The natural product hybrid of Syringolin A and Glidobactin A synergizes proteasome inhibition potency with subsite selectivity

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Electronic Supplementary Information

Supplementary Figures

Supplementary Experimental Procedures

Supplementary Figures

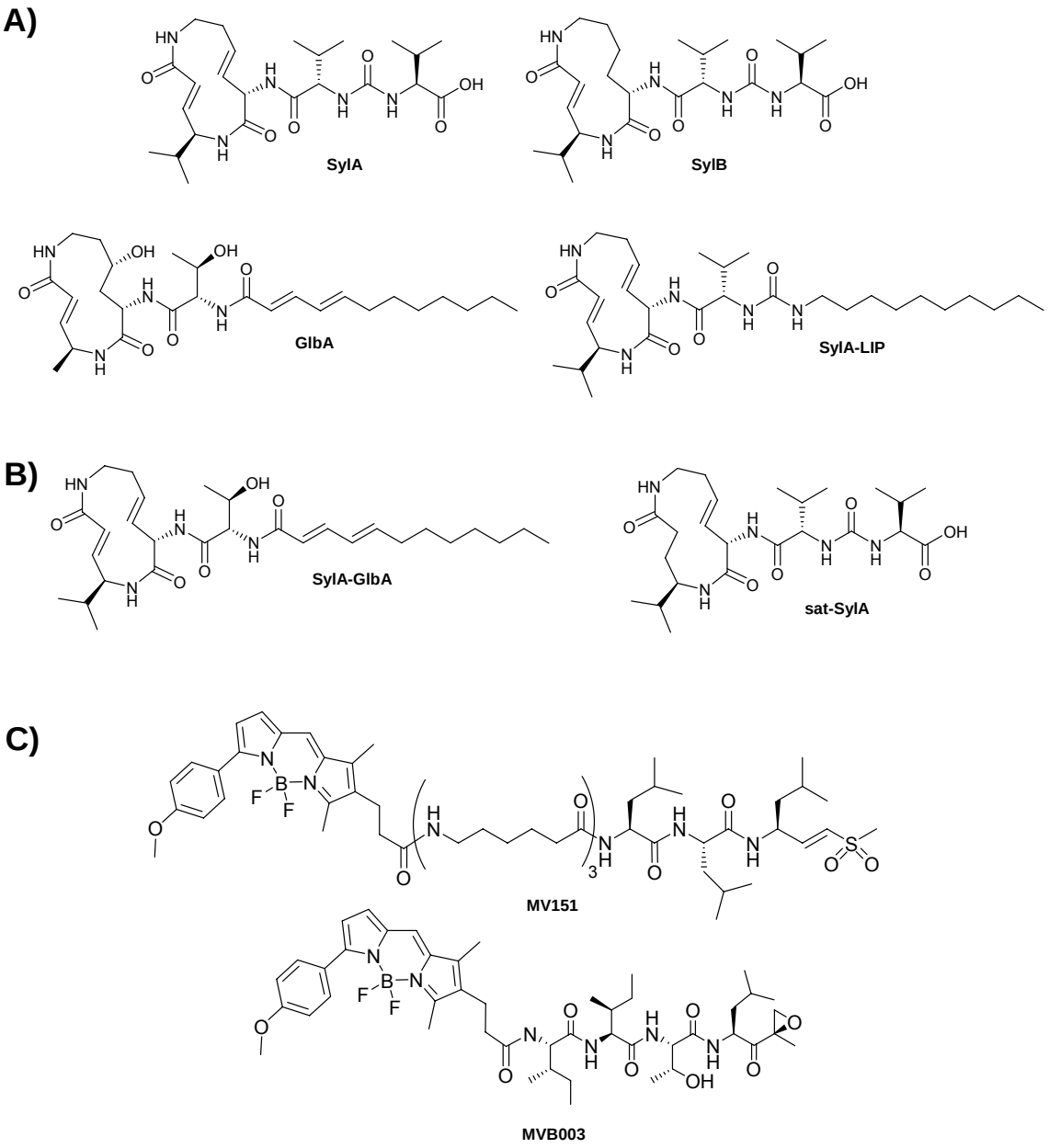


Figure S1. Chemical structures of syrbactins and activity-based probes (ABPs) used throughout this study. **A)** Structures of the previously described natural products Syringolin A (**SylA**), Syringolin B (**SylB**), Glidobactin A (**GlbA**) and the model compound **SylA-LIP**. **B)** Structure of the direct Syringolin A / Glidobactin A hybrid **SylA-GlbA** and the control compound **sat-SylA** in which the reactive double bond is reduced to a saturated single bond. Both compounds have been prepared for the first time during this study. **C)** Chemical structures of the ABPs MV151 and MVB003 used in the profiling experiments.

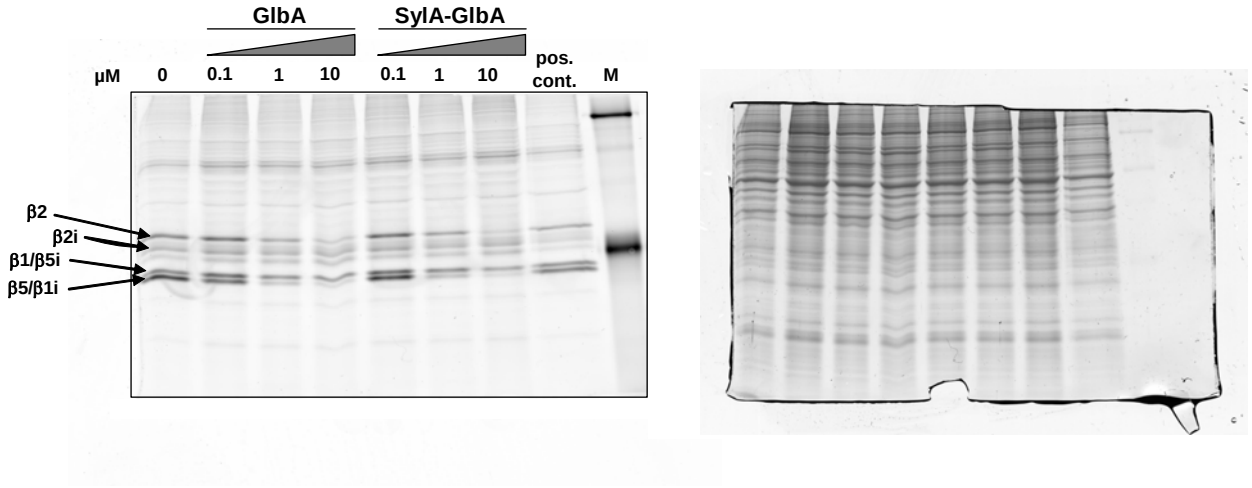


Figure S2. *in vivo* labelling of living Jurkat cells with varying concentrations of GlbA or SylA-GlbA, with 10 µg protein/lane and MV151 as ABP (left panel) and corresponding Coomassie stain (right panel) as loading control. M = 25 and 75 kDa marker lane. pos. cont. = 1 µM Ada-Ahx_3-L_3-VS

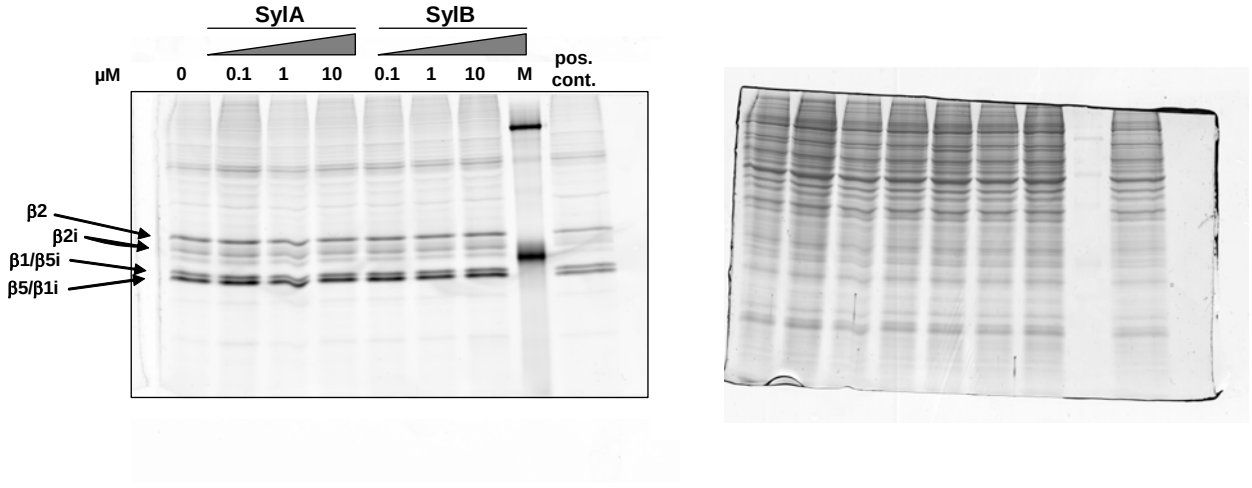
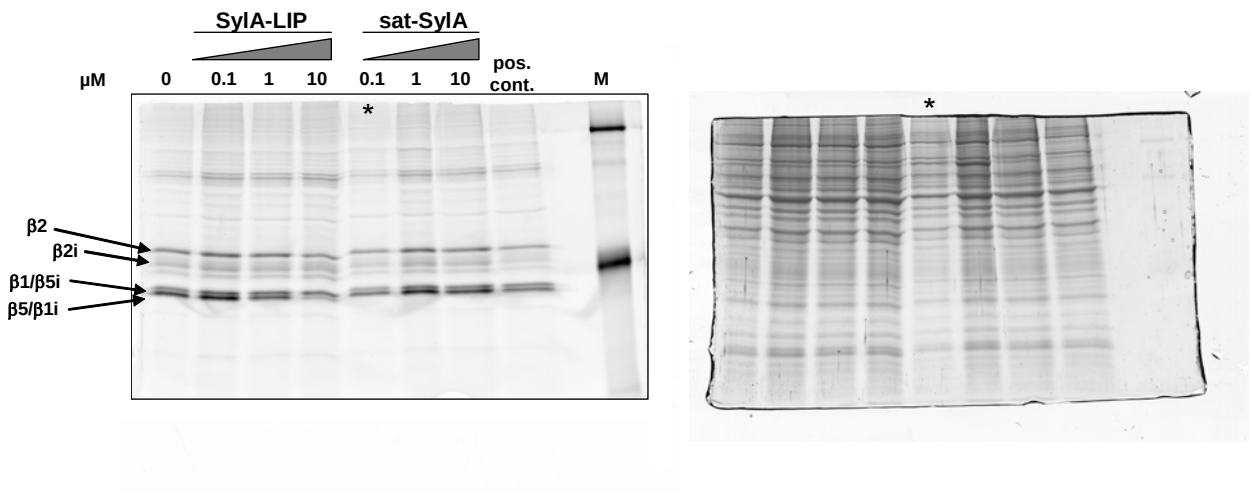


Figure S3. *in vivo* labelling of living Jurkat cells with varying concentrations of SylA or SylB, with 10 µg protein/lane and MV151 as ABP (left panel) and corresponding Coomassie stain (right panel) as loading control. M = 25 and 75 kDa marker lane. pos. cont. = 1 µM Ada-Ahx_3-L_3-VS



* less protein loaded

Figure S4. *in vivo* labelling of living Jurkat cells with varying concentrations of SylA-LIP or sat-SylA, with 10 µg protein/lane and MV151 as ABP (left panel) and corresponding Coomassie stain (right panel) as loading control. M = 25 and 75 kDa marker lane. pos. cont. = 1 µM Ada-Ahx$_3$-L$_3$-VS

Supplementary Experimental Conditions

General information

Unless otherwise noted, all reagents and solvents were purchased from Acros, Fluka, Sigma, Aldrich or Merck and used without further purification. Dry solvents were purchased as anhydrous reagents from commercial suppliers.

LC-MS analyses were performed on an HPLC system from Agilent (1200 series) with a Eclipse XDB-C18, 5 µm (column dimensions: 150 × 4.60 mm) column from Agilent and a Thermo Finnigan LCQ Advantage Max ESI-Spectrometer. As buffer eluents, 0.1% formic acid in water (buffer A) and 0.1% formic acid in acetonitrile (buffer B) were used at a flow of 1 mL/min. The following gradient program was employed: 0 min / 90% A / 10% B, 1 min / 90% A / 10% B, 10 min / 0% A / 100% B, 12 min / 0% A / 100% B, 15 min / 90% A / 10% B.

Preparative HPLC was conducted on a Varian HPLC system (Pro Star 215) with a VP 250/21 Nucleosil C18PPN-column from Macherey-Nagel. The corresponding gradient is described in the synthesis section.

Nuclear magnetic resonance (NMR) spectra were taken on a Varian Mercury 400 system (400 MHz for ^{1}H- and 100 MHz for ^{13}C-NMR). ^{1}H NMR spectra are reported in the following manner: chemical shifts calculated with reference to solvent standards based on tetramethylsilane, multiplicity (s, singulet; d, doublet; t, triplet; q, quartet; m, multiplet), coupling constant(s) in Hz, and number of protons.

TLC analyses were performed with TLC aluminium sheets 20 × 20 cm silica gel 60 F254 from Merck.

HRMS measurements were performed on a LC-HR/ESI-FTMS machine from Thermo Electron Corporation.

Experimental details of the synthesis of SylA-GlbA

*Synthesis of (2E),(4E) Dodecadienoic ethyl ester (**2**)*

N,*N*-Diisopropylamine (1.64 mL, 11.7 mmol) was dissolved under argon in THF (2.5 mL) in a 25 mL flame-dried flask and cooled to -78 °C. To this mixture was slowly added a 2 M solution of *n*-butyllithium in cyclohexane (3.9 mL, 7.8 mmol). The mixture was stirred at -78 °C for 10 minutes and at 0 °C for 15 minutes. After cooling again to -78 °C, (*E*)-triethyl 4-phosphonocrotonate (1.46 g, 1 mL, 5.8 mmol) was slowly added and the resulting mixture stirred for 15 min. Octanal (500 mg, 610 µL, 3.9 mmol) was added dropwise and stirring was

continued for 15 min at -78 °C. The mixture was allowed to warm to room temperature and stirred for further 2.5 hours. The reaction was quenched by addition of a saturated aq. NH_4Cl solution, extracted twice with a mixture of ethyl acetate/cyclohexane (1:1) and the combined organic layers were dried over Na_2SO_4, filtered and concentrated to dryness. The crude product was purified by flash column chromatography (1% ethyl acetate in cyclohexane) to afford 58.5 mg of (2*E*),(4*E*) Dodecadienoic ethyl ester (67% yield).

TLC (33% ethyl acetate in cyclohexane): Rf = 0.52.
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.25 (dd, J = 15.5, 10.0 Hz, 1 H), 6.07-6.20 (m, 2 H), 5.77 (d, J = 15.4 Hz, 1 H), 4.19 (q, J = 7.1 Hz, 2 H), 2.15-2.16 (m, 2 H), 1.40-1.43 (m, 2 H), 1.28 (t, J = 7.1 Hz, 3 H), 1.24-1.33 (m, 8 H), 0.88 (t, J = 6.9 Hz, 3 H).
^{13}C NMR (100 MHz, $CDCl_3$): δ 167.51, 145.29, 144.93, 128.54, 119.36, 60.34, 33.20, 31.98, 29.34, 29.30, 28.94, 22.84, 14.53, 14.27.
GC/MS (EI): *m/z* calcd for $C_{14}H_{24}O_2$ 224.18; found 224.

*Synthesis of (2E),(4E) Dodecadienoic acid (**3**)*
In a 10 mL flask, (2*E*),(4*E*) Dodecadienoic ethyl ester (**2**, 47 mg, 210 µmol, 1 eq.) was dissolved in a methanol/water (1:1, 3 mL) mixture and a solution of lithium hydroxide (15 mg, 630 µmol) in water (0.5 mL) was added. The resulting mixture was heated to 50 °C for one hour and then acidified by addition of a 1 M aq. HCl solution (1 mL). Methanol was removed by distillation under reduced pressure and the remaining mixture was extracted three times with DCM. The combined organic layers were dried over Na_2SO_4, filtered and concentrated to dryness to yield 33.7 mg of (2*E*),(4*E*) Dodecadienoic acid (**3**) as a colorless solid (82% yield).
TLC (33% ethyl acetate in cyclohexane): Rf = 0.35.
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.35 (m, 1 H), 6.17-6.21 (m, 2 H), 5.78 (d, J = 15.2 Hz, 1 H), 2.14-2.21 (m, 2 H), 1.37-1.48 (m, 2 H), 1.23-1.35 (m, 8 H), 0.88 (t, J = 6.9 Hz, 3 H).
^{13}C NMR (100 MHz, $CDCl_3$): δ 172.90, 147.65, 146.40, 128.30, 118.31, 33.16, 31.85, 29.24, 29.17, 28.72, 22.72, 14.16.
HRMS (ESI): *m/z* calcd for $C_{12}H_{20}O_2H^+$ $[M + H]^+$ 197.1536; found 197.1536.

*Synthesis of [(2R)-tert-Butoxy-(1S)-((5S)-isopropyl-2,7-dioxo-1,6-diaza-cyclododeca-(3E),(9E)-dien-(8S)-ylcarbamoyl)-propyl]-carbamic acid tert-butyl ester (**5**)*

Boc-(L)-Thr(O*t*Bu)-OH (19 mg, 66 µmol, 1.2 eq.), **4** (17.9 mg, 55 µmol, 1 eq.), PyBop (43 mg, 82 µmol, 1.5 eq.) and HOAt (12 mg, 82 µmol, 1.5 eq.) were dissolved in *N*,*N*-dimethylformamide (2.0 mL) in a 10 mL flask. The solution was cooled to 0 °C and *N*,*N*-diisopropylethylamine (20 µL, 110 µmol, 2 eq.) was added. The reaction was stirred for 40 minutes at room temperature. After concentration to dryness, the crude product was purified by flash column chromatography (5% methanol in dichloromethane) to yield 17.3 mg of [(2*R*)-*tert*-Butoxy-(1S)-((5S)-isopropyl-2,7-dioxo-1,6-diaza-cyclododeca-(3*E*),(9*E*)-dien-(8*S*)-ylcarbamoyl)-propyl]-carbamic acid *tert*-butyl ester (**5**) as a colorless solid (62% yield).

TLC (10% methanol in dichloromethane): Rf = 0.54.
HPLC: t_R = 8.56 min.
^{1}H NMR (400 MHz, d_6-DMSO): δ 8.06-8.12 (m, 2 H), 7.45 (t, J = 7.1 Hz, 1 H), 6.68 (dd, J = 15.5, 5.5 Hz, 1 H), 6.14 (d, J = 9.3 Hz, 1 H), 6.10 (d, J = 15.5 Hz, 1 H), 5.61 (dt, J = 15.6, 7.8 Hz, 1 H), 5.42 (dd, J = 16.0, 7.7 Hz, 1 H), 4.94 (dd, J = 7.6, 7.6 Hz, 1 H), 4.05-4.12 (m, 1 H), 3.95 (dd, J = 9.2, 3.0 Hz, 1 H), 3.83-3.90 (m, 1 H), 3.08-3.23 (m, 2 H), 2.22-2.31 (m, 1 H), 1.90-2.04 (m, 1 H), 1.69-1.77 (m, 1 H), 1.39 (s, 9 H), 1.07 (s, 9 H), 1.02 (d, J = 6.1 Hz, 3 H), 0.93 (d, J = 6.7 Hz, 3 H), 0.91 (d, J = 6.7 Hz, 3 H).
^{13}C NMR (100 MHz, d_6-DMSO): δ 169.38, 168.70, 166.19, 155.10, 143.14, 132.94, 126.30, 121.46, 78.28, 73.41, 67.67, 58.95, 55.48, 53.30, 42.62, 34.90, 31.35, 28.09, 28.00, 19.66, 19.59, 19.20.
HRMS (ESI): *m/z* calcd for $C_{26}H_{44}O_6N_4H^+$ $[M + H]^+$ 509.3334, found 509.3332.

*Synthesis of Dodeca-(2E),(4E)-dienoic acid [(2R)-hydroxy-(1S)-((5S)-isopropyl-2,7-dioxo-1,6-diaza-cyclododeca-(3E),(9E)-dien-(8S)-ylcarbamoyl)-propyl]-amide (**SylA-GlbA**, **6**)*

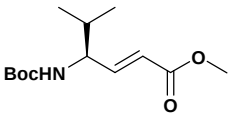
In a 10 mL flask was dissolved [(2*R*)-*tert*-Butoxy-(1*S*)-((5*S*)-isopropyl-2,7-dioxo-1,6-diaza-cyclododeca-(3*E*),(9*E*)-dien-(8*S*)-ylcarbamoyl)-propyl]-carbamic acid *tert*-butyl ester (**5**, 14 mg, 27 µmol, 1 eq.) in a mixture of trifluoroacetic acid in dichloromethane (1:4, 1 mL). After 30 minutes, the mixture was concentrated to dryness and used in the next reaction without further purification (TLC (2% triethylamine + 15% methanol in dichloromethane): Rf = 0.27; HRMS (ESI): *m/z* calcd for $C_{17}H_{28}O_4N_4H^+$ $[M + H]^+$ 353.2183, found 353.2184).
The crude ammonium salts were dissolved in dichloromethane (1.5 mL) and (2*E*),(4*E*) Dodecadienoic acid (**3**, 7 mg, 33 µmol, 1.2 eq.), PyBop (22 mg, 41 µmol, 1.5 eq.), HOAt (6 mg, 41 µmol, 1.5 eq.) and *N*,*N*-diisopropylethylamine (20 µL, 110 µmol, 4 eq.) were added

successively. The reaction was stirred overnight at room temperature. After concentration to dryness, the crude product was purified by preparative HPLC (buffer A: 0.1% TFA in water, buffer B: 0.1% TFA in acetonitrile, gradient program: 0 min / 85% A / 15% B → 10 min / 85% A / 15% B → 30 min / 60% A / 40% B → 50 min / 40% A / 60% B → 60 min / 0% A / 100% B → 80 min / 0% A / 100% B) to yield 3.6 mg of [(2*R*)-hydroxy-(1*S*)-((5*S*)-isopropyl-2,7-dioxo-1,6-diaza-cyclododeca-(3*E*),(9*E*)-dien-(8*S*)-ylcarbamoyl)-propyl]-amide (**SylA-GlbA**, **6**) as a colorless solid (25% yield).

TLC (2% acetic acid + 10% methanol in dichloromethane): Rf = 0.39.
HPLC: t_R = 9.30 min.
^{1}H NMR (400 MHz, d_6-DMSO): δ 8.09 (d, J = 8.9 Hz, 1 H), 7.90 (d, J = 7.1 Hz, 1 H), 7.88 (d, J = 8.5 Hz, 1 H), 7.45 (t, J = 6.9 Hz, 1 H), 6.99 (dd, J = 15.0, 10.4 Hz, 1 H), 6.68 (dd, J = 15.4, 5.3 Hz, 1 H), 6.04-6.22 (m, 4 H), 5.59 (dt, J = 15.5, 7.6 Hz, 1 H), 5.43 (dd, J = 15.9, 7.4 Hz, 1 H), 4.89 (dd, J = 7.3, 7.3 Hz, 1 H), 4.35 (dd, J = 8.7, 4.3 Hz, 1 H), 4.04-4.11 (m, 1 H), 3.93-4.00 (m, 1 H), 3.09-3.25 (m, 2 H), 2.23-2.32 (m, 1 H), 2.09-2.16 (m, 2 H), 1.93-2.03 (m, 1 H), 1.68-1.78 (m, 1 H), 1.33-1.42 (m, 2 H), 1.22-1.30 (m, 8 H), 1.03 (d, J = 6.3 Hz, 3 H), 0.94 (d, J = 6.7 Hz, 3 H), 0.91 (d, J = 6.7 Hz, 3 H), 0.86 (t, J = 6.9 Hz, 3 H).
^{13}C NMR (150 MHz, d_6-DMSO): δ 169.70, 169.02, 166.32, 165.45, 143.27, 142.06, 39.70, 133.22, 128.56, 126.10, 123.08, 121.52, 66.82, 57.90, 55.56, 53.59, 42.58, 5.00, 32.23, 31.50, 31.21, 28.53, 28.48, 28.35, 22.06, 19.82, 19.75, 19.25, 13.95.
HRMS (ESI): *m/z* calcd for $C_{29}H_{46}O_5N_{44}H^+$ $[M + H]^+$ 531.3541, found 531.3535.

Synthesis of sat-SylA

Synthesis of (4S)-tert-Butoxycarbonylamino-5-methyl-hex-(2E)-enoic acid methyl ester



Oxalyl chloride (3.75 g, 2.49 mmol, 1.5 eq) was dissolved under argon in DCM (50 mL) *tert*-butyl-(*S*)-1-hydroxy-3-methylbutan-2-ylcarbamate (4 g, 19.68 mmol, 1 eq.) was dissolved under argon in DCM (50 mL) and cooled to -78 °C. To this solution DMSO (4.61 g, 59.07 mmol, 3 eq.) was added slowly and the mixture was stirred for 10 min. *tert*-Butyl-(*S*)-1-hydroxy-3-methylbutan-2-ylcarbamate (4g, 19.68 mmol, 1 eq.), dissolved in DCM (10 mL), was added and stirring was continued for 1 h at -78 °C. DIPEA (7.63 g, 59 mmol, 3 eq.) was added and the mixture was stirred for 2 h at RT. Methyl (triphenylphosphoranylidene)acetate

(9.87 g, 29.53 mmol, 1.5 eq.) dissolved in DCM (10 mL) was added to the mixture and stirring was continued over night at RT.

The solvent was reduced to half the volume under reduced pressure. The mixture was washed with a 10% aq. $KHSO_4$ solution, a 5% aq. $NaHCO_3$ solution and with brine. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The resulting crude product was purified by flash column chromatography (15% ethyl acetate in cyclohexane) to afford 4.6 g of (4*S*)-*tert*-Butoxycarbonylamino-5-methyl-hex-(2*E*)-enoic acid methyl ester as colorless crystals (yield 91%).

TLC (33% ethyl acetate in cyclohexane): Rf = 0.58.

HPLC (gradient 1): t_R = 8.93 min.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.86 (dd, J = 15.6, 5.6 Hz, 1 H), 5.92 (dd, J = 15.6, 1.6 Hz, ^{1}H), 4.50-4.62 (m, 1 H), 4.11-4.23 (m, 1 H), 3.73 (s, 3 H), 1.80-1.91 (m, 1 H), 1.44 (s, 9 H), 0.93 (d, J = 6.8 Hz, 3 H), 0.90 (d, J = 6.8 Hz, 3 H).

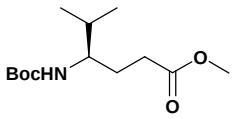
^{13}C NMR (100 MHz, $CDCl_3$): δ 166.75, 155.42, 147.74, 121.19, 79.78, 56.79, 51.66, 32.32, 28.44, 18.92, 18.10.

mp: 60.1-62.3 °C (ethyl acetate/cyclohexane).

GC-MS: t_R = 4.40 min, *m/z* = 257.

HRMS (ESI)*: m/z* calcd for $C_{13}H_{23}O_4NH^+$ $[M + H]^+$ 258.1700, found 258.1704.

Synthesis of (4R)-tert-Butoxycarbonylamino-5-methyl-hexanoic acid methyl ester



(4*S*)-*tert*-Butoxycarbonylamino-5-methyl-hex-(2*E*)-enoic acid methyl ester (1.8 g, 7 mmol, 1 eq.) was dissolved in MeOH (23 mL). To this solution was added 30%wt palladium/charcoal (11 mg, 0.35 mmol, 0.05 eq.), the flask was flushed with hydrogen and stirred overnight under hydrogen atmosphere. The mixture was filtered over a plug of Celite® and washed with dichloromethane. Concentration to dryness afforded 1.6 g (4*R*)-*tert*-Butoxycarbonylamino-5-methyl-hexanoic acid methyl ester as a colorless solid (94%).

TLC (10% ethyl acetate in cyclohexane): Rf = 0.28.

HPLC (gradient 2): t_R = 9.93 min.

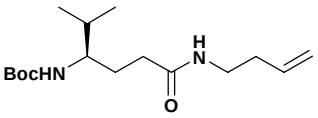
^{1}H NMR (400 MHz, $CDCl_3$): δ 4.30 (d, J = 9.8 Hz, 1 H), 3.64 (s, 3 H), 3.35-3.45 (m, 1 H), 2.34 (t, J = 7.5 Hz, 2 H), 1.76-1.86 (m, 1 H), 1.62-1.72 (m, 1 H), 1.49-1.60 (m, 1 H), 1.40 (s, 9 H),

0.88 (d, J = 6.8 Hz, 3 H), 0.86 (d, J = 6.9 Hz, 3 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 174.28, 156.00, 78.99, 55.45, 51.63, 32.62, 31.26, 28.43, 27.68, 19.04, 17.84.

HRMS (ESI): *m/z* calcd for $C_{13}H_{25}O_4NH^+$ $[M + H]^+$ 258.169, found 258.170.

Synthesis of [(1R)-(2-But-3-enylcarbamoyl-ethyl)-2-methyl-propyl]-carbamic acid tert-butyl ester



(4*R*)-*tert*-Butoxycarbonylamino-5-methyl-hexanoic acid methyl ester (1.8 g, 6.94 mmol, 1 eq.) was dissolved in MeOH/H_2O (10:1, 40 mL). A solution of lithium hydroxide (875 mg, 20.86 mmol, 3 eq.) in H_2O (3.5 mL) was added at 0 °C and the mixture was stirred for 30 minutes at RT. After concentration to dryness the resulting solid was dissolved in a 10% aq. $KHSO_4$ solution and extracted with chloroform. The combined organic layers were dried over Na_2SO_4 and concentrated to yield 1.635 g (6.66 mmol, 96%) of the free acid as a colorless solid (TLC (33% ethyl acetate in cyclohexane): Rf = 0.37; HPLC: t_R = 8.39 min; HRMS (ESI): *m/z* calcd for $C_{12}H_{23}O_4NH^+$ $[M + H]^+$ 246.1700, found 246.1699). The resulting acid (850 mg, 3.46 mmol, 1 eq.), 3-butenylamine hydrochloride (448 mg, 4.16 mmol, 1.2 eq.), and PyBop (2.7 g, 5.18 mmol, 1.5 eq.) were dissolved in DCM (15 mL) and DIPEA (1.2 mL, 9 mmol, 2.5 eq.) was added at 0 °C. The resulting mixture was stirred overnight at RT, stopped by acidification (1M aq. HCl) and the resulting mixture was extracted with chloroform. The combined organic phases were washed with a 5% aq. $NaHCO_3$ solution, dried over Na_2SO_4 and concentrated to dryness. The crude product was purified by flash column chromatography (30% ethyl acetate in cyclohexane) to yield 867 mg [(1*R*)-(2-But-3-enylcarbamoyl-ethyl)-2-methyl-propyl]-carbamic acid *tert*-butyl ester as a colorless solid (84% yield).

TLC (33% ethyl acetate in cyclohexane): Rf = 0.34.

HPLC: t_R = 9.15 min.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.29 (br s, 1 H), 5.77 (ddt, J = 17.0, 10.2, 6.8 Hz, 1 H), 5.02-5.12 (m, 2 H), 4.41 (d, J = 9.8 Hz, 1 H), 3.25-3.43 (m, 3 H), 2.10-2.30 (m, 4 H), 1.77-1.86 (m, 1 H), 1.63-1.72 (m, 1 H), 1.50-1.60 (m, 1 H), 1.42 (s, 9 H), 0.89 (d, J = 6.8 Hz, 3 H), 0.86 (d, J = 6.9 Hz, 3 H).

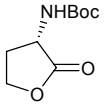
^{13}C NMR (100 MHz, $CDCl_3$): δ 173.08, 156.76, 135.50, 116.99, 79.29, 55.31, 38.67,

33.80, 32.66, 29.22, 28.47, 19.23, 17.82.

GC-MS: t_R = 5.41 min, *m/z* = 298.

HRMS (ESI): *m/z* calcd for $C_{16}H_{30}O_3N_2H^+$ $[M + H]^+$ 299.2329, found 299.2325.

Synthesis of Boc-homoserine lactone



Homoserine (5 g, 42 mmol, 1. eq.) was dissolved under argon in TFA (15 mL) and stirred for 12 h at RT. The solvent was removed under reduced pressure and toluene was added and concentrated to dryness again. This procedure was repeated three times to remove remaining traces of TFA. The resulting white solid of deprotected homoserine lactone was used without further purification (4.15 g, 41.13 mmol, 98%).

Homoserine lactone (4.15 g, 41.13 mmol, 1 eq.) and di-*tert*-butyl dicarbonate (8.95 g, 41.13 mmol, 1 eq.) were solved in DCM (75 mL) and cooled to 0 °C. To this solution was slowly added triethylamine (17.2 mL, 123.15 mmol, 3 eq.). The resulting mixture was stirred for 10 min at 0 °C and for 12 h at RT. The organic phase was washed with H_2O (75 mL), 1M aq. HCl (75 mL), dried over Na_2SO_4 and concentrated to dryness. The crude product was purified by flash column chromatography (33% ethyl acetate in cyclohexane) to yield 7.84 g of Boc-homoserine lactone as a colorless solid (94%).

TLC (33% ethyl acetate in cyclohexane): Rf = 0.19.

HPLC: t_R = 3.82 min.

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.09 (br s, 1 H), 4.43 (t, J = 8.6 Hz, 1 H), 4.33 (br s, 1 H), 4.24 (ddd, J = 11.4, 9.3, 5.9 Hz, 1 H), 2.75 (s, 1 H), 2.21 (d, J = 12.1 Hz, 1 H), 1.45 (s, 9 H).

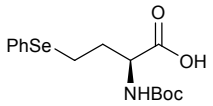
^{13}C NMR (100 MHz, $CDCl_3$): δ 161.69, 147.01, 65.95, 53.63, 50.48, 30.93, 28.49.

mp: 127.6-131.2 °C (ethyl acetate/cyclohexane).

IR KBr. $\tilde{\nu}$: 3358 (s), 2982 (m), 1777 (s), 1684 (s), 1530 (s), 1452 (s), 1376 (s), 1296 (s), 1259, (s), 1163 (m), 1006 (s), 614 (s).

HRMS (ESI): *m/z* calcd for $C_9H_{15}NO_4H^+$ $[M + H]^+$ 202.107, found 202.107.

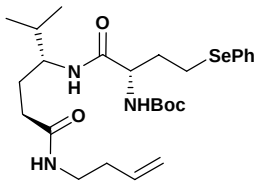
Synthesis of (2S)-tert-Butoxycarbonylamino-4-phenylselanyl-butyric acid



Sodium borohydride (113 mg, 2.9 mmol, 1.2 eq.) was dissolved under argon in dry DMF (5 mL). A solution of diphenyl diselenide (776 mg, 2.48 mmol, 1 eq.) in dry DMF (4 mL) and a solution of Boc-homoserine lactone (0.5 g, 2.48 mmol, 1 eq.) in dry DMF (4 mL). were added and the resulting mixture was heated to 100 °C for 90 min. The mixture was cooled to 0 °C, methanol (5 mL) was added and stirred for an additional hour. The solvents were removed under reduced pressure and the remaining residue was partitioned between diethyl ether (100 mL) and 80 mM aq. NaOAc buffer (pH 5.0, 100 mL). The aqueous layer was extracted with diethyl ether (3x 50 mL). The combined organic layers were dried over Na_2SO_4 and concentrated to dryness. The crude product was purified by flash column chromatography (33% ethyl acetate in cyclohexane) to yield 973 mg (2*S*)-*tert*-Butoxycarbonylamino-4-phenylselanyl-butyric acid as a colorless solid (86% yield).

TLC (50% ethyl acetate in cyclohexane): Rf = 0.64.
HPLC: t_R = 9.54 min.
^{1}H NMR (400 MHz, $CDCl_3$): δ 9.20 (br s, 1 H), 7.48-7.52 (m, 2 H), 7.23-7.28 (m, 3 H), 5.07 (d, J = 6.4 Hz, 1 H), 4.35 (br s, 1 H), 2.93 (t, J = 8.0 Hz, 2 H), 2.16-2.32 (m, 1 H), 1.98-2.15 (m, 1 H), 1.44 (s, 9 H).
^{13}C NMR (100 MHz, $CDCl_3$): δ 155.81, 155.6, 109.98, 101.84, 95.02, 66.3, 66.0, 30.07, 29.33, 28.58.

Synthesis of {(1S)-[(1R)-(2-But-3-enylcarbamoyl-ethyl)-2-methyl-propylcarbamoyl]-3-phenylselanyl-propyl}-carbamic acid tert-butyl ester



[(1*R*)-(2-But-3-enylcarbamoyl-ethyl)-2-methyl-propyl]-carbamic acid *tert*-butyl ester (585, 1.96 mmol, 1 eq.) was dissolved in a TFA/DCM (1:3, 10 mL) mixture. After 30 min, the mixture was evaporated to dryness to afford 380 mg (1.91 mmol, 98%) of the corresponding ammonium salts as a colorless solid (TLC (2% triethylamine + 50% ethyl acetate in cyclohexane): Rf = 0.21; HPLC: t_R = 2.35 min; HRMS (ESI): *m/z* calcd for $C_{11}H_{22}ON_2H^+$

$[M + H]^+$ 199.1805, found 199.1798).

The deprotected intermediate (790 mg, 3.98 mmol, 1 eq.), (2*S*)-*tert*-Butoxycarbonylamino-4-phenylselanyl-butyric acid (1.71 g, 4.78 mmol, 1.2 eq.), PyBop (3.11 g, 5.97 mmol, 1.5 eq.) and HOAt (814 mg, 5.98 mmol, 1.5 eq.) were dissolved in DCM (20 mL). DIPEA (1 mL, 5.99 mmol, 2 eq.) was added at 0 °C and the resulting mixture was stirred for 2 h at RT. The reaction was stopped with the addition of 1M aq. HCl and the mixture was extracted with chloroform (3x 75 mL). The organic phases were washed with a 5% aq. $NaHCO_3$ solution, dried over Na_2SO_4 and concentrated to dryness under reduced pressure. The crude product was purified by flash column chromatography (50% ethyl acetate in cyclohexane) to yield 1.65 g of {(1*S*)-[(1*R*)-(*2*-But-3-enylcarbamoyl-ethyl)-2-methyl-propylcarbamoyl]-3-phenylselanyl propyl}-carbamic acid *tert*-butyl ester as a colorless solid (77% yield).

TLC (60% ethyl acetate in cyclohexane): Rf = 0.37.

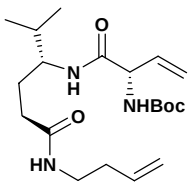
HPLC: t_R = 10.40 min.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.46-7.51 (m, 2 H), 7.23-7.28 (m, 3 H), 6.36 (br s, 1 H), 6.20 (d, J = 9.6 Hz, 1 H), 5.76 (ddt, J = 17.0, 10.2, 6.8 Hz, 1 H), 5.13 (d, J = 7.7 Hz, 1 H), 5.02-5.11 (m, 2 H), 4.18 (td, J = 7.3, 6.5 Hz, 1 H), 3.65-3.73 (m, 1 H), 3.30-3.40 (m, 1 H), 3.18-3.28 (m, 1 H), 2.93 (t, J = 7.4 Hz, 2 H), 2.21-2.28 (m, 2 H), 2.11- 2.21 (m, 2 H), 1.93-2.09 (m, 2 H), 1.83-1.92 (m, 1 H), 1.64-1.75 (m, 1 H), 1.47-1.59 (m, 1 H), 1.43 (s, 9 H), 0.86 (d, J = 6.7 Hz, 3 H), 0.85 (d, J = 6.8 Hz, 3 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 172.91, 171.97, 155.85, 135.47, 132.90, 129.58, 129.30, 127.30, 117.00, 80.62, 55.06, 54.03, 38.78, 33.76, 33.37, 32.32, 28.68, 28.37, 23.77, 19.29, 17.87.

HRMS (ESI): *m/z* calcd for $C_{26}H_{41}O_4N_3SeH^+$ $[M + H]^+$ 540.2335, found 540.2327.

Synthesis of {(1S)-[(1R)-(2-But-3-enylcarbamoyl-ethyl)-2-methyl-propylcarbamoyl]- allyl}-carbamic acid tert-butyl ester

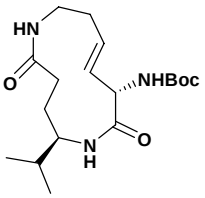


{(1*S*)-[(1*R*)-(*2*-But-3-enylcarbamoyl-ethyl)-2-methyl-propylcarbamoyl]-3-phenylselanyl propyl}-carbamic acid *tert*-butyl ester (847 mg, 1.57 mmol, 1 eq.) was dissolved in DCM (80 mL). H_2O_2 (30% in H_2O, 10 mL) and DIPEA (10 mL) were added and the resulting mixture was heated to 50 °C for 3h. The reaction was quenched by addition of a saturated aq. $CuSO_4$

solution. Addition of ethyl acetate (50 mL) and a 10% aq. $KHSO_4$ solution (50 mL) generated a biphasic mixture which was separated in a funnel. The organic phase was washed with a 5% aq. $NaHCO_3$ solution (50 mL) and brine (50 mL), dried over Na_2SO_4, filtered and concentrated to dryness. The crude product was purified by flash column chromatography (60% ethyl acetate in cyclohexane) to yield 437 mg of {(1*S*)-[(1*R*)-(2-But-3-enylcarbamoyl-ethyl)-2-methyl-propylcarbamoyl]-allyl}-carbamic acid *tert*-butyl ester as a colorless solid (73% yield).

TLC (60% ethyl acetate in cyclohexane): Rf = 0.19.
HPLC: t_R = 8.64 min.
^{1}H NMR (400 MHz, $CDCl_3$): δ 6.28 (br s, 1 H), 6.11 (d, J = 9.5 Hz, 1 H), 5.92 (ddd, J = 17.0, 10.4, 6.5 Hz, 1 H), 5.77 (ddt, J = 17.0, 10.3, 6.8 Hz, 1 H), 5.38 (ddd, J = 17.2, 1.4, 0.7 Hz, 1 H), 5.30 (ddd, J = 10.3, 1.3, 0.8 Hz, 1 H), 5.27-5.31 (m, 1 H), 5.02-5.12 (m, 2 H), 4.54-4.62 (m, 1 H), 3.68-3.77 (m, 1 H), 3.31-3.42 (m, 1 H), 3.20-3.30 (m, 1 H), 2.23-2.30 (m, 2 H), 2.05-2.21 (m, 2 H), 1.84-1.95 (m, 1 H), 1.67-1.77 (m, 1 H), 1.52-1.62 (m, 1 H), 1.44 (s, 9 H), 0.89 (d, J = 6.7 Hz, 3 H), 0.87 (d, J = 6.7 Hz, 3 H).
^{13}C NMR (100 MHz, $CDCl_3$): δ 172.78, 170.48, 155.49, 135.50, 133.87, 118.35, 116.97, 80.54, 57.84, 54.19, 38.73, 33.81, 33.33, 32.43, 28.62, 28.38, 19.26, 17.99.
HRMS (ESI): *m/z* calcd for $C_{20}H_{35}O_4N_3H^+$ $[M + H]^+$ 382.2700, found 382.2704.

Synthesis of ((5R)-Isopropyl-2,7-dioxo-1,6diaza-cyclododec-(9E)-en-(8S)-yl)-carbamic acid tert-butyl ester



{(1*S*)-[(1*R*)-(2-But-3-enylcarbamoyl-ethyl)-2-methyl-propylcarbamoyl]-allyl}-carbamic acid *tert*-butyl ester (260 mg, 682 µmol, 1 eq.) was dissolved under argon in toluene (340 mL) and heated to 90 °C. A solution of Grubbs' 2nd generation catalyst (87 mg, 102 µmol, 0.15 eq.) in toluene (25 mL) was added over 8 hours with a syringe pump to the preheated mixture. The resulting solution was stirred for further 10 h at 90 °C. After concentration to dryness, the crude product was purified by flash column chromatography (70% ethyl acetate in cyclohexane) to afford 105 mg of ((5*R*)-Isopropyl-2,7-dioxo-1,6-diaza-cyclododec-(9*E*)-en-(8*S*)-yl)-carbamic acid *tert*-butyl ester as a light brown solid (44% yield).

TLC (80% ethyl acetate in cyclohexane): Rf = 0.19.

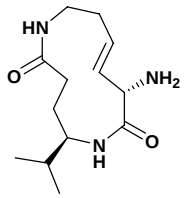
HPLC: t_R = 7.91 min.

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.99 (d, J = 7.5 Hz, 1 H), 5.76-5.86 (m, 1 H), 5.71 (d, J = 5.1 Hz, 1 H), 5.57 (d, J = 7.7 Hz, 1 H), 5.15 (ddd, J = 14.8, 9.7, 0.7 Hz, 1 H), 4.36-4.44 (m, 1 H), 3.89-4.02 (m, 1 H), 3.56-3.65 (m, 1 H), 2.79-2.87 (m, 1 H), 2.35-2.48 (m, 1 H), 2.09-2.21 (m, 1 H), 1.85-1.96 (m, 2 H), 1.75-1.84 (m, 1 H), 1.53-1.61 (m, 1 H), 1.41 (s, 9 H), 0.89 (d, J = 6.9 Hz, 3 H), 0.88 (d, J = 6.8 Hz, 3 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 174.25, 170.58, 154.87, 131.73, 130.62, 79.50, 57.52, 56.47, 37.54, 35.12, 32.99, 32.37, 28.46, 21.95, 18.63, 18.32.

HRMS (ESI): *m/z* calcd for $C_{18}H_{31}O_4N_3H^+$ $[M + H]^+$ 354.2387, found 354.2388.

Synthesis of (8S)-Amino-(5R)-isopropyl-1,6-diaza-cyclododec-(9E)-ene-2,7-dione



((5*R*)-Isopropyl-2,7-dioxo-1,6diaza-cyclododec-(9*E*)-en-(8*S*)-yl)-carbamic acid *tert*-butyl ester. (50 mg, 141 µmol, 1 eq.) was dissolved in a TFA/DCM (1:3, 3.5 mL) mixture. After 30 min, the mixture was evaporated to dryness to afford 51 mg of (8*S*)-Amino-(5*R*)-isopropyl-1,6-diaza-cyclododec-(9*E*)-ene-2,7-dione as a colorless solid (>98% yield).

TLC (2% triethylamine + 5% methanol in dichloromethane): Rf = 0.34.

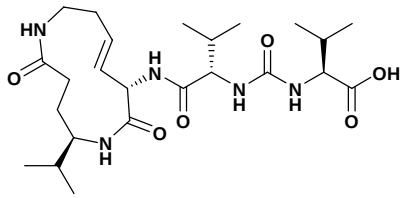
HPLC: t_R = 4.25 min.

^{1}H NMR (400 MHz, CD_3OD): δ 6.93 (d, J = 8.8 Hz, 1 H), 6.03 (ddd, J = 15.3, 11.1, 4.4 Hz, 1 H), 5.37 (ddd, J = 15.1, 10.0, 0.8 Hz, 1 H), 4.38 (d, J = 10.0 Hz, 1 H), 3.87 (td, J = 13.7, 3.4 Hz, 1 H), 3.55-3.63 (m, 1 H), 2.88 (ddd, J = 13.6, 4.7, 2.2 Hz, 1 H), 2.35-2.50 (m, 2 H), 1.94-2.17 (m, 3 H), 1.74-1.83 (m, 1 H), 1.66 (ddd, J = 12.1, 7.7, 3.9 Hz, 1 H), 0.94 (d, J = 6.9 Hz, 6 H).

^{13}C NMR (100 MHz, CD_3OD): δ 175.98, 169.52, 138.05, 126.46, 58.24, 56.89, 37.84, 35.29, 34.38, 32.92, 23.76, 19.00, 18.93.

HRMS (ESI): *m/z* calcd for $C_{13}H_{23}O_2N_3H^+$ $[M + H]^+$ 254.1863, found 254.1863.

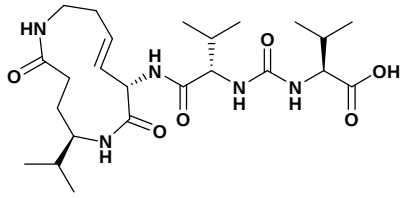
Synthesis of (2S)-{(3S)-[1-((5R)-Isopropyl-2,7-dioxo-1,6-diaza-cyclododec-(9E)-en-(8S)-ylcarbamoyl)-2-methyl-propyl]-ureido}-3-methyl-butyric acid methyl ester



(2*S*)-[3-((1*S*)-Methoxycarbonyl-2-methyl-propyl)-ureido]-3-methyl-butyric acid (16 mg, 57 µmol, 1.05 eq.), (8*S*)-Amino-(5*R*)-isopropyl-1,6-diaza-cyclododec-(9*E*)-ene-2,7-dione trifluoroacetic salt (20 mg, 54 µmol, 1 eq.), PyBop (31 mg, 59 µmol, 1.1 eq.) and HOAt (8 mg, 59 µmol, 1.1 eq.) were dissolved in DCM (5.0 mL). The solution was cooled to 0 °C and DIPEA (28 µL, 162 µmol, 3 eq.) was added. The reaction was stirred for 40 min at RT. After concentration to dryness, the crude product was purified by flash column chromatography (10% MeOH in DCM) to yield 21 mg of (2*S*)-{(3*S*)-[1-((5*R*)-Isopropyl-2,7-dioxo-1,6-diaza-cyclododec-(9*E*)-en-(8*S*)-ylcarbamoyl)-2 methyl-propyl]-ureido}-3-methyl-butyric acid methyl ester as a colorless solid (75% yield).

TLC (10% MeOH in DCM): Rf = 0.58.
HPLC: t_R = 7.42 min.
^{1}H NMR (400 MHz, $CD_3OD/CDCl_3$ = 1:4): δ 5.77 (ddd, J = 15.2, 11.1, 3.9 Hz, 1 H), 5.19 (dd, J = 15.3, 9.6 Hz, 1 H), 4.62 (d, J = 9.8 Hz, 1 H), 4.21 (d, J = 5.0 Hz, 1 H), 4.00 (d, J = 6.2 Hz, 1 H), 3.83 (ddd, J = 13.4, 13.4, 3.6 Hz, 1 H), 3.68 (s, 3 H), 3.48-3.55 (m, 1 H), 2.75-2.82 (m, 1 H), 2.33-2.41 (m, 1 H), 2.26-2.33 (m, 1 H), 1.85-2.11 (m, 5 H), 1.66-1.73 (m, 1 H), 1.55-1.61 (m, 1 H), 0.91 (d, J = 6.7 Hz, 3 H), 0.90 (d, J = 6.8 Hz, 3 H), 0.84-0.88 (m, 12 H).
^{13}C NMR (100 MHz, $CD_3OD/CDCl_3$ = 1:4): δ 174.91, 174.18, 172.76, 171.74, 158.98, 133.16, 129.11, 59.26, 58.56, 57.14, 56.77, 37.39, 34.44, 33.49, 32.38, 31.47, 31.26, 29.95, 23.15, 19.33, 19.18, 18.82, 18.55, 17.72, 17.67.
HRMS (ESI): *m/z* calcd for $C_{25}H_{43}O_6N_5H^+$ $[M + H]^+$ 510.3286, found 510.3282.

*Synthesis of (2S)-{(3S)-[1-((5R)-Isopropyl-2,7-dioxo-1,6-diaza-cyclododec-(9E)-en-(8S)-ylcarbamoyl)-2-methyl-propyl]-ureido}-3-methyl-butyric acid, (**sat-SylA**)*



(2*S*)-{(3*S*)-[1-((5*R*)-Isopropyl-2,7-dioxo-1,6-diaza-cyclododec-(9*E*)-en-(8*S*)-ylcarbamoyl)-2

methyl-propyl]-ureido}-3-methyl-butyric acid methyl ester (21 mg, 41 µmol, 1 eq.) and aluminium chloride (55 mg, 410 µmol, 10 eq.) were dissolved under argon in ethanethiol/chloroform (1:1, 3 mL). The resulting mixture was stirred for 1 h at RT. After quenching the reaction by addition of 0.1 M aq. HCl, the mixture was concentrated to dryness and the remaining residue was purified by preparative HPLC (buffer A: 0.1% TFA in water, buffer B: 0.1% TFA in acetonitrile: gradient program: 0 min / 90% A / 10% B → 10 min / 90% A / 10% B → 30 min / 70% A / 30% B → 50 min / 40% A / 60% B → 60 min / 0% A / 100% B → 80 min / 0% A / 100% B) to yield 12.6 mg of (2*S*)-{(3*S*)-[1-((5*R*)-Isopropyl-2,7-dioxo-1,6-diaza-cyclododec-(9*E*)-en-(8*S*)-ylcarbamoyl)-2-methyl-propyl]-ureido}-3-methyl-butyric acid, (**sat-SylA**):

TLC (2% CH_3COOH + 15% MeOH in DCM): Rf = 0.42.
HPLC: t_R = 6.60 min.
^{1}H NMR (400 MHz, d_6-DMSO): δ 12.39 (br s, 1 H), 7.86 (d, J = 6.5 Hz, 1 H), 7.54 (d, J = 8.4 Hz, 1 H), 6.65 (d, J = 9.3 Hz, 1 H), 6.31 (d, J = 8.8 Hz, 1 H), 6.27 (d, J = 8.9 Hz, 1 H), 5.66 (ddd, J = 15.0, 10.9, 3.8 Hz, 1 H), 5.20 (dd, J = 14.9, 9.8 Hz, 1 H), 4.62 (dd, J = 9.5, 6.6 Hz, 1 H), 3.95-4.03 (m, 2 H), 3.67-3.80 (m, 1 H), 3.39-3.50 (m, 1 H), 2.64-2.72 (m, 1 H), 2.16-2.27 (m, 2 H), 1.75-2.02 (m, 5 H), 1.54-1.64 (m, 1 H), 1.42-1.52 (m, 1 H), 0.75-0.89 (m, 18 H).
^{13}C NMR (100 MHz, d_6-DMSO): δ 174.08, 172.51, 170.90, 170.48, 157.63, 130.97, 129.88, 57.50, 57.43, 55.86, 55.20, 36.22, 33.75, 32.89, 31.71, 31.01, 30.24, 22.86, 19.26 (2 carbons), 18.93, 18.54, 17.62, 17.46.
HRMS (ESI): *m/z* calcd for $C_{24}H_{41}O_6N_5H^+$ $[M + H]^+$ 496.3130, found 496.3127.

Experimental conditions of competitive ABPP of HEK cell lysates

10 mL of 20 µg Hek cell lysate proteins were first incubated at 37 °C for an hour with different concentrations of syrbactins, followed by addition of MV151 to a final concentration of 1 µM. The resulting mixture was incubated for one hour, the proteins were denatured by boiling for min in SB and resolved on a 12.5% SDS-PAGE. For fluorescence detection, a Typhoon scanncer with the settings excitation at 523 and emission at 580 nm nm was used.

Experimental conditions of competitive ABPP of thymus cell lysates

Profiling was performed as described in B.I. Florea *et al.*, Chem. Biol. 2010, *in press*. For the

preparation of the thymus cell lysates, three month old mice were used.

Experimental condition of in vivo profiling of Jurkat cells

10^6 cells were incubated with varying concentrations of syrbactins for 3 hours in the incubator at 37 °C, followed by a 2 hour incubation with 5 μM MV151. The cells were washed with PBS, lysed and separated on 12.5% SDS-PAGE. For the positive control, 1 μM of the proteasome inhibitor Ada-Ahx_3-L_3-VS was added prior to MV151 labelling. For fluorescence detection, a Typhoon scanner with the settings excitation at 523 and emission at 580 nm was used.