Electronic Supplementary Information (ESI)

Inter-chain acyl transfer reaction in a peptide six-helical bundle: a chemical method for regulating the interaction between peptides or proteins

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Experimental Section

Peptide synthesis, purification, and identification

Peptides were synthesized using a CS-Bio 136 automated peptide synthesizer (CS Bio Co., Menlo Park, CA) with a standard solid phase Fmoc chemistry protocol. All protected amino acids used were purchased from GL Biochem Ltd. (Shanghai, China). Rink amide resin (0.38 ~ 0.45 mmol/g, Nankai Hecheng S&T Co. Ltd., Tianjin, China) was used. Coupling of the amino acids was achieved using *O*-benzotriazol-1-yl-*N*,*N*,*N*',*N*'-tetramethyl-uronium hexafluorophosphate (HBTU, GL Biochem, Shanghai, China) and diisopropylethylamine (DIEA, Acrose) as an activator and an active base, respectively, in N,N-dimethylformamide (DMF) solution. The Fmoc protective group was removed using 20% piperidine/DMF. Between every coupling or Fmoc removal, the resin was washed five times with DMF, and three times with dichloromethane (DCM). The carboxyl termini were amidated upon cleavage from the resin, and the amino termini were capped with acetic acid

anhydride. The peptides were cleaved from the resin and de-protected with Reagent K, which contained 82.5% trifluoroacetic acid, 5% thioanisole, 5% m-cresol, 5% water, and 2.5% ethanedithiol. The crude products were precipitated with cold diethyl ether and lyophilized.

For peptides with the side-chain thioester, Fmoc-L-glutamic acid O-Allyl ester (Fmoc-Glu(OAll)-OH) was used in the thioester modified site. The O-Allyl group was removed by 1eq tetrakis(triphenylphosphine)palladium(0) with 10eq 5,5-dimethyl-1,3-cyclohexanedione, as a scavenger in the DCM/THF (1:1) solution. Then the resin was washed five times with 0.5% DIEA in DMF, and five times with 1M Sodium diethyldithiocarbamate in DMF. 4 eq 1-Ethyl-3-(3-dimethyllaminopropyl)carbodiimide hydrochloride (EDC) and 4eq benzyl mercaptan were added to the resin for thioester formation. Finally, the resin cleaved by Reagent K. The crude peptide products were purified by preparative reverse phase HPLC using a Waters preparative HPLC system (PrepLC 4000): gradient elution of 30–50% solvent B in solvent A (0.1% trifluoroacetic acid in H_2O; solvent A and 0.1% trifluoroacetic acid in 70%CH_3CN/H_2O; solvent B) over 60 min at 16 ml/min on a Waters X-bridge C8, 10μm, 19.5mm* 250mm column. Analytical RP-HPLC was performed on a RP-C8 column (Zorbax Eclipse XDB-C8, 5 μm, 4.6 x 150 mm) with gradient elution of 5–100% solvent B in solvent A over 25 min at a flow rate of 1 ml/min. Compounds were detected by UV absorption at 220 nm with SHIMADZU SPD-10A. All peptides were purified to > 95% purity. The molecular weight of the peptides was confirmed by MALDI-TOF-MS (Autoflex III, Bruker Daltonics).

Native polyacrylamide gel electrophoresis (N-PAGE)

Tris-glycine gels (20%) and BayGene Mini Cell were used for N-PAGE. N-peptide solutions were incubated with PBS at the indicated concentrations at 37 °C for 30 min before addition of C-peptide (final concentration of N- and C-peptide = 50 μM). After

incubation at 37 °C for 30 min, the samples were mixed with Tris-glycine native sample buffer (Invitrogen, Carlsbad, CA) at a ratio of 1:1, and then loaded onto the gels (30 μl/well). Gel electrophoresis was carried out (120 V constant voltage, room temperature, 2.5 h), and the gel was then stained with Bio-red Bio-Safe Coomassie Stain.

Circular Dichroism (CD) spectroscopy

N-peptide was incubated with C peptide at 37 °C for 30 min in PBS, pH 7.2 (final concentration of N-peptide and C-peptide = 10 μM). The mixture was then cooled to room temperature. CD spectra were acquired at room temperature (Biologic MOS-450: 4.0 nm bandwidth, 0.1 nm resolution, 0.1 cm path length, 4.0 s response time, and 50 nm/min scanning speed). The spectra were corrected by subtraction of the solvent blank. Thermal midpoint analysis was performed to determine the temperature (T_m) at which 50% of the 6HB would decompose. The temperature was controlled by a Bio-logic TCU250 system. The final concentration of N- and C-peptide was 1 μM in PBS, pH 7.2. CD spectra were monitored at 222 nm from 20 °C to 90 °C (2 °C/min).

Analysis the covalent bond formation between C- and N-peptide by Tricine-SDS polyacrylamide gel electrophoresis (Tricine-SDS-PAGE)

20% polyacrylamide gels and BayGene Mini Cell were used for Tricine-SDS-PAGE. Cathode buffer was 0.1M Tricine, 0.1M Tris and 1% SDS, and anode was 0.2M Tris. N-peptide solutions were incubated with PBS at the indicated concentrations at 37°C for 30 min before addition of C-peptide (final concentration of N- and C-peptide = 50 μM). After incubation at 37°C for 0~48 hr, the samples were mixed with Tris-SDS-glycine sample buffer (Invitrogen, Carlsbad, CA) at a ratio of 1:1 and then loaded onto the gels (20 μl/well). Gel electrophoresis was firstly carried out at 30 V

constant voltage in room temperature for 1 h, then carried out at 150 V constant voltage in room temperature for 2 h. The gel was then stained with Bio-red Bio-Safe Coomassie Stain.

HPLC for determining the covalent bond formation between C- and N-peptide

Reactions were carried out in 1.5 mL Eppendorf tubes. A standard solution of 50 μM Tryptophan (Trp) was prepared, and stock solutions of N- and C-peptide were prepared by dissolving the appropriate peptide in PBS (pH 7.2). In a typical experiment, the N-peptide and C-peptide were incubated at a 1:1 ratio at room temperature, and then PBS (pH 7.2) and Trp solution were added. The typical final concentration of the internal Trp standard, C peptide, and N peptide was 25 μM. The reaction mixture was incubated at 37 °C for 0 to 48 h. At the indicated times, a portion of the 50 μL reaction mixture was removed, and then quenched immediately with 5 μL 10% TFA solution. Samples were frozen at -78 °C prior to HPLC analysis. Reverse-phase analytical HPLC was performed using a RP-C8 column (Agilent Zorbax Eclipse XDB-C8, 5 μm, 4.6 x 150 mm) connected to an Agilent 1200 series HPLC system with an automatic sampler. Binary gradients of solvents A and B were employed at a flow rate of 1 mL/min, with monitoring at 280 nm. Peptide concentrations were determined by comparison to the internal Trp standard.

We also tested the thioester transfer reaction at 70 °C above the Tm of 6HB, and the reaction was monitored in the same way and the HPLC profile were presented in Figure S4. Separated N36 and C34E(SBn)5H9 solutions were preheated to 70 ℃for 30 minutes, then mixed together and monitored the reaction at 70 ℃C34E(SBn)5H9 was rapidly decomposed at 70 ℃and most degraded after incubate at 70 ℃for 2 hours . However, very small amount of inter chain covalent adduct was observed, suggesting that inter chain thioester transfer reaction occurred at temperature above the 6HB Tm and the reaction rate was higher than that at 37 ℃

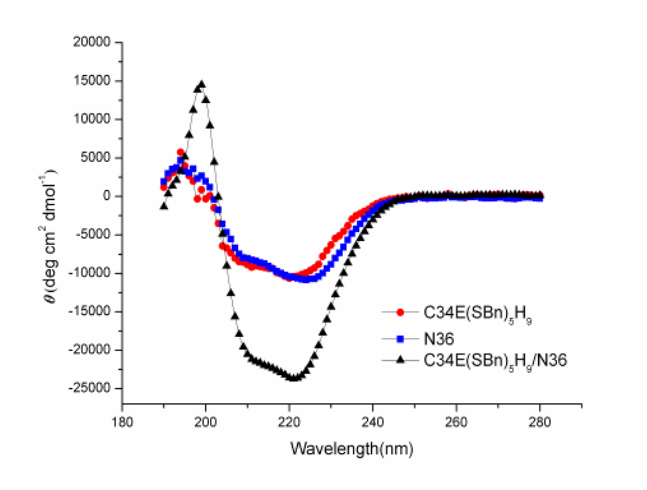

Fig. S1 CD spectroscopy of N36, C34E(SBn)$_5$H$_9$ and their complex (6HB). The final concentration of each peptide in PBS was 10 μM.

Table S1. *α*-helical content and *T*m of C- and N- peptides and 6HBs

	α-helical content (%)	*T*m (°C)
N36	32.4	
N36A$_{29}$	36.1	
NP$_{25\text{-}34}$	4.0	
C34	13.2	
C34E$_5$H$_9$	11.7	
C34E(SBn)$_5$	31.9	
C34 E(SBn)$_5$H$_9$	31.2	
C34/N36	76.4	56
C34 E$_5$H$_9$/N36	89.2	58
C34 E(SBn)$_5$/N36	90.3	57
C34 E(SBn)$_5$H$_9$/N36	71.5	55
C34 E(SBn)$_5$H$_9$/N36A$_{29}$	75.3	55
C34 E(SBn)$_5$H$_9$/NP$_{25\text{-}34}$	10.2	-

The data were all determined after 30min co- incubation of C- and N-peptides

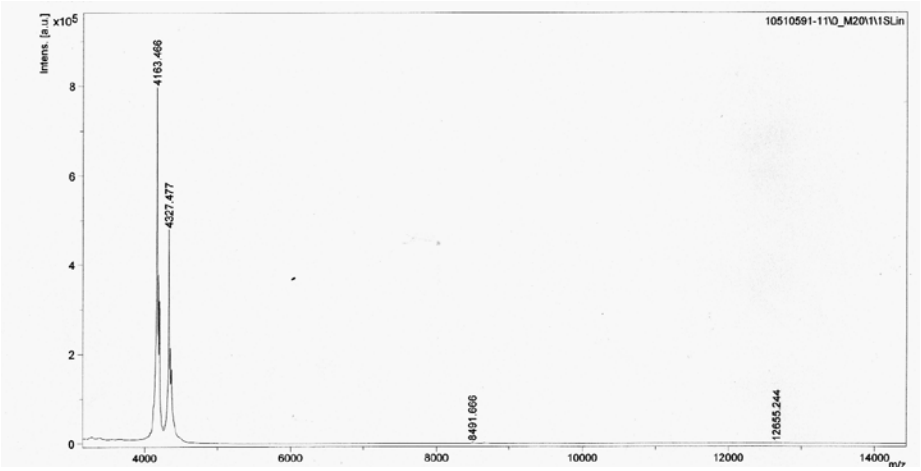

Fig. S2 MALDI-TOF-MS of $C34E_5H_9$/N36 after 48 h co-incubation

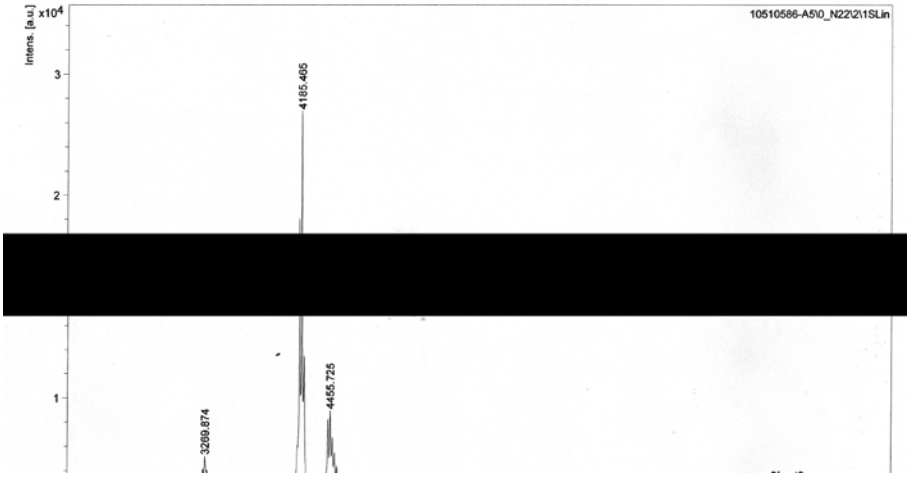

Fig. S3 MALDI-TOF-MS of $C34E(SBn)_5H_9$/N36 with 0.5%SDS after 48 h co-incubation

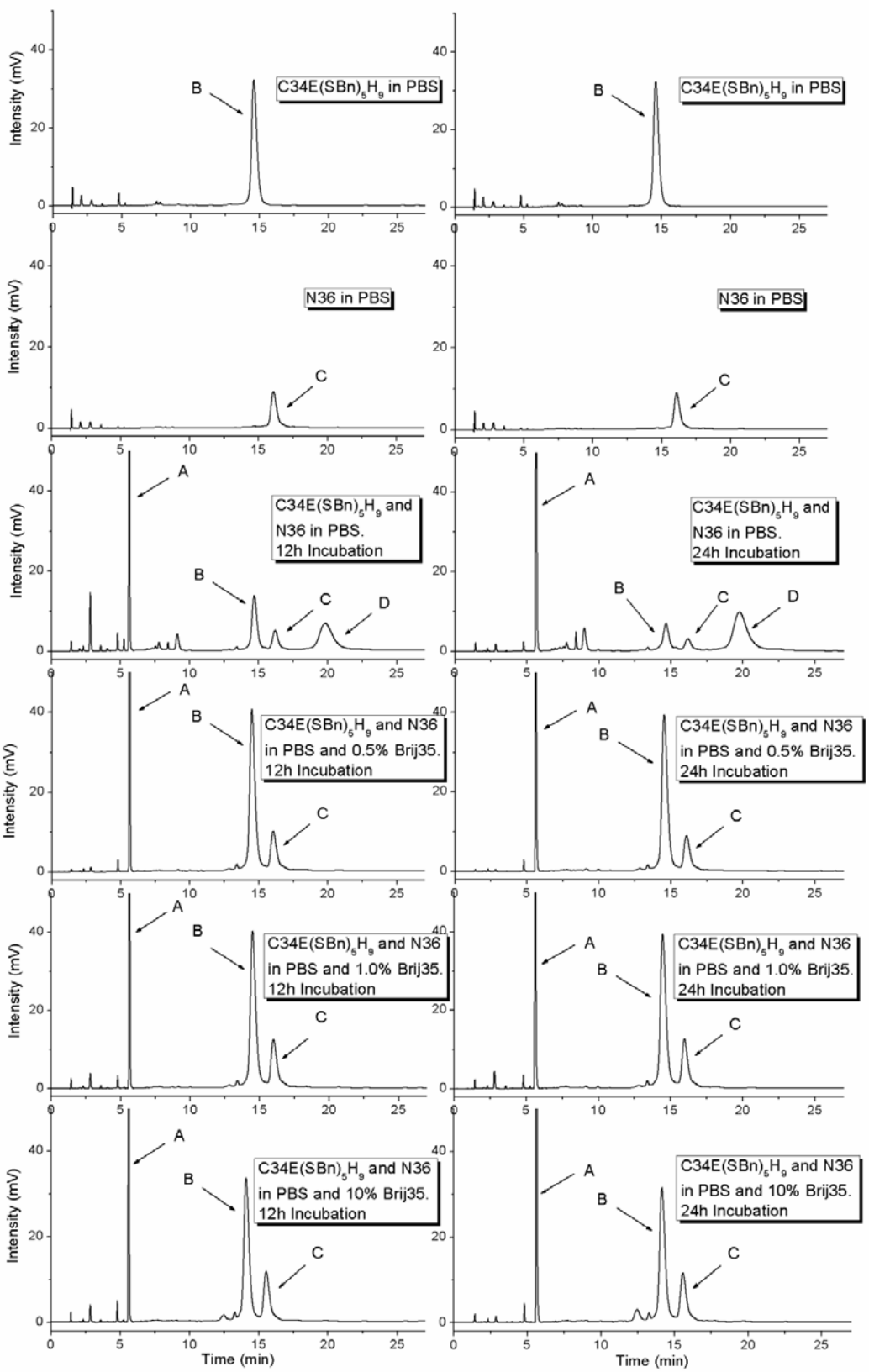

Fig. S4. HPLC analysis of peptides with or without Brij35. Samples were incubated at 37°C in PBS (pH 7.2) or in PBS plus Brij35 at different concentrations. A: Trp, B: $C34E(SBn)_5H_9$, C: N36, D: Acyl transferred adduct. When Brij35 was to PBS, no acyl transfer adduct was formed.

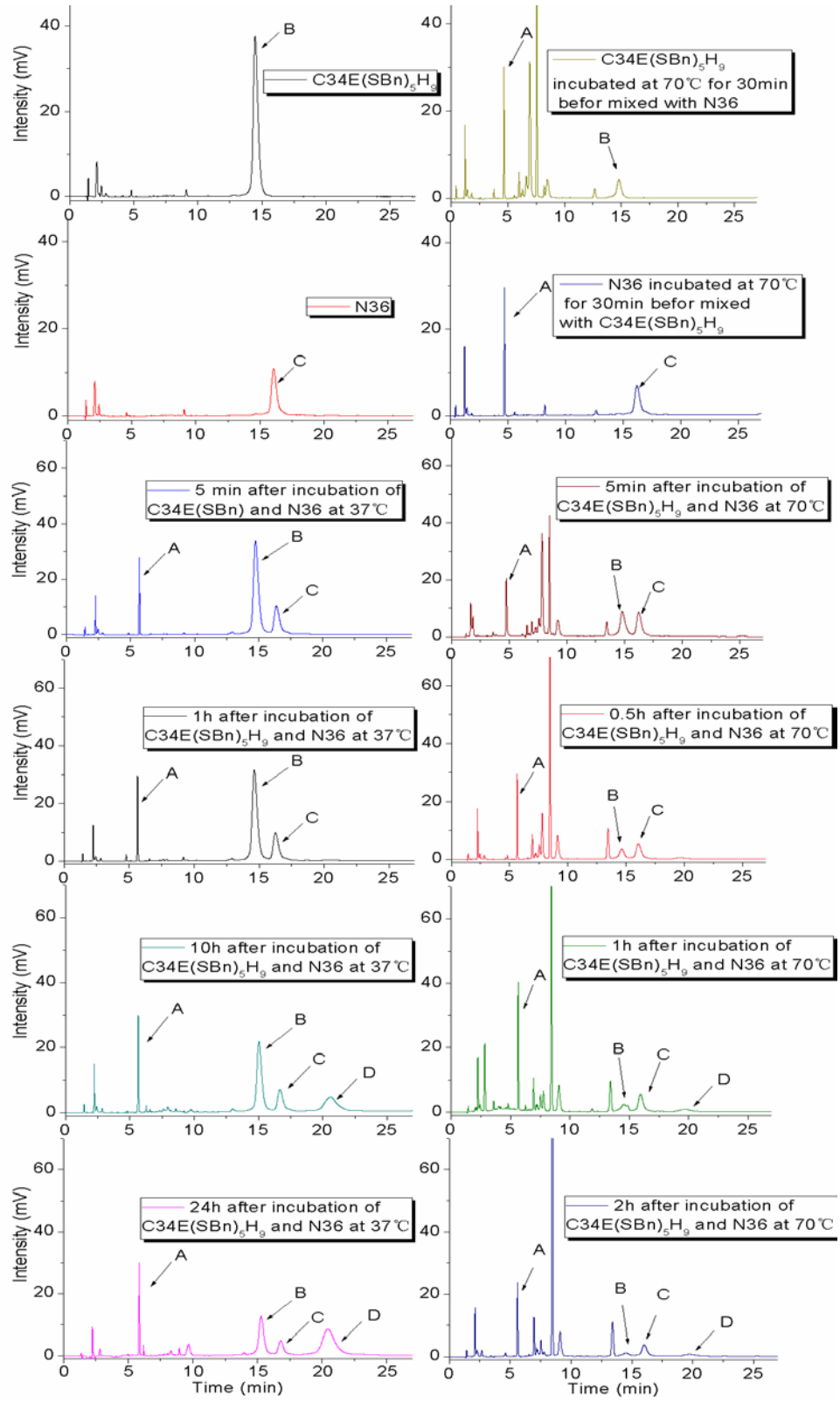

Fig.S5 HPLC analysis of thioeser transfer reaction between N36 and $C34E(SBn)_5H_9$ at 37 ^oC (left) and 70 ^oC (right). Peak A, B, C D were from tryptophan inner reference, $C34E(SBn)_5H_9$, N36 and $C34E(SBn)_5H_9$/N36 covalent adduct, respectively. These experiments were performed by using another RP C8 column.