Supporting Information

**Aromatic capping surprisingly stabilizes furan moieties in peptides against acidic degradation during resin cleavage**

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Materials and Methods

**Products**
Polystyrene PHB (Wang resin, capacity ~0.99 mmol/g, mesh 100-200) and 2-Chlorotrityl chloride resin (capacity ~1.55 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 was obtained from Acros. DMF peptide synthesis grade and N-methylpyrrolidon (NMP) were purchased from Biosolve. Dichloromethane (DCM) and N,N-diisopropylethylamine (DIPEA) were obtained from Aldrich. Trifluoroacetic acid (TFA) was obtained from Iris Biotech GmbH. All chemicals were used without further purification. All reagents used for automated peptide synthesis were peptidesynthesis grade.

**Peptide syntheses**
For manual peptide synthesis, reactions were performed in a peptide vessel 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/rtBu strategy using PyBOP/DIPEA or DIC/HOBt couplings.
Automated peptide syntheses were performed on a 24-reactor block SYRO Multiple Peptide Synthesizer equipped with a vortexing unit (Multisynetch, Witten, Germany). Peptides were synthesized by standard Fmoc/rtBu strategy using HBTU/DIPEA couplings.

**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 H2O (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. Alternatively, a Phenomenex Clarity column (250 x 4.6mm, 5μm at 50°C) as used with 0,1M TEAA with 5% MeCN (A) and MeCN(B). The column was flushed for 3 min with 100% A, then a gradient from 0 to 100% B over 60 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 NH4OAc in H2O (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.
**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 synthesis on 2-Chlorotrityl chloride resin**

The resin is preswollen in DCM for 30 min and then filtered off. A solution of amino acid (1.2 equiv) and DIPEA (4.8 equiv) in dry DCM is added to the resin. The reaction is shaken for 2 h. The resin is filtered off, treated with a mixture of DCM, DIPEA and MeOH (17:1:2), and washed with DMF (3 x 30 s), MeOH (3 x 30 s) and DCM (3 x 30 s). 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 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).

**Cleavage of the furan modified peptides**

TFA in combination with the appropriate scavengers is added to the resin. The resin is shaken for 1 h for short peptides (< 8 amino acids) or for 1.5 h for longer peptides (≥ 8 amino acids). The resin is then filtered off and washed three times with neat TFA. The combined filtrates are evaporated and the peptide is precipitated by adding a 10-20 fold excess of cold MTBE-ether. The mixture is centrifugated at 0°C and the supernatants is carefully removed. The precipitated peptide is then dissolved in water and lyophilized to obtain a powder.
Spectral data of cleaved furan modified peptides

HPLC analysis after acidolytic cleavage of Fur-Val-Glu(tBu)-Asp(tBu)-Arg(Pbf)-Thr-Val-Asp(tBu)-Val-His(Trt)-Ile-Arg(Pbf)-Arg(Pbf)-Leu-Arg(Pbf)-Lys(Boc)-Ala-Leu-Glu(tBu)-Pro-Gly-R (R = 2-Cl Trt resin) with TFA, DTT and H₂O

HPLC analysis after acidolytic cleavage of Fur-Ala-His(Trt)-Asn(Trt)-Leu-Ala-R (R = Wang resin) with TFA, DTT and H₂O
HPLC analysis after acidolytic cleavage of Fur-Ala-His(Trt)-Asn(Trt)-Leu-Ala-R (R = Wang resin) with TFA, TIS and H₂O

**compound 3**

Chemical Formula: C_{29}H_{42}N_{8}O_{9}
Exact Mass: 646.31 g mole⁻¹
Molecular Weight: 646.69 g mole⁻¹
MS (m/z): 647.2 [M+H]+, 771.3 [unknown impurity]

**compound 4**

Chemical Formula: C_{29}H_{46}N_{8}O_{9}
Exact Mass: 650.34 g mole⁻¹
Molecular Weight: 650.72 g mole⁻¹
MS (m/z): 326.1 [M+2H]²⁺, 651.1 [M+H]^+
MS spectra from LC-MS after acidolytic cleavage of Fmoc-Leu-FurAla-Gly-Lys(Boc)-Val-R (R = Wang resin)

**compound 6:**

Chemical Formula: C_{41}H_{54}N_{6}O_{9}
Exact Mass: 774.39523 g mole^{-1}
Molecular Weight: 774.90226 g mole^{-1}
MS (m/z): 775.2 [M+H]^+

**compound 7:**

Chemical Formula: C_{41}H_{56}N_{6}O_{10}
Exact Mass: 778.42653 g mole^{-1}
Molecular Weight: 778.93402 g mole^{-1}
MS (m/z): 793.2 [M+H]^+
compounds 8/9:

Chemical Formula: $C_{41}H_{56}N_6O_9$
Exact Mass: 776.41088 g mole$^{-1}$
Molecular Weight: 776.91814 g mole$^{-1}$
MS (m/z): 777.2 [M+H]$^+$

compound 10:

Chemical Formula: $C_{41}H_{58}N_6O_9$
Exact Mass: 778.42653 g mole$^{-1}$
Molecular Weight: 778.93402 g mole$^{-1}$
MS (m/z): 779.2 [M+H]$^+$
Compounds 11/12:

Chemical Formula: C_{45}H_{64}N_{6}O_{11}S_{2}
Exact Mass: 928,40745 \ g \ mole^{-1}
Molecular Weight: 929,15326 \ g \ mole^{-1}
MS (m/z): 929,4 \ [M+H]^{+}, 911,3 \ [M+H-H_{2}O]^{+}

Compound 13:

is out of range for the LC-MS system, a simple ESI-MS of the crude peptide after acidolytic cleavage with TFA/DTT (95:5) reveals the formed side-products:

Chemical Formula: C_{49}H_{74}N_{6}O_{13}S_{4}
Exact Mass: 1082,41967 \ g \ mole^{-1}
Molecular Weight: 1083,40426 \ g \ mole^{-1}
MS (m/z): 775,4 \ [6+H]^{+}; 929,4 \ [11/12+H]^{+}; 1083,3 \ [13+H]^{+}
HPLC analysis after acidolytic cleavage of Acetyl-Val-Glu(tBu)-Asp(tBu)-Arg(Pbf)-Thr-Val-Asp(tBu)-Val-His(Trr)-Ile-Arg(Pbf)-Arg(Pbf)-Leu-Arg(Pbf)-Lys(Boc)-Ala-Leu-Glu(tBu)-Pro-Gly-Fur-Ala-R (R = Rink Amide AM resin)

Chemical Formula: C_{110}H_{185}N_{37}O_{32}
Exact Mass: 2536.40 g mol^{-1}
Molecular Weight: 2537.87 g mol^{-1}
MS (m/z): 2538.0 [M+H]^+, 2521.4 [M+H-H_2O]^+
Oxidation of peptide 14

To 1ml of peptide 14 in MilliQ (394 µM) was added 10 µL of a NBS solution (39.4 mM). The reaction is analyzed after 4 hours by MALDI-TOF.

Chemical Formula: C_{110}H_{185}N_{37}O_{33}

Exact Mass: 2552.39 g mole\(^{-1}\)

Molecular Weight: 2553.9 g mole\(^{-1}\)

MALDI-TOF: 2553.9 [M+H]\(^+\), 2536.9 [M+H-H\(_2\)O]\(^+\)
HPLC analysis acidolytic cleavage of Acetyl-Val-Glu(tBu)-Asp(tBu)-Fur-Ala-Thr-Val-Asp(tBu)-Val- 
His(Trt)-Ile-Arg(Pbf)-Arg(Pbf)-Leu-Arg(Pbf)-Lys(Boc)-Ala-Leu-Glu(tBu)-Pro-Gly-R (R = Rink Amide 
AM resin)

Chemical Formula: C_{104}H_{173}N_{33}O_{31}
Exact Mass: 2380.30 g mole^{-1}
Molecular Weight: 2381.69 g mole^{-1}
MS (m/z): 2382.5 [M+H]^+

![HPLC graph with peak at 214 nm wavelength](image)
Oxidation of peptide 15

To 1ml of peptide 15 in MilliQ (420 µM) was added 10.1 µL of a NBS solution (41.6 mM). The reaction is analyzed after 4 hours by MALDI-TOF.

Chemical Formula: C₁₀₅H₁₇₃N₃₃O₃₂
Exact Mass: 2396.29 g mole⁻¹
Molecular Weight: 2397.7 g mole⁻¹
MALDI-TOF: 2398.8 [M+H]⁺, 2382.9 [15+H]⁺
HPLC analysis after acidolytic cleavage of Acetyl-Val-Glu(tBu)-Asp(tBu)-Arg(Pbf)-Thr-Val-Asp(tBu)-FurAla-His(Trt)-Ile-Arg(Pbf)-Arg(Pbf)-Leu-Arg(Pbf)-Lys(Boc)-Ala-Leu-Glu(tBu)-Pro-Gly-R (R = Rink Amide AM resin)

Chemical Formula: C_{105}H_{176}N_{36}O_{31}

Exact Mass: 2437.33 g mole\(^{-1}\)

Molecular Weight: 2438.74 g mole\(^{-1}\)

MS (m/z): 2440.1 [M+H]\(^+\)
Oxidation of peptide 16

To 1ml of peptide 16 in MilliQ (410 µM) was added 9.8 µl of a NBS solution (41.6 mM). The reaction is analyzed after 4 hours by MALDI-TOF.

Chemical Formula: C_{105}H_{176}N_{36}O_{32}
Exact Mass: 2453.33 g mole^{-1}
Molecular Weight: 2454.7 g mole^{-1}
MALDI-TOF: 2456.2 [M+H]^+, 2439.0 [16+H]^+ or [M-H_2O+H]^+
HPLC analysis after acidolytic cleavage of Acetyl-Val-Glu(tBu)-Asp(tBu)-Arg(Pbf)-Thr-Val-Asp(tBu)-Val-FurAla-Ile-Arg(Pbf)-Arg(Pbf)-Leu-Arg(Pbf)-Lys(Boc)-Ala-Leu-Glu(tBu)-Pro-Gly-R \( (R = \text{Rink Amide AM resin}) \)

Chemical Formula: \( C_{104}H_{178}N_{34}O_{31} \)

Exact Mass: 2399.34 g \text{mole}^{-1}

Molecular Weight: 2400.74 g \text{mole}^{-1}

MS (m/z): 2402.4 [M+H]^{+}
Oxidation of peptide 17

To 250 µl of peptide 17 in MilliQ (417 µM) was added 8 µl of a NBS solution (20.8 mM). The reaction is analyzed after 4 hours by MALDI-TOF.

Chemical Formula: C_{104}H_{178}N_{34}O_{32}

Exact Mass: 2415.33 g mole\(^{-1}\)

MW: 2416.7 g mole\(^{-1}\)

MALDI-TOF: 2415.5 [M+H]\(^+\), 2398.9 [M-H\_2O+H]\(^+\)
HPLC analysis after acidolytic cleavage of Acetyl-Val-Glu(tBu)-Asp(tBu)-Arg(Pbf)-Thr-Val-Asp(tBu)-
Val-His(Trt)-Ile-Arg(Pbf)-FurAla-Leu-Arg(Pbf)-Lys(Boc)-Ala-Leu-Glu(tBu)-Pro-Gly-R (R = Rink
Amide AM resin)

Chemical Formula: C_{104}H_{173}N_{33}O_{31}
Exact Mass: 2380.30 \text{ g mole}^{-1}
Molecular Weight: 2381.69 \text{ g mole}^{-1}
MS (m/z): 2383.4 [M+H]^+

Voyager Spec #1[BP = 2383.4, 23578]
Oxidation of peptide 18

To 250 µl of peptide 18 in MilliQ (420 µM) was added 5 µl of a NBS solution (20.8 mM). The reaction is analyzed after 4 hours by MALDI-TOF.

Chemical Formula: C_{104}H_{173}N_{33}O_{32}

Exact Mass: 2396.29 g mole$^{-1}$

MW: 2397.7 g mole$^{-1}$

MALDI-TOF: 2398.0 [M+H]$^+$, 2381.2 [M-H$_2$O+H]$^+$

![MALDI-TOF spectrum](image)
HPLC analysis after acidolytic cleavage of Acr-FurAla-Thr(tBu)-Pro-Lys(Boc)-Arg(Pbf)-Pro-Arg(Pbf)-Gly-Arg(Pbf)-Pro-Lys(Boc)-Lys(Boc)-R (R = Rink Amide AM resin)

Chemical Formula: C_{78}H_{120}N_{26}O_{15}
Exact Mass: 1660.94 g mole\(^{-1}\)
Molecular Weight: 1661.95 g mole\(^{-1}\)
MS (m/z): 1662.6 [M+H]\(^{+}\)
HPLC analysis after acidolytic cleavage of Napht-FurAla-Trp(Boc)-Ser(tBu)-His(Trt)-Pro-Gln(Trt)-Phe-Glu(OtBu)-Lys(Boc)-R (R = 2-Cl Trt resin)

Chemical Formula: $C_{68}H_{80}N_{14}O_{16}$
Exact Mass: 1348.59 g mole$^{-1}$
Molecular Weight: 1349.45 g mole$^{-1}$
LC-MS: $t_R=15.073 \text{min}$ (1349.4=[M+H]$^+$, 675.3=[M+2H]$^{2+}$/2)
**HPLC analysis after acidolytic cleavage of Acr-Gly-FurAla-R (R = Rink Amide AM resin)**

![Chemical Structure](image)

Chemical Formula: C\textsubscript{23}H\textsubscript{20}N\textsubscript{4}O\textsubscript{4}

Exact Mass: 416.15 g mole\(^{-1}\)

Molecular Weight: 416.43 g mole\(^{-1}\)

RPHPLC: \(t_R=35.1\) min (Clarity)

LC-MS: \(t_R=12.307\) (417.1=[M+H]\(^{+}\))

**RPHPLC**

**LC-MS**
Oxidation of peptide 23 into 24

To 1 ml of peptide 23 in MilliQ (240 µM) was added 4.3 µl of a NBS solution (55.6 mM). The reaction is analyzed after 1 hours by RPHPLC and ESI-MS.

Chemical Formula: C_{23}H_{20}N_{4}O_{5}

Exact Mass: 432.14 g mole\(^{-1}\)

MW: 432.43 g mole\(^{-1}\)

RPHPLC: t\(_R\)=28.6 min; 28.8 min

ESI-MS: 452.3 [M+H₂O+H]\(^+\), 434.4 [M+H]\(^+\)