Use of Homoserinyl *γ*-Aldehyde–Containing Peptides in Solid-Phase Reductive Amination

Dimitrios Tolis,[a] Dimitra Magkafa [a] and Spyridon Mourtas *,[a]

[a] Department of Chemistry, University of Patras, 26510 Rio Patras, Greece.

E-mail: s.mourtas@upatras.gr

HPLC and NMR data

1. Synthesis of peptide aldehydes

1.1. HPLC data

Peptide 9a
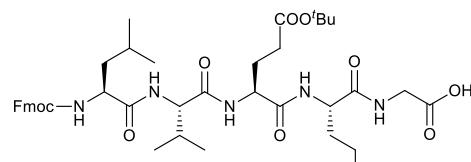

9a: Fmoc-Leu-Val-Glu(*t*Bu)-Hse-Gly-OH
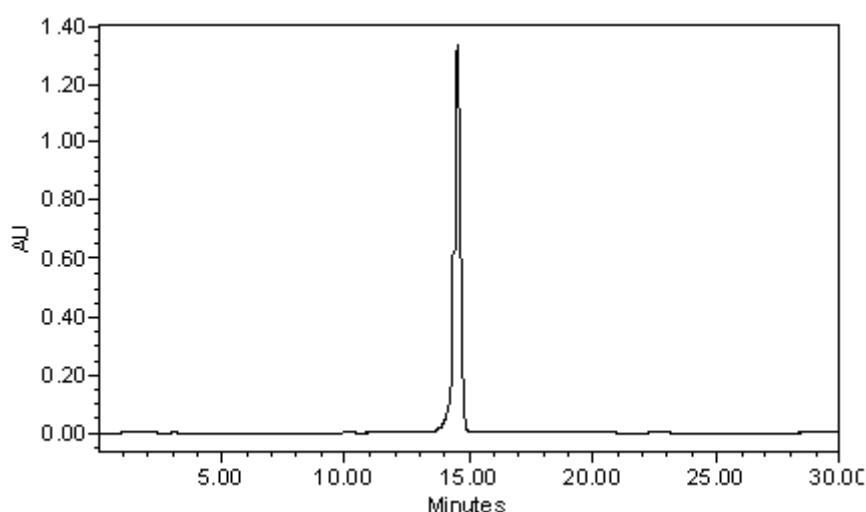
Figure S1: Analytical HPLC of crude Fmoc-Leu-Val-Glu(*t*Bu)-Hse-Gly-OH (**9a**); column: Purospher RP-8e, 5 μm, 250-4 mm; gradient: 20% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 265 nm; t_R = 14.1 min (**9a**). The eluted peak was collected and subjected to ESI-MS m/z: 9: [M+H]+ calc.: 796.41 found: 796.43.

Peptide 10
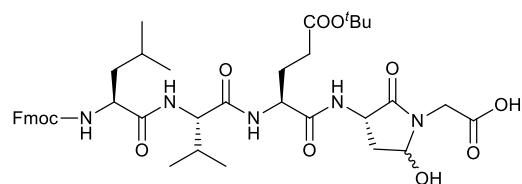
10: γ-hydroxy lactam peptide;
Derived from Fmoc-Leu-Val-Glu(^{t}Bu)-Hse(AL)-Gly-OH
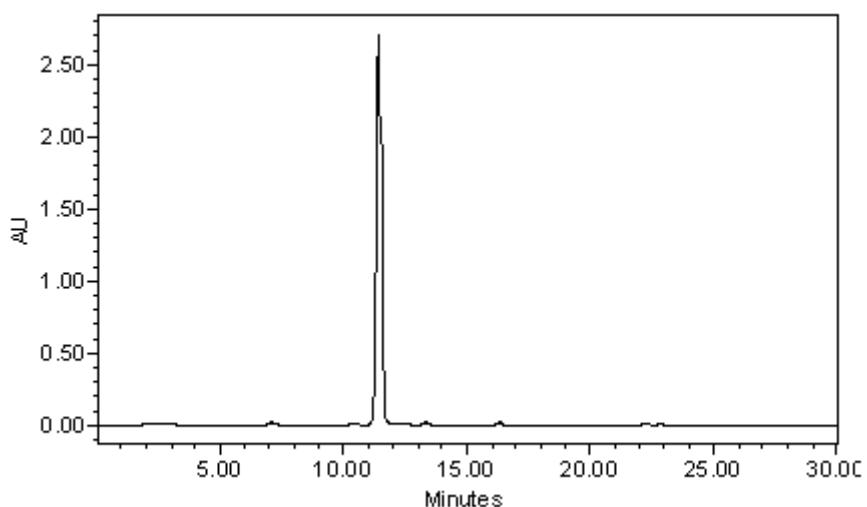
Figure S2: Analytical HPLC of crude *γ*-hydroxy lactam peptide **10**; column: Lichrosphere RP-18, 5 µm, 125–4 mm; gradient: 40% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 265 nm; t_R = 11.2 min (**10**). The eluted peak was collected and subjected to ESI-MS: m/z [M+H]$^+$ calc.: 794.40; found: 794.40.

Peptide 9b
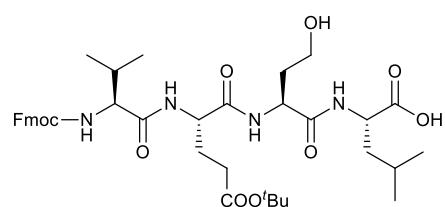
**9b**: Fmoc-Val-Glu(^{t}Bu)-Hse-Leu-OH
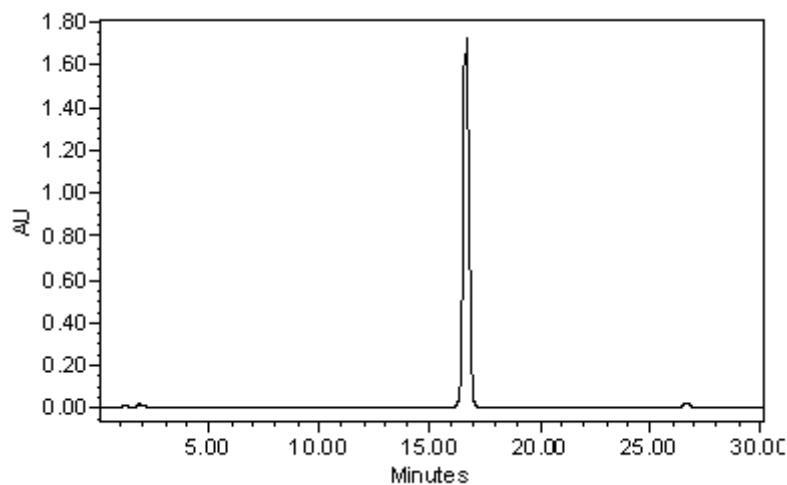
Figure S3: Analytical HPLC of crude Fmoc-Val-Glu(^{t}Bu)-Hse-Leu-OH (**9b**); column: Lichrosphere RP-18, 5 µm, 125–4 mm; gradient: 20% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 265 nm; t_R = 16.4 min (**9b**). The eluted peak was collected and subjected to ESI-MS: m/z [M+H]$^+$ calc. 739.39; found: 739.40.

Peptide 11
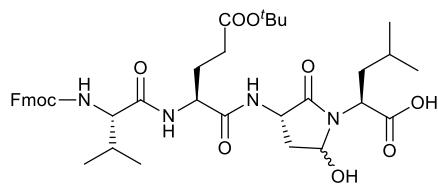
11: γ-hydroxy lactam of Fmoc-Val-Glu(^{t}Bu)-Hse(AL)-Leu-OH
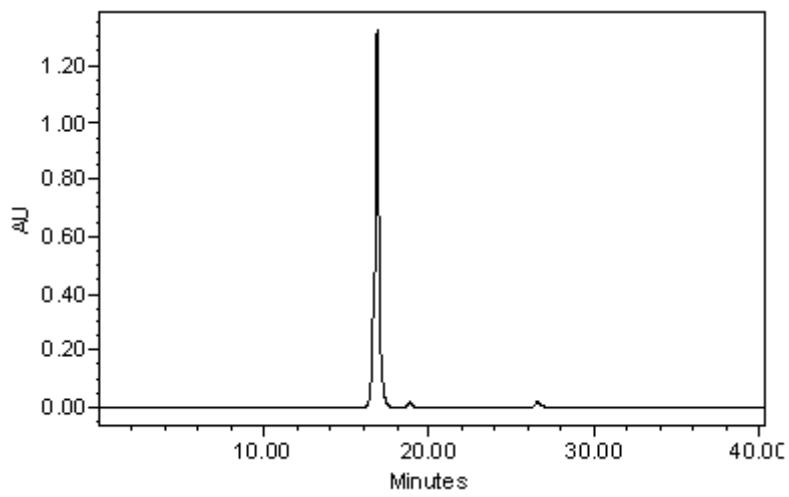
Figure S4: Analytical HPLC of crude *γ*-hydroxy lactam peptide **11**; column: Lichrosphere RP-18, 5 μm, 125–4 mm; gradient: 20% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 265 nm; t_R = 16.6 min (**11**). The eluted peak was collected and subjected to ESI-MS: m/z [M+H]${}^+$ calc. 737.38; found: 737.39.

Peptide 13
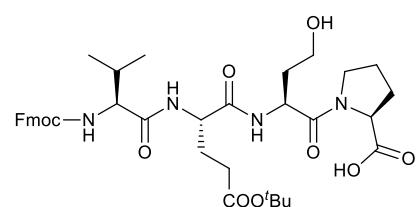
**13**: Fmoc-Val-Glu(*t*Bu)-Hse-Pro-OH
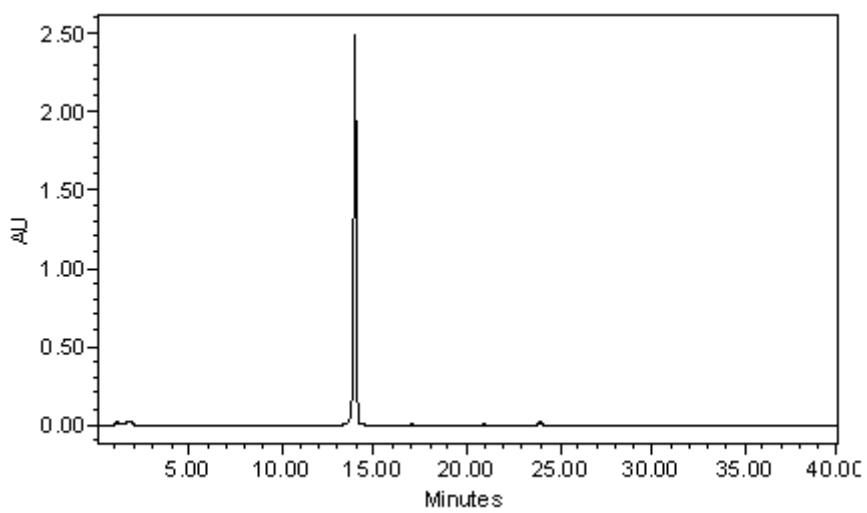
Figure S5: Analytical HPLC of crude Fmoc-Val-Glu(*t*Bu)-Hse-Pro-OH (**13**); column: Lichrosphere RP-18, 5 μm, 125–4 mm; gradient: 20% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 265 nm; t_R = 13.8 min (**13**). The eluted peak was collected and subjected to ESI-MS: m/z [M+H]$^+$ calc. 723.36 found: 723.37.

Peptide 14
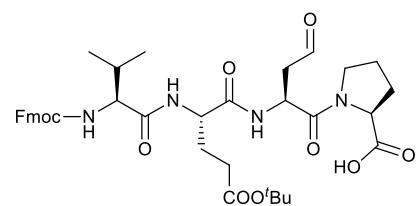
**14**: Fmoc-Val-Glu(^{t}Bu)-Hse(AL)-Pro-OH
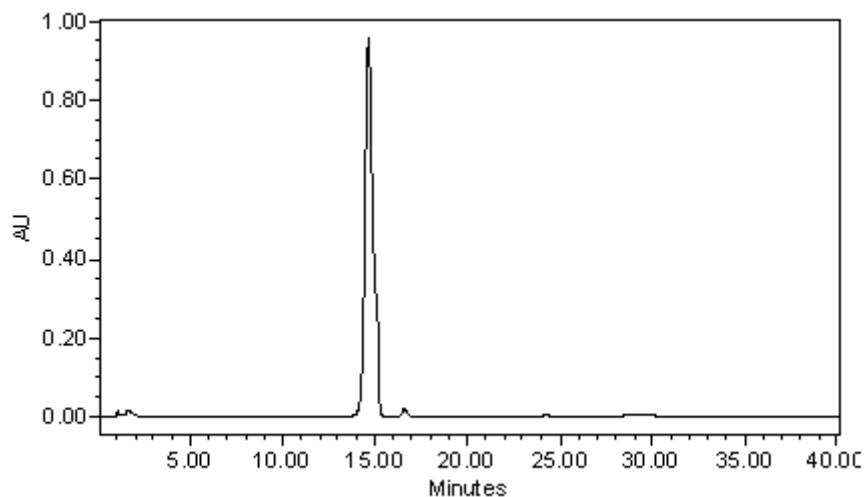
Figure S6: Analytical HPLC of crude Fmoc-Val-Glu(^{t}Bu)-Hse-Pro-OH (**14**); column: Lichrosphere RP-18, 5 µm, 125–4 mm; gradient: 20% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 265 nm; t_R = 14.3 min (**14**). The eluted peak was collected and subjected to ESI-MS: m/z [M+H]$^+$ calc. 721.34; found: 721.35.

Peptide 16, 17
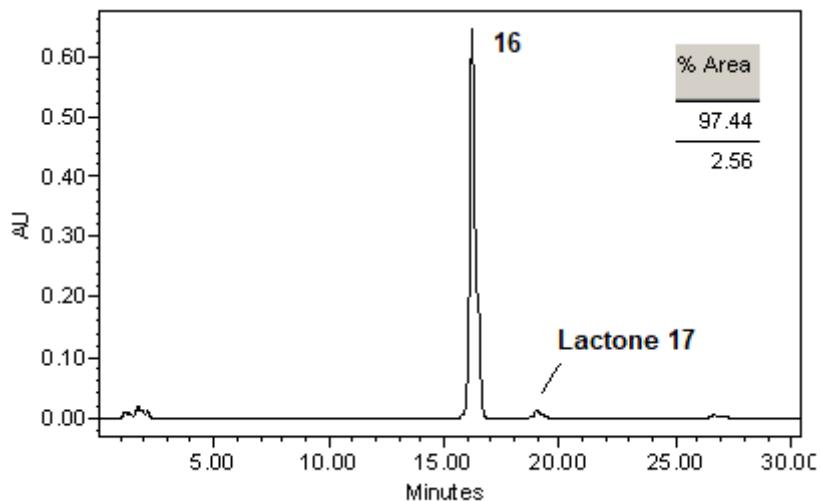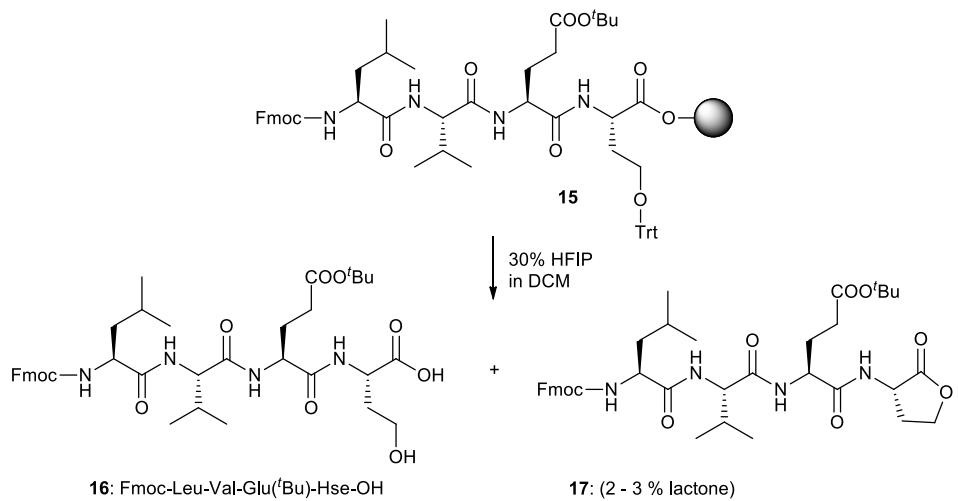
Figure S7: Analytical HPLC of crude Fmoc-Leu-Val-Glu(^{t}Bu)-Hse-OH (**16**); column: Lichrosphere RP-18, 5 µm, 125–4 mm; gradient: 20% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 265 nm; t_R = 16.3 min (**16**), 18.9 min (**17**). The eluted peaks were collected and subjected to ESI-MS. **16**: m/z [M+H]$^+$ calc. 739.39; found: 739.41 **17:** m/z [M+H]$^+$ calc. 721.38; found: 721.39.

Peptide 18, 19

16: Fmoc-Leu-Val-Glu(^{t}Bu)-Hse-OH   [containing 17 (2 -3 %)]

DMP oxidation

18: Fmoc-Leu-Val-Glu(^{t}Bu)-Hse(AL)-OH

19: γ-hydroxy lactone peptide

+

17: Lactone (2 -3 %)
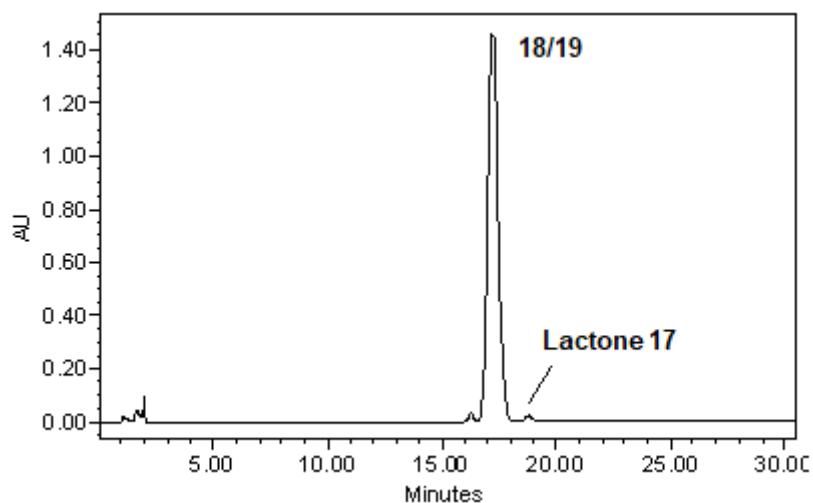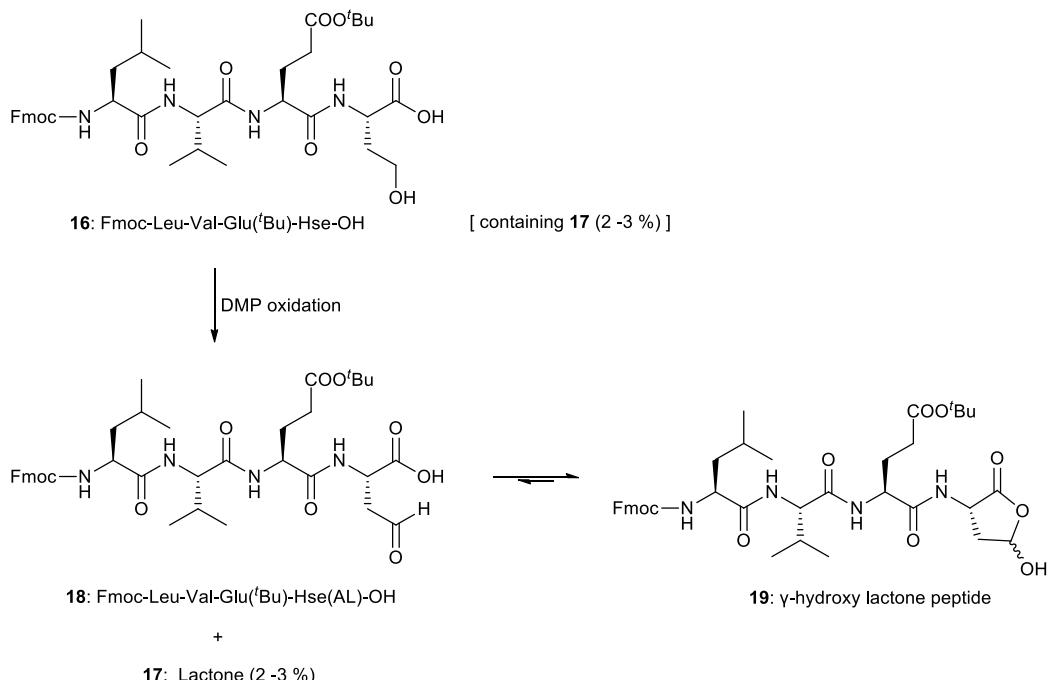
Figure S8: Analytical HPLC of crude *γ*-hydroxy lactone peptide (**18**) (derived from Fmoc-Leu-Val-Glu(^{t}Bu)-Hse(AL)-OH peptide **18**); column: Lichrosphere RP-18, 5 μm, 125–4 mm; gradient: 20% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 265 nm; t_R = 16.9 min (**18/19**). The eluted peaks were collected and subjected to ESI-MS. **18/19**: m/z [M+H]$^+$ calc. 737.38; found: 737.39.

Peptide 20 [containing ~10% lactone (17) and ~10% bis-^{t}Bu product (21)]

16: Fmoc-Leu-Val-Glu(^{t}Bu)-Hse-OH

+

17: Lactone (2-3%)

^{t}BuOC(=NH)CCl$_3$
in DCM/NMP (9:1)

20: Fmoc-Leu-Val-Glu(^{t}Bu)-Hse-O^{t}Bu

+

21: Fmoc-Leu-Val-Glu(^{t}Bu)-Hse(^{t}Bu)-O^{t}Bu (~10 %)

+

17: Lactone (2-3%)
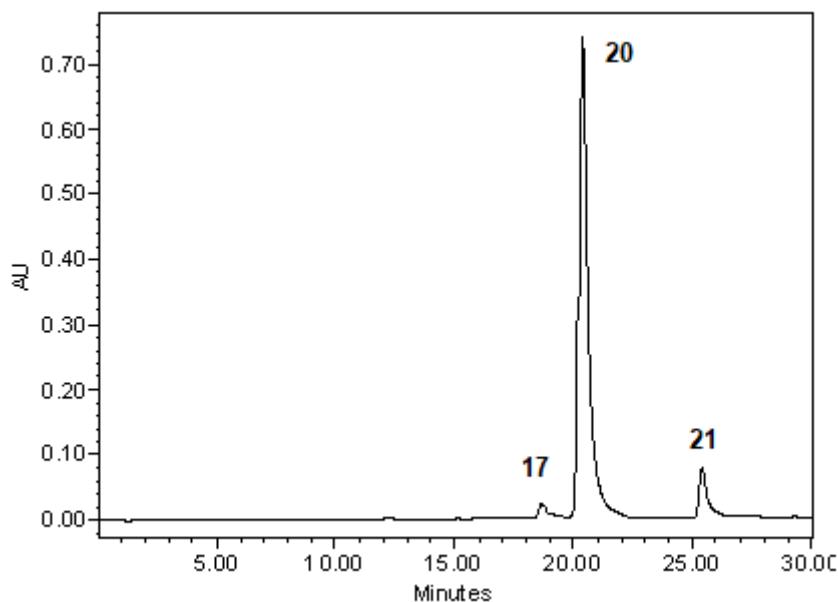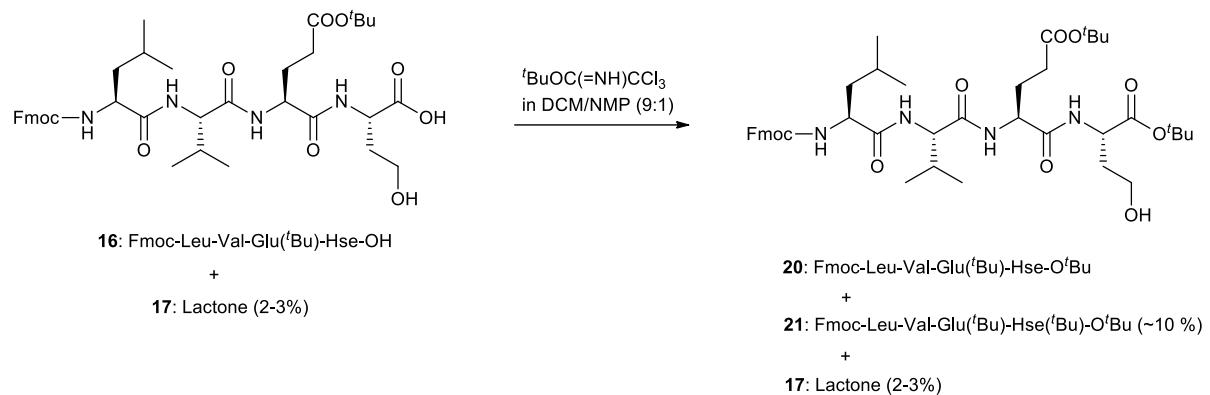
Figure S9: Analytical HPLC of Fmoc-Leu-Val-Glu(^{t}Bu)-Hse-O^{t}Bu (**20**) (containing 2–3% lactone **17** and 10% bis-O^{t}Bu **21**); column: Lichrosphere RP-18, 5 μm, 125–4 mm; gradient: 20% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 265 nm; 18.9 min (**17**), 20.9 min (**20**), 25.9 min (**21**)]; The eluted peaks were collected and subjected to ESI-MS. **20:** m/z [M+H]$^+$ calc. 795.45; found: 795.44; **21:** m/z [M+H]$^+$ calc. 851.52; found: 851.51.

Peptide 22

20: Fmoc-Leu-Val-Glu(^{t}Bu)-Hse-O^{t}Bu
+
21: Fmoc-Leu-Val-Glu(^{t}Bu)-Hse(^{t}Bu)-O^{t}Bu (~10%)
+
17: Lactone (2-3%)

DMP oxiadation

22: Fmoc-Leu-Val-Glu(^{t}Bu)-Hse(AL)-O^{t}Bu
+
21: Fmoc-Leu-Val-Glu(^{t}Bu)-Hse(^{t}Bu)-O^{t}Bu (~10%)
+
17: Lactone (2-3%)
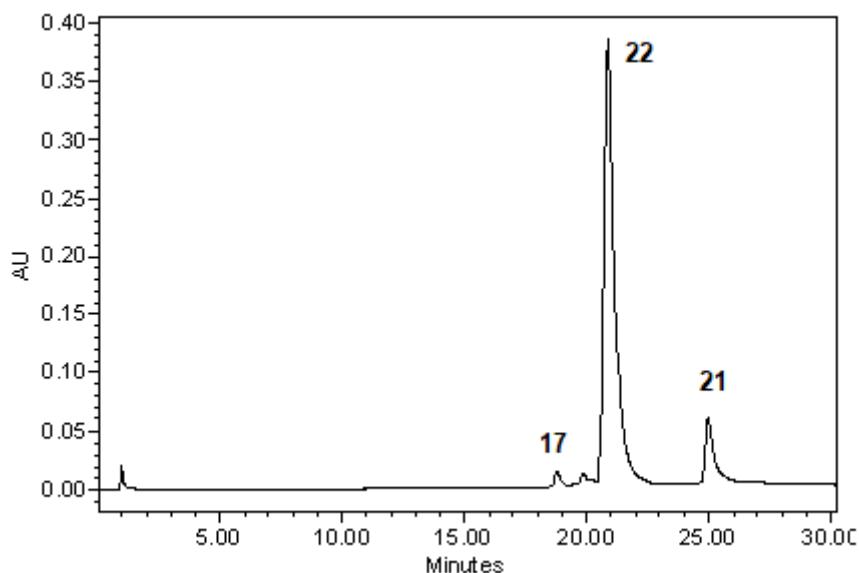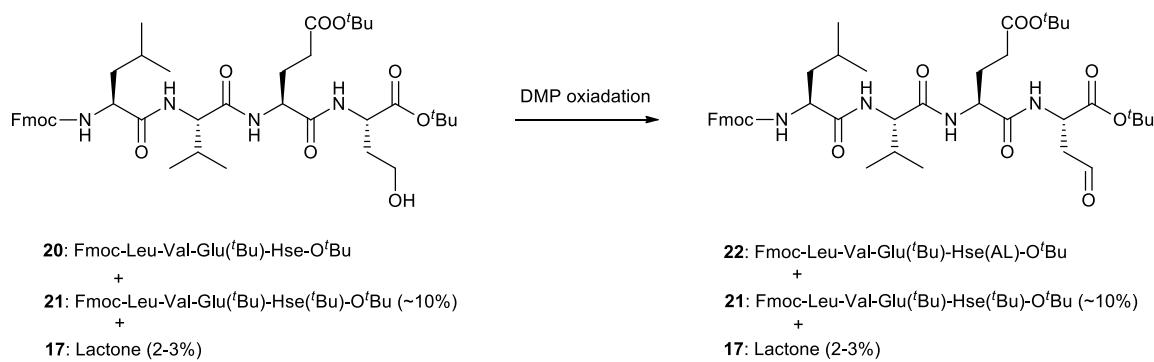
**Figure S10:** Analytical HPLC of Fmoc-Leu-Val-Glu(^{t}Bu)-Hse(AL)-O^{t}Bu (**22**) (containing ~10% lactone **17** and bis-O^{t}Bu **21**); column: Lichrosphere RP-18, 5 µm, 125–4 mm; gradient: 20% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 265 nm; 18.9 min (**17**), 21.5 min (**22**), 25.9 min (**21**)]; The eluted peaks were collected and subjected to ESI-MS. **22:** m/z [M+H]$^+$ calc. 793.44; found: 793.44.

1.2. ^{1}H / ^{13}C-NMR data

γ-hydroxy lactam of
Fmoc-Leu-Val-Glu(^{t}Bu)-HseAL-Gly-OH (**10**)

γ-hydroxy lactam of
Fmoc-Val-Glu(^{t}Bu)-HseAL-Leu-OH (**11**)

Fmoc-Val-Glu(^{t}Bu)-Hse-Pro-OH (**13**)

-CH$_2$OH

Fmoc-Val-Glu(^{t}Bu)-HseAL-Pro-OH (**14**)

aldehyde-H

-CH$_2$CHO

f1 (ppm)
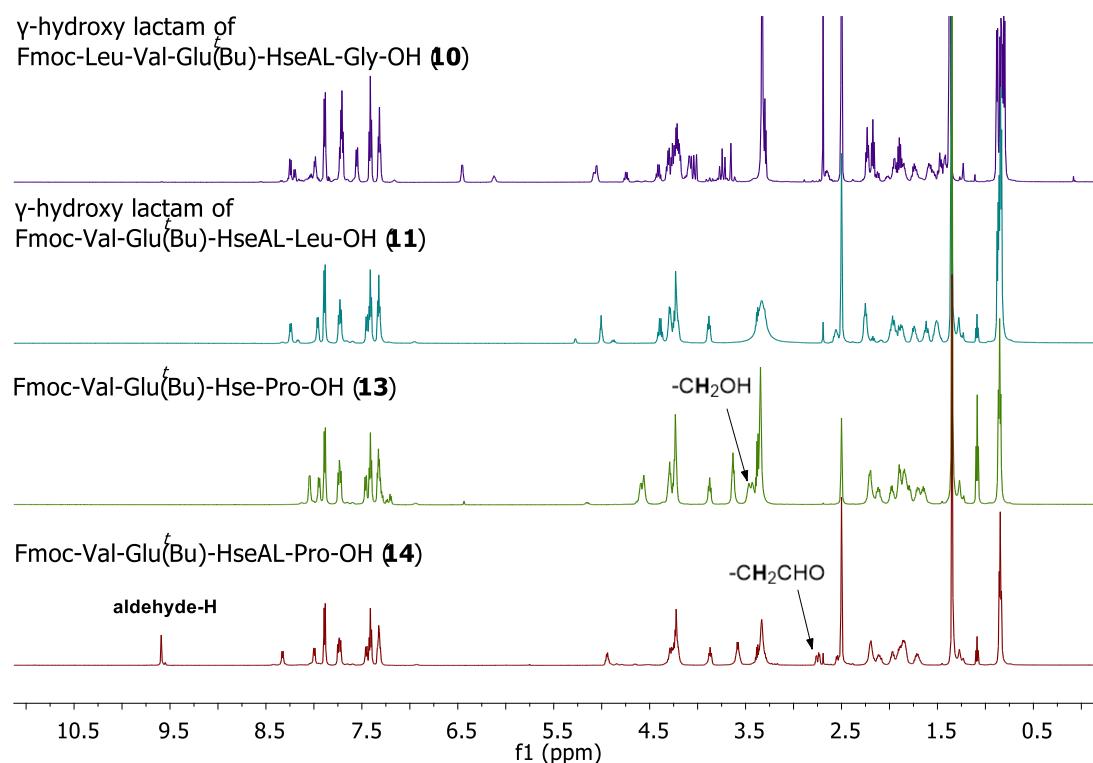
Figure S11: ^{1}H-NMR (DMSO-d$_6$) of *γ*-hydroxy lactam of Fmoc-Leu-Val-Glu(^{t}Bu)-Hse(AL)-Gly-OH (**10**), *γ*-hydroxy lactam of Fmoc-Leu-Val-Glu(^{t}Bu)-Hse(AL)-Leu-OH (**11**), Fmoc-Leu-Val-Glu(^{t}Bu)-Hse-Pro-OH (**13**), Fmoc-Leu-Val-Glu(^{t}Bu)-Hse(AL)-Pro-OH (**14**).
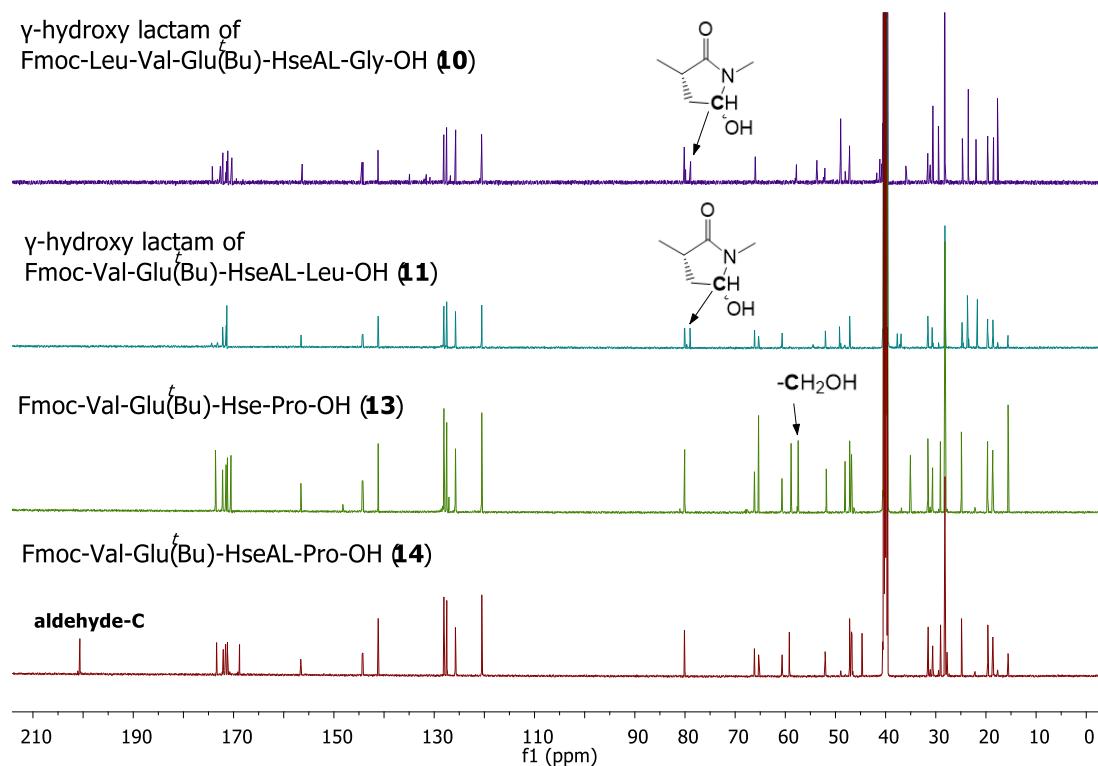
Figure S12: ^{13}C-NMR (DMSO-d$_6$) of γ-hydroxy lactam of Fmoc-Leu-Val-Glu(^{t}Bu)-Hse(AL)-Gly-OH (**10**), γ-hydroxy lactam of Fmoc-Leu-Val-Glu(^{t}Bu)-Hse(AL)-Leu-OH (**11**), Fmoc-Leu-Val-Glu(^{t}Bu)-Hse-Pro-OH (**13**), Fmoc-Leu-Val-Glu(^{t}Bu)-Hse(AL)-Pro-OH (**14**).

Figure S13: ^{13}C-NMR (DMSO-d$_6$) (Expanded area 61–33 ppm) of Fmoc-Leu-Val-Glu(^{t}Bu)-Hse-Pro-OH (**13**) and Fmoc-Leu-Val-Glu(^{t}Bu)-Hse(AL)-Pro-OH (**14**).
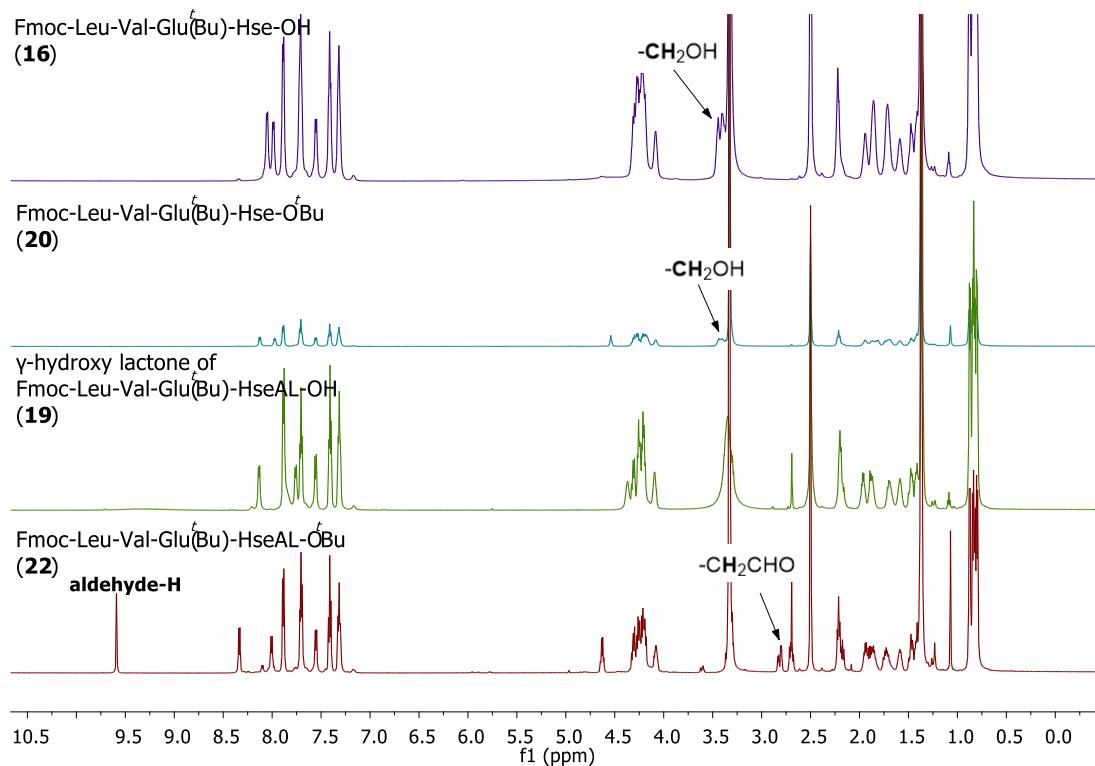
Figure S14: ^{1}H-NMR (DMSO-d_6) of Fmoc-Leu-Val-Glu(^{t}Bu)-Hse-OH (**16**), Fmoc-Leu-Val-Glu(^{t}Bu)-Hse-O^{t}Bu (**20**), C-terminal homoserinyl γ-aldehyde peptide (**18**) in equilibrium with the C-terminal γ-hydroxy lactone peptide (**19**), the ^{t}butylated C-terminal homoserinyl γ-aldehyde peptide (**22**).
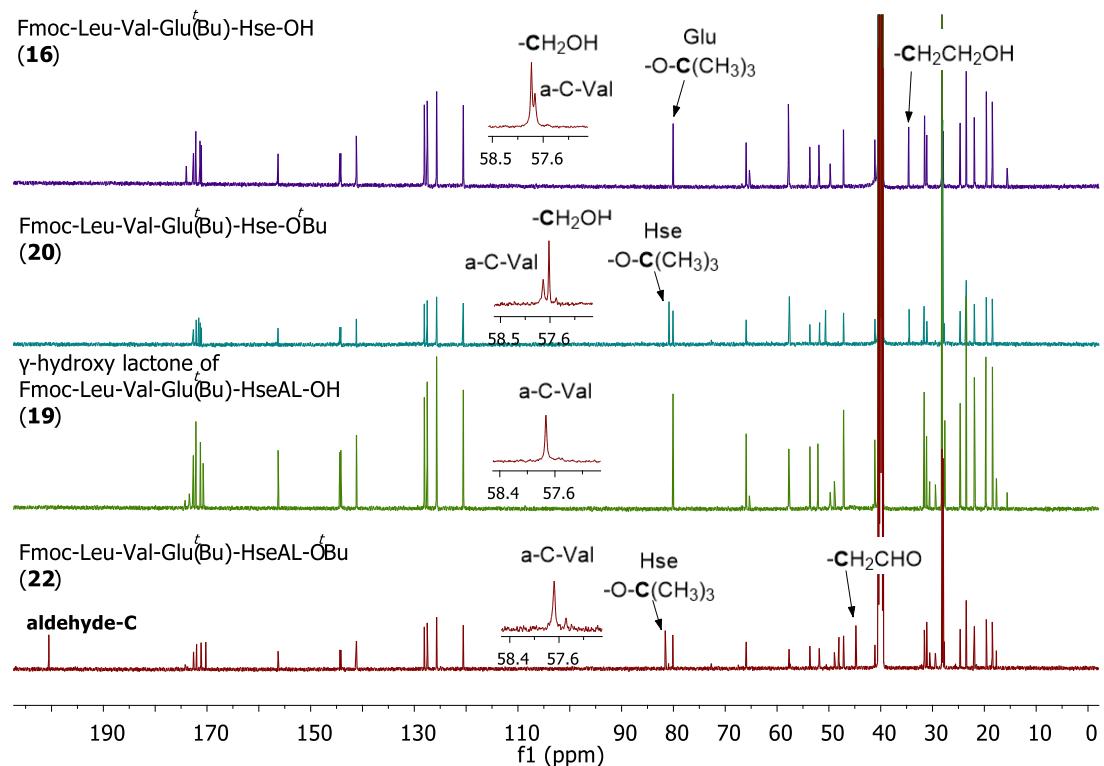
Figure S15: ^{13}C-NMR (DMSO-d$_6$) of Fmoc-Leu-Val-Glu(^{t}Bu)-Hse-OH (**16**), Fmoc-Leu-Val-Glu(^{t}Bu)-Hse-O^{t}Bu (**20**), C-terminal homoserinyl γ-aldehyde peptide (**18**) in equilibrium with the C-terminal γ-hydroxy lactone peptide **19**, the ^{t}butylated C-terminal homoserinyl γ-aldehyde peptide **22**.

2. Reductive Amination

### Reductive amination between 10, 11 and resin 23 (No products were formed)
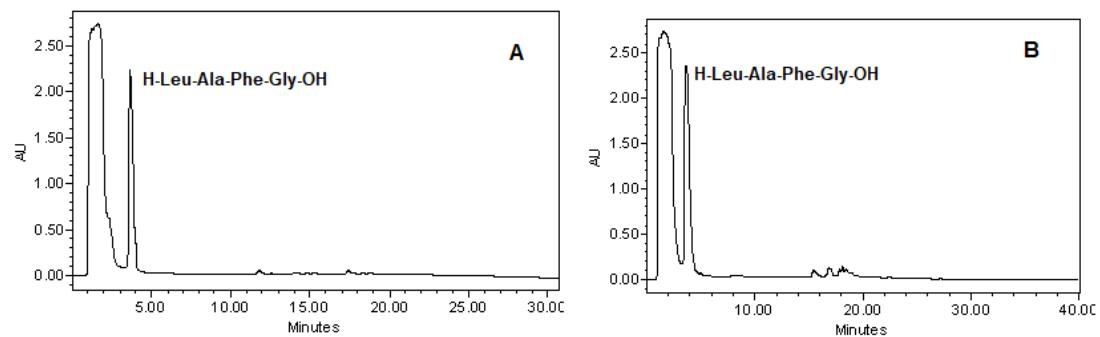
Figure S16: Analytical HPLC of the crude product obtained by the reductive amination of **10** and **11** with resin **23** (after cleavage from the resin); column: Lichrosphere RP-18, 5 μm, 125–4 mm; gradient: 20% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 214 nm; No product was identified.

Formation of peptide 27 (through reductive amination between 14 and resin 23)
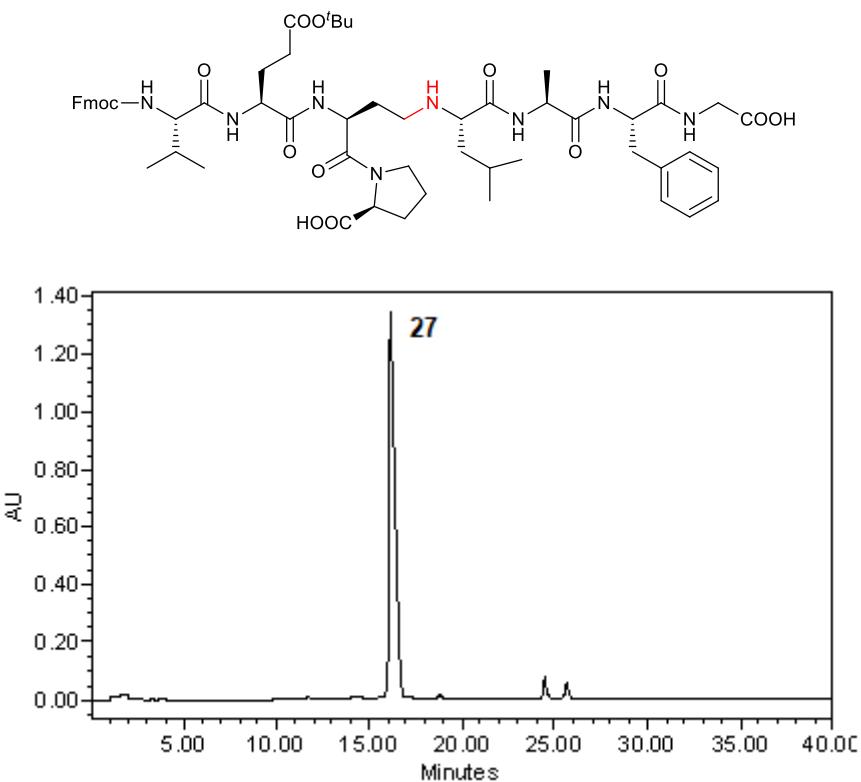
**Figure S17:** Analytical HPLC of crude Fmoc-Val-Glu(^{t}Bu)-Hse(-Pro-OH)-β-ψ[CH$_2$-NH]-Leu-Ala-Phe-Gly-OH (**27**); column: Lichrosphere RP-18, 5 µm, 125–4 mm; gradient: 20% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 265 nm; 15.9 min (**27**); The eluted peak was collected and subjected to ESI-MS: m/z [M+H]$^+$ calc. 1111.57; found: 1111.58.

Formation of peptide 28 (through reductive amination between 18/19 and resin 23)
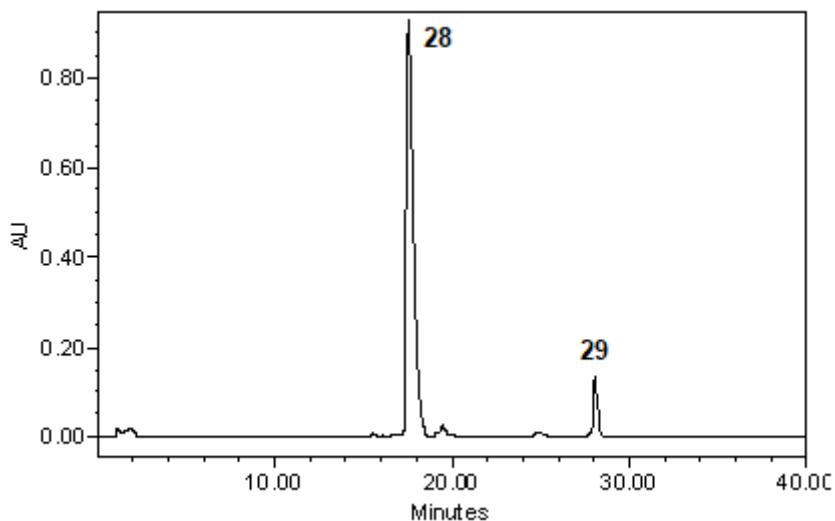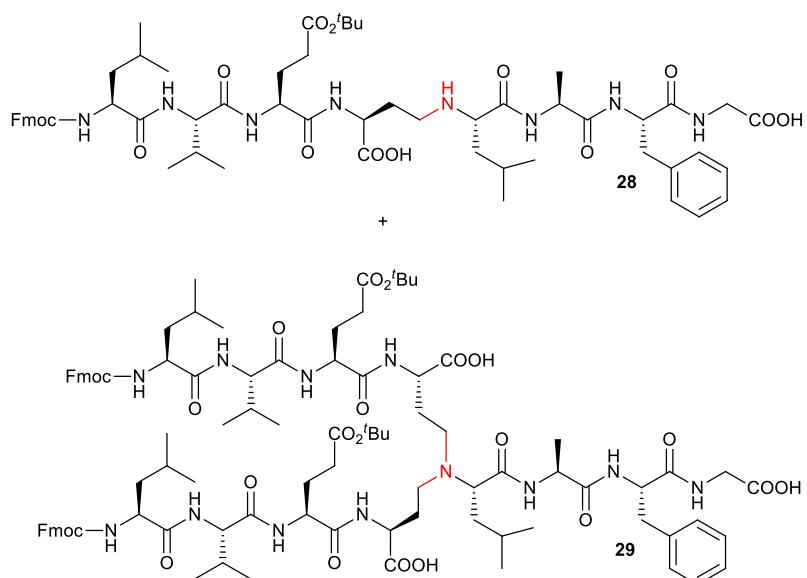
Figure S18: Analytical HPLC of **28** (and 15.0% **29**) obtained by the reductive amination of **18/19** with resin **23**; column: Lichrosphere RP-18, 5 µm, 125–4 mm; gradient: 20% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 265 nm; 17.5 min (**28**), 28.1 min (**29**); The eluted peaks were collected and subjected to ESI-MS. **28:** m/z [M+H]$^+$ calc. 1127.60; found: 1127.62; **29:** m/z [M+H]$^+$ calc. 1847.98; found: 1848.99.

Peptide 30 (through reductive amination between 22 and resin 23)
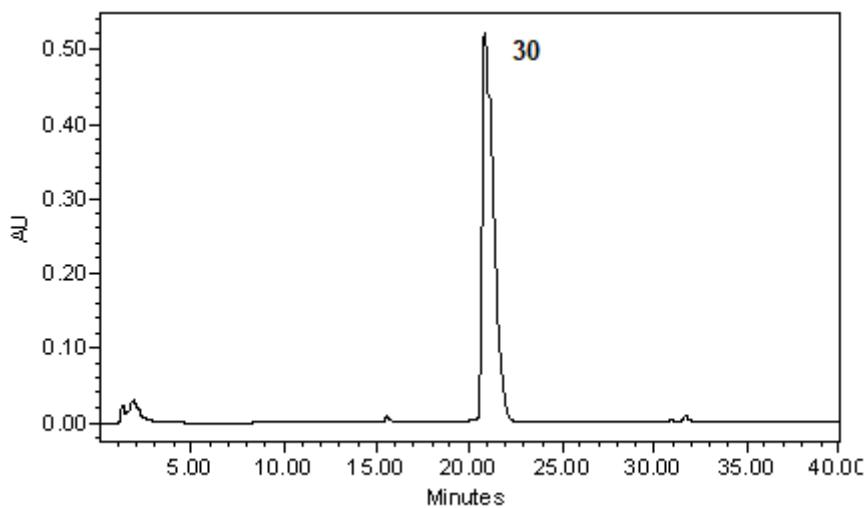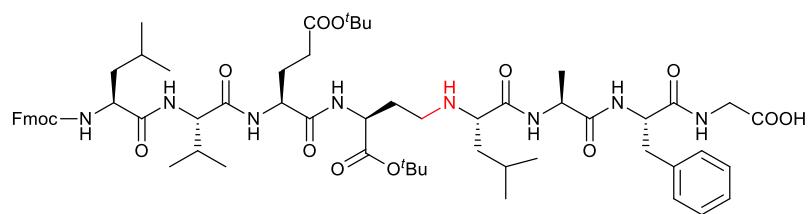
Figure S19: Analytical HPLC of peptide Fmoc-Leu-Val-Glu(^{t}Bu)-Hse(α-^{t}Bu)-β-ψ[CH$_2$-NH]-Leu-Ala-Phe-Gly-OH (**30**) (obtained by the reductive amination of **22** with resin **23**); column: Lichrosphere RP-18, 5 μm, 125–4 mm; gradient: 20% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 265 nm; 21.0 min (**30**); The eluted peak was collected and subjected to ESI-MS: m/z [M+H]$^+$ calc. 1183.67; found: 1183.69

3. Cleaved peptides from resins 32, 33, 34 and the corresponding non-Boc-protected precursors
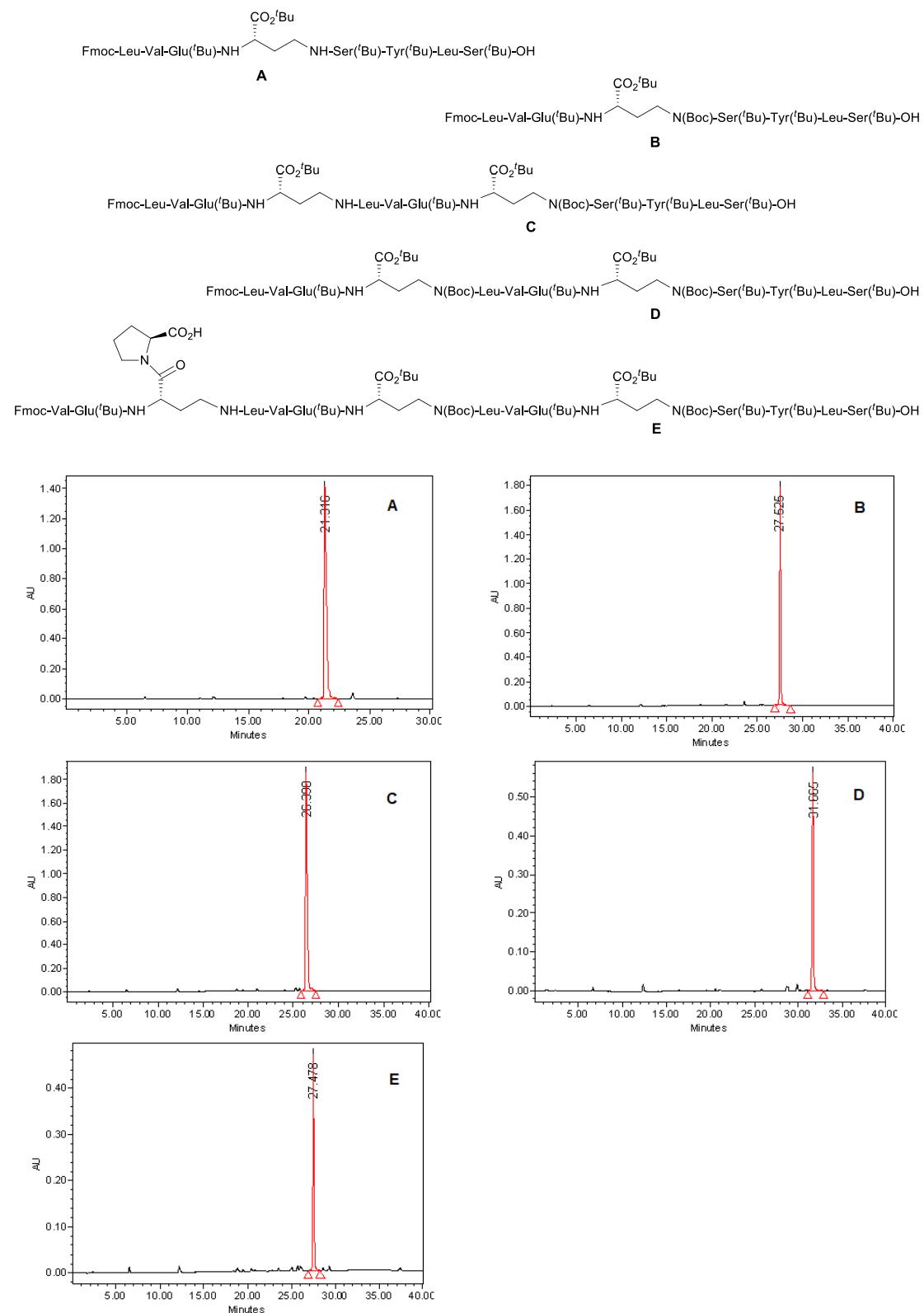

Figure S20: Analytical HPLC of the resin-cleaved crude peptides **A** – **E** during the synthesis of **34**, after treatment of the resins with DCM/TFE/AcOH (7:2:1); column: Column: Purospher RP-8e, 5 μm, 125–4 mm; gradient: 20% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV

detection at 265 nm; The eluted peaks were collected and subjected to ESI-MS: **A**: m/z [M+H]$^+$ calc. 1413.85; found: 1413.82; **B**: m/z [M+H]$^+$ calc. 1513.91; found: 1513.89; **C**: m/z [M+H]$^+$ calc. 2068.27 and [M+2H]$^{+2}$ 1034.64; found: [M+H]$^+$ 2068.25 and [M+2H]$^{+2}$ 1034.61; **D**: m/z [M+H]$^+$ calc. 2168.33 and [M+2H]$^{+2}$ 1084.67; found: [M+H]$^+$ 2168.30 and [M+2H]$^{+2}$ 1084.69; **E**: m/z [M+H]$^+$ calc. 2650.60 and [M+2H]$^{+2}$ 1325.80; found: [M+H]$^+$ 2650.62 and [M+2H]$^{2+}$ 1325.79.

## 4. Lactone formation

4.1 HPLC data
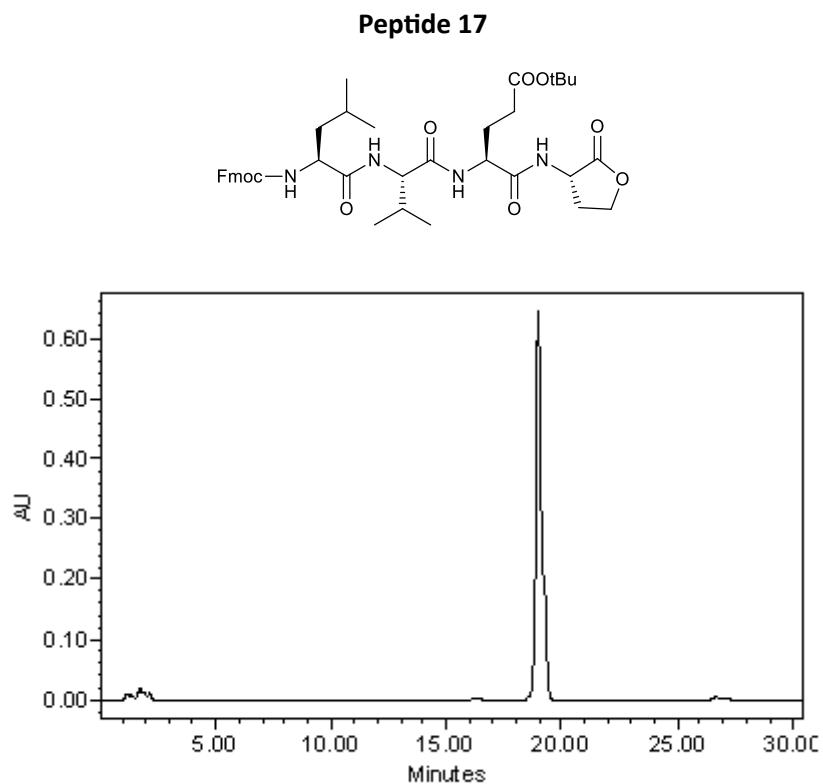
Peptide 17

Figure S21: Analytical HPLC of γ-lactone peptide (**17**) derived from Fmoc-Leu-Val-Glu(^{t}Bu)-Hse-OH (**16**) after acidic treatment with 0.1% TFA; column: Lichrosphere RP-18, 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; 18.9 min (**17**); The eluted peak was collected and subjected to ESI-MS: m/z [M+H]$^+$ calc. 721.38; found: 721.40.

4.2 ^{1}H/^{13}C-NMR data
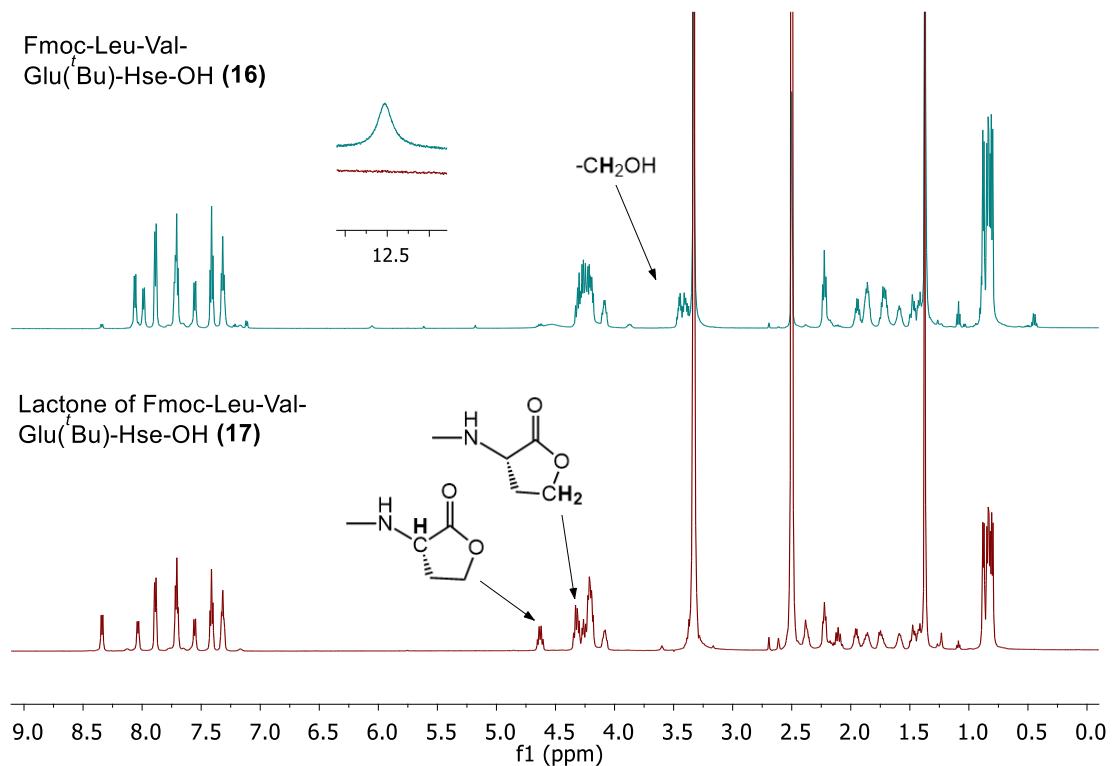
Figure S22: ^{1}H-NMR (DMSO-d$_6$) of Fmoc-Leu-Val-Glu(^{t}Bu)-Hse-OH (**16**) and Lactone of Fmoc-Leu-Val-Glu(^{t}Bu)-Hse-OH (**17**).
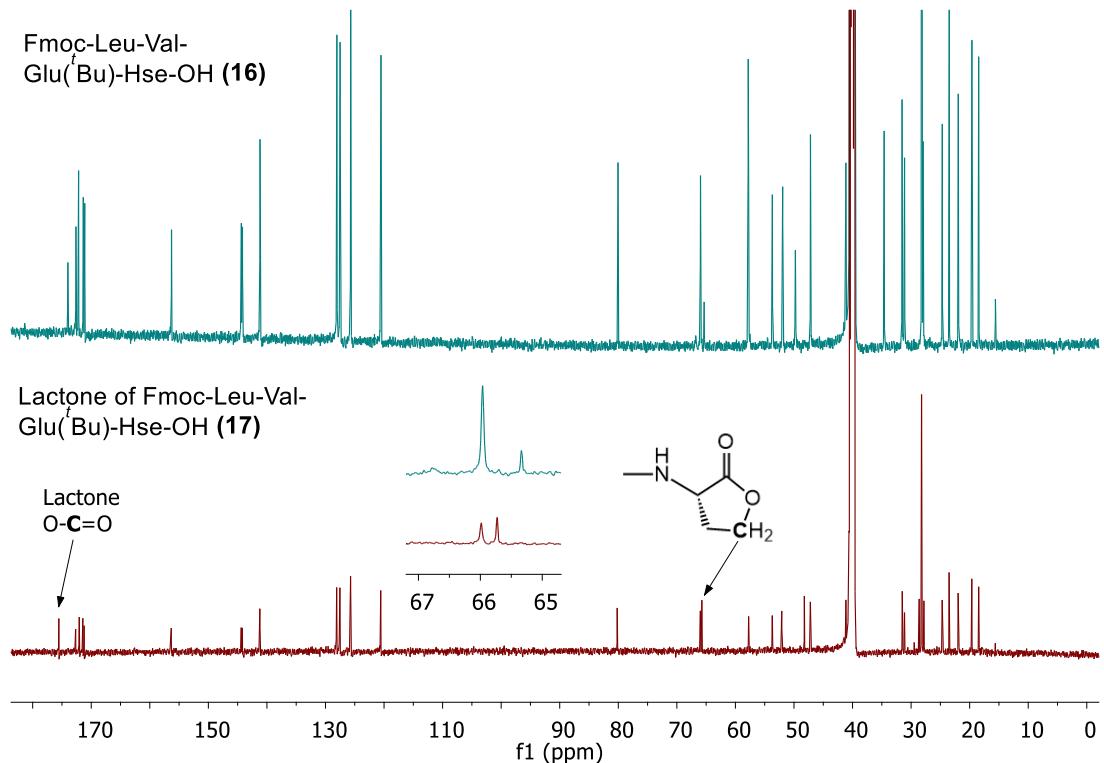
**Figure S23:** ^{1}H-NMR (DMSO-d$_6$) of Fmoc-Leu-Val-Glu(^{t}Bu)-Hse-OH (**16**) and Lactone of Fmoc-Leu-Val-Glu(^{t}Bu)-Hse-OH (**17**).

5. Racemization study during lactone formation
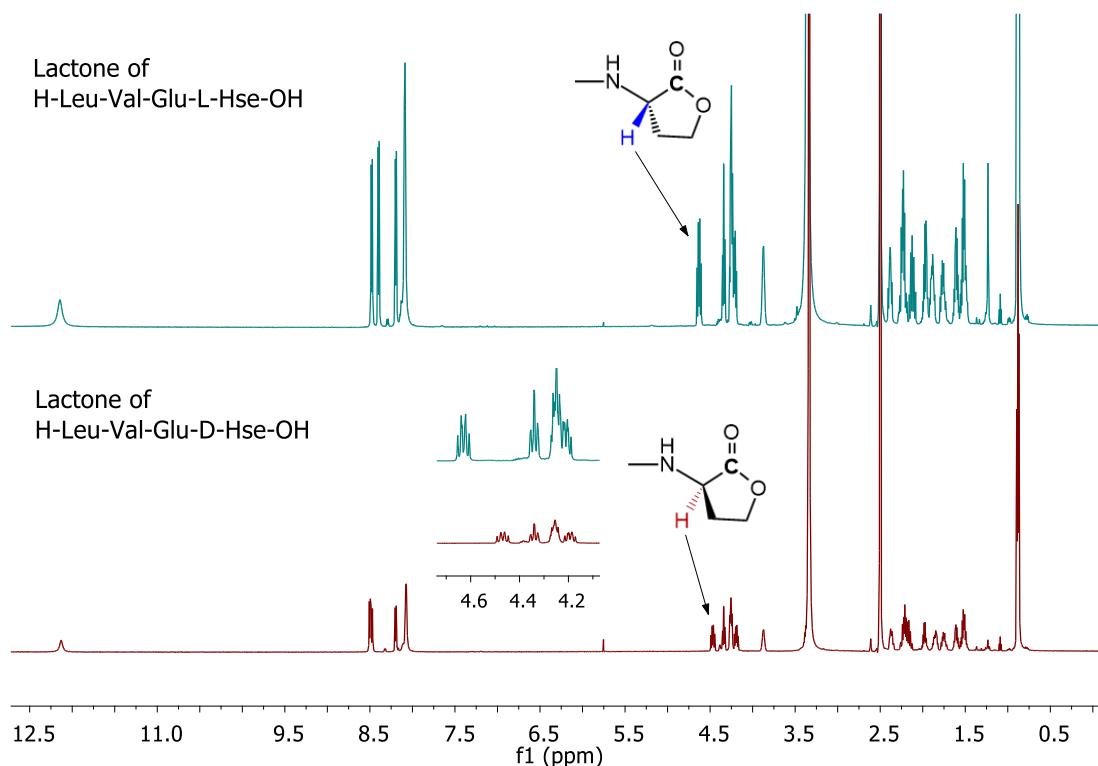
Figure S24: ^{1}H-NMR (DMSO-d_6) of H-Leu-Val-Glu-L-Hse-OH **(A)** and H-Leu-Val-Glu-D-Hse-OH **(B)**.

6. Treatment of resin 15 with 30% HFIP in DCM and TFE/DCM/TES (30/65/5) study

Fmoc-Leu-Val-Glu(tBu)-Hse(Trt)-O—◯

15

Fmoc-Leu-Val-Glu(tBu)-Hse-OH

16

Fmoc-Leu-Val-Glu(tBu)—N(H)—[lactone structure]

17

Fmoc-Leu-Val-Glu(tBu)-Hse(Trt)-OH

34
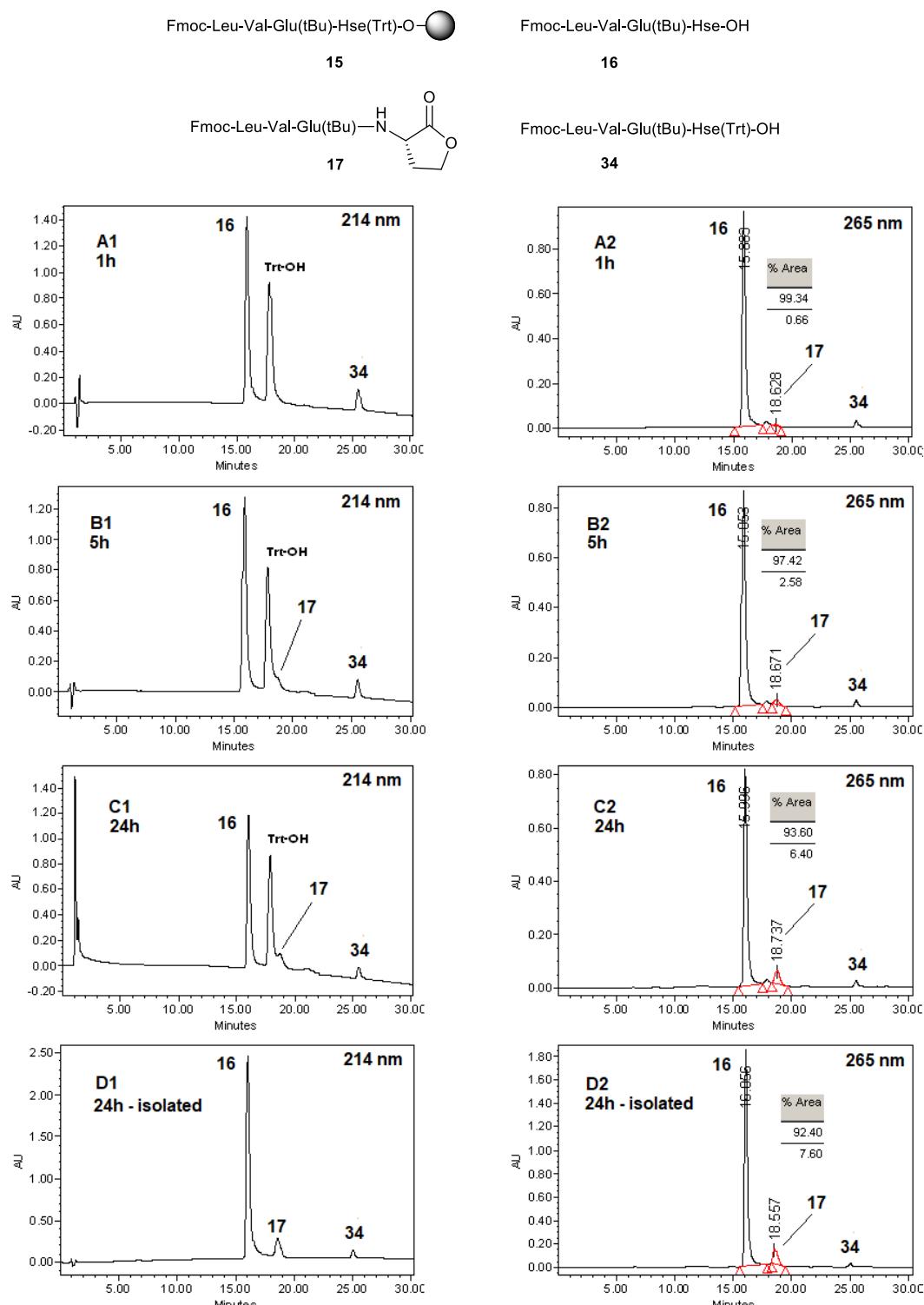
Figure S25: Analytical HPLC during the treatment of resin **15** with 30% HFIP (15 mL/gr resin); **(A)** at 1 h; **(B)** at 5 h; **(C)** at 24 h; **(D)** isolated solid; column: Lichrosphere RP-18, 5 μm, 125–4 mm; gradient: 20% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 214 nm (**A1–D1**) and 264 nm (**A2–D2**); The eluted peaks were collected and subjected to ESI-MS. **16**: m/z [M+H]$^+$ calc. 739.39; found: 739.40; **17:** m/z [M+H]$^+$ calc. 721.38; found: 721.39; **34:** m/z [M+H]$^+$ calc. 981.50; found: 981.49.
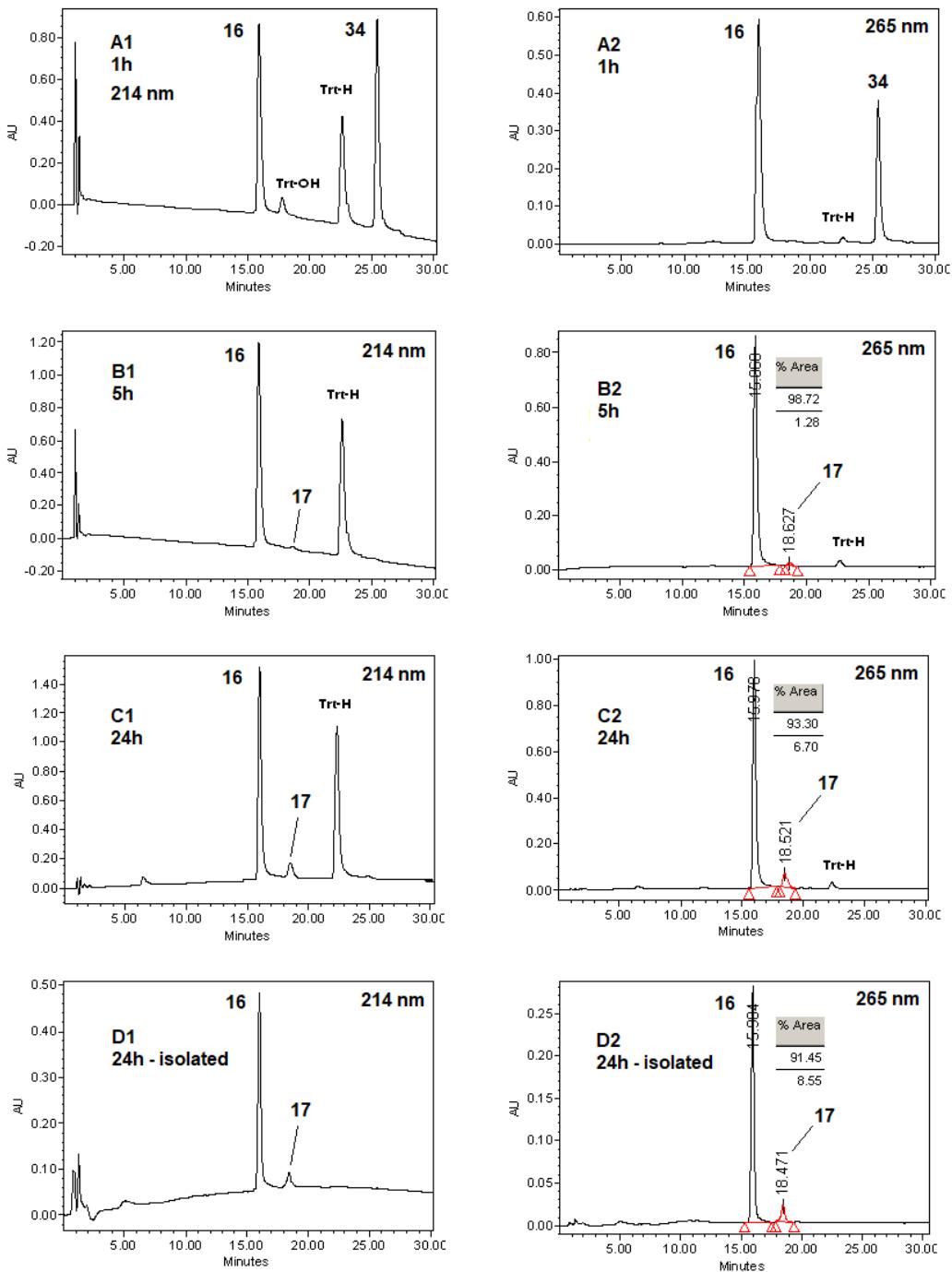
Figure S26: Analytical HPLC during the treatment of resin **15** with TFE/DCM/TES (30/65/5) (15 mL/gr resin); **(A)** at 1 h; **(B)** at 5 h; **(C)** at 24 h; **(D)** isolated solid; column: Lichrosphere RP-18, 5 μm, 125–4 mm; gradient: 20% to 100% AcCN in water (both containing 0.08% TFA) in 30 min; flow rate: 1 mL/min; UV detection at 214 nm (**A1–D1**) and 264 nm (**A2–D2**); The eluted peaks were collected and subjected to/identified by ESI-MS.