Supporting Information

Accelerated Fmoc Solid-phase Synthesis of Peptides with Aggregation-disrupting Backbones

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1. General Information..............................................................................................................................................S2
   a. Materials
   b. HPLC
   c. Air-bath heating SPPS
   d. Mass spectrometry

2. Experimental Section..............................................................................................................................................S3
   a. Organic synthesis
   b. Supporting figures
1. General Information

a. Materials

All reagents and solvents were purchased from Sinopharm Chemical Reagent Co., Ltd., Alfa Aesar China Co., Ltd., &K Chemical Co., Ltd. THF was distilled from sodium/diphenyl ketone immediately prior to use. DMF was distilled under reduced pressure from sodium sulfate and stored over 4 Å molecular sieves. CH₂Cl₂, pyridine and Et₃N were distilled from calcium hydrate immediately prior to use. All other commercially available reagents and solvents were used as received without further purification unless otherwise indicated. All organic extracts were dried over sodium sulfate or magnesium sulfate. TLC was carried out on plates pre-coated with silica gel 60 F254 (250 layer thickness). Visualization was accomplished using UV light, iodine vapors, ninhydrin solution, permanganate solution and/or phosphomolybdic acid (PMA) solution. Flash column chromatographic purification of products was accomplished using forced-flow chromatography on Silica Gel (300-400 mesh on large-scale or 200-300 mesh on small-scale). Fmoc-protected amino acids were purchased from GL Biochem (Shanghai) Co., Ltd. Rinkamide AM polystyrene resins (1% DVB, 100-200 mesh, loading = 0.34 mmol/g) were purchased from Tianjian Nankai HECHENG S&T Co., Ltd.

b. HPLC

Analytical HPLC was run on a SHIMADZU (Prominence LC-20AT) instrument using an analytical column (Grace Vydac “Peptide C18”, 250 × 4.6 mm, 5 μm particle size, flow rate 1.2 mL/min, rt). Analytical injections were monitored at 214 nm and 254 nm. Semi preparative HPLC was run on a SHIMADZU (Prominence LC-20AT) instrument using a semi preparative column (Grace Vydac “Peptide C18”, 250 × 10 mm, 10 μm particle size, flow rate 4.0 mL/min). Solvent A was 0.1% TFA in acetonitrile, and solvent B was 0.1% TFA in water. Both solvents were filtered through 0.22 μm filter paper and sonicated for 20 min before use.

c. Air-bath heating SPPS

Screw-cap glass peptide synthesis reaction vessels were attained from commercial sources. The SPPS glasswares were immobilized on a rotary shaker (130 rpm) during reaction.

Fmoc-protected Rinkamide AM or hydrazide resins were initially swelled in DCM/DMF (1/1) for 10 min before use.

Fmoc deprotection was conducted using 20% piperidine (0.1 M Oxyma)/DMF (1 min + 9 min). The resin was washed with 5× DMF, 5× DCM and 5× DMF.

Arg coupling was started by pouring a preactivated (30 sec at RT) a solution of 4 eq Fmoc-Arg(Phb)-OH (0.1 M in DMF) with 3.6 eq HCTU and 8 eq DIEA to the resin. The reaction mixture was kept at RT for 45 min. Coupling of amino acids other than Arg was started by pouring a preactivated (5 min at RT) solution of 4 eq protected amino acid (0.1 M in DMF) with 4 eq DIC and 4 eq Oxyma to the resin. His(Trt) coupling was kept at RT for 45 min. Cys(Trt) coupling was kept at 50°C (air-bath heating) for 20 min. Other residue couplings were kept at 75°C (air-bath heating) for 20 min.

At the end of SPPS, the resin was cleaved with modified Reagent K, TFA cocktail (5% H₂O, 5% thioanisole, 2.5% EDT). After 2-3 h, the resin was washed with an equal volume of TFA once. Combined TFA eluents were concentrated under nitrogen blow. The crude peptides were obtained through precipitation with ice cold ether and 4°C centrifugation at 5000 rpm for 2 min. The peptide pellet was dissolved in 0.1% TFA containing CH₃CN/H₂O (1/1), characterized by analytical HPLC and ESI-MS, and purified, if necessary, by semi preparative HPLC before lyophilization.

d. Mass spectrometry

ESI mass spectra were measured on Agilent 6210 Time of Flight Mass Spectrometer or a Bruker Daltonics DataAnalysis 3.0 workstation.
2. Experimental Section
a. Organic Synthesis

To a solution of 6-hydroxy-1,3-benzoxathiol-2-one (3.4 g, 20 mmol) in DMF (20 mL) was added cesium carbonate (19.6 g, 60 mmol) and iodomethane (5 mL, 80 mmol) and the mixture was stirred at 40°C overnight. After cooling to room temperature the precipitate was filtered off and washed with EtOAc. The combined filtrate was then washed with ice-cold ether and purified using the same procedure as that for ordinary crude peptides.

DMF (7.7 mL, 100 mmol) was cooled in an ice-bath and treated with freshly distilled phosphorous oxychloride (9.1 mL, 100 mmol) and the mixture was stirred at 0°C for 30 min until the white solid appeared. The reaction mixture was then treated with a solution of 10 in DMF (20 mL) and stirred at 90°C overnight. After cooling to room temperature the reaction was quenched with diluted water cautiously. The solid was filtered off and washed with EtOAc. The combined filtrate was washed with saturated aq. NaHCO₃ and brine, dried, and evaporated to give 11 as a clear brown oil which could be used without further purification.

To a solution of 11 in CH₂Cl₂ (20 mL), cooled to 0°C was added boron tribromide (3.85 mL, 40 mmol) dropwise and the mixture was stirred at 0°C for 2 h. The reaction mixture was quenched with diluted water cautiously at 0°C. The precipitate was carefully filtered off and washed with CH₂Cl₂. The filtrate was washed with brine, dried and evaporated to give the crude product as a brown solid. Flash chromatography [Petroleum ether:EtOAc = 20:1] gave the salicylaldehyde 12 (2.6 g, 13.1 mmol, 62% for three steps) as a yellowish solid.

To a solution of salicylaldehyde 12 (2.6 g, 13.1 mmol) in AcOH (20 mL), NaIO₄ (2.78 g, 13.1 mmol) was added and the mixture was stirred at RT for 2 h. The solution was concentrated and the product was purified through flash chromatographed [Petroleum ether:EtOAc = 10:1 -> 4:1 -> 1:1 -> 0:1] to give 13 (1.66 g, 7.8 mmol, 60%) as a yellowish solid. 1H NMR (400 MHz, CDCl₃): δ(ppm) 11.70 (s, 1H), 9.79 (s, 1H), 7.98 (s, 1H), 6.45 (s, 1H), 3.91 (s, 3H), 2.73 (s, 3H). 13C NMR (100 MHz, CDCl₃): δ(ppm) 194.6, 166.6, 161.5, 131.1, 125.7, 115.5, 99.7, 56.6, 41.7. ESI-MS (positive): 215.5 (observed, M+H); 214.2 (calculated, M).

b. Supporting figures

![Figure S1](image1.png)

**Figure S1** 2-Hydroxy-4-methoxy-5-(methylthio)benzaldehyde (Hmsb).

![Scheme S1](image2.png)

**Scheme S1** Deprotection of Hmsb with reducing TFA cocktail (2.5% H₂O, 2.5% TIPS, 2.5% EDT, 1% Bu₄NI) at 0°C 30 min, then RT 2 hr. The crude product can be precipitated with ice-cold ether and purified using the same procedure as that for ordinary crude peptides.
Scheme S2  Synthesis of auxiliary Hmsb.

Figure S2  Racemization test of Ac-Cys[L/D]-(Hmsb)Ala-Lys-NH₂.
Figure S3  Crude HPLC and ESI-MS of model peptides.
A) H-Met(O)-Leu-(Hmsb)Leu-Lys-Ser-NH$_2$;
B) H-Met(O)-Asn-(Hmsb)Asn-Lys-Ser-NH$_2$;
C) H-Met(O)-Ile-(Hmsb)Gly-Lys-Ser-NH$_2$;
D) H-Met(O)-Ile-(Hmsb)Ala-Lys-Ser-NH$_2$;
E) H-Met(O)-Phe-(Hmsb)Phe-Lys-Ser-NH$_2$;
F) H-Met(O)-Pro-(Hmsb)Gln-Lys-Ser-NH$_2$;
G) H-Met(O)-Arg-(Hmsb)Arg-Lys-Ser-NH$_2$;
H) H-Met(O)-Trp-(Hmsb)Tyr-Lys-Ser-NH$_2$;
Figure S4  Air-bath heating shaker.

Compound 13 (\(^1\)H NMR)

Compound 13 (\(^{13}\)C NMR)
Compound 13 (ESI-MS)