Electronic Supplementary Material (ESI) for RSC Medicinal Chemistry.

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1. General Information

1.01 Materials for peptide synthesis and rat serum stability

All reagents were acquired as reagent grade and used without further purification. Solvents for RP-HPLC were purchased as RP-HPLC grade and used without further purification**.** 4-Methylmorpholine (NMM), piperidine, *N*,*N*′-diisopropylcarbodiimide (DIC), 1,2-ethanedithiol (EDT), triisopropylsilane (TIPS), formic acid, 2,4,6-trimethylpyridine (*sym*-collidine), *N*-methyl-2-pyrrolidone (NMP), rat serum, vinyl butyrate, vinyl decanoate, vinyl stearate, vinyl pivalate and vinyl 4-*tert*-butylbenzoate were purchased from Merck (St. Louis, MO, USA). Vinyl palmitate was purchased from TCI (Tokyo, Japan). Aminomethyl polystyrene (AM-PS) resin was synthesized "in house" as described.[1] 2-Chlorotrityl chloride polystyrene resin was purchased from Chempep (Florida, USA). Fmoc (Fmoc = 9-Fluorenylmethoxycarbonyl) protected amino acids were purchased from CS Bio (Shanghai, China) with the following side chain protection, Fmoc-Arg(Pbf)-OH (Pbf = 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl), Fmoc-Asp(O^{t}Bu)-OH (^{t}Bu = *tert*-butyl), Fmoc-Cys(Trt)-OH (Trt = triphenylmethyl), Fmoc-Glu(O^{t}Bu)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH (Boc = *tert*-butyloxycarbonyl), Fmoc-Phe-OH, Fmoc-Pro-OH, Fmoc-Ser(^{t}Bu)-OH, Fmoc-Thr(^{t}Bu)-OH and Fmoc-Val-OH. *O*-(7-Azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluroniumhexafluoro phosphate (HATU), 3-(tritylthio)propionic acid and 6-chloro-1-hydroxybenzotriazole (6-Cl-HOBt) were purchased from CS Bio. Fmoc-Thr(^{t}Bu)-HMPP (HMPP = hydroxymethylphenoxy propionic acid) was purchased from Polypeptide (Strasbourg, France). Trifluoroacetic acid (TFA) was purchased from Oakwood Chemicals (Estill, SC, USA). *N,N*-dimethylformamide (DMF, AR grade) and acetonitrile (CH_3CN, HPLC and LCMS grade) were purchased from Merck Millipore (Berlington, MA, USA).

1.02 Materials for bioluminescence assay

Human cerebral microvascular endothelial cells (hCMVEC) were purchased from Applied Biological Materials (Canada) and cultured in T-75 Falcon flasks from Corning (NY, USA). The collagen-I growth substrate was purchased from Gibco, Thermofisher (Waltham, MA, USA) and modified Hyclone M199 from GE Healthcare (Logan, UT, USA) was used as a growth media. Supplemental ingredients added to M199 include 10 % FBS (Moregate, NZ), 1.0 µg/mL hydrocortisone (Sigma, St Louis, MO, USA), 10 µg/mL heparin (Sigma), 3 ng/mL human FGF (Novus Biologicals, Centennial, CO, USA), 1.0 ng/mL human EGF (Novus Biologicals), 80 nM dibutyryl cyclic adenosine monophosphate (Sigma), GlutaMAX (Gibco), and Penicillin-Streptomycin mix (Gibco). 0.05% Trypsin- Ethylenediaminetetra-acetic acid (EDTA) dissociation reagent was purchased from Gibco. NaCl, KCl, $NaHCO_3$, NaH_2PO_4, $MgCl_2$, $CaCl_2$, sodium-(*D*)-gluconate, (*D*)-glucose, potassium-(*D*)-gluconate, *N*-methyl-(*D*)-glucamine (NMDG) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Quantification of ATP levels by bioluminescence assay was performed in Costar 96 well plates (Corning) using the ATP Determination Kit, A22066, from Invitrogen (Carlsbad, CA, USA). Cx43 gap-junction and hemichannel pan-inhibitor carbenoxolone disodium salt was purchased from Sigma-Aldrich and dissolved in sterile Milli-Q water. Sterile Normal Ringers (NR) and Hypoxic-Acidic Ion-shifted Ringers (HAIR) solutions were made at 10X concentration in reverse osmosis (RO) water and sterilised by autoclaving. On the day of experimentation, these were adjusted to 1X solutions and the non-autoclavable solutes added before pH adjustment.

Normal Ringers		Hypoxic-Acidic Ion-shifted Ringers	
As 10× stock for autoclaving		*As 10× stock for autoclaving*	
NaCl	124 mM	NaCl	34 mM
KCl	3 mM	-	-
$NaHCO_3$	26 mM	$NaHCO_3$	13 mM
NaH_2PO_4	1 mM	NaH_2PO_4	3 mM
$MgCl_2$	1.5 mM	$MgCl_2$	1 mM
-	-	Na (*D*)-gluconate	1.5 mM
On day of experiment[a] *dilute to 1X in* H_2O		*On day of experiment*[a] *dilute to 1X in* H_2O	
$CaCl_2$	1.3 mM	$CaCl_2$	0.13 mM
(*D*)-glucose	10 mM	K (*D*)-gluconate	65 mM
-	-	*N*-methyl-(*D*)-glucamine (NMDG)	38 mM
pH adjust to 7.4.		pH adjust to 6.6, bubble through N_2, 5 min.	

Table 1. Preparation of Normal Ringer (NR) and Hypoxic, Acidic, Ion-shifted Ringers (HAIR) solution. [a] the following chemicals added to the 1X stock solution

1.03 Peptide synthesis

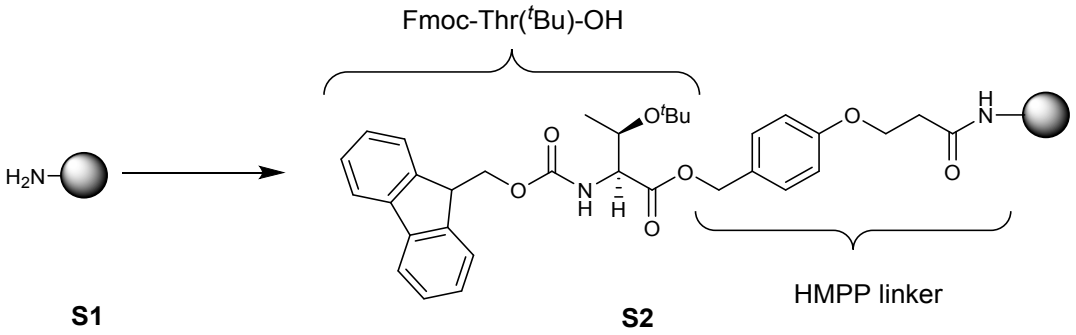
Fmoc-solid phase peptide synthesis (SPPS) was carried out using a Tribute® automated peptide synthesizer (Gyros Protein technologies, Tuscon AZ, USA).

1.04 Analytical, Semi-preparative HPLC and Mass Spectroscopy

Analytical RP-HPLC and semi-preparative RP-HPLC was performed on a Thermo Scientific (Waltham, MA, USA) Dionex Ultimate 3000 HPLC equipped with a four channel UV Detector at 210, 225, 254 and 280 nm. The peptide synthesis and CLipPA reaction monitoring was done using either Phenomenex® (Torrance, CA, USA) C18 Gemini analytical column (5 µm, 110 Å, 4.6 × 150 mm) at a flow rate of 1 mL/min and Phenomenex® (Torrance, CA, USA) semi-preparative C18 Gemini column (5 µm, 110 Å, 10 × 250 mm) at a flow rate of 4 mL/min. The monitoring of rat serum stability assays was done using Waters® (Milford, MA, USA) C18 symmetry analytical column (3.5 µm, 100 Å, 4.6 × 75 mm). The solvent system used was A (0.1% TFA in Milli-Q water and B (0.1% TFA in CH_3CN). Low-resolution mass spectroscopy was acquired on an Agilent (Santa Clara, CA, USA) Technologies 1260 Infinity LC equipped with an Agilent Technologies 6120 Quadrupole mass spectrometer. Peptides were purified using semi-preparative HPLC with a suitably adjusted gradient of 5% B to 95% B and fractions were collected, analyzed by analytical RP-HPLC or ESI-MS, pooled and lyophilized. Yields refer to chromatographically homogeneous materials.

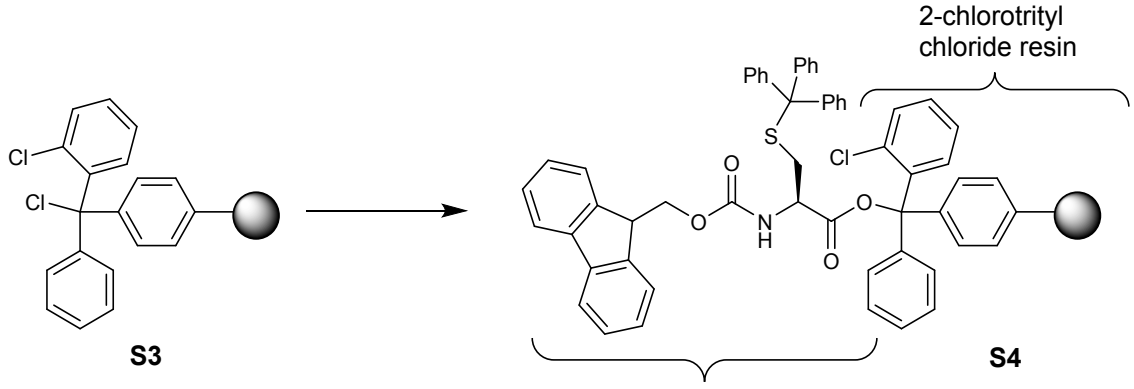
2. General Procedure for 9-fluorenylmethoxycarbonyl (Fmoc) Solid Phase Peptide Synthesis (SPPS)

Method 1: General procedure for loading of Fmoc-Thr(*t*Bu)-HMPP linker to the resin:



To aminomethyl polystyrene resin **S1** (106 mg, 0.1 mmol, loading: 0.94 mmol/g) pre-swollen in CH_2Cl_2 (5 mL, 20 min), was added Fmoc-Thr(*t*Bu)-4-(hydroxymethyl)phenoxy propionic acid (HMPP) (115 mg, 2.0 equiv., 0.2 mmol) in DCM:DMF (1 mL, 9:1 *v*/*v*) followed by addition of DIC (31 µL, 2.0 equiv., 0.2 mmol). The reaction mixture was gently agitated at room temperature for 2 h. The resin was filtered and washed with DMF (3 × 3 mL) after which a negative Kaiser test confirmed successful coupling.

Method 2: General procedure for loading of Fmoc-Cys(Trt)-OH to 2-chlorotrityl chloride resin:



To 2-chlorotrityl chloride polystyrene resin **S3** (123 mg, 0.1 mmol, loading: 0.81 mmol/g) pre-swollen in CH_2Cl_2 (5 mL, 20 min), was added Fmoc-Cys(Trt)-OH (293 mg, 5.0 equiv, 0.5 mmol) in DCM (1 mL) followed by addition of DIPEA (174 µL, 10 equiv, 1.0 mmol). The reaction mixture was gently agitated at room temperature overnight. The resin was filtered and treated with DCM:MeOH:DIPEA (2 × 5 mL, 80:15:5 *v*/*v*/*v*) at room temperature for 10 min to cap any unreacted 2-chlorotrityl chloride resin then washed with DMF (3 × 3 mL).

Method 3: General procedure for iterative Fmoc-SPPS for foupling of following Fmoc-amino acids; Fmoc-Arg(Pbf)-OH, Fmoc-Asp(O^{t}Bu)-OH, Fmoc-Glu(O^{t}Bu)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Phe-OH, Fmoc-Pro-OH, Fmoc-Ser(^{t}Bu)-OH, Fmoc-Thr(^{t}Bu)-OH, Fmoc-Val-OH and 3-(tritylthio)propionic acid

Solid phase peptide synthesis was performed on the resulting Fmoc-Thr(^{t}Bu)-HMPP linker resin **S2** or Fmoc-Cys(Trt)-2-chlorotrityl resin **S4** at room temperature *via* Fmoc-SPPS using a Tribute® automated peptide synthesizer. The reaction sequences and reagents for peptide elongation are summarized in Table 2.

Steps	Reagents[a]	Duration[b]
Fmoc deprotection	20% piperidine in DMF (4 mL, *v*/*v*)	7 min × 2
Resin washing	DMF (4 mL)	0.5 min × 4
Activation of amino acid	5.0 eq. of Fmoc-AA-OH, 4.75 eq. HATU, 0.5 M NMM in DMF (2 mL *v*/*v*, 10 eq.)	2 min
Amino acid coupling		60 min
Resin capping	20% acetic anhydride in DMF (1 mL, *v*/*v*)	1 min
Resin washing	DMF (4 mL)	0.5 min × 2

Table 2. Reaction sequences and reagents used for Tribute® automated peptide synthesizer for peptide synthesis.

[a] Regents usage are based on 0.1 mmol peptide synthesis scale. [b] The reaction was conducted at the room temperature.

Method 4: General procedure for coupling of Fmoc-Cys(Trt)-OH and Fmoc-Cys(Acm)-OH using racemization free conditions

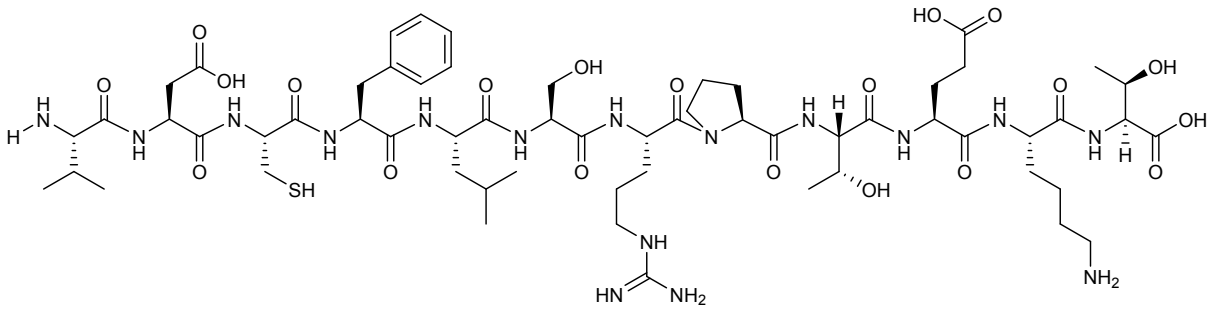
For the attachment of either Fmoc-Cys(Trt)-OH or Fmoc-Cys(Acm)-OH, a double coupling procedure using racemization-free conditions was performed at room temperature using the following reagents: Fmoc-Cys(Trt)-OH / Fmoc-Cys(Acm)-OH (5.0 equiv.), HATU (4.75 equiv.) HOAt, (4.75 equiv.) 2,4,6-trimethylpyridine (8.0 equiv.), DCM:DMF (6 mL, 1:1 *v*/*v*). Following the double coupling, unreacted amines were then capped by treatment with 20% acetic anhydride in DMF (1 mL, *v*/*v*) for 1 min.

Method 5: General procedure for TFA-mediated peptidyl-resin cleavage

Upon completion of the peptide synthesis using **Method 3** and **Method 4** the final Fmoc group was removed yielding peptidyl-resin, which was successively washed with DMF (4 × 8 mL), CH_2Cl_2 (4 × 5 mL) and dried under vacuum prior to peptide cleavage from the resin. The peptidyl-resin was treated with TFA/TIPS/H_2O/EDT (10 mL, 91.5:1.0:5.0:2.5 *v*/*v*/*v*/*v*) at room temperature for 2 h. The resin was separated from the supernatant by filtration followed by evaporation of TFA filtrate *via* N_2 gas. The peptide was precipitated in cold diethyl ether then isolated by centrifugation (4000 rpm, 3 × 10 min). The precipitated product was dissolved in CH_3CN/H_2O containing 0.1% TFA (10 mL, 1:1 *v*/*v*), and lyophilized to afford the crude peptide.

3. Synthesis of native Peptide5 (1) and peptide scaffolds (2)-(8)

3.01 Synthesis of Peptide5 (1) : H_2N-VDCFLSRPTEKT-OH



(**1**)

Fmoc-Thr(*t*Bu)-HMPP resin **S2** was prepared using **Method 1** which was followed by peptide elongation on resin using **Method 3**. Fmoc-Cys(Trt)-OH was coupled to peptidyl-resin using **Method 4**. Upon the completion of peptide synthesis, the peptidyl-resin was cleaved using **Method 5** to afford crude peptide (**1**) (121 mg). (**Figure S1**).

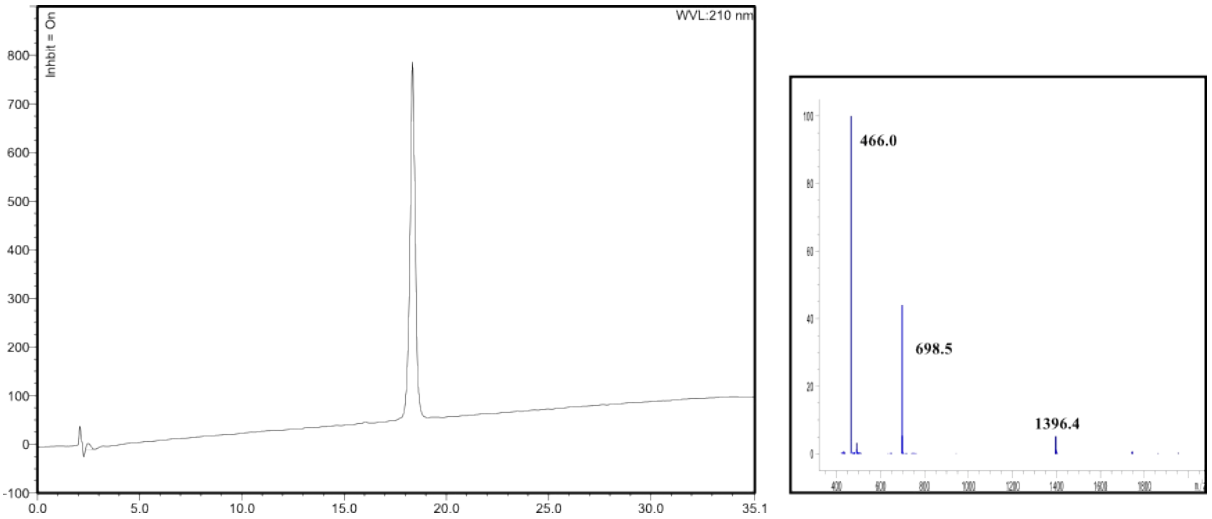
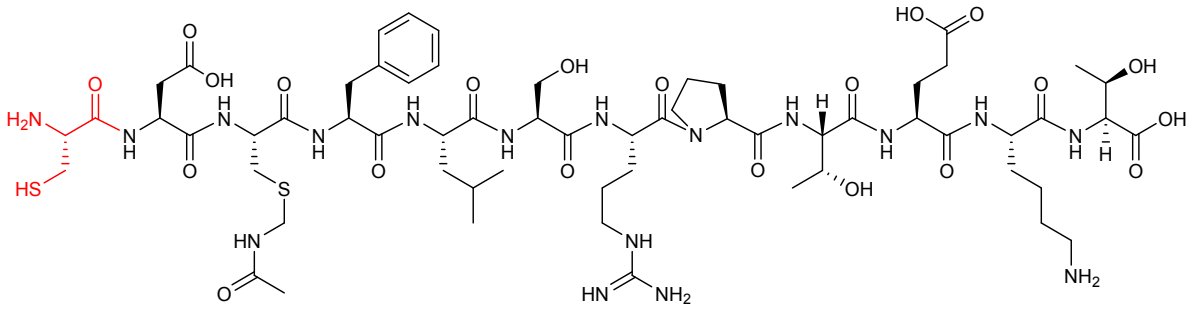


Figure S1. HPLC of crude peptide monitored at 210 nm and observed ESI-MS of crude peptide (**1**). Observed ESI-MS of the main peak for crude peptide (**1**), calcd. Mass: 1395.6, found 1396.4 $[M+1H]^{+1}$, 698.5 $[M+2H]^{+2}$, 466.0 $[M+3H]^{+3}$. Deconvolution affords a mass of 1395.1 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 µ; 4.6 x 150 mm) and a linear gradient of 5-35% B in 30 min, *ca*. 1% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

3.02 Synthesis of peptide scaffold (2) : H_2N-CDC(Acm)FLSRPTEKT-OH



(**2**)

Fmoc-Thr(ᵗBu)-HMPP resin **S2** was prepared using **Method 1** which was followed by peptide elongation on resin using **Method 3**. Fmoc-Cys(Trt)-OH and Fmoc-Cys(Acm)-OH were coupled to peptidyl-resin using **Method 4**. Upon the completion of peptide synthesis, the peptidyl-resin was cleaved using **Method 5** to afford crude peptide (**2**) (138 mg) which was used in the next step without further purification (**Figure S2**).

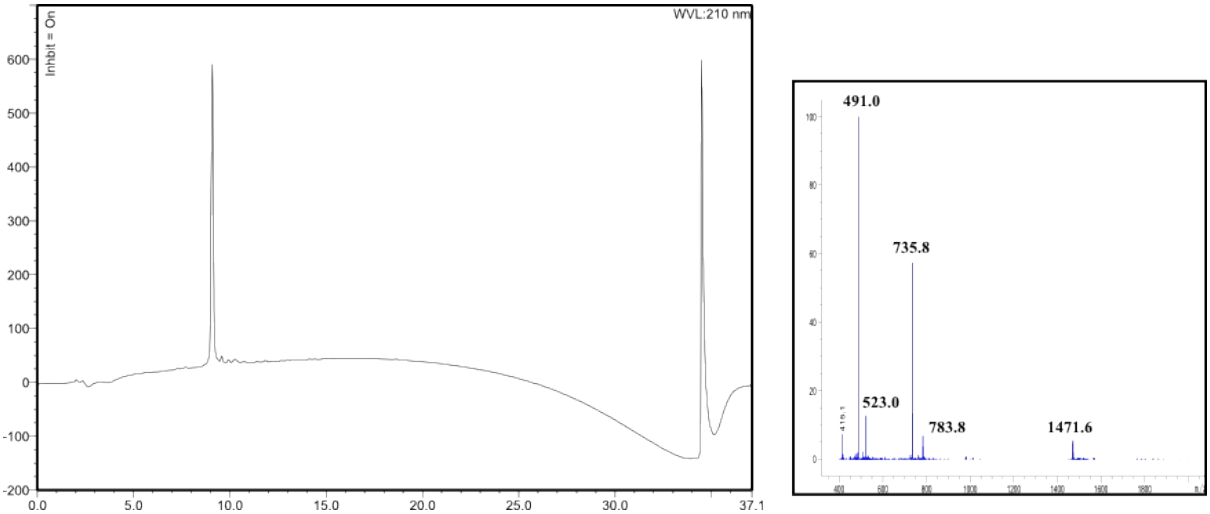
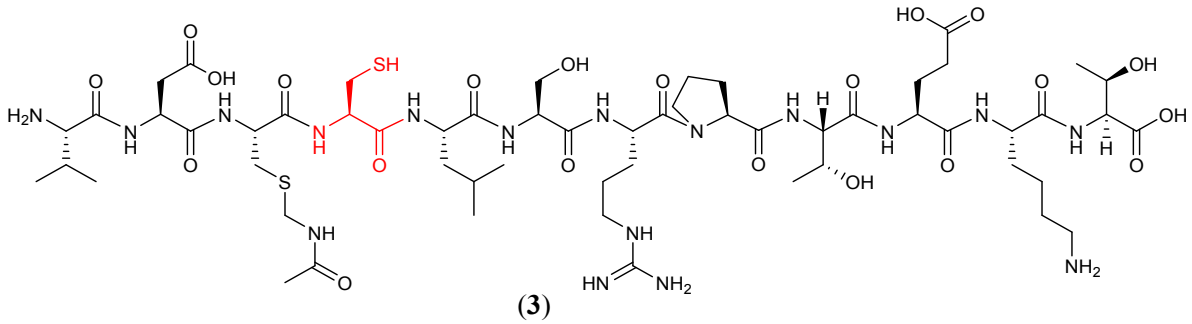


Figure S2. HPLC of crude peptide monitored at 210 nm and observed ESI-MS of crude peptide (**2**). Observed ESI-MS of the main peak for crude peptide (**2**), calcd. Mass: 1470.5, found 1471.6 $[M+1H]^{+1}$, 735.8 $[M+2H]^{+2}$, 491.0 $[M+3H]^{+3}$, where 783.8 $[M+2H]^{+2}$ and 523.0 $[M+3H]^{+3}$ corresponds to +96 Da due to the presence of trifluoroacetate adduct. Deconvolution affords a mass of 1470.1 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 µ; 4.6 x 150 mm) and a linear gradient of 5-35% B in 30 min, *ca*. 1% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

3.03 Synthesis of peptide scaffold (3) : H_2N-VDC(Acm)CLSRPTEKT-OH



(**3**)

Fmoc-Thr(ᵗBu)-HMPP resin **S2** was prepared using **Method 1** which was followed by peptide elongation on resin using **Method 3**. Fmoc-Cys(Trt)-OH and Fmoc-Cys(Acm)-OH were coupled to peptidyl-resin using **Method 4**. Upon the completion of peptide synthesis, the peptidyl-resin was cleaved using **Method 5** to afford crude peptide (**3**) (138 mg) which was used in the next step without further purification (**Figure S3**).

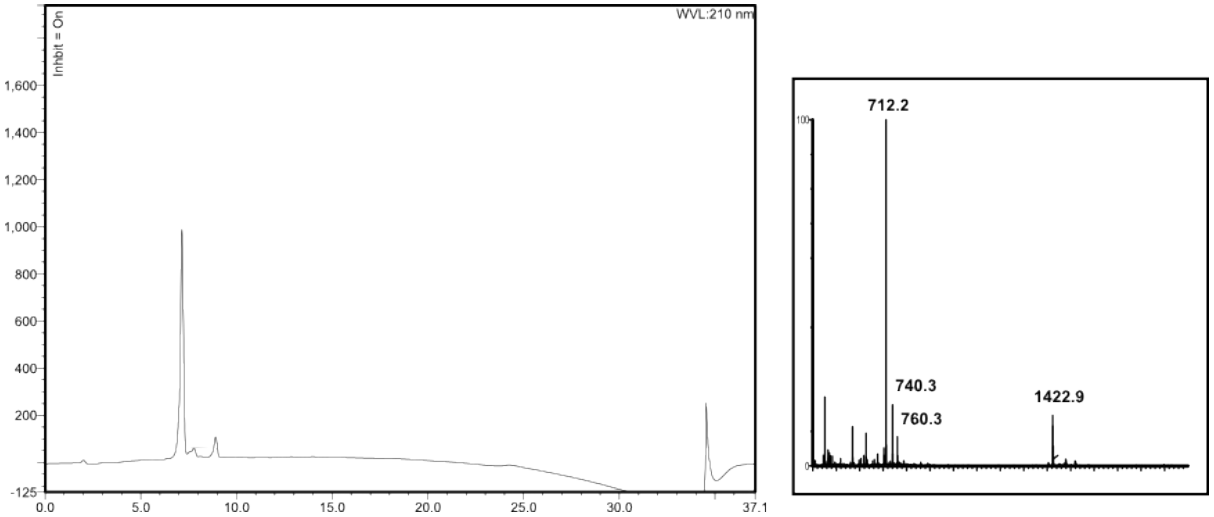
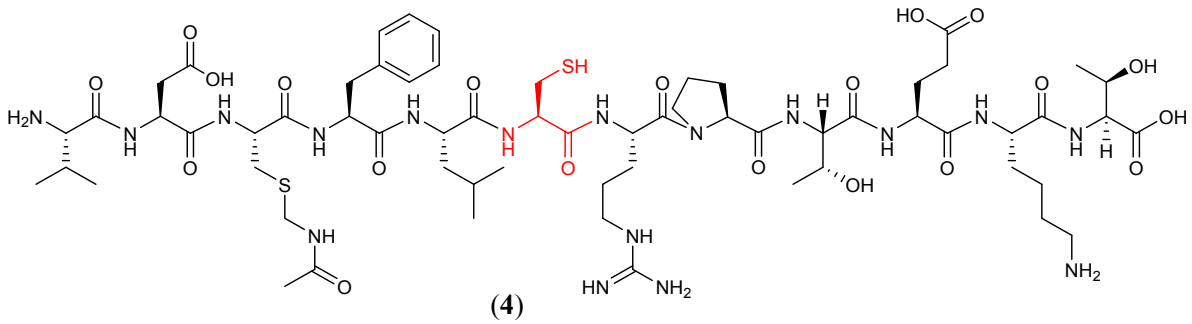


Figure S3. HPLC of crude peptide monitored at 210 nm and observed ESI-MS of crude peptide (**3**). Observed ESI-MS of the main peak for crude peptide (**3**), calcd. Mass: 1422.5, found 1422.9 $[M+1H]^{+1}$, 712.2 $[M+2H]^{+2}$. Deconvolution affords a mass of 1422.2 Da, where 740.3 $[M+2H]^{+2}$ corresponds to +56 Da due to the presence of *tert*-butyl adduct and 760.3 $[M+2H]^{+2}$ corresponds to +96 Da due to the presence of trifluoroacetate adduct. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 μ; 4.6 x 150 mm) and a linear gradient of 5-35% B in 30 min, *ca*. 1% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

3.04 Synthesis of peptide scaffold (4) : H_2N-VDC(Acm)FLCRPTEKT-OH



(**4**)

Fmoc-Thr(ᵗBu)-HMPP resin **S2** was prepared using **Method 1** which was followed by peptide elongation on resin using **Method 3**. Fmoc-Cys(Trt)-OH and Fmoc-Cys(Acm)-OH were coupled to peptidyl-resin using **Method 4**. Upon the completion of peptide synthesis, the peptidyl-resin was cleaved using **Method 5** to afford crude peptide (**4**) (135 mg) which was used in the next step without further purification (**Figure S4**).

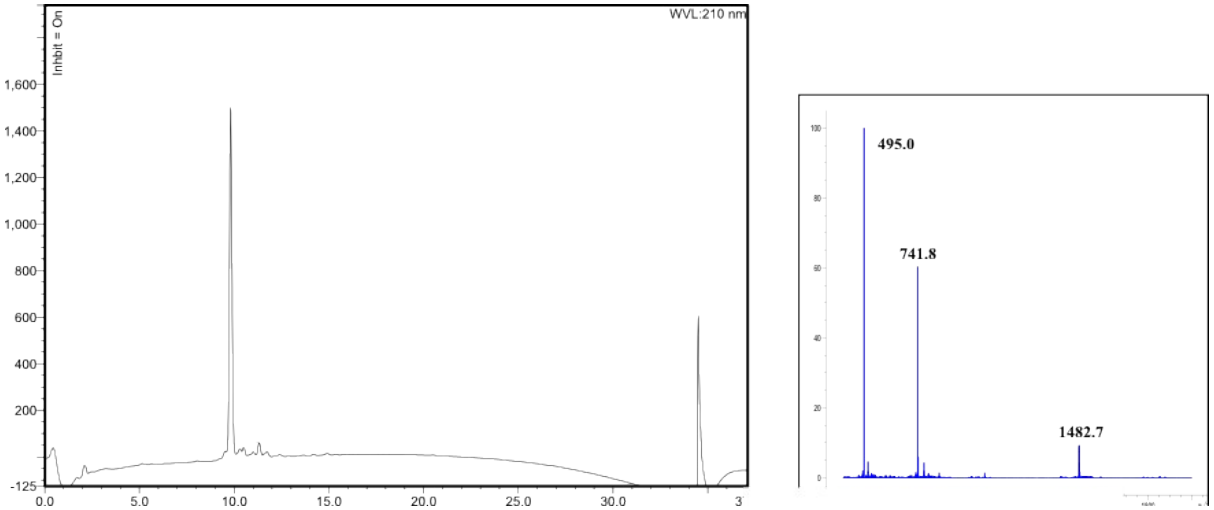
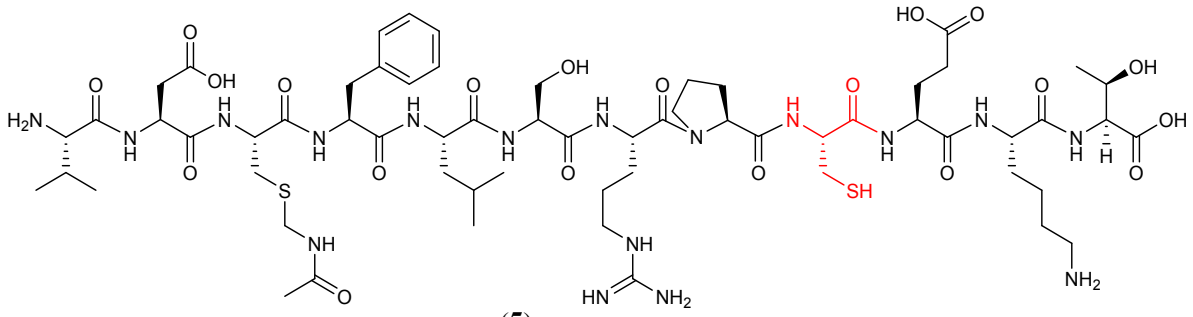


Figure S4. HPLC of crude peptide monitored at 210 nm and observed ESI-MS of crude peptide (**4**). Observed ESI-MS of the main peak for crude peptide (**4**), calcd. Mass: 1482.6, found 1482.7 $[M+1H]^{+1}$, 741.8 $[M+2H]^{+2}$, 495.0 $[M+3H]^{+3}$. Deconvolution affords a mass of 1481.8 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 µ; 4.6 x 150 mm) and a linear gradient of 5-35% B in 30 min, *ca*. 1% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

3.05 Synthesis of peptide scaffold (5) : H_2N-VDC(Acm)FLSRPCEKT-OH



(**5**)

Fmoc-Thr(ᵗBu)-HMPP resin **S2** was prepared using **Method 1** which was followed by peptide elongation on resin using **Method 3**. Fmoc-Cys(Trt)-OH and Fmoc-Cys(Acm)-OH were coupled to peptidyl-resin using **Method 4**. Upon the completion of peptide synthesis, the peptidyl-resin was cleaved using **Method 5** to afford crude peptide (**5**) (137 mg) which was used in the next step without further purification (**Figure S5**).

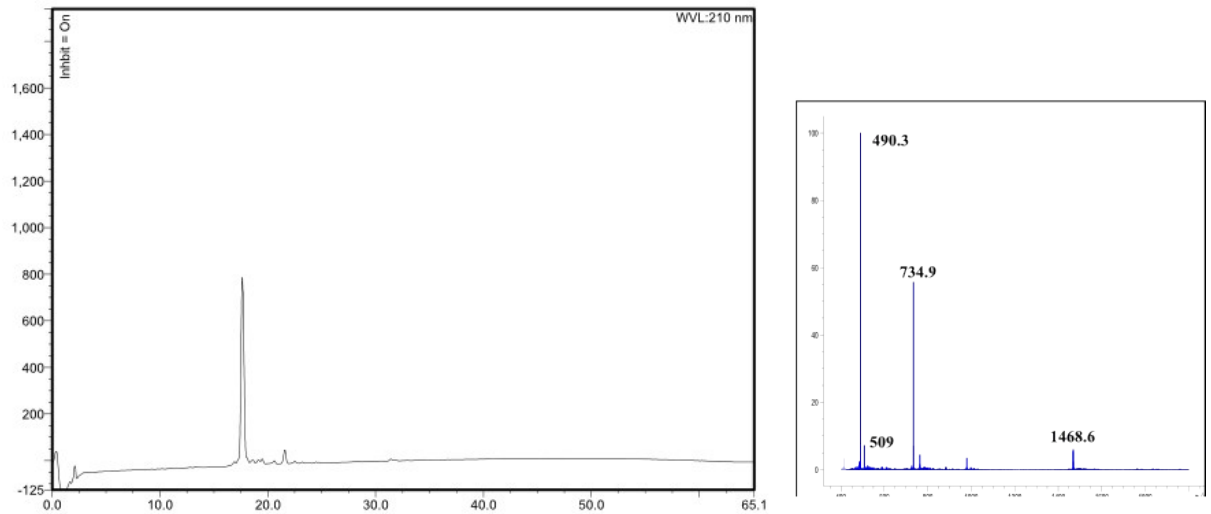
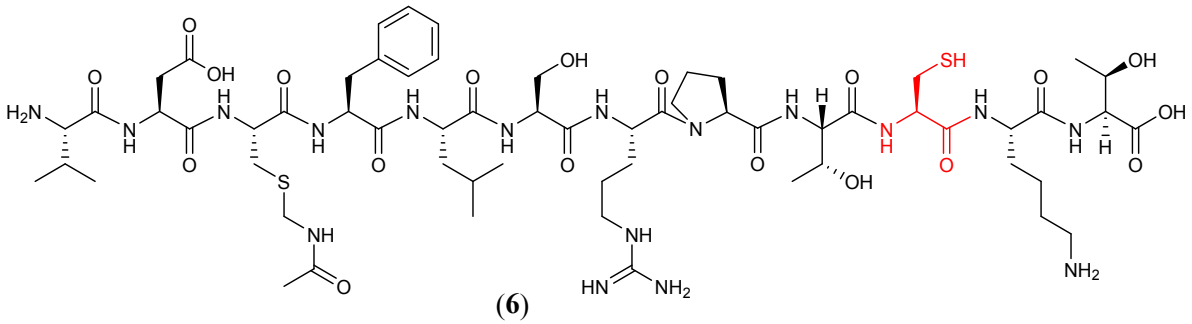


Figure S5. HPLC of crude peptide monitored at 210 nm and observed ESI-MS of crude peptide (**5**). Observed ESI-MS of the main peak for crude peptide (**5**), calcd. Mass: 1468.5, found 1468.6 $[M+1H]^{+1}$, 734.9 $[M+2H]^{+2}$, 490.3 $[M+3H]^{+3}$, where 509.0 $[M+3H]^{+3}$ corresponds to +56 Da due to the presence of *tert*-butyl adduct. Deconvolution affords a mass of 1473.8 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 µ; 4.6 x 150 mm) and a linear gradient of 5-95% B in 60 min, *ca*. 1% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

3.06 Synthesis of peptide scaffold (6) : H_2N-VDC(Acm)FLSRPTCKT-OH



(**6**)

Fmoc-Thr(ᵗBu)-HMPP resin **S2** was prepared using **Method 1** which was followed by peptide elongation on resin using **Method 3**. Fmoc-Cys(Trt)-OH and Fmoc-Cys(Acm)-OH were coupled to peptidyl-resin using **Method 4**. Upon the completion of peptide synthesis, the peptidyl-resin was cleaved using **Method 5** to afford crude peptide (**6**) (137 mg) which was used in the next step without further purification (**Figure S6**).

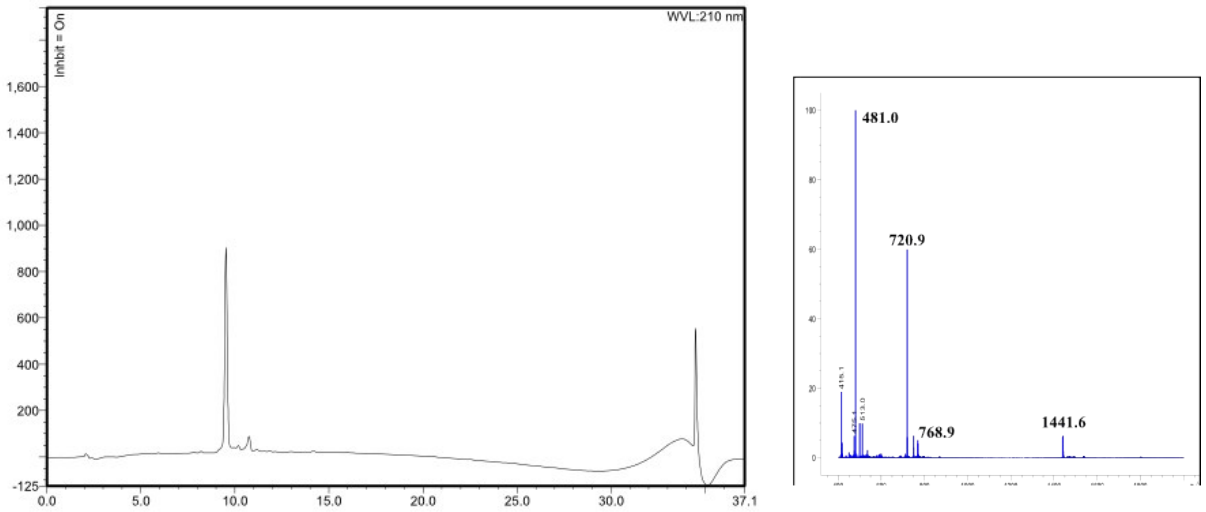
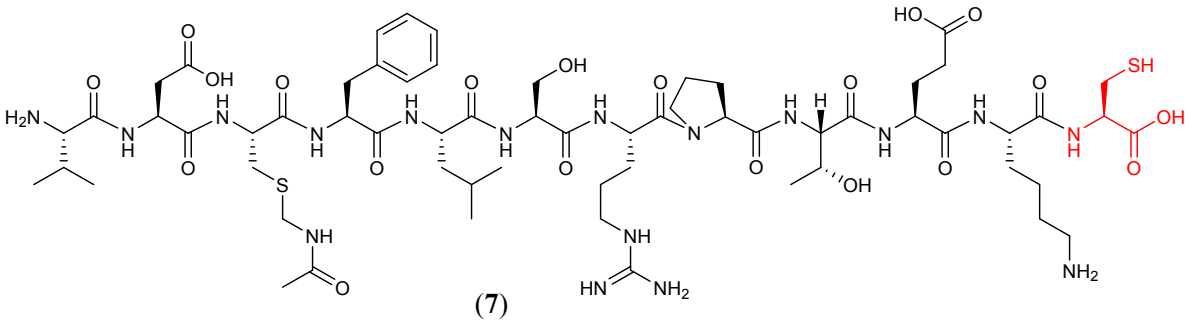


Figure S6. HPLC of crude peptide monitored at 210 nm and observed ESI-MS of crude peptide (**6**). Observed ESI-MS of the main peak for crude peptide (**6**), calcd. Mass: 1440.5, found 1441.6 $[M+1H]^{+1}$, 720.9 $[M+2H]^{+2}$, 481.0 $[M+3H]^{+3}$, where 768.9 $[M+2H]^{+2}$ corresponds to +96 Da due to the presence of trifluoroacetate adduct. Deconvolution affords a mass of 1440.1 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 µ; 4.6 x 150 mm) and a linear gradient of 5-35% B in 30 min, *ca*. 1% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

3.07 Synthesis of peptide scaffold (7) : H_2N-VDC(Acm)FLSRPTEKC-OH



(**7**)

Fmoc-Cys(Trt)-2-chlorotrityl polystyrene resin **S4** was prepared using **Method 2** which followed by peptide elongation on resin using **Method 3**. The resin was taken out from Tribute® automated peptide synthesizer for the coupling of Fmoc-Cys(Acm)-OH using **Method 4**. Upon the completion of peptide synthesis, the peptidyl-resin was taken out and cleaved using **Method 5** to afford crude peptide (**7**) (132 mg) which was used in the next step without further purification (**Figure S7**).

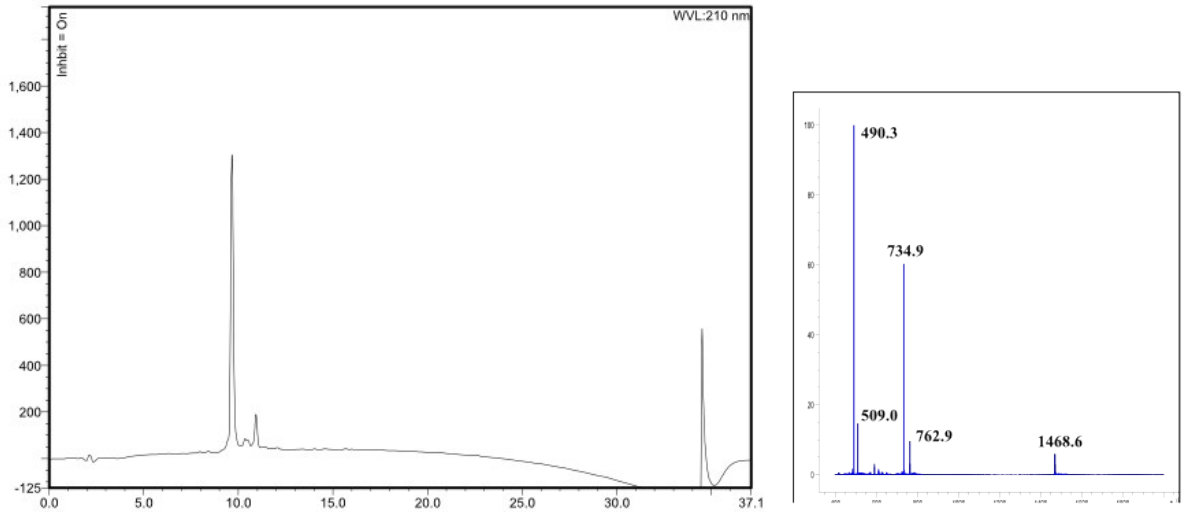
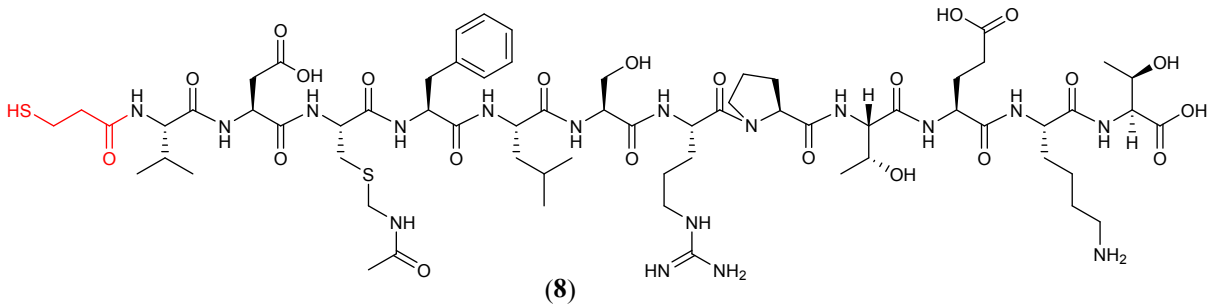


Figure S7. HPLC of crude peptide monitored at 210 nm and observed ESI-MS of crude peptide (**7**). Observed ESI-MS of the main peak for crude peptide (**7**), calcd. Mass: 1468.5, found 1468.6 $[M+1H]^{+1}$, 734.9 $[M+2H]^{+2}$, 490.3 $[M+3H]^{+3}$, where 762.9 $[M+2H]^{+2}$ and 509.3 $[M+3H]^{+3}$ corresponds to +56 Da due to the presence of *tert*-butyl adduct. Deconvolution affords a mass of 1467.8 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 µ; 4.6 x 150 mm) and a linear gradient of 5-35% B in 30 min, *ca*. 1% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

3.08 Synthesis of peptide scaffold (8) : $HS(CH_2)_2CO$-VDC(Acm)FLSRPTEKT-OH



(**8**)

Fmoc-Thr(ᵗBu)-HMPP resin **S2** was prepared using **Method 1** which followed by peptide elongation on resin using **Method 3**. The resin was taken out from Tribute® automated peptide synthesizer for the coupling of Fmoc-Cys(Acm)-OH using **Method 4**. Upon the completion of peptide synthesis, the peptidyl-resin was taken out and cleaved using **Method 5** to afford crude peptide (**8**) (142 mg) which was used in the next step without further purification. (**Figure S8**).

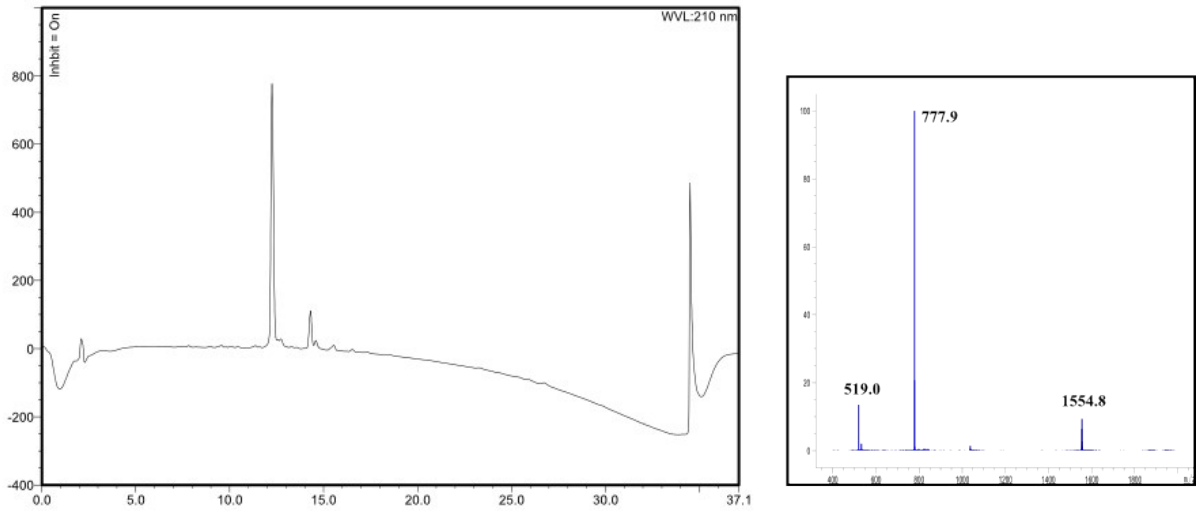


Figure S8. HPLC of crude peptide monitored at 210nm and observed ESI-MS of crude peptide (**8**). Observed ESI-MS of the main peak for crude peptide (**8**), calcd. Mass: 1554.7, found 1554.8 $[M+1H]^{+1}$, 777.9 $[M+2H]^{+2}$, 519.0 $[M+3H]^{+3}$. Deconvolution affords a mass of 1553.9 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 μ; 4.6 x 150 mm) and a linear gradient of 5-35% B in 30 min, *ca*. 1% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

4. General Procedure for CLipPA (Cysteine Lipidation on a Peptide or Amino acid) and removal of Acm protecting group

Method 6: General procedure for CLipPA reaction of peptide scaffolds (2)-(8)

Peptide (c = 10 mM, 3.0 μmol, Vol_{total} = 300 μL) was dissolved in either **Stock solution 1** or **Stock solution 2** followed by addition of **Stock solution 3** and the addition of trifluoroacetic acid (5% *v*/*v* of total final volume, Vol = 15 μL). The reaction mixture was irradiated at wavelength of 365 nm using a UV lamp at room temperature before a sample was taken for LC-MS analysis at 30 minute intervals. An analytical sample was prepared by dilution with NMP and analyzed using an analytical Gemini C-18 column (Phenomenex®, 5 μ, 4.6 x 150 mm) at 1.0 mL/min. Upon completion of the reaction, peptide was precipitated by addition of cold diethyl ether and recovered by centrifugation, followed by two times diethyl ether washing. Peptide was dissolved with acetonitrile:water (1:1 *v*/*v*) containing 0.1% TFA and lyophilized. The crude peptide used to the next step without further purification.

Peptide concentration = 10 mM (3.0 μmol, Vol_{total} = 300 μL)
The total final volume to make 10 mM peptide concentration refers to inclusion of volume used for NMP, reagents and trifluoroacetic acid.

Stock solution 1: solid vinyl ester used
DMPA (3 eq., 9.0 μmol, 2.3 mg) in degassed NMP (100 μL)
Vinyl ester (70 eq., 210 μmol) in degassed NMP (91 μL)

Stock solution 2: liquid vinyl ester used
DMPA (3 eq., 9.0 μmol, 2.3 mg) in degassed NMP (191 μL – Volume for vinyl ester)
Vinyl ester (70 eq., 210 μmol) added as a neat solution

Stock solution 3:
Tert-nonylmercaptan (80 eq., 240 μmol, Vol = 45 μL)
Ttriisopropylsilane (80 eq., 240 μmol, Vol = 49 μL)

Examples:
For the preparation of reaction mixture where solid vinyl ester used:
300 μL = 100 μL NMP + 91 μL NMP + 45 μL thiol + 49 μL TIPS + 15 μL TFA

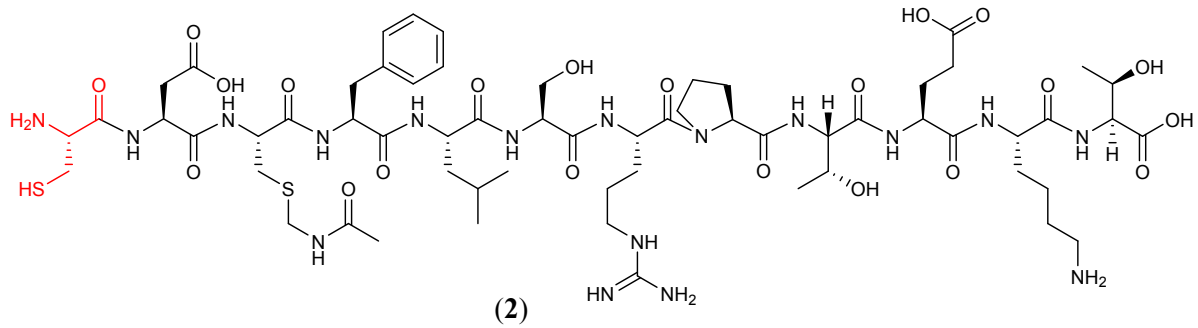
For the preparation of reaction mixture where liquid vinyl ester used:
300 μL = volume for vinyl ester + (191 μL – Volume for vinyl ester) + 45 μL thiol + 49 μL TIPS + 15 μL TFA

Method 7: Removal of acetamidomethyl (Acm) protecting group of sulfhydryl side chain of cysteine residue

Crude Acm protected peptide was dissolved in acetonitrile:water (1:1 *v*/*v*) containing 0.1% TFA (1.0 mM) followed by addition of AgOAc (50 molar equiv. per Acm protecting group). The reaction mixture was left overnight with agitation at room temperature. To quench unreacted amount of AgOAc, dithiothreitol (300 molar equivalents per Acm protecting group) was added in 6.0 M guanidine hydrochloride (5 mL) and the reaction mixture left for 1 h with agitation at room temperature. The peptide solution was diluted with water containing 0.1% TFA to give approx. 0.5 to 1.0 M final concentration of guanidine hydrochloride and filtered through using a Phenomenex® RC membrane filter (26 mm, 0.45 μm). The collected peptide solution was lyophilized and then purified by RP-HPLC.

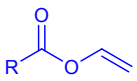
5. Synthesis of *S*-Lipopeptide Analogues (9), (16), (23), (30), (37) and (44) using peptide scaffold (2)

S-Lipopeptide analogues (**9**), (**16**), (**23**), (**30**), (**37**) and (**44**) were prepared using CLipPA reaction with peptide scaffold (**2**) and vinyl esters using **Method 6** followed by Acm removal of ^{3}Cys residue using **Method 7**.

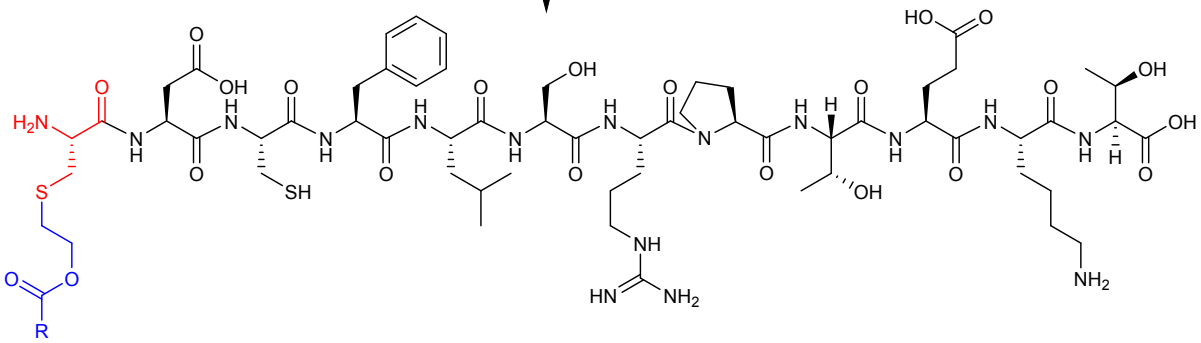


(**2**)

i) CLipPA



ii) Acm removal



(**9**) R = propyl
(**16**) R = nonyl
(**23**) R = pentadecyl
(**30**) R = heptadecyl
(**37**) R = *tert*-butyl
(**44**) R = 4-*tert*-butylphenyl

5.01 Synthesis of *S*-lipopeptide (9)

Peptide scaffold (**2**) (10 mg, 6.8 µmol) was used to obtain *S*-lipopeptide (**9**) (6.4 mg, 62% yield).

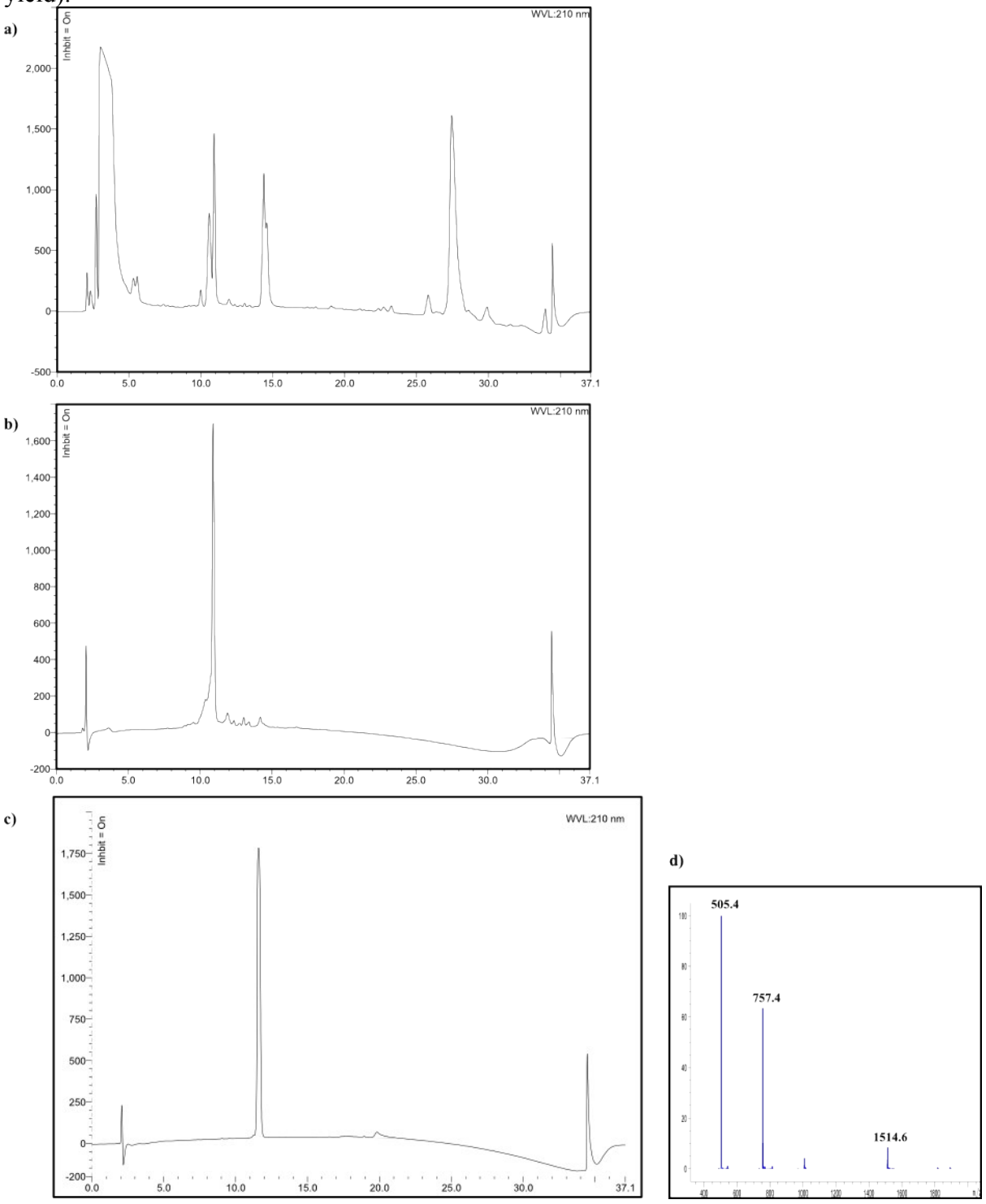


Figure S9. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**9**). a) HPLC chromatogram of CLipPA reaction monitored at 214 nm at t = 60 min 365 nm UV irradiation; b) HPLC chromatogram of reaction mixture monitored at 214 nm after diethyl ether work-up; c) HPLC chromatogram of purified peptide (**9**), after Acm removal, monitored at 214 nm; d) Observed ESI-MS for peptide (**9**), calcd. Mass: 1513.8, found 1514.6 $[M+1H]^{+1}$, 757.4 $[M+2H]^{+2}$, 505.4 $[M+3H]^{+3}$. Deconvolution affords a mass of 1513.2 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 µ; 4.6 x 150 mm) and a linear gradient of 5-95% B in 30 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

5.02 Synthesis of *S*-lipopeptide (16)

Peptide scaffold (**2**) (10 mg, 6.8 µmol) was used to obtain *S*-lipopeptide (**16**) (7.0 mg, 64% yield).

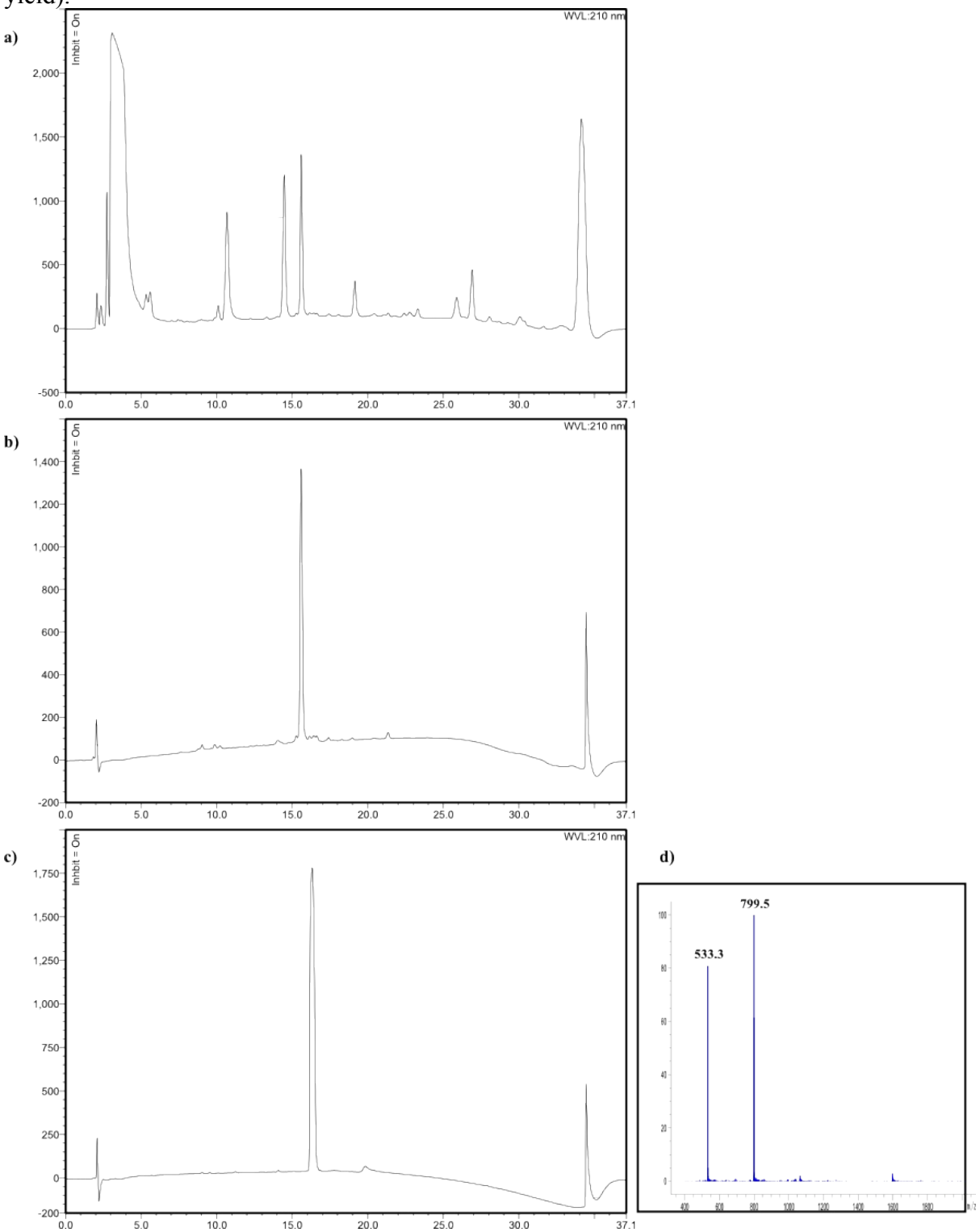


Figure S10. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**16**). a) HPLC chromatogram of CLipPA reaction monitored at 214 nm at t = 60 min 365 nm UV irradiation; b) HPLC chromatogram of reaction mixture monitored at 214 nm after diethyl ether work-up; c) HPLC chromatogram of purified peptide (**16**), after Acm removal, monitored at 214 nm; d) Observed ESI-MS for peptide (**16**), calcd. Mass: 1597.9, found 799.5 $[M+2H]^{+2}$, 533.3 $[M+3H]^{+3}$. Deconvolution affords a mass of 1596.9 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 µ; 4.6 x 150 mm) and a linear gradient of 5-95% B in 30 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

5.03 Synthesis of *S*-lipopeptide (23)

Peptide scaffold (**2**) (10 mg, 6.8 µmol) was used to obtain *S*-lipopeptide (**23**) (6.3 mg, 55% yield).

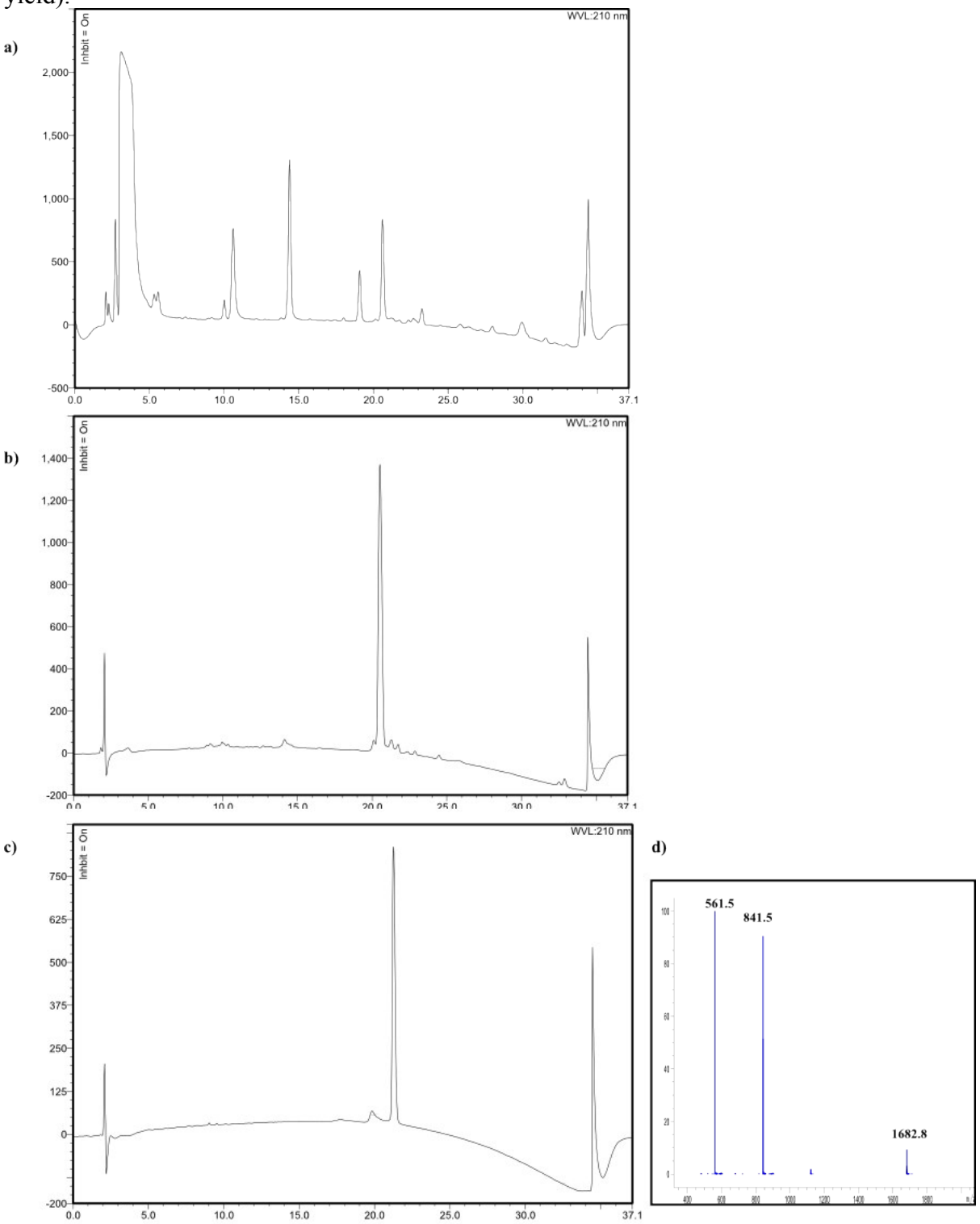


Figure S11. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**23**). a) HPLC chromatogram of CLipPA reaction monitored at 214 nm at t = 60 min 365 nm UV irradiation; b) HPLC chromatogram of reaction mixture monitored at 214 nm after diethyl ether work-up; c) HPLC chromatogram of purified peptide (**23**), after Acm removal, monitored at 214 nm; d) Observed ESI-MS for peptide (**23**), calcd. Mass: 1682.1, found 1682.8 $[M+1H]^{+1}$, 841.5 $[M+2H]^{+2}$, 561.5 $[M+3H]^{+3}$. Deconvolution affords a mass of 1681.4 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 µ; 4.6 x 150 mm) and a linear gradient of 5-95% B in 30 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

5.04 Synthesis of *S*-lipopeptide (30)

Peptide scaffold (**2**) (10 mg, 6.8 µmol) was used to obtain *S*-lipopeptide (**30**) (6.2 mg, 53% yield).

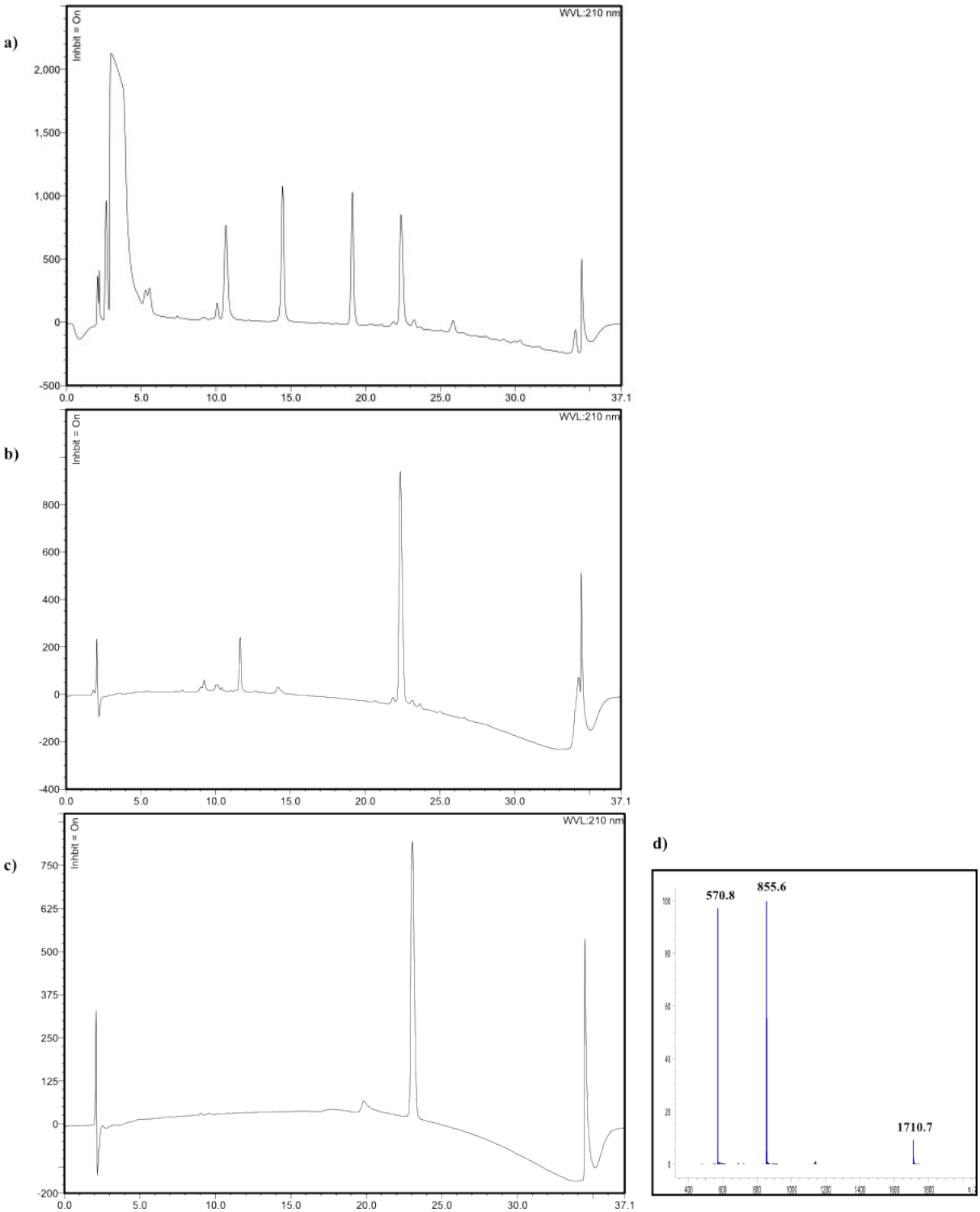


Figure S12. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**30**). a) HPLC chromatogram of CLipPA reaction monitored at 214 nm at t = 60 min 365 nm UV irradiation; b) HPLC chromatogram of reaction mixture monitored at 214 nm after diethyl ether work-up; c) HPLC chromatogram of purified peptide (**30**), after Acm removal, monitored at 214 nm; d) Observed ESI-MS for peptide (**30**), calcd. Mass: 1710.1, found 1710.7 $[M+1H]^{+1}$, 855.6 $[M+2H]^{+2}$, 570.8 $[M+3H]^{+3}$. Deconvolution affords a mass of 1709.4 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 µ; 4.6 x 150 mm) and a linear gradient of 5-95% B in 30 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

5.05 Synthesis of *S*-lipopeptide (37)

Peptide scaffold (**2**) (10 mg, 6.8 µmol) was used to obtain *S*-lipopeptide (**37**) (5.7 mg, 55% yield).

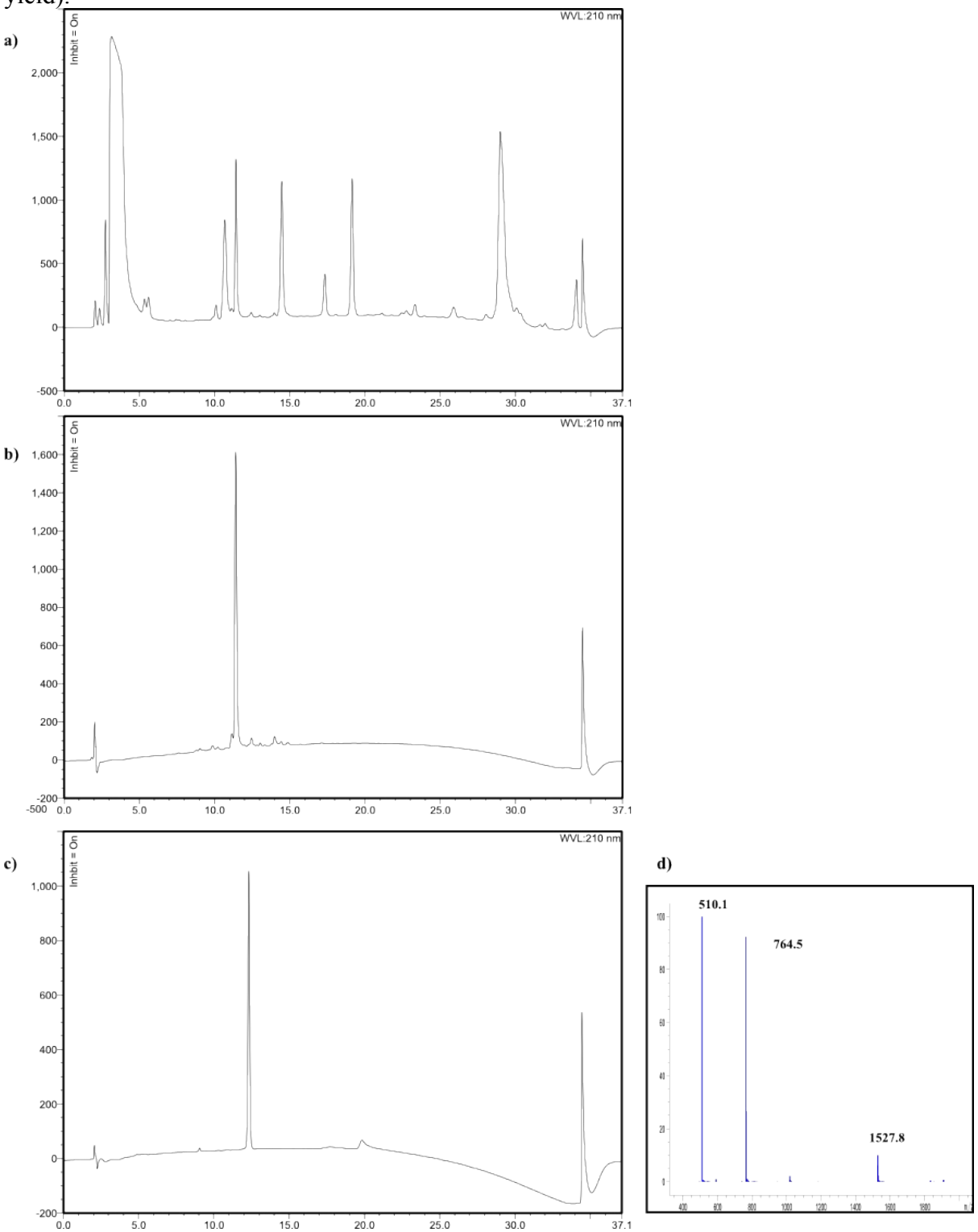


Figure 13. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**37**). a) HPLC chromatogram of CLipPA reaction monitored at 214 nm at t = 60 min 365 nm UV irradiation; b) HPLC chromatogram of reaction mixture monitored at 214 nm after diethyl ether work-up; c) HPLC chromatogram of purified peptide (**37**), after Acm removal, monitored at 214 nm; d) Observed ESI-MS for peptide (**37**), calcd. Mass: 1527.8, found 1527.8 $[M+1H]^{+1}$, 764.5 $[M+2H]^{+2}$, 510.1 $[M+3H]^{+3}$. Deconvolution affords a mass of 1527.0 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 µ; 4.6 x 150 mm) and a linear gradient of 5-95% B in 30 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

5.06 Synthesis of *S*-lipopeptide (44)

Peptide scaffold (**2**) (10 mg, 6.8 µmol) was used to obtain *S*-lipopeptide (**44**) (6.3 mg, 58% yield).

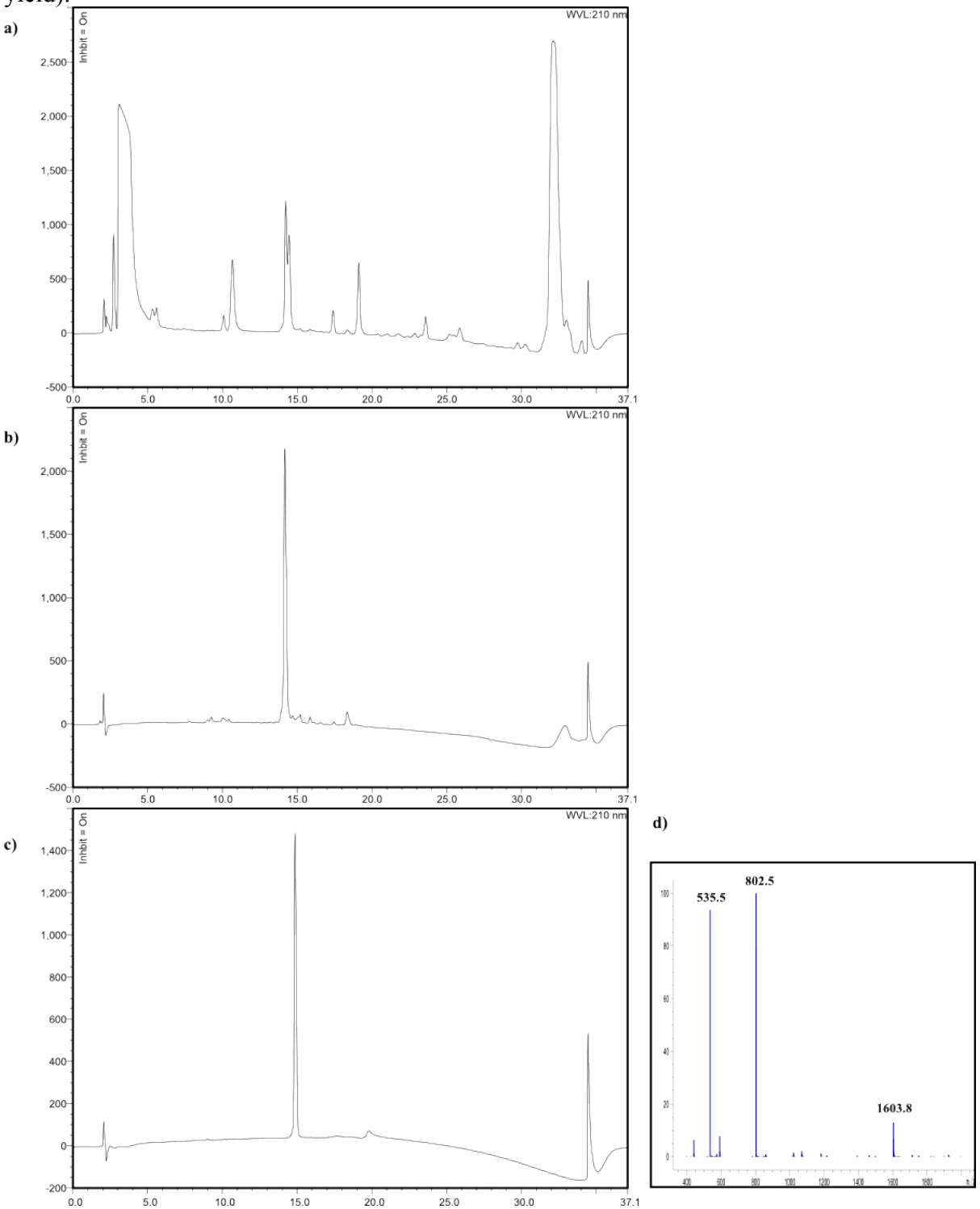
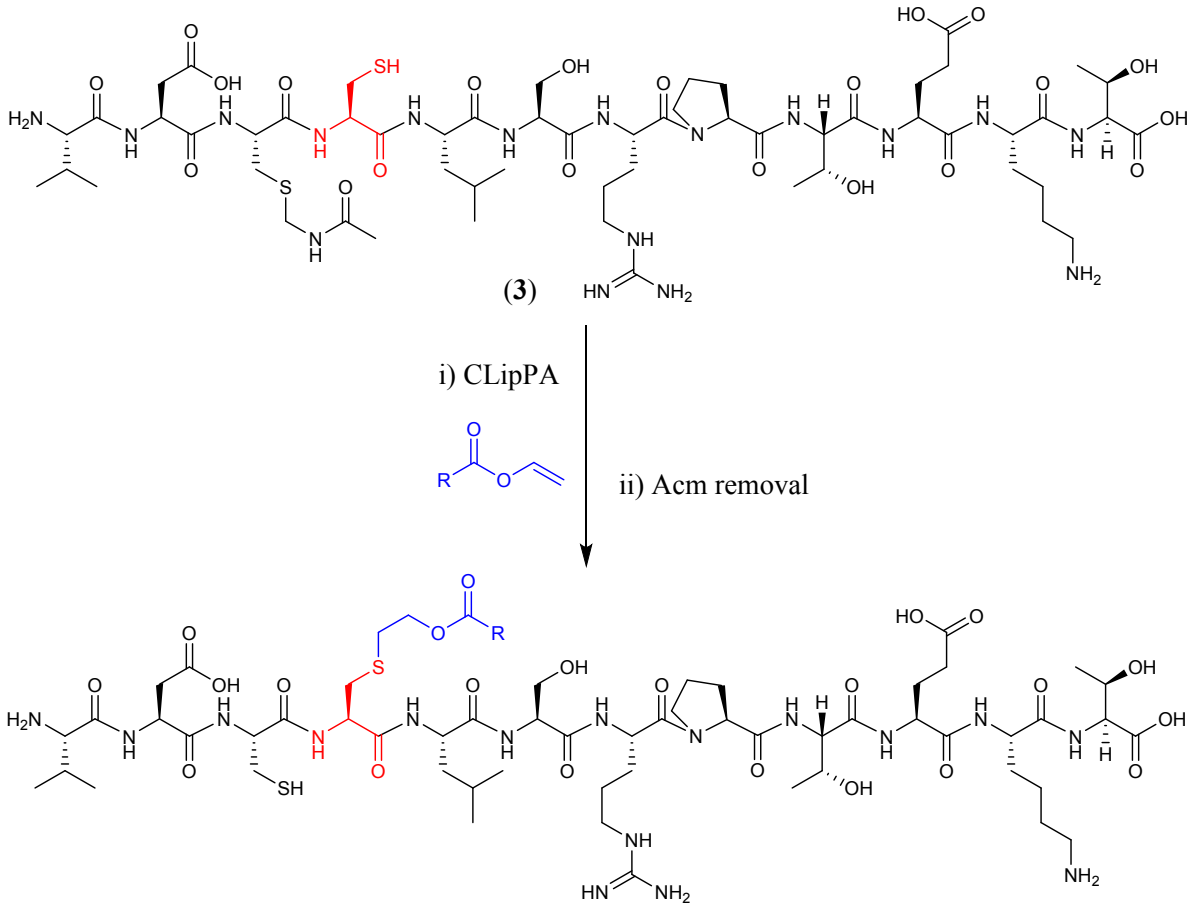


Figure S14. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**44**). a) HPLC chromatogram of CLipPA reaction monitored at 214 nm at t = 60 min 365 nm UV irradiation; b) HPLC chromatogram of reaction mixture monitored at 214 nm after diethyl ether work-up; c) HPLC chromatogram of purified peptide (**44**), after Acm removal, monitored at 214 nm; d) Observed ESI-MS for peptide (**44**), calcd. Mass: 1603.6, found 1603.8 $[M+1H]^{+1}$, 802.5 $[M+2H]^{+2}$, 535.5 $[M+3H]^{+3}$. Deconvolution affords a mass of 1603.1 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 µ; 4.6 x 150 mm) and a linear gradient of 5-95% B in 30 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

6. Synthesis of *S*-Lipopeptide Analogues (10), (17), (24), (31), (38) and (45) using peptide scaffold (3)

S-Lipopeptide analogues (**10**), (**17**), (**24**), (**31**), (**38**) and (**45**) were prepared using CLipPA reaction with peptide scaffold (**3**) and vinyl esters using **Method 6** followed by Acm removal of ^{3}Cys residue using **Method 7**.



(**10**) R = propyl
(**17**) R = nonyl
(**24**) R = pentadecyl
(**31**) R = heptadecyl
(**38**) R = *tert*-butyl
(**45**) R = 4-*tert*-butylphenyl

6.01 Synthesis of *S*-lipopeptide (10)

Peptide scaffold (**3**) (10 mg, 7.03 µmol) was used to obtain *S*-lipopeptide (**10**) (6.7 mg, 65% yield).

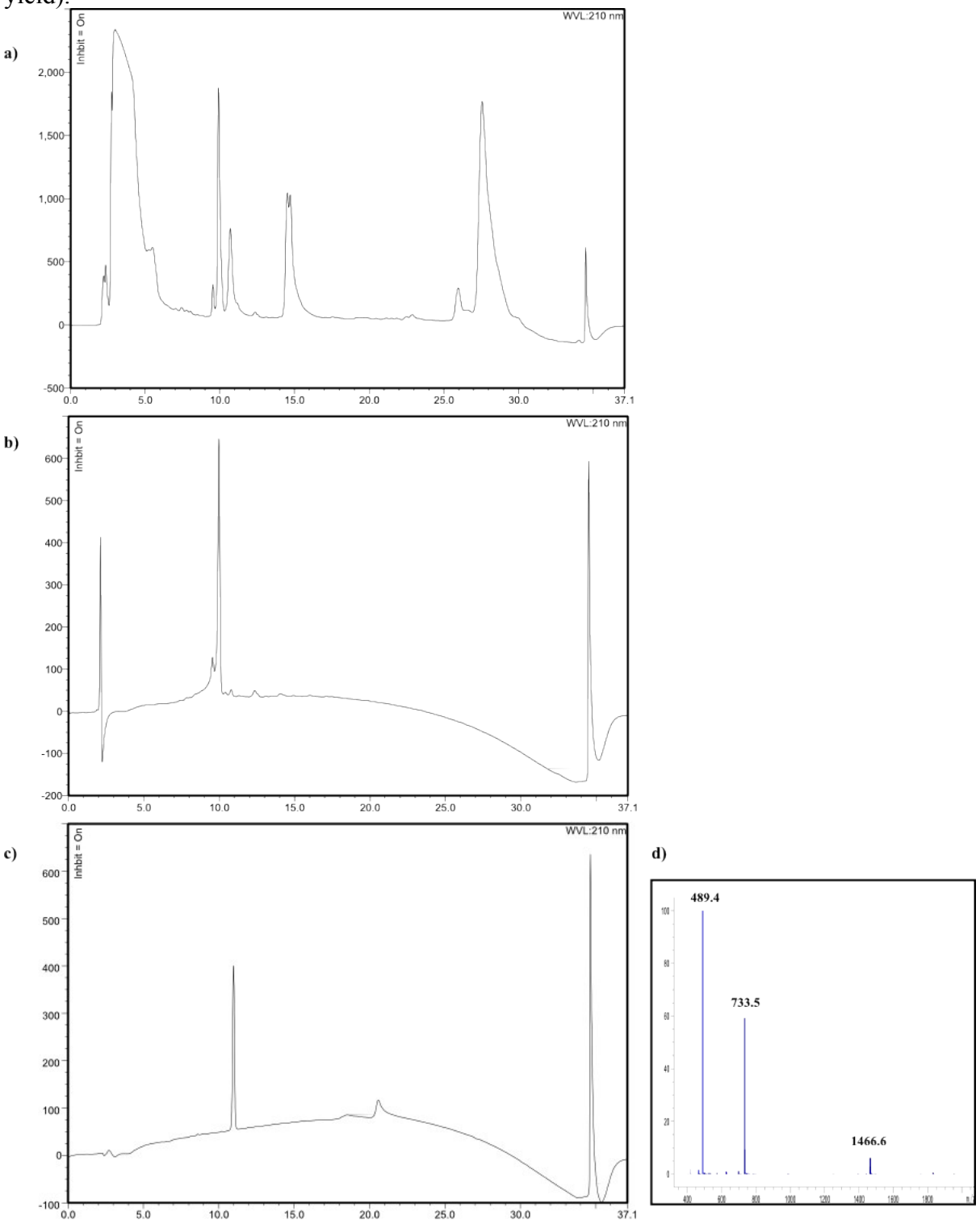


Figure S15. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**10**). a) HPLC chromatogram of CLipPA reaction monitored at 214 nm at t = 60 min 365 nm UV irradiation; b) HPLC chromatogram of reaction mixture monitored at 214 nm after diethyl ether work-up; c) HPLC chromatogram of purified peptide (**10**), after Acm removal, monitored at 214 nm; d) Observed ESI-MS for peptide (**10**), calcd. Mass: 1465.7, found 1466.66$[M+1H]^{+1}$, 733.5 $[M+2H]^{+2}$, 489.4 $[M+3H]^{+3}$. Deconvolution affords a mass of 1465.3 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 µ; 4.6 x 150 mm) and a linear gradient of 5-95% B in 30 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

6.02 Synthesis of *S*-lipopeptide (17)

Peptide scaffold (**3**) (10 mg, 7.03 µmol) was used to obtain *S*-lipopeptide (**17**) (6.9 mg, 63% yield).

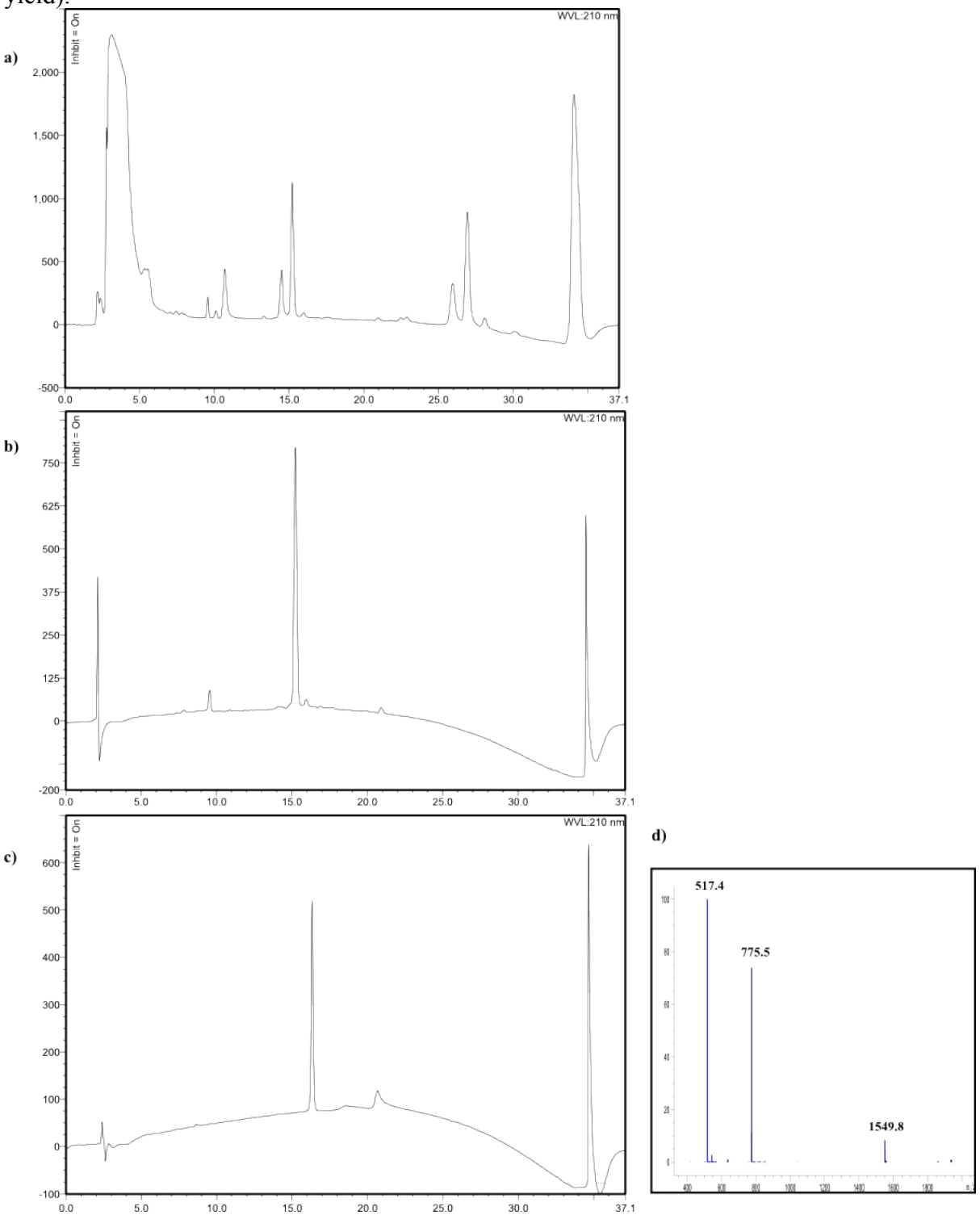


Figure S16. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**17**). a) HPLC chromatogram of CLipPA reaction monitored at 214 nm at t = 60 min 365 nm UV irradiation; b) HPLC chromatogram of reaction mixture monitored at 214 nm after diethyl ether work-up; c) HPLC chromatogram of purified peptide (**17**), after Acm removal, monitored at 214 nm; d) Observed ESI-MS for peptide (**17**), calcd. Mass: 1549.9, found 1549.8 $[M+1H]^{+1}$, 775.5 $[M+2H]^{+2}$, 517.4 $[M+3H]^{+3}$. Deconvolution affords a mass of 1549.0 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 µ; 4.6 x 150 mm) and a linear gradient of 5-95% B in 30 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

6.03 Synthesis of *S*-lipopeptide (24)

Peptide scaffold (**3**) (10 mg, 7.03 µmol) was used to obtain *S*-lipopeptide (**24**) (7.1 mg, 62% yield).

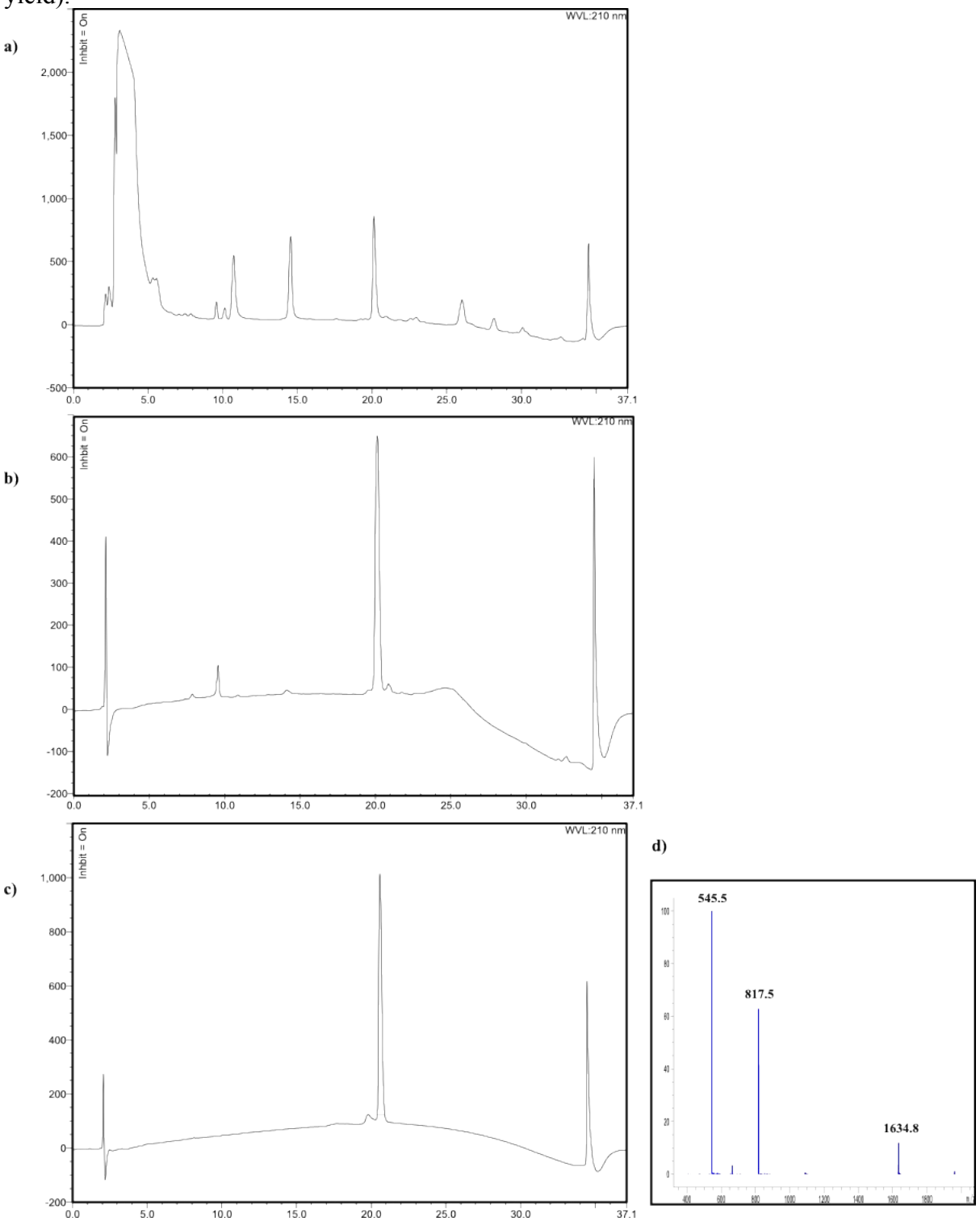


Figure S17. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**24**). a) HPLC chromatogram of CLipPA reaction monitored at 214 nm at t = 60 min 365 nm UV irradiation; b) HPLC chromatogram of reaction mixture monitored at 214 nm after diethyl ether work-up; c) HPLC chromatogram of purified peptide (**24**), after Acm removal, monitored at 214 nm; d) Observed ESI-MS for peptide (**24**), calcd. Mass: 1634.0, found 1634.8 $[M+1H]^{+1}$, 817.5 $[M+2H]^{+2}$, 845.5 $[M+3H]^{+3}$. Deconvolution affords a mass of 1633.4 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 µ; 4.6 x 150 mm) and a linear gradient of 5-95% B in 30 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

6.04 Synthesis of *S*-lipopeptide (31)

Peptide scaffold (**3**) (10 mg, 7.03 µmol) was used to obtain *S*-lipopeptide (**31**) (5.3 mg, 45% yield).

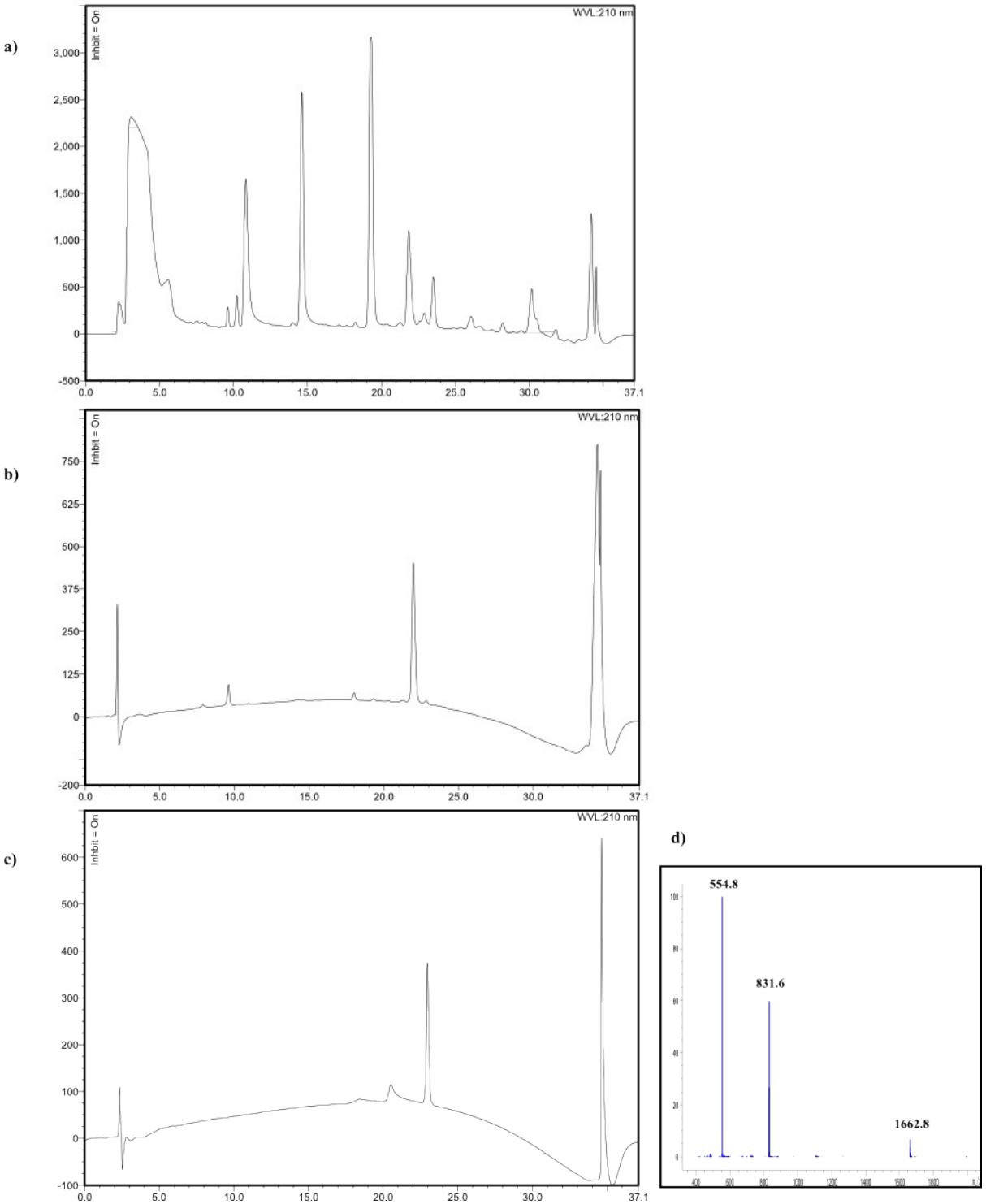


Figure S18. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**31**). a) HPLC chromatogram of CLipPA reaction monitored at 214 nm at t = 60 min 365 nm UV irradiation; b) HPLC chromatogram of reaction mixture monitored at 214 nm after diethyl ether work-up; c) HPLC chromatogram of purified peptide (**31**), after Acm removal, monitored at 214 nm; d) Observed ESI-MS for peptide (**31**), calcd. Mass: 1662.1, found 1662.8 $[M+1H]^{+1}$, 831.6 $[M+2H]^{+2}$, 554.8 $[M+3H]^{+3}$. Deconvolution affords a mass of 1661.5 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 µ; 4.6 x 150 mm) and a linear gradient of 5-95% B in 30 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

6.05 Synthesis of *S*-lipopeptide (38)

Peptide scaffold (**3**) (10 mg, 7.03 µmol) was used to obtain *S*-lipopeptide (**38**) (6.6 mg, 64% yield).

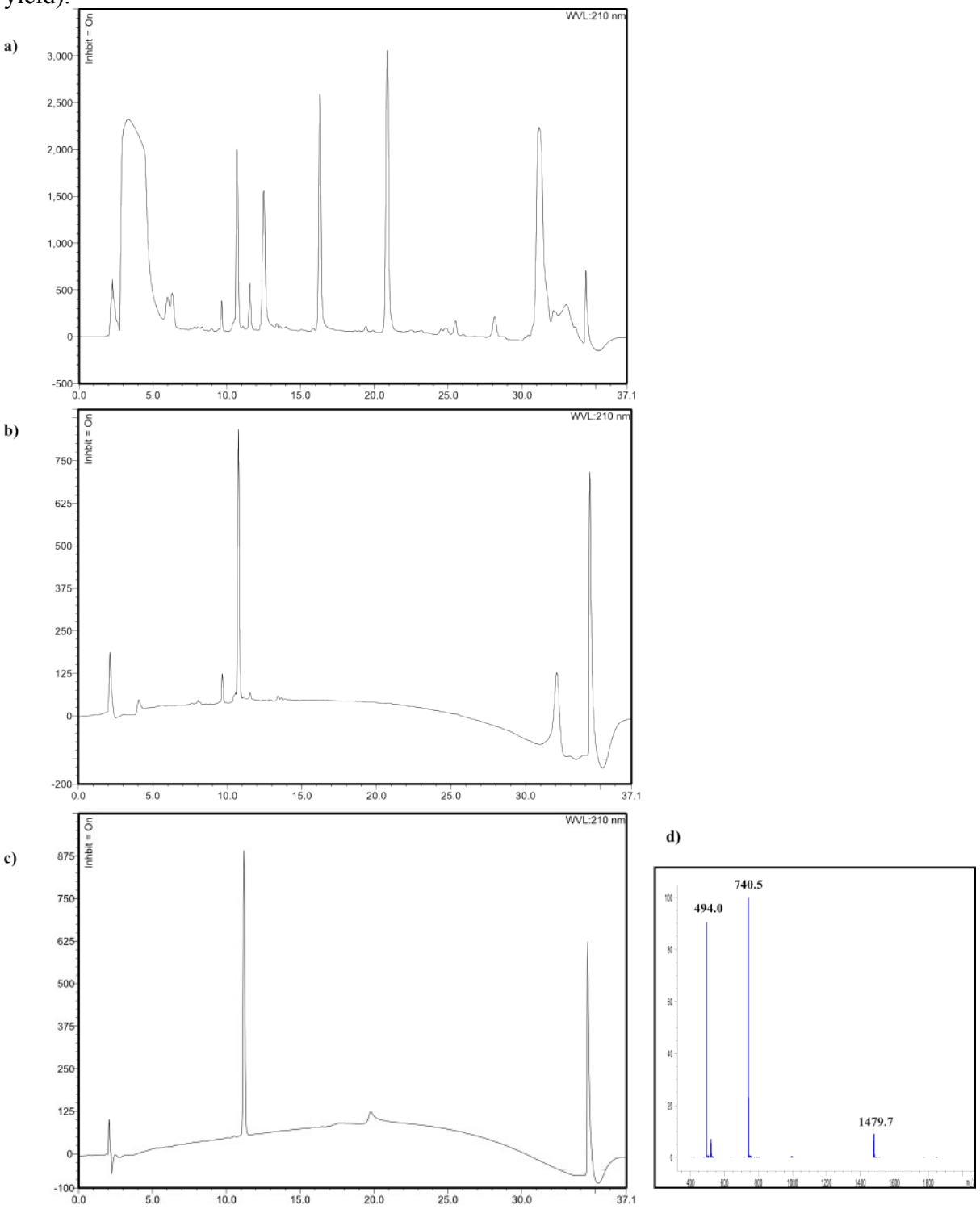


Figure S19. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**38**). a) HPLC chromatogram of CLipPA reaction monitored at 214 nm at t = 60 min 365 nm UV irradiation; b) HPLC chromatogram of reaction mixture monitored at 214 nm after diethyl ether work-up; c) HPLC chromatogram of purified peptide (**38**), after Acm removal, monitored at 214 nm; d) Observed ESI-MS for peptide (**38**), calcd. Mass: 1479.7, found 1479.7 $[M+1H]^{+1}$, 740.5 $[M+2H]^{+2}$, 494.0 $[M+3H]^{+3}$. Deconvolution affords a mass of 1478.9 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 µ; 4.6 x 150 mm) and a linear gradient of 5-95% B in 30 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

6.06 Synthesis of *S*-lipopeptide (45)

Peptide scaffold (**3**) (10 mg, 7.03 µmol) was used to obtain *S*-lipopeptide (**45**) (5.3 mg, 62% yield).

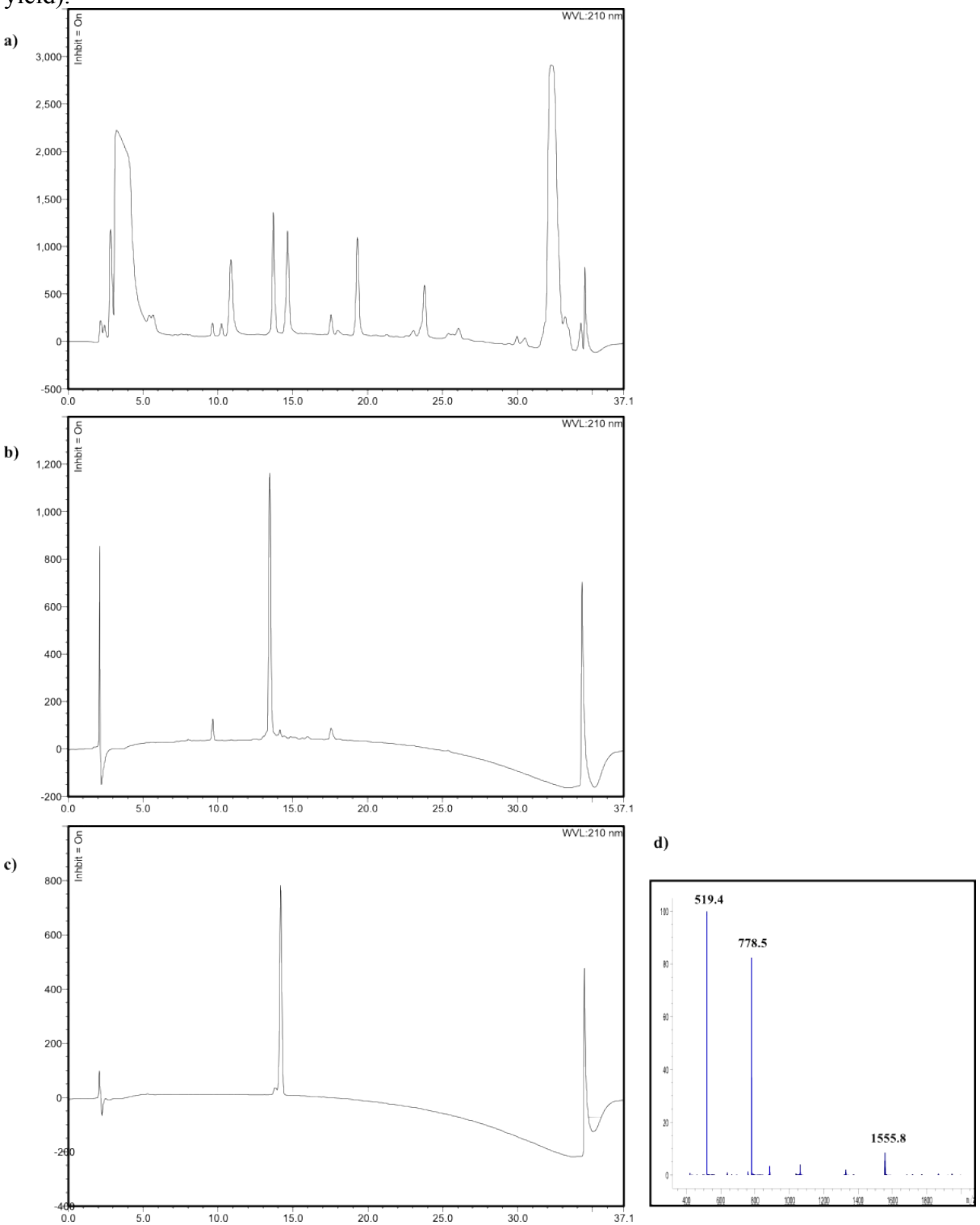
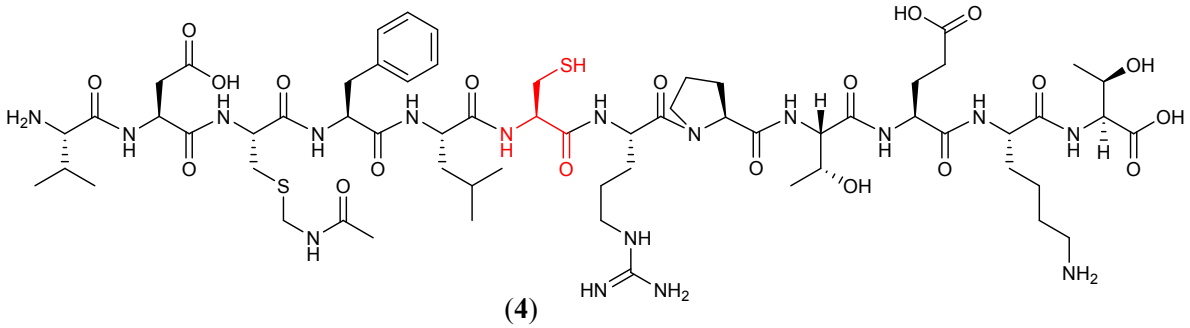


Figure S20. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**45**). a) HPLC chromatogram of CLipPA reaction monitored at 214 nm at t = 60 min 365 nm UV irradiation; b) HPLC chromatogram of reaction mixture monitored at 214 nm after diethyl ether work-up; c) HPLC chromatogram of purified peptide (**45**), after Acm removal, monitored at 214 nm; d) Observed ESI-MS for peptide (**45**), calcd. Mass: 1555.5, found 1555.8 $[M+1H]^{+1}$, 778.5 $[M+2H]^{+2}$, 519.4 $[M+3H]^{+3}$. Deconvolution affords a mass of 1555.0 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 µ; 4.6 x 150 mm) and a linear gradient of 5-95% B in 30 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

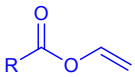
7. Synthesis of *S*-Lipopeptide Analogues (11), (18), (25), (32), (39) and (46) using peptide scaffold (4)

S-Lipopeptide analogues (**11**), (**18**), (**25**), (**32**), (**39**) and (**46**) were prepared using CLipPA reaction with peptide scaffold (**4**) and vinyl esters using **Method 6** followed by Acm removal of ^{3}Cys residue using **Method 7**.

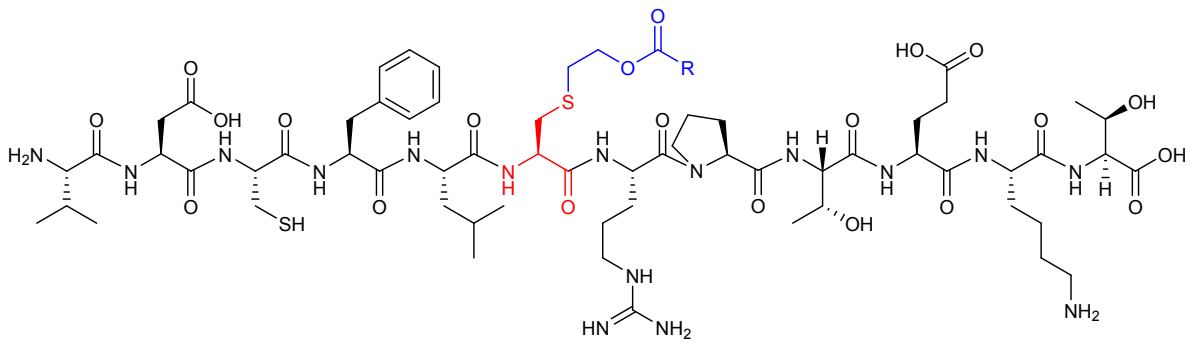


(**4**)

i) CLipPA



ii) Acm removal



(**11**) R = propyl
(**18**) R = nonyl
(**25**) R = pentadecyl
(**32**) R = heptadecyl
(**39**) R = *tert*-butyl
(**46**) R = 4-*tert*-butylphenyl

7.01 Synthesis of *S*-lipopeptide (11)

Peptide scaffold (**4**) (10 mg, 6.74 µmol) was used to obtain *S*-lipopeptide (**11**) (5.3 mg, 52% yield).

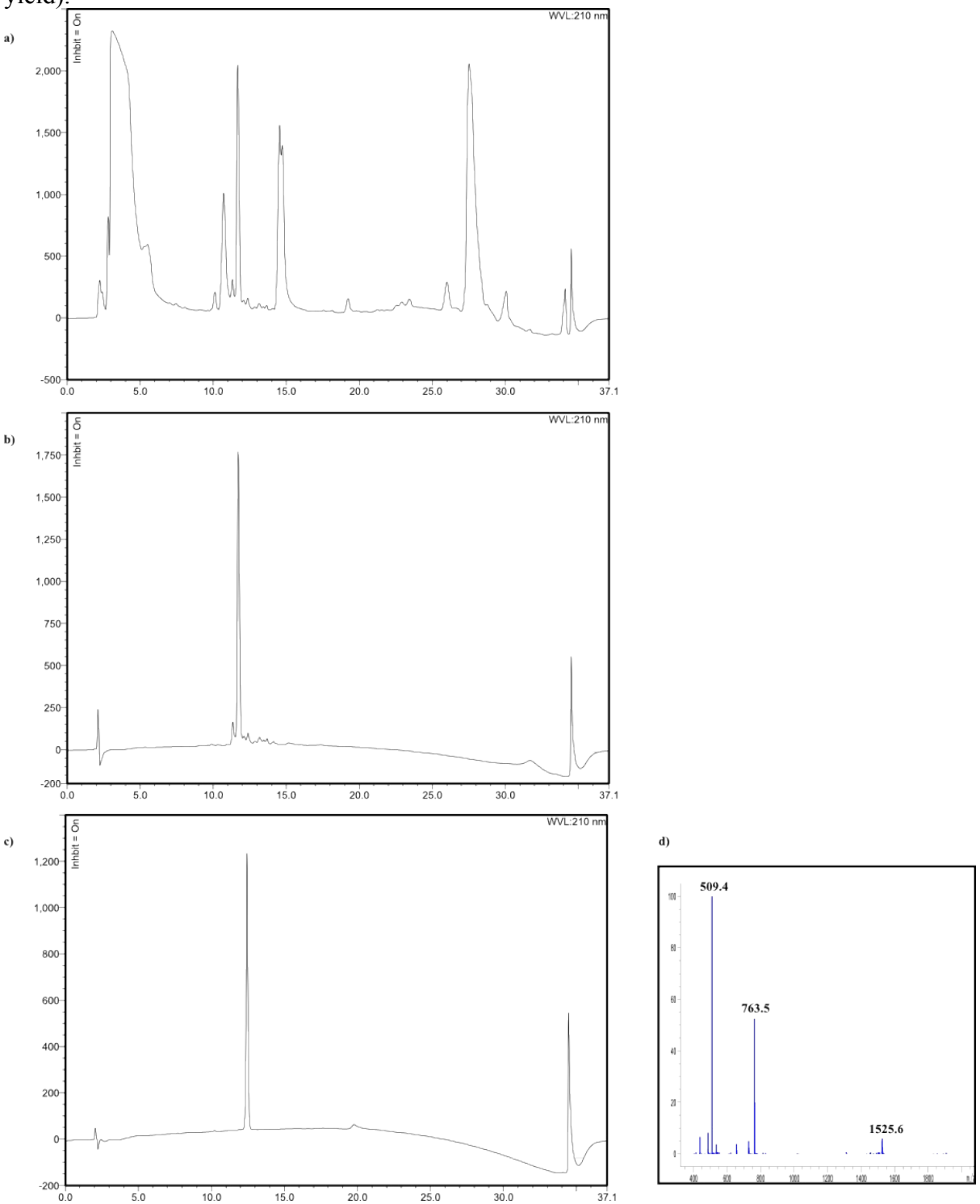


Figure S21. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**11**). a) HPLC chromatogram of CLipPA reaction monitored at 214 nm at t = 60 min 365 nm UV irradiation; b) HPLC chromatogram of reaction mixture monitored at 214 nm after diethyl ether work-up; c) HPLC chromatogram of purified peptide (**11**), after Acm removal, monitored at 214 nm; d) Observed ESI-MS for peptide (**11**), calcd. Mass: 1525.8, found 1525.6 $[M+1H]^{+1}$, 763.5 $[M+2H]^{+2}$, 509.4 $[M+3H]^{+3}$. Deconvolution affords a mass of 1524.9 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 µ; 4.6 x 150 mm) and a linear gradient of 5-95% B in 30 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

7.02 Synthesis of *S*-lipopeptide (18)

Peptide scaffold (**4**) (10 mg, 6.74 µmol) was used to obtain *S*-lipopeptide (**18**) (6.3 mg, 58% yield).

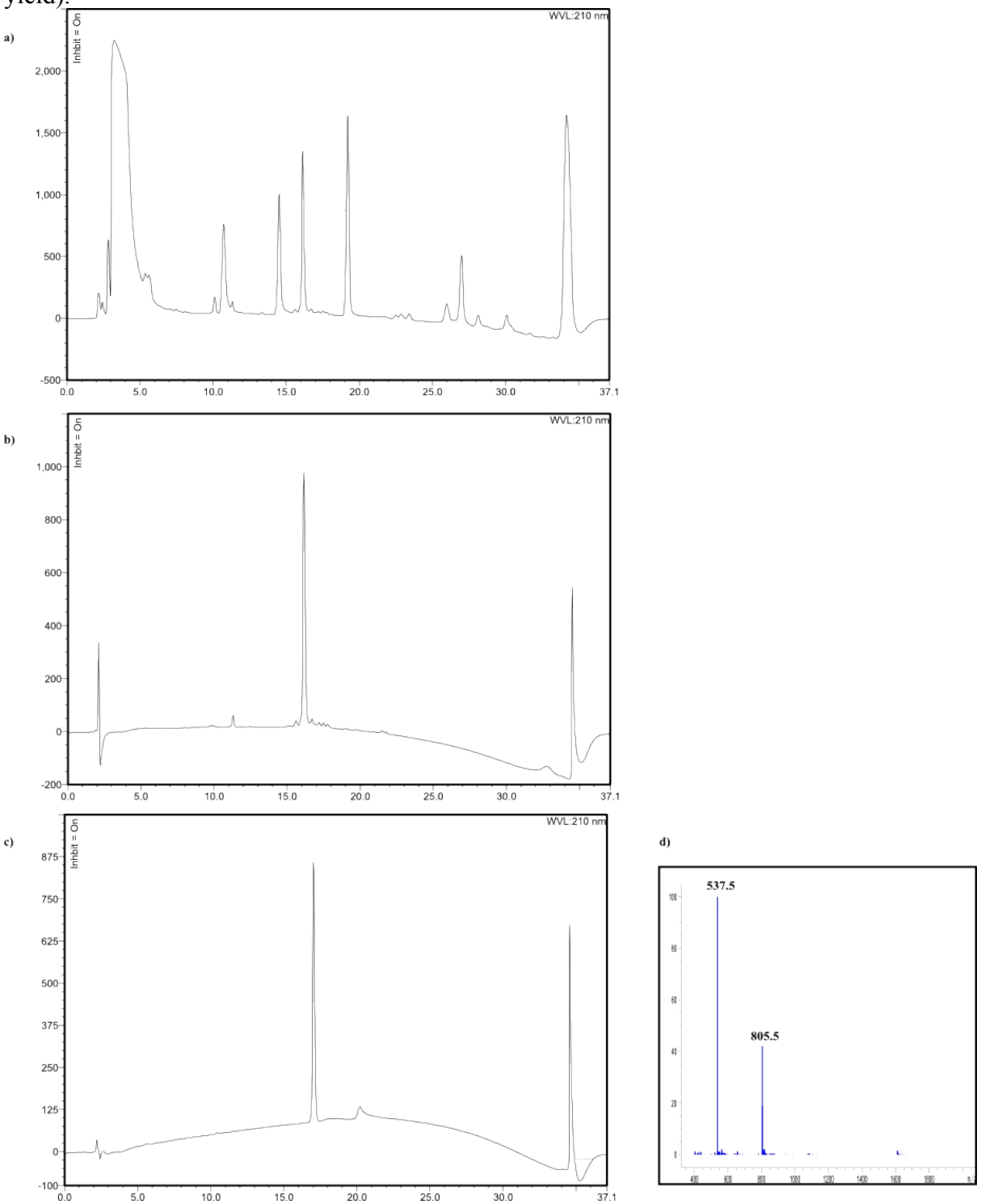


Figure S22. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**18**). a) HPLC chromatogram of CLipPA reaction monitored at 214 nm at t = 60 min 365 nm UV irradiation; b) HPLC chromatogram of reaction mixture monitored at 214 nm after diethyl ether work-up; c) HPLC chromatogram of purified peptide (**18**), after Acm removal, monitored at 214 nm; d) Observed ESI-MS for peptide (**18**), calcd. Mass: 1609.9, found 805.5 $[M+2H]^{+2}$, 537.5 $[M+3H]^{+3}$. Deconvolution affords a mass of 1609.3 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 µ; 4.6 x 150 mm) and a linear gradient of 5-95% B in 30 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

7.03 Synthesis of *S*-lipopeptide (25)

Peptide scaffold (**4**) (10 mg, 6.74 µmol) was used to obtain *S*-lipopeptide (**25**) (6.8 mg, 60% yield).

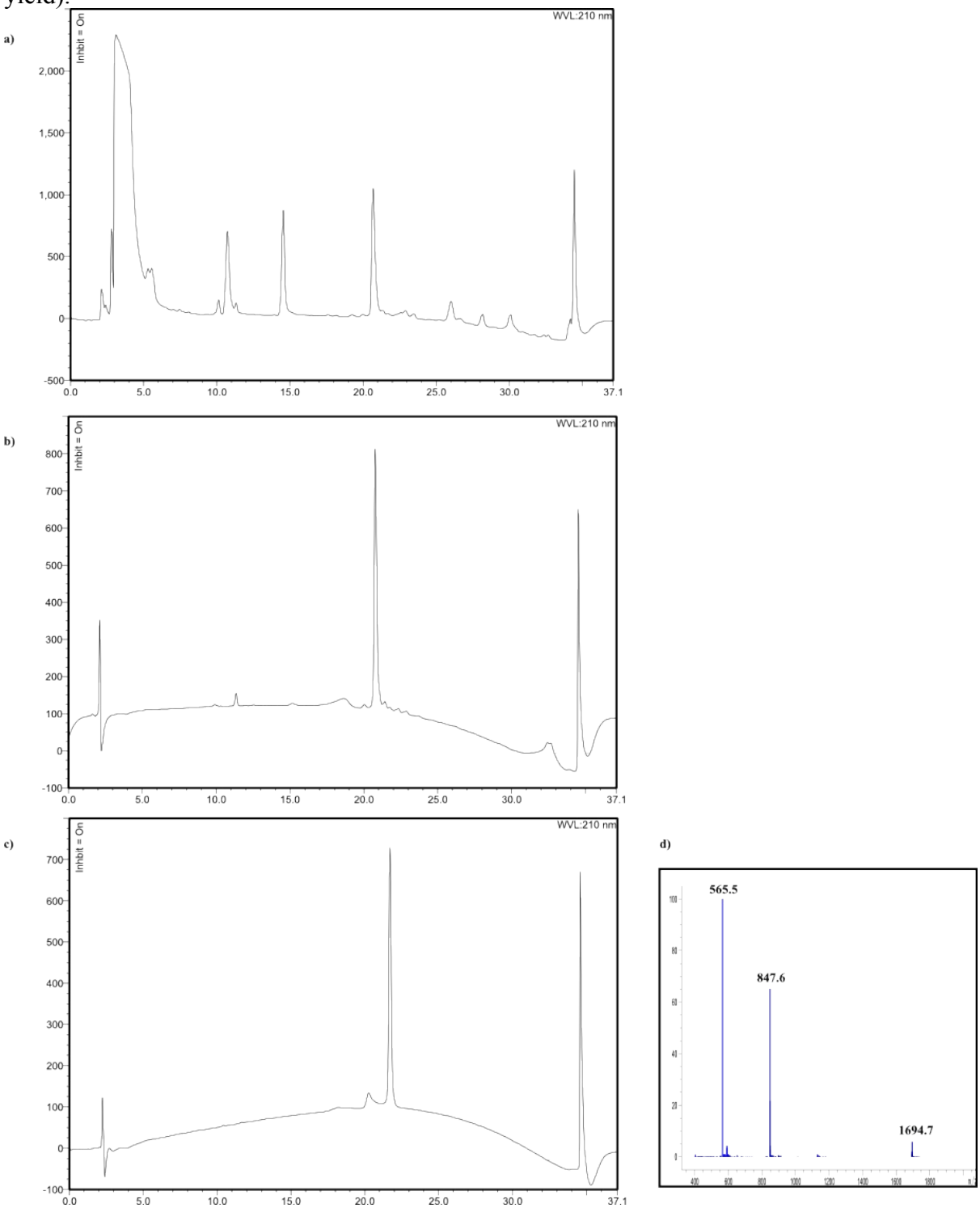


Figure S23. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**25**). a) HPLC chromatogram of CLipPA reaction monitored at 214 nm at t = 60 min 365 nm UV irradiation; b) HPLC chromatogram of reaction mixture monitored at 214 nm after diethyl ether work-up; c) HPLC chromatogram of purified peptide (**25**), after Acm removal, monitored at 214 nm; d) Observed ESI-MS for peptide (**25**), calcd. Mass: 1694.1, found 1694.7 $[M+1H]^{+1}$, 847.6 $[M+2H]^{+2}$, 565.5 $[M+3H]^{+3}$. Deconvolution affords a mass of 1693.5 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 µ; 4.6 x 150 mm) and a linear gradient of 5-95% B in 30 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

7.04 Synthesis of *S*-lipopeptide (32)

Peptide scaffold (**4**) (10 mg, 6.74 µmol) was used to obtain *S*-lipopeptide (**32**) (6.4 mg, 55% yield).

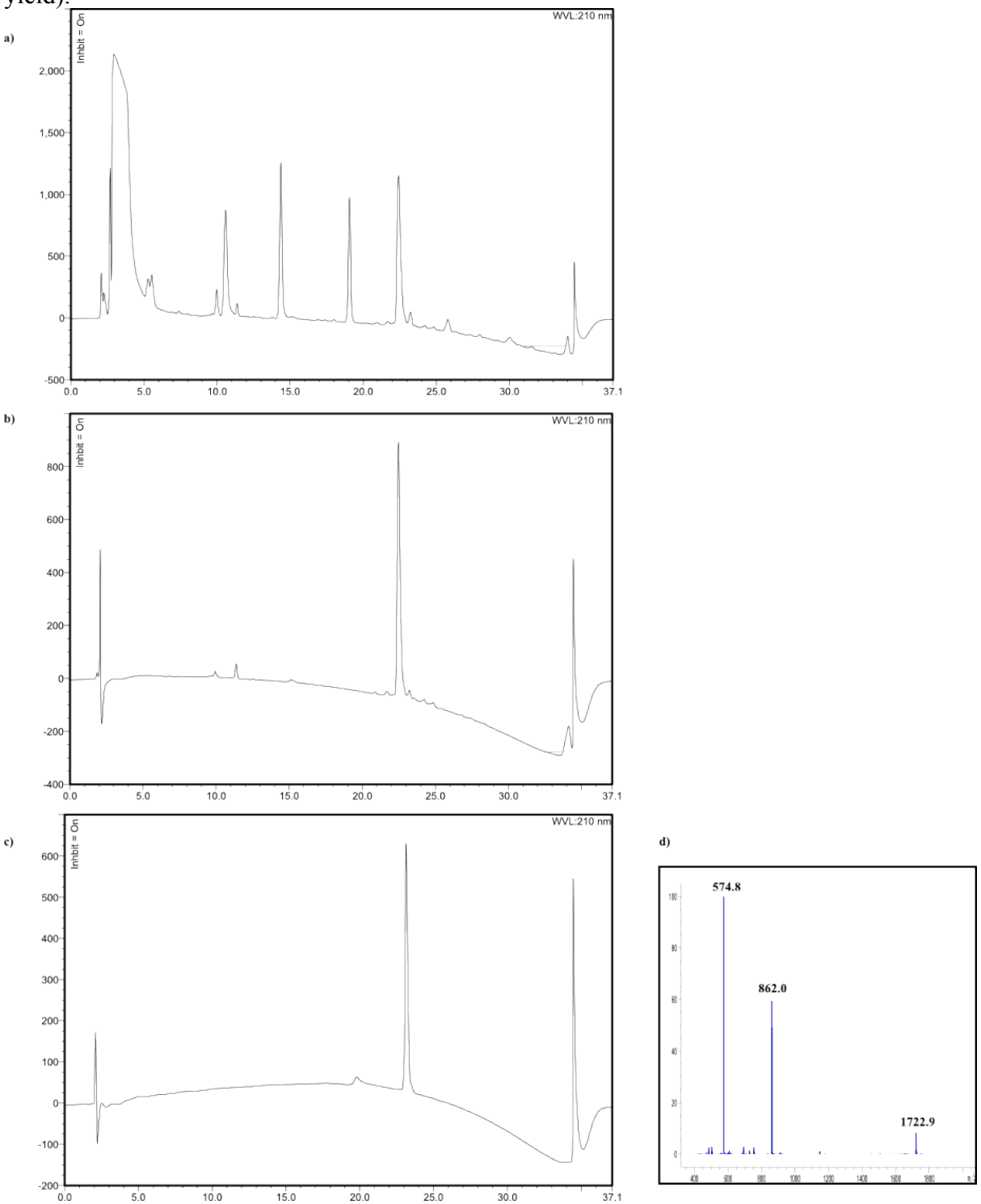


Figure S24. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**32**). a) HPLC chromatogram of CLipPA reaction monitored at 214 nm at t = 60 min 365 nm UV irradiation; b) HPLC chromatogram of reaction mixture monitored at 214 nm after diethyl ether work-up; c) HPLC chromatogram of purified peptide (**32**), after Acm removal, monitored at 214 nm; d) Observed ESI-MS for peptide (**32**), calcd. Mass: 1722.2, found 1722.9 $[M+1H]^{+1}$, 862.0 $[M+2H]^{+2}$, 574.8 $[M+3H]^{+3}$. Deconvolution affords a mass of 1721.8 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 µ; 4.6 x 150 mm) and a linear gradient of 5-95% B in 30 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

7.05 Synthesis of *S*-lipopeptide (39)

Peptide scaffold (**4**) (10 mg, 6.74 µmol) was used to obtain *S*-lipopeptide (**39**) (5.1 mg, 49% yield).

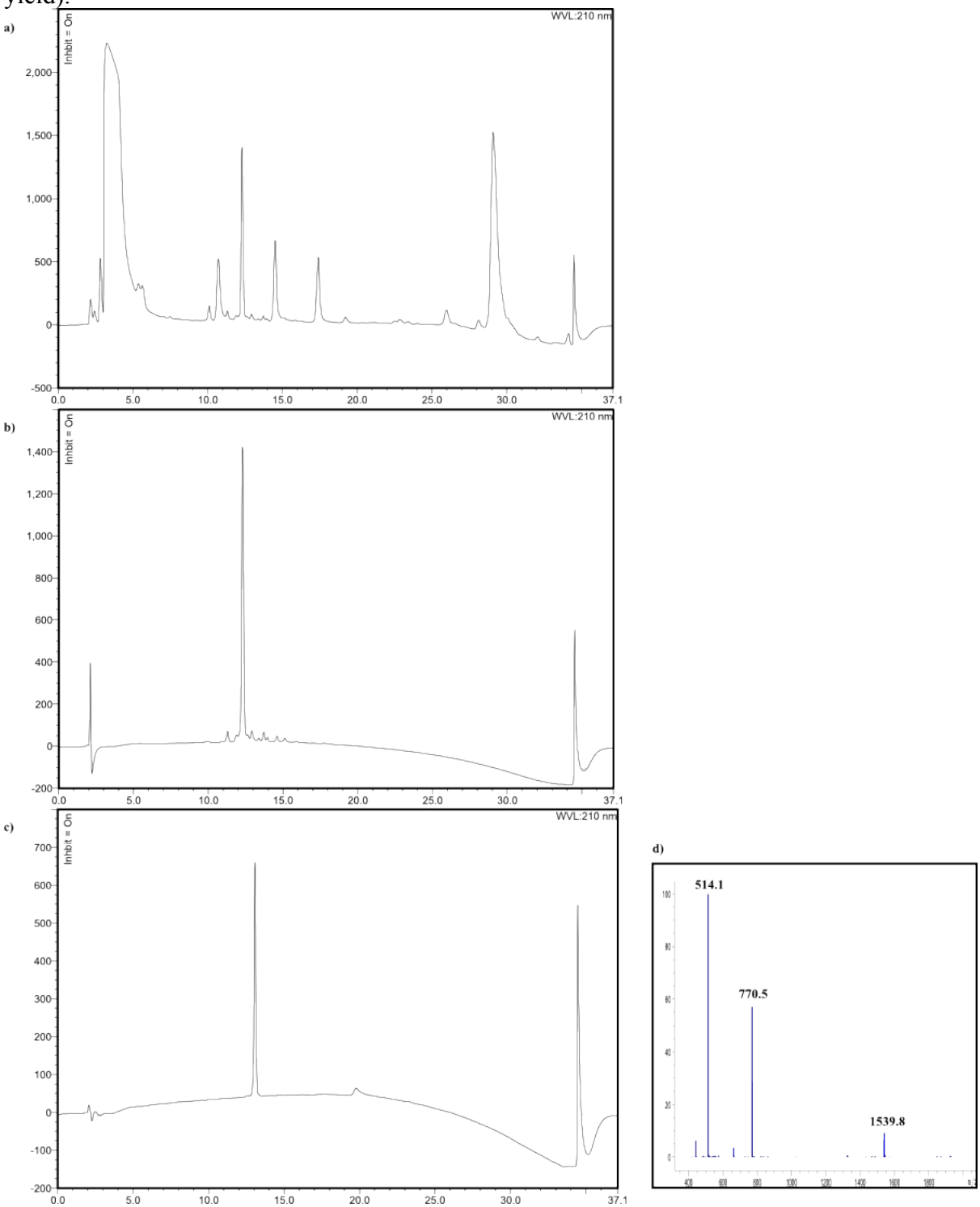


Figure S25. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**39**). a) HPLC chromatogram of CLipPA reaction monitored at 214 nm at t = 60 min 365 nm UV irradiation; b) HPLC chromatogram of reaction mixture monitored at 214 nm after diethyl ether work-up; c) HPLC chromatogram of purified peptide (**39**), after Acm removal, monitored at 214 nm; d) Observed ESI-MS for peptide (**39**), calcd. Mass: 1539.8, found 1539.8 $[M+1H]^{+1}$, 770.5 $[M+2H]^{+2}$, 514.1 $[M+3H]^{+3}$. Deconvolution affords a mass of 1539.0 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 µ; 4.6 x 150 mm) and a linear gradient of 5-95% B in 30 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

7.06 Synthesis of *S*-lipopeptide (46)

Peptide scaffold (**4**) (10 mg, 6.74 μmol) was used to obtain *S*-lipopeptide (**46**) (6.0 mg, 55% yield).

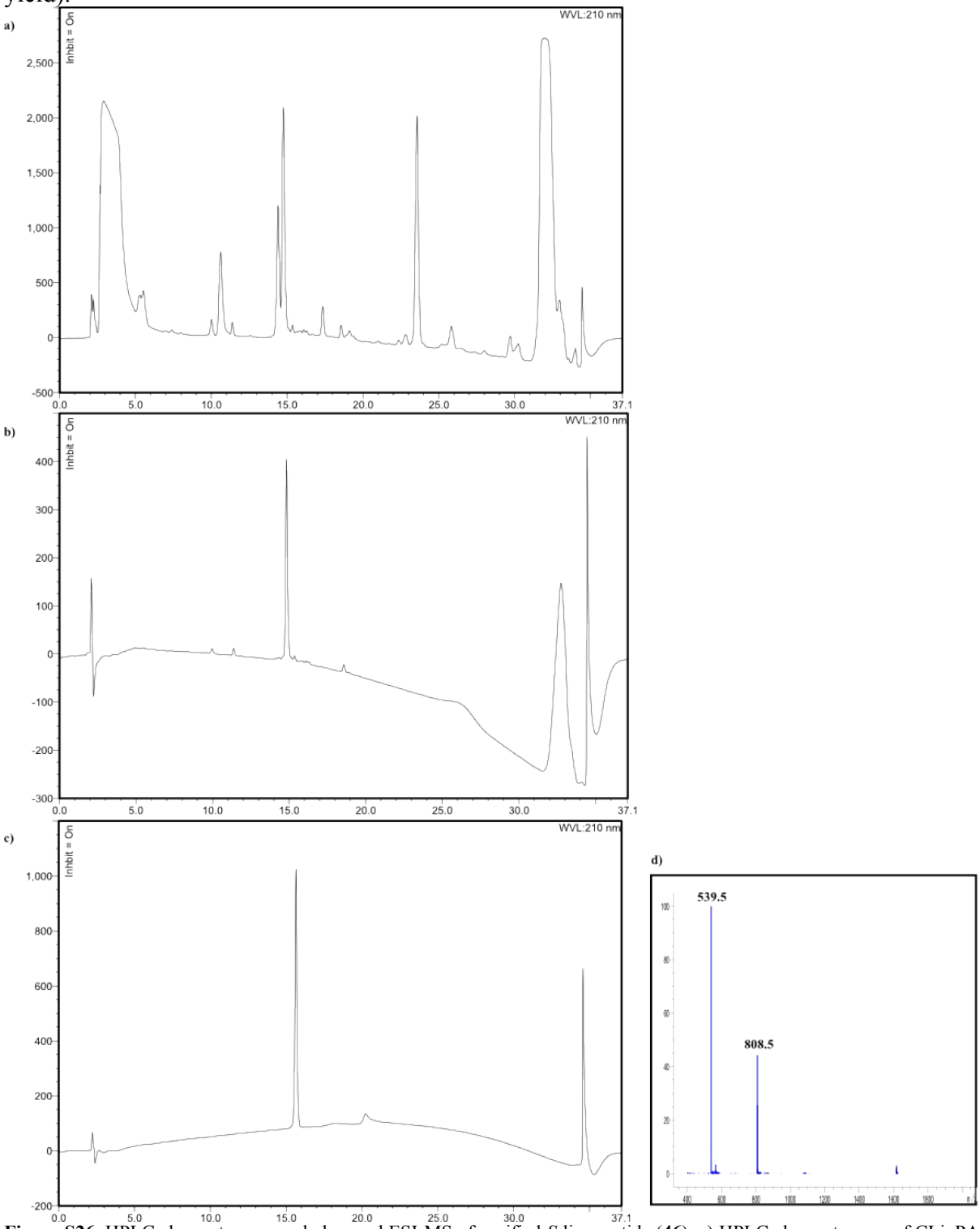
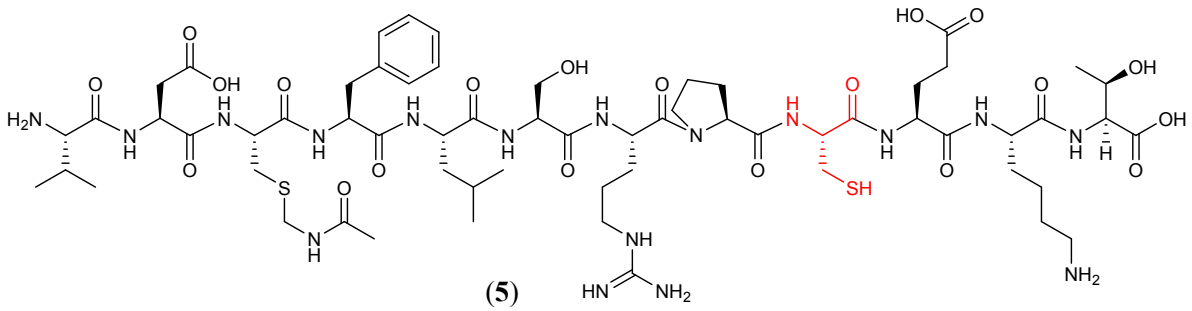


Figure S26. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**46**). a) HPLC chromatogram of CLipPA reaction monitored at 214 nm at t = 60 min 365 nm UV irradiation; b) HPLC chromatogram of reaction mixture monitored at 214 nm after diethyl ether work-up; c) HPLC chromatogram of purified peptide (**46**), after Acm removal, monitored at 214 nm; d) Observed ESI-MS for peptide (**46**), calcd. Mass: 1615.6, found 808.5 $[M+2H]^{+2}$, 539.5 $[M+3H]^{+3}$. Deconvolution affords a mass of 1615.3 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 μ; 4.6 x 150 mm) and a linear gradient of 5-95% B in 30 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

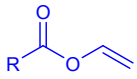
8. Synthesis of *S*-Lipopeptide Analogues (12), (19), (26), (33), (40) and (47) using peptide scaffold (5)

S-Lipopeptide analogues (**12**), (**19**), (**26**), (**33**), (**40**) and (**47**) were prepared using CLipPA reaction with peptide scaffold (**5**) and vinyl esters using **Method 6** followed by Acm removal of ^{3}Cys residue using **Method 7**.

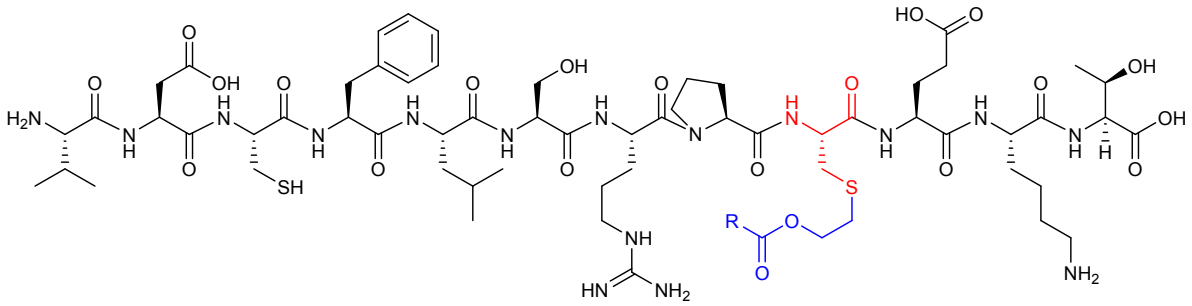


(**5**)

i) CLipPA



ii) Acm removal



(**12**) R = propyl
(**19**) R = nonyl
(**26**) R = pentadecyl
(**33**) R = heptadecyl
(**40**) R = *tert*-butyl
(**47**) R = 4-*tert*-butylphenyl

8.01 Synthesis of *S*-lipopeptide (12)

Peptide scaffold (**5**) (10 mg, 6.81 µmol) was used to obtain *S*-lipopeptide (**12**) (5.7 mg, 55% yield).

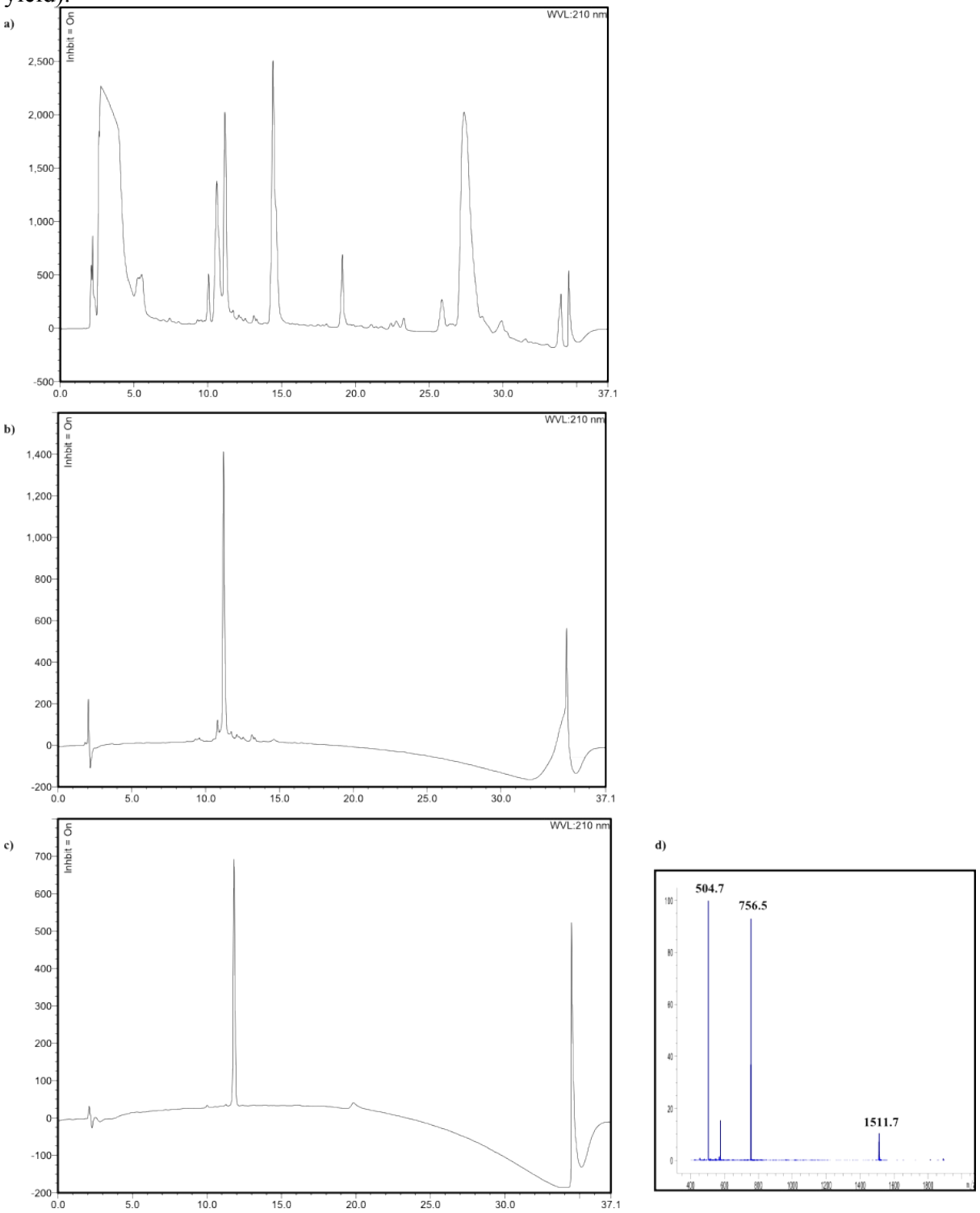


Figure S27. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**12**). a) HPLC chromatogram of CLipPA reaction monitored at 214 nm at t = 60 min 365 nm UV irradiation; b) HPLC chromatogram of reaction mixture monitored at 214 nm after diethyl ether work-up; c) HPLC chromatogram of purified peptide (**12**), after Acm removal, monitored at 214 nm; d) Observed ESI-MS for peptide (**12**), calcd. Mass: 1511.8, found 1511.7 $[M+1H]^{+1}$, 756.5 $[M+2H]^{+2}$, 504.7 $[M+3H]^{+3}$. Deconvolution affords a mass of 1510.9 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 µ; 4.6 x 150 mm) and a linear gradient of 5-95% B in 30 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

8.02 Synthesis of *S*-lipopeptide (19)

Peptide scaffold (**5**) (10 mg, 6.81 µmol) was used to obtain *S*-lipopeptide (**19**) (5.4 mg, 51% yield).

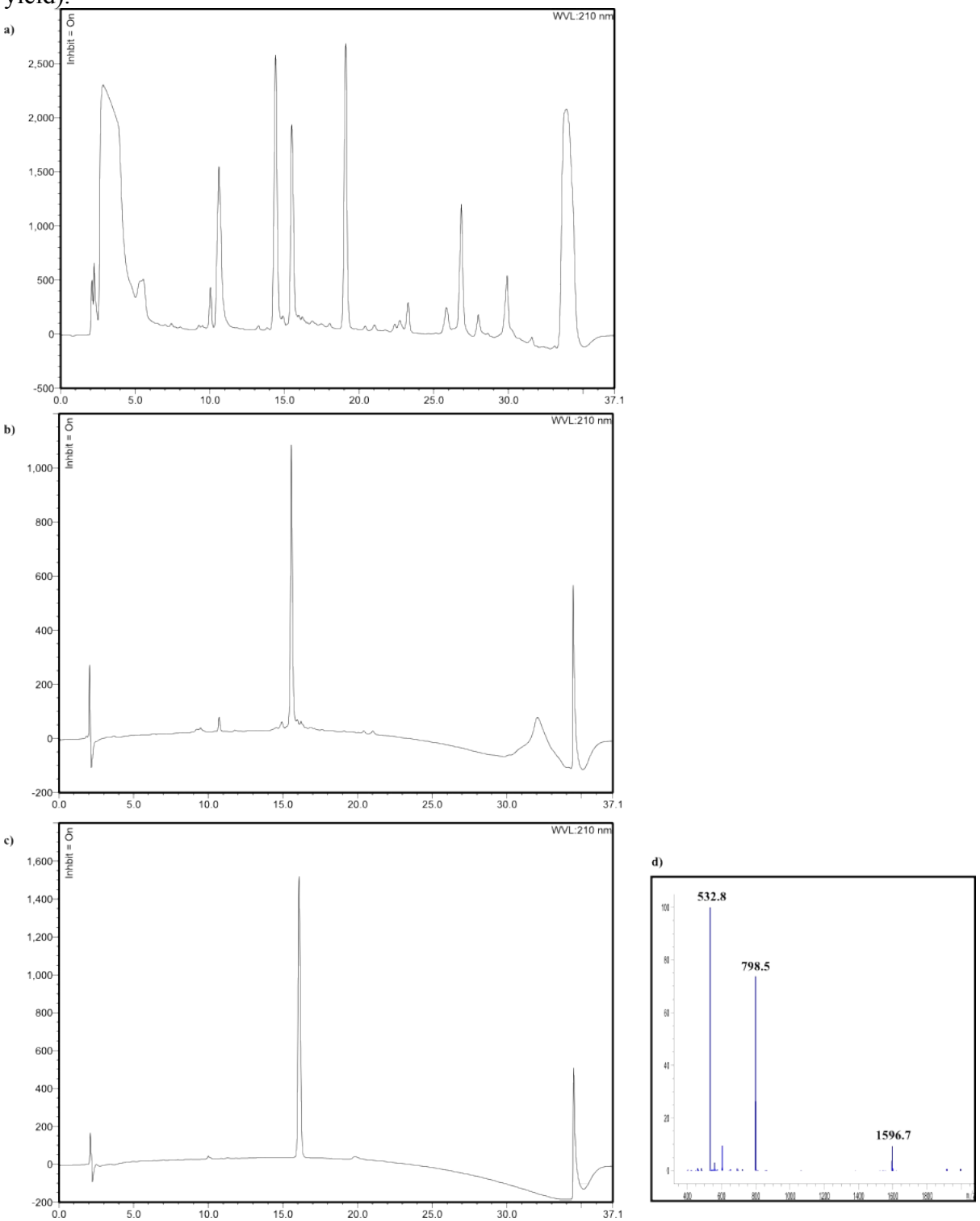


Figure S28. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**19**). a) HPLC chromatogram of CLipPA reaction monitored at 214 nm at t = 60 min 365 nm UV irradiation; b) HPLC chromatogram of reaction mixture monitored at 214 nm after diethyl ether work-up; c) HPLC chromatogram of purified peptide (**19**), after Acm removal, monitored at 214 nm; d) Observed ESI-MS for peptide (**19**), calcd. Mass: 1549.9, found 1549.8 $[M+1H]^{+1}$, 775.5 $[M+2H]^{+2}$, 517.4 $[M+3H]^{+3}$. Deconvolution affords a mass of 1549.0 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 µ; 4.6 x 150 mm) and a linear gradient of 5-95% B in 30 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

8.03 Synthesis of *S*-lipopeptide (26)

Peptide scaffold (**5**) (10 mg, 6.81 µmol) was used to obtain *S*-lipopeptide (**26**) (6.3 mg, 55% yield).

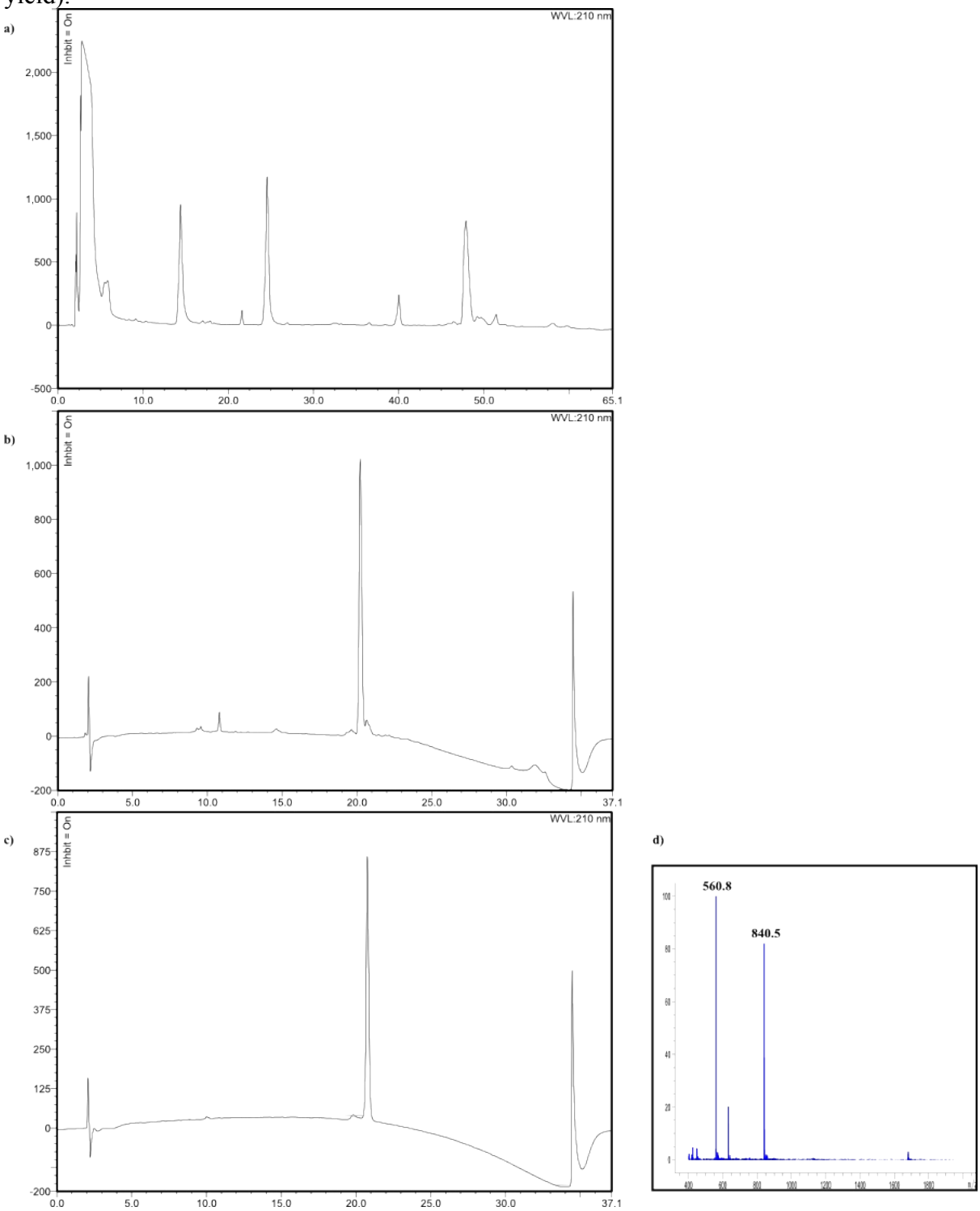


Figure S29. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**26**). a) HPLC chromatogram of CLipPA reaction monitored at 214 nm at t = 60 min 365 nm UV irradiation; b) HPLC chromatogram of reaction mixture monitored at 214 nm after diethyl ether work-up; c) HPLC chromatogram of purified peptide (**26**), after Acm removal, monitored at 214 nm; d) Observed ESI-MS for peptide (**26**), calcd. Mass: 1680.1, found 840.5 $[M+2H]^{+2}$, 560.8 $[M+3H]^{+3}$. Deconvolution affords a mass of 1679.2 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 µ; 4.6 x 150 mm) and a linear gradient of 5-65% B in 60 min, *ca* 1% B per min at a flow rate of 1.0 mL/min for (a) and 5-95% B in 30 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min for (b)–(c). Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

8.04 Synthesis of *S*-lipopeptide (33)

Peptide scaffold (**5**) (10 mg, 6.81 µmol) was used to obtain *S*-lipopeptide (**33**) (7.2 mg, 62% yield).

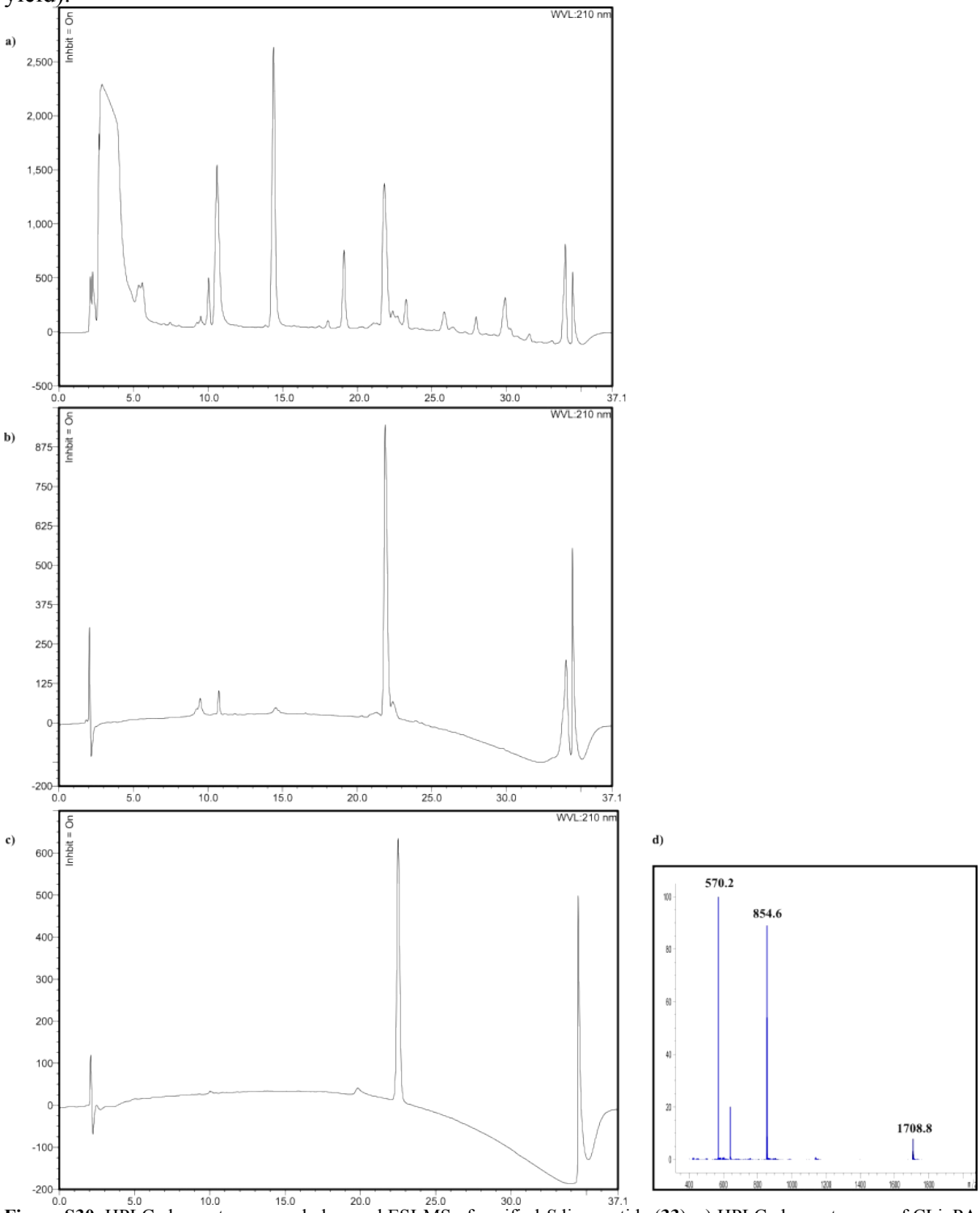


Figure S30. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**33**). a) HPLC chromatogram of CLipPA reaction monitored at 214 nm at t = 60 min 365 nm UV irradiation; b) HPLC chromatogram of reaction mixture monitored at 214 nm after diethyl ether work-up; c) HPLC chromatogram of purified peptide (**33**), after Acm removal, monitored at 214 nm; d) Observed ESI-MS for peptide (**33**), calcd. Mass: 1708.2, found 1708.8 $[M+1H]^{+1}$, 854.6 $[M+2H]^{+2}$, 570.2 $[M+3H]^{+3}$. Deconvolution affords a mass of 1707.5 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 µ; 4.6 x 150 mm) and a linear gradient of 5-95% B in 30 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

8.05 Synthesis of *S*-lipopeptide (40)

Peptide scaffold (**5**) (10 mg, 6.81 µmol) was used to obtain *S*-lipopeptide (**40**) (5.5 mg, 53% yield).

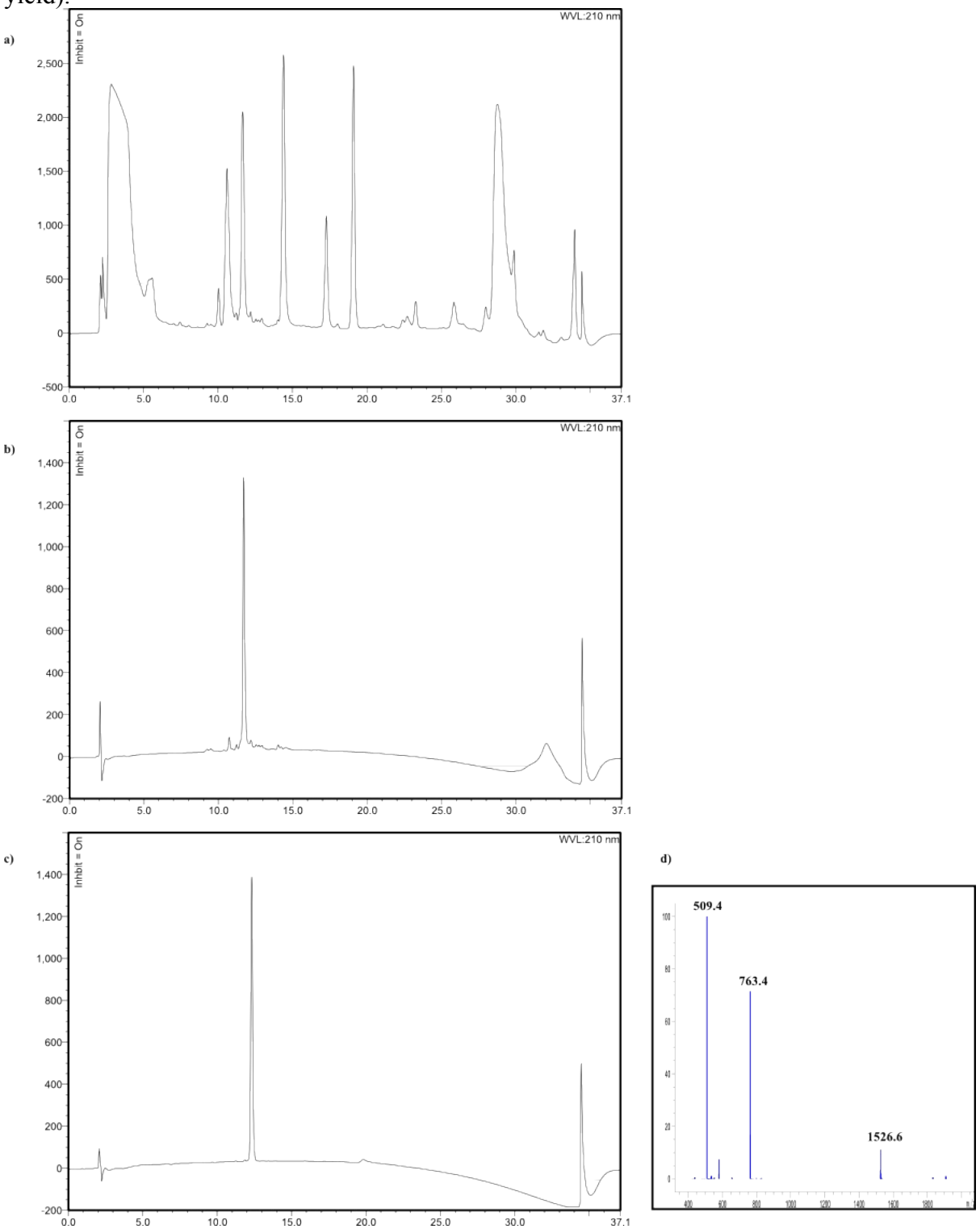


Figure S31. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**40**). a) HPLC chromatogram of CLipPA reaction monitored at 214 nm at t = 60 min 365 nm UV irradiation; b) HPLC chromatogram of reaction mixture monitored at 214 nm after diethyl ether work-up; c) HPLC chromatogram of purified peptide (**40**) , after Acm removal, monitored at 214 nm; d) Observed ESI-MS for peptide (**40**), calcd. Mass: 1525.8, found 1526.6 $[M+1H]^{+1}$, 763.4 $[M+2H]^{+2}$, 509.4 $[M+3H]^{+3}$. Deconvolution affords a mass of 1525.2 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 µ; 4.6 x 150 mm) and a linear gradient of 5-95% B in 30 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

8.06 Synthesis of *S*-lipopeptide (47)

Peptide scaffold (**5**) (10 mg, 6.81 μmol) was used to obtain *S*-lipopeptide (**47**) (6.6 mg, 61% yield).

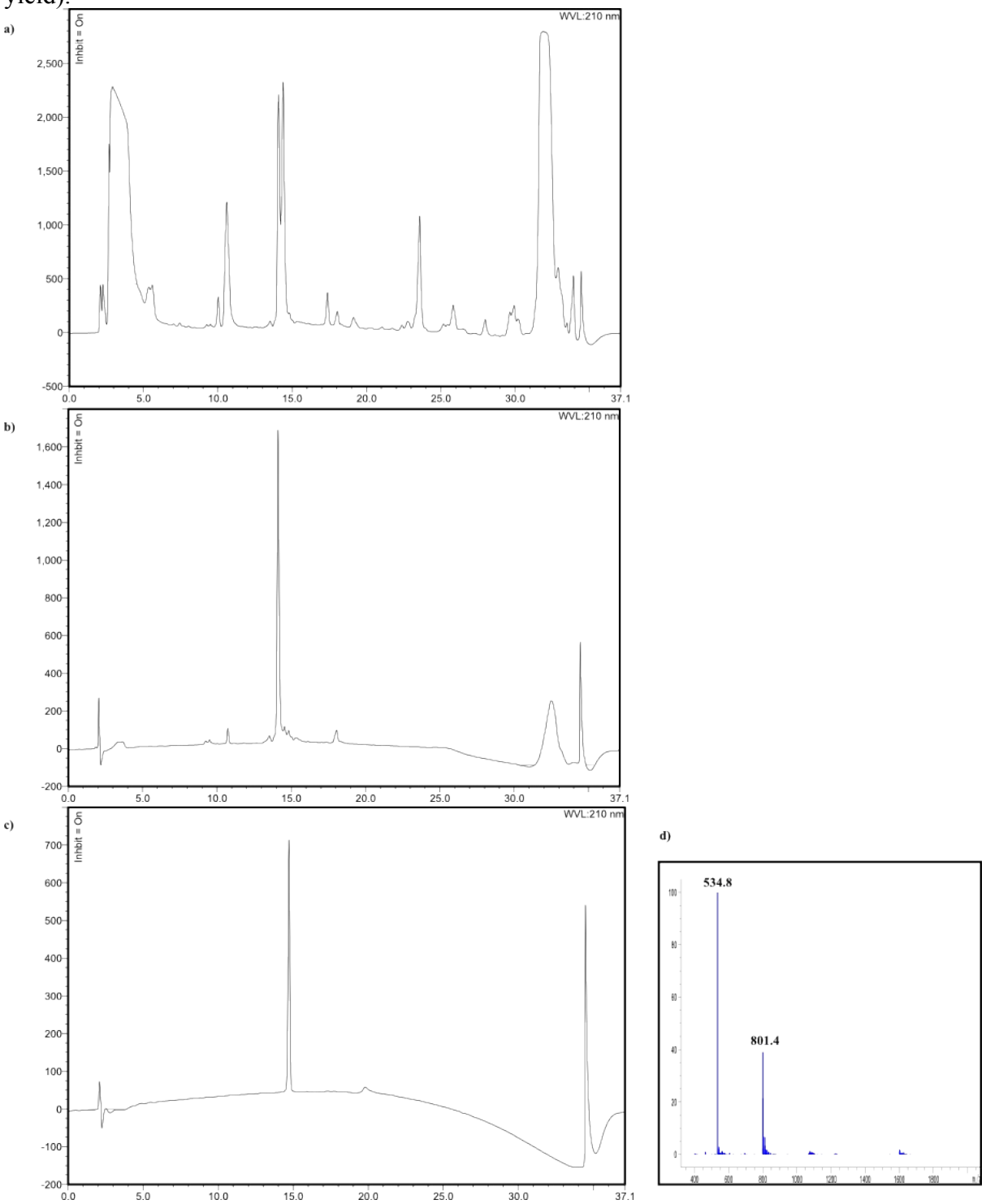
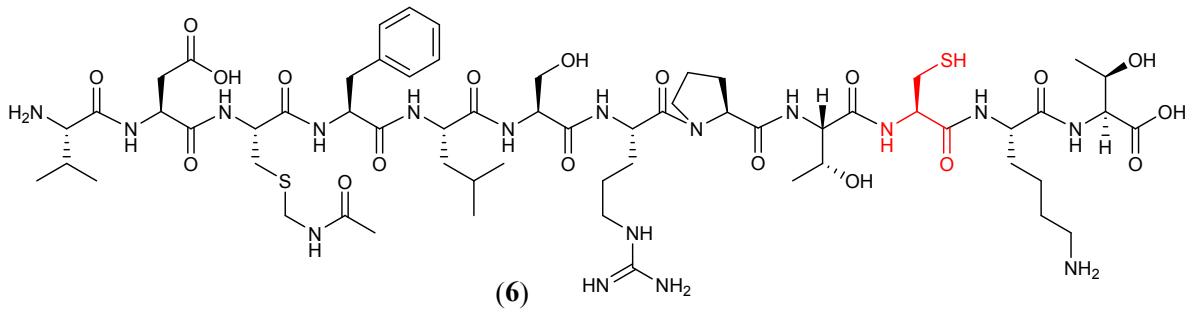


Figure S32. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**47**). a) HPLC chromatogram of CLipPA reaction monitored at 214 nm at t = 60 min 365 nm UV irradiation; b) HPLC chromatogram of reaction mixture monitored at 214 nm after diethyl ether work-up; c) HPLC chromatogram of purified peptide (**47**), after Acm removal, monitored at 214 nm; d) Observed ESI-MS for peptide (**47**), calcd. Mass: 1601.6, found 801.4 $[M+2H]^{+2}$, 534.8 $[M+3H]^{+3}$. Deconvolution affords a mass of 1601.1 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 μ; 4.6 x 150 mm) and a linear gradient of 5-95% B in 30 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

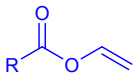
9. Synthesis of *S*-Lipopeptide Analogues (13), (20), (27), (34), (41) and (48) using peptide scaffold (6)

S-Lipopeptide analogues (**13**), (**20**), (**27**), (**34**), (**41**) and (**48**) were prepared using CLipPA reaction with peptide scaffold (**6**) and vinyl esters using **Method 6** followed by Acm removal of ^{3}Cys residue using **Method 7**.

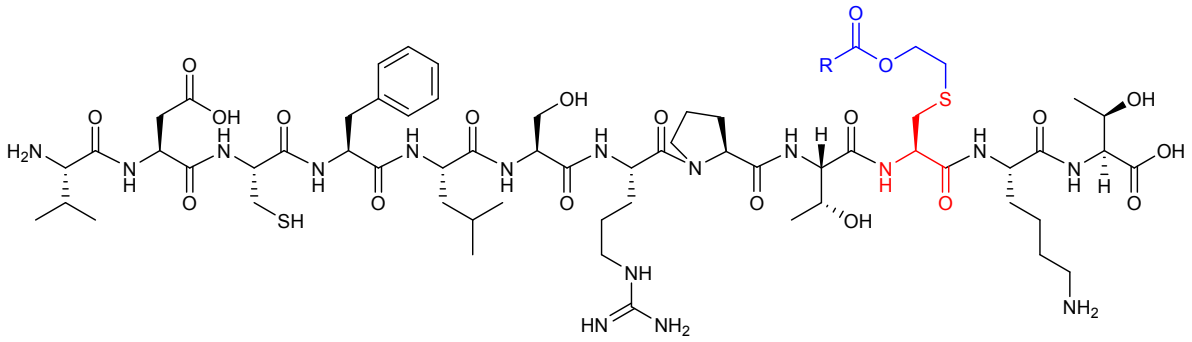


(**6**)

i) CLipPA



ii) Acm removal



(**13**) R = propyl
(**20**) R = nonyl
(**27**) R = pentadecyl
(**34**) R = heptadecyl
(**41**) R = *tert*-butyl
(**48**) R = 4-*tert*-butylphenyl

9.01 Synthesis of *S*-lipopeptide (13)

Peptide scaffold (**6**) (10 mg, 6.94 µmol) was used to obtain *S*-lipopeptide (**13**) (6.0 mg, 58% yield).

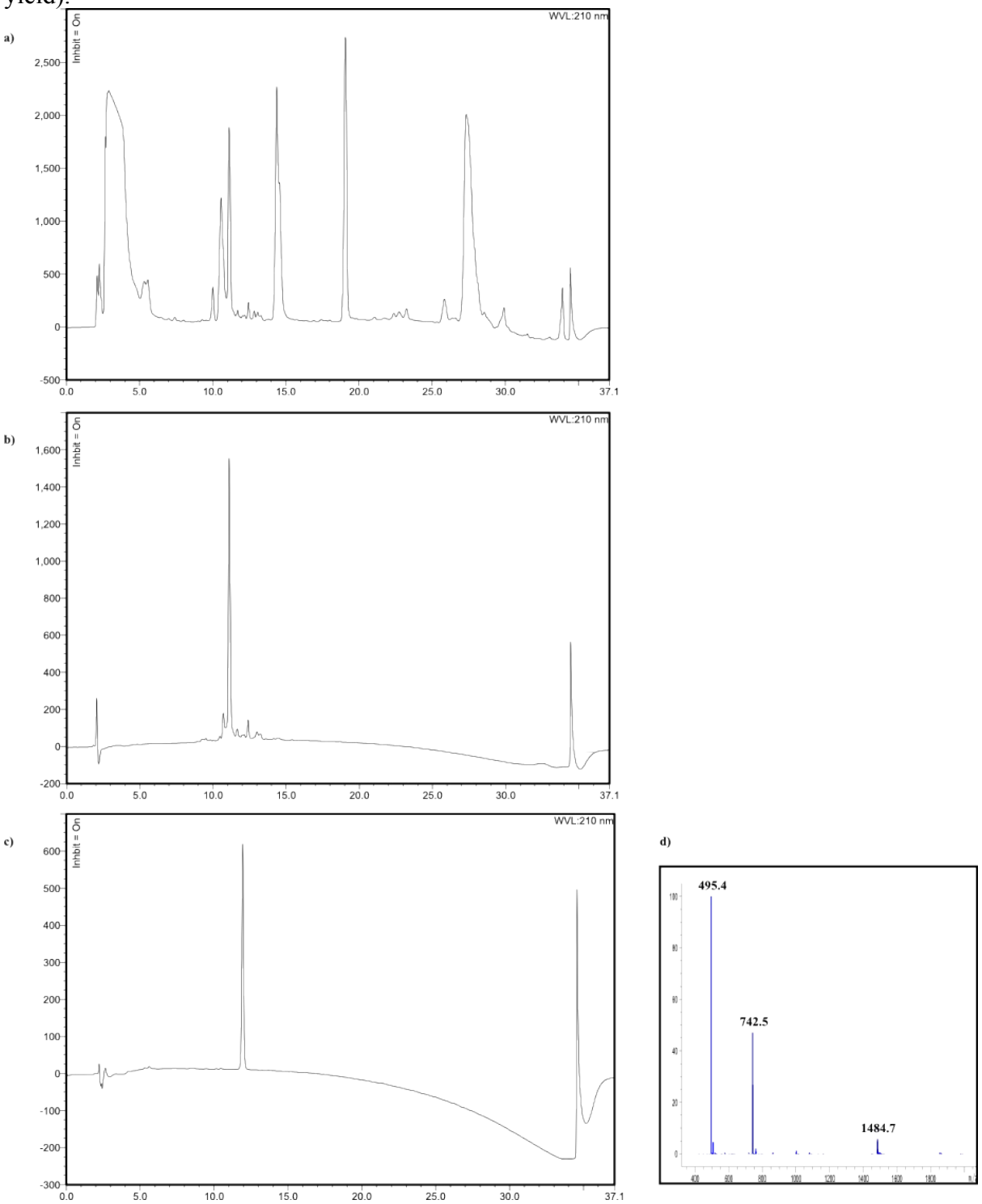


Figure S33. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**13**). a) HPLC chromatogram of CLipPA reaction monitored at 214 nm at t = 60 min 365 nm UV irradiation; b) HPLC chromatogram of reaction mixture monitored at 214 nm after diethyl ether work-up; c) HPLC chromatogram of purified peptide (**13**), after Acm removal, monitored at 214 nm; d) Observed ESI-MS for peptide (**13**), calcd. Mass: 1483.8, found 1484.7 $[M+1H]^{+1}$, 742.5 $[M+2H]^{+2}$, 495.4 $[M+3H]^{+3}$. Deconvolution affords a mass of 1483.3 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 µ; 4.6 x 150 mm) and a linear gradient of 5-95% B in 30 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

9.02 Synthesis of *S*-lipopeptide (20)

Peptide scaffold (**6**) (10 mg, 6.94 μmol) was used to obtain *S*-lipopeptide (**20**) (5.7 mg, 52% yield).

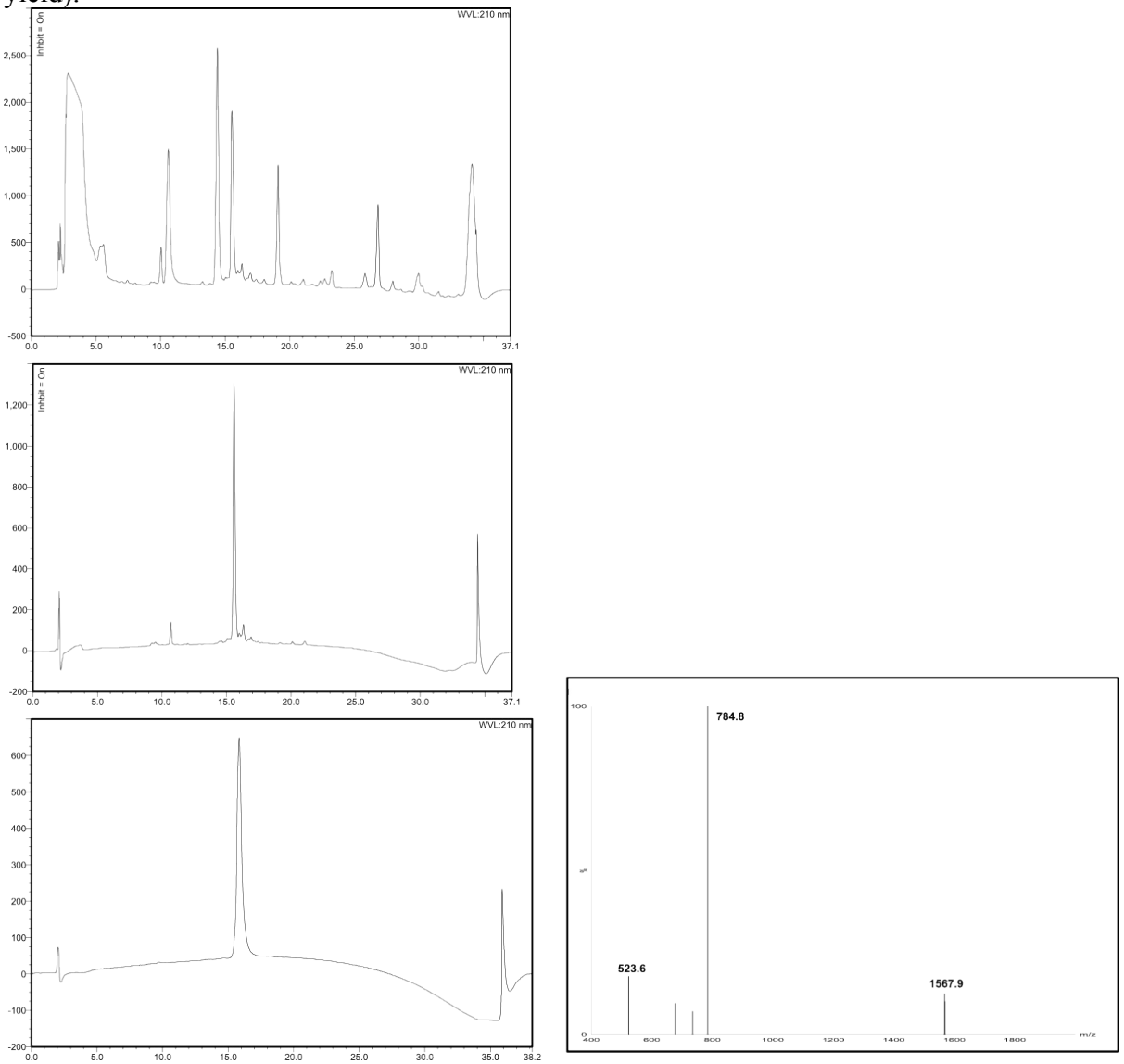


Figure S34. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**20**). a) HPLC chromatogram of CLipPA reaction monitored at 214 nm at t = 60 min 365 nm UV irradiation; b) HPLC chromatogram of reaction mixture monitored at 214 nm after diethyl ether work-up; c) HPLC chromatogram of purified peptide (**20**), after Acm removal, monitored at 214 nm; d) Observed ESI-MS for peptide (**20**), calcd. Mass: 1567.9, found 1567.9 $[M+1H]^{+1}$, 784.8 $[M+2H]^{+2}$, 523.6 $[M+3H]^{+3}$. Deconvolution affords a mass of 1567.4 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 μ; 4.6 x 150 mm) and a linear gradient of 5-95% B in 30 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

9.03 Synthesis of *S*-lipopeptide (27)

Peptide scaffold (**6**) (10 mg, 6.94 μmol) was used to obtain *S*-lipopeptide (**27**) (5.8 mg, 51% yield).

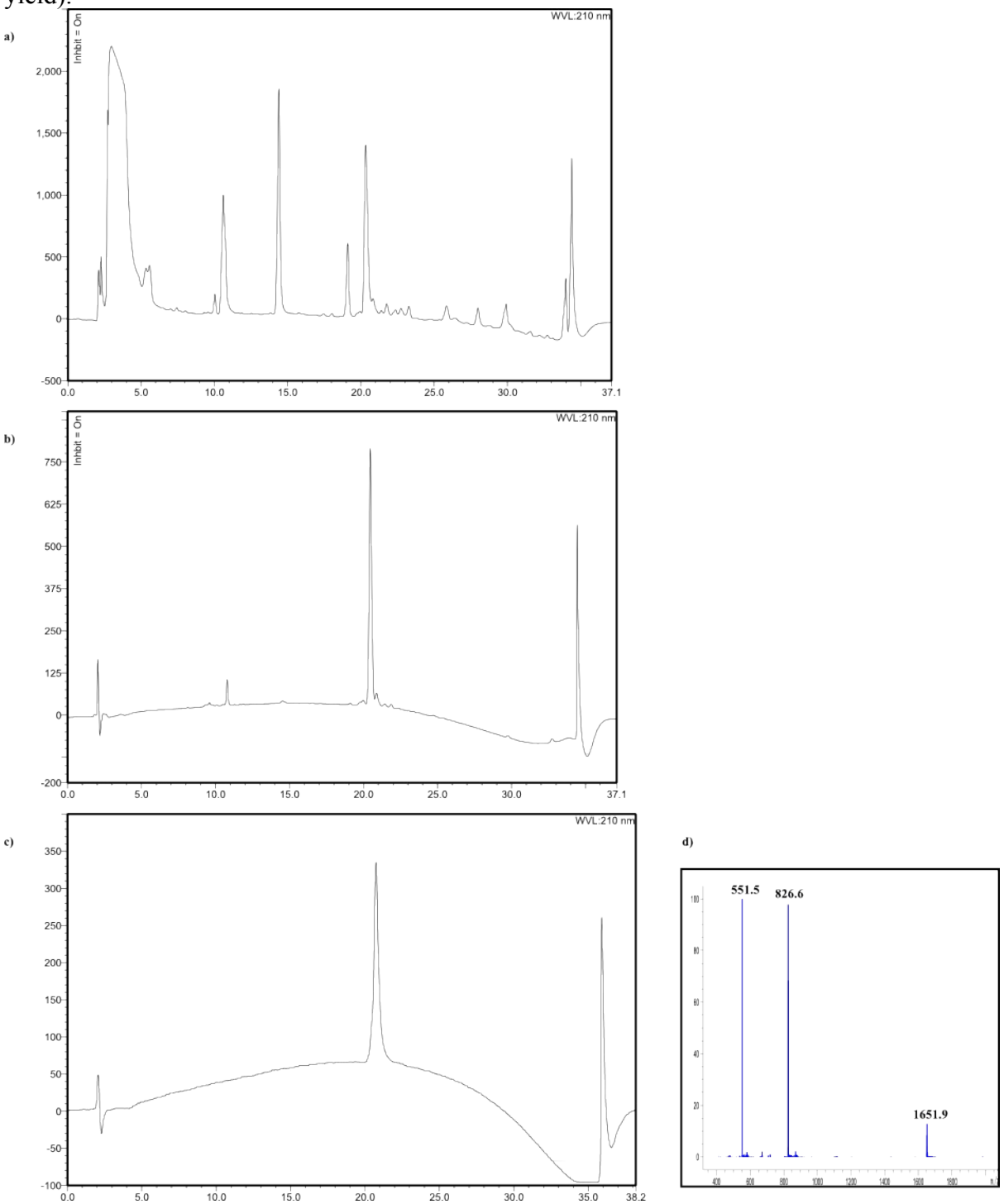


Figure S35. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**27**). a) HPLC chromatogram of CLipPA reaction monitored at 214 nm at t = 60 min 365 nm UV irradiation; b) HPLC chromatogram of reaction mixture monitored at 214 nm after diethyl ether work-up; c) HPLC chromatogram of purified peptide (**27**), after Acm removal, monitored at 214 nm; d) Observed ESI-MS for peptide (**27**), calcd. Mass: 1652.1, found 1651.9 $[M+1H]^{+1}$, 826.6 $[M+2H]^{+2}$, 551.5 $[M+3H]^{+3}$. Deconvolution affords a mass of 1651.2 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 μ; 4.6 x 150 mm) and a linear gradient of 5-95% B in 30 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

9.04 Synthesis of *S*-lipopeptide (34)

Peptide scaffold (**6**) (10 mg, 6.94 μmol) was used to obtain *S*-lipopeptide (**34**) (5.9 mg, 51% yield).

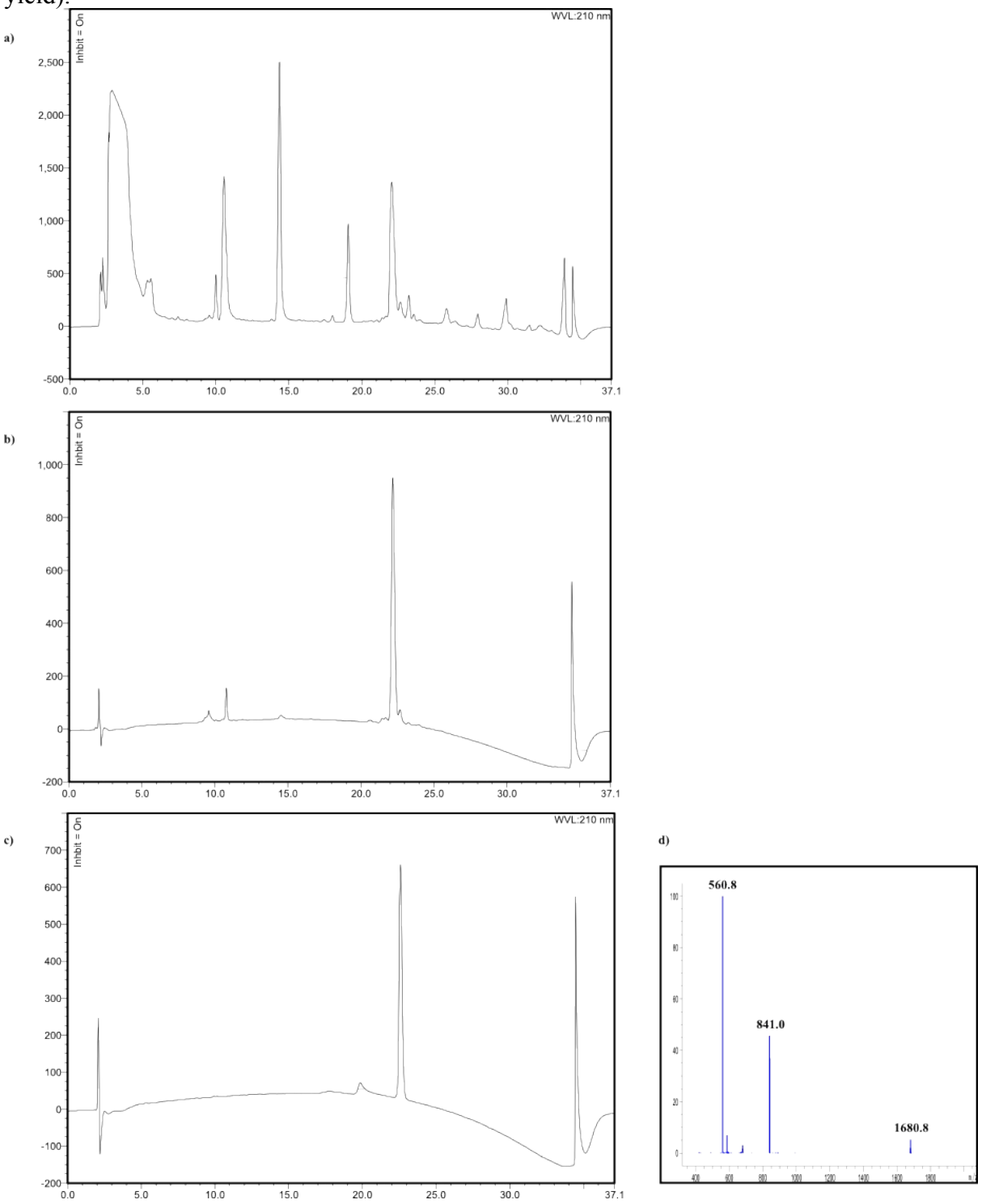


Figure S36. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**34**). a) HPLC chromatogram of CLipPA reaction monitored at 214 nm at t = 60 min 365 nm UV irradiation; b) HPLC chromatogram of reaction mixture monitored at 214 nm after diethyl ether work-up; c) HPLC chromatogram of purified peptide (**34**), after Acm removal, monitored at 214 nm; d) Observed ESI-MS for peptide (**34**), calcd. Mass: 1680.2, found 1680.8 $[M+1H]^{+1}$, 841.0 $[M+2H]^{+2}$, 560.8 $[M+3H]^{+3}$. Deconvolution affords a mass of 1679.7 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 μ; 4.6 x 150 mm) and a linear gradient of 5-95% B in 30 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

9.05 Synthesis of *S*-lipopeptide (41)

Peptide scaffold (**6**) (10 mg, 6.94 μmol) was used to obtain *S*-lipopeptide (**41**) (5.7 mg, 55% yield).

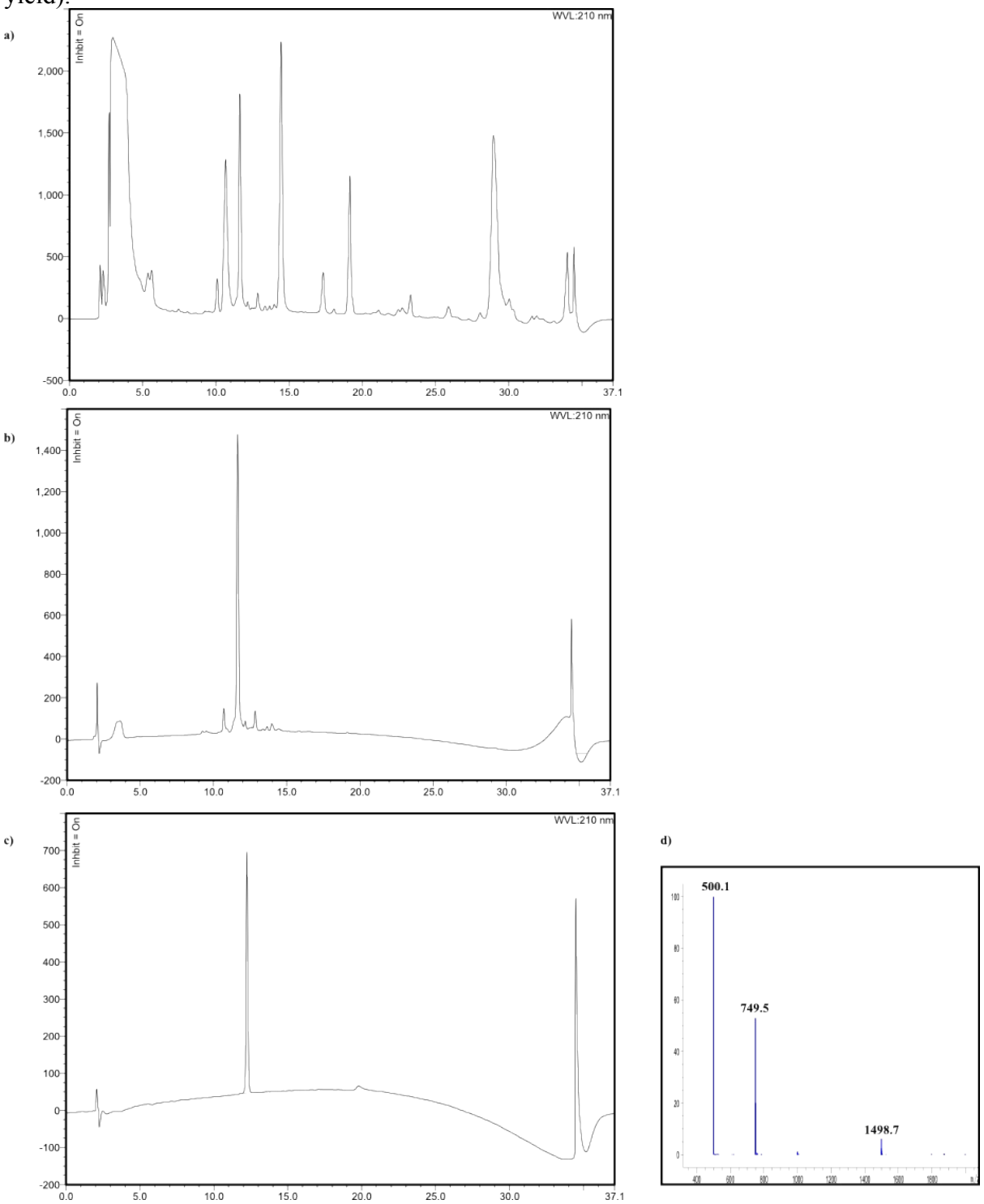


Figure S37. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**41**). a) HPLC chromatogram of CLipPA reaction monitored at 214 nm at t = 60 min 365 nm UV irradiation; b) HPLC chromatogram of reaction mixture monitored at 214 nm after diethyl ether work-up; c) HPLC chromatogram of purified peptide (**41**), after Acm removal, monitored at 214 nm; d) Observed ESI-MS for peptide (**41**), calcd. Mass: 1497.8, found 1498.7 $[M+1H]^{+1}$, 749.5 $[M+2H]^{+2}$, 500.1 $[M+3H]^{+3}$. Deconvolution affords a mass of 1497.3 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 μ; 4.6 x 150 mm) and a linear gradient of 5-95% B in 30 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

9.06 Synthesis of *S*-lipopeptide (48)

Peptide scaffold (**6**) (10 mg, 6.94 μmol) was used to obtain *S*-lipopeptide (**48**) (5.6 mg, 51% yield).

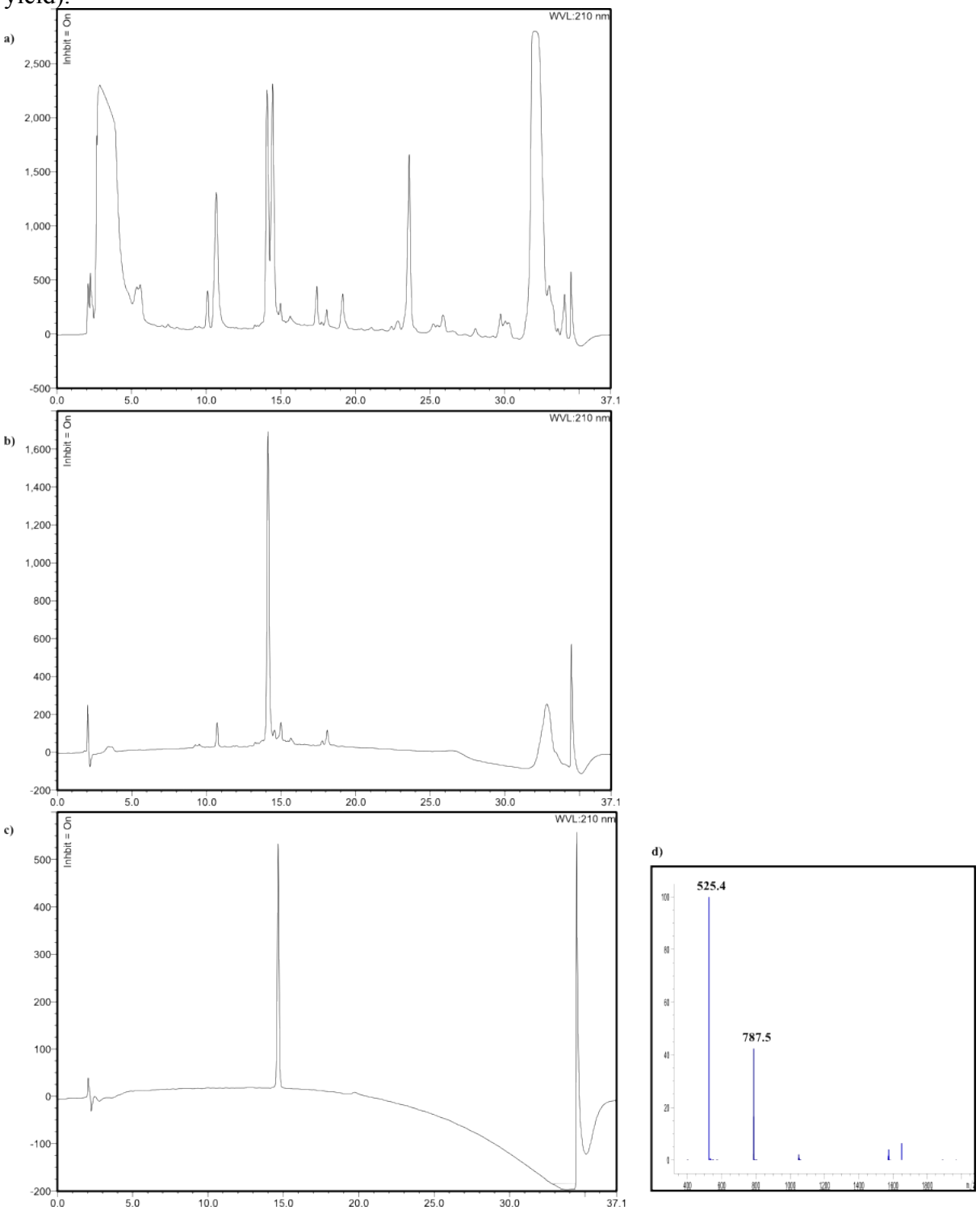
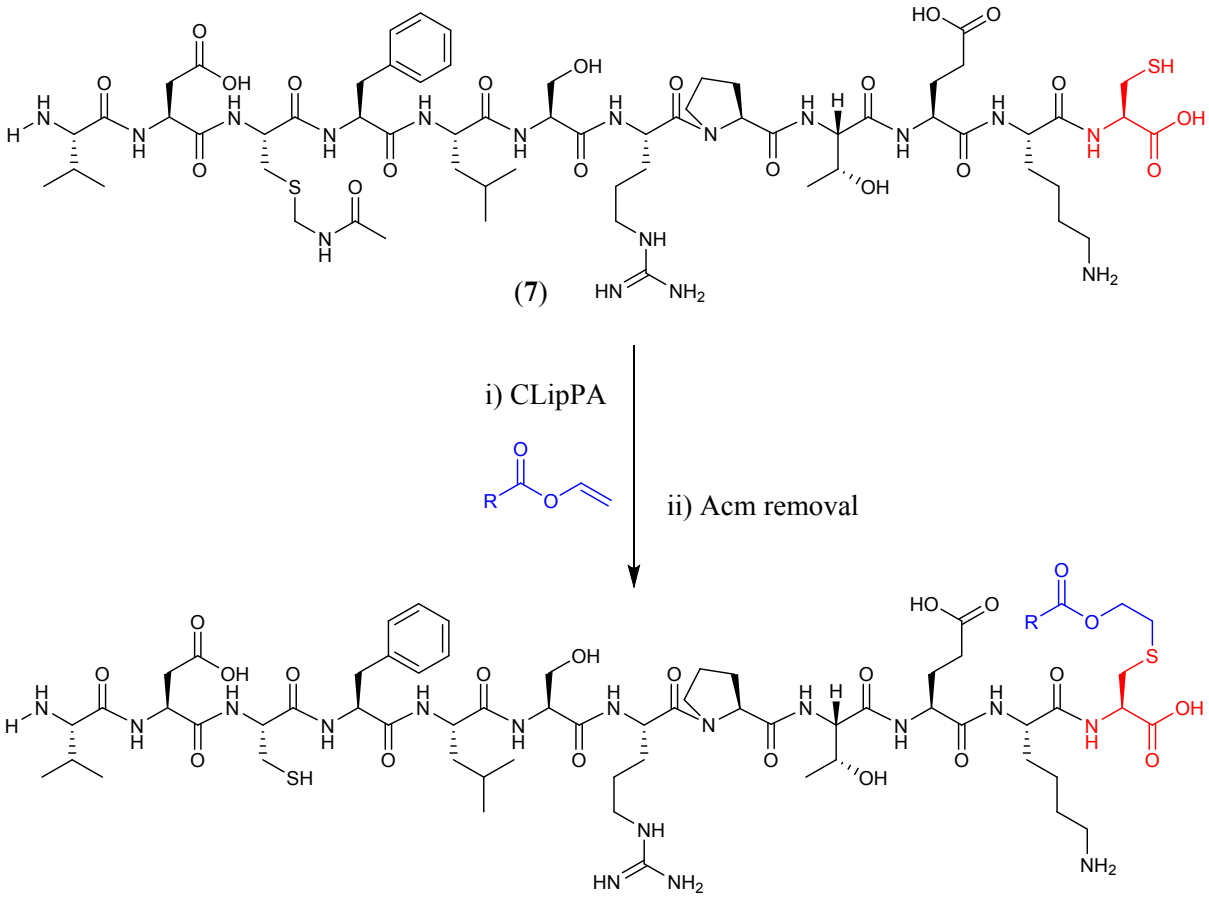


Figure S38. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**48**). a) HPLC chromatogram of CLipPA reaction monitored at 214 nm at t = 60 min 365 nm UV irradiation; b) HPLC chromatogram of reaction mixture monitored at 214 nm after diethyl ether work-up; c) HPLC chromatogram of purified peptide (**48**), after Acm removal, monitored at 214 nm; d) Observed ESI-MS for peptide (**48**), calcd. Mass: 1573.6, found 787.5 $[M+2H]^{+2}$, 525.4 $[M+3H]^{+3}$. Deconvolution affords a mass of 1573.1 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 μ; 4.6 x 150 mm) and a linear gradient of 5-95% B in 30 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

10. Synthesis of *S*-Lipopeptide Analogues (14), (21), (28), (35), (42) and (49) using peptide scaffold (7)

S-Lipopeptide analogues (**14**), (**21**), (**28**), (**35**), (**42**) and (**49**) were prepared using CLipPA reaction with peptide scaffold (**2**) and vinyl esters using **Method 6** followed by Acm removal of ^{3}Cys residue using **Method 7**.



10.01 Synthesis of *S*-lipopeptide (14)

Peptide scaffold (**7**) (10 mg, 6.81 µmol) was used to obtain *S*-lipopeptide (**14**) (6.2 mg, 60% yield).

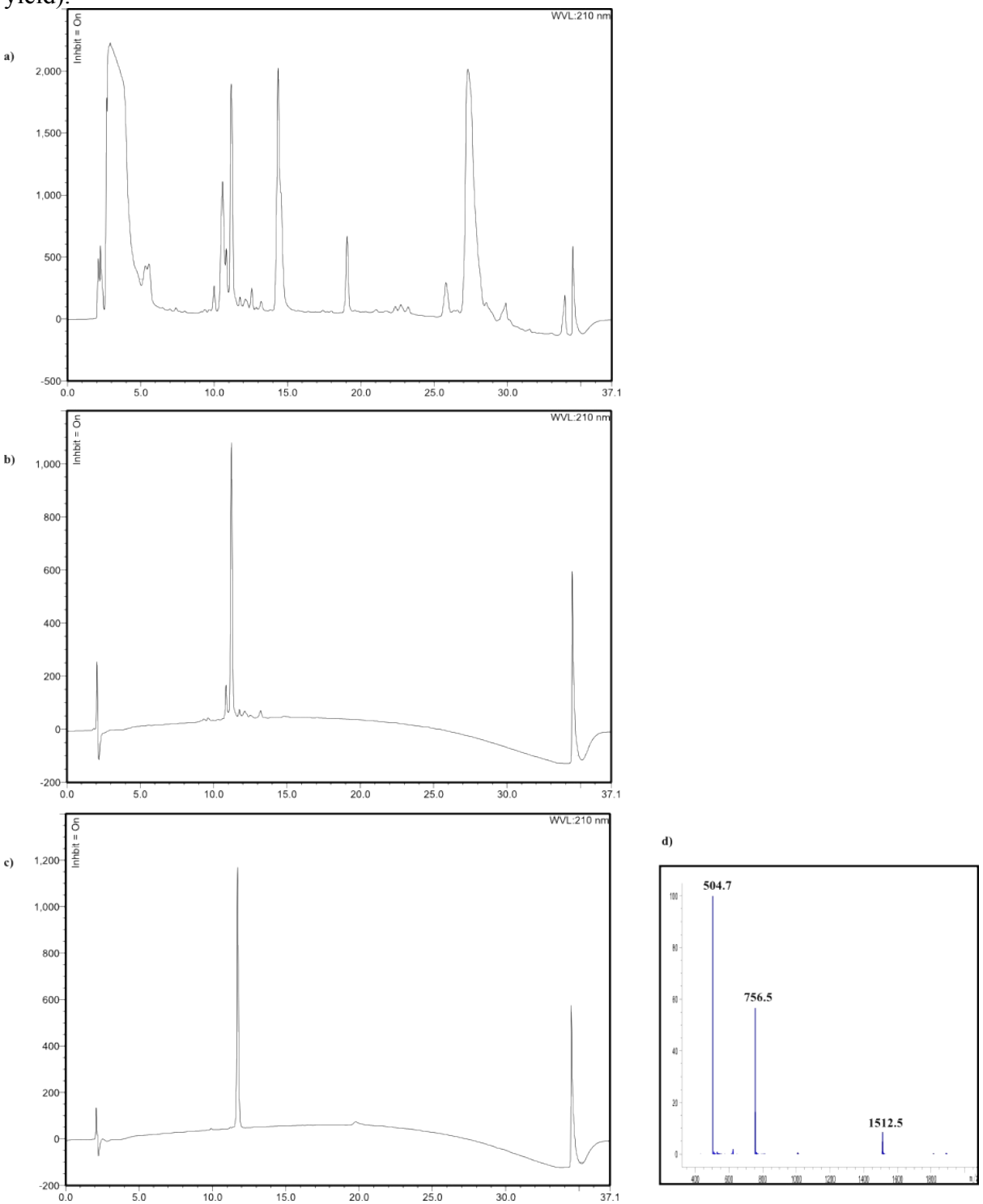


Figure S39. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**14**). a) HPLC chromatogram of CLipPA reaction monitored at 214 nm at t = 60 min 365 nm UV irradiation; b) HPLC chromatogram of reaction mixture monitored at 214 nm after diethyl ether work-up; c) HPLC chromatogram of purified peptide (**14**), after Acm removal, monitored at 214 nm; d) Observed ESI-MS for peptide (**14**), calcd. Mass: 1511.8, found 1512.5 $[M+1H]^{+1}$, 756.5 $[M+2H]^{+2}$, 504.7 $[M+3H]^{+3}$. Deconvolution affords a mass of 1511.2 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 µ; 4.6 x 150 mm) and a linear gradient of 5-95% B in 30 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

10.02 Synthesis of *S*-lipopeptide (21)

Peptide scaffold (**7**) (10 mg, 6.81 µmol) was used to obtain *S*-lipopeptide (**21**) (6.2 mg, 57% yield).

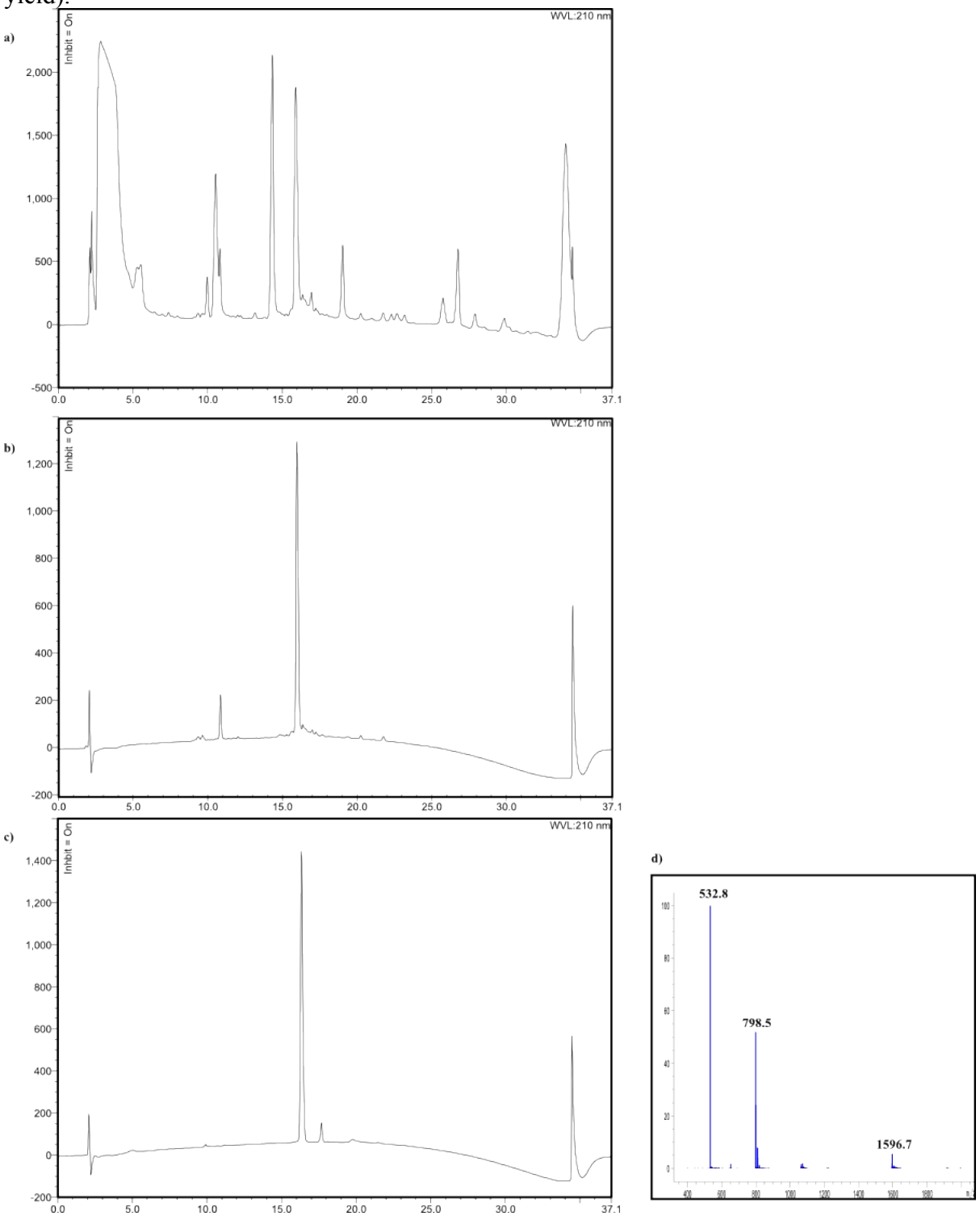


Figure S40. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**21**). a) HPLC chromatogram of CLipPA reaction monitored at 214 nm at t = 60 min 365 nm UV irradiation; b) HPLC chromatogram of reaction mixture monitored at 214 nm after diethyl ether work-up; c) HPLC chromatogram of purified peptide (**21**), after Acm removal, monitored at 214 nm; d) Observed ESI-MS for peptide (**21**), calcd. Mass: 1595.9, found 1596.7 $[M+1H]^{+1}$, 798.5 $[M+2H]^{+2}$, 532.8 $[M+3H]^{+3}$. Deconvolution affords a mass of 1595.4 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 µ; 4.6 x 150 mm) and a linear gradient of 5-95% B in 30 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

10.03 Synthesis of *S*-lipopeptide (28)

Peptide scaffold (**7**) (10 mg, 6.81 μmol) was used to obtain *S*-lipopeptide (**28**) (5.8 mg, 51% yield).

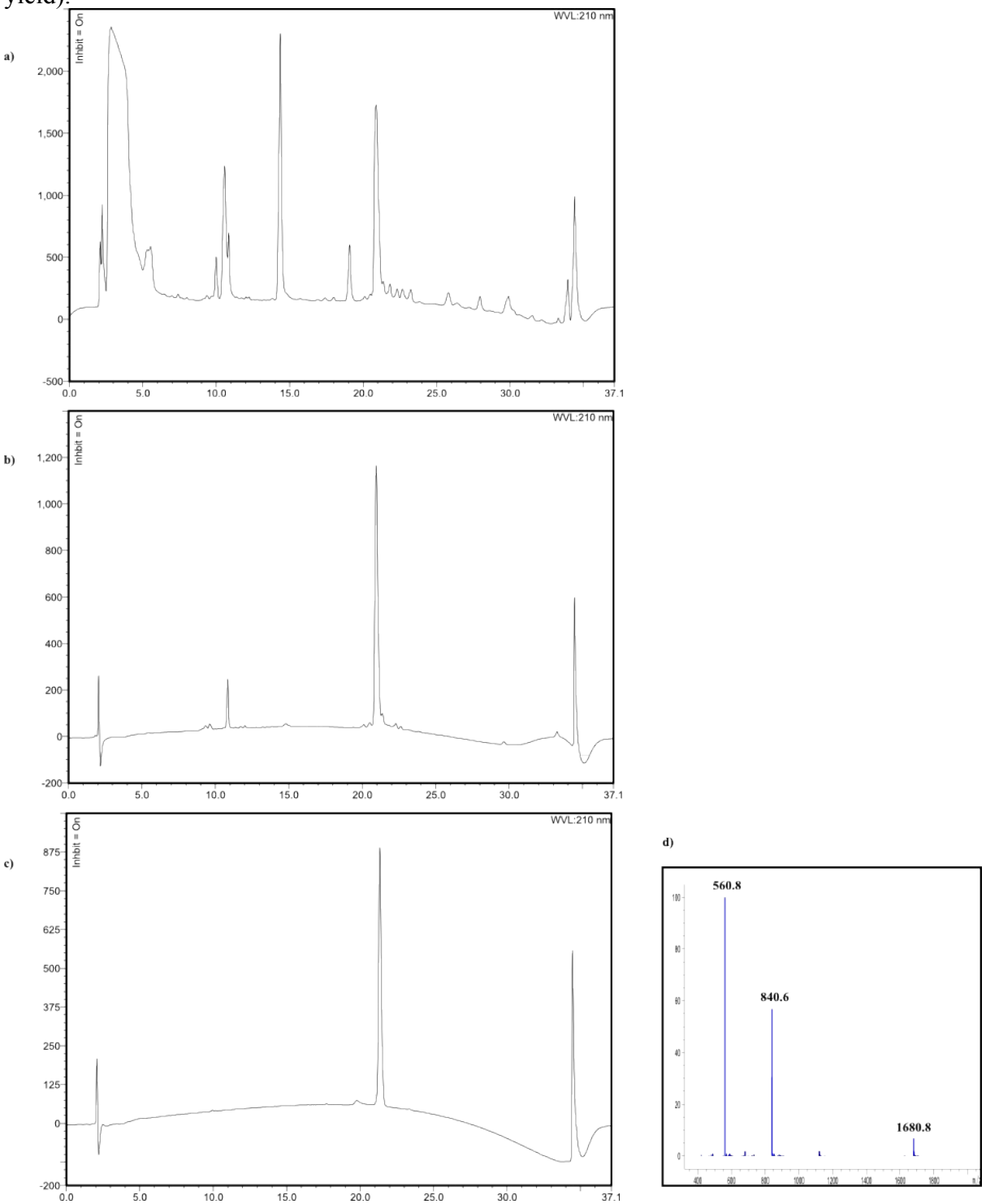


Figure S41. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**28**). a) HPLC chromatogram of CLipPA reaction monitored at 214 nm at t = 60 min 365 nm UV irradiation; b) HPLC chromatogram of reaction mixture monitored at 214 nm after diethyl ether work-up; c) HPLC chromatogram of purified peptide (**28**), after Acm removal, monitored at 214 nm; d) Observed ESI-MS for peptide (**28**), calcd. Mass: 1680.1, found 1680.8 $[M+1H]^{+1}$, 840.6 $[M+2H]^{+2}$, 560.8 $[M+3H]^{+3}$. Deconvolution affords a mass of 1679.5 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 μ; 4.6 x 150 mm) and a linear gradient of 5-95% B in 30 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

10.04 Synthesis of *S*-lipopeptide (35)

Peptide scaffold (**7**) (10 mg, 6.81 μmol) was used to obtain *S*-lipopeptide (**35**) (6.6 mg, 57% yield).

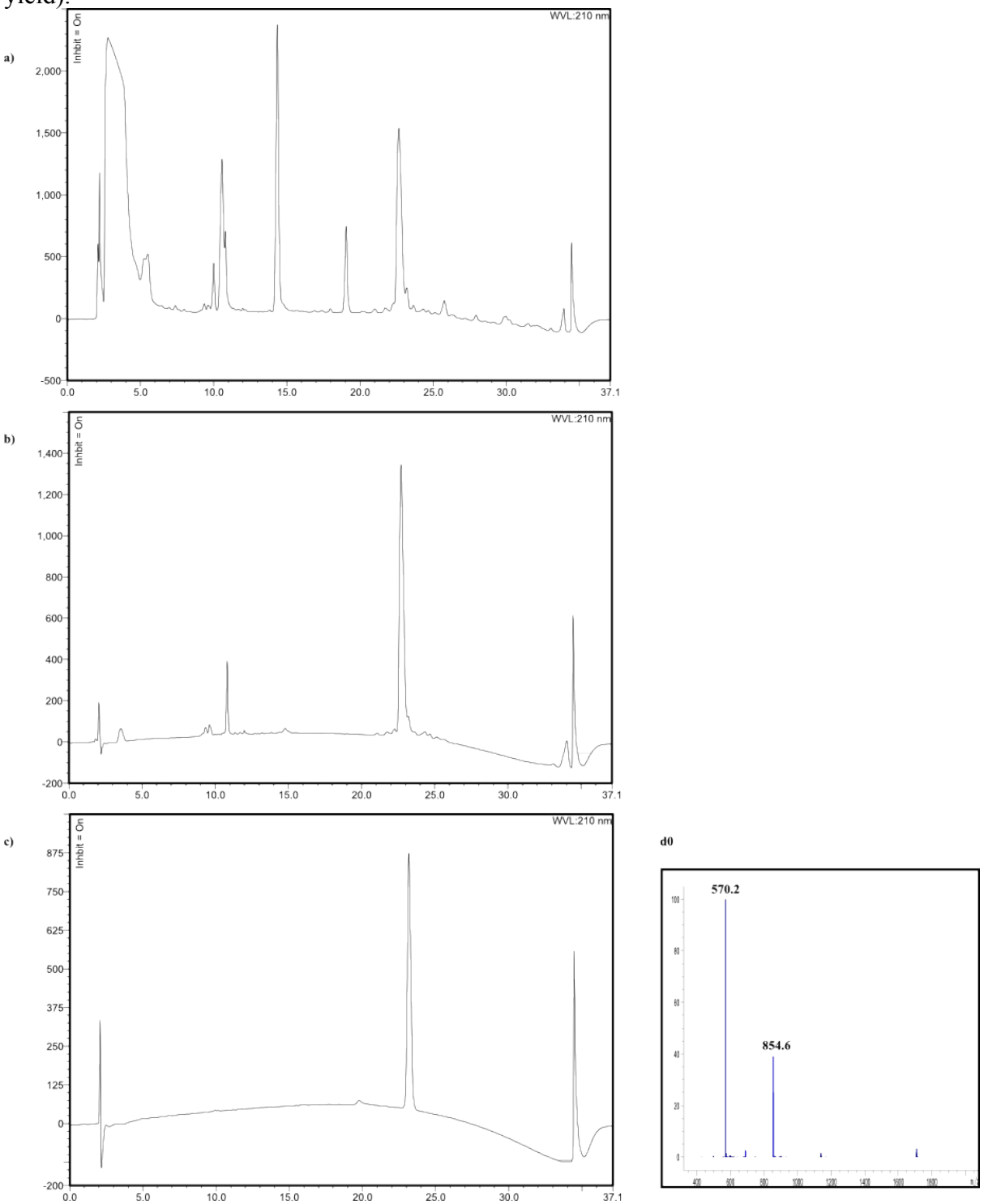


Figure S42. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**35**). a) HPLC chromatogram of CLipPA reaction monitored at 214 nm at t = 60 min 365 nm UV irradiation; b) HPLC chromatogram of reaction mixture monitored at 214 nm after diethyl ether work-up; c) HPLC chromatogram of purified peptide (**35**), after Acm removal, monitored at 214 nm; d) Observed ESI-MS for peptide (**35**), calcd. Mass: 1708.2, found 854.6 $[M+2H]^{+2}$, 570.2 $[M+3H]^{+3}$. Deconvolution affords a mass of 1707.4 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 μ; 4.6 x 150 mm) and a linear gradient of 5-95% B in 30 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

10.05 Synthesis of *S*-lipopeptide (42)

Peptide scaffold (**7**) (10 mg, 6.81 μmol) was used to obtain *S*-lipopeptide (**42**) (6.0 mg, 58% yield).

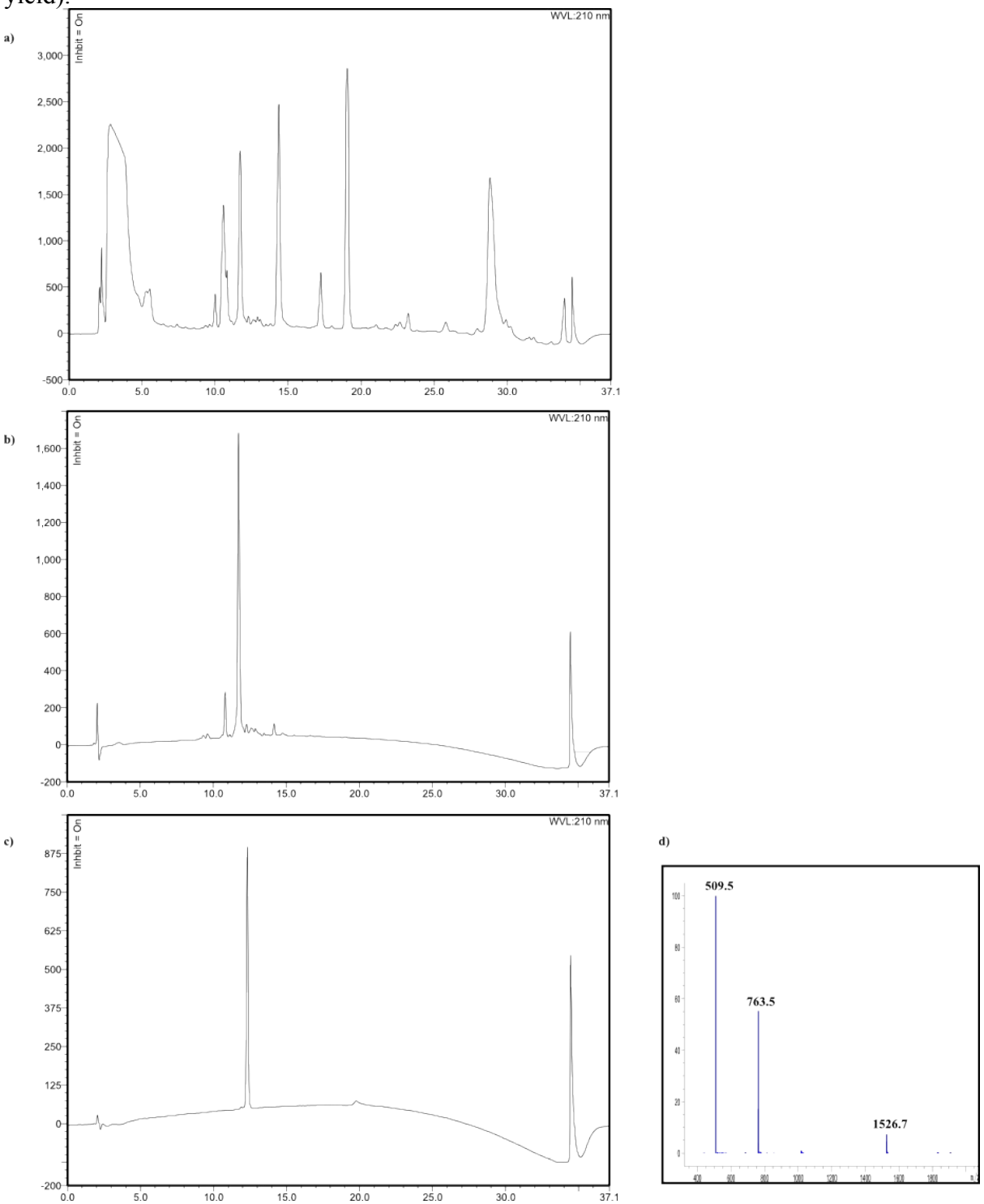


Figure S43. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**42**). a) HPLC chromatogram of CLipPA reaction monitored at 214 nm at t = 60 min 365 nm UV irradiation; b) HPLC chromatogram of reaction mixture monitored at 214 nm after diethyl ether work-up; c) HPLC chromatogram of purified peptide (**42**), after Acm removal, monitored at 214 nm; d) Observed ESI-MS for peptide (**42**), calcd. Mass: 1525.8, found 1526.7 $[M+1H]^{+1}$, 763.5 $[M+2H]^{+2}$, 509.5 $[M+3H]^{+3}$. Deconvolution affords a mass of 1525.4 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 μ; 4.6 x 150 mm) and a linear gradient of 5-95% B in 30 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

10.06 Synthesis of *S*-lipopeptide (49)

Peptide scaffold (**7**) (10 mg, 6.81 µmol) was used to obtain *S*-lipopeptide (**49**) (5.8 mg, 53% yield).

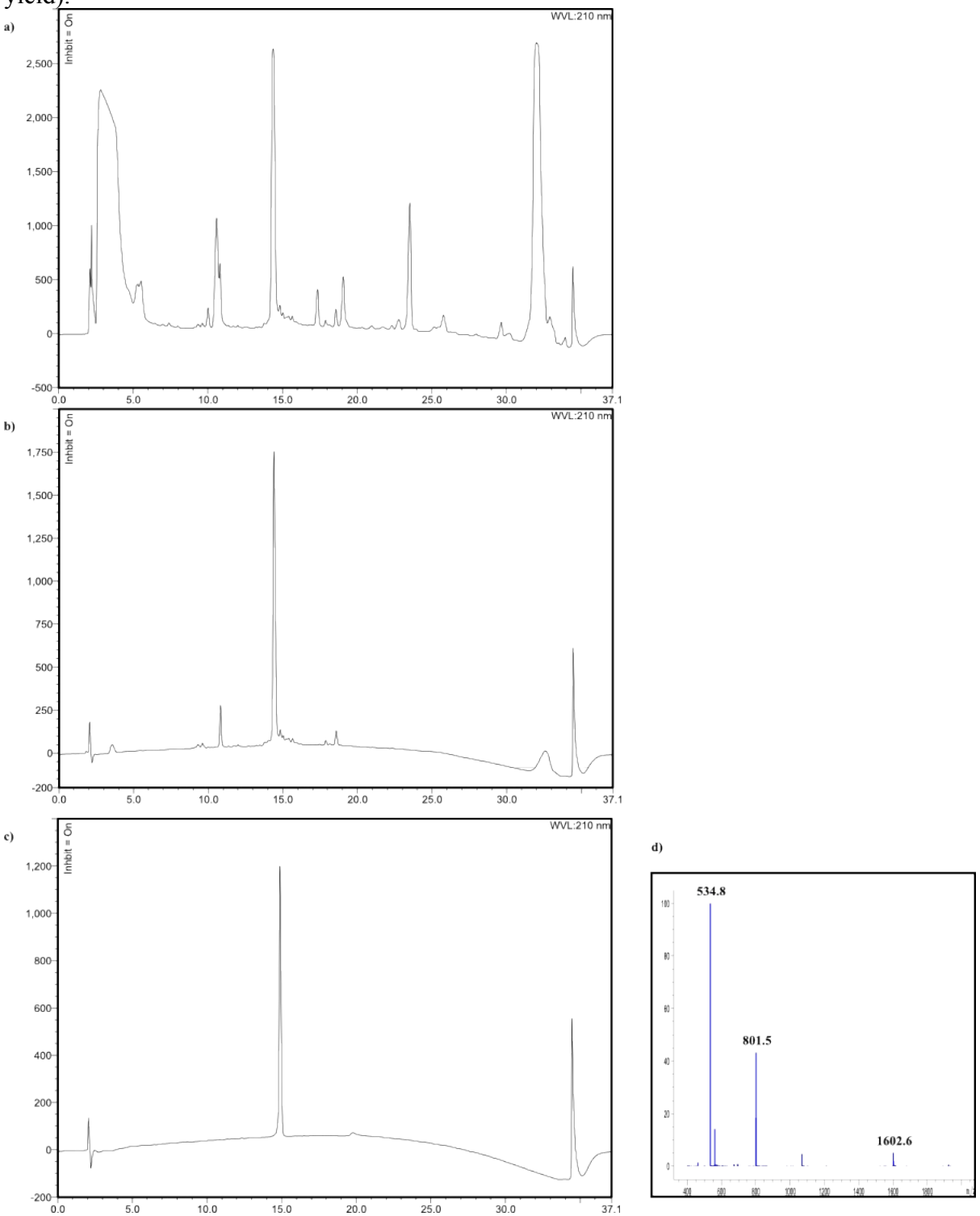
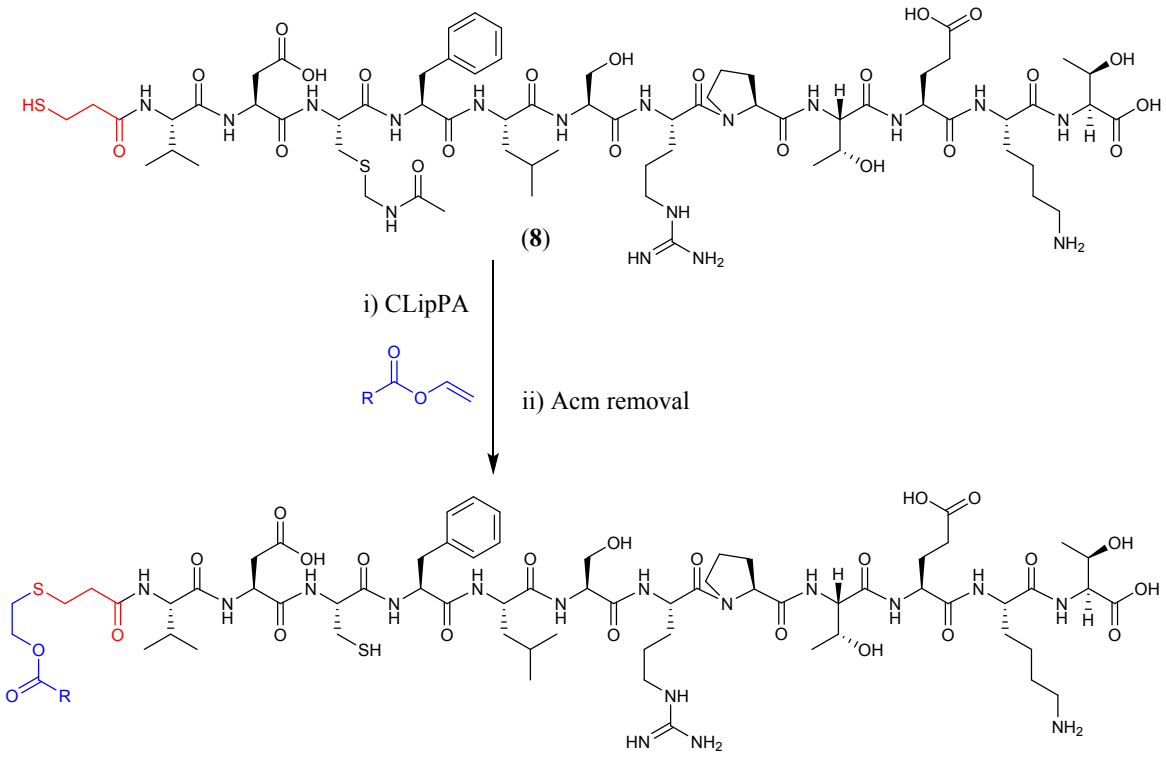


Figure S44. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**49**). a) HPLC chromatogram of CLipPA reaction monitored at 214 nm at t = 60 min 365 nm UV irradiation; b) HPLC chromatogram of reaction mixture monitored at 214 nm after diethyl ether work-up; c) HPLC chromatogram of purified peptide (**49**), after Acm removal, monitored at 214 nm; d) Observed ESI-MS for peptide (**49**), calcd. Mass: 1601.6, found 1602.6 $[M+1H]^{+1}$, 801.5 $[M+2H]^{+2}$, 534.8 $[M+3H]^{+3}$. Deconvolution affords a mass of 1601.3 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 µ; 4.6 x 150 mm) and a linear gradient of 5-95% B in 30 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

11. Synthesis of *S*-Lipopeptide Analogues (15), (22), (29), (36), (43) and (50) using peptide scaffold (8)

S-Lipopeptide analogues (**15**), (**22**), (**29**), (**36**), (**43**) and (**50**) were prepared using CLipPA reaction with peptide scaffold (**8**) and vinyl esters using **Method 6** followed by Acm removal of ^{3}Cys residue using **Method 7**.



(**15**) R = propyl
(**22**) R = nonyl
(**29**) R = pentadecyl
(**36**) R = heptadecyl
(**43**) R = *tert*-butyl
(**50**) R = 4-*tert*-butylphenyl

11.01 Synthesis of *S*-lipopeptide (15)

Peptide scaffold (**8**) (10 mg, 6.43 µmol) was used to obtain *S*-lipopeptide (**15**) (5.3 mg, 52% yield).

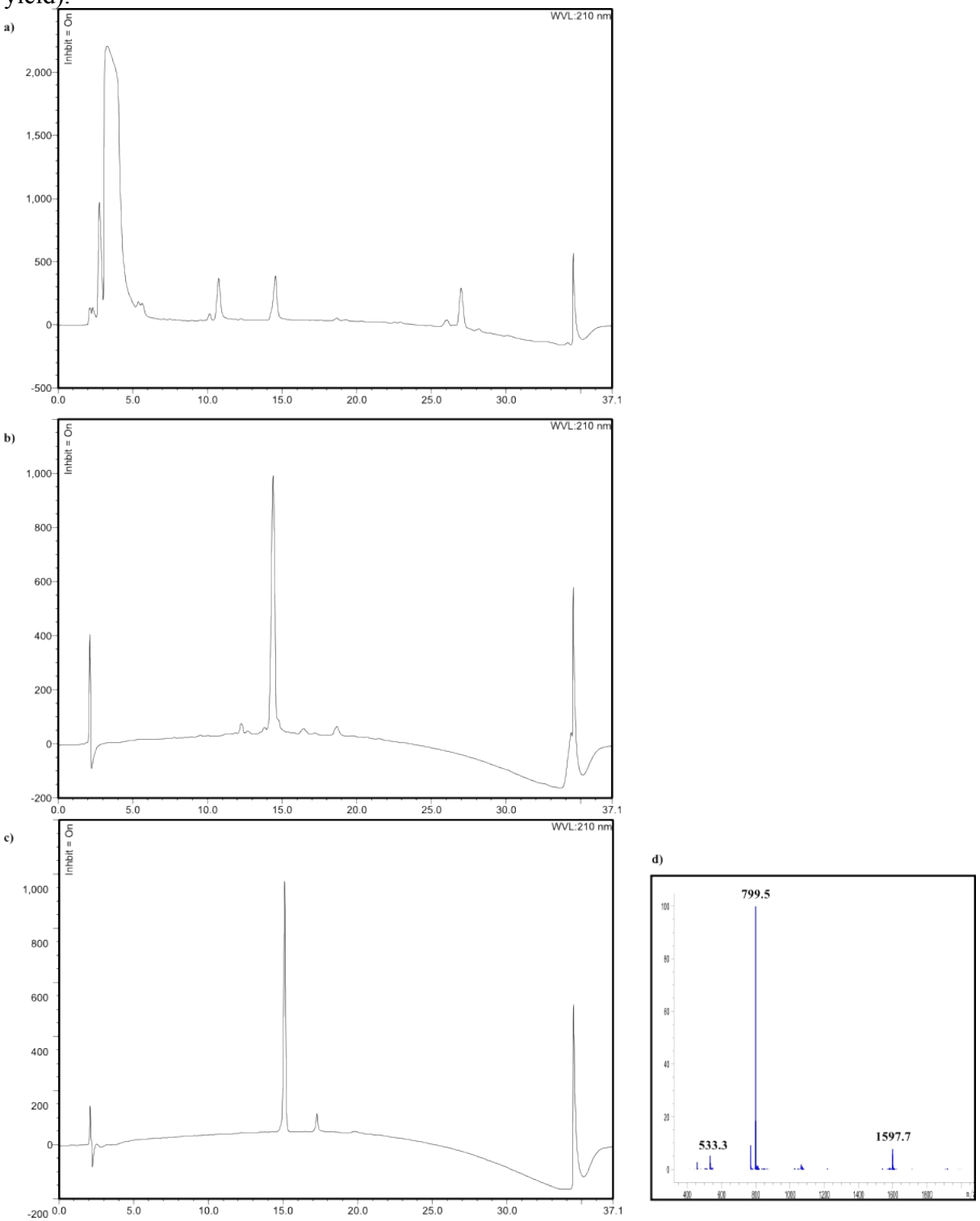


Figure S45. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**15**). a) HPLC chromatogram of CLipPA reaction monitored at 214 nm at t = 60 min 365 nm UV irradiation; b) HPLC chromatogram of reaction mixture monitored at 214 nm after diethyl ether work-up; c) HPLC chromatogram of purified peptide (**15**), after Acm removal, monitored at 214 nm; d) Observed ESI-MS for peptide (**15**), calcd. Mass: 1597.7, found 1597.7 $[M+1H]^{+1}$, 799.5 $[M+2H]^{+2}$, 533.3 $[M+3H]^{+3}$. Deconvolution affords a mass of 1596.9 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 µ; 4.6 x 150 mm) and a linear gradient of 5-65% B in 20 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

11.02 Synthesis of *S*-lipopeptide (22)

Peptide scaffold (**8**) (10 mg, 6.43 µmol) was used to obtain *S*-lipopeptide (**22**) (5.9 mg, 55% yield).

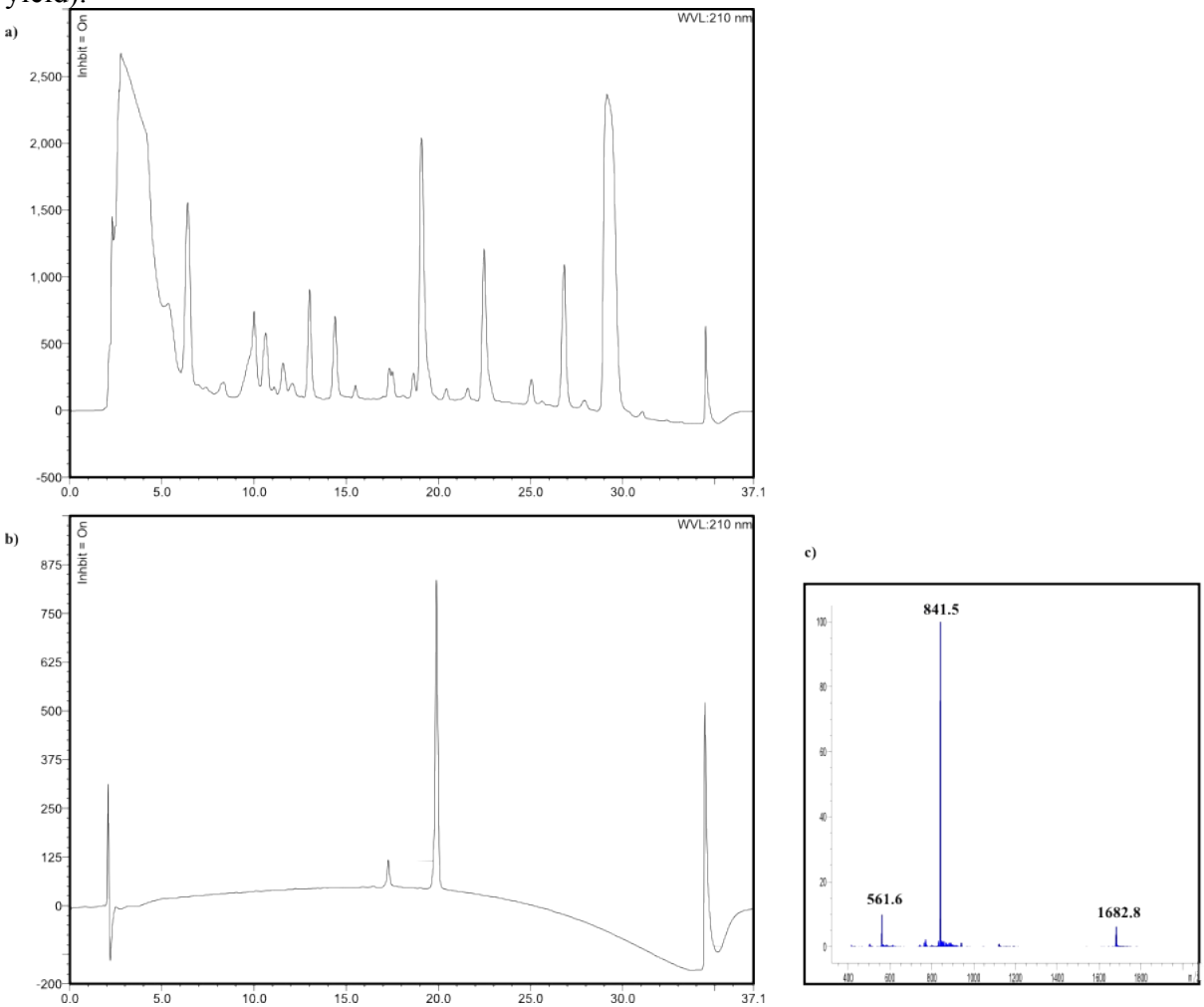


Figure S46. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**22**). a) HPLC chromatogram of CLipPA reaction monitored at 214 nm at t = 60 min 365 nm UV irradiation; b) HPLC chromatogram of purified peptide (**22**), after Acm removal, monitored at 214 nm; c) Observed ESI-MS for peptide (**22**), calcd. Mass: 1681.8, found 1682.8 $[M+1H]^{+1}$, 841.5 $[M+2H]^{+2}$, 561.6 $[M+3H]^{+3}$. Deconvolution affords a mass of 1681.5 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 µ; 4.6 x 150 mm) and a linear gradient of 5-95% B in 30 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

11.03 Synthesis of *S*-lipopeptide (29)

Peptide scaffold (**8**) (10 mg, 6.43 µmol) was used to obtain *S*-lipopeptide (**29**) (5.9 mg, 52% yield).

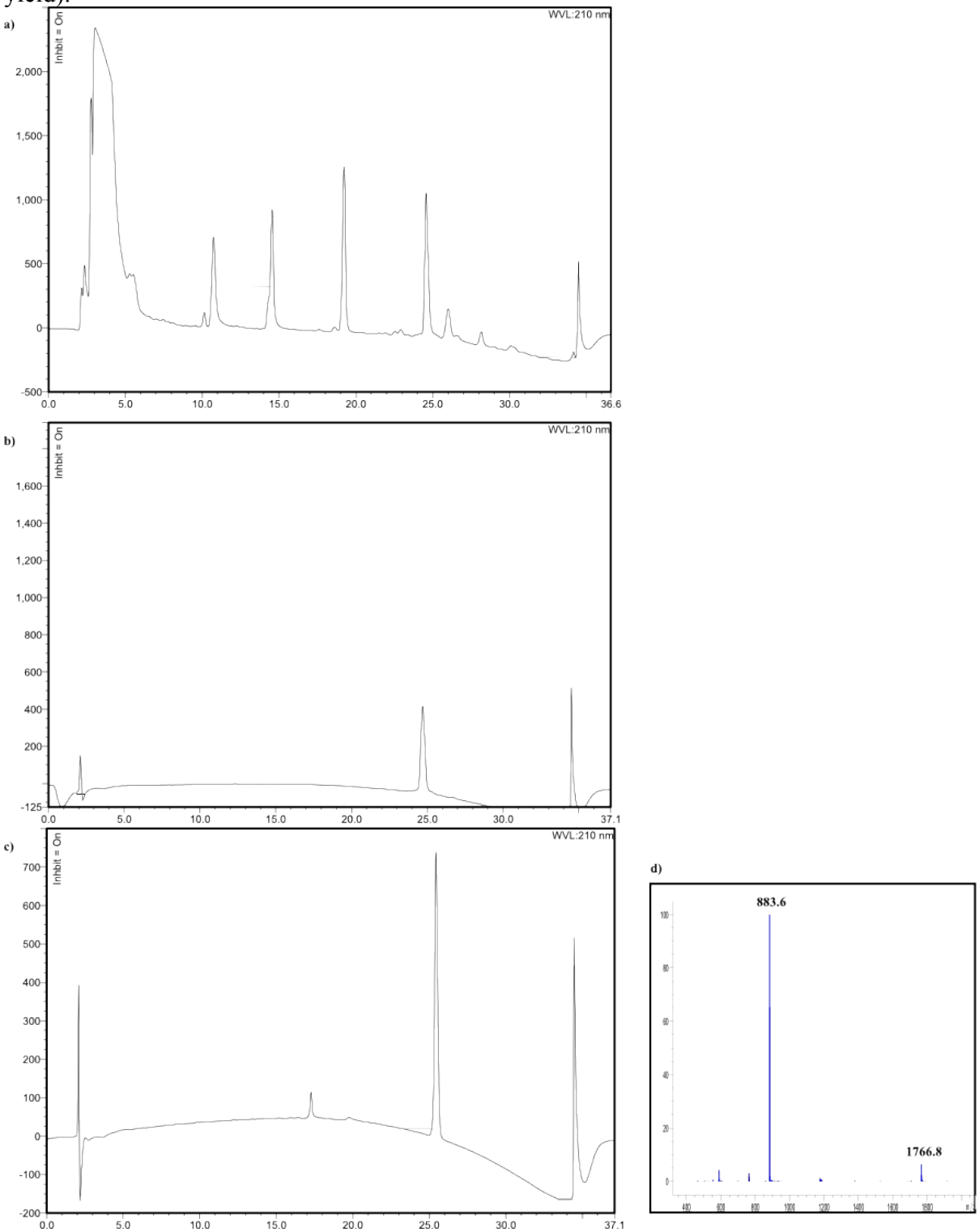


Figure S47. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**29**). a) HPLC chromatogram of CLipPA reaction monitored at 214 nm at t = 60 min 365 nm UV irradiation; b) HPLC chromatogram of reaction mixture monitored at 214 nm after diethyl ether work-up; c) HPLC chromatogram of purified peptide (**29**), after Acm removal, monitored at 214 nm; d) Observed ESI-MS for peptide (**29**), calcd. Mass: 1765.9, found 1766.8 $[M+1H]^{+1}$, 883.6 $[M+2H]^{+2}$. Deconvolution affords a mass of 1765.5 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 µ; 4.6 x 150 mm) and a linear gradient of 5-95% B in 30 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

11.04 Synthesis of *S*-lipopeptide (36)

Peptide scaffold (**8**) (10 mg, 6.43 μmol) was used to obtain *S*-lipopeptide (**36**) (5.9 mg, 51% yield).

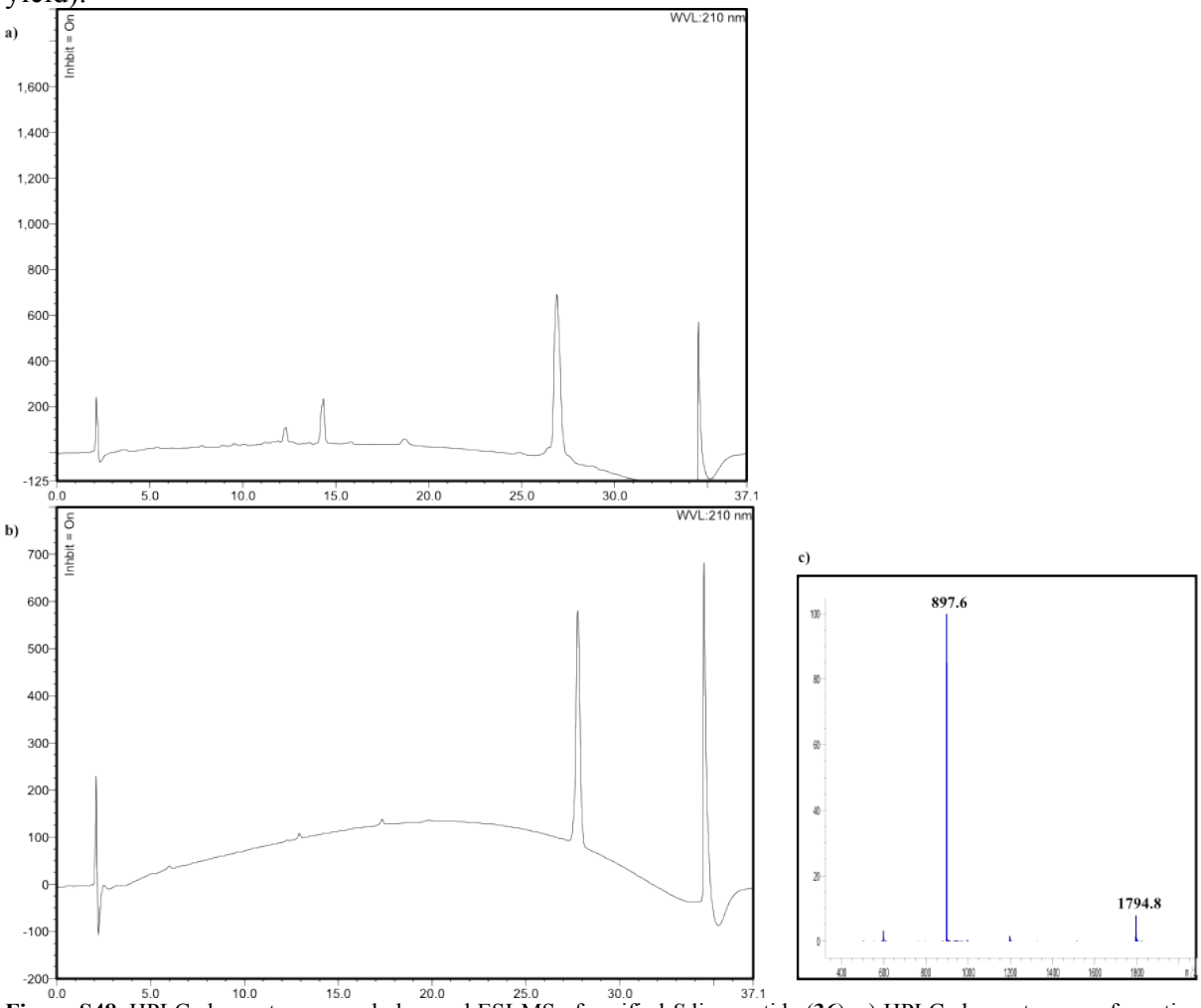


Figure S48. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**36**). a) HPLC chromatogram of reaction mixture monitored at 214 nm after diethyl ether work-up; b) HPLC chromatogram of purified peptide (**36**), after Acm removal, monitored at 214 nm; c) Observed ESI-MS for peptide (**36**), calcd. Mass: 1794.2, found 1794.8 $[M+1H]^{+1}$, 897.6 $[M+2H]^{+2}$. Deconvolution affords a mass of 1793.5 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 μ; 4.6 x 150 mm) and a linear gradient of 5-95% B in 30 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

11.05 Synthesis of *S*-lipopeptide (43)

Peptide scaffold (**8**) (10 mg, 6.43 µmol) was used to obtain *S*-lipopeptide (**43**) (5.3 mg, 51% yield).

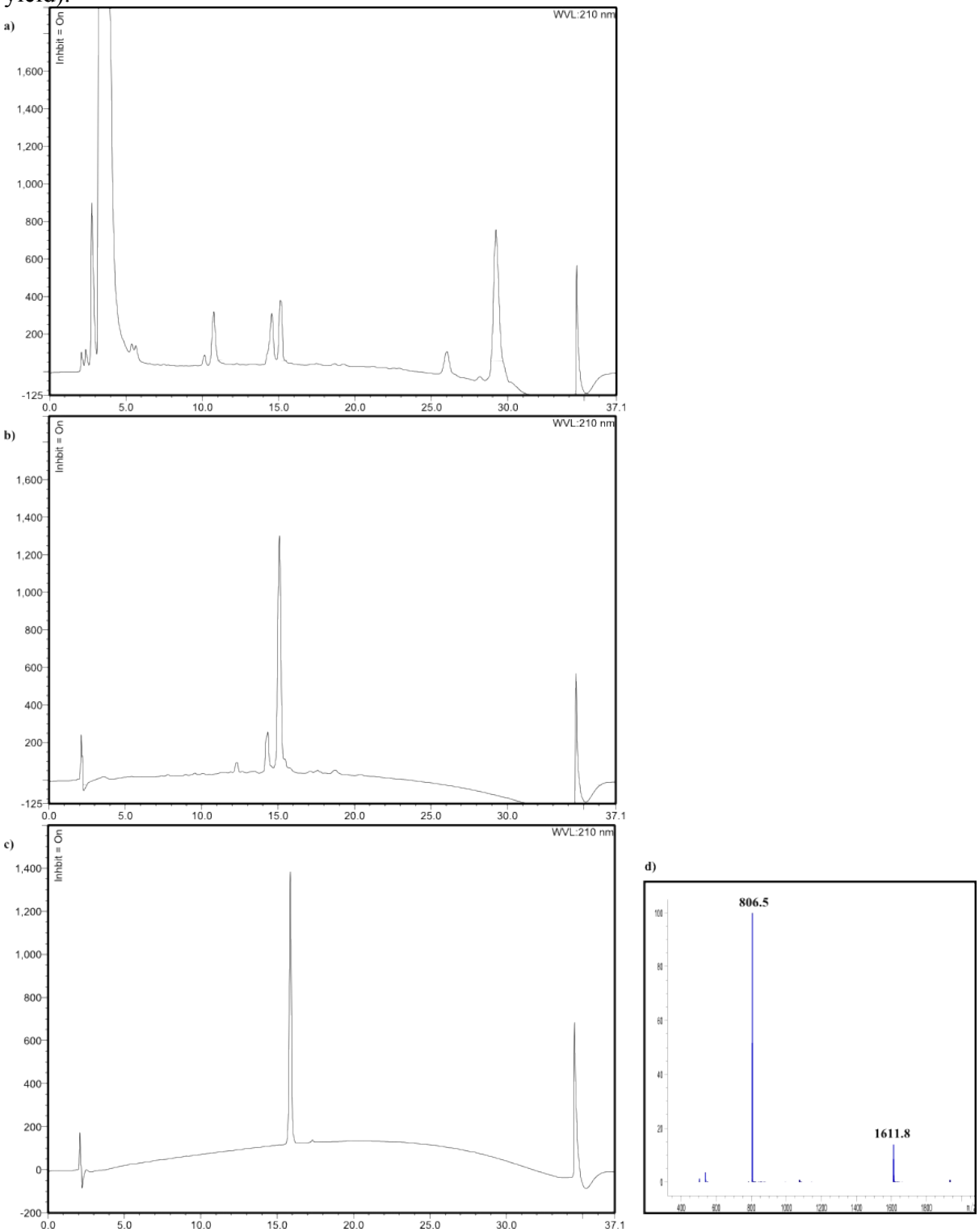


Figure S49. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**43**). a) HPLC chromatogram of CLipPA reaction monitored at 214 nm at t = 60 min 365 nm UV irradiation; b) HPLC chromatogram of reaction mixture monitored at 214 nm after diethyl ether work-up; c) HPLC chromatogram of purified peptide (**43**), after Acm removal, monitored at 214 nm; d) Observed ESI-MS for peptide (**43**), calcd. Mass: 1611.9, found 1611.8 $[M+1H]^{+1}$, 806.5 $[M+2H]^{+2}$. Deconvolution affords a mass of 1610.9 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 µ; 4.6 x 150 mm) and a linear gradient of 5-95% B in 30 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

11.06 Synthesis of *S*-lipopeptide (50)

Peptide scaffold (**8**) (10 mg, 6.43 µmol) was used to obtain *S*-lipopeptide (**50**) (6.0 mg, 55% yield).

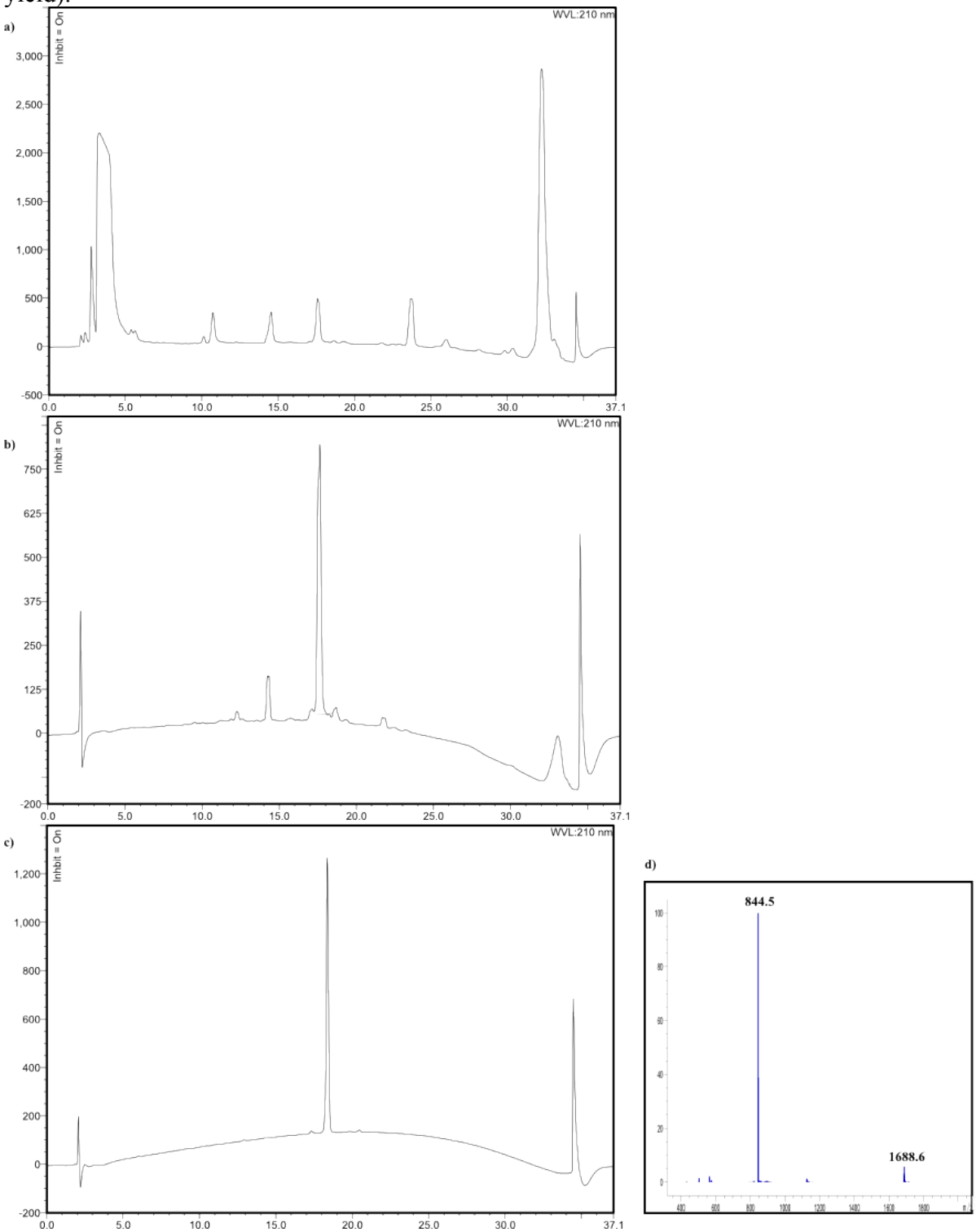


Figure S50. HPLC chromatogram and observed ESI-MS of purified *S*-lipopeptide (**50**). a) HPLC chromatogram of CLipPA reaction monitored at 214 nm at t = 60 min 365 nm UV irradiation; b) HPLC chromatogram of reaction mixture monitored at 214 nm after diethyl ether work-up; c) HPLC chromatogram of purified peptide (**50**), after Acm removal, monitored at 214 nm; d) Observed ESI-MS for peptide (**50**), calcd. Mass: 1687.9, found 1688.6 $[M+1H]^{+1}$, 844.5 $[M+2H]^{+2}$. Deconvolution affords a mass of 1687.3 Da. Chromatographic separations were performed using a Phenomenex® C18 Gemini column (5 µ; 4.6 x 150 mm) and a linear gradient of 5-95% B in 30 min, *ca*. 3% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*).

12. Bioluminescence Assay of *S*-Lipopeptide (9)-(50)

Method 8: Human cerebral microvascular endothelial cell culture

Human cerebral microvascular endothelial cells (hCMVEC) were cultured at 37 ^{o}C and 5 % CO_2 in collagen I-coated (1 μg/cm^2) T-75 flasks in 7-8 mL of modified M199 growth media replenished every 2-3 days. Cells were monitored daily by light microscopy for culture health and sterility. Upon reaching 80-90 % confluency, cultures were washed in phosphate-buffered saline (PBS) and passaged by gentle dissociation in 0.05 % trypsin for 2 min before being split in a 1:4 - 1:6 ratio in fresh media for continuation of culture or plated for bioluminescence experimental studies. All experiments were conducted in cultures within passages 8-17, where we have observed consistent cell growth profiles and formation of endothelial monolayers.

Method 9: General procedure for inducing hemichannel opening and bioluminescence ATP assay

Dissociated hCMVECs were plated at 2 x 10^6 / well into 96 well plates coated with 1 μg/cm^2 collagen-I and incubated for 48 hours to allow formation of a confluent monolayer. For the practical purposes of sample handling and controlling the duration of exposure to treatment, each assay contained a set of twelve conditions. Of these, 8 were lipopeptides of interest and 4 were control groups common to all assays performed; normal Ringers solution (NR), hypoxic, acidic, ion-shifted Ringers solution (HAIR), HAIR + Peptide5 (**1**), and HAIR + carbenoxolone (CBX). These controls allowed for consistency across assays when collating data on the large number of *S*-lipopeptides screened. For each assay plate, lipopeptides were selected to ensure a range of modification type and placement to avoid any bias that may occur in a particular single experiment. Each treatment group was carried out in duplicate within the assay plate.

On the day of experimentation, cells were washed four times in fresh NR (37 ^{o}C) to remove culture media and then twice more in either NR or HAIR. The cells were then left in a final 200 μL of the respective solutions. HAIR was either applied alone, containing 10 μM CBX, 10 μM Peptide5 (**1**), or 10 μM *S*-lipopeptide (**9-50**) and all conditions incubated for a further 2 h. Following this, supernatant samples were then diluted 1:10 in firefly luciferase ATP detection mixture in an opaque 96 well plates. Each sample was detected in triplicate to aid accuracy in plate reading. The detection plate was immediately taken for reading in a CLARIOstar® spectrophotometric plate reader (BMG Labtech, Germany). The bioluminescence at 560 nm of the ATP-dependent luciferin-luciferase reaction was sampled five times between 7 and 21 minutes after the initiation of the detection reaction to allow for detection to occur during the plateau period of the reaction. An ATP standard curve (0 - 50 nM) was generated and run during every detection to allow the conversion of bioluminescence units to a molar concentration.
Assays were repeated until each *S*-lipopeptide had been assayed in 3 separate experiments (n=3). To collate the large set of experimental data, concentrations were expressed relative to the injury-only HAIR control (100%) within each assay and the mean value of the 3 experiments calculated. The values presented for NR, HAIR, HAIR + Peptide5 (**1**), and HAIR + CBX controls are the product of n=16 repeats as these were present in all assays performed.

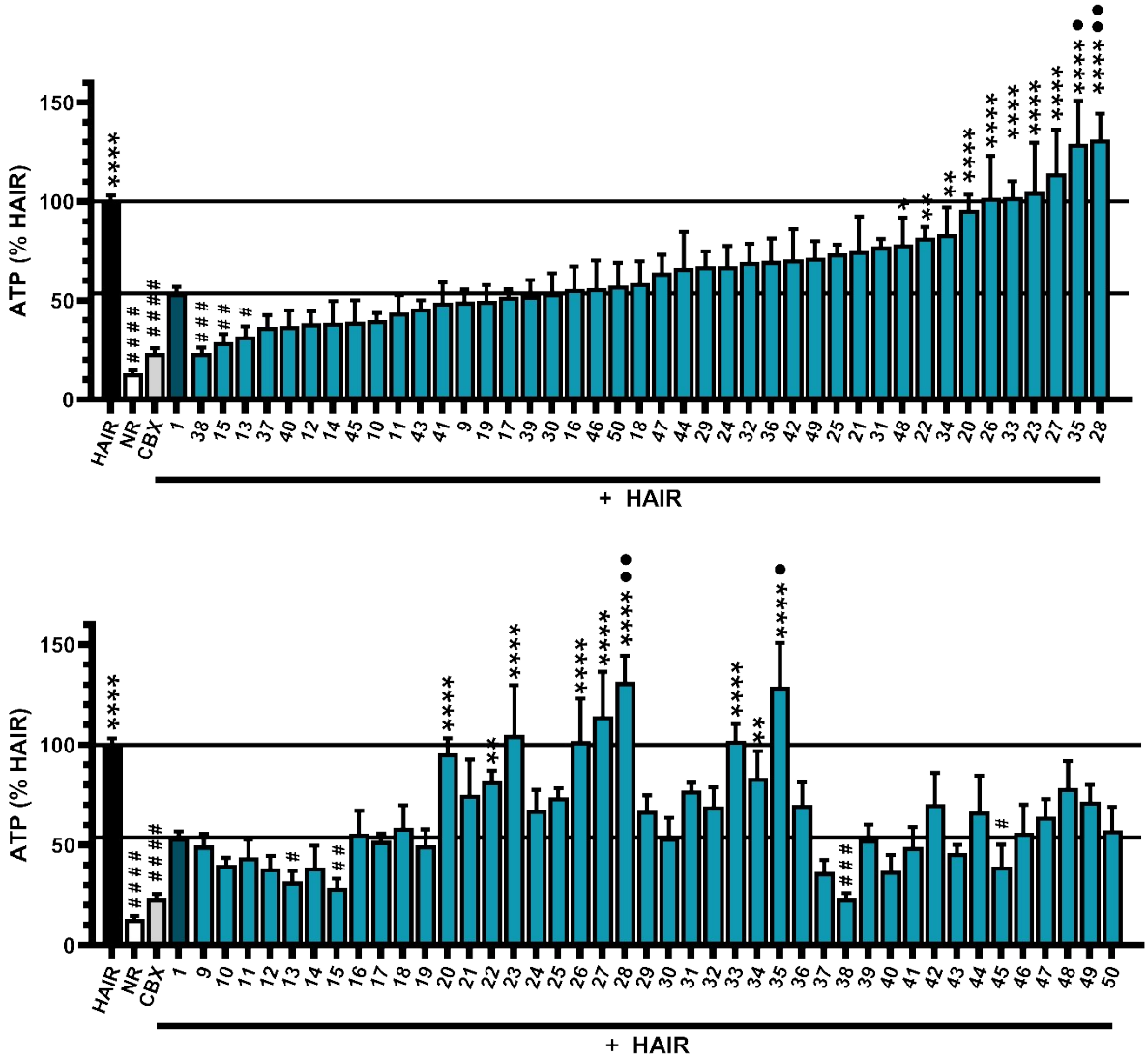


Figure S51: *S*-lipopeptide analogues (**9**)-(**50**) demonstrate variable bioactivity in *in vitro* ischaemic injury model. A line at 53.75 % on each figure indicates the level of native Peptide5 (**1**) control. A line at 100 % on each figure indicates the level of HAIR control. All values represent mean ± SEM % relative to untreated injury (HAIR). n=3 (*S*-lipopeptides), and n=16 (HAIR, NR, HAIR + CBX, HAIR + **1**). One-way ANOVA with multiple comparisons vs Peptide5 **(1)**, * $p < 0.05$, ** $p < 0.01$, **** $p < 0001$. * indicates a change above Peptide5 **(1)**, # indicates a change below Peptide5. ● indicates a change above HAIR control.

13. Rat serum stability assay

Method 10: General procedure for rat serum stability assay[2,3]

S-Lipopeptide analogues (**15**), (**17**), (**25**), (**34**), (**38**) and (**45**) were dissolved in Milli-Q water at a concentration of 1 mg/50 µL (from a peptide stock solution concentration: 20 mg/L in Milli-Q water). Each of 20 mg/L stock solution of analogues were incubated with 25% rat serum (v/v) in PBS buffer (pH 7.4) to make up a final peptide concentration of 150 µg/L. Incubation was carried at 37° C and a 100 µL sample was taken out at the time interval of 0, 2, 4, 6, 8, 10, 15, 20, 40, 60 min for each replicate. The sample at each time-point was quenched by the addition of 100 µL MeCN and 25 µL of 15% (*w*/*v*) aqueous trichloroacetic acid solution to precipitate serum proteins. The mixture was stored at 4° C for overnight. An HPLC sample was prepared by centrifugation at 14500 rpm for 20 minutes using a microcentrifuge. 100 µL of supernatant was removed and analyzed by reverse-phase HPLC using an analytical C18 symmetry column (Waters®, 3.5 µm, 100 Å, 4.6 × 75 mm). The assays were performed in triplicate. The relative amount of remaining peptide (measured in % relative to the t = 0 min control) was analyzed by integration of the area underneath the desired peptide peak monitored at 210 nm.

14. References

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