Site-Selective Chemical Cleavage of Peptide Bonds

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Supporting Information

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Table S1: Optimization studies for serine cyclization on Fmoc-Gly-Ala-Ser-Phe-Ala-Gly, 1a.

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<sup>a</sup>Reaction conditions: peptide (1 equiv), DSC (5-20 equiv), DIEA (5-20 equiv) and a crystal of DMAP in DMF were stirred overnight at room temperature. Purified by HPLC. <sup>b</sup>Calculated by HPLC.

Figure S1. Serine-selective cleavage of 1a using DSC under neutral aqueous solution.
**Figure S2.** Acetylation of lysine-side chain under DSC reaction conditions followed by decarboxylation to free-lysine side chain: Cyclization of peptides containing serine and unprotected lysine; acetylation of lysine under DSC conditions gave (2h); Cleavage of peptide with acetylated lysine under buffer conditions gave N-terminal carboxylated lysine fragment 3h and C-terminal fragment 4. N-terminal carboxylated lysine fragment 3h eventually underwent decarboxylation to generate free-lysine side-chain fragment 3h’.
Figure S3. Cyclization and cleavage of peptide containing reduced or free cysteine under the reaction conditions. Cyclization to five membered thiazolidinone ring was observed at cysteine residue followed by cleavage at N-terminus of cysteine to generate thiazolidinone modified C-terminal fragment Thz-Phe-Arg-Phe-Gly-NH₂ and N-terminal fragment Fmoc-Gly-Ala-OH.

Fmoc-Gly-Ala-Thz-Phe-Arg-Phe-Gly-NH₂, LCMS: \( m/z \) 1004.40 (calcd \([M+H]^{+} = 1004.20\), 502.1 (calcd \([ (M+2)/2 ]^{+} = 502.6\)). Purity: >95% (HPLC analysis at 254 nm). Retention time:
19.52 min.


**Thz-Phe-Arg-Phe-Gly-NH$_2$ (C-terminal fragment).** LCMS: $m/z$ 654.2 (calcd $[M+H]^+ = 654.27$). Purity: >95% (HPLC analysis at 254 nm). Retention time: 13.47 min.

HPLC spectra of the cyclized starting material and the crude reaction mixture after incubation in buffer solution; the product peak is labeled.
N-terminal fragment

thiazolidinone containing C-terminal fragment
Figure S4. Cyclization and cleavage of peptide (12) containing glutamic acid and serine under the reaction conditions. Cyclization to five membered ring was observed at both serine and glutamic acid followed by cleavage at both serine and glutamic acid. Although, both serine and glutamic acid underwent cyclization but time based studies monitored by HPLC and LC/MS showed that cleavage at serine is kinetically favorable as compared to cleavage at glutamic acid.
Figure S5. Peptide (1i) containing aspartic acid and serine under the reaction conditions. Side chain of aspartic acid forms intermediate with DSC but the formation of strained four-membered ring was not observed at Asp. On hydrolysis intermediate at Asp cleaves off to generate free side-chain at Asp and cleavage of peptide chain was observed only at serine residue.
**Figure S6. Reaction of peptide with free carboxylic group, Fmoc-FGSG-OH under the reaction conditions** (a) Cyclization of peptide, Fmoc-FGSG-OH with DSC gave both serine cyclized product (A1); MS analysis $m/z$ 615.40 (calcd $[M+H]^+$ = 615.20) and N-hydroxy succinimide derivative of serine cyclized product (A2); MS analysis $m/z$ 712.34 (calcd $[M+H]^+$ = 712.22) under mass spectrometer (MS) analysis. But under the HPLC conditions: 0.1% FA (v/v) in water (solvent A): 0.1% FA (v/v) acetonitrile (solvent B); gradient 0-80 %, 0.1% FA (v/v) acetonitrile in 25 min, flow rate = 1.0 mL/min, *only one peak was observed at 20.2 min which corresponds to serine cyclized product (A1) as analyzed by MS. This is because in presence of water N-hydroxy succinimide derivative undergoes hydrolysis to give the free carboxylic group.* (b) After addition of buffer, cleavage was observed at N-terminus of serine and generated N-terminal fragment Fmoc Gly and C-terminal fragment Oxd-Gly-Phe-OH with intact carboxylic group. *In buffer N-hydroxy succinimide derivative undergoes hydrolysis to give the free carboxylic group.*
**Fmoc-Gly-Oxd-Gly-Phe-OH (A1).** LCMS: \( m/z \) 615.40 (calcd \([M+H]^+\) = 615.20). Purity: >95% (HPLC analysis at 254 nm). Retention time: 20.2 min.

**Fmoc-Gly-OH (N-terminal fragment).** LCMS: \( m/z \) 298.06 (calcd \([M+H]^+\) = 298.1), 320.05 (calcd \([M+Na]^+\) = 320.2). Purity: >95% (HPLC analysis at 254 nm). Retention time: 18.8 min.

**Oxd-Gly-Phe-OH (C-terminal fragment).** LCMS: \( m/z \) 336.01 (calcd \([M+H]^+\) = 336.11), 358.1 (calcd \([M+Na]^+\) = 358.2). Purity: >95% (HPLC analysis at 254 nm). Retention time: 10.5 min.

HPLC spectra of the cyclized starting material and the crude reaction mixture after incubation in buffer solution; the product peak is labeled.
Figure S7. Serine-selective peptide bond cleavage of amyloid-β peptide, with mutated β-alanine, a well-known mutation responsible for various age-related disorders such as cataract and Alzheimer’s. HPLC traces of linear peptide 18, cyclic peptide 18a, and cleavage
products; N-terminal fragment **18b** and C-terminal fragment **18c**. Inset shows MS spectra corresponding to each peak in the HPLC spectra (**18, 18a, 18b, 18c** and **18d**). N-terminal fragment **18b** eventually underwent decarboxylation to generate free side-chain lysine fragment **18d** with retention time of 14.1. Reaction conditions = linear peptide **18** (1 equiv), DSC (20 equiv), DIEA (20 equiv) and crystal of DMAP in DMF followed by cleavage with 0.1 M phosphate buffer (pH 7.5), 37 °C.

Figure S8. Serine-selective peptide bond cleavage of completely unprotected peptide unprotected peptide SGISGPLS, a fragment of antimicrobial Bovine β-defensin 13. HPLC traces of linear peptide and cleavage products; N-terminal fragment OxdGI and the C-terminal fragment OxdGPL.
Methods

I. General. All commercial materials (Aldrich, Fluka, Nova) were used without further purification. All solvents were reagent grade or HPLC grade (Fisher). Anhydrous THF, diethyl ether, CH₂Cl₂, and DMF were obtained from a dry solvent system (passed through column of alumina) and used without further drying. All reactions were performed under air in round bottom flask. Yields refer to chromatographically pure compounds; % yield were obtained by comparison of HPLC peak areas of products and starting material. HPLC was used to monitor reaction progress.

Materials. Fmoc-amino acids were obtained from Nova Biochem is under (EMD Millipore Corporation)(Billerica, Massachusetts) and CreoSalus (Louisville, Kentucky). Rink amide resin was obtained from ChemPep Inc (Wellington, Florida). N,N,N',N'-Tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU) was obtained from CreoSalus (Louisville, Kentucky). N,N'-Disuccinimidyl carbonate (DSC) was obtained from Nova Biochem, under (EMD Millipore Corporation) (Billerica, Massachusetts). 4-Dimethylaminopyridine (DMAP): Merck KGaA (Darmstadt, Germany). N,N-Dimethylformamide (DMF): Macron Fine Chemicals (Center Valley, Pennsylvania). Dichloroethane (DCE), acetonitrile, N,N- Diisopropylethylamine (DIEA), N,N'-diisopropylcarbodiimide (DPC), were purchased from (EMD Millipore Corporation)(Billerica, Massachusetts). Piperidine was purchased from Alfa Aesar (Ward Hill, Massachusetts). Trifluoroacetic acid (TFA) was purchased from VWR 100 Matsonford Road Radnor, PA. Diethyl Ether: Sigma Aldrich (St. Louis, Missouri). Water was purified using a Millipore MilliQ water purification system.

NMR:
Proton NMR spectra were recorded on a 600 MHz spectrometer and carbon NMR spectra on a 151 MHz, spectrometer at ambient temperature. All NMR chemical shifts (δ) are referenced in ppm relative to residual solvent or internal tetramethylsilane. ¹H NMR chemical shifts referenced to residual DMSO-δ₆ at 2.50 ppm, and ¹³C NMR chemical shifts referenced to DMSO-δ₆ at 39.52 ppm. Carbon NMR spectra are proton decoupled. NMR spectral data are reported as chemical shift (multiplicity, coupling constants (J), integration). Multiplicity is reported as follows: singlet (s), broad singlet (bs), doublet (d), doublet of doubles (dd), doublet of triplet (td), triplet (t) and multiplet (m). Coupling constant (J) in Hertz (Hz).
HPLC

**Semi-Preparative HPLC:**

Preparative HPLC chromatography (HPLC) was performed on Beckman Coulter equipped with System Gold 168 detector and 125P solvent module HPLC with a 10 mm C-18 reversed-phase column. All separations involved a mobile phase of 0.1% FA (v/v) in water (solvent A) and 0.1% FA (v/v) in acetonitrile (solvent B). Semi-preparative HPLC method using a linear gradient of 0–80% acetonitrile in 0.1% aqueous FA over 30 min at room temperature with a flow rate of 3.0 mL min⁻¹. The eluent was monitored by absorbance at 220 nm and 254 nm unless otherwise noted.

**Analytical HPLC:**

Analytical HPLC chromatography (HPLC) was performed on an Agilent 1200 series HPLC equipped with a 4.6 mm C-18 reversed-phase column. All separations involved mobile phase of 0.1% FA (v/v) in water (solvent A) and 0.1% FA (v/v) in acetonitrile (solvent B). Peptide compositions were evaluated by analytical reverse phase HPLC using a gradient of 0.1% FA in acetonitrile versus 0.1% FA in water. Analytical HPLC method using a linear gradient of 0–80% 0.1% FA (v/v) acetonitrile in 0.1% aqueous FA over 30 min at room temperature with a flow rate of 1.0 mL min⁻¹. The eluent was monitored by absorbance at 254 nm unless otherwise noted.

**LCMS:**

Mass spectrometry was performed using ultra high performance liquid chromatography-mass spectrometry using the Agilent 1100 Series LCMSD VL MS Spectrometer.

**Fmoc Solid-Phase Peptide Synthesis.** Peptides were synthesized manually on a 0.25 mm scale using Rink amide resin. Fmoc–group was deprotected using 20% piperidine–DMF for 20 min to obtain a deprotected peptide-resin. Fmoc-protected amino acids (1.25mm) were sequentially coupled on the resin using a HBTU (1.25mm) and DIEA (1.25mm) for 2 h at room temperature. Peptides were synthesized using standard protocols. The peptide was cleaved from the resin using a cocktail of 95:2.5:2.5, trifluoroacetic acid:triisopropyl silane:water for 2 h. The resin was removed by filtration and the resulting solution was concentrated. The oily residue was tritutated with diethyl ether to obtain a white suspension. The resulting solid was purified by HPLC.
II. Serine Cyclization and NMR Spectra:

General Procedure for cyclization: To a 5-mL round-bottom flask containing peptide (2-20mg (1 equiv.) in 0.5-2 mL dimethylformamide (DMF) was added a solution of DSC (5-20 equiv.), DIEA (5-20 equiv.) and crystal of DMAP in DMF (0.2-0.5 mL). The mixture was stirred at room temperature for 10 h. The reaction was concentrated under vacuum and resulting peptide was dissolved in 1:1 mixture of water and acetonitrile and purified by HPLC. The purified fractions were lyophilized to afford cyclized peptide as a white powder; yield: (60-80 %).

HPLC Trace: Retention time = 15.37, Purity: >95% (HPLC analysis at 254 nm)
HRMS: $m/z$ 384.155, (calcd [M+H]$^+$ = 384.148)

NMR

$^1$H NMR chemical shifts referenced to residual DMSO-$d_6$ at 2.50 ppm, and $^{13}$C NMR chemical shifts referenced to DMSO-$d_6$ at 39.52 ppm.

$^1$H NMR: (600 MHz, DMSO-$d_6$) $\delta$ 7.89 (d, $J$ = 7.5 Hz, 2H), 7.81 (d, $J$ = 8.0 Hz, 1H), 7.71 (d, $J$ = 7.5 Hz, 2H), 7.55 (t, $J$ = 6.0 Hz, 1H), 7.42 (t, $J$ = 7.4 Hz, 2H), 7.33 (t, $J$ = 7.4 Hz, 2H), 7.27 (s, 1H), 7.11 (s, 1H), 4.87 (br s, 1H), 4.29 (d, $J$ = 6.8 Hz, 2H), 4.26 – 4.17 (om, 2H), 3.72 (dd, $J$ = 16.8, 6.0 Hz, 1H), 3.66 (dd, $J$ = 16.8, 6.0 Hz, 1H), 3.61 (dd, $J$ = 10.5, 5.4 Hz, 1H), 3.55 (dd, $J$ = 10.5, 4.8 Hz, 1H).

$^{13}$C NMR: (151 MHz, DMSO-$d_6$) $\delta$ 171.9, 169.0, 156.5, 143.8, 140.7, 127.6, 127.1, 125.2, 120.1, 65.8, 61.7, 54.9, 46.6, 43.5.
HPLC Trace: Retention time = 18.3 min, Purity: >95% (HPLC analysis at 254 nm)
HRMS: $m/z$ 432.116, (calcd $[M+Na]^+ = 432.121$)

**NMR**

$^1$H NMR chemical shifts referenced to residual DMSO-$d_6$ at 2.50 ppm, and $^{13}$C NMR chemical shifts referenced to DMSO-$d_6$ at 39.52 ppm.

$^1$H NMR (600 MHz, DMSO-$d_6$) $\delta$ 7.90 (d, $J = 7.5$ Hz, 2H), 7.75 (s, 1H), 7.72 (d, $J = 7.4$ Hz, 2H), 7.64 (t, $J = 6.0$ Hz, 1H), 7.42 (t, $J = 7.5$ Hz, 2H), 7.39 (s, 1H), 7.33 (t, $J = 7.4$ Hz, 2H), 4.73 (dd, $J = 9.1$, 3.1 Hz, 1H), 4.60 (t, $J = 9.0$ Hz, 1H), 4.36 – 4.15 (m, 6H).

$^{13}$C NMR (151 MHz, DMSO-$d_6$) $\delta$ 169.9, 169.1, 156.4, 153.5, 143.8, 140.7, 127.6, 127.1, 125.2, 120.1, 66.8, 65.8, 55.4, 46.6, 44.1.
This structure was consistent with all 1D and 2D NMR data: $^1$H, $^{13}$C COSY, (H / C) HSQC, (H / N) HSQC, (H / C) HMBC, and NOESY.

$^1$H NMR (599.62 MHz, DMSO-d$_6$)

Presaturation used for water suppression.

Water peak suppressed.
III. Cleavage of cyclized peptides:

General procedure for the cleavage of peptides: To a cyclized peptide (2a-j), 1 mL phosphate buffer (pH 7.5) was added. The reaction was stirred at 37 °C, and monitored by analytical HPLC at regular intervals. The reaction mixture was lyophilized, purified by HPLC and isolated. HPLC: 0.1% FA (v/v) in water (solvent A); 0.1% FA (v/v) acetonitrile (solvent B); gradient 0-80 %, 0.1% FA (v/v) acetonitrile in 25 min, flow rate = 1.0 mL/min, detection wavelength 254 nm.

IV. Ring opening of modified C-terminal fragment to original fragment with serine residue at the terminus:
**General procedure for ring opening modified Serine fragment:** To a cyclized peptide (IV), sodium methoxide (NaOMe) in methanol (MeOH) was added. The reaction was stirred at 37 °C, and monitored by analytical HPLC at regular intervals. It resulted in ring opening and generated peptide (V). We also observed the formation of methyl ester peptide by cleavage at N-terminal of modified serine (IV) with NaOMe/MeOH. Treatment of peptide (V) with hydrazine and aqueous potassium hydroxide (KOH) generated original fragment back (III), analyzed by analytical HPLC. We also observed the formation of cleavage products from (V) by using aq. KOH. The reaction mixture was lyophilized, purified by HPLC and isolated.


OAc-Gly-Ser(N-methoxide)-Phe-Arg-NH₂ (V). LCMS: m/z 565.2 (calcd [M+H]⁺ = 565.5). Purity: >95% (HPLC analysis at 254 nm). Retention time: 7.3 min.

OAc-Gly-Ser-Phe-Arg-NH₂ (III). LCMS: m/z 507.2 (calcd [M+H]⁺ = 507.5), 529.2 (calcd [M+Na]⁺ = 529.5). Purity: >95% (HPLC analysis at 254 nm). Retention time: 5.8 min.
HPLC spectra of the purified cyclized starting material (IV) and purified ring opened by sodium methoxide/methanol (V) and ring opened original fragment (III) by treatment with aqueous potassium hydroxide.

Peptide Characterization and HPLC Traces
**Fmoc-Gly-Ala-Ser-Phe-Ala-Gly-NH₂ (1a).** LCMS: m/z 730.20 (calcd [M+H]⁺ = 730.01), Purity: >95% (HPLC analysis at 254 nm). Retention time: 17.85 min.

**Fmoc-Gly-Ala-Oxd-Phe-Ala-Gly-NH₂ (2a).** LCMS: m/z 756.40 (calcd [M+H]⁺ = 756.10), 778.5 (calcd [M+Na]⁺ = 778.0). Purity: >95% (HPLC analysis at 254 nm). Retention time: 17.9 min.

**Fmoc-Gly-Ala-OH (3a).** LCMS: m/z 369.20 (calcd [M+H]⁺ = 369.13). Purity: >95% (HPLC analysis at 254 nm). Retention time: 18.6 min.

**Oxd-Phe-Ala-Gly-NH₂ (4).** LCMS: m/z 406.20 (calcd [M+H]⁺ = 406.0), 428.2 (calcd [M+Na]⁺ = 428.3). Purity: >95% (HPLC analysis at 254 nm). Retention time: 8.9 min.

HPLC spectra of the cyclized starting material and the crude reaction mixture after incubation in buffer solution; the product peak is labeled.


S25
Oxa-Phe-Ala-Gly-NH$_2$ (4). LCMS: $m/z$ 406.0 (calcd $[M+H]^{+} = 406.1$), 428.0 (calcd $[M+Na]^{+} = 428.2$). Purity: >95% (HPLC analysis at 254 nm). Retention time: 6.9 min.

HPLC spectra of the cyclized starting material and the crude reaction mixture after incubation in buffer solution; the product peak is labeled
Fmoc-Gly-Met-Oxd-Phe-Ala-Gly-NH$_2$ (2c). LCMS: $m/z$ 816.0 (calcd $[M+H]^+$ = 816.20), 408.2 (calcd $(M+2)/2]^+$ = 408.8). Purity: >95% (HPLC analysis at 254 nm). Retention time: 21.8 min.


HPLC spectra of the cyclized starting material and the crude reaction mixture after incubation in buffer solution; the product peak is labeled


HPLC spectra of the cyclized starting material and the crude reaction mixture after incubation in buffer solution; the product peak is labeled


**Oxd-Phe-Ala-Gly-NH₂ (4).** LCMS: m/z 406.0 (calcd [M+H]⁺ = 406.1), 428.0 (calcd [M+Na]⁺ = 428.2). Purity: >95% (HPLC analysis at 254 nm). Retention time: 9.8 min.

HPLC spectra of the cyclized starting material and the crude reaction mixture after incubation in buffer solution; the product peak is labeled.


HPLC spectra of the cyclized starting material and the crude reaction mixture after incubation in buffer solution; the product peak is labeled.
phosphate buffer (pH -7.5), 37 °C
**Fmoc-Gly-Val-Oxd-Phe-Ala-Gly-NH₂ (2g)**. LCMS: $m/z$ 784.60 (calcd $[M+H]^+ = 784.58$), 392.5 (calcd [(M+2)/2]$^+ = 392.79$). Purity: >95% (HPLC analysis at 254 nm). Retention time: 16.6 min.

**Fmoc-Gly-Val-OH (3g)**. LCMS: $m/z$ 397.1 (calcd $[M+H]^+ = 397.18$). Purity: >95% (HPLC analysis at 254 nm). Retention time: 15.6 min.

**Oxd-Phe-Ala-Gly-NH₂ (4)**. LCMS: $m/z$ 406.0 (calcd $[M+H]^+ = 406.1$), 428.0 (calcd $[M+Na]^+ = 428.2$). Purity: >95% (HPLC analysis at 254 nm). Retention time: 6.8 min.

HPLC spectra of the cyclized starting material and the crude reaction mixture after incubation in buffer solution; the product peak is labeled

![HPLC spectra of cyclized starting material and crude reaction mixture](image-url)


HPLC spectra of the cyclized starting material and the crude reaction mixture after incubation in buffer solution; the product peak is labeled.
**Fmoc-Gly-Asp(cyc)-Oxd-Phe-Ala-Gly-NH₂ (2i).** LCMS: \( m/z \) 782.10 (calcd \([M+H]^+ = 782.53\)), 391.1 (calcd \([(M+2)/2]^+ = 391.76\)). Purity: >95\% (HPLC analysis at 254 nm). Retention time: 19.8 min.

**Fmoc-Gly-Asp-OH (3i).** LCMS: \( m/z \) 413.1 (calcd \([M+H]^+ = 413.14\)). Purity: >95\% (HPLC analysis at 254 nm). Retention time: 18.8 min.

**Oxd-Phe-Ala-Gly-NH₂ (4).** LCMS: \( m/z \) 406.0 (calcd \([M+H]^+ = 406.1\)), 428.0 (calcd \([M+Na]^+ = 428.2\)). Purity: >95\% (HPLC analysis at 254 nm). Retention time: 9.8 min.

HPLC spectra of the cyclized starting material and the crude reaction mixture after incubation in buffer solution; the product peak is labeled.

In case of Asp containing peptide, cyclization of Asp was observed after treatment with DSC but after buffer addition Asp ring opens again to give the original fragment. Details about the ring cyclization and opening of Asp are under study in our lab.
**Fmoc-Gly-Pro-Oxd-Phe-Ala-Gly-NH$_2$ (2j).** LCMS: $m/z$ 782.20 (calcd $[M+H]^+$ = 782.56), 391.5 (calcd $[(M+2)/2]^+$ = 391.78). Purity: >95% (HPLC analysis at 254 nm). Retention time: 19.55 min.

**Fmoc-Gly-Pro-OH (3j).** LCMS: $m/z$ 395.1 (calcd $[M+H]^+$ = 395.16). Purity: >95% (HPLC analysis at 254 nm). Retention time: 18.54 min.

**Oxd-Phe-Ala-Gly-NH$_2$ (4).** LCMS: $m/z$ 406.0 (calcd $[M+H]^+$ = 406.1), 428.0 (calcd $[M+Na]^+$ = 428.2). Purity: >95% (HPLC analysis at 254 nm). Retention time: 8.9 min.

HPLC spectra of the cyclized starting material and the crude reaction mixture after incubation in buffer solution; the product peak is labeled.
**Fmoc-Gly-Ala-Oxd-Phe-Arg-Phe-Gly-NH\(_2\) (5a).** LCMS: m/z 1003.0 (calcd [M+H]\(^+\) = 1003.2), 502.0 (calcd [(M+2)/2]\(^+\) = 501.91). Purity: >95% (HPLC analysis at 254 nm). Retention time: 19.6 min.

**Fmoc-Gly-Ala-OH (5b).** LCMS: m/z 369.2 (calcd [M+H]\(^+\) = 369.13). Purity: >95% (HPLC analysis at 254 nm). Retention time: 19.89 min.

**Oxd-Phe-Arg-Phe-Gly-NH\(_2\) (5c).** LCMS: m/z 652.3 (calcd [M+H]\(^+\) = 652.71), 674.2 (calcd [M+Na]\(^+\) = 674.71). Purity: >95% (HPLC analysis at 254 nm). Retention time: 13.3 min.

HPLC spectra of the cyclized starting material and the crude reaction mixture after incubation in buffer solution; the product peak is labeled.

Fmoc-Ala-OH (6b). LCMS: m/z 312.0 (calcd [M+H]$^+$ = 312.07). Purity: >95% (HPLC analysis at 254 nm). Retention time: 12.9 min.

Oxd-Phe-Val-Gly-Ala-OH (6c). LCMS: m/z 506.3 (calcd [M+H]$^+$ = 506.51), 528.2 (calcd [M+Na]$^+$ = 528.51). Purity: >95% (HPLC analysis at 254 nm). Retention time: 5.06 min.

HPLC spectra of the cyclized starting material and the crude reaction mixture after incubation in buffer solution; the product peak is labeled.


HPLC spectra of the cyclized starting material and the crude reaction mixture after incubation in buffer solution; the product peak is labeled.


Oxd-Val-Arg-Phe-Ala-OH (8c). LCMS: $m/z$ 605.3 (calcd $[M+H]^+$ = 605.65), 627.4 (calcd
$\left[M+Na\right]^+ = 627.65$). Purity: $>95\%$ (HPLC analysis at 254 nm). Retention time: 8.71 min.


HPLC spectra of the cyclized starting material and the crude reaction mixture after incubation in buffer solution; the product peak is labeled.


HPLC spectra of the starting material and the crude reaction mixture after incubation in buffer solution; the product peak is labeled.
m/z = 532.7

9b

9c

m/z = 532.7


HPLC spectra of the cyclized starting material and the crude reaction mixture after incubation in buffer solution; the product peak is labeled.
Fmoc-Cys-Gly-Arg-Arg-Ala-Cys-Gly-Oxd-Phe-Ala-Gly-NH₂, disulfide bond (11a). LCMS: 
\( m/z \) 1330.1 (calcd \([M+H]^+ = 1330.24\)), 665.5 (calcd \([M+2/2]^+ = 665.62\)). Purity: >95% (HPLC analysis at 254 nm). Retention time: 13.1 min.

Fmoc-Cys-Gly-Arg-Arg-Ala-Cys-Gly-OH, disulfide bond (11b). LCMS: \( m/z \) 942.5 (calcd \([M+H]^+ = 942.84\)), 471.7 (calcd \([M+2/2]^+ = 471.92\)). Purity: >95% (HPLC analysis at 254 nm). Retention time: 12.2 min.

Oxd-Phe-Ala-Gly-NH₂ (11c). LCMS: \( m/z \) 406.2 (calcd \([M+H]^+ = 406.39\)), Purity: >95% (HPLC analysis at 254 nm). Retention time: 5.8 min.

HPLC spectra of the starting material and the crude reaction mixture after incubation in buffer solution; the product peak is labeled.

**Fmoc-Arg-Ala-OH (12b).** LCMS: m/z 468.09 (calcd [M+H]⁺ = 468.26). Purity: >95% (HPLC analysis at 254 nm). Retention time: 12.5 min.


**Oxd-Gly-Phe-NH₂ (12d).** LCMS: m/z 335.2 (calcd [M+H]⁺ = 335.32), 357.4 (calcd [M+Na]⁺ = 357.32). Purity: >95% (HPLC analysis at 254 nm). Retention time: 8.98 min.

HPLC spectra of the starting material and the crude reaction mixture after incubation in buffer solution; the product peak is labeled.
Fmoc-Arg-Pro-Pro-Gly-Phe-Oxd-Pro-Phe-Arg-NH₂ (13a). LCMS: m/z 1308.3 (calcd [M+H]^+ = 1308.22), 654.3 (calcd [(M+2)/2]^+ = 654.61). Purity: >95% (HPLC analysis at 254 nm). Retention time: 16.2 min.

Fmoc-Arg-Pro-Pro-Gly-Phe-OH (13b). LCMS: m/z 795.3 (calcd [M+H]^+ = 795.64). Purity: >95% (HPLC analysis at 254 nm). Retention time: 14.9 min.

Oxd-Pro-Phe-Arg-NH₂ (13c). LCMS: m/z 531.3 (calcd [M+H]^+ = 531.57), Purity: >95% (HPLC analysis at 254 nm). Retention time: 7.28 min.

HPLC spectra of the cyclized starting material and the crude reaction mixture after incubation in buffer solution; the product peak is labeled


HPLC spectra of the cyclized starting material and the crude reaction mixture after incubation in buffer solution; the product peak is labeled
OAc-Ala-Val-Ala-Pro-Ala-Ala-Oxd-Ile-Val-Ala-NH₂ (15a). LCMS: m/z 937.1 (calcd [M+H]^+ = 937.02), Purity: >95% (HPLC analysis at 254 nm). Retention time: 10.9 min.

OAc-Ala-Val-Ala-Pro-Ala-Ala-OH (15b). LCMS: m/z 541.4 (calcd [M+H]^+ = 541.56). Purity: >95% (HPLC analysis at 254 nm). Retention time: 8.2 min.

Oxd-Ile-Val-Ala-NH₂ (15c). LCMS: m/z 414.3 (calcd [M+H]^+ = 414.46), Purity: >95% (HPLC analysis at 254 nm). Retention time: 6.1 min.

HPLC spectra of the cyclized starting material and the crude reaction mixture after incubation in buffer solution; the product peak is labeled.
OAc-Ala-Val-Ala-Pro-βAla-Ala-Oxd-Ile-Val-Ala-NH₂ (16a). LCMS: m/z 937.1 (calcd [M+H]⁺ = 937.02), 975.1 (calcd [M+K]⁺ = 975.02). Purity: >95% (HPLC analysis at 254 nm). Retention time: 10.6 min.

OAc-Ala-Val-Ala-Pro-βAla-Ala-OH (16b). LCMS: m/z 541.4 (calcd [M+H]⁺ = 541.56). Purity: >95% (HPLC analysis at 254 nm). Retention time: 7.6 min.

Oxd-Ile-Val-Ala-NH₂ (15c). LCMS: m/z 414.3 (calcd [M+H]⁺ = 414.46), Purity: >95% (HPLC analysis at 254 nm). Retention time: 5.9 min.

HPLC spectra of the starting material and the crude reaction mixture after incubation in buffer solution; the product peak is labeled


Oxd-(N-methyl)Leu-Gly-NH₂ (17c). LCMS: m/z 315.2 (calcd [M+H]⁺ = 315.3), 337.1 (calcd [M+Na]⁺ = 337.3) Purity: >95% (HPLC analysis at 254 nm). Retention time: 8.88 min

HPLC spectra of the cyclized starting material and the crude reaction mixture after incubation in buffer solution; the product peak is labeled.
References: