Electronic Supplementary Information (ESI)

Synthesis and Biological Evaluation of Vioprolide B and its Dehydrobutyrine-Glycine Analogue

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Content:

1 Supplementary Figures

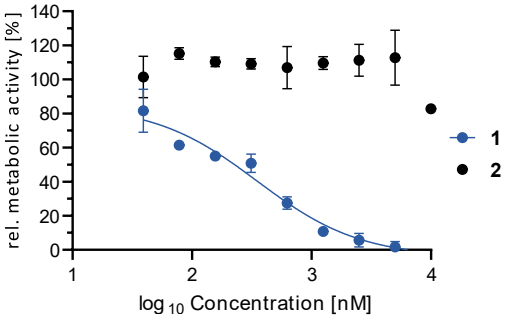


Figure S1: Dose-dependent inhibition of HeLa cell proliferation by compounds **1** (Vioprolide B) and **2** (Dhb-Gly analogue). IC_{50} is determined by MTT assay with 72 h treatment and calculated as 351 nM (78-527 nM 95% confidence interval) for **1** and > 10 µM for **2**. Data points result from four biologically independent experiments performed in three technical replicates.

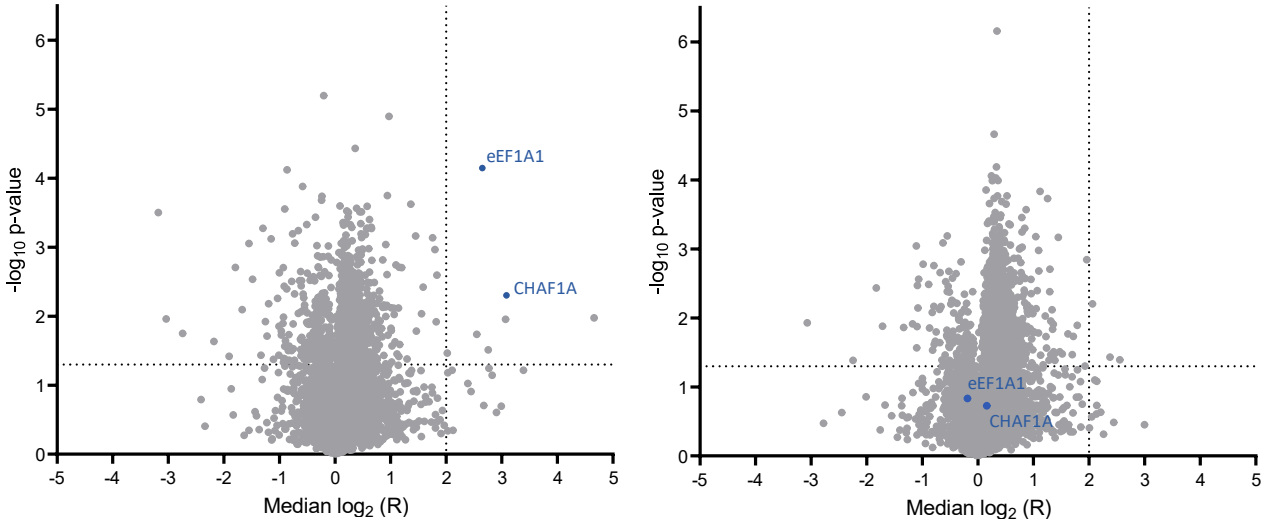


Figure S2: Volcano plots of the competitive isotopically labelled desthiobiotin azide (isoDTB)- activity-based protein profiling (ABPP). In brief, identical sample sets of Jurkat cells were treated *in situ* with compound **1** (shown in **A**) or compound **2** (shown in **B**) respective DMSO as control, lysed, incubated with cysteine-reactive iodoacetamide alkyne (**IAA**) and clicked to isotopically labelled light (compound-treated) respective heavy tags (DMSO-treated). Samples are combined (in **A**: treatment with **1** and DMSO, in **B**: treatment with **2** and DMSO), enriched, digested with trypsin, eluted from streptavidin beads and quantified by LC-MS/MS. The difference in MS1 signal intensity between heavy (compound-treated, control) and light (DMSO-treated) labelled peptides is represented by the competition ratio *R*. The volcano plots represent the median $\log_2(R)$ and the significance ($-\log_{10}(p)$ obtained by a one-sample t-test) for all quantified cysteines. All data result from at least three biologically independent replicates.

2 Biochemical Methods

Cell culture

Jurkat cells were cultured in RPMI 1640 (*Sigma*) containing 10% (v/v) fetal bovine serum (FBS) (*Sigma*), pyruvate and L-glutamine (2 mM final concentration). HeLa cells were grown in Dulbecco's Modified Eagle Medium (DMEM high glucose, 4.5 g/L; *Sigma*), supplemented with 10% (v/v) FBS and L-glutamine (2 mM final concentration). HeLa cells were detached with Accutase (*Thermo Fisher Scientific*). Cell numbers were determined by mixing cell suspension with trypan blue [1:1 (v/v)], followed by counting living cells in a *Neubauer*-improved cell counting chamber. All cells were incubated at 37°C with 5% CO_2 at constant humidity. Pre-warmed sterile phosphate-buffered saline (PBS, 136.9 mM NaCl, 10.1 mM Na_2HPO_4, 2.7 mM KCl, 1.8 mM KH_2PO_4 in ddH_2O, pH 7.4) was used to wash cells between passages.

MTT cytotoxicity assays

HeLa cells were seeded at a density of 4000 cells per well (200 µL) in a transparent, flat-bottomed 96-well plate (*Thermo Fisher Scientific*). Cells were grown overnight in a humidified atmosphere at 37 °C and 5% CO_2 to allow the cells to adhere to the surface. Subsequently, the medium was aspirated and replaced by fresh medium supplemented with compound in concentrations ranging from 39 nM to 10 µM or 0.1% DMSO as a control. The cells were incubated at 37 °C, 5% CO_2 for 72 h. For the determination of metabolic activity, 20 µL 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H- tetrazolium bromide solution (MTT, 5mg/mL in PBS, *Sigma*) was added to each well, and the cells were incubated at 37 °C, 5% CO_2 for 3 h. Thereafter, the medium was aspirated, and the violet formazan crystals were dissolved in 200 µL DMSO per well under shaking (300 r.p.m., 25 min).

Jurkat cell suspension was seeded (16,000 cells/well in 50 µL) in 96-well plates (transparent Nunc round-bottomed, *Thermo Fisher Scientific*). Compound (50 µL/well, 0.1% final DMSO concentration) in varying concentrations or DMSO respectively was added in RPMI-1640 medium containing 10% (v/v) FBS in triplicates and cells incubated (37°C, 5% CO_2, 48 h). Subsequently, cells were supplemented with 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide solution (MTT) (20 µL/well, 5 mg/mL in PBS, *Sigma*) and incubated (37°C, 5% CO_2, 2 h). For cell lysis, triplex lysis buffer (50 µL, 10% (w/v) SDS, 0.012 M HCl,

2% (v/v) isobutanol) was added to each well, pipetted up and down and incubated (37°C, 5% CO_2, o.n.).

The absorbance was measured at 570 nm for both cell lines with a reference wavelength of 630 nm using an Infinite F200 pro plate reader (*Tecan*). Four biological replicates, each consisting of three technical replicates, were measured for each data point. Cell viability was normalised with respect to the DMSO control and fitted as log(inhibitor) vs. response variable slope (four parameters) non-linear regression using *GraphPad* Prism 10.01. Cytotoxicity is reported as the IC_{50} value, the concentration at which 50% viability is reached.

3 Isotopically Labelled Desthiobiotin Azide (isoDTB)-Activity-Based Protein Profiling (ABPP) experiments

The experiment was performed according to a modified literature procedure.[1, 2] IsoDTB tags were synthesised as previously described.[2]

Labelling, Lysis and Click Reaction of Jurkat Cells

For labelling experiments, 6×10^6 Jurkat cells were resuspended in 2 mL RPMI medium (without additives) and treated with Vioprolide B (**1**), Vioprolide Dhb-Gly analogue (**2**), or DMSO (10 µM final compound concentration, 1% final DMSO concentration). After incubation (37 °C, 5% CO_2, 1 h), cells were centrifuged (500 × g, 5 min), medium was aspirated, cells were washed with PBS (1 × 2 mL), and incubated (r. t., 5 min) with pre-cooled lysis buffer (300 µL with 1% NP-40, 1% sodium deoxycholate in 0.1% sodium dodecyl sulfate (SDS) in PBS). After lysis by sonication (5 cycles, 10 s, 50% intensity, Sonopuls HD 2070 ultrasonic rod, *Bandelin electronic GmbH*) with cooling breaks on ice, lysates were clarified by centrifugation (700 × g, 4 °C, 30 min) and supernatants were transferred into fresh tubes. Protein concentration was determined using the ROTI Quant universal Kit (*Carl Roth*) and adjusted to 200 µg proteome in lysis buffer (200 µL final volume). Standardised lysates were incubated (room temperature (r. t.), 1 h) with iodoacetamide alkyne (IAA, 4 µL, 50 mM stock) before being subjected to the copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction. 28 µL click master mix [4 µL TCEP (tris(2-carboxyethyl)phosphine) (52 mM, dd H_2O stock), 12 µL Tris(benzyltriazolylmethyl)amine (TBTA) (1.7 mM, 4:1 tBuOH:DMSO stock), 4 µL $CuSO_4$ (50 mM, dd H_2O stock), 4 µL light or heavy isoDTB-azide (50 mM), 4 µL 10% SDS in PBS] were added and incubated (r. t., 1 h) in the dark. DNAase I (1 µL, *Roche*, 04716728001) was added, and the samples were incubated (37 °C, 30 min).

Mass spectrometry (MS) Sample Preparation

Heavy and light tagged samples were combined (1:1) and subsequently incubated (r.t., 1,000 rpm, 5 min) with magnetic beads (SpeedBead magnetic carboxylate, *Cytiva*, 651 521 050 502 50, 20 µL and 451 521 050 502 50, 20 µL) before ethanol (400 µL) was added. The samples were incubated again (r.t., 1,000 rpm, 5 min). Samples were placed on a magnetic rack, supernatants were removed, beads were washed with 80% ethanol in water (3 × 400 µL), and subsequently resuspended in urea (2 M in 0.5% SDS in PBS). Samples were reduced with

dithiothreitol (200 mM in water, 10 µL), incubated (65 °C, 15 min), alkylated with iodoacetamide (384 mM in water, 10 µL), and incubated (37 °C, 1,000 rpm, 30 min). After the addition of ethanol (400 µL) and followed incubation (r. t., 1,000 rpm, 5 min), samples were placed on a magnetic rack, supernatants were removed, beads were washed with 80% ethanol in water (3 × 400 µL) and resuspended in urea (2 M in 0.5% SDS in PBS). Protein digestion was conducted using trypsin incubation (2 µg, *Promega*, V511C, 37 °C, 200 rpm, overnight).

The next day, the supernatants were transferred into fresh tubes and peptide elution from the beads was conducted with two-fold incubation (37°C, 1,000 rpm, 30 min) with DMSO (2% in water, 50 µL). Supernatants were pooled, pre-washed (PBS) streptavidin agarose beads (*Thermo Scientific*, 10733315, 50 µL beads in 1.2 mL PBS) were added, and the suspension was incubated (r. t., 1 h, continuous rotation). Samples were centrifuged (1,000 × g, 3 min), supernatants were removed by aspiration, and beads were resuspended in PBS (600 µL). Samples were transferred onto centrifuge columns (*Thermo Scientific*, 11894131) and washed over several steps (2 × 600 µL PBS, 3 × 600 µL water, 3 × 600 µL 50% acetonitrile (MeCN) in water). Peptides were eluted with 0.1% trifluoroacetic acid (TFA) in 50% MeCN in water (1 × 200 µL, then 2 × 70 µL) and completed by centrifugation (3,000 × g, 3 min). The solvent was removed with a rotating vacuum concentrator (45 °C). Peptides were dissolved in 0.1% TFA in water (30 µL) under sonication (3 min) and filtered through pre-equilibrated (0.1 % TFA in water) centrifugal filters (*Merck*, UFC30GVNB) at (17,000 × g, 2 min).

Sample Analysis by LC-MS/MS

Peptide quantification was performed using an HPLC-MS/MS system consisting of a Vanquish Neo UHPLC (*Thermo Fisher Scientific*) coupled to an Orbitrap Eclipse Tribrid instrument (*Thermo Fisher Scientific*). The Vanquish Neo UHPLC was equipped with a PepMap™ Neo 5 µm C18 300 µm × 5 mm Trap Cartridge (*Thermo Fisher Scientific*) and operated in a Trap-and-Elute-Injection mode whereby the samples were loaded to the cartridge before the separation started with a flow rate of 400 nL/min using buffer A (0.1% formic acid (FA) in water) and buffer B (0.1% FA in acetonitrile). Peptide separation is achieved using an Aurora UltimateTM separation column (3rd generation, 25 cm, nanoflow UHPLC compatible, *ionoptics*) at 40 °C coupled to a Nanospray Flex Ion Source (*Thermo Fisher Scientific*). The HPLC method comprises 75 min and starts with a gradient from 5% to 40% buffer B over a period of 60 min, followed by a second gradient up to 60% buffer B within 5 min and an isocratic period of 10 min at 90% buffer B. Separation column washing and equilibration are conducted with enabled fast equilibration, equilibration factor “3” at 5% buffer B. Trap column washing and equilibration

are conducted with enabled fast wash and equilibration together with zebra wash (2 wash cycles, automatic equilibration factor). The Orbitrap Eclipse mass spectrometer was run with an internal real-time mass calibration using user-defined lock mass (positive, m/z = 445.12003) and operated in data-dependent acquisition mode. The full MS scans were collected in the orbitrap at a resolution of 120,000 and an AGC target of 4e5 with automated maximum injection time in a scan range of 300-1500 m/z. For MS2 scans, the TOP10 intense ions with a charge state of 2-7 were selected with a minimum intensity threshold of 5e3 and enabled isotope exclusion and dynamic exclusion for 30 s whereby peaks with charge state 1 or unassigned charge state were excluded. MS2 spectra were collected at a resolution of 15,000 and an AGC target of 5e4 with automated maximum injection time. Isolation in the quadrupole was conducted using a window of 1.6 m/z. Higher-energy collision-induced dissociation (HCD) with normalised collision energy of 30% was used to generate the fragments which were detected in the orbitrap. Data acquisition was performed using *Thermo Scientific* Foundation software version 3.1sp9 and Xcalibur version 4.6.

Data Analysis

Acquired raw files were converted into mzML format using the MSConvert tool (version: 3.0.21193-ccb3e0136) of the ProteoWizard software[3] (version: 3.0.21193 64bit). Standard settings were used with vendor´s peak picking enabled. Further analysis was performed with FragPipe interface (version: 20.0) with MSFragger (version 3.8)[4-10], Philosopher (version: 5.0.0)[11], IonQuant (version 1.9.8)[12] and Python (version 3.7.3). Every experiment (Scheme 3B, Figure S2A, Figure S2B) was analysed in a different run. The global settings were set to "0"(=auto) RAM and "4" for parallelism. A "closed search" was conducted with the following settings: The FASTA database for homo sapiens (proteome identifier UP0 000 056 40) was downloaded on the 9th of February 2023 from uniport.org[13] whereas reverse sequences were added manually. The precursor mass tolerance was set from "-20" to "20" ppm, with a fragment mass tolerance of "20" ppm. The calibration and optimisation were set to "mass calibration and parameter optimisation" and the isotope error to "0/1/2". For protein digestion, an "enzymatic" cleavage was entered with enabled clip N-term and "trypsin" as enzyme name and the trypsin cleavage rules (cut after "KR", but no cleavage after "P" and "1" missing cleavage, sense "C"). Peptide length was given with 6 to 50 in a peptide mass range of 500-5,000 Da. Variable modifications were given with a maximum of "3" per peptide and a maximum of "5,000" combinations and all modifications were enabled in the first search. The following settings were used as mass delta at the respective site: 15.9949 (M), 42.0106 ([^), 561.3387 (C), 567.3462

(C). The fixed modification is enabled for all amino acids with 0.0, except for cysteine (57.02146). Mass offsets and labile modifications were disabled and all other settings for MSFragger were kept as default. Run validation tools were activated and Cristal-C was disabled. The following settings for Rescoring using deep learning prediction were chosen: MSBooster, prediction of RT and spectra prediction was enabled whereas correlated features were not activated. PSM validation was set to percolator with a min probability of "0.5" and "--only psms --no terminate --post-processing-tdc" as command line options. ProteinProphet was enabled with "--maxppmdiff 2000000" and FDR filter and reports are activated to generate reports using "--sequential --prot 0.01" as filter. Other settings for the FDR filter, PTM-Shepherd and O-Pair were disabled. For MS1Quantification, Run MS1 quant, IonQuant and MaxLFQ were enabled with MaxLFQ min ions set to "2". The masses for labelling were given with C561.3387 for light and C567.3462 for heavy. Re-quantification was activated, match between runs (MBR) and intensity normalisation across the runs were not enabled. Feature detection and peak tracing were conducted with min. scans of "3" and a min. isotopes of "2", using a tolerance of 10 ppm for m/z, 0.4 min for RT and 0.05 $1/k_D$ for IM. The other settings for MS1 quantification were kept as default. Run TMT- Integrator for isobaric labelling-based Quantification and spectral library generation was disabled.

For downstream data analysis, the "ion_label_quant.tsv" files of the individual replicates were analysed separately. The "Modified peptide" was generated as either the "Light Modified Peptide" or the "Heavy Modified Peptide" based on the entry with the higher Intensity, for each entry. The masses of probe modification in the "Modified Peptide" were replaced by an "*" and the mass of carbamidomethylation ([57.0215]) in this entry was deleted, if present. The full protein sequence was linked into the table. Based on this information, all peptide sequences that do not occur exactly once in the protein were excluded and the residue number of the modified residue was determined. The "identifier" was generated in the format "UniProtCode"_C_"residue number", where C is the one letter code of the modified amino acid cysteine. For each "identifier", the median "Log2 ratio HL", which is the log2 transformed ratio of the heavy and light channels, was determined as median of the "Log2 ratio HL" column of all corresponding ions and renamed "Log2R_replicate name". If several different "Modified peptides" were detected for the same "Identifier", the "Modified Peptide" with the shortest sequence was kept. For all identifiers, the data for all replicates was now combined into one table. If different "Modified peptides" were detected for the same "Identifier" in the different replicates, the "Modified Peptide" with the shortest sequence was kept. The average of the Log2R values of the individual replicates was calculated and named "Log2R_Average" and

only kept, if it was quantified in at least 2 out of 4 replicates. Biological replicates which showed dominantly no content (NaN) were considered to be empty due to handling error and excluded for further evaluation.

Statistical analysis was performed according to a published procedure[2] using the Perseus[14] software (version 1.6.80). The data for each replicate of the same condition were loaded into Perseus before the rows were filtered based on at least two valid values. A two-sided one-sample t-test was conducted against a value of $\log_2 (R) = 0$. The median values of $\log_2 (R)$ and $- \log_{10} (p)$ derived from Perseus were used for the visualisation in a volcano plot. Proteins were called a "hit" if the median ratio $\log_2 (R)$ was above 2 and the statistical significance p was smaller than 0.05. The UniProt[13] identifier is linked to the data based on peptide sequence.

4 Equipment and general synthetic methods

All reactions sensitive to air or moisture were conducted in flame-dried glassware under a positive pressure of argon using standard Schlenk techniques. Dry tetrahydrofuran (THF) and dichloromethane (CH_2Cl_2) were obtained from an *M. Braun* MB-SPS 800 solvent purification system. Other dry solvents were acquired in the highest purity available and used without further purification.

Flash **chromatography** was conducted on silica 60 (*Merck*, Grain size: 40-63 µm, Pore size: 60 Å) using the specified eluent mixtures. For automated flash chromatography, a *Büchi* C-815 Flashmaster with pre-packed *Biotage* columns (*Biotage* Sfär Silica D Duo 60 µm; size as specified in protocol) was employed. Thin layer chromatography (TLC) was performed on silica-coated glass plates (*Merck*, silica 60 F_{254}) with detection by UV (λ = 254 and 366 nm) or by staining with a potassium permanganate ($KMnO_4$) or ninhydrin (Nin) solution followed by heat treatment.

IR spectra were recorded on a Perkin Elmer Frontier FT/IR (ATR), with signal intensities designated as s (strong), m (medium), w (weak).

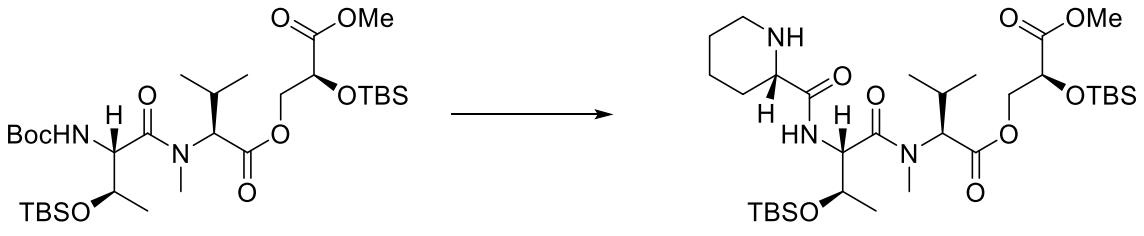
^{1}H- and ^{13}C-NMR spectra were recorded on a *Bruker* AVHD-400, AVHD-500, or an AVHD-500cr spectrometer. Deuterated chloroform, MeOH, and DMSO were obtained from *Deutero* and used as solvents. The specific residual proton signal served as internal standard for spectrum calibration. Apparent multiplets resulting from accidental equality of coupling constants to those of magnetically non-equivalent protons are denoted as virtual (*virt.*). The relative configuration of products and the multiplicity of the ^{13}C-NMR signals were determined by two-dimensional NMR spectra (COSY, HSQC, HMBC, NOESY). If relevant, the ratio of the rotamers is specified, and the distinguishable signals are labeled (R1, R2). If the signals of a pair of rotamers overlap, the labeling is omitted. The determination of specific optical rotation values was carried out using an ADP440+ polarimeter (*Bellingham+Stanley*) at room temperature and a wavelength of 589 nm (Na-D-line).

High-resolution mass spectroscopy (HRMS) was performed on a *Thermo Fischer Scientific* LTQ Orbitrap XL (ESI).

A Melting Point M-565 instrument from *Büchi* was used for the determination of **melting points**.

5 Synthetic procedures and NMR spectra

_N_-Boc-L-Pip-L-Thr-(_N_-Me)L-Val-L-Gls(_O_-TBS)-_O_-Me (5)



3 **5**

$C_{32}H_{63}N_3O_8Si_2$
M = 674.04 g/mol

Boc-Deprotection:

A solution of peptolide **3** (500 mg, 754 µmol, 1.00 equiv.) in CH_2Cl_2 (2.6 mL) cooled to 0 °C is added dropwise with TFA (0.65 mL). After stirring for three hours at this temperature, CH_2Cl_2 (15 mL) is added, and the solvent is removed under reduced pressure at room temperature. The resulting crude product is dried by azeotropic distillation (PhMe, 2 × 15 mL) and used in the following reaction without further purification

Peptide coupling:

Boc-L-Pip (303 mg, 1.32 mmol, 1.75 equiv.) and HOAt (185 mg, 1.36 mmol, 1.80 equiv.) are dissolved in CH_2Cl_2 (2.5 mL) and added at 0 °C to EDC·HCl (260 mg, 1.36 mmol, 1.80 equiv.) and DIPEA (128 µL, 97.5 mg, 754 µmol, 1.00 equiv.). Subsequently, the crude product of the deprotected peptoid is dissolved in CH_2Cl_2 (6.6 mL) and slowly added dropwise to the reaction mixture. After the reaction is stirred and warmed to room temperature over 16 hours, it is quenched by the addition of water (10 mL) and CH_2Cl_2 (30 mL). The phases are separated, and the organic phase is washed with 10% citric acid (20 mL), $NaHCO_3$ (20 mL), and NaCl solution (15 mL). Then, the organic phase is dried over Na_2SO_4 and filtered, before the solvent is removed under reduced pressure. After the crude product is purified by column chromatography (silica gel: Ø 3 cm, ↕ 20 cm, n-pentane/EtOAc = 6/1 → 5/1 → 4/1), peptolide **4** (312 mg, 404 µmol, 54% over 2 steps) is obtained as a yellow oil.

Boc-Deprotection:

To a solution of the peptolide **4** (3.07 g, 3.97 mmol, 1.00 equiv.) in CH_2Cl_2 (70 mL) at 0 °C, 2,6-lutidine (6.91 mL, 6.37 g, 59.5 mmol, 15.0 equiv.) and TMSOTf (7.88 mL, 9.70 g, 43.6 mmol, 11.0 equiv.) are added dropwise successively, and the reaction mixture is stirred at this temperature for seven hours. After this time, the reaction is quenched by the addition of $NaHCO_3$ solution (100 mL) and the phases are separated. The aqueous phase is extracted with EtOAc (3 × 50 mL), and the combined organic phases are dried over Na_2SO_4 and filtered. The solvent is removed under reduced pressure, and the residue is purified by column chromatography (silica gel: Ø 5 cm, ↕ 20 cm, CH_2Cl_2/MeOH = 100/1 → 50/1 → 25/1). The Amine **5** (2.38 g, 3.52 mmol, 89%) is obtained as a colorless oil

TLC: R_f = 0.15 (CH_2Cl_2/MeOH = 20/1) [$KMnO_4$, Nin: purple].

Rotamer ratio: R1/R2 ≈ 95/5.

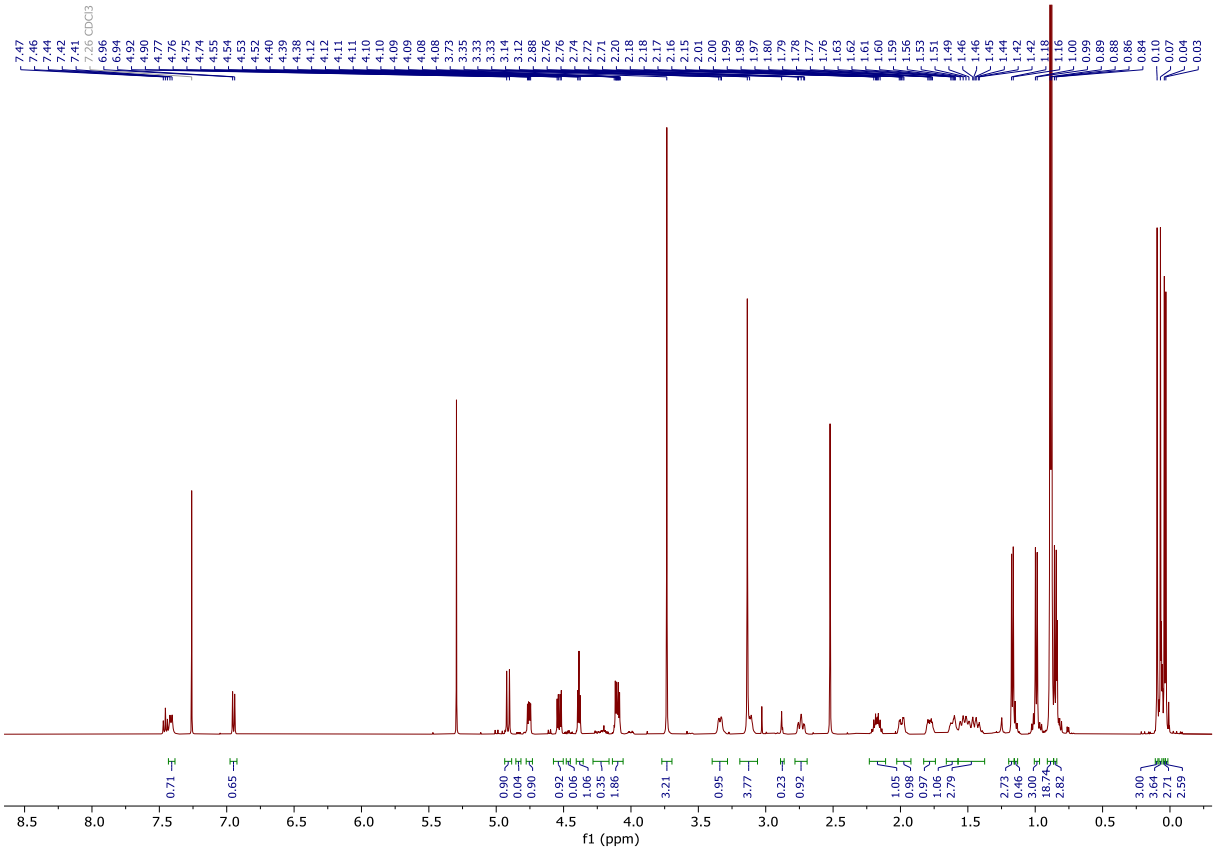
^{1}H-NMR (500 MHz, $CDCl_3$, 298 K): δ (ppm) = 7.41 (d, ^3J = 7.8 Hz, 1H, Pip NH), 6.95 (d, ^3J = 7.6 Hz, 1H, Thr NH), 4.92 (d, ^3J = 10.2 Hz, 1H, Val NMeC*H* R1), 4.78 (dd, ^3J = 9.2 Hz, ^3J = 3.2 Hz, 1H, Thr C*H*NH R2), 4.74 (dd, ^3J = 7.9 Hz, ^3J = 4.1 Hz, 1H, Thr C*H*NH R1), 4.53 (dd, ^3J = 11.1 Hz, ^3J = 4.5 Hz, 1H, Gls CH*H* R1), 4.46 – 4.43 (m, 1H, Gls CH*H* R2) 4.39 (*virt*. t, $^3J \approx {}^3J$ = 4.6 Hz, 1H, Gls CH), 4.30 – 4.16 (m, 3H, Val NMeC*H* R2, Thr C*H*CH_3 R2, Gls C*H*H R2), 4.13 – 4.06 (m, 2H, Gls C*H*H R1, Thr C*H*CH_3 R1), 3.74 (s, 3H, CO_2CH_3), 3.26 (dd, ^3J = 9.9 Hz, ^3J = 3.3 Hz, 1H, Pip CH), 3.14 (s, 3H, Val NCH_3 R1), 3.06 (*virt*. dt, ^2J = 12.1 Hz, $^3J \approx {}^3J$ = 4.0 Hz, 1H, Pip NCH*H*), 2.88 (s, 3H, NCH_3 R2), 2.72 – 2.67 (m, 1H, Pip NC*H*H), 2.34 – 2.23 [m, 1H, C*H*$(CH_3)_2$], 2.17 [*virt*. dsept, ^3J = 10.2 Hz, $^3J \approx {}^3J$ = 6.6 Hz, 1H, C*H*$(CH_3)_2$], 2.00 – 1.90 (m, 1H, Pip CHCH*H*), 1.82 – 1.72 (m, 1H, Pip $CHCH_2$C*H*H), 1.61 – 1.53 (m, 1H, Pip NCH_2CH*H*), 1.52 – 1.35 (m, 3H, Pip NCH_2C*H*H, Pip CHC*H*HC*H*H), 1.17 (d, ^3J = 6.2 Hz, 3H, Thr CH_3 R1), 1.14 (d, ^3J = 6.3 Hz, 3H, Thr CH_3 R2), 1.02 (d, ^3J = 6.1 Hz, 3H, Val CH_3 R2), 0.99 (d, ^3J = 6.6 Hz, 3H, Val CH_3 R1), 0.96 (d, ^3J = 6.6 Hz, 3H, Val CH_3 R2), 0.94 – 0.89 [m, 9H, $SiC(CH_3)_3$]$^{\lozenge}$, 0.90 – 0.88 [m, 9H, $SiC(CH_3)_3$]$^{\lozenge}$, 0.85 (d, ^3J = 6.8 Hz, 3H, Val CH_3 R1), 0.10 (s, 3H, $SiCH_3$), 0.07 (s, 3H, $SiCH_3$), 0.05 (s, 3H, $SiCH_3$), 0.04 (s, 3H, $SiCH_3$).

$^{\lozenge}$Signals overlap.

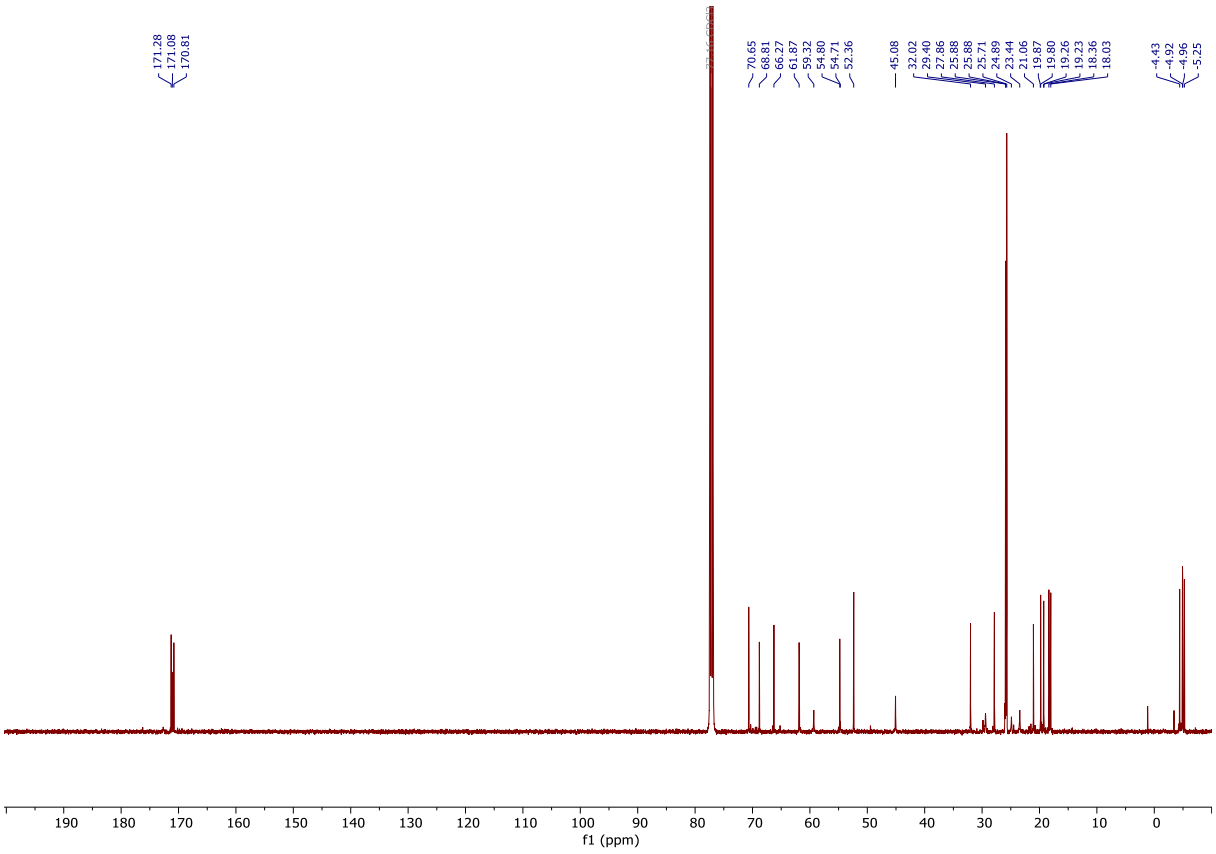
^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 171.3 (s, Thr CO)$^{\blacklozenge}$, 171.1 (s, CO_2CH_3)$^{\blacklozenge}$, 171.1 (s, Pip CO)$^{\blacklozenge}$, 170.8 (s, Val CO)$^{\blacklozenge}$, 70.7 (d, Gls CH), 68.8 (d, Thr *C*HOTBS), 66.3 (t, Gls CH_2), 61.9 (d, Val NMe*C*H), 59.3 (d, Pip CH), 54.8 (d, Thr CHNH), 52.4 (q, CO_2CH_3), 45.1 (t, Pip NCH_2), 32.0 (q, Val NCH_3 R1), 29.4 (q, Val NCH_3 R2), 27.9 [d, Val *C*H$(CH_3)_2$ R1], 25.9 [q, SiC(*C*H$_3$)$_3$], 25.9 (t, Pip NCH*C*H$_2$), 25.7 [q, SiC(*C*H$_3$)$_3$], 24.9 (t, Pip CHCH$_2$*C*H$_2$), 23.4 (q, Thr CH_3), 21.1 (t, Pip NCH$_2$*C*H$_2$), 19.7 (q, CHCH$_3$*C*H$_3$), 19.3 (q, CH*C*H$_3$CH$_3$), 18.4 [s, Si*C*$(CH_3)_3$], 18.0 [s, Si*C*$(CH_3)_3$], −4.4 (q, $SiCH_3$), −4.9 (q, $SiCH_3$), −5.0 (q, $SiCH_3$), −5.3 (q, $SiCH_3$).

$^{\blacklozenge}$Assignment interchangeable.

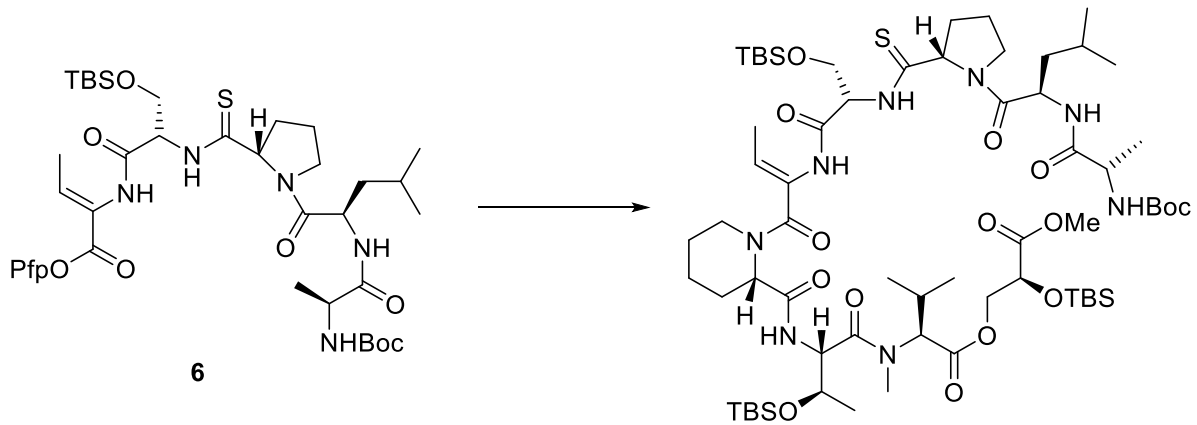
^{1}H-NMR:



^{13}C-NMR:



***N*-Boc-L-Ala-D-Leu-L-ThioPro-L-Ser(*O*-TBS)-(*Z*)-Dhb-Pro-L-Thr(*O*-TBS)-(*N*-Me)L-Val-L-Gls(*O*-TBS)-*O*-Me (*Z*-7)**



6

***Z*-7**

$C_{64}H_{118}N_8O_{15}SSi_3$

M = 1356.00 g/mol

To a solution of pentafluorophenyl ester **6** (412 mg, 469 µmol, 1.00 equiv.) in THF (3 mL), a solution of peptide **5** (347 mg, 515 µmol, 1.10 equiv.) in THF (3 mL) is added, and the reaction mixture is stirred at room temperature for 15 minutes. Subsequently, it is heated to 50 °C and stirred at this temperature for 18 hours. After cooling the reaction solution to room temperature, the solvent is removed under reduced pressure, and the residue is purified by column chromatography (silica gel: Ø 3 cm, ↕ 20 cm, n-pentane/EtOAc = 5/1 → 4/1 → 3/1 → 2/1). This process yields nonapeptide *Z*-**7** (302 mg, 222 µmol, 48%) as a colorless solid.

TLC: R_f = 0.62 (*n*-Pentan/EtOAc = 2/1) [UV, $KMnO_4$].

M.p.: 86 °C.

Specific rotation: $[\alpha]_D^{RT} = -170.0$ (c = 10.0, $CHCl_3$).

IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3283 (m, OH), 2957 (m, -(CH_2)−H), 2931 (m, -(CH_2)−H), 1744 (s, C=O), 1651 (s, C=O), 1530 (s), 1442 (m), 1389 (s, -(CH)−H), 1365 (m), 1254 (m), 1160 (m, C=O), 1104 (m), 1006 (m), 838 (m), 779 (m).

Rotamer ratio: R1/R2 ≈ 71/29.

^{1}H-NMR (500 MHz, $CDCl_3$, 298 K): δ (ppm) = 8.85 (d, ^3J = 7.6 Hz, 1H, Ser NH), 8.26 (s, 1H, Dhb NH R1), 8.13 – 8.01 (m, 3H, Leu NH R2, Dhb NH R2, Thr NH R2), 7.84 (d, ^3J = 7.2 Hz, 1H, Thr NH R1), 7.70 (d, ^3J = 8.5 Hz, 1H, Leu NH R1), 7.53 (d, ^3J = 8.4 Hz, 1H, Ala NH R1), 6.98 (d, ^3J = 8.4 Hz, 1H, Ala NH R2), 5.54 (q, ^3J = 6.9 Hz, 1H, Dhb CH R2), 5.32 – 5.12 (m, 3H, Dhb CH R1, Ser CH, Pip CH R2), 5.07 – 4.85 (m, 4H, Val CH R1, ThioPro CH, Pip CH R1, Thr C*H*NH R2), 4.71 – 3.87 (m, 11H, Thr C*H*NH R1, Leu CH, Ala CH, Pip NCH*H*, ThioPro NCH*H*, Ser CH*H*, Gls CH, Gls CH_2, Thr C*H*OH, Val CH R2), 3.77 – 3.66 (m, 4H, CO_2CH_3, Ser C*H*H R1), 3.65 – 3.51 (m, 2H, ThioPro NC*H*H, Ser C*H*H R2), 3.19 – 3.06 (m, 4H, Val CH_3 R1, Pip NC*H*H), 2.89 (s, 3H, Val CH_3 R2), 2.55 – 2.36 (m, 3H, ThioPro CHCH*H*, Pip CHCH*H*), 2.32 – 1.94 [m, ThioPro CHC*H*H, Pip CHC*H*H, ThioPro NCH_2CH_2, Val C*H*(CH_3)$_2$], 1.83 (d, ^3J = 6.9 Hz, 3H, Dhb CH_3 R1), 1.78 – 1.65 [m, 5H, Dhb CH_3 R2, Pip NCH_2CHH, Leu C*H*(CH_3)$_2$ R2], 1.65 – 1.51 [m, 3H, Leu C*H*(CH_3)$_2$, Pip NCH_2CHH, Pip $CHCH_2CHH$], 1.49 – 1.32 [m, 12H, OC(CH_3)$_3$, Leu CH_2, Pip $CHCH_2CHH$], 1.31 – 1.11 (m, 9H, Ala CH_3, Thr CH_3, Val CH_3), 1.04 – 0.73 [m, 36H, Val CH_3, SiC(CH_3)$_3$, Leu (CH_3)$_2$], 0.12 – 0.03 (m, 18H, $SiCH_3$).

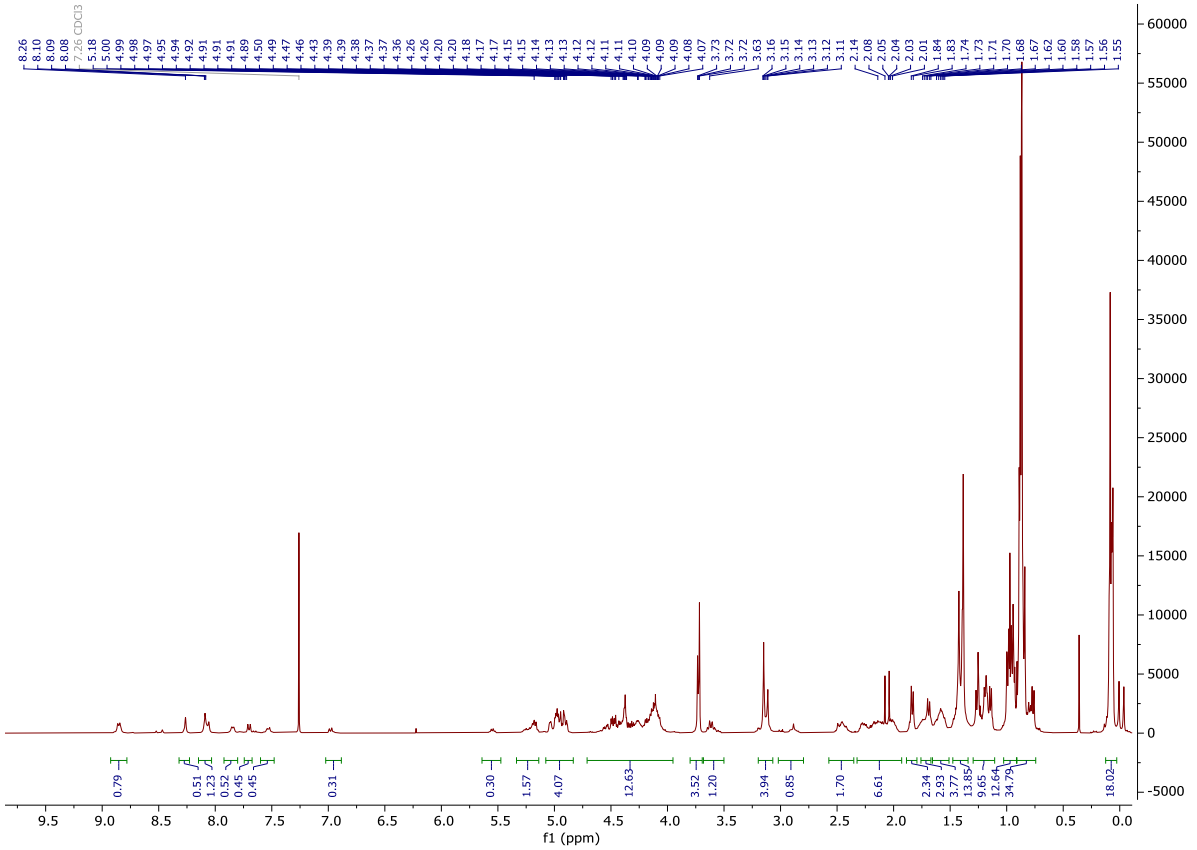
^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 204.0 (s, CS R2), 203.9 (s, CS R1), 174.8 (s, Ala CO R2), 174.6 (s, Ala CO R1), 173.4 (s, Leu CO R2), 173.3 (s, Leu CO R1), 172.5 (s, Val CO R1), 172.2 (s, Val CO R2), 171.8 (s, Gls CO R2), 171.3 (s, Gls CO R1), 170.7 (s, Thr CO R2)♦, 170.5 (s, Thr CO R1)♣, 170.1 (s, Pip CO R2)♦, 169.9 (s, Pip CO R1)♣, 169.8 (s, Ser CO R2)♦, 169.7 (s, Ser CO R1)♣, 169.5 (s, Dhb CO R1)♣, 169.4 (s, Dhb CO R2)♦, 157.3 (s, $NHCO_2$ R2), 157.2 (s, $NHCO_2$ R1), 129.6 (s, Dhb *C*CH R2), 128.3 (s, Dhb *C*CH R1), 119.6 (d, Dhb CH R1), 117.0 (d, Dhb CH R2), 79.4 [s, O*C*$(CH_3)_3$ R1], 79.2 [s, O*C*$(CH_3)_3$ R2], 70.6 (d, Gls CH R1), 70.0 (d, ThioPro CH R1), 69.6 (d, Gls CH R2), 69.3 (d, ThioPro CH), 67.5 (d, Thr CHOH), 67.0 (d, Thr CHOH), 66.3 (t, Gls CH_2 R1), 65.3 (t, Gls CH_2 R2), 62.1 (d, Val NMe*C*H R1), 61.9 (d, Val NMe*C*H R2), 61.4 (t, Ser CH_2 R2), 61.2 (d, Ser CH_2 R1), 61.1 (t, Ser CH R1), 60.7 (d, Ser CH R2), 56.3 (d, Thr CHNH R1), 55.5 (d, Thr CHNH R2), 53.6 (d, Pip CH R2), 53.5 (d, Pip CH R1), 52.7 (q, CO_2CH_3 R2), 52.4 (q, CO_2CH_3 R1), 50.1 (d, Leu CHNH R2), 49.8 (d, Leu CHNH R1), 49.5 (d, Ala CH R1), 49.3 (d, Ala CH R2), 48.5 (t, ThioPro NCH_2 R1), 47.9 (t, ThioPro NCH_2 R2), 47.5 (t, Pip NCH_2 R1), 47.1 (t, Pip NCH_2 R2), 40.6 (t, Leu CH_2 R1), 40.4 (t, Leu CH_2 R2), 33.2 (t, ThioPro CH*C*H_2 R2), 33.1 (t, ThioPro CH*C*H_2 R1), 32.1 (q, Val NCH_3 R1), 29.8 (q, Val NCH_3 R2), 28.4 [q, OC(*C*$H_3)_3$], 27.7 [d, Val *C*H$(CH_3)_2$ R1], 26.1 [d, Val *C*H$(CH_3)_2$ R2], 25.9 (t, Pip CHCH_2*C*H_2 R2), 25.8 [q, SiC(*C*$H_3)_3$], 25.7 (t, Pip CHCH_2*C*H_2 R1), 25.3 (t, Pip CH*C*H_2), 25.0 [d, Leu *C*H$(CH_3)_2$ R2], 24.8 [d, Leu *C*H$(CH_3)_2$ R1]♠, 24.7 (t, ThioPro NCH_2*C*H_2)♠, 23.5 (q, Leu CH_3), 22.0 (q, Leu CH_3), 21.3 (t, Pip NCH_2*C*H_2), 20.1 (q, Thr CH_3 R2), 19.9 (q, Thr CH_3 R1), 19.6 (q, Val CH_3 R2), 19.1 [s, Si*C*$(CH_3)_3$], 18.5 (q, Val CH_3 R2), 18.3 (q, Val CH_3 R1), 18.1 [s, Si*C*$(CH_3)_3$], 15.7 (q, Ala CH_3 R1), 15.4 (q, Ala CH_3 R2), 12.2 (q, Dhb CH_3 R1), 12.0 (q, Dhb CH_3 R2), −3.5 [q, Si$(CH_3)_2$], −5.0 [q, Si$(CH_3)_2$], −5.3 [q, Si$(CH_3)_2$].

♦/♣/♠ Assignment interchangeable.

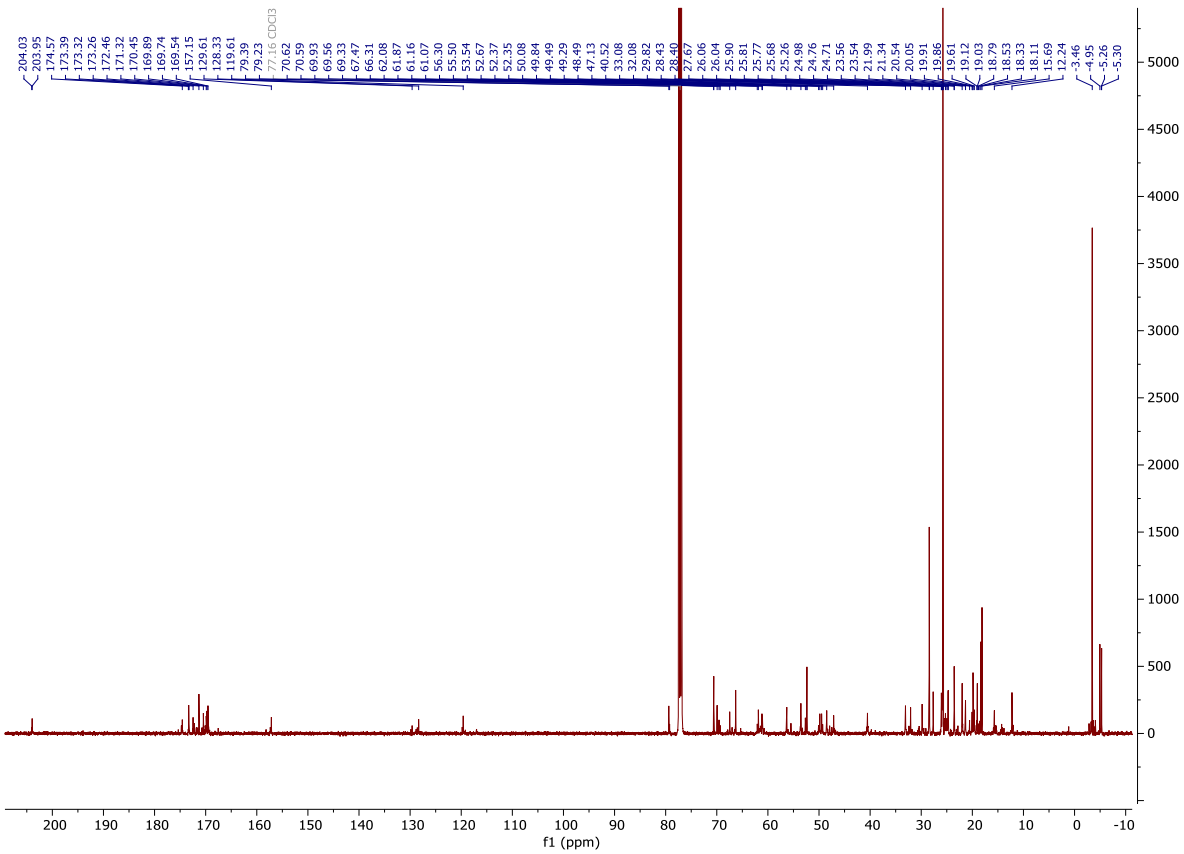
HR-MS (ESI): $[C_{64}H_{118}N_8O_{15}SSi_3+H]^+$ calculated: 1355.7818; found: 1355.7795.

$[C_{64}H_{118}N_8O_{15}SSi_3+Na]^+$ calculated: 1377.7637; found: 1377.7615.

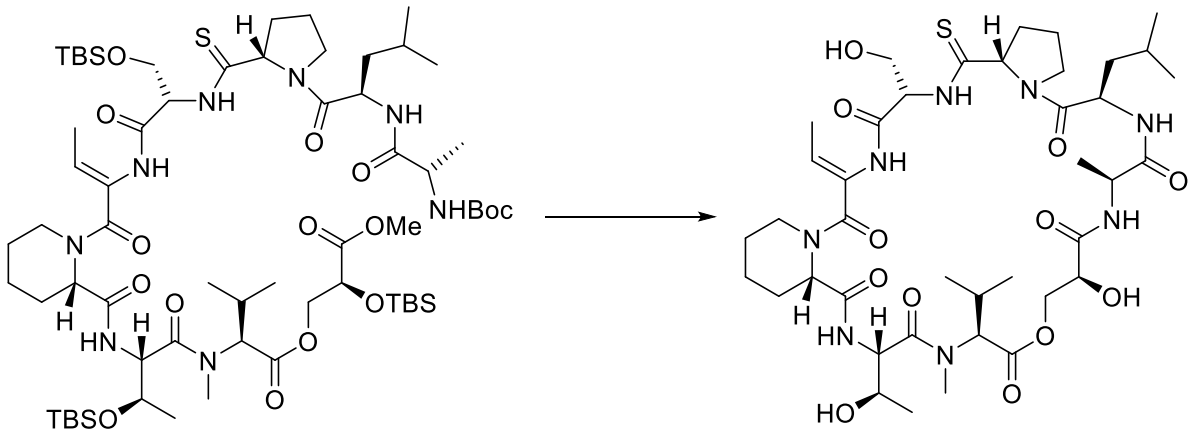
^{1}H-NMR:



^{13}C-NMR:



(*Z*)-Macrocycle (*Z*-8)



Z-7 *Z*-8

$C_{40}H_{64}N_8O_{12}S$

M = 881.06 g/mol

Ester Hydrolysis:

A solution of nonapeptide *Z*-**7** (335 mg, 247 µmol, 1.00 equiv.) in DCE (28 mL) is treated with Me_3SnOH (357 mg, 1.98 mmol, 8.00 equiv.) and the reaction mixture is stirred at 80 °C for 48 hours. Subsequently, the mixture is cooled to room temperature, and a 5% citric acid solution (15 mL) is added. The emulsion is stirred for 10 minutes, the resulting phases are separated, and the aqueous phase is extracted with EtOAc (2 × 25 mL). The combined organic phases are washed with 5% citric acid solution (2 × 20 mL) and NaCl solution (20 mL), dried over Na_2SO_4, and filtered. The residue is purified by column chromatography (silica gel: Ø 2 cm, ↕ 18 cm, CH_2Cl_2/MeOH/AcOH = 100/1/0.01 → 20/1/0.01) to remove residual tin reagent, yielding a colorless solid (190 mg).

Boc Deprotection:

The obtained solid is dissolved in CH_2Cl_2 (9 mL) and cooled to 0 °C, then treated with TFA (2.4 mL) and stirred at this temperature for 90 minutes. After adding water (10 mL), the aqueous phase is adjusted to pH = 7 using 1 M sodium hydroxide and $NaHCO_3$ solution and extracted with EtOAc (2 × 40 mL). The combined organic phases are washed with NaCl solution (30 mL), dried over Na_2SO_4, and filtered. The solvent is removed under reduced pressure, and the residue is dried by azeotropic distillation (PhMe, 2 × 15 mL). The resulting yellowish solid is used in the next reaction without further purification.

Macrolactamization:

A solution of the yellowish solid in CH_2Cl_2 (5 mL) is slowly added over four hours to a solution of HATU (127 mg, 387 µmol, 1.57 equiv.) and 2,4,6-collidine (82.1 µL, 75.1 mg, 620 µmol, 2.51 equiv.) in CH_2Cl_2 (55 mL). The reaction mixture is stirred for 16 hours, and then water (25 mL) is added. The phases are separated, the aqueous phase is saturated with solid NaCl, and extracted with CH_2Cl_2 (2 × 25 mL). The combined organic phases are washed with 10% citric acid (25 mL), $NaHCO_3$ (25 mL), and NaCl solution, and the washing solutions are back-extracted with CH_2Cl_2 (10 mL each). The combined organic phases are dried over Na_2SO_4, filtered, and the solvent is removed under reduced pressure. The crude product is dissolved in dry MeCN (10 mL) and filtered through Celite to remove inorganic salts. After washing the filter cake several times with MeCN, the solvent is removed under reduced pressure. The resulting yellowish resin (172 mg) is used in the next reaction without further purification.

TBS Deprotection:

A solution of the yellowish resin in dry MeCN (21 mL) is cautiously treated with HF (48% in water, 1.09 mL) and stirred at room temperature for 24 hours. The reaction is quenched by adding Na2CO3 solution (20 mL), and the aqueous phase is extracted with EtOAc (3 × 15 mL). The combined organic phases are washed with NaCl solution (20 mL), dried over Na_2SO_4, and filtered. Before completely removing the solvent, Celite is added. The Celite loaded with the crude product is purified by column chromatography (silica gel: Ø 2 cm, ↕ 17 cm, CH_2Cl_2/MeOH = 75/1 → 50/1 → 40/1 → 30/1 → 20/1 → 10/1). Approximately 94 mg of a colorless solid are obtained, which is still impure. This is further purified by semi-preparative HPLC (*Phenomenex* Luna 5u Silica, 20 × 250 mm, ^{i}PrOH/*n*-Heptane = 20/80, r.t., 15 mL·min^{-1}, 215 nm, t_R (*Z*-**8**) = 12.5 min, Injection concentration: 94 mg mL^{-1}). After lyophilization (distilled water), the macrocycle *Z*-**8** (55.5 mg, 63.0 µmol, 26% over 4 steps) is obtained as an amorphous, colorless solid.

TLC: R_f = 0.48 (CH_2Cl_2/MeOH = 9/1) [UV, $KMnO_4$].

M.p.: 176 °C.

Specific rotation: $[\alpha]_D^{RT} = -182.0$ (c = 10.0, $CHCl_3$).

IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3292 (m, OH), 2961 (m, -(CH_2)−H), 1747 (s, C=O), 1639 (s, C=O), 1524 (s), 1441 (m), 1261 (m), 1192 (m), 1158 (m, C=O), 1065 (m), 753 (m), 665 (m).

Rotamer ratio: R1/R2 ≈ 90/10.

^{1}H-NMR (500 MHz, $CDCl_3$, 298 K): δ (ppm) = 8.64 (s, 2H, Dhb NH R1, Ser NH R2), 8.58 (s, 1H, Dhb NH R2), 8.46 (d, ^{3}J = 8.4 Hz, 1H, Ser NH R1), 7.77 (d, ^{3}J = 10.0 Hz, 1H, Thr NH R2), 7.64 (d, ^{3}J = 9.3 Hz, 1H, Ala NH R2), 7.57 (d, ^{3}J = 8.9 Hz, 2H, Ala NH R1, Leu NH R1), 7.48 (d, ^{3}J = 8.5 Hz, 1H, Thr C*H*NH R1), 6.85 (s, 1H, Leu NH R2), 5.54 (q, ^{3}J = 7.0 Hz, 1H, Dhb CH R1), 5.40 – 5.35 (m, 1H, Ser CH R2), 5.27 (q, ^{3}J = 7.0 Hz, 1H, Dhb CH R2), 5.23 – 5.19 (m, 1H, Pip CH R2), 5.18 – 5.12 (m, 1H, Ser CH R1), 5.04 – 5.00 (m, 1H, Pip CH R1), 4.99 – 4.94 (m, 2H, ThioPro CH, Thr CH R2), 4.90 – 4.81 (m, 3H, Leu CH, Gls CH*H* R2, Gls C*H*H R2), 4.75 – 4.59 (m, 3H, Thr C*H*NH R1, Ala CH, Gls CH*H* R1), 4.53 – 4.46 (m, 1H, Gls CH R2), 4.43 – 4.35 (m, 2H, Ser CH*H*, Gls CH R1), 4.33 – 4.25 (m, 2H, Pip NCH*H*, Gls C*H*H R1), 4.20 (d, ^{3}J = 6.8 Hz, 1H, Val NMeC*H* R2), 4.13 – 4.02 (m, 2H, Val NMeC*H* R1, ThioPro NCH*H* R1), 4.02 – 3.92 (m, 2H, ThioPro NCH*H* R2, Thr C*H*OH), 3.71 (dt, ^{2}J = 10.4 Hz, ^{3}J = 7.2 Hz, 1H, ThioPro NC*H*H), 3.62 – 3.55 (m, 1H, Ser CH*H*), 3.17 (s, 3H, Val CH_3 R1), 3.08 – 2.99 (m, 1H, Pip NC*H*H), 2.78 (s, 3H, Val CH_3 R2), 2.51 – 2.33 [m, 3H, ThioPro CHCH*H*, Val C*H*$(CH_3)_2$ R1, Pip CHCH*H*], 2.29 – 2.19 [m, 2H, ThioPro NCHCH*H*, Val C*H*$(CH_3)_2$ R2], 2.08 – 1.98 (m, 1H, Pip CHC*H*H), 1.82 (d, ^{3}J = 7.0 Hz, 3H, Dhb CH_3), 1.79 – 1.66 (m, 4H, Pip NCH_2CH_2, ThioPro NCH_2CH_2), 1.65 – 1.55 [m, 2H, Leu CH*H*, Leu C*H*$(CH_3)_2$], 1.53 (d, ^{3}J = 7.0 Hz, 3H, Ala CH_3), 1.51 – 1.41 (m, 3H, Leu C*H*H, Pip $CHCH_2CH_2$), 1.37 (d, ^{3}J = 6.9 Hz, 3H, Ala CH_3 R2), 1.31 (d, ^{3}J = 6.4 Hz, 3H, Thr CH_3 R1), 1.15 (d, ^{3}J = 6.3 Hz, 3H, Thr CH_3 R2), 1.08 – 1.04 (m, 3H, Val CH_3 R2), 1.01 (d, ^{3}J = 6.5 Hz, 3H, Val CH_3 R1), 0.92 [m, 9H, Val CH_3, Leu CH(C*H*$_3)_2$].

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 204.1 (s, CS R2), 203.4 (s, CS R1), 173.8 (s, Ala CO R1), 173.3 (s, Ala CO R2), 173.0 (s, Thr CO R2), 172.9 (s, Leu CO R2), 172.6 (s, Leu CO R1), 171.4 (s, Gls CO R2), 171.2 (s, Thr CO R1) ♦, 171.1 (s, Val CO R1) ♦, 171.1 (s, Pip CO)♦, 170.8 (s, Gls CO R1), 170.6 (s, Ser CO R1), 169.6 (s, Ser CO R2), 169.4 (s, Val CO R2), 169.1 (s, Dhb CO R2), 168.4 (s, Dhb CO R1), 129.1 (s, Dhb *C*CH R1), 128.4 (s, Dhb *C*CH R2), 121.6 (d, Dhb CH R1), 117.4 (d, Dhb CH R2), 70.9 (d, Gls CH R1), 70.2 (d, Gls CH R2), 69.6 (d, ThioPro CH), 68.2 (d, Val NMe*C*H R2), 66.2 (d, Thr CHOH), 66.2 (d, Val NMe*C*H R1), 65.9 (t, Gls CH_2 R1), 65.7 (t, Gls CH_2 R2), 61.0 (t, Ser CH_2 R2), 60.1 (d, Ser CH R2), 60.0 (t, Ser CH_2 R1), 59.8 (d, Ser CH R1), 55.6 (d, Thr CHNH R1), 53.9 (d, Pip CH R1), 53.8 (d, Pip CH R2), 52.1 (d, Thr CHNH R2), 50.3 (d, Leu CHNH R2), 49.5 (d, Leu CHNH R1), 48.6 (t, ThioPro NCH_2), 48.4 (d, Ala CH R1), 48.0 (d, Ala CH R2), 47.3 (t, Pip NCH_2 R1), 46.7 (t, Pip NCH_2 R2), 40.6 (t, Leu CH_2 R1), 40.2 (t, Leu CH_2 R2), 34.4 (q, Val NCH_3 R1), 33.0 (t, ThioPro

CH*C*H$_2$ R2), 32.4 (t, ThioPro CH*C*H$_2$ R1), 29.6 (q, Val NCH$_3$ R2), 27.0 [d, Val *C*H(CH$_3$)$_2$ R2], 26.7 [d, Val *C*H(CH$_3$)$_2$ R1], 25.4 (t, ThioPro NCH$_2$*C*H$_2$ R2), 25.3 (t, ThioPro NCH$_2$*C*H$_2$ R1)♣, 25.1 (t, Pip CHCH$_2$*C*H$_2$)♣, 24.8 [d, Leu *C*H(CH$_3$)$_2$]♠, 24.7 (t, Pip CH*C*H$_2$ R1)♠, 24.5 (t, Pip CH*C*H$_2$ R2), 23.2 (q, Leu CH$_3$ R2), 22.9 (q, Leu CH$_3$ R1), 22.6 (q, Leu CH$_3$ R1), 22.1 (q, Leu CH$_3$ R2), 21.4 (t, Pip NCH$_2$*C*H$_2$ R2), 21.2 (t, Pip NCH$_2$*C*H$_2$ R1), 20.5 (q, Val CH$_3$ R1), 20.5 (q, Thr CH$_3$ R1), 19.8 (q, Thr CH$_3$ R2), 19.7 (q, Val CH$_3$ R2), 19.5 (q, Ala CH$_3$), 19.0 (q, Val CH$_3$), 18.8 (q, Ala CH$_3$ R2), 18.3 (q, Val CH$_3$ R2), 12.4 (q, Dhb CH$_3$ R1), 12.0 (q, Dhb CH$_3$ R2).

♦/♣/♠ Assignment interchangeable.

HR-MS (ESI): [C$_{40}$H$_{64}$N$_8$O$_{12}$S+H]$^+$ calculated: 881.4437; found: 881.4420.

[C$_{40}$H$_{64}$N$_8$O$_{12}$S+Na]$^+$ calculated: 903.4256; found: 903.4237.

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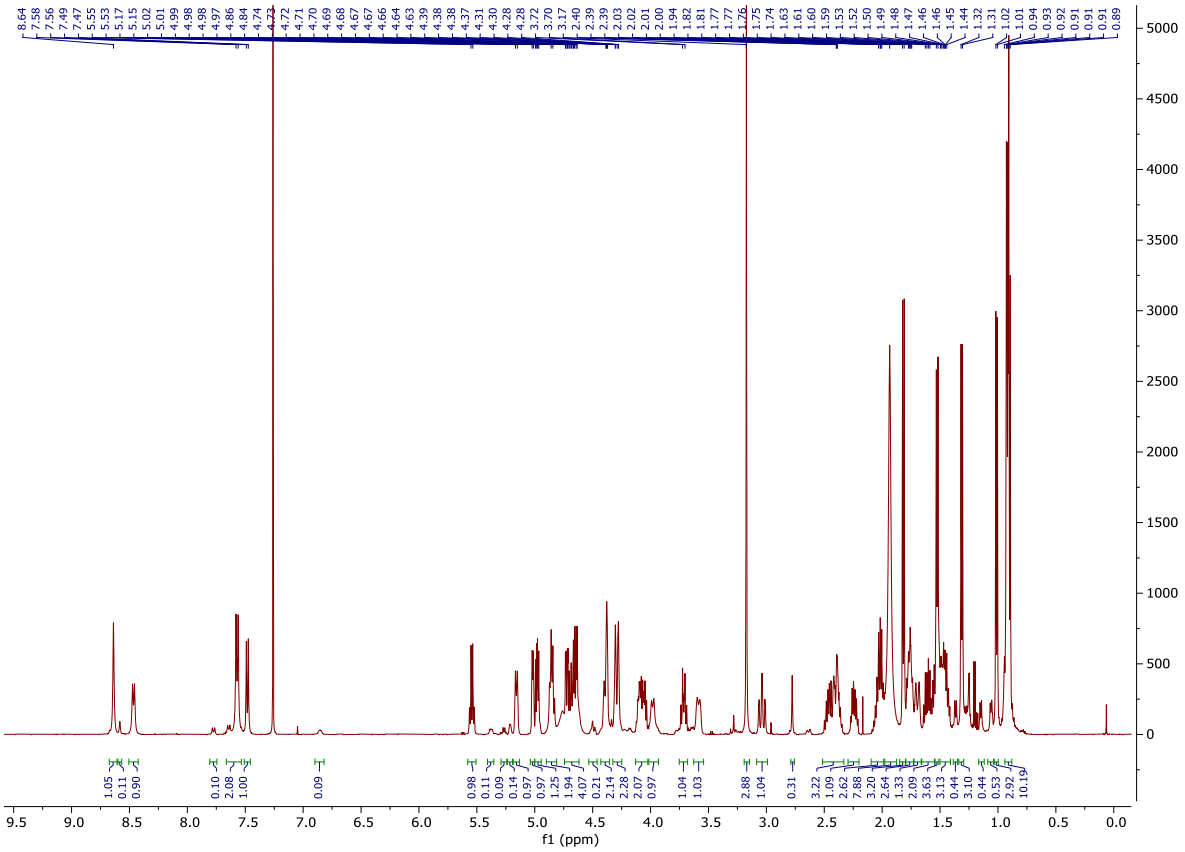
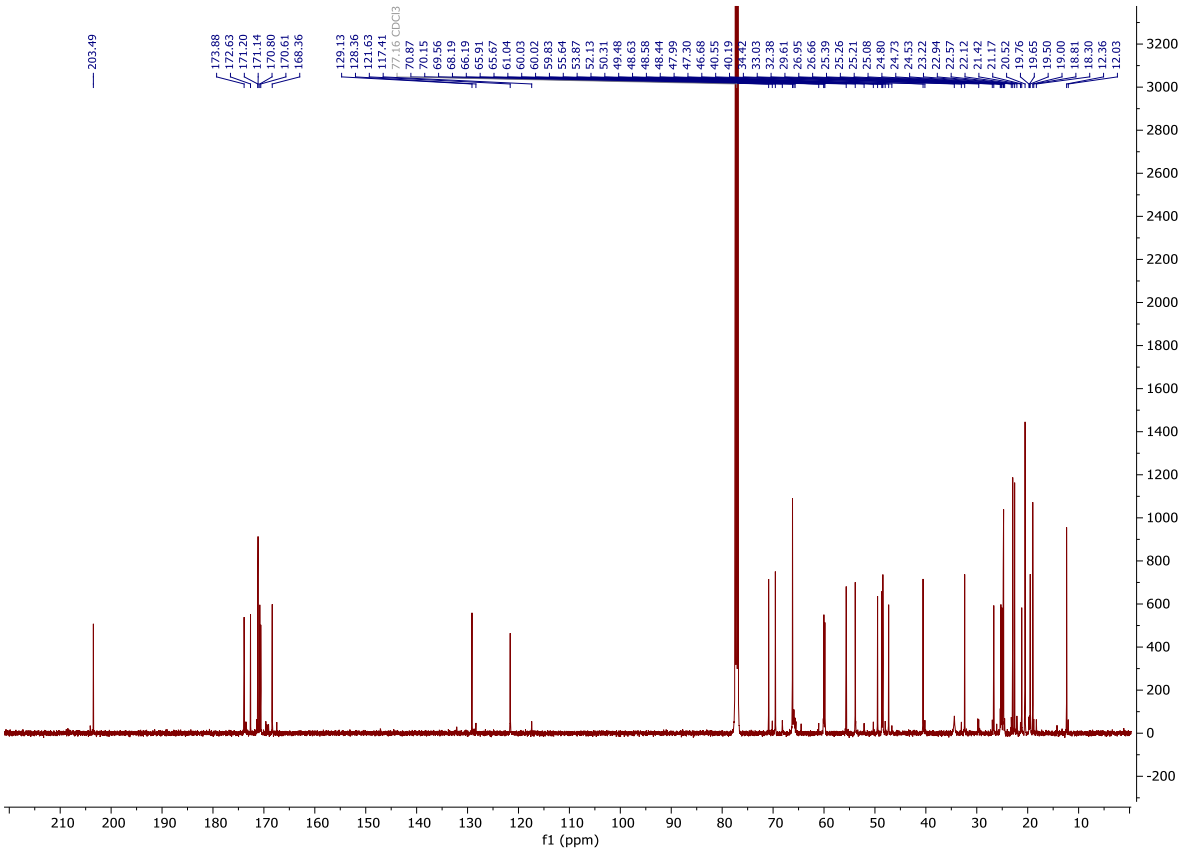
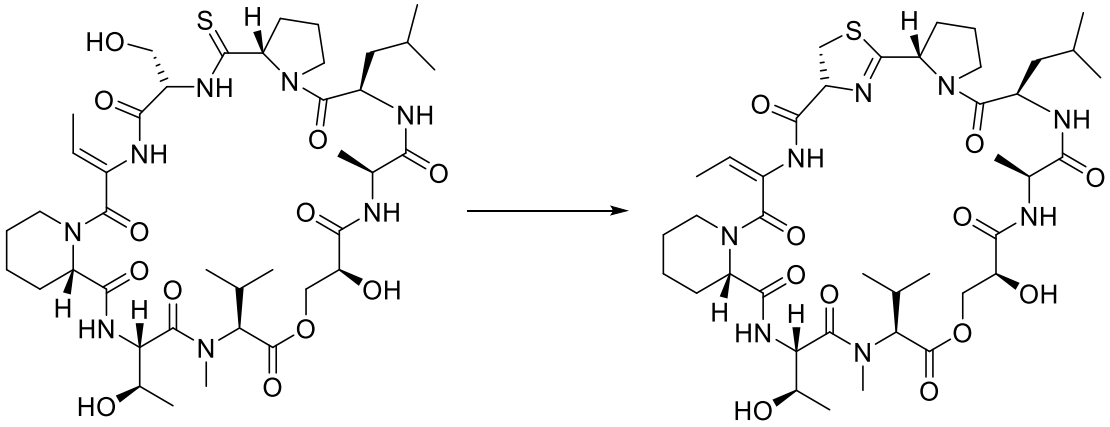


^{13}C-NMR:



Vioprolide B (1)



Z-**8**

1

$C_{40}H_{62}N_8O_{11}S$

M = 863.04 g/mol

Cyclization:

A solution of macrocycle *Z*-**8** (25 mg, 28.4 µmol, 1.00 equiv.) in THF (2.2 mL) is placed in a Schlenk flask containing PPh_3 (11.2 mg, 42.6 µmol, 1.50 equiv.). To this solution, a solution of DIAD (8.36 µL, 8.61 mg, 42.6 µmol, 1.50 equiv.) in THF (0.6 mL) is slowly added dropwise, and the resulting yellow solution is stirred at room temperature for 22 hours. Subsequently, a small amount of silica gel is added to the reaction solution, and the solvent is removed under reduced pressure. The silica gel loaded with the crude product is purified by column chromatography (silica gel: Ø 1 cm, ↕ 14 cm, CH_2Cl_2/MeOH = 30/1 → 20/1 → 10/1), yielding approximately 28 mg of a colorless solid, still contaminated with residues of starting materials and reagents. This is further purified by semi-preparative HPLC (*Phenomenex* Luna 5u Silica, 20 × 250 mm, ^{i}PrOH/*n*-Heptane = 50/50, r.t., 15 mL·min^{-1}, 215 nm, t_R = 10.1 min, Injection concentration: 28 mg·mL^{-1}). After lyophilization (distilled water), (*Z*)-vioprolide B (15.5 mg, 18.0 µmol, 63%) is obtained as an amorphous, colorless solid.

TLC: R_f = 0.42 (CH_2Cl_2/MeOH = 9/1) [UV, $KMnO_4$].

Iodination:

A solution of (*Z*)-vioprolide B (9.00 mg, 10.4 µmol, 1.00 equiv.) in CH_2Cl_2 (0.3 mL) is treated with NIS (2.23 mg, 9.91 µmol, 0.95 equiv.) and DABCO (1.29 mg, 11.5 µmol, 1.10 equiv.), and the yellow reaction solution is stirred for five hours under light exclusion. Subsequently, it is diluted with EtOAc (30 mL). It is sequentially washed with 10% citric acid (15 mL), $NaHCO_3$ (15 mL), 1 M $Na_2S_2O_3$ (15 mL), and NaCl solution (20 mL). Each washing solution is saturated with solid NaCl before use and back-extracted with EtOAc (10 mL each) after use. The combined organic phases are dried over Na_2SO_4, filtered, and the solvent is removed under reduced pressure. The residue is purified by a quick column chromatography (silica gel: Ø 1 cm, ↕ 8 cm, EtOAc → CH_2Cl_2/MeOH = 20/1 → 10/1). This yields a colorless solid (14 mg), which is used in the next reaction without further purification.

TLC: R_f = 0.51 (CH_2Cl_2/MeOH = 9/1) [UV, $KMnO_4$].

Hydrodeiodination:

To a suspension of palladium on activated carbon (5 wt% Pd, 11.5 mg) in MeOH (0.9 mL), NEt_3 (1.73 µL, 1.27 mg, 12.5 µmol, 1.20 equiv.) is added. After exchanging the argon atmosphere with a hydrogen atmosphere according to the general procedure, the suspension is stirred at room temperature for 45 minutes. The iodinated crude product is dissolved in MeOH (0.9 mL) and also placed under a hydrogen atmosphere following the general procedure. This solution is added to the suspension and stirred for three hours under exclusion of light. The reaction is terminated by removing the hydrogen balloon and purging the reaction solution with argon for five minutes. After filtering the suspension over Celite and washing the filter cake with EtOAc (20 mL), the combined filtrate is washed with 1 M $Na_2S_2O_3$ solution (15 mL). The washing solution is saturated with solid NaCl before use and back-extracted with EtOAc (3 × 15 mL) after use. Subsequently, the combined organic phases are washed with NaCl solution (25 mL), dried over Na_2SO_4, filtered, and the solvent is removed under reduced pressure. The residue is purified by column chromatography (silica gel: Ø 1 cm, ↕ 9 cm, CH_2Cl_2/MeOH = 50/1 → 25/1 → 10/1), yielding approximately 8 mg of a colorless solid, still contaminated with residues of starting materials and reagents. This is further purified by semi-preparative HPLC (*Phenomenex* Luna 5u Silica, 20 × 250 mm, ^{i}PrOH/*n*-Heptane = 20/80, r.t., 15 mL·min^{-1}, 215 nm, t_R (**1**) = 12.5 min, Injection concentration: 8 mg·mL^{-1}). After lyophilization (distilled water), Vioprolide B (**1**, 3.15 mg, 3.65 µmol, 35%) is obtained as an amorphous, colorless solid.

TLC: R_f = 0.45 (CH_2Cl_2/MeOH = 9/1) [UV, $KMnO_4$].

M.p.: 126 °C.

Specific rotation: $[\alpha]_D^{RT} = -42.0$ (c = 10.0, $CHCl_3$).

IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3292 (m, OH), 2992 (m, -(CH_2)−H), 2928 (m, -(CH_2)−H), 1742 (s, C=O), 1643 (s, C=O), 1517 (s), 1445 (m), 1376 (s, -(CH)−H), 1261 (m), 1196 (m), 1164 (m, C=O), 1021 (m), 797 (m).

Rotamer ratio: R1/R2 ≈ 57/43.

^{1}H-NMR (500 MHz, $CDCl_3$, 292 K): δ (ppm) = 9.32 (s, 1H, Dhb NH R2), 8.77 (s, 1H, Dhb NH R1), 8.35 (d, ^3J = 9.9 Hz, 1H, Thr NH R1), 7.78 (d, ^3J = 7.8 Hz, 1H, Thr NH R2), 7.28 − 7.22 (m, 1H, Ala NH R2)*, 7.08 (d, ^3J = 9.8 Hz, Ala NH R1), 6.68 (d, ^3J = 10.0 Hz, Leu

NH R2), 6.24 (d, ^3J = 9.9 Hz, Leu NH R1), 5.69 (q, ^3J = 7.2 Hz, 1H, Dhb CH R2), 5.57 (d, ^3J = 7.2 Hz, 1H, Dhb CH R1), 5.24 (d, ^3J = 5.2 Hz, 1H, Pip CH R1), 5.21 – 5.17 (m, 1H, Pip CH R2), 5.11 (dd, ^3J = 8.5 Hz, ^3J = 5.4 Hz, 1H, ThioPro CH R2), 5.09 – 5.04 (m, 1H, Thz CH), 4.99 (dd, ^3J = 8.4 Hz, ^3J = 5.0 Hz, 1H, ThioPro CH R1), 4.95 (d, ^3J = 10.1 Hz, 1H, Thr C*H*NH R1)$^{\diamond}$, 5.03 – 4.91 (m, 1H, Leu CH R2)$^{\diamond}$, 4.91 – 4.85 (m, 1H, Leu CH R1), 4.81 (d, ^3J = 10.8 Hz, 1H, Gls CH*H* R2), 4.79 – 4.73 (m, 1H, Ala CH R2), 4.72 – 4.67 (m, 2H, Ala CH R1, Thr C*H*NH R2), 4.65 – 4.55 (m, 2H, Gls CH R1, Gls C*H*H R1), 4.41 – 4.36 (m, 1H, Gls CH R2), 4.28 (d, ^3J = 10.6 Hz, 1H, Val NMeC*H* R1), 4.24 – 4.13 (m, 2H, Thr C*H*OH, Gls CH*H* R1), 4.10 – 4.01 (m, 2H, Gls C*H*H R2, Pip NCH*H*), 3.88 – 3.77 (m, 2H, ThioPro NCH*H* R1, Thz CH*H* R2), 3.72 – 3.62 (m, 1H, ThioPro NC*H*H), 3.60 – 3.56 (m, 2H, Thz CH*H* R1, Thz C*H*H R1), 3.56 – 3.53 (m, 1H, ThioPro NCH*H* R2), 3.52 (s, 3H, Val NCH_3 R1), 3.49 – 3.46 (m, 1H, Thz C*H*H R2), 3.40 – 3.32 (m, 1H, Pip NC*H*H R1), 3.29 – 3.23 (m, 1H, Val NMeC*H* R2), 3.18 – 3.09 (m, 1H, Pip NC*H*H R2), 2.78 – 2.70 (m, 4H, Val NCH_3 R2, Pip CHC*H*H R1), 2.60 – 2.51 [m, 2H, Pip CHC*H*H R2, Val C*H*$(CH_3)_2$ R1], 2.40 – 2.29 (m, 1H, ThioPro CHC*H*H), 2.27 – 2.17 [m, 2H, Val C*H*$(CH_3)_2$ R2, ThioPro $CHCH_2CHH$], 2.17 – 2.10 (m, 1H, ThioPro CHCH*H* R1), 2.09 – 1.95 (m, 2H, ThioPro CHCH*H* R2, ThioPro $CHCH_2CHH$), 1.87 – 1.71 (m, 5H , Dhb CH_3, Pip $CHCH_2CHH$, Pip NCH_2CHH R1)$^{\blacklozenge}$, 1.72 – 1.66 (m, 1H, Pip NCH_2CHH R2)$^{\blacklozenge}$, 1.57 – 1.48 [m, 5H, Pip $CHCH_2CHH$, Pip NCH_2CHH, Pip CHCH*H*, Leu CH*H*, Leu C*H*$(CH_3)_2$], 1.46 – 1.37 (m, 1H, Leu C*H*H R1)$^{\diamond}$, 1.40 (d, ^3J = 6.8 Hz, 3H, Ala CH_3 R2)$^{\diamond}$, 1.33 (d, ^3J = 7.1 Hz, 3H, Ala CH_3 R1), 1.30 – 1.19 (m, 4H, Thr CH_3 R2, Leu C*H*H R2), 1.17 – 1.11 (m, 6H, Val $CHCH_3CH_3$ R1, Thr CH_3 R1), 1.06 (d, ^3J = 6.5 Hz, 3H, Val $CHCH_3CH_3$ R2), 0.98 – 0.80 [m, 9H, Val $CHCH_3CH_3$, Leu $CH(CH_3)_2$].

*Signal and residual solvent proton signal overlap.

$^{\diamond}$Signals overlap.

$^{\blacklozenge}$Assignment interchangeable.

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 181.1 (s, NCS R2), 180.5 (s, NCS R1), 172.9 (s, Thr CO R1), 172.4 (s, Thr CO R2), 171.7 (s, Ala CO), 171.5 (s, Leu CO R1), 171.4 (s, Gls CO R1), 171.2 (s, Thz CO R2, Leu CO R2), 170.8 (s, Gls CO R2), 170.2 (s, Pip CO R2), 169.6 (s, Val CO R1), 169.5 (s, Thz CO R1), 169.3 (s, Val CO R2), 169.2 (s, Pip CO R1), 167.4 (s, Dhb CO R1), 166.1 (s, Dhb CO R2), 128.8 (s, Dhb *C*CH R2), 128.7 (s, Dhb *C*CH R1), 118.6 (d, Dhb CH R2), 115.7 (d, Dhb CH R1), 77.8 (d, Thz CH R1), 76.8 (d, Thz CH R2)$^{\#}$, 71.7 (d, Gls CH R2), 70.1 (d, Val NMe*C*H R2), 69.5 (d, Gls CH R1), 68.0 (d, Thr CHOH R1), 66.8 (d,

Thr CHOH R2), 66.3 (t, Gls CH_2 R2), 65.7 (t, Gls CH_2 R1), 65.4 (d, Val NMe*C*H R1), 60.2 (d, ThioPro CH R2), 60.2 (d, ThioPro CH R1), 56.2 (d, Thr CHNH R2), 53.9 (d, Pip CH R1), 53.1 (d, Pip CH R2), 51.8 (d, Thr CHNH R1), 49.1 (d, Leu CH R1), 48.9 (d, Leu CH R2), 48.0 (t, ThioPro NCH_2 R2), 47.7 (t, ThioPro NCH_2 R1) [◊], 47.7 (d, Ala CH R2) [◊], 47.6 (d, Ala CH R1) [◊], 46.3 (t, Pip NCH_2 R2), 46.2 (t, Pip NCH_2 R1), 40.7 (q, Val CH_3 R2), 40.2 (t, Leu CH_2 R2), 40.0 (t, Leu CH_2 R1), 37.8 (t, Thz CH_2 R2), 36.5 (t, Thz CH_2 R1), 30.4 (t, ThioPro CH*C*H_2 R1), 29.7 (q, Val CH_3 R1), 29.5 (t, ThioPro CH*C*H_2 R2), 29.0 [d, Val *C*H(CH_3)$_2$ R2], 27.7 [d, Val *C*H(CH_3)$_2$ R1], 26.7 (t, Pip NCH*C*H_2 R1), 26.1 (t, Pip NCH*C*H_2 R2), 25.7 (t, Pip NCH_2*C*H_2 R2), 25.6 (t, Pip NCH_2*C*H_2 R1), 25.5 (t, ThioPro NCH_2*C*H_2 R2), 25.3 (t, ThioPro NCH_2*C*H_2 R1), 24.7 [d, Leu *C*H(CH_3)$_2$ R1], 24.4 [d, Leu *C*H(CH_3)$_2$ R2], 22.9 (q, Leu CH_3 R1), 22.8 (q, Leu CH_3 R2), 22.6 (q, Leu CH_3 R2), 22.5 (q, Leu CH_3 R1), 21.7 (q, Val CH_3 R1), 21.2 (t, Pip CHCH_2*C*H_2 R1), 21.1 (t, Pip CHCH_2*C*H_2 R2), 20.1 (q, Thr CH_3 R2), 19.7 (q, Thr CH_3 R1), 19.7 (q, Val CH_3 R1), 19.6 (q, Val CH_3 R2), 19.4 (q, Val CH_3 R2), 19.1 (q, Ala CH_3 R2), 18.0 (q, Ala CH_3 R1), 13.7 (q, Dhb CH_3 R2), 13.1 (q, Dhb CH_3 R1).

[#]Signal and residual solvent proton signal overlap. Chemical shifts originates from the HMBC spectrum. The assignment to the rotamers is interchangeable.

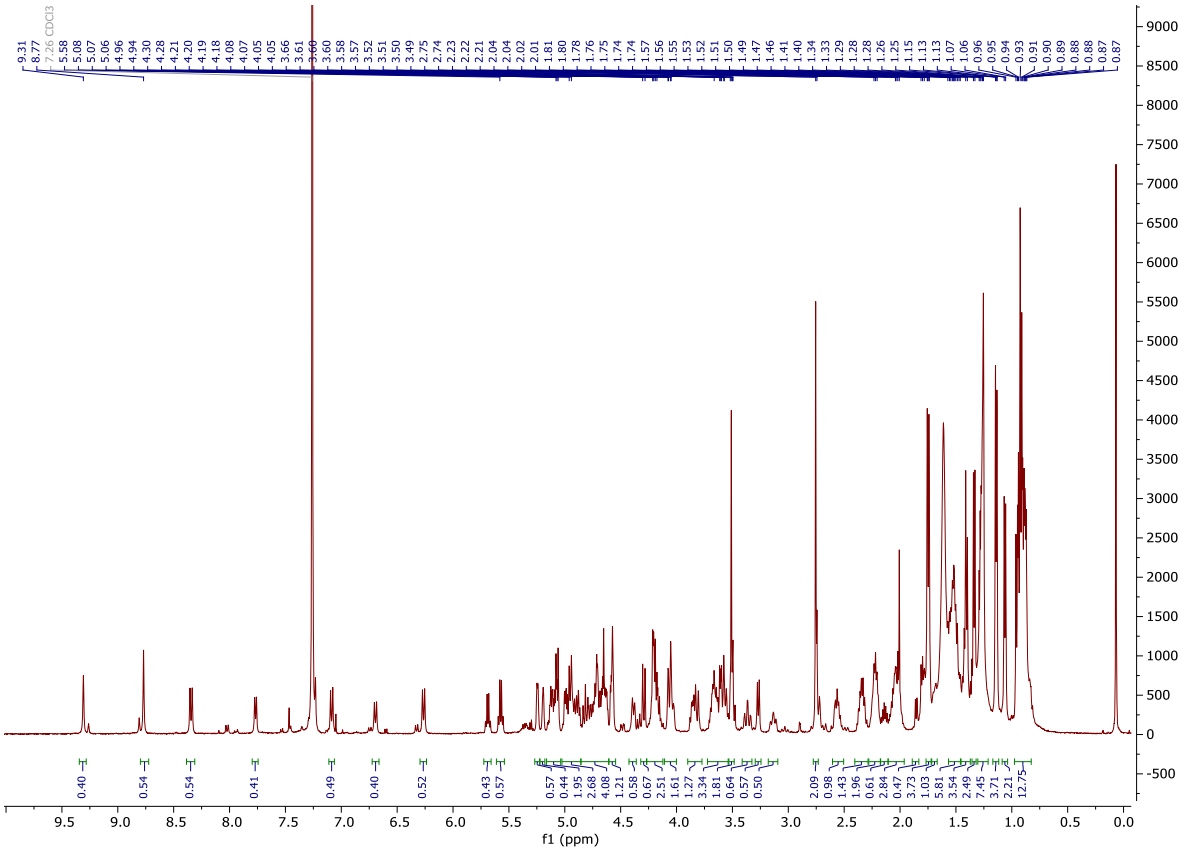
[◊]Assignment interchangeable

HR-MS (ESI): $[C_{40}H_{62}N_8O_{11}S+H]^+$ calculated: 863.4332; found: 863.4325.

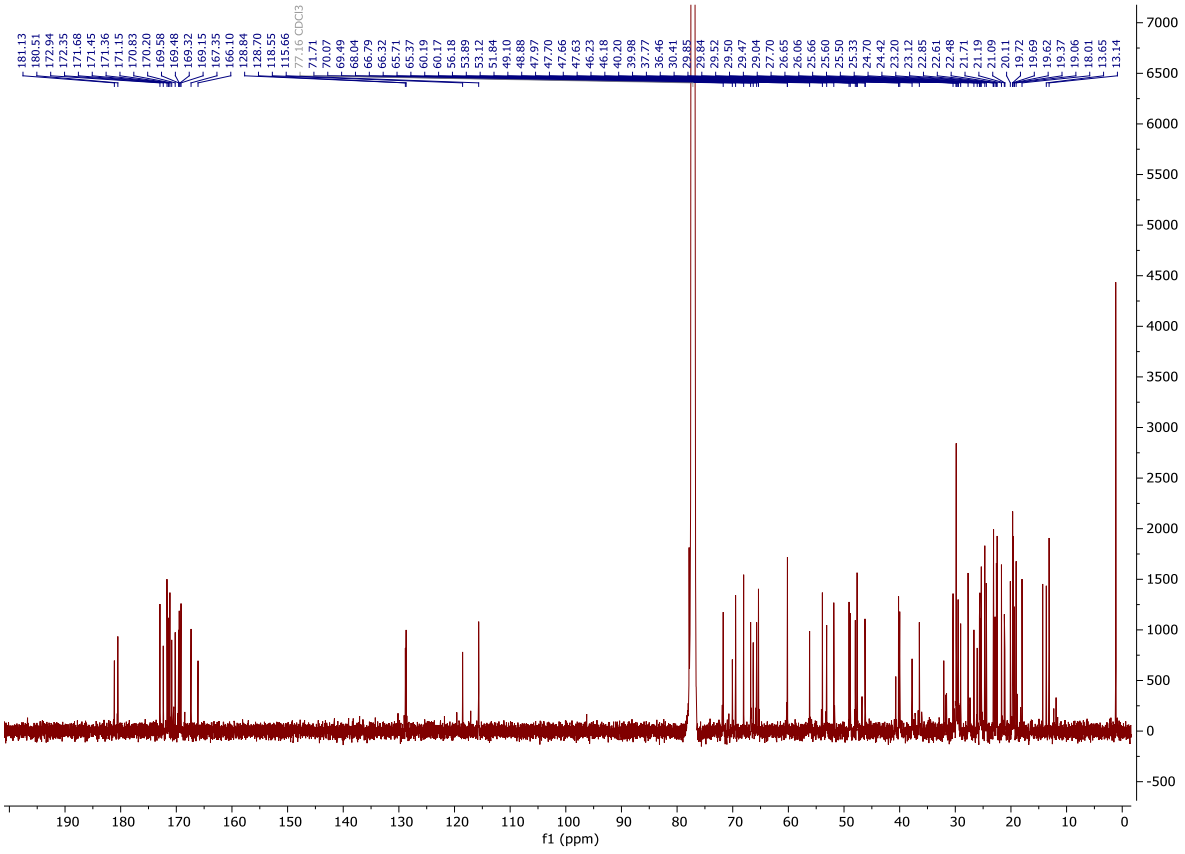
$[C_{40}H_{62}N_8O_{11}S+Na]^+$ calculated: 885.4151; found: 885.4132.

The spectroscopic data is in line with literature values.[18]

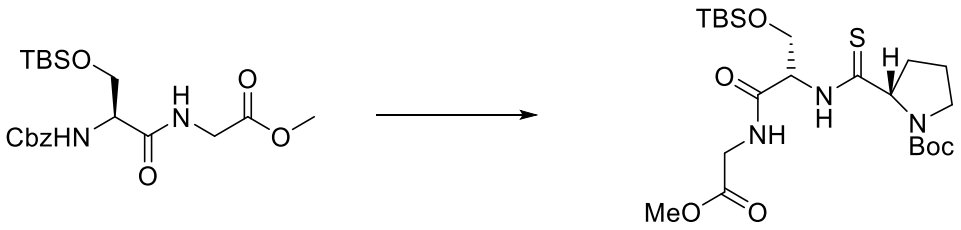
^{1}H-NMR:



^{13}C-NMR:



***N*-Boc- L-ThioPro -L-Ser(*O*-TBS)-Gly-*O*-Me**



9

$C_{22}H_{41}N_3O_6SSi$
M = 503.73 g/mol

Cbz deprotection:

To a solution of dipeptide **9** (2.00 g, 4.71 mmol, 1.00 equiv.) in MeOH (47 mL) and EtOAc (47 mL), palladium on activated carbon (10 wt% Pd, 200 mg) is added, and the argon atmosphere is replaced with a hydrogen atmosphere according to the general procedure. The suspension is stirred at room temperature for 26 hours, then the hydrogen balloon is removed, and the reaction solution is purged with argon for five minutes. Subsequently, the suspension is filtered over Celite, and the filter cake is washed with EtOAc (40 mL). The solvent is removed under reduced pressure, yielding a colorless solid (1.39 g), which is used in the next reaction without further purification.

Thioacylation:

The colorless solid is dissolved in THF (11 mL), and at 0 °C, it is slowly treated with a solution of benzotriazole **10** (1.77 g, 4.71 mmol, 1.00 equiv.) in THF (5.5 mL). After stirring the reaction solution at this temperature for 30 minutes, the solvent is removed at room temperature under reduced pressure, and the residue is dissolved in Et_2O (50 mL). The ethereal solution is washed with 10% citric acid solution (30 mL), with the washing solution back-extracted with Et_2O (20 mL). The combined organic phases are washed with $NaHCO_3$ solution (2 × 30 mL), with the two basic washing solutions also combined and back-extracted with Et2O (20 mL). Subsequently, the combined organic phases are washed with NaCl solution (50 mL), dried over Na_2SO_4, and filtered. The solvent is removed under reduced pressure, and the crude product is purified by column chromatography (silica gel: Ø 5 cm, ↕ 20 cm, *n*-pentane/EtOAc = 4/1 → 3/1 → 2/1 → 1/1). This yields the tripeptide (1.74 g, 3.45 mmol, 73% over 2 steps) as a yellow solid.

TLC: R_f = 0.78 (*n*-Pentane/EtOAc = 1/1) [UV, $KMnO_4$].

M.p.: 72 °C.

Specific rotation: $[\alpha]_D^{RT} = -72.0$ (c = 10.0, $CHCl_3$).

IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3329 (m, OH), 2980 (m, -(CH_2)−H), 1747 (s, C=O), 1672 (s, C=O), 1517 (s), 1394 (s, -(CH)−H), 1368 (m), 1210 (m), 1162 (m, C=O), 1129 (m), 840 (m), 778 (m).

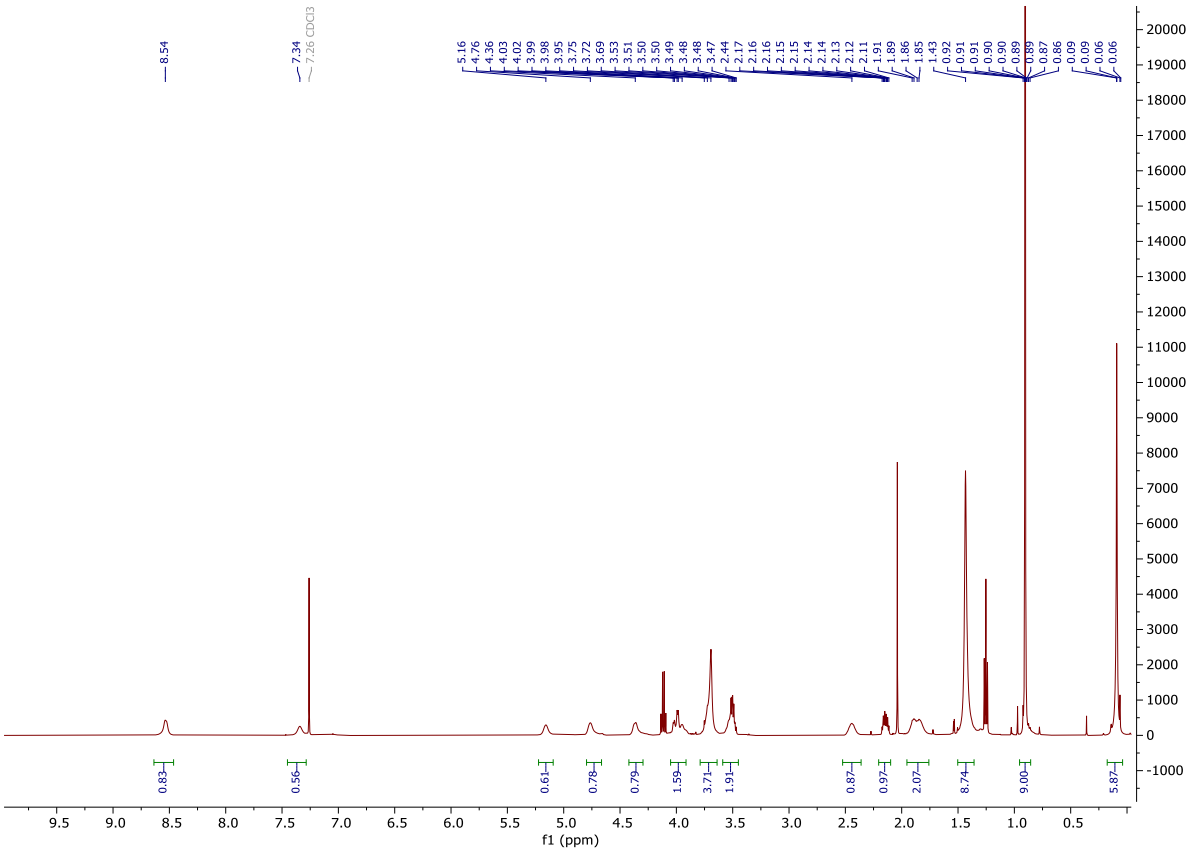
^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 8.54 (s, 1H, Ser NH), 7.34 (s, 1H, Gly NH), 5.16 (s, 1H, Ser CH), 4.76 (s, 1H, ThioPro CH), 4.36 (s, 1H, Ser CH*H*), 4.05 – 3.87 (m, 2H, Gly CH_2), 3.78 – 3.61 (m, 4H, CO_2CH_3, Ser C*H*H), 3.54 – 3.46 (m, 2H, ThioPro NCH_2), 2.41 – 2.33 (m, 1H, ThioPro CHCH*H*), 2.14 (ddt, ^2J = 12.6 Hz, ^3J = 6.7 Hz, ^3J = 5.2 Hz, 1H, ThioPro CHC*H*H), 1.93 – 1.76 (m, 2H, ThioPro $CHCH_2CH_2$), 1.43 [s, 9H, $OC(CH_3)_2$], 0.90 [s, 9H, $SiC(CH_3)_3$], 0.08 (s, 6H, $SiCH_3$).

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 204.1 (s, NCS), 169.5 (s, Ser CO), 169.2 (s, Gly CO), 156.2 (s, NCO_2), 81.6 [s, $OC(CH_3)_3$], 69.5 (d, ThioPro CH), 62.2 (t, Ser CH_2), 58.8 (d, Ser CH), 52.2 (q, CO_2CH_3), 48.2 (t, ThioPro NCH_2), 41.6 (t, Gly CH_2), 33.6 (t, ThioPro $CHCH_2$), 28.4 [q, $OC(CH_3)_3$], 25.9 [q, $SiC(CH_3)_3$], 24.5 (t, NCH_2CH_2), 18.3 [s, $SiC(CH_3)_3$], −5.3 (q, $SiCH_3$), −5.6 (q,$SiCH_3$).

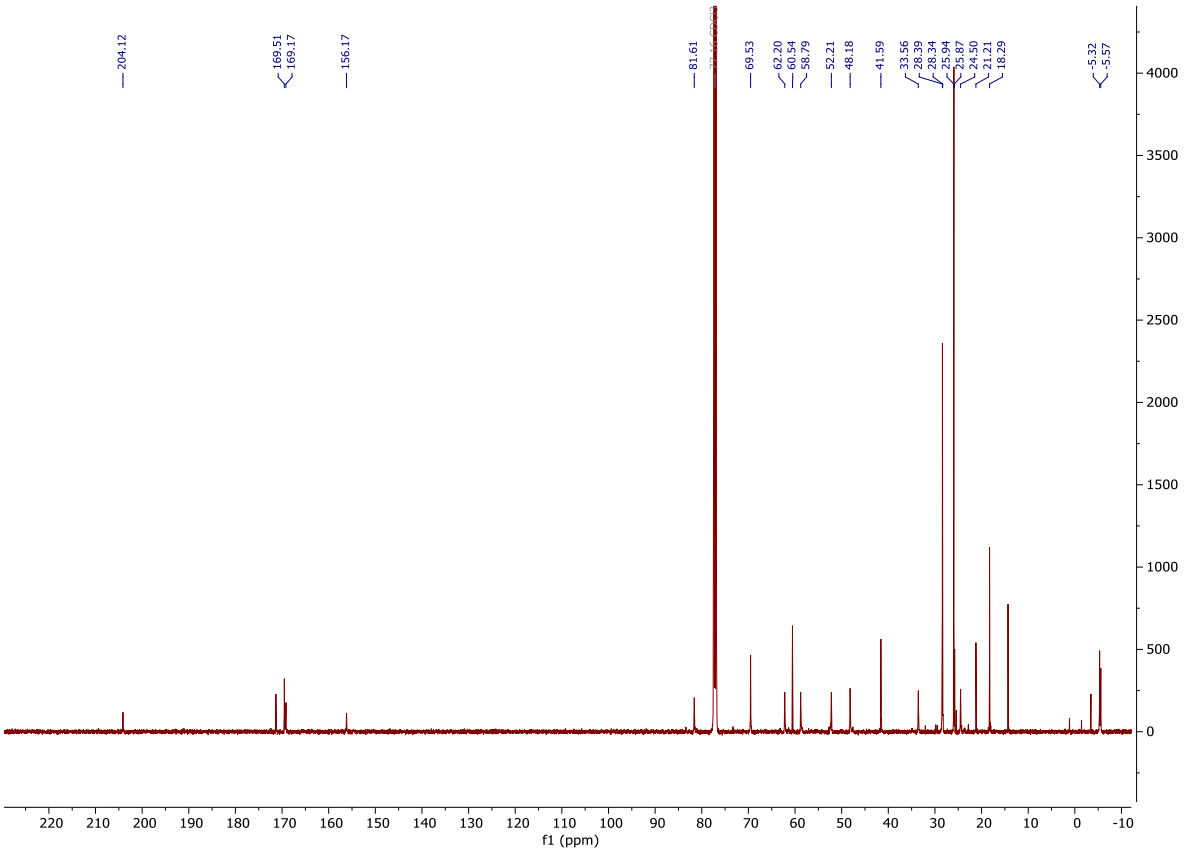
HR-MS (ESI): $[C_{22}H_{41}N_3O_6SSi+H]^+$ calculated: 504.2558; found: 504.2554.

$[C_{22}H_{41}N_3O_6SSi+Na]^+$ calculated: 526.2377; found: 526.2374.

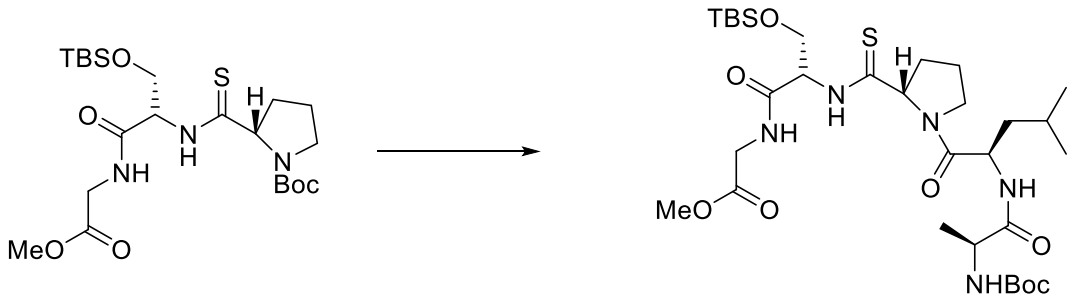
^{1}H-NMR:



^{13}C-NMR:



***N*-Boc-L-Ala-D-Leu-L-ThioPro-L-Ser(*O*-TBS)-L-Sec(*Se*-Ph)-*O*-Me (13)**



13

$C_{31}H_{57}N_5O_8SSi$

M = 687.97 g/mol

Boc deprotection:

A solution of tripeptide (1.81 g, 3.59 mmol, 1.00 equiv.) in CH_2Cl_2 (40 mL) is slowly treated, one after another at 0 °C, with 2,6-lutidine (3.34 mL, 3.08 g, 28.8 mmol, 8.00 equiv.) and TMSOTf (3.10 mL, 3.81 g, 17.2 mmol, 4.77 equiv.). After stirring at 0 °C for six hours, the reaction is quenched by adding $NaHCO_3$ solution (40 mL), and the phases are separated. The aqueous phase is extracted with EtOAc (3 × 40 mL), and the combined organic phases are dried over Na_2SO_4 and filtered. The solvent is removed under reduced pressure, and the residue is dried by azeotropic distillation (PhMe, 3 × 25 mL). As a result, the deprotected tripeptide (1.45 g) is obtained as a colorless solid, which is used in the subsequent reaction without further purification.

Peptide coupling:

The crude product and Ala-Leu dipeptide (1.19 g, 3.95 mmol, 1.10 equiv.) are dissolved in CH_2Cl_2 (72 mL), and at 0 °C, they are incrementally treated with HATU (1.50 g, 3.95 mmol, 1.10 equiv.) and dropwise with 2,4,6-collidine (479 µL, 0.43 g, 3.59 mmol, 1.00 equiv.). After stirring the reaction solution at room temperature for 24 hours, the solvent is removed under reduced pressure, and the residue is treated with Et_2O (50 mL). The resulting precipitate is filtered, and the filter cake is washed with Et_2O (30 mL). Subsequently, the combined filtrates are washed with $NaHCO_3$ (40 mL) and NaCl solution (40 mL), dried over Na_2SO_4, filtered, and the solvent is removed under reduced pressure. The residue is purified by column

chromatography (silica gel: Ø 7 cm, ↕ 20 cm, CH2Cl2/MeOH = 99/1), yielding the pentapeptide **13** (1.47 g, 3.87 mmol, 98% over 2 steps) as a yellowish solid.

TLC: R_f = 0.30 (*n*-Pentane/EtOAc = 1/1) [$KMnO_4$].

M.p.: 76 °C.

Specific rotation: $[\alpha]_D^{RT} = -20.0$ (c = 10.0, $CHCl_3$).

IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3316 (m, OH), 2959 (m, -(CH_2)−H), 2931 (m, -(CH_2)−H), 1745 (s, C=O), 1652 (s, C=O), 1520 (s), 1451 (m), 1368 (m), 1247 (m), 1168 (m, C=O), 1065 (m), 859 (m), 759 (m).

Rotamer ratio: R1/R2 ≈ 88/12.

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 8.57 (d, ^3J = 7.4 Hz, 1H, Ser NH), 7.31 (s, 1H, Leu NH), 7.28 – 7.22 (m, 1H, Gly NH)*, 6.15 (d, ^3J = 8.3 Hz, 1H, Ala NH), 5.19 – 5.13 (m, 1H, Ser CH), 4.93 (dd, ^3J = 8.7 Hz, ^3J = 3.7 Hz, 1H, ThioPro CH), 4.60 – 4.54 (m, 1H, Leu CH R2), 4.47 – 4.37 (m, 1H, Leu CH R1), 4.33 – 4.21 (m, 2H, Ala CH, Ser CH*H*), 4.19 (d, ^2J = 17.7 Hz, 1H, Gly CH*H* R2)◊, 4.16 (d, ^2J = 17.7 Hz, 1H, Gly C*H*H R1)◊, 4.10 – 4.02 (m, 2H, Ser C*H*H, ThioPro NCH*H*), 3.75 (s, 3H, CO_2CH_3), 3.71 (d, ^2J = 17.7 Hz, 1H, Gly C*H*H R2)◊, 3.67 (d, ^2J = 17.7 Hz, 1H, Gly CH*H* R1)◊, 3.65 – 3.55 (m, 1H, ThioPro NC*H*H), 2.45 – 2.35 (m, 1H, ThioPro CHCH*H*), 2.35 – 2.27 (m, 1H, ThioPro CHC*H*H), 2.04 – 1.91 (m, 2H, ThioPro $CHCH_2CH_2$), 1.73 – 1.63 [m, 1H, Leu C*H*(CH_3)$_2$], 1.62 – 1.47 (m, 2H, Leu CH_2), 1.44 [s, 9H, OC(CH_3)$_2$ R2]◊, 1.43 [s, 9H, OC(CH_3)$_2$ R1]◊, 1.30 (d, ^3J = 7.1 Hz, 3H, Ala CH_3), 1.00 – 0.93 (m, 6H, Leu CH_3), 0.88 [s, 9H, SiC(CH_3)$_3$ R1], 0.87 [s, 9H, SiC(CH_3)$_3$ R2], 0.11 (s, 6H, $SiCH_3$).

* Signal and residual solvent proton signal overlap.

◊ Signals overlap.

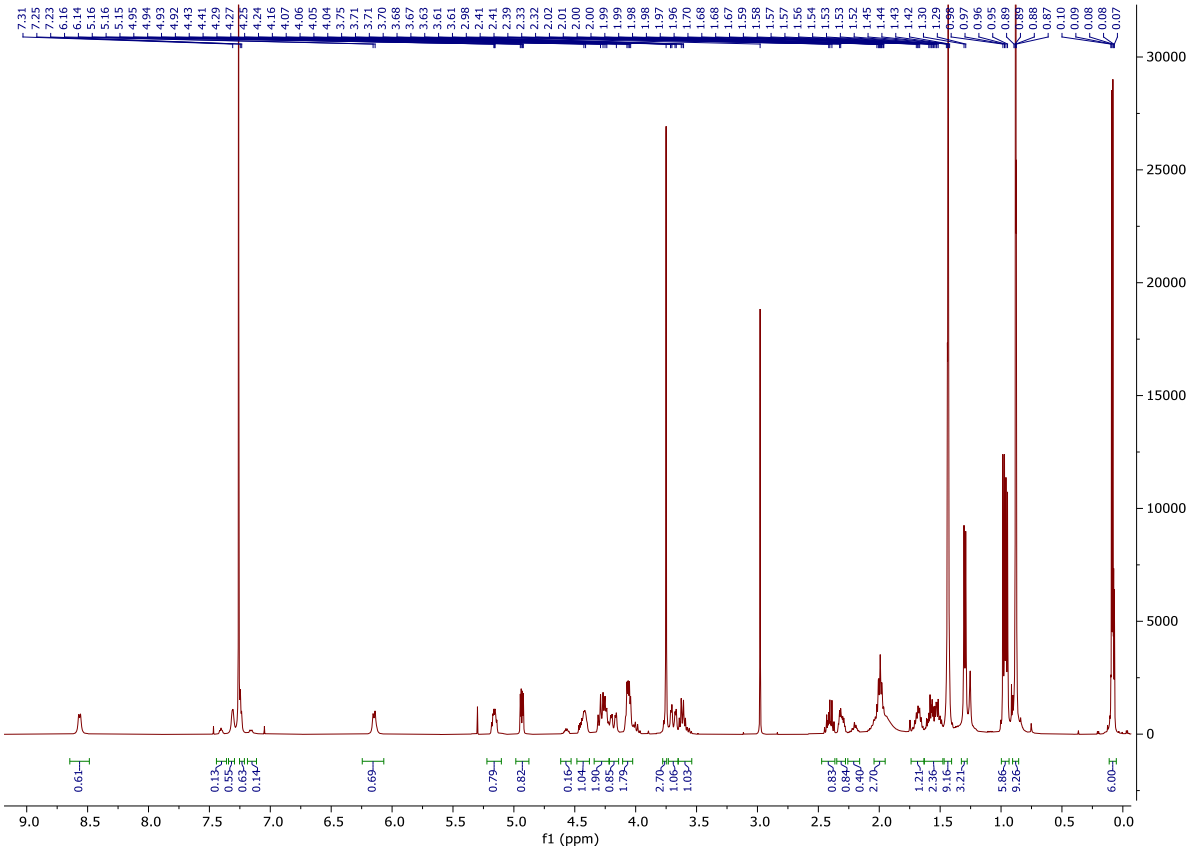
^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 203.5 (s, NCS R1), 203.1 (s, NCS R2), 174.6 (s, Ala CO R2), 174.3 (s, Ala CO R1), 172.9 (s, Leu CO R2), 172.8 (s, Leu CO R1), 171.4 (s, Gly CO R2), 171.3 (s, Gly CO R1), 169.4 (s, Ser CO R1), 169.1 (s, Ser CO R2), 158.1 (s, NCO_2 R2) 156.9 (s, NCO_2 R1), 80.7 [s, O*C*(CH_3)$_3$ R2], 80.4 [s, O*C*(CH_3)$_3$ R1], 69.3 (d, ThioPro CH R1), 69.2 (d, ThioPro CH R2), 62.5 (t, Ser CH_2 R2), 61.8 (t, Ser CH_2 R1), 61.6 (d, Ser CH R2), 60.4 (d, Ser CH R1), 52.5 (q, CO_2CH_3) , 50.5 (d, Ala CH R1), 49.8 (d, Ala CH R2), 49.1 (d,

Leu CH R1), 48.9 (d, Leu CH R2), 48.5 (t, ThioPro NCH_2 R2), 48.5 (t, ThioPro NCH_2 R1), 41.6 (t, Gly CH_2 R2), 41.2 (t, Gly CH_2 R1), 40.2 (t, Leu CH_2), 33.2 (t, ThioPro $CHCH_2$ R1), 32.9 (t, ThioPro $CHCH_2$ R2), 28.4 [q, $OC(CH_3)_3$ R1], 28.4 [q, $OC(CH_3)_3$ R2], 25.9 [q, $SiC(CH_3)_3$ R2], 25.8 [q, $SiC(CH_3)_3$ R1], 24.9 [d, Leu $CH(CH_3)_2$ R1], 24.8 [d, Leu $CH(CH_3)_2$ R2], 24.5 (t, ThioPro NCH_2CH_2 R1), 24.2 (t, ThioPro NCH_2CH_2 R2), 23.5 (q, Leu CH_3 R1), 23.4 (q, Leu CH_3 R2), 22.2 (q, Leu CH_3 R1), 22.1 (q, Leu CH_3 R2), 18.3 [s, $SiC(CH_3)_3$ R1], 18.1 [s, $SiC(CH_3)_3$ R2], 17.1 (q, Ala CH_3 R2), 16.1 (q, Ala CH_3 R1), −3.5 (q, $SiCH_3$ R1), −3.5 (q, $SiCH_3$ R2), −5.2 (q, $SiCH_3$ R1), −5.3 (q,$SiCH_3$ R2).

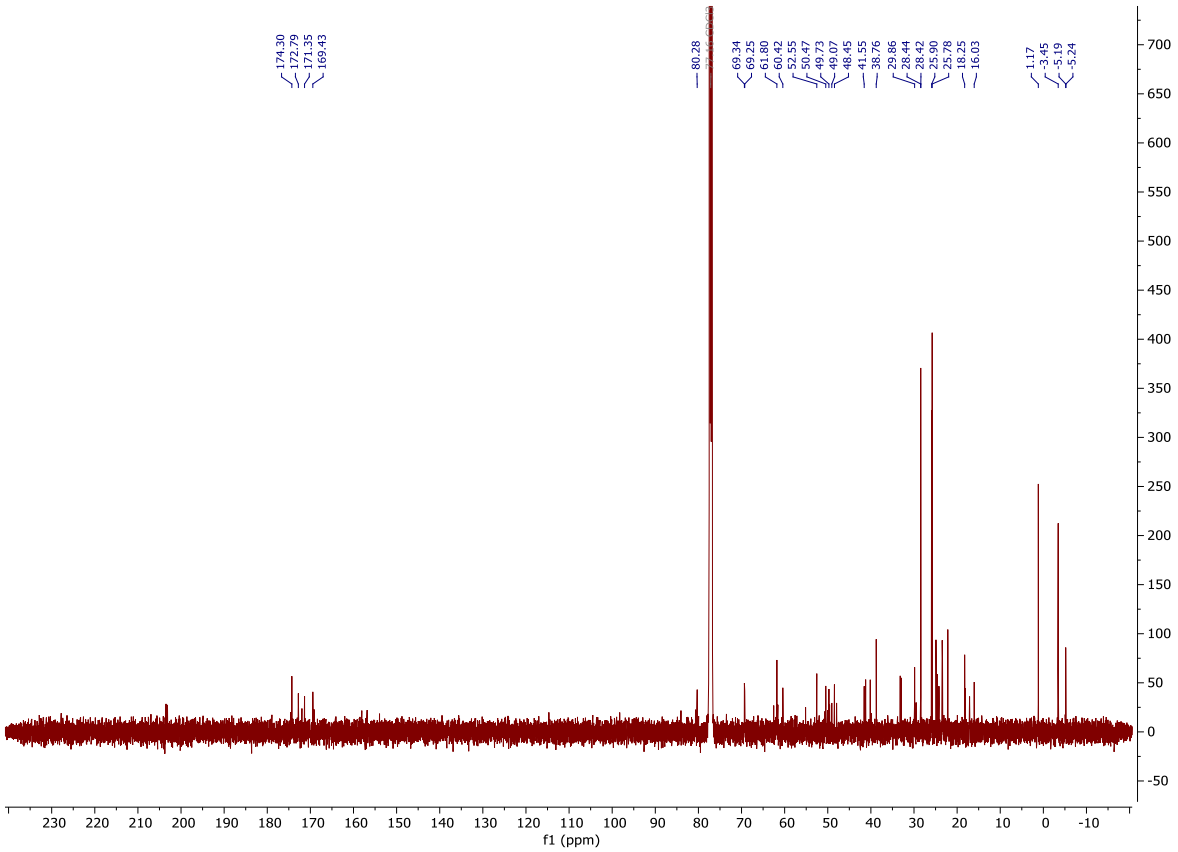
HR-MS (ESI): $[C_{31}H_{57}N_5O_8SSi+H]^+$ calculated: 688.3770; found: 688.3760.

$[C_{31}H_{57}N_5O_8SSi+Na]^+$ calculated: 710.3589; found: 710.3576.

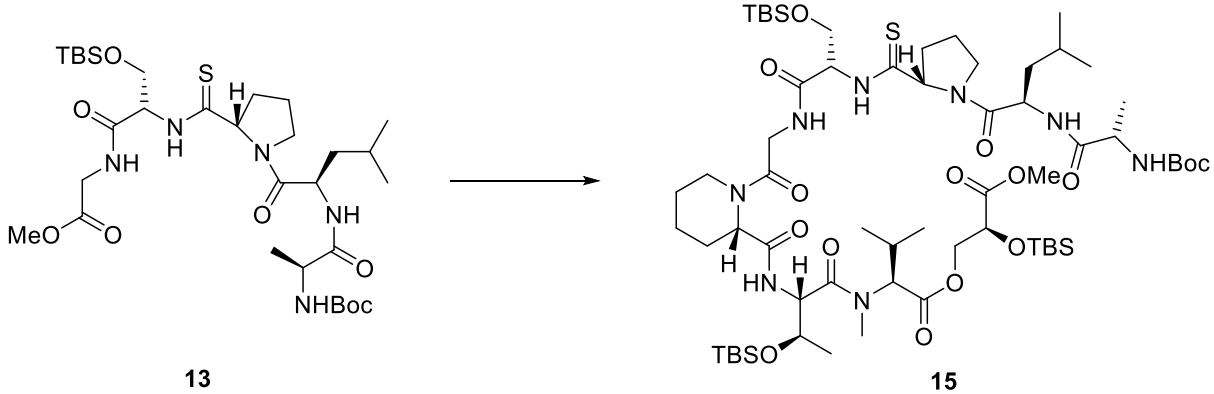
^{1}H-NMR:



^{13}C-NMR:



_N_-Boc-L-Ala-D-Leu-L-ThioPro-L-Ser(_O_-TBS)-Gly-L-Pip-L-Thr(_O_-TBS)-(_N_-Me)L-Val-L-Gls(_O_-TBS)-_O_-Me (15)



13

15

$C_{62}H_{116}N_8O_{15}SSi_3$

M = 1329.97 g/mol

Saponification:

To a solution of pentapeptide **13** (102 mg, 148 µmol, 1.00 equiv.) in THF (2 mL) and water (0.4 mL), $LiOH \cdot H_2O$ (15.5 mg, 370 µmol, 2.50 equiv.) is added at 0 °C. The reaction solution is stirred at this temperature for six hours before terminating the reaction by adding water (15 mL) and Et_2O (10 mL). The phases are separated, and the organic phase is extracted with water (10 mL). The combined aqueous phases are adjusted to pH = 3 using a 10% citric acid solution and extracted with EtOAc (3 × 20 mL). Subsequently, the combined organic phases are dried over Na_2SO_4 and filtered. The solvent is removed under reduced pressure, and the residue is dried by azeotropic distillation (PhMe, 15 mL). As a result, the free carboxylic acid is obtained as a colorless solid, which is used in the following reaction without further purification.

Esterification and Fragment Coupling:

The crude product is dissolved in CH_2Cl_2 (1.2 mL) and initially treated with C_6F_5OH (35.5 mg, 193 µmol, 1.30 equiv.) before EDC·HCl (37.0 mg, 193 µmol, 1.30 equiv.) is added dropwise at 0 °C. After stirring the suspension at this temperature for 30 minutes, it is gradually warmed to room temperature with stirring over 23 hours.

Subsequently, a solution of peptide **5** (110 mg, 163 µmol, 1.10 equiv.) in CH_2Cl_2 (1.8 mL) is slowly added to the reaction solution, followed by stirring for an additional 16 hours. The reaction is terminated by removing the solvent under reduced pressure. The residue is then purified by column chromatography (silica gel: Ø 2 cm, ↕ 16 cm, n Pentane/EtOAc = 5/1 → 4/1 → 3/1 → 2/1 → 1/1), yielding the nonapeptide **15** (105 mg, 78.9 µmol, 53% over 3 steps) as a colorless solid.

TLC: R_f = 0.41 (*n*-Pentan/EtOAc = 2/1) [$KMnO_4$].

M.p.: 92 °C.

Specific rotation: $[\alpha]_D^{RT} = -76.0$ (c = 10.0, $CHCl_3$).

IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3311 (m, OH), 2955 (m, -(CH_2)−H), 2931 (m, -(CH_2)−H), 1744 (s, C=O), 1649 (s, C=O), 1513 (s), 1463 (m), 1364 (s, -(CH)−H), 1254 (m), 1163 (m, C=O), 1020 (m), 838 (m), 780 (m).

Rotamer ratio: R1/R2 ≈ 84/16.

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 8.56 (d, ^{3}J = 7.1 Hz, 1H, Ser NH), 7.32 – 7.26 (m, 1H, Leu NH)*, 7.22 – 7.14 (m, 1H, Gly NH), 6.74 (d, ^{3}J = 8.2 Hz, 1H, Thr NH), 6.59 (d, ^{3}J = 8.9 Hz, 1H, Ala NH), 5.23 (s, 1H, Pip CH R1), 5.20 – 5.14 (m, 1H, Ser CH R2), 5.13 – 5.07 (m, 1H, Ser CH R1), 5.00 – 4.91 (m, 2H, ThioPro CH, Thr C*H*OH), 4.82 – 4.73 (m, 1H, Thr C*H*NH R1), 4.76 – 4.71 (m, 1H Thr C*H*NH R2), 4.58 – 4.48 (m, 5H, Pip CH R2, Leu CH R1, Gls CH*H*, Gls CH R2, Gly CH*H* R2), 4.44 – 4.40 (m, 1H, Leu CH R2), 4.39 – 4.35 (m, 1H, Gls CH R1), 4.31 – 4.15 (m, 3H, Gly CH*H* R1, Ser CH*H*, Ala CH), 4.13 – 4.08 (m, 2H, Val CH, Gls C*H*H), 4.05 – 3.95 (m, 2H, ThioPro NCH*H*, Ser C*H*H), 3.94 – 3.86 (m, 1H, Gly C*H*H), 3.72 (s, 3H, CO_2CH_3), 3.67 – 3.52 (m, 2H, ThioPro NC*H*H, Pip NCH*H*), 3.33 – 3.26 (m, 1H, Pip NC*H*H), 3.10 (s, 3H, Val NCH_3), 2.43 – 2.22 (m, 3H, ThioPro CHCH_2, Pip CHCH*H* R2), 2.21 – 2.09 [m, 2H, Pip CHCH*H* R1, Val C*H*$(CH_3)_2$], 2.02 – 1.91 (m, 2H, ThioPro NCH_2CH_2), 1.75 – 1.48 (m, 7H, Leu CH_2, Pip CHC*H*HCH_2, Pip NCH_2CH_2), 1.42 [s, 10H, C*H*$(CH_3)_2$, $OC(CH_3)_3$], 1.33 – 1.27 (m, 3H, Ala CH_3), 1.20 – 1.14 (m, 3H, Thr CH_3), 1.01 − 0.91 (m, 9H, Val CH_3, 2 x Leu CH_3), 0.91 – 0.78 [m, 30H, 3 x $SiC(CH_3)_3$, Val CH_3], 0.10 – 0.06 (m, 12H, 4 x $SiCH_3$), 0.04 – 0.01 (m, 6H, 2 x $SiCH_3$).

* Signal and residual solvent proton signal overlap.

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): *δ* (ppm) = 203.3 (s, NCS R1), 203.2 (s, NCS R2), 174.3 (s, Ala CO R1), 174.1 (s, Ala CO R2), 172.8 (s, Thr CO R2), 172.7 (s, Thr CO R1), 172.3 (s, Pip CO R2), 172.2 (s, Pip CO R1), 171.2 (s, Leu CO R1)♣, 171.1 (s, Leu CO R2)♦, 170.8 (s, Gls CO R1)♣, 170.7 (s, Gls CO R2)♦, 170.2 (s, Ser CO R1)♣, 170.1 (s, Ser CO R2)♦, 169.2 (s, Gly CO R2)♦, 168.6 (s, Gly CO R1)♣, 168.5 (s, Val CO R2)♦, 168.2 (s, Val CO R1)♣, 156.5 (s, NCO_2 R1), 156.4 (s, NCO_2 R2), 80.2 [s, O*C*$(CH_3)_3$ R2], 79.9 [s, O*C*$(CH_3)_3$ R1], 70.6 (d, Gls CH R1), 69.7 (d, Gls CH R2), 69.1 (d, Thr CHOH R2), 68.9 (d, Thr CHOH R1), 68.7 (d, Val NMe*C*H R2), 68.5 (d, Val NMe*C*H R1), 66.2 (t, Gls CH_2 R2), 66.1 (t, Gls CH_2 R1), 61.9 (t, Ser CH_2 R1), 61.8 (t, Ser CH_2 R2), 61.6 (d, ThioPro CH R1), 61.5 (d, Ser CH R1), 61.3 (d, ThioPro CH R2), 60.7 (d, Ser CH R2), 55.3 (d, Thr CHNH R2), 54.9 (d, Thr CHNH R1), 53.1 (d, Pip CH R2), 52.8 (d, Pip CH R1), 52.3 (q, CO_2CH_3), 50.0 (s, Leu CH R1), 49.9 (s, Leu CH R2), 49.5 (d, Ala CH R1), 49.4 (d, Ala CH R2), 48.3 (t, ThioPro NCH_2 R2), 48.1 (t, ThioPro NCH_2 R1), 43.6 (t, Pip NCH_2 R2). 43.3 (t, Pip NCH_2 R1), 41.6 (t, Gly CH_2 R1), 41.1 (t, Gly CH_2 R2), 40.4 (t, Leu CH_2 R2), 40.3 (t, Leu CH_2 R1), 33.2 (t, ThioPro CH*C*H_2 R1), 32.9 (t, ThioPro CH*C*H_2 R2), 31.9 (q, Val NCH_3 R2), 31.7 (q, Val NCH_3 R1), 28.5 [q, OC(*C*$H_3)_3$ R1], 28.4 [q, OC(*C*$H_3)_3$ R2], 27.9 [d, Val *C*H$(CH_3)_2$ R2], 27.8 [d, Val *C*H$(CH_3)_2$ R1], 25.9 [q, SiC(*C*$H_3)_3$ R1],

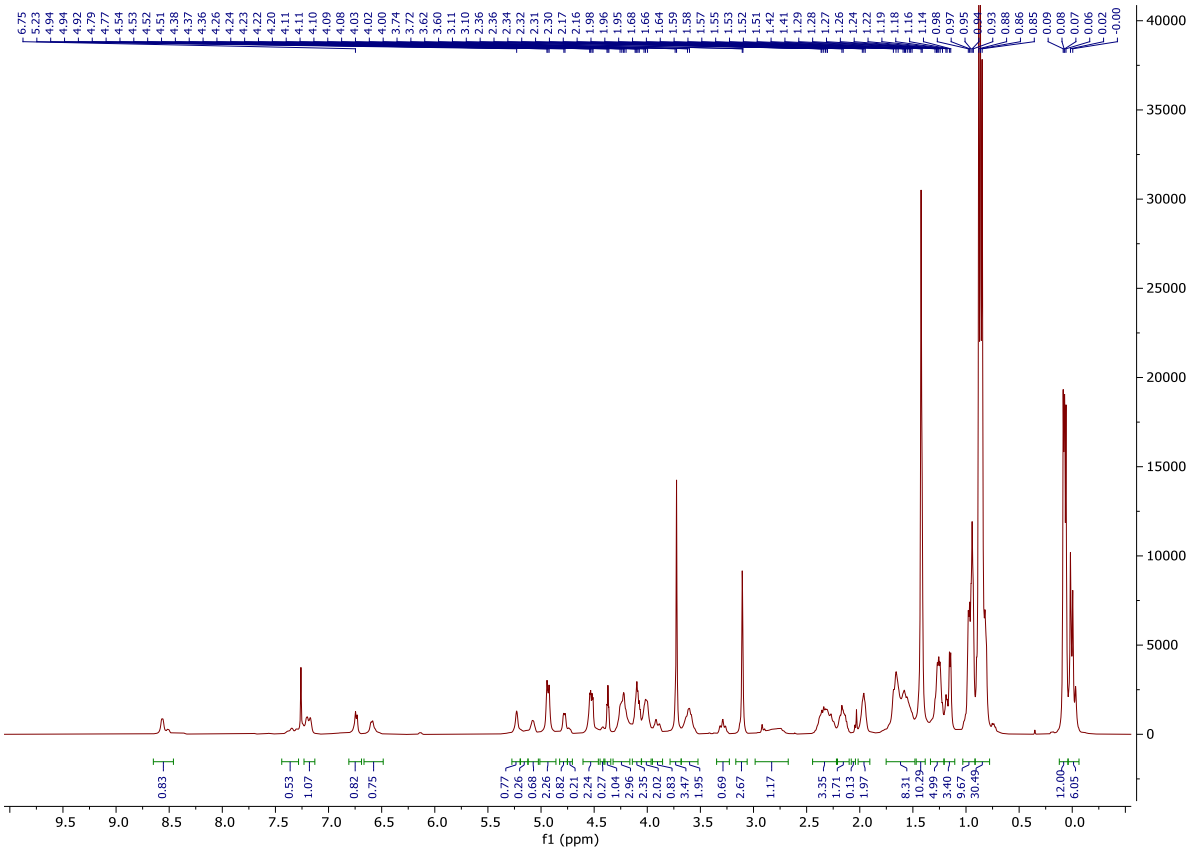
25.9 [q, SiC(CH_3)$_3$ R2], 25.9 [q, SiC(CH_3)$_3$ R1]♣, 25.8 [q, SiC(CH_3)$_3$ R2], 25.8 (t, Pip CHCH$_2$$CH_2$ R1)♣, 25.7 [q, SiC(CH_3)$_3$], 25.4 (t, Pip CHCH$_2$$CH_2$ R1), 25.3 (t, Pip CHCH_2 R2), 24.8 (Pip CHCH_2 R1), 24.6 [d, Leu $CH(CH_3)_2$ R2], 24.4 (t, ThioPro NCH$_2$$CH_2$ R2 24.3 [d, Leu $CH(CH_3)_2$ R1], 24.2 (t, ThioPro NCH$_2$$CH_2$ R1), 23.4 (q, Leu CH_3 R1), 23.3 (q, Leu CH_3 R2), 22.2 (q, Leu CH_3 R2), 22.1 (q, Leu CH_3 R1), 21.3 (q, Thr CH_3 R1), 21.2 (q, Thr CH_3 R2), 20.5 (Pip NCH$_2$$CH_2$ R2), 20.4 (Pip NCH$_2$$CH_2$ R1), 19.7 (q, Val CH_3 R1), 19.7 (q, Val CH_3 R2), 19.2 (q, Val CH_3 R1) , 19.1 (q, Val CH_3 R2), 18.3 [s, SiC(CH_3)$_3$ R1], 18.3 [s, SiC(CH_3)$_3$ R1], 18.2 [s, SiC(CH_3)$_3$ R2], 18.1 [s, SiC(CH_3)$_3$ R2], 18.0 [s, SiC(CH_3)$_3$ R1], 18.0 [s, SiC(CH_3)$_3$ R2], 17.2 (q, Ala CH_3 R2), 16.7 (q, Ala CH_3 R1), −3.5 (q, $SiCH_3$ R1), −4.6 (q, $SiCH_3$ R2), −5.0 (q, $SiCH_3$ R1), −5.2 (q, $SiCH_3$ R2), −5.3 (q, $SiCH_3$ R2), −5.3 (q, $SiCH_3$ R1).

♦/♣/♠ Assignment interchangeable.

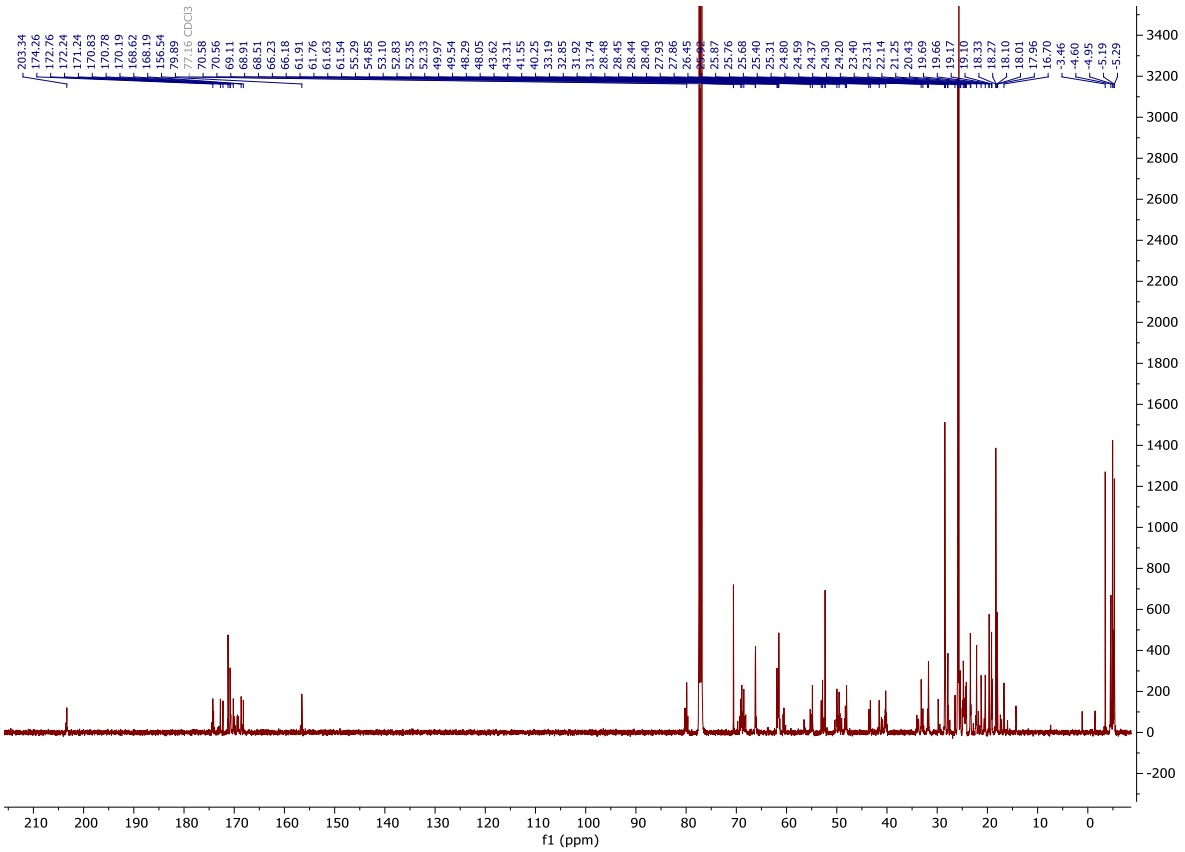
HR-MS (ESI): [$C_{62}H_{116}N_8O_{15}SSi_3$+H]$^+$ calculated: 1329.7662; found: 1329.7636.

[$C_{62}H_{116}N_8O_{15}SSi_3$+Na]$^+$ calculated: 1351.7481; found: 1351.7448.

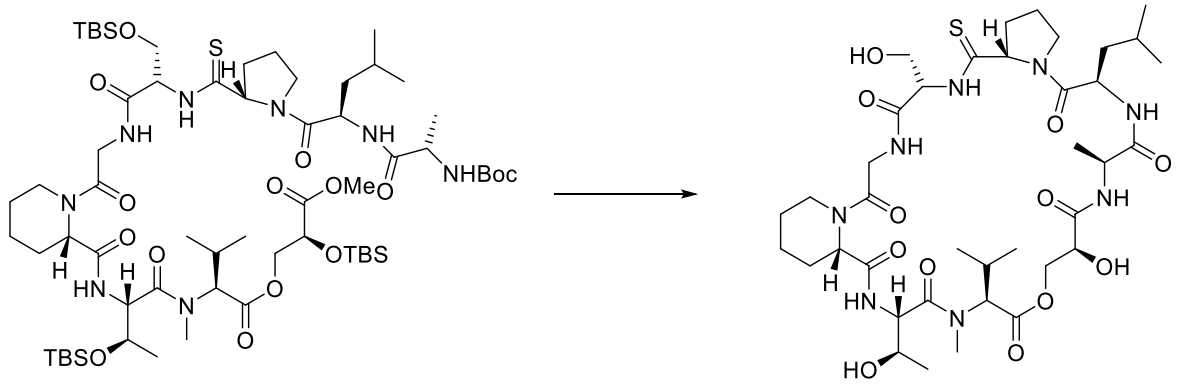
^{1}H-NMR:



^{13}C-NMR:



Macrocycle 16



15

16

$C_{38}H_{62}N_8O_{12}S$

M = 855.02 g/mol

Ester Cleavage:

A solution of nonapeptide **15** (409 mg, 308 µmol, 1.00 equiv.) in DCE (34 mL) is treated with Me_3SnOH (357 mg, 1.98 mmol, 8.00 equiv.) and the reaction mixture is stirred at 80 °C for 48 hours. Subsequently, the mixture is cooled to room temperature, and 5% citric acid solution (20 mL) is added. The emulsion is stirred for 15 minutes, the resulting phases are separated, and the aqueous phase is extracted with EtOAc (2 × 30 mL). The combined organic phases are washed with 5% citric acid solution (2 × 20 mL), and the combined wash solutions are back-extracted with EtOAc (20 mL). Then, the combined organic phases are washed with NaCl solution (20 mL), dried over Na_2SO_4, and filtered. The residue is purified by column chromatography (silica gel: Ø 2 cm, ↕ 16 cm, CH_2Cl_2/MeOH/AcOH = 100/1/0.01 → 75/1/0.01 → 50/1/0.01 → 25/1/0.01) to remove residual tin reagent. A colorless solid (414 mg) is obtained.

Boc Deprotection:

The obtained solid is dissolved in CH_2Cl_2 (20 mL) and cooled to 0 °C, then treated with TFA (5 mL). The solution is stirred at this temperature for three hours. After the addition of water (20 mL), the aqueous phase is adjusted to pH = 7 using 1 M sodium hydroxide and $NaHCO_3$ solution. The phases are separated, and the aqueous phase is saturated with solid NaCl and extracted with EtOAc (2 × 60 mL). The combined organic phases are washed with NaCl solution (50 mL), dried over Na_2SO_4, and filtered before removing the solvent under reduced pressure. The residue is then dried by azeotropic distillation with PhMe (2 × 20 mL). The resulting yellowish solid (207 mg) is used in the subsequent reaction without further purification.

Macrolactamization:

A solution of the yellowish solid in CH_2Cl_2 (15 mL) is slowly added dropwise over four hours to a solution of HATU (320 mg, 843 µmol, 2.74 eq.) and 2,4,6 Collidin (179 µL, 163 mg, 1.35 mmol, 4.38 eq.) in CH_2Cl_2 (120 mL). The reaction solution is stirred for 18 hours before adding water (70 mL). The phases are separated, the aqueous phase is saturated with solid NaCl, and extracted with CH_2Cl_2 (2 × 50 mL). The combined organic phases are washed with 10% citric acid solution (70 mL), $NaHCO_3$ (70 mL), and NaCl solution (70 mL), and the washing

solutions are counter-extracted with CH_2Cl_2 (each 10 mL). Subsequently, the combined organic phases are dried over Na_2SO_4, filtered, and the solvent is removed under reduced pressure. The residue is dissolved in dry MeCN (20 mL) and filtered through Celite to remove inorganic salts. After washing the filter cake several times with MeCN, the solvent is removed under reduced pressure from the combined filtrates. The resulting yellowish resin (216 mg) is used in the following reaction without further purification.

TBS Deprotection:

A solution of the yellowish resin in dry MeCN (45 mL) is carefully treated with HF (48% in water, 2.45 mL), and the reaction solution is stirred at room temperature for 26 hours. The reaction is quenched by adding Na_2CO_3 solution (50 mL), and the aqueous phase is extracted with EtOAc (3 × 50 mL). The combined organic phases are washed with NaCl solution (100 mL), dried over Na_2SO_4, and filtered before most of the solvent is removed under reduced pressure. Before complete solvent removal, Celite is added. The Celite loaded with the crude product is purified by column chromatography (silica gel: Ø 2 cm, ↕ 22 cm, CH_2Cl_2/MeOH = 50/1 → 40/1 → 30/1 → 20/1 → 10/1), yielding approximately 93 mg of a colorless solid that is still impure. This is further purified by semi-preparative HPLC (Phenomenex Luna 5u Silica, 20 × 250 mm, ^{i}PrOH/*n*-Heptane = 20/80, r.t., 15 mL·min^{-1}, 215 nm, t_R (**16**) = 30.3 min, injection concentration: 93 mg·mL^{-1}). After lyophilization (with ultra-pure water), macrocycle **16** (30.9 mg, 36.1 µmol, 12% over 4 steps) is obtained as an amorphous, colorless solid.

TLC: R_f = 0.41 (CH_2Cl_2/MeOH = 9/1) [UV, $KMnO_4$].

M.p.: 171 °C.

Specific rotation: $[\alpha]_D^{RT} = -76.0$ (c = 10.0, $CHCl_3$).

IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3325 (m, OH), 2959 (m, -(CH_2)−H), 1742 (s, C=O), 1637 (s, C=O), 1515 (s), 1446 (m), 1262 (m), 1193 (m), 1149 (m, C=O), 1123 (m), 1063 (m), 1018 (m), 753 (m), 666 (m).

Rotamer ratio: R1/R2 ≈ 59/41.

^{1}H-NMR (500 MHz, $CDCl_3$, 298 K): δ (ppm) = 8.49 (d, ^3J = 7.9 Hz, 1H, Ser NH R2), 8.26 (s, 1H, Ser NH R1), 7.89 (t, ^3J = 5.9 Hz, 1H, Gly NH R1), 7.74 (d, ^3J = 8.7 Hz, Leu NH R1), 7.66

(s, 1H, Ala NH R2), 7.52 (d, ^3J = 9.2 Hz, 1H, Ala NH R1), 7.35 (s, 1H, Gly NH R2), 7.17 – 7.11 (m, 1H, Thr NH R2), 7.06 (d, ^3J = 8.4 Hz, 1H, Thr NH R1)$^{\lozenge}$, 7.09 – 7.02 (m, 1H, Leu NH R2)$^{\lozenge}$, 5.38 (s, 1H, Pip CH R2), 5.20 (d, ^3J = 5.5 Hz, 1H, Pip CH R1), 5.17 – 5.11 (m, 1H, Ser CH R2), 5.08 – 5.03 (m, 1H, Ser CH R1), 5.03 – 5.00 (m, 1H, Ala CH R1), 4.97 (d, ^3J = 8.8 Hz, ^3J = 4.9 Hz, 1H, ThioPro CH R1), 4.87 (d, ^3J = 8.8 Hz, 1H, Thr C*H*NH R2), 4.80 (dd, ^3J = 11.6 Hz, ^3J = 3.8 Hz, 1H, Gls CH*H* R1), 4.79 (dd, ^2J = 15.9 Hz, ^3J = 5.9 Hz, Gly CH*H* R1), 4.68 – 4.62 (m, 1H, Ala CH R1), 4.61 – 4.57 (m, 1H, Gly CH*H* R2), 4.55 (dd, ^3J = 8.4 Hz, ^3J = 1.9 Hz, 1H, Thr C*H*NH R1), 4.50 (s, 1H, Gls CH R2), 4.46 – 4.39 (m, 1H, Val CH R2), 4.36 (dd, ^3J = 11.5 Hz, ^3J = 5.7 Hz, 1H, Ser CH*H*), 4.34 – 4.29 (m, 1H, Gls CH R1), 4.28 – 4.21 (m, 1H, Val CH R1), 4.13 – 3.99 (m, 4H, ThioPro NCH*H*, Pip NCH*H*, Gls C*H*H, Thr C*H*OH), 3.91 – 3.84 (m, 1H, Ser C*H*H R2), 3.79 – 3.70 (m, 1H, ThioPro NC*H*H), 3.61 – 3.54 (m, 1H, Ser C*H*H R1), 3.50 (dd, ^2J = 15.9 Hz, ^3J = 5.0 Hz, Gly C*H*H), 3.29 – 3.21 (m, 1H, Pip NC*H*H R2), 3.15 (td, ^2J = 13.4 Hz, ^3J = 2.6 Hz, Pip NC*H*H R1), 3.07 (s, 3H, CO_2CH_3 R1), 2.77 (s, 3H, CO_2CH_3 R2), 2.52 – 2.37 (m, 1H, ThioPro CHCH*H*), 2.36 – 2.25 [m, 3H, Val C*H*(CH_3)$_2$, Pip CHCH_2], 2.25 – 2.16 (m, 1H, ThioPro CHC*H*H), 2.06 – 1.98 (m, 2H, ThioPro NCH_2CH_2), 1.85 – 1.66 (m, 4H, Leu CH*H*, Pip NCH_2CH_2, Pip $CHCH_2CH$*H*), 1.64 (d, ^3J = 7.0 Hz, 3H, Ala CH_3 R1), 1.59 – 1.40 (m, 3H, Leu C*H*HC*H*(CH_3)$_2$, Pip $CHCH_2CH$H), 1.35 – 1.29 (m, 3H, Ala CH_3 R2), 1.28 – 1.22 (m, 3H, Thr CH_3 R1), 1.17 (d, ^3J = 6.3 Hz, 3H, Thr CH_3 R2), 1.08 (d, ^3J = 6.6 Hz, 3H, Val $CHCH_3CH_3$ R2), 0.99 (d, ^3J = 6.5 Hz, 3H, Val $CHCH_3CH_3$ R1), 0.95 – 0.81 [m, 9H, Val $CHCH_3CH_3$, Leu $CH(CH_3)_2$].

$^{\lozenge}$Signals overlap.

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 204.1 (s, CS R2), 203.4 (s, CS R1), 173.8 (s, Ala CO R1), 173.3 (s, Ala CO R2), 173.1 (s, Leu CO R2), 172.9 (s, Leu CO R1), 171.7 (s, Pip CO R2)$^{\clubsuit}$, 171.5 (s, Pip CO R1)$^{\clubsuit}$, 171.4 (s, Thr CO)$^{\clubsuit}$, 171.0 (s, Gls CO)$^{\blacklozenge}$, 170.9 (s, Val CO)$^{\blacklozenge}$, 170.8 (s, Gly CO R1)$^{\blacklozenge}$, 170.4 (s, Gly CO R2)$^{\blacklozenge}$, 169.3 (s, Ser CO), 71.0 (d, Gls CH R1), 70.6 (d, Gls CH R2), 69.6 (d, ThioPro CH R1), 68.9 (d, ThioPro CH R2), 67.8 (d, Thr CHOH R2), 67.0 (t, Gls CH_2 R2), 66.3 (t, Gls CH_2 R1), 65.6 (d, Thr CHOH R1), 65.6 (d, Val NMe*C*H R2), 64.5 (d, Val NMe*C*H R1), 62.3 (t, Ser CH_2 R2), 60.9 (d, Ser CH_2 R1), 59.9 (t, Ser CH), 55.5 (d, Thr CHNH R1), 53.5 (d, Pip CH R2), 53.0 (d, Pip CH R1), 52.0 (d, Thr CHNH R2), 50.4 (d, Leu CHNH R2), 49.2 (d, Leu CHNH R1), 48.8 (t, ThioPro NCH_2), 48.6 (d, Ala CH), 48.4 (d, ThioPro NCH_2 R2), 44.5 (t, Pip NCH_2 R1), 43.6 (t, Pip NCH_2 R2), 41.7 (t, Leu CH_2 R2), 41.4 (t, Leu CH_2 R1), 40.3 (t, Gly CH_2 R1), 39.6 (t, Gly CH_2 R2), 32.7 (q, Val NCH_3 R1), 32.7 (t,

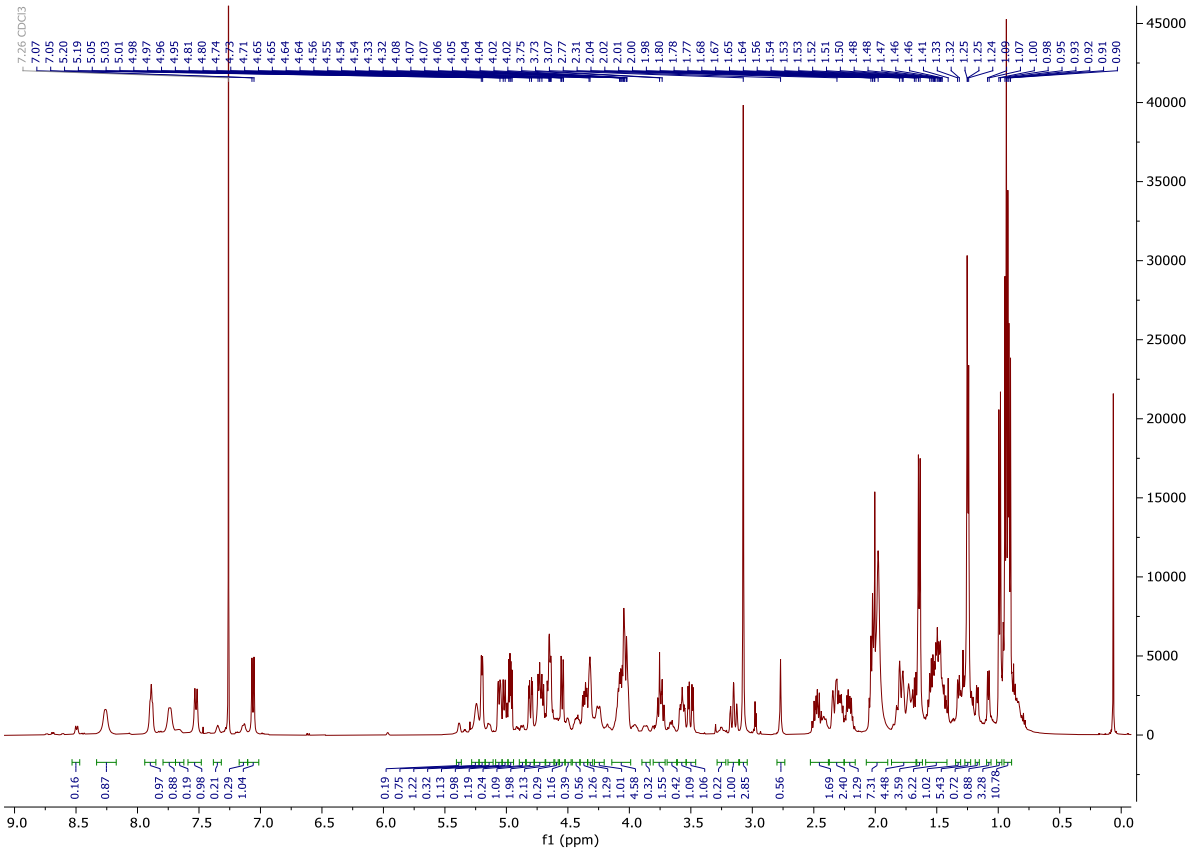
ThioPro CH*C*H$_2$ R1), 32.1 (t, ThioPro CH*C*H$_2$ R2), 29.9 (q, Val NCH$_3$ R2), 26.5 [d, Val *C*H(CH$_3$)$_2$ R1], 26.1 [d, Val *C*H(CH$_3$)$_2$ R2], 25.5 (t, Pip CHCH$_2$*C*H$_2$ R1), 25.4 (t, Pip CHCH$_2$*C*H$_2$ R2), 24.9 [d, Leu *C*H(CH$_3$)$_2$]♠, 24.8 (t, Pip CH*C*H$_2$)♠, 24.6 (t, ThioPro CH$_2$*C*H$_2$ R1)♠, 24.4 (t, ThioPro CH$_2$*C*H$_2$ R2)♠, 23.4 (q, Leu CH$_3$ R2), 23.0 (q, Leu CH$_3$ R1), 22.8 (q, Leu CH$_3$ R2), 22.7 (q, Leu CH$_3$ R1), 20.7 (q, Thr CH$_3$ R1), 20.4 (t, Pip NCH$_2$*C*H$_2$), 20.1 (q, Val CH$_3$ R1), 20.0 (q, Val CH$_3$ R2), 19.9 (q, Ala CH$_3$ R1), 19.8 (q, Thr CH$_3$ R2), 19.1 (q, Val CH$_3$), 16.8 (q, Ala CH$_3$ R2).

♦/♣/♠ Assignment interchangeable.

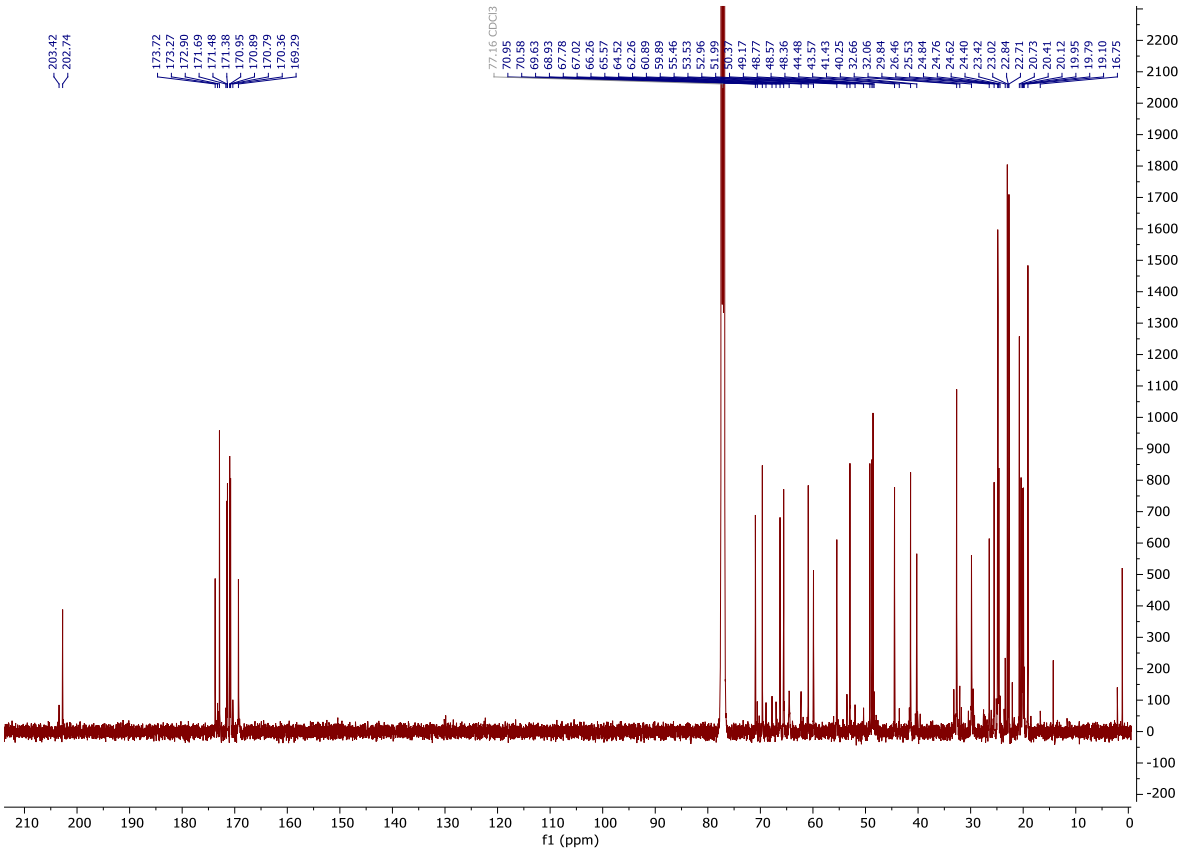
HR-MS (ESI): [C$_{38}$H$_{62}$N$_8$O$_{12}$S+H]$^+$ calculated: 855.4281; found: 855.4262.

[C$_{38}$H$_{62}$N$_8$O$_{12}$S+Na]$^+$ calculated: 877.4100; found: 877.4081.

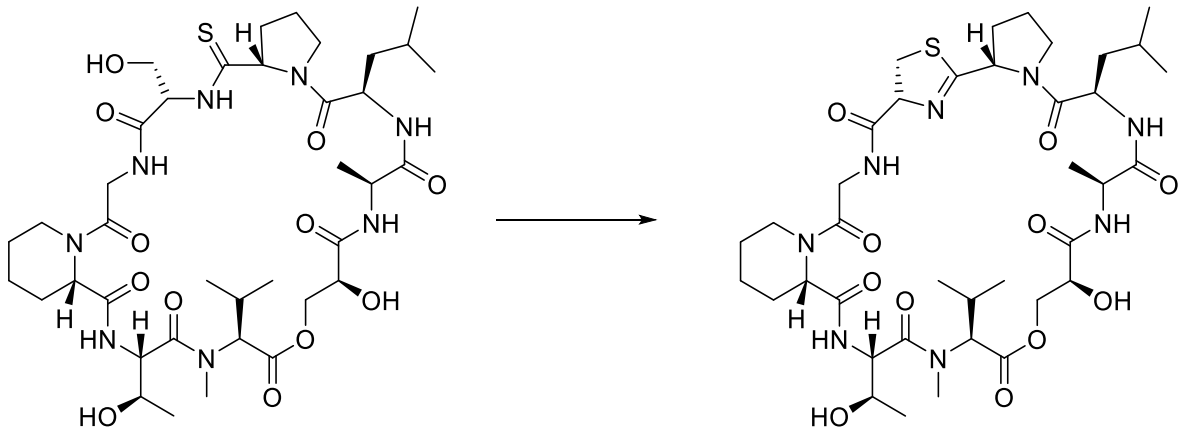
^{1}H-NMR:



^{13}C-NMR:



Gly-Vioprolide B (2)



16

2

$C_{38}H_{60}N_8O_{11}S$

M = 837.00 g/mol

A solution of macrocycle **16** (5.00 mg, 5.85 µmol, 1.00 equiv.) in THF (1 mL) is treated with *Burgess* reagent (8.51 mg, 35.7 µmol, 6.11 equiv.), and the suspension is stirred for two hours at 60 °C. Subsequently, NaCl solution (20 mL) is added to terminate the reaction. The mixture is extracted with EtOAc (3 × 15 mL), and the combined organic phases are dried over Na_2SO_4 and filtered. The solvent is removed under reduced pressure, yielding approximately 9 mg of a colorless solid, which is still contaminated with starting materials and reagents. This is further purified by semi-preparative HPLC (*Phenomenex* Luna 5u Silica, 20 × 250 mm, ^{i}PrOH/*n*-Heptane = 30/70, r.t., 15 mL·min−1, 215 nm, t_R (**2**) = 14.9 min, injection concentration: 9 mg·mL−1). After lyophilization (using ultra-pure water), Gly Vioprolid B (**2**, 4.06 mg, 4.85 µmol, 83%) is obtained as an amorphous, colorless solid.

TLC: R_f = 0.38 (CH_2Cl_2/MeOH = 9/1) [UV, $KMnO_4$].

M.p.: 159 °C.

Specific rotation: $[\alpha]_D^{RT}$ = −126.0 (*c* = 10.0, $CHCl_3$).

IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3326 (m, OH), 2960 (m, -(CH_2)−H), 1742 (s, C=O), 1639 (s, C=O), 1520 (s), 1447 (m), 1262 (m), 1124 (m, C=O), 1017 (m), 753 (m).

Rotamer ratio: R1/R2 ≈ 59/41.

^{1}H-NMR (500 MHz, $CDCl_3$, 298 K): δ (ppm) = 8.10 (s, 1H, Gly NH R1), 7.92 (s, 1H, Thr NH R2), 7.67 (s, 1H, Ala NH R1), 7.59 (d, ^3J = 9.3 Hz, 1H, Thr NH R1), 7.47 (s, 1H, Gly NH R2), 7.25 (s, 1H, Ala NH R2)*, 7.16 (s, 1H, Leu NH R2), 7.08 (d, ^3J = 8.5 Hz, 1H, Leu NH R1), 5.41 – 5.37 (m, 1H, Pip CH R2), 5.21 (d, ^3J = 5.5 Hz, 1H, Pip CH R1), 4.94 (q, ^3J = 7.6 Hz, 1H, Ala CH R1), 4.87 (d, ^3J = 8.9 Hz, 1H, Thr C*H*NH R2), 4.81 – 4.61 (m, 3H, Gls CH*H* R1, Gly CH*H*, Leu CH), 4.57 (d, ^3J = 8.9 Hz, 1H, Thr C*H*NH R1), 4.52 – 4.39 (m, 5H, Gls CH*H* R2, Ala CH R2, ThioPro CH, Val CH R1, Thz CH R2), 4.36 – 4.25 (m, 3H, Thz CH R1, Pip NCH*H* R1, Gls C*H*H R2), 4.25 – 4.17 (m, 1H, Val CH R2), 4.13 – 3.94 (m, 5H, Gls C*H*H R1, Gls CH, Thr C*H*OH, Thz CH*H*, ThioPro NCH*H*), 3.78 – 3.72 (m, 1H, Pip NCH*H* R2), 3.69 – 3.62 (m, 2H, ThioPro NC*H*H R1, Pip NC*H*H R2), 3.60 – 3.46 (m, 3H, Gly C*H*H, ThioPro NC*H*H R2, Pip NC*H*H R1), 3.19 – 3.11 (m, 1H, Thz C*H*H), 3.07 (s, 3H, Val NCH_3 R1), 2.78 (s, 3H, Val NCH_3 R2), 2.36 – 2.23 [m, 5H, Val C*H*(CH_3)$_2$, ThioPro CHCH_2 R2, Pip NCHCH_2], 2.18 – 1.96 (m, 5H, ThioPro CHCH_2 R1, Pip NCH_2C*H*H, ThioPro $CHCH_2$CH_2), 1.86 – 1.65 (m, 3H, Pip NCH_2CH*H*, Leu CH*H*CH(CH_3)$_2$, Pip $CHCH_2$CH*H*), 1.62 (d,

^{3}J = 6.9 Hz, 3H, Ala CH_3 R1), 1.60 – 1.38 (m, 3H, Leu C*H*HC*H*$(CH_3)_2$, Pip $CHCH_2CHH$), 1.32 (d, ^{3}J = 7.1 Hz, 3H, Ala CH_3 R2), 1.23 (d, 3H, ^{3}J = 6.4 Hz, 3H, Thr CH_3 R1), 1.17 (d, ^{3}J = 6.4 Hz, 3H, Thr CH_3 R2), 1.11 – 1.05 (m, 3H, Val $CHCH_3CH_3$ R2), 0.99 (d, ^{3}J = 6.5 Hz, 3H, Val $CHCH_3CH_3$ R1), 0.96 – 0.76 [m, 9H, Val $CHCH_3CH_3$, Leu $CH(CH_3)_2$].

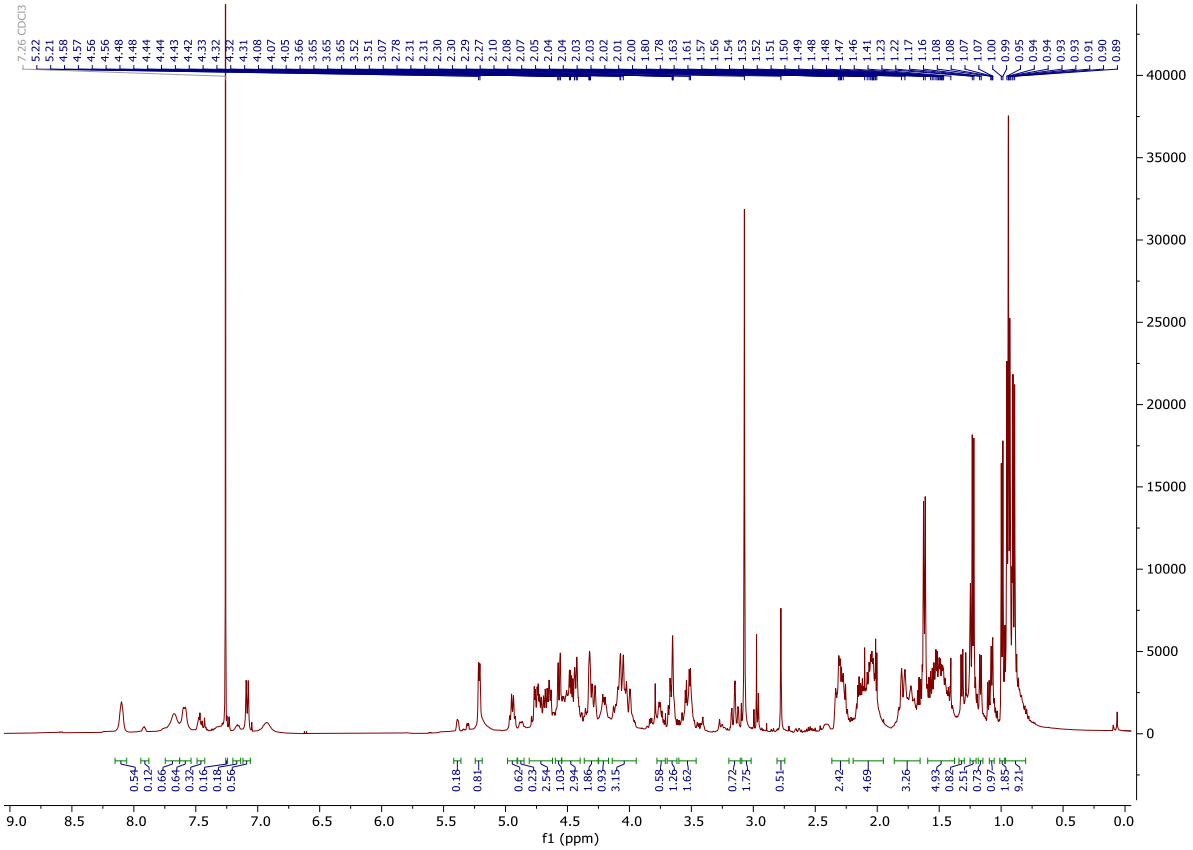
*Signal and residual solvent proton signal overlap.

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 173.8 (s, NCS R1), 173.2 (s, NCS R2), 172.9 (s, Thr CO R2), 172.8 (s, Ala CO R2), 172.8 (s, Thr CO R1), 172.8 (s, Ala CO R1), 172.0 (s, Gls CO R2), 171.4 (s, Gls CO R1), 171.3 (s, Thz CO R1), 171.2 (s, Thz CO R2), 171.1(s, Leu CO R1)♣, 171.0 (s, Val CO R1)♣, 170.8 (s, Pip CO R1), 170.8 (s, Gly CO R1), 170.6 (s, Leu CO R2)♦, 170.5 (s, Pip CO R2)♦, 170.4 (s, Gly CO R2)♦, 169.5 (s, Val CO R2), 71.0 (d, Thz CH R1), 70.7 (d, Thz CH R2), 67.8 (d, Thr CHOH R1), 67.1 (d, Thr CHOH R2), 66.9 (t, Gls CH_2 R2), 66.3 (t, Gls CH_2 R1), 65.6 (d, Gls CH R1), 65.5 (d, Gls CH R2), 62.7 (d, ThioPro CH R2), 61.9 (d, ThioPro CH R1), 61.4 (t, Pip NCH_2 R1), 61.3 (t, Pip NCH_2 R2), 55.3 (d, Thr CHNH R1), 54.8 (d, Val NMe*C*H R1), 53.9 (d, Val NMe*C*H R2), 53.5 (d, Pip CH R2), 52.8 (d, Pip CH R1), 52.2 (d, Thr CHNH R2), 50.8 (d, Ala CH R2), 49.4 (d, Ala CH R1), 48.4 (Leu CH), 48.1 (t, ThioPro NCH_2 R1), 47.7 (t, ThioPro NCH_2 R2), 44.4 (t, Thz CH_2 R1), 43.6 (t, Thz CH_2 R2), 41.2 (t, Leu CH_2), 40.3 (t, Gly CH_2 R1), 39.5 (t, Gly CH_2 R2), 39.1 (q, Val NCH_3 R2), 29.8 (t, ThioPro NCH*C*H_2 R1), 29.4 (q, Val NCH_3 R1), 29.2 (t, ThioPro NCH*C*H_2 R2), 28.4 [d, Val *C*H$(CH_3)_2$ R2], 26.5 [d, Val *C*H$(CH_3)_2$ R1], 25.7 (t, Pip NCH*C*H_2 R2), 25.5 (t, Pip NCH*C*H_2 R1), 25.4 (t, ThioPro NCH_2*C*H_2 R2), 25.0 (ThioPro NCH_2*C*H_2 R1), 24.9 (t, Pip NCH_2*C*H_2), 24.7 [d, Leu *C*H$(CH_3)_2$], 23.4 (q, Leu CH_3 R2), 23.1 (q, Leu CH_3 R1), 22.6 (q, Leu CH_3 R1), 22.0 (q, Leu CH_3 R2), 21.3 (q, Val CH_3 R2), 20.6 (q, Thr CH_3 R1), 20.4 (q, Val CH_3 R1), 20.2 (t, Pip $CHCH_2$*C*H_2 R1), 20.2 (q, Thr CH_3 R2), 19.8 (q, Ala CH_3 R1), 19.7 (q, Pip $CHCH_2$*C*H_2 R2), 19.2 (q, Val CH_3 R2), 19.1 (q, Val CH_3 R1), 18.6 (q, Ala CH_3 R2).

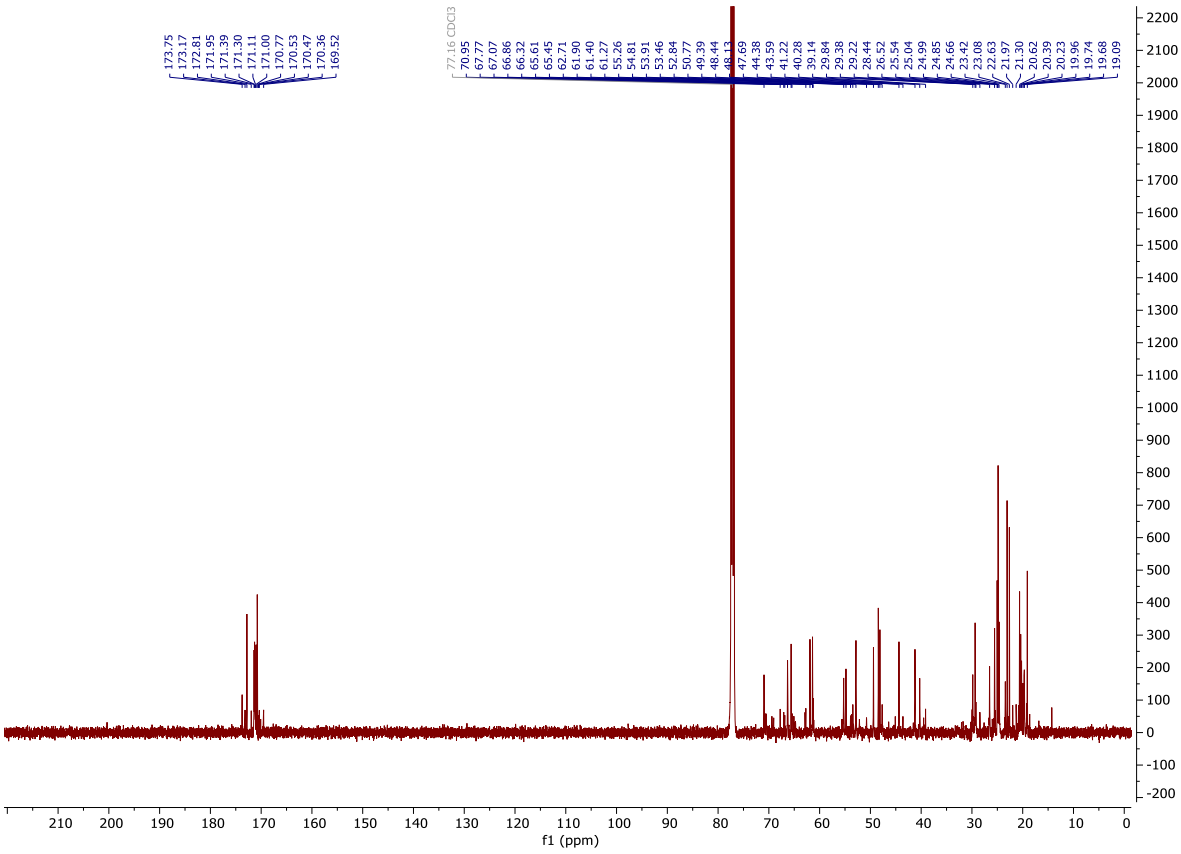
♦/♣ Assignment interchangeable.

HR-MS (ESI): $[C_{38}H_{60}N_8O_{11}S+H]^+$ calculated: 837.4175; found: 837.4160.

^{1}H-NMR:



^{13}C-NMR:



6 Comparison of the NMR spectra

Table 1: Comparison of the 1H-NMR spectra of Vioprolide B (**1**) and Gly-Vioprolide B (**2**) with indication of the chemical shift (δ), coupling constants (J), and multiplicities (in parentheses).

	Vioprolide B (1)		**Gly-Vioprolide B (2)**	
(Amino-)acid Proton	δ [ppm]	J [Hz]	δ [ppm]	J [Hz]
Ala				
NH	7.08 (d)	9.8	7.67 (s)	-
	7.28 – 7.22 (m)	-	7.25 (s)	-
α-H	4.72 – 4.67 (m)	-	4.94 (q)	7.6
	4.79 – 4.73 (m)	-	4.52 – 4.39 (m)	-
β-H_3	1.33 (d)	7.1	1.62 (d)	6.9
	1.40 (d)	6.8	1.32 (d)	7.1
Leu				
NH	6.24 (d)	9.9	7.08 (d)	8.5
	6.68 (d)	10.0	7.16 (s)	-
α-H	4.91 – 4.85 (m)	-	4.81 – 4.61 (m)	-
	5.03 – 4.91 (m)	-	-	-
β-H_a	1.46 – 1.37 (m)	-	1.86 – 1.65 (m)	-
	1.30 - 1.19 (m)	-	-	-
β-H_b	1.57 – 1.48 (m)	-	1.60 – 1.38 (m)	-
γ-H	1.57 – 1.48 (m)	-	1.60 – 1.38 (m)	-
δ-H_3	0.98 – 0.80 (m)	-	0.96 – 0.76 (m)	-
Pro				
α-H	4.99 (dd)	8.4, 5.0	4.52 – 4.39 (m)	-
	5.11 (dd)	8.5, 5.4	-	-
β-H_a	2.40 – 2.29 (m)	-	2.36 – 2.23 (m)	-
β-H_b	2.17 – 2.10 (m)	-	2.18 – 1.96 (m)	-
	2.09 – 1.95 (m)	-	-	-
γ-H_a	2.27 – 2.17 (m)	-	2.18 – 1.96 (m)	-
γ-H_b	2.09 – 1.95 (m)	-	2.18 – 1.96 (m)	-
δ-H_a	3.72 – 3.62 (m)	-	3.69 – 3.62 (m)	-
	-	-	3.60 – 3.46 (m)	-
δ-H_b	3.88 – 3.77 (m)	-	4.13 – 3.94 (m)	-
	3.56 – 3.53 (m)	-	-	-
Thz				
α-H	5.09 – 5.04 (m)	-	4.36 – 4.25 (m)	-
	-	-	4.52 – 4.39 (m)	-
β-H_a	3.60 – 3.56 (m)	-	4.13 – 3.94 (m)	-
	3.88 – 3.77 (m)	-	-	-
β-H_b	3.60 – 3.56 (m)	-	3.19 – 3.11 (m)	-
	3.49 – 3.46 (m)	-	-	-
Dhb/Gly				
NH	8.77 (s)	-	8.10 (s)	-
	9.32 (s)	-	7.47 (s)	-

	Vioprolid B (4)		Gly-Vioprolid B (96)	
(Amino-)acid Proton	δ [ppm]	J [Hz]	δ [ppm]	J [Hz]
Pip				
α-H	5.24 (d)	5.2	5.21 (d)	5.5
	5.21 – 5.17 (m)	-	5.41 – 5.37 (m)	-
β-H_a	2.78 – 2.70 (m)	-	2.36 – 2.23 (m)	-
	3.18 – 3.09 (m)	-	-	-
β-H_b	1.57 – 1.48 (m)	-	2.36 – 2.23 (m)	-
γ-H_a	1.87 – 1.71 (m)	-	1.86 – 1.65 (m)	-
γ-H_b	1.57 – 1.48 (m)	-	1.60 – 1.38 (m)	-
δ-H_a	1.87 – 1.71 (m)	-	2.18 – 1.96 (m)	-
	1.72 – 1.66 (m)	-	-	-
δ-H_b	1.57 – 1.48 (m)	-	1.86 – 1.65 (m)	-
ε-H_a	4.10 – 4.01 (m)	-	4.36 – 4.25 (m)	-
	-	-	3.78 – 3.72 (m)	-
ε-H_b	3.40 – 3.32 (m)	-	3.60 – 3.46 (m)	-
	3.18 – 3.09 (m)	-	3.69 – 3.62 (m)	-
Thr				
NH	8.35 (d)	9.9	7.59 (d)	9.3
	7.78 (d)	7.8	7.92 (s)	-
α-H	4.95 (d)	10.1	4.57 (d)	8.9
	4.72 – 4.67 (m)	-	4.87 (d)	8.9
β-H	4.24 – 4.13 (m)	-	4.13 – 3.94 (m)	-
γ-H_3	1.17 – 1.11 (m)	-	1.23 (d)	6.4
	1.30 – 1.19 (m)	-	1.17 (d)	6.4
Val				
N-CH_3	3.52 (s)	-	3.07 (s)	-
	2.78 – 2.70 (m)	-	2.78 (s)	-
α-H	4.28 (d)	10.6	4.52 – 4.39 (m)	-
	3.29 – 3.23 (m)	-	4.25 – 4.17 (m)	-
β-H	2.60 – 2.51 (m)	-	2.36 – 2.23 (m)	-
	2.27 – 2.17 (m)	-	-	-
γ-H_3	1.17 – 1.11 (m)	-	0.99 (d)	6.5
	1.06 (d)	6.5	1.11 – 1.05 (m)	-
γ-H_3	0.98 – 0.80 (m)	-	0.96 – 0.76 (m)	-
Gls				
α-H	4.65 – 4.55 (m)	-	4.13 – 3.94 (m)	-
	4.41 – 4.36 (m)	-	-	-
β-H_a	4.65 – 4.55 (m)	-	4.13 – 3.94 (m)	-
	4.10 – 4.01 (m)	-	4.36 – 4.25 (m)	-
β-H_b	4.24 – 4.13 (m)	-	4.81 – 4.61 (m)	-
	4.81 (d)	10.8	4.52 – 4.39 (m)	-

Table 2: Comparison of the 13C-NMR spectra of Vioprolide B (**1**) and Gly-Vioprolide B (**2**).

	Vioprolide B (1)	**Gly-Vioprolide B (2)**
(Amino-)acid Carbon atom	δ [ppm]	δ [ppm]
Ala		
CO	171.7	172.8
C-α	47.6, 47.7	49.4, 50.8
C-β	18.0, 19.1	19.8, 18.6
Leu		
CO	171.5, 171.2	171.1, 170.6
C-α	49.1, 48.9	48.4
C-β	40.0, 40.2	41.2
C-γ	24.7, 24.4	24.7
C-δ	22.9, 22.8	23.4, 23.1
C-δ’	22.6, 22.5	22.6, 22.0
Pro		
CO	180.5, 181.1	173.8, 173.2
C-α	60.2	61.9, 62.7
C-β	30.4, 29.5	29.8, 29.2
C-γ	25.3, 25.5	25.0, 25.4
C-δ	47.7, 48.0	48.1, 47.7
Thz		
CO	169.5, 171.2	171.3, 171.2
C-α	77.8, 76.8	71.0, 70.7
C-β	36.5, 37.8	44.4, 43.6
Pip		
CO	169.2, 170.2	170.8, 170.5
C-α	53.9, 53.1	52.8, 53.5
C-β	26.7, 26.1	25.5, 25.7
C-γ	21.1, 21.2	20.2, 19.7
C-δ	25.6, 25.5	24.9
C-ε	46.2, 46.3	61.4, 61.3
Thr		
CO	172.9, 172.4	172.8, 172.9
C-α	51.8, 56.2	55.3, 52.2
C-β	68.0, 66.8	67.8, 67.1
C-γ	19.7, 20.1	20.6, 20.2
Val		
CO	169.6, 169.3	171.0, 169.5
N-CH_3	29.7, 40.7	29.4, 39.1
C-α	65.4, 70.1	54.8, 53.9
C-β	27.7, 29.0	26.5, 28.4
C-γ	21.7, 19.6	20.4, 21.3
C-γ’	19.7, 19.4	19.1, 19.2
Gls		
CO	171.4, 170.8	171.4, 172.0
C-α	69.5, 71.7	65.6, 65.5
C-β	65.7, 66.3	66.3, 66.9

7 Data Availability

Proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE[15] partner repository and can be accessed using the identifier PXD053104 (reviewer account username reviewer_pxd053104@ebi.ac.uk, password SNidZt2JyMry).

The primary data of the NMR spectra have been deposited to Zenodo and can be accessed using the DOI: 10.5281/zenodo.11576894

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