Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supporting Information

A multi-functional peptide as an HIV-1 entry inhibitor based on self-concentration, recognition, and covalent attachment

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Experimental

Synthesis of Fmoc-Asp(OH)-OChol

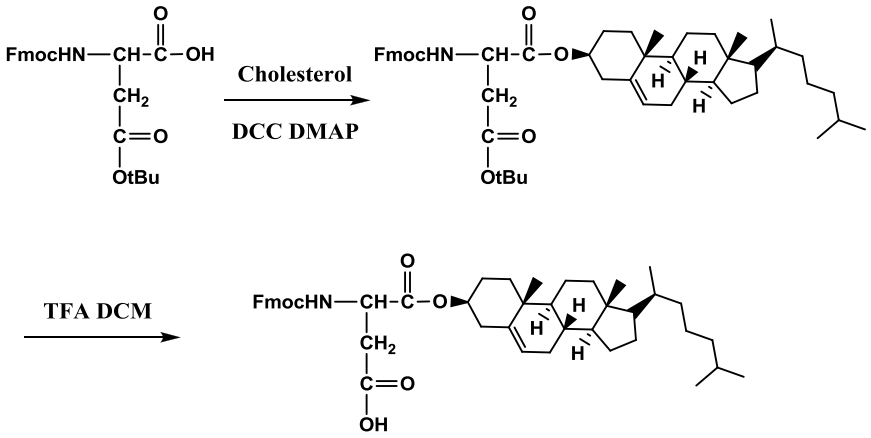
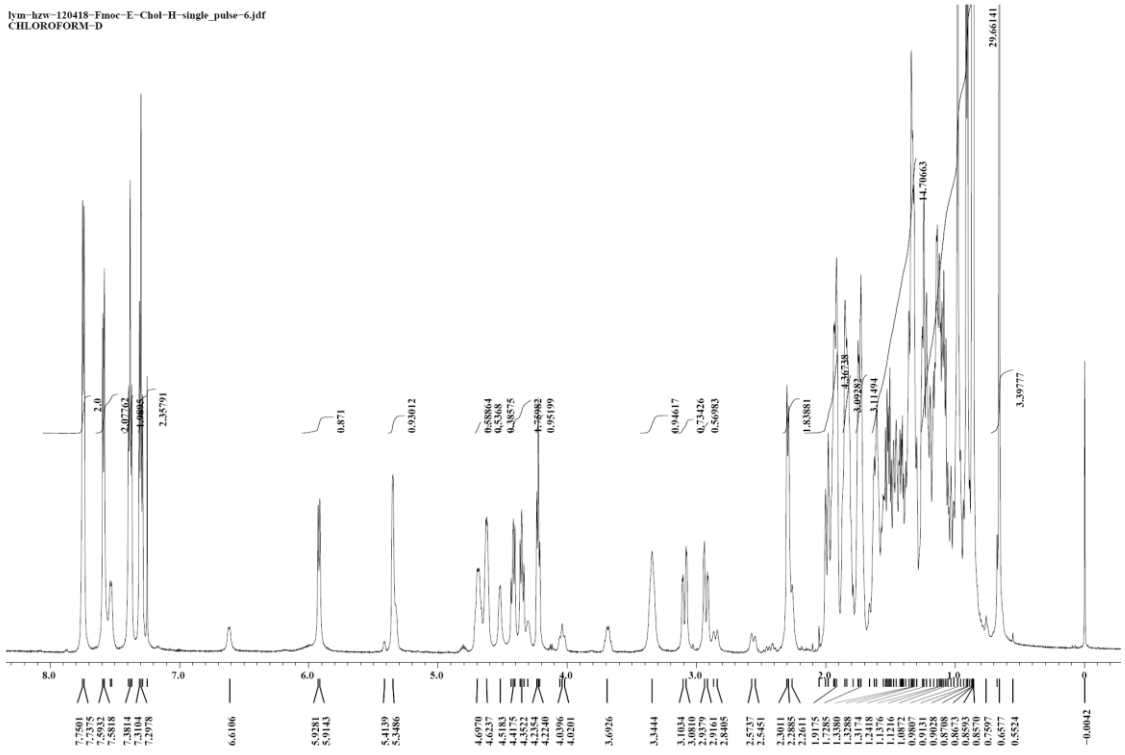


Fig. S1. Protocol for Fmoc-Asp(OH)-OChol building block synthesis

^{1}H NMR (600MHz, $CDCl_3$):



^{13}C NMR (600MHz, $CDCl_3$):

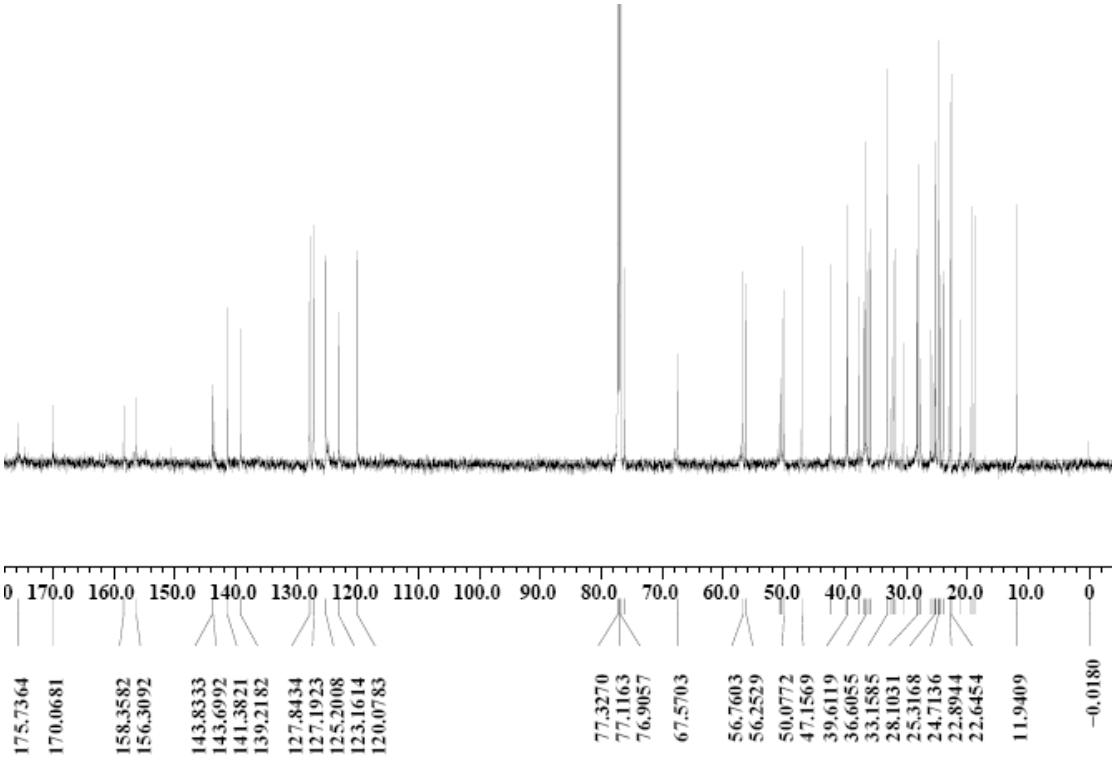


Fig. S2. ^{1}H and ^{13}C NMR spectroscopy of Fmoc-Asp(OH)-Chol measured by 600M NMR in the solvent $CDCl_3$.

^{1}H NMR (600MHz, $CDCl_3$):
δ= 0.66-2.01 (m, H, cholesterol back bone); 2.29 (d, 2H, -OCH$\underline{CH_2}$C=, cholesterol); 2.92-3.10 (q, 2H, -CH-$\underline{CH_2}$-C=O); 3.34 (m, 1H, -COOC$\underline{H}$-, cholesterol); 4.22 (t, 1H, -$\underline{CH}$-CH_2-, Fmoc); 4.35-4.42 (m, 2H, -CH-$\underline{CH_2}$-, Fmoc); 4.62 (m, 1H, -CO$\underline{OH}$); 5.35 (m, 1H, -C=$\underline{CH}$-, cholesterol); 5.93 (d,1H, -$\underline{NH}$-); 7.30-7.75 (m, 8H, Fmoc, Bzl)
Formula: $C_{46}H_{61}NO_6$
Mass calculated: 723.45
Mass observed: 746.7 ($M+Na^+$)
Melting point: 74-76 ^{0}C

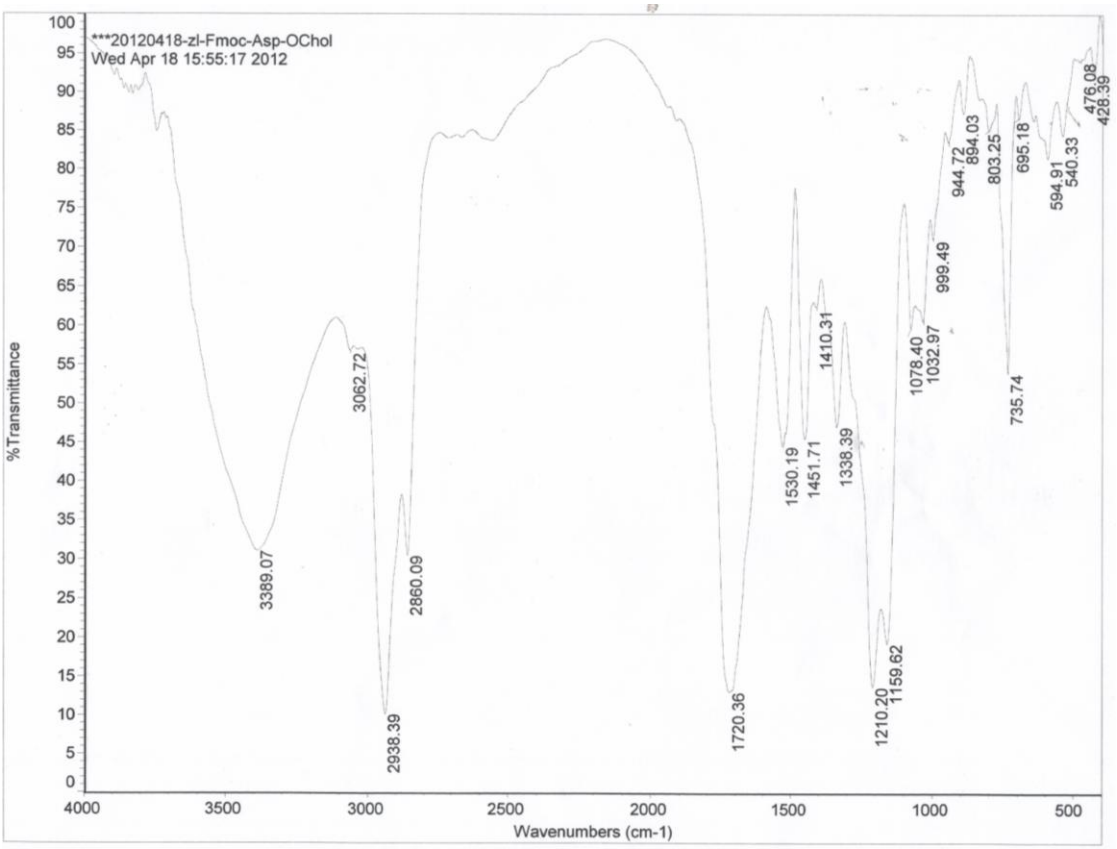


Fig. S3. The IR spectra of Fmoc-Asp(OH)-OChol.

Table S1. Molecular weight and HPLC purity of synthesized peptides

Peptides	Sequence	Mass calculated	Mass observed	HPLC purity [c]
C34	Ac-WMEWDREINNYTSLIHSLIEESQNQQEKNEQELL-$CONH_2$	4287.0125	4287.9243[a]	95.1%
NCS-C34	Ac-WMEWK(NCS)REINNYTSLIHSLIEESQNQQEKNEQELL-$CONH_2$	4342.0423	4342.9238 [a]	93.5%
C34-Chol	Ac-WMEWDREINNYTSLIHSLIEESQNQQEKNEQELL-GSGN-Chol	4970.4801	1244.1011 $(M+4H^+)$[b]	97.4%
NCS-C34-Chol	Ac-WMEWK(NCS)REINNYTSLIHSLIEESQNQQEKNEQELL-GSGN-Chol	5026.4885	1258.3667 $(M+4H^+)$ [b]	93.1%
NCS-C34-Chol K655R	Ac-WMEWK(NCS)REINNYTSLIHSLIEESQNQQERNEQELL-GSGN-Chol	5054.4947	1034.4884 $(M+4H^+ + Na^+)$ [b]	95.0%
N36	Ac-SGIVQQQNNLLRAIEAQQHLLQLTVWGIRQLQARIL-$CONH_2$	4161.3612	4163.3443 [a]	97.7%

[a], mass observed from MALDI-TOF. [b], mass observed from ESI-QTOF. [c], HPLC purities were recorded by C-8 or polar CN column with a flow rate of 0.8 $mL \cdot min^{-1}$.

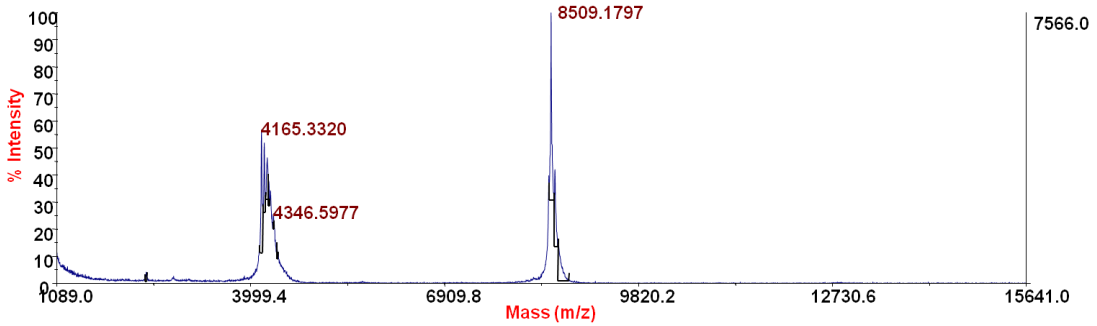


Fig. S4. MALDI-TOF result of N36/NCS-C34 covalent dimmer. The sample was prepared by co-incubation of N36 and NCS-C34 in PBS buffer at 37 ^{0}C for 12 h. After incubation, N36 and NCS-C34 formed a dimmer covalently and completely in HPLC. The peak which has retention time 19 min show the covalent dimmer of N36 and NCS-C34.

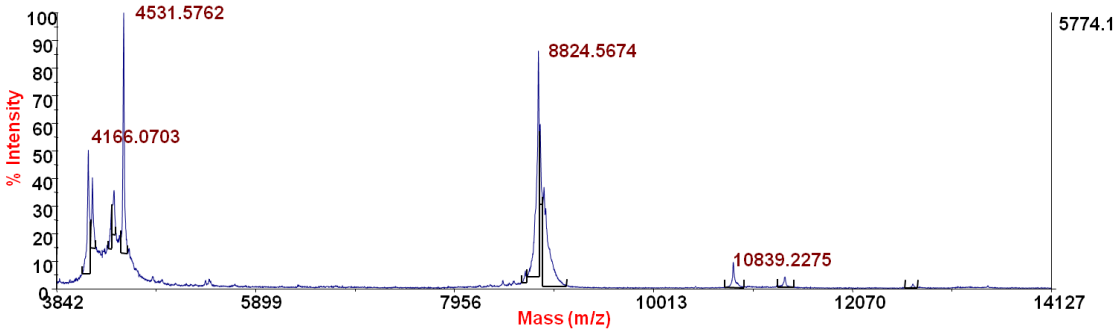


Fig. S5. MALDI-TOF result of N36/NCS-C34-Chol covalent dimmer with cholesterol degradation. The sample was prepared by co-incubation of N36 and NCS-C34-Chol in PBS buffer at 37 ^{0}C for 48 h. After incubation, N36 and NCS-C34-Chol formed a dimmer covalently and completely in HPLC. However, the MALDI-TOF data indicated that cholesterol group has been degraded. The truncated peptide without cholesterol was represented in retention time 23 min in analytical HPLC.

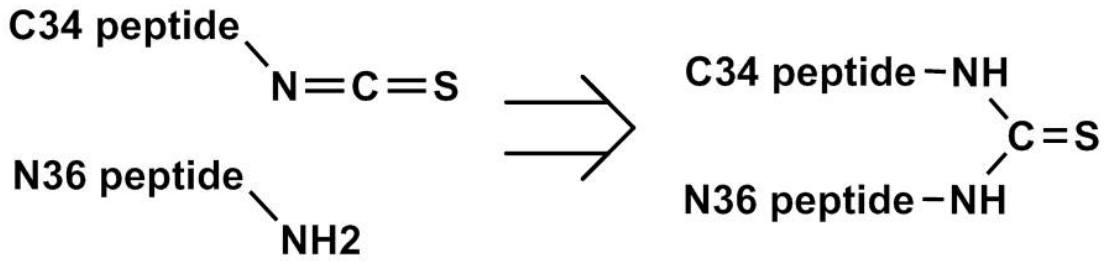


Fig. S6. The chemical process of the covalent reaction.