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Supporting Information

An efficient metal-free desulfurization strategy mediated by Togni-II Reagent

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I. General Information

Materials and Methods

All commercial materials (Sigma Aldrich, Acros, TCI, Adamas, J&K, GL Biochem, *etc*.) were used without further purification. All solvents were reagent grade or HPLC grade (Oceanpak, Merck). Anhydrous CH_2Cl_2 and *N,N*-dimethyl formamide were commercially available and were purified and dried by passing through a PURE SOLV® solvent purification system (Innovative Technology, Inc.). All reactions were performed under an atmosphere of dry argon (g). Filtration for crude peptide was performed using a Bulk GHP Acrodisc® 13 mm syringe filter with 0.2 μm GHP membrane. Ultra-pure argon (≥ 99.999%) was used in all desulfurization reactions. Yields are calculated after chromatographical purification unless otherwise stated.

^{1}H NMR spectra were recorded at 400 MHz at ambient temperature with $CDCl_3$ (Cambridge Isotope Laboratories, Inc.) as the solvent unless otherwise stated. ^{13}C NMR spectra were recorded at 100.0 MHz at ambient temperature with $CDCl_3$ as the solvent unless otherwise stated. Chemical shifts are reported in parts per million relatives to $CDCl_3$ (1H, δ 7.26; 13C, δ 77.0). Data for ^{1}H NMR are reported as follows: chemical shift, integration, multiplicity (app = apparent, par obsc = partially obscure, ovrlp = overlapping, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet) and coupling constants. All ^{13}C NMR spectra were recorded with complete proton decoupling. Optical rotations were recorded on an AUTOPOL III digital polarimeter at 589 nm and are recorded as $[\alpha]_D^{25}$ (concentration in grams/100 mL solvent). Analytical thin layer chromatography was performed using Merck TLC silica gel 60-F254 glass plates. Flash chromatography was performed using 200-300 mesh silica gel (Qingdao Haiyang Chemical Co., Ltd.).

HPLC

All HPLC separations involved a mobile phase of 0.05% (*v/v*) TFA in water (solvent A) and 0.04% (*v/v*) TFA in acetonitrile (solvent B).

Analytical LC-MS(SQD) analyses were performed using a Waters Alliance e2695

Separations Module and a Waters 2489 UV/Visible (UV/Vis) Detector equipped with an Agilent C18 column (5.0 μm, 4.6 × 150 mm) or an Exsil Analytical C18 column (5.0 μm, 2.1 × 150 mm) at a flow rate of 0.4 mL/min, or with a Proto-300 C4 column (5.0 μm, 2.1 × 150 mm) at a flow rate of 0.2 mL/min. The wavelengths of UV-detector were set to 210 nm and 220 nm.

Preparative HPLC separations were performed using a Hanbon Sci. & Tech. NP7005C solvent delivery system and a Hanbon Sci. & Tech. NU3010C UV detector equipped with a Exsil Pure 300 C18 column (10.0 μm, 20 × 250 mm) at a flow rate of 15 mL/min, or a Proto-300 C4 column (10 μm, 20 × 250 mm) at a flow rate of 16 mL/min. The wavelengths of UV-detector were set to 210 nm and 220 nm.

II. General Procedures

2.1 Preparation of pre-loaded resins for solid phase peptide synthesis

Pre-load Fmoc-$NHNH_2$ to 2-chlorotritylchloride (CTC) resin

To a mixture of Fmoc-hydrazine (0.5 equiv) and CTC resin in dry DCM (approx. 10 mL per gram of resin) was added DIEA (4.0 equiv) dropwise. The reaction mixture was agitated for 2 hours. The resin was collected and washed with 17/2/1 (*v*/*v*/*v*) of DCM/MeOH/DIEA (× 3), DCM (× 3), DMF (× 3), DCM (× 3), and dried in vacuo overnight at room temperature before the loading test.

Pre-load Fmoc-$NHNH_2$ to Trityl-OH ChemMatrix® resin

The trityl-OH ChemMatrix® resin (1.0 equiv.) was swelled with dry DCM (approx. 15 mL per gram of resin) for 10 min in a vessel for solid phase peptide synthesis. Then dichloro sulfoxide (10 equiv.) was added dropwise. The vessel was shaked overnight at room temperature. Drained the resin and rinsed it with dry DCM (×6) then with 2% DIEA in DCM (×4) to get chlorinated trityl ChemMatrix® resin.

The chlorinated trityl ChemMatrix® resin then was swelled with dry DCM for 10 min and cooled to 0 ℃. Fmoc-$NHNH_2$ (4.0 equiv.) and DIEA (10 equiv.) in cosolvent of

DMF/DCM (5:1, approx. 6 mL per gram of resin) was added dropwise to the preswelled resin. The reaction was agitated for 22 hours. The resin was treated with a small amount of methanol (approx. 200 μl per gram of resin) for 15 min and then washed with DMF (×3), H_2O (×3), DMF (×3), MeOH (×3), Et_2O (×3) and dried in vacuo for 24 hours before the loading test.

Determination of resin loading

Dry Fmoc-hydrazine resin (approx. 5 μmol with respect to Fmoc) was weighted into a clean test tube ($Weight_{resin/mg}$), followed by the addition of 2 mL of 2% DBU in DMF. The mixture was agitated gently for 1 hour, and then diluted to 10 mL with acetonitrile. 2 mL of the resulting solution was taken out and diluted to 25 mL as the test solution. A reference solution was prepared in the same manner without the addition of resin.

Two matched silica UV cells were filled with reference solution to blank the U.V. spectrophotometer. The solution in one of the silica UV cells was changed to the test solution after washing with the test solution for three times. The optical density at 304 nm was recorded for three times and the average value was calculated as Abs_{sample}. The Fmoc loading of resin could be calculated using the equation below.

$$\text{Fmoc loading (mmol/g)} = Abs_{sample} \times 16.4 / Weight_{resin/mg}$$

2.2 Automated solid-phase peptide synthesis

Automated peptide synthesis was performed on a CEM peptide synthesizer (CEM LibertyBlue) or a CS Bio peptide synthesizer (CX136XT).

Peptides were synthesized following the general protocol on a CEM peptide synthesizer using DMF as solvent, deblock for 2 min in piperidine/DMF (20/80, *v/v*), couple for 5 min using excess amino acids (4 equiv) and DIC/Oxyma (1:1, 4 equiv) as coupling reagents. The coupling cycle was repeated as needed for amino acids after steric hindered residues such as Pro, Ile, Thr and Val.

Peptide synthesis was performed following the general protocol on a CS Bio peptide synthesizer (CX136XT): using DMF as solvent, deblocking (5 min × 2) in

piperidine/DMF (20:80, v/v), couple for 20 min using HATU/HOBt (1:1) as coupling reagent, for amino acids after steric hindered residues, the coupling cycle was repeated as needed.

The following $^{\alpha}N$-Fmoc or $^{\alpha}N$-Boc-protected amino acids from Novabiochem, GL Biochem or CS Bio were employed in SPPS: Fmoc-Ala-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Asp(O^{t}Bu)-OH, Fmoc-Cys(Trt)-OH, Fmoc-Cys(Acm)-OH, Fmoc-Glu(O^{t}Bu)-OH, Fmoc-Gly-OH, Fmoc-His(Trt)-OH, Fmoc-Ile-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Met-OH, Fmoc-Nle-OH, Fmoc-Phe-OH, Fmoc-Pro-OH, Fmoc-Ser(^{t}Bu)-OH, Fmoc-Thr(^{t}Bu)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Tyr-OH, Fmoc-Val-OH, Boc-Gly-OH, Fmoc-Thz-OH.

The CTC resin (1.19 mmol/g) and Fmoc-Val-Wang resin (0.42 mmol/g) employed in SPPS was purchased from GL Biochem. The Rink amide-MBHA resin (0.35 mmol/g) was purchased from CS Bio. The employed trityl-OH ChemMatrix® resin (0.30 mmol/g) employed was purchased from PCAS-BioMatrix.

2.3 Loading glycan modified amino acid on peptide

Fmoc-Asn(β-Ac$_3$GlcNAc)-OH[1] and Fmoc-Thr(α-Ac$_3$GalNAc)-OH[2] were chemically synthesized following previous procedure. The coupling procedure: using DMF as solvent, deblock for 5 min (× 2) in piperidine/DMF (20/80, v/v), couple for 20 min using Fmoc-Asn(β-Ac$_3$GlcNAc)-OH or Fmoc-Thr(α-Ac$_3$GalNAc)-OH (1.5 equiv), DIEA (3 equiv) was used as base and HATU/HOAt (1:1, 1.5 equiv) as coupling reagents. The next coupling cycle was repeated.

2.4 Removal of acetyl protections of carbohydrate on peptide

Upon completion of the automated synthesis on a 0.05 mmol scale, the Ac-protected glycopeptide resin was washed into a peptide synthesis vessel by using DCM. To the resin was added 10 mL of hydrazine hydrate solution in DMF (5%, *v*/*v*) and the resulting

[1] Robert E. T.; Bun C.; Leo R.; Katrina A. J.; Richard J. P. *Angew. Chem. Int. Ed.* 2013, **52**, 9723-9727.

[2] a) S. D. Kuduk, J. B. Schwarz, X.-T. Chen, P. W. Glunz, D. Sames, G. Ragupathi, P. O. Livingston, and S. J. Danishefsky, *J. Am. Chem. Soc.*, 1998, **120**, 12474 –12485; b) I. Tavernaro, S. Hartmann, L. Sommer, H. Hausmann, C. Rohner, M. Ruehl, A. Hoffmann-Roeder and S. Schlecht, *Org. Biom. Chem.*, 2015, **13**, 81–97.

mixture was agitated at room temperature for 16 hours. The resin was collected and washed with DMF (× 3), DCM (× 3), DMF (× 3), MeOH (× 3), DCM (× 3), and was ready for global deprotection.

2.5 Preparation of peptidyl acids, peptidyl amides and peptidyl hydrazides

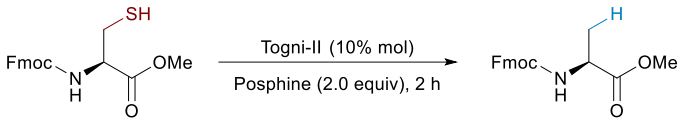
Upon completion of the automated synthesis on a 0.05 mmol scale, the peptide resin was washed into a peptide synthesis vessel by using DCM. Resin cleavage and global deprotection was performed under the treatment of TFA/TIS/H_2O (95:2.5:2.5, *v/v/v*) solution for 2 hours. The resin was then removed by filtration, and the filtrate was concentrated under a nitrogen atmosphere. The resulting residue was washed with cold diethyl ether to give a white solid, which was then dissolved in a mixture of acetonitrile and water. The resulting solution was ready for HPLC purification after filtration.

2.6 Metal-free desulfurization

To a solution of the cysteine / thiol-aspartate / thiol-glutamate containing peptide (~ 1 mg) in 200 μL of degassed buffer (6 M Gn·HCl, 200 mM Na_2HPO_4, pH 7.0) was added 200 μL of 0.5 M neutral TCEP solution (0.5 M TCEP·HCl dissolved in water, adjusting pH to 7.0 with NaOH solution), 50 μL of 2-methyl-2-propanethiol and 100 μL of radical initiator Togni-II (0.2 M in degassed methanol) or VA-044 (0.1 M in degassed water). Unless otherwise stated, the reaction mixture was stirred at 37 °C for 2 hours, quenched by addition of CH_3CN/H_2O/AcOH (10/85/5, *v/v/v*) and further purified using HPLC.

III. Extended Data

Table S1. Desulfurization of model substrate Fmoc-L-Cys-OMe with different phosphine reagents[a]

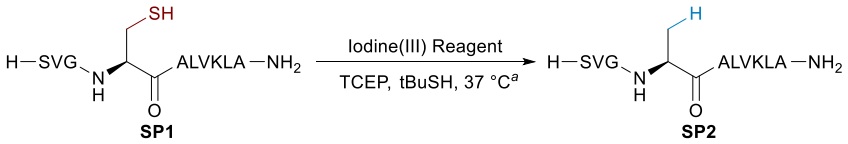


1 2

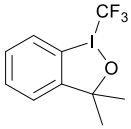
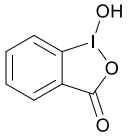
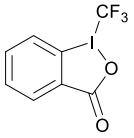
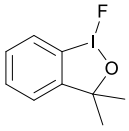
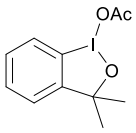
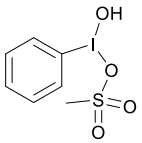
Entry	Posphine	Solvent	Yield (%)
1	TOP	DCM	93
2	PPh_3	DCM	<5%
3	$P(OEt)Ph_2$	DCM	<5%

[a]Reaction conditions: 10% Togni-II in degassed DCM, phosphine reagent, **1** (1.0 equiv.), 25 ˚C, Ar atmosphere, 2 h.

Table S2. Desulfurization of model peptide **SP1** under 37 ˚C induced by several kinds of commercially available iodine(III) reagents

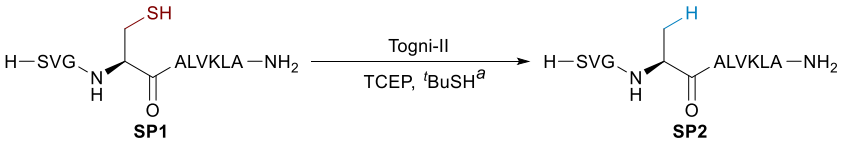


SP1 SP2

Entry	Iodine(III) Reagent	Time	Result[b]	Entry	Iodine(III) Reagent	Time	Result
1		2 h	Full converted	4		4 h	Not full converted
2		1 h	Full converted	5[c]		5 h	Full converted
3		4 h	Not full converted	6		4 h	Not full converted

[a]Reaction conditions: 200 µl Buffer (6 M Gn·HCl, 200 mM Na_2HPO_4, pH 7.0), 200 µl neutral TECP solution (500 mM), 50 µL ^{t}BuSH, 100 µL iodine(III) reagents (200 mM in MeOH), 37 ˚C; [b]The reactions were monitored by LC-MS; [c]The temperature was increased to 50 ˚C.

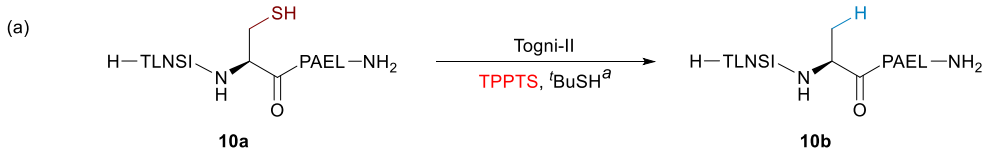
Table S3. Togni-II mediated desulfurization of model peptide **SP1** under different temperature[a]



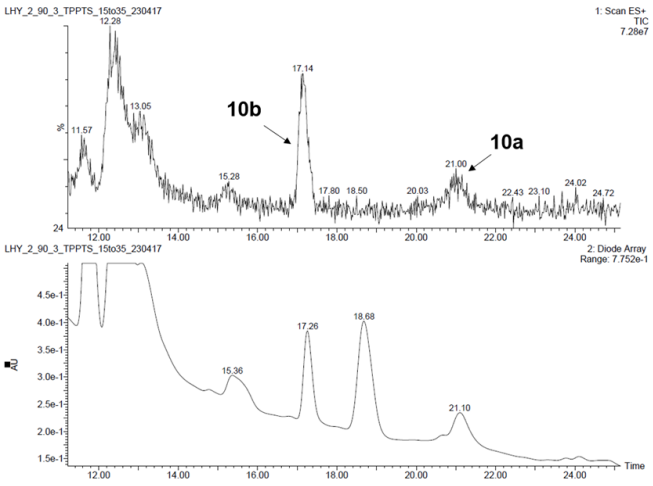
Entry	Togni-II	TCEP	Temperature	Time	Result[b]
1	Added	Added	50 ˚C	30 min	Full converted
2	Added	Added	37 ˚C	1 h	Full converted
3	Added	Added	25 ˚C	24 h	Not full converted
4	No	Added	37 ˚C	4 h	Not full converted
5	Added	No	37 ˚C	2 h	S-trifluoromethylated

[a]Reaction conditions: 200 µl Buffer (6 M Gn·HCl, 200 mM Na_2HPO_4, pH 7.0), 200 µl neutral TECP solution (500 mM), 50 µL ^{t}BuSH, 100 µL Togni-II (200 mM in MeOH), 37 ˚C; [b]The reactions were monitored by LC-MS.

Scheme S1. Desulfurization of model peptide **10a** with TPPTS.

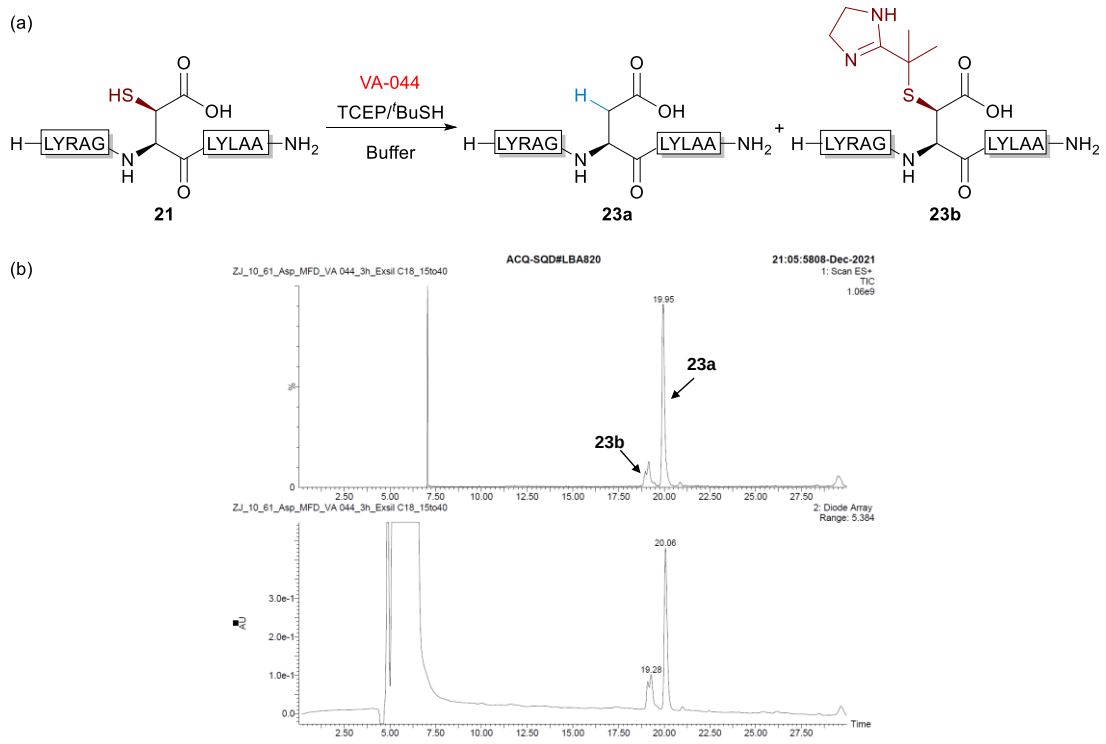


(b)



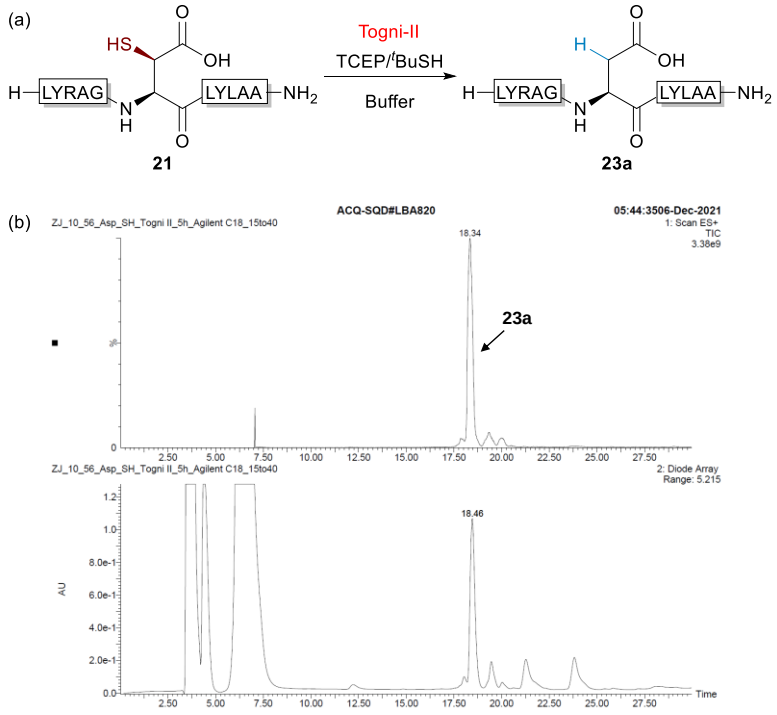
(a) Desulfurization of model peptide **10a** with TPPTS, [a] conditions: 200 µl Buffer (6 M Gn·HCl, 200 mM Na_2HPO_4, pH 7.0), 200 µl neutral TPPTS solution (500 mM in H_2O); 50 µL ^{t}BuSH; 100 µL Togni-II (200 mM in MeOH), 37 °C, 5 h; (b) The LCMS trace of the reaction, in which **10a** was not completely consumed after 5 h.

Scheme S2. Desulfurization of Asp($^{\beta}$SH) induced by VA-044 resulted in some radical adduct



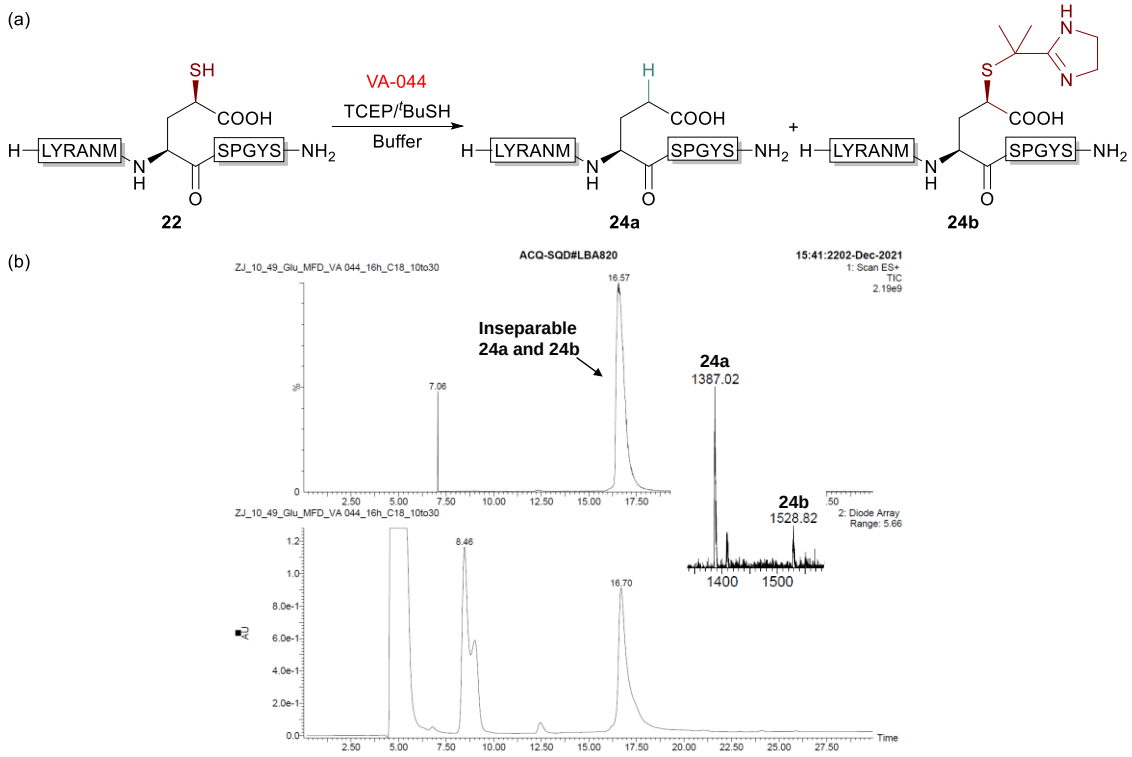
(a) VA-044 induced desulfurization of peptide **21**, conditions: degassed MeCN/H_2O (1:1), neutral TECP solution (500 mM), ^{t}BuSH, VA-044 (100 mM), 37 ˚C, 3 h; (b) The LCMS trace of the reaction, in which radical adduct **23b** was observed.

Scheme S3. Desulfurization of Asp($^{\beta}$SH) induced by Togni-II



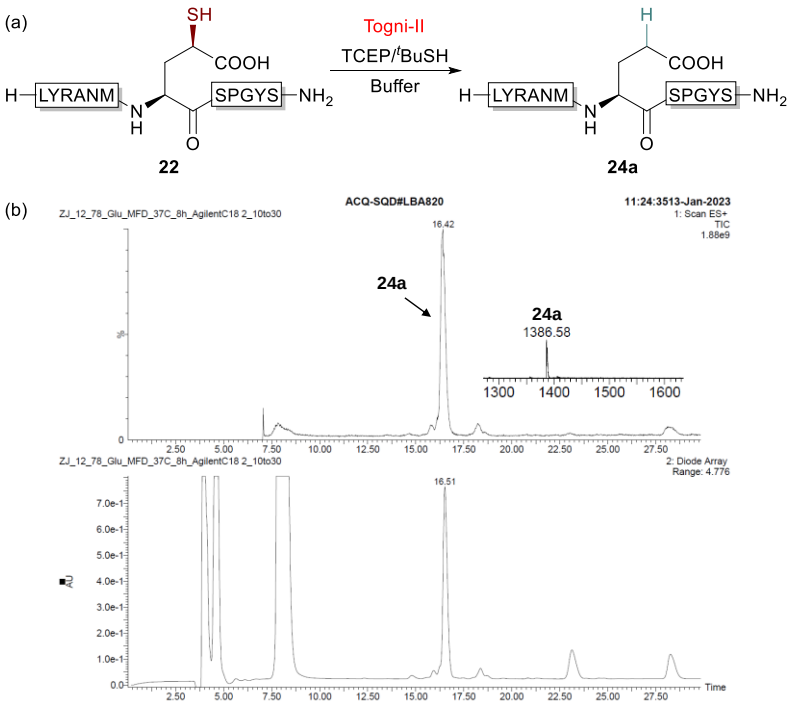
(a) Togni-II induced desulfurization of peptide **21**, conditions: Buffer (6 M Gn·HCl, 200 mM Na_2HPO_4, pH 7.0), neutral TECP solution (500 mM), ^{t}BuSH, Togni-II (200 mM in MeOH), 50 ˚C, 5 h; (b) The LCMS trace of the reaction, in which no radical adduct was observed.

Scheme S4. Desulfurization of Glu(ᵞSH) induced by VA-044 resulted in some inseparable radical adduct



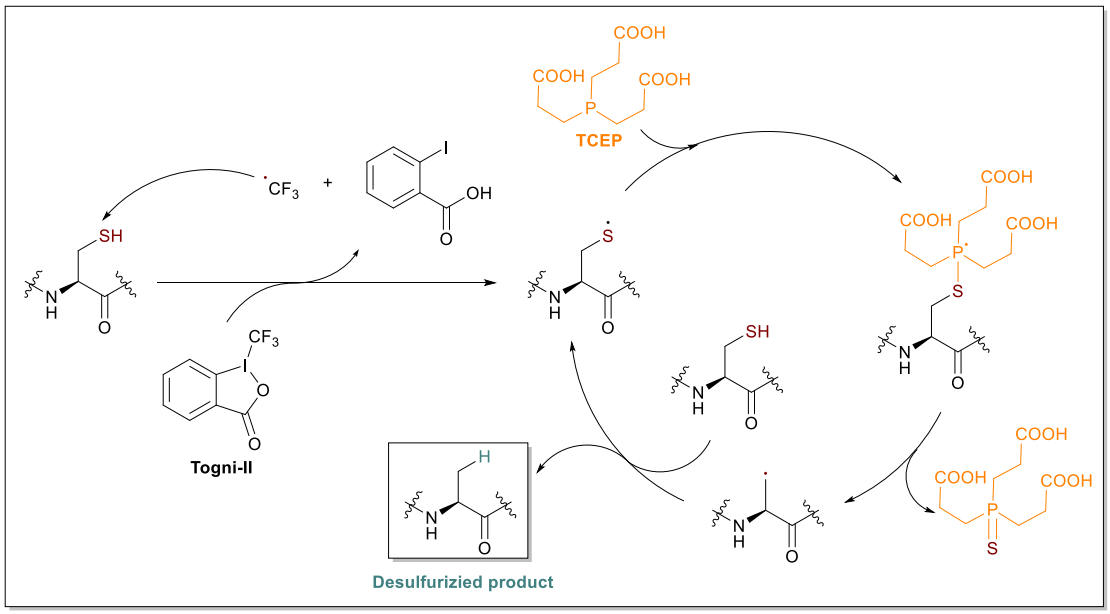
(a) VA-044 induced desulfurization of peptide **22**, conditions: Buffer (6 M Gn·HCl, 200 mM Na_2HPO_4, pH 7.0), neutral TECP solution (500 mM), glutathione (40 mM), VA-044 (100 mM), 65 ˚C, 16 h; (b) The LCMS trace and the mass signal of the reaction, in which inseparable radical adduct **24b** was observed.

Scheme S5. Desulfurization of Glu(ᵞSH) induced by Togni-II

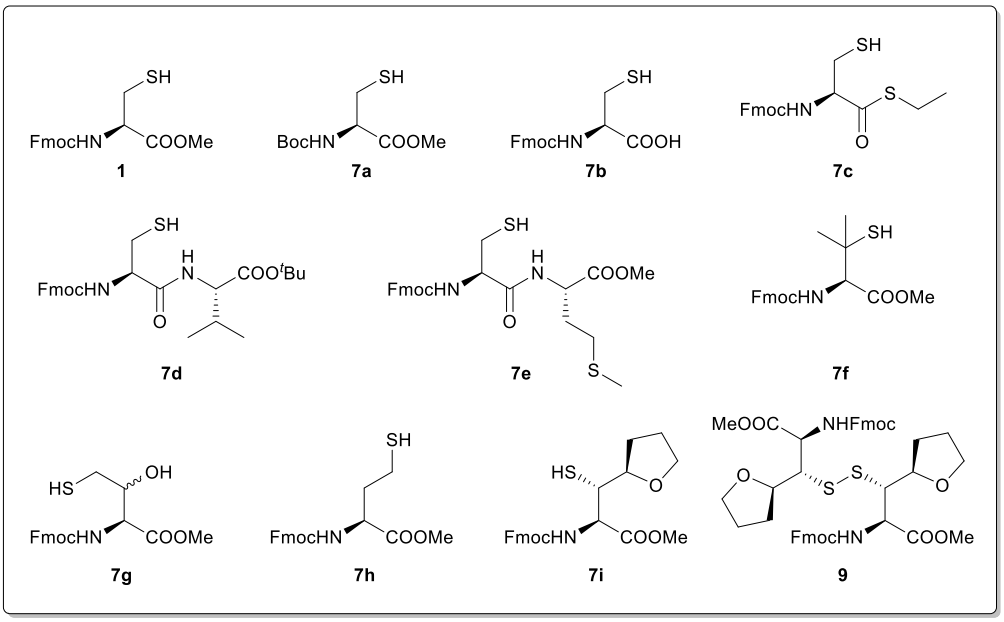


(a) Togni-II induced desulfurization of peptide **22**, conditions: Buffer (6 M Gn·HCl, 200 mM Na_2HPO_4, pH 7.0), neutral TECP solution (500 mM), ᵗBuSH, Togni-II (200 mM in MeOH), 37 ˚C, 8 h; (b) The LCMS trace of the reaction, in which no radical adduct was observed.

Scheme S6. Proposed mechanisms for Togni-II reagent mediated desulfurization

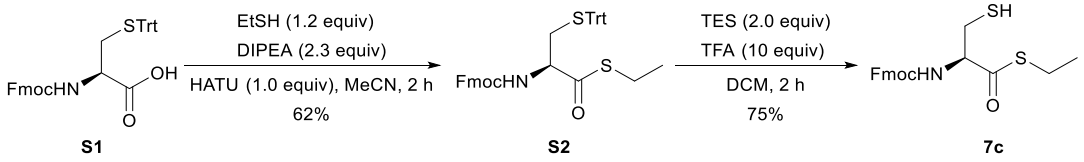


IV. Preparation and Characterization of Amino Acid Substrates for Desulfurization



The substrates **1**, **7a** and **7b** were synthesized according to the previously reported procedures[3].

Synthesis of Compound 7c



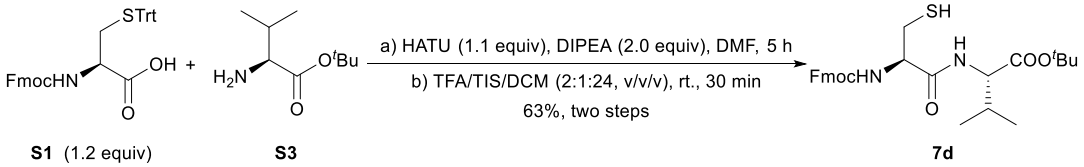
The solution of compound **S1** (500 mg, 0.85 mmol) and HATU (324 mg, 0.85 mmol) in anhydrous acetonitrile (2.8 ml) was cooled to 0 ˚C, then DIPEA (342 µl, 1.96 mmol) and ethanethiol (77 µl, 1.02 mmol) were added under argon atmosphere. The solution was stirred at room temperature for 2 h. The solvent was removed under reduced pressure after full conversion of starting material, and the residual mixture was diluted by EtOAc, washed with brine, dried over Na_2SO_4 and filtered. The filtrate was

[3] a) N. Stellenboom, R. Hunter and M. R. Caira, *Tetrahedron*, 2010, **66**, 3228-3241; b) Y. A. Lin, J. M. Chalker and B. G. Davis, *J. Am. Chem. Soc.*, 2010, **132**, 16805-16811; c) J. E. Montgomery, J. A. Donnelly, S. W. Fanning, T. E. Speltz, X. Shangguan, J. S. Coukos, G. L. Greene and R. E. Moellering, *J. Am. Chem. Soc*, 2019, **141**, 16374-16381.

concentrated in vacuo and the resulting crude mixture was purified using flash column chromatography (petroleum ether/EtOAc = 9/1) to give the product **S2** (335 mg, 62%).

Compound **S2** (295 mg, 0.47 mmol) was dissolved in DCM (23 ml), triethylsilane (150 μl, 0.94 mmol) and trifluoroacetic acid (361 μl, 4.69 mmol) were added dropwise at room temperature and stirred for 20 min. After the reaction finished, the solvent was removed under reduced pressure and the residues were purified by column chromatography (petroleum ether/EtOAc = 5/1) to afford the desired product **7c** (137 mg, 75%), white solid. m.p. 110-111 °C. $[\alpha]_D^{20}$ -0.15 (c, 1.37 in $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, J = 7.6 Hz, 2H, Ar-CH), 7.61 (dd, J = 7.6, 4.0 Hz, 2H, Ar-CH), 7.39 (t, J = 7.6 Hz, 2H, Ar-CH), 7.31 (t, J = 7.6 Hz, 2H, Ar-CH), 5.69 (d, J = 9.2 Hz, 1H, -NH), 4.69-4.64 (m, 1H), 4.50 (dd, J = 10.8, 6.8 Hz, 1H), 4.42 (dd, J = 10.8, 6.8 Hz, 1H), 4.24 (t, J = 7.2 Hz, 1H), 3.17-3.10 (m, 1H), 2.92 (q, J = 7.2 Hz, 2H), 2.87-2.79 (m, 1H), 1.37 (dd, J = 10.0, 7.2 Hz, 1H), 1.26 (t, J = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.2, 155.9, 144.0, 143.8, 141.6, 141.6, 128.0, 128.0, 127.3, 127.3, 125.3, 120.2, 67.5, 61.4, 47.4, 27.5, 23.9, 14.6; HRMS (ESI): *m/z* calcd for $C_{20}H_{21}NO_3S_2Na^+$ $[M+Na]^+$: 410.0855; observed: 410.0834.

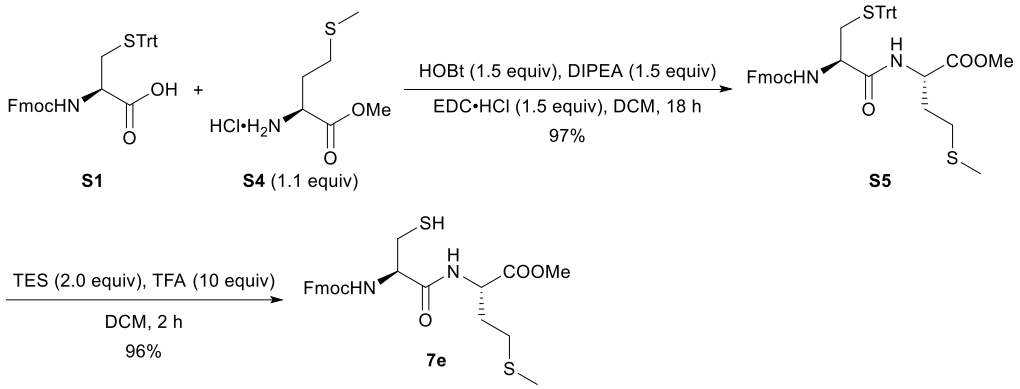
Synthesis of Compound 7d



To a solution of compound **S1** (190 mg, 0.32 mmol) and compound **S3** (47 mg, 0.27 mmol) in anhydrous DMF (3.0 ml) was added the HATU (113 mg, 0.29 mmol) and DIPEA (94 μl, 0.54 mmol) under argon atmosphere. After stirred at room temperature for 5 h, the reaction was diluted by EtOAc, washed with brine, dried over Na_2SO_4 and filtered. The filtrate was concentrated in vacuo and the resulting crude mixture was purified using flash column chromatography (petroleum ether/EtOAc = 5/1). The obtained compound was treated with TFA/TIS/DCM (2/1/24, v/v/v, 12.8 ml) for 30 min

under room temperature. After the reaction finished, the solvent was removed under reduced pressure and the residues were purified by column chromatography (Toluene/EtOAc = 10/1) to afford the desired product **7d** (85 mg, 63%, two steps) as white solid. m.p. 67-68 °C. $[\alpha]_D^{20}$ +1.2 (c, 1.25 in $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.6 Hz, 2H, Ar-CH), 7.57 (d, *J* = 7.6 Hz, 2H, Ar-CH), 7.39 (t, *J* = 7.6 Hz, 2H, Ar-CH), 7.30 (t, *J* = 7.6 Hz, 2H, Ar-CH), 6.62 (d, *J* = 8.8 Hz, 1H, -NH), 5.73 (d, *J* = 7.2 Hz, 1H, -NH), 4.48-4.37 (m, 4H), 4.22 (t, *J* = 6.8 Hz, 1H), 3.11-2.02 (m, 1H), 2.77-2.67 (m, 1H), 2.20-2.12 (m, 1H), 1.68 (t, *J* = 8.4 Hz, 1H), 1.44 (s, 9H, 3-CH3), 0.90 (dd, *J* = 6.8, 11.6 Hz, 6H, 2-CH3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 169.6, 141.5, 128.0, 127.3, 125.2, 120.3, 82.5, 67.5, 58.1, 47.3, 31.4, 28.2, 19.2, 17.8; HRMS (ESI): m/z calcd for $C_{27}H_{34}N_2O_5SNa^+$ $[M+Na]^+$: 521.2081; observed: 521.2075.

Synthesis of Compound 7e

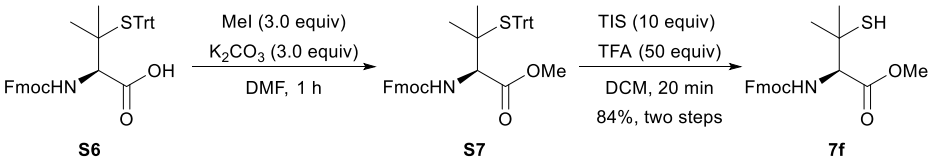


To a solution of compound **S1** (500 mg, 0.85 mmol) and compound **S4** (222 mg, 1.1 mmol) in anhydrous DCM was added the 1-hydroxybenzotriazole (173 mg, 1.28 mmol), DIPEA (223 μl, 1.28 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (245 mg, 1.28 mmol) under argon atmosphere. The solution was stirred at room temperature for 18 h. The reaction was diluted by EtOAc, washed with brine, dried over Na_2SO_4 and filtered. The filtrate was concentrated in vacuo and the resulting crude mixture was purified using flash column chromatography (petroleum ether/EtOAc = 3/1) to give the compound **S5** (644 mg, 97%).

Obtained compound **S5** was dissolved in anhydrous DCM (44 ml), triethylsilane (280 μl, 1.76 mmol) and trifluoroacetic acid (677 μl, 8.80 mmol) were added dropwise at

room temperature under argon atmosphere and stirred for 20 min. After the reaction finished, the solvent was removed under reduced pressure and the residues were purified by column chromatography (DCM/MeOH = 50/1) to afford the desired product **7e** (413 mg, 96%) as white solid. m.p. 107-108 °C. $[\alpha]_D^{20}$ +2.6 (c, 1.02 in $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.6 Hz, 2H, Ar-CH), 7.57 (d, *J* = 7.6 Hz, 2H, Ar-CH), 7.39 (t, *J* = 7.6 Hz, 2H, Ar-CH), 7.31 (t, *J* = 7.2 Hz, 2H, Ar-CH), 6.92 (d, *J* = 6.0 Hz, 1H, -NH), 5.71 (d, *J* = 6.8 Hz, 1H, -NH), 4.71-4.66 (m, 1H), 4.49-4.45 (m, 1H), 4.42-4.37 (m, 2H), 4.21 (t, *J* = 6.4 Hz, 1H), 3.74 (s, 3H), 3.08-3.01 (m, 1H), 2.76-2.69 (m, 1H), 2.49 (t, *J* = 7.2 Hz, 2H), 2.22-2.12 (m, 1H), 2.05 (s, 3H), 2.02-1.95 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.1, 169.7, 156.1, 143.9, 143.8, 141.5, 141.5, 128.0, 127.3, 127.3, 125.2, 125.2, 120.3, 120.2, 67.5, 56.3, 52.9, 52.1, 47.3, 31.3, 30.2, 27.2, 15.7; HRMS (ESI): m/z calcd for $C_{24}H_{28}N_2O_5S_2Na^+$ $[M+Na]^+$: 511.1332; observed: 511.1323.

Synthesis of Compound 7f

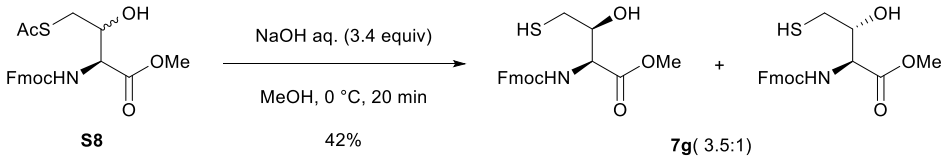


S6 **S7** **7f**

To a solution of compound **S6** (150 mg, 0.24 mmol) and K_2CO_3 (101 mg, 0.73 mmol) in anhydrous DMF was added iodomethane (46 µl, 0.73 mmol) under argon atmosphere. The solution was stirred at room temperature for 1 h. The reaction was diluted by EtOAc, washed with brine, dried over Na_2SO_4, filtered and concentrated. The crude material was redissolved in anhydrous DCM, triisopropylsilane (491 µl, 2.4 mmol) and trifluoroacetic acid (939 µl, 12.2 mmol) were added dropwise at room temperature under argon atmosphere and stirred for 20 min. After the reaction finished, the solvent was removed under reduced pressure and the residues were purified by column chromatography (petroleum ether/EtOAc = 6/1) to afford the desired product **7f** (69 mg, 84%, two steps) as white slurry. $[\alpha]_D^{20}$ -2.8 (c, 0.6 in $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) *δ* 7.75 (d, *J* = 7.6 Hz, 2H, Ar-CH), 7.58 (d, *J* = 6.8 Hz, 2H, Ar-CH), 7.39 (t, *J* =

7.2 Hz, 2H, Ar-CH), 7.30 (td, *J* = 7.6, 1.2 Hz, 2H, Ar-CH), 5.73 (d, *J* = 10.0 Hz, 1H, -NH), 4.48-4.34 (m, 3H), 4.22 (t, *J* = 6.8 Hz, 1H), 3.75 (s, 3H), 1.96 (s, 1H), 1.48 (s, 3H), 1.37 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.2, 151.2, 144.0, 143.9, 141.5, 128.0, 127.3, 125.3, 120.2, 120.2, 67.5, 62.8, 52.4, 47.4, 31.1, 29.6; HRMS (ESI): *m/z* calcd for $C_{21}H_{23}NO_4SNa^+$ $[M+Na]^+$: 408.1240; observed: 408.1244.

Synthesis of Compound 7g

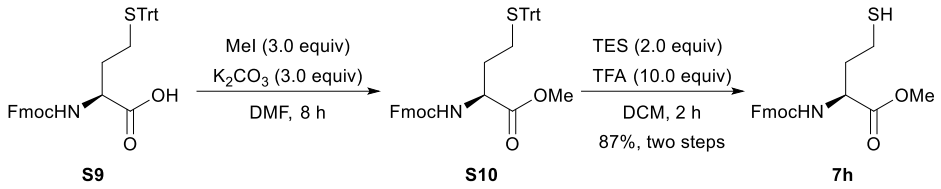


S8 **7g**(3.5:1)

Compound **S8**[4] (52 mg, 0.12 mmol) was dissolved in MeOH (2 ml) and cooled to 0 ˚C, then 0.2 N NaOH (2 ml) was added dropwise and stirred at 0 ˚C for 20 min. 1 N HCl was added carefully to neutralize the reaction mixture at 0 ˚C. The mixture was diluted by EtOAc, washed with brine, dried over Na_2SO_4, filtered and concentrated. The residues were then purified by flash column chromatography (petroleum ether/EtOAc = 3/1) to give the desired product **7g** (19 mg, 42%) as white solid. m.p. 103-104 °C. $[\alpha]_D^{20}$ +12.4 (c, 0.72 in $CHCl_3$), 3.5:1 d.r. determinded by ^{1}H NMR; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.6 Hz, 2H, Ar-CH), 7.60-7.56 (m, 2H, Ar-CH), 7.39 (t, *J* = 7.6 Hz, 2H, Ar-CH), 7.33-7.29 (m, 2H, Ar-CH), 5.57 (d, *J* = 9.6 Hz, 1H, -NH), 4.57 (d, *J* = 9.2 Hz, 1H), 4.51-4.39 (m, 2H), 4.21 (t, *J* = 6.8 Hz, 1H), 4.15-3.91 (m, 1H), 3.77 (s, 3H), 3.27-2.79 (m, 1H), 2.72-2.65 (m, 1H), 2.54-2.46 (m, 1H), 1.55 (t, *J* = 8.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.2, 156.9, 144.0, 143.7, 141.5, 141.5, 128.0, 128.0, 127.3, 125.3, 125.2, 120.2, 120.2, 73.3, 67.4, 57.5, 56.7, 53.1, 47.4, 28.6, 28.3; HRMS (ESI): *m/z* calcd for $C_{20}H_{21}NO_5SNa^+$ $[M+Na]^+$: 410.1033; observed: 410.1056.

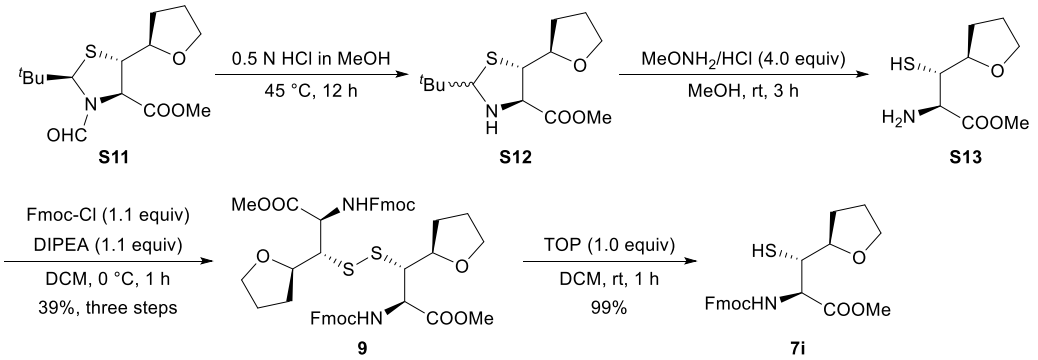
[4] J. Chen, P. Wang, J. Zhu, Q. Wan and S. J. Danishefsky, *Tetrahedron*, 2010, **66**, 2277-2283.

Synthesis of Compound 7h



To a solution of compound **S9** (205 mg, 0.34 mmol) and K_2CO_3 (142 mg, 1.02 mmol) in anhydrous DMF at 0 ˚C was added iodomethane (46 µl, 0.73 mmol). The solution was warmed to room temperature and stirred for 8 h under argon atmosphere. After the full conversion of **S9**, the reaction was diluted by EtOAc, washed with brine, dried over Na_2SO_4, filtered and concentrated. The crude material was redissolved in anhydrous DCM, triethylsilane (96 µl, 0.6 mmol) and trifluoroacetic acid (237 µl, 3.1 mmol) were added dropwise under argon atmosphere and stirred for 20 min. Then the solvent was removed under reduced pressure and the residues were purified by column chromatography (petroleum ether/EtOAc = 4/1) to afford the desired product **7h** (111 mg, 87%, two steps) as white solid. m.p. 100-101 °C. $[\alpha]_D^{20}$ +7.1 (c, 0.98 in $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, J = 7.6 Hz, 2H, Ar-CH), 7.58 (d, J = 6.4 Hz, 2H, Ar-CH), 7.39 (t, J = 7.6 Hz, 2H, Ar-CH), 7.30 (t, J = 7.6 Hz, 2H, Ar-CH), 5.41 (d, J = 8.4 Hz, 1H, -NH), 4.56-4.50 (m, 1H), 4.46-4.38 (m, 2H), 4.20 (t, J = 6.8 Hz, 1H), 3.74 (s, 3H), 2.60-2.46 (m, 2H), 2.16-2.08 (m, 1H), 1.99-1.90 (m, 1H), 1.53 (t, J = 8.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.6, 156.1, 144.0, 143.8, 141.5, 141.5, 127.9, 127.2, 125.2, 125.2, 120.2, 120.1, 67.1, 52.8, 52.8, 47.3, 37.1, 20.8; HRMS (ESI): *m/z* calcd for $C_{20}H_{21}NO_4SNa^+$ $[M+Na]^+$: 394.1083; observed: 394.1074.

Synthesis of Compounds 7i and 9



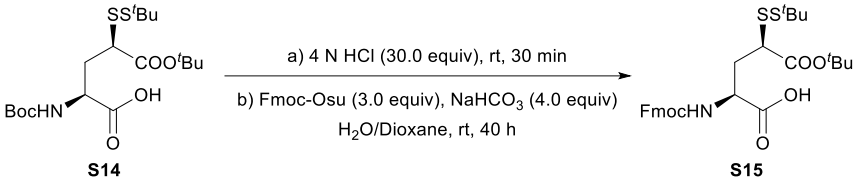
Compound **S11**[5] (117 mg, 0.39 mmol) was dissolved in 0.5 N HCl in MeOH, and the mixture was stirred at 45 ºC for 12 hours. After the reaction completed, the solvent was removed under reduced pressure, then diluted by EtOAc, washed with brine, dried over Na_2SO_4, filtered and concentrated. Next, to the solution of this crude in MeOH, $MeONH_2$·HCl (113 mg, 1.35 mmol) was added. The reaction mixture was stirred at room temperature for 3 h. Then the solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography (petroleum ether/EtOAc = 2/1 to DCM/MeOH = 10/1) to give compound **S13**. To a solution of compound **S13** in DCM at 0 ˚C was added DIPEA (37 µl, 0.20 mmol) and Fmoc-Cl (55 mg, 0.20 mmol). The reaction mixture was stirred at 0 ˚C for 1 h. This residue was purified by column chromatography (petroleum ether/EtOAc = 4/1) to give product **9** (65 mg, 39%, three steps) as white slurry. $[\alpha]_D^{20}$ +0.16 (c, 1.24 in $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, J = 7.6 Hz, 4H, Ar-CH), 7.59 (d, J = 7.2 Hz, 4H, Ar-CH), 7.38 (t, J = 7.6 Hz, 4H, Ar-CH), 7.29 (t, J = 7.2 Hz, 4H, Ar-CH), 5.89 (d, J = 9.6 Hz, 2H, -NH), 4.93 (dd, J = 9.2, 2.4 Hz, 2H), 4.44-4.34 (m, 4H), 4.22 (t, J = 7.2 Hz, 2H), 3.98-3.93 (m, 2H), 3.91-3.85 (m, 2H), 3.83-3.77 (m, 2H), 3.75 (s, 6H), 3.38 (dd, J = 7.6, 2.4 Hz, 2H), 2.10-2.02 (m, 2H), 1.97-1.79 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.4, 156.3, 144.1, 143.9, 141.5, 127.9, 127.3, 127.3, 125.3, 125.3, 120.2, 78.9, 68.7, 67.4, 62.0, 55.6, 52.9, 47.4, 30.2, 26.2; HRMS (ESI): *m/z* calcd for $C_{46}H_{48}N_2O_{10}S_2Na^+$ $[M+Na]^+$: 875.2643; observed: 875.2615.

To a solution of compound **9** (27 mg, 0.032 mmol) in DCM was added TOP (11 mg, 0.029 mmol) and stirred at room temperature for 30 min. Then the solvent was removed and the residue was purified by column chromatography (petroleum ether/EtOAc = 3/1) to give product **7i** (27.3 mg, 99%) as white slurry. $[\alpha]_D^{20}$ +1.29 (c, 2.7 in $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, J = 7.6 Hz, 2H, Ar-CH), 7.60 (dd, J = 7.2, 4.8 Hz, 2H, Ar-CH), 7.38 (t, J = 7.6 Hz, 2H, Ar-CH), 7.30 (t, J = 7.6 Hz, 2H, Ar-CH), 5.56 (d, J = 9.6 Hz, 1H, -NH), 5.08 (d, J = 9.6 Hz, 1H), 4.48-4.36 (m, 2H), 4.24 (t, J = 6.8 Hz,

[5] H. Yin, M. Zheng, H. Chen, S. Wang, Q. Zhou, Q. Zhang and P. Wang, *J. Am. Chem. Soc.*, 2020, **142**, 14201-14209.

1H), 3.87-3.78 (m, 3H), 3.76 (s, 3H), 3.25 (dt, *J* = 9.2, 2.4 Hz, 1H), 2.13-2.07 (m, 1H), 1.93-1.77 (m, 3H), 1.42 (d, *J* = 9.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6, 156.3, 144.2, 144.0, 141.5, 141.5, 127.9, 127.3, 125.4, 125.3, 120.2, 120.2, 80.0, 68.9, 67.5, 56.2, 52.9, 47.4, 46.7, 30.8, 26.1; HRMS (ESI): *m/z* calcd for $C_{23}H_{25}NO_5SNa^+$ $[M+Na]^+$: 450.1346; observed: 450.1330.

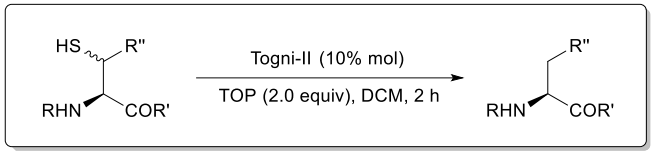
Synthesis of the (2S, 4R)-Fmoc-Glu(O^{t}Bu, $^\gamma$SS^{t}Bu)-OH (S15)



Compound **S14**[6] (310 mg, 0.73 mmol) was dissolved in 4 N HCl in 1,4-dioxane (5.5 ml, 22 mol) under an argon atmosphere. The reaction was stirred at room temperature for 30 min. The solvent was blown off with a stream of argon. The resulting residue was transferred to a flask containing Fmoc-OSu (741 mg, 2.20 mol) and $NaHCO_3$ (246 mg, 2.93 mmol), and the solvent 1,4-dioxane/water (9 ml/3 ml) was added. The reaction was stirred at room temperature for 40 h and then quenched with 1 N aqueous HCl at 0 ˚C, then the mixture was diluted by EtOAc, washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was then purified by flash column chromatography (petroleum ether/EtOAc = 3/1) to give the desired product **S15** (180 mg, 45%, two steps) as white soild. m.p. 68-69 °C. $[\alpha]_D^{20}$ -104 (c, 0.1 in MeOH). R_f = 0.32 (petroleum ether/EA/AcOH = 66/33/1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.6 Hz, 2H, Ar-CH), 7.59 (d, *J* = 7.2 Hz, 2H, Ar-CH), 7.39 (t, *J* = 7.2 Hz, 2H, Ar-CH), 7.31 (t, *J* = 7.2 Hz, 2H, Ar-CH), 5.32 (d, *J* = 8.4 Hz, 1H, -NH), 4.56-4.44 (m, 2H), 4.33 (m, 1H), 4.21 (t, *J* = 6.8 Hz, 1H), 3.43 (t, *J* = 7.6 Hz, 1H), 2.47-2.26 (m, 2H), 1.48 (s, 9H, ^{t}Bu), 1.33 (s, 9H, ^{t}Bu); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.2, 170.5, 156.2, 143.7, 141.2, 127.7, 127.1, 125.1, 125.0, 119.9, 82.6, 67.4, 52.9, 52.8, 48.3, 47.0, 33.4, 29.9, 28.0; HRMS (ESI): *m/z* calcd for $C_{28}H_{34}NO_6S_2^-$ $[M-H]^-$: 544.1833; observed: 450.1836.

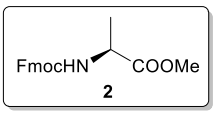
[6] Y. Gui, L. Qiu, Y. Li, H. Li and S. Dong, *J. Am. Chem. Soc.*, 2016, **138**, 4890-4899.

V. General Procedure of Togni-II Induced Desulfurization



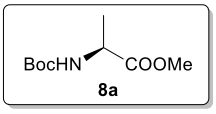
To a solution of Togni-II (0.1 equiv) in degassed DCM (solvent degassed with argon for 15 min) the TOP (2.0 equiv) was added under argon. The solution of sulfur-containing amino acid (1.0 equiv) in degassed DCM (in total 0.1 M) was added dropwise into the mixture. The mixture was stirred at room temperature under argon for 2 h. Then the reaction mixture was diluted with EtOAc, washed with brine, dried over Na_2SO_4, concentrated. The residue was purified by flash column chromatography to give the desired product.

Fmoc-Ala-OMe (2)



The reaction was performed with **1** (15.0 mg, 0.042 mmol), Togni-II (1.3 mg, 0.0042 mmol), TOP (31.1 mg, 0.08 mmol) in anhydrous DCM (0.4 mL), and the desired product **2** (12.7 mg, 93%) was obtained after purification by column chromatography (petroleum ether/EtOAc = 5/1) as white solid. R_f = 0.48 (petroleum ether/EA = 3/1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, J = 7.6 Hz, 2H, Ar-H), 7.58 (t, J = 6.8 Hz, 2H, Ar-H), 7.39 (t, J = 7.2 Hz, 2H, Ar-H), 7.30 (t, J = 8.0 Hz, 2H, Ar-H), 5.34 (d, J = 8.0 Hz, 1H, -NH), 4.42-4.34 (m, 3H), 4.21 (t, J = 6.8 Hz, 1H), 3.75 (s, 1H), 1.42 (d, J = 7.2 Hz, 3H). Analytical data for **2** was essentially the same as reported in the literature.[7]

Boc-Ala-OMe (8a)

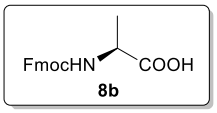


The reaction was performed with **7a** (25.7 mg, 0.11 mmol), Togni-II (3.5 mg, 0.01 mmol), TOP (80.9 mg, 0.22 mmol) in anhydrous DCM (1.1 ml), and the desired product **8a** (20.0 mg, 90%) was obtained after purification by column chromatography (petroleum ether/EtOAc = 8/1) as colorless oil. $[\alpha]_D^{20}$ 6.75 (c, 0.1 in $CHCl_3$), R_f = 0.52 (Toluene/EtOAc = 5/1). ^{1}H

[7] a) L. Konnert, F. Lamaty, J. Martinez and E. Colacino, *J Org Chem*, 2014, **79**, 4008-4017; b) M. Shibasaki, N. Kumagai and S. Adachi, *Synlett.*, 2017, **29**, 301-305.

NMR (400 MHz, $CDCl_3$) δ 5.02 (bs, 1H, -NH), 4.32-4.26 (m, 1H), 3.72 (s, 3H), 1.42 (s, 9H), 1.36 (d, J = 7.2 Hz, 3H). Analytical data for **8a** was essentially the same as reported in the literature.[8]

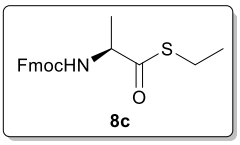
Fmoc-Ala-OH (8b)



8b

The reaction was performed with **7b** (15.0 mg, 0.044 mmol), Togni-II (1.4 mg, 0.0044 mmol), TOP (32.4 mg, 0.09 mmol) in MeOH/H_2O (1/1) cosolvent (0.4 ml), and the desired product **8b** (12.4 mg, 91%) was obtained after purification by column chromatography (petroleum ether/EtOAc/AcOH = 2/1/0.05) as white solid. $[\alpha]_D^{20}$ 3.11 (c, 1.8 in $CHCl_3$). R_f = 0.48 (DCM/MeOH/AcOH = 10/1/0.05). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, J = 7.6 Hz, 2H, Ar-H), 7.58-7.52 (m, 2H, Ar-H), 7.36 (t, J = 7.6 Hz, 2H, Ar-H), 7.28 (t, J = 8.0 Hz, 2H, Ar-H), 6.50 (s, 1H), 5.36 (d, J = 7.2 Hz, 1H), 4.47-4.34 (m, 3H), 4.20 (t, J = 7.2 Hz, 1H), 1.45 (d, J = 7.2 Hz, 3H). Analytical data for **8b** was essentially the same as reported in the literature.[9]

Fmoc-Ala-SEt (8c)



8c

The reaction was performed with **7c** (30 mg, 0.0774 mmol), Togni-II (1.94 mg, 0.0055 mmol), TOP (45.5 mg, 0.123 mmol) in anhydrous DCM (0.6 mL), and the desired product **8c** (24.9 mg, 90%) was obtained after purification by column chromatography (petroleum ether/EtOAc = 7/1) as white solid. m.p. 130-131 °C. $[\alpha]_D^{20}$ -0.85 (c, 0.7 in $CHCl_3$). R_f = 0.39 (petroleum ether/EA = 7/1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, J = 7.2 Hz, 2H, Ar-H), 7.60 (t, J = 7.2 Hz, 2H, Ar-H), 7.39 (t, J = 7.6 Hz, 2H, Ar-H), 7.30 (t, J = 7.2 Hz, 2H, Ar-H), 5.23 (d, J = 8.4 Hz, 1H, -NH), 4.50-4.42 (m, 2H), 4.35 (dd, J = 10.8, 7.2 Hz, 1H), 4.22 (t, J = 6.8 Hz, 1H), 2.88 (q, J = 7.6 Hz, 2H), 1.40 (d, J = 7.2 Hz, 3H), 1.24 (t, J = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.5, 155.7, 144.1, 143.9, 141.5, 127.9, 127.3, 125.3, 125.3, 120.2, 67.3, 56.9, 47.4, 23.5, 19.3, 14.7; HRMS (ESI): *m/z* calcd for $C_{20}H_{21}NO_3SNa^+$ $[M+Na]^+$: 378.1134; observed: 378.1120.

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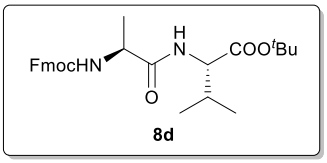
[8] E. Speckmeier, M. Klimkait and K. Zeitler, *J. Org. Chem.*, 2018, **83**, 3738-3745.

[9] E. Speckmeier and K. Zeitler, *ACS Catalysis*, 2017, **7**, 6821-6826.

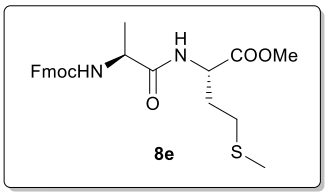
Fmoc-Ala-Val-O^{t}Bu (8d)



8d

The reaction was performed with **7d** (15.0 mg, 0.03 mmol), Togni-II (1.0 mg, 0.003 mmol), TOP (23.2 mg, 0.06 mmol) in anhydrous DCM (0.3 mL), and the desired product **8d** (13.0 mg, 93%) was obtained after purification by column chromatography (petroleum ether/EtOAc = 3/1) as white solid. $[\alpha]_D^{20}$ -2.8 (c, 0.74 in $CHCl_3$). R_f = 0.31 (petroleum ether/EA = 3/1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, J = 7.2 Hz, 2H, Ar-H), 7.57 (d, J = 7.6 Hz, 2H, Ar-H), 7.38 (t, J = 7.2 Hz, 2H, Ar-H), 7.29 (t, J = 7.6 Hz, 2H, Ar-H), 6.40 (d, J = 8.8 Hz, 1H, -NH), 5.44 (d, J = 7.6 Hz, 1H, -NH), 4.43-4.36 (m, 3H), 4.28 (t, J = 6.8 Hz, 1H), 4.20 (t, J = 6.8 Hz, 1H), 2.19-2.11 (m, 1H), 1.44 (s, 9H, -^{t}Bu), 1.40 (d, J = 7.2 Hz, 3H, -CH_3), 0.88 (dd, J = 6.8, 11.2 Hz, 6H, 2-CH_3). Analytical data for **8d** was essentially the same as reported in the literature.[10]

Fmoc-Ala-Met-OMe (8e)



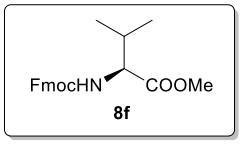
8e

The reaction was performed with **7e** (30.0 mg, 0.0614 mmol), Togni-II (1.94 mg, 0.006 mmol), triethylphospine (14.5 mg, 0.12 mmol) in anhydrous DCM (0.6 mL), and the desired product **8e** (25.4 mg, 91%) was obtained after purification by column chromatography (petroleum ether/EtOAc = 3/2) as white solid. $[\alpha]_D^{20}$ 2.30 (c, 1.2 in $CHCl_3$). R_f = 0.29 (DCM/MeOH = 50/1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, J = 7.6 Hz, 2H, Ar-CH), 7.57 (d, J = 7.2 Hz, 2H, Ar-CH), 7.38 (t, J = 7.2 Hz, 2H, Ar-CH), 7.29 (t, J = 7.6 Hz, 2H, Ar-CH), 6.73 (bs, 1H, -NH), 5.43 (bs, 1H, -NH), 4.71-4.66 (m, 1H), 4.42-4.37 (m, 2H), 4.31-4.24 (m, 1H), 4.20 (t, J = 6.8 Hz, 1H), 3.73 (s, 3H), 2.47 (t, J = 7.6 Hz, 2H), 2.20-2.10 (m, 1H), 2.03 (s, 3H), 2.00-1.94 (m, 1H), 1.39 (d, J = 7.2 Hz, 3H). Analytical data for **8e** was essentially the same as reported in the literature.[11]

[10] A. Bach, J. N. Eildal, N. Stuhr-Hansen, R. Deeskamp, M. Gottschalk, S. W. Pedersen, A. S. Kristensen and K. Stromgaard, *J. Med. Chem.*, 2011, **54**, 1333-1346.

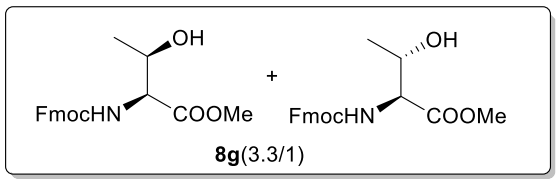
[11] R. M. de Figueiredo, J.-S. Suppo, C. Midrier and J.-M. Campagne, *Adv. Synth. Catal.*, 2017, **359**, 1963-1968.

Fmoc- Val-OMe (8f)



The reaction was performed with **7f** (17.0 mg, 0.044 mmol), Togni-II (2.8 mg, 0.009 mmol), TOP (32.7 mg, 0.09 mmol) in anhydrous DCM (0.4 mL), and the desired product **8f** (13.4 mg, 86%) was obtained after purification by column chromatography (petroleum ether/EtOAc/Toluene = 10/1/10) as white slurry. $[\alpha]_D^{20}$ -0.96 (c, 1.2 in $CHCl_3$). R_f = 0.25 (PE/EA /Toluene = 10/1/10). ^1H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, J =7.6 Hz, 2H, ArCH), 7.59 (dd, J = 7.2, 2.4 Hz, 2H, ArCH), 7.39 (t, J = 7.6 Hz, 2H, ArCH), 7.32-7.28 (m, 2H, ArCH), 5.29 (d, J = 9.2 Hz, 1H, NH), 4.43-4.35 (m, 2H, $COCH_2$), 4.30 (dd, J = 9.2, 5.2 Hz, 1H, CH), 4.22 (t, J = 7.2 Hz, 1H), 3.74 (s, 3H, OCH_3), 2.20-2.11 (m, 1H, $CH(CH_3)_2$), 0.96 (d, J = 6.8 Hz, 3H, $CH(CH_3)_2$), 0.90 (d, J = 6.8 Hz, 3H, $CH(CH_3)_2$). Analytical data for **8f** was essentially the same as reported in the literature.[12]

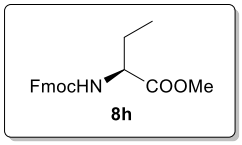
Fmoc-Thr- OMe (8g)



The reaction was performed with **7g** (9.9 mg, 0.026 mmol), Togni-II (0.8 mg, 0.0025 mmol), TOP (18.9 mg, 0.05 mmol) in anhydrous DCM (0.3 mL), and the desired product **8g** (7.6 mg, 84%) was obtained after purification by column chromatography (petroleum ether/EtOA = 2/1) as white slurry. $[\alpha]_D^{20}$ -5.47 (c, 1.1 in $CHCl_3$). R_f = 0.58 (Toluene /EA = 1/1), 3.3:1 d.r. determinded by ^1H NMR. ^1H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, J = 7.6 Hz, 2H), 7.61-7.57 (m, 2H), 7.39 (t, J = 7.2 Hz, 2H), 7.32-7.28 (m, 2H), 5.58 (d, J = 9.2 Hz, 1H), 4.50-4.40 (m, 2H), 4.37-4.32 (m, 1H), 4.23 (t, J = 7.6 Hz, 1H), 3.77 (s, 3H), 2.02 (brs, 1H), 1.24 (d, J = 6.4 Hz, 3H); HRMS (ESI): *m/z* calcd for $C_{20}H_{21}NO_5Na^+$ $[M+Na]^+$: 378.1312; observed: 378.1317. Analytical data for **8g** was essentially the same as reported in the literature.[13]

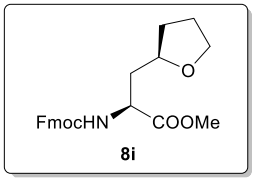
[12] C. A. Brotherton and E. P. Balskus, *J. Am. Chem. Soc.*, 2013, **135**, 3359-3362.
[13] T. Ghosh, A. Mukherji and P. K. Kancharla, *Org. Lett.*, 2019, **21**, 3490-3495.

Methyl (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)butanoate (8h)



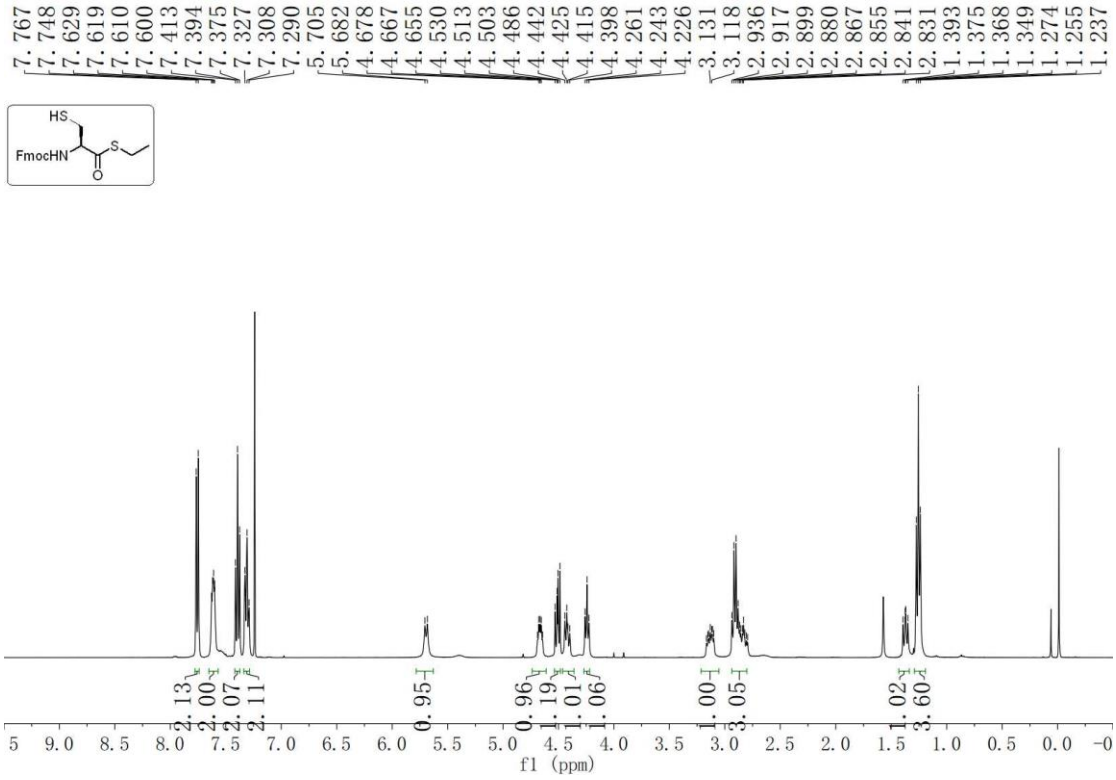
The reaction was performed with **7h** (30.0 mg, 0.081 mmol), Togni-II (5.2 mg, 0.016 mmol), TOP (59.9 mg, 0.16 mmol) in anhydrous DCM (0.8 mL), and the desired product **8h** (25.2 mg, 92%) was obtained after purification by column chromatography (petroleum ether/EtOA = 7/1) as white soild. m.p. 108-109 °C. $[\alpha]_D^{20}$ 1.43 (c, 1.1 in $CHCl_3$). R_f = 0.31 (petroleum ether/EA = 5/1). ^1H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, J = 7.6 Hz, 2H, Ar-CH), 7.59 (dd, J = 7.2, 3.6 Hz, 2H, Ar-CH), 7.39 (t, J = 7.6 Hz, 2H, Ar-CH), 7.30 (t, J = 7.6 Hz, 2H, Ar-CH), 5.30 (d, J = 8.0 Hz, 1H, -NH), 4.39 (d, J = 7.2 Hz, 2H) 4.35-4.32 (m, 1H), 4.22 (t, J = 7.2 Hz, 1H), 3.74 (s, 3H), 1.92-1.84 (m, 1H), 1.77-1.66 (m, 1H), 0.92 (t, J = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.1, 156.1, 144.1, 144.0, 141.5, 127.9, 127.3, 125.3, 120.2, 67.2, 55.1, 52.6, 47.4, 26.0, 9.7; HRMS (ESI): *m/z* calcd for $C_{20}H_{21}NO_4Na^+$ $[M+Na]^+$: 362.1363; observed: 362.1366.

Methyl (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-((R)-tetrahydrofuran-2-yl)propanoate (8i)

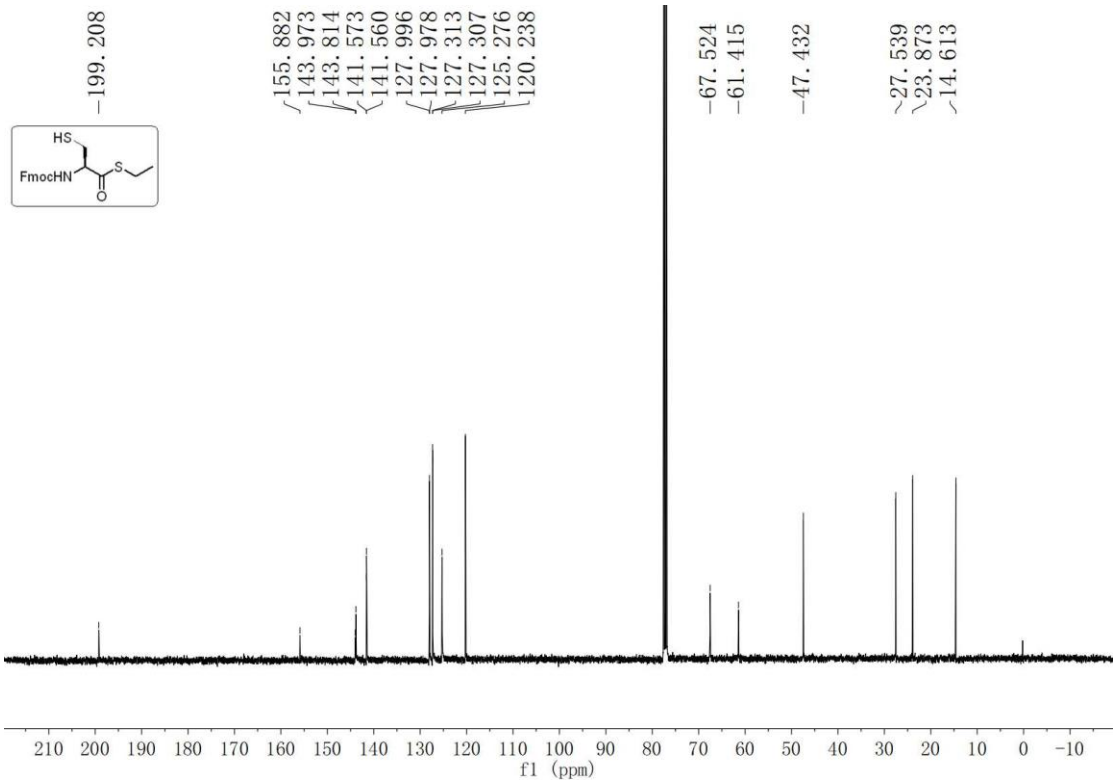


A：The reaction was performed with **7i** (20.0 mg, 0.047 mmol), Togni-II (1.5 mg, 0.004 mmol), TOP (34.7 mg, 0.09 mmol) in anhydrous DCM (0.5 mL), and the desired product **8i** (15.4 mg, 83%) was obtained after purification by column chromatography (petroleum ether/EtOA = 4/1). **B**：The reaction was performed with **9** (15.0 mg, 0.017 mmol), Togni-II (1.1 mg, 0.003 mmol), TOP (26.0 mg, 0.07 mmol) in anhydrous DCM (0.35 mL), the desired product **8i** (11.7 mg, 84%) was obtained after purification. $[\alpha]_D^{20}$ +1.34 (c, 1.2 in $CHCl_3$), R_f = 0.50 (petroleum ether/EA = 2/1); ^1H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, J = 7.6 Hz, 2H, Ar-CH), 7.61 (t, J = 8.0 Hz, 2H, Ar-CH), 7.38 (t, J = 7.2 Hz, 2H, Ar-CH), 7.30 (t, J = 7.2 Hz, 2H, Ar-CH), 6.20 (d, J = 8.8 Hz, 1H, -NH), 4.55-4.50 (m, 1H), 4.38 (dd, J = 10.4, 7.2 Hz, 1H), 4.31 (dd, J = 10.4, 7.2 Hz, 1H), 4.24 (t, J = 7.2 Hz, 1H), 3.92-3.84 (m, 2H), 3.79-3.68 (m, 4H), 2.11-1.98 (m, 2H), 1.94-1.81 (m, 3H), 1.53-1.44 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.9, 156.4, 144.3, 144.1, 141.5, 127.8, 127.2, 125.5, 125.4, 120.1, 76.7, 68.3, 67.3, 53.1, 52.6, 47.4, 37.0, 32.2, 25.6; HRMS (ESI): *m/z* calcd for $C_{23}H_{25}NO_5Na^+$ $[M+Na]^+$: 418.1625; observed: 418.1625.

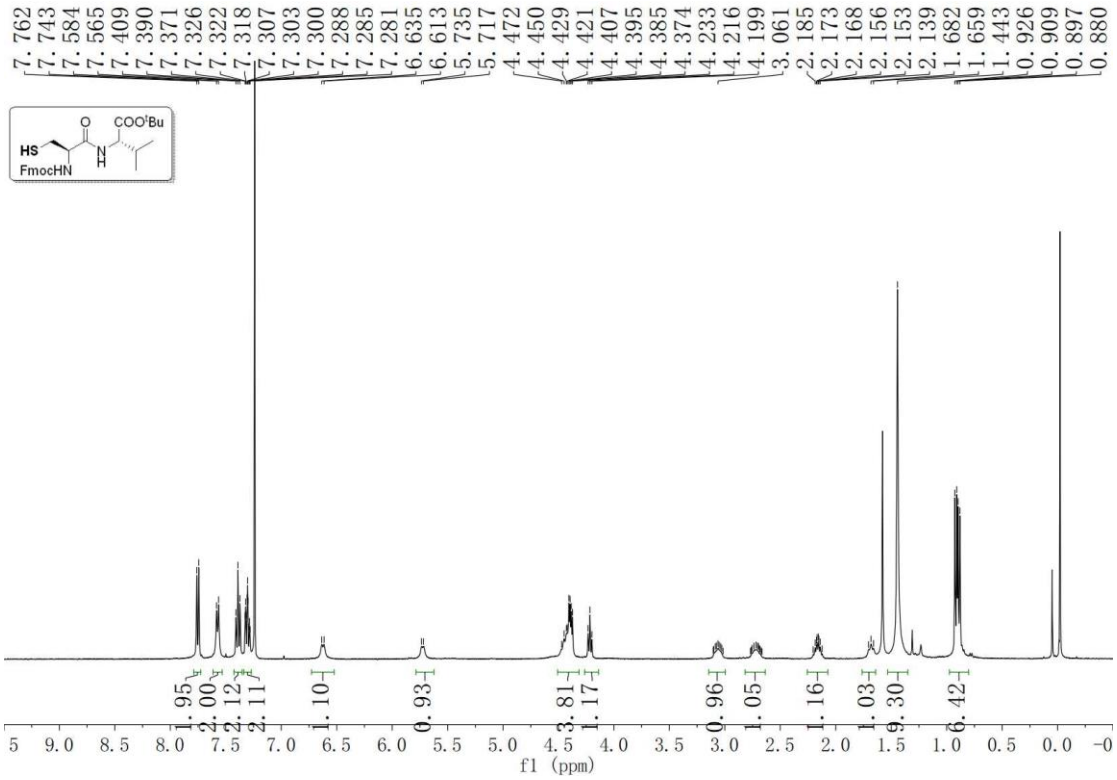
VI. NMR Spectrum of Amino Acid Substrates and Derivatives



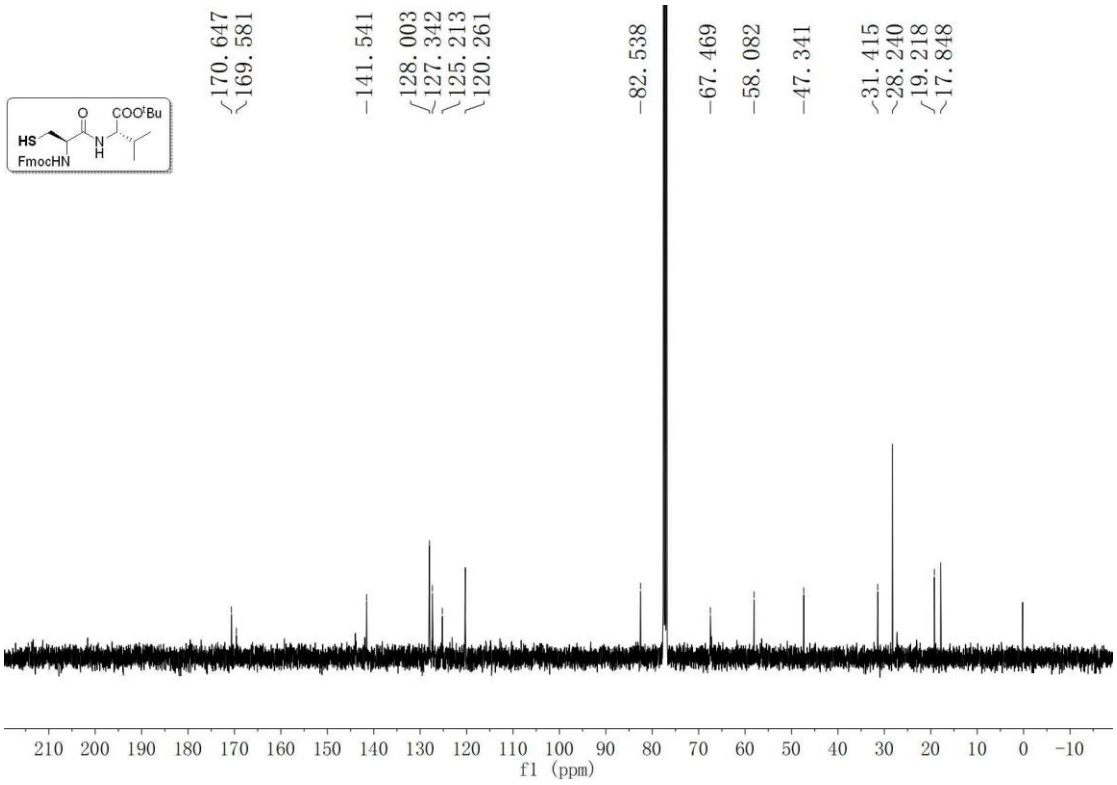
^{1}H NMR spectrum of **7c** in $CDCl_3$, 400 MHz



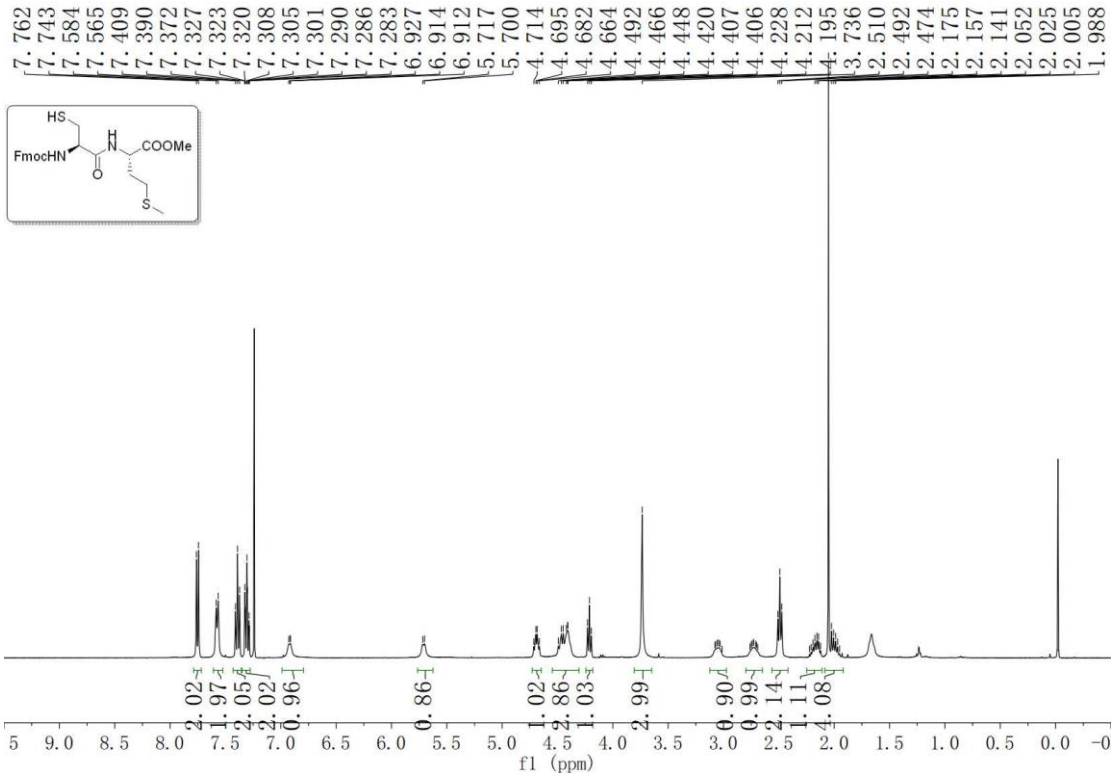
^{13}C NMR spectrum of **7c** in $CDCl_3$, 100 MHz



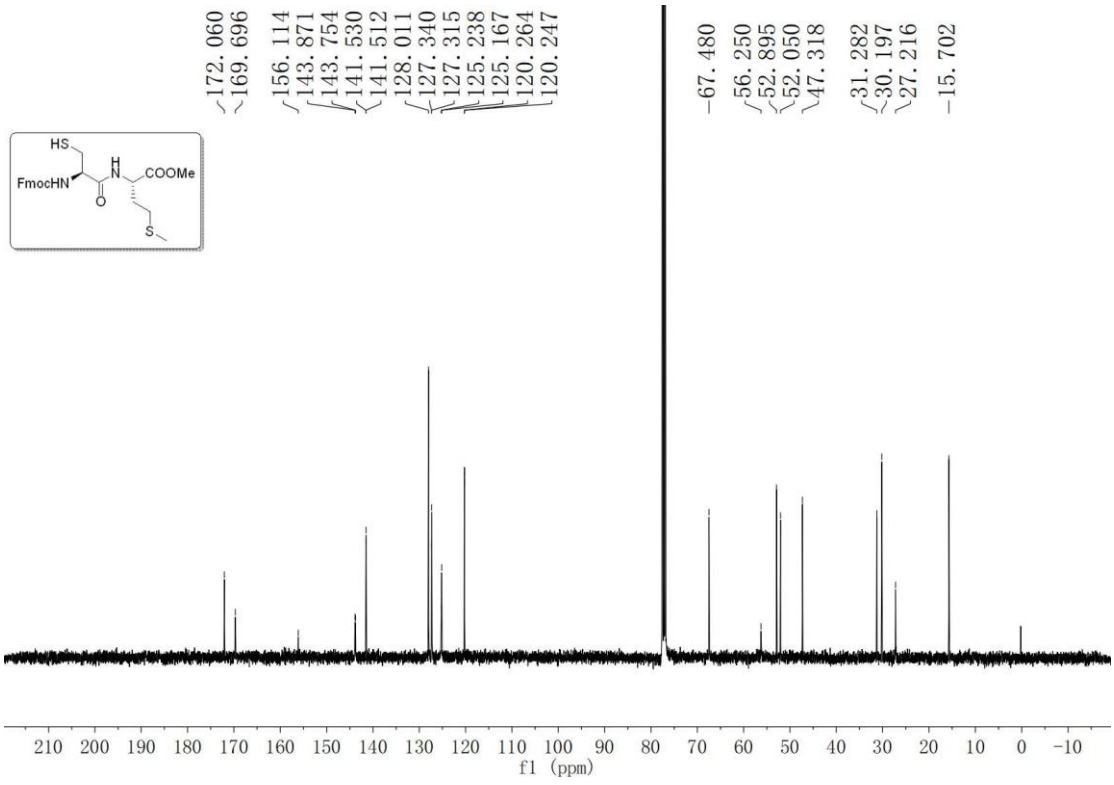
^{1}H NMR spectrum of **7d** in $CDCl_3$, 400 MHz



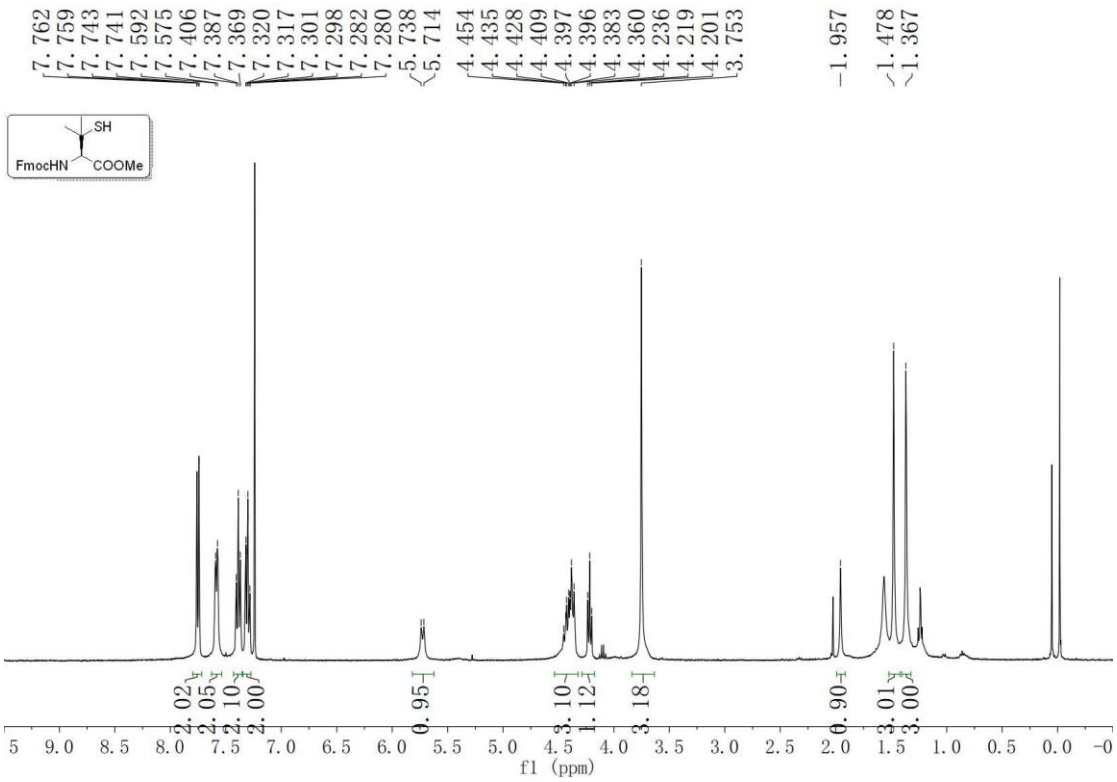
^{13}C NMR spectrum of **7d** in $CDCl_3$, 100 MHz



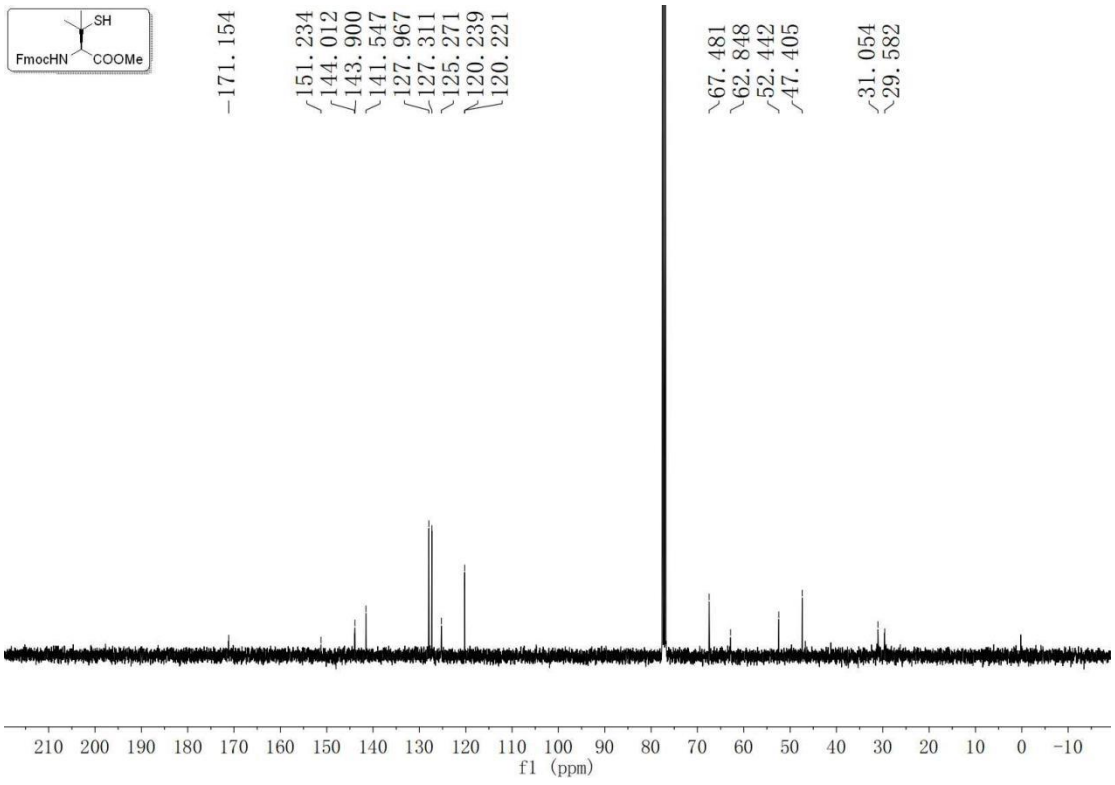
^{1}H NMR spectrum of **7e** in $CDCl_3$, 400 MHz



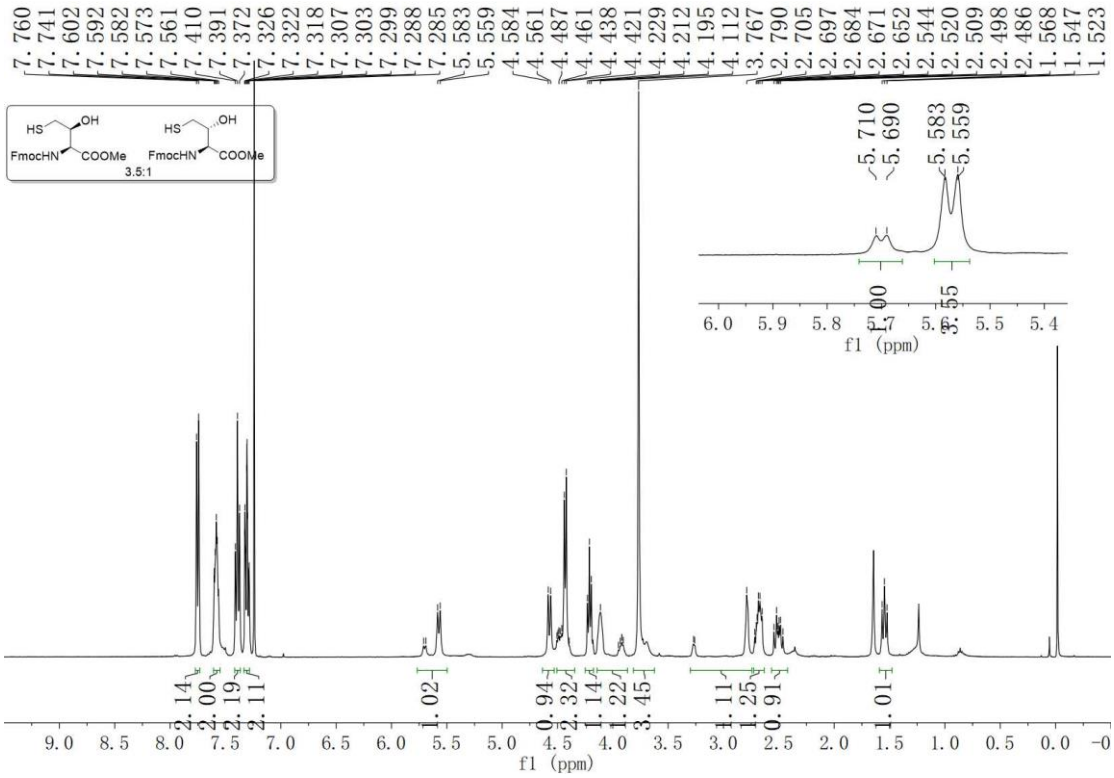
^{13}C NMR spectrum of **7e** in $CDCl_3$, 100 MHz



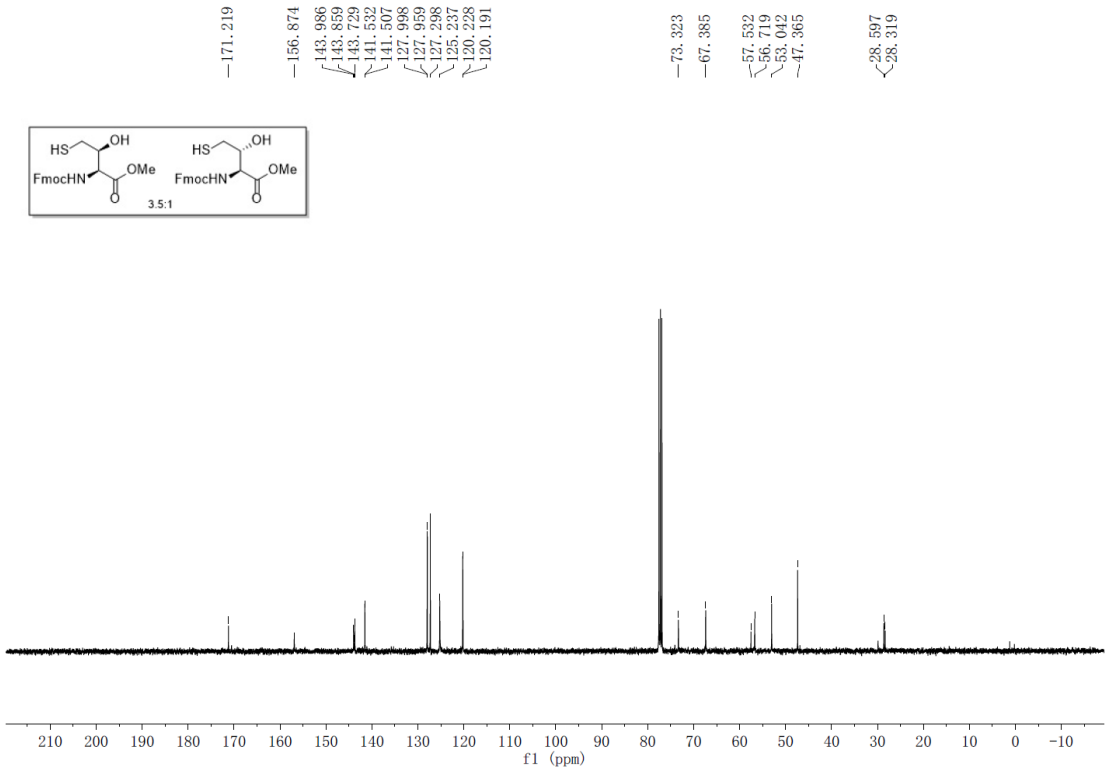
^{1}H NMR spectrum of **7f** in $CDCl_3$, 400 MHz



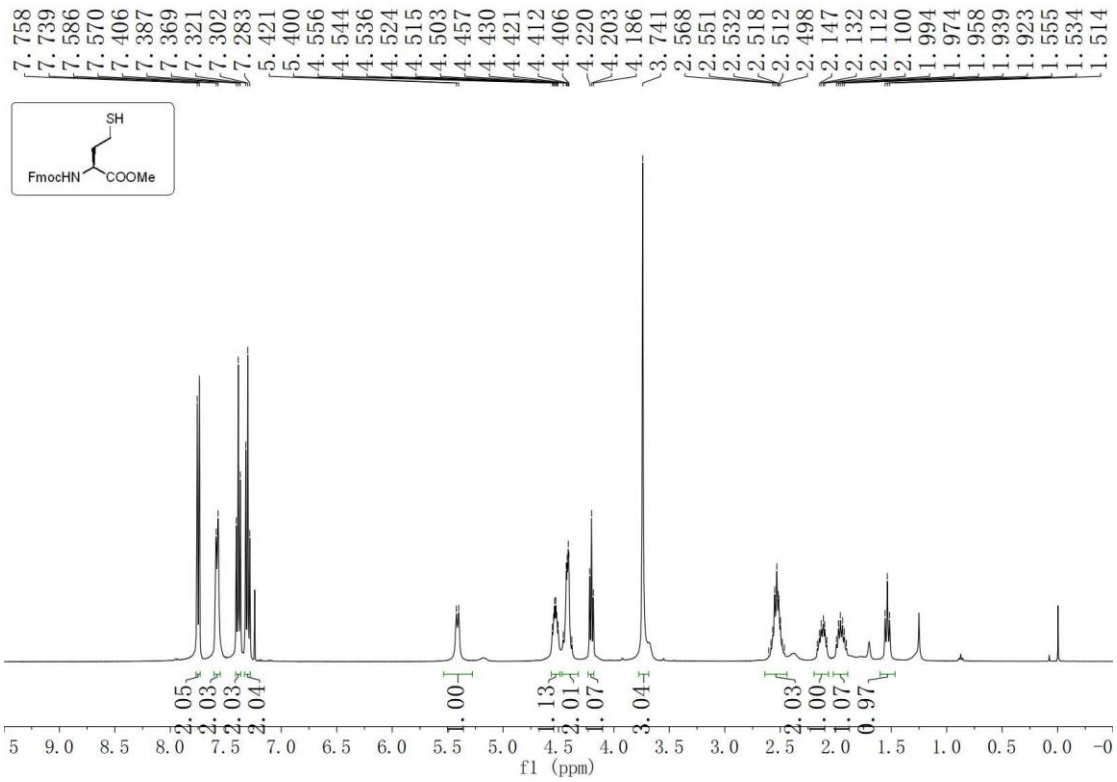
^{13}C NMR spectrum of **7f** in $CDCl_3$, 100 MHz



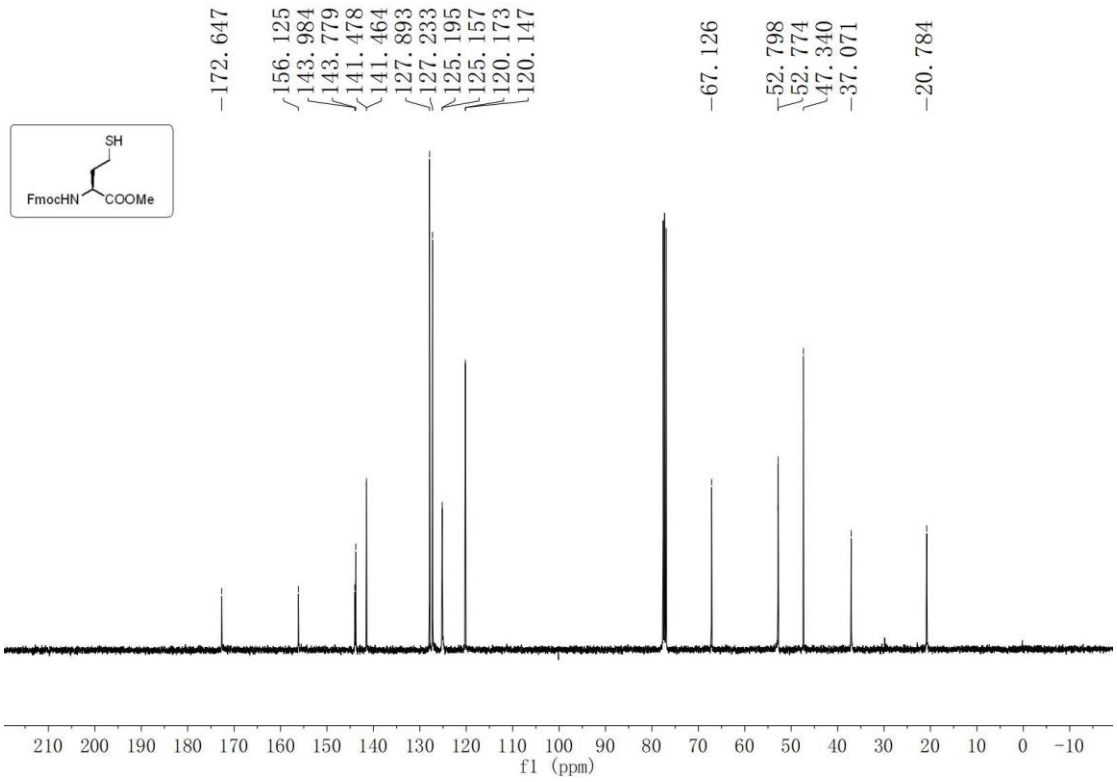
^{1}H NMR spectrum of **7g** in $CDCl_3$, 400 MHz



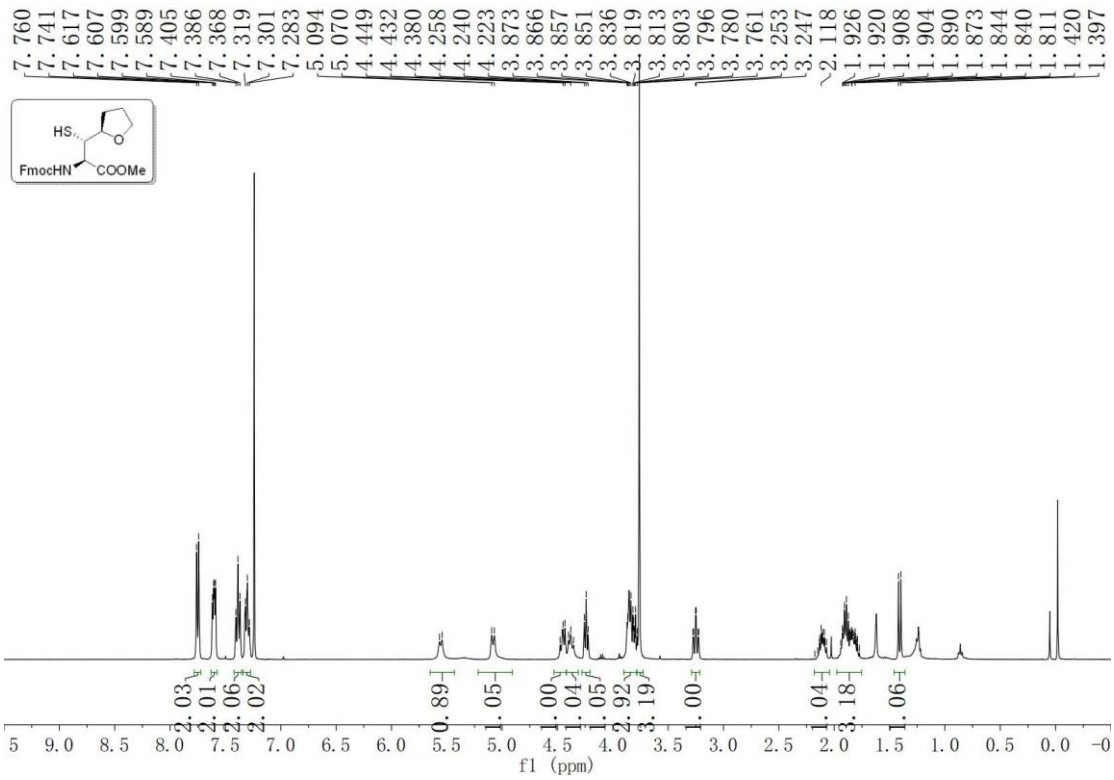
^{13}C NMR spectrum of **7g** in $CDCl_3$, 100 MHz



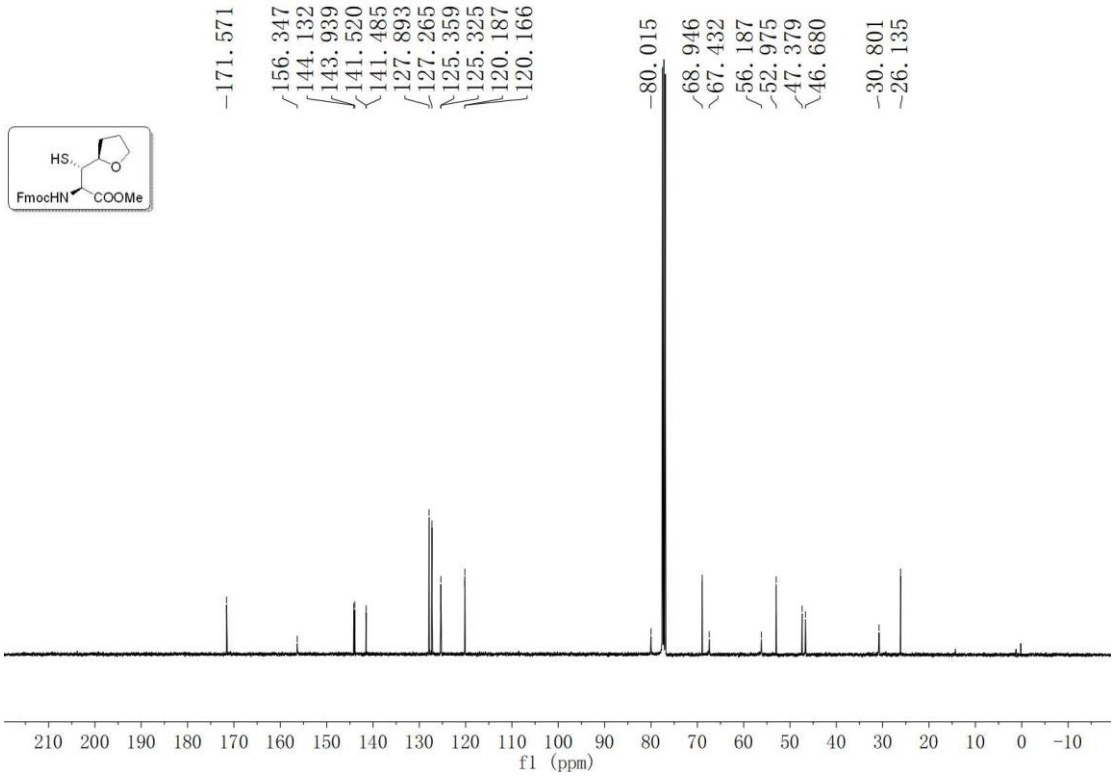
^{1}H NMR spectrum of **7h** in $CDCl_3$, 400 MHz



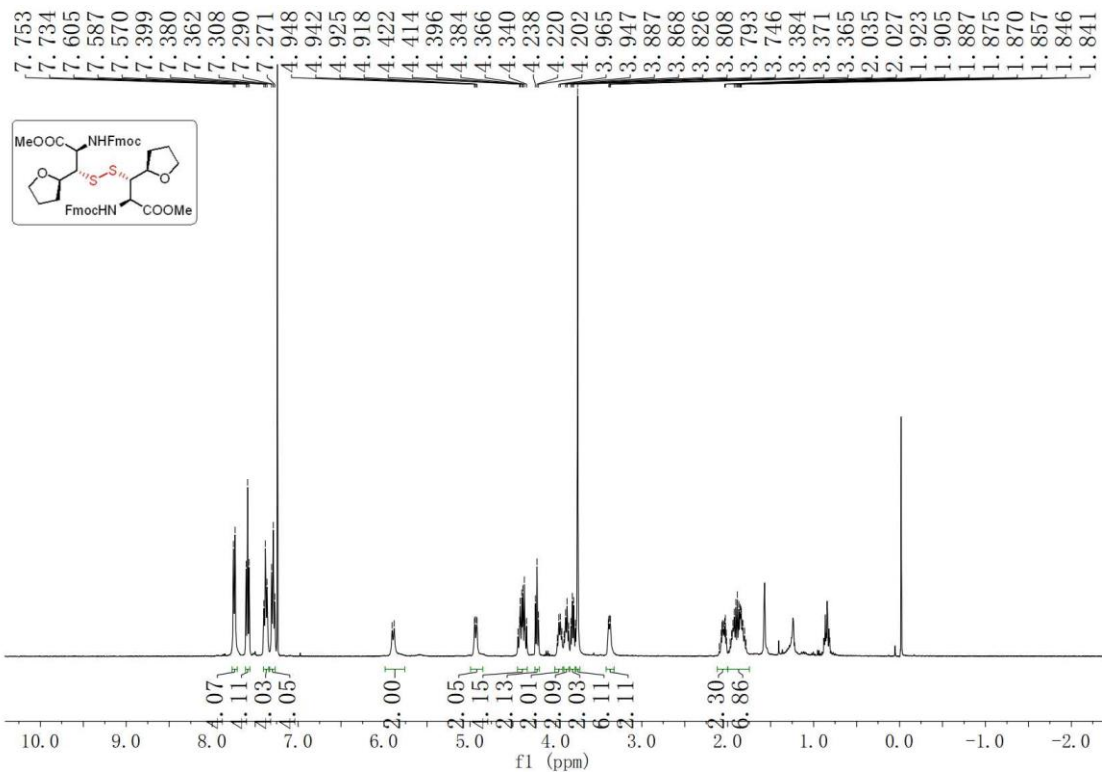
^{13}C NMR spectrum of **7h** in $CDCl_3$, 100 MHz



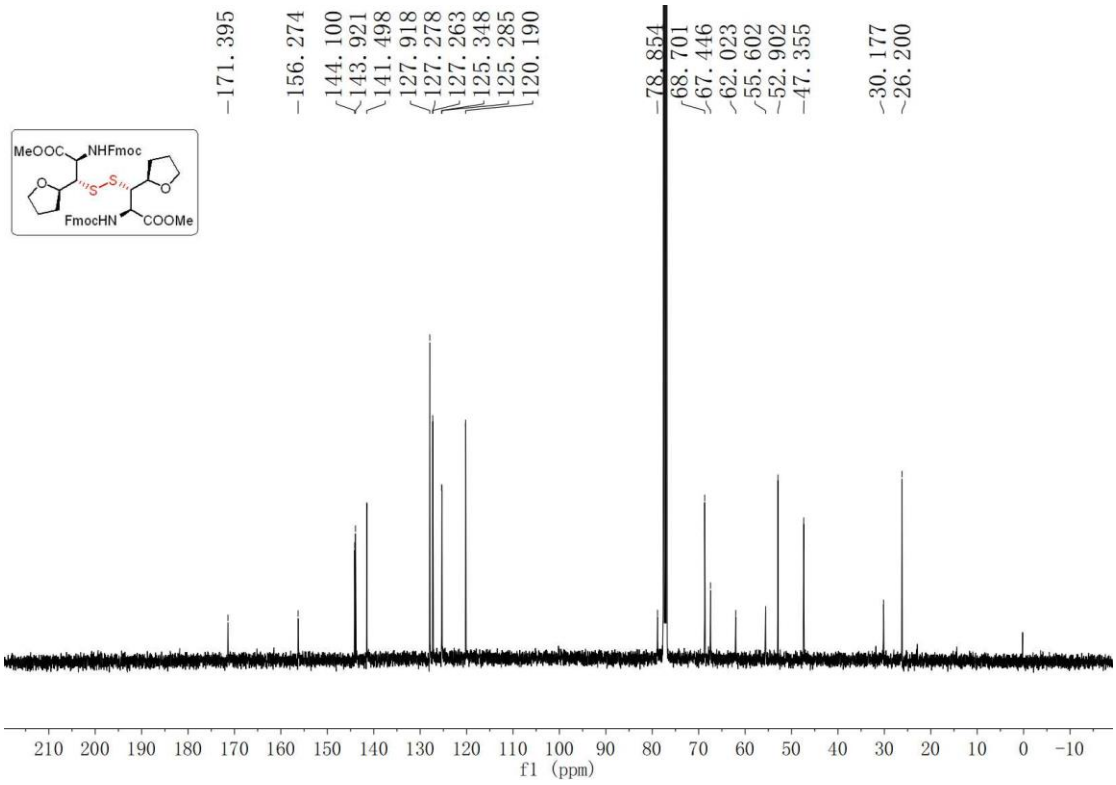
^{1}H NMR spectrum of **7i** in $CDCl_3$, 400 MHz



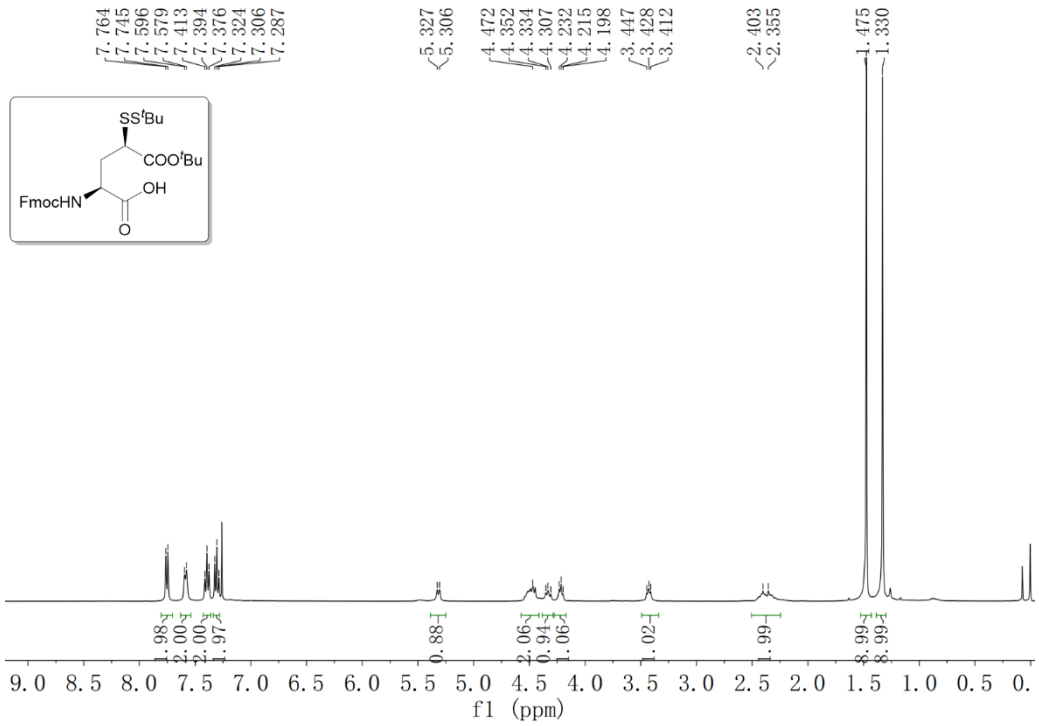
^{13}C NMR spectrum of **7i** in $CDCl_3$, 100 MHz



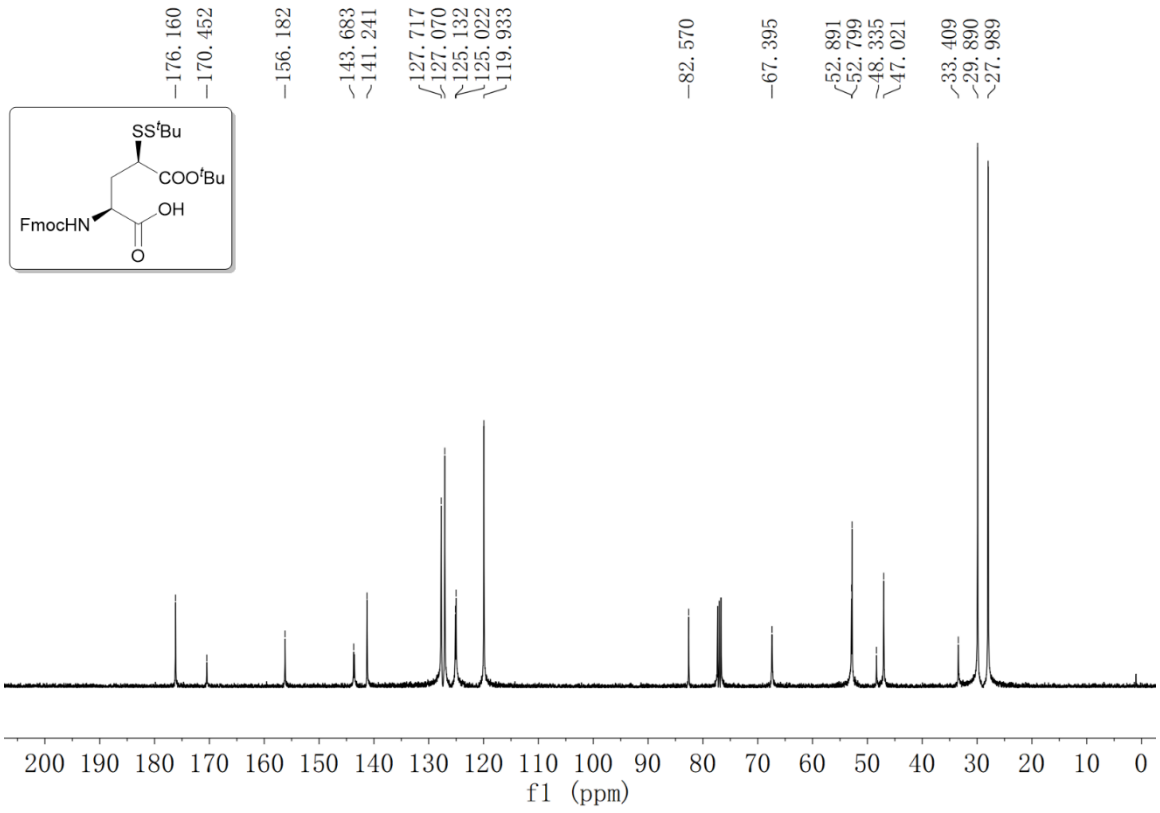
^{1}H NMR spectrum of **9** in $CDCl_3$, 400 MHz



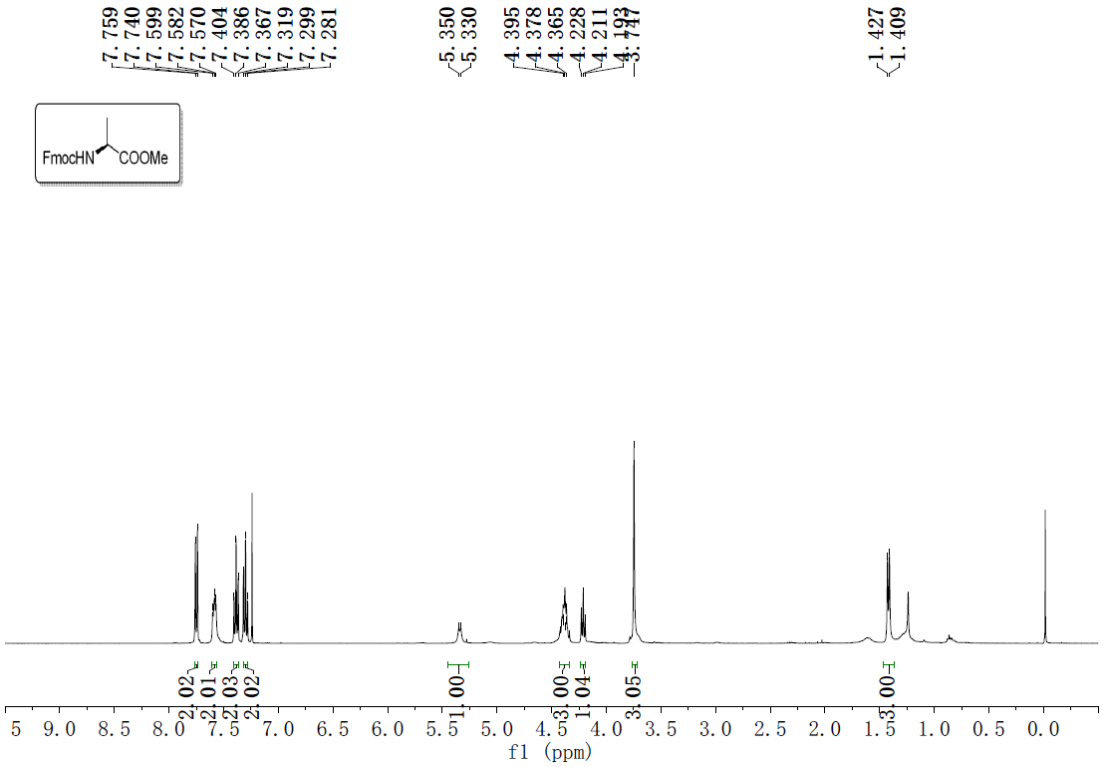
^{13}C NMR spectrum of **9** in $CDCl_3$, 100 MHz



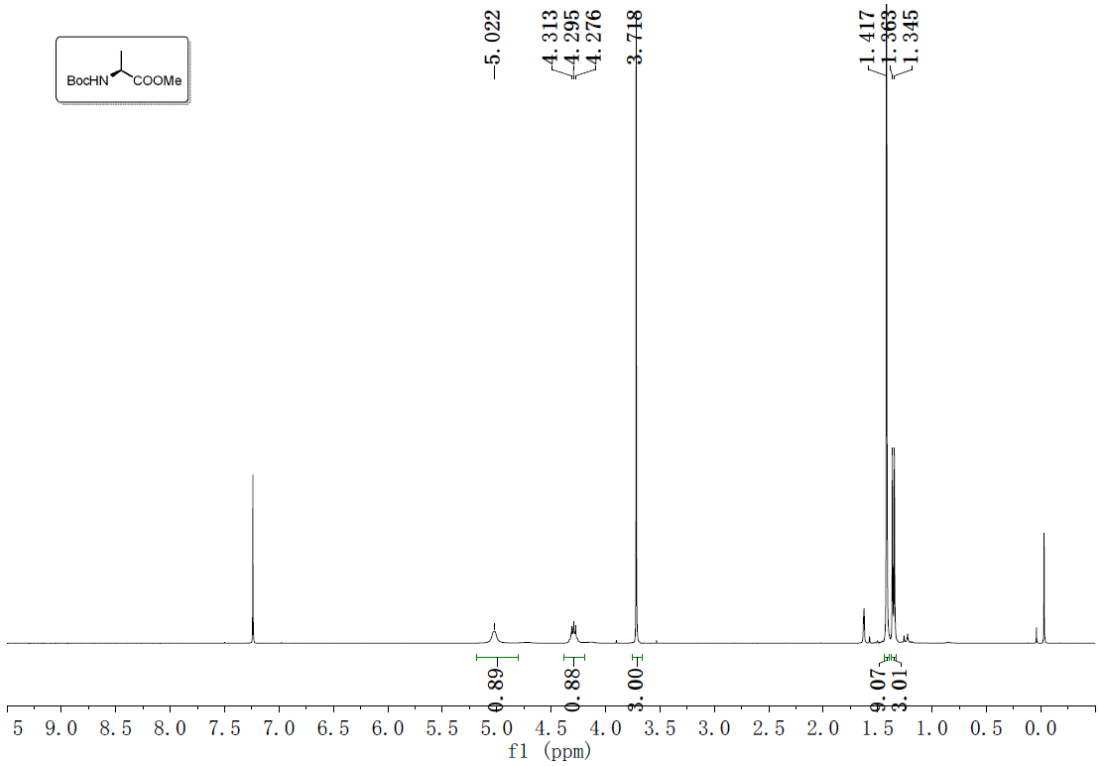
^{1}H NMR spectrum of **S15** in $CDCl_3$, 400 MHz



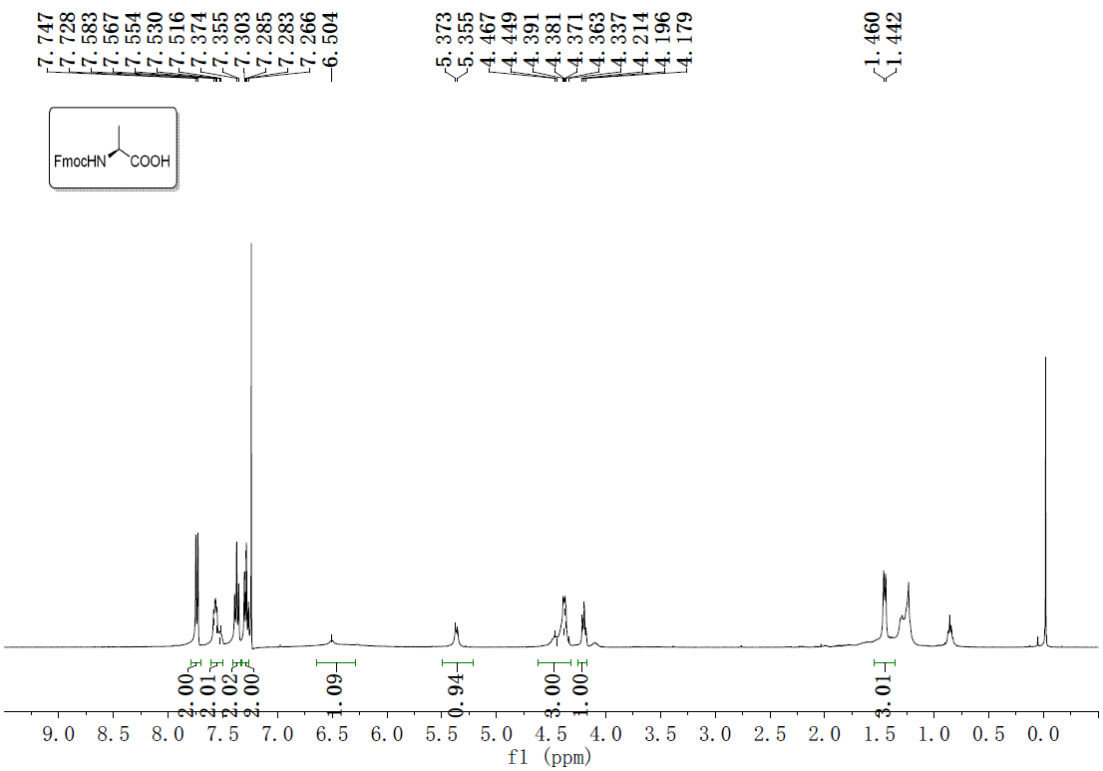
^{13}C NMR spectrum of **S15** in $CDCl_3$, 100 MHz



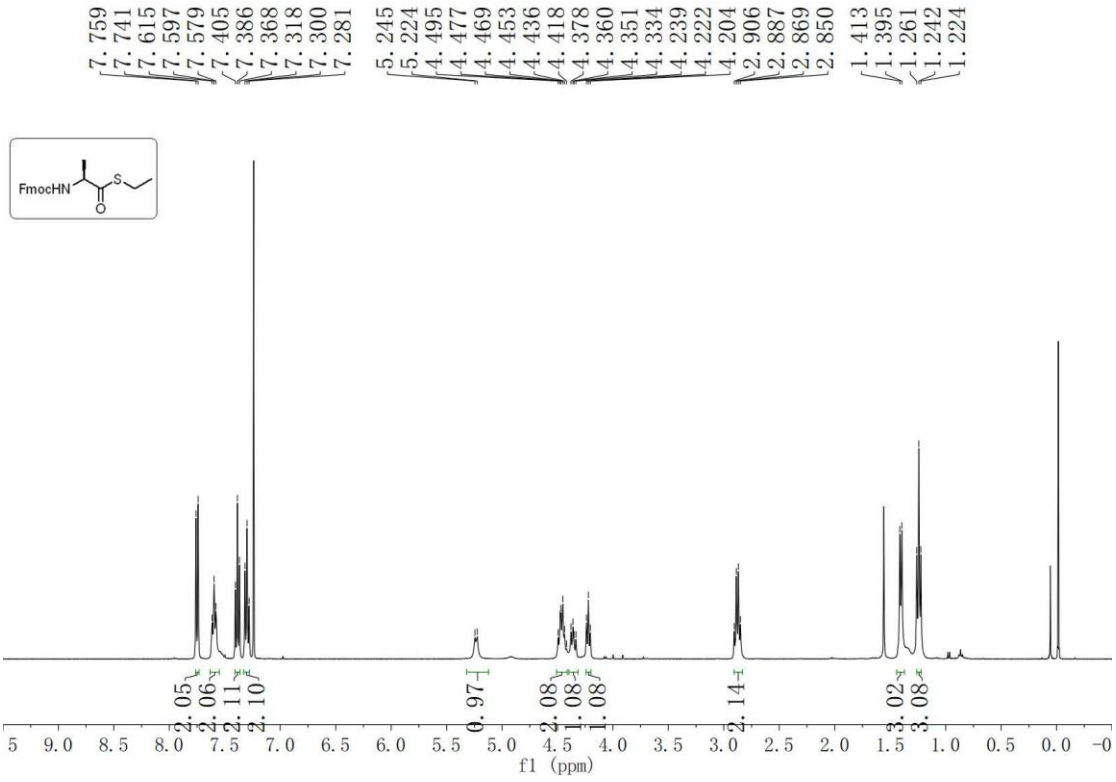
^{1}H NMR spectrum of **2** in $CDCl_3$, 400 MHz



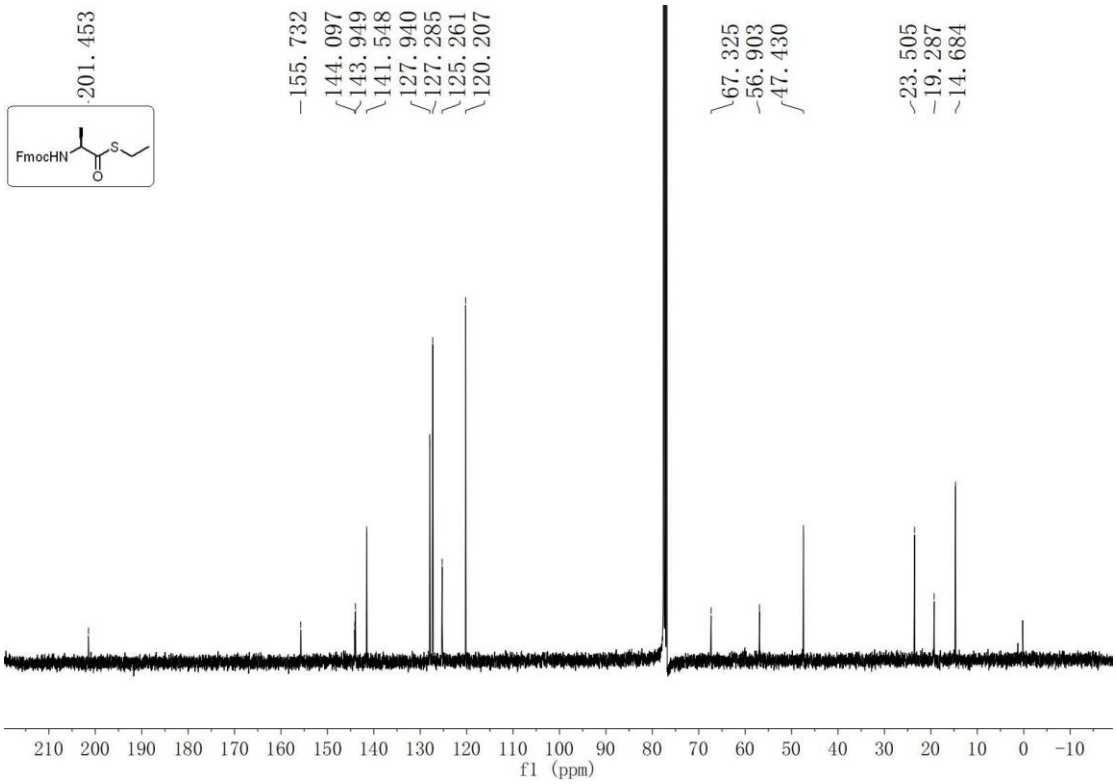
^{1}H NMR spectrum of **8a** in $CDCl_3$, 400 MHz



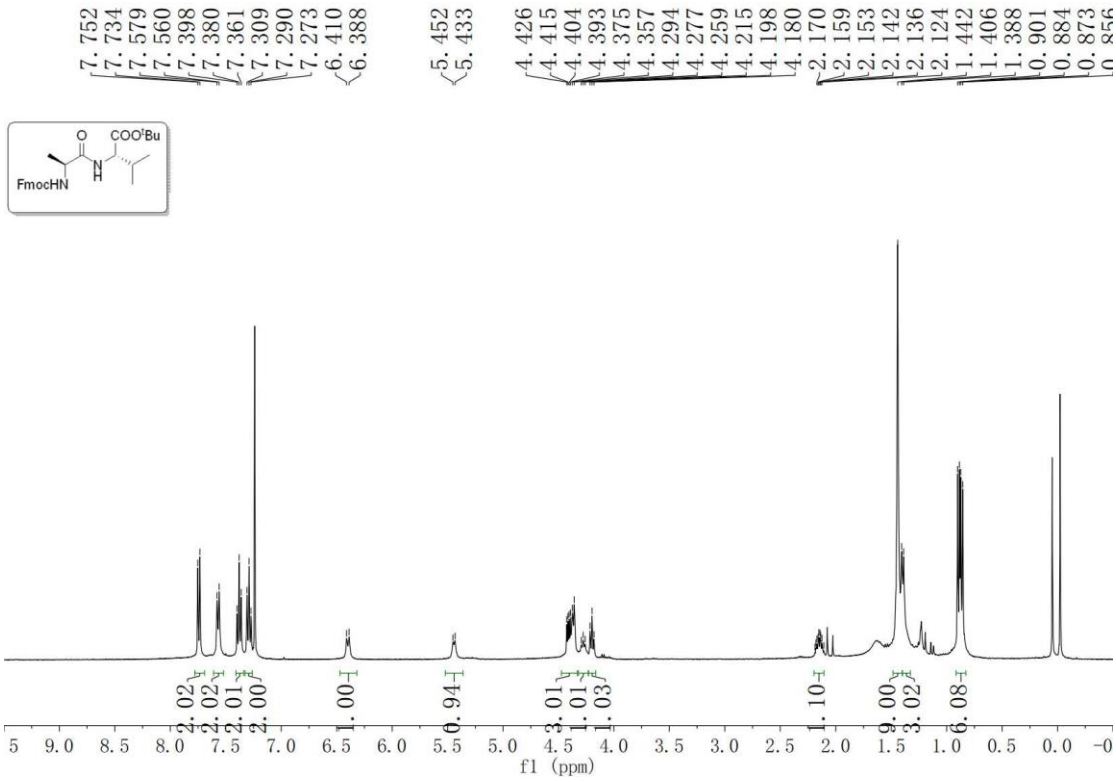
^{1}H NMR spectrum of **8b** in $CDCl_3$, 400 MHz



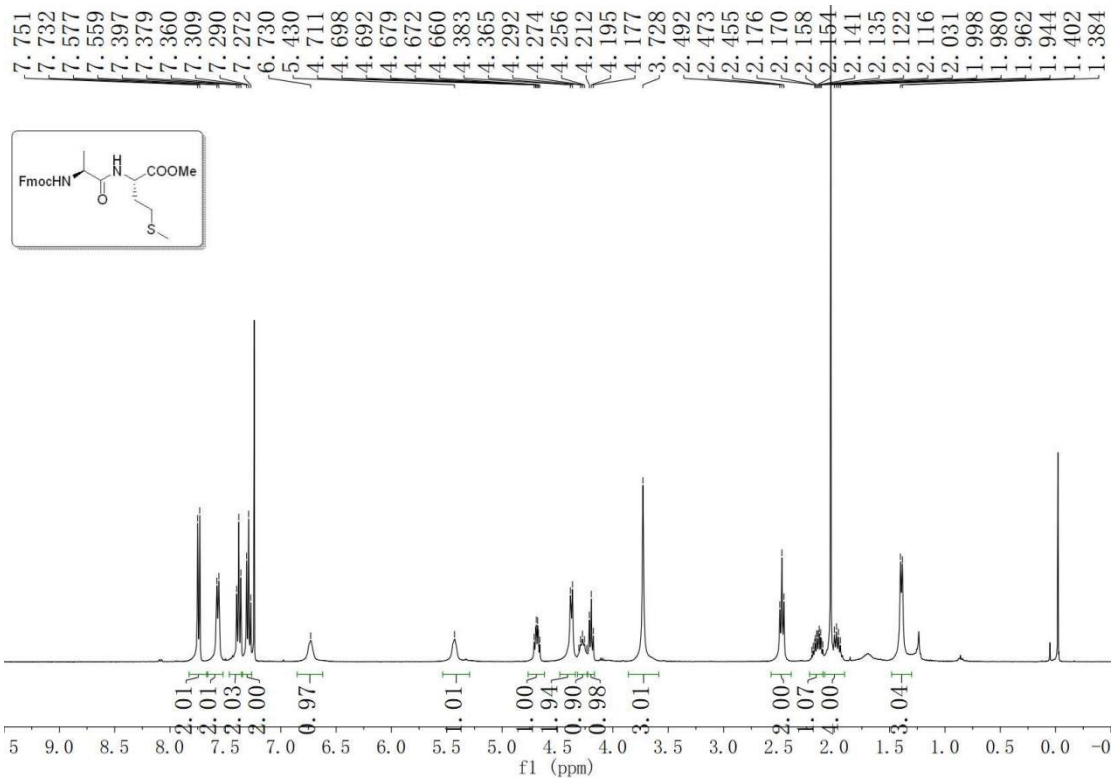
^{1}H NMR spectrum of **8c** in $CDCl_3$, 400 MHz



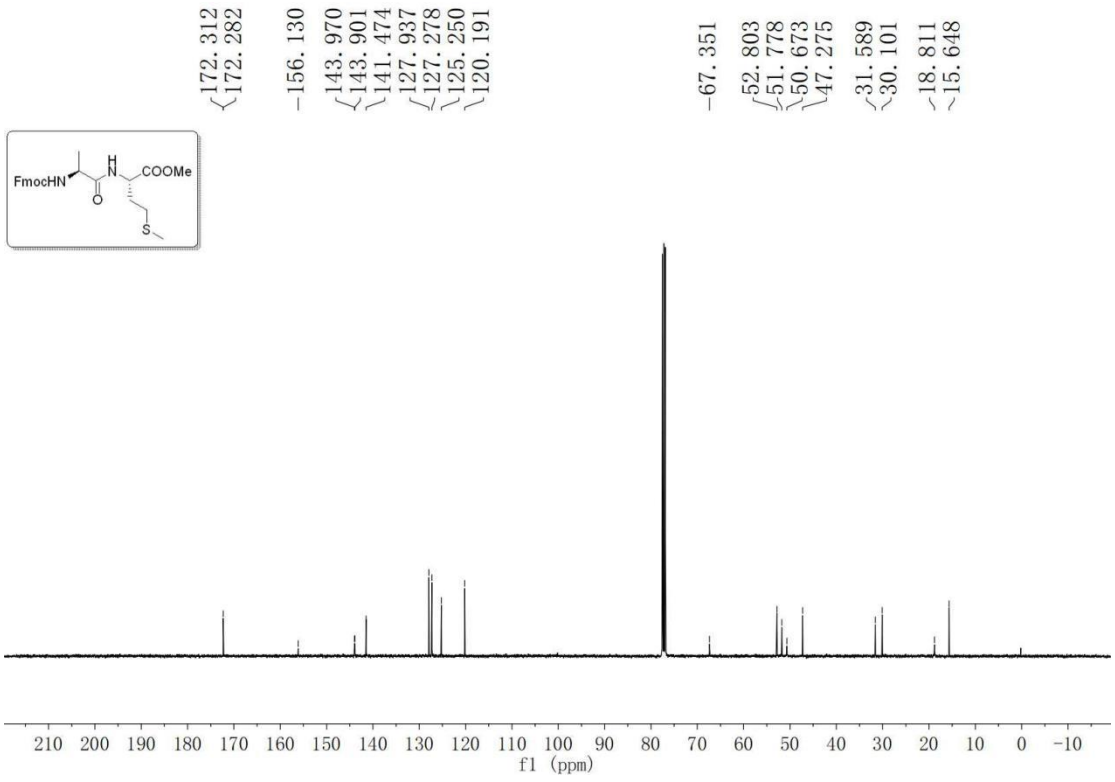
^{13}C NMR spectrum of **8c** in $CDCl_3$, 100 MHz



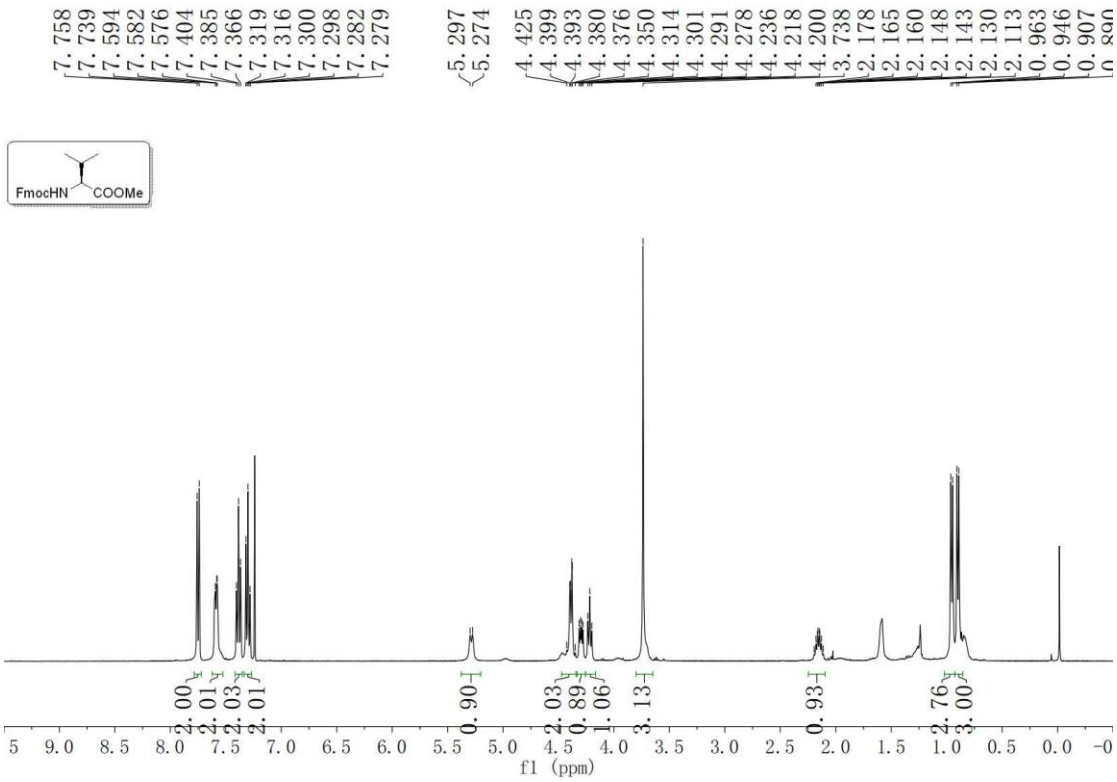
^{1}H NMR spectrum of **8d** in $CDCl_3$, 400 MHz



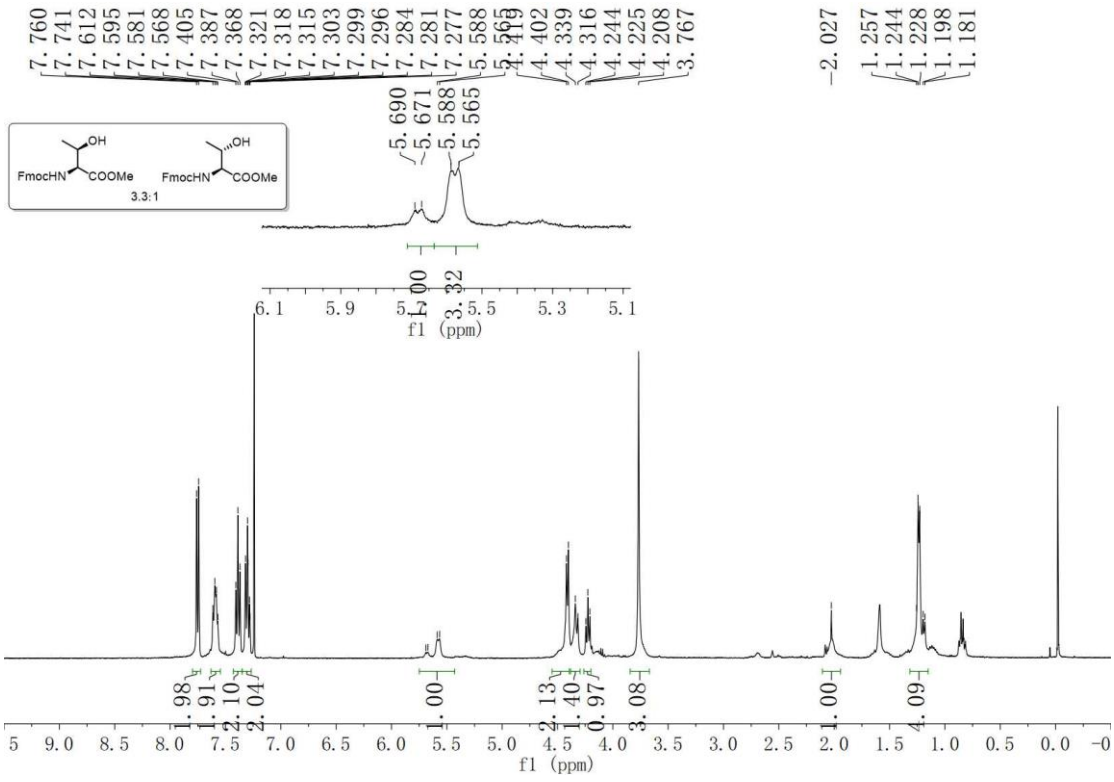
^{1}H NMR spectrum of **8e** in $CDCl_3$, 400 MHz



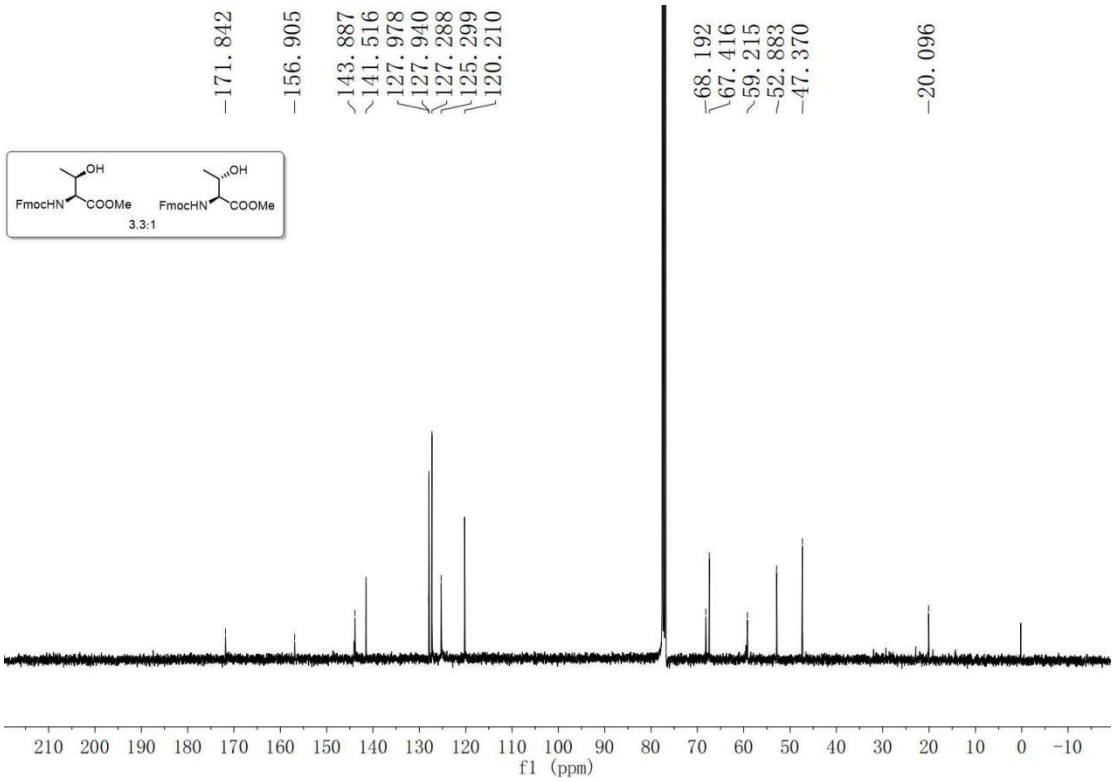
^{13}C NMR spectrum of **8e** in $CDCl_3$, 100 MHz



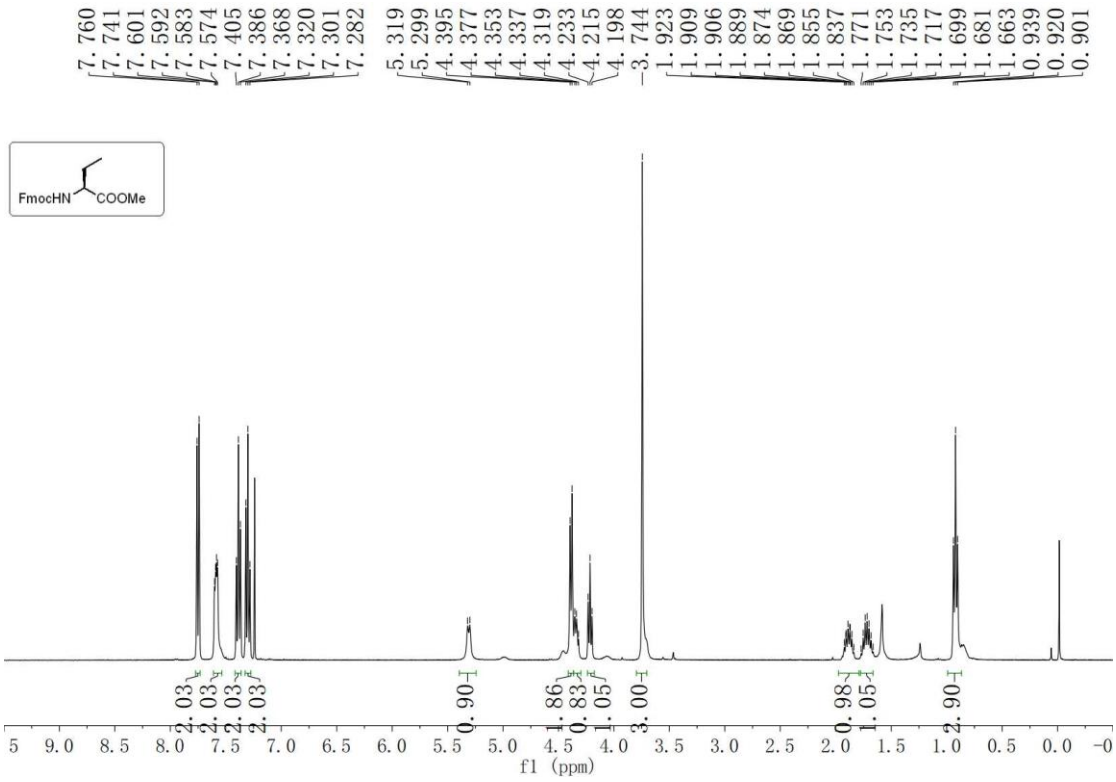
^{1}H NMR spectrum of **8f** in $CDCl_3$, 400 MHz



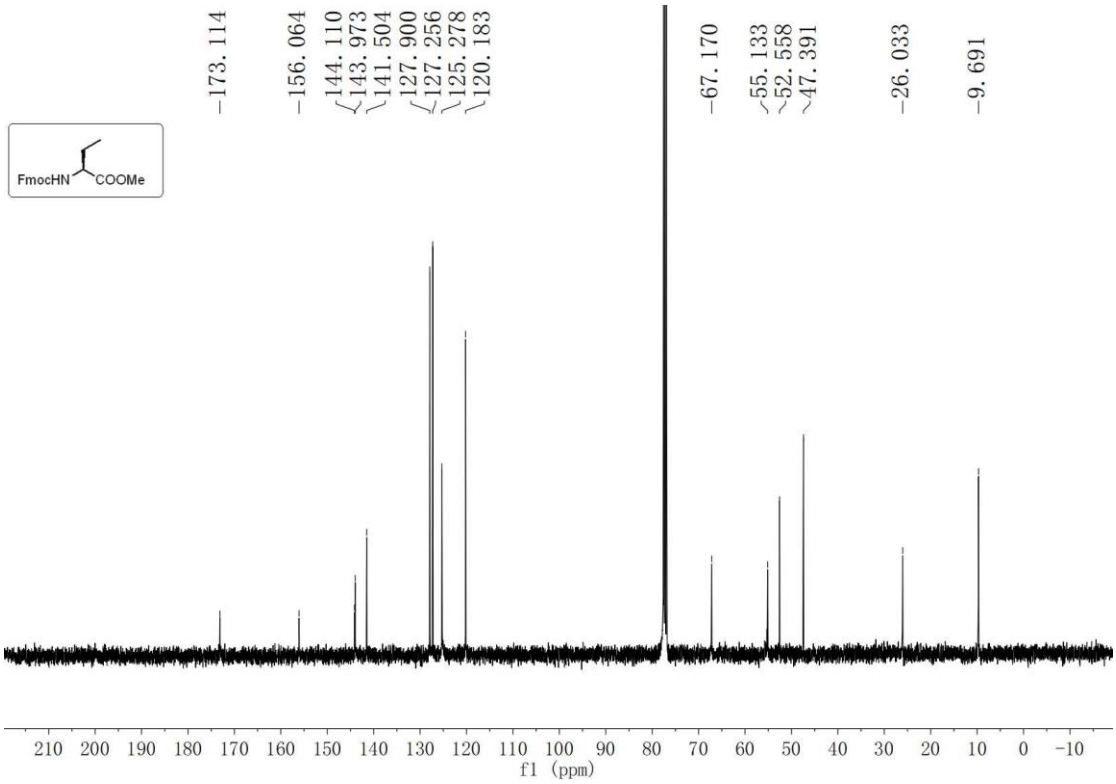
^{1}H NMR spectrum of **8g** in $CDCl_3$, 400 MHz



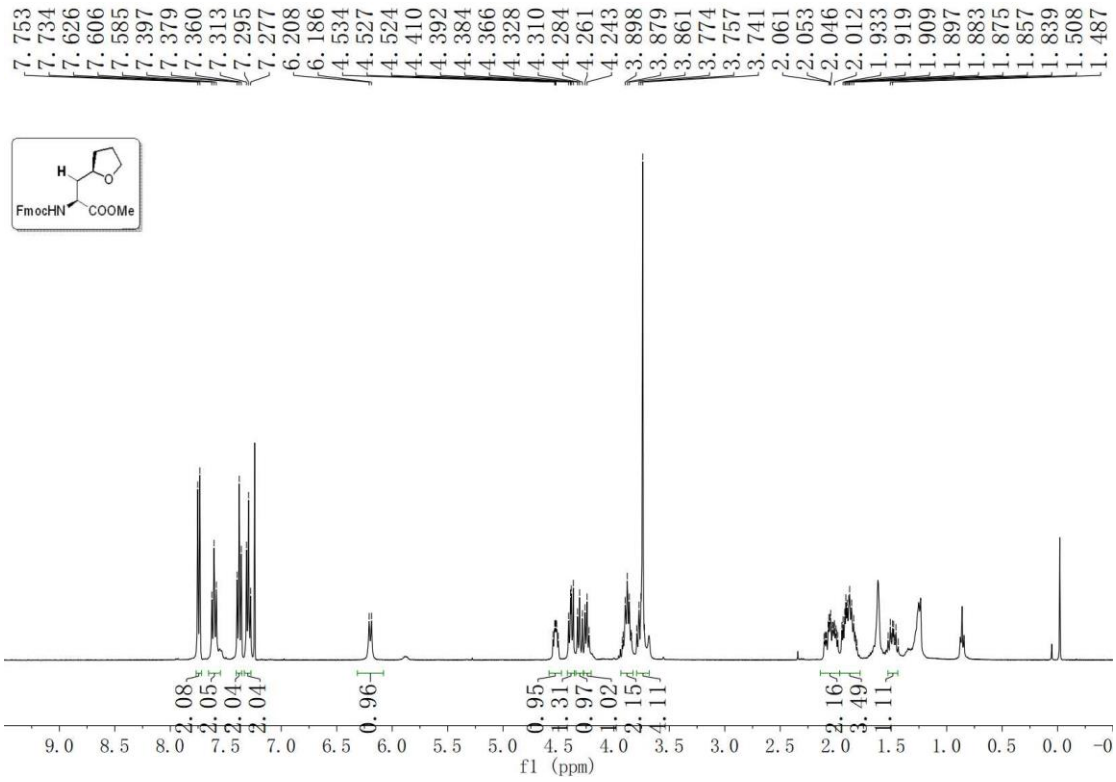
^{13}C NMR spectrum of **8g** in $CDCl_3$, 100 MHz



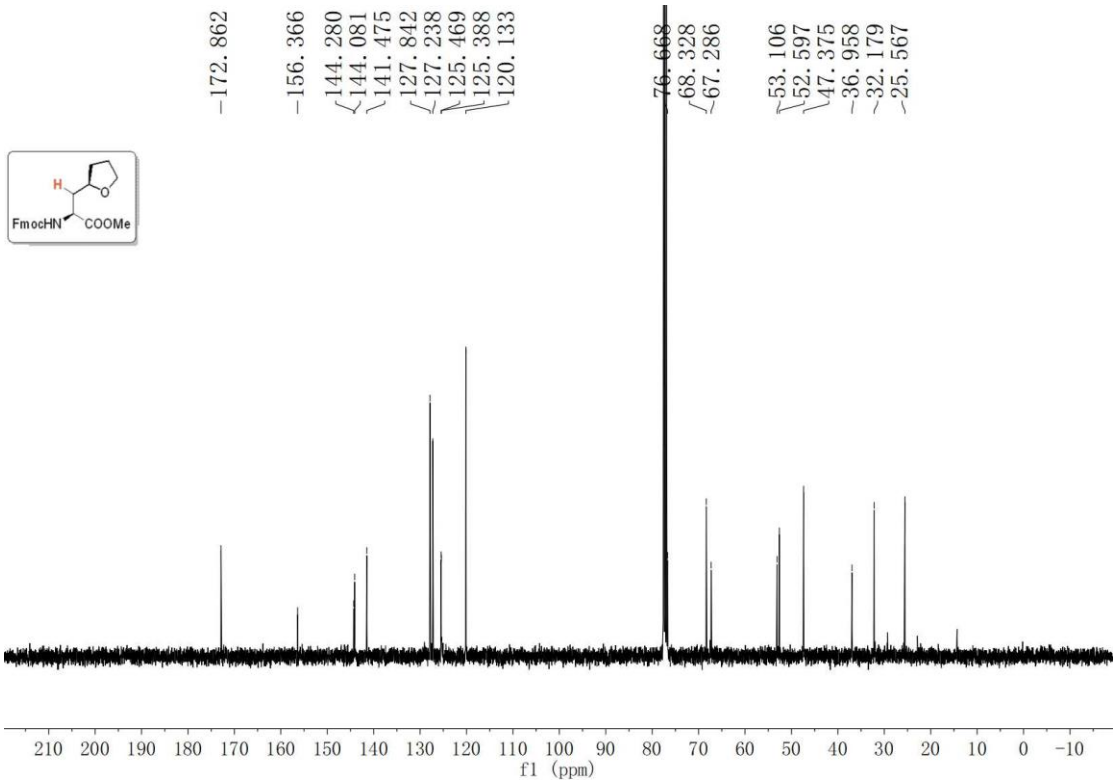
^{1}H NMR spectrum of **8h** in $CDCl_3$, 400 MHz



^{13}C NMR spectrum of **8h** in $CDCl_3$, 100 MHz



^{1}H NMR spectrum of **8i** in $CDCl_3$, 400 MHz



^{13}C NMR spectrum of **8i** in $CDCl_3$, 100 MHz

VII. Preparation and Characterization of Peptides[14]

*Model Peptide **SP1***

H−SVGCALVKLA−NH_2

SP1

Chemical Formula: $C_{42}H_{78}N_{12}O_{11}S$
Exact Mass: 958.56
Molecular Weight: 959.22

Peptide **SP1** was prepared according to General Procedure **2.2** & **2.5** using Rink amide MBHA resin (0.35 mmol/g, 0.05 mmol) and other standard $^{\alpha}N$-Fmoc amino acids. After global deprotection using TFA/TIS/H_2O (95/2.5/2.5, *v/v/v*), the crude peptide was dissolved in 10 mL of CH_3CN/H_2O/AcOH (35/60/5, *v/v/v*) and further purified using RP-HPLC (linear gradient 17-30% solvent B over 30 min, Exsil Pure 300 C18 column). Product eluted at 18.0-19.5 min. The fractions were collected and lyophilized to provide peptide **SP1** (26.9 mg, 56%) as a fluffy white solid.

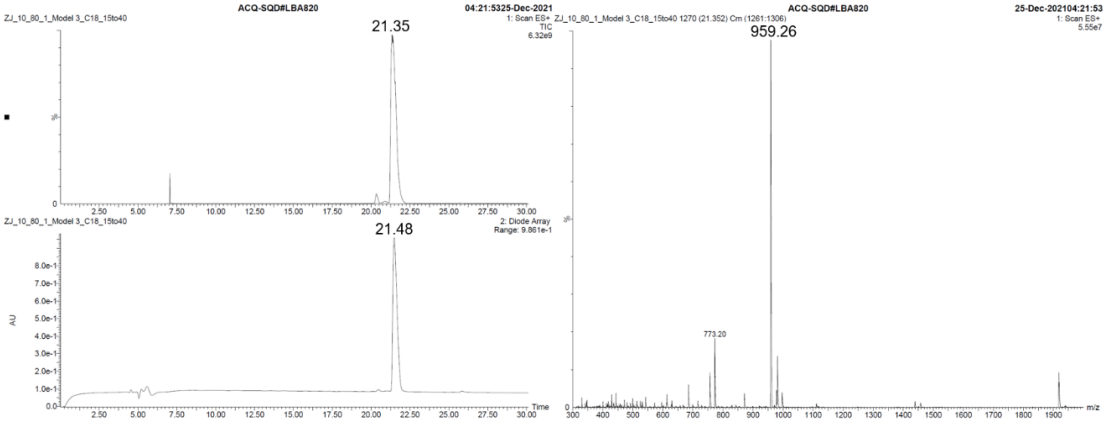


Figure S1. HPLC-MS analysis of peptide **SP1**. Left: MS (Top) and UV (Bottom) traces (Linear gradient of 15-40% solvent B over 30 min, Agilent C18 column, t_R = 21.4 min); Right: ESI-MS data. Calcd for $C_{42}H_{78}N_{12}O_{11}S$: 959.22 Da (average isotopes), $[M+H]^+$ *m/z* = 959.57; observed: 959.26.

*Model Peptide **10a***

H−TLNSICPAEL−NH_2

10a

Chemical Formula: $C_{45}H_{78}N_{12}O_{15}S$
Exact Mass: 1058.54
Molecular Weight: 1059.25

[14] The fragmentation signals of some peptides in ESI-MS data may result from the high source and desolvation temperature of mass spectrometers.

Peptide **10a** was prepared according to General Procedure **2.2 & 2.5** using Rink amide HMBA resin (0.35 mmol/g, 0.05 mmol) and other standard $^{\alpha}N$-Fmoc amino acids. After global deprotection using TFA/TIS/H_2O (95/2.5/2.5, *v/v/v*), the crude peptide was dissolved in 9 mL of CH_3CN/H_2O/AcOH (33/62/5, *v/v/v*) and further purified using RP-HPLC (linear gradient 15-30% solvent B over 30 min, Exsil Pure 300 C18 column). Product eluted at 18.0-19.5 min. The fractions were collected and lyophilized to provide peptide **10a** (15.8 mg, 30%) as a fluffy white solid.

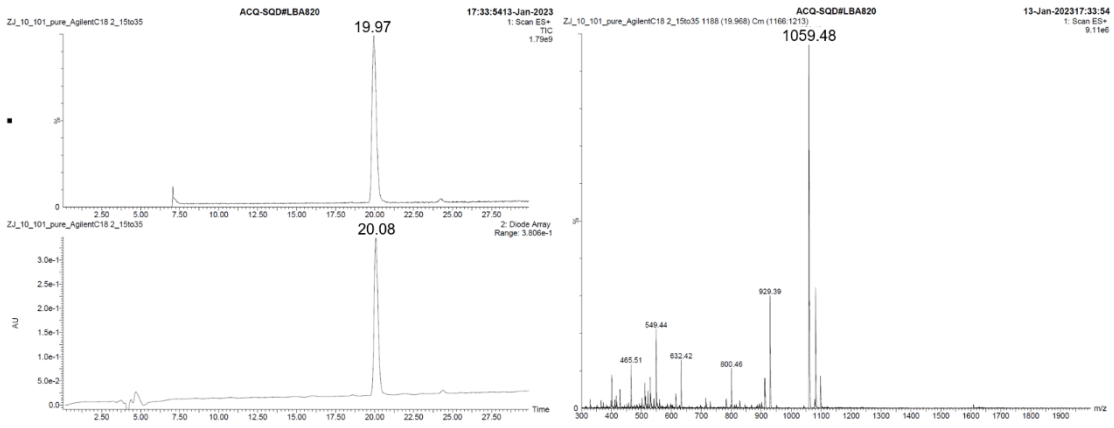


Figure S2. HPLC-MS analysis of peptide **10a**. Left: MS (Top) and UV (Bottom) traces (Linear gradient of 20-45% solvent B over 30 min, Agilent C18 column, t_R = 20.1 min); Right: ESI-MS data. Calcd for $C_{45}H_{78}N_{12}O_{15}S$: 1059.25 Da (average isotopes), $[M+H]^+$ *m/z* = 1059.55; observed: 1059.48.

*Desulfurized Peptide **10b***

H−TLNSIAPAEL−NH_2
10b

Chemical Formula: $C_{45}H_{78}N_{12}O_{15}$
Exact Mass: 1026.57
Molecular Weight: 1027.19

Peptide **10a** (1.04 mg, 1.01 μmol) was subjected to the MFD conditions following General Procedure **2.6** as described previously. The reaction was quenched by addition of CH_3CN/H_2O/AcOH (10/85/5, *v/v/v*) and further purified using RP-HPLC (linear gradient 15-30% solvent B over 30 min, Exsil Pure 300 C18 column). Product eluted at 15.0-16.2 min. The fractions were collected and lyophilized to provide peptide **10b** (0.80 mg, 79%) as a fluffy white solid.

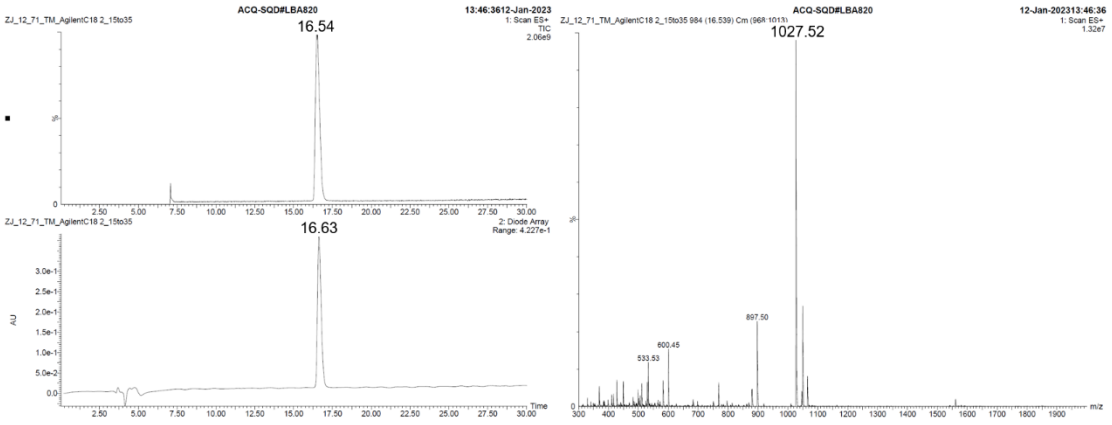


Figure S3. HPLC-MS analysis of peptide **10b**. Left: MS (Top) and UV (Bottom) traces (Linear gradient of 15-35% solvent B over 30 min, Agilent C18 column, t_R = 16.6 min); Right: ESI-MS data. Calcd for $C_{45}H_{78}N_{12}O_{15}$: 1027.19 Da (average isotopes), $[M+H]^+$ *m/z* = 1027.58; observed: 1027.52.

*Model Peptide **11a***

H−THMSYCGAEN−NH_2

11a

Chemical Formula: $C_{44}H_{66}N_{14}O_{16}S_2$
Exact Mass: 1110.42
Molecular Weight: 1111.21

Peptide **11a** was prepared according to General Procedure **2.2 & 2.5** using Rink amide HMBA resin (0.35 mmol/g, 0.05 mmol) and other standard $^{\alpha}N$-Fmoc amino acids. After global deprotection using TFA/TIS/H_2O (95/2.5/2.5, *v/v/v*), the crude peptide was dissolved in 8 mL of CH_3CN/H_2O/AcOH (30/65/5, *v/v/v*) and further purified using RP-HPLC (linear gradient 5-25% solvent B over 30 min, Exsil Pure 300 C18 column). Product eluted at 15.7-17.0 min. The fractions were collected and lyophilized to provide peptide **11a** (24.2 mg, 44%) as a fluffy white solid.

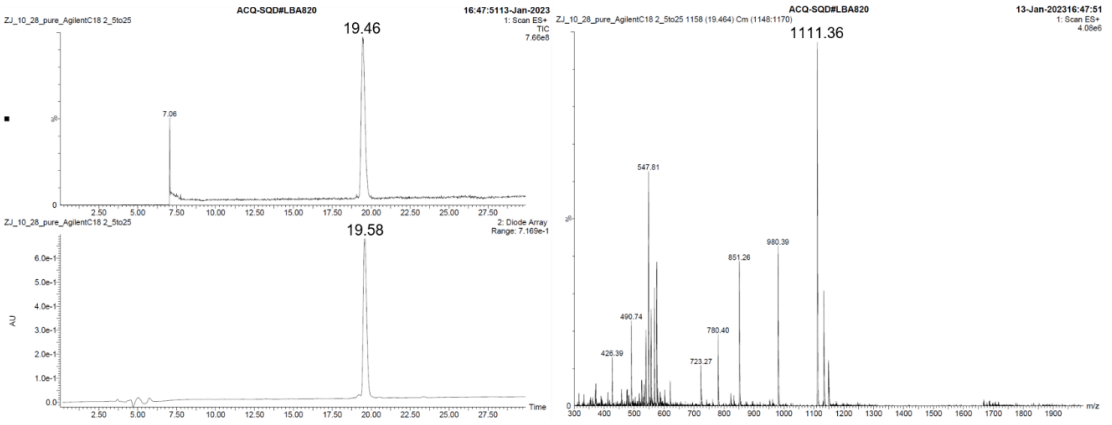


Figure S4. HPLC-MS analysis of peptide **11a**. Left: MS (Top) and UV (Bottom) traces (Linear gradient of 5-25% solvent B over 30 min, Agilent C18 column, t_R = 19.6 min); Right: ESI-MS data. Calcd for $C_{44}H_{66}N_{14}O_{16}S_2$: 1111.21 Da (average isotopes), $[M+H]^+$ *m/z* = 1111.43; observed: 1111.36.

*Desulfurized Peptide **11b***

H−THMSYAGAEN−NH_2

11b

Chemical Formula: $C_{44}H_{66}N_{14}O_{16}S$
Exact Mass: 1078.45
Molecular Weight: 1079.15

Peptide **11a** (2.02 mg, 1.82 µmol) was subjected to the MFD conditions following General Procedure **2.6** as described previously. The reaction was quenched by addition of CH_3CN/H_2O/AcOH (10/85/5, *v/v/v*) and further purified using RP-HPLC (linear gradient 5-20% solvent B over 30 min, Exsil Pure 300 C18 column). Product eluted at 17.5-18.5 min. The fractions were collected and lyophilized to provide peptide **11b** (1.61 mg, 82%) as a fluffy white solid.

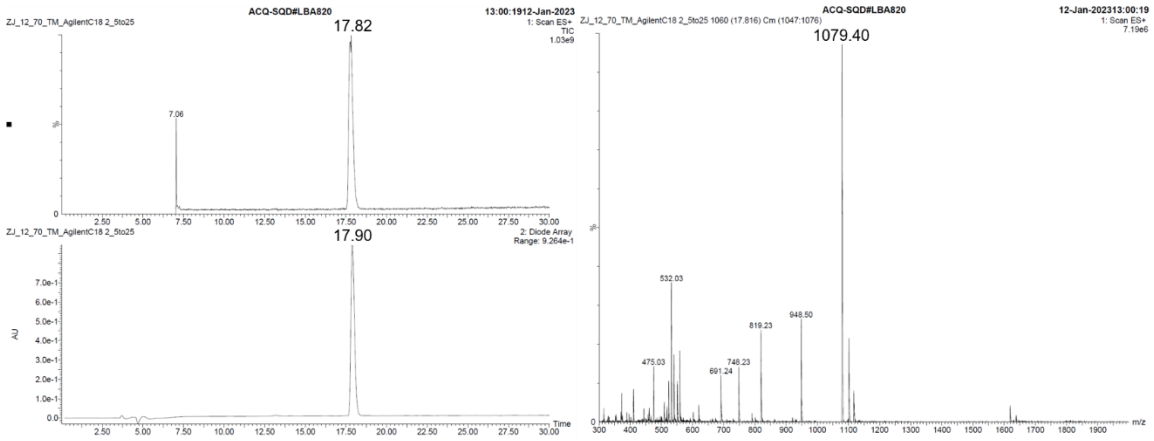


Figure S5. HPLC-MS analysis of peptide **11b**. Left: MS (Top) and UV (Bottom) traces (Linear gradient of 5-25% solvent B over 30 min, Agilent C18 column, t_R = 17.9 min); Right: ESI-MS data. Calcd for $C_{44}H_{66}N_{14}O_{16}S$: 1079.15 Da (average isotopes), $[M+H]^+$ *m/z* = 1079.46; observed: 1079.40.

*Model Peptide **12a***

H−TLWSICPAEL−NH_2

12a

Chemical Formula: $C_{52}H_{82}N_{12}O_{14}S$
Exact Mass: 1130.58
Molecular Weight: 1131.36

Peptide **12a** was prepared according to General Procedure **2.2 & 2.5** using Rink amide-MBHA resin (0.35 mmol/g, 0.05 mmol) and other standard $^{\alpha}N$-Fmoc amino acids. After global deprotection using TFA/TIS/H_2O (95/2.5/2.5, *v/v/v*), and the crude peptide was dissolved in 15 mL of CH_3CN/H_2O (20/80, *v/v*) and further purified using RP-HPLC (linear gradient 20-45% solvent B over 30 min, Exsil Pure 300 C18 column). Product eluted at 18.3-19.3 min. The fractions were collected and lyophilized to provide peptide **12a** (14.63 mg, 26%) as a fluffy white solid.

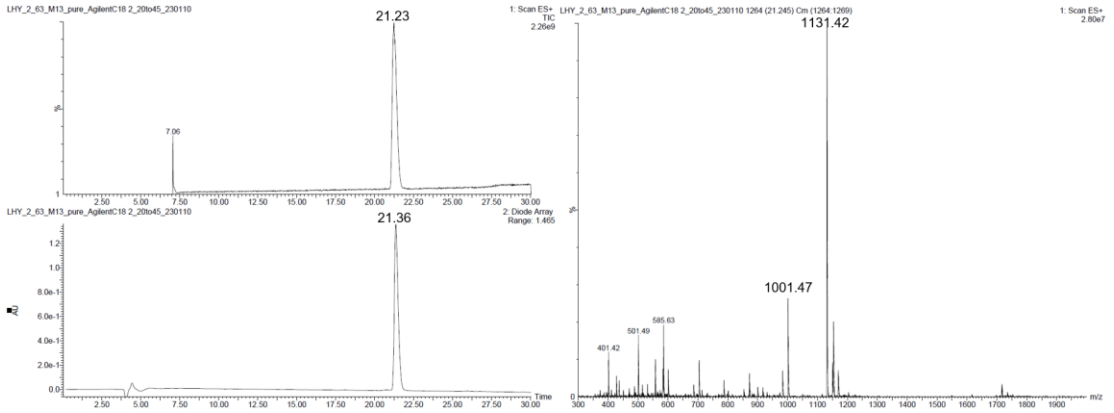


Figure S6. HPLC-MS analysis of peptide **12a**. Left: MS (Top) and UV (Bottom) traces (Linear gradient of 20-45% solvent B over 30 min, Agilent C18 column, t_R = 21.4 min); Right: ESI-MS data. Calcd for $C_{52}H_{82}N_{12}O_{14}S$: 1131.36 Da (average isotopes), $[M+H]^+$ *m/z* = 1131.59; observed: 1131.42.

*Desulfurized Peptide **12b***

H−TLWSIAPAEL−NH_2

12b

Chemical Formula: $C_{52}H_{82}N_{12}O_{14}$
Exact Mass: 1098.61
Molecular Weight: 1099.30

Peptide **12a** (1.02 mg, 0.90 µmol) was subjected to the MFD conditions following General Procedure **2.6** as described previously. The reaction was quenched by addition of CH_3CN/H_2O/AcOH (10/85/5, *v*/*v*/*v*) and further purified using RP-HPLC (linear gradient 20-45% solvent B over 30 min, Exsil Pure 300 C18 column). Product eluted at 16.3-17.1 min. The fractions were collected and lyophilized to provide peptide **12b** (0.94 mg, 95%) as a fluffy white solid.

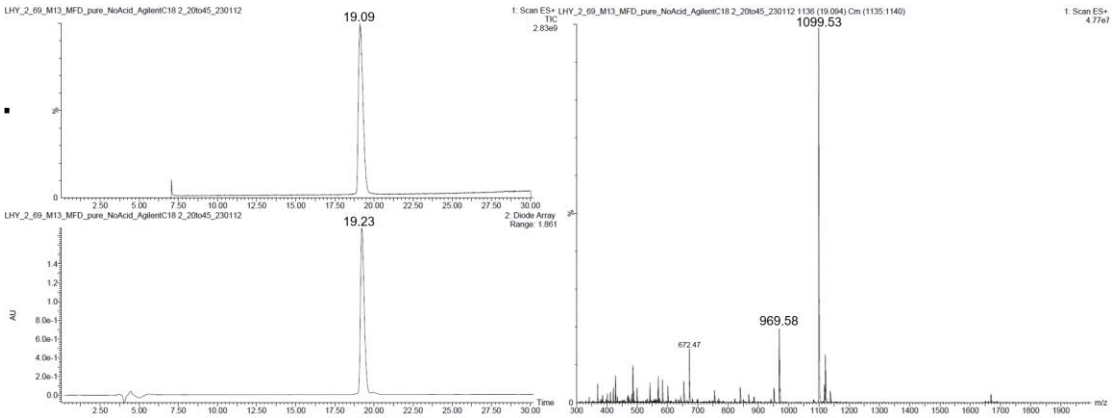


Figure S7. HPLC-MS analysis of peptide **12b**. Left: MS (Top) and UV (Bottom) traces (Linear gradient of 20-45% solvent B over 30 min, Agilent C18 column, t_R = 19.2 min); Right: ESI-MS data. Calcd for $C_{52}H_{82}N_{12}O_{14}$: 1099.30 Da (average isotopes), $[M+H]^+$ *m/z* = 1099.62; observed: 1099.53.

*Model peptide **13a***

H−SVGCTCVTPI−NH_2
13a

Chemical Formula: $C_{40}H_{71}N_{11}O_{13}S_2$
Exact Mass: 977.47
Molecular Weight: 978.19

Peptide **13a** was prepared according to General Procedure **2.2 & 2.5** using Rink amide-MBHA resin (0.35 mmol/g, 0.05 mmol) and other standard $^{\alpha}N$-Fmoc amino acids. After global deprotection using TFA/TIS/H_2O (95/2.5/2.5, *v/v/v*), the crude peptide was dissolved in 10 mL of CH_3CN/H_2O/AcOH (30/65/5, *v/v*) and further purified using RP-HPLC (linear gradient 10-25% solvent B over 30 min, Exsil Pure 300 C18 column). Product eluted at 16.5-17.5 min. The fractions were collected and lyophilized to provide peptide **13a** (9.3 mg, 19%) as a fluffy white solid.

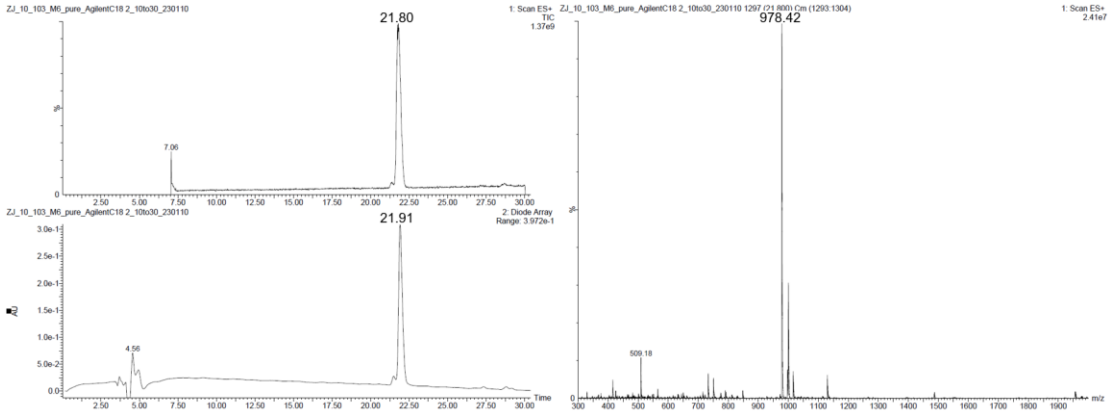


Figure S8. HPLC-MS analysis of peptide **13a**. Left: MS (Top) and UV (Bottom) traces (Linear gradient of 10-30% solvent B over 30 min, Agilent C18 column, t_R = 21.9 min); Right: ESI-MS data. Calcd for $C_{40}H_{71}N_{11}O_{13}S_2$: 978.19 Da (average isotopes), $[M+H]^+$ *m/z* = 978.48; observed: 978.42.

*Desulfurized Peptide **13b***

H−SVGATAVTPI−NH_2
13b

Chemical Formula: $C_{40}H_{71}N_{11}O_{13}$
Exact Mass: 913.52
Molecular Weight: 914.07

Peptide **13b** (0.90 mg, 0.92 µmol) was subjected to the MFD conditions following General Procedure **2.6** as described previously. The reaction was quenched by addition of CH_3CN/H_2O/AcOH (10/85/5, *v/v/v*) and further purified using RP-HPLC (linear gradient 10-25% solvent B over 30 min, Exsil Pure 300 C18 column). Product eluted at 17.6-18.3 min. The fractions were collected and lyophilized to provide peptide **13b** (0.70 mg, 83%) as a fluffy white solid.

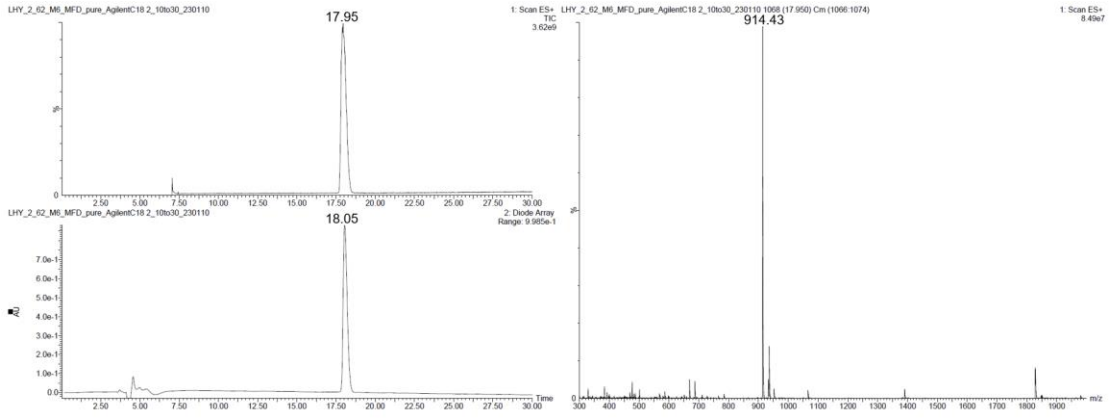


Figure S9. HPLC-MS analysis of peptide **13b**. Left: Left: MS (Top) and UV (Bottom) traces (Linear gradient of 10-30% solvent B over 30 min, Agilent C18 column, t_R = 18.0 min); Right: ESI-MS data. Calcd for $C_{40}H_{71}N_{11}O_{13}$: 914.07 Da (average isotopes), $[M+H]^+$ *m/z* = 914.53; observed: 914.43.

*Model peptide **14a***

H−SVGCTC(Acm)VTPI—NH_2

14a

Chemical Formula: $C_{43}H_{76}N_{12}O_{14}S_2$
Exact Mass: 1048.50
Molecular Weight: 1049.27

Peptide **14a** was prepared according to General Procedure **2.2 & 2.5** using Rink amide-MBHA resin (0.35 mmol/g, 0.05 mmol) and other standard $^{\alpha}N$-Fmoc amino acids. After global deprotection using TFA/TIS/H_2O (95/2.5/2.5, *v/v/v*), the crude peptide was dissolved in 15 mL of CH_3CN/H_2O (20/80, *v/v*) and further purified using RP-HPLC (linear gradient 8-30% solvent B over 30 min, Exsil Pure 300 C18 column). Product eluted at 17.6-18.3 min. The fractions were collected and lyophilized to provide peptide **9** (13.0 mg, 25%) as a fluffy white solid.

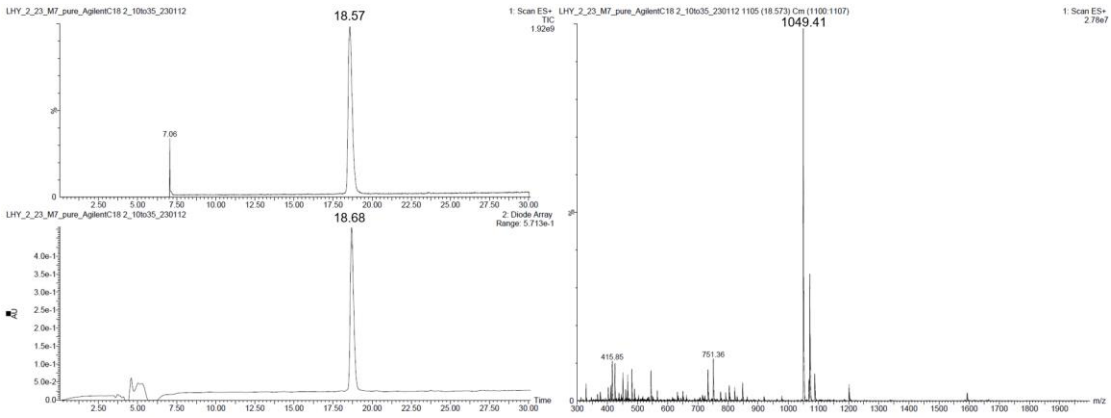


Figure S10. HPLC-MS analysis of peptide **14a**. Left: MS (Top) and UV (Bottom) traces (Linear gradient of 10-35% solvent B over 30 min, Agilent C18 column, t_R = 18.6 min); Right: ESI-MS data. Calcd for $C_{43}H_{76}N_{12}O_{14}S_2$: 1049.27 Da (average isotopes), $[M+H]^+$ *m/z* = 1049.51; observed: 1049.41.

*Desulfurized Peptide **14b***

H−SVGATC(Acm)VTPI—NH_2

14b

Chemical Formula: $C_{43}H_{76}N_{12}O_{14}S$
Exact Mass: 1016.53
Molecular Weight: 1017.21

Peptide **14a** (1.07 mg, 0.80 µmol) was subjected to the MFD conditions following General Procedure **2.6** as described previously. The reaction was quenched by addition of CH_3CN/H_2O/AcOH (10/85/5, *v*/*v*/*v*) and further purified using RP-HPLC (linear gradient 8-30% solvent B over 30 min, Exsil Pure 300 C18 column). Product eluted at 14.9-15.5 min. The fractions were collected and lyophilized to provide peptide **14b** (1.00 mg, 96%) as a fluffy white solid.

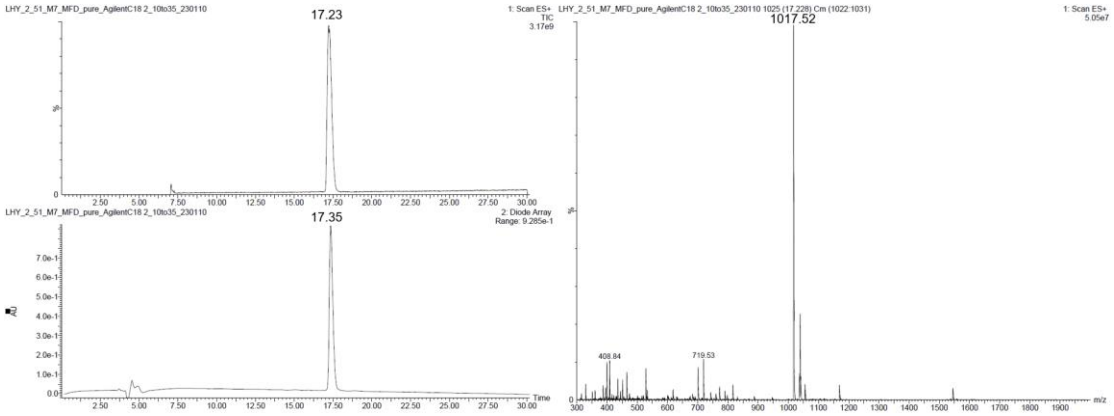


Figure S11. HPLC-MS analysis of peptide **14b**. Left: MS (Top) and UV (Bottom) traces (Linear gradient of 10-35% solvent B over 30 min, Agilent C18 column, t_R = 17.3 min); Right: ESI-MS data. Calcd for $C_{40}H_{72}N_{11}O_{13}$: 1017.21 Da (average isotopes), $[M+H]^+$ *m/z* = 1017.54; observed: 1017.52.

*Model peptide **15a***

H−ThzHRSYCGALN—NH_2

15a

Chemical Formula: $C_{46}H_{71}N_{17}O_{13}S_2$
Exact Mass: 1133.49
Molecular Weight: 1134.30

Peptide **15a** was prepared according to General Procedure **2.2 & 2.5** using Rink amide-MBHA resin (0.35 mmol/g, 0.05 mmol), Fmoc-Thz-OH and other standard $^{\alpha}N$-Fmoc amino acids. After global deprotection using TFA/TIS/H_2O (95/2.5/2.5, *v*/*v*/*v*), the crude peptide was purified using RP-HPLC (linear gradient 8-30% solvent B over 30 min, Exsil Pure 300 C18 column). Product eluted at 14.7-15.3 min. The fractions were collected and lyophilized to provide peptide **15a** (17.5 mg, 21%) as a fluffy white solid.

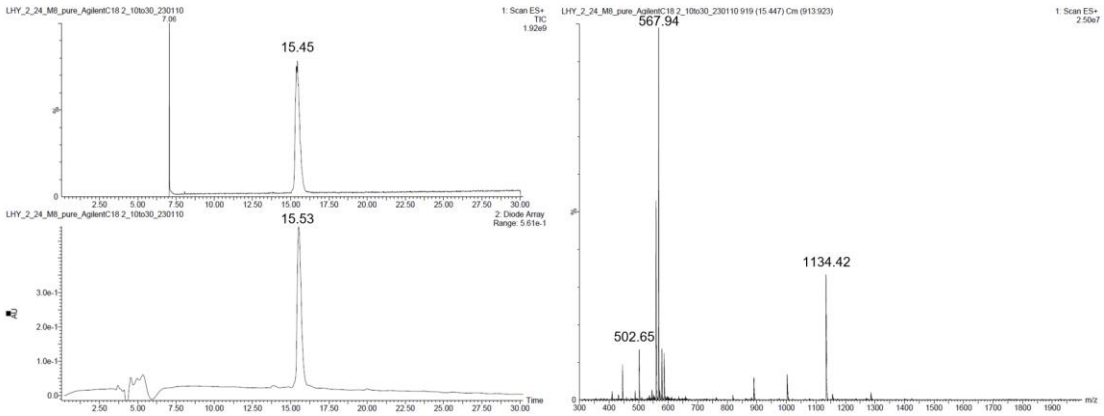


Figure S12. HPLC-MS analysis of peptide **15a**. Left: MS (Top) and UV (Bottom) traces (Linear gradient of 10-30% solvent B over 30 min, Agilent C18 column, t_R = 15.5 min); Right: ESI-MS data. Calcd for $C_{46}H_{71}N_{17}O_{13}S_2$: 1134.30 Da (average isotopes), $[M+H]^+$ *m/z* = 1134.50, $[M+2H]^{2+}$ *m/z* = 567.75; observed: 1134.42, 567.94.

*Desulfurized Peptide **15b***

H−ThzHRSYAGALN—NH_2
15b

Chemical Formula: $C_{46}H_{71}N_{17}O_{13}S$
Exact Mass: 1101.51
Molecular Weight: 1102.24

Peptide **15a** (1.08 mg, 0.95 μmol) was subjected to the MFD conditions following General Procedure **2.6** as described previously. The reaction was quenched by addition of CH_3CN/H_2O/AcOH (10/85/5, *v*/*v*/*v*) and further purified using RP-HPLC (linear gradient 8-25% solvent B over 30 min, Exsil Pure 300 C18 column). Product eluted at 14.8-22.4 min. The fractions were collected and lyophilized to provide peptide **15b** (0.78 mg, 74%) as a fluffy white solid.

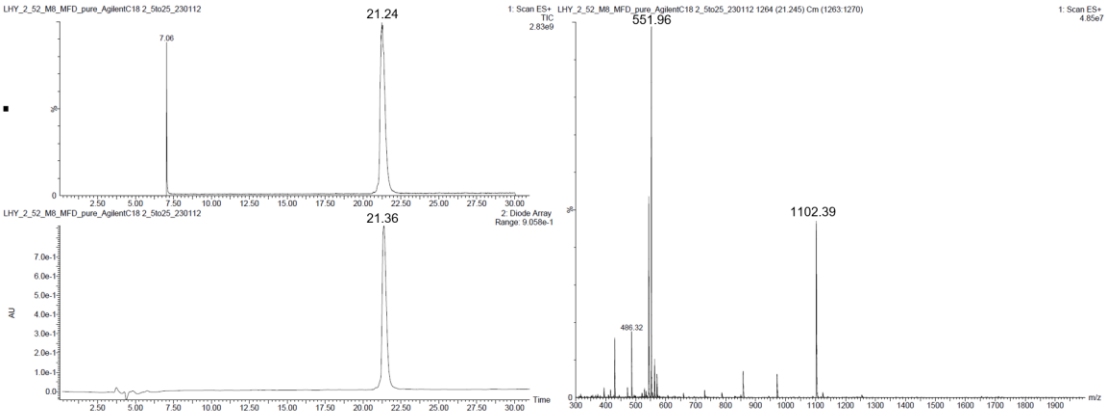


Figure S13. HPLC-MS analysis of peptide **15b**. Left: MS (Top) and UV (Bottom) traces (Linear gradient of 10-30% solvent B over 30 min, Agilent C18 column, t_R = 21.3 min); Right: ESI-MS data. Calcd for $C_{46}H_{71}N_{17}O_{13}S$: 1101.51 Da (average isotopes), $[M+H]^+$ *m/z* = 1102.52; $[M+2H]^{2+}$ *m/z* = 551.76; observed:1102.39, 551.96.

*Model Peptide **16a***

H−TLNSICPAEL−$NHNH_2$

16a

Chemical Formula: $C_{45}H_{79}N_{13}O_{15}S$
Exact Mass: 1073.55
Molecular Weight: 1074.26

Peptide **16a** was prepared according to General Procedure **2.2 & 2.5** using Fmoc-NHNH-CTC resin (0.42 mmol/g, 0.05 mmol) and other standard $^{\alpha}N$-Fmoc amino acids. After global deprotection using TFA/TIS/H_2O (95/2.5/2.5, *v/v/v*), the crude peptide was purified using RP-HPLC (linear gradient 10-35% solvent B over 30 min, Exsil Pure 300 C18 column). Product eluted at 18.7-19.3 min. The fractions were collected and lyophilized to provide peptide **16a** (16.6 mg, 31%) as a fluffy white solid.

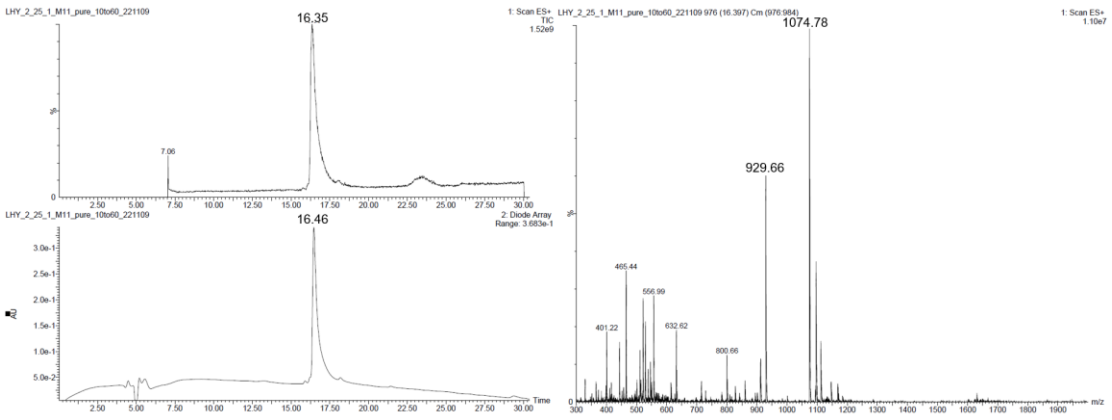


Figure S14. HPLC-MS analysis of peptide **16a**. Left: MS (Top) and UV (Bottom) traces (Linear gradient of 10-60% solvent B over 30 min, Exsil C18 column, t_R = 16.4 min); Right: ESI-MS data. Calcd for $C_{45}H_{79}N_{13}O_{15}S$: 1074.26 Da (average isotopes), $[M+H]^+$ *m/z* = 1074.56; observed: 1074.78.

*Desulfurized Peptide **16b***

H−TLNSIAPAEL−$NHNH_2$

16b

Chemical Formula: $C_{45}H_{79}N_{13}O_{15}$
Exact Mass: 1041.58
Molecular Weight: 1042.20

Peptide **16a** (1.13 mg, 1.05 µmol) was subjected to the MFD conditions following General Procedure **2.6** as described previously. The reaction was quenched by addition of CH_3CN/H_2O/AcOH (10/85/5, *v/v/v*) and further purified using RP-HPLC (linear gradient 10-30% solvent B over 30 min, Exsil Pure 300 C18 column). Product eluted at 18.8-19.4 min. The fractions were collected and lyophilized to provide peptide **16b** (0.63 mg, 57%) as a fluffy white solid.

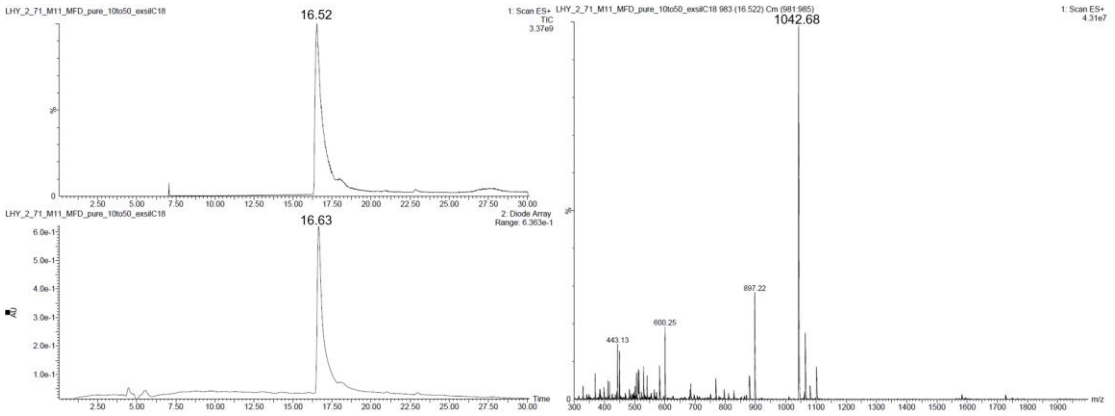


Figure S15. HPLC-MS analysis of peptide **16b**. Left: MS (Top) and UV (Bottom) traces (Linear gradient of 10-50% solvent B over 30 min, Exsil C18 column, t_R = 16.6 min); Right: ESI-MS data. Calcd for $C_{45}H_{79}N_{13}O_{15}$: 1042.20 Da (average isotopes), $[M+H]^+$ *m/z* = 1042.59; observed: 1042.68.

*Model Peptide **17a***

Biotin—FSTNPATV CA-NH_2

17a

Chemical Formula: $C_{53}H_{82}N_{14}O_{16}S_2$
Exact Mass: 1234.55
Molecular Weight: 1235.44

Peptide **17a** was prepared according to General Procedure **2.2 & 2.5** using Rink amide-MBHA resin (0.35 mmol/g, 0.05 mmol), D-Biotin and other standard $^{\alpha}$*N*-Fmoc amino acids. After global deprotection using TFA/TIS/H_2O (95/2.5/2.5, *v/v/v*), the crude peptide was dissolved in 15 mL of CH_3CN/H_2O (20/80, *v/v*) and further purified using RP-HPLC (linear gradient 15-35% solvent B over 30 min, Exsil Pure 300 C18 column). Product eluted at 20.5-21.5 min. The fractions were collected and lyophilized to provide peptide **17a** (16.8 mg, 27%) as a fluffy white solid.

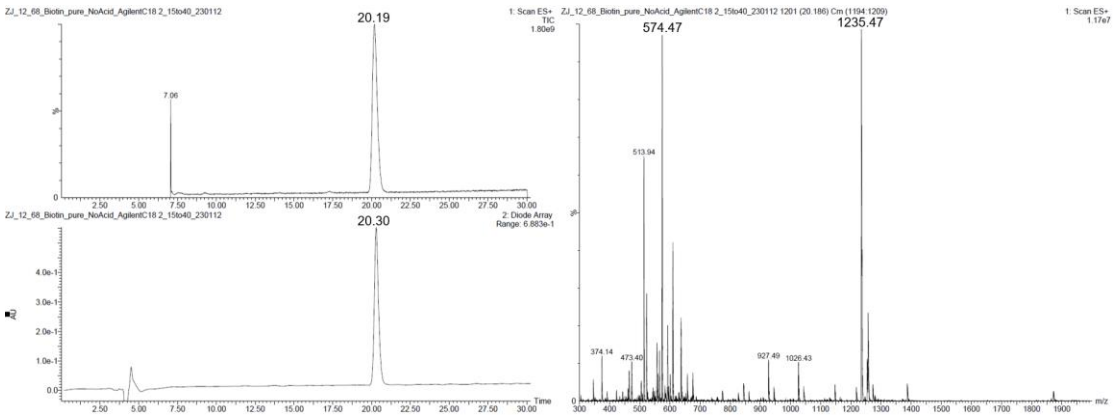


Figure S16. HPLC-MS analysis of peptide **17a**. Left: MS (Top) and UV (Bottom) traces (Linear gradient of 15-40% solvent B over 30 min, Agilent C18 column, t_R = 20.3 min); Right: ESI-MS data. Calcd for $C_{53}H_{82}N_{14}O_{16}S_2$: 1235.44 Da (average isotopes), $[M+H]^+$ *m/z* =1235.56; observed: 1235.47.

*Desulfurized Peptide **17b***

Biotin−FSTNPATV AA−NH_2

17b

Chemical Formula: $C_{53}H_{82}N_{14}O_{16}S$
Exact Mass: 1202.58
Molecular Weight: 1203.38

Peptide **17a** (0.94 mg, 0.76 µmol) was subjected to the MFD conditions following General Procedure **2.6** as described previously. The reaction was quenched by addition of CH_3CN/H_2O/AcOH (10/85/5, *v*/*v*/*v*) and further purified using RP-HPLC (linear gradient 15-35% solvent B over 30 min, Exsil Pure 300 C18 column). Product eluted at 18.7-19.4 min. The fractions were collected and lyophilized to provide peptide **17b** (0.63 mg, 69%) as a fluffy white solid.

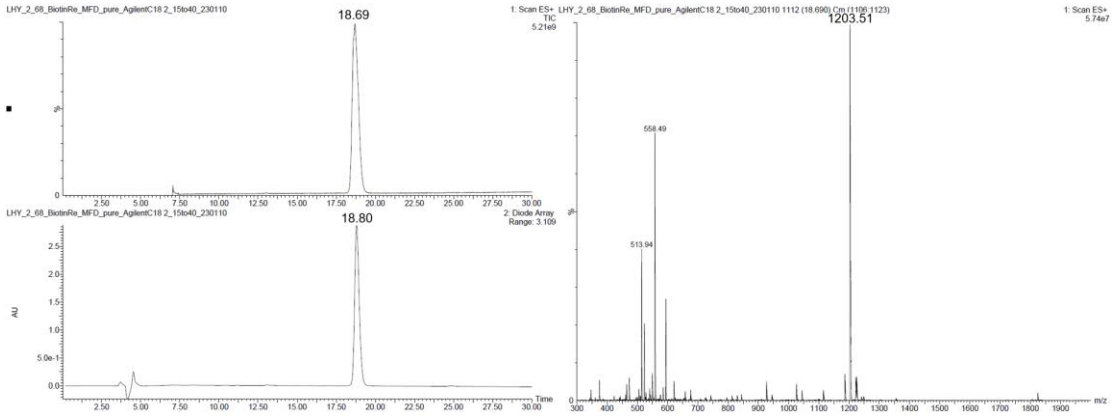


Figure S17. HPLC-MS analysis of peptide **17b**. Left: MS (Top) and UV (Bottom) traces (Linear gradient of 15-40% solvent B over 30 min, Agilent C18 column, t_R = 18.8 min); Right: ESI-MS data. Calcd for $C_{53}H_{82}N_{14}O_{16}S$: 1203.38 Da (average isotopes), $[M+H]^+$ *m/z* = 1203.59; observed: 1203.51.

*Model Peptide **18a***

H−PHFS(PO_3H_2)YCGALN−NH_2

18a

Chemical Formula: $C_{50}H_{71}N_{14}O_{16}PS$
Exact Mass: 1186.46
Molecular Weight: 1187.23

Peptide **18a** was prepared according to General Procedure **2.2 & 2.5** using Rink amide-MBHA resin (0.35 mmol/g, 0.05 mmol), Fmoc-Ser(HPO_3Bzl)-OH and other standard $^{\alpha}$*N*-Fmoc amino acids. After global deprotection using TFA/TIS/H_2O (95/2.5/2.5, *v*/*v*/*v*), the crude peptide was dissolved in 15 mL of CH_3CN/H_2O (20/80, *v*/*v*) and further purified using RP-HPLC (linear gradient 8-35% solvent B over 30 min, Exsil Pure 300 C18 column). Product eluted at 17.9-18.6 min. The fractions were collected and lyophilized to provide peptide **18a** (9.2 mg, 16%) as a fluffy white solid.

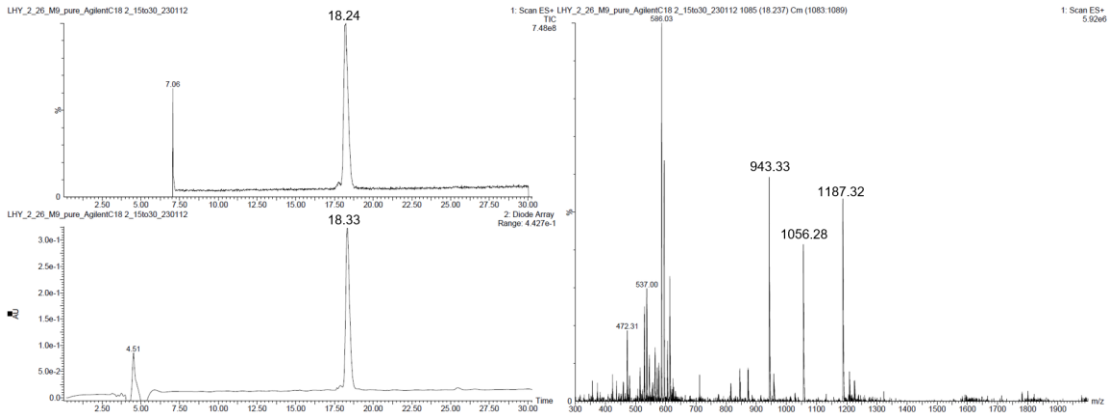


Figure S18. HPLC-MS analysis of peptide **18a**. Left: MS (Top) and UV (Bottom) traces (Linear gradient of 15-30% solvent B over 30 min, Agilent C18 column, t_R = 18.3 min); Right: ESI-MS data. Calcd for $C_{50}H_{71}N_{14}O_{16}PS$: 1187.23 Da (average isotopes), $[M+H]^+$ *m/z* = 1187.47; observed: 1187.32.

*Desulfurized Peptide **18b***

H−PHFS(PO$_3$H$_2$)YAGALN−NH$_2$

18b

Chemical Formula: $C_{50}H_{71}N_{14}O_{16}P$
Exact Mass: 1154.49
Molecular Weight: 1155.17

Peptide **18a** (1.05 mg, 0.88 µmol) was subjected to the MFD conditions following General Procedure **2.6** as described previously. The reaction was quenched by addition of CH_3CN/H_2O/AcOH (10/85/5, *v*/*v*/*v*) and further purified using RP-HPLC (linear gradient 10-35% solvent B over 30 min, Exsil Pure 300 C18 column). Product eluted at 17.8-18.4 min. The fractions were collected and lyophilized to provide peptide **18b** (0.73 mg, 71%) as a fluffy white solid.

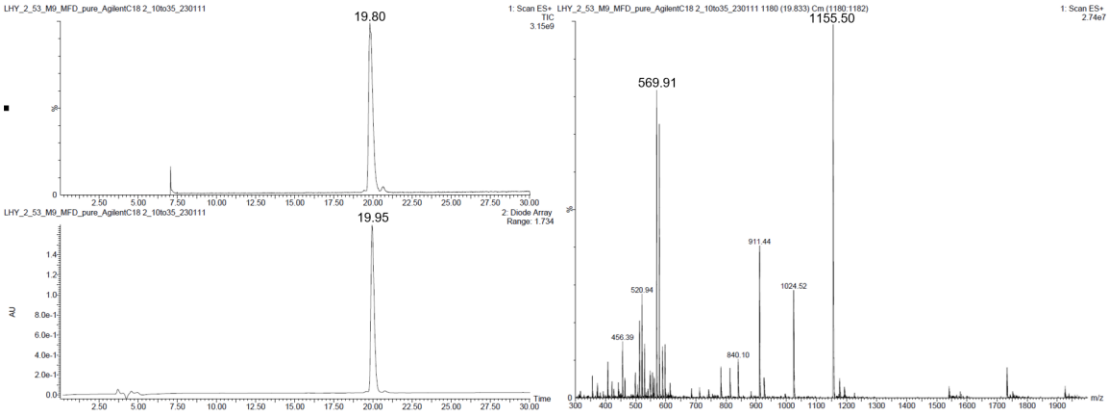


Figure S19. HPLC-MS analysis of peptide **18b**. Left: MS (Top) and UV (Bottom) traces (Linear gradient of 10-35% solvent B over 30 min, Agilent C18 column, t_R = 19.9 min); Right: ESI-MS data. Calcd for $C_{50}H_{71}N_{14}O_{16}P$: 1155.17 Da (average isotopes), $[M+H]^+$ *m/z* = 1155.50; observed: 1155.50.

*Model Peptide **19a***

H−PHFT(α-GalNAc)YCGALN−NH_2

19a

Chemical Formula: $C_{59}H_{85}N_{15}O_{18}S$
Exact Mass: 1323.59
Molecular Weight: 1324.48

Peptide **19a** was prepared according to General Procedure **2.2**, **2.3**, **2.4 & 2.5** using Rink amide-MBHA resin (0.35 mmol/g, 0.05 mmol), Fmoc-Thr(α-Ac_3GalNAc)-OH was and other standard $^{\alpha}$*N*-Fmoc amino acids. After global deprotection using TFA/TIS/H_2O (95/2.5/2.5, *v/v/v*), the crude peptide was dissolved in 15 mL of CH_3CN/H_2O (20/80, *v/v*) and further purified using RP-HPLC (linear gradient 10-35% solvent B over 30 min, Exsil Pure 300 C18 column). Product eluted at 18.8-19.4 min. The fractions were collected and lyophilized to provide peptide **19a** (19.8 mg, 30%) as a fluffy white solid.

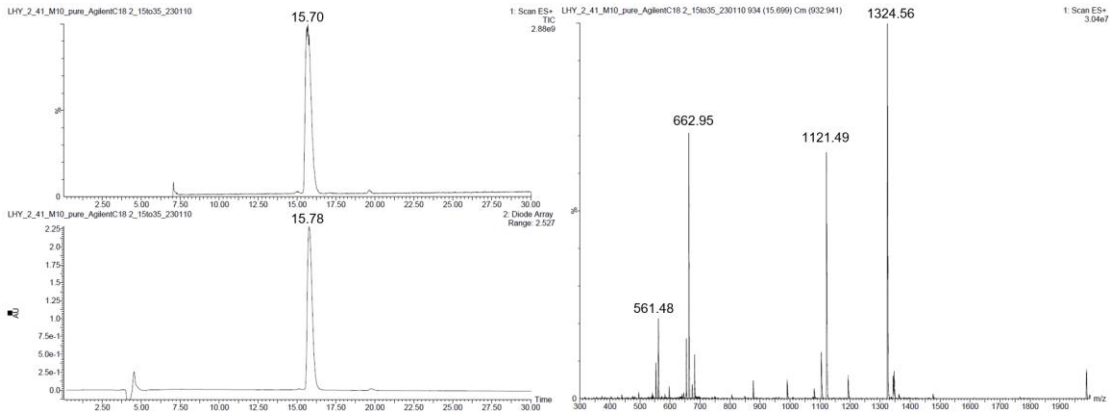


Figure S20. HPLC-MS analysis of peptide **19a**. Left: MS (Top) and UV (Bottom) traces (Linear gradient of 15-30% solvent B over 30 min, Agilent C18 column, t_R = 15.7 min); Right: ESI-MS data. Calcd for $C_{59}H_{85}N_{15}O_{18}S$: 1324.48 Da (average isotopes), $[M+H]^+$ *m/z* = 1324.60; observed: 1324.56.

*Desulfurized Peptide **19b***

H−PHFT(α-GalNAc)YAGALN−NH_2

19b

Chemical Formula: $C_{59}H_{85}N_{15}O_{18}$
Exact Mass: 1291.62
Molecular Weight: 1292.42

Peptide **19a** (1.07 mg, 0.80 μmol) was subjected to the MFD conditions following General Procedure **2.6** as described previously. The reaction was quenched by addition of CH_3CN/H_2O/AcOH (10/85/5, *v/v/v*) and further purified using RP-HPLC (linear gradient 10-35% solvent B over 30 min, Exsil Pure 300 C18 column). Product eluted at 16.2-16.8 min. The fractions were collected and lyophilized to provide peptide **19b** (0.86 mg, 82%) as a fluffy white solid.

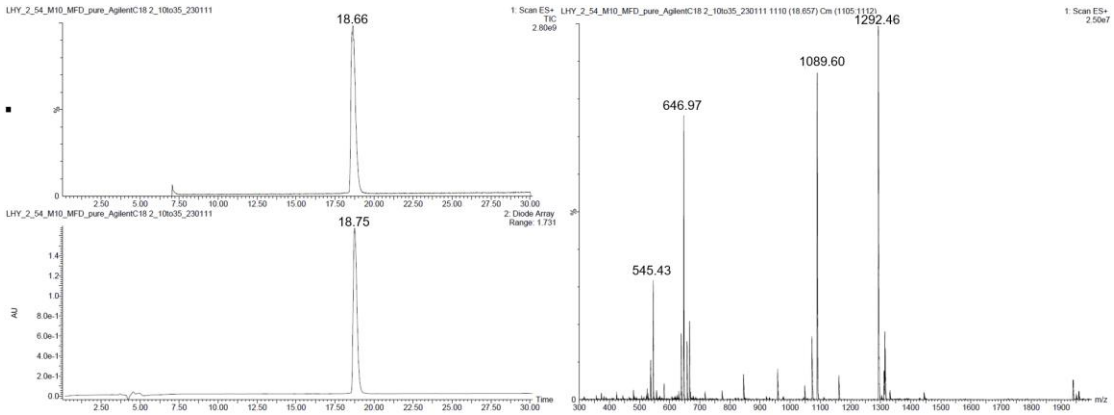
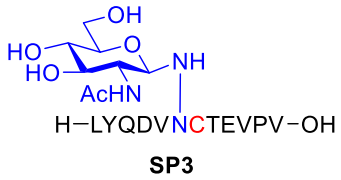


Figure S21. HPLC-MS analysis of peptide **19b**. Left: MS (Top) and UV (Bottom) traces (Linear gradient of 10-35% solvent B over 30 min, Agilent C18 column, t_R = 18.7 min); Right: ESI-MS data. Calcd for $C_{59}H_{85}N_{15}O_{18}$: 1292.42 Da (average isotopes), $[M+H]^+$ *m/z* = 1292.63; observed: 1292.46.

*N-GlcNAc Modified Peptide **SP3***



SP3

Chemical Formula: $C_{68}H_{107}N_{15}O_{26}S$
Exact Mass: 1581.72
Molecular Weight: 1582.74

Glycopeptide **SP3** was prepared according to General Procedure**2.2**, **2.3**, **2.4 & 2.5** using Fmoc-Val-Wang resin (0.26 mmol/g, 0.05 mmol), Fmoc-Asn(Ac_3GlcNAc)-OH and other standard $^{\alpha}$*N*-Fmoc amino acids. After global deprotection using TFA/TIS/H_2O (95/2.5/2.5, *v/v/v*), the crude peptide was dissolved in 12 mL of CH_3CN/H_2O/AcOH (30/65/5, *v/v/v*) and further purified using RP-HPLC (linear gradient 15-30% solvent B over 30 min, Exsil Pure 300 C18 column). Product eluted at 17.5-19.0 min. The fractions were collected and lyophilized to provide peptide **SP3** (10.5 mg, 13%) as a fluffy white solid.

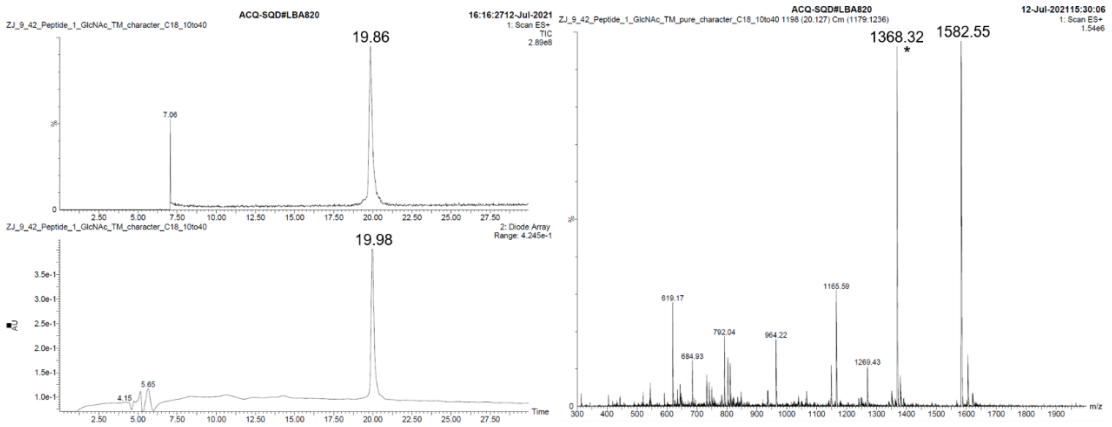
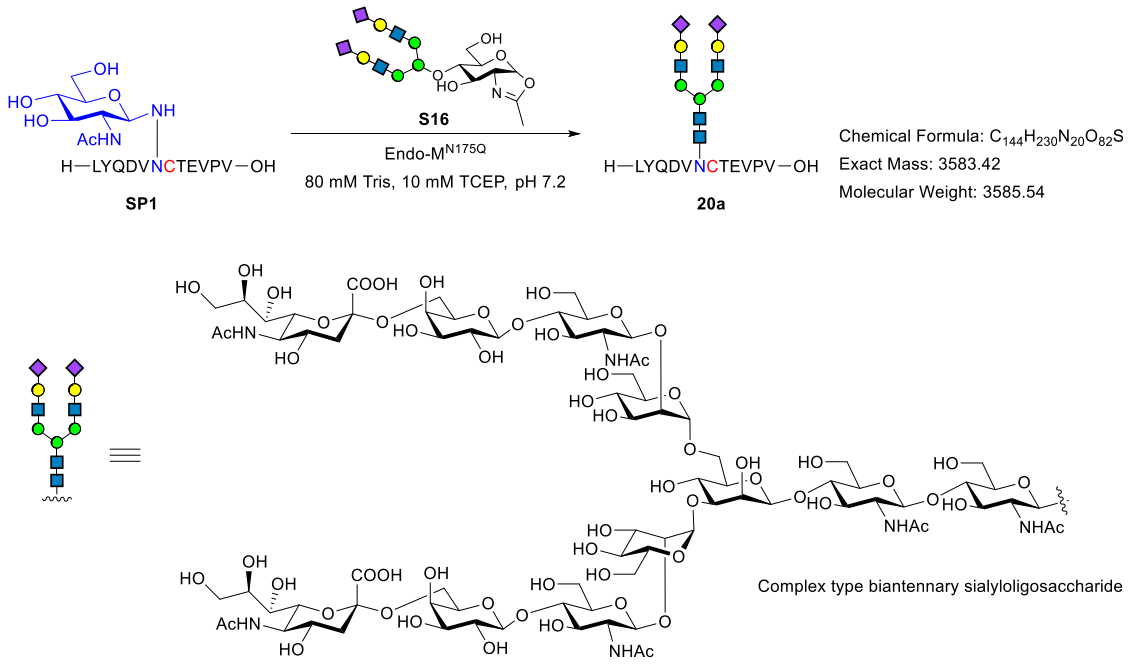


Figure S22. HPLC-MS analysis of peptide **SP3**. Left: MS (Top) and UV (Bottom) traces (Linear gradient of 10-40% solvent B over 30 min, Exsil Pure 300 C18 column, t_R = 19.9 min); Right: ESI-MS data. Calcd for $C_{68}H_{107}N_{15}O_{26}S$: 1582.74 Da (average isotopes), $[M+H]^+$ *m/z* = 1582.73; observed: 1582.55. *Signals denote GlcNAc dissociated species in mass.

*Model Peptide **20a***



A solution of GlcNAc-modified peptide **SP3** (1.02 mg, 0.63 μmol) and sialyl biantennary complex type *N*-glycan oxazoline[15] (SCT-ox, **s16**, 2.58 mg, 1.26 μmol) in 127 μL of Tris buffer (80 mM, 10 mM TCEP·HCl, pH 7.2) was incubated with Endo-M^{N175Q} [16] (3.2 μL, 20.1 mg/mL) at room temperature for 3 hours. One more SCT-ox

[15] Oxazoline **s16** was prepared according to the published procedures: F. Tang, L.-X. Wang and W. Huang, *Nat. Protoc.* 2017, **12**, 1702-1721. The plasmid encoding wild-type Endo-M was kindly provided by Prof. Wei Huang.
[16] The glycosynthase Endo-M^{N175Q} was prepared according to the published procedures: M. Umekawa, C. Li, T.

(2.58 mg, 1.26 μmol) in Tris buffer and Endo-M^{N175Q} (3.2 μL, 20.1 mg/mL) were added and stirred for another 3 hours. The reaction was diluted by addition of 2.0 mL of CH_3CN/H_2O/AcOH (20/75/5, v/v/v), and further purified using RP-HPLC (linear gradient 10-30% solvent B over 30 min, Exsil Pure 300 C18 column). Product eluted at 19.2-20.0 min. The fractions were collected and lyophilized to provide peptide **20a** (0.85 mg, 37%) as a fluffy white solid.

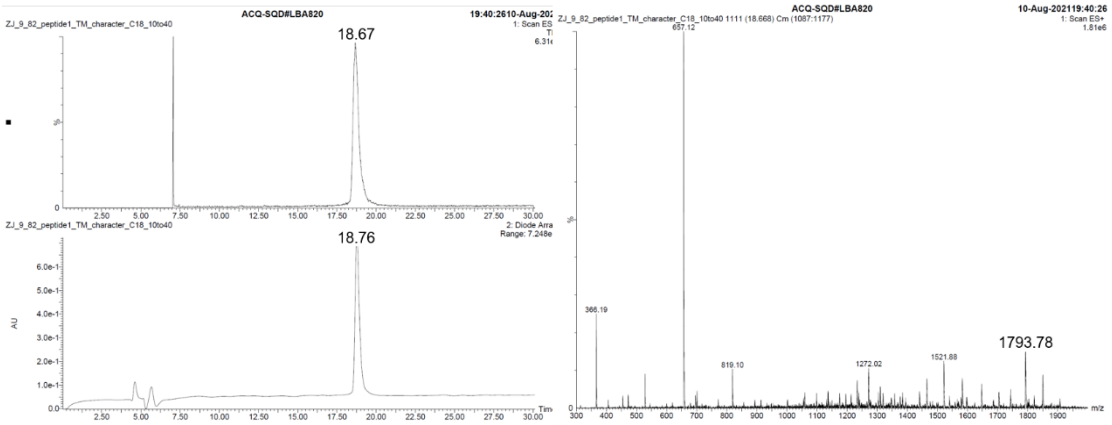
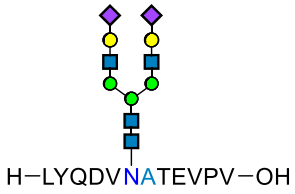


Figure S23. HPLC-MS analysis of peptide **20a**. Left: MS (Top) and UV (Bottom) traces (Linear gradient of 10-40% solvent B over 30 min, Exsil Pure 300 C18 column, t_R = 18.7 min); Right: ESI-MS data. Calcd for $C_{144}H_{230}N_{20}O_{82}S$: 3585.54 Da (average isotopes), $[M+2H]^{2+}$ *m/z* = 1793.22; observed: 1793.78.

*Desulfurized Peptide **20b***



20b

Chemical Formula: $C_{144}H_{230}N_{20}O_{82}$
Exact Mass: 3551.44
Molecular Weight: 3553.48

Peptide **20a** (0.85 mg, 0.24 μmol) was subjected to the MFD conditions following General Procedure **2.6** as described previously. The reaction was stirred for 5 hours and quenched by addition of CH_3CN/H_2O/AcOH (10/85/5, *v*/*v*/*v*) and further purified using RP-HPLC (linear gradient 10-30% solvent B over 30 min, Exsil Pure 300 C18 column). Product eluted at 17.5-18.5 min. The fractions were collected and lyophilized to provide peptide **20b** (0.60 mg, 71%) as a fluffy white solid.

Higashiyama, W. Huang, H. Ashida, K. Yamamoto and L.-X. Wang, *J. Biol. Chem.* 2010, **285**, 511-521.

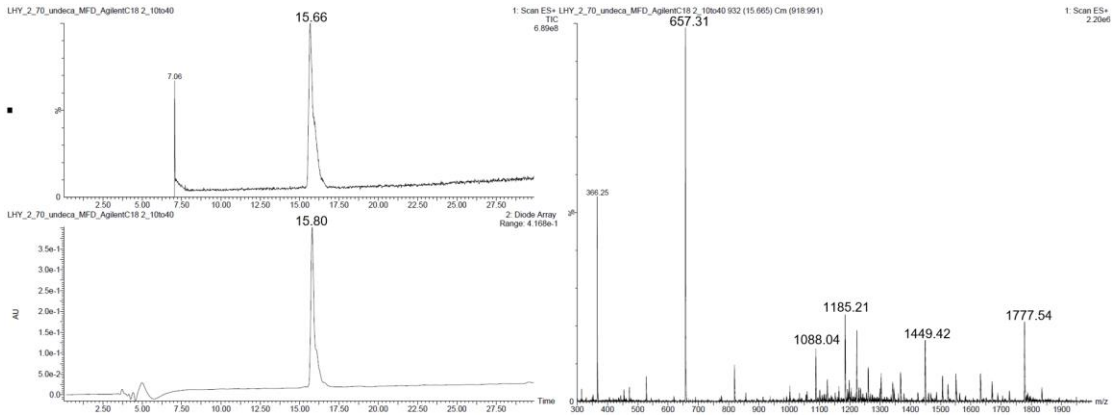
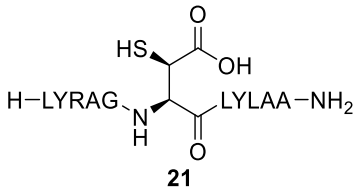


Figure S24. HPLC-MS analysis of peptide **20b**. Left: MS (Top) and UV (Bottom) traces (Linear gradient of 10-40% solvent B over 30 min, Agilent C18 column, t_R = 15.7 min); Right: ESI-MS data. Calcd for $C_{144}H_{230}N_{20}O_{82}$: 3553.48 Da (average isotopes), $[M+2H]^{2+}$ *m/z* = 1777.23, $[M+3H]^{3+}$ *m/z* = 1185.16; observed: 1777.54, 1185.21.

*β-thiol-Asp Containing Peptide **21***



21

Chemical Formula: $C_{57}H_{89}N_{15}O_{15}S$
Exact Mass: 1255.64
Molecular Weight: 1256.49

Peptide **21** was prepared according to General Procedure **2.2 & 2.5** using Rink amide-MBHA resin (0.35 mmol/g, 0.05 mmol), Fmoc-Asp(O^{t}Bu)($^\beta$SS^{t}Bu)-OH[17] and other standard $^\alpha$*N*-Fmoc amino acids. After global deprotection using TFA/TIS/H_2O (95/2.5/2.5, *v/v/v*), the crude peptide dissolved in 12 mL of CH_3CN/H_2O/AcOH (25/70/5, *v/v/v*) and further purified using RP-HPLC (linear gradient 25-40% solvent B over 30 min, Exsil Pure 300 C18 column). The fractions were collected and lyophilized to obtain precursor with S^{t}Bu protection group. Then it was treated with 20 mM TCEP (pH 7.2) for 10 hours and further purified using RP-HPLC (linear gradient 15-40% solvent B over 30 min, Exsil Pure 300 C18 column). Product eluted at 17.0-18.0 min. The fractions were collected and lyophilized to provide peptide **21** (12.9 mg, 21%) as a fluffy white solid.

[17] The β-thio-Asp derivative was prepared following our previous reported procedures: Dong, Suwei; Li, Jingwei and Li, Yaohao, Aspartic acid derivative, preparing method, and application in solid-phase polypeptide synthesis, *China Pat.*, CN106167457A, 2016.

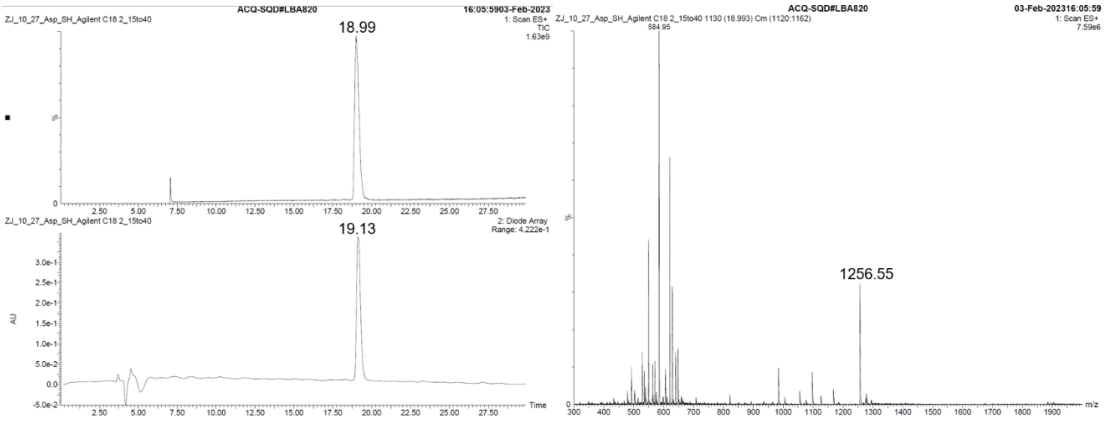
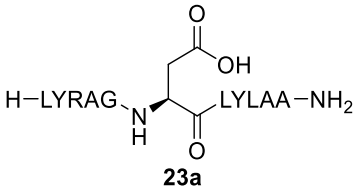


Figure S25. HPLC-MS analysis of peptide **21**. Left: MS (Top) and UV (Bottom) traces (Linear gradient of 15-40% solvent B over 30 min, Agilent C18 column, t_R = 19.1 min); Right: ESI-MS data. Calcd for $C_{57}H_{89}N_{15}O_{15}S$: 1256.49 Da (average isotopes), $[M+H]^+$ *m/z* = 1256.65; observed:1256.55.

*Desulfurized Asp Containing Peptide **23a***



23a

Chemical Formula: $C_{57}H_{89}N_{15}O_{15}$
Exact Mass: 1223.67
Molecular Weight: 1224.43

Peptide **21** (1.12 mg, 0.89 μmol) was subjected to the MFD conditions following General Procedure **2.6** as described previously. The reaction was stirred for 5 hours under 50 ˚C, quenched by addition of CH_3CN/H_2O/AcOH (10/85/5, *v*/*v*/*v*) and further purified using RP-HPLC (linear gradient 10-35% solvent B over 30 min, Exsil Pure 300 C18 column). Product eluted at 21.0-22.0 min. The fractions were collected and lyophilized to provide peptide **23a** (0.91 mg, 83%) as a fluffy white solid.

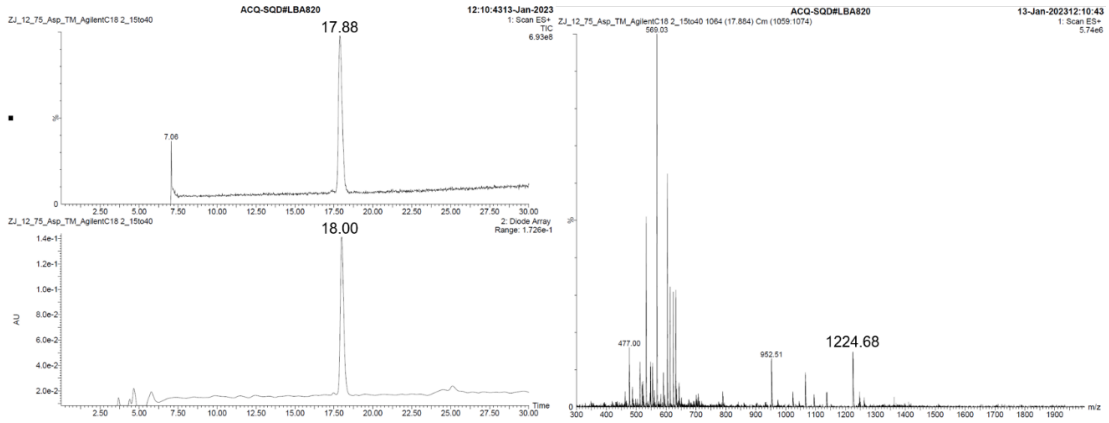
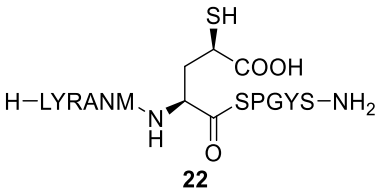


Figure S26. HPLC-MS analysis of peptide **23a**. Left: MS (Top) and UV (Bottom) traces (Linear gradient of 15-40% solvent B over 30 min, Agilent C18 column, t_R = 18.0 min); Right: ESI-MS data. Calcd for $C_{57}H_{89}N_{15}O_{15}$: 1224.43 Da (average isotopes), $[M+H]^+$ *m/z* = 1224.67; observed:1224.68.

*γ-thiol-Glu Containing Peptide **22***



22

Chemical Formula: $C_{60}H_{91}N_{17}O_{19}S_2$
Exact Mass: 1417.61
Molecular Weight: 1418.61

Peptide **22** was prepared according to General Procedure **2.2 & 2.5** using Rink amide-MBHA resin (0.35 mmol/g, 0.05 mmol), Fmoc-Glu(O^{t}Bu)($^\gamma$SS^{t}Bu)-OH (**S15**) and other standard $^\alpha$*N*-Fmoc amino acids. After global deprotection using TFA/TIS/H_2O (95/2.5/2.5, *v/v/v*), the crude peptide dissolved in 10 mL of CH_3CN/H_2O/AcOH (20/75/5, *v/v/v*) and further purified using RP-HPLC (linear gradient 10-40% solvent B over 30 min, Exsil Pure 300 C18 column). The fractions were collected and lyophilized to obtain precursor with S^{t}Bu protection group. Then it was treated with 20 mM TCEP (pH 7.2) for 10 hours and further purified using RP-HPLC (linear gradient 10-25% solvent B over 30 min, Exsil Pure 300 C18 column). Product eluted at 16.0-17.5 min. The fractions were collected and lyophilized to provide peptide **22** (16.3 mg, 23%) as a fluffy white solid.

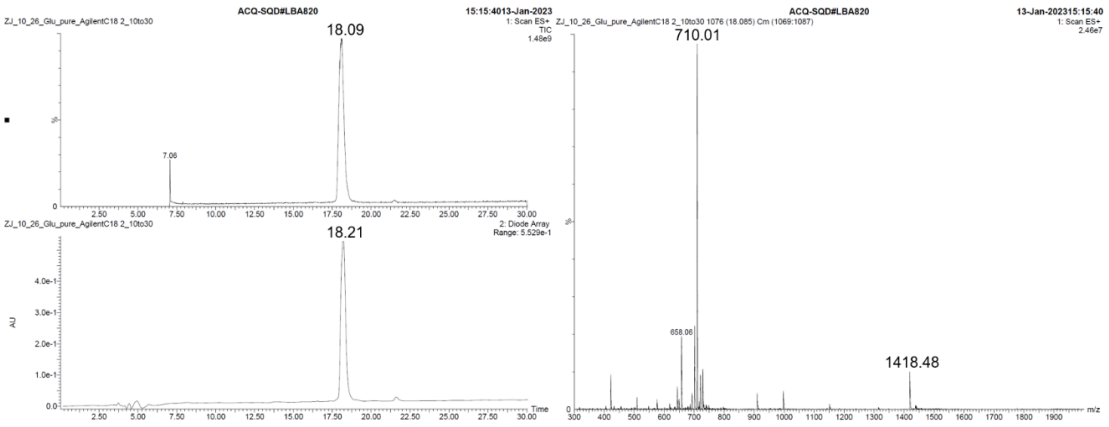
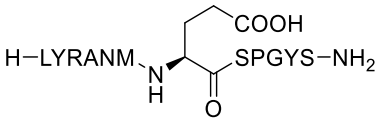


Figure S27. HPLC-MS analysis of peptide **22**. Left: MS (Top) and UV (Bottom) traces (Linear gradient of 10-30% solvent B over 30 min, Agilent C18 column, t_R = 18.2 min); Right: ESI-MS data. Calcd for $C_{60}H_{91}N_{17}O_{19}S_2$: 1418.61 Da (average isotopes), $[M+H]^+$ *m/z* = 1418.62, $[M+2H]^{2+}$ *m/z* = 709.81; observed:1418.48, 710.01.

*Desulfurized Glu Containing Peptide **24a***



24a

Chemical Formula: $C_{60}H_{91}N_{17}O_{19}S$
Exact Mass: 1385.64
Molecular Weight: 1386.55

Peptide **22** (1.15 mg, 0.81 μmol) was subjected to the MFD conditions following General Procedure **2.6** as described previously. The reaction was stirred for 8 hours under 37 ˚C, quenched by addition of CH_3CN/H_2O/AcOH (10/85/5, *v*/*v*/*v*) and further purified using RP-HPLC (linear gradient 10-20% solvent B over 30 min, Exsil Pure 300 C18 column). Product eluted at 20.0-21.0 min. The fractions were collected and lyophilized to provide peptide **24a** (0.98 mg, 87%) as a fluffy white solid.

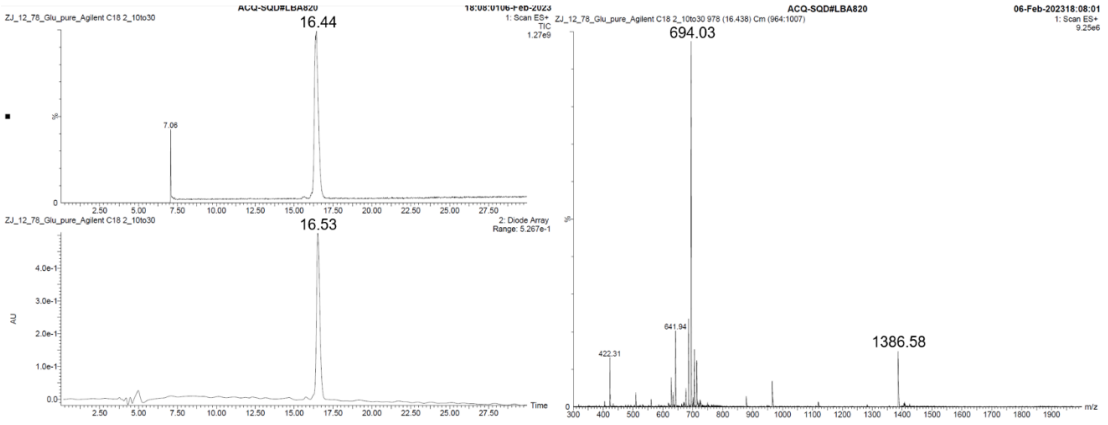
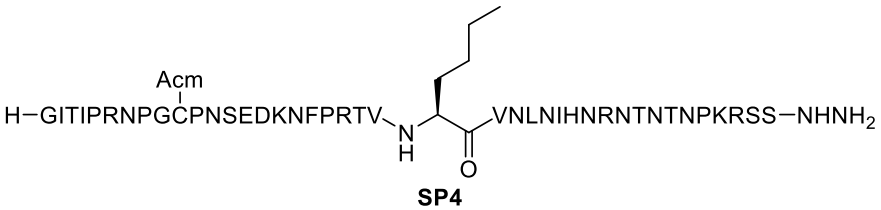


Figure S28. HPLC-MS analysis of peptide **24a**. Left: MS (Top) and UV (Bottom) traces (Linear gradient of 10-30% solvent B over 30 min, Agilent C18 column, t_R = 16.5 min); Right: ESI-MS data. Calcd for $C_{60}H_{91}N_{17}O_{19}S$: 1386.55 Da (average isotopes), $[M+H]^+$ *m/z* = 1386.65, $[M+2H]^{2+}$ *m/z* = 693.83; observed:1386.58, 694.03.

*Peptide IL-17A(1-41) (**SP4**)*



SP4

Chemical Formula: $C_{196}H_{325}N_{69}O_{62}S$
Exact Mass: 4669.41
Molecular Weight: 4672.24

Peptide **SP4** was prepared according to General Procedure **2.2 & 2.5** using Trityl-$NHNH_2$ ChemMatrix resin (0.28 mmol/g, 0.05 mmol), Fmoc-Nle-OH and other standard $^{\alpha}N$-Fmoc amino acids. After global deprotection using TFA/TIS/H_2O

(95/2.5/2.5, *v/v/v*), the crude peptide was dissolved in 12 mL of CH_3CN/H_2O/AcOH (35/60/5, *v/v/v*) and further purified using RP-HPLC (linear gradient 20-30% solvent B over 30 min, Proto 300 C4 column). Product eluted at 15.5-17.0 min. The fractions were collected and lyophilized to provide peptide **SP4** (82.8 mg, 35%) as a fluffy white solid.

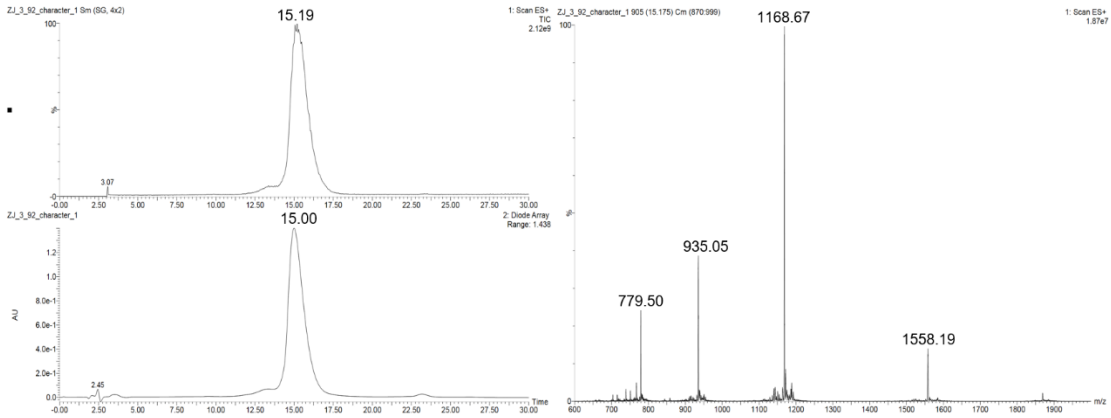
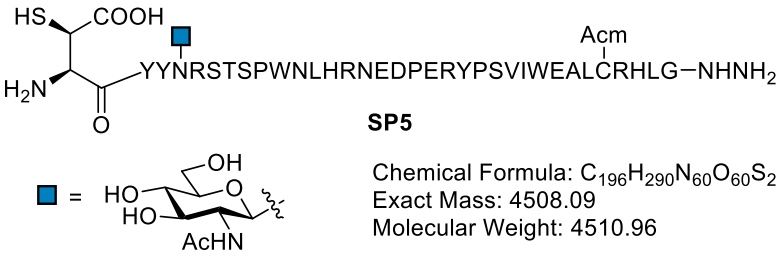


Figure S29. HPLC-MS analysis of peptide **SP4**. Left: MS (Top) and UV (Bottom) traces (Linear gradient of 20-30% solvent B over 30 min, PROTO-300 C4 column, t_R = 15.7 min); Right: ESI-MS data. Calcd for $C_{196}H_{325}N_{69}O_{62}S$: 4672.24 Da (average isotopes), $[M+3H]^{3+}$ *m/z* = 1558.15, $[M+4H]^{4+}$ *m/z* = 1169.11, $[M+5H]^{5+}$ *m/z* = 935.09, $[M+6H]^{6+}$ *m/z* = 779.41; observed:1558.19, 1168.67, 935.05, 779.50.

*Peptide IL-17A(42-75) (**SP5**)*



Peptide **SP5** was prepared according to General Procedure **2.2**, **2.3**, **2.4 & 2.5** using Trityl-$NHNH_2$ ChemMatrix resin (0.28 mmol/g, 0.05 mmol), Fmoc- Asn(Ac_3GlcNAc)-OH, Boc-Asp(^{t}Bu, $^\beta$STmob)-OH[18] and other standard $^\alpha N$-Fmoc amino acids. Then the resin was subjected to a cleavage cocktail TFA/TIS/H_2O (95/2.5/2.5, *v/v/v*) to get global deprotection. The crude material was dissolved in a solution of CH_3CN/H_2O/AcOH

[18] For the synthetic route of thiol-Asp derivative, see: R. E. Thompson, B. Chan, L. Radom, K. A. Jolliffe and R. J. Payne, *Angew. Chem. Int. Ed*. **2013**, *52*, 9723-9727.

(40/55/5, *v/v/v*), and was further purified using preparative RP-HPLC (linear gradient 15-30% solvent B over 30 min, Proto 300 C4 column), and the product eluted at 17.0-19.0 min. The fractions were collected, and concentrated via lyophilization to provide peptide **SP5** (36.7 mg, 16%) as a fluffy white solid.

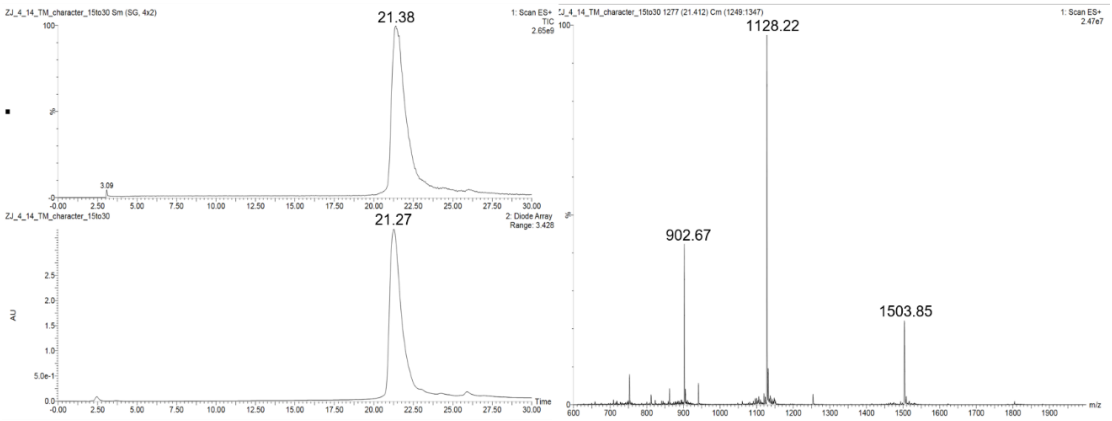
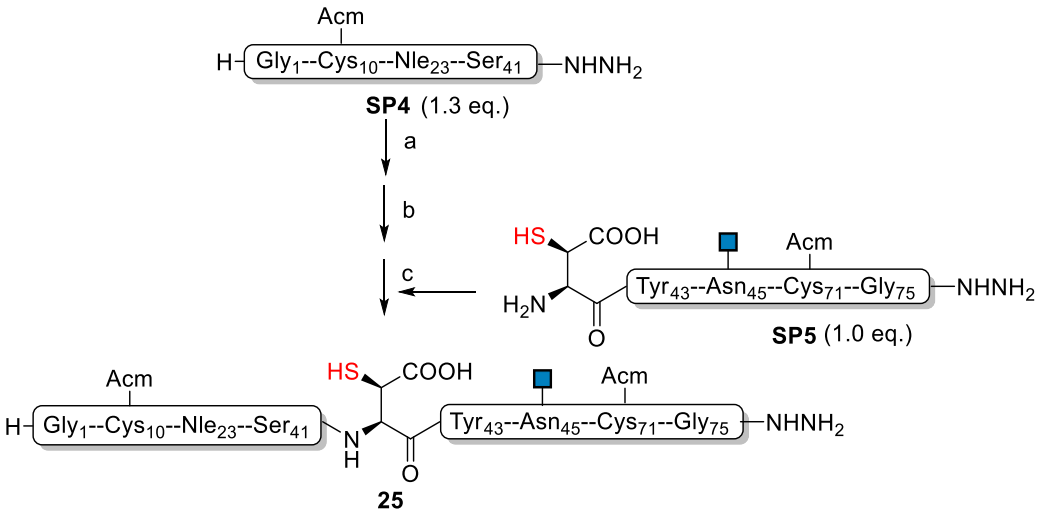


Figure S30. HPLC-MS analysis of peptide **SP5**. Left: MS (Top) and UV (Bottom) traces (Liner gradient of 15-30% solvent B over 30 min, PROTO-300 C4, t_R = 21.3 min); Right: ESI-MS data. Calcd for $C_{196}H_{290}N_{60}O_{60}S_2$: 4510.96 Da (average isotopes), $[M+3H]^{3+}$ *m/z* = 1504.37, $[M+4H]^{4+}$ *m/z* = 1128.53, $[M+5H]^{5+}$ *m/z* = 903.03, $[M+6H]^{6+}$ *m/z* = 752.52; observed:1503.85, 1128.22, 902.67.

*Thiol-Derived Peptide IL-17A(1-75) (**25**): Ligation reaction between IL-17A(1-41) (**SP4**) and IL-17A(42-75) (**SP5**)*



Scheme S5. Assembly of Western Part IL-17A(1-75). Conditions: (a) 6 M Gn·HCl, 200 mM NaH_2PO_4, pH 3~4, -15 °C, 15 min; (b) 200 mM $NaNO_2$, pH 6.5, -15 °C, 15 min; (c) 6 M Gn·HCl, 200 mM Na_2HPO_4, 200 mM MPAA, pH 7.0, **SP5**, pH adjusted to ~6.9, r.t. (■ = GlcNAc), 37 °C, overnight.

14.16 mg (3.04 μmol) of IL-17A(1-41) (**SP4**, 1.3 equiv) was dissolved in 0.5 mL of buffer A (6 M Gn·HCl, 200 mM NaH_2PO_4, pH 3~4), the resulting solution was transferred into -15 °C bath, and 106 μL of 200 mM $NaNO_2$ (7 equiv) was added dropwise. After oxidized for 15 min, the system was treated with 0.5 mL of buffer B (6 M Gn·HCl, 200 mM Na_2HPO_4, 200 mM MPAA, pH 7.0) and stirred for 5 min. 10.54 mg (2.34 μmol) of IL-17A(42-75) (**SP5,** 1.0 equiv) was dissolved in 1.0 mL of buffer B and then added to the mixture. After adjusting the pH to 6.9 with 2.0 M NaOH, the resulting reaction was warmed to room temperature and monitored by LC-MS. After 13 h, the same volume of buffer C (6 M Gn·HCl, 200 mM NaH_2PO_4, 150 mmol TCEP·HCl, pH 6.3) was added and reacted for 20 min. The reaction was quenched with 4 mL of MeCN/H_2O/AcOH (30/65/5, *v/v/v*) solution. The crude mixture was purified using preparative HPLC (20-30% solvent B over 30 min, Beim Brueckle C4 column). The fractions containing pure peptide were collected, and concentrated via lyophilization to afford IL-17A(1-75) (**25**) (13.83 mg, 65%) as a fluffy white solid.

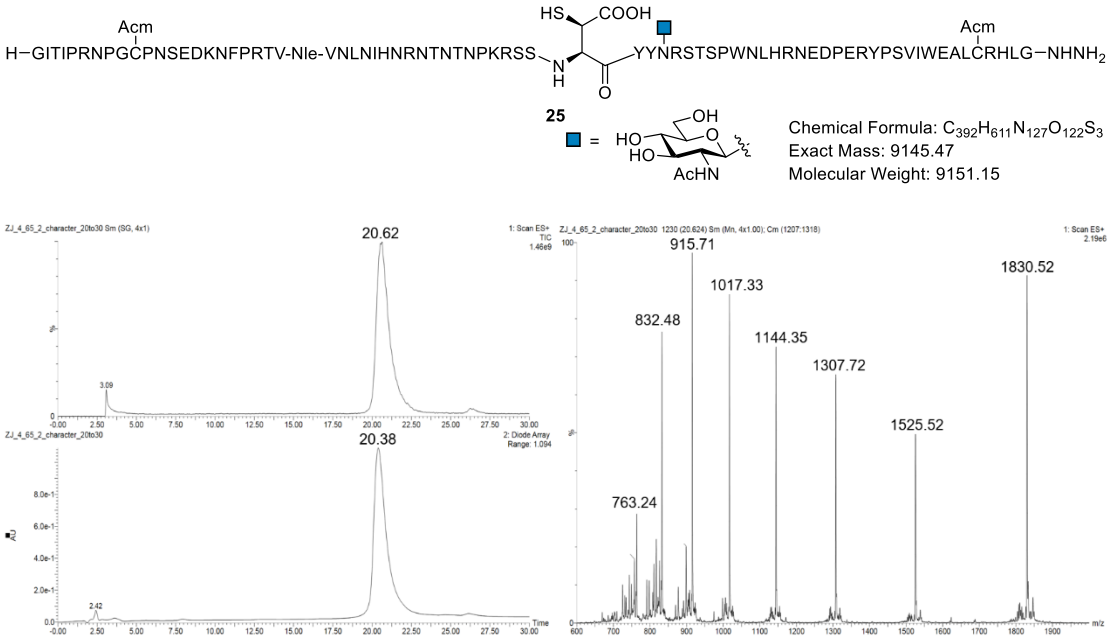
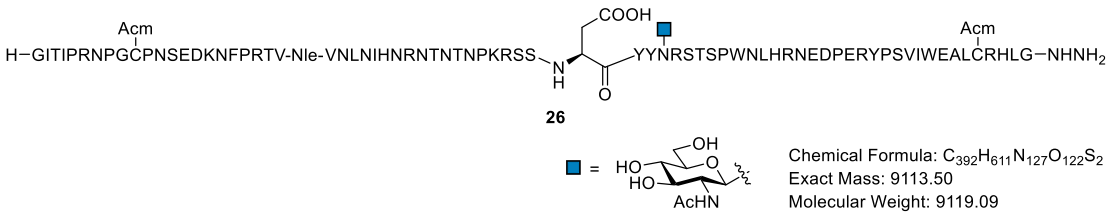


Figure S31. HPLC-MS analysis of peptide **25**. Left: MS (Top) and UV (Bottom) traces (Liner gradient of 20-30% solvent B over 30 min, PROTO-300 C4 column, t_R = 20.5 min); Right: ESI-MS data. Calcd for $C_{392}H_{611}N_{127}O_{122}S_3$: 9151.15 Da (average isotopes), $[M+5H]^{5+}$ *m/z* = 1830.90, $[M+6H]^{6+}$ *m/z* = 1525.92, $[M+7H]^{7+}$ *m/z* = 1308.08, $[M+8H]^{8+}$ *m/z* = 1144.69, $[M+9H]^{9+}$ *m/z* = 1017.62, $[M+10H]^{10+}$ *m/z* = 915.96, $[M+11H]^{11+}$ *m/z* = 832.78, $[M+12H]^{12+}$ *m/z* = 763.46; observed:1830.52, 1525.52, 1307.72, 1144.35, 1017.33, 915.71, 832.48, 763.24.

*Native Peptide IL-17A(1-75) (**26**): Desulfurization of **25** Using Togin-II Reagent*



Following General Procedure **2.6** as described previously, thiol-containing peptide IL-17A(1-75) (**23**, 1.62 mg, 0.18 μmol) was dissolved in 300.0 μL of buffer (6 M Gn·HCl, 200 mM NaH_2PO_4, pH 8.4) under an argon atmosphere, followed by the addition of 300.0 μL of 0.5 M bond-breaker® TCEP solution (Pierce), 50.0 μL of 2-methyl-2-propanethiol and 80.0 μL of radical initiator (0.2 M Togin-II reagent in MeOH). The reaction was stirred at 50 °C and monitored using LC-MS. Upon completion after 10 h, the reaction was diluted with CH_3CN/H_2O/AcOH (30/65/5, v/v/v), and further purified using HPLC. The fractions containing pure peptide were collected, and concentrated via lyophilization to afford desulfurized IL-17A(1-75) (**26**) (1.35 mg, 84%) as a fluffy white solid.

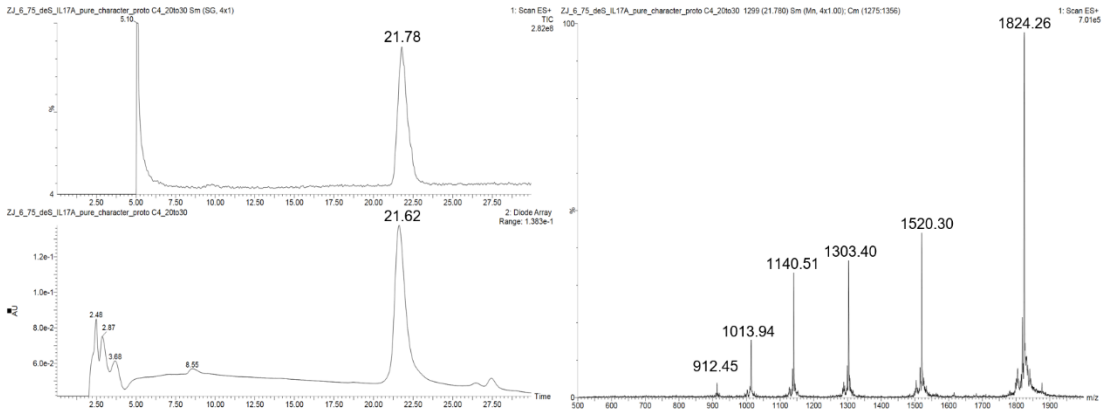


Figure S32. HPLC-MS analysis of peptide **26**. Left: MS (Top) and UV (Bottom) traces (Liner gradient of 20-30% solvent B over 30 min, PROTO-300 C4 column, t_R = 21.7 min); Right: ESI-MS data. Calcd for $C_{392}H_{611}N_{127}O_{122}S_2$: 9119.09 Da (average isotopes), $[M+5H]^{5+}$ *m/z* = 1824.51, $[M+6H]^{6+}$ *m/z* = 1520.59, $[M+7H]^{7+}$ *m/z* = 1303.51, $[M+8H]^{8+}$ *m/z* = 1140.70, $[M+9H]^{9+}$ *m/z* = 1014.06, $[M+10H]^{10+}$ *m/z* = 912.76, $[M+11H]^{11+}$ *m/z* = 829.87, $[M+12H]^{12+}$ *m/z* = 760.80; observed:1824.26, 1520.30, 1303.40, 1140.51, 1013.94, 912.45.