# Supporting information

# Green solvent mixture for ultrasound-assistant solid-phase peptide synthesis: a fast versatility method and its applications in flow and natural products synthesis

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# 1. General information

All commercially available reagents including the substrates were used as received. Column chromatography purifications were performed by flash chromatography using Merck silica gel 60. The reversed-phase medium pressure liquid chromatography (RP-MPLC) performed on Santai Science Inc. SepaBean® machine T with SW-5222-040-SP C18 26 × 185 mm column. The semi-preparative high-performance liquid chromatography (SP-HPLC) performed on Agilent 1260 with Nouryon Kromasil® C18 10 × 250 mm column. $^{1}$H NMR, and $^{13}$C NMR spectra were recorded using Q.One Instruments Quantum-I 400 M spectrometer. $^{1}$H NMR and $^{13}$C NMR chemical shifts were reported in parts per million (ppm) downfield from tetramethylsilane. Coupling constants (*J*) are reported in Hertz (Hz). The residual solvent peak was used as an internal reference: $^{1}$H NMR (chloroform δ 7.26) and $^{13}$C NMR (chloroform δ 77.16). The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. HRMS were obtained on Waters Xevo G2-XS QTof. UPLC-MS spectra were acquired on an Agilent Technologies 1290 Infinity LC equipped with an Agilent Technologies 6470 Quadrupole mass spectrometer. HPLC-UV spectra were acquired on an Agilent Technologies 1260.

**HPLC and UPLC-MS methods**

**Method A:**

Buffer A: 0.1% TFA in $H_2O$; Buffer B: 0.1% TFA in MeCN.
Ratio: 10-95% B in 10 min.
Flow rate: 1 mL/min
Column: ProntoSIL® C18 5 × 150 mm column, Bischoff Chromatography
Record wave length: 215 nm

**Method B:**

Buffer A: 0.1% TFA in $H_2O$; Buffer B: 0.1% TFA in MeCN.
Ratio: 20-80% B in 20 min.
Flow rate: 1 mL/min
Column: ProntoSIL® C18 5 × 150 mm column, Bischoff Chromatography
Record wave length: 215 nm

**Method C:**

Buffer A: 0.1% TFA in $H_2O$; Buffer B: 0.1% TFA in MeCN.
Ratio: 10-35% B in 6 min, 35-95% B from 6 min to 10 min, 95-10% B from 10 min to 13 min.
Flow rate: 1 mL/min
Column: ProntoSIL® C18 5 × 150 mm column, Bischoff Chromatography
Record wave length: 215 nm

**Method D:**

Buffer A: 0.1% TFA in $H_2O$; Buffer B: 0.1% TFA in MeCN.
Ratio: 10-60% B in 20 min, 60-95% B from 20 min to 25 min.
Flow rate: 1 mL/min
Column: ProntoSIL® C18 5 × 150 mm column, Bischoff Chromatography
Record wave length: 215 nm

**Method E:**

Buffer A: 0.1% TFA in $H_2O$; Buffer B: 0.1% TFA in MeCN.
Ratio: 10-90% B in 13 min, 90-10% from 13 min to 15 min.
Flow rate: 1 mL/min
Column: ProntoSIL® C18 5 × 150 mm column, Bischoff Chromatography
Record wave length: 215 nm

**Method F:**

Buffer A: 0.1% TFA in $H_2O$; Buffer B: 0.1% TFA in MeCN.
Ratio: 20-40% B in 15 min, keep 40% B from 20 min to 25 min, 40-80% B from 20 min to 25 min, 80-20% B from 25 min to 30 min.
Flow rate: 1 mL/min
Column: ProntoSIL® C18 5 × 150 mm column, Bischoff Chromatography
Record wave length: 215 nm

**UPLC-MS Method G:**

Buffer A: 0.1% TFA in $H_2O$; Buffer B: 0.1% TFA in MeCN.
Ratio: 10-60% B in 5 min, 60-95% B from 5 min to 7 min, keep 95% from 7 min to 9 min, 95-10% B from 9 min to 10 min.
Flow rate: 0.4 mL/min
Column: NanoChrom 2.1 × 50 mm, 3 μm column

## 2. Solubility assessments

For the solubility assessment of Fmoc-amino acids, coupling reagents and *N*,*N'*-diisopropylurea (DIU), 0.5 mmol of compound were weighed in 10 mL centrifuge tube. The designated solvent system (1 mL) was then added to the solid to reach a concentration of 0.5 M, and the vial was sonicated for 10 min before visual inspection. A clear solution was noted as soluble (S) while cloudy solution or solution containing insoluble material were diluted further by adding 1 mL of solvent to the centrifuge tube to reach a concentration of 0.25 M. The mixture was again sonicated for 10 min before visual inspection. A clear solution was noted as soluble at 0.25 M (S (0.25 M)) while cloudy solutions or solutions containing insoluble material were diluted further by adding 2 mL of solvent to the centrifuge tube to reach a concentration of 0.125 M. A clear solution was noted as soluble at 0.125 M (S (0.125 M)) while cloudy solutions or solutions containing insoluble material were noted as insoluble (I).

**Table S1.** Solubility screening of 10 other typical amino acids in solvents mixture

| Solvent | Fmoc-AA(PG)-OH | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Val** | **Ala** | **Leu** | **Ile** | **Gly** | **Thr(*t*Bu)** | **Cys(Trt)** | **Lys(Boc)** | **Aib** | **Pro** |
| **NCP:An** 1:1 | S | S | S | S | S | S | S | S | S | S |
| **NFM:An** 1:1 | S | S | S | S | S | S | S | S | S | S |
| **NOP:An** 1:1 | S | S | S | S | S | S | S | S | S | S |

**Fig. S1.** Representative 10 examples of solubility of Fmoc-amino acids in NFM/An 1:1

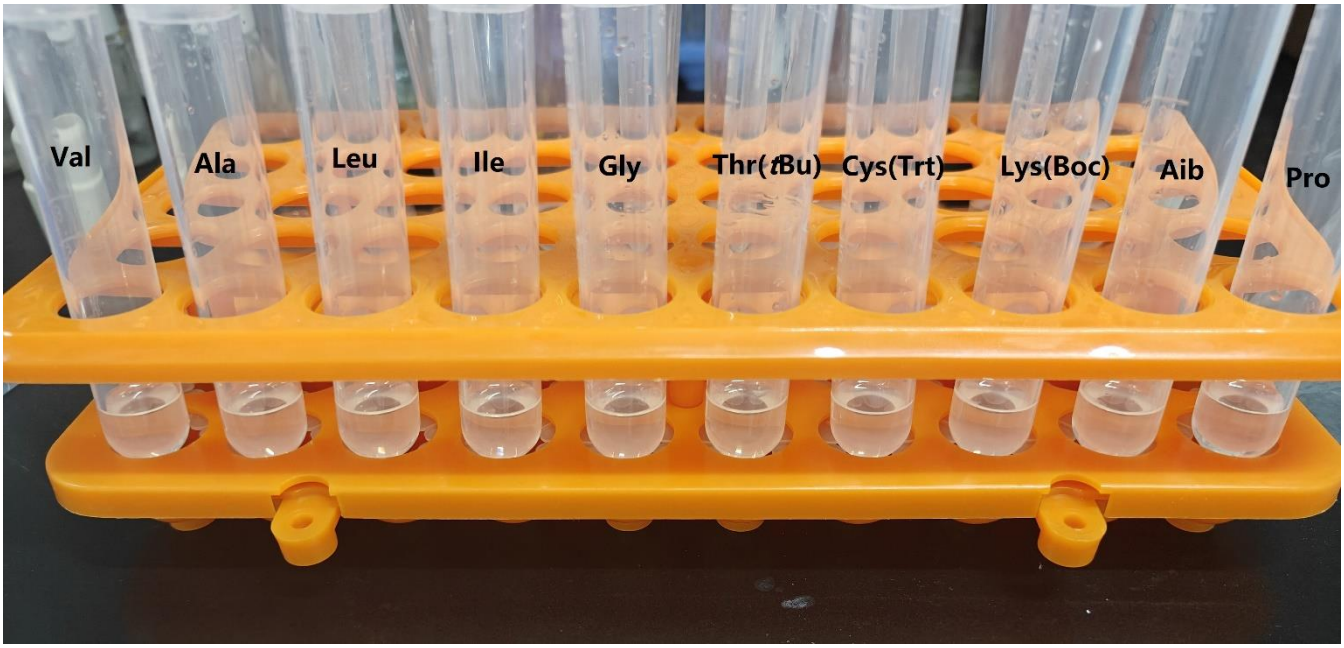



**Fig. S2. Solubility of the main coupling reagents combinations: (**A**) OxymaPure /DIC, (**B**) OxymaB/DIC, (**C**) HATU/DIPEA, and (**D**) PyAOP/DIPEA**

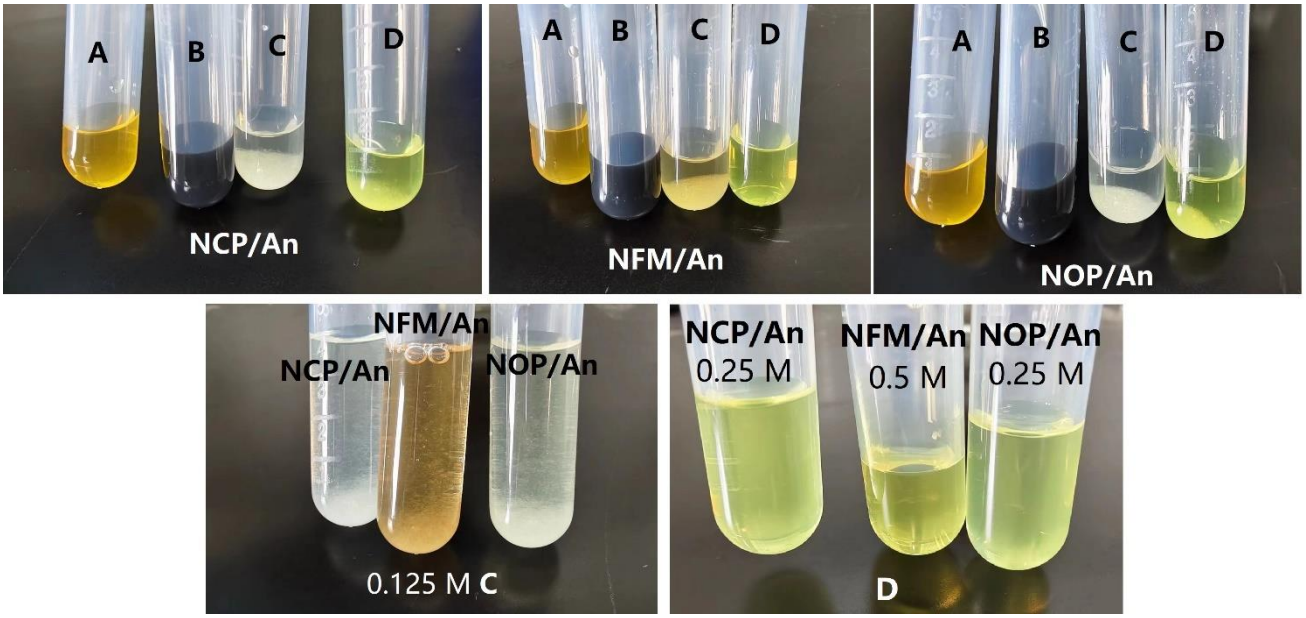
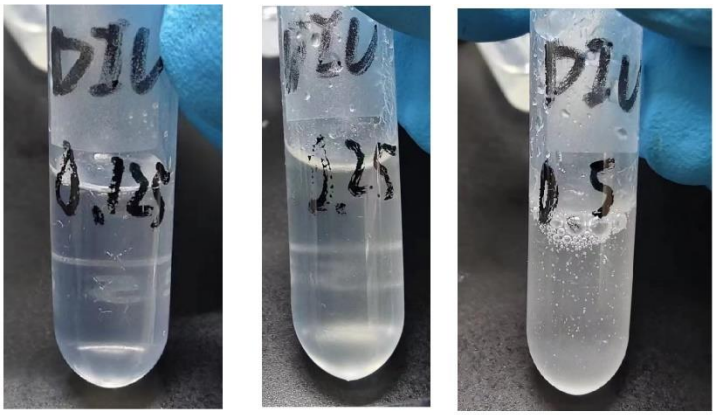


**Fig. S3** Solubility of the byproduct diisopropylurea (DIU) in NFM/An




## 3. Swelling tests

Each resin (1.0 g) was dispersed into the indicated solvent (10 mL) in a graduated syringe (30 mL), fitted with a polypropylene fritted disc at room temperature. The resin was shortly stirred with a spatula and then allowed to sonicate at room temperature for 30 mins. The solvent was drained by $N_2$ gas, after which the final resin volume was recorded. All the measurements were performed in triplicate and the data were reported as the medium value.

**Table S2.** Physical parameters of resins evaluated for swelling tests

| Resin | Bead size (mesh) | Loading (mmol/g) | Cross-linking (%) |
|---|---|---|---|
| PS-Wang | 100-200 | 1.08 | 1 |
| PS-2-CTC-Cl | 100-200 | 1.02 | 1 |
| PS-MHBA-Rink | 100-200 | 0.92 | 1 |

**Table S3.** Records for the swelling measurements of the reported resins with the related standard deviation values

| Solvent | Swell 1 | Swell 2 | Swell 3 | Swelling | STD |
|---|---|---|---|---|---|
| | | **PS-Wang resin** | | | |
| DMF | 6.0 | 6.0 | 6.2 | 6.1 | 0.09 |
| NFM/An 1:1 | 6.4 | 6.1 | 6.4 | 6.3 | 0.14 |
| NOP/An 1:1 | 8.0 | 7.9 | 7.9 | 7.9 | 0.05 |
| NCP/An 1:1 | 6.5 | 6.4 | 6.8 | 6.6 | 0.17 |
| | | **PS-2-CTC** | | | |
| DMF | 4.8 | 4.7 | 4.7 | 4.7 | 0.05 |
| NFM/An 1:1 | 5.0 | 5.1 | 50 | 5.0 | 0.05 |
| NOP/An 1:1 | 6.0 | 5.7 | 5.6 | 5.8 | 0.17 |
| NCP/An 1:1 | 6.1 | 5.7 | 5.9 | 5.9 | 0.16 |
| | | **PS-MBHA-Rink** | | | |
| DMF | 5.1 | 4.9 | 5.1 | 5.0 | 0.09 |
| NFM/An 1:1 | 5.8 | 5.9 | 6.1 | 5.9 | 0.12 |
| NOP/An 1:1 | 4.8 | 4.8 | 4.5 | 4.7 | 0.14 |
| NCP/An 1:1 | 7.1 | 7.0 | 7.2 | 7.1 | 0.08 |

## 4. Viscosity measurement

The solvent viscosities were determined using a NDJ-8S viscometer (Lichen). The cylinder-shaped spindle (0# rotor) was used at the rotation of 60 rpm. The multi-point (10-fold determination) method was used for the viscosity measurement. All measurements were performed at 20 °C in triplicates.

**Table S4.** Viscosity measurement data

| Sample name | viscosity（mPa·s） | | | measurement conditions |
|---|---|---|---|---|
| | 1 | 2 | 3 | |
| NFM-Anisole 1:1 | 2.45 | 2.43 | 2.44 | 0# rotor；60 RPM |

## 5. Melting point check

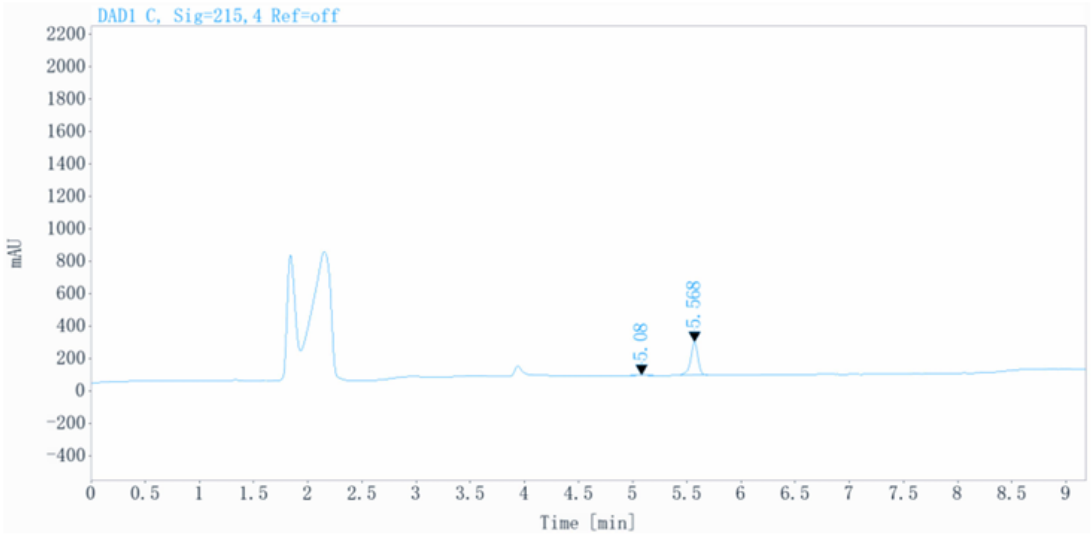
4 mL of NFM/An mixture solvents was added in a 10 mL centrifuge tube. The centrifuge tube was frozen in a refrigerator under -18 $^{o}$C for 12 h, and the status of solvent mixture was confirmed by visual inspection.

**Fig. S4** NFM/An 1:1mixture at -18 $^{o}$C frozen after 12 h

## 6. Coupling reactions kinetics in solid phase

The overall process has been modified from literature[1]. Fmoc-Gly-OH (129.5 mg, 0.436 mmol, 1.3 equiv.) and OxymaB (80.6 mg,0.436 mmol, 1.3 equiv.) were dissolved in the indicated solvent (5 mL). DIC (55.0 mg,0.436 mmol, 1.3 equiv.) was added. The clear solution was then directly added to the reactor containing pre-swelled peptide resin H-Pro-Phe-Ala-PS-2-CTC (0.5 g, loading = 0.67 mmol/g). The progress of the peptide bond formation was carried out in NFM/An under sonication and monitored by analyzing a reaction suspension (0.1 mL) at set time points (t = 0, 1, 3, 5, 7, 10, 20, and 60 min.). The withdrawn resin suspension aliquots were immediately transferred to a fritted syringe (1 mL) and filtered. The resin was further washed with NFM/An (2 × 2 mL) and subjected to Fmoc-removal using 5 % (v/v) DBU in NFM/An (2 min). After Fmoc-removal, the resin was filtered and washed with NFM/An and IPA (3 × 10 mL, alternating). About 20 mg dried resin was treated with 0.2 mL of TFA and agitated for 15 min at ambient temperature. The cleaved peptide solution was diluted with 0.8 mL MeCN and then subjected to HPLC analysis (**Method A**).

**Fig. S5.** Amino acid coupling kinetics study. HPLC analysis (λ = 215 nm) of retrieved peptide after 10 min coupling reaction in NFM/An




**Signal:** DAD1 C, Sig=215,4 Ref=off

| RT [min] | Type | Width [min] | Area | Height | Area% | Name |
|---|---|---|---|---|---|---|
| 5.080 | MM | 0.0798 | 20.1861 | 4.2175 | 2.0395 | |
| 5.568 | MM | 0.0784 | 969.5575 | 206.0839 | 97.9605 | |
| | | Sum | 989.7436 | | | |

**Fig. S6.** Amino acid coupling kinetics study. HPLC analysis (λ = 215 nm) of retrieved peptide after 1, 3, 5, 7, 10, 20, 60 min coupling reaction in NFM/An

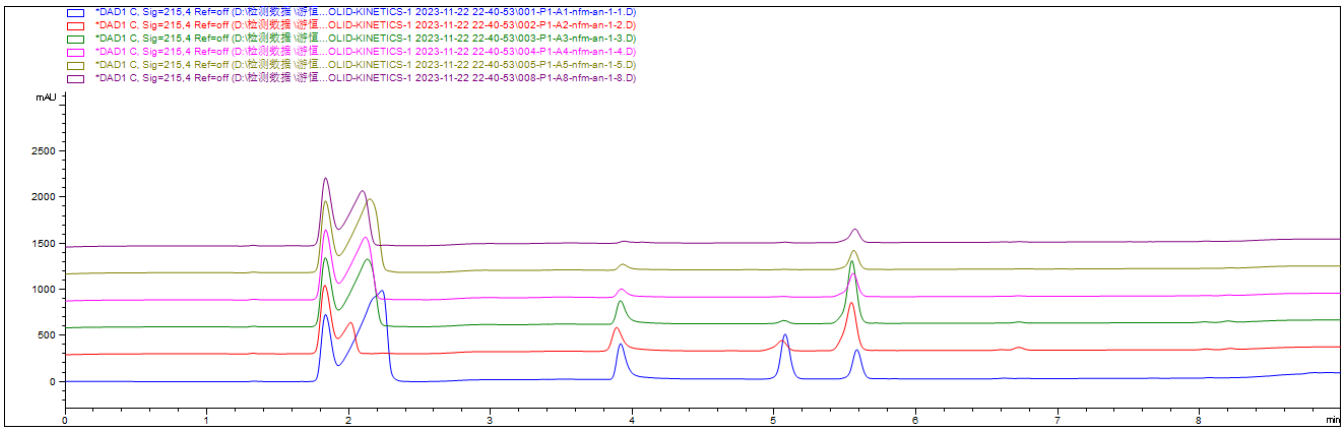
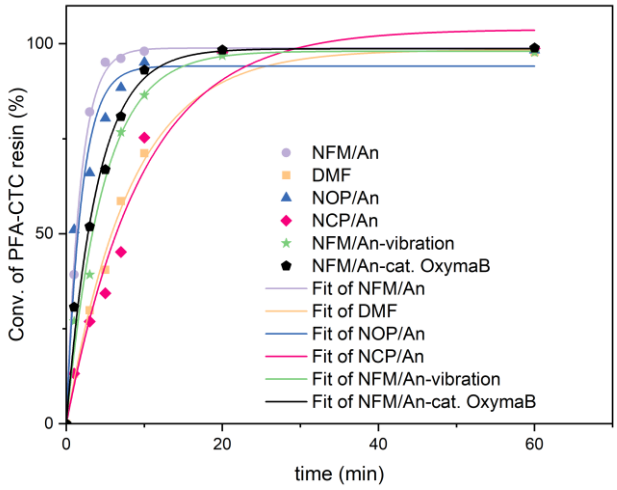


**Table S5.** Data for coupling kinetics experiments performed in various conditions depicting % completion of the coupling reaction over time.

| Time | % Coupling | | | | | |
|---|---|---|---|---|---|---|
| (min) | NFM/An | DMF | NOP/An | NCP/An | NFM/An-vibration | NFM/An-cat. OxymaB |
| 0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 1 | 39.3 | 13.2 | 51.1 | 13.2 | 27.0 | 30.7 |
| 3 | 82.1 | 29.9 | 66.0 | 26.9 | 39.3 | 51.9 |
| 5 | 95.1 | 40.5 | 80.4 | 34.4 | 66.8 | 66.9 |
| 7 | 96.2 | 58.6 | 88.5 | 45.2 | 76.8 | 80.9 |
| 10 | 98.1 | 71.2 | 95.1 | 75.3 | 86.5 | 93.1 |
| 20 | 98.0 | NA | 98.1 | 97.7 | 96.9 | 98.4 |
| 60 | 98.3 | 97.9 | 98.3 | 98.8 | 97.7 | 98.9 |

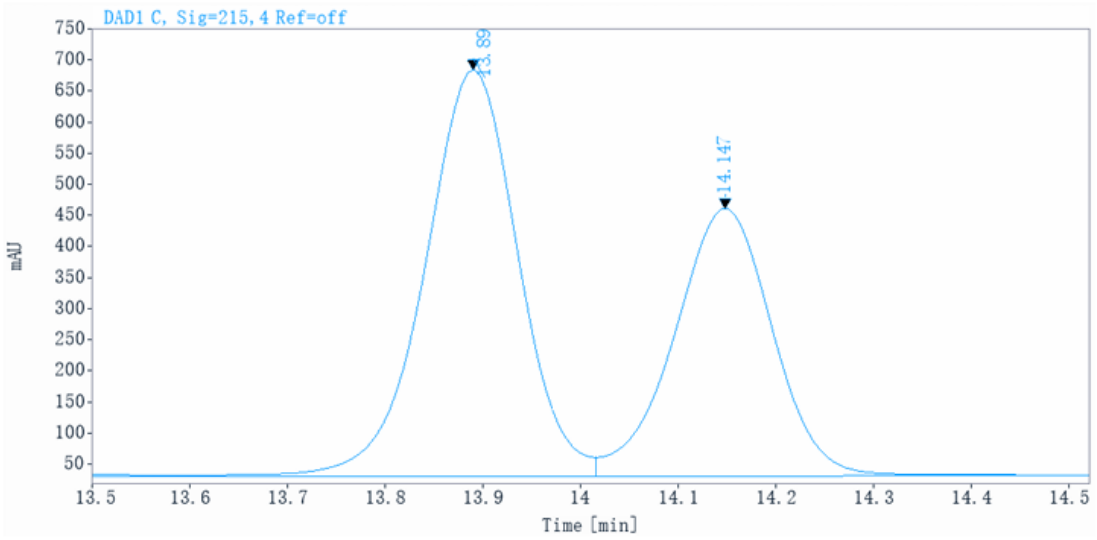
**Fig. S7** Experimental (markers) and simulated (lines) reaction kinetics for coupling reaction under different conditions. The conversion of tri- to tetrapeptide was quantified by HPLC analysis after Fmoc-removal, resin wash and peptide cleavage.




## 7. Epimerization tests

Fmoc-Phg-OH (0.22 mmol) was dissolved in a glass vial with 2 mL of solvents. The desired coupling reagent combination (0.22 mmol) was then added (OxymaPure/DIC, OxymaB/DIC, and HOBt/DIC). After 5 minutes of preactivation, H-Pro-$NH_2$ (0.2 mmol) was added. The solution was sonicated at room temperature. After 1 h from the beginning of the reaction, an aliquot (80 µL) of the solution was diluted with 0.5 mL of a 1: 2 $CH_3CN/H_2O$ mixture and injected into an HPLC system (**Method B**), in order to monitor the conversion and the racemization ratio.

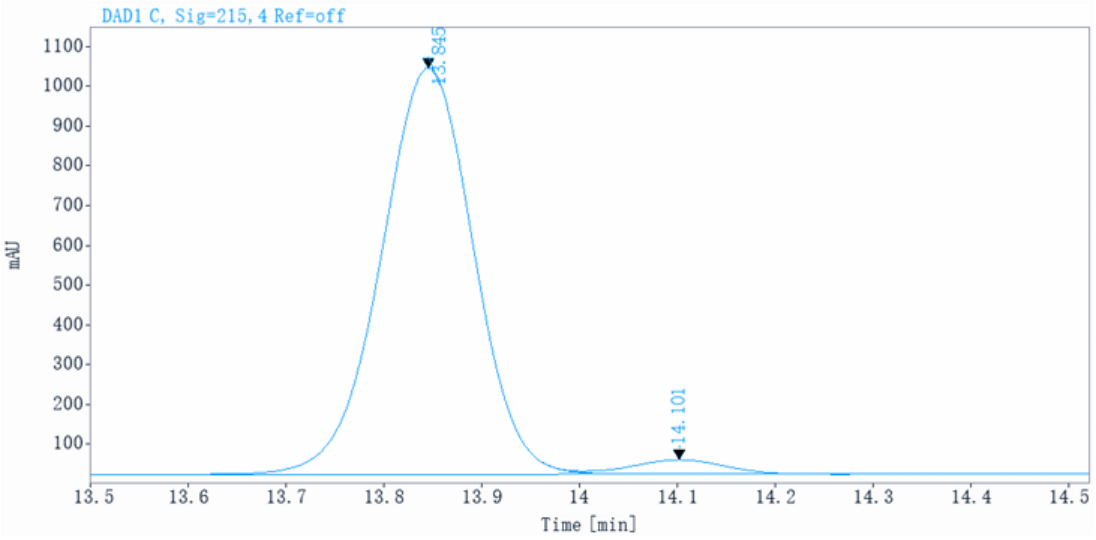
**Fig. S8.** Chromatogram of Fmoc-rac-Phg-Pro-$NH_2$, liquid phase synthesis with OxymaB by using racemic Fmoc-Phg-OH in DMF




**Signal:** DAD1 C, Sig=215,4 Ref=off

| RT [min] | Type | Width [min] | Area | Height | Area% | Name |
|---|---|---|---|---|---|---|
| 13.890 | VV R | 0.1029 | 4379.6235 | 654.8573 | 59.0904 | |
| 14.147 | VB | 0.1099 | 3032.1125 | 431.6858 | 40.9096 | |
| | | Sum | 7411.7361 | | | |

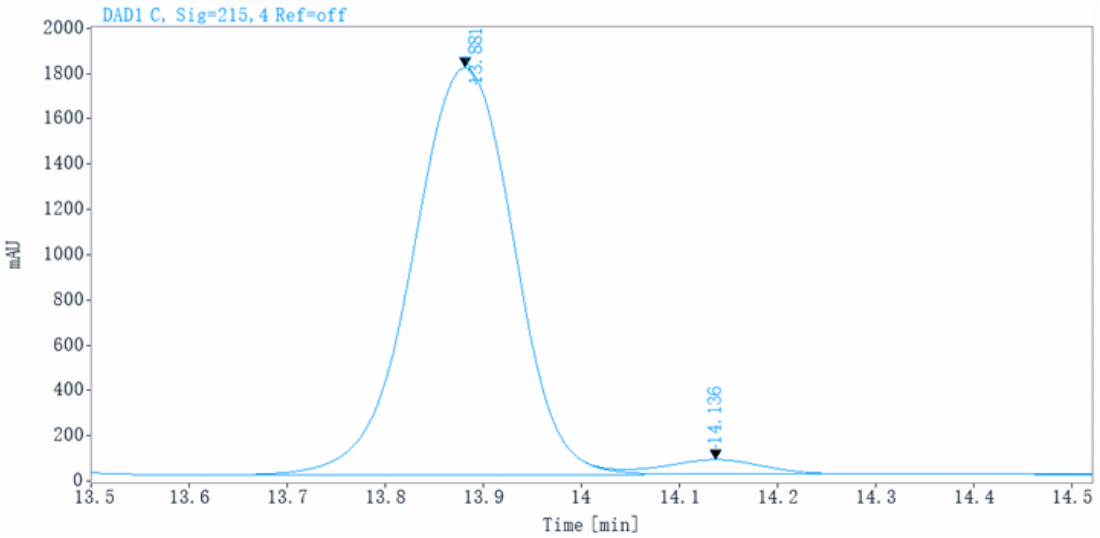
**Fig. S9.** Chromatogram of Fmoc-Phg-Pro-$NH_2$, liquid phase synthesis with OxymaB in NFM




**Signal:** DAD1 C, Sig=215,4 Ref=off

| RT [min] | Type | Width [min] | Area | Height | Area% | Name |
|---|---|---|---|---|---|---|
| 13.845 | VV R | 0.1005 | 6763.7778 | 1024.2996 | 96.3981 | |
| 14.101 | VV E | 0.1054 | 252.7262 | 37.1162 | 3.6019 | |
| | | Sum | 7016.5041 | | | |

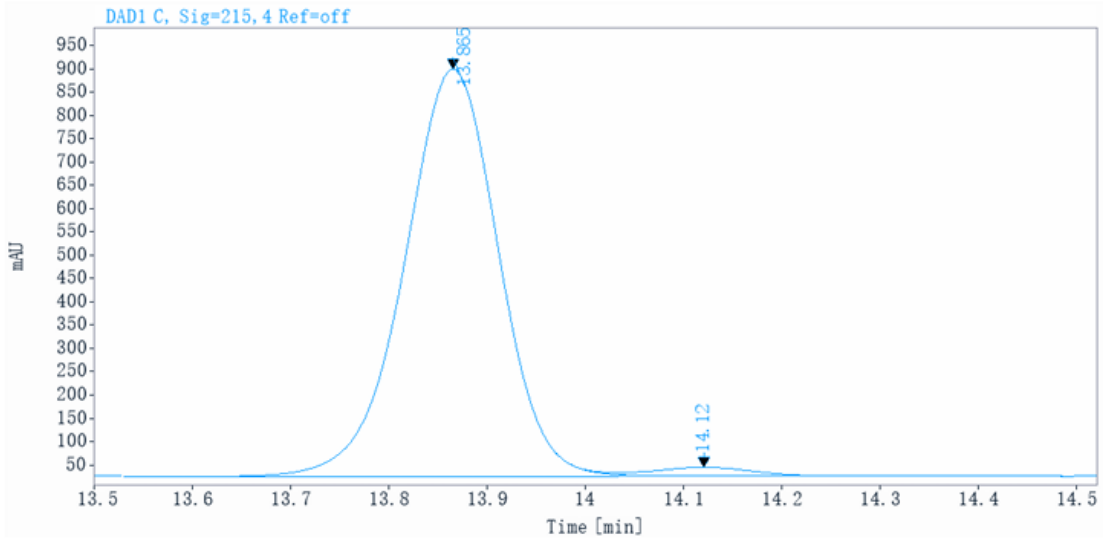
**Fig. S10.** Chromatogram of Fmoc-Phg-Pro-$NH_2$, liquid phase synthesis with OxymaB in DMF




**Signal:** DAD1 C, Sig=215,4 Ref=off

| RT [min] | Type | Width [min] | Area | Height | Area% | Name |
|---|---|---|---|---|---|---|
| 13.881 | BV R | 0.1134 | 12908.9111 | 1800.3788 | 96.3283 | |
| 14.136 | VV E | 0.1121 | 492.0391 | 66.6868 | 3.6717 | |
| | | Sum | 13400.9503 | | | |

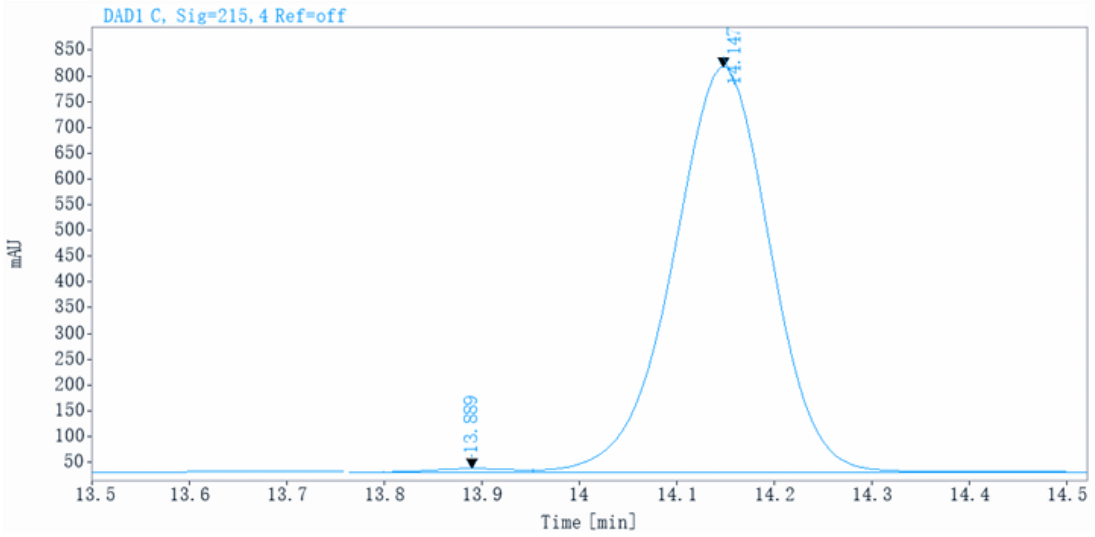
**Fig. S11.** Chromatogram of Fmoc-Phg-Pro-$NH_2$, liquid phase synthesis with OxymaB in NFM/An




**Signal:** DAD1 C, Sig=215,4 Ref=off

| RT [min] | Type | Width [min] | Area | Height | Area% | Name |
|---|---|---|---|---|---|---|
| 13.865 | BV R | 0.1014 | 5833.1313 | 876.7205 | 97.4998 | |
| 14.120 | VV E | 0.1146 | 149.5782 | 19.6999 | 2.5002 | |
| | | Sum | 5982.7096 | | | |

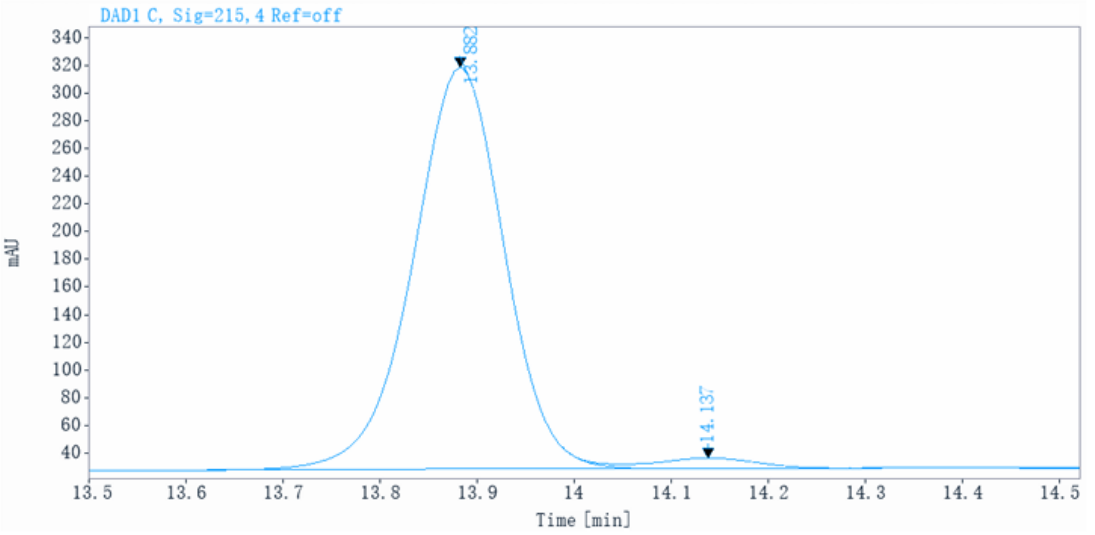
**Fig. S12.** Chromatogram of Fmoc-D-Phg-Pro-$NH_2$, liquid phase synthesis with OxymaB in NFM/An




**Signal:** DAD1 C, Sig=215,4 Ref=off

| RT [min] | Type | Width [min] | Area | Height | Area% | Name |
|---|---|---|---|---|---|---|
| 13.889 | MF | 0.1051 | 52.9523 | 8.3997 | 0.9567 | |
| 14.147 | FM | 0.1157 | 5481.8237 | 789.4664 | 99.0433 | |
| | | Sum | 5534.7761 | | | |

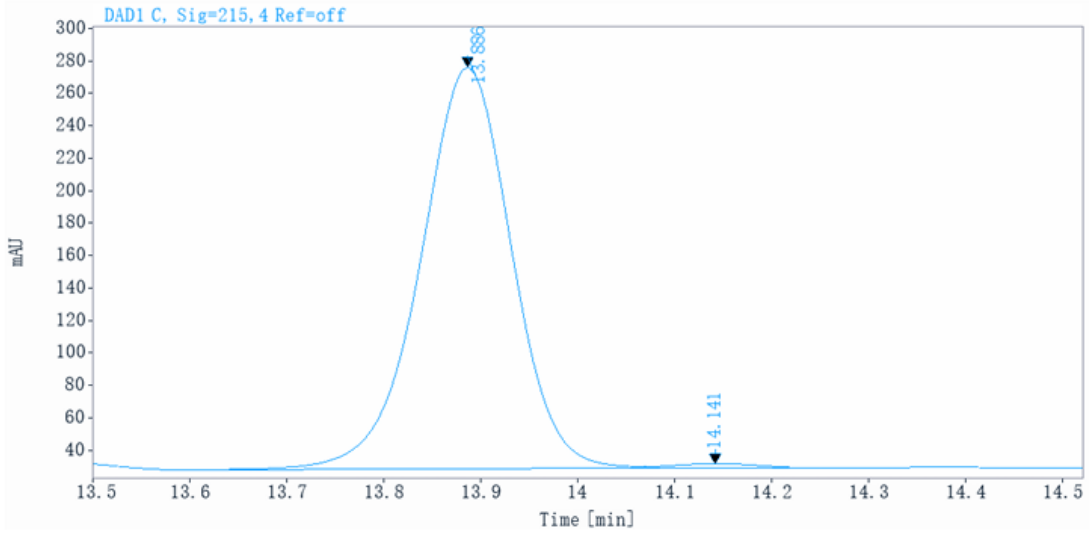
**Fig. S13.** Chromatogram of Fmoc-Phg-Pro-$NH_2$, liquid phase synthesis with OxymaPure in NFM/An




**Signal:** DAD1 C, Sig=215,4 Ref=off

| RT [min] | Type | Width [min] | Area | Height | Area% | Name |
|---|---|---|---|---|---|---|
| 13.882 | BV R | 0.1027 | 1917.3374 | 291.5707 | 96.7227 | |
| 14.137 | VV E | 0.1172 | 64.9650 | 8.1347 | 3.2773 | |
| | | Sum | 1982.3024 | | | |

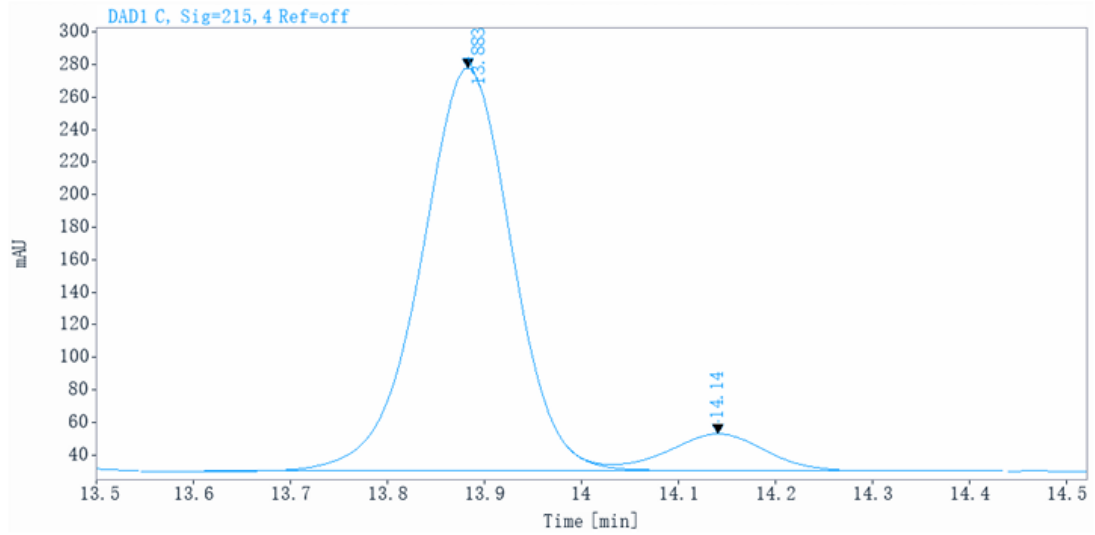
**Fig. S14.** Chromatogram of Fmoc-Phg-Pro-$NH_2$, liquid phase synthesis with catalytic amount of OxymaB in NFM/An




**Signal:** DAD1 C, Sig=215,4 Ref=off

| RT [min] | Type | Width [min] | Area | Height | Area% | Name |
|---|---|---|---|---|---|---|
| 13.886 | MF | 0.1087 | 1620.4355 | 248.4585 | 98.7570 | |
| 14.141 | FM | 0.1099 | 20.3947 | 3.0932 | 1.2430 | |
| | | Sum | 1640.8303 | | | |

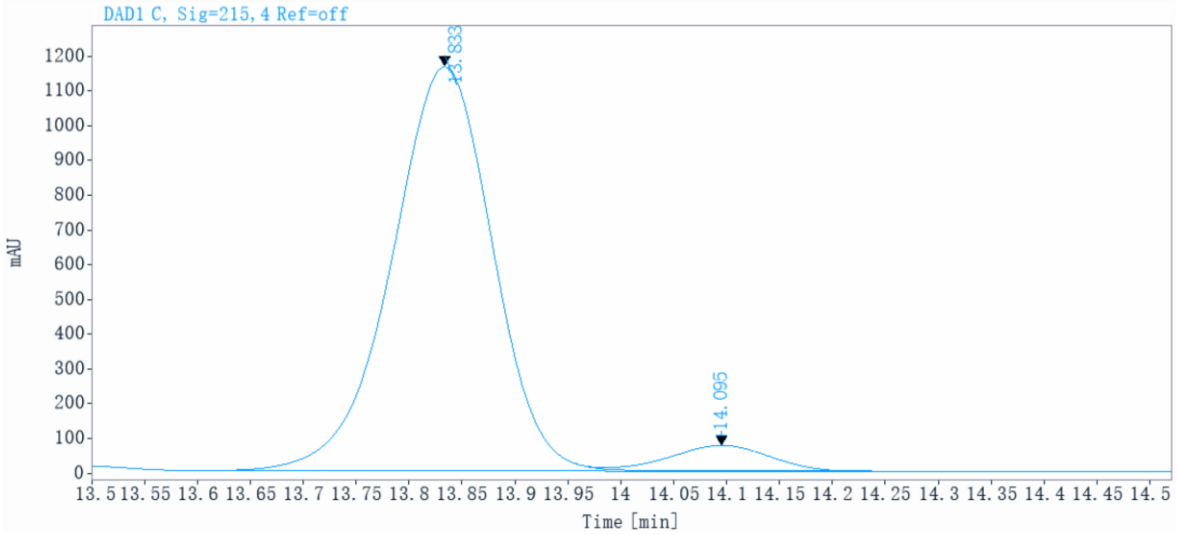
**Fig. S15.** Chromatogram of Fmoc-Phg-Pro-$NH_2$, liquid phase synthesis with HOBt in NFM/An




**Signal:** DAD1 C, Sig=215,4 Ref=off

| RT [min] | Type | Width [min] | Area | Height | Area% | Name |
|---|---|---|---|---|---|---|
| 13.883 | BV R | 0.1000 | 1618.3219 | 248.5504 | 91.0659 | |
| 14.140 | VB E | 0.1070 | 158.7675 | 22.8597 | 8.9341 | |
| | | Sum | 1777.0894 | | | |

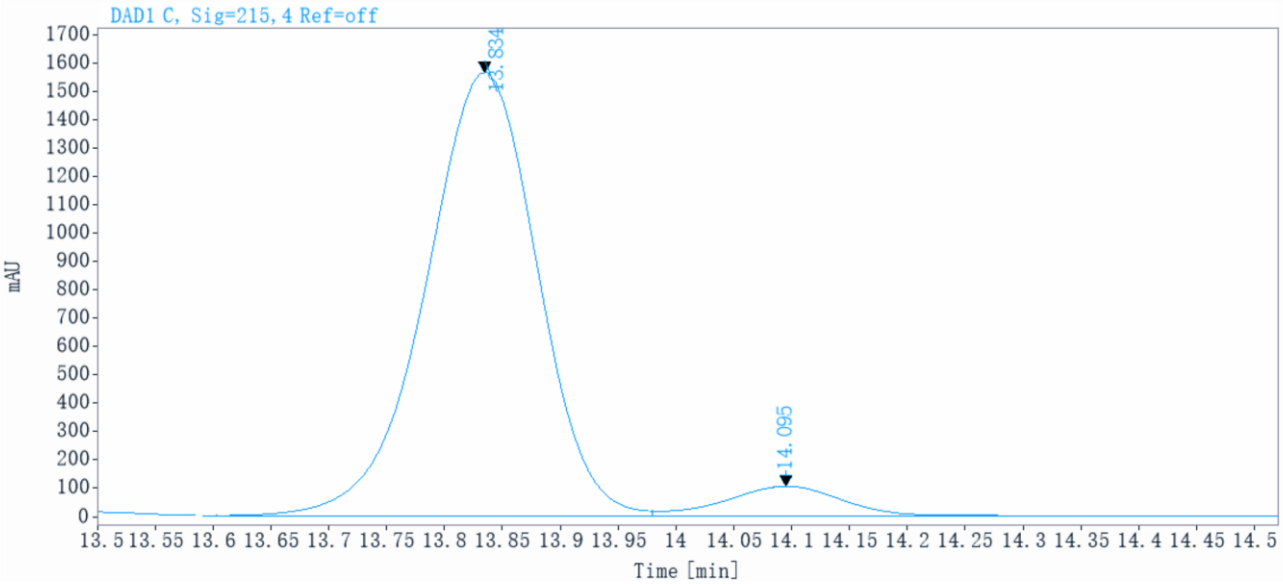
**Fig. S16.** Chromatogram of Fmoc-Phg-Pro-$NH_2$, liquid phase synthesis with HATU in NFM/An




**Signal:** DAD1 C, Sig=215,4 Ref=off

| RT [min] | Type | Width [min] | Area | Height | Area% | Name |
|---|---|---|---|---|---|---|
| 13.833 | BV R | 0.1030 | 7691.0186 | 1165.5574 | 93.8138 | |
| 14.095 | VB E | 0.1051 | 507.1532 | 74.7977 | 6.1862 | |
| | | Sum | 8198.1718 | | | |

**Fig. S17.** Chromatogram of Fmoc-Phg-Pro-$NH_2$, liquid phase synthesis with PyAOP in NFM/An



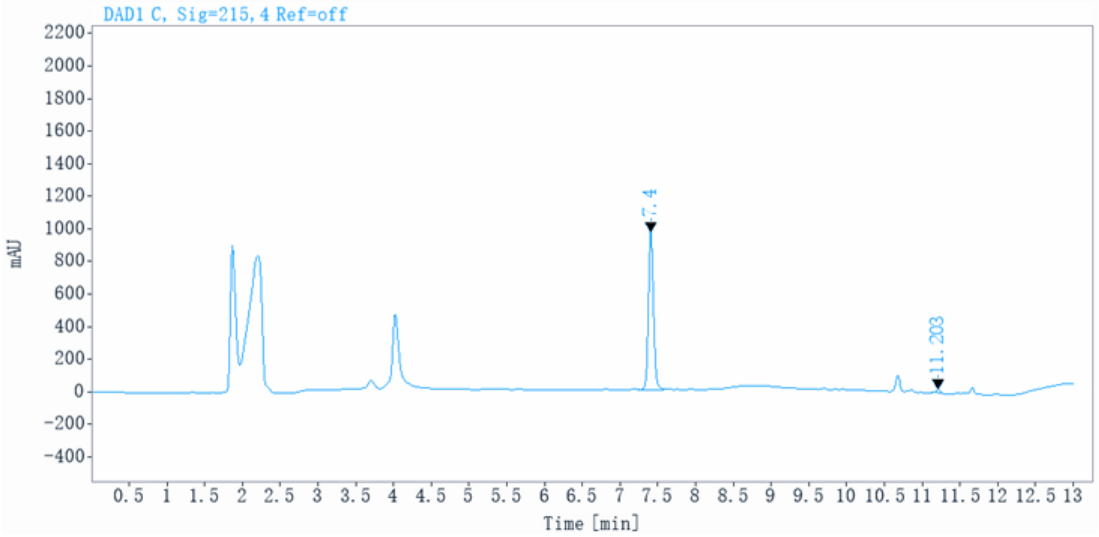

**Signal:** DAD1 C, Sig=215,4 Ref=off

| RT [min] | Type | Width [min] | Area | Height | Area% | Name |
|---|---|---|---|---|---|---|
| 13.834 | MF | 0.1111 | 10460.6270 | 1569.0165 | 93.3483 | |
| 14.095 | FM | 0.1175 | 745.3865 | 105.7464 | 6.6517 | |
| | | Sum | 11206.0135 | | | |

# 8. Deprotection tests

The total progress is modified from literature[1]. A solution of 5% (v/v) DBU in the designated solvent (1 mL) was added to a reactor (5 mL syringe) containing pre-swelled (2 × in the candidate solvent) Fmoc-Ala-Phe-Phe-Ala-CTC (0.1 g, loading = 0.45 mmol/g) under sonication for 1 mins. The peptide cleavage was performed by treating 100 mg of peptidyl resin with 1 mL of TFA/$H_2O$ (95:5, v/v) mixture for 2 h at ambient temperature. 50 μL of cleavage solution was diluted with MeCN (1 mL) and directly subjected for HPLC analysis. (**Method C**).

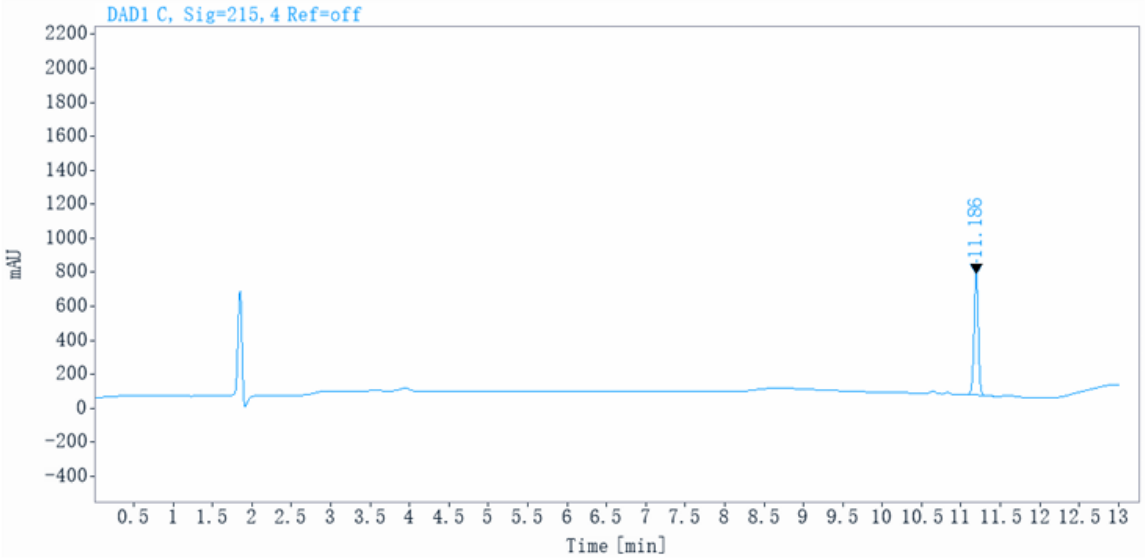
**Fig. S18.** Chromatogram of H-Ala-Phe-Phe-Ala-OH, after 1 mins deprotection with 5% DBU in NFM/An




**Signal:** DAD1 C, Sig=215,4 Ref=off

| RT [min] | Type | Width [min] | Area | Height | Area% | Name |
|---|---|---|---|---|---|---|
| 7.400 | MM | 0.0790 | 4621.1934 | 975.2328 | 98.2574 | |
| 11.203 | MM | 0.0604 | 81.9564 | 22.6158 | 1.7426 | |
| | | Sum | 4703.1498 | | | |

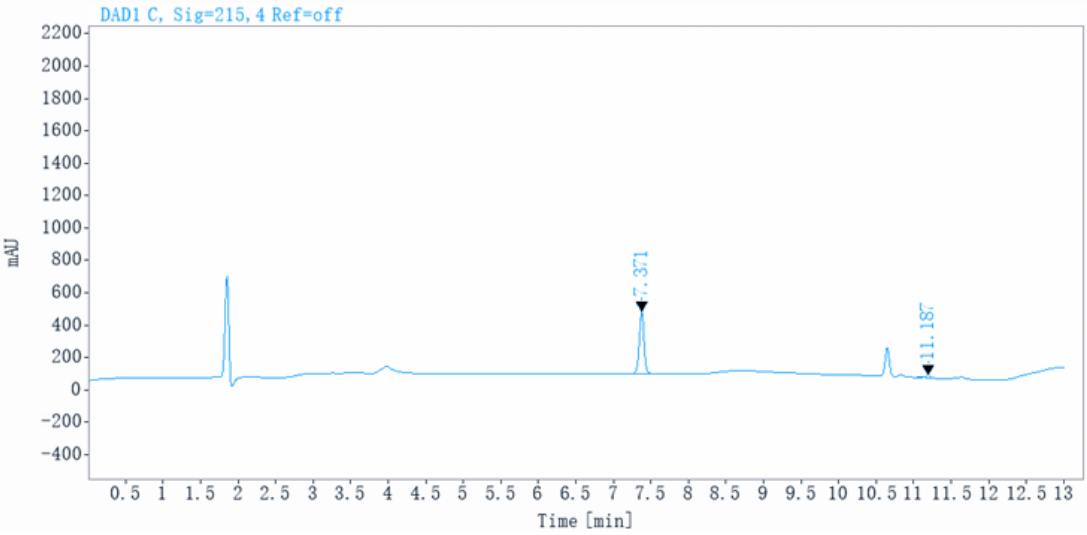
**Fig. S19.** Chromatogram of Fmoc-Ala-Phe-Phe-Ala-OH before deprotection




Signal: DAD1 C, Sig=215,4 Ref=off

| RT [min] | Type | Width [min] | Area | Height | Area% | Name |
|---|---|---|---|---|---|---|
| 11.186 | BBA | 0.0569 | 2644.1243 | 718.7830 | 100.0000 | |
| | | Sum | 2644.1243 | | | |

**Fig. S20.** Chromatogram of H-Ala-Phe-Phe-Ala-OH, after 20 mins deprotection with 20% 4-methylpiperidine in DMF




Signal: DAD1 C, Sig=215,4 Ref=off

| RT [min] | Type | Width [min] | Area | Height | Area% | Name |
|---|---|---|---|---|---|---|
| 7.371 | BBA | 0.0676 | 1705.7898 | 385.6819 | 95.9009 | |
| 11.187 | MM | 0.0678 | 72.9101 | 17.9312 | 4.0991 | |
| | | Sum | 1778.6999 | | | |

## 9. First loading tests

The first loading of the resin was quantified *via* the following procedure: a defined amount (about 20 mg) of the dry resin was weighed in a volumetric flask, and subsequently, the flask was filled to 100 mL

with 20% 4-methyl piperidine in DMF to remove the Fmoc-protecting group. The sample was shaken at 23 °C and kept for 3 h. The absorbance of the respective solution was measured with a Shimadzu UV-2600 spectrophotometer. A blank (20% 4-methyl piperidine in DMF) was read, and subsequently, the absorbance of the released dibenzofulvene−4-methyl piperidine adduct in solution was quantified spectrophotometrically at 289.8 nm[2]. The described procedure was performed threefold, and the obtained resin substitution values were combined to a mean value (max. tolerated deviation from measured values: ±0.02 mmol/g).

Based on the following formula, the calculation of the resin substitution was made

$$S = \frac{Abs \times V \times 1000}{\varepsilon \times l \times m}$$

where $S$ = substitution [mmol/g], $Abs$ = absorbance at 289.8 nm, $V$ = final volume [mL], $\varepsilon$ = extinction coefficient (6089 L $mol^{-1}$ $cm^{-1}$), $l$ = light path length [cm], and $m$ = resin weight [mg].

**Table S6.** Data for first loading experiments performed in various conditions for different resin and peptides.

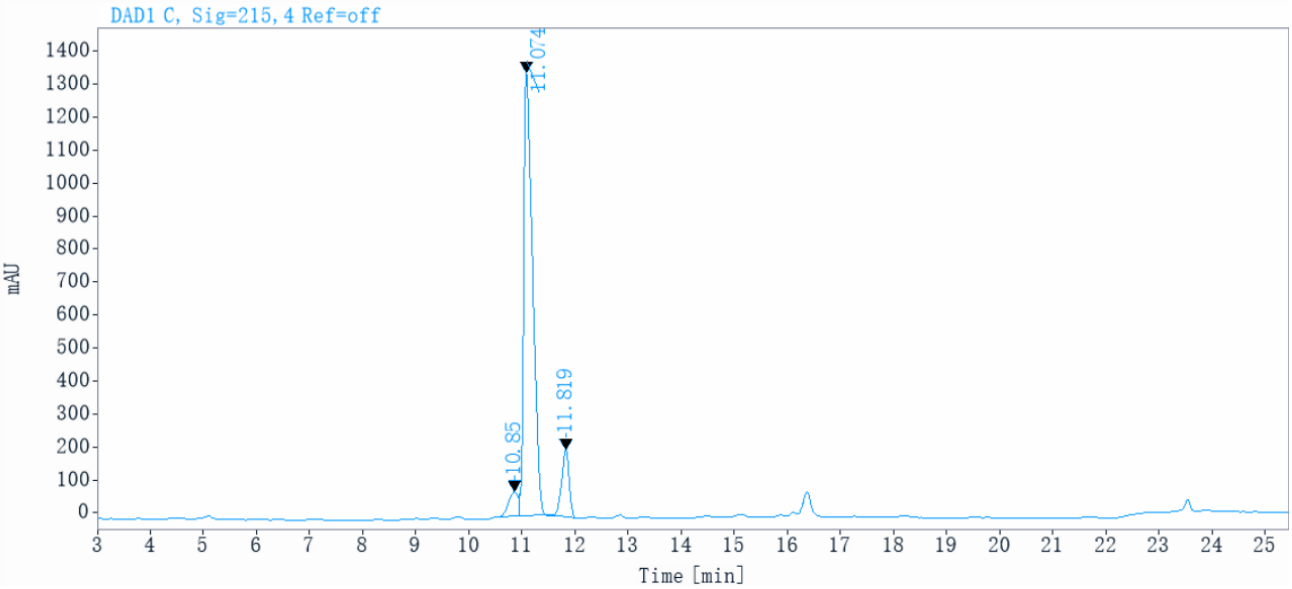
| Resins | AAs | Mass (mg) | Abs | S (mmol/g) | maximum theoretical value (mmol/g) | Loading efficiency (%) |
|---|---|---|---|---|---|---|
| **CTC** | | | | | | |
| NFM-AN | Fmoc-Pro-OH | 20.4 | 0.712 | 0.573 | 0.792 | 72 |
| Traditional | Fmoc-Pro-OH | 20.2 | 0.684 | 0.556 | 0.792 | 70 |
| NFM-AN | Fmoc-Ala-OH | 24.0 | 0.853 | 0.584 | 0.809 | 72 |
| Traditional | Fmoc-Ala-OH | 20.2 | 0.673 | 0.547 | 0.809 | 68 |
| NFM-AN | Fmoc-Leu-OH | 22.3 | 0.785 | 0.578 | 0.782 | 74 |
| Traditional | Fmoc-Leu-OH | 21.2 | 0.738 | 0.572 | 0.782 | 73 |
| NFM-AN | Fmoc-Phe-OH | 21.3 | 0.687 | 0.530 | 0.762 | 70 |
| Traditional | Fmoc-Phe-OH | 20.3 | 0.735 | 0.595 | 0.762 | 78 |
| NFM-AN | Fmoc-Glu-OAll | 19.8 | 0.698 | 0.579 | 0.749 | 77 |
| Traditional | Fmoc-Glu-OAll | 19.4 | 0.658 | 0.557 | 0.749 | 74 |
| **PS-Wang** | | | | | | |
| NFM-AN | Fmoc-Leu-OH | 20.3 | 0.814 | 0.659 | 0.793 | 83 |
| Traditional | Fmoc-Leu-OH | 20.6 | 0.846 | 0.674 | 0.793 | 85 |
| NFM-AN | Fmoc-Pro-OH | 19.2 | 0.854 | 0.730 | 0.803 | 91 |
| Traditional | Fmoc-Pro-OH | 19.2 | 0.822 | 0.689 | 0.803 | 86 |
| PS-MHBA-Rink | | | | | | |
| NFM-AN | Fmoc-Leu-OH | 18.8 | 0.801 | 0.700 | 0.833 | 84 |
| Traditional | Fmoc-Leu-OH | 21.6 | 0.974 | 0.741 | 0.833 | 89 |
| NFM-AN | Fmoc-Pro-OH | 19.6 | 0.875 | 0.733 | 0.844 | 87 |
| Traditional | Fmoc-Pro-OH | 26.3 | 1.193 | 0.745 | 0.844 | 88 |

# 10. SPPS of challenging model peptides on US-GSPPS

Challenging model peptides manual syntheses were carried out at 30-35 °C under sonication in polyethylene syringes fitted with a polyethylene porous disc and connected to a nitrogen source to remove excess reagents and solvents. 0.2 g of preloaded Fmoc-resin was utilized. After the swelling of the resin in 2 mL of NFM/An, the Fmoc protective group was removed by 5% DBU in NFM/An (1 times × 2 mL, 2 min) and then the resin was washed with the selected solvent (3 times × 1.5 mL, 1 min each). Corresponding Fmoc-protected amino acids (three-fold excess with respect to the loading of the resin) were pre-activated by OxymaB (0.6 equiv. with respect to the loading of the resin) and DIC (three-fold excess of the reagents with respect to the loading of the resin) for 1 minutes and coupled to the resin for 10 minutes. In the case of both Aib residues in the sequence, the coupling of Fmoc-Aib-OH was repeated a second time. After each coupling step, the resin was washed with the EtOH and NFM/An (3 times each solvent × 2 mL, 0.5 min each). And then, the Fmoc protective group was removed by treating the peptide resin with 5% DBU in NFM/An (1 times × 2 mL, 2 min) and the resin was washed with the selected solvent (3 times × 1.5 mL, 1 min each). After the Fmoc cleavage of the N-terminal amino group, the peptide resin was further washed with EtOH (3 times × 2 mL, 1 min each) and dried under a vacuum for 12 hours. The dry peptide resin was suspended in 5 mL of the TFA/TIS/$H_2O$ (95/2.5/2.5 v/v/v) mixture and stirred for 2 h. The resin was filtered off, washed with TFA (1 time × 1 mL, 1 min) and diisopropylether (25 mL) cooled to 4 °C was added to the solution dropwise. The peptide was filtered and

dried in vacuo to obtain crude peptide that was directly analysed by HPLC (Method D or Method E) and LC-MS.

**Fig. S21.** HPLC and LCMS Chromatogram of H-Y-Aib-Aib-FL-OH, manual SPPS in NFM/An on PS-2-CTC-Cl resin




**Signal:** DAD1 C, Sig=215,4 Ref=off

| RT [min] | Type | Width [min] | Area | Height | Area% | Name |
|---|---|---|---|---|---|---|
| 10.850 | MF | 0.2220 | 1004.3253 | 75.4159 | 5.3932 | |
| 11.074 | FM | 0.1928 | 15578.4121 | 1346.5510 | 83.6559 | |
| 11.819 | BB | 0.1543 | 2039.2817 | 204.5023 | 10.9509 | |
| | | Sum | 18622.0192 | | | |

| Peak no. | RT (min) | Compound | Relative area |
|---|---|---|---|
| 1 | 10.850 | H-Y-Aib-Aib-L-$OH_2$ (des-F, m/z = 465) | 5.4 |
| 2 | 11.074 | Target molecule: H-Y-Aib-Aib-FL-$OH_2$ | 83.6 |
| 3 | 11.819 | H-Y-Aib-FL-$OH_2$ (des-Aib, m/z = 527) | 11.0 |

Target molecule: H-Y-Aib-Aib-FL-$OH_2$

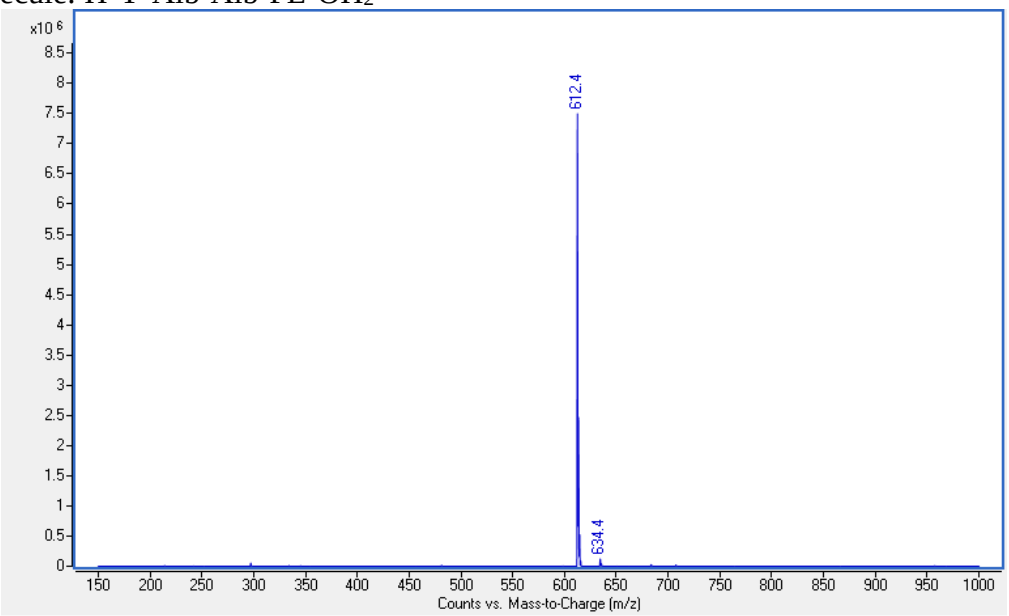
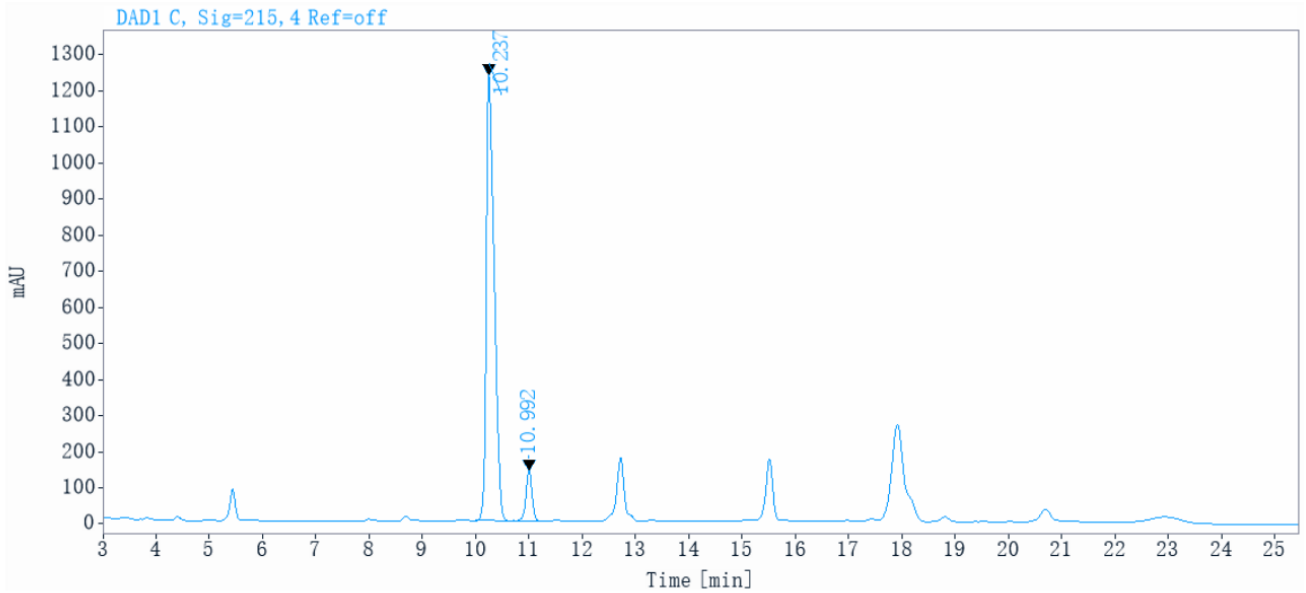


**Fig. S22.** HPLC and LCMS Chromatogram of H-Y-Aib-Aib-FL-$NH_2$, manual SPPS in NFM/An on PS-MBHA-Rink resin




**Signal:** DAD1 C, Sig=215,4 Ref=off

| RT [min] | Type | Width [min] | Area | Height | Area% | Name |
|---|---|---|---|---|---|---|
| 10.237 | BV | 0.1598 | 12681.7979 | 1235.1094 | 91.8796 | |
| 10.992 | VB | 0.1218 | 1120.8356 | 139.4028 | 8.1204 | |
| | | Sum | 13802.6334 | | | |

| Peak no. | RT (min) | Compound | Relative area |
|---|---|---|---|
| 1 | 10.237 | Target molecule: H-Y-Aib-Aib-FL-$NH_2$ | 91.9 |
| 2 | 10.992 | H-Y-Aib-FL-$NH_2$ (des-Aib, m/z = 526) | 8.1 |

The other impurities are non-peptide impurities. They likely originate from the cleavage of the MBHA-rink-resin.

Target molecule: H-Y-Aib-Aib-FL-$NH_2$

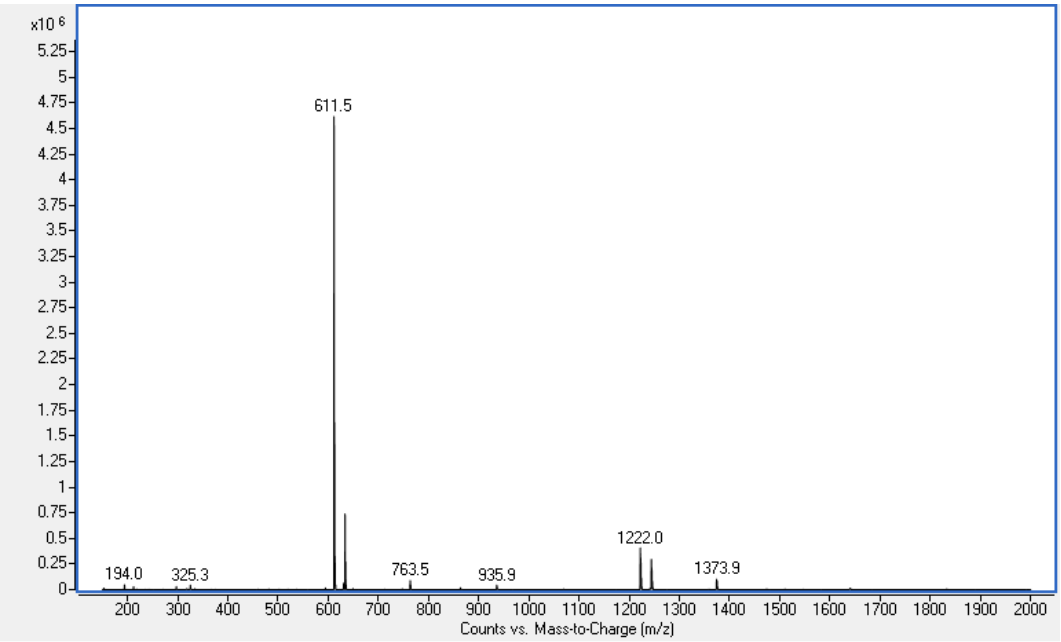
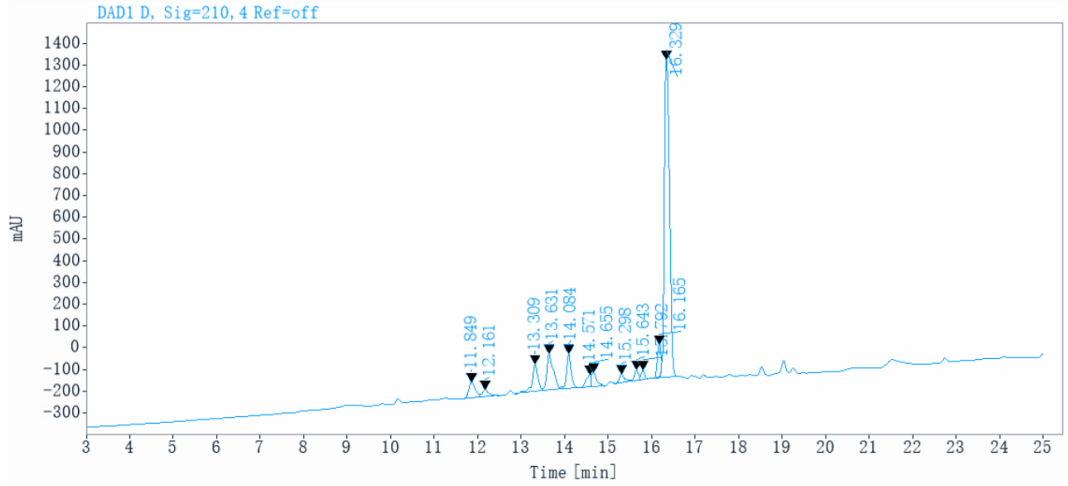


**Fig. S23.** HPLC and LCMS Chromatogram of H-WFTTLISTIM-$NH_2$, manual SPPS in NFM/An on PS-MBHA-Rink resin




**Signal:** DAD1 D, Sig=210,4 Ref=off

| RT [min] | Type | Width [min] | Area | Height | Area% | Name |
|---|---|---|---|---|---|---|
| 11.849 | BV | 0.1573 | 763.0270 | 72.2017 | 3.7919 | |
| 12.161 | VB | 0.1559 | 349.5153 | 32.3841 | 1.7369 | |
| 13.309 | VV R | 0.1295 | 1219.1917 | 128.7038 | 6.0588 | |
| 13.631 | VV R | 0.1452 | 1833.2378 | 170.0186 | 9.1103 | |
| 14.084 | VB | 0.1076 | 1150.7218 | 160.5105 | 5.7185 | |
| 14.571 | BV | 0.1033 | 423.5881 | 56.5889 | 2.1050 | |
| 14.655 | VB | 0.1177 | 552.8438 | 66.0255 | 2.7474 | |
| 15.298 | VV | 0.1376 | 406.5672 | 42.5051 | 2.0204 | |
| 15.643 | VV | 0.0969 | 325.5832 | 50.6822 | 1.6180 | |
| 15.792 | VB | 0.0866 | 243.0668 | 43.9034 | 1.2079 | |
| 16.165 | BV E | 0.0865 | 845.5976 | 152.8350 | 4.2022 | |
| 16.329 | VB R | 0.1318 | 12009.8340 | 1461.8309 | 59.6828 | |
| | | Sum | 20122.7743 | | | |

| Peak no. | RT (min) | Compound | Relative area |
|---|---|---|---|
| 1 | 11.849 | des-TTIM, m/z = 878 | 3.8 |
| 2 | 12.161 | des-T, m/z = 1080 | 1.7 |
| 3 | 13.309 | H-WFTTLISTIM-$NH_2$ + tBu (+ tBu, m/z = 1268) | 6.1 |
| 4 | 13.631 | H-WFTTLISTIM-$NH_2$+ tBu (+ tBu, m/z = 1268) | 9.1 |
| 5 | 14.084 | H-WFTTLISTIM-$NH_2$ + tBu (+ tBu, m/z = 1268) | 5.7 |
| 6 | 14.571 | des-L or I, m/z = 1098 | 2.1 |
| 7 | 14.655 | | 2.7 |
| 8 | 15.298 | | 2.0 |
| 9 | 15.643 | H-FTTLISTIM-$NH_2$+ tBu (des-W + tBu, m/z = 1082) | 1.6 |
| 10 | 15.792 | des-L or I, m/z = 1098 | 1.2 |
| 11 | 16.165 | des-T, m/z = 1110 | 4.2 |
| 12 | 16.329 | Target molecule: H-WFTTLISTIM-$NH_2$ | 59.7 |

Target molecule: H-WFTTLISTIM-$NH_2$

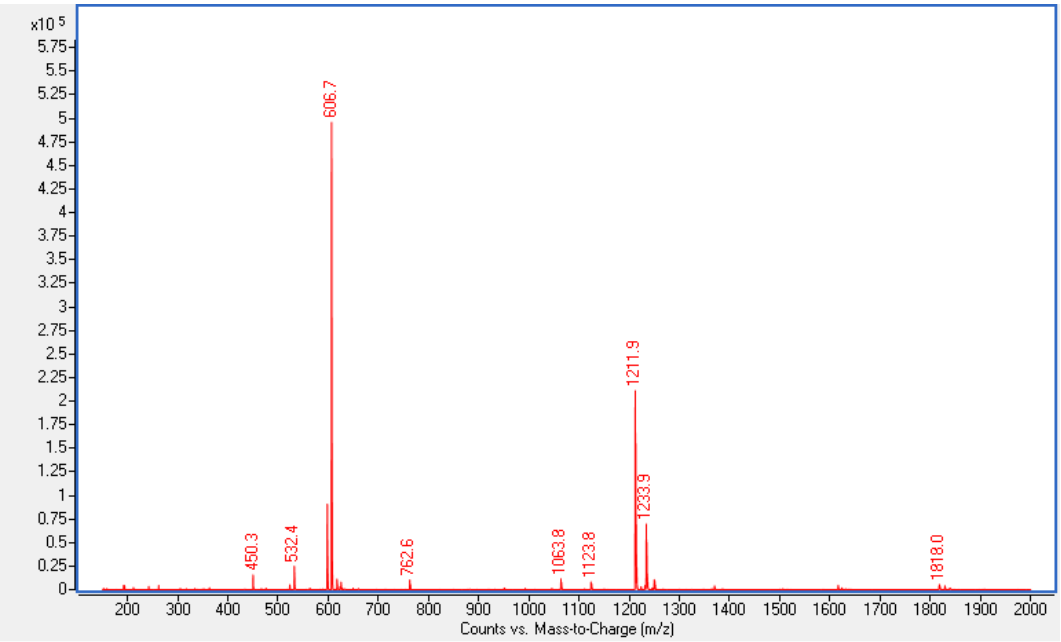
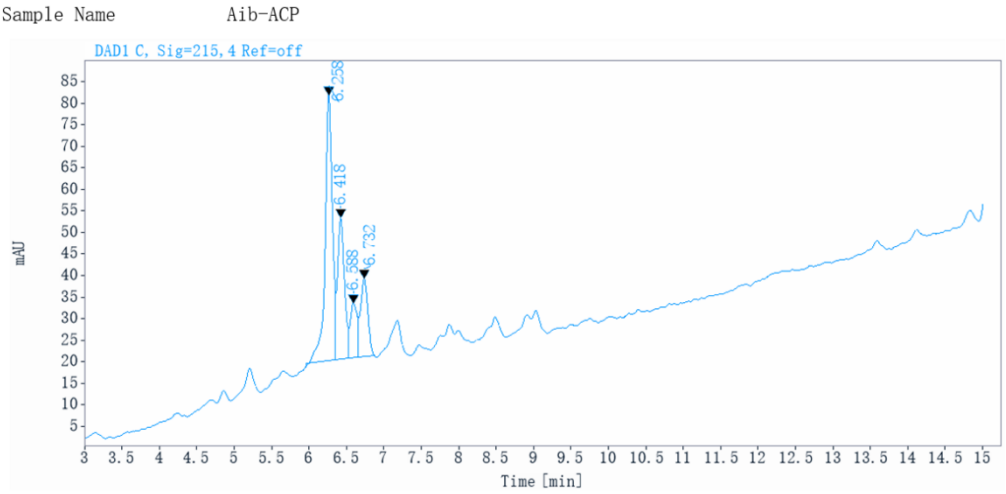


**Fig. S24.** HPLC and LCMS Chromatogram of H-VQ-Aib-Aib-IDYING-$NH_2$, manual SPPS in NFM/An on PS-MBHA-Rink resin

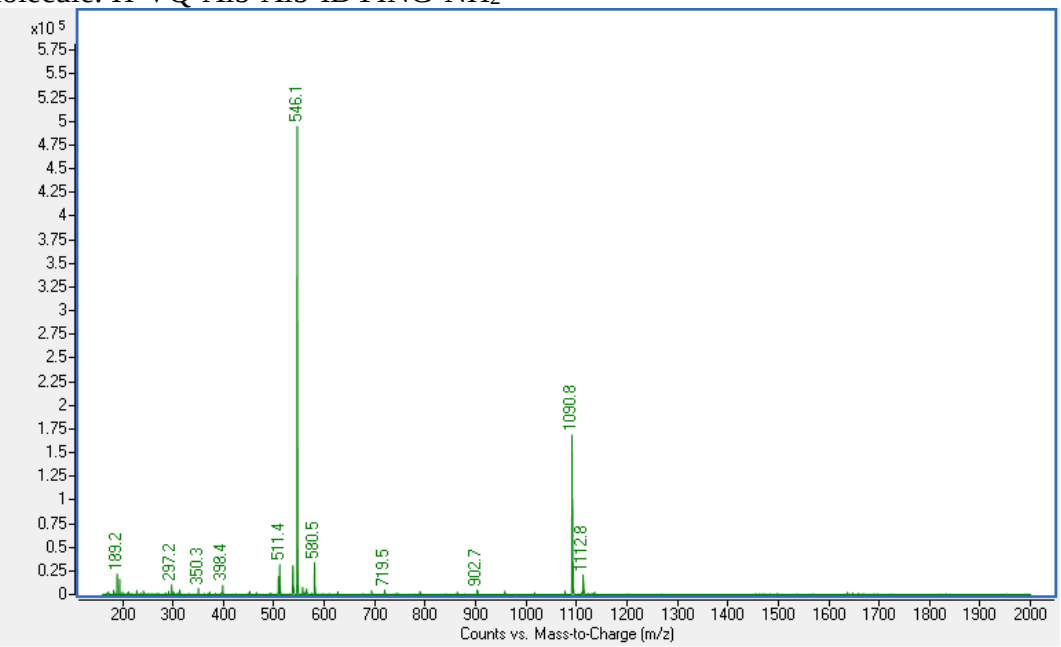



**Signal:** DAD1 C, Sig=215,4 Ref=off

| RT [min] | Type | Width [min] | Area | Height | Area% | Name |
|---|---|---|---|---|---|---|
| 6.258 | MF | 0.1145 | 425.1526 | 61.8625 | 50.6263 | |
| 6.418 | MF | 0.1054 | 208.6690 | 33.0107 | 24.8479 | |
| 6.588 | MF | 0.1064 | 81.4703 | 12.7607 | 9.7013 | |
| 6.732 | FM | 0.1138 | 124.4936 | 18.2391 | 14.8245 | |
| | | Sum | 839.7856 | | | |

| Peak no. | RT (min) | Compound | Relative area |
|---|---|---|---|
| 1 | 6.258 | Target molecule: H-VQ-Aib-Aib-IDYING-$NH_2$ | 50.6 |
| 2 | 6.418 | H-VQ-Aib-IDYING-$NH_2$ (des-Aib, m/z = 1005) | 24.8 |
| 3 | 6.588 | H-V-Aib-Aib-IDYING-$NH_2$ (des-Q, m/z = 962) | 9.7 |
| 4 | 6.732 | H-V-Aib-Aib-IDYIN-$NH_2$ (des-GQ, m/z = 905) | 14.8 |

Target molecule: H-VQ-Aib-Aib-IDYING-$NH_2$

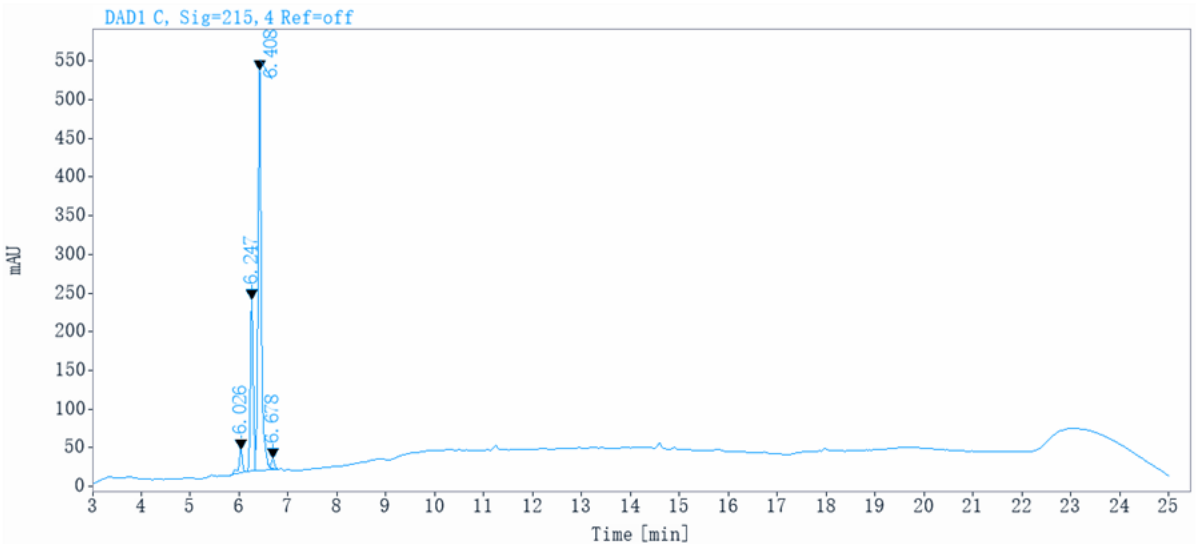



# 11. Application of US-GSPPS in the total synthesis of natural product

## 11.1. Total synthesis of linear natural product

Total synthesis of natural product were carried out at 30-35 °C under sonication in polyethylene syringes fitted with a polyethylene porous disc and connected to a nitrogen source to remove excess reagents and solvents. 0.2 g of preloaded Fmoc-resin was utilized. After the swelling of the resin in 2 mL of NFM/An, the Fmoc protective group was removed by 5% DBU in NFM/An (2 times × 2 mL, 2 min each) and then the resin was washed with the selected solvent (3 times × 1.5 mL, 1 min each). Corresponding Fmoc-protected amino acids (three-fold excess with respect to the loading of the resin) were pre-activated by OxymaB (0.6 equiv. with respect to the loading of the resin) and DIC (three-fold excess of the reagents with respect to the loading of the resin) for 3 minutes and coupled to the resin for 10 minutes. After each coupling step, the Fmoc protective group was removed by treating the peptide resin with 5% DBU in NFM/An (2 times × 2 mL, 2 min each) and the resin was washed with the EtOH and NFM/An (3 times × 2 mL, 1 min each). After the Fmoc cleavage of the N-terminal amino group, the peptide resin was further washed with EtOH (3 times × 2 mL, 1 min each) and dried under a vacuum for 12 hours. The dry peptide resin was suspended in 5 mL of the TFA/TIS/$H_2O$ (95/2.5/2.5 v/v/v) mixture and stirred for 2 h. The resin was filtered off, washed with TFA (1 time × 1 mL, 1 min) and MTBE (25 mL) cooled to 4 °C was added to the solution dropwise. The peptide was filtered and dried in vacuo to obtain crude peptide that was directly analysed by HPLC (Method D or Method E). And the total yield was calculated after the RP-MPLC purification.

**Fig. S25.** HPLC Chromatogram of AFNIHNRNLL, manual SPPS in NFM/An on PS-CTC-Cl resin




**Signal:** DAD1 C, Sig=215,4 Ref=off

| RT [min] | Type | Width [min] | Area | Height | Area% | Name |
|---|---|---|---|---|---|---|
| 6.026 | VB R | 0.0667 | 153.9278 | 30.1247 | 4.2731 | |
| 6.247 | BV | 0.0652 | 937.7894 | 222.1529 | 26.0332 | |
| 6.408 | VV R | 0.0712 | 2448.8992 | 518.0298 | 67.9818 | |
| 6.678 | VB E | 0.0679 | 61.6715 | 13.8526 | 1.7120 | |
| | | Sum | 3602.2879 | | | |

| Peak no. | RT (min) | Compound | Relative area |
|---|---|---|---|
| 1 | 6.026 | H-ANIHNRNLL-OH (des-F, m/z = 1065) | 4.3 |
| 2 | 6.247 | des-N, m/z = 1098 | 26.0 |
| 3 | 6.408 | Target molecule: H-AFNIHNRNLL-OH | 68.0 |
| 4 | 6.678 | H-AFNIHNNLL-OH (des-R, m/z = 1056) | 1.7 |

**Fig. S26.** HRMS Chromatogram of AFNIHNRNLL, manual SPPS in NFM/An on PS-CTC-Cl resin, $[M+2H]^{2+}$

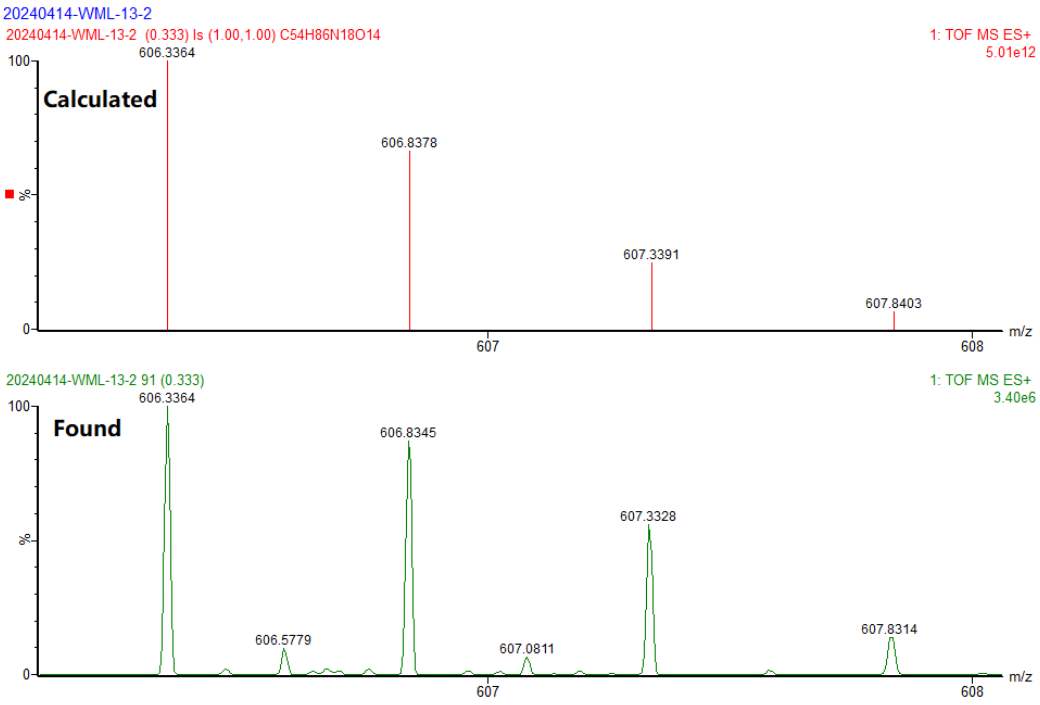
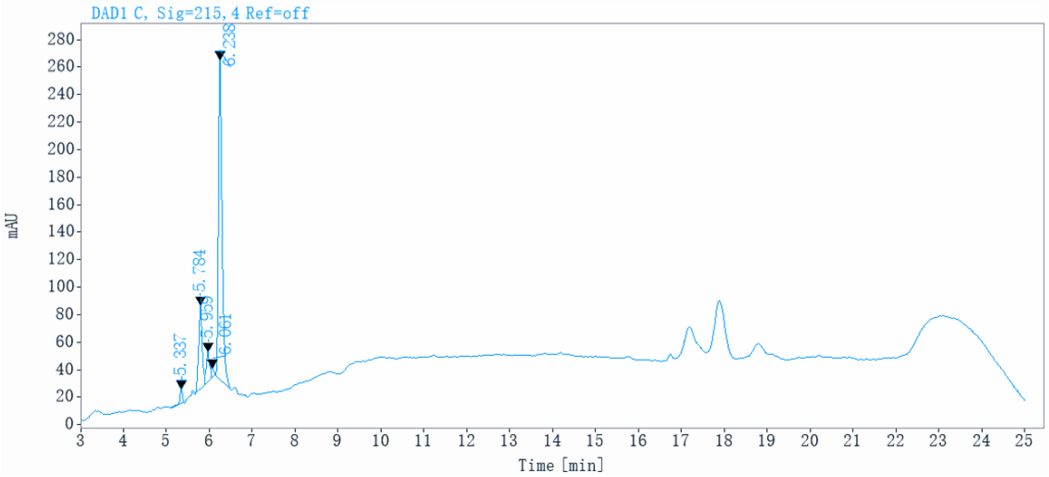


**Fig. S27.** HPLC Chromatogram of AVLVDKQCPD, manual SPPS in NFM/An on PS-CTC-Cl resin

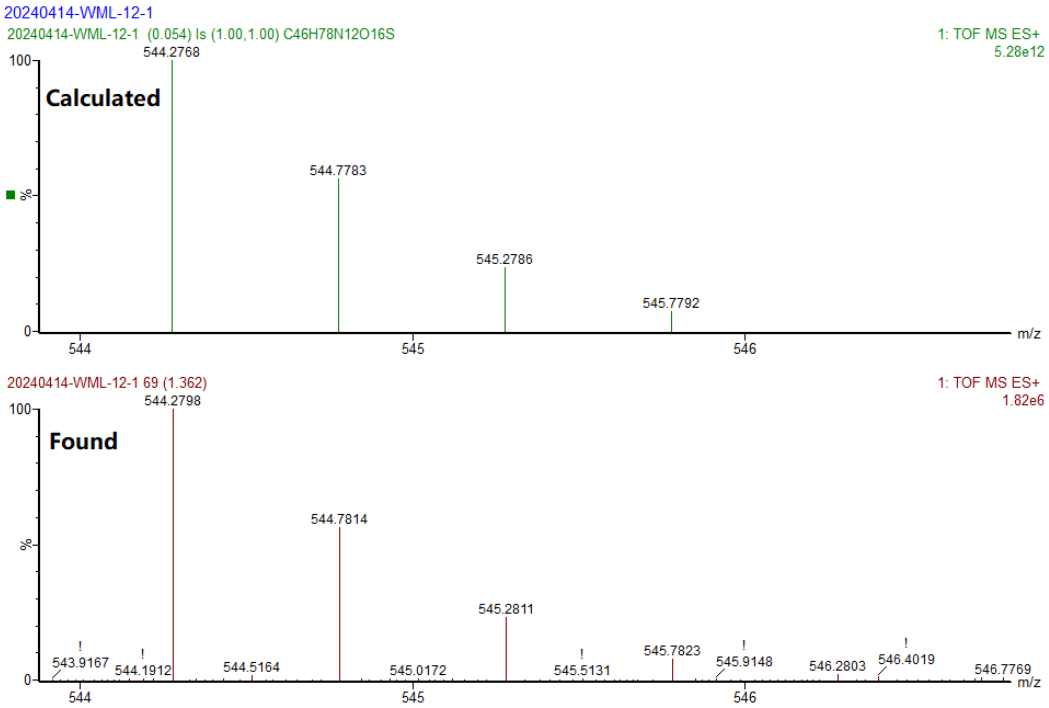



**Signal:** DAD1 C, Sig=215,4 Ref=off

| RT [min] | Type | Width [min] | Area | Height | Area% | Name |
|---|---|---|---|---|---|---|
| 5.337 | BB | 0.0657 | 44.2883 | 10.8363 | 2.2953 | |
| 5.784 | BV | 0.0846 | 349.6281 | 61.2559 | 18.1199 | |
| 5.959 | VV | 0.0808 | 122.6269 | 22.0890 | 6.3553 | |
| 6.061 | VB | 0.0635 | 28.0978 | 6.9018 | 1.4562 | |
| 6.238 | BB | 0.0893 | 1384.8895 | 233.0185 | 71.7734 | |
| | | Sum | 1929.5307 | | | |

| Peak no. | RT (min) | Compound | Relative area |
|---|---|---|---|
| 1 | 5.337 | des-VQC-OH, m/z = 757 | 2.3 |
| 2 | 5.784 | | 18.1 |
| 3 | 5.959 | | 6.4 |
| 4 | 6.061 | des-V-OH, m/z = 988 | 1.5 |
| 5 | 6.238 | Target molecule: H-AVLVDKQCPD-OH | 71.8 |

**Fig. S28.** HRMS Chromatogram of AVLVDKQCPD, manual SPPS in NFM/An on PS-CTC-Cl resin, $[M+2H]^{2+}$



**11.2.Total synthesis of Acremonamide**

Total synthesis of natural product **Acremonamide** were carried out at 30-35 °C under sonication in polyethylene syringes fitted with a polyethylene porous disc and connected to a nitrogen source to remove excess reagents and solvents.

**US-GSPPS:** 0.2 g of preloaded Fmoc-resin was utilized. After the swelling of the resin in 2 mL of NFM/An, the Fmoc protective group was removed by 5% DBU in NFM/An (2 times × 2 mL, 2 min each) and then the resin was washed with the selected solvent (3 times × 1.5 mL, 1 min each). Corresponding Fmoc-protected amino acids (three-fold excess with respect to the loading of the resin) were pre-activated by OxymaB (0.6 equiv. with respect to the loading of the resin) and DIC (three-fold excess of the reagents with respect to the loading of the resin) for 3 minutes and coupled to the resin for 10 minutes. After each coupling step, the Fmoc protective group was removed by treating the peptide resin with 5% DBU in NFM/An (2 times × 2 mL, 2 min each) and the resin was washed with the EtOH and NFM/An (3 times × 2 mL, 1 min each).

**US-GSPPS on-resin esterification:** a solution of *R*-Hiv (2 equiv., 48.2 mg, 0.408 mmol), DMAP (0.2 equiv., 5.0 mg, 0.041 mmol), and DIC (2 equiv., 64 µL, 0.408 mmol) in NFM/An was added into Fmoc-deprotected resin syringe. The syringe was sonicated under 30-35 °C for 30 minutes.

**Cleavage:** After the Fmoc cleavage of the N-terminal amino group, the peptide resin was further washed with EtOH (3 times × 2 mL, 1 min each) and dried under a vacuum for 12 hours. The dry peptide resin was suspended in 5 mL of the TFA/TIS/$H_2O$ (95/2.5/2.5 v/v/v) mixture and stirred for 2 h. The resin was filtered off, washed with TFA (1 time × 1 mL, 1 min) and MTBE (25 mL) cooled to 4 °C was added to the solution dropwise. The linear peptide was purified by RP-MPLC as a white solid (79.4 mg, 64% yield).

**Solution phase macrocyclization:** HATU (3 equiv., 0.24 mmol, 91 mg) and DIPEA (6 equiv., 0.48 mmol, 81 µL) was added into a solution of linear peptide precursor (48.8 mg, 0.08 mmol) in NFM/An (20 mL). The mixture was allowed to sonicate at room temperature for 1 h and then quenched with saturated aqueous solution of $NH_4Cl$ (20 mL). The aqueous layer was extracted with EA (3 ×10 mL). The combined organic layers were washed with brine (20 mL), dried over anhydrous $Na_2SO_4$, filtered, and concentrated under reduced pressure. Purification of the crude product was performed by RP-MPLC to obtain the **Acremonamide** as pale yellow solid (24.2 mg, 51% yield).

**Fig. S29** LCMS of the on-resin esterification

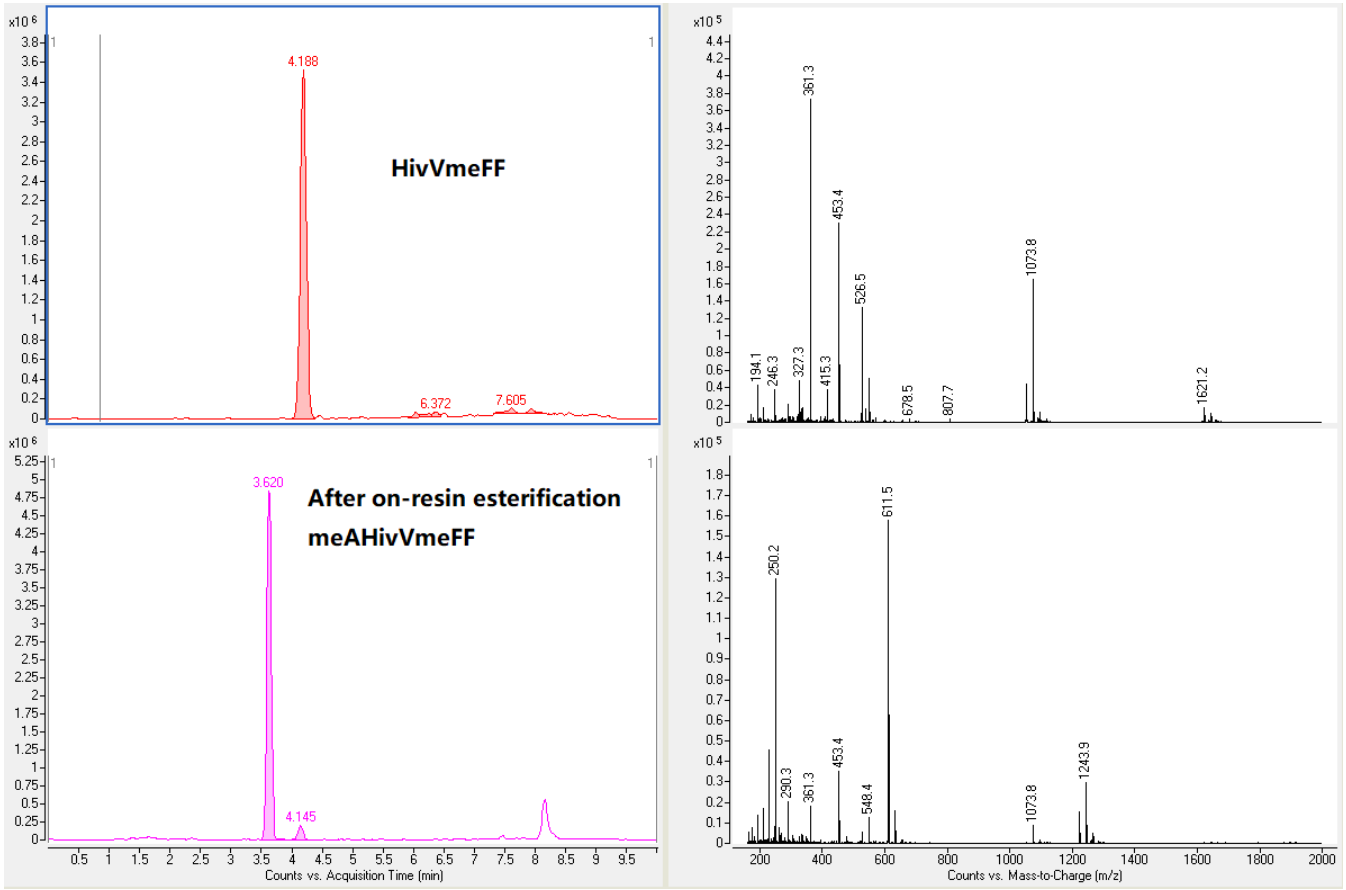
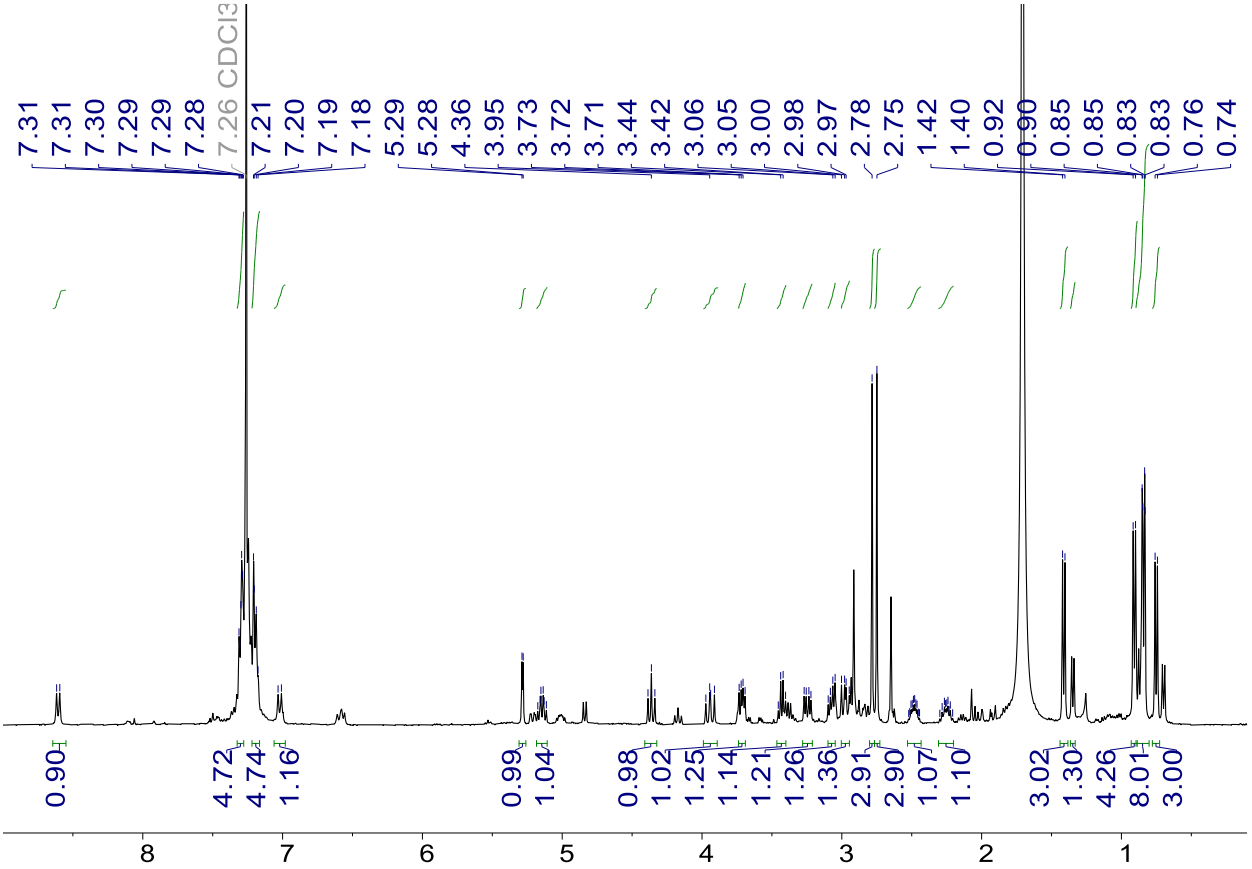


**Fig. S30.** $^{1}$H NMR spectrum of Acremonamide's major conformer ($CDCl_3$, 298 K, 400 MHz)




The ratio of major : minor = 3 : 1.3

**Fig. S31.** $^{13}$C NMR spectrum of Acremonamide's major/minor conformer ($CDCl_3$, 298 K, 100 MHz)

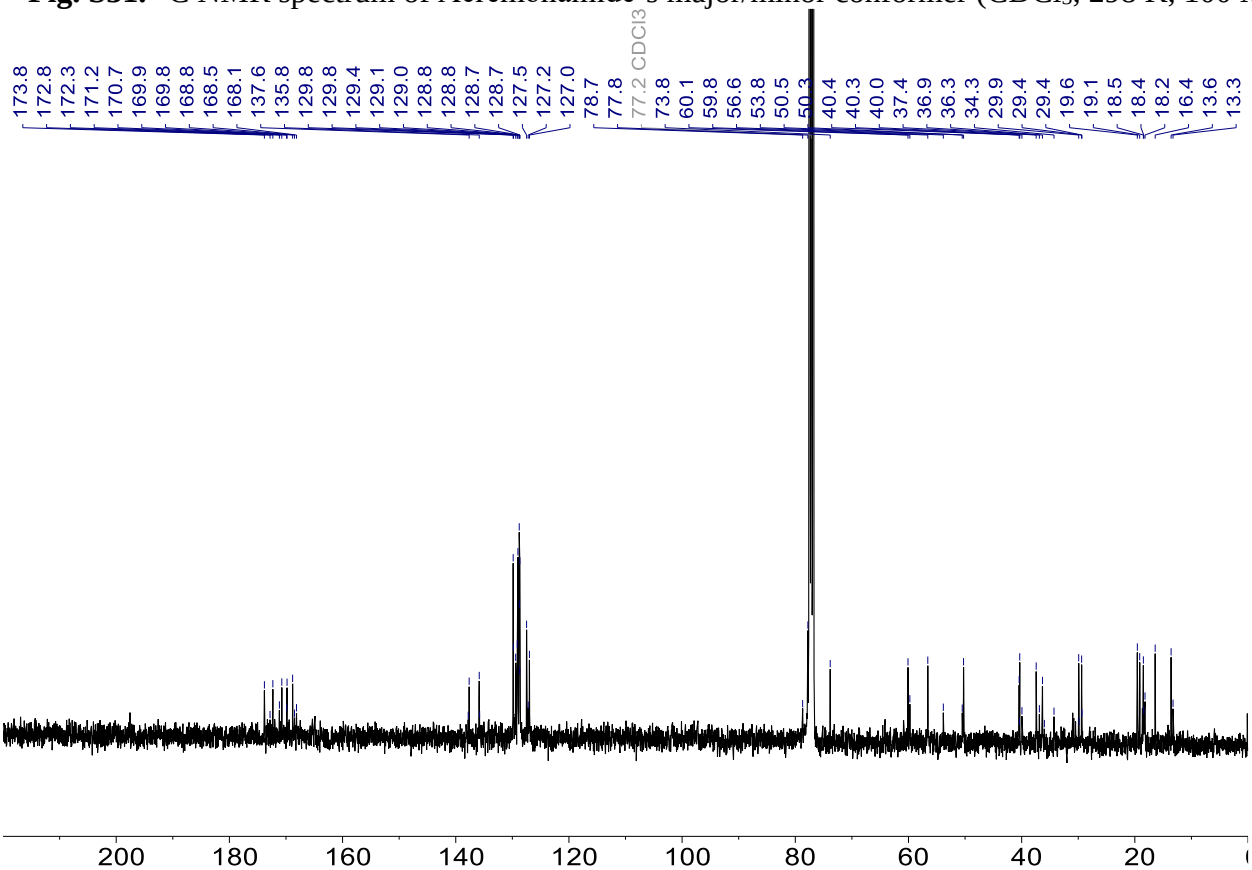


**Table S7.** $^{1}H$ and $^{13}C$ NMR spectral data comparison of Acremonamide's major conformer in $CDCl_3$

| | | Reported | | Synthesized | |
|---|---|---|---|---|---|
| | **position, type** | **δC** | **δH, multb (J in Hz)** | **δC** | **δH, multb (J in Hz)** |
| *N*-Me-Ala | 1, C | 169.6 | | 169.8 | |
| | 2, CH | 59.9 | 3.41 , q (7.0) | 60.1 | 3.43 , q (6.9) |
| | 3, $CH_3$ | 13.5 | 1.4 , d (7.0) | 13.6 | 1.41 , d (6.9) |
| Phe | 4, C | 172.2 | | 172.3 | |
| | 5, CH | 50.0 | 5.14 , m | 50.3 | 5.14 , td (6.4, 9.0) |
| | 6, $CH_2$ | 40.3 | 3.05 , dd (13.5, 6.5) | 40.4 | 3.07 , dd (13.4, 6.4) |
| | | | 2.96 , dd (13.5, 9.0) | | 2.97 , dd (13.2, 8.9) |
| | 7, C | 135.7 | | 135.8 | |
| | 8, 8′, CH | 129.6 | 7.24 , overlaped | 129.8 | 7.24 , overlaped |
| | 9, 9′, CH | 128.5 | 7.24 , overlaped | 128.7 | 7.24 , overlaped |
| | 10, CH | 127.2 | 7.24 , overlaped | 127.5 | 7.24 , overlaped |
| | 5-NH | | 8.59 , d (9.0) | | 8.60 , d (9.0) |
| *N*-Me-Phe | 11, C | 170.5 | | 170.7 | |
| | 12, CH | 73.7 | 3.69 , m | 73.8 | 3.71 , td (11.2, 5.8) |
| | 13, $CH_2$ | 36.1 | 3.94 , dd (13.5, 11.0) | 36.3 | 3.94 , dd (13.6, 10.9) |
| | | | 3.22 , dd (13.5, 6.0) | | 3.25 , dd (13.5, 5.9) |
| | 14, C | 137.5 | | 137.6 | |
| | 15, 15′, CH | 128.7 | 7.19 , overlaped | 128.8 | 7.19 , overlaped |
| | 16, 16′, CH | 128.6 | 7.19 , overlaped | 128.8 | 7.19 , overlaped |
| | 17, CH | 126.7 | 7.19 , overlaped | 127.0 | 7.19 , overlaped |
| Val | 18, C | 173.5 | | 173.8 | |
| | 19, CH | 56.4 | 4.35 , t (10.0) | 56.6 | 4.36 , t (9.8) |
| | 20, CH | 29.8 | 2.26 , m | 29.9 | 2.25 , m |
| | 21, CH | 18.3 | 0.89 , d (7.0) | 18.5 | 0.91 , d (6.8) |
| | 22, $CH_3$ | 19.5 | 0.84 , d (6.5) | 19.6 | 0.84 , d (6.9) |
| | 19-NH | | 6.95 , d (9.5) | | 7.02 , d (9.7) |
| *R*-Hiv | 23, C | 168.6 | | 168.8 | |
| | 24, CH | 77.9 | 5.28 , d (3.0) | 77.9 | 5.28 , d (3.4) |
| | 25, CH | 29.2 | 2.49 , m | 29.4 | 2.48 , pd (3.2, 6.8, 7.2) |
| | 26, $CH_3$ | 16.1 | 0.7 , d (6.5) | 16.4 | 0.75 , d (6.7) |
| | 27, CH | 19.1 | 0.82 , d (7.0) | 19.1 | 0.84 , d (6.9) |
| | 28, $CH_3$ | 37.3 | 2.74 , s | 37.4 | 2.75 , s |
| | 29, $CH_3$ | 40.0 | 2.74 , s | 40.3 | 2.78 , s |

**Fig. S32.** $^{1}$H NMR spectrum of Acremonamide's minor conformer ($CDCl_3$, 298 K, 400 MHz)

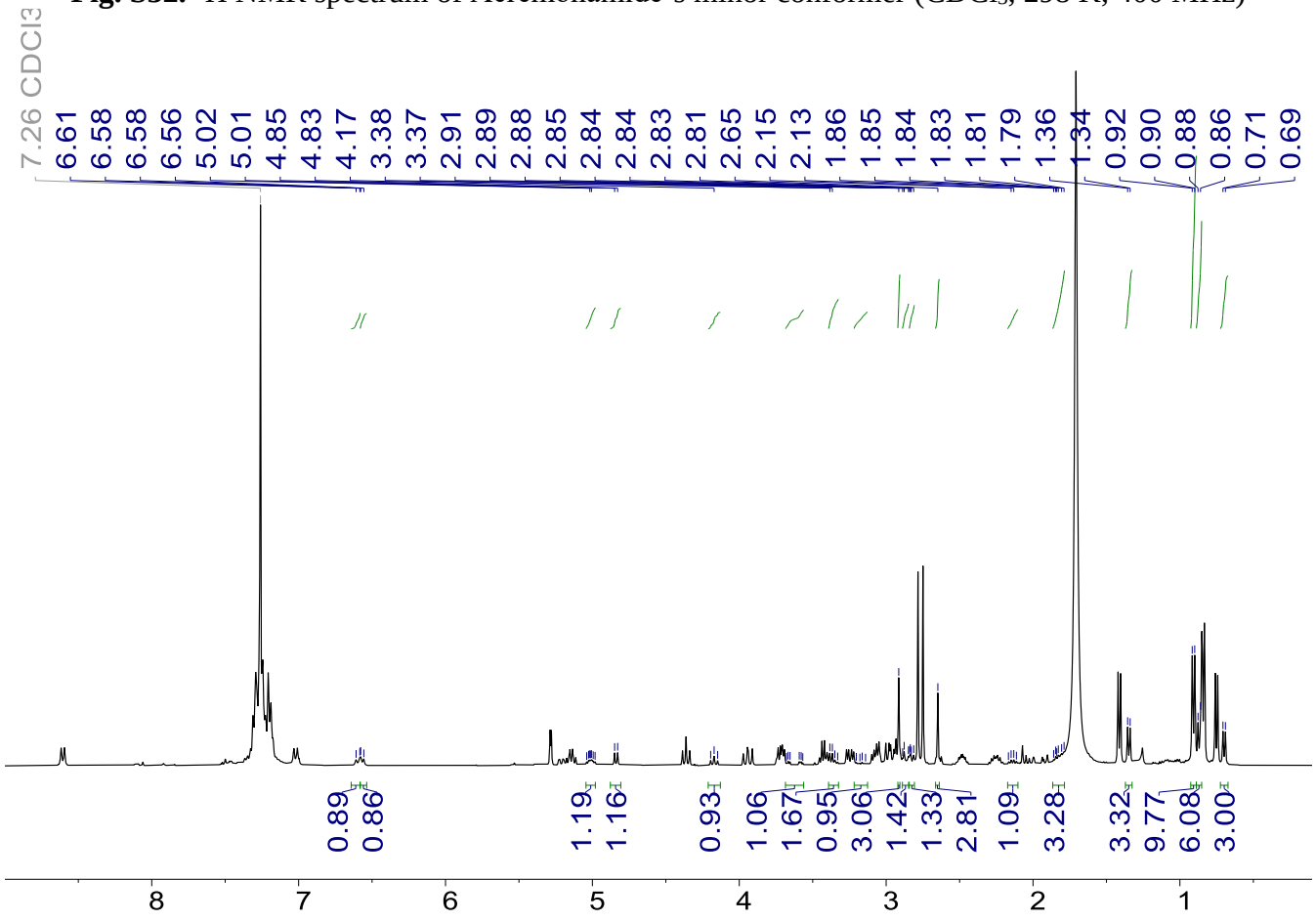
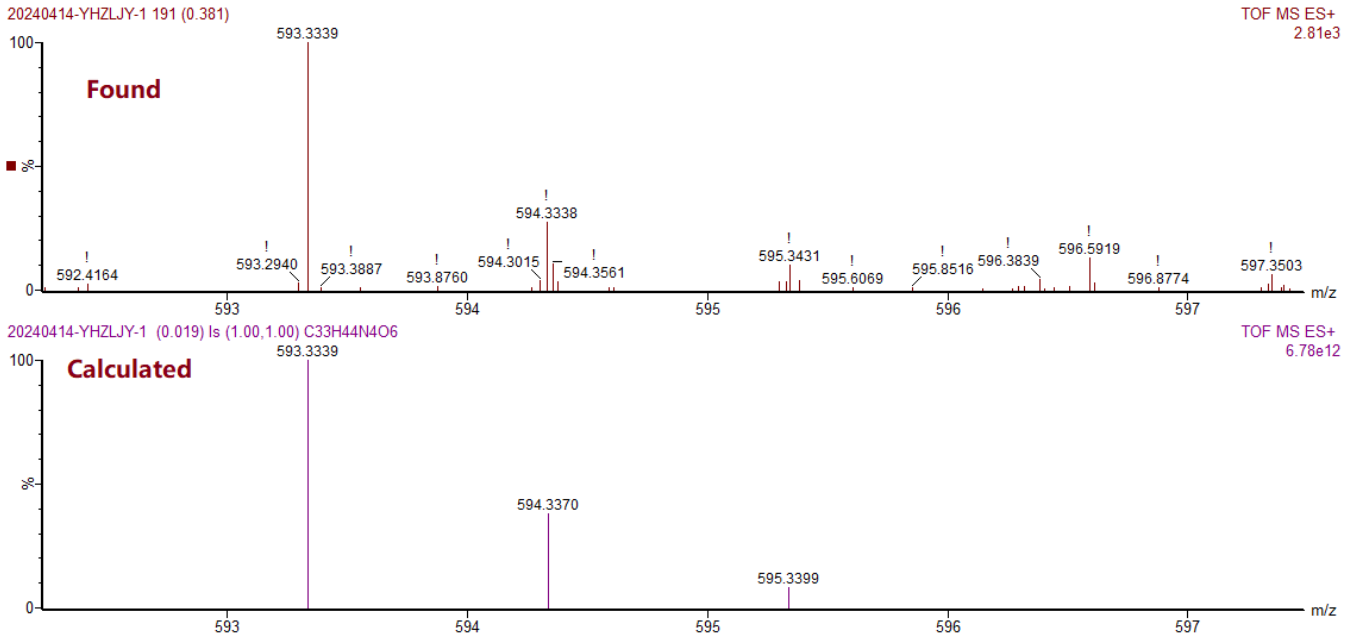


**Table S8.** $^{1}H$ and $^{13}C$ NMR spectral data comparison of Acremonamide's minor conformer in $CDCl_3$

| | | Reported | | Synthesized | |
|---|---|---|---|---|---|
| | **position, type** | **δC** | **δH, multb (J in Hz)** | **δC** | **δH, multb (J in Hz)** |
| *N*-Me-Ala | 1, C | 169.7 | | 169.9 | |
| | 2, CH | 59.5 | 3.35 , q (7.0) | 59.8 | 3.36 , q (7.1) |
| | 3, $CH_3$ | 13.5 | 1.34 , d (6.7) | 13.3 | 1.34 , d (6.9) |
| Phe | 4, C | 172.2 | | 172.3 | |
| | 5, CH | 50.5 | 4.96 , m | 50.5 | 5.01 , m |
| | 6, $CH_2$ | 40.3 | 2.87 , m | 40.4 | 2.91 , m |
| | | | 2.82 , m | | 2.86 , m |
| | 7, C | 135.5 | | 135.8 | |
| | 8, 8′, CH | 129.2 | 7.24 , overlaped | 129.8 | 7.24 , overlaped |
| | 9, 9′, CH | 128.5 | 7.24 , overlaped | 128.7 | 7.24 , overlaped |
| | 10, CH | 127.2 | 7.24 , overlaped | 127.5 | 7.24 , overlaped |
| | 5-NH | | 8.59 , d (9.0) | | 6.60 , d (10.1) |
| *N*-Me-Phe | 11, C | 170.9 | | 171.2 | |
| | 12, CH | 73.7 | 3.69 , m | 73.8 | 3.62 , m |
| | 13, $CH_2$ | 36.1 | 3.94 , dd (13.5, 11.0) | 36.0 | 3.94 , overlaped |
| | | | 3.22 , dd (13.5, 6.0) | | 3.17 , m |
| | 14, C | 137.7 | | 137.8 | |
| | 15, 15′, CH | 128.9 | 7.19 , overlaped | 129.4 | 7.19 , overlaped |
| | 16, 16′, CH | 128.6 | 7.19 , overlaped | 129.1 | 7.19 , overlaped |
| | 17, CH | 127.0 | 7.19 , overlaped | 127.2 | 7.19 , overlaped |
| Val | 18, C | 172.8 | | 172.8 | |
| | 19, CH | 53.6 | 4.18 , t (9.5) | 53.8 | 4.17 , t (9.5) |
| | 20, CH | 29.8 | 1.8 , m | 29.9 | |
| | 21, CH | 18.3 | 0.88 , d (6.7) | 18.4 | 0.91 , overlaped |
| | 22, $CH_3$ | 19.5 | 0.85 , d (6.8) | 19.6 | 0.87 , d (6.7) |
| | 19-NH | | 6.51 , d (7.4) | | 6.57 , d (8.4) |
| *R*-Hiv | 23, C | 168.2 | | 168.5 | |
| | 24, CH | 78.5 | 4.8 , d (8.0) | 78.7 | 4.84 , d (8.2) |
| | 25, CH | 29.2 | 2.12 , m | 29.4 | 2.14 , m |
| | 26, $CH_3$ | 18.2 | 0.69 , d (6.8) | 18.2 | 0.70 , d (6.7) |
| | 27, CH | 19.1 | 0.82 , d (7.0) | 19.1 | 0.87 , d (6.7) |
| | 28, $CH_3$ | 37.2 | 2.6 , s | 36.9 | 2.65 , s |
| | 29, $CH_3$ | 40.1 | 2.9 , s | 40.0 | 2.91 , s |

**Fig. S33.** HRMS of spectral data of Acremonamide $[M+H]^{1+}$



### 11.3.Total synthesis of desotamide B

Total synthesis of natural product **desotamide B** were carried out at 30-35 °C under sonication in polyethylene syringes fitted with a polyethylene porous disc and connected to a nitrogen source to remove excess reagents and solvents.

**US-GSPPS:** 0.1 g of preloaded Fmoc-resin was utilized. After the swelling of the resin in 2 mL of NFM/An, the Fmoc protective group was removed by 5% DBU in NFM/An (2 times × 2 mL, 2 min each) and then the resin was washed with the selected solvent (3 times × 1.5 mL, 1 min each). Corresponding Fmoc-protected amino acids (three-fold excess with respect to the loading of the resin) were pre-activated by OxymaB (0.6 equiv. with respect to the loading of the resin) and DIC (three-fold excess of the reagents with respect to the loading of the resin) for 3 minutes and coupled to the resin for 10 minutes. After each coupling step, the Fmoc protective group was removed by treating the peptide resin with 5% DBU in NFM/An (2 times × 2 mL, 2 min each) and the resin was washed with the EtOH and NFM/An (3 times × 2 mL, 1 min each).

**Alloc deprotection:** a solution of $Pd(PPh_3)_4$ (0.1 equiv., 0.0102 mmol, 12 mg) and $PhSiH_3$ (5 equiv., 0.51 mmol, 64 µL) in NFM/An was added into the resin syringe. The syringe was sonicated under 30-35 °C for 10 minutes. The reaction solution was removed by nitrogen gas, and the resin was washed with the EtOH and NFM/An (3 times × 2 mL, 1 min each).

**On-resin macrocyclization:** PyAOP (3 equiv., 0.31 mmol, 162 mg) and NMM (6 equiv., 0.61 mmol, 68 µL) was added into the resin syringe. The reaction was carried out under 30-35 °C for 10 minutes.

**Cleavage:** After the on-resin macrocyclization, the peptide resin was further washed with EtOH (3 times × 2 mL, 1 min each) and dried under a vacuum for 12 hours. The dry peptide resin was suspended in 5 mL of the TFA/TIS/$H_2O$ (95/2.5/2.5 v/v/v) mixture and stirred for 2 h. The resin was filtered off, washed with TFA (1 time × 1 mL, 1 min). The cleavage solution was dried by bubble nitrogen gas and then purified by RP-MPLC to obtain the **desotamide B** as a white solid (21.6 mg, 31% yield).

**Fig. S34.** $^{1}$H NMR spectrum of of desotamide B (d6-DMSO, 298 K, 400 MHz)

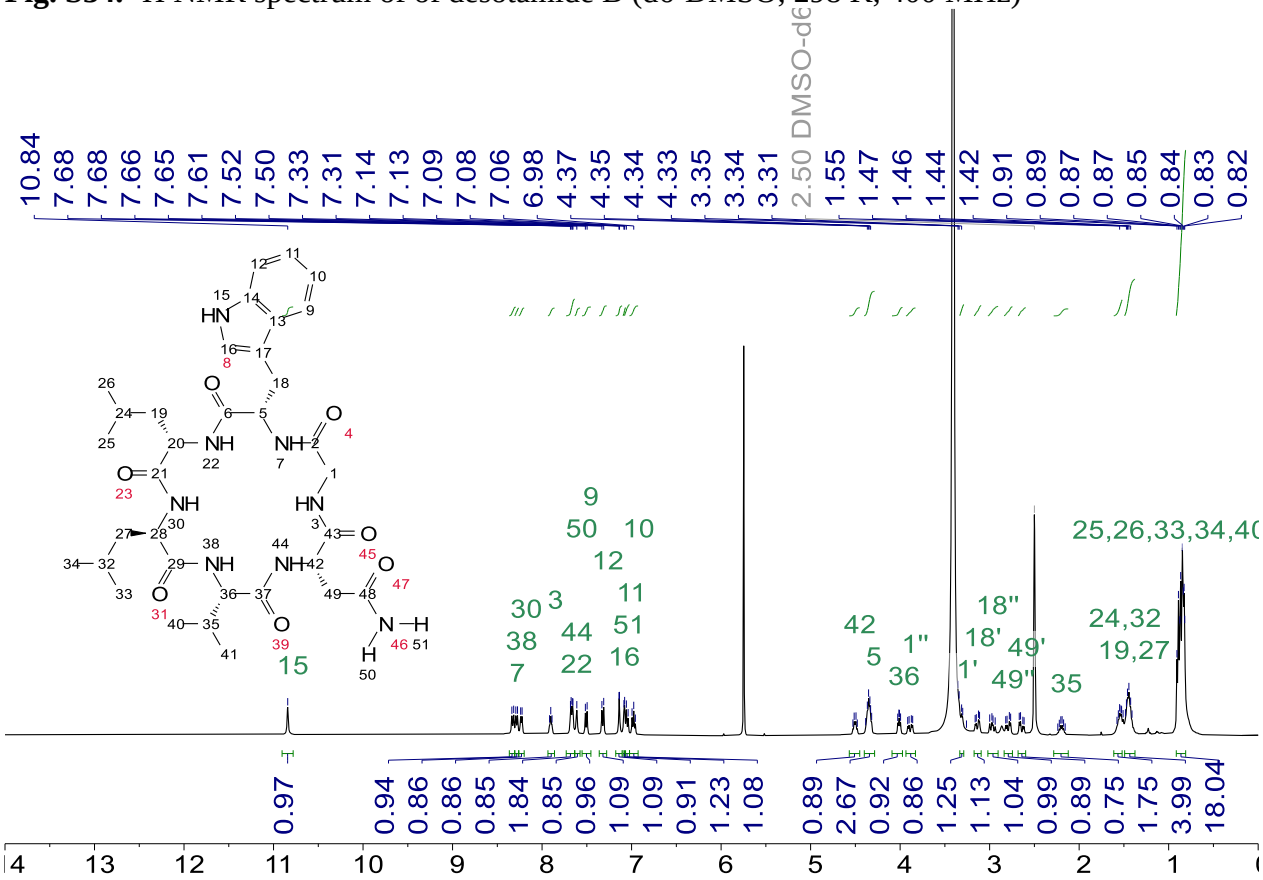



**Fig. S35.**$^{13}$C NMR spectrum of of desotamide B (d6-DMSO, 298 K, 100 MHz)

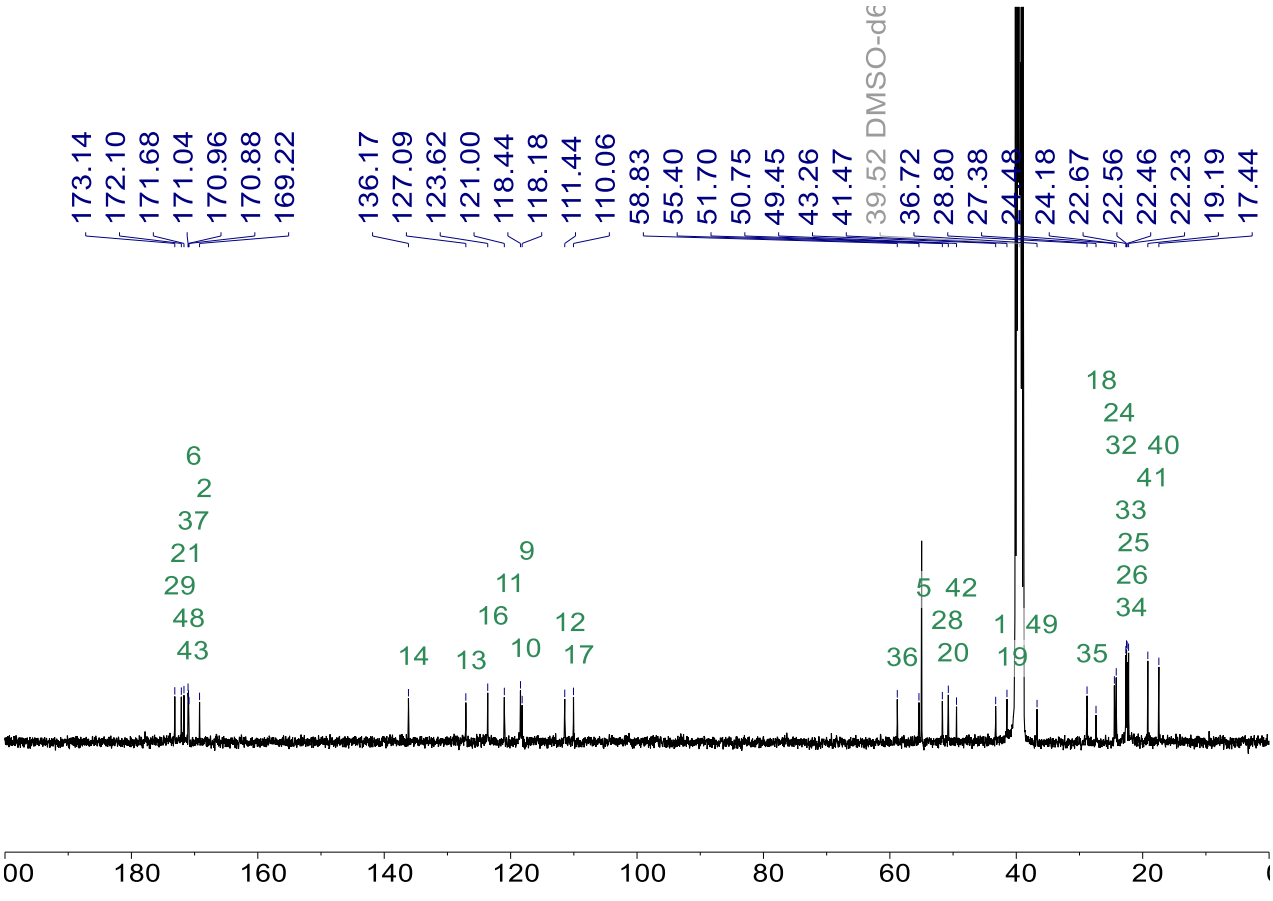
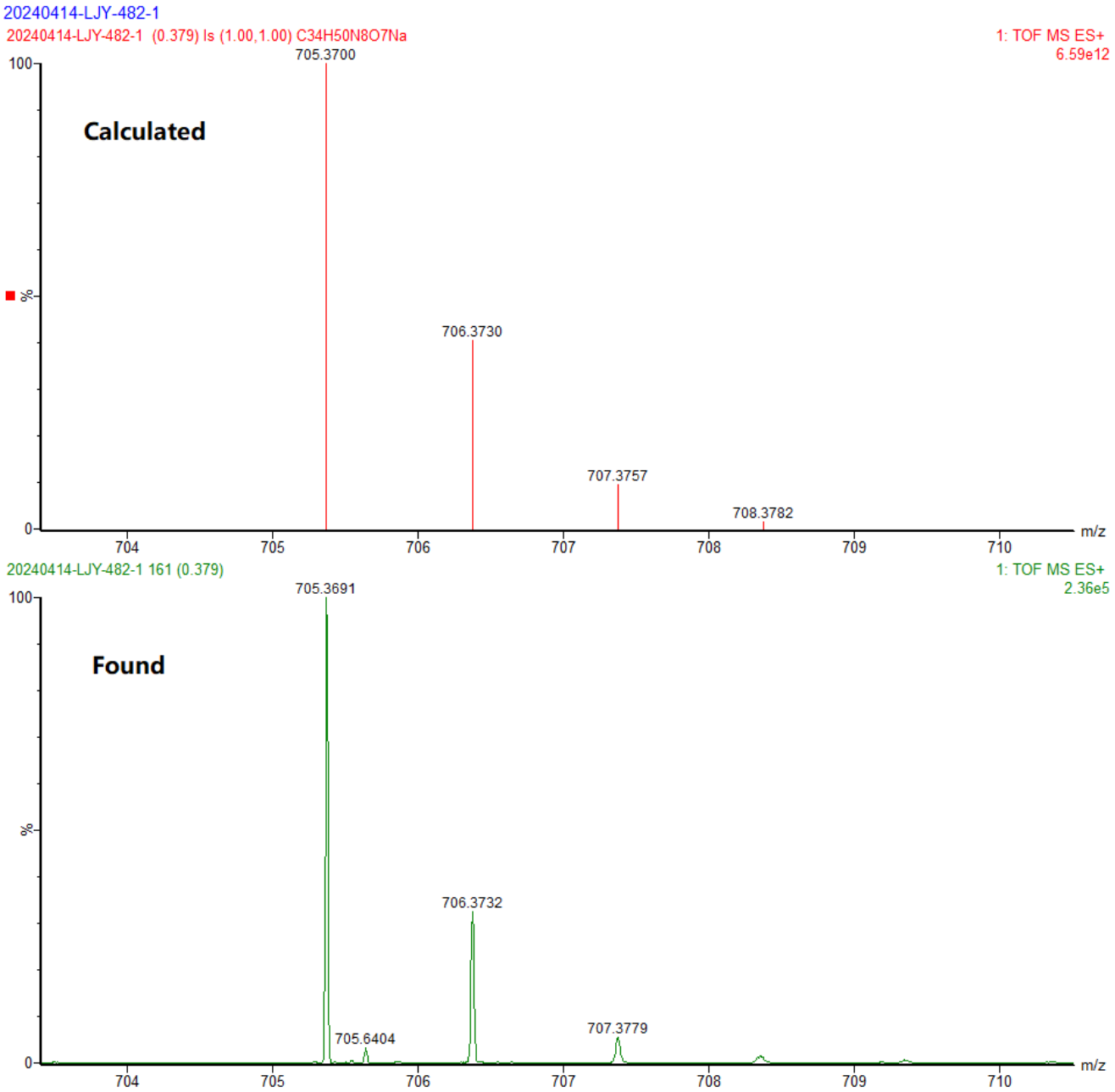


**Table S9.** $^{1}H$ and $^{13}C$ NMR spectral data comparison of desotamide B in d6-DMSO

| | | Reported | | Synthesized | |
|---|---|---|---|---|---|
| | **position** | **δC, type** | **δH, multb (J in Hz)** | **δC, type** | **δH, multb (J in Hz)** |
| Gly | 2, C | 169.0 | | 169.2 | |
| | 1, $CH_2$ | 43.1 | 3.90 , dd (16.0, 6.0) | 43.3 | 3.89 , dd (16.0, 6.0) |
| | | | 3.35 , dd (16.0, 5.0) | | 3.31 , dd (16.0, 4.9) |
| | 3, NH | | 7.89 , t (5.0) | | 7.90 , t (5.5) |
| Trp | 6, C | 170.8 | | 170.9 | |
| | 5, CH | 55.2 | 4.34 , m | 55.4 | 4.35 , m |
| | 18, $CH_2$ | 27.1 | 3.15 , dd (14.5, 4.5) | 27.4 | 3.14 , dd (14.7, 4.9) |
| | | | 2.98 , dd (14.5, 10.0) | | 2.97 , dd (14.7, 9.8) |
| | 7, NH | | 8.32 , d (7.5) | | 8.33 , d (8.2) |
| | 15, NH | | 10.82 , s | | 10.84 , s |
| | 16, CH | 123.4 | 7.15 , s | 123.6 | 7.14 , s |
| | 17, C | 109.9 | | 110.1 | |
| | 13, C | 126.9 | | 127.1 | |
| | 9, CH | 117.9 | 7.51 , d (7.5) | 118.2 | 7.51 , d (7.8) |
| | 10, CH | 118.2 | 6.98 , t (7.5) | 118.4 | 6.98 , t (7.4) |
| | 11, CH | 120.7 | 7.06 , t (7.0) | 121.0 | 7.06 , m |
| | 12, CH | 111.2 | 7.33 , d (8.0) | 111.4 | 7.32 , d (8.1) |
| | 14, C | 136.0 | | 136.2 | |
| Leu1 | 21, C | 171.5 | | 172.1 | |
| | 20, CH | 50.6 | 4.37 , m | 50.8 | 4.46 , m |
| | 19, $CH_2$ | 41.2 | 1.55 , m | 41.5 | 1.55 , m |
| | | | 1.45 , m | | 1.45 , m |
| | 24, CH | 24.3 | 1.43 , m | 24.5 | 1.43 , m |
| | 25, $CH_3$ | 22.4 | 0.89 , d (6.5) | 22.2 | 0.87 , d (6.5) |
| | 26, $CH_3$ | 22.5 | 0.90 , d (6.5) | 22.5 | 0.87 , d (6.5) |
| | 22, NH | | 7.71 , d (7.0) | | 7.67 , d (8.2) |
| Leu2 | 29, C | 172.9 | | 173.1 | |
| | 28, CH | 51.5 | 4.37 , m | 51.7 | 4.35 , m |
| | 27, $CH_2$ | 39.5 | 1.45 , m | 39.5 | 1.45 , m |
| | 32, CH | 24.0 | 1.55 , m | 24.2 | 1.55 , m |
| | 33, $CH_3$ | 22.0 | 0.83 , d (5.0) | 22.6 | 0.85 , d (5.0) |
| | 34, $CH_3$ | 22.2 | 0.85 , d (4.0) | 22.7 | 0.85 , d (4.0) |
| | 30, NH | | 8.18 , d (5.5) | | 8.23 , d (6.4) |
| Val | 37, C | 170.5 | | 171.0 | |
| | 36, CH | 58.8 | 4.01 , dd (7.0, 5.5) | 58.8 | 4.01 , dd (7.6, 5.1) |
| | 35, CH | 28.6 | 2.19 , m | 28.8 | 2.20 , m |
| | 40, $CH_3$ | 17.3 | 0.86 , d (7.0) | 17.4 | 0.86 , d (7.0) |
| | 41, $CH_3$ | 19.0 | 0.86 , d (7.0) | 19.2 | 0.86 , d (7.0) |
| | 38, NH | | 8.26 , d (7.0) | | 8.28 , d (7.6) |
| Asn | 43, C | 170.8 | | 171.0 | |
| | 42, CH | 49.3 | 4.52 , dd (13.5, 6.0) | 49.5 | 4.50 , dd (13.2, 5.8) |
| | 49, $CH_2$ | 36.6 | 2.78 , dd (16.0, 5.5) | 36.7 | 2.79 , dd (16.0, 5.8) |
| | | | 2.64 , dd (16.0, 5.5) | | 2.64 , dd (16.0, 5.6) |
| | 48, C | 171.8 | | 171.7 | |
| | 44, NH | | 7.70 , d (7.0) | | 7.67 , d (8.2) |
| | 50, $NH_2$ | | 7.58 , s | | 7.61 , s |
| | 51, $NH_2$ | | 7.05 , s | | 7.09 , s |

**Fig. S36.** HRMS of spectral data of desotamide B $[M+Na]^{1+}$




## 12. US-GSPPS in continuous-flow devices

Rink amide MBHA-polystyrene resin (0.05 g, loading = 1.02 mmol $g^{-1}$) was placed in a PEEK column with two polyethylene porous disc. The resin was previously swelled with NFM/An (0.5 mL) for 0.5 h. The PEEK column was then placed in line within the sonication bath (30-35 °C). Four solutions, including wash solution NFM/An, deprotection solution 5% DBU in NFM/An, coupling combinations solution 3/6/3 eq. AAs/DIC/OxymaB, and EtOH were selected by a 6-port switching valve and then feed into the PEEK column by a HPLC pump. A pressure regulator was set in the end of the reaction devices.

The HPLC pump flow rate was kept in 1 mL/min. Before each coupling cycle, 4 min deprotection, 5 min wash step was carried out to remove the Fmoc protection group. A coupling combination solution of corresponding AAs 3/6/3 eq. AAs/DIC/OxymaB (0.15 mmol) was prepared in 1.2 mL NFM/An, and then feed into the PEEK column. After each coupling, 5 min NFM/An wash step was carried out. After the final Fmoc deprotection, the 6-port switching valve was switched to EtOH solution and feed into the PEEK column to wash the resin.

After the final EtOH wash step, the resin was removed from the PEEK column and dried in vacuo. The dry peptide resin was suspended in 5 mL of the TFA/TIS/$H_2O$ (95/2.5/2.5 v/v/v) mixture and stirred for 2 h. The resin was filtered off, washed with TFA (1 time × 1 mL, 1 min) and MTBE (25 mL) cooled to 4 °C was added to the solution dropwise. The peptide was filtered and dried in vacuo to obtain crude peptide that was directly analysed by HPLC (Method D and Method E).

**Fig. S37. PEEK column**

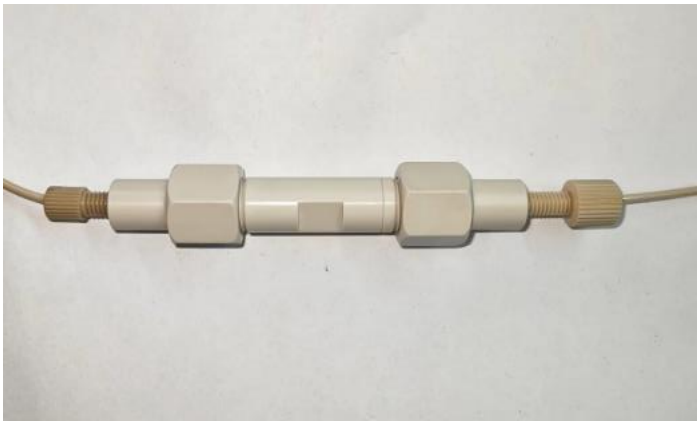


**Fig. S38. continuous-flow devices for US-GSPPS**

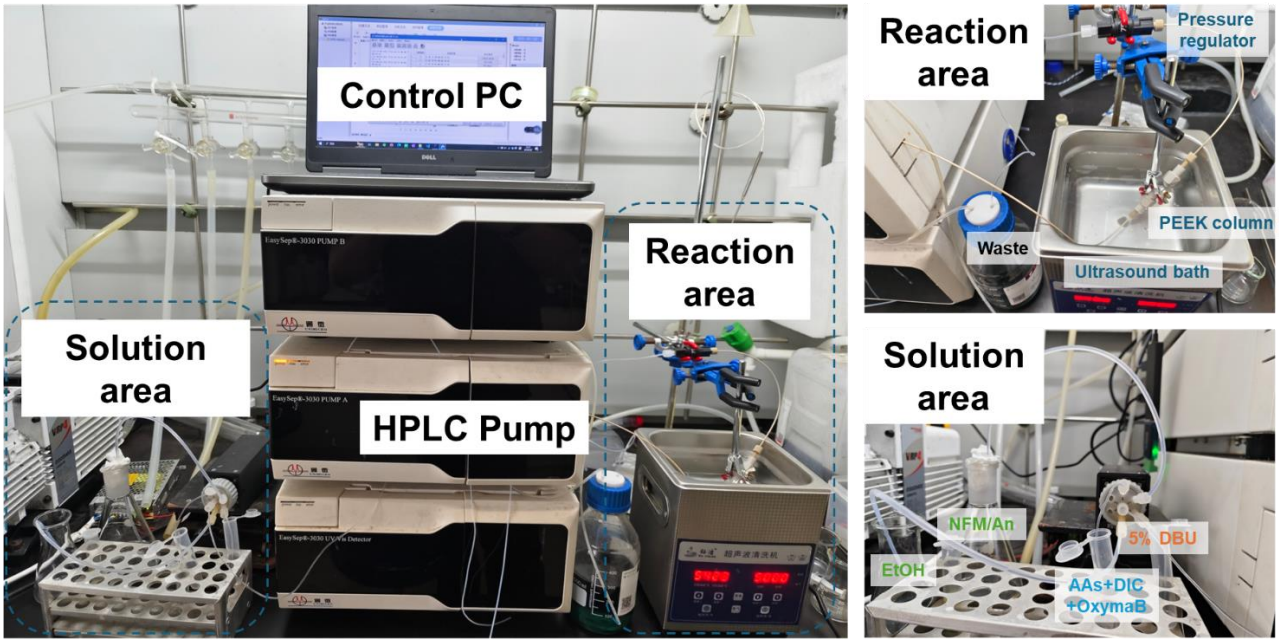
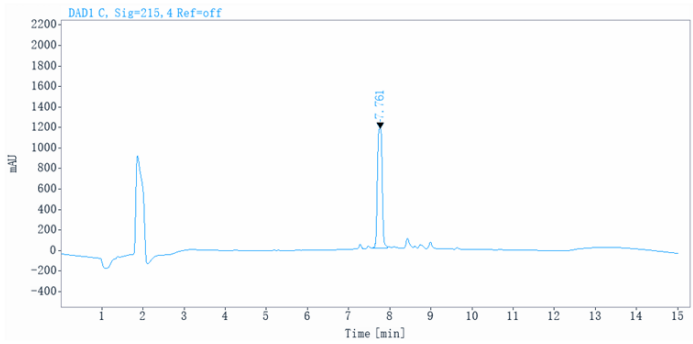


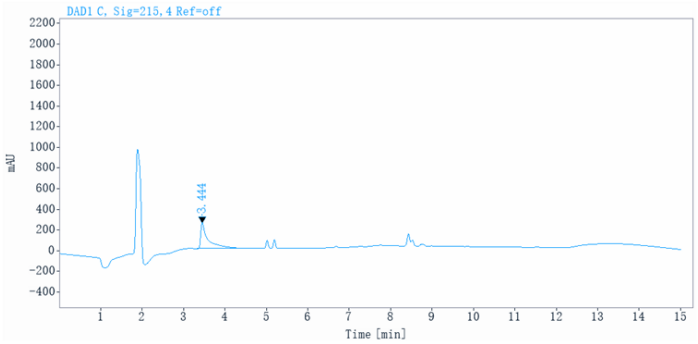

**Fig. S39.** Chromatogram of Fmoc-A-$NH_2$, starting material




Signal: DAD1 C, Sig=215,4 Ref=off

| RT [min] | Type | Width [min] | Area | Height | Area% | Name |
|---|---|---|---|---|---|---|
| 7.761 | MM | 0.1328 | 9286.2363 | 1165.3322 | 100.0000 | |
| | | Sum | 9286.2363 | | | |

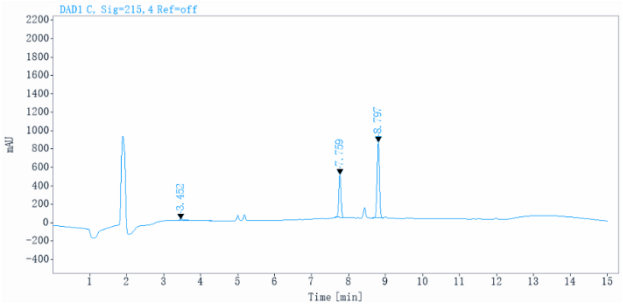
**Fig. S40.** Chromatogram of H-A-$NH_2$, after batch deprotection




Signal: DAD1 C, Sig=215,4 Ref=off

| RT [min] | Type | Width [min] | Area | Height | Area% | Name |
|---|---|---|---|---|---|---|
| 3.444 | MM | 0.2150 | 3218.4033 | 249.4368 | 100.0000 | |
| | | Sum | 3218.4033 | | | |

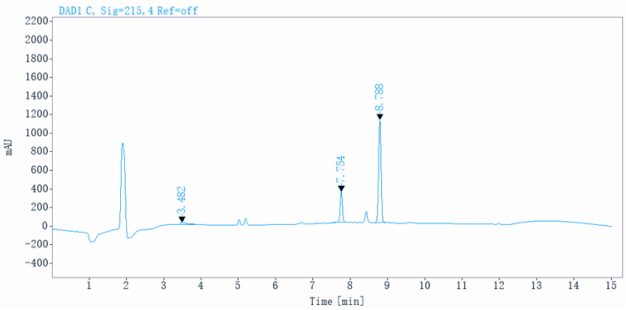
**Fig. S41.** Chromatogram of Fmoc-FA-$NH_2$ after 2 min flow deprotection and flow coupling (0.25 M)




Signal: DAD1 C, Sig=215,4 Ref=off

| RT [min] | Type | Width [min] | Area | Height | Area% | Name |
|---|---|---|---|---|---|---|
| 3.452 | MM | 0.3341 | 310.9604 | 15.5112 | 5.3507 | |
| 7.759 | MM | 0.0685 | 1935.9955 | 471.0859 | 33.3125 | |
| 8.797 | MM | 0.0714 | 3564.6641 | 831.5262 | 61.3368 | |
| | | Sum | 5811.6199 | | | |

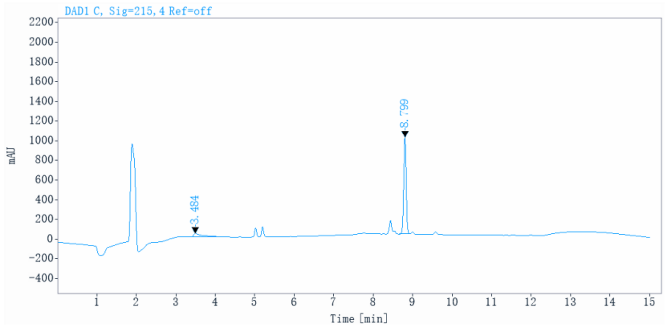
**Fig. S42.** Chromatogram of Fmoc-FA-$NH_2$ after 3 min flow deprotection and flow coupling (0.25 M)




Signal: DAD1 C, Sig=215,4 Ref=off

| RT [min] | Type | Width [min] | Area | Height | Area% | Name |
|---|---|---|---|---|---|---|
| 3.482 | MM | 0.2706 | 432.7310 | 26.6490 | 6.2167 | |
| 7.754 | MM | 0.0680 | 1361.5924 | 333.6208 | 19.5610 | |
| 8.788 | MM | 0.0772 | 5166.4355 | 1115.2150 | 74.2223 | |
| | | Sum | 6960.7590 | | | |

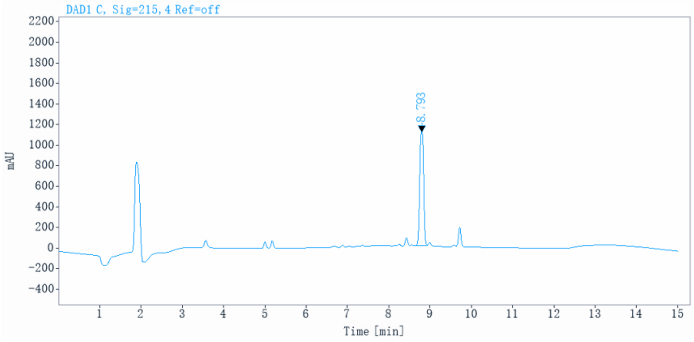
**Fig. S43.** Chromatogram of Fmoc-FA-$NH_2$ after 4 min flow deprotection and flow coupling (0.25 M)




Signal: DAD1 C, Sig=215,4 Ref=off

| RT [min] | Type | Width [min] | Area | Height | Area% | Name |
|---|---|---|---|---|---|---|
| 3.484 | MM | 0.2645 | 586.3951 | 36.9517 | 12.1213 | |
| 8.799 | MM | 0.0713 | 4251.3472 | 994.4354 | 87.8787 | |
| | | Sum | 4837.7423 | | | |

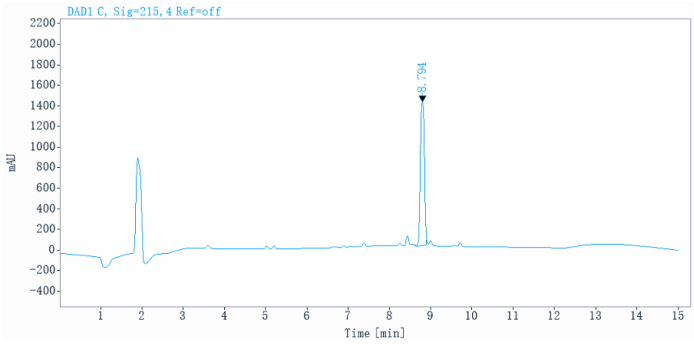
**Fig. S44.** Chromatogram of Fmoc-FA-$NH_2$ after 4 min flow deprotection and flow coupling (0.125 M)




Signal: DAD1 C, Sig=215,4 Ref=off

| RT [min] | Type | Width [min] | Area | Height | Area% | Name |
|---|---|---|---|---|---|---|
| 8.793 | MM | 0.1105 | 7356.3042 | 1109.4960 | 100.0000 | |
| | | Sum | 7356.3042 | | | |

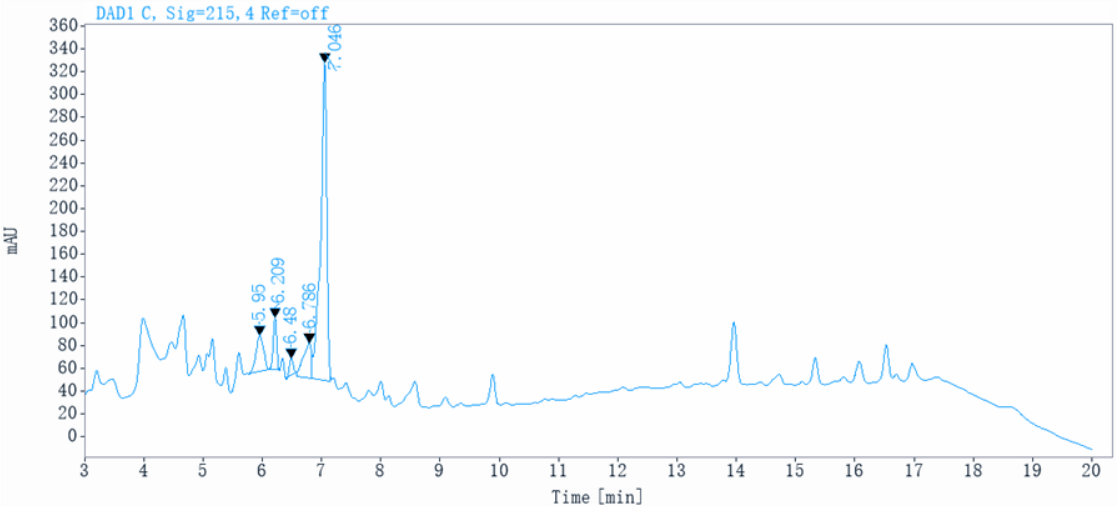
**Fig. S45.** Chromatogram of Fmoc-FA-$NH_2$ after 4 min flow deprotection and flow coupling (0.125 M)




Signal: DAD1 C, Sig=215,4 Ref=off

| RT [min] | Type | Width [min] | Area | Height | Area% | Name |
|---|---|---|---|---|---|---|
| 8.794 | MM | 0.1127 | 9445.6514 | 1397.0487 | 100.0000 | |
| | | Sum | 9445.6514 | | | |

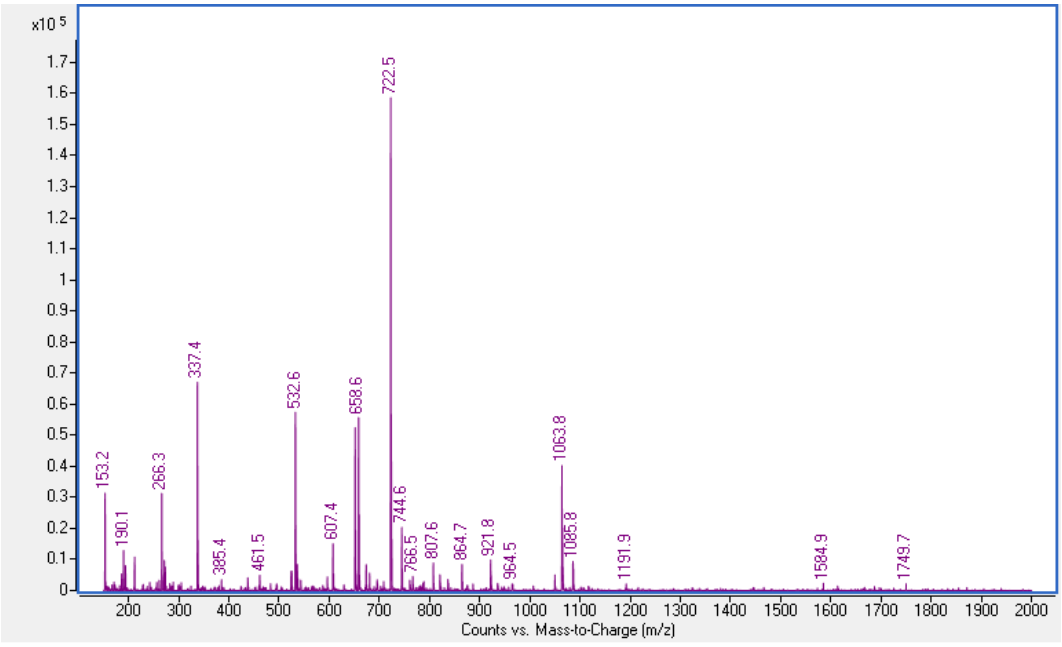
**Fig. S46.** HPLC and LCMS Chromatogram of ACP after 4 min flow deprotection and flow coupling (0.125 M)




**Signal:** DAD1 C, Sig=215,4 Ref=off

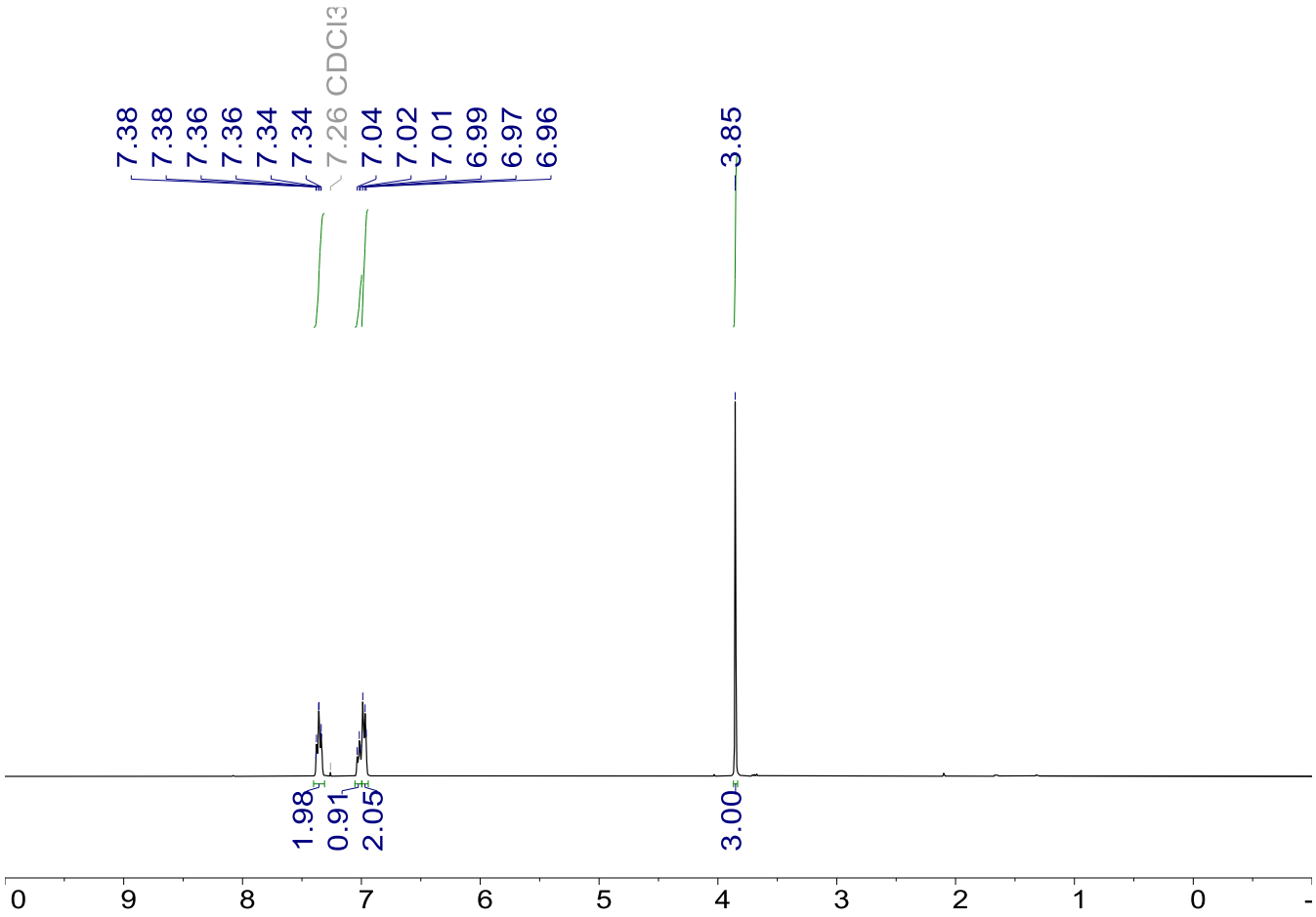
| RT [min] | Type | Width [min] | Area | Height | Area% | Name |
|---|---|---|---|---|---|---|
| 5.950 | MM | 0.1493 | 286.0463 | 31.9321 | 10.0002 | |
| 6.209 | MM | 0.0665 | 184.3770 | 46.2354 | 6.4458 | |
| 6.480 | MM | 0.0732 | 64.9481 | 14.7841 | 2.2706 | |
| 6.786 | MF | 0.1611 | 297.9409 | 30.8307 | 10.4160 | |
| 7.046 | FM | 0.1210 | 2027.0983 | 279.2650 | 70.8674 | |
| | | Sum | 2860.4107 | | | |

| Peak no. | RT (min) | Compound | Relative area |
|---|---|---|---|
| 1 | 5.950 | | 10.0 |
| 2 | 6.209 | | 6.4 |
| 3 | 6.480 | | 2.3 |
| 4 | 6.786 | | 10.4 |
| 5 | 7.046 | Target molecule: H-VQAAIDYING-$NH_2$ | 70.9 |



## 13. NMR spectra of Recycled Green Solvents

**Fig. S47.** NMR spectrum of Anisole




## 14. The comparison regarding time, cost, and purity among US-GSPPS, US-SPPS, MW-SPPS, and normal-SPPS

We summarize the time, cost, and purity data of US-GSPPS, US-SPPS, MW-SPPS, and normal-SPPS. The results were showed in Table S10. And the detail of the time and cost calculation was shown in follows.

**Table S10.** The comparison regarding time, cost, and purity among US-GSPPS, US-SPPS, MW-SPPS, and normal-SPPS

| Entry | Peptides | Method | Time (min) | Cost (RMB) | Purity (%) |
|---|---|---|---|---|---|
| 1 | H-WFTTLISTIM-$NH_2$ | **US-GSPPS** | **122** | **59.78** | **59.7** |
| 2 | (JR 10-mer) | US-SPPS | 116.5 | 94.53 | 66[3] |
| 3 | | MW-SPPS (90 ℃[4]) | 44.9 | 100.72 | 57[4] |
| 4 | | Normal-SPPS | 1130 | 96.38 | 57[1] |

**Calculation detail:**

1. US-GSPPS:

**Time** (the calculation method is refer to literature[3])**:**

10 × 10 minutes coupling + 11 × 2 minutes Fmoc-deprotection = 122 minutes

**Cost:**

Each peptide coupling per mmol:

| **Compound** | **Supplier** | **Amount[b]** | **Price[a]** | **Total cost** (RMB) |
|---|---|---|---|---|
| NFM (CAS:4394-85-8) | Tansoole Adamas | 36.25 mL | 104 RMB /500 mL | 7.54 |
| Anisole (CAS:100-66-3) | Tansoole Greagent | 36.25 mL | 70 RMB /500 mL | 5.075 |
| EtOH (CAS: 64-17-5) | Tansoole Greagent | 30 mL | 625 RMB /25 L | 0.75 |
| Oxyma-B (CAS: 5417-13-0) | HighFine | 0.111 g | 560 RMB /100 g | 0.6216 |
| DIC (CAS: 693-13-0) | Tansoole Adamas | 0.45 mL | 74 RMB /100 mL | 0.333 |
| DBU (CAS: 6674-22-2) | Tansoole Adamas | 0.5 g | 98 RMB /500 g | 0.098 |

[a] The price was inquired from the supplier at 2024/07/31. [b] The amount include 2 times wash, 2times deprotection, and 1 times coupling.

In this case, the Fmoc-protected amino acids (Supplier: Bide Pharmatech) was three-fold excess, and the total amino acids cost is 45.36 RMB.

**Therefore, the total cost by using US-GSPPS is 59.78 RMB/mmol.**

2. US-SPPS:

**Time:**

10 × 10 minutes coupling + 11 × (1+0.5) minutes Fmoc-deprotection = 116.5 minutes

**Cost:**

Each peptide coupling per mmol:

| **Compound** | **Supplier** | **Amount[b]** | **Price[a]** | **Total cost** (RMB) |
|---|---|---|---|---|
| DMF (CAS: 68-12-2) | Tansoole Adamas | 178 mL | 38 RMB /500 mL | 13.53 |
| DCM (CAS: 75-09-2) | Tansoole Greagent | 120 mL | 420 RMB /25 L | 2.02 |
| DIPEA (CAS: 7087-68-5) | Tansoole Adamas | 1.35 mL | 29 RMB /100 mL | 0.39 |
| Oxyma (CAS: 3849-21-6) | Tansoole Adamas | 0.568 g | 49 RMB /100 g | 0.28 |
| COMU (CAS: 1075198-30-9) | Tansoole Adamas | 1.712 g | 670 RMB /100 g | 11.47 |
| Piperidine (CAS: 110-89-4) | Tansoole Greagent | 12 mL | 265 RMB | 6.36 |

| | | | | |
|---|---|---|---|---|
| | | | /500 mL | |

[a] The price was inquired from the supplier at 2024/07/31. [b] The amount include 2 times wash, 2times deprotection, and 1 times coupling.

In this case, the Fmoc-protected amino acids (Supplier: Bide Pharmatech) was four-fold excess, and the total amino acids cost is 60.48 RMB.

**Therefore, the total cost by using US-GSPPS is 94.53 RMB/mmol.**

3. MW-SPPS:

**Time:**

10 × (2.75) minutes coupling + 11 × (1.58) minutes Fmoc-deprotection = 44.9 minutes

**Cost:**

Each peptide coupling per mmol:

| **Compound** | **Supplier** | **Amount[b]** | **Price[a]** | **Total cost** (RMB) |
|---|---|---|---|---|
| DMF (CAS: 68-12-2) | Tansoole Adamas | 221 mL | 38 RMB /500 mL | 16.80 |
| Oxyma (CAS: 3849-21-6) | Tansoole Adamas | 0.71 g | 49 RMB /100 g | 0.35 |
| DIC (CAS: 693-13-0) | Tansoole Adamas | 0.75 mL | 74 RMB /100 mL | 0.56 |
| Piperidine (CAS: 110-89-4) | Tansoole Greagent | 14 mL | 265 RMB /500 mL | 7.42 |

[a] The price was inquired from the supplier at 2024/07/31. [b] The amount include 2 times wash, 2times deprotection, and 1 times coupling.

In this case, the Fmoc-protected amino acids (Supplier: Bide Pharmatech) was five-fold excess, and the total amino acids cost is 75.60 RMB.

**Therefore, the total cost by using US-GSPPS is 100.72 RMB/mmol.**

4. Normal-SPPS:

**Time:**

10 × 80 minutes coupling + 11 × (15+15) minutes Fmoc-deprotection = 1130 minutes

**Cost:**

Each peptide coupling per mmol:

| **Compound** | **Supplier** | **Amount[b]** | **Price[a]** | **Total cost** (RMB) |
|---|---|---|---|---|
| DMF (CAS: 68-12-2) | Tansoole Adamas | 420.8 mL | 38 RMB /500 mL | 31.98 |
| DCM (CAS: 75-09-2) | Tansoole Greagent | 26 mL | 420 RMB /25 L | 0.44 |
| Oxyma (CAS: 3849-21-6) | Tansoole Adamas | 0.568 g | 49 RMB /100 g | 0.28 |
| DIC (CAS: 693-13-0) | Tansoole Adamas | 0.60 mL | 74 RMB /100 mL | 0.44 |
| Piperidine (CAS: 110-89-4) | Tansoole Greagent | 5.2 mL | 265 RMB /500 mL | 2.76 |

[a] The price was inquired from the supplier at 2024/07/31. [b] The amount include 2 times wash, 2times deprotection, and 1 times coupling.

In this case, the Fmoc-protected amino acids (Supplier: Bide Pharmatech) was four-fold excess, and the total amino acids cost is 60.48 RMB.

**Therefore, the total cost by using US-GSPPS is 96.38 RMB/mmol.**

## Supporting References


(1) Martin, V.; Jadhav, S.; Egelund, P. H. G.; Liffert, R.; Castro, H. J.; Krüger, T.; Haselmann, K. F.; Quement, S. T. L.; Albericio, F.; Dettner, F.; Lechner, C.; Schönleber, R.; Pedersen, D. S. Harnessing Polarity and Viscosity to Identify Green Binary Solvent Mixtures as Viable Alternatives to DMF in Solid-Phase Peptide Synthesis. *Green Chem.* **2021**, *23* (9), 3295–3311. https://doi.org/10.1039/D1GC00603G.

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