A type of RMBL resin and its preparation method and application in solid-phase peptide synthesis

Technical field

This invention relates to the field of solid-phase peptide synthesis technology, featuring the preparation of the specially designed RMBL resins, namely a kind of intramolecular acyl transfer linker loaded resins, and its application in solid-phase peptide synthesis.

Background technology

Peptides, with great physiological activity and biocompatibility, are an important class of compounds in nature. Peptide drugs have advantages of better safety, reliability and targeting specificity compared to small molecule drugs. Nowadays, solid-phase peptide synthesis strategy has become the main method for peptide synthesis since its invention in 1963, and most common peptide sequences can be efficiently synthesized utilizing this method. Because of the tendency of natural peptides to be degraded by enzymes, thus losing their activity, it has become a research hotspot to improve metabolic stability of peptide drugs. Inspired by the unique structures of cyclic peptide natural products, researchers have developed a variety of strategies to improve stability of peptides, among which cyclization and insertion of unnatural amino acids are the two predominant ones. It has been verified that pharmacokinetic parameters of peptides, namely serum stability, membrane permeability and oral bioavailability, can be significantly enhanced upon introduction of sterically hindered amino acids into the native sequences.

Sterically hindered amino acids consist of two major categories. The first is N-alkylated amino acids, among which N-methylated amino acids occupies the majority. According to existing researches, reactivity of secondary amines with carboxylic acids is only 1/10 to 1/100 of that of primary amines, making it challenging to achieve condensation with ideal efficiency under conventional SPPS conditions such as DIC/HATU. The other type is α,α-disubstituted amino acids

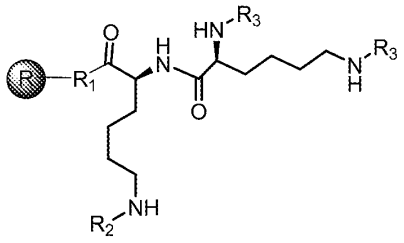
represented by Aib, Ac3c, Ac5c, etc. Steric hinderance of acyl transfer increases dramatically when α-C is double substituted, thus decreasing condensation efficiency. Currently, accelerating the formation of sterically hindered amide bonds via generation of more active intermediates is the simplest method to improve condensation efficiency, while limitations still exist. For example, the condensation reagent BTC, which is commonly utilized for construction of *N*-methyl amide bonds, needs to be dissolved in anhydrous THF, and carboxylic activation process should be conducted at 0 ℃ before the in-situ generated acyl chloride is transferred to the resin for subsequent condensation at room temperature. Severe conditions, poor controllability, demands for low temperatures and multi-step complex operations make it inconducive to industrial production. As a consequence, it's of great importance to develop a mild, controllable and easy-to-handle strategy for the synthesis of sterically hindered peptides.

Summary of the invention

This invention solves the problem of low condensation efficiency caused by two-phase acyl transfer process in conventional SPPS by developing a new type of intramolecular acyl transfer linker (RMBL) loaded resin. The proposed linker comprises two essential components, respectively a peptide synthesis handle and multiple carboxyl activating sites, allowing acyl transfer to proceed in an intramolecular manner. This invention is applied to solid-phase peptide synthesis, especially for the syntheses of two typical sterically hindered peptide categories.

The technical proposal of this invention is presented as follows.

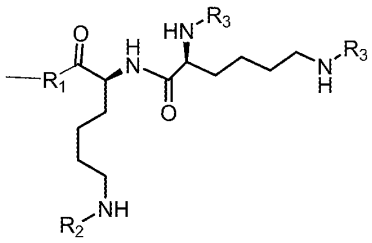
A kind of intramolecular acyl transfer linker (RMBL) loaded resin, the structure of which comprises two essential components, respectively a peptide synthesis handle and multiple carboxyl activating sites. Structure of the resin is presented in Formula (I).



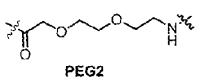
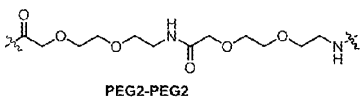
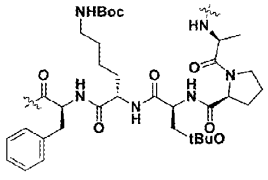
Formula (I)

Connecting H to  gives AM resin, preferably selected from any one of AM resin, AM PEGA resin and AM PEGMatrix resin. When RMBL needs to be cleaved from the resin: connecting H to  gives cleavable resin, preferably selected from any one of Rink resin, HMPA resin and HMPB resin.

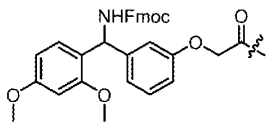
Ribosome-Mimicking Bifunctional Linker (RMBL) refers to a functional group connected to :



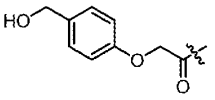
R_1 can be short PEG chains or short peptides, or be set directly to none. When C-terminus of R_1 ends up with -COOH, it can be connected to  via amide condensation. When N-terminus of R_1 ends up with $-NH_2$, it can be connected to backbone Lys of RMBL via amide condensation. R_1 can be preferably selected from any one of the structures below:

none , 
PEG2 , 
PEG2-PEG2 , 
APSKF (containing side chains)

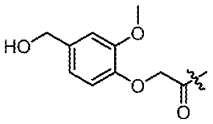
R_2 is the peptide anchoring point, preferably selected from any one of the structures below:



Rink linker ,

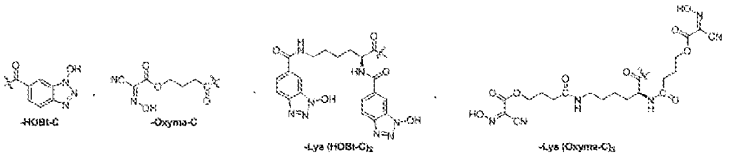


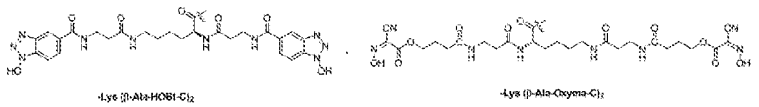
HMPA linker ,

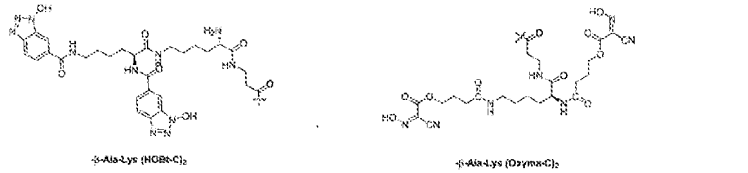


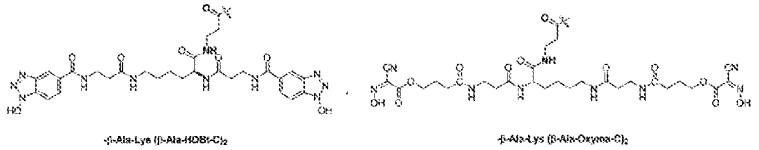
HMPB linker

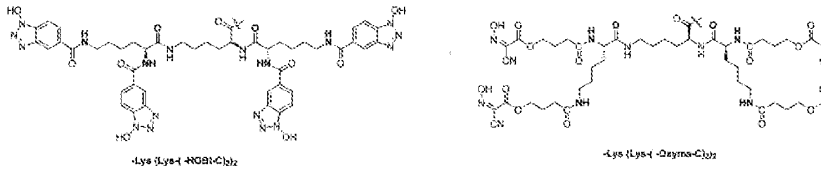
R_3 is an activating reagent analogs-containing fragment. When C-terminus of R_3 is -COOH, it can be connected to backbone Lys of RMBL, and β-Ala can be inserted as spacers before connection of activators. R_3 is preferably selected from any one of the structures below:

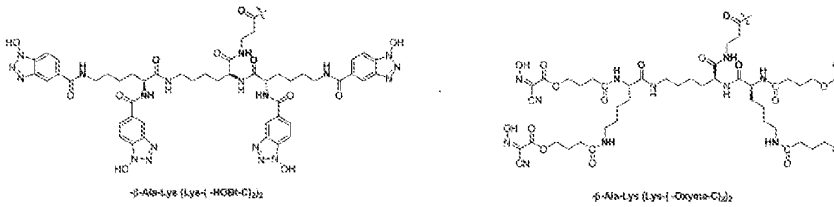

-HOBt-C
-Oxyma-C
-Lys (HOBt-C)2
-Lys (Oxyma-C)2

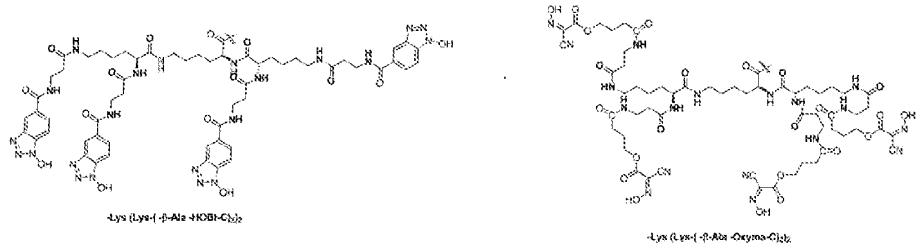

-Lys (β-Ala-HOBt-C)2
-Lys (β-Ala-Oxyma-C)2

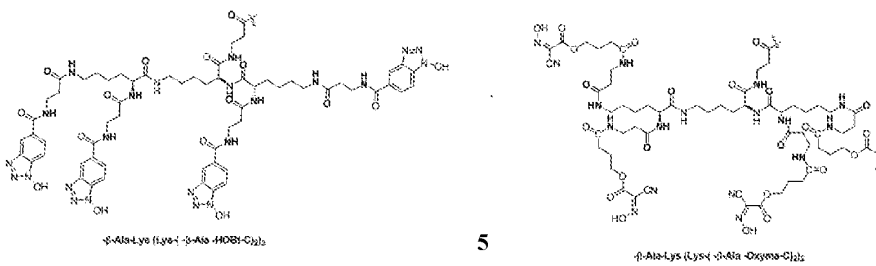

-β-Ala-Lys (HOBt-C)2
-β-Ala-Lys (Oxyma-C)2


-β-Ala-Lys (β-Ala-HOBt-C)2
-β-Ala-Lys (β-Ala-Oxyma-C)2


-Lys (Lys-(-HOBt-C)2)2
-Lys (Lys-(-Oxyma-C)2)2


-β-Ala-Lys (Lys-(-HOBt-C)2)2
-β-Ala-Lys (Lys-(-Oxyma-C)2)2


-Lys (Lys-(-β-Ala -HOBt-C)2)2
-Lys (Lys-(-β-Ala -Oxyma-C)2)2

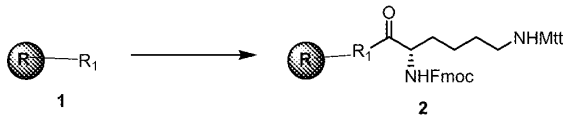

-β-Ala-Lys (Lys-(-β-Ala -HOBt-C)2)2
-β-Ala-Lys (Lys-(-β-Ala -Oxyma-C)2)2

Brief synthetic route of the intramolecular acyl transfer linker loaded resin (RMBL resin) is presented as below:

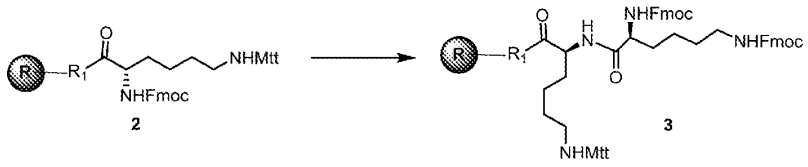
(1)



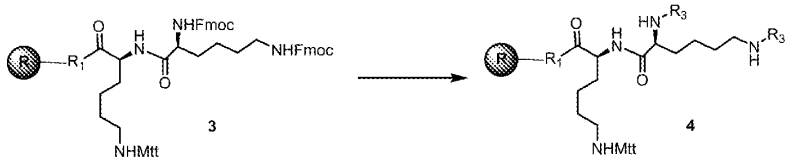
(2)



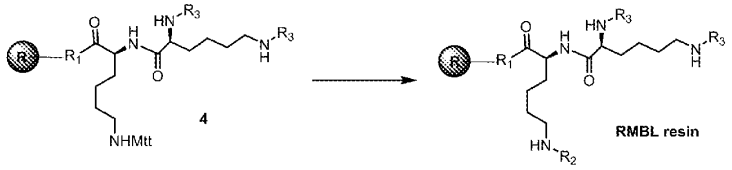
(3)



(4)



(5)



Preferably, a specific synthetic route of the intramolecular acyl transfer linker loaded resin (RMBL resin) is presented as below:

(1) The resin  is selected as the starting material. After pre-swelling, R_1 is connected to it to afford compound **1**.

(2) Fmoc of compound 1 is removed, followed by connection of Fmoc-Lys(Mtt)-OH to it to afford compound **2**.

(3) Fmoc of compound 3 is removed, followed by connection of Fmoc-Lys(Fmoc)-OH to it to afford compound **3**.

(4) Fmoc of compound **3** is removed, followed by connection of R_3 to it to afford compound **4**.

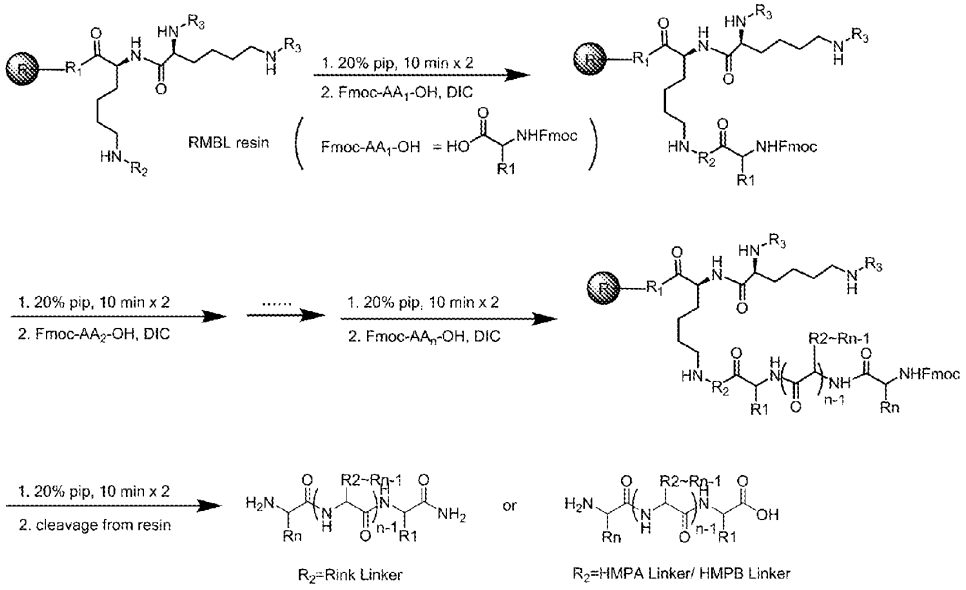
(5) Mtt of compound **4** is removed, followed by connection of R_2 to it to afford RMBL resin.

Average amino substitution degree of RMBL resin measured by amino substitution degree determination method is 0.27~0.39 mmol/g.

Preferably, resin with R_3=-β -Ala-Lys (Lys-(-β-Ala -HOBt-C)$_2$)$_2$ is named as HOBt RMBL resin, and resin with R_3=-β-Ala-Lys (Lys-(-β-Ala -Oxyma-C)$_2$)$_2$ is named as Oxyma RMBL resin.

RMBL resins mentioned above are applicable to solid-phase peptide synthesis.

Synthetic route of peptides utilizing HOBt/Oxyma RMBL resins developed in this invention is presented as below:



n represents the number of amino acids in the peptide, n>1; R_n represents side chain R group of the n^{th} amino acid, and R_{n-1} represents side chain R group of the $(n-1)^{th}$ amino acid.

Preferably, peptides to be synthesized can be common peptides, model peptides containing N-methyl or α,α-disubstituted amino acids, and natural products of sterically hindered peptides and their derivatives.

Compared to current peptide synthesis technologies, this innovation has following advantages:

The intramolecular acyl transfer linker (RMBL) loaded resin (namely RMBL resin) developed in this invention can be directly applied to solid-phase peptide synthesis using standard operating methods. In each coupling cycle, no additional activating reagent is required except for DIC. Acyl transfer process is launched on resin via an intramolecular mechanism, which makes condensation efficiency much higher than that of conventional methods, especially for the two aforementioned categories of sterically hindered peptides. Reagents involved in the whole peptide synthesis procedure do not require anhydrous treatment and the operating protocol is compatible with various experimental conditions. Most peptides can be synthesized with high purity under mild conditions at room temperature when the reaction time is set to 2 h per aa.

Description of figures:

Figure 1 is characterization results of RMBL connected to resin by LC-MS. For example, RMBL is loaded on Rink resin to afford RMBL resin, which is subject to cleavage operation to afford crude RMBL, the N-terminus of which ends up with -$CONH_2$. The crude RMBL is dissolved in solvents and injected into LC-MS instrument to launch its characterization. Peak area ratio of the product in LC at the wavelength of 214 nm demonstrates synthesis quality of RMBL resin, and MS data measured at corresponding retention time verifies the structure accuracy of proposed RMBL. In this example: R_1= PEG2-PEG2, R_2=Rink linker， R_3= -β -Ala-Lys (Lys-(-β-Ala -HOBt-C)$_2$)$_2$.

Figure 2 is the principle diagram of RMBL improving the condensation efficiency of sterically hindered amino acids.

Figure 3 is HPLC spectra of angiotensin II synthesized by Oxyma RMBL resin, HOBt RMBL resin.

Figure 4 is HPLC spectra of tetrapeptide QWRK synthesized by Oxyma RMBL resin, HOBt RMBL resin.

Figure 5 is HPLC spectra of model peptide 1 synthesized by Oxyma RMBL resin, HOBt RMBL resin and commercially available Rink resins.

Figure 6 is HPLC spectra of model peptide 2 synthesized by Oxyma RMBL resin, HOBt RMBL resin and commercially available Rink resin.

Figure 7 is HPLC spectra of model peptide 3 synthesized by Oxyma RMBL resin, HOBt RMBL resin and commercially available Rink resin.

Figure 8 is HPLC spectra of model peptide 4 synthesized by Oxyma RMBL resin, HOBt RMBL resin and commercially available Rink resin.

Figure 9 is HPLC spectra of model peptide 5 synthesized by Oxyma RMBL resin, HOBt RMBL resin and commercially available Rink resin.

Figure 10 is HPLC spectra of model peptide 6 synthesized by Oxyma RMBL resin, HOBt RMBL resin and commercially available Rink resin.

Figure 11 is HPLC spectra of model peptide 7 synthesized by Oxyma RMBL resin, HOBt RMBL resin and commercially available Rink resin.

Figure 12 is HPLC spectra of model peptide 8 synthesized by Oxyma RMBL resin, HOBt RMBL resin and commercially available Rink resin.

Figure 13 is HPLC spectra of model peptide 9 synthesized by Oxyma RMBL resin, HOBt RMBL resin and commercially available Rink resin.

Figure 14 is HPLC spectra of model peptide 10 synthesized by Oxyma RMBL resin, HOBt RMBL resin and commercially available Rink resin.

Figure 15 is HPLC spectra of model peptide 11 synthesized by Oxyma RMBL resin, HOBt RMBL resin and commercially available Rink resin.

Figure 16 is HPLC spectra of model peptide 12 synthesized by Oxyma RMBL resin, HOBt RMBL resin and commercially available Rink resin.

Figure 17 is HPLC spectrum of model peptide 13 synthesized by Oxyma RMBL resin.

Figure 18 is HPLC spectrum of model peptide 14 synthesized by Oxyma RMBL resin.

Figure 19 is HPLC spectrum of model peptide 15 synthesized by Oxyma RMBL resin.

Figure 20 is HPLC spectrum of model peptide 16 synthesized by Oxyma RMBL resin.

Figure 21 is HPLC spectrum of model peptide 17 synthesized by Oxyma RMBL resin.

Figure 22 is HPLC spectrum of model peptide 18 synthesized by Oxyma RMBL resin.

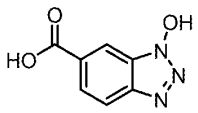
Figure 23 is HPLC spectrum of destrusin B linear peptide synthesized by Oxyma RMBL resin.

Figure 24 is HPLC spectrum of [2s,3s-Hmp]-Ab L linear peptide synthesized by Oxyma RMBL resin.

Figure 25 is HPLC spectrum ofMeLeu-Cs A linear peptide synthesized by Oxyma RMBL resin.

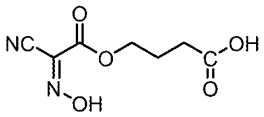
Specific implementation methods:

The following embodiments are further explanations of this invention, not limitations of it.



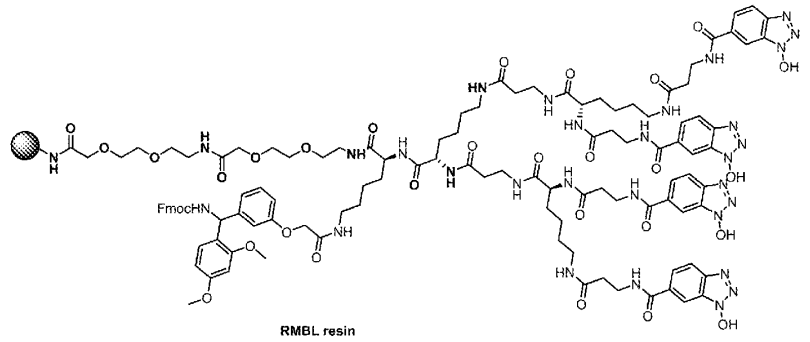
HOBt-C

HOBt-C is synthesized using the method reported in literature (Langmuir, 2005, 21, 71-78.)

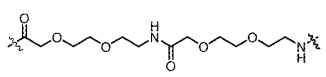
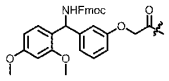


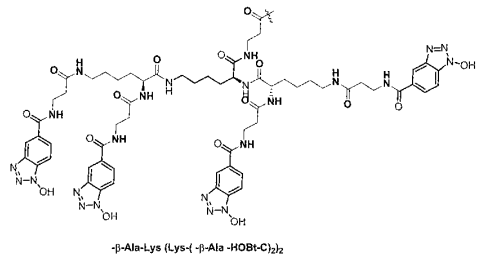
Oxyma-C

Oxyma-C is synthesized following embodiments 1-5.



HOBt RMBL Rink resin is synthesized following embodiments 6-13.

(R_1=  , R_2=  , R_3=



)

When R_3= -β -Ala-Lys (Lys-(-β-Ala -Oxyma-C)$_2$)$_2$, Oxyma RMBL Rink resin is synthesized following procedures provided in embodiments 6-13.

When R_2=HMPA Linker, HOBt RMBL HMPA resin is synthesized following procedures provided in embodiments 6-13.

When R_2=HMPA Linker, R_3= -β -Ala-Lys (Lys-(-β-Ala -Oxyma-C)$_2$)$_2$, Oxyma RMBL HMPA resin is synthesized following procedures provided in embodiments 6-13.

When R_2=HMPB Linker, HOBt RMBL HMPB resin is synthesized following procedures provided in embodiments 6-13.

When R_2=HMPB Linker, R_3= -β -Ala-Lys (Lys-(-β-Ala -Oxyma-C)$_2$)$_2$, Oxyma RMBL HMPB resin is synthesized following procedures provided in embodiments 6-13.

Tetrapeptide QWRK, angiotensin II and twelve model peptides are synthesized using Oxyma/HOBt RMBL Rink resin following embodiment 14.

Tetrapeptide QWRK, angiotensin II and twelve model peptides are synthesized

for control using Rink resin following embodiment 15.

The diester peptide fragment 1 in the natural product destruxin B is synthesized following embodiments 16-19.

Destruxin B linear peptide is synthesized using Oxyma RMBL HMPA resin following embodiment 20.

The natural product destruxin B is synthesized via cyclization of destruxin B linear peptide following embodiment 21.

The diester peptide fragment 2 in the natural product [2s,3s-Hmp]-Ab L is synthesized following embodiments 22-25.

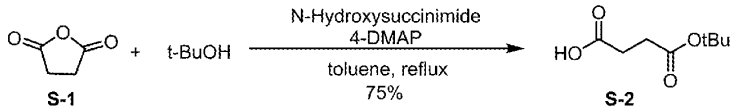
[2s,3s-Hmp]-Ab L linear peptide is synthesized using Oxyma RMBL HMPA resin following embodiment 26.

The natural product derivative [2s,3s-Hmp]-Ab L is synthesized via cyclization of [2s,3s-Hmp]-Ab L linear peptide following embodiment 27.

MeLeu-Cs A linear peptide is synthesized using Oxyma RMBL HMPA resin following embodiment 28.

The natural product derivative MeLeu-Cs A is synthesized via cyclization of MeLeu-Cs A linear peptide following embodiment 29.

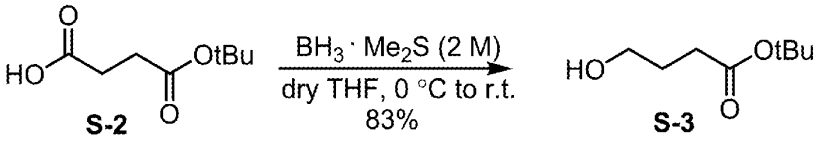
Embodiment 1:



To a solution of succinic anhydride **S-1** (3 g, 30 mmol, 1 eq.) in dry toluene (30 mL) is added N-hydroxysuccinimide (1.03 g, 9 mmol, 0.3 eq.) and 4-dimethylaminopyridine (0.35 g, 3 mmol, 0.1 eq.). After stirring for 10 min, tert-butanol (3.5 mL, 36 mmol, 1.2 eq.) is injected. The reaction is heated to reflux for 24 h until **S-1** is consumed. The cooled solution is diluted with 20 mL ethyl acetate and washed with 10% aqueous citric acid and saturated aqueous NaCl. The combined organic extracts are dried over anhydrous Na_2SO_4, filtered, and concentrated to afford **S-2** as a white solid (3.9 g, 75%).

^{1}H NMR (400 MHz, Chloroform-d) δ 2.62 (ddd, J = 7.3, 6.0, 1.3 Hz, 2H), 2.54 (ddd, J = 7.4, 6.0, 1.3 Hz, 2H), 1.44 (s, 9H).^{13}C NMR (101 MHz, $CDCl_3$) δ 178.3, 171.4, 81.0, 77.3, 77.2, 77.0, 76.7, 30.1, 29.1, 28.0. IR (film, cm^{-1}): 3690.09, 3662.79, 2981.13, 2902.65, 2876.76, 1726.92, 1712.40, 1405.23, 1371.21, 1253.56, 1225.73, 1152.31, 1076.25, 1047.41, 897.59.

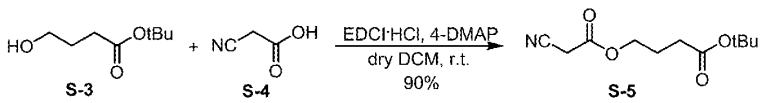
Embodiment 2:



To a solution of **S-2**(40 mg, 0.23 mmol, 1 eq.) in 0.5 mL anhydrous THF is added 0.5 mL BH_3Me_2S (2 M) at 0 °C. The solution was then allowed to warm to room temperature and stirred for 6 h. The reaction is quenched with H_2O and washed with saturated aqueous NaCl. The combined organic phase is dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue is purified by flash chromatography (petroleum ether: ethyl acetate =20:1) to afford **S-3** as colorless oil (30 mg, 83%).

^{1}H NMR (500 MHz, Chloroform-d) δ 3.66 (t, J = 6.1 Hz, 2H), 2.34 (t, J = 7.1 Hz, 2H), 1.89 – 1.78 (m, 2H), 1.44 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.4, 80.5, 77.3, 77.0, 76.8, 62.3, 32.5, 28.1, 27.8. HRMS (ESI, m/z) calcd for C8H16O3Na+: $[M+Na]^+$: 183.0992, found 183.0990. IR (film, cm^{-1}): 3489.73, 3431.20, 3371.85, 1698.22, 1691.25, 1728.67, 1367.64, 1150.96, 423.52.

Embodiment 3:

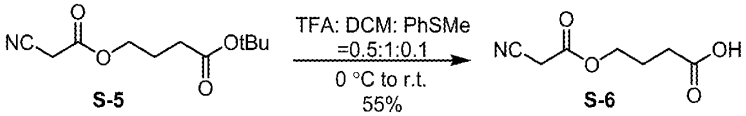


To a solution of cyanoacetic acid **S-4**(895mg, 10.5 mmol, 1.2 eq.) in 20 mL dry DCM is added 4-DMAP (102.9 mg, 0.88 mmol, 0.1 eq.) andEDCI·HCl (2.02 g, 10.5 mmol, 1.2 eq.). The atmosphere is replaced with N_2 and the system is cooled down to 0℃, followed by addition of a diluted solution of **S-3** (1.55 g, 8.75 mmol, 1 eq.) in 10 mL dry DCM. The reaction is stirred at room temperature. The reaction is monitored by TLC until completion. The mixture is extracted, washed, dried over anhydrous

Na_2SO_4, and concentrated. The residue is purified by flash chromatography (petroleum ether: ethyl acetate =30:1) to afford **S-5** as colorless oil (1.78 g, 90%).

^1H NMR (500 MHz, Chloroform-*d*) δ 4.23 (t, *J* = 6.4 Hz, 2H), 3.45 (s, 2H), 2.31 (t, *J* = 7.3 Hz, 2H), 1.96 (q, *J* = 6.8 Hz, 2H), 1.43 (s, 9H).^{13}C NMR (126 MHz, $CDCl_3$) δ 171.9, 163.0, 113.0, 80.9, 77.4, 77.2, 76.9, 66.2, 31.8, 28.2, 28.2, 24.8, 23.9. HRMS (ESI, m/z) calcd for $C_{11}H_{17}NO_4Na^+$: $[M+Na]^+$: 250.1050, found 250.1046. IR (film, cm^{-1}): 3689.82, 3663.01, 2989.84, 2981.67, 2902.45, 2876.78, 1405.25, 1254.46, 1076.24, 1047.37, 898.35.

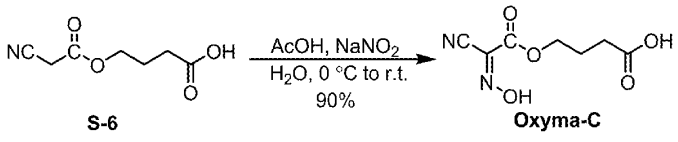
Embodiment 4:



To a mixed solvent of TFA:DCM:PhSMe (volume ratio 0.5:1:0.1, 15 mL) is slowly added **S-5** (1.2 g, 5.28 mmol) in 5 mL dry DCM at 0 °C. The reaction is allowed to proceed at 0 °C overnight. The reaction is monitored by TLC until completion. The solution is concentrated under reduced pressure, and the residue is purified by flash chromatography (dichloromethane: acetone=30:1) to afford **S-6** as colorless oil (498 mg, 55%).

^1H NMR (400 MHz, Chloroform-*d*) δ 4.29 (t, *J* = 6.3 Hz, 2H), 3.46 (s, 2H), 2.50 (t, *J* = 7.1 Hz, 2H), 2.06 (q, *J* = 6.6 Hz, 2H).^{13}C NMR (101 MHz, $CDCl_3$) δ 178.4, 163.0, 113.0, 77.5, 77.2, 76.8, 65.9, 30.4, 24.8, 23.5. HRMS (ESI, m/z) calcd for $C_7H_8NO_4^-$: $[M-H]^-$:170.0453, found 170.0447. IR (film, cm^{-1}): 3276.39, 2936.85, 2831.91, 2265.46, 1976.02, 1742.06, 1393.56, 1336.90, 1263.82, 1185.30, 1091.43, 1025.40, 934.46, 518.39.

Embodiment 5:

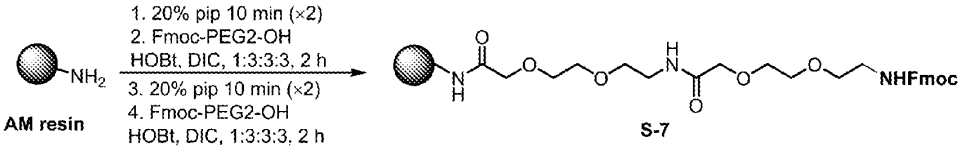


To a solution of **S-6** (500 mg, 2.9 mmol, 1 eq.) in water is added sodium nitrite (219 mg, 5.8 mmol, 2 eq.) and AcOH (315 μL, 7.25 mmol, 2.5 eq.) at 0 °C. The

reaction is warmed to room temperature and stirred for 6 h. The reaction is monitored by TLC until completion. The aqueous layer is extracted by ethyl acetate. The combined organic layer is washed with water, saturated aqueous NaCl and dried over anhydrous Na_2SO_4, and concentrated to afford **Oxyma-C** as a yellow solid (520 mg, 90%).

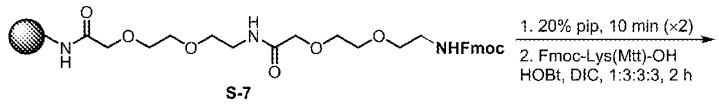
^{1}H NMR (500 MHz, DMSO-d_6) δ 15.06 (s, 1H), 12.16 (s, 1H), 4.28 (t, J = 6.5 Hz, 2H), 2.34 (t, J = 7.3 Hz, 2H), 1.94 – 1.83 (m, 2H).^{13}C NMR (126 MHz, DMSO) δ 173.8, 158.4, 125.8, 108.8, 65.5, 40.0, 39.9, 39.9, 39.8, 39.7, 39.6, 39.5, 39.4, 39.4, 39.2, 39.0, 29.8, 23.4. HRMS (ESI, m/z) calcd for $C_7H_7N_2O_5^-$: [M-H]$^-$: 199.0433 found 199.0430. IR (film, cm^{-1}): 3733.30, 3689.50, 3663.11, 2990.29, 2902.51, 2876.81, 1737.28, 1708.35, 1406.13, 1254.37, 1224.75, 1075.65, 1048.45, 898.69.

Embodiment 6:

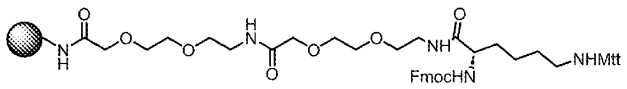


A mixture of DCM:DMF=1:1 is added to AM resin (0.5 g) in a 25 mL SPPS vessel to allow fully swelling. The solvent is filtered, and the resin is washed with DMF for 6 times, followed by treatment with 5 mL 20% piperidine for 10 min (x2) to remove Fmoc protecting groups. The piperidine solution is filtered and the resin is washed with DMF for 6 times. Fmoc-PEG2-OH（288 mg, 3 eq.）is added to the vessel followed by addition of DMF (5 mL) and DIC (3 eq.). The reaction is carried out at room temperature for 2 h, and the resin is washed with DMF adequately. Repeating the deprotection and condensation protocols as mentioned above, another Fmoc-PEG2-OH is connected to afford **S-7**.

Embodiment 7:



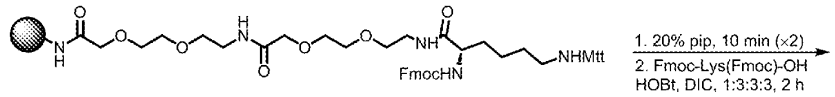
S-7



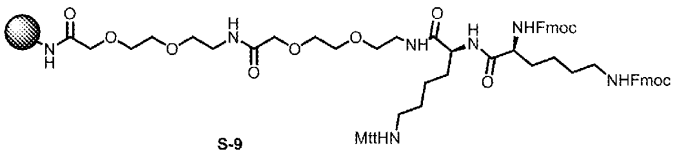
S-8

S-7 is treated with 5 mL 20% piperidine for 10 min (x2) to remove Fmoc protecting groups. The piperidine solution is filtered and the resin is washed with DMF for 6 times. Fmoc-Lys(Mtt)-OH (468 mg, 3 eq.) is added to the vessel followed by addition of DMF (5 mL) and DIC (3 eq.). The reaction is carried out at room temperature for 2 h, and then the resin is washed with DMF to afford **S-8**.

Embodiment 8:



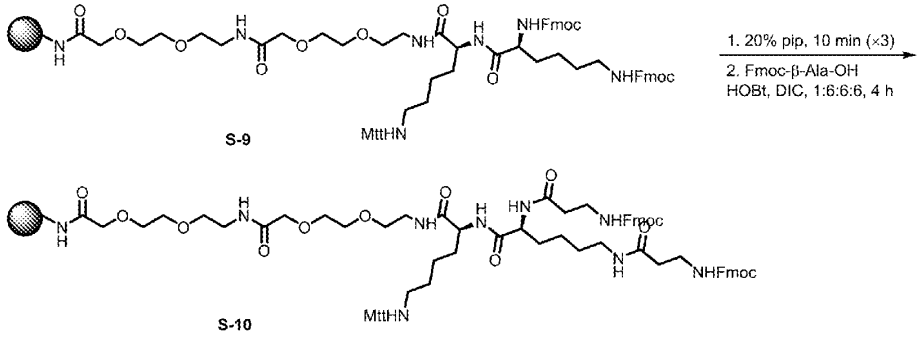
S-8



S-9

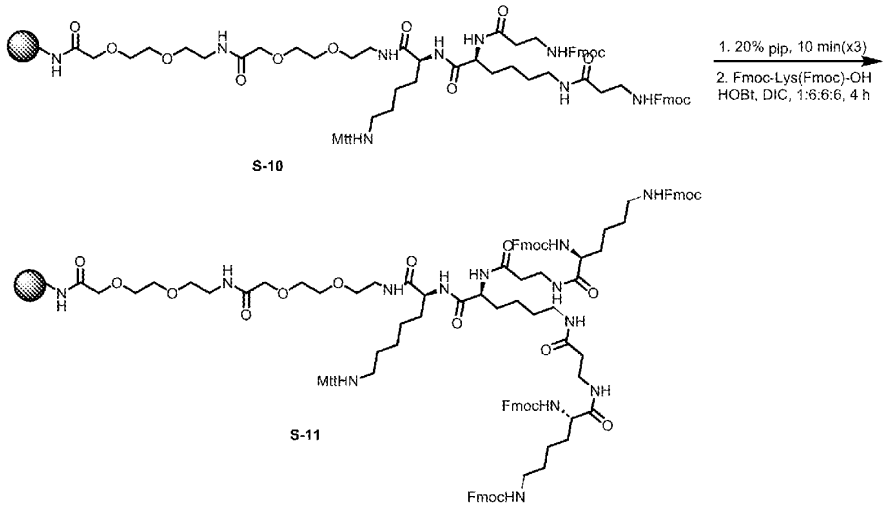
S-8 is treated with 5 mL 20% piperidine for 10 min (x2) to remove Fmoc protecting groups. The piperidine solution is filtered and the resin is washed with DMF for 6 times. Fmoc-Lys(Fmoc)-OH (442 mg, 3 eq.) is added to the vessel followed by addition of DMF (5 mL) and DIC (3 eq.). The reaction is carried out at room temperature for 2 h, and then the resin is washed with DMF to afford **S-9**.

Embodiment 9:



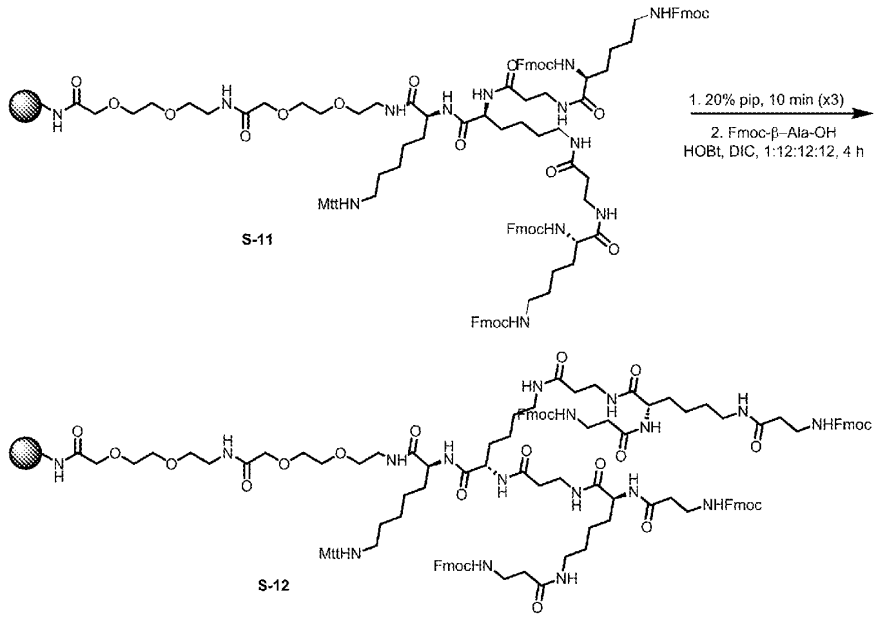
S-9 is treated with 5 mL 20% piperidine for 10 min (x3) to remove Fmoc protecting groups. The piperidine solution is filtered and the resin is washed with DMF for 6 times. Fmoc-*β*-Ala -OH (466 mg, 6 eq.) is added to the vessel followed by addition of DMF (5 mL) and DIC (6 eq.). The reaction is carried out at room temperature for 4 h, and then the resin is washed with DMF to afford **S-10**.

Embodiment 10:



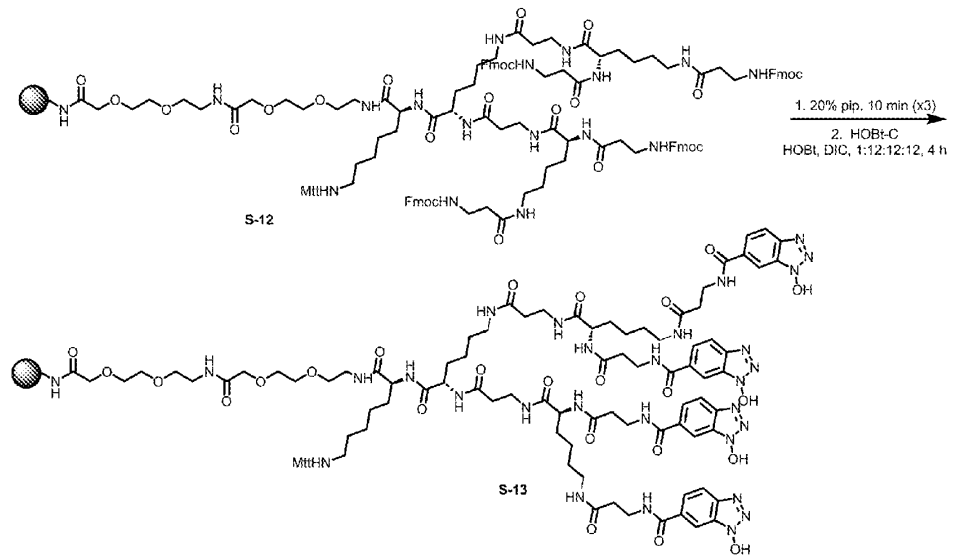
S-10 is treated with 5 mL 20% piperidine for 10 min (x3) to remove Fmoc protecting groups. The piperidine solution is filtered and the resin is washed with DMF for 6 times. Fmoc-Lys(Fmoc)-OH (884 mg, 6 eq.) is added to the vessel followed by addition of DMF (5 mL) and DIC (6 eq.). The reaction is carried out at room temperature for 4 h, and then the resin is washed with DMF to afford **S-11**.

Embodiment 11:



S-11 is treated with 5 mL 20% piperidine for 10 min (x3) to remove Fmoc protecting groups. The piperidine solution is filtered and the resin is washed with DMF for 6 times. Fmoc-β-Ala -OH (932 mg, 12 eq.) is added to the vessel followed by addition of DMF (5 mL) and DIC (12 eq.). The reaction is carried out at room temperature for 4 h, and then the resin is washed with DMF to afford **S-12**.

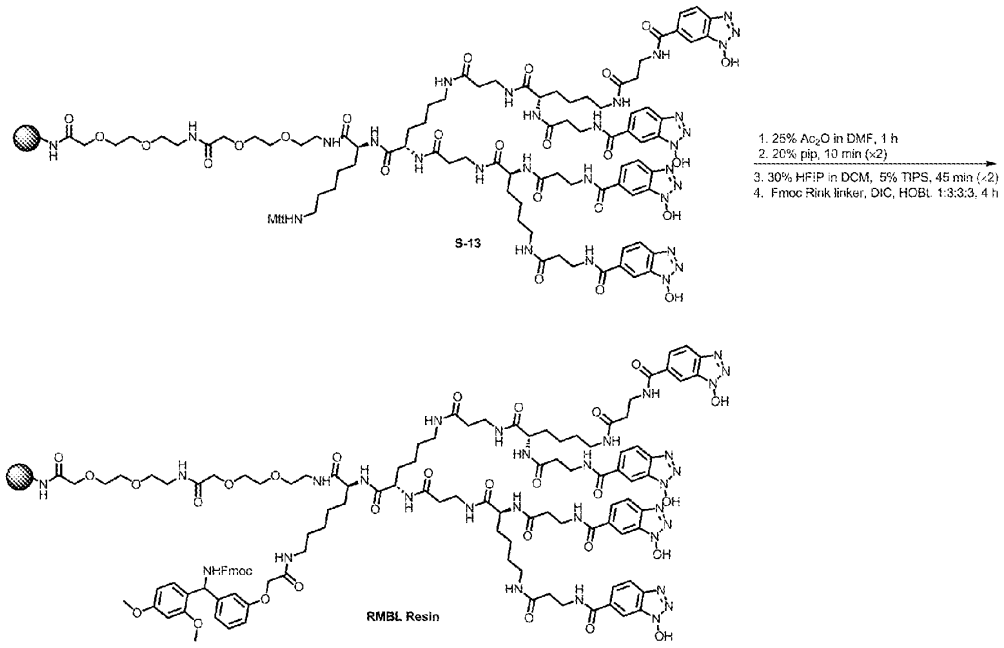
Embodiment 12:



S-12 is treated with 5 mL 20% piperidine for 10 min (x3) to remove Fmoc

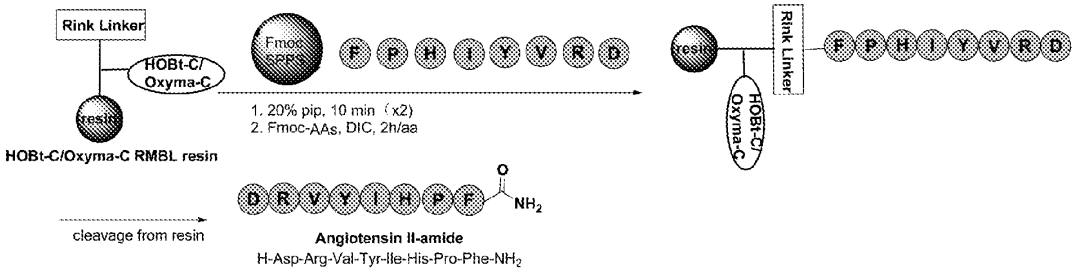
protecting groups. The piperidine solution is filtered and the resin is washed with DMF for 6 times. HOBt-C (600 mg, 12 eq.) is added to the vessel followed by addition of DMF (5 mL) and DIC (12 eq.). The reaction is carried out at room temperature for 4 h, and then the resin is washed with DMF to afford **S-13**.

Embodiment 13:



S-13 is treated with 5 mL 25% Ac_2O in DMF for 2 h to cap unreacted amino groups. The solution is filtered and the resin is washed with DMF for 6 times. The resulting resin is treated with 5 mL 20% piperidine for 10 min (x2). The solution is filtered and the resin is washed with DMF for 6 times. The resulting resin is treated with 30% HFIP and 5% TIPS in DCM (5 mL) for 45 min (x2) to remove Mtt protecting groups. The solution is filtered and washed with DCM for 3 times, DMF for 3 times. Fmoc-Rink linker (405 mg, 3 eq.) is added to the vessel followed by addition of DMF (5 mL) and DIC (3 eq.). The reaction is carried out at room temperature for 4 h, and then the resin is washed with DMF to afford HOBt-C RMBL Rink resin.

Embodiment 14:



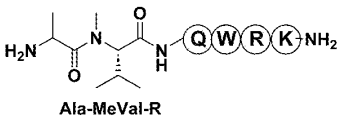
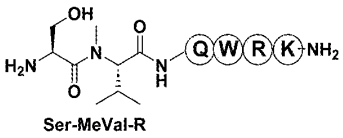
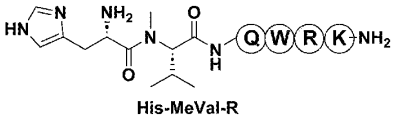
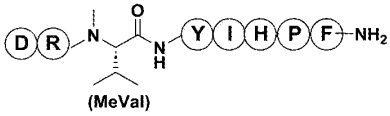
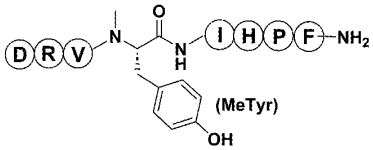
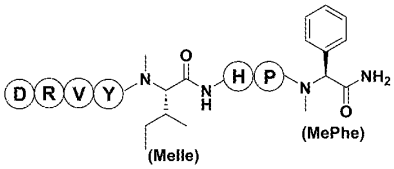
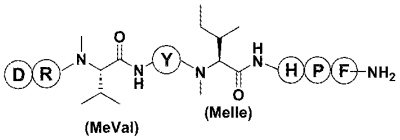
Taking synthesis of angiotensin II as an example. HOBt-C / Oxyma-C RMMR Rink resin (50 mg) is swelled with a mixture of DCM:DMF=1:1 (2.5 mL) for 30 min. Then the solvent is filtered and the resin is washed with DMF for 6 times. Removal of Fmoc groups is carried out by treatment with 20% piperidine in DMF (2.5 mL) for 10 min (x2). The solution is filtered and the resin is washed with DMF for 6 times. Fmoc-Phe-OH is added to the vessel followed by addition of DMF (1 mL) and DIC, and concentration of Fmoc-Phe-OH and DIC should be set as 0.2 M. The reaction is carried out at room temperature for 2 h. The solution is filtered and the resin is washed with DMF for 6 times The resulting resin is treated with 20% piperidine in DMF (2.5 mL) for 10 min (x2) to remove Fmoc groups. The solution is filtered and the resin is washed with DMF for 6 times. The condensation and deprotection cycle is repeated to realize connection of Fmoc-Pro-OH, Fmoc-His-OH, Fmoc-Ile-OH, Fmoc-Tyr-OH, Fmoc-Val-OH, Fmoc-Arg-OH and Fmoc-Asp-OH. The resulting resin is shrunk with DCM and Et_2O. Final peptide cleavage from the resin is carried out by treatment with a cleavage cocktail of TFA:TIPS:H_2O=94:5:1 (v/v/v, 1 mL) for 2 h at room temperature. The resulting mixture is filtered and the filtrate is evaporated to remove most solvents. Cold Et_2O (5 mL) is added to precipitate the crude peptide. The crude peptide is dissolved in a mixture of H_2O:ACN=1:1 and is subjected to HPLC analysis (C18 column) to gain purity data determined by peak area ratio.

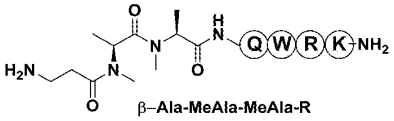
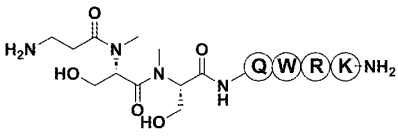
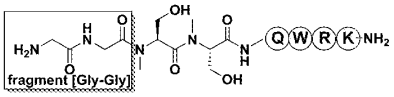
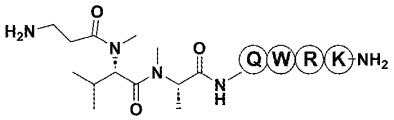
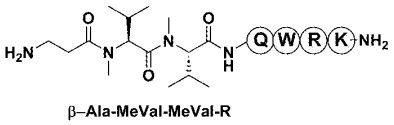
Tetrapeptide QWRK synthesized using HOBt-C RMBL Rink resin gives purity of 86%. Angiotensin II synthesized using HOBt-C RMBL Rink resin gives purity of 96%. Tetrapeptide QWRK synthesized using Oxyma-C RMBL Rink resin gives purity of 91%. Angiotensin II synthesized using Oxyma-C RMBL Rink resin gives purity of 98%.

Other model peptides are synthesized using Oxyma/ HOBt RMBL Rink resin following embodiment 14.

Purity data of twelve N-methyl amino acid-containing peptides are listed in 3rd and 4th column of Table 1.

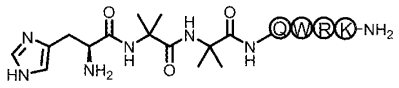
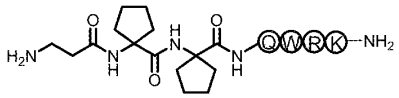
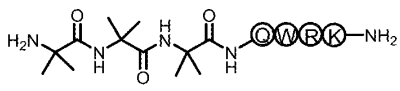
Table 1

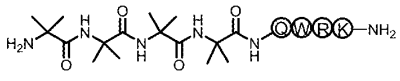
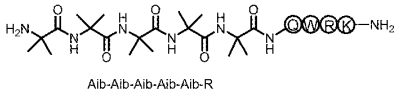
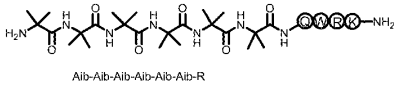
Entry	Structure of model peptide	HOBt-C RMBL resin [a]	Oxyma-C RMBL resin [b]	Rink resin [c]
1	 Ala-MeVal-R	89%	94%	13%
2	 Ser-MeVal-R	87%	98%	3%
3	 His-MeVal-R	65%	86%	6%
4	 (MeVal)	65%	83%	0%
5	 (MeTyr)	94%	96%	0%
6	 (MeIle) (MePhe)	78%	93%	0%
7	 (MeVal) (MeIle)	75%	92%	0%

8	 β–Ala-MeAla-MeAla-R	70%	75%	25%
9	 β–Ala-MeSer-MeSer-R	85%	95%	20%
10	 [Gly-Gly]-MeSer-MeSer-R	93%	95%	0%
11	 β–Ala-MeVal-MeAla-R	40%	56%	15%
12	 β–Ala-MeVal-MeVal-R	20%	48% 60% [d]	3%

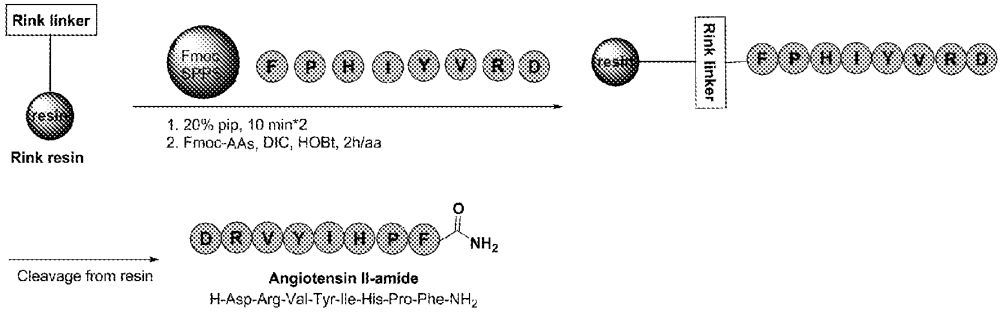
Purity data of six α,α-disubstituted amino acid-containing peptides are listed in table 2.

Table 2

Entry	Model peptides	Oxyma-C RMBL resin [b]
13	 His-Aib-Aib-R	96%
14	 bAla-Ac$_5$c-Ac$_5$c-R	92%
15	 Aib-Aib-Aib-R	96%

16	 Aib-Aib-Aib-Aib-R	95%
17	 Aib-Aib-Aib-Aib-Aib-R	95%
18	 Aib-Aib-Aib-Aib-Aib-Aib-R	95%

Embodiment 15:



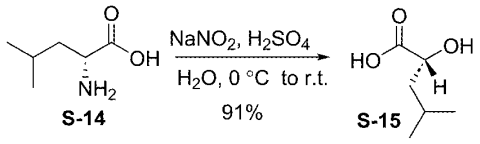
Taking synthesis of angiotensin II as an example. Rink resin (50 mg) is swelled with a mixture of DCM:DMF=1:1 (2.5 mL) for 30 min. The solvent is filtered and the resin is washed with DMF for 6 times. Removal of Fmoc groups is carried out by treatment with 20% piperidine in DMF (2.5 mL) for 10 min (x2). Fmoc-Phe-OH and HOBt are added to the vessel followed by addition of DMF (1 mL) and DIC, and concentration of Fmoc-Phe-OH, HOBt and DIC should be set as 0.2 M. The reaction is carried out at room temperature for 2 h. The solution is filtered and the resin is washed with DMF for 6 times The resulting resin is treated with 20% piperidine in DMF (2.5 mL) for 10 min (x2) to remove Fmoc groups. The solution is filtered and the resin is washed with DMF for 6 times. The condensation and deprotection cycle is repeated to realize connection of Fmoc-Pro-OH, Fmoc-His-OH, Fmoc-Ile-OH, Fmoc-Tyr-OH, Fmoc-Val-OH, Fmoc-Arg-OH and Fmoc-Asp-OH. The resulting resin is shrunk with DCM and Et_2O. Final peptide cleavage from the resin is carried out by treatment with a cleavage cocktail of TFA:TIPS:H_2O=94:5:1 (v/v/v, 1 mL) for 2 h at

room temperature. The resulting mixture is filtered and the filtrate is evaporated to remove most solvents. Cold Et_2O (5 mL) is added to precipitate the crude peptide. The crude peptide is dissolved in a mixture of H_2O:ACN=1:1 and is subjected to HPLC analysis (C18 column) to gain purity data determined by peak area ratio.

Tetrapeptide QWRK synthesized using Rink resin gives purity of 87%. Angiotensin II synthesized using Rink resin gives purity of 95%.

Other model peptides (1-12) are synthesized using Rink resin following embodiment 15, and purity data are listed in the 5th column of Table 1.

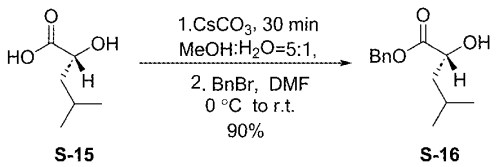
Embodiment 16:



D-Ile (4 g, 30.5 mmol, 1 eq.) is dissolved in 80 mL 1M aqueous sulfuric acid, and the solution is cooled to 0°C. Then, $NaNO_2$ (4.2 g, 61 mmol, 2 eq.) is dissolved in 60 mL water and added dropwise to the system. After the addition is complete, the reaction is allowed to proceed at 0°C for 6 hours before being transferred to room temperature. The reaction is monitored by TLC until completion. Afterward, ethyl acetate is added for extraction. The organic phase is washed with water and saturated aqueous NaCl, then dried over Na_2SO_4 to afford **S-15** (3.69 g, 91%).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.33 – 4.27 (m, 1H), 1.92 (tdd, *J* = 13.3, 8.1, 6.6 Hz, 1H), 1.69 – 1.57 (m, 2H), 0.97 (d, *J* = 6.6 Hz, 7H).^{13}C NMR (101 MHz, $CDCl_3$) δ 180.7, 77.5, 77.2, 76.8, 69.1, 43.4, 24.6, 23.3, 21.6. HRMS (ESI, m/z) calcd for $C_6H_{11}O_3^-$: [M-H]$^-$:131.0708 found 131.0699. IR (film, cm^{-1}): 3378.12, 2958.65, 2872.72, 1718.33, 1648.18, 1469.50, 1387.81, 1369.53, 1270.16, 1219.56, 1140.96, 1084.63, 915.74. $[\alpha]_D^{25}$ +6.0 (c 0.3, $CHCl_3$).

Embodiment 17:

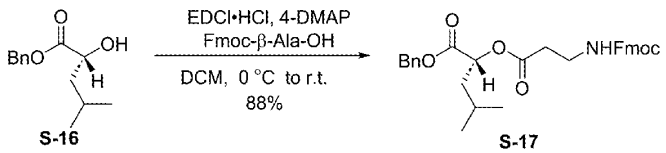


S-15 S-16

S-15 (200 mg, 1.52 mmol, 1 eq.) is dissolve in a mixed solvent of MeOH:H_2O= 9:1 (v/v). Then, Cs_2CO_3 (0.25 g, 0.8 mmol, 0.5 eq.) is added and the reaction is allowed to proceed at room temperature for 30 minutes before the solvent is evaporated from the system. The resulting mixture is redissolved in DMF and the system is cooled to 0°C, then benzyl bromide (180 μL, 1.6 mmol, 1.05 eq.) is slowly added to the flask. After the addition is complete, the reaction mixture is transferred back to room temperature. The reaction is monitored by TLC until completion. After concentration, the mixture is dilute with ethyl acetate, and the organic phase is washed with water and saturated aqueous NaCl, then dried over Na_2SO_4. The residue is purified by flash chromatography (petroleum ether: ethyl acetate =8:1) to afford **S-16** (301 mg, 90%).

^{1}HNMR (400 MHz, Chloroform-*d*) δ 7.43 – 7.30 (m, 5H), 5.21 (d, *J* = 1.4 Hz, 2H), 4.24 (ddd, *J* = 8.4, 6.0, 4.9 Hz, 1H), 2.61 (d, *J* = 6.0 Hz, 1H), 1.89 (dq, *J* = 8.0, 6.6 Hz, 1H), 1.61 – 1.54 (m, 2H), 0.94 (dd, *J* = 6.7, 4.5 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.7, 135.2, 128.7, 128.5, 128.3, 77.4, 77.0, 76.7, 69.2, 67.3, 43.4, 24.4, 23.2, 21.6. HRMS (ESI, m/z) calcd for $C_{13}H_{19}O_3^+$: $[M+H]^-$:223.1329 found 223.1325. IR (film, cm^{-1}): 3479.06, 3066.80, 3034.68, 2956.94, 2870.75, 1734.89, 1498.28, 1456.11, 1368.69, 1267.29, 1196.23, 1139.09, 1084.78, 1003.47, 748.15, 697.24. $[\alpha]_D^{25}$ +13.0 (c 0.3, $CHCl_3$).

Embodiment 18:



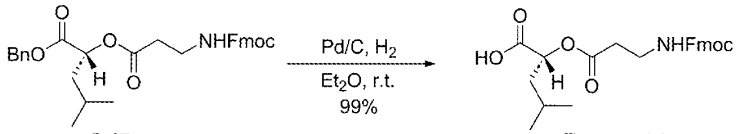
S-16 S-17

Fmoc-β-Ala-OH (93 mg, 0.42 mmol, 1.5 eq.) is dissolved in 1 mL anhydrous DCM. EDCI·HCl (57 mg, 0.63 mmol, 1.5 eq.) and 4-DMAP (4 mg, 0.04 mmol, 0.1 eq.) are added at 0 ℃. Subsequently, **S-16** is dissolved in 1 mL anhydrous DCM and

added dropwise to the flask. After the addition is complete, the reaction is allowed to proceed at room temperature. The reaction is monitored by TLC until completion. The organic phase is washed with water and saturated aqueous NaCl, dried with Na_2SO_4, and concentrated. The residue is purified by column chromatography (petroleum ether: ethyl acetate= 10:1) to afford **S-17** (114 mg,88%).

^{1}H NMR (400 MHz, Chloroform-d) δ 7.76 (dt, J = 7.6, 1.0 Hz, 2H), 7.61 (d, J = 7.4 Hz, 2H), 7.46 – 7.26 (m, 9H), 5.60 (s, 1H), 5.24 – 5.11 (m, 3H), 4.37 (d, J = 7.3 Hz, 2H), 4.22 (t, J = 7.2 Hz, 1H), 3.53 (q, J = 6.1 Hz, 2H), 2.63 (td, J = 5.8, 2.5 Hz, 2H), 1.86 – 1.64 (m, 3H), 0.93 (t, J = 6.6 Hz, 6H). ^{13}C NMR (101 MHz, CDCl3) δ 172.1, 170.9, 156.5, 144.1, 144.1, 141.4, 135.3, 128.8, 128.7, 128.4, 127.8, 127.2, 125.3, 120.1, 77.5, 77.2, 76.8, 71.4, 67.4, 67.0, 47.4, 39.7, 37.0, 34.8, 24.8, 23.1, 21.7. HRMS (ESI, m/z) calcd for $C_{31}H_{34}NO_6^+$: $[M+H]^+$:516.2381 found 516.2378. IR (film, cm^{-1}): 3362.46, 3065.70, 2957.48, 1740.49, 1523.13, 1449.95, 1372.32, 1249.59, 1173.96, 1143.98, 1078.86, 1009.68, 758.75, 740.94, 697.50. $[\alpha]_D^{25}$ +12.2 (c 0.3, $CHCl_3$).

Embodiment 19:



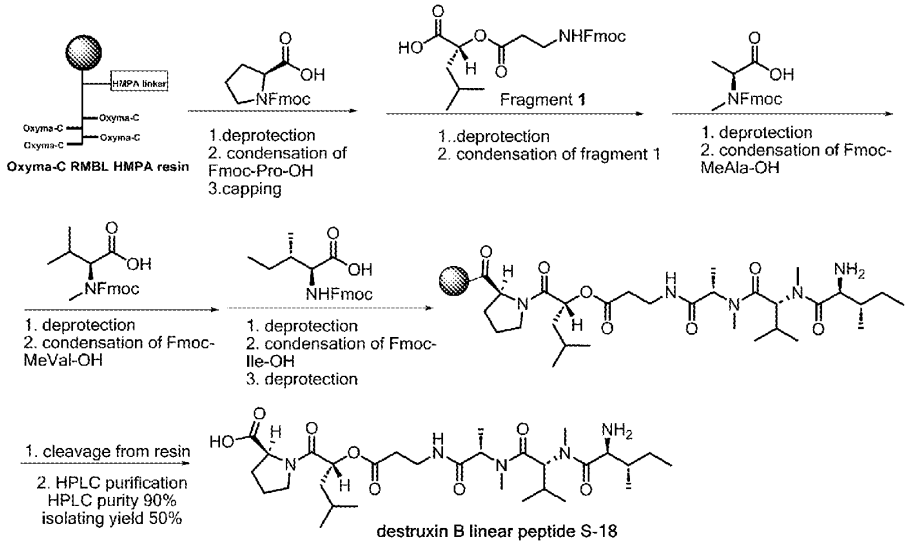
S-17 **Fragment 1**

S-17 (1.78 g, 3.45 mmol, 1 eq.) is dissolved in ethanol, then Pd/C (88 mg, 10% m%) is added. The reaction is carried out under H_2 atmosphere using a H_2 gasbag. The reaction is monitored by TLC until completion. The mixture is filtered through celite and washed multiple times with ethanol. The filtrate is concentrated and dried under reduced pressure to yield **Fragment 1** (1.47 g, 99%).

^{1}H NMR (500 MHz, Chloroform-d) δ 7.77 (d, J = 7.6 Hz, 2H), 7.57 (dd, J = 24.0, 7.1 Hz, 2H), 7.40 (t, J = 7.5 Hz, 2H), 7.31 (t, J = 7.5 Hz, 2H), 5.59 (t, J = 6.4 Hz, 1H), 5.20 – 5.01 (t, 1H), 4.48 – 4.34 (m, 2H), 4.23 (t, J = 7.2 Hz, 1H), 3.53 (q, J = 5.4, 4.7 Hz, 2H), 2.71 – 2.53 (m, 2H), 1.86 – 1.65 (m, 3H), 0.94 (dd, J = 14.0, 6.2 Hz, 6H). ^{13}C NMR (126 MHz, CDCl3) δ 175.5, 172.2, 156.6, 144.1, 141.4, 127.8, 127.2, 125.3,

125.3, 120.1, 77.4, 77.2, 76.9, 71.2, 67.1, 47.3, 39.6, 36.9, 34.6, 24.8, 23.1, 21.6. HRMS (ESI, m/z) calcd for $C_{24}H_{26}NO_6^-$: $[M-H]^-$:424.1760 found 424.1768. IR (film, cm^{-1}): 3661.54, 3065.89, 2959.81, 2871.18, 1738.51, 1525.32, 1448.17, 1378.21, 1340.87, 1273.36, 1253.74, 1178.77, 1134.45, 1077.65, 1007.64, 737.67. $[\alpha]_D^{25}$ +11.8 (c 0.3, $CHCl_3$).

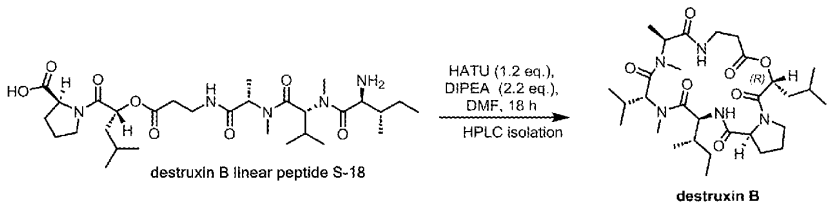
Embodiment 20:



Oxyma RMBL HMPA resin (200 mg) is swelled with a mixture of DCM:DMF=1:1 (5 mL) for 30 min. The solvent is filtered and the resin is washed with DMF for 6 times. Removal of Fmoc groups is carried out by treatment with 20% piperidine in DMF (5 mL) for 10 min (x2). The resin is then washed for 6 times with DMF. Fmoc-Pro-OH (0.4 mmol) is added to the vessel followed by addition of DMF (2 mL) and DIC (0.4 mmol), The reaction is carried out at room temperature for 2 h. The solution is filtered and the resin is washed with DMF for 6 times. The resulting resin is treated with 20% piperidine in DMF (2.5 mL) for 10 min (x2) to remove Fmoc groups. The solution is filtered and the resin is washed with DMF for 6 times. The condensation and deprotection cycle is repeated to realize connection of **fragment 1**, Fmoc-MeAla-OH, Fmoc-MeVal-OH and Fmoc-Ile-OH. Specially, the resin should be treated with a mixture of Ac_2O: pyridine: DMF=2.5:1:6.5 (v/v/v, 5 mL) to cap unreacted HMPA linkers after condensation of Fmoc-Pro-OH. The final

resin-peptide complex is shrunk with DCM and Et_2O. Final peptide cleavage from the resin is carried out by treatment with a cleavage cocktail of TFA:TIPS:H_2O=94:5:1 (v/v/v, 3 mL) for 2h at room temperature, followed by concentration under vacuum to yield crude product. The crude product is dissolved in MeOH and is subjected to HPLC purification (C18) to afford pure target peptide. Purity of the crude product is 90%. The elution solvent system is 1% TFA in H_2O (phase A) and MeCN (phase B), and the product is eluted at 15 min (40% MeCN). After concentration and lyophilization, the linear peptide **S-18** is obtained as a pure product (17 mg, 50%).

Embodiment 21:

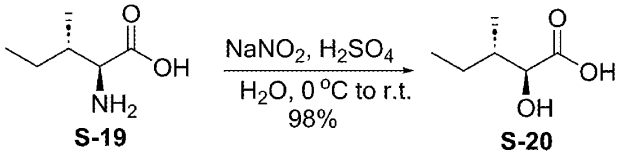


HATU (4 mg, 0.011 mmol, 1.5 eq.) is dissolved in 50 mL anhydrous DMF under N_2 atmosphere. DIPEA (2.5 μL, 0.015 mmol, 2 eq.) is then added dropwise. The linear peptide S-18 (4.5 mg, 0.007 mmol, 1 eq.) is dissolved in 100 mL anhydrous DMF and is added to the system using a syringe pump at a rate of 5 mL/h. The reaction is allowed to proceed at room temperature for 12 hours. The solution is concentrated and the crude product is dissolved in methanol and directly subjected to HPLC purification to afford destruxin B as a white solid (3.2 mg, 75%).

^{1}H NMR (600 MHz, Chloroform-d) δ 8.24 (d, J = 10.1, 1H), 7.18 (d, J = 9.2 Hz, 1H), 5.18 (q, J = 6.8 Hz, 1H), 4.93 (d, J = 10.9 Hz, 1H), 4.90 – 4.85 (m, 2H), 4.67 (d, J = 7.1 Hz, 1H), 4.07 – 4.00 (m, 1H), 3.95 – 3.88 (m, 1H), 3.47 – 3.43 (m, 1H), 3.22 (s, 3H), 3.11 – 3.05 (m, 1H), 2.73 (s, 3H), 2.70 – 2.64 (m, 1H), 2.65 – 2.55 (m, 1H), 2.50 – 2.45 (m, 1H), 2.34 – 2.28 (m, 1H), 2.15 – 2.03 (m, 1H), 2.00 – 1.90 (m, 3H), 1.90 – 1.80 (m, 1H), 1.50 – 1.35 (m, 2H), 1.25 – 1.30 (1H, m) 1.30 – 1.25(d, J = 6.8 Hz, 3H), 1.28 – 1.24 (m, 1H), 0.99 (d, J = 6.7 Hz, 3H), 0.94 (t, J = 7.0 Hz, 6H), 0.89 (d, J = 6.6 Hz, 3H), 0.85 (m, 6H). ^{13}C NMR (150 MHz, Chloroform-d) δ 173.8, 173.5, 171.2, 171.1, 169.9, 169.7, 71.9, 60.7, 58.2, 55.5, 53.7, 46.5, 38.9, 37.4, 34.4, 33.3, 30.8, 28.9, 28.2, 27.2, 24.5, 24.4, 24.1, 23.3, 21.4, 19.9, 19.6, 15.3, 15.2, 11.3. HRMS

(ESI, m/z) calcd for $C_{30}H_{52}N_5O_7$: $[M+H]^+$: 594.3861, found 594.3853; IR ($CHCl_3$) 3386,3302,2965,2929,2875,1781,1730,1672,1626,1545,1517,1442,1409,1377,1339,1272,1178, 1094 cm^{-1}; $[\alpha]_D^{25}$ –236 (c 0.3, MeOH).

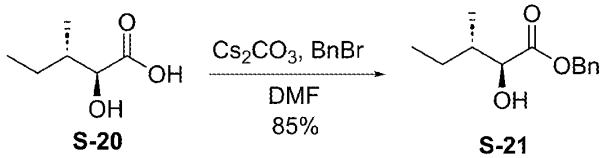
Embodiment 22:



S-19 (2.6 g, 20 mmol, 1 eq.) is weighed into a round-bottom flask and dissolved in 25 mL 1 M aqueous H_2SO_4, after which the temperature is cooled to 0°C. Subsequently, $NaNO_2$ is dissolved in 25 mL water and added dropwise to the flask. After a period of time, the temperature is allowed to rise to room temperature for the reaction to continue. The reaction is monitored by TLC until completion. After extraction with EA, the organic phase is washed with saturated aqueous NaCl and then concentrated to afford **S-20** as white solid (3.76 g, 98%).

^1H NMR (400 MHz, Chloroform-*d*) δ 4.13 (d, *J* = 3.6 Hz, 1H), 1.86 (p, *J* = 7.3, 3.8 Hz, 1H), 1.41 (m, 1H), 1.33 – 1.26 (m, 1H), 1.00 (d, *J* = 6.9 Hz, 3H), 0.91 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 179.0, 75.0, 39.0, 23.8, 15.5, 11.9. HRMS (ESI, m/z) calcd for $C_6H_{11}O_3^-$: $[M-H]^-$: 131.0714 found 131.0709.IR (film, cm^{-1}): 3597.72, 3465.34, 2966.93, 2931.24, 2878.43, 1719.20, 1461.23, 1382.25, 1244.56, 1213.27, 1136.95, 1072.61, 1045.85, 1016.18, 960.95, 745.33, 677.09. $[\alpha]_D^{25}$ -11.0 (c 0.5, $CHCl_3$).

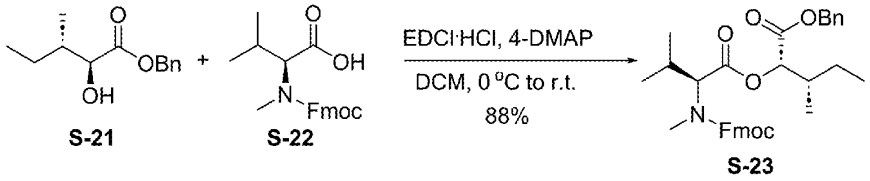
Embodiment 23:



S-20 (3 g, 23 mmol, 1 eq.) is dissolved in a mixture of MeOH: H_2O=9:1 (v/v, 60 mL) in a round-bottom flask, followed by the addition of Cs_2CO_3. After the reaction is complete, the solvent is evaporated and the residue is redissolved in anhydrous DMF

followed by slow addition of BnBr (3.26 mL, 25.2 mmol, 1.1 eq.). The reaction is monitored by TLC until completion. Saturated aqueous NH_4Cl is added to quench the reaction. After extraction with EA, the organic phase is washed with saturated aqueous NaCl, dried over Na_2SO_4, and concentrated. The product is then purified by column chromatography (PE: EA=15:1) to yield **S-21** as colorless liquid (3.55 g, 85%).

Embodiment 24:

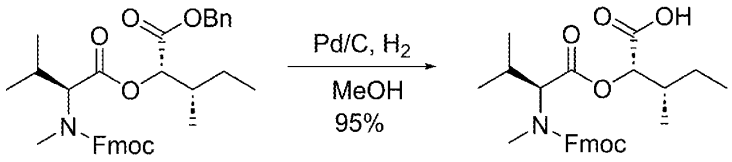


S-22 (4.59 g, 15 mmol, 1.5 eq.) is dissolved in 35 mL anhydrous DCM, and EDCI·HCl (4.27 g, 15 mmol, 1.5 eq.) and 4-DMAP (122 mg, 1 mmol, 0.1 eq.) are added. Subsequently, **S-21** (2.22 g, 10 mmol, 1 eq.) is dissolved in 30 mL anhydrous DCM and added dropwise to the system. After addition is complete, the reaction is allowed to proceed at room temperature. The reaction is monitored by TLC until completion, after which the organic phase is washed with water and saturated aqueous NaCl. The organic phases are combined, dried with Na_2SO_4, and concentrated. The product is purified by column chromatography (PE: EA = 22:1) to yield **S-23** as light yellow oily liquid (5.72 g, 88%).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.76 (dd, *J* = 7.7, 3.4 Hz, 2H), 7.60 (q, *J* = 6.9, 5.5 Hz, 2H), 7.47 – 7.28 (m, 9H), 5.23 (d, *J* = 5.5 Hz, 0.33H), 5.20 (d, *J* = 5.5 Hz, 0.67H), 5.14 (d, *J* = 1.5 Hz, 0.67H), 5.11 (d, *J* = 1.5 Hz, 0.34H), 4.94 (d, *J* = 4.4 Hz, 0.54H), 4.89 (d, *J* = 4.5 Hz, 0.35H), 4.57 (d, *J* = 10.4 Hz, 0.54H), 4.54 – 4.37 (m, 2H), 4.24 (m, 1.37H), 2.92 (s, 2H), 2.88 (s, 1H), 2.21 (m, 0.58H), 2.16 – 2.06 (m, 0.37H), 1.99 (m, 1H), 1.49 – 1.34 (m, 1H), 1.28 – 1.15 (m, 1H), 1.04 (d, *J* = 6.5 Hz, 2H), 0.96 – 0.80 (m, 9H), 0.75 (d, *J* = 6.7 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.0, 170.4, 169.3, 169.2, 157.0, 156.3, 144.2, 144.2, 144.0, 143.9, 141.5, 141.5, 135.4, 128.7, 128.6, 128.5, 127.8, 127.2, 127.2, 127.2, 125.2, 125.1, 125.1, 125.0, 120.1, 120.1, 76.8, 67.8, 67.8, 67.1, 67.0, 64.3, 64.1, 47.4, 36.7, 36.6, 30.7, 30.6, 27.9, 27.6, 24.6,

19.7, 19.7, 19.2, 18.9, 15.6, 11.6. HRMS (ESI, m/z) calcd for $C_{34}H_{39}NO_6^+$: $[M+H]^+$: 558.2850 found 558.2843.IR (film, cm^{-1}): 3694.39, 3631.10, 3544.32, 2968.29, 2877.09, 1745.32, 1704.01, 1449.73, 1401.19, 1299.27, 1257.43, 1194.91, 1148.03, 1112.04, 1047.15, 740.51. $[\alpha]_D^{25}$ -50.6 (c 0.5, $CHCl_3$).

Embodiment 25:

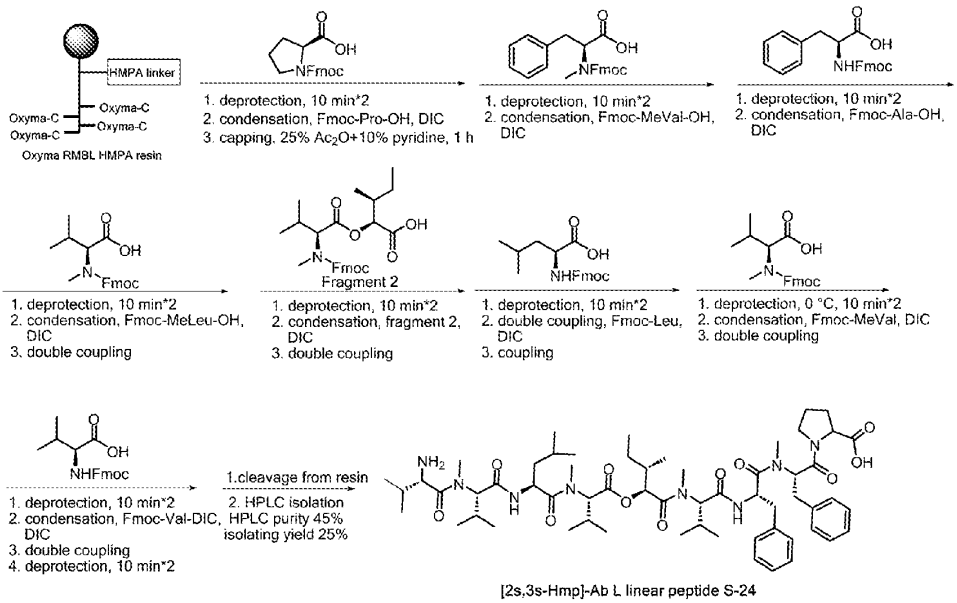


S-23 **Fragment 2**

S-23 (5 g, 7.6 mmol, 1 eq.) is dissolved in 60 mL methanol, then Pd/C (500 mg, 10% m%) is added. The reaction is carried out under H_2 atmosphere using a H_2 gasbag. The reaction is monitored by TLC until completion. The mixture is filtered through celite and the filtrate is concentrated and dried under reduced pressure to yield **Fragment 2** (3.55 g, 95%).

^1H NMR (500 MHz, Chloroform-*d*) δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.65 – 7.54 (m, 2H), 7.39 (dt, *J* = 8.3, 4.9 Hz, 2H), 7.34 – 7.28 (m, 2H), 4.94 (d, *J* = 4.3 Hz, 0.59H), 4.87 (d, *J* = 4.2 Hz, 0.44H), 4.53 (dd, *J* = 10.4, 4.7 Hz, 1H), 4.50 – 4.39 (m, 2H), 4.25 (m, 1H), 4.17 (d, *J* = 10.7 Hz, 0.43H), 2.92 (s, 2H), 2.88 (s, 1H), 2.24 (m, 0.61H), 2.09 (m, 0.37H), 2.00 (m, 1H), 1.49 (m, 1H), 1.37 – 1.19 (m, 1H), 1.07 (d, *J* = 6.6 Hz, 2H), 1.00 – 0.86 (m, 9H), 0.73 (d, *J* = 6.7 Hz, 1H).^{13}C NMR (126 MHz, $CDCl_3$) δ 174.0, 171.0, 170.4, 157.1, 156.4, 144.2, 144.1, 144.0, 143.9, 141.5, 127.8, 127.2, 125.1, 125.1, 125.0, 125.0, 120.1, 120.1, 76.5, 76.4, 67.9, 67.8, 64.4, 64.3, 47.4, 36.6, 36.5, 30.9, 30.7, 27.9, 27.6, 24.6, 19.7, 19.2, 18.9, 15.5, 11.6. HRMS (ESI, m/z) calcd for $C_{27}H_{33}NO_6^+$: $[M+H]^+$: 468.2381 found 468.2373. IR (film, cm^{-1}): 3693.02, 3598.48, 2969.78, 2901.96, 2877.10, 1746.18, 1706.47, 1664.92, 1482.03, 1448.57, 1406.08, 1301.51, 1255.18, 1196.78, 1153.86, 1074.23, 1048.09, 740.35, 675.89. $[\alpha]_D^{25}$ -37.4 (c 0.5, $CHCl_3$).

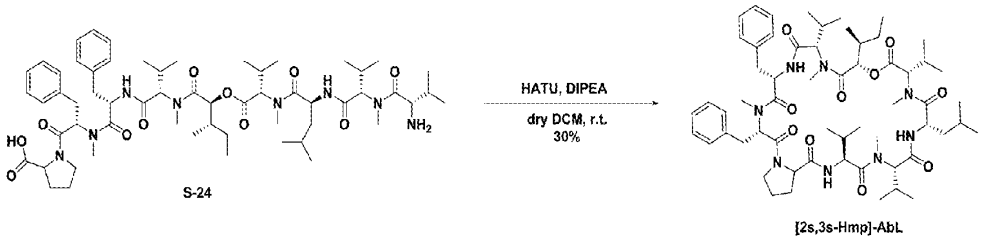
Embodiment 26:



Oxyma RMBL HMPA resin (200 mg) is swelled with a mixture of DCM:DMF=1:1 (5 mL) for 30 min. The solvent is filtered and the resin is washed with DMF for 6 times. Removal of Fmoc groups is carried out by treatment with 20% piperidine in DMF (5 mL) for 10 min (x2). The resin is then washed 6 times with DMF. Fmoc-Pro-OH is added to the vessel followed by addition of DMF (2 mL) and DIC, and concentration of Fmoc-Pro-OH and DIC should be set as 0.2 M. The reaction is carried out at room temperature for 2 h. When double coupling is required, same amount of reagents are added to the drained resin and the reaction is allowed to proceed for 4 h. The solution is filtered and the resin is washed with DMF for 6 times. The resulting resin is treated with 20% piperidine in DMF (5 mL) for 10 min (x2) to remove Fmoc groups. The solution is filtered and the resin is washed with DMF for 6 times. The condensation and deprotection cycle is repeated to realize connection of Fmoc-MeVal-OH、Fmoc-Phe-OH, Fmoc-MeVal-OH, **Fragment 2** and Fmoc-Leu-OH. Specially, the resin should be treated with a mixture of Ac_2O: pyridine: DMF=2.5:1:6.5 (v/v/v, 5 mL) to cap unreacted HMPA linkers after condensation of Fmoc-Pro-OH. The drained resin is treated with 20% piperidine at 0 ℃ for 1 min (x2), and is then washed with DMF for 6 times. Then, connection of Fmoc-MeVal-OH and Fmoc-Val-OH is carried out following the aforementioned cycle. The resulting resin is

shrunk with DCM and Et_2O. Final peptide cleavage from the resin is carried out by treatment with a cleavage cocktail of TFA:TIPS:H_2O=94:5:1 (v/v/v, 3 mL) for 2h at room temperature, followed by concentration under vacuum to yield crude product. The crude product is dissolved in MeOH and is subjected to HPLC purification (C18) to afford pure target peptide. Purity of the crude product is 45%. The elution solvent system is 1% TFA in H_2O (phase A) and MeCN (phase B), and the product is eluted at 21 min (90% MeCN). After concentration and lyophilization, the linear peptide **S-24** is obtained as a pure product (16 mg, 25%).

Embodiment 27:



HATU (3 mg, 0.008 mmol, 2 eq.) is dissolved in 6 mL anhydrous DCM under N_2 atmosphere. DIPEA (2.5 μL, 0.012 mmol, 2 eq.) is then added dropwise. The linear peptide **S-24** (4 mg, 0.004 mmol, 1 eq.) is dissolved in 8 mL anhydrous DCM and is added to the system using a syringe pump at a rate of 1.5 mL/h. The reaction is allowed to proceed at room temperature for 12 hours. The solution is concentrated and the crude product is dissolved in methanol and directly subjected to HPLC purification to afford [2s,3s-Hmp]-Ab L as a white solid (1.5 mg, 50%).

^{1}H NMR (600 MHz, Chloroform-*d*)

	Literature Data （Conformation A）	Our data （Conformation A）	Literature Data （Conformation B）	Our data （Conformation B）
Hmp				
2-CH	5.05 (1H, d, 4.4)	5.03 (1H, d, 4.5)	5.15-5.20 (1H, m)	5.19 – 5.13 (1H, m)
3-CH	1.72-1.85(1H, m)	1.85-1.70 (1H, m)	1.72-1.85 (1H, m)	1.85-1.70 (1H, m)
4-CH_2	1.45-1.55(1H, m)	1.45-1.55(1H, m)	1.45-1.55 (1H, m)	1.45-1.55 (1H, m)
	1.61-1.71 (1H, m)	1.61-1.71 (1H, m)	1.28-1.32 (1H, m)	1.28-1.32 (1H, m)
4-CH_3	0.90-1.00 (3H, m)	1.00-0.80 (3H, m）	0.90-1.00 (3H, m)	1.00-0.80 (3H, m）

5-CH_3	0.79-0.89(3H, m)	1.00-0.80 (3H, m）	0.79-0.89 (3H, m)	1.00-0.80 (3H, m）
MeVal				
α-CH	5.15-5.20(1H, m)	5.19 – 5.12 (1H, m)	3.28-3.34 (1H, m)	3.29 (1H, m)
β-CH	2.17-2.26(1H, m)	2.18-2.24 (1H, m)	2.27-2.32(1H, m)	2.33-2.26 (1H, m)
γ-CH_3	0.79-0.89(3H, m)	1.00-0.80 (3H, m）	0.79-0.89(3H, m)	1.00-0.80 (3H, m）
γ-CH_3	0.90-1.00(3H, m)	1.00-0.80 (3H, m）	0.90-1.00 (3H, m)	1.00-0.80 (3H, m）
N-CH_3	2.76(3H, s)	2.75 (3H, s)	3.20 (3H, s)	3.18 (3H, s)
Phe				
α-CH	5.34 (1H, q, 7.8)	5.32 (1H, q. 8.0)	5.10-5.15(1H, m)	5.14-5.11 (1H, m)
β-CH_2	3.20-3.29(1H, m)	3.27-3.20(1H, m)	3.30-3.40 (1H, m)	3.34-3.28 (1H, m)
$C_γ$ (ar.)			2.59-2.62 (1H, m)	2.63 – 2.57 (1H, m)
$C_δ$(ar.)	2.72-2.77(1H, m)	2.70-2.76(1H, m)	7.43 (2H, d, 7.4)	7.40 (2H, d, 8.0)
$C_ε$(ar.)	7.23 (2H, d, 4.4)	7.24 – 7.19 (2H, m)	7.26-7.33 (2H, m)	7.35-7.29 (2H, m)
$C_ζ$(ar.)	7.26-7.33(2H, m)	7.35 – 7.29 (2H, m)	7.26-7.33(1H, m)	7.35-7.29 (2H, m)
NH	8.28(1H, d, 8.95)	8.24 (1H, d, 9.0)	9.10 (1H, d, 9.7)	9.06 (1H, d, 9.6)
MePhe				
α-CH	2.95-3.03 (1H, m)	2.98 (1H, m)	3.62(1H, dd, 11.9, 2.7)	3.63 – 3.58 (1H, m)
β-CH_2	3.05-3.16(1H, m)	3.12 – 3.07 (1H, m)	2.95-3.03 (1H, m)	3.05-2.96 (1H, m)
	2.72-2.77 (1H, m)	2.71-2.77(1H, m)	3.38-3.50(1H, m)	3.40 (1H, m)
$C_γ$ (ar.)				
$C_δ$(ar.)	7.14(2H, d,7.0)	7.14 – 7.09 (2H, d, 7.2)	6.22(2H, d, 7.7)	6.21 (2H, d, 7.4)
$C_ε$(ar.)	7.26-7.33 (2H m)	7.35 – 7.29 (2H, m)	7.05 (2H, t, 7.0)	7.05 – 6.97 (2H, m)
$C_ζ$(ar.)	7.26-7,33 (1H m)	7.35 – 7.29 (1H, m)	7.26-7.33 (1H, m)	7.35 – 7.29 (1H, m)
N-CH_3	3,04(3H, s)	3.07 (3H, s)	2.40 (3H, s)	2.39 (3H, s)
Pro				
α-CH	4.20(1H, t, 8.7)	4.23-4.16 (1H, t, 8.75)	4.69 (1H, d, 7.7)	4.67 (1H, t, 7.7)
β-CH_2	3.00-3.05 (2H, m)	3.05-2.95（2H, m)	2.27-2.33 (1H, m)	2.33-2.26 (1H, m)
			0.79-0.89(1H, m)	0.79-0.89(1H, m)
γ-CH_2	2.06-2.13(1H, m)	2.12-2.05 (1H, m)	2.06-2.13 (1H, m)	2.12-2.07 (1H, m)
	1.50-1.70 (1H, m)	1.50-1.70 (1H, m)	1.50-1.70 (1H, m)	1.72-1.50 (1H, m)
δ-CH_2	3.30-3,38 (1H, m)	3.40-3.35 (1H, m)	4.34 (1H, t, 8.4)	4.32 (1H, t, 8.8)
	3.39-3.50 (1H, m)	3.50-3.42 (1H, m)	3.65-3.72 (1H, m)	3.72 – 3.65 (1H, m)

Val				
α-CH	4.40(1H, t, 9.2)	4.43-4.36 (1H, m)	4.45 (1H, t, 10.7)	4.45 (1H, m)
β-CH	2.17-2.26(1H, m)	2.25-2.17 (1H, m)	2.46-2.55 (1H, m)	2.52-2.45 (1H, m)
γ-CH_3	0.79-0.89 (3H, m)	1.00-0.80 (3H,m）	0.79-0.89 (3H, m)	1.00-0.80 (3H,m）
γ-CH_3	0.90-1.00 (3H, m)	1.00-0.80 (3H,m）	0.90-1.00(3H, m)	1.00-0.80 (3H,m）
NH	9.18(1H, d, 8.8)	9.16 (1H, d, 8.5)	7.78 (1H, d, 8.6)	7.76 (1H, d, 8.8)
MeVal2				
α-CH	5.25(1H, t, 11.4)	5.23 (1H, t, 11.2)	5.25 (1H, t, 11.4)	5.23 (1H, t, 11)
β-CH	2.27-2,33(1H, m)	2.33-2.26 (1H, m)	2.35-2.39 (1H, m)	2.40-2.34 (1H, m)
γ-CH_3	0.79-0.89(3H, m)	1.00-0.80 (3H,m）	0.79-0.89(3H, m)	1.00-0.80 (3H, m）
γ-CH_3	0.90-1.00 (3H, m)	1.00-0.80 (3H,m）	0.90-1.00 (3H, m)	1.00-0.80 (3H, m）
N-CH_3	3.27 (3H, s)	3.24 (3H, s)	3.34 (3H, s)	3.33 (3H, s)
Leu				
α-CH	4.78(1H, q, 5.8)	4.77 (1H, q, 6.0)	4.85 (1H, q, 7.8)	4.83 (1H, t, 7.6)
β-CH_2	1.72-1.85 (1H, m)	1.72-1.85 (1H, m)	1.72-1.85(1H, m)	1.85-1.72 (1H, m)
	1.09-1.39 (1H, m)	1.09-1.39 (1H, m)	1.09-1.39 (1H, m)	1.09-1.39 (1H, m)
γ-CH	1.50-1.70(1H, m)	1.50-1.70(1H, m)	1.50-1.70 (1H, m)	1.50-1.70 (1H, m)
δ-CH_3	0.90-1.00(3H, m)	1.00-0.80 (3H, m)	0.90-1.00(3H, m)	1.00-0.80 (3H, m）
δ-CH_3	0.90-1.00(3H, m)	1.00-0.80 (3H, m)	0.90-1.00(3H, m)	1.00-0.80 (3H, m）
NH	7.75(1H, d, 7.8)	7.72 (1H, d, 8.1)	7.98(1H, d, 8.6)	7.95 (1H, d, 8.5)
MeVal3				
α-CH	4.97(1H, d, 11.2)	4.96 (1H, d, 11.2)	3.36-3.40 (1H, m)	3.40-3.35 (1H, m)
β-CH	2.27-2.33(1H, m)	2.33-2.26 (1H, m)	2.27-2.33(1H, m)	2.34-2.27 (1H, m)
γ-CH_3	0.79-0.89 (3H, m)	1.00-0.80 (3H,m）	0.79-0.89(3H, m)	1.00-0.80 (3H,m）
γ-CH_3	0.90-1.00 (3H, m)	1.00-0.80 (3H,m）	0.90-1.00(3H, m)	1.00-0.80 (3H,m）
N-CH_3	2.88 (3H, s)	2.88 (3H, s)	2.82(3H, s)	2.88 (3H, s)

^{13}C NMR (126 MHz, $CDCl_3$)

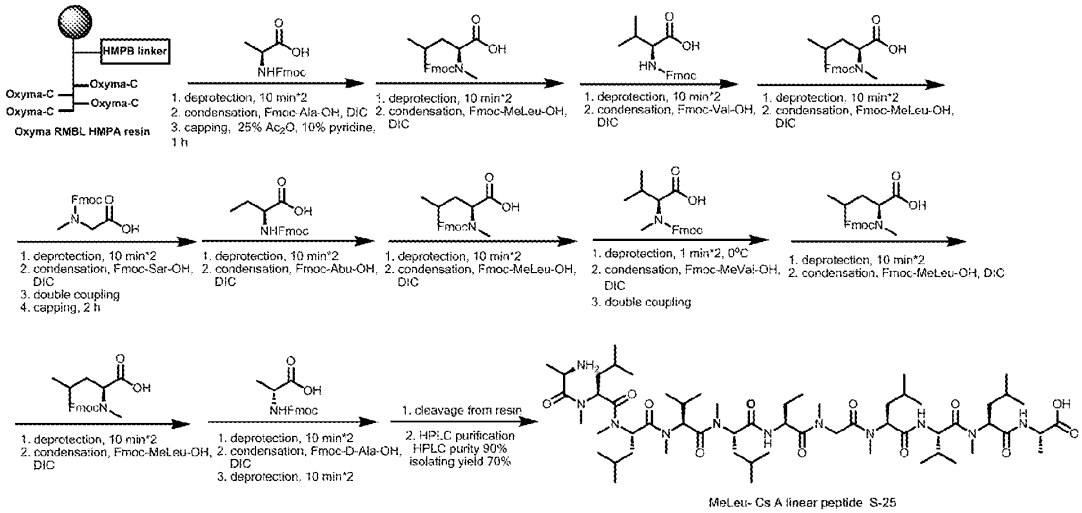
Literature Data	Our data	Literature Data	Our data	Literature Data	Our data
172.92	172.8	76.9	76.8	30.6	30.6
172.87	172.7	74.9	74.7	30.6	30.5
172.3	172.2	74.5	74.4	29.7	29.5
171.8	171.6	71	70.9	26.6	26.6
171.3	171.1	63.7	63.5	26.5	26.4

170.9	170.7	62.5	63.3	26.41	26.4
170.7	170.5	61.6	62.0	26.4	26.3
170.6	170.5	61.5	61.7	25.2	25.0
170.3	170.2	61.5	61.7	25	25.0
170.1	169.9	61.4	61.6	24.3	24.8
170	169.8	61.1	61.3	24.1	24.2
170	169.7	59.3	59.2	23.6	23.4
169.9	169.6	56.4	56.3	23.3	23.1
169.6	169.4	56.3	56.1	23.1	22.9
169.5	169.4	51.1	50.9	22.9	22.8
168.8	168.7	50.1	49.9	22.6	22.7
168.8	168.7	49.6	49.4	22.3	22.5
168.8	168.6	47.7	47.6	22.1	22.2
137.9	137.7	46.5	46.4	20.9	20.7
137.9	137.6	41.8	41.6	20.7	20.6
137.3	137.2	41.5	41.3	19.9	19.8
135.3	135.1	40.6	40.4	19.8	19.6
130.6	130.4	39.8	39.6	19.6	19.4
129.8	129.6	39.5	39.3	19.5	19.3
129.7	129.6	39.4	39.2	19.2	19.1
129.4	129.2	39.2	39.1	19.1	19.0
129.4	129.2	39.1	39.0	18.9	18.8
129	128.9	36.8	36.6	18.7	18.5
129	128.8	36.7	36.6	18.4	18.3
128.7	128.5	36.2	36.0	18.2	18.1
128	128.4	36.1	36.0	15.6	15.4
127.2	127.7	32.6	32.5	15.4	15.3
126.9	127.0	31.4	31.2	14.42	14.3
126.9	127.0	30.8	30.8	14.35	14.3
172.92	172.8	30.8	30.7	11.9	11.7
172.87	172.7	76.9	76.8	11.8	11.6
172.3	172.2	74.9	74.7	30.6	30.6

171.8	171.6	74.5	74.4	30.6	30.5
171.3	171.1	71	70.9	29.7	29.5

HRMS (ESI, m/z) calcd for $C_{59}H_{91}N_8O_{10}^+$: $[M+H]^+$: 1071.6853 found 1071.6855. IR ($CHCl_3$) 3311, 3258, 3031, 2962, 2872, 1728, 1671, 1628, 1527, 1489, 1459, 1411, 1271, 1202, 1123, 1081, 1037, 917, 868, 731, 699, 648, 605, 546, 511, 482 cm^{-1}. $[\alpha]_D^{25}$ –174 (c 0.3, $CHCl_3$).

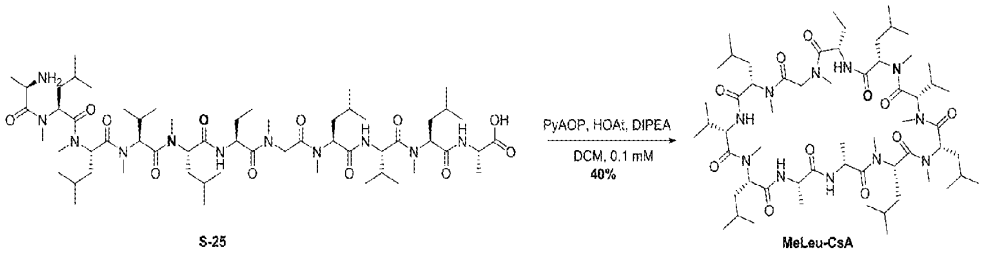
Embodiment 28:



Oxyma RMBL HMPA resin (50 mg) is swelled with a mixture of DCM:DMF=1:1 (5 mL) for 30 min. The solvent is filtered and the resin is washed with DMF for 6 times. Removal of Fmoc groups is carried out by treatment with 20% piperidine in DMF (5 mL) for 10 min (x2). The resin is then washed for 6 times with DMF. Fmoc-Ala-OH is added to the vessel followed by addition of DMF (1 mL) and DIC, and concentration of Fmoc-Ala-OH and DIC should be set as 0.2 M. The reaction is carried out at room temperature for 2 h. When double coupling is required, same amount of reagents are added to the drained resin and the reaction is allowed to proceed for 4 h. The solution is filtered and the resin is washed with DMF for 6 times. The resulting resin is treated with 20% piperidine in DMF (5 mL) for 10 min (x2) to remove Fmoc groups. The solution is filtered and the resin is washed with DMF for 6 times. The condensation and deprotetion cycle is repeated to realize connection of Fmoc-MeLeu-OH, Fmoc-Val-OH, Fmoc-MeLeu-OH, Fmoc-Sar-OH, Fmoc-Abu-OH

and Fmoc-MeLeu-OH. Specially, the resin should be treated with a mixture of Ac_2O: pyridine: DMF=2.5:1:6.5 (v/v/v, 1 mL) to cap unreacted HMPA linkers after condensation of Fmoc-Pro-OH. The drained resin is treated with 20% piperidine at 0 ℃ for 1 min (x2), and is then washed with DMF 6 times. Then, connection of Fmoc-MeVal-OH, Fmoc-MeLeu-OH,Fmoc-MeLeu-OH, Fmoc-D-Ala-OH is carried out following the aforementioned cycle. The resulting resin is shrunk with DCM and Et_2O. Final peptide cleavage from the resin is carried out by treatment with a cleavage cocktail of TFA: DCM=1: 99 (v/v/v, 1 mL) for 2 min (x8) at room temperature, followed by concentration under vacuum to yield crude MeLeu-Cs A. The crude product is dissolved in MeOH and is subjected to HPLC purification (C18) to afford pure target peptide. Purity of the crude product is 90%. The elution solvent system is 1% TFA in H_2O (phase A) and MeCN (phase B), and the product is eluted at 23 min (80% MeCN). After concentration and lyophilization, the linear peptide **S-24** is obtained as a pure product (11 mg, 70%).

Embodiment 29:



S-25 MeLeu-CsA

PyAOP (6 mg, 0.01 mmol, 2 eq.) is dissolved in 10 mL anhydrous DCM under N_2 atmosphere. DIPEA (3 μL, 0.013 mmol, 2 eq.) is then added dropwise. The linear peptide **S-25** (6 mg, 0.0052 mmol, 1 eq.) is dissolved in 15 mL anhydrous DCM and is added to the system using a syringe pump at a rate of 3 mL/h. The reaction is allowed to proceed at room temperature for 12 hours. The solution is concentrated and the crude product is dissolved in methanol and directly subjected to HPLC purification to afford MeLeu-CsA as a white solid (2.2 mg, 40%).

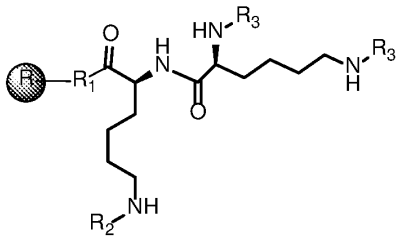
^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.46 (d, *J* = 9.8 Hz, 1H), 8.05 (d, *J* = 6.9 Hz, 1H), 7.50 (t, *J* = 7.9 Hz, 2H), 5.69 (dd, *J* = 11.2, 4.2 Hz, 1H), 5.34 (dd, *J* = 11.6, 3.9

Hz, 1H), 5.19 (dd, *J* = 10.8, 5.0 Hz, 1H), 5.16 – 5.07 (m, 3H), 4.94 (td, *J* = 9.3, 5.7 Hz, 1H), 4.89 – 4.81 (m, 1H), 4.75 – 4.65 (m, 2H), 4.42 (p, *J* = 7.1 Hz, 1H), 3.41 (s, 3H), 3.36 (s, 3H), 3.27 (s, 3H), 3.19 (s, 3H), 3.16 (d, *J* = 13.7 Hz, 1H), 3.09 (s, 3H), 2.68 (d, *J* = 0.9 Hz, 6H), 2.43 (m, 1H), 2.20-2.08 (m, 3H), 2.07 – 1.91 (m, 2H), 1.84 – 1.74 (m, 1H), 1.73 – 1.52 (m, 5H), 1.45 (m, 2H), 1.37 – 1.32 (m, 5H), 1.28 – 1.24 (m, 5H), 1.22 – 1.18 (m, 1H), 1.08 – 0.99 (m, 9H), 0.94 (dd, *J* = 6.7, 4.6 Hz, 6H), 0.87 (m, 24H), 0.73 (d, *J* = 6.5 Hz, 3H), 0.69 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.15, 173.87, 173.51, 173.22, 172.12, 171.93, 171.61, 171.27, 171.11, 171.02, 170.55, 77.58, 77.37, 77.16, 58.71, 57.51, 55.67, 55.44, 55.31, 54.45, 50.22, 49.02, 48.63, 48.21, 44.98, 42.67, 40.99, 39.68, 39.61, 39.25, 37.85, 36.52, 32.11, 31.74, 31.57, 31.54, 30.33, 30.21, 30.13, 30.01, 25.24, 25.09, 24.85, 24.78, 24.66, 24.21, 24.18, 24.15, 24.06, 23.85, 22.48, 22.24, 21.59, 21.35, 20.72, 20.05, 18.86, 18.63, 18.08, 15.42, 11.49, 10.27. HRMS (ESI, m/z) calcd for $C_{59}H_{107}N_{11}O_{11}^+$ $[M+H]^+$: 1146.8224 found 1146.8220. IR (film, cm^{-1}): 3320, 3057, 2960, 2926, 2871, 1738, 1681, 1626, 1536, 1470, 1412, 1383, 1300, 1202, 1207, 1172, 1131, 1094, 1051, 952, 920, 798, 727, 648, 555. $[\alpha]_D^{25}$ –214 (c 0.2, $CHCl_3$).

It should be noted that the aforementioned examples are preferred embodiments of the present invention and are not intended to limit the scope of protection of the invention. Any equivalent substitutions or alternatives made on the basis of the above are also within the scope of protection of the invention.

权 利 要 求

1、 An RMBL resin, characterized in that, its structure is as shown in Formula (I):



Formula (I)

Wherein,  is connected with H to form an amine methyl (AM) resin or a cleavable peptide synthesis resin;

R_1 is none;

or a short PEG chain with a carboxyl group at the C-terminus which can condense with  and an amino group at the N-terminus which can subsequently condense with the carboxyl group of the backbone lysine;

or a short peptide with a carboxyl group at the C-terminus which can condense with  and an amino group at the N-terminus which can subsequently condense with the carboxyl group of the backbone lysine;

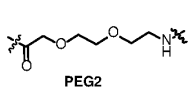
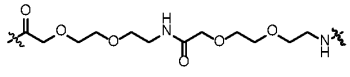
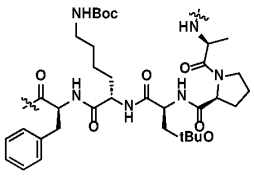
R_2 is a peptide synthesis handle;

R_3 is a fragment with an activator analog, with a carboxyl group at the C-terminus connected to OH, which can condense with the amino group of the backbone Lys, and β-alanine can be used as a spacer inserted between the amide bonds.

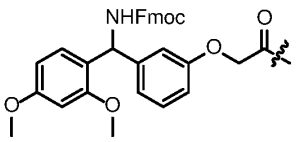
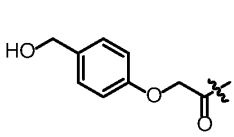
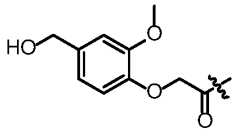
2、 The RMBL resin as claimed in claim 1, characterized in that,  connected with H is selected from: AM resin, AM PEGA resin, AM PEGMatrix resin.

3、 The RMBL resin as claimed in claim 1, characterized in that,  connected with H is selected from: Rink resin, HMPA resin, HMPB resin.

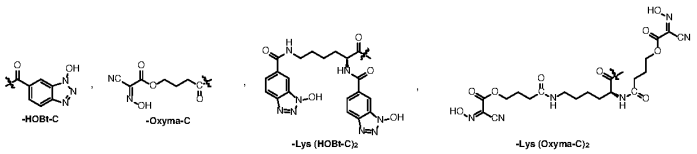
4、 The RMBL resin as claimed in claim 1, characterized in that, the R_1 is selected from:

none ,  PEG2 ,  PEG2-PEG2 ,  APSKF (containing side chains) .

5、The RMBL resin as claimed in claim 1, characterized in that, the R_2 is selected from:

 Rink linker ,  HMPA linker ,  HMPB linker .

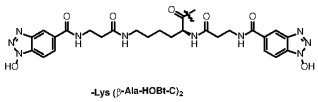
6、The RMBL resin as claimed in claim 1, characterized in that, the R_3 is selected from:

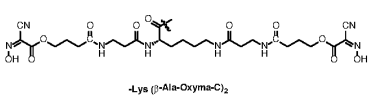

-HOBt-C

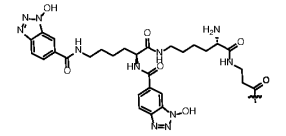
-Oxyma-C

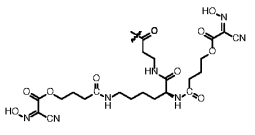
-Lys (HOBt-C)$_2$

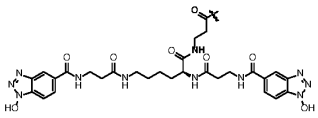
-Lys (Oxyma-C)$_2$

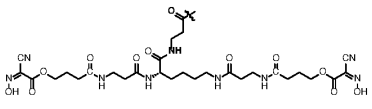

-Lys (β-Ala-HOBt-C)$_2$

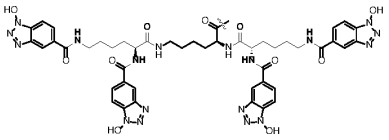

-Lys (β-Ala-Oxyma-C)$_2$

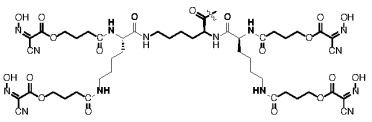

β-Ala-Lys (HOBt-C)$_2$

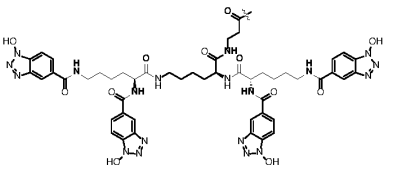

β-Ala-Lys (Oxyma-C)$_2$

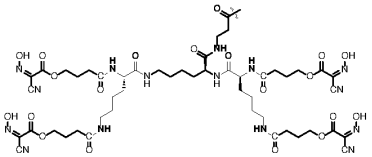

β-Ala-Lys (β-Ala-HOBt-C)$_2$

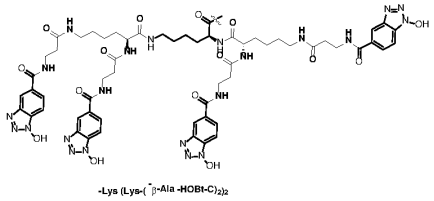

β-Ala-Lys (β-Ala-Oxyma-C)$_2$

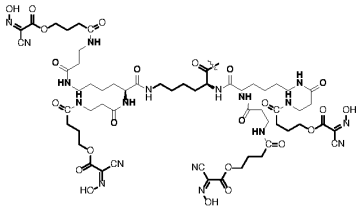

-Lys (Lys-(-HOBt-C)$_2$)$_2$

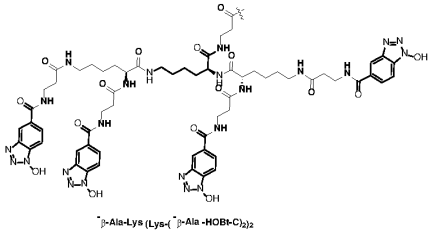

-Lys (Lys-(-Oxyma-C)$_2$)$_2$

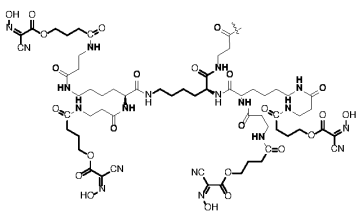

β-Ala-Lys (Lys-(-HOBt-C)$_2$)$_2$


β-Ala-Lys (Lys-(-Oxyma-C)$_2$)$_2$


-Lys (Lys-(β-Ala -HOBt-C)$_2$)$_2$

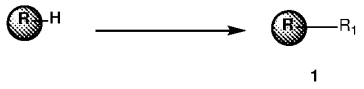

-Lys (Lys-(β-Ala -Oxyma-C)$_2$)$_2$


β-Ala-Lys (Lys-(β-Ala -HOBt-C)$_2$)$_2$


β-Ala-Lys (Lys-(β-Ala -Oxyma-C)$_2$)$_2$

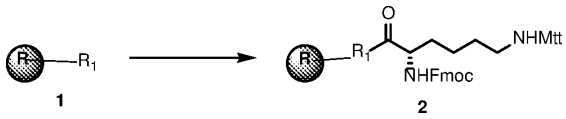
7、 A preparation method for the RMBL resin as claimed in claims 1-6, characterized in that, the synthesis route is as follows:

（1）



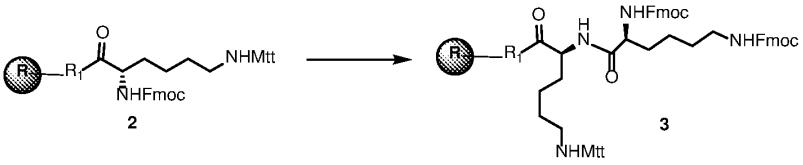
1

（2）



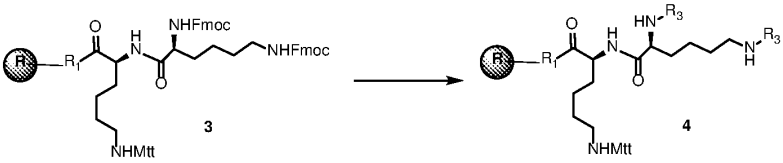
1 2

（3）



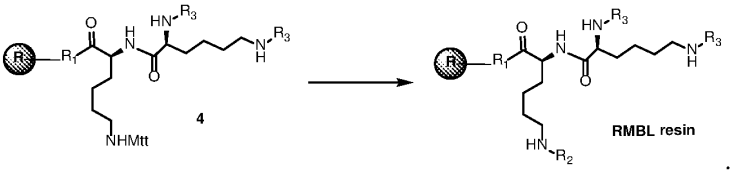
2 3

（4）



3 4

（5）



4 RMBL resin

8、 The preparation method as claimed in claim 7, characterized in that, the specific synthesis steps are:

(1) Starting with  resin as the initial raw material, condense R_1 to obtain compound **1**;

(2) After removing the N-9-fluorenylmethoxycarbonyl protecting group from compound **1**, condense N-9-fluorenylmethoxycarbonyl-N'-methyltrityl-L-lysine to obtain compound **2**;

(3) After removing the N-9-fluorenylmethoxycarbonyl protecting group from

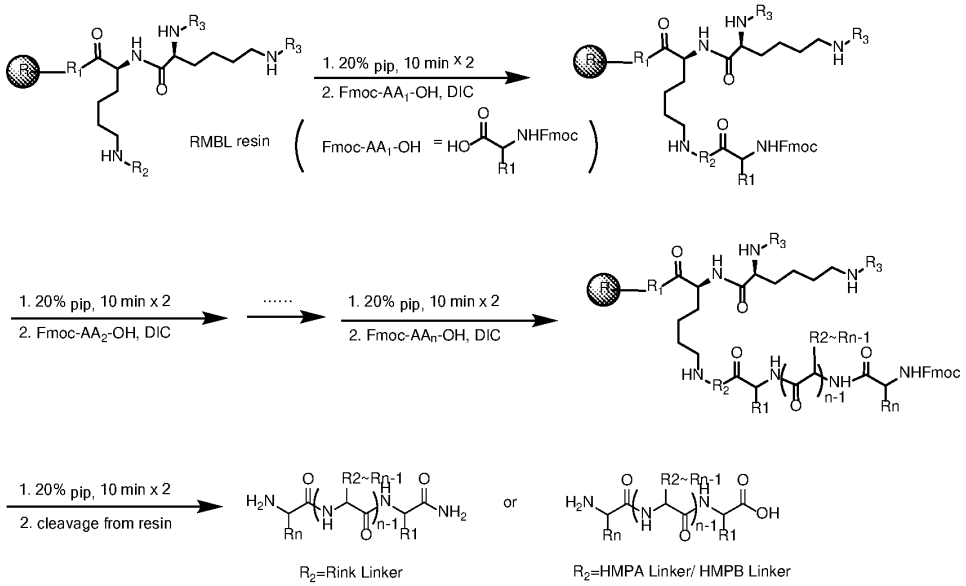
compound **2**, condense N,N'-9-fluorenylmethoxycarbonyl-L-lysine to obtain compound **3**;

(4) After removing the N-9-fluorenylmethoxycarbonyl protecting group from compound **3**, condense each amide bond between R_3 from the C-terminal to obtain compound **4**;

(5) After removing the N-methyltrityl protecting group from compound 4, condense R_2 to obtain the RMBL resin.

9、The application of the RMBL resin as claimed in claims 1-6 in solid-phase peptide synthesis.

10、The application as claimed in claim 9, characterized in that, the route for synthesizing peptides with the RMBL resin is:



Wherein, n represents the number of amino acids in the synthesized peptide sequence, n>1; R_{n-1} refers to the R group of the side chain of the $(n-1)^{th}$ amino acid, and R_n refers to the R group of the side chain of the n^{th} amino acid.