nature synthesis

Supplementary information

https://doi.org/10.1038/s44160-024-00570-0

O-to-*O* acyl transfer for epimerization-free peptide C-terminal salicylaldehyde ester synthesis

In the format provided by the authors and unedited

Table of Contents

1. General Information

All commercially available amino acids and coupling reagents (purchased from Aldrich and GL Biochem) were used without further purification. All solvents in reagent grade (RCI) or HPLC grade (DUKSAN) were used without purification. Anhydrous dichloromethane (DCM) was freshly distilled from calcium hydride (CaH_2) before use.

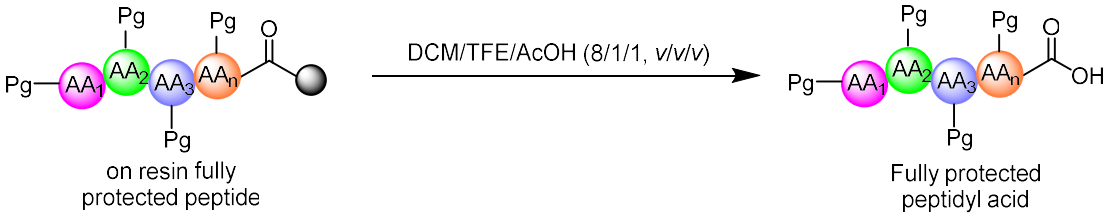
Analytical TLC was performed on E. Merck silica gel 60 F254 plates. Silica flash column chromatography was performed on E. Merck 230-400 mesh silica gel 60. Visualization on TLC was achieved by use of UV light (254 nm). ^{1}H and ^{13}C NMR spectra were recorded on Bruker 400 MHz spectrometer in $CDCl_3$, CD_3CN or DMSO-d_6 with tetramethylsilane (TMS) as internal standard. The chemical shifts are expressed in ppm and coupling constants are given in Hz. Data for ^{1}H NMR are recorded as follows: chemical shift (δ, ppm), multiplicity (s = singlet; d = doublet; t = triplet; m = multiplet), coupling constant (Hz), integration. Data for ^{1}H NMR, ^{13}C NMR are reported in terms of chemical shift (δ, ppm). Waters UPLC H-class system equipped with an ACQUITY UPLC photodiode array detector and a Waters SQ Detector 2 mass spectrometer using a Waters ACQUITY BEH C18 column (1.7 μm, 130 Å, 2.1 × 50 mm) at a flow rate of 0.4 mL/min. Preparative HPLC was performed on a Waters system, using a Vydac 218TPTM C18 column (10 μm, 22 × 250 mm) at a flow rate of 10 mL/min or a Vydac 218TPTM C18 column (10 μm, 30 × 250 mm) at a flow rate of 20 mL/min. Mobile phases of HPLC used are as followed: Solvent A: 0.1% TFA (*v*/*v*) in acetonitrile; Solvent B: 0.1% TFA (*v*/*v*) in water.

2. General Experimental Procedures

2.1 Fmoc-based Solid-phase Peptide Synthesis (SPPS)

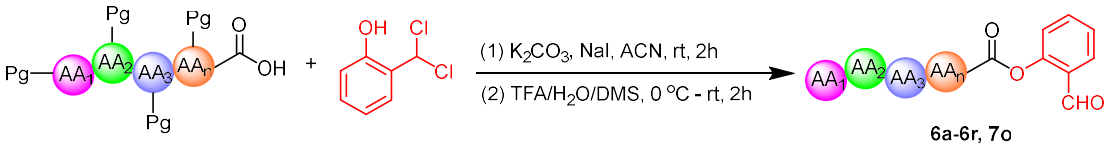
The solid phase peptide synthesis was carried out manually using 2-chloro-trityl resin (GL Biochem, loading capacity: 0.65 mmol/g or 1.1 mmol/g). 2-chloro-trityl chloride resin was swollen in dry CH_2Cl_2 for 30 min and then it washed with CH_2Cl_2 (5 mL × 3). After that, a solution of Fmoc-Xaa-COOH (4.0 equiv. relative to resin loading capacity) and DIEA (8.0 equiv. relative to resin capacity) in CH_2Cl_2 was added and the resin was shaken at room temperature for 2 h to load the first amino acid. Then the resin was washed with DMF (5 mL × 3), CH_2Cl_2 (5 mL × 3) and subsequently treated with a solution of CH_2Cl_2/CH_3OH/DIEA (17:2:1, *v*/*v*/*v*, 5 mL) for 1 h to capping. The resin was washed with DMF (5 mL × 3), CH_2Cl_2 (5 mL × 3), and DMF (5 mL × 3). Finally, it was submitted to iterative peptide assembly (Fmoc-SPPS). The deblock solution was a mixture of 20/80 (*v*/*v*) of piperidine/DMF. The following Fmoc amino acids and Boc amino acids from GL Biochem were employed: *FmocNH*-Ala-*COOH*, *FmocNH*-Cys(Acm)-*COOH*, *FmocNH*-Asp(O*t*Bu)-*COOH*, *FmocNH*-Glu(O*t*Bu)-*COOH*, *FmocNH*-Phe-*COOH*, *FmocNH*-Gly-*COOH*, *FmocNH*-His(Trt)-*COOH*, *FmocNH*-Ile-*COOH*, *FmocNH*-Lys(Boc)-*COOH*, *FmocNH*-Leu-*COOH*, *FmocNH*-Met-*COOH*, *FmocNH*-Asn(Trt)-*COOH*, *FmocNH*-Pro-*COOH*, *FmocNH*-Gln(Trt)-*COOH*, *FmocNH*-Arg(Pbf)-*COOH*, *FmocNH*-Ser(*t*Bu)-*COOH*, *FmocNH*-Thr(*t*Bu)-*COOH*, *FmocNH*-Val-*COOH*, *FmocNH*-Trp(Boc)-*COOH*, *FmocNH*-Tyr(*t*Bu)-*COOH*, *FmocNH*-His(Boc)-*COOH*, *BocNH*-Glu(O*t*Bu)-*COOH*, *BocNH*-Ser(*t*Bu)-*COOH*, *BocNH*-Thr(*t*Bu)-*COOH*, *BocNH*-Gly-*COOH*, *BocNH*-Gln(Trt)-*COOH*, *BocNH*-Phe-*COOH*, *BocNH*-Pro-*COOH* and *BocNH*-Ala-*COOH*. The resin was washed with DMF (5 mL × 3), CH_2Cl_2 (5 mL × 3), and DMF (5 mL × 3). For the coupling step, a solution of Fmoc protected amino acid or Boc protected amino acid (4.0 equiv. according to the resin capacity), HATU (4.0 equiv.) and DIEA (8.0 equiv.) in DMF was gently agitated with the resin at room temperature for 1 h. The resin was washed with DMF (5 mL × 3), CH_2Cl_2 (5 mL × 3), and DMF (5 mL × 3). Mass analysis was performed with a Waters 3100 mass spectrometer equipped with an electrospray ionization source (ESI).

2.2 Cleavage fully protected peptide from 2-chloro-trityl chloride resin



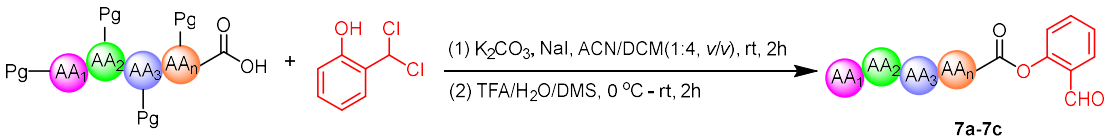
The on-resin fully protected peptide, obtained as described in the Fmoc-SPPS section, was subjected to mild acidic cleavage cocktail (5-10 mL) of CH_2Cl_2/AcOH/trifluoroethanol (8/1/1, *v*/*v*/*v*), 3 times for 60 min each. Following filtration, the resulting cleavage solutions were combined and concentrated to give crude protected peptide bearing the free carboxylic acid at the C-terminus.

2.3 General procedure for synthesis of C-terminus short peptide (5 to 6 amino acids) SAL esters 6a-6r and 7o



A solution of DCP reagent (2.0 equiv.) in ACN was added dropwise slowly (dropwise over about 40 min) to a flask containing the crude fully protected peptidyl acid (1.0 equiv.), K_2CO_3 (6.5 equiv or 10.0 equiv), NaI (24.0 equiv) and ACN (3 to 10 mM concentration) at room temperature under an Ar atmosphere. Then the mixture was stirred at room temperature for 2 h. After that, the mixture was filtered, and the filter cake was washed with ACN and DCM. The filtration was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*) was added. The mixture was then allowed to warm to room temperature and was stirred for a further 2 h. TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged. The precipitate was pelleted, and the ether was subsequently decanted. The resulting solid was purified by HPLC and lyophilization to give the short peptide SAL esters **6a-6r** and **7o** as white solid.

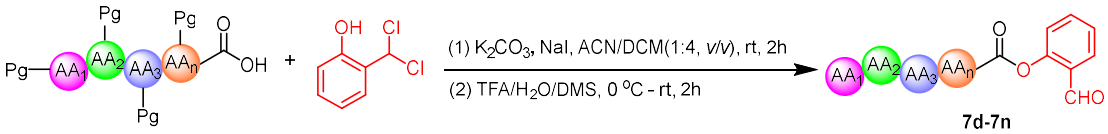
2.4 General procedure for synthesis of C-terminus short peptide (7 to 9 amino acids) SAL esters 7a-7c



7a-7c

A solution of DCP reagent (3.0 equiv.) in ACN was added dropwise slowly (dropwise over about 40 min) to a flask containing the crude fully protected peptidyl acid (1.0 equiv), K_2CO_3 (10.0 equiv), NaI (24.0 equiv) and ACN/DCM (1/4 *v*/*v*, 2 to 4 mM concentration) at room temperature under an Ar atmosphere. Then the mixture was stirred at room temperature for 2 h. After that, the mixture was filtered, and the filter cake was washed with ACN and DCM. The filtration was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*) was added. The mixture was allowed to warm to room temperature and stirred for a further 2 h. TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged. The precipitate was pelleted, and the ether was subsequently decanted. The resulting solid was purified by HPLC and lyophilization to give the short peptide SAL esters **7a-7c** as white solid.

2.5 General procedure for synthesis of C-terminus long peptide (more than 9 amino acids) SAL esters 7d-7n



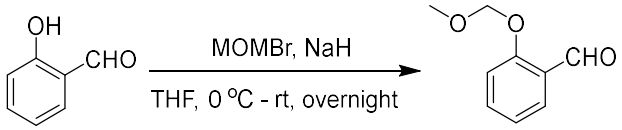
7d-7n

A solution of DCP reagent (5.0 equiv) in ACN was added dropwise slowly (dropwise over 40 min) to a flask containing the crude fully protected peptidyl acid (1.0 equiv), K_2CO_3 (24.0 equiv), NaI (30.0 equiv) and mL ACN/DCM (1:4 *v*/*v*, 1 to 3 mM concentration) at room temperature under an Ar atmosphere. Then the mixture was stirred at room temperature for 2 h. After that, the reaction solvent was poured into the other flask (left solid salt in the flask). The solvent was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*) was added. The mixture was allowed to warm to room temperature and stirred for a further 2 h.

TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged. The precipitate was pelleted, and the ether was subsequently decanted. The resulting solid was purified by HPLC and lyophilization to give the long peptide SAL esters **7d-7n** as white solid.

3. Synthesis of the DCP (2-(dichloromethyl)phenol)

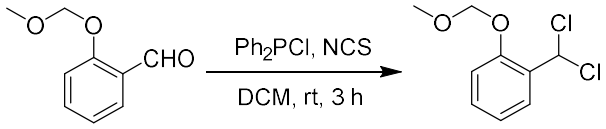
3.1 Synthesis of 1-a



1-a

A solution of salicylaldehyde (12.2 g, 0.1 mol) in dry THF (20 mL) was added slowly to a flask containing NaH (60% in oil, 6.0 g, 0.15 mol) and 240 mL dry THF at 0 °C under an Ar atmosphere. After stirring at room temperature for 1 h, the bromo(methoxy)methane (18.8g, 0.15 mol) in dry THF (20 mL) was added dropwise to the resulting suspension at 0 °C, and stirred overnight at room temperature. The reaction mixture was diluted with ethyl acetate and quenched by saturated NH_4Cl. Then the organic layer was washed with brine, dried over anhydrous $NaSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane /DCM = 2/1 to 1/1) to give the product **1-a** (15.4 g, 92.8% yield) as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.53 (s, 1H), 7.87 (d, J = 8.0 Hz, 1H), 7.56 (t, J = 8.0 Hz, 1H), 7.24 (d, J = 8.0 Hz, 1H), 7.11 (t, J = 8.0 Hz, 1H), 5.32 (s, 2H), 3.55 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.8, 159.7, 135.9, 128.4, 125.5, 121.9, 115.0, 94.6, 56.5. ESI-HRMS calcd. for $C_9H_{10}O_3Na$ $[M+Na]^+$ m/z=189.0522, found 189.0516.

3.2 Synthesis of 1-aa

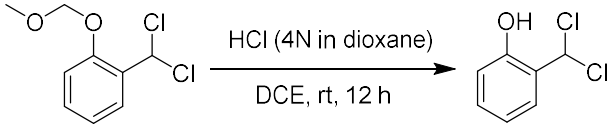


1-a **1-aa**

To a solution of NCS (2.68g, 20.0 mmol) in DCM (60 ml) was added Ph_2PCl (4.42 g, 20.0 mmol). Then a solution of **1-a** (1.66 g, 10.0 mmol) in DCM (5 mL) was added at room temperature. The

mixture was stirred at room temperature for 3 h, then added DCM (200 mL) to dilute the mixture. The mixture was washed with saturated solution of $NaHCO_3$ (200 mL × 2) and brine (200 mL), dried over anhydrous Na_2SO_4, filtered and concentrated in *vacuo* to left some solvent (about 20 mL). The crude product was purified by silica gel column chromatography (First using 0.1% Et_3N (in hexane, 500 mL) to elute the column and elute the column with 500 mL hexane, then added the solution of the crude product (in 20 mL DCM and 50 mL hexane) to the column, finally using hexane/DCM = 2.5/1 to 2/1 elute the column to give the product **1aa** (1.02 g, 46.2% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (d, J = 8.0 Hz, 1H), 7.35 (t, J = 8.0 Hz, 1H), 7.24 (s, 1H), 7.13 (d, J = 8.0 Hz, 1H), 7.12 (t, J = 8.0 Hz, 1H), 5.29 (s, 2H), 3.53 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.2, 131.1, 129.3, 128.1, 122.3, 114.1, 94.5, 66.5, 56.4. EI-HRMS calcd. for $C_9H_{10}Cl_2O_2$ $[M]^+$ m/z=220.0058, found 220.0055.

3.3 Synthesis of DCP (2-(dichloromethyl)phenol)



1-aa DCP (**1**)

To a solution of **1aa** (265.2 mg, 1.2 mmol) in dry DCE (10 mL) was added HCl (4N in dioxane, 1.5 mL, 6.0 mmol), the mixture was stirred at room temperature under an Ar atmosphere for 12 h. The mixture was concentrated under in *vacuo* to left a little solvent (about 1 mL), then added DCM (50 mL) to the flask. The residual HCl in the mixture was blown off with argon for 30 min, then the mixture was washed with water and brine quickly (as the DCP is not stable in the water, so it needs to be washed quickly). The organic layer was dried over anhydrous $NaSO_4$ and concentrated in *vacuo* to left some solvent (about 2 mL), then added dry DCM to the flask containing DCP until the concentration is 25 mmol/L. The solution of DCP in dry DCM will be used to the next step without further purification. Fortunately, the solution of DCP in dry DCM showed good stability at 0 °C for at least 7 days as indicated by NMR. After 7 days, only 2.6% of DCP was hydrolyzed (see Figure S1 and Figure S2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, J = 8.0 Hz, 1H), 7.27 (t, J = 8.0 Hz, 1H), 7.16 (s, 1H), 7.02 (t, J = 8.0 Hz, 1H), 6.78 (d, J = 8.0 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.7, 131.2, 128.1, 126.9, 121.1, 115.9, 67.03. EI-HRMS calcd. for $C_7H_6Cl_2O$ $[M]^+$

m/*z*=175.9796, found 175.9790.

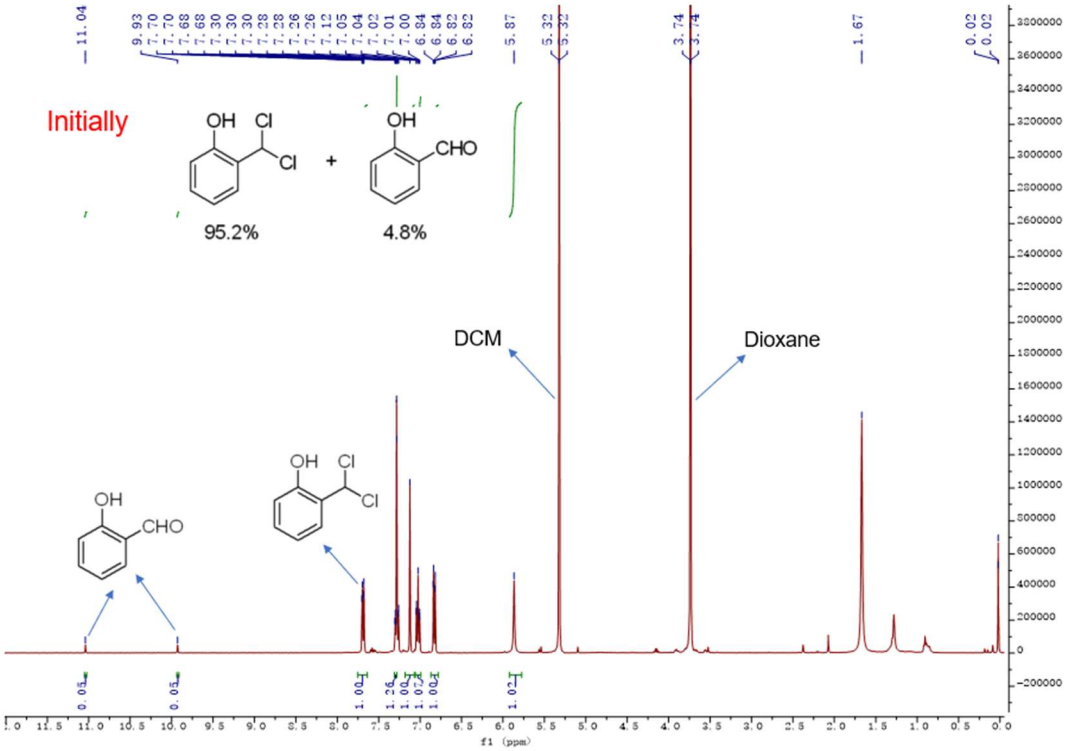


Figure S1. Initial NMR (400 MHz, $CDCl_3$) of the DCP in dry DCM.

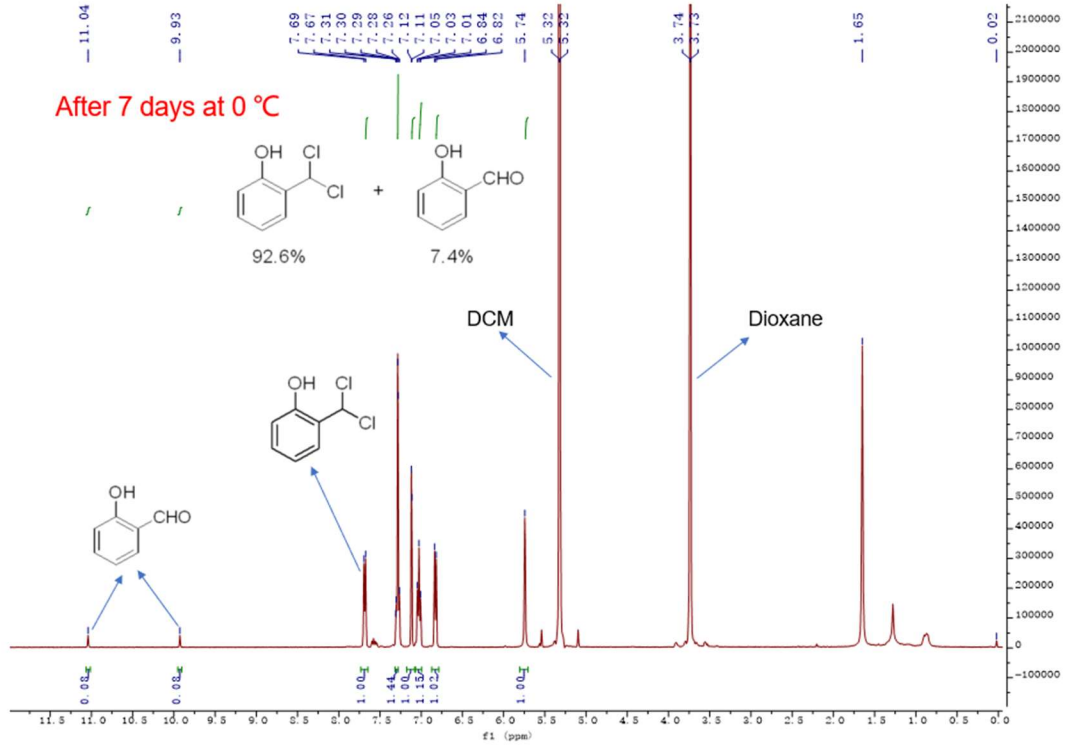
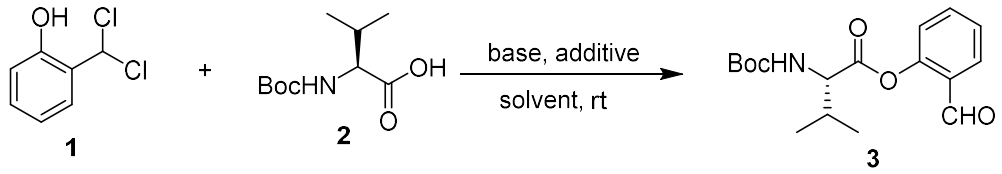


Figure S2. NMR (400 MHz, $CDCl_3$) of the DCP in dry DCM.

4. Synthesis of SAL ester 3

4.1 Table 1. Condition screening of the reaction.[a]

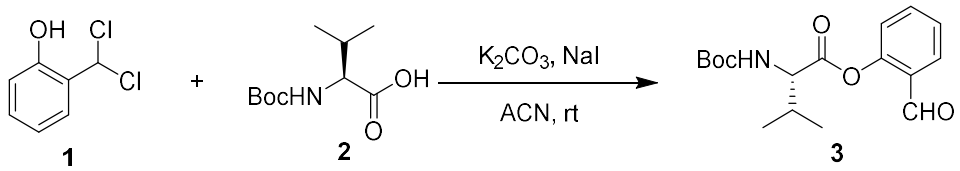


Entry	Base	Additive	Solvent	Time	Yield (%)[b]
1	K_2CO_3	-	CH_2Cl_2	3 h	19
2	K_2CO_3	-	$CHCl_3$	3 h	31
3	K_2CO_3	-	$CHCl_3$	12 h	18
4	K_2CO_3	-	CCl_4	3 h	12
5	K_2CO_3	-	DCE	3 h	trace
6	K_2CO_3	-	Dioxane	3 h	trace
7	K_2CO_3	NaI (2.0 equiv)	$CHCl_3$	3 h	31
8	K_2CO_3	NaI (2.0 equiv)	THF	3 h	trace
9	K_2CO_3	NaI (2.0 equiv)	EA	3 h	18
10	K_2CO_3	NaI (2.0 equiv)	acetone	3 h	39
11	K_2CO_3	-	ACN	3 h	52
12	K_2CO_3	NaI (2.0 equiv)	ACN	3 h	53
13	NaOH	NaI (2.0 equiv)	ACN	3 h	n.d.
14	Et_3N	NaI (2.0 equiv)	ACN	3 h	n.d.
15	K_2CO_3	NaI (8.0 equiv)	ACN	3 h	59
16	K_2CO_3	NaI (16.0equiv)	ACN	3 h	68
17	K_2CO_3	NaI (24.0 equiv)	ACN	3 h	72
18	K_2CO_3	NaI (32.0 equiv)	ACN	3 h	72
19	K_2CO_3	KI (24.0 equiv)	ACN	3 h	53
20	K_2CO_3	*n*-Bu_4NI (24.0 equiv)	ACN	3 h	n.d.
21	K_2CO_3	NH_4I (24.0 equiv)	ACN	3 h	n.d.
22	Na_2CO_3	NaI (24.0 equiv)	ACN	3 h	24
23	Et_3N	NaI (24.0 equiv)	ACN	3 h	n.d.
24	DABCO[c]	NaI (24.0 equiv)	ACN	3 h	n.d.
25	K_2CO_3	NaBArF[d] (24.0 equiv)	ACN	3 h	51
26	K_2CO_3	Cesibor[e] (24.0 equiv)	ACN	3 h	trace

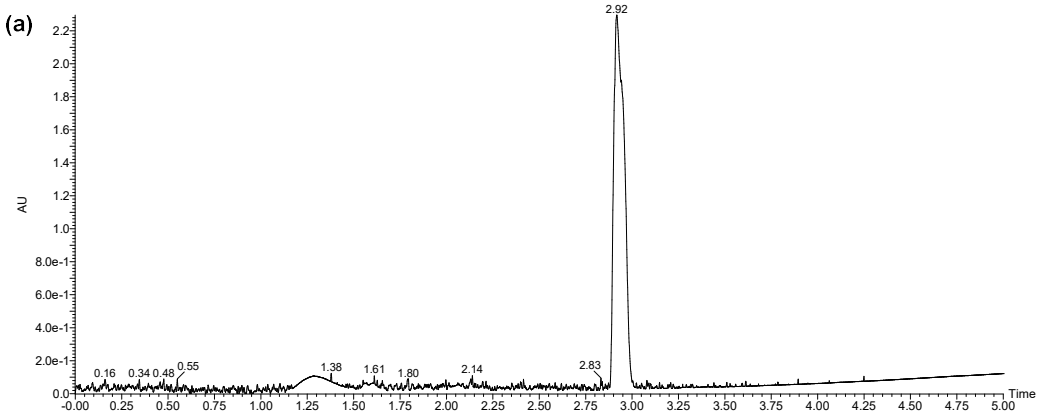
[a] Reaction conditions: **1** (0.2 mmol, 2.0 equiv.), **2** (0.1 mmol, 1.0 equiv.), base (0.65 mmol, 6.5 equiv.) in 5 mL solvent at rt. [b] Isolated yield. [c] DABCO is triethylenediamine. [d] NaBARF is sodium

tetrakis[3,5-bis(trifluoromethyl)phenyl]borate. [e] Cesibor is sodium tetrakis(4-fluorophenyl)borate.

4.2 Synthesis of SAL ester 3



A solution of crude DCP reagent (0.2 mmol) in ACN (0.5 mL) was added dropwise slowly (dropwise over about 40 min, 4 drops after every 2 min) to a flask containing *BocNH*-Val-*COOH* (21.7 mg, 0.1 mmol), K_2CO_3 (89.7 mg 0.65 mmol), NaI (360.0 mg, 2.40 mmol) and ACN (5 mL) at room temperature under an Ar atmosphere. After stirring at room temperature for 2 h, the mixture was filtered, and the filter cake was washed with ACN (10 mL) and DCM (10 mL). The filtration was concentrated and purified by silica gel column chromatography to afford **3** (23.2 mg, 72.2%) as white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.20 (s, 1H), 7.94 (d, *J* = 8.0 Hz, 1H), 7.66 (t, *J* = 8.0 Hz, 1H), 7.42 (t, *J* = 8.0 Hz, 1H), 7.22 (d, *J* = 8.0 Hz, 1H), 5.12 (d, *J* = 8.0 Hz, 1H), 4.54-4.51 (m, 1H), 2.44-2.37 (m, 1H), 1.49 (s, 9H), 1.12 (d, *J* = 8.0 Hz, 3H), 1.08 (d, *J* = 8.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.5, 171.1, 155.8, 151.9, 135.3, 129.9, 128.2, 126.7, 123.2, 80.3, 59.1, 30.8, 28.3, 19.4, 17.7.



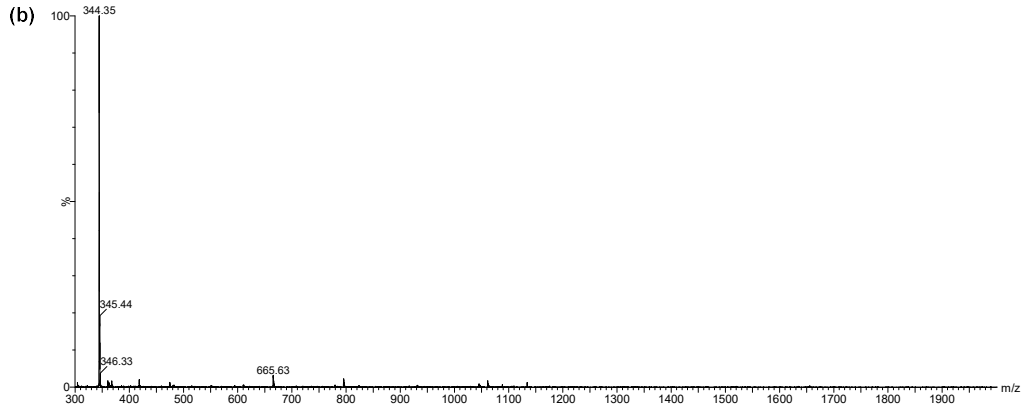
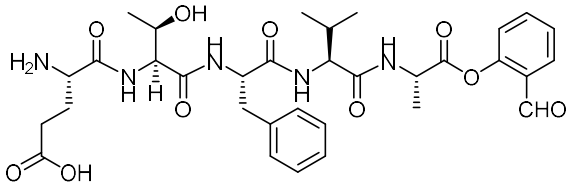


Figure S3. (a) UV trace from analytical RP-UPLC of **3**. Gradient: 40-95% ACN/H_2O containing 0.1% TFA over 5 min at a flow rate of 0.4 mL/min. (c) ESI-MS of **3**. ESI-MS calcd. for $C_{17}H_{23}NO_5Na$ $[M+Na]^+$ m/z=344.36, found 344.35.

5. Synthesis of C-terminus peptide SAL esters

5.1 *H_2N*-ETFVA-*CO*-SAL ester (6a)



A solution of crude DCP reagent (0.1 mmol) in ACN (0.5 mL) was added dropwise slowly (dropwise over about 40 min, 4 drops after every 2 min) to a flask containing the crude fully protected peptidyl acid *BocHN*-Glu(O*t*Bu)-Thr(*t*Bu)-Phe-Val-Ala-*COOH* (prepared according to general experimental procedure **2.1** and **2.2**) (38.9 mg, 0.05 mmol), K_2CO_3 (45.0 mg 0.33 mmol), NaI (180.0 mg, 1.20 mmol) and ACN (5 mL) at room temperature under an Ar atmosphere. After stirring at room temperature for 2 h, the mixture was filtered, and the filter cake was washed with ACN (10 mL) and DCM (10 mL). The filtration was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*, 5 mL) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged. The precipitate was pelleted, and the ether was subsequently decanted. The resulting solid was purified by HPLC and lyophilization to give the peptide SAL ester **6a** (24.7 mg, 73.7%) as white solid.

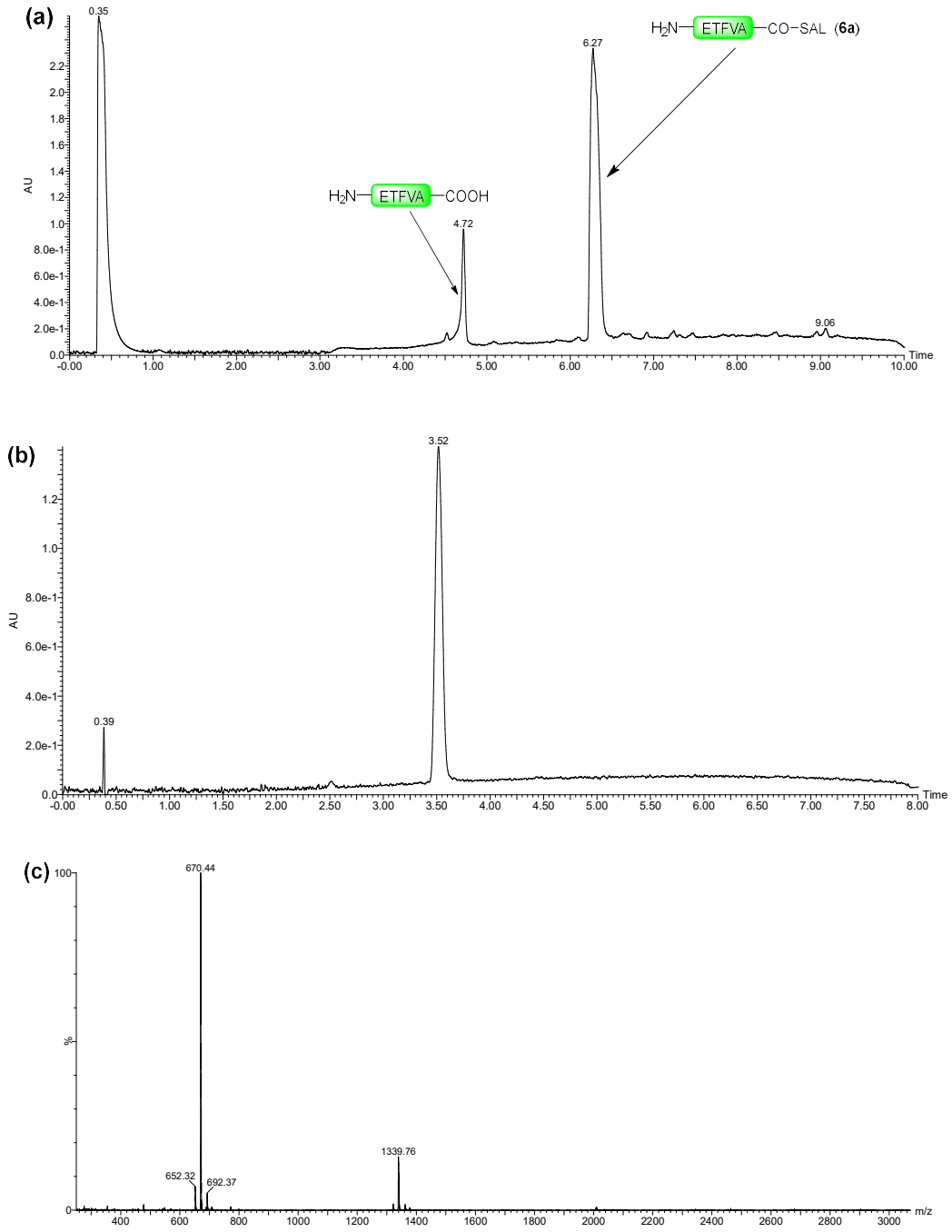
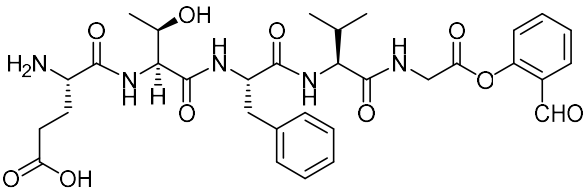
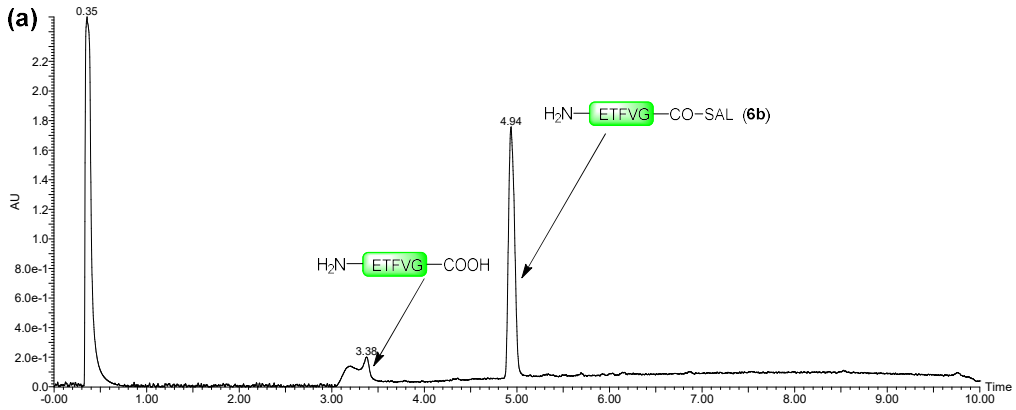


Figure S4. (a) UV trace from analytical RP-UPLC of crude reaction mixture. Gradient: 10-60% ACN/H_2O containing 0.1% TFA over 10 min at a flow rate of 0.4 mL/min. (b) UV trace from analytical RP-UPLC of **6a**. Gradient: 20-60% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. (c) ESI-MS of **6a**. ESI-MS calcd. for $C_{33}H_{44}N_5O_{10}$ $[M+H]^+$ $m/z = 670.74$, found 670.44.

5.2 *H_2N*-ETFVG-*CO*-SAL ester (6b)



A solution of crude DCP reagent (0.1 mmol) in ACN (0.5 mL) was added dropwise slowly (dropwise over about 40 min, 4 drops after every 2 min) to a flask containing the crude fully protected peptidyl acid *BocHN*-Glu(O*t*Bu)-Thr(*t*Bu)-Phe-Val-Gly-*COOH* (prepared according to general experimental procedure **2.1** and **2.2**) (38.2 mg, 0.05 mmol), K_2CO_3 (45.0 mg 0.33 mmol), NaI (180.0 mg, 1.20 mmol) and ACN (5 mL) at room temperature under an Ar atmosphere. After stirring at room temperature for 2 h, the mixture was filtered, and the filter cake was washed with ACN (10 mL) and DCM (10 mL). The filtration was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*, 5 mL) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged. The precipitate was pelleted, and the ether was subsequently decanted. The resulting solid was purified by HPLC and lyophilization to give the peptide SAL ester **6b** (20.8 mg, 63.5%) as white solid.



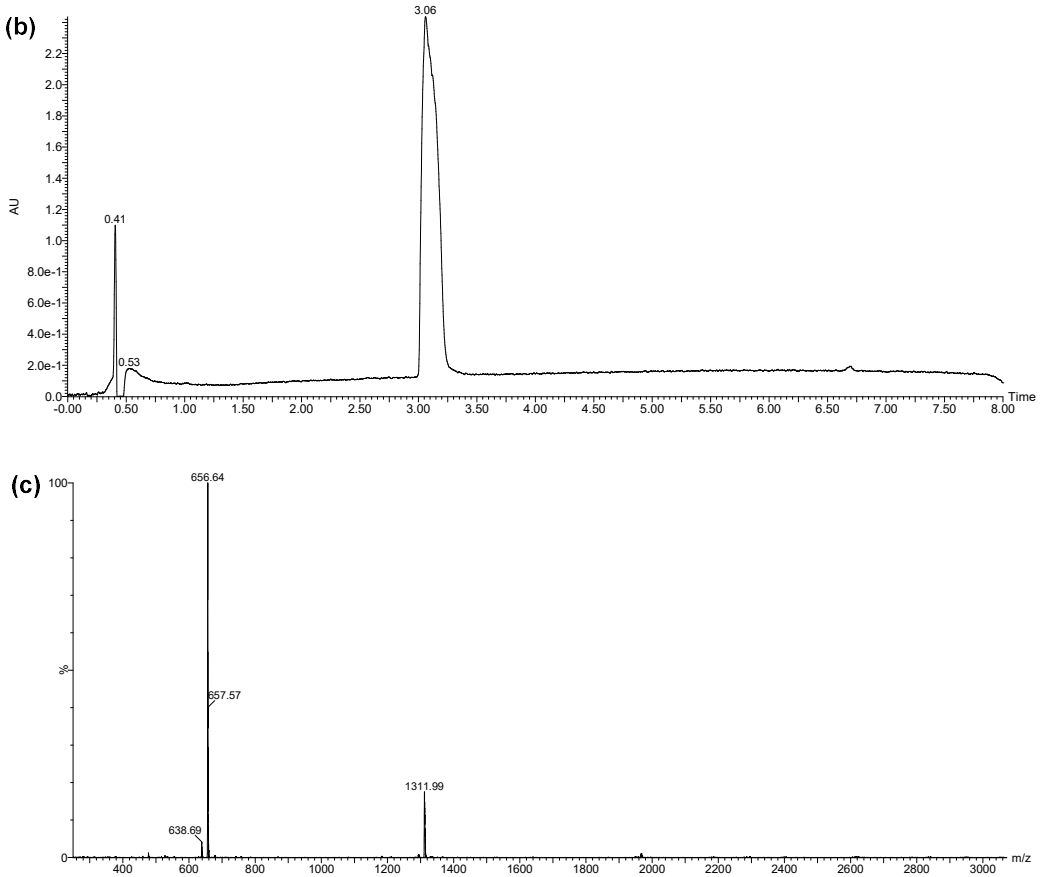
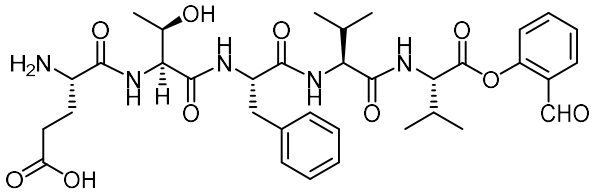


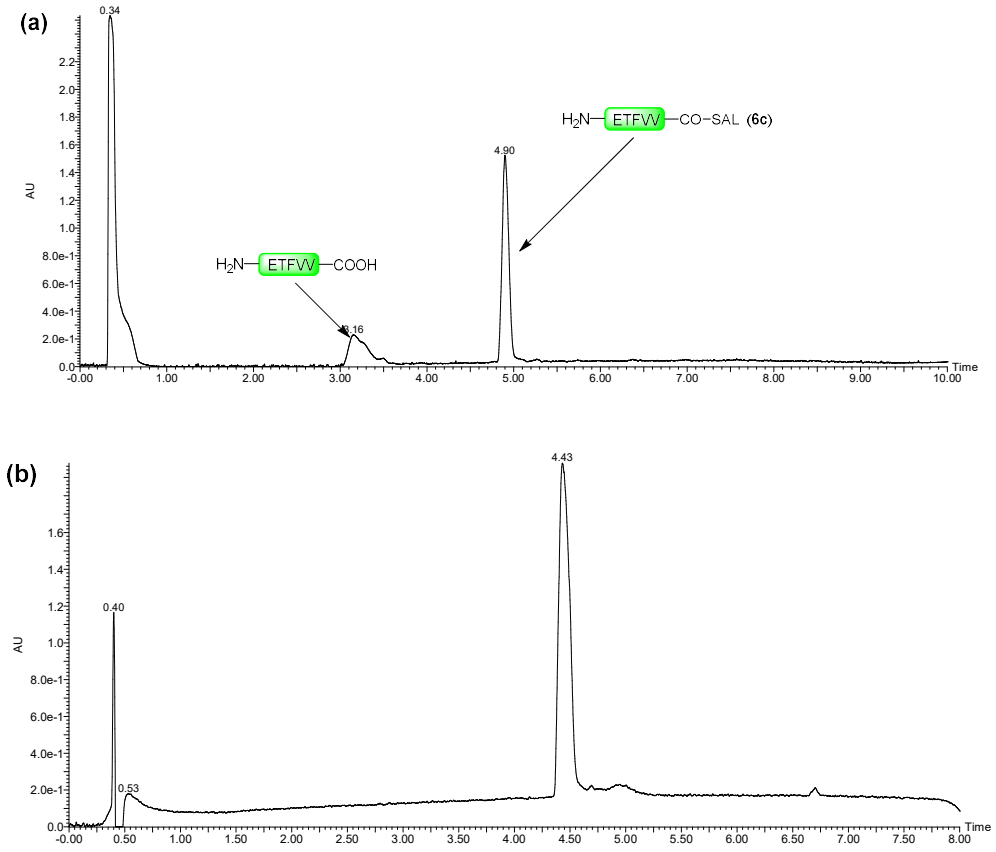
Figure S5. (a) UV trace from analytical RP-UPLC of crude reaction mixture. Gradient: 20-60% ACN/H_2O containing 0.1% TFA over 10 min at a flow rate of 0.4 mL/min. (b) UV trace from analytical RP-UPLC of **6b**. Gradient: 25-60% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. (c) ESI-MS of **6b**. ESI-MS calcd. for $C_{32}H_{42}N_5O_{10}$ $[M+H]^+$ $m/z = 656.71$, found 656.64.

5.3 *H_2N*-ETFVV-*CO*-SAL ester (6c)



A solution of crude DCP reagent (0.1 mmol) in ACN (0.5 mL) was added dropwise slowly (dropwise over about 40 min, 4 drops after every 2 min) to a flask containing the crude fully protected peptidyl acid *BocHN*-Glu(O*t*Bu)-Thr(*t*Bu)-Phe-Val-Val-*COOH* (prepared according to

general experimental procedure **2.1** and **2.2**) (40.3 mg, 0.05 mmol), K_2CO_3 (45.0 mg 0.33 mmol), NaI (180.0 mg, 1.20 mmol) and ACN (5 mL) at room temperature under an Ar atmosphere. After stirring at room temperature for 2 h, the mixture was filtered, and the filter cake was washed with ACN (10 mL) and DCM (10 mL). The filtration was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*, 5 mL) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged. The precipitate was pelleted, and the ether was subsequently decanted. The resulting solid was purified by HPLC and lyophilization to give the peptide SAL ester **6c** (17.7 mg, 50.7%) as white solid.



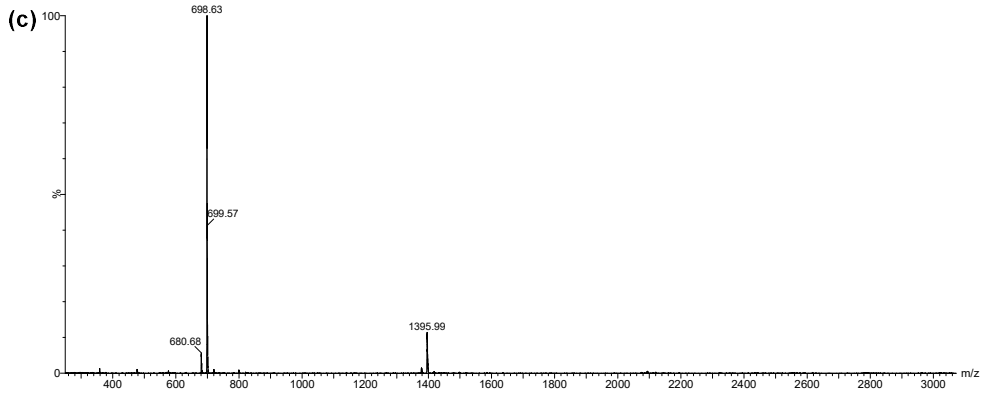
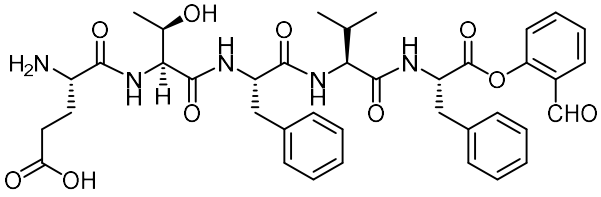


Figure S6. (a) UV trace from analytical RP-UPLC of crude reaction mixture. Gradient: 30-60% ACN/H_2O containing 0.1% TFA over 10 min at a flow rate of 0.4 mL/min. (b) UV trace from analytical RP-UPLC of **6c**. Gradient: 20-60% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. (c) ESI-MS of **6c.** ESI-MS calcd. for $C_{35}H_{48}N_5O_{10}$ $[M+H]^+$ m/z = 698.79, found 698.63.

5.4 *H_2N*-ETFVF-*CO*-SAL ester (6d)



A solution of crude DCP reagent (0.1 mmol) in ACN (0.5 mL) was added dropwise slowly (dropwise over about 40 min, 4 drops after every 2 min) to a flask containing the crude fully protected peptidyl acid *BocHN*-Glu(O*t*Bu)-Thr(*t*Bu)-Phe-Val-Phe-*COOH* (prepared according to general experimental procedure **2.1** and **2.2**) (42.7 mg, 0.05 mmol), K_2CO_3 (45.0 mg 0.33 mmol), NaI (180.0 mg, 1.20 mmol) and ACN (5 mL) at room temperature under an Ar atmosphere. After stirring at room temperature for 2 h, the mixture was filtered, and the filter cake was washed with ACN (10 mL) and DCM (10 mL). The filtration was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*, 5 mL) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged. The precipitate was pelleted, and the ether was subsequently decanted. The resulting solid was purified by HPLC and lyophilization to give the peptide SAL ester **6d** (28.7 mg, 77.0%)

as white solid.

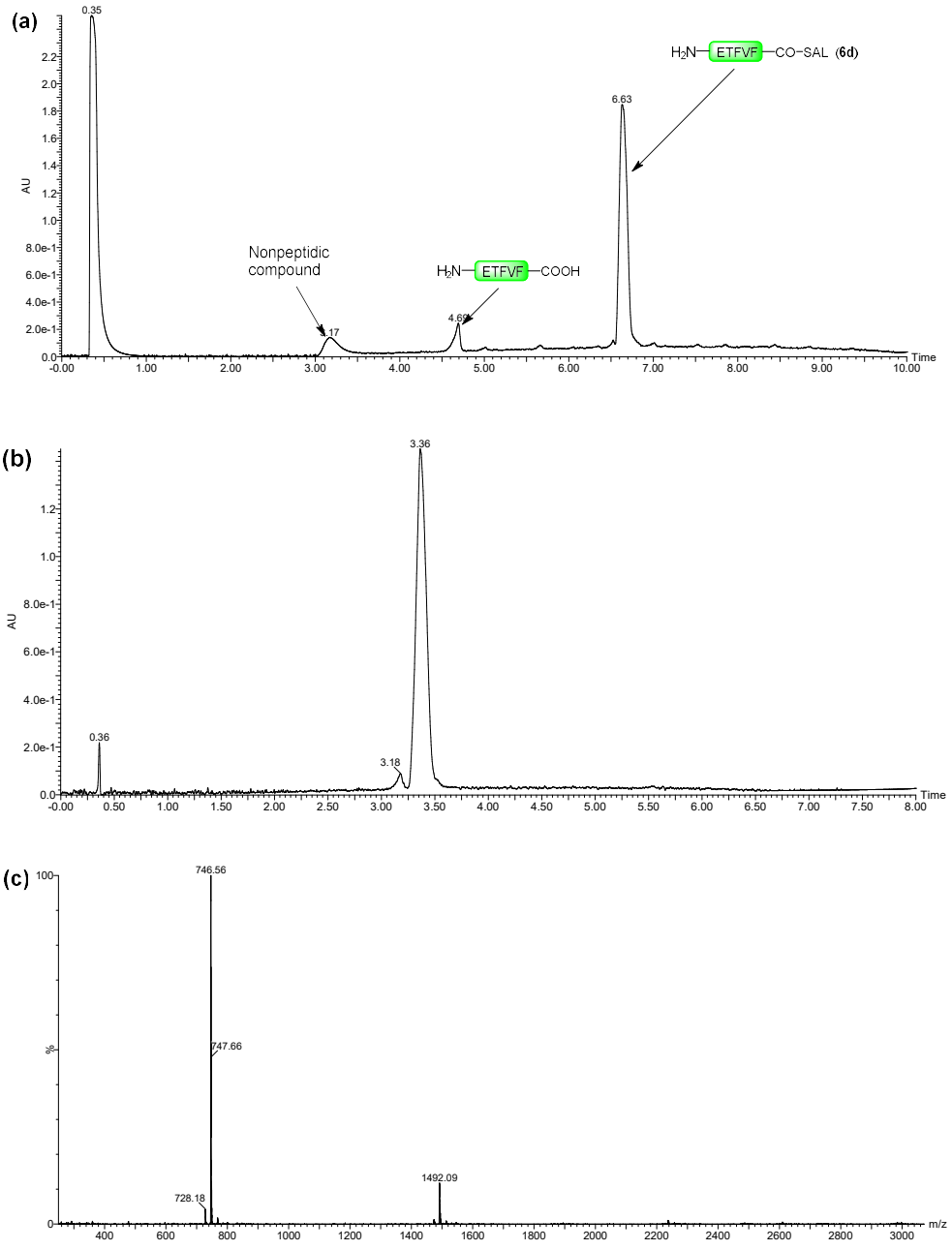
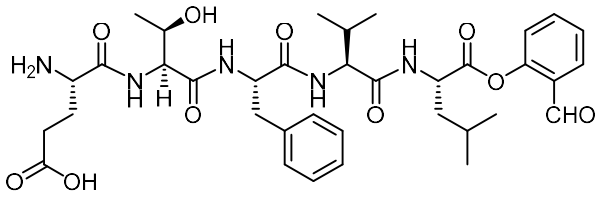
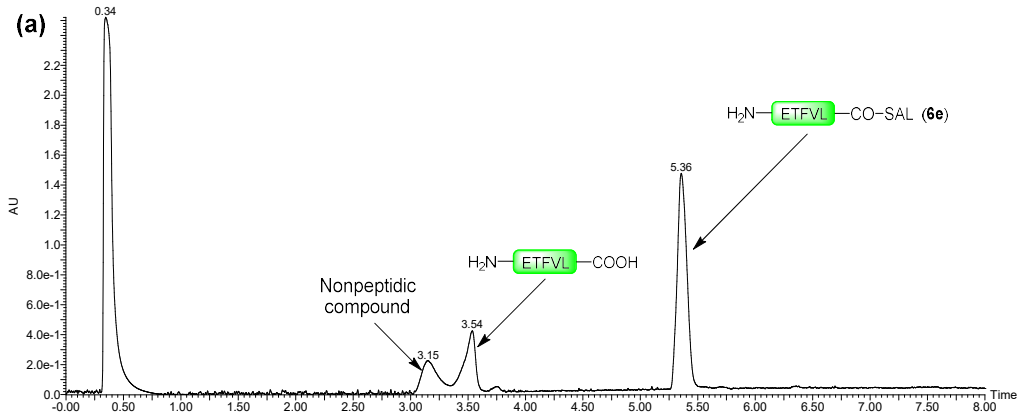


Figure S7. (a) UV trace from analytical RP-UPLC of crude reaction mixture. Gradient: 20-60% ACN/H_2O containing 0.1% TFA over 10 min at a flow rate of 0.4 mL/min. (b) UV trace from analytical RP-UPLC of **6d**. Gradient: 30-60% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. (c) ESI-MS of **6d**. ESI-MS calcd. for $C_{39}H_{48}N_5O_{10}$ $[M+H]^+$ m/z = 746.84, found 746.56.

5.5 *H₂N*-ETFVL-*CO*-SAL ester (6e)



A solution of crude DCP reagent (0.1 mmol) in ACN (0.5 mL) was added dropwise slowly (dropwise over about 40 min, 4 drops after every 2 min) to a flask containing the crude fully protected peptidyl acid *BocHN*-Glu(O*t*Bu)-Thr(*t*Bu)-Phe-Val-Leu-*COOH* (prepared according to general experimental procedure **2.1** and **2.2**) (41.0 mg, 0.05 mmol), K_2CO_3 (45.0 mg 0.33 mmol), NaI (180.0 mg, 1.20 mmol) and ACN (5 mL) at room temperature under an Ar atmosphere. After stirring at room temperature for 2 h, the mixture was filtered, and the filter cake was washed with ACN (10 mL) and DCM (10 mL). The filtration was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*, 5 mL) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged. The precipitate was pelleted, and the ether was subsequently decanted. The resulting solid was purified by HPLC and lyophilization to give the peptide SAL ester **6e** (21.8 mg, 61.2%) as white solid.



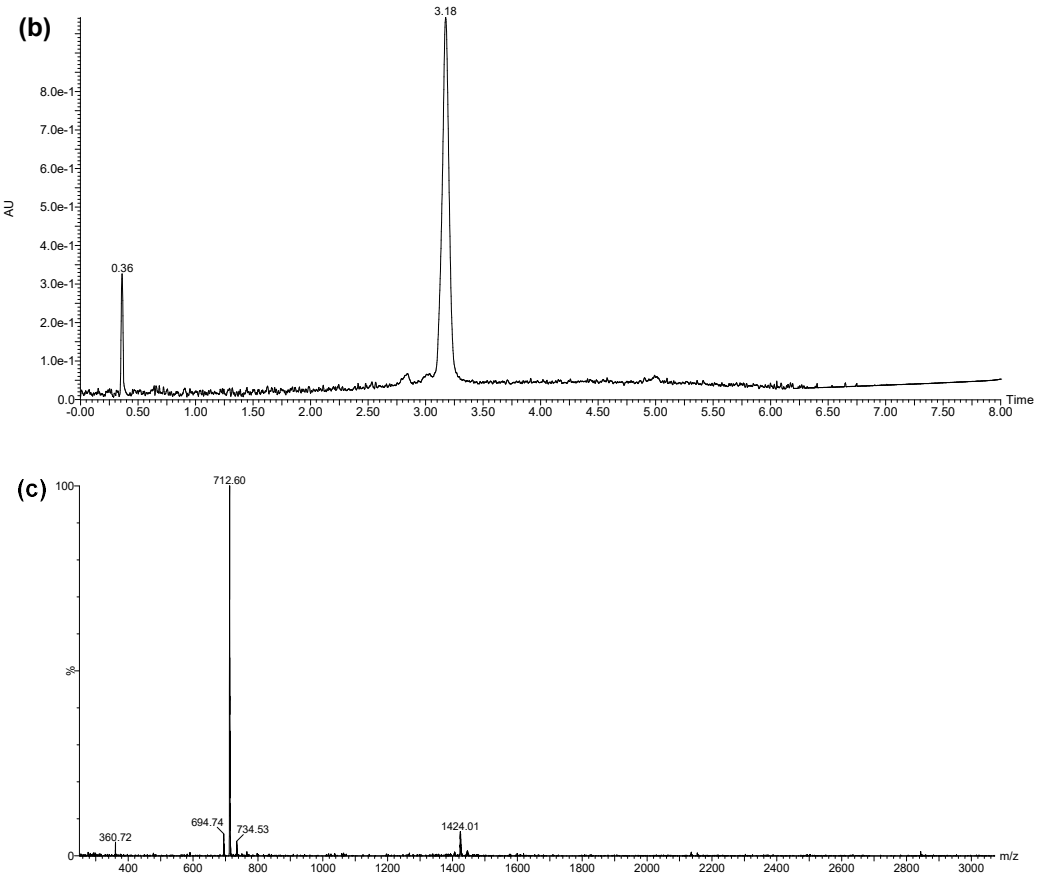
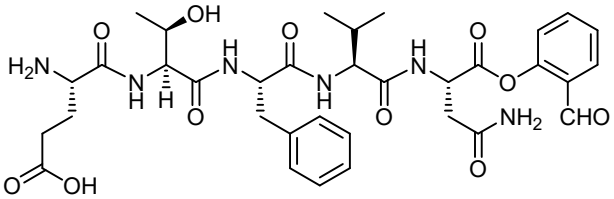


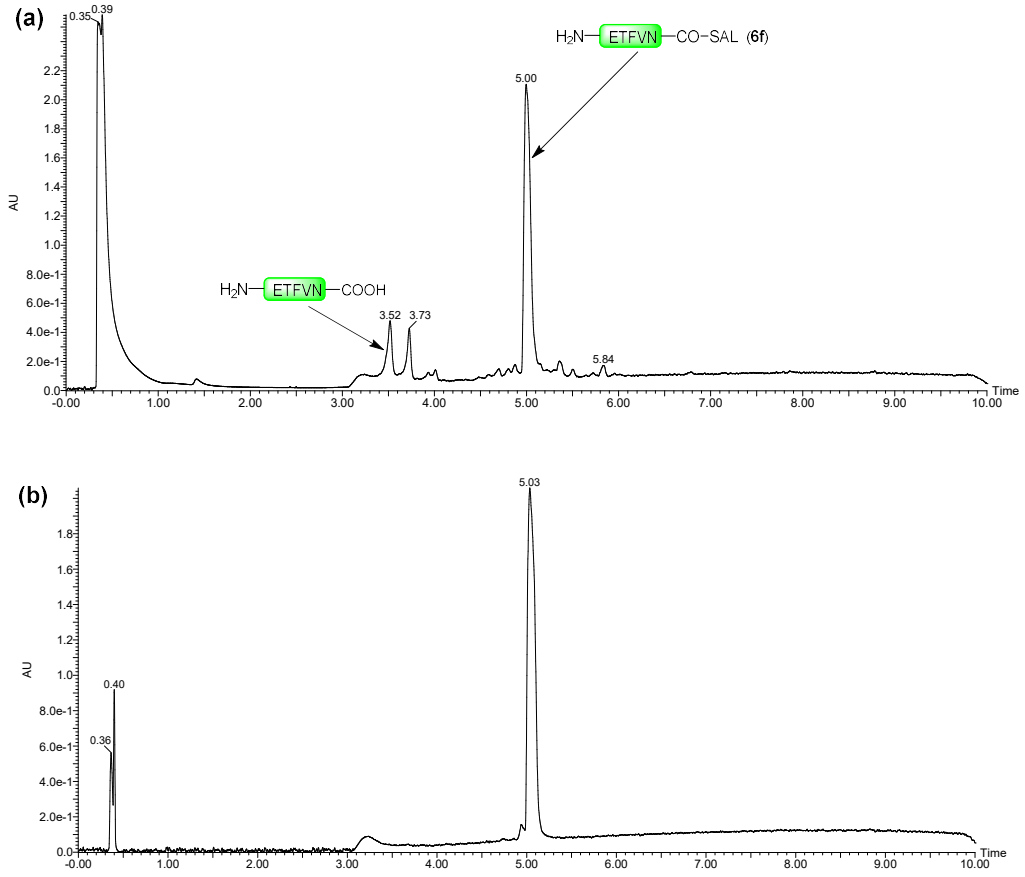
Figure S8. (a) UV trace from analytical RP-UPLC of crude reaction mixture. Gradient: 30-60% ACN/H_2O containing 0.1% TFA over 10 min at a flow rate of 0.4 mL/min. (b) UV trace from analytical RP-UPLC of **6e**. Gradient: 30-70% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. (c) ESI-MS of **6e**. ESI-MS calcd. for $C_{36}H_{50}N_5O_{10}$ $[M+H]^+$ m/z = 712.82, found 712.60.

5.6 *H_2N*-ETFVN-*CO*-SAL ester (6f)



A solution of crude DCP reagent (0.1 mmol) in ACN (0.5 mL) was added dropwise slowly (dropwise over about 40 min, 4 drops after every 2 min) to a flask containing the crude fully protected peptidyl acid *BocHN*-Glu(O*t*Bu)-Thr(*t*Bu)-Phe-Val-Asn(Trt)-*COOH* (prepared

according to general experimental procedure **2.1** and **2.2**) (53.2 mg, 0.05 mmol), K_2CO_3 (45.0 mg 0.33 mmol), NaI (180.0 mg, 1.20 mmol) and ACN (5 mL) at room temperature under an Ar atmosphere. After stirring at room temperature for 2 h, the mixture was filtered, and the filter cake was washed with ACN (10 mL) and DCM (10 mL). The filtration was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*, 5 mL) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged. The precipitate was pelleted, and the ether was subsequently decanted. The resulting solid was purified by HPLC and lyophilization to give the peptide SAL ester **6f** (19.4 mg, 54.5%) as white solid.



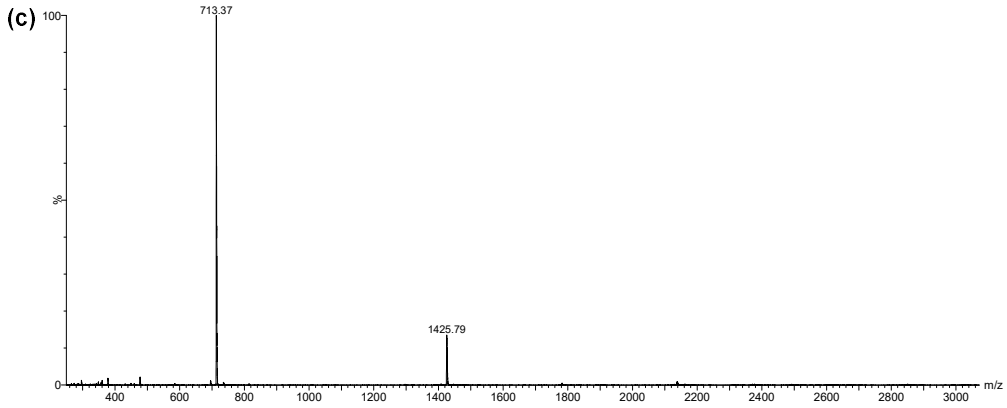
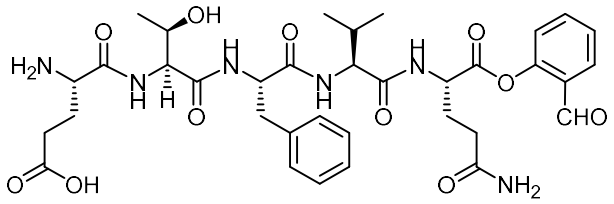


Figure S9. (a) UV trace from analytical RP-UPLC of crude reaction mixture. Gradient: 15-60% ACN/H_2O containing 0.1% TFA over 10 min at a flow rate of 0.4 mL/min. (b) UV trace from analytical RP-UPLC of **6f**. Gradient: 15-60% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. (c) ESI-MS of **6f**. ESI-MS calcd. for $C_{34}H_{45}N_6O_{11}$ $[M+H]^+$ m/z = 713.76, found 713.37.

5.7 *H_2N*-ETFVQ-*CO*-SAL ester (6g)



A solution of crude DCP reagent (0.1 mmol) in ACN (0.5 mL) was added dropwise slowly (dropwise over about 40 min, 4 drops after every 2 min) to a flask containing the crude fully protected peptidyl acid *BocHN*-Glu(O*t*Bu)-Thr(*t*Bu)-Phe-Val-Gln(Trt)-*COOH* (prepared according to general experimental procedure **2.1** and **2.2**) (53.9 mg, 0.05 mmol), K_2CO_3 (45.0 mg 0.33 mmol), NaI (180.0 mg, 1.20 mmol) and ACN (5 mL) at room temperature under an Ar atmosphere. After stirring at room temperature for 2 h, the mixture was filtered, and the filter cake was washed with ACN (10 mL) and DCM (10 mL). The filtration was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*, 5 mL) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged. The precipitate was pelleted, and the ether was subsequently decanted. The resulting solid was purified by HPLC and lyophilization to give the peptide SAL

ester **6g** (21.2 mg, 58.3%) as white solid.

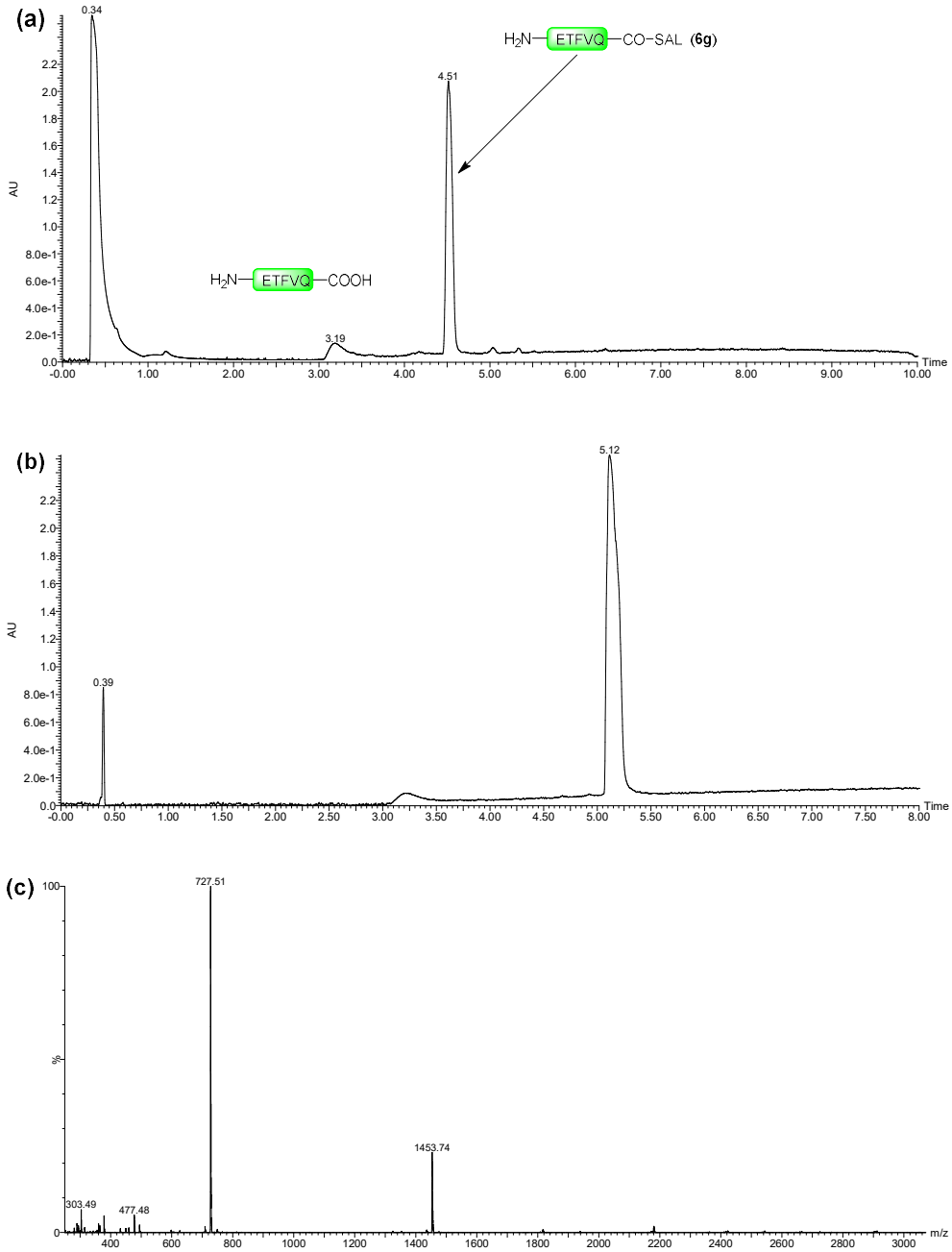
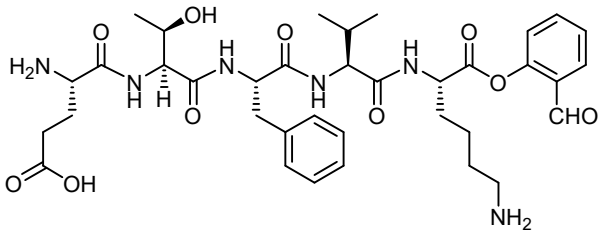
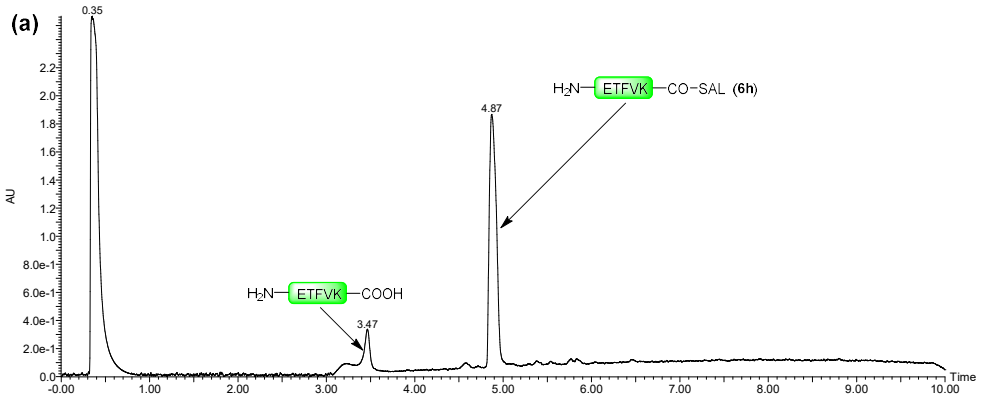


Figure S10. (a) UV trace from analytical RP-UPLC of crude reaction mixture. Gradient: 30-60% ACN/H_2O containing 0.1% TFA over 10 min at a flow rate of 0.4 mL/min. (b) UV trace from analytical RP-UPLC of **6g**. Gradient: 20-60% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. (c) ESI-MS of **6g**. ESI-MS calcd. for $C_{35}H_{47}N_6O_{11}$ $[M+H]^+$ m/z = 727.79, found 727.51.

5.8 *H₂N*-ETFVK-*CO*-SAL ester (6h)



A solution of crude DCP reagent (0.1 mmol) in ACN (0.5 mL) was added dropwise slowly (dropwise over about 40 min, 4 drops after every 2 min) to a flask containing the crude fully protected peptidyl acid *BocHN*-Glu(O*t*Bu)-Thr(*t*Bu)-Phe-Val-Lys(Boc)-*COOH* (prepared according to general experimental procedure **2.1** and **2.2**) (46.8 mg, 0.05 mmol), K_2CO_3 (45.0 mg 0.33 mmol), NaI (180.0 mg, 1.20 mmol) and ACN (5 mL) at room temperature under an Ar atmosphere. After stirring at room temperature for 2 h, the mixture was filtered, and the filter cake was washed with ACN (10 mL) and DCM (10 mL). The filtration was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*, 5 mL) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged. The precipitate was pelleted, and the ether was subsequently decanted. The resulting solid was purified by HPLC and lyophilization to give the peptide SAL ester **6h** (21.9 mg, 60.3%) as white solid.



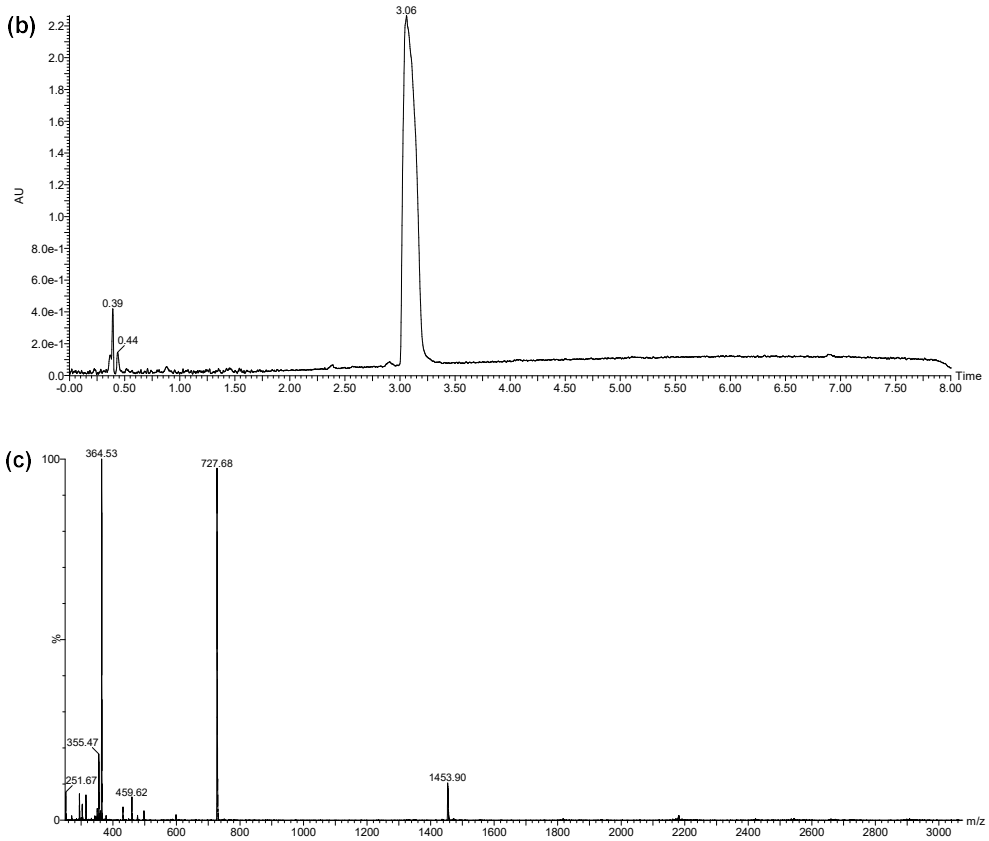
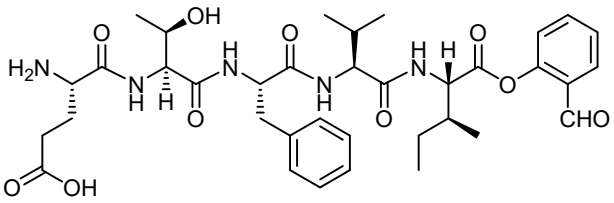


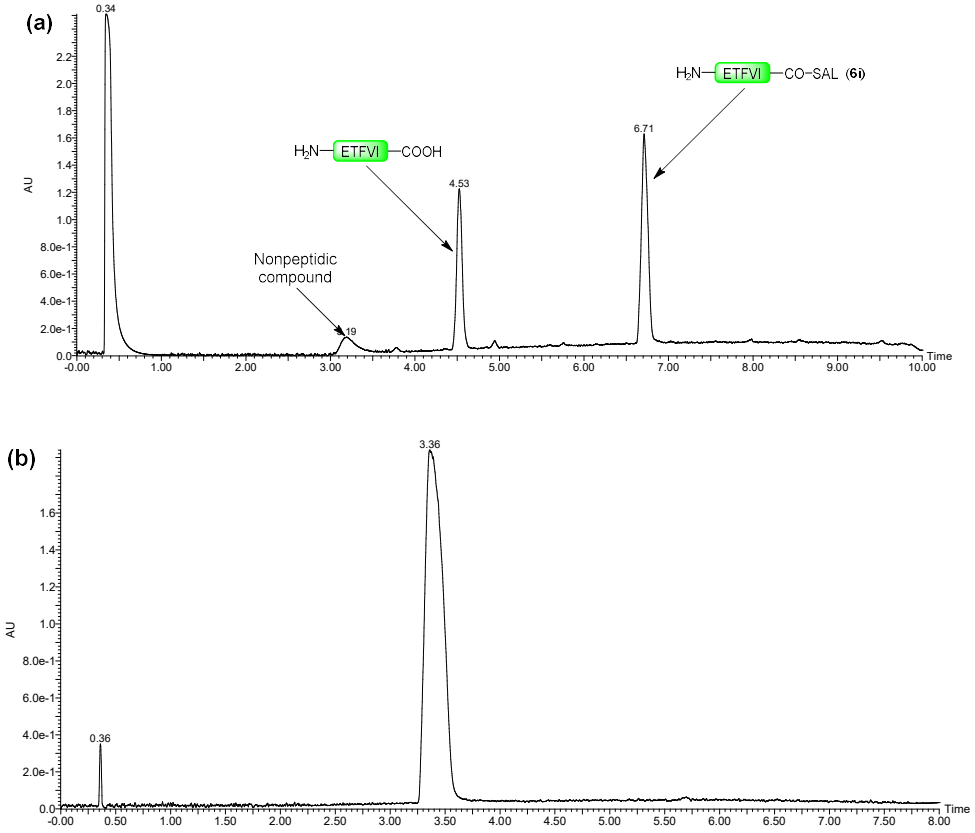
Figure S11. (a) UV trace from analytical RP-UPLC of crude reaction mixture. Gradient: 15-60% ACN/H_2O containing 0.1% TFA over 10 min at a flow rate of 0.4 mL/min. (b) UV trace from analytical RP-UPLC of **6h**. Gradient: 20-60% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. (c) ESI-MS of **6h**. ESI-MS calcd. for $C_{36}H_{51}N_6O_{10}$ $[M+H]^+$ $m/z = 727.84$, found 727.68.

5.9 *H_2N*-ETFVI-*CO*-SAL ester (6i)



A solution of crude DCP reagent (0.1 mmol) in ACN (0.5 mL) was added dropwise slowly (dropwise over about 40 min, 4 drops after every 2 min) to a flask containing the crude fully

protected peptidyl acid *BocHN*-Glu(O*t*Bu)-Thr(*t*Bu)-Phe-Val-Ile-*COOH* (prepared according to general experimental procedure **2.1** and **2.2**) (41.0 mg, 0.05 mmol), K_2CO_3 (45.0 mg 0.33 mmol), NaI (180.0 mg, 1.20 mmol) and ACN (5 mL) at room temperature under an Ar atmosphere. After stirring at room temperature for 2 h, the mixture was filtered, and the filter cake was washed with ACN (10 mL) and DCM (10 mL). The filtration was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*, 5 mL) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged. The precipitate was pelleted, and the ether was subsequently decanted. The resulting solid was purified by HPLC and lyophilization to give the peptide SAL ester **6i** (16.4 mg, 46.8%) as white solid.



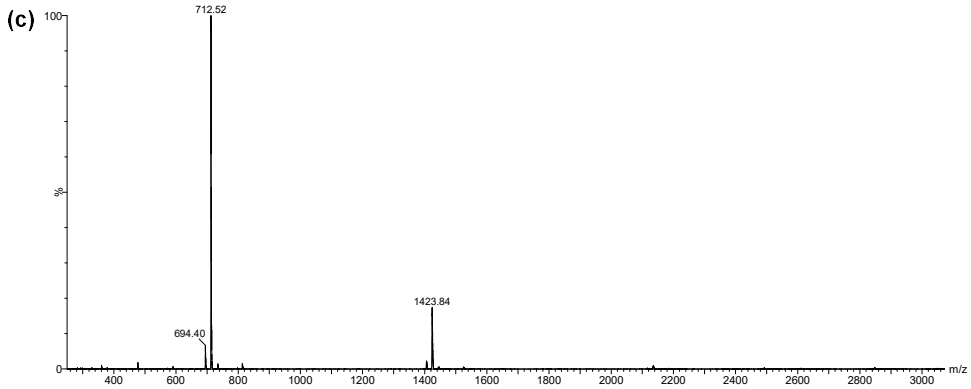
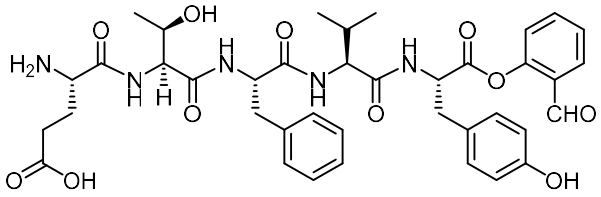


Figure S12. (a) UV trace from analytical RP-UPLC of crude reaction mixture. Gradient: 20-60% ACN/H_2O containing 0.1% TFA over 10 min at a flow rate of 0.4 mL/min. (b) UV trace from analytical RP-UPLC of **6i**. Gradient: 30-60% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. (c) ESI-MS of **6i**. ESI-MS calcd. for $C_{36}H_{50}N_5O_{10}$ $[M+H]^+$ m/z = 712.82, found 712.52.

5.10 *H_2N*-ETFVY-*CO*-SAL ester (6j)



A solution of crude DCP reagent (0.1 mmol) in ACN (0.5 mL) was added dropwise slowly (dropwise over about 40 min, 4 drops after every 2 min) to a flask containing the crude fully protected peptidyl acid *BocHN*-Glu(O*t*Bu)-Thr(*t*Bu)-Phe-Val-Tyr(*t*Bu)-*COOH* (prepared according to general experimental procedure **2.1** and **2.2**) (46.3 mg, 0.05 mmol), K_2CO_3 (45.0 mg 0.33 mmol), NaI (180.0 mg, 1.20 mmol) and ACN (5 mL) at room temperature under an Ar atmosphere. After stirring at room temperature for 2 h, the mixture was filtered, and the filter cake was washed with ACN (10 mL) and DCM (10 mL). The filtration was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*, 5 mL) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged. The precipitate was pelleted, and the ether was subsequently decanted. The resulting solid was purified by HPLC and lyophilization to give the peptide SAL

ester **6j** (27.3 mg, 71.7%) as white solid.

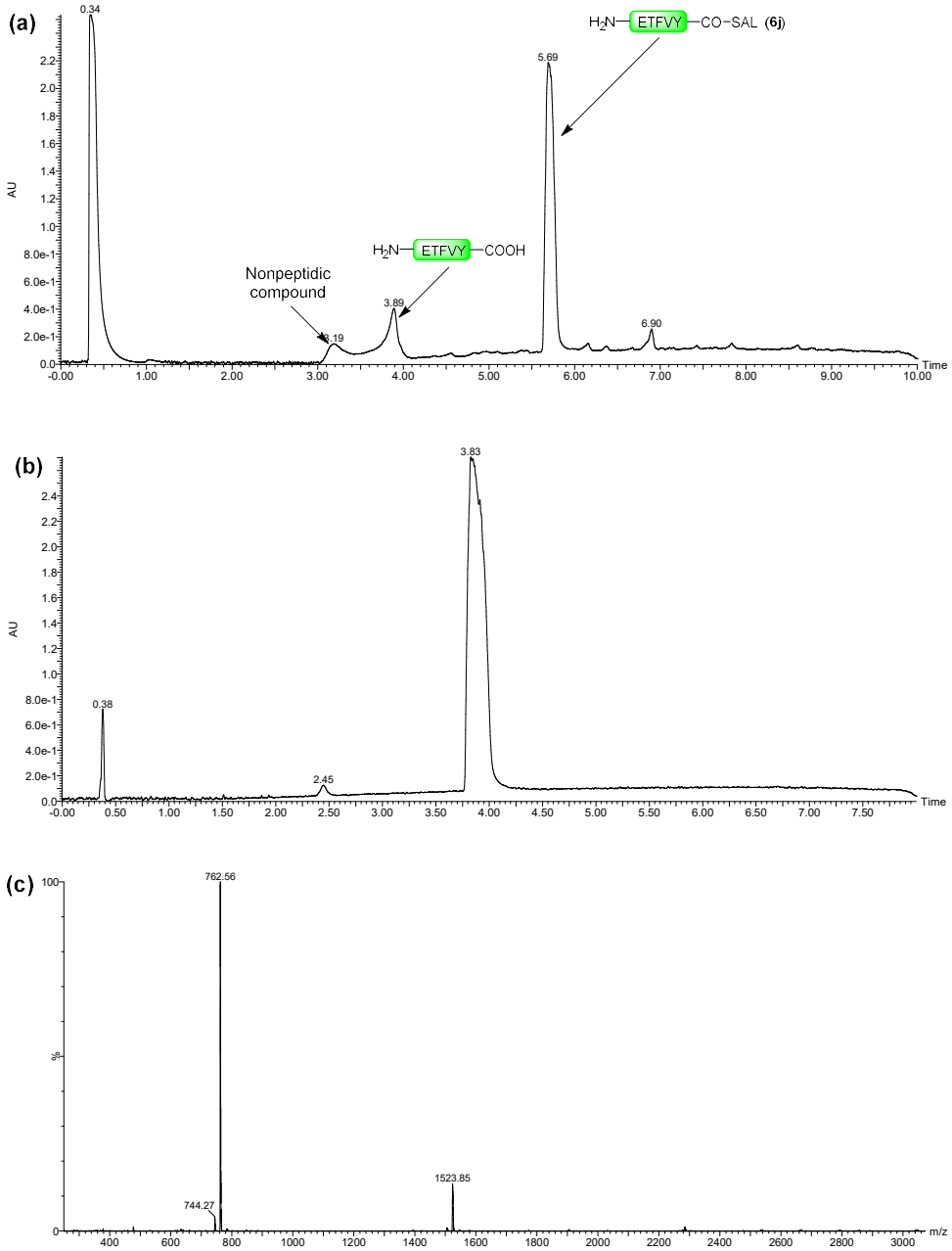
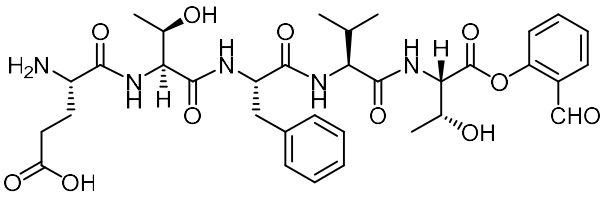
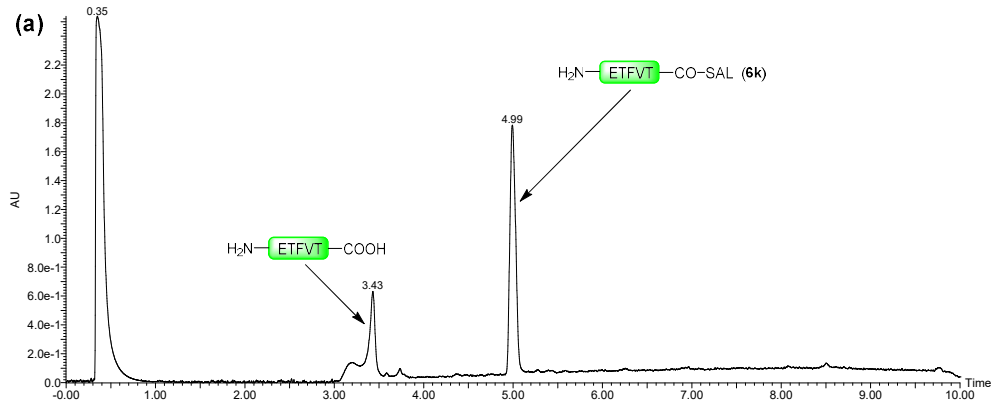


Figure S13. (a) UV trace from analytical RP-UPLC of crude reaction mixture. Gradient: 20-60% ACN/H_2O containing 0.1% TFA over 10 min at a flow rate of 0.4 mL/min. (b) UV trace from analytical RP-UPLC of **6j**. Gradient: 25-60% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. (c) ESI-MS of **6j**. ESI-MS calcd. for $C_{39}H_{48}N_5O_{11}$ $[M+H]^+$ m/z = 762.84, found 762.56.

5.11 *H₂N*-ETFVT-*CO*-SAL ester (6k)



A solution of crude DCP reagent (0.1 mmol) in ACN (0.5 mL) was added dropwise slowly (dropwise over about 40 min, 4 drops after every 2 min) to a flask containing the crude fully protected peptidyl acid *BocHN*-Glu(O*t*Bu)-Thr(*t*Bu)-Phe-Val-Thr(*t*Bu)-*COOH* (prepared according to general experimental procedure **2.1** and **2.2**) (43.2 mg, 0.05 mmol), K_2CO_3 (45.0 mg 0.33 mmol), NaI (180.0 mg, 1.20 mmol) and ACN (5 mL) at room temperature under an Ar atmosphere. After stirring at room temperature for 2 h, the mixture was filtered, and the filter cake was washed with ACN (10 mL) and DCM (10 mL). The filtration was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*, 5 mL) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged. The precipitate was pelleted, and the ether was subsequently decanted. The resulting solid was purified by HPLC and lyophilization to give the peptide SAL ester **6k** (18.6 mg, 53.2%) as white solid.



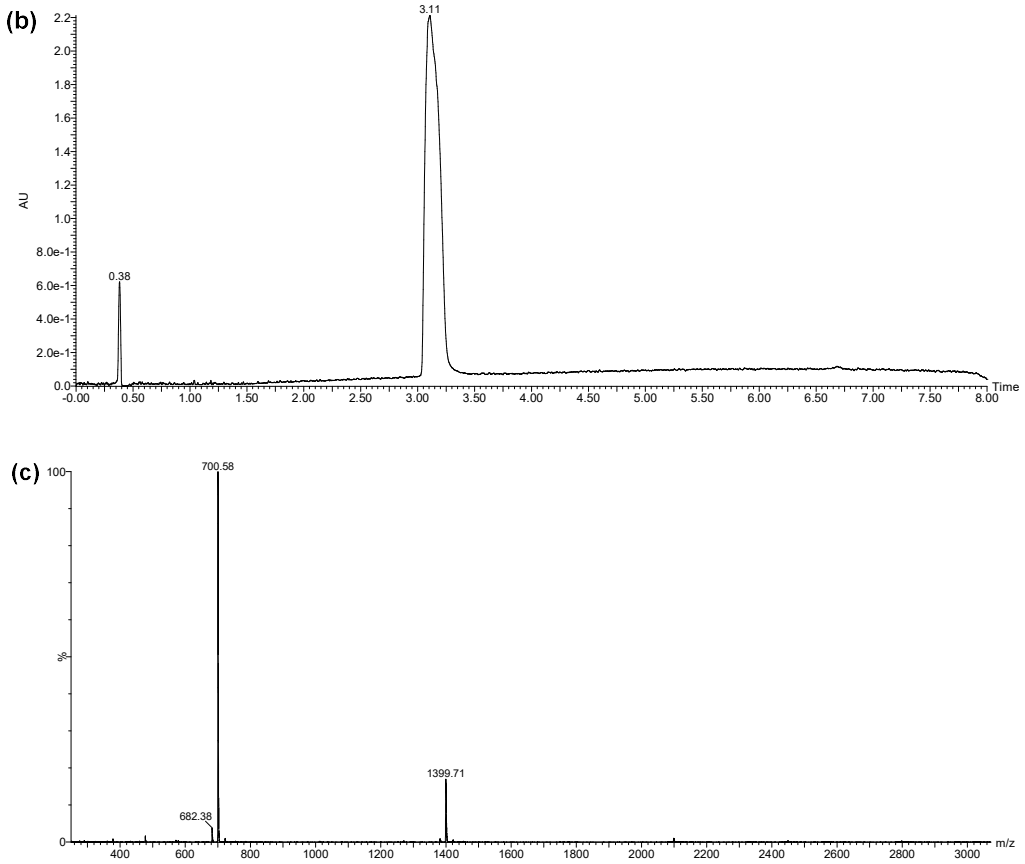
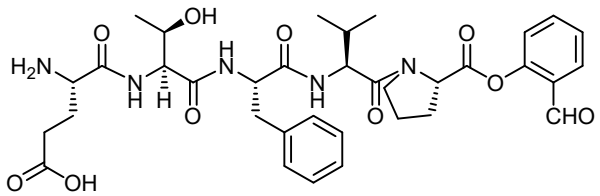


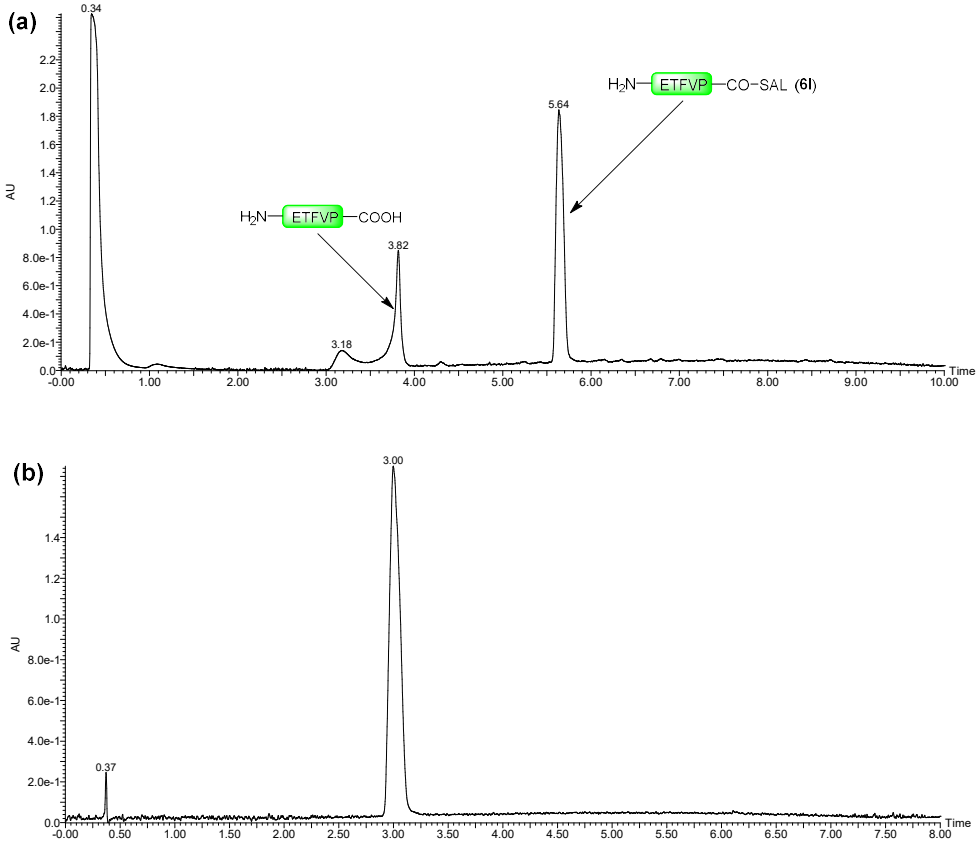
Figure S14. (a) UV trace from analytical RP-UPLC of crude reaction mixture. Gradient: 20-60% ACN/H_2O containing 0.1% TFA over 10 min at a flow rate of 0.4 mL/min. (b) UV trace from analytical RP-UPLC of **6k**. Gradient: 25-60% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. (c) ESI-MS of **6k**. ESI-MS calcd. for $C_{34}H_{46}N_5O_{11}$ $[M+H]^+$ m/z = 700.76, found 700.58.

5.12 *H_2N*-ETFVP-*CO*-SAL ester (6l)



A solution of crude DCP reagent (0.1 mmol) in ACN (0.5 mL) was added dropwise slowly (dropwise over about 40 min, 4 drops after every 2 min) to a flask containing the crude fully protected peptidyl acid *BocHN*-Glu(O*t*Bu)-Thr(*t*Bu)-Phe-Val-Pro-*COOH* (prepared according to

general experimental procedure **2.1** and **2.2**) (40.2 mg, 0.05 mmol), K_2CO_3 (45.0 mg 0.33 mmol), NaI (180.0 mg, 1.20 mmol) and ACN (5 mL) at room temperature under an Ar atmosphere. After stirring at room temperature for 2 h, the mixture was filtered, and the filter cake was washed with ACN (10 mL) and DCM (10 mL). The filtration was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*, 5 mL) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged. The precipitate was pelleted, and the ether was subsequently decanted. The resulting solid was purified by HPLC and lyophilization to give the peptide SAL ester **6l** (19.8 mg, 56.9%) as white solid.



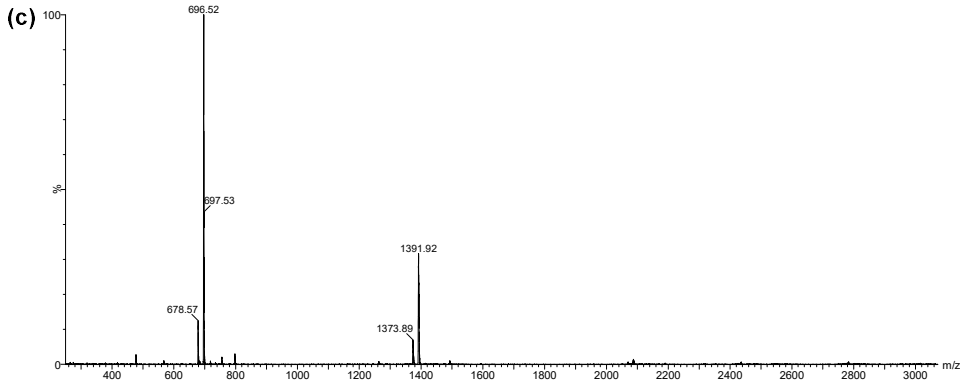
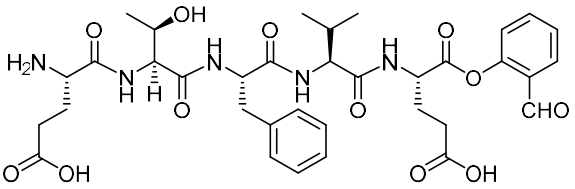


Figure S15. (a) UV trace from analytical RP-UPLC of crude reaction mixture. Gradient: 20-60% ACN/H_2O containing 0.1% TFA over 10 min at a flow rate of 0.4 mL/min. (b) UV trace from analytical RP-UPLC of **6l**. Gradient: 30-60% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. (c) ESI-MS of **6l**. ESI-MS calcd. for $C_{35}H_{46}N_5O_{10}$ $[M+H]^+$ m/z = 696.78, found 696.52.

5.13 *H_2N*-ETFVE-*CO*-SAL ester (6m)



A solution of crude DCP reagent (0.1 mmol) in ACN (0.5 mL) was added dropwise slowly (dropwise over about 40 min, 4 drops after every 2 min) to a flask containing the crude fully protected peptidyl acid *BocHN*-Glu(O*t*Bu)-Thr(*t*Bu)-Phe-Val-Glu(*t*Bu)-*COOH* (prepared according to general experimental procedure **2.1** and **2.2**) (44.6 mg, 0.05 mmol), K_2CO_3 (45.0 mg 0.33 mmol), NaI (180.0 mg, 1.20 mmol) and ACN (5 mL) at room temperature under an Ar atmosphere. After stirring at room temperature for 2 h, the mixture was filtered, and the filter cake was washed with ACN (10 mL) and DCM (10 mL). The filtration was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*, 5 mL) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged. The precipitate was pelleted, and the ether was subsequently decanted. The resulting solid was purified by HPLC and lyophilization to give the peptide SAL

ester **6m** (20.2 mg, 55.5%) as white solid.

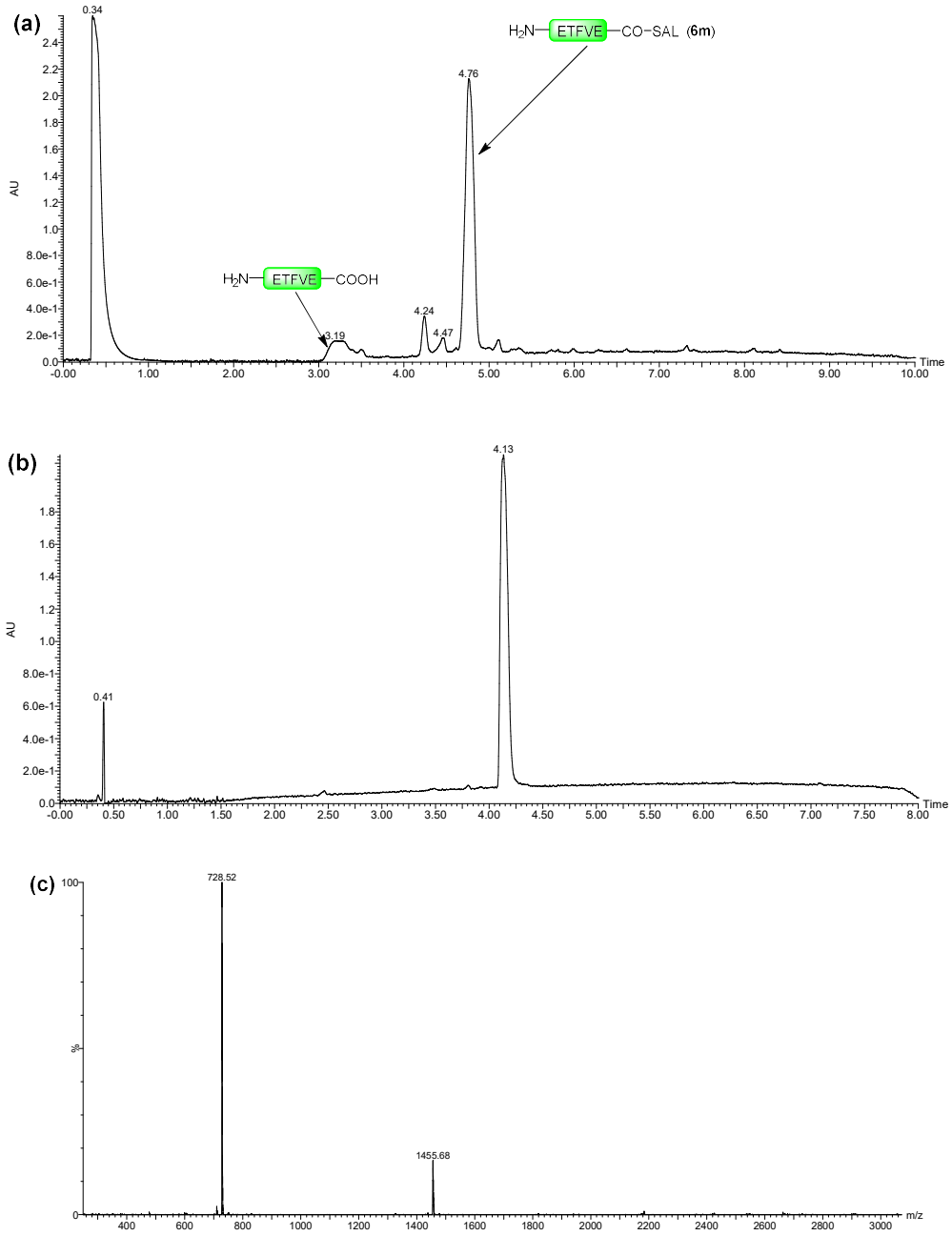
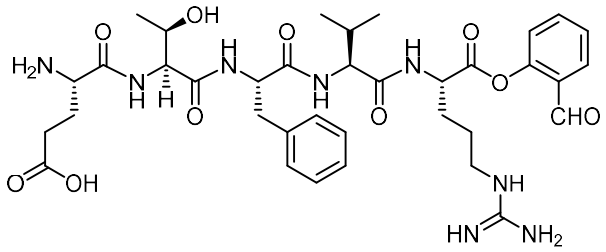
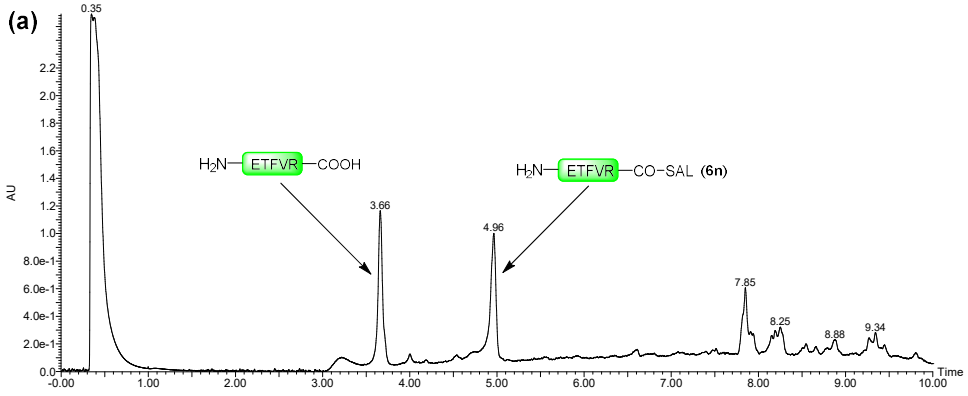


Figure S16. (a) UV trace from analytical RP-UPLC of crude reaction mixture. Gradient: 20-60% ACN/H_2O containing 0.1% TFA over 10 min at a flow rate of 0.4 mL/min. (b) UV trace from analytical RP-UPLC of **6m**. Gradient: 15-60% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. (c) ESI-MS of **6m**. ESI-MS calcd. for $C_{35}H_{46}N_5O_{12}$ $[M+H]^+$ $m/z = 728.78$, found 728.52.

5.14 *H₂N*-ETFVR-*CO*-SAL ester (6n)



A solution of crude DCP reagent (0.1 mmol) in ACN (0.5 mL) was added dropwise slowly (dropwise over about 40 min, 4 drops after every 2 min) to a flask containing the crude fully protected peptidyl acid *BocHN*-Glu(O*t*Bu)-Thr(*t*Bu)-Phe-Val-Arg(Pbf)-*COOH* (prepared according to general experimental procedure **2.1** and **2.2**) (55.8 mg, 0.05 mmol), K_2CO_3 (45.0 mg 0.33 mmol), NaI (180.0 mg, 1.20 mmol) and ACN (5 mL) at room temperature under an Ar atmosphere. After stirring at room temperature for 2 h, the mixture was filtered, and the filter cake was washed with ACN (10 mL) and DCM (10 mL). The filtration was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*, 5 mL) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged. The precipitate was pelleted, and the ether was subsequently decanted. The resulting solid was purified by HPLC and lyophilization to give the peptide SAL ester **6n** (15.2 mg, 40.3%) as white solid.



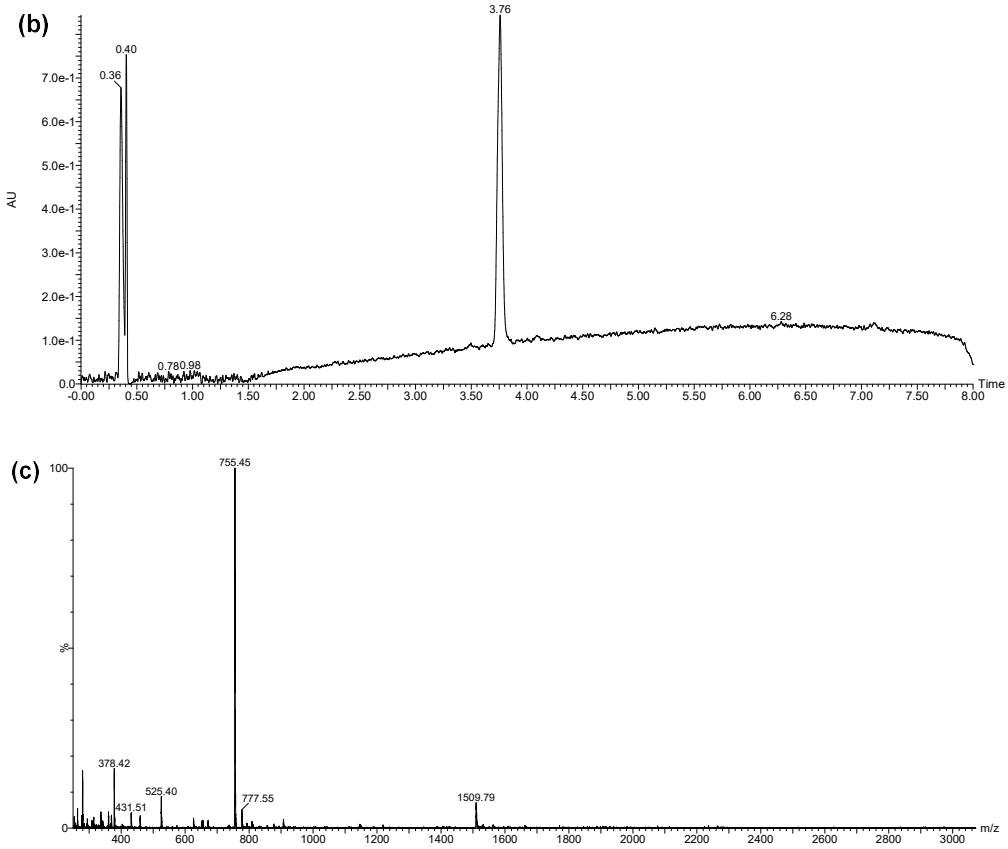
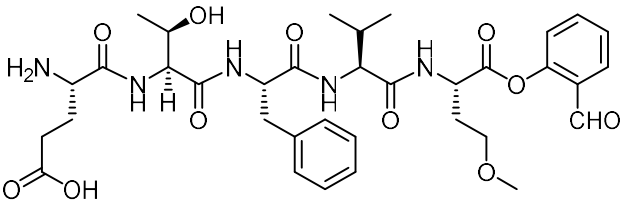


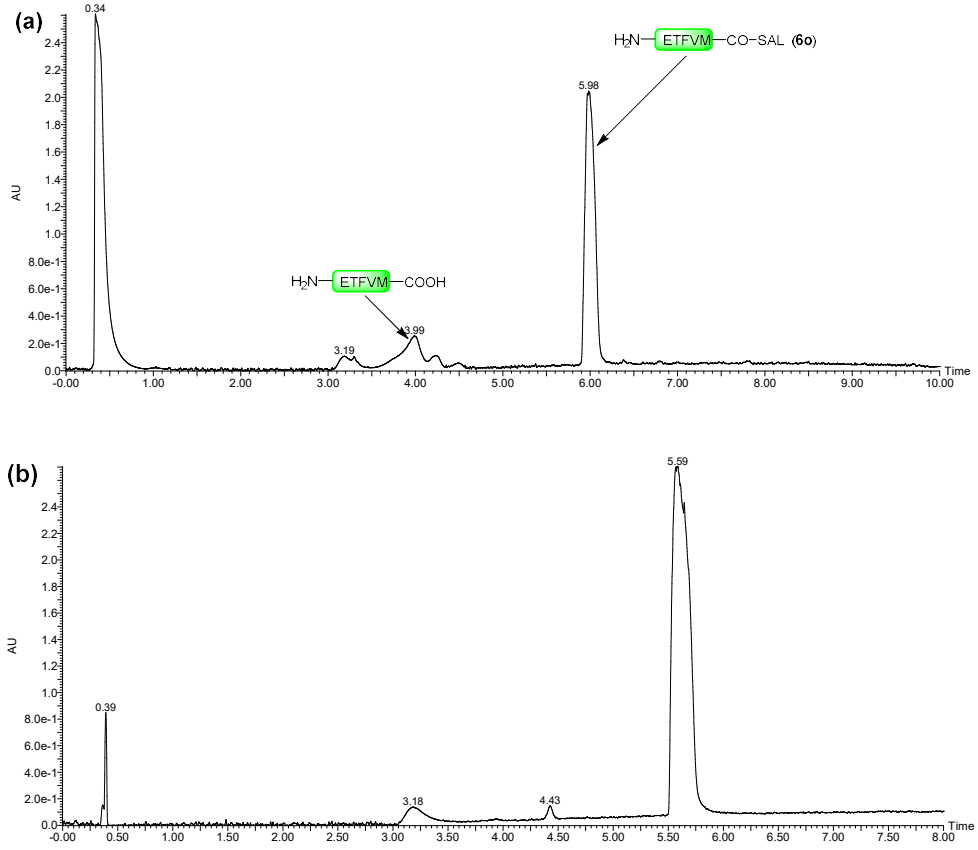
Figure S17. (a) UV trace from analytical RP-UPLC of crude reaction mixture. Gradient: 15-60% ACN/H_2O containing 0.1% TFA over 10 min at a flow rate of 0.4 mL/min. (b) UV trace from analytical RP-UPLC of **6n**. Gradient: 10-60% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. (c) ESI-MS of **6n**. ESI-MS calcd. for $C_{36}H_{51}N_8O_{10}$ $[M+H]^+$ m/z =755.85, found 755.45.

5.15 *H_2N*-ETFVM-*CO*-SAL ester (6o)



A solution of crude DCP reagent (0.1 mmol) in ACN (0.5 mL) was added dropwise slowly (dropwise over about 40 min, 4 drops after every 2 min) to a flask containing the crude fully protected peptidyl acid *BocHN*-Glu(O*t*Bu)-Thr(*t*Bu)-Phe-Val-Met-*COOH* (prepared according to

general experimental procedure **2.1** and **2.2**) (42.0 mg, 0.05 mmol), K_2CO_3 (45.0 mg 0.33 mmol), NaI (180.0 mg, 1.20 mmol) and ACN (5 mL) at room temperature under an Ar atmosphere. After stirring at room temperature for 2 h, the mixture was filtered, and the filter cake was washed with ACN (10 mL) and DCM (10 mL). The filtration was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*, 5 mL) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged. The precipitate was pelleted, and the ether was subsequently decanted. The resulting solid was purified by HPLC and lyophilization to give the peptide SAL ester **6o** (23.6 mg, 64.7%) as white solid.



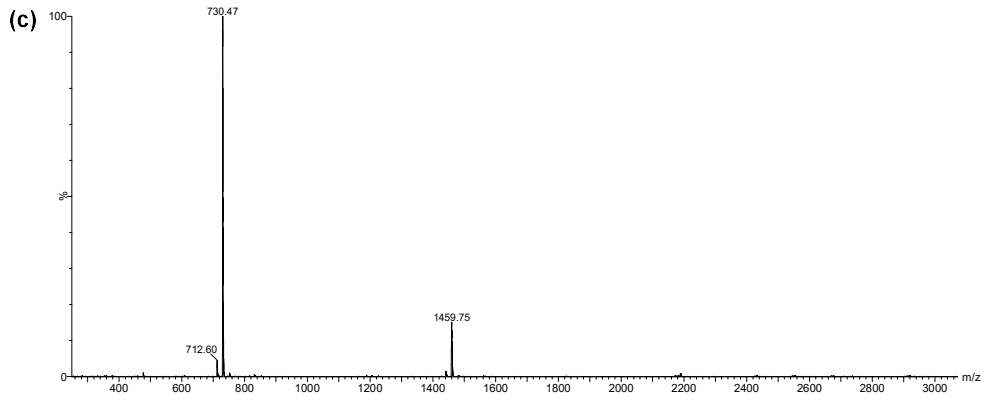
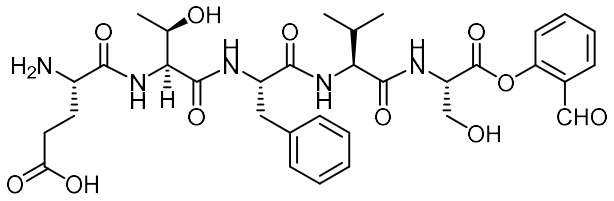


Figure S18. (a) UV trace from analytical RP-UPLC of crude reaction mixture. Gradient: 20-60% ACN/H_2O containing 0.1% TFA over 10 min at a flow rate of 0.4 mL/min. (b) UV trace from analytical RP-UPLC of **6o**. Gradient: 15-60% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. (c) ESI-MS of **6o**. ESI-MS calcd. for $C_{35}H_{48}N_5O_{10}S$ $[M+H]^+$ m/z = 730.85, found 730.47.

5.16 *H_2N*-ETFVS-*CO*-SAL ester (6p)



A solution of crude DCP reagent (0.1 mmol) in ACN (0.5 mL) was added dropwise slowly (dropwise over about 40 min, 4 drops after every 2 min) to a flask containing the crude fully protected peptidyl acid *BocHN*-Glu(O*t*Bu)-Thr(*t*Bu)-Phe-Val-Ser(*t*Bu)-*COOH* (prepared according to general experimental procedure **2.1** and **2.2**) (42.5 mg, 0.05 mmol), K_2CO_3 (45.0 mg 0.33 mmol), NaI (180.0 mg, 1.20 mmol) and ACN (5 mL) at room temperature under an Ar atmosphere. After stirring at room temperature for 2 h, the mixture was filtered, and the filter cake was washed with ACN (10 mL) and DCM (10 mL). The filtration was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*, 5 mL) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged. The precipitate was pelleted, and the ether was subsequently decanted. The resulting solid was purified by HPLC and lyophilization to give the peptide SAL

ester **6p** (20.7 mg, 60.4%) as white solid.

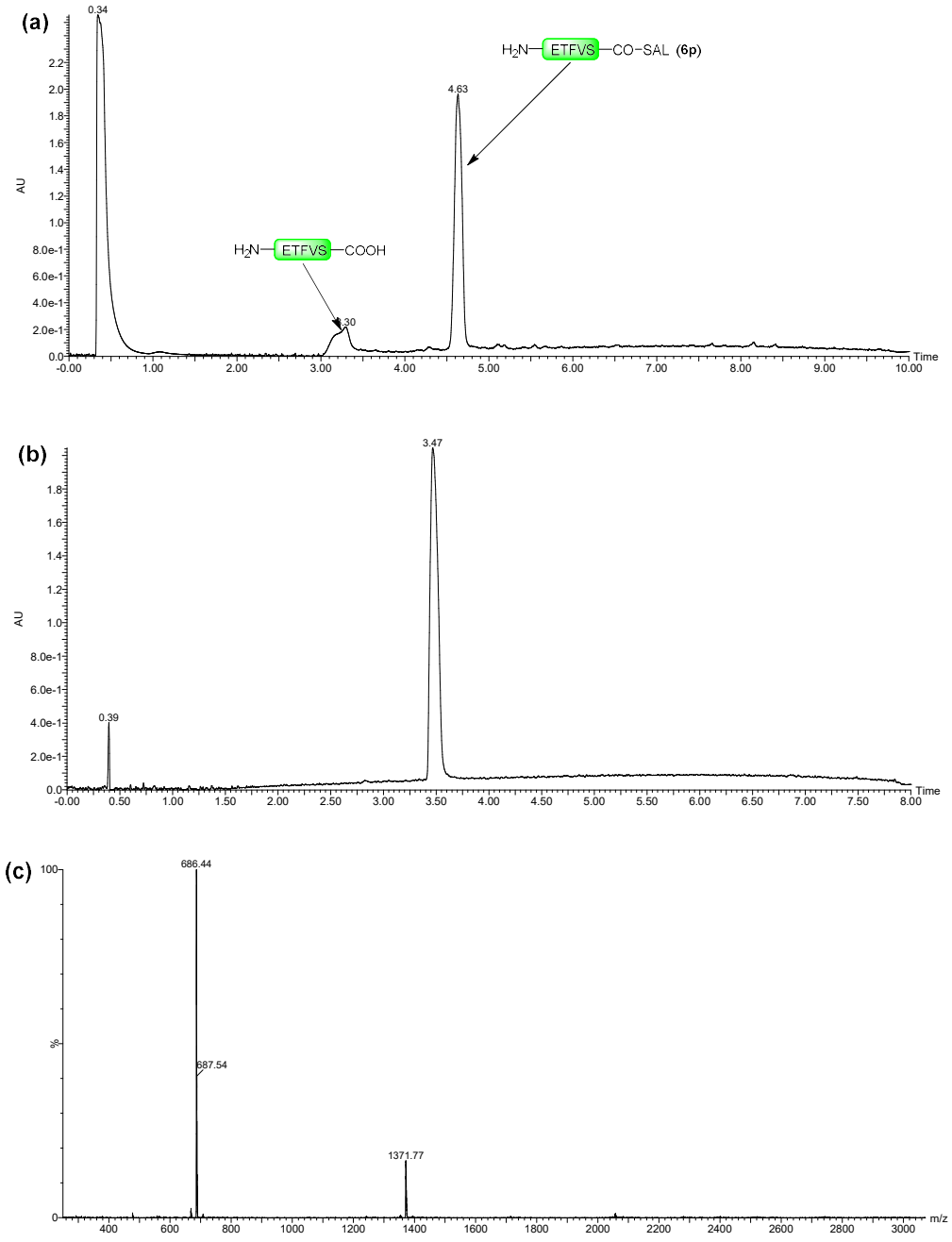
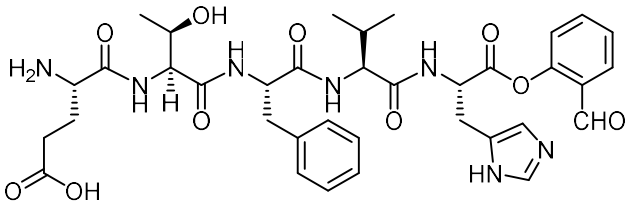
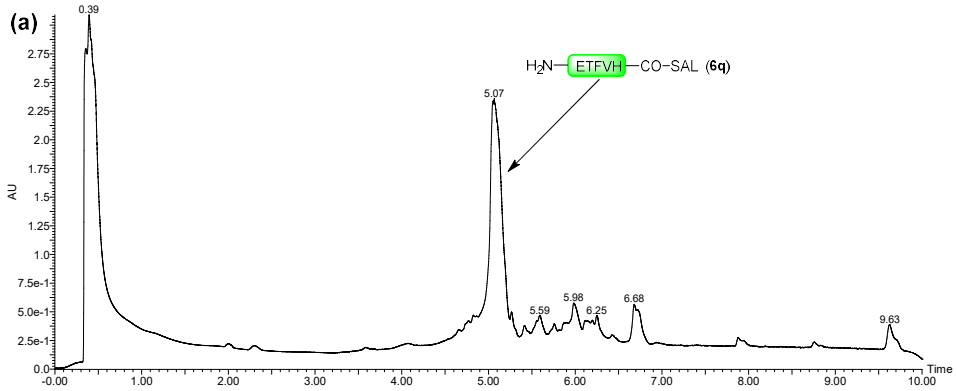


Figure S19. (a) UV trace from analytical RP-UPLC of crude reaction mixture. Gradient: 20-60% ACN/H_2O containing 0.1% TFA over 10 min at a flow rate of 0.4 mL/min. (b) UV trace from analytical RP-UPLC of **6p**. Gradient: 15-60% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. (c) ESI-MS of **6p**. ESI-MS calcd. for $C_{33}H_{44}N_5O_{11}$ $[M+H]^+$ m/z = 686.74, found 686.44.

5.17 *H₂N*-ETFVH-*CO*-SAL ester (6q)



A solution of crude DCP reagent (0.1 mmol) in ACN (0.5 mL) was added dropwise slowly (dropwise over about 40 min, 4 drops after every 2 min) to a flask containing the crude fully protected peptidyl acid *BocHN*-Glu(O*t*Bu)-Thr(*t*Bu)-Phe-Val-His(Trt)-*COOH* (prepared according to general experimental procedure **2.1** and **2.2**) (54.3 mg, 0.05 mmol), K_2CO_3 (45.0 mg 0.33 mmol), NaI (180.0 mg, 1.20 mmol) and ACN (5 mL) at room temperature under an Ar atmosphere. After stirring at room temperature for 2 h, the mixture was filtered, and the filter cake was washed with ACN (10 mL) and DCM (10 mL). The filtration was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*, 5 mL) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged. The precipitate was pelleted, and the ether was subsequently decanted. The resulting solid was purified by HPLC and lyophilization to give the peptide SAL ester **6q** (18.7 mg, 50.8%) as white solid.



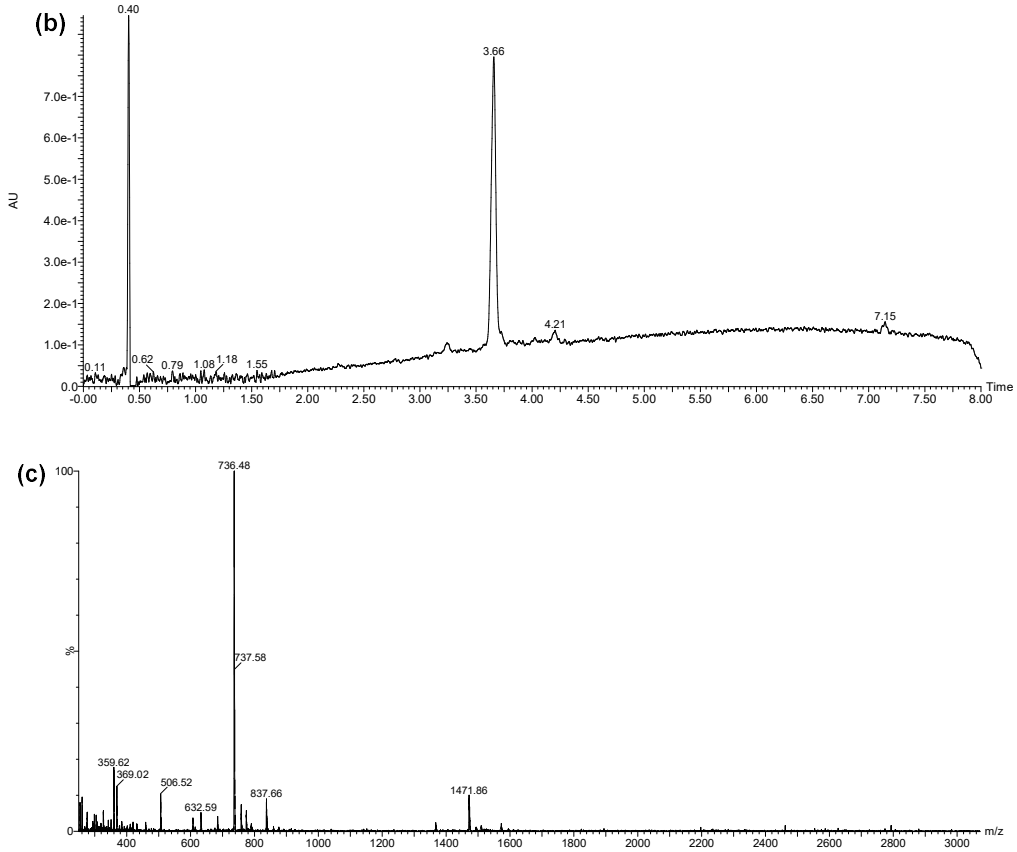
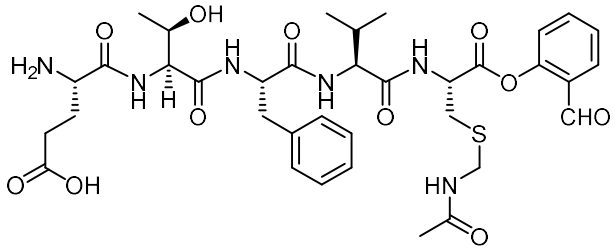


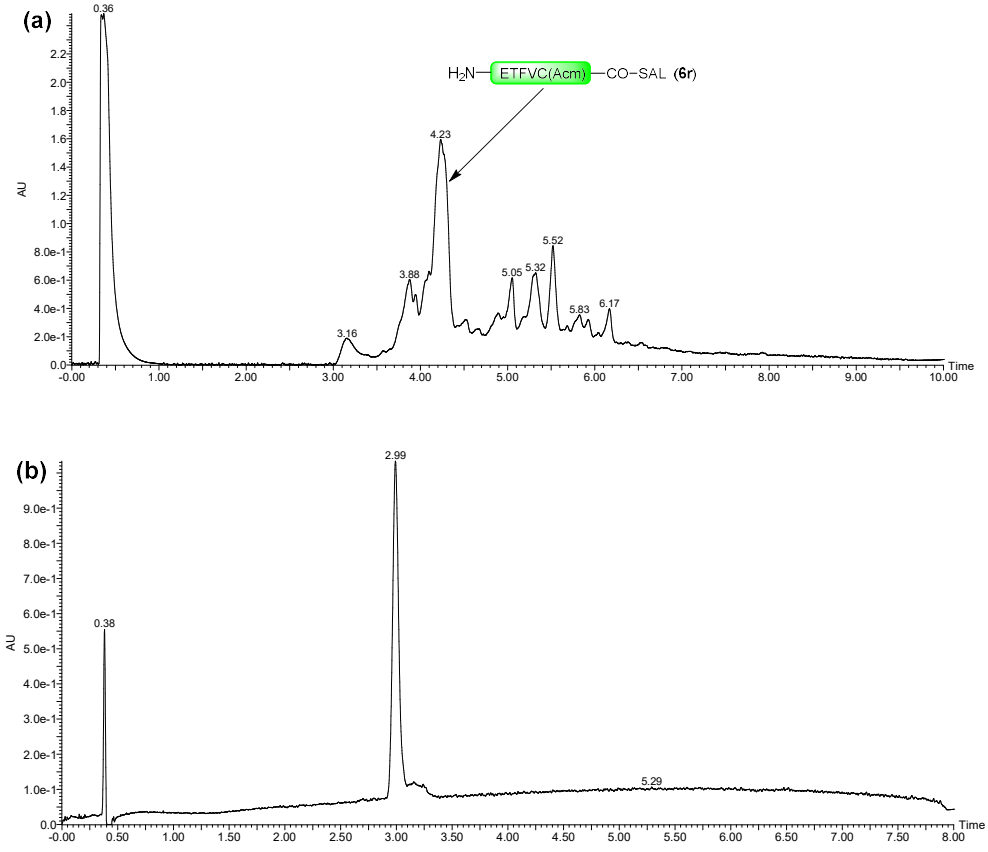
Figure S20. (a) UV trace from analytical RP-UPLC of crude reaction mixture. Gradient: 10-60% ACN/H_2O containing 0.1% TFA over 10 min at a flow rate of 0.4 mL/min. (b) UV trace from analytical RP-UPLC of **6q**. Gradient: 10-60% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. (c) ESI-MS of **6q**. ESI-MS calcd. for $C_{36}H_{46}N_7O_{10}$ $[M+H]^+$ m/z = 736.80, found 736.48.

5.18 *H_2N*-ETFVC(Acm)-*CO*-SAL ester (6r)



A solution of crude DCP reagent (0.1 mmol) in ACN (0.5 mL) was added dropwise slowly (dropwise over about 40 min, 4 drops after every 2 min) to a flask containing the crude fully

protected peptidyl acid *BocHN*-Glu(O*t*Bu)-Thr(*t*Bu)-Phe-Val-Cys(Acm)-*COOH* (prepared according to general experimental procedure **2.1** and **2.2**) (44 mg, 0.05 mmol), K_2CO_3 (45.0 mg 0.33 mmol), NaI (180.0 mg, 1.20 mmol) and ACN (5 mL) at room temperature under an Ar atmosphere. After stirring at room temperature for 2 h, the mixture was filtered, and the filter cake was washed with ACN (10 mL) and DCM (10 mL). The filtration was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*, 5 mL) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged. The precipitate was pelleted, and the ether was subsequently decanted. The resulting solid was purified by HPLC and lyophilization to give the peptide SAL ester **6r** (13.6 mg, 35.2%) as white solid.



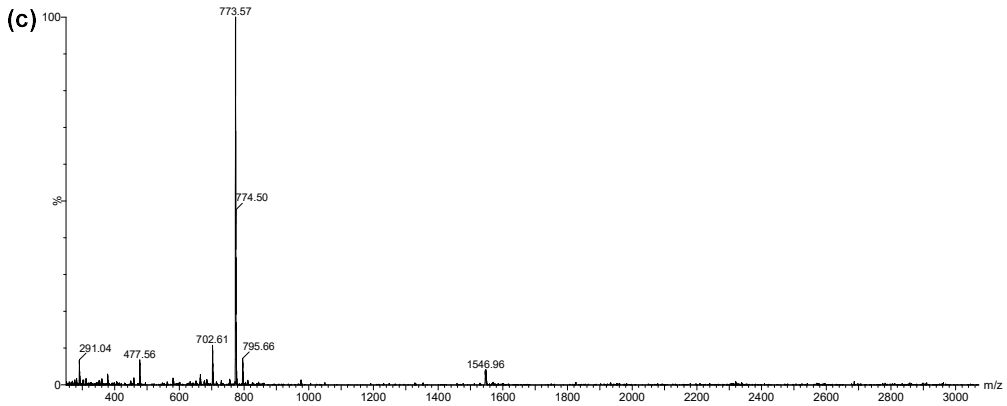
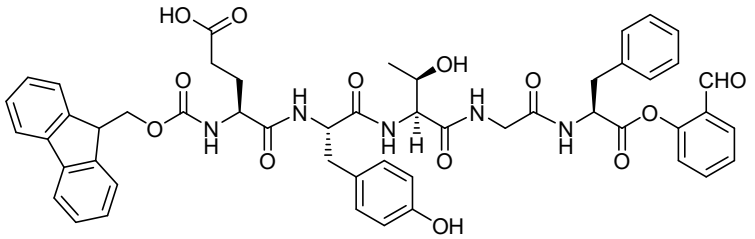


Figure S21. (a) UV trace from analytical RP-UPLC of crude reaction mixture. Gradient: 25-60% ACN/H_2O containing 0.1% TFA over 10 min at a flow rate of 0.4 mL/min. (b) UV trace from analytical RP-UPLC of **6r**. Gradient: 20-60% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. (c) ESI-MS of **6r**. ESI-MS calcd. for $C_{36}H_{49}N_6O_{11}S$ $[M+H]^+$ $m/z = 773.88$, found 773.57.

5.19 *FmocHN*-EYTGF-CO-SAL ester (7o)



A solution of crude DCP reagent (0.06 mmol) in ACN (0.4 mL) was added dropwise slowly (dropwise over about 40 min, 4 drops after every 2 min) to a flask containing the crude fully protected peptidyl acid *FmocHN*-Glu(O*t*Bu)-Tyr(*t*Bu)-Thr(*t*Bu)-Gly-Phe-*COOH* (prepared according to general experimental procedure **2.1** and **2.2**) (30.2 mg, 0.03 mmol), K_2CO_3 (27.5 mg 0.2 mmol), NaI (108.0 mg, 0.72 mmol) and ACN (10 mL) at room temperature under an Ar atmosphere. After stirring at room temperature for 2 h, the mixture was filtered, and the filter cake was washed with ACN (10 mL) and DCM (10 mL). The filtration was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*, 4 mL) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged. The precipitate was pelleted, and the ether was subsequently

decanted. The resulting solid was purified by HPLC and lyophilization to give the peptide SAL ester **7o** (14.3 mg, 50.6%) as white solid.

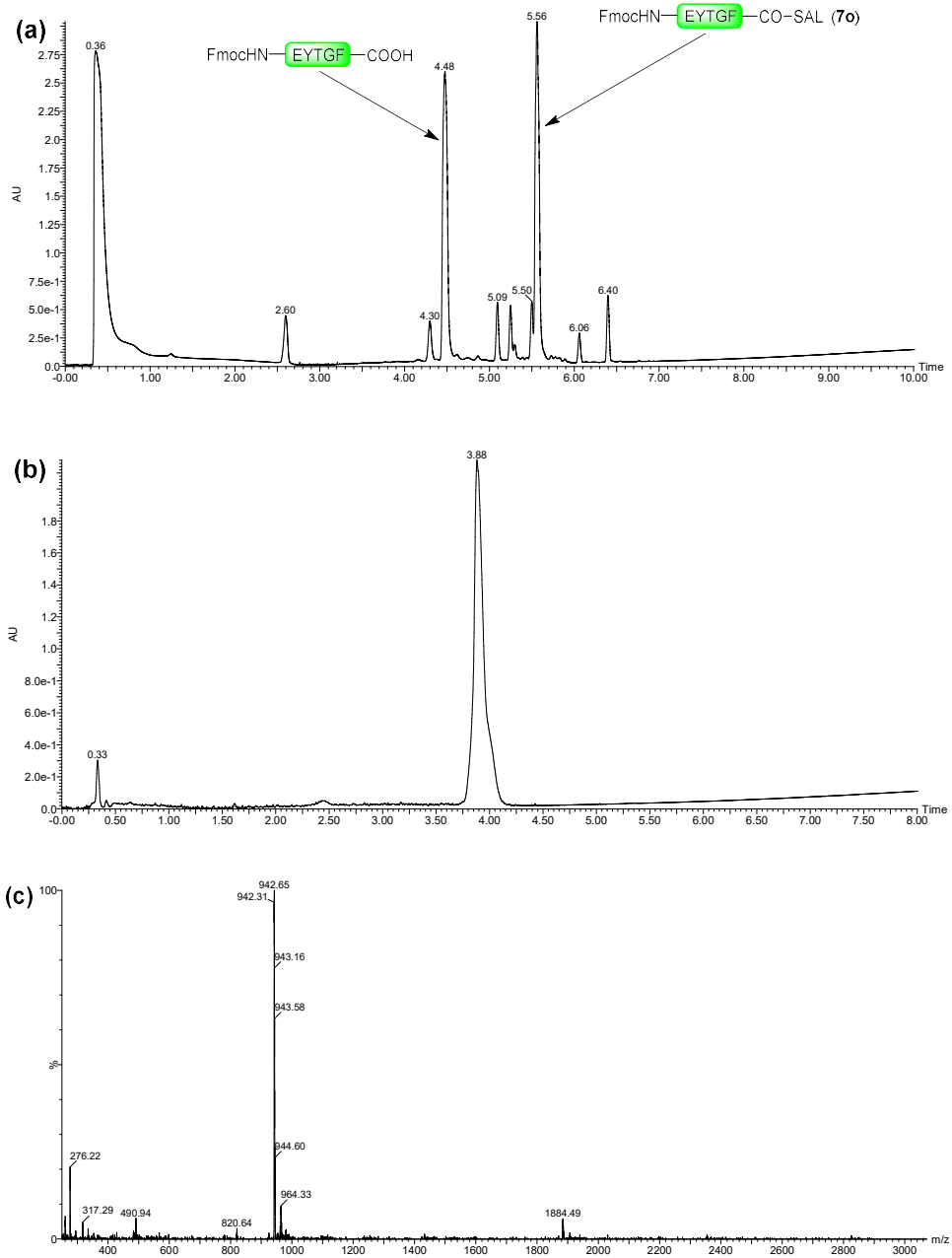
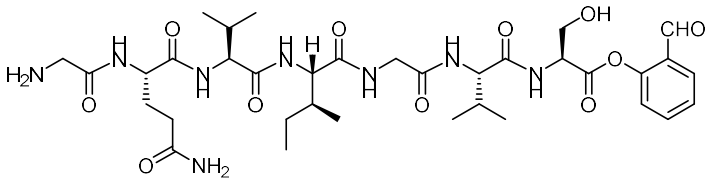
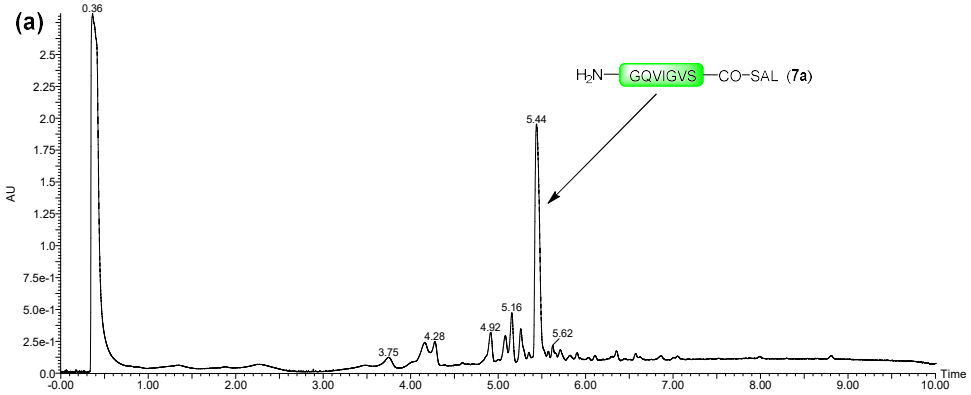


Figure S22. (a) UV trace from analytical RP-UPLC of crude reaction mixture. Gradient: 35-90% ACN/H_2O containing 0.1% TFA over 10 min at a flow rate of 0.4 mL/min. (b) UV trace from analytical RP-UPLC of **7o**. Gradient: 40-90% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. (c) ESI-MS of **7o**. ESI-MS calcd. for $C_{51}H_{52}N_5O_{13}$ $[M+H]^+$ $m/z = 942.99$, found 942.65.

5.20 *H_2N*-GQVIGVS-*CO*-SAL ester (7a)



A solution of crude DCP reagent (0.12 mmol) in ACN (0.5 mL) was added dropwise slowly (dropwise over about 40 min, 4 drops after every 2 min) to a flask containing the crude fully protected peptidyl acid *BocHN*-Gly-Gln(Trt)-Val-Ile-Gly-Val-Ser(*t*Bu)-*COOH* (prepared according to general experimental procedure **2.1** and **2.2**) (42.3 mg, 0.04 mmol), K_2CO_3 (55.2 mg 0.33 mmol), NaI (144.0 mg, 1.20 mmol) and ACN/DCM (1:4, 10 mL) at room temperature under an Ar atmosphere. After stirring at room temperature for 2 h, the mixture was filtered, and the filter cake was washed with ACN (10 mL) and DCM (10 mL). The filtration was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*, 5 mL) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged. The precipitate was pelleted, and the ether was subsequently decanted. The resulting solid was purified by HPLC and lyophilization to give the peptide SAL ester **7a** (13.6 mg, 44.6%) as white solid.



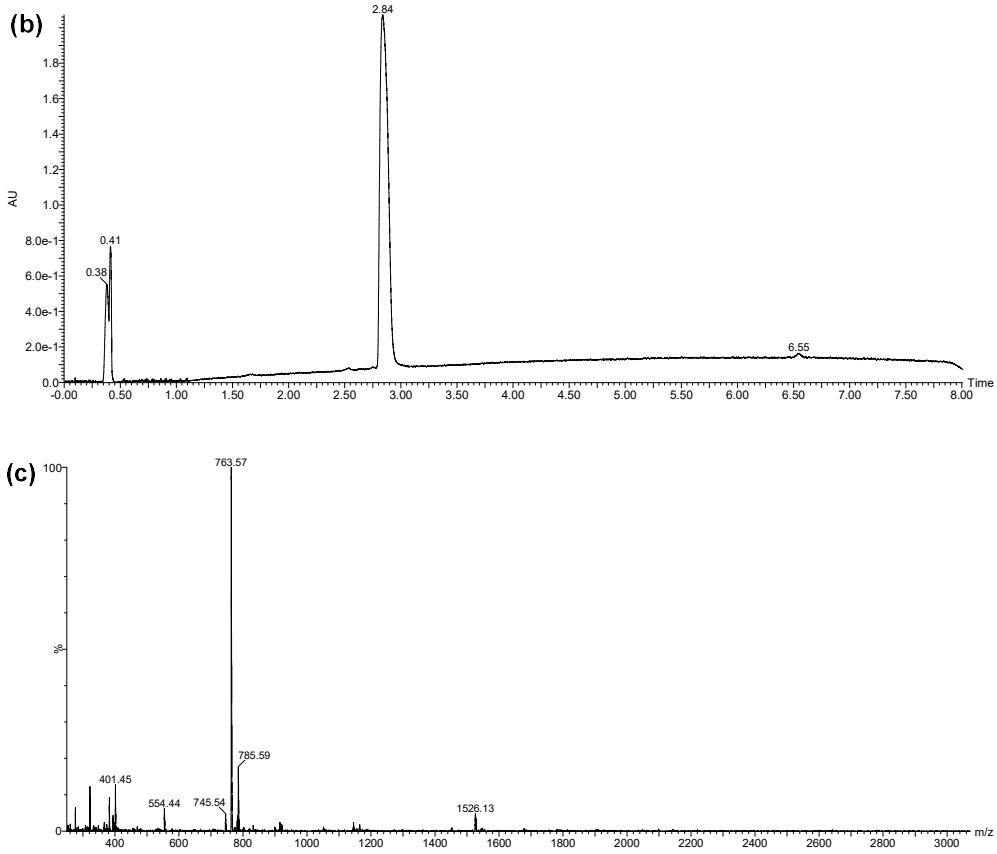
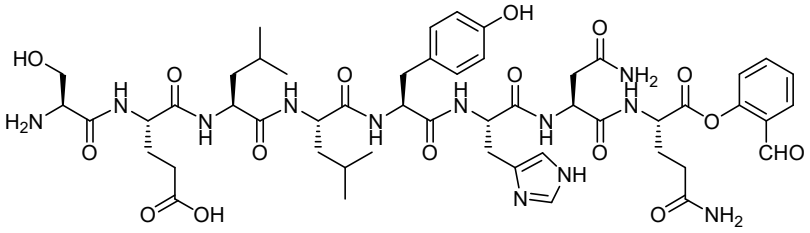


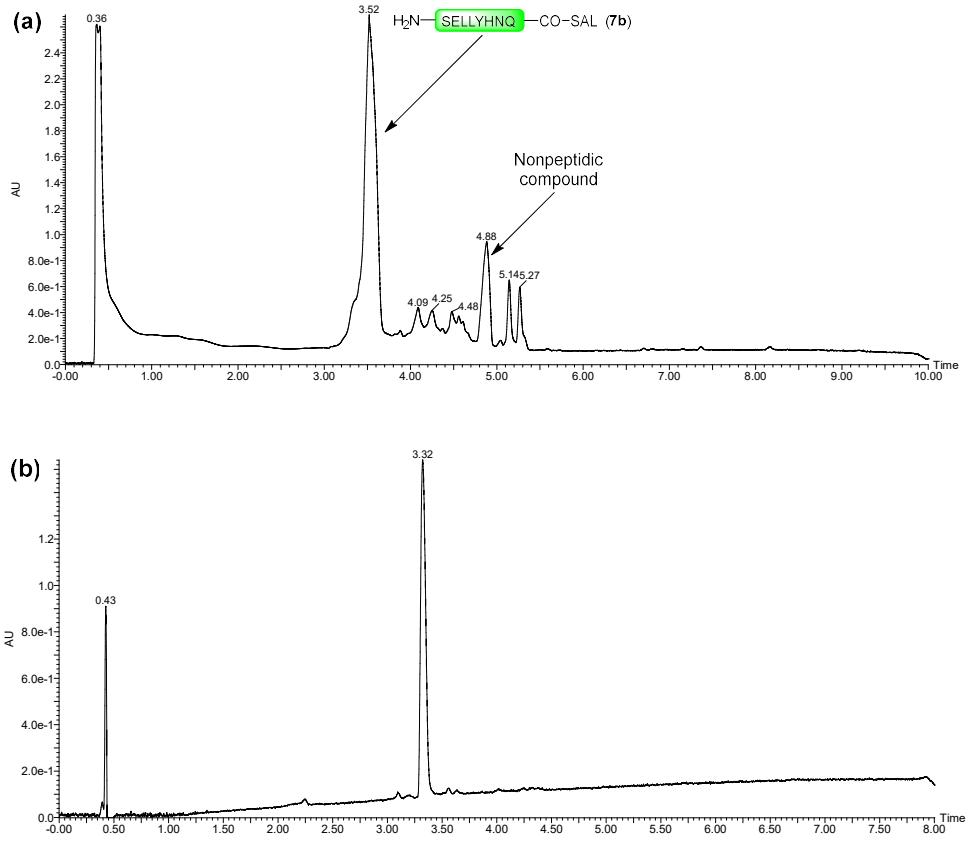
Figure S23. (a) UV trace from analytical RP-UPLC of crude reaction mixture. Gradient: 10-60% ACN/H_2O containing 0.1% TFA over 10 min at a flow rate of 0.4 mL/min. (b) UV trace from analytical RP-UPLC of **7a**. Gradient: 20-60% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. (c) ESI-MS of **7a**. ESI-MS calcd. for $C_{35}H_{55}N_8O_{11}$ $[M+H]^+$ m/z = 763.89, found 763.57.

5.21 *H_2N*-SELLYHNQ-*CO*-SAL ester (7b)



A solution of crude DCP reagent (0.12 mmol) in ACN (0.5 mL) was added dropwise slowly (dropwise over about 40 min, 4 drops after every 2 min) to a flask containing the crude fully protected peptidyl acid *BocHN*-Ser(*t*Bu)-Glu(O*t*Bu)-Leu-Leu-Tyr(*t*Bu)-His(Trt)-Asn(Trt)-

Gln(Trt)-*COOH* (prepared according to general experimental procedure **2.1** and **2.2**) (80.0 mg, 0.04 mmol), K_2CO_3 (55.2 mg 0.33 mmol), NaI (144.0 mg, 1.20 mmol) and ACN/DCM (1:4, 10 mL) at room temperature under an Ar atmosphere. After stirring at room temperature for 2 h, the mixture was filtered, and the filter cake was washed with ACN (10 mL) and DCM (10 mL). The filtration was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*, 5 mL) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged. The precipitate was pelleted, and the ether was subsequently decanted. The resulting solid was purified by HPLC and lyophilization to give the peptide SAL ester **7b** (22.6 mg, 51.0%) as white solid.



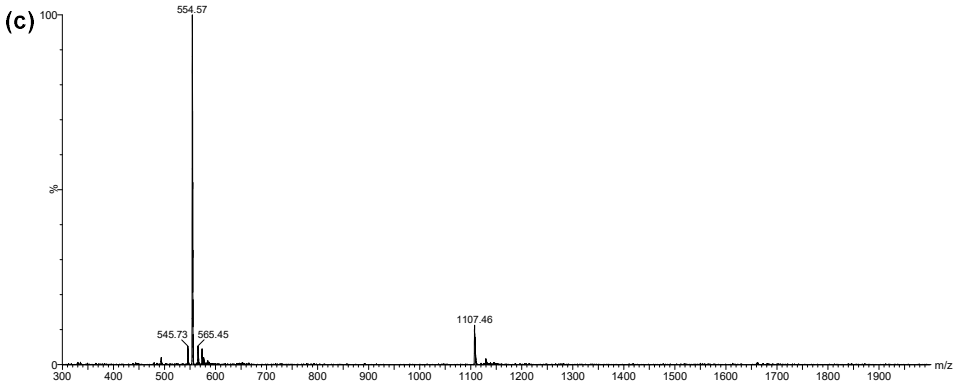
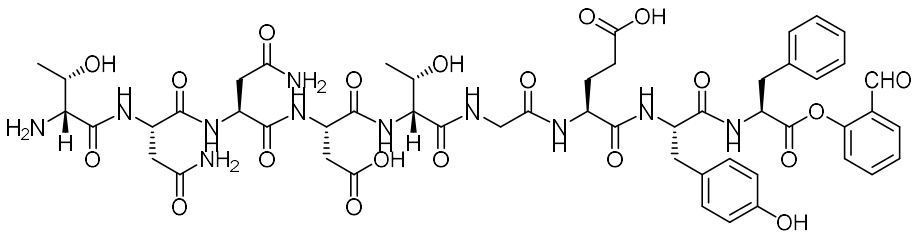


Figure S24. (a) UV trace from analytical RP-UPLC of crude reaction mixture. Gradient: 20-60% ACN/H_2O containing 0.1% TFA over 10 min at a flow rate of 0.4 mL/min. (b) UV trace from analytical RP-UPLC of **7b**. Gradient: 10-50% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. (c) ESI-MS of **7b**. ESI-MS calcd. for $C_{51}H_{71}N_{12}O_{16}$ $[M+H]^+$ m/z = 1108.20, found 1107.46.

5.22 *H_2N*-TNNDTGEYF-*CO*-SAL ester (7c)



A solution of crude DCP reagent (0.12 mmol) in ACN (0.5 mL) was added dropwise slowly (dropwise over about 40 min, 4 drops after every 2 min) to a flask containing the crude fully protected peptidyl acid *BocHN*-Thr(*t*Bu)-Asn(Trt)-Asn(Trt)-Asp(O*t*Bu)-Thr(*t*Bu)-Gly-Glu(O*t*Bu)-Tyr(*t*Bu)-Phe-*COOH* (prepared according to general experimental procedure **2.1** and **2.2**) (77.0 mg, 0.04 mmol), K_2CO_3 (55.2 mg 0.33 mmol), NaI (144.0 mg, 1.20 mmol) and ACN/DCM (1:4, 10 mL) at room temperature under an Ar atmosphere. After stirring at room temperature for 2 h, the mixture was filtered, and the filter cake was washed with ACN (10 mL) and DCM (10 mL). The filtration was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*, 5 mL) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged. The

precipitate was pelleted, and the ether was subsequently decanted. The resulting solid was purified by HPLC and lyophilization to give the peptide SAL ester **7c** (31.5 mg, 67.7%) as white solid.

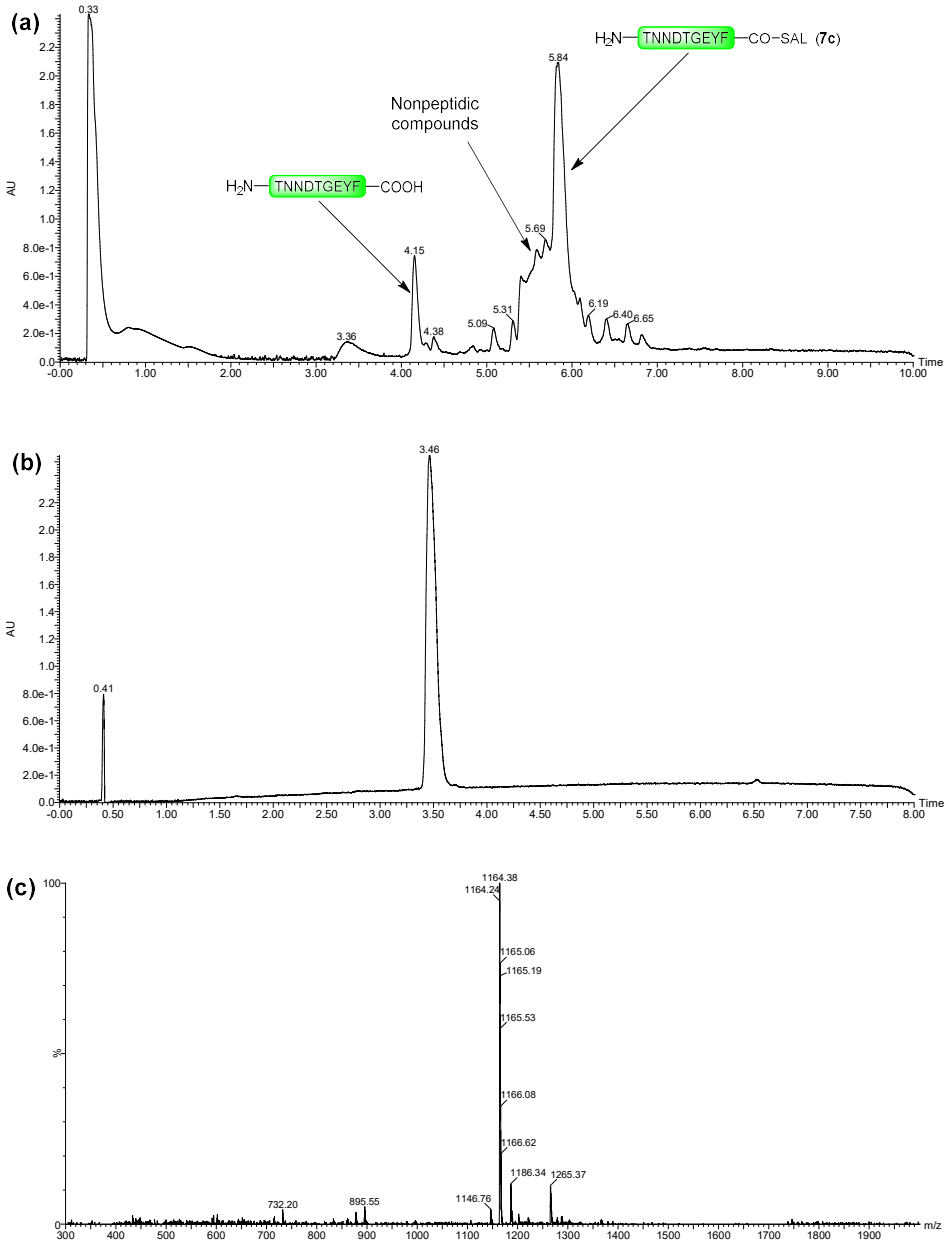
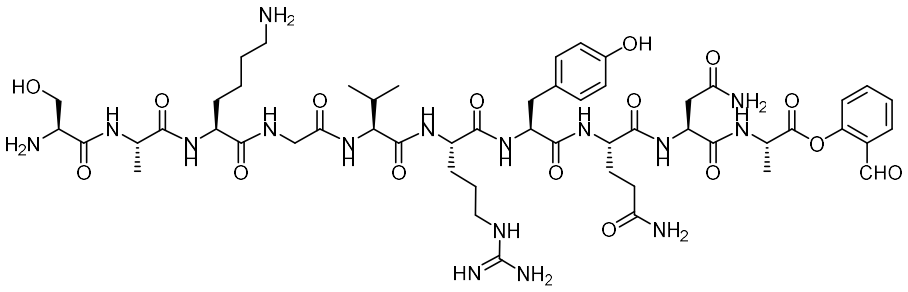
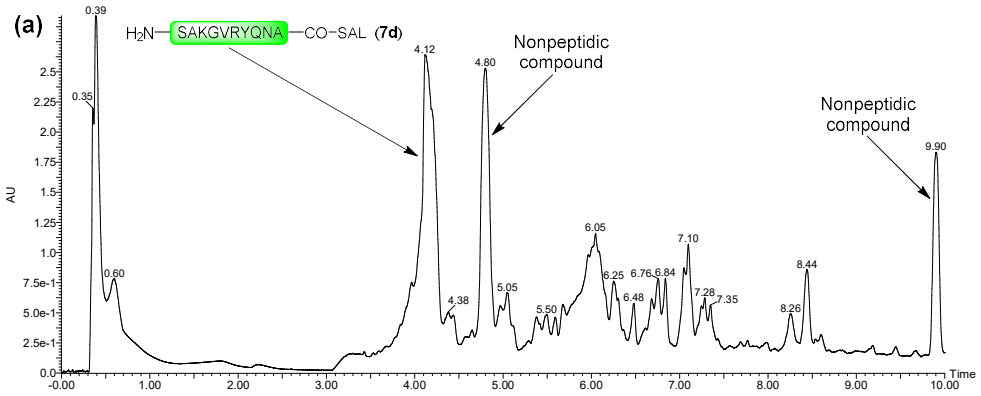


Figure S25. (a) UV trace from analytical RP-UPLC of crude reaction mixture. Gradient: 10-60% ACN/H_2O containing 0.1% TFA over 10 min at a flow rate of 0.4 mL/min. (b) UV trace from analytical RP-UPLC of **7c**. Gradient: 15-60% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. (c) ESI-MS of **7c**. ESI-MS calcd. for $C_{52}H_{66}N_{11}O_{20}$ $[M+H]^+$ $m/z = 1165.15$, found 1164.38.

5.23 *H₂N*-SAKGVRYQNA-*CO*-SAL ester (7d)



A solution of DCP reagent (0.15 mmol) in ACN (0.5 mL) was added dropwise slowly (dropwise over 40 min) to a flask containing the crude fully protected peptidyl acid *BocHN*-Ser(*t*Bu)-Ala-Lys(Boc)-Gly-Val-Arg(Pbf)-Tyr(*t*Bu)-Gln(Trt)-Asn(Trt)-Ala-*COOH* (prepared according to general experimental procedure **2.1** and **2.2**) (64.3 mg, 0.03 mmol), K_2CO_3 (99.4 mg, 0.72 mmol), NaI (135 mg, 0.9 mmol) and mL ACN/DCM (1:4, 15 mL) at room temperature under an Ar atmosphere. Then the mixture was stirred at room temperature for 2 h. After that, the reaction solvent was poured into the other flask (left solid salt in the flask). The solvent was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged. The precipitate was pelleted, and the ether was subsequently decanted. The resulting solid was purified by HPLC and lyophilization to give the long peptide SAL ester **7d** (19.8 mg, 55.2%) as white solid.



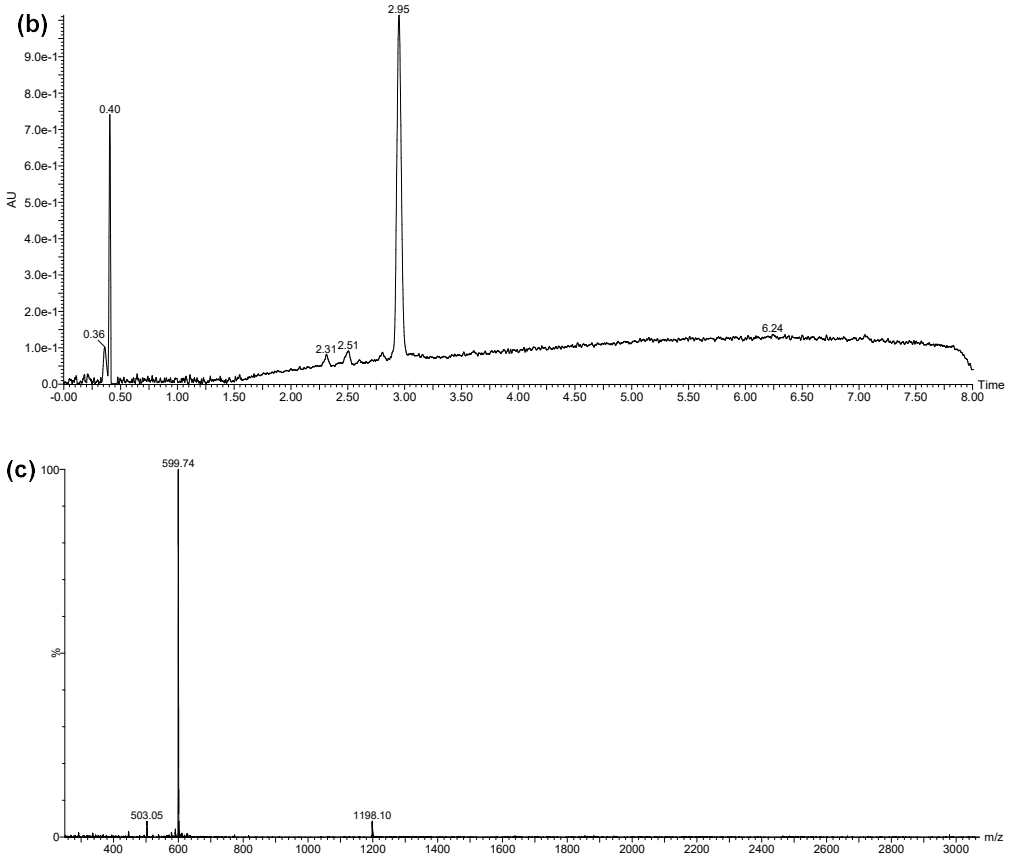
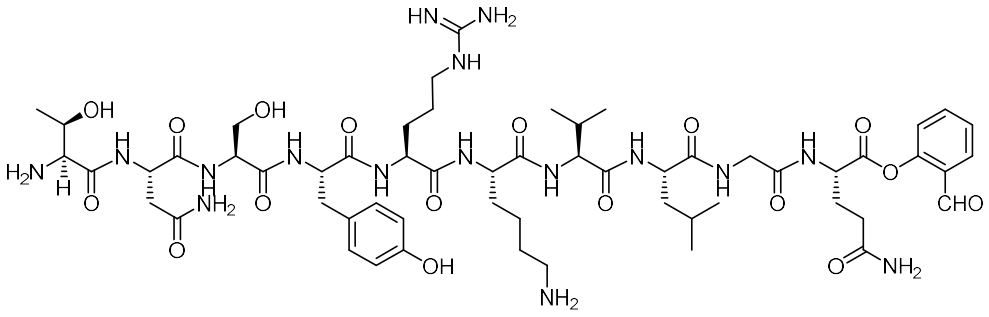
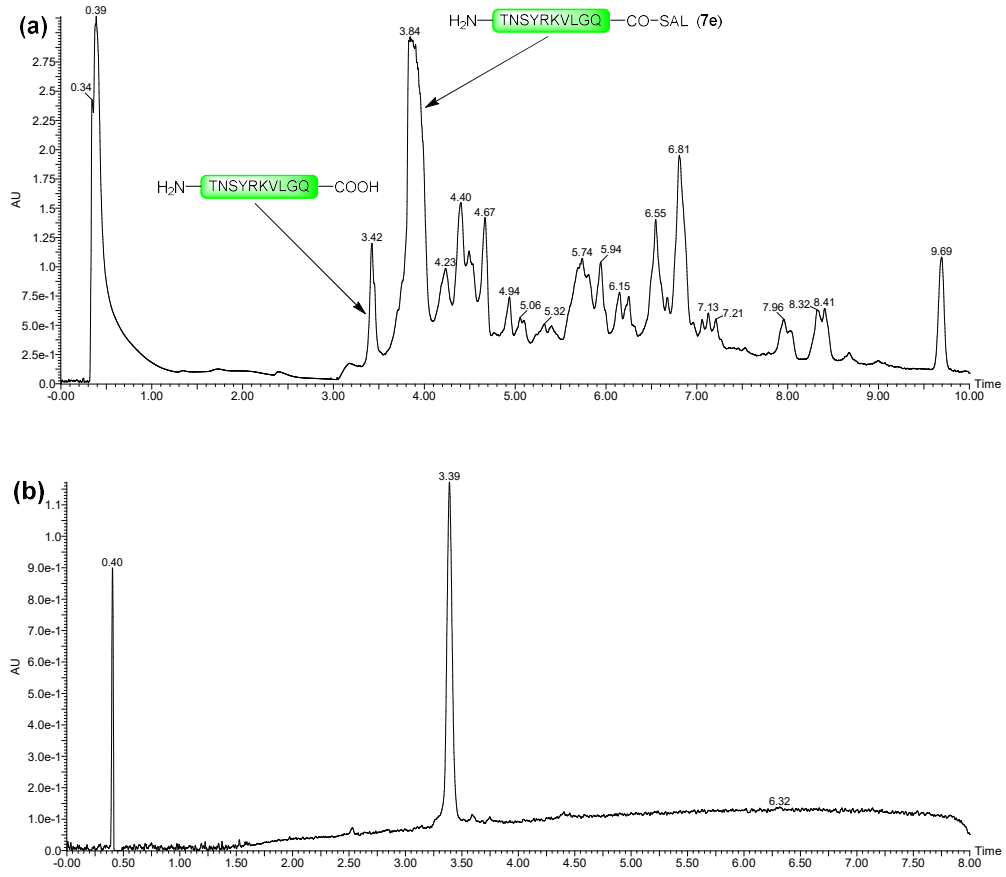


Figure S26. (a) UV trace from analytical RP-UPLC of crude reaction mixture. Gradient: 15-60% ACN/H_2O containing 0.1% TFA over 10 min at a flow rate of 0.4 mL/min. (b) UV trace from analytical RP-UPLC of **7d**. Gradient: 10-60% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. (c) ESI-MS of **7d**. ESI-MS calcd. for $C_{53}H_{81}N_{16}O_{16}$ $[M+H]^+$ m/z = 1198.33, found 1198.10.

5.24 *H_2N*-TNSYRKVLGQ-*CO*-SAL ester (7e)



A solution of DCP reagent (0.15 mmol) in ACN (0.5 mL) was added dropwise slowly (dropwise over 40 min) to a flask containing the crude fully protected peptidyl acid *BocHN*-Thr(tBu)-Asn(Trt)-Ser(*t*Bu)-Tyr(*t*Bu)-Arg(Pbf)-Lys(Boc)-Val-Leu-Gly-Gln(Trt)-*COOH* (prepared according to general experimental procedure **2.1** and **2.2**) (68.1 mg, 0.03 mmol), K_2CO_3 (99.4 mg, 0.72 mmol), NaI (135 mg, 0.9 mmol) and mL ACN/DCM (1:4, 15 mL) at room temperature under an Ar atmosphere. Then the mixture was stirred at room temperature for 2 h. After that, the reaction solvent was poured into the other flask (left solid salt in the flask). The solvent was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged. The precipitate was pelleted, and the ether was subsequently decanted. The resulting solid was purified by HPLC and lyophilization to give the long peptide SAL ester **7e** (14.9 mg, 39.1%) as white solid.



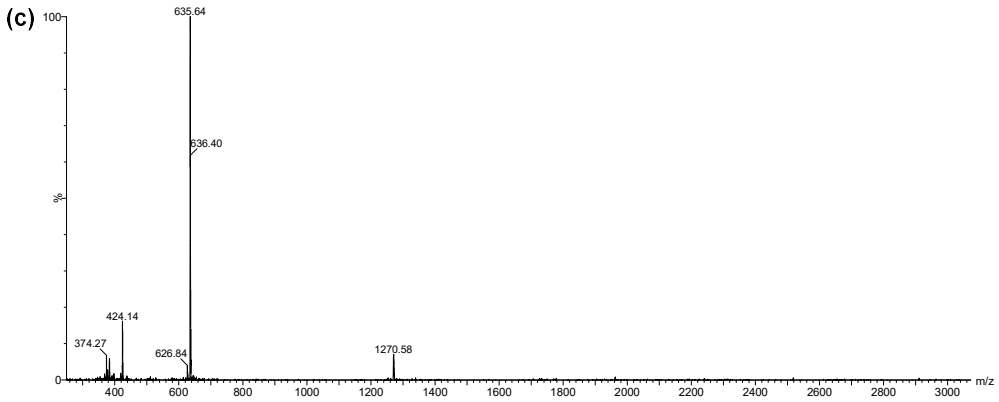
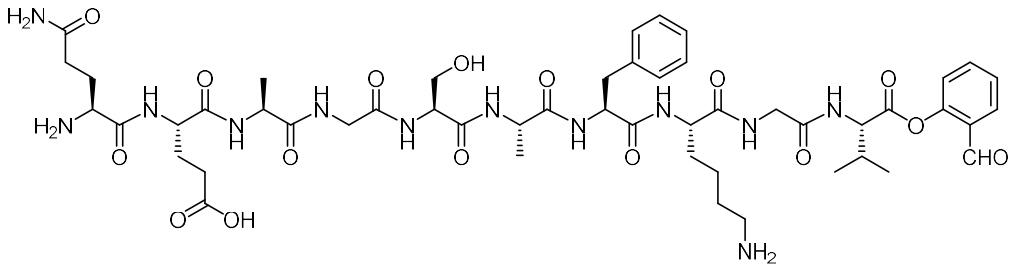


Figure S27. (a) UV trace from analytical RP-UPLC of crude reaction mixture. Gradient: 20-60% ACN/H_2O containing 0.1% TFA over 10 min at a flow rate of 0.4 mL/min. (b) UV trace from analytical RP-UPLC of **7e**. Gradient: 10-60% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. (c) ESI-MS of **7e**. ESI-MS calcd. for $C_{57}H_{89}N_{16}O_{17}$ $[M+H]^+$ m/z = 1270.43, found 1270.58.

5.25 *H_2N*-QEAGSAFKGV-*CO*-SAL ester (7f)



A solution of DCP reagent (0.15 mmol) in ACN (0.5 mL) was added dropwise slowly (dropwise over 40 min) to a flask containing the crude fully protected peptidyl acid *BocHN*-Gln(Trt)-Glu(O*t*Bu)-Ala-Gly-Ser(*t*Bu)-Ala-Phe-Lys(Boc)-Gly-Val-*COOH* (prepared according to general experimental procedure **2.1** and **2.2**) (46.4 mg, 0.03 mmol), K_2CO_3 (99.4 mg, 0.72 mmol), NaI (135 mg, 0.9 mmol) and mL ACN/DCM (1:4, 15 mL) at room temperature under an Ar atmosphere. Then the mixture was stirred at room temperature for 2 h. After that, the reaction solvent was poured into the other flask (left solid salt in the flask). The solvent was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged. The precipitate was pelleted, and the ether was subsequently

decanted. The resulting solid was purified by HPLC and lyophilization to give the long peptide SAL ester **7f** (15.2 mg, 46.2%) as white solid.

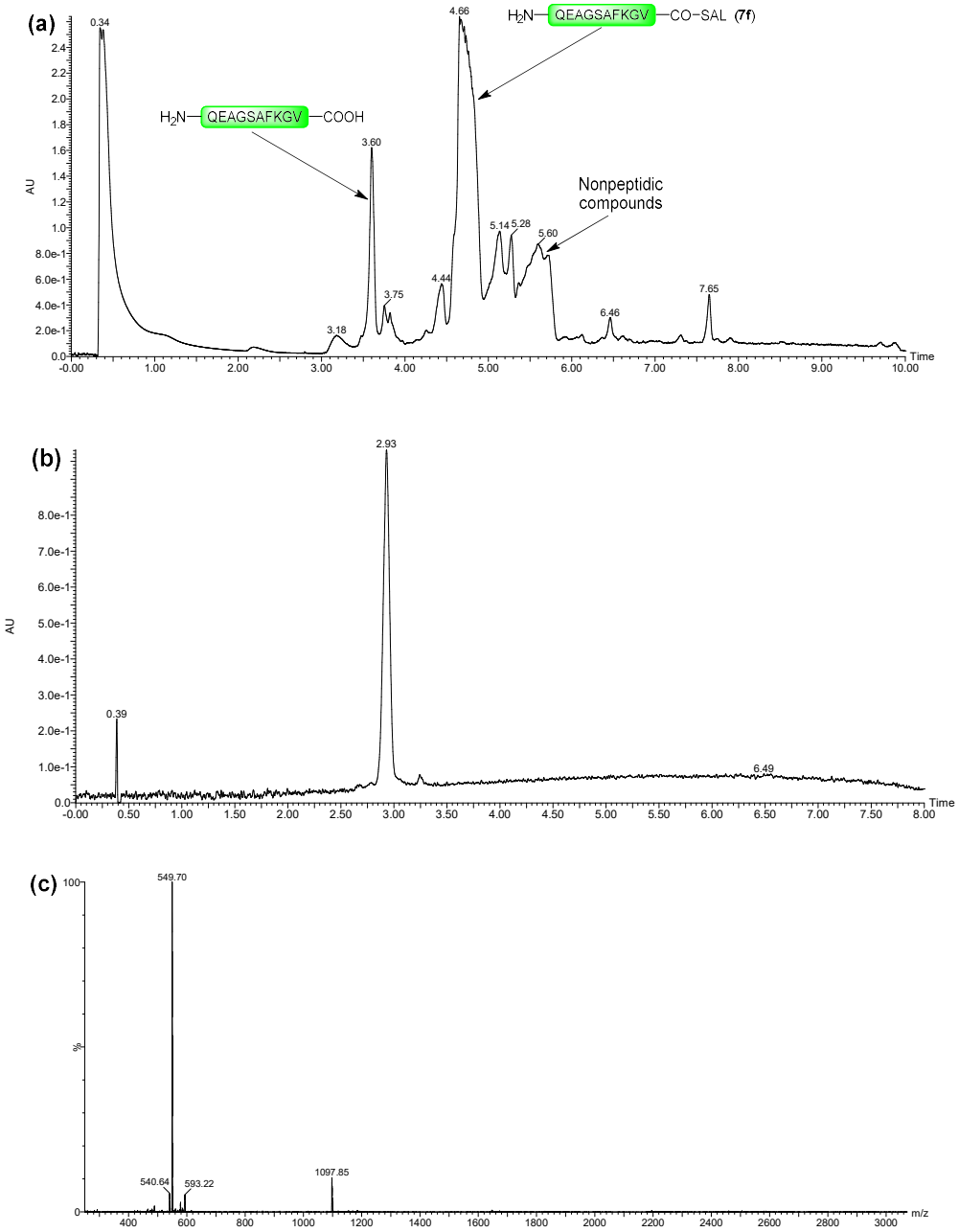
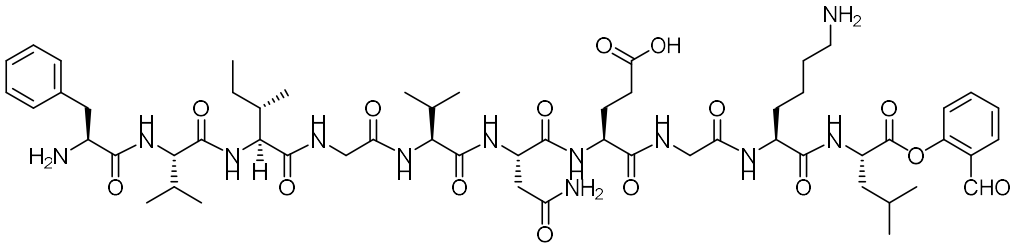
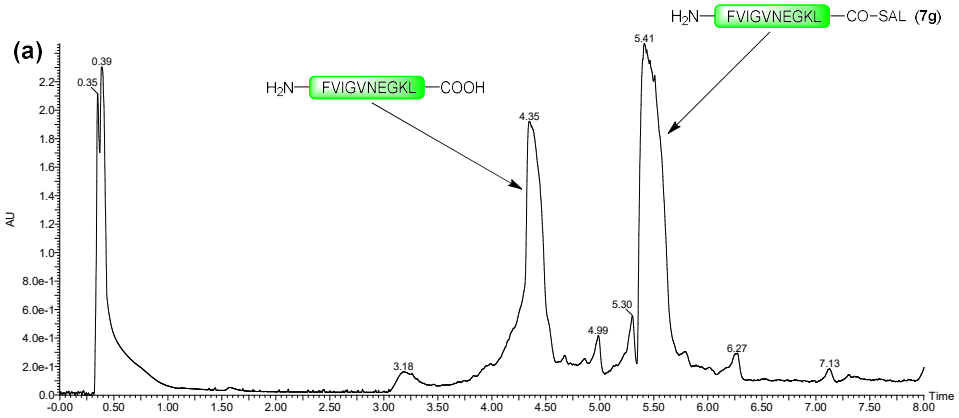


Figure S28. (a) UV trace from analytical RP-UPLC of crude reaction mixture. Gradient: 20-60% ACN/H_2O containing 0.1% TFA over 10 min at a flow rate of 0.4 mL/min. (b) UV trace from analytical RP-UPLC of **7f**. Gradient: 25-60% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. (c) ESI-MS of **7f**. ESI-MS calcd. for $C_{50}H_{73}N_{12}O_{16}$ $[M+H]^+$ m/z = 1098.20, found 1097.85.

5.26 *H_2N*-FVIGVNEGKL-*CO*-SAL ester (7g)



A solution of DCP reagent (0.15 mmol) in ACN (0.5 mL) was added dropwise slowly (dropwise over 40 min) to a flask containing the crude fully protected peptidyl acid *BocHN*-Phe-Val-Ile-Gly-Val-Asn(Trt)-Glu(O*t*Bu)-Gly-Lys(Boc)-Leu-*COOH* (prepared according to general experimental procedure **2.1** and **2.2**) (47.2 mg, 0.03 mmol), K_2CO_3 (99.4 mg, 0.72 mmol), NaI (135 mg, 0.9 mmol) and mL ACN/DCM (1:4, 15 mL) at room temperature under an Ar atmosphere. Then the mixture was stirred at room temperature for 2 h. After that, the reaction solvent was poured into the other flask (left solid salt in the flask). The solvent was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged. The precipitate was pelleted, and the ether was subsequently decanted. The resulting solid was purified by HPLC and lyophilization to give the long peptide SAL ester **7g** (12.9 mg, 36.5%) as white solid.



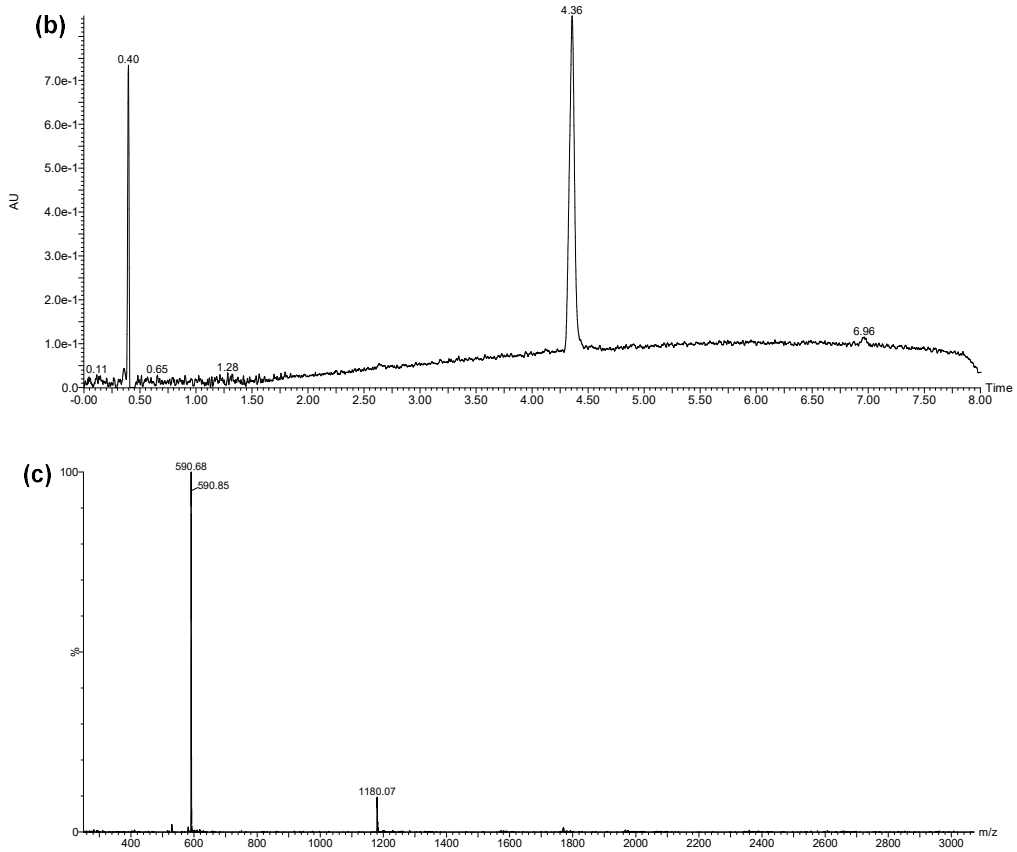
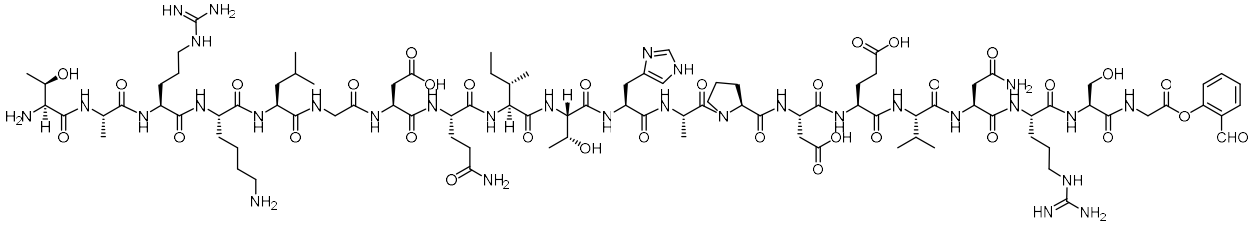


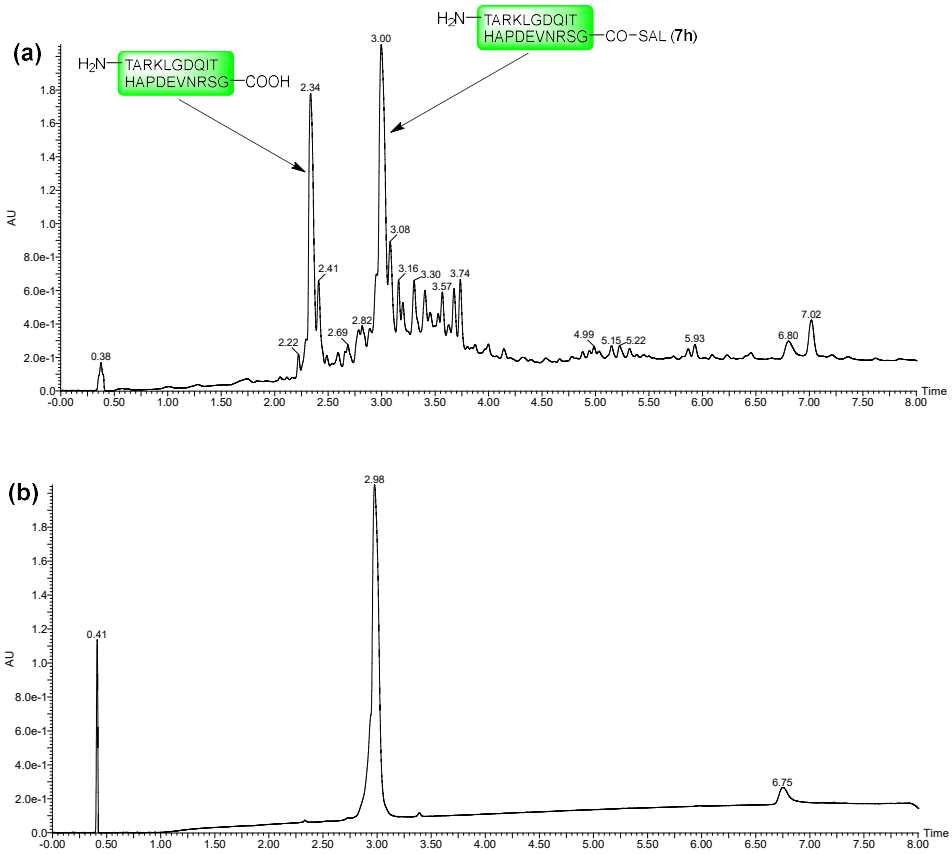
Figure S29. (a) UV trace from analytical RP-UPLC of crude reaction mixture. Gradient: 20-60% ACN/H_2O containing 0.1% TFA over 10 min at a flow rate of 0.4 mL/min. (b) UV trace from analytical RP-UPLC of **7g**. Gradient: 25-60% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. (c) ESI-MS of **7g**. ESI-MS calcd. for $C_{57}H_{87}N_{12}O_{15}$ $[M+H]^+$ m/z = 1180.39, found 1180.07.

5.27 *H_2N*-TARKLGDQITHAPDEVNRSG-*CO*-SAL ester (7h)



A solution of DCP reagent (0.05 mmol) in ACN (0.3 mL) was added dropwise slowly (dropwise over 40 min) to a flask containing the crude fully protected peptidyl acid *BocHN*-Thr(*t*Bu)-Ala-

Arg(Pbf)-Lys(Boc)-Leu-Gly-Asp(O*t*Bu)-Gln(Trt)-Ile-Thr(*t*Bu)-His(Trt)-Ala-Pro-Asp(O*t*Bu)-Glu(O*t*Bu)-Val-Asn(Trt)-Arg(Pbf)-Ser(*t*Bu)-Gly-*COOH* (prepared according to general experimental procedure **2.1** and **2.2**) (77.5 mg, 0.02 mmol), K_2CO_3 (66.2 mg, 0.48 mmol), NaI (90.0 mg, 0.6 mmol) and mL ACN/DCM (1:4, 15 mL) at room temperature under an Ar atmosphere. Then the mixture was stirred at room temperature for 2 h. After that, the reaction solvent was poured into the other flask (left solid salt in the flask). The solvent was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged. The precipitate was pelleted, and the ether was subsequently decanted. The resulting solid was purified by HPLC and lyophilization to give the long peptide SAL ester **7h** (14.2 mg, 31.3%) as white solid.



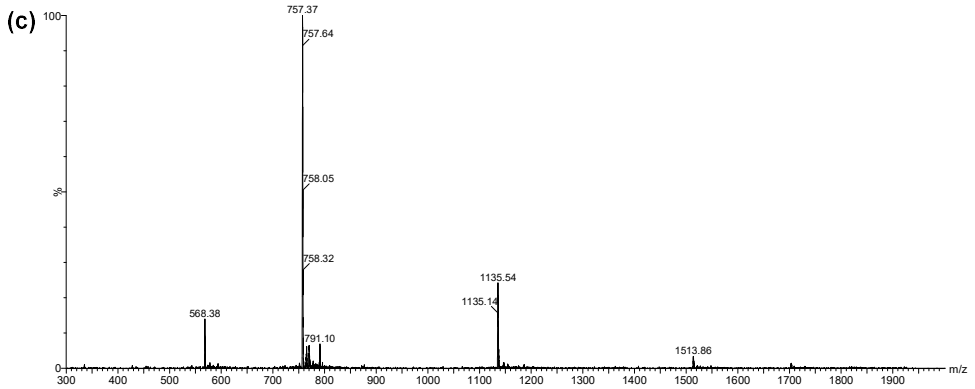
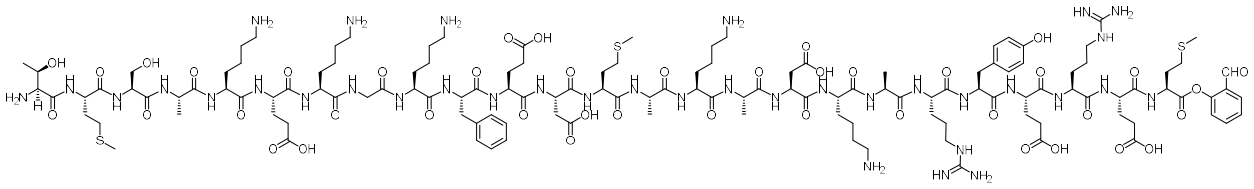


Figure S30. (a) UV trace from analytical RP-UPLC of crude reaction mixture. Gradient: 10-60% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. (b) UV trace from analytical RP-UPLC of **7h**. Gradient: 10-50% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. (c) ESI-MS of **7h**. ESI-MS calcd. for $C_{96}H_{155}N_{31}O_{33}$ $[M+2H]^{2+}$ m/z = 1135.73, found 1135.54; $[M+3H]^{3+}$ m/z =757.49, found 757.37; $[M+4H]^{4+}$ m/z = 568.37, found 568.38.

5.28 *H_2N*-TMSAKEKGKFEDMAKADKARYEREM-*CO*-SAL ester (7i, from HMGB1 (51-75))



A solution of DCP reagent (0.1 mmol) in ACN (0.5 mL) was added dropwise slowly (dropwise over 40 min) to a flask containing the crude fully protected peptidyl acid *BocHN*-Thr(*t*Bu)-Met-Ser(*t*Bu)-Ala-Lys(Boc)-Glu(O*t*Bu)-Lys(Boc)-Gly-Lys(Boc)-Phe-Glu(O*t*Bu)-Asp(O*t*Bu)-Met-Ala-Lys(Boc)-Ala-Asp(O*t*Bu)-Lys(Boc)-Ala-Arg(Pbf)-Tyr(*t*Bu)-Glu(O*t*Bu)-Arg(Pbf)-Glu(O*t*Bu)-Met-*COOH* (prepared according to general experimental procedure **2.1** and **2.2**) (91.2 mg, 0.02 mmol), K_2CO_3 (66.3 mg, 0.48 mmol), NaI (90 mg, 0.6 mmol) and mL ACN/DCM (1:4, 20 mL) at room temperature under an Ar atmosphere. Then the mixture was stirred at room temperature for 2 h. After that, the reaction solvent was poured into the other flask (left solid salt in the flask). The solvent was concentrated, and the resulting residue was placed in an ice bath,

then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged. The precipitate was pelleted, and the ether was subsequently decanted. The resulting solid was purified by HPLC and lyophilization to give the long peptide SAL ester **7i** (18.4 mg, 30.1%) as white solid.

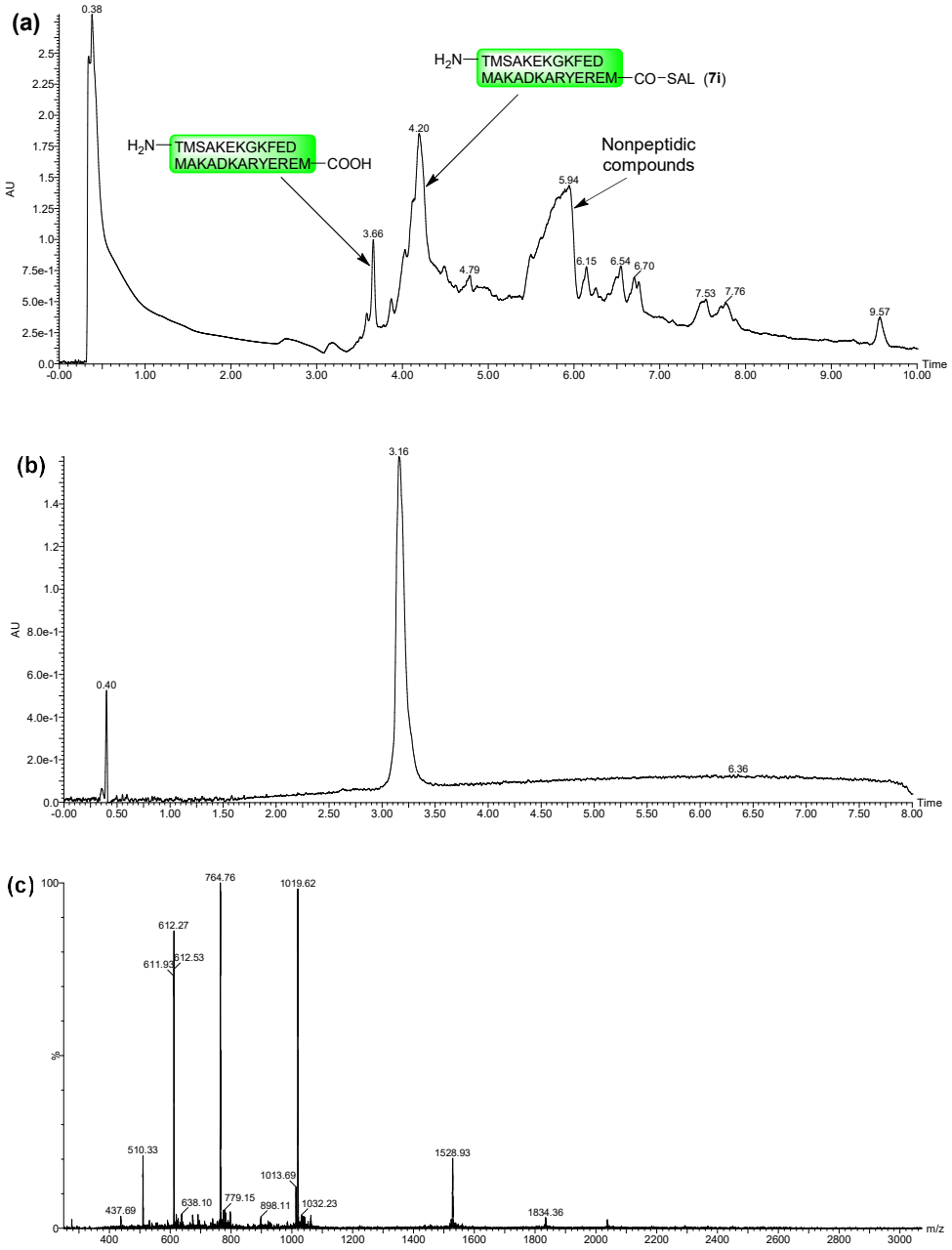
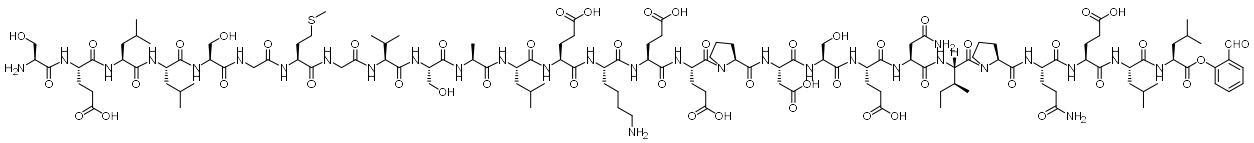


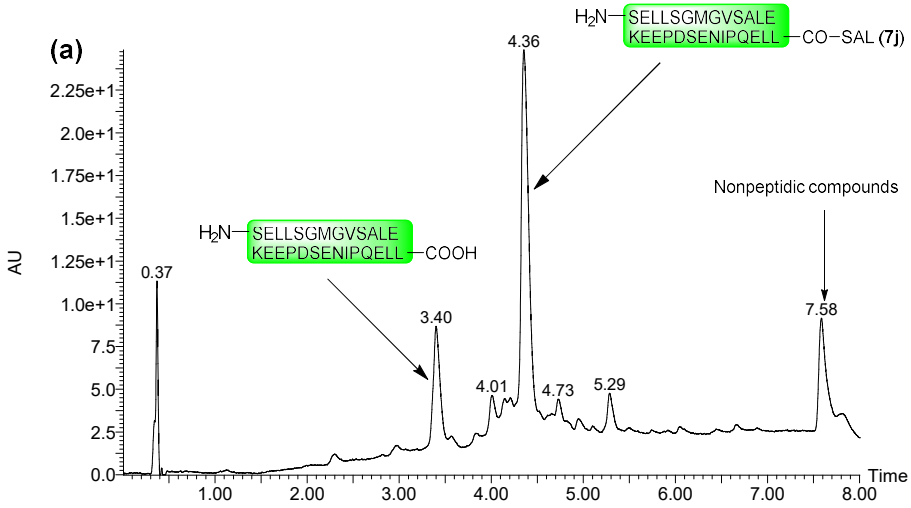
Figure S31. (a) UV trace from analytical RP-UPLC of crude reaction mixture. Gradient: 20-60% ACN/H_2O containing 0.1% TFA over 10 min at a flow rate of 0.4 mL/min. (b) UV trace from analytical RP-UPLC of **7i**. Gradient: 15-60% ACN/H_2O containing 0.1% TFA over 8 min at a

flow rate of 0.4 mL/min. (c) ESI-MS of **7i**. ESI-MS calcd. for $C_{131}H_{210}N_{36}O_{42}S_3$ $[M+2H]^{2+}$ m/z = 1528.75, found 1528.93; $[M+3H]^{3+}$ m/z = 1019.50, found 1019.62; $[M+4H]^{4+}$ m/z = 764.88, found 764.76; $[M+5H]^{5+}$ m/z = 612.10, found 612.27.

5.29 *H₂N*-SELLSGMGVSALEKEEPDSENIPQELL-*CO*-SAL ester (7j, from SKP2 (30-56))



A solution of DCP reagent (0.15 mmol) in ACN (0.75 mL) was added dropwise slowly (dropwise over 40 min) to a flask containing the crude fully protected peptidyl acid *BocHN*-Ser(*t*Bu)-Glu(O*t*Bu)-Leu-Leu-Ser(*t*Bu)-Gly-Met-Gly-Val-Ser(*t*Bu)-Ala-Leu-Glu(O*t*Bu)-Lys(Boc)-Glu(O*t*Bu)-Glu(O*t*Bu)-Pro-Asp(O*t*Bu)-Ser(*t*Bu)-Glu(O*t*Bu)-Asn(Trt)-Ile-Pro-Gln(Trt)-Glu(O*t*Bu)-Leu-Leu-*COOH* (prepared according to general experimental procedure **2.1** and **2.2**) (126.5 mg, 0.03 mmol), K_2CO_3 (99.4 mg, 0.72 mmol), NaI (135 mg, 0.9 mmol) and ACN/DCM (1:4, 15 mL) at room temperature under an Ar atmosphere. Then the mixture was stirred at room temperature for 2 h. After that, the reaction solvent was poured into the other flask (left solid salt in the flask). The solvent was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged. The precipitate was pelleted, and the ether was subsequently decanted. The resulting solid was purified by HPLC and lyophilization to give the long peptide SAL ester **7j** (29.3 mg, 32.4%) as white solid.



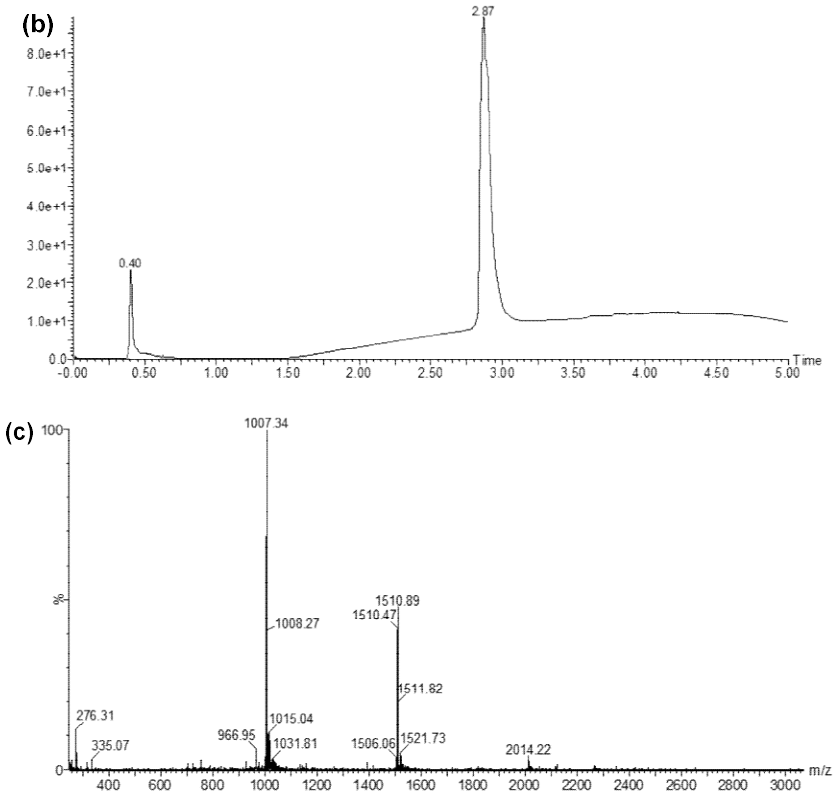
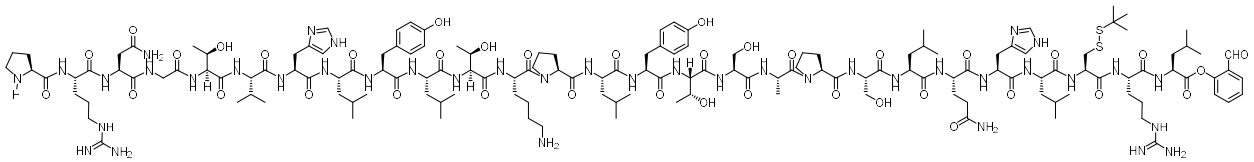


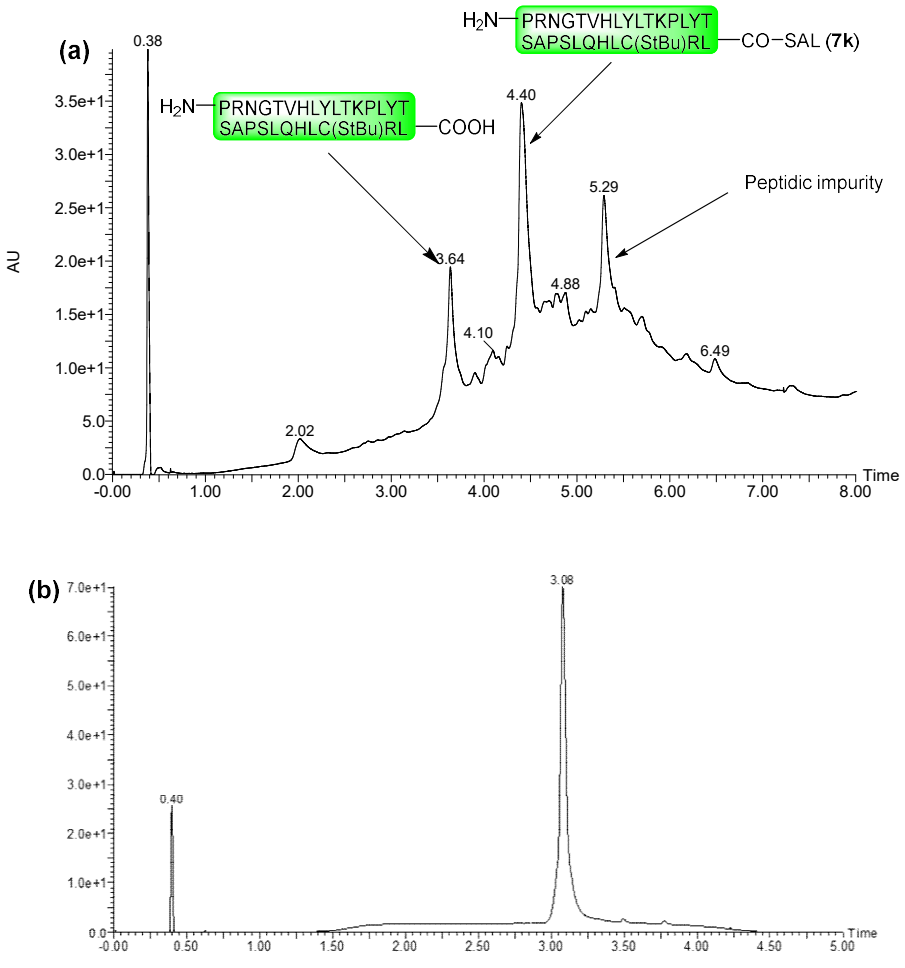
Figure S32. (a) UV trace from analytical RP-UPLC of crude reaction mixture. Gradient: 20-60% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. (b) UV trace from analytical RP-UPLC and its ESI-MS of **7j**. Gradient: 5-95% ACN/H_2O containing 0.1% TFA over 5 min at a flow rate of 0.4 mL/min. (c) ESI-MS of **7j**. ESI-MS calcd. for $C_{131}H_{208}N_{30}O_{49}S$ $[M+2H]^{2+}$ m/z = 1510.67, found 1510.89; $[M+3H]^{3+}$ m/z = 1007.44, found 1007.34.

5.30 *H_2N*-PRNGTVHLYLTKPLYTSAPSLQHLC(StBu)RL-*CO*-SAL ester (7k, from SOCS2 (143-169))



A solution of DCP reagent (0.2 mmol) in ACN (0.75 mL) was added dropwise slowly (dropwise over 40 min) to a flask containing the crude fully protected peptidyl acid *BocHN*-Pro-Arg(Pbf)-Asn(Trt)-Gly-Thr(*t*Bu)-Val-His(Trt)-Leu-Tyr(*t*Bu)-Leu-Thr(*t*Bu)-Lys(Boc)-Pro-Leu-Tyr(*t*Bu)-

Thr(*t*Bu)-Ser(*t*Bu)-Ala-Pro-Ser(*t*Bu)-Leu-Gln(Trt)-His(Trt)-Leu-Cys(S*t*Bu)-Arg(Pbf)-Leu-*COOH* (prepared according to general experimental procedure **2.1** and **2.2**) (209.4 mg, 0.04 mmol), K_2CO_3 (132.5 mg, 0.96 mmol), NaI (180 mg, 1.20 mmol) and ACN/DCM (1:4, 20 mL) at room temperature under an Ar atmosphere. Then the mixture was stirred at room temperature for 2 h. After that, the reaction solvent was poured into the other flask (left solid salt in the flask). The solvent was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged. The precipitate was pelleted, and the ether was subsequently decanted. The resulting solid was purified by HPLC and lyophilization to give the long peptide SAL ester **7k** (26.1 mg, 20.0%) as white solid.



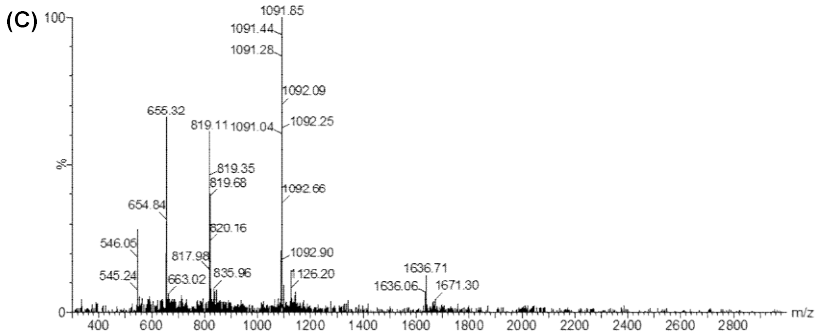
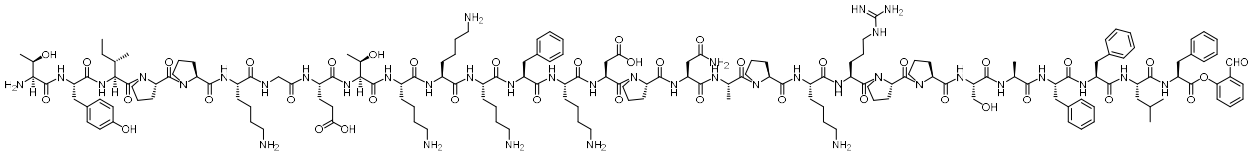


Figure S33. (a) UV trace from analytical RP-UPLC of crude reaction mixture. Gradient: 20-60% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. (b) UV trace from analytical RP-UPLC and its ESI-MS of **7k**. Gradient: 5-95% ACN/H_2O containing 0.1% TFA over 5 min at a flow rate of 0.4 mL/min. (c) ESI-MS of **7k**. ESI-MS calcd. for $C_{150}H_{236}N_{40}O_{38}S_2$ $[M+2H]^{2+}$ *m*/*z* =1636.95, found 1636.71; $[M+3H]^{3+}$ *m*/*z* =1091.63, found 1091.85; $[M+4H]^{4+}$ *m*/*z* =818.98, found 819.11; $[M+5H]^{5+}$ *m*/*z* =655.38, found 655.32; $[M+6H]^{6+}$ *m*/*z* = 546.31, found 546.05.

5.31 *H_2N*-TYIPPKGETKKKFKDPNAPKRPPSAFFLF-*CO*-SAL ester (7l, from HMGB1 (77-105))



A solution of DCP reagent (0.1 mmol) in ACN (0.5 mL) was added dropwise slowly (dropwise over 40 min) to a flask containing the crude fully protected peptidyl acid *BocHN*-Thr(*t*Bu)-Tyr(*t*Bu)-Ile-Pro-Pro-Lys(Boc)-Gly-Glu(O*t*Bu)-Thr(*t*Bu)-Lys(Boc)-Lys(Boc)-Lys(Boc)-Phe-Lys(Boc)-Asp(O*t*Bu)-Pro-Asn(Trt)-Ala-Pro-Lys(Boc)-Arg(Pbf)-Pro-Pro-Ser(*t*Bu)-Ala-Phe-Phe-Leu-Phe-*COOH* (prepared according to general experimental procedure **2.1** and **2.2**) (97.0 mg, 0.02 mmol), K_2CO_3 (66.3 mg, 0.48 mmol), NaI (90 mg, 0.6 mmol) and mL ACN/DCM (1:4, 20 mL) at room temperature under an Ar atmosphere. Then the mixture was stirred at room temperature for 2 h. After that, the reaction solvent was poured into the other flask (left solid salt in the flask). The solvent was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*) was added. After global deprotection for 2 h, TFA

was blown off and the oily residue was triturated with diethyl ether and centrifuged. The precipitate was pelleted, and the ether was subsequently decanted. The resulting solid was purified by HPLC and lyophilization to give the long peptide SAL ester **7l** (25.3 mg, 37.0%) as white solid.

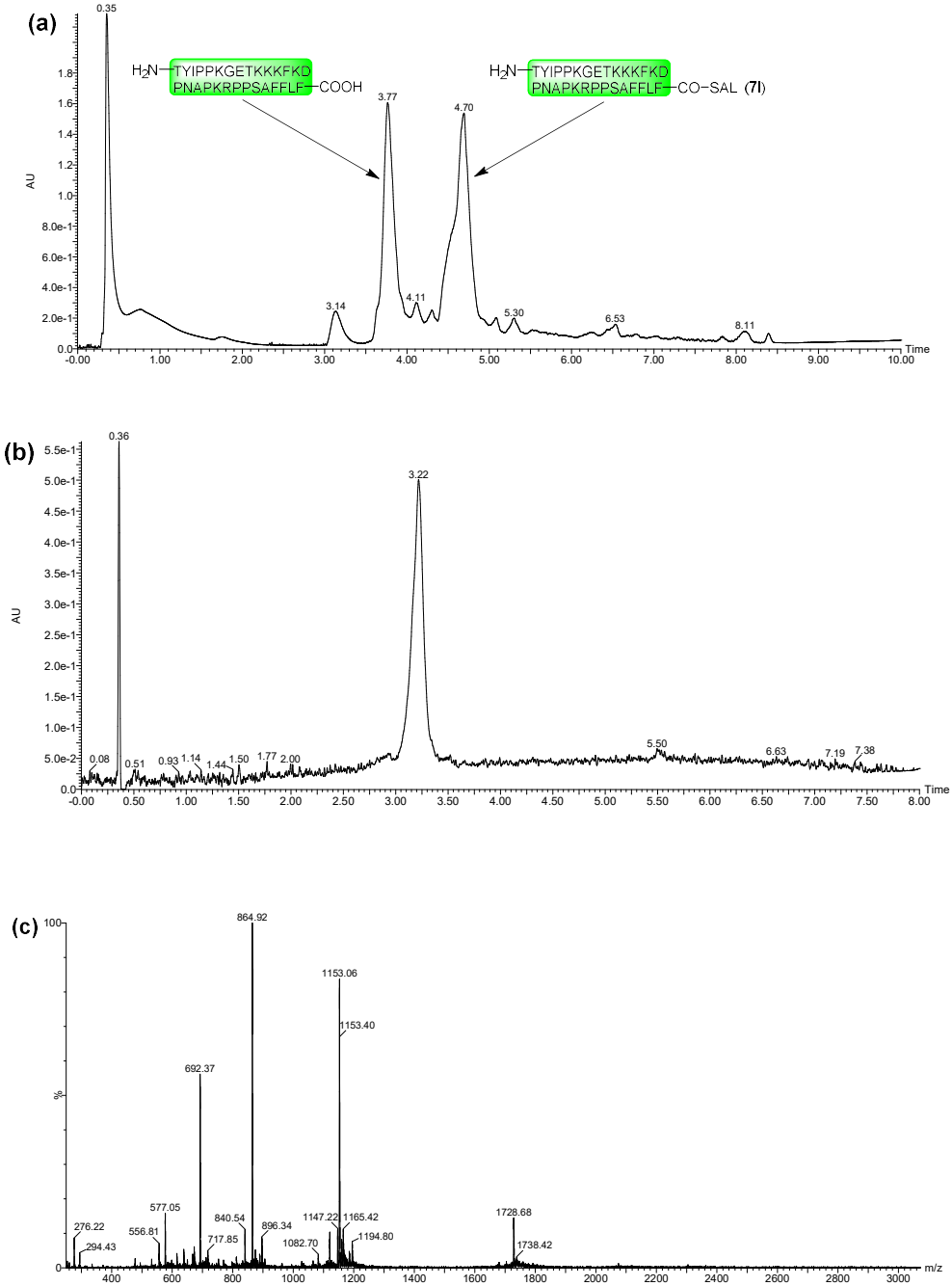
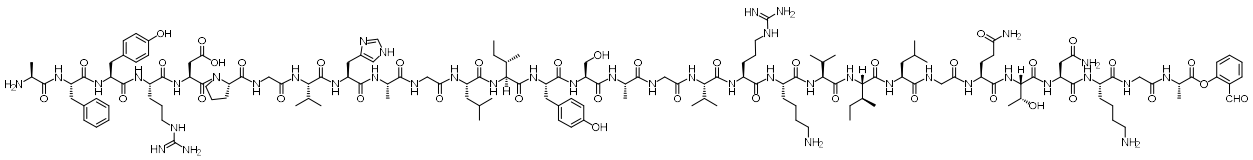


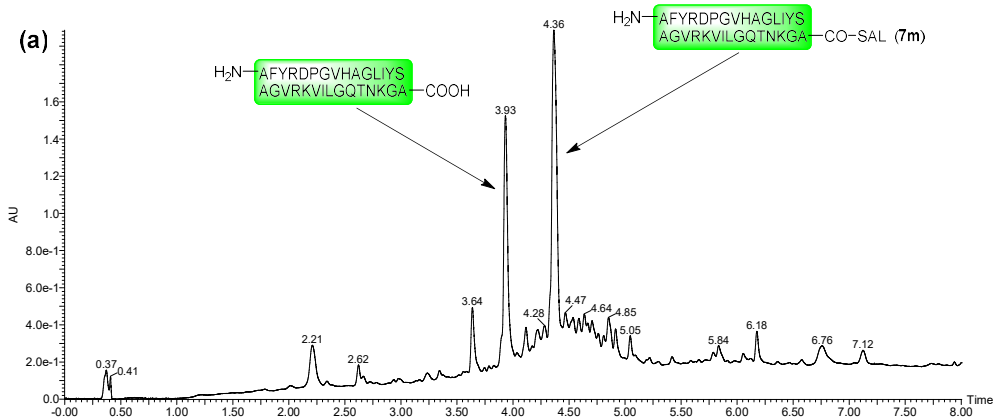
Figure S34. (a) UV trace from analytical RP-UPLC of crude reaction mixture. Gradient: 30-70% ACN/H_2O containing 0.1% TFA over 10 min at a flow rate of 0.4 mL/min. (b) UV trace from analytical RP-UPLC and its ESI-MS of **7l**. Gradient: 30-60% ACN/H_2O containing 0.1% TFA

over 8 min at a flow rate of 0.4 mL/min. (c) ESI-MS of **7l**. ESI-MS calcd. for $C_{168}H_{251}N_{39}O_{40}$ $[M+2H]^{2+}$ m/z = 1728.54, found 1728.68; $[M+3H]^{3+}$ m/z = 1152.69, found 1153.06; $[M+4H]^{4+}$ m/z = 864.77, found 864.92; $[M+5H]^{5+}$ m/z = 692.01, found 692.37.

5.32 *H_2N*-AFYRDPGVHAGLIYSAGVRKVILGQTNKGA -*CO*-SAL ester (7m)



A solution of DCP reagent (0.05 mmol) in ACN (0.3 mL) was added dropwise slowly (dropwise over 40 min) to a flask containing the crude fully protected peptidyl acid *BocHN*-Ala-Phe-Tyr(*t*Bu)-Arg(Pbf)-Asp(O*t*Bu)-Pro-Gly-Val-His(Trt)-Ala-Gly-Leu-Ile-Tyr(*t*Bu)-Ser(*t*Bu)-Ala-Gly-Val-Arg(Pbf)-Lys(Boc)-Val-Ile-Leu-Gly-Gln(Trt)-Thr(*t*Bu)-Asn(Trt)-Lys(Boc)-Gly-Ala-*COOH* (prepared according to general experimental procedure **2.1** and **2.2**) (49.2 mg, 0.01 mmol), K_2CO_3 (33.1 mg, 0.24 mmol), NaI (45.0 mg, 0.3 mmol) and mL ACN/DCM (1:4, 10 mL) at room temperature under an Ar atmosphere. Then the mixture was stirred at room temperature for 2 h. After that, the reaction solvent was poured into the other flask (left solid salt in the flask). The solvent was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged. The precipitate was pelleted, and the ether was subsequently decanted. The resulting solid was purified by HPLC and lyophilization to give the long peptide SAL ester **7m** (9.2 mg, 28.2%) as white solid.



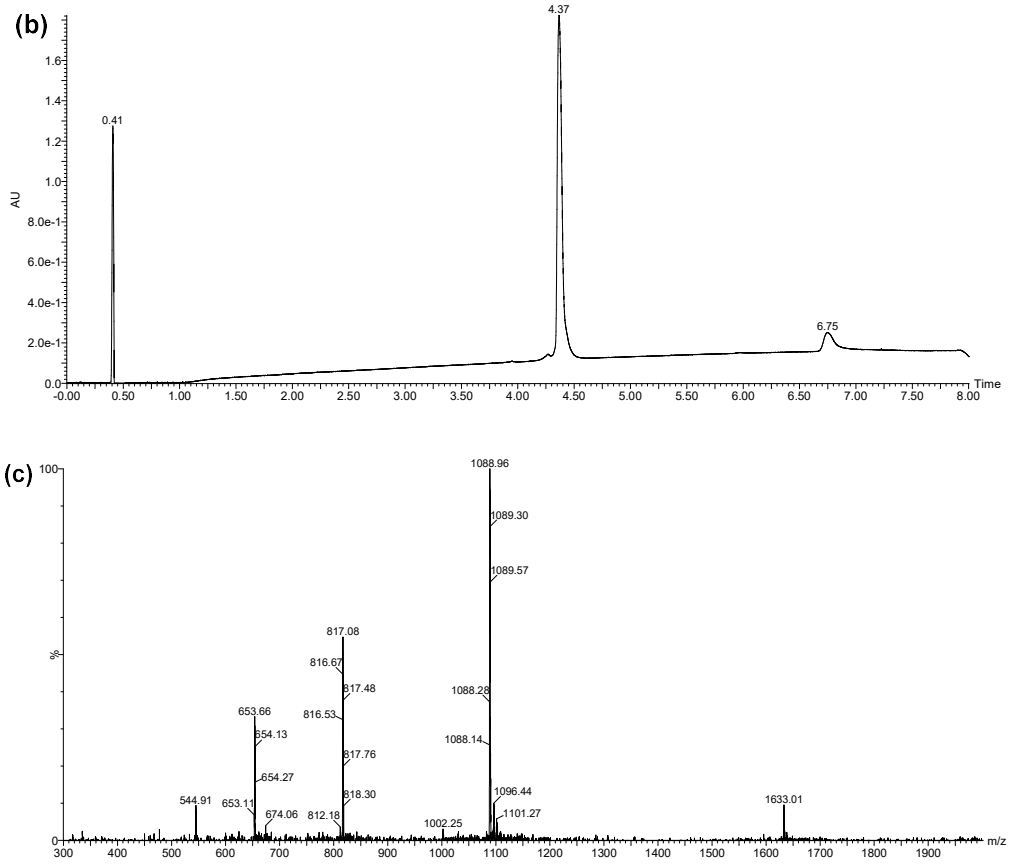
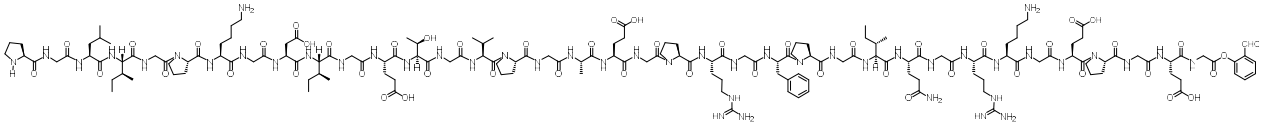


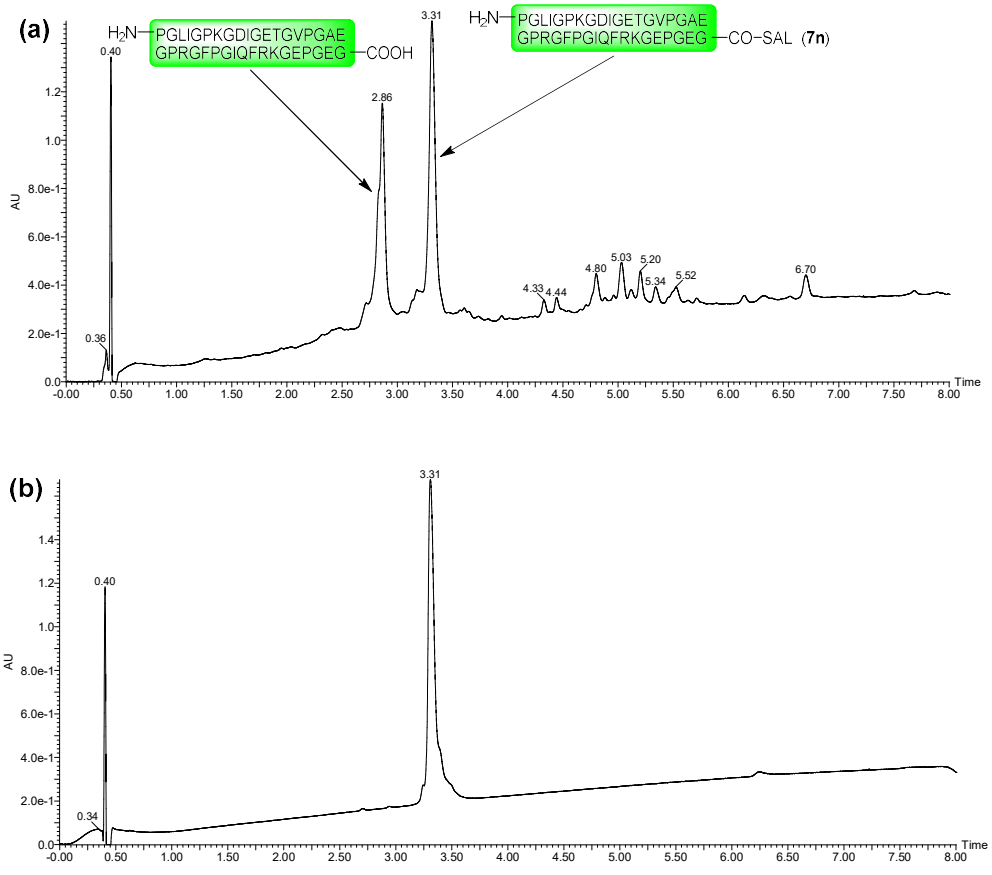
Figure S35. (a) UV trace from analytical RP-UPLC of crude reaction mixture. Gradient: 10-50% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. (b) UV trace from analytical RP-UPLC of **7m**. Gradient: 10-50% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. (c) ESI-MS of **7m**. ESI-MS calcd. for $C_{150}H_{234}N_{42}O_{40}$ $[M+2H]^{2+}$ m/z = 1632.88, found 1633.01; $[M+3H]^{3+}$ m/z = 1088.92, found 1088.96; $[M+4H]^{4+}$ m/z = 816.93, found 817.08; $[M+5H]^{5+}$ m/z =653.74, found 653.66.

5.33 *H_2N*-PGLIGPKGDIGETGVPGAEGPRGFPGIQGRKGEPGEG-*CO*-SAL ester (7n, from Adiponectin (71-107))



A solution of DCP reagent (0.05 mmol) in ACN (0.3 mL) was added dropwise slowly (dropwise over 40 min) to a flask containing the crude fully protected peptidyl acid *BocHN*-Pro-Gly-Leu-Ile-Gly-Pro-Lys(Boc)-Gly-Pro-Lys(Boc)-Gly-Asp(O*t*Bu)-Ile-Gly-Glu(O*t*Bu)-Thr(*t*Bu)-Gly-Val-

Pro-Gly-Ala-Glu(O*t*Bu)-Gly-Pro-Arg(Pbf)-Gly-Phe-Pro-Gly-Ile-Gln(Trt)-Gly-Arg(Pbf)-Lys(Boc)-Gly-Glu(O*t*Bu)-Pro-Gly-Glu(O*t*Bu)-Gly-*COOH* (prepared according to general experimental procedure **2.1** and **2.2**) (49.3 mg, 0.01 mmol), K_2CO_3 (33.1 mg, 0.24 mmol), NaI (45.0 mg, 0.3 mmol) and mL ACN/DCM (1:4, 10 mL) at room temperature under an Ar atmosphere. Then the mixture was stirred at room temperature for 2 h. After that, the reaction solvent was poured into the other flask (left solid salt in the flask). The solvent was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged. The precipitate was pelleted, and the ether was subsequently decanted. The resulting solid was purified by HPLC and lyophilization to give the long peptide SAL ester **7n** (9.9 mg, 27.1%) as white solid.



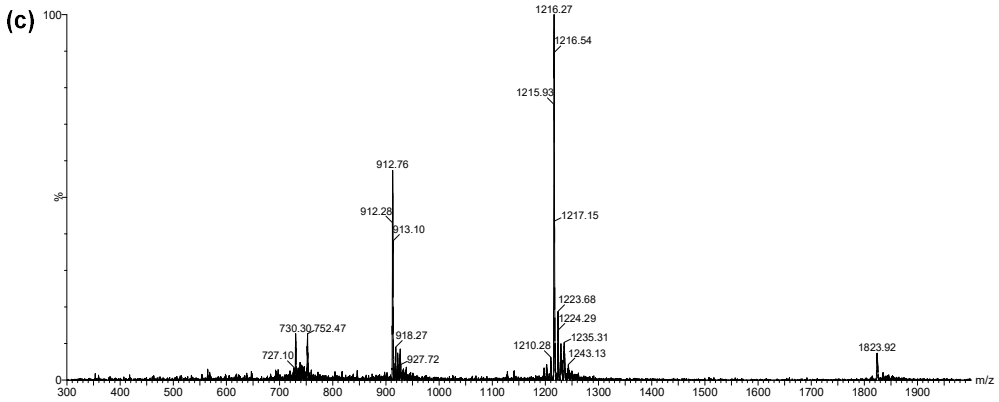


Figure S36. (a) UV trace from analytical RP-UPLC of crude reaction mixture. Gradient: 15-50% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. (b) UV trace from analytical RP-UPLC of **7n**. Gradient: 15-50% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. (c) ESI-MS of **7n**. ESI-MS calcd. for $C_{161}H_{252}N_{46}O_{51}$ $[M+2H]^{2+}$ m/z = 1824.02, found 1823.92; $[M+3H]^{3+}$ m/z = 1216.35, found 1216.27; $[M+4H]^{4+}$ m/z = 912.51, found 912.76.

5.34 The sequence of the protein HMGB1, SKP2, SOCS2 and Adiponectin

Sequence of high mobility group protein B1 (**HMGB1,** 215 amino acids, UniProt entry: B1MTB0)

```
                10                  20                  30                  40                  50                  60
MGKGDPKKPR GKMSSYAFFV QTCREEHKKK HPDASVNFSE FSKKCSERWK TMSAKEKGKF
                70                  80                  90                 100                 110                 120
EDMAKADKAR YEREMKTYIP PKGETKKKFK DPNAPKRPPS AFFLFCSEYR PKIKGEHPGL
               130                 140                 150                 160                 170                 180
SIGDVAKKLG EMWNNTAADD KQPYEKKAAK LKEKYEKDIA AYRAKGKPDA AKKGVVKAEK
               190                 200                 210
SKKKKEEEED EEDEEDEEEE EDEEDEDEEE DDDDE
```

Sequence of S-phase kinase-associated protein 2 (**SKP2**, 424 amino acids, UniProt entry: Q13309)

```
                10                  20                  30                  40                  50                  60
MHRKHLQEIP DLSSNVATSF TWGWDSSKTS ELLSGMGVSA LEKEEPDSEN IPQELLSNLG
                70                  80                  90                 100                 110                 120
HPESPPRKRL KSKGSDKDFV IVRRPKLNRE NFPGVSWDSL PDELLLGIFS CLCLPELLKV
               130                 140                 150                 160                 170                 180
SGVCKRWYRL ASDESLWQTL DLTGKNLHPD VTGRLLSQGV IAFRCPRSFM DQPLAEHFSP
               190                 200                 210                 220                 230                 240
FRVQHMDLSN SVIEVSTLHG ILSQCSKLQN LSLEGLRLSD PIVNTLAKNS NLVRLNLSGC
               250                 260                 270                 280                 290                 300
```

SGFSEFALQT LLSSCSRLDE LNLSWCFDFT EKHVQVAVAH VSETITQLNL SGYRKNLQKS
310 320 330 340 350 360
DLSTLVRRCP NLVHLDLSDS VM LKNDCFQE FFQLNYLQHL SLSRCYDIIP ETLLELGEIP
370 380 390 400 410 420
TLKTLQVFGI VPDGTLQLLK EALPHLQINC SHFTTIARPT IGNKKNQEIW GIKCRLTLQK

PSCL

Sequence of suppressor of cytokine signaling 2 (**SOCS2**, 198 amino acids, UniProt entry: O14508)

10 20 30 40 50 60
MTLRCLEPSG NGGEGTRSQW GTAGSAEEPS PQAARLAKAL RELGQTGWYW GSMTVNEAKE
70 80 90 100 110 120
KLKEAPEGTF LIRDSSHSDY LLTISVKTSA GPTNLRIEYQ DGKFRLDSII CVKSKLKQFD
130 140 150 160 170 180
SVVHLIDYYV QMCKDKRTGP EAPRNGTVHL YLTKPLYTSA PSLQHLCRLT INKCTGAIWG
190
LPLPTRLKDY LEEYKFQV

Sequence of **Adiponectin** (244 amino acids, UniProt entry: Q15848)

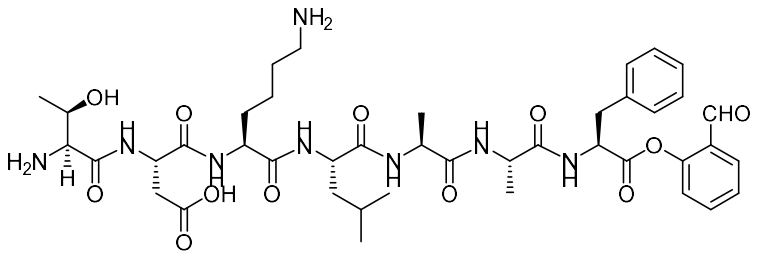
10 20 30 40 50 60
MLLLGAVLLL LALPGHDQET TTQGPGVLLP LPKGACTGWM AGIPGHPGHN GAPGRDGRDG
70 80 90 100 110 120
TPGEKGEKGD PGLIGPKGDI GETGVPGAEG PRGFPGIQGR KGEPGEGAYV YRSAFSVGLE
130 140 150 160 170 180
TYVTIPNMPI RFTKIFYNQQ NHYDGSTGKF HCNIPGLYYF AYHITVYMKD VKVSLFKKDK
190 200 210 220 230 240
AMLFTYDQYQ ENNVDQASGS VLLHLEVGDQ VWLQVYGEGE RNGLYADNDN DSTFTGFLLY

HDTN

6. Study of the epimerization issue at the C-terminal amino acid

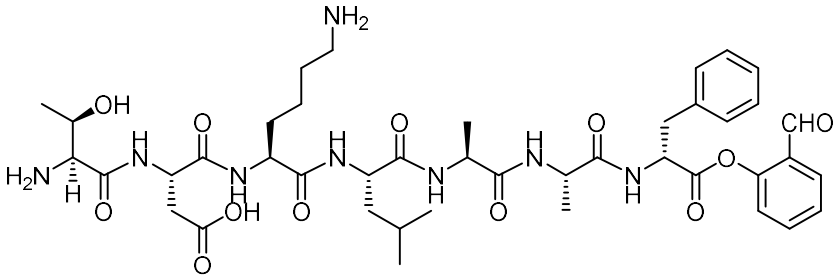
Both epimeric *H_2N*-TDKLAAF-*CO*-SAL ester **(S8a)** and *H_2N*-TDKLAAf-*CO*-SAL ester **(S9a)** were synthesized according to general experimental procedure **2.1**, **2.2** and **2.4**.

6.1 Crude peptide: *H_2N*-TDKLAAF-*CO*-SAL ester (S8a)



A solution of crude DCP reagent (0.12 mmol) in ACN (0.5 mL) was added dropwise slowly (dropwise over about 40 min, 4 drops after every 2 min) to a flask containing the crude fully protected peptidyl acid *BocHN*-Thr(*t*Bu)-Asp(O*t*Bu)-Lys(Boc)-Leu-Ala-Ala-Phe-*COOH* (prepared according to general experimental procedure **2.1** and **2.2**) (46.3 mg, 0.04 mmol), K_2CO_3 (55.2 mg 0.33 mmol), NaI (144.0 mg, 1.20 mmol) and ACN/DCM (1:4, 10 mL) at room temperature under an Ar atmosphere. After stirring at room temperature for 2 h, the mixture was filtered, and the filter cake was washed with ACN (10 mL) and DCM (10 mL). The filtration was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*, 5 mL) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged to obtain the crude peptide *H_2N*-TDKLAAF-*CO*-SAL ester (**S8a**).

6.2 Crude peptide: *H_2N*-TDKLAAf-*CO*-SAL ester (S9a)



A solution of crude DCP reagent (0.12 mmol) in ACN (0.5 mL) was added dropwise slowly (dropwise over about 40 min, 4 drops after every 2 min) to a flask containing the crude fully protected peptidyl acid *BocHN*-Thr(*t*Bu)-Asp(O*t*Bu)-Lys(Boc)-Leu-Ala-Ala-phe-*COOH* (prepared according to general experimental procedure **2.1** and **2.2**) (46.3 mg, 0.04 mmol), K_2CO_3 (55.2 mg 0.33 mmol), NaI (144.0 mg, 1.20 mmol) and ACN/DCM (1:4, 10 mL) at room temperature under an Ar atmosphere. After stirring at room temperature for 2 h, the mixture was filtered, and the filter cake was washed with ACN (10 mL) and DCM (10 mL). The filtration was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*, 5 mL) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged to obtain the crude peptide *H_2N*-TDKLAAf-*CO*-SAL ester (**S9a**).

Crude peptide **S8a** and **S9a** and a co-injection of a mixture of crude peptide **S8a** and **S 9a** were analyzed separately by UPLC-MS. No epimerization was observed for our strategy.

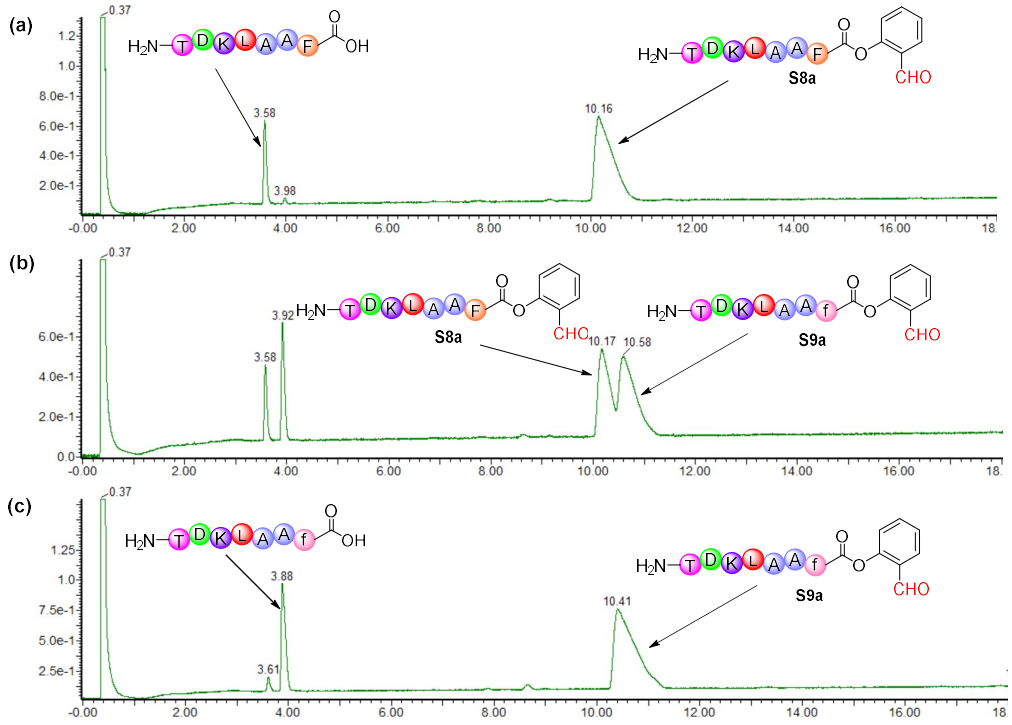


Figure S37. UV trace from UPLC-MS analysis of crude **S8a**, a mixture of crude **S8a** and **S9a** and crude **S9a** for epimerization study: gradient 20-30% CH_3CN/H_2O containing 0.1% TFA over 18 min at a flow rate of 0.4 mL/min.

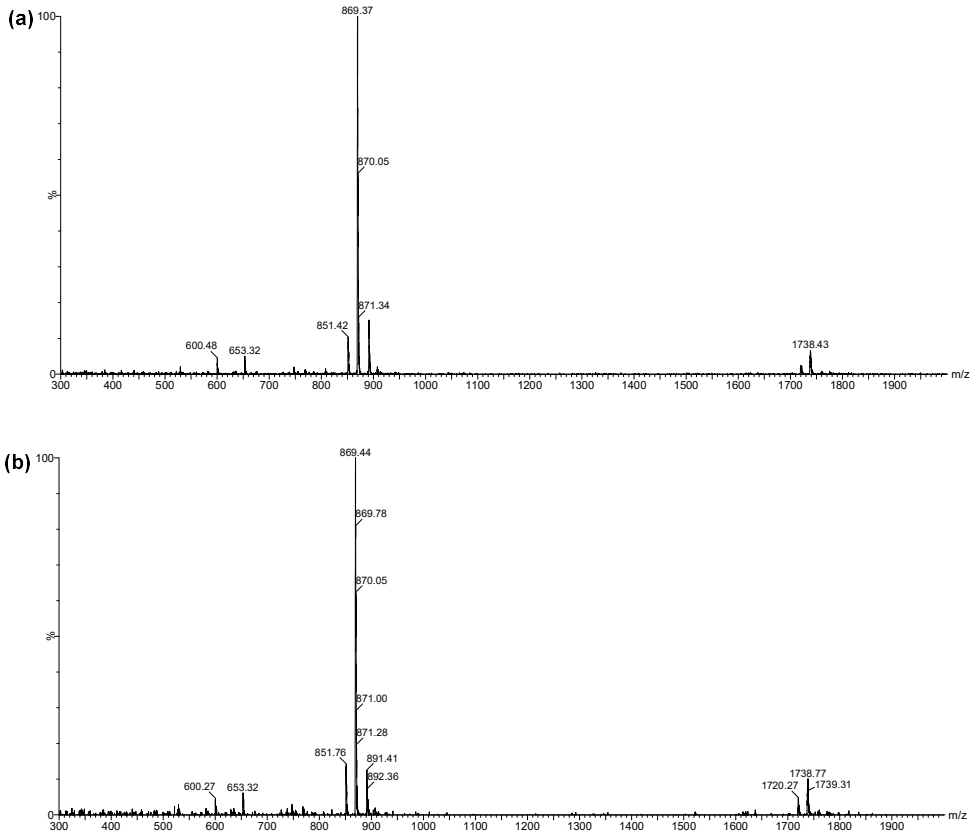
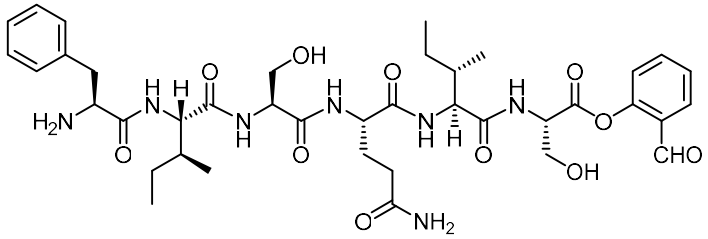


Figure S38. (a) ESI-MS of **S8a**. ESI-MS calcd. for $C_{42}H_{61}N_8O_{12}$ $[M+H]^+$ m/z = 869.99, found 869.37. (b) ESI-MS of **S9a**. ESI-MS calcd. for $C_{42}H_{61}N_8O_{12}$ $[M+H]^+$ m/z = 869.99, found 869.44.

Both epimeric *H_2N*-FISQIS-*CO*-SAL ester **(S8b)** and *H_2N*-FISQIs-*CO*-SAL ester **(S9b)** were synthesized according to general experimental procedure **2.1**, **2.2** and **2.3**.

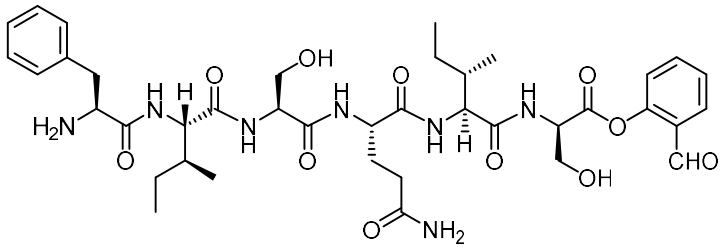
6.3 Crude peptide: *H_2N*- FISQIS-*CO*-SAL ester (S8b)



A solution of crude DCP reagent (0.1 mmol) in ACN (0.5 mL) was added dropwise slowly (dropwise over about 40 min, 4 drops after every 2 min) to a flask containing the crude fully protected peptidyl acid *BocHN*-Phe-Ile-Ser(*t*Bu)-Gln(Trt)-Ile-Ser(*t*Bu)-*COOH* (prepared

according to general experimental procedure **2.1** and **2.2**) (57.4 mg, 0.05 mmol), K_2CO_3 (45.0 mg 0.33 mmol), NaI (180.0 mg, 1.20 mmol) and ACN (5 mL) at room temperature under an Ar atmosphere. After stirring at room temperature for 2 h, the mixture was filtered, and the filter cake was washed with ACN (10 mL) and DCM (10 mL). The filtration was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*, 5 mL) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged to obtain the crude peptide *H_2N*-FISQIS-*CO*-SAL ester (**S8b**).

6.4 Crude peptide: *H_2N*- FISQIs-*CO*-SAL ester (S9b)



A solution of crude DCP reagent (0.1 mmol) in ACN (0.5 mL) was added dropwise slowly (dropwise over about 40 min, 4 drops after every 2 min) to a flask containing the crude fully protected peptidyl acid *BocHN*-Phe-Ile-Ser(*t*Bu)-Gln(Trt)-Ile-ser(*t*Bu)-*COOH* (prepared according to general experimental procedure **2.1** and **2.2**) (57.4 mg, 0.05 mmol), K_2CO_3 (45.0 mg 0.33 mmol), NaI (180.0 mg, 1.20 mmol) and ACN (5 mL) at room temperature under an Ar atmosphere. After stirring at room temperature for 2 h, the mixture was filtered, and the filter cake was washed with ACN (10 mL) and DCM (10 mL). The filtration was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*, 5 mL) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged to obtain the crude peptide *H_2N*-FISQIs-*CO*-SAL ester (**S9b**).

Crude peptide **S8b** and **S9b** and a co-injection of a mixture of crude peptide **S8b** and **S9b** were analyzed separately by UPLC-MS. No epimerization was observed for our strategy.

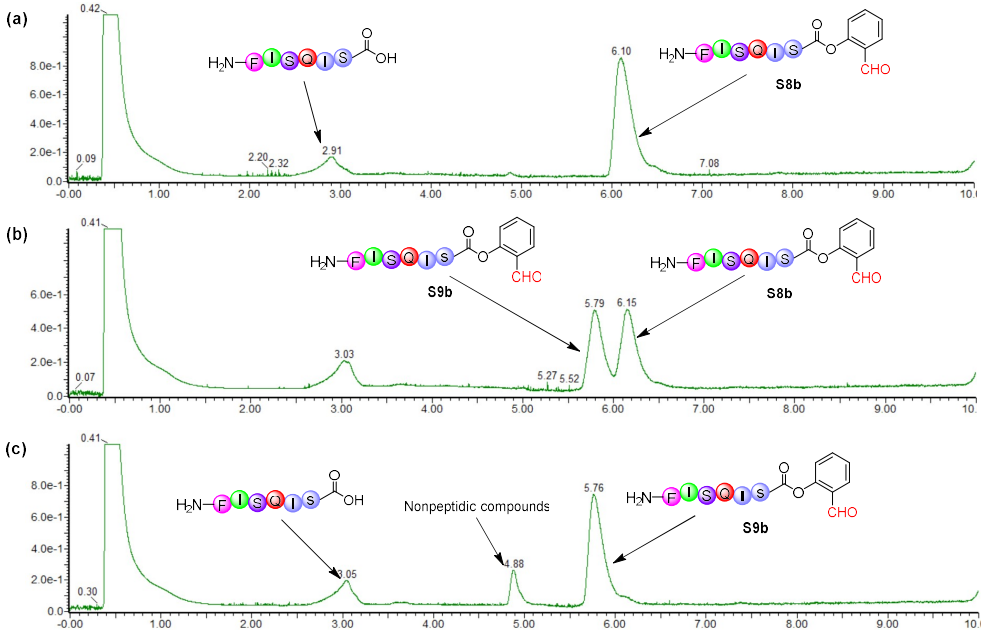


Figure S39. UV trace from UPLC-MS analysis of crude **S8b**, a mixture of crude **S8b** and **S9b** and crude **S9b** for epimerization study: gradient 20-30% CH_3CN/H_2O containing 0.1% TFA over 10 min at a flow rate of 0.4 mL/min.

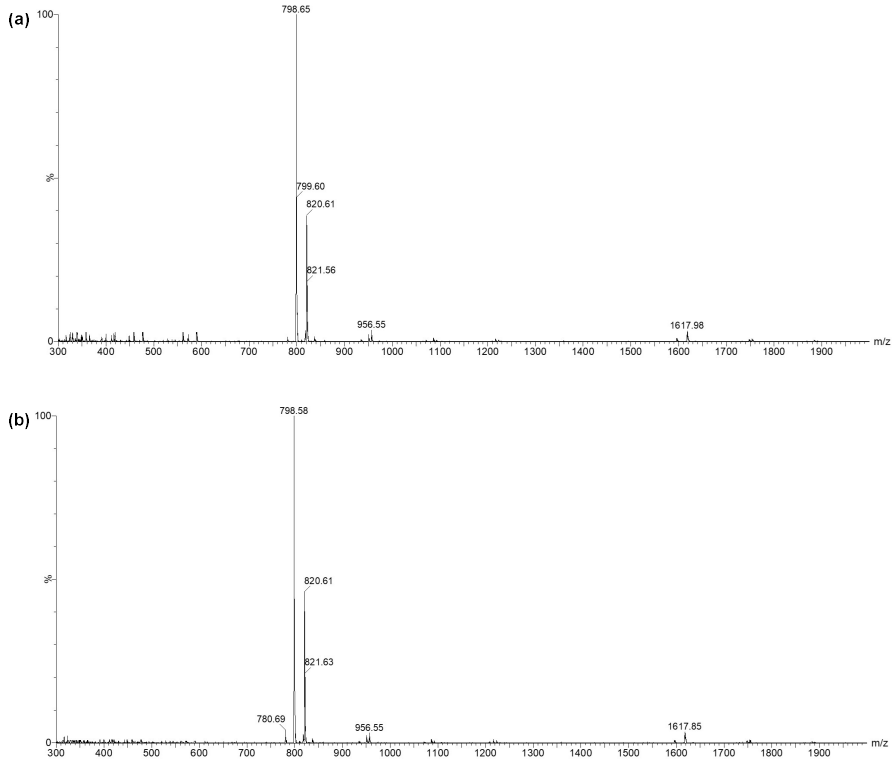
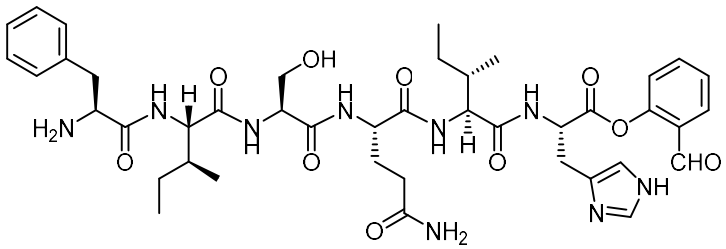


Figure S40. (a) ESI-MS of **S8b**. ESI-MS calcd. for $C_{39}H_{56}N_7O_{11}$ $[M+H]^+$ m/z = 798.91, found 798.65. (b) ESI-MS of **S9b**. ESI-MS calcd. for $C_{39}H_{56}N_7O_{11}$ $[M+H]^+$ m/z = 798.91, found 798.58.

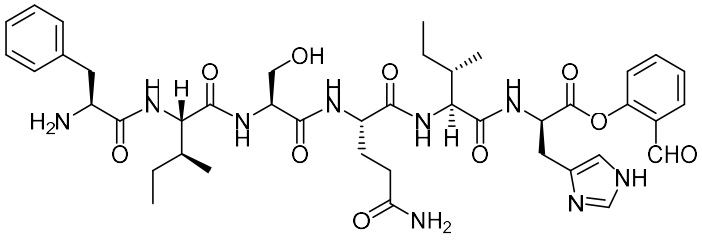
Both epimeric *H₂N*-FISQIH-*CO*-SAL ester **(S8c)** and *H₂N*- FISQIh-*CO*-SAL ester **(S9c)** were synthesized according to general experimental procedure **2.1**, **2.2** and **2.3**.

6.5 Crude peptide: *H₂N*- FISQIH-*CO*-SAL ester (S8c)



A solution of crude DCP reagent (0.1 mmol) in ACN (0.5 mL) was added dropwise slowly (dropwise over about 40 min, 4 drops after every 2 min) to a flask containing the crude fully protected peptidyl acid *BocHN*-Phe-Ile-Ser(*t*Bu)-Gln(Trt)-Ile-His(Trt)-*COOH* (prepared according to general experimental procedure **2.1** and **2.2**) (69.2 mg, 0.05 mmol), K_2CO_3 (45.0 mg 0.33 mmol), NaI (180.0 mg, 1.20 mmol) and ACN (5 mL) at room temperature under an Ar atmosphere. After stirring at room temperature for 2 h, the mixture was filtered, and the filter cake was washed with ACN (10 mL) and DCM (10 mL). The filtration was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*, 5 mL) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged to obtain the crude peptide *H₂N*-FISQIH-*CO*-SAL ester (**S8c**).

6.6 Crude peptide: *H₂N*- FISQIh-*CO*-SAL ester (S9c)



A solution of crude DCP reagent (0.1 mmol) in ACN (0.5 mL) was added dropwise slowly (dropwise over about 40 min, 4 drops after every 2 min) to a flask containing the crude fully protected peptidyl acid *BocHN*-Phe-Ile-Ser(*t*Bu)-Gln(Trt)-Ile-his(Trt)-*COOH* (prepared according to general experimental procedure **2.1** and **2.2**) (69.2 mg, 0.05 mmol), K_2CO_3 (45.0 mg 0.33 mmol),

NaI (180.0 mg, 1.20 mmol) and ACN (5 mL) at room temperature under an Ar atmosphere. After stirring at room temperature for 2 h, the mixture was filtered, and the filter cake was washed with ACN (10 mL) and DCM (10 mL). The filtration was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*, 5 mL) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged to obtain the crude peptide *H_2N*-FISQIh-*CO*-SAL ester (**S9c**).

Crude peptide **S8c** and **S9c** and a co-injection of a mixture of crude peptide **S8c** and **S9c** were analyzed separately by UPLC-MS. No epimerization was observed for our strategy.

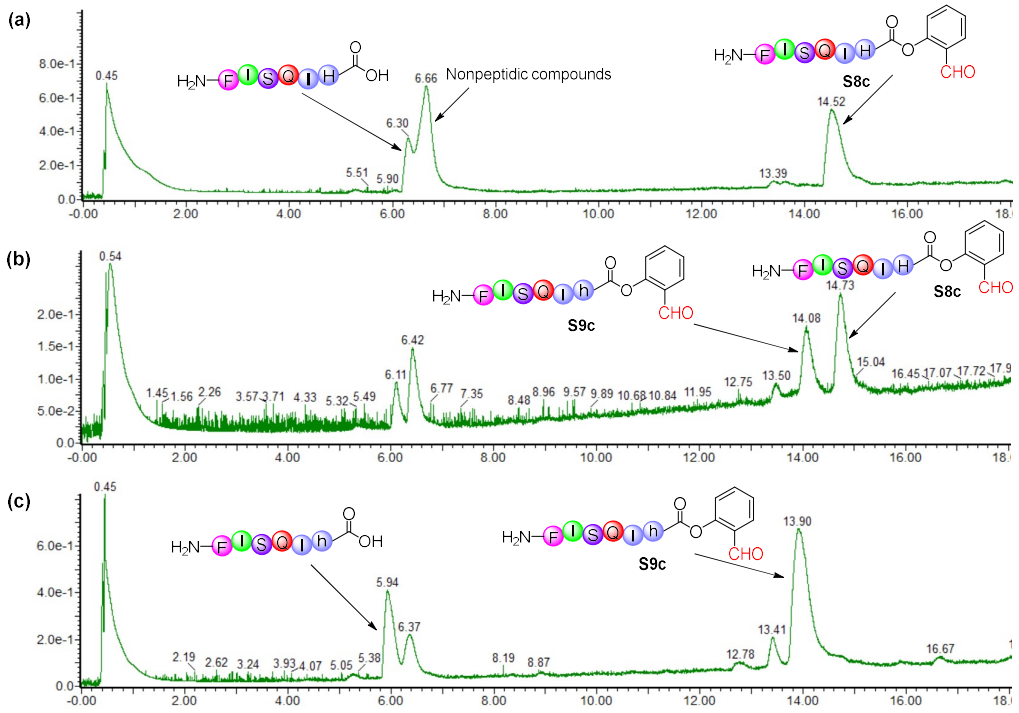


Figure S41. UV trace from UPLC-MS analysis of crude **S8c**, a mixture of crude **S8c** and **S9c** and crude **S9c** for epimerization study: gradient 10-25% CH_3CN/H_2O containing 0.1% TFA over 10 min at a flow rate of 0.4 mL/min.

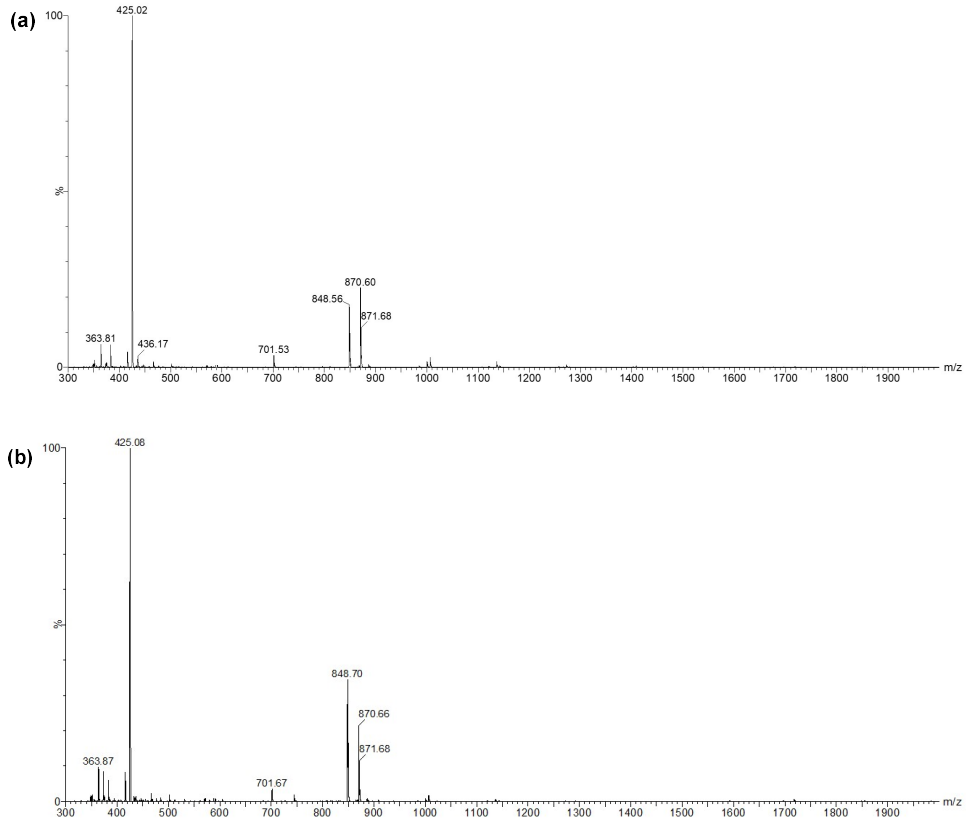
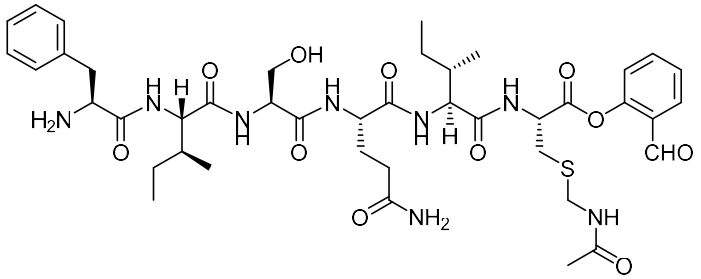


Figure S42. (a) ESI-MS of **S8c**. ESI-MS calcd. for $C_{42}H_{58}N_9O_{10}$ $[M+H]^+$ m/z = 848.97, found 848.56. (b) ESI-MS of **S9c**. ESI-MS calcd. for $C_{39}H_{56}N_7O_{11}$ $[M+H]^+$ m/z = 848.97, found 848.70.

Both epimeric *H_2N*-FISQIH-*CO*-SAL ester **(S8d)** and *H_2N*- FISQIh-*CO*-SAL ester **(S9d)** were synthesized according to general experimental procedure **2.1**, **2.2** and **2.3**.

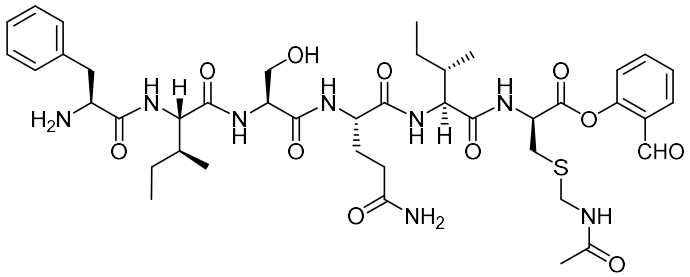
6.7 Crude peptide: *H_2N*- FISQIC(Acm)-*CO*-SAL ester (S8d)



A solution of crude DCP reagent (0.1 mmol) in ACN (0.5 mL) was added dropwise slowly (dropwise over about 40 min, 4 drops after every 2 min) to a flask containing the crude fully

protected peptidyl acid *BocHN*-Phe-Ile-Ser(*t*Bu)-Gln(Trt)-Ile-Cys(Acm)-*COOH* (prepared according to general experimental procedure **2.1** and **2.2**) (58.9 mg, 0.05 mmol), K_2CO_3 (45.0 mg 0.33 mmol), NaI (180.0 mg, 1.20 mmol) and ACN (5 mL) at room temperature under an Ar atmosphere. After stirring at room temperature for 2 h, the mixture was filtered, and the filter cake was washed with ACN (10 mL) and DCM (10 mL). The filtration was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*, 5 mL) was added. After global deprotection for 2 h, the mixture was triturated with diethyl ether and centrifuged to obtain the crude peptide *H_2N*-FISQIC(Acm)-*CO*-SAL ester (**S8d**).

6.8 Crude peptide: *H_2N*- FISQIc(Acm)-*CO*-SAL ester (S9d)



A solution of crude DCP reagent (0.1 mmol) in ACN (0.5 mL) was added dropwise slowly (dropwise over about 40 min, 4 drops after every 2 min) to a flask containing the crude fully protected peptidyl acid *BocHN*-Phe-Ile-Ser(*t*Bu)-Gln(Trt)-Ile-cys(Acm)-*COOH* (prepared according to general experimental procedure **2.1** and **2.2**) (58.9 mg, 0.05 mmol), K_2CO_3 (45.0 mg 0.33 mmol), NaI (180.0 mg, 1.20 mmol) and ACN (5 mL) at room temperature under an Ar atmosphere. After stirring at room temperature for 2 h, the mixture was filtered, and the filter cake was washed with ACN (10 mL) and DCM (10 mL). The filtration was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*, 5 mL) was added. After global deprotection for 2 h, the mixture was triturated with diethyl ether and centrifuged to obtain the crude peptide *H_2N*-FISQIc(Acm)-*CO*-SAL ester (**S9d**).

Crude peptide **S8d** and **S9d** and a co-injection of a mixture of crude peptide **S8d** and **S9d** were analyzed separately by UPLC-MS. No epimerization was observed for our strategy.

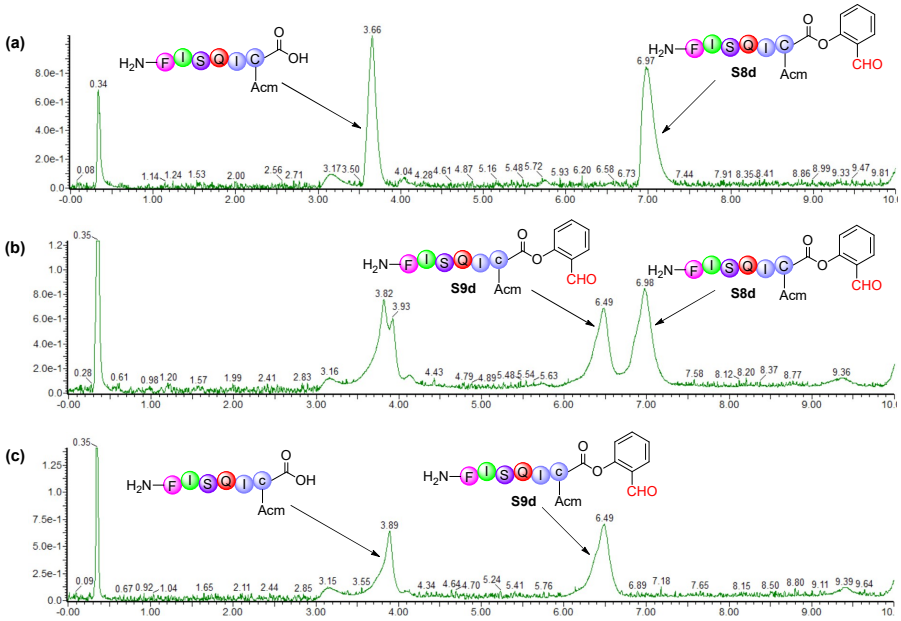


Figure S43. UV trace from UPLC-MS analysis of crude **S8d**, a mixture of crude **S8d** and **S9d** and crude **S9d** for epimerization study: gradient 20-30% CH_3CN/H_2O containing 0.1% TFA over 10 min at a flow rate of 0.4 mL/min.

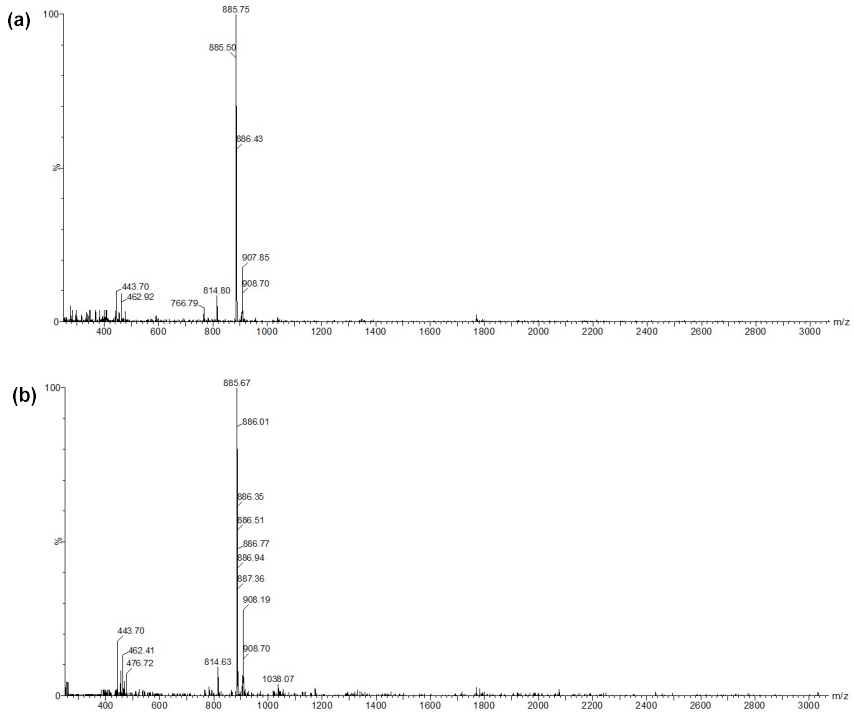
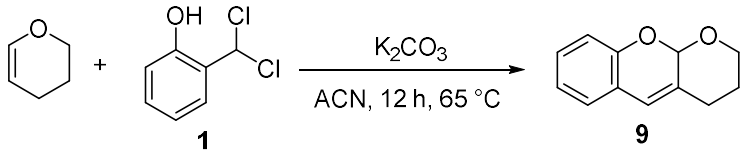


Figure S44. (a) ESI-MS of **S8d**. ESI-MS calcd. for $C_{42}H_{58}N_9O_{10}$ $[M+H]^+$ m/z = 886.05, found 885.75. (b) ESI-MS of **S9d**. ESI-MS calcd. for $C_{39}H_{56}N_7O_{11}$ $[M+H]^+$ m/z = 886.05, found 885.67.

7. Control experiment and proposed reaction pathway

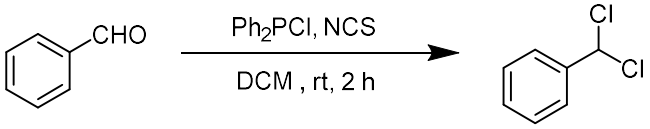
7.1 Synthesis of compound 15 via Diels-Alder reaction



1 **9**

To a solution of crude DCP reagent (0.4 mmol) in ACN (8 mL) was added 3,4-dihydro-2H-pyran (201.6 mg, 2.4 mmol) and K_2CO_3 (180mg, 1.3 mmol). The mixture was stirred at 65 ℃ for 12 h. Then the mixture was filtered, the filtrate was concentrated and purified by silica gel flash chromatography to afford **9** (15.9 mg, 21.1% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.6 (s, 1H), 7.13 (t, *J* = 8.0 Hz, 1H), 7.00 (d, *J* = 8.0 Hz, 1H), 6.92-6.88 (m, 2H), 6.29 (s, 1H), 5.93 (s, 1H), 4.18-4.14 (m, 1H), 3.91-3.84 (m, 1H), 2.59-2.55 (m, 1H), 2.44-2.36 (m, 1H), 1.95-1.89 (m, 1H), 1.84-1.79 (m, 1H). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 150.9, 131.8, 128.6, 126.2, 121.5, 119.3, 117.9, 115.4, 98.4, 66.7, 30.8, 29.3. ESI-HRMS calcd. for $C_{12}H_{13}O_2$ $[M+H]^+$ *m*/*z*=189.0910, found 189.0918.

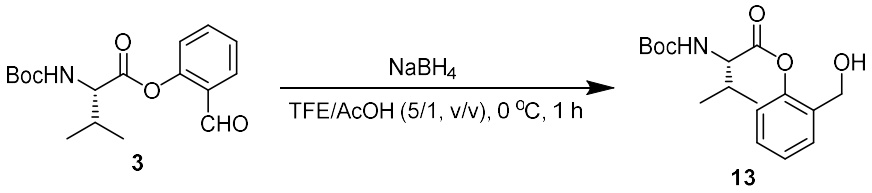
7.2 Synthesis of dichloromethylbenzene



10

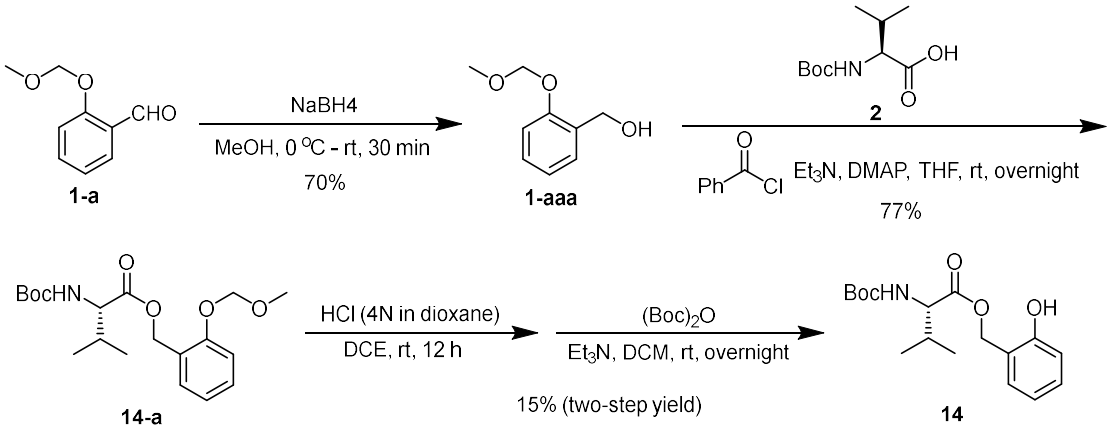
To a solution of NCS (2.68g, 20.0 mmol) in DCM (60 ml) was added Ph_2PCl (4.42 g, 20.0 mmol). Then a solution of benzaldehyde (1.06 g, 10.0 mmol) in DCM (5 mL) was added at room temperature. The mixture was stirred at room temperature for 2 h. The solvent was removed under reduced pressure and the obtained crude product was purified by silica gel flash chromatography to afford dichloromethylbenzene **10** (1.01 g, 62.7% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62-7.59 (m, 2H), 7.46-7.39 (m, 3H), 6.74 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.4, 129.9, 128.8, 126.1, 71.8. EI-HRMS calcd. for $C_7H_6Cl_2$ $[M]^+$ *m*/*z*=159.9847, found 159.9838.

7.3 Synthesis of 2-(hydroxymethyl)phenyl (*tert*-butoxycarbonyl)-L-valinate (13)



To a solution of **3** (80.3 mg, 0.25 mmol) in 5 mL of TFE/AcOH(5/1, *v*/*v*) was added $NaBH_4$ (95.0 mg, 2.5 mmol) at 0 ºC. The mixture was stirred at 0 ºC for 1 h. Then DCM (20 mL) was added and the mixture was filtered. The filtrate was concentrated and purified by silica gel flash chromatography to afford **13** (18.1 mg, 22.3% yield) and mixture of **13** and **14** (46.6 mg, 57.7% yield). ^{1}H NMR (400 MHz, CD_3CN) δ 7.53 (d, *J* = 8.0 Hz, 1H), 7.37-7.30 (m, 2H), 7.06 (d, *J* = 8.0 Hz, 1H), 5.81 (d, *J* = 4.0 Hz, 1H), 4.54 (d, *J* = 4.0 Hz, 2H), 4.25 (t, *J* = 8.0 Hz, 1H), 3.29 (t *J* = 8.0 Hz, 1H), 2.33-2.27 (m, 1H), 1.47 (s, 9H), 1.10 (d, *J* = 4.0 Hz, 3H), 1.06 (d, *J* = 4.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 171.2, 156.4, 147.7, 135.0, 128.1, 128.0, 126.3, 122.0, 78.9, 60.3, 58.0, 29.8, 28.6, 19.6, 19.0. ESI-MS calcd. for $C_{17}H_{25}NO_5Na$ $[M+Na]^+$ *m*/*z*=346.38, found 346.27.

7.4 Synthesis of 2-hydroxybenzyl (*tert*-butoxycarbonyl)-L-valinate (14)



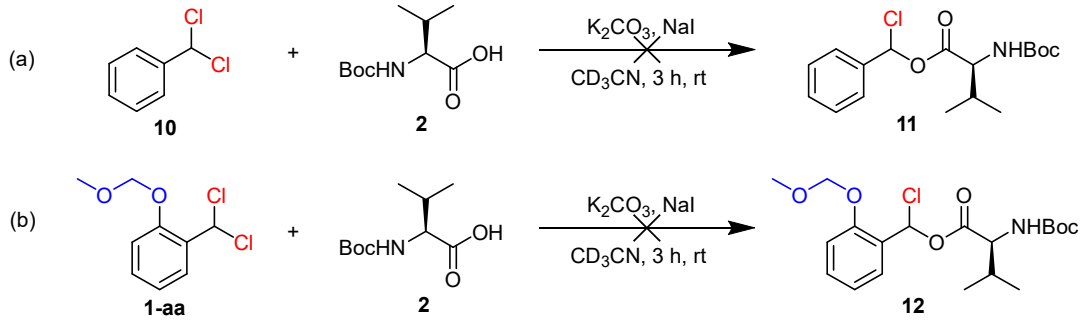
To a solution of **1-a** (1.66 g, 10.0 mmol) in MeOH (30 mL) was added $NaBH_4$ (456.0 mg, 12.0 mmol) at 0 ºC. The reaction was allowed to warm to room temperature and stirred at room temperature for 30 min. The reaction was quenched with cold water and extracted with EA. The organic layer was dried over anhydrous Na_2SO_4, concentrated, and purified by silica gel flash

chromatography to afford **1-aaa** (1.18 g, 70.2% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (d, *J* = 8.0 Hz, 1H), 7.28 (t, *J* = 8.0 Hz, 1H), 7.13 (d, *J* = 8.0 Hz, 1H), 7.04 (t, *J* = 8.0 Hz, 1H), 5.27 (s, 2H), 4.74 (s, 2H), 3.52 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.0, 130.0, 128.9, 128.8, 122.0, 114.1, 94.6, 61.6, 56.2.

To a solution of **1-aaa** (1.01 g, 6.0 mmol) in dry THF (30 mL) was added **2** (1.31 g, 6.0 mmol), benzoyl chloride (0.84 g, 6.0 mmol), Et_3N (1.21 g, 12.0 mmol) and DMAP (0.18 g, 1.5 mmol). The mixture was stirred overnight at room temperature. The reaction was quenched with water and extracted with EA. The organic layer was dried over anhydrous Na_2SO_4, concentrated, and purified by silica gel flash chromatography to afford **14-a** (1.70 g, 77.2% yield). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 7.36 (d, *J* = 8.0 Hz, 1H), 7.31 (t, *J* = 8.0 Hz, 1H), 7.20 (d, *J* = 8.0 Hz, 1H), 7.12 (d, *J* = 8.0 Hz, 1H), 6.99 (t, *J* = 8.0 Hz, 1H), 5.24 (dd, *J* = 8.0 Hz, 4.0 Hz, 2H), 5.13 (dd, *J* = 16.0 Hz, 12.0 Hz, 2H), 3.88 (t, *J* = 8.0 Hz, 1H), 3.39 (s, 3H), 2.04-1.97 (m, 1H), 1.38 (s, 9H), 0.86 (d, *J* = 8.0 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 172.4, 156.2, 156.1, 130.1, 130.0, 125.0, 121.7, 114.5, 94.4, 78.6, 61.8, 59.9, 56.1, 30.1, 28.6, 19.4, 18.7.

To a solution of **14-a** (367.2 mg, 1.0 mmol) in dry DCE (10 mL) was added HCl (4N in dioxane, 1.25 mL, 5.0 mmol), the mixture was stirred at room temperature under an Ar atmosphere for 12 h. The mixture was concentrated under in *vacuo* to give the crude product. Then dissolved the crude product in DCM (10 mL), added $(Boc)_2O$ (436.4 mg, 2.0 mmol) and Et_3N (708.1 mg, 7.0 mmol). The mixture was stirred overnight at room temperature. Then added water to the reaction mixture and extracted with EA. The organic layer was dried over anhydrous Na_2SO_4, concentrated and purified by silica gel flash chromatography to afford **14** (48.8 mg, 15.1% yield). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 9.69 (s, 1H), 7.23 (d, *J* = 8.0 Hz, 1H), 7.18-7.13 (m, 2H), 6.84 (d, *J* = 8.0 Hz, 1H), 6.77 (t, *J* = 8.0 Hz, 1H), 5.06 (dd, 28.0 Hz, 16.0 Hz, 2H), 3.87 (t, *J* = 8.0 Hz, 1H), 2.03-1.98 (m, 1H), 1.38 (s, 9H), 0.86 (d, *J* = 4.0 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 172.5, 156.2, 155.8, 130.1, 129.8, 122.4, 119.1, 115.5, 78.6, 61.9, 59.9, 30.1, 28.6, 19.4, 18.8. ESI-MS calcd. for $C_{17}H_{25}NO_5Na$ $[M+Na]^+$ *m*/*z*=346.38, found 346.07.

7.5 Attempts of a-chloroester formation via S_N2 reaction



Dichloromethylbenzene **10** (32.2mg, 0.2 mmol) or MOM protected DCP **1-aa** (44.2 mg, 0.2 mmol), **2** (21.7 mg, 0.1 mmol), K_2CO_3 (89.7 mg 0.65 mmol), NaI (360.0 mg, 2.40 mmol) and CD_3CN (5 mL) at room temperature under an Ar atmosphere. After stirring at room temperature for 3 h, the mixture was filtered and did the ^1H NMR tests of crude mixture of reaction directly (Figure S36 and Figure S38). In order to compare with the ^1H NMR of crude mixture of reaction, we also did the ^1H NMR tests of the dichloromethylbenzene and MOM protected DCP **1-aa** using CD_3CN as solvent (Figure S35 and Figure S37). During the reaction, no new signal peak of the aromatic ring was observed and the dichloromethylbenzene or MOM protected DCP **1-aa** was still in the reaction mixture (Figure S36 and Figure S38). It indicated that the *BocNH*-Val-*COOH* **2** couldn't reacted with dichloromethylbenzene under the condition of K_2CO_3 and NaI in the solvent of CD_3CN via S_N2 reaction.

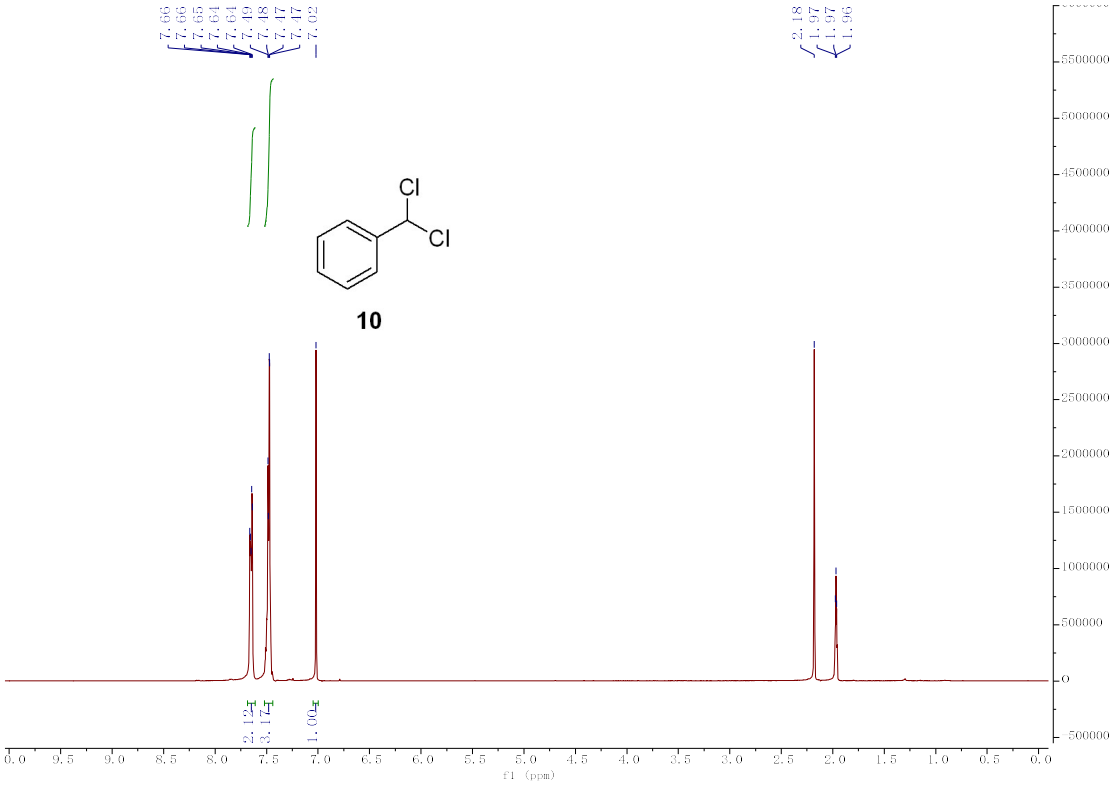


Figure S45. ^{1}H NMR (400 MHz, CD_3CN) of dichloromethyl benzene

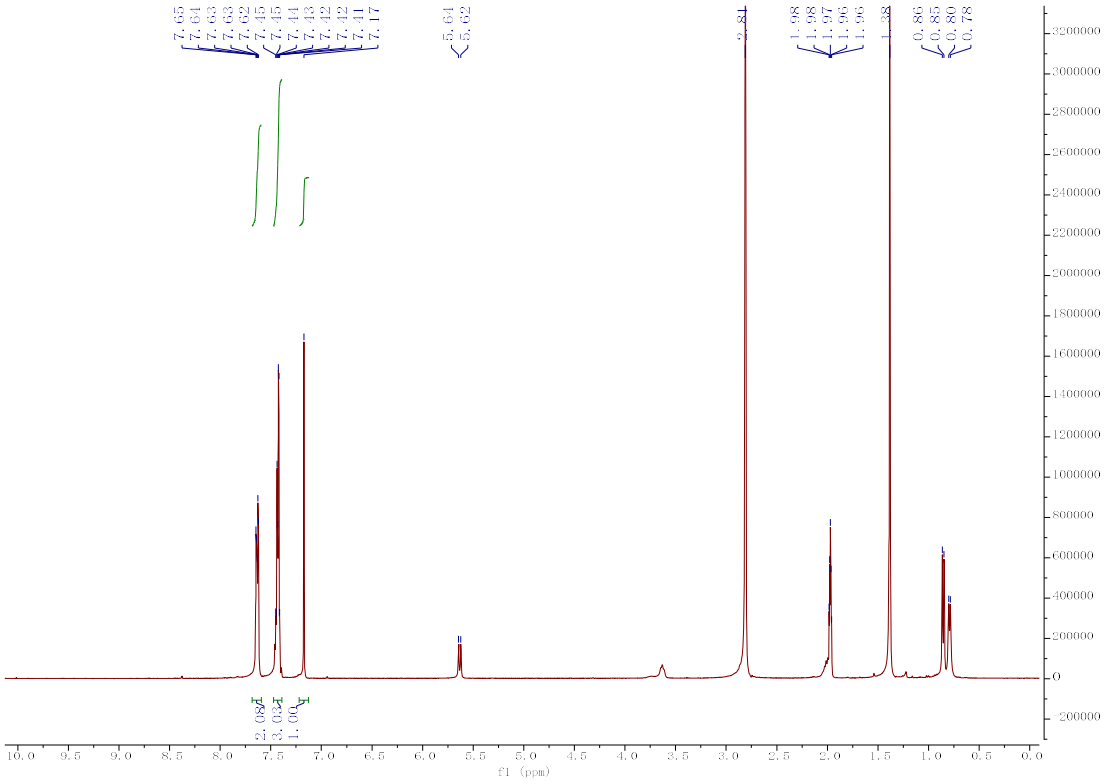


Figure S46. ^{1}H NMR (400 MHz, CD_3CN) of the crude mixture of **reaction a**

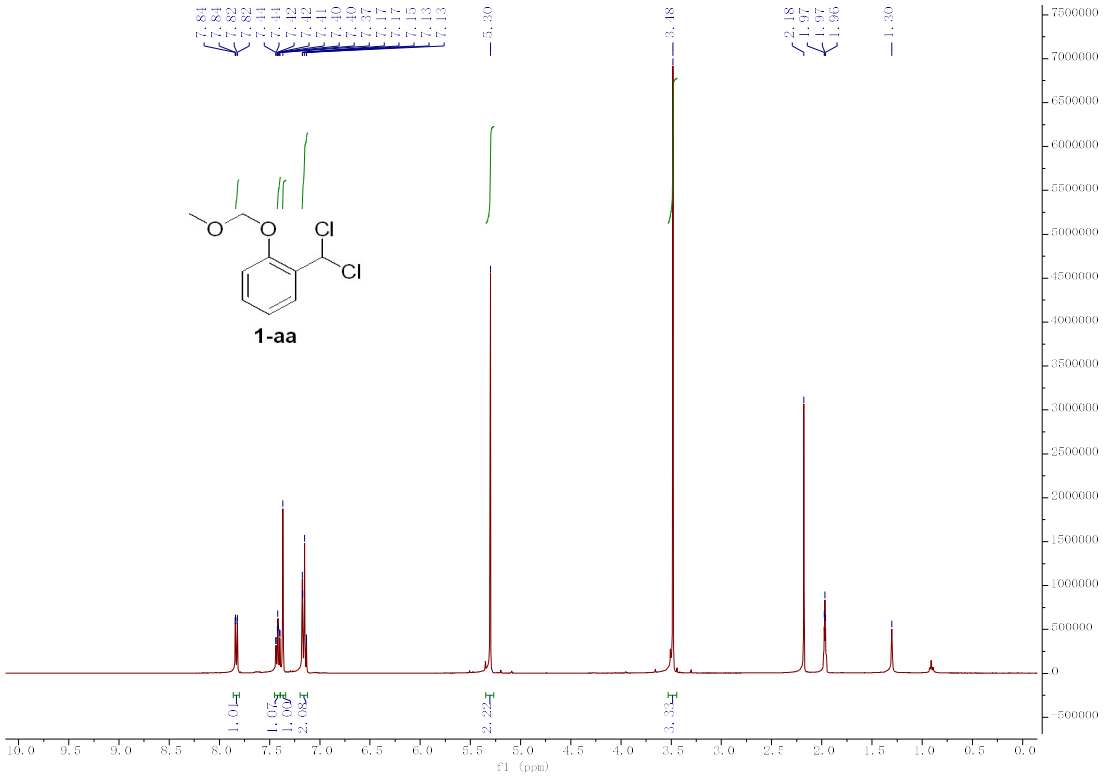


Figure S47. ^{1}H NMR (400 MHz, CD_3CN) of **1-aa**

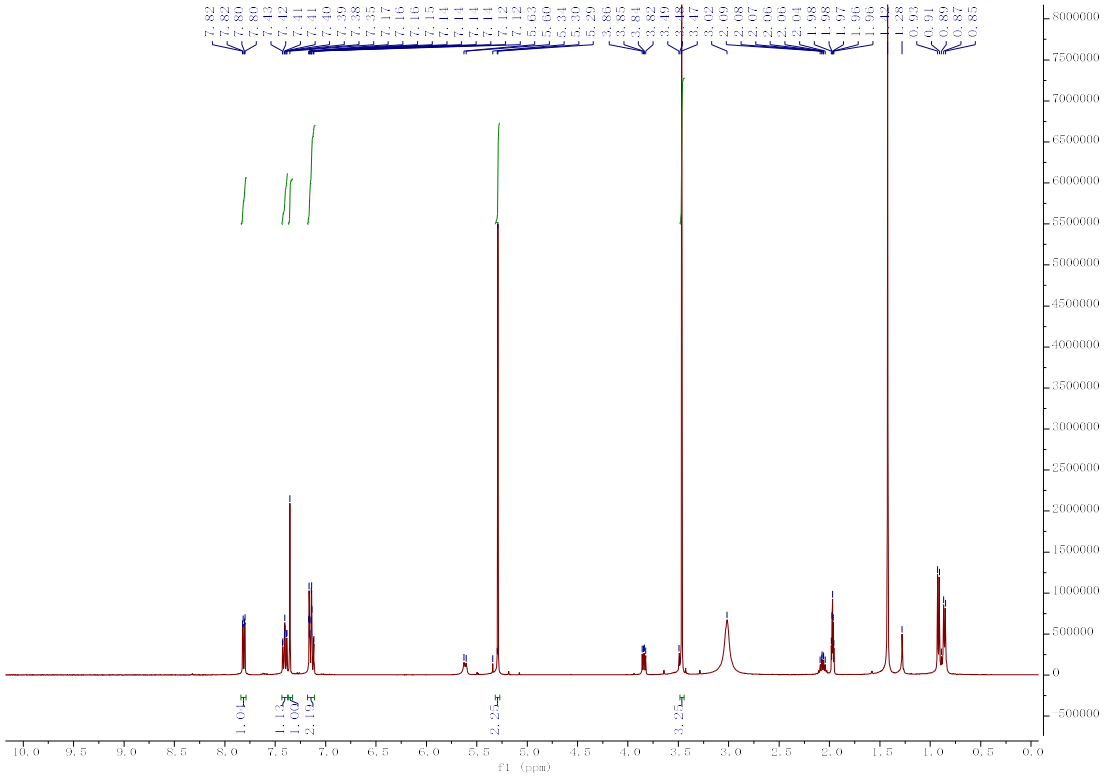
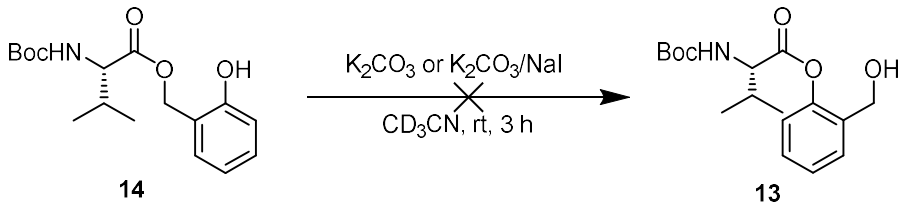


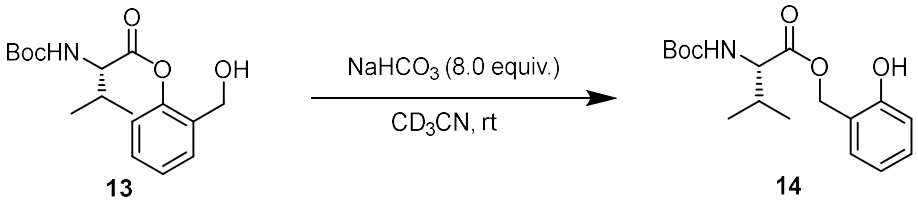
Figure S48. ^{1}H NMR (400 MHz, CD_3CN) of the crude mixture of **reaction b**

7.6 Attempts of formation of 13 from 14 under basic conditions



14 (3.2mg, 0.01 mmol) K_2CO_3 (9.0 mg 0.065 mmol), NaI (36.0 mg, 0.24 mmol) or no NaI and CD_3CN (0.5 mL) at room temperature under an Ar atmosphere. After stirring at room temperature for 3 h, the mixture was filtered and did the ^1H NMR tests of crude mixture of reaction directly**.** No product **13** was observed in the in situ NMR study.

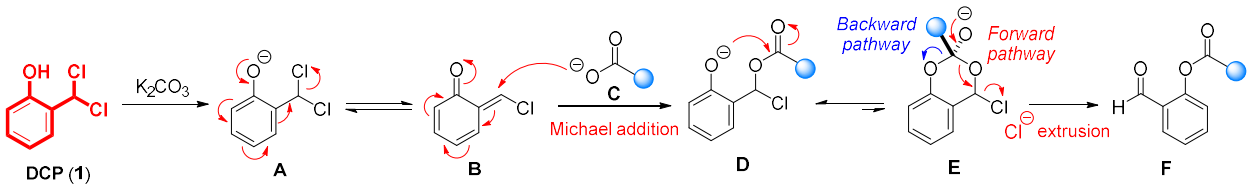
7.7 Formation of 14 from 13 under $NaHCO_3$ condition[a]



Entry	Time	Formation of **14** (%)[b]	Entry	Time	Formation of **14** (%)[b]
1	0 h	3% / 3% / 3%	7	6 h	81% / 80% / 81%
2	1 h	11% / 12% / 11%	8	7 h	88% / 89% / 89%
3	2 h	20% / 20% / 21%	9	8 h	93% / 93% / 93%
4	3 h	34% / 35% / 35%	10	9 h	96% / 96% / 96%
5	4 h	52% / 51% / 51%	11	10 h	99% / 99% /99%
6	5 h	67% / 66% / 67%			

[a] Reaction conditions: **13** (0.03 mmol, 1.0 equiv.), $NaHCO_3$ (0.24 mmol, 8.0 equiv.) in 0.5 mL solvent at rt. [b] Three parallel reactions were done under the same conditions. The formation of **14** was tested by crude ^1H NMR (CD_3CN).

7.8 proposed reaction pathway



The proposed mechanism was illustrated in the above figure. The DCP reagent **1**, The DCP reagent **1**, when added to the reaction mixture containing side chain protected peptide C-terminal carboxylate **C** (formed by deprotonation), K_2CO_3 and NaI, is deprotonated to form the phenolate **A**, which undergoes conjugate elimination to give rise to the highly reactive *o*-quinone methide (*o*-QM) intermediate **B**. We also tried to trap the *o*-QM using electron-rich dienophile 3,4-dihydropyran **8**, and the inverse-electron-demand Diels-Alder (IEEDA) adduct **9** (after HCl elimination) was isolated under basic condition (Figure 5b). The reaction of *o*-QM generated via photolysis with electron-rich dienophiles has been reported, and the possibility of Prins cyclization of salicylaldehyde and 3,4-dihydropyran which needs Lewis acid catalysis can be excluded. The Michael-type 1,4-addition of **C** to **B** provides the α-chloroester intermediate **D** (in phenolate form). As the other possible pathway, the formation of **D** via the direct S_N2 reaction between **C** and **A** was excluded by the control experiments (Figure 5c). No substitution products **11** or **12** were observed in the *in situ* NMR study under the K_2CO_3/NaI conditions using benzal chloride **10** and **1-aa** respectively. In the next step, the tetrahedral intermediate **E**, the common intermediate of the *O*-to-*O* acyl transfer that equilibrates with **D**, undergoes synergistic $C_{carboxyl}$-$O_{benzylic}$ bond cleavage/Cl^- extrusion to give SAL aldehyde **F** as the final product. During the reaction optimization, we observed the addition of excess amount of NaI (up to 24.0 equiv) significantly improve the conversion of reaction. Since I^- has been widely used as additive/catalyst for its good nucleophilicity and leaving capability, it is proposed that the high concentration of I^- from the solubilized NaI serves as a scavenger of *o*-quinone methide **B** and suppresses its decomposition by generating corresponding adduct in equilibrium manner. The high solubility of NaI in MeCN (249 mg/g) that ensures enough concentration of I^- is critical for the reaction. Changing NaI to tetrakis(3,5-bis(trifluoromethyl)phenyl)borate (NaBARF) failed to improve the yield, which excludes other contributions of Na^+. To further understand the *O*-to-*O* acyl transfer process, we synthesized phenolic ester **13** and benzylic ester **14** respectively. As expected, **13** showed a high tendency of acyl transfer and was difficult to purify due to the simultaneous formation of **14**. The

partially purified **13** (contaminated by 3% of **14**) underwent the *O*-to-*O* acyl transfer under mild basic conditions ($NaHCO_3$) to give rise to **14** (Figure 5d), while no formation of **13** from **14** was observed by *in situ* NMR under the K_2CO_3 or K_2CO_3/NaI conditions

8. Density functional theory (DFT) calculations

Computational methods: All calculations were performed with the Gaussian 09 program[1]. Pruned integration grids with 99 radial shells and 590 angular points per shell were used. Geometry optimizations of all minima and transition structures were carried out using the M06-2X functional[2] with the 6-311+G(d,p) basis set[3] in MeCN (ε = 35.688) solvent with the SMD[4] model. Frequency calculations at the same level were performed to confirm that each stationary point was either a minimum or a transition structure and to evaluate its zero-point energy and the thermal corrections at 298 K. Unless specified, transition-state structures were confirmed to connect their corresponding reactants and products by intrinsic reaction coordinate (IRC) calculations[5,6]. A standard state of 298 K and 1 mol/L was used for calculating thermal corrections[7]. The energy profile was drawn according to Gibbs free energies in solution (ΔG_{sol}). The computed structures were illustrated using CYLView[8]. Our conformation searching combines our initial conjecture and optimization of a molecular structure with a subsequent procedural conformation search. We then used the Gaussian 09 program to optimize the ten conformations with the ten lowest energy conformations (less than all), and the resulting different conformations are shown in SI. Conformational searching was applied to transition states and intermediates by the CREST program[9,10].

8.1 Discussion on the establishment of the reaction model

In the optimal condition of this reaction, a large excess of K_2CO_3 (6.5 eq.) and NaI (24 eq.) were remarkable. We tried to analyze the optimization process to help us build the corresponding model. Here are some notable points:

1. The change of K_2CO_3 to other bases (6.5 eq.) in optimal conditions results in a significant decrease in yield (Na_2CO_3: 24%, Et_3N: no product, DABCO: no product).
2. The increase in yield was obvious only when a large number of NaI was added

(no NaI: 52%, 2.0 eq. NaI: 53%, 8.0 eq. NaI: 59%, 16.0 eq. NaI: 68%, 24.0 eq. NaI: 72%).

3. The additional sodium salts (Cesibor and NaBArF) with good solubility in MeCN causes the reaction to be reduced (NaBArF: 51%) or even the desired product was trace (Cesibor), such reductions are observed for additional I source (NH_4I: no product, *n*-Bu_4NI: no product, KI: 53%).

4. According to our early research, the solvent DMF causes high yield and racemization of the substrate simultaneously.

Based on points 1 and 2, we realized that K_2CO_3, as the base, is the determining factor in our reaction. Points 2 and 3 indicate that adding NaI can increase reaction yield from 52% to 72%. The reason for this was not known and we did not consider it at this moment in our calculations. Point 4 suggests that the molecular aggregation of K_2CO_3 could significantly reduce reactivity compared to monomer K_2CO_3. According to the above discussion, a K_2CO_3-assisted *O*-to-*O* acyl transfer was hypothesized to simulate the actual reaction system.

8.2 The molecular aggregation of K_2CO_3

Even though we did not consider how phenol is protonated in our calculations, we tried to give a rough understanding of how this could happen. We computed the relative stability of monomer K_2CO_3, dimer $(K_2CO_3)_2$, and tetramer $(K_2CO_3)_4$. The calculated free energy of formation (ΔG_f) and optimized structures are shown in **Figure S49**.

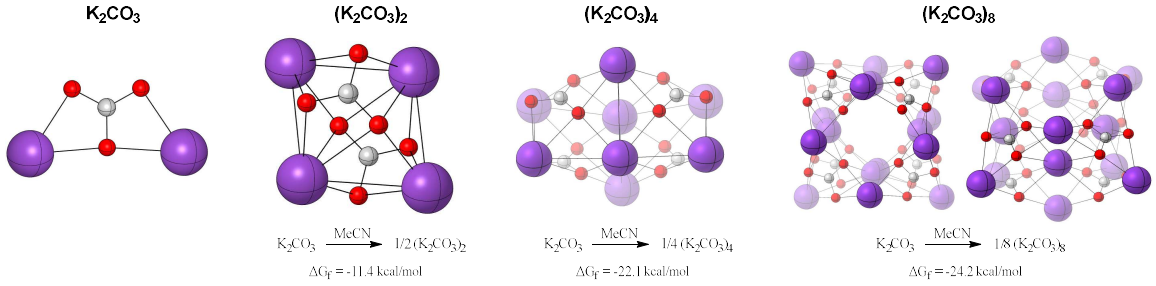


Figure S49. Molecular structures and free energy of formation of monomer K_2CO_3, dimer $(K_2CO_3)_2$, and tetramer $(K_2CO_3)_4$

The above calculations from monomeric K2CO3 to tetramers and octamers are similar (**Figure**

S49). [11,12]. Therefore, we consider the thermodynamics from DCP to **IN1** from tetramers and octamers (**Figure S50**). Phenol to form phenolate by base should be exergonic. However, our calculations shown in **Figure S50** demonstrated that these processes are endergonic and not favored. Therefore, we proposed that DCP molecule could react on the surface of potassium carbonate clusters or solid potassium carbonate in the actual system. For simplicity, we choose one potassium carbonate in our potential energy surface. Adding more K_2CO_3 could have different computed numbers but this would not change the mechanism of the present reaction.

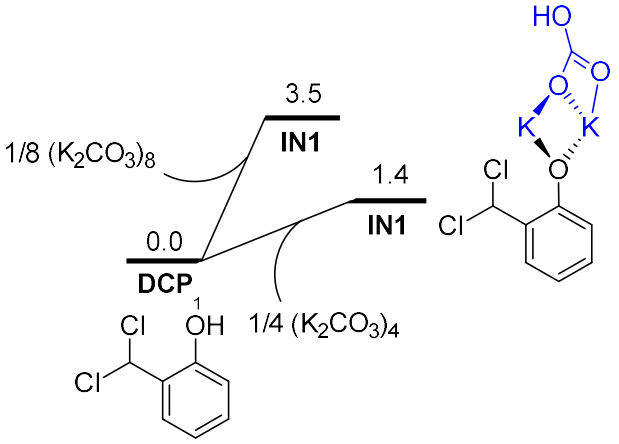


Figure S50. The energy profile of proton transfer

8.3 Details about TS4

In the process of searching **TS4**, we tried various combinations of K and O atoms and carried out a potential energy surface scan (by gradually changing the O2-C(acyl) bond distance). Our calculation showed that the whole potential energy surface was cliff-like, due to the departure of Cl^- (**Figure S51**). We checked the vibrational mode of **TS4**, finding that there is a corresponding bond breaking of the C-Cl bond and the O2-C(acyl) bond. This suggested that we located the correct transition state for this synergetic bond breaking and forming.

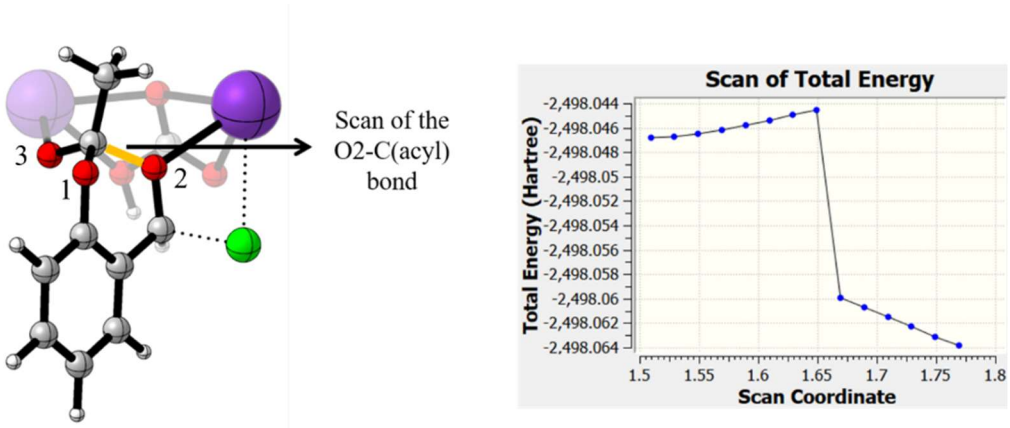


Figure S51. The scan of the O2-C(acyl) bond.

8.4 Description of chloride anion departure

From the simple but intuitive analysis in **Figure S52**, it can be seen that during the acyl transfer of DCP reagent, weaker and more stable chloride ions are generated (this is also a process of entropy increase). While 2-(chloromethyl)phenol generates stronger and unstable alkoxy anions from moderately alkaline phenol hydroxyl anions, which is endergonic (ΔG = 27.6 kcal/mol). The former process of forming KCl is also very helpful for the reaction.

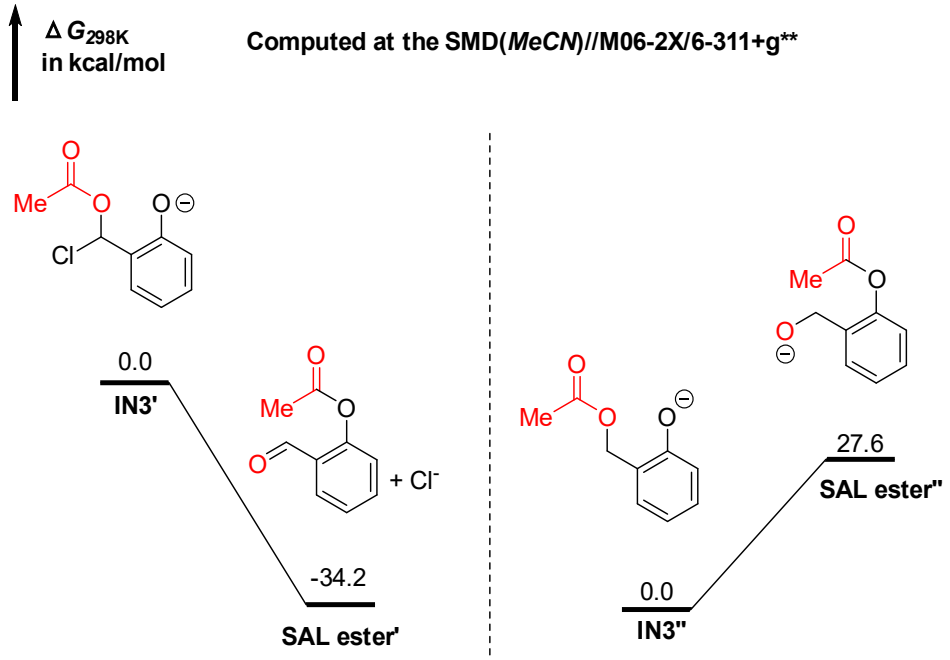


Figure S52. The Gibbs free energy profiles of IN3', SAL ester', IN3" and SAL ester".

8.5 **Discussion on the potential energy surface without $KHCO_3$**

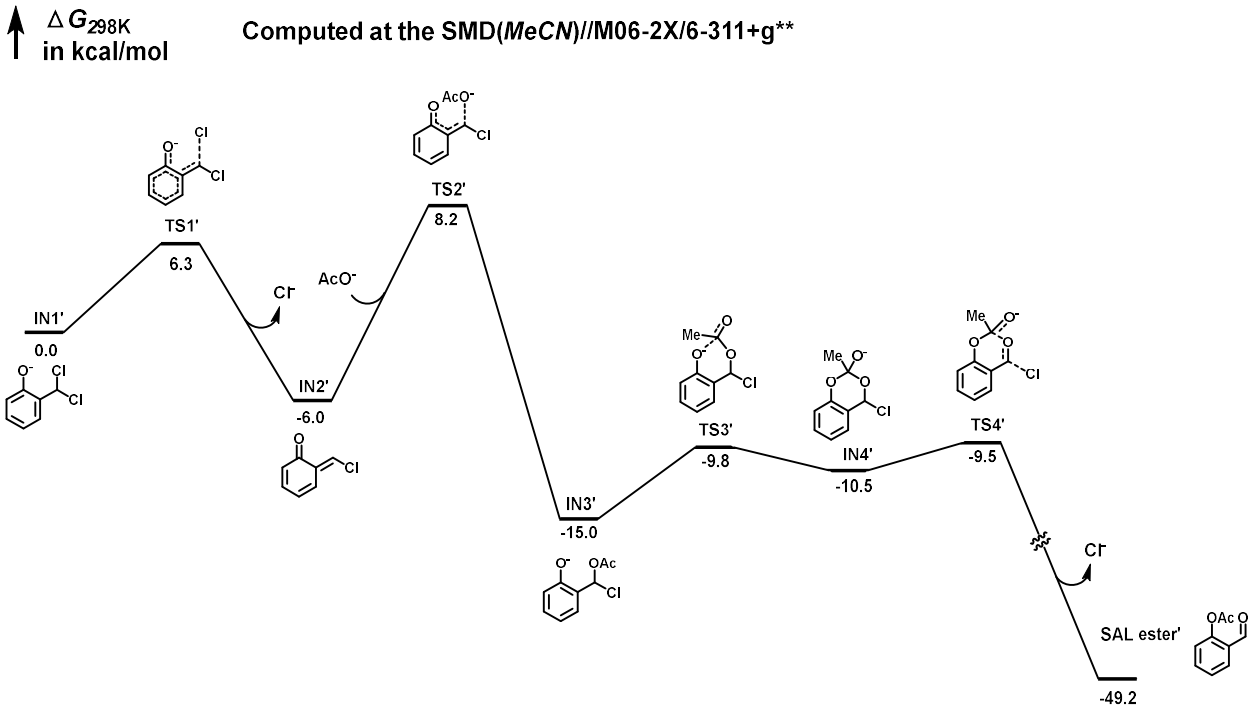


Figure S53. The Gibbs free energy profiles of anion-mediated O-to-O acyl transfer.

We proposed that using DABCO or NEt_3 generates anionic species of **IN1'**, which could also give the same product as the reaction using K_2CO_3. We computed the corresponding energy surface starting from **IN1'**, which is given in **Figure S53**. Firstly, **IN1'** is generated by base-assisted deprotonation of DCP reagent. Then entrusting Cl^- anion produced neutral species *o*-QM, **IN2'** with an activation free energy of 6.3 kcal/mol (**IN1'** to **TS1'**). The Michael addition of AcO^- converts the neutral species **IN2'** to anionic species **IN3'**. This step requires 14.2 kcal/mol. The followed processes (**IN3'**, **TS3', IN4', TS4', SAL)** are easy and exergonic.

We compare **Figures 6** and **Figure S53**, proposing that the Michael addition step is the key to the reaction. Using DABCO or NEt_3, this Michael addition step requires an activation free energy of 14.2 kcal/mol (from **IN2'** to **TS2'**), while using K_2CO_3, this is 7.0 kcal/mol (from **IN2** to **TS2**). The former is much slower than the latter and we hypothesize that, some unknown side reactions between *o*-QM and other species present in the reaction system could become favored in the former case (these side reactions could destroy the in situ generated **IN2'**). Experimentally using DABCO

or NEt_3 gave complex mixture, supporting such as hypothesis (using bulky organic base such as proton sponge gave similar results). While using K_2CO_3, the Michael addition requires only 7 kcal/mol, faster than the diffusion-controlled reaction[13]. Consequently, the side reactions to destroy **IN2** by species present in the reaction are not competitive. Therefore, we propose that K_2CO_3 can help the Michael addition and this could be key to the present reaction. Using Na_2CO_3 can also help the reaction too, even though the yield was only 24%. We propose that the basicity of Na_2CO_3 compared to K_2CO_3 could influence the reaction.

8.6 **Discussion of other important processes**

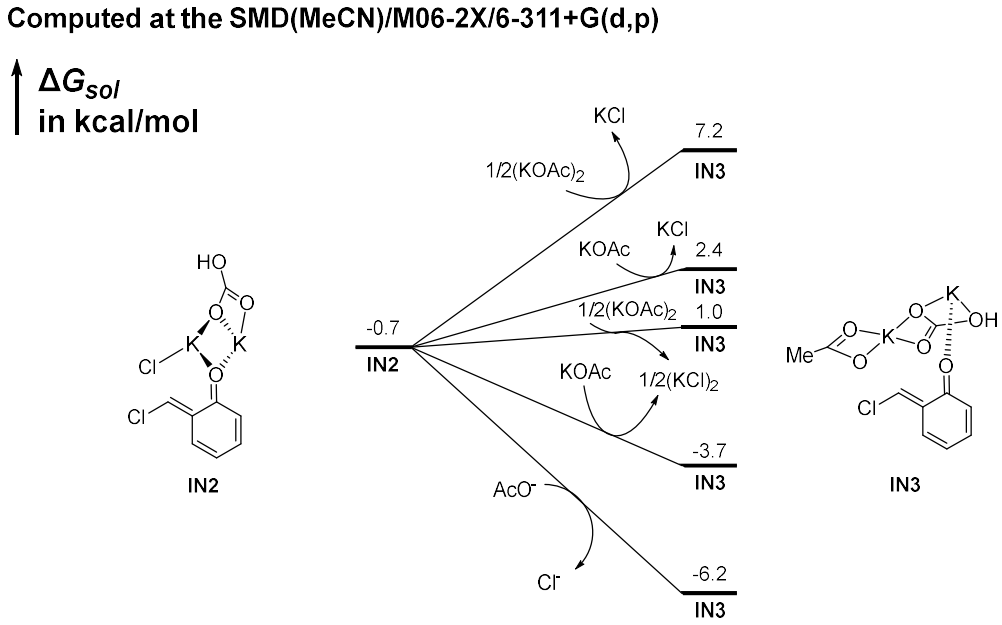


Figure S54. The Gibbs free energy profiles of the exchange of chloride ions and acetate ions.

The species involved in the ligand exchange of **IN2** to **IN3** could be $(KOAc)_n$ or free AcO^-. It is difficult to know the form of $(KOAc)_n$ in solution. As show in **Figure S54**, we here computed several possible forms and found that the thermodynamic of ligand exchange using different KOAc varies from -3.7 to 7.2 kcal/mol, but ligand exchange with AcO^- is more exergonic. Therefore, it is reasonable to have such ligand exchange with OAc^-.

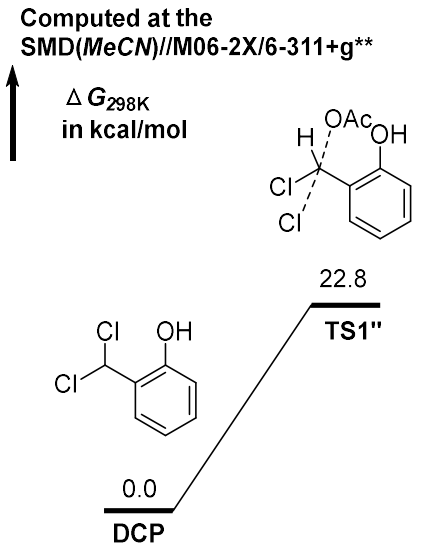


Figure S55. The process of direct nucleophilic attack on DCP reagent by acetate group.

We also considered a direct nucleophilic attack on the DCP reagent by the acetate group, which has an activation free energy of 22.8 kcal/mol (**Figure S55**). This suggests that such a process is difficult to compete with the K_2CO_3-assisted process we reported.

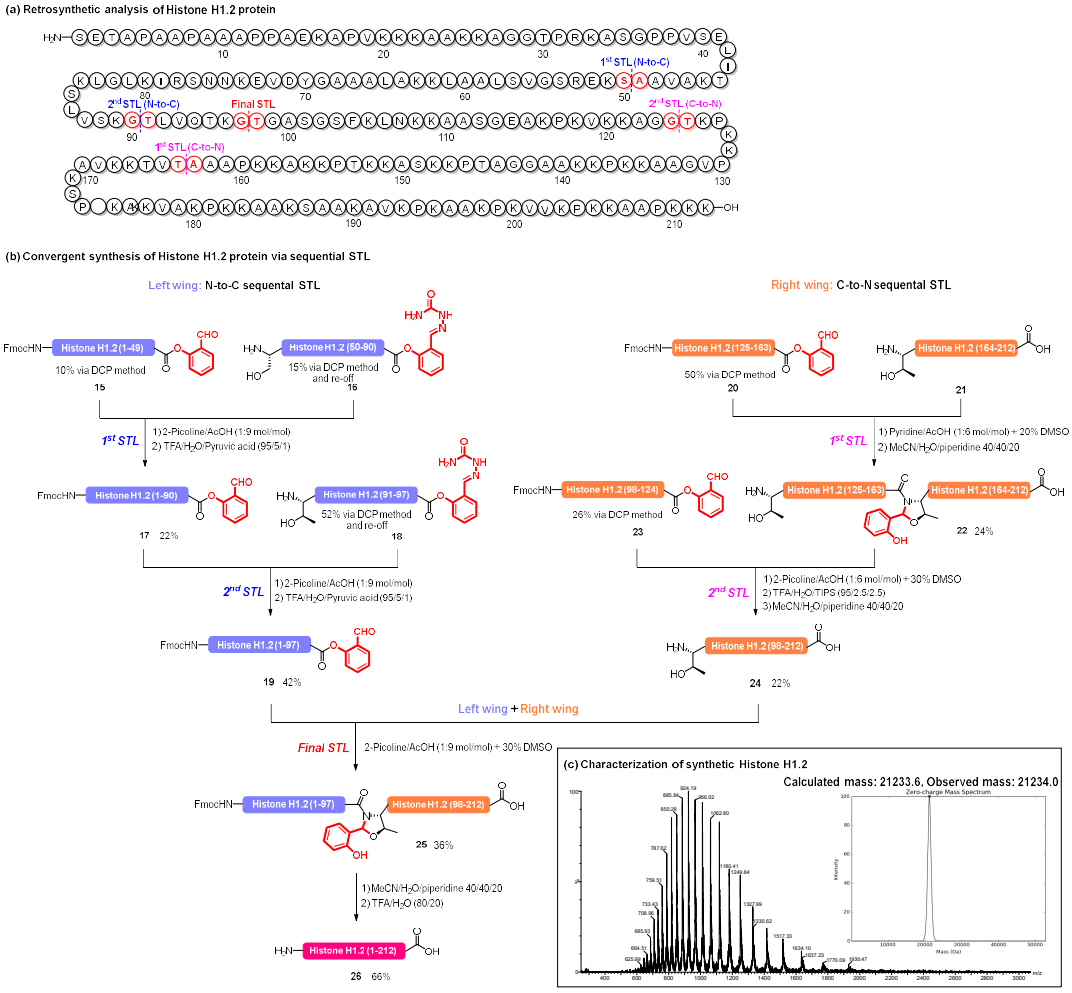
8.7 Energy data

	G_{sol}[a]		G_{sol}[a]
AcO^-	-228.575272	**$(K_2CO_3)_2$**	-2927.666893
Cl^-	-460.388722	**$(K_2CO_3)_4$**	-5855.392740
DCP	-1265.863526	**$(K_2CO_3)_8$**	-11710.809487
IN1'	-1265.401202	**$(KCl)_2$**	-2120.527335
IN1	-2729.708741	**KCl**	-1060.255386
IN1_conformer1	-2729.707238	**$(KOAc)_2$**	-1656.863605
IN1_conformer2	-2729.706939	**KOAc**	-828.416516
IN2'	-805.025080	**SAL ester"**	-573.959586
IN2	-2729.709906	**SAL ester'**	-573.280502
IN2_conformer1	-2729.709120	**SAL ester**	-2497.967168
IN3"	-574.003587	**SAL ester_conformer1**	-2497.964392

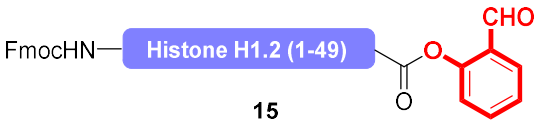
IN3'	-1033.611732	**SAL ester_conformer2**	-2497.966674
IN3	-2497.905106	**SAL ester_conformer3**	-2497.965137
IN3_conformer1	-2497.903953	**SAL ester_conformer4**	-2497.966433
IN3_conformer2	-2497.903873	**SAL ester_conformer5**	-2497.966913
IN3_conformer3	-2497.904857	**TS1''**	-1494.399510
IN3_conformer4	-2497.904116	**TS1'**	-1265.391230
IN3_conformer5	-2497.903964	**TS1**	-2729.698302
IN4'	-1033.604415	**TS1_conformer1**	-2729.696878
IN4	-2497.936653	**TS2'**	-1033.574711
IN4_conformer1	-2497.934270	**TS2**	-2497.893997
IN4_conformer2	-2497.934261	**TS2_conformer1**	-2497.892892
IN5	-2497.918582	**TS3'**	-1033.603315
IN5_conformer1	-2497.915210	**TS3**	-2497.911744
IN6	-2497.913160	**TS3_conformer1**	-2497.909207
IN6_conformer1	-2497.912897	**TS4'**	-1033.602821
IN6_conformer2	-2497.912917	**TS4**	-2497.911683
K_2CO_3	-1463.815295	**TS4_conformer1**	-2497.911338

[a] SMD(MeCN)/M06-2X/6-311+G(d,p) (hartree)

9. Total synthesis of Histone H1.2



9.1 Synthesis of 15



15

A solution of DCP reagent (0.5 mmol) in ACN (1 mL) was added dropwise slowly (dropwise over 40 min) to a flask containing the crude fully protected peptidyl acid *FmocHN*–Histone H1.2 (2-49)A–*COOH* (prepared according to general experimental procedure **2.1** and **2.2**) (619.2 mg, 0.1 mmol), K_2CO_3 (331.2 mg, 2.4mmol), NaI (450

mg, 3.0 mmol) and mL ACN/DCM (1:4, 100 mL) at room temperature under an Ar atmosphere. Then the mixture was stirred at room temperature for 2 h. After that, the reaction solvent was poured into the other flask (left solid salt in the flask). The solvent was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged. The precipitate was pelleted, and the ether was subsequently decanted. The resulting solid was purified by HPLC and lyophilization to give the peptide SAL ester **15** (50.3 mg, 10.0%) as white solid.

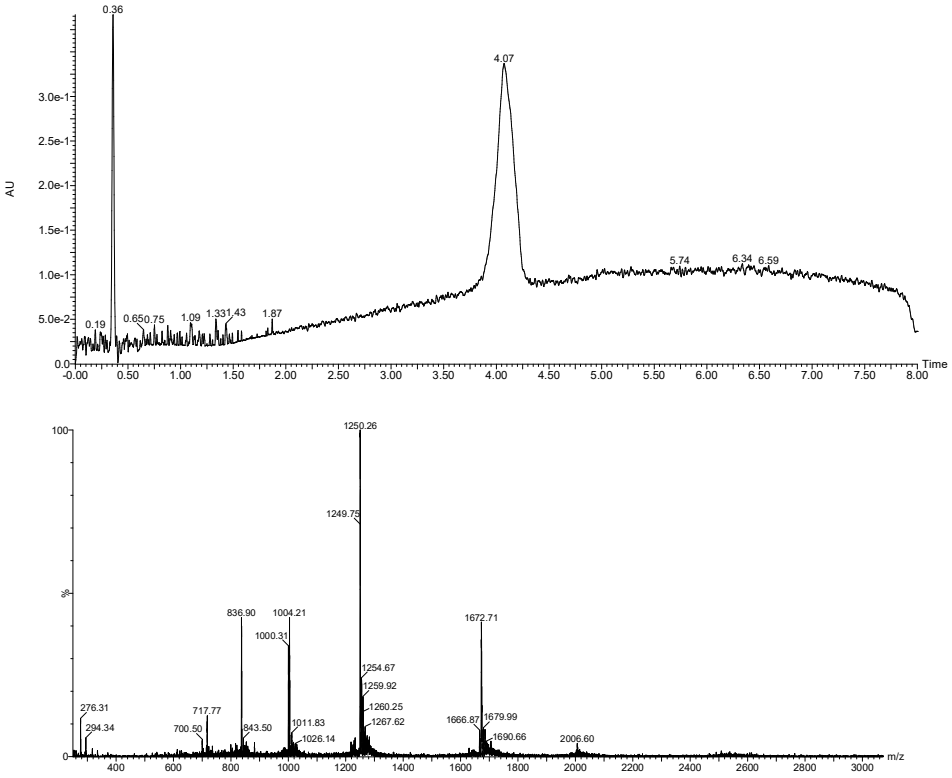
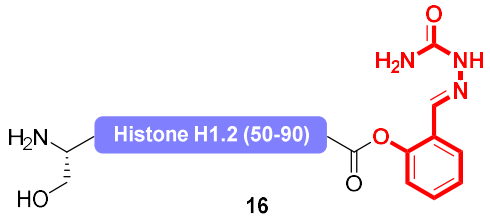


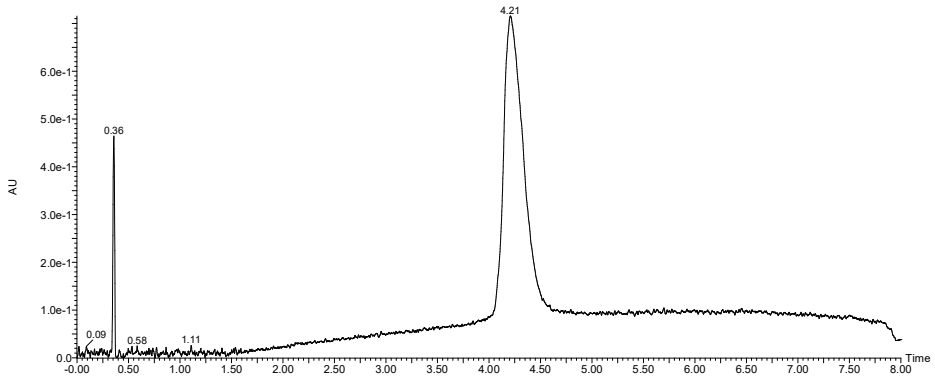
Figure S56. UV trace from analytical RP-UPLC and its ESI-MS of **15**. Gradient: 20-60% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. ESI-MS calcd. for $C_{230}H_{373}N_{60}O_{65}$ $[M+3H]^{3+}$ *m*/*z* = 1672.95, found 1672.71; $[M+4H]^{4+}$ *m*/*z* = 1254.96, found 1254.67; $[M+5H]^{5+}$ *m*/*z* = 1004.17, found 1004.21; $[M+6H]^{6+}$ *m*/*z* = 836.98, found 836.90.

9.2 Synthesis of 16



16

A solution of DCP reagent (0.5 mmol) in ACN (1 mL) was added dropwise slowly (dropwise over 40 min) to a flask containing the crude fully protected peptidyl acid *BocHN*–Histone H1.2 (51-90)G–*COOH* (prepared according to general experimental procedure **2.1** and **2.2**) (655.4 mg, 0.1 mmol), K_2CO_3 (331.2 mg, 2.4mmol), NaI (450 mg, 3.0 mmol) and mL ACN/DCM (1:4, 100 mL) at room temperature under an Ar atmosphere. Then the mixture was stirred at room temperature for 2 h. After that, the reaction solvent was poured into the other flask (left solid salt in the flask). The solvent was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged. The precipitate was pelleted, and the ether was subsequently decanted. The resulting solid was dissolved in MeCN/H_2O/TFA (250:250:0.5, *v*/*v*/*v*, 20 mL), then added semicarbazide hydrochloride (44.6 mg, 0.4 mmol). After 30 min, the mixture was purified by HPLC and lyophilization to give the peptide SAL ester **16** (66.3 mg, 15.0%) as white solid.



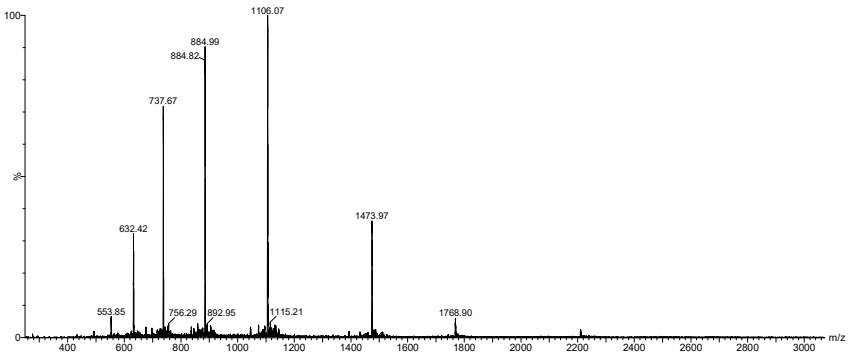
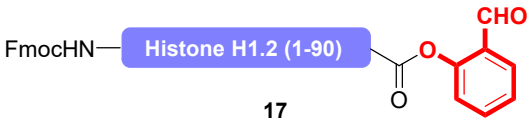


Figure S57. UV trace from analytical RP-UPLC and its ESI-MS of **16**. Gradient: 20-60% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. ESI-MS calcd. for $C_{194}H_{336}N_{59}O_{58}$ $[M+3H]^{3+}$ m/z = 1474.38, found 1473.97; $[M+4H]^{4+}$ m/z = 1106.04, found 1106.07; $[M+5H]^{5+}$ m/z = 885.03, found 884.99; $[M+6H]^{6+}$ m/z = 737.69, found 737.67; $[M+7H]^{7+}$ m/z = 632.45, found 632.42.

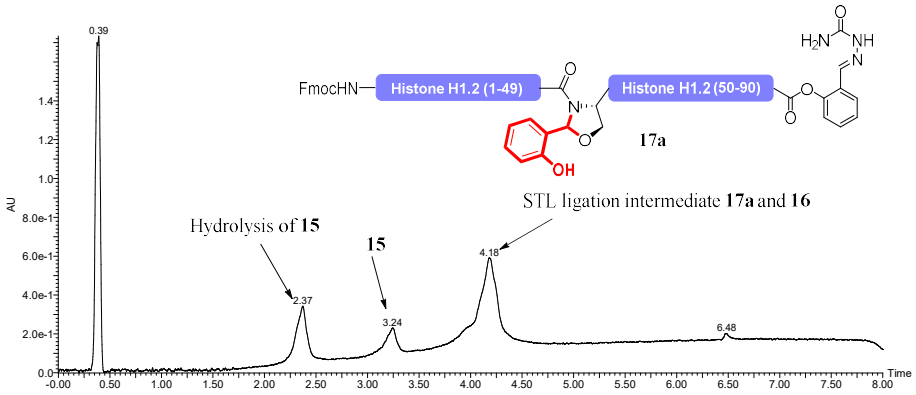
9.3 Synthesis of 17



17

15 (34.8 mg, 6.9 μmol) and **16** (20.0 mg, 4.6 μmol) were dissolved in 2-picoline/acetic acid (1/9, mol/mol) at a concentration of 5 mM at room temperature. The reaction mixture was stirred at room temperature for 16 h. Then, the solvent was removed under condensed air and washed with diethyl ether and centrifuged. The residue was treated with 1.8 mL TFA/H_2O/Pyruvic acid (95/5/1, *v*/*v*/*v*) for 1 h. Then, TFA was blown off and the residue was washed with diethyl ether and centrifuged. Finally, the product was purified by preparative reverse-phase HPLC using gradient of 20% ACN/H_2O to 60% ACN/H_2O over 45 min. After lyophilization, 9.4 mg of product **17** was obtained in an isolated yield of 22.1%.

(a) Ligation



(b) Acidolysis

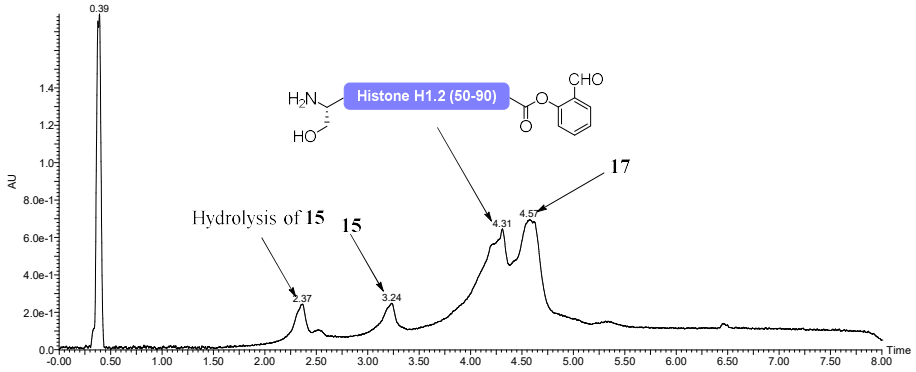


Figure S58. UV trace from analytical RP-UPLC of Ser/Thr ligation reaction mixture between **15** and **16**. (a) Ser/Thr ligation at 16 h. Gradient: 20-60% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. (b) Acidolysis at 1 h. Gradient: 20-60% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min.

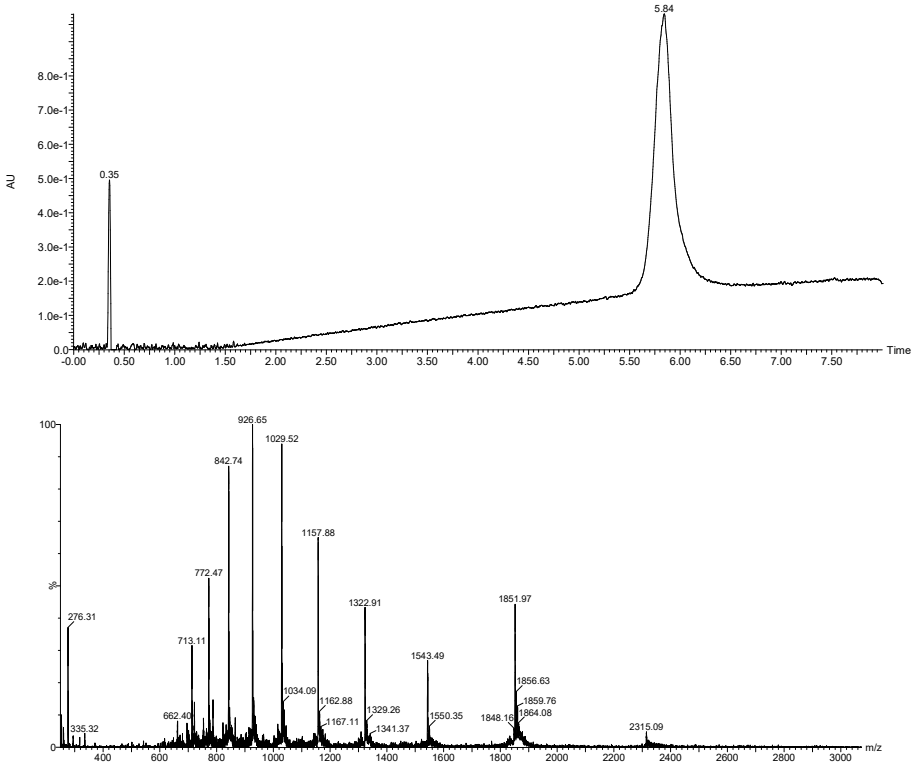
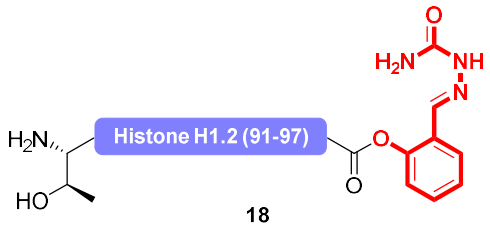


Figure S59. UV trace from analytical RP-UPLC and its ESI-MS of **17**. Gradient: 20-50% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. ESI-MS calcd. for $C_{416}H_{699}N_{116}O_{121}$ $[M+5H]^{11+}$ m/z = 1852.36, found 1851.97; $[M+6H]^{6+}$ m/z = 1543.80, found 1543.49; $[M+7H]^{7+}$ m/z = 1323.40, found 1322.91, $[M+8H]^{8+}$ m/z = 1158.10, found 1157.88, $[M+ 9H]^{9+}$ m/z = 1029.54, found 1029.52, $[M+10H]^{10+}$ m/z = 926.68, found 926.65, $[M+11H]^{11+}$ m/z = 842.53, found 842.74, $[M+12H]^{12+}$ m/z = 772.40, found 772.47, $[M+13H]^{13+}$ m/z = 712.06, found 713.11.

9.4 Synthesis of 18



18

A solution of crude DCP reagent (0.27 mmol) in ACN (1.0 mL) was added dropwise

slowly (dropwise over about 40 min, 8 drops after every 2 min) to a flask containing the crude fully protected peptidyl acid *BocHN*–Histone H1.2 (91-97)G–*COOH* (prepared according to general experimental procedure **2.1** and **2.2**) (117.1 mg, 0.09 mmol), K_2CO_3 (124.2 mg 0.90 mmol), NaI (144.0 mg, 2.16 mmol) and ACN (20 mL) at room temperature under an Ar atmosphere. After stirring at room temperature for 2 h, the mixture was filtered, and the filter cake was washed with ACN (20 mL) and DCM (20 mL). The filtration was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*, 10 mL) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged. The precipitate was pelleted, and the ether was subsequently decanted. The resulting solid was dissolved in MeCN/H_2O/TFA (250:250:0.5, *v*/*v*/*v*, 20 mL), then added semicarbazide hydrochloride (40.2 mg, 0.36 mmol). After 30 min, the mixture was purified by HPLC and lyophilization to give the peptide SAL ester **18** (42.5 mg, 52.1%) as white solid.

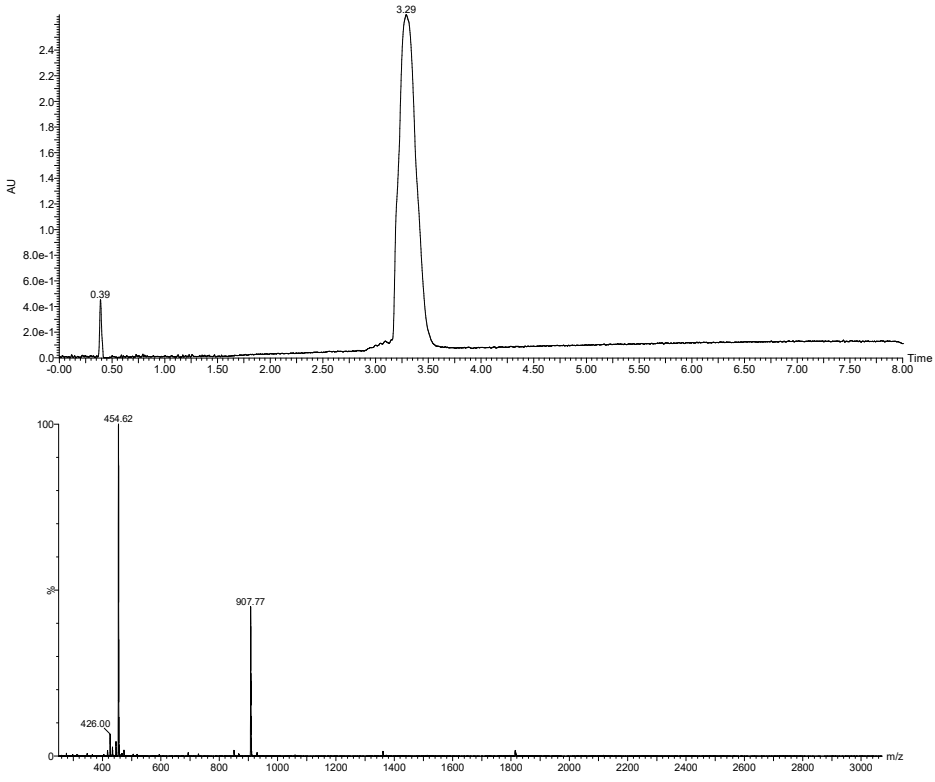
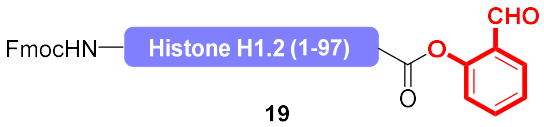


Figure S60. UV trace from analytical RP-UPLC and its ESI-MS of **18**. Gradient: 10-

50% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. ESI-MS calcd. for $C_{40}H_{67}N_{12}O_{12}$ $[M+H]^+$ m/z = 908.05, found 907.77.

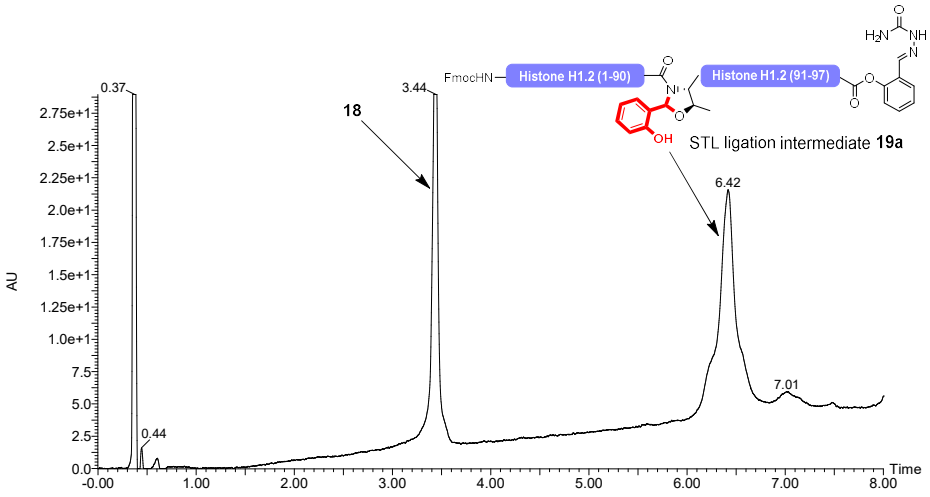
9.5 Synthesis of 19



19

17 (5.5 mg, 0.6 μmol) and **18** (2.2 mg, 2.4 μmol) were dissolved in 2-picoline/acetic acid (1/9, mol/mol) at a concentration of 5mM at room temperature. The reaction mixture was stirred at room temperature for 2 h. Then, the solvent was removed under condensed air and washed with diethyl ether and centrifuged. The residue was treated with 200 uL TFA/H_2O/Pyruvic acid (95/5/1, *v*/*v*/*v*) for 1 h. Then, TFA was blown off and the residue was washed with diethyl ether and centrifuged. Finally, the product was purified by preparative reverse-phase HPLC using gradient of 20% ACN/H_2O to 60% ACN/H_2O over 45 min. After lyophilization, 2.5 mg of product **19** was obtained in an isolated yield of 42.0%.

(a) Ligation



(b) Acidolysis

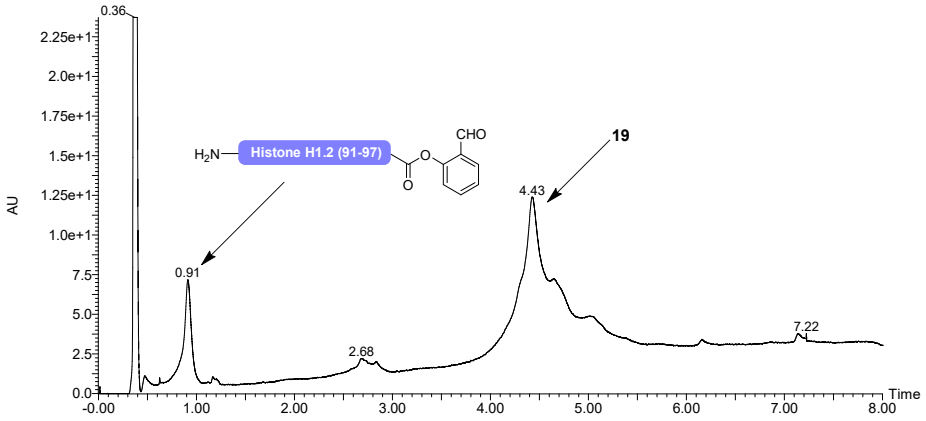


Figure S61. UV trace from analytical RP-UPLC of Ser/Thr ligation reaction mixture between **17** and **18**. (a) Ser/Thr ligation at 2 h. Gradient: 10-50% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. (b) Acidolysis at 1 h. Gradient: 20-60% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min.

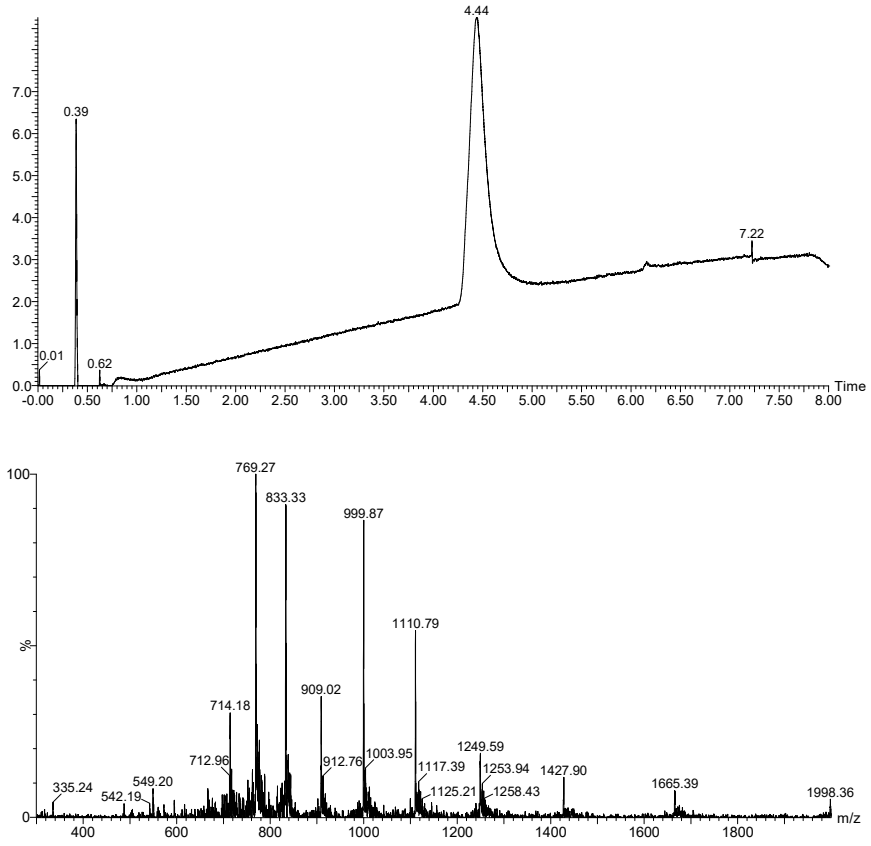
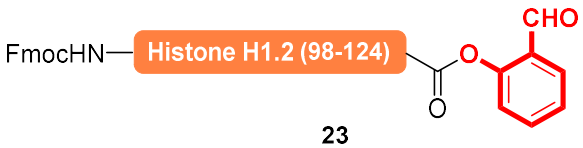


Figure S62. UV trace from analytical RP-UPLC and its ESI-MS of **19**. Gradient: 20-

60% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. ESI-MS calcd. for $C_{448}H_{751}N_{125}O_{131}$ $[M+5H]^{11+}$ m/z = 1997.94, found 1998.36; $[M+6H]^{6+}$ m/z = 1665.11, found 1665.39; $[M+7H]^{7+}$ m/z = 1427.38, found 1427.90, $[M+8H]^{8+}$ m/z = 1249.09, found 1249.59, $[M+ 9H]^{9+}$ m/z = 1110.41, found 1110.79, $[M+10H]^{10+}$ m/z = 999.47, found 999.87, $[M+11H]^{11+}$ m/z = 908.70, found 909.02, $[M+12H]^{12+}$ m/z = 833.06, found 833.33, $[M+13H]^{13+}$ m/z = 769.05, found 769.27, $[M+14H]^{14+}$ m/z = 714.19, found 714.18.

9.6 Synthesis of 23



23

A solution of DCP reagent (0.2 mmol) in ACN (1 mL) was added dropwise slowly (dropwise over 40 min) to a flask containing the crude fully protected peptidyl acid *FmocHN*–Histone H1.2(99-124)G–*COOH* (prepared according to general experimental procedure **2.1** and **2.2**) (500.0mg, 0.12 mmol), K_2CO_3 (397.4mg, 2.88mmol), NaI (540.0mg, 3.60mmol) and ACN/DCM (1:4, 40 mL) at room temperature under an Ar atmosphere. Then the mixture was stirred at room temperature for 2 h. After that, the reaction solvent was poured into the other flask (left solid salt in the flask). The solvent was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged. The precipitate was pelleted, and the ether was subsequently decanted. The resulting solid was purified by HPLC and lyophilization to give the peptide SAL ester **23** (45.2 mg, 25.6%) as white solid.

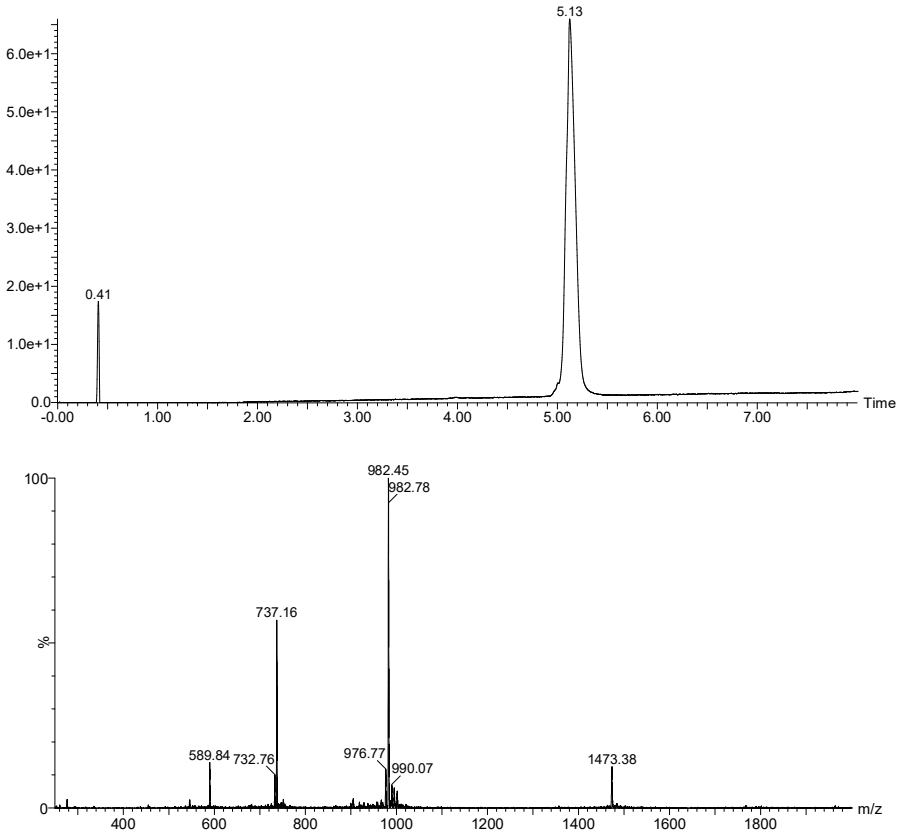
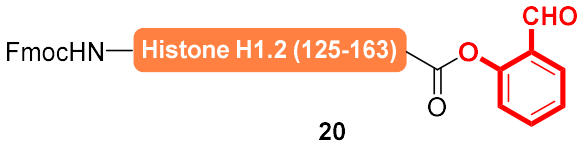


Figure S63. UV trace from analytical RP-UPLC and its ESI-MS of **23**. Gradient: 10-50% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. ESI-MS calcd. for $C_{136}H_{211}N_{35}O_{38}$ $[M+2H]^{2+}$ m/z = 1473.20, found 1473.38; $[M+3H]^{3+}$ m/z = 982.46, found 982.45; $[M+4H]^{4+}$ m/z = 737.10, found 737.16; $[M+5H]^{5+}$ m/z = 589.88, found 589.84.

9.7 Synthesis of 20



20

A solution of DCP reagent (0.2 mmol) in ACN (1 mL) was added dropwise slowly (dropwise over 40 min) to a flask containing the crude fully protected peptidyl acid *FmocHN*–Histone H1.2(126-163)A–*COOH* (prepared according to general experimental procedure **2.1** and **2.2**) (224.84 mg, 0.04 mmol), K_2CO_3 (132.5 mg,

0.96mmol), NaI (180 mg, 1.20 mmol) and ACN/DCM (1:4, 40 mL) at room temperature under an Ar atmosphere. Then the mixture was stirred at room temperature for 2 h. After that, the reaction solvent was poured into the other flask (left solid salt in the flask). The solvent was concentrated, and the resulting residue was placed in an ice bath, then the cold TFA/H_2O/DMS (90:5:5, *v*/*v*/*v*) was added. After global deprotection for 2 h, TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged. The precipitate was pelleted, and the ether was subsequently decanted. The resulting solid was purified by HPLC and lyophilization to give the peptide SAL ester **20** (67.9 mg, 40.2%) as white granular solid.

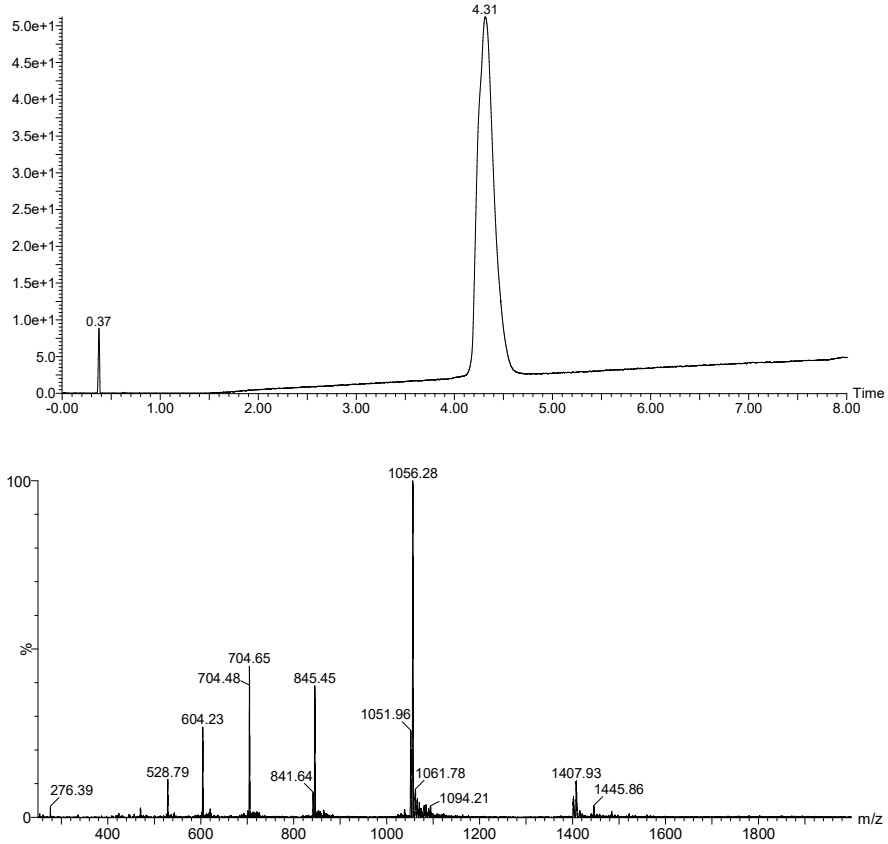
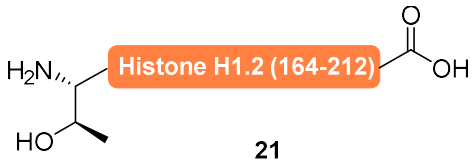


Figure S64. UV trace from analytical RP-UPLC and its ESI-MS of **20**. Gradient: 10-50% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. ESI-MS calcd. for $C_{198}H_{332}N_{54}O_{47}$ $[M+3H]^{3+}$ m/z = 1408.06, found 1407.93; $[M+4H]^{4+}$ m/z = 1056.29, found 1056.28; $[M+5H]^{5+}$ m/z = 845.23, found 845.45; $[M+6H]^{6+}$ m/z = 704.52, found 704.65; $[M+7H]^{7+}$ m/z =604.02, found 604.23, $[M+8H]^{8+}$ m/z = 528.65,

found 528.79.

9.8 Synthesis of 21



21

The crude fully protected peptidyl acid *BocHN*–Histone H1.2 (165-213)–*COOH* was synthesized on 2-chlorotrityl chloride resin according to the general experimental procedure **2.1**. The cleavage and all global deprotection mixture was a mixture of TFA//H_2O/TIPS (95/2.5/2.5, vol/vol/vol/vol). The product was purified by preparative reverse-phase HPLC using gradient of 10% ACN/H_2O to 40% ACN/H_2O over 45 min. After lyophilization, product **21** was obtained as white solid.

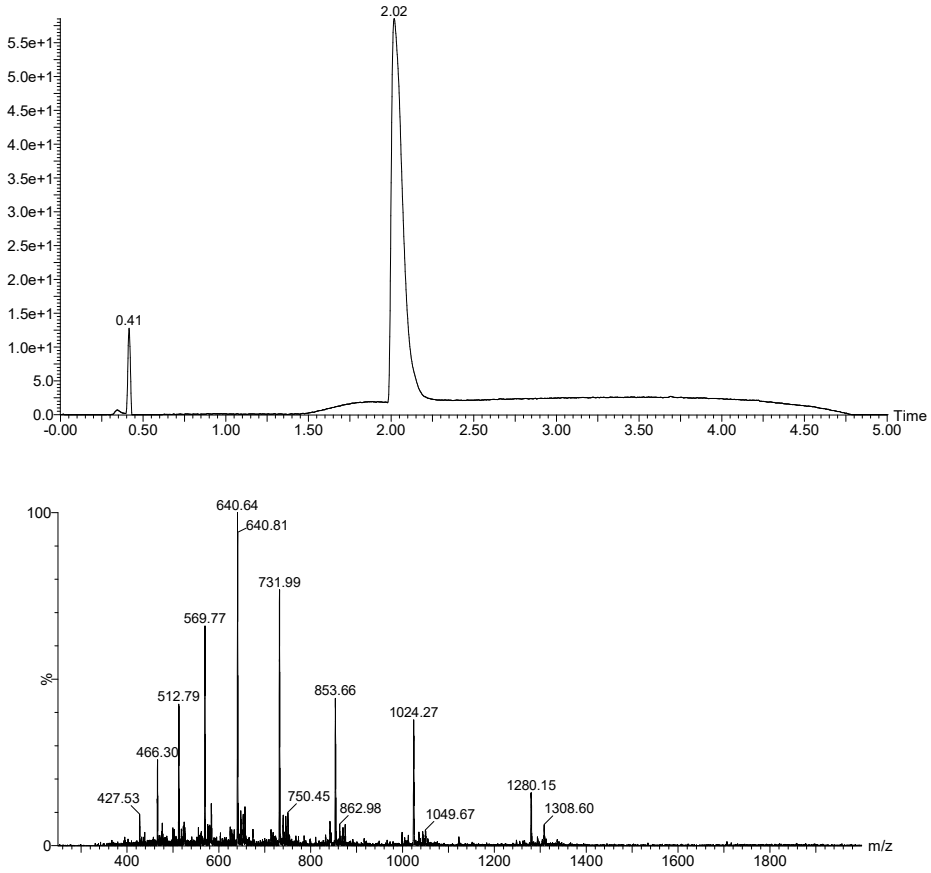
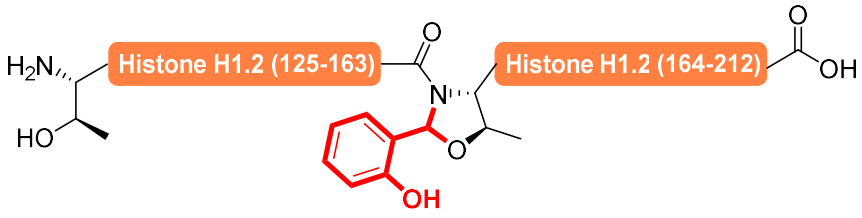


Figure S65. UV trace from analytical RP-UPLC and its ESI-MS of **21**. Gradient: 5-95%

ACN/H_2O containing 0.1% TFA over 5 min at a flow rate of 0.4 mL/min. ESI-MS calcd. for $C_{236}H_{434}N_{70}O_{54}$ $[M+4H]^{4+}$ m/z = 1280.13, found 1280.15; $[M+5H]^{5+}$ m/z = 1024.34, found 1024.27; $[M+6H]^{6+}$ m/z = 853.78, found 853.66; $[M+7H]^{7+}$ m/z = 731.96, found 731.99, $[M+8H]^{8+}$ m/z = 640.59, found 640.64, $[M+ 9H]^{9+}$ m/z = 569.52, found 569.77, $[M+10H]^{10+}$ m/z =512.67, found 512.79, $[M+11H]^{11+}$ m/z =466.15, found 466.30, $[M+12H]^{12+}$ m/z = 427.39, found 427.53.

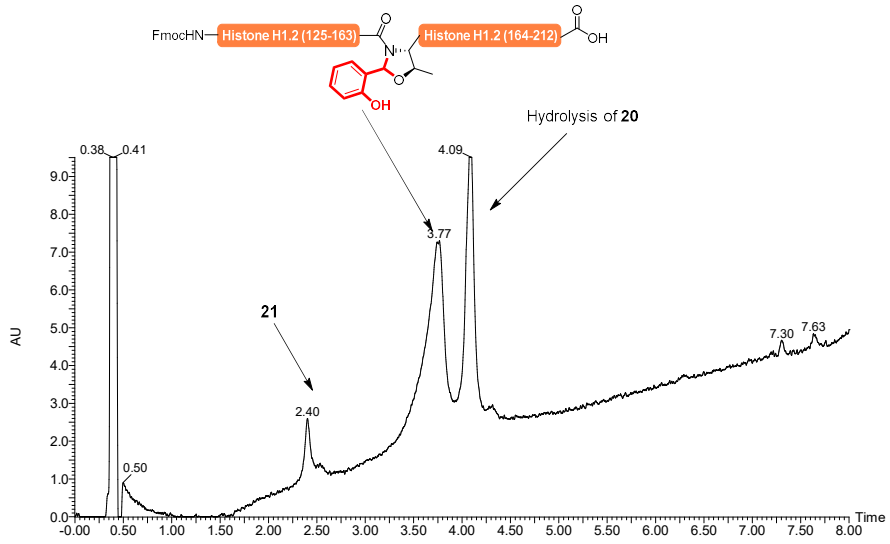
9.9 Synthesis of 22



22

20 (18.9 mg, 3.9 μmol) and **21** (24.1 mg, 5.7 μmol) were dissolved in pyridine/acetic acid (1/6, mol/mol) and 20% DMSO at a concentration of 10 mM at room temperature. The reaction mixture was stirred at room temperature for 5h. Then, the solvent was removed under condensed air and washed with diethyl ether and centrifuged. The residue was treated with 156 uL ACN/H_2O (1/1, *v*/v) with 20% piperidine for 20min. Then, the product was purified by preparative reverse-phase HPLC using gradient of 10% ACN/H_2O to 50% ACN/H_2O over 45 min. After lyophilization, 8.5 mg of product **22** was obtained in an isolated yield of 24.1%.

(a) Ligation



(b) Fmoc removal

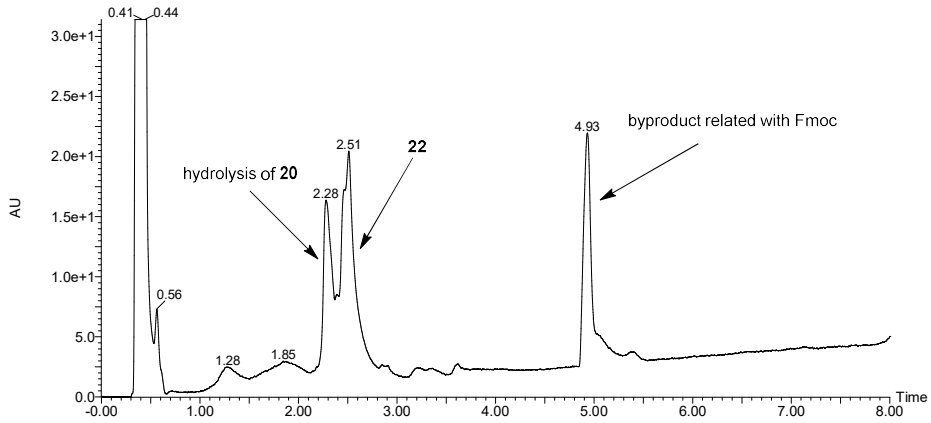


Figure S66. UV trace from analytical RP-UPLC of Ser/Thr ligation reaction mixture between **20** and **21**. (a) Ser/Thr ligation at 5 h. Gradient: 10-50% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. (b) Fmoc removal at 20 min. Gradient: 10-50% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min.

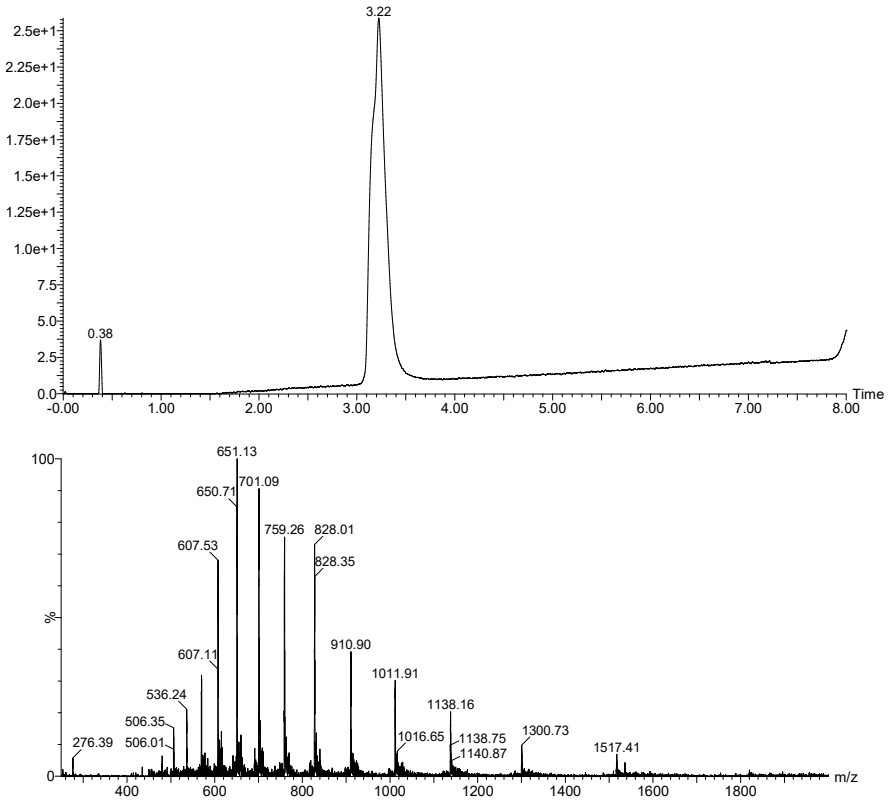
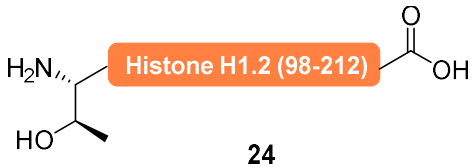


Figure S67. UV trace from analytical RP-UPLC and its ESI-MS of **22**. Gradient: 10-50% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. ESI-MS calcd. for $C_{419}H_{754}N_{124}O_{98}$ $[M+6H]^{6+}$ m/z = 1517.24, found 1517.41; $[M+7H]^{7+}$ m/z = 1300.63, found 1300.73, $[M+8H]^{8+}$ m/z = 1138.18, found 1138.16, $[M+ 9H]^{9+}$ m/z = 1011.82, found 1011.91, $[M+10H]^{10+}$ m/z = 910.74, found 910.90, $[M+11H]^{11+}$ m/z = 828.04, found 828.01, $[M+12H]^{12+}$ m/z = 759.12, found 759.26, $[M+13H]^{13+}$ m/z = 700.80, found 701.09; $[M+14H]^{14+}$ m/z = 650.81, found 651.13; $[M+13H]^{14+}$ m/z = 607.49, found 607.53.

9.10 Synthesis of 24

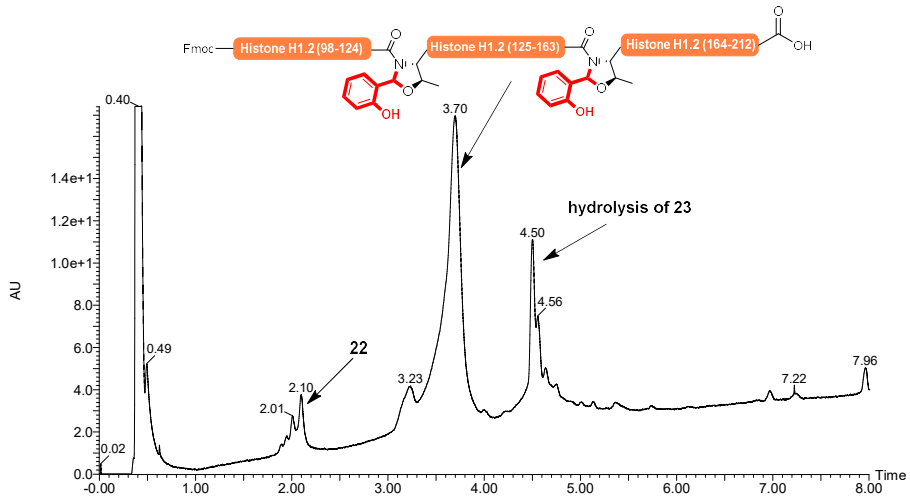


24

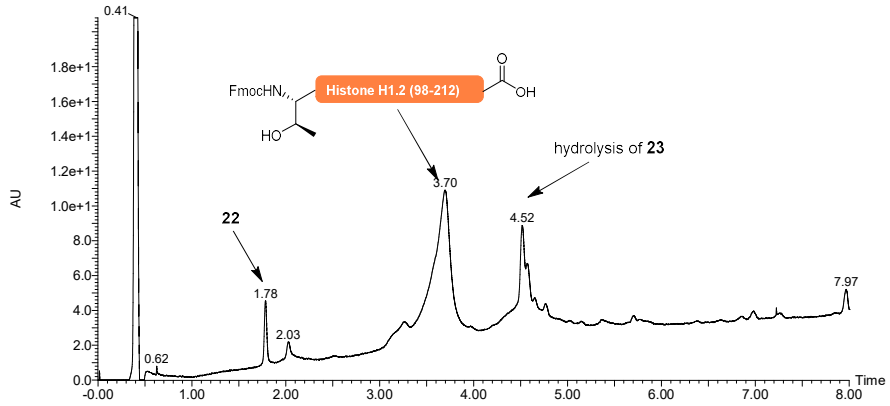
23 (17.8 mg, 6.1 μmol) and **22** (27.7 mg, 3.1 μmol) were dissolved in 2-picoline/acetic

acid (1/6, mol/mol) and 30% DMSO at a concentration of 5 mM at room temperature. The reaction mixture was stirred at room temperature for 6 h. Then, the solvent was removed under condensed air and washed with diethyl ether and centrifuged. The residue was treated with 350 uL TFA/H_2O/TIPS (95/2.5/2.5, *v*/*v*/*v*) for 1 h. TFA was blown off and the reside was washed with diethyl ether and centrifuged. Direct Fmoc removal will result in overlap between product and starting materials. Finally, product with N-terminal Fmoc was purified by preparative reverse-phase HPLC using gradient of 10% ACN/H_2O to 50% ACN/H_2O over 45 min. The N-terminal with Fmoc protected product was lyophilized and then treated with 700 uL ACN/H_2O (1/1, *v*/*v*) with 20% piperidine for 20 min. Then, the product was purified by second time preparative reverse-phase HPLC using gradient of 10% ACN/H_2O to 50% ACN/H_2O over 45 min. After lyophilization, 3.0 mg of product **24** was obtained in an isolated yield of 21.6%.

(a) Ligation



(b) Acidolysis



(c) Fmoc removal

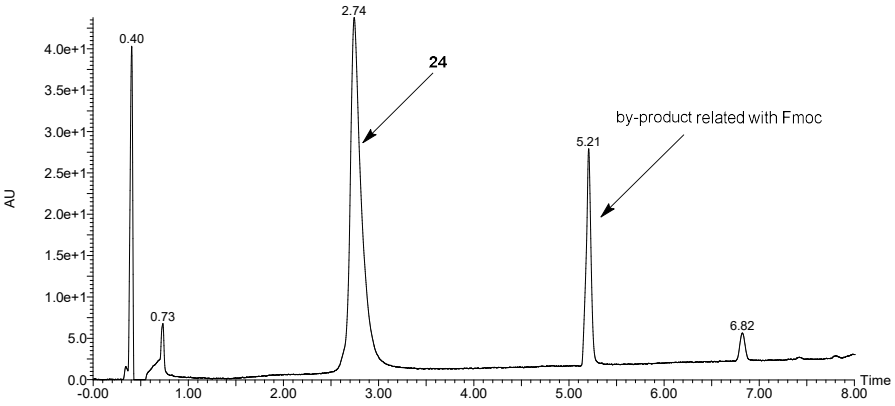


Figure S68. UV trace from analytical RP-UPLC of Ser/Thr ligation reaction mixture between **22** and **23** (a) Ser/Thr ligation at 6h. Gradient: 10-50% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. (b) Acidolysis at 1 h. Gradient: 10-50% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. (c) Fmoc removal at 20 min. Gradient: 10-50% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min.

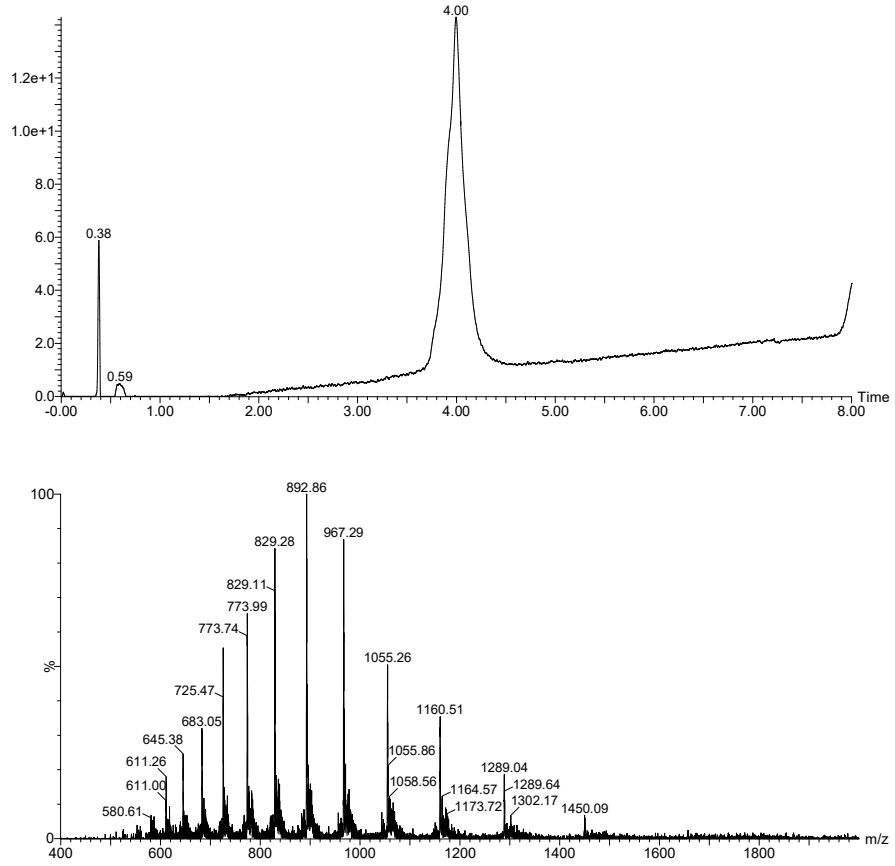
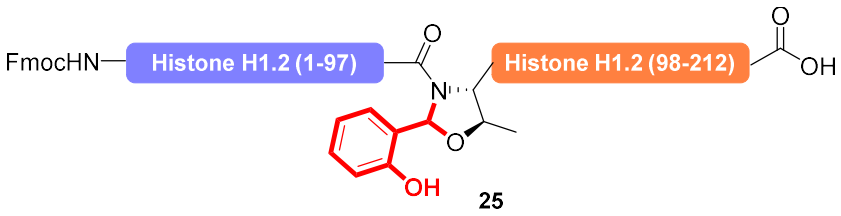


Figure S69. UV trace from analytical RP-UPLC and its ESI-MS of **24**. Gradient: 10-50% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. ESI-MS calcd. for $C_{526}H_{945}N_{159}O_{131}$ $[M+8H]^{8+}$ m/z = 1450.17, found 1450.09, $[M+ 9H]^{9+}$ m/z = 1289.15, found 1289.04, $[M+10H]^{10+}$ m/z = 1160.33, found 1160.51, $[M+11H]^{11+}$ m/z = 1054.94, found 1055.26, $[M+12H]^{12+}$ m/z = 967.11, found 967.29, $[M+13H]^{13+}$ m/z = 892.79, found 892.86; $[M+14H]^{14+}$ m/z = 829.10, found 829.28; $[M+15H]^{15+}$ m/z = 773.89, found 773.99; $[M+16H]^{16+}$ m/z = 725.58, found 725.47; $[M+17H]^{17+}$ m/z = 682.96, found 683.05; $[M+18H]^{18+}$ m/z = 645.07, found 645.38; $[M+19H]^{19+}$ m/z =611.18 , found 611.26.

9.11 Synthesis of 25



19 (9.1 mg, 0.91 μmol) and **24** (7.4 mg, 0.64 μmol) were dissolved in 2-picoline/acetic acid (1/9, mol/mol) and 30% DMSO at a concentration of 2 mM at room temperature. The reaction mixture was stirred at room temperature for 14 h. Then, the solvent was removed under condensed air and washed with diethyl ether and centrifuged. The resulting solid was purified by preparative reverse-phase HPLC using gradient of 20% ACN/H_2O to 60% ACN/H_2O over 45 min. After lyophilization, 4.9 mg of product **25** was obtained in an isolated yield of 35.6%.

Ligation

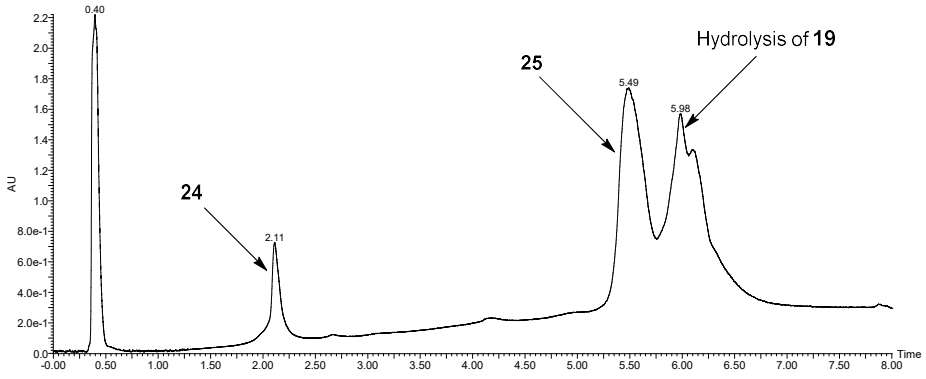


Figure S70. UV trace from analytical RP-UPLC of Ser/Thr ligation reaction mixture between **19** and **24**. (a) Ser/Thr ligation at 14 h. Gradient: 10-50% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min.

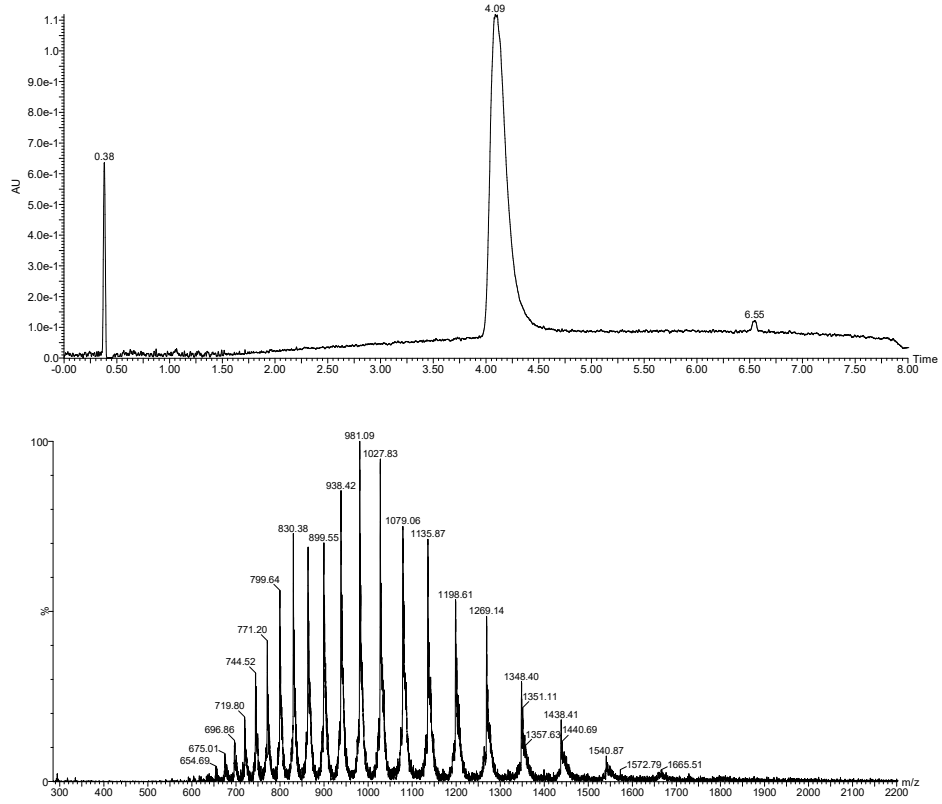


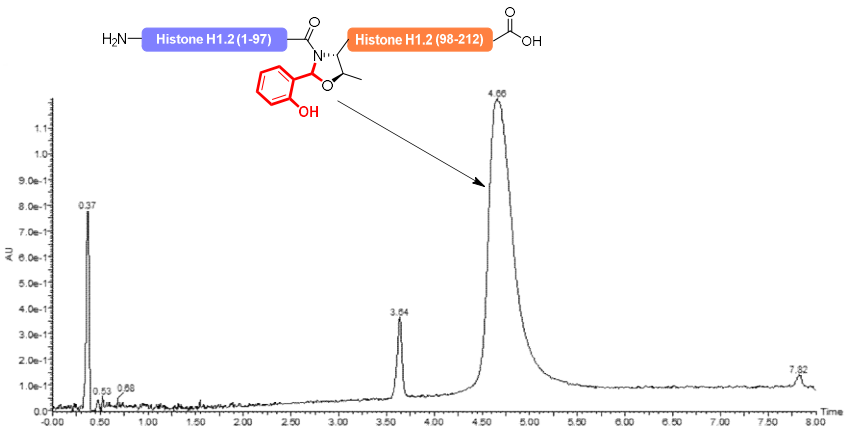
Figure S71. UV trace from analytical RP-UPLC and its ESI-MS of **25**. Gradient: 20-60% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. ESI-MS calcd. for $C_{974}H_{1784}N_{284}O_{261}$ $[M+14H]^{14+}$ m/z = 1540.99, found 1540.87; $[M+15H]^{15+}$ m/z = 1438.33, found 1438.41; $[M+16H]^{16+}$ m/z = 1348.50, found 1348.40; $[M+17H]^{17+}$ m/z = 1269.23, found 1269.14; $[M+18H]^{18+}$ m/z =1198.78, found 1198.61; $[M+19H]^{19+}$ m/z =1135.74, found 1135.87; $[M+20H]^{20+}$ m/z = 1079.00, found 1079.06; $[M+21H]^{21+}$ m/z =1027.67, found 1027.83; $[M+22H]^{22+}$ m/z = 981.01, found 981.09; $[M+23H]^{23+}$ m/z = 938.39, found 938.42; $[M+24H]^{24+}$ m/z = 899.33, found 899.55; $[M+25H]^{25+}$ m/z = 863.40, found 863.40; $[M+26H]^{26+}$ m/z = 830.23, found 830.38; $[M+27H]^{27+}$ m/z = 799.52, found 799.64; $[M+28H]^{28+}$ m/z = 771.01, found 771.20; $[M+29H]^{29+}$ m/z = 744.45, found 744.52; $[M+30H]^{30+}$ m/z = 719.67, found 719.80; $[M+31H]^{31+}$ m/z = 696.48, found 696.86; $[M+32H]^{32+}$ m/z = 674.75, found 675.01; $[M+33H]^{33+}$ m/z = 654.33, found 654.69.

9.12 Synthesis of 26

26

25 (2.2 mg, 0.10 umol) was treated with 50 uL ACN/H_2O/piperidine (4/4/2, *v*/*v*/v) for 20min. The result mixture was lyophilization to give the white solid. The white solid was treated with 50 uL TFA/H_2O (80/20, v/v) for 30 min. TFA was blown off and the oily residue was triturated with diethyl ether and centrifuged. The precipitate was pelleted, and the ether was subsequently decanted. The resulting solid was purified by HPLC and lyophilization to give final product **26** (1.4 mg, 66.0%) as white solid.

(a) Fmoc removal



(b) Acidolysis

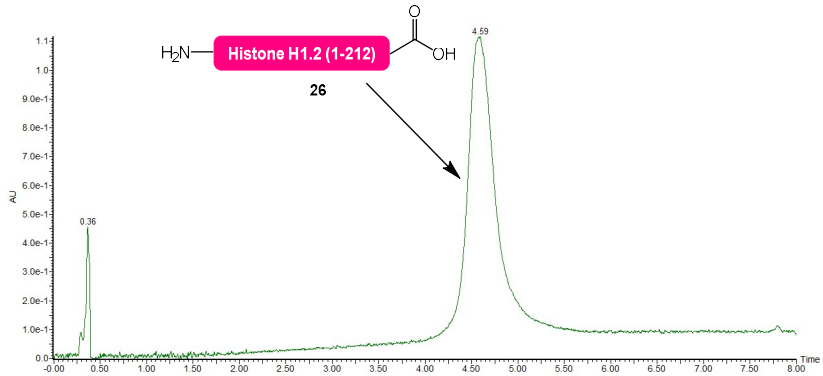


Figure S72. UV trace from analytical RP-UPLC of deFmoc and acidolysis of **25**. (a) Fmoc removal at 20 min. Gradient: 20-50% ACN/H_2O containing 0.1% TFA over 8

min at a flow rate of 0.4 mL/min. (b) Acidolysis at 30 min. Gradient: 20-50% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min.

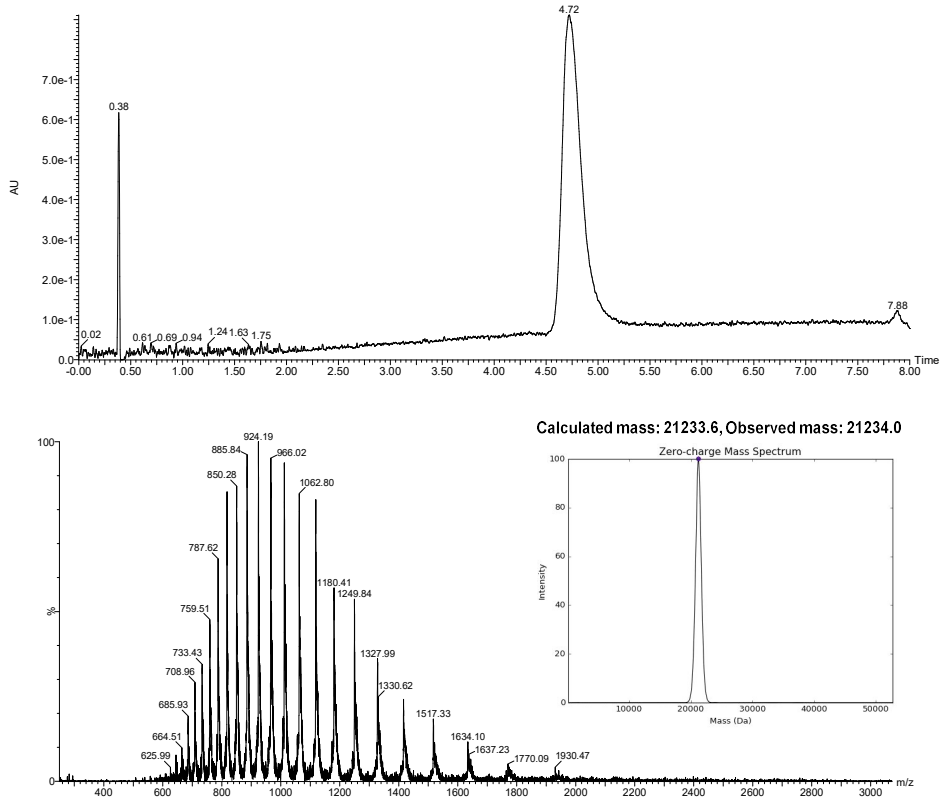


Figure S73. UV trace from analytical RP-UPLC and its deconvolution of mass spectra of protein **26**. Gradient: 20-50% ACN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. ESI-MS calcd. for $C_{952}H_{1692}N_{284}O_{258}$ $[M+12H]^{12+}$ m/z =1770.47, found 1770.09; $[M+ 13H]^{13+}$ m/z = 1634.36, found1634.10; $[M+14H]^{14+}$ m/z = 1517.69, found1517.33; $[M+15H]^{15+}$ m/z = 1416.56, found 1416.22; $[M+16H]^{16+}$ m/z = 1328.10, found 1327.99; $[M+17H]^{17+}$ m/z = 1250.04, found1249.84; $[M+18H]^{18+}$ m/z =1180.65, found 1180.41; $[M+19H]^{19+}$ m/z =1118.56, found 1118.51; $[M+20H]^{20+}$ m/z = 1062.68, found 1062.80; $[M+21H]^{21+}$ m/z =1012.13, found 1012.20; $[M+22H]^{22+}$ m/z = 966.17, found 966.02; $[M+23H]^{23+}$ m/z = 924.20, found 924.19; $[M+24H]^{24+}$ m/z = 885.74, found 885.84; $[M+25H]^{25+}$ m/z = 850.35, found 850.28; $[M+26H]^{26+}$ m/z = 817.68, found 817.68; $[M+27H]^{27+}$ m/z = 787.43, found 787.62; $[M+28H]^{28+}$ m/z = 759.34, found 759.51; $[M+29H]^{29+}$ m/z = 733.19, found 733.43; $[M+30H]^{30+}$ m/z = 708.79, found 708.96; $[M+31H]^{31+}$ m/z = 685.96, found 685.93.

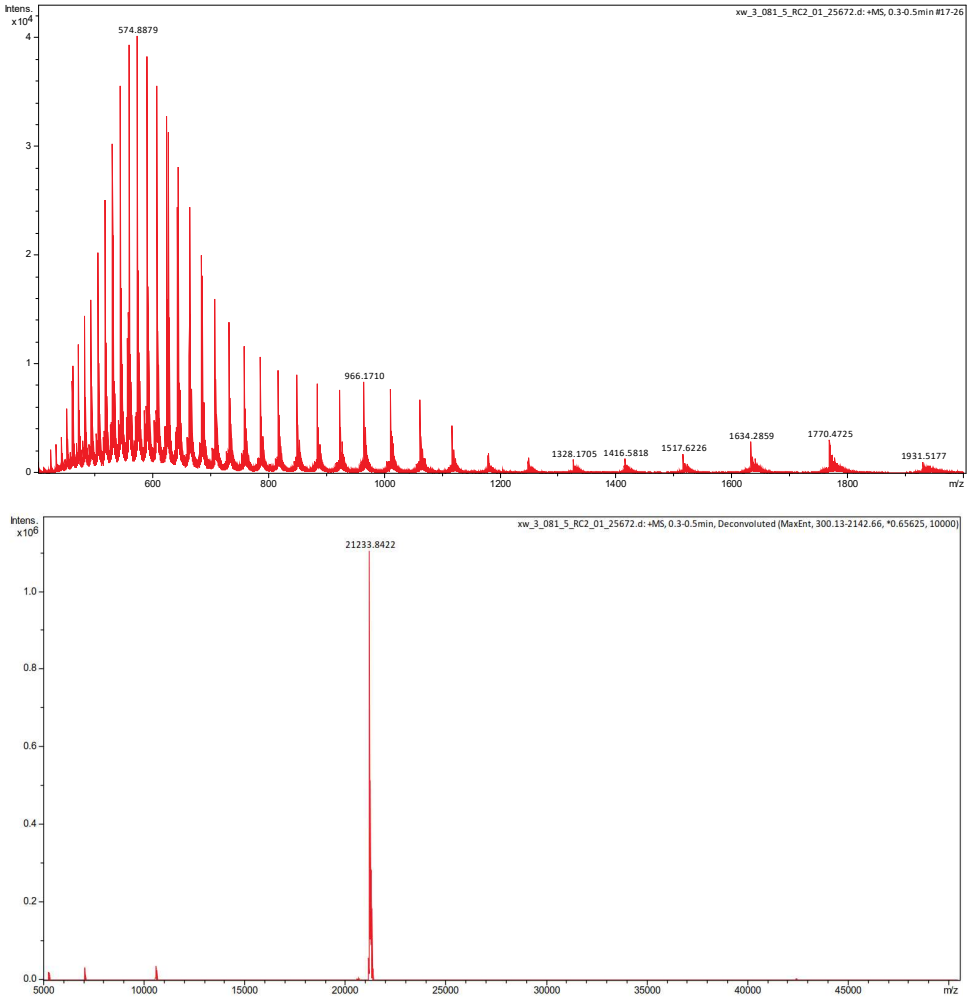
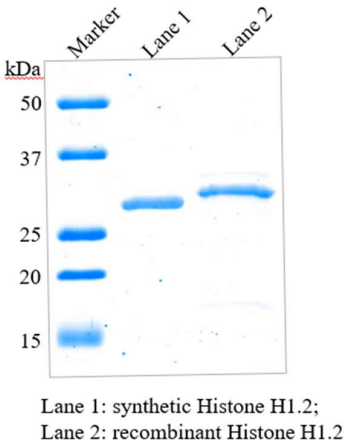


Figure S74. ESI-HRMS and its deconvolution of mass spectra of protein **26**



Lane 1: synthetic Histone H1.2;
Lane 2: recombinant Histone H1.2

Figure S75. SDS-PAGE of synthetic Histone H1.2 and recombinant expressed Histone H1.2 (with His_6 tag at C-terminal)

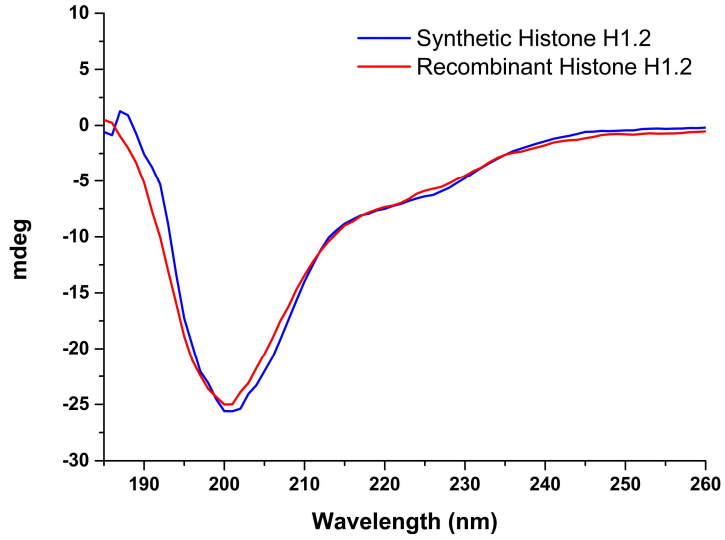


Figure S76. CD of Synthetic Histone H1.2 and recombinant expressed Histone H1.2**.** The Synthesized human Histone H1.2 and Rosetta 2 (DE3) Competent Cell expressed Histone H1.2 (with His_6 tag at C-terminal) were dissolved in 0.5 mL phosphate buffer (10 mM Na_2HPO_4, 1.7 mM KH_2PO_4, 137mM NaF, pH 7.4), respectively. The concentration of the synthetic one was 0.273 mg/mL and the recombinant one was 0.261 mg/mL (The protein concentration was determined by Thermo Scientific NanoDrop UV-Vis spectrophotometers). The CD spectrum was measured by a J-815 circular dichroism spectrometer (JASCO), each sample was scanned for 1 time at room temperature using phosphate buffer (10 mM Na_2HPO_4, 1.7 mM KH_2PO_4, 137mM NaF, pH 7.4) as blank in a 0.1 cm cell.

CD data of Synthetic Histone H1.2

wavelength	mdeg
260	-0.18756
259	-0.23435
258	-0.21841
257	-0.26482
256	-0.27626
255	-0.29849
254	-0.26372
253	-0.28367
252	-0.32494
251	-0.43476
250	-0.42972
249	-0.46527
248	-0.49814

247	-0.4753
246	-0.54881
245	-0.57211
244	-0.75221
243	-0.92067
242	-1.04124
241	-1.18667
240	-1.39703
239	-1.6066
238	-1.83025
237	-2.04917
236	-2.29013
235	-2.62367
234	-2.98094
233	-3.38215
232	-3.79829
231	-4.25309
230	-4.75387
229	-5.18335
228	-5.63577
227	-5.9481
226	-6.27599
225	-6.39224
224	-6.5838
223	-6.80845
222	-7.07134
221	-7.25479
220	-7.50869
219	-7.65343
218	-7.94872
217	-8.09774
216	-8.46044
215	-8.83898
214	-9.41581
213	-10.0805
212	-11.189
211	-12.5417
210	-13.9666
209	-15.6708
208	-17.4106
207	-19.1274
206	-20.7981
205	-22.0469
204	-23.2898
203	-24.075
202	-25.3811
201	-25.6028
200	-25.5912
199	-24.548

198	-23.1022
197	-22.0734
196	-19.6769
195	-17.2778
194	-13.3296
193	-8.86615
192	-5.31788
191	-3.73973
190	-2.56589
189	-0.69976
188	0.912201
187	1.27302
186	-0.86041
185	-0.5833

CD data of recombinant expressed Histone H1.2

wavelength	mdeg
260	-0.52309
259	-0.56821
258	-0.59213
257	-0.67656
256	-0.70741
255	-0.71737
254	-0.72825
253	-0.69169
252	-0.75412
251	-0.81287
250	-0.78438
249	-0.7525
248	-0.7828
247	-0.8484
246	-1.01377
245	-1.12701
244	-1.27923
243	-1.31151
242	-1.39595
241	-1.52565
240	-1.76305
239	-1.9509
238	-2.1328
237	-2.3301
236	-2.43363
235	-2.60996
234	-2.91127
233	-3.29633
232	-3.76707
231	-4.08042
230	-4.51452

229	-4.84072
228	-5.23009
227	-5.55287
226	-5.71674
225	-5.89704
224	-6.1581
223	-6.62086
222	-6.97843
221	-7.22345
220	-7.3425
219	-7.57358
218	-7.83911
217	-8.14967
216	-8.64691
215	-8.98675
214	-9.69693
213	-10.4097
212	-11.254
211	-12.274
210	-13.4351
209	-14.6889
208	-16.2237
207	-17.4714
206	-19.0585
205	-20.4802
204	-21.7623
203	-23.1
202	-23.9018
201	-24.9863
200	-25.0166
199	-24.3412
198	-23.6168
197	-22.4269
196	-21.0622
195	-18.8892
194	-15.9239
193	-13.0366
192	-10.0509
191	-7.75282
190	-5.16969
189	-3.31551
188	-1.99442
187	-0.94713
186	0.237411
185	0.497379

9.13 Expression of Histone H1.2

The expressed Histone H1.2 sample used as standard in SDS-PAGE analysis and CD spectrometry was prepared as follows. The plasmids VB900124-6412zdn_Histone H1.2_His6 were introduced into *E. coli* Rosetta 2 (DE3) competent cells via a transformation. The transformed cells were then streaked onto a selection plate containing ampicillin (100 µg/mL) and incubated overnight at 37 ºC. From the resulting plate, a single colony was selected and used to establish a starter culture in Lysogeny broth (LB) liquid medium supplemented with ampicillin (100 µg/mL). The starter culture was subsequently inoculated at a 1:100 dilution into LB liquid medium with ampicillin (100 µg/mL) for further incubation at 37 ºC. Once the optical density (O.D.) of the cell suspension reached 0.6, 1 mM IPTG was added, and the cells were incubated at 4 ºC for an additional 12 hours.

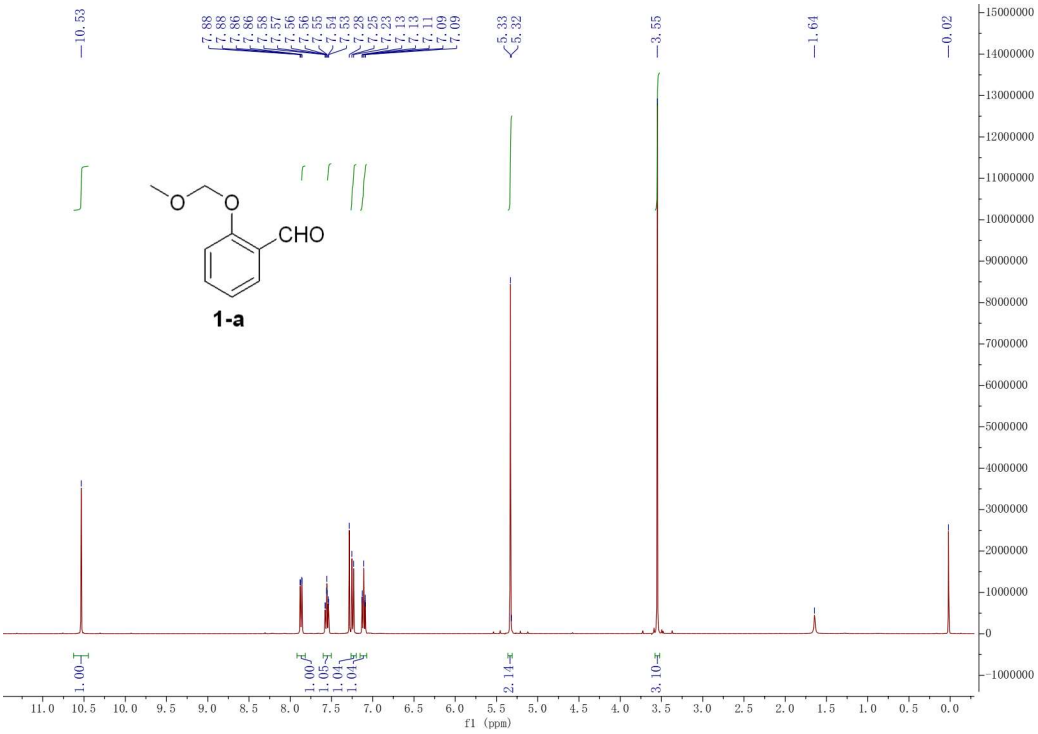
The cells were collected by centrifugation and then suspended in lysis buffer (50 mM Tris-HCl, 500 mM NaCl, 5% Glycerol, pH 8.0). To induce cell lysis, the suspension was sequentially passed through a homogenizer and a high-pressure cell crusher. Subsequently, the resulting lysate was subjected to centrifugation at 15000 rpm for 30 minutes at 4 ºC. After centrifugation, the resulting supernatant was combined with 1 mL of nickel gel in a 125 mL mixing bottle. The mixture was incubated for 40 minutes at 4 °C. Subsequently, the suspension was introduced into a gravity chromatography column to separate the nickel gel from the supernatant. The nickel gel was then resuspended using lysis buffer within the gravity chromatography column and washed with a washing buffer (50 mM Tris-HCl, 500 mM NaCl, 5% Glycerol, 40 mM IMD, pH 8.0) at a volume 30 to 50 times that of the gel. The protein of interest was subsequently eluted using an elution buffer (500 mM Tris-HCl, 500 mM NaCl, 5% Glycerol, 250 mM IMD, pH 8.0) at a volume 3 to 5 times that of the gel. Finally, the eluted proteins were finally concentrated using a Millipore Centrifugal Filter with a molecular weight cutoff (MWCO) of 10 kDa.

The sequence of the expressed Histone H1.2 is MSETAPAAPAAAPPAEKAPVKKKAAKKAGGTPRKASGPPVSELITKAVAASK

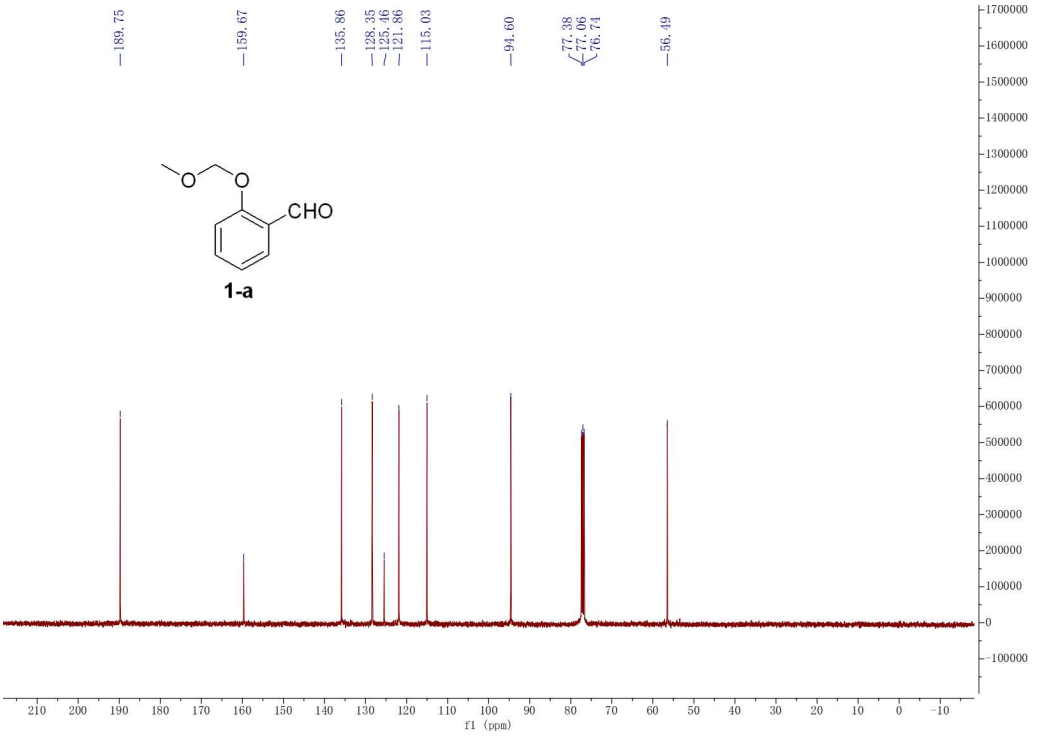
ERSGVSLAALKKALAAAGYDVEKNNSRIKLGLKSLVSKGTLVQTKGTGASGS
FKLNKKAASGEAKPKVKKAGGTKPKKPVGAAKKPKKAAGGATPKKSAKKT
PKKAKKPAAATVTKKVAKSPKKAKVAKPKKAAKSAAKAVKPKAAKPKVVK
PKKAAPKKKLEHHHHHH (221 AAs, His6 at C-terminus with LE as spacer).

10.^{1}H and ^{13}C NMR spectra

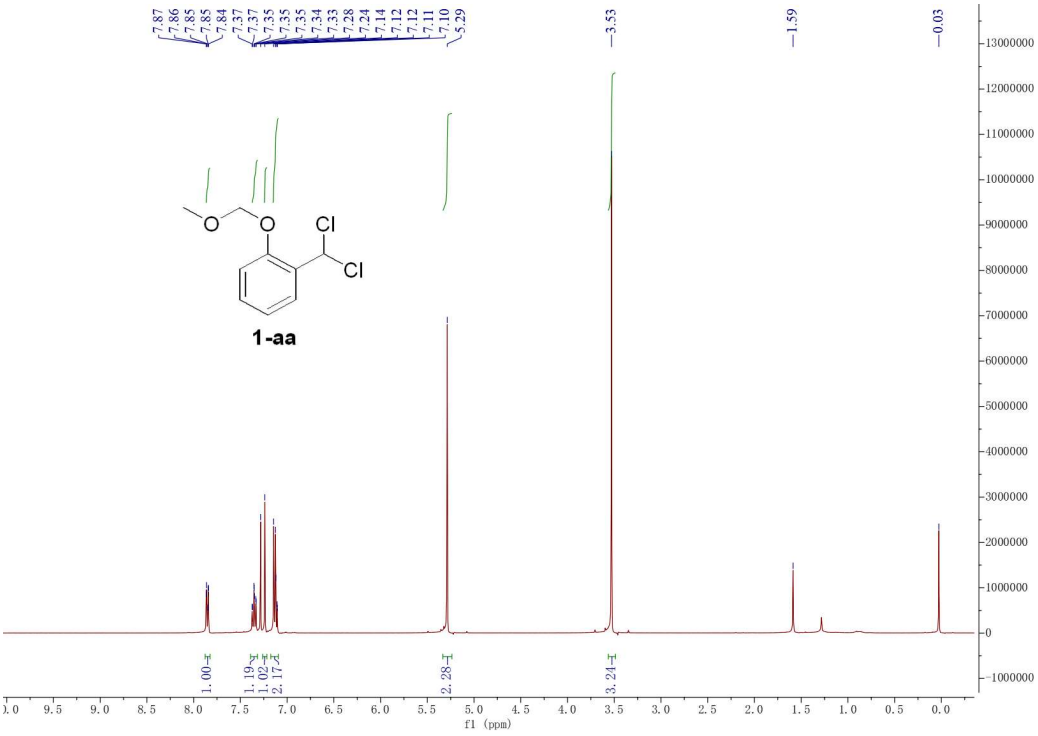
^{1}H NMR (400 MHz, $CDCl_3$) of **1-a**



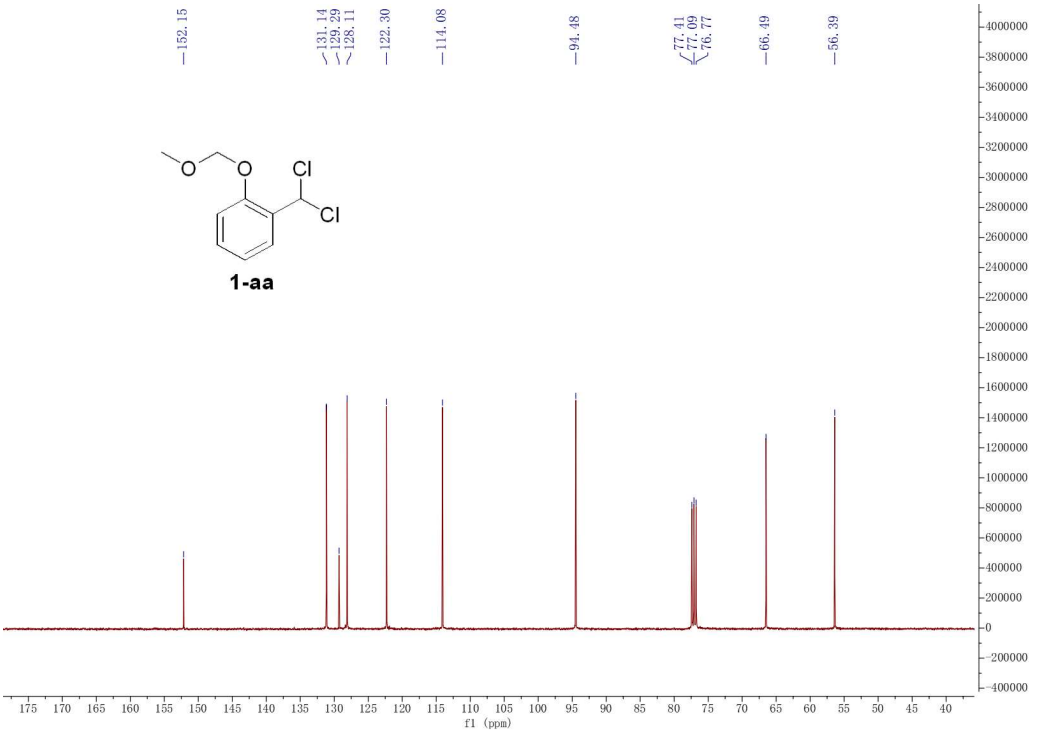
^{13}C NMR (100 MHz, $CDCl_3$) of **1-a**



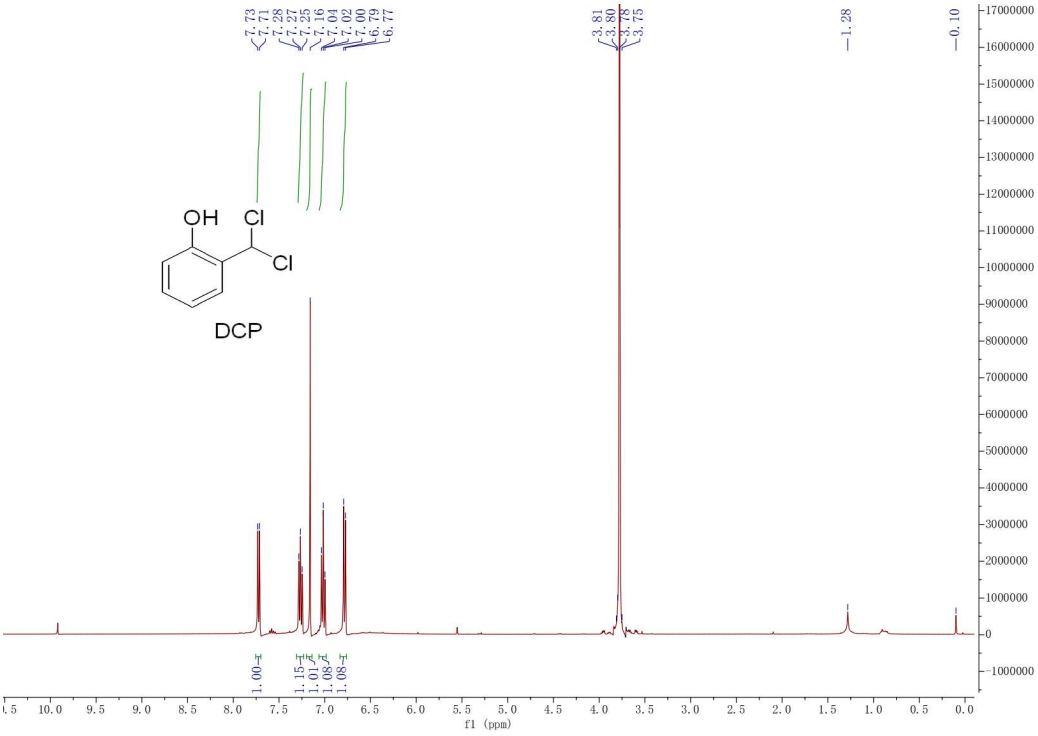
^{1}H NMR (400 MHz, $CDCl_3$) of **1-aa**



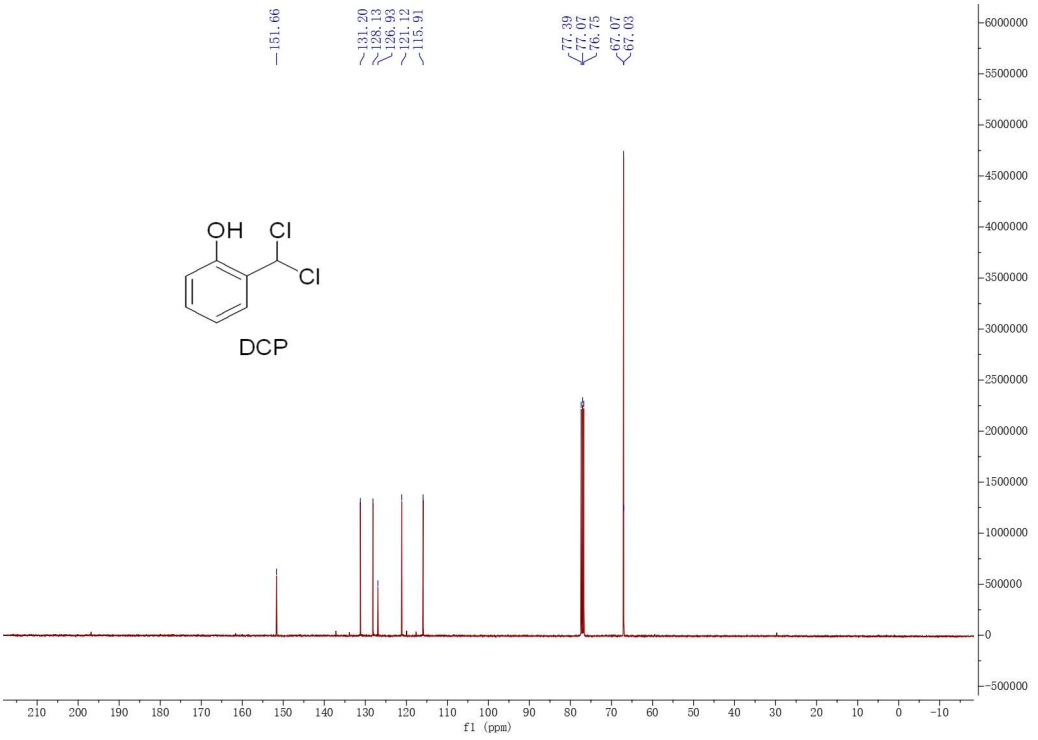
^{13}C NMR (100 MHz, $CDCl_3$) of **1-aa**



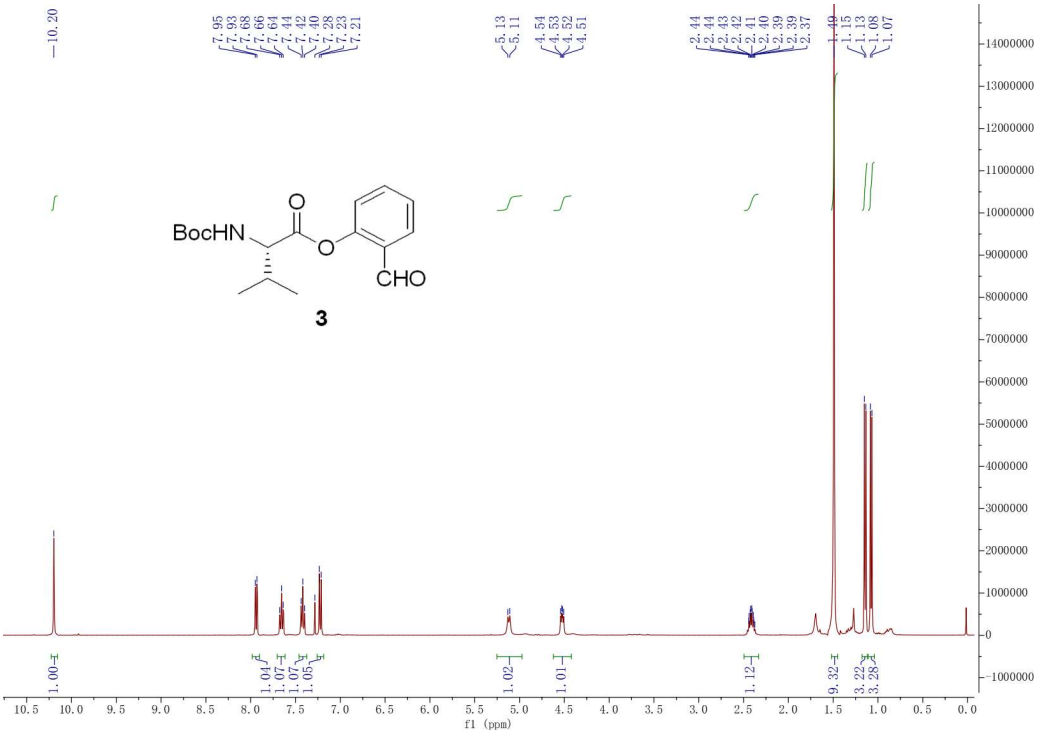
^{1}H NMR (400 MHz, $CDCl_3$) of DCP



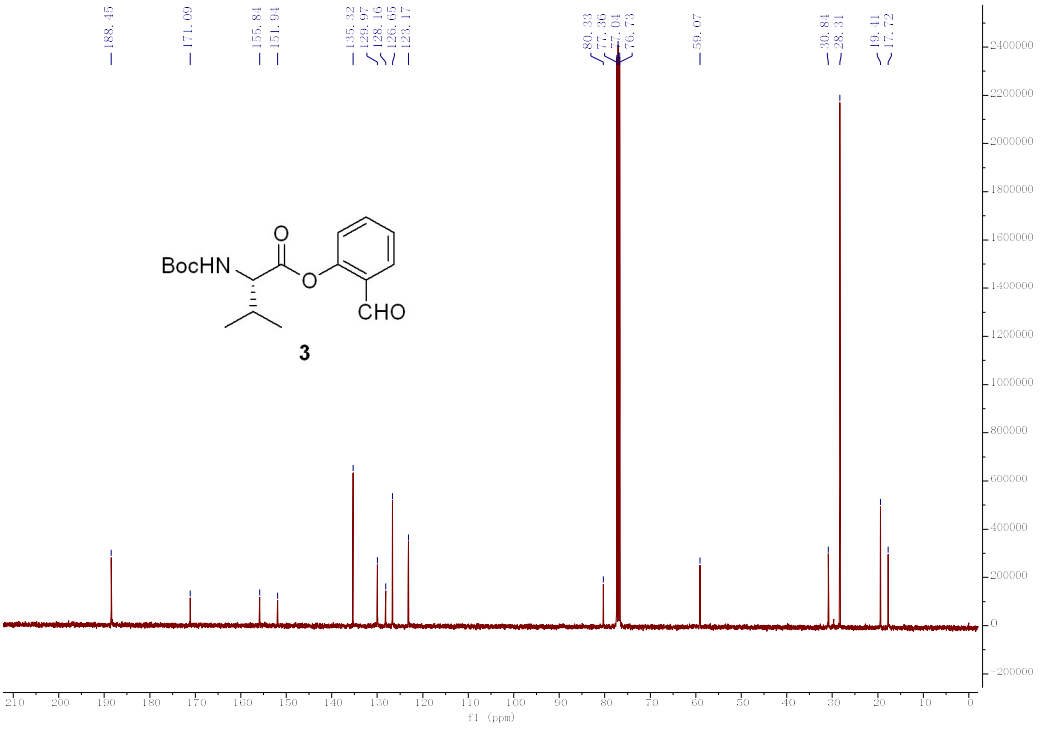
^{13}C NMR (100 MHz, $CDCl_3$) of DCP



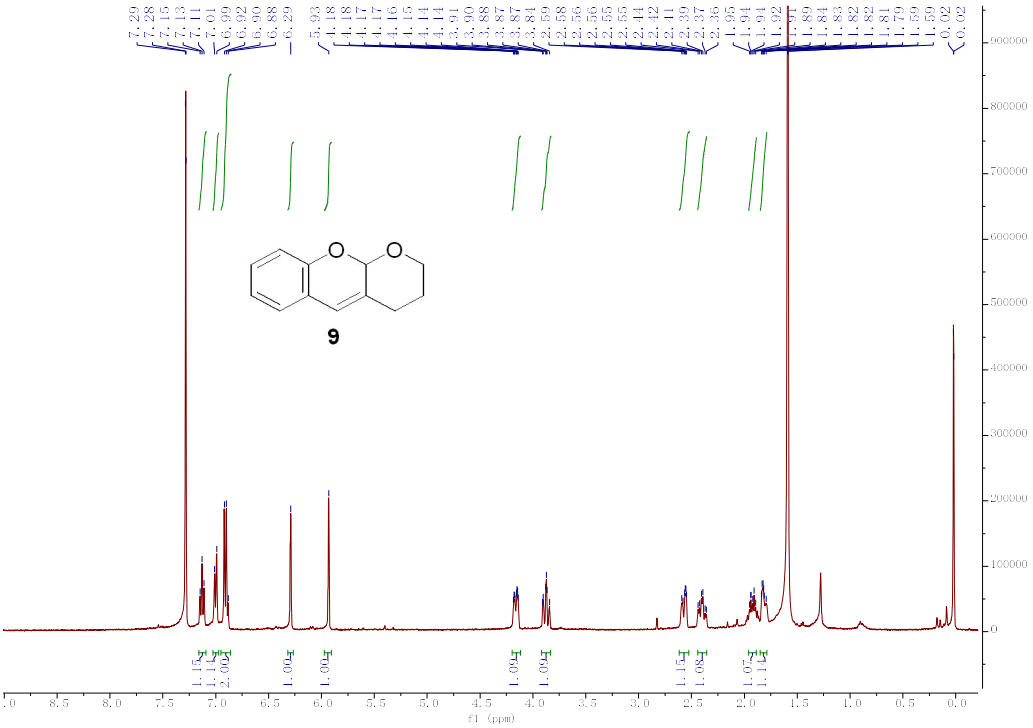
^{1}H NMR (400 MHz, $CDCl_3$) of **3**



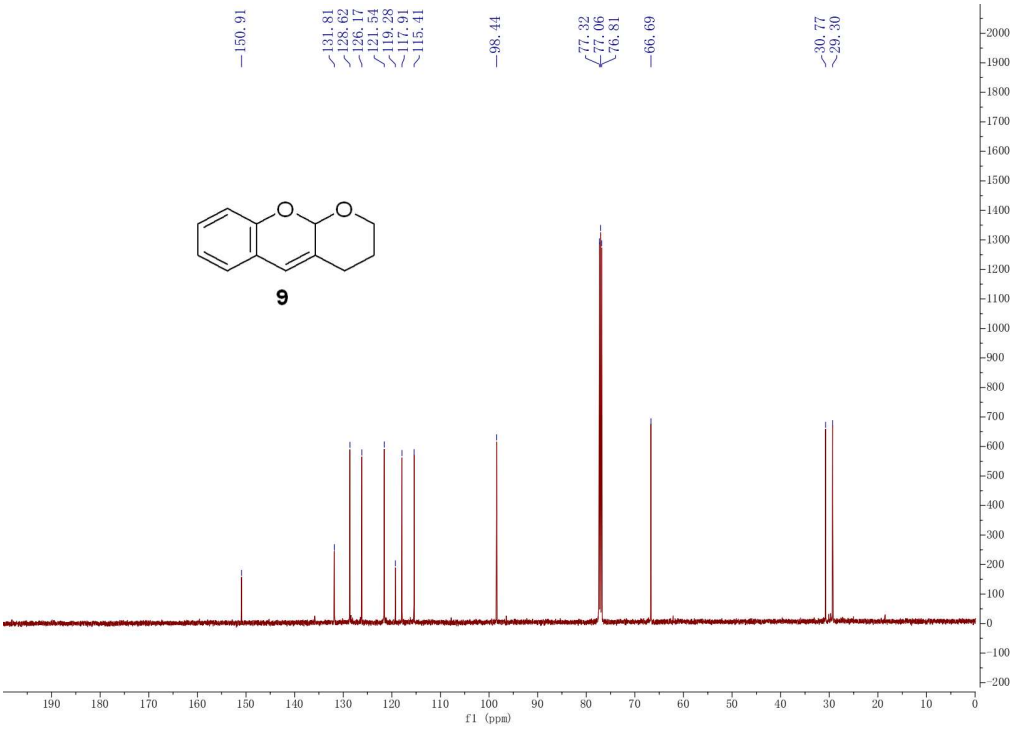
^{13}C NMR (100 MHz, $CDCl_3$) of **3**



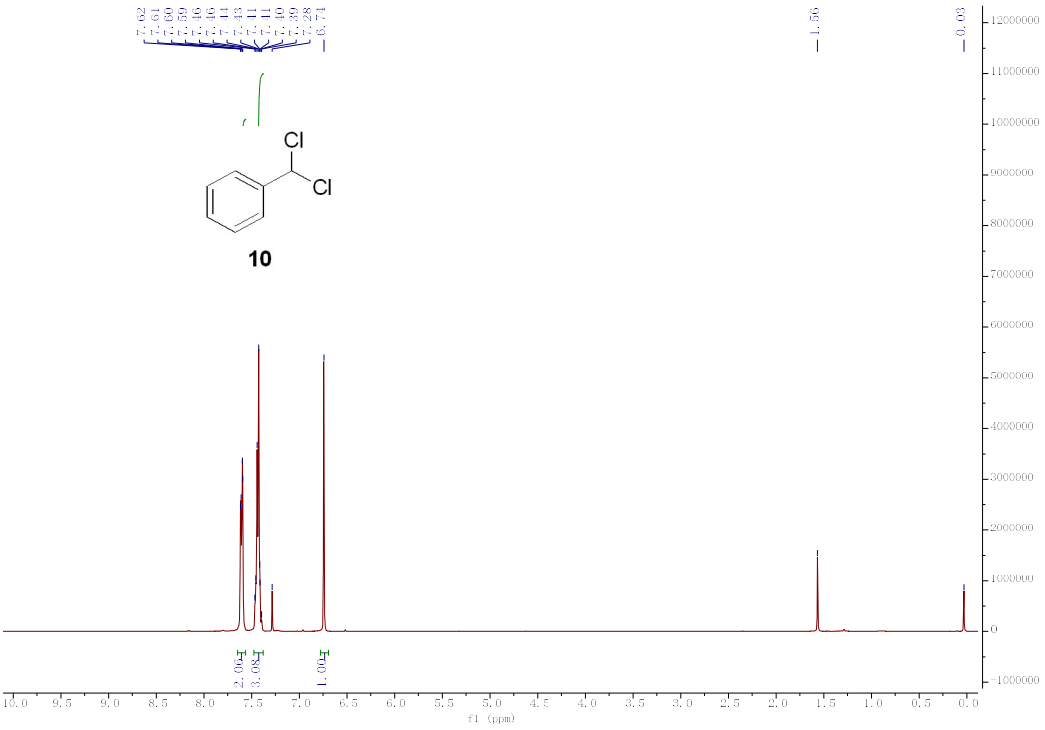
^{1}H NMR (400 MHz, $CDCl_3$) of **9**



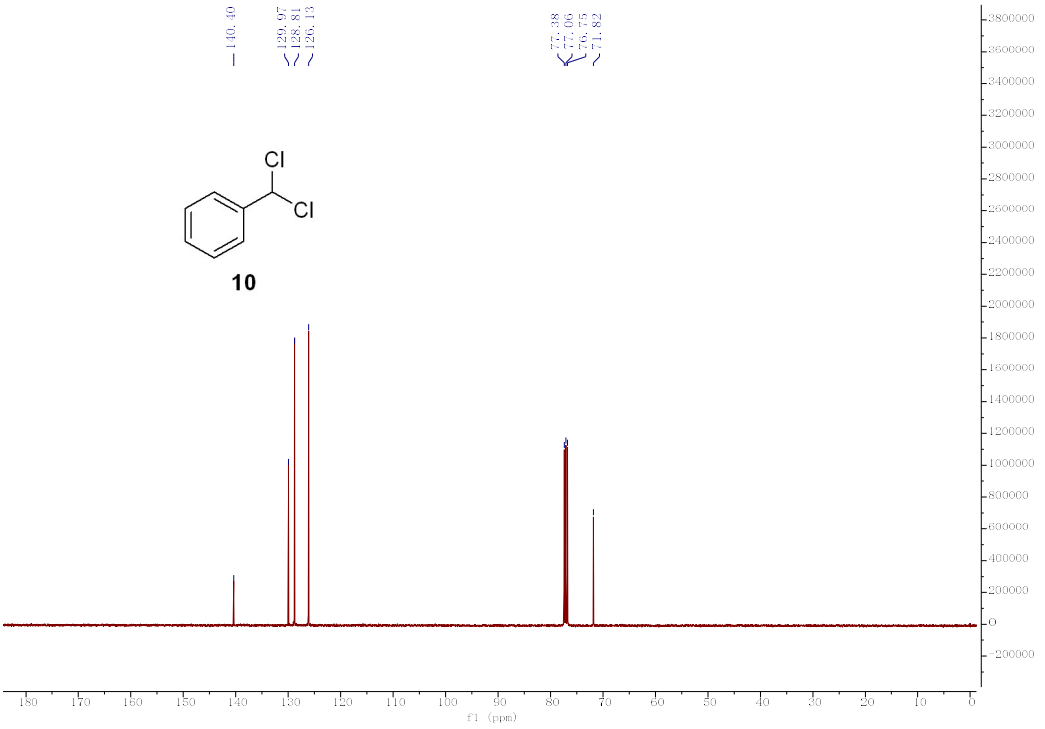
^{13}C NMR (100 MHz, $CDCl_3$) of **9**



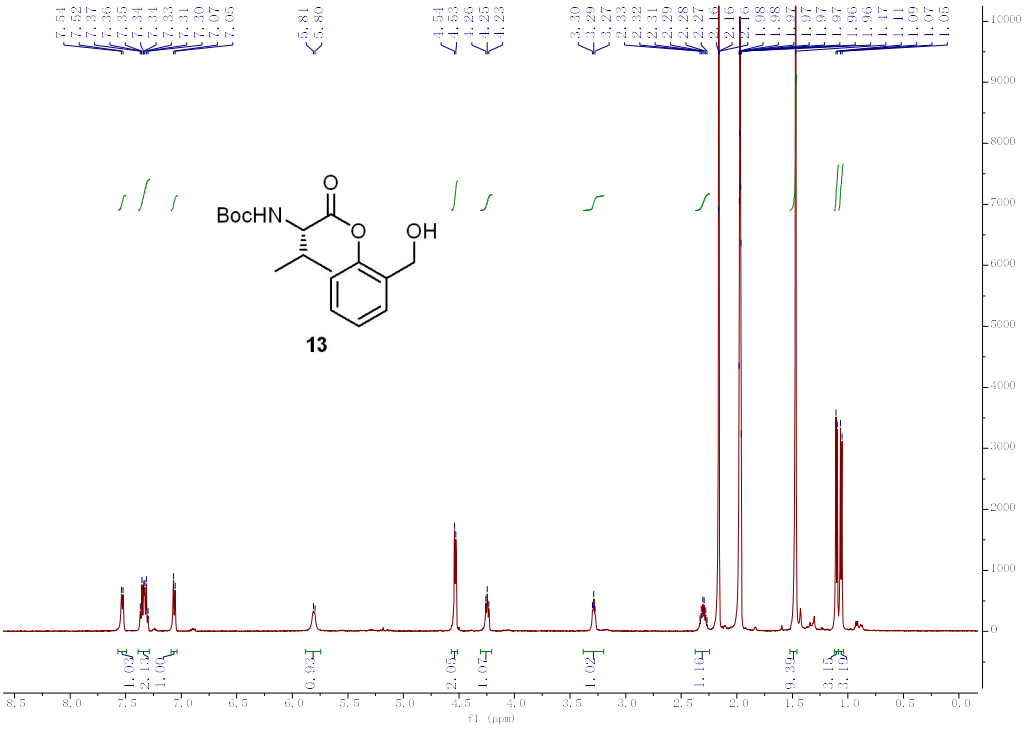
^{1}H NMR (400 MHz, $CDCl_3$) of dichloromethylbenzene **10**



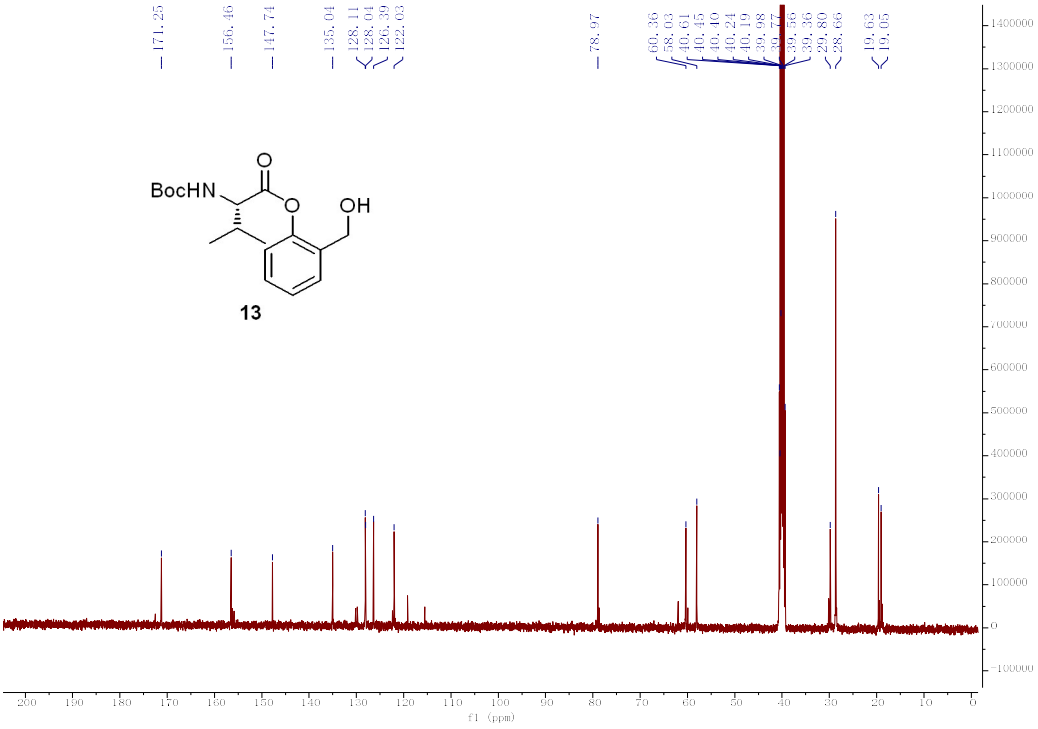
^{13}C NMR (100 MHz, $CDCl_3$) of dichloromethylbenzene **10**



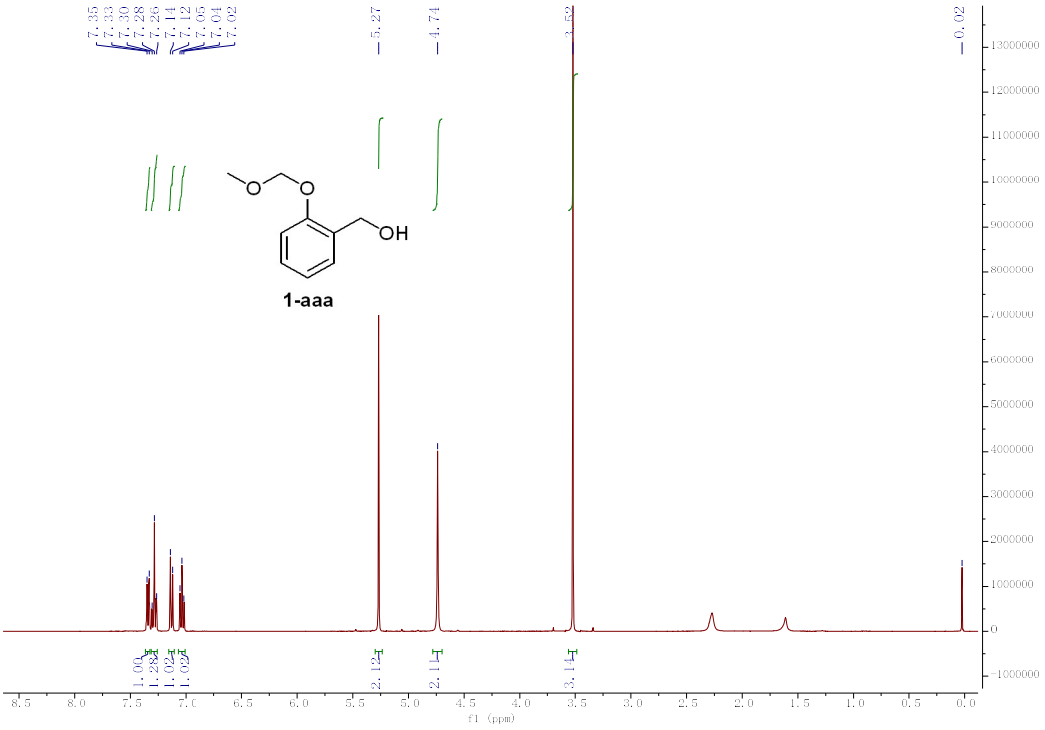
^{1}H NMR (400 MHz, CD_3CN) of **13**



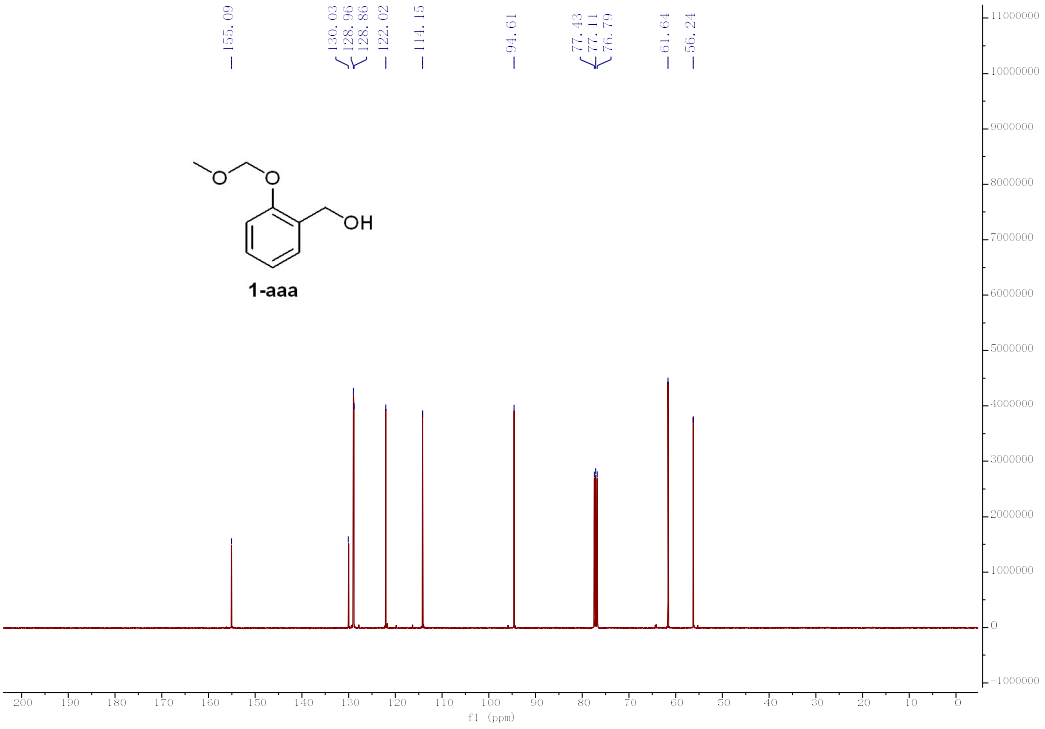
^{13}C NMR (100 MHz, DMSO-d_6) of **13**



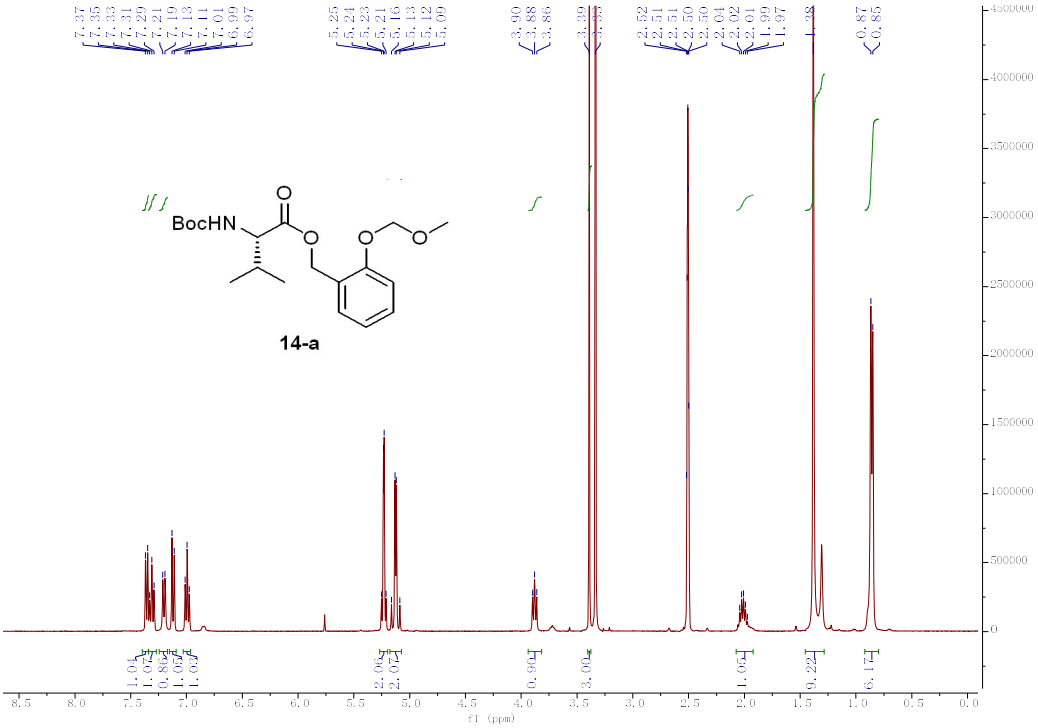
^{1}H NMR (400 MHz, $CDCl_3$) of **1-aaa**



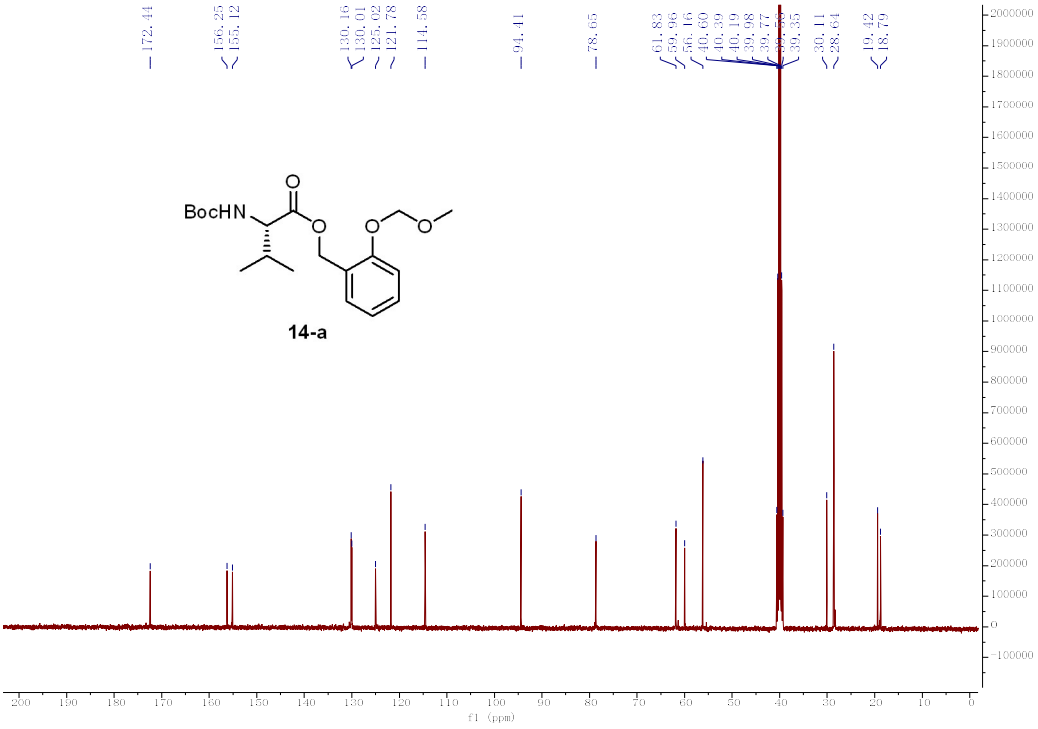
^{13}C NMR (100 MHz, $CDCl_3$) of **1-aaa**



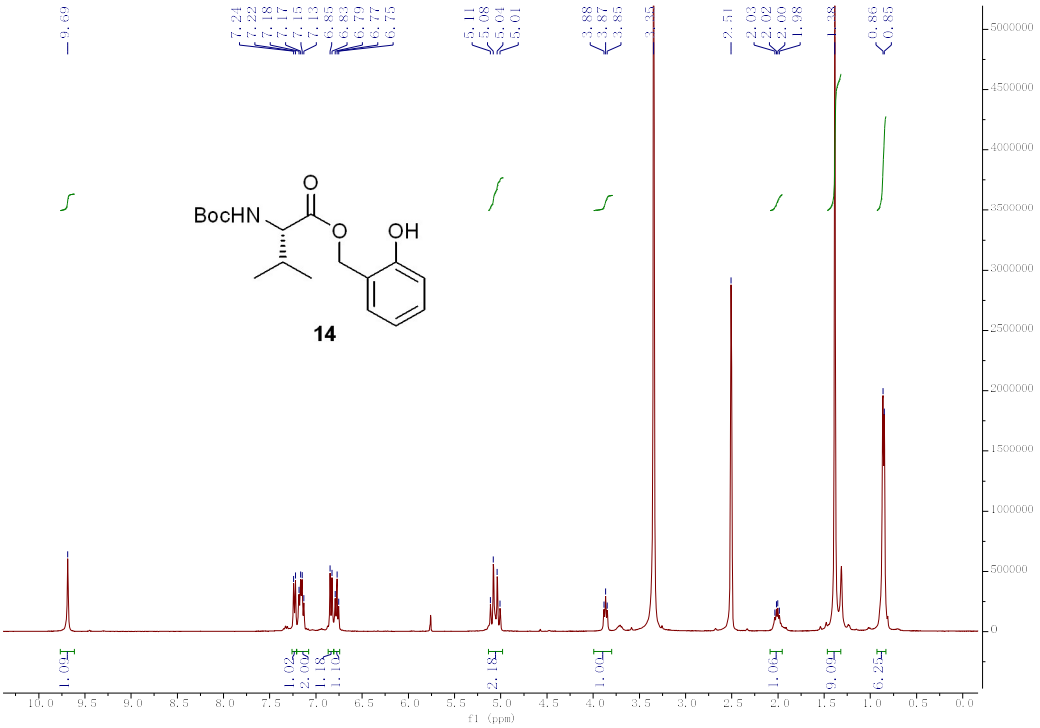
^{1}H NMR (400 MHz, DMSO-d_6) of **14-a**



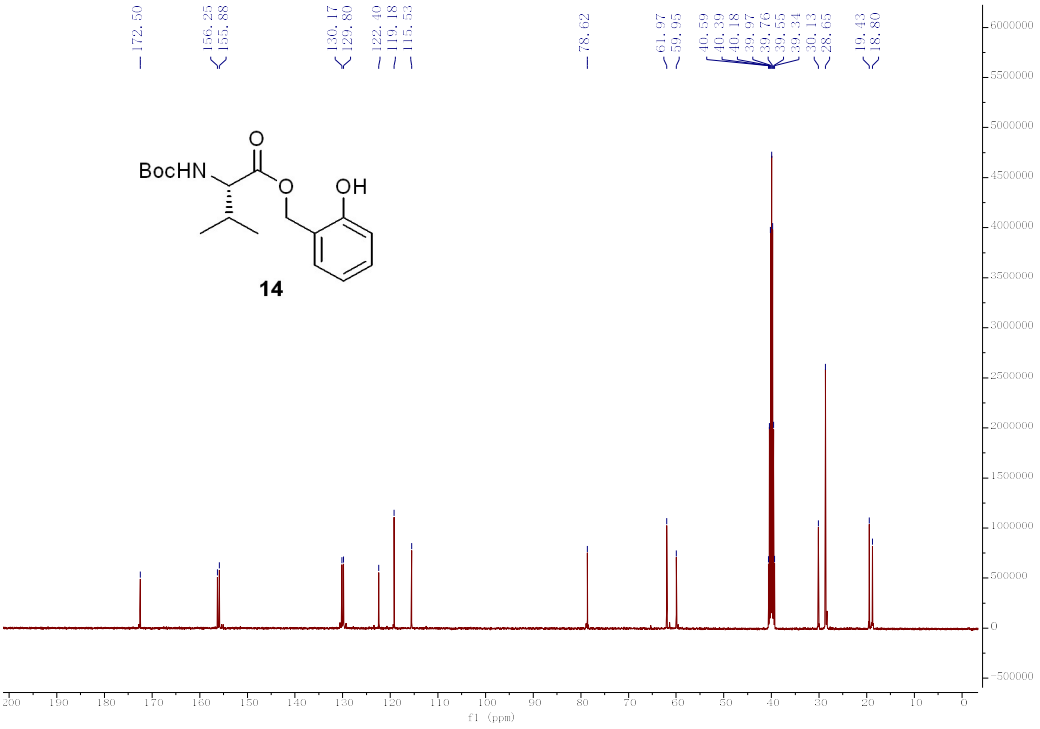
^{13}C NMR (100 MHz, DMSO-d_6) of **14-a**



^{1}H NMR (400 MHz, DMSO-d_6) of **14**



^{13}C NMR (100 MHz, DMSO-d_6) of **14**



11.References

1. McClelland, R. A. & Santry, L. Reactivity of tetrahedral intermediates. *Acc. Chem. Res.* **16**, 394-399 (1983). Kürti, L. & Czakó, B. Strategic applications of named reactions in organic synthesis: background and detailed mechanisms. Elsevier Academic Press: Amsterdam, 2005. Gaussian 09, Revision E.01, Frisch, M. J., Trucks, G. W., Schlegel, H. B., Scuseria, G. E., Robb, M. A., Cheeseman, J. R., Scalmani, G., Barone, V., Mennucci, B., Petersson, G. A., Nakatsuji, H., Caricato, M., Li, X., Hratchian, H. P., Izmaylov, A. F., Bloino, J., Zheng, G., Sonnenberg, J. L., Hada, M., Ehara, M., Toyota, M., Fukuda, R., Hasegawa, J., Ishida, M., Nakajima, T., Honda, Y., Kitao, O., Nakai, H., Vreven, T., Montgomery, J. A. Jr., Peralta, J. E., Ogliaro, F., Bearpark, M., Heyd, J. J., Brothers, E., Kudin, K. N., Staroverov, V. N., Keith, T., Kobayashi, R., Normand, J., Raghavachari, K., Rendell, A., Burant, J. C., Iyengar, S. S., Tomasi, J., Cossi, M., Rega, N., Millam, J. M., Klene, M., Knox, J. E., Cross, J. B., Bakken, V., Adamo, C., Jaramillo, J., Gomperts, R., Stratmann, R. E., Yazyev, O., Austin, A. J., Cammi, R., Pomelli, C., Ochterski, J. W., Martin, R. L., Morokuma, K., Zakrzewski, V. G., Voth, G. A., Salvador, P., Dannenberg, J. J., Dapprich, S., Daniels, A. D., Farkas, O., Foresman, J. B., Ortiz, J. V., Cioslowski, J., and Fox, D. J. Gaussian, Inc. Wallingford CT. (2013).
2. Zhao, Y. & Truhlar, D. G. The M06 suite of density functionals for main group thermochemistry, thermochemical kinetics, noncovalent interactions, excited states, and transition elements: two new functionals and systematic testing of four M06-class functionals and 12 other functionals. *Theor. Chem. Acc.* **120**, 215-241 (2008).
3. Blaudeau, J.-P., McGrath, M. P., Curtiss, L. A. & Radom, L. Extension of Gaussian-2 (G2) theory to molecules containing third-row atoms K and Ca. *J. Chem. Phys.* **107**, 5016-5021 (1997).
4. Marenich, A. V., Cramer, C. J. & Truhlar, D. G. Universal solvation model based on solute electron density and on a continuum model of the solvent defined by the bulk dielectric constant and atomic surface tensions. *J. Phys. Chem. B* **113**, 6378-6396 (2009).

5. Fukui, K. Formulation of the reaction coordinate. *J. Phys. Chem.* **74**, 4161-4163 (1970).
6. Fukui, K. The path of chemical reactions - the IRC approach. *Acc. Chem. Res.* **14**, 363-368 (1981).
7. Keith, J. A. & Carter, E. A. Quantum chemical benchmarking, validation, and prediction of acidity constants for substituted pyridinium ions and pyridinyl radicals. *J. Chem. Theory Comput.* **8**, 3187-3206 (2012).
8. Legault, C. Y. CYLview, 1.0b; Université de Sherbrooke. (2009).
9. Pracht, P. & Grimme, S. Calculation of absolute molecular entropies and heat capacities made simple. *Chem. Sci.* **12**, 6551-6568 (2021).
10. Pracht, P., Bohle, F. & Grimme, S. Automated exploration of the low-energy chemical space with fast quantum chemical methods. *Phys. Chem. Chem. Phys.* **22**, 7169-7192 (2020).
11. Xu, H., Muto, K., Yamaguchi, J., Zhao, C., Itami, K., Musaev, D. G. Key mechanistic features of Ni-catalyzed C–H/C–O biaryl coupling of azoles and naphthalen-2-yl pivalates. *J. Am. Chem. Soc.* **136**, 14834-14844 (2014).
12. Li, B., Wang, M., Fang, S. & Liu, J. DFT study on the mechanism of palladium(0)-catalyzed reaction of aryl iodides, norbornene, and di-*tert*-butyldiaziridinone. *Organometallics* **38**, 2189-2198 (2019).
13. Zhou, Y. & Yu, Z. 1,5-X insertions of free alkylidene carbenes: a theoretical study. *Asian J. Org. Chem.* **12**, e202300440 (2023).