Supporting Information

Toward Tryptathionine-stapled One-Bead-One-Compound (OBOC) Libraries: Solid Phase Synthesis of a Bioactive Octretoate Analog

Antoine Blanc[†, ‡], Mihajlo Todorovic[†], Iulia Dude[£], Helen Merkens[£], François Bénard[£], David M Perrin[†, *]

[†]Chemistry Department, UBC, 2036 Main Mall, Vancouver, V6T-1Z1 Canada

[£]Molecular Oncology, British Columbia Cancer Agency Research Centre, 675 West10th Avenue, Vancouver, BC, V5Z 1L3, Canada

[‡]Present Addresses: Department of Biochemistry and Chemistry, SUNY Oneonta, 108 Ravine Pkwy, Oneonta, NY 13820, USA

* E-mail: dperrin@chem.ubc.ca

1. Introductory Section

All reactions requiring the exclusion of air and/or moisture were conducted in flame-dried glassware under an argon atmosphere. Dry solvents such as DCM, DMF, EtOAc and MeCN were prepared by allowing HPLC grade solvents to remain in contact with flamed-dried 3 Å or 4 Å molecular sieves.[1] Reactions requiring a set temperature were performed using a mineral oil bath and a temperature controlled hot plate (IKA Ceramag Midi equipped with an IKA ETS-D4 Fuzzy thermometer). Reactions conducted below room temperature were performed in an ice/water bath (~ 4°C) or dry ice/acetone bath (-78°C). Solvents were evaporated under reduced pressure using a Büchi rotary evaporator. Lyophilisation of water, water/MeCN mixture with or without formic acid proceeded by sublimation below 200 mTorr after freezing the solution in a dry ice/acetone bath. A brine solution refers to a saturated NaCl $_{(aq)}$ solution. 2-Chlorotrityl chloride resin (CTC) refers to merrifield chloro resin (chloromethyl-copoly(styrene-1%)-divinylbenzene, 100-200 mesh, loading of 1.00-1.60 mmole/g). All reagents and solvents were purchased at the highest quality grade from commercial suppliers and used without prior purification unless otherwise stated. All chemicals and products were stored at 4^{o}C or –20^{o}C under argon. Reaction progress was followed by thin layer chromatography (TLC) on alumina backed 0.25 mm silica gel 60 aluminium sheets containing F-254 indicator. TLC analyte retention factor was reported as R_f that refers to a ratio-to-solvent front measured on plates. Visualization on TLC was monitored by different methods; using a UV lamp at 254 and 365 nm; iodine crystals embedded in silica; potassium permanganate; cerium molybdate (Hanessian's stain); ninhydrin; dinitrophenylhydrazine (DNP); bromocresol green; cinnamaldehyde according to literature procedures.[2] For acid-labile compounds, TLC plates were pre-run in 1% TEA together with the noted solvent system. Analytical RP-HPLC was performed using an Agilent 1100 HPLC, equipped with a photodiode array detector using reverse phase semi-prep columns C_{18} (4.6 x 250 mm, 5 µm, 80 Å) at a flow rate of 1 ml/min with absorbance detection at 229 nm (amide), 265 nm (Fmoc) and/or 280 nm (HPI), 290 nm (tryptathionine) or 301 nm (Fmoc). Linear gradient was achieved by combining eluents A and B as the mobile phase. eluent A was 0.1% (*v*/*v*) formic acid (FA) in Milli-Q water and eluent B was 0.1% (*v*/*v*) formic acid (FA) in MeCN. RP-HPLC

analyte chromatogram retention time was reported as t_r. The % of eluent B at which the analyte eluted relative to the retention time value was calculated as followed: (t_r - 12) • elution gradient, in which 12 min represented the column dead time in minutes and the elution gradient corresponded to (% eluent B final - % eluent B initial)/(time final – time initial). Flash chromatography was performed on silica gel F60 (230-400 mesh, 43-60 μm). For acid labile compounds, the silica gel was resuspended in 1:3 (*v*/*v*) TEA/ 10% (*v*/*v*) EtOH in DCM and stirred overnight, then filtered and washed with various organic solvents and air dried overnight. Manual reverse phase column chromatography was performed on Sep-Pak (C18) gel (Waters, Delaware). Pressure was applied manually using a syringe. Elution was achieved with a dropwise flow rate with various proportions of 0.1% (*v*/*v*) formic acid (FA) in Milli-Q water and 0.1% (*v*/*v*) formic acid (FA) in MeCN as the mobile phase. The product elution was monitored by TLC, ESI-MS, and/or RP-HPLC (18). Purification by RP-HPLC was performed on same system as the analytical RP-HPLC (C18) described above. Low-resolution mass spectrometry (LR-MS) and high-resolution mass spectrometry (HR-MS) in electrospray ionization (ESI) mode were acquired using positive or negative ionization mode in MeOH or MeCN on a Waters ZQ equipped with ESCI ion source and on a Micromass LCT time-of-flight spectrometer respectively. Nuclear magnetic resonance spectra were recorded in deuterated solvents. Proton (^{1}H-NMR), and carbon (^{13}C-NMR) nuclear magnetic resonance spectra were recorded using a 400 MHz and 100 MHz NMR spectrometer respectively using standard pulse sequences. Chemical shifts for all spectra were reported in parts per million (ppm) relative to tetramethylsilane referenced to the residual solvent peak signal. UV spectra were recorded on a spectrophotometer in 1 mL quartz cuvettes. Colorimetric resin analysis was performed by a Kaiser or Green Malachite test according to literature procedures.[3] Resin loading with Fmoc protected amino acids was determined by Fmoc cleavage and quantification of the released dibenzofulvene (DBF) groups.[4, 5]

2 Experimental Details Section

2.1 General Fmoc/CAM Solid-phase Peptide Synthesis (SPPS) Procedure on TentaGel Macrobeads with Tartrate-Based Linker

Unless stated otherwise, the procedure was performed by manual solid-phase peptide synthesis carried out in a Zeba^{TM} spin desalting column (Pierce, 5 mL) with vortex mixing on TentaGel macrobeads amine resin using standard Fmoc chemistry. If a mini Zeba^{TM} spin desalting column (Pierce, 0.5 mL) was used, the volumes were scaled down to between one ninth and one tenth and reagent equivalents were scaled accordingly.

Swelling and loading procedure: The resin (0.35 g, ~ 87 µmol) was filtered with 1:1:3 (*v*/*v*/*v*) dry DMF/dry MeCN/dry EtOAc (10 mL). The resin was resuspended in 1:1:3 (*v*/*v*/*v*) dry DMF/dry MeCN/dry EtOAc (6 mL), shaken for 30 minutes, drained by filtration and the procedure was repeated two more times with fresh solvent. The resin was resuspended in a solution of clicked dipeptide on tartrate-based linker **SI-11** (4 equivalents, 0.06 M), PyBOP (5 eq.), HOBt hydrate (4 eq.) and DIPEA (30 eq.) in 1:1:3 (*v*/*v*/*v*) dry DMF/dry MeCN/dry EtOAc to give pH ~ 9.5. After 5 hours 30 minutes of shaking in the dark, a resin sample was taken and a Kaiser test applied showing qualitatively a low resin loading. Consequently, additional PyBOP (11 eq.) and DIPEA (10 eq.) was added to the mixture of resin to yield a pH ~ 9.5 mixture which was further shaken overnight in the dark. The resin was drained by filtration and further filtered with 1:1:3 (*v*/*v*/*v*) dry DMF/dry MeCN/dry EtOAc (10 ml). The resin was resuspended in 1:1:3 (*v*/*v*/*v*) dry DMF/dry MeCN/dry EtOAc (6 mL), shaken, drained by filtration and the procedure was repeated six more times with fresh solvent. The resin was capped as described in the Fmoc-protected amino acid coupling procedure to yield a loading of 0.12 mmol/g (*Fmoc loading test*).

Fmoc-deprotection procedure: If the previous step was not done in DMF then the resin was shaken 3 x 10 minutes in dry DMF and drained by filtration each time. The resin (0.35 g) was resuspended in clear solution of 20% (*v*/*v*) diethylamine in dry DMF (5 mL) at pH ~ 11, shaken for 2 hours 30 minutes, drained by filtration and filtered with dry DMF (15

ml). The resin was resuspended in dry DMF (6 mL), shaken, drained by filtration and the procedure was repeated six more times with fresh solvent. The filtration and the shaking procedures were repeated using 1:1 (*v*/*v*) EtOH/EtOAc, dry MeCN, dry EtOAc, dry DCM. A Kaiser test was performed. If the following step was peptide coupling, then the resin was further washed with 1:1:3 (*v*/*v*/*v*) dry DMF/dry MeCN/dry EtOAc.

Fmoc-protected amino acid coupling procedure: The resin (0.35 g) was resuspended in a clear yellow solution comprising the noted N^{α}-Fmoc-protected amino acid (5 equivalents, 0.08 M), Oxyma Pure (5 eq.), COMU (5 eq.), DIPEA (12 eq.) in 1:1:3 (*v*/*v*/*v*) dry DMF/dry MeCN/dry EtOAc at pH ~ 10. The resin was shaken for a maximum of 90 min. Upon completion of reaction, estimated by Kaiser test, the resin was drained by filtration. The resin was washed according to the following procedure: The resin was filtered with 1:1:3 (*v*/*v*/*v*) dry DMF/dry MeCN/dry EtOAc (15 ml). The resin was resuspended in 1:1:3 (*v*/*v*/*v*) dry DMF/dry MeCN/dry EtOAc (6 mL), shaken, drained by filtration and the procedure was repeated six more times with fresh solvent. A Kaiser test and *Fmoc loading test* were performed. Following each coupling step, a capping step was performed as follows. The resin (0.35 g) was resuspended in 1:2:2 (*v*/*v*/*v*) Ac_2O/TMP/dry EtOAc (5 mL) at pH ~ 7 and shaken for 1 h, then drained by filtration. The resin was washed according to the following procedure: the resin was filtered with 1:1:3 (*v*/*v*/*v*) dry DMF/dry MeCN/dry EtOAc (15 ml). The resin was resuspended in 1:1:3 (*v*/*v*/*v*) dry DMF/dry MeCN/dry EtOAc (6 mL), shaken, drained by filtration and the procedure was repeated six more times with fresh solvent. The filtration and the shaking procedures were repeated using dry MeCN, dry EtOAc, dry DCM. If the following step was Fmoc-deprotection, then the resin was further washed with dry DMF otherwise the resin was stored dry at 4^{o}C.

HPI derivatives coupling procedure[6–9]: The resin (0.35 g) was resuspended in a clear yellow solution of 0.33 M Boc-HPI in dry EtOAc (4 equivalents, 0.06 M final), Oxyma Pure (4 eq.), COMU (4 eq.), DIPEA (11 eq.) in 1:1:3 (*v*/*v*/*v*) dry DMF/dry MeCN/dry EtOAc at pH ~ 10. The resin was shaken overnight in the dark and drained by filtration. The resin was washed as done for Fmoc-protected amino acid coupling procedure and stored dry at 4^{o}C.

Tartrate acetonide deprotection: If the resin was already swollen by treatment with a polar organic solvent from previous step, the resin was then filtered in AcOH (10 mL). The resin (0.35 g) was resuspended in AcOH (6 mL), shaken and drained by filtration and the procedure was repeated four more times with fresh solvent. The dry resin or swollen resin in AcOH was resuspended in 95:2.5:2.5 (*v*/*v*/*v*) TFA/ H_2O/TIS (5 mL), shaken for 2 hours in the dark and the solution was drained by filtration. The resin was washed according to the following procedure: the resin was filtered with 1:9 (*v*/*v*) toluene/AcOH (20 mL). The resin was resuspended in 1:9 (*v*/*v*) toluene/AcOH (6 mL), shaken, drained by filtration and the procedure was repeated five more times with fresh solvent. The filtration and the shaking procedures were repeated using 2:8 (*v*/*v*) EtOH/EtOAc or CPME, MeCN, EtOAc, DCM. At this stage, the resin was either dried over P_2O_5 under vacuum in a desiccator and used the following day or stored at 4^{o}C. However, if the resin was directly used for the tartrate oxidative cleavage, then the resin was further washed with MeCN prior to use.

Alkylation of cysteine thiol: The Vaccuum-P_2O_5 dried and argon flushed free diol resin (~ 1.5 mg, ~ 20 dry beads, ~ 0.18 µmole) in a mini Zeba^{TM} spin desalting column (Pierce, 0.5 mL) was resuspended in a solution of 12 mM bromoacetamide (33 eq.), 0.7 mM tetrabutylammonium iodide (TBAI) (2 eq.), 1.7 mM (tris(2-carboxyethyl)phosphine) (TCEP) (5 eq.) in dry DMF (0.5 mL) of pH ~ 4, followed by addition of a solution of 500 mM DIPEA (15 eq.) in dry DMF (0.006 mL) of pH ~ 11 to give a final pH ~ 8 clear, pale yellow, solution. The resulting mixture was shaken for 1 h 30 min under argon in the dark and drained by gravity under an argon layer, the previous step was repeated once and the resin was drained by filtration. The resin was filtered with DMF (3 mL). The resin was resuspended in DMF (0.6 mL), shaken, drained by filtration and the procedure was repeated four more times with fresh solvent. The filtration and the shaking procedures were repeated using 1:1 (*v*/*v*) EtOH/EtOAc, MeCN, EtOAc, DCM and the resin was dried under vacuum.

Oxidative cleavage of products from tartrate-based linker resin: The resin (0.22 g, ~ 26 µmole) was filtered in 1:1:4 (*v*/*v*/*v*) *tert*-BuOH/AcOH/Milli-Q water (20 mL) pH ~ 1. The resin was resuspended in 1:1:4 (*v*/*v*/*v*) *tert*-BuOH/AcOH/Milli-Q water (6 mL), shaken for 30 minutes, drained by filtration and the procedure was repeated two more times with

fresh solvent. The resin was resuspended in a solution of 35 mM $NaIO_4$ (7 eq.) in 1:1:4 (*v*/*v*/*v*) *tert*-BuOH/AcOH/Milli-Q water pH ~ 1, and shaken for 10 min in the dark. The supernatant was collected by filtration. The resin was drained by filtration with 1:1:4 (*v*/*v*/*v*) *tert*-BuOH/AcOH/Milli-Q water (10 mL) and the filtrate was pooled with the previous filtrate. The resin was resuspended in 1:1:4 (*v*/*v*/*v*) *tert*-BuOH/AcOH/Milli-Q water (6 mL), shaken and drained by filtration and the filtrate was pooled with the previous one. The shaking procedure was repeated seven more times with fresh solvent. Then, the pooled filtrate was treated with dimethyl sulfide (33 equivalents relative to $NaIO_4$) to quench excess sodium *meta*-periodate and the mixture turned out to be a clear brown solution. Products were further collected from the resin by repeating the filtration and the shaking procedures with MeCN. To the brown turbid mixture was added a solution of 0.1 M aqueous sodium thiosulfate (7 equivalents relative to $NaIO_4$) to quenched iodine by-products until the mixture turned out to be clear and colorless. When no additional *N*-glyoxyloyl and in some case amines and/or fluorescent containing products eluting from resin were detected by TLC (2,4-Dinitrophenylhydrazine dye for *N*-glyoxyloyl, ninhydrin dye for amine), the volatiles were evaporated under reduced pressure to afford a *N*-glyoxyloyl peptide as a crude white solid. In some cases, the resin was further washed, as described above, with DCM and the filtrate pooled with the previous filtrate. The resin was stored in either 1:1:4 (*v*/*v*/*v*) *tert*-BuOH/AcOH/Milli-Q water, MeCN or DCM at 4^{o}C for a night. The following day, the filtrate was analyzed by TLC/LR-ESI-MS to make sure no additional product was left on resin. If product was detected, then the resin was washed with the previous solvent system used until all the product was recovered and no more products could be eluted.

Oximation of *N*-glyoxyloyl peptide with *O*-benzylhydroxylammonium chloride: In several cases of small scale reactions (~ 20 dry beads, 1.5 mg, ~ 0.18 µmole), the *N*-glyoxyloyl crude peptide mixture from the previous step was not evaporated to dryness but instead the volume was reduced to 0.5 mL or less by rotoevaporation and directly resuspended in the oximation mixture to yield the desired oxime according to the procedure applied to larger scale (0.22 g, ~ 26 µmole) as follows. The dry *N*-glyoxyloyl crude peptide was dissolved in a solution of MeCN (10 mL), saturated $KH_2PO_{4(aq)}$ (10 mL) buffered to pH ~

6 with 1 M $NaOH_{(aq)}$. To the mixture, O-benzylhydroxylammonium chloride (0.034 g, 0.210 mmol, ~ 8 eq.), 1,4-phenylenediamine hydrochloride (0.114 g, 0.631 mmol, ~ 24 eq.), MeCN (15 mL), Milli-Q (5 mL), saturated $KH_2PO_{4(aq)}$ (6.5 mL) buffered to pH ~ 6 with 1 M $NaOH_{(aq)}$ and additional 1 M $NaOH_{(aq)}$ (3 mL) were added to give a clear pale yellow solution at pH ~ 6.5. The resulting mixture was stirred for 14 h in the dark and evaporated to dryness under reduced pressure to afford *N*-glyoxyloyl crude peptide as a white solid. However, in the case of the small scale (~ 20 beads), the reaction mixture was diluted with 0.1% FA (*v*/*v*) in Milli-Q to lower the proportion of MeCN to under 5% in volume. Otherwise, the *N*-glyoxyloyl crude peptide white solid was dissolved in 0.1% FA (*v*/*v*) of 1.5:8.5 (*v*/*v*) MeCN/Milli-Q water. In both cases the crude mixture was loaded on Sep-Pak (C18) column (Waters, Delaware) pre-equilibrated with 0.1% FA (*v*/*v*) in Milli-Q water and purified by manual reverse phase column chromatography with increasing proportions of 0.1% FA (*v*/*v*) in MeCN against 0.1% FA (*v*/*v*) in Milli-Q water as described in *General Experimental Information*. The crude was further purified by RP-HPLC (C18) with gradient of 20% to 100% of 0.1% FA (*v*/*v*) in MeCN against 0.1% FA (*v*/*v*) in Milli-Q water over 30 min as described in *General Experimental Information*. Then further purification was performed by RP-HPLC (C18) with gradient of 20% to 100% of 0.1% FA (*v*/*v*) in MeCN against 0.1% FA (*v*/*v*) in Milli-Q water over 18 min at 2 ml/min. Oximes were reported to undergo hydrolysis upon freeze-thaw cycling.[10]

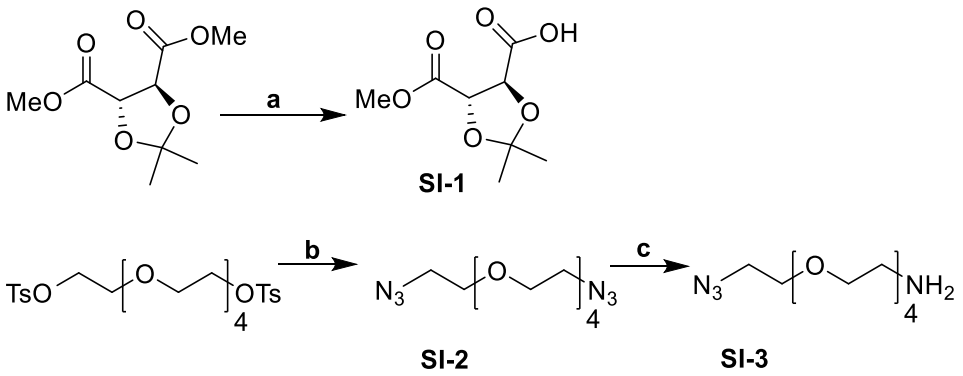
2.2 General Savige-Fontana Reaction Procedure on TentaGel Macrobeads[6–9]

The dry resin (0.35 g, ~ 38 µmol) was resuspended in TFA (5 mL), shaken for 4 h 30 min to 5 h in the dark and drained by filtration. The resin was washed according to the following procedure: the resin was filtered with 1:9 (*v*/*v*) Toluene/AcOH (15 ml). The resin was resuspended in 1:9 (*v*/*v*) Toluene/AcOH (6 mL), shaken, drained by filtration and the procedure was repeated nine more times with fresh solvent. The filtration and the shaking procedures were repeated using 1:1 (*v*/*v*) toluene/EtOAc, MeCN, 1:1 (*v*/*v*) EtOH/EtOAc, MeCN, EtOAc and DCM. A Kaiser test was performed, and the resin was stored dry at 4°C. No alkylated by-products were observed by MS analysis due to residual

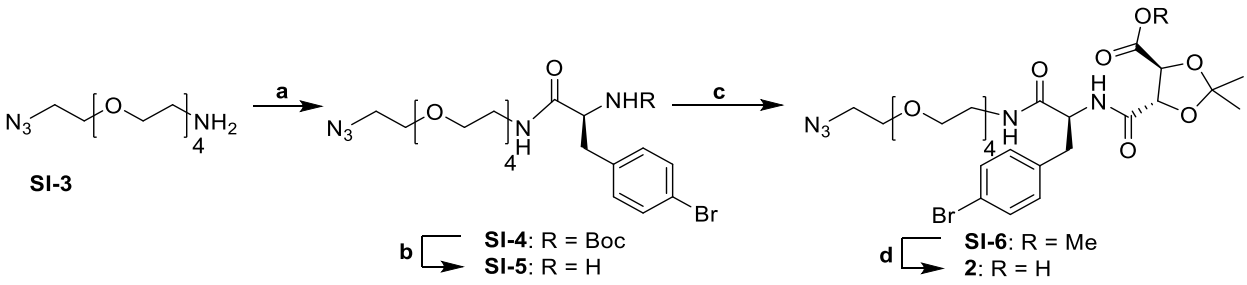
carbocations which may have happened without presence of triisopropylsilane in the washes. Note, all residual acetic acid must be washed out of the resin prior to amide bond formation.

2.3 Detailed Synthetic Procedures

2.3.1 Tartrate-Based Linker Synthesis



Scheme *S1*. First part of linker synthesis. Reagents and conditions: **a**) KOH, MeOH, 4°C to RT, 2 h, 60 %; **b**) NaN_3, TBAI, Na_2CO_3 anhydrous DMF, Ar, temperature ~ 60°C, reaction time ~ 2 h, yield ~ 96%; **c**) PPh_3, 85% (*w*/*w*) $H_3PO_{4(aq.)}$, water, CPME, 4°C, 22 h, 72%.



Scheme *S2*. Linker synthesis. Reagents and conditions: **a**) N^{α}-Boc-Phe(4-Br)-OH, Oxyma, COMU, dry EtOAc, dry MeCN, DIEA, Ar, 4°C to RT, 22 h, 63%; **b**) 1:4 (*v*/*v*) TFA/DCM, 4°C to RT, 3 h 30 min; **c**) Tartrate methyl ester (**SI-1**), COMU, Oxyma, DIEA, dry EtOAc, dry MeCN, 4°C to RT, Ar, 68 % (over two steps); **d**) 3 M NaOH in MeOH, 9:1 (*v*/*v*) DCM/MeOH, 2 h, 4°C to RT, 90 %.

(4*S*,5*S*)-2, 2-Dimethyl-1, 3-dioxolane-4, 5-dicarboxylate-4-methyl ester (SI-1). To a light clear pale yellow solution of dimethyl (4*S*,5*S*)-2,2-dimethyl-1,3-dioxolane-4,5-dicarboxylate-dimethyl ester (5.02 g, 22.9 mmol) in 1:10 (*v*/*v*) MeCN/H_2O (560 mL), 5M $KOH_{(aq)}$ (126 mL, 25.2 mmol) was added dropwise. The solution was cooled in an ice/water bath and stirred for 1 hour. The solution cooled in ice/water bath was acidified to pH ~ 2 with saturated aqueous orthophosphoric acid and extracted with EtOAc (12 x 40 mL). The organic layers were pooled, dried over anhydrous $MgSO_4$, filtered and evaporated to dryness under reduced pressure to afford (4*S*,5*S*)-2,2-dimethyl-1,3-dioxolane-4,5-dicarboxylate-4-methyl ester (**SI-1**) (3.18 g, 15.6 mmol), **Scheme *S1***, as a thick clear gum in 68% yield, R_f ~ 0.25 (0.3:1:3.7:5 AcOH/Acetone/Heptane/EtOAc). LR-MS (ESI-single quadrupole) *m/z*: calcd for $C_8H_{11}O_6$ $[M - H]^-$: 203.1, found 203.3. **^{1}H-NMR** (300 MHz, CD_2Cl_2): δ 7.00 (br s, 1H), 4.83 (d, *J* = 5.2 Hz, 1H), 4.79 (d, *J* = 5.2 Hz, 1H), 3.81 (s, 3H), 1.48 (s, 3H), 1.46 (s, 3H).

1,14-Diazido-3,6,9,12-tetraoxatetradecane (SI-2). A solution of 3,6,9,12-tetraoxatetradecane-1,14-diyl bis(4-methylbenzenesulfonate) (5.02 g, 9.18 mmol), TBAI (0.180 g, 0.460 mmol), and Na_2CO_3 (0.990 g, 9.20 mmol) dry DMF (200 mL) was treated with NaN_3 (1.79 g, 27.5 mmol) under argon. The reaction mixture was warmed up to 70°C and stirred overnight for 20 h. The reaction mixture was cooled in ice/water bath, diluted with saturated $Na_2CO_{3\,(aq)}$ (400 mL) and extracted with EtOAc (5 x 100 mL). Organic layers were pooled, dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure to afford 1,14-diazido-3,6,9,12-tetraoxatetradecane (**SI-2**) (2.60 g; 9.03 mmol), **Scheme *S1***, as a clear yellow oil in 98% yield, R_f ~ 0.25 (1:9 Heptane/Et_2O). HR-MS (ESI-TOF) *m/z*: calcd for $C_{10}H_{20}N_6O_4Na$ $[M + Na]^+$: 311.1444, found 311.1442. **^{1}H-NMR** (300 MHz, CD_2Cl_2): δ 3.67-3.61 (m, 16H), 3.38 (t, *J* = 5.2 Hz, 4H). **^{13}C-NMR** (75 MHz, APT, CD_2Cl_2): δ 70.7 (**C**H_2), 70.7 (**C**H_2), 70.6 (**C**H_2), 70.0 (**C**H_2), 50.0 (**C**H_2).

14-Azido-3,6,9,12-tetraoxatetradecane-1-amine (SI-3). A solution of 1,14-diazido-3,6,9,12-tetraoxatetradecane (**SI-2**) (2.50 g, 8.70 mmol) in $H_3PO_{4(aq)}$/$KH_2PO_{4(aq)}$ (180 mL) pH ~1 cooled in ice/water bath was treated dropwise with a solution of PPh_3 (2.23 g, 8.7 mmol) in CPME (90 mL) under vigorous stirring. The resulting turbid biphasic mixture was stirred overnight at room temperature, the aqueous layer decanted, and the latter

extracted with CPME (3 x 60 mL). The subsequent aqueous layer was basified to pH ~ 12 with 1 N NaOH and extracted with DCM (5 x 60 mL). The organic layers were pooled, dried over anhydrous $MgSO_4$ and evaporated under reduced pressure to afford 14-azido-3,6,9,12-tetraoxatetradecane-1-amine (**SI-3**) (1.50 g, 5.72 mmol), **Scheme *S1***, as a thick yellow oil in 66% yield, R_f ~ 0.2 (0.1:2:7.9 TEA/EtOH/DCM). HR-MS (ESI-TOF) *m/z*: calcd for $C_{10}H_{23}N_4O_4$ $[M + H]^+$: 263.1719, found 263.1722. Contaminated with $OPPh_3$ and S.M.: **^{1}H-NMR** (300 MHz, CD_2Cl_2): δ 3.67-3.57 (m, 16H), 3.47-3.44 (t, *J* = 5.2 Hz, 2H), 3.40-3.36 (t, *J* = 5.1 Hz, 2H). **^{13}C-NMR** (75 MHz, APT, CD_2Cl_2): δ 73.8 (**C**H_2), 72.8 (**C**H_2), 70.9 (**C**H_2), 70.7 (**C**H_2), 70.6 (**C**H_2), 70.5 (**C**H_2), 70.4 (**C**H_2), 70.4 (**C**H_2), 70.3 (**C**H_2), 70.0 (**C**H_2), 61.5 (**C**H_2), 50.9 (**C**H_2), 42.0 (**C**H_2), 40.2 (**C**H_2).

(2*S*)-3-(4'-Bromophenyl)-2-(([(2''-methyl-2''-propanyl)oxy]carbonyl)amino)-1-*N*-(14'''-azido-3''',6''',9''',12'''-tetraoxatetradecane-1'''-)-propanamide (**SI-4**). 1-Amino-14-azido-3,6,9,12-tetraoxatetradecane (**SI-3**) (1.50 g, 5.72 mmol), COMU (3.43 g, 8.00 mmol), Oxyma Pure (0.896 g, 6.30 mmol) and (2*S*)-3-(4'-bromophenyl)-2-(([(2''-methyl-2''-propanyl)oxy]carbonyl)amino)-propanoic acid (2.16 g, 6.30 mmol) were dissolved in dry 4:1 (*v*/*v*) EtOAc/MeCN (55 mL) and cooled in an ice/water bath. To the yellow clear mixture, DIPEA (3.50 mL, 20.0 mmol) was added and the mixture was stirred at room temperature for 18 hours. The clear dark orange reaction mixture was diluted with saturated $Na_2CO_{3(aq)}$ (150 mL) and extracted with EtOAc (5 x 50 mL). The organic layers were pooled, dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure to yield a thick yellow gum that was purified by flash chromatography on silica (4:2:4 to 1:1:0 EtOAc/Heptane/DCM) to afford (2*S*)-3-(4'-bromophenyl)-2-(([(2''-methyl-2''-propanyl)oxy]carbonyl)amino)-1-*N*-(14'''-azido-3''',6''',9''',12'''-tetraoxatetradecane-1'''-)-propanamide (**SI-4**) (1.68 g, 2.86 mmol), **Scheme *S2***, as a gummy yellow solid in 50% yield, R_f ~ 0.1 (4:6 EtOAc/Heptane). HR-MS (ESI-TOF) *m/z*: calcd for $C_{24}H_{38}N_5O_7BrNa$ $[M + Na]^+$: 610.1852, found 610.1860. Contaminated with *N*,*N*-dimethylmorpholine-4-carboxamide: **^{1}H-NMR** (300 MHz, CD_2Cl_2): δ 7.43 (d, *J* = 8.3 Hz, 2H), 7.11 (d, *J* = 8.3, 2H), 6.48 (br s, 1H), 5.19 (br s, 1H), 4.33-4.26 (m, 1H), 3.67-3.37 (m, 20H), 3.05 (dd, *J* =6.4; 13.1 Hz, 1H), 2.94 (dd, *J* =6.4; 13.1 Hz, 1H), 1.39 (s, 9H). **^{13}C-NMR** (75 MHz, APT, CD_2Cl_2): δ 170.8 (**C**), 155.2 (**C**), 136.4 (**C**), 131.6 (**C**H), 131.4 (**C**H),

120.6 (**C**), 79.8 (**C**), 70.7 (**C**H_2), 70.7 (**C**H_2), 70.6 (**C**H_2), 70.4 (**C**H_2), 70.0 (**C**H_2), 69.7 (**C**H_2), 66.6 (**C**H_2), 55.6 (**C**H), 50.9 (**C**H_2), 47.5 (**C**H_2), 39.4 (**C**H_2), 38.3 (**C**H_2), 28.1 (**C**H_3).

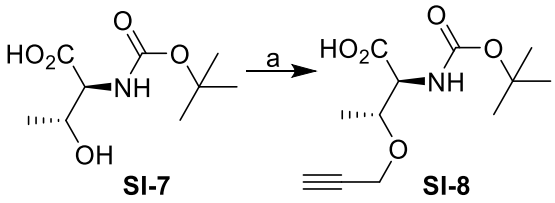
(4*S*,5*S*)-2,2-Dimethyl-1,3-dioxolane-4-carboxylate methyl ester-5-*N*-{(2'*S*)-3'-(4''-bromophenyl)-1'-*N'*-(14''''-azido-3'''',6'''',9'''',12''''-tetraoxatetradecane-1''''-)-propanamide)}-carboxamide (SI-6). A clear solution of (2*S*)-3-(4'-bromophenyl)-2-(([(2''-methyl-2''-propanyl)oxy]carbonyl)amino)-1-*N*-(14'''-azido-3''',6''',9''',12'''-tetraoxatetradecane-1'''-)-propanamide (**SI-4**) (1.35 g, 2.3 mmol) in DCM (100 mL) was treated with TFA (50 mL). The resulting pale-yellow clear solution was stirred for 2 hours and evaporated under reduced pressure to yield thick yellow oil/gum. The crude product (**18**) was used without further purification. The crude product (**SI-5**) was dissolved in 1:1:1 (v/v/v) dry EOAc/dry MeCN/ dry DMF (22 mL), cooled to 4^{o}C and DIPEA (0.8 ml, 4.6 mmol) was added. A solution of 1-methyl-2,3-*O*-isopropylidene-D-tartrate monoester (**SI-1**) (0.63 g, 2.51 mmol) in dry EtOAc (15 mL) was added to the mixture, followed by COMU (1.17 g, 2.7 mmol), Oxyma (0.325 g, 2.28 mmol) and DIPEA (1.4 mL, 8 mmol) resuting in a solution of pH ~ 9. The solution was stirred at RT overnight, and diluted with EtOAc (150 mL). The organic layer was washed with sat KH_2PO_4 (aq) (3 x 50 mL), sat $NaHCO_3$(aq) (8 x 50 mL) and brine (3 x 50 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered and the volatiles were evaporated to dryness under reduced pressure to yield a thick yellow oil, that was purified by flash chromatography on silica (1:1 EtOAc/Heptane to EtOAc) to afford (4*S*,5*S*)-2,2-dimethyl-1,3-dioxolane-4-carboxylate methyl ester-5-*N*-{(2'*S*)-3'-(4''-bromophenyl)-1'-*N'*-(14''''-azido-3'''',6'''',9'''',12''''-tetraoxatetradecane-1''''-)-propanamide)}-carboxamide (**SI-6**) (1.38 g, 2.04 mmol), **Scheme *S2***, as a thick yellow gum in 89% yield, R_f ~ 0.1 (1:9 Heptane/EtOAc. HR-MS (ESI-TOF) *m/z*: calcd for $C_{27}H_{40}N_5O_{10}BrNa$ $[M + Na]^+$: 696.1856, found 696.1858. **^{1}H-NMR** (300 MHz, CD_2Cl_2): δ 7.43 (d, *J* = 8.3 Hz, 2H), 7.11 (d, *J* = 8.3, 3H), 6.55 (br s, 1H), 4.68 (d, *J* = 5.3 Hz, 1H), 4.63-4.58 (m, 1H), 4.53 (d, *J* = 5.2 Hz, 1H), 3.79 (s, 3H), 3.66-3.38 (m, 20H), 3.10 (dd, *J* = 6.9; 14.7 Hz, 1H), 2.99 (dd, *J* = 6.9; 14.7 Hz, 1H), 1.41 (s, 3H), 1.36 (s, 3H). **^{13}C-NMR** (75 MHz, APT, CD_2Cl_2): δ 170.6 (**C**), 169.3 (**C**), 169.2 (**C**), 135.8 (**C**), 131.7 (**C**H), 131.3 (**C**H), 120.9 (**C**), 113.5 (**C**), 77.9 (**C**H), 77.6 (**C**H), 70.7 (**C**H_2), 70.7 (**C**H_2), 70.6 (**C**H_2), 70.4 (**C**H_2), 70.0 (**C**H_2), 69.5 (**C**H_2), 66.6 (**C**H_2),

53.7 (**C**H), 52.8 (**C**H_3), 50.9 (**C**H_2), 39.5 (**C**H_2), 38.1 (**C**H_2), 29.8 (**C**H_2), 26.4 (**C**H_3), 26.0 (**C**H_3).

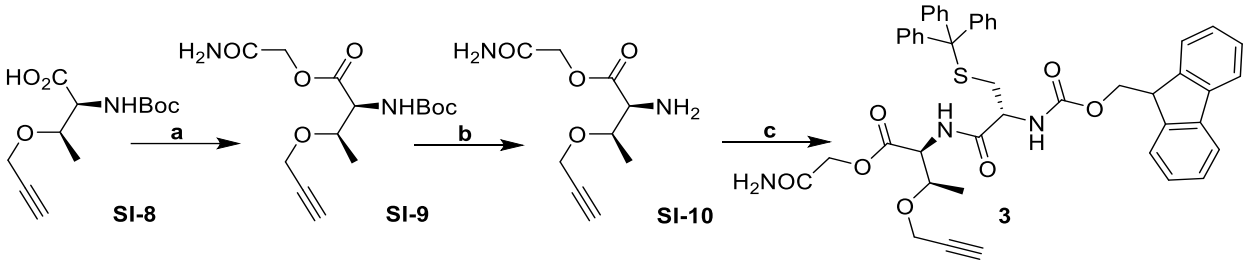
(4*S*,5*S*)-2,2-Dimethyl-1,3-dioxolane-4-carboxylic acid-5-*N*-{(2'*S*)-3'-(4''-bromophenyl)-1'-*N'*-(14''''-azido-3'''',6'''',9'''',12''''-tetraoxatetradecane-1''''-)-propanamide)}-carboxamide (2). A clear pale yellow solution of (4*S*,5*S*)-2,2-dimethyl-1,3-dioxolane-4-carboxylate methyl ester-5-*N*-{(2'*S*)-3'-(4''-bromophenyl)-1'-*N'*-(14''''-azido-3'''',6'''',9'''',12''''-tetraoxatetradecane-1''''-)-propanamide)}-carboxamide (**SI-6**) (1.37 g, 2.04 mmol) in 9:1 (*v*/*v*) DCM/MeOH at 4^{o}C was treated dropwise with a clear solution of 1N NaOH in MeOH (12.25 mL, 12.24 mmol). The resulting solution was stirred for 1 h, cooled in ice/water bath, acidified to pH ~ 4 with acetic acid and evaporated under reduced pressure to yield a crude white solid. The crude product was dissolved in saturated $KH_2PO_{4(aq)}$ (200 mL), acidified to pH ~ 3 with aqueous saturated *ortho*-phosphoric acid and extracted with EtOAc (5 x 30 mL). The organic layers were pooled, dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure to afford ((4*S*,5*S*)-2,2-dimethyl-1,3-dioxolane-4-carboxylic acid-5-*N*-{(2'*S*)-3'-(4''-bromophenyl)-1'-*N'*-(14''''-azido-3'''',6'''',9'''',12''''-tetraoxatetradecane-1''''-)-propanamide)}-carboxamide (**2**) (1.35 g, 2.04 mmol), **Scheme *S2***, as a thick yellow gum in quantitative yield, R_f ~ 0.3 (0.2:0.8:9 AcOH/EtOH/DCM). HR-MS (ESI-TOF) *m/z*: calcd for $C_{26}H_{37}N_5O_{10}Br$ $[M - H]^-$: 658.1724, found 658.1720. **^{1}H-NMR** (300 MHz, CD_2Cl_2): δ 7.45 (d, *J* = 8.4 Hz, 2H, Ar**H**$^{Phe(Br)}$), 7.40 (d, *J* = 8.5, 1H, N**H**), 7.12 (d, *J* = 8.4 Hz, 2H, Ar**H**$^{Phe(Br)}$), 6.62 (br s, 1H, N**H**), 4.71 (q, *J* = 7.0 Hz, 1H, α-**H**$^{Phe(Br)}$), 4.52 (d, *J* = 8.5 Hz, 1H, α-**H**^{Tartrate}), 4.36 (d, *J* = 8.5 Hz, 1H, α-**H**^{Tartrate}), 3.66-3.37 (m, 21H, **H**^{PEG}), 3.16 (dd, *J* = 7.0; 13.8 Hz, 1H, β-**H**$^{Phe(Br)}$), 3.04 (dd, *J* = 7.0; 13.8 Hz, 1H, β-**H**$^{Phe(Br)}$), 1.49 (s, 3H, CC**H**$_3$), 1.38 (s, 3H, CC**H**$_3$). **^{13}C-NMR** (75 MHz, APT, CD_2Cl_2): δ 170.9 (**C**), 169.3 (**C**), 168.2 (**C**), 135.3 (**C**), 131.8 (**C**H$^{Phe(Br)}$), 131.3 (**C**H$^{Phe(Br)}$), 121.2 (**C**), 113.3 (**C**CH$_3$), 76.5 (α-**C**H^{Tartrate}), 75.9 (α-**C**H^{Tartrate}), 70.69 (**C**H$_2^{PEG}$), 70.65 (**C**H$_2^{PEG}$), 70.54 (**C**H$_2^{PEG}$), 70.49 (**C**H$_2^{PEG}$), 70.3 (**C**H$_2^{PEG}$), 70.0 (**C**H$_2^{PEG}$), 69.4 (**C**H$_2^{PEG}$), 53.9 (α-**C**H$^{Phe(Br)}$), 50.9 (**C**H$_2$ ^{PEG}), 39.7 (β-**C**H$_2^{Phe(Br)}$), 38.1 (**C**H$_2^{PEG}$), 29.8 (**C**H$_2$), 26.1 (C**C**H$_3$), 25.8 (C**C**H$_3$). Assignation established by ^{1}H-^{1}H homonuclear correlation spectroscopy (COSY) and ^{1}H-^{13}C HSQC (Heteronucleiar Single-Quantum Correlation) experiment which reveals a 2D

heteronucleiar chemical shift correlation map between directly bonded ^{1}H and X-heteronuclei (^{13}C in our case).

2.3.2 Propargylated Dipeptide Synthesis



Scheme *S3*. Threonine propargylation. Reagents and conditions: **a**) *i*) 60% (*w*/*w*) NaH in mineral oil added by portion, dry DMF, 4ºC, 90 min, Ar; *ii*) 80% (w/v) propargylbromide in toluene (dropwise over ~ 20 min), then stirred ~ 6 h, 4ºC; yield: 50%.



Scheme *S4*. Octreotate propargylated dipeptide precursor synthesis. Reagents and conditions: **a**) *i*) Cs_2CO_3, 3:7 (*v*/*v*) water/EtOH, ~ 1 h; *ii*) dried against P_2O_5 under vac, ~ 3 h; *iii*) 2-bromoacetamide, TBAI, 1:1 (*v*/*v*) dry MeCN/dry DMF, 70ºC, 18 h 30 min, Ar, 90%; **b**) 1:4 (*v*/*v*) TFA/DCM, ~ 1 h 30 min; **c**) *i*) DIPEA, dry EtOAc/dry MeCN, Ar, 4ºC; *ii*) N^{α}-Fmoc-Cys(Trt)-OH, Oxyma, COMU, DIPEA, 20 h, 4ºC to RT, Ar, 68% (over two steps).

(2*S*, 3*R*)- 2-(([(2'-Methyl-2'-propanyl)oxy]carbonyl)amino)-3-hydroxybutanoic acid (SI-7). A clear solution of di-*tert*-butyl dicarbonate (5.90 g, 27.0 mmol) dissolved in THF (60 mL) was added dropwise to a clear solution of (2*S*, 3*R*)-2-amino-3-hydroxybutanoic acid (2.57g, 21.6 mmol) and Na_2CO_3 (4.80 g 45.4 mmol) in H_2O (45 mL) at 4ºC. The reaction mixture was left to warm up to room temperature overnight, then was cooled to 4ºC and acidified to pH ~ 3 with saturated aqueous orthophosphoric acid solution. The resulting aqueous mixture was extracted with EtOAc (5 x 30 mL), the organic layers were pooled, washed with saturated $KH_2PO_{4(aq)}$ (1 x 30 mL) and dried over anhydrous $MgSO_4$.

The organic layer was filtered and evaporated under reduced pressure to yield a thick yellow gum. The crude product was purified by flash chromatography on silica (0.1:0.1:9.8 to 0.1:0.7:9.2 AcOH/EtOH/DCM) using dry loading method to afford (2*S*, 3*R*)- 2-(([(2'-methyl-2'-propanyl)oxy]carbonyl)amino)-3-hydroxybutanoic acid (**SI-7**) (4.40 g, 20.0 mmol), **Scheme *S3***, as a gummy white solid in 92% yield, R_f ~ 0.1 (0.1:0.4:9.5 AcOH/EtOH/DCM. LR-MS (ESI-single quadrupole) *m/z*: calcd for $C_9H_{17}NO_5Na$ $[M + Na]^+$: 242.1, found 242.4. **^{1}H-NMR** (300 MHz, CD_2Cl_2): δ 1.25 (d, *J* = 3.3 Hz, 3H, γ-**H**), 1.46 (s, 9H, γ-**H**), 4.33 (m, 2H, α-**H**, β-**H**), 5.68 (s, 1H, N**H**), 6.19-6.57 (broad s, 3H, **H**$_2$O+O**H**).

(2*S*, 3*R*)- 2-(([(2'-Methyl-2'-propanyl)oxy]carbonyl)amino)-3-(prop-2''-yn-1''-yloxy)butanoic acid (SI-8). Vaccuum-P_2O_5 dried and argon flushed (2*S*, 3*R*)- 2-(([(2'-methyl-2'-propanyl)oxy]carbonyl)amino)-3-hydroxybutanoic acid (**SI-7**) (4.18 g, 19.1 mmol) was dissolved in dry DMF (80 mL) and cooled to 4^{o}C. Under argon, to the clear solution, 60% (*w*/*w*) NaH in mineral oil (2.26 g, 57.3 mmol) was added in three equivalent portions resulting in a foamy white mixture which was stirred for 1.5 hours at 4^{o}C before a solution of 80% (*w*/*v*) propargyl bromide in toluene (3.37 mL, 22.9 mmol) was added dropwise and further stirred for an additional 3.5 hours at 4^{o}C resulting in a clear light brown mixture. The mixture was stirred at 4^{o}C for 5 h, slowly diluted with water resulting in a solution of pH > 14, extracted with 1:1 (*v*/*v*) Heptane/CPME (3 x 50 mL) and acidified with saturated aqueous *ortho*-phosphoric acid solution to pH ~ 2.5 at 4^{o}C. The resulting aqueous mixture was extracted with EtOAc (7 x 50 mL), the organic layers were pooled, dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure to yield a thick yellow gum. The crude was purified by flash chromatography on silica (0.2:8:1.8 to 0.2:3:6.8 AcOH/Hept/$CHCl_3$) using dry loading method to afford (2*S*, 3*R*)- 2-(([(2'-methyl-2'-propanyl)oxy]carbonyl)amino)-3-(prop-2''-yn-1''-yloxy)butanoic acid (**SI-8**) (2.54 g, 9.90 mmol), **Scheme *S3***, as a gummy yellow solid in 52% yield, R_f ~ 0.1 (0.2:1:8.8 AcOH/Hept/$CHCl_3$). HR-MS (ESI-TOF) *m/z*: calcd for $C_{12}H_{19}NO_5Na$ $[M + Na]^+$: 280.1161, found 280.1164. Contaminated with ~ 15% of Boc-Thr-OH: **^{1}H-NMR** (300 MHz, CD_2Cl_2): δ 10.62 (br s, 1H, CO_2**H**), 5.40 (d, *J* = 9.4 Hz, 1H, N**H**), 4.55-4.09 (m, 4H, α-**H**, β-**H**, OC**H**$_2$C), 2.52 (br s, 1H, CC**H**), 1.45 (s, 9H, **H**$^{tert\text{-Bu}}$), 1.24 (d, *J* = 6 Hz, 3H, γ-**H**). **^{13}C-NMR** (100 MHz, APT, CD_2Cl_2): δ 174.9 (**C**O_2H), 156.4 (**C**ONH), 80.3 (**C**$^{tert\text{-Bu}}$), 79.4 (CH_2**C**CH),

74.6 (**C** or **C**H_2 but assigned as CH_2C**C**H)*****, 74.4 (β-**C**H), 58.1 (α-**C**H), 56.4 (**C**H_2CCH), 28.1 (**C**H_3*tert*-Bu), 15.9 (γ-**C**H_3); spectrum is in accordance with literature.[11] ***** Based on correlation between signal at 74.64 ppm {^{13}C} and 2.52 ppm {^{1}H} established by ^{1}H-^{13}C HSQC (Heteronucleiar Single-Quantum Correlation) experiment which reveals a 2D heteronucleiar chemical shift correlation map between directly-bonded ^{1}H and X-heteronuclei (^{13}C in our case).

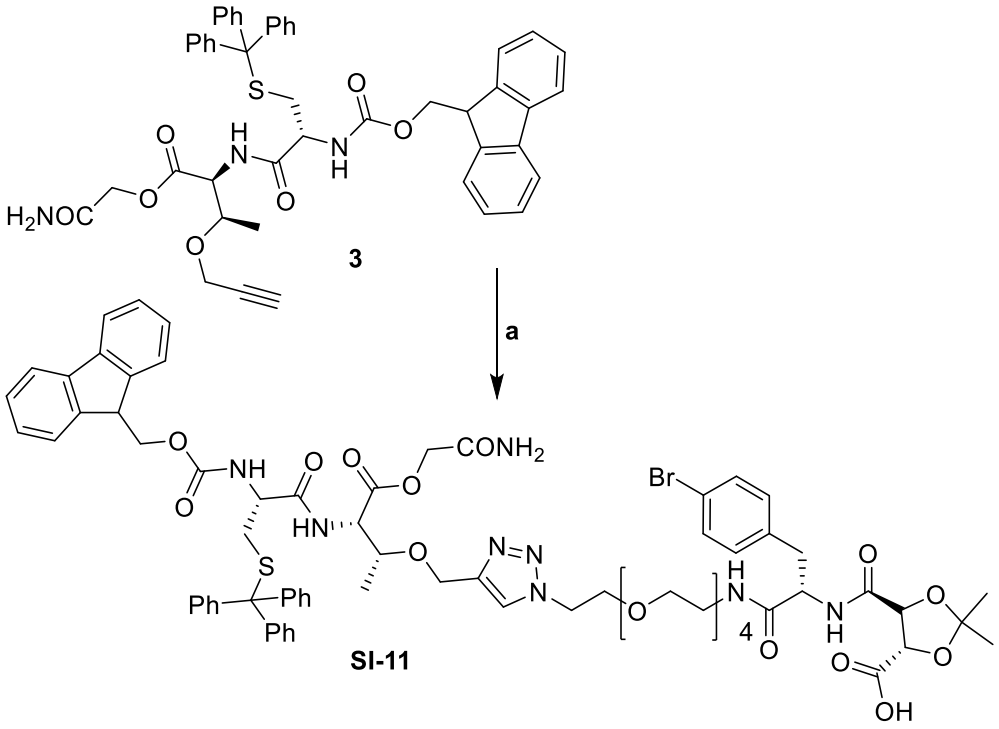
(2*S*, 3*R*) – 1 - (Ethanamide - 2' – yl -) – 2 - (([(2'' – methyl - 2'' – propanyl) oxy] carbonyl) amino) -3 - (prop - 2''' – yn - 1''' – yloxy) - butanoate ester (SI-9). To a solution of (2*S*, 3*R*)- 2-(([(2'-methyl-2'-propanyl)oxy]carbonyl)amino)-3-(prop-2''-yn-1''-yloxy)butanoic acid (**SI-8**) (2.54 g, 9.90 mmol) in 2:1 (*v*/*v*) EtOH/H_2O (36 mL),Cs_2CO_3 (1.60 g, 4.5 mmol) was added. The resulting mixture was stirred for 1 hour before being evaporated to dryness under reduced pressure. The dry salt was resuspended in EtOH, evaporated to dryness under reduced pressure and the cycle was repeated two more times, followed by three additional times using toluene. The resulting threonine cesium carbonate salt was further dried over P_2O_5 for 4 hours under vacuum. Under argon, to the dry salt was added TBAI (0.368 g, 1.00 mmol), 2-bromoacetamide (4.09 g, 26.7 mmol) and dry 1:1 (*v*/*v*) MeCN/DMF (100 mL) and the resulting clear light-yellow mixture was stirred at 70^{o}C overnight. The reaction mixture was cooled to room temperature, diluted with EtOAc (100 mL), washed with brine (3 x 50 mL), and the organic layer was dried over anhydrous $MgSO_4$. The resulting solution was filtered and evaporated under reduced pressure to yield a white solid purified by flash chromatography on silica (4:6 to 6:4 EtOAc/Hept) to afford (2*S*, 3*R*)-1-(ethanamide-2'-yl-)-2-(([(2''-methyl-2''-propanyl)oxy]carbonyl)amino)-3-(prop-2'''-yn-1'''-yloxy)butanoate ester (**SI-9**) (2.70 g, 8.59 mmol), **Scheme *S4***, as a white solid in 87% yield, R_f ~ 0.2 (4:6 EtOAc/Heptane). HR-MS (ESI-TOF) *m/z*: calcd for $C_{14}H_{22}N_2O_6Na$ $[M + Na]^+$: 337.1376, found 337.1374. Contaminated with trace amount of bromoacetamide: **^{1}H-NMR** (300 MHz, CD_2Cl_2): δ 6.68 (br s, 1H, N**H**), 6.54 (br s, 1H, N**H**), 6.32 (br s, 1H, N**H**), 5.46 (d, *J* = 8.2 Hz, 1H, N**H**), 4.71 (d, *J* = 15.1 Hz, 1H, OC**H**$_2^{CAM}$), 4.55 (d, *J* = 15.1 Hz, 1H, OC**H**$_2^{CAM}$), 4.37 (dd, *J* = 3.0; 8.7 Hz, 1H, α-**H**), 4.32-4.24 (m, 1H, β-**H**), 4.22 (dd, *J* = 2.5; 15.8 Hz, 1H, OC**H**$_2$CCH), 4.12 (dd, *J* = 2.5; 15.8 Hz, 1H, OC**H**$_2$CCH), 3.85 (s, 0.74H, 2-bromoacetamide), 2.55 (t, *J* = 2.5

Hz, 1H, $OCH_2CC\mathbf{H}$), 1.44 (s, 9H, $\mathbf{H}^{tert\text{-}Bu}$), 1.25 (d, *J* = 6.1 Hz, 3H, γ-**H**). **^{13}C-NMR** (100 MHz, APT, CD_2Cl_2): δ 174.7 ($\mathbf{C}OO^{Thr}$), 169.8 ($\mathbf{C}ONH^{CAM}$), 156.5 ($\mathbf{C}ONH^{Boc}$), 80.4 ($\mathbf{C}^{tert\text{-}Bu}$), 79.1 ($CH_2\mathbf{C}CH$), 75.0 (**C** or $\mathbf{C}H_2$ but assigned as $CH_2C\mathbf{C}H$)*****, 73.9 (β-**C**H), 63.1 ($\mathbf{C}H_2^{CAM}$), 58.5 (α-**C**H), 56.0 ($\mathbf{C}H_2CCH$), 28.1 ($\mathbf{C}H_3^{tert\text{-}Bu}$), 15.5 (γ-$\mathbf{C}H_3$). ***** Based on correlation between signal at 74.97 ppm {^{13}C} and 2.55 ppm {^{1}H} established by ^{1}H-^{13}C HSQC (Heteronucleiar Single-Quantum Correlation) experiment which reveals a 2D heteronucleiar chemical shift correlation map between directly bonded ^{1}H and X-heteronuclei (^{13}C in our case).

N*$^{\alpha}$-Fmoc-*S*$^{\beta}$-trityl-cysteinyl-*O*$^{\beta}$-propargyl-threonine carbamoylmethyl ester (3)**. To a solution of (2*S*, 3*R*)-1-(ethanamide-2'-yl-)-2-(([(2''-methyl-2''-propanyl)oxy]carbonyl)amino)-3-(prop-2'''-yn-1'''-yloxy)butanoate ester (**SI-9**) (2.61 g, 8.30 mmol) in DCM (100 mL), TFA (50 mL) was added. The resulting pale yellow clear solution was stirred for 2 hours and evaporated under reduced pressure to yield thick yellow oil/gum, **Scheme *S4. The crude product (**SI-10**) was used without further purification, dissolved in dry 4:1 (*v*/*v*) EtOAc/MeCN (100 mL) under argon and cooled to 4^{o}C. To the subsequent clear yellow pale solution was added DIPEA (2.89 mL, 16.6 mmol) to raise the pH, Oxyma Pure (1.30 g, 9.10 mmol), COMU (4.62 g, 10.8 mmol), *N*$^{\alpha}$-Fmoc-Cys(Tr)-OH (5.33 g, 9.10 mmol), and additional DIPEA (3.30 mL, 19.1 mmol) to reach pH ~ 9 at 4^{o}C. The ensuing clear yellowish solution was stirred for 5 hours at room temperature resulting in a dark orange solution that was diluted with EtOAc (100 mL), then washed with cold saturated $NaHCO_{3(aq)}$ (3 x 50 mL), saturated $KH_2PO_{4(aq)}$ (3 x 50 mL) and brine (3 x 50 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure to yield a brown foamy solid that was purified by NH_4OH-quenched flash chromatography on silica (3:2:5 to 5:2:3 EtOAC/DCM/Heptane) to afford a brown solid. [Prior to use, the NH_4OH-quenched silica was flushed with 1:9 AcOH/EtOAc (1L), 0.1:4:5.9 AcOH/EtOAc/Heptane and 300mL 3:2:5 EtOAc/DCM/Heptane (300 mL)]. The brown solid was dissolved in EtOAc (200 mL), washed with saturated $Ca_2CO_{3(aq)}$ (6 x 80 mL), brine (3 x 80 mL) and was dried over anhydrous $MgSO_4$. The organic layer was further washed with saturated $Na_2CO_{3(aq)}$ (3 x 80 mL), brine (3 x 80 mL), dried over anhydrous $MgSO_4$ and evaporated under reduced

pressure to afford N^{α}-Fmoc-S^{β}-trityl-cysteinyl-O^{β}-propargyl-threonine carbamoylmethyl ester (**3**) (4.69 g, 6.06 mmol), **Scheme *S4***, as a beige solid in 73% yield over two steps, R_f ~ 0.2 (5:2:3 EtOAc/DCM/Heptane). RP-HPLC: t_r = 42.07 min (A_{301} 98%) with gradient of 40% to 78% solvent B over 20 min, 78% to 100% over 40 min at a flow rate of 1 mL/min; UV-Vis spectrum with λ_{max} = 214, 264 and 300 nm. HR-MS (ESI-TOF) *m/z*: calcd for $C_{46}H_{43}N_3O_7SNa$ $[M + Na]^+$: 804.2719, found 804.2710. Contaminated with EtOAc and Heptane:**^{1}H-NMR** (300 MHz, CD_2Cl_2): δ 7.78 (t, *J* = 6.3 Hz, 2H, Ar**H**^{Fmoc}), 7.59 (d, *J* = 7.4 Hz, 2H, Ar**H**^{Fmoc}), 7.46-7.22 (m, 19H, Ar**H**$^{Fmoc+Tr}$), 6.57 (d, *J* = 7.9 Hz, 1H, N**H**), 5.40 (br s, 1H, N**H**), 5.07 (d, *J* = 7.4 Hz, 1H, N**H**), 4.64 (d, *J* = 15.8 Hz, 1H, OC**H**$_2^{CAM}$), 4.53 (dd, *J* = 3.1; 8.4 Hz, 1H, α-**H**^{Cys}), 4.46-4.06 (m, 8H, OC**H**$_2^{CAM}$, α-**H**^{Thr}, β-**H**^{Thr}, OC**H**$_2$CCH, C**H**$_2^{Fmoc}$, C**H**^{Fmoc}), 2.71 (dd, J = 7.6; 13.5 Hz, 1H, β-**H**^{Cys}), 2.62 (dd, J = 5.5; 13.5 Hz, 1H, β-**H**^{Cys}), 2.45 (t, *J* = 2.3 Hz, 1H, OCH$_2$CC**H**), 1.57 (s, 9H, **H**$^{tert\text{-}Bu}$), 1.16 (d, *J* = 6.6 Hz, 3H, γ-**H**). Contaminated with EtOAc and Heptane: **^{13}C-NMR** (75 MHz, APT, CD_2Cl_2): δ 171.1 (**C**OO^{Thr}), 169.1 (**C**ONH^{CAM}), 168.8 (**C**ONH^{Cys}), 156.0 (CONH^{Fmoc}), 144.4 (**C**^{Tr}), 143.9 (**C**^{Fmoc}), 143.9 (**C**^{Fmoc}), 141.4 (**C**^{Fmoc}), 141.4 (**C**^{Fmoc}), 129.6 (**C**H^{Tr}), 128.3 (**C**H^{Tr}), 127.9 (**C**H^{Tr}), 127.2 (**C**H^{Fmoc}), 127.1 (**C**H^{Fmoc}), 125.1 (**C**H^{Fmoc}), 125.07 (**C**H^{Fmoc}), 120.1 (Ar**C**$^{Phe(Br)}$), 79.0 (CH$_2$**C**CH), 75.1 (**C** or **C**H$_2$ but assigned as CH$_2$C**C**H)*****, 73.7 (β-**C**H^{Thr}), 67.5 (**C**^{Tr}), 67.1 (**C**H$_2^{Fmoc}$), 63.3 (**C**H$_2^{CAM}$), 57.1 (α-**C**H^{Thr}), 56.1 (**C**H$_2$CCH), 54.2 (α-**C**H^{Cys}), 47.2 (**C**H^{Fmoc}), 33.6 (β-**C**H$_2^{Cys}$), 15.6 (γ-**C**H$_3$). ***** Based on correlation between signal at 75.07 ppm {^{13}C} and 2.45 ppm {^{1}H} established by ^{1}H-^{13}C HSQC (Heteronucleiar Single-Quantum Correlation) experiment which reveals a 2D heteronucleiar chemical shift correlation map between directly bonded ^{1}H and X-heteronuclei (^{13}C in our case).

2.3.3 CuAAC of Linker and Octreotate Dipeptide Precursor



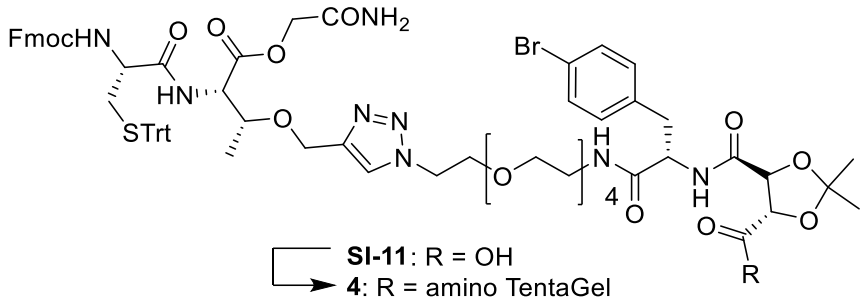
Scheme *S5*. CuAAC of linker and octreotate dipeptide precursor. Reagents and conditions: **a**) azido tartrate-based linker (**2**), 0.1 eq. $CuSO_4$, 0.44 eq. ascorbic acid, 0.22 eq. Cs_2CO_3, CPME, water, RT, Ar, 17 h, 77%.

***N*$^{\alpha}$- Fmoc - *S*$^{\beta}$ – trityl – cysteinyl - *O*$^{\beta}$ - [(1 - { *N*’’’’ - (1’’ - *N*’’ - { 14’, 3’, 6’, 9’, 12’ – tetraoxatetradecane - 1’ } - (2’’ *S*) - 3’’ - (4’’’ – bromophenyl) – propenamide) - 5’’’’ – carboxamide - (4*S*’’’’ , 5*S*’’’’) - 2’’’’ , 2’’’’ -dimethyl - 1’’’’ , 3’’’’ – dioxolane - 4’’’’ - carboxylic acid } - 1*H* - 1, 2, 3 – triazol – 4 – yl) methoxy] - threonine carbamoylmethyl ester (SI-11)**. Under argon, (4*S*,5*S*)-2,2-dimethyl-1,3-dioxolane-4-carboxylic acid-5-{(2’*S*)-3’-(4’’-bromophenyl)-1’-*N*’-(14’’’’-azido-3’’’’,6’’’’,9’’’’,12’’’’-tetraoxatetradecane-1’’’’-)-propanamide)}-carboxamide (**2**) (0.308 g, 0.470 mmol) was dissolved in partially degassed (argon bubbling) CPME and treated with *N*$^{\alpha}$-Fmoc-*S*$^{\beta}$-trityl-cysteinyl-*O*$^{\beta}$-propargyl-threonine carbamoylmethyl ester (**3**) (0.369 g, 0.47 mmol). The clear yellow solution was treated with a clear blue solution of $CuSO_4$ hexahydrate (0.0115 g, 0.046 mmol) in partially degassed (argon bubbled) milli-Q water (0.37 mL) followed by a clear solution of ascorbic acid (0.0242 g, 0.137 mmol) and Cs_2CO_3 (0.0224

g, 0.068 mmol) in partially degassed (argon bubbled) milli-Q water (1.56 mL). The resulting bi-phasic mixture was vigorously stirred for 3 hours under argon, diluted with DCM (100 mL), and washed with saturated $KH_2PO_{4(aq)}$ (3 x 30 mL) as well as brine (3 x 30 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure to yield an off white solid that was purified by flash chromatography on silica (0.2:9.8 to 0.2:0.8:9 AcOH/EtOH/DCM) to afford *N*$^{\alpha}$-Fmoc-*S*$^{\beta}$-trityl-cysteinyl-*O*$^{\beta}$-[(1-{*N*''''-(1''-*N*''-{14', 3',6',9',12'-tetraoxatetradecane-1'}-(2''*S*)-3''-(4'''-bromophenyl)-propanamide)-5''''-carboxamide-(4*S''''*,5*S''''*)-2'''',2''''-dimethyl-1'''',3''''-dioxolane-4''''-carboxylic acid}-1*H*-1,2,3-triazol-4-yl)methoxy]-threonine carbamoylmethyl ester (**SI-11**) contaminated with acetic acid as a gummy yellow solid. The product was dissolved in DCM (100 mL), washed once with brine (30 mL), dried over anhydrous MgSO4, filtered, dried under reduced pressure to afford the title compound (**SI-11**) (0.31 g, 0.215 mmol) in 46 % yield, **Scheme *S5***, as a white solid, R_f ~0.2 (0.2:0.8:9 AcOH/EtOH/DCM). RP-HPLC: t_r = 39.65 min (A_{301} 100%) with gradient of 40% to 78% solvent B over 20 min, 78% to 100% over 20 min at a flow rate of 1 mL/min; UV-Vis spectrum with λ_{max} = 224, 264 and 300 nm. HR-MS (ESI-TOF) *m/z*: calcd for $C_{72}H_{81}N_8O_{17}SBrNa$ $[M + Na]^+$: 1463.4521, found 1463.4509. Contaminated with AcOH/EtOH: **^{1}H-NMR** (300 MHz, CD_2Cl_2): δ 7.78 (dd, *J* = 5.8; 7.3 Hz, 2H, Ar**H**^{Fmoc}), 7.67 (s, 1H, CO_2**H**), 7.59 (d, *J* = 7.1 Hz, 2H, Ar**H**^{Fmoc}), 7.43-7.20 (m, 22H, Ar**H**$^{Fmoc+Tr+Phe(Br)+triazole}$), 7.10 (d, *J* = 8.4 Hz, 2H, Ar**H** $^{Phe(Br)}$), 6.95 (d, *J* = 8.3 Hz, 1H, N**H**), 6.81 (br s, 1H, N**H**), 6.25 (br s, 1H, N**H**), 5.32 (br s, 1H, OC**H**$_2$-triazole, overlap with DCM peak), 5.28 (d, *J* = 7.4 Hz, 1H, N**H**), 4.78-4.62 (m, 3H, OC**H**$_2^{CAM}$, **H**$^{\alpha\text{-Phe(Br)}}$, **H**$^{\alpha-tartrate}$), 4.55-4.32 (m, 9H, **H**$^{\alpha-tartrate}$, α-**H**^{Cys}, OC**H**$_2^{CAM}$, α-**H**^{Thr}, C**H**$_2^{Fmoc}$, C**H**^{Fmoc}), 4.23 (t, *J* = 7.0 Hz, 2H, OC**H**$_2^{PEG}$), 3.94-3.83 (m, 3H, β-**H**^{Thr}, NC**H**$_2^{PEG}$), 3.58-3.38 (m, 16H, (OC**H**$_2$C**H**$_2$)$_3^{PEG}$, NC**H**$_2$C**H**$_2^{PEG}$), 3.15 (dd, *J* = 6.3; 14.8 Hz, 1H, **H**$^{\beta\text{-Phe(Br)}}$), 2.99 (dd, *J* = 7.0; 14.8 Hz, 1H, **H**$^{\beta\text{-Phe(Br)}}$), 2.73-2.59 (m, 2H, β-**H**^{Cys}), 1.46 (s, 3H, C**H**$_3^{iPr}$), 1.35 (s, 3H, C**H**$_3^{iPr}$), 1.19 (d, *J* = 5.3 Hz, 3H, γ-**H**). Contaminated with heptane: **^{13}C-NMR** (75 MHz, APT, CD_2Cl_2): δ 171.3 (**C**OO^{Thr}), 170.4 (**C**OOH^{tartrate}), 170.3 (**C**ONH^{tartrate}), 169.2 (**C**ONH^{Cys}, **C**ONH$^{Phe(Br)}$, **C**ONH^{CAM}), 156.1 (**C**ONH^{Fmoc}), 144.6 (**C**^{Tr}, **C**-4 triazole), 144.1 (**C**^{Fmoc}), 144.0 (**C**^{Fmoc}), 141.4 (**C**^{Fmoc}, **C**^{Fmoc}), 135.8 (Ar**C**$^{Phe(Br)}$), 131.7 (Ar**C**H$^{Phe(Br)}$), 131.5 (Ar**C**H$^{Phe(Br)}$), 129.7

(**C**H^{Tr}), 128.3 (**C**H^{Tr}), 127.9 (**C**H^{Tr}), 127.3 (**C**H^{Fmoc}), 127.1 (**C**H^{Fmoc}), 125.3 (2 x **C**H^{Fmoc}), 121.0 (Ar**C**$^{Phe(Br)}$), 120.1 (**C**H-5^{triazole}), 113.4 (O**C**^{iPr}O), 77.3 (2 x α-**C**H^{tartrate}), 74.1 (β-**C**H^{Thr}), 70.4 (3 x O**C**H$_2$^{PEG}), 70.3 (O**C**H$_2$^{PEG}), 69.6 (O**C**H$_2$^{PEG}), 69.3 (O**C**H$_2$^{PEG}), 67.3 (**C**^{Tr}), 63.1 (**C**H$_2$^{CAM}), 61.8 (**C**H$_2$^{Fmoc}), 57.1 (α-**C**H^{Thr}), 54.4 (O**C**H$_2$-triazole, DCM peak), 54.1 (α-**C**H^{Cys}), 53.8 (α-**C**H$^{Phe(Br)}$), 50.4 (O**C**H$_2$^{PEG}), 47.2 (**C**H^{Fmoc}), 39.6 (N**C**H$_2$^{PEG}), 37.9 (β-**C**H$_2$$^{Phe(Br)}$), 34.0 (β-**C**H$_2$^{cys}), 29.9 (**C**H$_3$^{iPr}), 29.8 (N**C**H$_2$^{PEG}), 26.2 (**C**H$_3$^{iPr}),15.8 (γ-**C**H$_3$^{Thr}). Assignation established by ^{1}H-^{1}H homonuclear correlation spectroscopy (COSY) and ^{1}H-^{13}C HSQC (Heteronucleiar Single-Quantum Correlation) experiment which reveals a 2D heteronucleiar chemical shift correlation map between directly-bonded ^{1}H and X-heteronuclei (^{13}C in our case). However, chemical shifts were not resolvable from each other in several cases.

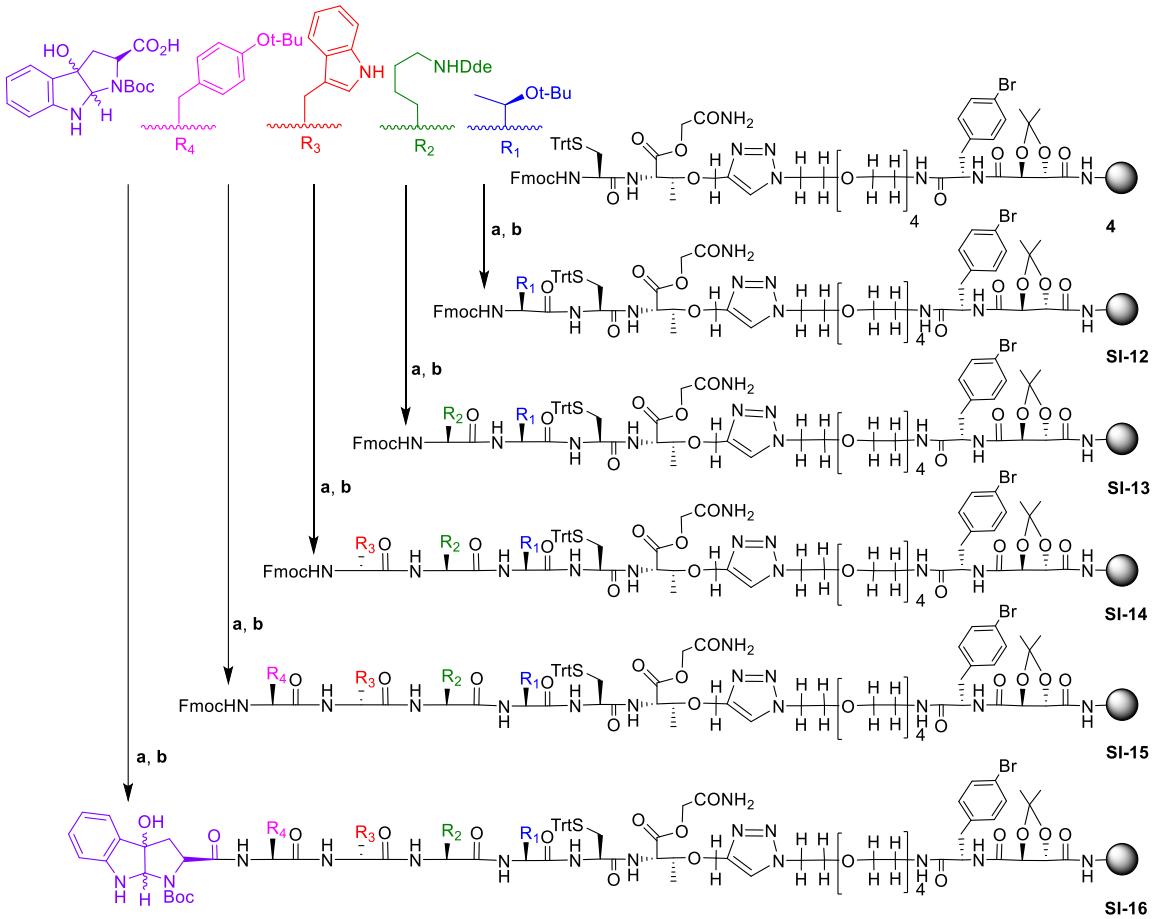
2.3.4 Fmoc/Cam-SPPS of [Ttn]-Octreotate



Scheme *S6*. Loading of dipeptide-linker on solid phase. Reagents and conditions: **a**) *i*) Macrobead TentaGel amine resin (0.25 mmol/g for loading with *N*^{α}-Fmoc-Gly-OH from RAPP Polymere), 1:1:3 (*v*/*v*/*v*) dry DMF/dry MeCN/dry EtOAc, DIPEA, PyBOP, HOBt•H$_2$O, pH ~ 9, ON; *ii*) 1:2:2 (*v*/*v*/*v*) Ac$_2$O/TMP/dry EtOAc, 1 h, pH ~ 7. Loading of 0.12 mmol/g; 60 % yield relative to resin commercial loading (Fmoc-Gly-OH).

***N*^{α}-Fmoc-*S*^{β}-trityl-cysteinyl-*O*^{β}-[(1-{*N*''''-([1''-*N*''-{14', 3',6',9',12'-tetraoxatetradecane-1'}-(2''*S*)-3''-(4'''-bromophenyl)-propanamide)-5''''-carboxamide-(4*S*'''',5*S*'''')-2'''',2''''-dimethyl-1'''',3''''-dioxolane-4''''-N''''-TentaGel MB-carboxamide}-1*H*-1,2,3-triazol-4-yl)methoxy]-threonine carbamoylmethyl ester (4)**. Prepared according to the *general Fmoc/CAM Solid-phase peptide synthesis (SPPS) procedure on TentaGel microbeads* using *N*^{α}-Fmoc-*S*^{β}-trityl-cysteinyl-*O*^{β}-[(1-{*N*''''-(1''-*N*''-

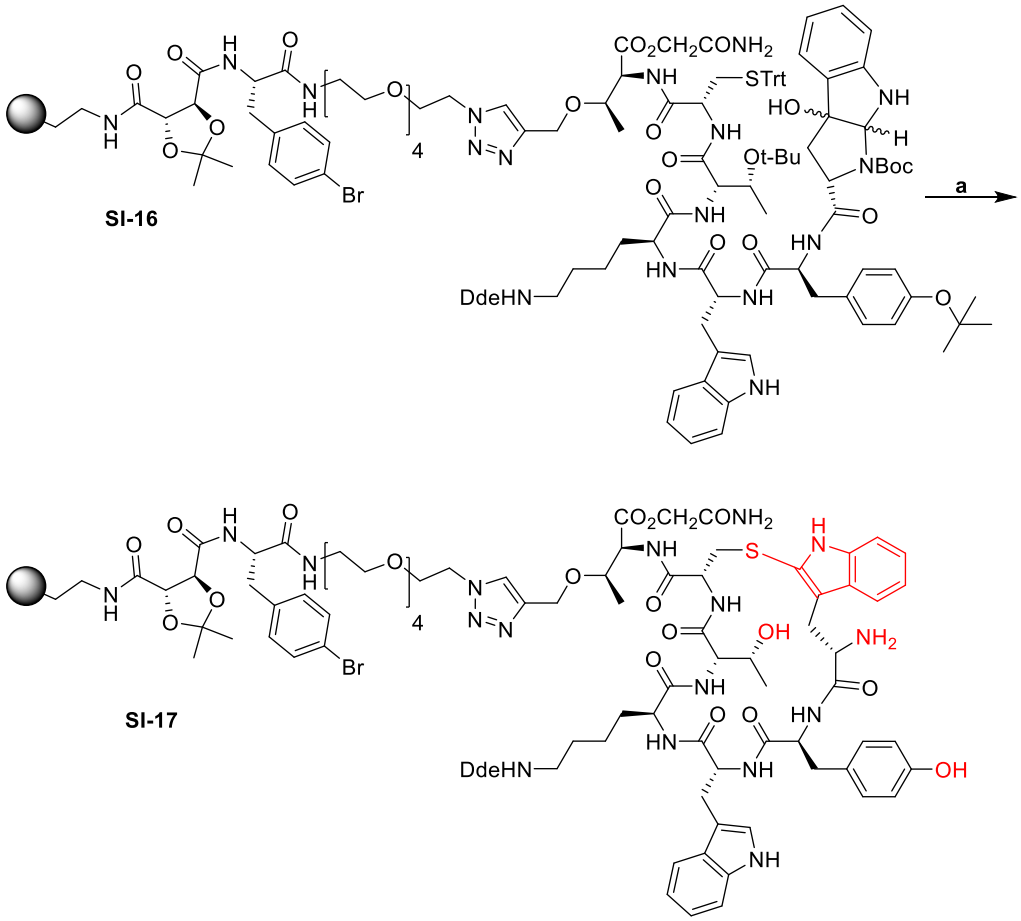
{14', 3',6',9',12'-tetraoxatetradecane-1'}-(2''*S*)-3''-(4'''-bromophenyl)-propanamide)-5''''-carboxamide-(4*S*'''',5*S*'''')-2'''',2''''-dimethyl-1'''',3''''-dioxolane-4''''-carboxylic acid}-1*H*-1,2,3-triazol-4-yl)methoxy]-threonine carbamoylmethyl ester (**SI-11**). Starting with 0.087 mmol of TentaGel macrobead amine (loading of 0.25 mmol/g with Fmoc-Gly-OH from Rapp Polymere) and resulting in 0.053 mmol (0.12 mmol/g loading) of N^{α}-Fmoc-S^{β}-trityl-cysteinyl-O^{β}-[(1-{*N*''''-([1''-*N*''-{14', 3',6',9',12'-tetraoxatetradecane-1'}-(2''*S*)-3''-(4'''-bromophenyl)-propanamide)-5''''-carboxamide-(4*S*'''',5*S*'''')-2'''',2''''-dimethyl-1'''',3''''-dioxolane-4''''-*N*''''-TentaGel MB-carboxamide}-1*H*-1,2,3-triazol-4-yl)methoxy]-threonine carbamoylmethyl ester (**4**), a conversion of 61% using PyBOP was achieved from loading values (**Scheme *S6***).



Scheme *S7*. SPPS of (Ttn)-TATE precursor. Reagents and conditions: **a**) 20% (*v*/*v*) DEA in dry DMF, pH ~ 11, 2 h 30 min; **b**) *i*) N^{α}-Fmoc-Xaa-OH or Boc-HPI[6], COMU, Oxyma, DIPEA, 1:1:3 (*v*/*v*/*v*) dry DMF/dry MeCN/dry EtOAc, 45 min to 1 h, pH ~ 9; *ii*) 1:2:2 (*v*/*v*/*v*)

Ac_2O/TMP/dry EtOAc, 1 h, pH ~ 7, 76% yield over 6 steps. Macrobead TentaGel amine resin was used.

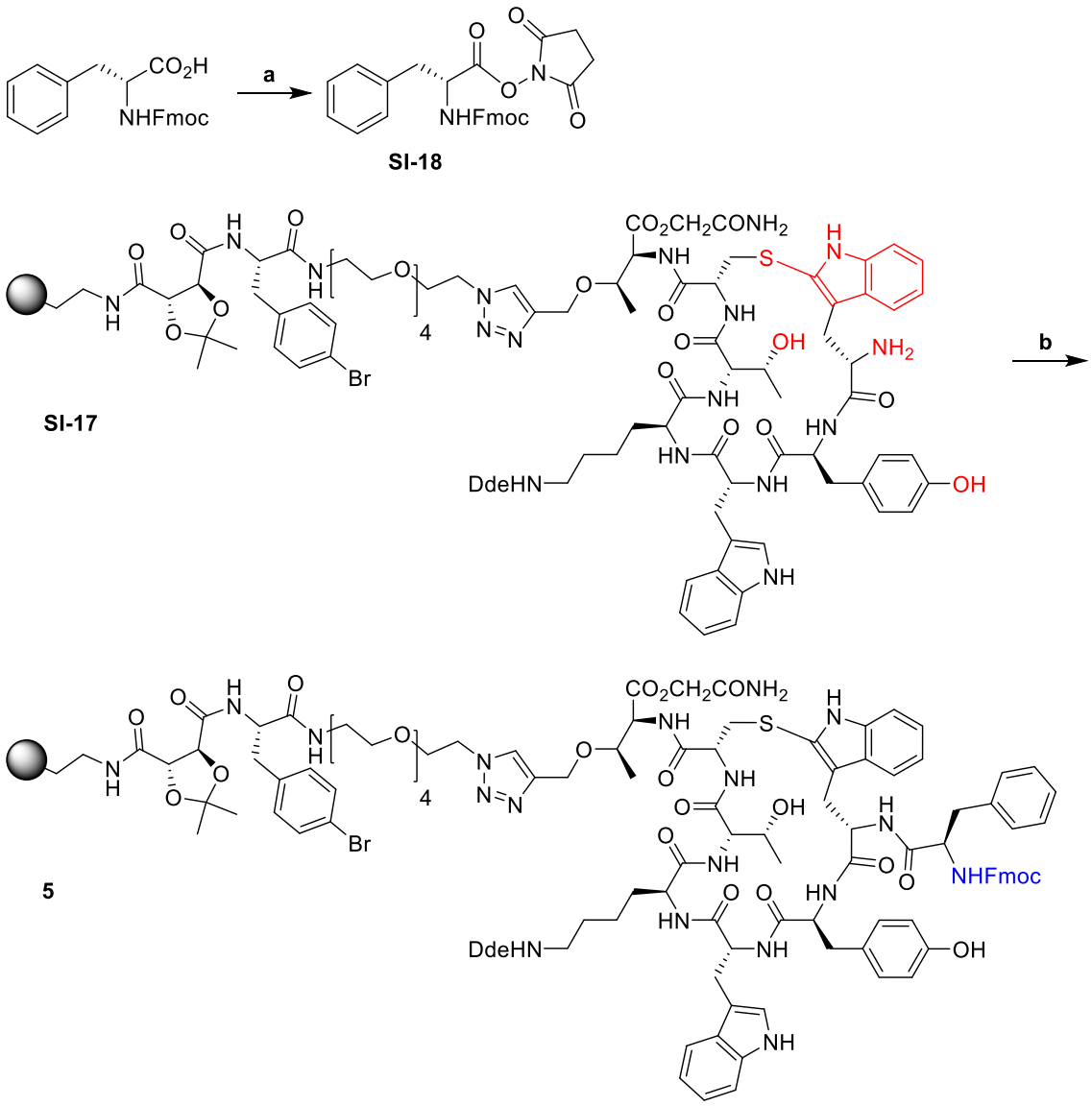
N*-1-(*Tert*-butoxycarbonyl)-3*a*-hydroxypyrrolo[2,3-*b*]indole-2-carboxamide (*syn-cis* and *anti-cis*)-4-*O-tert*-butyl-tyrosinyl-D-tryptophanyl-N^{ε}-1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl-lysinyl-O^{β}-*tert*-butyl-threonyl-S^{β}-trityl-cysteinyl-O^{β}-[(1-{*N*''''-([1''-*N*''-{14', 3',6',9',12'-tetraoxatetradecane-1'}-(2''*S*)-3''-(4'''-bromophenyl)-propanamide)-5''''-carboxamide-(4*S''''*,5*S''''*)-2'''',2''''-dimethyl-1'''',3''''-dioxolane-4''''-*N*''''-TentaGel MB-carboxamide}-1*H*-1,2,3-triazol-4-yl)methoxy]-threonine carbamoylmethyl ester (SI16)**. Prepared according to *general Fmoc/CAM Solid-phase peptide synthesis (SPPS) procedure on TentaGel macrobeads* from dipeptide on tartrate-based linker **4**. The conversion of N^{α}-Fmoc-D-tryptophanyl-N^{ε}-1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl-lysinyl-O^{β}-*tert*-butyl-threonyl-S^{β}-trityl-cysteinyl-O^{β}-[(1-{*N*''''-([1''-*N*''-{14', 3',6',9',12'-tetraoxatetradecane-1'}-(2''*S*)-3''-(4'''-bromophenyl)-propanamide)-5''''-carboxamide-(4*S''''*,5*S''''*)-2'''',2''''-dimethyl-1'''',3''''-dioxolane-4''''-*N*''''-TentaGel MB-carboxamide}-1*H*-1,2,3-triazol-4-yl)methoxy]-threonine carbamoylmethyl ester (**SI-14**) after D-Trp coupling was 87% (0.046 mmol). Around 12% of the resin **SI-14** (0.006 mmol) was used for various tests and the remaining 0.040 mmol of resin **SI-14** was used to prepare *N*-1-(*tert*-Butoxycarbonyl)-3*a*-hydroxypyrrolo[2,3-*b*]indole-2-carboxamide (*syn-cis* and *anti-cis*)-4-*O-tert*-butyl-tyrosinyl-D-tryptophanyl-N^{ε}-1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl-lysinyl-O^{β}-*tert*-butyl-threonyl-S^{β}-trityl-cysteinyl-O^{β}-[(1-{*N*''''-([1''-*N*''-{14', 3',6',9',12'-tetraoxatetradecane-1'}-(2''*S*)-3''-(4'''-bromophenyl)-propanamide)-5''''-carboxamide-(4*S''''*,5*S''''*)-2'''',2''''-dimethyl-1'''',3''''-dioxolane-4''''-*N*''''-TentaGel MB-carboxamide}-1*H*-1,2,3-triazol-4-yl)methoxy]-threonine carbamoylmethyl ester (**SI-16**), **Scheme *S7. The last loading quantification was done on N^{α}-Fmoc-4-*O-tert*-butyl-tyrosinyl-D-tryptophanyl-N^{ε}-1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl-lysinyl-O^{β}-*tert*-butyl-threonyl-S^{β}-trityl-cysteinyl-O^{β}-[(1-{*N*''''-([1''-*N*''-{14', 3',6',9',12'-tetraoxatetradecane-1'}-(2''*S*)-3''-(4'''-bromophenyl)-propanamide)-5''''-carboxamide-(4*S''''*,5*S''''*)-2'''',2''''-dimethyl-1'''',3''''-dioxolane-4''''-*N*''''-TentaGel MB-carboxamide}-1*H*-1,2,3-triazol-4-yl)methoxy]-threonine carbamoylmethyl ester (**SI-15**) to give an overall conversion of 76 % (0.035 mmol), **Scheme *S7***.



Scheme *S8*. Savige-Fontana tryptathionylation of Boc-HPI-heptapeptide on TentaGel resin. Reagents and conditions: **a**) TFA, 5 h.

H-(2-Mercapto-tryptophanyl-tyrosyl-D-tryptophanyl-*N*$^{\varepsilon}$-1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl-lysinyl-threonyl-cysteinyl)cyclic sulfide-*O*$^{\beta}$-[(1-{*N*''''-(1''-*N*''-{14', 3',6',9',12'-tetraoxatetradecane-1'}-(2''*S*)-3''-(4'''-bromophenyl)-propanamide)-2''''-(benzyloxyimino)acetamide}-1*H*-1,2,3-triazol-4-yl)methoxy]-threonine carbamoylmethyl ester (SI-17). Prepared according to *general Fmoc/CAM Solid-phase peptide synthesis (SPPS) procedure on TentaGel microbeads* from dipeptide on tartrate-based linker **4** to yield *N*-1-(*tert*-butoxycarbonyl)-3*a*-hydroxypyrrolo[2,3-*b*]indole-2-carboxamide (*syn-cis* and *anti-cis*)-4-*O*-*tert*-butyl-tyrosinyl-D-tryptophanyl-*N*$^{\varepsilon}$-1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl-lysinyl-*O*$^{\beta}$-*tert*-butyl-threonyl-*S*$^{\beta}$-trityl-cysteinyl-*O*$^{\beta}$-[(1-{*N*''''-([1''-*N*''-{14', 3',6',9',12'-tetraoxatetradecane-1'}-(2''*S*)-3''-(4'''-

bromophenyl)-propanamide)-5’’’’-carboxamide-(4*S’’’’*,5*S’’’’*)-2’’’’,2’’’’-dimethyl-1’’’’,3’’’’-dioxolane-4’’’’-*N*’’’’-TentaGel MB-carboxamide}-1*H*-1,2,3-triazol-4-yl)methoxy]-threonine carbamoylmethyl ester (**SI-16**) to which was applied the *General Savige-Fontana reaction procedure on TentaGel macrobeads* yielding H-(2-mercapto-tryptophanyl-tyrosyl-D-tryptophanyl-N^{ε}-1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl-lysinyl-threonyl-cysteinyl)cyclic sulfide-O^{β}-[(1-{*N*’’’’-([1’’-*N*’’-{14’, 3’,6’,9’,12’-tetraoxatetradecane-1’}-(2’’*S*)-3’’-(4’’’-bromophenyl)-propanamide)-5’’’’-carboxamide-(4*S’’’’*,5*S’’’’*)-2’’’’,2’’’’-dimethyl-1’’’’,3’’’’-dioxolane-4’’’’-*N*’’’’-TentaGel MB-carboxamide}-1*H*-1,2,3-triazol-4-yl)methoxy]-threonine carbamoylmethyl ester (**SI-17**), **Scheme *S8***.



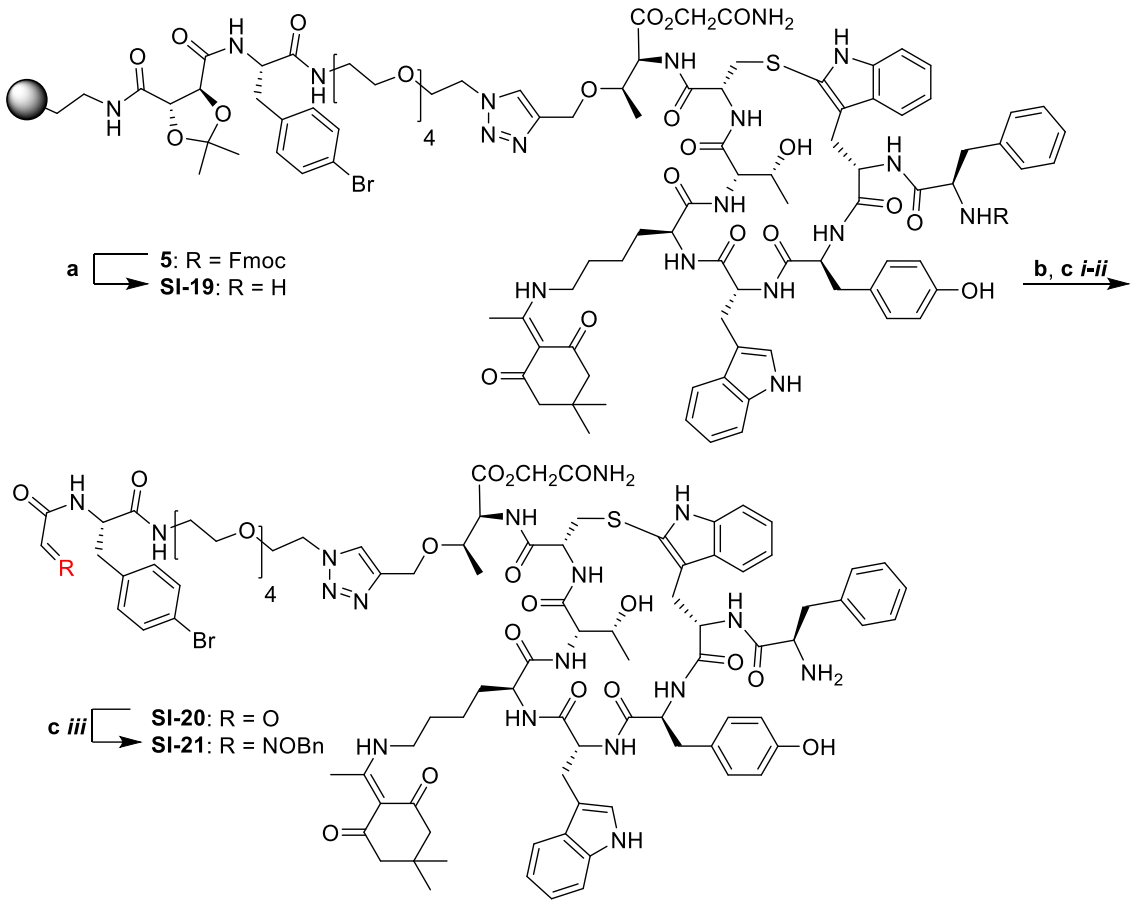
Scheme *S9*. Acylation of (Ttn)-TATE precursor. Reagents and conditions: **a**) *N*$^{\alpha}$-Fmoc-D-Phe-OH, DSC, TMP, 1:1 (*v*/*v*) dry CPME/dry MeCN, 22 h, w-up and use as crude; **b**) *N*$^{\alpha}$-Fmoc-D-Phe-OSu (**SI-18**), DIPEA, 1:2:2 (*v*/*v*/*v*) dry DCM/dry MeCN/dry EtOAc, 4 h 30 min, pH ~ 8.

(2,5-Dioxopyrrolidin-1-yl) (2*R*)-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-3-phenylpropanoate (**SI-18**). (2*R*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-phenylpropanoic acid (0.100 g, 0.206 mmol) and *N*,*N*'-disuccinimidyl carbonate (0.073 g, 0.284 mmol) were dissolved in 1:1 (*v*/*v*) dry MeCN/CPME at 4^{o}C. To the clear mixture, 2,4,6-trimethylpyridine (0.075 mL, 0.568 mmol) was added, and the mixture was stirred at room temperature for 18 h and additionnal *N*,*N*'-disuccinimidyl carbonate (0.018 g, 0.091 mmol) was added. The resulting clear pale-yellow solution was stirred for 3 hours, diluted with 1:1 (*v*/*v*) EtOAc/Heptane, washed with saturated $KH_2PO_{4(aq)}$ (3 x 30 mL) and brine (3 x 30 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure to afford (2,5-dioxopyrrolidin-1-yl) (2*R*)-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-3-phenylpropanoate (**SI-18**) (0.108 g, 0.222 mmol), **Scheme *S9***, as a white solid in 86%, R_f ~ 0.3 (6:4 EtOAc/Heptane). HR-MS (ESI-TOF) *m/z*: calcd for $C_{28}H_{24}N_2O_6Na$ [M + Na]$^{+}$: 507.1532, found 507.1537. **^{1}H-NMR** (300 MHz, CD_2Cl_2): 7.80 (d, *J* = 7.8 Hz, 2H), 7.57 (d, *J* = 7.0 Hz, 2H), 7.42 (t, *J* = 7.8 Hz, 2H), 7.35-7.30 (m, 7H), 5.29 (d, *J* = 8.8 Hz, 1H), 5.03-4.97 (m, 1H), 4.47-4.33 (m, 2H), 4.22 (d, *J* = 6.7 Hz, 1H), 3.35 (dd, *J* = 6.0; 14.4 Hz, 1H), 3.21 (dd, *J* = 6.7; 14.4 Hz, 1H), 2.84 (s, 4H).

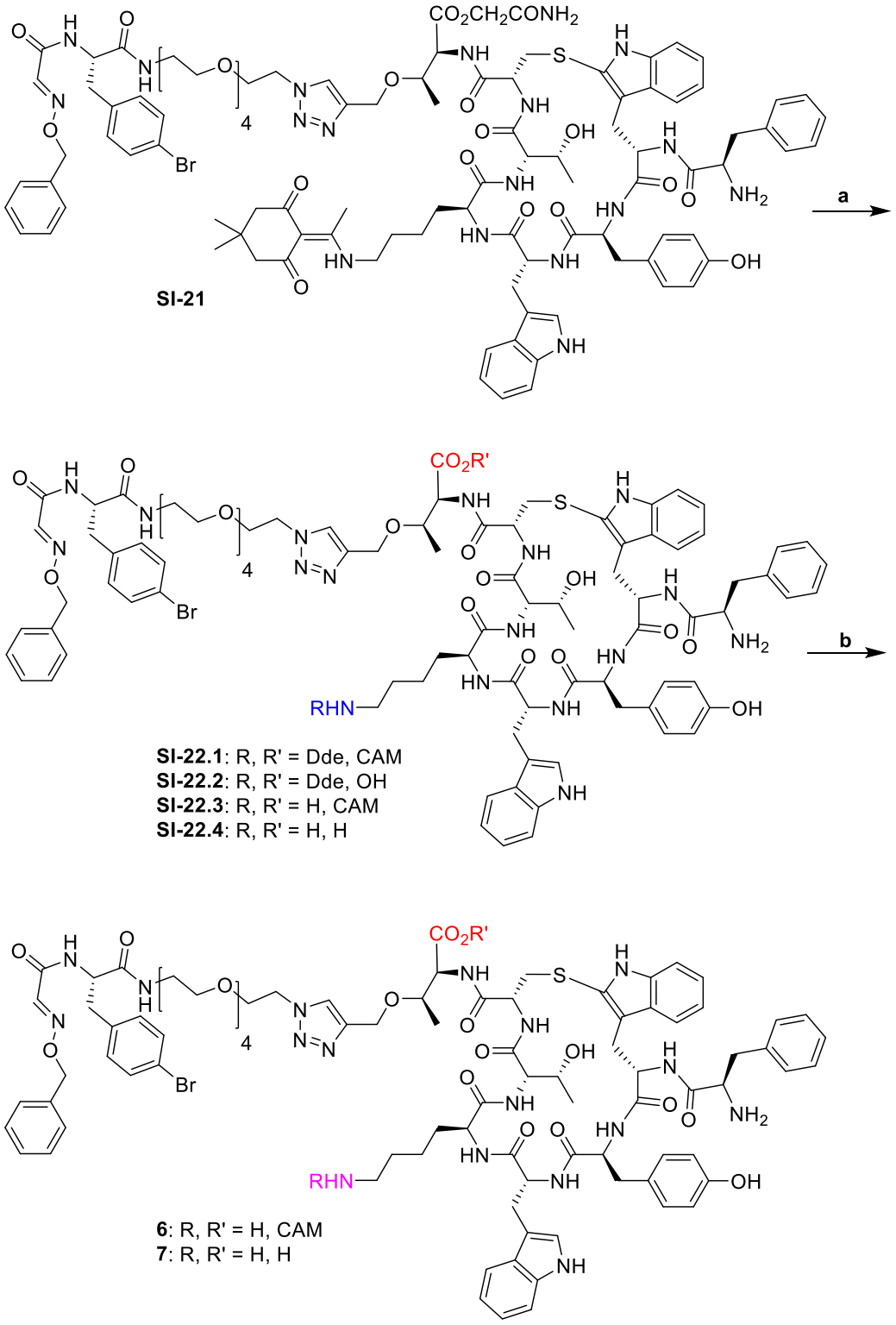
***N*$^{\alpha}$-Fmoc -D-phenylalanyl-(2-mercapto-tryptophanyl-tyrosyl-D-tryptophanyl-*N*$^{\varepsilon}$-1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl-lysinyl-threonyl-cysteinyl)cyclic sulfide-*O*$^{\beta}$-[(1-{*N*''''-([1''-*N*''-{14', 3',6',9',12'-tetraoxatetradecane-1'}-(2''*S*)-3''-(4'''-bromophenyl)-propanamide)-5''''-carboxamide-(4*S*'''',5*S*'''')-2'''',2''''-dimethyl-1'''',3''''-dioxolane-4''''-*N*''''-TentaGel MB-carboxamide}-1*H*-1,2,3-triazol-4-yl)methoxy]-threonine carbamoylmethyl ester (5)**. Prepared according to *general Fmoc/CAM Solid-phase peptide synthesis (SPPS) procedure on TentaGel macrobeads* from dipeptide on tartrate-based linker **4** and *General Savige-Fontana reaction procedure on TentaGel microbeads* until (2-mercapto-tryptophanyl-tyrosyl-D-tryptophanyl-*N*$^{\varepsilon}$-1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl-lysinyl-threonyl-cysteinyl)cyclic sulfide-*O*$^{\beta}$-

[(1-{*N*’’’’-([1’’-*N*’’-{14’, 3’,6’,9’,12’-tetraoxatetradecane-1’}-(2’’*S*)-3’’-(4’’’-bromophenyl)-propanamide)-5’’’’-carboxamide-(4*S’’’’*,5*S’’’’*)-2’’’’,2’’’’-dimethyl-1’’’’,3’’’’-dioxolane-4’’’’-*N*’’’’-TentaGel MB-carboxamide}-1*H*-1,2,3-triazol-4-yl)methoxy]-threonine carbamoylmethyl ester (**SI-17**). Loading procedure: The resin **SI-17** (0.386 g, ~ 39 µmole) was filtered with 1:2:2 (*v*/*v*/*v*) dry DCM/dry MeCN/dry EtOAc (10 mL). The resin was resuspended in 1:2:2 (*v*/*v*/*v*) dry DCM/dry MeCN/dry EtOAc (6 mL), shaken for 30 minutes, drained by filtration and the procedure was repeated two more times with fresh solvent. The resin was resuspended in 57 mM DIPEA in 1:2:2 (*v*/*v*/*v*) dry DCM/dry MeCN/dry EtOAc (6 mL), shaken, drained by filtration resulting in filtrate of pH ~ 9. All acetic acid from previous washing step (Savige-Fontana) should have been washed-out. The free base resin was resuspended with a solution of crude (2,5-dioxopyrrolidin-1-yl) (2*R*)-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-3-phenylpropanoate (**SI-18**) (0.068 g, 0.14 mmol) and DIPEA (0.012 mL, 0.07 mmol) in 1:2:2 (*v*/*v*/*v*) dry DCM/dry MeCN/dry EtOAc (5 mL), shaken in the dark for 12 h and drained by filtration (pH ~ 7). The resin was filtered with 1:2:2 (*v*/*v*/*v*) dry DCM/dry MeCN/dry EtOAc (15 mL). The resin was resuspended in 1:2:2 (*v*/*v*/*v*) dry DCM/dry MeCN/dry EtOAc (6 mL), shaken, drained by filtration and the procedure was repeated five more times with fresh solvent. The filtration and the shaking procedures were repeated using 1:1 (*v*/*v*) EtOH/EtOAc, MeCN and DCM and a Kaiser test was performed to afford N^{α}-Fmoc -D-phenylalanyl-(2-mercapto-tryptophanyl-tyrosyl-D-tryptophanyl-N^{ε}-1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl-lysinyl-threonyl-cysteinyl)cyclic sulfide-O^{β}-[(1-{*N*’’’’-([1’’-*N*’’-{14’, 3’,6’,9’,12’-tetraoxatetradecane-1’}-(2’’*S*)-3’’-(4’’’-bromophenyl)-propanamide)-5’’’’-carboxamide-(4*S’’’’*,5*S’’’’*)-2’’’’,2’’’’-dimethyl-1’’’’,3’’’’-dioxolane-4’’’’-*N*’’’’-TentaGel MB-carboxamide}-1*H*-1,2,3-triazol-4-yl)methoxy]-threonine carbamoylmethyl ester (**5**) in 0.10 mmol/g (*Fmoc loading test*), **Scheme *S9***. The resin was dried under vacuum and stored at 4°C. We employed Fmoc-Phe-OSu (**SI-18**) instead of standard coupling reagents to avoid side reaction on the deprotected side chain.[12]

2.3.5 Oxidative Cleavage of Protected [Ttn]-Octreotate from Resin, Oxination and Deprotection



Scheme *S10*. Cleavage of protected (Ttn)-TATE from Macrobead TentaGel amine resin. and oxination. Reagents and conditions: **a**) 20% DEA (*v*/*v*) in dry DMF pH ~ 11, 2 h 30 min; **b**) 95:2.5:2.5 (*v*/*v*/*v*) TFA/water/TIS, 2 h; **c**) One Pot: *i*) 1:1:4 (*v*/*v*/*v*) AcOH/*t*-BuOH/water, $NaIO_4$, pH ~ 1, 10 min; *ii*) dimethylsulfide, ~ 5 min, 1 M aqueous sodium thiosulfate ($Na_2S_2O_3$); *iii*) $BnONH_3$•Cl, 1,4-phenylenediamine hydrochloride, MeCN, aqueous KH_2PO_4, NaOH, pH ~ 6.5, ON; *iv*) Sep-Pak (C18) in 0.1% FA water/MeCN; *v*) RP-HPLC (C18) in 0.1% FA water/MeCN.



Scheme *S11*. CAM and Dde deprotection of the benzyl oxime (Ttn)-octraotate in solution. Reagents and conditions: **a**) 0.2 M Cs_2CO_3, 4:6 (*v*/*v*) water/DMF, ~ 8 h, pH ~ 11 then

0.1% FA water quenched and lyophilized; **b**) ~ 1% (v/v) hydrazine hydrate in dry DMF, water, ~ 1 h, then 0.1% FA water quenched, Sep-Pak (C18) in 0.1% FA water/MeCN and RP-HPLC (C18) in 0.1% FA water/MeCN.

H -D – Phenylalanyl - (2 – mercapto – tryptophanyl – tyrosyl – D – tryptophanyl - *N*$^{\varepsilon}$ – 1 - (4, 4 – dimethyl - 2, 6 – dioxocyclohex – 1 – ylidene) ethyl – lysinyl – threonyl – cysteinyl) cyclic sulfide - *O*$^{\beta}$ -[(1 - { *N*’’’’ - (1’’ - *N*’’ - { 14’, 3’, 6’, 9’, 12’ – tetraoxatetradecane - 1’ } - (2’’*S*) - 3’’ - (4’’’ – bromophenyl) – propenamide) - 2’’’’ - (benzyloxyimino) acetamide } - 1*H* - 1, 2, 3 – triazol – 4 – yl) methoxy] - threonine (7)/carbamoylmethyl ester (6). Fmoc-deprotection procedure: *N*$^{\alpha}$-Fmoc -D-phenylalanyl-(2-mercapto-tryptophanyl-tyrosyl-D-tryptophanyl-*N*$^{\varepsilon}$-1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl-lysinyl-threonyl-cysteinyl)cyclic sulfide-*O*$^{\beta}$-[(1-{*N*’’’’-([1’’-*N*’’-{14’, 3’,6’,9’,12’-tetraoxatetradecane-1’}-(2’’*S*)-3’’-(4’’’-bromophenyl)-propanamide)-5’’’’-carboxamide-(4*S*’’’’,5*S*’’’’)-2’’’’,2’’’’-dimethyl-1’’’’,3’’’’-dioxolane-4’’’’-*N*’’’’-TentaGel MB-carboxamide}-1*H*-1,2,3-triazol-4-yl)methoxy]-threonine carbamoylmethyl ester (**5**) (0.162 g, ~ 16 µmol) was resuspended in a clear solution of 20% (*v*/*v*) diethylamine in dry DMF (6 mL) at pH ~ 11, shaken for 2 hours 30 minutes, drained by filtration and washed with dry DMF (15 ml). The resin was resuspended in dry DMF (6 mL), shaken, drained by filtration and the procedure was repeated six more times with fresh solvent. The filtration and the shaking procedures were repeated using MeCN, 1:1 (*v*/*v*) EtOH/EtOAc, 1:9 (*v*/*v*) AcOH/EtOAc, dry DCM. A Kaiser test was performed to afford H-D-phenylalanyl-(2-mercapto-tryptophanyl-tyrosyl-D-tryptophanyl-*N*$^{\varepsilon}$-1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl-lysinyl-threonyl-cysteinyl)cyclic sulfide-*O*$^{\beta}$-[(1-{*N*’’’’-([1’’-*N*’’-{14’, 3’,6’,9’,12’-tetraoxatetradecane-1’}-(2’’*S*)-3’’-(4’’’-bromophenyl)-propanamide)-5’’’’-carboxamide-(4*S*’’’’,5*S*’’’’)-2’’’’,2’’’’-dimethyl-1’’’’,3’’’’-dioxolane-4’’’’-*N*’’’’-TentaGel MB-carboxamide}-1*H*-1,2,3-triazol-4-yl)methoxy]-threonine carbamoylmethyl ester (**SI-19**) as an ammonium acetate salt, that was dried under vacuum and stored at 4^{o}C. The latter is also identified as CAM/Dde protected (Ttn)-TATE on resin (**SI-19**), **Scheme *S10***. From the CAM/Dde protected (Ttn)-TATE on resin (**SI-19**) was recovered the corresponding H-D-phenylalanyl-(2-mercapto-tryptophanyl-tyrosyl-D-tryptophanyl-*N*$^{\varepsilon}$-1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl-lysinyl-threonyl-cysteinyl)cyclic sulfide-*O*$^{\beta}$-[(1-{*N*’’’’-(1’’-*N*’’-

{14', 3',6',9',12'-tetraoxatetradecane-1'}-(2''*S*)-3''-(4'''-bromophenyl)-propanamide)-2''''-(benzyloxyimino)acetamide}-1*H*-1,2,3-triazol-4-yl)methoxy]-threonine carbamoylmethyl ester (**SI-21**) solution according to *the tartrate acetonide deprotection procedure*, *the oxidative cleavage of products from tartrate-based linker resin procedure* and *the oxime of N-glyoxyloyl peptide with O-benzylhydroxylammonium chloride procedure* to afford the corresponding oxime *O*-benzyloxime **SI-21** of the CAM/Dde protected (Ttn)-TATE, **Scheme *S10***. LR-MS (MALDI-TOF) *m/z*: calcd for $C_{100}H_{123}N_{18}O_{22}SBrNa$ [M + Na]$^+$: 2061.8:2063.8, found 2062.1:2064.1. RP-HPLC: t_r = 25.56 min (A_{290} 66%), with gradient of 20% to 100% solvent B over 30 min at a flow rate of 1 mL/min; UV-Vis spectrum with λ_{max} = 224, 292 nm with expected double shoulder. However, the latter was only partially purified and isolated in several RP-HPLC (C18) fractions before being deprotected. CAM deprotection procedure in solution: The dry H-D-phenylalanyl-(2-mercapto-tryptophanyl-tyrosyl-D-tryptophanyl-N^{ε}-1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl-lysinyl-threonyl-cysteinyl)cyclic sulfide-O^{β}-[(1-{*N*''''-(1''-*N*''-{14', 3',6',9',12'-tetraoxatetradecane-1'}-(2''*S*)-3''-(4'''-bromophenyl)-propanamide)-2''''-(benzyloxyimino)acetamide}-1*H*-1,2,3-triazol-4-yl)methoxy]-threonine carbamoylmethyl ester (**SI-21**) (~ 0.32 µmol) partially purified was dissolved in a clear solution of 0.2 M Cs_2CO_3 (~ 50 eq.) in 4:6 (*v*/*v*) Milli-Q water/DMF, pH ~ 12 and mixed for 8 h in the dark. The reaction was followed by RP-HPLC(C18)-UV by sampling ~ 1% of the reaction volume, then diluted 50 x with 0.1% FA in 2:8 (v/v) MeCN/Milli-Q water before injection on RP-HPLC(C18). The candidate peaks were collected, lyophilized and analyzed by low resolution MALDI-TOF. Upon near completion of the reaction, the mixture was diluted with 0.1% formic acid (6 equivalents relative to Cs_2CO_3) in 2:8 (*v*/*v*) MeCN/water and lyophilized to afford a mixture of fully and partially CAM/Dde deprotected (Ttn)-TATE (**SI-22**), **Scheme *S11***. Dde deprotection procedure in solution: The crude powder **SI-22** was dissolved in a clear solution of approximately 0.07 M hydrazine (~ 4.5 eq.) in 1:9 (*v*/*v*) Milli-Q water/DMF corresponding approximately to 0.4% (*v*/*v*) of hydrazine. The pale yellow clear mixture was stirred between 1 h to 1 h 30 min in the dark and the reaction was followed by RP-HPLC(C18)-UV as previously done. Upon completion of the reaction, the mixture was diluted 100 x, in volume, with a solution of 0.1% FA in Milli-Q water and

either stored at -80°C or carried to the purification step. The crude mixture was directly loaded on a 0.1% FA in Milli-Q water pre-equilibrated Sep-Pak (C18) and purified, followed by RP-HPLC (C18) purification, both as described in *General Experimental Information*, to afford H-D-phenylalanyl-(2-mercapto-tryptophanyl-tyrosyl-D-tryptophanyl-lysinyl-threonyl-cysteinyl)cyclic sulfide-O^{β}-[(1-{*N*''''-(1''-*N*''-{14', 3',6',9',12'-tetraoxatetradecane-1'}-(2''*S*)-3''-(4'''-bromophenyl)-propanamide)-2''''-(benzyloxyimino)acetamide}-1*H*-1,2,3-triazol-4-yl)methoxy]-threonine carbamoylmethyl ester also called CAM protected *O*-benzyloxime-(Ttn)-TATE (**6**) (32 nmol, 0.00025% yield) and the H-D-phenylalanyl-(2-mercapto-tryptophanyl-tyrosyl-D-tryptophanyl-lysinyl-threonyl-cysteinyl)cyclic sulfide-O^{β}-[(1-{*N*''''-(1''-*N*''-{14', 3',6',9',12'-tetraoxatetradecane-1'}-(2''*S*)-3''-(4'''-bromophenyl)-propanamide)-2''''-(benzyloxyimino)acetamide}-1*H*-1,2,3-triazol-4-yl)methoxy]-threonine (**7**) also called *O*-benzyloxime-(Ttn)-TATE (181 nmol, 0.001% yield), **Scheme *S11***. Yields were relative to the starting material fully protected (Ttn)-TATE resin (**5**) (0.162 g, 0.016 mmol) and thus over 6 steps. Oximes were reported to undergo hydrolysis upon freeze-thaw cycling.[10] CAM protected *O*-benzyloxime-(Ttn)-TATE (**6**): RP-HPLC (C18): t_r = 11.13 min (A_{290} 100%), with gradient of 20% to 100% solvent B over 18 min at 2 mL/min; UV-Vis spectrum with λ_{max} = 223, 285, 290, 304 nm. HR-MS (ESI-TOF) m/z calcd for $C_{90}H_{113}N_{18}O_{20}S$ $[M + 2H]^{2+}$: 938.3641:939.3646, found 938.3644:939.3547. *O*-benzyloxime-(Ttn)-TATE (**7**): RP-HPLC (C18): t_r = 11.38 min (A_{290} 100%), with gradient of 20% to 100% solvent B over 18 min at 2 mL/min; UV-Vis spectrum with λ_{max} = 226, 284, 290, 302 nm. HR-MS (ESI-TOF) m/z calcd for $C_{88}H_{110}N_{17}O_{19}S$ $[M + 2H]^{2+}$: 909.8534:910.8538, found 909.8524:910.8485. Procedure for quantifying (Ttn)-TATE[13]: All pipetmen used were calibrated by gravimetry using water. A solution of 5 mM N^{α}-Ac-Cys-OH (NAC) and *o*-phthalaldehyde (OPA) in borate buffer, called OPA-NAC buffer reagent, was prepared by mixing 50 mM N^{α}-Ac-Cys-OH (NAC) in Milli-Q water (25 mL, 1.25 mmol), 50 mM *o*-phthalaldehyde (OPA) in EtOH (25 mL, 1.25 mmol) and borate buffer (200 mL). The borate buffer was prepared by dissolving *ortho*-boric acid (6.18 g, 99.95 mmol) and NaOH (2.8 g, 70 mmol) in Milli-Q water (1 L) to give pH ~ 9.5. A standard (calibration) curve was prepared by measuring the absorbance at 335 nm of various but exact volumes (5.00, 10.0, 20.0, 30.0, 40.0 and

50.0 µL) of 7 mM hexylamine and phenylalanine in 2:8 (*v*/*v*) MeCN/Milli-Q water diluted with OPA-NAC buffer reagent (3.000 mL). All volumes and absorbance measurements were done in duplicate to give a linear regression curve of A_{335} = 11839x + 0.0298 with R^2 = 0.999 where A_{335} is the absorbance measured at 335 nm and x is the corresponding molar concentration according to Beer-Lambert law (l = 1 cm). Solutions of *O*-benzyloxime-(Ttn)-TATE derivatives were prepared in 2:8 (*v*/*v*) MeCN/water, samples were taken, diluted in OPA-NAC buffer reagent (0.750 mL) and absorbance at 335 nm was measured. All volumes and absorbance measurements were done in duplicate and linear regression formulas were used to deduce the amounts of the *O*-benzyloxime-(Ttn)-TATE derivative.

Additional Note 1. Several by-products were isolated by RP-HPLC from the crude reaction mixture following oximation and the corresponding structures were tentatively assigned from UV-VIS spectrum and MALDI-TOF-MS (**Figure *S1***). In addition, tryptathionine, tryptathionine sulfoxide and tryptathionine sulfone were identified in these by-products. Various truncates and tryptophan dimerization were also detected in low proportion to the desired product. Overall, the synthesis by SPPS gave clean results with the major contaminations arising from Savige-Fontana reaction and oximation reaction (**Figure *S1***). However, other possible by-product could be avoided with the Boc protection of the tryptophan indole's nitrogen.

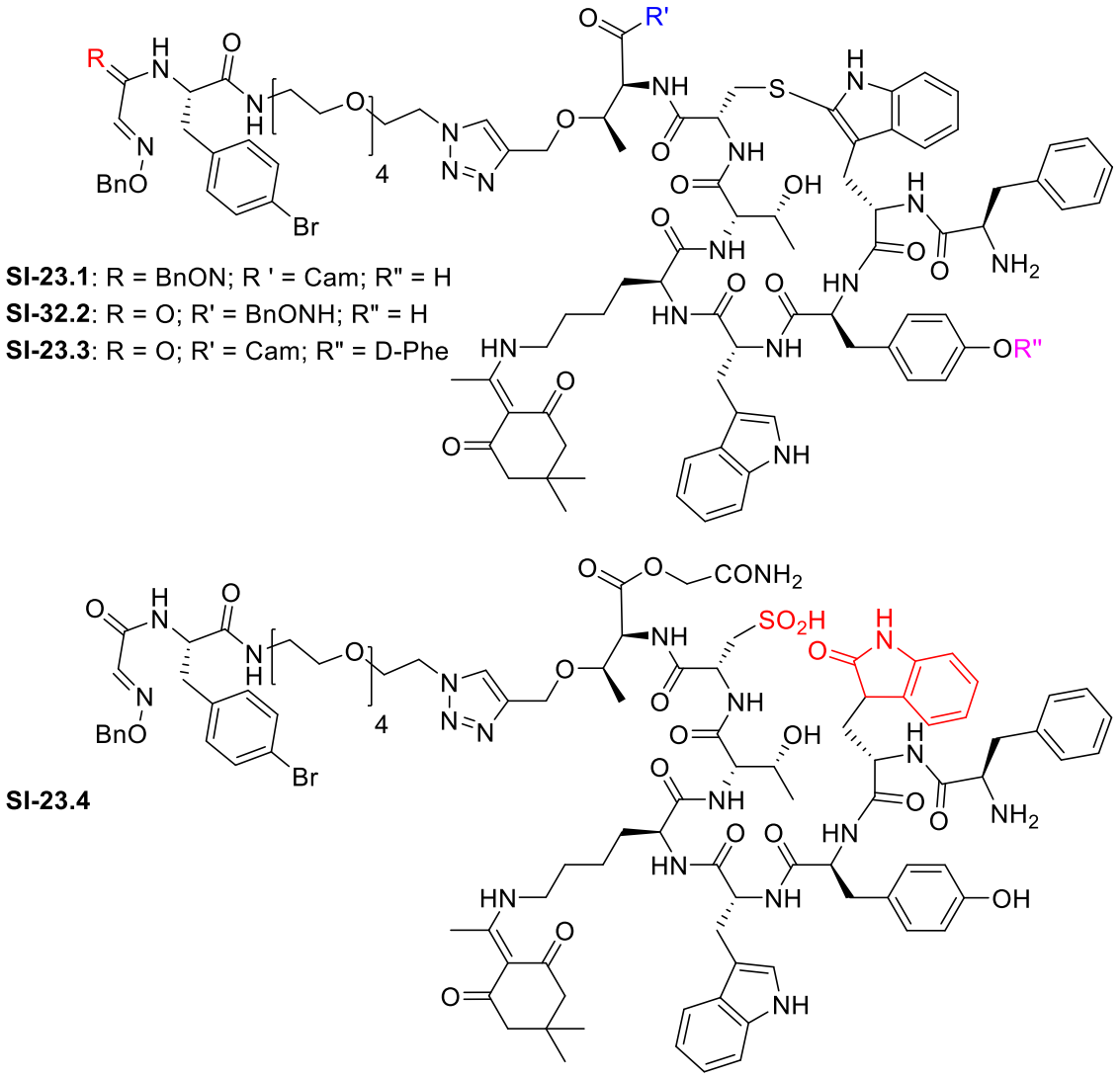
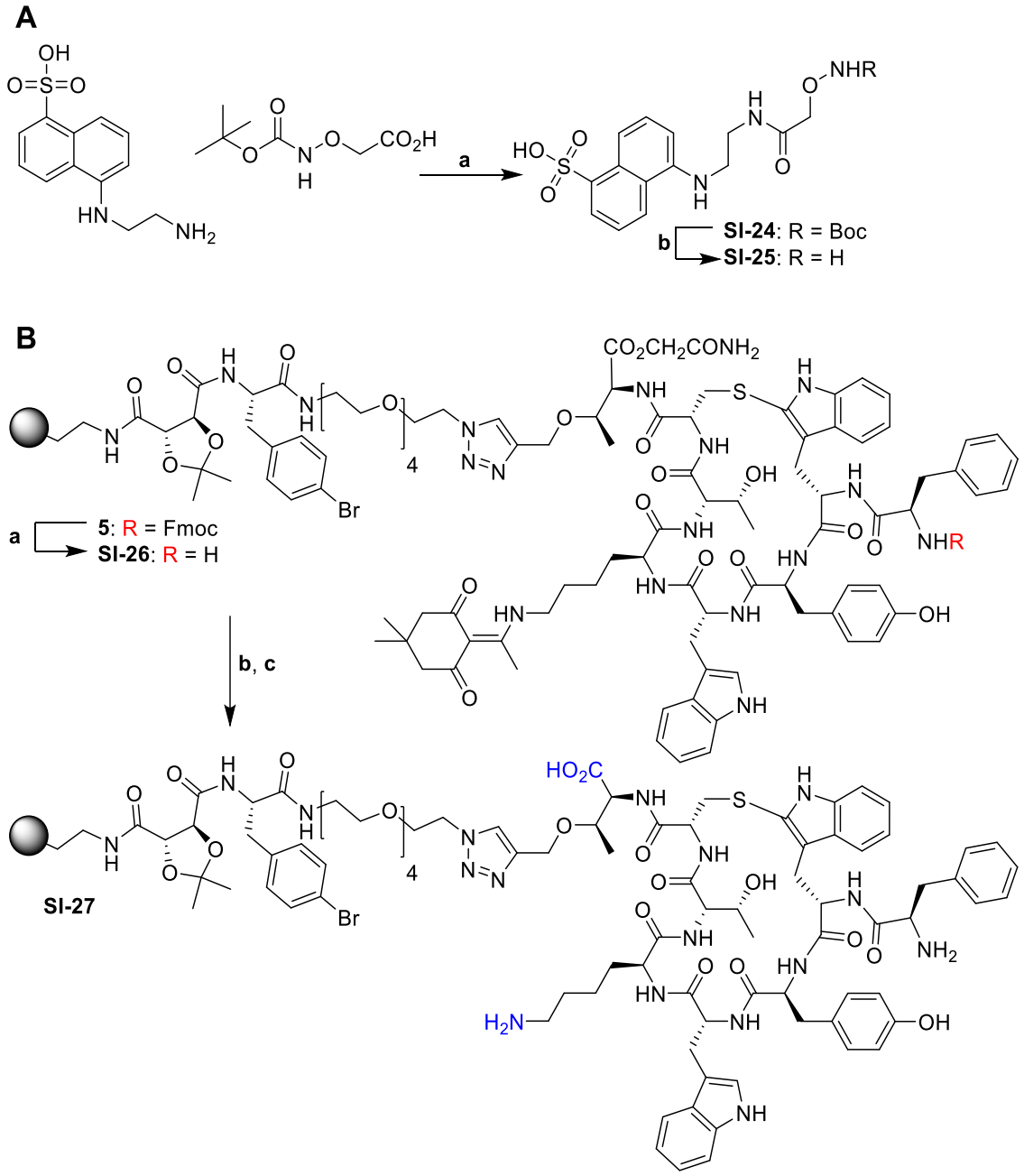


Figure *S1*. Suggested by-products from RP-HPLC-UV-Vis and MALDI-TOF-MS analysis.

Additional Note 2. The benzyloxime-CAM/Dde protected (Ttn)-TATE (**SI-21**), along with its tryptathionine oxidized counterparts, were exposed, in solution, to 0.2 M Cs_2CO_3 in 4:6 (*v*/*v*) water/DMF and the reaction was followed by RP-HPLC (C18)/MALDI-TOF. The optimal time for CAM ester saponification, with minimal side reactions, was found between 6 and 8 hours but 100% hydrolysis could not be reached. Longer exposure to base led to a complex mixture of Dde deprotected peptides with or without CAM and the loss of oxime with or without CAM and Dde. With did not find by-product coming from CAM ester amidation by the Lys side chain. The reaction was quenched with 0.1% FA water and lyophilized to yield a crude mixture of benzyloxime-(Ttn)-TATE (**7**) fully

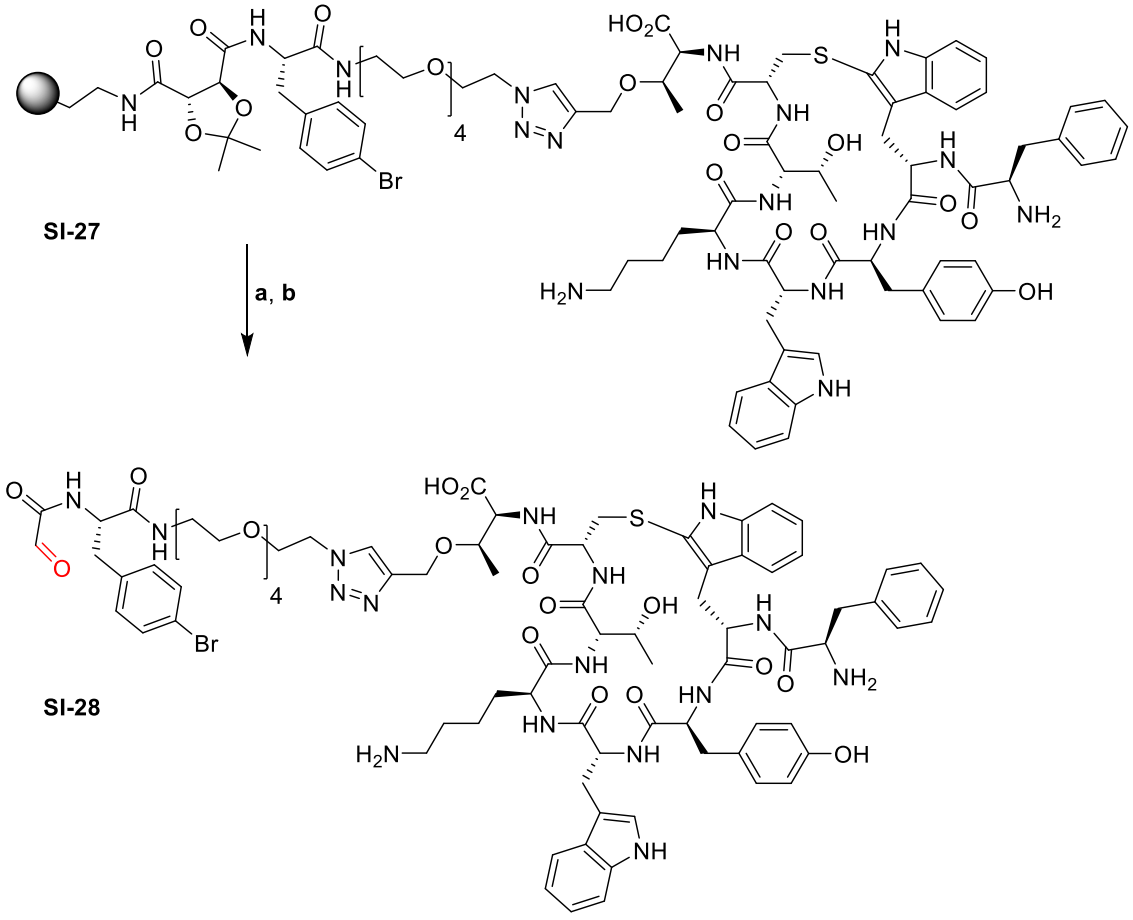
deprotected mixed with derivatives having either Dde, CAM or both (**Scheme *S11***). Following CAM hydrolysis, the hydrazinolysis of the Dde protecting group on lysine of the crude product (**SI-22**) was achieved as follows (**Scheme *S11***): exposure to approximately 4 equivalents of 0.15 M hydrazine, for a period of 30 min to 1 hour, in DMF/water yielded the CAM protected benzyloxime-(Ttn)-TATE (**6**) and two compounds (**7** and **7.2**) with the expected UV spectrum and the expected MS data for benzyloxime-(Ttn)-TATE but different retention times on RP-HPLC. The two compounds (**7** and **7.2**) were not further characterized but could be epimers of the peptide or, more likely, *cis*/*trans* configurational isomers of the oxime. Oximes are usually in equilibrium as a mixture of *cis* (*Z*)- and *trans* (*E*)-isomer.[14] However, the *trans* configuration with the O-N bond opposite to the C-C bond in the oxime (C=N bond) is favored based on steric arguments.[15] In all cases, the desired product was mixed with the corresponding tryptathionine sulfoxide (the thiol bridge is oxidized to the sulfoxide) and tryptathionine sulfone (the thiol bridge is oxidized to the sulfone) bridge. The overall yields of benzyloxime-(Ttn)-TATE were low due to significant loss of product during the different stages of synthesis, including the analytical procedures, tests and purification. However, most by-products appear to have formed during the oximation stage, which gave us confidence in the overall purity of the product on the beads.

2.3.6 Oxidative Cleavage of Fully Deprotected [Ttn]-Octreotate from Resin and Oximation



Scheme *S12A & B*. Synthesis of EDANS and deprotection of (Ttn)-TATE on Macrobead TentaGel amine resin. Reagents and conditions: Panel **A**: **a**) DIC, Oxyma, 1:3 (*v*/*v*) dry DMF/dry MeCN, 24 h, pH ~ 5, 60%; **b**) 1:4 (*v*/*v*) TFA/DCM, ~ 1 h. Panel **B**: **a**) 20% DEA

(*v*/*v*) in dry DMF, pH ~ 11, 2 h 30 min; **b**) 0.2 M Cs_2CO_3, 6:4 (*v*/*v*) water/DMF, 14 h, pH ~ 11; **c**) 1% (*v*/*v*) hydrazine hydrate in dry DMF, 1 h 30 min.



Scheme *S13*. Cleavage of fully deprotected (Ttn)-TATE from Macrobead TentaGel amine resin. Reagents and conditions: **a**) 95:2.5:2.5 (*v*/*v*/*v*) TFA/water/TIS, 2 h; **b**) One Pot: *i*) 1:1:4 (*v*/*v*/*v*) AcOH/*t*-BuOH/water, $NaIO_4$, pH ~ 1, 10 min; *ii*) dimethylsulfide, ~ 5 min; 1 M aqueous sodium thiosulfate ($Na_2S_2O_3$).

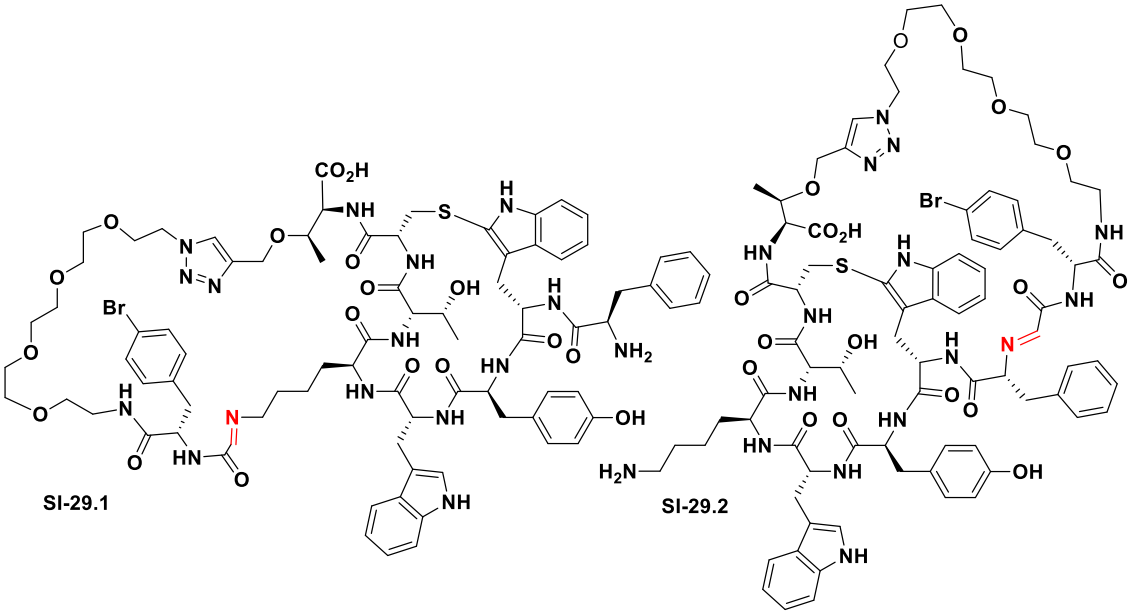
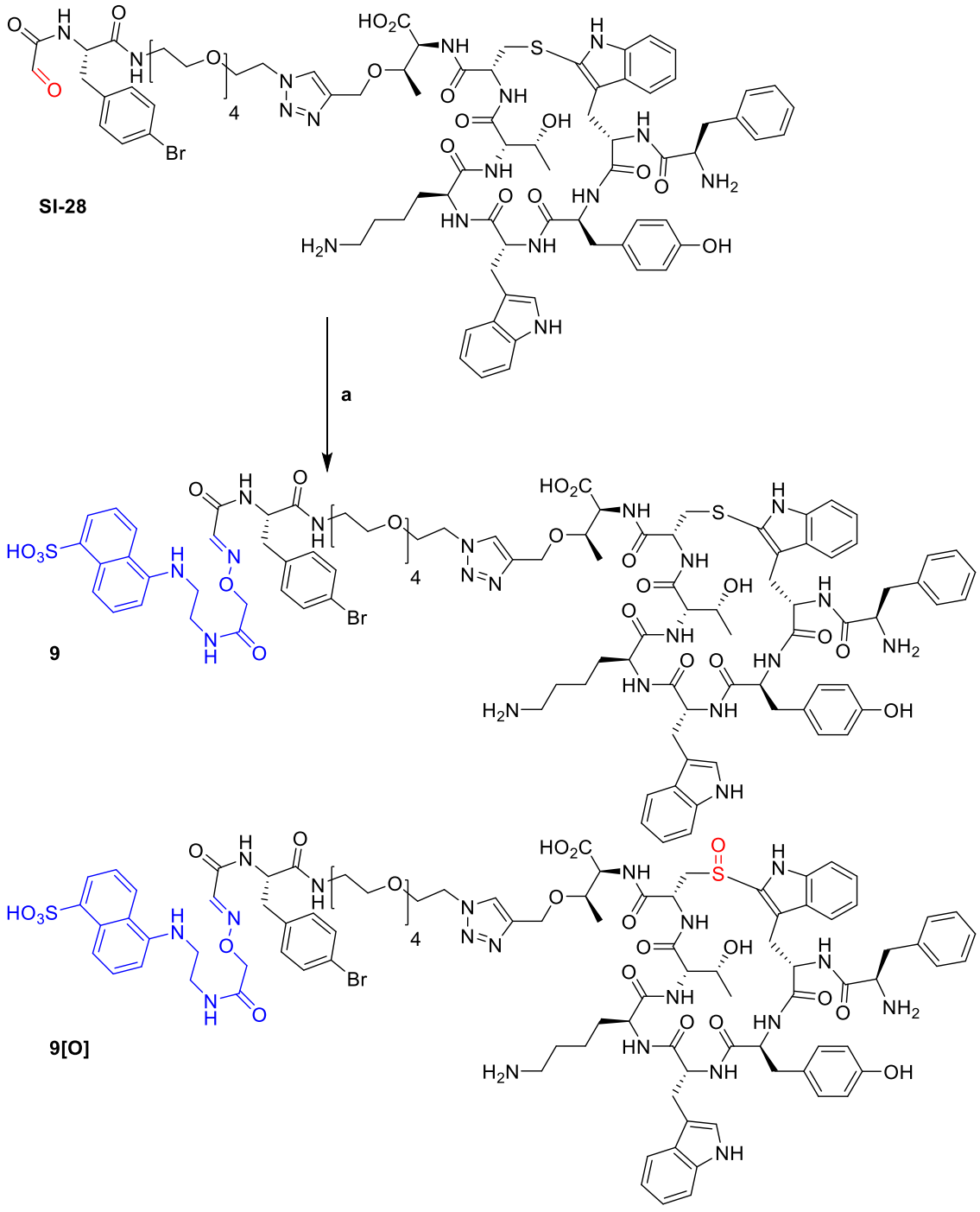


Figure *S2*. Intramolecular imine-(Ttn)-TATE (**SI-29**) found in crude product.



Scheme *S14*. Oximation of the bicyclic imino-(Ttn)-TATE. Reagents and conditions: **a**) Freshly deprotected under argon H-Aoa-EDANS (**SI-25**), 1,4-phenylenediamine hydrochloride, MeCN, AcOH, *t*-BuOH, NaOH, pH ~ 5, 21 h; *iv*) Sep-Pak (C18) in 0.1% FA water/MeCN; *v*) RP-HPLC (C18) in 0.1% FA water/MeCN.

N*-[5''-(2'-Aminoethyl)-1''-naphthalenesulfonic)-[({[(2-methyl-2-propanyl)oxy]carbonyl}amino)oxy]acetamide** (**SI-24**). Under argon, a yellow clear solution of [({[(2-methyl-2-propanyl)oxy]carbonyl}amino)oxy]acetic acid (0.102 g, 0.533 mmol), 5-[(2-aminoethyl)amino]-1-naphthalenesulfonic acid (0.169 g, 0.586 mmol), Oxyma Pure (0.083 g, 0.586 mmol) and DIC (0.092 mL, 0.586 mmol) in 1:3 (v/v) dry DMF/dry MeCN (28 mL) was stirred for 24 h in the dark. The mixture was evaporated to dryness under reduced pressure to afford a crude orange solid that was purified by flash chromatography on silica (7:3 DCM/EtOAc to 2:4:4 EtOH/DCM/EtOH), repurified by manual reverse phase column chromatography on Sep-Pak C18 gel (Waters, Delaware) as described in *General Experimental Information*, then repurified by flash chromatography on silica (1:5:4 EtOH/DCM/EtOAc to 3:7 EtOH/DCM) to afford *N*-[5''-(2'-aminoethyl)-1''-naphthalenesulfonic)-[({[(2-methyl-2-propanyl)oxy]carbonyl}amino)oxy]acetamide (**SI-24**) (0.078 g, 0.178 mmol), also called *N*-4-Boc-Aoa-EDANS, **Scheme *S12A, as a white solid in 33% yield, R_f ~ 0.10 (2:3:5 EtOH/EtOAc/DCM). RP-HPLC (C18): t_r = 20.75 min (A_{341} 100%) with gradient of 20% to 100% solvent B over 32 min at a flow rate of 1 mL/min; UV-Vis spectrum with λ_{max} = 215, 252, 340 nm. HR-MS (ESI-TOF) *m/z*: calcd for $C_{19}H_{24}N_3O_7S$ $[M - H]^-$: 438.1335, found: 438.1333. **^{1}H-NMR** (400MHz, MeOD + D_2O): δ 8.05 (d, *J* = 8.7 Hz, 1H), 7.99 (t, *J* = 6.8 Hz, 2H), 7.40-7.35 (m, 2H), 6.68 (d, *J* = 7.7 Hz, 1H), 3.55 (t, *J* = 5.9 Hz, 2H), 3.37 (t, *J* = 5.9 Hz, 2H), 1.24 (s, 9H). **^{13}C-NMR** (100 MHz, APT, $CDCl_3$): δ 173.0 (**C**), 145.3 (**C**), 141.1 (**C**), 131.2 (**C**), 129.2 (**C**H), 127.3 (**C**H), 126.0 (**C**), 125.9 (**C**H), 124.5 (**C**H), 116.8 (**C**H), 106.6 (**C**H), 84.6 (**C**), 76.5 (**C**), 44.8 (**C**H_2), 39.5 (**C**H_2), 28.7 (**C**H_3); spectrum is in accordance with literature values[16]

N*-[5''-(2'-Aminoethyl)-1''-naphthalenesulfonic)-[(amino)oxy] acetamide** (**SI-25**). Under argon, *N*-4-Boc-Aoa-EDANS (**SI-24**) (0.078 g, 0.177 mol) was dissolved in 0.02:0.58:0.4 (*v*/*v*/*v*) Milli-Q water/DCM/TFA (5 mL) and stirred for 2 h in the dark. The orange clear mixture was evaporated to dryness under reduced pressure and flushed with argon to afford the crude *N*-[5''-(2'-aminoethyl)-1''-naphthalenesulfonic)-[(amino)oxy]acetamide (**SI-25**), also called H-Aoa-EDANS, **Scheme *S12A, as an orange solid that was used immediately without further manipulations. HR-MS (ESI-TOF) *m/z*:

calcd for $C_{14}H_{16}N_3O_5S$ [M - H]$^-$: 338.0811, found: 338.0804. RP-HPLC (C18): t_r = 5.85 min (A_{342} 96%) with gradient of 20% to 100% solvent B over 18 min at a flow rate of 2 mL/min; UV-Vis spectrum with λ_{max} = 212, 252, 338 nm. RP-HPLC (C18): t_r = 7.53 min (A_{342} 94%) with gradient of 10% to 100% solvent B over 21 min at a flow rate of 2 mL/min; UV-Vis spectrum with λ_{max} = 212, 250, 338 nm.

H-D-Phenylalanyl-(2-mercapto-tryptophanyl-tyrosyl-D-tryptophanyl-*N*$^{\varepsilon}$-1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl-lysinyl-threonyl-cysteinyl)cyclic sulfide-*O*$^{\beta}$-[(1-{*N*''''-(1''-*N*''-{14', 3',6',9',12'-tetraoxatetradecane-1'}-(2''*S*)-3''-(4'''-bromophenyl)-propanamide)-2''''-(3'''''-oxyimino-*N*-[5'''''''-(2''''''-aminoethyl)-1''''''-naphthalenesulfonic]acetamide)acetamide}-1*H*-1,2,3-triazol-4-yl)methoxy]-threonine (9). Fmoc-deprotection procedure: *N*$^{\alpha}$-Fmoc -D-phenylalanyl-(2-mercapto-tryptophanyl-tyrosyl-D-tryptophanyl-*N*$^{\varepsilon}$-1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl-lysinyl-threonyl-cysteinyl)cyclic sulfide-*O*$^{\beta}$-[(1-{*N*''''-([1''-*N*''-{14', 3',6',9',12'-tetraoxatetradecane-1'}-(2''*S*)-3''-(4'''-bromophenyl)-propanamide)-5''''-carboxamide-(4*S*'''',5*S*'''')-2'''',2''''-dimethyl-1'''',3''''-dioxolane-4''''-*N*''''-TentaGel MB-carboxamide}-1*H*-1,2,3-triazol-4-yl)methoxy]-threonine carbamoylmethyl ester (**5**) (0.21 g, ~ 21 µmole) was resuspended in a clear solution composed of 20% (*v*/*v*) diethylamine in dry DMF (6 mL) at pH ~ 11, shaken for 2 hours 30 minutes, drained by filtration and filtered with dry DMF (15 ml). The resin was resuspended in dry DMF (6 mL), shaken, drained by filtration and the procedure was repeated six more times with fresh solvent. The filtration and the shaking procedures were repeated using MeCN, 1:1 (*v*/*v*) EtOH/EtOAc, 1:9 (*v*/*v*) AcOH/EtOAc, dry DCM. A Kaiser test was performed to afford D-phenylalanyl-(2-mercapto-tryptophanyl-tyrosyl-D-tryptophanyl-*N*$^{\varepsilon}$-1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl-lysinyl-threonyl-cysteinyl)cyclic sulfide-*O*$^{\beta}$-[(1-{*N*''''-([1''-*N*''-{14', 3',6',9',12'-tetraoxatetradecane-1'}-(2''*S*)-3''-(4'''-bromophenyl)-propanamide)-5''''-carboxamide-(4*S*'''',5*S*'''')-2'''',2''''-dimethyl-1'''',3''''-dioxolane-4''''-*N*''''-TentaGel MB-carboxamide}-1*H*-1,2,3-triazol-4-yl)methoxy]-threonine carbamoylmethyl ester (**SI-26**), also called CAM/Dde-(Ttn)-TATE resin (**SI-26**), **Scheme *S12B***, as an ammonium acetate salt, was dried under vacuum and stored at 4^{o}C. CAM/Dde deprotection procedure on solid phase: The CAM/Dde-(Ttn)-TATE resin (**SI-26**) was filtered with 4:6 (*v*/*v*) Milli-Q water/DMF (10

mL). The resin was resuspended in 4:6 (*v*/*v*) Milli-Q water/DMF (6 mL), shaken for 30 minutes, drained by filtration and the procedure was repeated two more times with fresh solvent. The resin was resuspended in 0.2 M Cs_2CO_3 (~ 42 eq.) of 3:4 (*v*/*v*) Milli-Q water/DMF (6 mL) pH ~ 12, shaken in the dark for 14 h and drained by filtration. The resin was filtered with 1:1 (*v*/*v*) Milli-Q water/DMF (20 mL). The resin was resuspended in 1:1 (*v*/*v*) Milli-Q water/DMF (6 mL), shaken, drained by filtration and the washing procedure was repeated nine more times with fresh solvent. The filtration and the shaking procedures were repeated using Milli-Q water, 1:1 (*v*/*v*) EtOH/Milli-Q water, 0.1% FA in 1:1 (*v*/*v*) DMF/Milli-Q water, 1:1 (*v*/*v*) EtOH/DMF, DMF. The resin was resuspended in 0.99% (*v*/*v*) hydrazine hydrate (~ 35 eq.) of DMF, shaken for 1 h 30 min and drained by filtration. The resin was rinsed with DMF (20 mL), resuspended in DMF (6 mL), shaken, drained by filtration and the procedure was repeated six more times with fresh solvent. The filtration and the shaking procedures were repeated using 1:1 (*v*/*v*) DMF/Milli-Q water, 0.1% FA in 1:1 (*v*/*v*) DMF/Milli-Q water, 1:1 (*v*/*v*) EtOH/DMF, EtOH, DMF, MeCN, DCM. The resin was stored wet in DCM at 4°C to afford D-phenylalanyl-(2-mercapto-tryptophanyl-tyrosyl-D-tryptophanyl-lysinyl-threonyl-cysteinyl)cyclic sulfide-O^{β}-[(1-{*N*''''-([1''-*N*''-{14', 3',6',9',12'-tetraoxatetradecane-1'}-(2''*S*)-3''-(4'''-bromophenyl)-propanamide)-5''''-carboxamide-(4*S*'''',5*S*'''')-2'''',2''''-dimethyl-1'''',3''''-dioxolane-4''''-*N*''''-TentaGel MB-carboxamide}-1*H*-1,2,3-triazol-4-yl)methoxy]-threonine (**SI-27**), also called (Ttn)-TATE on resin **SI-27** (**Scheme *S12B***). From (Ttn)-TATE on resin **SI-27** (0.183 g) was recovered the corresponding *N*-glyoxyloyl **SI-28** as well as the cyclic imines **SI-29** in solution according to according to *the tartrate acetonide deprotection procedure*, *the oxidative cleavage of products from tartrate-based linker resin procedure*, **Scheme *S13***. Crude *N*-glyoxyloyl-(Ttn)-TATE (**SI-28**): LR-MS (ESI-High Capacity ion Trap) *m/z*: calcd for $C_{81}H_{103}N_{16}O_{19}BrS$ $[M + 2H]^{+2}$: 857.3:858.3, found: 857.8:858.8. RP-HPLC (C18): t_r = 10.05 min (A_{290} 55.4%), with gradient of 20% to 100% solvent B over 18 min at 2 mL/min; UV-Vis spectrum with λ_{max} = 226, 284, 290, 304 nm. Crude cyclic imines-(Ttn)-TATE (**SI-29**): LR-MS (ESI-TOF) *m/z*: calcd for $C_{81}H_{100}N_{16}O_{18}BrS$ $[M + H]^{+}$: 1695.6:1697.6, found: 1695.7:1697.7. RP-HPLC (C18): t_r = 9.41 min (A_{290} 96%), with gradient of 20% to 100% solvent B over 18 min at 2 mL/min; UV-Vis spectrum with λ_{max} = 224, 284, 290, 302 nm.

Oxiamation of *N*-glyoxyloyl peptide and imines mixture with H-Aoa-EDANS: The argon flushed crude H-Aoa-EDANS (**SI-25**) was immediately dissolved in 40:5:18 (*v*/*v*/*v*) Milli-Q water/AcOH/*tert*-BuOH (buffered to pH ~ 5 with 4 N $NaOH_{(aq)}$) (4 mL) and transferred to the argon flushed dry *N*-glyoxyloyl **SI-28** and cyclic imines **SI-29**. To the argon flushed round bottom flask, from which H-Aoa-EDANS (**SI-25**) was transferred, was added 40:5:18 (*v*/*v*/*v*) Milli-Q water/AcOH/*tert*-BuOH (buffered to pH ~ 5 with 4 N $NaOH_{(aq)}$) (6 mL), transferred to the previous mixture and stirred in the dark. The procedure was repeated with NaOH basified Milli-Q water pH ~ 12 (5 x 5 mL), MeCN (3 x 5 mL) and 40:5:18 (*v*/*v*/*v*) Milli-Q water/AcOH/*tert*-BuOH (buffered to pH ~ 5 with 4 N $NaOH_{(aq)}$) (2 x 5 mL). The reaction was followed by RP-HPLC(C18)-UV by sampling ~ 0.03% of the reaction volume, then diluted 4 x, in volume, with 0.1% FA in 2:8 (v/v) MeCN/Milli-Q water before injection on RP-HPLC(C18). The pH ~ 5, orange, clear mixture was stirred in the dark under argon for 4 h 15 min before 1,4-phenylenediamine hydrochloride (0.079 g, 0.435 mmol) was added to the mixture and stirred for 21 h overall. The mixture was evaporated under reduced pressure to dryness and the crude solid was purified on a 0.1% FA in Milli-Q water pre-equilibrated Sep-Pak (C18) column as described in *General Experimental Information*. The product was further purified by RP-HPLC (C18) as described in *General Experimental Information* but applying a gradient of 20% to 100% of 0.1% FA (*v*/*v*) in MeCN against 0.1% FA (*v*/*v*) in Milli-Q water over 18 min at 2 ml/min. Further purification was performed by RP-HPLC (C18) applying a gradient of 30% to 100% of 0.1% FA (*v*/*v*) in MeCN against 0.1% FA (*v*/*v*) in Milli-Q water over 16 min at 2 ml/min to afford, **Scheme *S14***, as white lightly pink powders, H-D-Phenylalanyl-(2-mercapto-tryptophanyl-tyrosyl-D-tryptophanyl-N^{ε}-1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl-lysinyl-threonyl-cysteinyl)cyclic sulfide-O^{β}-[(1-{*N*’’’’-(1’’-*N*’’-{14’, 3’,6’,9’,12’-tetraoxatetradecane-1’}-(2’’*S*)-3’’-(4’’’-bromophenyl)-propanamide)-2’’’’-(3’’’’’-oxyimino-*N*-[5’’’’’’-(2’’’’’-aminoethyl)-1’’’’’’-naphthalenesulfonic]acetamide)acetamide}-1*H*-1,2,3-triazol-4-yl)methoxy]-threonine (**9**), also called EDANS-oxime-(Ttn)-TATE (**9**) (1.3 µmole) and H-D-Phenylalanyl-(2-mercapto-tryptophanyl-tyrosyl-D-tryptophanyl-N^{ε}-1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl-lysinyl-threonyl-cysteinyl)cyclic sulfoxide-O^{β}-[(1-{*N*’’’’-(1’’-*N*’’-{14’, 3’,6’,9’,12’-tetraoxatetradecane-1’}-(2’’*S*)-3’’-(4’’’-bromophenyl)-

propanamide)-2’’’’-(3’’’’’-oxyimino-*N*-[5’’’’’’’-(2’’’’’’-aminoethyl)-1’’’’’’’-naphthalenesulfonic]acetamide)acetamide}-1*H*-1,2,3-triazol-4-yl)methoxy]-threonine EDANS-oxime-(Ttn[O])-TATE (**9[O]**) (0.26 µmole) in 6% and 1.2% yield respectively over 5 steps from the protected (Ttn)-TATE on resin **5** (0.21 g, 0.021 mmol) starting material. Oximes were reported to undergo hydrolysis upon freeze-thaw cycling.[10] EDANS-oxime-(Ttn[O])-TATE (**9[O]**): RP-HPLC (C18): t_r = 10.21 min (A_{290} 100%), with gradient of 20% to 100% solvent B over 18 min at 2 mL/min; UV-Vis spectrum with λ_{max} = 223, 253, 283, 290, 302 and 338 nm. LR-MS (ESI-High Capacity ion Trap) *m/z*: calcd for $C_{95}H_{118}N_{19}O_{24}BrS_2$ $[M_{[O]} + 2H]^{+2}$: 1025.9:1026.9, found: 1025.9:1026.9; *m/z*: calcd for $C_{95}H_{117}N_{19}O_{25}BrS_2Na$ $[M_{2[O]} + H + Na]^{+2}$: 1044.9:1045.9, found: 1044.9:1045.9. EDANS-oxime-(Ttn)-TATE (**9**): RP-HPLC (C18): t_r = 10.39 min (A_{290} 99%), with gradient of 20% to 100% solvent B over 18 min at 2 mL/min; UV-Vis spectrum with λ_{max} = 223, 255, 283, 290, 302 and 340 nm. LR-MS (ESI-High Capacity ion Trap) *m/z*: calcd for $C_{95}H_{117}N_{19}O_{23}BrS_2$ $[M + H]^{+}$: 2034.7:2036.7, found: 2034.7:2036.7; *m/z*: calcd for $C_{95}H_{117}N_{19}O_{23}BrS_2Na$ $[M + H + Na]^{+2}$: 1028.9:1029.9, found: 1029.9:1029.9. <u>EDANS-(Ttn)-TATE quantification procedure</u>: All pipetmen used were calibrated by gravimetry using water. A standard (calibration) curve was prepared by measuring the absorbance at 340 nm of solutions of various, but exact, concentrations of EDANS (λ_{max} = 252, 335 nm) in 0.1% FA 4:6 (*v*/*v*) MeCN/Milli-Q water. All volumes and absorbance measurements were done in duplicate to give a linear regression curve of A_{340} = 4728.4x - 0.0134 with R^2 = 0.998 where A_{340} is the absorbance measured at 340 nm and x is the corresponding molar concentration according to Beer-Lambert law (l = 1 cm) (**Figure *S3***). Solutions of EDANS-(Ttn)-TATE derivatives were prepared in 0.1% FA in 2:8 (*v*/*v*) MeCN/water, samples were taken, diluted in 0.1% FA 4:6 (*v*/*v*) MeCN/Milli-Q water and absorbance at 340 nm was measured. All volumes and absorbance measurements were done in duplicate and the linear regression formulas were used to deduce the number of EDANS-(Ttn)-TATE derivatives.

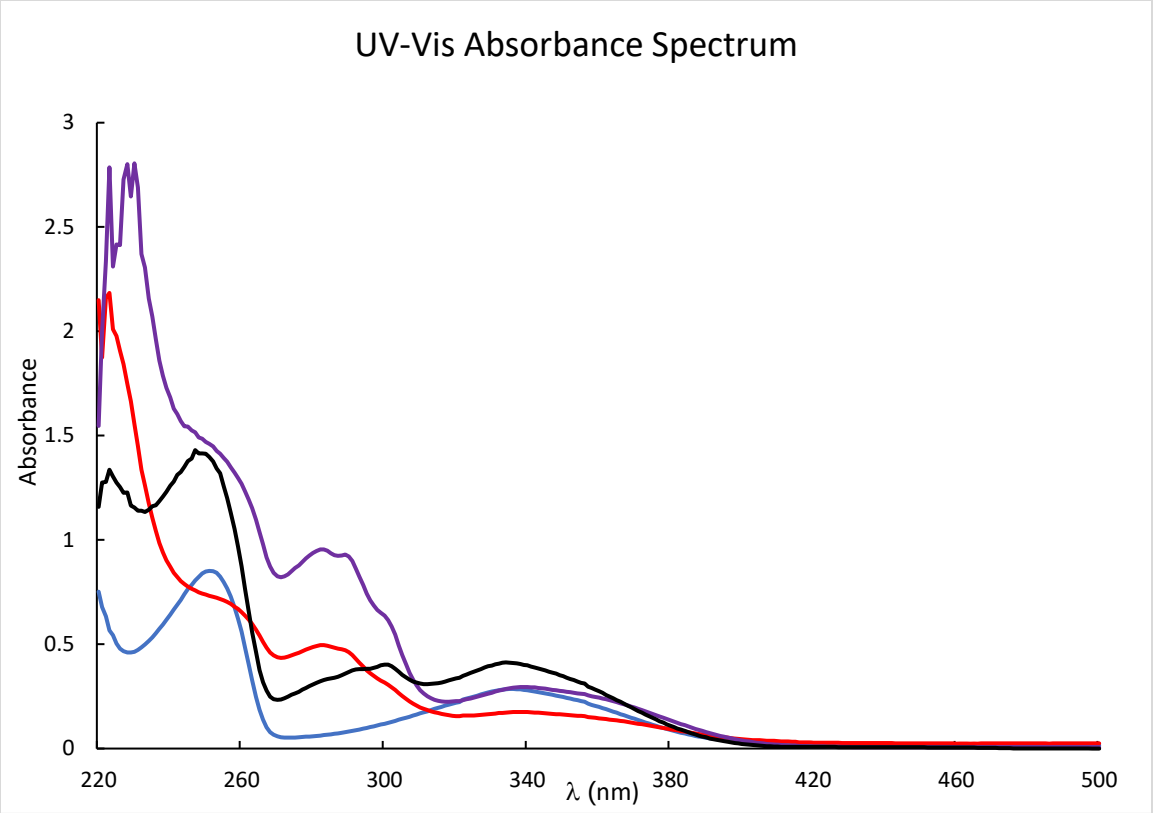


Figure *S3* Representative UV-Vis absorbance spectra of EDANS-Oxime-(Ttn)-TATE and its oxidized counterpart. Spectra taken in 0.1% FA 4:6 (*v*/*v*) water/MeCN of 62 µM EDANS-oxime-(Ttn)-TATE (**9**) in purple, 32 µM EDANS-oxime-(Ttn[O])-TATE (**9[O]**) in red and 50 µM EDANS in blue. Spectrum of a 1:1 mixture of trytptathionine N^{α}-D-Phe-(Trp-Tyr-Cys)c-OH[6] and EDANS at 55 µM in 0.1% FA 0.5:9.5 (*v*/*v*) MeCN/water in black. The 1:1 mixture of tryptathionine N^{α}-D-Phe-(Trp-Tyr-Cys)c-OH[6] and EDANS absorbance values at their respective λ_{max} were different relative to the EDANS-oxime-(Ttn)-TATE (**9**). The tryptathionine absorbance increased by around two times in the latter case due to the additive absorbance of the tryptophan and the tryptathionine found in EDANS-oxime-(Ttn)-TATE (**9**).

2.4 Synthesis of Ttn-TATE on 2-CTC Resin

Peptide Synthesis. The dried resin contained in a ZEBA Desalt spin column was solvated in 5 mL DMF and shaken for at least 30 minutes. The solvent was drained and the resin was resuspended in 5 mL 2:8 piperidine/DMF (0.5M Oxyma) and shaken for 5 minutes. The solvent was drained and the resin was resuspended in 5 mL 2:8 piperidine/DMF (0.5M Oxyma) and shaken for a further 10 minutes. The solvent was drained and the resin was washed with seven 5 mL portions of DMF, with shaking and draining. The resin was then Kaiser tested and if a positive result (purple or brown) was observed, the beads were then resuspended in a 5mL solution of DMF which already contained 4 equivalents each of Fmoc-Xaa, Oxyma and COMU and ~11 equivalents DIEA. The resin was then shaken for 1-3 hours at room temperature. Double coupling was done for Fmoc-D-Phe-OH to couple to the secondary amine of the Hpi. The solvent was drained and the resin was washed with five 5 mL portions of DMF, with shaking and draining. The resin was then Kaiser tested and if a negative result (no change in coloration) was observed, the beads were then resuspended in a 5 mL solution of 1:2:2 Ac_2O/Collidine/EtOAc and shaken for 20 minutes at room temperature. If the result of the Kaiser test remained positive, another coupling with same Fmoc amino acid was performed. Once the capping was complete, the solvent was drained and the resin was washed with five 5 mL portions of DMF, with shaking and draining. The resin was then washed with DCM and left to dry under reduced pressure if couplings were complete for the day or resuspended in 2:8 piperidine/DMF (0.5 M Oxyma) for the next Fmoc deprotection.

Global Deprotection, Cleavage and Tryptathionylation. To the spin column containing the free-base resin bound peptide was added 5 mL TFA and the beads were shaken at room temperature for 2 hours. The solution was then filtered into a round bottom flask containing 0.25 mL 1:1 v/v TIS/H_2O and the filtrate was evaporated under reduced pressure to yield a brown or pinkish solid. The last traces of TFA were removed by two successive rounds of co-evaporation with DCM. The crude peptide was then triturated with three 10 mL portions of Et_2O and allowed to air dry overnight to yield an off-white powder. Purified by reversed-phased HPLC on C18 with 0.1% formic acid H_2O/MeCN gradient elution.

H-D-Phe-cyclo(tryptathio)-[Trp-Tyr-D-Trp-Lys-Thr-Cys]-Thr-OH (1). Purification was performed according to the described procedure in the present section. HPLC method (2 mL/min, Agilent Eclipse XDB-C18 9.4 x 250 mm): 90:10 – 60:40 (H_2O/MeCN, 0.1% formic acid) 0-19 min: t_R = 16.3 min; λ_{max} 290 nm HRMS-ESI (m/z) $[M+H]^+$ calcd. for $C_{57}H_{70}N_{11}O_{12}S$, 1132.4926; found 1132.4914. Isolated yield: 7%.

2.5 Off-Bead Bioactivity of [Ttn]-TATE

2.5.1 *In Vitro* SSTR-2 receptor binding assays

Filtration competitive binding assays were performed in 96-wells filtration plates (MultiScreen, Millipore) to measure the binding affinity of the various oxime-(Ttn)-TATEs. The quantified oxime-(Ttn)-TATE was aliquoted in exact amount, lyophilized and dissolved in the appropriate buffer. Membrane (0.2 µg) from Chinese Hamster Ovary cells (CHO-K1) transfected with sst_{2a} receptor (Perkin Elmer, Canada) was used for the assay per Perkin Elmer protocol. Membrane, 0.05 nM of [^{125}I]Tyr-somatostatin-14 (Perkin Elmer, Canada) and increasing concentration of 10^{-5} to 10^{-12} M of the oxime (Ttn)-TATE in an assay buffer (25 mM Hepes pH 7.4, 10 mM $MgCl_2$, 1 mM $CaCl_2$ and 0.5% (*w*/*v*) BSA) were mixed in 96-well GF/B filter plate in triplicates. After incubation under agitation at 27 ˚C for 60 minutes, the wells were washed 6 times with ice-cold 50 mM TRIS-HCl pH 7.8.

Membrane filters were punched and was measured, by gamma emission (gamma counter Cobra-II Auto Gamma, Canberra Packard Canada), the residual amount of radio-labeled [^{125}I]Tyr-somatostatin-14 non-covalently bound to the membrane solubilized sst_{2a} receptor. The experiment was repeated in triplicate. Data were fitted to a one-site competition model (GraphPad Prism 6.1 software) to calculate the inhibition constant (K_i).

2.5.2 Cell culture

The SST receptor-positive rat pancreatic carcinoma (AR42J) cell lines was purchased from American Type Culture Collection (ATCC, Manassas, VA) and provided by Professor Francois Benard laboratory (BC cancer). All manipulations were carried out in a sterile and cell culture grade environment including equipment, chemicals, solutions, media and supplements. Cells were cultured in DMEM/F-12 medium supplemented with 20% FBS and 100 units/mL of penicillin as well as 100 µg/mL of streptomycin under incubation in 95% air: 5% CO_2 at 37ºC. Medium was replaced every 5 days. Sub-culturing was achieved as followed: From 80% confluent cells in 75 cm^2 flask, the medium was removed and discarded. The cell layer was briefly rinsed with phosphate buffer saline (PBS) pH 7.4, then 0.25% (w/v) Trypsin-0.53 mM EDTA solution (1 to 2 mL) was added and incubated in 95% air: 5% CO_2 at 37ºC until cell layer dispersed (minimum exposure time, usually within 5 to 15 minutes). To the flask was added 6.0 to 8.0 mL of complete growth medium and the cell suspension was transferred to a centrifuge tube by gently pipetting. They were spun at approximately 125 x g for 5 to 10 minutes; the supernatant was discarded, and the cell pellet was re-suspended in a known volume of freshly growth medium. The concentration of cells was determined and distributed in a new flask with a sub-cultivation ratio of 1:3 to 1:4.

2.5.3 Fluorescent binding assay with Ar42J cells

All manipulations were carried out in a sterile and cell culture grade environment including equipment, chemicals, solutions, media and supplements. Stock labeling solutions were made from lyophilized EDANS-oxime-(Ttn)-TATE (**9**) (0.2 mg, 0.115 µmole) and EDANS-oxime-(Ttn[O])-TATE (**9[O]**) (0.04 mg, 0.0193 µmole) dissolved in 7 mL and 1 mL of PBS (1x) to give clear solutions of 16.5 µM and 19.3 µM respectively and used immediately.

The stock blocking solution was prepared from *N*-azidoacetate-TATE (38.7 mg, 34 µmole) dissolved in PBS (1x) (0.5 mL) and serum free DMEM/F-12 media (2.5 mL) to yield a 11 mM clear solution and used immediately. Prior to the staining experiment, the Ar42J cells were detached from culture dishes with trypsin/EDTA as previously described but dispersed in 10^5 cells/well of LabTek 8-chamber slides (Nunc Inc. Rochester, NY), working volume of 400 µL and incubated overnight in DMEM/F-12 medium supplemented with 20% FBS and 100 units/mL of penicillin as well as 100 µg/mL of streptomycin in 95% air: 5% CO_2 at 37ºC. Then, to each well of LabTek 8-chamber slides containing Ar42J cells, cell medium was replaced with appropriate volume of serum free DMEM/F-12 medium and the blocking agent *N*-azidoacetate-TATE to yield different concentrations (0 mM, 2.20 mM or 11.0 mM) and incubated for 10 min. Then, to each well of LabTek 8-chamber slides containing Ar42J cells, the appropriate volume of stock labeling solutions was added to yield different concentrations of labeling solution (0 mM, 4.12 µM, 4.82 µM or 8.25 µM) as well as blocking solution (0 mM, 1.38 mM or 2.75 mM) and Ar42J cells in resulting mixtures were incubated in 95% air: 5% CO_2 at 37ºC for 80 min in a working volume of 400 µL. From each well of the LabTek 8-chamber slides containing Ar42J cells, the solution was replaced by PBS (400 µL), gently shaken and solution was discarded. The washing procedure was repeated two additional times. To each well of the LabTek 8-chamber slides containing Ar42J cells, was added 4% (*w*/*v*) paraformaldehyde in PBS (400 µL) and incubated for 15 min at RT. The solutions were discarded, replaced by PBS (400 µL) and solutions were removed. The washing procedure was repeated two additional times. The cells were immediately imaged with a Leica SP5 X Laser Scanning Confocal Microscope (inverted), objective HC PL APO 10x/0.40 CS oo/0.17 /A, objective HC PL APO 40x/1.25-0.75 oil CS, excitation laser of 405 nm, emission filter Long Pass (415 nm-690 nm).

3 Experimental Data

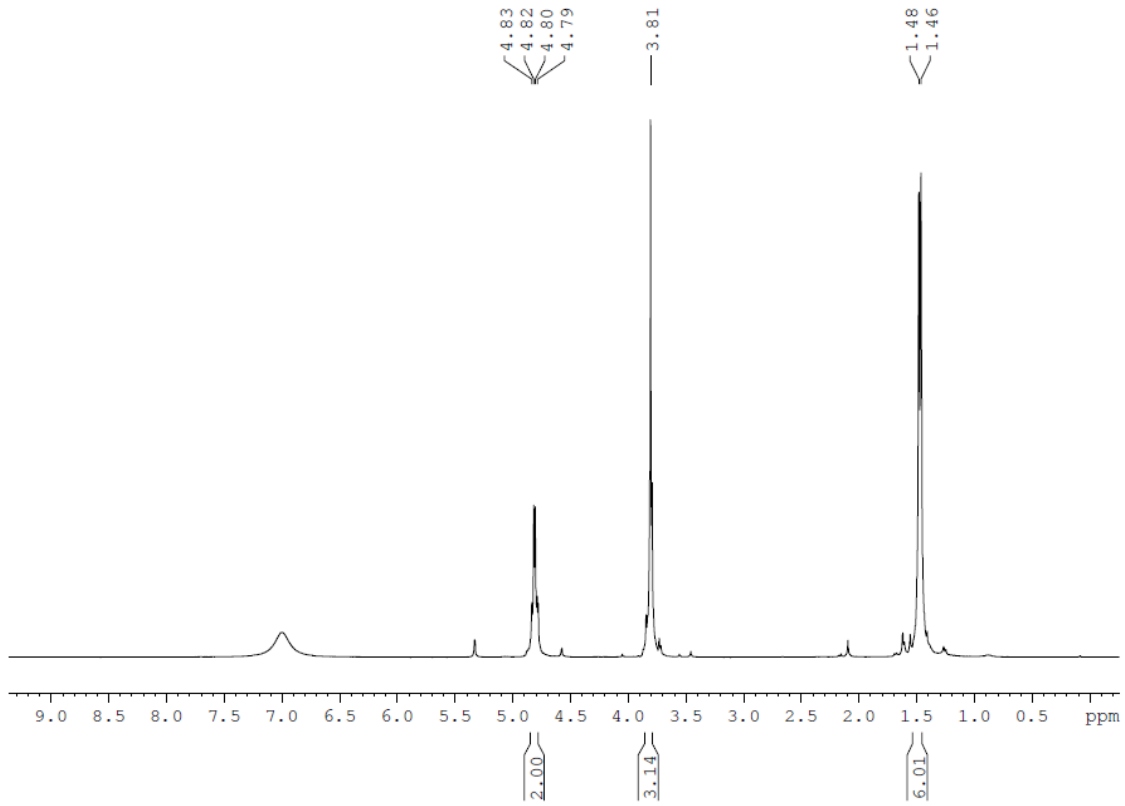


Figure *S5*. ^{1}H-NMR spectrum (300 MHz, CD_2Cl_2) of 1-methyl-2,3-*O*-isopropylidene-D-tartrate monoester (**SI-1**).

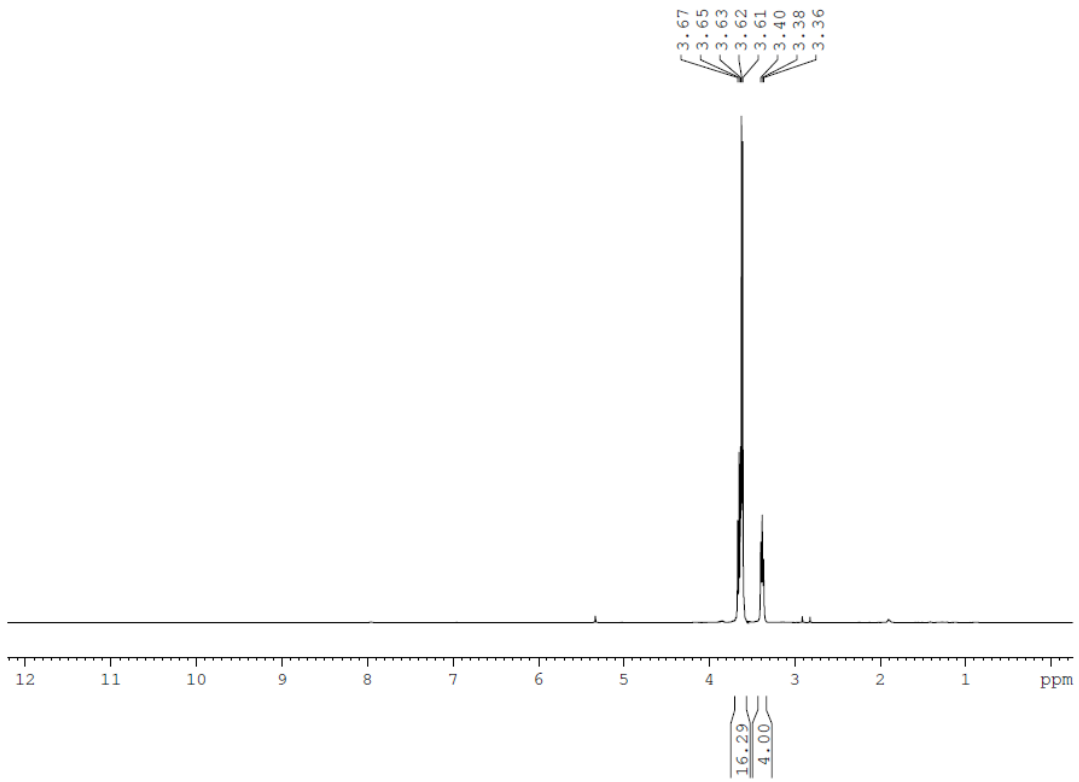


Figure *S6*. ^{1}H-NMR spectrum (300 MHz, CD_2Cl_2) of 1,14-diazido-3,6,9,12-tetraoxatetradecane (**SI-2**).

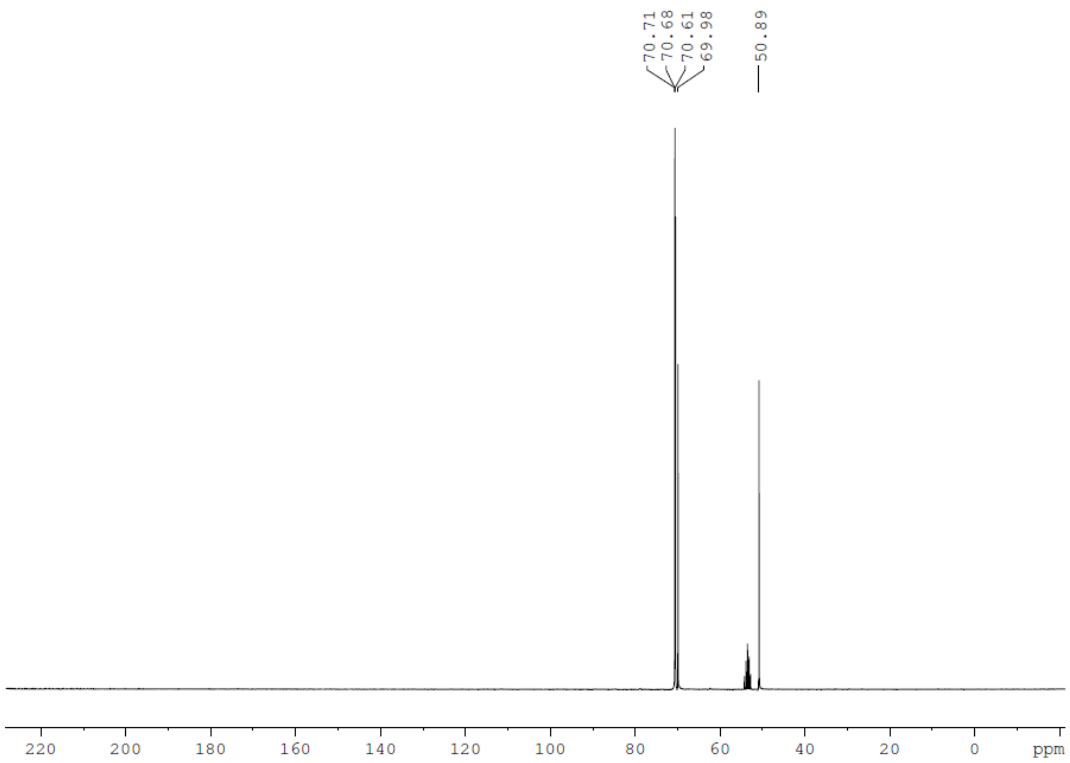


Figure *S7*. ^{13}C-NMR spectrum (75 MHz, APT, CD_2Cl_2) of 1,14-diazido-3,6,9,12-tetraoxatetradecane (**SI-2**).

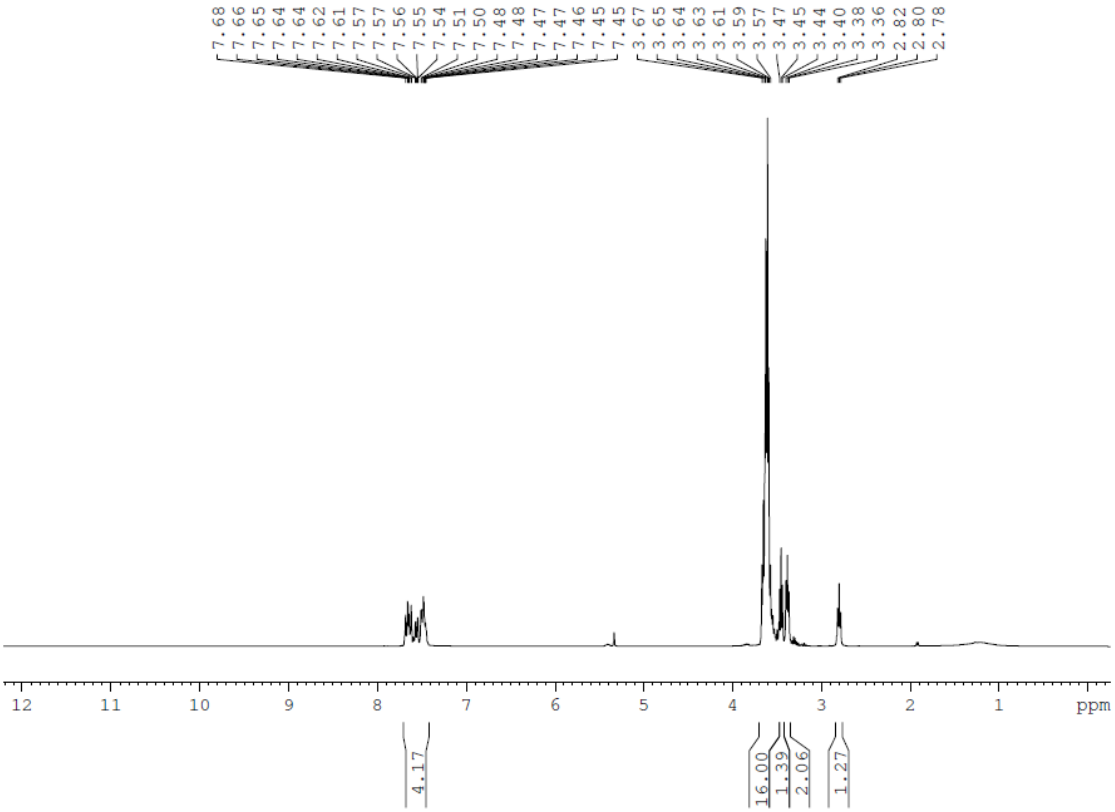


Figure *S8*. ^{1}H-NMR spectrum (300 MHz, CD_2Cl_2) of 14-azido-3,6,9,12-tetraoxatetradecane-1-amine (**SI-3**).

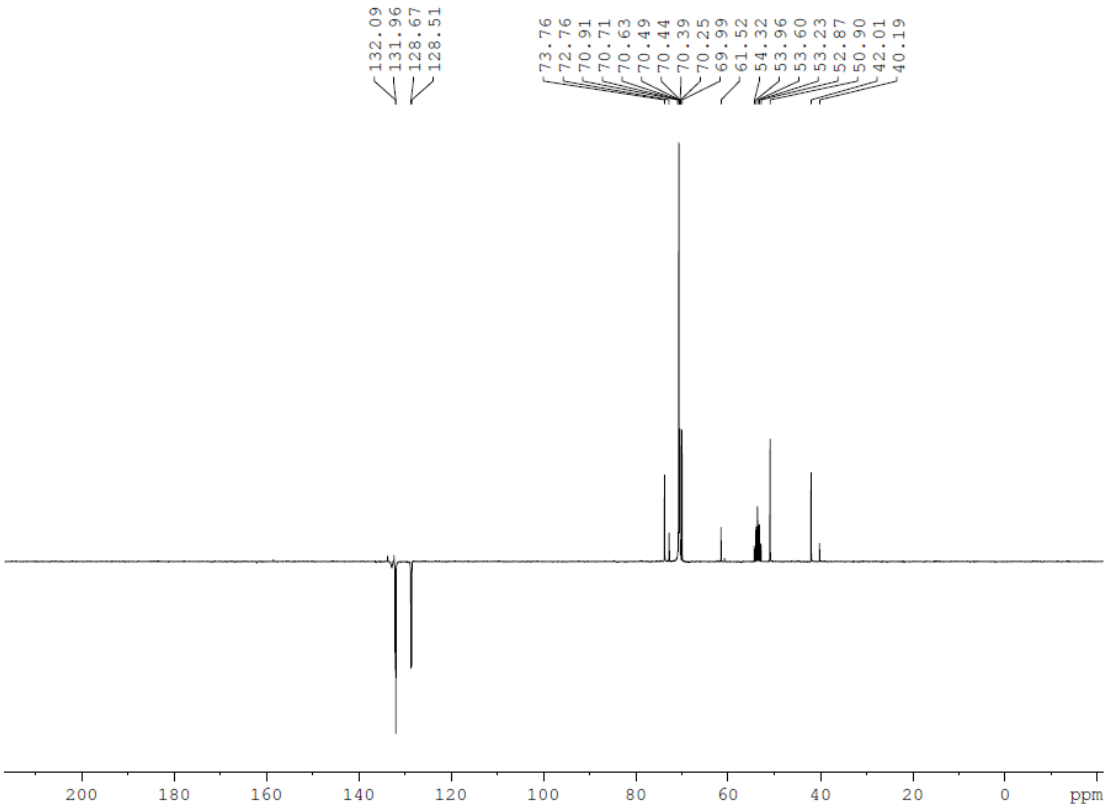


Figure *S9*. ^{13}C-NMR spectrum (75 MHz, APT, CD_2Cl_2) of 14-azido-3,6,9,12-tetraoxatetradecane-1-amine (**SI-3**).

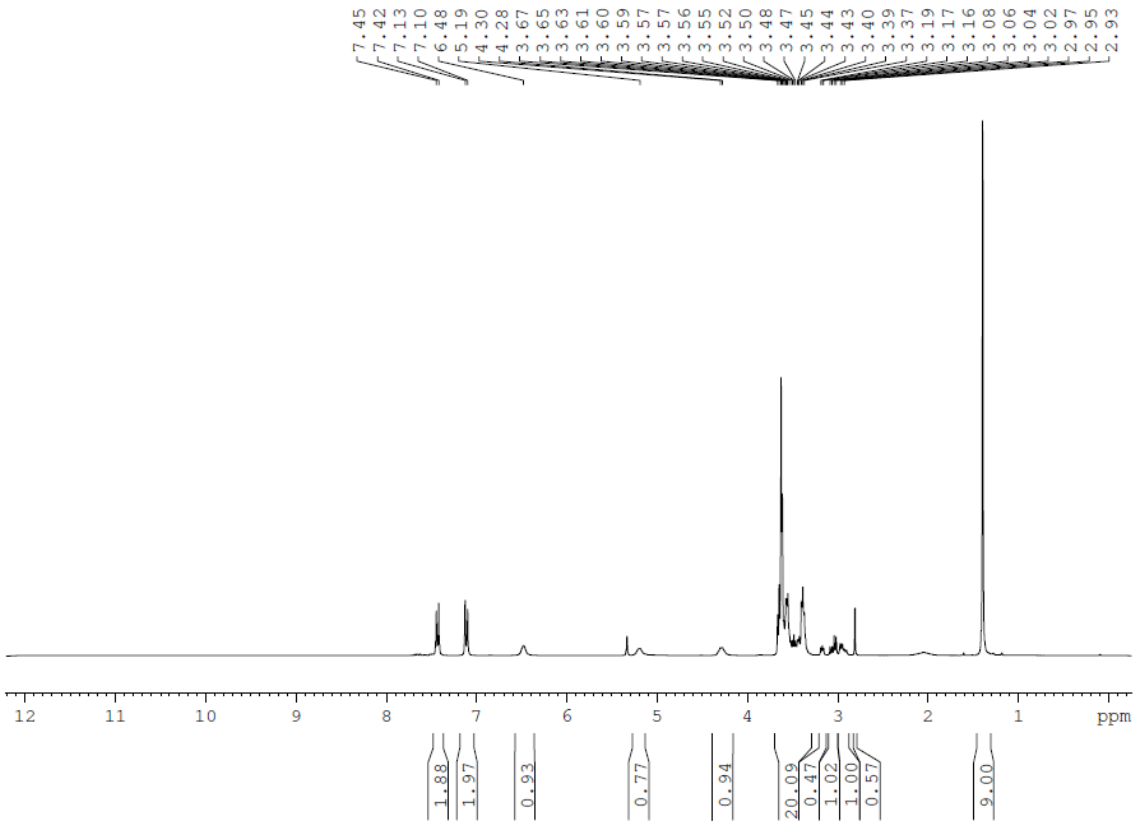


Figure *S10*. ^{1}H-NMR spectrum (300 MHz, CD_2Cl_2) of (2*S*)-2-([(2'-methyl-2'-propanyl)oxy]carbonylamino)-3-(4-bromophenyl)propanoate-1'-amido-(14'-azido-3',6',9',12'-tetraoxatetradecane) (**SI-4**).

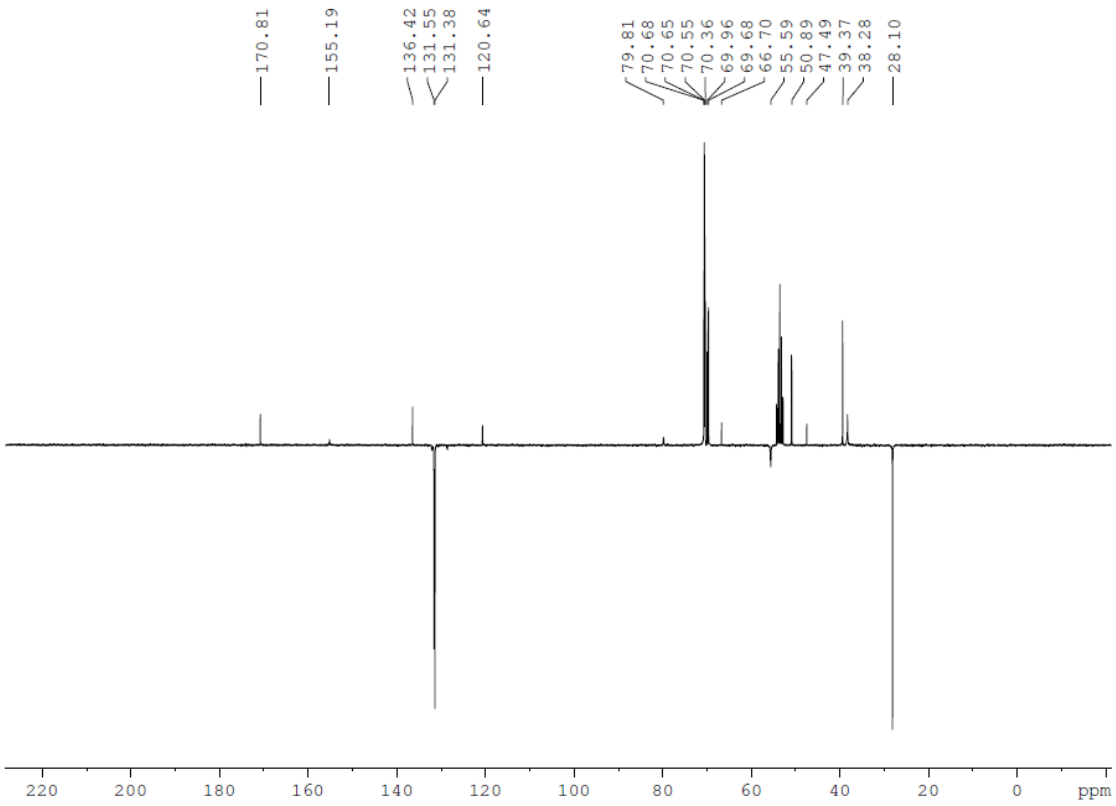


Figure *S11*. ^{13}C-NMR spectrum (75 MHz, APT, CD_2Cl_2) of (2*S*)-2-([(2'-methyl-2'-propanyl)oxy]carbonylamino)-3-(4-bromophenyl)propanoate-1'-amido-(14'-azido-3',6',9',12'-tetraoxatetradecane) (**SI-5**).

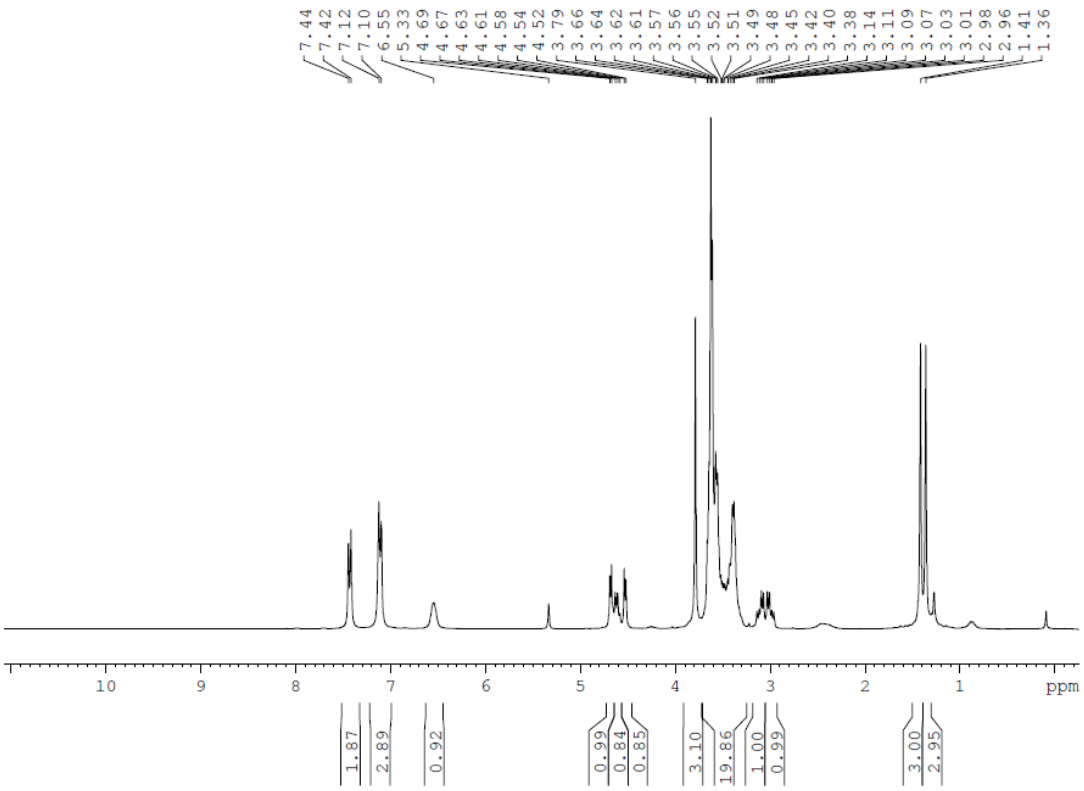


Figure *S12*. ^{1}H-NMR spectrum (300 MHz, CD_2Cl_2) of 2-[(1'-methyl ester-2',3'-*O*-isopropylidene-D-tartrate)- (2*S*)-2-amido)-3-(4'-bromophenyl)propanoate]-1'-amido-(14'-azido-3',6',9',12'-tetraoxatetradecane) (**SI-6**).

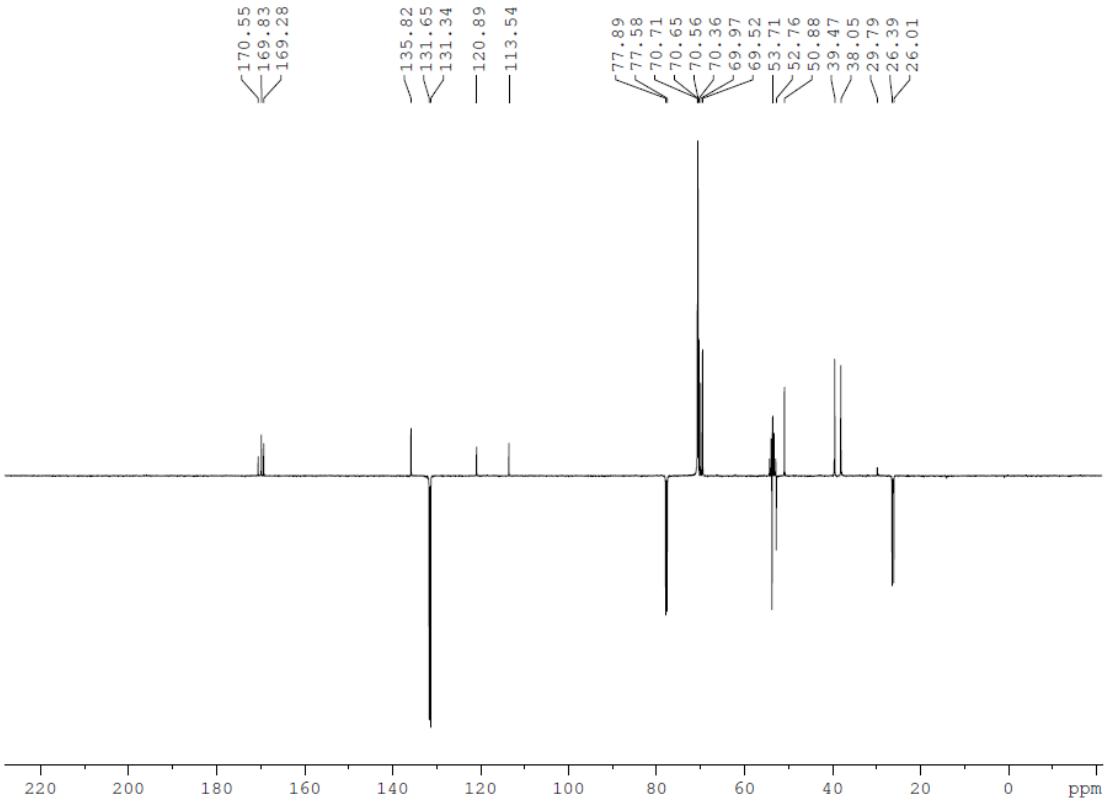


Figure *S13*. ^{13}C-NMR spectrum (75 MHz, APT, CD_2Cl_2) of 2-[(1'-methyl ester-2',3'-*O*-isopropylidene-D-tartrate)- (2*S*)-2-amido)-3-(4'-bromophenyl)propanoate]-1'-amido-(14'-azido-3',6',9',12'-tetraoxatetradecane) (**SI-6**).

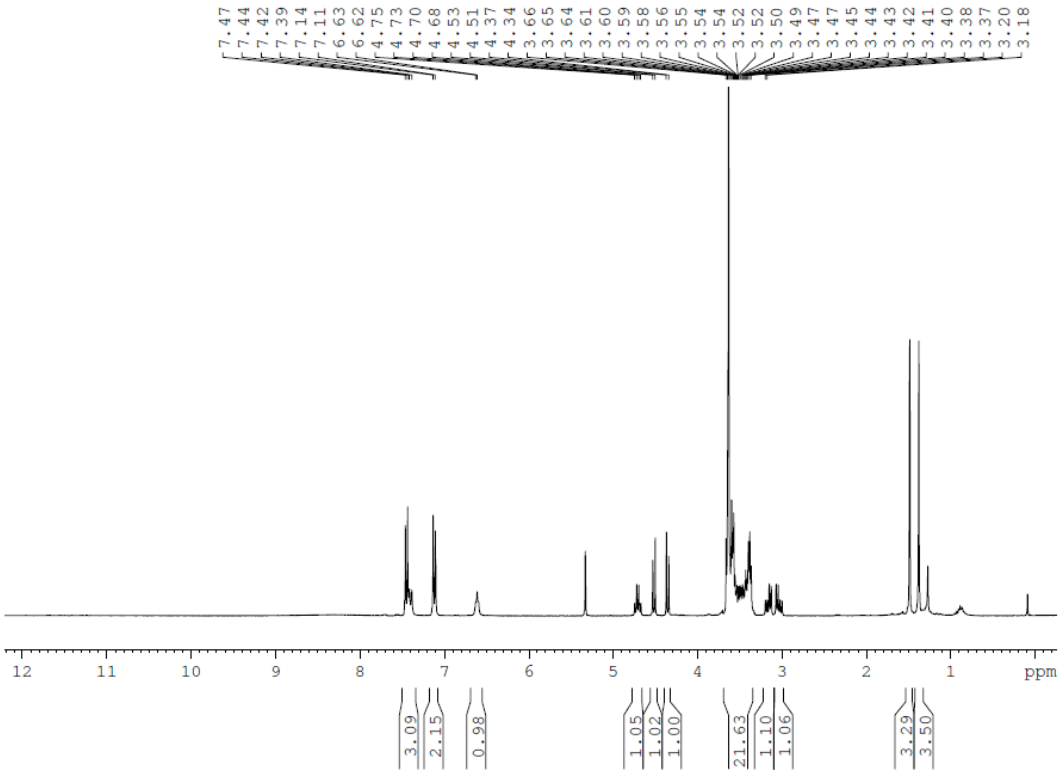


Figure *S14.* ^{1}H-NMR spectrum (300 MHz, CD_2Cl_2) of 2-[(2',3'-*O*-isopropylidene-D-tartrate)- (2*S*)-2-amido)-3-(4'-bromophenyl)propanoate]-1'-amido-(14'-azido-3',6',9',12'-tetraoxatetradecane) (**2**).

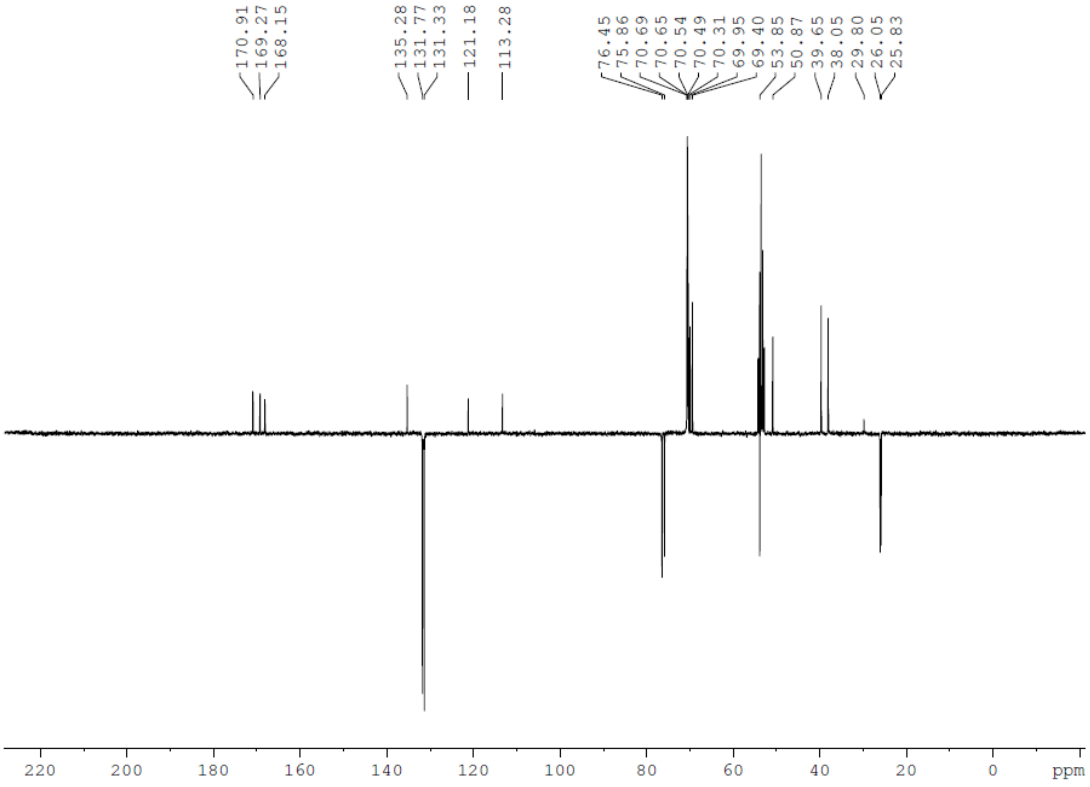


Figure *S15.* ^{13}C-NMR spectrum (75 MHz, CD_2Cl_2) of 2-[(2',3'-*O*-isopropylidene-D-tartrate)- (2*S*)-2-amido)-3-(4'-bromophenyl)propanoate]-1'-amido-(14'-azido-3',6',9',12'-tetraoxatetradecane) (**2**).

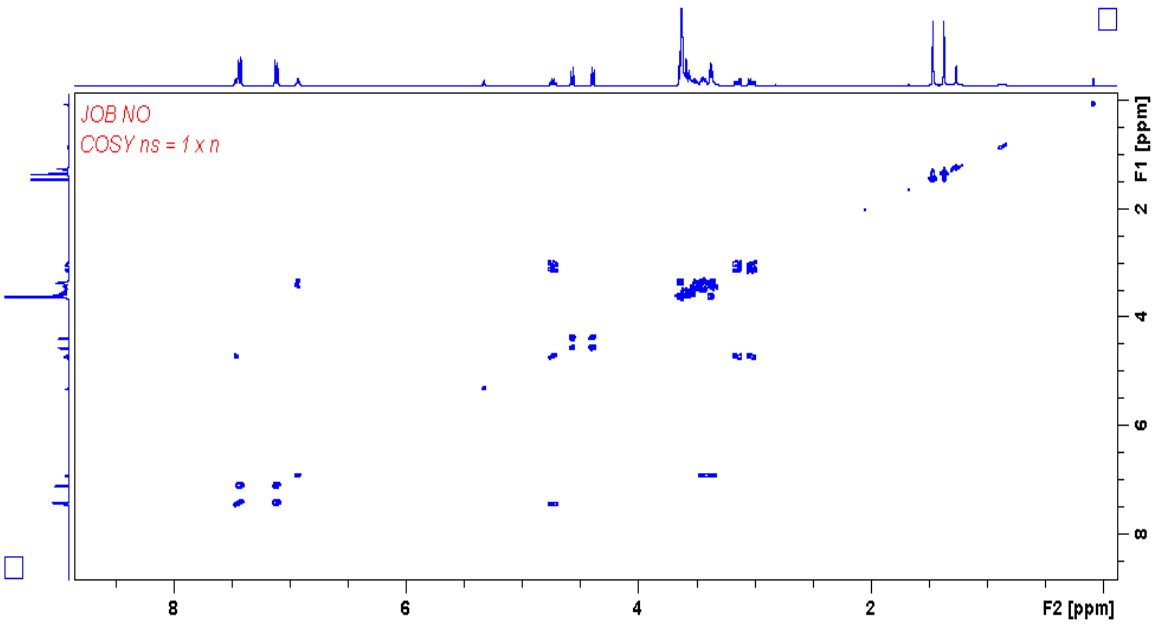


Figure *S16.* COSY-NMR spectrum (400 MHz, CD_2Cl_2) of 2-[(2',3'-*O*-isopropylidene-D-tartrate)- (2*S*)-2-amido)-3-(4'-bromophenyl)propanoate]-1'-amido-(14'-azido-3',6',9',12'-tetraoxatetradecane) (**2**).

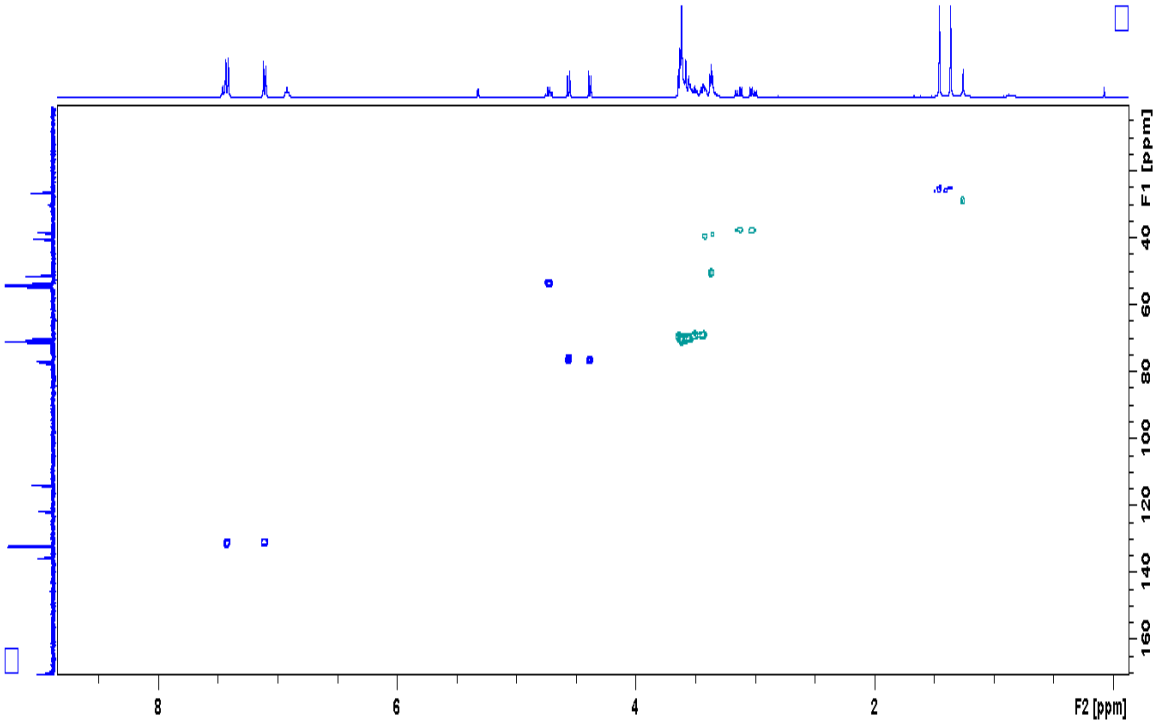


Figure *S17.* HSQC-NMR spectrum (400 MHz, CD_2Cl_2) of 2-[(2',3'-*O*-isopropylidene-D-tartrate)- (2*S*)-2-amido)-3-(4'-bromophenyl)propanoate]-1'-amido-(14'-azido-3',6',9',12'-tetraoxatetradecane) (**2**).

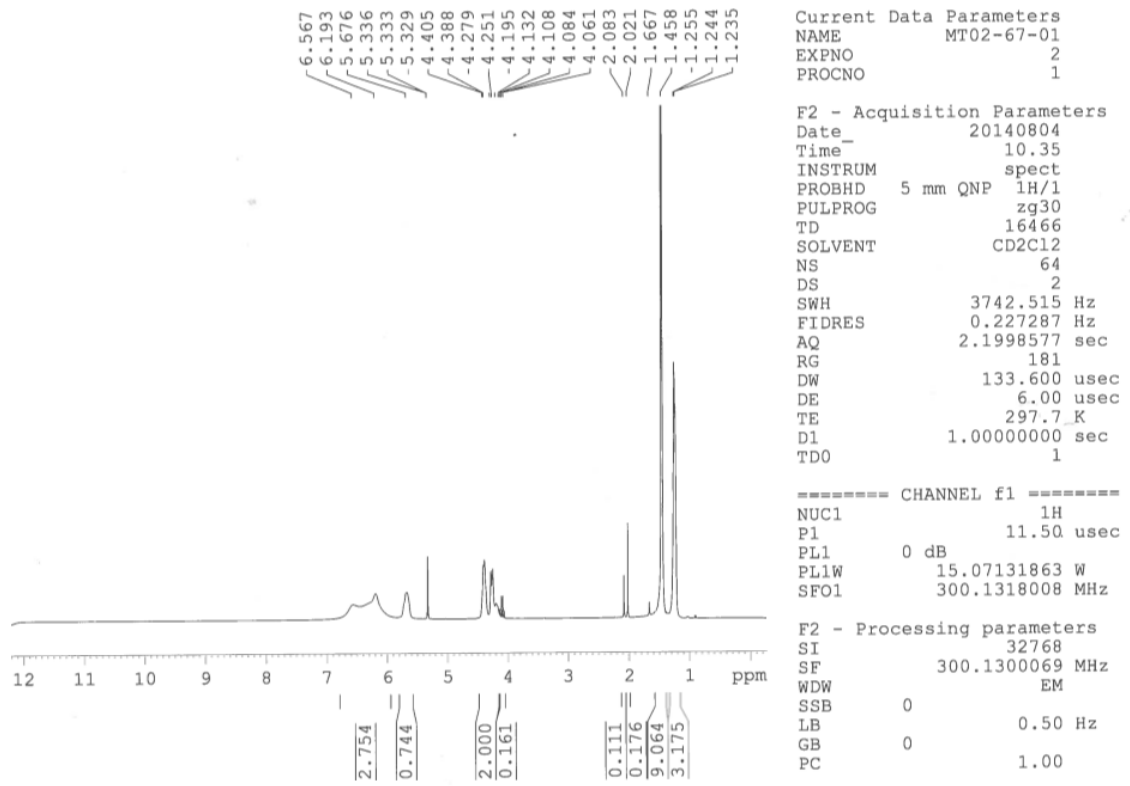


Figure *S18*. ^{1}H-NMR spectrum (300 MHz, CD_2Cl_2) of (2*S*,3*R*)-2-[(*tert*-butoxycarbonyl)amino]-3-hydroxybutanoic acid (**SI-7**).

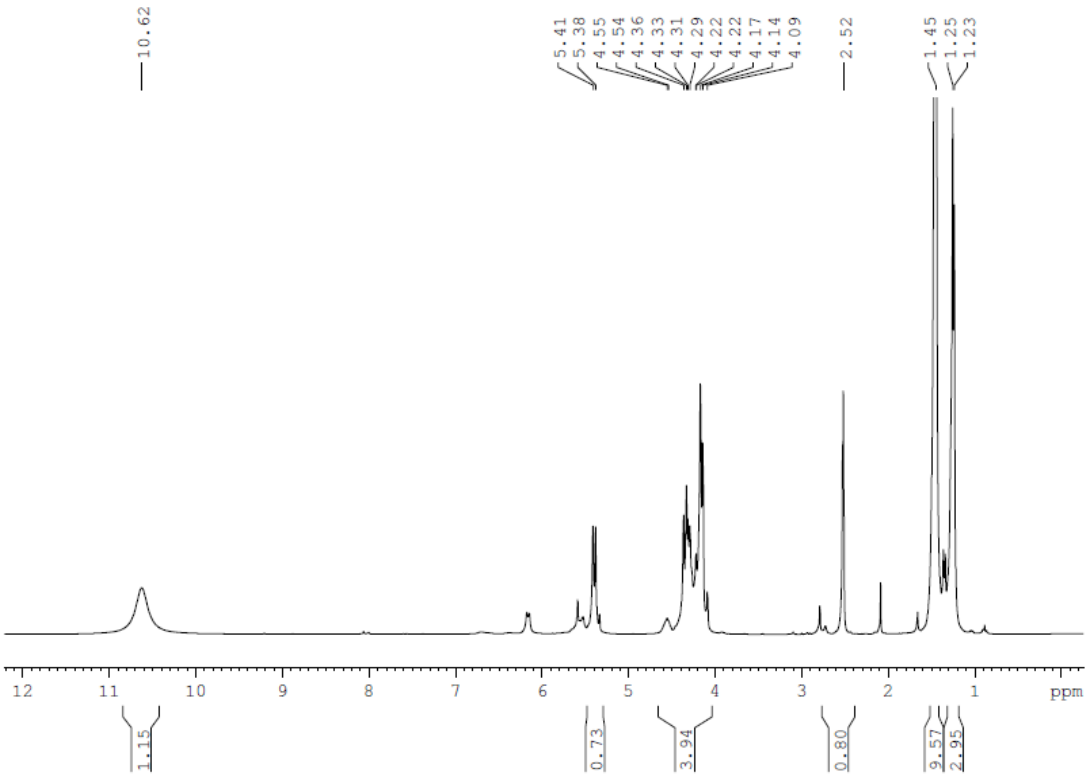


Figure *S19*. ^{1}H-NMR spectrum (300 MHz, CD_2Cl_2) of (2*S*,3*R*)-2-((*tert*-butoxycarbonyl)amino)-3-(prop-2-yn-1-yloxy)butanoic acid (**SI-8**).

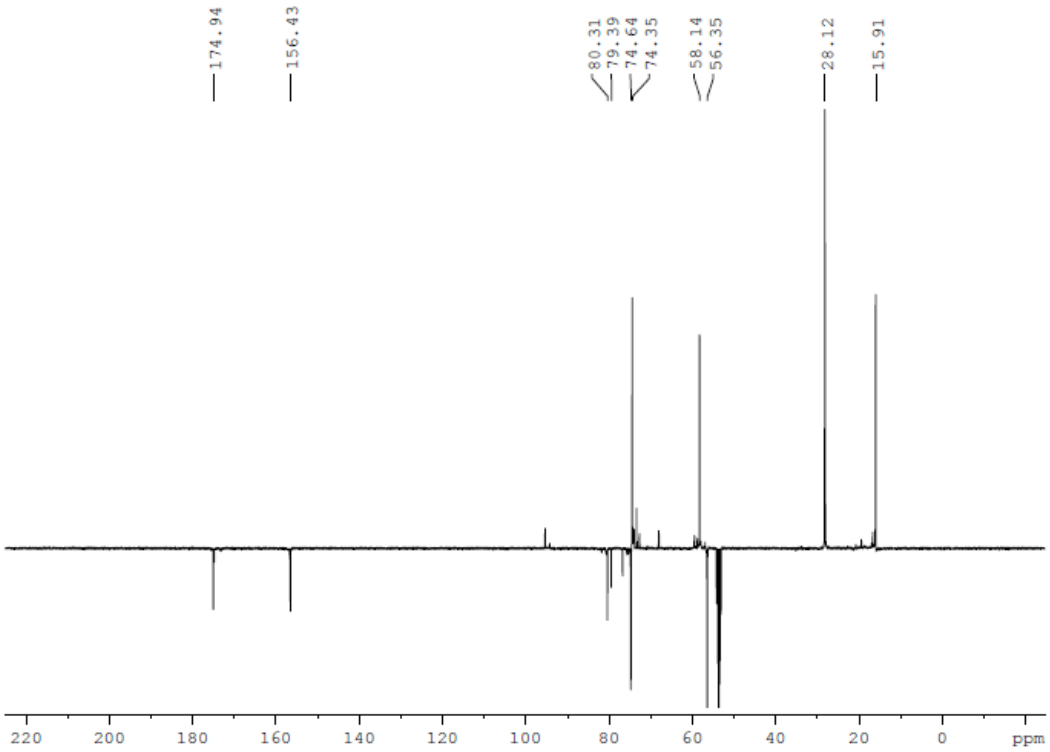


Figure *S20.* ^{13}C-NMR spectrum (100 MHz, APT, CD_2Cl_2) of (2*S*,3*R*)-2-((*tert*-butoxycarbonyl)amino)-3-(prop-2-yn-1-yloxy)butanoic acid (**SI-8**).

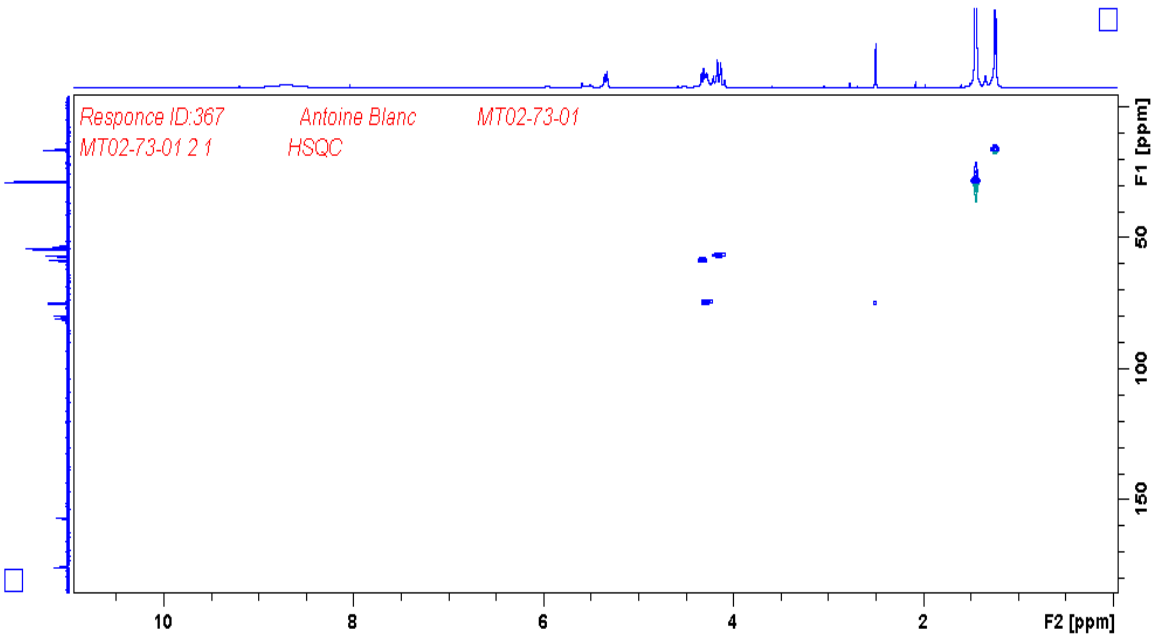


Figure *S21.* HSQC-NMR spectrum (400 MHz, CD_2Cl_2) of (2*S*,3*R*)-2-((*tert*-butoxycarbonyl)amino)-3-(prop-2-yn-1-yloxy)butanoic acid (**S-8**).

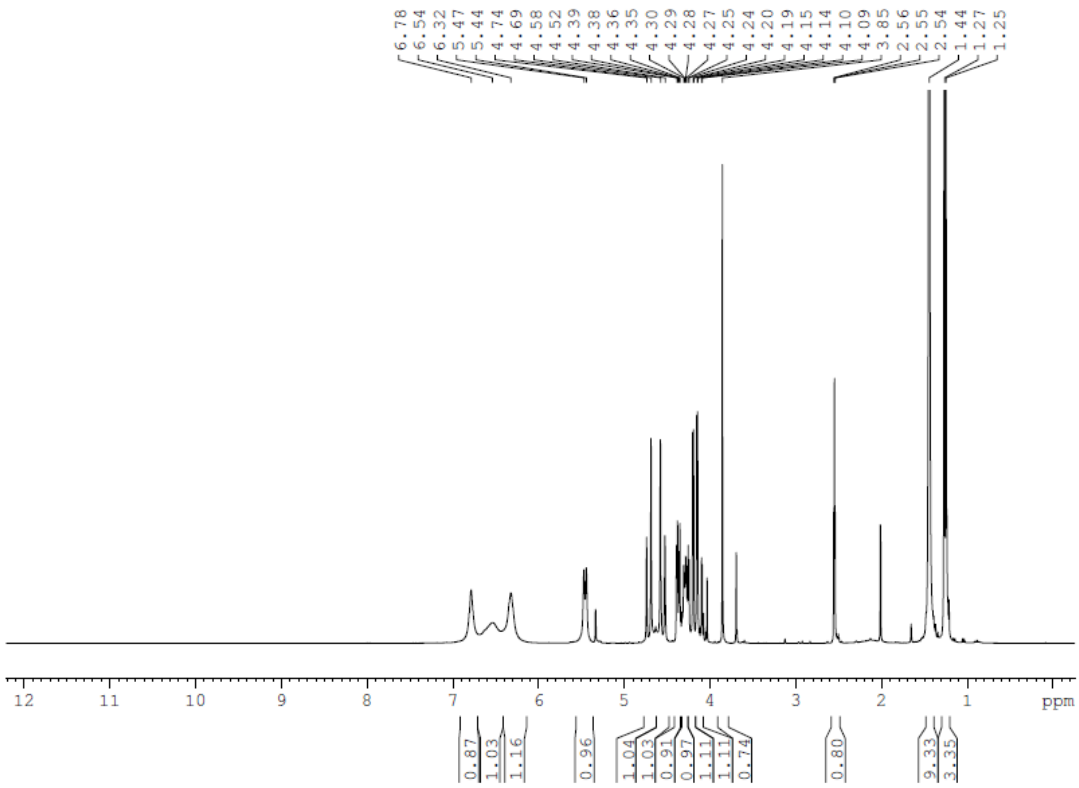


Figure *S22*. ^{1}H-NMR spectrum (300 MHz, CD_2Cl_2) of (2*S*,3*R*)-2-((*tert*-butoxycarbonyl)amino)-3-(prop-2-yn-1-yloxy)butanoate carbamoylmethyl ester (**SI-9**).

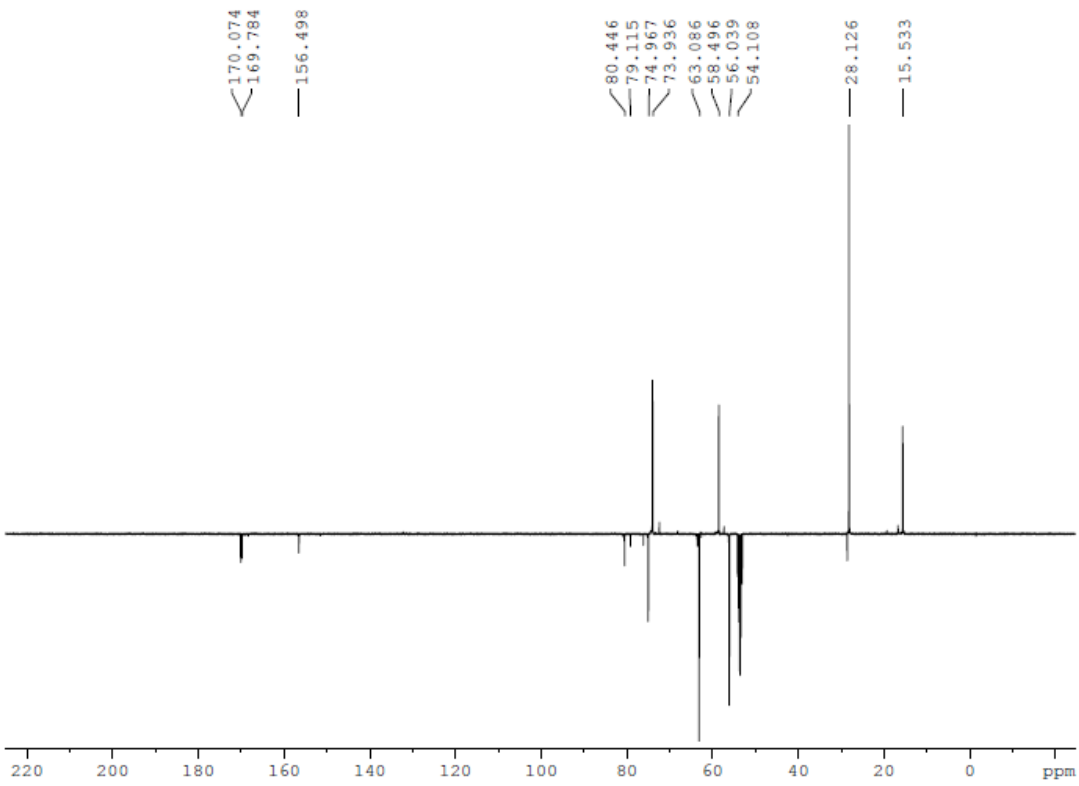


Figure *S23*. ^{13}C-NMR spectrum (100 MHz, APT, CD_2Cl_2) of (2*S*,3*R*)-2-((*tert*-butoxycarbonyl)amino)-3-(prop-2-yn-1-yloxy)butanoate carbamoylmethyl ester (**SI-9**).

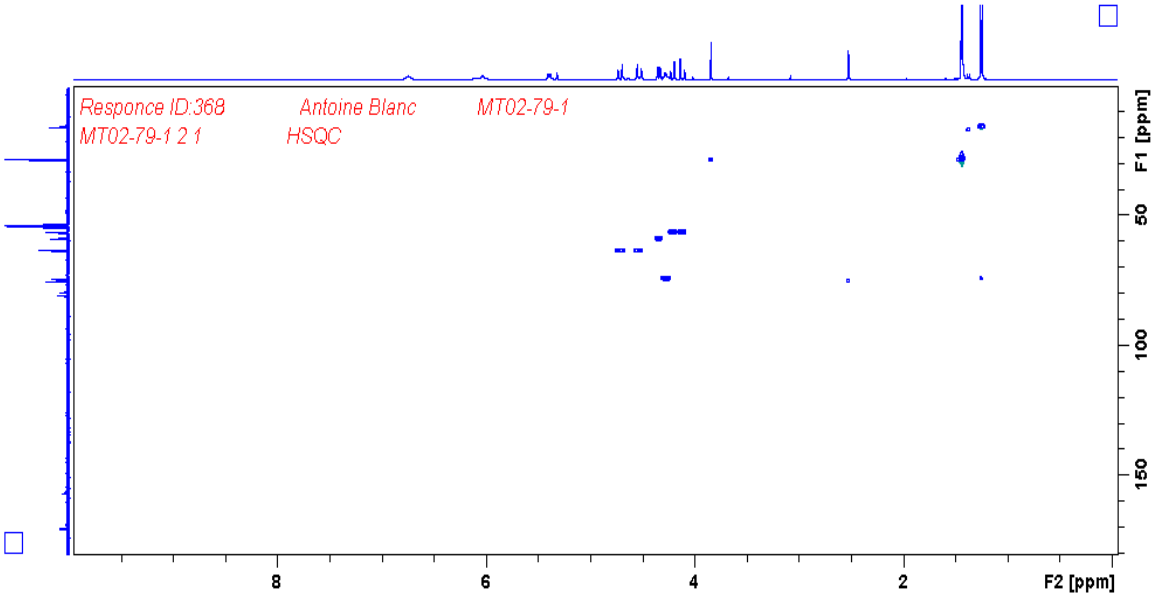


Figure *S24.* HSQC-NMR spectrum (400 MHz, CD_2Cl_2) of (2*S*,3*R*)-2-((*tert*-butoxycarbonyl)amino)-3-(prop-2-yn-1-yloxy)butanoate carbamoylmethyl ester (**SI-9**).

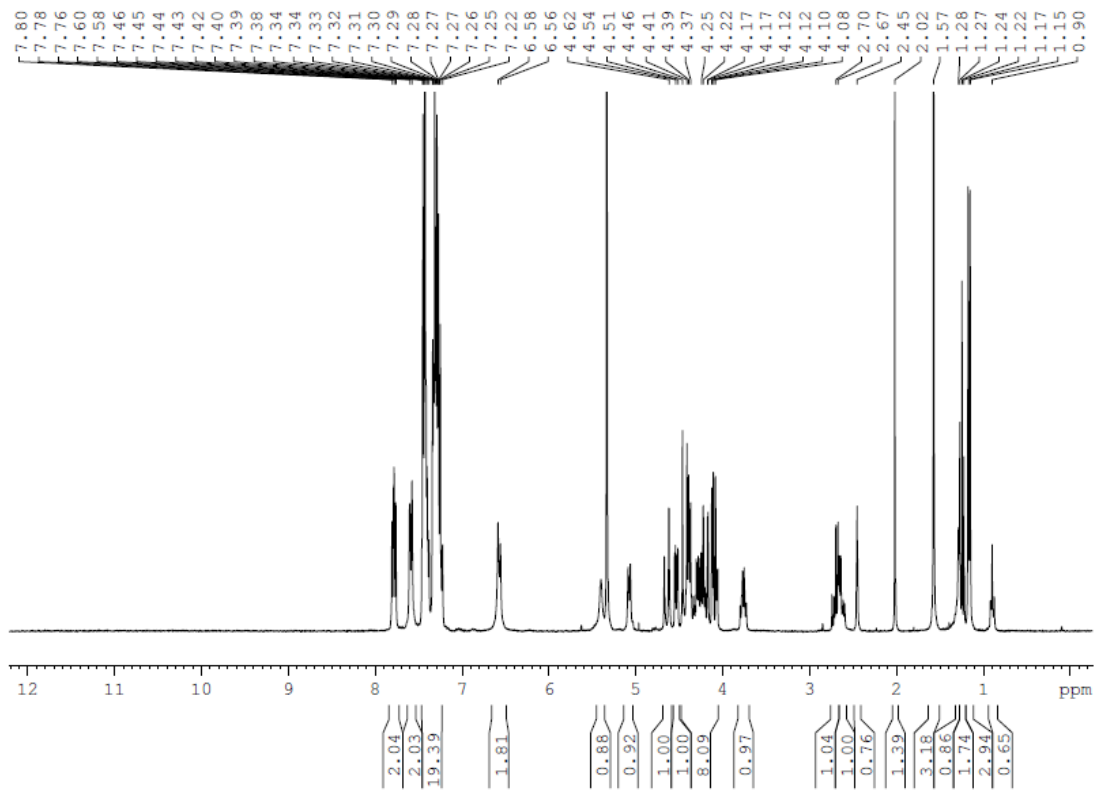


Figure *S25*. ^{1}H-NMR spectrum (300 MHz, CD_2Cl_2) of *N*$^{\alpha}$-Fmoc-*S*$^{\beta}$-trityl-cysteinyl-*O*$^{\beta}$-propargyl-threonine carbamoylmethyl ester (**3**).

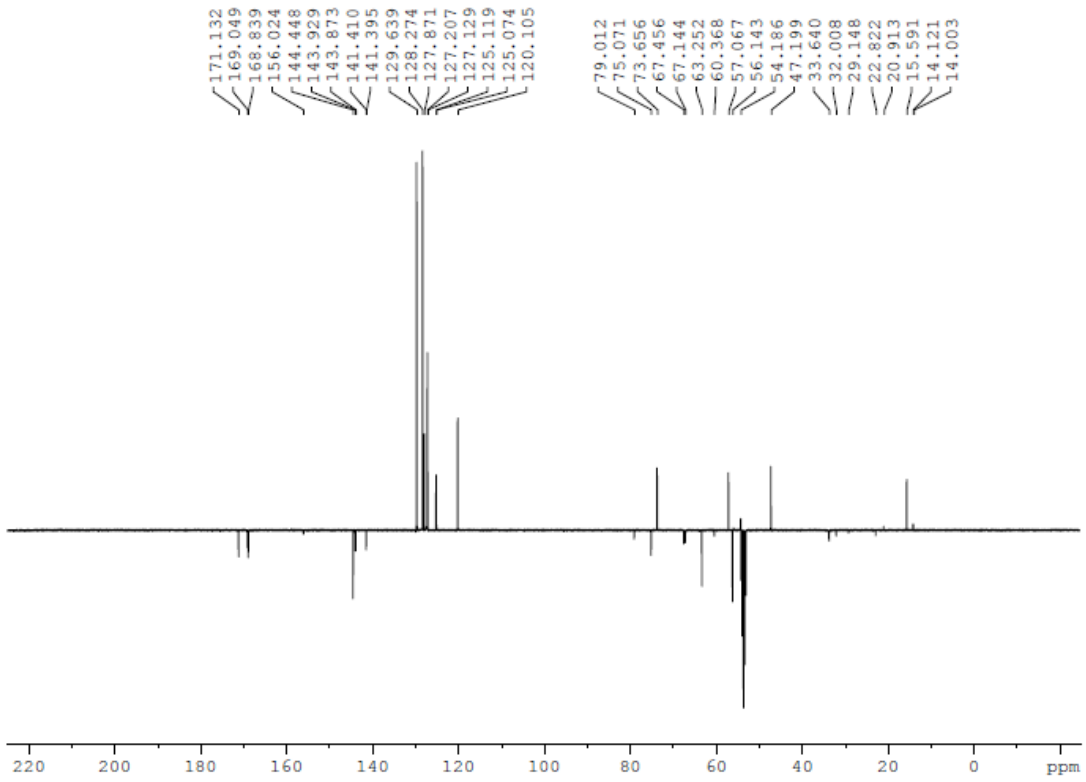


Figure *S26*. ^{13}C-NMR spectrum (100 MHz, APT, CD_2Cl_2) of *N*$^{\alpha}$-Fmoc-*S*$^{\beta}$-trityl-cysteinyl-*O*$^{\beta}$-propargyl-threonine carbamoylmethyl ester (**3**).

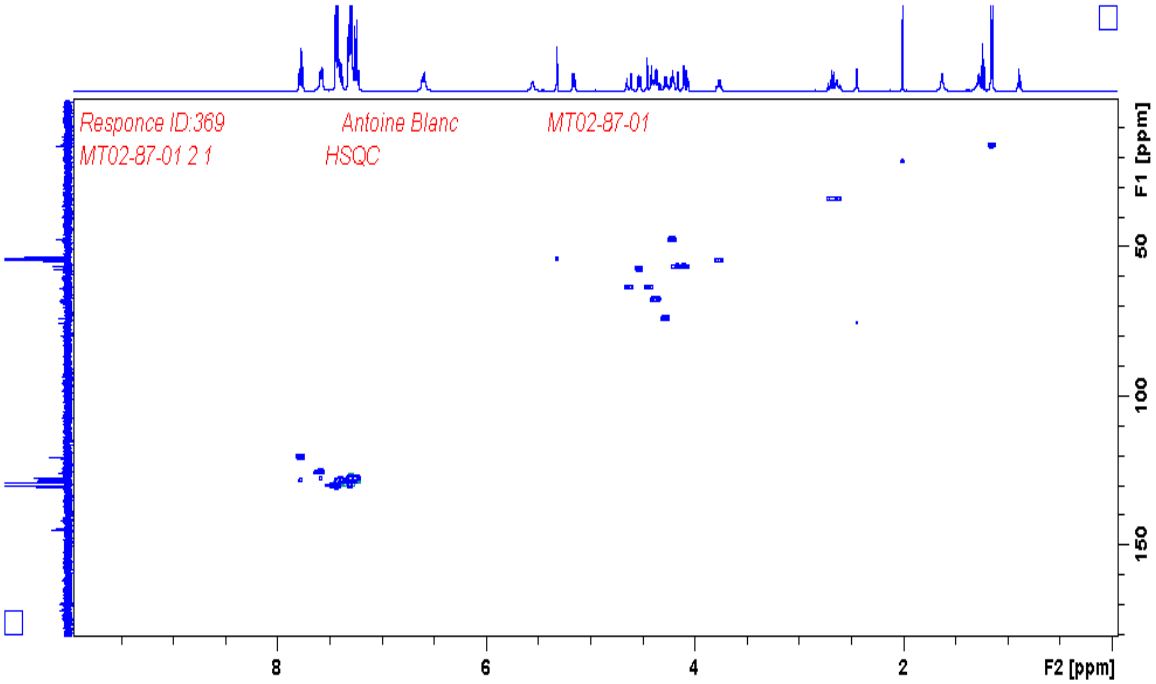


Figure *S27*. HSQC-NMR spectrum (400 MHz, CD_2Cl_2) of N^{α}-Fmoc-S^{β}-trityl-cysteinyl-O^{β}-propargyl-threonine carbamoylmethyl ester (**3**).

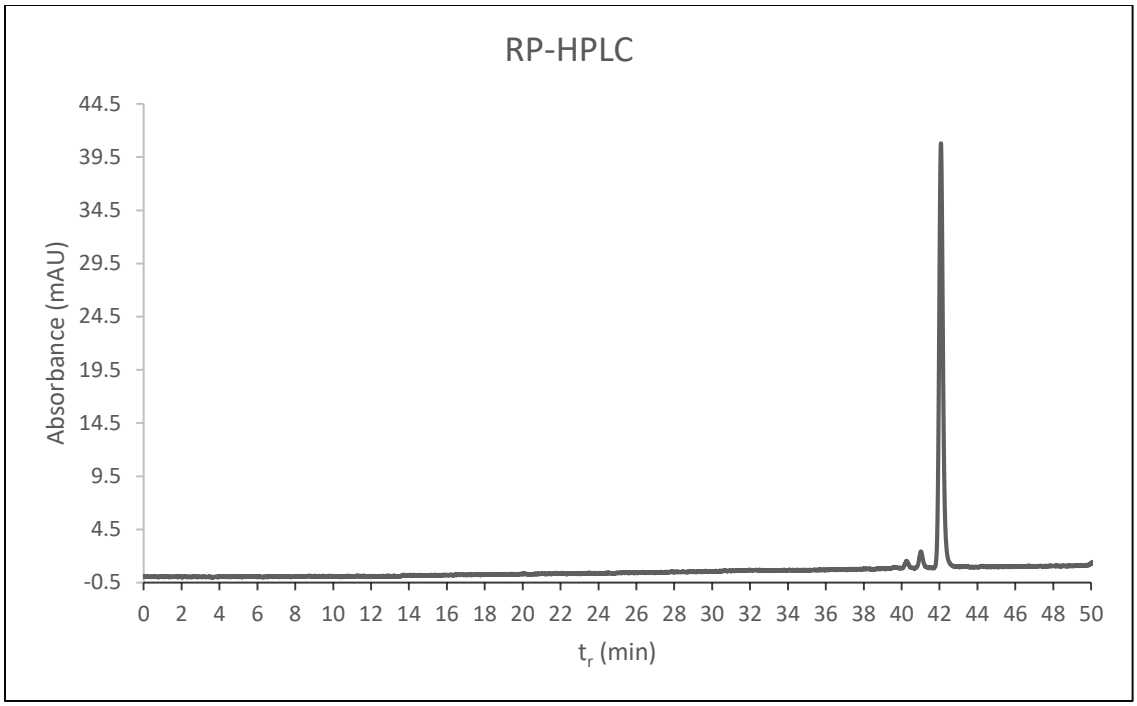


Figure *S28a.* RP-HPLC (C18) chromatogram of N^{α}-Fmoc-S^{β}-trityl-cysteinyl-O^{β}-propargyl-threonine carbamoylmethyl ester (**3**). Gradient of 40% to 78% solvent B over 20 min, 78% to 100% over 40 min at a flow rate of 1 mL/min; t_r = 42.07 min (A_{301} 98%).

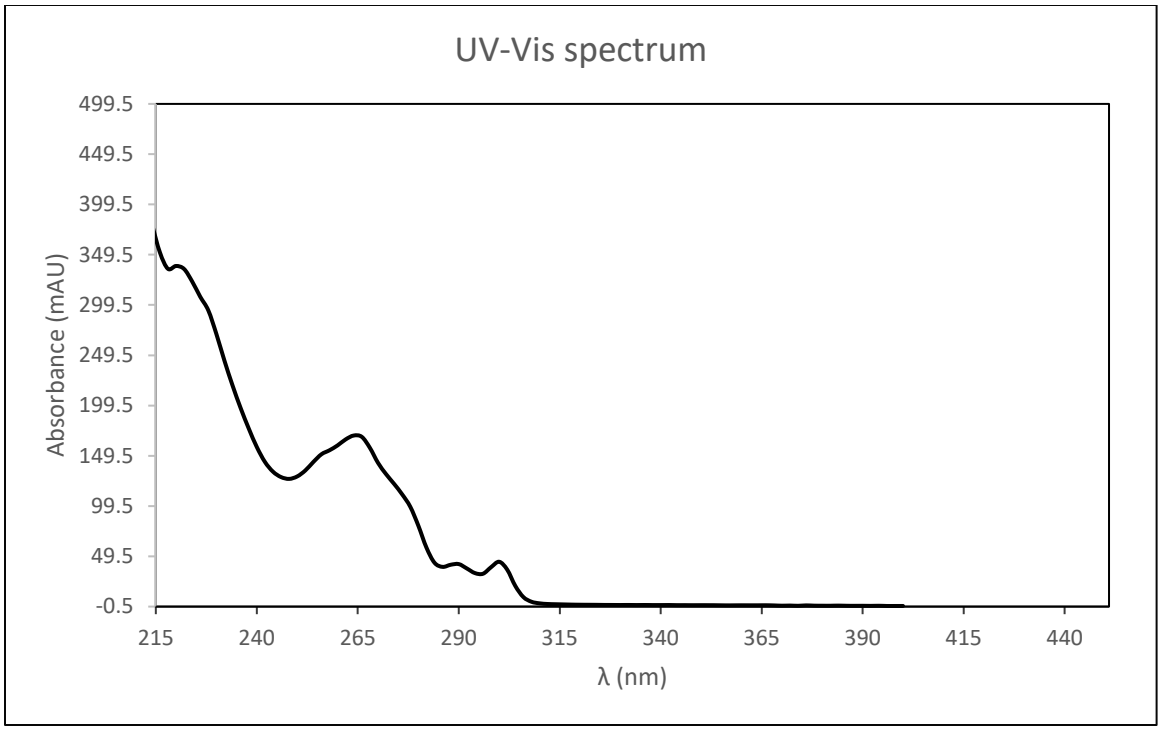


Figure *S28b*. UV-Vis spectrum of *N*$^{\alpha}$-Fmoc-*S*$^{\beta}$-trityl-cysteinyl-*O*$^{\beta}$-propargyl-threonine carbamoylmethyl ester (**3**). UV-Vis spectra were taken with the RP-HPLC diode array detectors directly in the RP-HPLC flow cells at t_r = 42.07 min (83% eluent B). Analyte concentration unknown.

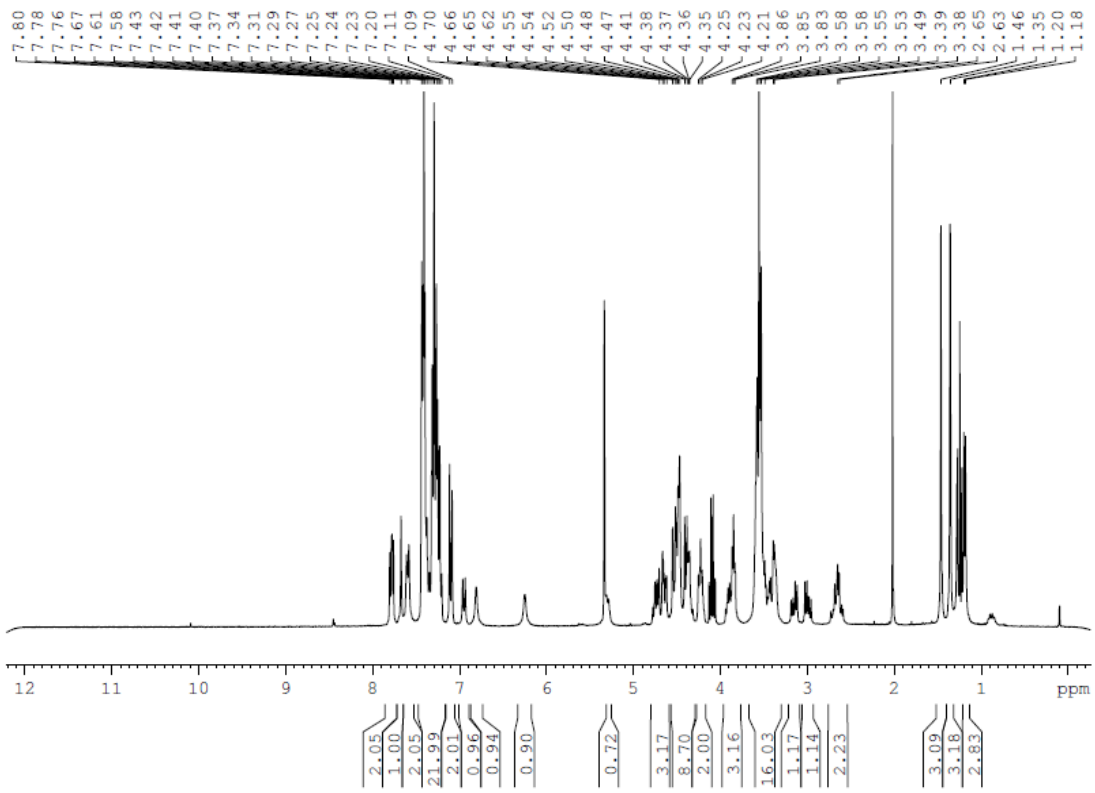


Figure *S29*. ^{1}H-NMR spectrum (300 MHz, CD_2Cl_2) of *N*$^{\alpha}$-Fmoc-*S*$^{\beta}$-trityl-cysteinyl-*O*$^{\beta}$-(4-methyl-1-(*N*-(PEG$_4$-NHCO-Phe(4-Br)-NHCO-1-(2,3-*O*-isopropylidene)-D-tartrate-OH))-[1,2,3]triazolyl)-threonine carbamoylmethyl ester (**SI-11**).

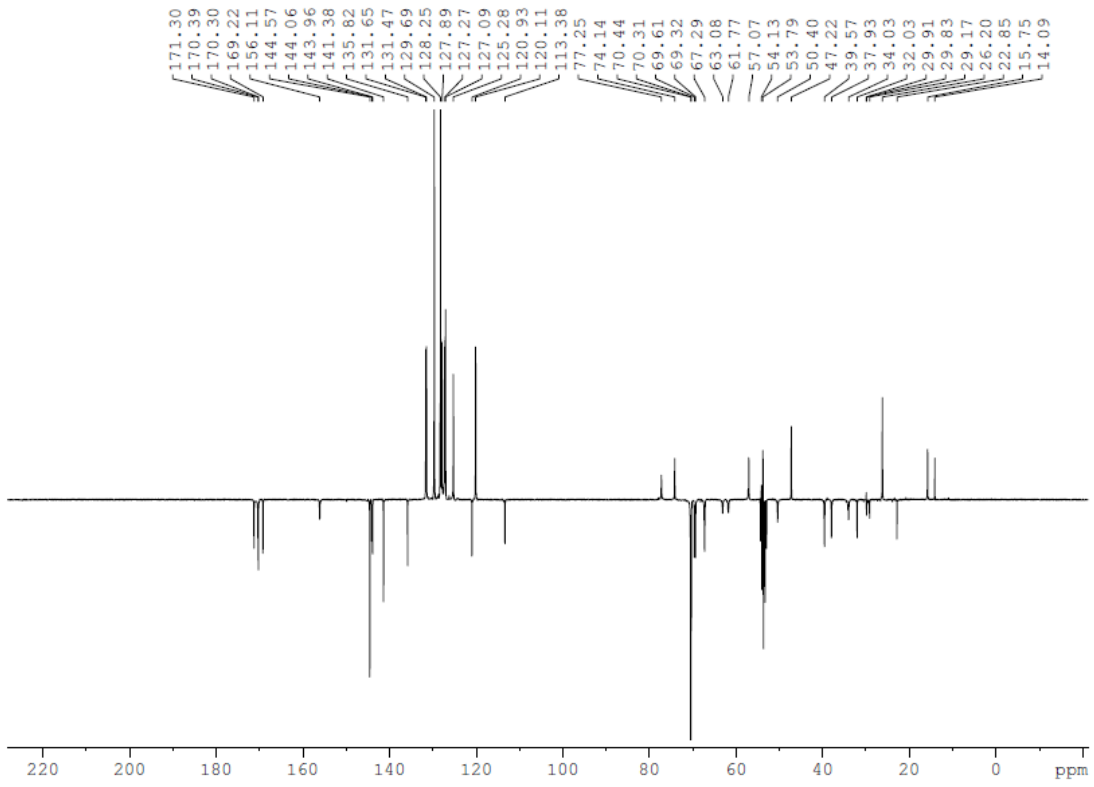


Figure *S30*. ^{13}C-NMR spectrum (75 MHz, APT, CD_2Cl_2) of *N*$^{\alpha}$-Fmoc-*S*$^{\beta}$-trityl-cysteinyl-*O*$^{\beta}$-(4-methyl-1-(*N*-(PEG$_4$-NHCO-Phe(4-Br)-NHCO-1-(2,3-*O*-isopropylidene)-D-tartrate-OH))-[1,2,3]triazolyl)-threonine carbamoylmethyl ester (**SI-11**).

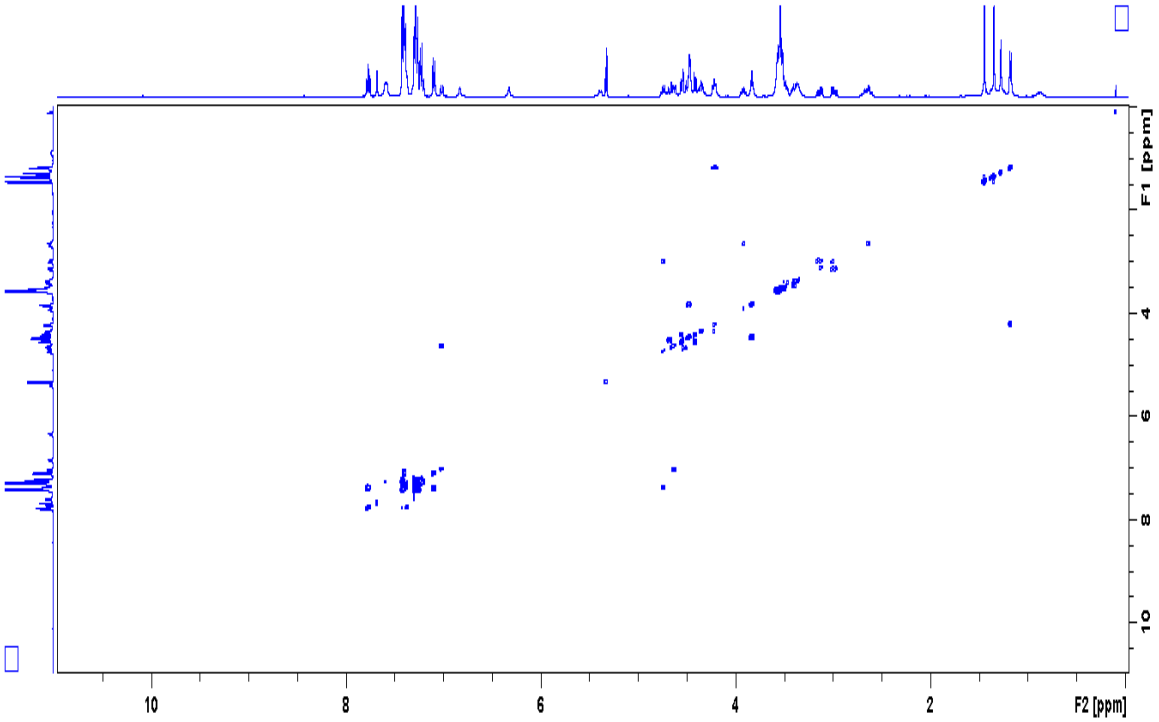


Figure *S31*. COSY-NMR spectrum (400 MHz, CD_2Cl_2) of N^{α}-Fmoc-S^{β}-trityl-cysteinyl-O^{β}-(4-methyl-1-(*N*-(PEG_4-NHCO-Phe(4-Br)-NHCO-1-(2,3-*O*-isopropylidene)-D-tartrate-OH))-[1,2,3]triazolyl)-threonine carbamoylmethyl ester (**SI-11**).

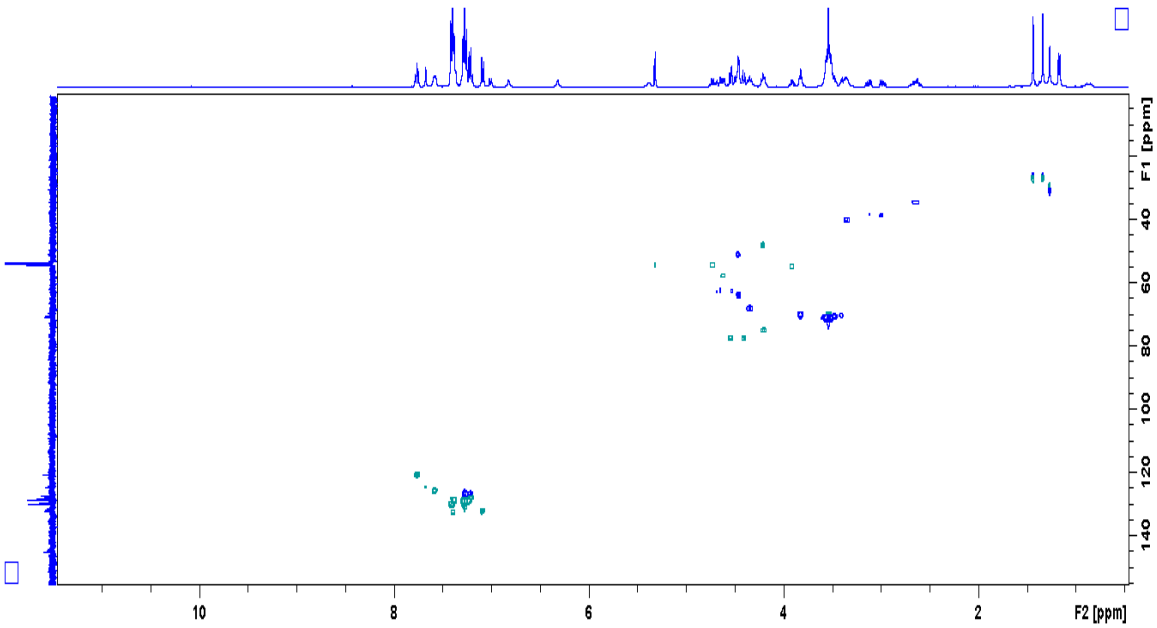


Figure *S32*. HSQC-NMR spectrum (400 MHz, CD_2Cl_2) of *N*$^{\alpha}$-Fmoc-*S*$^{\beta}$-trityl-cysteinyl-*O*$^{\beta}$-(4-methyl-1-(*N*-(PEG$_4$-NHCO-Phe(4-Br)-NHCO-1-(2,3-*O*-isopropylidene)-D-tartrate-OH))-[1,2,3]triazolyl)-threonine carbamoylmethyl ester (**SI-11**).

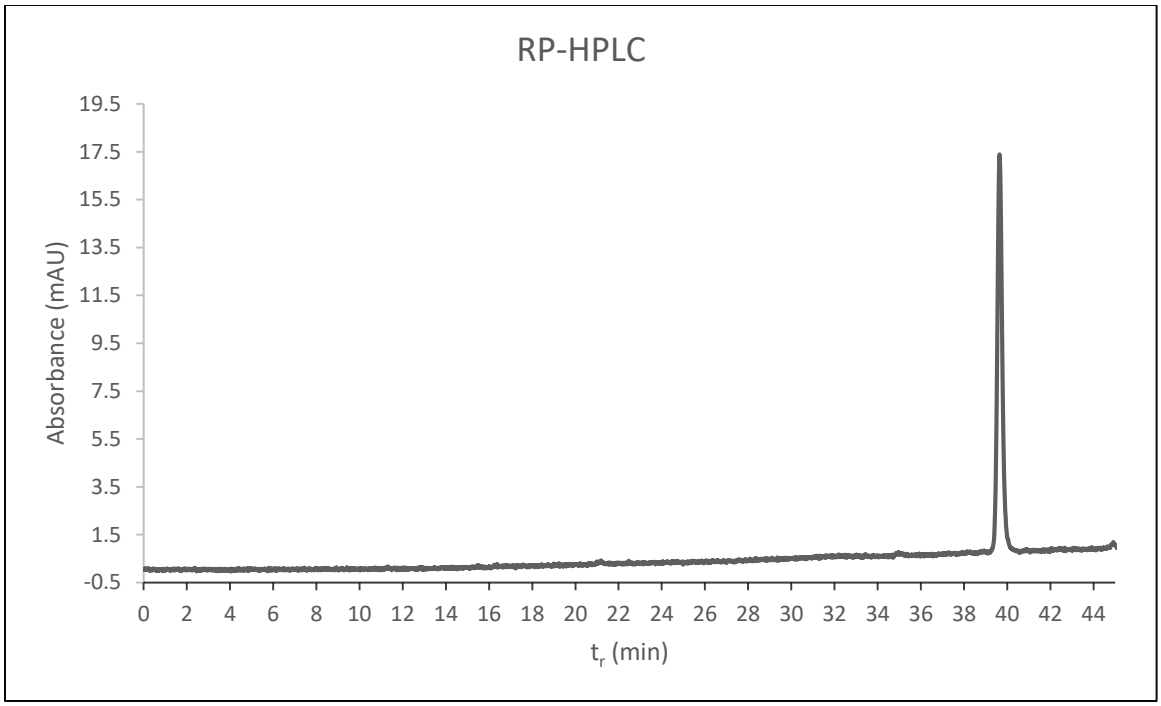


Figure *S33a*. RP-HPLC (C18) chromatogram of *N*$^{\alpha}$-Fmoc-*S*$^{\beta}$-trityl-cysteinyl-*O*$^{\beta}$-(4-methyl-1-(*N*-(PEG$_4$-NHCO-Phe(4-Br)-NHCO-1-(2,3-*O*-isopropylidene)-D-tartrate-OH))-[1,2,3]triazolyl)-threonine carbamoylmethyl ester (**SI-11**). Gradient of 40% to 78% solvent B over 20 min, 78% to 100% over 20 min at a flow rate of 1 mL/min; t_r = 39.65 min (A_{301} 100%).

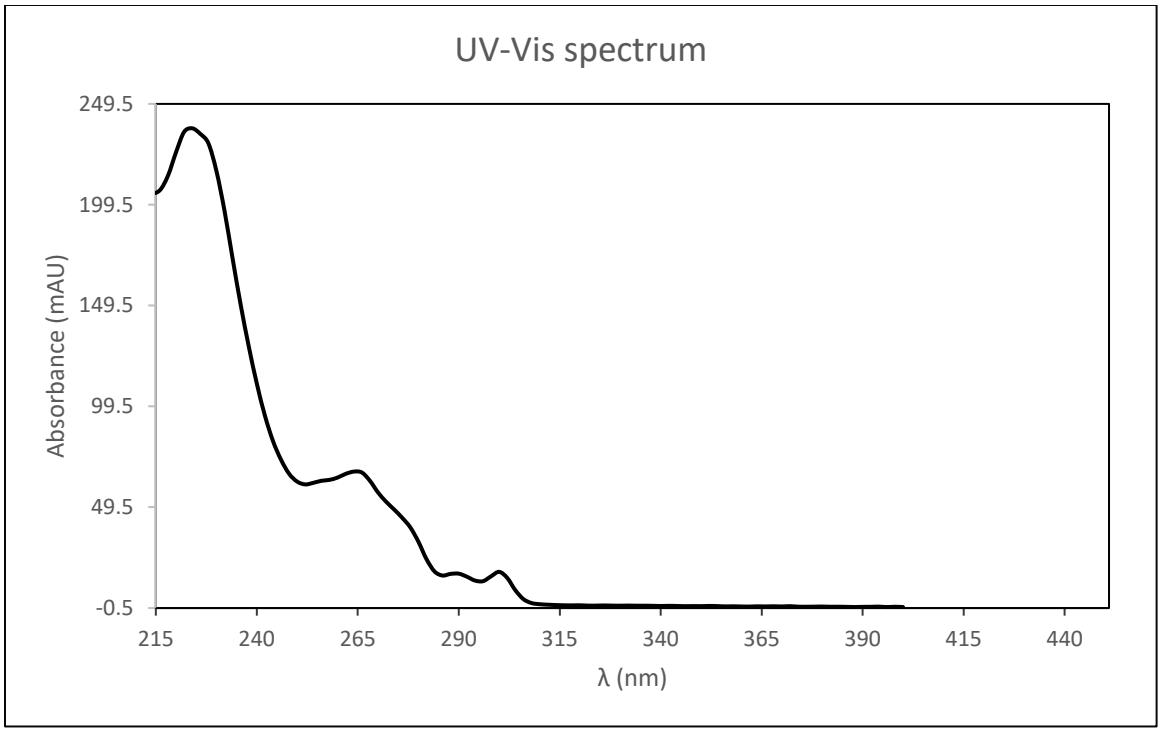


Figure *S33b*. UV-Vis spectrum of *N*$^{\alpha}$-Fmoc-*S*$^{\beta}$-trityl-cysteinyl-*O*$^{\beta}$-(4-methyl-1-(*N*-(PEG$_4$-NHCO-Phe(4-Br)-NHCO-1-(2,3-*O*-isopropylidene)-D-tartrate-OH))-[1,2,3]triazolyl)-threonine carbamoylmethyl ester (**SI-11**). UV-Vis spectra were taken with the RP-HPLC diode array detectors directly in the RP-HPLC flow cells at t_r = 39.65 min (82% eluent B). Analyte concentration unknown.

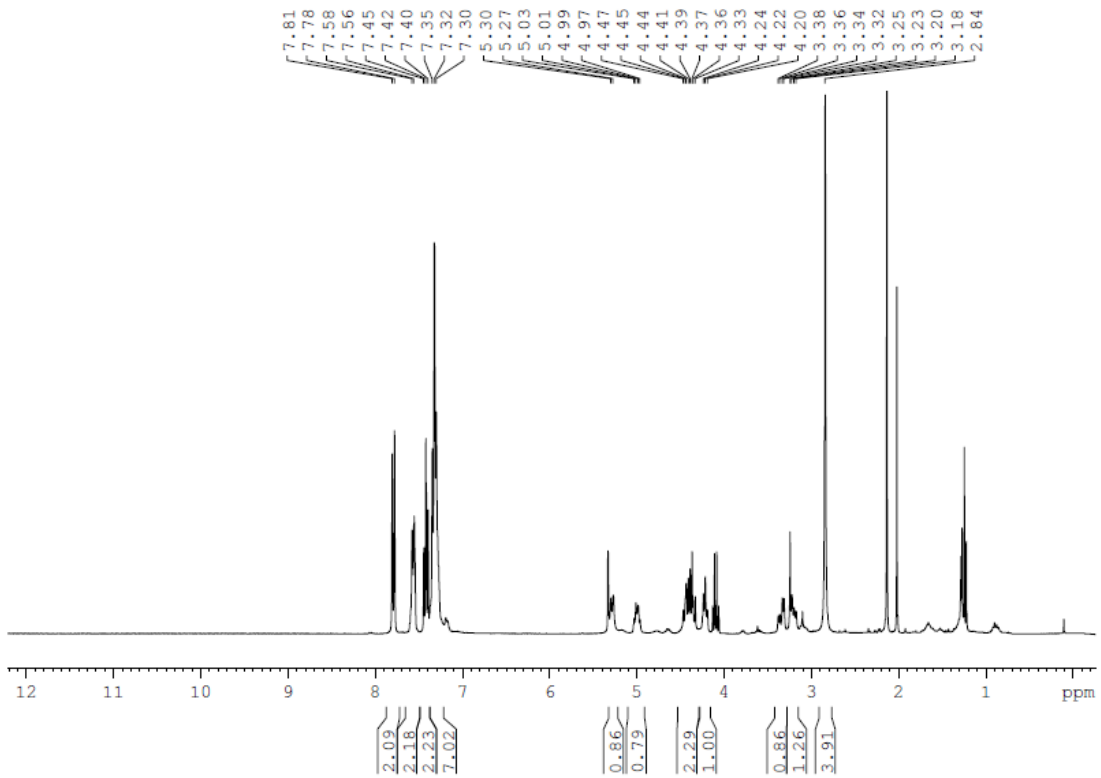


Figure *S34*. ^{1}H-NMR spectrum (300 MHz, CD_2Cl_2) of (2*S*)-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-3-phenylpropanoate succinimidyl ester (**SI-18**).

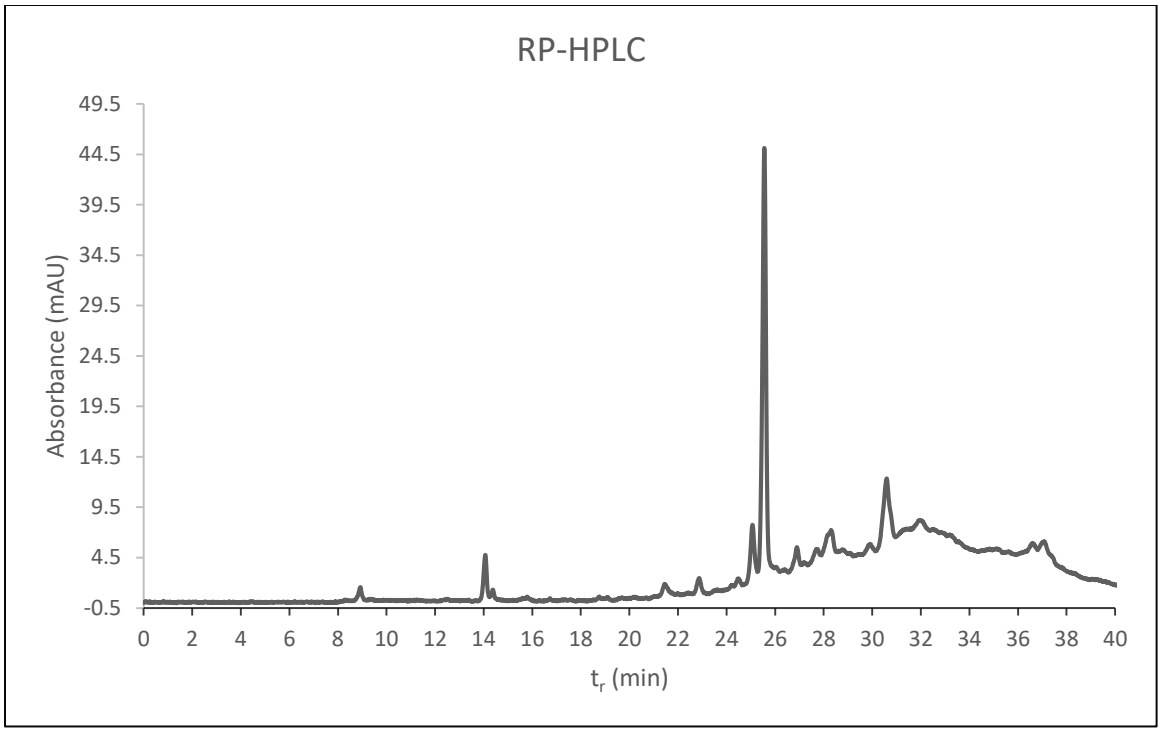


Figure *S35a*. RP-HPLC (C18) chromatogram of semi-purified D-phenylalanyl-(2-mercapto-tryptophanyl-tyrosyl-D-tryptophanyl-N^{ε}-1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl-lysinyl-threonyl-cysteinyl)cyclic sulfide -O^{β}-(4-methyl-1-(*N*-(PEG_4-NHCO-Phe(4-Br)-NHCO-(*O*-benzyloxime))-[1,2,3]triazolyl)-threonine carbamoylmethyl ester (**SI-21**). Gradient of 20% to 100% solvent B over 30 min at a flow rate of 1 mL/min; t_r = 25.56 min (A_{290} 66%).

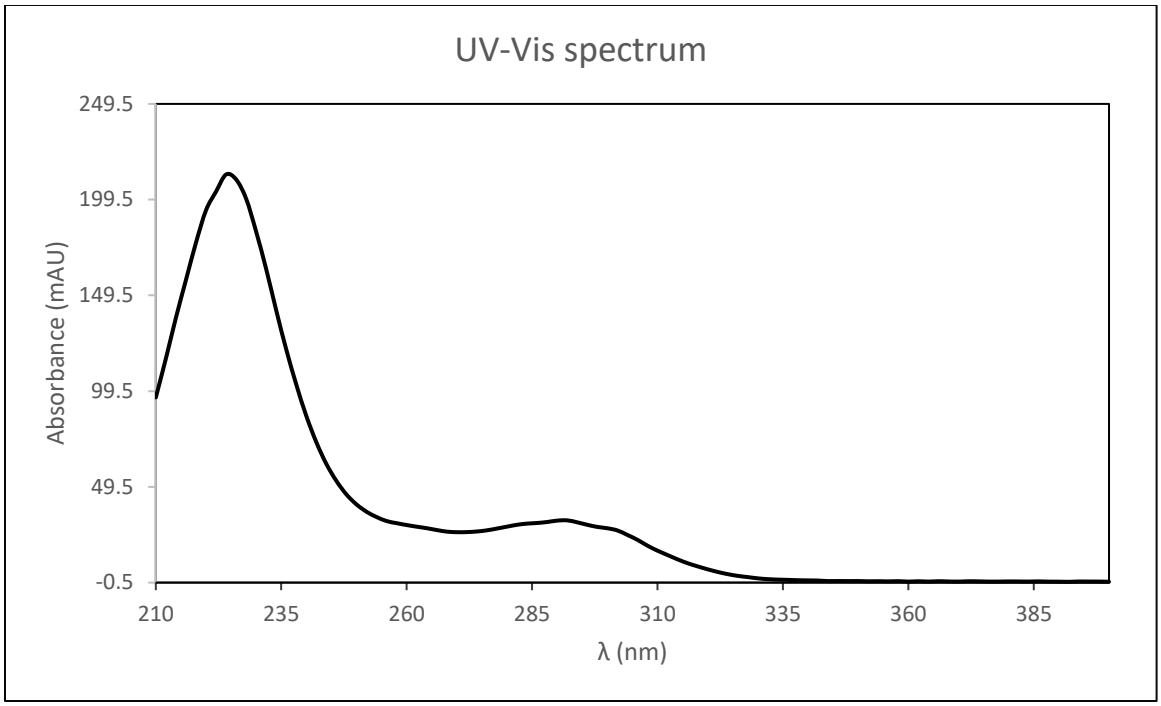


Figure *S35b*. Representative UV spectrum of D-phenylalanyl-(2-mercapto-tryptophanyl-tyrosyl-D-tryptophanyl-N^{ε}-1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl-lysinyl-threonyl-cysteinyl)cyclic sulfide -O^{β}-(4-methyl-1-(*N*-(PEG$_4$-NHCO-Phe(4-Br)-NHCO-(*O*-benzyloxime))-[1,2,3]triazolyl)-threonine carbamoylmethyl ester (**SI-21**). UV-Vis spectra were taken with the RP-HPLC diode array detectors directly in the RP-HPLC flow cells at t_r = 25.56 min (56% eluent B). Analyte concentration unknown.

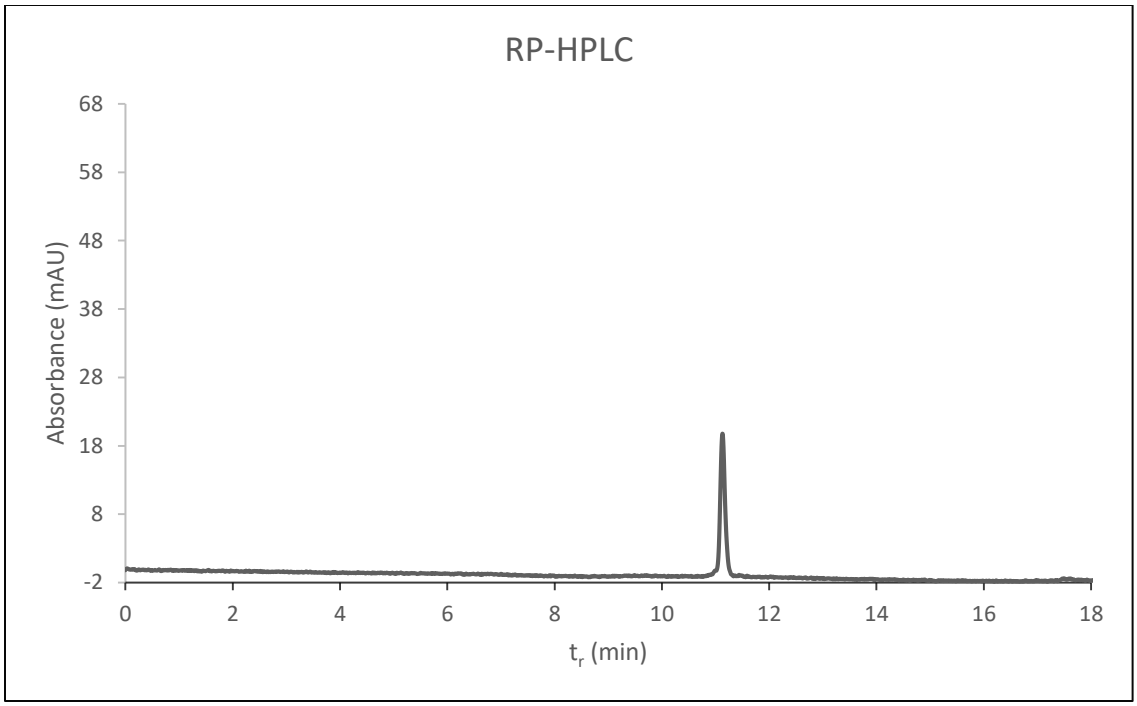


Figure *S36a*. RP-HPLC (C18) chromatogram of D-phenylalanyl-(2-mercapto-tryptophanyl-tyrosyl-D-tryptophanyl-lysinyl-threonyl-cysteinyl)cyclic sulfide-*O*$^{\beta}$-(4-methyl-1-(*N*-(PEG$_4$-NHCO-Phe(4-Br)-NHCO-(*O*-benzyloxime))-[1,2,3]triazolyl)-threonine carbamoylmethyl ester (**6**). Gradient of 20% to 100% solvent B over 18 min at 2 mL/min; t_r = 11.13 min (A_{290} 100%).

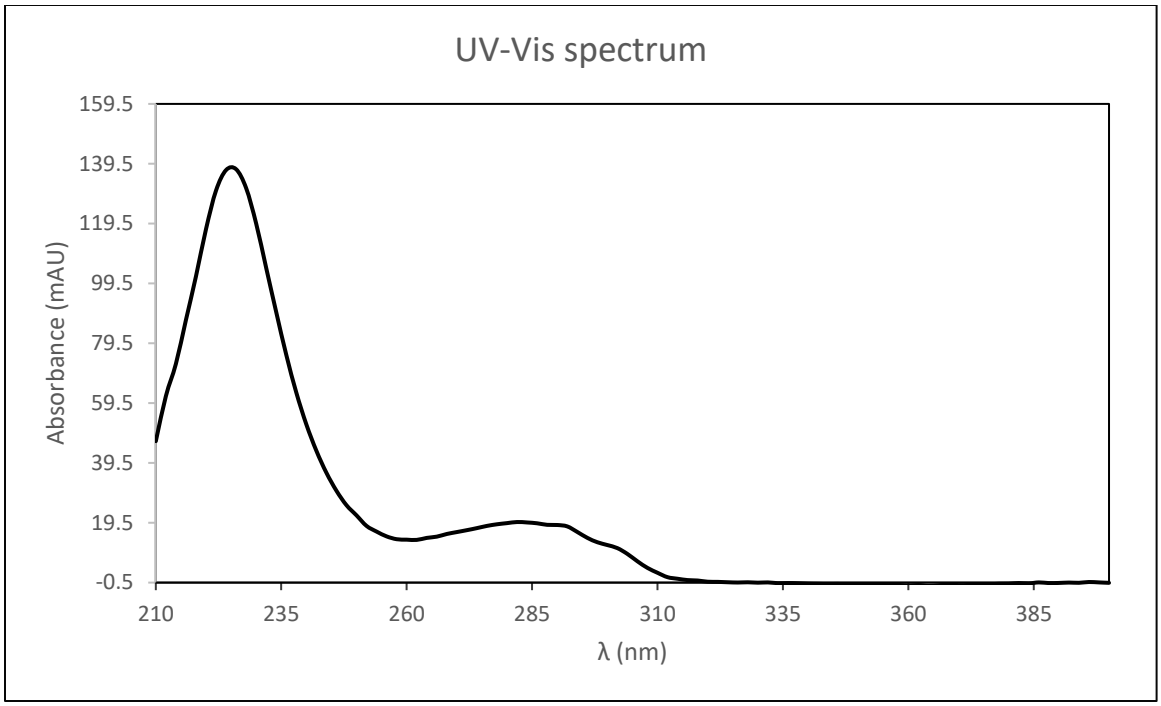


Figure *S36b*. Representative UV spectrum of of D-phenylalanyl-(2-mercapto-tryptophanyl-tyrosyl-D-tryptophanyl-lysinyl-threonyl-cysteinyl)cyclic sulfide-*O*$^{\beta}$-(4-methyl-1-(*N*-(PEG$_4$-NHCO-Phe(4-Br)-NHCO-(*O*-benzyloxime))-[1,2,3]triazolyl)-threonine carbamoylmethyl ester (**6**).UV-Vis spectra were taken with the RP-HPLC diode array detectors directly in the RP-HPLC flow cells at t_r = 11.13 min (43% eluent B). Analyte concentration unknown.

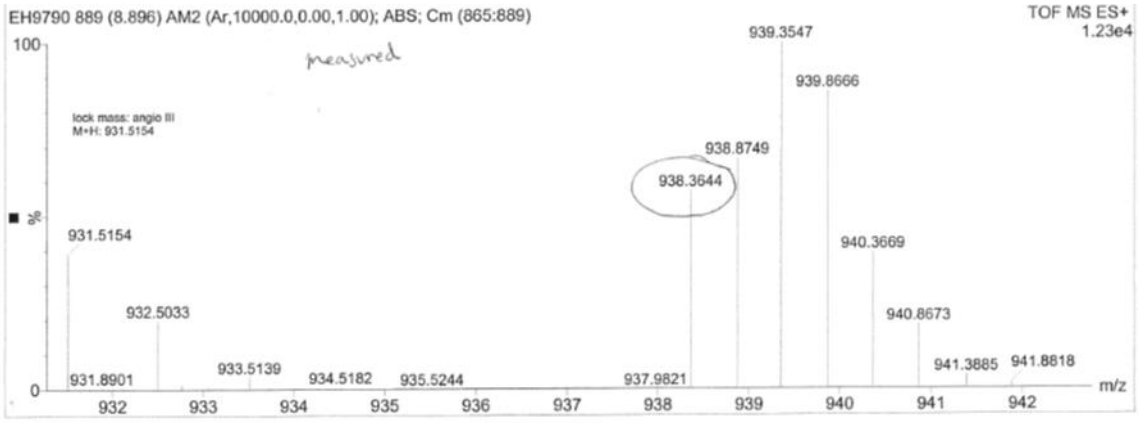


Figure *S36c*. HR-MS (ESI-TOF) of D-phenylalanyl-(2-mercapto-tryptophanyl-tyrosyl-D-tryptophanyl-lysinyl-threonyl-cysteinyl)cyclic sulfide-O^{β}-(4-methyl-1-(*N*-(PEG$_4$-NHCO-Phe(4-Br)-NHCO-(*O*-benzyloxime))-[1,2,3]triazolyl)-threonine carbamoylmethyl ester (**6**).

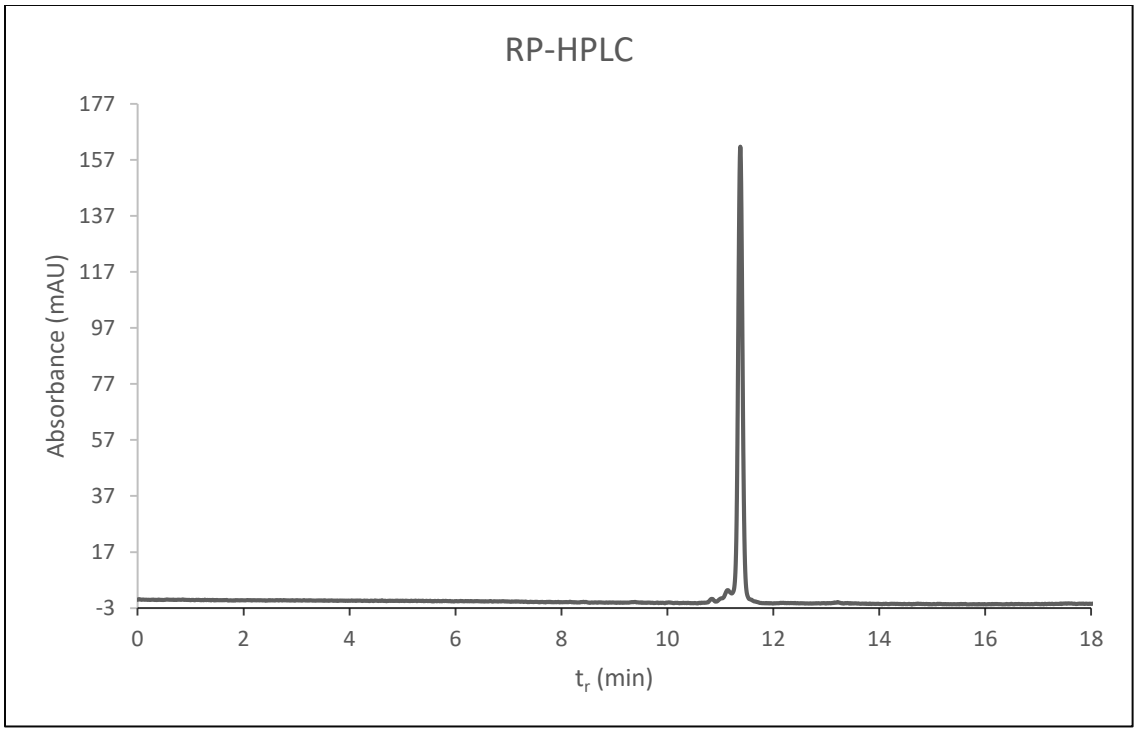


Figure *S37a*. RP-HPLC (C18) chromatogram of D-phenylalanyl-(2-mercapto-tryptophanyl-tyrosyl-D-tryptophanyl-lysinyl-threonyl-cysteinyl)cyclic sulfide-*O*$^{\beta}$-(4-methyl-1-(*N*-(PEG$_4$-NHCO-Phe(4-Br)-NHCO-(*O*-benzyloxime))-[1,2,3]triazolyl)-threonine (**7**). Gradient of 20% to 100% solvent B over 18 min at 2 mL/min; t_r = 11.38 min (A_{290} 100%).

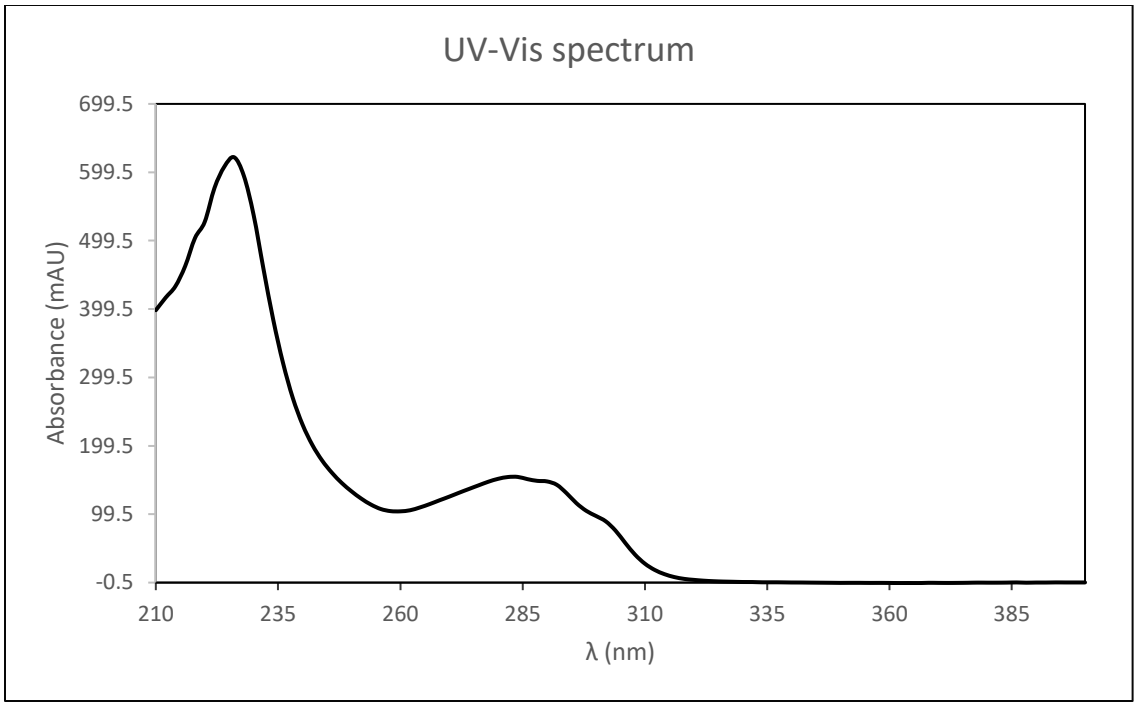


Figure *S37b*. UV-Vis spectrum of D-phenylalanyl-(2-mercapto-tryptophanyl-tyrosyl-D-tryptophanyl-lysinyl-threonyl-cysteinyl)cyclic sulfide-*O*$^{\beta}$-(4-methyl-1-(*N*-(PEG$_4$-NHCO-Phe(4-Br)-NHCO-(*O*-benzyloxime))-[1,2,3]triazolyl)-threonine (**7**). UV-Vis spectra were taken with the RP-HPLC diode array detectors directly in the RP-HPLC flow cells at t_r = 11.38 min (44% eluent B). Analyte concentration unknown.

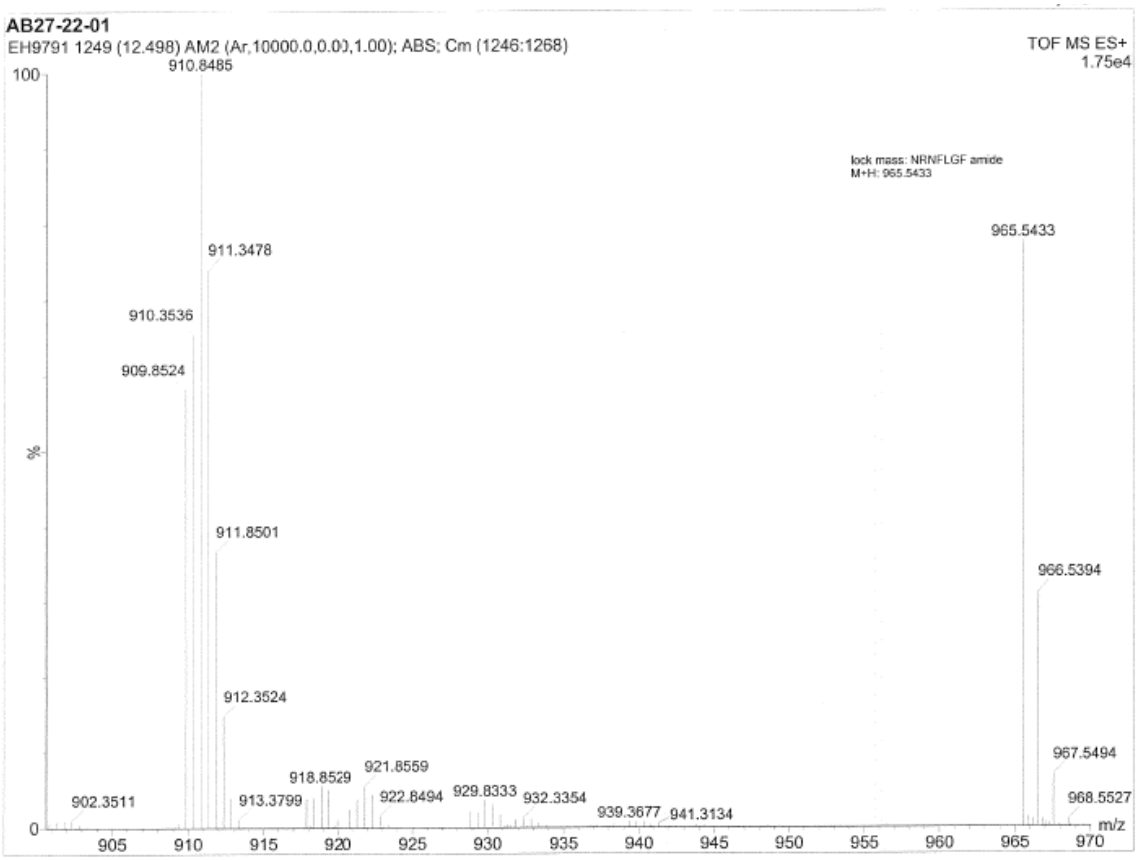


Figure *S37c*. HR-MS (ESI-TOF) of D-phenylalanyl-(2-mercapto-tryptophanyl-tyrosyl-D-tryptophanyl-lysinyl-threonyl-cysteinyl)cyclic sulfide-O^{β}-(4-methyl-1-(*N*-(PEG$_4$-NHCO-Phe(4-Br)-NHCO-(*O*-benzyloxime))-[1,2,3]triazolyl)-threonine (**7**).

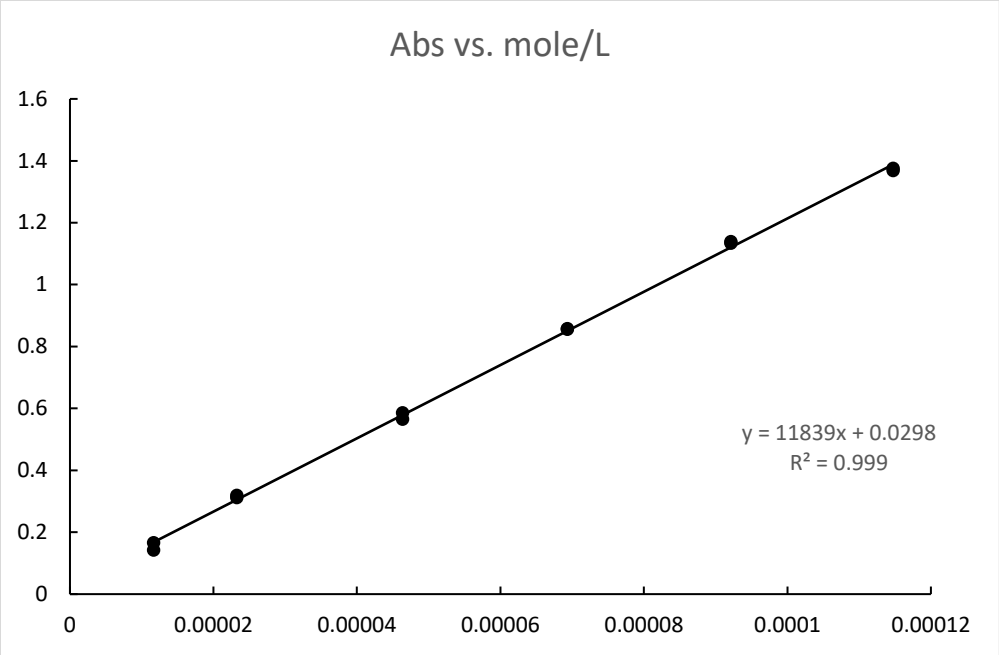


Figure *S38*. Linear regression curve of hexylamine and phenylalanine UV-Vis absorbance. A_{335} = 11839X + 0.0298 with R^2 = 0.999 where A_{335} is the absorbance measured at 335 nm in 2:8 (*v*/*v*) MeCN/Milli-Q water diluted with OPA-NAC borate eluent reagent. X is the corresponding molar concentration of 2 equivalents of free amine according to Beer-Lambert law (l = 1 cm).

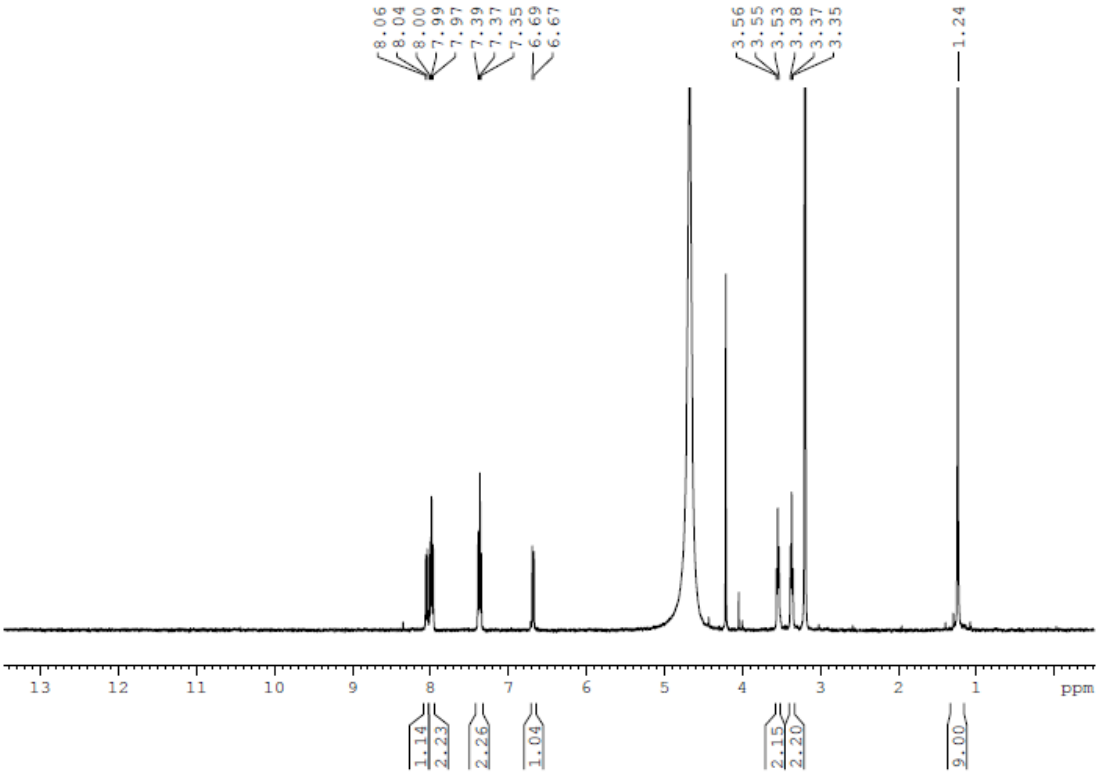


Figure *S39*. ^{1}H-NMR spectrum (400 MHz, D_2O + CD_3OD) of 5-(2-(((*N*$^{\alpha}$-(*tert*-butoxycarbonyl)amino)oxyacetyl)amino)ethylamino)naphthalene-1-sulfonic acid (**SI-24**).

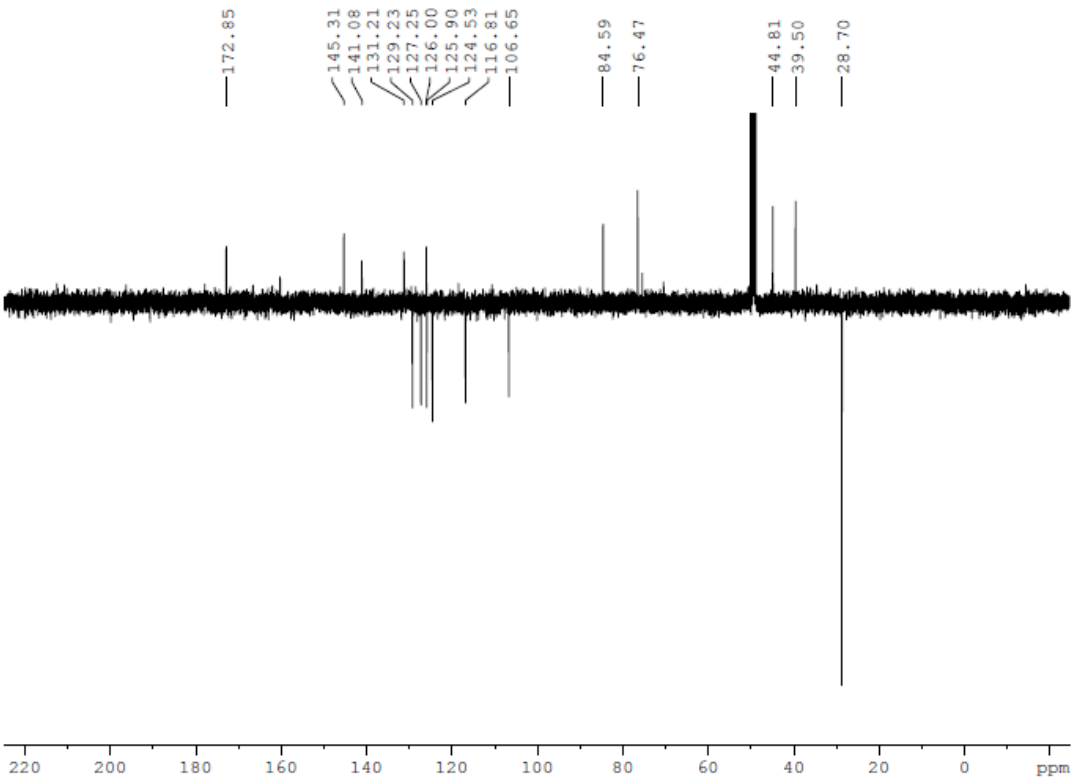


Figure *S40*. ^{13}C-NMR spectrum (100 MHz, APT, D_2O + CD_3OD) of 5-(2-(((*N*$^{\alpha}$-(*tert*-butoxycarbonyl)amino)oxyacetyl)amino)ethylamino)naphthalene-1-sulfonic acid (**SI-24**).

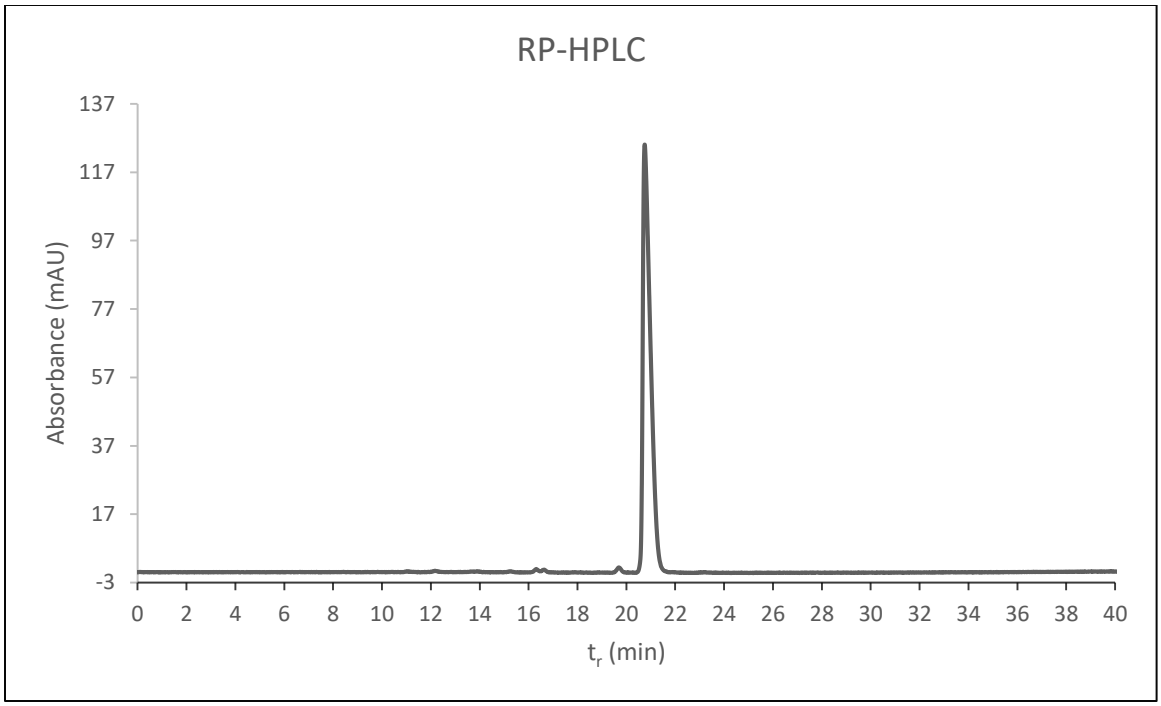


Figure *S41a*. RP-HPLC (C18) chromatogram of 5-(2-(((*N*$^{\alpha}$-(*tert*-butoxycarbonyl)amino)oxyacetyl)amino)ethylamino)naphthalene-1-sulfonic acid (**SI-24**). Gradient of 20% to 100% solvent B over 32 min at a flow rate of 1 mL/min; t_r = 20.75 min (A_{341} 100%).

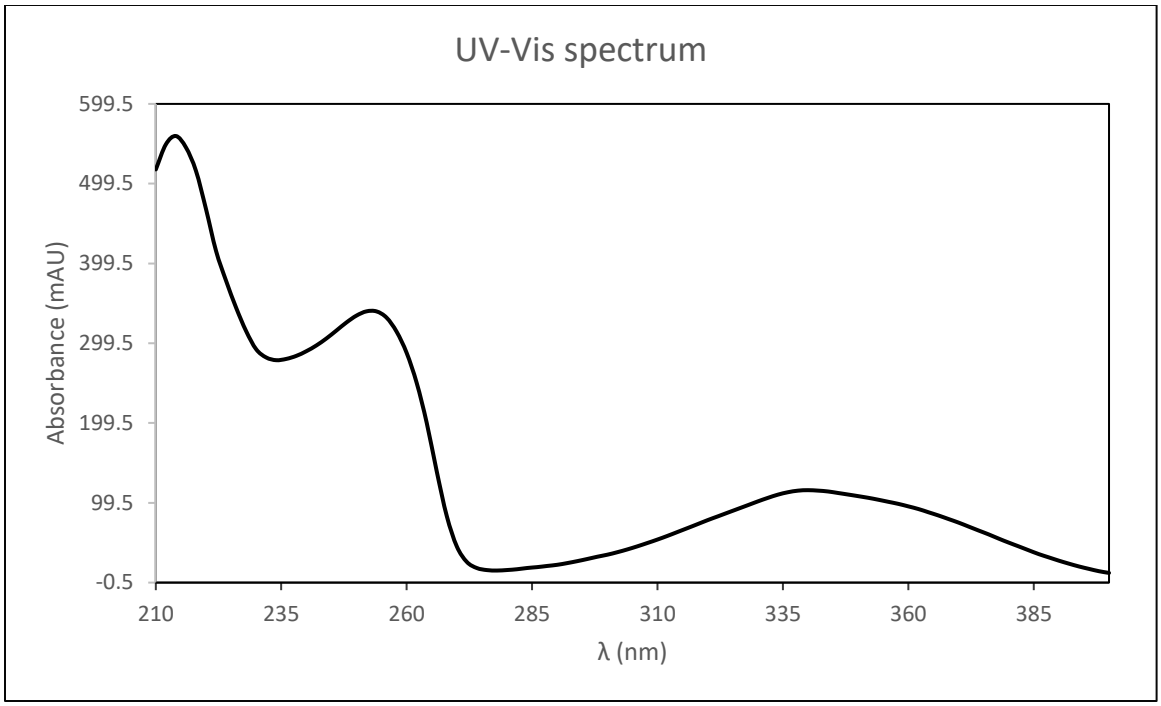


Figure *S41b*. UV-Vis spectrum of 5-(2-(((*N*$^{\alpha}$-(*tert*-butoxycarbonyl)amino)oxyacetyl)amino)ethylamino)naphthalene-1-sulfonic acid (**SI-24**). UV-Vis spectra were taken with the RP-HPLC diode array detectors directly in the RP-HPLC flow cells at t_r = 20.75 min (42% eluent B). Analyte concentration unknown.

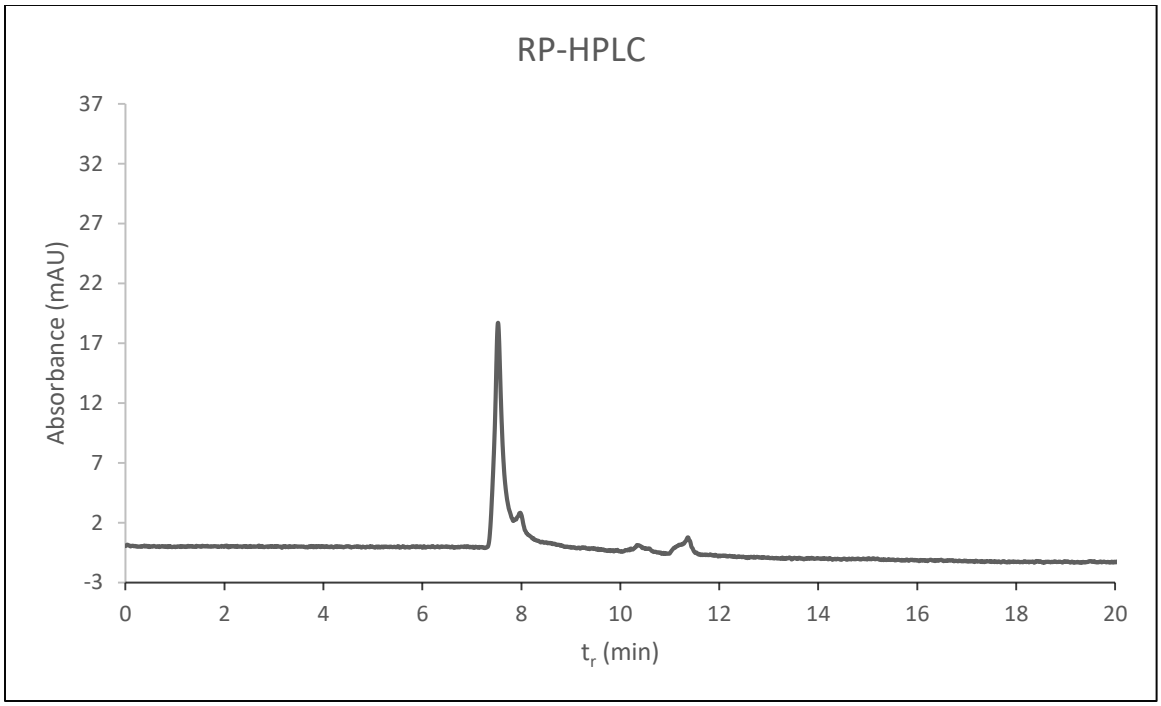


Figure *S42a*. RP-HPLC (C18) chromatogram of 5-(2-((amino)oxyacetyl)amino)ethylamino)naphthalene-1-sulfonic acid (**SI-25**). Gradient of 10% to 100% solvent B over 21 min at 2 mL/min; t_r = 7.53 min (A_{342} 94%).

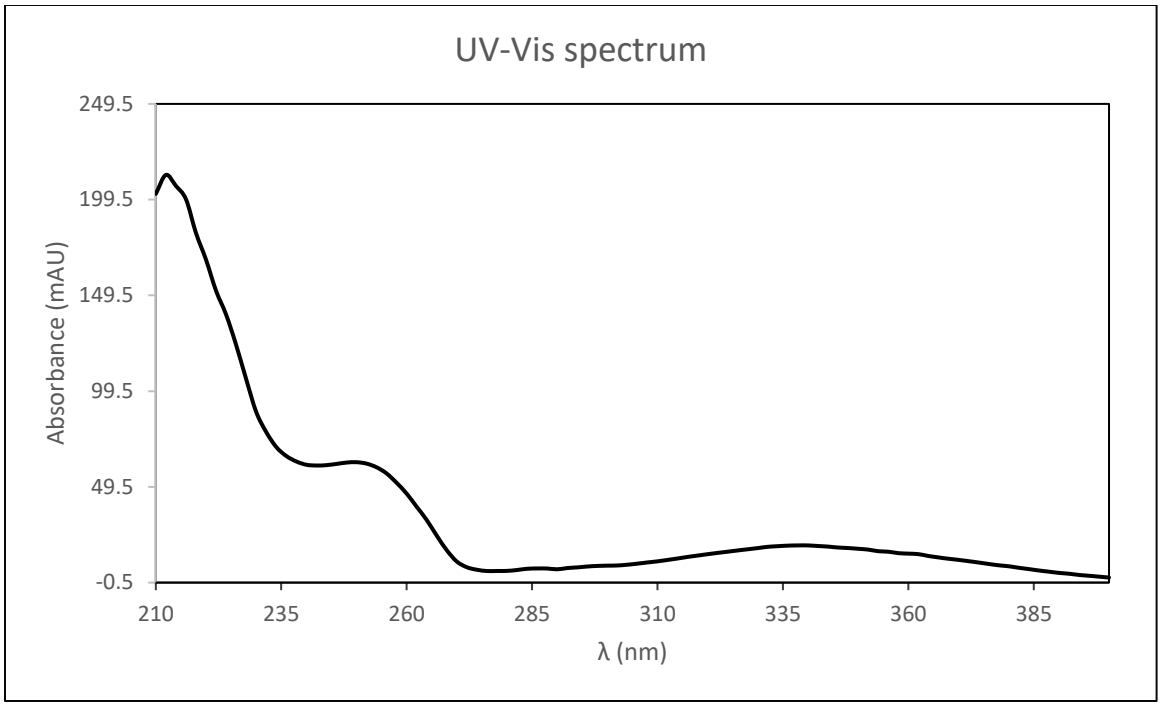


Figure *S42b*. UV-Vis spectrum of 5-(2-((amino)oxyacetyl)amino)ethylamino)naphthalene-1-sulfonic acid (**SI-25**). UV-Vis spectra were taken with the RP-HPLC diode array detectors directly in the RP-HPLC flow cells at t_r = 7.53 min (16% eluent B). Analyte concentration unknown.

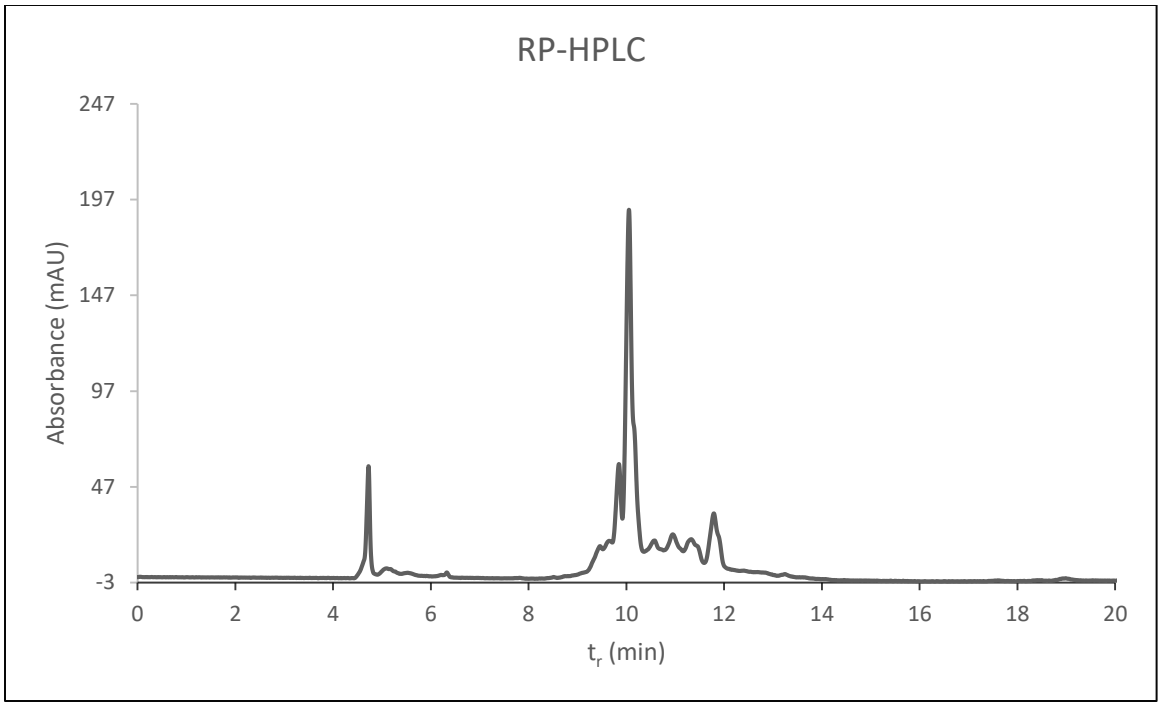


Figure *S43a*. RP-HPLC (C18) chromatogram of crude D-phenylalanyl-(2-mercapto-tryptophanyl-tyrosyl-D-tryptophanyl-lysinyl-threonyl-cysteinyl)cyclic sulfide-O^{β}-(4-methyl-1-(N-(PEG_4- NHCO-Phe(4-Br)-NHCO-(*N*-glyoxyloyl))-[1,2,3]triazolyl)-threonine (**SI-28**). Gradient of 20% to 100% solvent B over 18 min at 2 mL/min; t_r = 10.05 min (A_{290} 55.4%).

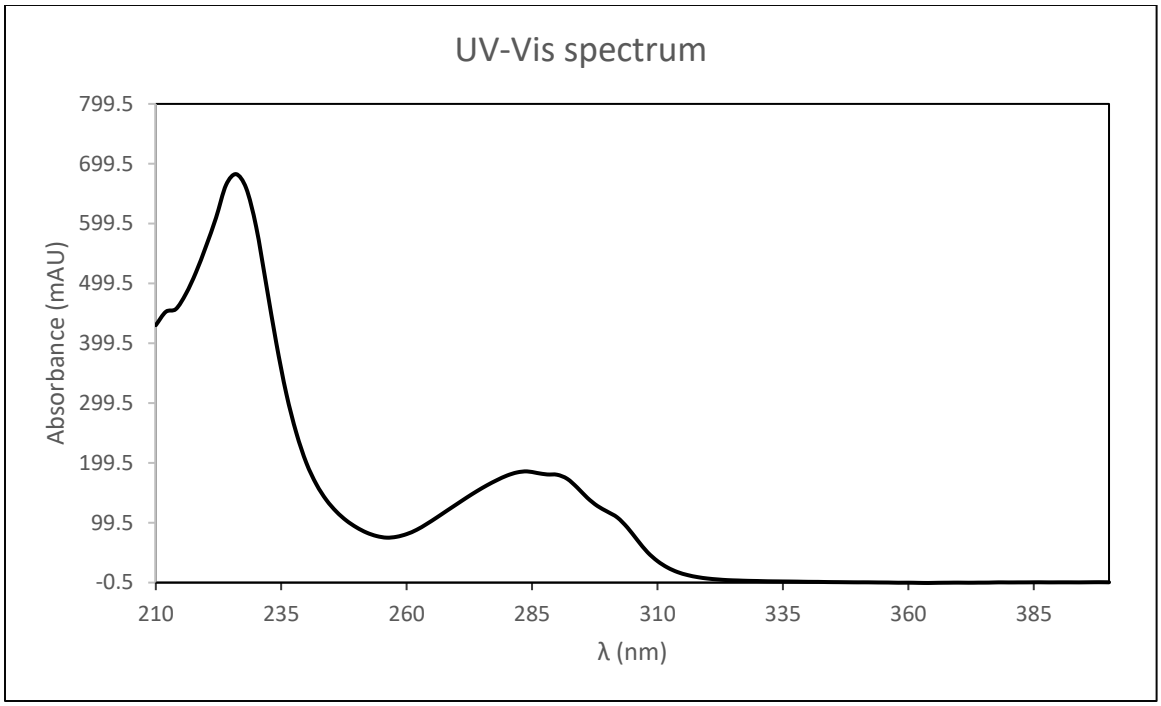


Figure *S43b*. UV-Vis spectrum of crude D-phenylalanyl-(2-mercapto-tryptophanyl-tyrosyl-D-tryptophanyl-lysinyl-threonyl-cysteinyl)cyclic sulfide-O^{β}-(4-methyl-1-(N-(PEG_4-NHCO-Phe(4-Br)-NHCO-(*N*-glyoxyloyl))-[1,2,3]triazolyl)-threonine (**SI-28**). UV-Vis spectra were taken with the RP-HPLC diode array detectors directly in the RP-HPLC flow cells at t_r = 10.05 min (38% eluent B). Analyte concentration unknown.

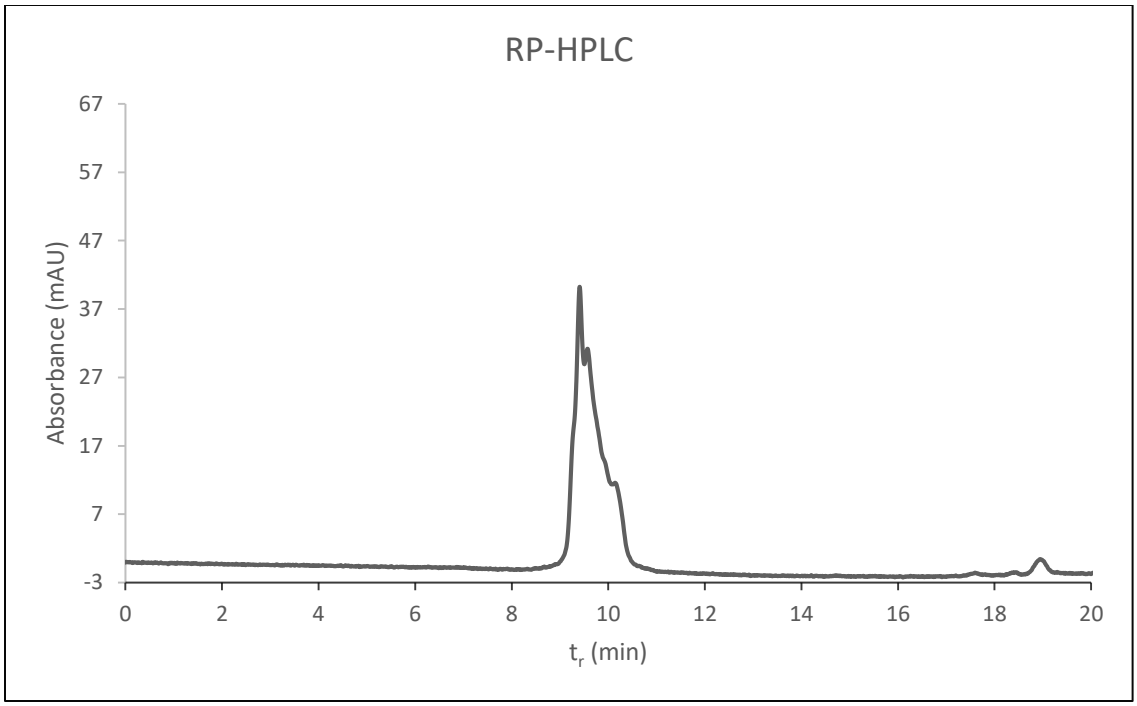


Figure *S44a*. RP-HPLC (C18) chromatogram of crude D-phenylalanyl-(2-mercapto-tryptophanyl-tyrosyl-D-tryptophanyl-lysinyl-threonyl-cysteinyl)cyclic sulfide-*O*$^{\beta}$-(4-methyl-1-(N-(PEG$_4$-NHCO-Phe(4-Br)-NHCO-(imine))-[1,2,3]triazolyl)-threonine (**SI-29**). Gradient of 20% to 100% solvent B over 18 min at 2 mL/min; t_r = 9.41 min (A_{290} 96%).

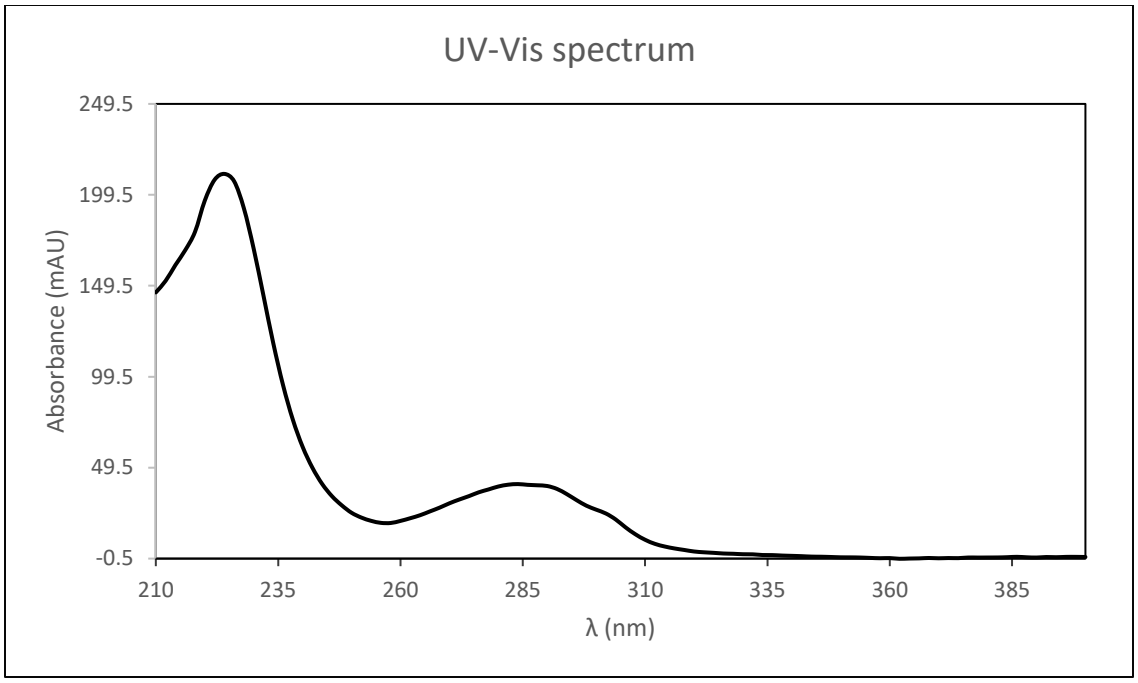


Figure *44b*. UV-Vis spectrum of crude D-phenylalanyl-(2-mercapto-tryptophanyl-tyrosyl-D-tryptophanyl-lysinyl-threonyl-cysteinyl)cyclic sulfide-*O*$^{\beta}$-(4-methyl-1-(N-(PEG$_4$- NHCO-Phe(4-Br)-NHCO-(imine))-[1,2,3]triazolyl)-threonine (**SI-29**). UV-Vis spectra were taken with the RP-HPLC diode array detectors directly in the RP-HPLC flow cells at t_r = 9.41 min (35% eluent B). Analyte concentration unknown.

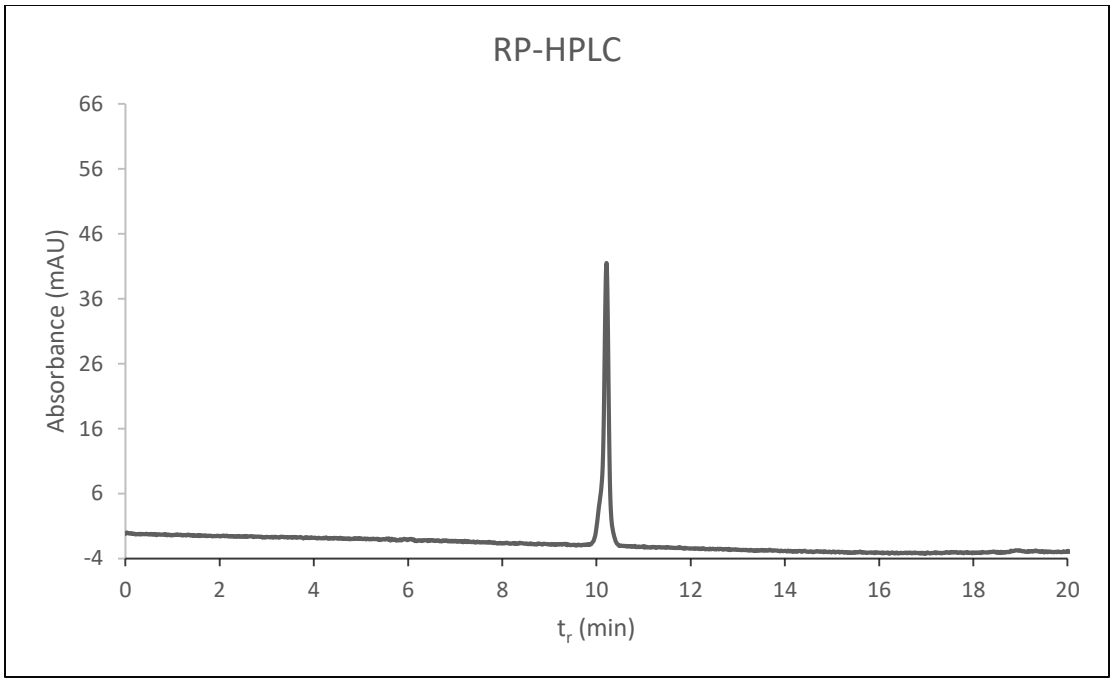


Figure *45a*. RP-HPLC (C18) chromatogram of D-phenylalanyl-(2-mercapto-tryptophanyl-tyrosyl-D-tryptophanyl-lysinyl-threonyl-cysteinyl)cyclic sulfoxide-O^{β}-(4-methyl-1-(*N*-(PEG$_4$- NHCO-Phe(4-Br)- NHCO-(Aoa-EDANS oxime))-[1,2,3]triazolyl)-threonine (**9[O]**). Gradient of 20% to 100% solvent B over 18 min at 2 mL/min; t_r = 10.21 min (A_{290} 100%).

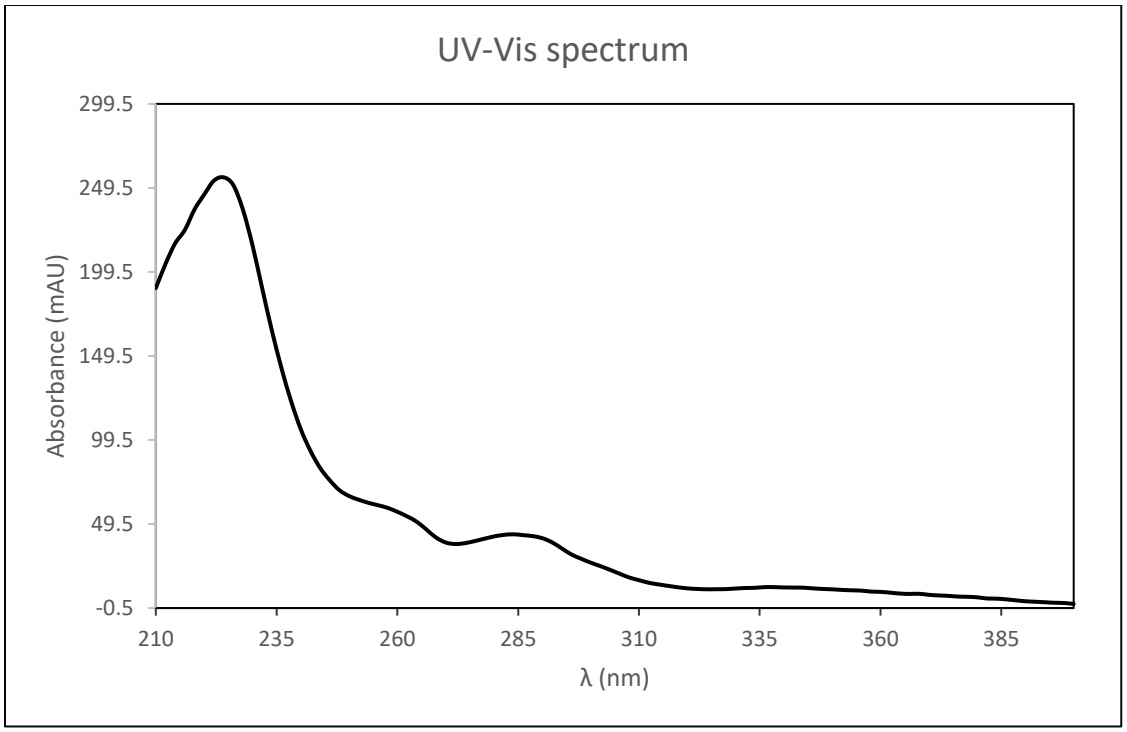


Figure *S45b*. UV-Vis spectrum of D-phenylalanyl-(2-mercapto-tryptophanyl-tyrosyl-D-tryptophanyl-lysinyl-threonyl-cysteinyl)cyclic sulfoxide-*O*$^{\beta}$-(4-methyl-1-(*N*-(PEG_4- NHCO-Phe(4-Br)- NHCO-(Aoa-EDANS oxime))-[1,2,3]triazolyl)-threonine (**9[O]**). UV-Vis spectra were taken with the RP-HPLC diode array detectors directly in the RP-HPLC flow cells at t_r = 10.21 min (39% eluent B). Analyte concentration unknown.

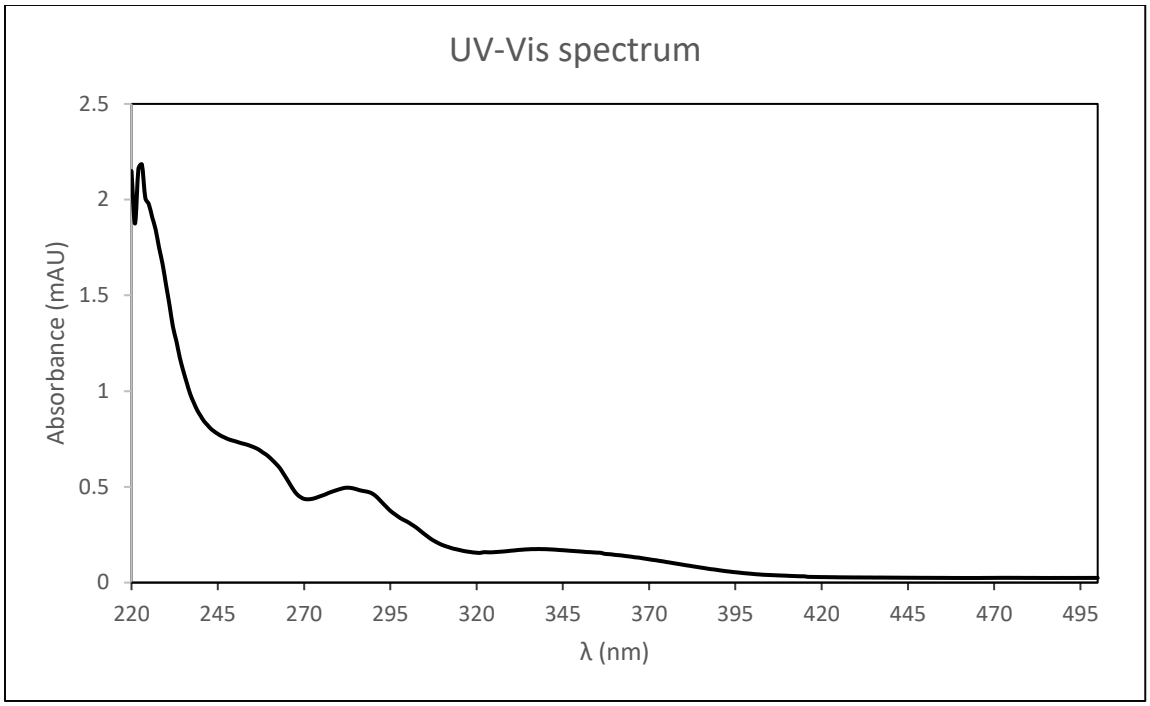


Figure *S45c*. UV-Vis spectrum, from spectrophotometer, of 32µM D-phenylalanyl-(2-mercapto-tryptophanyl-tyrosyl-D-tryptophanyl-lysinyl-threonyl-cysteinyl)cyclic sulfoxide-O^{β}-(4-methyl-1-(*N*-(PEG_4-NHCO-Phe(4-Br)-NHCO-(Aoa-EDANS oxime))-[1,2,3]triazolyl)-threonine (**9[O]**) in 0.1%FA 4:6 (*v*/*v*) MeCN/Milli-Q water.

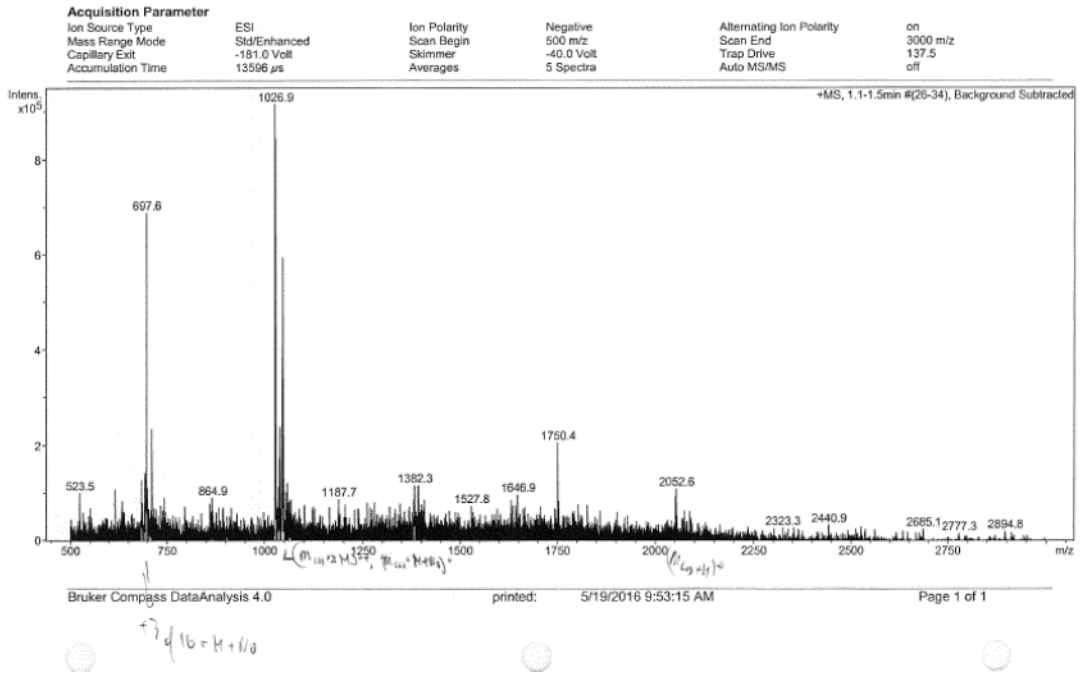


Figure *S45d*. LR-MS (ESI-High Capacity ion Trap) of D-phenylalanyl-(2-mercapto-tryptophanyl-tyrosyl-D-tryptophanyl-lysinyl-threonyl-cysteinyl)cyclic sulfoxide-O^{β}-(4-methyl-1-(*N*-(PEG$_4$- NHCO-Phe(4-Br)- NHCO-(Aoa-EDANS oxime))-[1,2,3]triazolyl)-threonine (**9[O]**).

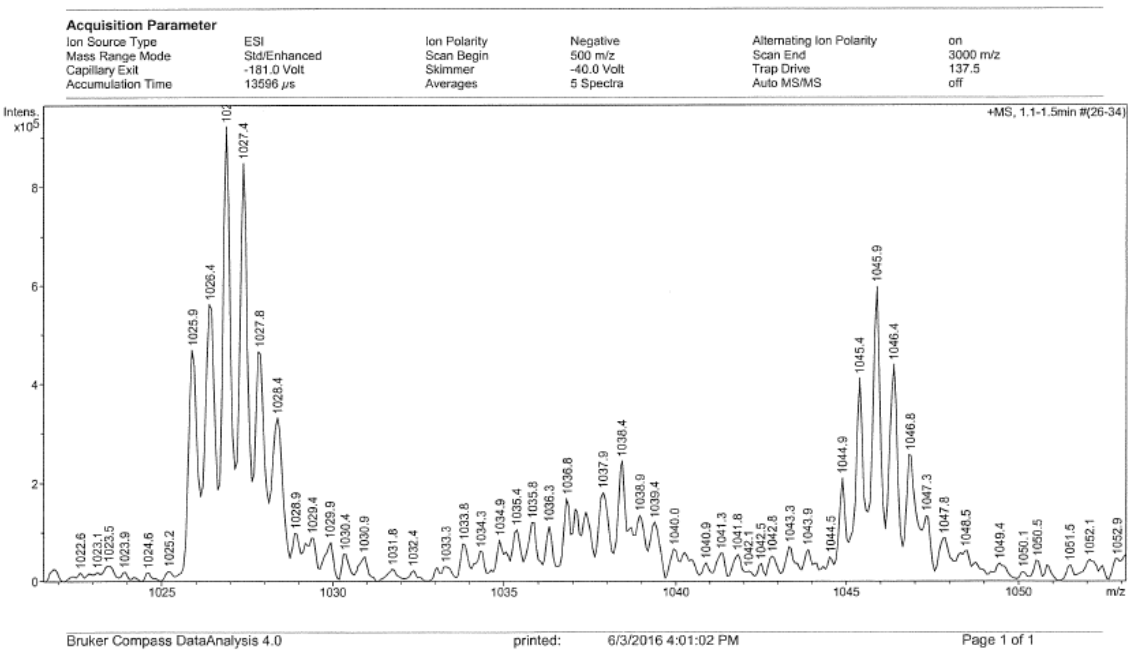


Figure *S45e*. Zoom in LR-MS (ESI-High Capacity ion Trap) of D-phenylalanyl-(2-mercapto-tryptophanyl-tyrosyl-D-tryptophanyl-lysinyl-threonyl-cysteinyl)cyclic sulfoxide-O^{β}-(4-methyl-1-(*N*-(PEG$_4$- NHCO-Phe(4-Br)- NHCO-(Aoa-EDANS oxime))-[1,2,3]triazolyl)-threonine (**9[O]**).

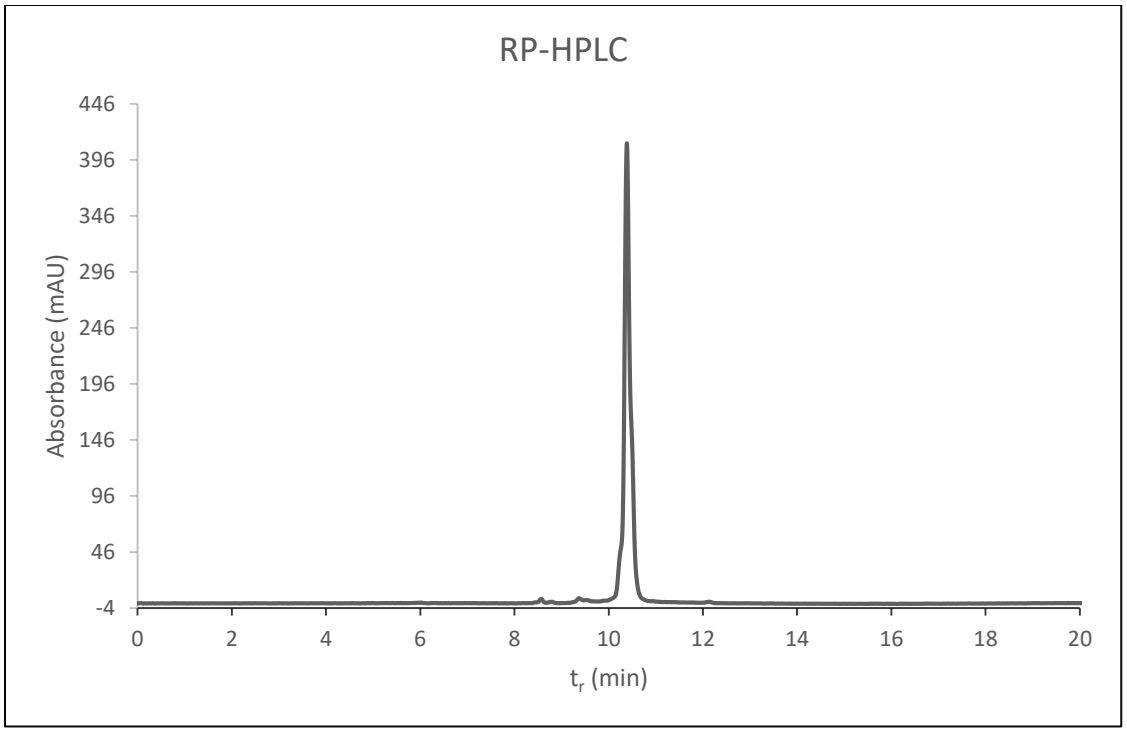


Figure *S46a*. RP-HPLC (C18) chromatogram of D-phenylalanyl-(2-mercapto-tryptophanyl-tyrosyl-D-tryptophanyl-lysinyl-threonyl-cysteinyl)cyclic sulfide-O^{β}-(4-methyl-1-(*N*-(PEG_4- NHCO-Phe(4-Br)- NHCO-(Aoa-EDANS oxime))-[1,2,3]triazolyl)-threonine (**9**). Gradient of 20% to 100% solvent B over 18 min at 2 mL/min; t_r = 10.39 min (A_{290} 99%).

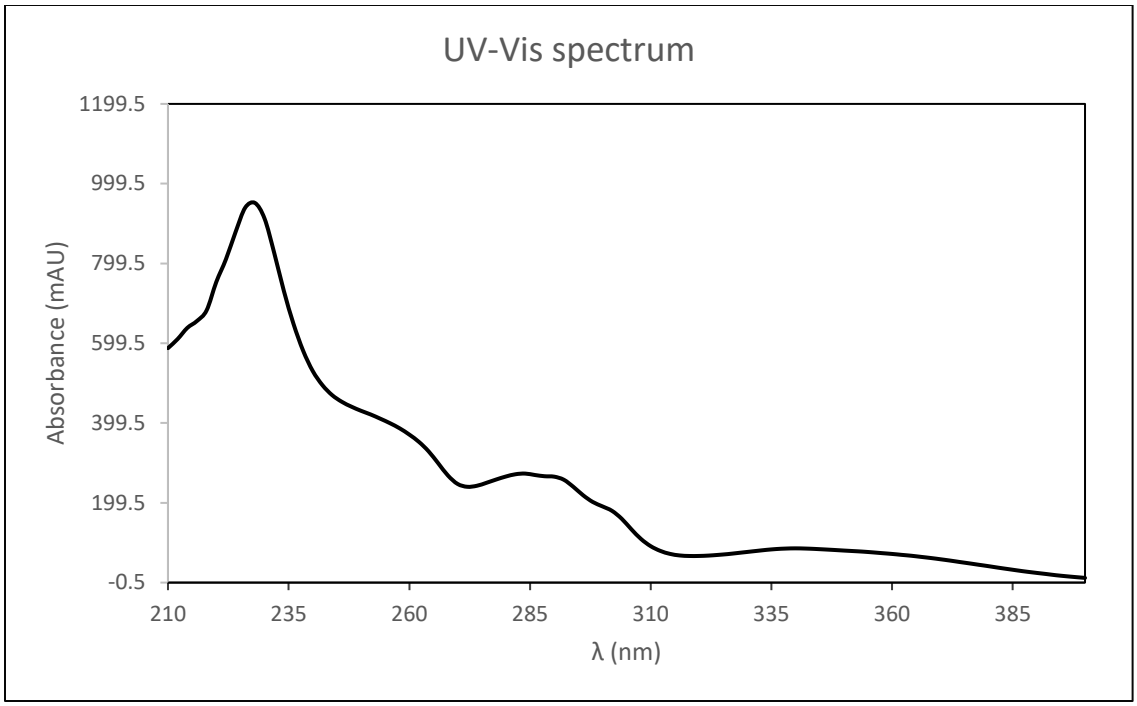


Figure *S46b*. UV-Vis spectrum, from on-line RP-HPLC (C18) detector, of D-phenylalanyl-(2-mercapto-tryptophanyl-tyrosyl-D-tryptophanyl-lysinyl-threonyl-cysteinyl)cyclic sulfide-O^{β}-(4-methyl-1-(*N*-(PEG_4-NHCO-Phe(4-Br)-NHCO-(Aoa-EDANS oxime))-[1,2,3]triazolyl)-threonine (**9**). UV-Vis spectra were taken with the RP-HPLC diode array detectors directly in the RP-HPLC flow cells at t_r = 10.39 min (40% eluent B). Analyte concentration unknown.

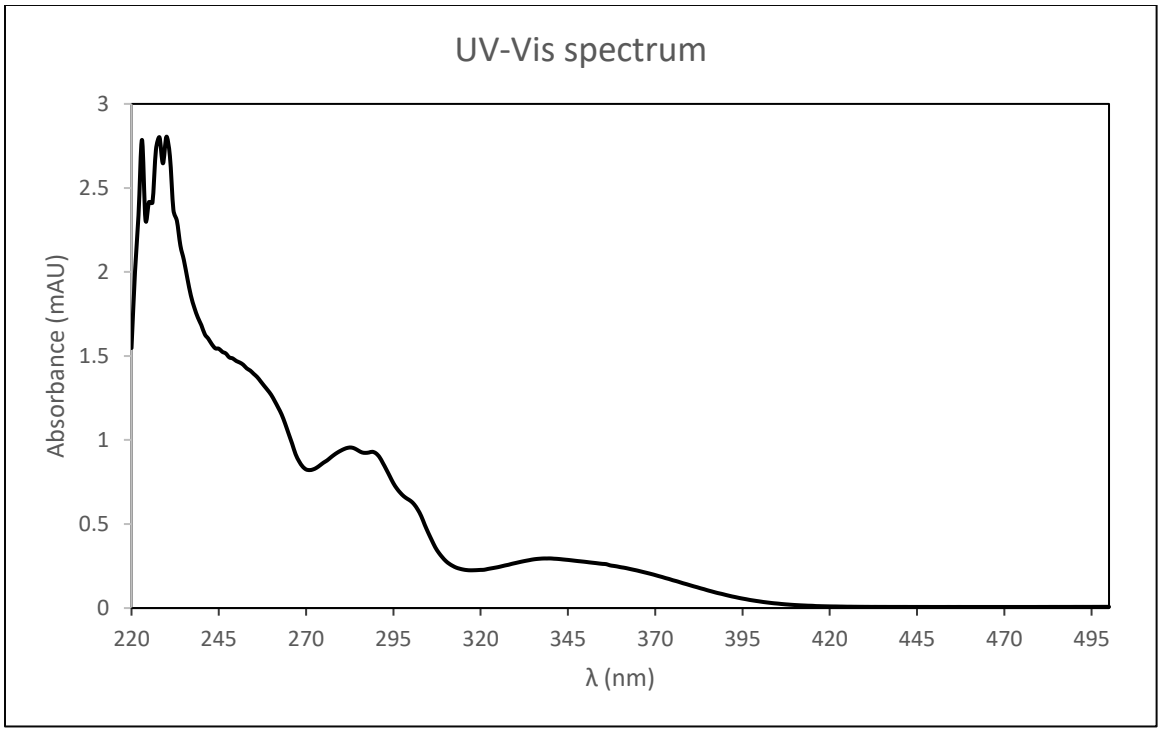


Figure *S46c*. UV-Vis spectrum, from spectrophotometer, of 62 µM D-phenylalanyl-(2-mercapto-tryptophanyl-tyrosyl-D-tryptophanyl-lysinyl-threonyl-cysteinyl)cyclic sulfide-O^{β}-(4-methyl-1-(*N*-(PEG_4- NHCO-Phe(4-Br)- NHCO-(Aoa-EDANS oxime))-[1,2,3]triazolyl)-threonine (**9**) in 0.1%FA 4:6 (*v*/*v*) MeCN/Milli-Q water.

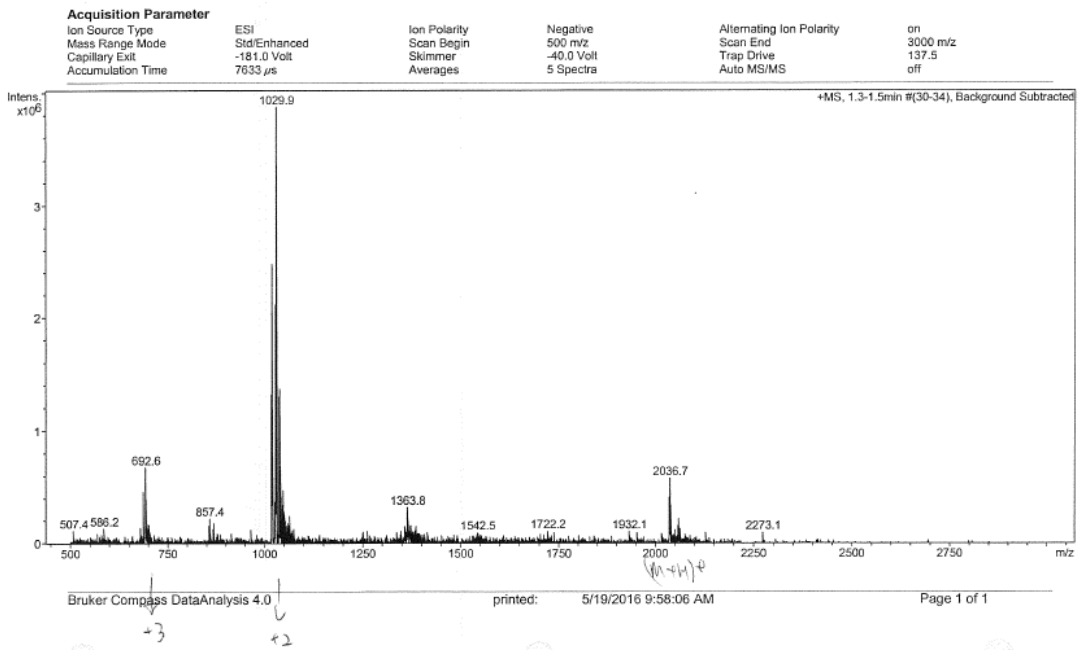


Figure *S46d*. LR-MS (ESI-High Capacity ion Trap) of D-phenylalanyl-(2-mercapto-tryptophanyl-tyrosyl-D-tryptophanyl-lysinyl-threonyl-cysteinyl)cyclic sulfide-*O*$^{\beta}$-(4-methyl-1-(*N*-(PEG$_4$- NHCO-Phe(4-Br)- NHCO-(Aoa-EDANS oxime))-[1,2,3]triazolyl)-threonine (**9**).

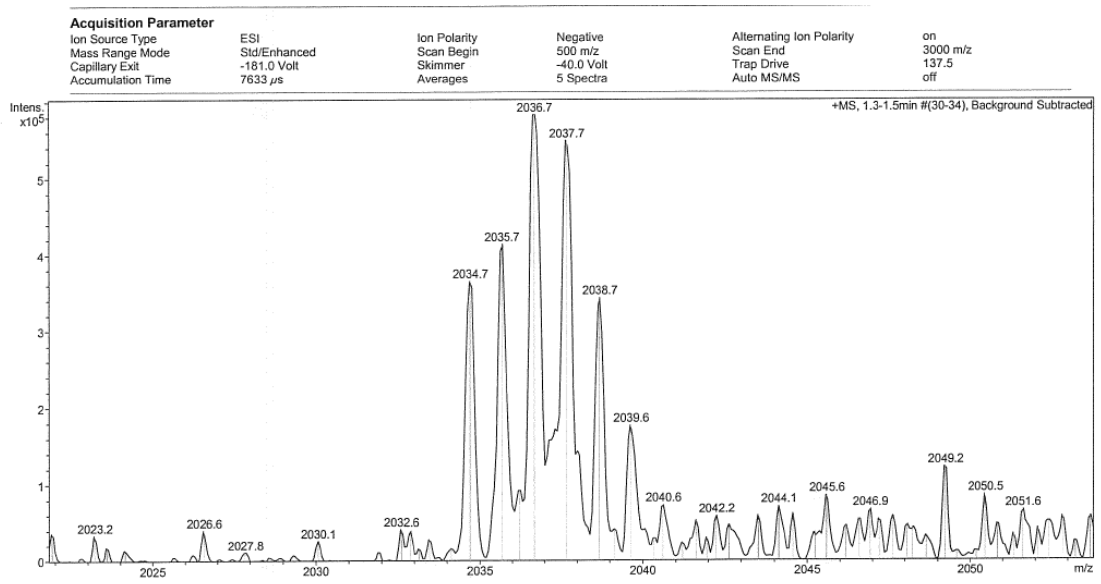


Figure *S46e*. Zoom in LR-MS (ESI-High Capacity ion Trap) of D-phenylalanyl-(2-mercapto-tryptophanyl-tyrosyl-D-tryptophanyl-lysinyl-threonyl-cysteinyl)cyclic sulfide-O^{β}-(4-methyl-1-(*N*-(PEG_4- NHCO-Phe(4-Br)- NHCO-(Aoa-EDANS oxime))-[1,2,3]triazolyl)-threonine (**9**).

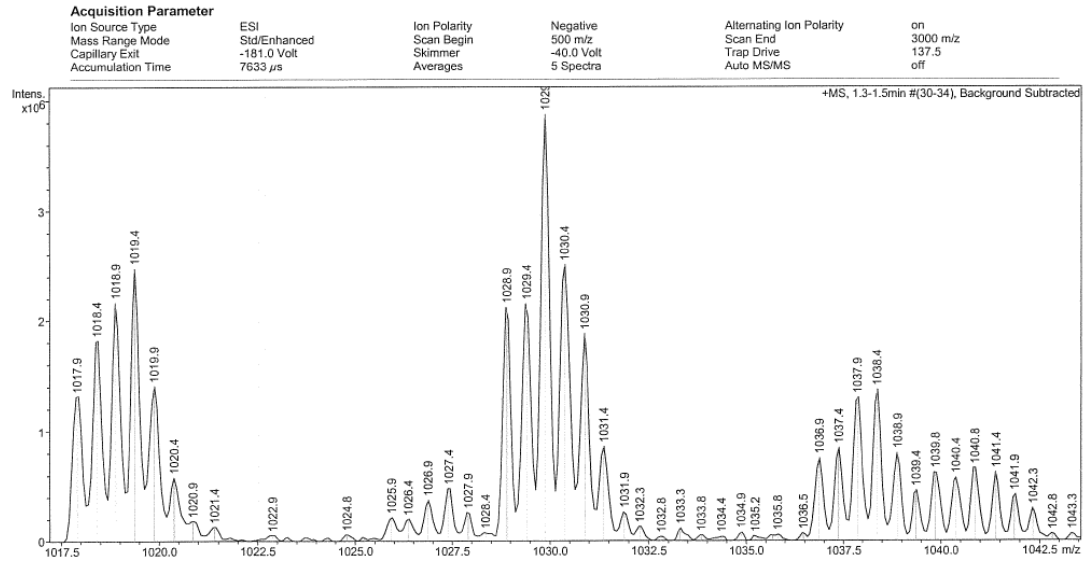


Figure *S46f*. Zoom in LR-MS (ESI-High Capacity ion Trap) of D-phenylalanyl-(2-mercapto-tryptophanyl-tyrosyl-D-tryptophanyl-lysinyl-threonyl-cysteinyl)cyclic sulfide-*O*$^{\beta}$-(4-methyl-1-(*N*-(PEG$_4$- NHCO-Phe(4-Br)- NHCO-(Aoa-EDANS oxime))-[1,2,3]triazolyl)-threonine (**9**).

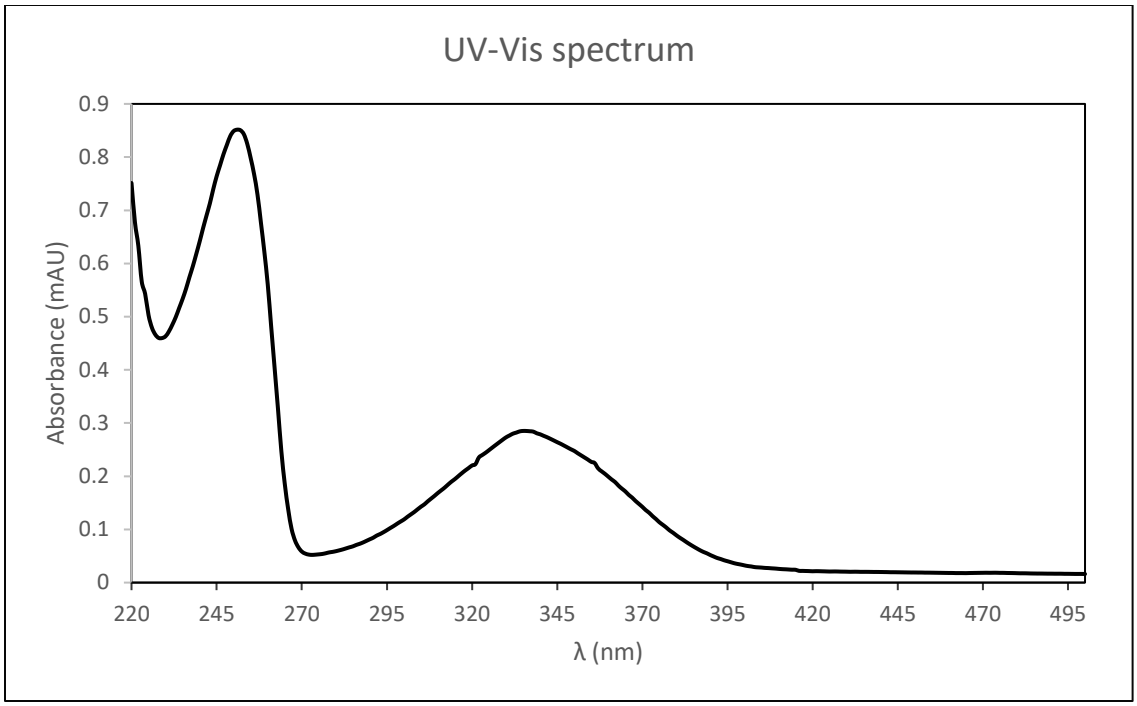


Figure *S47*. UV-Vis spectrum of 50 µM EDANS in 0.1%FA 4:6 (*v*/*v*) MeCN/Milli-Q water.

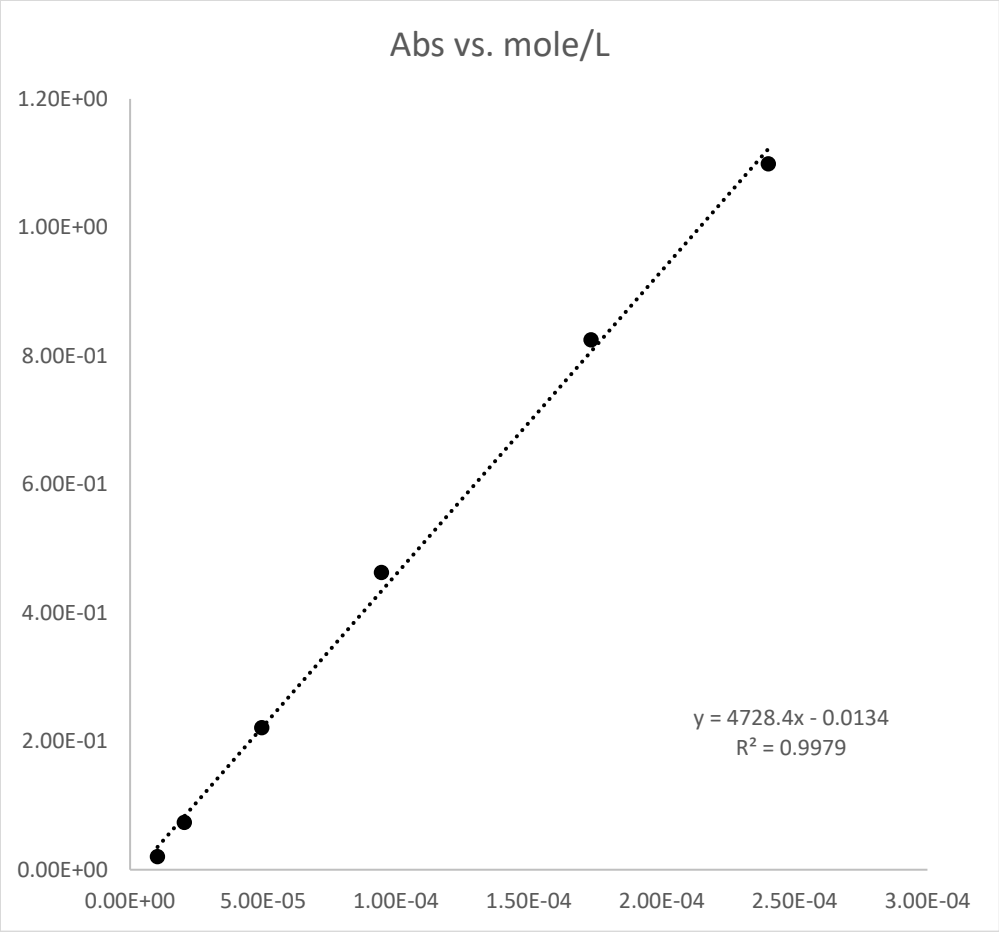
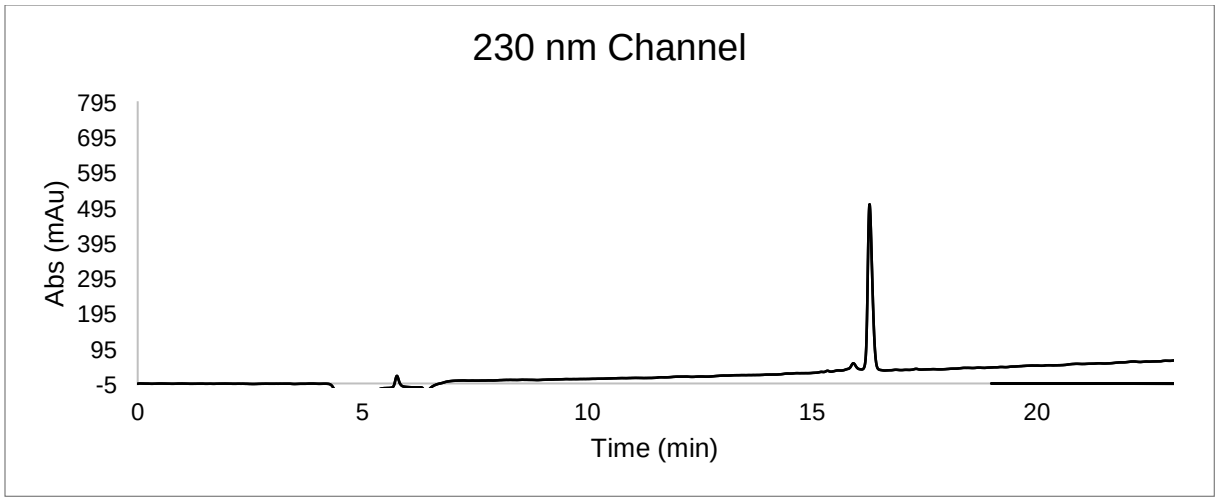
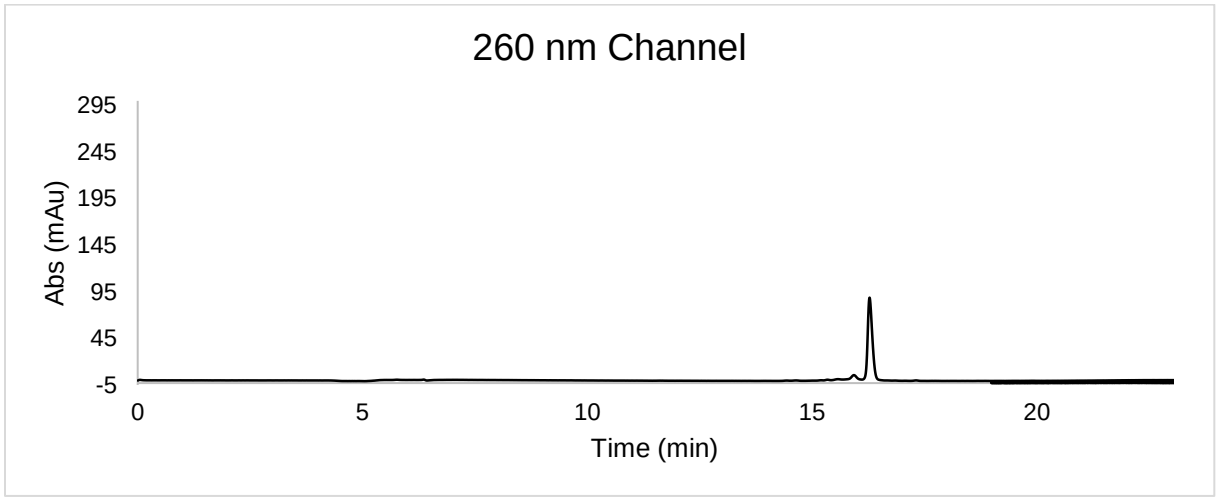


Figure *S48*. Linear regression curve of EDANS UV-Vis absorbance. A_{340} = 4728.4X - 0.0134 with R^2 = 0.998 where A_{340} is the absorbance measured at 340 nm in 0.1%FA 4:6 (*v*/*v*) MeCN/Milli-Q water. X is the corresponding molar concentration of EDANS according to Beer-Lambert law (l = 1 cm).


230 nm Channel
795
695
595
495
395
295
195
95
-5
Abs (mAu)
0
5
10
15
20
Time (min)


260 nm Channel
295
245
195
145
95
45
-5
Abs (mAu)
0
5
10
15
20
Time (min)

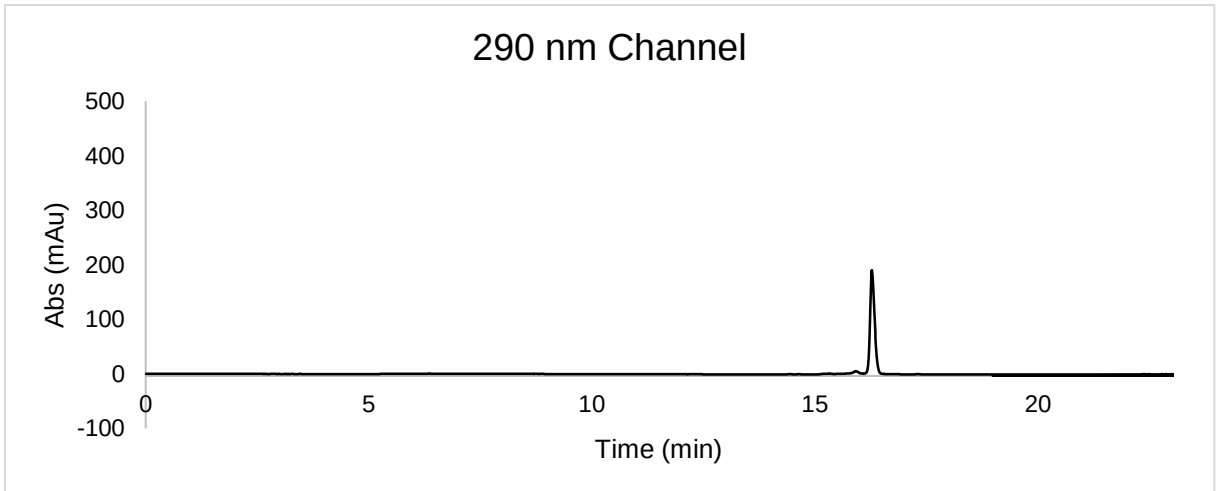

290 nm Channel
500
400
300
200
100
0
-100
Abs (mAu)
0
5
10
15
20
Time (min)

Figure *S49*. RP-HPLC (C18) chromatogram of H-D-Phe-cyclo(tryptathio)-[Trp-Tyr-D-Trp-Lys-Thr-Cys]-Thr-OH (**10**). Gradient of 10% to 40% solvent B over 19 min at 2 mL/min; t_r = 16.3 min (A_{290} 100%).

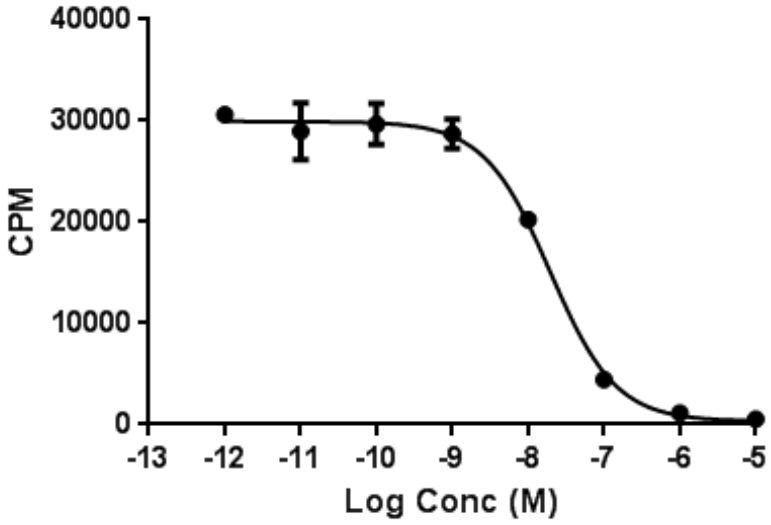


Figure *S50*. Displacement curve of [^{125}I]Tyr-somatostatin-14 by *O*-benzyloxime-(Ttn)-TATE-Cam (**6**). K_i: 18.7 nM (n=3).

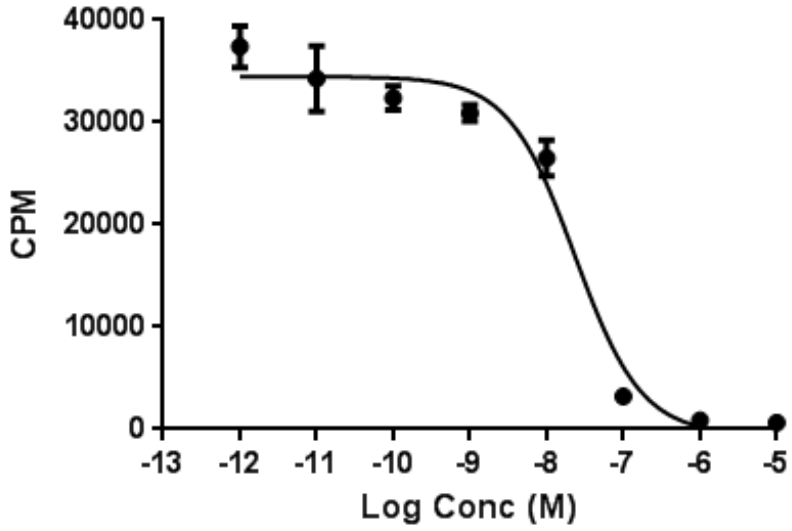


Figure *S51*. Displacement curve of [125I]Tyr-somatostatin-14 by *O*-benzyloxime-(Ttn)-TATE (**7**). K_i: 22.5 nM (n=3).

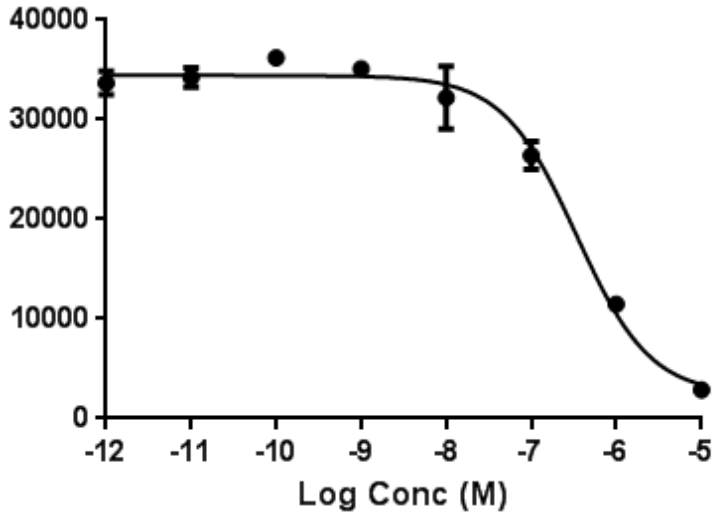


Figure *S52*. Displacement curve of [125I]Tyr-somatostatin-14 by EDANS-oxime-(Ttn[O])-TATE (**9[O]**). K_i: 327.1 nM (n=3).

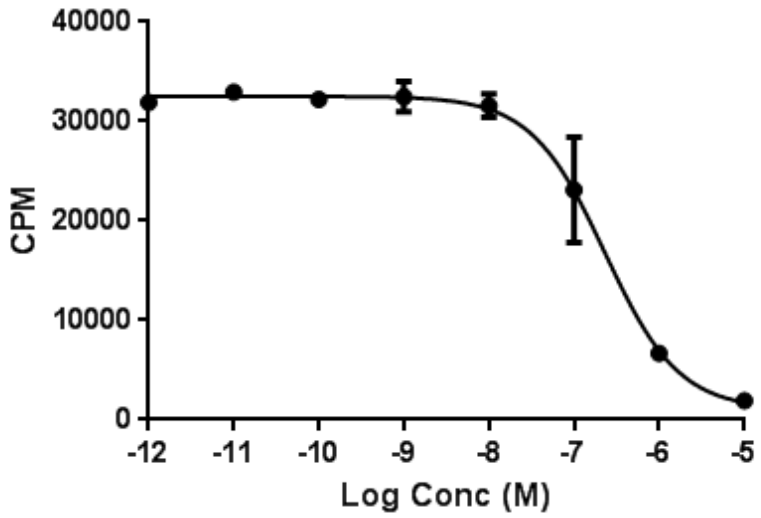


Figure *S53*. Displacement curve of [125I]Tyr-somatostatin-14 by EDANS-oxime-(Ttn)-TATE (**9**). K_i: 221.7 nM (n=3).

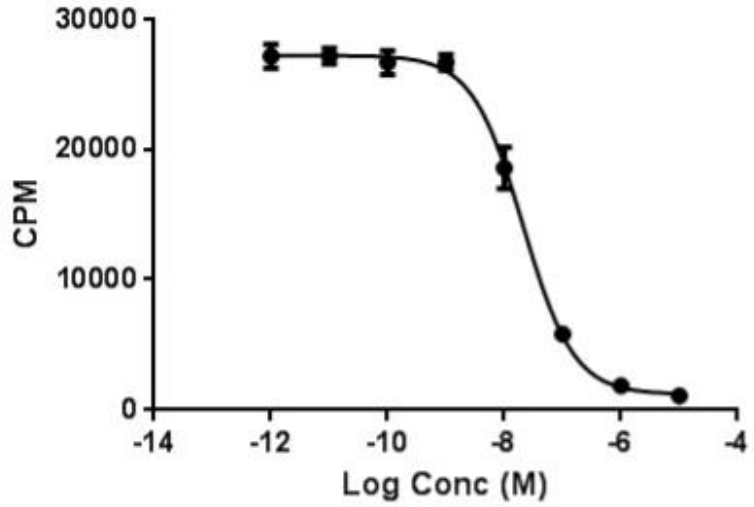


Figure *S54*. Displacement curve of [125I]Tyr-somatostatin-14 by H-D-Phe-cyclo(tryptathio)-[Trp-Tyr-D-Trp-Lys-Thr-Cys]-Thr-OH (**1**). K_i: 20 nM (n=3).

4 Bibliography

(1) Williams, D. B. G.; Lawton, M. *J. Org. Chem.* **2010**, *75* (24), 8351–8354.

(2) Ryder, D. J. *J. Label. Compd. Radiopharm.* **1991**, *29* (9), 1097–1097.

(3) Gaggini, F.; Porcheddu, A.; Reginato, G.; Rodriquez, M.; Taddei, M. *J. Comb. Chem.* **2004**, *6* (5), 805–810.

(4) Gude, M.; Ryf, J.; White, P. D. *Lett. Pept. Sci.* **2002**, *9* (4), 203–206.

(5) Várady, L.; Rajur, S. B.; Nicewonger, R. B.; Guo, M.; Ditto, L. *J. Chromatogr. A* **2000**, *869* (1), 171–179.

(6) Blanc, A.; Xia, F.; Todorovic, M.; Perrin, D. M. *Amino Acids* **2017**, *49* (2), 407–414.

(7) Matinkhoo, K.; Pryyma, A.; Todorovic, M.; Patrick, B. O.; Perrin, D. M. *J. Am. Chem. Soc.* **2018**, *140* (21), 6513–6517.

(8) Blanc, A.; Dietrich, D. J.; Perrin, D. M. *Pept. Sci.* **2018**, *111* (1), e24050.

(9) Blanc, A.; Todorovic, M.; Perrin, D. M. *Chem. Commun.* **2019**, *55* (3), 385–388.

(10) Agten, S. M.; Suylen, D. P. L.; Hackeng, T. M. *Bioconjug. Chem.* **2015**.

(11) Henseler, A. H.; Ayats, C.; Pericàs, M. A. *Adv. Synth. Catal.* **2014**, *356* (8), 1795–1802.

(12) Vrettos, E. I.; Sayyad, N.; Mavrogiannaki, E. M.; Stylos, E.; Kostagianni, A. D.; Papas, S.; Mavromoustakos, T.; Theodorou, V.; Tzakos, A. G. *RSC Adv.* **2017**, *7* (80), 50519–50526.

(13) Hernández, M. J. M.; Camañas, R. M. V.; Alvarez-Coque, M. C. G. *Microchem. J.* **1990**, *42* (3), 288–293.

(14) Haney, C. M.; Loch, M. T.; Horne, W. S. *Chem. Commun.* **2011**, *47* (39), 10915–10917.

(15) Marriott, P.; Trapp, O.; Shellie, R.; Schurig, V. *J. Chromatogr. A* **2001**, *919* (1), 115–126.

(16) Takaoka, Y.; Tsutsumi, H.; Kasagi, N.; Nakata, E.; Hamachi, I. *J. Am. Chem. Soc.* **2006**, *128* (10), 3273–3280.