Dethioacylation by Sirtuins 1–3: Considerations for Drug Design using Mechanism-based Sirtuin inhibition

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Figure S14. Chemical stability of compound 4 in assay buffer, analyzed by LC-MS after 2h incubation at 37 °C. (A) Structure and calculated m/z of compound 4, and its possible thioamide-to-oxoamide conversion product, and deacylated product. (B) Total ion chromatogram (TIC, grey) and diode array trace (red). (C) Extracted ion chromatograms for m/z corresponding to compound 4 (red), and its possible thioamide-to-oxoamide conversion product (grey), and deacylated product (black). (D) m/z display of TIC at t = 2.77-2.98 min, the relevant ion is displayed in the red box. (E) m/z display of TIC at t = 3.15-3.81 min, representing a ghost peak of the LC-MS system. The relevant ions are displayed in the red boxes.
Figure S15. Chemical stability of compound 5 in assay buffer, analyzed by LC-MS after 2h incubation at 37 °C. (A) Structure and calculated m/z of compound 5, and its possible thioamide-to-oxoamide conversion product, and deacylated product. (B) Total ion chromatogram (TIC, grey) and diode array trace (red). (C) Extracted ion chromatograms for m/z corresponding to compound 5 (red), and its possible thioamide-to-oxoamide conversion product (grey), and deacylated product (black). (D) m/z display of TIC at $t = 2.05-2.46$ min, the relevant ion is displayed in the red box. (E) m/z display of TIC at $t = 2.81-3.33$ min, representing a ghost peak of the LC-MS system. The relevant ions are displayed in the red boxes.
**Figure S16.** Chemical stability of compound 6 in assay buffer, analyzed by LC-MS after 2h incubation at 37 °C. (A) Structure and calculated m/z of compound 6, and its possible thioamide-to-oxoamide conversion product, and deacylated product. (B) Total ion chromatogram (TIC, grey) and diode array trace (red). (C) Extracted ion chromatograms for m/z corresponding to compound 6 (red), and its possible thioamide-to-oxoamide conversion product (grey), and deacylated product (black). (D) m/z display of TIC at $t = 2.66$-$3.00$ min, the relevant ion is displayed in the red box. (E) m/z display of TIC at $t = 3.15$-$3.81$ min, representing a ghost peak of the LC-MS system. The relevant ions are displayed in the red boxes.
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Figure S18. Chemical stability of compound 11 in assay buffer, analyzed by LC-MS after 2h incubation at 37 °C. (A) Structure and calculated m/z of compound 11, and its possible thioamide-to-oxoamide conversion product, and deacylated product. (B) Total ion chromatogram (TIC, grey) and diode array trace (red). (C) Extracted ion chromatograms for m/z corresponding to compound 11 (red), and its possible thioamide-to-oxoamide conversion product (grey), and deacylated product (brown and black). (D) m/z display of TIC at t = 2.39-2.63 min, the relevant ion is displayed in the red box. (E) m/z display of TIC at t = 3.15-3.81 min, representing a ghost peak of the LC-MS system. The relevant ions are displayed in the red boxes.
Figure S19. Chemical stability of compound 12 in assay buffer, analyzed by LC-MS after 2h incubation at 37 °C. (A) Structure and calculated m/z of compound 12, and its possible thioamide-to-oxoamide conversion product, and deacylated product. (B) Total ion chromatogram (TIC, grey) and diode array trace (red). (C) Extrated ion chromatograms for m/z corresponding to compound 12 (red), and its possible thioamide-to-oxoamide conversion product (grey), and deacylated product (brown and black). (D) m/z display of TIC at $t_r=2.39-2.63$ min, the relevant ion is displayed in the red box. (E) m/z display of TIC at $t_r=3.15-3.81$ min, representing a ghost peak of the LC-MS system. The relevant ions are displayed in the red boxes.

Figure S20. Chemical stability of compounds 1-6 (A) and S5-S7 (B) in human male serum (0-24h).
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Figure S21. Representative images of Western blot analysis of whole-cell lysates (HEK293) after 6h treatment with inhibitors EX-527, 1, 2, 5, 9, and Sireal2 (25 μM), co-treated with TSA (1 μM), from at least two independent experiments. (A) Full image of membranes showing levels of Ac-p53 (K382, purple) and vinculin (loading control, red), analyzed at 700 nm (anti-rabbit IgG secondary antibody). (B) Full image of membranes showing levels of p53 (blue), analyzed at 800 nm (anti-mouse IgG secondary antibody). (C). Overlay of 700 nm and 800 nm channels, showing levels of Ac-p53 (K382, red at ~50 kDa) and vinculin (loading control, red at ~125 kDa), and p53 (green). (D) Bar graph representing the relative levels of Ac-p53/vinculin (loading control), error bars represent the standard error of the mean based on data from two independent experiments performed in duplicate.
General experimentals
All reagents and solvents were of analytical grade and used without further purification as obtained from commercial suppliers. Anhydrous solvents were obtained from a PureSolv-system. Reactions were conducted under an atmosphere of nitrogen whenever anhydrous solvents were used. All reactions were monitored by thin-layer chromatography (TLC) using silica gel coated plates (analytical SiO$_2$-60, F-254). TLC plates were visualized under UV light and by dipping in either (a) a solution of potassium permanganate (10 g/L), potassium carbonate (67 g/L) and sodium hydroxide (0.83 g/L) in H$_2$O, (b) a solution of ninhydrin (3 g/L) in 3% acetic acid in H$_2$O (v/v), or (c) a solution of molybdato-phosphoric acid (12.5 g/L) and cerium(IV)sulfate (5 g/L) in 3% conc. sulfuric acid in H$_2$O (v/v) followed by heating with a heat gun. Vacuum liquid chromatography (VLC) was performed with silica gel 60 (particle size 15-40 µm). After column chromatography, appropriate fractions were pooled and dried at high vacuum (<2 mbar) for at least 12 h to give obtained products in high purity (>95%) unless otherwise stated. Evaporation of solvents was carried out under reduced pressure at a temperature below 40 °C. UPLC-MS analyses were performed on a Phenomenex Kinetex column (1.7 µm, 50×2.10 mm) using a Waters Acquity ultra high-performance liquid chromatography (UPLC) system. Gradient A with eluent I (0.1% HCOOH in H$_2$O) and eluent II (0.1% HCOOH in MeCN) rising linearly from 0% to 95% of II during t = 0.00-5.20 min was applied at a flow rate of 0.6 mL/min. Preparative reversed-phase HPLC purification was performed on a C18 Phenomenex Luna column (5 µm, 100 Å, 250×20 mm) using an Agilent 1260 LC system equipped with a diode array UV detector and an evaporative light scattering detector (ELSD). Gradient B with eluent III (H$_2$O/MeCN/TFA, 95:5:0.1) and eluent IV (0.1% TFA in MeCN) rising linearly from 0% to 95% of IV during t = 5-45 min or t = 5-65 min, at a flow rate of 20 mL/min. High-resolution mass spectrometry (HRMS) measurements were recorded either on a maXis G3 quadrupole time-of-flight (TOF) mass spectrometer (Bruker Daltonics, Bremen, Germany) equipped with an electrospray (ESI) source or on an Agilent 1290 UHPLC equipped with a diode array detector and coupled to Agilent 6550 QTOF mass spectrometer operated in positive electrospray or on a Bruker Solarix WR by either matrix assisted laser desorption/ionization, or electrospray ionization (ESI). Nuclear magnetic resonance (NMR) spectra were recorded either on a Bruker Avance III HD equipped with a cryogenically cooled probe (1H NMR and 13C NMR recorded at 600 and 151 MHz, respectively) or a Bruker Avance III (1H NMR, 13C NMR and 19F NMR recorded at 400, 101, and 377 MHz, respectively). All spectra were recorded at 298 K. Chemical shifts are reported in ppm relative to deuterated solvent as internal standard (δ$_{\text{H}}$ DMSO-$d_6$ 2.50 ppm; δ$_{\text{C}}$ DMSO-$d_6$ 39.52 ppm). Assignments of NMR spectra are based on 2D correlation spectroscopy (COSY, HSQC and HMBC spectra).
**Fluorescence-based in vitro sirtuin deacylation assays.** Materials: SIRT1 (aa 193-741 with N-terminal GST-tag, ≥60% purity; #50012), SIRT2 (aa 50-356 with C-terminal His-tag, ≥90% purity; #50013), and SIRT3 (aa 102-399 with N-terminal GST-tag; ≥64% purity; #50014) were acquired from BPS Biosciences (San Diego, CA). Purities were based on SDS-PAGE and Coomassie blue stain according to the supplier, and all enzyme concentrations given were based on the stock concentrations determined by the supplier. Sirtuin substrates where acquired in previous reports: Ac-Gln-Pro-Lys-Lys(Ac)-AMC (oxo-10), Ac-Gln-Pro-Lys-Lys(pro)-AMC (oxo-11), Ac-Gln-Pro-Lys-Lys(but)-AMC (oxo-12). Assay buffer was prepared as described in Biomol International product sheets BML-KI-143; [http://www.enzolifesciences.com/BML-AK500/flur-de-lys-hdac-fluorometric-activity-assay-kit/](http://www.enzolifesciences.com/BML-AK500/flur-de-lys-hdac-fluorometric-activity-assay-kit/) Tris HCl (50 mM), NaCl (137 mM), KCl (2.7 mM), MgCl₂ (1 mM), pH 8.0) with addition of BSA (1.0 mg/mL) unless stated otherwise. Trypsin (10,000 units/mg, TPCK treated from bovine pancreas, Sigma-Aldrich; #T1426) was purchased from Sigma-Aldrich (Steinheim, Germany). All chemicals and solvents were of analytical grade were and used without further purification as obtained from commercial suppliers.

All reactions were performed in black low binding 96-well microtiter plates (Corning half area wells), with duplicate series in each assay and each assay performed at least twice. All reactions were performed in assay buffer, with appropriate concentrations of substrates and inhibitors obtained by dilution from 10-55 mM stock solutions in either water or DMSO, and appropriate concentration of enzyme obtained by dilution of the stock provided by the supplier. DMSO concentration in the final assay solution did not exceed 1% (v/v) and control wells without either enzyme (negative control) or inhibitor (positive control) were included in each plate. Plates were analyzed using a FLUOstar Omega microplate reader with excitation at 360 nm and detecting emission at 460 nm. Fluorescence measurements (RFU) were converted to [AMC] concentrations based on an [AMC]-fluorescence standard curve, and all data analysis was performed using GraphPad Prism (vers. 8.1.2).

**End-point inhibition assays:** End-point inhibition assays were performed as previously described. In short, relevant substrate, co-substrate (NAD⁺), and inhibitor were added to each well, and the experiment initiated by addition of a freshly prepared solution of relevant sirtuin, for a final volume of 25 μL per well. The following final concentrations were used: SIRT enzyme (100 nM or 250 nM), oxo-10 (50 μM), NAD⁺ (500 μM), and inhibitor (1/10/100 μM or 3-fold dilution series for concentration response assays). The plate was incubated at 37 °C for 60 min, then a solution of trypsin and NAM (25 μL, 5 mg/mL and 4 mM, respectively; final concentration 2.5 mg/mL and 2 mM, respectively) was added and the assay development was allowed to proceed for 90 min at RT, before fluorescence measurement and calculation of residual activity. For concentration-response assays, IC₅₀ values were obtained by fitting the resulting data to the concentration-response equation using GraphPad Prism.

**End-point pre-incubation assays:** SIRT1 and inhibitor was pre-incubated with or without NAD⁺ for 30 min at 37 °C in a total volume of 40 μL, prior to addition of oxo-10 (and NAD⁺ if excluded in pre-incubation), for a final volume of 45 μL. For pre-incubation excluding NAD⁺, the following concentrations were used: SIRT1 (113 nM during pre-incubation, giving 100 nM after substrate and NAD⁺ addition), inhibitor (1.0, 2.0 μM or 100 μM), oxo-10 (0/50 μM) and NAD⁺ (0/500 μM); For pre-incubation including NAD⁺, the following concentrations were used: SIRT2 (113 nM during pre-incubation, giving 100 nM after substrate and NAD⁺ addition), inhibitor (1.0, 2.0 μM or
100 μM), substrate (0/50 μM) and NAD+ (563/500 μM). The plate was incubated at 37 °C for 30 min, then a solution of trypsin and NAM (45 μL, 5.0 mg/mL and 4 mM, respectively; final concentration 2.5 mg/mL and 2 mM, respectively) was added and the assay development was allowed to proceed for 90 min at RT, before fluorescence measurement and calculation of residual activity.

**End-point sirtuin deacylation assays:** End-point sirtuin deacylation assays were performed as previously described. In short, relevant substrate and co-substrate (NAD+) were added to each well, and the experiment initiated by addition of a freshly prepared solution of relevant sirtuin, for a final volume of 25 μL per well. The following final concentrations were used: SIRT enzyme (100 nM or 250 nM), substrate (50 μM), and NAD+ (500 μM). The plate was incubated at 37 °C for 60 min, then a solution of trypsin and NAM (25 μL, 5 mg/mL and 4 mM, respectively; final concentration 2.5 mg/mL and 2 mM, respectively) was added and the assay development was allowed to proceed for 90 min at RT, before fluorescence measurement and calculation of residual activity. For concentration-response assays, IC50 values were obtained by fitting the resulting data to the concentration-response equation using GraphPad Prism.

**End-point assays using whole-cell lysates:** HEK293 cells where seeded in 10 cm plates. Upon reaching approx. 80-90% confluence the cells where lysed and whole-cell lysates was prepared as previously described. Relevant substrate were added to the wells, and the experiment initiated by addition of the freshly prepared lysate (~65 μg per well), for a final volume of 25 μL per well and substrate concentrations of 50 μM. The plate was incubated at 37 °C for 2 h, then a solution of trypsin and NAM (25 μL, 5 mg/mL and 4 mM, respectively; final concentration 2.5 mg/mL and 2 mM, respectively) was added and the assay development was allowed to proceed for 90 min at RT, before fluorescence measurement and calculation of residual activity.

**UPLC-based in vitro sirtuin deacylation assays.** All reactions were performed in Eppendorf tubes, with duplicate series in each assay and each assay performed at least twice. All reactions were performed in assay buffer without BSA, and the following final concentrations were used: SIRT enzyme (100 nM), compound (15 μM), and NAD+ (500 μM). The reaction tubes were incubated at 37 °C under agitation and aliquotes (80 μL) were taken at time points (15 min, 30 min, 60 min) and quenched with HCO2H/MeOH (6:94, 20 μL). The samples were filtered (0.50 μm) before analysis on an analytical reverse phase ultra-performance liquid chromatography (UPLC) (Waters, Acquity) system with a reverse phase C18 column equipped with a C18 column (Acquity UPLC BEH C18, 1.7 μm, 130 Å, 2.1×50 mm), a C8 column (C8 Acquity UPLC BEH column, 1.7 μm, 130 Å, 100×2.10 mm), and a diode array UV detector. Sample were analyzed using a binary buffer system consisting of H2O/CH3CN/TFA (A, 95:5:0.1; B, 5:95:0.1) rising linearly from 5% to 95% of B during t = 7 min at a flow rate of 0.45 mL/min. Integration of the area under the curve (AUC) of the UV absorbance (280 nm) for the peaks corresponding to the compound- and the deacetylated product were used to calculate the sirtuin catalyzed conversion. NOTE: Compound 7 was could not be detected on either column and was therefore excluded from the assay.
**Chemical stability assays.** Serum stability: 400 µL human male serum (Sigma-Aldrich; #H4522) was incubated at 37 °C for 15 mins. The serum was spiked with a DMSO-stock solution of the respective inhibitor to reach a final concentration of 150 µM. The mixture was shaken at 750 rpm in an incubator at 37 °C. Aliquot (45 µL) were taken out at time points (0 min, 15 min, 30 min, 1 h, 2 h, 4 h, 6 h and 24 h) and quenched with 50 µL 6M urea and incubated for 10 min at 4 °C. 100 µL ice-cold acetonitrile was added to the serum and incubated for another >10 min at 4 °C. The samples were centrifuged for 90 min at 20,000 g and diluted with 100 µL H2O, and filtered (0.50 µm) before analysis on analytical HPLC and subsequent integration of the AUC of the recovered compound over time. Half lives (t½) were determined using GraphPad Prism and fitted to a one-exponential decay equation assuming first-order kinetics. Each assay was performed at least twice.

Buffer stability: A DMSO-stock solution of compound was diluted to a final concentration of 40 µM in assay buffer without BSA. The mixture was agitated at 750 rpm in an incubator at 37 °C and aliquotes (100 µL) were taken out at time points (0 h, 1 h, 2 h, 4 h and 24 h) and diluted with MeCN (100 µL). The samples were filtered (0.50 µm) before analysis analyzed by UPLC-MS. The total ion chromatograms (TIC) were analyzed by displaying extracted ion chromatograms (EIC) of m/z [M+H]+ values of the compounds, and their possible deacylated products, as well as the possible thioamide/thiourea conversion to the corresponding amide/urea analogs.

**Cell culture.** HEK293 (ATCC; #CRL-1573) cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM, Thermo Fisher Scientific; #11965118) containing 10% (v/v) FBS (Thermo Fisher Scientific; #26140079) and 1% penicillin-streptomycin (Sigma-Aldrich; #P4333). Cells where cultured at 37 °C with 5% CO2 in a humidified incubator and sub-cultured every 2-4 days.

**Compound treatment of HEK293 cells.** HEK293 cells were seeded in 12-well plates add a density of 100,000 cells per well and incubated overnight. Upon reaching approx. 80-90% confluency, the cells were treated with 25 µM of either **EX-527** (Sigma-Aldrich #E7034), 1, 2 or respective volume of DMSO together with 1 µM TSA for 6 h (DMSO control w/o TSA was included). After incubation, cells where washed in phosphate-buffered saline (PBS, pH 7.4, Thermo Scientific; #10010023) and collected in ice-cold radio immunoprecipitation assay buffer (RIPA buffer, 20 mM TRIS base, 150 mM NaCl, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulphate, 0.1% triton-X-100, 1 mM EDTA, 1 mM EGTA, pH 7.4) buffer containing protease- (Sigma-Aldrich; #P8340) and phosphatase (Sigma-Aldrich; #P0044 and #P5726) inhibitors, 10 µM TSA (Selleckchem; #S1045), 10 mM nicotinamide (NAM, Sigma-Aldrich; #N5535) and 10 mM sodium butyrate (Sigma-Aldrich; #303410). Cells were lysed by sonication (10 intermittent pulses with a probe sonicator) after which samples were centrifuged at 4 °C and 14,000 g for 1 h. Supernatants were collected and protein concentration was determined using a Bicinchoninic Acid Kit for Protein Determination (BCA assay, Sigma-Aldrich # BCA1). Proteins (30 µg per well) were separated on 4-12% SDS-PAGE gels with MOPS running buffer (50 mM MOPS, 50 mM Tris base, 0.1% SDS, 1 mM EDTA, pH 7.7, Thermo Fisher Scientific; #NP0001) at 120 V. Protein bands were transferred onto PVDF membranes using an iBlot 2 dry blotting transfer system (Thermo Fisher Scientific). Membranes were blocked in Odyssey blocking solution TBS (Li-Cor; #927-50000) for 1 h at RT and then probed using monoclonal anti-mouse p53 (1:1000; #2524), anti-rabbit acetyl-p53 (Lys382) and anti-rabbit vinculin (1:1000; #13901) antibodies from Cell Signaling Technology (Beverly, MA) overnight at 4 °C with constant shaking. After 3×5 min
washes in Tris-buffered saline with 0.1% tween-20, the membranes were incubated with anti-rabbit or anti-mouse IgG secondary antibody from Li-Cor (Lincoln, NE) at a 1:15000 dilution for 1 h at RT under constant shaking. All antibodies were diluted in Odyssey Blocking Buffer TBS with 0.1% tween-20. Membranes were washed again 3×5 min and dried before visualization using the Odyssey Fc Imaging System. Band intensities were determined using Image Studio Lite (vers. 5.2.5).

Synthesis of compounds 3-12, TA, TB, oxo-1, and S8-S13.

**Benzyl (S)-(6-ethanethioamido-1-oxo-1-(phenylamino)hexan-2-yl)carbamate (TA).**<sup>7-8</sup> Cbz-Lys(Boc)-OH (400 mg, 1.05 mmol) was dissolved in 6.0 mL CH₂Cl₂. TFA (6.0 mL) was added to the solution and the reaction mixture that was stirred for 2 h at ambient temperature and then concentrated under reduced pressure. Excess TFA was removed by co-evaporation with CH₂Cl₂:toluene (1:1, v/v) to afford a colorless oil (623 mg), tentatively assigned as the TFA salt of Cbz-Lys-OH (UPLC-MS t<sub>r</sub> 0.76 min, m/z 281.1; [M+H]<sup>+</sup>*, C<sub>14</sub>H<sub>12</sub>N<sub>2</sub>O<sub>4</sub>, Calcd 281.1), which was used without further purification. A solution of K₂CO₃ (0.6 mL, 10% (w/v)) in H₂O was added to a solution of the crude oil (123 mg, ~0.21 mmol) in ethanol (5.0 mL). Ethyl dithioacetate (41 mg, 0.34 mmol) was added and the reaction mixture was stirred overnight. Additional ethyl dithioacetate (20 mg, 0.17 mmol) was added to the reaction mixture and stirred for 1 h and then the reaction mixture was concentrated under reduced pressure. The residue was dissolved in 10 mL H₂O and acidified to pH 2 with aq. HCl (2 M) and extracted with CH₂Cl₂ (3×20 mL). The combined organic layer was washed with brine (50 mL), dried over MgSO₄ and concentrated under reduced pressure, affording a colorless solid (36 mg), tentatively assigned as N²-((benzyl oxy)carbonyl)-N⁶-ethanethioli-L-lysine (UPLC-MS t<sub>r</sub> 1.79 min, m/z 339.2; [M+H]<sup>+</sup>*, C<sub>15</sub>H<sub>12</sub>N<sub>2</sub>O<sub>3</sub>S<sup>+</sup>, Calcd 339.1), which was used without further purification. The colorless solid (36 mg, ~0.11 mmol) was redisolved in anhydrous DMF (3.0 mL) and cooled to 0 °C. HOBt (11 mg, 0.08 mmol), iPr₂NEt (42 mg, 0.33 mmol), aniline (11 mg, 0.12 mmol) and then HATU (38 mg, 0.10 mmol) were added and the reaction mixture was stirred for 30 min at 0 °C and then at ambient temperature for 30 min. H₂O (2.0 mL) was added followed by preparative reversed-phase HPLC purification of the reaction mixture to afford the desired thioamide TA (30 mg, 35% over three steps) as a colorless fluffy material after lyophilization. <sup>1</sup>H NMR (600 MHz, DMSO-d<sub>6</sub>) δ 9.99 (s, 1H, CO<sub>Lys</sub>NH), 9.94 (t, J = 5.5 Hz, 1H, NH<sub>Lys</sub>), 7.60 (d, J = 7.9 Hz, 2H, H<sub>2pm</sub>, H<sub>6pm</sub>), 7.55 (d, J = 7.9 Hz, 1H, NH<sub>Lys</sub>), 7.39-7.12 (m, 7H, H<sub>Ar,Cbz</sub>, H<sub>3ph</sub>, H<sub>5ph</sub>), 7.05 (t, J = 7.3 Hz, 1H, H<sub>4pm</sub>), 5.03 (s, 2H, CH<sub>2,Cbz</sub>), 4.13 (td, J = 8.6, 5.2 Hz, 1H, H<sub>α,Lys</sub>), 3.52-3.40 (m, 2H, H<sub>β,Lys</sub>), 2.36 (s, 3H, (C=S)CH₃), 1.74-1.50 (m, 4H, H<sub>δ,Lys</sub>, H<sub>ε,Lys</sub>), 1.46-1.27 (m, 2H, H<sub>γ,Lys</sub>). <sup>13</sup>C NMR (151 MHz, DMSO-d<sub>6</sub>) δ 198.8 (C=S), 171.0 (CO<sub>Lys</sub>), 156.1 (CO<sub>Cbz</sub>), 138.9 (C<sub>1pm</sub>), 137.0 (C<sub>1Ar,Cbz</sub>), 128.7 (C<sub>3pm</sub>, C<sub>5pm</sub>), 128.3 (C<sub>3Ar,Cbz</sub>, C<sub>5Ar,Cbz</sub>), 127.8 (C<sub>4Ar,Cbz</sub>), 127.7 (C<sub>2Ar,Cbz</sub>, C<sub>6Ar,Cbz</sub>, 123.3 (C<sub>4ph</sub>), 119.2 (C<sub>2ph</sub>, C<sub>6ph</sub>), 65.4 (CH<sub>2,Cbz</sub>), 55.5 (C<sub>α,Lys</sub>), 45.2 (C<sub>ε,Lys</sub>), 32.8 ((C=S)CH₃), 31.5 (C<sub>δ,Lys</sub>), 26.9 (C<sub>β,Lys</sub>), 23.2 (C<sub>γ,Lys</sub>). UPLC-MS t<sub>r</sub> 1.89 min, m/z 414.2 ([M+H]<sup>+</sup>*, C<sub>22</sub>H₂₂N₃O₅S<sup>+</sup>, Calcd 414.3). CAS-RN 1125842-09-2. Data in agreement with litterature.<sup>9</sup>
**N²-(tert-butoxycarbonyl)-N⁶-propionyl-L-lysine (S8).** Trimethylsilyl chloride (272 mg, 2.50 mmol) and iPr₂NEt (1.29 g, 10.0 mmol) were added to a solution of Boc-Lys-OH (616 mg, 2.50 mmol) in THF (40 mL). The reaction mixture was sonicated for 3 min after which propionyl chloride (342 mg, 3.70 mmol) was added, resulting in immediate formation of a colorless precipitate. The reaction mixture was stirred at ambient temperature for 1 h, followed by addition of aq. citric acid (12% w/v, 40 mL). The reaction mixture was concentrated under reduced pressure to approx. 40 mL and extracted with EtOAc (2×40 mL). The combined organic layer was washed with brine (80 mL), dried over MgSO₄ and concentrated under reduced pressure. The crude residue was purified by VLC (0-95% EtOAc and 0.25% AcOH in heptane), affording the desired amide S8 (555 mg, 73%) as a colorless foam. TLC (10% MeOH in CH₂Cl₂ with 0.25% AcOH) Rₜ = 0.3. ¹H NMR (600 MHz, DMSO-d₆) δ 12.37 (br s, 1H, COOH), 7.69 (t, J = 5.7 Hz, 1H, NH), 6.99 (d, J = 8.0 Hz, 1H, NH₂), 3.85-3.79 (m, 1H, H₆), 3.04-2.96 (m, 2H, H₆), 2.04 (q, J = 7.6 Hz, 2H, NH₂COCH₂), 1.69-1.49 (m, 2H, H₆), 1.41-1.23 (m, 13H, C(CH₃)₃, H₆, H₆), 0.98 (t, J = 7.6 Hz, 3H, CH₂CH₃). ¹³C NMR (151 MHz, DMSO-d₆) δ 174.2 (COOH), 172.6 (NH₂CO), 155.6 (COBoc), 77.9 (C(CH₃)₃Boc), 53.4 (C₆), 38.1 (C₆), 30.4 (C₆), 28.8 (C₆), 28.5 (NH₂COCH₂), 28.2 (C(CH₃)₃Boc), 23.0 (C₆), 10.0 (CH₂CH₃). Two sets of signals (approximately 5:1) were detectable due to rotamers. Only peaks for the major rotamer is given. UPLC-MS tr = 1.23 min, m/z 302.2 ([M+H]⁺, C₁₄H₂₇N₂O₅⁺, Calcd 303.2). CAS-RN 1416785-82-4.

**N²-(tert-butoxycarbonyl)-N⁶-propanethioyl-L-lysine (S9).** Lawesson’s reagent (351 mg, 0.87 mmol) was added to a solution of S8 (436 mg, 1.44 mmol) in THF (40 mL). The reaction mixture was stirred at ambient temperature for 4 h, then additional Lawesson’s reagent (120 mg, 0.30 mmol) was added and the reaction mixture was stirred at 40 °C for 1 h. Aq. citric acid/sodium citrate (1 M, pH = 4, 100 mL) was added and the reaction mixture concentrated to approx. 100 mL and extracted with EtOAc (3×80 mL). The combined organic layer was washed with brine (80 mL), dried over MgSO₄, and concentrated under reduced pressure. The crude residue was purified by VLC (10-50% EtOAc and 0.25% AcOH in heptane), affording the desired thioamide S9 (348 mg, 76%) as a colorless oil. TLC (10% MeOH in CH₂Cl₂ with 0.25% AcOH): Rₜ = 0.7. ¹H NMR (600 MHz, DMSO-d₆) δ 12.39 (br s, 1H, COOH), 9.85 (t, J = 5.3 Hz, 1H, NH), 7.02 (d, J = 8.0 Hz, 1H, NH₂), 3.87-3.80 (m, 1H, H₆), 3.49-3.40 (m, 2H, H₆), 2.53-2.48 (m, 2H, CH₂CH₃, overlap with solvent peak), 1.69-1.60 (m, 1H, H₆,Ha), 1.58-1.46 (m, 3H, H₆,Ha, H₆,Ha), 1.40-1.23 (m, 11H, C(CH₃)₃, H₆,Ha), 1.14 (t, J = 7.5 Hz, 3H, CH₂CH₃). ¹³C NMR (151 MHz, DMSO-d₆) δ 204.9 (NH₂CS), 174.2 (COOH), 156.6 (COBoc), 77.9 (C(CH₃)₃Boc), 53.4 (C₆), 44.9 (C₆), 38.2 (CH₂CH₃), 30.4 (C₆), 28.2 (C(CH₃)₃Boc), 26.8 (C₆), 23.2 (C₆), 13.9 (CH₂CH₃). Two sets of signals (approximately 9:1) were detectable due to rotamers. Only peaks for the major rotamer is given. UPLC-MS tr = 1.58 min, m/z 319.4 ([M+H]⁺, C₁₄H₂₇N₂O₅S⁺, Calcd 319.2); HRMS m/z 341.1507 ([M+Na]⁺, C₁₄H₂₆Na₂N₂O₅S⁺, Calcd 341.1511).
Benzyl (S)-1-(((S)-3-(1H-indol-3-yl)-1-(isopropylamino)-1-oxopropan-2-ylamino)-1-oxo-6-propanethioamidohexan-2-yl)carbamate (3). Compound S9 (277 mg, 0.87 mmol), was dissolved in CH2Cl2/TFA/thioanisole (1:1:0.02, v/v, 16 mL) and stirred at ambient temperature for 1 h. The reaction mixture was concentrated under reduced pressure and excess TFA was removed by coevaporation: toluene (2×20 mL). The crude mixture was redissolved in H2O/1,4-dioxane (1:1, v/v, 10 mL), followed by addition of NaHCO3 (0.48 g, 5.71 mmol). The pH was adjusted to 8-9 with aq. NaOH (2 M), then Cbz-OSu (422 mg, 1.69 mmol) was added and the reaction was stirred for 2 h at ambient temperature. The 1,4-dioxane was removed under reduced pressure and the resulting solution was acidified with aq. HCl (2 M, 20 mL). The aqueous solution was extracted with EtOAc (3×40 mL) and the combined organic layer was washed with brine (2×40 mL), dried over MgSO4, and concentrated under reduced pressure, affording a colorless solid (624 mg), tentatively assigned as N2-((benzzyloxy)carbonyl)-N7-propanethiolyl-L-lysine (UPLC-MS *t* 1.72 min, *m/z* 352.3; [M+H]+, C17H28N2O4S+, Calcd 352.2), which was used without further purification. The colorless solid (58 mg, ~0.08 mmol) was dissolved in anhydrous DMF (3.0 mL) and cooled to 0 °C. HOBt (16 mg, 0.12 mmol), iPr2NEt (93 mg, 0.72 mmol) and then HATU (38 mg, 0.10 mmol) were added and the reaction mixture was stirred at 0 °C for 30 min and then at ambient temperature for 2.5 h. H2O (2.0 mL) was added followed by preparative reversed-phase HPLC purification of the reaction mixture to afford the desired amide 3 (2 mg, 4% over 3 steps) as a colorless fluffy material after lyophilization. 1H NMR (600 MHz, DMSO-d6) δ 10.79 (d, *J* = 2.6 Hz, 1H, NHIndole), 9.84 (t, *J* = 5.4 Hz, 1H, NHβ,Trp), 7.85 (d, *J* = 8.1 Hz, 1H, NHα,Trp), 7.65 (d, *J* = 7.7 Hz, 1H, COTrpNH), 7.55 (d, *J* = 7.9 Hz, 1H, H4Indole), 7.41 (d, *J* = 8.1 Hz, 1H, NHα,Lys), 7.39-7.22 (m, 6H, Hα,β,Trp, H7Indole), 7.10 (d, *J* = 2.4 Hz, 1H, H2Indole), 7.04 (t, *J* = 7.5 Hz, 1H, H6Indole), 6.95 (t, *J* = 7.5 Hz, 1H, H5Indole), 5.06-4.98 (m, 2H, CH2,Cbz), 4.45 (td, *J* = 7.8, 6.1 Hz, 1H, Hα,Trp), 3.95 (td, *J* = 8.5, 5.1 Hz, 1H, Hα,Lys), 3.77 (h, *J* = 6.4 Hz, 1H, CH3Pr), 3.43-3.33 (m, 2H, Hε,Lys, overlap with residual H2O), 3.06 (mABX, *J* = 14.6, 6.1 Hz, 1H, Hβ,Trp), 2.96 (mABX, *J* = 14.6, 7.6 Hz, Hβ,Trp), 2.53-2.48 (m, 2H, NHCSCH2, overlap with solvent peak), 1.60-1.42 (m, 4H, Hβ,Lys, Hδ,Lys), 1.30-1.18 (m, Hε,Lys), 1.14 (t, *J* = 7.5 Hz, 3H, NHCSCH2H3), 0.99 (d, *J* = 6.6 Hz, 3H, CH3,Pr,A), 0.90 (d, *J* = 6.6 Hz, 3H, CH3,Pr,B). 13C NMR (151 MHz, DMSO-d6) δ 204.9 (C=S), 171.5 (COIndole), 170.0 (COTrp), 156.0 (CO,Cbz), 137.0 (C1Ar,Cbz), 136.0 (C7Indole), 128.3 (C3Ar,Cbz), 127.8 (C4Ar,Cbz), 127.6 (C2Ar,Cbz), 127.4 (C3Indole), 123.5 (C2Indole), 120.8 (C6Indole), 118.5 (C4Indole), 118.1 (C5Indole), 111.1 (C7Indole), 109.8 (C3Indole), 65.4 (CH2,Cbz), 54.8 (Cα,Lys), 53.3 (Cα,Trp), 45.0 (Cγ,Lys), 40.4 (CHPr), 38.1 (NHCSCH2H3), 31.6 (C6,Lys), 27.9 (Cβ,Trp), 26.8 (C5,Lys), 23.0 (Cγ,Lys), 22.3 (CH3,Pr,A), 22.1 (CH3,Pr,B), 13.9 (NHCSCH2H3). UPLC-MS *t* 1.99 min, *m/z* 580.4 ([M+H]+, C31H42N6O4S+, Calcd 580.3); HRMS *m/z* 602.2771 ([M+Na]+, C31H44N6NaO4S+, Calcd 602.2777).
**N²-(tert-butoxycarbonyl)-N⁶-butyryl-L-lysine (S10).** By the method described for S8, the titled compound was synthesized using trimethylsilyl chloride (272 mg, 2.50 mmol), iPr₂NEt (1.29 g, 10.0 mmol), Boc-Lys-OH (616 mg, 2.50 mmol), and butyryl chloride (394 mg, 3.70 mmol). The crude residue was purified by VLC (0-4% MeOH and 0.25% AcOH in CH₂Cl₂), affording the desired amide S10 (608 mg, 77%) as a colorless foam. TLC (10% MeOH in CH₂Cl₂ with 0.25% AcOH) Rᵣ = 0.3. ¹H NMR (600 MHz, DMSO-d₆) δ 12.37 (br s, 1H, COOH), 7.71 (t, J = 5.7 Hz, 1H, NH₂), 6.98 (d, J = 8.0 Hz, 1H, NH₂), 3.85-3.78 (m, 1H, H₃), 3.05-2.96 (m, 2H, H₄), 2.01 (t, J = 7.6 Hz, 2H, NH₂COCH₂), 1.69-1.59 (m, 1H, H₅,Lys), 1.57-1.46 (m, 3H, H₆,Lys,B, CH₂CH₃), 1.42-1.22 (m, 13H, C(CH₃)₃, Hₓ,Lys, H₆,Lys), 0.84 (t, J = 7.4 Hz, 3H, CH₂CH₃). ¹³C NMR (151 MHz, DMSO-d₆) δ 174.2 (COOH), 171.7 (CH₂CO), 156.6 (COBOC), 77.9 (C(CH₃)₃BOC), 53.4 (Cₒ), 38.1 (Cₓ), 37.4 (HNCOCH₂), 30.4 (Cₘ), 28.8 (Cₘ), 28.2 (C(CH₃)₃BOC), 23.0 (Cₜ), 18.7 (CH₂CH₃), 13.6 (CH₂CH₃). Two sets of signals (approximately 8:1) were detectable due to rotamers. Only peaks for the major rotamer is given. UPLC-MS tᵣ 1.36 min, m/z 317.3 ([M+H]⁺, C₁₅H₂₆N₂O₄S⁺, Calcd 317.2). CAS-RN 14608-98-1.

**N⁶-butanethioyl-N²-(tert-butoxycarbonyl)-L-lysine (S11).** By the method described for S9, the titled compound was synthesized using S10 (482 mg, 1.52 mmol) and Lawesson’s reagent (551 mg, 1.36 mmol). The crude residue was purified by VLC (0-4% MeOH and 0.25% AcOH in CH₂Cl₂), affording the desired thioamide S11 (337 mg, 67%) as a colorless oil. TLC (10% MeOH in CH₂Cl₂ with 0.25% AcOH): Rᵣ = 0.6. ¹H NMR (600 MHz, DMSO-d₆) δ 12.44 (br s, 1H, COOH), 9.89 (t, J = 5.6 Hz, 1H, NH₂), 7.03 (d, J = 8.1 Hz, 1H, NH₂), 3.90-3.80 (m, 1H, H₆), 3.54-3.41 (m, 2H, H₄), 2.53-2.48 (m, 2H, C=CH₂, overlap with solvent peak), 1.73-1.50 (m, 6H, H₅,Lys, H₆,Lys, CH₂CH₃), 1.43-1.28 (m, 11H, C(CH₃)₃, H₆,Lys), 0.86 (t, J = 7.4 Hz, 3H, CH₂CH₃). ¹³C NMR (151 MHz, DMSO-d₆) δ 203.4 (C=S), 174.2 (COOH), 156.6 (COBOC), 77.9 (C(CH₃)₃BOC), 53.4 (Cₒ), 46.9 (C=SCH₂), 44.9 (Cₓ), 30.4 (Cₘ), 28.2 (C(CH₃)₃BOC), 26.8 (Cₘ), 23.2 (Cₜ), 22.3 (CH₂CH₃), 13.1 (CH₂CH₃). Two sets of signals (approximately 8:1) were detectable due to rotamers. Only peaks for the major rotamer is given. UPLC-MS tᵣ 1.63 min, m/z 333.3 ([M+H]⁺, C₁₅H₂₆N₂O₄S⁺, Calcd 333.2). HRMS m/z 333.1843 ([M+H]⁺, C₁₅H₂₆N₂O₄S⁺, Calcd 333.1843).

**Benzyl ((S)-1-(((S)-3-(1H-indol-3-yl)-1-(isopropylamino)-1-oxopropan-2-yl)amino)-6-butanethioamido-1-oxohexan-2-yl)carbamate (4).** Compound S11 (300 mg, 0.90 mmol), was dissolved in CH₂Cl₂/TFA/thioanisole (1:1:0.02, v/v, 16 mL) and stirred at ambient temperature for 1 h. The reaction mixture was concentrated under reduced pressure and excess TFA was removed by coevaporations with CH₂Cl₂/toluene (1:1, v/v, 2×20 mL). The residue was redissolved in H₂O/1,4-dioxane (1:1, v/v, 10 mL), followed by addition of NaHCO₃ (0.48 g, 5.71 mmol). The pH was adjusted to 8-9 with NaOH (2 M). Cbz-Osu (422 mg, 1.69 mmol) was added and the reaction mixture was stirred at ambient temperature for 2 h. The 1,4-dioxane was removed under reduced pressure and the resulting solution was acidified with HCl (2 M, 20 mL) and extracted with EtOAc (3×40 mL). The combined organic layer was washed with brine (2×40 mL), dried over MgSO₄, and concentrated under reduced pressure, affording a colorless solid (562 mg), tentatively assigned as N⁶-(benzyloxy)carbonyl-N⁶-butanethioyl-L-lysine (UPLC-MS tᵣ 1.80 min, m/z 367.3; [M+H]⁺, C₁₇H₂₅N₂O₄S⁺, Calcd 367.2), which was used without further
purification. The colorless solid (50 mg, ~0.08 mmol) was redissolved in anhydrous DMF (2.0 mL) and cooled to 0 °C. HOBT (13 mg, 0.10 mmol), 3 (50 mg, 0.14 mmol), HATU (38 mg, 0.10 mmol) were added and the reaction was stirred at 0 °C for 30 min and then at ambient temperature overnight. H₂O (2.0 mL) was added followed by preparative reversed-phase HPLC purification of the reaction mixture to afford the desired amide 4 (12 mg, 25% over 3 steps) as a colorless fluffy material after lyophilization.

1H NMR (600 MHz, DMSO-d₆) δ 10.79 (d, J = 2.4 Hz, 1H, NH₇Indole), 9.85 (t, J = 5.4 Hz, 1H, NH₇Lys), 7.86 (d, J = 8.1 Hz, 1H, NH₆-Trp), 7.66 (d, J = 7.7 Hz, 1H, COTrpNH), 7.56 (d, J = 7.9 Hz, 1H, H₃Indole), 7.42 (d, J = 7.9.1 Hz, 1H, NH₇Lys), 7.38-7.22 (m, 6H, H₇Ar,Cbz, H₇Indole), 7.11 (d, J = 2.4 Hz, 1H, H₂Indole), 7.06-7.02 (m, 1H, H₆Indole), 6.99-6.93 (m, 1H, H₅Indole), 5.07-4.98 (m, 2H, CH₂,Cbz), 4.46 (td, J = 7.8, 6.2 Hz, 1H, H₆-Trp), 3.96 (td, J = 8.5, 5.1 Hz, 1H, H₇Lys), 3.77 (h, J = 6.4 Hz, 1H, CH₃Pr), 3.43-3.38 (m, 2H, H₇Lys), 3.06 (mABX, J = 14.6, 6.1 Hz, 1H, H₆-Trp,A), 2.97 (mABX, 1H, J = 14.6, 7.6 Hz, H₆-Trp,B), 2.53-2.48 (m, 2H, C=SCH₂, overlap with solvent peak), 1.66 (h, J = 7.4 Hz, 2H, C=SCH₂CH₂CH₃), 1.60-1.42 (m, 4H, H₃Lys, H₇Lys), 1.34-1.16 (m, 2H, H₇Lys), 1.00 (d, J = 6.6 Hz, 3H, CH₂Pr,A), 0.91 (d, J = 6.6 Hz, 3H, CH₂Pr,B), 0.84 (t, J = 7.4 Hz, 3H, NH₇CSCH₂CH₂CH₃). 13C NMR (151 MHz, DMSO-d₆) δ 203.3 (C=S), 171.4 (CO₂Lys), 170.0 (CO₂Cba), 156.0 (CO₂Cbz), 137.0 (C1Ar,Cbz), 136.0 (C7aIndole), 128.3 (C3a,Cbz, C5a,Cbz), 127.8 (C4a,Cbz), 127.6 (C2a,Cbz, C6a,Cbz), 127.4 (C3aIndole), 123.5 (C2aIndole), 122.8 (C6Indole, 118.5 (C4Indole), 118.1 (C5Indole), 111.1 (C7aIndole), 109.8 (C3Indole), 65.4 (CH₂,Cbz), 54.8 (CO₂Lys), 53.3 (C₆-Trp), 46.8 (NH₇CSCH₂), 44.9 (C₂,Lys), 40.4 (CH₂Pr), 31.5 (C₇a,Lys), 27.9 (C₆-Trp), 26.8 (C₅a,Lys), 23.0 (C₇a,Lys), 22.2 (CH₂Pr,A), 22.1 (CH₃Pr,B), 13.0 (NH₇CSCH₂CH₂CH₃). UPLC-MS tᵣ 2.09 min, m/z 595.4 ([M+H]⁺, C₃₂H₄₄N₂O₄S⁺, Calcd 594.3); HRMS m/z 594.3107 ([M+H]⁺, C₃₂H₄₄N₂O₄S⁺, Calcd 594.3109).

**Benzyl (S)-(6-butanethioamido-1-oxo-1-(phenylamino)hexan-2-yl)carbamate (TB).** Crude N²-((benzyloxy)carbonyl)-N²-propanethioamido-l-lysine (50 mg, ~0.08 mmol), HATU (38 mg, 0.10 mmol) and aniline (18 mg, 0.20 mmol), were dissolved in anhydrous DMF (2.0 mL) and cooled to 0 °C. HATU (38 mg, 0.10 mmol) was added and the reaction was stirred at 0 °C for 1 h. Then, additional aniline (36 mg, 0.40 mmol) and HATU (18 mg, 0.10 mmol) were added to the reaction mixture and the reaction stirred at ambient temperature for 1 h. H₂O (2.0 mL) was added followed by preparative reversed-phase HPLC purification of the reaction mixture to afford the desired amide TB (4 mg, 9%) as a colorless fluffy material after lyophilization.

1H NMR (600 MHz, DMSO-d₆) δ 9.99 (s, 1H, CO₂LysNH), 9.87 (t, J = 5.5 Hz, 1H, NH₇Lys), 7.59 (d, J = 8.0 Hz, 2H, H₄Ph₂, H₆Ph), 7.53 (d, J = 7.9 Hz, 1H, NH₇Lys), 7.42-7.19 (m, 7H, H₇Ar,Cbz, H₃Ph, H₅Ph), 7.05 (t, J = 7.4 Hz, 1H, H₄Ph), 5.03 (s, 2H, CH₂,Cbz), 4.13 (td, J = 8.6, 5.2 Hz, 1H, H₇Lys), 3.55-3.39 (m, 2H, H₇Lys), 2.47 (t, J = 7.4 Hz, 2H, (C=S)CH₂), 1.76-1.50 (m, 6H, H₆Lys, H₆Lys, (C=S)CH₂CH₂), 1.48-1.27 (m, 2H, H₇Lys), 0.82 (t, J = 7.4 Hz, 3H, CH₃). 13C NMR (151 MHz, DMSO-d₆) δ 203.4 (C=S), 171.0 (CO₂Lys), 156.1 (CO₂Cbz), 138.9 (C1Ph), 137.0 (C₁Ar,Cbz), 128.7 (C₄Ph, C₅Ph), 128.3 (C₃Ar,Cbz, C5a,Cbz), 127.8 (C₄Ar,Cbz), 127.7 (C₃Ar,Cbz, C6a,Cbz), 123.3 (C₄Ph), 119.2 (C₂Ph, C₆Ph), 65.4 (CH₂,Cbz), 55.3 (C₆a,Lys), 46.9 ((C=S)CH₂), 44.9 (C₂,Lys), 31.5 (C₇a,Lys), 26.9 (C₅a,Lys), 23.1 (C₇a,Lys), 22.3 (CH₂CH₃), 13.0 (CH₃). UPLC-MS tᵣ 1.94 min, m/z 442.3 ([M+H]⁺, C₂₃H₃₂N₂O₄S⁺, Calcd 442.2); CAS-RN 1429749-39-2.
Benzyl ((S)-1-(((S)-3-(1H-indol-3-yl)-1-(isopropylamino)-1-oxopropan-2-yl)amino)-1-oxo-6-thioureidohecan-2-yl)carbamate (5). A solution of S1 (17 mg, 0.03 mmol) dissolved and iPr2NEt (27 mg, 0.21 mmol) in anhydrous DMF (5.0 mL) was added dropwise to a solution of S2 (10 mg, 0.03 mmol) in anhydrous DMF (1.0 mL) at 0 °C over 5 min. The reaction mixture was stirred at 0 °C for 10 min and then at ambient temperature for 20 min. Ammonia in MeOH (7M, 100 μL) was added and the reaction mixture was stirred for 10 min at ambient temperature. H2O (2.0 mL) was added and preparative reversed-phase HPLC purification of the reaction mixture afforded the desired thiourea 5 (10 mg, 52%) as a colorless fluffy material after lyophilization. 1H NMR (600 MHz, DMSO-d6) δ 10.79 (d, J = 2.3 Hz, 1H, NHindole), 7.86 (d, J = 8.2 Hz, 1H, NHα,Trp), 7.65 (d, J = 7.7 Hz, 1H, COTrpNH), 7.62-7.48 (m, 2H, H4indole, NHε,Lys), 7.43 (d, J = 7.9 Hz, 1H, NHε,Lys), 7.39-7.22 (m, 6H, HAr,Cbz, H7indole), 7.11 (d, J = 2.3 Hz, 1H, H2indole), 7.04 (t, J = 7.6 Hz, 1H, H6indole), 6.96 (t, J = 7.3 Hz, 1H, H5indole), 5.07-4.98 (m, 2H, CH2,Cbz), 4.45 (td, J = 7.8, 6.0 Hz, 1H, Hα,Trp), 3.94 (q, J = 7.5, Hβ,Lys), 3.78 (hept, J = 6.8 Hz, 1H, CHβPr), 3.28 (br s, 2H, Hε,Lys), 3.06 (mABX, J = 14.6, 6.0 Hz, 1H, Hβ,Trp,A), 2.97 (mABX, J = 14.6, 7.7 Hz, 1H, Hβ,Trp,B), 1.59-1.12 (m, 6H, Hβ,Lys, Hε,Lys, H6,Lys), 1.00 (d, J = 6.6 Hz, 3H, CH3,Pr,A), 0.91 (d, J = 6.6 Hz, 3H, CH3,Pr,B). 13C NMR (151 MHz, DMSO-d6) δ 183.1 (C=O), 171.5 (CO,Lys), 170.1 (CO,Trp), 158.3 (q, J = 36.4 Hz, residual CO,TFA), 156.0 (CO,Cbz), 137.0 (C1Ar,Cbz), 136.0 (C7indole), 128.4 (C3Ar,Cbz, C5Ar,Cbz), 127.8 (C4Ar,Cbz), 127.7 (C2Ar,Cbz, C6Ar,Cbz), 127.4 (C3indole), 123.5 (C2indole), 120.8 (C6indole), 118.4 (C4indole), 118.1 (C5indole), 115.5 (q, J = 290.6 Hz, residual CF3,TFA), 111.2 (C7indole), 109.8 (C3indole), 65.5 (CH2,Cbz), 55.0 (Ca,Lys), 53.3 (Ca,Trp), 43.8 (Ca,Lys), 40.4 (CHβPr), 31.6 (Cβ,Lys), 28.5 (Cδ,Lys), 27.9 (Cβ,Trp), 22.9 (Cγ,Lys), 22.2 (CH3,Pr,A), 22.1 (CH3,Pr,B). UPLC-MS tR 1.71 min, m/z 567.4 ([M+H]+, C29H38N8O8S+, Calcd 567.3); HRMS m/z 567.2742 ([M+H]+, C29H38N8O8S+, Calcd 567.2748).

Benzyl ((10S,13S)-13-((1H-indol-3-yl)methyl)-16-methyl-11,14-dioxo-4-thioxo-3,5,12,15-tetraazaheptadecan-10-yl)carbamate (6). By the method described for S9, the titled compound was synthesized using S1 (12 mg, 0.02 mmol), S2 (20 mg, 0.07 mmol) and using ethylamine (70% in H2O, 180 μL, 0.23 mmol). Preparative reversed-phase HPLC purification afforded the desired thiourea 6 (12 mg, 87%) as a colorless fluffy material after lyophilization. 1H NMR (600 MHz, DMSO-d6) δ 10.79 (d, J = 2.6 Hz, 1H, NHindole), 7.86 (d, J = 8.1 Hz, 1H, NHα,Trp), 7.65 (d, J = 7.7 Hz, 1H, COTrpNH), 7.56 (d, J = 7.7 Hz, 1H, H4indole), 7.42 (d, J = 7.9 Hz, 1H NHα,Lys), 7.38-7.22 (m, 8H, NHCH2CH3, NHε,Lys, HAr,Cbz, H7indole), 7.10 (d, J = 2.4 Hz, 1H, H2indole), 7.01-7.08 (m, 1H, H6indole), 6.98-6.93 (m, 1H, H5indole), 5.07-4.97 (m, 2H, CH2,Cbz), 4.45 (td, J = 7.8, 6.1 Hz, 1H, Hα,Trp), 3.95 (td, J = 8.5, 5.1 Hz, Hε,Lys), 3.77 (hept, J = 6.7 Hz, 1H, CHβPr), 3.36-3.30 (m, 4H, NHCH2CH3, H6,Lys, overlap with residual H2O), 3.06 (mABX, J = 14.6, 6.1 Hz, 1H, Hβ,Trp,A), 2.96 (mABX, J = 14.6, 7.7 Hz, 1H, Hβ,Trp,B), 1.59-1.51 (m, Hβ,Lys,A), 1.50-1.34 (m, 3H, Hγ,Lys,B, H6,Lys), 1.33-1.12 (m, 2H, Hε,Lys), 1.05 (t, J = 7.2 Hz, 3H, NHCH2CH3), 1.00 (d, J = 6.6 Hz, 3H, CH3,Pr,A), 0.91 (d, J = 6.6 Hz, 3H, CH3,Pr,B). 13C NMR (151 MHz, DMSO-d6) δ 171.5 (CO,Lys), 170.0 (CO,Trp), 156.0 (CO,Cbz), 137.0 (C1Ar,Cbz), 136.0 (C7indole), 128.3 (C3Ar,Cbz, C5Ar,Cbz), 127.8 (C4Ar,Cbz), 127.7 (C2Ar,Cbz, C6Ar,Cbz), 127.4 (C3indole), 123.5 (C2indole), 120.8 (C6indole), 118.5 (C4indole), 118.1 (C5indole), 111.1 (C7indole), 109.8 (C3indole), 65.4 (CH2,Cbz), 54.9 (Ca,Lys), 53.3 (Ca,Trp), 43.3 (Ca,Lys), 40.4 (CHβPr), 38.1 (NHCH2CH3), 31.6 (Cβ,Lys), 28.5 (Cδ,Lys), 27.9 (Cβ,Trp), 22.9 (Cγ,Lys), 22.2 (CH3,Pr,A), 22.1 (CH3,Pr,B), 14.5 NHCH2CH3. The peak for Cε,Lys was broad
and of low intensity and the peak for C=S was not visible in $^{13}$C NMR, probably due to fast quadrupolar relaxation via the nearby $^{14}$N-nuclei. UPLC-MS $t_r$ 1.98 min, $m/z$ 595.5 ([M+H]$^+$, C$_{31}$H$_{43}$N$_6$O$_4$S$^+$, Calcd 595.3); HRMS $m/z$ 617.2881 ([M+Na]$^+$, C$_{31}$H$_{42}$Na$_2$NaO$_4$S$^+$, Calcd 617.2886).

### Benzyl ((5S,8S)-5-((1H-indol-3-yl)methyl)-2-methyl-4,7-dioxo-14-thioxo-3,6,13,15-tetraazanonadecan-8-yl)carbamate (7).

A solution of butylamine (10 mg, 0.13 mmol) and iPr$_2$NEt (100 mg, 0.78 mmol) in anhydrous CH$_2$Cl$_2$ (5.0 mL) was added dropwise to a solution of S2 (37 mg, 0.13 mmol) in anhydrous CH$_2$Cl$_2$ (8.0 mL) at 0 °C. The reaction mixture was stirred for 15 min at 0 °C and then at ambient temperature for 2 h. The reaction mixture was concentrated under reduced pressure and the crude residue was redissolved in DMF (2 mL). iPr$_2$NEt (100 mg, 0.78 mmol) and S1 (10 mg, 0.019 mmol) were added and the reaction mixture was stirred at ambient temperature for 2 h. H$_2$O (1.0 mL) was added preparative reversed-phase HPLC purification of the reaction mixture afforded the desired thiourea 7 (5 mg, 42%) as a colorless fluffy material after lyophilization. $^1$H NMR (600 MHz, DMSO-$d_6$) δ 10.79 (d, $J$ = 2.4 Hz, 1H, NH$_{indole}$), 7.86 (d, $J$ = 8.1 Hz, 1H, NH$_{Trp}$), 7.66 (d, $J$ = 7.7 Hz, 1H, CO$_{Trp}$), 7.42 (d, $J$ = 7.9 Hz, 1H NH$_{Lys}$), 7.56 (d, $J$ = 7.9 Hz, 1H, H$_4$), 7.38-7.22 (m, 8H, NH$_{Trp}$, NH$_{Lys}$, H$_{Ar, Cbz}$, H$_7$), 7.11 (d, $J$ = 2.3 Hz, 1H, H$_2$), 7.04 (t, $J$ = 7.4 Hz, 1H, H$_6$), 6.96 (t, $J$ = 7.4 Hz, 1H, H$_9$), 5.07-4.98 (m, 2H, CH$_2$), 4.45 (q, $J$ = 7.3 Hz, 1H, H$_{A,Lys}$), 3.78 (h, $J$ = 6.9 Hz, 1H, CH$_{in}$), 3.30 (br s, 2H, H$_{Lys}$, overlap with residual H$_2$O), 3.06 (m, $J$ = 14.6, 6.1 Hz, 1H, H$_{Trp}$), 2.97 (m, $J$ = 14.6, 7.6 Hz, 1H, H$_{Trp}$), 1.60-1.35 (m, 6H, H$_{Trp}$, H$_{Lys}$, CH$_2$), 1.33-1.10 (m, 6H, H$_{Trp}$, CH$_2$), 1.00 (d, $J$ = 6.6 Hz, 3H, CH$_3$), 0.93-0.82 (m, 6H, CH$_3$, CH$_2$), 1.33 (s, 1H, NH$_{indole}$), 118.5 (CO$_{Lys}$), 170.0 (CO$_{Trp}$), 158.2 (q, $J$ = 36.3 Hz, CO$_{TFA}$), 156.0 (CO$_{Cbnz}$), 137.0 (C$_{1Ar,Cbnz}$), 136.0 (C$_{7Ar}$), 128.4 (C$_{4Ar,Cbnz}$), 127.8 (C$_{2Ar,Cbnz}$, C$_{6Ar,Cbnz}$), 127.4 (C$_{3Ar}$), 123.5 (C$_{2Indole}$), 120.8 (C$_{6Indole}$), 118.5 (C$_{4Indole}$), 118.1 (C$_{5Indole}$), 115.7 (q, $J$ = 291.8 Hz, CF$_{3,TFA}$), 111.1 (C$_{7Indole}$), 109.8 (C$_{Indole}$), 103.4 (CH$_2$), 54.9 (C$_{O,Lys}$), 53.3 (C$_{O,Trp}$), 43.2 (C$_{Lys}$), 40.4 (CH$_3$), 31.6 (C$_{Lys}$), 30.9 (CH$_2$CH$_2$), 29.0 (CH$_2$CH$_2$), 28.5 (C$_{O,Lys}$), 27.9 (C$_{Trp}$), 22.9 (C$_{Lys}$), 22.2 (CH$_3$), 22.1 (CH$_3$), 19.6 (CH$_2$), 13.7 (CH$_2$CH$_2$), The peak for C$_{Lys}$ was broad and of low intensity and the peak for C$_{Trp}$ was not visible in $^{13}$C NMR, probably due to fast quadrupolar relaxation via the nearby $^{14}$N-nuclei. UPLC-MS $t_r$ 1.98 min, $m/z$ 623.5 ([M+H]$^+$, C$_{33}$H$_{47}$N$_6$O$_4$S$^+$, Calcd 623.3); HRMS $m/z$ 645.3196 ([M+Na]$^+$, C$_{33}$H$_{48}$Na$_2$NaO$_4$S$^+$, Calcd 645.3199).

### Benzyl ((S)-1-(((S)-3-((1H-indol-3-yl)-1-(isopropylamino)-1-oxopropan-2-yl)amino)-6-(2,2-difluoroacetamido)-1-oxohexan-2-yl)carbamate (8).

Difluoroacetic anhydride (8 mg, 0.05 mmol) in DMF (0.5 mL) was added dropwise to a solution of S1 (8 mg, 0.02 mmol) and iPr$_2$NEt (13 mg, 0.10 mmol) in DMF (2.0 mL) at 0 °C. The reaction was stirred at ambient temperature for 1 h and H$_2$O (2.0 mL) was added to the reaction mixture. Preparative reversed-phase HPLC purification of the reaction mixture afforded the desired $\alpha$-fluoroamide 8 (4 mg, 46%) as a colorless fluffy material after lyophilization. $^1$H NMR (600 MHz, DMSO-$d_6$) δ 10.79 (s, 1H, NH$_{indole}$), 8.73 (t, $J$ = 5.7 Hz, 1H, NH$_{Trp}$), 7.85 (d, $J$ = 8.1 Hz, 1H, NH$_{Trp}$), 7.65 (d, $J$ = 7.7 Hz, 1H,
**Benzyl \(((\text{S})-1-\{(\text{S})-3-(1\text{-H-indol-3-yl})\text{-1-(isopropylamino)-1-oxopropan-2-yl)amino\}-1\text{-oxo-6-(2,2,2-trifluoroacetamido)hexan-2-yl})\text{carbamate} \) (9). Compound S1 (58 mg, 0.11 mmol) was dissolved in MeCN/CH2Cl2 (1:1, v/v, 4.0 mL) and cooled to 0 °C. iPr2NEt (88 mg, 0.68 mmol) and trifluoroacetic anhydride (23 mg, 0.11 mmol) was added and the reaction mixture was stirred at 0 °C for 30 min. Additional trifluoroacetic anhydride (2×23 mg, 0.22 mmol) was added and the reaction mixture was stirred for 1 h. The CH2Cl2 was removed by a stream of N2, and H2O (2.0 mL) was added to the reaction mixture followed by preparative reversed-phase HPLC purification of the reaction mixture to afford the desired α-fluoroamide 9 (13 mg, 19%) as a colorless fluffy material after lyophilization. 1H NMR (600 MHz, DMSO-\(d_6\)) \(\delta\) 10.78 (br s, 1H, NH\text{Indole}), 9.37 (t, J = 5.7 Hz, 1H, NH\text{Lys}), 7.84 (d, J = 8.2 Hz, 1H, NH\text{O-Trp}), 7.64 (d, J = 7.7 Hz, 1H, CO\text{Trp-NH}), 7.55 (d, J = 7.9 Hz, 1H, H\text{Indole}), 7.42 (d, J = 7.9 Hz, 1H, NH\text{Lys}), 7.39-7.23 (m, 6H, H\text{Ar-Cbz}, H\text{Indole}), 7.10 (d, J = 2.4 Hz, 1H, H\text{Indole}), 7.06-7.01 (m, 1H, H\text{Indole}), 6.98-6.92 (m, 1H, H\text{Gly}), 5.07-4.97 (m, 2H, CH\text{Cz}), 4.45 (q, J = 7.4, 1H, H\text{O-Trp}), 3.94 (td, J = 8.6, 5.4 Hz, 1H, H\text{Lys}), 3.78 (hept, J = 6.7 Hz, 1H, CH\text{Pr}), 3.11 (q, J = 6.8 Hz, 2H, H\text{Lys}), 3.06 (m, 1H, J = 14.6, 6.0 Hz, H\text{Gly}), 2.96 (m, 1H, J = 14.6, 7.7 Hz, H\text{Gly}), 1.84-1.50 (m, 1H, H\text{Lys}), 1.43-1.19 (m, 3H, H\text{Lys}), 0.99 (d, J = 6.6 Hz, 3H, CH\text{Pr}), 0.91 (d, J = 6.5 Hz, 3H, CH\text{Pr}). 13C NMR (151 MHz, DMSO-\(d_6\)) \(\delta\) 171.5 (CO\text{Lys}), 170.0 (CO\text{Trp}), 156.1 (q, J = 35.5 Hz, CO\text{CF3}), 156.0 (CO\text{Cz}), 137.0 (C\text{Ar-Cbz}), 136.0 (C\text{Indole}), 138.4 (C\text{N-Cbz}, C\text{N-Cbz}), 128.8 (C\text{Ar-Cbz}, C\text{Gly}), 127.7 (C\text{Gly}), 127.4 (C\text{Gly}), 121.8 (C\text{Gly}), 111.8 (C\text{Gly}), 109.8 (C\text{Gly}), 108.6 (t, J = 246.6 Hz, CO\text{Gly}), 105.4 (CH\text{Cz}), 54.9 (C\text{Lys}), 53.3 (C\text{Trp}), 40.4 (CH\text{Pr}), 38.4 (C\text{Lys}), 31.4 (C\text{Lys}), 28.3 (C\text{Lys}), 27.9 (C\text{Gly}), 22.7 (C\text{Lys}), 22.2 (CH\text{Pr}), 22.1 (CH\text{Pr}). 19F NMR (376 MHz, DMSO-\(d_6\)) \(\delta\) -74.3 (s, CF\text{3}, TFA), -126.1 (s, CO\text{Gly}). UPLC-MS \(m/z\) 586.4 ([M+H]+, C\text{H}3\text{F}2\text{N}5\text{O}5, Calcd 586.3); HRMS \(m/z\) 608.2652 ([M+Na]+, C\text{H}3\text{F}3\text{N}5\text{Na}2O5, Calcd 608.2660).
Benzyl ((S)-1-((S)-3-(1H-indol-3-yl)-1-(isopropylamino)-1-oxopropan-2-yl)amino)-6-acetamido-1-oxohexan-2-yl)carbamate (oxo-1). Acetic anhydride (0.50 mL, 5.28 mmol) was added dropwise to a solution of S1 (10 mg, 0.02 mmol) in DMF (2.0 mL) at 0 °C. The reaction mixture was stirred at ambient temperature for 1 h after which H2O (2.0 mL) was added to the reaction mixture. Preparative reversed-phase HPLC purification of the reaction mixture afforded the desired amide oxo-1 (7 mg, 65%) as a colorless fluffy material after lyophilization.

1H NMR (600 MHz, DMSO-d6) δ 10.80 (d, J = 2.3 Hz, 1H, NHindole), 7.84 (d, J = 8.1 Hz, 1H, NH,Trp), 7.76 (t, J = 5.6 Hz, 1H, NH,Lys), 7.65 (d, J = 7.7 Hz, 1H, COtrp, NH), 7.56 (d, J = 7.9 Hz, 1H, H4indole), 7.41 (d, J = 7.8 Hz, 1H, NH,Lys), 7.40-7.22 (m, 6H, HAcbz, H7indole), 7.10 (d, J = 2.3 Hz, 1H, H2indole), 7.04 (t, J = 7.6 Hz, 1H, H6indole), 6.95 (t, J = 7.3 Hz, 1H, H5indole), 5.07-4.98 (m, 2H, CH2cbz), 4.45 (td, J = 7.9, 6.0 Hz, 1H, H0trp), 3.93 (td, J = 8.5, 5.2 Hz, 1H, Ha,llys), 3.78 (hept, J = 6.7 Hz, 1H, CHPr), 3.06 (mabx, J = 14.6, 6.0 Hz, 1H, H6trp,a), 2.99-2.92 (m, 3H, Hcbz, Lys, Hcbz, trp,b), 1.77 (s, 3H, COCH3), 1.56-1.41 (m, 2H, Hβllys), 1.36-1.10 (m, 4H, Hγ,llys, Hδ,llys, γ,llys), 1.00 (d, J = 6.6 Hz, 3H, CH3,pr,b), 0.91 (d, J = 6.6 Hz, 3H, CH3,pr,b), 13C NMR (151 MHz, DMSO-d6) δ 171.5 (COlys), 170.0 (COtrp), 168.9 (CH3), 156.0 (COcbz), 136.9 (C1ar,cbz), 136.0 (C7a,indole), 128.3 (C3ar,cbz, C5ar,cbz), 127.8 (C4ar,cbz), 127.6 (C2ar,cbz), 127.4 (C3a,indole), 123.5 (C2,indole), 120.8 (C6,indole), 118.5 (C4,indole), 118.1 (C5,indole), 111.1 (C7,indole), 109.8 (C3,indole), 65.5 (CH2,cbz), 54.9 (C2,lys), 53.3 (C3,lys), 40.4 (CHPr), 38.3 (C2,lys), 31.5 (C1,lys), 28.8 (C5,lys), 27.8 (C4, lys), 22.9 (C3, lys), 22.6 (COCH3), 22.2 (CH3,pr,b), 22.1 (CH3,pr,b). UPLC-MS tR 1.70 min, m/z 550.4 ([M+H]+, C30H40N6O5+; calcd 550.3); HRMS m/z 550.3018 ([M+H]+, C30H40N6O5+; calcd 550.3024).

N2-N2-acetyl-N5-trityl-L-glutamyl-L-prolyl-N5-(tert-butoxycarbonyl)-L-lysine (S12). 2-chlorotritryl chloride resin (2.50 g, 100-200 mesh, 1.10 mmol/g loading) was swelled in CH2Cl2 (25 mL) for 30 min and then washed with CH2Cl2 (2×10 mL). A mixture of Fmoc-Lys(Boc)-OH (2.58 g, 5.50 mmol) and iPr2NET (1.42 g, 11.0 mmol) in DMF (3.4 mL) was added to the resin and agitated for 3 h. The resin was drained and capped with CH2Cl2/MeOH/lutidine (7.4:2:1.0:5.0, 26.3 mL) for 1h and washed with DMF (4×10 mL) and CH2Cl2 (4×10 mL). The resin was dried overnight under high vacuum and loading was determined to 0.60 mmol/g, measured spectrophotometrically by quantifying the amount of Fmoc released upon cleavage of a small sample.10 Ac-Gln(Trt)-Pro-Lys(Boc)-resin was synthesized by SPPS, using Fmoc-Pro-OH and Fmoc-Gln(Trt)-OH. Cleavage from resin was performed with CH2Cl2/MeOH/hexafluoroisopropanol (8:1:1, v/v, 25 mL), and the reaction mixture was concentrated under reduced pressure, affording the desired carboxylic acid S12 (217 mg, 32% based on resin loading) as a colorless solid, which was used without further purification.1H NMR (600 MHz, DMSO-d6) δ 12.45 (br s, COOH, 1H), 8.58 (s, 1H, NHPh3), 8.09-7.98 (m, 2H, NHb,llys, NHa,llys), 7.30-7.23 (m, 6H, H3, Ph, H5, Ph), 7.22-7.14 (m, 9H, H2Ph, H4Ph, H6Ph), 6.72 (t, J = 5.7 Hz, 1H, NHa,llys), 5.15 (t, J = 6.7 Hz, 1H, CHCF3), residual hexafluoroisopropanol, 4.42-4.36 (m, 1H, Ha, Gin), 4.33 (dd, J = 8.6, 3.6 Hz, 1H, Hp, Pro), 4.12-4.01 (m, 1H, Hb, Lys), 3.48-3.38 (m, 1H, Hb, Pro,a), 3.36-3.26 (m, 1H, Hb, Pro,b, overlap with residual water), 2.902.80 (m, 2H, Hc,llys), 2.48-2.41 (m, 1H, Hb, Gin,a), 2.35-2.23 (m, 1H, Hb, Gin,b), 2.02-1.92 (m, 1H, Hb, Pro,a), 1.87-1.69 (m, 8H, Hb, Pro,a, Hb, Gin,a, Hb, Pro, COCH3), 1.69-1.56 (m, 2H, Hb, Gin,b, Hb, Lys,a), 1.56-1.46 (m, 1H, Hc,Lys,b), 1.36 (s, 9H, C(CH3)3), 1.33-1.19 (m, 4H, Hc, Lys, Hc, Lys).13C NMR (151 MHz, DMSO) δ 173.5 (CO2H), 171.6 (COb,Gln, 171.4 (COa,Pro), 170.1 (COa,Gln), 169.0 (COCH3), 155.5 (COC(CH3)3), 144.9 (C1,Ph), 128.5 (C2,Ph, C6,Ph), 127.4 (C3,Ph,
Tert-butyl ((S)-6-(((S)-6-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-1-((4-methyl-2-oxo-2H-chromen-7-yl)amino)-1-oxohexan-2-yl)amino)-5-((S)-1-((N²-acetyl-N²-trityl-L-glutaminy1)pyrrolidine-2-carboxamido)-6-oxohexyl)carbamate (S13). Compound S12 (1.80 g, 2.38 mmol), S4 (1.53 g, 2.40 mmol), HOBt (395 mg, 2.92 mmol) and iPr₂NEt (631 mg, 4.88 mmol) were dissolved in DMF/CH₂Cl₂ (1:2, v/v, ζ 36 mL) and cooled to 0 °C. EDC (560 mg, 2.92 mmol) was added and the reaction mixture was stirred for 5 min at 0 °C and was then stirred for 3 h at ambient temperature. The reaction mixture was diluted with CH₂Cl₂ (300 mL) and washed with brine (300 mL), aq. HCl (0.5 M, 3×250 mL), saturated aq. NaHCO₃ (3×250 mL), and brine (2×250 mL). The organic phase was dried over Na₂SO₄ and concentrated under reduced pressure, affording the desired amide S13 (2.56 g, 85%) as a colorless solid, which was used without further purification. ¹H NMR (600 MHz, DMSO-d₆) δ 10.28 (s, 1H, NHAMC), 8.57 (s, 1H, NHTrt), 8.05 (d, J = 7.7 Hz, 1H, NH₂Gin), 8.00-7.94 (m, 2H, NH₂Lys(Fmoc), NH₂Lys(Boc)), 7.87 (d, J = 7.7 Hz, 2H, H₄Fmoc, H₅Fmoc), 7.77 (d, J = 2.0 Hz, 1H, H₈AMC), 7.70-7.62 (m, 3H, H₅AMC, H₁Fmoc, H₈Boc), 7.52-7.47 (m, 1H, H₆Fmoc), 7.39 (t, J = 7.4 Hz, 2H, H₃Fmoc, H₆Fmoc), 7.35-7.09 (m, 18H, H₂Fmoc, H₇Fmoc, NH₋Lys(Fmoc), H₋Trt), 6.68 (t, J = 5.7 Hz, 1H, NH₋Lys(Boc), 6.24 (d, J = 1.3 Hz, 1H, H₃AMC), 5.75 (s, 1H, residuel CH₃Cl), 4.43-4.36 (m, 1H, H₋Gin), 4.35-4.29 (m, 2H, H₋Pro, H₋Lys(Fmoc)), 4.29-4.20 (m, 2H, CH₂Fmoc), 4.20-4.11 (m, 2H, H₉Fmoc, H₋Lys(Boc)), 3.44-3.38 (m, 1H H₋Pro,A), 3.31-3.25 (m, 1H H₋Pro,B, overlap with residual H₂O), 3.01-2.92 (m, 2H, H₋Lys(Fmoc)), 2.89 (s, 3H, NCH₃, residual DMF), 2.87-2.81 (m, 2H, H₋Lys(Boc)), 2.73 (s, 3H, NCH₃, Residual DMF), 2.48-2.42 (m, 1H, H₋Gin,A), 2.35 (s, 3H, CH₃AMC), 2.32-2.24 (m, 1H, H₋Gin,B), 2.05-1.93 (m, 1H, H₋Pro,A), 1.91-1.11 (m, 29H, H₋Lys(Fmoc), H₋Lys(Boc), H₋Lys(Boc), H₋Pro,B, H₋Pro,A, H₋Pro,B, H₋Pro,A, H₋Lys(Fmoc), H₋Lys(Boc), CH₃-acetyl, C(CH₃)₂Boc), 13C NMR (151 MHz, DMSO) δ 171.8 (CO₋Gin), 171.7 (CO₋Pro), 171.6 (CO₋Lys(Fmoc)), 171.3 (CO₋Lys(Boc)), 170.4 (CO₋Gin), 169.1 (COCH₃), 162.3 (CO₋DMTr), 160.0 (C₋AMC), 156.0 (CO₋Fmoc), 155.5 (CO₋Boc), 153.6 (C₋AMC), 153.0 (C₋AMC), 144.8 (C₋Trt), 144.90 (C₋AMC), 143.86 (C₋AMC), 142.1 (C₋AMC), 140.7 (C₋AMC), 140.7 (C₋AMC), 128.5 (C₋Trt, C₋Trt), 127.5 (C₋Fmoc, C₋Boc), 127.4 (C₋Trt, C₋Trt), 127.0 (C₋Fmoc, C₋Boc), 126.2 (C₋Trt), 125.9 (C₋AMC), 125.09 (C₋Fmoc/C₋Fmoc), 125.07 (C₋Fmoc/C₋Fmoc), 120.1 (C₋Fmoc, C₋Fmoc), 115.2 (C₋AMC), 115.1 (C₋AMC), 112.3 (C₋AMC), 105.6 (C₋AMC), 77.3 (C₋Ph), 69.2 (C₋AMC), 65.2 (CH₂Fmoc), 59.3 (C₋Pro), 54.9 (CH₂Cl₂), 53.7 (CO₋Lys(Fmoc)), 52.7 (CO₋Lys(Boc)), 49.7 (CO₋Gin), 46.7 (C₋Fmoc), 46.6 (C₋Pro), 35.8 (NCH₃, residual DMF), 32.0 (C₋Gin), 31.3 (C₋Lys(Fmoc)), 31.3 (C₋Lys(Boc)), 30.8 (NCH₃, residual DMF), 29.2 (C₋Lys(Fmoc)), 29.1 (C₋Lys(Boc)), 29.0 (C₋Pro), 28.2 (C₋AMC), 27.4 (C₋Gin), 24.3 (C₋Pro), 22.70 (C₋Lys(Fmoc)), 22.67 (C₋Lys(Boc)), 22.3 (CH₃-acetyl), 17.9 (C₋AMC). Two sets of signals (approximately 5:1) were detectable due to rotamers. Only peaks for the major rotamer is given. AMC=7-amino-4-methylcoumarin. UPLC-MS tₑ 1.96 min, m/z 756.4 ([M+H]+, C₄₂H₆₃N₅O₈⁺, Calcd 756.4).
(S)-1-(acetyl-L-glutamyl)-N-((S)-6-amino-1-((S)-6-ethanethioamido-1-(4-methyl-2-oxo-2H-chromen-7-yl)amino)-1-oxohexan-2-yl)amino)-1-oxohexan-2-y]pyrrolidine-2-carboxamide (10). Octanethiol (1.80 mL, 10.4 mmol), DBU (10 mg, 0.06 mmol) and piperidine (205 μL, 2.1 mmol) were added to a solution of S13 (2.56 g, 2.03 mmol) in DMF (37 mL). The reaction mixture was stirred for 3 h at ambient temperature and was then concentrated down to 4 mL under reduced pressure. Ice-cold Et2O was added, forming a colorless precipitate, which was triturated with ice-cold Et2O (3×40 mL) affording a colorless solid (1.90 g, 90%), tentatively assigned as tert-butyl ((S)-5-((S)-1-(N-acetyl-N-trityl-L-glutamyl)pyrrolidine-2-carboxamido)-6-((S)-6-amino-1-((4-methyl-2-oxo-2H-chromen-7-yl)amino)-1-oxohexan-2-yl)amino)-6-oxohexyl)carbamate (UPLC-MS tr 2.01 min, m/z 1041.5; [M+H]+, C83H73NaO10S, Calcd 1041.5), which was used without further purification. To a solution of the colorless solid (38 mg, ~0.04 mmol) in DMF (2.0 mL) was added ethyl dithioacetate (6 mg, 0.05 mmol) and iPr2NEt (7 mg, 0.06 mmol). The reaction mixture was stirred for 45 min at ambient temperature and was then concentrated under reduced pressure. The crude residue was dissolved in CH2Cl2 (1.5 mL), then TFA (1.0 mL) and TIPS (125 μL, 0.6 mmol) was added. The reaction mixture was stirred for 20 min at ambient temperature and was then concentrated under reduced pressure. Preparative reversed-phase HPLC purification of the crude residue afforded the desired thioamide 10 (2 mg, 5% from S13), as a colorless fluffy material after lyophilization. 1H NMR (600 MHz, DMSO-d6) δ 10.37 (s, 1H, NHAMC), 9.96 (t, J = 5.4 Hz, 1H, NH2Lys(thioacetyl)), 8.11-8.04 (m, 3H, NH2Gln, NH2Lys NH2Lys(thioacetyl)), 7.81 (d, J = 2.0 Hz, 1H, H8AMC), 7.75-7.64 (m, 4H, H5AMC, NH3+), 7.48 (dd, J = 8.7, 2.1 Hz, 1H, H6AMC), 7.33 (s, 1H, CONH2Gln,A), 6.81 (s, 1H, CONH2Gln,b), 6.27 (d, J = 1.4 Hz, 1H, H3AMC), 4.46 (td, J = 8.2, 5.9 Hz, 1H, H2,Gln), 4.42-4.32 (m, 2H, H2,Pro, H2,Lys(thioacetyl)), 4.31-4.22 (m, 1H, H2,Lys), 3.71-3.61 (m, 2H, H3,Pro, overlap with residual H2O), 3.49-3.41 (m, 2H, H3,Lys(thioacetyl), overlap with residual H2O), 2.82-2.72 (m, 2H, H2,Lys), 2.40 (d, J = 1.3 Hz, 3H, CH3,AMC), 2.36 (s, 3H, CH3,thioacetyl), 2.19-2.01 (m, 3H, H2,Gln, H3,Pro,A), 1.94-1.47 (m, 16H, H2,Lys(thioacetyl), H2,Lys(thioacetyl), H2,Lys, H2,Lys, H3,Pro,B, H3,Pro, H2,Gln, CH3,acetyl), 1.45-1.27 (m, 4H, H2,Lys, H2,Lys(thioacetyl)). 13C NMR (151 MHz, DMSO) δ 198.8 (C=S), 173.9 (CO2,Gln), 171.9 (CO2,Lys), 171.8 (CO2,Pro), 171.4 (CO2,Lys(thioacetyl)), 170.5 (CO2,Gln), 169.2 (COCH2), 160.0 (C2AMC), 158.1 (q, J = 31.4 Hz, CO2TFA), 153.6 (C8aAMC), 153.1 (C4AMC), 142.1 (C7AMC), 126.0 (C5AMC), 116.6 (q, J = 294.5 Hz, CF3,TFA), 115.3 (C6AMC), 115.2 (C4aAMC), 112.4 (C3AMC), 105.7 (C8AMC), 59.5 (Cα,Pro), 53.7 (Cα,Lys(thioacetyl)), 52.3 (Cα,Lys), 49.9 (Cα,Gln), 46.9 (C5,Pro), 45.2 (C5,Pro), 38.8 (C1,Lys), 32.8 (CH3(thioacetyl), 31.4 (Cβ,Lys(thioacetyl)), 31.1 (Cβ,Lys), 31.0 (Cγ,Gln), 29.1 (C6,Pro), 27.1 (Cβ,Gln), 26.9 (C5,Lys), 26.5 (C6,Lys), 24.5 (C5,Pro), 23.1 (Cγ,Lys(thioacetyl), 22.3 (Cγ,Gln), 22.1 (CH3,acetyl), 18.0 (CH3,AMC). Two sets of signals (approximately 5:1) were detectable due to rotamers. Only peaks for the major rotamer is given. AMC=7-amino-4-methylcoumarin. UPLC-MS tr 1.13 min, m/z 757.1 ([M+H]+, C36H33NaO6S+, Calcd 757.4). HRMS m/z 757.3700 ([M+Na]+, C36H35NaO6S+, Calcd 757.3702).
(S)-1-(acetyl-L-glutaminy1)-N-((S)-6-amino-1-(((S)-1-((4-methyl-2-oxo-2H-chroman-7-yl)amino)-1-oxo-6-propanethioamidohexan-2-yl)amino)-1-oxohexan-2-yl)pyrrolidine-2-carboxamide (11). Pyridine (133 mg, 1.68 mmol) was added to a solution of S9 (50 mg, 0.16 mmol) and 7-amino-4-methylcoumarin (33 mg, 0.19 mmol) in anhydrous THF (3.0 mL) at 0 °C. POCl₃ (89 mg, 0.58 mmol) was added and the reaction mixture was stirred at 0 °C for 1 h. The reaction mixture was poured onto aq. saturated NaHCO₃/ice (25 mL) and extracted with EtOAc (2×25 mL). The combined organic layer was washed with aq. HCl (2×25 mL) and aq. saturated NaHCO₃ (2×25 mL), dried over Na₂SO₄ and concentrated under reduced pressure. The residue was redissolved in CH₂Cl₂ (3.0 mL) and TIPS (0.25 mL, 1.22 mmol) and TFA (2.0 mL) were added. The reaction mixture was stirred at ambient temperature for 25 min and was then concentrated under reduced pressure. Excess TFA was removed by coevaporations: CH₂Cl₂/toluene (1:1, 2×100 mL) affording a colorless solid (66 mg), tentatively assigned as the TFA salt of (S)-2-amino-N-(4-methyl-2-oxo-2H-chromen-7-yl)-6-propanethioamidohexanamide (UPLC-MS tᵣ 1.22 min, m/z 375.7; [M+H]⁺, C₁₉H₂₁N₂O₃S⁺. Calcd 376.2), which was used without further purification. The colorless solid (66 mg, ~0.13 mmol), S12 (122 mg, 0.16 mmol), HOBT (28 mg, 0.20 mmol), and iPr₂NEt (35 mg, 0.27 mmol) were dissolved in CH₂Cl₂ (3.0 mL) and cooled to 0 °C. EDC (39 mg, 0.20 mmol) was added and the reaction mixture was stirred for 5 min at 0 °C and was then stirred overnight at ambient temperature. The reaction mixture was concentrated under reduced pressure and the crude residue was dissolved in EtOAc (50 mL) and washed with aq. KHSO₄ (5%, w/v, 3×30 mL), saturated aq. NaHCO₃ (3×30 mL), and brine (2×30 mL). The organic phase was dried over Na₂SO₄ and concentrated under reduced pressure, affording a colorless solid (144 mg) tentatively assigned as tert-butyl ((S)-5-(((S)-1-((N-5-acetyl-N⁵-trityl-L-glutaminy1)pyrrolidine-2-carboxamido)-6-(((S)-1-((4-methyl-2-oxo-2H-chromen-7-yl)amino)-1-oxo-6-propanethioamidohexan-2-yl)amino)-6-oxoheptyl)carbamate (UPLC-MS tᵣ 2.20 min, m/z 1113.4; [M+H]⁺, C₉₁H₇₇N₃O₁₄S⁺. Calcd 1113.5). The colorless solid (144 mg) was dissolved in CH₂Cl₂ (1.5 mL) and TIPS (125 µL, 0.61 mmol) and TFA (1.0 mL) were added. The reaction mixture was stirred for 30 min at ambient temperature and was then concentrated under reduced pressure. Excess TFA was removed by coevaporations: CH₂Cl₂/toluene (1:1, v/v, 25 mL), MeCN/toluene (1:1, v/v, 25 mL) and MeCN (25 mL). Preparative reversed-phase HPLC purification of the crude residue afforded the desired amide 11 (19 mg, 16% over three steps), as a colorless fluffy material after lyophilization. ¹H NMR (600 MHz, DMSO-d₆) δ 10.37 (s, 1H, NH AMC), 9.88 (t, J = 5.4 Hz, 1H, NH₆-Lys(thiopropionyl)), 8.10-8.04 (m, 3H, NH₆,Gln, NH₆,Lys NH₆,Lys(thiopropionyl)), 7.81 (d, J = 2.0 Hz, 1H, H₈AMC), 7.73 (d, J = 8.7 Hz, 1H, H₅AMC), 7.67 (br s, 3H, NH₃⁺), 7.47 (dd, J = 8.7, 2.1 Hz, 1H, H₆AMC), 7.32 (s, 1H, CONH₂,Gln,A), 6.81 (s, 1H, CONH₂,Gln,B), 6.27 (d, J = 1.4 Hz, 1H, H₃AMC), 4.46 (td, J = 8.2, 5.9 Hz, 1H, H₆,Gln), 4.39-4.32 (m, 2H, H₆,Pro, H₆,Lys(thiopropionyl)), 4.31-4.22 (m, 1H, H₃,Gln), 3.67-3.62 (m, 2H, H₆,Pro, overlap with residual H₂O), 3.52-3.41 (m, 2H, H₆,Lys(thiopropionyl), overlap with residual H₂O), 2.85-2.72 (m, 2H, H₆,Lys), 2.48 (d, J = 7.5 Hz, 2H, CH₂,thiopropionyl), 2.40 (d, 1.1 Hz, 3H, CH₃,AMC), 2.20-1.96 (m, 3H, H₆,Gln, H₆,Pro), 1.94-1.46 (m, 16H, H₆,Lys(thiopropionyl), H₆,Lys(thiopropionyl), H₆,Lys, H₆,Pro,B, H₇,Pro, H₆,Gln, CH₃,acetyl), 1.45-1.27 (m, 4H, H₇,Lys, H₇,Lys(thiopropionyl)), 1.12 (t, J = 7.5 Hz, 3H, CH₃,thiopropionyl). ¹³C NMR (151 MHz, DMSO) δ 204.9 (C=S), 173.9 (CO₅,Gln), 171.8 (CO₄,Lys), 171.7 (CO₄,Pro), 171.3 (CO₃,Lys(thiopropionyl)), 170.5 (CO₂,Gln), 169.1 (COCH₃), 160.0 (C₂AMC), 158.0 (q, J = 32.6 Hz, CO(TFA)), 153.6 (C₈AAMC), 153.1 (C₄AMC), 142.1 (C₇AMC), 126.0 (C₅AMC), 116.7 (q, J = 297.7 Hz, CF₃,TFA), 115.2 (C₆AMC), 115.1 (C₄AAMC), 112.3 (C₃AMC), 105.7 (C₈AMC), 59.4 (C₆,Pro), 53.6 (C₅,Pro), 53.5 (C₄,Pro)
(C2,Lys thiopropionyl), 52.2 (C3,Lys), 49.9 (C3,Gln), 46.9 (C6,Pro), 45.0 (C12,Lys thiopropionyl), 38.8 (CH2 thiopropionyl), 38.1 (C13,Lys), 31.4 (C3,Gln), 31.1 (C7,Lys thiopropionyl), 29.1 (C6,Pro), 27.1 (C3,Gln), 26.9 (C12,Lys thiopropionyl), 26.5 (C8,Lys), 24.5 (C2,Pro), 23.0 (C3,Lys thiopropionyl), 22.3 (C12,Lys), 22.1 (CH3 acetyl), 18.0 (CH3 AMC), 13.9 (CH3 thiopropionyl). Two sets of signals (approximately 5:1) were detectable due to rotamers. Only peaks for the major rotamer is given. UPLC-MS tr 1.25 min, m/z 771.3 ([M+H]+, C39H55N06S+; Calcld 771.4). HRMS m/z 771.3852 ([M+Na]+, C40H57N06S+; Calcld 771.3858). AMC=7-amino-4-methylcoumarin.

(S)-1-(acetyl L-glutaminy])-N-((S)-6-amino-1-((S)-6-butanethioamido-1-((4-methyl-2-oxo-2H-chromen-7-yl)amino)-1-oxohexan-2-yl)pyrrolidine-2-carboxamide (12). Pyridine (31 mg, 0.40 mmol) was added to a solution of S11 (31 mg, 0.09 mmol) and 7-amino-4-methylcoumarin (33 mg, 0.19 mmol) in anhydrous THF (2.0 mL) at 0 °C. POCl3 (89 mg, 0.58 mmol) was added and the reaction mixture was stirred at 0 °C for 1 h. The reaction mixture was poured onto aq. saturated NaHCO3/ice (25 mL) and extracted with EtOAc (2×25 mL). The combined organic layer was washed with 0.07 [M+H]+, C20H26N3O3S+; Calcld 390.2), which was used without further purification. The colorless solid (35 mg, ∼0.07 mmol), S12 (66 mg, 0.09 mmol), HOBt (14 mg, 0.10 mmol), and HOBt (14 mg, 0.10 mmol) were dissolved in CH2Cl2 (3.0 mL) and cooled to 0 °C. EDC (20 mg, 0.10 mmol) was added and the reaction mixture was stirred for 5 min at 0 °C and was then stirred overnight at ambient temperature. The reaction mixture was concentrated under reduced pressure and the crude residue was dissolved in EtOAc (50 mL) and washed with sat. KHSO4 (5%, w/v, 3×30 mL), saturated aq. NaHCO3 (3×30 mL), and brine (2×30 mL). The organic phase was dried over Na2SO4 and concentrated under reduced pressure. The residue was redissolved in CH2Cl2 (3.0 mL) and TFA (2.0 mL) were added. The reaction mixture was stirred at ambient temperature for 25 min and was then concentrated under reduced pressure. Excess TFA was removed by coevaporations with CH2Cl2/toluene (1:1, v/v, 2×100 mL) affording a colorless solid (35 mg), tentatively assigned as the TFA salt of (S)-2-amino-6-butanethioamido-6-[(4-methyl-2-oxo-2H-chromen-7-yl)hexanamide (UPLC-MS tr 1.38 min, m/z 389.8; [M+H]+, C20H26N3O3S+; Calcld 390.2), which was used without further purification. The colorless solid (35 mg, ∼0.07 mmol), S12 (66 mg, 0.09 mmol), HOBt (14 mg, 0.10 mmol), and HOBt (14 mg, 0.10 mmol) were dissolved in CH2Cl2 (3.0 mL) and cooled to 0 °C. EDC (20 mg, 0.10 mmol) was added and the reaction mixture was stirred for 5 min at 0 °C and was then stirred overnight at ambient temperature. The reaction mixture was concentrated under reduced pressure and the crude residue was dissolved in EtOAc (50 mL) and washed with sat. KHSO4 (5%, w/v, 3×30 mL), saturated aq. NaHCO3 (3×30 mL), and brine (2×30 mL). The organic phase was dried over Na2SO4 and concentrated under reduced pressure, affording a colorless solid (82 mg) tentatively assigned as tert-butyl ((S)-5-((S)-1-((Nε-acetylimino)-6-tryl L-glutaminy]-pyrrolidine-2-carboxamido)-6-(((S)-6-butanethioamido-1-((4-methyl-2-oxo-2H-chromen-7-yl)amino)-1-oxohexan-2-yl)amino)-6-oxohexyl)carbamate (UPLC-MS tr 2.25 min, m/z 1127.4; [M+H]+, C52H78N10O16S2+; Calcld 1127.6). The colorless solid (82 mg) was dissolved in CH2Cl2 (1.5 mL) and TIPS (125 μL, 0.61 mmol) and TFA (1.0 mL) were added. The reaction mixture was stirred for 30 min at ambient temperature and was then concentrated under reduced pressure. Excess TFA was removed by coevaporations: CH2Cl2/toluene (1:1, v/v, 25 mL), MeCN/toluene (1:1, v/v, 25 mL) and MeCN (25 mL). Preparative reversed-phase HPLC purification of the crude residue afforded the desired amide 12 (9 mg, 14%), as a colorless fluffy material after lyophilization. 1H NMR (600 MHz, DMSO-d6) δ 10.38 (s, 1H, NHAMC), 9.89 (s, J = 5.4 Hz, 1H, NH2 Lys thiobutyrly), 8.11-8.02 (m, 3H, NH2 Gln, NH2 Lys NH2 Lys thiobutyrly), 7.81 (d, J = 2.0 Hz, 1H, H6 AMC), 7.76-7.65 (m, 4H, H5 AMC, NH3+), 7.48 (d, J = 8.7, 2.0 Hz, 1H, H6 AMC), 7.33 (s, 1H, CONH2 Gin A), 6.81 (s, 1H, CONH2 Gin B), 6.27 (d, J = 1.4 Hz, 1H, H3 AMC), 4.46 (t, J = 8.2, 5.9 Hz, 1H, HA Gin), 4.43-4.30 (m, 2H, HA Pro, HA Lys thiobutyrly), 4.31-4.23 (m, 1H, HA Lys), 3.71-3.59 (m, 2H, HA Pro), 3.53-3.40 (m, 2H, HA Lys thiobutyrly), 2.84-2.71 (m, 1H, H2 Lys thiobutyrly), 1.27-1.08 (m, 18H, 18CH3), 0.86-0.70 (m, 18H, 18CH3).
2H, H₆,Lys), 2.46 (t, J = 7.4 Hz, 2H, CH₂CH₂CH₃), 2.40 (d, J = 1.3 Hz, 3H, CH₃,AMC), 2.21-1.98 (m, 3H, H₀,Gln, H₆,Pro), 1.94-1.45 (m, 18H, H₆,Lys(thiobutyryl), H₁,Lys(thiobutyryl), H₈,Lys, H₉,Lys, H₁₀,Pro,B, H₁₁,Pro, H₁₂,Gln, CH₂,acetyl, CH₂CH₂CH₃), 1.43-1.28 (m, 4H, H₁,Lys, H₁₂,Lys(thiobutyryl)), 0.81 (t, J = 7.4 Hz, 3H, CH₃, thiobutyryl). ¹³C NMR (151 MHz, DMSO) δ 203.4 (C=S), 173.9 (CO₀,Gln), 171.8 (CO₀,Lys), 171.7 (CO₀,Pro), 171.3 (CO₀,Lys(thiobutyryl)), 170.5 (CO₀,Gln), 169.2 (COCH₃), 160.0 (C₂AMC), 158.10 (q, J = 33.0 Hz, CO₇,Pro), 153.6 (C₈AMC), 153.1 (C₄AMC), 142.1 (C₇AMC), 125.9 (C₅AMC), 116.57 (q, J = 295.2 Hz, CF₃,Pro), 115.2 (C₆AMC), 115.1 (C₄aAMC), 112.3 (C₃AMC), 105.7 (C₇AMC), 59.4 (C₀,Pro), 53.7 (C₀,Lys(thiobutyryl)), 52.2 (C₆,Lys), 49.9 (C₀,Gln), 46.92 (CH₂CH₂CH₃), 46.85 (C₅,Pro), 44.9 (C₆,Lys(thiobutyryl)), 38.8 (C₅,Lys), 31.4 (C₇,Gln), 31.1 (C₉,Lys), 31.0 (C₈,Lys(thiobutyryl)), 29.1 (C₅,Pro), 27.1 (C₂,Pro), 26.9 (C₆,Lys(thiobutyryl)), 26.5 (C₉,Lys), 24.5 (C₇,Pro), 23.0 (C₇,Lys(thiobutyryl)), 22.3 (C₄,Pro, CH₂CH₂CH₃), 22.1 (CH₃,acetyl), 18.0 (CH₃,AMC), 13.0 (CH₃, thiobutyryl). Two sets of signals (approximately 5:1) were detectable due to rotamers. Only peaks for the major rotamer is given. AMC=7-amino-4-methylcoumarin. UPLC-MS tᵣ 1.28 min, m/z 785.2 [(M+H)+, C₃₈H₅₇N₅O₉S⁺, Calcd 785.4). HRMS m/z 785.4009 [(M+Na)+, C₃₉H₅₉NaO₁₇S⁺, Calcd 785.415).

Supporting references

NMR spectra of compounds 3-12, oxo-1, TA, TB, and S8-S13.

$^1$H and $^{13}$C spectra of compound 3.
$^1$H and $^{13}$C spectra of compound 4.
$^1$H and $^{13}$C spectra of compound 5
\(^1\)H and \(^{13}\)C spectra of compound 6.
$^1$H and $^{13}$C spectra of compound 7.
$^1$H and $^{13}$C spectra of compound 8.
$^{19}$F spectra of compound 8.
$^1\text{H}$ and $^{13}\text{C}$ spectra of compound 9.
$^{19}\text{F}$ spectra of compound 9.
$^1$H and $^{13}$C spectra of compound 10.
$^1$H and $^{13}$C spectra of compound 11
$^1$H and $^{13}$C spectra of compound 12.
$^1$H and $^{13}$C spectra of compound oxo-1.

[Diagram of the chemical structure and spectra]
$^1$H and $^{13}$C spectra of TA.
$^1$H and $^{13}$C spectra of TB.
\(^1\)H and \(^{13}\)C spectra of compound S8.
$^1$H and $^{13}$C spectra of compound S9.
$^1$H and $^{13}$C spectra of compound S10.
$^1$H and $^{13}$C spectra of compound S11.
$^{1} \text{H} \text{ and } ^{13} \text{C} \text{ spectra of S12.}$
$^1$H and $^{13}$C spectra of S13.