Abbreviations: CTC, 2-chlorotrityl chloride ; DIPEA, *N*,*N*-diisopropylethylamine; DMF, dimethylformamide; ESI-MS, electrospray ionization mass spectrometry; Fmoc, Fluorenylmethyloxycarbonyl; HATU, (1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate); HOBt, 1-Hydroxybenzo-triazole; HPLC, high performance liquid chromatography; MeOH, methanol; NMM, *N*-Methylmorpholine; PyBroP, bromotripyrrolidinophosphonium hexafluorophosphate; SPPS, solid phase peptide synthesis, TFA, trifluoroacetic acid; UPLC, ultra-high performance liquid chromatography; UV, ultra-violet.

Synthesis of peptides: Peptides were synthesized in glass vessels using standard Fmoc-based solid phase peptide synthesis (SPPS) techniques.

HPLC purification: Method A: The crude peptide was purified using a Waters 4000 system connected to a Waters Delta-Pak™ C18, 15 micron, 100 Å reverse-phase HPLC column (25 × 200mm) eluting with a solvent gradient of A:B (21:79 to 4:96) over 60 minutes at a flow rate of 20 mL/min (solvent A: 0.1% TFA in water; solvent B: 0.1% TFA in 80% acetonitrile and 20% water). The desired product fractions were combined, and the peptide was repurified using the same HPLC system except with a gradient of A:B (27:73 to 7:93) over 60 minutes at a flow rate of 20 mL/min.

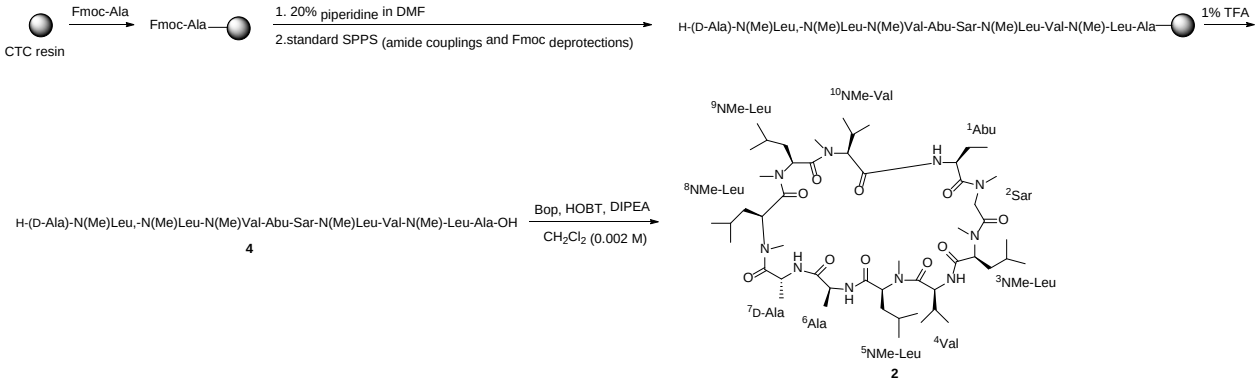
Method B: The crude peptide was purified using a Waters 4000 system connected to a Phenomenex Luna™ C18, 10 micron, 100 Å reversed-phase HPLC column (25 × 200mm) eluting with a solvent gradient of A:B (55:45 to 10:90) over 60 minutes at a flow rate of 20 mL/min (solvent A: 0.1% TFA in water; solvent B: 0.1% TFA in 80% acetonitrile and 20% water). The desired product fractions were combined, and the peptide was repurified using the same HPLC system except with a gradient of A:B (53:47 to 20:80) over 60 minutes at a flow rate of 20 mL/min.

HPLC purity of peptides: Pure peptides were analyzed using a HP1090 system with a 4.6 x 150 mm Phenomenex C18 (2), 5 micron 100 Å (4.6x150 mm) column eluting with a solvent gradient A:C, where solvent A: 0.1% TFA in water and solvent C: 0.09% TFA in acetonitrile:water (4:1), over 20 minutes at a flow rate of 1.0 mL/min. The specific retention time, UV purity (220 nm), and solvent gradient are described for peptides **2** and **3**.

High resolution mass spectrometry: Accurate Mass Spectrometry analyses were conducted on an Agilent 6220 TOF mass spectrometer (Agilent Technologies, Wilmington, DE) in positive or negative electrospray mode. The system was calibrated to greater than 1 ppm accuracy across the mass range prior to analyses according to manufacturer's specifications. The samples were separated using UHPLC on an Agilent 1200 (Agilent Technologies, Wilmington, DE) system prior to mass spectrometric analysis. The resulting spectra were automatically lock mass corrected and the target mass ions and any confirming adducts (Na^+, NH_4^+) were extracted and combined as a chromatogram. The mass accuracy was calculated for all observed isotopes against the theoretical mass ions derived from the chemical formula using MassHunter software (Agilent Technologies, Wilmington, DE).

Notes: We thank James Xu and Villa Zheng (Chinese Peptide Company) for peptide synthesis support.

Synthesis of peptide **2**



Synthesis of the Fmoc-Ala-CT resin

The CTC resin (10 g, 11 mmol) was suspended in dichloromethane (~ 50 mL). To the suspension was added Fmoc-Ala-OH (2.18 g, 7 mmol) and DIPEA (8.73 mL, 50 mmol). The mixture was stirred gently at rt for 3 h. To the mixture was then added MeOH (10 mL), and resulting mixture was stirred at rt for 0.5 h. Reaction mixture was filtered, and the solid was washed sequentially with DMF (3 x 100 mL), dichloromethane (3 x 100 mL) and MeOH (3 x 100 mL). The resulting washed solid was dried under vacuum overnight to furnish 13 g of Fmoc-Ala-CT resin. Loading level of the Fmoc-Ala-CT resin was established to be 0.3 mmol/g

via standard UV absorption method upon cleavage of small aliquots of the product resin (Shimadzu UV-1601, wavelength = 289.5 nm).

Note: The CTC resin was purchased from Tianjin Nankai Hecheng Science & Technology Co., Ltd with the following specifications: 1.1mmol/g loading level, 100-200 mesh size, 1% DVB (divinylbenzene) cross linking.

Amide couplings and Fmoc-deprotections

Fmoc-Ala-CT resin (0.5 mmol, 1.67 g) was swelled in DMF (30 mL) for 2 h, then 20% piperidine in DMF (30 mL) was added. The mixture was kept at rt for 0.5 h while a stream of nitrogen was bubbled through it. The mixture was filtered, and the peptidyl resin was washed with DMF (5×30 mL) to give H-Ala-CTC resin. Fmoc-N(Me)Leu-OH (0.366 g, 1 mmol), HATU (0.36 g, 0.633 mmol) and DMF (5 mL) were added followed by NMM (0.22 mL, 2 mmol). The suspension was kept at rt for 50 min while a stream of nitrogen was bubbled through it. The Kaiser ninhydrin test was used to indicate reaction completion. Upon completion, the peptidyl resin was washed with DMF (3×30 mL). 20% piperidine in DMF (30 mL) was added into the resin. After 0.5 h at rt, the suspension was filtered, and the resin was washed with DMF. The following amino acids were sequentially coupled to the resin bound peptide followed by Fmoc deprotection: Fmoc-^{4}Val, Fmoc-^{3}N(Me)Leu, Fmoc-^{2}Sar, Fmoc-^{1}Abu, Fmoc-^{10}N(Me)Val, Fmoc-^{9}N(Me)Leu, Fmoc-^{8}NMe Leu, and Fmoc-^{7}DAla. In several cases, the initial amide coupling did not proceed to completion, so two or three coupling iterations were necessary. Once the coupling was deemed complete, Fmoc deprotection with 20% piperidine (in DMF) was performed. The table below summarizes the amidation conditions for each Fmoc-amino acid.

Fmoc-amino acid	Coupling agent	Base	solvent	Reaction time (h)
^{4}Val (3 mmol)	HATU	NMM	DMF	1
^{4}Val (3 mmol)	HATU	NMM	DMF	1
^{3}N(Me)Leu (1 mmol)	HATU	NMM	DMF	1
^{2}Sar (3 mmol)	HATU	NMM	DMF	1
^{2}Sar (3 mmol)	PyBroP	DIPEA	THF	2
^{1}Abu (3 mmol)	HATU	NMM	DMF	1

[1]Abu (3 mmol)	PyBroP	DIPEA	THF	2
[1]Abu (3 mmol)	PyBroP	DIPEA	THF	2
[10]N(Me)Val (1 mmol)	HATU	NMM	DMF	1
[9]N(Me)Leu (3 mmol)	HATU	NMM	DMF	1
[9]N(Me)Leu (3 mmol)	PyBroP	DIPEA	THF	2
[8]N(Me)Leu (3 mmol)	HATU	NMM	DMF	1
[8]N(Me)Leu (3 mmol)	PyBroP	DIPEA	THF	2
[7]D-Ala (3 mmol)	HATU	NMM	DMF	1
[7]D-Ala (3 mmol)	PyBrop	DIPEA	THF	2

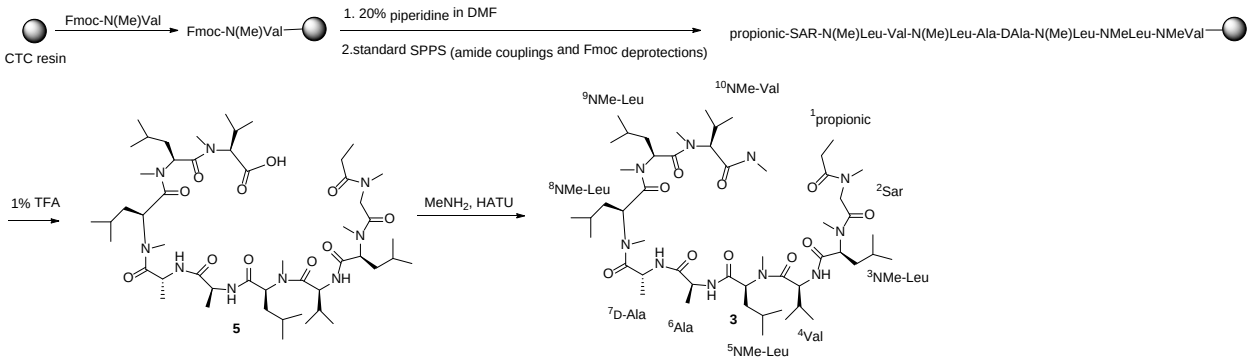
coupling conditions: Fmoc-amino acid:HATU:NMM (1:0.95:1), Fmoc-amino acid:Pyrop:DIPEA (1:1:1.7).

amidations involving Fmoc-[3]N(Me)Leu and Fmoc-[10]N(Me)Val required only one coupling round.

*Peptide cleavage, final synthesis steps, and purification of peptide **2***

After the last amide coupling and subsequent Fmoc deprotection, the peptidyl resin was washed with methanol (2 x 30 mL), dichloromethane (2 x 30 mL), and methanol (2 x 30 mL). The peptidyl resin was then dried under vacuum overnight. 1% TFA in dichloromethane (35 mL) was added to the resin, and the mixture was shaken for 30 min and then filtered. The solution was neutralized to pH 7 with DIPEA, and this neutralized solution was concentrated under reduced pressure to deliver crude peptide **4**. Crude peptide **4** was dissolved in dichloromethane (250 mL). To the resulting solution was added Bop (0.663 g, 1.5 mmol), DIPEA (0.322 mL, 2.5 mmol), and HOBT (0.203 g, 1.5 mmol). The reaction mixture was stirred overnight. Dichloromethane was removed under reduced pressure to afford crude peptide **2**. The crude peptide was purified using reverse phase HPLC Method A. The combined desired fractions were then lyophilized to afford peptide **2** (56.5 mg, 10.7% based on Fmoc-Ala-CT resin). UV purity (220 nm) = 95.8% (retention time = 10.79 min, solvent gradient A:C, 18:82 to 8:92); HRMS (ESI) *m/z* calc'd for $C_{52}H_{94}N_{10}O_{10}Na$:1041.7047, found 1041.7042.

Synthesis of peptide **3**



Synthesis of the Fmoc-NMeVal-CT resin

The CTC resin (2 g, 2.0 mmol) was suspended in dichloromethane (~ 50 mL). To the suspension was added Fmoc-NMe-Val-OH (0.7 g, 2 mmol) and DIPEA (1.75 mL, 10 mmol). The mixture was shaken at room temperature for 3 h. To the mixture was then added MeOH (2 mL), and the resulting mixture was stirred at rt for 0.5 h. Reaction mixture was filtered, and the solid was washed sequentially with DMF (3 x 20 mL), dichloromethane (3 x 20 mL) and MeOH (3 x 20 mL). The washed solid was dried under vacuum overnight to furnish 2.5 g of Fmoc-N(Me)-Val-CT resin. Loading level of the Fmoc-N(Me)-Val-CT resin was established to be 0.36 mmol/g via standard UV absorption method upon cleavage of small aliquots of the product resin (Shimadzu UV-1601, wavelength = 289.5 nm).

Note: The CTC resin was purchased from Tianjin Nankai Hecheng Science & Technology Co., Ltd with the following specifications: 1.1mmol/g loading level, 100-200 mesh size, 1% DVB (divinylbenzene) cross linking.

Amide couplings and Fmoc-deprotections

Fmoc-N(Me)Val-CT resin (0.6 mmol, 1.666 g) was swelled in DMF (30 mL) for 2 h, then 20% piperidine in DMF (30 mL) was added. The mixture was kept at rt for 0.5 h while a stream of nitrogen was bubbled through it. The mixture was filtered, and the peptidyl resin was washed with DMF (5×30 mL) to give H-N(Me)Val-CTC resin. PyBroP (0.803 g, 1.8 mmol), DIPEA (0.627 mL, 3.6 mmol) and THF (6 mL) were added. After 10 min, Fmoc-NMeLeu-OH (0.662 g, 1.8 mmol) was added to the mixture. The suspension was kept at rt for 2 h an agitated with a stream of nitrogen. Additional amounts of PyBroP (1.8 mmol) and DIPEA (3.6 mmol) were added to the mixture. After an additional 2 h, the coupling went to completion (based on the Kaiser ninhydrin test). The peptidyl resin was washed with DMF (3×30 mL), and 20% piperidine in DMF (30 mL) was added into the resin. After 0.5 h at rt, the suspension was filtered, and the resin was washed with DMF. The following amino acids were coupled sequentially to the resin bound peptide followed by Fmoc deprotection: Fmoc-^{8}N(Me)Leu, Fmoc-^{7}D-Ala, Fmoc-^{6}Ala, Fmoc-^{5}N(Me)-Leu, Fmoc-^{4}Val, Fmoc-^{3}N(Me)Leu, Fmoc-^{2}Sar, and ^{1}propionic acid. In several cases, the initial amide coupling did not proceed to completion, so two or three coupling iterations were necessary. Once the coupling was deemed complete, Fmoc

deprotection with 20% piperidine (in DMF) was performed. The table below summarizes the amidation conditions for each Fmoc-amino acid.

Fmoc-amino acid	Coupling agent	Base	solvent	Reaction time (h)
^{8}N(Me)Leu (1.1 mmol)	PyBroP	DIPEA	THF	2
^{8}N(Me)Leu (1.1 mmol)	PyBroP	DIPEA	THF	2
^{7}D-Ala (1.1 mmol)	PyBroP	DIPEA	THF	2
^{7}D-Ala (1.1 mmol)	PyBroP	DIPEA	THF	2
^{6}Ala (3.2 mmol)	HATU	NMM	DMF	1
^{5}N(Me)Leu (1.2 mmol)	HATU	NMM	DMF	1
^{4}Val (1.2 mmol)	PyBroP	DIPEA	THF	2
^{4}Val (1.2 mmol)	PyBroP	DIPEA	THF	2
^{3}N(Me)Leu (0.7 mmol)	HATU	NMM	DMF	1
^{2}Sar (1.1 mmol)	PyBroP	DIPEA	THF	2
^{2}Sar (1.1 mmol)	PyBroP	DIPEA	THF	2
propionic acid (2.2 mmol)	HATU	NMM	DMF	1

coupling conditions: Fmoc-amino acid:HATU:NMM (1:0.95:1), Fmoc-amino acid:PyroP:DIPEA (1:1:1.7).

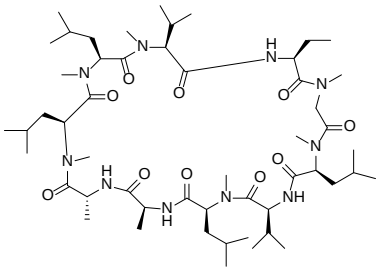
Note: The amidations involving Fmoc-^{6}Ala, Fmoc-^{5}N(Me)-Leu, Fmoc-^{3}N(Me)Leu, and propionic acid required only one coupling round. Fmoc-deprotection with 20%piperidine in DMF then occurred except in the case with propionic acid.

*Peptide cleavage, final synthesis steps, and purification of peptide **3***

After the last amide coupling with propionic acid, the peptidyl resin was washed with methanol (2 x 30 mL), dichloromethane (2 x 30 mL), and methanol (2 x 30 mL). The peptidyl resin was then dried under vacuum overnight. 1% TFA in dichloromethane (40 mL) was added to the resin, and the mixture was shaken for 60 min and then filtered. The solution was neutralized to pH 7 with DIPEA, and this neutralized solution was concentrated under reduced pressure to deliver crude peptide **5**. It was then purified by reverse phase HPLC, and the desired fractions were combined and lyophilized to deliver **5** (236 mg). DMF (2 mL) was added to peptide **5** followed by methylamine hydrochloride (31 mg, 0.46 mmol), HATU (88 mg, 0.23 mmol), and NMM (0.152 mL, 1.38 mmol). The reaction mixture was then shaken for 60 min. The mixture was then concentrated under reduced pressure to give crude peptide **3.** The crude peptide was purified via reverse phase HPLC Method B to deliver peptide **3** (21 mg, 3.3% based on the Fmoc-N(Me)Val-

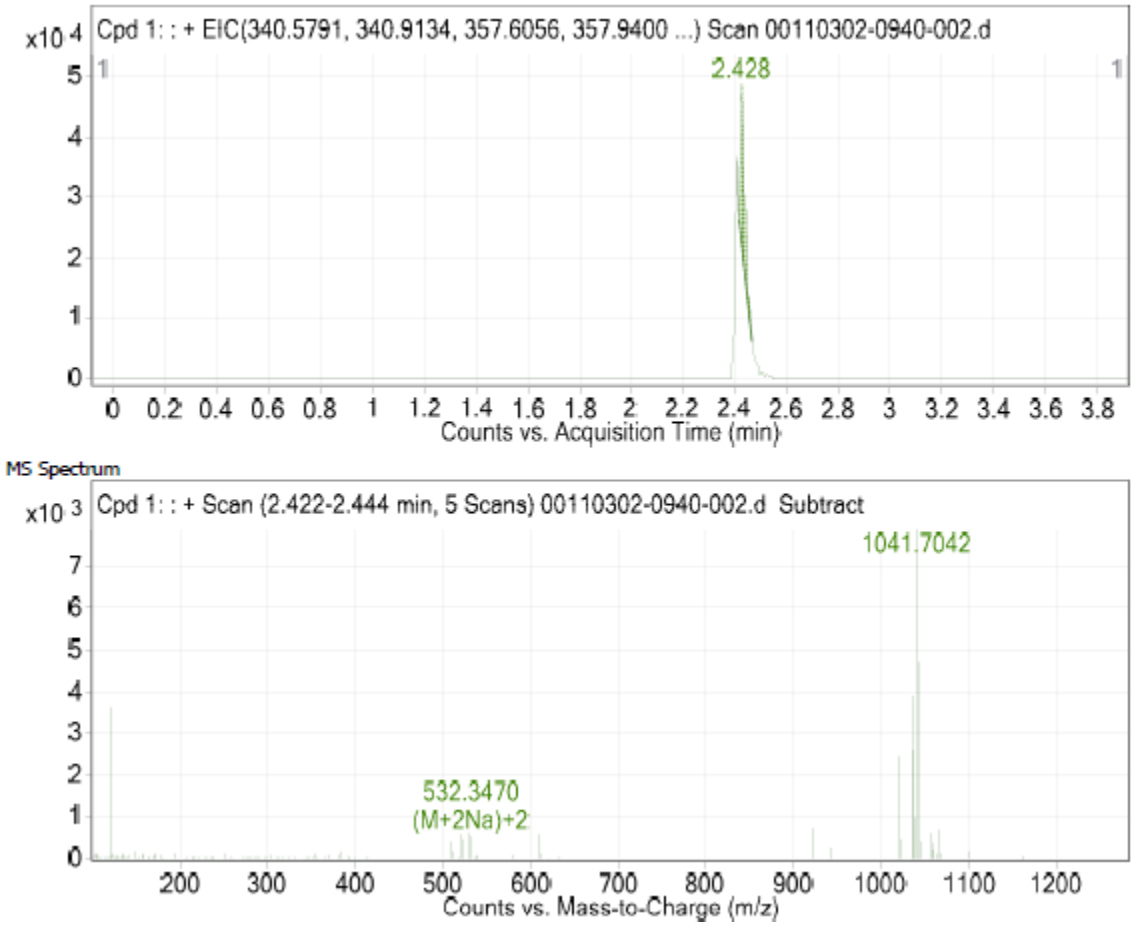
CT resin). UV purity (220 nm) = 95.3% (retention time = 9.88 min, solvent gradient A:C, 30:70 to 20:80); HRMS (ESI) *m/z* calc'd for $C_{52}H_{96}N_{10}O_{10}Na$:1043.7203, found 1043.7200.

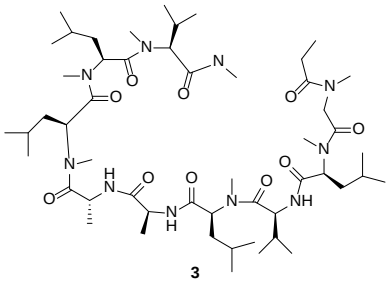
uPLC/HRMS traces



2

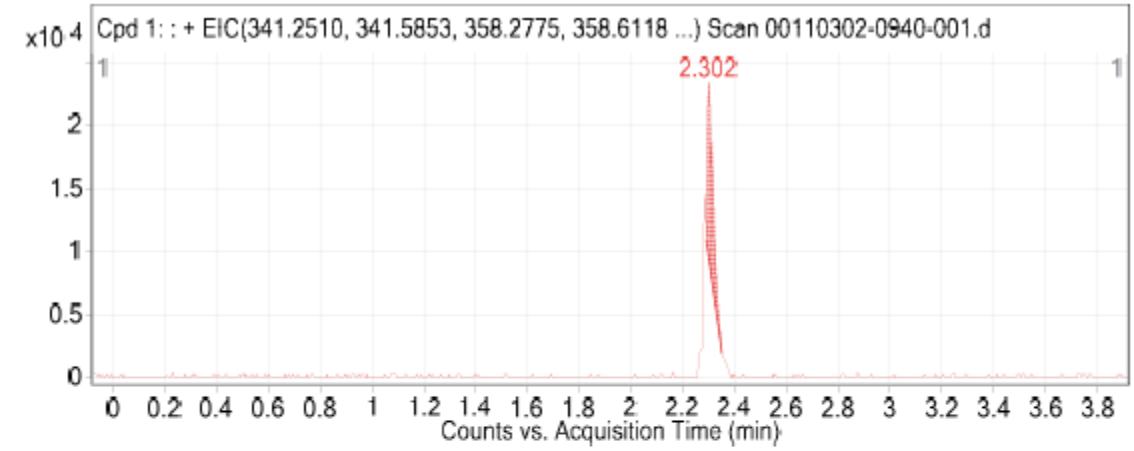
HRMS (ESI) *m/z* calc'd for $C_{52}H_{94}N_{10}O_{10}Na$:1041.7047, found 1041.7042





3

HRMS (ESI) *m/z* calc'd for $C_{52}H_{96}N_{10}O_{10}Na$:1043.7203, found 1043.7200



MS Spectrum