Peptide-Directed Solid-Phase Reductive Amination

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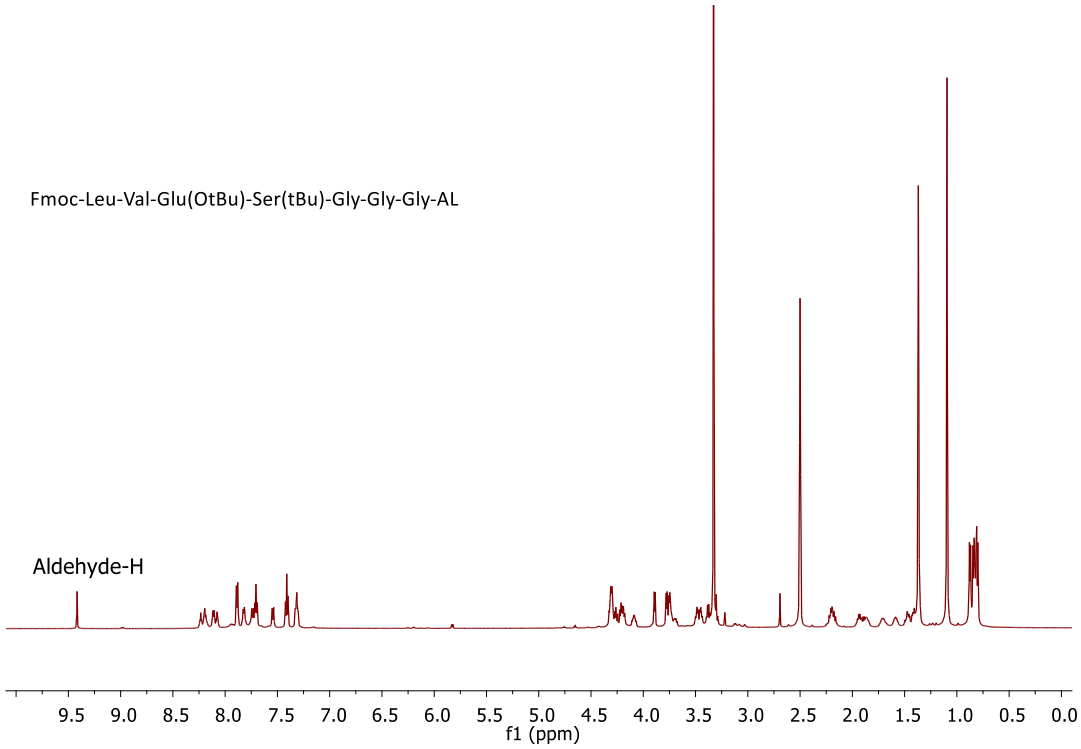
SUPPLEMENTARY INFORMATION

Table S1. Fmoc-protected peptide aldehydes **4a–4g** synthesized [1,2]

A/A	Fmoc-peptide aldehydes	m/z Calc.	m/z found $[M+H]^+$
4a	Fmoc-Leu-Val-Glu(O^{t}Bu)-Ser(^{t}Bu)-Gly-Gly-Gly-AL	935.50	936.49
4b	Fmoc-Ala-Ser(^{t}Bu)-Ser(^{t}Bu)-Aib-AL	666.36	667.38
4c	Fmoc-Ala-Ser(^{t}Bu)-Ser(^{t}Bu)-Leu-AL	694.39	695.41
4d	Fmoc-Ala-Ser(^{t}Bu)-Ser(^{t}Bu)-Ile-AL	694.39	695.42
4e	Fmoc-Ala-Ser(^{t}Bu)-Ser(^{t}Bu)-Phe-AL	728.38	729.40
4f	Fmoc-Cys(Trt)-Ala-Lys(Boc)-Val-AL	967.46	968.49
4g	Fmoc-Ser(^{t}Bu)-Tyr(^{t}Bu)-Leu-Ser(^{t}Bu)-Thr(^{t}Bu)-AL	999.59	1000.58

[1] NMR and HPLC of the crude **4a–4g** are presented in **Figures S1–S14**.
[2] The eluted peaks from each HPLC analysis corresponding to the products were collected and subjected to ESI-MS.



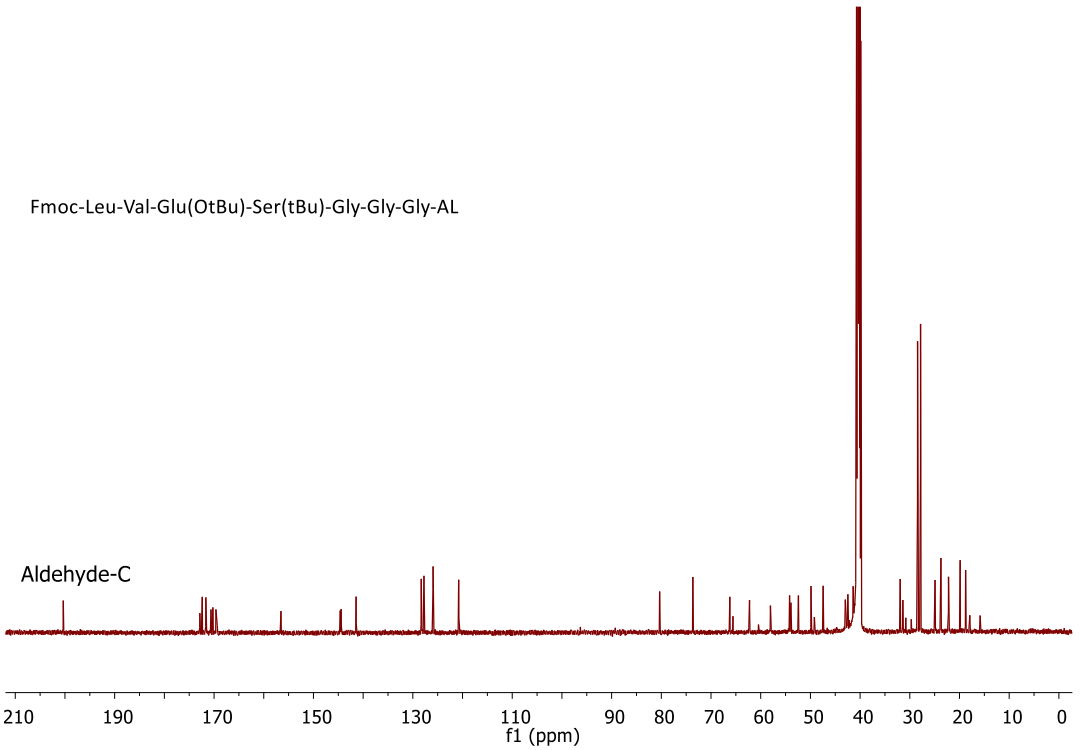


Figure S1. ^{1}H, ^{13}C-NMR data of crude **Fmoc-Leu-Val-Glu(O^{t}Bu)-Ser(^{t}Bu)-Gly-Gly-Gly-AL** (**4a**) in DMSO-d_6

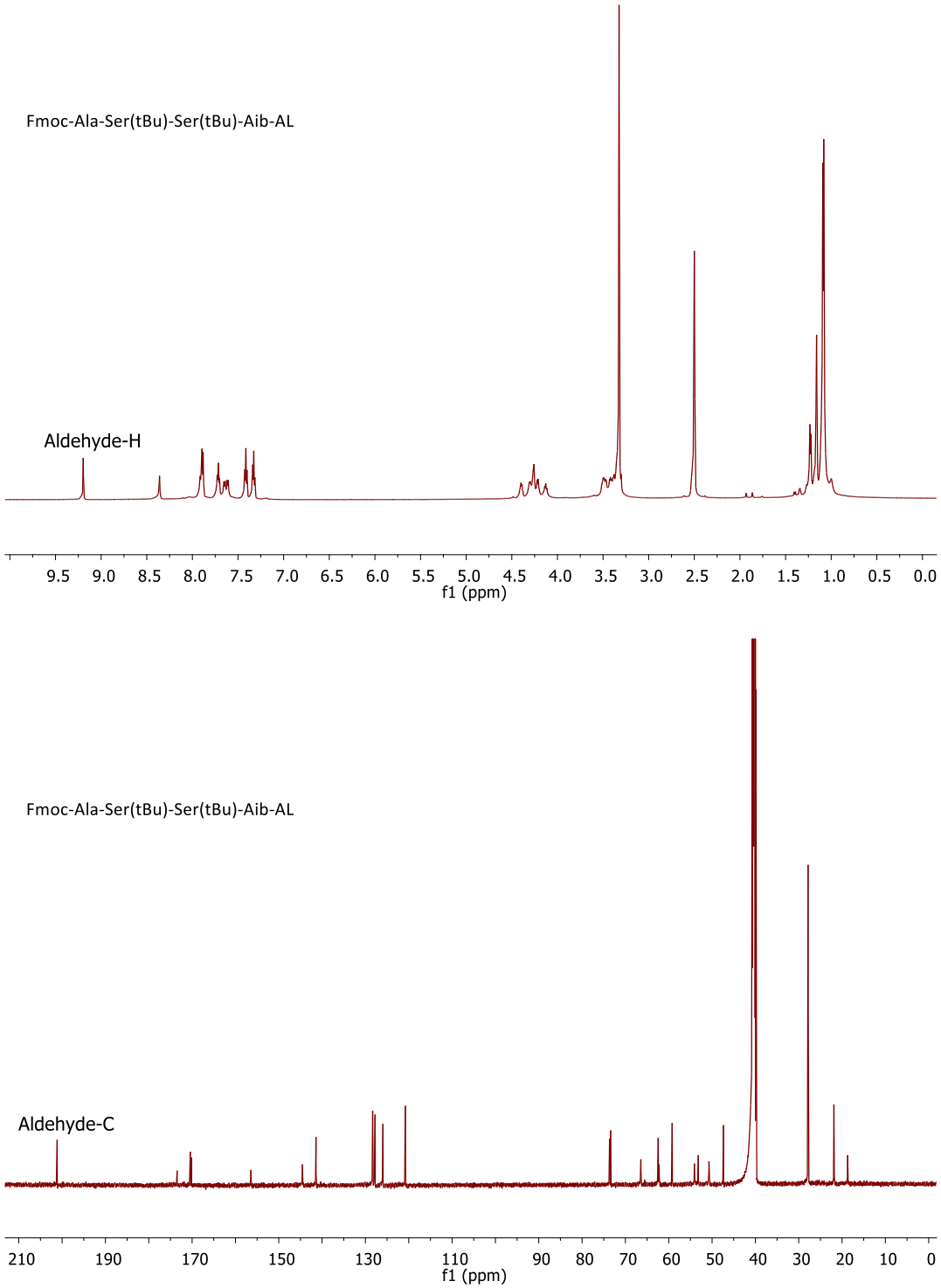


Figure S2. ^{1}H, ^{13}C-NMR data of crude **Fmoc-Ala-Ser(ᵗBu)-Ser(ᵗBu)-Aib-AL** (**4b**) in DMSO-d_6

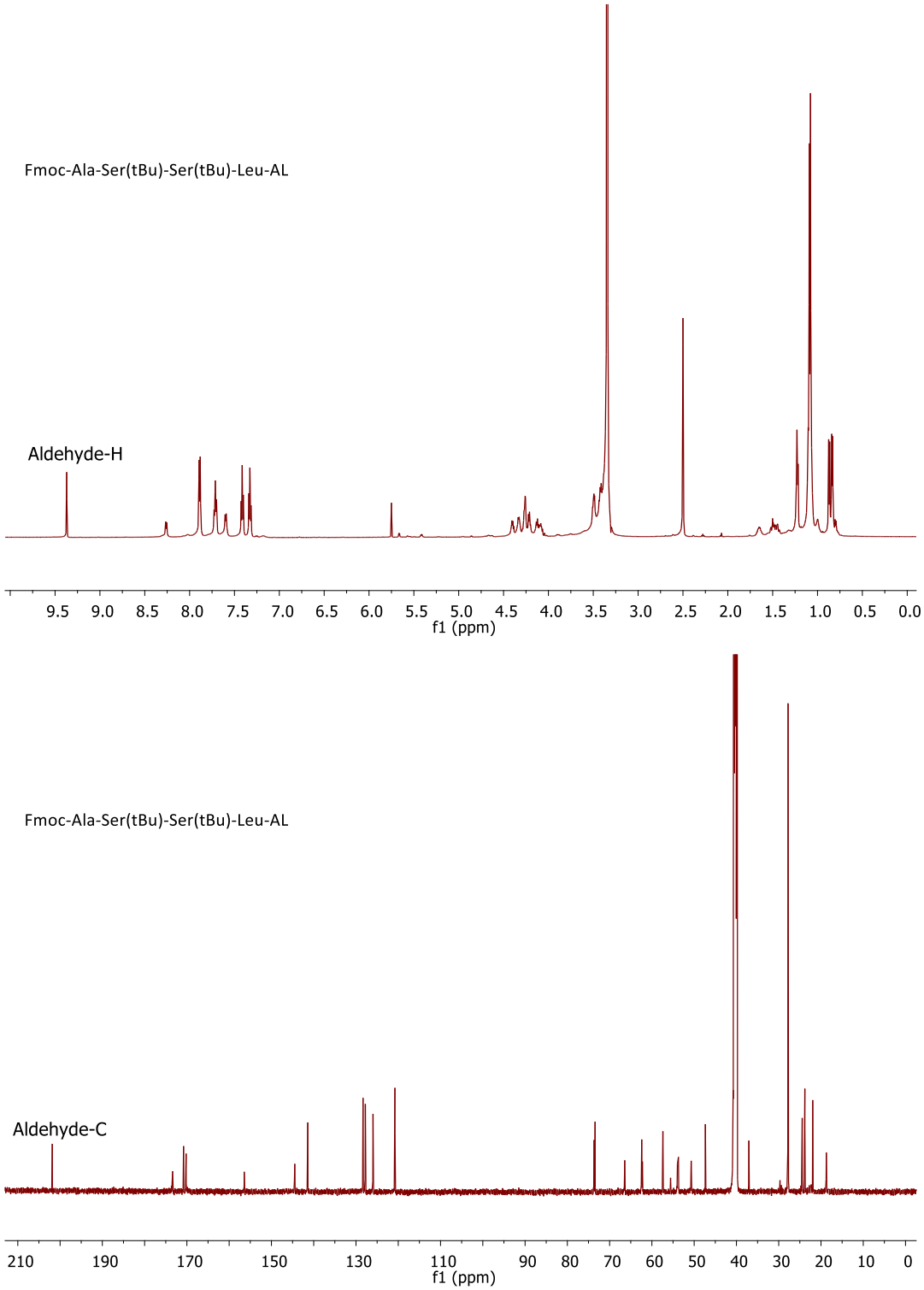


Figure S3. ^{1}H, ^{13}C-NMR data of crude **Fmoc-Ala-Ser(ᵗBu)-Ser(ᵗBu)-Leu-AL** (**4c**) in DMSO-d_6

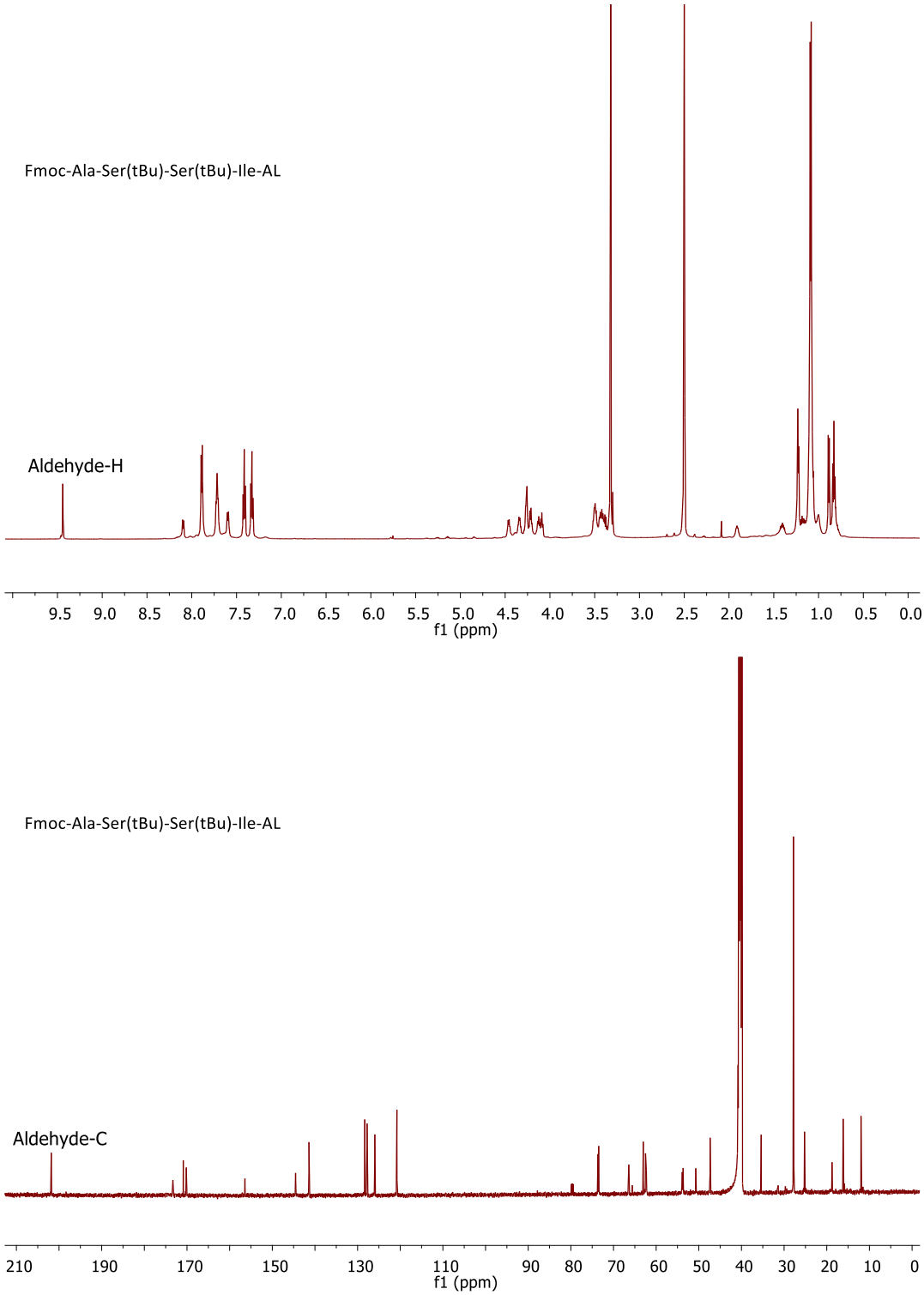


Figure S4. ^{1}H, ^{13}C-NMR data of crude **Fmoc-Ala-Ser(ᵗBu)-Ser(ᵗBu)-Ile-AL** (**4d**) in DMSO-d_6

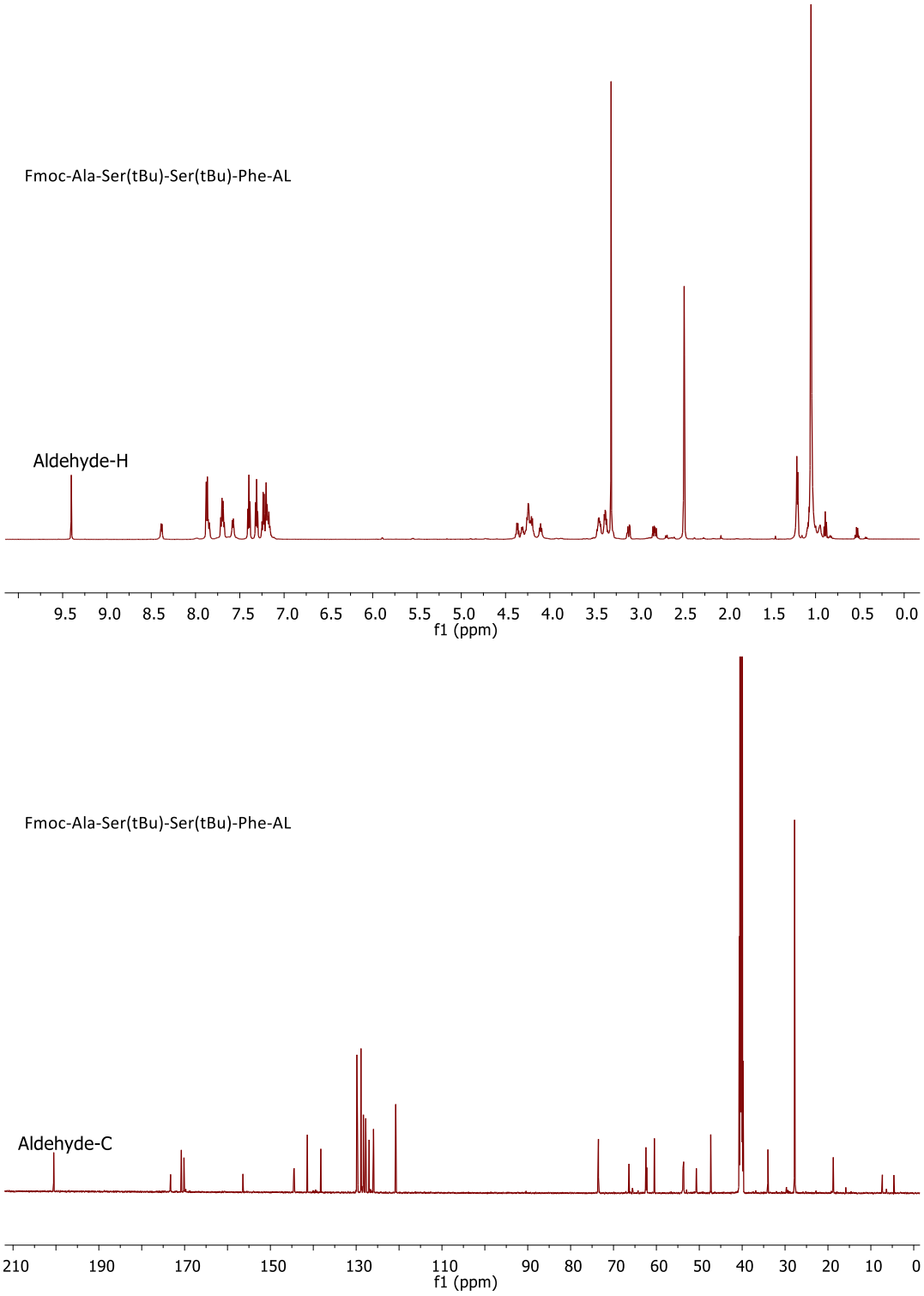


Figure S5. ^{1}H, ^{13}C-NMR data of crude **Fmoc-Ala-Ser(ᵗBu)-Ser(ᵗBu)-Phe-AL** (**4e**) in DMSO-d_6

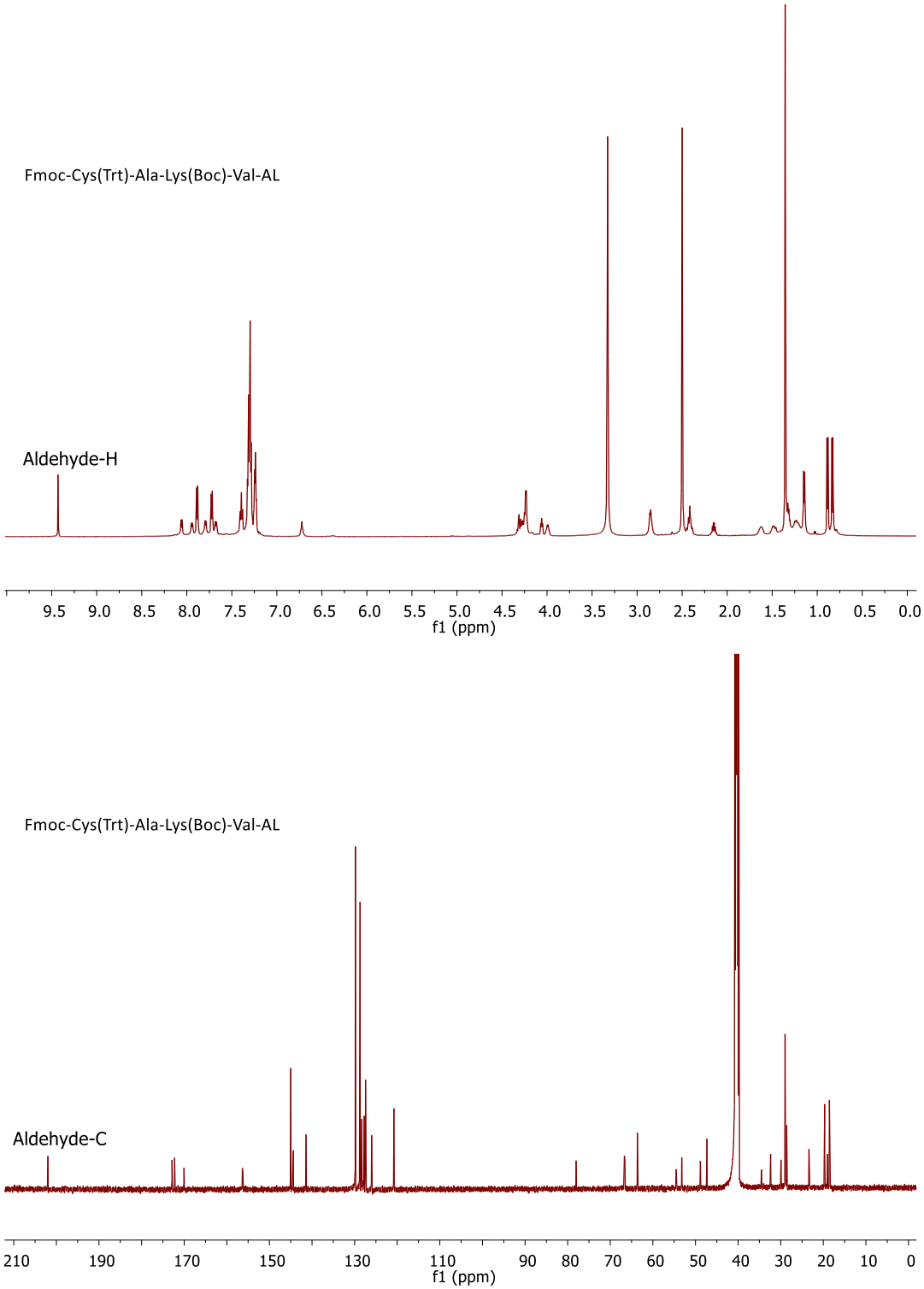


Figure S6. ^{1}H, ^{13}C-NMR data of crude **Fmoc-Cys(Trt)-Ala-Lys(Boc)-Val-AL** (**4f**) in DMSO-d_6

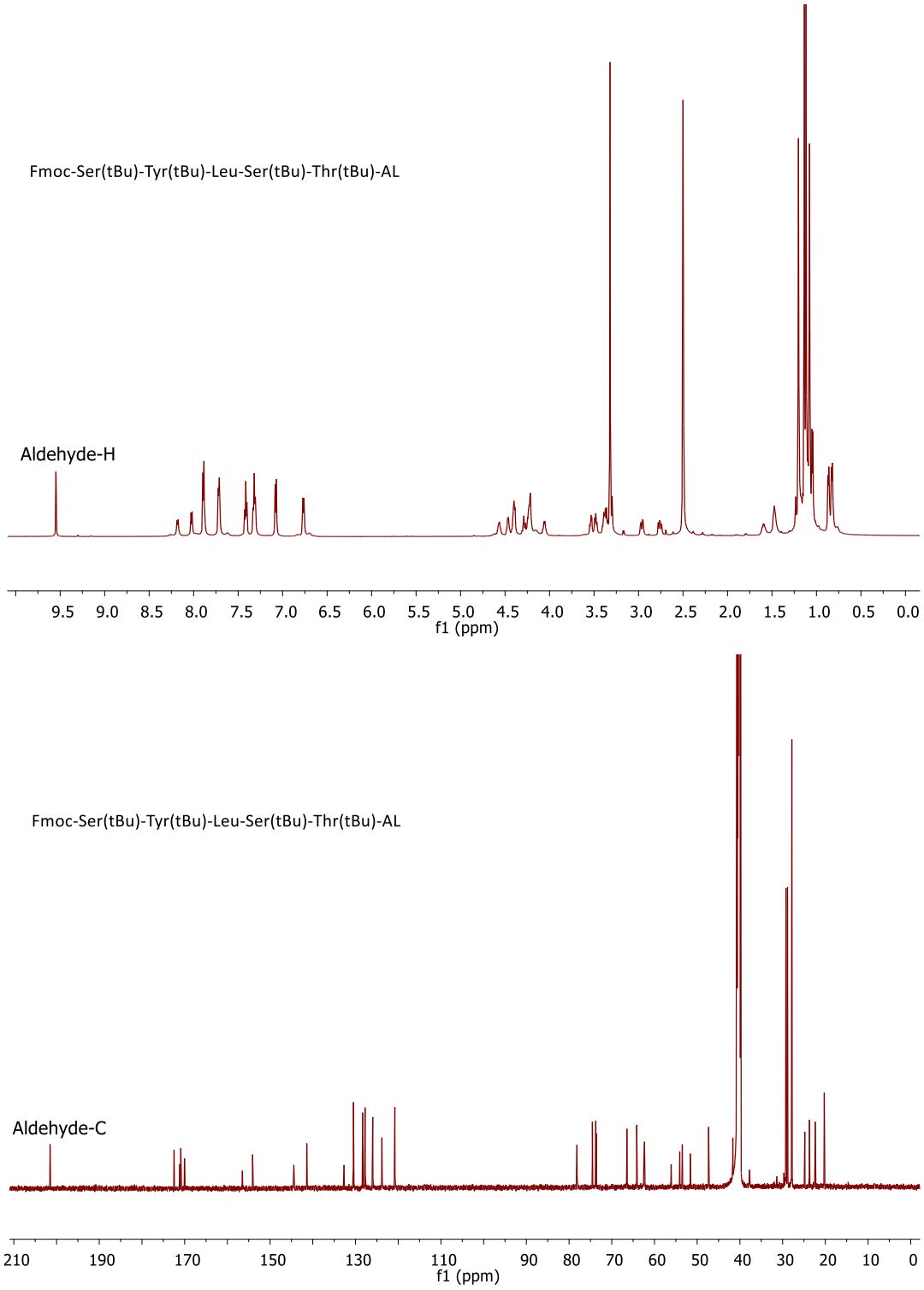


Figure S7. ^{1}H, ^{13}C-NMR data of crude **Fmoc-Ser(ᵗBu)-Tyr(ᵗBu)-Leu-Ser(ᵗBu)-Thr(ᵗBu)-AL** (**4g**) in DMSO-d_6

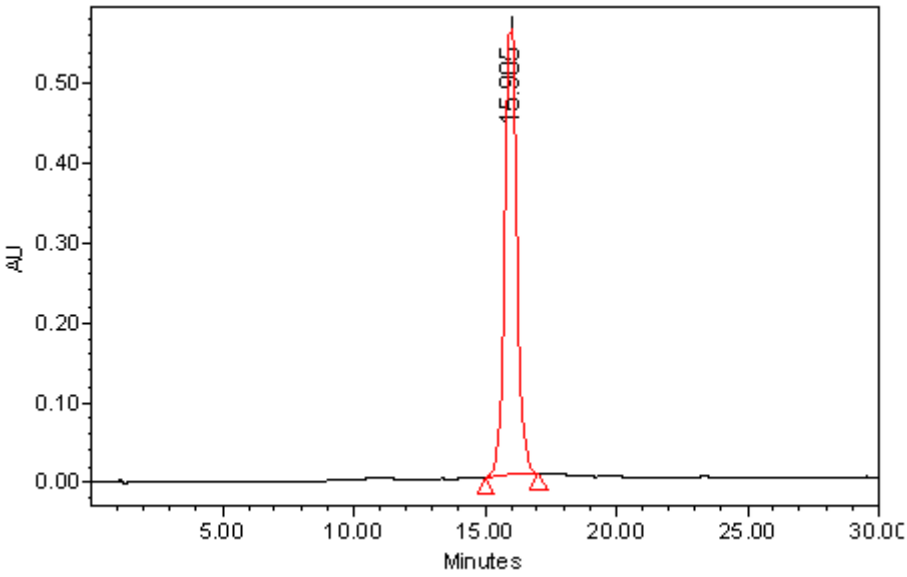


Figure S8. Analytical HPLC of crude **Fmoc-Leu-Val-Glu(O^{t}Bu)-Ser(^{t}Bu)-Gly-Gly-Gly-AL** (**4a**); Column: Purospher RP-8e, 5 μm, 125–4 mm; gradient: 20% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 265 nm.

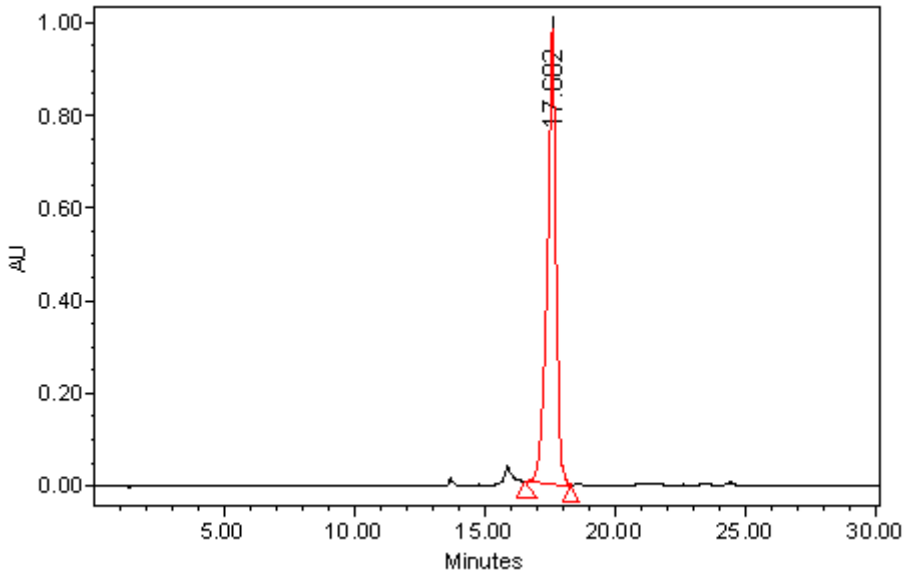


Figure S9. Analytical HPLC of crude **Fmoc-Ala-Ser(^{t}Bu)-Ser(^{t}Bu)-Aib-AL** (**4b**); Column: Purospher RP-8e, 5 μm, 125–4 mm; gradient: 20% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 265 nm.

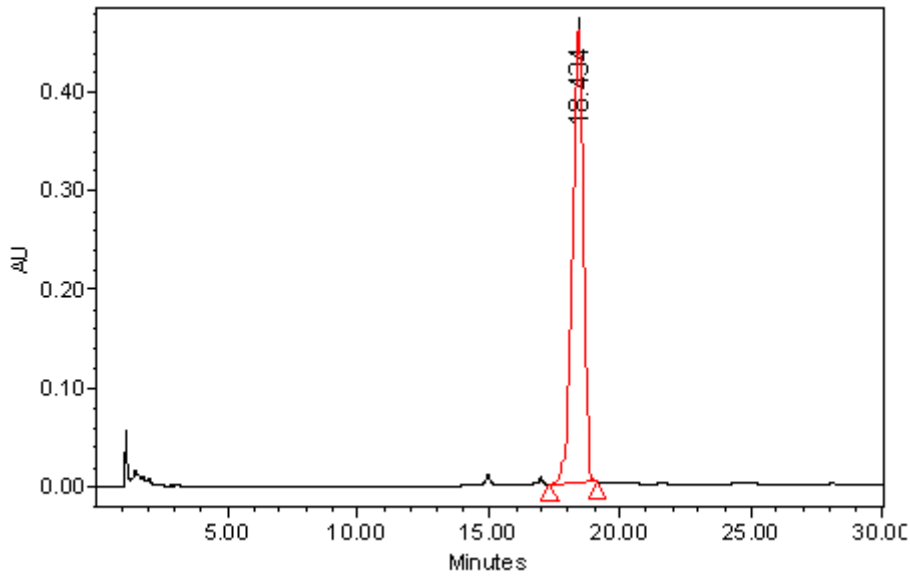


Figure S10. Analytical HPLC of crude **Fmoc-Ala-Ser(ᵗBu)-Ser(ᵗBu)-Leu-AL** (**4c**); Column: Purospher RP-8e, 5 μm, 125–4 mm; gradient: 20% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 265 nm.

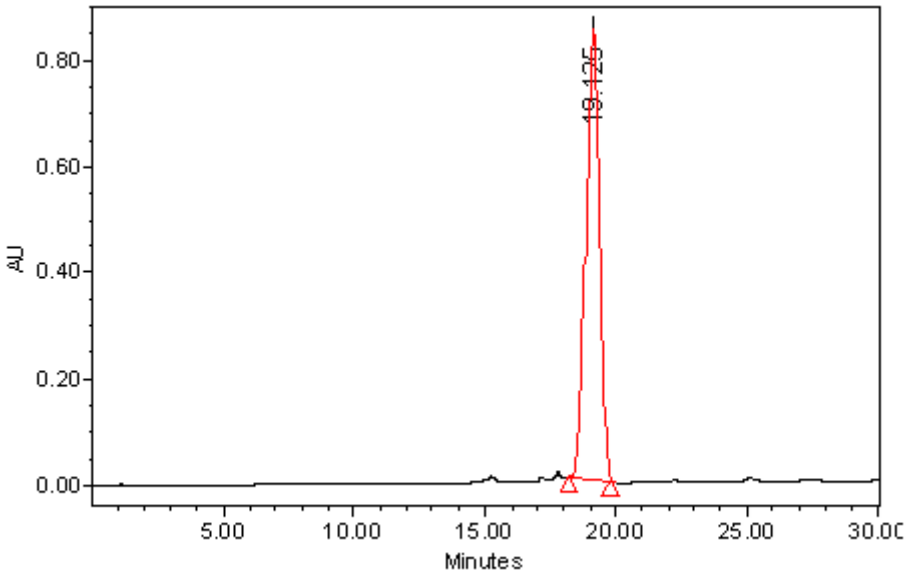


Figure S11. Analytical HPLC of crude **Fmoc-Ala-Ser(ᵗBu)-Ser(ᵗBu)-Ile-AL** (**4d**); Column: Purospher RP-8e, 5 μm, 125–4 mm; gradient: 20% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 265 nm.

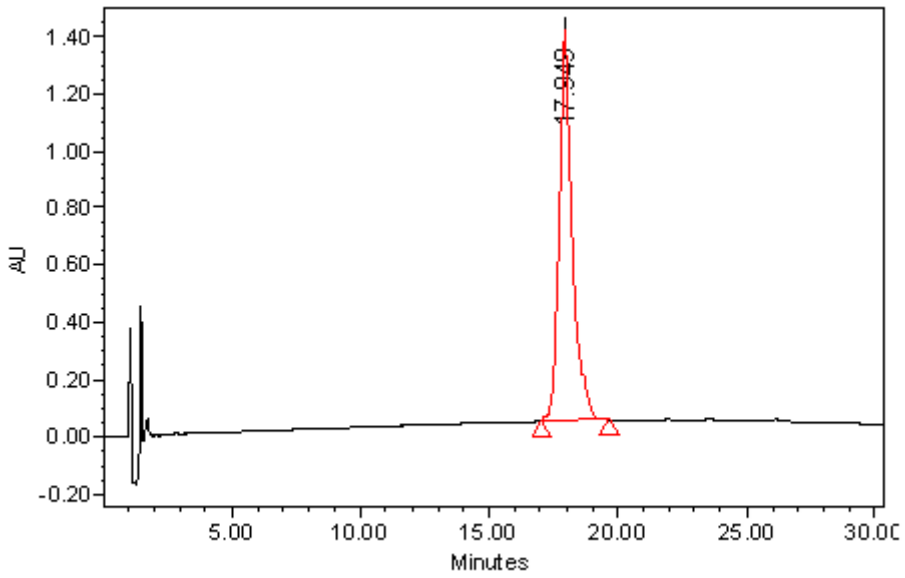


Figure S12. Analytical HPLC of crude **Fmoc-Ala-Ser(ᵗBu)-Ser(ᵗBu)-Phe-AL** (**4e**); Column: Purospher RP-8e, 5 μm, 125–4 mm; gradient: 20% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 265 nm.

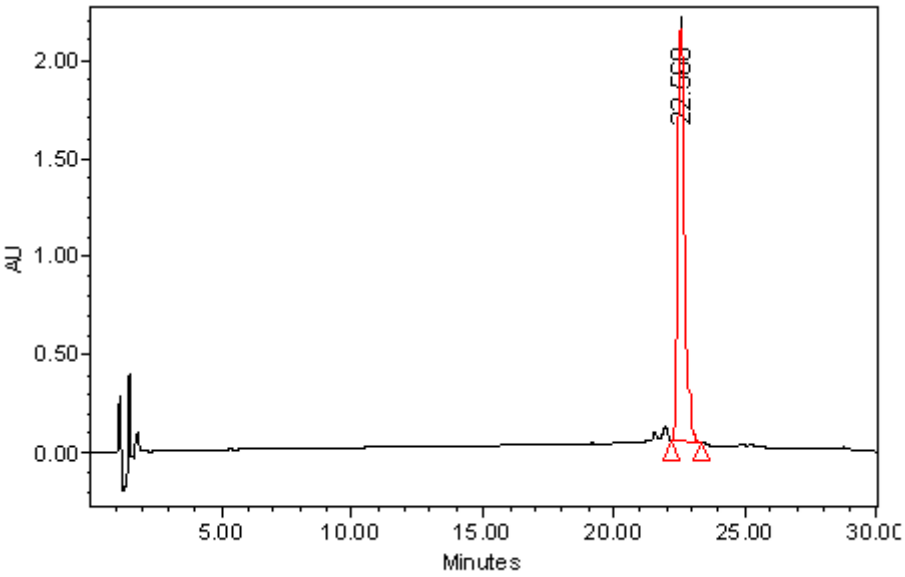


Figure S13. Analytical HPLC of crude **Fmoc-Cys(Trt)-Ala-Lys(Boc)-Val-AL** (**4f**); Column: Purospher RP-8e, 5 μm, 125–4 mm; gradient: 20% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 265 nm.

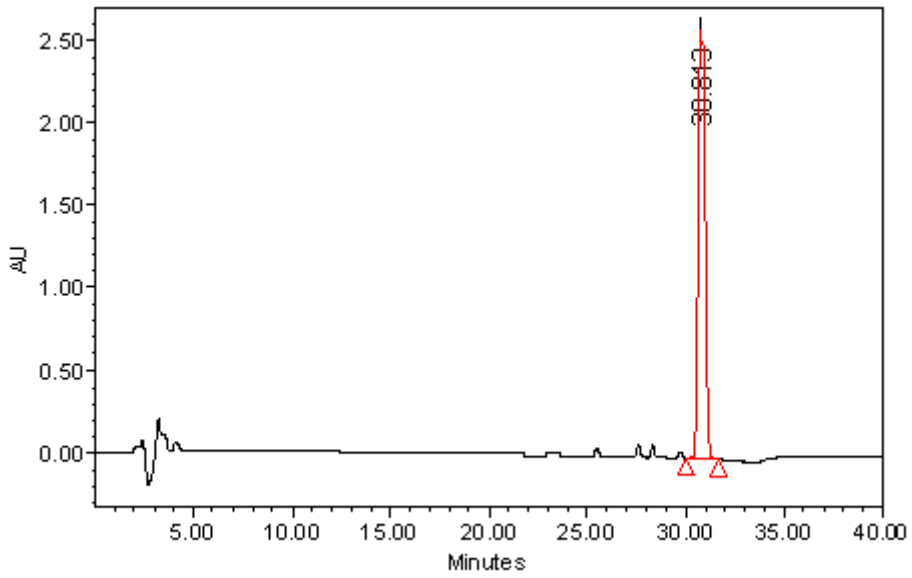


Figure S14. Analytical HPLC of crude **Fmoc-Ser(ᵗBu)-Tyr(ᵗBu)-Leu-Ser(ᵗBu)-Thr(ᵗBu)-AL** (**4g**); Column: YMC-Triart C18, 12 nm, S-5 μm, 250–4.6 mm; gradient: 40% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 265 nm.

Table S2. Peptide fragments **8a–8g** synthesized by SPFC method A (Scheme 2)[1], and the corresponding epimerization levels (% Epimer) [2,3]

Method of Preparation	Product obtained	% Epimer[3]	m/z calc.	m/z found (ESI-MS) $[M+H]^+$
Fmoc-Leu-Val-Glu(O^{t}Bu)-Ser(^{t}Bu)-Gly-Gly-**Gly-AL 4a +** H-Leu-Ala-Phe-Gly-O-CLTR	Fmoc-Leu-Val-Glu(O^{t}Bu)-Ser(^{t}Bu)-Gly-Gly-**Gly-Ψ[CH_2–NH]**-Leu-Ala-Phe-Gly-OH **8a**	-	1325.73	1326.82
Fmoc-Ala-Ser(^{t}Bu)-Ser(^{t}Bu)-**Aib-AL 4b +** H-Leu-Ala-Phe-Gly-O-CLTR	Fmoc-Ala-Ser(^{t}Bu)-Ser(^{t}Bu)-**Aib-Ψ[CH_2–NH]**-Leu-Ala-Phe-Gly-OH **8b**	-	1056.59	1057.62
Fmoc-Ala-Ser(^{t}Bu)-Ser(^{t}Bu)-**Leu-AL 4c +** H-Asp(O^{t}Bu)-Tyr(^{t}Bu)-Trp(Boc)-Gly-O-CLTR	Fmoc-Ala-Ser(^{t}Bu)-Ser(^{t}Bu)-**Leu-Ψ[CH_2–NH]**-Asp(O^{t}Bu)-Tyr(^{t}Bu)-Trp(Boc)-Gly-OH **8c.1**	2.1% 2.2%	1429.78	1430.76
Fmoc-Ala-Ser(^{t}Bu)-Ser(^{t}Bu)-**Leu-AL 4c +** H-Ser(^{t}Bu)-Tyr(^{t}Bu)-Leu-Ser(^{t}Bu)-O-CLTR	Fmoc-Ala-Ser(^{t}Bu)-Ser(^{t}Bu)-**Leu-Ψ[CH_2–NH]**-Ser(^{t}Bu)-Tyr(^{t}Bu)-Leu-Ser(^{t}Bu)-OH **8c.2**	2.0% 2.1%	1314.81	1315.81
Fmoc-Ala-Ser(^{t}Bu)-Ser(^{t}Bu)-**Ile-AL 4d +** H-Ser(^{t}Bu)-Tyr(^{t}Bu)-Leu-Ser(^{t}Bu)-O-CLTR	Fmoc-Ala-Ser(^{t}Bu)-Ser(^{t}Bu)-**Ile-Ψ[CH_2–NH]**-Ser(^{t}Bu)-Tyr(^{t}Bu)-Leu-Ser(^{t}Bu)-OH **8d**	3.3% 3.6% 4.3%	1314.81	1315.98
Fmoc-Ala-Ser(^{t}Bu)-Ser(^{t}Bu)-**Phe-AL 4e +** H-Leu-Ala-Phe-Gly-O-CLTR	Fmoc-Ala-Ser(^{t}Bu)-Ser(^{t}Bu)-**Phe-Ψ[CH_2–NH]**-Leu-Ala-Phe-Gly-OH **8e**	2.1% 2.2%	1118.61	1119.64
Fmoc-Cys(Trt)-Ala-Lys(Boc)-**Val-AL 4f +** H-Ser(^{t}Bu)-Tyr(^{t}Bu)-Leu-Ser(^{t}Bu)-O-CLTR	Fmoc-Cys(Trt)-Ala-Lys(Boc)-**Val-Ψ[CH_2–NH]**-Ser(^{t}Bu)-Tyr(^{t}Bu)-Leu-Ser(^{t}Bu)-OH **8f** [4]	6.0% 6.2% 7.0%	1587.87	1588.92
Fmoc-Ser(^{t}Bu)-Tyr(^{t}Bu)-Leu-Ser(^{t}Bu)-**Thr(^{t}Bu)-AL 4g +** H-Leu-Ala-Phe-Gly-O-CLTR	Fmoc-Ser(^{t}Bu)-Tyr(^{t}Bu)-Leu-Ser(^{t}Bu)-**Thr(^{t}Bu)-Ψ[CH_2–NH]**-Leu-Ala-Phe-Gly-OH **8g**	0.7% 0.8%	1389.82	1390.93

[1] 15 min imine formation in THF/MeOH (5:1) at rt; 1+1 h reduction with $NaBH_3CN$ in THF–1% AcOH at rt.

[2] HPLC of the crude **8a–8g** are presented in **Figures S15–S22**.

[3] The eluted peaks from each HPLC analysis corresponding to the products were collected and subjected to ESI-MS.

[4] In the case of Fmoc-Cys(Trt)-Ala-Lys(Boc)-**Val-Ψ[CH_2–N(Boc)]**-Ser(^{t}Bu)-Tyr(^{t}Bu)-Leu-Ser(^{t}Bu)-OH (**8f + Boc**) the epimerization was measured at 5.7%.

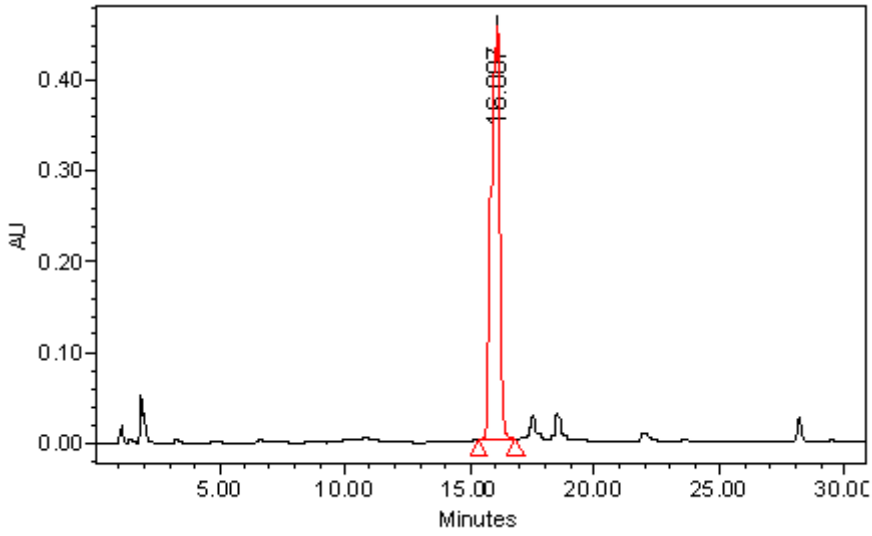


Figure S15. Analytical HPLC of crude **Fmoc-Leu-Val-Glu(O^{t}Bu)-Ser(^{t}Bu)-Gly-Gly-Gly-Ψ[CH_2–NH]-Leu-Ala-Phe-Gly-OH** (**8a**); Column: Purospher RP-8e, 5 μm, 125–4 mm; gradient: 20% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 265 nm.

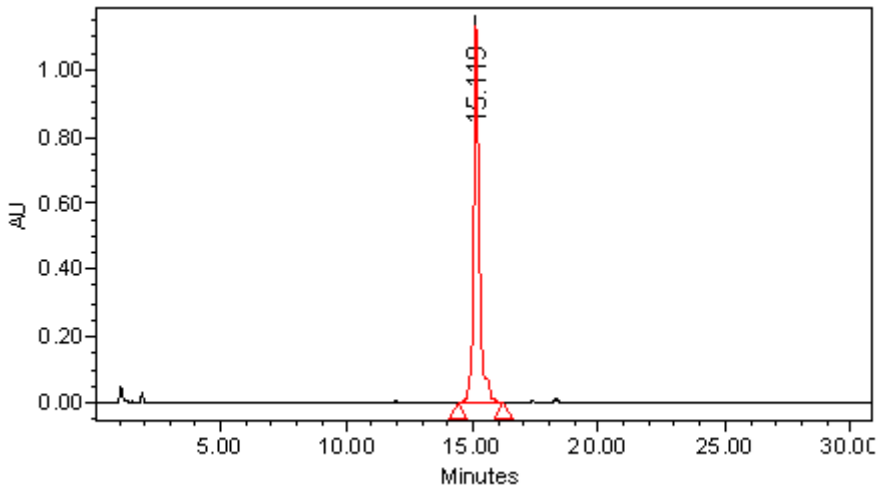


Figure S16. Analytical HPLC of crude **Fmoc-Ala-Ser(ᵗBu)-Ser(ᵗBu)-Aib-Ψ[CH₂–NH]-Leu-Ala-Phe-Gly-OH** (**8b**); Column: Purospher RP-8e, 5 μm, 125–4 mm; gradient: 20% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 265 nm.

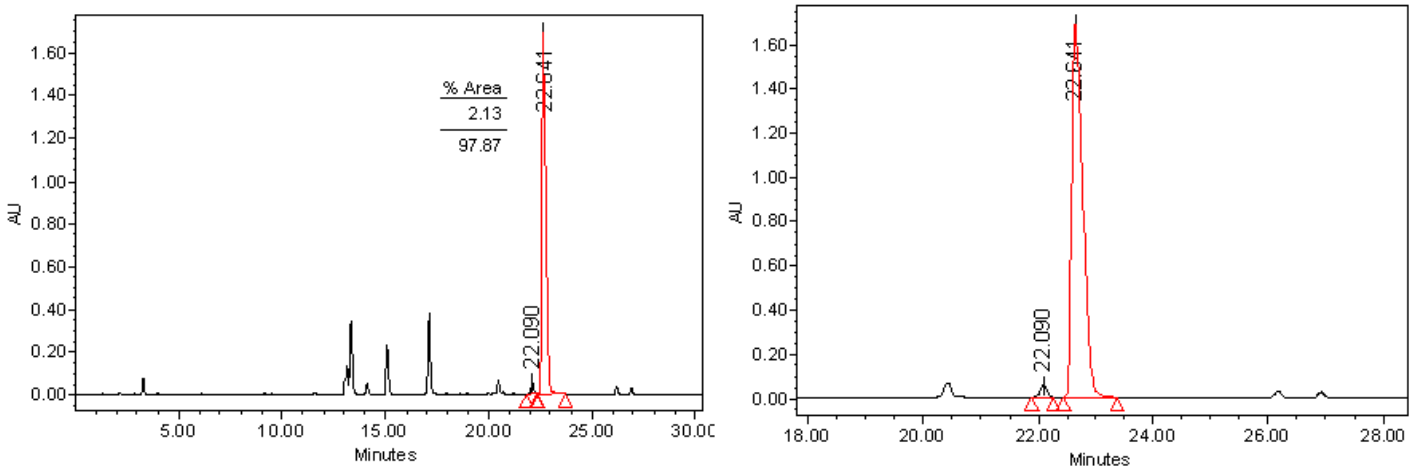


Figure S17. Analytical HPLC of crude **Fmoc-Ala-Ser(ᵗBu)-Ser(ᵗBu)-Leu-Ψ[CH₂–NH]-Asp(OᵗBu)-Tyr(ᵗBu)-Trp(Boc)-Gly-OH (8c.1)**; Column: Purospher RP-8e, 5 μm, 125–4 mm; gradient: 20% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 265 nm.

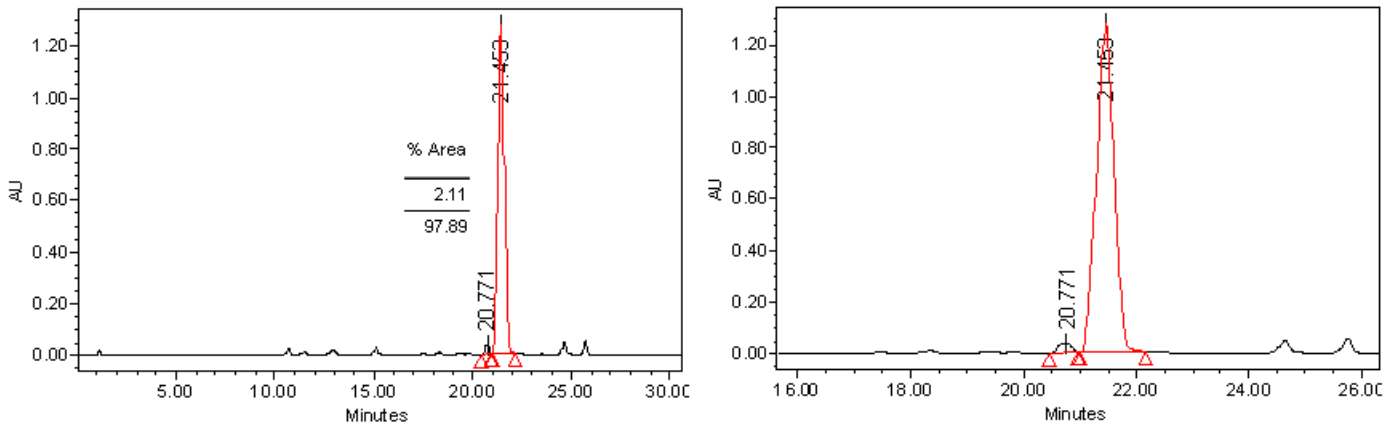


Figure S18. Analytical HPLC of crude **Fmoc-Ala-Ser(ᵗBu)-Ser(ᵗBu)-Leu-Ψ[CH_2–NH]-Ser(ᵗBu)-Tyr(ᵗBu)-Leu-Ser(ᵗBu)-OH** (**8c.2**); Column: Purospher RP-8e, 5 μm, 125–4 mm; gradient: 20% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 265 nm.

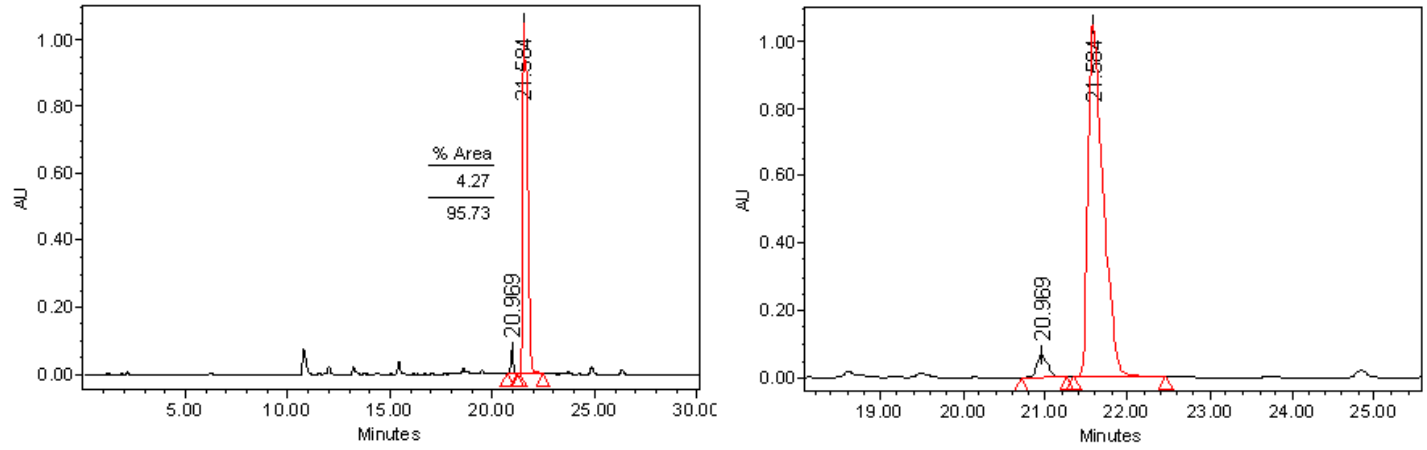


Figure S19. Analytical HPLC of crude **Fmoc-Ala-Ser(ᵗBu)-Ser(ᵗBu)-Ile-Ψ[CH_2–NH]-Ser(ᵗBu)-Tyr(ᵗBu)-Leu-Ser(ᵗBu)-OH** (**8d**); Column: Purospher RP-8e, 5 μm, 125–4 mm; gradient: 20% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 265 nm.

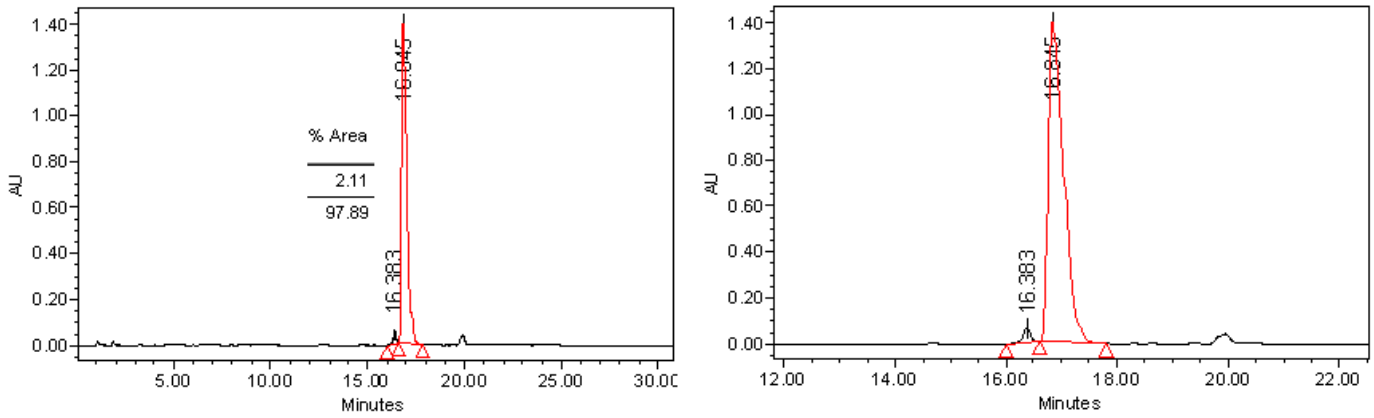


Figure S20. Analytical HPLC of crude **Fmoc-Ala-Ser(ᵗBu)-Ser(ᵗBu)-Phe-Ψ[CH_2–NH]-Leu-Ala-Phe-Gly-OH** (**8e**); Column: Purospher RP-8e, 5 μm, 125–4 mm; gradient: 20% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 265 nm.

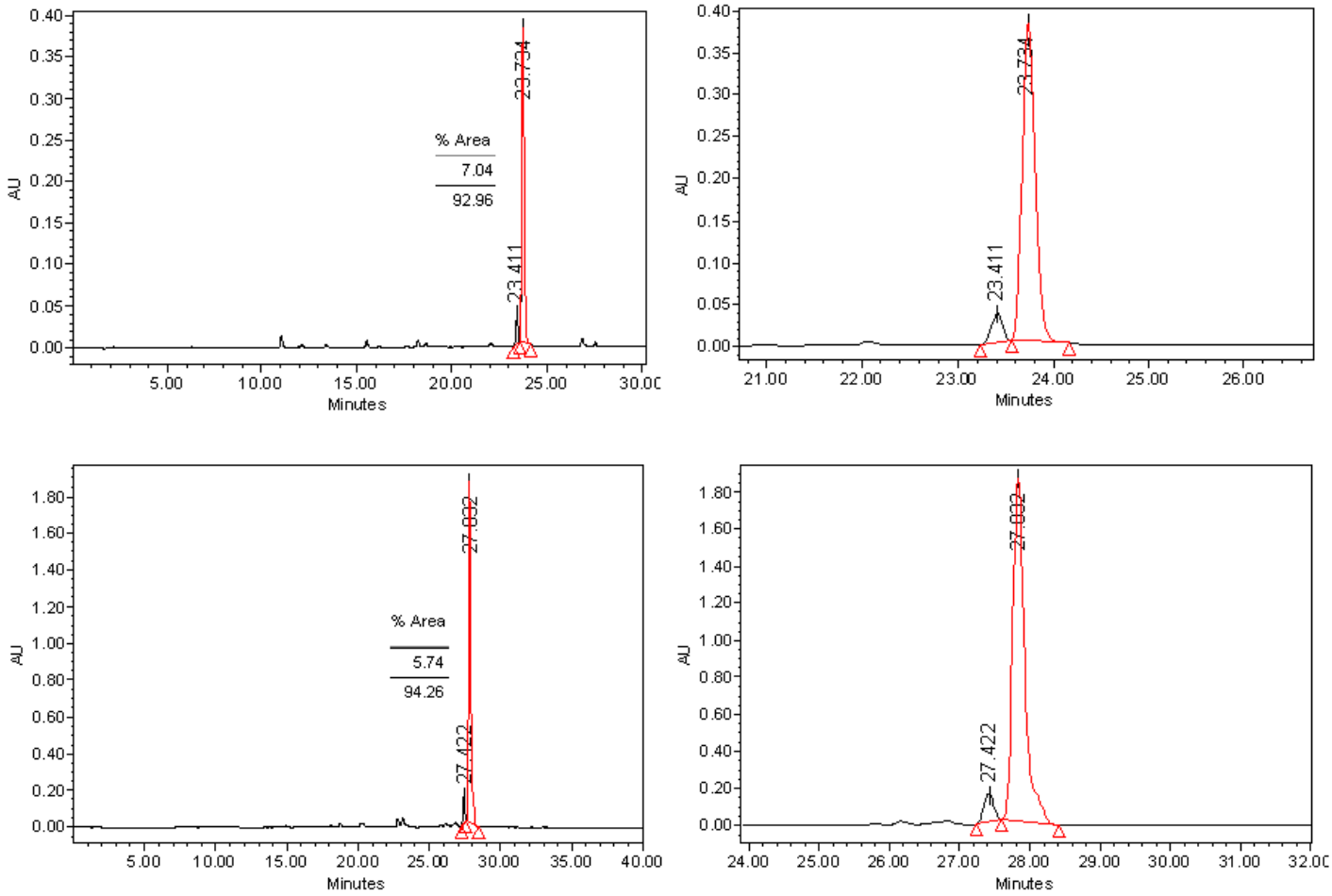


Figure S21. Analytical HPLC of crude **Fmoc-Cys(Trt)-Ala-Lys(Boc)-Val-Ψ[CH_2–NH]-Ser(ᵗBu)-Tyr(ᵗBu)-Leu-Ser(ᵗBu)-OH** (**8f**) and **Fmoc-Cys(Trt)-Ala-Lys(Boc)-Val-Ψ[CH_2–N(Boc)]-Ser(ᵗBu)-Tyr(ᵗBu)-Leu-Ser(ᵗBu)-OH** (**8f+Boc**); Column: Purospher RP-8e, 5 μm, 125–4 mm; gradient: 20% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 265 nm.

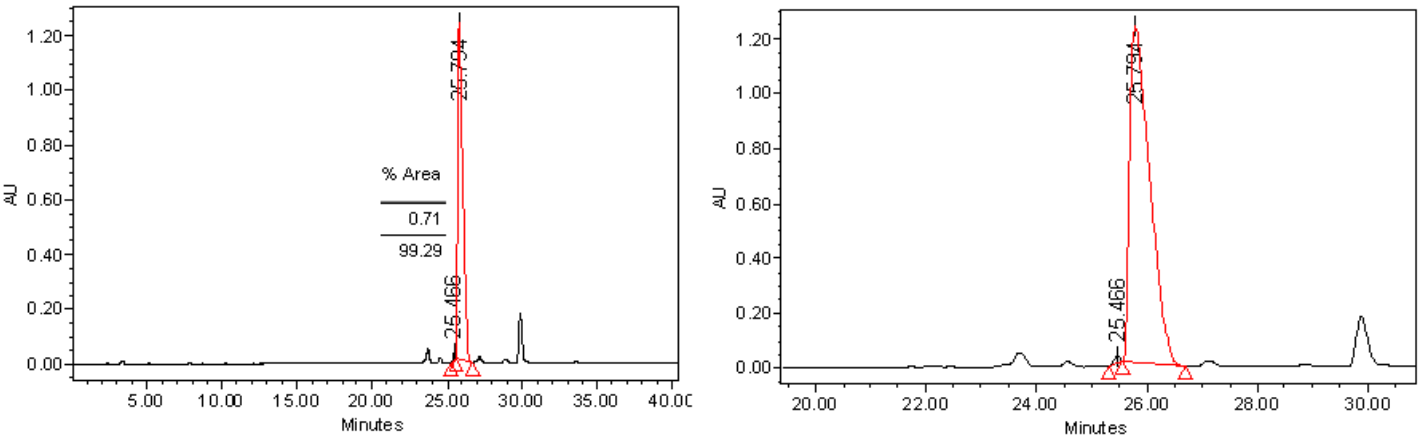


Figure S22. Analytical HPLC of crude **Fmoc-Ser(ᵗBu)-Tyr(ᵗBu)-Leu-Ser(ᵗBu)-Thr(ᵗBu)-Ψ[CH_2–NH]-Leu-Ala-Phe-Gly-OH** (**8g**); Column: YMC-Triart C18, 12 nm, S-5 μm, 250–4.6 mm; gradient: 40% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 265 nm.

Table S3. Percentage epimerization (% Epimer) of peptide fragments **8c.2–8g** obtained after suspending the corresponding resin-bound imines **6c.2–6g** in THF or THF–1% AcOH for 24 h at rt [1]

Resin-bound peptidyl imine sequence	Ψ[CH_2–NH]-containing peptide sequence	% Epimer in THF after 24h	% Epimer in THF–1% AcOH
Fmoc-Ala-Ser(^{t}Bu)-Ser(^{t}Bu)-**Leu-[CH=N]**-Ser(^{t}Bu)-Tyr(^{t}Bu)-Leu-Ser(^{t}Bu)-O-CLTR **6c.2**	Fmoc-Ala-Ser(^{t}Bu)-Ser(^{t}Bu)-**Leu-Ψ[CH_2–NH]**-Ser(^{t}Bu)-Tyr(^{t}Bu)-Leu-Ser(^{t}Bu)-OH **8c.2**	15.0%	47.5%
Fmoc-Ala-Ser(^{t}Bu)-Ser(^{t}Bu)-**Ile-[CH=N]**-Ser(^{t}Bu)-Tyr(^{t}Bu)-Leu-Ser(^{t}Bu)-O-CLTR **6d**	Fmoc-Ala-Ser(^{t}Bu)-Ser(^{t}Bu)-**Ile-Ψ[CH_2–NH]**-Ser(^{t}Bu)-Tyr(^{t}Bu)-Leu-Ser(^{t}Bu)-OH **8d**	7.4% [2]	46.1%
Fmoc-Ala-Ser(^{t}Bu)-Ser(^{t}Bu)-**Phe-[CH=N]**-Leu-Ala-Phe-Gly-O-CLTR **6e**	Fmoc-Ala-Ser(^{t}Bu)-Ser(^{t}Bu)-**Phe-Ψ[CH_2–NH]**-Leu-Ala-Phe-Gly-OH **8e**	24.8%	
Fmoc-Cys(Trt)-Ala-Lys(Boc)-**Val-[CH=N]**-Ser(^{t}Bu)-Tyr(^{t}Bu)-Leu-Ser(^{t}Bu)-O-CLTR **6f**	Fmoc-Cys(Trt)-Ala-Lys(Boc)-**Val-Ψ[CH_2–NH]**-Ser(^{t}Bu)-Tyr(^{t}Bu)-Leu-Ser(^{t}Bu)-OH **8f**	16.2%	49.1%
Fmoc-Ser(^{t}Bu)-Tyr(^{t}Bu)-Leu-Ser(^{t}Bu)-**Thr(^{t}Bu)-[CH=N]**-Leu-Ala-Phe-Gly-O-CLTR **6g**	Fmoc-Ser(^{t}Bu)-Tyr(^{t}Bu)-Leu-Ser(^{t}Bu)-**Thr(^{t}Bu)-Ψ[CH_2–NH]**-Leu-Ala-Phe-Gly-OH **8g**	4.1%	

[1] HPLC of the crude epimerized **8c.2–8g** are presented in **Figures S23–S27**. All experiments were performed at least three times and this is the maximum measured values.

[2] An additional 24 h resin suspension in THF (total 48 h) increased epimerization from 7.4% to 13.8%.

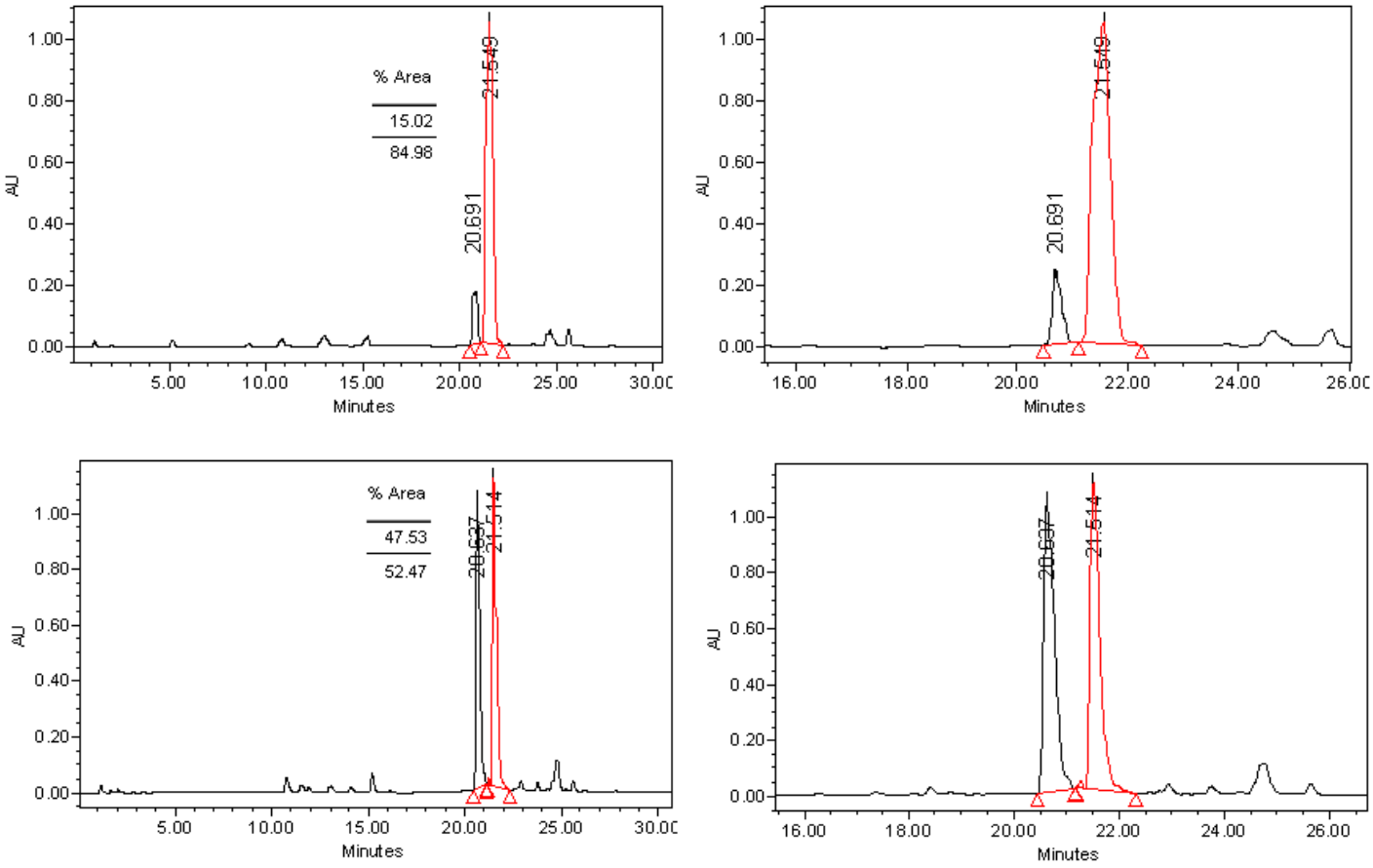


Figure S23. Analytical HPLC of crude **Fmoc-Ala-Ser(^{t}Bu)-Ser(^{t}Bu)-Leu-Ψ[CH_2–NH]-Ser(^{t}Bu)-Tyr(^{t}Bu)-Leu-Ser(^{t}Bu)-OH** (**8c.2**) after 24 h suspension of the corresponding imine in THF and THF–1% AcOH; Column: Purospher RP-8e, 5 μm, 125–4 mm; gradient: 20% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 265 nm.

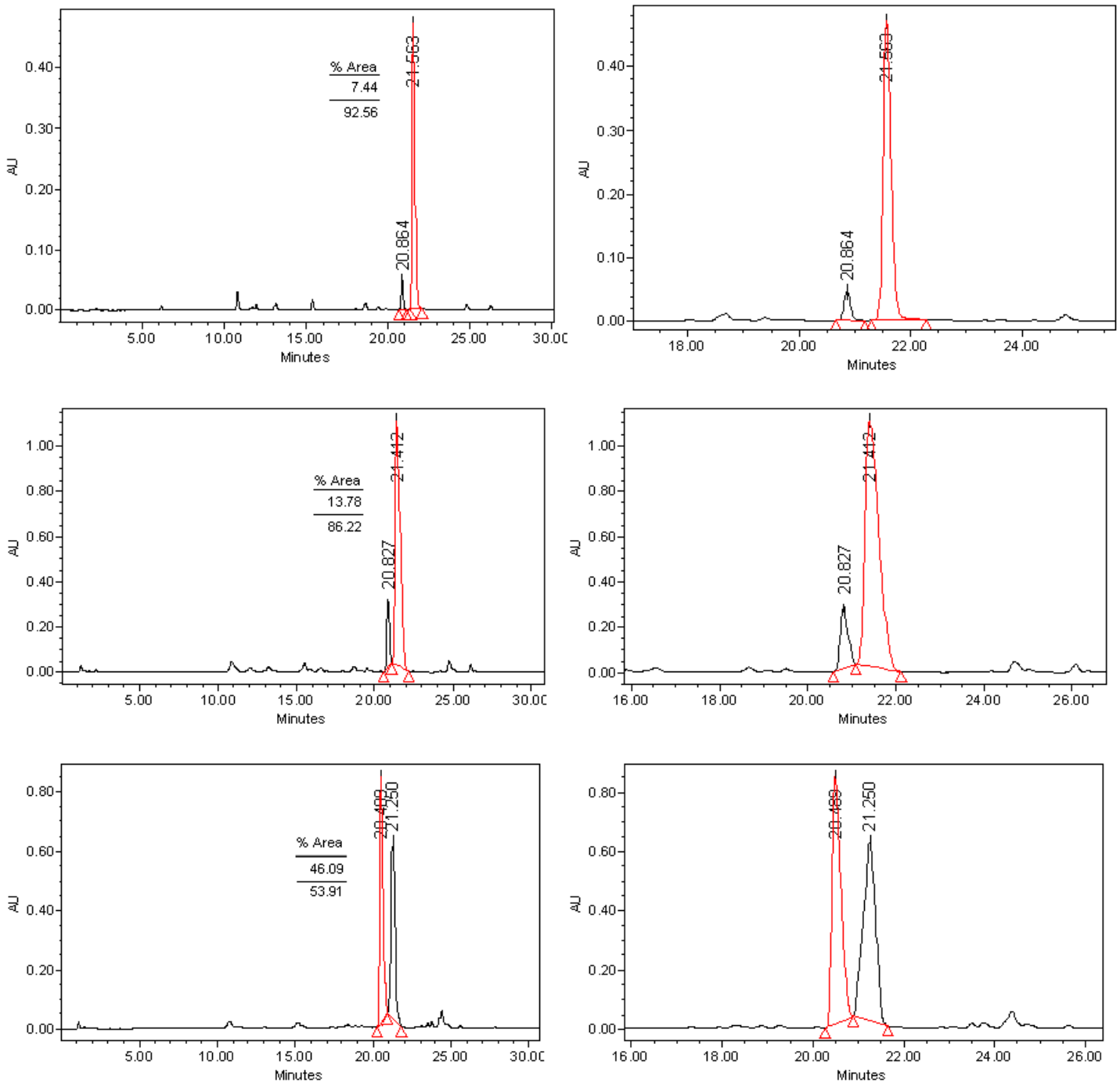


Figure S24. Analytical HPLC of crude **Fmoc-Ala-Ser(ᵗBu)-Ser(ᵗBu)-Ile-Ψ[CH_2–NH]-Ser(ᵗBu)-Tyr(ᵗBu)-Leu-Ser(ᵗBu)-OH** (**8d**) after 24 h and 2x24 h suspension of the corresponding imine in THF and 24 h suspension of the imine in THF–1% AcOH; Column: Purospher RP-8e, 5 μm, 125–4 mm; gradient: 20% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 265 nm.

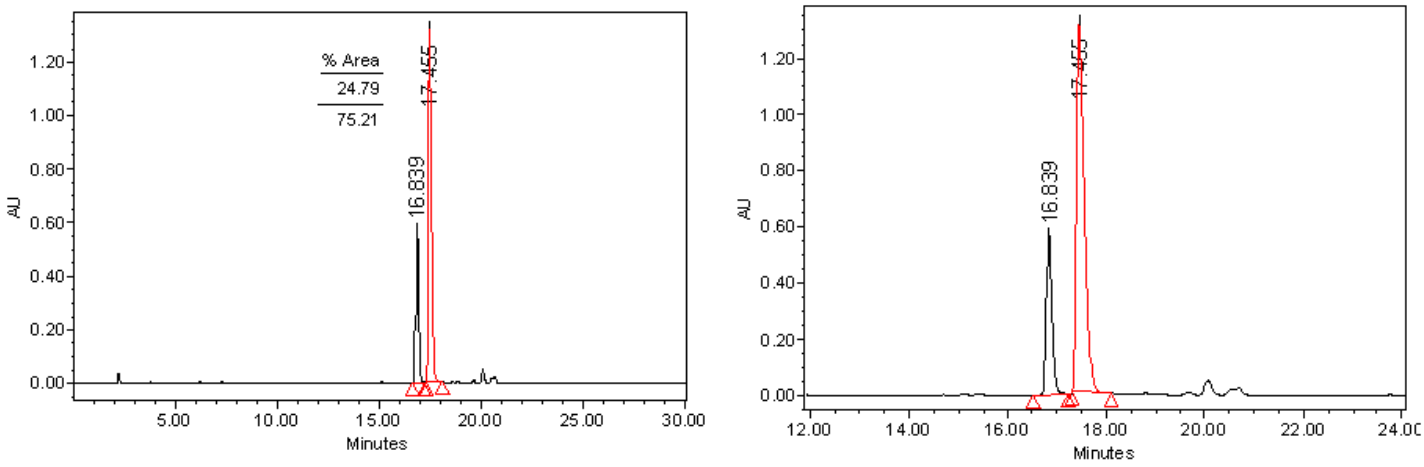


Figure S25. Analytical HPLC of crude **Fmoc-Ala-Ser(ᵗBu)-Ser(ᵗBu)-Phe-Ψ[CH₂–NH]-Leu-Ala-Phe-Gly-OH (8e)** after 24 h suspension of the corresponding imine in THF; Column: Purospher RP-8e, 5 μm, 125–4 mm; gradient: 20% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 265 nm.

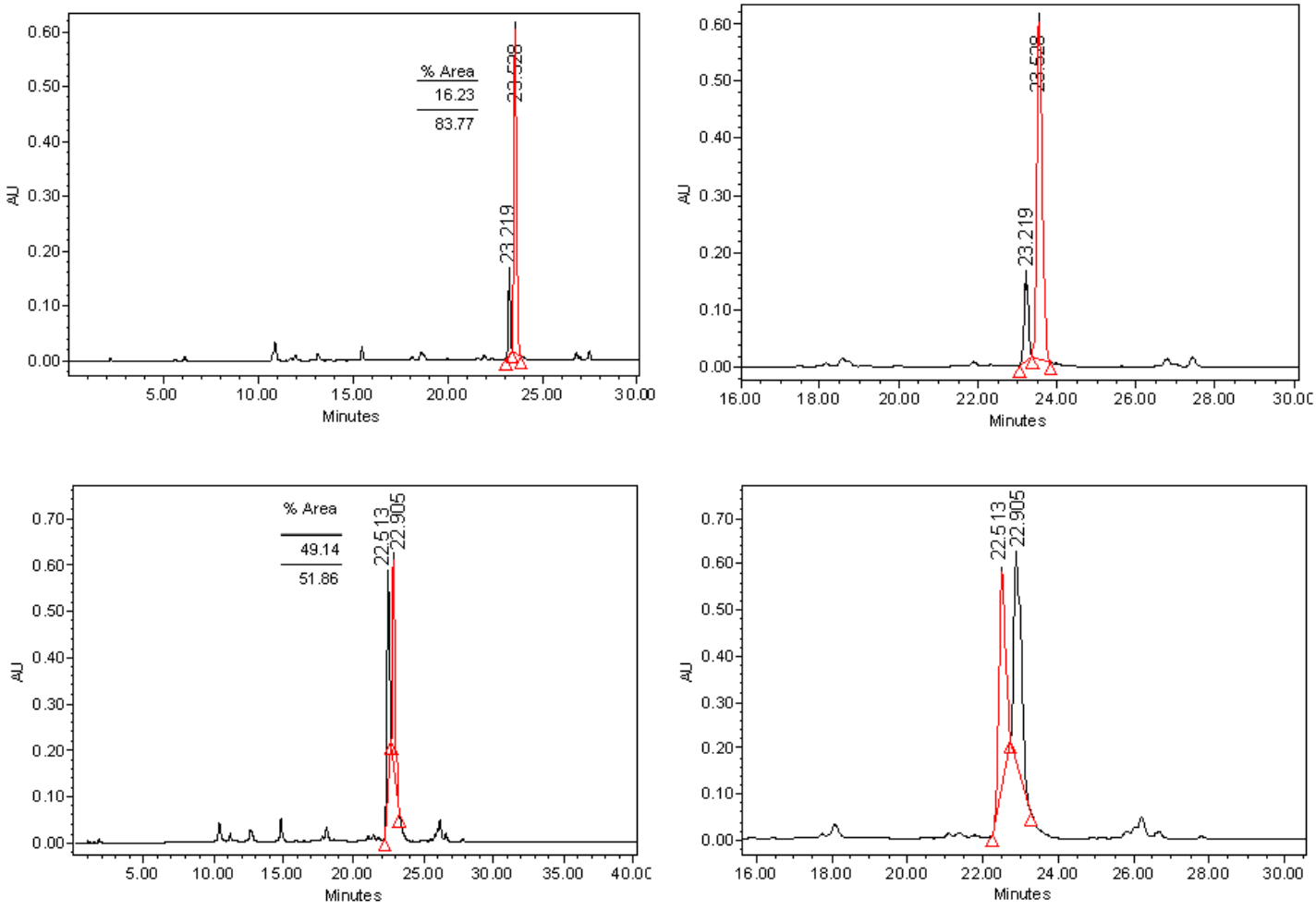


Figure S26. Analytical HPLC of crude **Fmoc-Cys(Trt)-Ala-Lys(Boc)-Val-Ψ[CH₂–NH]-Ser(ᵗBu)-Tyr(ᵗBu)-Leu-Ser(ᵗBu)-OH (8f)** after 24 h suspension of the corresponding imine in THF and THF**–**1% AcOH; Column: Purospher RP-8e, 5 μm, 125–4 mm; gradient: 20% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 265 nm.

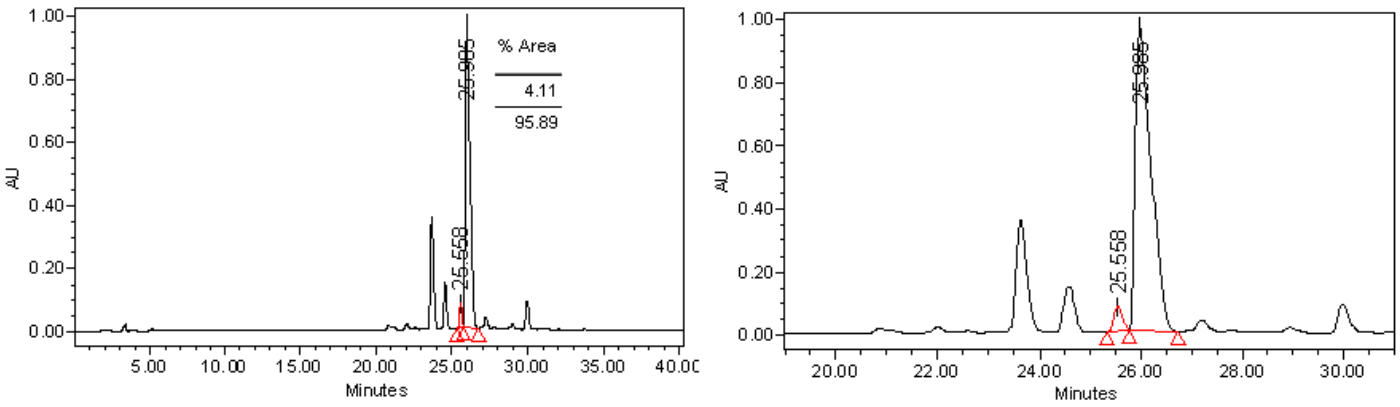


Figure S27. Analytical HPLC of crude **Fmoc-Ser(ᵗBu)-Tyr(ᵗBu)-Leu-Ser(ᵗBu)-Thr(ᵗBu)-Ψ[CH₂–NH]-Leu-Ala-Phe-Gly-OH (8g)** after 24 h suspension of the corresponding imine in THF; Column: YMC-Triart C18, 12 nm, S-5 μm, 250–4.6 mm; gradient: 40% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 265 nm.

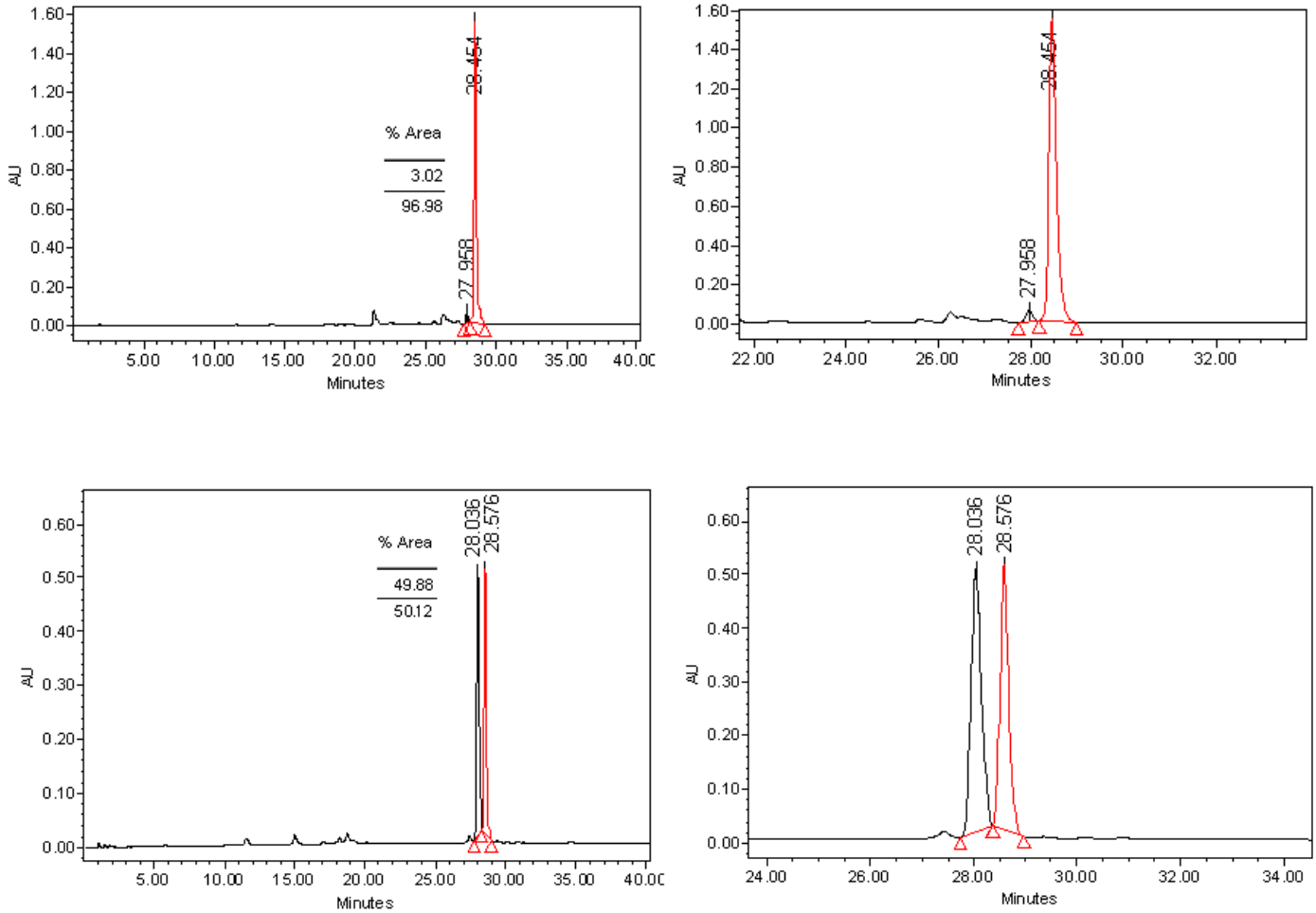


Figure S28. Analytical HPLC of crude **Fmoc-Cys(Trt)-Ala-Lys(Boc)-Val-Ψ[CH₂–N(Boc)]-Ser(ᵗBu)-Tyr(ᵗBu)-Leu-Ser(ᵗBu)-OH (10f)** prepared by *Method B* (**Scheme 4**) and after 24 h suspension of the corresponding imine in THF–1% AcOH; Column: Purospher RP-8e, 5 μm, 125–4 mm; gradient: 20% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 265 nm.

Table S4. Peptide fragments **13a–13e** synthesized *via* Boc-protection and Direct approaches illustrated in Scheme 5 [1,2]

Reaction performed	Final peptide sequence after cleavage from the resin	Method	m/z calc.	m/z found $[M+H]^+$
Fmoc-Ala-Ser(^{t}Bu)-Ser(^{t}Bu)-**Leu-AL** + H-Ser(^{t}Bu)-Tyr(^{t}Bu)-Leu-Ser(^{t}Bu)-**Thr(^{t}Bu)-Ψ[CH_2–N(Boc)]**-Leu-Ala-Phe-Gly-O-CLTR	Fmoc-Ala-Ser(^{t}Bu)-Ser(^{t}Bu)-**Leu-Ψ[CH_2–NH]**-Ser(^{t}Bu)-Tyr(^{t}Bu)-Leu-Ser(^{t}Bu)-**Thr(^{t}Bu)-Ψ[CH_2–N(Boc)]**-Leu-Ala-Phe-Gly-OH **13a**	Boc-protection approach	1946.20	1947.18
Fmoc-Ala-Ser(^{t}Bu)-Ser(^{t}Bu)-**Aib-AL** + H-Ala-Ser(^{t}Bu)-Ser(^{t}Bu)-**Phe-Ψ[CH_2–N(Boc)]**-Leu-Ala-Phe-Gly-O-CLTR	Fmoc-Ala-Ser(^{t}Bu)-Ser(^{t}Bu)-**Aib-Ψ[CH_2–NH]**-Ala-Ser(^{t}Bu)-Ser(^{t}Bu)-**Phe-Ψ[CH_2–N(Boc)]**- Leu-Ala-Phe-Gly-OH **13b**	Boc-protection approach	1646.96	1648.01
Fmoc-Ala-Ser(^{t}Bu)-Ser(^{t}Bu)-**Leu-Ψ[CH_2–NH]**-Ser(^{t}Bu)-Tyr(^{t}Bu)-Leu-Ser(^{t}Bu)-**Thr(^{t}Bu)-Ψ[CH_2–N(Boc)]**-Leu-Ala-Phe-Gly-O-CLTR + **Boc_2O**/DIPEA	Fmoc-Ala-Ser(^{t}Bu)-Ser(^{t}Bu)-**Leu-Ψ[CH_2–N(Boc)]**-Ser(^{t}Bu)-Tyr(^{t}Bu)-Leu-Ser(^{t}Bu)-**Thr(^{t}Bu)-Ψ[CH_2–N(Boc)]**-Leu-Ala-Phe-Gly-OH **13c**	Boc-protection approach	2046.26	2047.20
Fmoc-Ala-Ser(^{t}Bu)-Ser(^{t}Bu)-**Aib-AL** + H-Ala-Ser(^{t}Bu)-Ser(^{t}Bu)-**Phe-Ψ[CH_2–NH]**-Leu-Ala-Phe-Gly-O-CLTR	Fmoc-Ala-Ser(^{t}Bu)-Ser(^{t}Bu)-**Aib-Ψ[CH_2–NH]**-Ala-Ser(^{t}Bu)-Ser(^{t}Bu)-**Phe-Ψ[CH_2–NH]**-Leu-Ala-Phe-Gly-OH **13d**	Direct approach	1546.91	1547.89
Fmoc-Ala-Ser(^{t}Bu)-Ser(^{t}Bu)-**Phe-AL** + H-Ala-Ser(^{t}Bu)-Ser(^{t}Bu)-**Leu-Ψ[CH_2–NH]**-Ser(^{t}Bu)-Tyr(^{t}Bu)-Leu-Sert(^{t}Bu)-O-CLTR	Fmoc-Ala-Ser(^{t}Bu)-Ser(^{t}Bu)-**Phe-Ψ[CH_2–NH]**-Ala-Ser(^{t}Bu)-Ser(^{t}Bu)-**Leu-Ψ[CH_2–NH]**-Ser(^{t}Bu)-Tyr(^{t}Bu)-Leu-Sert(^{t}Bu)-OH **13e**	Direct approach	1805.12	1806.13

[1] HPLC of the crude products (and intermediates) are presented in **Figures S29–S35**.

[2] The eluted peaks from each HPLC analysis corresponding to the products were collected and subjected to ESI-MS.

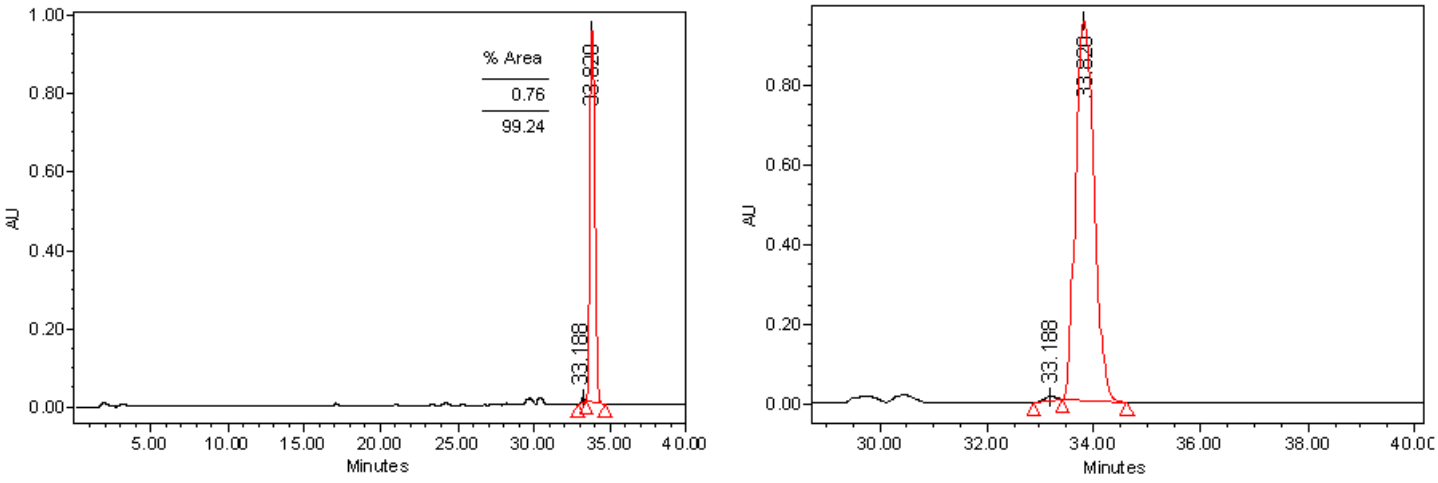


Figure S29. Analytical HPLC of crude fragment A+B in the Ψ[CH_2–N(Boc)] protected form: **Fmoc-Ser(^{t}Bu)-Tyr(^{t}Bu)-Leu-Ser(^{t}Bu)-Thr(^{t}Bu)-Ψ[CH_2–N(Boc)]-Leu-Ala-Phe-Gly-OH (the D-diastereomer was also identified in the Boc-protected form)**; Column: YMC-Triart C18, 12 nm, S-5 μm, 250–4.6 mm; gradient: 50% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 265 nm.

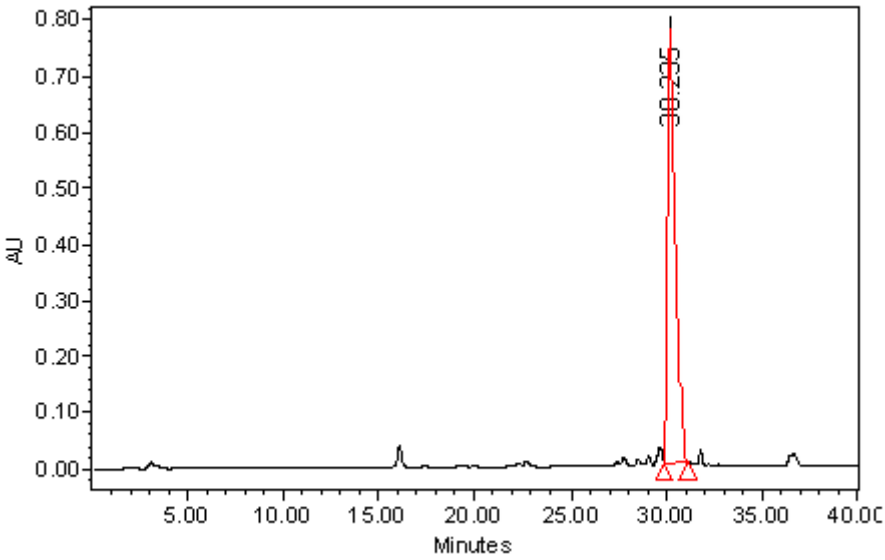


Figure S30. Analytical HPLC of crude fragment A+B+C (Boc-approach): **Fmoc-Ala-Ser(ᵗBu)-Ser(ᵗBu)-Leu-Ψ[CH_2–NH]-Ser(ᵗBu)-Tyr(ᵗBu)-Leu-Ser(ᵗBu)-Thr(ᵗBu)-Ψ[CH_2–N(Boc)]-Leu-Ala-Phe-Gly-OH (13a)**; Column: YMC-Triart C18, 12 nm, S-5 μm, 250–4.6 mm; gradient: 50% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 265 nm.

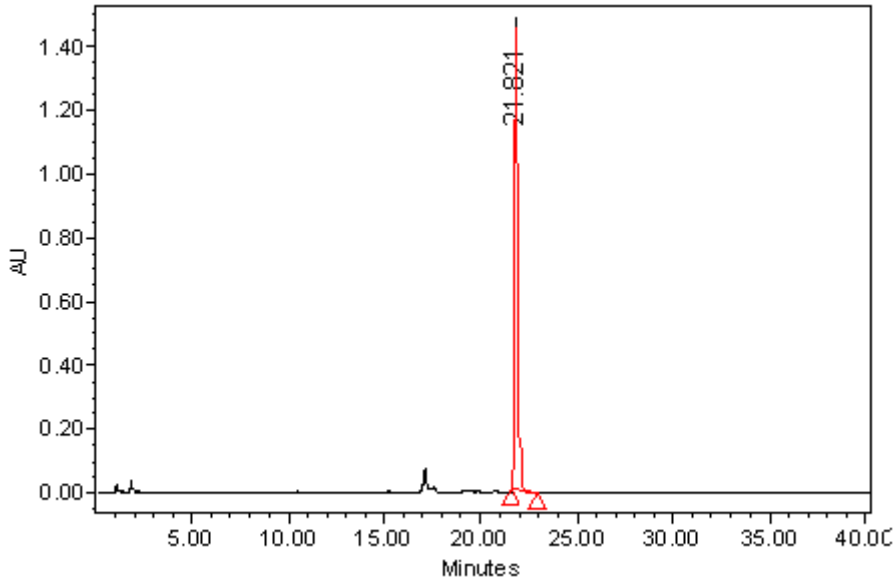


Figure S31. Analytical HPLC of crude fragment A+B in the Ψ[CH_2–N(Boc)] protected form: **Fmoc-Ala-Ser(ᵗBu)-Ser(ᵗBu)-Phe-Ψ[CH_2–N(Boc)]-Leu-Ala-Phe-Gly-OH**; Column: Purospher RP-8e, 5 μm, 125–4 mm; gradient: 20% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 265 nm.

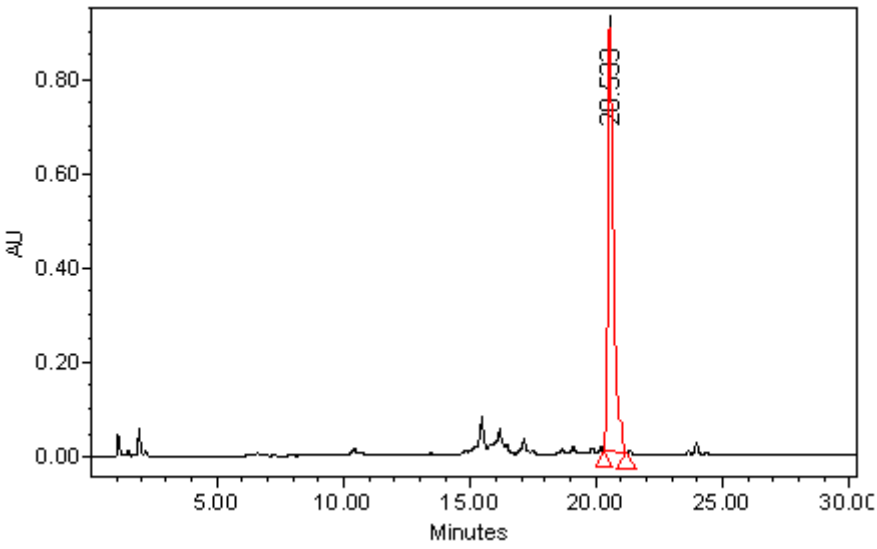


Figure S32. Analytical HPLC of crude fragment A+B+C (Boc-approach): **Fmoc-Ala-Ser(ᵗBu)-Ser(ᵗBu)-Aib-Ψ[CH_2–NH]-Ala-Ser(ᵗBu)-Ser(ᵗBu)-Phe-Ψ[CH_2–N(Boc)]-Leu-Ala-Phe-Gly-OH (13b)**; Column: Purospher RP-8e, 5 μm, 125–4 mm; gradient: 20% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 265 nm.

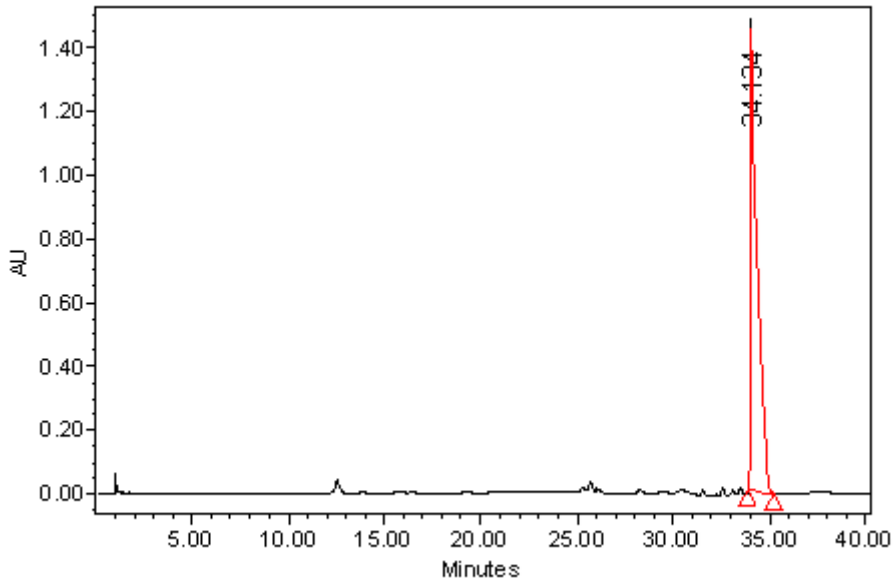


Figure S33. Analytical HPLC of crude fragment A+B+C (Boc-approach): **Fmoc-Ala-Ser(ᵗBu)-Ser(ᵗBu)-Leu-Ψ[CH_2–N(Boc)]-Ser(ᵗBu)-Tyr(ᵗBu)-Leu-Ser(ᵗBu)-Thr(ᵗBu)-Ψ[CH_2–N(Boc)]-Leu-Ala-Phe-Gly-OH (13c)**; Column: YMC-Triart C18, 12 nm, S-5 μm, 250–4.6 mm; gradient: 60% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 265 nm.

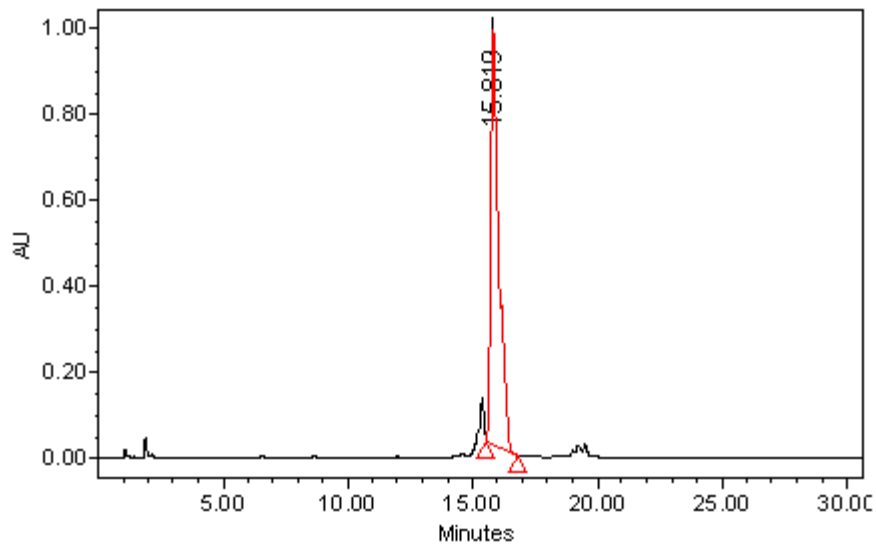


Figure S34. Analytical HPLC of crude fragment A+B+C (Direct approach): **Fmoc-Ala-Ser(ᵗBu)-Ser(ᵗBu)-Aib-Ψ[CH_2–NH]-Ala-Ser(ᵗBu)-Ser(ᵗBu)-Phe-Ψ[CH_2–NH]-Leu-Ala-Phe-Gly-OH (13d)**; Column: Purospher RP-8e, 5 μm, 125–4 mm; gradient: 20% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 265 nm.

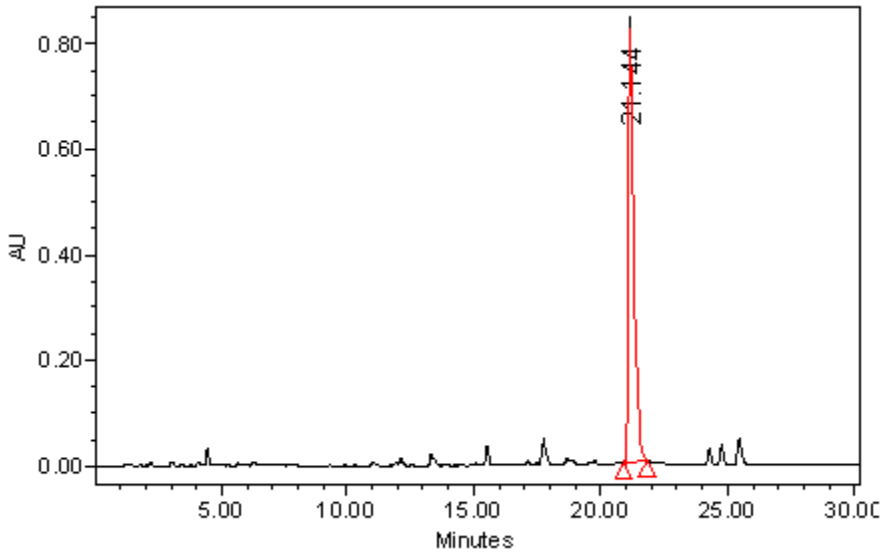
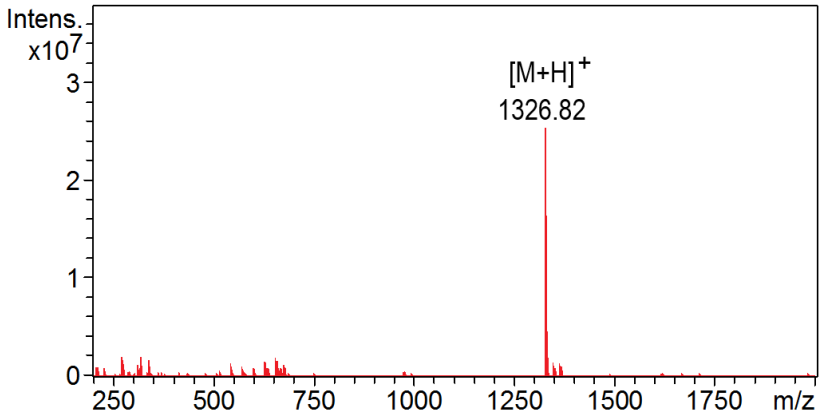


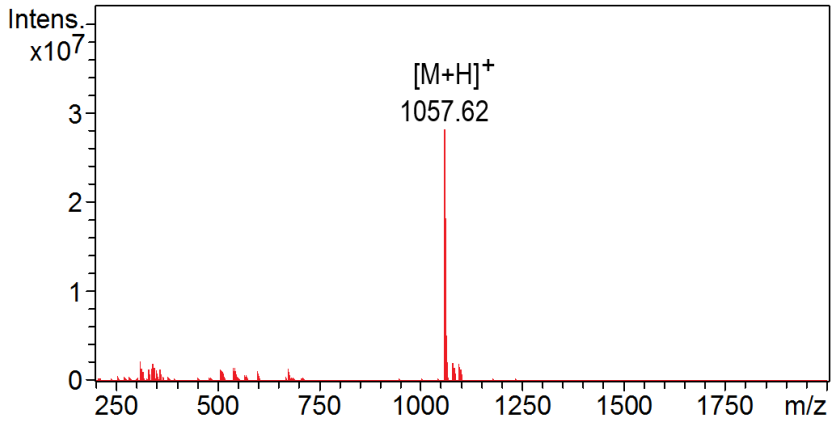
Figure S35. Analytical HPLC of crude fragment A+B+C (Direct approach): **Fmoc-Ala-Ser(ᵗBu)-Ser(ᵗBu)-Phe-Ψ[CH_2–NH]-Ala-Ser(ᵗBu)-Ser(ᵗBu)-Leu-Ψ[CH_2–NH]-Ser(ᵗBu)-Tyr(ᵗBu)-Leu-Sert(ᵗBu)-OH (13e)**; Column: Purospher RP-8e, 5 μm, 125–4 mm; gradient: 20% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 265 nm.

ESI-MS of the products obtained by solid-phase reductive amination

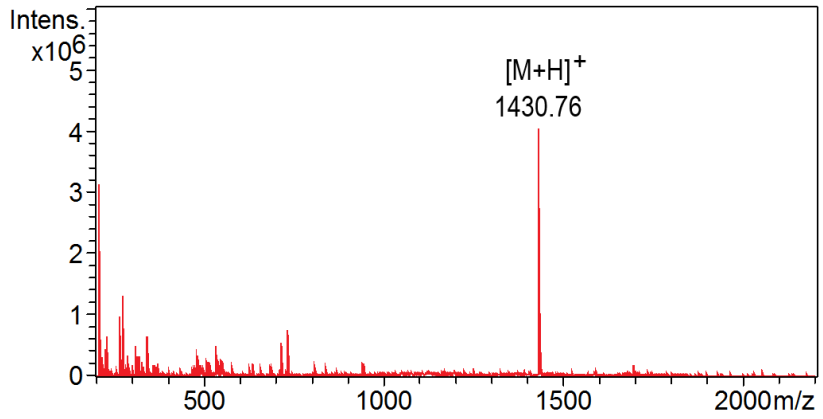
Fmoc-Leu-Val-Glu(O^{t}Bu)-Ser(^{t}Bu)-Gly-Gly-Gly-Ψ[CH_2–NH]-Leu-Ala-Phe-Gly-OH 8a



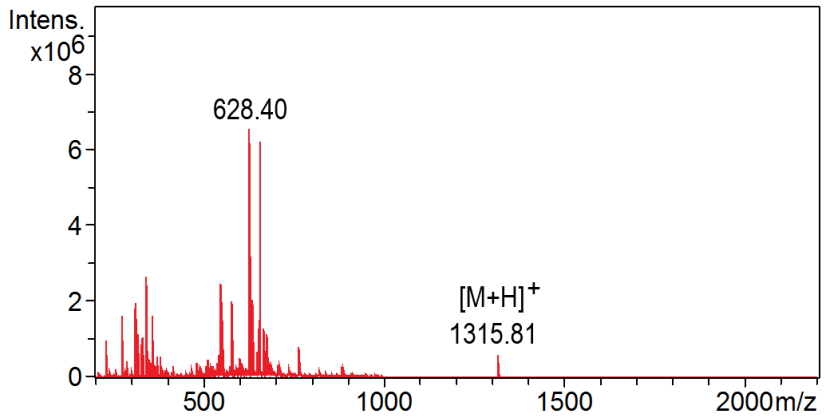
Fmoc-Ala-Ser(^{t}Bu)-Ser(^{t}Bu)-Aib-Ψ[CH_2–NH]-Leu-Ala-Phe-Gly-OH 8b



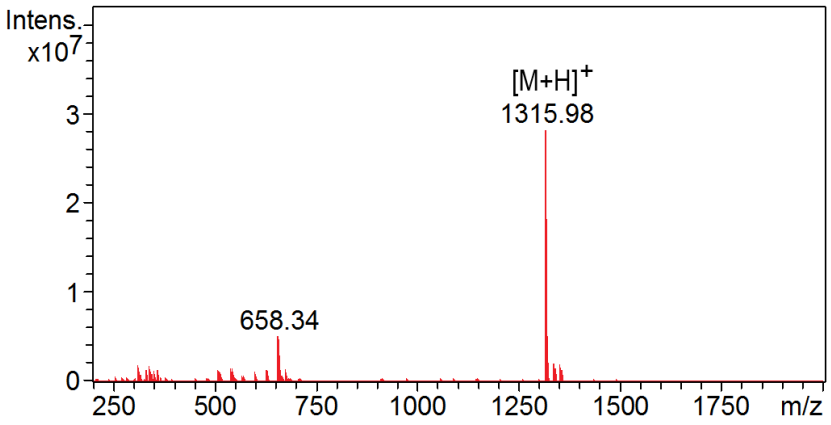
Fmoc-Ala-Ser(^{t}Bu)-Ser(^{t}Bu)-Leu-Ψ[CH_2–NH]-Asp(O^{t}Bu)-Tyr(^{t}Bu)-Trp(Boc)-Gly-OH 8c.1



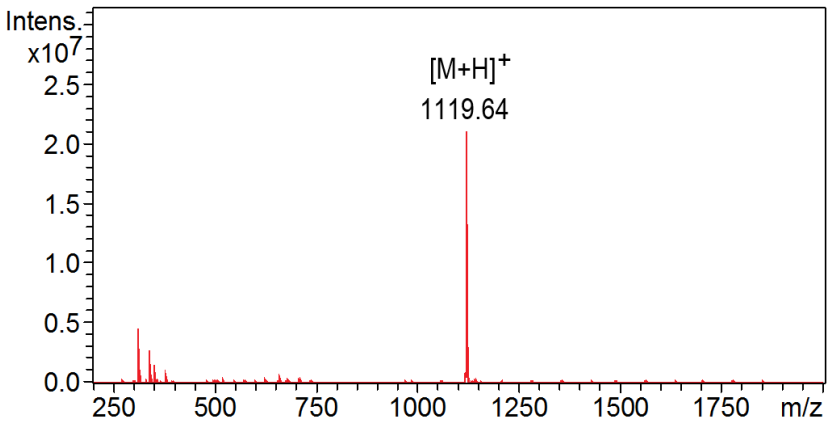
Fmoc-Ala-Ser(ᵗBu)-Ser(ᵗBu)-Leu-Ψ[CH_2–NH]-Ser(ᵗBu)-Tyr(ᵗBu)-Leu-Ser(ᵗBu)-OH 8c.2



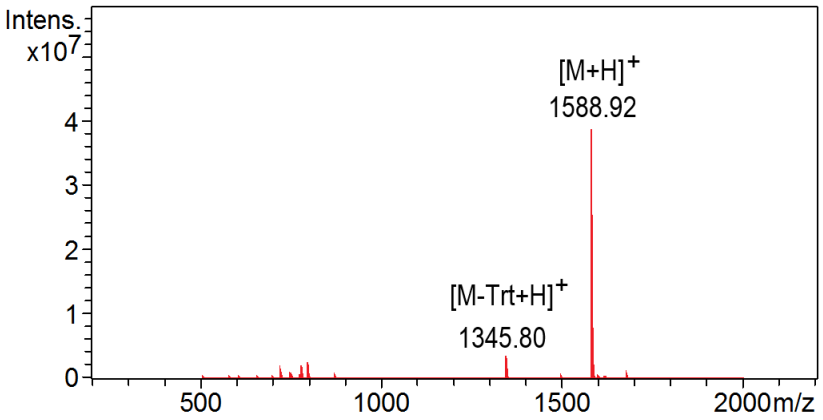
Fmoc-Ala-Ser(ᵗBu)-Ser(ᵗBu)-Ile-Ψ[CH_2–NH]-Ser(ᵗBu)-Tyr(ᵗBu)-Leu-Ser(ᵗBu)-OH 8d



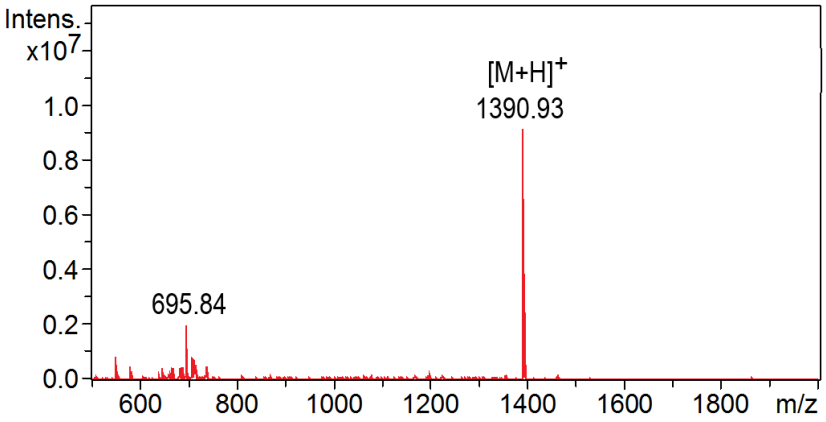
Fmoc-Ala-Ser(ᵗBu)-Ser(ᵗBu)-Phe-Ψ[CH_2–NH]- Leu-Ala-Phe-Gly-OH 8e



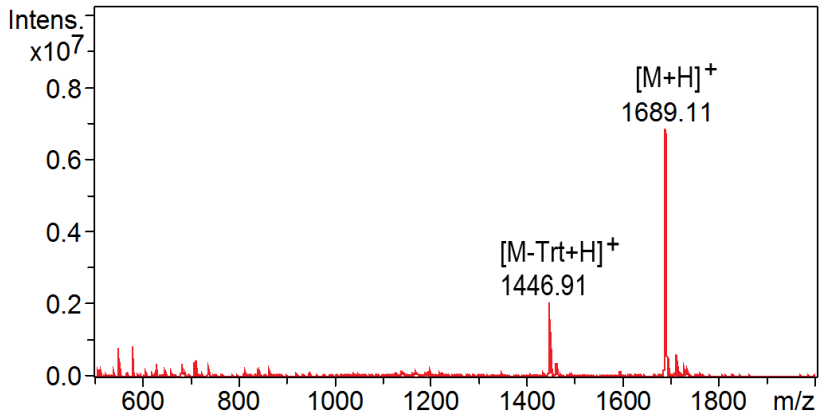
Fmoc-Cys(Trt)-Ala-Lys(Boc)-Val-Ψ[CH_2–NH]-Ser(^{t}Bu)-Tyr(^{t}Bu)-Leu-Ser(^{t}Bu)-OH 8f



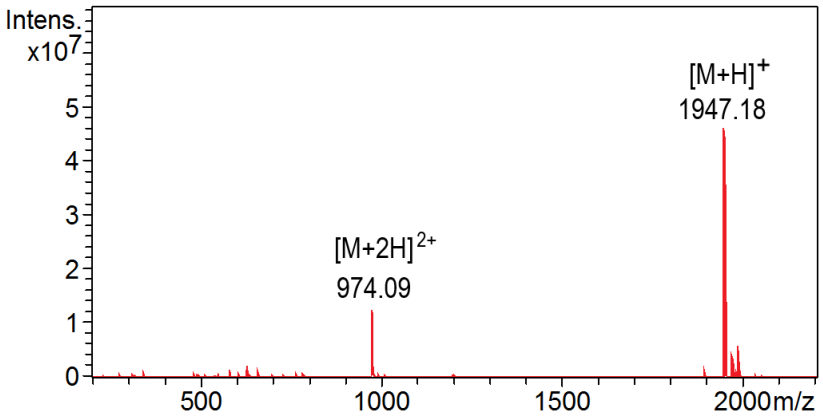
Fmoc-Ser(^{t}Bu)-Tyr(^{t}Bu)-Leu-Ser(^{t}Bu)-Thr(^{t}Bu)-Ψ[CH_2–NH]-Leu-Ala-Phe-Gly-OH 8g



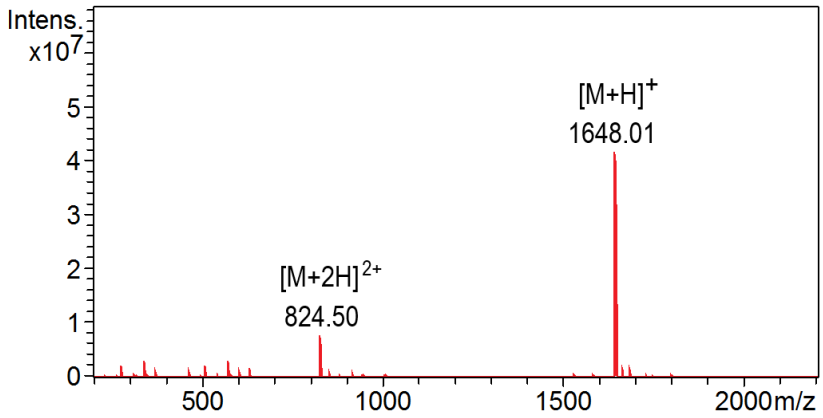
Fmoc-Cys(Trt)-Ala-Lys(Boc)-Val-Ψ[CH_2–N(Boc)]-Ser(^{t}Bu)-Tyr(^{t}Bu)-Leu-Ser(^{t}Bu)-OH 10f



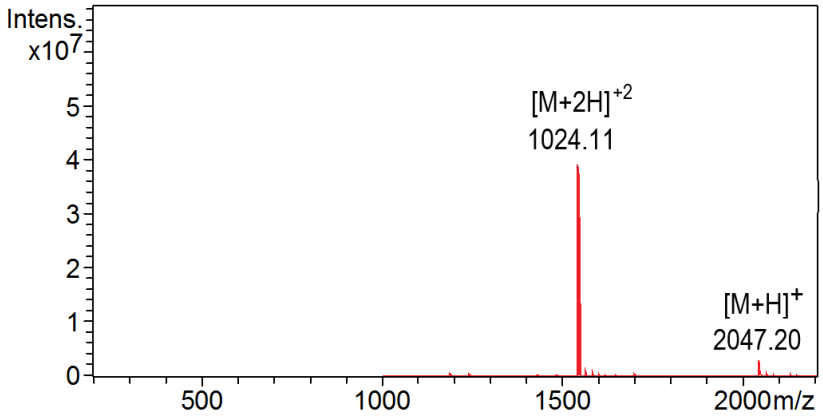
Fmoc-Ala-Ser(ᵗBu)-Ser(ᵗBu)-Leu-Ψ[CH_2–NH]-Ser(ᵗBu)-Tyr(ᵗBu)-Leu-Ser(ᵗBu)-Thr(ᵗBu)-Ψ[CH_2–N(Boc)]-Leu-Ala-Phe-Gly-OH 13a



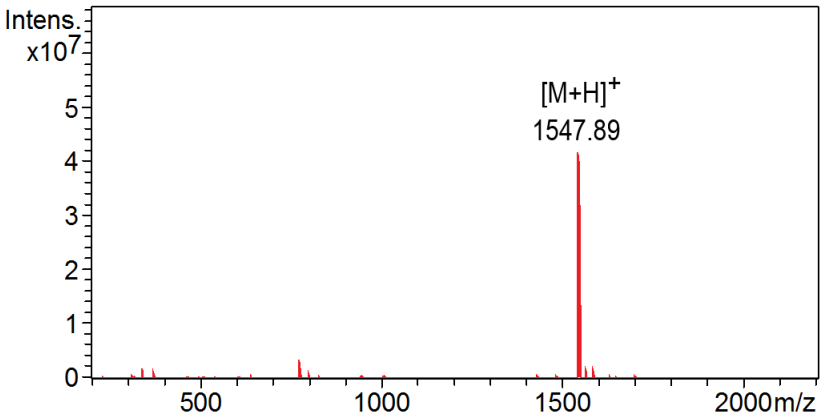
Fmoc-Ala-Ser(ᵗBu)-Ser(ᵗBu)-Aib- Ψ[CH_2–NH]-Ala-Ser(ᵗBu)-Ser(ᵗBu)-Phe-Ψ[CH_2–N(Boc)]- Leu-Ala-Phe-Gly-OH 13b



Fmoc-Ala-Ser(ᵗBu)-Ser(ᵗBu)-Leu-Ψ[CH_2–N(Boc)]-Ser(ᵗBu)-Tyr(ᵗBu)-Leu-Ser(ᵗBu)-Thr(ᵗBu)-Ψ[CH_2–N(Boc)]-Leu-Ala-Phe-Gly-OH 13c



Fmoc-Ala-Ser(ᵗBu)-Ser(ᵗBu)-Aib-Ψ[CH₂–NH]-Ala-Ser(ᵗBu)-Ser(ᵗBu)-Phe-Ψ[CH₂–NH]-Leu-Ala-Phe-Gly-OH 13d



Fmoc-Ala-Ser(ᵗBu)-Ser(ᵗBu)-Phe-Ψ[CH₂–NH]-Ala-Ser(ᵗBu)-Ser(ᵗBu)-Leu-Ψ[CH₂–NH]-Ser(ᵗBu)-Tyr(ᵗBu)-Leu-Sert(ᵗBu)-OH 13e