Supplementary information

Catalog

1. Materials and Reagents

Rink amide AM resin, HCTU, HATU, PyAop, HOAt, DIEA were bought from CS Bio, GL Biochem (Shanghai, China), Adamas (Shanghai, China). (DMF), dichloromethane (DCM), dimethyl sulfoxide (DMSO), N-methyl-2-pyrrolidone (NMP) and anhydrous diethyl ether were purchased from Sinopharm Chemical Reagent. Thioanisole and trifluoroacetic acid (TFA) (HPLC grade) were purchased from J&K Scientific (Beijing, China). TLC was executed on plates pre-coated with silica gel 60 F254 (250layer thickness). We used UV light (254 nm), permanganate solution to achieve visualization. MP AM Resin, TCA Deblock, ACN Washing, CAP- A, CAP-B, Activator, SOL,Oxidizing, DMT-dT-CE phosphoramodite, DMT-dC-CE phosphoramodite, DMT-dA-CE phosphoramodite, DMT-dG-CE phosphoramodite were bought from Hebei DNA chem Biotechnology Co., Ltd.

2. HPLC-MS

2.1 peptide characterization

Analytical HPLC was run on a SHIMADZU (Prominence LC-20AT) instrument using analytical column (Grace Vydac "Protein & Peptide C18", 250 × 4.6 mm, 5 μm particle size, flow rate 0.80 mL/min, R.T.). The gradient is 10%-99%. Analytical samples 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 3mL/min, rt). The gradient is 1%-99%. Solution A was 0.08 % trifluoroacetic acid in acetonitrile, and solution B was 0.1 % trifluoroacetic acid in ddH_2O.

2.2 Oligonucleotide and peptide- oligonucleotide characterization

Analytical HPLC was run on a SHIMADZU (Prominence LC-20AT) instrument using analytical column (Grace Vydac "Protein & Peptide C18", 250 × 4.6 mm, 5 μm particle size, flow rate 0.80mL/min, R.T.). The gradient is 1%-99%. Analytical samples were monitored at 260 nm and 280 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 3mL/min, rt). The gradient is 1%-81%. Solution A was pure acetonitrile, and solution B was 0.1M TEAA

0.10M TEAA: Add 6ml glacial acetic acid, 100ml ddH_2O water, and 13.75ml ethylamine into the beaker successively, and then ddH_2O to adjust the volume to 2L. Finally, the pH of solution is adjusted to 7.

3. The removal of Boc from the model peptide

3.1 Removal of Boc from model peptide LWFA

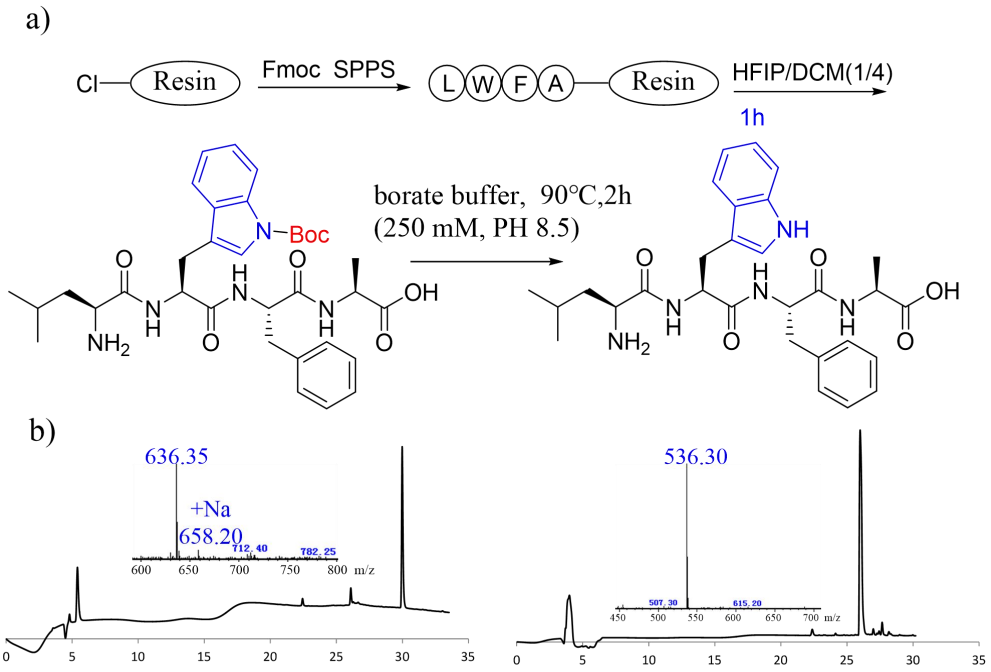


Fig. S1. Synthesis and removal of Boc protecting group from model peptide **1**: LW(Boc)FA

Model peptide **1** was prepared by standard solid-phase peptide synthesis on chorine resin. Chorine resin (0.1 mmol, 312.5 mg, 1.0 equiv.) is added to the reaction tube. The synthesis steps are as follows:

(1) 20% piperidine was added to the reaction tube to remove Fmoc, and the reaction was performed twice for 5 min each time. After the reaction, resin was washed with DMF and acetonitrile three times each. Fmoc-Ala-OH (0.4 mmol, 124.5 mg, 4.0 equiv.) and DIEA (0.8 mmol, 132.0 μl, 8.0 equiv.) were dissolved in DCM and added to the reaction tube. The mixture was stirred at rt for 12 h. After the reaction, CPG was washed with DMF and acetonitrile three times each.

(2) 20% piperidine was added to the reaction tube to remove Fmoc. The reaction was performed twice for 5 min each time. After the reaction, resin was washed with DMF and acetonitrile three times each. HCTU (0.4 mmol, 165.5 mg, 4.0 equiv), Fmoc-Phe-OH (0.4 mmol, 155.0 mg, 4.0 equiv.) and DIEA (0.8 mmol, 132 μl, 8.0 equiv.) were dissolved in DMF and added to the reaction tube. The mixture was stirred at rt for 40 min. After the reaction, CPG was washed with DMF and acetonitrile three times each.

(3) Repeat the previous step and attach the remaining two amino acids (Tryptophan and leucine) to the resin.

(4) 20% piperidine was added to the reaction tube to remove Fmoc, and the reaction was

performed twice for 5 min each time. The model peptide was released from the resin by the reaction of 20% HFIP (methylene chloride) with the resin for 1 h, while Boc of tryptophan remained on the model peptide. (M:636.35)

(5) The model peptide containing Boc was dissolved in borate buffer (250 mM, pH8.50) at 90℃ for 2 h, and the reaction solution was analyzed by HPLC and MS.(M:536.30) The Boc was successfully removed from the peptide.

3.2 Removal of Boc from model peptide HLWFA

3.2.1 Removal of Boc from model peptide HLWFA

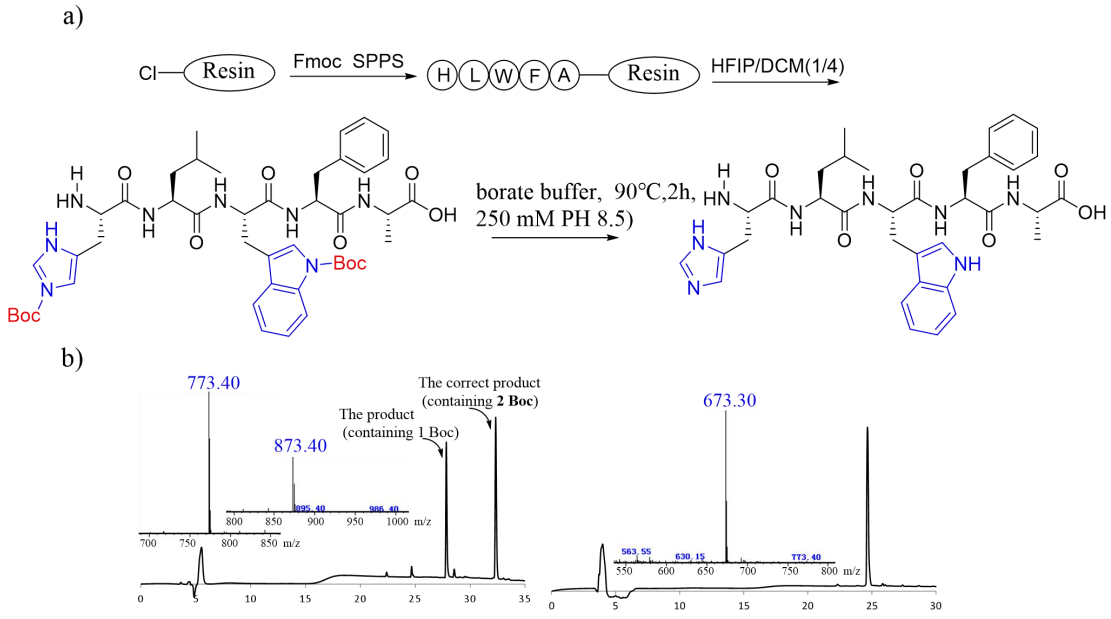


Fig. S2. Synthesis and removal of Boc protecting group from model peptide **3**: H(Boc)LW(Boc)FA**.**

Model peptide **3** was prepared by standard solid-phase peptide synthesis on chlorine resin. Chlorine resin (0.1 mmol, 312.5 mg, 1.0 equiv.) is added to the reaction tube. The specific synthesis steps are as follows:

(1) 20% piperidine was added to the reaction tube to remove Fmoc, and the reaction was performed twice for 5 min each time. After the reaction, resin was washed with DMF and acetonitrile three times each. Fmoc-Ala-OH (0.4 mmol, 124.5 mg, 4.0 equiv.) and DIEA (0.8 mmol, 132.0 μl, 8.0 equiv.) were dissolved in DCM and added to the reaction tube. The mixture was stirred at rt for 12 h. After the reaction, CPG was washed with DMF and acetonitrile three times each.

(2) 20% piperidine was added to the reaction tube to remove Fmoc. The reaction was performed twice for 5 min each time. After the reaction, resin was washed with DMF and acetonitrile three

times each. HCTU (0.4 mmol, 165.50 mg, 4.0 equiv.), Fmoc-Phe-OH (0.4 mmol, 155.0 mg, 4.0 equiv.) and DIEA (0.8 mmol, 132.0 μl, 8.0 equiv.) were dissolved in DMF and added to the reaction tube. The mixture was stirred at rt for 40 min. After the reaction, CPG was washed with DMF and acetonitrile three times each.

(3) Repeat the previous step 2) and attach the remaining three amino acids (tryptophan, leucine and histidine) to the resin.

(4) 20% piperidine was added to the reaction tube to remove Fmoc, and the reaction was performed twice for 5 min each time. The fully protected model peptide HLWFA was released from the resin by the reaction of 20% HFIP (methylene chloride) with the resin for 1 h. w HPLC analysis showed that there were two products. The results of MS showed that one product (M:873.4) was correct and contained two Boc, while the other product (M:773.4) contained only one Boc. The reason may be that HFIP caused one of the Boc to disappear.

(5) The model peptide containing Boc was dissolved in borate buffer (250 mM, pH8.5) at 90℃ for 2 h, and the reaction solution was analyzed by HPLC and MS.(M:673.30) All Boc were successfully removed from the peptide.

3.2.2 Stability of Fmoc-His(Boc)-OH by treatment of TCA

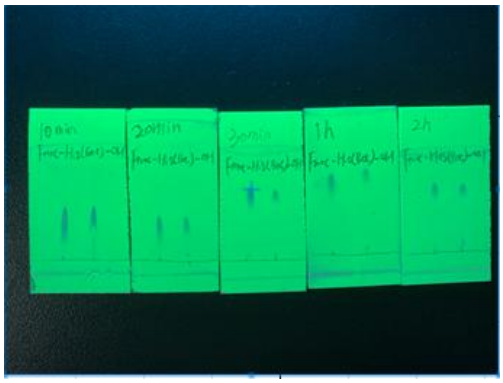


Fig. S3. Stability (TLC analysis) of Fmoc-His(Boc)-OH by treatment of TCA

The DMTr group needs to be removed by weak acid TCA in every step of oligonucleotide synthesis, so it is necessary to verify whether TCA will remove Boc from histidine. Fmoc-His(Boc)-OH was dissolved by ethyl acetate as reference. Then Fmoc-His(Boc)-OH was dissolved with TCA and stirred. After 10 minutes, 20 minutes, 30 minutes, 1 hour and 2 hours, the Fmoc-His(Boc)-OH dissolved with TCA was analyzed by thin layer chromatography (TLC) (CH_2Cl_2: MeOH = 10:1). The reference was Fmoc-His(Boc)-OH dissolved by ethyl acetate. Fmoc-His(Boc)-OH dissolved by ethyl acetate was in the same position as Fmoc-His(Boc)-OH dissolved by TCA, and the Fmoc-His(Boc)-OH remained stable after 2h TCA treatment. This is only a preliminary judgment, and more accurate conclusions need to be analyzed by HPLC analysis.

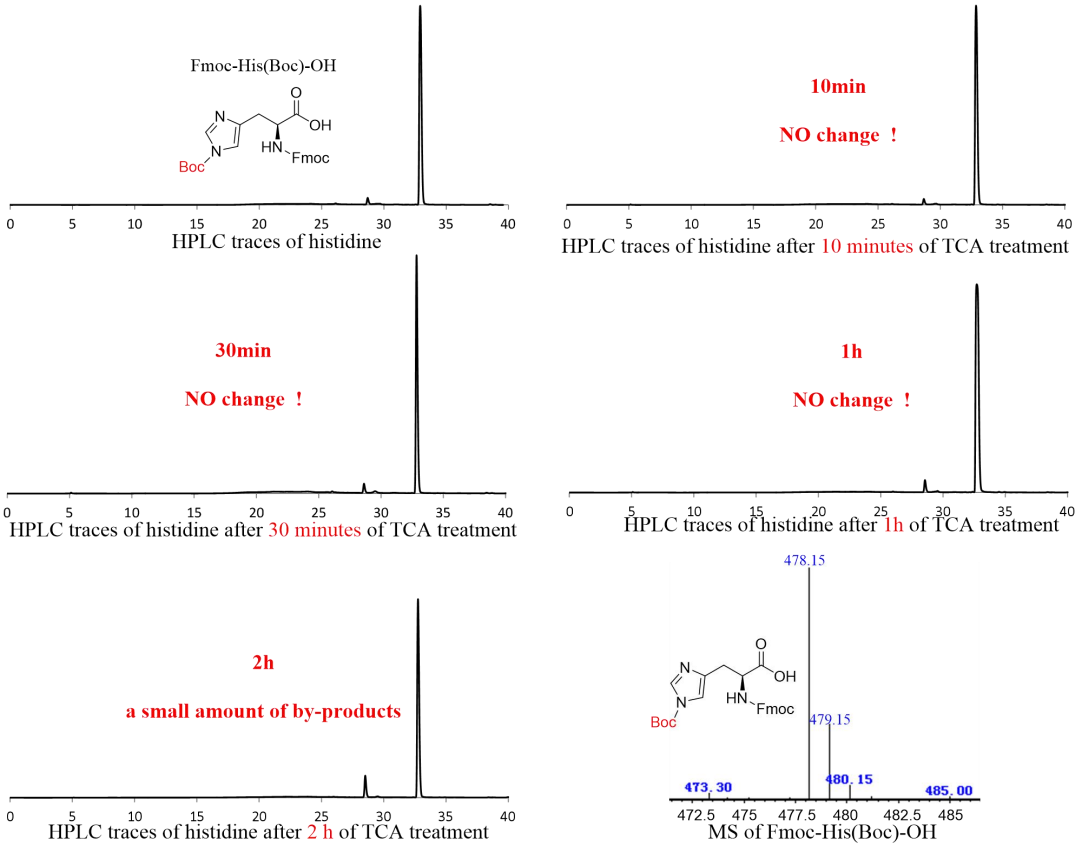


Fig. S4. Stability of Fmoc-His(Boc)-OH to TCA, with the treatment time of 10 min, 30 min, 1 hour, and 2 hours.

Fmoc-His(Boc)-OH was dissolved in acetonitrile and then analyzed by HPLC. The gradient is 10%-99% in 40 min. Analytical samples were monitored at 260 nm and 280 nm. Solution A was pure acetonitrile, and solution B was 0.1M TEAA (Refer to 2.2).

Fmoc-His(Boc)-OH was dissolved in acetonitrile and then analyzed by HPLC. The chromatography was single peak and the mass spectrometry (478.15) was correct. Then the Fmoc-His(Boc)-OH was dissolved with TCA and stirred for 10 minutes, then the white solid was precipitated by adding petroleum ether, repeated twice, and finally analyzed by HPLC. The results showed that Fmoc-His(Boc)-OH was stable after 10min treatment of TCA. Fmoc-His(Boc)-OH was stirred in the TCA for 30 min, 1 hour and 2 hours respectively. The subsequent operation was consistent with the previous operation. The results showed that Fmoc-His(Boc)-OH was stable after 1h treatment of TCA, and a small amount of by-products were produced after 2 h treatment of TCA.

3.3 Removal of Boc from model peptide FARL

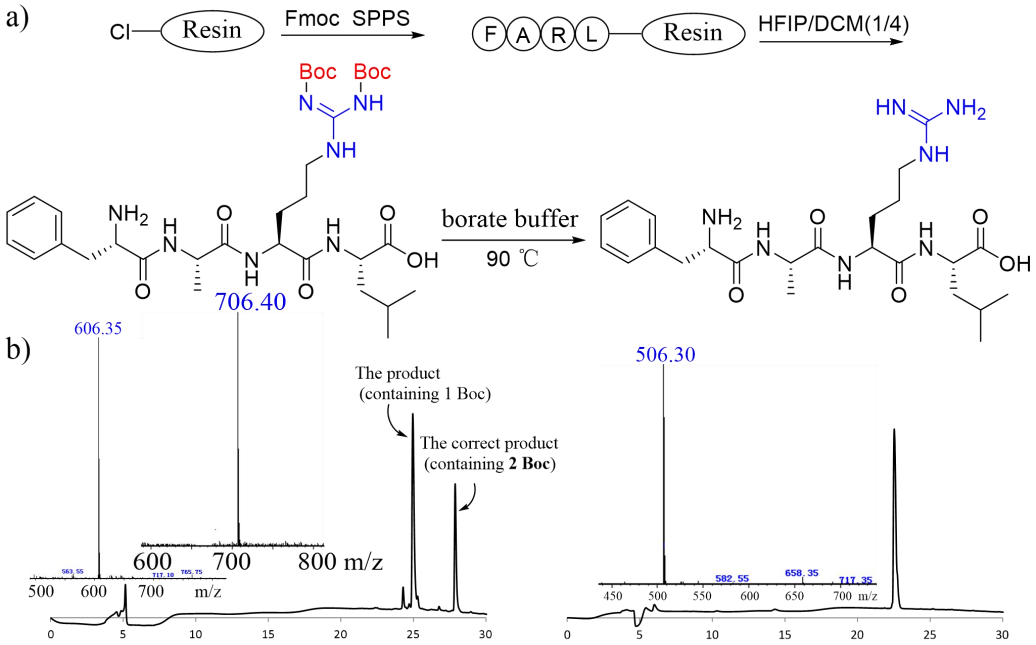


Fig. S5. Synthesis and removal of Boc protecting group from model peptide **5.**

Model peptide **5** was prepared by standard solid-phase peptide synthesis on chlorine resin. Chlorine resin (0.1 mmol, 312.5 mg, 1.0 equiv.) is added to the reaction tube. The synthesis steps are as follows:

(1) 20% piperidine was added to the reaction tube to remove Fmoc, and the reaction was performed twice for 5 min each time. After the reaction, resin was washed with DMF and acetonitrile three times each. Fmoc-Leu-OH (0.4 mmol, 141.4 mg, 4.0 equiv.) and DIEA (0.8 mmol, 132.0 μl, 8.0 equiv.) were dissolved in DCM and added to the reaction tube. The mixture was stirred at rt for 12 h. After the reaction, CPG was washed with DMF and acetonitrile three times each.

(2) 20% piperidine was added to the reaction tube to remove Fmoc. The reaction was performed twice for 5 min each time. After the reaction, resin was washed with DMF and acetonitrile three times each. PyAop (0.4 mmol, 208.6 mg, 4.0 equiv.), HOAt (54.4 mg, 0.4 mmol, 3.8 equiv.), Fmoc-Arg(Boc)$_2$-OH (0.4 mmol, 238.7 mg, 4.0 equiv.) and DIEA (0.8 mmol, 132.0 μl, 8.0 equiv.) were dissolved in DMF and added to the reaction tube. The mixture was stirred at rt for 12h. After the reaction, resin was washed with DMF and acetonitrile three times each.

(3) 20% piperidine was added to the reaction tube to remove Fmoc. The reaction was performed twice for 5 min each time. After the reaction, resin was washed with DMF and acetonitrile three times each. HCTU (0.4 mmol, 165.5 mg, 4.0 equiv.), Fmoc-Ala-OH (0.4 mmol, 124.5 mg, 4.0 equiv.) and DIEA (0.8 mmol, 132.0 μl, 8.0 equiv.) were dissolved in DMF and added to the reaction tube. The mixture was stirred at rt for 40 min. After the reaction, CPG was washed with DMF and acetonitrile three times each.

(4) Repeat the previous step (3) and attach the remaining amino acids (Phenylalanine) to the resin.

(5) 20% piperidine was added to the reaction tube to remove Fmoc, and the reaction was performed twice for 5 min each time. The fully protected model peptide FARL was released from the resin by the reaction of 20% HFIP (methylene chloride) with the resin for 1 h. HPLC analysis showed that there were two products. The results of MS showed that one product (M:706.30) was correct and contained two Boc, while the other product (M:606.35) contained only one Boc. The reason may be that HFIP caused one of the Boc to disappear.

(6) The model peptide containing Boc was dissolved in borate buffer (250 mM, pH8.5) at 90℃ for 2 h, and the reaction solution was analyzed by HPLC and MS.(M:506.30). All Boc were successfully removed from the peptide.

3.4 Removal of Boc from model peptide WHFARL

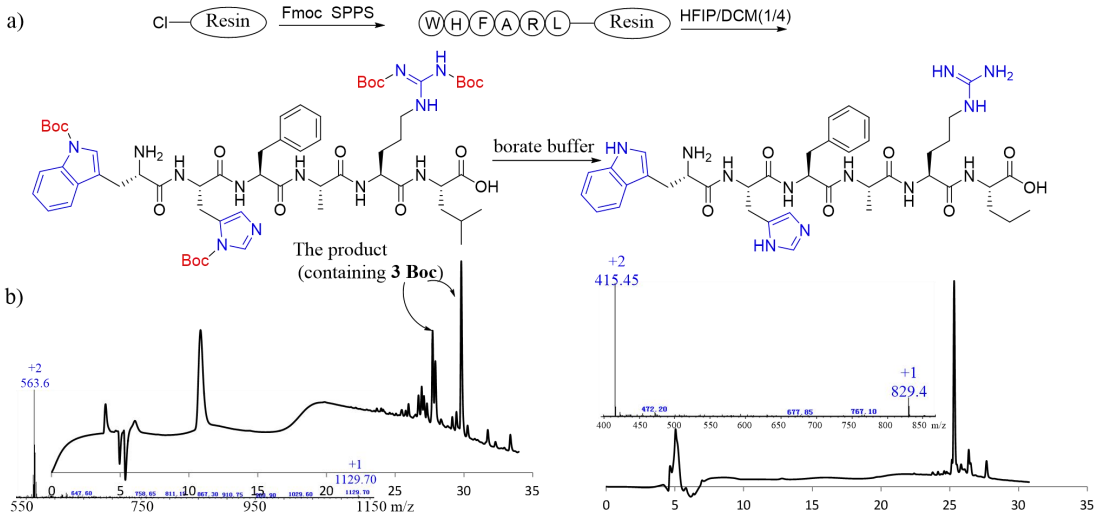


Fig. S6. Synthesis and removal of Boc protecting group from model peptide **7.**

Model peptide **7** was prepared by standard solid-phase peptide synthesis on chlorine resin. Chlorine resin (0.1 mmol, 312.5 mg, 1.0 equiv.) is added to the reaction tube. The specific synthesis steps are as follows:

(1) 20% piperidine was added to the reaction tube to remove Fmoc, and the reaction was performed twice for 5 min each time. After the reaction, resin was washed with DMF and acetonitrile three times each. Fmoc-Leu-OH (0.4 mmol, 141.4 mg, 4.0 equiv.) and DIEA (0.8 mmol, 132.0 μl, 8.0 equiv.) were dissolved in DCM and added to the reaction tube. The mixture was stirred at rt for 12 h. After the reaction, CPG was washed with DMF and acetonitrile three times each.

(2) 20% piperidine was added to the reaction tube to remove Fmoc. The reaction was performed twice for 5 min each time. After the reaction, resin was washed with DMF and acetonitrile three times each. PyAop (0.4 mmol, 208.6 mg, 4.0 equiv.), HOAt (0.4 mmol, 54.4 mg, 4.0 equiv.), Fmoc-Arg-OH (0.4 mmol, 238.7 mg, 4.0 equiv.) and DIEA (0.8 mmol, 132.0 μl, 8.0 equiv.) were

dissolved in DMF and added to the reaction tube. The mixture was stirred at rt for 12h. After the reaction, CPG was washed with DMF and acetonitrile three times each.

(3) 20% piperidine was added to the reaction tube to remove Fmoc. The reaction was performed twice for 5 min each time. After the reaction, resin was washed with DMF and acetonitrile three times each. HCTU (0.4 mmol, 157.2 mg, 4.0 equiv.), Fmoc-Ala-OH (0.4 mmol, 125.0 mg, 4.0 equiv.) and DIEA (0.8 mmol, 132.0 μl, 8.0 equiv.) were dissolved in DMF and added to the reaction tube. The mixture was stirred at rt for 40 min. After the reaction, CPG was washed with DMF and acetonitrile three times each.

(4) Repeat the previous step (3) and attach the remaining amino acids (Phenylalanine, histidine andtryptophan) to the resin.

(5) 20% piperidine was added to the reaction tube to remove Fmoc, and the reaction was performed twice for 5 min each time. The fully protected model peptide FARL was released from the resin by the reaction of 20% HFIP (methylene chloride) with the resin for 1 h. HPLC analysis showed that there were three products and their molecular weight is 1129, indicating that the polypeptide contains 3 Boc.

(6) The model peptide containing Boc was dissolved in borate buffer (250 mM, pH8.5) at 90℃ for 2 h, and the reaction solution was analyzed by HPLC and MS.(M:829.40). All Boc were successfully removed from the peptide.

3.5 Removal of Boc (borate buffer pH=8.5) from model peptide LKFA

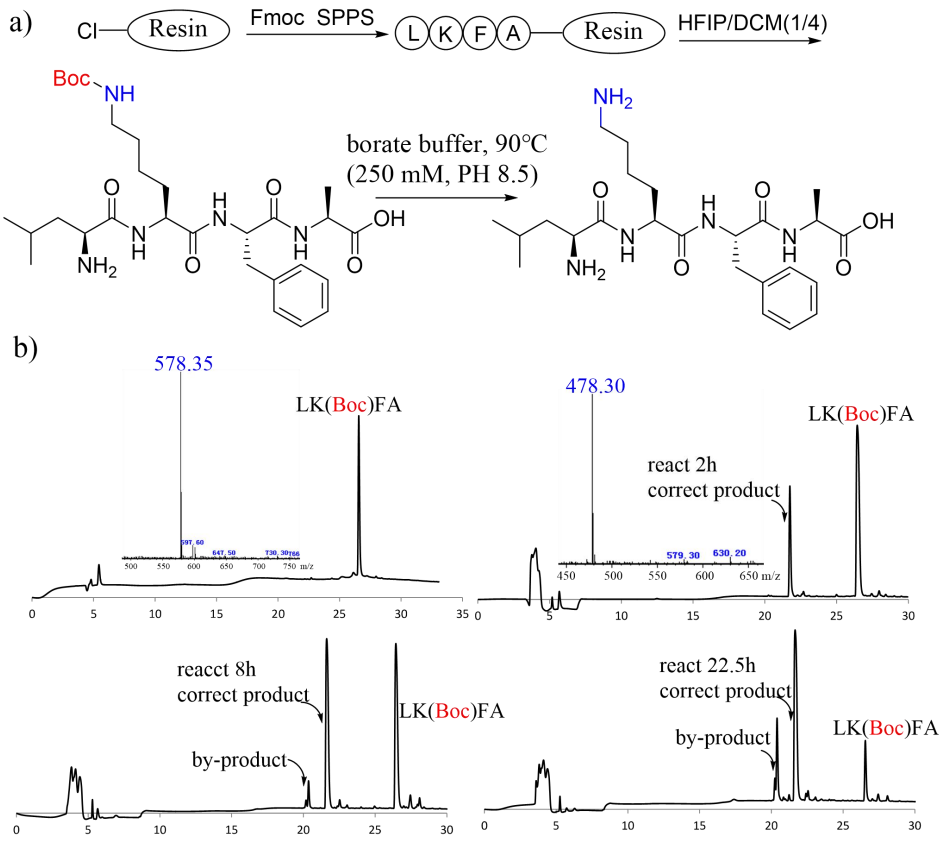


Fig. S7 Removal of Boc (borate buffer, pH8.50) from model peptide LKFA

Model peptide LKFA was prepared by standard solid-phase peptide synthesis on chlorine resin. Chlorine resin (0.1 mmol, 312.5 mg, 1.0 equiv.) is added to the reaction tube. The specific synthesis steps are as follows:

(1) 20% piperidine was added to the reaction tube to remove Fmoc, and the reaction was performed twice for 5 min each time. After the reaction, resin was washed with DMF and acetonitrile three times each. Fmoc-Ala-OH (0.4 mmol, 124.5 mg, 4.0 equiv.) and DIEA (0.8 mmol, 132.0 μl, 8.0 equiv.) were dissolved in DCM and added to the reaction tube. The mixture was stirred at rt for 12 h. After the reaction, CPG was washed with DMF and acetonitrile three times each.

(2) 20% piperidine was added to the reaction tube to remove Fmoc. The reaction was performed twice for 5 min each time. After the reaction, resin was washed with DMF and acetonitrile three times each. HCTU (0.4 mmol, 165.5 mg, 4.0 equiv.), Fmoc-Phe-OH (0.4 mmol, 155.0 mg, 4.0 equiv.) and DIEA (0.8 mmol, 132.0 μl, 8.0 equiv.) were dissolved in DMF and added to the reaction tube. The mixture was stirred at rt for 40 min. After the reaction, resin was washed with DMF and acetonitrile three times each.

(4) Repeat the previous step (3) and attach the remaining amino acids (Lysine, leucine) to the resin.

(5) 20% piperidine was added to the reaction tube to remove Fmoc, and the reaction was performed twice for 5 min each time. The fully protected model peptide LKFA was released from the resin by the reaction of 20% HFIP (methylene chloride) with the resin for 1 h. HPLC analysis showed correct products and it's molecular weight is 578.35, indicating that the peptide contains 1 Boc.

(6) The model peptide LKFA containing 1 Boc was dissolved in borate buffer (250 mM, pH8.5) at 90℃, and the reaction solution was analyzed by HPLC and MS.(M:478.30). After 2 hours of reaction, about one-third of Boc was removed.After 8 h of reaction, half of Boc was removed; After reaction 22.5, most Boc was removed. However, a long time side reaction results in a partial byproduct in front of the main peak, which has the same molecular weight as the main peak.

3.6 Removal of Boc (borate buffer pH8.3) from model peptide LKFA

To further investigate the byproducts in Fig. S7, we prepared a short peptide, LkFA, containing a D-Lys in the sequence. By HPLC analyzing, we found that the retention time of LkFA overlapped with one of byproduct. However, we still can't be sure it happens to Lys because there are still three other amino acid residues, Leu, Phe, and Ala.

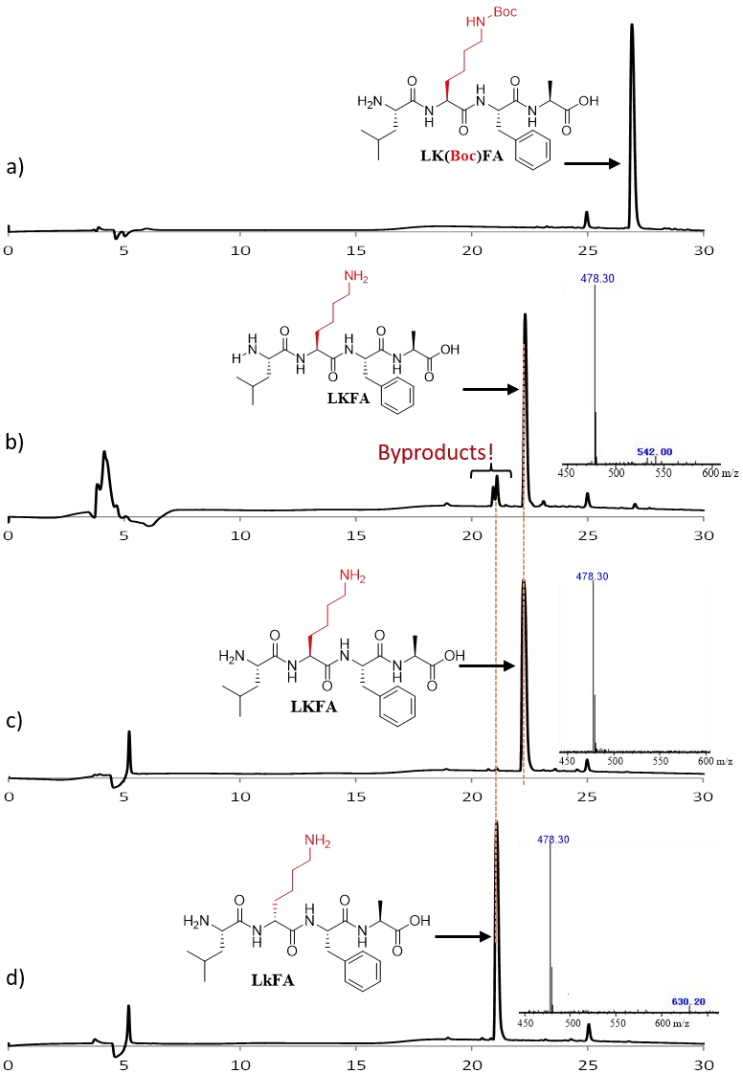


Fig. S8. Removal of Boc protecting group from LK(Boc)FA. a) after 0-hour treatment of LK(Boc)FA in borate buffer (90°C, pH8.3); b) after 15-hour treatment of LK(Boc)FA in borate buffer (90°C, pH8.3); c) HPLC chromatogram of LKFA prepared by Fmoc SPPS; d) HPLC chromatogram of LkFA prepared by Fmoc SPPS (k is D-Lys).

4. The removal of tBu from the model peptide

4.1 Removal of tBu from model peptide WDGFAL

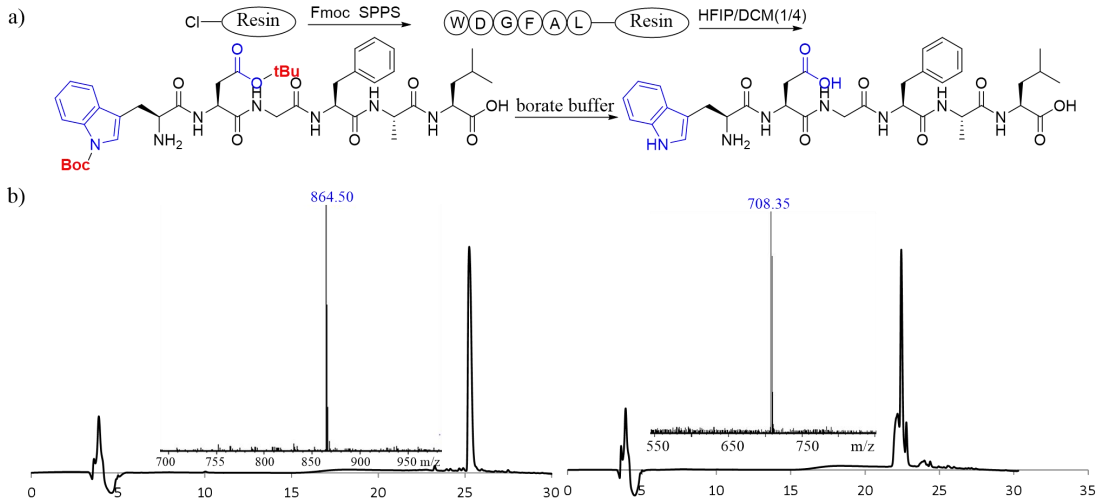


Fig. S9 Synthesis and removal of Boc protecting group from model peptide **9**

Model peptide was prepared by standard solid-phase peptide synthesis on chlorine resin. Chlorine resin (0.1 mmol, 312.5 mg, 1.0 equiv.) is added to the reaction tube. The specific synthesis steps are as follows:

(1) 20% piperidine was added to the reaction tube to remove Fmoc, and the reaction was performed twice for 5 min each time. After the reaction, resin was washed with DMF and acetonitrile three times each. Fmoc-Leu-OH (0.4 mmol, 141.3 mg, 4.0 equiv.) and DIEA (0.8 mmol, 132.0 μl, 8.0 equiv.) were dissolved in DCM and added to the reaction tube. The mixture was stirred at rt for 12 h. After the reaction, CPG was washed with DMF and acetonitrile three times each.

(2) 20% piperidine was added to the reaction tube to remove Fmoc, and the reaction was performed twice for 5 min each time. After the reaction, resin was washed with DMF and acetonitrile three times each. HCTU (0.4 mmol, 165.5 mg, 4.0 equiv.), Fmoc-Ala-OH (0.4 mmol, 124.5 mg, 4.0 equiv.) and DIEA (0.8 mmol, 132.0 μl, 8.0 equiv.) were dissolved in DCM and added to the reaction tube. The mixture was stirred at rt for 40min. After the reaction, CPG was washed with DMF and acetonitrile three times each.

(3) Repeat the previous step (3) and attach the remaining amino acids (Phenylalanine, Glycine, aspartic acid, tryptophan)

(4) 20% piperidine was added to the reaction tube to remove Fmoc, and the reaction was performed twice for 5 min each time. The fully protected model peptide FARL was released from the resin by the reaction of 20% HFIP (methylene chloride) with the resin for 1 h. HPLC showed that Boc and tBu remained on the peptide. (M:864.5)

(5) The model peptide containing Boc,tBu was dissolved in borate buffer (250 mM pH8.5) at 90℃ for 2 h, and the reaction solution was analyzed by HPLC and MS.(M:708.35) The Boc,tBu was successfully removed from the peptide.

4.2 Removal of tBu from model peptide WEGFAL

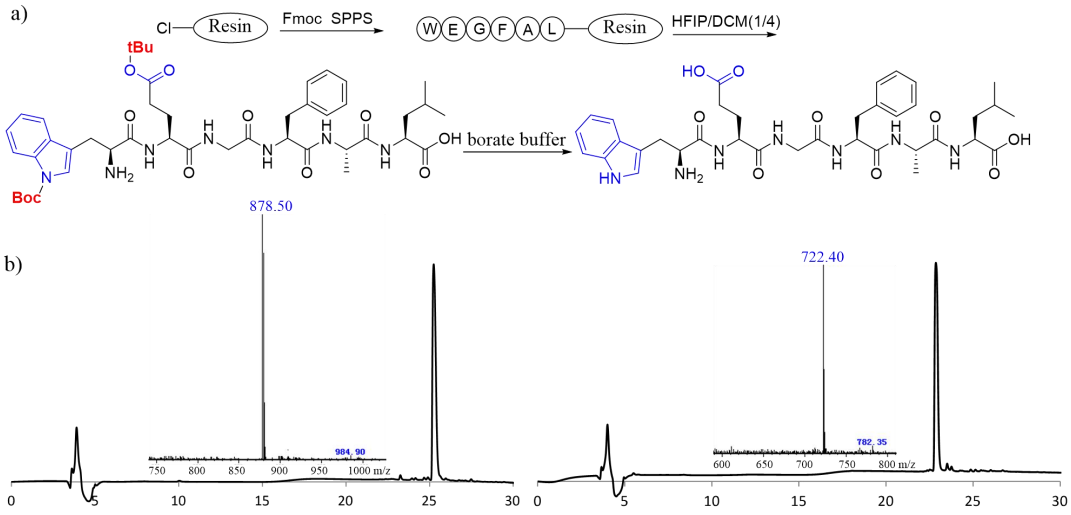


Fig. S10 Synthesis and removal of Boc protecting group from model peptide **11**

Model peptide was prepared by standard solid-phase peptide synthesis on chlorine resin. Chlorine resin (0.1 mmol, 312.0 mg 1.0 equiv.) is added to the reaction tube. The specific synthesis steps are as follows:

(1) 20% piperidine was added to the reaction tube to remove Fmoc, and the reaction was performed twice for 5 min each time. After the reaction, resin was washed with DMF and acetonitrile three times each. Fmoc-Leu-OH (0.4 mmol, 141.4 mg, 4.0 equiv.) and DIEA (0.8 mmol, 132.0 μl, 8.0 equiv.) were dissolved in DCM and added to the reaction tube. The mixture was stirred at rt for 12 h. After the reaction, CPG was washed with DMF and acetonitrile three times each.

(2) 20% piperidine was added to the reaction tube to remove Fmoc, and the reaction was performed twice for 5 min each time. After the reaction, resin was washed with DMF and acetonitrile three times each. HCTU (0.4 mmol, 165.5 mg, 4.0 equiv.), Fmoc-Ala-OH (0.4 mmol, 124.5 mg, 4.0 equiv.) and DIEA (0.8 mmol, 132.0 μl, 8.0 equiv.) were dissolved in DCM and added to the reaction tube. The mixture was stirred at rt for 40min . After the reaction, CPG was washed with DMF and acetonitrile three times each.

(3) Repeat the previous step (3) and attach the remaining amino acids (Phenylalanine, Glycine, aminoglutaric acid, tryptophan)

(4) 20% piperidine was added to the reaction tube to remove Fmoc, and the reaction was performed twice for 5 min each time. The fully protected model peptide FARL was released from

the resin by the reaction of 20% HFIP (methylene chloride) with the resin for 1 h. HPLC showed that Boc and tBu remained on the peptide. (M:878.5)

(5) The model peptide containing Boc,tBu was dissolved in borate buffer (250 mM, pH8.5) at 90℃ for 4 h, and the reaction solution was analyzed by HPLC and MS.(M:722.40) The Boc,tBu was successfully removed from the peptide.

4.3 Removal of tBu from model peptide SGFAL,TGFAL, and YGFAL

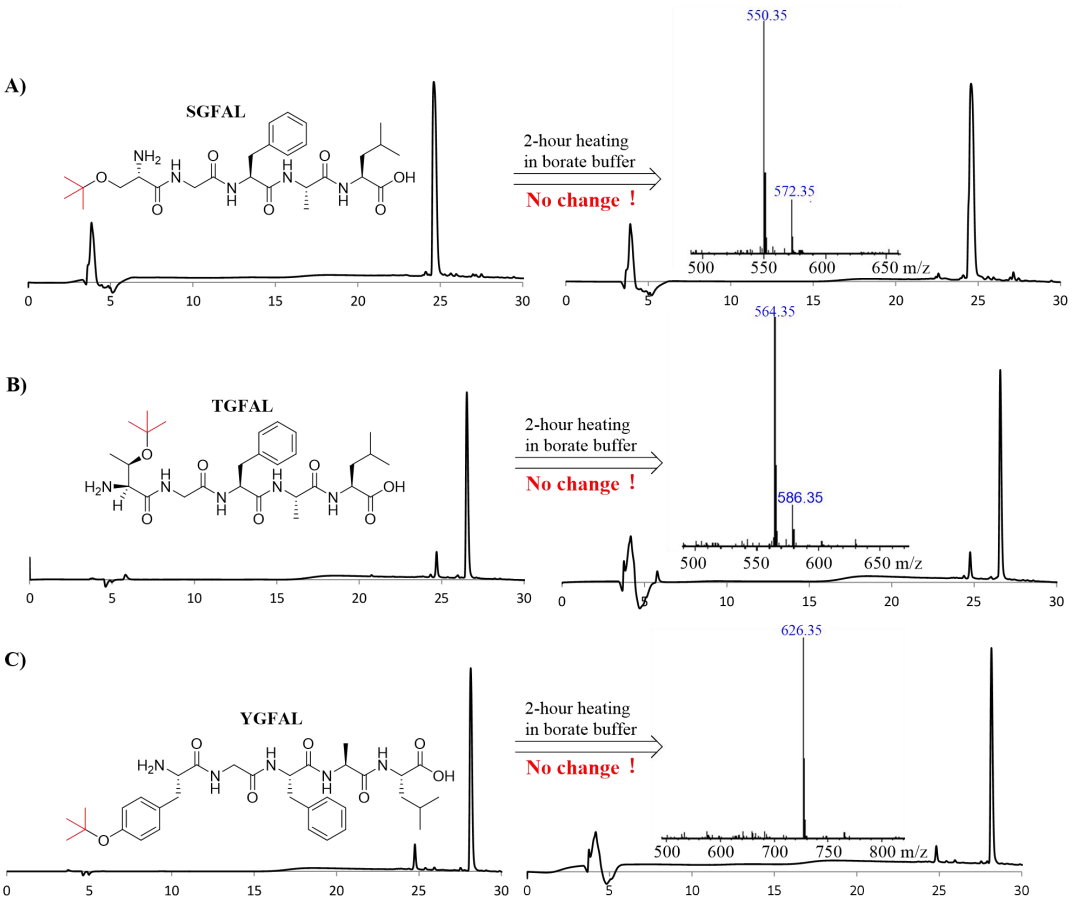


Fig. S11 Removal of tBu from model peptide SGFAL, TGFAL, and YGFAL

(1) Model peptide SGFAL, TGFAL, and YGFAL was prepared by standard solid-phase peptide synthesis on chlorine resin. The fully protected model peptide was released from the resin by the reaction of 20% HFIP (methylene chloride) with the resin for 1 h. HPLC and MS showed that tBu remained on the peptide. (M:550.35, 564.35,626.35).

(2) The model peptide SGFAL, TGFAL, and YGFAL containing tBu was dissolved in borate buffer (250mM, pH8.5) at 90℃ for 2 h, Unfortunately, the borate buffer could not remove the tBu protective groups of the side chains of serine, threonine and tyrosine.

4.4 Removal of TBS from model peptide SDLSK, TDLSK, and YGFAL

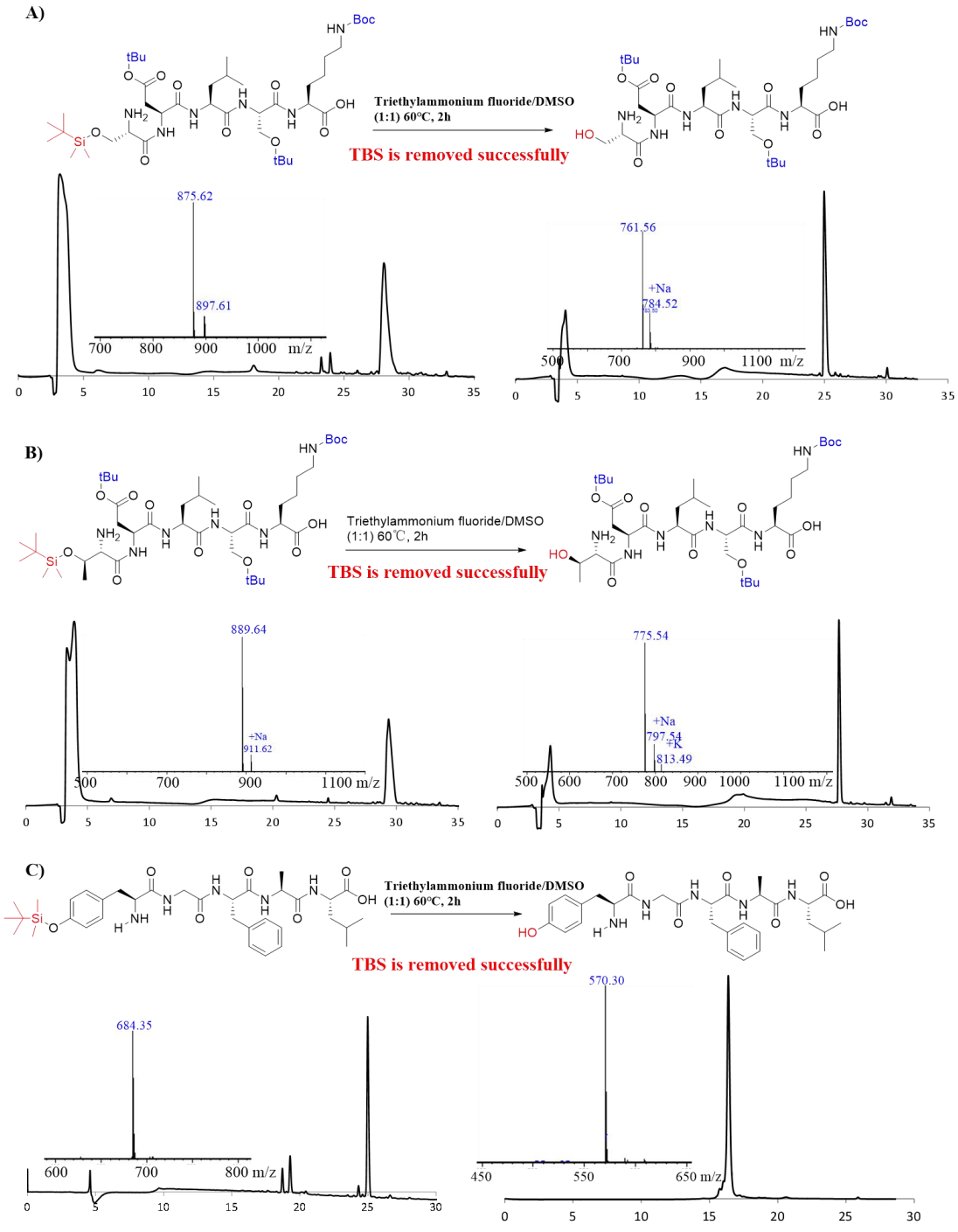


Fig. S12 Removal of TBS from model peptide SDLSK, TDLSK, and YGFAL

The tert-butyl groups of serine, threonine, and tyrosine cannot be removed by borate buffers, so it is necessary to find a protective group that is compatible with the synthesis

peptide-oligonucleotides. TBS is a commonly used hydroxyl protective group with relatively mild deprotective conditions, so it can be considered for use.

(1) Model peptide SDLSK, TDLSK, and YGFAL was prepared by standard solid-phase peptide synthesis on chlorine resin. The first amino acid is protected by TBS, the others are normal. The fully protected model peptide was released from the resin by the reaction of 20% HFIP (methylene chloride) with the resin for 1 h. HPLC and MS showed that TBS remained on the peptide. (M:875.62, 889.64, 684.35).

(2) The model peptide SGFAL, TGFAL, and YGFAL containing TBS was dissolved in a mixture of triethylamine trihydrofluorate and DMSO (1:1) at 60℃ for 2 h, and the reaction solution was analyzed by HPLC and MS.(M:761.56, 775.54, 570.30). The TBS was successfully removed from the peptide.

(3) Synthesis of POCs containing serine, threonine and tyrosine is carried out later.

5. Stability of oligonucleotide in borate buffer at 90℃

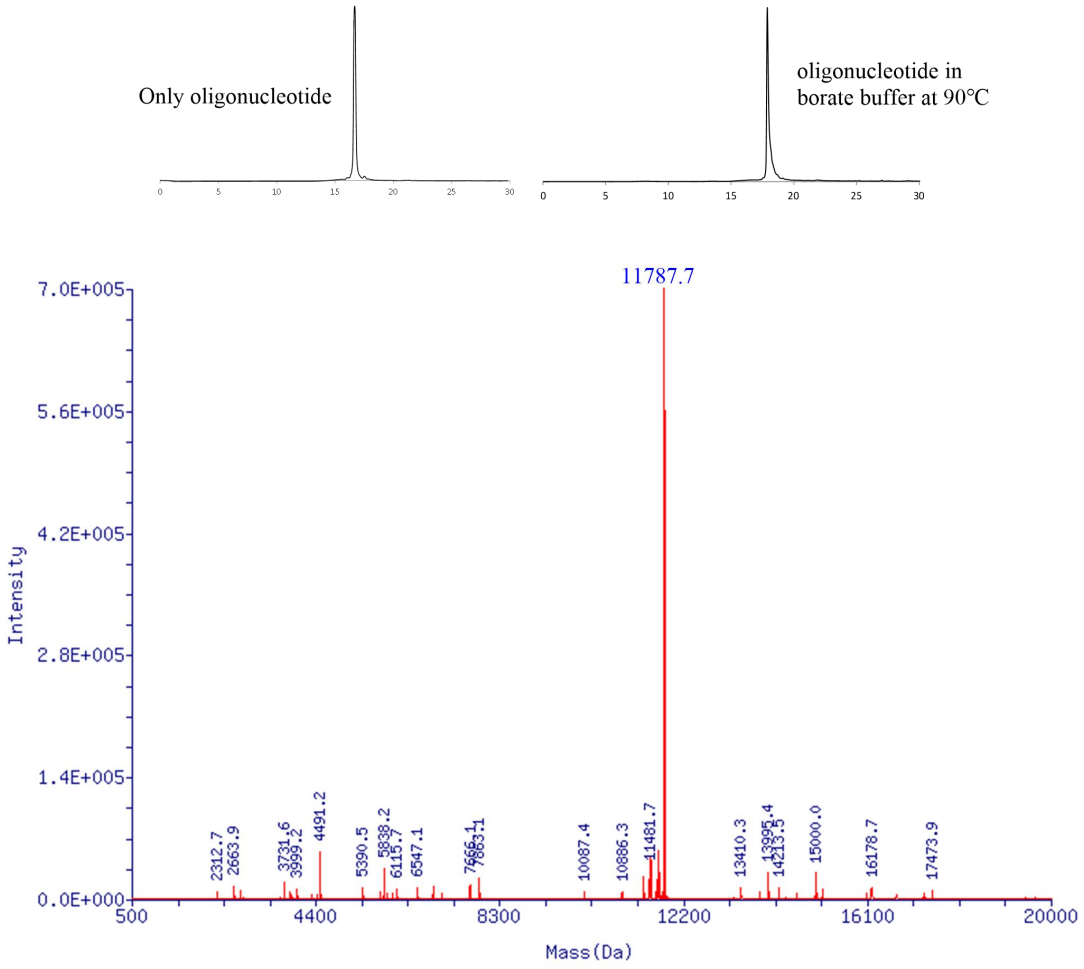


Fig. S13 HPLC trace and MS of oligonucleotide

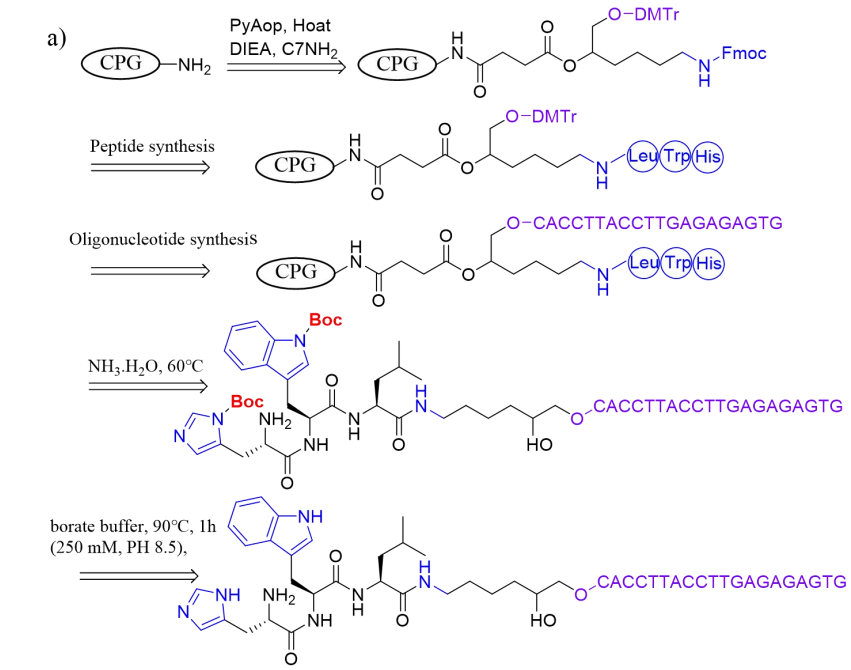
(1) Oligonucleotide (GCTAGTCTATTGCTGACGTCACTCACCTGTCAGTATAC) was synthesis by standard CE-phosphoramidite chemistry on DNA synthesis apparatus. [5′-DMT-dN-3′-P(OCE)NiPr2, dN) T, A(Bz), C(Bz), G(iBu)].

(2) The oligonucleotide was released from the CPG by the reaction of ammonia water (60 ℃) for 2 h, then precipitated with anhydrous ethanol.

(3) The oligonucleotide was dissolved in borate buffer (250 mM, pH8.5) at 90℃ for 5 h, and the reaction solution was analyzed by HPLC and MS (M:11787.7). The HPLC trace of oligonucleotide is the same as the original oligonucleotide, and the MS result is also correct.

6. Synthesis of oligonucleotide-peptide conjugates.

6.1 Synthesis of POC-1



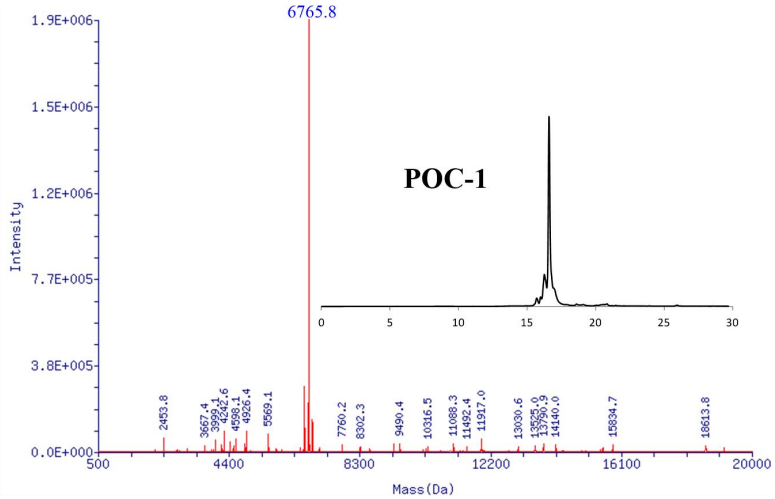


Fig. S14 Chemical Synthesis and analysis of **POC-1**

POC-1 was prepared by standard solid-phase peptide synthesis on CPG resin, followed by oligonucleotide synthesis on the same solid support. The specific synthesis steps are as follows:

(1) 250 mg of CPG was put into the reaction tube, 20.6 mg HCTU and 38.9 mg C7-NH_2 were put into a 10ml centrifuge tube, and 2.0 ml DMF was added for dissolution, then 17.0 μl DIEA was added into the centrifuge tube, and finally poured into the reaction tube for 1.5 hours. After the reaction, CPG was washed with DMF and acetonitrile three times each. Take 1ml acetic anhydride, 1ml DIEA, and finally use DMF to volume to 10ml. Block the unreacted amino group with configured acetic anhydride twice for 5 min each time.

(2) 20% piperidine was added to the reaction tube to remove Fmoc, and the reaction was performed twice for 5 min each time. After the reaction, CPG was washed with DMF and acetonitrile three times each. HCTU (0.20 mmol, 82.7 mg 4.0 equiv.), Fmoc-Leu-OH (0.2 mmol, 70.6 mg, 4.0 equiv.) and DIEA (0.4 mmol, 66.0 μl, 40.0 equiv.) were dissolved in DMF and added to the reaction tube. The mixture was stirred at rt for 40min. After the reaction, CPG was washed with DMF and acetonitrile three times each.

(3) Repeat the previous step (2) and attach the remaining amino acids (tryptophan, histidine)

(4) Oligonucleotide (CACCTTACCTTGAGAGAGTG) was synthesis by standard CE-phosphoramidite chemistry on DNA synthesis apparatus. [5′-DMT-dN-3′-P(OCE)NiPr2, dN) T, A(Bz), C(Bz), G(iBu)].

(5) 20% piperidine was added to the reaction tube to remove Fmoc, and the reaction was performed twice for 5 min each time. Then, CPG was washed with DMF and acetonitrile three times each. TCA was added to the reaction tube (15s) to remove DMTr, CPG was washed with acetonitrile three times. Repeat 5-10 times until there is no color left.

(6)The crude POC-1 was released from the CPG by the reaction of ammonia water (60℃) for 1 h, then precipitated with anhydrous ethanol.

(7) The crude POC-1 containing Boc was dissolved in borate buffer (250 mM, pH8.5) at 90℃ for 2 h, and the reaction solution was analyzed by HPLC and MS.(M:6765.8) The Boc was successfully removed from the **POC-1**.

6.2 Synthesis of POC-2

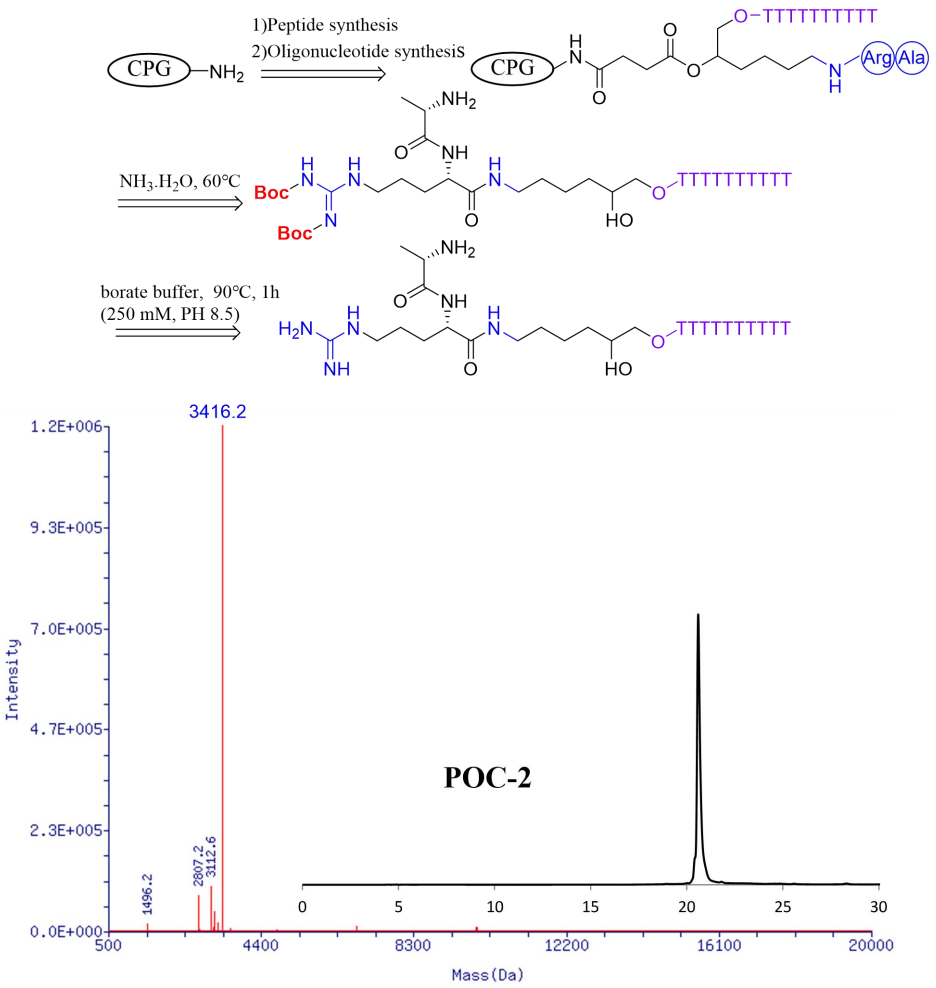


Fig. S15 Chemical Synthesis and analysis of **POC-2**

POC-2 was prepared by standard solid-phase peptide synthesis on CPG, followed by oligonucleotide synthesis on the same solid support. The specific synthesis steps are as follows:

(1) 250mg of CPG was put into the reaction tube, 20.6 mg HCTU and 38.9 mg C7-NH_2 were put into a 10ml centrifuge tube, and 2.0 ml DMF was added for dissolution, then 17.0 μl DIEA was added into the centrifuge tube, and finally poured into the reaction tube for 1.5 hours. After the reaction, CPG was washed with DMF and acetonitrile three times each. Take 1ml acetic anhydride, 1ml DIEA, and finally use DMF to volume to 10ml. Block the unreacted amino group with configured acetic anhydride twice for 5 min each time.

(2) 20% piperidine was added to the reaction tube to remove Fmoc, and the reaction was performed twice for 5 min each time. After the reaction, resin was washed with DMF and acetonitrile three times each. HCTU (0.2 mmol, 82.7 mg, 4.0 equiv.), Fmoc-Arg(Boc_2)-OH (0.2 mmol, 119.3 mg, 4.0 equiv.) and DIEA (0.4 mmol, 66.0 μl, 8.0 equiv.) were dissolved in DMF and added to the reaction tube. The mixture was stirred at rt for 1.5h. After the reaction, CPG was washed with DMF and acetonitrile three times each. PyAop (0.2 mmol, 104.2 mg, 4.0 equiv.), Fmoc-Arg(Boc_2)-OH (0.2 mmol, 119.3 mg, 4.0 equiv)and DIEA (0.4 mmol, 66.0 μl, 8.0 equiv.) were dissolved in DMF and added to the reaction tube. The mixture was stirred at rt for 12h.

(4) Oligonucleotide (TTTTTTTTTT) was synthesis by standard CE-phosphoramidite chemistry on DNA synthesis apparatus. [5′-DMT-dN-3′-P(OCE)NiPr2, dN) T, A(Bz), C(Bz), G(iBu)].

(5) 20% piperidine was added to the reaction tube to remove Fmoc, and the reaction was performed twice for 5 min each time. Then, CPG was washed with DMF and acetonitrile three times each. TCA was added to the reaction tube (15s) to remove DMTr, CPG was washed with acetonitrile three times. Repeat 5-10 times until there is no color left.

(6)The crude **POC-2** was released from the CPG by the reaction of ammonia water (60 ℃) for 1 h, then precipitated with anhydrous ethanol.

(7) The crude POC-2 containing Boc was dissolved in borate buffer (250 mM, pH8.5) at 90℃ for 2 h, and the reaction solution was analyzed by HPLC and MS.(M:3416.2) The Boc was successfully removed from the **POC-2**.

6.3 Synthesis of POC-3

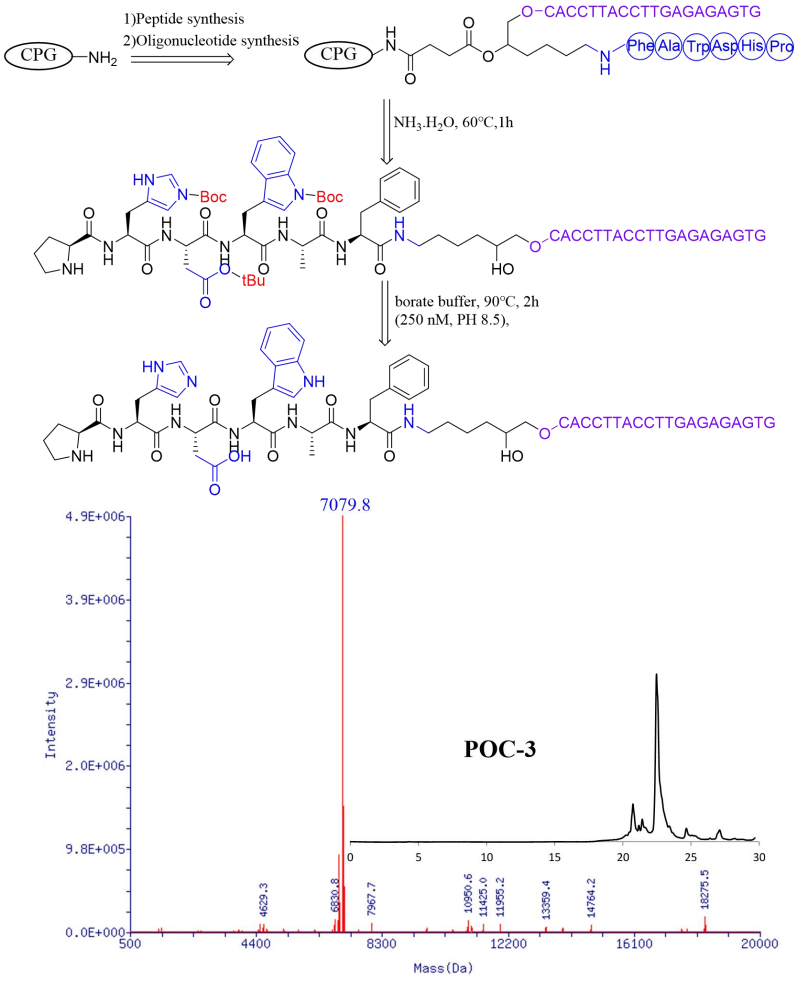


Fig. S16 Chemical Synthesis and analysis of **POC-3**

POC-3 was prepared by standard solid-phase peptide synthesis on CPG, followed by oligonucleotide synthesis on the same solid support. The specific synthesis steps are as follows:

(1) 250 mg of CPG was put into the reaction tube, 20.6 mg HCTU and 38.9 mg C7-NH_2 were put

into a 10.0 ml centrifuge tube, and 2.0 ml DMF was added for dissolution, then 17.0 μl DIEA was added into the centrifuge tube, and finally poured into the reaction tube for 1.5 hours. After the reaction, CPG was washed with DMF and acetonitrile three times each. Take 1ml acetic anhydride, 1ml DIEA, and finally use DMF to volume to 10ml. Block the unreacted amino group with configured acetic anhydride twice for 5 min each time

(2) 20% piperidine was added to the reaction tube to remove Fmoc, and the reaction was performed twice for 5 min each time. After the reaction, resin was washed with DMF and acetonitrile three times each. HCTU (0.2 mmol, 82.7 mg, 4.0 equiv.), Fmoc-Phe-OH (0.2 mmol, 77.4 mg, 4.0 equiv.) and DIEA (0.4 mmol, 66.0 μl, 8.0 equiv.) were dissolved in DMF and added to the reaction tube. The mixture was stirred at rt for 40min. After the reaction, CPG was washed with DMF and acetonitrile three times each.

(3) Repeat the previous step (2) and attach the remaining amino acids (Alanine, tryptophan, aspartic acid, histidine, proline).

(4) Oligonucleotide (CACCTTACCTTGAGAGAGTG) was synthesis by standard CE-phosphoramidite chemistry on DNA synthesis apparatus. [5′-DMT-dN-3′-P(OCE)NiPr2, dN) T, A(Bz), C(Bz), G(iBu)].

(5) 20% piperidine was added to the reaction tube to remove Fmoc, and the reaction was performed twice for 5 min each time. Then, CPG was washed with DMF and acetonitrile three times each. TCA was added to the reaction tube (15s) to remove DMTr, CPG was washed with acetonitrile three times. Repeat 5-10 times until there is no color left.

(6)The crude POC-3 was released from the CPG by the reaction of ammonia water (60 ℃) for 1 h, then precipitated with anhydrous ethanol.

(7) The crude POC-3 containing Boc,tBu was dissolved in borate buffer (250 mM, pH8.5) at 90℃ for 2 h, and the reaction solution was analyzed by HPLC and MS.(M:7079.8) The Boc,tBu was successfully removed from the **POC-3**.

6.4 Synthesis of POC-4

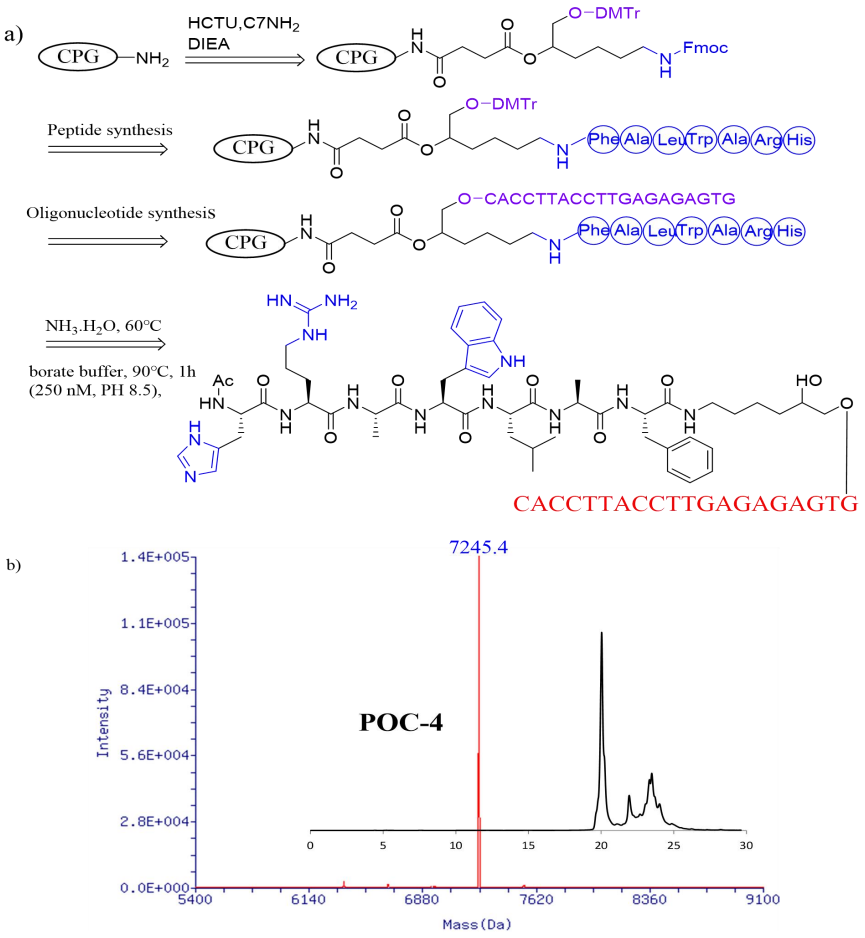


Fig. S17 Chemical Synthesis and analysis of **POC-4**

POC-4 was prepared by standard solid-phase peptide synthesis on CPG, followed by oligonucleotide synthesis on the same solid support. The specific synthesis steps are as follows:

(1) 250 mg of CPG was put into the reaction tube, 20.6 mg HCTU and 38.9 mg C7-NH_2 were put into a 10ml centrifuge tube, and 2ml DMF was added for dissolution, then 17ul DIEA was added into the centrifuge tube, and finally poured into the reaction tube for 1.5 hours. After the reaction, CPG was washed with DMF and acetonitrile three times each. Take 1ml acetic anhydride, 1ml DIEA, and finally use DMF to volume to 10ml. Block the unreacted amino group with configured acetic anhydride twice for 5 min each time

(2) 20% piperidine was added to the reaction tube to remove Fmoc, and the reaction was performed twice for 5 min each time. After the reaction, resin was washed with DMF and acetonitrile three times each. HCTU (0.2 mmol, 82.7 mg, 4.0 equiv.), Fmoc-Phe-OH (0.2 mmol, 77.4 mg, 4.0 equiv) and DIEA (0.4 mmol, 66.0 μl, 8.0 equiv.) were dissolved in DMF and added to the reaction tube. The mixture was stirred at rt for 40min. After the reaction, CPG was washed with DMF and acetonitrile three times each.

(3) Repeat the previous step (2) and attach the remaining amino acids (alanine, leucine, tryptophan, alanine, arginine, histidine); Arginine is synthesized according to the method in POC-2.

(4) Oligonucleotide (CACCTTACCTTGAGAGAGTG) was synthesis by standard

CE-phosphoramidite chemistry on DNA synthesis apparatus. [5′-DMT-dN-3′-P(OCE)NiPr2, dN) T, A(Bz), C(Bz), G(iBu)].

(5) 20% piperidine was added to the reaction tube to remove Fmoc, and the reaction was performed twice for 5 min each time. Then, CPG was washed with DMF and acetonitrile three times each. TCA was added to the reaction tube (15s) to remove DMTr, CPG was washed with acetonitrile three times. Repeat 5-10 times until there is no color left.

(6)The crude **POC-4** was released from the CPG by the reaction of ammonia water for (60 ℃) 1 h, then precipitated with anhydrous ethanol.

(7) The crude **POC-4** containing Boc was dissolved in borate buffer (250mM PH8.5) at 90℃ for 2 h, and the reaction solution was analyzed by HPLC and MS.(M:7079.8) The Boc was successfully removed from the **POC-4**.

6.5 Synthesis of POC-5

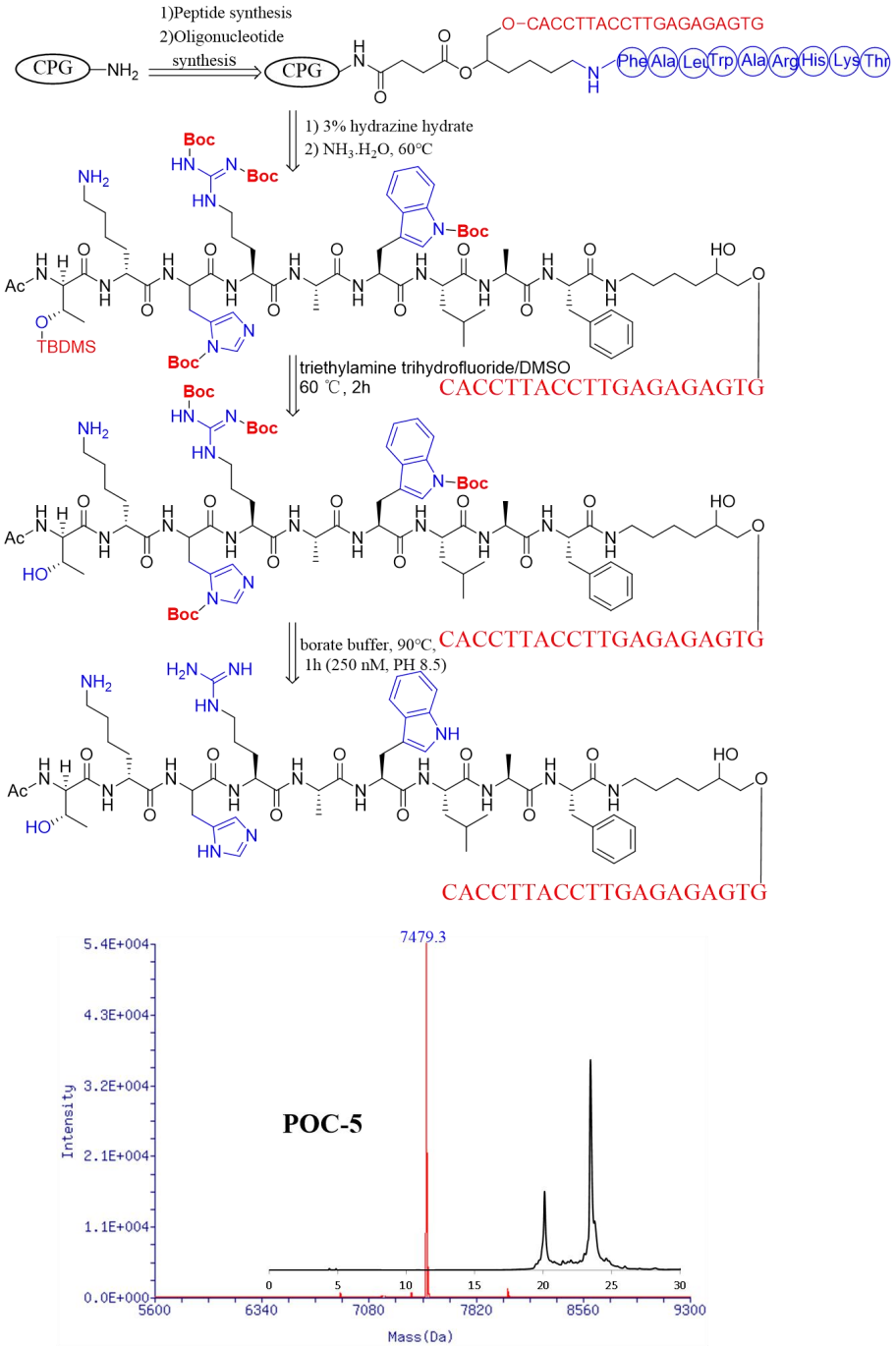


Fig. S18 Chemical Synthesis and analysis of **POC-5**

POC-5 was prepared by standard solid-phase peptide synthesis on CPG, followed by oligonucleotide synthesis on the same solid support. The specific synthesis steps are as follows:
(1) 250 mg of CPG was put into the reaction tube, 20.6 mg HCTU and 38.9 mg C7-NH_2 were put into a 10ml centrifuge tube, and 2ml DMF was added for dissolution, then 17ul DIEA was added into the centrifuge tube, and finally poured into the reaction tube for 1.5 hours. After the reaction, CPG was washed with DMF and acetonitrile three times each. Take 1ml acetic anhydride, 1ml

DIEA, and finally use DMF to volume to 10ml. Block the unreacted amino group with configured acetic anhydride twice for 5 min each time

(2) 20% 20% piperidine was added to the reaction tube to remove Fmoc, and the reaction was performed twice for 5 min each time. After the reaction, resin was washed with DMF and acetonitrile three times each. HCTU (0.2 mmol, 82.7 mg, 4.0 equiv.), Fmoc-Phe-OH (0.2 mmol, 77.4 mg, 4.0 equiv.) and DIEA (0.4 mmol, 66.0 μl, 8.0 equiv.) were dissolved in DMF and added to the reaction tube. The mixture was stirred at rt for 40min. After the reaction, CPG was washed with DMF and acetonitrile three times each.

(3) Repeat the previous step (2) and attach the remaining amino acids (alanine, leucine, tryptophan, alanine, arginine, histidine); Arginine is synthesized according to the method in **POC-2**.

(4) Oligonucleotide (CACCTTACCTTGAGAGAGTG) was synthesis by standard CE-phosphoramidite chemistry on DNA synthesis apparatus. [5′-DMT-dN-3′-P(OCE)NiPr2, dN) T, A(Bz), C(Bz), G(iBu)].

(5） 3% hydrazine hydrat was added to the reaction tube to remove ivDde, and the reaction was performed four times for 5 min each time.

(6) 20% piperidine was added to the reaction tube to remove Fmoc, and the reaction was performed twice for 5 min each time. Then, CPG was washed with DMF and acetonitrile three times each. TCA was added to the reaction tube (15s) to remove DMTr, CPG was washed with acetonitrile three times. Repeat 5-10 times until there is no color left.

(7) The crude POC-5 was released from the CPG by the reaction of ammonia water for (60 ℃) 1 h, then precipitated with anhydrous ethanol.

(8) Triethylamine trihydrofluoride/DMSO (1/1 60℃ 2h) was added to the reaction tube to remove TBDMS, then it is precipitated with anhydrous ethanol

(9) Finally the crude **POC-5** containing Boc was dissolved in borate buffer (250 mM, pH8.5) at 90℃ for 2 h, and the reaction solution was analyzed by HPLC and MS.(M:7479.3) The Boc was successfully removed from the **POC-5**.

6.6 Synthesis of POC-6

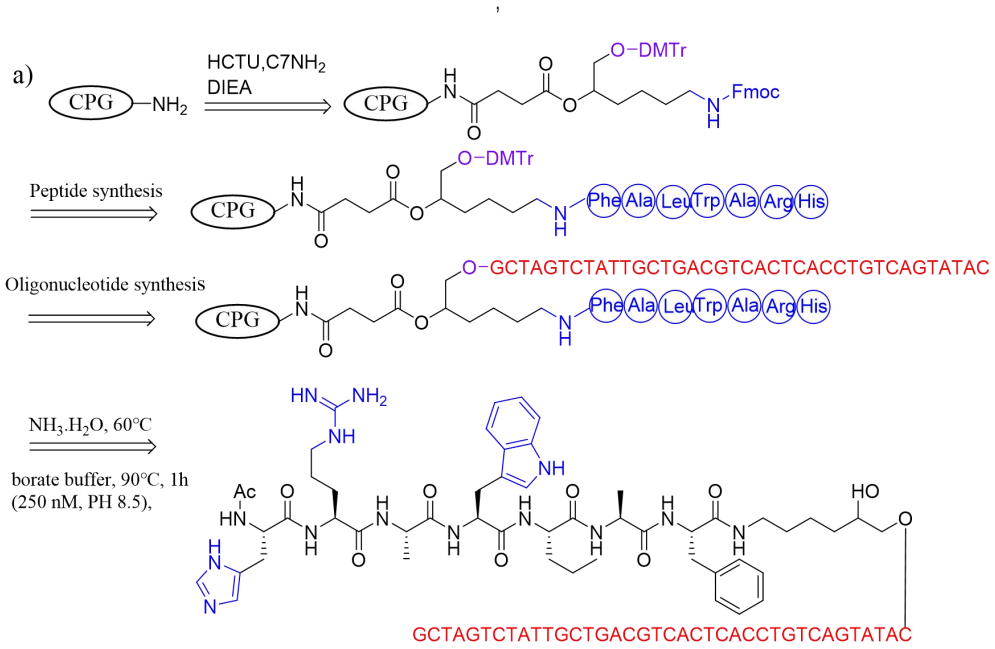


Fig. S19 Chemical Synthesis and analysis of **POC-6**

POC-6 was prepared by standard solid-phase peptide synthesis on CPG, followed by oligonucleotide synthesis on the same solid support. The specific synthesis steps are as follows:

(1) 250 mg of CPG was put into the reaction tube, 20.6 mg HCTU and 38.9 mg C7-NH_2 were put into a 10ml centrifuge tube, and 2.0 ml DMF was added for dissolution, then 17.0 μl DIEA was added into the centrifuge tube, and finally poured into the reaction tube for 1.5 hours. After the reaction, CPG was washed with DMF and acetonitrile three times each. Take 1ml acetic anhydride, 1ml DIEA, and finally use DMF to volume to 10ml. Block the unreacted amino group with configured acetic anhydride twice for 5 min each time

(2) 20% piperidine was added to the reaction tube to remove Fmoc, and the reaction was performed twice for 5 min each time. After the reaction, resin was washed with DMF and

acetonitrile three times each. HCTU (0.2 mmol, 82.7 mg, 4.0 equiv.), Fmoc-Phe-OH (0.2 mmol, 77.4 mg, 4.0 equiv.) and DIEA (0.4 mmol, 66.0 μl, 8.0 equiv.) were dissolved in DMF and added to the reaction tube. The mixture was stirred at rt for 40min. After the reaction, CPG was washed with DMF and acetonitrile three times each.

(3) Repeat the previous step (2) and attach the remaining amino acids (alanine, leucine, tryptophan, alanine, arginine, histidine); Arginine is synthesized according to the method in POC-2.

(4) Oligonucleotide (GCTAGTCTATTGCTGACGTCACTCACCTGTCAGTATAC) was synthesis by standard CE-phosphoramidite chemistry on DNA synthesis apparatus. [5′-DMT-dN-3′-P(OCE)NiPr2, dN) T, A(Bz), C(Bz), G(iBu)].

(5) 20% piperidine was added to the reaction tube to remove Fmoc, and the reaction was performed twice for 5 min each time. Then, CPG was washed with DMF and acetonitrile three times each. TCA was added to the reaction tube (15s) to remove DMTr, CPG was washed with acetonitrile three times. Repeat 5-10 times until there is no color left.

(6)The crude **POC-6** was released from the CPG by the reaction of ammonia water (60℃) for 1 h, then precipitated with anhydrous ethanol.

(7) The crude **POC-6** containing Boc was dissolved in borate buffer (250 mM, pH8.5) at 90℃ for 2 h, and the reaction solution was analyzed by HPLC and MS.(M:12710.7) The Boc was successfully removed from the **POC-6**.

6.7 Synthesis of POC-7

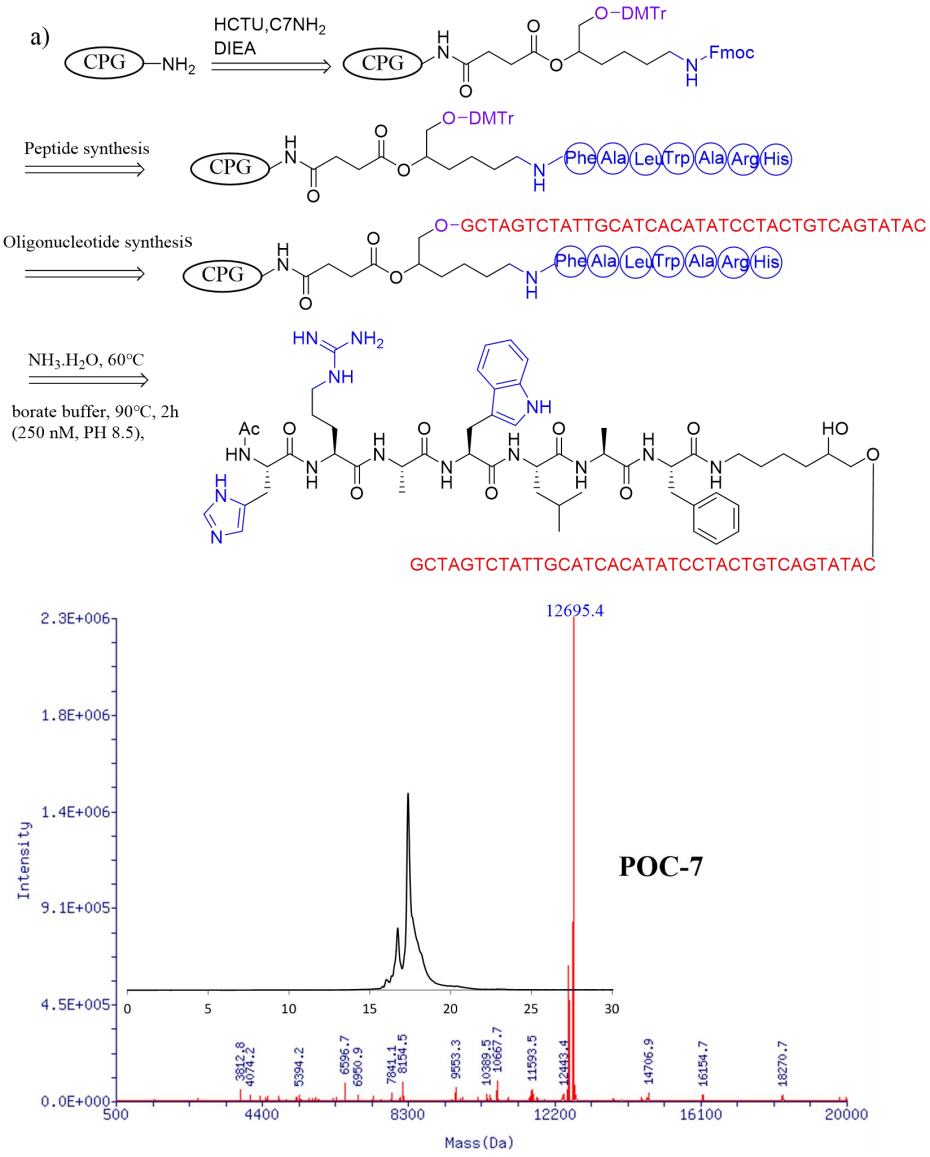


Fig. S20 Chemical Synthesis and analysis of **POC-7**

POC-7 was prepared by standard solid-phase peptide synthesis on CPG, followed by oligonucleotide synthesis on the same solid support. The specific synthesis steps are as follows:

(1) 250 mg of CPG was put into the reaction tube, 20.6 mg HCTU and 38.9 mg C7-NH_2 were put into a 10ml centrifuge tube, and 2ml DMF was added for dissolution, then 17ul DIEA was added into the centrifuge tube, and finally poured into the reaction tube for 1.5 hours. After the reaction, CPG was washed with DMF and acetonitrile three times each. Take 1ml acetic anhydride, 1ml DIEA, and finally use DMF to volume to 10ml. Block the unreacted amino group with configured acetic anhydride twice for 5 min each time

(2) 20% piperidine was added to the reaction tube to remove Fmoc, and the reaction was performed twice for 5 min each time. After the reaction, resin was washed with DMF and acetonitrile three times each. HCTU (0.2 mmol, 82.7 mg, 4.0 equiv.), Fmoc-Phe-OH (0.2 mmol,

77.4 mg, 4.0 equiv.) and DIEA (0.4 mmol, 66.0 μl, 8.0 equiv.) were dissolved in DMF and added to the reaction tube. The mixture was stirred at rt for 40min. After the reaction, CPG was washed with DMF and acetonitrile three times each.

(3) Repeat the previous step (2) and attach the remaining amino acids (alanine, leucine, tryptophan, alanine, arginine, histidine); Arginine is synthesized according to the method in **POC-2**.

(4) Oligonucleotide (GCTAGTCTATTGCATCACATATCCTACTGTCAGTATAC) was synthesis by standard CE-phosphoramidite chemistry on DNA synthesis apparatus. [5′-DMT-dN-3′-P(OCE)NiPr2, dN) T, A(Bz), C(Bz), G(iBu)].

(5) 20% piperidine was added to the reaction tube to remove Fmoc, and the reaction was performed twice for 5 min each time. Then, CPG was washed with DMF and acetonitrile three times each. TCA was added to the reaction tube (15s) to remove DMTr, CPG was washed with acetonitrile three times. Repeat 5-10 times until there is no color left.

(6)The crude **POC-7** was released from the CPG by the reaction of ammonia water for (60℃) 1 h, then precipitated with anhydrous ethanol.

(7) The crude **POC-7** containing Boc was dissolved in borate buffer (250 mM, pH8.5) at 90℃ for 2 h, and the reaction solution was analyzed by HPLC and MS.(M:12695.4) The Boc was successfully removed from the **POC-7**.

6.8 Synthesis of POC-8

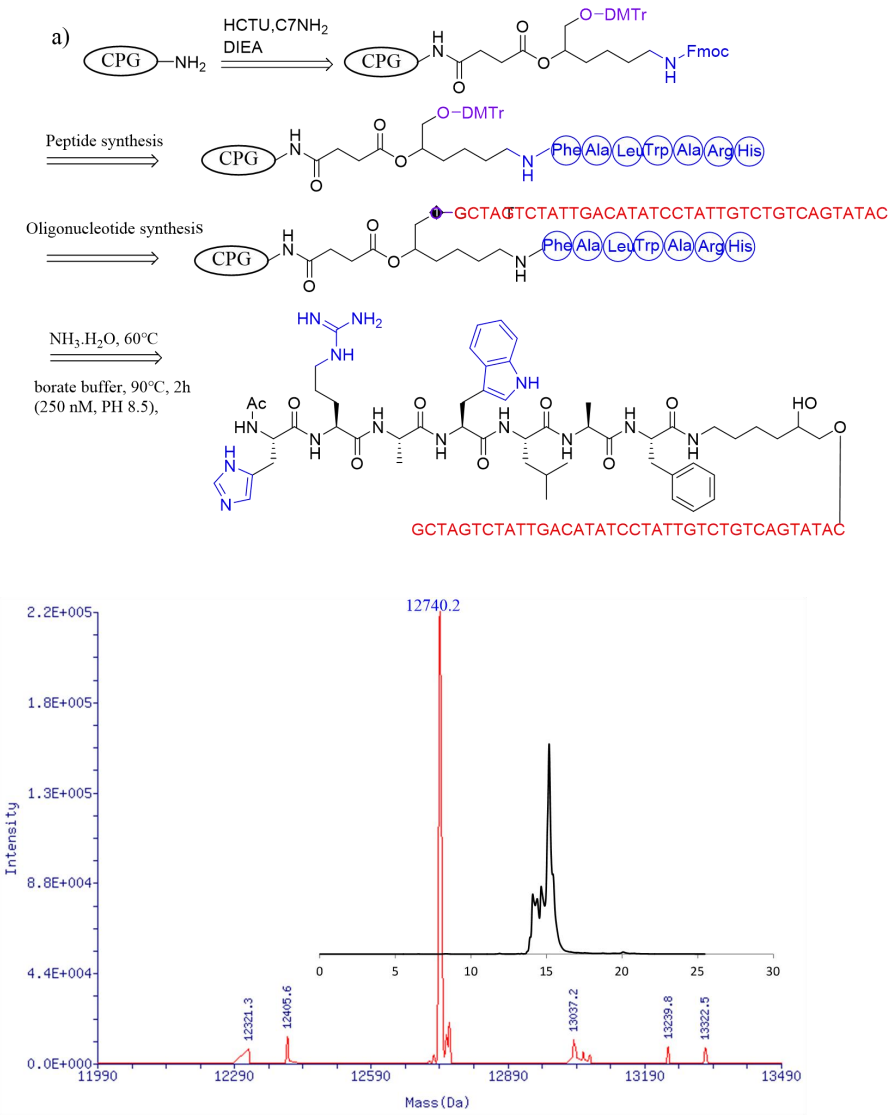


Fig. S21 Chemical Synthesis and analysis of **POC-8**

POC-8 was prepared by standard solid-phase peptide synthesis on CPG, followed by oligonucleotide synthesis on the same solid support. The specific synthesis steps are as follows:

(1) 250 mg of CPG was put into the reaction tube, 20.6 mg HCTU and 38.9 mg C7-NH_2 were put into a 10ml centrifuge tube, and 2ml DMF was added for dissolution, then 17.0 μl DIEA was added into the centrifuge tube, and finally poured into the reaction tube for 1.5 hours. After the reaction, CPG was washed with DMF and acetonitrile three times each. Take 1.0 ml acetic anhydride, 1ml DIEA, and finally use DMF to volume to 10.0 ml. Block the unreacted amino group with configured acetic anhydride twice for 5 min each time

(2) 20% piperidine was added to the reaction tube to remove Fmoc, and the reaction was performed twice for 5 min each time. After the reaction, resin was washed with DMF and acetonitrile three times each. HCTU (0.2 mmol, 82.7 mg, 4.0 equiv.), Fmoc-Phe-OH (0.2 mmol, 77.4 mg, 4.0 equiv) and DIEA (0.4 mmol, 66.0 μl, 8.0 equiv.) were dissolved in DMF and added

to the reaction tube. The mixture was stirred at rt for 40min. After the reaction, CPG was washed with DMF and acetonitrile three times each.

(3) Repeat the previous step (2) and attach the remaining amino acids (alanine, leucine, tryptophan, alanine, arginine, histidine); Arginine is synthesized according to the method in **POC-2**.

(4) Oligonucleotide (GCTAGTCTATTGACATATCCTATTGTCTGTCAGTATAC) was synthesis by standard CE-phosphoramidite chemistry on DNA synthesis apparatus. [5′-DMT-dN-3′-P(OCE)NiPr2, dN) T, A(Bz), C(Bz), G(iBu)].

(5) 20% piperidine was added to the reaction tube to remove Fmoc, and the reaction was performed twice for 5 min each time. Then, CPG was washed with DMF and acetonitrile three times each. TCA was added to the reaction tube (15s) to remove DMTr, CPG was washed with acetonitrile three times. Repeat 5-10 times until there is no color left.

(6)The crude **POC-8** was released from the CPG by the reaction of ammonia water for (60℃) 1 h, then precipitated with anhydrous ethanol.

(7) The crude **POC-8** containing Boc was dissolved in borate buffer (250 mM, pH8.5) at 90℃ for 2 h, and the reaction solution was analyzed by HPLC and MS.(M:12740.2) The Boc was successfully removed from the **POC-8**.

6.9 Synthesis of POC-9

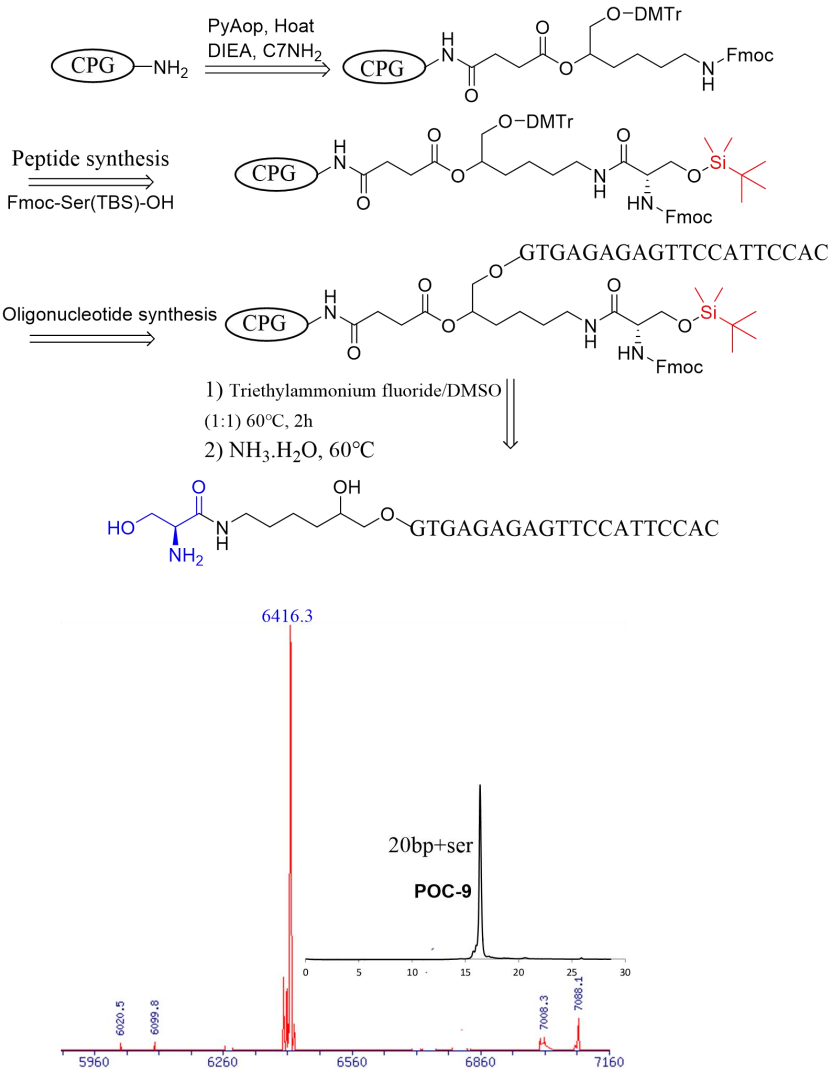


Fig. S22 Chemical Synthesis and analysis of **POC-9**

POC-9 was prepared by standard solid-phase peptide synthesis on CPG, followed by oligonucleotide synthesis on the same solid support. The hydroxyl group of serine is protected by TBS, after the synthesis, **POC-9** was removed from CPG with ammonia (60℃, 2h) and precipitated with anhydrous ethanol to obtain white solid. The white solid was dissolved in a mixture of triethylamine trihydrofluorate and DMSO, and the TBS was removed by reaction at 60℃ for 2h. Finally, the final product was precipitated by anhydrous ethanol and analyzed by HPLC and MS.(M:6416.3) The TBS was successfully removed from the **POC-9**, and the integrity of oligonucleotides was not affected when TBS was removed.

6.10 Synthesis of POC-10

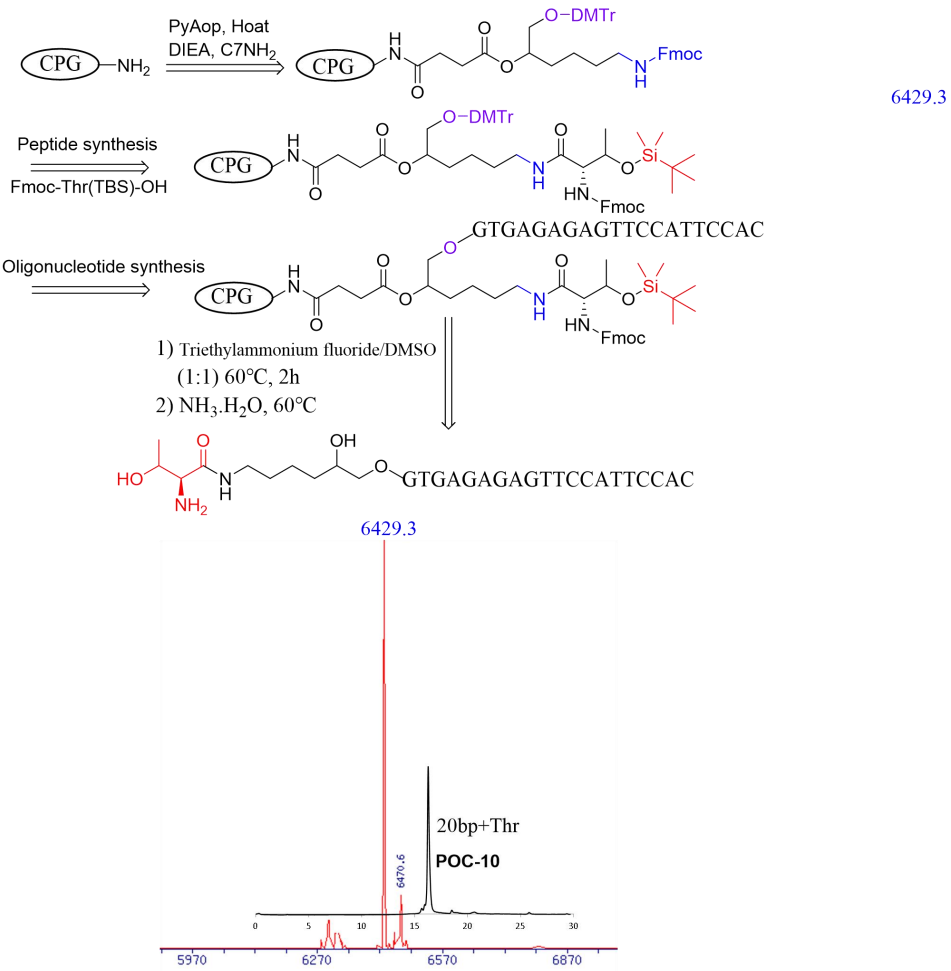


Fig. S23 Chemical Synthesis and analysis of **POC-10**

POC-10 was prepared by standard solid-phase peptide synthesis on CPG, followed by oligonucleotide synthesis on the same solid support. The hydroxyl group of threonine is protected by TBS, after the synthesis, POC-10 was removed from CPG with ammonia (60℃, 2h) and precipitated with anhydrous ethanol to obtain white solid. The white solid was dissolved in a mixture of triethylamine trihydrofluorate and DMSO, and the TBS was removed by reaction at 60℃ for 2h. Finally, the final product was precipitated by anhydrous ethanol and analyzed by HPLC and MS (M:6429.3). The TBS was successfully removed from the **POC-10**, and the integrity of oligonucleotides was not affected when TBS was removed.

6.11 Synthesis of POC-11

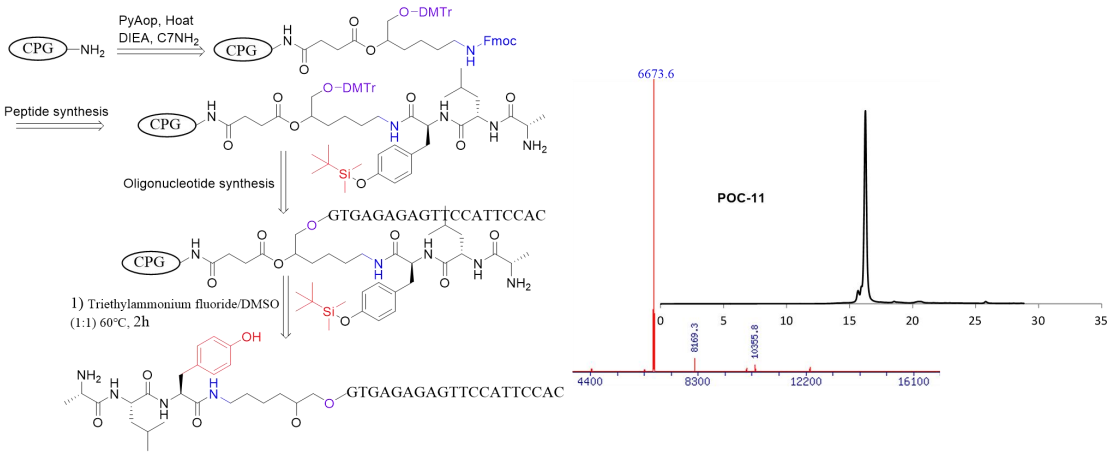


Fig. S24 Chemical Synthesis and analysis of **POC-11**

POC-11 was prepared by standard solid-phase peptide synthesis on CPG, followed by oligonucleotide synthesis on the same solid support. The hydroxyl group of tyrosine is protected by TBS, after the synthesis, **POC-11** was removed from CPG with ammonia (60℃, 2h) and precipitated with anhydrous ethanol to obtain white solid. The white solid was dissolved in a mixture of triethylamine trihydrofluorate and DMSO, and the TBS was removed by reaction at 60℃ for 2h. Finally, the final product was precipitated by anhydrous ethanol and analyzed by HPLC and MS (M:6673.6). The TBS was successfully removed from the **POC-11**, and the integrity of oligonucleotides was not affected when TBS was removed.

7. The PCR reaction of POC-6, POC-7, POC-8 and their sequencing

Following selection experiments, the DNA of the oligonucleotide-compound conjugates was amplified by PCR. In a first PCR run, the Illumina Nextera Transposase Adapters were introduced using 10 μL eluted DNA-conjugates, 5 μl of Taq DNA polymerase (Thermo Fisher Scientific), 1x Taq Buffer (100 mM Tris-HCl (pH = 8.8 at 25 °C), 500 mM KCl, 0.8% (v/v) Nonidet P40, Thermo Fisher Scientific), 3 mM $MgCl_2$, 0.625 mM of each dNTP (dATP, dGTP, dTTP, and dCTP, corresponding to 2.5 mM of the mixture of dNTPs) and 1 μM of each primer in a total reaction volume of 40 μL. The PCR program started with pre-denaturation at 95 °C for 3 min, followed by denaturation for 30 s at 95 °C, annealing for 30 s at 55 °C, and elongation for 30 s at 72 °C. After 25 cycles in total, the time for elongation was prolonged to 5 min. The PCR products were analyzed by agarose gel electrophoresis (4% agarose gel) and then purified using the QIAquick PCR Purification Kit (Qiagen). The DNA concentration was determined by UV spectroscopy and barcoded oligonucleotide sequences from different selection experiments were pooled. Gel-purified Sfi I/Not I-digested DNA and pCantab 5E was ligated after the determination of the optimum insert/vector ratio (10/1). Transforming the ligation mix into TG1 yields colonies on

plates, and then colonies were sequenced.

Primer

F:TATAGGCCCAGCCGGCCGCTAGTCTATTG
R:CCAAATTGCGGCCGCGTATACTGACAG