc. for [M + H]^+ 678.40.
5) **6e** c(ALKFAK) was isolated in 39% yield (0.5 mg). ESI-MS (m/z): obs. 659.17, calc. for [M + H]^+ 659.42.

6) **6f** c(ALKFAV) was isolated in 55% yield (0.7 mg). ESI-MS (m/z): obs. 630.08, calc. for [M + H]^+ 630.40.
The synthesis of WT-Kalata S and Se-Kalata S analogue

The Kalata S and its seleno-analogue, Se-Kalata S, were prepared by making first the linear peptides followed by cyclization and oxidative folding.

WT-Kalata S
GLPVCGETCV GGTCPGCS CSWPVCTR

Se-Kalata S
GLPVCGETCV GGTCPGUS CSWPVCTR

The sequence of linear WT-Kalata S and Se-Kalata S (U = Sec)

WT-Kalata S
CSCSW PVCTR NGLPV CGETC VGGTC NTPG

Se-Kalata S
USCSW PVCTR NGLPV CGETC VGGTC NTPG

Figure S4. Analytical HPLC and ESI-MS of linear Kalata S-NHNH₂

Figure S5. Analytical HPLC and ESI-MS of cyclized reduced WT-Kalata S
Figure S6. Analytical HPLC (XSelect C18 column: 3.5 μm, 130 Å, 4.6 × 150 mm at 220 nm) and ESI-MS of oxidative folding of reduced WT-Kalata S

Figure S7. Analytical HPLC and ESI-MS of Sez-Kalata S-MPAA

Figure S8. Analytical HPLC and ESI-MS of reduced Se-Kalata S
Figure S9. Analytical HPLC (XSelect C18 column: 3.5 μm, 130 Å, 4.6 × 150 mm at 220 nm) and ESI-MS of oxidative folding of reduced Se-Kalata S
**Supplementary Figures**

**Figure S10.** Analytical HPLC (using XSelect C18 column (3.5 μm, 130 Å, 4.6 × 150 mm) at 220 nm) and ESI-MS of M1 (obs. 697.92, calc. for [M + H]^+ 698.28).

**Figure S11.** Analytical HPLC (using XSelect C18 column (3.5 μm, 130 Å, 4.6 × 150 mm) at 220 nm) and ESI-MS of M1' (obs. 1368.33, calc. for [M + H]^+ 1368.39).

**Figure S12.** Analytical HPLC(XSelect C18 column: 3.5 μm, 130 Å, 4.6 × 150 mm at 220 nm) and ESI-MS of Sec-LKFAG-MPAA dimer (obs. 1668.67, calc. for [M + H]^+ 1668.78).
Figure S13. Analytical HPLC (XSelect C18 column: 3.5 μm, 130 Å, 4.6 × 150 mm at 220 nm) and ESI-MS of c(ULKFAG) (obs. 668.00, calc. for [M + H]+ 668.27).

Figure S14. Analytical HPLC (XSelect C18 column: 3.5 μm, 130 Å, 4.6 × 150 mm at 220 nm) and ESI-MS of Sec-LKFAT-MPAA dimer (obs. 1756.50, calc. for [M + H]+ 1756.88).

Figure S15. Analytical HPLC (XSelect C18 column: 3.5 μm, 130 Å, 4.6 × 150 mm at 220 nm) and ESI-MS of c(ULKFAT) (obs. 711.92, calc. for [M + H]+ 711.74).
Figure S16. Analytical HPLC (XSelect C18 column: 3.5 μm, 130 Å, 4.6 × 150 mm at 220 nm) and ESI-MS of Sec-LKFAA-MPAA dimer (obs. 1696.58, calc. for [M + H]+ 1696.83).

Figure S17. Analytical HPLC (XSelect C18 column: 3.5 μm, 130 Å, 4.6 × 150 mm at 220 nm) and ESI-MS of c(ULKFAA) (obs. 681.92, calc. for [M + H]+ 681.72).

Figure S18. Analytical HPLC (XSelect C18 column: 3.5 μm, 130 Å, 4.6 × 150 mm at 220 nm) and ESI-MS of Sec-LKFAF-MPAA dimer (obs. 1846.58, calc. 1848.02).
Figure S19. Analytical HPLC (XSelect C18 column: 3.5 μm, 130 Å, 4.6 × 150 mm at 220 nm) and ESI-MS of c(ULKFAF) (obs. 758.00, calc. for [M + H]+ 757.81) and c(ULKFAF) + MPAA (obs. 925.83, calc. for [M + H]+ 924.32).

Figure S20. Analytical HPLC (XSelect C18 column: 3.5 μm, 130 Å, 4.6 × 150 mm at 220 nm) and ESI-MS of Sec-LKFAK-MPAA dimer (obs. 1809.89, calc. 1810.01).

Figure S21. Analytical HPLC (XSelect C18 column: 3.5 μm, 130 Å, 4.6 × 150 mm at 220 nm) and ESI-MS of c(ULKFAK) (obs. 738.92, calc. for [M + H]+ 738.81).
Figure S22. Analytical HPLC (XSelect C18 column: 3.5 μm, 130 Å, 4.6 × 150 mm at 220 nm) and ESI-MS of Sec-LKFAV-MPAA dimer (obs. 1752.75, calc. for [M + H]^+ 1752.94).

Figure S23. Analytical HPLC (XSelect C18 column: 3.5 μm, 130 Å, 4.6 × 150 mm at 220 nm) and ESI-MS of c(ULKFAV) (obs. 710.00, calc. for [M + H]^+ 710.31) and c(ULKFAV) + MPAA (obs. 877.83, calc. for [M + H]^+ 876.32).

Figure S24. Analytical HPLC (XSelect C18 column: 3.5 μm, 130 Å, 4.6 × 150 mm at 220 nm) and ESI-MS of pure WT-Kalata S-NHNH₂ (obs. 2914.54, calc. 2914.21).
Figure S25. Analytical HPLC (XSelect C18 column: 3.5 μm, 130 Å, 4.6 × 150 mm at 220 nm) and ESI-MS of Sez-Kalata S-NHNH₂ (obs. 2974.27, calc. 2974.16).

Figure S26. Circular Dichroism (CD) spectra in the far-UV for WT-Kalata S and Se-Kalata S (30 μM for each protein in buffer solution (50 mM NH₄HCO₃; 10 mM reduced GSH, pH 7.5 containing 50% iPrOH)) at 25 °C.
Figure S27. HR-MS analysis of WT-Kalata S. a. The simulated HR-MS of reduced form of WT-Kalata S with chemical formula C_{116}H_{177}N_{35}O_{39}S_{6} is shown; b. The deconvoluted HR-MS of WT-Kalata S. The observed (deconvoluted) and simulated isotopic patterns are identical (±3.2 ppm).
Figure S28. HR-MS analysis of Se-Kalata S. a. The simulated HR-MS of reduced form of Se-Kalata S with chemical formula $\text{C}_{116}\text{H}_{177}\text{N}_{35}\text{O}_{39}\text{S}_5\text{Se-}\text{Na}$ is shown; b. The deconvoluted HR-MS of Se-Kalata S. The observed (deconvoluted) and simulated isotopic patterns are identical ($\pm 6.2$ ppm).

References