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* Corresponding author: christoph.nitsche@anu.edu.au

List of abbreviations

Boc	*tert*-butyloxycarbonyl protecting group
DCM	dichloromethane
DIPEA	diisopropylethylamine
DMF	dimethylformamide
DMSO-d_6	deuterated dimethyl sulfoxide
DTT	dithiothreitol
EDT	ethanedithiol
Fmoc	fluorenylmethoxycarbonyl protecting group
HATU	hexafluorophosphate azabenzotriazole tetramethyl uronium
HMBC	heteronuclear multiple bond correlation
HOBT	hydroxybenzotriazole
HRMS-ESI	high resolution mass spectrometry electrospray ionisation
HSQC	heteronuclear single quantum coherence
LCMS	liquid chromatography mass spectrometry
NMM	*N*-methyl morpholine
NMR	nuclear magnetic resonance
RP-HPLC	reverse phase high performance liquid chromatography
rt	room temperature
SPPS	solid-phase peptide synthesis
tBu	*tert*-butyl protecting group
TCEP	tris(2-carboxyethyl)phosphine
TFAA	trifluoroacetate anhydride
TFA	trifluoroacetic acid
TIPS/TIS	triisopropylsilane
Tris	tris(hydroxymethyl)aminomethane
Trt	trityl protecting group

Materials

Unless otherwise noted, all materials used in this study were purchased from commercial sources and used as received: Rink amide resin (~0.6 mmol/g, Auspep, Australia), Fmoc-AA-OH (GL Biochem, China; AK Scientific, USA; Ambeed; USA, Sigma-Aldrich, USA; ChemSupply, Australia), piperidine (Sigma-Aldrich, USA), *N*-methyl morpholine (Sigma-Aldrich, USA), HATU (AK Scientific, USA), 3,4-diaminobenzoic acid (EGA-Chemie, Spain), isoamyl nitrite (Sigma-Aldrich, USA). Solvents were purchased from ChemSupply, Australia; Supelco, USA; Univar, USA. Deuterated solvents were obtained from Cambridge Isotope Laboratories (USA).

Instrumentation and analytical methods

Thin-Layer Chromatography (TLC)

TLC analyses were performed on pre-coated aluminium-backed silica sheets (Merck TLC Silica gel 60 F_{254}) using UV visualisation (254 nm) or staining. The retention factor (R_f) was calculated based on the distance the compound has travelled compared to the solvent front.

LCMS method

The sample was analysed using an Agilent 1260/ 6120 LCMS system equipped with an Eclipse XDB-C_{18} column (1.8 μm, 2.1 mm × 50 mm) at a gradient of 20 – 95% B for 20 min, unless otherwise specified. Mass characterisation was confirmed using the tandem mass spectrometer appended to the LCMS system. Solvent A for the LCMS was ultrapure water containing 0.1% TFA and solvent B was MeCN containing 0.1% TFA.

RP-HPLC method

The samples were purified using a Waters HPLC system on an SymmetryPrep™ C_{18} column (7 μm, 150mm × 19mm) at a gradient of 20 – 90% solvent B for 20 min. Solvent A for the RP-HPLC was water containing 0.1% TFA and solvent B was MeCN containing 0.1% TFA.

Flash column chromatography

Small molecules were purified using a Biotage® Isolera™ One system equipped with a Biotage SNAP Ultra silica gel cartridge (silica; *n*-hexane:ethyl acetate).

HRMS-ESI method

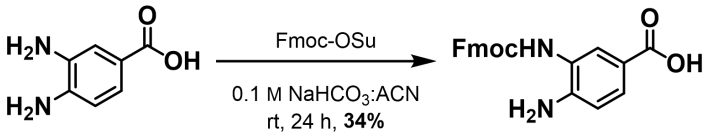
High resolution electrospray ionisation mass spectrometry (HRMS-ESI) analyses were performed at the ANU Joint Mass Spectrometry Facility on a Thermo Scentific Orbitrap Elite mass spectrometer (Thermo Fisher Scientific, Delaware, USA) equipped with a HESI-II electrospray ionisation source coupled to an Ulti-Mate 3000 UHPLC (Thermo Scientific). An isocratic elution mode was used to deliver the sample into MS by direct infusion. The scan range was set to *m*/*z* 150–2000 and was performed on the orbitrap FTMS mass analyser at a resolution of 120000. The spray voltage was set at 2.5 kV, and the source heater temperature at 300 °C. The data was analysed using Freestyle software. Elemental composition reports were produced within 3 ppm error.

NMR spectroscopy

Small molecule NMR spectra were recorded on a Bruker Avance III 400 MHz equipped with a 5 mm Bruker probe head (PA BBO 400S1 BBF-H-D-05 Z SP) and peptide NMR spectra were recorded on a Bruker 800 MHz NMR equipped with a cryoprobe. Chemical shifts are reported in parts per million (ppm) and were referenced internally to the solvent. Spectra were recorded at 318 K. NMR spectra of peptide **1** (1.2 mg) were recorded in D_2O (200 μL, 8.5 mM). Resonances were assigned from COSY, [^{1}H,^{13}C]-HSQC, and [^{1}H,^{13}C]-HMBC spectra. All NMR spectra were processed using the Mnova NMR software (Mestrelab, Spain).

Synthesis of Fmoc-Dbz-OH

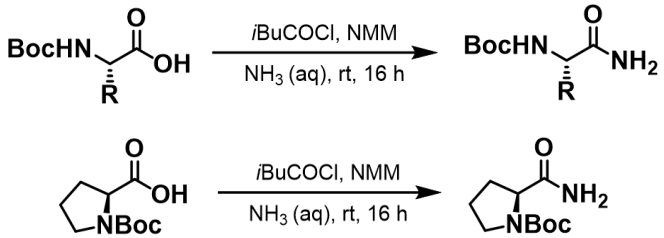
Following the literature procedure,[1] to a magnetically stirred solution of 3,4-diaminobenzoic acid (2.0 g, 13.6 mmol) in $NaHCO_3$ (0.1 M in MeCN/H_2O, 1:1 *v/v*, 80 mL) was added *N*-(9-fluorenylmethyloxycarbonyloxy) succinimide (4.6 g, 13.6 mmol) in small portions. The reaction mixture was stirred at room temperature for 24 h, neutralised with 1 M HCl(aq) until a precipitate formed. The precipitate was collected via suction filtration and washed with diethyl ether (3 × 40 mL), *n*–hexane (3 × 40 mL), then methanol (3 × 40 mL) and dried under reduced pressure to afford Fmoc-Dbz-OH as a grey solid (1.7 g, 34%). The material was used in the next step without further purification.



Scheme S1. Preparation of Fmoc-Dbz-OH

General procedure for Boc-amino amide synthesis (procedure 1)

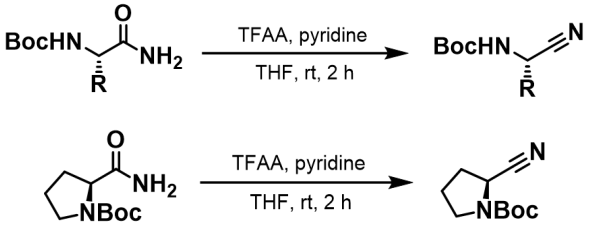
Following the literature procedure,[2] Boc-protected amino acids (38 mmol, 1 equiv.) were solved in THF (100 mL, 0.38 mol/L). The mixture was cooled down to −10 ℃ and NMM (76 mmol, 2 equiv.) and *i*BuCOCl were added. After stirring at −10 °C for 30 mins, the mixture was treated with aqueous 28 – 30% ammonia solution (9 mL, 70 mmol, 1.8 equiv.) and stirred at room temperature overnight. The solvent was removed under reduced pressure and the crude material was dissolved in DCM (125 mL), washed with HCl solution (3 × 200 mL), water (3 × 200 mL), brine (3 × 200 mL) and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure and the product was purified using flash column chromatography (silica; 50 – 100% ethyl acetate: *n*-hexane containing 0.1% formic acid). The products were collected, and excess solvents were evaporated to afford **7a** – **11a**.



Scheme S2. Preparation of Boc-aminoamide **7a** – **11a**.

General procedure for Boc-amino nitrile synthesis (procedure 2)

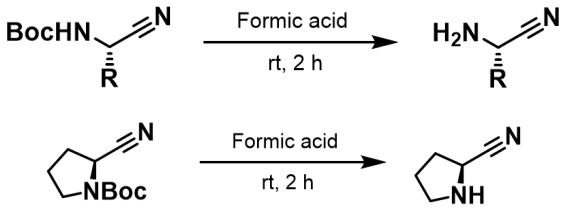
Following the literature procedure,[3] amino amides **7a** – **11a** (26 mmol, 1 equiv.) were added to dry THF (100 mL) and cooled to −10 °C. The mixture was treated with pyridine (78 mmol, 3 equiv.) and TFAA (39 mmol, 1.5 equiv.), and the resulting mixture was stirred for 4 h at room temperature. The sample was concentrated, then re-dissolved in ethyl acetate (100 mL). The organic phase was washed with 1 M $NHCO_3$ (3 × 100 mL), brine (3 × 100 mL), and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure and the product was purified using flash column chromatography (silica; 1:3 *v/v* ethyl acetate: *n*-hexane containing 0.1% formic acid). The product was collected, and the solvent was evaporated under reduced pressure to afford Boc-amino nitriles **7b** – **11b**.



Scheme S3. Preparation of Boc-aminonitrile **7b** – **11b**.

General procedure for amino nitrile synthesis (procedure 3)

Boc-amino nitriles **7b** – **11b** (0.62 mmol, 1 equiv.) were dissolved in concentrated formic acid (5 mL, 0.12 mol/L) and magnetically stirred at room temperature for 2 h. Afterwards the solvent was removed under reduced pressure to afford products **7** – **11** as they formic acid salts. The material was directly used in the next step without further purification.



Scheme S4. Preparation of aminonitrile **7** – **11** (obtained as formic acid salts).

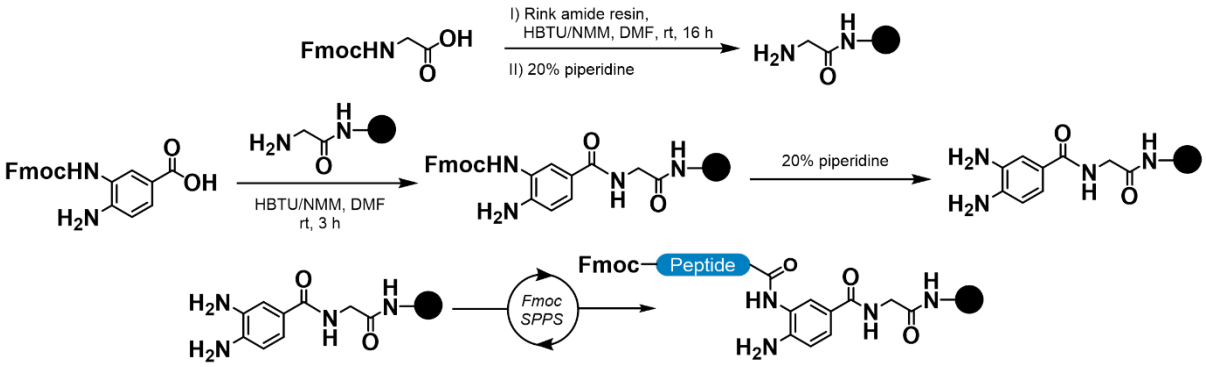
General procedure for Fmoc-SPPS (procedure 4)

Preparation of the Dawson linker

Fmoc-protected Rink amide resin (capacity ~0.6 mmol/g) was swollen in DMF for 30 min, treated with 20% piperidine in DMF (2 × 5 min) and washed with DMF (3 × 3 mL), DCM (3 × 3 mL) and then DMF (3 × 3 mL). A solution of Fmoc-Gly-OH (1.5 equiv., 0.2 mmol), HATU (1.5 equiv., 0.2 mmol), HOBt (1.5 equiv., 0.2 mmol), and NMM (1.5 equiv., 0.2 mmol) in DMF (3 mL) was added to the resin and agitated for 16 h. The Fmoc-protecting group was removed as described above and the resin was subjected to a solution of Fmoc-Dbz-OH (1.5 equiv., 0.2 mmol), HATU (1.5 equiv., 0.2 mmol) and NMM (1.5 equiv. 0.2 mmol) in DMF (3 mL) and agitated for 3 h.

Peptide synthesis

The modified resin (1 equiv., 0.1 mmol) was swollen in DMF for 30–60 min at room temperature, drained and then washed with DCM (3 × 3 mL) and DMF (3 × 3 mL). The resin was treated with 20% piperidine in DMF *v/v* (2 mL, 2 × 5 min), drained and washed with DMF (3 × 3 mL), DCM (3 × 3 mL) and then DMF (3 × 3 mL). The resin was loaded with the first amino acid from a concoction made of Fmoc-protected amino acid (1.5 equiv., 0.2 mmol), HATU (1.5 equiv., 0.2 mmol), HOBt (1.5 equiv., 0.2 mmol) and NMM (1.5 equiv., 0.2 mmol) in DMF (final concentration 0.1 M). The mixture was agitated for 2 h. After agitation, the resin was drained, washed with DMF (3 × 3 mL), DCM (3 × 3 mL) and then DMF (3 × 3 mL). Amino acid coupling and Fmoc deprotection were repeated as described above until the desired sequence was completed. After the final washing step, the resin was dried under a gentle stream of N_2 gas.

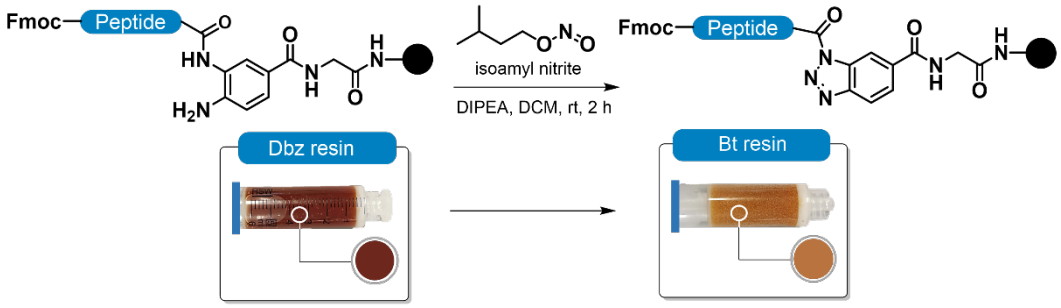


Scheme S5. Preparation of peptide sequences on the modified resin.

General procedure for linker activation (procedure 5)

Preparation of the benzotriazole linker

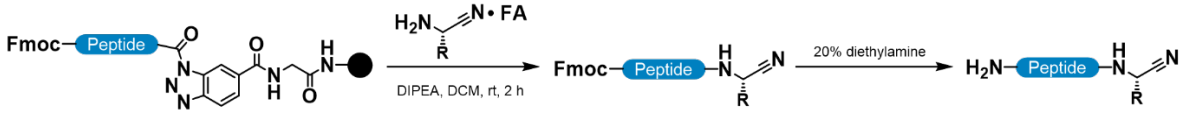
The resin-bound Dbz-peptide was swollen in DCM for 1 h and washed with DMF (3 × 3 mL), DCM (3 × 3 mL) and then DMF (3 × 3 mL). The modified resin was treated with a solution of isoamyl nitrite (10 equiv., 1.5 mmol) in DMF (3 mL) and then agitated for 12 h. The resin was washed with DMF (3 × 2 mL), DCM (3 × 2 mL) and then DMF (3 × 2 mL).



Scheme S6. Activation of the C-terminus.

General procedure for nitrile peptide synthesis (procedure 6)

The modified resin was subjected to a solution of amino nitriles **7** – **11** (0.6 mmol, 4 equiv.) and DIPEA (1.2 mmol, 8 equiv.) in DCM (2 mL), and agitated at room temperature for 4 h. The solution was collected, and the resin was washed with DCM (3 × 5 mL). The mixture was concentrated under reduced pressure and treated with 20% diethylamine in DCM *v/v* for 30 min. The peptide was immediately triturated with diethyl ether (30 mL) and the pellet was collected by centrifugation. The diethyl ether was decanted to afford the crude linear nitrile peptides **1a** – **6a** as an amorphous solid.



Scheme S7. Preparation of linear nitrile peptides **1a** – **6a**.

General procedure for peptide sidechain deprotection (procedure 7)

Peptides **1a** – **6a** (0.16 mmol, 1 equiv.) were treated to a concoction of TFA/TIPS/EDT in DCM (5:2.5:2.5:90 *v/v/v/v,* 10 mL), and agitated at room temperature for 1 h. The solvent was removed under reduced pressure, and the crude mixture was triturated with ice-cold diethyl ether (30 mL). The white precipitate was centrifuged, and the diethyl ether was decanted. The crude peptide was directly used in the next step without further purification.

General procedure for peptide thiazoline cyclisation (procedure 8)

Peptides **1b** – **6b** (0.16 mmol, 1 equiv.) were dissolved in a solution of 10 mM Tris·HCl buffer, pH 7.5 (2.5 mL) and 4 mM TCEP (2.5 mL) at room temperature. The mixture was agitated for 20 h and the progression of the reaction was monitored with HRMS and analytical LCMS. The material was directly used in the next step without further purification.

General procedure for the oxidation of thiazoline peptides (procedure 9)

Peptides **1c** – **6c** (0.16 mmol, 1 equiv.) were dissolved in MeCN (2 mL) before adding crushed pellets of NaOH (0.47 mmol, 3 equiv.). The mixture was agitated under air for 24 h. After concentrating the sample, the macrocyclic peptides **1** – **5** were purified following standard the RP-HPLC method. The fractions containing product were collected and lyophilised to afford peptides **1** – **5** as white solids.

Table S1. Calculated and observed high resolution *m/z* values of investigated amino acids and peptides.

Codes	Molecular formula	Calculated $[M+H]^+$	Observed $[M+H]^+$	Calculated $[M+Na]^+$	Observed $[M+Na]^+$
1a	$C_{59}H_{71}N_9O_8S$	1066.5218	1066.5189	1088.5038	1088.5011
1b	$C_{35}H_{49}N_9O_6S$	724.3599	724.3593	746.3419	746.3409
1c	$C_{35}H_{46}N_8O_6S$	707.3333	707.3321	729.3153	729.3139
1	$C_{35}H_{44}N_8O_6S$	705.3177	705.3181	727.2997	727.3000
2a	$C_{53}H_{67}N_9O_8S$	990.4905	990.4875	1012.4725	1012.4701
2b	$C_{29}H_{45}N_9O_6S$	648.3286	648.3266	670.3106	670.3087
2c	$C_{29}H_{42}N_8O_6S$	631.3020	631.3008	653.2840	653.2826
2	$C_{29}H_{40}N_8O_6S$	629.2864	629.2845	651.2684	651.2663
3a	$C_{56}H_{73}N_9O_8S$	1032.5375	1032.5355	1054.5195	1054.5178
3b	$C_{32}H_{51}N_9O_6S$	690.3755	690.3736	712.3575	712.3554
3c	$C_{32}H_{48}N_8O_6S$	673.3490	673.3466	695.3310	695.3284
3	$C_{32}H_{46}N_8O_6S$	671.3333	671.3344	693.3153	693.3163
4a	$C_{48}H_{60}N_8O_5S$			883.4299	883.4298
4b	$C_{29}H_{46}N_8O_5S$	690.3755	690.3762		
4c	$C_{29}H_{43}N_7O_5S$	602.3118	602.3143	624.2938	624.2962
4	$C_{29}H_{41}N_7O_5S$	600.2962	600.2970	622.2782	622.2789
5a	$C_{56}H_{66}N_8O_7S$	995.4847	995.4847		
5b	$C_{32}H_{44}N_8O_5S$	653.3227	653.3223		
5c	$C_{32}H_{41}N_7O_5S$	636.2962	636.2965	658.2782	658.2784
5	$C_{32}H_{39}N_7O_5S$	634.2805	634.2829	656.2625	656.2650
6a	$C_{50}H_{61}N_9O_6S$	1016.5062	1016.5058	1038.4882	1038.4877
6b	$C_{31}H_{47}N_9O_6S$	674.3442		696.3262	696.3237
6c	$C_{31}H_{44}N_8O_6S$	657.3177	657.3177	679.2997	679.2998
6	$C_{31}H_{42}N_8O_6S$	655.3020	655.2986	677.2840	677.2815
7a	$C_{14}H_{20}N_2O_3$			287.1372	287.1379
7b	$C_{14}H_{18}N_2O_2$			269.1266	269.1273
7	$C_9H_{10}N_2$	147.0916	147.0921		
8a	$C_8H_{16}N_2O_3$			211.1059	211.1057
8b	$C_8H_{14}N_2O_2$	171.1128	171.1144		
8	$C_3H_6N_2$	71.0603	71.0604		
9a	$C_{11}H_{22}N_2O_3$			253.1528	253.1532
9b	$C_{11}H_{20}N_2O_2$			235.1417	235.1415
9	$C_6H_{12}N_2$	113.1073	113.1071		
10a	$C_{11}H_{22}N_2O_3$			253.1522	253.1525
10b	$C_{11}H_{20}N_2O_2$			235.1422	235.1419
10	$C_6H_{12}N_2$	113.1072	113.1076		
11a	$C_{10}H_{18}N_2O_3$			237.1209	237.1212
11b	$C_{10}H_{16}N_2O_2$			219.1104	219.1108
11	$C_5H_8N_2$	97.0760	97.0763		

LCMS data for cyclic thiazole peptides (1 – 6)

Peptide 1

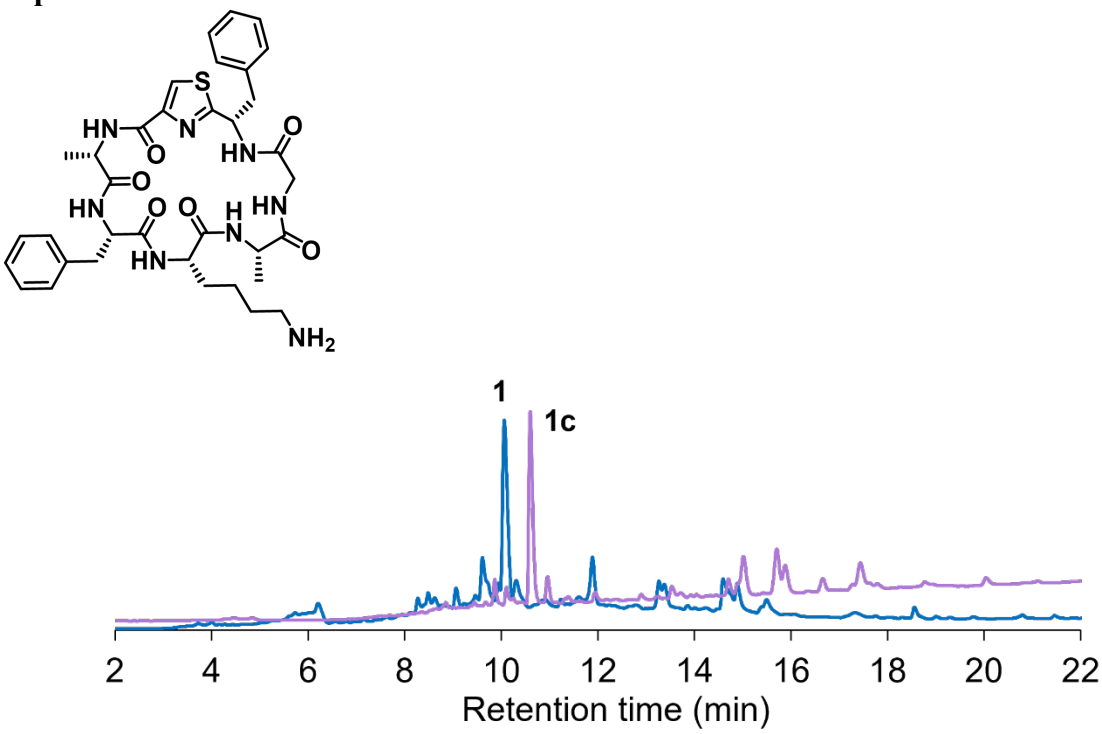


Figure S1. Oxidation of peptide **1c** to **1**. Superimposed chromatograms (254 nm) of crude peptide **1** (thiazole) and its precursor **1c** (thiazoline). No purification was performed at any step.

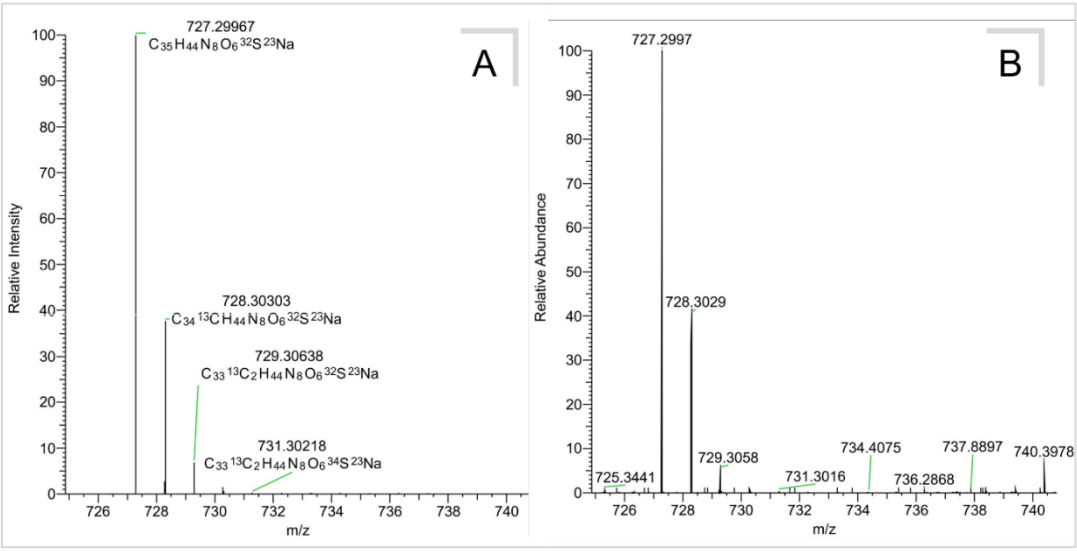
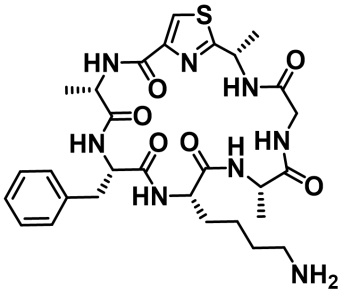


Figure S2. Isotopic signature of peptide **1** (calculated for $C_{35}H_{44}N_8O_6SNa^+$ $[M+Na]^+$). Samples were analysed following the HRMS-ESI method. The isotope model is shown in (A), and the observed isotopes are shown in (B).

Peptide 2



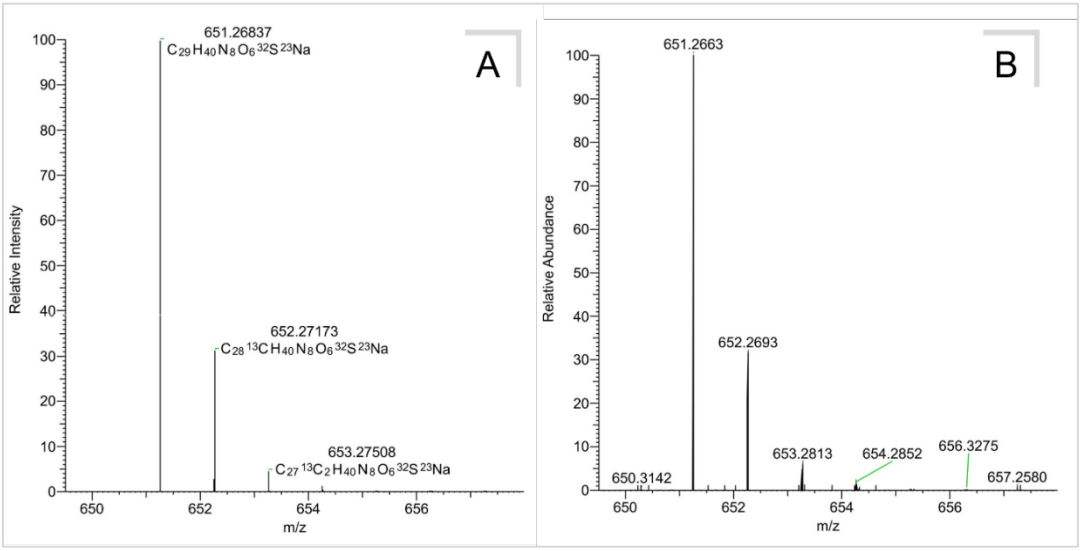


Figure S3. Isotopic signature of peptide **2** (calculated for $C_{29}H_{40}N_8O_6SNa^+$ $[M+Na]^+$). Samples were analysed following the HRMS-ESI method. The isotope model is shown in (A), and the observed isotopes are shown in (B).

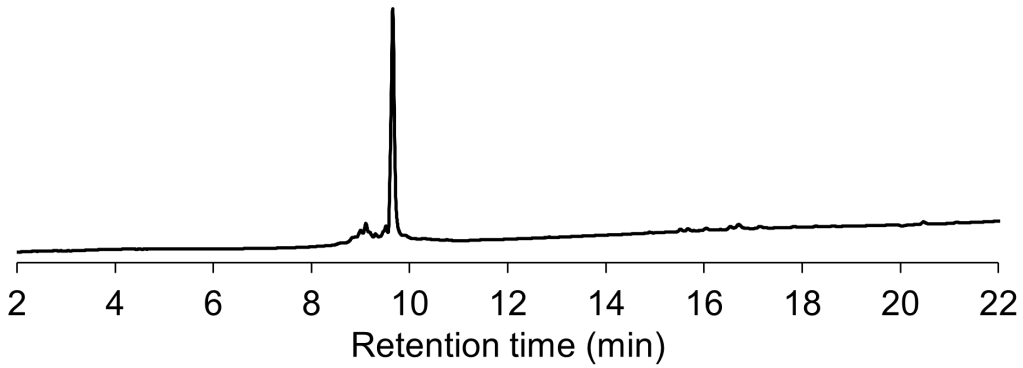
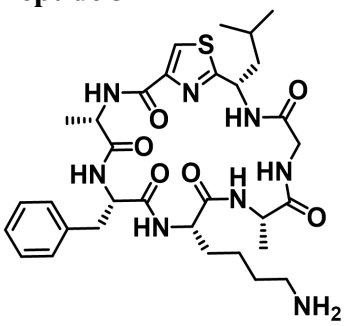


Figure S4. Chromatogram (254 nm) of purified peptide **2**. The peptide was analysed following the LCMS method.

Peptide 3



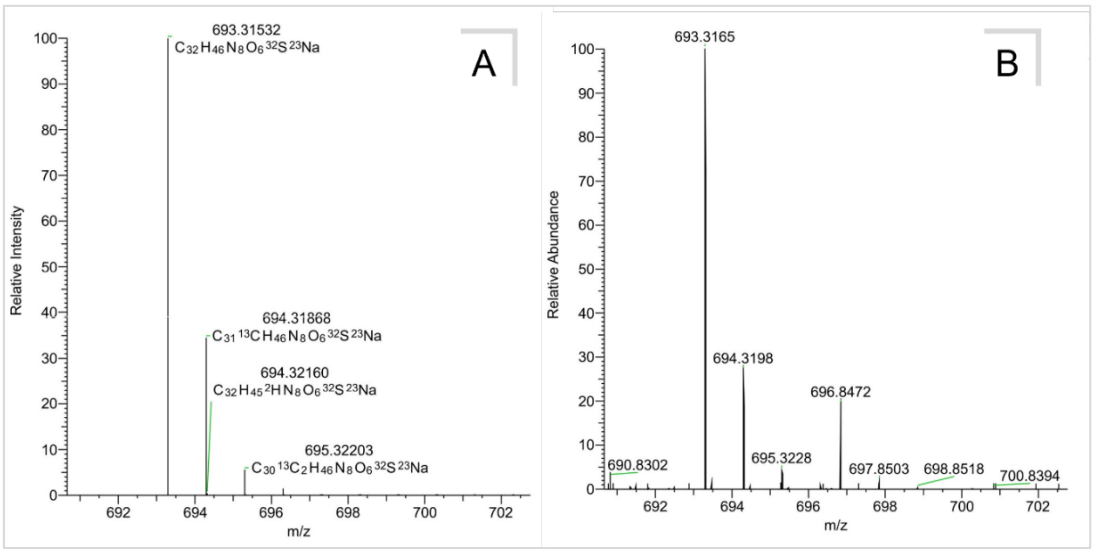


Figure S5. Isotopic signature of peptide **3** (calculated for $C_{32}H_{46}N_8O_6SNa^+$ $[M+Na]^+$). Samples were analysed following the HRMS-ESI method. The isotope model is shown in (A), and the observed isotopes are shown in (B).

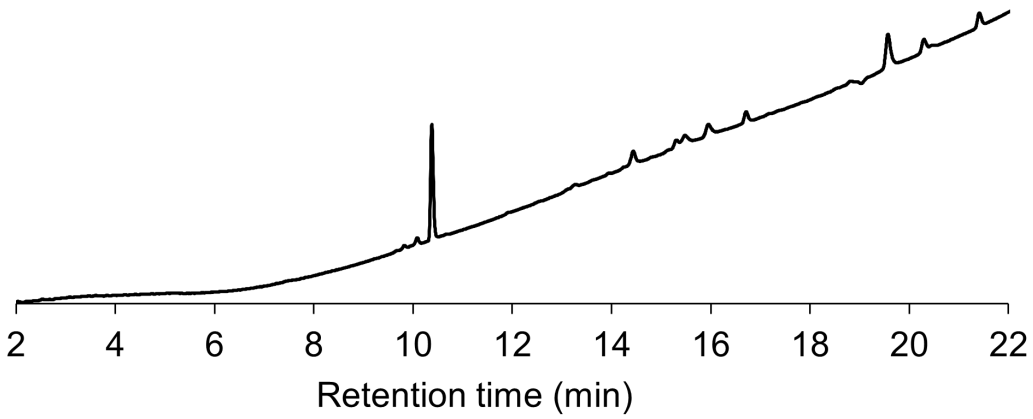
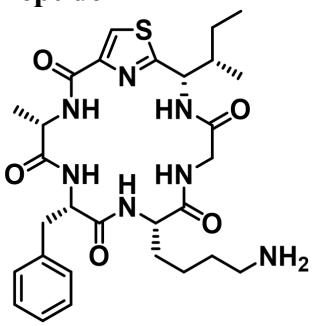


Figure S6. Chromatogram (254 nm) of purified peptide **3**. The peptide was analysed following the LCMS method.

Peptide 4



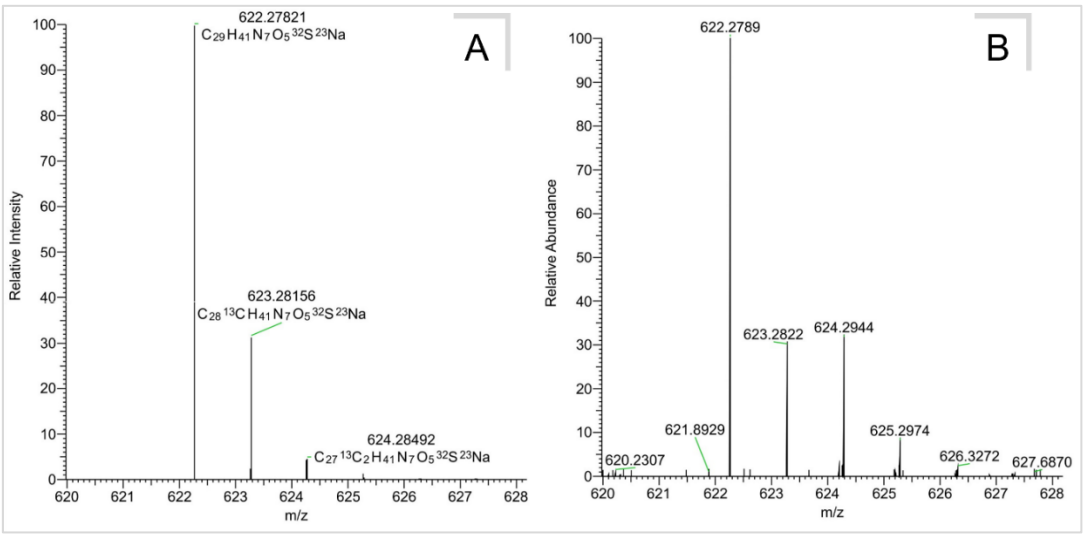


Figure S7. Isotopic signature of peptide **4** (calculated for $C_{29}H_{41}N_7O_5SNa^+$ $[M+Na]^+$). Samples were analysed following the HRMS-ESI method. The isotope model is shown in (A), and the observed isotopes are shown in (B).

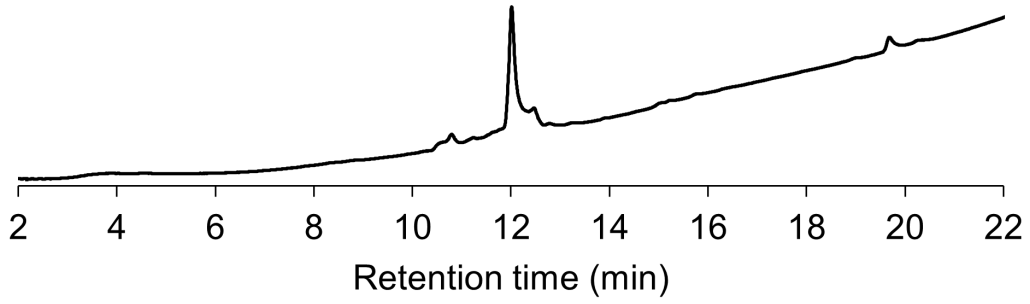
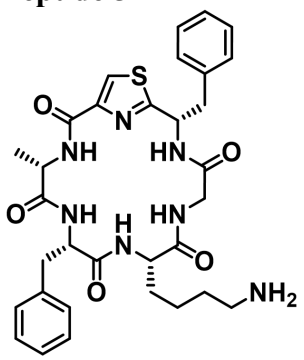


Figure S8. Chromatogram (254 nm) of purified peptide **4**. The peptide was analysed following the LCMS method.

Peptide 5



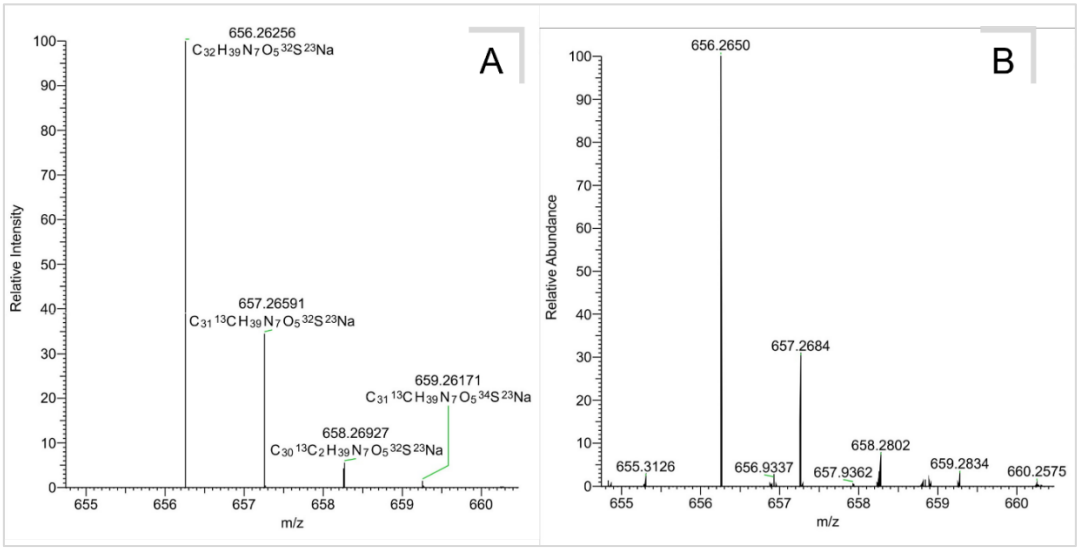


Figure S9. Isotopic signature of peptide **5** (calculated for $C_{32}H_{39}N_7O_5SNa^+$ $[M+Na]^+$). Samples were analysed following the HRMS-ESI method. The isotope model is shown in (A), and the observed isotopes are shown in (B).

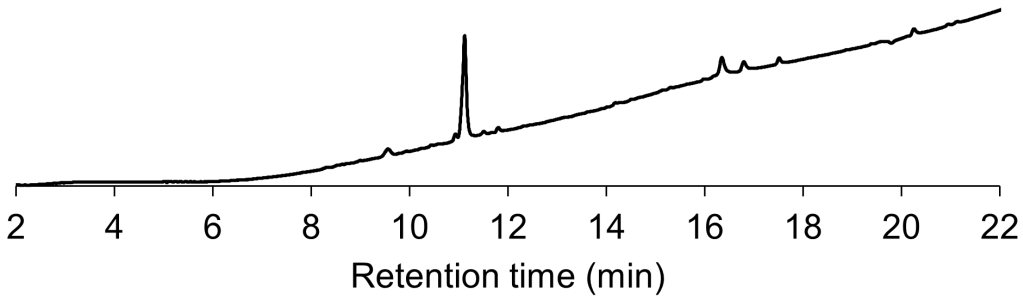
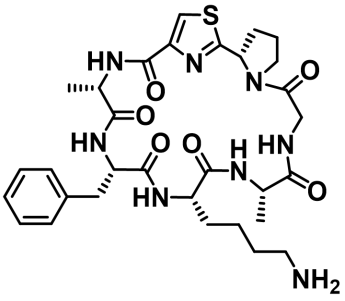


Figure S10. Chromatogram (254 nm) of purified peptide **5**. The peptide was analysed following the LCMS method.

Attempted peptide 6



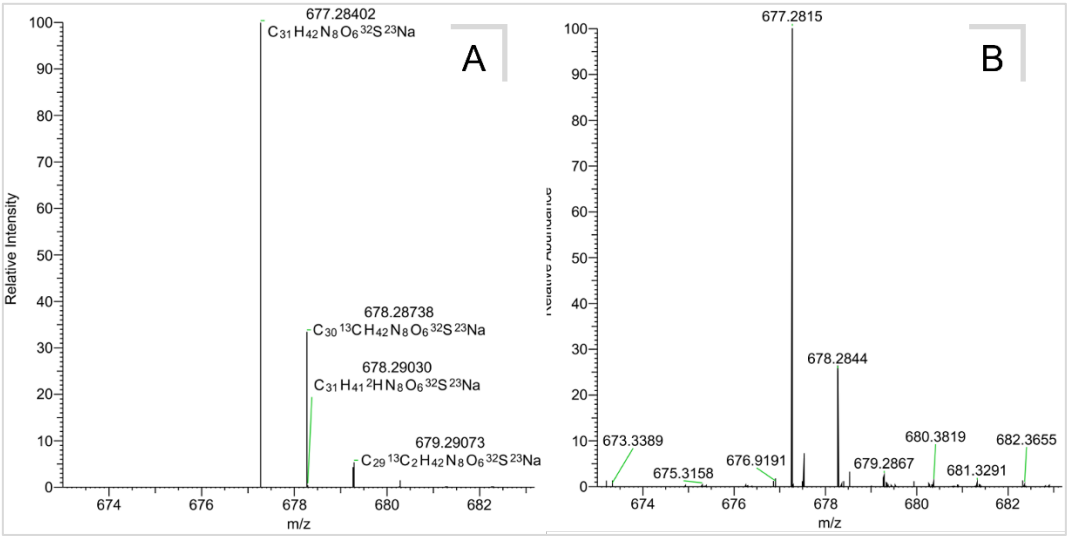


Figure S11. Isotopic signature of peptide **6** (calculated for $C_{31}H_{42}N_8O_6SNa^+$ $[M+Na]^+$). Samples were analysed following the HRMS-ESI method. The isotope model is shown in (A), and the observed isotopes are shown in (B).

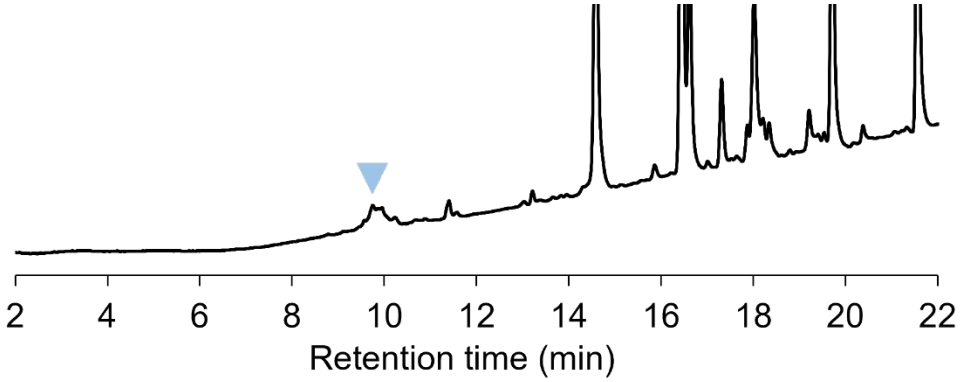
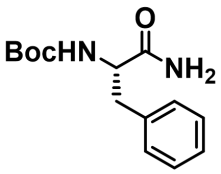


Figure S12. Chromatogram (254 nm) of crude peptide **6**. The peptide was analysed following the LCMS method. The indicated peak contains a mixture of **6** and **6c** indicating incomplete oxidation and low overall yield, preventing isolation **6**.

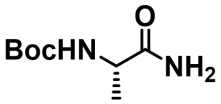
Characterisation data for small molecules (7a,b – 11a,b)

Boc-Phe-NH_2 (7a)



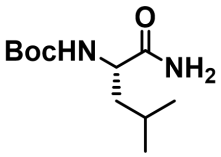
Product was obtained as a white solid (8.6 g, 92%). R_f = 0.72 (n-hexane: ethyl acetate, 1:1 *v/v*). **^{1}H NMR (400 MHz, DMSO-*d*$_6$)** 7.41 (s, 1H), 7.37 – 7.21 (m, 5H), 7.06 (s, 1H), 6.84 (d, *J* = 8.7 Hz, 1H), 4.15 (td, *J* = 9.4, 4.2 Hz, 1H), 3.01 (dd, *J* = 13.7, 4.4 Hz, 1H), 2.78 (dd, *J* = 13.8, 10.2 Hz, 1H), 1.35 (s, 9H) ppm. **^{13}C NMR (101 MHz, DMSO-*d*$_6$)** δ 174.1, 155.7, 138.8, 129.6, 128.4, 126.6, 78.4, 56.1, 38.0, 28.6 ppm. **HRMS-ESI (m/z):** calculated for $C_{14}H_{20}N_2O_3Na^+$ $[M+Na]^+$ 287.1372; found 287.1379.

Boc-Ala-NH_2 (8a)



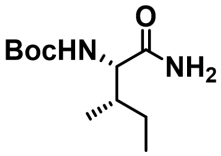
The product was obtained as a white solid (2.2 g, 31%). R_f = 0.70 (n-hexane: ethyl acetate, 1:1 *v/v*). **^{1}H NMR (400 MHz, DMSO- *d*$_6$)** δ 7.20 (s, 1H), 6.91 (s, 1H), 6.75 (d, *J* = 7.8 Hz, 1H), 3.89 (t, *J* = 7.4 Hz, 1H), 1.38 (s, 9H), 1.16 (d, *J* = 7.2 Hz, 3H) ppm. **^{13}C NMR (101 MHz, DMSO-*d*$_6$)** δ 175.2, 155.5, 78.3, 49.9, 28.7, 18.8 ppm. **HRMS-ESI (m/z):** calculated for $C_8H_{16}N_2O_3Na^+$ $[M+Na]^+$ 211.1059; found 211.1057.

Boc-Leu-NH_2 (9a)



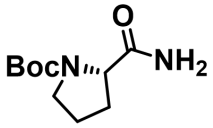
The product was obtained as a white solid (8.2 g, 94%). R_f = 0.75 (*n*-hexane: ethyl acetate, 1:1 *v/v*). **^{1}H NMR (400 MHz, DMSO-*d*$_6$)** δ 7.19 (s, 1H), 6.90 (s, 1H), 6.74 (d, *J* = 8.5 Hz, 1H), 3.88 (td, *J* = 9.1, 5.4 Hz, 1H), 1.67 – 1.52 (m, 2H), 1.38 (m, 10H), 0.86 (dd, *J* = 8.1, 6.6 Hz, 6H) ppm. **^{13}C NMR (101 MHz, DMSO-*d*$_6$)** δ 175.2, 155.8, 78.3, 53.1, 41.4, 28.7, 24.8, 23.5, 21.9 ppm. **HRMS-ESI (m/z):** calculated for $C_{11}H_{22}N_2O_3Na^+$ $[M+Na]^+$ 253.1528; found 253.1532.

Boc-Ile-NH_2 (10a)



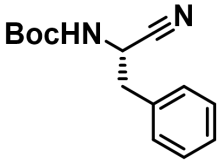
The product was obtained as a white solid (6.2 g, 71%). R_f = 0.75 (*n*-hexane: ethyl acetate, 1:1 *v/v*). **^{1}H NMR (400 MHz, DMSO-*d*$_6$)** *δ* 7.25 (s, 1H), 6.98 (s, 1H), 6.54 (d, *J* = 9.1 Hz, 1H), 3.81 – 3.67 (m, 1H), 1.93 – 1.58 (m, 2H), 1.38 (s, 9H), 1.14 – 1.00 (m, 1H), 0.90 – 0.79 (m, 6H) ppm.**^{13}C NMR (101 MHz, DMSO-*d*$_6$)** *δ* 174.0, 155.8, 78.4, 59.1, 36.9, 28.6, 24.8, 15.9, 11.5 ppm. **HRMS-ESI (m/z):** calculated for $C_{11}H_{22}N_2O_3Na^+$ $[M+Na]^+$ 253.1522; found 253.1525.

Boc-Pro-NH_2 (11a)



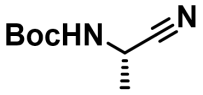
The product was obtained as a white solid (3.7 g, 45%). R_f = 0.82 (*n*-hexane: ethyl acetate, 1:1 *v/v*). **^{1}H NMR (400 MHz, DMSO-*d*$_6$) *δ*** 7.27 (d, *J* = 12.8 Hz, 1H), 6.88 (d, *J* = 17.3 Hz, 1H), 4.00 (ddd, *J* = 12.1, 8.6, 3.3 Hz, 1H), 3.37 (dd, *J* = 10.2, 5.4 Hz, 1H), 3.26 (dt, *J* = 10.2, 6.7 Hz, 1H), 2.08 (ddd, *J* = 18.3, 8.8, 3.6 Hz, 1H), 1.89 – 1.69 (m, 3H), 1.34 (s, 9H).**^{13}C NMR (101 MHz, DMSO-*d*$_6$)** *δ* 175.1, 153.8, 78.8, 60.0, 46.8, 31.5, 28.5, 23.6 ppm. **HRMS-ESI (m/z):** calculated for $C_{10}H_{18}N_2O_3Na^+$ $[M+Na]^+$ 237.1209; found 237.1212.

Tert-butyl (_S_)-(1-cyano-2-phenylethyl)carbamate (7b)



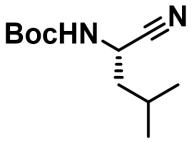
The product was obtained as a white solid (5.7 g, 89%). R_f = 0.78 (n-hexane: ethyl acetate, 4:1 *v/v*). **^{1}H NMR (400 MHz, DMSO-*d*$_6$)** δ 7.82 (d, *J* = 8.2 Hz, 1H), 7.37 – 7.20 (m, 5H), 4.64 (q, *J* = 8.1 Hz, 1H), 3.05 (d, *J* = 7.9 Hz, 2H), 1.37 (s, 9H) ppm.**^{13}C NMR (101 MHz, DMSO-*d*$_6$)** δ 155.1, 136.1, 129.8, 128.8, 127.5, 120.0, 79.8, 44.0, 37.9, 28.5 ppm. **HRMS-ESI (m/z):** calculated for $C_{14}H_{18}N_2O_2Na^+$ $[M+Na]^+$ 269.1266; found 269.1273.

Tert-butyl (_S_)-(1-cyanoethyl)carbamate (8b)



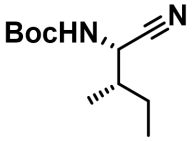
The product was obtained as a white solid (1.2 g, 27%). R_f = 0.65 (n-hexane: ethyl acetate, 4:1 *v/v*). **^{1}H NMR (400 MHz, DMSO-*d*$_6$)** δ 7.73 (d, *J* = 8.9 Hz, 1H), 4.50 (p, *J* = 7.5 Hz, 1H), 1.41 (s, 9H), 1.38 (dd, *J* = 7.2, 1.2 Hz, 3H) ppm. **^{13}C NMR (101 MHz, DMSO-*d*$_6$)** δ 155.1, 121.2, 79.7, 37.8, 28.5, 18.8 ppm. **HRMS-ESI (m/z):** calculated for $C_8H_{14}N_2O_2^+$ $[M+H]^+$ 171.1128; found 171.1144.

Tert-butyl (_S_)-(1-cyano-3-methylbutyl)carbamate (9b)



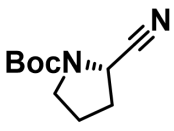
The product was obtained as a white solid (4.2 g, 78%). R_f = 0.85 (n-hexane: ethyl acetate, 4:1 *v/v*). **^{1}H NMR (400 MHz, DMSO- *d*$_6$)** δ 7.73 (d, *J* = 8.2 Hz, 1H), 4.43 (q, *J* = 7.9 Hz, 1H), 1.72 – 1.58 (m, 3H), 1.41 (s, 9H), 0.89 (dd, *J* = 6.2, 4.1 Hz, 6H) ppm. **^{13}C NMR (101 MHz, DMSO- *d*$_6$)** δ 155.2, 120.5, 79.7, 40.8, 40.7, 28.5, 24.7, 22.3, 22.1 ppm. **HRMS-ESI (m/z):** calculated for $C_{11}H_{20}N_2O_2Na^+$ $[M+Na]^+$ 235.1417; found 235.1415.

Tert-butyl ((1_S_,2_S_)-1-cyano-2-methylbutyl)carbamate (10b)



The product was obtained as a white solid (3.5 g, 64%). A colourless crystal of compound **10b** was obtained by slow evaporation from 1:3 *v/v* ethyl acetate:n-hexane solution. R_f = 0.85 (n-hexane: ethyl acetate, 4:1 *v/v*). **^{1}H NMR (400 MHz, DMSO-*d*$_6$)** *δ* 7.79 (d, *J* = 8.4 Hz, 1H), 4.35 (t, *J* = 7.8 Hz, 1H), 1.78 – 1.66 (m, 1H), 1.41 (m, 10H), 1.16 (dt, *J* = 13.0, 7.5 Hz, 1H), 0.98 (d, *J* = 6.8 Hz, 3H), 0.85 (t, J = 7.4 Hz, 3H) ppm. **^{13}C NMR (101 MHz, DMSO-*d*$_6$)** *δ* 155.4, 119.4, 79.7, 47.5, 37.1, 28.5, 25.4, 15.5, 11.1 ppm. **HRMS-ESI (m/z):** calculated for $C_{11}H_{20}N_2O_2^+$ $[M+Na]^+$ 235.1422; found 235.1419.

Tert-butyl (_S_)-2-cyanopyrrolidine-1-carboxylate (11b)



The product was obtained as a yellow oil (1.4 g, 27%). R_f = 0.91 (n-hexane: ethyl acetate, 4:1 *v/v*). **^{1}H NMR (400 MHz, DMSO-*d*$_6$)** *δ* 4.62 (dd, *J* = 8.0, 3.3 Hz, 1H), 3.36 (d, *J* = 6.6 Hz, 1H), 3.30 – 3.18 (m, 1H), 2.30 – 2.07 (m, 2H), 2.02 – 1.85 (m, 2H), 1.44 (s, 9H) ppm. **^{13}C NMR (101 MHz, DMSO-*d*$_6$)** *δ* 153.6, 120.2, 80.4, 47.3, 46.4, 31.5, 28.4, 24.8 ppm. **HRMS-ESI (m/z):** calculated for $C_{10}H_{16}N_2O_2Na^+$ $[M+Na]^+$ 219.1104; found 219.1108.

Crystal structure determination (10b)

Single crystals of **10b** were grown from 1:3 *v/v* ethyl acetate: *n*-hexane solution as colourless prism-shaped crystals. Suitable crystals were selected and the crystals mounted on MiTeGen holders in oil on a SuperNova, Dual, Cu at home/near, HyPix diffractometer. The crystals were kept at 150.01(10) K during data collection. Using Olex2,[4] the structures were solved with the SHELXT[5] structure solution program using Intrinsic Phasing and refined with the SHELXL[6] refinement package using Least Squares minimization of F^2.

Crystal data for compound **10b**: $C_{11}H_{20}N_2O_2$, M_r = 212.29, monoclinic, $P2_1$ (No. 4), a = 5.12630(10) Å, b = 10.4781(3) Å, c = 12.0133(3) Å, β = 98.138(2)°, V = 638.78(3) Å^3, T = 150.01(10) K, Z = 2, Z' = 1, μ(Cu K$_\alpha$) = 0.612, 6805 reflections measured, 2578 unique (R_{int} = 0.0356) which were used in all calculations. The final wR_2 was 0.1284 (all data) and R_1 was 0.0464 (I≥2 σ(I)). Flack parameter was 0.03(15), with the absolute configuration CIF entry set as "rmad" on the basis of this determination from the anonymous dispersion features of the data and the *s*-Bu substituent that is inferred to have a fixed absolute configuration throughout the synthetic scheme.

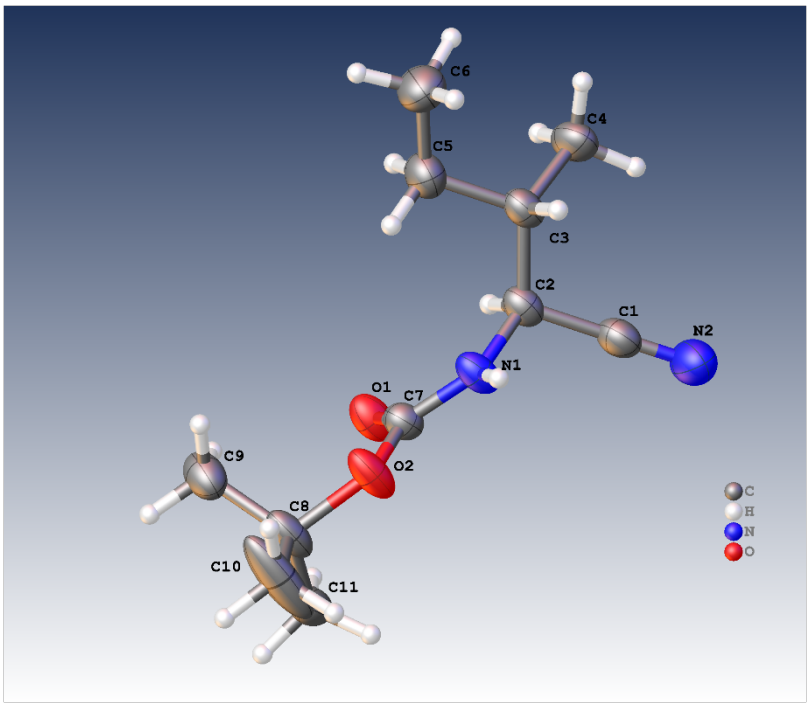


Figure S13. Molecular structure of **10b** showing atom labelling scheme (atomic displacement parameters shown at 50% probability level, intermolecular hydrogen-bonding not shown for clarity).

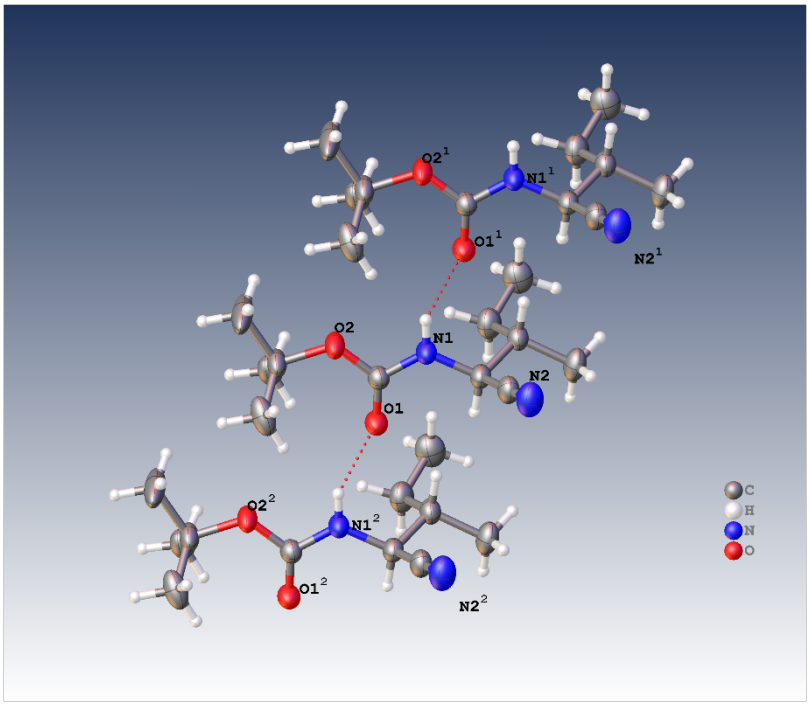


Figure S14. Crystal structure of **10b** showing a portion of the polymeric chains formed by intermolecular hydrogen-bonding (atomic displacement parameters shown at 50% probability level, partial atom labelling scheme given, symmetry operator [1] denotes –1+*x*, *y*, *z*, [2] denotes 1+*x*, *y*, *z*).

NMR spectra for small molecules (7a,b – 11a,b)

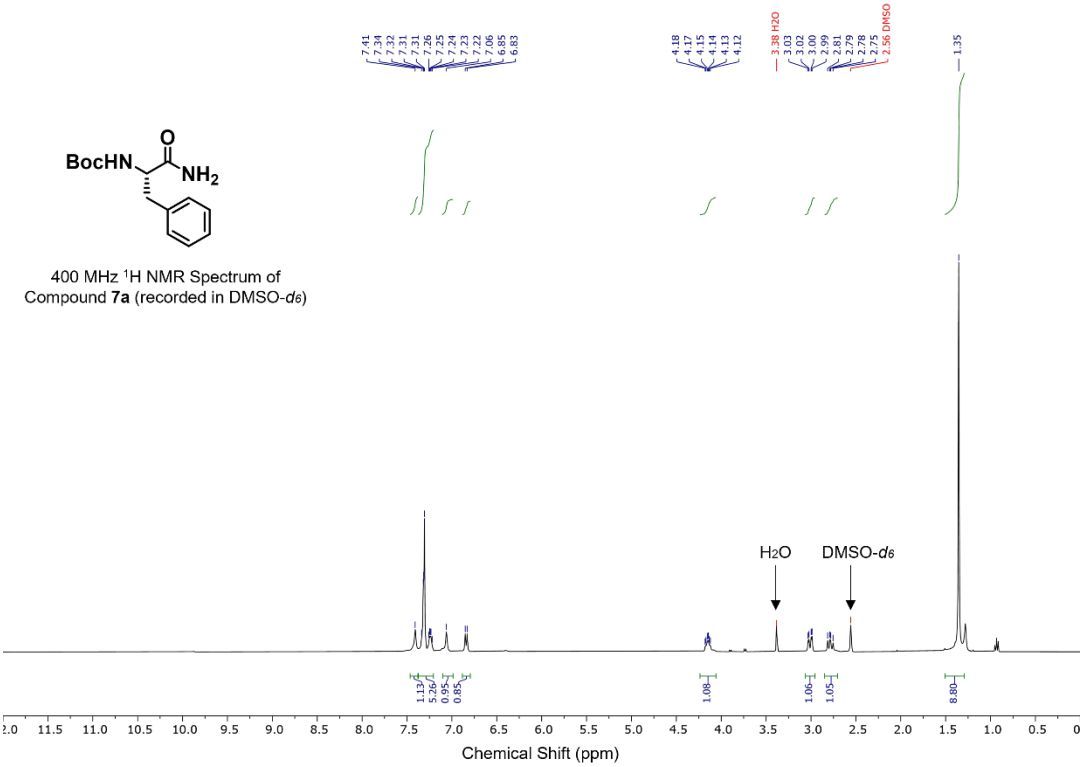


Figure S15. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of compound **7a**.

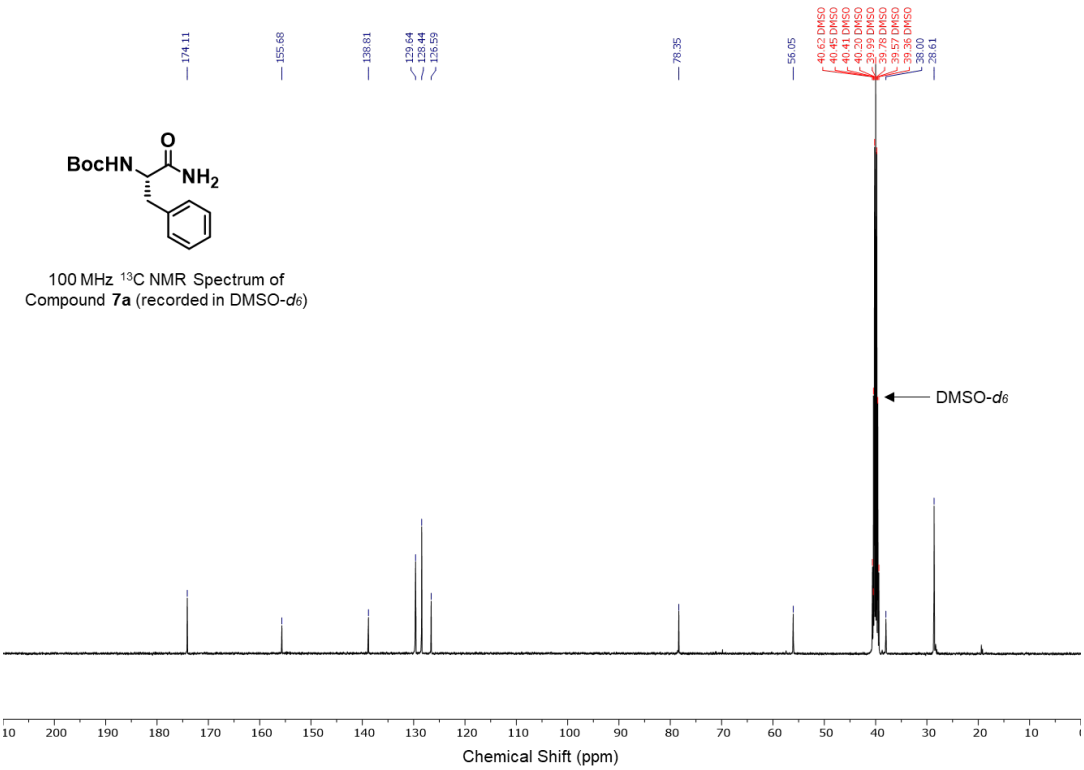


Figure S16.^{13}C NMR spectrum (100 MHz, DMSO-d_6) of compound **7a**.

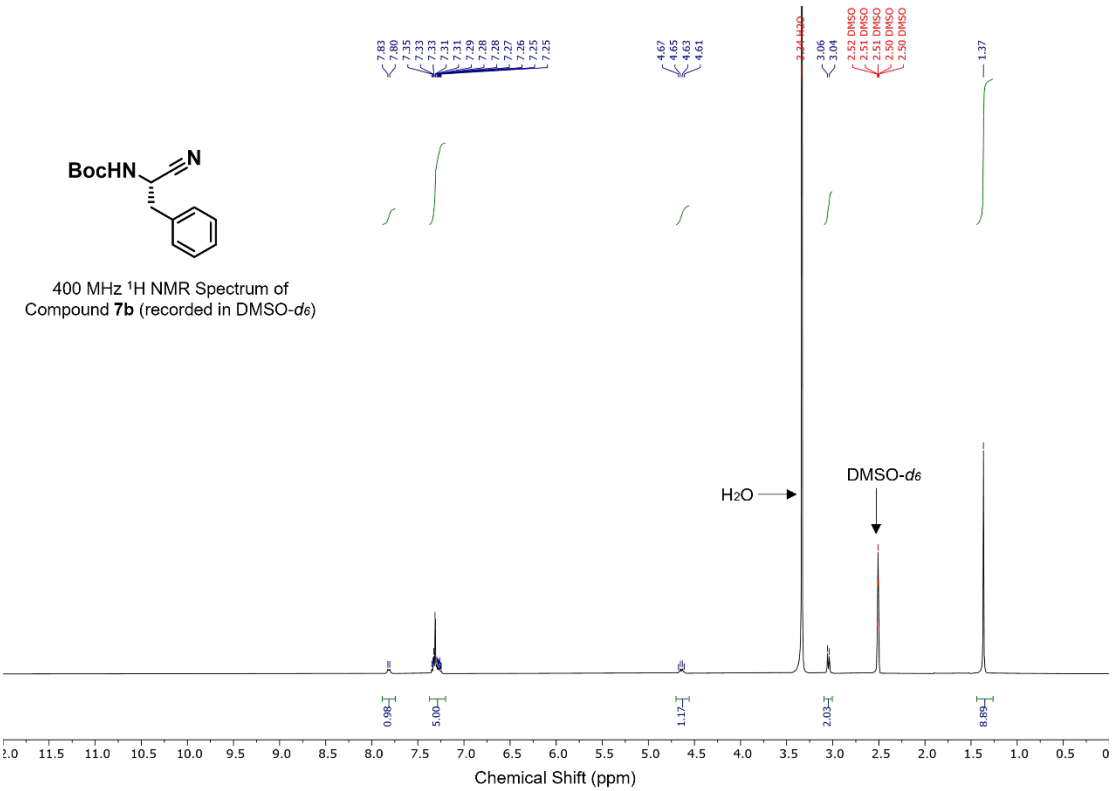


Figure S17. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of compound **7b**.

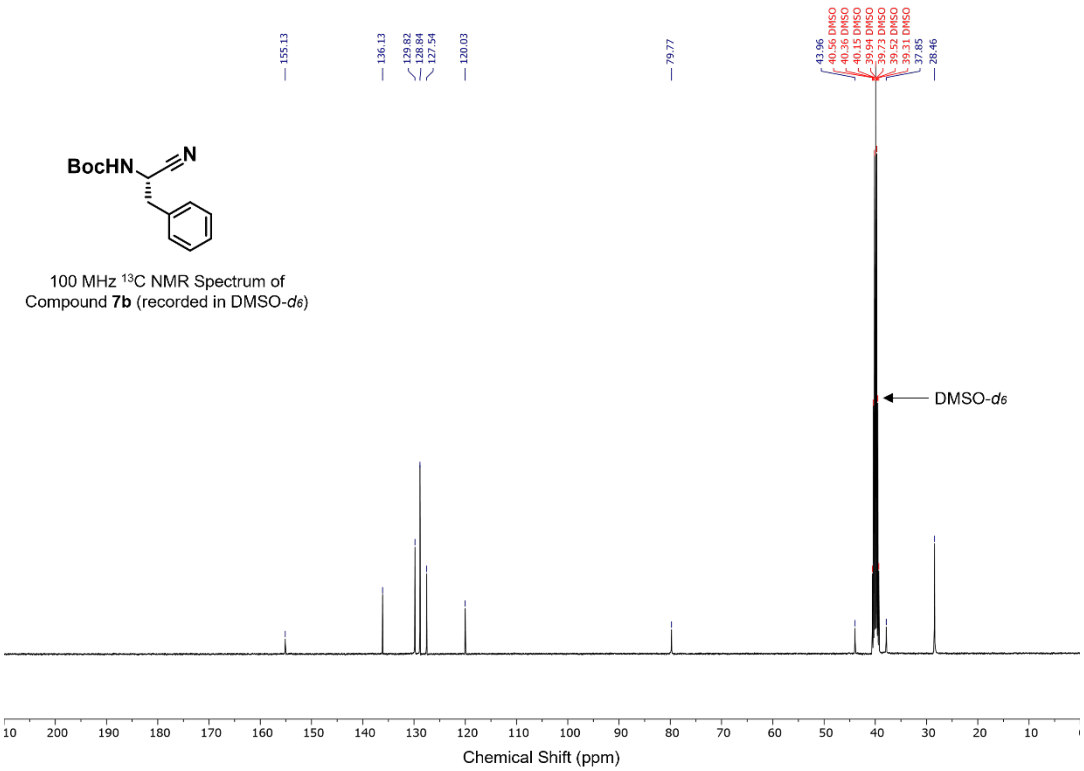


Figure S18. ^{13}C NMR spectrum (100 MHz, DMSO-d_6) of compound **7b**.

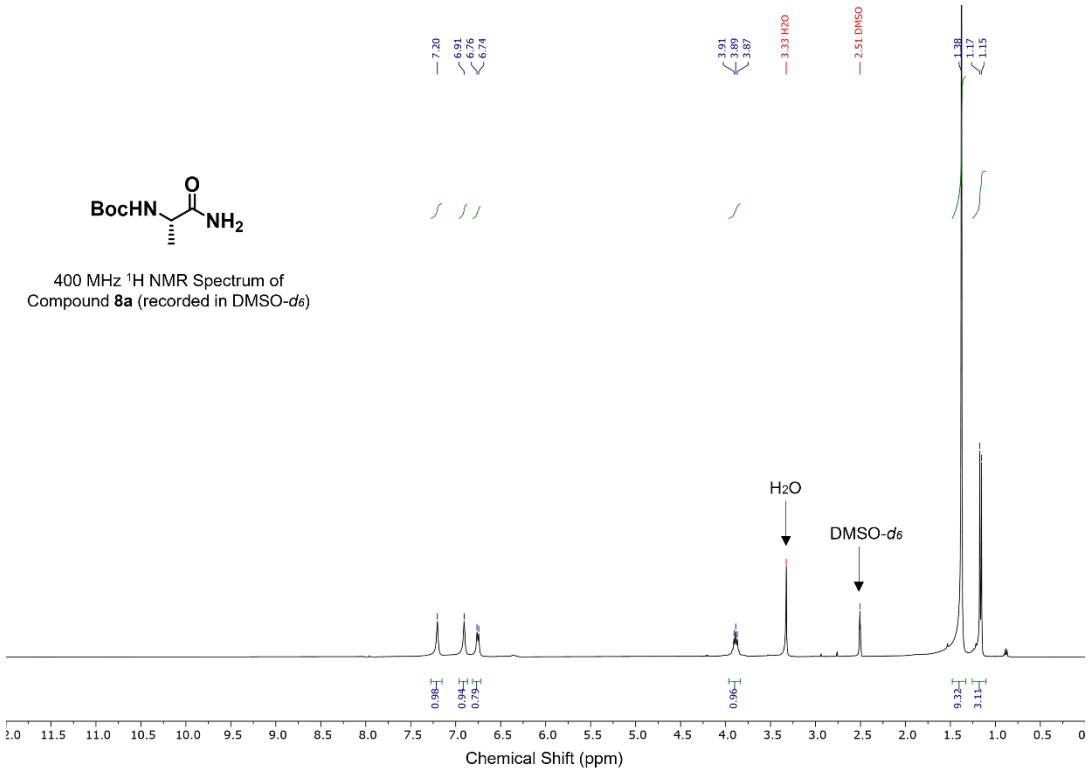


Figure S19. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of compound **8a**.

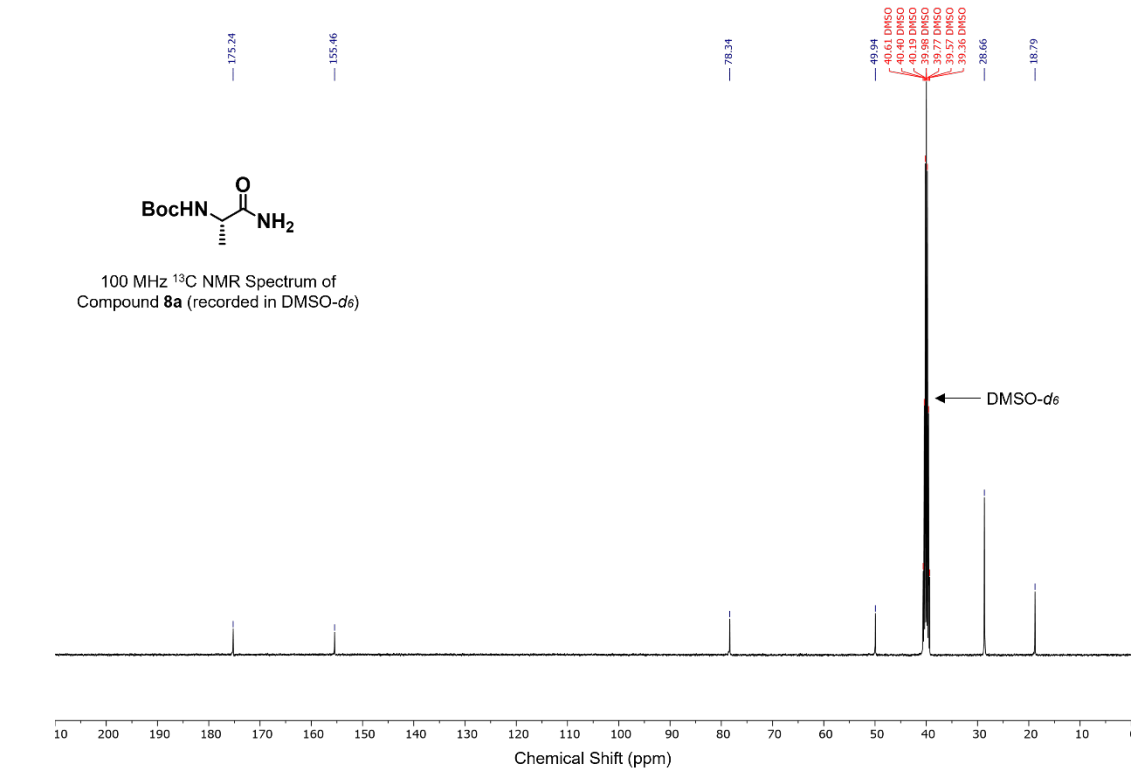


Figure S20. ^{13}C NMR spectrum (100 MHz, DMSO-d_6) of compound **8a**.

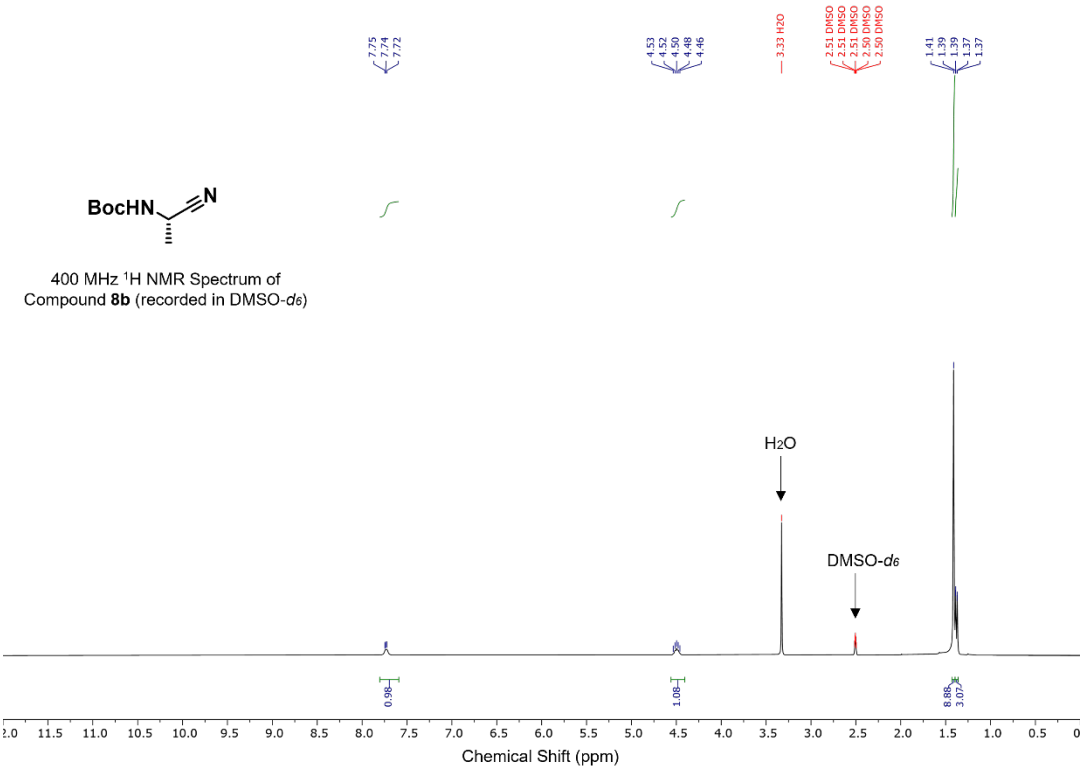


Figure S21. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of compound **8b**.

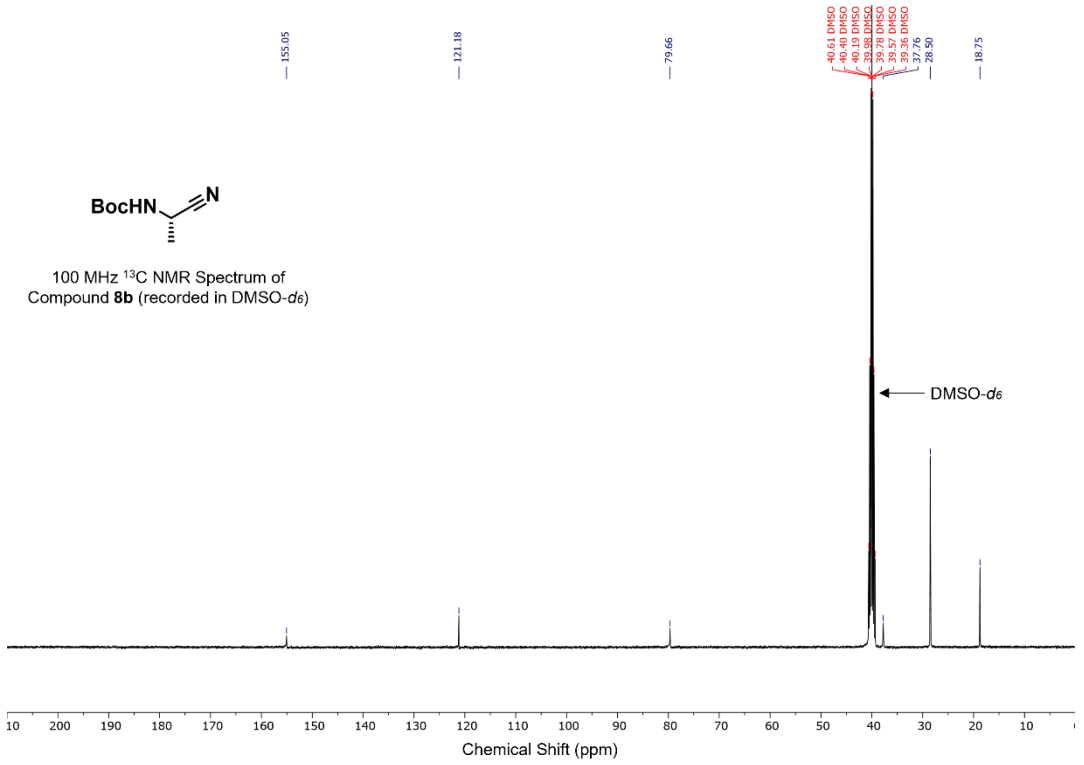


Figure S22. ^{13}C NMR spectrum (100 MHz, DMSO-d_6) of compound **8b**.

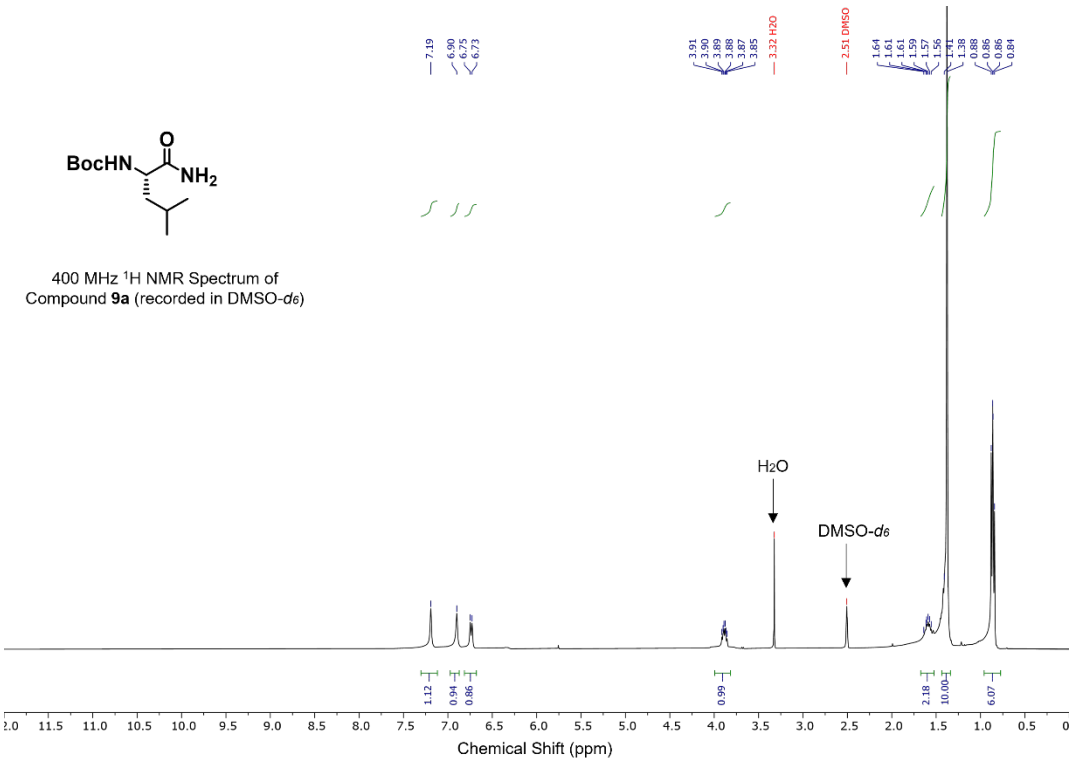


Figure S23. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of compound **9a**.

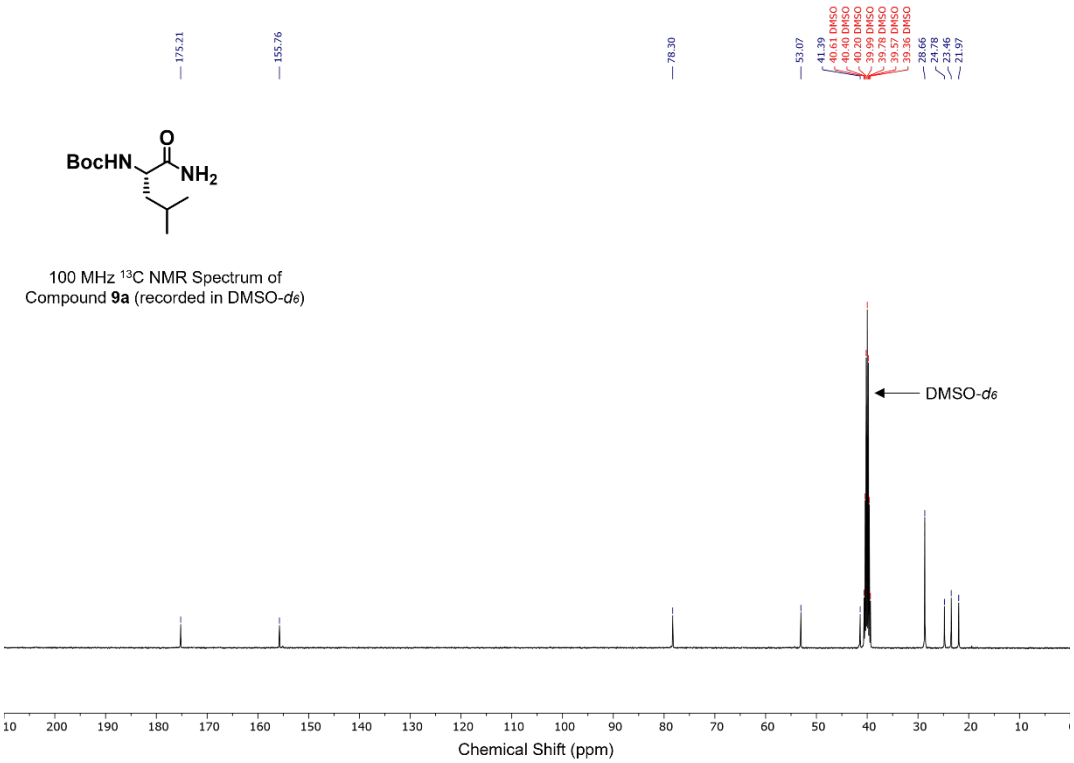


Figure S24. ^{13}C NMR spectrum (100 MHz, DMSO-d_6) of compound **9a**.

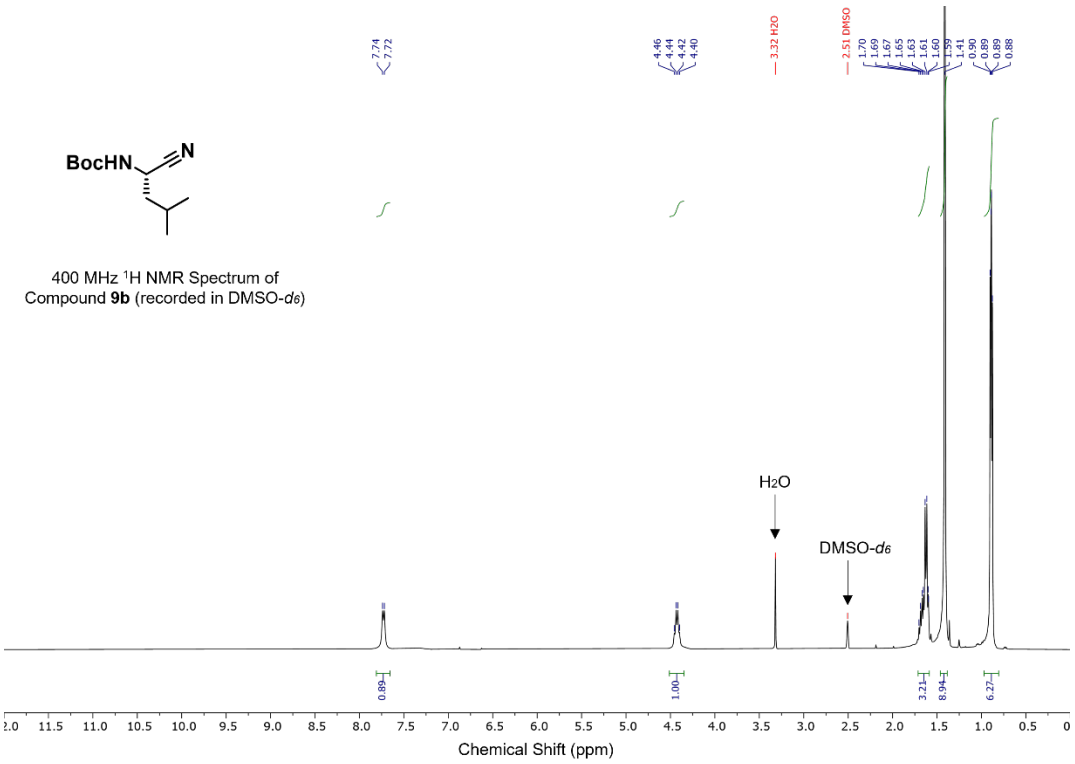


Figure S25. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of compound **9b**.

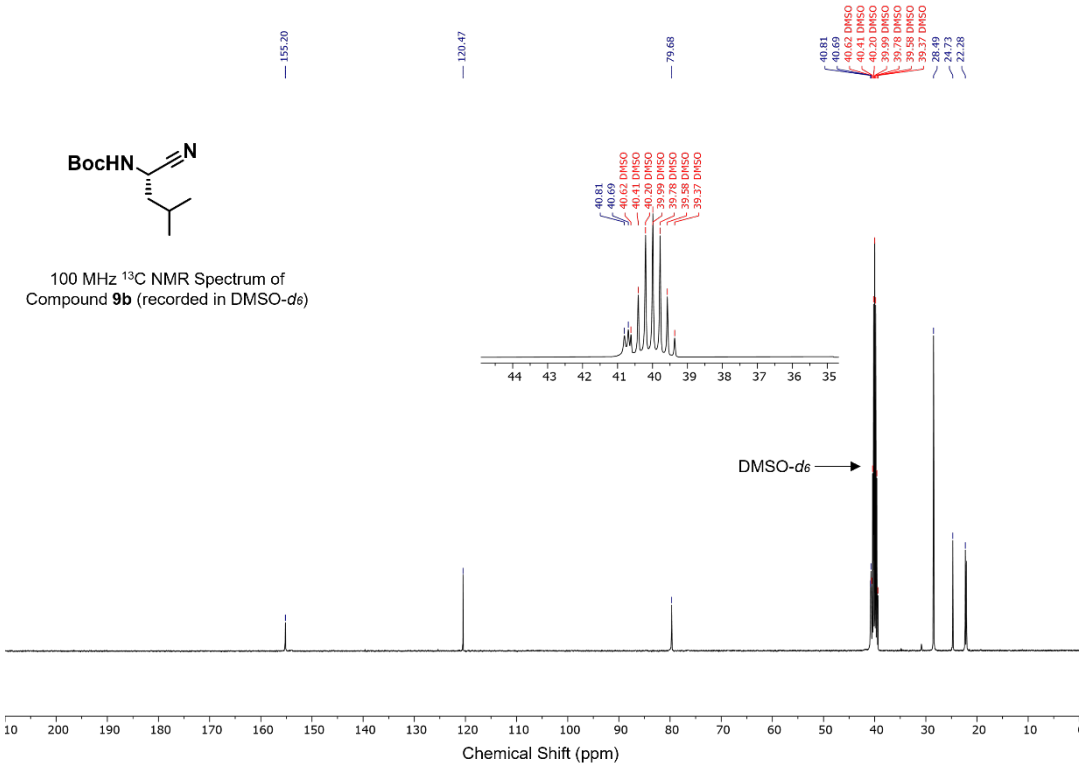


Figure S26. ^{13}C NMR spectrum (100 MHz, DMSO-d_6) of compound **9b**.

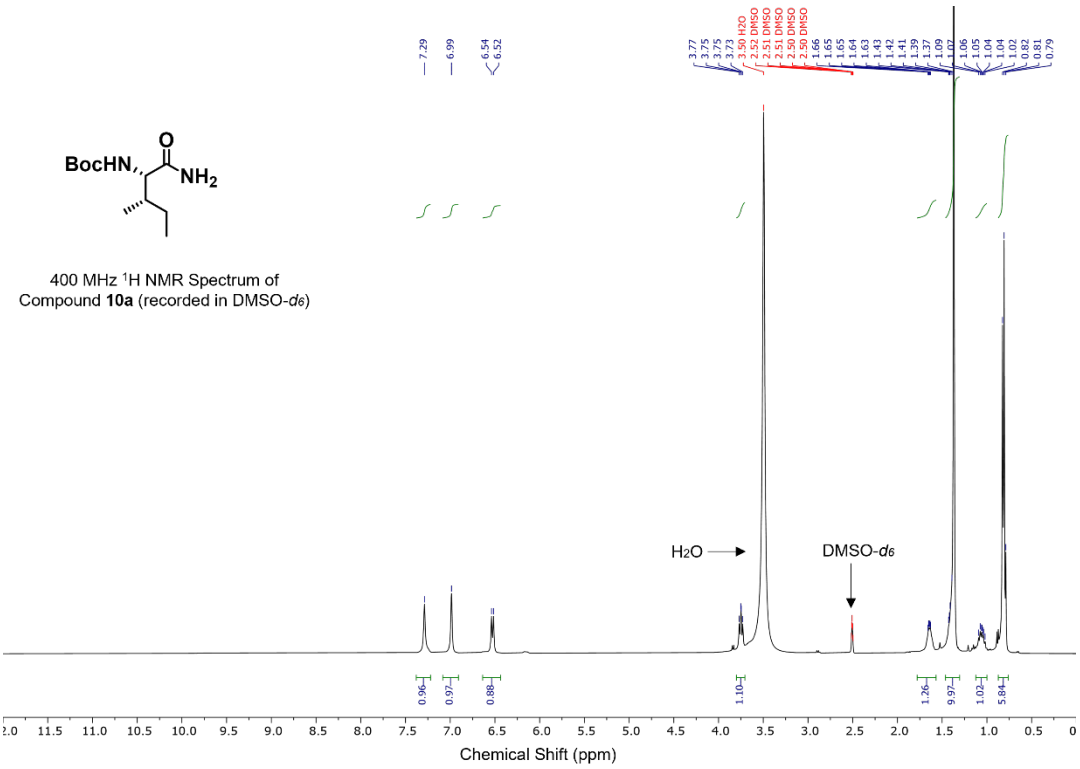


Figure S27. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of compound **10a**.

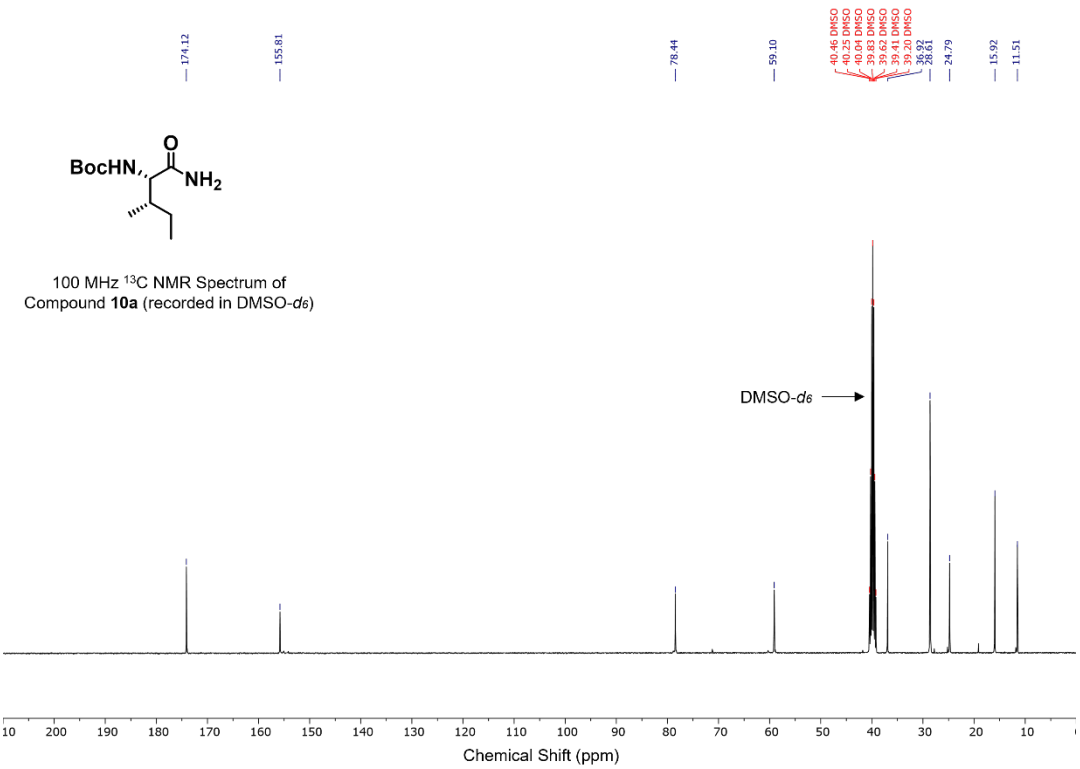


Figure S28. ^{13}C NMR spectrum (100 MHz, DMSO-d_6) of compound **10a**.

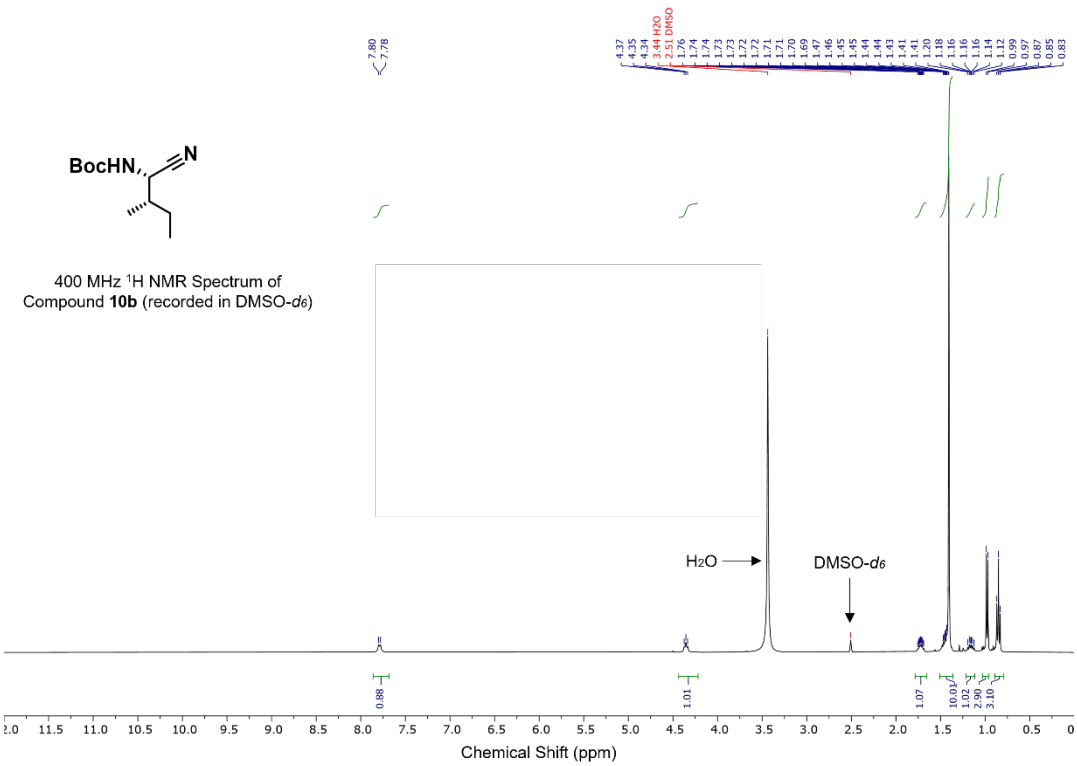


Figure S29. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of compound **10b**.

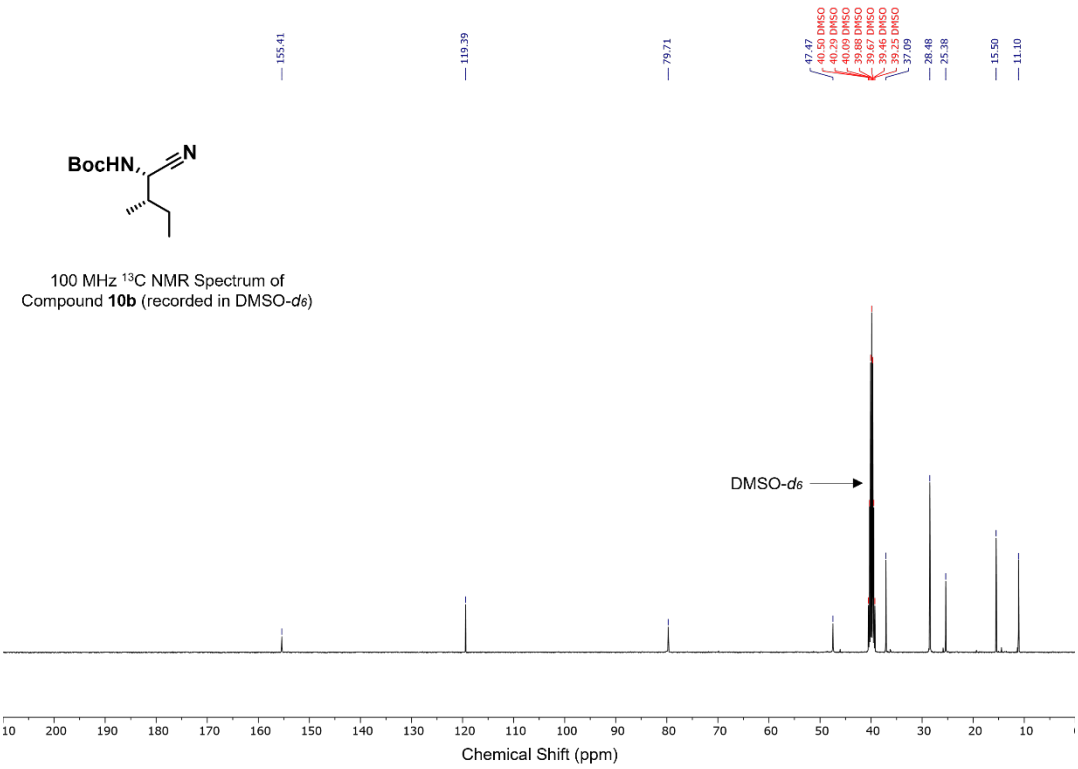


Figure S30. ^{13}C NMR spectrum (100 MHz, DMSO-d_6) of compound **10b**.

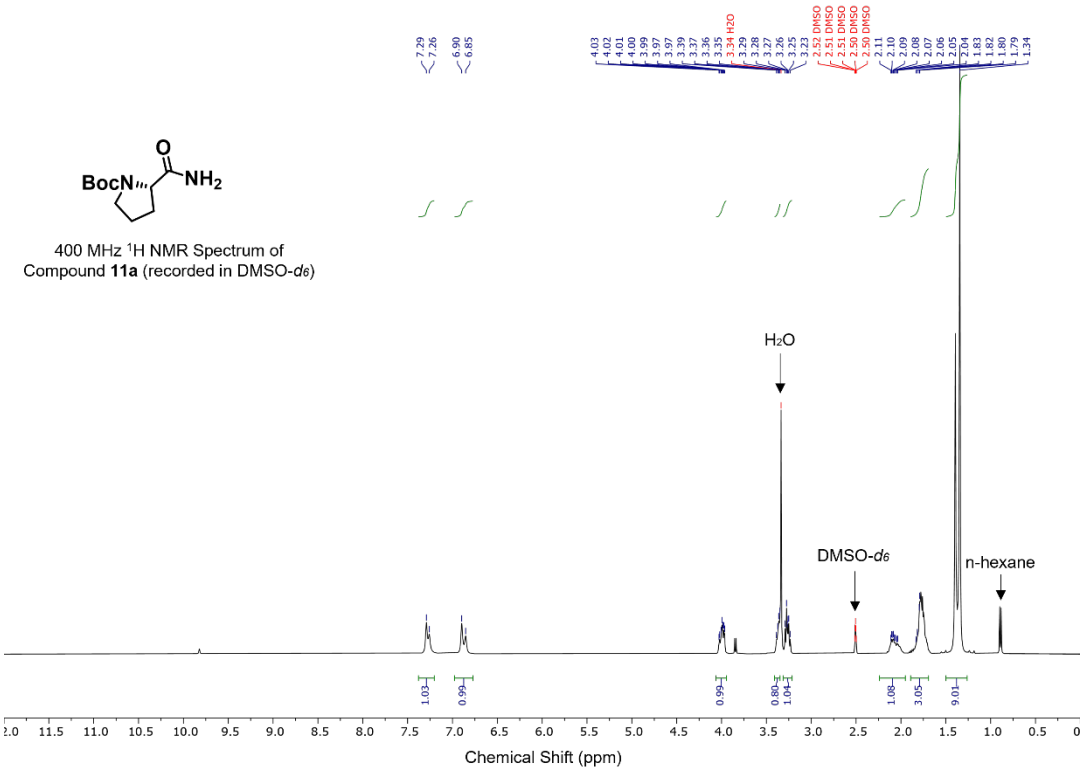


Figure S31. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of compound **11a**.

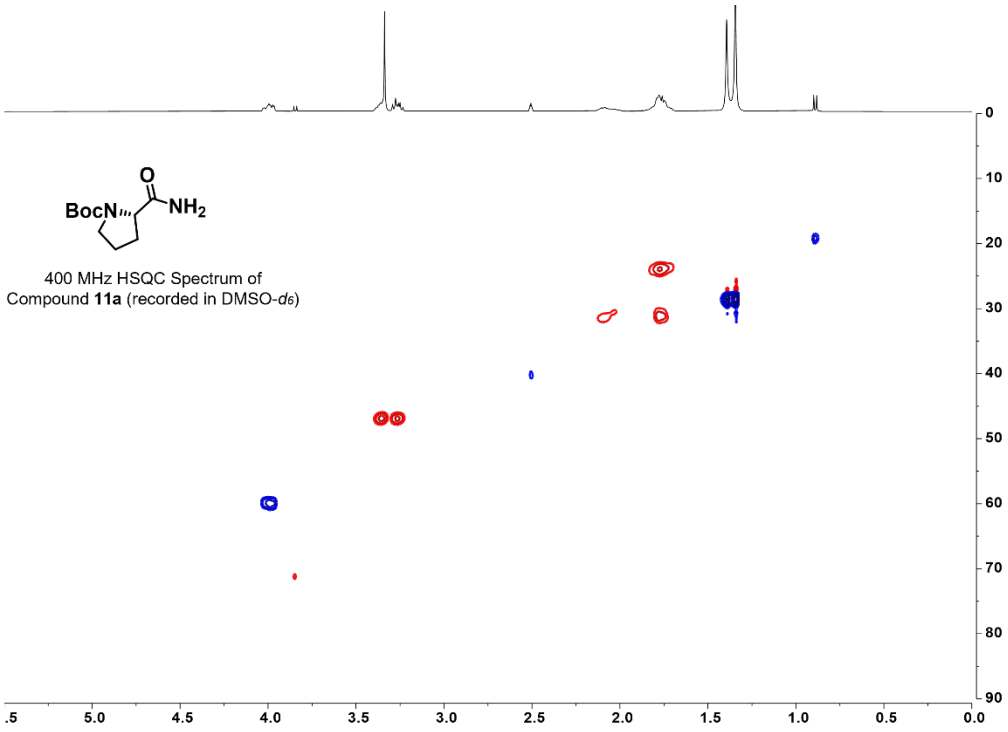


Figure S32. HSQC spectrum (400 MHz, DMSO-d_6) of compound **11a**.

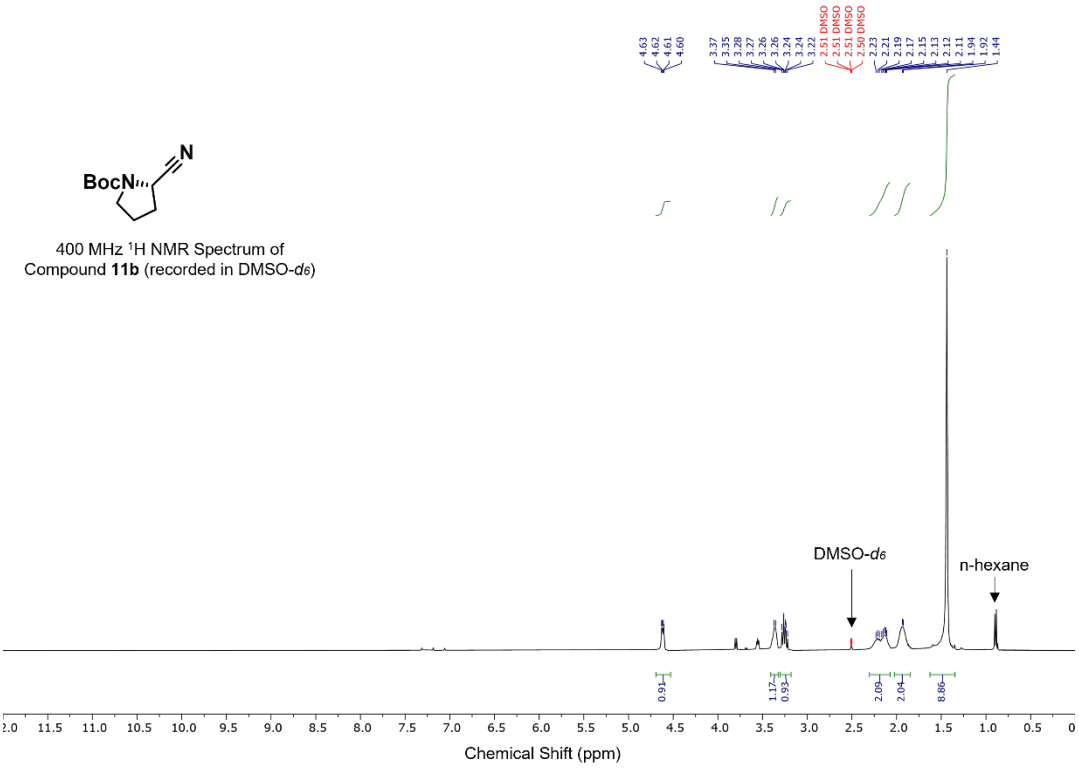


Figure S33. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of compound **11b**.

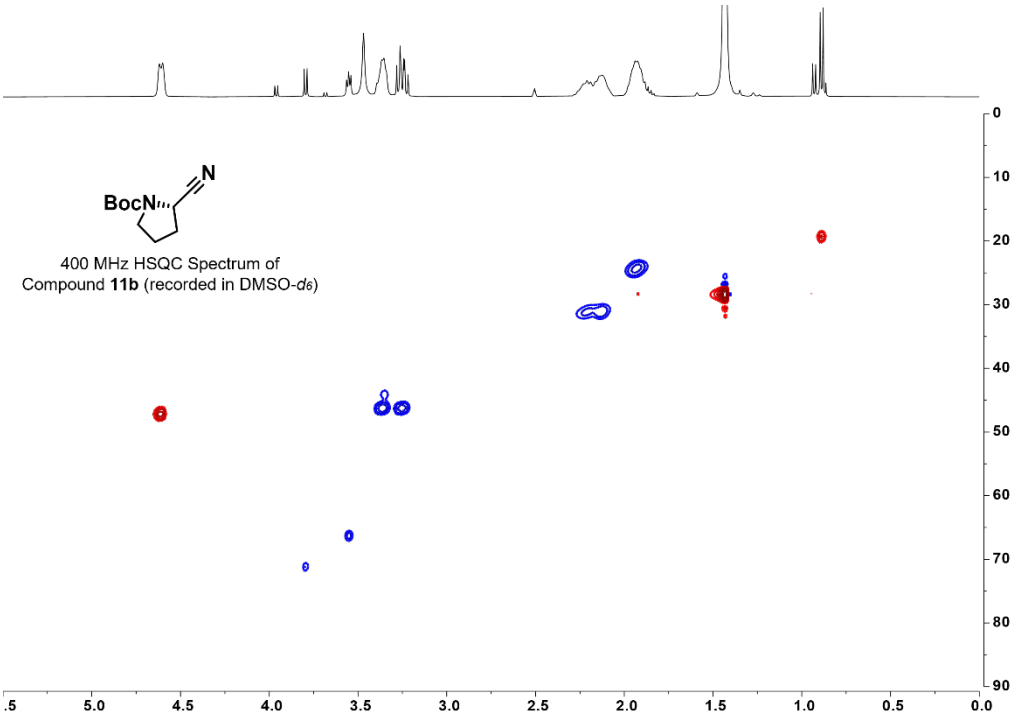
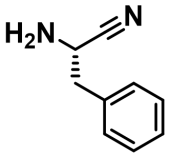


Figure S34. HSQC spectrum (400 MHz, DMSO-d_6) of compound **11b**.

HRMS data for compounds 7 – 11

(*S*)-2-amino-3-phenylpropanenitrile (formic acid salt) (7)



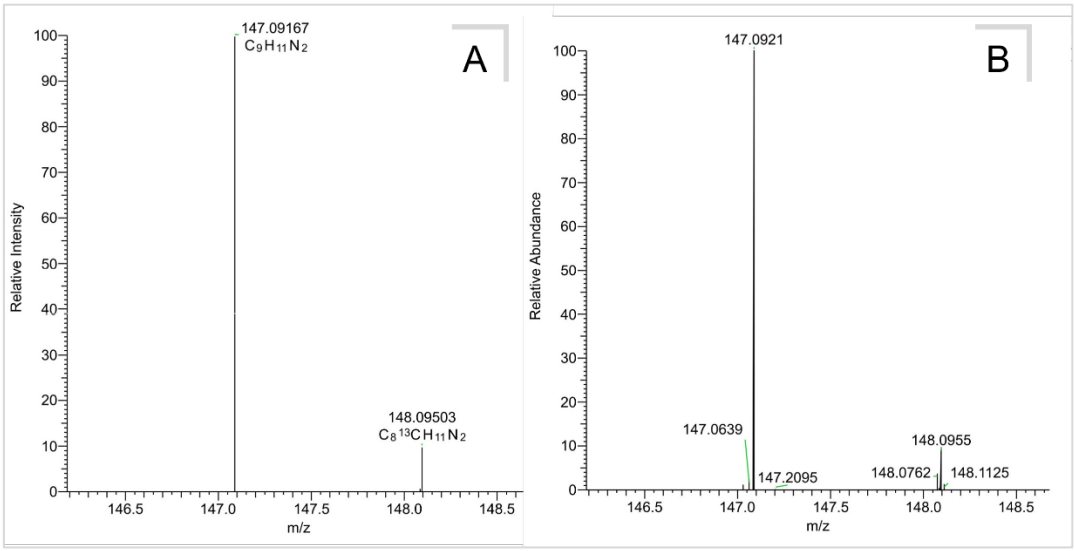
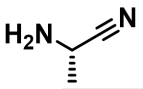


Figure S35. Isotopic signature of compound **7** (calculated for $C_9H_{11}N_2^+$ $[M+H]^+$). Samples were analysed following the HRMS-ESI method. The isotope model is shown in (A), and the observed isotopes are shown in (B).

(S)-2-aminopropanenitrile (formic acid salt) (8)



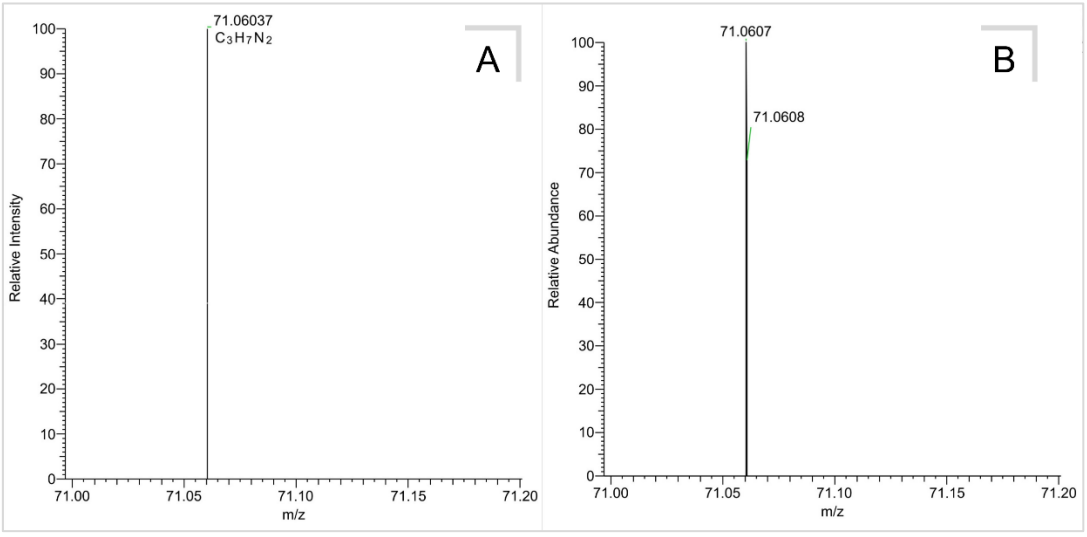


Figure S36. Isotopic signature of compound **8** (calculated for $C_3H_7N_2^+$ $[M+H]^+$). Samples were analysed following the HRMS-ESI method. The isotope model is shown in (A), and the observed isotopes are shown in (B).

(*S*)-2-amino-4-methylpentanenitrile (formic acid salt) (9)

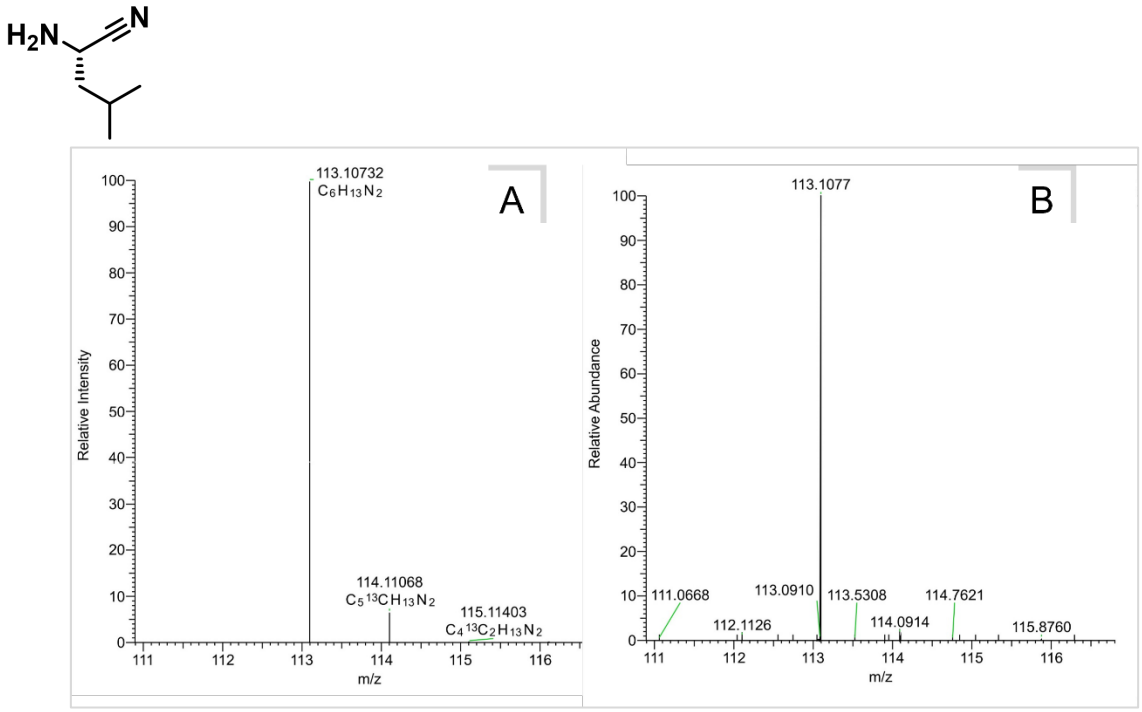


Figure S37. Isotopic signature of compound **9** (calculated for $C_6H_{13}N_2^+$ $[M+H]^+$). Samples were analysed following the HRMS-ESI method. The isotope model is shown in (A), and the observed isotopes are shown in (B).

(2*S*,3*S*)-2-amino-3-methylpentanenitrile (formic acid salt) (10)

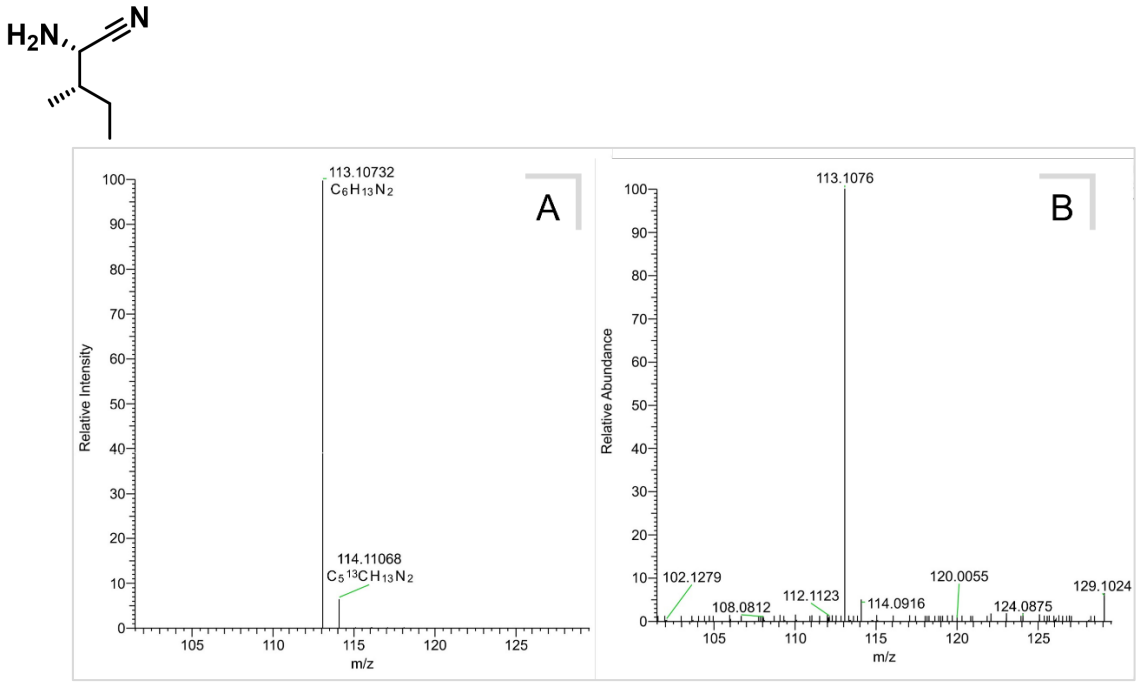
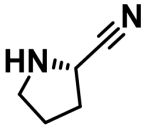


Figure S38. Isotopic signature of compound **10** (calculated for $C_6H_{13}N_2^+$ $[M+H]^+$). Samples were analysed following the HRMS-ESI method. The isotope model is shown in (A), and the observed isotopes are shown in (B).

(*S*)-pyrrolidine-2-carbonitrile (formic acid salt) (11)



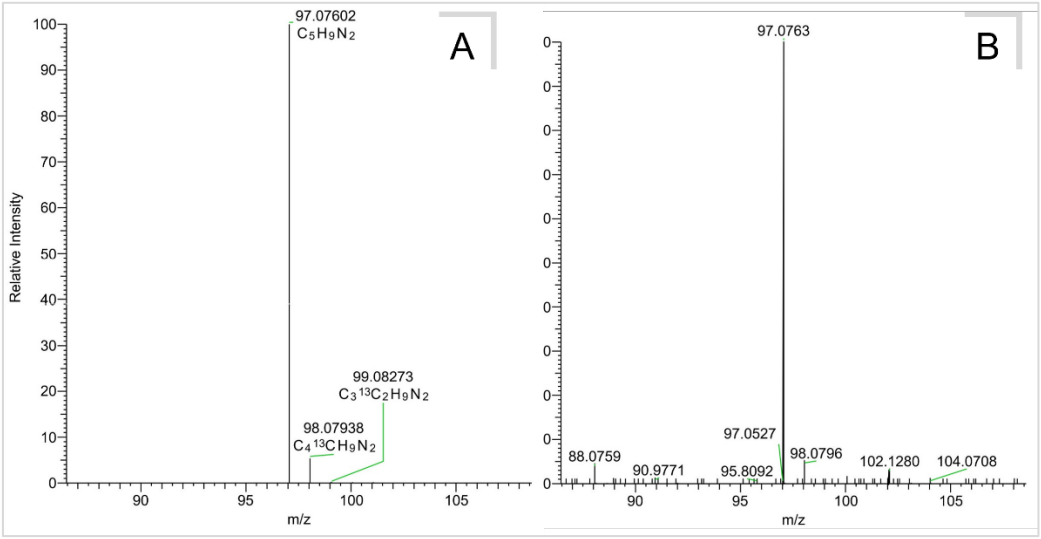
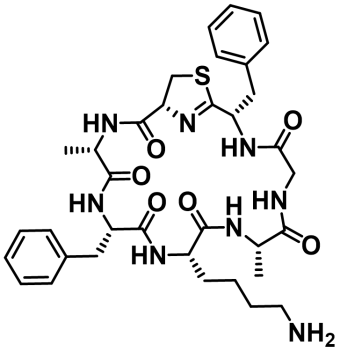


Figure S39. Isotopic signature of compound **11** (calculated for $C_5H_9N_2^+$ $[M+H]^+$). Samples were analysed following the HRMS-ESI method. The isotope model is shown in (A), and the observed isotopes are shown in (B).

LCMS data for crude cyclic thiazoline peptides (1c – 6c)

Peptide 1c



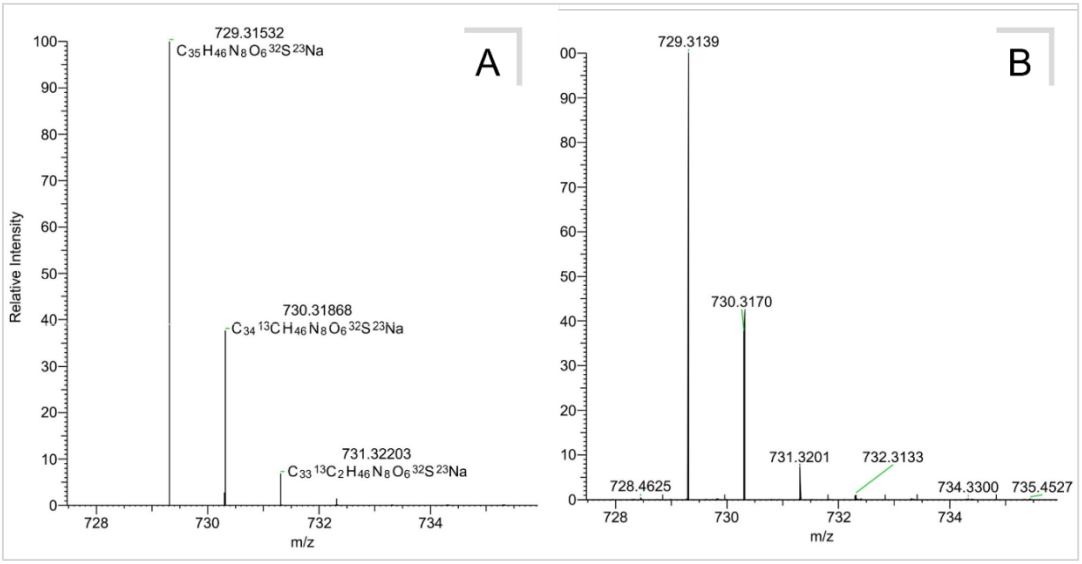


Figure S40. Isotopic signature of peptide **1c** (calculated for $C_{35}H_{46}N_8O_6SNa^+$ $[M+Na]^+$). Samples were analysed following the HRMS-ESI method. The isotope model is shown in (A), and the observed isotopes are shown in (B).

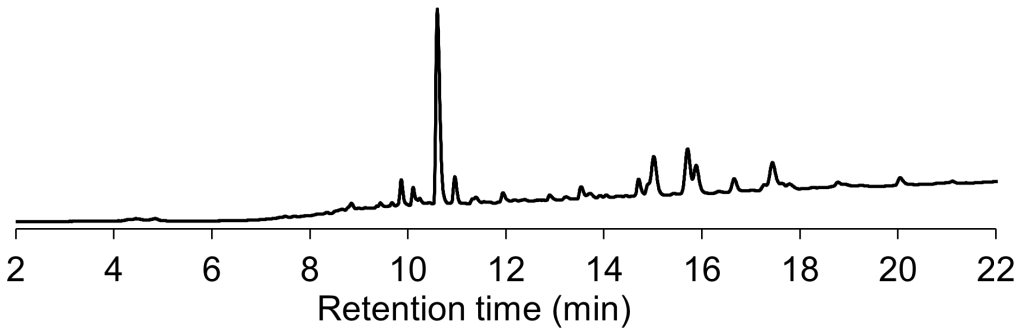
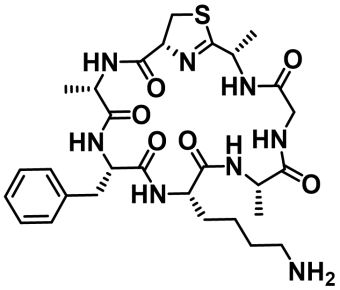


Figure S41. Chromatogram (254 nm) of crude peptide **1c**. The peptide was analysed following the LCMS method.

Peptide 2c



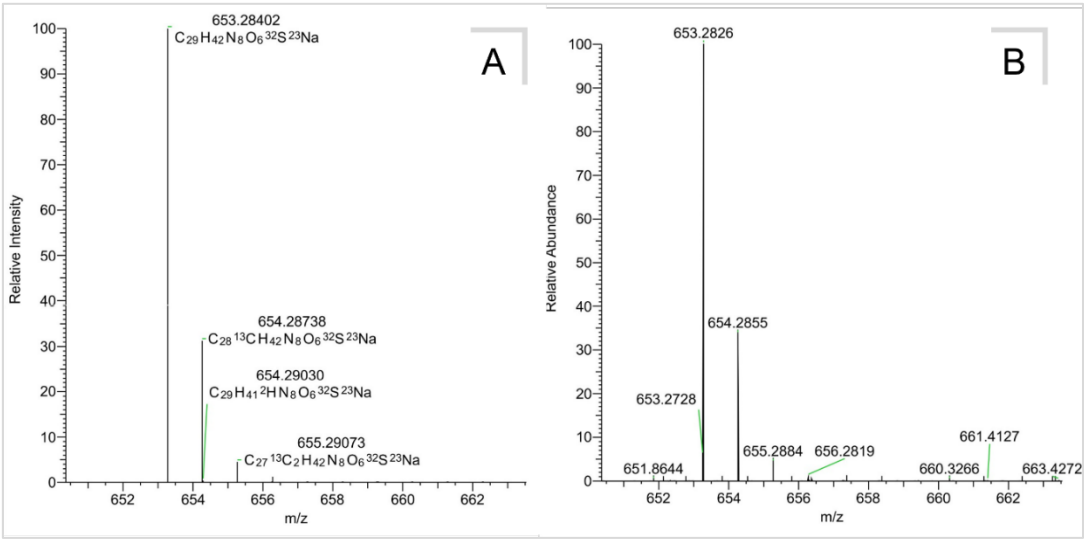


Figure S42. Isotopic signature of peptide **2c** (calculated for $C_{29}H_{42}N_8O_6SNa^+$ $[M+Na]^+$). Samples were analysed following the HRMS-ESI method. The isotope model is shown in (A), and the observed isotopes are shown in (B).

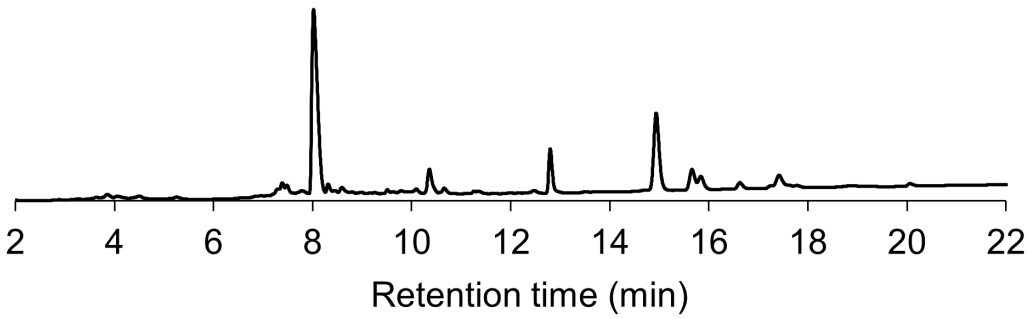
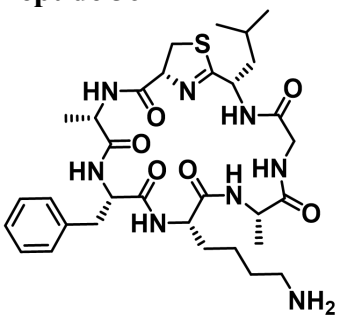


Figure S43. Chromatogram (254 nm) of crude peptide **2c**. The peptide was analysed following the LCMS method.

Peptide 3c



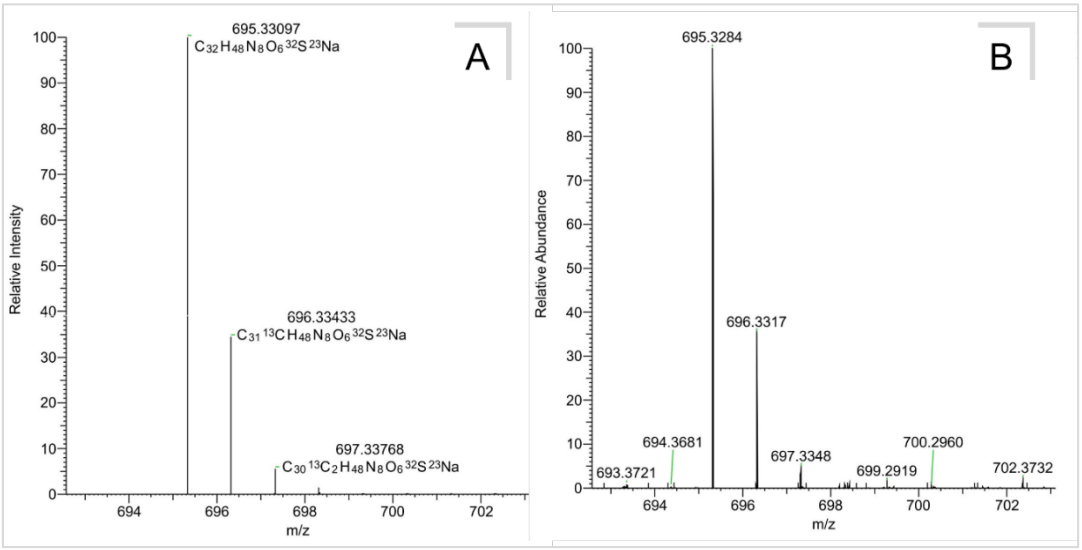


Figure S44. Isotopic signature of peptide **3c** (calculated for $C_{32}H_{48}N_8O_6SNa^+$ $[M+Na]^+$). Samples were analysed following the HRMS-ESI method. The isotope model is shown in (A), and the observed isotopes are shown in (B).

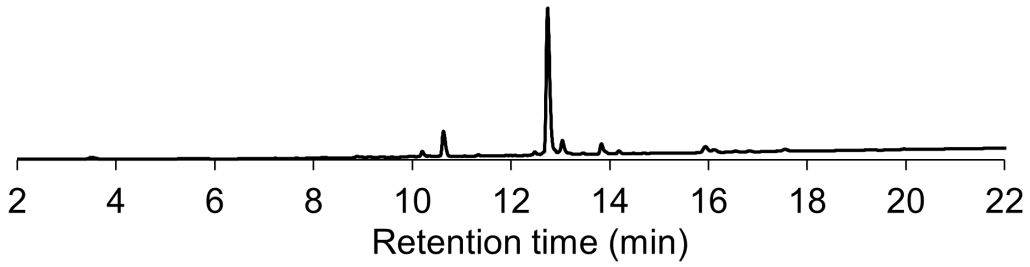
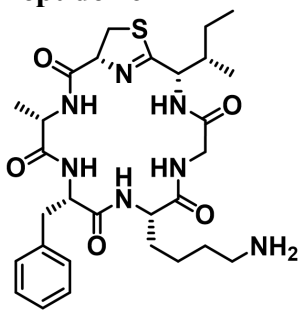


Figure S45. Chromatogram (254 nm) of crude peptide **3c**. The peptide was analysed following the LCMS method.

Peptide 4c



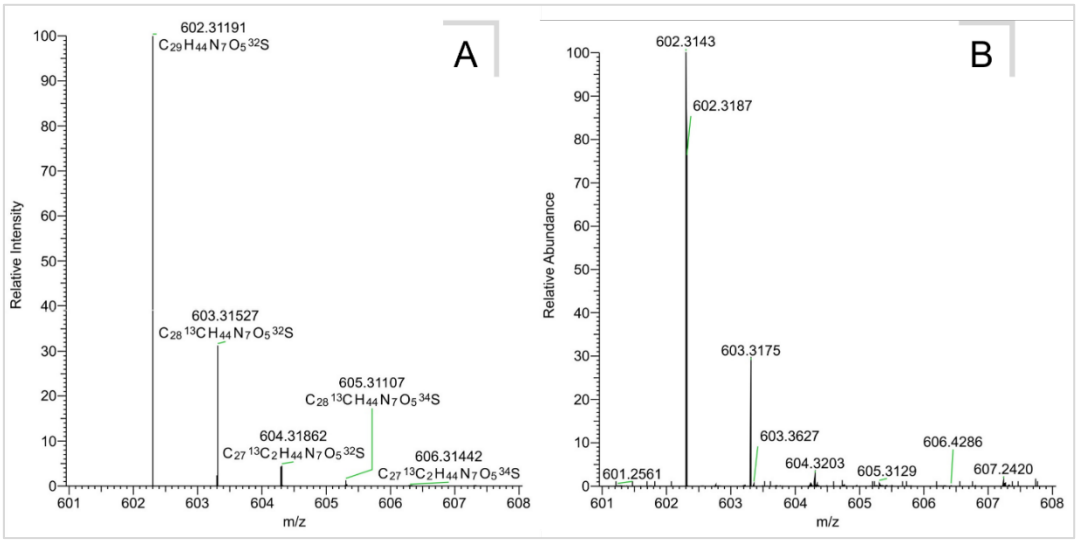


Figure S46. Isotopic signature of peptide **4c** (calculated for $C_{29}H_{44}N_7O_5S^+$ $[M+H]^+$). Samples were analysed following the HRMS-ESI method. The isotope model is shown in (A), and the observed isotopes are shown in (B).

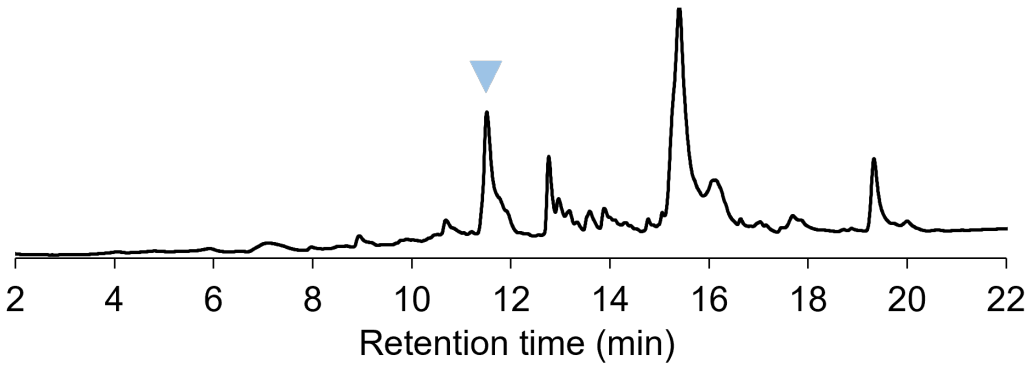
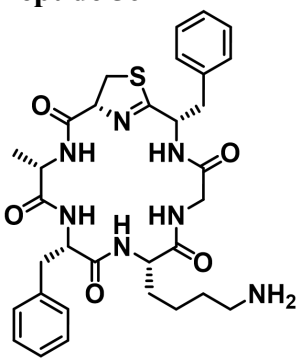


Figure S47. Chromatogram (254 nm) of crude peptide **4c**. The peptide was analysed following the LCMS method.

Peptide 5c



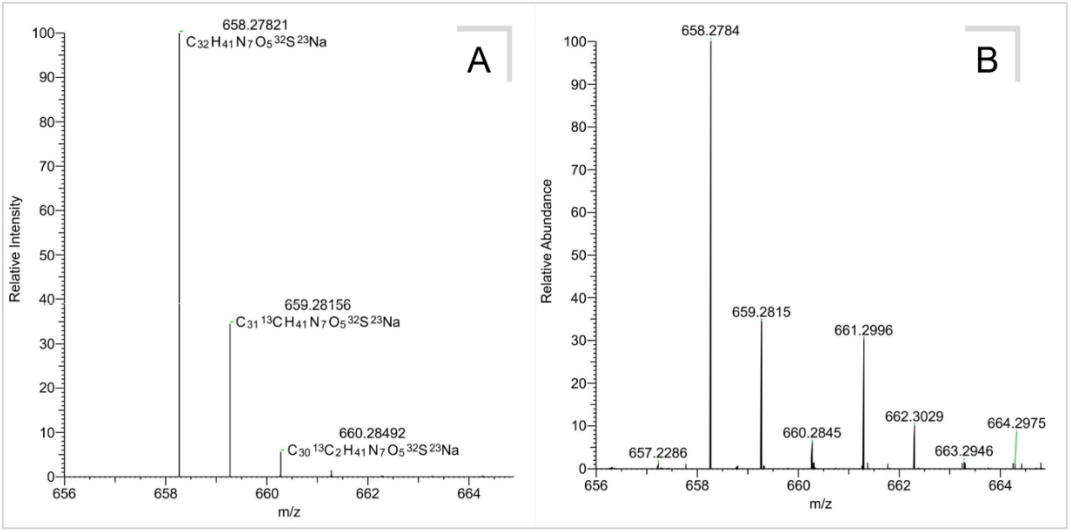


Figure S48. Isotopic signature of peptide **5c** (calculated for $C_{32}H_{41}N_7O_5SNa^+$ $[M+Na]^+$). Samples were analysed following the HRMS-ESI method. The isotope model is shown in (A), and the observed isotopes are shown in (B).

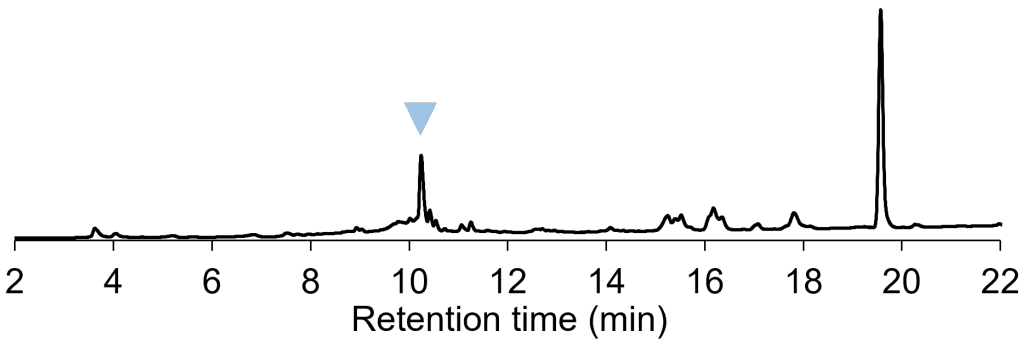
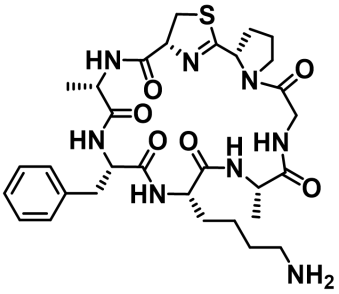


Figure S49. Chromatogram (254 nm) of crude peptide **5c**. The peptide was analysed following the LCMS method.

Peptide 6c



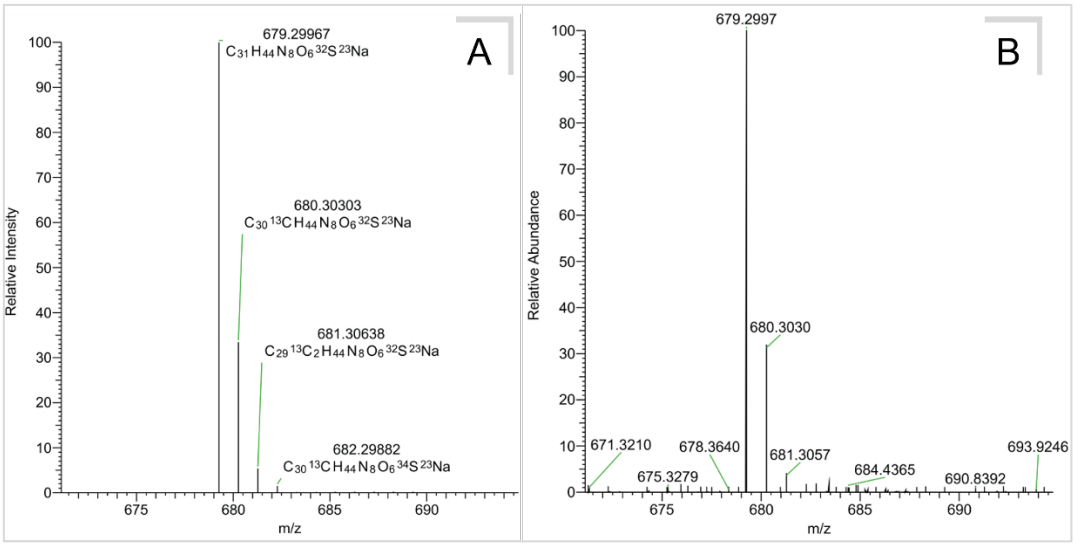


Figure S50. Isotopic signature of peptide **6c** (calculated for $C_{31}H_{44}N_8O_6SNa^+$ $[M+Na]^+$). Samples were analysed following the HRMS-ESI method. The isotope model is shown in (A), and the observed isotopes are shown in (B).

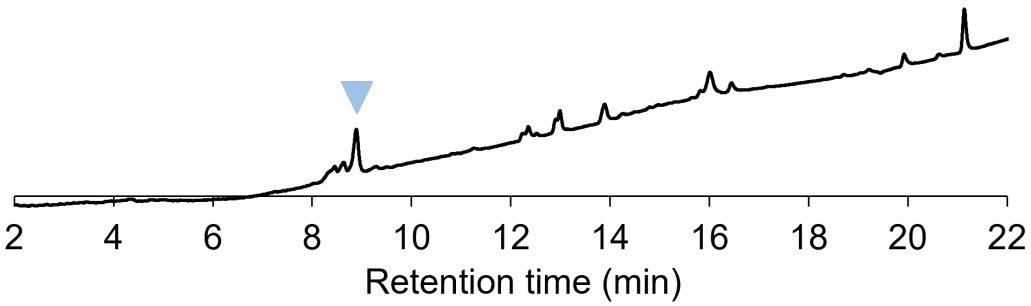


Figure S51. Chromatogram (254 nm) of crude peptide **6c**. The peptide was analysed following the LCMS method.

References

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4. Dolomanov, O. V., Bourhis, L. J., Gildea, R. J, Howard, J. A. K. and Puschmann, H., *J. Appl. Cryst.* 2009, **42**, 339–341.
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6. Sheldrick, G. M. *Acta Cryst.* 2015, **C71**, 3–8.