Electronic Supplementary Information for

Late-stage macrolactonisation enabled by tandem acyl transfers followed by desulphurisation

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Contents

A. General information

Materials

All reagents and solvents were obtained from Peptide Institute, Inc. (Osaka, Japan), FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan), Tokyo Chemical Industry Co., Ltd (Tokyo, Japan), Nacalai Tesque, Inc. (Kyoto, Japan), Watanabe Chemical Industries, Ltd (Hiroshima, Japan), Merck KGaA (Darmstadt, Germany), Combi-Blocks Inc. (San Diego, USA), Sigma-Aldrich Co. LLC (St Louis, MO), and CEM Corporation (North Carolina, USA).

NMR

NMR spectra were measured by Bruker AV400N at 400 MHz or Bruker AV500N at 500 MHz frequency for ^{1}H, and Bruker AV400N at 100 MHz or Bruker AV500N at 125 MHz frequency for ^{13}C using the resonances of chloroform (δ_H 7.26 and δ_C 77.0) or DMSO (δ_H 2.49 and δ_C 39.7) as internal references. Chemical shifts were reported in parts per million (ppm) on the δ scale from an internal standard (NMR descriptions: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad). Coupling constants, *J*, are reported Hertz.

HPLC, MS

Each peptide was characterized by MS analysis as described below. Mass spectra were recorded on a Waters MICROMASS®LCT PREMIERTM (ESI-TOF) or a LC-MS (Shimadzu, Japan, Prominence-I LC-2030, LCMS-2020) and a COSMOSIL $5C_{18}$-AR-II analytical column (Nacalai Tesque, Japan, 4.6 × 250 mm, flow rate 1 mL min^{-1}) were used, and eluting products were detected by UV at 220 nm and MS. For HPLC analysis and separation, HPLC was carried out on HITACHI L-7150 with an L-2400 detector or Waters Alliance 2695 Separations Module with ELS 2420 System using a COSMOSIL $5C_{18}$-AR-II analytical column (Nacalai Tesque, 4.6 × 250 mm, flow rate 1.0 mL min^{-1}), a COSMOSIL $5C_{18}$-AR-II semipreparative column (Nacalai Tesque, 10 × 250 mm, flow rate 3.0 mL min^{-1}), or a COSMOSIL $5C_{18}$- AR-II preparative column (Nacalai Tesque, 20 × 250 mm, flow rate 10 mL min^{-1}) eluting with a linear gradient system (solvent A: 0.1% TFA in H_2O, solvent B: 0.1% TFA in MeCN), and eluting products were detected by UV at 220 nm.

Cyclisation and desulphurisation (reaction under anaerobic conditions)

Argon gas was sparged into the buffer for cyclisation and desulphurisation for 30 min with sonication. The reaction was carried out under inert gas atmosphere with the use of the flesh deoxygenated buffer.

B. Fmoc solid-phase peptide synthesis (SPPS)

B-1. Synthesis of peptide acid

Loading on 2-chlorotrityl chloride resin

2-Chlorotrityl chloride resin (copolystyrene-1% DVD, 100–200 mesh) was swollen in DMF for 10 min. A solution of Fmoc-Val-OH (2 equiv.) and *i*Pr_2EtN (2 equiv.) in DMF was added to resin and stirred for 3 h at rt. The completed resin was filtered off and was washed with DMF (×3), CH_2Cl_2 (×3). Treatment of the resin with CH_3OH/iPrEtN/CH_2Cl_2 (17:2:1 v/v/v) for 1 h, followed by washing with DMF (×3), CH_2Cl_2 (×3), afforded the requisite Fmoc-Val-Cl-Trt resin.

Quantification of resin loading

The resulting resin was treated with a solution of 20% piperidine in DMF (3 mL) and stirred for 15 min. The absorbance of the piperidine-fulvenne adduct (λ = 290 nm, ε = 1.65 M−1 cm−1) was measured using Beckman DU800 Spectorophotometer to estimate the loading of the amino acid.

Deprotection of Fmoc group

Treatment of peptide resins with 20% piperidine in DMF for 10 min at rt followed by washing with DMF (×5) was used for the Fmoc removal.

Coupling of Amino acids

A solution of Fmoc-AA-OH (4.0 equiv.), *N*,*N*-diisopropylcarbodiimide (DIPCI) (4.0 equiv.) and 1-hydroxybenzotriazole monohydrate (HOBt·H_2O) (4.0 equiv.) in DMF (0.2 M of Fmoc-AA-OH) were added to the resin with reaction at rt for 2 h. After washing of the resin with DMF (×3), completion of the coupling reaction was checked by the Kaiser ninhydrin test. The coupling was repeated until the Kaiser test became negative. The following side-chain protected amino acids were employed: Arg(Pbf), Thr(*t*Bu), Tyr(*t*Bu), Gln(Trt), Lys(Boc), Glu(*t*Bu), Ser(*t*Bu).

Acidic cleavage of the protected resin with all side-chain protections removed and the purification of the resulting peptides

Deprotection of acid-labile protecting groups with concomitant release of peptides from the resin was achieved using a cocktail of TFA–triethylsilane (TES)–H_2O (95:2.5:2.5, (v/v)) (50 μL/1 mg resin) at rt for 2 h. After the resin was filtered off, concentrated by N_2 gas and cold diethyl ether (Et_2O) was added to the filtrate, and the precipitate was collected by centrifugation. The obtained precipitate was washed with cold Et_2O and dissolved in CH_3CN aq. containing 0.1%(v/v) TFA, then purified using preparative HPLC.

Acidic cleavage of the protected resin with side-chain protections remaining

Treatment of the protected peptide resin with 30%(v/v) HFIP in CH_2Cl_2, with additional reaction at rt for 2 h, followed by concentration of the reaction, afforded the residue containing the side-chain protected peptides. Dissolving of the resulting residue, followed by addition of cold Et_2O, gave side-chain protected peptide acid which was then brought to next step without further purification.

B-2. Synthesis of thioester peptides

Hydrazination of 2-chlorotrityl chloride resin

2-Chlorotrityl chloride resin (copolystyrene-1% DVD, 100-200 mesh, 500 mg) was swollen in DMF for 15 min. Well swollen resin was treated with a solution of $NH_2NH_2 \cdot H_2O$ (0.15 mL)-Et_3N (0.30 mL) in DMF (4 mL) at rt for 2 h. The reaction was quenched by the addition of methanol and the resulting resin was washed with DMF (×3) to afford the requisite hydrazine 2-Cl Trt resin.

Preparative of peptide hydrazide

Protected peptide resin for peptide hydrazides were constructed on the hydrazine 2-Cl Trt resin using Fmoc SPPS. Except for the coupling of Fmoc-Thr(STrt)-OH (**1**), coupling conditions mentioned above were used for all Fmoc amino acid. Release of peptide hydrazide with concomitant removal of side-chain protections was also performed according to the procedure described above.

Coupling of Fmoc-Thr(STrt)-OH (1)

A solution of Fmoc-Thr(STrt)-OH (**1**) (2.0 equiv. 0.2 M), *N,N*-diisopropylcarbodiimide (DIPCI) (2.0 equiv.) and 1-hydroxybenzotriazole monohydrate ($HOBt \cdot H_2O$) (2.0 equiv.) in DMF was added to the resin and stirred at rt for 12 h. The resin was then washed with DMF (×3). Completion of the coupling reaction was checked by the Kaiser ninhydrin test. The coupling was repeated until the Kaiser test became negative.

Conversion of peptide hydrazides to the corresponding thioester peptides

The resulting peptide hydrazide was dissolved in buffer (6 M Gn·HCl, 200 mM sodium phosphate, pH 3.5). To the solution was added 10 %(w/w) $NaNO_2$ aq. at −10 °C (final peptide concentration: 5 mM) with additional reaction at −10 °C for 30 min. Then, 400 mM MESNa in the same buffer was added to the reaction and the pH was adjusted at 6.0 with 6 M NaOH (final peptide concentration is 2.5 mM). After being incubated for 30 min (in the case of Val hydrazide, 60 min) at rt, the reaction was quenched by the addition of TCEP·HCl (8.0 equiv.), and the resulting mixture was directly subjected to HPLC purification.

C. Synthesis of Fmoc-Thr(STrt)-OH (1)

Methyl (*2S,3S*)-2-([{(9*H*-fluoren-9-yl)methoxy}carbonyl]amino)-3-hydroxy-4-(triphenylmethylthio) butanoate (4)

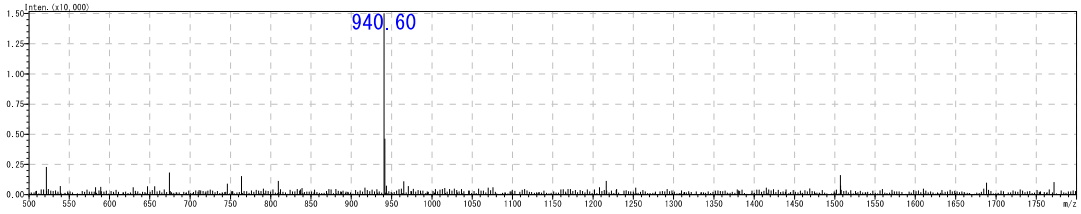
Fmoc protected *syn*-epoxide **3** was synthesised according to the previous report.[ref. S1] To the solution of **3** (1.3 g, 3.7 mmol) in MeOH (50 mL, 74 mM) were added triphenylmethyl thiol (1.1 g, 4.0 mmol) and *N,N*-diisopropylethylamine (0.70 mL, 4.0 mmol) at rt. After being stirred for 3 h, the reaction mixture was diluted with EtOAc and quenched by the addition of sat. NH_4Cl aq. The resulting organic layer was washed with sat. NH_4Cl aq., dried over $MgSO_4$, and concentrated under reduced pressure. The obtained residue was purified by flash silica gel column chromatography (Hex:EtOAc) to afforded **4** (2.0 g, 3.2 mmol, 87%) as colourless amorphous. **^{1}H-NMR** (400 MHz, $CDCl_3$): δ=7.77 (d, *J* = 6.7 Hz, 2H), 7.58 (t, *J* = 8.1 Hz, 2H), 7.44–7.17 (m, 21H), 5.37 (d, *J* = 9.4 Hz, 1H), 4.44–4.32 (m, 2H), 4.22–4.17 (m, 2H), 3.69–3.6463 (brs, 3H), 3.42 (m, 1H), 2.59–2.54 (m, 1H), 2.50–2.44 (m, 1H). **^{13}C-NMR** (100 MHz, $CDCl_3$): δ=170.9, 156.9, 144.4, 143.8, 143.6, 141.3, 129.5, 128.1, 128.0, 127.8, 127.1, 127.0, 125.1, 120.0, 69.4, 67.4, 57.1, 52.7, 47.1, 35.9; HRMS (ESI-TOF) *m/z*: $[M+Na]^+$ Calcd for $C_{39}H_{35}NNaO_5S$ 652.2128; Found 652.2155.

(2*S*,3*S*)-2-{([{(9*H*-fluoren-9-yl)methoxy}carbonyl]amino)-3-hydroxy-4-(triphenylmethylthio)butanoate (1)

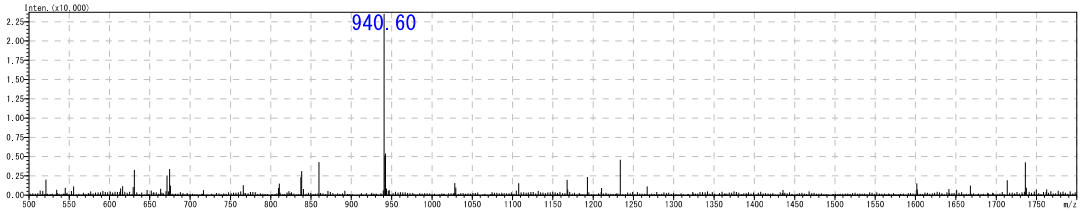
To a solution of **4** (1.0 g, 1.6 mmol) in dichloroethane (53 mL, 30 mM) was added Me_3SnOH (0.92 g, 4.8 mmol) at rt and the resulting mixture was heated to 60 °C. After being stirred for 3 h, the reaction mixture was allowed to cool to rt and then concentrated under reduced pressure. The obtained residue was dissolved in EtOAc and washed with sat. NH_4Cl aq. The resulting organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The obtained residue was purified by silica gel column chromatography ($CHCl_3$:MeOH) to afford **1** (0.82 g, 1.3 mmol, 84%) as colourless amorphous. **^{1}H-NMR** (400 MHz, $CDCl_3$): δ=7.76 (d, *J* = 6.8 Hz, 2H), 7.58 (t, *J* = 8.1 Hz, 2H), 7.43–7.17 (m, 23H), 5.34 (d, *J* = 9.2 Hz, 1H), 4.45–4.41 (m, 1H), 4.37–4.32 (m, 1H), 4.20 (m, 2H), 3.47 (m, 1H), 2.59–2.55 (m, 1H), 2.50–2.45 (m, 1H). **^{13}C-NMR** (100 MHz, $CDCl_3$): δ=174.6, 156.9, 144.3, 143.7, 143.5, 141.3, 129.5, 128.1, 128.0, 127.8, 127.1, 127.0, 125.1, 120.0, 69.3, 67.5, 56.9, 47.1, 35.6; HRMS (ESI-TOF) *m/z*: $[M+Na]^+$ Calcd for $C_{38}H_{33}NNaO_5S$ 638.1972; Found 638.1994.

D. Characterisation of synthetic peptide substrates (R = $CH_2CH_2SO_3H$)

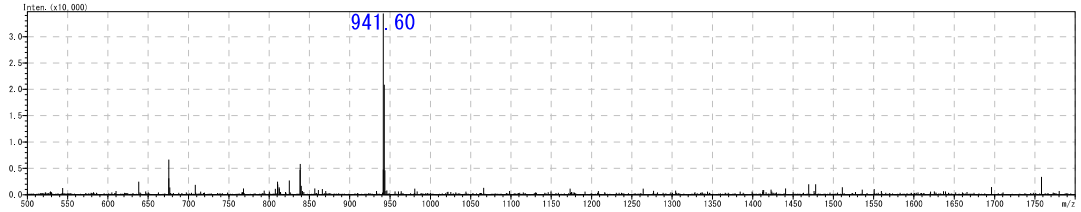
*Ac-Thr(SH)-**TRLQYA**-NHNH$_2$* (**5a**) (6.4 mg, 5.5 μmol, 27% isolated yield). Analytical HPLCconditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min, Retention time (RT) = 15.2 min. Preparative HPLC conditions: linear gradient of solvent B in solvent A, 8% to 18% over 30 min, RT = 25.1 min. LRMS (ESI-Q) *m/z*: $[M+H]^+$ calcd for $C_{39}H_{66}N_{13}O_{12}S$ 940.5, found 940.6.



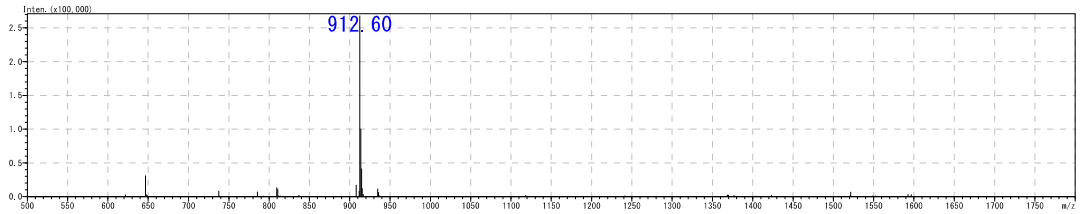
*Ac-Thr(SH)-**TRLQY**-D-**A**-NHNH$_2$* (**5b**) (11 mg, 9.5 μmol, 26% isolated yield). Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min, Retention time (RT) = 15.2 min. Preparative HPLC conditions: linear gradient of solvent B in solvent A, 8% to 18% over 30 min, RT = 24.9 min. LRMS (ESI-Q) *m/z*: $[M+H]^+$ calcd for $C_{39}H_{66}N_{13}O_{12}S$ 940.5, found 940.6.



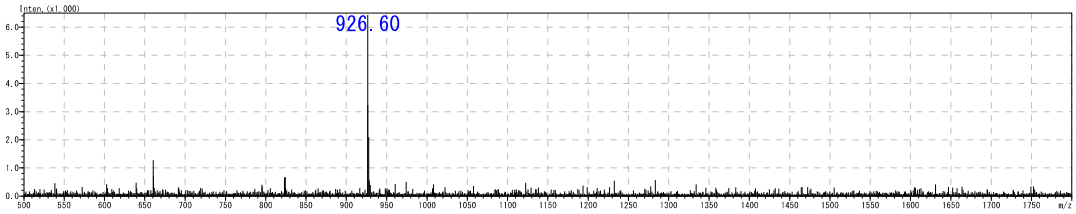
*Ac-Thr(SH)-**TRLEYA**-NHNH$_2$* (**5c**) (26 mg, 22 μmol, 37% isolated yield). Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min, Retention time (RT) = 14.8 min. Preparative HPLC conditions: linear gradient of solvent B in solvent A, 8% to 18% over 30 min, RT = 17.1 min. LRMS (ESI-Q) *m/z*: $[M+H]^+$ calcd for $C_{39}H_{65}N_{12}O_{13}S$ 941.5, found 941.6.



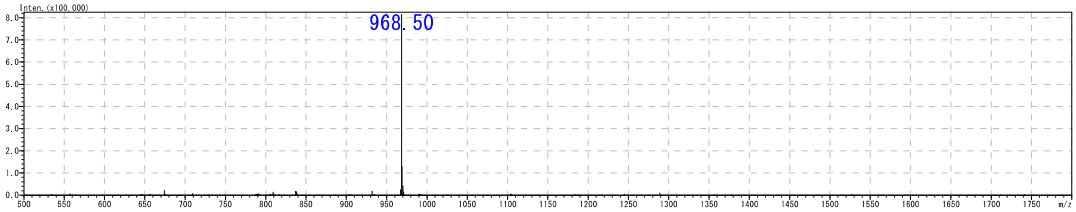
*Ac-Thr(SH)-**TKLQYA**-NHNH$_2$* (**5d**) (22 mg, 20 μmol, 33% isolated yield). Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min, Retention time (RT) = 14.1 min. Preparative HPLC conditions: linear gradient of solvent B in solvent A, 10% to 20% over 30 min, RT = 19.7 min. LRMS (ESI-Q) *m/z*: $[M+H]^+$ calcd for $C_{39}H_{66}N_{11}O_{12}S$ 912.5, found 912.6.



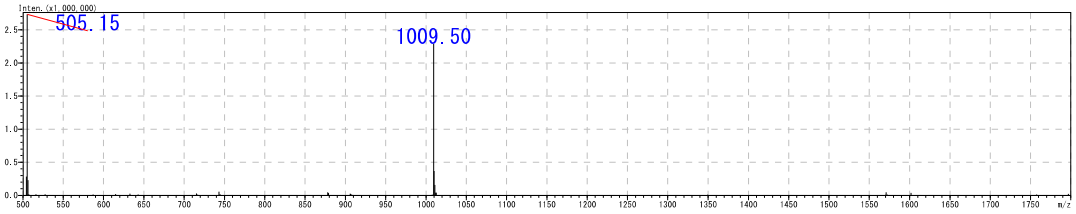
*Ac-Thr(SH)-**SRLQYA**-NHNH₂* (**5e**) (11 mg, 9.4 µmol, 15% isolated yield). Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min, Retention time (RT) = 14.2 min. Preparative HPLC conditions: linear gradient of solvent B in solvent A, 8% to 18% over 30 min, RT = 14.2 min. LRMS (ESI-Q) *m/z*: $[M+H]^+$ calcd for $C_{38}H_{64}N_{13}O_{12}S$ 926.5, found 926.6.



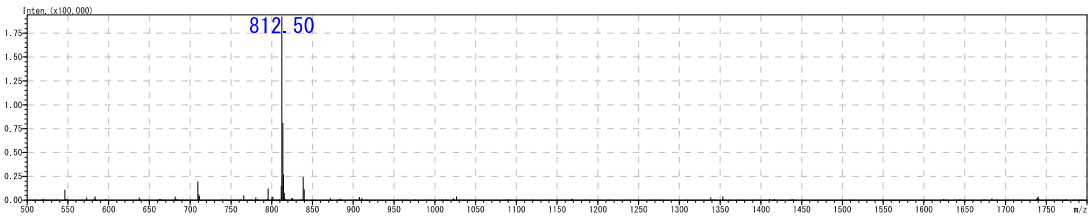
*Ac-Thr(SH)-**TRLQYV**-NHNH₂* (**5f**) (29 mg, 25 µmol, 12% isolated yield). Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min, Retention time (RT) = 15.6 min. Preparative HPLC conditions: linear gradient of solvent B in solvent A, 8% to 18% over 30 min, RT = 27.5 min. LRMS (ESI-Q) *m/z*: $[M+H]^+$ calcd for $C_{41}H_{70}N_{13}O_{12}S$ 968.5, found 968.5.



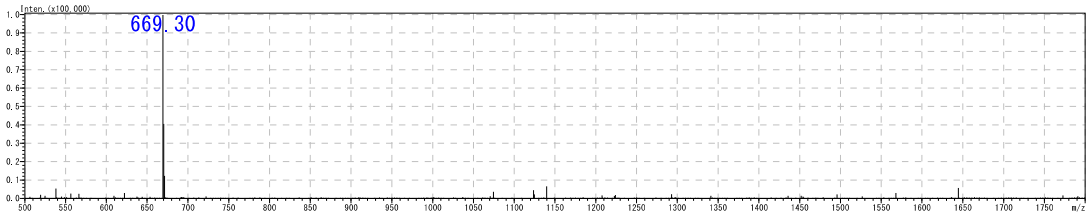
*H-pGlu-Thr(SH)-**TRLQYA**-NHNH₂* (**5g**) (44 mg, 35 µmol, 18% isolated yield). Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min, Retention time (RT) = 13.4 min. Preparative HPLC conditions: linear gradient of solvent B in solvent A, 10% to 20% over 30 min, RT = 26.9 min. LRMS (ESI-Q) *m/z*: $[M+H]^+$ calcd for $C_{42}H_{68}N_{14}O_{13}S$ 1009.5, found 1009.5.



*Ac-Thr(SH)-**TRLYA**-NHNH₂* (**5h**) (7.2 mg, 6.9 µmol, 17% isolated yield). Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min, Retention time (RT) = 14.5 min. Semi-preparative HPLC conditions: linear gradient of solvent B in solvent A, 8% to 18% over 30 min, RT = 13.8 min. LRMS (ESI-Q) *m/z*: $[M+H]^+$ calcd for $C_{34}H_{58}N_{11}O_{10}S$ 812.4, found 812.5.

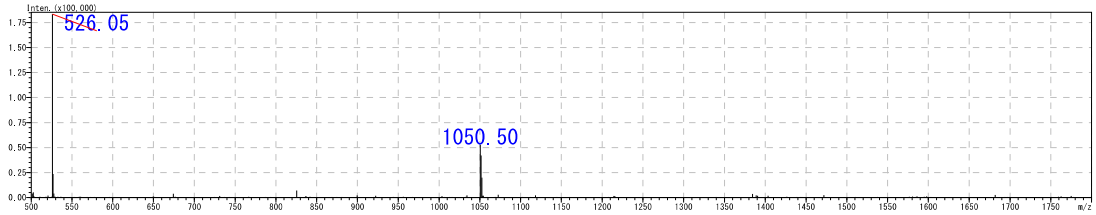


*Ac-Thr(SH)-**ARYA**-NHNH*$_2$ (**5i**) (10 mg, 13 μmol, 27% isolated yield). Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min, Retention time (RT) = 11.1 min. Semi-preparative HPLC conditions: linear gradient of solvent B in solvent A, 5% to 15% over 30 min, RT = 13.3 min. LRMS (ESI-Q) *m/z*: $[M+H]^+$ calcd for $C_{27}H_{45}N_{10}O_8S$ 669.3, found 669.3.

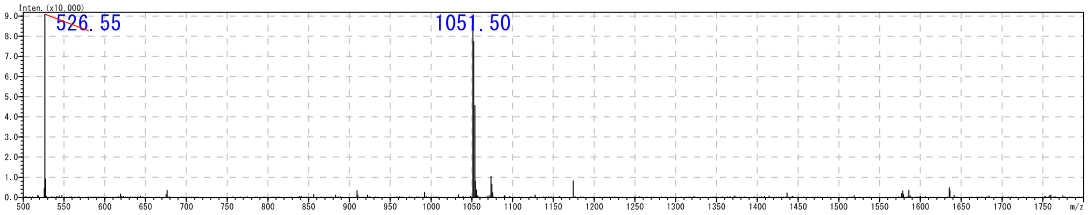


*Ac-Thr(SH)-**TRLQYA**-SR* (**6a**) (3.2 mg, 3.1 μmol, 56% isolated yield). Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min, Retention time (RT) = 16.0 min. Semi-preparative HPLC conditions: linear gradient of solvent B in solvent A, 10% to 20% over 30 min, RT = 27.3 min. LRMS (ESI-Q) *m/z*: $[M+H]^+$ calcd for $C_{41}H_{68}N_{11}O_{15}S_3$ 1050.4, found 1050.5. MS spectrum was shown in Figure S9.

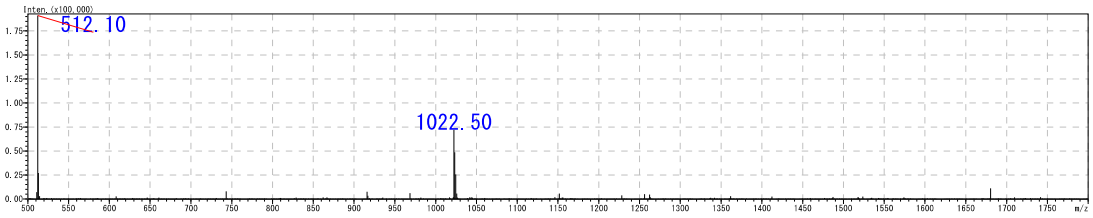
*Ac-Thr(SH)-**TRLQY**-D-**A**-SR* (**6b**) (3.8 mg, 3.2 μmol, 50% isolated yield). Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min, Retention time (RT) = 17.2 min. Semi-preparative HPLC conditions: linear gradient of solvent B in solvent A, 10% to 20% over 30 min, RT = 28.4 min. LRMS (ESI-Q) *m/z*: $[M+H]^+$ calcd for $C_{41}H_{68}N_{11}O_{15}S_3$ 1050.4, found 1050.5.



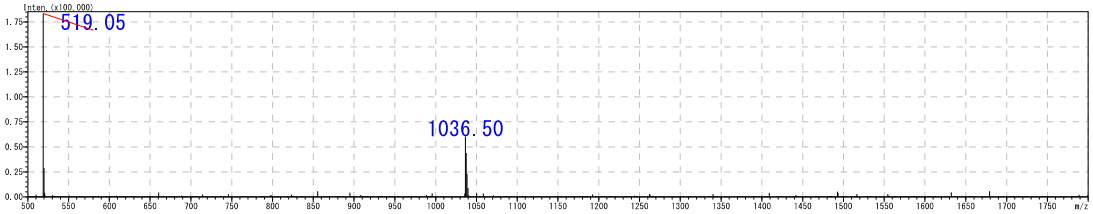
*Ac-Thr(SH)-**TRLEYA**-SR* (**6c**) (11 mg, 9.7 μmol, 44% isolated yield). Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min, Retention time (RT) = 16.8 min. Semi-preparative HPLC conditions: linear gradient of solvent B in solvent A, 10% to 20% over 30 min, RT = 24.9 min. LRMS (ESI-Q) *m/z*: $[M+H]^+$ calcd for $C_{41}H_{67}N_{10}O_{16}S_3$ 1051.4, found 1051.5.



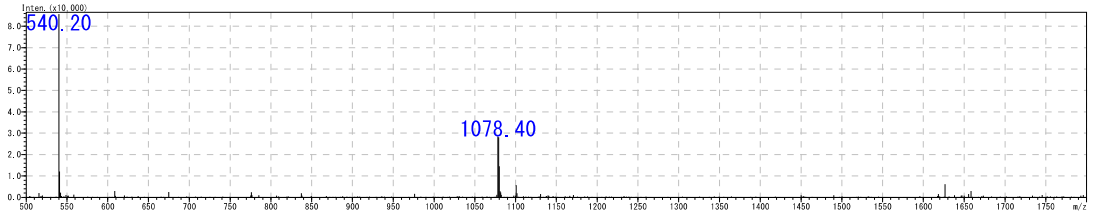
*Ac-Thr(SH)-**TKLQYA**-SR* (**6d**) (8.9 mg, 7.8 µmol, 40% isolated yield). Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min, Retention time (RT) = 15.8 min. Semi-preparative HPLC conditions: linear gradient of solvent B in solvent A, 10% to 20% over 30 min, RT = 26.6 min. LRMS (ESI-Q) *m/z*: $[M+H]^+$ calcd for $C_{41}H_{68}N_9O_{15}S_3$ 1022.4, found 1022.5.



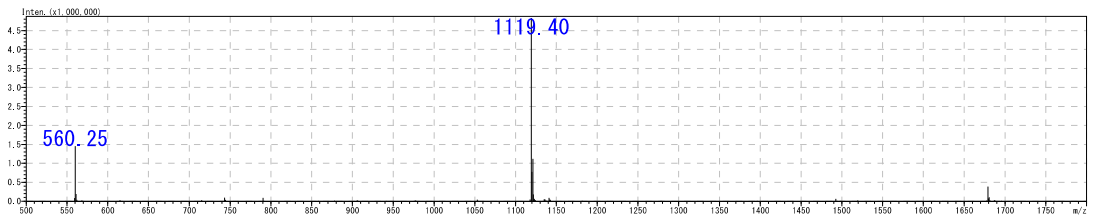
*Ac-Thr(SH)-**SRLQYA**-SR* (**6e**) (5.3 mg, 4.6 µmol, 49% isolated yield). Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min, Retention time (RT) = 15.5 min. Semi-preparative HPLC conditions: linear gradient of solvent B in solvent A, 10% to 20% over 30 min, RT = 25.8 min. LRMS (ESI-Q) *m/z*: $[M+H]^+$ calcd for $C_{40}H_{66}N_{11}O_{15}S_3$ 1036.4, found 1036.3.



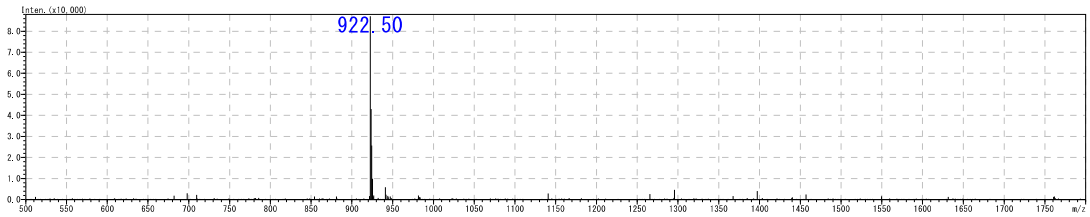
*Ac-Thr(SH)-**TRLQYV**-SR* (**6f**) (15 mg, 12 µmol, 50% isolated yield). Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min, Retention time (RT) = 18.0 min. Semi-preparative HPLC conditions: linear gradient of solvent B in solvent A, 10% to 25% over 30 min, RT = 28.0 min. LRMS (ESI-Q) *m/z*: $[M+H]^+$ calcd for $C_{43}H_{72}N_{11}O_{15}S_3$ 1078.4, found 1078.4.



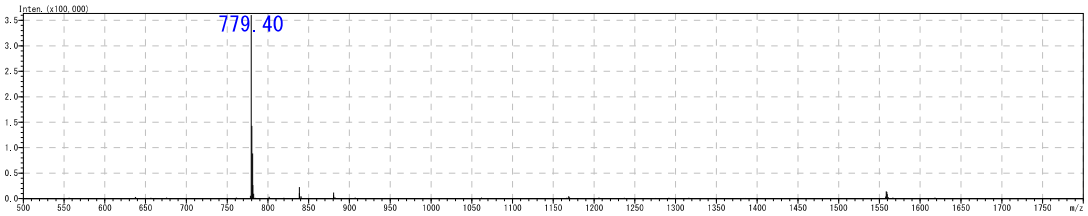
*H-pGlu-Thr(SH)-**TRLQYA**-SR* (**6g**) (3.7 mg, 3.0 µmol, 50% isolated yield). Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min, Retention time (RT) = 15.2 min. Semi-preparative HPLC conditions: linear gradient of solvent B in solvent A, 10% to 20% over 30 min, RT = 23.8 min. LRMS (ESI-Q) *m/z*: $[M+H]^+$ calcd for $C_{42}H_{68}N_{14}O_{13}S$ 1119.4, found 1119.4.



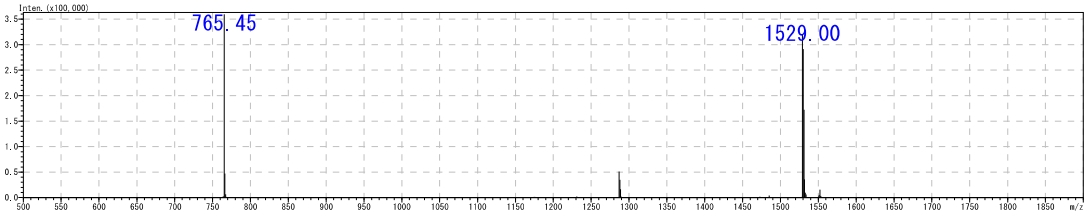
*Ac-Thr(SH)-**TRLYA**-SR* (**6h**) (4.6 mg, 4.5 μmol, 64% isolated yield). Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min, Retention time (RT) = 16.8 min. Semi-preparative HPLC conditions: linear gradient of solvent B in solvent A, 10% to 20% over 30 min, RT = 25.1 min. LRMS (ESI-Q) *m/z*: $[M+H]^+$ calcd for $C_{36}H_{60}N_9O_{13}S_3$ 922.3, found 922.5.



*Ac-Thr(SH)-**ARYA**-SR* (**6i**) (6.4 mg, 7.2 μmol, 54% isolated yield). Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min, Retention time (RT) = 12.7 min. Semi-preparative HPLC conditions: linear gradient of solvent B in solvent A, 5% to 25% over 30 min, RT = 29.0 min. LRMS (ESI-Q) *m/z*: $[M+H]^+$ calcd for $C_{29}H_{47}N_8O_{11}S_3$ 779.3, found 779.4.



*Ac-Thr(OH)- **T**(tBu)**R**(Pbf)**LQ**(Trt)**Y**(tBu)**V**-OH* (**6f'**). Analytical HPLC conditions: linear gradient of solvent B in solvent A, 55% to 95% over 30 min, Retention time (RT) = 17.9 min. LRMS (ESI-Q) *m/z*: $[M+H]^+$ calcd for $C_{81}H_{114}N_{11}O_{16}S$ 1528.8, found 1529.0.



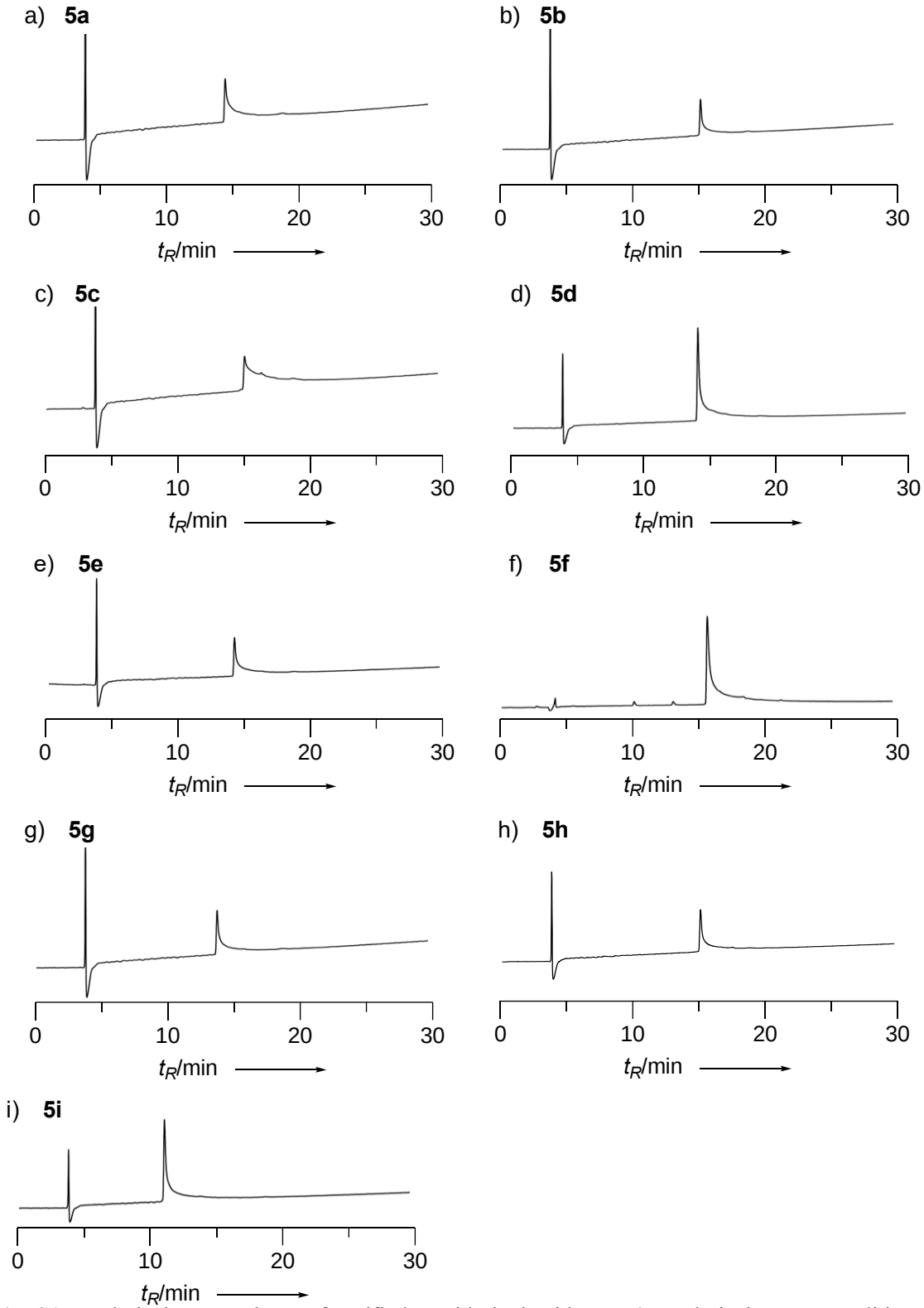


Fig. S1. Analytical HPLC charts of purified peptide hydrazide **5a**–**5i**. Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min. Generally, HPLC analyses of peptide hydrazides on ODS column afford broad HPLC trace.

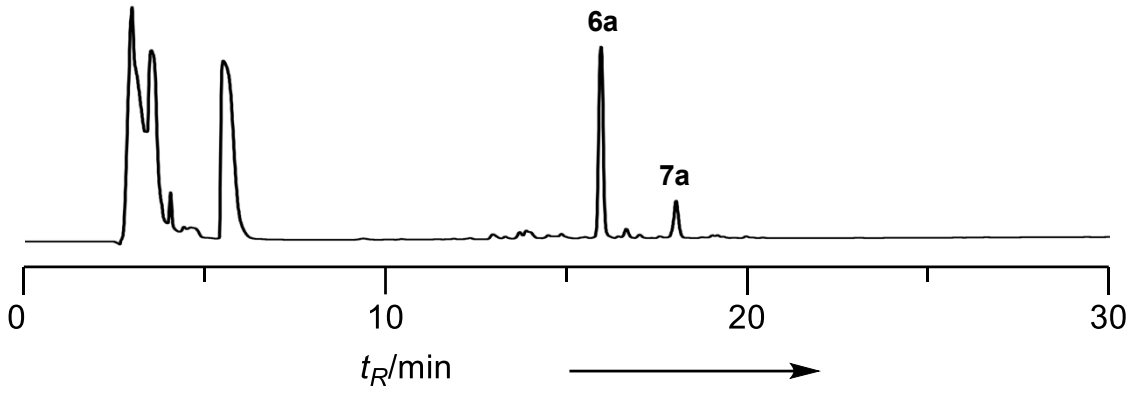


Fig. S2. Analytical HPLC chart of the conversion from **5a** to **6a**. Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min.

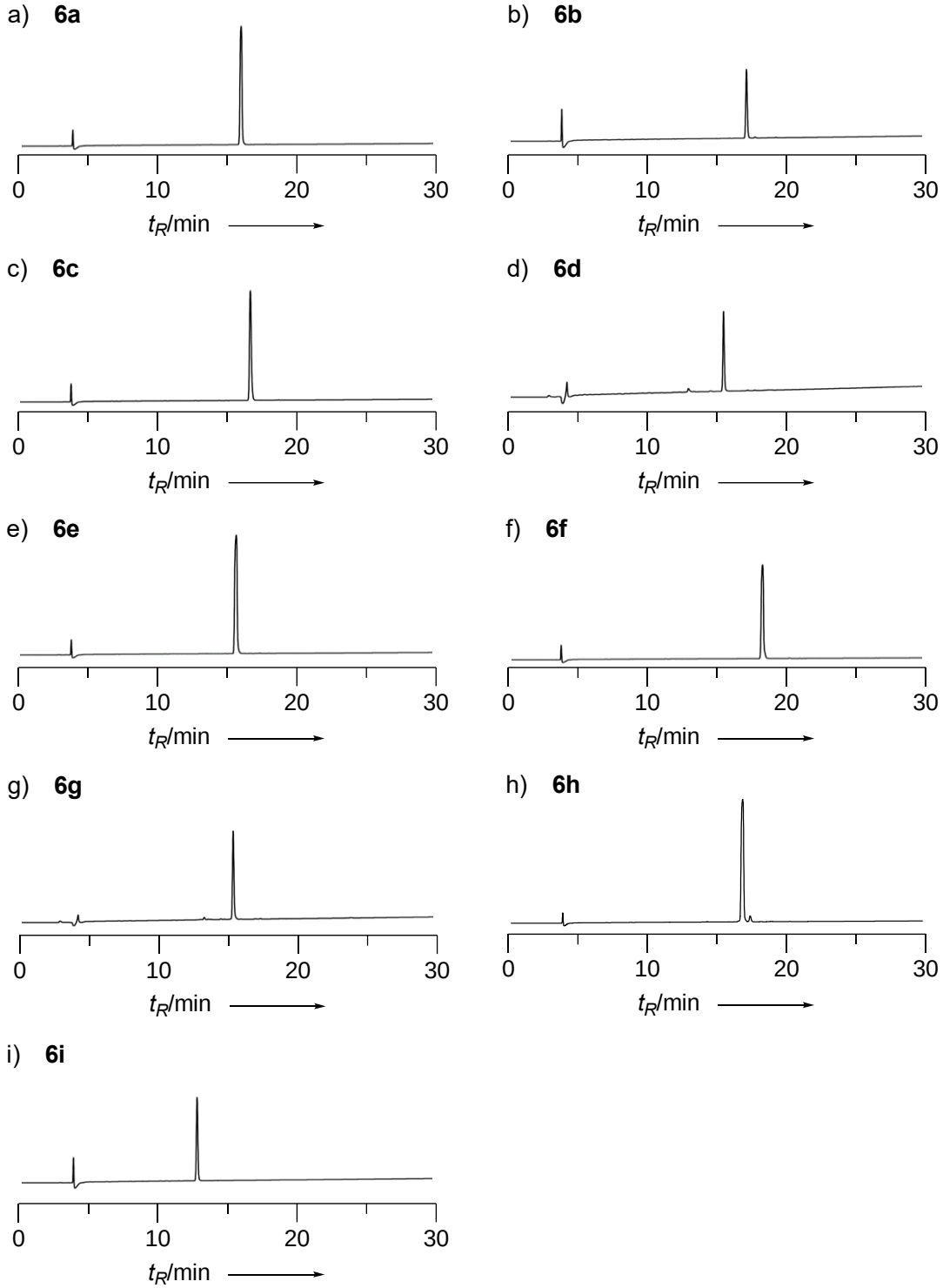
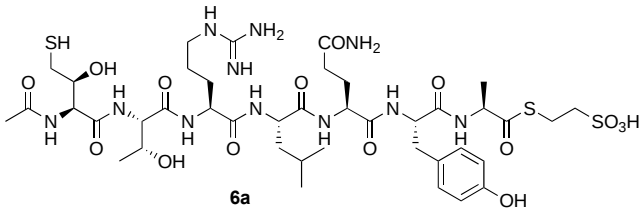


Fig. S3. Analytical HPLC chart of purified peptide thioesters **6a–6i**. Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min.

E. Optimisation of the one-pot/tandem acyl transfer–desulphrisation reaction and the application of the optimum to the macrolactonisation of 6a

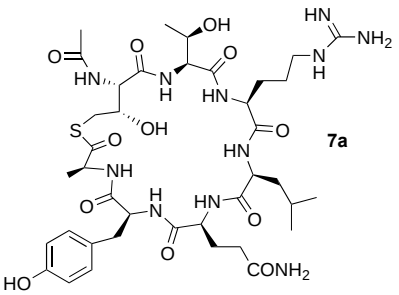
E-1. Optimisation of the pH conditions for (thio)lactone formation

For the optimisation of the pH for (thio)lactone formation, the following examination was conducted. Substrate peptide thioester **6a** (0.050 μmol) was dissolved in a degassed aqueous buffer containing 6 M Gn·HCl–0.2 M Na phosphate (50 μL) at various pH (5.5, 6.0, 6.5 and 7.0). The reaction mixtures were stirred at 37 °C for 2 h under anaerobic conditions and the progress of the reactions was analyzed by HPLC. HPLC traces (Fig. S4) indicated that the efficient conversion of **6a** to (thio)lactone peptide required the reaction over at pH 6.5 although a small amount of epimerisation material (**7b**, discussed later) was formed. Consequently, the reaction under 1 mM concentration at pH 6.5, 37 °C was tentatively fixed the optimum for the initial cyclisation.



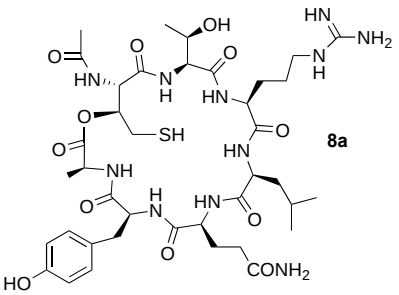
6a

Peptide **6a**: Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min, RT = 16.0 min. LRMS (ESI-Q) *m/z*: $[M+H]^+$ calcd for $C_{41}H_{68}N_{11}O_{15}S_3$ 1050.4, found 1050.4.



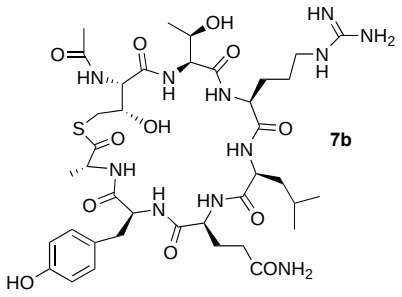
7a

Peptide **7a**: Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min, RT = 18.0 min. LRMS (ESI-Q) *m/z*: $[M+H]^+$ calcd for $C_{39}H_{62}N_{11}O_{12}S$ 908.4, found 908.5.



8a

Peptide **8a**: Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min, RT = 19.9 min. LRMS (ESI-Q) *m/z*: $[M+H]^+$ calcd for $C_{39}H_{62}N_{11}O_{12}S$ 908.4, found 908.5.



Epimerised by-product **7b**: Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min, RT = 17.5 min. LRMS (ESI-Q) *m/z*: $[M+H]^+$ calcd for $C_{39}H_{62}N_{11}O_{12}S$ 908.4, found 908.5.

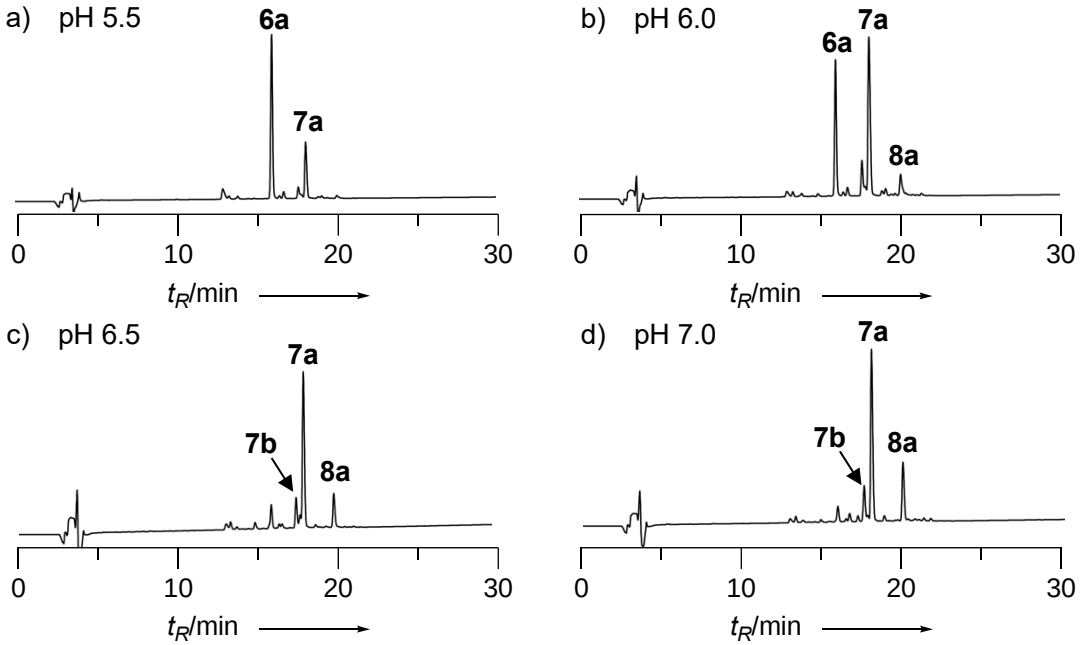


Fig. S4. Analytical HPLC charts of sequential intramolecular S–S and S–O acyl transfer reaction at pH5.5 (a), 6.0 (b), 6.5 (c) and 7.0 (d). Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min.

E-2. Optimisation of reaction temperature for (thio)lactone formation

Substrate peptide **6a** (0.050 μmol) was dissolved in a degassed aqueous buffer containing 6 M Gn·HCl–0.2 M Na phosphate at pH 6.5. The reaction mixture was stirred at 50 °C for 2 h under anaerobic conditions. Progress of each reaction was analyzed by HPLC.

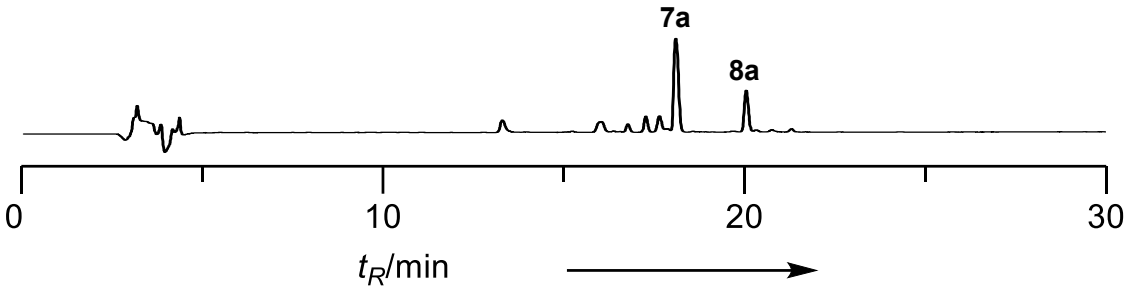


Fig. S5. Analytical HPLC chart of sequential intramolecular S–S and S–O acyl transfer reactions (reaction at 50 °C). Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min.

E-3. Optimisation of peptide concentration for (thio)lactone formation

Substrate peptide **6a** (0.050 μmol) was dissolved in a degassed aqueous buffer containing 6 M Gn·HCl–0.2 M Na phosphate at pH 6.5 (10 or 5 μL, 5 or 10 mM, respectively). Each reaction mixture was stirred at 37 °C for 2 h under anaerobic conditions. Progress of each reaction was analyzed by HPLC.

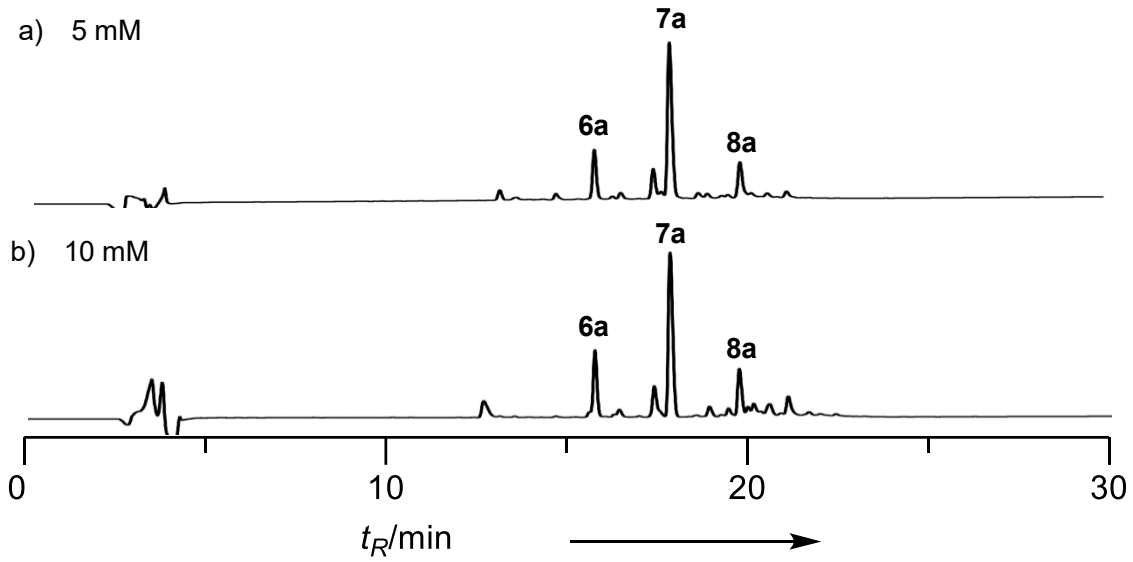


Fig. S6. Analytical HPLC charts of sequential intramolecular S–S and S–O acyl transfer reactions (peptide concentration: 5 mM (a) and 10 mM (b)). Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min.

Optimum conditions for initial (thio)lactone formation.

According to the results described in E-1 and E-2, the optimum conditions for initial (thio)lactone formation was fixed as followed. Linear thioester peptides (1 mM) were incubated in degassed 6 M Gn·HCl–0.2 M Na phosphate buffer (pH 6.5) at 37 °C under anaerobic conditions for the initial (thio)lactone formation.

E-4. Optimisation of desulphurisation of a mixture of thio- and oxylactones under radical-mediated conditions

After the preliminary evaluation of several desulphurisation reaction, the reaction using *t*-BuSH, TCEP and VA-044 in 6 M Gn·HCl–0.2 M Na phosphate buffer was set for the standard desulphurisation conditions. The equilibrium shift from thiolactone **7a** to oxylactone **8a** during the desulphurisation step is critically important for the progress of the overall reaction, therefore, the reaction pH is one contributing factor for the reaction progress. Actually, the preliminary examination showed that the desulphurisation at pH 7.4 is suitable for the reaction while the optimum pH for the initial cyclisation is pH 6.5. Other indispensable factor is to carry out the reaction under anaerobic conditions. To the reaction mixture (1 mM peptide **6a**), obtained under optimum conditions for initial (thio)lactone formation, was added the same volume of desulphurisation buffer (10%(v/v) *t*-BuSH, 520 mM TCEP·HCl, 2.5 mM VA-044·2HCl in 6 M Gn·HCl–0.2 M Na phosphate, pH 7.5; final reaction mixture was containing 5%(v/v) *t*-BuSH, 260 mM TCEP·HCl, 1.25 mM VA-044·2HCl at pH 7.4 and peptide concentration was 0.5 mM). After being stirred at 37 °C for 24 h under aerobic (Fig. S6 (a)) or anaerobic (Fig. S6 (b)) conditions, the reaction were analyzed by HPLC.

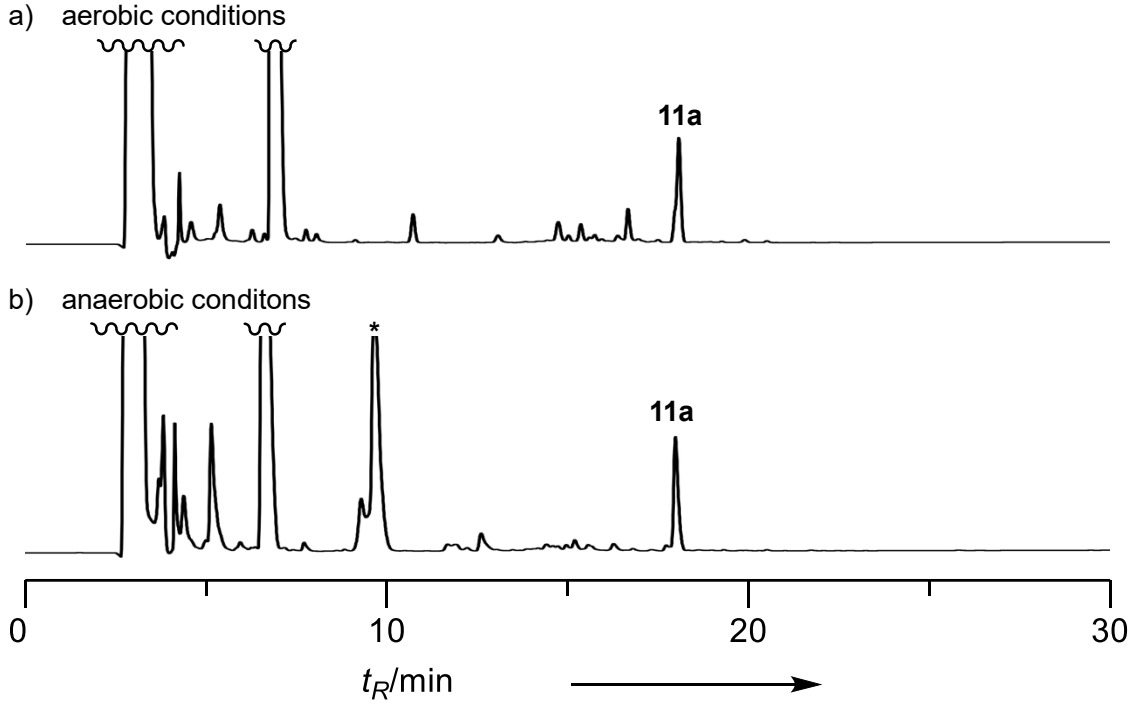


Fig. S7. Analytical HPLC chart of the desulphurisation reaction under aerobic condition (a) and anaerobic condition (b). Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min.

Typical macrolactonisation reactions under optimum conditions

According to the results described in E-3, the optimum conditions for macrolactonisation was fixed as followed. To the reaction mixture, obtained under optimum conditions for initial (thio)lactone formation, was added the same volume of desulphurisation buffer (10%(v/v) *t*-BuSH, 520 mM TCEP·HCl, 2.5 mM VA-044·2HCl in 6 M Gn·HCl–0.2 M Na phosphate, pH 7.5; final reaction mixture was containing 5%(v/v) *t*-BuSH, 260 mM TCEP·HCl, 1.25 mM VA-044·2HCl at pH 7.4 and peptide concentration was 0.5 mM). After being stirred at 37 °C for 24 h under anaerobic conditions for desulphurisation reaction.

E-5. Desulphurisation of the cyclisation mixture and isolation of the resulting lactone peptide 11a (Table 1, entry 1)

According to the typical macrolactonisation reactions under optimum conditions. The desired desulphurized lactone peptide **11a** was obtained in 63% isolated yield (1.0 mg, 1.1 μmol) by semi-preparative HPLC purification of the reaction mixture.

Peptide **11a**: Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min, RT = 18.0 min. LRMS (ESI-Q) *m/z*: $[M+H]^+$ calcd for $C_{39}H_{62}N_{11}O_{12}$ 876.5, found 876.6.

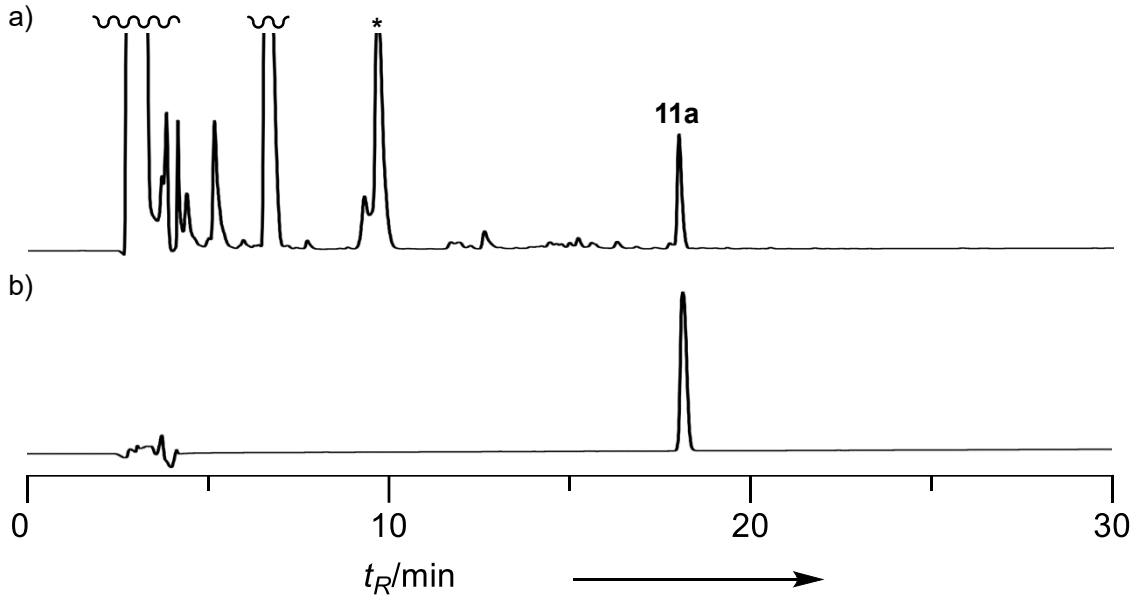


Fig. S8. Analytical HPLC chart of crude reaction mixture of desulphurisation (a) and purified desulphurized lactone peptide **11a** (b). Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min.

Fig. S9. MS spectra of peptide thioester **6a** (a), thiolactone **7a** (b), oxylactone **8a** (c) and desulphurized lactone peptide **11a** (d). Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min.

Table S1. 1D and 2D NMR data for **11a** in DMSO-d_6.

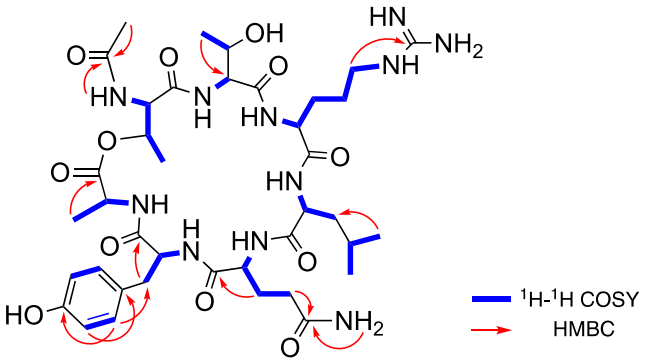
C/H	δ_H (J in Hz)	δ_C
Ac		
CH_3	1.94 (3H, s)	22.6
CO	-	170.4
Thr[1]		
1NH	8.15 (1H, d, 7.4)	-
1α	4.61 (1H, dd, 7.4, 2.1)	55.6
1β	5.21 (1H, m)	70.5
1γ	1.17 (3H, d, 6.5)	16.4
CO	-	169.7
Thr[2]		
2NH	8.08 (1H, d, 8.0)	-
2α	4.24 (1H, m)	58.9
2β	4.20 (1H, m)	67.0
2γ	1.02 (3H, d, 6.4)	20.2
CO	-	171.7[a]
Arg[3]		
3NH	7.68 (1H, m)	-
3α	4.18 (1H, m)	53.5
3β	1.82, 1.60 (each 1H, m)	28.5
3γ	1.52 (2H, m)	25.3
3δ	3.08 (2H, m)	40.5
3ε	7.67 (1H, m)	-
3ζ	-	157.0
3η	7.11 (2H, brs)	-
CO	-	171.5[a]
Leu[4]		
4NH	7.81 (1H, brs)	-
4α	4.17 (1H, m)	51.7
4β	1.57 (2H, m)	24.5
4γ	1.49 (1H, m)	40.0[b]
4δ–1	0.84 (3H, d, 6.5)	21.3
4δ–2	0.87 (3H, d, 6.5)	23.3
CO	-	170.7[a]
Gln[5]		

5NH	8.01 (1H, brs)	-
5α	3.82 (1H, m)	54.8
5β	1.85 (2H, m)	26.3
5γ	1.97 (2H, m)	31.6
5δ	-	174.0
5ε	7.23, 6.78 (each 1H, brs)	-
CO	-	172.4
Tyr[6]		
6NH	7.71 (1H, m)	-
6α	4.20 (1H, m)	55.1
6β	2.95 (1H, dd, 14.3, 5.1),	36.2
	2.74 (1H, dd, 14.3, 9.5)	
6γ	-	127.9
6δ	6.97 (2H, d, 8.4)	130.2
6ε	6.64 (2H, d, 8.4)	115.2
6ζ	-	156.4
6η	nd	-
CO		171.1
Ala[7]		
7NH	7.97 (1H, brs)	-
7α	4.14 (1H, m)	48.8
7β	1.26 (3H, d, 7.1)	16.8
CO	-	171.1

[a]signals may be interchangeable

[b]overlapped with a signal of DMSO-d_6.

nd: not detected



Selected 2D NMR correlations for **11a**.

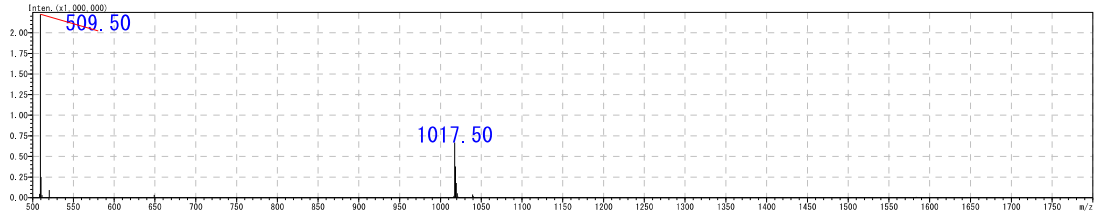
F. Characterisation of thiolactone 7a and oxylactone 8a

F-1. Oxidation of a mixture of 7a and 8a obtained under the optimum conditions for (thio)lactone formation

Peptide thioester **6a** (1 mM, 50 μL) was subjected to tandem acyl transfer under optimum conditions. To the reaction mixture was added 40 mM 2,2'-dipyridyl disulphide in 6 M Gn·HCl–0.2 M Na phosphate buffer (50 μL), and the reaction mixture was stirred at 37 °C for 2 h (Fig. S10 (a)) or 24 h (Fig. S10 (b)) under anaerobic conditions. After being stirring at 37 °C, the reaction progress was detected by HPLC.

After the complete conversion of a mixture of **7a** and **8a** to **9a**, 40 mM TCEP·HCl and 40 mM Na ascorbate in a degassed Na phosphate buffer (50 μL) was added to the resulting mixture containing heterodisulphide peptide **9a**. The reaction mixture was stirred at 37 °C for 15 min (Fig. S10 (c)) and 2 h (Fig. S10 (d)) under anaerobic conditions and analyzed by HPLC.

Peptide **9a**: Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min, RT = 23.0 min. LRMS (ESI-Q) *m/z*: $[M+H]^+$ calcd for $C_{44}H_{65}N_{12}O_{12}S_2$ 1017.4, found 1017.5.



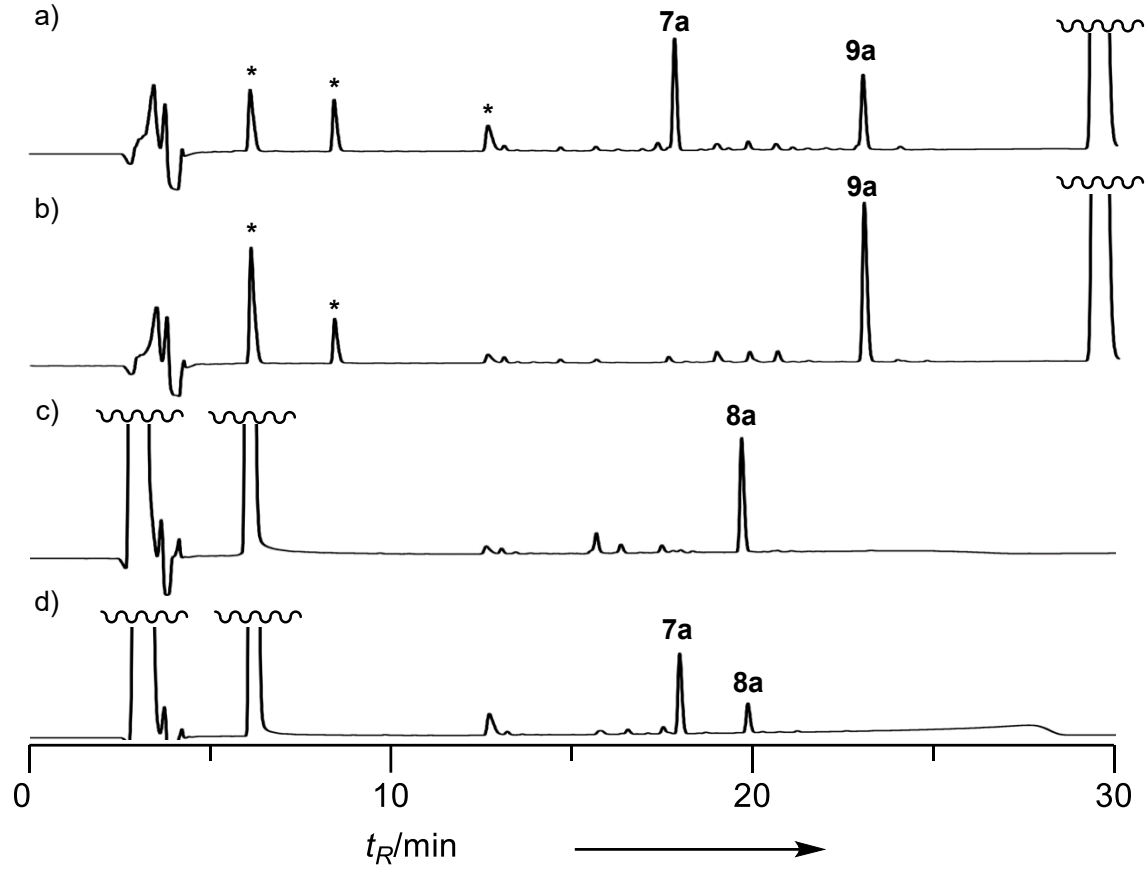


Fig. S10. Analytical HPLC charts of oxidation with 2,2'-dipyridyl disulphide for 2 h (a) and 24 h (b). HPLC analysis of the reduction with TCEP · HCl for 15 min (c) and 2 h (d). Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min. *nonpeptidyl compounds.

F-2. NCL-mediated conversion of the mixture of 7a and 8a

Peptide thioester **6a** (1 mM, 50 μL) was subjected to tandem acyl transfer under optimum conditions. To the reaction mixture was added cysteine hydrochloride (5 μmol) in 6 M Gn·HCl–0.2 M Na phosphate (50 μL) containing 80 mM TCEP·HCl and 60 mM MPAA. The resulting mixture was adjusted to pH 5.0 by adding 6 M NaOH and stirred at 37 °C for 13 h, under anaerobic conditions. The reaction was analyzed by HPLC.

Peptide **10a**: Analytical HPLC condition: linear gradient of solvent B in solvent A, 5% to 45% over 30 min, RT = 17.9 min. LRMS (ESI-Q) *m/z*: $[M+H]^+$ calcd for $C_{42}H_{69}N_{12}O_{14}S_2$ 1029.4, found 1029.5.

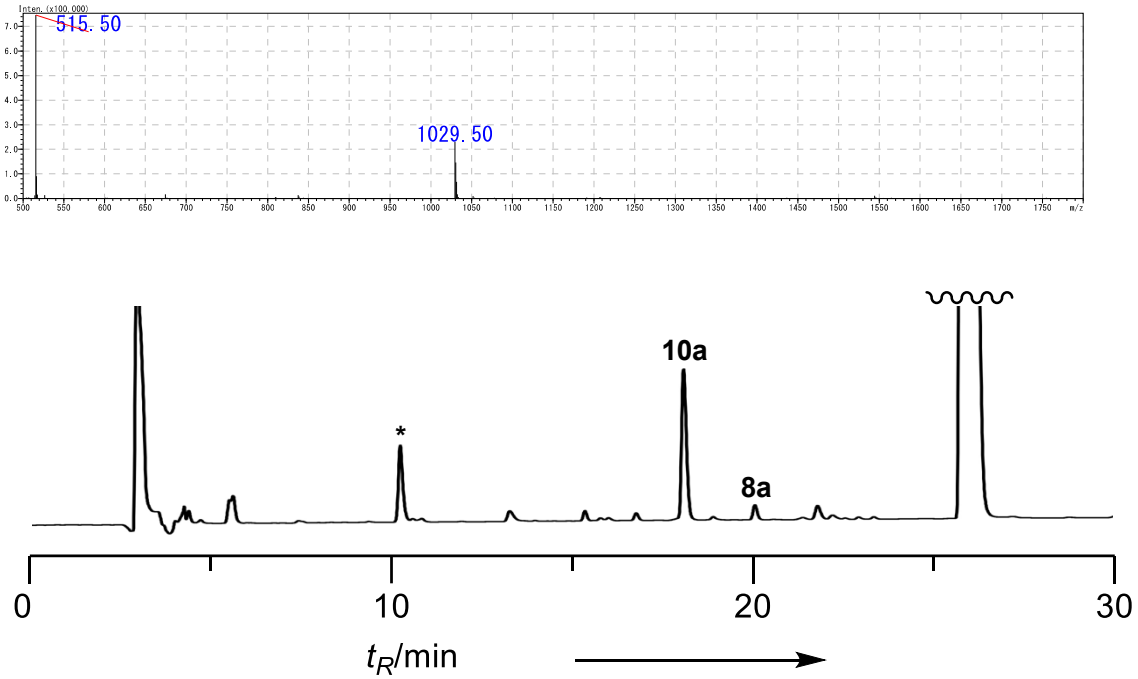


Fig. S11. Analytical HPLC charts of NCL. Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min.

G. Examination of epimerisation during the macrolactonisation of 6a

Epimerisation of a C-terminal amino acid during the tandem acyl-transfer/desulphurisation protocol was evaluated the comparative HPLC analyses of samples obtained by the subjection of the C-terminal L-Ala (**6a**) and D-Ala (**6b**) peptides to the typical macrolactonisation reaction under optimum conditions mentioned above. Comparison of HPLC traces (Fig. S12 (a) vs 12 (b) and S12 (c) vs 12 (d)) indicated that the cyclisation step accompanied small extent of epimerisation (ca 5%).

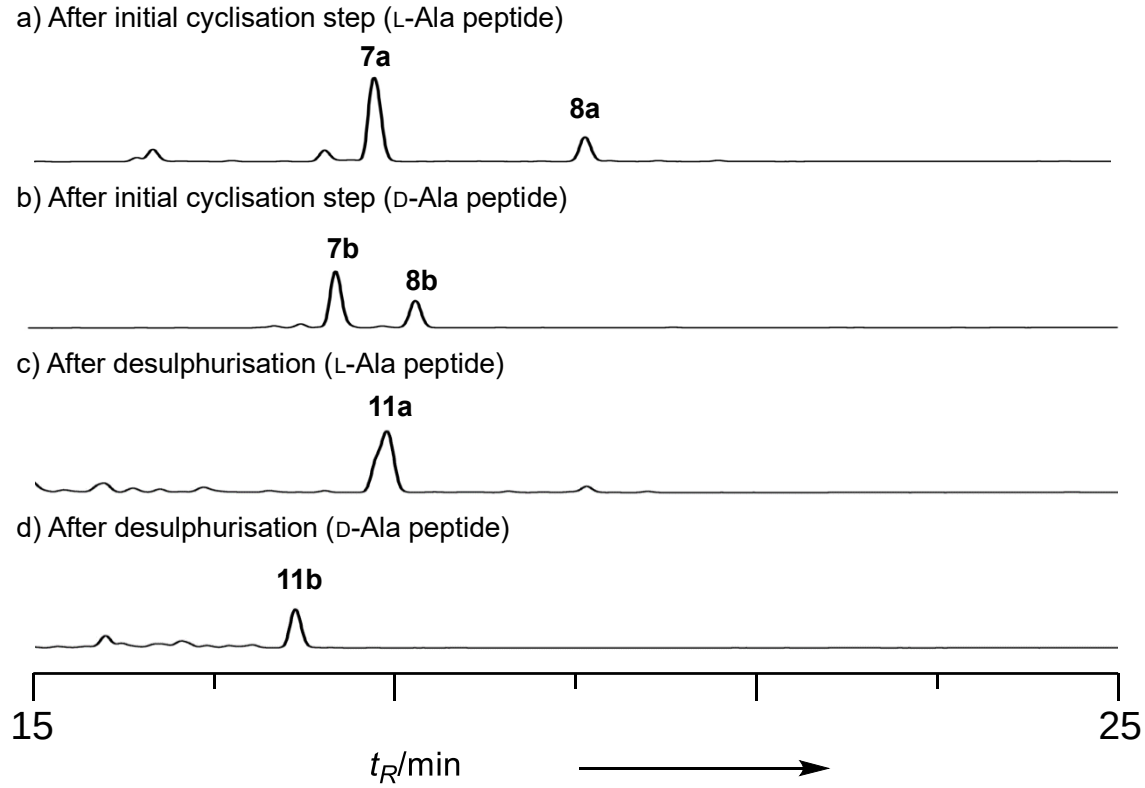
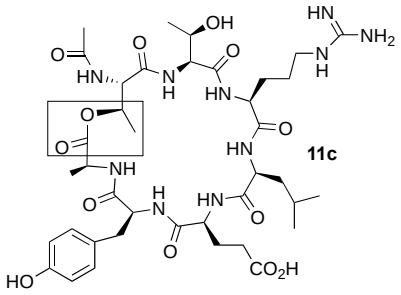


Fig. S12. Analytical HPLC chart of sequential intramolecular S–S and S–O acyl transfer reaction of L-Ala peptide (a) and D-Ala peptide (b). Desulphurisation of L-Ala peptide (c) and D-Ala peptide (d). Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min.

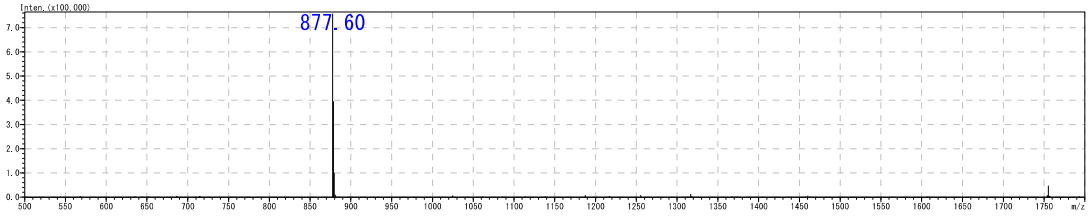
H. Scope of one-pot acyl transfer–desulphrisation reaction

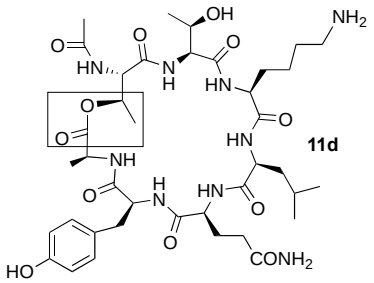
According to the typical macrolactonisation reaction under optimum conditions, linear peptide thioesters (**6c**–**6e**, **6g**) listed in Table 1 were converted to the corresponding lactone peptides (**11c**–**11e**, **11g**).



11c

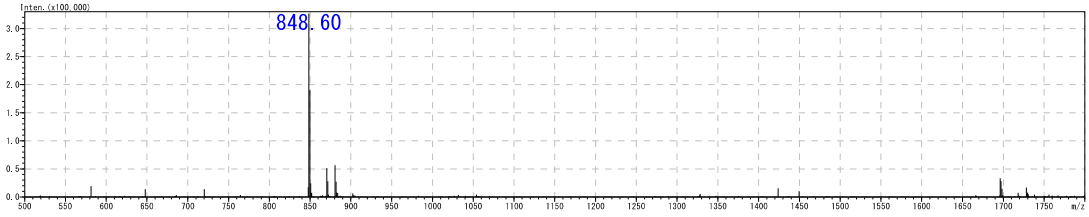
Semi-preparative HPLC conditions of peptide **11c**: linear gradient of solvent B in solvent A, 15% to 25% over 30 min, RT = 22.1 min. LRMS (ESI-Q) *m/z*: $[M+H]^+$ calcd for $C_{39}H_{61}N_{10}O_{13}$ 877.4, found 877.6.

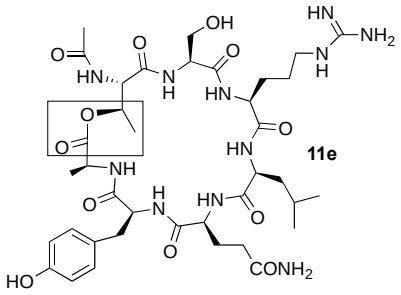




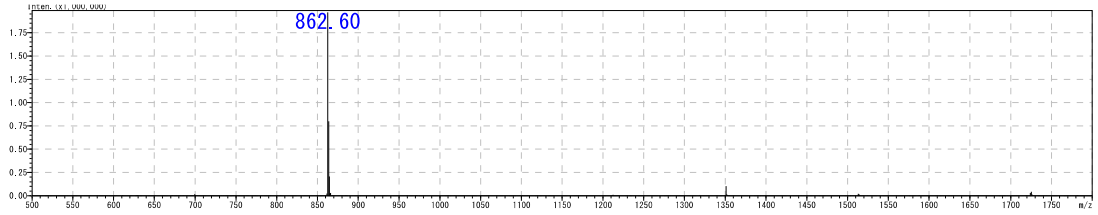
11d

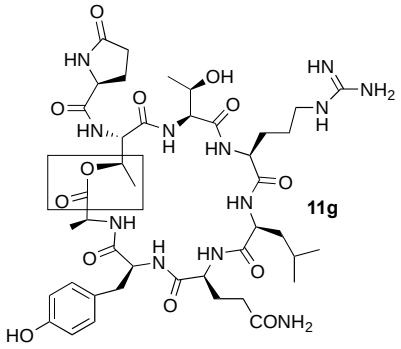
Semi-preparative HPLC conditions of peptide **11d**: linear gradient of solvent B in solvent A, 14% to 24% over 30 min, RT = 22.2 min. LRMS (ESI-Q) *m/z*: $[M+H]^+$ calcd for $C_{39}H_{62}N_9O_{12}$ 848.5, found 848.6.



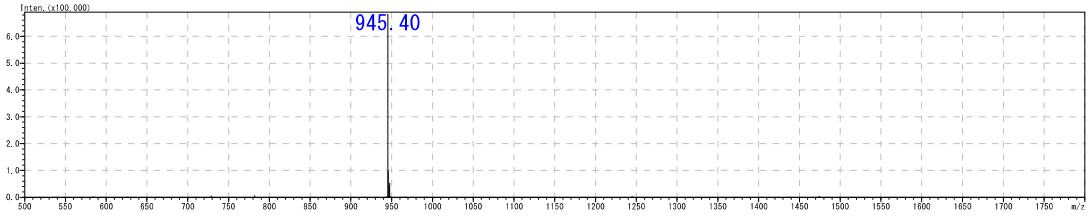


Semi-preparative HPLC conditions of peptide **11e**: linear gradient of solvent B in solvent A, 14% to 24% over 30 min, RT = 19.6 min. LRMS (ESI-Q) *m/z*: $[M+H]^+$ calcd for $C_{38}H_{60}N_{11}O_{12}$ 862.4, found 862.6.





Semi-preparative HPLC conditions of peptide **11g**: linear gradient of solvent B in solvent A, 10% to 20% over 30 min, RT = 26.8 min. LRMS (ESI-Q) *m/z*: $[M+H]^+$ calcd for $C_{42}H_{65}N_{12}O_{13}$ 945.5, found 945.4.



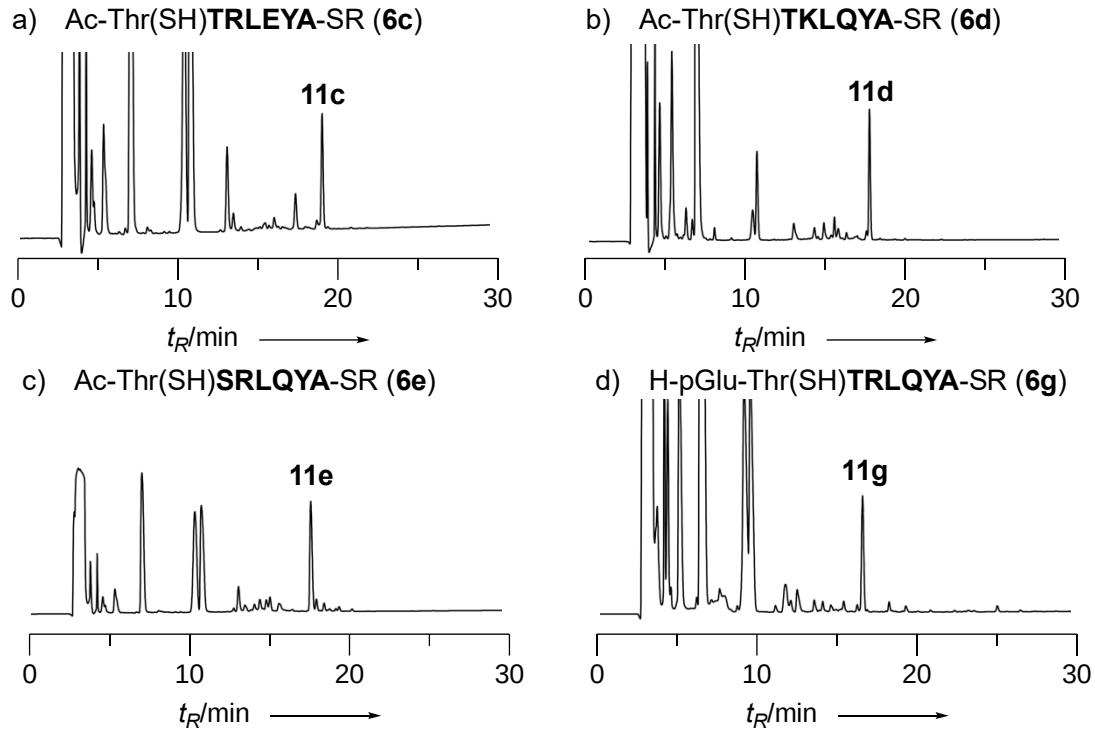


Fig. S13. Analytical HPLC charts of crude reaction mixture of desulpurisation. The substrate linear peptide thioesters were **6c** (a), **6d** (b), **6e** (c) and **6g** (d). Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min.

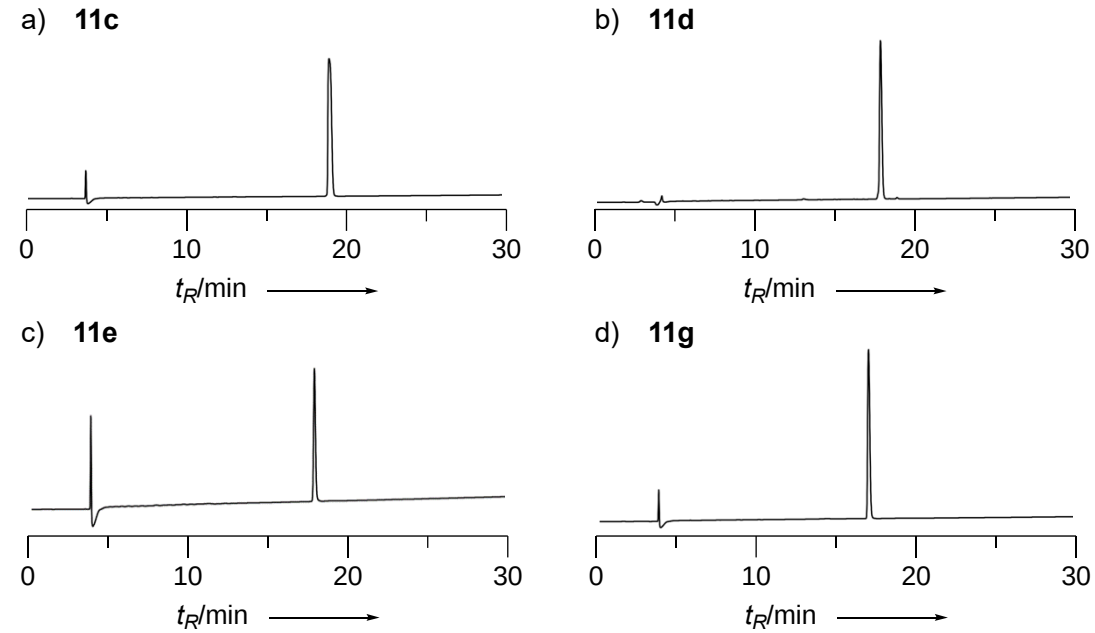


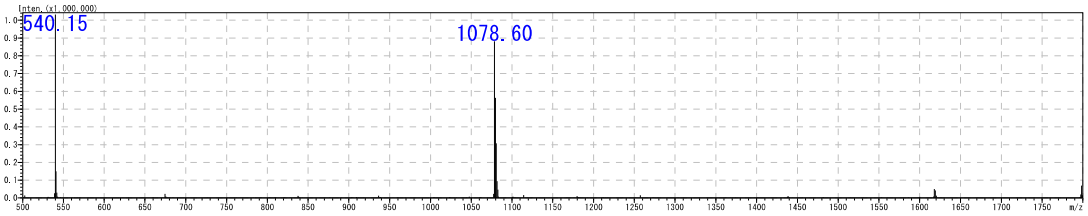
Fig. S14. Analytical HPLC charts of purified desulphurized lactone peptide **11c–11e**, **11g**. Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min.

I. Synthesis of Val-Thr macrolactone peptide 11f

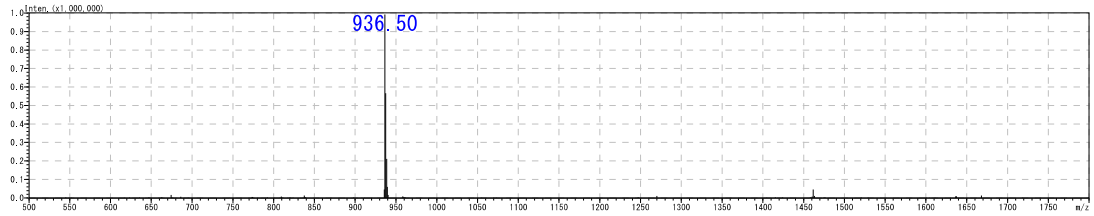
I-1. Macrolactonisation of C-terminal Val thioester peptide 6f

Peptide thioester **6f** was subjected to tandem acyl transfer under the optimum conditions for initial (thio)lactone formation for 30 h at 37 °C to afford a mixture of **6f**, thiolactone **7f** and oxylactone **8f** (Fig. S15 (a) and 15 (b)). Although small amount of **6f** remained, the degassed desulphurisation buffer (40 mM MESNa, 520 mM TCEP·HCl, 2.5 mmol VA-044·2HCl in 6 M Gn·HCl–0.2 M Na phosphate, pH 7.5) was added to the reaction mixture. After being stirred for 24 h, the reaction mixture was purified using semi-preparative HPLC to afford the desired desulphurized lactone peptide **11f** (1.4 mg, 1.4 μmol, 34% isolated yield) with a by-product **12**, a desulphurized linear peptide ring-opened through S–S acyl transfer with MESNa.

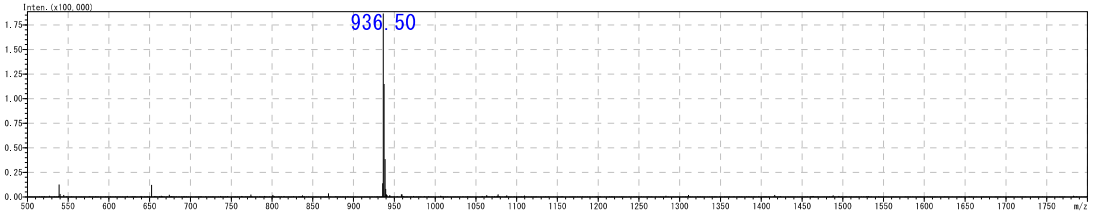
Peptide **6f**: Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min, RT = 17.9 min. LRMS (ESI-Q) *m/z*: $[M+H]^+$ calcd for $C_{43}H_{72}N_{11}O_{15}S_3$ 1078.4, found 1078.6.



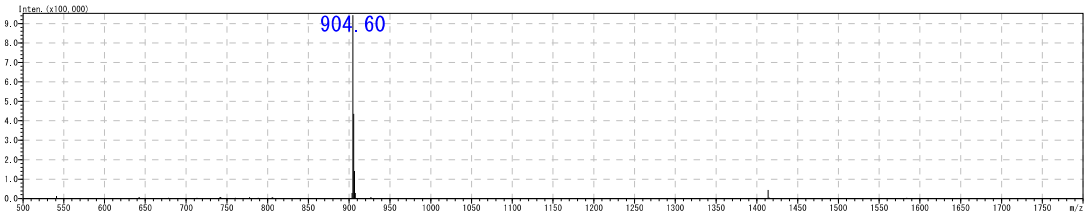
Peptide **7f**: Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min, RT = 20.8 min. LRMS (ESI-Q) *m/z*: $[M+H]^+$ calcd for $C_{41}H_{66}N_{11}O_{12}S$ 936.5, found 936.5.



Peptide **8f**: Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min, RT = 22.5 min. LRMS (ESI-Q) *m/z*: $[M+H]^+$ calcd for $C_{41}H_{66}N_{11}O_{12}S$ 936.5, found 936.5.



Peptide **11f**: Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min, RT = 21.0 min. LRMS (ESI-Q) *m/z*: $[M+H]^+$ calcd for $C_{39}H_{62}N_{11}O_{12}S$ 904.5, found 904.6.



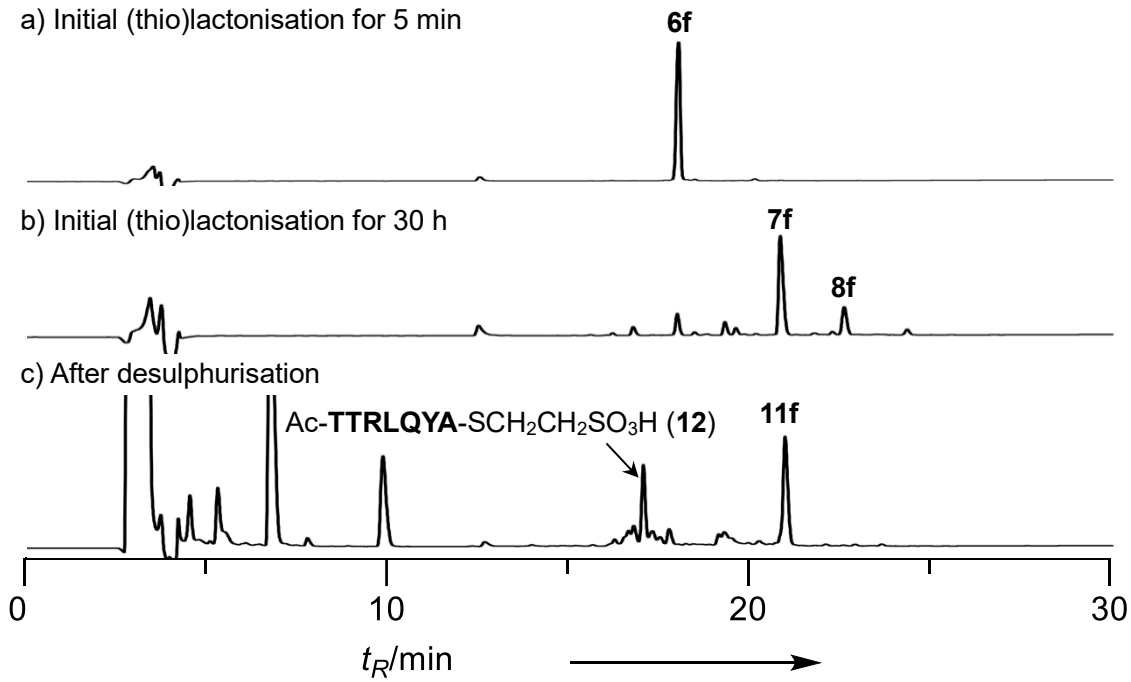


Fig. S15. Analytical HPLC charts of sequential S–S and S–O acyl transfer reaction at 5 min (a) and 30 h (b). HPLC analysis of the crude reaction mixture of desulphurisation (c). Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min.

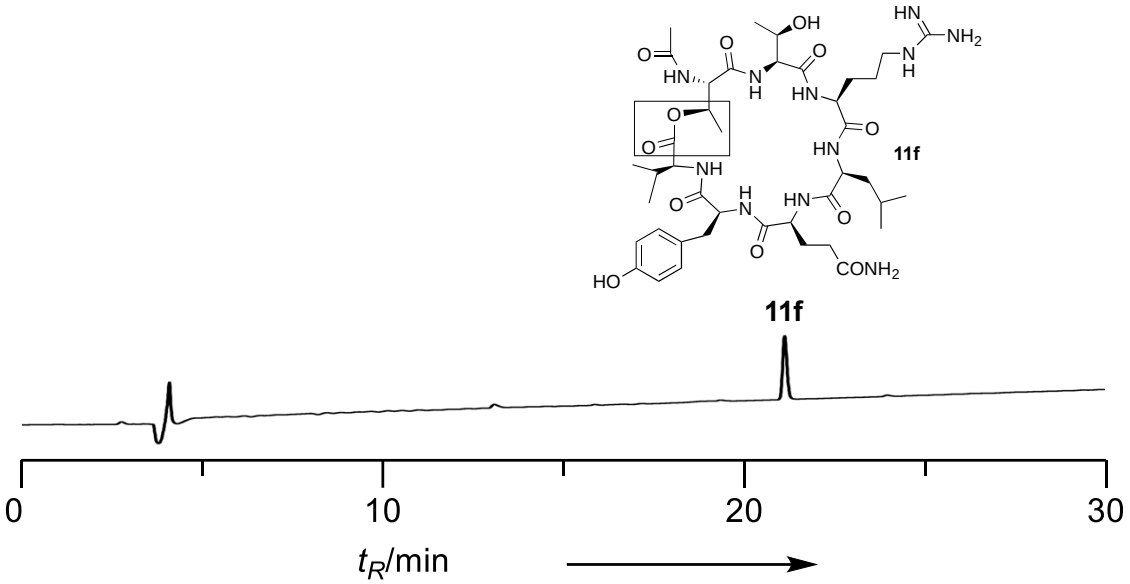
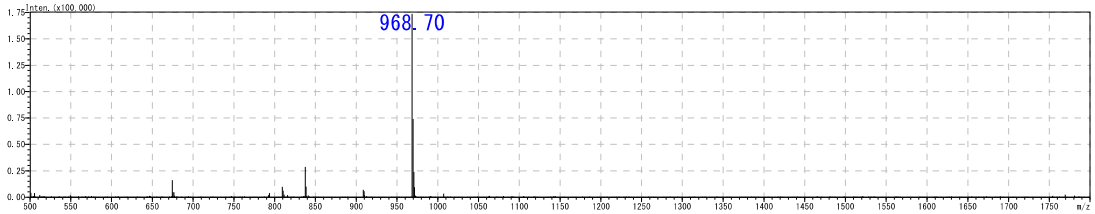


Fig. S16. Analytical HPLC charts of purified desulphurized lactone peptide **11f**. Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min.

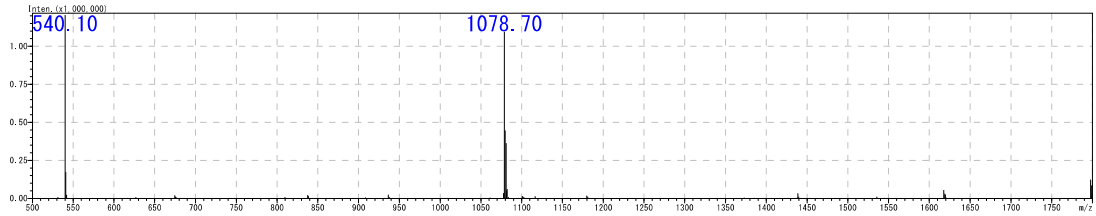
I-2. Evaluation of epimerisation during macrolactonisation of 6f

Epimerisation of C-terminal Val peptide **6f** was also evaluated by a procedure similar to that employed for the epimerisation check of the C-terminal Ala peptide **6a** although the cyclisation and subsequent desulphurisation, under reaction conditions used for the preparation of **11f,** reached incomplete. In contrast to the Ala peptide **6a**, the macrolactonisation of the C-terminal Val peptide accompanied no epimerisation. At present, the reason for such a difference in epimerisation remains to be disclosed. C-terminal D-Val peptide thioester was obtained by above procedure.

*Ac-Thr(SH)-**TRLQY**-D-**V**-NHNH$_2$* (**5h'**) (3.3 mg, 2.7 μmol, 9.4% isolated yield). Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min, Retention time (RT) = 17.0 min. Semi-preparative HPLC conditions: linear gradient of solvent B in solvent A, 8% to 20% over 30 min, RT = 25.6 min. LRMS (ESI-Q) *m/z*: $[M+H]^+$ calcd for $C_{41}H_{70}N_{13}O_{12}S$ 968.5, found 968.7.



*Ac-Thr(SH)-**ARYAQY**-D-**V**-SR* (**6h'**) (1.1 mg, 0.96 μmol, 35% isolated yield). Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min, Retention time (RT) = 20.0 min. Semi-preparative HPLC conditions: linear gradient of solvent B in solvent A, 10% to 25% over 30 min, RT = 29.2 min. LRMS (ESI-Q) *m/z*: $[M+H]^+$ calcd for $C_{43}H_{72}N_{11}O_{15}S_3$ 1078.4, found 1078.7.



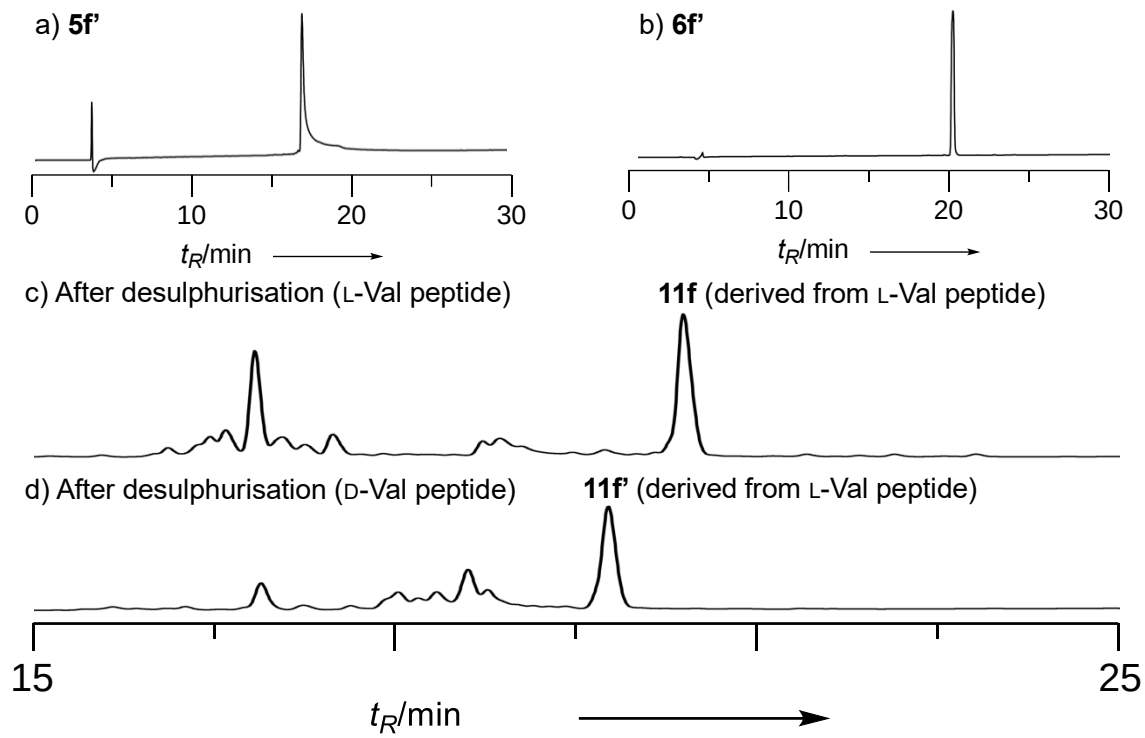


Fig. S17. Analytical HPLC chart of purified peptide hydrazide (a) and peptide thioester (b); the crude reaction mixture of desulphurisation of L-Val **11f** (c) and D-Val **11f'** (d). Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min.

J. Examination of macrolactonisation of side chain protected peptide 6f' under conventional condition

The linear protected peptide acid **6f'** was synthesized using the general procedure and subjected to macrolactonisation according to established protocol.[ref. S2] To a solution of peptide acid **6f'** (0.76 μmol, 1 mM) in CH_2Cl_2 (700 μL) was added a solution of 2-methyl-6-nitrobenzoic anhydride (MNBA) (0.91 μmol), 4-dimethylaminopyridine (DMAP) (1.8 μmol) and triethylamine (1.7 μmol) in CH_2Cl_2 (60 μL) at rt. After being stirred at rt for 24 h, the reaction mixture was diluted with 50%(v/v) CH_3CN aq. The resulting aqueous layer was analyzed using HPLC. In the analysis results, the desired protected lactone peptide peak was not detected, and several peaks corresponding to the molecular wight of the linear substrate peptide acid **6f'** were detected.

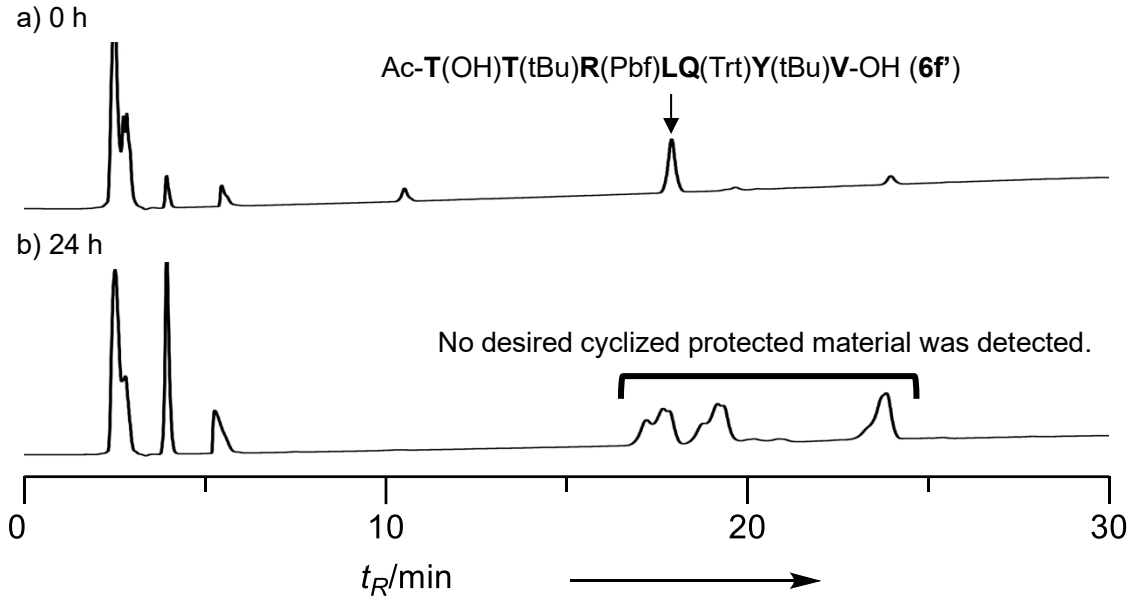
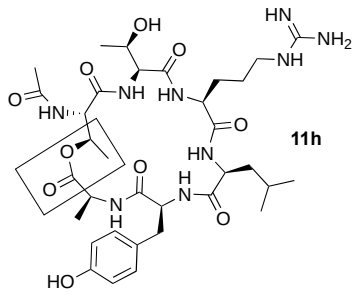


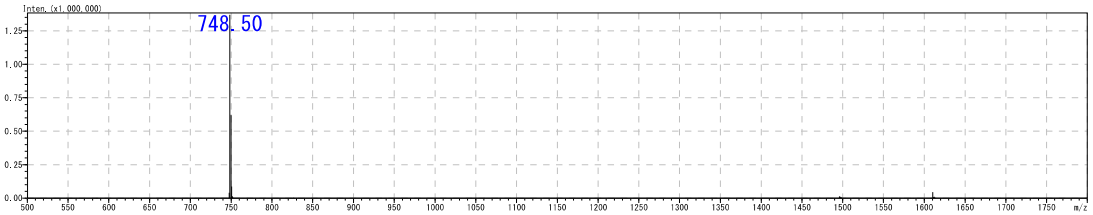
Fig. S18. Analytical HPLC chart of condensation reaction at 0 h (a) and 24 h (b). Analytical HPLC conditions: linear gradient of solvent B in solvent A, 55% to 95% over 30 min.

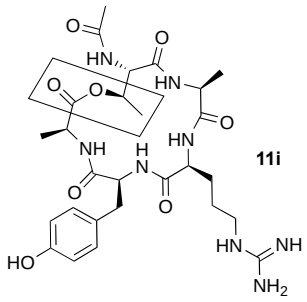
K. Evaluation of the effect of ring size for macrolactonisation

Substrate peptide thioester **9h**, **9i** were dissolved in a degassed aqueous buffer containing 6 M Gn·HCl–0.2 M Na phosphate at pH 7.0. After being stirred at 37 °C for 3 h under anaerobic conditions, the resulting mixture was subjected to desulphurisation under optimum conditions. The linear peptide thioester was converted to the corresponding lactone peptides **11h** (0.75 mg, 1.5 μmol, 57%isolated), **11i** (0.59 mg, 0.82 μmol, 53%isolated yield), but those of four- and three-residue were resulted in complex mixtures at cyclisation step.

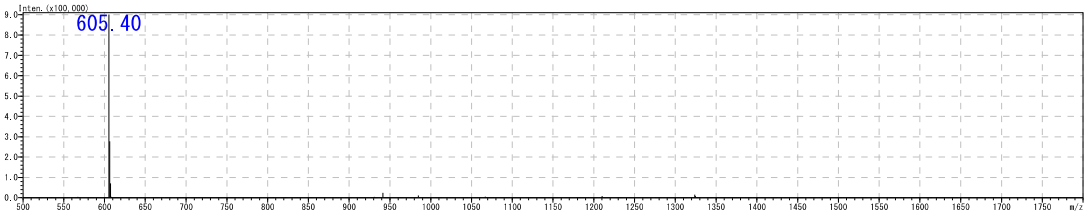


Semi-preparative HPLC conditions of peptide **11h**: linear gradient of solvent B in solvent A, 15% to 25% over 30 min, RT = 26.3 min. LRMS (ESI-Q) *m/z*: $[M+H]^+$ calcd for $C_{34}H_{54}N_9O_{10}$ 748.4, found 748.5.





Semi-preparative HPLC conditions of peptide **11i**: linear gradient of solvent B in solvent A, 8% to 18% over 30 min, RT = 21.0 min. LRMS (ESI-Q) *m/z*: $[M+H]^+$ calcd for $C_{27}H_{41}N_8O_8$ 605.3, found 605.4.



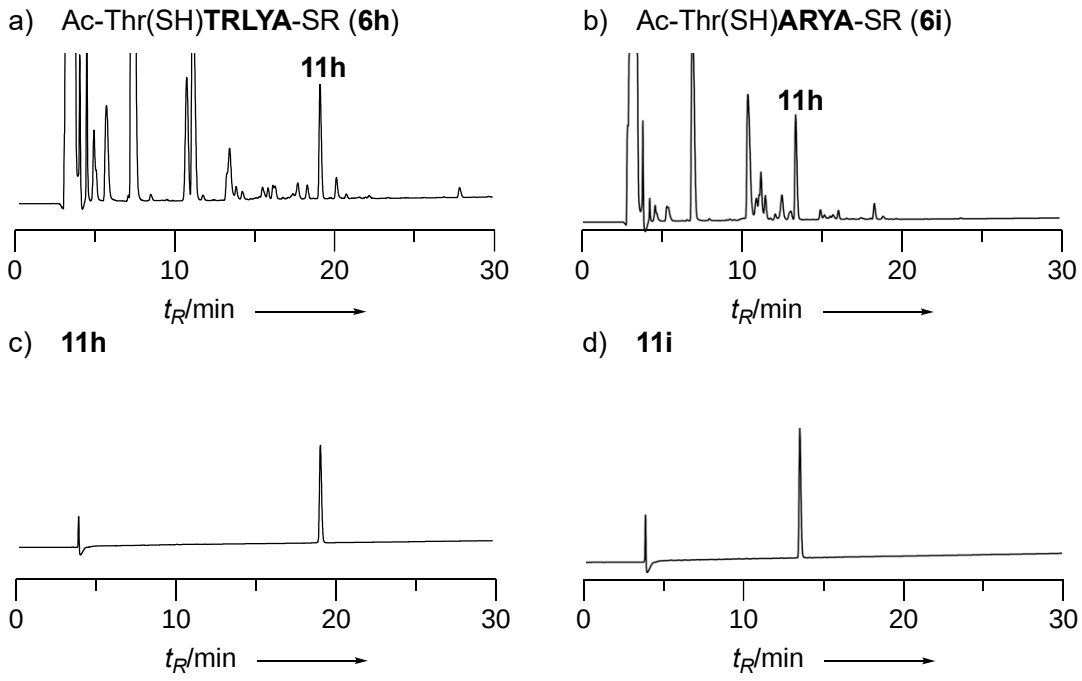
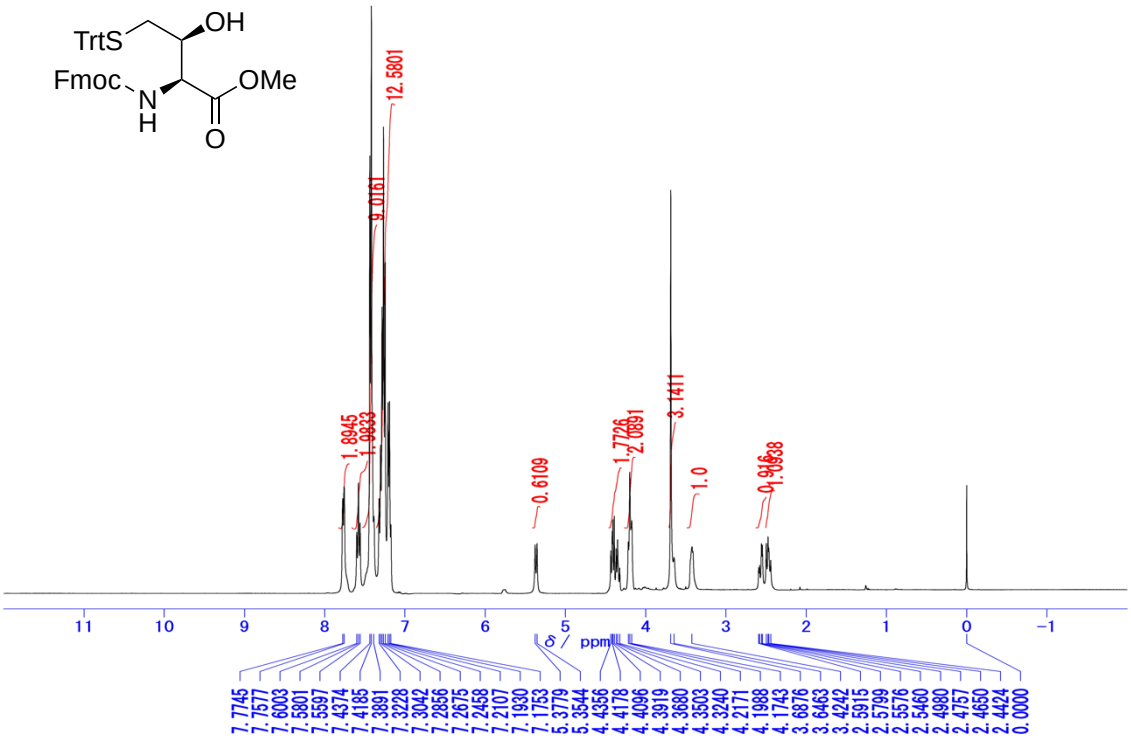


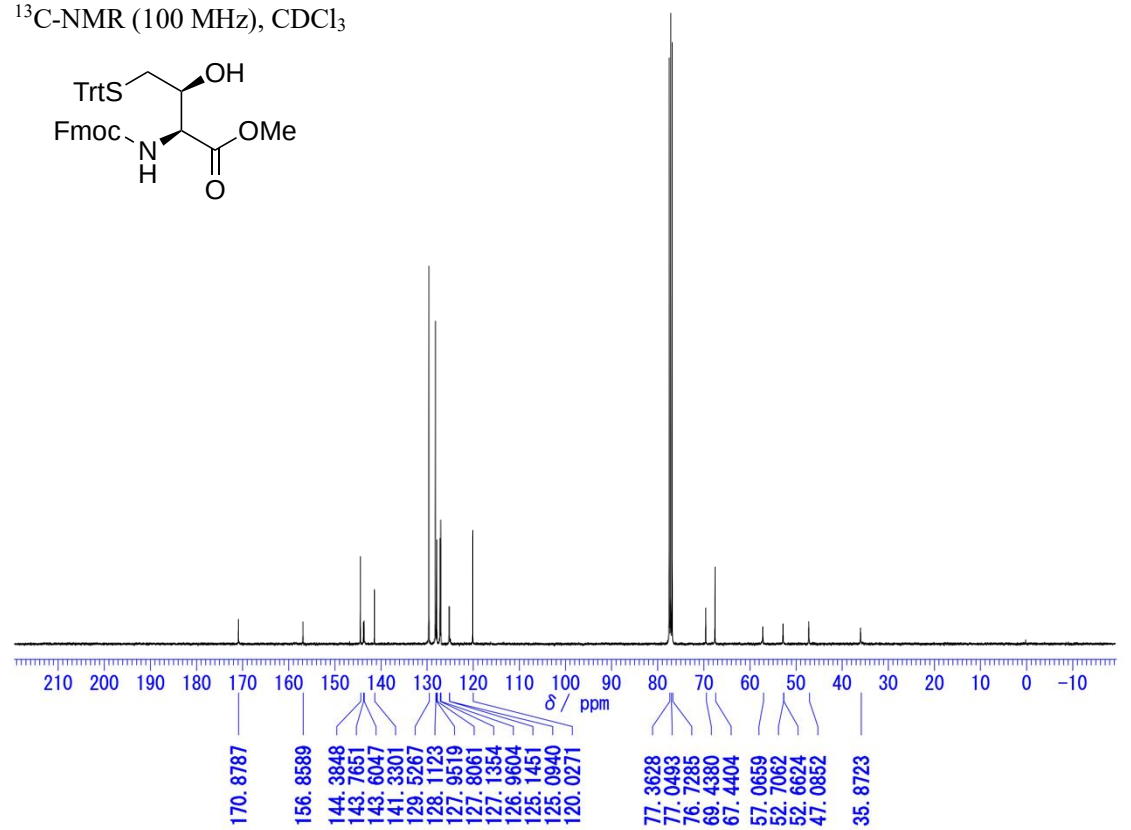
Fig. S19. Analytical HPLC chart of crude reaction mixture of desulphurisation. The substrate linear peptide thioesters were **6h** (a) and **6i** (b). HPLC analysis of purified desulphurized lactone peptide **11h** (c) and **11i** (d). Analytical HPLC conditions: linear gradient of solvent B in solvent A, 5% to 45% over 30 min.

L. NMR spectra

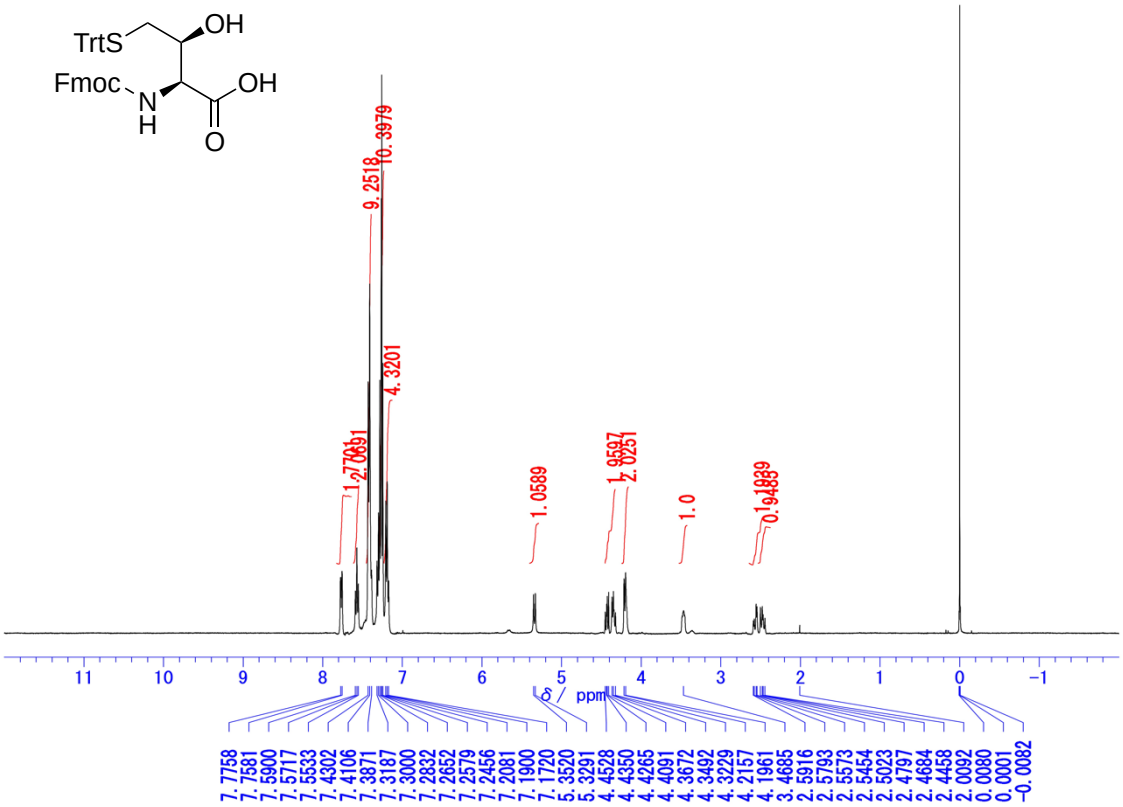
^{1}H-NMR (400 MHz), $CDCl_3$



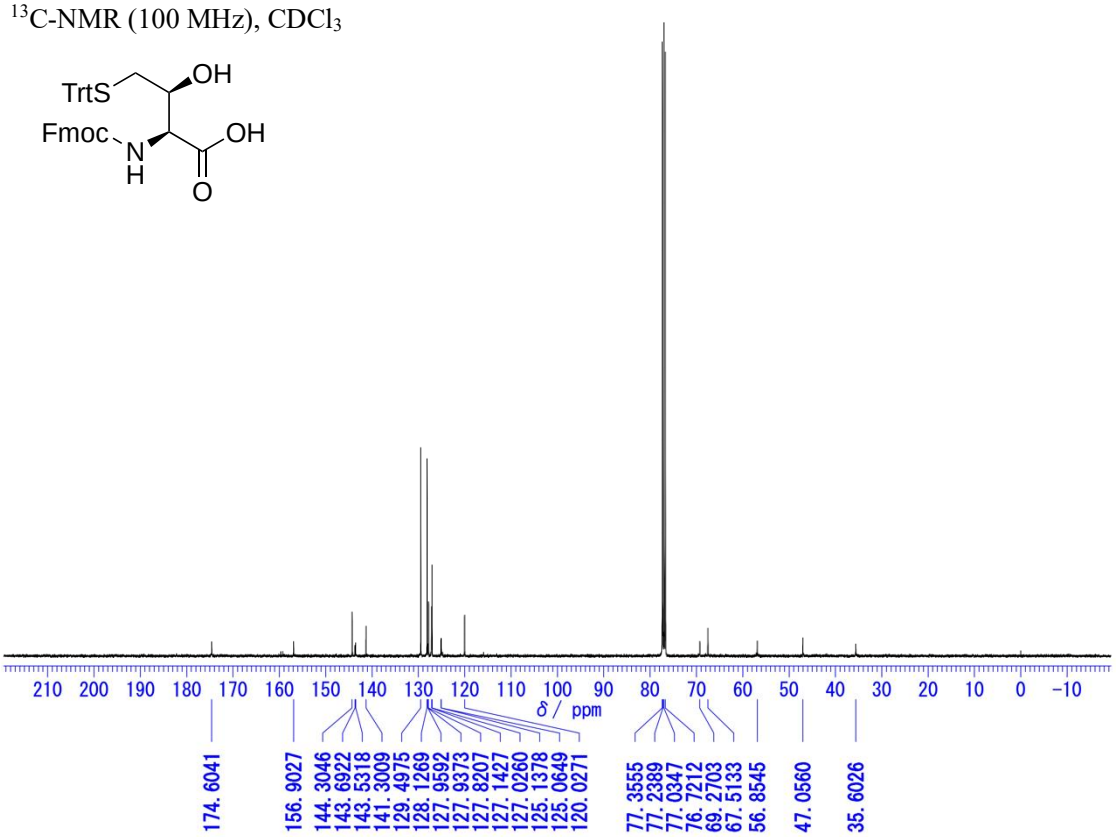
^{13}C-NMR (100 MHz), $CDCl_3$



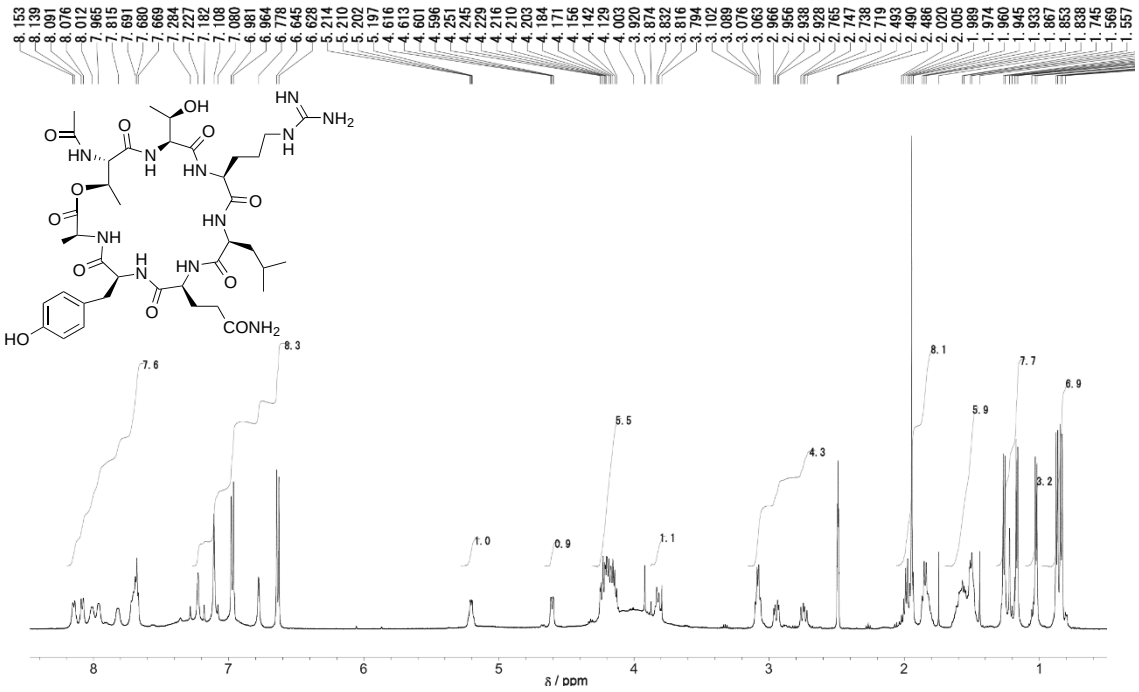
^{1}H-NMR (400 MHz), $CDCl_3$



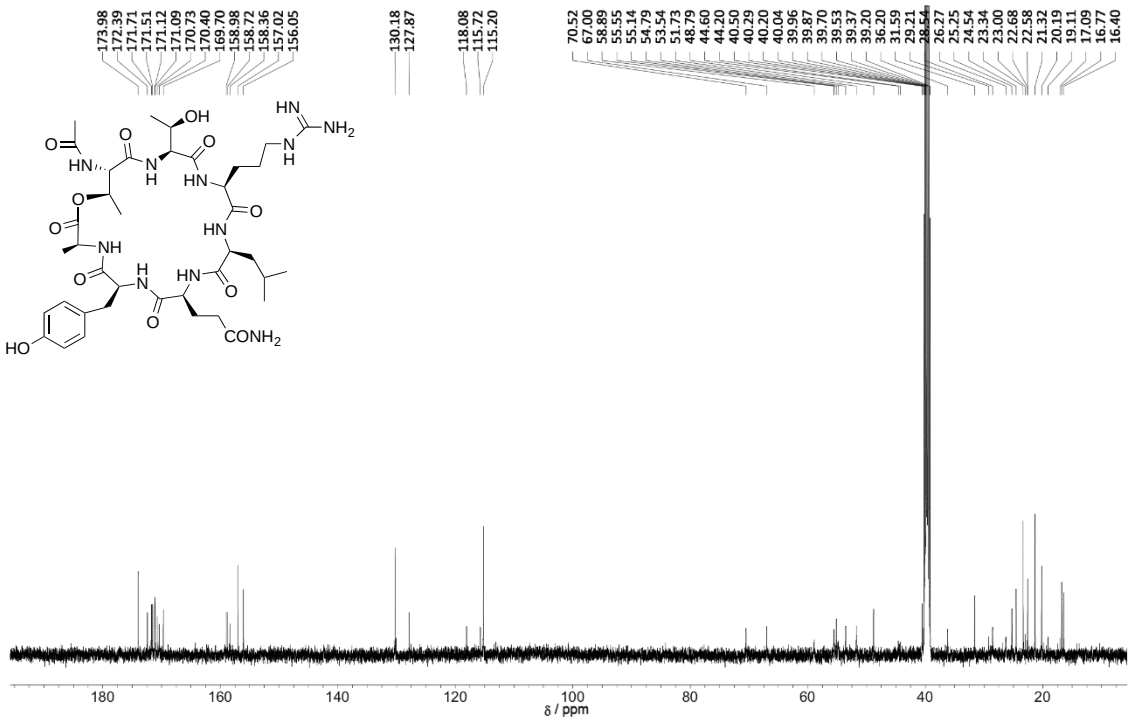
^{13}C-NMR (100 MHz), $CDCl_3$



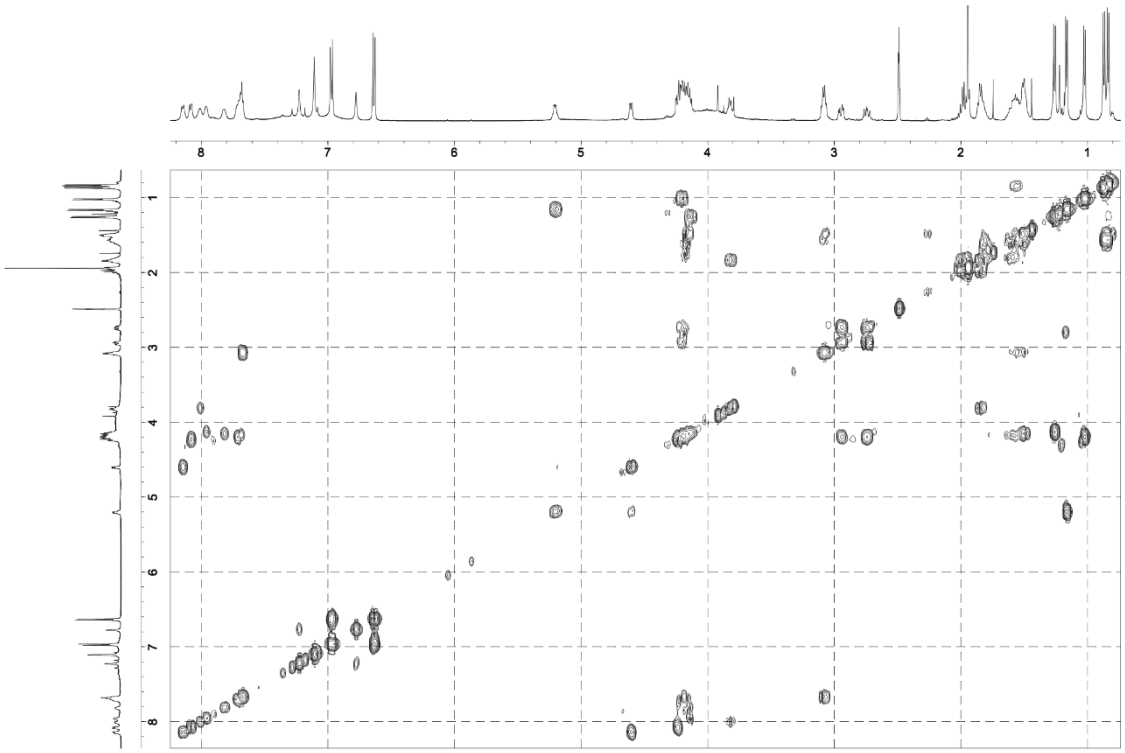
^{1}H-NMR (500 MHz), DMSO-d_6



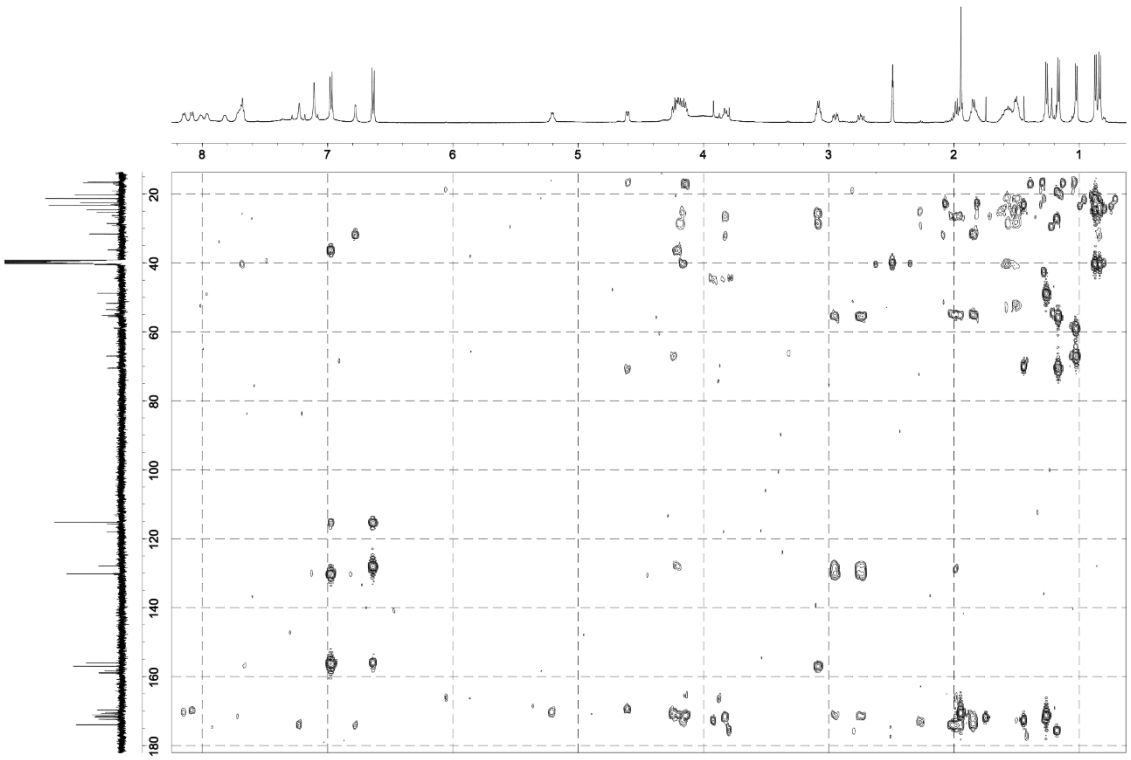
^{13}C-NMR (125 MHz), DMSO-d_6



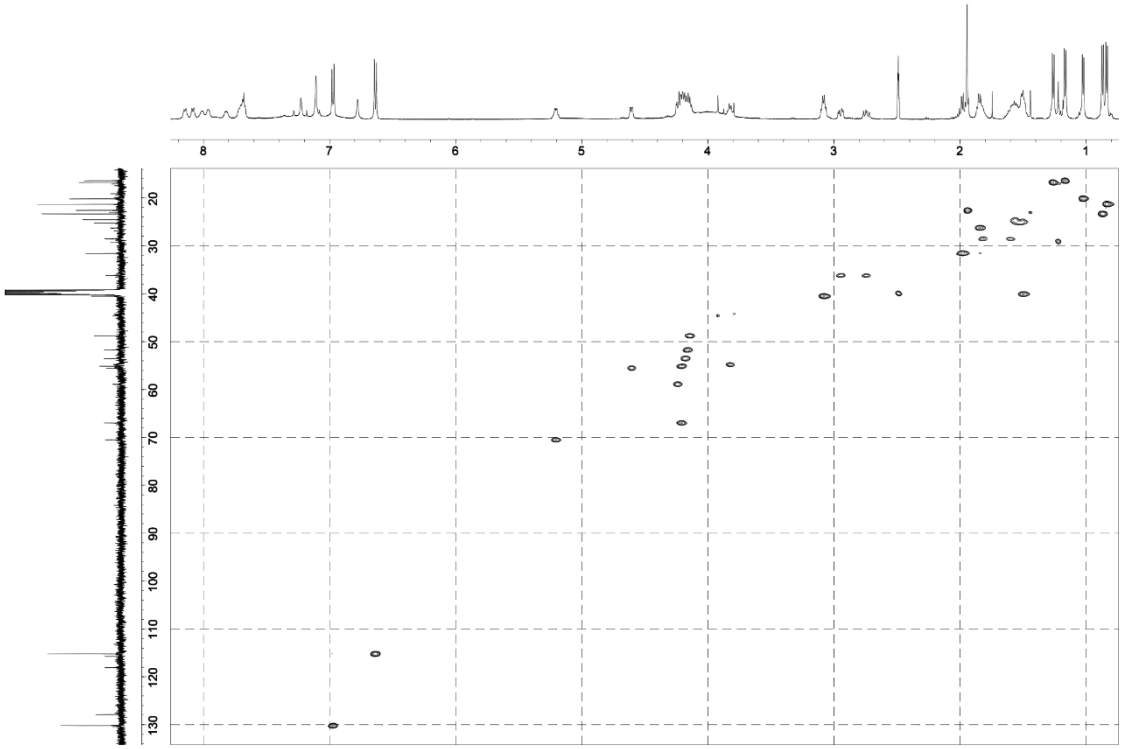
^{1}H-^{1}H COSY spectrum of **11a** (500 MHz), DMSO-d_6



HSQC spectrum of **11a** (500 MHz), DMSO-d_6



HMBC spectrum of **11a** (500 MHz), DMSO-d_6



M. Reference

S1. J. Chen, P. Wang, J. Zhu, Q. Wan, S. J. Danishefsky *Tetrahedron* **2010**, *66*, 2277–2283.

S2. I. Shiina,* M. Kubota, R. Ibuka, *Tetrahedron Lett*. **2002**, *43*, 7535–7539.