Supporting Information

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

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1. General Information

a. Materials

All reagents and solvents were purchased from Sinopharm Chemical Reagent Co., Ltd., Alfa Aesar China Co., Ltd., J&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. CH_2Cl_2, pyridine and Et_3N were distilled from calcium hydride 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. Rink amide 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 Rink amide 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(Pbf)-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% H_2O, 5% thioanisole, 2.5% EDT). After 2-3 h, the resin was washed with an equal volume of TFA once. Combined TFA elutents 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 CH_3CN/H_2O (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 brine, dried, and evaporated to give **10** as a clear brown oil which could be used without further purification.

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. $NaHCO_3$ and brine, dried, and evaporated to give **11** as brown solid, which could be used without further purification.

To a solution of **11** in CH_2Cl_2 (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 CH_2Cl_2. 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), $NaIO_4$ (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. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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

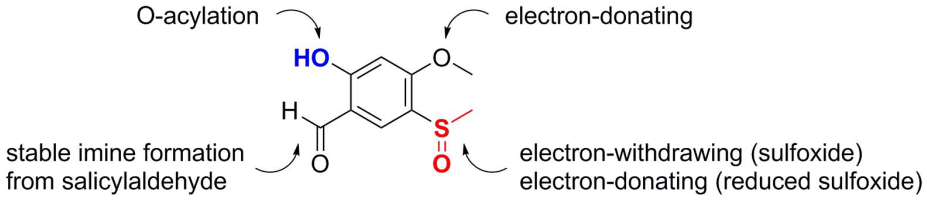
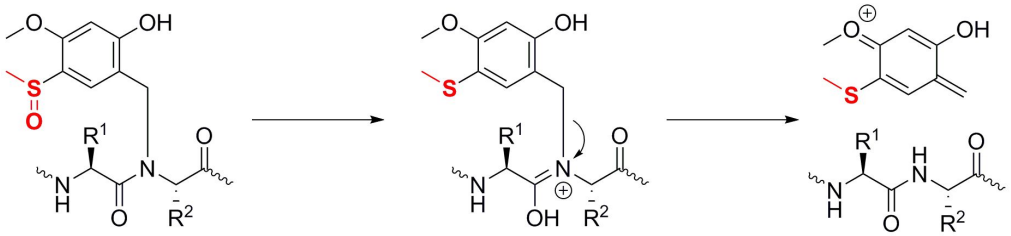
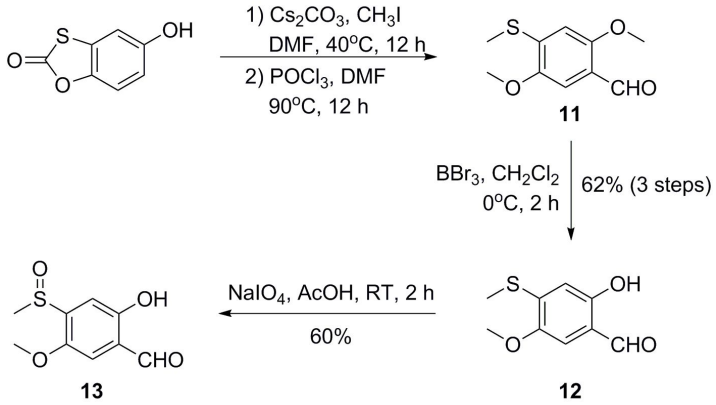


Figure S1 2-Hydroxy-4-methoxy-5-(methylthio)benzaldehyde (Hmsb).



Scheme S1 Deprotection of Hmsb with reducing TFA cocktail (2.5% H_2O, 2.5% TIPS, 2.5% EDT, 1% Bu_4NI) 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.

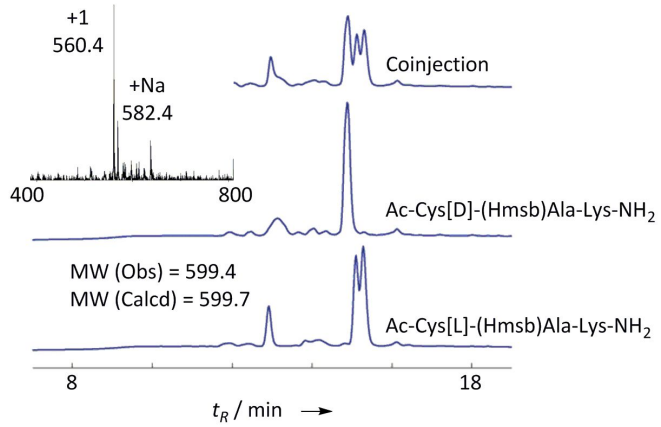
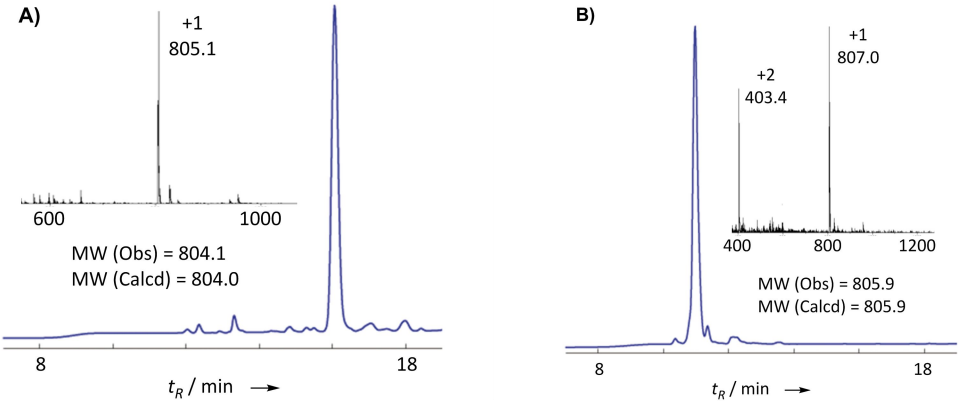


Figure S2 Racemization test of Ac-Cys[L/D]-(Hmsb)Ala-Lys-NH_2.



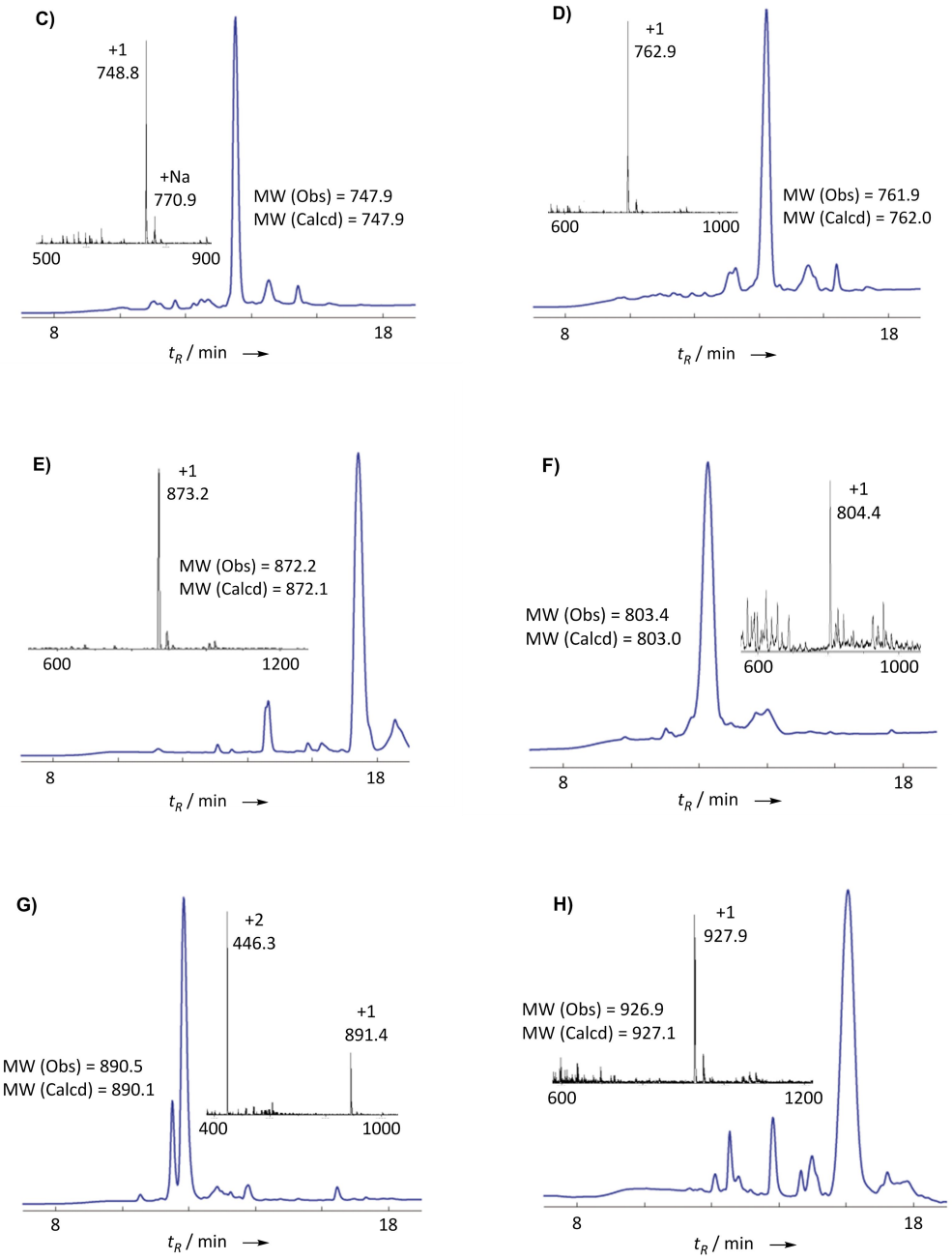


Figure S3 Crude HPLC and ESI-MS of model peptides.

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;

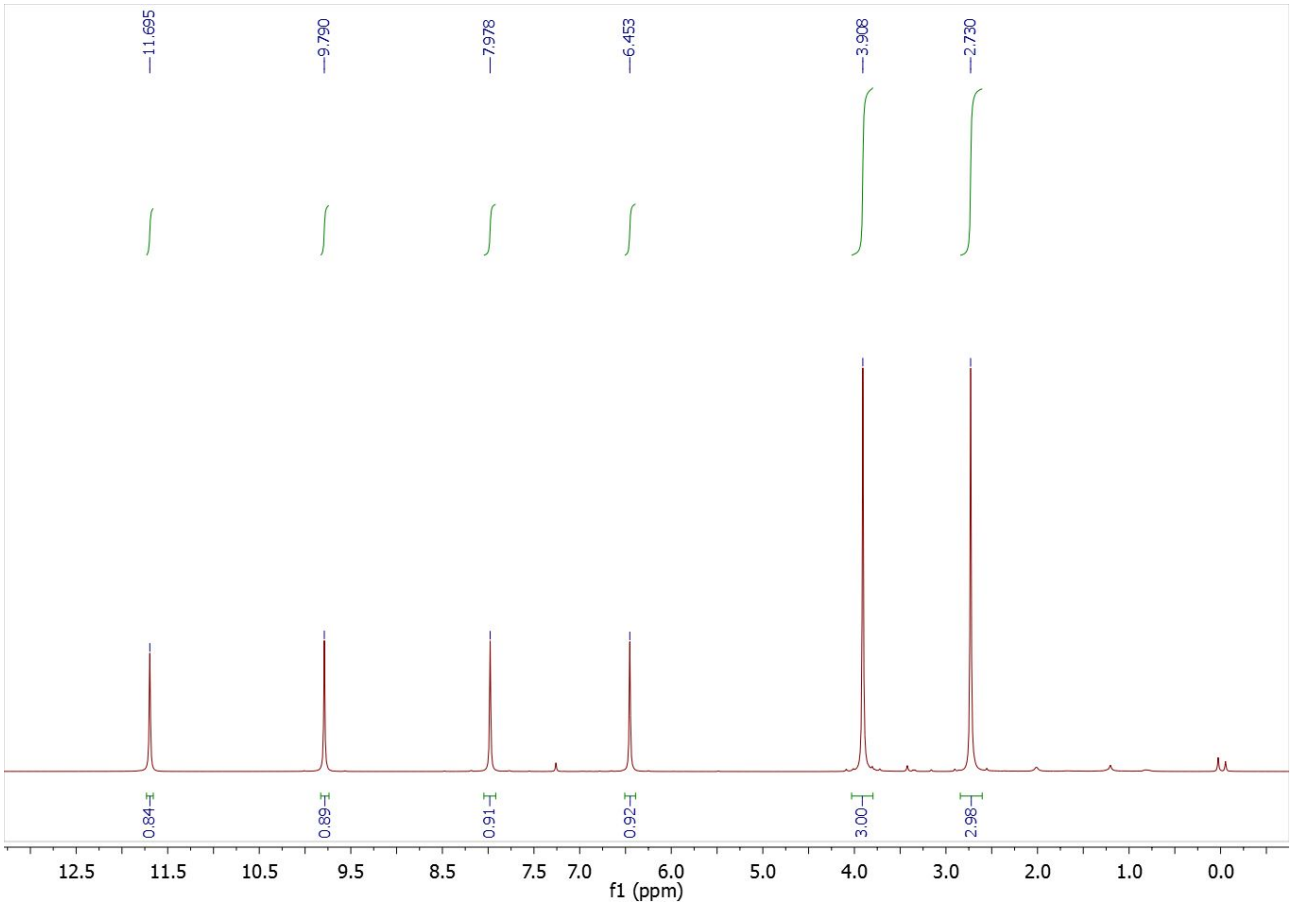
F) H-Met(O)-Pro-(Hmsb)Gln-Lys-Ser-NH_2;

G) H-Met(O)-Arg-(Hmsb)Arg-Lys-Ser-NH_2;

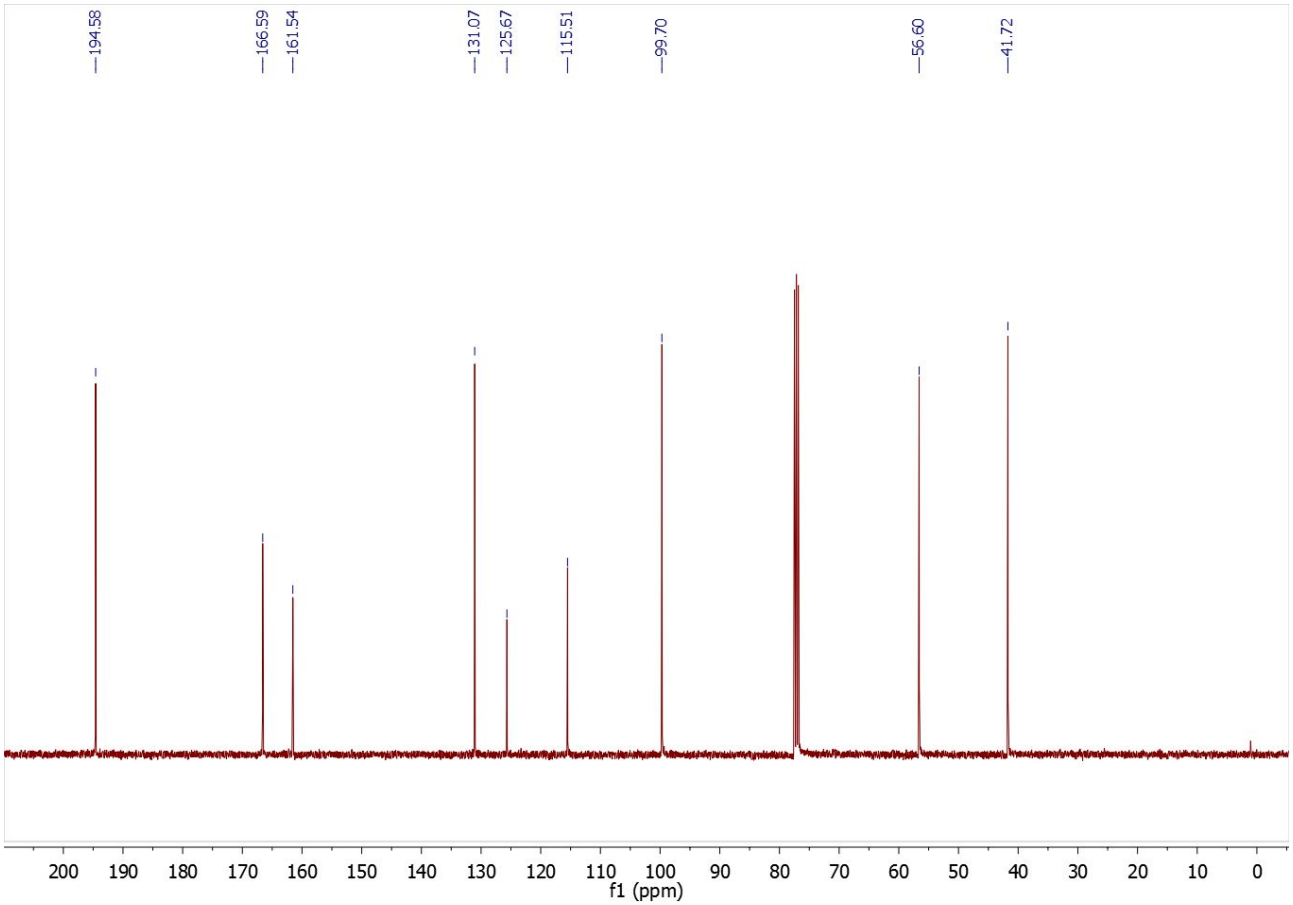
H) H-Met(O)-Trp-(Hmsb)Tyr-Lys-Ser-NH_2;

Figure S4 Air-bath heating shaker.

Compound **13** (^{1}H NMR)



Compound **13** (^{13}C NMR)



Compound **13** (ESI-MS)