Supporting Information

From DNA cross-linking to peptide labeling: on the versatility of the furan-oxidation-conjugation strategy

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Materials and Methods

Products

TentaGel S NH_2 (capacity ~0.25 mmol/g, 90 µm) and Polystyrene PHB (Wang resin, capacity ~0.99 mmol/g, mesh 100-200) were obtained from Iris Biotech GmbH. Rink Amide AM resin was obtained from Novabiochem. All amino acids and coupling reagents were purchased from Novabiochem. L-amino acids were used throughout the synthesis. 4-dimethylaminopyridine (DMAP) was purchased from Acros. *N,N*-dimethylformamide (DMF) extra dry with molecular sieves and tetrahydrofuran (THF) were obtained from Acros. DMF peptide synthesis grade and *N*-methylpyrrolidon (NMP) were purchased from Biosolve. Dichloromethane (DCM) and *N,N*-di*iso*propylethylamine (DIPEA) were obtained from Aldrich. Trifluoroacetic acid (TFA) was obtained from Iris Biotech GmbH. 1-Aminopyrene was purchased from Aldrich. Sodium cyanoborohydride was obtained from Acros. All chemicals were used without further purification. All reagents used for automated peptide synthesis were peptide synthesis grade.

Peptide syntheses

For manual peptide synthesis, reactions were performed in a peptide vessel protected against light and comprising a sintered glass funnel and a 3-way stopcock for easy filtration and washing or in plastic vials equipped with a sintered filter. The solid phase reactions were performed on a shaker (Selecta Vibromatic) at 200 U/min or on a Yellow Line TTS 2 vortexer at 1200 rpm. Peptides were synthesized by standard Fmoc/*t*Bu strategy using PyBOP/DIPEA couplings.

Automated peptide syntheses were performed on a 24-reactor block SYRO Multiple Peptide Synthesizer equipped with a vortexing unit (Multisyntech, Witten, Germany). Peptides were synthesized by standard Fmoc/*t*Bu strategy using HBTU/DIPEA couplings.

Photolyses were carried out on a small scale with a 4W Bioblock Scientific compact UV lamp set at 365 nm.

Analyses

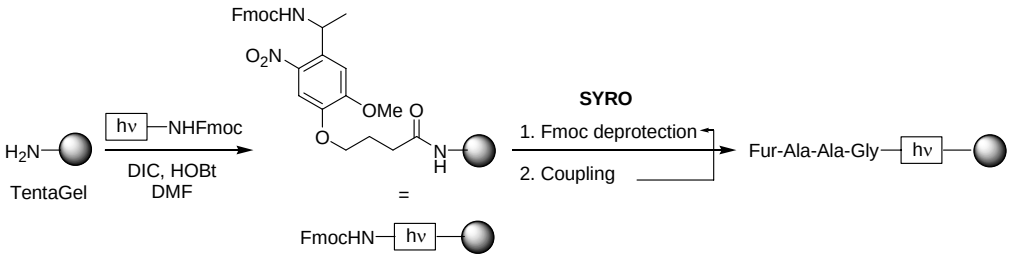
ESI-MS spectra were recorded using an LCQ ion trap mass spectrometer (Finnigan MAT).

RP-HPLC analyses were performed on an Agilent 1100 Series instrument with a Phenomenex Luna C18(2) column (250 x 4.6 mm, 5 µm at 35 °C). A flow rate of 1 ml/min was used with the following solvent systems: 0.1% TFA in H_2O (A) and MeCN (B). The column was flushed for 3 min with 100% A, then a gradient from 0 to 100% B over 15 min was used, followed by 5 min of flushing with 100% B.

LC-MS data were collected on an Agilent 1100 Series instrument with a Phenomenex Luna C18(2) column (250 x 4.6 mm, 5 µm at 35 °C) connected to an ESMSD type VL mass detector using the following solvent systems: 5 mM NH_4OAc in H_2O (A) and MeCN (B). The column was flushed with 100% A for 2 min, then a gradient from 0 to 100% B over 15 min was used, followed by 5 min of flushing with 100% B.

^1H NMR spectra were recorded in MeOD at 300 MHz (Bruker Avance 300). *J* values are given in Hz.

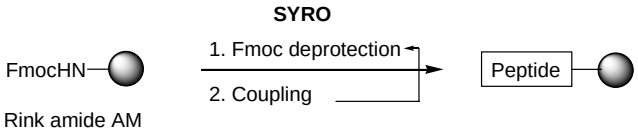
General procedure for peptide synthesis on TentaGel resin with a photolabile linker



Before using the TentaGel resin for synthesis it is derivatised with a photocleavable linker. For that purpose TentaGel resin is preswollen in DMF for 30 min. The resin is filtered off. HOBt (3 equiv) and DIC (3 equiv) are added to a solution of photocleavable linker (3 equiv) in DMF. After 30 min of preactivation this reaction mixture is added to the resin. The reaction mixture is shaken overnight. The resin is filtered off and washed with DMF (3 x 30 s), MeOH (3 x 30 s) and DCM (3 x 30 s). The resin is then dried under vacuum.

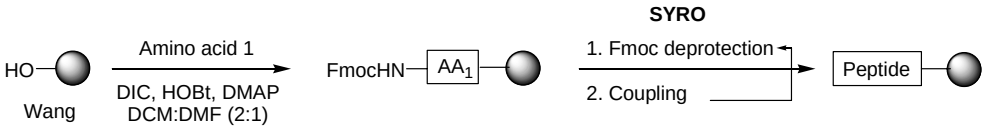
After preloading the resin with the photocleavable linker, the peptide synthesis is performed on an automated peptide synthesizer using the following protocols for Fmoc deprotection and coupling.
Fmoc deprotection: A solution of 40% piperidine in DMF is added to the resin. The resin is shaken for 3 min and filtered off. Then a solution of 20% piperidine in DMF is added to the resin. The reaction mixture is shaken for 12 min. The resin is filtered off and washed with DMF (6 x 30 s)
Coupling: 5 equiv of a 0.5 M solution of amino acid in DMF, 5 equiv of a 0.5 M solution of HBTU in DMF and 10 equiv of a 2.0 M solution of DIPEA in NMP are added to the resin. The reaction mixture is shaken for 40 min. The resin is filtered off and washed with DMF (4 x 30 s).

General procedure for peptide synthesis on Rink amide AM resin



The resin is preswollen in DMF for 30 min and then filtered off. Peptide synthesis is performed on an automated peptide synthesizer using the following protocols for Fmoc deprotection and coupling.
Fmoc deprotection: A solution of 40% piperidine in DMF is added to the resin. The resin is shaken for 3 min and filtered off. Then a solution of 20% piperidine in DMF is added to the resin. The reaction mixture is shaken for 12 min. The resin is filtered off and washed with DMF (6 x 30 s)
Coupling: 5 equiv of a 0.5 M solution of amino acid in DMF, 5 equiv of a 0.5 M solution of HBTU in DMF and 10 equiv of a 2.0 M solution of DIPEA in NMP are added to the resin. The reaction mixture is shaken for 40 min. The resin is filtered off and washed with DMF (4 x 30 s).

General procedure for peptide synthesis on Wang resin



The resin is preswollen in DCM for 30 min and then filtered off. HOBt (5 equiv), DIC (5 equiv) and DMAP (0.3 equiv) are added to a solution of amino acid (5 equiv) in DCM: DMF (2:1). After 30 min of preactivation, this mixture is added to the resin. The reaction is shaken for 3 h. The resin is filtered off and washed with DMF (3 x 30 s), MeOH (3 x 30 s) and DCM (3 x 30 s). The coupling is repeated using the same protocol. After preloading the resin with the first amino acid, peptide synthesis is performed on an automated peptide synthesizer using the following protocols for Fmoc deprotection and coupling.
Fmoc deprotection: A solution of 40% piperidine in DMF is added to the resin. The resin is shaken for 3 min and filtered off. Then a solution of 20% piperidine in DMF is added to the resin. The reaction mixture is shaken for 12 min. The resin is filtered off and washed with DMF (6 x 30 s)
Coupling: 5 equiv of a 0.5 M solution of amino acid in DMF, 5 equiv of a 0.5 M solution of HBTU in DMF and 10 equiv of a 2.0 M solution of DIPEA in NMP are added to the resin. The reaction mixture is shaken for 40 min. The resin is filtered off and washed with DMF (4 x 30 s).

General procedure for peptide labeling on TentaGel with photolabile linker

Oxidation
A solution of NBS (3 equiv) in THF:acetone:H_2O 5:4:2 (200 µL) is added to the resin (10 mg). The reaction mixture is shaken for 5 h. After 5 h, the resin is filtered and washed with DCM (5 x 30 s). The resin is then dried under vacuum.

Reductive amination
A solution of 1-aminopyrene (30 equiv) in THF (100 µL) and a solution of sodium cyanoborohydride (60 equiv) in THF:H_2O 1:1 (100 µL) are added to the resin. Subsequently 50 µL of a 15% CH_3COOH/H_2O solution is added. The reaction mixture is shaken for 4 h. After 4 h reaction time, the resin is filtered and extensively washed with DCM (3 x 30 s), DMF (3 x 30 s), MeOH (3 x 30 s), DCM (3 x 30 s), diethylether (3 x 30 s) and DCM (3 x 30 s) respectively. The resin is then dried under vacuum.

Cleavage from the resin
Approximately 1 mg resin is suspended in 100 µL MeCN in a small glass test tube. The test tube placed at 1 cm under a UV lamp and irradiated at 365 nm for 3 hours. After 3 h the solution is collected for HPLC, ES-MS and LC-MS analysis.

General procedure for peptide labeling on acid sensitive resins

Oxidation
A solution of NBS (3 equiv) in THF:acetone:H_2O 5:4:2 (200 µL) is added to the resin (10 mg). The reaction mixture is shaken for 5 h. After 5 h, the resin is filtered and washed with DCM (5 x 30 s). The resin is then dried under vacuum.

Reductive amination
A solution of 1-aminopyrene (1 equiv) in THF (100 µL) and a solution of sodium cyanoborohydride (1 equiv) in THF:H_2O 1:1 (100 µL) are added to the resin. Subsequently 50 µL of a 15% CH_3COOH/H_2O solution is added. The reaction mixture is shaken for 1 h. After 1 h reaction time, the resin is filtered and extensively washed with DCM (3 x 30 s), DMF (3 x 30 s), MeOH (3 x 30 s), DCM (3 x 30 s), diethylether (3 x 30 s) and DCM (3 x 30 s) respectively. The resin is then dried under vacuum.

Cleavage from the resin
A cleavage mixture of TFA:TIS:H_2O 95:2.5:2.5 (200 µL) is added to the resin. The reaction mixture is shaken for 1 h. After 1 h the resin is filtered off and washed with DCM (3 x 30 s). The filtrate is evaporated and dried on the oil pump. For cysteine-containing peptides, an extra deprotection step to remove the S*t*Bu group should be performed before cleavage. For that purpose, the resin is treated with 2-mercaptoethanol in DMF (1:1). After 8 days, the resin is filtered off and washed with DMF (3 x 30 s), MeOH (3 x 30 s) and DCM (3 x 30 s). The deprotected peptide can then be cleaved as described above. The crude residue after cleavage is dissolved in methanol for HPLC, ES-MS and LC-MS analysis. The relevant chromatograms and spectra are listed below.

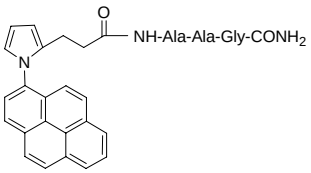
Large scale labeling of Fur-Ala-Ala-Gly-Rink and NMR analysis of the labeled peptide

A solution of NBS (3 equiv, 32.2 mg) in THF:acetone:H_2O 5:4:2 (1 mL) is added to the resin (100 mg, loading 0.66 mmol/g). The reaction mixture is shaken for 5 h. After 5 h, the resin is filtered and washed with DCM (5 x 30 s). The resin is dried under vacuum. A solution of 1-aminopyrene (1 equiv, 14.3 mg) in THF (1 mL) and a solution of sodium cyanoborohydride (1 equiv, 4.4 mg) in THF:H_2O 1:1 (1 mL) are then added to the resin. Subsequently 500 µL of a 15% CH_3COOH/H_2O solution is added. The reaction mixture is shaken for 1 h. After 1 h reaction time, the resin is filtered and extensively washed with DCM (3 x 30 s), DMF (3 x 30 s), MeOH (3 x 30 s), DCM (3 x 30 s), diethylether (3 x 30 s) and DCM (3 x 30 s) respectively. The resin is then dried under vacuum. Cleavage of the labeled peptide from the resin is perfomed by adding a cleavage mixture of TFA:TIS:H_2O 95:2.5:2.5 (2 mL) to the resin. The reaction mixture is shaken for 1 h. After 1 h the resin is filtered off and washed with DCM (3 x 30 s). The filtrate is evaporated and dried on the oil pump. The peptide (33.9 mg) is recovered in good purity with a yield of 95 % and is further purified with RP-HLC for NMR analysis.

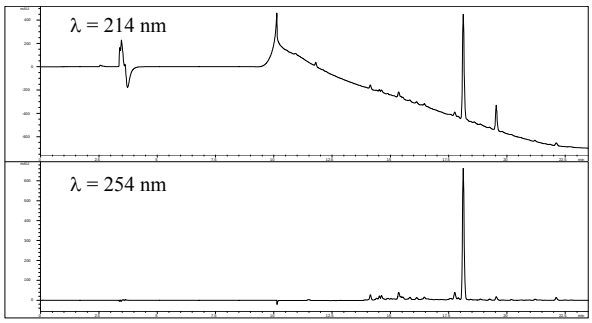
1H NMR δ_H (300 MHz; MeOD) 8.34 (3H, m, ArH) , 8.22 (2H, m, ArH), 8.14 (2H, m, ArH), 8.02 (1H, m, ArH), 7.50 (1H, m, ArH), 6.92 (1H, d, pyrole), 6.35 (1H, t, pyrole), 6.24 (1H, d, pyrole), 4.17 (2H, m, Ala CH), 3.83 (1H, d, Gly CH), 3.70 (1H, d, Gly CH), 2.65 (2H, m, CH_2), 2.42 (2H, m, CH_2), 1.29 (6H, m, $2CH_3$)

Spectral Data

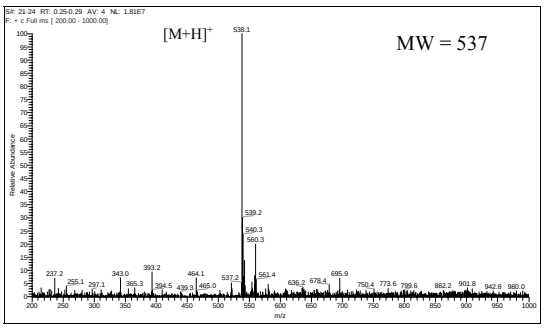
Spectral data after labeling, acidolytic cleavage and side-chain deprotection of Fur-Ala-Ala-Gly-R (Table 1; Entry 5)



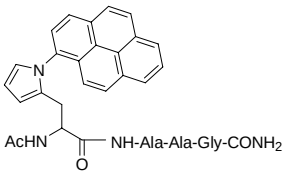
RP-HPLC analysis of crude cleavage product



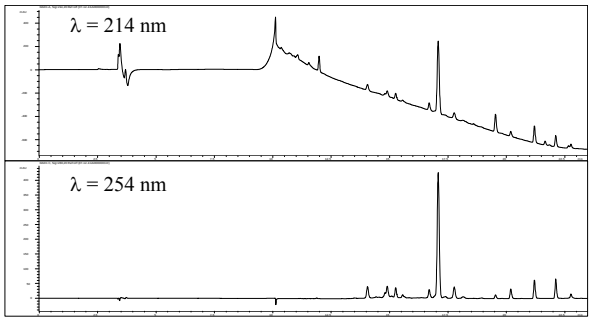
ESI-MS analysis of crude cleavage product



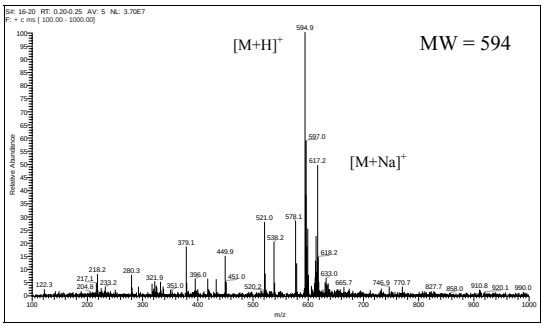
Spectral data after labeling, acidolytic cleavage and side-chain deprotection of Ac-FurAla-Ala-Ala-Gly-R (Table 2; Entry 1)



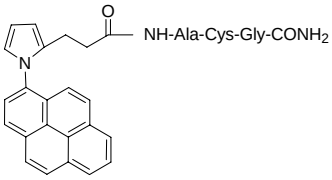
RP-HPLC analysis of crude cleavage product



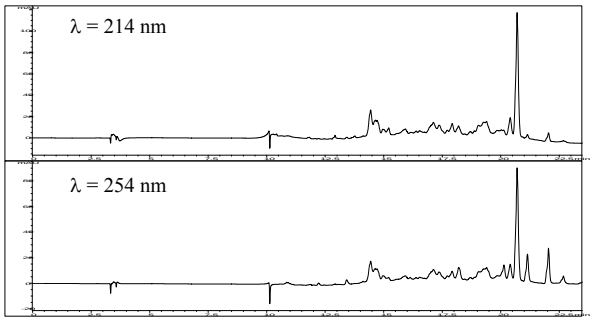
ESI-MS analysis of crude cleavage product



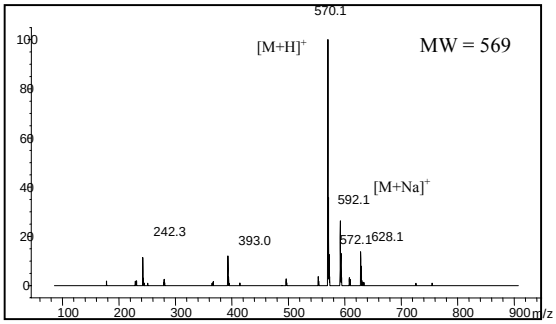
Spectral data after labeling, acidolytic cleavage and side-chain deprotection of Fur-Ala-Cys(StBu)-Gly-R (Table 2; Entry 2)



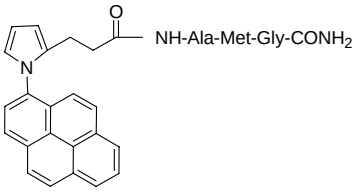
RP-HPLC analysis of crude cleavage product



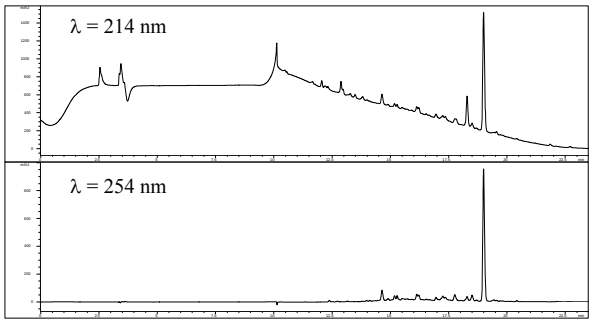
ESI-MS analysis of crude cleavage product



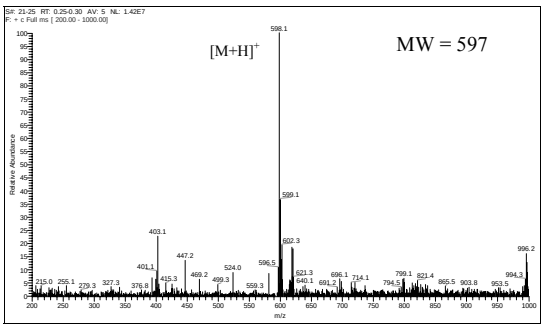
Spectral data after labeling, acidolytic cleavage and side-chain deprotection of Fur-Ala-Met-Gly-R (Table 2; Entry 3)



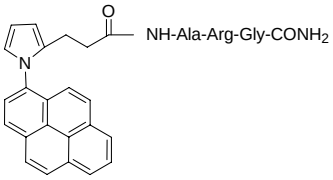
RP-HPLC analysis of crude cleavage product



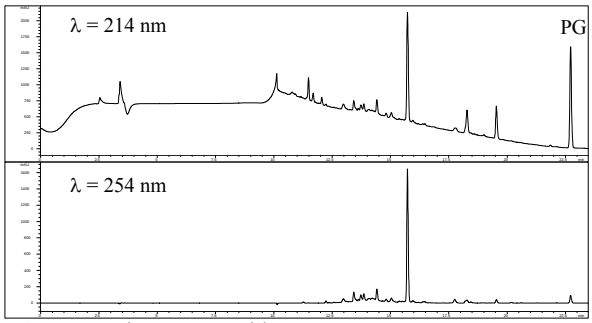
ESI-MS analysis of crude cleavage product



Spectral data after labeling, acidolytic cleavage and side-chain deprotection of Fur-Ala-Arg(Pbf)-Gly-R (Table 2; Entry 4)

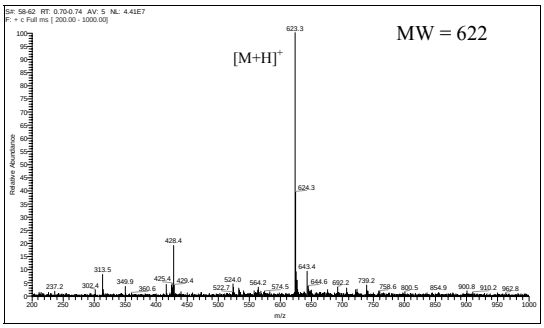


RP-HPLC analysis of crude cleavage product

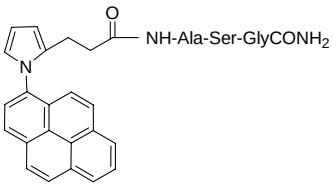


PG = Protecting group residue.

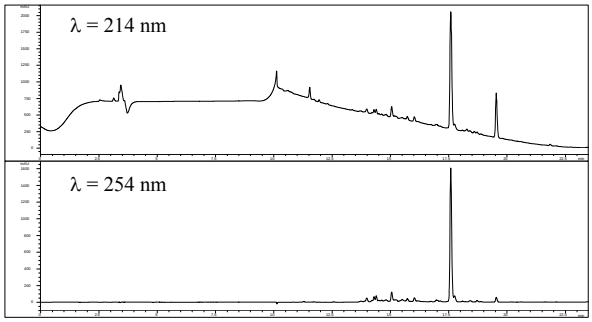
ESI-MS analysis of crude cleavage product



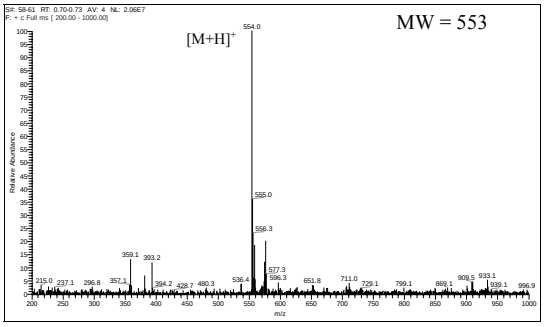
Spectral data after labeling, acidolytic cleavage and side-chain deprotection of Fur-Ala-Ser(tBu)-Gly-R (Table2; Entry 5)



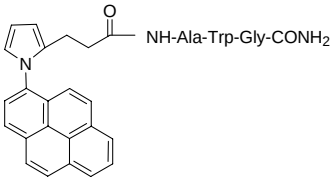
RP-HPLC analysis of crude cleavage product



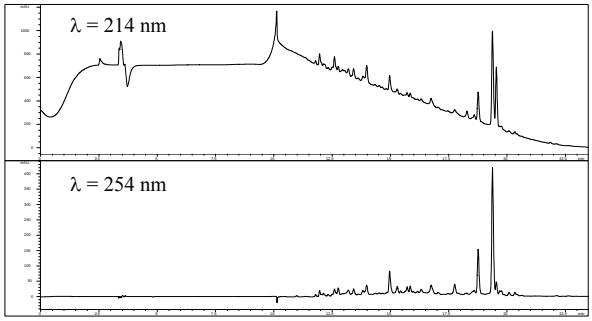
ESI-MS analysis of crude cleavage product



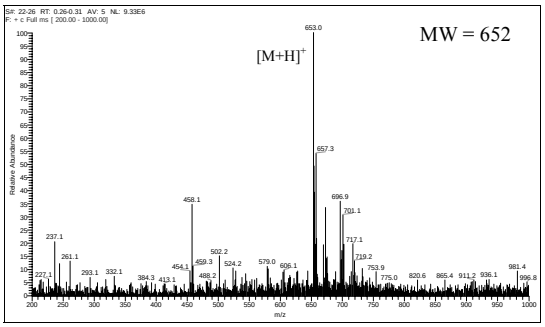
Spectral data after labeling, acidolytic cleavage and side-chain deprotection of Fur-Ala-Trp(Boc)-Gly-R (Table 2; Entry 6)



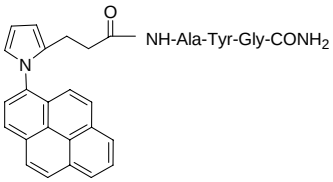
RP-HPLC analysis of crude cleavage product



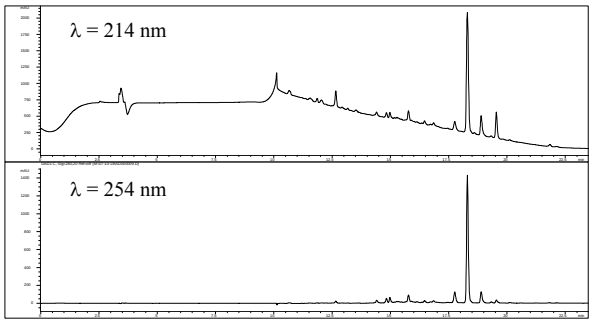
ESI-MS analysis of crude cleavage product



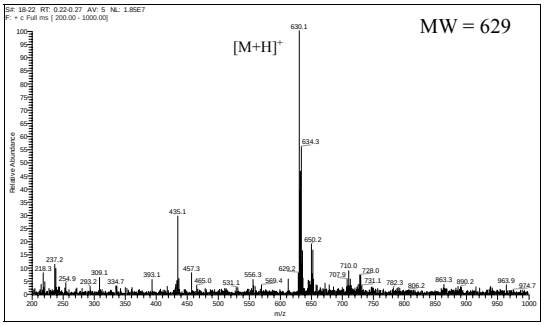
Spectral data after labeling, acidolytic cleavage and side-chain deprotection of Fur-Ala-Tyr(tBu)-Gly-R (Table 2; Entry 7)



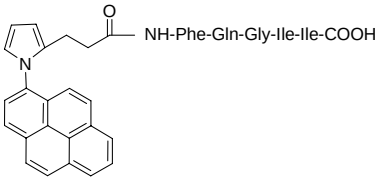
RP-HPLC analysis of crude cleavage product



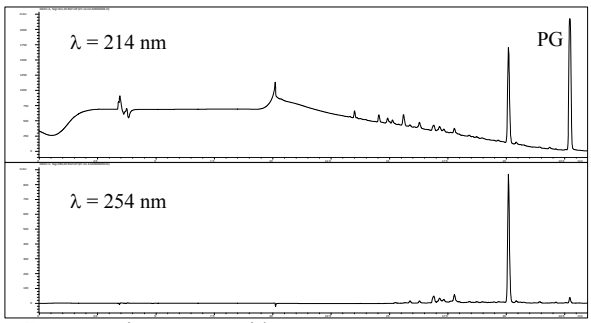
ESI-MS analysis of crude cleavage product



Spectral data after labeling, acidolytic cleavage and side-chain deprotection of Fur-Phe-Gln(Trt)-Gly-Ile-Ile-W (Table2; Entry 8)

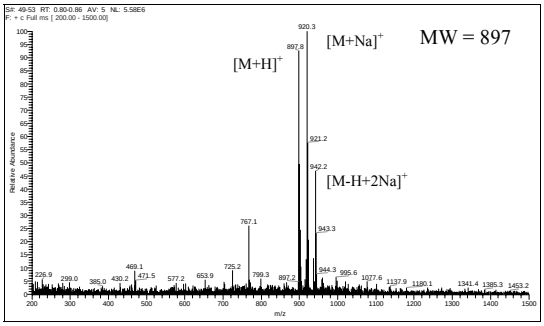


RP-HPLC analysis of crude cleavage product

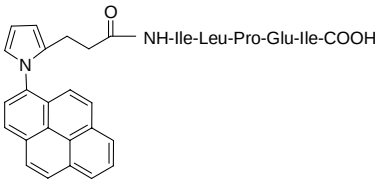


PG = Protecting group residue

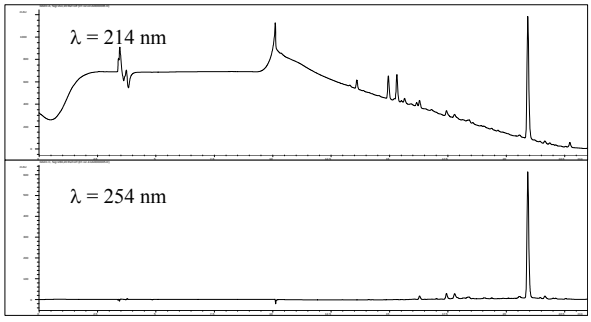
ESI-MS analysis of crude cleavage product



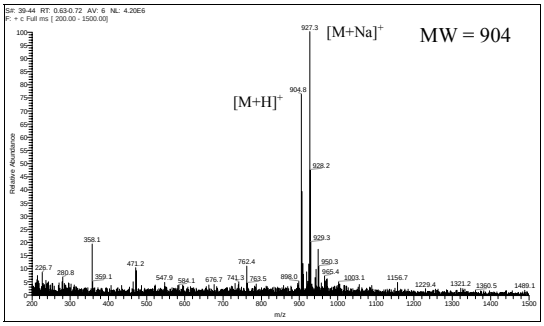
Spectral data after labeling, acidolytic cleavage and side-chain deprotection of Fur-Ile-Leu-Pro-Glu(tBu)-Ile-W (Table2; Entry 9)



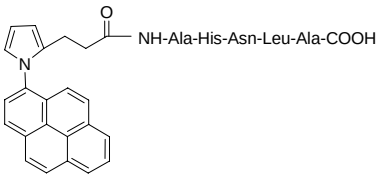
RP-HPLC analysis of crude cleavage product



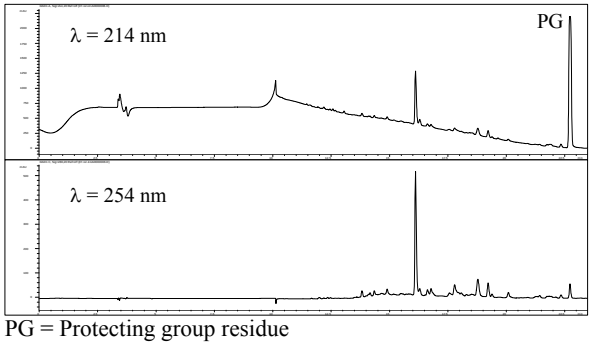
ESI-MS analysis of crude cleavage product



Spectral data after labeling, acidolytic cleavage and side-chain deprotection of Fur-Ala-His(Trt)-Asn(Trt)-Leu-Ala-W (Table 2; Entry 10)

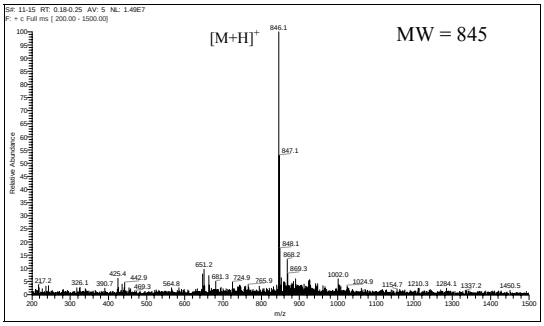


RP-HPLC analysis of crude cleavage product

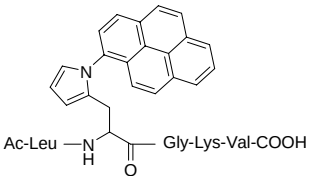


PG = Protecting group residue

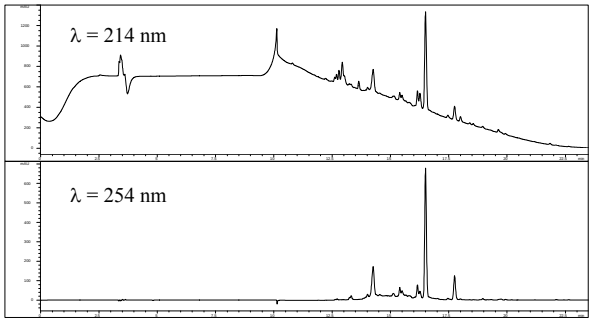
ESI-MS spectrum of crude cleavage product



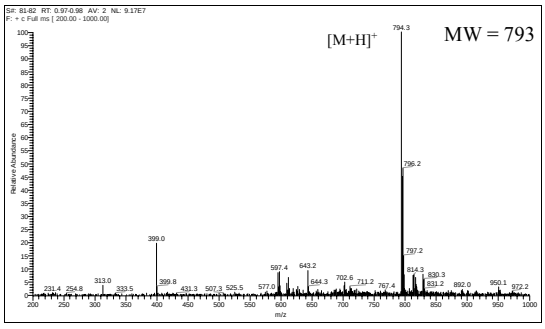
Spectral data after labeling and acidolytic cleavage and side-chain deprotection of AcLeu-FurAla-Gly-Lys(Boc)-Val-W (Table 2; Entry 11)



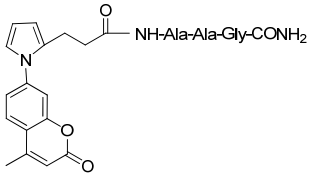
RP-HPLC analysis of crude cleavage product



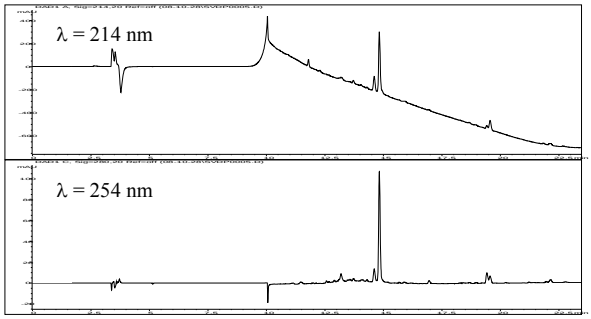
ESI-MS analysis of crude cleavage product



Spectral data after labeling with 7-amino-4-methylcoumarin, acidolytic cleavage and side-chain deprotection of Fur-Ala-Ala-Gly-R



RP-HPLC analysis of crude cleavage product



ESI-MS analysis of crude cleavage product