Supporting information for:

Thiol-Ene Photoimmobilization of Chymotrypsin on Polysiloxane Gels for Enzymatic Peptide Synthesis

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General Considerations

Poly[3-mercaptopropylmethyilsiloxane] (PMMS, SMS-992, $M_W$ 4000-7000, 95 cst) was purchased from Gelest Inc. Poly(ethylene glycol)diacrylate (average $M_n$~700) was purchased from Aldrich Inc. 2,2-Dimethoxy-2-phenylacetophenone (DMPA), chymotrypsin (from bovine pancreas), N-benzoyl-L-tyrosine ethyl ester (Bz-Tyr-OEt), N-acetyl-L-phenylalanine ethyl ester (Ac-Phe-OEt), N-acetyl-L-tryptophan ethyl ester (Ac-Trp-OEt), L-phenylalaninamide (Phe-NH$_2$), L-tyrosine amide (Tyr-NH$_2$), glycinamide (Gly-NH$_2$), alaninamide (Ala-NH$_2$), anhydrous organic solvents (hexane, THF, toluene, DMF, DMSO) were purchased from Aladdin (Shanghai) Inc. All flash chromatography were performed using Macherey-Nagel MN Kieselgel 60 (0.063-1.2 mm).

All $^1$H NMR spectra were obtained using a Bruker HW500 MHz spectrometer (AVANCE AV-500) and recorded in DMSO-$d_6$ (internal reference 2.50 ppm).

Fourier transform infrared (FT-IR) spectra were obtained on a Nicolet 5700 FT-IR spectrometer.

Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry was performed on an AB Sciex 5800 MALDI-TOF spectrometry. Protein solution (0.5-1.0 mg/mL) was mixed with an equal volume of matrix (0.5 mL of water, 0.5 mL of acetonitrile, 2 µL of trifluoroacetic acid, and 8.0 mg of 4-hydroxy-3,5-dimethoxycinnamic acid), and 2 µL of the resulting mixture was spotted on the plate target.

An ultraviolet (UV) lamp (230V, 0.20A, $\lambda = 365$ nm, LUYOR-3109, Shanghai LUYOR Instrument Co, Ltd) was used to irradiate the samples to perform the photocrosslinking reaction.
**Enzyme activity assay**

Chymotrypsin (from bovine pancreas, lyophilized powder) activity is determined by hydrolyzing N-benzoyl-L-tyrosine ethyl ester (BTEE). Unit of enzyme activity (U)\(^1\) is defined as: 1 mg of protein hydrolyzes 1.0 μmol of BTEE per min at pH 7.8 at 25 °C. The assay mixture was composed of 1.42 mL of Tris–HCl buffer (80 mM, pH 7.8), 1.4 mL of 1.18 mM BTEE and 0.08 ml of 2 M CaCl\(_2\). After addition of 0.1 mL of enzyme solution, the reaction was carried out at 25 °C for 3 min. The suspension was immediately separated by an external magnetic field of 0.5 T and the absorbance of the solution was measured at 256 nm. The specific activity was calculated as follows:

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\Delta A
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Specific activity (U/mg min) = \(\frac{0.964 \times E_w \times 3 \times 3}{\Delta A}\) = 48 units/mg protein

where \(\Delta A\) was the absorbance change of the solution at 256 nm, \(E_w\) represented the amount of enzyme contained in 0.1 ml of enzyme solution, 0.964 was the molar extinction coefficient of N-benzoyl-L-tyrosine at 256 nm.

**Enzyme modification**

Chymotrypsin (2.00 g, 0.08 mmol of protein, 1.04 mmol of –NH\(_2\) groups) was dissolved in 200 mL phosphate buffer (0.1 M, pH 8.0). 10-Undecenoyl chloride (3.24 g, 16 mmol, the molar ratio of enzyme to 10-undecenoyl chloride is 1:200) dissolved in dichloromethane (20 mL) was added dropwise to the above phosphate buffer solution.\(^2\) The two-phase reaction mixture was vigorously stirred at 25 °C and the pH value was maintained at 7.5~8.0 (pH 7.5~8.0 was chosen to increase the nucleophilicity of the amino groups of lysine fragments in the protein towards 10-undecenoyl chloride) by the addition of 0.1 M sodium hydroxide solution. After 36 h, the vinyl-modified proteins were purified by dialysis against phosphate buffer (0.1 M, pH 8.0) for 48 h using a dialysis cube with molecular weight cut-off (MWCO) of 5000 Da, followed by lyophilization for 48 h.

**Typical preparation procedure of PMMS-CT**
As shown in Figure S1, vinyl-modified proteins chymotrypsin (50.00 mg), PMMS (100.00 mg, 0.75 mmol of –SH groups), poly (ethylene glycol) diacrylate (140.00 mg, 0.2 mmol, as cross-linker), DMPA (10 mg, as photo-initiator), and dichloromethane (2 mL) were added in a 10 mL transparent vial tube. The mixture was ultrasonicated for 2 min to ensure a homogeneous dispersion, and then UV irradiated at 365 nm at room temperature for 2 min. The biocatalyst gel was carefully crushed to small particles, washed 3 times with dichloromethane to remove any unreacted chymotrypsin and DMPA, and dried in a fume hood overnight, to provide the polysiloxane-based biocatalyst gel PMMS-CT (274.00 mg) which was stored for future uses.

![Figure S1](image-url)  
*Figure S1.* Typical preparation procedure of biopolymer PMMS-CT catalyst.
Enzymatic synthesis of dipeptide Bz-Tyr-Phe-NH₂

In a 10 mL round-bottom flask, acyl donor N-benzoyl-L-tyrosine ethyl ester (Bz-Tyr-OEt, 250.8 mg, 0.8 mmol), and acyl acceptor L-Phenylalaninamide (Phe-NH₂, 262.4 mg, 1.6 mmol), distilled water (40 μL, 1.0 vol%), PMMS-CT (100.00 mg, ca. 17.2 mg chymotrypsin immobilized) were added in 4 mL hexane/THF (v/v= 1:1). The reaction mixture was stirred at 37 °C for 3 h.[5] The crude product mixture was dissolved in MeOH (40 mL) and PMMS-CT was separated by filtration. The resulting solvent was evaporated and the crude product was first well-dispersed in benzene, then filtered, washed with benzene and water, and finally dried under vacuum to give the dipeptide Bz-Tyr-Phe-NH₂ (337.9 mg, Yield: 98%, m.p. = 256-259 °C) as a white solid. Bz-Tyr-Phe-NH₂: ¹H-NMR (300 MHz, DMSO) δH 8.51 (1 H, d, J = 8.2 Hz), 8.06 (1 H, d, J = 8.1 Hz), 7.77 (2 H, d, J = 7.2 Hz), 7.54 – 7.38 (3 H, m), 7.28 – 7.10 (5 H, m), 7.06 (2 H, d, J = 8.4 Hz), 6.61 (2 H, d, J = 8.3 Hz), 5.33 (1 H, s), 4.46 (2 H, m), 3.15 – 2.65 (5 H, m). ¹³C NMR (75 MHz, DMSO): δ 173.16, 171.66, 166.80, 156.13, 138.22, 134.52, 131.80, 130.51, 129.71, 128.82, 128.66, 128.47, 127.87, 126.69, 115.32, 55.80, 54.21, 38.10, 36.46. ESI-MS m/z: 430.2 [M - H]⁺, calculated for Bz-Tyr-Phe-NH₂, 431.2.

Figure S2. ¹H-NMR spectrum of Bz-Tyr-Phe-NH₂ in DMSO.
Figure S3. $^{13}$C-NMR spectrum of Bz-Tyr-Phe-NH$_2$ in DMSO.

Figure S4. ESI-MS data of Bz-Tyr-Phe-NH$_2$. 
Peptide synthesis catalyzed by PMMS-CT in low-water organic solvent

In a 10 mL round-bottom flask, distilled water (40 μL, 1.0 vol%), PMMS-CT (100 mg, ca. 17.2 mg chymotrypsin immobilized), 0.8 mmol acyl donor (Bz-Tyr-OEt, Ac-Phe-OEt, or Ac-Trp-OEt) and 1.6 mmol acyl acceptor (Phe-NH₂ or Tyr-NH₂) were added into 4 mL hexane/ tetrahydrofuran (v/v= 1:1). The reaction mixture was stirred at 37 °C for 3 h. The crude product mixture was dissolved in MeOH (40 mL) and PMMS-CT was separated by filtration. The resulting solvent was evaporated and the crude product was purified by flash column chromatography to give the desired product.

Ac-Trp-Phe-NH₂ (257.40 mg, yield: 82%, m.p. = 206-208 °C) as a white solid, purified by flash column chromatography using ethyl acetate/petroleum ether (v/v= 1/2) as the eluent. ^1H-NMR (300 MHz, DMSO) δ_H 8.03 (1 H, d, J 7.8), 7.83 (1 H, d, J 8.1), 7.55 (0 H, d, J 7.7), 7.36 – 7.20 (2 H, m), 7.13 – 6.94 (5 H, m), 6.64 (2 H, d, J 8.2), 5.75 (0 H, s), 4.47 (1 H, s), 4.34 (1 H, d, J 5.1), 2.88 (4 H, ddd, J 43.3, 34.5, 6.4), 2.09 (2 H, s). ^13C NMR (75 MHz, DMSO): δ 174.62, 173.22, 171.25, 139.64, 137.87, 131.16, 131.02, 130.03, 129.82, 129.11, 128.26, 128.02, 125.23, 122.63, 120.11, 120.01, 113.09, 112.03, 63.07, 55.57, 55.48, 39.19, 29.15, 24.32. ESI-MS m/z: 391.2 [M - H]⁺, calculated for Ac-Trp-Phe-NH₂, 392.2.

Figure S5. ESI-MS data of Ac-Trp-Phe-NH₂.
Figure S6. $^1$H-NMR spectrum of Ac-Trp-Phe-NH$_2$.

Figure S7. $^1$H-NMR spectrum of Ac-Trp-Phe-NH$_2$. 
Bz-Tyr-Tyr-NH₂ (311.40 mg, yield: 87%, m.p. = 250-252 °C) as a white solid, purified by flash column chromatography using ethyl acetate as the eluent. ¹H-NMR (500 MHz, DMSO) δ H 8.54 (1 H, t, J 11.8), 8.05 – 7.89 (1 H, m), 7.80 (2 H, d, J 7.5), 7.48 (3 H, d, J 7.6), 7.08 (2 H, d, J 8.3), 7.05 – 7.00 (2 H, m), 6.62 (4 H, dd, J 16.6, 8.3), 4.66 – 4.54 (1 H, m), 4.40 (1 H, dd, J 13.4, 8.3), 2.84 (4 H, d, J 7.6). ¹³C NMR (75 MHz, DMSO): δ 174.64, 172.87, 168.20, 157.58, 157.47, 135.92, 133.24, 133.08, 129.98, 129.17, 116.67, 57.20, 55.87, 37.83, 37.34. ESI-MS m/z: 446.2 [M - H]⁻, calculated for Bz-Tyr-Tyr-NH₂, 447.2.

**Figure S8.** ¹H-NMR spectrum of Bz-Tyr-Tyr-NH₂ in DMSO.
Figure S9. $^{13}$C-NMR spectrum of Bz-Tyr-Tyr-NH$_2$ in DMSO.

Figure S10. ESI-MS data of Bz-Tyr-Tyr-NH$_2$. 
Ac-Phe-Tyr-NH₂ (251.20 mg, yield: 85%, m.p. =249-252 °C) as a white solid, purified by flash column chromatography using ethyl acetate/petroleum ether (v/v= 1/2) as the eluent. \( ^1 \)H-NMR (300 MHz, DMSO) \( \delta \) 8.07 (1 H, d, \( J \) 8.2), 7.99 – 7.80 (1 H, m), 7.34 – 7.08 (5 H, m), 6.99 (3 H, d, \( J \) 8.4), 6.63 (2 H, d, \( J \) 8.3), 5.74 (0 H, s), 4.53 – 4.21 (2 H, m), 2.91 (3 H, dt, \( J \) 11.6, 5.8), 2.80 – 2.56 (3 H, m), 2.08 (3 H, s). 

\( ^{13} \)C NMR (75 MHz, DMSO): \( \delta \)174.64, 172.76, 171.02, 157.59, 139.82, 131.90, 130.88, 129.78, 127.96, 116.66, 55.89, 55.85, 38.54, 24.22. ESI-MS m/z: 368.2 [M - H], calculated for Ac-Phe-Tyr-NH₂, 369.2.

**Figure S11.** \(^1\)H-NMR spectrum of Ac-Phe-Tyr-NH₂ in DMSO.
**Figure S12.** $^{13}$C-NMR spectrum of Ac-Phe-Tyr-NH$_2$ in DMSO.

**Figure S13.** ESI-MS data of Ac-Phe-Tyr-NH$_2$. 
Ac-Trp-Tyr-NH₂ (267.90 mg, yield: 82%) as a yellow oil, purified by flash column chromatography using ethyl acetate/petroleum ether (v/v= 1/1) as the eluent. 

$^1$H-NMR (300 MHz, DMSO) $\delta$ 8.03 (1 H, d, $J$ = 7.8 Hz), 7.83 (1 H, d, $J$ = 8.1 Hz), 7.55 (1 H, d, $J$ = 7.7 Hz), 7.36 – 7.20 (2 H, m), 7.13 – 6.94 (5 H, m), 6.64 (2 H, d, $J$ = 8.2 Hz), 4.47 (1 H, s), 4.34 (1 H, d, $J$ = 5.1 Hz), 2.88 (4 H, m), 2.09 (2 H, s).

$^{13}$C NMR (75 MHz, DMSO): $\delta$ 175.35, 173.44, 172.16, 157.56, 137.90, 132.00, 129.52, 129.50, 129.02, 128.85, 125.48, 125.34, 122.91, 122.81, 117.17, 116.83, 53.60, 50.46, 29.07, 28.85, 24.42, 24.17, 23.95. ESI-MS m/z: 407.2 [M - H], calculated for Ac-Trp-Tyr-NH₂, 408.2.

Figure S14. $^1$H-NMR spectrum of Ac-Trp-Tyr-NH₂ in DMSO.
**Figure S15.** $^{13}$C-NMR spectrum of Ac-Trp-Tyr-NH$_2$ in DMSO.

**Figure S16.** ESI-MS data of Ac-Trp-Tyr-NH$_2$. 
Ac-Phe-Phe-NH$_2$ (223.30 mg, yield: 79%) as a white solid, purified by flash column chromatography using ethyl acetate/petroleum ether (v/v= 1/2) as the eluent. $^1$H-NMR (600 MHz, DMSO) $\delta$ 8.12 (1 H, m), 8.08 (1 H, m), 7.24 (6 H, m), 7.21 – 7.15 (4 H, m), 4.47 – 4.37 (2 H, m), 2.97 (2 H, m), 2.75 (3 H, m), 1.72 (3 H, s). $^{13}$C NMR (75 MHz, DMSO): $\delta$ 174.46, 172.84, 170.99, 139.80, 139.63, 131.00, 130.87, 129.82, 129.78, 128.02, 55.83, 55.55, 39.28, 39.02, 24.22. ESI-MS m/z: 352.2 [M - H]$^-$, calculated for Ac-Phe-Phe-NH$_2$, 353.2.

**Figure S17.** $^1$H-NMR spectrum of Ac-Phe-Phe-NH$_2$ in DMSO.
**Figure S18.** $^{13}$C-NMR spectrum of Ac-Phe-Phe-NH$_2$ in DMSO.

**Figure S19.** ESI-MS data of Ac-Phe-Phe-NH$_2$. 
References


