SUPPORTING INFORMATION

Convenient Synthesis of Thioamidated Peptides and Proteins

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Materials:

Fmoc- and orthogonally protected amino acids, 1-hydroxybenzotriazole (HOBt), O-(6-chlorobenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HCTU), 2-Chlorotriethylchloride polystyrene (2Cl-TCP) were purchased from GL Biochem, Shanghai. 4-Nitro-*o*-phenylenediamine, Lawesson's reagent, sodium nitrite, *N,N'*-Diisopropylcarbodiimide (DIC), *N,N*-diisopropylethylamine (DIPEA), trifluoroacetic acid (TFA), trifluoroethanol (TFE), triisopropylsilane (TIPS), dimethylformamide (DMF), dichloromethane (DCM), ethyl acetate (EtOAc), hydrochloric acid (HCl), sodium bicarbonate (NaHCO₃), sodium chloride (NaCl), sodium sulfate (Na₂SO₄), anhydrous tetrahydrofuran (THF), 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU), piperazine, glacial acetic acid, calcium hydride and piperidine were purchased from Sigma-Aldrich. All the above reagents were used as commercially supplied. Solvents for RP-HPLC were purchased as HPLC grade and used without further purification. Dichloromethane was dried with calcium hydride. All the other solvents were used as commercially supplied. All the reactions were performed in oven-dried glass apparatus. Reactions on solid-support were carried out in plastic syringes (10 ml) fitted with a frit column plate purchased from Torvix (USA).

Instrumentation:

High-resolution mass spectra were recorded on a Bruker Daltonics ESI Q-TOF (Maxis Impact) mass spectrometer. ESI mass spectra were recorded in positive ion mode on a HCTultra ETD II ion-trap spectrometer (Bruker Daltonics, Germany). Nuclear magnetic resonance (NMR) spectra were recorded in 500 MHz Bruker Avance spectrometer (Bruker, Karlsruhe, Germany). Circular Dichroism (CD) spectra were acquired on a JASCO-715 spectropolarimeter using 0.1 cm path-length cuvette. The CD

spectra were averaged over 3 scans and the baseline correction was done by subtraction of the spectrum with the appropriate blank solution. Analytical RP-HPLC was performed on a Shimadzu UFLC system equipped with Prominence Diode Array (PDA) detector using an analytical column (Phenomenex C18, 250 mm x 4.6 mm I.D., 5 μ m) at a flow rate of 1 mL min⁻¹. Purifications were performed using a semi-preparative column (Phenomenex C18, 250 mm x 10 mm I.D., 5 μ m) at a flow rate of 4 mL min⁻¹.

Methods:

Peptide synthesis

Peptides **4** to **10** were synthesized on 2Cl-TCP resin; **11** and **12** were synthesized on Rink Amide AM resin by using the standard Fmoc-based chemistry. All the peptides were synthesized manually. The C-terminal amino acid residue, Fmoc-Xaa-OH (1.25 equiv) was loaded on to the resin with 2.5 equiv DIPEA in anhydrous DCM at room temperature. After loading the first amino acid, the remaining unreacted trityl chloride groups bound to the solid-support were capped using methanol (200 μ l/100 mg resin) for 15 min. Next, the resin was thoroughly washed with DCM (3 times), 1:1 DCM-methanol (3 times) and methanol (3 times) and finally dried under vacuum. The loading capacity was estimated from the dry weight of the resin, which ranged from 0.4-0.6 mmol g⁻¹. The elongation of the rest of the peptide was performed on 150 mg (0.09-0.12 mmol) scale with DIC/HOBt as the coupling agents (2.5 equiv). Fmoc-deprotections for **4**, **6**, and **7** were carried out with 10% piperidine in DMF (60 s x 2) and in using 2% DBU + 5% piperazine in DMF for **9** and **10**.

Cleavage from the resin and global deprotection

Peptides **5a-5t** were cleaved-off from the resin under mild condition with the cleavage cocktail AcOH:TFE:DCM (3:1:6) for 1 h. The syringe was thoroughly washed with dichloromethane and the combined extracts were concentrated in vacuo. The residual acetic acid present in the mixture was co-evaporated with toluene to yield a light yellow powder. The light yellow mass was dried and dissolved in ACN: H₂O (1:1) and the crude purity was checked by RP-HPLC (solvent A: 0.1% TFA in water, solvent B: 0.1% TFA in acetonitrile, gradient 50-100% B solvent over 20 minutes).

The global deprotection of protected peptides **6-10** was carried out with TFA:DCM:TIPS:H₂O (62:31:3.5:3.5) for 35 min. Deprotected peptide was precipitated in chilled ether yielding the crude product and further it was purified by RP-HPLC (solvent A: 0.1% TFA in water, solvent B: 0.1% TFA in acetonitrile, gradient 15-45% B solvent over 20 minutes).

The global deprotection of protected proteins **11-12** was carried out with TFA: DCM: TIPS: H₂O (62:31:3.5:3.5) for 2 h. Deprotected crude protein was precipitated in chilled ether. The white solid was pelleted by centrifugation and dissolved in 7 M Guanidine HCl for purification using RP-HPLC (solvent A: 0.1% TFA in water, solvent B: 0.1% TFA in acetonitrile, gradient 20-50% B solvent over 40 minutes).

NMR Acquisition

The NMR spectra of **2c**, **2e'**, **2f**, **2i**, **2i'**, **2m**, **2q**, **2r**, **6**, and **7** were recorded in DMSO-*d*₆. **9** and **10** were dissolved in 20 mM sodium phosphate buffer (pH 7.4) with 10% D₂O and 0.1% TMSP as an internal standard ($\delta=0$ ppm). Standard Bruker pulse sequences zgpg30 for ¹H, mlevsgpph/dipsi2rcesgpph (60 ms mixing time) for TOCSY, and noesygpph (200 ms mixing time) for NOESY were used to acquire the NMR data. Two-dimensional data were obtained using 2048 data points in the direct dimension

and 512 data points in the indirect dimension. The NMR of compounds **2c**, **2e'**, **2f**, **2i**, **2i'**, **2m**, **2q**, **2r**, **6**, and **7** were obtained using a concentration of 1-3 mM at room temperature (25°C) and for Pin1 WW analogs **9** and **10**, the NMR were acquired at a concentration of 100 μ M at 15°C.

Circular Dichroism Spectroscopy

Far-UV CD spectra of **9-10** were recorded at 10 μ M concentration and **11-12** were recorded at 40 μ M concentration in 20 mM sodium phosphate buffer (pH 7.4) over a wavelength range of 190-400 nm with a scan rate of 100 nm per minute and data pitch of 0.5 nm.

Improved synthesis of the aminoanilides **1a-1t**

To a 0.1-0.3 M solution of Fmoc-Xaa-OH (1 equiv) in DMF, HCTU (1.2 equiv) and DIPEA (1.5 equiv) were added followed by 4-Nitro-*o*-phenylenediamine (1.1 equiv). The reaction was stirred vigorously for 6 h until TLC (Thin Layer Chromatography) indicated the complete consumption of Fmoc-Xaa-OH. The reaction mixture was poured into cold water and filtered using sintered disc filter-funnel. The yellow solid obtained after filtration was dissolved in ethyl acetate and washed with 1N HCl (2 times) and saturated NaHCO₃ (2 times). The organic fractions were combined, dried over Na₂SO₄ and evaporated to dryness, yielding respective aminoanilides **1a-1t** as a yellow solid. Crude HPLC was performed by reversed phase HPLC (solvent A: 0.1% TFA in water, solvent B: 0.1% TFA in methanol, gradient 80-100% B solvent over 20 minutes) using C₁₈ analytical column. It was used for the next step without further purification.

Improved synthesis of the thioaminoanilides **2a-2t**

To a 0.1-0.3 M solution of aminoanilide (**1a-1t**) in dry THF, 1.5 equiv of Lawesson's reagent was added. The reaction mixture was stirred at 30 °C for 6-8 h. The progress of

the reaction was monitored by TLC. After completion, the reaction mixture was filtered through sintered disc filter-funnel, evaporated to dryness and dissolved in EtOAc. The solution was washed with saturated NaHCO_3 (2 times), dried over Na_2SO_4 and evaporated to dryness, yielding the respective thioaminoanilides **2a-2t** as a yellow solid. Crude HPLC was performed by reversed phase HPLC (solvent A: 0.1% TFA in water, solvent B: 0.1% TFA in methanol, gradient 80-100% B solvent over 20 minutes) using C_{18} analytical column. It was used for the next step without further purification.

Improved synthesis of the thiobenzotriazolides 3a-3t

To a 0.1-0.3 M solution of thioaminoanilide (**2a-2t**) in glacial acetic acid containing 5 % water, 1.5 equiv of sodium nitrite was added in portions at 4 °C. The reaction mixture was stirred at 4 °C for 30 min. The progress of the reaction mixture was monitored by TLC. Upon completion, the reaction mixture was poured into cold water and centrifuged. The supernatant was discarded, and the light orange precipitate was dissolved in DCM. The DCM solution was washed with saturated NaHCO_3 (2 times), saturated NaCl (once) and dried over Na_2SO_4 . This solution was directly utilized for incorporating the thiobenzotriazolides **3a-3t** onto the solid support.

Incorporation of the thiobenzotriazolides on solid-support (5a-5t)

The Fmoc deprotected peptide **4** was swollen in dichloromethane in a fritted syringe prior to the coupling. The solution of thiobenzotriazolidine precursor (2 equiv with respect to the resin loading of **4**) in dichloromethane was taken into the syringe along with (0.5 equiv) of DIPEA. The syringe was rotated in a mechanical rotor for 45 min under protection from light. The step was repeated once more. Finally, the resin was washed 5× with dichloromethane. Crude HPLC was performed by reversed phase HPLC (solvent A: 0.1% TFA in water, solvent B: 0.1% TFA in acetonitrile, gradient 50-100% B solvent over 20 minutes) using C_{18} analytical column.

NMR characterisation of new compounds 2c, 2e', 2f, 2i, 2i', 2m, 2q, 2r, 6, and 7

(9*H*-fluoren-9-yl)methyl (*S*)-(1-((2-amino-5-nitrophenyl)amino)-4-oxo-1-thioxo-4-(tritylamino)butan-2-yl)carbamate (**2c**). ¹H NMR (500 MHz, (CD₃)₂SO) δ 11.34 (s, 1H), 8.75 (s, 1H), 7.93 (m, 3H), 7.74 (dd, *J* = 13.0, 8.2 Hz, 2H), 7.41 (q, *J* = 7.6, 2H), 7.34-7.14 (m, 14 H), 6.81-6.29 (m, 2H), 2.90 (m, 2H); ¹³C{¹H} NMR (125 MHz, (CD₃)₂SO) δ 206.3, 168.7, 155.9, 150.6, 144.6, 143.8, 143.6, 140.7, 135.1, 128.6, 127.7, 127.4, 127.1, 126.3, 125.4, 125.2, 124.9, 124.7, 122.2, 120.1, 113.9, 69.5, 65.9, 58.1, 46.6 ppm.

(9*H*-fluoren-9-yl)methyl (*S*)-(1-((2-amino-5-nitrophenyl)amino)-3-(*tert*-butylthio)-1-thioxopropan-2-yl)carbamate (**2e'**). ¹H NMR (500 MHz, (CD₃)₂SO) δ 11.42 (s, 1H), 7.98–7.86 (m, 4H), 7.75 (t, 2H), 7.42 (t, *J* = 7.7 Hz, 2H), 7.32 (m, 2H), 6.82–6.40 (m, 2H), 4.57 (q, *J* = 7.12 Hz, 1H), 4.30 (q, *J* = 8.0 Hz, 2 H), 4.25 (d, *J* = 6.1 Hz, 1H), 3.15–2.88 (m, 1H), 1.32 (s, 9 H); ¹³C{¹H} NMR (125 MHz, (CD₃)₂SO) δ 205.2, 155.9, 150.5, 143.8, 143.6, 140.7, 135.3, 127.7, 127.0, 125.4, 125.3, 125.0, 124.7, 122.3, 120.1, 113.9, 65.9, 61.2, 46.5, 42.4, 32.1, 30.7 ppm.

(9*H*-fluoren-9-yl)methyl (*S*)-(1-((2-amino-5-nitrophenyl)amino)-5-oxo-1-thioxo-5-(tritylamino)pentan-2-yl)carbamate (**2f**). ¹H NMR (500 MHz, (CD₃)₂SO) δ 11.29 (s, 1H), 8.64 (s, 1H), 7.90 (m, 3H), 7.73 (t, *J* = 9.4 Hz, 2H), 7.41 (t, *J* = 6.8 Hz, 2H), 7.35–7.15 (m, 16H), 6.81–6.30 (m, 3H), 4.41 (q, 1H), 4.32 (q, 1H), 4.25 (m, 2H), 4.09 (s, 1H), 1.99 (m, 2H); ¹³C{¹H} NMR (125 MHz, (CD₃)₂SO) δ 207.1, 171.3, 156.4, 150.7, 144.9, 143.8, 143.6, 140.7, 135.3, 128.5, 127.6, 127.5, 1

27.4, 127.1, 126.3, 125.4, 125.2, 125.0, 124.9, 122.3, 120.1, 113.8, 69.2, 65.9, 61.3, 46.6, 32.7, 29.7 ppm.

(9H-fluoren-9-yl)methyl (S)-(1-((2-amino-5-nitrophenyl)amino)-1-thioxo-3-(1-trityl-1H-imidazol-5-yl)propan-2-yl)carbamate (**2i**). ¹H NMR (500 MHz, (CD₃)₂SO) δ 11.30 (s, 1H), 7.96–7.86 (m, 4H), 7.75 (m, 3H), 7.70 (d, *J* = 8.1 Hz, 2H), 7.43–7.32 (m, 17H), 7.27 (m, 4H), 7.08 (m, 7H), 6.93–6.81 (m, 2H), 6.74 (d, *J* = 9.7, 3H), 4.35–4.15 (m, 2H), 3.12 (m, 2H), 3.02 (m, 2H); ¹³C{¹H} NMR (125 MHz, (CD₃)₂SO) δ 205.9, 155.6, 151.1, 143.7, 143.6, 142.0, 140.7, 137.6, 134.9, 129.3, 128.1, 128.0, 127.6, 127.0, 125.3, 124.9, 124.7, 122.2, 120.1, 119.6, 113.7, 65.8, 60.7, 46.6, 36.2 ppm.

tert-butyl (S)-5-(2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-((2-amino-5-nitrophenyl)amino)-3-thioxopropyl)-1H-imidazole-1-carboxylate (**2i'**). ¹H NMR (500 MHz, (CD₃)₂SO) δ 11.32 (s, 1H), 7.92–7.88 (m, 3H), 7.75–7.67 (m, 2H), 7.41 (m, 3H), 7.31 (m, 3H), 6.5 (s, 2H), 3.33 (s, 5H), 3.15 (m, 1H), 3.05 (m, 1H), 1.56 (s, 9H); ¹³C{¹H} NMR (125 MHz, (CD₃)₂SO) δ 206.2, 156.0, 150.7, 146.6, 143.7, 143.6, 140.7, 138.8, 136.7, 135.2, 127.6, 127.1, 127.0, 125.3, 125.2, 124.9, 124.6, 122.3, 120.1, 114.9, 113.8, 85.3, 65.9, 60.6, 46.5, 27.3 ppm.

(9H-fluoren-9-yl)methyl (S)-(1-((2-amino-5-nitrophenyl)amino)-4-(methylthio)-1-thioxobutan-2-yl)carbamate (**2m**). ¹H NMR (500 MHz, (CD₃)₂SO) δ 11.33 (s, 1H), 8.02 (d, *J* = 5.5 Hz, 1H), 7.93 (m, 2H), 7.74 (dd, *J* = 14.1, 8.2 Hz, 2H), 7.45–7.28 (m, 3H), 6.82–6.33 (m, 3H), 4.55 (q, 1H), 4.32 (q, 1H), 4.26 (m, 2H), 4.09 (s, 2H), 2.09 (m, 4H); ¹³C{¹H} NMR (125 MHz, (CD₃)₂SO) δ 207.0, 156.4, 156.0, 150.6, 143.8, 143.7, 143.5, 142.4, 140.7, 140.7, 135.2, 127.6, 1

27.0, 125.4, 125.2, 124.9, 124.8, 122.3, 120.1, 117.5, 113.9, 65.9, 65.6, 60.5, 48.8, 46.6, 33.4, 32.6, 29.7, 14.6 ppm.

(9*H*-fluoren-9-yl)methyl ((2*S*,3*R*)-1-((2-amino-5-nitrophenyl)amino)-3-(*tert*-butoxy)-1-thioxobutan-2-yl)carbamate (**2q**). ¹H NMR (500 MHz, (CD₃)₂SO) δ 10.89 (s, 1H), 8.01–7.86 (m, 2H), 7.75 (dd, *J* = 16.1, 8.2 Hz, 2H), 7.46–7.28 (m, 3H), 7.05 (d, *J* = 8.6 Hz, 1H), 6.85–6.39 (m, 3H), 4.55 (d, *J* = 9.4 Hz, 1H), 4.36 (d, *J* = 6.5 Hz, 2H), 4.27 (d, *J* = 6.3 Hz, 1H), 4.1 (s, 1H), 1.21 (s, 9H), 1.17 (d, *J* = 5.7 Hz, 3H); ¹³C{¹H} NMR (125 MHz, (CD₃)₂SO) δ 203.5, 156.6, 150.8, 144.3, 143.9, 141.2, 135.8, 128.4, 127.7, 125.7, 125.6, 125.1, 122.6, 120.7, 114.7, 74.9, 69.0, 66.5, 49.1, 47.2, 28.5, 19.8 ppm.

tert-butyl (*S*)-3-(2-(((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-((2-amino-5-nitrophenyl)amino)-3-thioxopropyl)-1*H*-indole-1-carboxylate (**2r**). ¹H NMR (500 MHz, (CD₃)₂SO) δ 11.3 (s, 1H), 8.17 (d, *J* = 6.85, 1H), 8.09–7.91 (d, 1H), 7.91–7.78 (m, 2H), 7.76–7.64 (m, 2H), 7.37 (m, 2H), 7.27 (m, 2H), 6.80–6.27 (m, 3H), 4.77 (m, 1H), 4.28 (s, 1H), 4.19 (s, 2H), 4.08 (s, 2H), 1.61 (s, 10H); ¹³C{¹H} NMR (125 MHz, (CD₃)₂SO) δ 206.9, 156.4, 150.6, 149.1, 143.7, 143.5, 140.7, 135.3, 134.7, 130.2, 127.6, 127.0, 125.4, 125.2, 125.0, 124.6, 124.5, 124.4, 122.4, 122.2, 120.1, 119.7, 116.2, 114.6, 113.8, 83.5, 66.0, 61.1, 46.5, 36.2, 27.6, 24.3 ppm.

((*S*)-4-amino-2-((*S*)-2-aminopropanamido)-4-oxobutanethiioyl)-*L*-valyl-*L*-arginylglycine (**6**). ¹H NMR (500 MHz, (CD₃)₂SO) δ 9.94 (d, *J* = 8.1 Hz, 1H), 8.73 (d, 1H), 8.11 (d, *J* = 8.3 Hz, 3H), 7.66 (s, 2H), 7.44 (s, 2H), 6.97 (s, 2H), 5.07 (q, 1H), 4.83 (t, *J* = 7.05 Hz, 1H), 4.28 (q, *J* = 7.08 Hz, 1H), 2.20 (m, 1H), 1.7 (m, 2H), 1.52 (m, 6H), 1.32 (d, *J* = 6.6 Hz, 6H), 0.89 (t, *J* = 5.9 Hz, 11H); ¹³C{¹H} NMR (125 MHz, (CD₃)₂SO)

δ 204.0, 171.5, 171.0, 170.8, 169.1, 169.0, 158.1, 157.8, 157.6, 156.9, 120.9, 118.5, 117.0, 116.1, 63.6, 55.6, 52.1, 48.0, 30.4, 29.0, 25.0, 18.9, 18.4, 17.1 ppm.

((*S*)-5-amino-2-((*S*)-2-aminopropanamido)-5-oxopentanethioyl)-*L*-valyl-*L*-arginylglycine (7). ^1H NMR (500 MHz, $(\text{CD}_3)_2\text{SO}$) δ 10.02 (d, $J = 8.9$ Hz, 1H), 8.64 (d, $J = 8.0$ Hz, 1H), 8.26 (t, 1H), 8.14 (d, $J = 8.1$ Hz, 1H), 8.08 (s, 1H), 7.57 (s, 1H), 7.38 (s, 1H), 6.88 (s, 1H), 4.85 (t, $J = 8.2$ Hz, 1H), 4.71 (q, $J = 7.4$ Hz, 1H), 4.32 (q, $J = 6.9$ Hz, 1H), 2.14 (m, 4H), 1.94-1.61 (m, 4H), 1.52 (m, 4H), 1.33 (d, $J = 6.4$ Hz, 4H), 0.89 (d, $J = 6.8$ Hz, 8H); $^{13}\text{C}\{^1\text{H}\}$ NMR (125 MHz, $(\text{CD}_3)_2\text{SO}$) δ 204.5, 173.9, 171.6, 171.0, 169.3, 169.0, 158.4, 158.2, 157.9, 157.7, 156.9, 120.8, 118.4, 116.1, 113.7, 63.5, 58.0, 52.1, 48.0, 31.4, 30.5, 30.4, 29.2, 24.9, 19.0, 18.5, 17.0 ppm.

Table S1. Crude yield of **1a-1t** and **2a-2t**

entry	amino acid	compound	yield (%) ^a	compound	yield (%) ^b
1	Ala	1a	84	2a	88
2	Arg(Pbf)	1b	96	2b	94
3	Asn(Trt)	1c	92	2c	96
4	Asp(tBu)	1d	92	2d	98
5	Cys(Trt)	1e	94	2e	86
6	Cys(tBu)	1e'	90	2e'	91
7	Gln(Trt)	1f	90	2f	91
8	Glu(tBu)	1g	94	2g	93
9	Gly	1h	94	2h	94
10	His(Trt)	1i	89	2i	90
11	His(Boc)	1i'	91	2i'	91
12	Ile	1j	94	2j	92
13	Leu	1k	94	2k	97
14	Lys(Boc)	1l	88	2l	93
15	Met	1m	89	2m	92
16	Phe	1n	86	2n	98
17	Pro	1o	70	2o	85
18	Ser(tBu)	1p	89	2p	93
19	Thr(tBu)	1q	97	2q	92
20	Trp(Boc)	1r	92	2r	88
21	Tyr(tBu)	1s	94	2s	96
22	Val	1t	94	2t	96

^ayield refer to isolated crude product of the reaction from starting material (amino acid)

to **1a-1t**

^byield refer to isolated crude product of the reaction from **1a-1t** to **2a-2t**

Figure S1.

A.

HPLC chromatogram of crude **4**.

B.

ESI-MS of peptide **4**.

Figure S2.

A.

HPLC chromatogram of crude **1a**.

B.

HPLC chromatogram of crude **2a**.

C.

HPLC chromatogram of crude **5a**.

D.

ESI-MS of peptide **5a**.

Figure S3.

A.

HPLC chromatogram of crude **1b**.

B.

HPLC chromatogram of crude **2b**.

C.

HPLC chromatogram of crude **5b**.

D.

ESI-MS of peptide **5b**.

Figure S4.

A.

HPLC chromatogram of crude **1c**.

B.

HPLC chromatogram of crude **2c**.

C.

D.

E.

HPLC chromatogram of crude **5c**.

F.

ESI-MS of peptide **5c**.

Figure S5.

A.

HPLC chromatogram of crude **1d**.

B.

HPLC chromatogram of crude **2d**.

C.

HPLC chromatogram of crude **5d**.

D.

ESI-MS of peptide **5d**.

Figure S6.

A.

HPLC chromatogram of crude **1e**.

B.

HPLC chromatogram of crude **2e**.

C.

HPLC chromatogram of crude **5e**.

D.

ESI-MS of peptide **5e**.

Figure S7.

A.

B.

C.

¹H-NMR spectrum in DMSO-*d*₆ of **2e'**.

D.

¹³C-NMR spectrum in DMSO-*d*₆ of **2e'**.

E.

HPLC chromatogram of crude **5e'**.

F.

ESI-MS of peptide **5e'**.

Figure S8.

A.

B.

C.

¹H-NMR spectrum in DMSO-*d*₆ of **2f**.

D.

¹³C-NMR spectrum in DMSO-*d*₆ of **2f**.

E.

HPLC chromatogram of crude **5f**.

F.

ESI-MS of peptide **5f**.

Figure S9.

A.

HPLC chromatogram of crude **1g**.

B.

HPLC chromatogram of crude **2g**.

C.

HPLC chromatogram of crude **5g**.

D.

ESI-MS of peptide **5g**.

Figure S10.

A.

HPLC chromatogram of crude **1h**.

B.

HPLC chromatogram of crude **2h**.

C.

HPLC chromatogram of crude **5h**

D.

ESI-MS of peptide **5h**.

Figure S11.

A.

HPLC chromatogram of crude **1i**.

B.

HPLC chromatogram of crude **2i**.

C.

¹H-NMR spectrum in DMSO-*d*₆ of **2i**.

D.

¹³C-NMR spectrum in DMSO-*d*₆ of **2i**.

Figure S12.

A.

HPLC chromatogram of crude **1i'**.

B.

HPLC chromatogram of crude **2i'**.

Chemical structure of compound 10 is shown above the spectrum. The structure is a thioamide derivative with a 4-nitrophenyl group, an Fmoc-protected amine, and a Boc-protected pyridine ring.

¹H NMR spectrum (CDCl₃) of compound 10. The x-axis represents chemical shift in ppm, ranging from 0 to 11. The spectrum shows several peaks, with integration values provided below the peaks: 1.0, 3.01, 2.06, 0.702, 5.29, 1.21, and 9.3. Chemical shifts are labeled above the peaks: 11.320, 8.54, 8.24, 8.01, 7.91, 7.87, 7.84, 7.74, 7.708, 7.693, 7.677, 7.424, 7.411, 7.395, 7.325, 7.310, 7.294, 6.788, 6.769, 6.489, 3.334, 3.163, 3.150, 3.134, 3.121, 3.064, 3.049, 3.035, 3.020, and 1.557.

¹H-NMR spectrum in DMSO-*d*₆ of **2i'**.

13C NMR spectrum of compound 10. The x-axis represents chemical shift in ppm, ranging from 220 to 0. The spectrum shows several peaks, with the most prominent ones at approximately 206.2, 156.0, 150.7, 146.7, 143.8, 143.7, 143.6, 140.7, 138.8, 136.7, 135.2, 127.7, 127.6, 127.1, 127.0, 125.4, 125.3, 125.2, 124.9, 124.6, 122.3, 120.1, 114.9, 113.9, 85.3, 65.9, 60.6, 46.5, and 27.3 ppm. The peak at 206.2 ppm is the highest, followed by a cluster of peaks between 120 and 160 ppm, and a smaller peak at 27.3 ppm.

 ^{13}C -NMR spectrum in DMSO- d_6 of **2i'**.

E.

HPLC chromatogram of crude **5i'**.

F.

ESI-MS of peptide **5i'**.

Figure S13.

A.

HPLC chromatogram of crude **1j**.

B.

HPLC chromatogram of crude **2j**.

C.

HPLC chromatogram of crude **5j**.

D.

ESI-MS of peptide **5j**.

Figure S14.

A.

HPLC chromatogram of crude **1k**.

B.

HPLC chromatogram of crude **2k**.

C.

HPLC chromatogram of crude **5k**.

D.

ESI-MS of peptide **5k**.

Figure S15.

A.

B.

C.

HPLC chromatogram of crude **5l**.

D.

ESI-MS of peptide **5l**.

Figure S16.

A.

HPLC chromatogram of crude **1m**.

B.

HPLC chromatogram of crude **2m**.

C.

¹H-NMR spectrum in DMSO-*d*₆ of **2m**.

D.

¹³C-NMR spectrum in DMSO-*d*₆ of **2m**.

E.

HPLC chromatogram of crude **5m**.

F.

ESI-MS of peptide **5m**.

Figure S17.

A.

B.

C.

HPLC chromatogram of crude **5n**.

D.

ESI-MS of peptide **5n**.

Figure S18.

A.

HPLC chromatogram of crude **10**.

B.

HPLC chromatogram of crude **20**.

C.

HPLC chromatogram of crude **5o**.

D.

ESI-MS of peptide **5o**.

Figure S19.

A.

B.

C.

HPLC chromatogram of crude **5p**.

D.

ESI-MS of peptide **5p**.

Figure S20.

A.

HPLC chromatogram of crude **1q**.

B.

HPLC chromatogram of crude **2q**.

C.

¹H-NMR spectrum in DMSO-*d*₆ of **2q**.

D.

¹³C-NMR spectrum in DMSO-*d*₆ of **2q**.

E.

HPLC chromatogram of crude **5q**.

F.

ESI-MS of peptide **5q**.

Figure S21.

A.

HPLC chromatogram of crude **1r**.

B.

HPLC chromatogram of crude **2r**.

Chemical structure of compound 10 is shown. The structure is a 4-nitrophenyl ring with an NH group at position 1, a C=S group at position 2, and a chiral center at position 3. The chiral center is bonded to an Fmoc group, a hydrogen atom, and a 1-Boc-2-phenylindol-3-ylmethyl group.

¹H NMR spectrum (CDCl₃) of compound 10. The spectrum shows peaks from 0 to 12 ppm. Integration values are provided below the peaks: 1.11, 2.39, 2.27, 2.33, 0.605, 1.98, 0.892, 1.65, 9.53, 2.19, and 10.2. A list of chemical shifts (δ) is provided at the top: 11.385, 8.177, 8.163, 8.071, 8.054, 7.932, 7.932, 7.890, 7.885, 7.874, 7.810, 7.743, 7.700, 7.682, 7.398, 7.383, 7.354, 7.305, 7.292, 7.277, 7.250, 7.233, 6.783, 6.764, 6.323, 4.780, 4.769, 4.756, 4.282, 4.199, 4.088, and 1.610.

D.

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E.

HPLC chromatogram of crude **5r**.

F.

ESI-MS of peptide **5r**.

Figure S22.

A.

HPLC chromatogram of crude **1s**.

B.

HPLC chromatogram of crude **2s**.

C.

HPLC chromatogram of crude **5s**.

D.

ESI-MS of peptide **5s**.

Figure S23.

A.

HPLC chromatogram of crude **1t**.

B.

HPLC chromatogram of crude **2t**.

C.

HPLC chromatogram of crude **5t**.

D.

ESI-MS of peptide **5t**.

Figure S24.

A.

HPLC chromatogram of crude **2c'**.

B.

HPLC chromatogram of crude **2f'**.

Figure S25.

A.

HPLC chromatogram of crude peptide **6** at 270 nm.

B.

HRMS of peptide **6**.

C.

D.

E.

TOCSY spectrum of peptide 6.

Figure S26.

A.

HPLC chromatogram of crude peptide 7 at 270 nm.

B.

HRMS of peptide 7.

C.

^1H -NMR spectrum in $\text{DMSO}-d_6$ of 7.

D.

^{13}C -NMR spectrum in $\text{DMSO}-d_6$ of **7**.

E.

TOCSY spectrum of peptide **7**.

Figure S27.

A.

Incorporation efficiency of a **3g** over the time as a DCM stock solution.

B.

Incorporation efficiency of a **3k** over the time as a DCM stock solution.

C.

Incorporation efficiency of a **3I** over the time as a DCM stock solution.

Figure S28.

HPLC chromatogram of crude peptide **8** with 10% piperidine (60s × 2) protocol.

ESI-MS of desired peptide **8**.

ESI-MS of **8a** (diastereomer obtained on epimerization at thionated phenylalanine)

ESI-MS of **Fmoc-8** (incomplete Fmoc deprotection of peptide **8**).

Figure S29.

A.

HPLC chromatogram of purified peptide 9.

B.

ESI-MS of purified peptide 9.

Circular dichroism spectrum of peptide **9**.

Figure S30.

HPLC chromatogram of purified peptide **10**.

B.

ESI-MS of purified peptide **10**.

C.

Circular dichroism spectrum of peptide **10**.

Figure S31.

^1H NMR overlay of pin1 WW variants **9** and **10**.

Figure S32.

A.

TOCSY spectrum of peptide **9**.

B.

NOESY spectrum of peptide **9**.

Table S2. Chemical shifts of Pin1 WW analogs.

Residues	10		9	
	H ^N	H α	H ^N	H α
K				
L				
P		4.88		4.87
P		4.38		4.37
G	8.81	4.04, 3.35	8.80	4.06, 3.34
W	7.4	5.28	7.40	5.27
E	9.84	4.85	9.83	4.85
K	8.97	4.40	8.97	4.37
R	8.89	4.49	8.87	4.51
M	8.19	5.33	8.19	5.34
S	8.98	4.73	8.97	4.74
N	-	-	-	-
G	-	-	-	-
R	8.06	4.78	8.00	4.79
V	8.64	4.62	8.83	4.87
Y	8.75	4.80	10.24	5.55
Y	9.15	5.29	9.19	5.31
F	9.38	5.66	9.40	5.67
N	8.16	4.45	8.19	4.46
H	8.19	4.11	8.21	4.14
I	8.34	3.86	8.32	3.86
T	7.42	4.12	7.41	4.13
N	8.10	4.14	8.10	4.15
A	7.16	4.44	7.17	4.47
S	8.36	6.09	8.39	6.08
Q	9.42	4.95	9.46	4.96
F	9.03	4.85		
E	8.09	4.36	8.10	4.36
R	8.60	2.78	8.58	2.72
P		3.91		3.91
S	8.24	4.30	8.24	4.32
G	7.96	3.77	7.96	3.82, 3.72

Figure S33.

A.

HPLC chromatogram of purified protein **11**.

B.

ESI-MS of purified protein **11**.

C.

Circular dichroism spectrum of protein 11.

Figure S34.

A.

HPLC chromatogram of purified protein 12.

B.

ESI-MS of purified protein 12.

C.

Circular dichroism spectrum of protein 12.