Supporting Information to

A Sustainable Chemo Enzymatic Approach to the Synthesis of Liraglutide

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General remarks

Materials

Unless stated otherwise, all chemicals were obtained from commercial suppliers and used without further purification. Anisole, dichloromethane (DCM), diisopropyl ether (DIPE), dimethyl carbonate (DMC), dimethyl sulfoxide (DMSO), ethyl acetate (EtOAc), N,N-dimethylformamide (DMF), N-butyl pyrrolidone (NBP), N-octyl pyrrolidone (NOP) were supplied by Sigma Aldrich, as well as HPLC-grade acetonitrile (ACN) and HPLC-grade water used for HPLC-MS analyses. The N-fluorenylmethyloxycarbonyl (Fmoc) amino acids were supplied by Fluorochem. The resins were supplied by Sigma Aldrich. Piperidine, Oxyma Pure®, N,N-diisopropylcarbodiimide (DIC), trifluoroacetic acid (TFA), diisopropylethylenamine (DIPEA), triisopropyl silane (TIS), and dithiothreitol (DTT) were purchased from Merck and Iris Biotech. The solid-phase synthesis of peptides (SPPS) was carried out manually in custom vessels (SPPS reactors) consisting of double-jacketed, temperature-controlled glass tubes fitted with a porous polyethylene disc and a valve. The SPPS reactors were connected to a vacuum source to allow removal of reagents and sovlents. The IKA EUROSTAR 20 digital overhead mechanical stirrer was employed at ~50 rpm. The Huber Minichiller 300 was employed for temperature control with H_2O + 0.1 g/L of Na_2CO_3 as the thermal fluid.

Analytical details

HPLC-MS analyses were performed on Agilent 1260 InfinityLab system coupled with Agilent InfinityLab LC/MSD ESI mass spectrometer (positive-ion mode, m/z = 100–2000 amu, fragmentor 30 V). The ChemStation software was used for data processing. Specific parameters used for the analyses are described in detail below.

Method 1: HPLC parameters for the analysis of H-(1-11)-CamFK-NH_2 2

Column: Agilent Poroshell 120, EC-C18 2.7µm, 4.6 x 100mm

Temperature: 35.0°C

Polarity: Positive Scan

UV: 214 nm

Injection volume: 10 µL

Mobile phase A: H_2O+0.08% TFA

Mobile phase B: ACN+0.08% TFA

Gradient: see Table S1

Table S1. Gradient of Analytical Method 1.

Time (min)	Mobile phase A (%)	Mobile phase B (%)	Flow (mL/min)
0.00	95.0	5.0	1.000
4.00	80.0	20.0	1.000
8.00	70.0	30.0	1.000
16.00	50.0	50.0	1.000
18.00	5.0	95.0	1.000
20.00	5.0	95.0	1.000
22.00	95.0	5.0	1.000
26.00	95.0	5.0	1.000

Method 2: HPLC parameters for the analysis of H-(12-31)-OH 3

Column: Phenomenex Luna 5 µm C18(2) 100 Å 250 x 4.6 mm

Temperature: 35.0°C

Polarity: Positive Scan

UV: 214 nm

Injection volume: 10 µL

Mobile phase A: H_2O+0.08% TFA

Mobile phase B: ACN+0.08% TFA

Gradient: See Table S2.

Table S2. Gradient of Analytical Method 2.

Time (min)	Mobile phase A (%)	Mobile phase B (%)	Flow (mL/min)
0.00	80.0	20.0	1.000
8.00	50.0	50.0	1.000
11.00	50.0	50.0	1.000
26.00	40.0	60.0	1.000
35.00	30.0	70.0	1.000
45.00	10.0	90.0	1.000
50.00	10.0	90.0	1.000
55.00	80.0	20.0	1.000
60.00	80.0	20.0	1.000

Method 3: HPLC parameters for the enzymatic coupling of 2 and 3 to form Liraglutide 1

Column: Waters Cortecs, $C18^{+}$, 2.7μm, 4.6 x 150mm

Temperature: 40.0°C

Polarity: Positive Scan

UV: 214 nm

Injection volume: 10 μL

Flow: 1.000 mL/min

Mobile phase A: H_2O+0.05% TFA

Mobile phase B: ACN+0.05% TFA

Gradient: See Table S3.

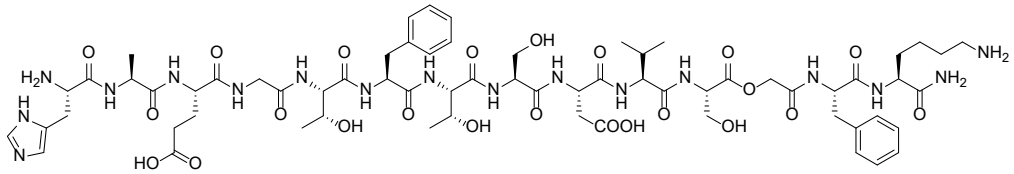
Table S3. Gradient of Analytical Method 3.

Time (min)	Mobile phase A (%)	Mobile phase B (%)	Flow (mL/min)
0.00	91.0	9.0	1.000
0.50	91.0	9.0	1.000
6.00	70.0	30.0	1.000
8.00	50.0	50.0	1.000
15.00	47.0	53.0	1.000
16.00	25.0	75.0	1.000
17.00	25.0	75.0	1.000
18.00	91.0	9.0	1.000
25.00	91.0	9.0	1.000

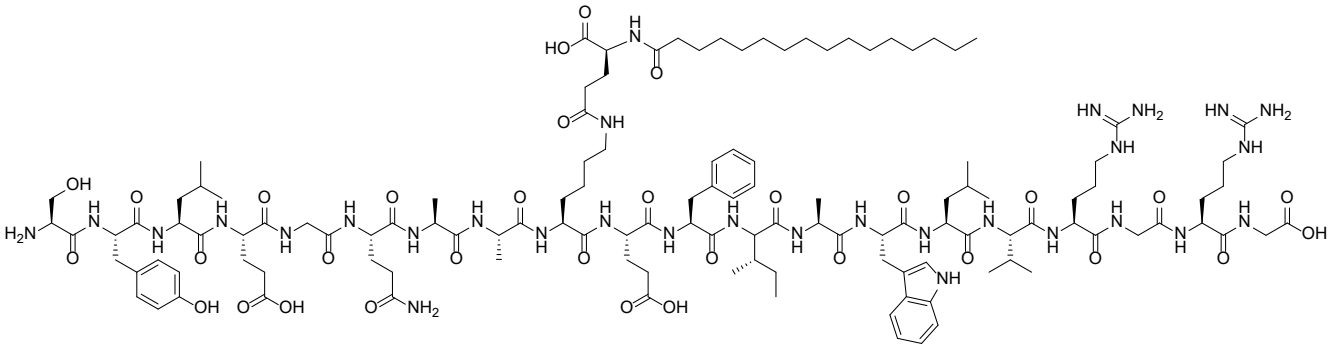
Synthetic Protocols

Below are reported the protocols for the solid-phase synthesis of peptide fragments H-(1-11)-CamFK-NH_2 **2** and H-(12-31)-OH **3**. Their structures are depicted in Figure S1.

Figure S1. Structures of peptide fragments H-(1-11)-CamFK-NH_2 **2** and H-(12-31)-OH **3**.



H-Liraglutide(1-11)-CamFK-NH_2
2



H-Liraglutide(12-31)-OH
3

Solid-phase synthesis of H-(1-11)-CamFK-NH_2 2 in DMF

The synthesis was performed on preloaded Fmoc-Lys(Boc)-Rink Amide resin (loading 0.62 mmol/g); all steps were carried out at 25°C. After swelling the resin with DMF (10 mL/g of resin, 30 min), the Fmoc protecting group was removed by treatment with a solution of piperidine 20% in DMF (2 times x 5 mL/g of resin, 10 min each) and with DMF (4 times x 5 mL/g of resin, 5 min each). Fmoc-Phe-OH and Oxyma Pure® (2.0 eq of each reagent with respect to the loading of the resin) were dissolved in DMF (5 mL/g of resin) and pre-activated with DIC (2.0 eq with respect to the loading of the resin) for 3 minutes. The coupling mixture was added to the resin and stirred for 90 minutes, followed by washes with DMF (3 times x 5 mL/g of resin, 5 min each). The steps of Fmoc-removal, washes and coupling were repeated until the target peptide sequence was achieved. Piperidine 20% in DMF was employed up to Phe^B included, while after Ser^{11} Fmoc-removal was performed with piperidine 10% in DMF + piperidine 5% in DMF (5 mL/g of resin, 10 min each). For each coupling, the selected Fmoc-AAx-OH, Oxyma Pure® and DIC (2.0 eq of each reagent with respect to the loading of the resin) were employed. The carboxyamidomethyl (Cam) ester was formed by coupling bromoacetic acid, Oxyma Pure® and DIC (respectively 2.0, 0.1, and 2.0 eq of the reagents with respect to the loading

of the resin) onto the resin according to the protocol described above. Subsequently, the Ser^{11} residue was introduced by adding KI (1.0 eq with respect to the loading of the resin) directly to the reaction vessel, followed by a solution of Fmoc-Ser(tBu)-OH and DIPEA (4.0 eq with respect to the loading of the resin) in DMF (5 mL/g of resin). This coupling step was performed for 24 hours. Boc-His(Trt)-OH was used to introduced His^{1} following the protocol described for Fmoc-amino acid residues. After the final coupling step, the resin was washed with DMF (3 times x 5 mL/g of resin, 5 min each), DCM (3 times x 5 mL/g of resin, 5 min each), and dried under vacuum for 12 hours. For cleavage, the resin was added gradually to a solution of TFA/TIS/H_2O 92.5/5.0/2.5 v/v/v % (10 mL/g of resin) at 10°C, the suspension was the stirred for 3 hours at room temperature. The resin was filtered, and diisopropyl ether (30 mL/g of resin) was added to the filtered solution at 10°C under vigorous stirring. The precipitated peptide was filtered, washed with ether (3 times x 5 mL/g of resin), and dried under vacuum to obtain **2**. The crude was analyzed via HPLC-MS using Method 1 (see Analytical details section reported above).

Table S4. Results for solid-phase synthesis of H-(1-11)-CamFK-NH_2 **2** in DMF.

Species	Peptide sequence	Area %	t_R (min)	rrt	m/z obs
Product	H-HAEGTFTSDVSCamFK-NH_2	74.629	7.842	1.000	1482.6
Ester hydrolysis	HO-GFK-NH_2	3.145	4.949	0.631	351.2
N,O-shifts	H-SCamFK-NH_2	1.577	4.572	0.583	438.2
	H-VSCamFK-NH_2	2.836	7.303	0.931	537.6
	H-HAEGT-OH	0.922	7.540	0.961	514.2
Deletion sequences	Des-Phe Des-Ser	3.667	8.074	1.030	1335.1 1395.6
	Des-SerCam	2.736	8.163	1.041	1337.5
Else	-	10.488	-	-	-

Figure S2. Chromatogram of H-(1-11)-CamFK-NH_2 **2**, solid-phase synthesis in DMF. All peaks between 3 and 18 minutes with Area % ≥ 0.5 were considered.

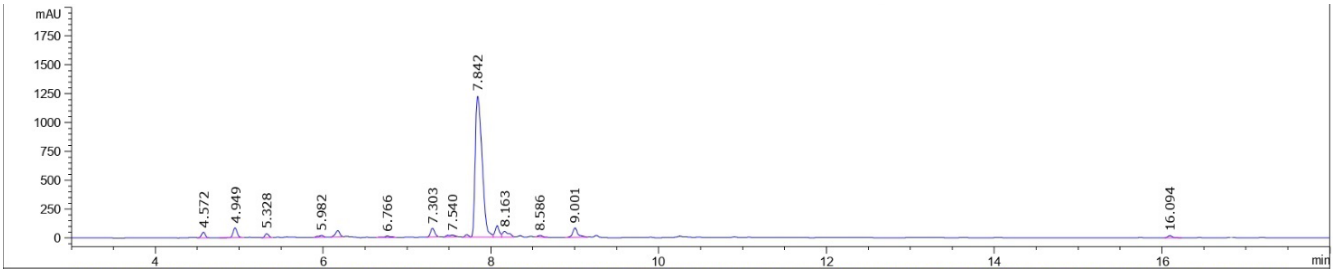


Figure S3. Mass chromatogram for peak at 7.842 min, m/z obs = 1482.6. Zoom on mass 1482.6.

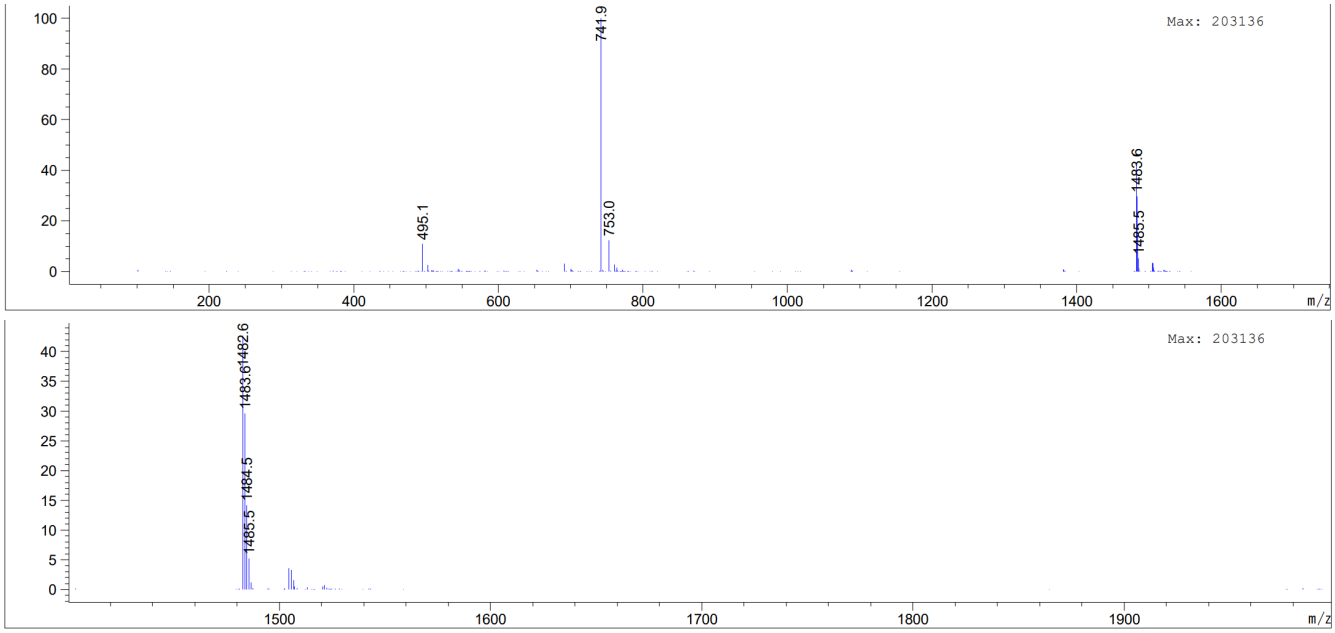


Figure S4. Mass chromatogram for peak at 4.949 min, m/z obs = 351.2.

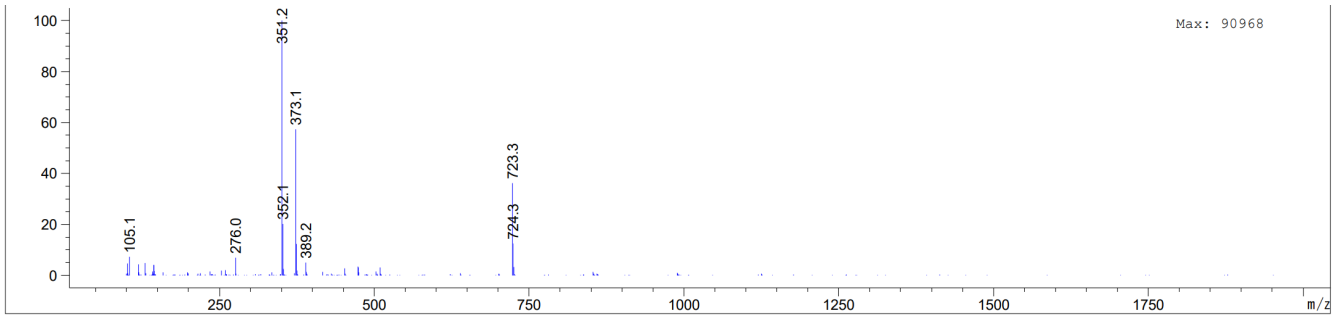


Figure S5. Mass chromatogram for peak at 4.572 min, m/z obs = 438.2.

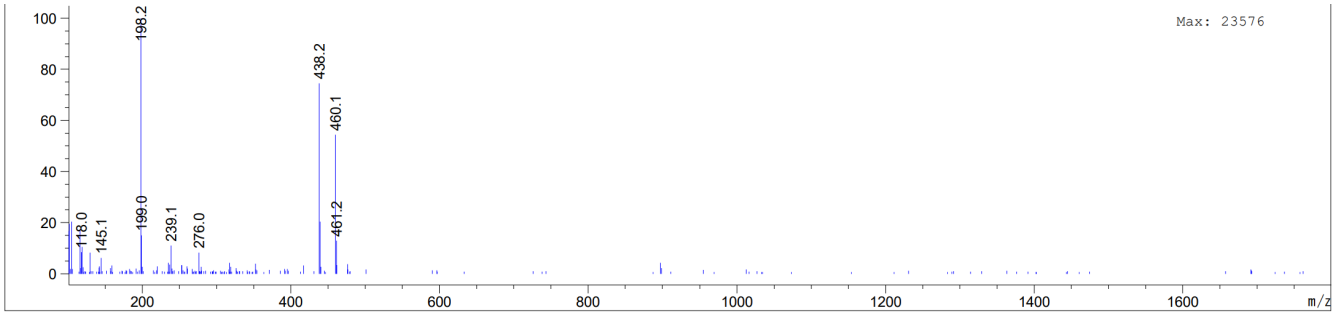


Figure S6. Mass chromatogram for peak at 7.303 min, m/z obs = 537.6.

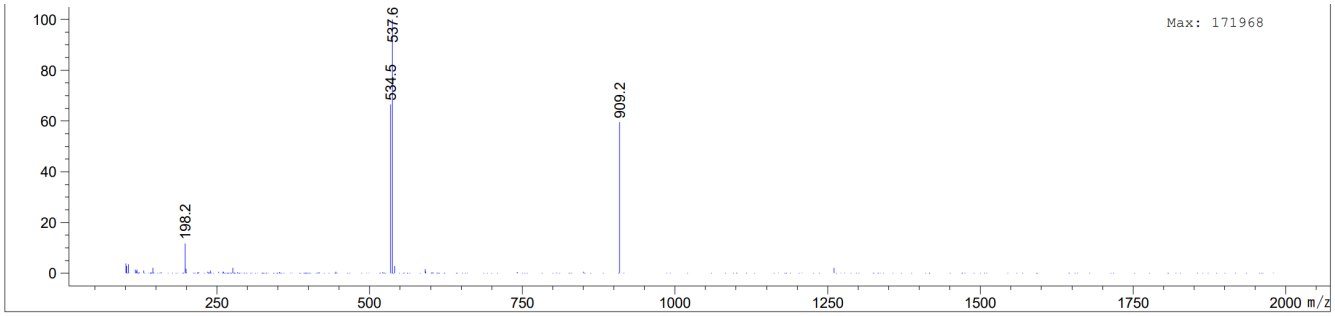


Figure S7. Mass chromatogram for peak at 7.540 min, m/z obs = 514.2.

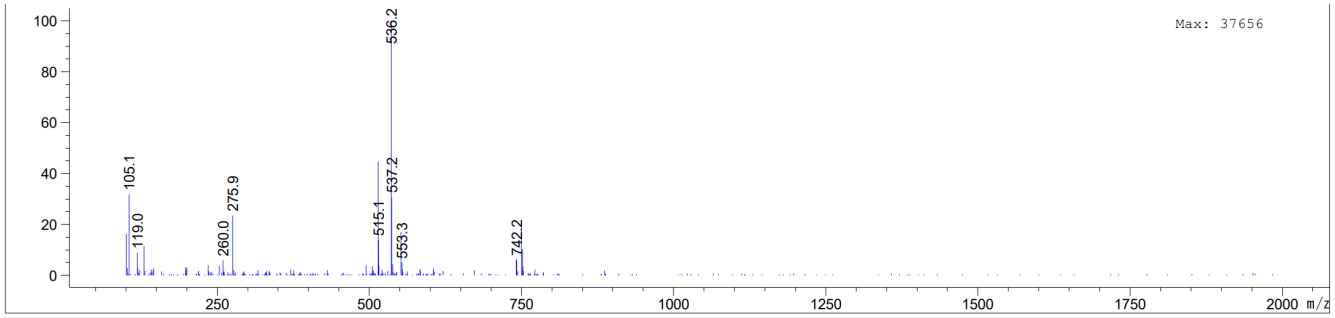


Figure S8. Mass chromatogram for peak at 8.074 min, m/z obs = 1335.1 and 1395.6.

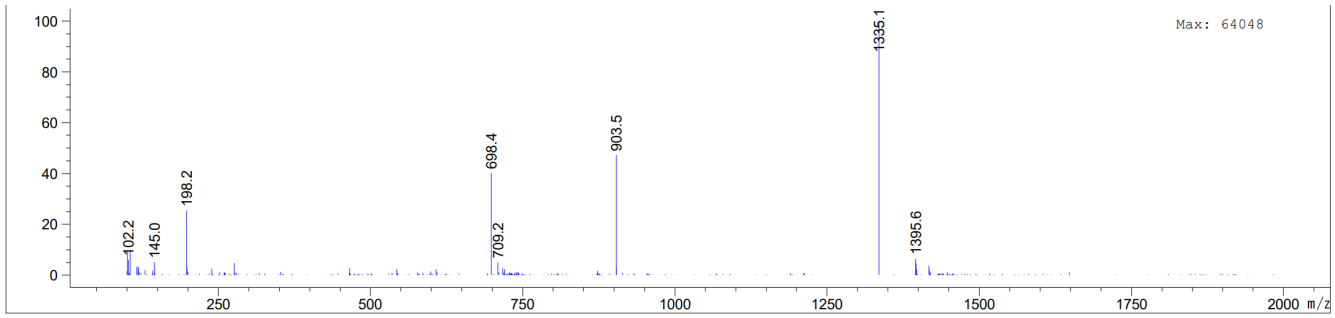
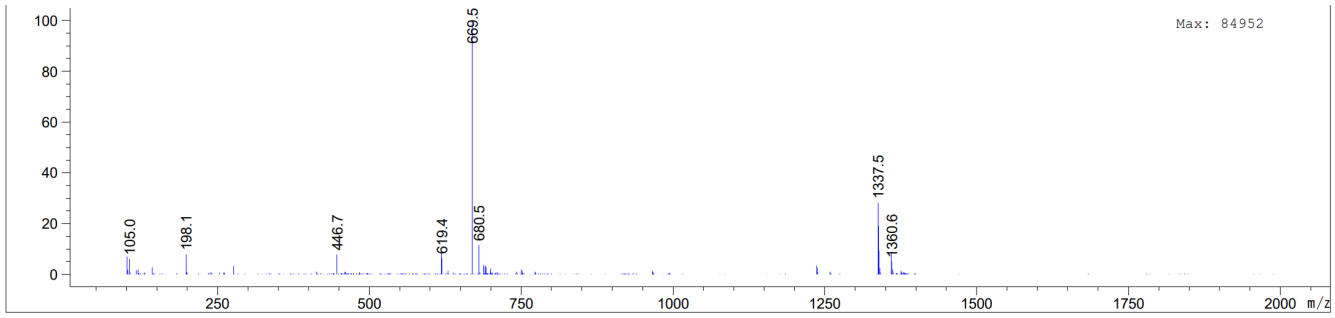


Figure S9. Mass chromatogram for peak at 8.163 min, m/z obs = 1337.5.



List of protocol modifications

Protocol modification **A**: elongation of reaction time in Fmoc-removal step.

Protocol modification **B**: Fmoc-removal step carried out at 40°C after the coupling of Thr^{5}.

Protocol modification **C**: All couplings repeated twice.

Protocol modification **D**: Elongation of reaction time for coupling of bromoacetic acid.

Protocol modification **E**: all steps at 40°C.

Protocol modification **F**: double or longer couplings. All steps at 40°C from Val^{27} (included) onwards.

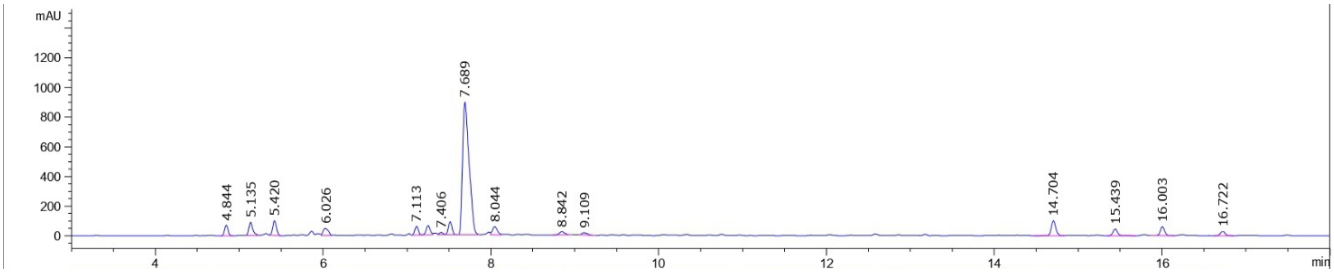
Solid-phase synthesis of H-(1-11)-CamFK-NH_2 2 in NOP/DMC (8:2), protocol modification A

The synthesis was performed on preloaded Fmoc-Lys(Boc)-Rink Amide resin (loading 0.62 mmol/g); all steps were carried out at 25°C. After swelling the resin with NOP/DMC (8:2) (10 mL/g of resin, 240 min), the Fmoc protecting group was removed by treatment with a solution of piperidine 20% in NOP/DMC (8:2) (2 times x 5 mL/g of resin, 15 min each) and with NOP/DMC (8:2) (4 times x 5 mL/g of resin, 5 min each). Fmoc-Phe-OH and Oxyma Pure® (2.0 eq of each reagent with respect to the loading of the resin) were dissolved in NOP/DMC (8:2) (5 mL/g of resin) and pre-activated with DIC (2.0 eq with respect to the loading of the resin) for 3 minutes. The coupling mixture was added to the resin and stirred for 90 minutes, followed by washes with NOP/DMC (8:2) (3 times x 5 mL/g of resin, 5 min each). The steps of Fmoc-removal, washes and coupling were repeated until the target peptide sequence was achieved. Piperidine 20% in NOP/DMC (8:2) was employed up to Phe^{B} included, while after Ser^{11} Fmoc-removal was performed with piperidine 10% in NOP/DMC (8:2) + piperidine 5% in NOP/DMC (8:2) (5 mL/g of resin, 15 min each). For each coupling, the selected Fmoc-AAx-OH, Oxyma Pure® and DIC (2.0 eq of each reagent with respect to the loading of the resin) were employed. The carboxyamidomethyl (Cam) ester was formed by coupling bromoacetic acid, Oxyma Pure® and DIC (respectively 2.0, 0.1, and 2.0 eq of the reagents with respect to the loading of the resin) onto the resin according to the protocol described above. Subsequently, the Ser^{11} residue was introduced by adding KI (1.0 eq with respect to the loading of the resin) directly to the reaction vessel, followed by a solution of Fmoc-Ser(tBu)-OH and DIPEA (4.0 eq with respect to the loading of the resin) in NOP/DMC (8:2) (5 mL/g of resin). This coupling step was performed for 24 hours. Boc-His(Trt)-OH was used to introduced His^{1} following the protocol described for Fmoc-amino acid residues. After the final coupling step, the resin was washed with NOP/DMC (8:2) (3 times x 5 mL/g of resin, 5 min each), DCM (3 times x 5 mL/g of resin, 5 min each), and dried under vacuum for 12 hours. For cleavage, the resin was added gradually to a solution of TFA/TIS/H_2O 92.5/5.0/2.5 v/v/v % (10 mL/g of resin) at 10°C, the suspension was the stirred for 3 hours at room temperature. The resin was filtered, and diisopropyl ether (30 mL/g of resin) was added to the filtered solution at 10°C under vigorous stirring. The precipitated peptide was filtered, washed with ether (3 times x 5 mL/g of resin), and dried under vacuum to obtain **2**. The crude was analyzed via HPLC-MS using Method 1 (see Analytical details section reported above).

Table S5. Results for solid-phase synthesis of H-(1-11)-CamFK-NH_2 **2** in NOP/DMC (8:2), protocol modification A.

Species	Peptide sequence	Area %	t_R (min)	rrt	m/z obs
Product	H-HAEGTFTSDVSCamFK-NH_2	60.938	7.689	1.000	1482.5
Ester hydrolysis	HO-GFK-NH_2	2.642	4.844	0.630	351.2
Deletion sequences	Des-SerCam	2.792	8.044	1.046	1337.4
Incomplete Fmoc removal	Fmoc-TFTSDVSCamFK-NH_2	2.730	16.003	2.081	1310.5
	Fmoc-TFTSDVFK-NH_2 Fmoc-FTSDVFK-NH_2	1.379	16.722	2.175	1165.4 1209.4
Derivatives of Fmoc-truncated sequences		6.994	14.704	1.913	1214.4
			15.439	2.008	1113.3
Epimers	-	5.345	7.113	0.925	741.9
	-		7.249	0.943	741.8
	-		7.406	0.963	741.9
Else	-	17.180	-	-	-

Figure S10. Chromatogram of H-(1-11)-CamFK-NH_2 **2**, solid-phase synthesis in NOP/DMC (8:2), protocol modification A. All peaks between 3 and 18 minutes with Area % ≥ 0.5 were considered.



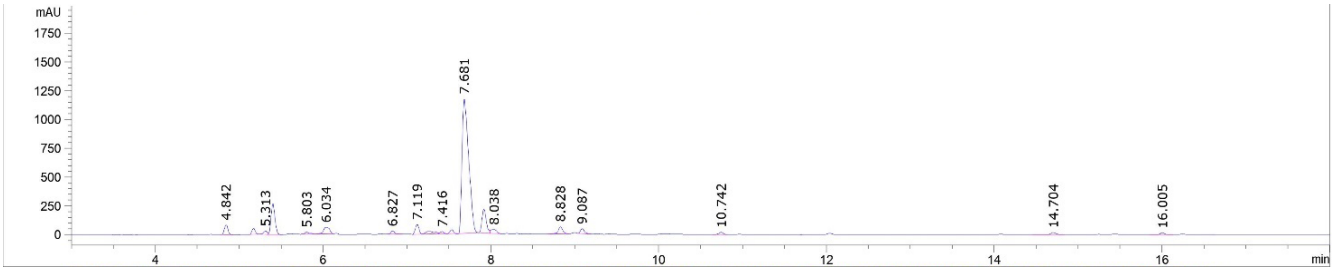
Solid-phase synthesis of H-(1-11)-CamFK-NH_2 2 in NOP/DMC (8:2), protocol modifications A and B

The synthesis was performed on preloaded Fmoc-Lys(Boc)-Rink Amide resin (loading 0.62 mmol/g); all steps were carried out at 25°C unless stated otherwise. After swelling the resin with NOP/DMC (8:2) (10 mL/g of resin, 240 min), the Fmoc protecting group was removed by treatment with a solution of piperidine 20% in NOP/DMC (8:2) (2 times x 5 mL/g of resin, 15 min each) and with NOP/DMC (8:2) (4 times x 5 mL/g of resin, 5 min each). Fmoc-Phe-OH and Oxyma Pure® (2.0 eq of each reagent with respect to the loading of the resin) were dissolved in NOP/DMC (8:2) (5 mL/g of resin) and pre-activated with DIC (2.0 eq with respect to the loading of the resin) for 3 minutes. The coupling mixture was added to the resin and stirred for 90 minutes, followed by washes with NOP/DMC (8:2) (3 times x 5 mL/g of resin, 5 min each). The steps of Fmoc-removal, washes and coupling were repeated until the target peptide sequence was achieved. Piperidine 20% in NOP/DMC (8:2) was employed up to Phe^B included, while after Ser^{11} Fmoc-removal was performed with piperidine 10% in NOP/DMC (8:2) + piperidine 5% in NOP/DMC (8:2) (5 mL/g of resin, 15 min each); finally, from Thr^5 (included) onwards piperidine 20% in NOP/DMC (8:2) was used at 40°C. For each coupling, the selected Fmoc-AAx-OH, Oxyma Pure® and DIC (2.0 eq of each reagent with respect to the loading of the resin) were employed. The carboxyamidomethyl (Cam) ester was formed by coupling bromoacetic acid, Oxyma Pure® and DIC (respectively 2.0, 0.1, and 2.0 eq of the reagents with respect to the loading of the resin) onto the resin according to the protocol described above. Subsequently, the Ser^{11} residue was introduced by adding KI (1.0 eq with respect to the loading of the resin) directly to the reaction vessel, followed by a solution of Fmoc-Ser(tBu)-OH and DIPEA (4.0 eq with respect to the loading of the resin) in NOP/DMC (8:2) (5 mL/g of resin). This coupling step was performed for 24 hours. Boc-His(Trt)-OH was used to introduced His^1 following the protocol described for Fmoc-amino acid residues. After the final coupling step, the resin was washed with NOP/DMC (8:2) (3 times x 5 mL/g of resin, 5 min each), DCM (3 times x 5 mL/g of resin, 5 min each), and dried under vacuum for 12 hours. For cleavage, the resin was added gradually to a solution of TFA/TIS/H_2O 92.5/5.0/2.5 v/v/v % (10 mL/g of resin) at 10°C, the suspension was the stirred for 3 hours at room temperature. The resin was filtered, and diisopropyl ether (30 mL/g of resin) was added to the filtered solution at 10°C under vigorous stirring. The precipitated peptide was filtered, washed with ether (3 times x 5 mL/g of resin), and dried under vacuum to obtain **2**. The crude was analyzed via HPLC-MS using Method 1 (see Analytical details section reported above).

Table S6. Results for solid-phase synthesis of H-(1-11)-CamFK-NH_2 **2** in NOP/DMC (8:2), protocol modifications A and B.

Species	Peptide sequence	Area %	t_R (min)	rrt	m/z obs
Product	H-HAEGTFTSDVSCamFK-NH_2	62.692	7.681	1.000	1482.6
Ester hydrolysis	HO-GFK-NH_2	2.386	4.842	0.630	351.2
N,O-shifts	H-HAEGT-OH	0.566	7.416	0.966	514.3
Deletion sequences	Des-Phe	2.936	6.034	0.786	1335.3
	Des-AspVal	0.905	6.827	0.889	634.8
	Des-Ser	6.978	7.915	1.030	1395.5
	Des-Ser-Des-AspVal	2.395	7.119	0.927	1181.3
Incomplete Fmoc removal	Fmoc-TFTSDVSCamFK-NH_2	0.547	16.005	2.084	1310.3
Derivatives of Fmoc-truncated sequences	-	0.721	14.704	1.914	1214.4
Epimers	-	3.332	7.253	0.944	741.8
			8.038	1.046	741.8
Else	-	19.873	-	-	-

Figure S11. Chromatogram of H-(1-11)-CamFK-NH_2 **2**, solid-phase synthesis in NOP/DMC (8:2), protocol modifications A and B. All peaks between 3 and 18 minutes with Area % ≥ 0.5 were considered.



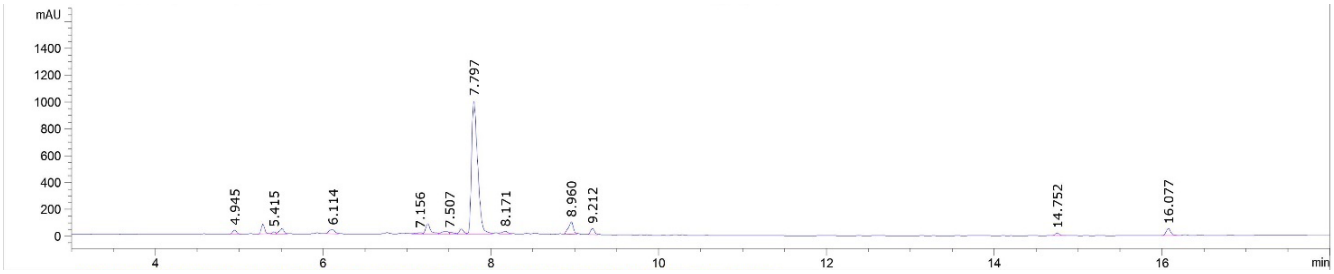
Solid-phase synthesis of H-(1-11)-CamFK-NH_2 2 in NOP/DMC (8:2), protocol modifications A, B, and C

The synthesis was performed on preloaded Fmoc-Lys(Boc)-Rink Amide resin (loading 0.62 mmol/g); all steps were carried out at 25°C unless stated otherwise. After swelling the resin with NOP/DMC (8:2) (10 mL/g of resin, 240 min), the Fmoc protecting group was removed by treatment with a solution of piperidine 20% in NOP/DMC (8:2) (2 times x 5 mL/g of resin, 15 min each) and with NOP/DMC (8:2) (4 times x 5 mL/g of resin, 5 min each). Fmoc-Phe-OH and Oxyma Pure® (2.0 eq of each reagent with respect to the loading of the resin) were dissolved in NOP/DMC (8:2) (5 mL/g of resin) and pre-activated with DIC (2.0 eq with respect to the loading of the resin) for 3 minutes. The coupling mixture was added to the resin and stirred for 90 minutes, followed by washes with NOP/DMC (8:2) (3 times x 5 mL/g of resin, 5 min each). The coupling was repeated a second time. The steps of Fmoc-removal, washes and double coupling were repeated until the target peptide sequence was achieved. Piperidine 20% in NOP/DMC (8:2) was employed up to Phe^B included, while after Ser^{11} Fmoc-removal was performed with piperidine 10% in NOP/DMC (8:2) + piperidine 5% in NOP/DMC (8:2) (5 mL/g of resin, 15 min each); finally, from Thr^5 (included) onwards piperidine 20% in NOP/DMC (8:2) was used at 40°C. For each coupling, the selected Fmoc-AAx-OH, Oxyma Pure® and DIC (2.0 eq of each reagent with respect to the loading of the resin) were employed; all couplings were performed twice. The carboxyamidomethyl (Cam) ester was formed by coupling bromoacetic acid, Oxyma Pure® and DIC (respectively 2.0, 0.1, and 2.0 eq of the reagents with respect to the loading of the resin) onto the resin according to the protocol described above. Subsequently, the Ser^{11} residue was introduced by adding KI (1.0 eq with respect to the loading of the resin) directly to the reaction vessel, followed by a solution of Fmoc-Ser(tBu)-OH and DIPEA (4.0 eq with respect to the loading of the resin) in NOP/DMC (8:2) (5 mL/g of resin). This coupling step was performed for 24 hours. The couplings of bromoacetic acid and Ser^{11} were not repeated a second time. Boc-His(Trt)-OH was used to introduced His^1 following the protocol described for Fmoc-amino acid residues. After the final coupling step, the resin was washed with NOP/DMC (8:2) (3 times x 5 mL/g of resin, 5 min each), DCM (3 times x 5 mL/g of resin, 5 min each), and dried under vacuum for 12 hours. For cleavage, the resin was added gradually to a solution of TFA/TIS/H_2O 92.5/5.0/2.5 v/v/v % (10 mL/g of resin) at 10°C, the suspension was the stirred for 3 hours at room temperature. The resin was filtered, and diisopropyl ether (30 mL/g of resin) was added to the filtered solution at 10°C under vigorous stirring. The precipitated peptide was filtered, washed with ether (3 times x 5 mL/g of resin), and dried under vacuum to obtain **2**. The crude was analyzed via HPLC-MS using Method 1 (see Analytical details section reported above).

Table S7. Results for solid-phase synthesis of H-(1-11)-CamFK-NH_2 **2** in NOP/DMC (8:2), protocol modifications A, B, and C.

Species	Peptide sequence	Area %	t_R (min)	rrt	m/z obs
Product	H-HAEGTFTSDVSCamFK-NH_2	69.923	7.797	1	1482.5
Ester hydrolysis	HO-GFK-NH_2	1.218	4.945	0.634	351.2
	H-HAEGTFTSDVS-OH	2.216	6.114	0.784	1150.4
N,O-shifts	H-HAEGT-OH	1.478	7.473	0.958	514.2
Deletion sequences	Des-SerCam	1.269	8.171	1.048	1337.5
Incomplete Fmoc removal	Fmoc-TFTSDVSCamFK-NH_2	2.891	16.077	2.062	1310.3
Derivatives of Fmoc-truncated sequences	-	0.989	14.752	1.892	1214.6
Epimers	-	4.323	7.248	0.93	741.5
Else	-	15.693	-	-	-

Figure S12. Chromatogram of H-(1-11)-CamFK-NH_2 **2**, solid-phase synthesis in NOP/DMC (8:2), protocol modifications A, B, and C. All peaks between 3 and 18 minutes with Area % ≥ 0.5 were considered.



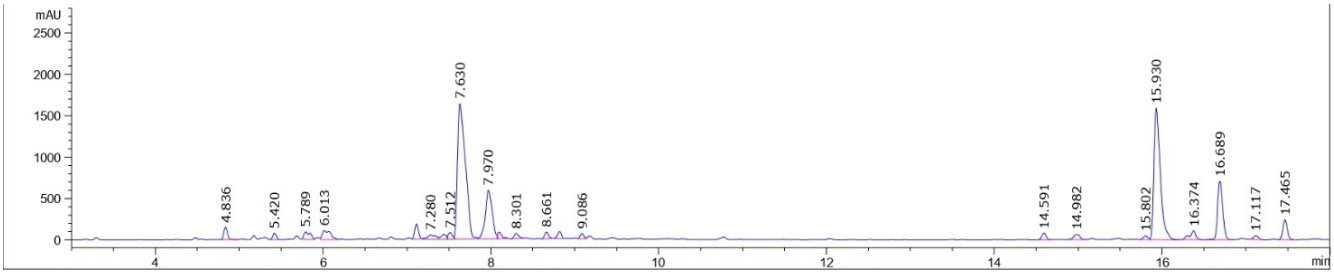
Solid-phase synthesis of H-(1-11)-CamFK-NH_2 2 in DMSO/EtOAc (1:9), protocol modification A

The synthesis was performed on preloaded Fmoc-Lys(Boc)-Rink Amide resin (loading 0.62 mmol/g); all steps were carried out at 25°C. After swelling the resin with DMSO/EtOAc (1:9) (10 mL/g of resin, 240 min), the Fmoc protecting group was removed by treatment with a solution of piperidine 20% in NOP/DMC (8:2) (2 times x 5 mL/g of resin, 15 min each) and with DMSO/EtOAc (1:9) (4 times x 5 mL/g of resin, 5 min each). Fmoc-Phe-OH and Oxyma Pure® (2.0 eq of each reagent with respect to the loading of the resin) were dissolved in DMSO/EtOAc (1:9) (5 mL/g of resin) and pre-activated with DIC (2.0 eq with respect to the loading of the resin) for 3 minutes. The coupling mixture was added to the resin and stirred for 90 minutes, followed by washes with DMSO/EtOAc (1:9) (3 times x 5 mL/g of resin, 5 min each). The steps of Fmoc-removal, washes and coupling were repeated until the target peptide sequence was achieved. Piperidine 20% in DMSO/EtOAc (1:9) was employed up to Phe^{B} included, while after Ser^{11} Fmoc-removal was performed with piperidine 10% in DMSO/EtOAc (1:9) + piperidine 5% in DMSO/EtOAc (1:9) (5 mL/g of resin, 15 min each). For each coupling, the selected Fmoc-AAx-OH, Oxyma Pure® and DIC (2.0 eq of each reagent with respect to the loading of the resin) were employed. The carboxyamidomethyl (Cam) ester was formed by coupling bromoacetic acid, Oxyma Pure® and DIC (respectively 2.0, 0.1, and 2.0 eq of the reagents with respect to the loading of the resin) onto the resin according to the protocol described above. Subsequently, the Ser^{11} residue was introduced by adding KI (1.0 eq with respect to the loading of the resin) directly to the reaction vessel, followed by a solution of Fmoc-Ser(tBu)-OH and DIPEA (4.0 eq with respect to the loading of the resin) in DMSO/EtOAc (1:9) (5 mL/g of resin). This coupling step was performed for 24 hours. Boc-His(Trt)-OH was used to introduced His^{1} following the protocol described for Fmoc-amino acid residues. After the final coupling step, the resin was washed with DMSO/EtOAc (1:9) (3 times x 5 mL/g of resin, 5 min each), DCM (3 times x 5 mL/g of resin, 5 min each), and dried under vacuum for 12 hours. For cleavage, the resin was added gradually to a solution of TFA/TIS/H_2O 92.5/5.0/2.5 v/v/v % (10 mL/g of resin) at 10°C, the suspension was the stirred for 3 hours at room temperature. The resin was filtered, and diisopropyl ether (30 mL/g of resin) was added to the filtered solution at 10°C under vigorous stirring. The precipitated peptide was filtered, washed with ether (3 times x 5 mL/g of resin), and dried under vacuum to obtain **2**. The crude was analyzed via HPLC-MS using Method 1 (see Analytical details section reported above).

Table S8. Results for solid-phase synthesis of H-(1-11)-CamFK-NH_2 **2** in DMSO/EtOAc (1:9), protocol modification A.

Species	Peptide sequence	Area %	t_R (min)	rrt	m/z obs
Product	H-HAEGTFTSDVSCamFK-NH_2	32.820	7.630	1.000	1482.5
Ester hydrolysis	HO-GFK-NH_2	1.431	4.836	0.634	351.2
	H-HAEGTFTSDVS-OH	2.314	6.013	0.788	1150.3
N,O-shifts	H-HAEGT-OH	1.194	7.333	0.961	514.2
Deletion sequences	Des-SerCam	11.052	7.970	1.045	1337.5
			8.661	1.135	1337.4
Incomplete Fmoc removal	Fmoc-TFTSDVSCamFK-NH_2	26.925	14.982	1.964	1310.4
			15.930	2.088	1310.5
	Fmoc-TFTSDVFK-NH_2 Fmoc-FTSDVSCamFK-NH_2	9.228	16.689	2.187	1165.4 1209.4
	Fmoc-TFTSDVFK-NH_2	0.555	17.117	2.243	1165.4
	Fmoc-FTSDVFK-NH_2	2.752	17.465	2.289	1064.3
Epimers	-	2.951	7.112	0.932	741.8
	-		7.280	0.954	741.8
Else	-	8.778	-	-	-

Figure S13. Chromatogram of H-(1-11)-CamFK-NH_2 **2**, solid-phase synthesis in DMSO/EtOAc (1:9), protocol modification A. All peaks between 3 and 18 minutes with Area % ≥ 0.5 were considered.



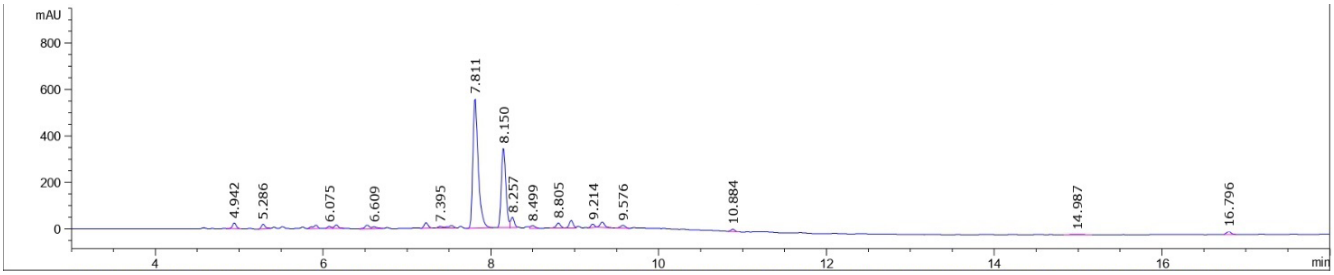
Solid-phase synthesis of H-(1-11)-CamFK-NH_2 2 in DMSO/EtOAc (1:9), protocol modifications A, B, and D

The synthesis was performed on preloaded Fmoc-Lys(Boc)-Rink Amide resin (loading 0.62 mmol/g); all steps were carried out at 25°C unless stated otherwise. After swelling the resin with DMSO/EtOAc (1:9) (10 mL/g of resin, 240 min), the Fmoc protecting group was removed by treatment with a solution of piperidine 20% in DMSO/EtOAc (1:9) (2 times x 5 mL/g of resin, 15 min each) and with DMSO/EtOAc (1:9) (4 times x 5 mL/g of resin, 5 min each). Fmoc-Phe-OH and Oxyma Pure® (2.0 eq of each reagent with respect to the loading of the resin) were dissolved in DMSO/EtOAc (1:9) (5 mL/g of resin) and pre-activated with DIC (2.0 eq with respect to the loading of the resin) for 3 minutes. The coupling mixture was added to the resin and stirred for 90 minutes, followed by washes with DMSO/EtOAc (1:9) (3 times x 5 mL/g of resin, 5 min each). The steps of Fmoc-removal, washes and coupling were repeated until the target peptide sequence was achieved. Piperidine 20% in DMSO/EtOAc (1:9) was employed up to Phe^B included, while after Ser^{11} Fmoc-removal was performed with piperidine 10% in DMSO/EtOAc (1:9) + piperidine 5% in DMSO/EtOAc (1:9) (5 mL/g of resin, 15 min each); finally, from Thr^5 (included) onwards piperidine 20% in DMSO/EtOAc (1:9) was used at 40°C. For each coupling, the selected Fmoc-AAx-OH, Oxyma Pure® and DIC (2.0 eq of each reagent with respect to the loading of the resin) were employed. The carboxyamidomethyl (Cam) ester was formed by coupling bromoacetic acid, Oxyma Pure® and DIC (respectively 2.0, 0.1, and 2.0 eq of the reagents with respect to the loading of the resin) onto the resin according to the protocol described above for 180 minutes. Subsequently, the Ser^{11} residue was introduced by adding KI (1.0 eq with respect to the loading of the resin) directly to the reaction vessel, followed by a solution of Fmoc-Ser(tBu)-OH and DIPEA (4.0 eq with respect to the loading of the resin) in DMSO/EtOAc (1:9) (5 mL/g of resin). This coupling step was performed for 24 hours. Boc-His(Trt)-OH was used to introduced His^1 following the protocol described for Fmoc-amino acid residues. After the final coupling step, the resin was washed with DMSO/EtOAc (1:9) (3 times x 5 mL/g of resin, 5 min each), DCM (3 times x 5 mL/g of resin, 5 min each), and dried under vacuum for 12 hours. For cleavage, the resin was added gradually to a solution of TFA/TIS/H_2O 92.5/5.0/2.5 v/v/v % (10 mL/g of resin) at 10°C, the suspension was the stirred for 3 hours at room temperature. The resin was filtered, and diisopropyl ether (30 mL/g of resin) was added to the filtered solution at 10°C under vigorous stirring. The precipitated peptide was filtered, washed with ether (3 times x 5 mL/g of resin), and dried under vacuum to obtain **2**. The crude was analyzed via HPLC-MS using Method 1 (see Analytical details section reported above).

Table S9. Results for solid-phase synthesis of H-(1-11)-CamFK-NH_2 **2** in DMSO/EtOAc (1:9), protocol modifications A, B, and D.

Species	Peptide sequence	Area %	t_R (min)	rrt	m/z obs
Product	H-HAEGTFTSDVSCamFK-NH_2	50.945	7.811	1.000	1482.5
Ester hydrolysis	HO-GFK-NH_2	1.421	4.942	0.633	351.2
	H-HAEGTFTSDVS-OH	0.576	6.075	0.778	1150.3
N,O-shifts	H-HAEGT-OH	1.194	7.333	0.961	514.2
Deletion sequences	Des-SerCam Des-Thr	25.826	8.150	1.043	1337.5 1381.5
Incomplete Fmoc removal	Fmoc-TFTSDVFK-NH_2	0.966	16.796	2.15	1165.4
Epimers	-	2.112	7.227	0.925	742
	-		7.395	0.947	741.8
Else	-	16.960	-	-	-

Figure S14. Chromatogram of H-(1-11)-CamFK-NH_2 **2**, solid-phase synthesis in DMSO/EtOAc (1:9), protocol modifications A, B, and D. All peaks between 3 and 18 minutes with Area % ≥ 0.5 were considered.



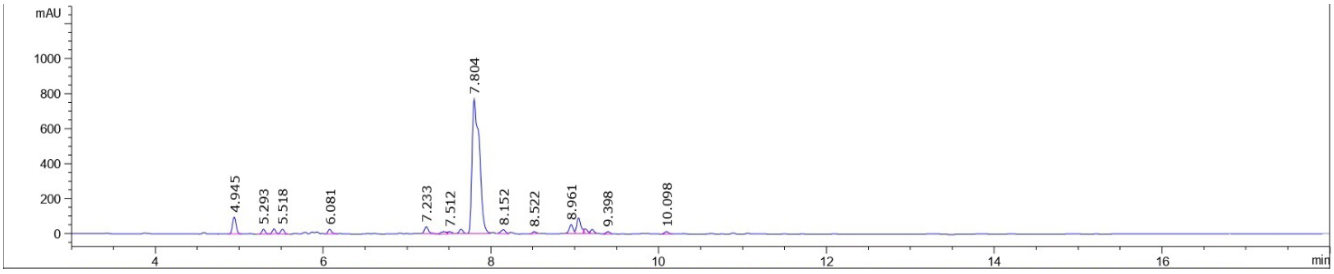
Solid-phase synthesis of H-(1-11)-CamFK-NH_2 2 in NBP/EtOAc (8:2), protocol modifications A and B

The synthesis was performed on preloaded Fmoc-Lys(Boc)-Rink Amide resin (loading 0.62 mmol/g); all steps were carried out at 25°C unless stated otherwise. After swelling the resin with NBP/EtOAc (8:2) (10 mL/g of resin, 240 min), the Fmoc protecting group was removed by treatment with a solution of piperidine 20% in NBP/EtOAc (8:2) (2 times x 5 mL/g of resin, 15 min each) and with NBP/EtOAc (8:2) (4 times x 5 mL/g of resin, 5 min each). Fmoc-Phe-OH and Oxyma Pure® (2.0 eq of each reagent with respect to the loading of the resin) were dissolved in NBP/EtOAc (8:2) (5 mL/g of resin) and pre-activated with DIC (2.0 eq with respect to the loading of the resin) for 3 minutes. The coupling mixture was added to the resin and stirred for 90 minutes, followed by washes with NBP/EtOAc (8:2) (3 times x 5 mL/g of resin, 5 min each). The steps of Fmoc-removal, washes and coupling were repeated until the target peptide sequence was achieved. Piperidine 20% in NBP/EtOAc (8:2) was employed up to Phe^B included, then from Ser^{11} Fmoc-removal was performed with piperidine 10% in NBP/EtOAc (8:2) + piperidine 5% in NBP/EtOAc (8:2) (5 mL/g of resin, 15 min each); finally, from Thr^5 (included) onwards piperidine 20% in NBP/EtOAc (8:2) was used at 40°C. For each coupling, the selected Fmoc-AAx-OH, Oxyma Pure® and DIC (2.0 eq of each reagent with respect to the loading of the resin) were employed. The carboxyamidomethyl (Cam) ester was formed by coupling bromoacetic acid, Oxyma Pure® and DIC (respectively 2.0, 0.1, and 2.0 eq of the reagents with respect to the loading of the resin) onto the resin according to the protocol described above. Subsequently, the Ser^{11} residue was introduced by adding KI (1.0 eq with respect to the loading of the resin) directly to the reaction vessel, followed by a solution of Fmoc-Ser(tBu)-OH and DIPEA (4.0 eq with respect to the loading of the resin) in NBP/EtOAc (8:2) (5 mL/g of resin). This coupling step was performed for 24 hours. Boc-His(Trt)-OH was used to introduced His^1 following the protocol described for Fmoc-amino acid residues. After the final coupling step, the resin was washed with NBP/EtOAc (8:2) (3 times x 5 mL/g of resin, 5 min each), DCM (3 times x 5 mL/g of resin, 5 min each), and dried under vacuum for 12 hours. For cleavage, the resin was added gradually to a solution of TFA/TIS/H_2O 92.5/5.0/2.5 v/v/v % (10 mL/g of resin) at 10°C, the suspension was the stirred for 3 hours at room temperature. The resin was filtered, and diisopropyl ether (30 mL/g of resin) was added to the filtered solution at 10°C under vigorous stirring. The precipitated peptide was filtered, washed with ether (3 times x 5 mL/g of resin), and dried under vacuum to obtain **2**. The crude was analyzed via HPLC-MS using Method 1 (see Analytical details section reported above).

Table S10. Results for solid-phase synthesis of H-(1-11)-CamFK-NH_2 **2** in NBP/EtOAc (8:2), protocol modifications A and B.

Species	Peptide sequence	Area %	t_R (min)	rrt	m/z obs
Product	H-HAEGTFTSDVSCamFK-NH_2	72.439	7.804	1	1482.5
Ester hydrolysis	HO-GFK-NH_2	4.484	4.945	0.634	351.2
	H-HAEGTFTSDVS-OH	1.222	6.081	0.779	1150.3
N,O-shifts	H-HAEGT-OH	1.579	7.443	0.927	514.2
Epimers	-	-	7.512	0.963	514.4
Else	-	18.019	-	-	-

Figure S15. Chromatogram of H-(1-11)-CamFK-NH_2 **2**, solid-phase synthesis in NBP/DMC (8:2), protocol modifications A and B. All peaks between 3 and 18 minutes with Area % ≥ 0.5 were considered.



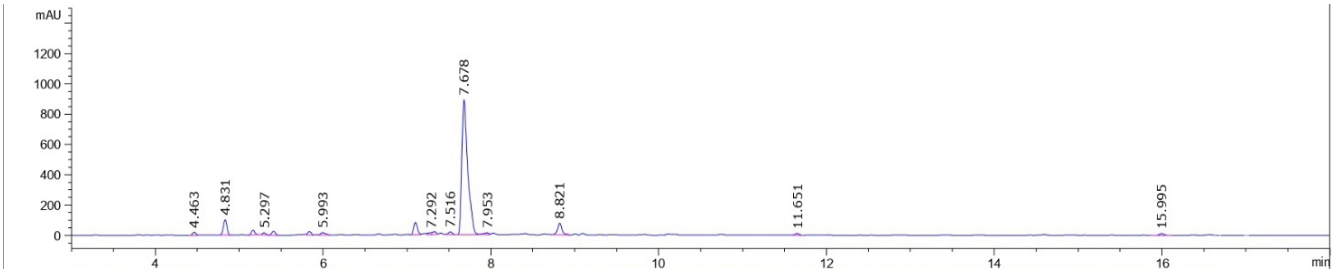
Solid-phase synthesis of H-(1-11)-CamFK-NH_2 2 in NBP/DMC (8:2), protocol modifications A and B

The synthesis was performed on preloaded Fmoc-Lys(Boc)-Rink Amide resin (loading 0.62 mmol/g); all steps were carried out at 25°C unless stated otherwise. After swelling the resin with NBP/DMC (8:2) (10 mL/g of resin, 240 min), the Fmoc protecting group was removed by treatment with a solution of piperidine 20% in NBP/DMC (8:2) (2 times x 5 mL/g of resin, 15 min each) and with NBP/DMC (8:2) (4 times x 5 mL/g of resin, 5 min each). Fmoc-Phe-OH and Oxyma Pure® (2.0 eq of each reagent with respect to the loading of the resin) were dissolved in NBP/DMC (8:2) (5 mL/g of resin) and pre-activated with DIC (2.0 eq with respect to the loading of the resin) for 3 minutes. The coupling mixture was added to the resin and stirred for 90 minutes, followed by washes with NBP/DMC (8:2) (3 times x 5 mL/g of resin, 5 min each). The steps of Fmoc-removal, washes and coupling were repeated until the target peptide sequence was achieved. Piperidine 20% in NBP/DMC (8:2) was employed up to Phe^{B} included, then from Ser^{11} Fmoc-removal was performed with piperidine 10% in NBP/DMC (8:2) + piperidine 5% in NBP/DMC (8:2) (5 mL/g of resin, 15 min each); finally, from Thr^{5} (included) onwards piperidine 20% in NBP/DMC (8:2) was used at 40°C. For each coupling, the selected Fmoc-AAx-OH, Oxyma Pure® and DIC (2.0 eq of each reagent with respect to the loading of the resin) were employed. The carboxyamidomethyl (Cam) ester was formed by coupling bromoacetic acid, Oxyma Pure® and DIC (respectively 2.0, 0.1, and 2.0 eq of the reagents with respect to the loading of the resin) onto the resin according to the protocol described above. Subsequently, the Ser^{11} residue was introduced by adding KI (1.0 eq with respect to the loading of the resin) directly to the reaction vessel, followed by a solution of Fmoc-Ser(tBu)-OH and DIPEA (4.0 eq with respect to the loading of the resin) in NBP/DMC (8:2) (5 mL/g of resin). This coupling step was performed for 24 hours. Boc-His(Trt)-OH was used to introduced His^{1} following the protocol described for Fmoc-amino acid residues. After the final coupling step, the resin was washed with NBP/DMC (8:2) (3 times x 5 mL/g of resin, 5 min each), DCM (3 times x 5 mL/g of resin, 5 min each), and dried under vacuum for 12 hours. For cleavage, the resin was added gradually to a solution of TFA/TIS/H_2O 92.5/5.0/2.5 v/v/v % (10 mL/g of resin) at 10°C, the suspension was the stirred for 3 hours at room temperature. The resin was filtered, and diisopropyl ether (30 mL/g of resin) was added to the filtered solution at 10°C under vigorous stirring. The precipitated peptide was filtered, washed with ether (3 times x 5 mL/g of resin), and dried under vacuum to obtain **2**. The crude was analyzed via HPLC-MS using Method 1 (see Analytical details section reported above).

Table S11. Results for solid-phase synthesis of H-(1-11)-CamFK-NH_2 **2** in NBP/DMC (8:2), protocol modifications A and B.

Species	Peptide sequence	Area %	t_R (min)	rrt	m/z obs
Product	H-HAEGTFTSDVSCamFK-NH_2	74.534	7.678	1	1482.4
Ester hydrolysis	HO-GFK-NH_2	5.058	4.831	0.629	351.2
N,O-shifts	H-SCamFK-NH_2	1.037	4.463	0.581	438.2
	H-HAEGT-OH	0.956	7.326	0.954	514.2
Deletion sequences	Fmoc-TFTSDVSCamFK-NH_2	0.821	15.995	2.083	1310.3
Epimers	-	4.981	7.100	0.925	741.9
	-		7.292	0.95	741.9
Else	-	12.613	-	-	-

Figure S16. Chromatogram of H-(1-11)-CamFK-NH_2 **2**, solid-phase synthesis in NBP/DMC (8:2), protocol modifications A and B. All peaks between 3 and 18 minutes with Area % ≥ 0.5 were considered.



Solid-phase synthesis of H-(12-31)-OH 3 in DMF

The synthesis was performed on preloaded Fmoc-Gly-MBH resin (loading 0.5 mmol/g); steps were carried out at 25°C up to Glu^{21} included, and 40°C from Ala^{19} onwards, unless stated otherwise. After swelling the resin with DMF (10 mL/g of resin, 30 minutes), the Fmoc protecting group was removed by treatment with a solution of piperidine 20% in DMF (2 times x 5 mL/g of resin, 10 minutes each) and washed with DMF (4 times x 5 mL/g of resin, 5 minutes each). Fmoc-Arg(Pbf)-OH and Oxyma Pure® (2.0 eq of each reagent with respect to the loading of the resin) were dissolved in DMF (5 mL/g of resin) and pre-activated with DIC (2.0 eq with respect to the loading of the resin) for 3 minutes. The coupling mixture was added to the resin and stirred for 90 minutes, followed by washes with DMF (3 times x 5 mL/g of resin, 5 minutes each). The steps of Fmoc-removal, washing and coupling were repeated until the target peptide sequence was achieved. For each coupling, the selected Fmoc-AAx-OH, Oxyma Pure® and DIC were employed; in some cases, the equivalents, duration of the coupling, double coupling and temperature were modified. For Arg^{28}, Val^{27}, Trp^{25}, Ala^{24}, Phe^{22}, Ala^{19}, and Gln^{17} 3.0 eq of each reagent with respect to the loading of the resin were employed. For Leu^{26}, Ile^{23}, and Glu^{21} the coupling time was shortened to 60 minutes and a double coupling was performed for 45 minutes. For Glu^{15} a double coupling (1.0 eq of each reagent with respect to the loading of the resin) of 60 minutes was performed; while for Ser^{12} the coupling time was shortened to 60 minutes and a double coupling (1.0 eq of each reagent with respect to the loading of the resin) of 45 minutes was added. For $Lys(GluPal)^{20}$ the coupling time was extended to 24 hours at 50°C. Details for each coupling step are summarized in Table S12. After the final coupling step, the resin was washed with DMF (3 times x 5 mL/g of resin, 5 minutes each), DCM (3 times x 5 mL/g of resin, 5 minutes each), and dried under vacuum for 12 hours. For cleavage, the resin was added gradually to a solution of TFA/TIS/H_2O/DTT 85.0/5.0/5.0/5.0 v/v/v/v % (10 mL/g of resin) at 10°C, the suspension was the stirred for 3 hours at room temperature. The resin was filtered, and diisopropyl ether (30 mL/g of resin) was added to the filtered solution at 10°C under vigorous stirring. The precipitated peptide was filtered, washed with ether (3 times x 5 mL/g of resin), and dried under vacuum to obtain **3**. The crude was analyzed via HPLC-MS using Method 2 (see Analytical details section reported above).

Table S12. Synthetic steps for H-(12-31)-OH **3** in DMF.

Coupling	Amino acid	Eq	Time (min)	T (°C)
30	Fmoc-Arg(Pbf)-OH	2	90	25
29	Fmoc-Gly-OH	2	90	25
28	Fmoc-Arg(Pbf)-OH	3	90	25
27	Fmoc-Val-OH	3	90	25
26	Fmoc-Leu-OH	2+2	60+45	25
25	Fmoc-Trp(Boc)-OH	3	90	25
24	Fmoc-Ala-OH	3	90	25
23	Fmoc-Ile-OH	2+2	60+45	25
22	Fmoc-Phe-OH	3	90	25
21	Fmoc-Glu(OtBu)-OH	2+2	60+45	25
20	Fmoc-Lys(γ-Glu(OtBu)Pal)-OH	2	24h	50
19	Fmoc-Ala-OH	3	90	40
18	Fmoc-Ala-OH	2	90	40
17	Fmoc-Gln(Trt)-OH	3	90	40
16	Fmoc-Gly-OH	2	90	40
15	Fmoc-Glu(OtBu)-OH	2+1	90+60	40
14	Fmoc-Leu-OH	2	90	40
13	Fmoc-Tyr(tBu)-OH	2	90	40
12	Fmoc-Ser(tBu)-OH	2+1	60+45	40

Table S13. Results for solid-phase synthesis of H-(12-31)-OH **3** in DMF.

Species		Area %	t_R (min)	rrt	m/z obs
Product	H-(12-31)-OH	65.296	20.466	1.000	1310.1
Deletion sequences	Des-Glu-LysGluPal	1.629	8.905	0.435	997.5
	Des-LysGluPal	1.906	9.909	0.484	1062.2
	Des-Leu(Ile)	2.161	14.935	0.730	1253.7
			20.258	0.990	1253.2
	Des-Phe	0.868	15.179	0.742	1236.3
	Des-Trp	1.124	15.636	0.764	1216.7
	Des-Ala	5.226	16.100	0.787	1274.7
			22.888	1.118	1274.1
	Des-Tyr Des-Glu Des-Gly	8.993	21.786	1.065	1228.3 1245.3 1281.8
	Des-Gln	2.758	27.949	1.366	1245.9
	Des-Arg	2.514	28.806	1.408	1231.7
	Des-Gly	1.588	32.030	1.565	1281.3
	Total	28.768	-	-	-
Epimers		1.085	20.085	0.981	1309.8
Else	-	4.851	-	-	-

Figure S17. Chromatogram of H-(12-31)-OH **3**, solid-phase synthesis in DMF. All peaks between 6 and 46 minutes with Area % ≥ 0.5 were considered.

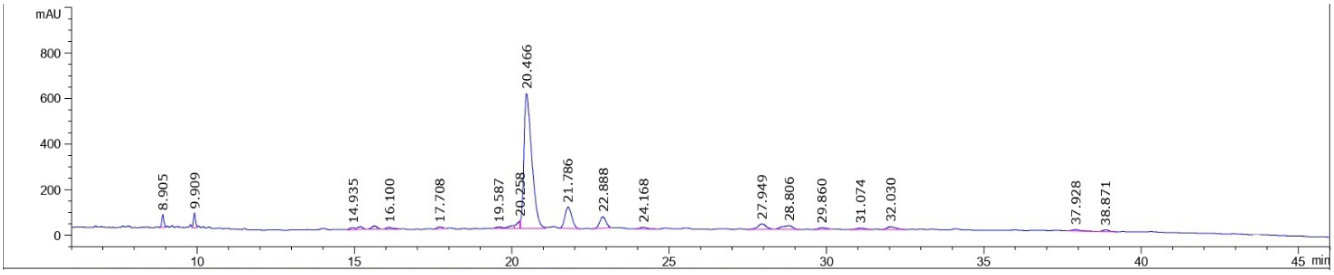


Figure S18. Mass chromatogram for peak at 20.466 min, m/z obs = 1310.1.

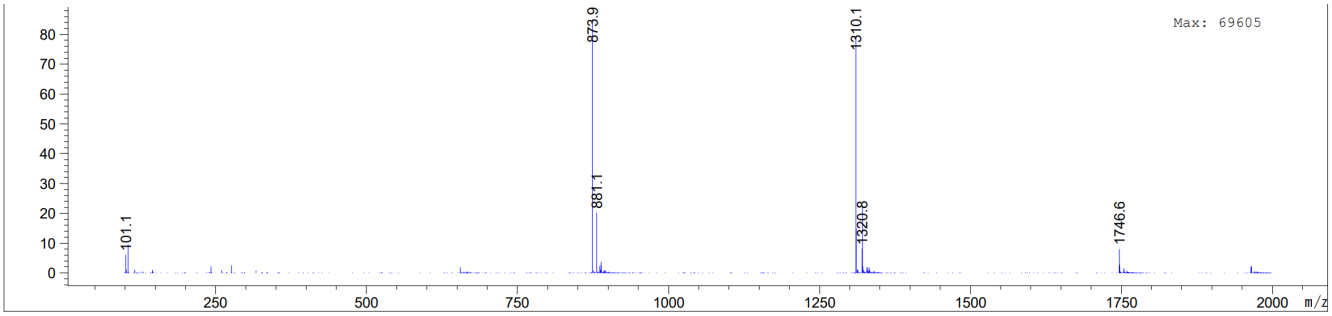


Figure S19. Mass chromatogram for peak at 8.905 min, m/z obs = 997.5. Zoom on mass 997.5.

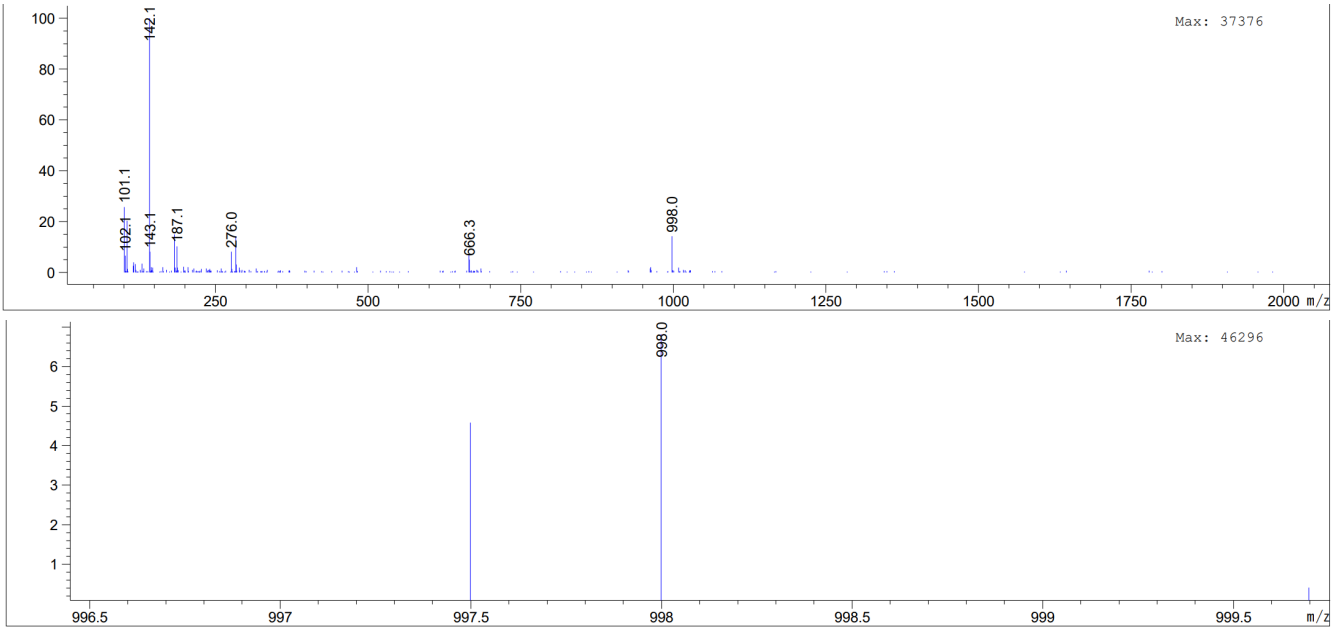


Figure S20. Mass chromatogram for peak at 9.909 min, m/z obs = 1062.2.

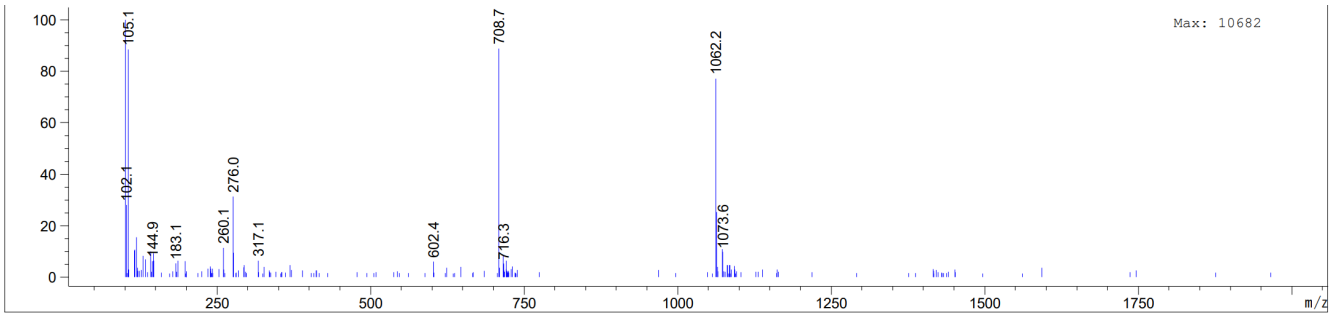


Figure S21. Mass chromatogram for peak at 14.935 min, m/z obs = 1253.7.

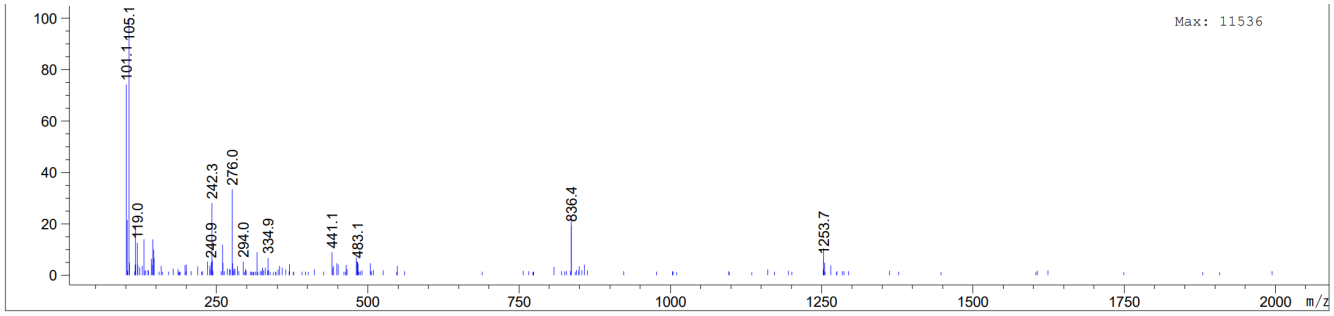


Figure S22. Mass chromatogram for peak at 20.258 min, m/z obs = 1253.2.

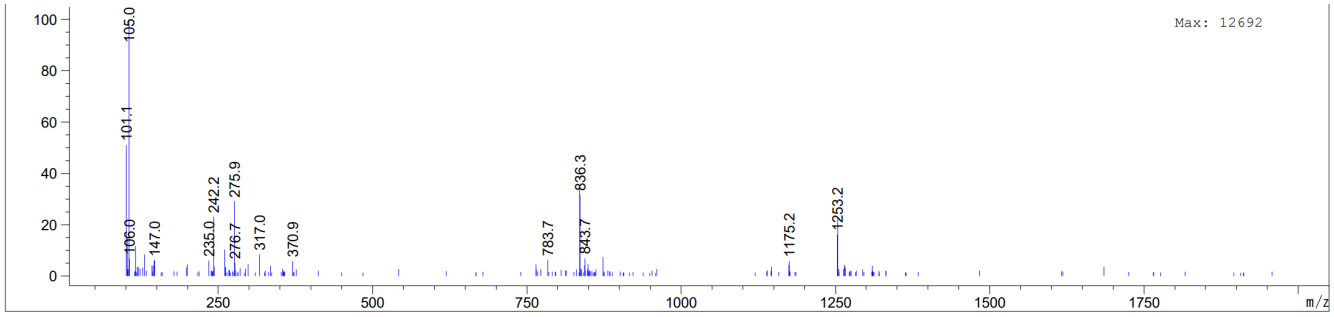


Figure S23. Mass chromatogram for peak at 15.179 min, m/z obs = 1236.3. Zoom on mass 1236.3.

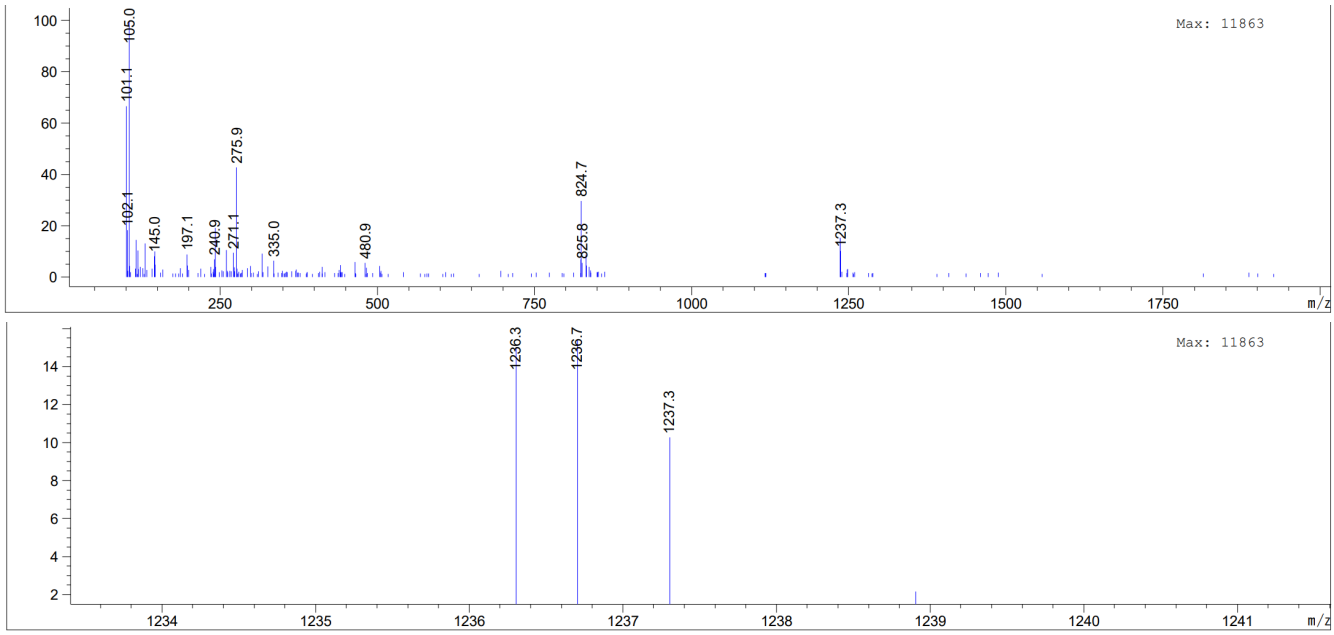


Figure S24. Mass chromatogram for peak at 15.636 min, m/z obs = 1216.7. Zoom on mass 1216.7.

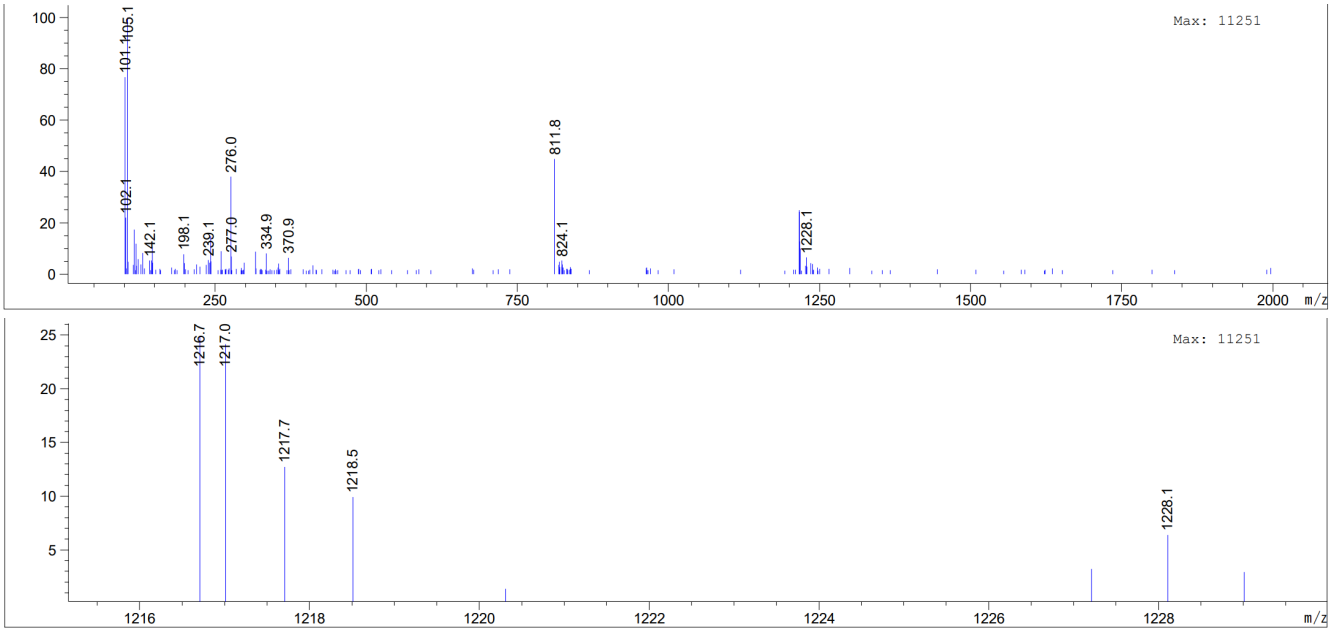


Figure S25. Mass chromatogram for peak at 16.100 min, m/z obs = 1274.1. Zoom on mass 1274.1.

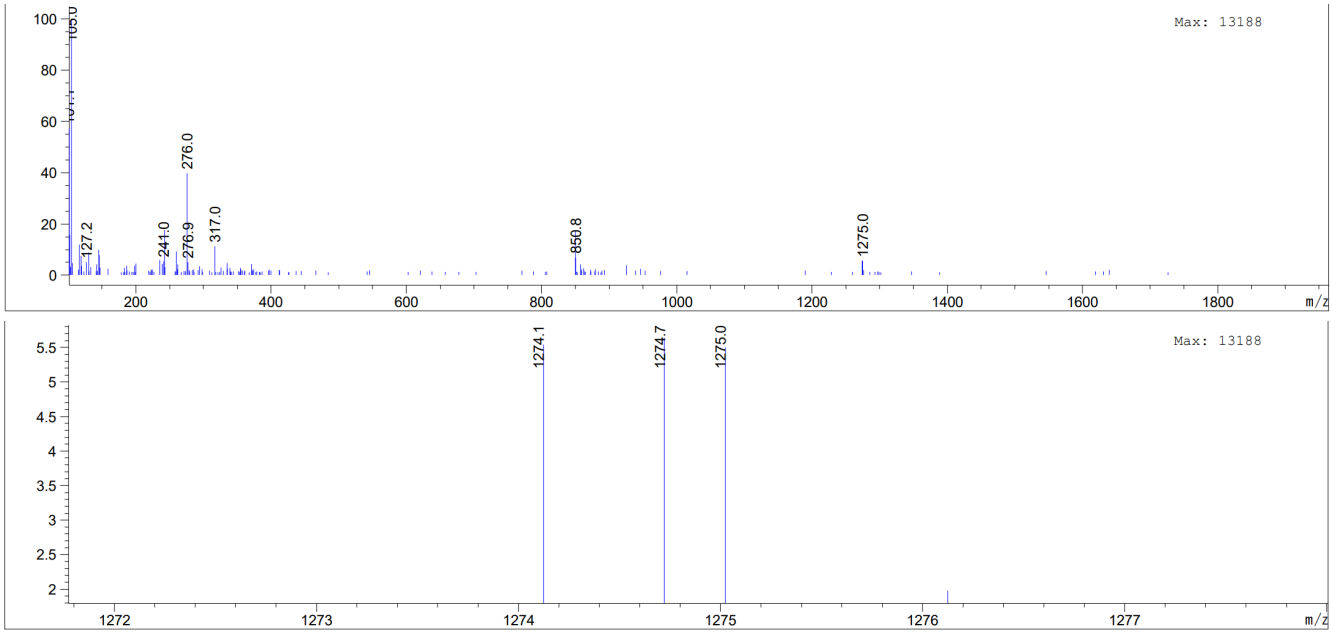


Figure S26. Mass chromatogram for peak at 22.888 min, m/z obs = 1274.1.

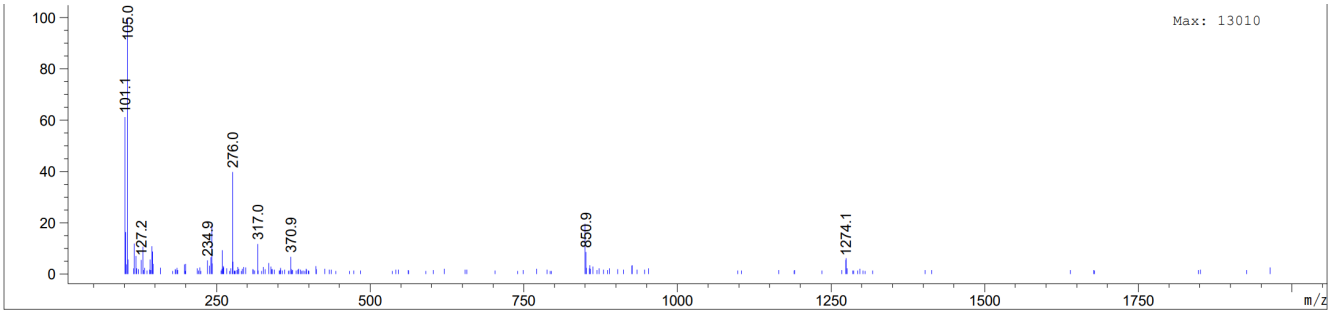


Figure S27. Mass chromatogram for peak at 21.786 min, m/z obs = 1228.3 and 1245.3 and 1281.8. Zoom on masses 1228.3 and 1245.3 and 1281.8.

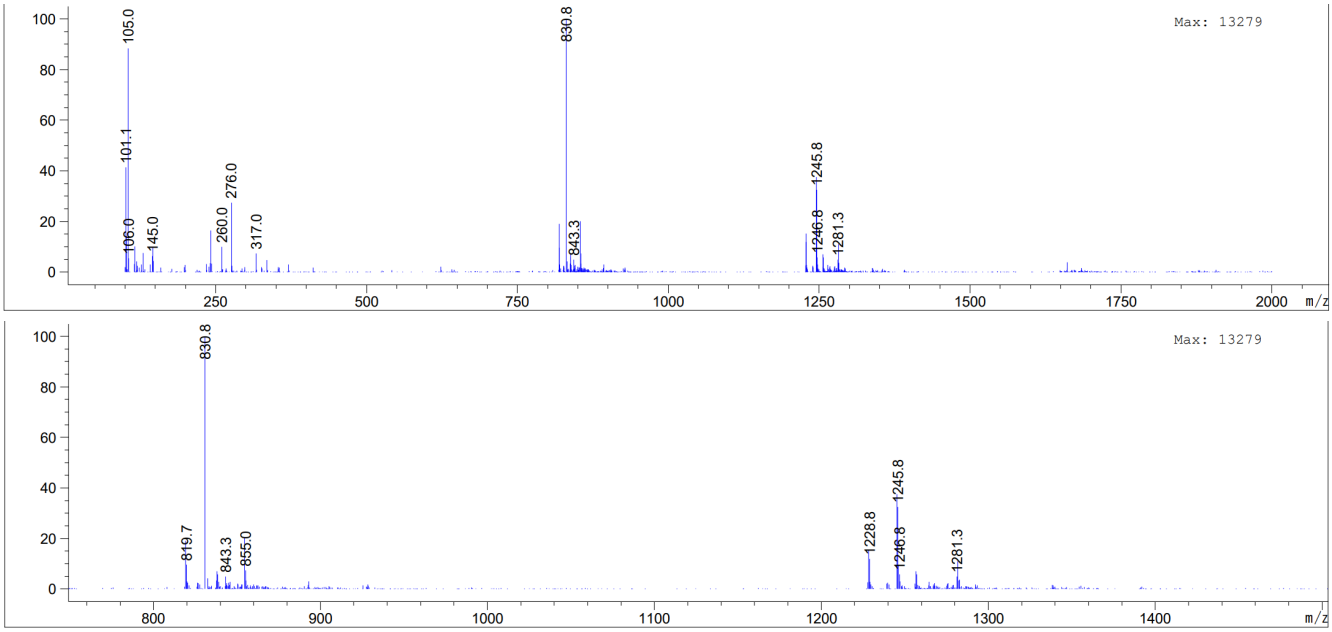


Figure S28. Mass chromatogram for peak at 27.949 min, m/z obs = 1245.9. Zoom on mass 1245.9.

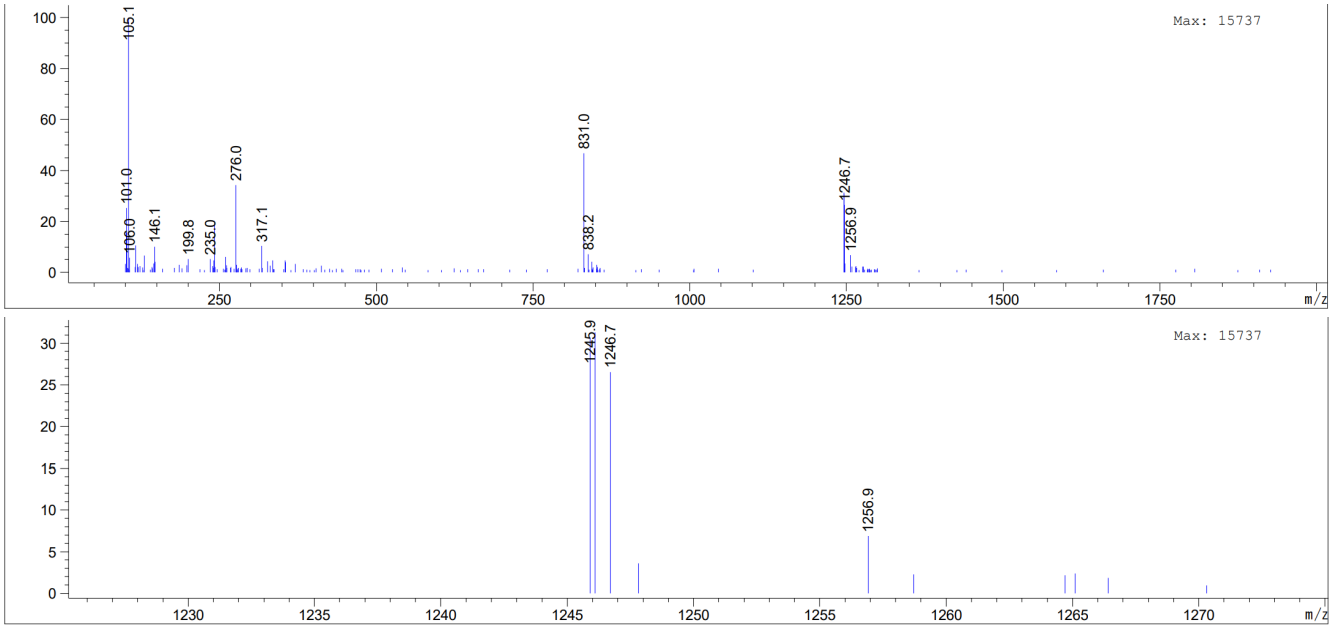


Figure S29. Mass chromatogram for peak at 28.806 min, m/z obs = 1231.7.

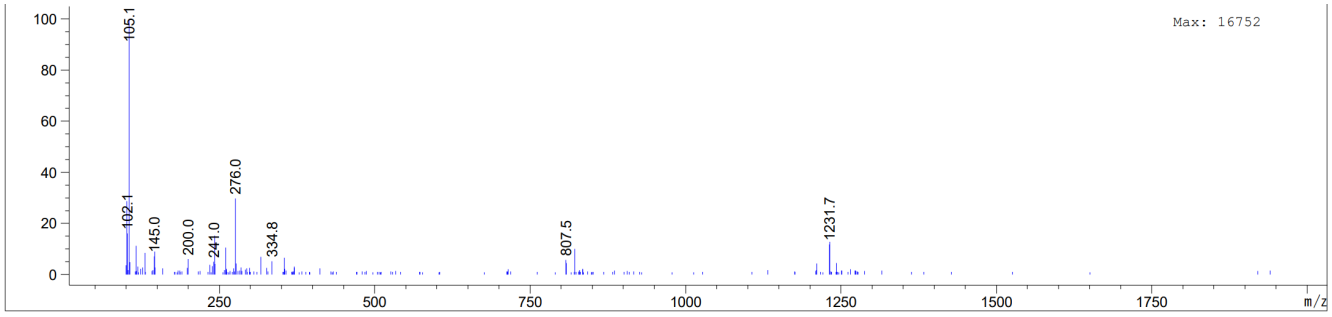


Figure S30. Mass chromatogram for peak at 32.030 min, m/z obs = 1281.3.

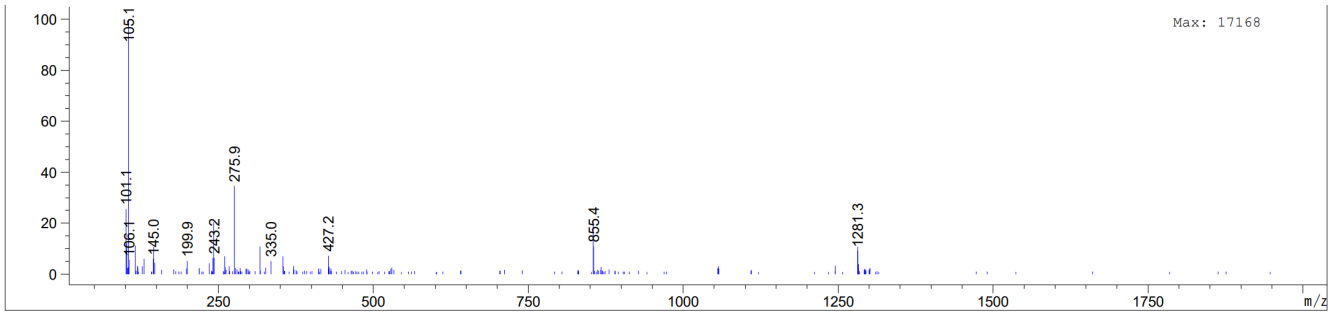
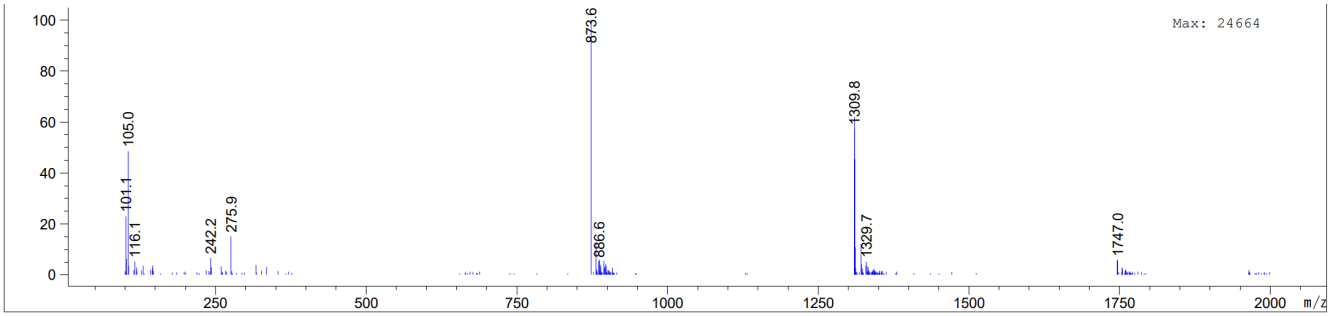


Figure S31. Mass chromatogram for peak at 20.085 min, m/z obs = 1309.8.



Solid-phase synthesis of H-(12-31)-OH 3 in NOP/DMC (8:2), protocol modification A

The synthesis was performed on preloaded Fmoc-Gly-MBH resin (loading 0.5 mmol/g); steps were carried out at 25°C up to Glu^{21} included, and 40°C from Ala^{19} onwards, unless stated otherwise. After swelling the resin with NOP/DMC (8:2) (10 mL/g of resin, 30 minutes), the Fmoc protecting group was removed by treatment with a solution of piperidine 20% in NOP/DMC (8:2) (2 times x 5 mL/g of resin, 15 minutes each) and washed with NOP/DMC (8:2) (4 times x 5 mL/g of resin, 5 minutes each). Fmoc-Arg(Pbf)-OH and Oxyma Pure® (2.0 eq of each reagent with respect to the loading of the resin) were dissolved in NOP/DMC (8:2) (5 mL/g of resin) and pre-activated with DIC (2.0 eq with respect to the loading of the resin) for 3 minutes. The coupling mixture was added to the resin and stirred for 90 minutes, followed by washes with NOP/DMC (8:2) (3 times x 5 mL/g of resin, 5 minutes each). The steps of Fmoc-removal, washing and coupling were repeated until the target peptide sequence was achieved. For each coupling, the selected Fmoc-AAx-OH, Oxyma Pure® and DIC were employed; in some cases, the equivalents, duration of the coupling, double coupling and temperature were modified. For Arg^{28}, Val^{27}, Trp^{25}, Ala^{24}, Phe^{22}, Ala^{19}, and Gln^{17} 3.0 eq of each reagent with respect to the loading of the resin were employed. For Leu^{26}, Ile^{23}, and Glu^{21} the coupling time was shortened to 60 minutes and a double coupling was performed for 45 minutes. For Glu^{15} a double coupling (1.0 eq of each reagent with respect to the loading of the resin) of 60 minutes was performed; while for Ser^{12} the coupling time was shortened to 60 minutes and a double coupling (1.0 eq of each reagent with respect to the loading of the resin) of 45 minutes was added. For $Lys(GluPal)^{20}$ the coupling time was extended to 24 hours at 50°C. Details for each coupling step are summarized in Table S14. After the final coupling step, the resin was washed with NOP/DMC (8:2) (3 times x 5 mL/g of resin, 5 minutes each), DCM (3 times x 5 mL/g of resin, 5 minutes each), and dried under vacuum for 12 hours. For cleavage, the resin was added gradually to a solution of TFA/TIS/H_2O/DTT 85.0/5.0/5.0/5.0 v/v/v/v % (10 mL/g of resin) at 10°C, the suspension was the stirred for 3 hours at room temperature. The resin was filtered, and diisopropyl ether (30 mL/g of resin) was added to the filtered solution at 10°C under vigorous stirring. The precipitated peptide was filtered, washed with ether (3 times x 5 mL/g of resin), and dried under vacuum to obtain **3**. The crude was analyzed via HPLC-MS using Method 2 (see Analytical details section reported above).

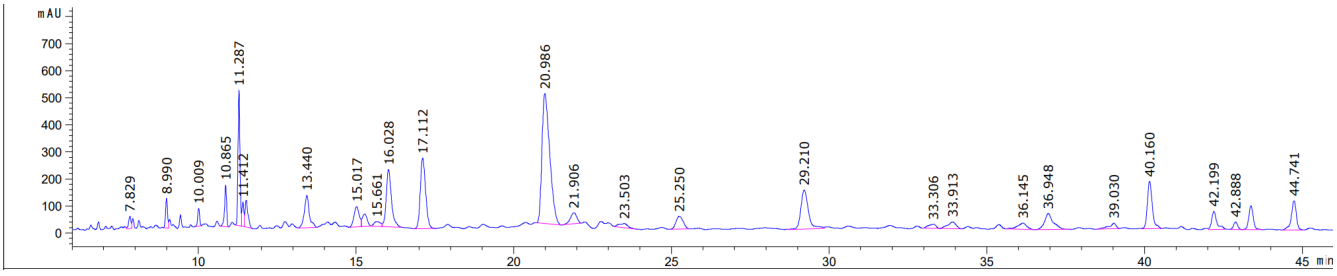
Table S14. Synthetic steps for H-(12-31)-OH **3** in NOP/DMC (8:2), protocol modification A.

Coupling	Amino acid	Eq	Time (min)	T (°C)
30	Fmoc-Arg(Pbf)-OH	2	90	25
29	Fmoc-Gly-OH	2	90	25
28	Fmoc-Arg(Pbf)-OH	3	90	25
27	Fmoc-Val-OH	3	90	25
26	Fmoc-Leu-OH	2+2	60+45	25
25	Fmoc-Trp(Boc)-OH	3	90	25
24	Fmoc-Ala-OH	3	90	25
23	Fmoc-Ile-OH	2+2	60+45	25
22	Fmoc-Phe-OH	3	90	25
21	Fmoc-Glu(OtBu)-OH	2+2	60+45	25
20	Fmoc-Lys(γ-Glu(OtBu)Pal)-OH	2	24h	50
19	Fmoc-Ala-OH	3	90	40
18	Fmoc-Ala-OH	2	90	40
17	Fmoc-Gln(Trt)-OH	3	90	40
16	Fmoc-Gly-OH	2	90	40
15	Fmoc-Glu(OtBu)-OH	2+1	90+60	40
14	Fmoc-Leu-OH	2	90	40
13	Fmoc-Tyr(tBu)-OH	2	90	40
12	Fmoc-Ser(tBu)-OH	2+1	60+45	40

Table S15. Results for solid-phase synthesis of H-(12-31)-OH **3** in NOP/DMC (8:2), protocol modification A.

Species		Area %	t_R (min)	rrt	m/z obs
Product	Lira(12-31)	24.868	20.986	1	1310.1
	+tBu	0.849	23.503	1.120	1337.4
Deletion sequences	Des-Glu-LysGluPal	1.431	8.990	0.428	997.7
	Des-LysGluPal	0.877	10.009	0.477	1062.5
	Des-Leu(Ile)	2.606	15.017	0.716	1253.3
	Des-Phe	1.485	15.273	0.728	1236.4
	Des-Gln-Phe	0.774	15.661	0.746	1172.2
	Des-Tyr Des-Glu	1.816	21.906	1.044	1228.4 1245.3
	Des-Ala	2.277	25.250	1.203	1273.7
	Des-Arg Des-Gln	7.191	29.210	1.392	1231.7 1246.2
	Des-Arg-Arg	3.438	36.948	36.948	1153.7
	Des-Gln-Lys(GluPal)	2.281	42.199	2.011	998.8
	Des-Gln-Ala	0.740	42.888	2.044	1210.1
	Total	24.916	-	-	-
N,O-shift	H-23-31-OH	0.960	39.030	1.860	1027.3
Else	-	48.406	-	-	-

Figure S32. Chromatogram of H-(12-31)-OH **3**, solid-phase synthesis in NOP/DMC (8:2), protocol modification A. All peaks between 6 and 46 minutes with Area % ≥ 0.5 were considered.



Solid-phase synthesis of H-(12-31)-OH 3 in NOP/DMC (8:2), protocol modifications A and E

The synthesis was performed on preloaded Fmoc-Gly-MBH resin (loading 0.5 mmol/g); all steps were carried out at 40°C. After swelling the resin with NOP/DMC (8:2) (10 mL/g of resin, 240 minutes), the Fmoc protecting group was removed by treatment with a solution of piperidine 20% in NOP/DMC (8:2) (2 times x 5 mL/g of resin, 15 minutes each) and washed with NOP/DMC (8:2) (4 times x 5 mL/g of resin, 5 minutes each). Fmoc-Arg(Pbf)-OH and Oxyma Pure® (2.0 eq of each reagent with respect to the loading of the resin) were dissolved in NOP/DMC (8:2) (5 mL/g of resin) and pre-activated with DIC (2.0 eq with respect to the loading of the resin) for 3 minutes. The coupling mixture was added to the resin and stirred for 90 minutes, followed by washes with NOP/DMC (8:2) (3 times x 5 mL/g of resin, 5 minutes each). The steps of Fmoc-removal, washing and coupling were repeated until the target peptide sequence was achieved. For each coupling, the selected Fmoc-AAx-OH, Oxyma Pure® and DIC were employed; in some cases, the equivalents, duration of the coupling, and double coupling were modified. For Arg^{28}, Val^{27}, Trp^{25}, Ala^{24}, Phe^{22}, Ala^{19}, and Gln^{17} 3.0 eq of each reagent with respect to the loading of the resin were employed. For Leu^{26}, Ile^{23}, and Glu^{21} the coupling time was shortened to 60 minutes and a double coupling was performed for 45 minutes. For Glu^{15} a double coupling (1.0 eq of each reagent with respect to the loading of the resin) of 60 minutes was performed; while for Ser^{12} the coupling time was shortened to 60 minutes and a double coupling (1.0 eq of each reagent with respect to the loading of the resin) of 45 minutes was added. For $Lys(GluPal)^{20}$ the coupling time was extended to 24 hours. Details for each coupling step are summarized in Table S16. After the final coupling step, the resin was washed with NOP/DMC (8:2) (3 times x 5 mL/g of resin, 5 min each), DCM (3 times x 5 mL/g of resin, 5 min each), and dried under vacuum for 12 hours. For cleavage, the resin was added gradually to a solution of TFA/TIS/H_2O/DTT 85.0/5.0/5.0/5.0 v/v/v/v % (10 mL/g of resin) at 10°C, the suspension was then stirred for 3 hours at room temperature. The resin was filtered, and diisopropyl ether (30 mL/g of resin) was added to the filtered solution at 10°C under vigorous stirring. The precipitated peptide was filtered, washed with ether (3 times x 5 mL/g of resin), and dried under vacuum to obtain **3**. The crude was analyzed via HPLC-MS using Method 2 (see Analytical details section reported above).

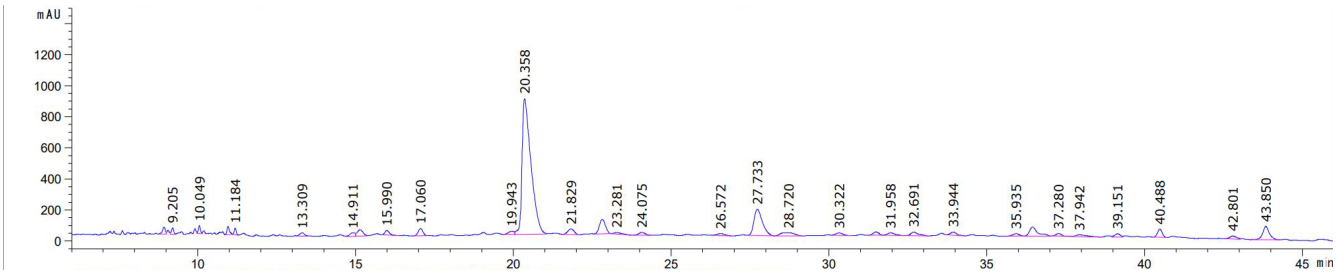
Table S16. Synthetic steps for H-(12-31)-OH **3** in NOP/DMC (8:2), protocol modifications A and E.

Coupling	Amino acid	Eq	Time (min)	T (°C)
30	Fmoc-Arg(Pbf)-OH	2	90	40
29	Fmoc-Gly-OH	2	90	40
28	Fmoc-Arg(Pbf)-OH	3	90	40
27	Fmoc-Val-OH	3	90	40
26	Fmoc-Leu-OH	2+2	60+45	40
25	Fmoc-Trp(Boc)-OH	3	90	40
24	Fmoc-Ala-OH	3	90	40
23	Fmoc-Ile-OH	2+2	60+45	40
22	Fmoc-Phe-OH	3	90	40
21	Fmoc-Glu(OtBu)-OH	2+2	60+45	40
20	Fmoc-Lys(γ-Glu(OtBu)Pal)-OH	2	24h	40
19	Fmoc-Ala-OH	3	90	40
18	Fmoc-Ala-OH	2	90	40
17	Fmoc-Gln(Trt)-OH	3	90	40
16	Fmoc-Gly-OH	2	90	40
15	Fmoc-Glu(OtBu)-OH	2+1	90+60	40
14	Fmoc-Leu-OH	2	90	40
13	Fmoc-Tyr(tBu)-OH	2	90	40
12	Fmoc-Ser(tBu)-OH	2+1	60+45	40

Table S17. Results for solid-phase synthesis of H-(12-31)-OH **3** in NOP/DMC (8:2), protocol modifications A and E.

Species		Area %	t_R (min)	rrt	m/z obs
Product	H-(12-31)-OH	53.349	20.358	1.000	1310.1
	+tBu	0.557	23.281	1.144	1337.9
	+Pbf	0.772	33.944	1.667	1435.7
Deletion sequences	Des-Glu-LysGluPal	0.914	8.928	0.439	997.7
	Des-Leu(Ile)	0.802	14.911	0.732	1253.3
	Des-Phe	1.630	15.145	0.744	1236.6
	Des-Ala	0.981	15.990	0.785	1274.6
	Des-Tyr	1.573	21.829	1.072	1228.8
	Des-Glu				1245.3
	Des-Gly				1281.2
	Des-Ala	4.691	22.817	1.121	1274.3
			31.495	1.547	1274.1
	Des-Gln	9.752	27.733	1.362	1246.2
	Des-Arg	1.886	28.720	1.411	1231.8
	Des-Gly-Arg	0.732	30.322	1.489	1203.2
	Des-Gln-Arg	3.458	36.456	1.791	1167.9
	Total	26.419	-	-	-
Epimers	-	0.844	19.943	0.980	1310.2
Else	-	18.060	-	-	-

Figure S33. Chromatogram of H-(12-31)-OH **3**, solid-phase synthesis in NOP/DMC (8:2), protocol modifications A and E. All peaks between 6 and 46 minutes with Area % ≥ 0.5 were considered.



Solid-phase synthesis of H-(12-31)-OH 3 in DMSO/EtOAc (1:9), protocol modifications A and F

The synthesis was performed on preloaded Fmoc-Gly-MBH resin (loading 0.5 mmol/g); steps were carried out at 25°C up to Arg^{28} included, and at 40°C from Val^{27} onwards. After swelling the resin with DMSO/EtOAc (1:9) (10 mL/g of resin, 120 minutes), the Fmoc protecting group was removed by treatment with a solution of piperidine 20% in DMSO/EtOAc (1:9) (2 times x 5 mL/g of resin, 15 minutes each) and washed with DMSO/EtOAc (1:9) (4 times x 5 mL/g of resin, 5 minutes each). Fmoc-Arg(Pbf)-OH and Oxyma Pure® (2.0 eq of each reagent with respect to the loading of the resin) were dissolved in DMSO/EtOAc (1:9) (5 mL/g of resin) and pre-activated with DIC (2.0 eq with respect to the loading of the resin) for 3 minutes. The coupling mixture was added to the resin and stirred for 90 minutes, followed by washes with DMSO/EtOAc (1:9) (3 times x 5 mL/g of resin, 5 minutes each). The steps of Fmoc-removal, washing and coupling were repeated until the target peptide sequence was achieved. For each coupling, the selected Fmoc-AAx-OH, Oxyma Pure® and DIC were employed; in some cases, the equivalents, duration of the coupling, and double coupling were modified. For Ala^{24}, Ala^{18}, Gly^{16}, Leu^{14}, and Tyr^{13} 3.0 eq of each reagent with respect to the loading of the resin were employed. For Arg^{28}, Val^{27}, Leu^{26}, Trp^{25}, Ile^{23}, Phe^{22}, Ala^{19}, and Ser^{12} the coupling time was shortened to 60 minutes and a double coupling was performed for 45 minutes. For Glu^{21}, Gln^{17}, and Glu^{15} a double coupling of 60 minutes was performed. For $Lys(GluPal)^{20}$ the coupling time was extended to 8 hours, and the coupling was repeated twice. Details for each coupling step are summarized in Table S18. After the final coupling step, the resin was washed with DMSO/EtOAc (1:9) (3 times x 5 mL/g of resin, 5 minutes each), DCM (3 times x 5 mL/g of resin, 5 minutes each), and dried under vacuum for 12 hours. For cleavage, the resin was added gradually to a solution of TFA/TIS/H_2O/DTT 85.0/5.0/5.0/5.0 v/v/v/v % (10 mL/g of resin) at 10°C, the suspension was then stirred for 3 hours at room temperature. The resin was filtered, and diisopropyl ether (30 mL/g of resin) was added dropwise to the filtered solution at 10°C under vigorous stirring. The precipitated peptide was filtered, washed with ether (3 times x 5 mL/g of resin), and dried under vacuum to obtain **3**. The crude was analyzed via HPLC-MS using Method 2 (see Analytical details section reported above).

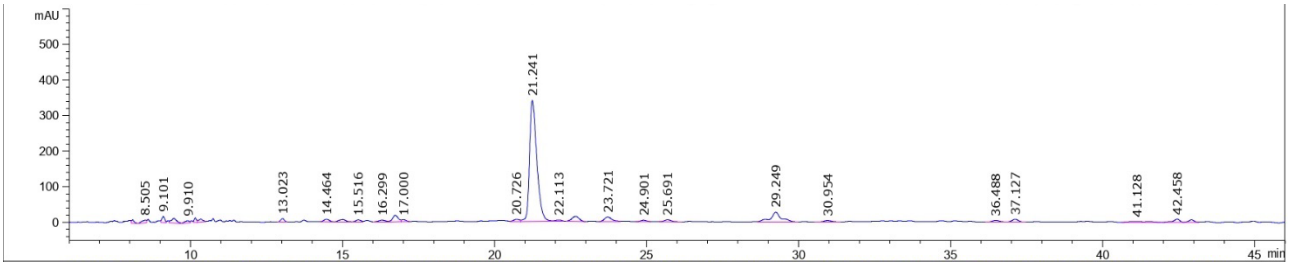
Table S18. Synthetic steps for H-(12-31)-OH **3** in DMSO/EtOAc (1:9), protocol modifications A and F.

Coupling	Amino acid	Eq	Time (min)	T (°C)
30	Fmoc-Arg(Pbf)-OH	2	90	25
29	Fmoc-Gly-OH	2	90	25
28	Fmoc-Arg(Pbf)-OH	2+2	60+45	25
27	Fmoc-Val-OH	2+2	60+45	40
26	Fmoc-Leu-OH	2+2	60+45	40
25	Fmoc-Trp(Boc)-OH	2+2	60+45	40
24	Fmoc-Ala-OH	3	90	40
23	Fmoc-Ile-OH	2+2	60+45	40
22	Fmoc-Phe-OH	2+2	60+45	40
21	Fmoc-Glu(OtBu)-OH	2+2	90+60	40
20	Fmoc-Lys(γ-Glu(OtBu)Pal)-OH	2+2	8h+8h	40
19	Fmoc-Ala-OH	2+2	60+45	40
18	Fmoc-Ala-OH	3	90	40
17	Fmoc-Gln(Trt)-OH	2+2	90+60	40
16	Fmoc-Gly-OH	3	90	40
15	Fmoc-Glu(OtBu)-OH	2+2	90+60	40
14	Fmoc-Leu-OH	3	90	40
13	Fmoc-Tyr(tBu)-OH	3	90	40
12	Fmoc-Ser(tBu)-OH	2+2	60+45	40

Table S19. Chromatogram of H-(12-31)-OH **3**, solid-phase synthesis in DMSO/EtOAc (1:9), protocol modifications A and F. All peaks between 6 and 46 minutes with Area % ≥ 0.5 were considered.

Species		t_R (min)	rrt	Area %	m/z obs
Product	H-(12-31)-OH	63.020	21.241	1.000	1310.1
	+Pbf	1.001	25.691	1.209	1435.6
Deletion sequences	Des-Glu-LysGluPal	1.271	9.101	0.428	997.8
	Des-LysGluPal	0.746	10.152	0.478	1062.2
	Des-Leu(Ile)-Ala	1.139	15.008	0.707	1217.8
	Des-Leu(Ile)	0.722	15.516	0.730	1253.4
	Des-Trp	0.834	16.299	0.767	1217.8
	Des-Ala	5.071	16.733	0.788	1274.3
			23.721	1.117	1274.1
	Des-Leu(Ile)	0.661	17.000	0.800	1253.2
	Des-Ser-Tyr	0.940	20.726	0.976	1184.3
	Des-Ser	0.621	22.113	1.041	1266.3
	Des-Tyr Des-Glu Des-Gly	2.587	22.663	1.067	1228.6 1245.1 1281.3
	Des-Gln Des-Arg	7.430	29.249	1.377	1245.7 1231.8
	Des-Gly-Arg	0.861	30.954	1.457	1203.7
	Des-Arg-Arg	1.181	37.127	1.748	1153.6
	Total	24.065	-	-	-
Else	-	11.914	-	-	-

Figure S34. Chromatogram of H-(12-31)-OH **3**, solid-phase synthesis in DMSO/EtOAc (1:9), protocol modifications A and F. All peaks between 6 and 46 minutes with Area % ≥ 0.5 were considered.



Solid-phase synthesis of H-(12-31)-OH 3 in NBP/DMC (8:2), protocol modifications A and F

The synthesis was performed on preloaded Fmoc-Gly-MBH resin (loading 0.5 mmol/g); steps were carried out at 25°C up to Arg^{28} included, and at 40°C from Val^{27} onwards. After swelling the resin with NBP/DMC (8:2) (10 mL/g of resin, 240 minutes), the Fmoc protecting group was removed by treatment with a solution of piperidine 20% in NBP/DMC (8:2) (2 times x 5 mL/g of resin, 15 minutes each) and washed with NBP/DMC (8:2) (4 times x 5 mL/g of resin, 5 minutes each). Fmoc-Arg(Pbf)-OH and Oxyma Pure® (2.0 eq of each reagent with respect to the loading of the resin) were dissolved in NBP/DMC (8:2) (5 mL/g of resin) and pre-activated with DIC (2.0 eq with respect to the loading of the resin) for 3 minutes. The coupling mixture was added to the resin and stirred for 90 minutes, followed by washes with NBP/DMC (8:2) (3 times x 5 mL/g of resin, 5 minutes each). The steps of Fmoc-removal, washing and coupling were repeated until the target peptide sequence was achieved. For each coupling, the selected Fmoc-AAx-OH, Oxyma Pure® and DIC were employed; in some cases, the equivalents, duration of the coupling, and double coupling were modified. For Ala^{24}, Ala^{18}, Gly^{16}, Leu^{14}, and Tyr^{13} 3.0 eq of each reagent with respect to the loading of the resin were employed. For Arg^{28}, Val^{27}, Leu^{26}, Trp^{25}, Ile^{23}, Phe^{22}, Ala^{19}, and Ser^{12} the coupling time was shortened to 60 minutes and a double coupling was performed for 45 minutes. For Glu^{21}, Gln^{17}, and Glu^{15} a double coupling of 60 minutes was performed. For $Lys(GluPal)^{20}$ the coupling time was extended to 8 hours, and the coupling was repeated twice. Details for each coupling step are summarized in Table S20. After the final coupling step, the resin was washed with NBP/DMC (8:2) (3 times x 5 mL/g of resin, 5 minutes each), DCM (3 times x 5 mL/g of resin, 5 minutes each), and dried under vacuum for 12 hours. For cleavage, the resin was added gradually to a solution of TFA/TIS/H_2O/DTT 85.0/5.0/5.0/5.0 v/v/v/v % (10 mL/g of resin) at 10°C, the suspension was then stirred for 3 hours at room temperature. The resin was filtered, and diisopropyl ether (30 mL/g of resin) was added dropwise to the filtered solution at 10°C under vigorous stirring. The precipitated peptide was filtered, washed with ether (3 times x 5 mL/g of resin), and dried under vacuum to obtain **3**. The crude was analyzed via HPLC-MS using Method 2 (see Analytical details section reported above).

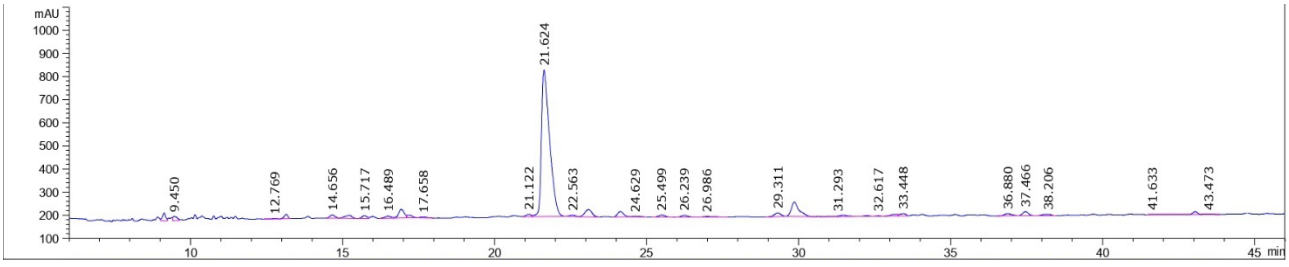
Table S20. Synthetic steps for H-(12-31)-OH **3** in NBP/DMC (8:2), protocol modifications A and F.

Coupling	Amino acid	Eq	Time (min)	T (°C)
30	Fmoc-Arg(Pbf)-OH	2	90	25
29	Fmoc-Gly-OH	2	90	25
28	Fmoc-Arg(Pbf)-OH	2+2	60+45	25
27	Fmoc-Val-OH	2+2	60+45	40
26	Fmoc-Leu-OH	2+2	60+45	40
25	Fmoc-Trp(Boc)-OH	2+2	60+45	40
24	Fmoc-Ala-OH	3	90	40
23	Fmoc-Ile-OH	2+2	60+45	40
22	Fmoc-Phe-OH	2+2	60+45	40
21	Fmoc-Glu(OtBu)-OH	2+2	90+60	40
20	Fmoc-Lys(γ-Glu(OtBu)Pal)-OH	2+2	8h+8h	40
19	Fmoc-Ala-OH	2+2	60+45	40
18	Fmoc-Ala-OH	3	90	40
17	Fmoc-Gln(Trt)-OH	2+2	90+60	40
16	Fmoc-Gly-OH	3	90	40
15	Fmoc-Glu(OtBu)-OH	2+2	90+60	40
14	Fmoc-Leu-OH	3	90	40
13	Fmoc-Tyr(tBu)-OH	3	90	40
12	Fmoc-Ser(tBu)-OH	2+2	60+45	40

Table S21. Results for solid-phase synthesis of H-(12-31)-OH **3** in NBP/DMC (8:2), protocol modifications A and F.

Species		Area %	t_R (min)	rrt	m/z obs
Product	H-(12-31)-OH	69.056	21.624	1.000	1310.2
	+Pbf	0.811	26.237	1.213	1435.8
Deletion sequences	Des-Glu-LysGluPal	1.403	9.125	0.422	997.6
	Des-Leu(Ile)-Ala	1.242	15.214	0.704	1217.6
	Des-Leu(Ile)	0.871	15.716	0.727	1253.9
	Des-Trp	0.828	16.489	0.763	1216.7
	Des-Ala	5.057	16.931	0.783	1274.3
			24.135	1.116	1274.3
	Des-Leu(Ile)	0.827	17.183	0.795	1253.3
	Des-Tyr Des-Glu Des-Gly	2.957	23.088	1.068	1228.3 1244.9 1281.2
	Des-Gln	1.367	29.309	1.355	1246.2
	Des-Arg	6.475	29.863	1.381	1231.8
	Des-Gly-Arg	0.865	31.460	1.455	1203.4
	Des-Arg-Arg	1.265	37.465	1.733	1153.8
	Total	23.157	-	-	-
Else	-	6.976	-	-	-

Figure S35. Chromatogram of H-(12-31)-OH **3**, solid-phase synthesis in NBP/DMC (8:2), protocol modifications A and F. All peaks between 6 and 46 minutes with Area % ≥ 0.5 were considered.



Enzymatic coupling of fragments 2 and 3 to form Liraglutide 1

Fragment **3** (1.0 eq, 100.8 mg based on Assay) was dissolved in Tricine buffer 50mM (5 mL) at pH 8.5, the pH was then adjusted to 11.0 with KOH 3M, and the solution was left stirring until complete dissolution. In another flask, fragment **2** (2.0 eq, 113.4 mg based on Assay) was dissolved in tricine buffer 50mM (6 mL) at pH 8.5, then TCEP (25 µL) was added to the solution of **2** and the mixture was stirred until complete dissolution. The solution of **3** was added to the solution of **2**, followed by the Liraligase enzyme (108 µL, 1.25% w/w with respect to **3**). The mixture was stirred at room temperature keeping the pH at 8.0 for 24 hours. Reaction progress was monitored via HPLC-MS using Method 3 (see Analytical method section reported above).

Table S22. Results of the enzymatic coupling of peptide fragments **2** and **3** to form Liraglutide **1** after 24 hours.

Species	Peptide sequence	t_R (min)	rrt	m/z obs	Area %
H-GFK-NH_2	HO-CamFK-NH_2	4.114	0.344	351.2	27.05
H-(1-11)-OH	H-HAEGTFTSDVS-OH	5.200	0.461	1150.4	31.49
Liraglutide	H-HAEGTFTSDVSSYLEGQAAK(EPal)EFIAWLVRGRG-OH	12.141	1.000	1250.9	34.58
Fragment 12-31	H-SYLEGQAAK(EPal)EFIAWLVRGRG-OH	13.492	1.030	1310.2	6.88

Figure S36. Chromatogram of the enzymatic coupling of **2** and **3** to form Liraglutide **1** after 24 hours.

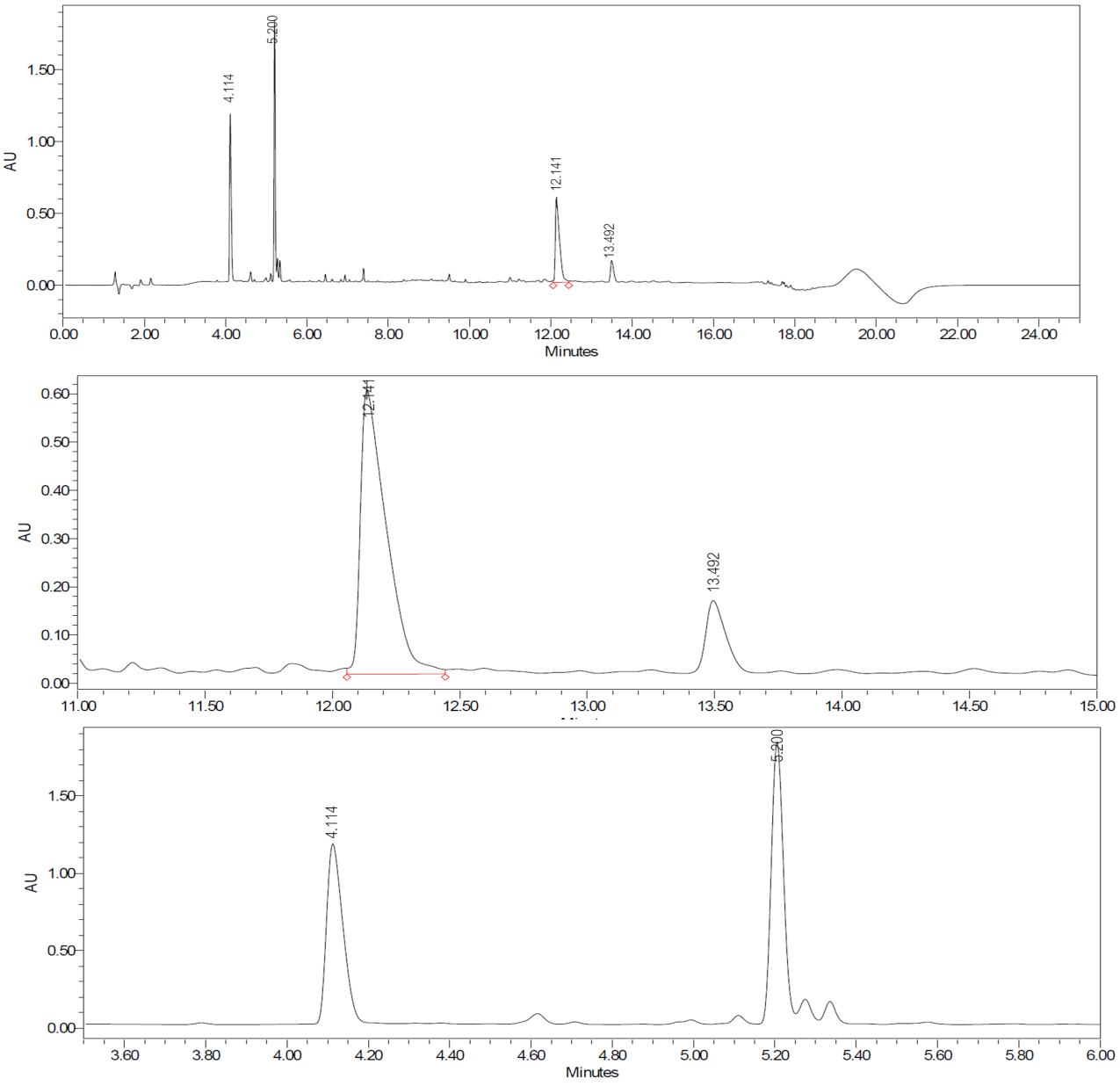


Figure S37. Mass chromatogram for peak at 4.114 min, m/z obs = 351.2.

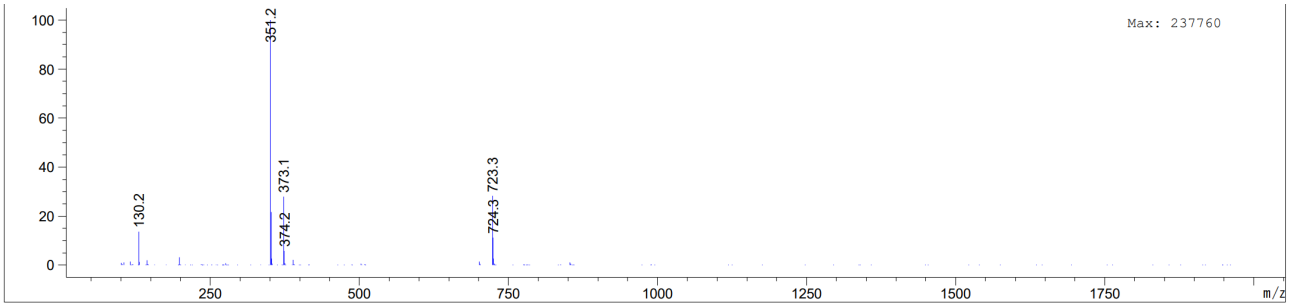


Figure S38. Mass chromatogram for peak at 5.200 min, m/z obs = 1150.4.

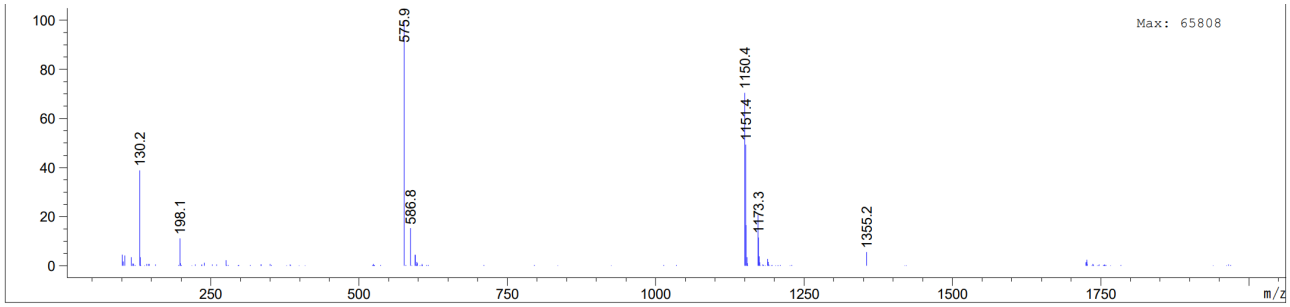


Figure S39. Mass chromatogram for peak at 12.141 min, m/z obs = 1250.9.

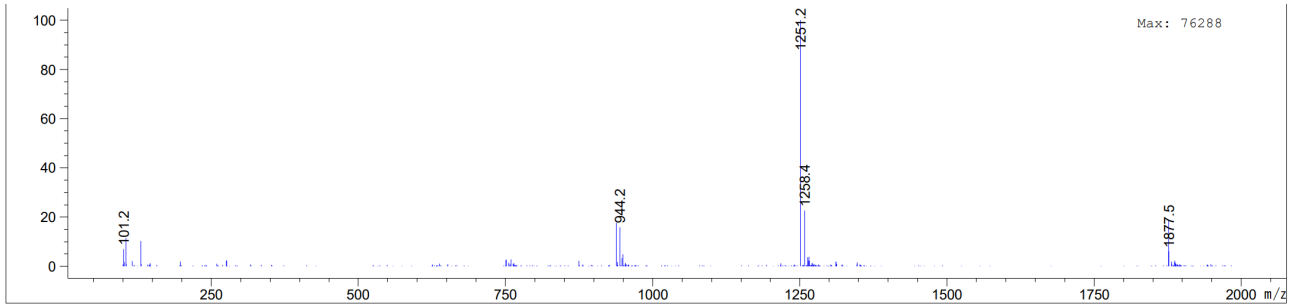
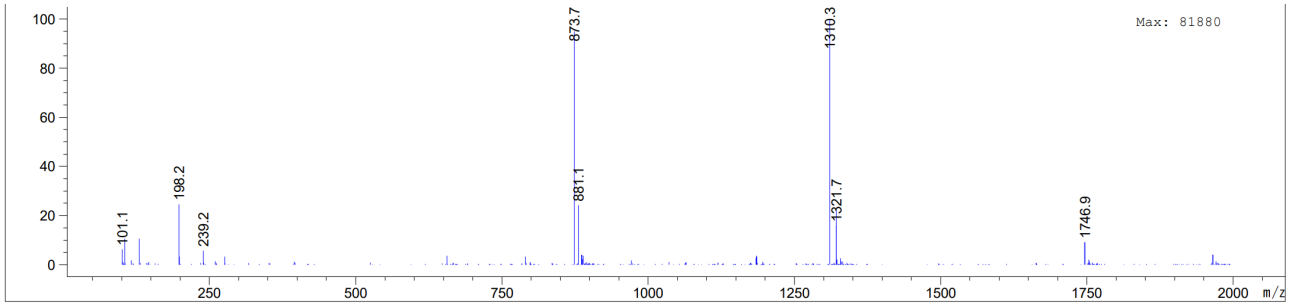


Figure S40. Mass chromatogram for peak at 13.492 min, m/z obs = 1310.2.



PMI calculation for solid-phase synthesis

Process Mass Intensity (PMI) is defined as the ratio between the total mass of materials employed for the synthesis and the mass of the isolated product, as follows:

$$PMI = \frac{\Sigma\, mass\ of\ materials}{mass\ of\ isolated\ product}$$

In the solid-phase synthesis of peptide fragments **2** and **3**, the materials employed for the synthesis include:

- Reagents employed for deprotection and coupling steps: amino acid residues (Fmoc-AAx(PG)-OH or Boc-AAx(PG)-OH), bromoacetic acid, Oxyma Pure, DIC, KI, DIPEA, piperidine
- Reagents and solvents employed for cleavage from the resin: TFA, TIS, DTT, H_2O, DCM, DIPE
- Solvents employed throughout the synthesis for swelling, coupling, deprotection and washing steps

The contribution of reagents employed for the coupling steps depends on the protocol, which was optimized for each solvent and therefore both the solvent and chemicals vary for each PMI calculation. For cleavage, the contribution is constant for the same peptide fragment.

This calculation yields the total PMI for each peptide fragment **2** and **3**, which is highly dependent on chain length. The value of PMI/aa can also be calculated as follows:

$$PMI/aa = \frac{\text{PMI}}{number\ of\ amino\ acid\ residues}$$

The recovery of solvents and piperidine used for deprotection can be considered, and the PMI consequently adjusted to PMI after recovery (PMIr):

$$PMIr = \frac{\Sigma\, mass\ of\ materials - \Sigma\, mass\ of\ recovered\ materials}{mass\ of\ isolated\ product}$$

Same as above, the process mass intensity can be expressed independently of chain length, as PMIr/aa:

$$PMIr/aa = \frac{\text{PMIr}}{number\ of\ amino\ acid\ residues}$$

The process mass intensity values (PMI, PMI/aa, PMIr, PMIr/aa) for all syntheses of **2** and **3** described in the Experimental Section are calculated below. Piperidine recovery accounts for the formation of the DBF-piperidine adduct, which was subtracted from the recoverable volume. The values of molecular weight, density, and recovery of the green solvent mixtures are calculated as follows:

Table S23. Molecular weight, density, and recovery for green solvent mixtures and base used in solid-phase synthesis of 2 and 3.

Species	MW (g/mol)	d (g/mL)	Recovery (%)
DMC	90.08	1.07	95
DMSO	78.13	1.1004	70
EtOAc	88.11	0.902	86
NBP	141.21	0.958	85
NOP	197.32	0.92	85
Piperidine	85.15	0.862	95
DMSO/EtOAc (1:9)	87.11	0.92	73.2
NBP/DMC (8:2)	130.98	0.9804	87
NOP/DMC (8:2)	175.87	0.95	87
NBP/EtOAc (8:2)	130.59	0.95	85.2

PMI calculation for solid-phase synthesis of 2

All calculations are reported on the same scale (1.0 g of resin, 0.62 mmol/g, 0.62 mmol); the amount of crude product was considered to be the same in all cases (0.673 g, yield = 73.25 %), based on the amount of product isolated in the solid-phase synthesis of **2** with DMF.

To allow for solvent and base recycling, the waste stream of deprotection steps (including the following washing steps) and coupling steps (including swelling and washing steps) were collected separately.

PMI calculation for solid-phase synthesis of 2 in DMF

Below is the total mass of reagents and solvents employed for swelling, deprotection, coupling, washes, and final cleavage of the peptide from the resin in the protocol for the synthesis of **2** in DMF.

Table S24. Overview of all reagents and solvents used for the SPPS of **2** in DMF.

Contribution	Species	MW (g/mol)	d (g/mL)	Eq	n (mmol)	V (mL)	M (g)	Rep	m_{tot} (g)
Resin	Fmoc-Lys(Boc)-Rink Amide			1.0	0.62		1.00		1.00
Deprotection	Piperidine	85.15	0.862			24.00	20.69		20.69
Coupling	Fmoc-Phe-OH	387.43	1.000	2.0	1.24		0.48	1	0.48
	BrAc	138.95	1.000	2.0	1.24		0.17	1	0.17
	Fmoc-Ser(tBu)-OH	383.45	1.000	4.0	2.48		0.95	1	0.95
	Fmoc-Val-OH	339.40	1.000	2.0	1.24		0.42	1	0.42
	Fmoc-Asp(tBu)-OH	411.45	1.000	2.0	1.24		0.51	1	0.51
	Fmoc-Ser(tBu)-OH	383.45	1.000	2.0	1.24		0.48	1	0.48
	Fmoc-Thr(tBu)-OH	397.43	1.000	2.0	1.24		0.49	1	0.49
	Fmoc-Phe-OH	387.43	1.000	2.0	1.24		0.48	1	0.48
	Fmoc-Thr(tBu)-OH	397.43	1.000	2.0	1.24		0.49	1	0.49
	Fmoc-Gly-OH	297.31	1.000	2.0	1.24		0.37	1	0.37
	Fmoc-Glu(OtBu)-OH	425.49	1.000	2.0	1.24		0.53	1	0.53
	Fmoc-Ala-OH•H_2O	329.30	1.000	2.0	1.24		0.41	1	0.41
	Boc-His(Trt)-OH	497.58	1.000	2.0	1.24		0.62	1	0.62
	Oxyma Pure	142.11	1.000	22.1	13.70		1.95		1.95
	DIC	126.20	0.815	24.0	14.88		1.88		1.88
	KI	166.00	1.000	1.0	0.62		0.10	1	0.10
	DIPEA	129.25	0.742	4.0	2.48		0.32	1	0.32
Cleavage	TFA	114.02	1.489			9.25	13.77	1	13.77
	TIS	158.36	0.773			0.50	0.39	1	0.39
	H_2O	18.02	0.997			0.25	0.25	1	0.25
	DIPE	102.17	0.725			45.00	32.63	1	32.63
	DCM	84.93	1.325			5.00	6.63	3	19.88
Total	DMF								572.06
Total sum									671.31

The PMI and PMI/aa can then be calculated as follows:

$$PMI = \frac{\Sigma\, mass\ of\ materials}{mass\ of\ isolated\ product} = \frac{671.31}{0.673} = 997.75$$

$$PMI/aa = \frac{\mathrm{PMI}}{number\ of\ amino\ acid\ residues} = \frac{997.75}{14} = 71.27$$

PMI calculation for solid-phase synthesis of 2 in NOP/DMC (8:2)

The synthesis of **2** in NOP/DMC (8:2) was performed according to different protocols, which implies different amounts of reagents and solvents. The process mass intensity values were calculated separately for the different protocols. Below is the total mass of reagents and solvents employed for swelling, deprotection, coupling, washing steps and final cleavage of the peptide from the resin in the protocol for the synthesis of **2** in NOP/DMC (8:2) with protocol modification **A** or both modifications **A** and **B**.

Table S25. Overview of all reagents and solvents used for the SPPS of **2** in NOP/DMC (8:2), protocol modifications A or A and B.

Contribution	Species	MW (g/mol)	d (g/mL)	Eq	n (mmol)	V (mL)	M (g)	Rep	m_{tot} (g)
Resin	Fmoc-Lys(Boc)-Rink Amide			1.0	0.62		1.00		1.00
Deprotection	Piperidine	85.15	0.862			16.50	14.22		14.22
Coupling	Fmoc-Phe-OH	387.43	1.000	2.0	1.24		0.48	1	0.48
	BrAc	138.95	1.000	2.0	1.24		0.17	1	0.17
	Fmoc-Ser(tBu)-OH	383.45	1.000	4.0	2.48		0.95	1	0.95
	Fmoc-Val-OH	339.40	1.000	2.0	1.24		0.42	1	0.42
	Fmoc-Asp(tBu)-OH	411.45	1.000	2.0	1.24		0.51	1	0.51
	Fmoc-Ser(tBu)-OH	383.45	1.000	2.0	1.24		0.48	1	0.48
	Fmoc-Thr(tBu)-OH	397.43	1.000	2.0	1.24		0.49	1	0.49
	Fmoc-Phe-OH	387.43	1.000	2.0	1.24		0.48	1	0.48
	Fmoc-Thr(tBu)-OH	397.43	1.000	2.0	1.24		0.49	1	0.49
	Fmoc-Gly-OH	297.31	1.000	2.0	1.24		0.37	1	0.37
	Fmoc-Glu(OtBu)-OH	425.49	1.000	2.0	1.24		0.53	1	0.53
	Fmoc-Ala-OH*H2O	329.30	1.000	2.0	1.24		0.41	1	0.41
	Boc-His(Trt)-OH	497.58	1.000	2.0	1.24		0.62	1	0.62
	Oxyma Pure	142.11	1.000	22.1	13.70		1.95		1.95
	DIC	126.20	0.815	24.0	14.88		1.88		1.88
	KI	166.00	1.000	1.0	0.62		0.10	1	0.10
	DIPEA	129.25	0.742	4.0	2.48		0.32	1	0.32
Cleavage	TFA	114.02	1.489			9.25	13.77	1	13.77
	TIS	158.36	0.773			0.50	0.39	1	0.39
	H_2O	18.02	0.997			0.25	0.25	1	0.25
	DIPE	102.17	0.725			45.00	32.63	1	32.63
	DCM	84.93	1.325			5.00	6.63	3	19.88
Total	NOP/DMC (8:2)					613.50			582.83
Total sum									675.60

The PMI and PMI/aa can then be calculated as follows:

$$PMI = \frac{\Sigma\, mass\ of\ materials}{mass\ of\ isolated\ product} = \frac{675.60}{0.67} = 1004.13$$

$$PMI/aa = \frac{\text{PMI}}{number\ of\ amino\ acid\ residues} = \frac{1004.13}{14} = 71.72$$

The PMIr can be calculated to consider recycling of NOP/DMC (8:2) (recovery 87%) as follows:

$$PMIr = \frac{\Sigma\, mass\ of\ materials\ -\ \Sigma\, mass\ of\ recovered\ materials}{mass\ of\ isolated\ product} = \frac{126.18}{0.67} = 187.54$$

$$PMIr/aa = \frac{\text{PMIr}}{number\ of\ amino\ acid\ residues} = \frac{187.54}{14} = 13.40$$

Since modification **C** introduced double couplings, a new value for process mass intensity must be calculated. Below is the total mass of reagents and solvents employed for swelling, deprotection, coupling, washing steps and final cleavage of the peptide from the resin in the protocol for the synthesis of **2** in NOP/DMC (8:2) with protocol modifications **A**, **B**, and **C**.

Table S26. Overview of all reagents and solvents used for the SPPS of **2** in NOP/DMC (8:2), protocol modifications **A**, **B**, and **C**.

Contribution	Species	MW (g/mol)	d (g/mL)	Eq	n (mmol)	V (mL)	M (g)	Rep	m_{tot} (g)
Resin	Fmoc-Lys(Boc)-Rink Amide			1.0	0.62		1.00		1.00
Deprotection	Piperidine	85.15	0.862			16.50	14.22		14.22
Coupling	Fmoc-Phe-OH	387.43	1.000	2.0	1.24		0.48	2	0.96
	BrAc	138.95	1.000	2.0	1.24		0.17	1	0.17
	Fmoc-Ser(tBu)-OH	383.45	1.000	4.0	2.48		0.95	1	0.95
	Fmoc-Val-OH	339.40	1.000	2.0	1.24		0.42	2	0.84
	Fmoc-Asp(tBu)-OH	411.45	1.000	2.0	1.24		0.51	2	1.02
	Fmoc-Ser(tBu)-OH	383.45	1.000	2.0	1.24		0.48	2	0.95
	Fmoc-Thr(tBu)-OH	397.43	1.000	2.0	1.24		0.49	2	0.99
	Fmoc-Phe-OH	387.43	1.000	2.0	1.24		0.48	2	0.96
	Fmoc-Thr(tBu)-OH	397.43	1.000	2.0	1.24		0.49	2	0.99
	Fmoc-Gly-OH	297.31	1.000	2.0	1.24		0.37	2	0.74
	Fmoc-Glu(OtBu)-OH	425.49	1.000	2.0	1.24		0.53	2	1.06
	Fmoc-Ala-OH•H_2O	329.30	1.000	2.0	1.24		0.41	2	0.82
	Boc-His(Trt)-OH	497.58	1.000	2.0	1.24		0.62	2	1.23
	Oxyma Pure	142.11	1.000	44.1	27.34		3.89		3.89
	DIC	126.20	0.815	46.0	28.52		3.60		3.60
	KI	166.00	1.000	1.0	0.62		0.10	1	0.10
	DIPEA	129.25	0.742	4.0	2.48		0.32	1	0.32
Cleavage	TFA	114.02	1.489			9.25	13.77	1	13.77
	TIS	158.36	0.773			0.50	0.39	1	0.39
	H_2O	18.02	0.997			0.25	0.25	1	0.25
	DIPE	102.17	0.725			45.00	32.63	1	32.63
	DCM	84.93	1.325			5.00	6.63	3	19.88
Total	NOP/DMC (8:2)					833.50			791.83
Total sum									893.54

The PMI and PMI/aa can then be calculated as follows:

$$PMI = \frac{\Sigma\, mass\ of\ materials}{mass\ of\ isolated\ product} = \frac{893.54}{0.67} = 1328.04$$

$$PMI/aa = \frac{\text{PMI}}{number\ of\ amino\ acid\ residues} = \frac{1328.04}{14} = 94.86$$

The PMIr can be calculated to consider recycling of NOP/DMC (8:2) (recovery 87%) as follows:

$$PMIr = \frac{\Sigma\, mass\ of\ materials\ -\ \Sigma\, mass\ of\ recovered\ materials}{mass\ of\ isolated\ product} = \frac{152.72}{0.67} = 226.98$$

$$PMIr/aa = \frac{\text{PMIr}}{number\ of\ amino\ acid\ residues} = \frac{226.98}{14} = 16.21$$

PMI calculation for solid-phase synthesis of 2 in DMSO/EtOAc (1:9)

Below is the total mass of reagents and solvents employed for swelling, deprotection, coupling, washing steps and final cleavage of the peptide from the resin in the protocol for the synthesis of **2** in DMSO/EtOAc (1:9) described above.

Table S27. Overview of all reagents and solvents used for the SPPS of **2** in DMSO/EtOAc (1:9).

Contribution	Species	MW (g/mol)	d (g/mL)	Eq	n (mmol)	V (mL)	M (g)	Rep	m_{tot} (g)
Resin	Fmoc-Lys(Boc)-Rink Amide			1.0	0.62		1.00		1.00
Deprotection	Piperidine	85.15	0.862			16.50	14.22		14.22
Coupling	Fmoc-Phe-OH	387.43	1.000	2.0	1.24		0.48	1	0.48
	BrAc	138.95	1.000	2.0	1.24		0.17	1	0.17
	Fmoc-Ser(tBu)-OH	383.45	1.000	4.0	2.48		0.95	1	0.95
	Fmoc-Val-OH	339.40	1.000	2.0	1.24		0.42	1	0.42
	Fmoc-Asp(tBu)-OH	411.45	1.000	2.0	1.24		0.51	1	0.51
	Fmoc-Ser(tBu)-OH	383.45	1.000	2.0	1.24		0.48	1	0.48
	Fmoc-Thr(tBu)-OH	397.43	1.000	2.0	1.24		0.49	1	0.49
	Fmoc-Phe-OH	387.43	1.000	2.0	1.24		0.48	1	0.48
	Fmoc-Thr(tBu)-OH	397.43	1.000	2.0	1.24		0.49	1	0.49
	Fmoc-Gly-OH	297.31	1.000	2.0	1.24		0.37	1	0.37
	Fmoc-Glu(OtBu)-OH	425.49	1.000	2.0	1.24		0.53	1	0.53
	Fmoc-Ala-OH•H_2O	329.30	1.000	2.0	1.24		0.41	1	0.41
	Boc-His(Trt)-OH	497.58	1.000	2.0	1.24		0.62	1	0.62
	Oxyma Pure	142.11	1.000	22.1	13.70		1.95		1.95
	DIC	126.20	0.815	24.0	14.88		1.88		1.88
	KI	166.00	1.000	1.0	0.62		0.10	1	0.10
	DIPEA	129.25	0.742	4.0	2.48		0.32	1	0.32
Cleavage	TFA	114.02	1.489			9.25	13.77	1	13.77
	TIS	158.36	0.773			0.50	0.39	1	0.39
	H_2O	18.02	0.997			0.25	0.25	1	0.25
	DIPE	102.17	0.725			45.00	32.63	1	32.63
	DCM	84.93	1.325			5.00	6.63	3	19.88
Total	DMSO/EtOAc (1:9)					613.50			565.55
Total sum									658.33

The PMI and PMI/residue can then be calculated as follows:

$$PMI = \frac{\Sigma\, mass\ of\ materials}{mass\ of\ isolated\ product} = \frac{658.33}{0.67} = 978.46$$

$$PMI/residue = \frac{\text{PMI}}{number\ of\ amino\ acid\ residues} = \frac{978.46}{14} = 69.89$$

The PMIr can be calculated to consider recycling of DMSO/EtOAc (1:9) (recovery 73.2%) as follows:

$$PMIr = \frac{\Sigma\, mass\ of\ materials\ -\ \Sigma\, mass\ of\ recovered\ materials}{mass\ of\ isolated\ product} = \frac{193.57}{0.67} = 287.70$$

$$PMIr/residue = \frac{\text{PMIr}}{number\ of\ amino\ acid\ residues} = \frac{287.70}{14} = 20.55$$

PMI calculation for solid-phase synthesis of 2 in NBP/EtOAc (8:2)

Below is the total mass of reagents and solvents employed for swelling, deprotection, coupling, washing steps and final cleavage of the peptide from the resin in the protocol for the synthesis of **2** in NBP/EtOAc (8:2) described above. The molecular weight and density of the green solvent mixture are calculated as well.

Table S28. Overview of all reagents and solvents used for the SPPS of **2** in NBP/EtOAc (8:2).

Contribution	Species	MW (g/mol)	d (g/mL)	Eq	n (mmol)	V (mL)	M (g)	Rep	m_{tot} (g)
Resin	Fmoc-Lys(Boc)-Rink Amide			1.0	0.62		1.00		1.00
Deprotection	Piperidine	85.15	0.862			16.50	14.22		14.22
Coupling	Fmoc-Phe-OH	387.43	1.000	2.0	1.24		0.48	1	0.48
	BrAc	138.95	1.000	2.0	1.24		0.17	1	0.17
	Fmoc-Ser(tBu)-OH	383.45	1.000	4.0	2.48		0.95	1	0.95
	Fmoc-Val-OH	339.40	1.000	2.0	1.24		0.42	1	0.42
	Fmoc-Asp(tBu)-OH	411.45	1.000	2.0	1.24		0.51	1	0.51
	Fmoc-Ser(tBu)-OH	383.45	1.000	2.0	1.24		0.48	1	0.48
	Fmoc-Thr(tBu)-OH	397.43	1.000	2.0	1.24		0.49	1	0.49
	Fmoc-Phe-OH	387.43	1.000	2.0	1.24		0.48	1	0.48
	Fmoc-Thr(tBu)-OH	397.43	1.000	2.0	1.24		0.49	1	0.49
	Fmoc-Gly-OH	297.31	1.000	2.0	1.24		0.37	1	0.37
	Fmoc-Glu(OtBu)-OH	425.49	1.000	2.0	1.24		0.53	1	0.53
	Fmoc-Ala-OH•H_2O	329.30	1.000	2.0	1.24		0.41	1	0.41
	Boc-His(Trt)-OH	497.58	1.000	2.0	1.24		0.62	1	0.62
	Oxyma Pure	142.11	1.000	22.1	13.70		1.95		1.95
	DIC	126.20	0.815	24.0	14.88		1.88		1.88
	KI	166.00	1.000	1.0	0.62		0.10	1	0.10
	DIPEA	129.25	0.742	4.0	2.48		0.32	1	0.32
Cleavage	TFA	114.02	1.489			9.25	13.77	1	13.77
	TIS	158.36	0.773			0.50	0.39	1	0.39
	H_2O	18.02	0.997			0.25	0.25	1	0.25
	DIPE	102.17	0.725			45.00	32.63	1	32.63
	DCM	84.93	1.325			5.00	6.63	3	19.88
Total	NBP/EtOAc (8:2)					613.50			580.86
Total sum									673.64

The PMI and PMI/residue can then be calculated as follows:

$$PMI = \frac{\Sigma\, mass\ of\ materials}{mass\ of\ isolated\ product} = \frac{673.64}{0.67} = 1001.22$$

$$PMI/residue = \frac{\text{PMI}}{number\ of\ amino\ acid\ residues} = \frac{1001.22}{14} = 71.52$$

The PMIr can be calculated to consider recycling of NBP/EtOAc (8:2) (recovery 85.2%) as follows:

$$PMIr = \frac{\Sigma\, mass\ of\ materials\ -\ \Sigma\, mass\ of\ recovered\ materials}{mass\ of\ isolated\ product} = \frac{135.26}{0.67} = 201.04$$

$$PMIr/residue = \frac{\text{PMIr}}{number\ of\ amino\ acid\ residues} = \frac{201.04}{14} = 14.36$$

PMI calculation for solid-phase synthesis of 2 in NBP/DMC (8:2)

Below is the total mass of reagents and solvents employed for swelling, deprotection, coupling, washing steps and final cleavage of the peptide from the resin in the protocol for the synthesis of **2** in NBP/DMC (8:2) described above.

Table S29. Overview of all reagents and solvents used for the SPPS of **2** in NBP/DMC (8:2).

Contribution	Species	MW (g/mol)	d (g/mL)	Eq	n (mmol)	V (mL)	M (g)	Rep	m_{tot} (g)
Resin	Fmoc-Lys(Boc)-Rink Amide			1.0	0.62		1.00		1.00
Deprotection	Piperidine	85.15	0.862			16.50	14.22		14.22
Coupling	Fmoc-Phe-OH	387.43	1.000	2.0	1.24		0.48	1	0.48
	BrAc	138.95	1.000	2.0	1.24		0.17	1	0.17
	Fmoc-Ser(tBu)-OH	383.45	1.000	4.0	2.48		0.95	1	0.95
	Fmoc-Val-OH	339.40	1.000	2.0	1.24		0.42	1	0.42
	Fmoc-Asp(tBu)-OH	411.45	1.000	2.0	1.24		0.51	1	0.51
	Fmoc-Ser(tBu)-OH	383.45	1.000	2.0	1.24		0.48	1	0.48
	Fmoc-Thr(tBu)-OH	397.43	1.000	2.0	1.24		0.49	1	0.49
	Fmoc-Phe-OH	387.43	1.000	2.0	1.24		0.48	1	0.48
	Fmoc-Thr(tBu)-OH	397.43	1.000	2.0	1.24		0.49	1	0.49
	Fmoc-Gly-OH	297.31	1.000	2.0	1.24		0.37	1	0.37
	Fmoc-Glu(OtBu)-OH	425.49	1.000	2.0	1.24		0.53	1	0.53
	Fmoc-Ala-OH•H_2O	329.30	1.000	2.0	1.24		0.41	1	0.41
	Boc-His(Trt)-OH	497.58	1.000	2.0	1.24		0.62	1	0.62
	Oxyma Pure	142.11	1.000	22.1	13.70		1.95		1.95
	DIC	126.20	0.815	24.0	14.88		1.88		1.88
	KI	166.00	1.000	1.0	0.62		0.10	1	0.10
	DIPEA	129.25	0.742	4.0	2.48		0.32	1	0.32
Cleavage	TFA	114.02	1.489			9.25	13.77	1	13.77
	TIS	158.36	0.773			0.50	0.39	1	0.39
	H_2O	18.02	0.997			0.25	0.25	1	0.25
	DIPE	102.17	0.725			45.00	32.63	1	32.63
	DCM	84.93	1.325			5.00	6.63	3	19.88
Total	NBP/DMC (8:2)					613.50			601.48
Total sum									694.25

The PMI and PMI/aa can then be calculated as follows:

$$PMI = \frac{\Sigma\, mass\ of\ materials}{mass\ of\ isolated\ product} = \frac{694.25}{0.67} = 1031.85$$

$$PMI/aa = \frac{\text{PMI}}{number\ of\ amino\ acid\ residues} = \frac{1031.85}{14} = 73.70$$

The PMIr can be calculated to consider recycling of NBP/DMC (8:2) (recovery 87%) as follows:

$$PMIr = \frac{\Sigma\, mass\ of\ materials\ -\ \Sigma\, mass\ of\ recovered\ materials}{mass\ of\ isolated\ product} = \frac{144.83}{0.67} = 215.26$$

$$PMIr/aa = \frac{\text{PMIr}}{number\ of\ amino\ acid\ residues} = \frac{215.26}{14} = 15.38$$

PMI calculation for solid-phase synthesis of 3

All calculations are reported on the same scale (1.0 g of resin, 0.5 mmol/g, 0.5 mmol); the amount of crude product was considered to be the same in all cases (0.316 g, yield = 63.19 %), based on the amount of product isolated in the solid-phase synthesis of **3** with DMF.

To allow for solvent and base recycling, the waste stream of deprotection steps (including the following washing steps) and coupling steps (including swelling and washing steps) were collected separately.

PMI calculation for solid-phase synthesis of 3 in DMF

Below is the total mass of reagents and solvents employed for swelling, deprotection, coupling, washes, and final cleavage of the peptide from the resin in the protocol for the synthesis of **3** in DMF described above.

Table S30. Overview of all reagents and solvents used for the SPPS of **3** in DMF.

Contribution	Species	MW (g/mol)	d (g/mL)	Eq	n (mmol)	V (mL)	M (g)	Rep	m_{tot} (g)
Resin	Fmoc-Gly-MBH resin			1.0	0.50		1.00		1.00
Deprotection	Piperidine	85.15	0.862			40.00	34.48		34.48
Coupling	Fmoc-Arg(Pbf)-OH	648.77	1.000	2.0	1.00		0.65	1	0.65
	Fmoc-Gly-OH	297.32	1.000	2.0	1.00		0.30	1	0.30
	Fmoc-Arg(Pbf)-OH	648.77	1.000	3.0	1.50		0.97	1	0.97
	Fmoc-Val-OH	339.39	1.000	3.0	1.50		0.51	1	0.51
	Fmoc-Leu-OH	353.41	1.000	2.0	1.00		0.35	2	0.71
	Fmoc-Trp(Boc)-OH	426.46	1.000	3.0	1.50		0.64	1	0.64
	Fmoc-Ala-OH•H_2O	329.34	1.000	3.0	1.50		0.49	1	0.49
	Fmoc-Ile-OH	353.41	1.000	2.0	1.00		0.35	2	0.71
	Fmoc-Phe-OH	387.43	1.000	3.0	1.50		0.58	1	0.58
	Fmoc-Glu(OtBu)-OH	425.47	1.000	2.0	1.00		0.43	2	0.85
	Fmoc-Lys(GluPal)-OH	792.06	1.000	2.0	1.00		0.79	1	0.79
	Fmoc-Ala-OH•H_2O	329.34	1.000	3.0	1.50		0.49	1	0.49
	Fmoc-Ala-OH•H_2O	329.34	1.000	2.0	1.00		0.33	1	0.33
	Fmoc-Gln(Trt)-OH	610.70	1.000	3.0	1.50		0.92	1	0.92
	Fmoc-Gly-OH	297.32	1.000	2.0	1.00		0.30	1	0.30
	Fmoc-Glu(OtBu)-OH	425.47	1.000	2.0	1.00		0.43	1.5	0.64
	Fmoc-Leu-OH	353.41	1.000	2.0	1.00		0.35	1	0.35
	Fmoc-Tyr(tBu)-OH	459.53	1.000	2.0	1.00		0.46	1	0.46
	Fmoc-Ser(tBu)-OH	383.44	1.000	2.0	1.00		0.38	1.5	0.58
	Oxyma Pure	142.11	1.000	53.0	26.50		3.77		3.77
	DIC	126.20	0.815	53.0	26.50		3.34		3.34
Cleavage	TFA	114.02	1.489			8.50	12.66	1	12.66
	TIS	158.36	0.773			0.50	0.39	1	0.39
	H_2O	18.02	0.997			0.50	0.50	1	0.50
	DTT	154.25	1.000			0.50	0.50	1	0.50
	DIPE	102.17	0.725			45.00	32.63	1	32.63
	DCM	84.93	1.325			5.00	6.63	3	19.88
Total	DMF								991.20
Total sum									1111.59

The PMI and PMI/residue can then be calculated as follows:

$$PMI = \frac{\Sigma\, mass\ of\ materials}{mass\ of\ isolated\ product} = \frac{1111.59}{0.83} = 1344.28$$

$$PMI/residue = \frac{\text{PMI}}{number\ of\ amino\ acid\ residues} = \frac{1344.28}{20} = 67.21$$

PMI calculation for solid-phase synthesis of 3 in NOP/DMC (8:2)

Below is the total mass of reagents and solvents employed for swelling, deprotection, coupling, washing steps and final cleavage of the peptide from the resin in the protocol for the synthesis of **3** in NOP/DMC (8:2) described above.

Table S31. Overview of all reagents and solvents used for the SPPS of **3** in NOP/DMC (8:2).

Contribution	Species	MW (g/mol)	d (g/mL)	Eq	n (mmol)	V (mL)	M (g)	Rep	m_{tot} (g)
Resin	Fmoc-Gly-MBH resin			1.0	0.50		1.00		1.00
Deprotection	Piperidine	85.15	0.862			40.00	34.48		34.48
Coupling	Fmoc-Arg(Pbf)-OH	648.77	1.000	2.0	1.00		0.65	1	0.65
	Fmoc-Gly-OH	297.32	1.000	2.0	1.00		0.30	1	0.30
	Fmoc-Arg(Pbf)-OH	648.77	1.000	3.0	1.50		0.97	1	0.97
	Fmoc-Val-OH	339.39	1.000	3.0	1.50		0.51	1	0.51
	Fmoc-Leu-OH	353.41	1.000	2.0	1.00		0.35	2	0.71
	Fmoc-Trp(Boc)-OH	426.46	1.000	3.0	1.50		0.64	1	0.64
	Fmoc-Ala-OH•H_2O	329.34	1.000	3.0	1.50		0.49	1	0.49
	Fmoc-Ile-OH	353.41	1.000	2.0	1.00		0.35	2	0.71
	Fmoc-Phe-OH	387.43	1.000	3.0	1.50		0.58	1	0.58
	Fmoc-Glu(OtBu)-OH	425.47	1.000	2.0	1.00		0.43	2	0.85
	Fmoc-Lys(GluPal)-OH	792.06	1.000	2.0	1.00		0.79	1	0.79
	Fmoc-Ala-OH•H_2O	329.34	1.000	3.0	1.50		0.49	1	0.49
	Fmoc-Ala-OH•H_2O	329.34	1.000	2.0	1.00		0.33	1	0.33
	Fmoc-Gln(Trt)-OH	610.70	1.000	3.0	1.50		0.92	1	0.92
	Fmoc-Gly-OH	297.32	1.000	2.0	1.00		0.30	1	0.30
	Fmoc-Glu(OtBu)-OH	425.47	1.000	2.0	1.00		0.43	1.5	0.64
	Fmoc-Leu-OH	353.41	1.000	2.0	1.00		0.35	1	0.35
	Fmoc-Tyr(tBu)-OH	459.53	1.000	2.0	1.00		0.46	1	0.46
	Fmoc-Ser(tBu)-OH	383.44	1.000	2.0	1.00		0.38	1.5	0.58
	Oxyma Pure	142.11	1.000	53.0	26.50		3.77		3.77
	DIC	126.20	0.815	53.0	26.50		3.34		3.34
Cleavage	TFA	114.02	1.489			8.50	12.66	1	12.66
	TIS	158.36	0.773			0.50	0.39	1	0.39
	H_2O	18.02	0.997			0.50	0.50	1	0.50
	DTT	154.25	1.000			0.50	0.50	1	0.50
	DIPE	102.17	0.725			45.00	32.63	1	32.63
	DCM	84.93	1.325			5.00	6.63	3	19.88
Total	NOP/DMC (8:2)					1050.00			997.50
Total sum									1117.89

The PMI and PMI/residue can then be calculated as follows:

$$PMI = \frac{\Sigma\, mass\ of\ materials}{mass\ of\ isolated\ product} = \frac{1117.89}{0.83} = 1351.90$$

$$PMI/residue = \frac{\text{PMI}}{number\ of\ amino\ acid\ residues} = \frac{1351.90}{20} = 67.60$$

The PMIr can be calculated to consider recycling of NOP/DMC (8:2) (recovery 87%) as follows:

$$PMIr = \frac{\Sigma\, mass\ of\ materials\ -\ \Sigma\, mass\ of\ recovered\ materials}{mass\ of\ isolated\ product} = \frac{166.39}{0.83} = 201.23$$

$$PMIr/residue = \frac{\text{PMIr}}{number\ of\ amino\ acid\ residues} = \frac{226.98}{14} = 10.06$$

PMI calculation for solid-phase synthesis of 3 in DMSO/EtOAc (1:9)

Below is the total mass of reagents and solvents employed for swelling, deprotection, coupling, washing steps and final cleavage of the peptide from the resin in the protocol for the synthesis of **3** in DMSO/EtOAc (1:9) described above.

Table S32. Overview of all reagents and solvents used for the SPPS of **3** in DMSO/EtOAc (1:9).

Contribution	Species	MW (g/mol)	d (g/mL)	Eq	n (mmol)	V (mL)	M (g)	Rep	m_{tot} (g)
Resin	Fmoc-Gly-MBH resin			1.0	0.50		1.00		1.00
Deprotection	Piperidine	85.15	0.862			40.00	34.48		34.48
Coupling	Fmoc-Arg(Pbf)-OH	648.77	1.000	2.0	1.00		0.65	1	0.65
	Fmoc-Gly-OH	297.32	1.000	2.0	1.00		0.30	1	0.30
	Fmoc-Arg(Pbf)-OH	648.77	1.000	2.0	1.00		0.65	2	1.30
	Fmoc-Val-OH	339.39	1.000	2.0	1.00		0.34	2	0.68
	Fmoc-Leu-OH	353.41	1.000	2.0	1.00		0.35	2	0.71
	Fmoc-Trp(Boc)-OH	426.46	1.000	2.0	1.00		0.43	2	0.85
	Fmoc-Ala-OH•H_2O	329.34	1.000	3.0	1.50		0.49	1	0.49
	Fmoc-Ile-OH	353.41	1.000	2.0	1.00		0.35	2	0.71
	Fmoc-Phe-OH	387.43	1.000	2.0	1.00		0.39	2	0.77
	Fmoc-Glu(OtBu)-OH	425.47	1.000	2.0	1.00		0.43	2	0.85
	Fmoc-Lys(GluPal)-OH	792.06	1.000	2.0	1.00		0.79	2	1.58
	Fmoc-Ala-OH•H_2O	329.34	1.000	2.0	1.00		0.33	2	0.66
	Fmoc-Ala-OH•H_2O	329.34	1.000	2.0	1.00		0.33	1	0.33
	Fmoc-Gln(Trt)-OH	610.70	1.000	2.0	1.00		0.61	2	1.22
	Fmoc-Gly-OH	297.32	1.000	3.0	1.50		0.45	1	0.45
	Fmoc-Glu(OtBu)-OH	425.47	1.000	2.0	1.00		0.43	2	0.85
	Fmoc-Leu-OH	353.41	1.000	3.0	1.50		0.53	1	0.53
	Fmoc-Tyr(tBu)-OH	459.53	1.000	3.0	1.50		0.69	1	0.69
	Fmoc-Ser(tBu)-OH	383.44	1.000	2.0	1.00		0.38	2	0.77
	Oxyma Pure	142.11	1.000	66.0	33.00		4.69		4.69
	DIC	126.20	0.815	66.0	33.00		4.16		4.16
Cleavage	TFA	114.02	1.489			8.50	12.66	1	12.66
	TIS	158.36	0.773			0.50	0.39	1	0.39
	H_2O	18.02	0.997			0.50	0.50	1	0.50
	DTT	154.25	1.000			0.50	0.50	1	0.50
	DIPE	102.17	0.725			45.00	32.63	1	32.63
	DCM	84.93	1.325			5.00	6.63	3	19.88
Total	DMSO/EtOAc (1:9)					1190.00			1096.99
Total sum									1222.25

The PMI and PMI/residue can then be calculated as follows:

$$PMI = \frac{\Sigma\, mass\ of\ materials}{mass\ of\ isolated\ product} = \frac{1222.25}{0.83} = 1478.10$$

$$PMI/residue = \frac{\text{PMI}}{number\ of\ amino\ acid\ residues} = \frac{1478.10}{20} = 73.91$$

The PMIr can be calculated to consider recycling of DMSO/EtOAc (1:9) (recovery 73.2%) as follows:

$$PMIr = \frac{\Sigma\, mass\ of\ materials\ -\ \Sigma\, mass\ of\ recovered\ materials}{mass\ of\ isolated\ product} = \frac{313.17}{0.83} = 378.73$$

$$PMIr/residue = \frac{\text{PMIr}}{number\ of\ amino\ acid\ residues} = \frac{378.73}{20} = 18.94$$

PMI calculation for solid-phase synthesis of 3 **in NBP/DMC (8:2)**

Below is the total mass of reagents and solvents employed for swelling, deprotection, coupling, washing steps and final cleavage of the peptide from the resin in the protocol for the synthesis of **3** in NBP/DMC (8:2) described above.

Table S33. Overview of all reagents and solvents used for the SPPS of **3** in NBP/DMC (8:2).

Contribution	Species	MW (g/mol)	d (g/mL)	Eq	n (mmol)	V (mL)	M (g)	Rep	m_{tot} (g)
Resin	Fmoc-Gly-MBH resin			1.0	0.50		1.00		1.00
Deprotection	Piperidine	85.15	0.862			40.00	34.48		34.48
Coupling	Fmoc-Arg(Pbf)-OH	648.77	1.000	2.0	1.00		0.65	1	0.65
	Fmoc-Gly-OH	297.32	1.000	2.0	1.00		0.30	1	0.30
	Fmoc-Arg(Pbf)-OH	648.77	1.000	2.0	1.00		0.65	2	1.30
	Fmoc-Val-OH	339.39	1.000	2.0	1.00		0.34	2	0.68
	Fmoc-Leu-OH	353.41	1.000	2.0	1.00		0.35	2	0.71
	Fmoc-Trp(Boc)-OH	426.46	1.000	2.0	1.00		0.43	2	0.85
	Fmoc-Ala-OH•H_2O	329.34	1.000	3.0	1.50		0.49	1	0.49
	Fmoc-Ile-OH	353.41	1.000	2.0	1.00		0.35	2	0.71
	Fmoc-Phe-OH	387.43	1.000	2.0	1.00		0.39	2	0.77
	Fmoc-Glu(OtBu)-OH	425.47	1.000	2.0	1.00		0.43	2	0.85
	Fmoc-Lys(GluPal)-OH	792.06	1.000	2.0	1.00		0.79	2	1.58
	Fmoc-Ala-OH•H_2O	329.34	1.000	2.0	1.00		0.33	2	0.66
	Fmoc-Ala-OH•H_2O	329.34	1.000	3.0	1.50		0.49	1	0.49
	Fmoc-Gln(Trt)-OH	610.70	1.000	2.0	1.00		0.61	2	1.22
	Fmoc-Gly-OH	297.32	1.000	3.0	1.50		0.45	1	0.45
	Fmoc-Glu(OtBu)-OH	425.47	1.000	2.0	1.00		0.43	2	0.85
	Fmoc-Leu-OH	353.41	1.000	3.0	1.50		0.53	1	0.53
	Fmoc-Tyr(tBu)-OH	459.53	1.000	3.0	1.50		0.69	1	0.69
	Fmoc-Ser(tBu)-OH	383.44	1.000	2.0	1.00		0.38	2	0.77
	Oxyma Pure	142.11	1.000	67.0	33.50		4.76		4.76
	DIC	126.20	0.815	67.0	33.50		4.23		4.23
Cleavage	TFA	114.02	1.489			8.50	12.66	1	12.66
	TIS	158.36	0.773			0.50	0.39	1	0.39
	H_2O	18.02	0.997			0.50	0.50	1	0.50
	DTT	154.25	1.000			0.50	0.50	1	0.50
	DIPE	102.17	0.725			45.00	32.63	1	32.63
	DCM	84.93	1.325			5.00	6.63	3	19.88
Total	NBP/DMC (8:2)					1190.00			1166.68
Total sum									1292.24

The PMI and PMI/residue can then be calculated as follows:

$$PMI = \frac{\Sigma\, mass\ of\ materials}{mass\ of\ isolated\ product} = \frac{1292.24}{0.83} = 1562.74$$

$$PMI/residue = \frac{\text{PMI}}{number\ of\ amino\ acid\ residues} = \frac{1562.74}{20} = 78.14$$

The PMIr can be calculated to consider recycling of NBP/DMC (8:2) (recovery 87%) as follows:

$$PMIr = \frac{\Sigma\, mass\ of\ materials\ -\ \Sigma\, mass\ of\ recovered\ materials}{mass\ of\ isolated\ product} = \frac{218.94}{0.83} = 264.77$$

$$PMIr/residue = \frac{\text{PMIr}}{number\ of\ amino\ acid\ residues} = \frac{264.77}{20} = 13.24$$

Table S34. Overview of process mass intensity values for all synthetic protocols.

Fragment	Solvent	Protocol modifications	PMI	PMI/aa	PMIr	PMIr/aa
1-11	DMF	-	997.75	71.27	-	-
	NOP/DMC (8:2)	**A** or **A** and **B**	1328.04	94.86	226.98	16.21
	NOP/DMC (8:2)	**A, B,** and **C**	1004.13	71.72	187.54	13.40
	DMSO/EtOAc (1:9)	**A** or **A, B,** and **D**	978.46	69.89	287.70	20.55
	NBP/EtOAc (8:2)	-	1001.22	71.52	201.04	14.36
	NBP/DMC (8:2)	-	1031.85	73.70	215.26	15.38
12-31	DMF	-	1344.28	67.21	-	-
	NOP/DMC (8:2)	-	1351.90	67.60	201.23	10.06
	NBP/DMC (8:2)	-	1562.74	78.14	264.77	13.24
	DMSO/EtOAc (1:9)	-	1478.10	73.91	378.73	18.94

Quantification of trifluoroacetic acid (TFA) in crude peptides

The TFA content (%) in crude peptides was quantified via ^{19}F-NMR with an internal standard. A known amount of crude peptide was weighed out, dissolved in the selected solvent and a known amount of standard was added. The amount of TFA is then calculated as follows:

$$TFA\ content\ (\%) = \frac{\int sample}{\int STD} \times \frac{\#F_{STD}}{\#F_{sample}} \times \frac{n_{STD} \times MW_{TFA}}{m_{sample}} \times 100$$

Fragment **2** was dissolved in H_2O while fragment **3** was dissolved in H_2O+0.5% NaOH 1M. 4-F-phenethylamine (MW=139.17 g/mol; d=1.061 g/mL; solution 0.5% v/v) was selected as the internal standard to match the solubility of the peptide fragments. In this case, $\#F_{STD}$=1 and $\#F_{sample}$=3; MW_{TFA}=114.02 g/mol. The results of TFA quantification are summarized in Table S35 and reported in detail below together with the ^{19}F-NMR spectra.

Table S35. TFA quantification via ^{19}F-NMR.

Entry	Fragment	Solvent	Protocol modifications	TFA content (%)
1	H-(1-11)-CamFK-NH_2	DMF		21.03
2		NOP/DMC (8:2)	**A**, **B**, **C**	19.11
3		DMSO/EtOAc (1:9)	**A**, **B**, **D**	21.77
4		NBP/EtOAc (8:2)		26.84
5		NBP/DMC (8:2)		30.98
6	H-(12-31)-OH	DMF		15.65
7		NOP/DMC (8:2)	**A**, **E**	21.01
8		NBP/DMC (8:2)		12.42
9		DMSO/EtOAc (1:9)		18.72

Figure S41. ^{19}F-NMR spectrum of fragment **2** in DMF.

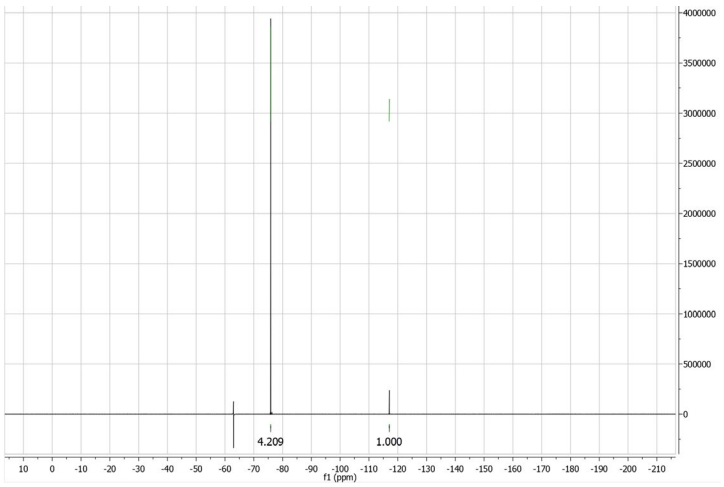


Table S36. TFA quantification for fragment **2** in DMF.

Entry	Fragment	∫sample	∫STD	STD (µL)	Sample (mg)	TFA content (%)
1	H-(1-11)-CamFK-NH_2	4.209	1.000	100	2.9	21.03

Figure S42. ^{19}F-NMR spectrum of fragment **2** in NOP/DMC (8:2), protocol modifications A, B, and C.

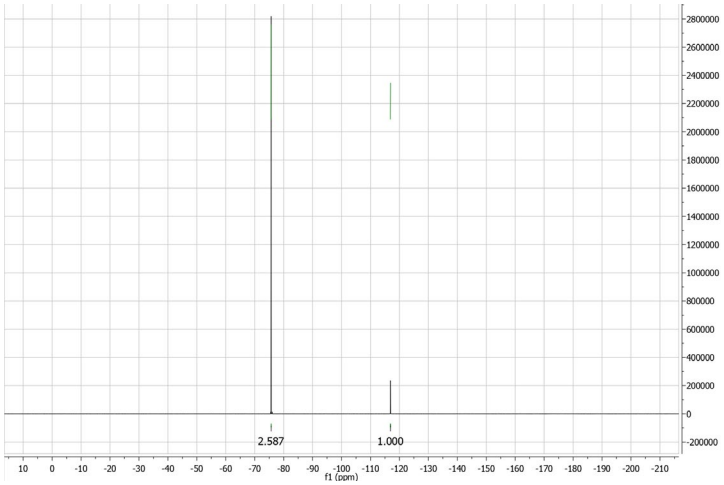


Table S37. TFA quantification for fragment **2** in NOP/DMC (8:2), protocol modifications A, B, and C.

Entry	Fragment	∫sample	∫STD	STD (µL)	Sample (mg)	TFA content (%)
2	H-(1-11)-CamFK-NH_2	2.587	1.000	200	3.8	19.11

Figure S43. ^{19}F-NMR spectrum of fragment **2** in DMSO/EtOAc (1:9), protocol modifications A, B, and D.

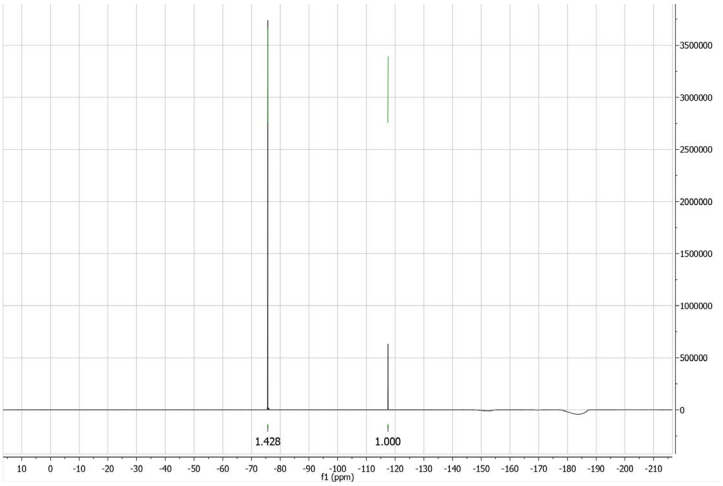


Table S38. TFA quantification for fragment **2** in DMSO/EtOAc (1:9), protocol modifications A, B, and D.

Entry	Fragment	∫sample	∫STD	STD (µL)	Sample (mg)	TFA content (%)
3	H-(1-11)-CamFK-NH_2	1.428	1.000	200	1.9	21.77

Figure S44. ^{19}F-NMR spectrum of fragment **2** in NBP/EtOAc (8:2).

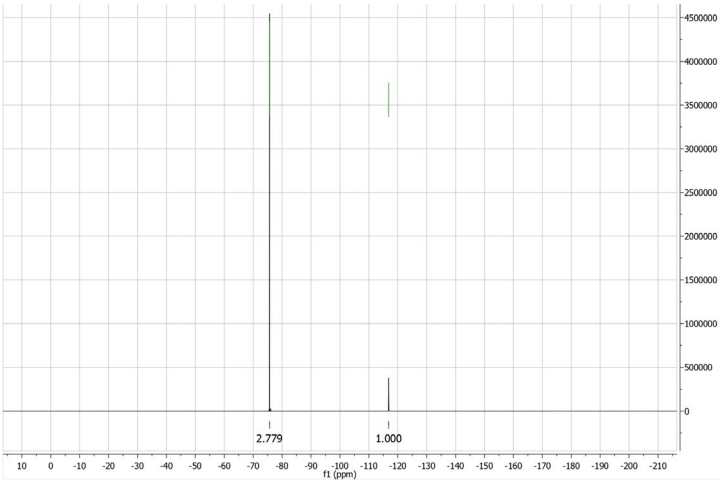


Table S39. TFA quantification for fragment **2** in NBP/EtOAc (8:2).

Entry	Fragment	∫sample	∫STD	STD (µL)	Sample (mg)	TFA content (%)
4	H-(1-11)-CamFK-NH_2	2.779	1.000	200	3.0	26.84

Figure S45. ^{19}F-NMR spectrum of fragment **2** in NBP/DMC (8:2).

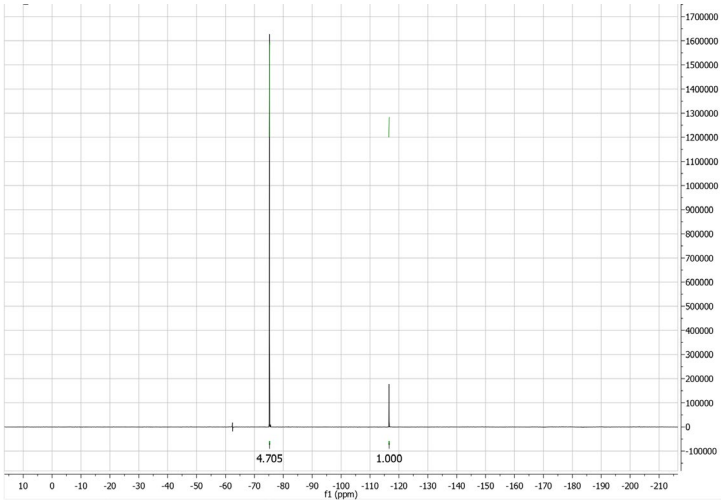


Table S40. TFA quantification for fragment **2** in NBP/DMC (8:2).

Entry	Fragment	∫sample	∫STD	STD (µL)	Sample (mg)	TFA content (%)
5	H-(1-11)-CamFK-NH_2	4.705	1.000	200	4.4	30.98

Figure S46. ^{19}F-NMR spectrum of fragment **3** in DMF

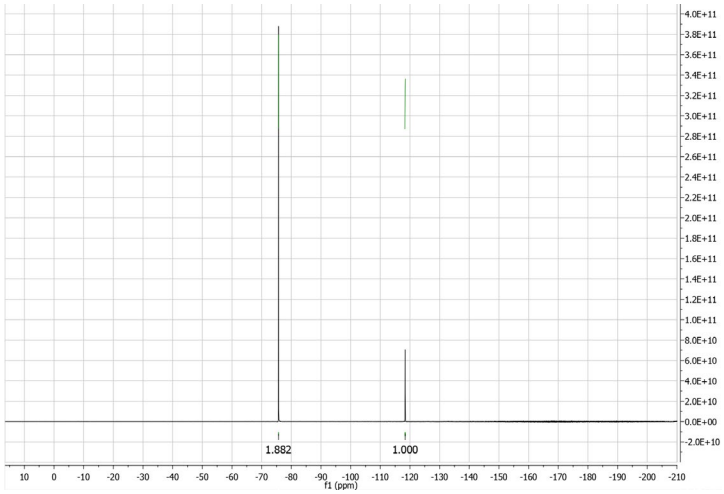


Table S41. TFA quantification for fragment **3** in DMF.

Entry	Fragment	∫sample	∫STD	STD (µL)	Sample (mg)	TFA content (%)
6	H-(12-31)-OH	1.882	1.000	200	3.3	16.52

Figure S47. ^{19}F-NMR spectrum of fragment **3** in NOP/DMC (8:2), protocol modifications **A** and **B**.

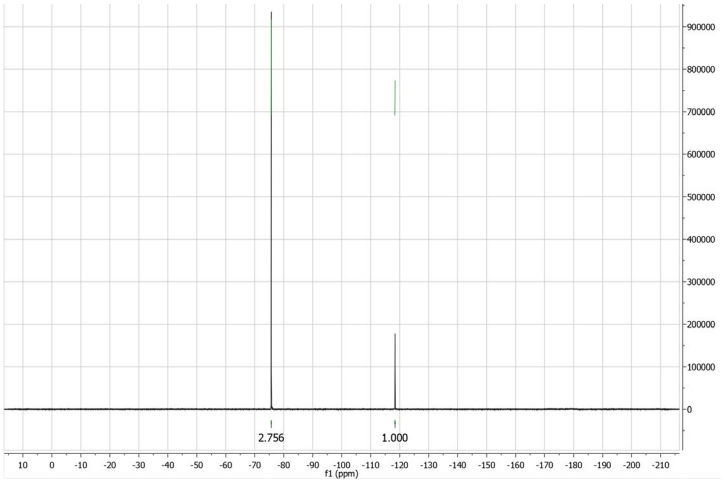


Table S42. TFA quantification for fragment **3** in NOP/DMC (8:2), protocol modifications A and B.

Entry	Fragment	∫sample	∫STD	STD (µL)	Sample (mg)	TFA content (%)
7	H-(12-31)-OH	2.756	1.000	200	3.8	21.01

Figure S48. ^{19}F-NMR spectrum of fragment **3** in NBP/DMC (8:2).

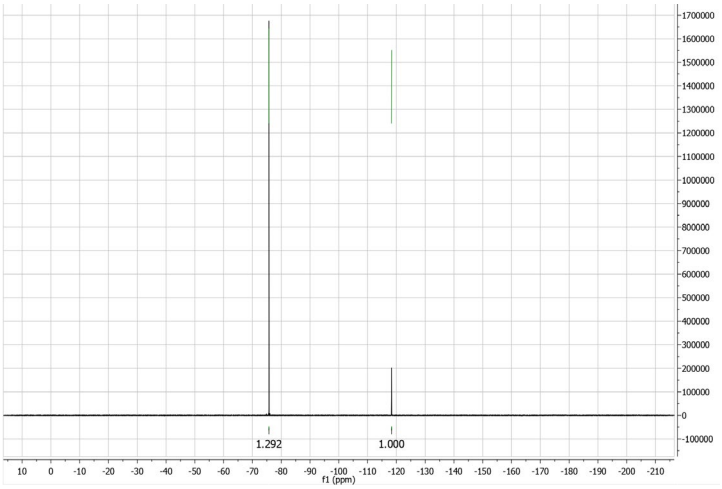


Table S43. TFA quantification for fragment **3** in NBP/DMC (8:2).

Entry	Fragment	∫sample	∫STD	STD (µL)	Sample (mg)	TFA content (%)
8	H-(12-31)-OH	1.292	1.000	200	3.0	12.48

Figure S49. ^{19}F-NMR spectrum of fragment **3** in DMSO/EtOAc (1:9).

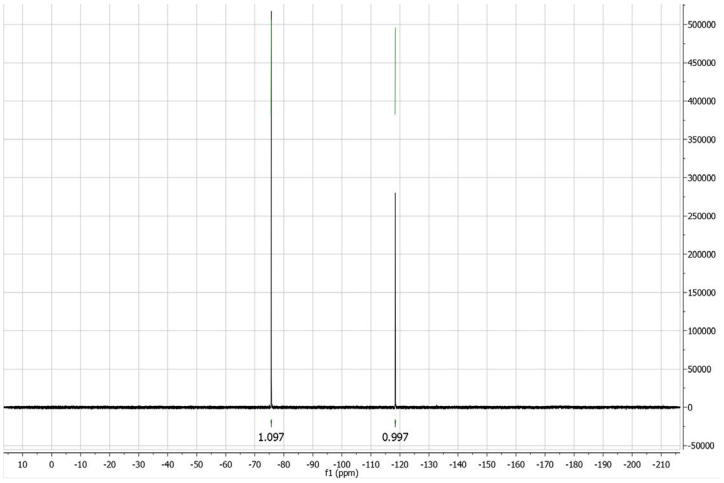


Table S44. TFA quantification for fragment **3** in NBP/DMC (8:2).

Entry	Fragment	∫sample	∫STD	STD (µL)	Sample (mg)	TFA content (%)
9	H-(12-31)-OH	1.097	1.000	200	1.7	18.69

Assay calculation in crude peptides

The Assay of crude peptide fragments was calculated via HPLC by comparison with titrated standard samples. The standard samples were injected with several concentrations and the absolute area was plotted to yield a calibration curve. The corresponding equation was then extrapolated and employed to determine the Assay of the synthesized fragment samples. For peptide **2**, both the standard and the samples to be determined were injected with Method 2. The results are summarized below:

Table S45. Injections of standard sample for the calibration curve of peptide **2**.

Entry	Amount (mg)	Volume (mL)	Area (mAU)	Titer
1	1.1	1.0	7266.6	66.0
2	2.6	1.6	9881.0	66.0
3	1.3	1.0	8329.4	66.0
4	2.3	1.0	12778.1	66.0

Figure S50. Calibration curve of peptide **2**.

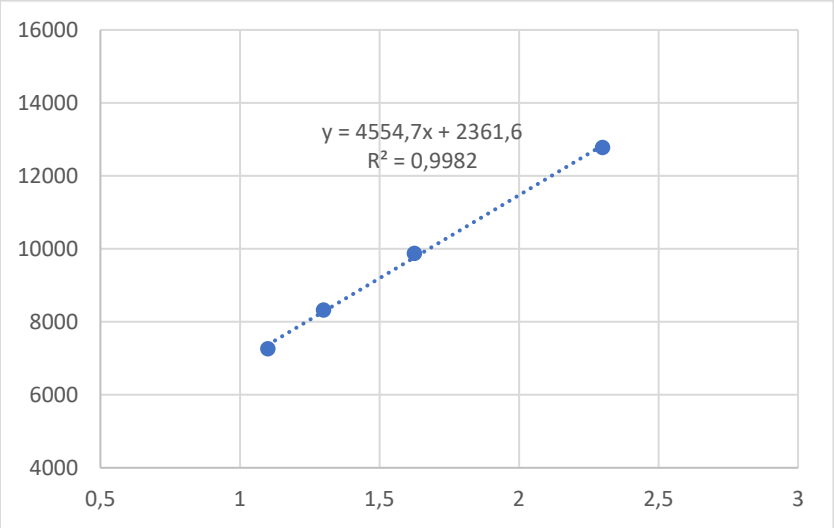


Table S46. Data from injections of peptide **2** samples for Assay quantification.

Entry	Solvent	Protocol modifications	Amount (mg)	Volume (mL)	Area (mAU)	Titer
1	DMF		1.8	1.0	7304.2	39.8
2	NOP/DMC (8:2)	**A, B, C**	1.4	1.0	5573.4	33.2
3	DMSO/EtOAc (1:9)	**A, B, D**	2.1	1.0	5578.1	22.2
4	NBP/EtOAc (8:2)		2.8	1.0	7333.6	25.7

5	NBP/DMC (8:2)		2.5	1.0	8514.6	35.7

Table S47. Injections of standard sample for the calibration curve of peptide **3**.

Entry	Amount (mg)	Volume (mL)	Area (mAU)	Titer
1	2.2	1.0	15353.7	76.3
2	1.4	1.0	9486.1	76.3
3	1.6	1.0	11540.7	76.3
4	2.5	1.5	8964.8	76.3

Figure S51. Calibration curve of peptide **3**.

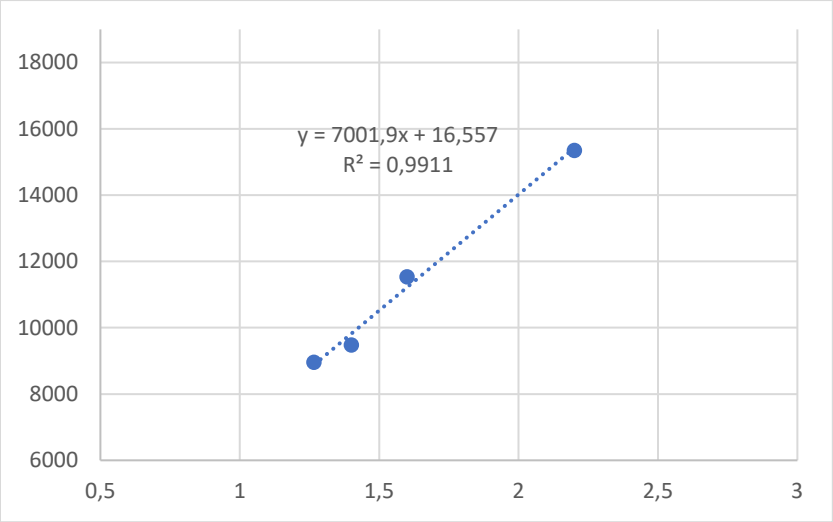


Table S48. Injections of samples of peptide **2** for Assay quantification.

Entry	Solvent	Protocol modifications	Amount (mg)	Volume (mL)	Area (mAU)	Titer
1	DMF		1.0	1.0	2828.6	37.8
2	NOP/DMC (8:2)	**A, E**	2.1	1.0	3876.9	23.2
3	NBP/DMC (8:2)		2.3	1.0	6249.2	31.9
4	DMSO/EtOAc (1:9)		1.6	1.0	4552.2	34.8