Supplementary Material for Chemical Communications

Facile synthesis of membrane-embedded peptides utilizing lipid bilayer-assisted chemical ligation

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Table S1 Optimization of native chemical ligation with Ile-Cys coupling site[a]

H-Asn-Val-Ser-Glu-Ala-Asp-Arg-Tyr-Ile-S-$(CH_2)_2$-CO-Ala-OH (CXCR4 (176-185)) —H-Cys-OH→ H-Asn-Val-Ser-Glu-Ala-Asp-Arg-Tyr-Ile-Cys-OH

Entry	Solvent + Additives (w/v %)	Temp (°C)	Thiol (v/v%)	Yields (%)[d] 24 h	54 h
1	Phosphate buffer[b]	37	PhSH (2%)	22	34
2	Phosphate buffer[b] 2% TCEP, 0.1% EDTA	20[c]	PhSH (2%)	50	71
3	Phosphate buffer[b] 2% TCEP, 0.1% EDTA	20[c]	$HSCH_2CH_2SO_3Na$ (2%, w/v)	23	52
4	Phosphate buffer[b] 2% TCEP, 0.1% EDTA	37	PhSH (2%)	62	88

[a] 1.5 equiv. of H-Cys-OH was used for the ligation reaction. [b] 0.2 mol dm^{-3}, pH 7.8, [c] under Ar. [d] Yields were estimated from HPLC peak areas.

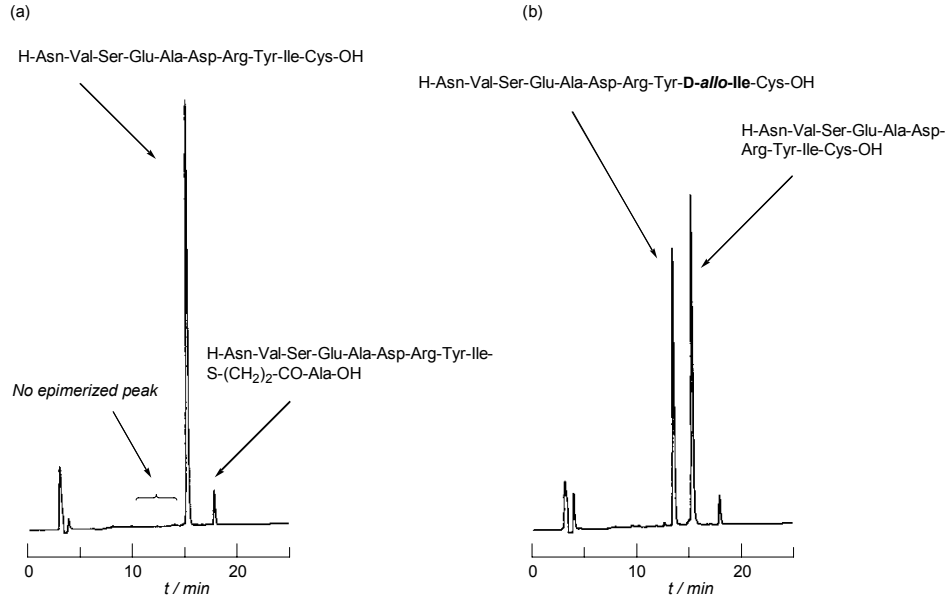

Fig. S1 HPLC analyses of model ligation reaction. (a) Monitor of ligation of the model Ile (2*S*,3*S*) thioester with cysteine under condition listed in Table S1, entry 4 (54 h). (b) Co-injection of crude ligated product and D-*allo*-Ile (2*R*,3*S*)-containing peptide. Column: Cosmosil $5C_{18}$ (4.6 x 250 mm); buffer A: 0.1% aq. TFA; buffer B: MeCN (0.1% TFA); linear gradient 20-35% B in A over 30 min; flow rate 1.0 cm^3 min^{-1}; detect 280 nm.

Comparison of above HPLC analyses (a) and (b) indicated that the coupling reaction proceeded without any accompanying epimerized product.

Representative Experimental Procedure for Chemical Ligation of TMD Peptides Embedded in Lipid Bilayer. A mixture of N-terminal Cys peptide (0.06 μmol), thiolester peptide (0.06 μmol) and palmitoyloleoyl phosphatidylcholine (POPC) (12.0 mmol) was dissolved in hexafluoro-2-propanol:$CHCl_3$ (1:4, 5 cm^3) or TFA (5 cm^3). Removal of solvents under reduced pressure gave the lipid/peptide mixed film on the surface of round-bottomed flask. The resulting mixed film was hydrated with 0.2 mol dm^{-3} phosphate buffer (pH 7.8, 1.2 cm^3) in the presence of tris(carboxyethyl)phosphine (2%, w/v) and EDTA (0.1%, w/v) and vortex-mixed. The mixture was subjected to ultrasonic treatment (1 min) followed by 10 freeze-thaw cycles to produce mutilamellar vesicles (MLVs). To the MLVs suspension (1.2 cm^3) was added thiophenol (24 mm^3) to initiate the ligation reaction, and the reaction was kept at 37 ºC. After 24 h reaction, reaction aliquot (50 mm^3) was treated with TFA (50 mm^3) to dissolve the peptides and lipid. The resulting solution was subjected to reversed phase HPLC analysis on C4 column using linear gradient elution [60-100 % B in A over 30 min; A: H_2O-HCO_2H (3:2); B: 2-propanol-HCO_2H (1:4); flow rate 0.65 cm^3 min^{-1}], and the eluted peaks were analyzed by ion-spray mass.

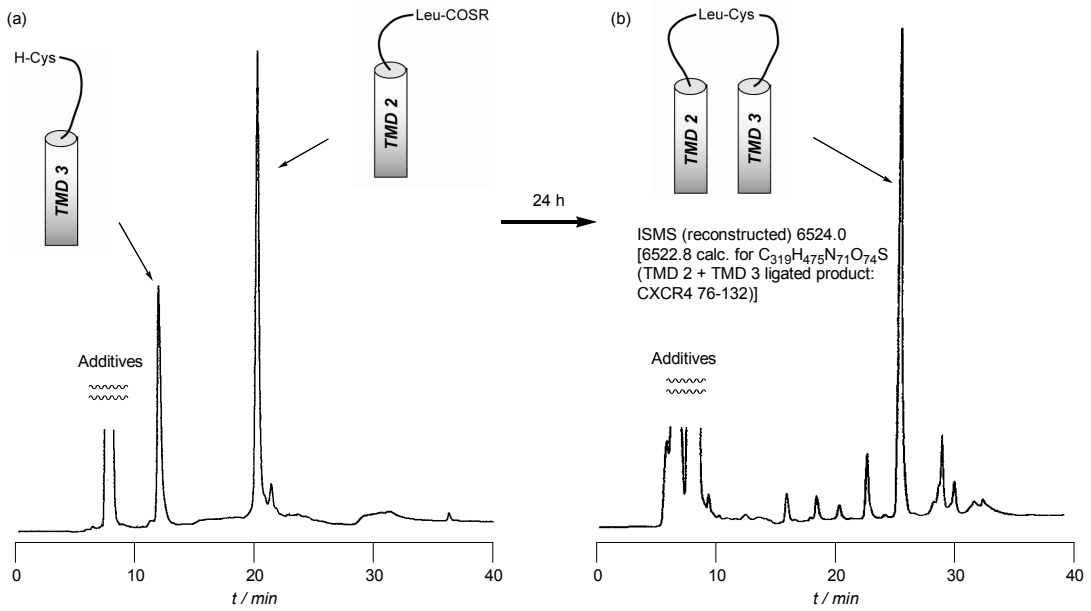

Fig. S2 HPLC analysis of ligation progress in the reaction of TMD 2 with TMD 3 under lipid bilayer assisted condition: (a) t = 0 h (reaction time). (b) t = 24 h. Column: Cosmosil $5C_4$ (4.6 x 150 mm); buffer A: H_2O-HCO_2H (3:2); B: 2-propanol-HCO_2H (1:4); linear garadient 60-100% B in A over 30 min; flow rate 0.65 cm^3min^{-1}; detect 280 nm.

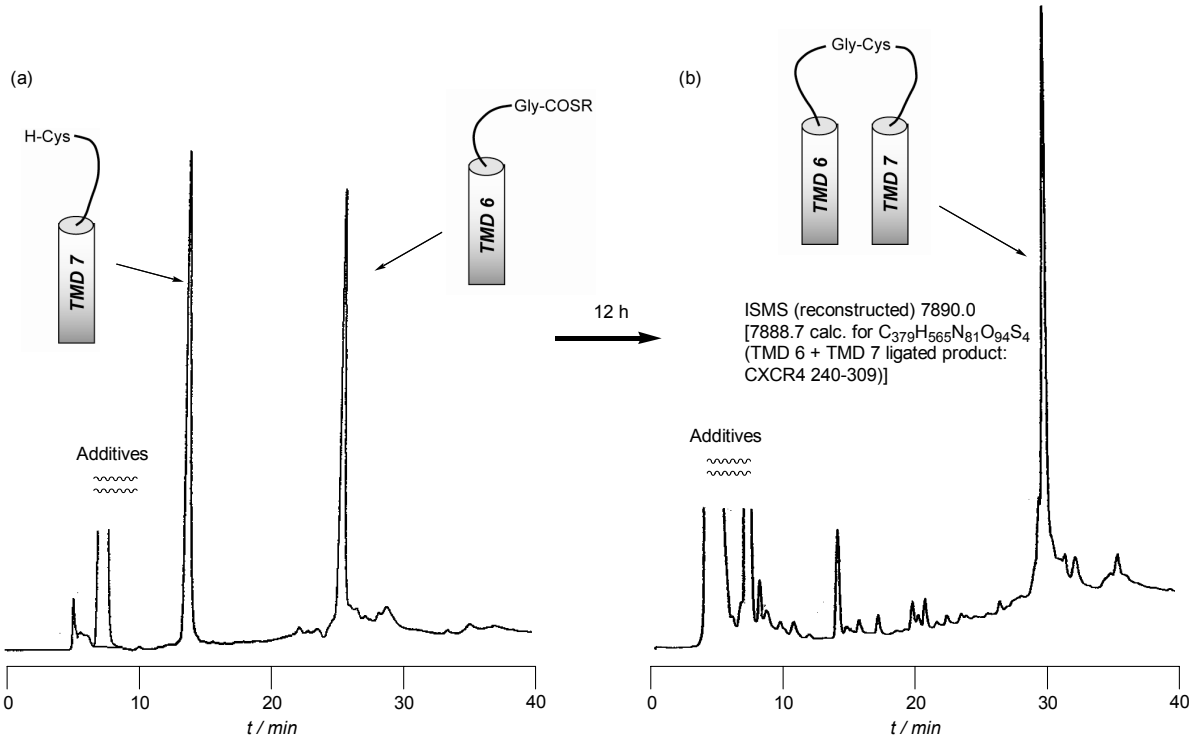

Fig. S3 HPLC analysis of ligation progress in the reaction of TMD 6 with TMD 7 under lipid bilayer assisted condition: (a) t = 0 h (reaction time). (b) t = 12 h. Column: Cosmosil $5C_4$ (4.6 x 150 mm); buffer A: H_2O-HCO_2H (3:2); B: 2-propanol-HCO_2H (1:4); linear garadient 60-100% B in A over 30 min; flow rate 0.65 cm^3min^{-1}; detect 280 nm.