Supporting information

First-in-class cyclic Temporin L analogue: design, synthesis and antimicrobial assessment

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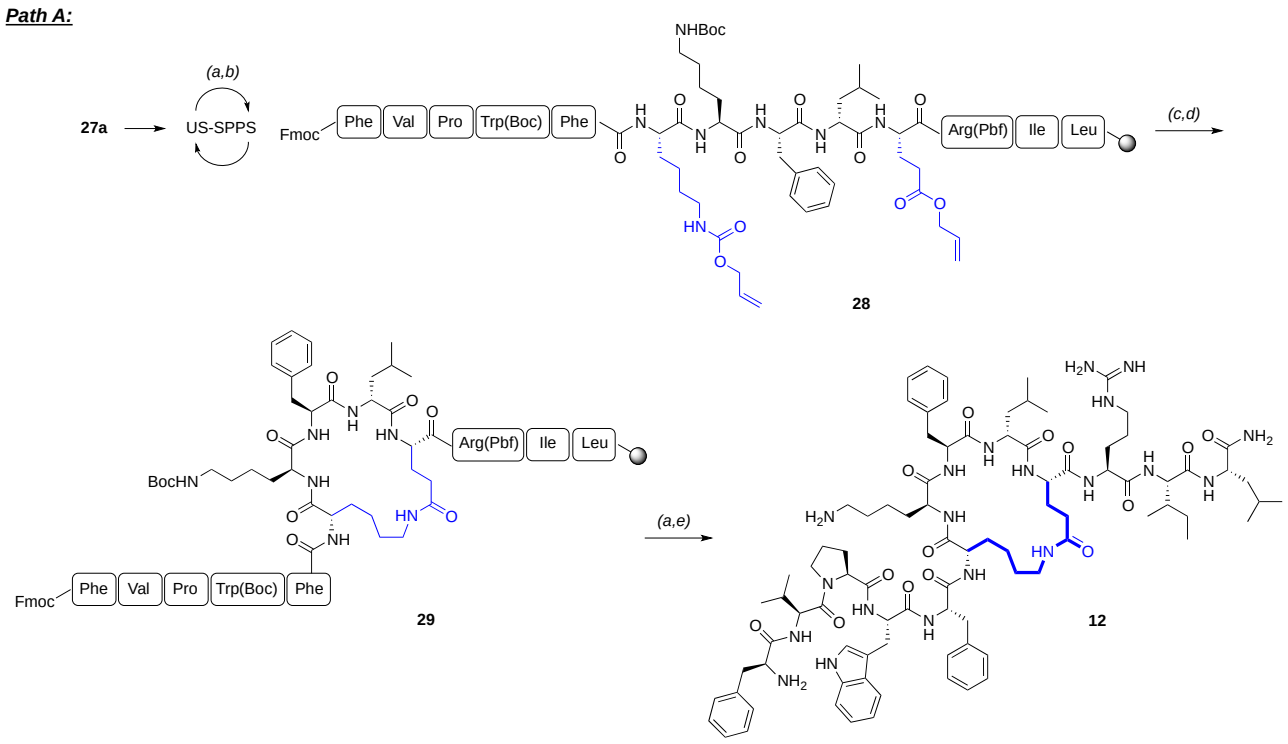
1. Synthetic paths for the achievement of key peptides **12**, **17**, **25** and **26**.



Scheme S1. (a) US-SPPS - Fmoc-deprotection: 20% piperidine in DMF, 0.5 + 1 min; (b) US-SPPS coupling: Fmoc-AA (2 equiv), COMU (2 equiv), Oxyma (2 equiv), DIEA (4 equiv), 5 min; (c) $Pd(PPh_3)_4$ (0.15 equiv), 1,3-dimethylbarbituric acid (3 equiv), DCM/DMF (3:2, *v/v*), 2 × 1 h; (d) PyAOP (2 equiv), HOAt (2 equiv), DIEA (4 equiv), DCM/DMF (1:1, *v/v*), 16 h; (e) TFA/TIS/H_2O (95:2.5:2.5, *v/v/v*), 3 h.

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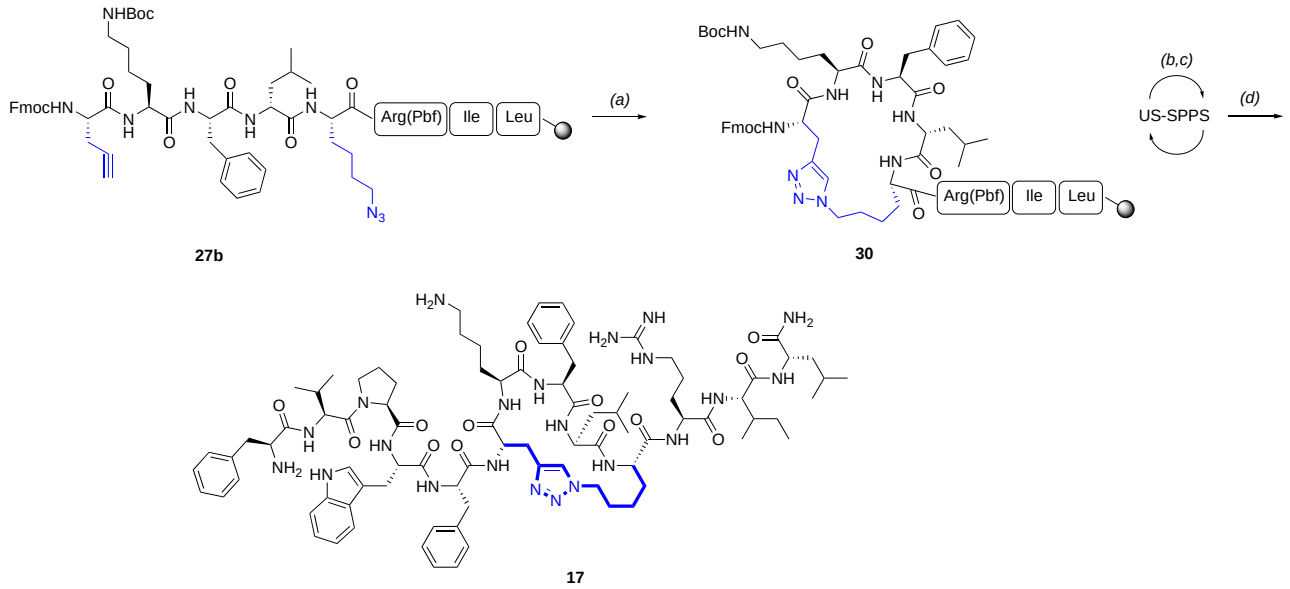
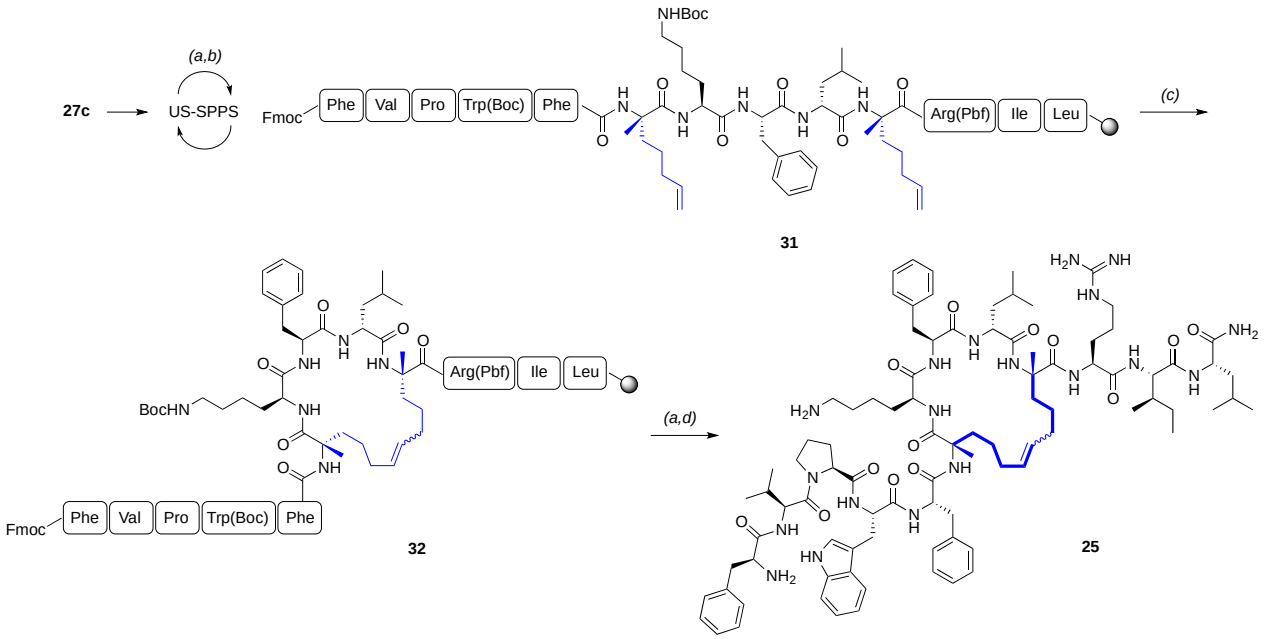


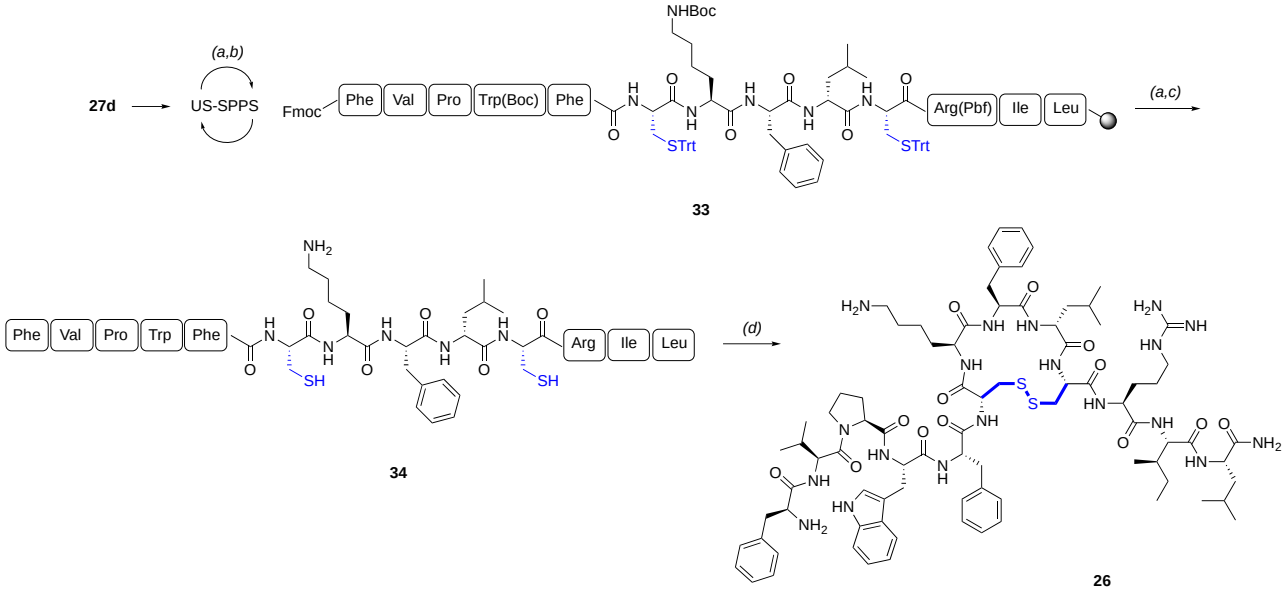
Scheme S2. (a) CuI (1 equiv), ascorbic acid (3 equiv), 2,4,6-collidine (5 equiv), DIEA (10 equiv), DMF, 3 × 1 h; (b) US-SPPS Fmoc-deprotection: 20% piperidine in DMF, 0.5 + 1 min; (c) US-SPPS coupling: Fmoc-AA (2 equiv), COMU (2 equiv), Oxyma (2 equiv), DIEA (4 equiv), 5 min; (d) TFA/TIS/H_2O (95:2.5:2.5, *v/v/v*), 3 h.

Path C:



Scheme S3. (a) US-SPPS Fmoc-deprotection: 20% piperidine in DMF, 0.5 + 1 min; (b) US-SPPS coupling: Fmoc-AA (2 equiv), COMU (2 equiv), Oxyma (2 equiv), DIEA (4 equiv), 5 min; (c) 6 mM solution (3 mL) of the Grubbs' 1st generation catalyst in DCE (20 mol% relative to the resin substitution), 3 × 2 h, under nitrogen; (d) TFA/TIS/H_2O (95:2.5:2.5, *v/v/v*), 3 h.

Path D:



Scheme S4. (a) US-SPPS Fmoc-deprotection: 20% piperidine in DMF, 0.5 + 1 min; (b) US-SPPS coupling: Fmoc-AA (2 equiv), COMU (2 equiv), Oxyma (2 equiv), DIEA (4 equiv), 5 min; (c) TFA/TIS/H_2O (95:2.5:2.5, *v/v/v*), 3 h; (d) NCS (1 equiv), H_2O for [peptide]= 0.5 mM, 15 min.

Table S1. Analytical data for peptides **10**-**26**.

ID	t_R (min)	Molecular Formula	$[M+H]^+_{calcd}$	$[M+2H]^+/2_{obs}$
10	11.6	$C_{82}H_{117}N_{20}O_{15}^+$	1621.9002	811.9556
11	11.4	$C_{79}H_{119}N_{20}O_{15}^+$	1587.9158	794.4611
12	13.9	$C_{89}H_{131}N_{20}O_{14}^+$	1704.0148	853.0122
13	11.3	$C_{79}H_{118}N_{20}O_{15}^+$	1586.9080	794.9626
14	14.7	$C_{74}H_{119}N_{19}O_{15}^+$	1513.9128	757.9648
15	11.6	$C_{82}H_{115}N_{22}O_{14}^+$	1631.8958	816.4502
16	11.3	$C_{79}H_{117}N_{22}O_{14}^+$	1597.9114	799.9601
17	14.5	$C_{89}H_{129}N_{22}O_{13}^+$	1714.0104	858.0162
18	13.2	$C_{79}H_{117}N_{22}O_{14}^+$	1597.9114	799.9601
19	11.7	$C_{74}H_{118}N_{21}O_{14}^+$	1524.9162	761.9532
20	12.1	$C_{85}H_{122}N_{20}O_{14}^+$	1646.9444	824.9761
21	11.4	$C_{79}H_{119}N_{20}O_{15}^+$	1587.9158	794.4587
22	11.6	$C_{85}H_{121}N_{22}O_{13}^+$	1657.9478	829.9784
23	11.2	$C_{79}H_{117}N_{22}O_{14}^+$	1597.9114	799.4593
24	13.9	$C_{89}H_{131}N_{20}O_{14}^+$	1704.0148	853.0121
25	14.9	$C_{92}H_{136}N_{19}O_{13}^+$	1715.0560	858.0627
26	14.2	$C_{84}H_{122}N_{19}O_{13}S_2^+$	1668.8905	835.8973

Table S2. ^{1}H NMR resonance assignments[a] of peptide **12** in SDS/DPC 9:1 solution

residue	NH (-Δδ/ΔT)[b]	$C^{\alpha}H$	$C^{\beta}H$	Others
Phe 1		4.43	3.23, 3.17	7.73(δ); 7.27 (ε)
Val 2	7.74 (3.7)	4.19	1.79	0.69, 0.63(γ)
Pro 3		4.39	2.26	2.01(γ); 3.70(δ)
Trp 4	7.56 (2.9)	4.29	3.26. 3.19	7.36(δ); 10.00, 7.21(ε); 7.29, 6.81(ζ); 6.88(η)
Phe 5	7.84 (nd)	4.19	2.89, 2.62	7.08(δ); 7.17(ε)
Lys* 6	7.84 (7.5)	3.85	1.56, 1.36	1.19(γ); 1.46(δ); 2.65(ε)
Lys 7	7.81 (2.9)	3.97	1.85	1.48(γ); 1.66(δ); 2.94(ε); 7.38(ζ)
Phe 8	8.19 (5.2)	4.24	3.07, 2.97	7.11(δ); 7.16(ε)
DLeu 9	8.63 (5.0)	3.69	1.78	1.387(γ); 0.70, 0.64(δ)
Glu*10	7,94 (2.9)	3.82	1.97	2.31, 2.20(γ)
Arg 11	7.96 (2.2)	4.07	1.97	1.70, 1.59(γ); 3.25, 3.13(δ); 7.26(ε)
Ile 12	7.51 (3.1)	3.91	1.72	1.04, 1.00, 0.84 (γ); 0.76(δ)
Leu 13	8.04 (6.0)	4.26	1.53	1.68(γ); 0.85(δ)

[a] Obtained at 25 °C, pH= 5.5, with TSP (δ 0.00 ppm) as reference shift. Chemical shifts are accurate to ±0.02 ppm. -Δδ/ΔT = temperature coefficients (ppb/K); nd: not determined. $CONH_2$ (C-terminal amide protons) 7.00, 6.86. *Cyclic residues.

Table S3. NOE derived upper limit constraints used for peptide **12** structure calculation.

1	PHE	HA	1	PHE	HB2	3.02
1	PHE	HA	1	PHE	HB3	3.02
1	PHE	HA	1	PHE	QD	5.64
1	PHE	HA	2	VAL	HN	2.74
1	PHE	HB2	2	VAL	HN	3.55
1	PHE	HB3	2	VAL	HN	3.55
1	PHE	QD	2	VAL	HN	6.88
1	PHE	QD	2	VAL	HA	7.22
1	PHE	QD	2	VAL	QG1	8.40
1	PHE	QD	2	VAL	QG2	8.40
1	PHE	QD	2	VAL	QQG	6.93
1	PHE	QE	2	VAL	HA	7.62
1	PHE	QE	2	VAL	QG1	8.65
1	PHE	QE	2	VAL	QG2	8.65
2	VAL	HN	2	VAL	HB	3.05
2	VAL	HN	2	VAL	QQG	4.15
2	VAL	HN	3	PRO	QD	6.16
2	VAL	HA	2	VAL	QG1	3.83
2	VAL	HA	2	VAL	QG2	3.83
2	VAL	HA	3	PRO	QD	3.87
2	VAL	HB	3	PRO	QD	5.45
2	VAL	HB	4	TRP	HD1	5.07
2	VAL	HB	4	TRP	HE1	4.85
2	VAL	QG1	4	TRP	HE1	5.78
2	VAL	QG2	4	TRP	HE1	5.78
2	VAL	QQG	4	TRP	HD1	5.35
2	VAL	QQG	4	TRP	HE1	4.95
3	PRO	HA	7	LYS	HN	3.92
3	PRO	QD	4	TRP	HD1	6.38
4	TRP	HN	4	TRP	HB2	3.42
4	TRP	HN	4	TRP	HB3	3.42
4	TRP	HN	4	TRP	QB	2.96
4	TRP	HN	4	TRP	HD1	3.36
4	TRP	HN	5	PHE	HN	4.17
4	TRP	HA	4	TRP	HB2	2.80
4	TRP	HA	4	TRP	HB3	2.80
4	TRP	HA	4	TRP	HD1	3.73
4	TRP	HA	4	TRP	HE3	3.89
4	TRP	HA	7	LYS	QB	4.49
4	TRP	HB2	4	TRP	HD1	3.61
4	TRP	HB3	4	TRP	HD1	3.61
4	TRP	QB	4	TRP	HD1	3.07
4	TRP	QB	4	TRP	HE3	3.38
5	PHE	HN	5	PHE	HB2	3.52
5	PHE	HN	5	PHE	HB3	3.52
5	PHE	HN	5	PHE	QB	3.01
5	PHE	HA	5	PHE	QD	6.85
5	PHE	HA	8	PHE	HN	5.50
6	LYSS	HN	8	PHE	HN	4.23
6	LYSS	HA	7	LYS	HN	3.39
6	LYSS	HA	9	DLEU	HN	3.76
6	LYSS	QB	6	LYSS	QE	4.06
7	LYS	HN	7	LYS	HA	2.80

7	LYS	HN	7	LYS	QB	3.62
7	LYS	HN	7	LYS	QG	5.20
7	LYS	HN	7	LYS	QD	5.17
7	LYS	HN	8	PHE	HN	3.08
7	LYS	HA	10	GLUS	HN	3.61
7	LYS	HA	10	GLUS	QG	4.03
7	LYS	QB	7	LYS	QE	7.25
7	LYS	QB	7	LYS	QZ	7.19
7	LYS	QG	7	LYS	QZ	7.19
8	PHE	HN	8	PHE	HB2	3.17
8	PHE	HN	8	PHE	HB3	3.17
8	PHE	HN	8	PHE	QD	7.62
8	PHE	HN	9	DLEU	HN	3.64
8	PHE	HA	8	PHE	HB2	2.93
8	PHE	HA	8	PHE	HB3	2.93
8	PHE	HA	8	PHE	QB	2.55
8	PHE	HA	8	PHE	QD	5.45
8	PHE	HA	8	PHE	QE	6.35
8	PHE	HA	11	ARG+	HN	3.76
8	PHE	HA	11	ARG+	QB	4.33
8	PHE	HA	12	ILE	HN	4.32
8	PHE	QB	9	DLEU	HN	3.40
8	PHE	QD	9	DLEU	HN	7.62
9	DLEU	HN	9	DLEU	QQD	5.35
9	DLEU	HN	10	GLUS	HN	3.36
9	DLEU	HA	9	DLEU	QD1	4.67
9	DLEU	HA	9	DLEU	QD2	4.67
9	DLEU	HA	9	DLEU	QQD	3.62
9	DLEU	HA	10	GLUS	HN	3.42
10	GLUS	HN	10	GLUS	QB	4.14
10	GLUS	HN	10	GLUS	HG2	3.33
10	GLUS	HN	10	GLUS	HG3	3.33
10	GLUS	HN	10	GLUS	QG	2.82
10	GLUS	HA	10	GLUS	HG2	3.79
10	GLUS	HA	10	GLUS	HG3	3.79
10	GLUS	HA	13	LEU	HN	3.61
10	GLUS	HA	14	CNH2	HN1	4.51
10	GLUS	HA	14	CNH2	HN2	4.85
11	ARG+	HN	11	ARG+	QB	4.11
11	ARG+	HN	12	ILE	HN	3.27
11	ARG+	HA	11	ARG+	HG2	3.76
11	ARG+	HA	11	ARG+	HG3	3.76
11	ARG+	HA	11	ARG+	QG	3.23
11	ARG+	HA	11	ARG+	QD	3.71
11	ARG+	HA	12	ILE	HN	3.64
11	ARG+	HA	14	CNH2	HN1	5.50
11	ARG+	QB	11	ARG+	HE	5.38
11	ARG+	QB	12	ILE	HN	4.24
11	ARG+	HG2	11	ARG+	HE	3.98
11	ARG+	HG3	11	ARG+	HE	3.98
11	ARG+	QG	11	ARG+	HE	3.37
11	ARG+	QG	12	ILE	HN	4.47
12	ILE	HN	12	ILE	HB	2.83
12	ILE	HN	12	ILE	QG2	4.67
12	ILE	HN	12	ILE	QG1	3.39
12	ILE	HN	12	ILE	QD1	5.54
12	ILE	HN	13	LEU	HN	2.74

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12 ILE  HN     13 LEU  QQD     7.63
12 ILE  HN     14 CNH2 HN1     5.50
12 ILE  HA     12 ILE  QG2     3.68
12 ILE  HA     12 ILE  QG1     3.31
12 ILE  HA     12 ILE  QD1     4.08
12 ILE  HA     13 LEU  HN      3.36
12 ILE  HB     13 LEU  HN      2.90
12 ILE  QG2    12 ILE  QG1     3.30
12 ILE  QG2    13 LEU  HN      4.76
13 LEU  HN     13 LEU  HA      2.86
13 LEU  HN     13 LEU  QB      4.18
13 LEU  HN     13 LEU  HG      2.43
13 LEU  HN     13 LEU  QQD     6.02
13 LEU  HN     14 CNH2 HN1     3.30
13 LEU  HN     14 CNH2 HN2     3.92
13 LEU  HA     13 LEU  HG      3.33
13 LEU  HA     13 LEU  QQD     4.59
13 LEU  QB     14 CNH2 HN1     6.38
13 LEU  HG     14 CNH2 HN1     4.17
13 LEU  HG     14 CNH2 HN2     4.72
13 LEU  QQD    14 CNH2 HN1     7.63
13 LEU  QQD    14 CNH2 HN2     7.63
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5. HPLC chromatograms of peptides **10-26:**

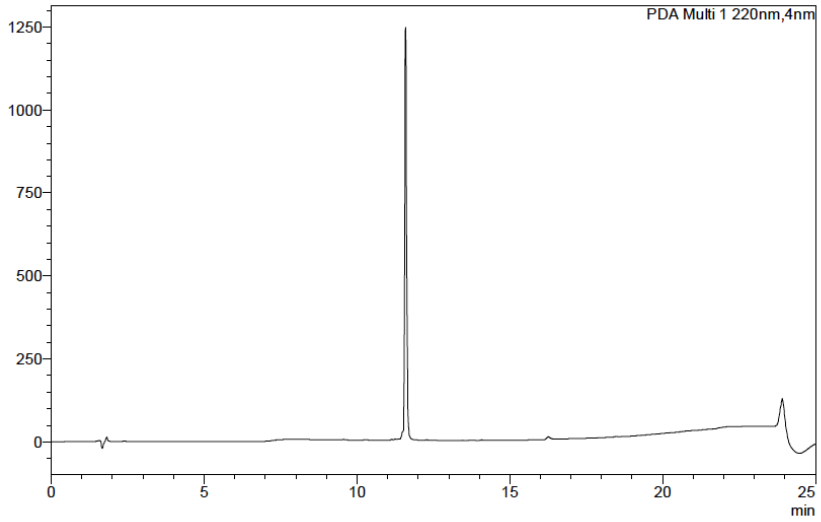

Figure S1. Chromatogram of **10** obtained by an analytical HPLC (Shimadzu UFLC SPD-M20A/DGU-20A3R/LC-20AD) equipped with a Phenomenex Kinetex C18 column (150 mm × 4.6 mm, 5 μm, 100 Å), and monitored by UV detection at 220 nm. t_R: 11.6 min [linear gradient 10-90% MeCN (0.1% TFA) in H_2O (0.1% TFA) over 20 min, flow rate of 1 mL/min].

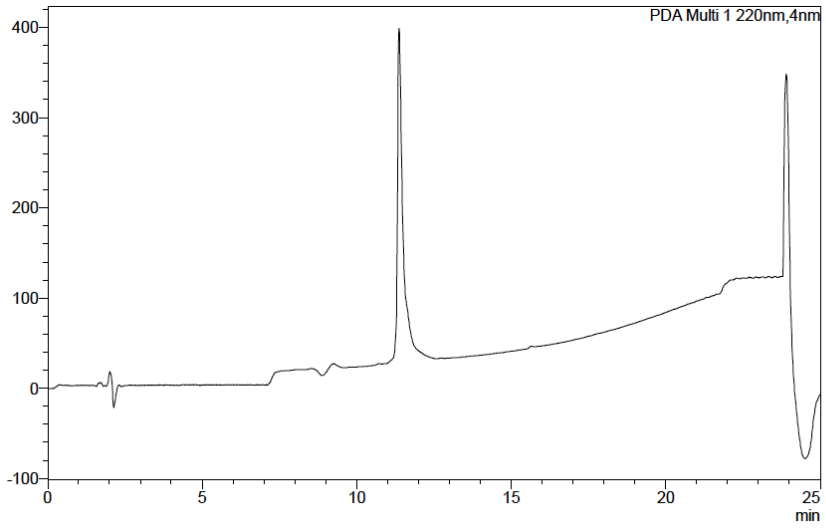

Figure S2. Chromatogram of **11** obtained by an analytical HPLC (Shimadzu UFLC SPD-M20A/DGU-20A3R/LC-20AD) equipped with a Phenomenex Kinetex C18 column (150 mm × 4.6 mm, 5 μm, 100 Å), and monitored by UV detection at 220 nm. t_R: 11.4 min [linear gradient 10-90% MeCN (0.1% TFA) in H_2O (0.1% TFA) over 20 min, flow rate of 1 mL/min].

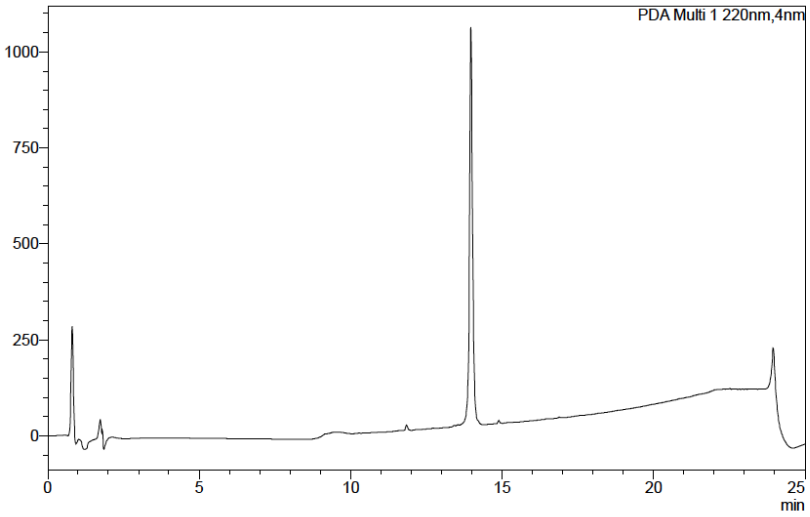

Figure S3. Chromatogram of **12** obtained by an analytical HPLC (Shimadzu UFLC SPD-M20A/DGU-20A3R/LC-20AD) equipped with a Phenomenex Kinetex C18 column (150 mm × 4.6 mm, 5 µm, 100 Å), and monitored by UV detection at 220 nm. t_R: 13.9 min [linear gradient 10-90% MeCN (0.1% TFA) in H_2O (0.1% TFA) over 20 min, flow rate of 1 mL/min].

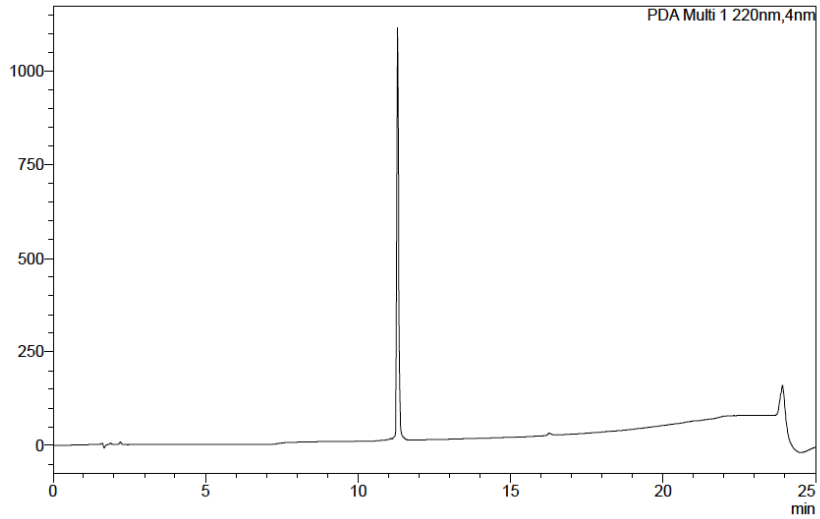

Figure S4. Chromatogram of **13** obtained by an analytical HPLC (Shimadzu UFLC SPD-M20A/DGU-20A3R/LC-20AD) equipped with a Phenomenex Kinetex C18 column (150 mm × 4.6 mm, 5 µm, 100 Å), and monitored by UV detection at 220 nm. t_R: 11.3 min [linear gradient 10-90% MeCN (0.1% TFA) in H_2O (0.1% TFA) over 20 min, flow rate of 1 mL/min].

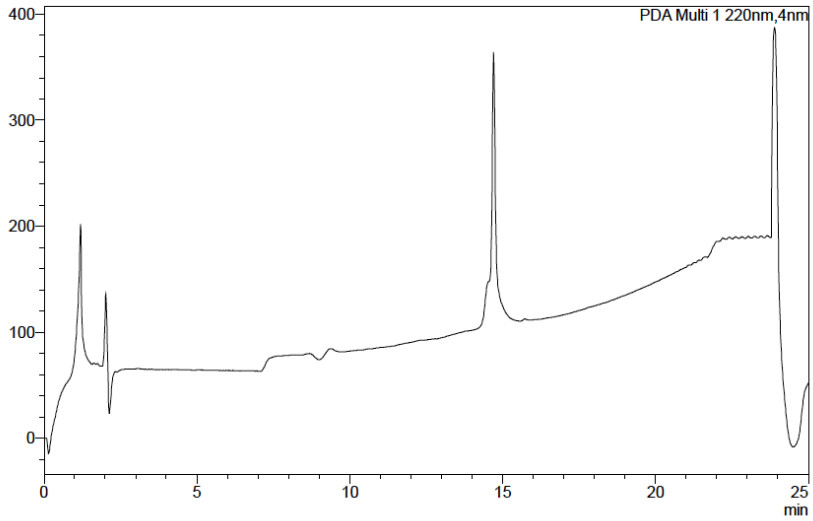

Figure S5. Chromatogram of **14** obtained by an analytical HPLC (Shimadzu UFLC SPD-M20A/DGU-20A3R/LC-20AD) equipped with a Phenomenex Kinetex C18 column (150 mm × 4.6 mm, 5 μm, 100 Å), and monitored by UV detection at 220 nm. t_R: 14.7 min [linear gradient 10-90% MeCN (0.1% TFA) in H_2O (0.1% TFA) over 20 min, flow rate of 1 mL/min].

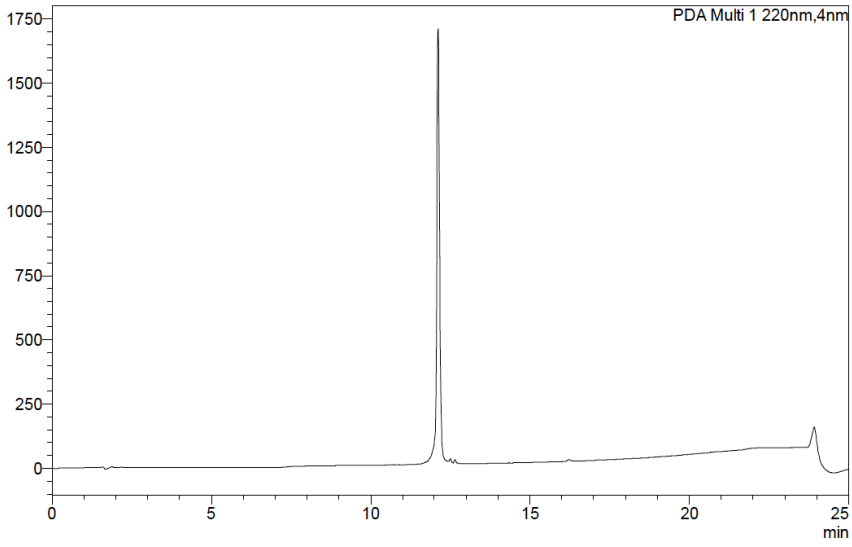

Figure S6. Chromatogram of **15** obtained by an analytical HPLC (Shimadzu UFLC SPD-M20A/DGU-20A3R/LC-20AD) equipped with a Phenomenex Kinetex C18 column (150 mm × 4.6 mm, 5 μm, 100 Å), and monitored by UV detection at 220 nm. t_R: 11.6 min [linear gradient 10-90% MeCN (0.1% TFA) in H_2O (0.1% TFA) over 20 min, flow rate of 1 mL/min].

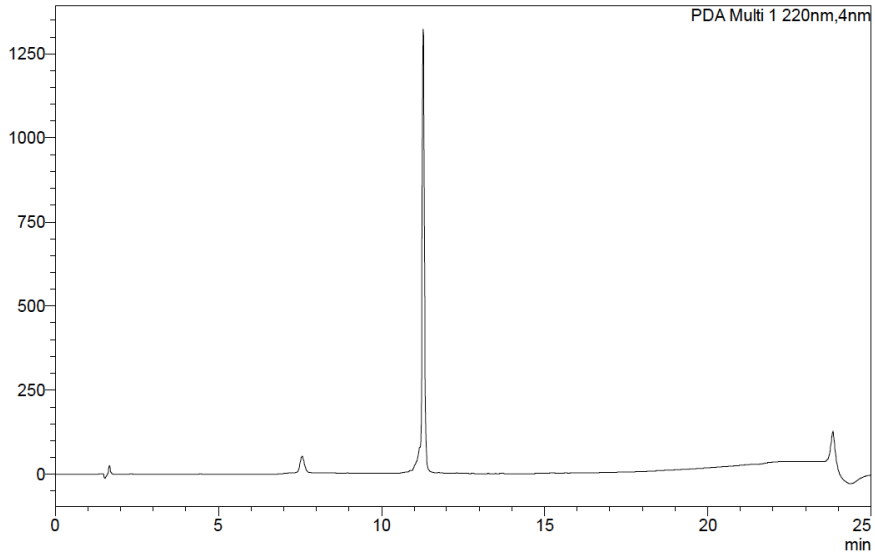

Figure S7. Chromatogram of **16** obtained by an analytical HPLC (Shimadzu UFLC SPD-M20A/DGU-20A3R/LC-20AD) equipped with a Phenomenex Kinetex C18 column (150 mm × 4.6 mm, 5 μm, 100 Å), and monitored by UV detection at 220 nm. t_R:11.3 min [linear gradient 10-90% MeCN (0.1% TFA) in H_2O (0.1% TFA) over 20 min, flow rate of 1 mL/min].

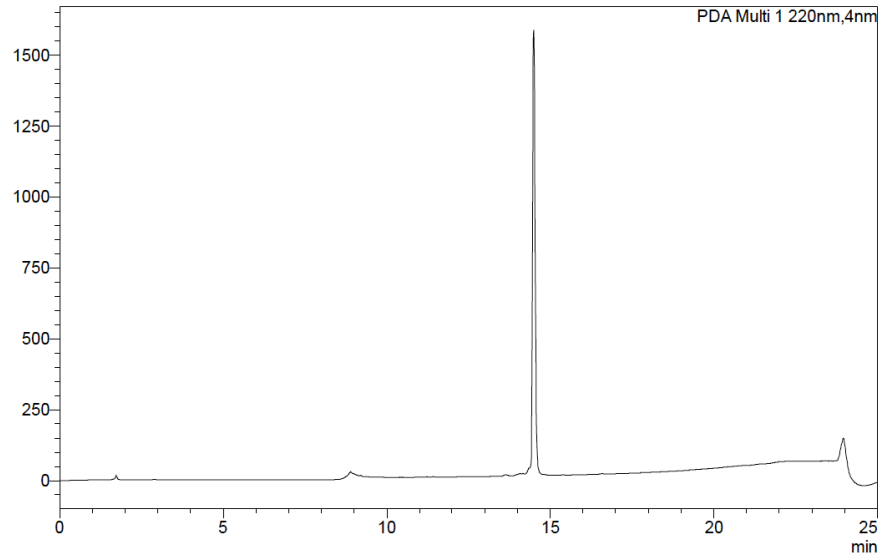

Figure S8. Chromatogram of **17** obtained by an analytical HPLC (Shimadzu UFLC SPD-M20A/DGU-20A3R/LC-20AD) equipped with a Phenomenex Kinetex C18 column (150 mm × 4.6 mm, 5 μm, 100 Å), and monitored by UV detection at 220 nm. t_R: 14.5 min [linear gradient 10-90% MeCN (0.1% TFA) in H_2O (0.1% TFA) over 20 min, flow rate of 1 mL/min].

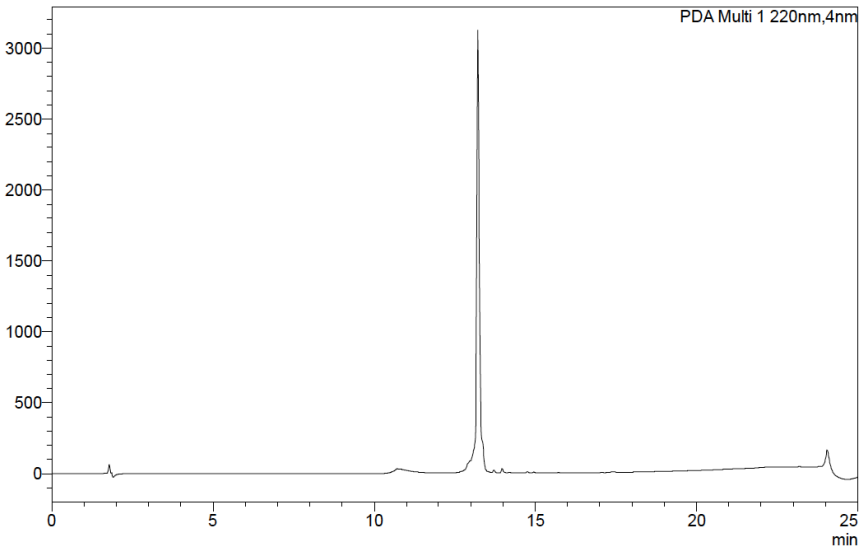

Figure S9. Chromatogram of **18** obtained by an analytical HPLC (Shimadzu UFLC SPD-M20A/DGU-20A3R/LC-20AD) equipped with a Phenomenex Kinetex C18 column (150 mm × 4.6 mm, 5 μm, 100 Å), and monitored by UV detection at 220 nm. t_R: 13.2 min [linear gradient 10-90% MeCN (0.1% TFA) in H_2O (0.1% TFA) over 20 min, flow rate of 1 mL/min].

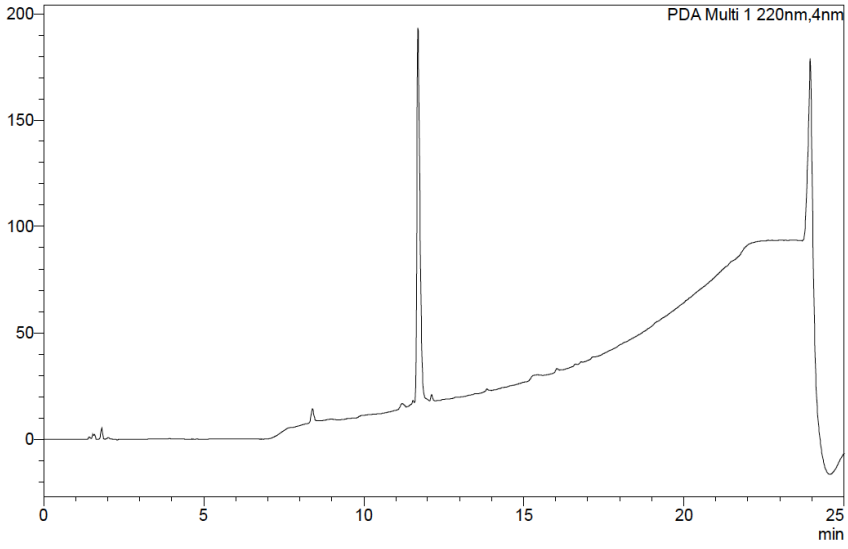

Figure S10. Chromatogram of **19** obtained by an analytical HPLC (Shimadzu UFLC SPD-M20A/DGU-20A3R/LC-20AD) equipped with a Phenomenex Kinetex C18 column (150 mm × 4.6 mm, 5 μm, 100 Å), and monitored by UV detection at 220 nm. t_R: 11.7 min [linear gradient 10-90% MeCN (0.1% TFA) in H_2O (0.1% TFA) over 20 min, flow rate of 1 mL/min].

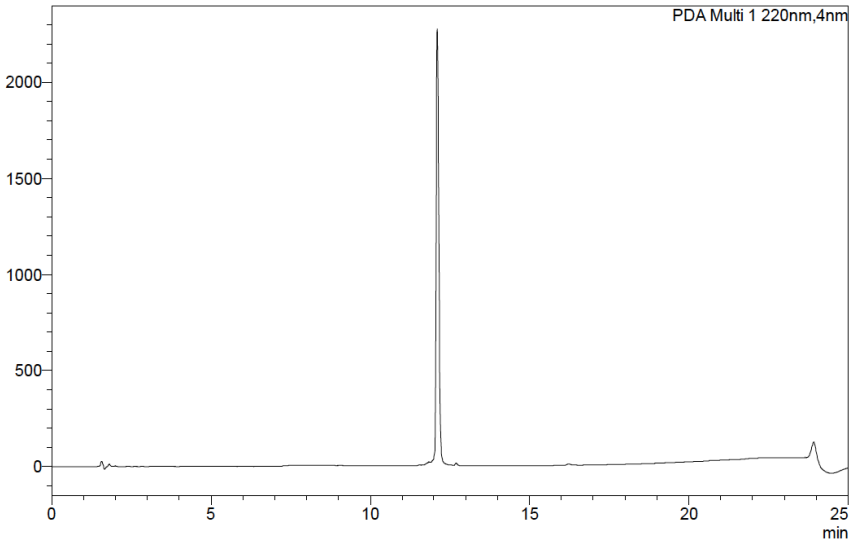

Figure S11. Chromatogram of **20** obtained by an analytical HPLC (Shimadzu UFLC SPD-M20A/DGU-20A3R/LC-20AD) equipped with a Phenomenex Kinetex C18 column (150 mm × 4.6 mm, 5 μm, 100 Å), and monitored by UV detection at 220 nm. t_R: 12.1 [linear gradient 10-90% MeCN (0.1% TFA) in H_2O (0.1% TFA) over 20 min, flow rate of 1 mL/min].

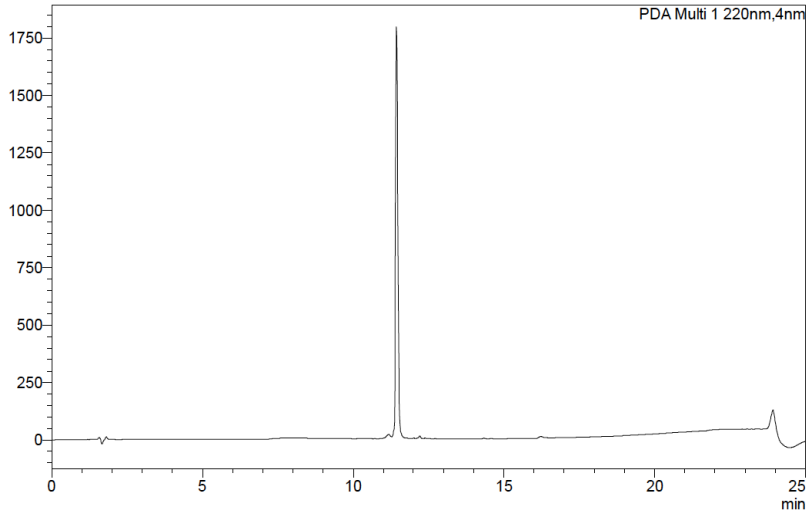

Figure S12. Chromatogram of **21** obtained by an analytical HPLC (Shimadzu UFLC SPD-M20A/DGU-20A3R/LC-20AD) equipped with a Phenomenex Kinetex C18 column (150 mm × 4.6 mm, 5 μm, 100 Å), and monitored by UV detection at 220 nm. t_R: 11.4 [linear gradient 10-90% MeCN (0.1% TFA) in H_2O (0.1% TFA) over 20 min, flow rate of 1 mL/min].

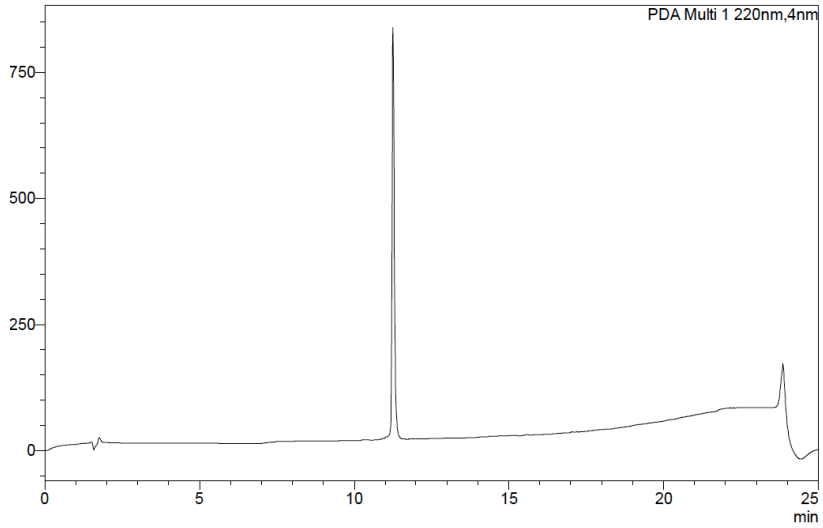

Figure S13. Chromatogram of **22** obtained by an analytical HPLC (Shimadzu UFLC SPD-M20A/DGU-20A3R/LC-20AD) equipped with a Phenomenex Kinetex C18 column (150 mm × 4.6 mm, 5 μm, 100 Å), and monitored by UV detection at 220 nm. t_R: 11.6 min [linear gradient 10-90% MeCN (0.1% TFA) in H_2O (0.1% TFA) over 20 min, flow rate of 1 mL/min].

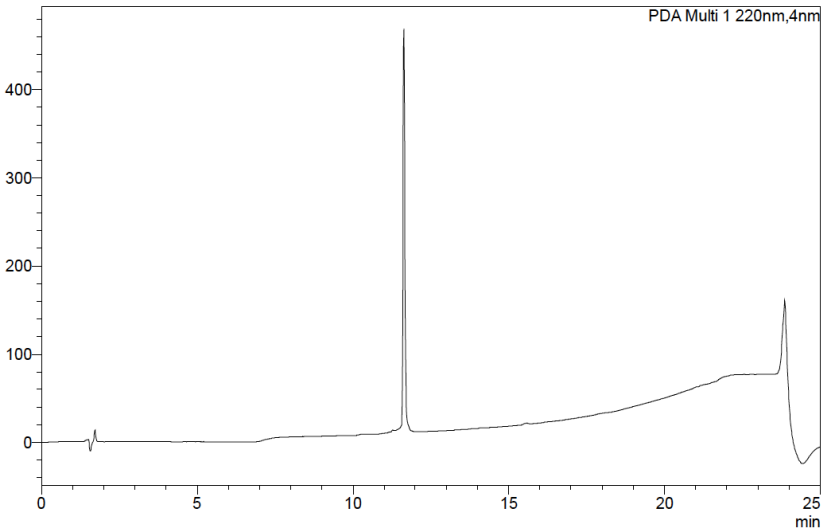

Figure S14. Chromatogram of **23** obtained by an analytical HPLC (Shimadzu UFLC SPD-M20A/DGU-20A3R/LC-20AD) equipped with a Phenomenex Kinetex C18 column (150 mm × 4.6 mm, 5 μm, 100 Å), and monitored by UV detection at 220 nm. t_R: 11.3 min [linear gradient 10-90% MeCN (0.1% TFA) in H_2O (0.1% TFA) over 20 min, flow rate of 1 mL/min].

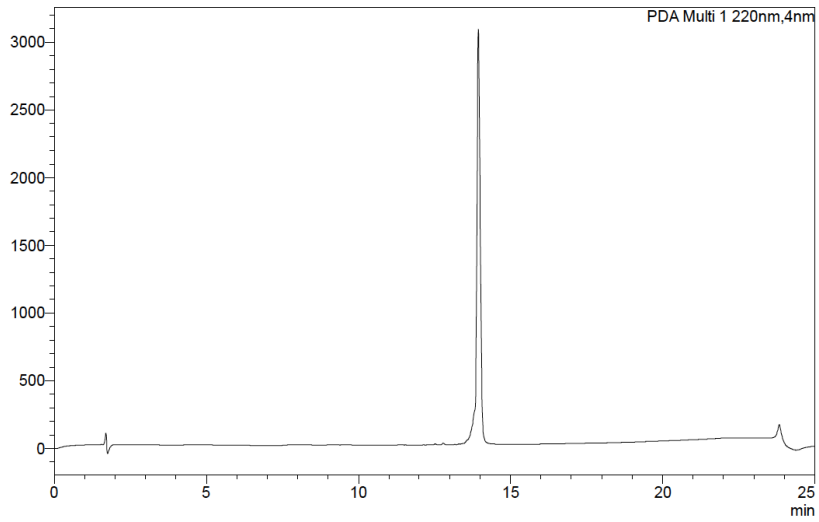

Figure S15. Chromatogram of **24** obtained by an analytical HPLC (Shimadzu UFLC SPD-M20A/DGU-20A3R/LC-20AD) equipped with a Phenomenex Kinetex C18 column (150 mm × 4.6 mm, 5 μm, 100 Å), and monitored by UV detection at 220 nm. t_R: 13.9 min [linear gradient 10-90% MeCN (0.1% TFA) in H_2O (0.1% TFA) over 20 min, flow rate of 1 mL/min].

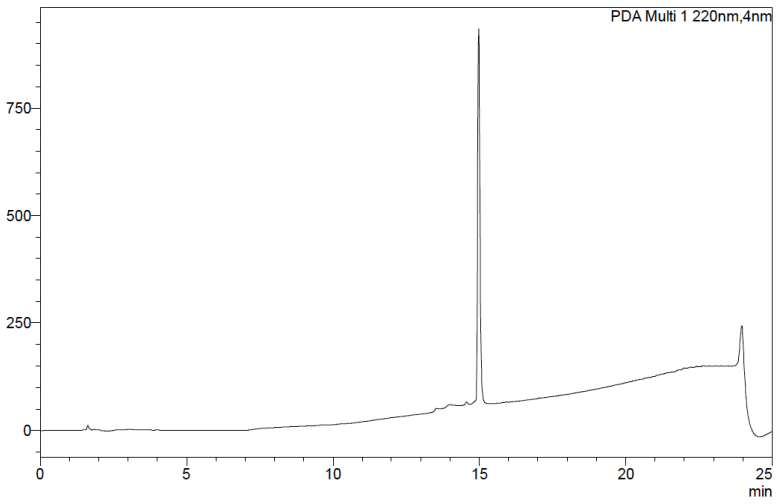

Figure S16. Chromatogram of **25** obtained by an analytical HPLC (Shimadzu UFLC SPD-M20A/DGU-20A3R/LC-20AD) equipped with a Phenomenex Kinetex C18 column (150 mm × 4.6 mm, 5 μm, 100 Å), and monitored by UV detection at 220 nm. t_R: 14.9 min [linear gradient 10-90% MeCN (0.1% TFA) in H_2O (0.1% TFA) over 20 min, flow rate of 1 mL/min].

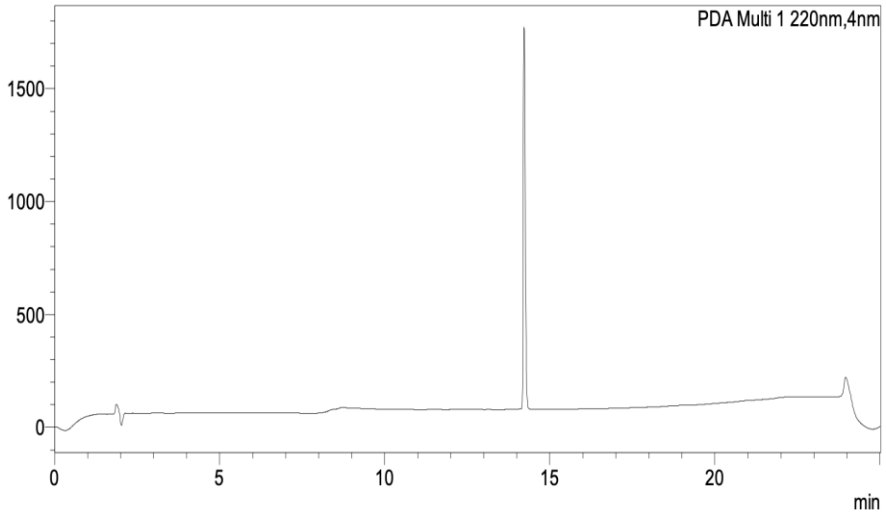

Figure S17. Chromatogram of **26** obtained by an analytical HPLC (Shimadzu UFLC SPD-M20A/DGU-20A3R/LC-20AD) equipped with a Phenomenex Kinetex C18 column (150 mm × 4.6 mm, 5 μm, 100 Å), and monitored by UV detection at 220 nm. t_R: 14.2 min [linear gradient 10-90% MeCN (0.1% TFA) in H_2O (0.1% TFA) over 20 min, flow rate of 1 mL/min].