

Supporting Information

for

Potent and fully non-competitive peptidomimetic inhibitor of multidrug resistance P-glycoprotein

Ophélie Arnaud^{§,∅}, Ali Koubeissi^{†,∅}, Laurent Ettouati^{†}, Raphaël Terreux[§], Ghina Alame[‡], Catherine Grenot[‡], Charles Dumontet[⊥], Attilio Di Pietro[§], Joëlle Paris[†] and Pierre Falson^{*§},*

TABLE OF CONTENTS.

Page S1: Table of contents

Page S2: General Synthetic Procedures

Page S3: Detailed Synthetic Procedures

Page S23: Figure SI 1. Typical experiment of fluorescence coupled to flux cytometry for quantifying the intracellular amount of mitoxantrone

General Synthetic Procedures.

General Methods. Solvents and reagents were of reagent grade and used without further purification. Melting points were taken on a Electrothermal 9200 apparatus or a Kofler bench and are uncorrected. Specific rotation $[\alpha]_D$ was recorded on Bellingham + Stanley Ltd ADP 220, Jasco P-1010 or Roussel-Jouan 71 polarimeters. IR spectra were determined on Perkin-Elmer Spectrum One FT-IR or Perkin-Elmer 1600 series FT-IR spectrometers (KBr or film). ^1H , ^{13}C and ^{19}F NMR spectra were measured on Bruker AC 200 (^1H 200.11 MHz; ^{13}C 50.29 MHz), Bruker ALS 300 (^1H 300.17 MHz), Bruker AM300 (^1H 300.17 MHz) or Bruker DRX 300 (^{13}C 75.44 MHz; ^{19}F 282.23 MHz) spectrometers (residual protonated solvent signal as internal reference). Elemental analysis was performed on all tested compounds by the Service Central d'Analyse, Vernaison, France and the results unless indicated in the experimental section are within $\pm 0.4\%$ of theoretical values. Electrospray mass spectra were recorded on a Thermo-Finnigan LCQ-Advantage in dichloromethane. Column chromatography was carried out with Merck silica gel 60 (40-63 Å) or SDS silica gel 60 (35-70 Å).

General procedure for peptide bond formation by chloroformate ester activation (GP-A). Acid (0.52 mmol) is dissolved in CH_2Cl_2 (5 mL) under argon atmosphere. The solution is cooled to $0\text{ }^\circ\text{C}$ and triethylamine (0.52 mmol) added dropwise over a period of 5 min. Ethyl chloroformate (50.52 mmol) is then added dropwise over a period of 5 min. The mixture is stirred at $0\text{ }^\circ\text{C}$ for 30 min, then a solution of free α -amine amino acid (0.52 mmol) in CH_2Cl_2 (4 mL) and triethylamine (0.52 mmol) at $0\text{ }^\circ\text{C}$ is added over a period of 5 min. The solution is stirred for 2 h at $0\text{ }^\circ\text{C}$ then warmed to room temperature and stirred overnight. Dichloromethane (5 mL) is added and the organic layer is washed with aqueous saturated sodium bicarbonate solution ($2 \times 10\text{ mL}$) and aqueous 1 M HCl ($2 \times 10\text{ mL}$). The organic layer is dried over anhydrous sodium sulfate, filtered and concentrated. The resulting product is purified by recrystallization or by flash chromatography on silica gel using appropriate solvent mixtures.

General procedure for one-pot double protection of amino acid (GP-B). A flask is charged with non-protected amino acid (4.2 mmol), NaOH (4.2 mmol), H_2O (5 mL) and CH_3CN (5 mL). *tert*-Butyl dicarbonate (4.62 mmol) is added and the resulting solution is stirred overnight at room temperature. The solvents are removed, and the flask contents are dried briefly under vacuum. The residue is triturated with Et_2O , dried under vacuum and dissolved in DMF (12 mL). Allyl bromide (4.62 mmol) or benzyl bromide (4.664 mmol) are added and the reaction mixture are stirred overnight at room temperature. The DMF is evaporated and the resulting residue is dissolved in EtOAc and poured into a separatory funnel. The organic layer is washed with saturated NaHCO_3 ($3 \times 25\text{ mL}$), H_2O (25 mL), 0.1 M KHSO_4 ($2 \times 25\text{ mL}$) and saturated NaCl (25 mL) and then dried over anhydrous sodium sulfate. The corresponding protected product is usually used without further purification.

General procedure for allyl deprotection of allyl esters (GP-C). A flask is charged with allyl ester (0.613 mmol) and CH_2Cl_2 (5 mL). Triphenylphosphine (0.030 mmol) and pyrrolidine (0.643 mmol) are added and the flask is flushed with argon. Tetrakis(triphenylphosphine)palladium(0) (0.0153 mmol) is then added and the mixture was stirred for 40 min. The solvents are removed by rotary evaporation and the residue is dissolved in EtOAc and poured into a separatory funnel. The organic layer is washed three times with 0.1 M KHSO_4 then with saturated NaCl, dried over anhydrous sodium sulfate, filtered and concentrated. The resulting acid compound is purified by flash chromatography on silica gel using appropriate solvent mixtures.

General procedure for esterification of alcohol derivatives (GP-D). To a solution of alcohol (1.162 mmol) in CH_2Cl_2 (10 mL) is added triethylamine (2.324 mmol) and benzyl or cyclohexanecarbonyl chloride (1.975 mmol) at $0\text{ }^\circ\text{C}$. After stirring overnight at room temperature, the mixture is poured into water (15 mL). The product is extracted with CH_2Cl_2 ($2 \times 20\text{ mL}$) and the organic layer is washed with water, dried over anhydrous sodium sulfate, filtered and concentrated as a yellow oil. The resulting crude cyclohexyl ester is purified by flash chromatography on silica gel using appropriate solvent mixtures.

General procedure for preparation of amino acid-derived aldehyde (GP-E). Lithium aluminum hydride (1.25 mmol) is added at $0\text{ }^\circ\text{C}$ to a stirred solution of the Weinreb amide (1 mmol) in Et_2O (6 mL). The mixture was stirred for 30 min at $0\text{ }^\circ\text{C}$ then hydrolyzed with 0.3 M KHSO_4 solution (5 mL).

The organic layer was separated and the aqueous phase was extracted three times with Et₂O. The organic phases were combined, washed three times successively with 3 M HCl solution, saturated sodium bicarbonate solution and brine, dried over anhydrous sodium sulfate, filtered and concentrated. The aldehyde product is purified by flash chromatography on silica gel using appropriate solvent mixtures.

General procedure for synthesis of aminomethylene dipeptide analogues (GP-F). The amino acid derived aldehyde (1.3 mmol) is dissolved in a mixture of methanol-acetic acid (99:1; 10 mL) containing the free α -amine amino acid (0.78 mmol). Sodium cyanoborohydride (1.04 mmol) is added portionwise during 45 min. After 24 h, the reaction mixture is cooled in an ice water bath, and a saturated sodium bicarbonate solution (60 mL) is added under stirring, followed by ethyl acetate (80 mL). The organic layer is collected, washed with water (40 mL), dried over anhydrous sodium sulfate, and concentrated in vacuo. The aminomethylene dipeptide is purified by flash chromatography on silica gel using appropriate solvent mixtures.

General procedure for preparation of acid derivatives from alkene compounds (GP-G). To a mixture of alkene derivative (0.244 mmol) in CH₃CN (4 mL) and NaIO₄ (1.708 mmol) in water (4 mL) is added at 0 °C RuCl₃·xH₂O (5 mg, 0.022 mmol). After stirring for 1 h at room temperature, water (10 mL) and Et₂O (10 mL) are added and the phases separated. The aqueous phase is re-extracted initially with Et₂O (15 mL) and then with EtOAc (15 mL). The combined organic phases are washed with water (25 mL), dried over anhydrous sodium sulfate, filtered and concentrated. The corresponding acid product is usually used without further purification or purified by flash chromatography on silica gel using appropriate solvent mixtures.

General procedure for synthesis of pyrrolidinyl esters (GP-H). A mixture of acid (10 mmol), *N*-hydroxysuccinimide (11 mmol), DMAP (1.1 mmol) and DCC (11 mmol) are dissolved in THF (50 mL) at 0 °C under nitrogen atmosphere. The reaction mixture is allowed to warm to room temperature and stirred for 48 h. The reaction mixture can be stored in the freezer overnight. After the precipitate is filtered out, the solvent is removed under reduced pressure. The pyrrolidinyl ester is purified by column chromatography on silica gel using appropriate solvent mixtures.

General procedure for peptide bond formation through pyrrolidinyl esters (GP-I). To a solution of pyrrolidinyl ester (2.538 mmol) and free α -amine amino acid (3.807 mmol) in DMF (60 mL), is added ethyl diisopropylamine (10.152 mmol). The reaction mixture is stirred overnight under argon atmosphere. Water (60 mL) and 1 M HCl (60 mL) are added. The mixture is then extracted with EtOAc (3×60 mL). The combined organic layers are washed with saturated solution of Na₂CO₃ (2×80 mL), 1 M HCl (2×80 mL), water (80 mL), brine (80 mL), dried over anhydrous Na₂SO₄, filtered and concentrated. The resulting product is usually purified by column chromatography on silica gel using appropriate solvent mixtures.

General procedure for benzylation of *N*^α-Boc protected amino-acid (GP-J). Commercial *N*^α-Boc protected amino acid (1.518 mmol) is dissolved in MeOH (9 mL), and H₂O (0.9 mL) is added. Cesium carbonate (20% aqueous solution) is added dropwise until pH ~8 (pH paper). After evaporation to dryness under reduced pressure, DMF (5 mL) is added and the solvent is removed under reduced pressure (water bath kept at 45 °C). DMF (5 mL) is added once more and removed under reduced pressure to afford a semisolid white material. DMF (5 mL) and benzyl bromide (1.745 mmol) are added, and the solution is stirred at room temperature for 4 h. Solvent is removed under reduced pressure and H₂O (20 mL) is added. The aqueous solution is extracted twice with EtOAc, and the combined organic extracts are dried over anhydrous sodium sulfate, filtered and concentrated. The resulting product is purified by recrystallization or by flash chromatography on silica gel using appropriate solvent mixtures.

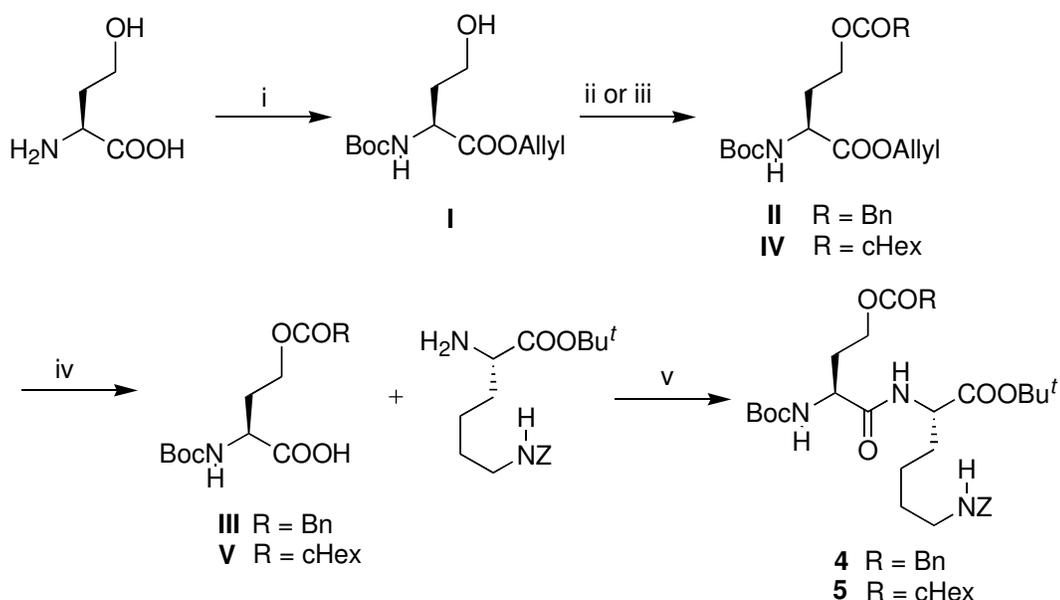
Detailed Synthetic Procedures

***N*^α-Boc-L-Asp(OBn)-L-Lys(Z)-OtBu (1).** According to the general procedure GP-A, **1** was obtained from commercial *N*^α-Boc-Asp(OBn)-OH and H-Lys(Z)-OtBu.HCl in 47% yield as a brown oil after purification by flash chromatography on silica gel (petroleum ether:ethyl acetate 7:3). [α]_D²⁷ +6.0 (*c* 1.00, CH₂Cl₂); IR (Film, cm⁻¹): 3336 (ν_{NH}), 3034 (ν_{CH}), 2978 (ν_{CH}), 1728 ($\nu_{\text{C=O}}$ ester), 1456 ($\nu_{\text{C=C}}$), 1368 (δ_{CH_3} *t*-Bu), 1247 ($\nu_{\text{C-C-O}}$ asym), 1160 ($\nu_{\text{O-C-C}}$ asym); ¹H NMR (300 MHz, CDCl₃, δ): 1.47 (s,

2C(CH₃)₃ Boc and *t*-Bu, 18H), 1.25-1.91 (m, 3CH₂ Lys, 6H), 2.89 (m, CH_{2β}, 2H), 3.18 (q, *J* = 6.3 Hz, CH₂NH, 2H), 4.44 (m, CH_α, 1H), 4.54 (m, CH_α, 1H), 5.10 (m, 2CH₂Ph, 4H), 5.64 (br, NH, 1H), 7.00 (m, NH, 1H), 7.35 (s, 2ArH, 10H); ¹³C NMR (CDCl₃, 75 MHz): 22.2, 22.4 (2C), 28.2, 28.3, 26.6 (6C), 29.5 (C), 32.4 (C), 40.9 (C), 52.9 (C), 53.1 (C), 67.1, 67.2 (2C), 81.0 (C), 82.5 (C), 128.4, 128.5, 128.6, 128.7, 128.8, 128.9, 129.0 (10C), 135.8 (C), 137.0 (C), 155.9, 156.8 (2C), 170.7, 171.3, 171.6 (3C); MS-ESI (*m/z*): 664.2 [M + Na]⁺; HRMS-ESI (*m/z*): [M + Na]⁺ calcd for C₃₄H₄₇N₃O₉Na, 664.3210; found, 664.32162.

***N*^α-Boc-L-Glu(OBn)-L-Lys(Z)-OtBu (2).** According to the general procedure GP-A, **2** was obtained from commercial *N*^α-Boc-Glu(OBn)-OH and H-Lys(Z)-OtBu.HCl in 45% yield as a white solid after purification by flash chromatography on silica gel (petroleum ether:ethyl acetate 7:3). Mp 80-82°C (lit. 79-81°C / ethanol + water); [α]_D²⁵ -3.0 (*c* 1.00, CH₂Cl₂); IR (KBr, cm⁻¹): 3325 (ν_{NH}); 3066 3034 (ν_{φCH}), 2978 2935 (ν_{CH}), 1729 (ν_{C=O} ester), 1456 (ν_{φC=C}), 1392 1368 (δ_{CH₃ *t*-Bu}), 1248 (ν_{C-C-O} asym), 1160 (ν_{O-C-C} asym); ¹H NMR (300 MHz, CDCl₃, δ): 1.43, 1.46 (2s, 2C(CH₃)₃ Boc and *t*-Bu, 18H), 1.32-1.84 (m, 3CH₂ Lys, 6H), 2.04 (m, CH_{2β} Glu, 2H), 2.50 (q, *J* = 6.5 Hz, CH_{2γ} Glu, 2H), 3.20 (q, *J* = 6.2 Hz, CH₂NH, 2H), 4.19 (m, CH_α, 1H), 4.44 (m, CH_α, 1H), 5.03 (br, NH, 1H), 5.11 (br, NH, 1H), 5.14 (s, CH₂Ph, 2H), 5.32 (s, CH₂Ph, 2H), 6.76 (d, *J* = 7.1 Hz, NH, 1H), 7.35 (s, 2ArH, 10H); ¹³C NMR (CDCl₃, 75 MHz): 22.5, 23.0 (2C), 28.4, 28.7 (6C), 29.5 (C), 30.8 (C), 32.3 (C), 40.9 (C), 52.9 (C), 53.9 (C), 66.9, 67.0 (2C), 80.4 (C), 82.5 (C), 128.5, 128.6, 128.7, 128.9, 129.0 (10C), 136.1 (C), 136.9 (C), 156.1, 156.9 (2C), 171.4, 171.8, 173.5 (3C); MS-ESI (*m/z*): 1333.1 [2M + Na]⁺, 678.2 [M + Na]⁺; HRMS-ESI (*m/z*): [M + Na]⁺ calcd for C₃₅H₄₉N₃O₉Na, 678.3367; found, 678.33598.

***N*^α-Boc-L-Glu(OtBu)-L-Lys(Z)-OtBu (3).** According to the general procedure GP-A, **3** was obtained from commercial *N*^α-Boc-Glu(OtBu)-OH and H-Lys(Z)-OtBu.HCl in 80% yield as a colorless oil after purification by flash chromatography on silica gel (petroleum ether:ethyl acetate 6:4). [α]_D²⁵ -9.0 (*c* 0.14, CH₂Cl₂); IR (Film, cm⁻¹): 3325 (ν_{NH}), 3067 3035 (ν_{φCH}), 2978 2933 (ν_{CH}), 1725 (ν_{C=O}), 1455 (ν_{φC=C}), 1392 1368 (δ_{CH₃ *t*-Bu}), 1250 (ν_{C-C-O} asym), 1156 (ν_{O-C-C} asym); ¹H NMR (300 MHz, CDCl₃, δ): 1.44, 1.46, 1.47 (s, 3C(CH₃)₃ Boc and 2*t*-Bu, 27H), 1.20-2.20 (m, 3CH₂ Lys and CH_{2β} Glu, 8H), 2.35 (m, CH_{2γ} Glu, 2H), 3.19 (q, *J* = 6.3 Hz, CH₂NH, 2H), 4.13 (m, CH_α, 1H), 4.44 (m, CH_α, 1H), 5.00 (br, NH, 1H), 5.11 (s, CH₂Ph, 2H), 5.30 (br, NH, 1H), 6.80 (d, *J* = 8.3 Hz, NH, 1H), 7.36 (s, ArH, 5H); ¹³C NMR (CDCl₃, 75 MHz): 22.5 (C), 28.6 (9C), 29.5, 30.0 (2C), 32.1, 32.3 (2C), 40.9 (C), 52.8 (C), 54.3 (C), 66.9 (C), 80.3 (C), 81.2 (C), 82.4 (C), 128.4, 128.5, 128.8 (5C), 137.0 (C), 156.1, 156.9 (2C), 171.4, 171.9, 173.0 (3C); MS-ESI (*m/z*): 1265.2 [2M + Na]⁺, 644.3 [M + Na]⁺; HRMS-ESI (*m/z*): [M + Na]⁺ calcd for C₃₂H₅₁N₃O₉Na, 644.3523; found, 644.35269.



Scheme 1. Synthesis of compounds 4 and 5. Reagents and Conditions. (i) a. $(\text{Boc})_2\text{O}$, NaOH, $\text{CH}_3\text{CN}/\text{H}_2\text{O}$, 24 h, RT. b. allyl bromide, DMF, 24 h, RT, 78%. (ii) PhCH_2COOH , DCC, DMAP, CH_2Cl_2 , 19 h, RT, 20%. (iii) cHexCOCl , Et_3N , 24 h, RT, 90%. (iv) $\text{Pd}(\text{Ph}_3)_4$, PPh_3 , CH_2Cl_2 , pyrrolidine, 40 min, RT, quant. yield for **III**, 70% for **V**. (v) a. ethyl chloroformate, Et_3N , CH_2Cl_2 , 30 min, 0 °C. b. H-Lys(Z)-OtBu.HCl, Et_3N , CH_2Cl_2 , 0 °C, 2 h – RT, 24 h, 88% for **4**, 77% for **5**.

(2S)-tert-Butoxycarbonylamino-4-hydroxy-butanoic acid allyl ester (I). According to the general procedure GP-B, **I** was obtained from commercial L-homoserine in 78% yield as a colorless viscous oil without further purification. IR (Film, cm^{-1}): 3382 (ν_{OH} and ν_{NH}), 2978 2935 (ν_{CH}), 1713 ($\nu_{\text{C=O}}$), 1454 ($\nu_{\text{C=C}}$), 1393 1368 (δ_{CH_3} *t*-Bu), 1252 ($\nu_{\text{C-C-O}}$ *asym*), 1167 ($\nu_{\text{O-C-C}}$ *asym*), 935 (δ_{CH} vinyl); ^1H NMR (300 MHz, CDCl_3 , δ): 1.47 (s, $\text{C}(\text{CH}_3)_3$ Boc, 9H), 1.65 (m, CH_β , 1H), 2.18 (m, CH_β , 1H), 3.70 (m, CH_2OH , 2H), 4.53 (m, CH_α , 1H), 4.67 (dd, $J = 1.1$ Hz and 5.6 Hz, $\text{CH}_2-\text{CH}=\text{CH}_2$, 2H), 5.28 (dd, $J = 1.3$ Hz and 9.0 Hz, $\text{CH}_2-\text{CH}=\text{CH}_2$, 1H), 5.35 (dd, $J = 1.5$ Hz and 15.8 Hz, $\text{CH}_2-\text{CH}=\text{CH}_2$, 1H), 5.41 (br, NH, 1H), 5.92 (m, $\text{CH}_2-\text{CH}=\text{CH}_2$, 1H).

(2S)-tert-Butoxycarbonylamino-4-phenylacetoxy-butanoic acid allyl ester (II). 174 mg of phenyl acetic acid (1.08 mmol) were dissolved in a minimal volume of CH_2Cl_2 containing *N* $^\alpha$ -Boc-Hse-OAllyl **I** (3.27 mmol, 849 mg). DCC (0.54 mmol) was then added followed 15 min later by DMAP (7 mg, 0.054 mmol) and the reaction kept under stirring for 3 h. DCC (110 mg, 0.54 mmol) was again added and the reaction was left under stirring for 16 h at room temperature. DCU was removed by filtration and the solvent evaporated. The crude product was dissolved in AcOEt and then washed three times with saturated 0.1 M HCl (25 mL), saturated NaHCO_3 (25 mL) and brine (25 mL). The organic layer was dried over anhydrous sodium sulfate, filtrated and concentrated. The resulting yellow oily residue was purified by flash chromatography on silica gel (petroleum ether:ethyl acetate 9:1) to yield 250 mg of **II** (20% yield) as a yellow oil. IR (Film, cm^{-1}): 3372 (ν_{NH}), 3065 3032 (ν_{CH}), 2978 2933 (ν_{CH}), 1739 1733 1714 ($\nu_{\text{C=O}}$), 1456 ($\nu_{\text{C=C}}$), 1392 1367 (δ_{CH_3} *t*-Bu), 1250 ($\nu_{\text{C-C-O}}$ *asym*), 1161 ($\nu_{\text{O-C-C}}$ *asym*), 935 (δ_{CH} vinyl); ^1H NMR (300 MHz, CDCl_3 , δ): 1.37 (s, $\text{C}(\text{CH}_3)_3$ Boc, 9H), 1.94 (m, CH_β , 1H), 2.12 (m, CH_β , 1H), 3.54 (s, CH_2Ph , 2H), 4.09 (m, $\text{CH}_{2\gamma}$, 2H), 4.36 (m, CH_α , 1H), 4.54 (t, $J = 1.5$ Hz, $\text{CH}_2-\text{CH}=\text{CH}_2$, 1H), 4.56 (t, $J = 1.3$ Hz, $\text{CH}_2-\text{CH}=\text{CH}_2$, 1H), 5.03 (d, $J = 7.9$ Hz, NH, 1H), 5.18 (dd, $J = 1.1$ Hz and 9.4 Hz, $\text{CH}_2-\text{CH}=\text{CH}_2$, 1H), 5.25 (dd, $J = 1.5$ Hz and 16.5 Hz, $\text{CH}_2-\text{CH}=\text{CH}_2$, 1H), 5.82 (m, $\text{CH}_2-\text{CH}=\text{CH}_2$, 1H), 7.24 (m, ArH, 5H).

(2S)-tert-Butoxycarbonylamino-4-phenylacetoxy-butanoic acid (III). According to the general procedure GP-C, **III** was obtained from **II** in quantitative yield as a yellow oil after purification by flash chromatography on silica gel (initially with petroleum ether:ethyl acetate 5:5 then with the same eluent +

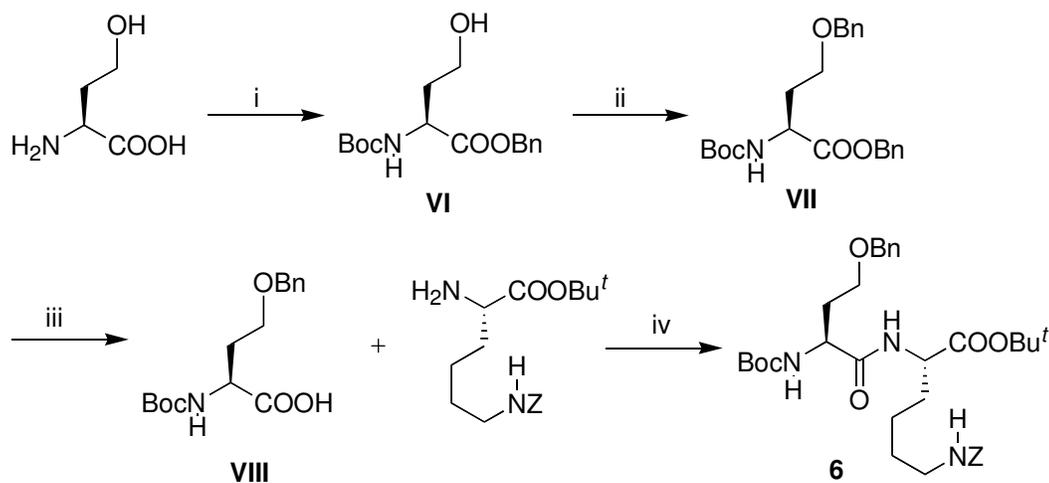
0.1% formic acid). IR (Film, cm^{-1}): 3379 (ν_{COOH} and ν_{NH}), 3033 (ν_{CH}), 2934 (ν_{CH}), 1716 ($\nu_{\text{C=O}}$), 1455 ($\nu_{\text{C=C}}$), 1394 1370 (δ_{CH_3} *t*-Bu), 1250 ($\nu_{\text{C-C-O}}$ asym), 1163 ($\nu_{\text{O-C-C}}$ asym); ^1H NMR (300 MHz, CDCl_3 , δ): 1.46 (s, $\text{C}(\text{CH}_3)_3$ Boc, 9H), 2.06 (m, CH_β , 1H), 2.23 (m, CH_β , 1H), 3.63 (s, CH_2Ph , 2H), 4.23 (m, $\text{CH}_{2\gamma}$, 2H), 4.43 (m, CH_α , 1H), 5.14 (d, $J = 7.5$ Hz, *NH*, 1H), 7.31 (m, ArH, 5H), 7.00-7.70 (br, *COOH*, 1H).

***N* ^{α} -Boc-L-Hse(COBn)-L-Lys(Z)-OtBu (4).** According to the general procedure GP-A, **4** was obtained from **III** and commercial H-Lys(Z)-OtBu.HCl in 88% yield as a colorless oil after purification by flash chromatography on silica gel (petroleum ether:ethyl acetate 6:4). $[\alpha]_D^{25}$ -3.0 (*c* 1.00, CH_2Cl_2); IR (Film, cm^{-1}): 3334 (ν_{NH}), 3066 3033 (ν_{CH}), 2978 2935 (ν_{CH}), 1732 ($\nu_{\text{C=O}}$ ester), 1667 ($\nu_{\text{C=O}}$ amide), 1456 ($\nu_{\text{C=C}}$), 1393 1369 (δ_{CH_3} *t*-Bu), 1249 ($\nu_{\text{C-C-O}}$ asym), 1160 ($\nu_{\text{O-C-C}}$ asym); ^1H NMR (300 MHz, CDCl_3 , δ): 1.45, 1.46 (2s, $2\text{C}(\text{CH}_3)_3$ Boc and *t*-Bu, 18H), 1.32-1.87 (m, 3CH_2 Lys, 6H), 1.94 (m, CH_β , 1H), 2.10 (m, CH_β , 1H), 3.18 (q, $J = 6.3$ Hz, CH_2NH , 2H), 3.65 (s, CH_2Ph OCOBn, 2H), 4.16 (m, $\text{CH}_{2\gamma}$ Hse, 2H), 4.44 (m, CH_α , 1H), 5.03 (br, *NH*, 1H), 5.11 (s, CH_2Ph Z, 2H), 6.74 (d, $J = 7.9$ Hz, *NH*, 1H), 7.28-7.37 (m, 2ArH, 10H); ^{13}C NMR (CDCl_3 , 75 MHz): 22.3 (C), 28.4, 28.7 (6C), 29.4, 31.6 (2C), 32.3 (C), 40.8 (C), 41.6 (C), 52.1 (C), 52.7 (C), 61.8 (C), 67.0 (C), 80.6 (C), 82.6 (C), 127.5, 128.5, 128.6, 128.9, 129.0, 129.7 (10C), 134.2 (C), 136.9 (C), 155.9, 156.9 (2C), 171.4, 171.5, 172.0 (3C); MS-ESI (*m/z*): 1333.0 $[\text{2M} + \text{Na}]^+$, 678.2 $[\text{M} + \text{Na}]^+$; HRMS-ESI (*m/z*): $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{35}\text{H}_{49}\text{N}_3\text{O}_9\text{Na}$, 678.3366; found, 678.33618.

(2*S*)-tert-Butoxycarbonylamino-4-cyclohexylcarbonyloxy-butanoic acid allyl ester (IV). According to the general procedure GP-D, **IV** was obtained from **I** in 90% yield as a yellow oil after purification by flash chromatography on silica gel (petroleum ether:ethyl acetate 9:1). IR (Film, cm^{-1}): 3369 (ν_{NH}), 2977 2934 (ν_{CH}), 1733 1716 ($\nu_{\text{C=O}}$), 1392 1368 (δ_{CH_3} *t*-Bu), 1248 ($\nu_{\text{C-C-O}}$ asym), 1166 ($\nu_{\text{O-C-C}}$ asym), 932 (δ_{CH} vinyl); ^1H NMR (300 MHz, CDCl_3 , δ): 1.19-1.37 (m, 2CH_2 cHex, 4H), 1.45 (s, $\text{C}(\text{CH}_3)_3$ Boc, 9H), 1.60-1.95 (m, 3CH_2 cHex, 6H), 2.04 (m, CH_β , 1H), 2.15-2.36 (m, CHCO cHex and CH_β , 2H), 4.15 (m, $\text{CH}_{2\gamma}$, 2H), 4.45 (m, CH_α , 1H), 4.65 (t, $J = 1.1$ Hz, $\text{CH}_2\text{-CH=CH}_2$, 1H), 4.66 (t, $J = 1.5$ Hz, $\text{CH}_2\text{-CH=CH}_2$, 2H), 5.17 (d, $J = 8.6$ Hz, *NH*, 1H), 5.28 (dd, $J = 1.3$ Hz and 9.0 Hz, $\text{CH}_2\text{-CH=CH}_2$, 1H), 5.36 (dd, $J = 1.5$ Hz and 15.8 Hz, $\text{CH}_2\text{-CH=CH}_2$, 1H), 5.92 (m, $\text{CH}_2\text{-CH=CH}_2$, 1H).

(2*S*)-tert-Butoxycarbonylamino-4-cyclohexylcarbonyloxy-butanoic acid (V). According to the general procedure GP-C, **V** was obtained from **IV** in 70% yield as a brown solid after purification by flash chromatography on silica gel (initially with petroleum ether:ethyl acetate 5:5 then with the same eluent + 0.1% formic acid). Mp 143-145°C; IR (KBr, cm^{-1}): 3386 3221 (ν_{COOH} and ν_{NH}), 2951 2928 (ν_{CH}), 1754 1722 1686 ($\nu_{\text{C=O}}$), 1394 1370 (δ_{CH_3} *t*-Bu), 1255 ($\nu_{\text{C-C-O}}$ asym), 1163 ($\nu_{\text{O-C-C}}$ asym); ^1H NMR (300 MHz, CDCl_3 , δ): 1.15-1.30 (m, 2CH_2 cHex, 4H), 1.46 (s, $\text{C}(\text{CH}_3)_3$ Boc, 9H), 1.60-1.95 (m, 3CH_2 cHex, 6H), 2.08 (m, CH_β , 1H), 2.19-2.35 (m, CHCO cHex and CH_β , 2H), 4.20 (m, $\text{CH}_{2\gamma}$, 2H), 4.42 (m, CH_α , 1H), 5.21 (d, $J = 8.2$ Hz, *NH*, 1H), 6.70-7.60 (br, *COOH*, 1H).

***N* ^{α} -Boc-L-Hse(COcHex)-L-Lys(Z)-OtBu (5).** According to the general procedure GP-A, **5** was obtained from **V** and commercial H-Lys(Z)-OtBu.HCl in 77% yield as viscous yellow oil after purification by flash chromatography on silica gel (petroleum ether:ethyl acetate 6:4). $[\alpha]_D^{26}$ -6.0 (*c* 1.00, CH_2Cl_2); IR (Film, cm^{-1}): 3325 (ν_{NH}) 3066 3034 (ν_{CH}), 2933 (ν_{CH}), 1731 ($\nu_{\text{C=O}}$ ester), 1454 ($\nu_{\text{C=C}}$), 1393 1368 (δ_{CH_3} *t*-Bu), 1248 ($\nu_{\text{C-C-O}}$ asym), 1161 ($\nu_{\text{O-C-C}}$ asym); ^1H NMR (300 MHz, CDCl_3 , δ): 1.44, 1.46 (s, $2\text{C}(\text{CH}_3)_3$ Boc and *t*-Bu, 18H), 1.20-2.20 (m, 3CH_2 Lys, 5CH_2 cHex and $\text{CH}_{2\beta}$ Hse, 18H), 2.31 (m, CHCO cHex, 1H), 3.19 (q, $J = 6.2$ Hz, CH_2NH , 2H), 4.05-4.23 (m, CH_α and $\text{CH}_{2\gamma}$ Hse, 3H), 4.45 (m, CH_α , 1H), 5.02 (br, *NH*, 1H), 5.10 (s, CH_2Ph , 2H), 5.25 (br, *NH*, 1H), 6.85 (d, $J = 7.9$ Hz, *NH*, 1H), 7.36 (s, ArH, 5H); ^{13}C NMR (CDCl_3 , 75 MHz): 22.3 (C), 25.8, 26.1 (3C), 28.4, 28.6 (6C), 29.3, 29.4 (2C), 31.5 (2C), 32.4 (C), 40.9 (C), 43.5 (C), 52.4 (C), 52.8 (C), 61.3 (C), 67.0 (C), 80.5 (C), 82.5 (C), 128.5, 128.6, 128.9 (5C), 136.9 (C), 155.9, 156.9 (2C), 171.5, 171.6, 176.5 (3C); MS-ESI (*m/z*): 1317.1 $[\text{2M} + \text{Na}]^+$, 670.3 $[\text{M} + \text{Na}]^+$; HRMS-ESI (*m/z*): $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{34}\text{H}_{53}\text{N}_3\text{O}_9\text{Na}$, 670.3679; found, 670.36846.



Scheme 2. Synthesis of compound 6. Reagents and Conditions. (i) a. (Boc)₂O, NaOH, CH₃CN/H₂O, 24 h, RT. b. benzyl bromide, DMF, 24 h, RT, 36%. (ii) benzyl bromide, Ag₂O, Ethyl ether, 24 h, RT, 17%. (iii) LiOH 0.5 M, THF, 24 h, RT, 74%. (iv) a. ethyl chloroformate, Et₃N, CH₂Cl₂, 30 min, 0 °C. b. H-Lys(Z)-OtBu.HCl, Et₃N, CH₂Cl₂, 0 °C, 2 h – RT, 24 h, 56%.

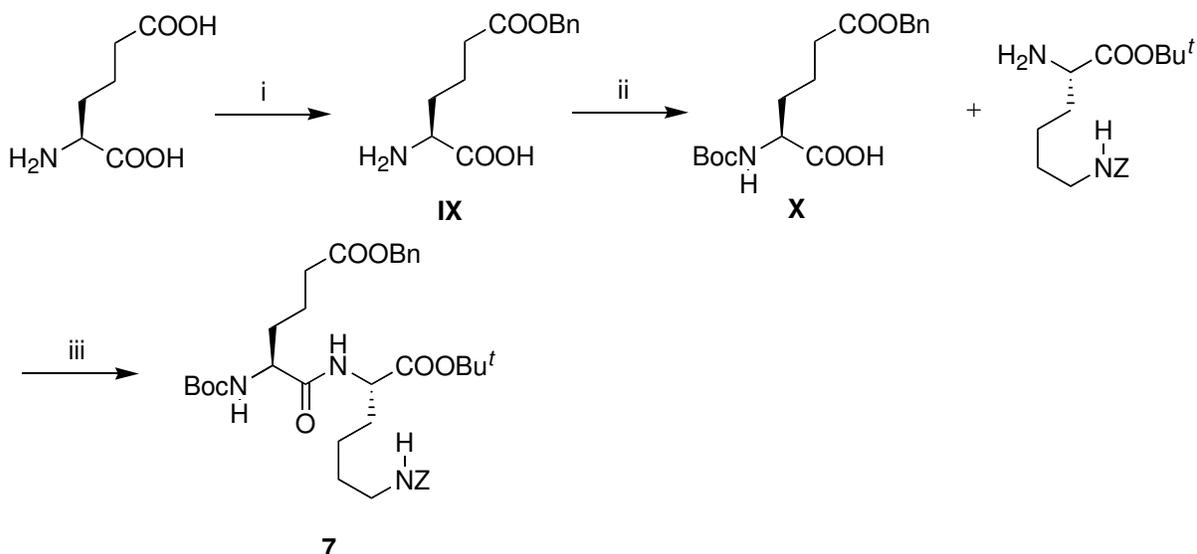
(S)-tert-Butoxycarbonylamino-4-hydroxy-butanoic acid benzyl ester (VI). According to the general procedure GP-B, **VI** was obtained from L-homoserine in 36% yield as a yellow oil after purification by flash chromatography on silica gel (petroleum ether:ethyl acetate 6:4). IR (Film, cm⁻¹): 3390 (ν_{OH} and ν_{NH}), 3066 3035 (ν_{φCH}), 2977 2934 (ν_{CH}), 1695 (ν_{C=O}), 1456 (ν_{φC=C}), 1392 1367 (δ_{CH₃ t-Bu}), 1254 (ν_{C-C-O asym}), 1163 (ν_{O-C-C asym}); ¹H NMR (300 MHz, CDCl₃, δ): 1.46 (s, C(CH₃)₃ Boc, 9H), 1.63 (m, CH_β, 1H), 2.18 (m, CH_β, 1H), 3.68 (m, CH₂OH, 2H), 4.55 (m, CH_α, 1H), 5.19 (AB, *J* = 13.0 Hz, CH₂Ph, 2H), 5.41 (d, *J* = 7.5 Hz, NH, 1H), 7.37 (s, ArH, 5H).

4-Benzyloxy-(2S)-tert-butoxycarbonylamino-butanoic acid benzyl ester (VII). To a solution of **VI** (769 mg, 2.5 mmol) in Et₂O (10 mL) was added Ag₂O (2.3 g, 10 mmol) and BnBr (2.1 mL, 17.5 mmol) at room temperature under an argon atmosphere and the mixture was stirred overnight. The mixture was filtered through a pad of celite and the filtrate was concentrated in vacuo. The precipitated derivative of γ-butyrolactone was isolated by filtration. The yellow viscous filtrate was then purified by flash chromatography on silica gel (petroleum ether:ethyl acetate 8:2) to afford 165 mg of **VII** (17% yield) as a yellow oil. IR (Film, cm⁻¹): 3406 (ν_{NH}), 3065 3033 (ν_{φCH}), 2977 2932 (ν_{CH}), 1739 1715 (ν_{C=O}), 1455 (ν_{φC=C}), 1391 1367 (δ_{CH₃ t-Bu}), 1250 (ν_{C-C-O asym}), 1161 (ν_{O-C-C asym}); ¹H NMR (300 MHz, CDCl₃, δ): 1.45 (s, C(CH₃)₃ Boc, 9H), 2.11 (m, CH_{2β}, 2H), 3.53 (m, CH_{2γ}, 2H), 4.43-4.51 (m+s, CH_α and CH₂Ph OBn, 3H), 5.12 (AB, *J* = 12.2 Hz, CH₂Ph CO₂Bn, 2H), 5.52 (d, *J* = 7.1 Hz, NH, 1H), 7.32-7.34 (m, 2ArH, 10H).

4-Benzyloxy-(2S)-tert-butoxycarbonylamino-butanoic acid (VIII). To a solution of **VII** (148 mg, 0.371 mmol) in THF (3 mL) was added 0.5 M aqueous LiOH (1.4 mL) at 0 °C. The mixture was stirred overnight at room temperature then Et₂O was added. The separated aqueous layer was acidified with 1 M KHSO₄ and extracted twice with ethyl acetate. The extracts were dried over anhydrous sodium sulfate, filtered and concentrated. The colorless oily residue was purified by flash chromatography on silica gel (initially with petroleum ether:ethyl acetate 5:5 then with the same eluent + 0.1% formic acid) to afford 85 mg of **VIII** (74% yield) as a colorless oil. IR (Film, cm⁻¹): 3325 (ν_{COOH} and ν_{NH}), 3033 (ν_{φCH}), 2979 2932 (ν_{CH}), 1716 (ν_{C=O}), 1454 (ν_{φC=C}), 1395 1369 (δ_{CH₃ t-Bu}), 1250 (ν_{C-C-O asym}), 1163 (ν_{O-C-C asym}); ¹H NMR (300 MHz, CDCl₃, δ): 1.46 (s, C(CH₃)₃ Boc, 9H), 2.15 (m, CH_{2β}, 2H), 3.67 (t, *J* = 4.7 Hz, CH_{2γ}, 2H), 4.39 (m, CH_α, 1H), 4.54 (AB, *J* = 11.6 Hz, CH₂Ph, 2H), 5.71 (d, *J* = 6.4 Hz, NH, 1H), 7.33 (m, ArH, 5H).

N^α-Boc-L-Hse(Bn)-L-Lys(Z)-OtBu (6). According to the general procedure GP-A, **6** was obtained from **VIII** and commercial H-Lys(Z)-OtBu.HCl in 56% yield as a colorless oil after purification by flash chromatography on silica gel (petroleum ether:ethyl acetate 6:4). [α]_D²⁶ -4.0 (c 1.00, CH₂Cl₂); IR (Film,

cm⁻¹): 3323 (ν_{NH}), 3065 3033 (ν_{CH}), 2977 2932 (ν_{CH}), 1666 ($\nu_{\text{C=O}}$ amide), 1455 ($\nu_{\text{C=C}}$), 1392 1367 (δ_{CH_3} *t*-Bu), 1250 ($\nu_{\text{C-C-O}}$ asym), 1159 ($\nu_{\text{O-C-C}}$ asym); ¹H NMR (300 MHz, CDCl₃, δ): 1.45, 1.46 (s, 2C(CH₃)₃ Boc and *t*-Bu, 18H), 1.20-1.80 (m, 3CH₂ Lys, 6H), 2.07 (m, CH_{2 β} Hse, 2H), 3.15 (q, *J* = 6.2 Hz, CH₂NH, 2H), 3.60 (br, CH_{2 γ} Hse, 2H), 4.26 (m, CH _{α} , 1H), 4.42 (m, CH _{α} , 1H), 4.51 (AB, *J* = 12.5 Hz, CH₂Ph OBn, 2H), 4.94 (br, NH, 1H), 5.09 (s, CH₂Ph Z, 2H), 5.71 (br, NH, 1H), 6.94 (d, *J* = 7.9 Hz, NH, 1H), 7.31-7.37 (m, 2ArH, 10H); ¹³C NMR (CDCl₃, 75 MHz): 22.5 (C), 28.4, 28.7 (6C), 29.4, 32.4, 32.5 (3C), 41.0 (C), 52.7 (C), 53.5 (C), 66.7 (C), 68.0 (C), 73.7 (C), 80.2 (C), 82.4 (C), 128.2, 128.4, 128.5, 128.8, 128.9 (10C), 137.0 (C), 138.3 (C), 156.1, 156.8 (2C), 171.5, 172.1 (2C); MS-ESI (*m/z*): 1277.1 [2M + Na]⁺, 650.2 [M + Na]⁺; HRMS-ESI (*m/z*): [M + Na]⁺ calcd for C₃₄H₄₉N₃O₈Na, 650.3417; found, 650.34240.



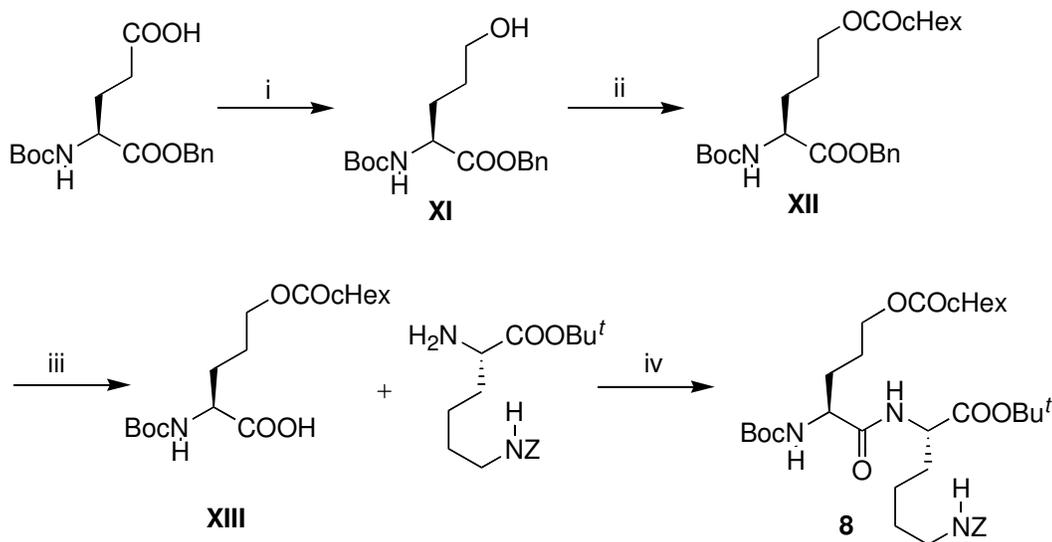
Scheme 3. Synthesis of compound 7. Reagents and Conditions. (i) PhCH₂OH, HCl 12 M, reflux, 1 h, 53%. (ii) (Boc)₂O, Et₃N, acetone/H₂O, 24 h, RT, 64%. (iii) a. ethyl chloroformate, Et₃N, CH₂Cl₂, 30 min, 0 °C. b. H-Lys(Z)-OtBu.HCl, Et₃N, CH₂Cl₂, 0° C, 2 h – RT, 24 h, 60%.

(2S)-Amino-hexanedioic acid 6-benzyl ester (IX). L- α -aminoadipic acid (400 mg, 2.5 mmol) was heated to 100 °C in benzyl alcohol (2.6 mL, 25 mmol) and 12 M HCl (210 μ L, 2.5 mmol) for 1 h. Et₂O was then added to precipitate the product which was filtered and dried. The product **IX** (336 mg, 53% yield) was obtained as a white solid. Mp 163-166°C (lit. 188 °C / hot water); IR (KBr, cm⁻¹): 3432 3215 (ν_{COOH} and ν_{NH_2}), 3000 (ν_{CH}), 2921 (ν_{CH}), 1735 ($\nu_{\text{C=O}}$), 1486 1456 ($\nu_{\text{C=C}}$), 1179 ($\nu_{\text{O-C-C}}$ asym); ¹H NMR (300 MHz, DMSO, δ): 1.55-1.87 (m, CH_{2 β} et CH_{2 γ} , 4H), 2.41 (t, *J* = 7.1 Hz, CH_{2 δ} , 2H), 3.88 (t, *J* = 6.0 Hz, CH _{α} , 1H), 5.09 (s, CH₂Ph, 2H), 7.35 (s, ArH, 5H), 8.35-8.52 (br, NH₂, 2H).

(2S)-tert-Butoxycarbonylamino-hexanedioic acid 6-benzyl ester (X). According to the general procedure GP-B, **X** was obtained from **IX** in 64% yield as a colorless oil with no purification needed. IR (Film, cm⁻¹): 3345 (ν_{COOH} and ν_{NH}), 3066 3034 (ν_{CH}), 2978 2936 (ν_{CH}), 1716 ($\nu_{\text{C=O}}$), 1456 ($\nu_{\text{C=C}}$), 1393 1369 (δ_{CH_3} *t*-Bu), 1248 ($\nu_{\text{C-C-O}}$ asym), 1167 ($\nu_{\text{O-C-C}}$ asym); ¹H NMR (300 MHz, CDCl₃, δ): 1.37 (s, C(CH₃)₃ Boc, 9H), 1.58-1.87 (m, CH_{2 β} and CH_{2 γ} , 4H), 2.33 (m, CH_{2 δ} , 2H), 4.23 (m, CH _{α} , 1H), 5.00 (br, NH, 1H), 5.04 (s, CH₂Ph, 2H), 7.28 (s, ArH, 5H).

N ^{α} -Boc-L-Aad(OBn)-L-Lys(Z)-OtBu (7). According to the general procedure GP-A, **7** was obtained from **X** and commercial H-Lys(Z)-OtBu.HCl in 60% yield as a colorless oil after purification by flash chromatography on silica gel (petroleum ether:ethyl acetate 7:3). [α]_D²³ -9.0 (*c* 1.00, CH₂Cl₂); IR (Film, cm⁻¹): 3324 (ν_{NH}), 3066 3034 (ν_{CH}), 2977 2935 (ν_{CH}), 1732 ($\nu_{\text{C=O}}$ ester), 1668 ($\nu_{\text{C=O}}$ amide), 1456 ($\nu_{\text{C=C}}$), 1392 1368 (δ_{CH_3} *t*-Bu), 1248 ($\nu_{\text{C-C-O}}$ asym), 1162 ($\nu_{\text{O-C-C}}$ asym); ¹H NMR (300 MHz, CDCl₃, δ): 1.44, 1.46 (2s, 2C(CH₃)₃ Boc and *t*-Bu, 18H), 1.30-1.72 (m, CH_{2 γ} Aad and 3CH₂ Lys, 8H), 1.84 (m, CH_{2 β} Aad, 2H), 2.37 (q, *J* = 5.9 Hz, CH_{2 δ} Aad, 2H), 3.18 (q, *J* = 5.9 Hz, CH₂NH, 2H), 4.05 (m, CH _{α} , 1H), 4.45 (m, CH _{α} , 1H), 5.06 (br, NH, 1H), 5.10 (s, CH₂Ph, 2H), 5.12 (s, CH₂Ph, 2H), 6.70 (d, *J* = 8.2

Hz, NH, 1H), 7.35 (s, 2ArH, 10H); ¹³C NMR (CDCl₃, 75 MHz): 21.3, 22.5, 29.5, 31.8, 32.2 (5C), 28.3, 28.7 (6C), 34.0 (C), 40.9 (C), 52.8 (C), 54.4 (C), 66.6 (C), 67.0 (C), 80.4 (C), 82.4 (C), 128.5, 128.6, 128.8, 128.9 (10C), 136.3 (C), 136.9 (C), 156.2, 156.9 (2C), 171.5, 172.3, 173.5 (3C); MS-ESI (*m/z*): 1361.0 [2M + Na]⁺, 692.2 [M + Na]⁺, 670.0 [M + H]⁺; HRMS-ESI (*m/z*): [M + Na]⁺ calcd for C₃₆H₅₁N₃O₉Na, 692.3523; found, 692.35189.



Scheme 4. Synthesis of compound 8. Reagents and Conditions. (i) a. ethyl chloroformate, Et₃N, CH₂Cl₂, 30 min, 0 °C. b. NaBH₄, H₂O, -10 °C, 1 h – RT, 2 h, 84%. (ii) cHexCOCl, Et₃N, CH₂Cl₂, 24 h, RT, 86%. (iii) H₂, Pd/C, EtOH, RT, 4 h, 83%. (iv) a. ethyl chloroformate, Et₃N, CH₂Cl₂, 30 min, 0 °C. b. H-Lys(Z)-OtBu.HCl, Et₃N, CH₂Cl₂, 0 °C, 2 h – RT, 24 h, 77%.

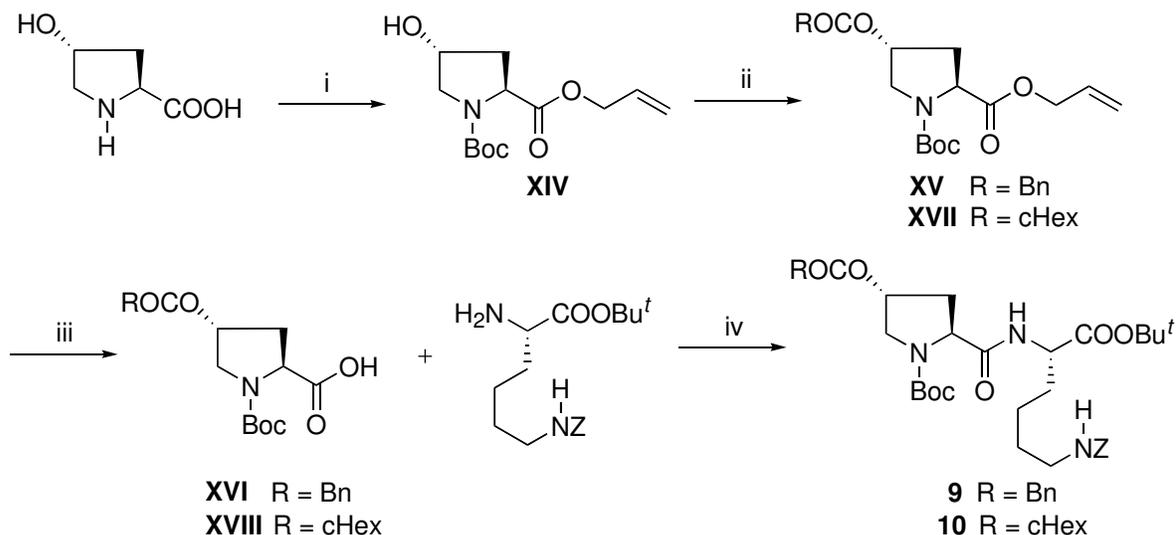
(2S)-tert-Butoxycarbonylamino-5-hydroxy-pentanoic acid benzyl ester (XI). To a solution of commercial N^α-Boc-Glu-OBn (503 mg, 1.5 mmol) in THF (10 mL) was added triethylamine (632 μL, 4.5 mmol) and ethyl chloroformate (430 μL, 4.5 mmol) at -10 °C under argon atmosphere. The mixture was stirred at -10 °C for 1 h and NaBH₄ (228 mg, 6 mmol) in water (8 mL) was added dropwise at -10 °C. The mixture was stirred at -10 °C for 1 h then at room temperature for 2 h. The reaction was quenched with 1 M aqueous KHSO₄ (20 mL) and the mixture was extracted three times with EtOAc. The extracts were washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The colorless oily residue was purified by flash chromatography on silica gel (petroleum ether:ethyl acetate 5:5) to afford 407 mg of XI (84% yield) as a colorless oil. IR (Film, cm⁻¹): 3375 (ν_{OH} and ν_{NH}), 3035 (ν_{φCH}), 2977 2933 (ν_{CH}), 1713 (ν_{C=O}), 1455 (ν_{φC=C}), 1392 1367 (δ_{CH₃ t-Bu}), 1253 (ν_{C-C-O asym}), 1166 (ν_{O-C-C asym}); ¹H NMR (300 MHz, CDCl₃, δ): 1.45 (s, C(CH₃)₃ Boc, 9H), 1.52-2.06 (m, CH_{2β}, CH_{2γ} and OH, 5H), 3.65 (t, *J* = 6.0 Hz, CH₂OH, 2H), 4.40 (m, CH_α, 1H), 5.19 (AB, *J* = 12.4 Hz, CH₂Ph and NH, 3H), 7.37 (s, ArH, 5H).

(2S)-tert-Butoxycarbonylamino-5-cyclohexylcarbonyloxy-pentanoic acid benzyl ester (XII). According to the general procedure GP-D, XII was obtained from XI in 86% yield as a colorless oil after purification by flash chromatography on silica gel (petroleum ether:ethyl acetate 8:2). IR (Film, cm⁻¹): 3368 (ν_{NH}), 3066 3034 (ν_{φCH}), 2933 (ν_{CH}), 1732 1716 (ν_{C=O}), 1454 (ν_{φC=C}), 1391 1367 (δ_{CH₃ t-Bu}), 1248 (ν_{C-C-O asym}), 1169 (ν_{O-C-C asym}); ¹H NMR (300 MHz, CDCl₃, δ): 1.2-1.4 (m, 2CH₂ cHex, 4H), 1.45 (s, C(CH₃)₃ Boc, 9H), 1.60-2.00 (m, CH_{2β}, CH_{2γ} and 3CH₂ cHex, 10H), 2.26 (m, CHCO cHex, 1H), 4.05 (t, *J* = 6.0 Hz, CH_{2δ}, 2H), 4.38 (m, CH_α, 1H), 5.07 (d, *J* = 8.6 Hz, NH, 1H), 5.19 (AB, *J* = 12.2 Hz, CH₂Ph, 2H), 7.37 (s, ArH, 5H).

(2S)-tert-Butoxycarbonylamino-5-cyclohexanecarbonyloxy-pentanoic acid (XIII). To a solution of benzyl ester XII (0.51 mmol) in ethanol (10 mL), was added 10% palladium on charcoal (11 mg, 1 part per weight per 40 parts by weight of benzyl ester). The reaction mixture was flushed with hydrogen gas bubbled through the mixture for 4 h at room temperature. The catalyst was removed by filtration, the solvent evaporated and the product dissolved in a saturated aqueous solution of sodium bicarbonate that

was washed with diethyl ether and acidified to pH 2-3 by addition of 1M HCl. The resulting solution was extracted twice with diethyl ether and the combined organic extracts were washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated. The resulting oil was dissolved in pentane and the solvent evaporated. This procedure was repeated four times in order to remove residual diethyl ether. Finally, the corresponding acid product **XIII** (145 mg) was obtained in 83% yield as a yellow oil without purification. IR (Film, cm^{-1}): 3350 (ν_{COOH} and ν_{NH}), 2933 (ν_{CH}), 1715 ($\nu_{\text{C=O}}$), 1394 1368 (δ_{CH_3} *t*-Bu), 1249 ($\nu_{\text{C-C-O}}$ *asym*), 1165 ($\nu_{\text{O-C-C}}$ *asym*); ^1H NMR (300 MHz, CDCl_3 , δ): 1.12-1.31 (m, 2CH_2 cHex, 4H), 1.35 (s, $\text{C}(\text{CH}_3)_3$ Boc, 9H), 1.52-2.00 (m, $\text{CH}_{2\beta}$, $\text{CH}_{2\gamma}$ and 3CH_2 cHex, 10H), 2.24 (m, CHCO cHex, 1H), 4.00 (t, $J = 6.0$ Hz, $\text{CH}_{2\delta}$, 2H), 4.27 (m, CH_α , 1H), 4.98 (d, $J = 7.9$ Hz, NH , 1H), 5.70-6.60 (br, COOH , 1H).

***N* ^{α} -Boc-L-Nva(δCOcHex)-L-Lys(Z)-O*t*Bu (**8**).** According to the general procedure GP-A, **8** was obtained from **XIII** and commercial H-Lys(Z)-O*t*Bu.HCl in 77% yield as a colorless oil after purification by flash chromatography on silica gel (petroleum ether:ethyl acetate 6:4). $[\alpha]_{\text{D}}^{26} +2.0$ (c 1.00, CH_2Cl_2); IR (Film, cm^{-1}): 3325 (ν_{NH}), 3066 3035 ($\nu_{\phi\text{CH}}$), 2933 (ν_{CH}), 1729 ($\nu_{\text{C=O}}$ ester), 1453 ($\nu_{\phi\text{C=C}}$), 1392 1368 (δ_{CH_3} *t*-Bu), 1249 ($\nu_{\text{C-C-O}}$ *asym*), 1160 ($\nu_{\text{O-C-C}}$ *asym*); ^1H NMR (300 MHz, CDCl_3 , δ): 1.44 and 1.47 (s, $2\text{C}(\text{CH}_3)_3$ Boc and *t*-Bu, 18H), 1.20-1.97 (m, 3CH_2 Lys, 5CH_2 cHex, $\text{CH}_{2\beta}$ and $\text{CH}_{2\gamma}$ Nva, 20H), 2.29 (m, CHCO cHex, 1H), 3.19 (q, $J = 5.7$ Hz, CH_2NH , 2H), 3.98-4.18 (m, CH_α and $\text{CH}_{2\delta}$ Nva, 3H), 4.45 (m, CH_α , 1H), 5.00 (br, NH , 1H), 5.11 (s, CH_2Ph , 2H), 6.64 (d, $J = 7.9$ Hz, NH , 1H), 7.35 (s, ArH, 5H); ^{13}C NMR (CDCl_3 , 75 MHz): 22.5 (C), 25.3 (C), 25.8, 26.1 (3C), 28.3, 28.7 (6C), 29.4, 29.5 (2C), 30.1 (2C), 32.3 (C), 40.9 (C), 43.6 (C), 52.8 (C), 54.2 (C), 63.7 (C), 67.0 (C), 80.4 (C), 82.4 (C), 128.4, 128.5, 128.8 (5C), 136.9 (C), 156.1, 156.9 (2C), 171.4, 172.2, 176.6 (3C); MS-ESI (m/z): 1345.2 [$2\text{M} + \text{Na}$]⁺, 684.3 [$\text{M} + \text{Na}$]⁺, 662.1 [$\text{M} + \text{H}$]⁺; HRMS-ESI (m/z): [$\text{M} + \text{Na}$]⁺ calcd for $\text{C}_{35}\text{H}_{55}\text{N}_3\text{O}_9\text{Na}$, 684.3836; found, 684.38378.



Scheme 5. Synthesis of compounds 9 and 10. Reagents and Conditions. (i) a. $(\text{Boc})_2\text{O}$, NaOH, $\text{CH}_3\text{CN}/\text{H}_2\text{O}$, 24 h, RT. b. allyl bromide, DMF, 24 h, RT, 97%. (ii) RCOCl (R = Bn, cHex), Et_3N , CH_2Cl_2 , 24 h, RT, 79% for **XV**, 84% for **XVII**. (iii) $\text{Pd}(\text{Ph}_3)_4$, PPh_3 , CH_2Cl_2 , pyrrolidine, 40 min, RT, quant. yield for **XVI**, 95% for **XVIII**. (iv) a. ethyl chloroformate, Et_3N , CH_2Cl_2 , 30 min, 0 °C. b. H-Lys(Z)-O*t*Bu.HCl, Et_3N , CH_2Cl_2 , 0 °C, 2 h – RT, 24 h, 65% for **9**, 69% for **10**.

(4*R*)-Hydroxy-pyrrolidine-1,(2*S*)-dicarboxylic acid 2-allyl ester 1-*tert*-butyl ester (XIV**).** According to the general procedure GP-B, **XIV** was obtained from commercial *trans*-4-hydroxy-L-proline in 97% yield as a colorless oil without purification. IR (Film, cm^{-1}): 3436 (ν_{OH}), 2978 2936 (ν_{CH}), 1748 1682 ($\nu_{\text{C=O}}$), 1415 ($\nu_{\text{C-C-O}}$ *asym*), 1368 (ν_{CH_3} *t*-Bu), 1159 ($\nu_{\text{O-C-C}}$ *asym*); ^1H NMR (300 MHz, CDCl_3 , δ): 1.42, 1.46 (2s, $\text{C}(\text{CH}_3)_3$ Boc 1st and 2nd conf., 9H), 2.09 (m, CH_β , 1H), 2.31 (m, CH_β , 1H), 3.55 (m, CH_2N , 2H), 4.40-4.51 (m, CH_α and CHOH , 2H), 4.65 (d, $J = 5.6$ Hz, $\text{CH}_2\text{-CH=CH}_2$, 2H), 4.26

(dd, $J = 0.7$ Hz and 10.1 Hz, $\text{CH}_2\text{-CH}=\text{CH}_2$, 1H), 5.84 (dd, $J = 0.9$ Hz and 18.2 Hz, $\text{CH}_2\text{-CH}=\text{CH}_2$, 1H), 5.91 (m, $\text{CH}_2\text{-CH}=\text{CH}_2$, 1H).

(4R)-Phenylacetoxy-pyrrolidine-1,(2S)-dicarboxylic acid 2-allyl ester 1-tert-butyl ester (XV).

According to the general procedure GP-D, **XV** was obtained from **XIV** in 79% yield as a colorless oil after purification by flash chromatography on silica gel (petroleum ether:ethyl acetate 8:2). IR (Film, cm^{-1}): 3065 3031 ($\nu_{\text{C-H}}$), 2978 2933 ($\nu_{\text{C-H}}$), 1744 1704 ($\nu_{\text{C=O}}$), 1455 ($\nu_{\text{C=C}}$), 1403 1367 (δ_{CH_3} *t*-Bu), 1258 ($\nu_{\text{C-C-O}}$ asym), 1157 ($\nu_{\text{O-C-C}}$ asym), 931 (δ_{CH} vinyl); ^1H NMR (300 MHz, CDCl_3 , δ): 1.44, 1.46 (2s, $\text{C}(\text{CH}_3)_3$ Boc 1st and 2nd conf., 9H), 2.20 (m, CH_β , 1H), 2.38 (m, CH_β , 1H), 3.62-3.76 (s+m, CH_2Ph and CH_2N , 4H), 4.32, 4.43 (2t, $J = 8.0$ Hz and 7.7 Hz, CH_α 1st and 2nd conf., 1H), 4.65 (m, $\text{CH}_2\text{-CH}=\text{CH}_2$, 2H), 5.22-5.38 (m, $\text{CH}_2\text{-CH}=\text{CH}_2$ and CHOCOBn , 3H), 5.92 (m, $\text{CH}_2\text{-CH}=\text{CH}_2$, 1H), 7.33 (m, ArH, 5H).

(4R)-Phenylacetoxy-pyrrolidine-1,(2S)-dicarboxylic acid 1-tert-butyl ester (XVI). According to the general procedure GP-C, **XVI** was obtained from **XV** in quantitative yield as a yellow oil after purification by flash chromatography on silica gel (initially with petroleum ether:ethyl acetate 5:5 then with the same eluent + 0.1% formic acid). IR (Film, cm^{-1}): 2980 (ν_{COOH}), 1732 ($\nu_{\text{C=O}}$), 1455 ($\nu_{\text{C=C}}$), 1369 (δ_{CH_3} *t*-Bu), 1257 ($\nu_{\text{C-C-O}}$ asym), 1160 ($\nu_{\text{O-C-C}}$ asym); ^1H NMR (300 MHz, CDCl_3 , δ): 1.46, 1.48 (2s, $\text{C}(\text{CH}_3)_3$ Boc 1st and 2nd conf., 9H), 2.40 (m, $\text{CH}_{2\beta}$, 2H), 3.60-3.72 (m+s, CH_2N and CH_2Ph , 4H), 4.32, 4.44 (2t, $J = 7.9$ Hz and 7.9 Hz, CH_α 1st and 2nd conf., 1H), 5.29 (m, CHOCOBn , 1H), 7.32 (m, ArH, 5H).

***N*^α-Boc-L-(4R)Hyp(COBn)-L-Lys(Z)-OtBu (9).** According to the general procedure GP-A, **9** was obtained from **XVI** and commercial H-Lys(Z)-OtBu.HCl in 65% yield as a colorless oil after purification by flash chromatography on silica gel (petroleum ether:ethyl acetate 5:5). $[\alpha]_D^{26}$ -9.0 (*c* 1.00, CH_2Cl_2); IR (Film, cm^{-1}): 3329 (ν_{NH}), 3065 3033 ($\nu_{\text{C-H}}$), 2978 2933 ($\nu_{\text{C-H}}$), 1695 ($\nu_{\text{C=O}}$ amide), 1455 ($\nu_{\text{C=C}}$), 1394 1368 (δ_{CH_3} *t*-Bu), 1251 ($\nu_{\text{C-C-O}}$ asym), 1158 ($\nu_{\text{O-C-C}}$ asym); ^1H NMR (300 MHz, CDCl_3 , δ): 1.44, 1.47 (2s, $\text{C}(\text{CH}_3)_3$ Boc 1st and 2nd conf., 9H), 1.34-1.69 (m, 3CH_2 Lys, 6H), 2.20 (m, CH_β , 1H), 2.45 (m, CH_β , 1H), 3.17 (m, CH_2NH , 2H), 3.55-3.63 (m+s, CH_2N and CH_2Ph , 4H), 4.28 (m, CH_α , 1H), 4.44 (m, CH_α , 1H), 5.10 (s, CH_2Ph Z, 2H), 5.25 (m, CH-O Hyp, 1H), 7.25-7.37 (m, 2ArH, 10H); ^{13}C NMR (CDCl_3 , 75 MHz): 22.6 (C), 28.4, 28.6 (6C), 29.4, 30.1 (2C), 32.4 (C), 40.9, 41.7 (2C), 52.9 (C), 58.9 (C), 60.8 (C), 66.9 (C), 73.7 (C), 81.1 (C), 82.4 (C), 127.6, 128.8, 129.1, 130.0 (10C), 133.9 (C), 137.1 (C), 155.5, 156.8 (2C), 171.2, 171.4, 171.6 (3C); MS-ESI (*m/z*): 1357.1 [$2\text{M} + \text{Na}$]⁺, 690.2 [$\text{M} + \text{Na}$]⁺; HRMS-ESI (*m/z*): [$\text{M} + \text{Na}$]⁺ calcd for $\text{C}_{36}\text{H}_{49}\text{N}_3\text{O}_9\text{Na}$, 690.3367; found, 690.33599.

(4R)-Cyclohexanecarbonyloxy-pyrrolidine-1,(2S)-dicarboxylic acid 2-allyl ester 1-tert-butyl ester (XVII). According to the general procedure GP-D-(GP-N), **XVII** was obtained from **XIV** in 84% yield as a colorless oil after purification by flash chromatography on silica gel (petroleum ether:ethyl acetate 8:2). IR (Film, cm^{-1}): 2977 2934 ($\nu_{\text{C-H}}$), 1733 1705 1699 ($\nu_{\text{C=O}}$), 1403 1368 (ν_{CH_3} *t*-Bu), 1248 ($\nu_{\text{C-C-O}}$ asym), 1163 ($\nu_{\text{O-C-C}}$ asym), 929 (ν_{CH} vinyl); ^1H NMR (300 MHz, CDCl_3 , δ): 1.21-1.38 (m, 2CH_2 cHex, 4H), 1.44, 1.47 (s, $\text{C}(\text{CH}_3)_3$ Boc 1st and 2nd conf., 9H), 1.61-1.91 (m, 3CH_2 cHex, 6H), 2.14-2.43 (m, $\text{CH}_{2\beta}$ and CHCO cHex, 3H), 3.63 (m, CH_2N , 2H), 4.35, 4.45 (2t, $J = 8.0$ Hz and 7.7 Hz, CH_α 1st and 2nd conf., 1H), 4.66 (m, $\text{CH}_2\text{-CH}=\text{CH}_2$, 2H), 5.23-5.38 (m, $\text{CH}_2\text{-CH}=\text{CH}_2$ and CH-O Hyp, 3H), 5.92 (m, $\text{CH}_2\text{-CH}=\text{CH}_2$, 1H).

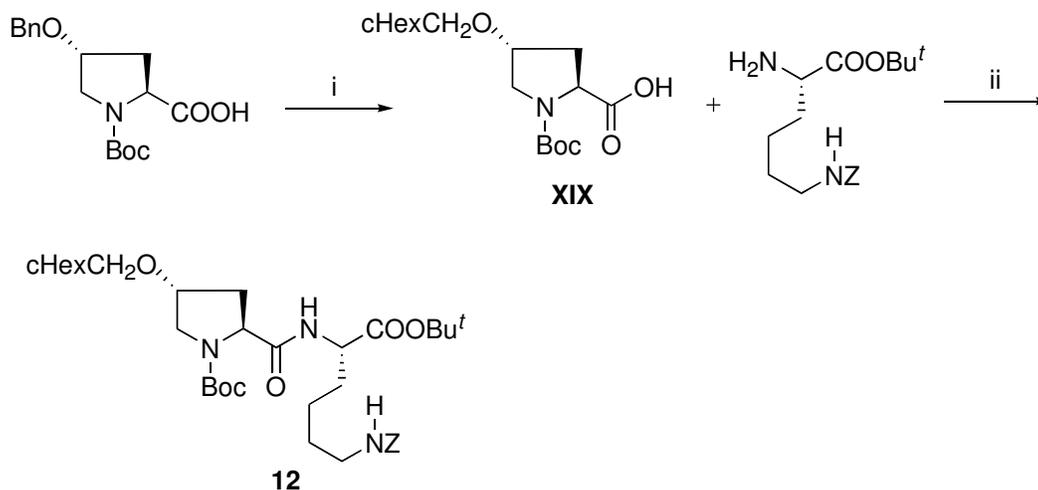
(4R)-Cyclohexanecarbonyloxy-pyrrolidine-1,(2S)-dicarboxylic 1-tert-butyl ester (XVIII).

According to the general procedure GP-C, **XVIII** was obtained from **XVII** in 95% yield as a yellow oil after purification by flash chromatography on silica gel (initially with petroleum ether:ethyl acetate 5:5 then with the same eluent + 0.1% formic acid). IR (Film, cm^{-1}): 3446 (ν_{COOH}), 2978 2933 ($\nu_{\text{C-H}}$), 1732 ($\nu_{\text{C=O}}$), 1368 (δ_{CH_3} *t*-Bu), 1249 ($\nu_{\text{C-C-O}}$ asym); ^1H NMR (300 MHz, CDCl_3 , δ): 1.25-1.41 (m, 2CH_2 cHex, 4H), 1.45, 1.49 (s, $\text{C}(\text{CH}_3)_3$ Boc 1st and 2nd conf., 9H), 1.63-1.90 (m, 3CH_2 cHex, 6H), 2.23-2.52 (m, $\text{CH}_{2\beta}$ and CHCO cHex, 3H), 3.65 (m, CH_2N , 2H), 4.36, 4.50 (2t, $J = 8.0$ Hz and 7.9 Hz, CH_α 1st and 2nd conf., 1H), 5.29 (d, $J = 15.4$ Hz, CH-O Hyp, 1H), 6.30-7.80 (br, COOH, 1H).

***N*^α-Boc-L-(4R)Hyp(COcHex)-L-Lys(Z)-OtBu (10).** According to the general procedure GP-A, **10** was obtained from **XVIII** and commercial H-Lys(Z)-OtBu.HCl in 69% yield as a viscous yellow oil

after purification by flash chromatography on silica gel (petroleum ether:ethyl acetate 6:4). $[\alpha]_D^{26}$ -11.0 (*c* 1.00, CH₂Cl₂); IR (Film, cm⁻¹): 3350 (ν_{NH}), 2933 (ν_{CH}), 1668 ($\nu_{\text{C=O}}$ amide), 1454 ($\nu_{\text{C=C}}$), 1394 (δ_{CH_3} *t*-Bu), 1248 ($\nu_{\text{C-C-O}}$ asym), 1160 ($\nu_{\text{O-C-C}}$ asym); ¹H NMR (300 MHz, CDCl₃, δ): 1.44, 1.47 (2s, C(CH₃)₃ Boc 1st and 2nd conf., 9H), 1.23-1.88 (m, 3CH₂ Lys and 5CH₂ cHex, 16H), 2.18-2.54 (m, CH_{2 β} and CHCO cHex, 3H), 3.19 (m, CH₂NH, 2H), 3.56 (br, CH₂N, 2H), 4.36 (m, CH _{α} , 1H), 4.45 (m, CH _{α} , 1H), 5.10 (AB, *J* = 12.4 Hz, CH₂Ph, 2H), 5.22 (br, CH-O Hyp, 1H), 7.05 (br, NH, 1H), 7.34 (m, ArH, 5H); ¹³C NMR (CDCl₃, 75 MHz): 21.4, 22.2, 22.6, 29.4, 30.1, 32.3, 32.9, 34.7 (8C), 28.3, 28.6 (6C), 37.3 (C), 43.3 (C), 53.1 (C), 59.0 (C), 60.4 (C), 60.8 (C), 72.8 (C), 73.6 (C), 81.1 (C), 82.4 (C), 128.1, 128.4, 128.5, 128.8 (5C), 137.0 (C), 155.6, 156.9 (2C), 171.3, 171.9, 175.8 (3C); MS-ESI (*m/z*): 1341.2 [2M + Na]⁺, 682.2 [M + Na]⁺, 660.0 [M + H]⁺; HRMS-ESI (*m/z*): [M + Na]⁺ calcd for C₃₅H₅₃N₃O₉Na, 682.3680; found, 682.36780.

***N*^α-Boc-L-(4*R*)Hyp(Bn)-L-Lys(Z)-OtBu (11).** According to the general procedure GP-A, **11** was obtained from commercial *N*^α-Boc-*trans*-4-Hyp(Bn)-OH and H-Lys(Z)-OtBu.HCl in 70% yield as a colorless oil after purification by flash chromatography on silica gel (petroleum ether:ethyl acetate 6:4). $[\alpha]_D^{26}$ -19.0 (*c* 1.00, CH₂Cl₂); IR (Film, cm⁻¹): 3325 (ν_{NH}), 3065 3033 (ν_{CH}), 2977 2934 (ν_{CH}), 1679 ($\nu_{\text{C=O}}$ amide), 1455 ($\nu_{\text{C=C}}$), 1394 1367 (ν_{CH_3} *t*-Bu), 1251 ($\nu_{\text{C-C-O}}$ asym), 1160 ($\nu_{\text{O-C-C}}$ asym); ¹H NMR (300 MHz, CDCl₃, δ): 1.35, 1.38 (2s, C(CH₃)₃ Boc 1st and 2nd conf., 9H), 1.23-1.78 (m, 3CH₂ Lys, 6H), 2.07 (m, CH _{β} , 1H), 2.32 (m, CH _{β} , 1H), 3.09 (m, CH₂NH, 2H), 3.44 (br, CH₂N, 2H), 4.24-4.37 (m, 2CH _{α} , 2H), 4.41 (d, *J* = 3.7 Hz, CH₂Ph Hyp, 2H), 4.96-5.05 (m, CH-O Hyp and CH₂Ph Z, 3H), 6.52 (br, NH, 1H), 6.97 (br, NH, 1H), 7.20-7.27 (m, 2ArH, 10H); ¹³C NMR (CDCl₃, 75 MHz): 22.6 (C), 28.4, 28.7 (6C), 29.4, 32.4 (2C), 34.7 (C), 40.9 (C), 52.4 (C), 59.2 (C), 60.4 (C), 66.9 (C), 71.6 (C), 77.3 (C), 80.8 (C), 82.3 (C), 128.0, 128.2, 128.5, 128.8 (10C), 137.1 (C), 138.3 (C), 155.7, 156.9 (2C), 171.6, 172.7 (2C); MS-ESI (*m/z*): 662.3 [M + Na]⁺, 540.2 [M + H - isobutene - CO₂]⁺; HRMS-ESI (*m/z*): [M + Na]⁺ calcd for C₃₅H₄₉N₃O₈Na, 662.3417; found, 662.34155.



Scheme 6. Synthesis of compound 12. (i) H₂, Rh/Al₂O₃, *i*PrOH, 24 h, RT, quant. yield. (ii) a. ethyl chloroformate, Et₃N, CH₂Cl₂, 30 min, 0 °C. b. H-Lys(Z)-OtBu.HCl, Et₃N, CH₂Cl₂, 0° C, 2 h – RT, 24 h, 67%.

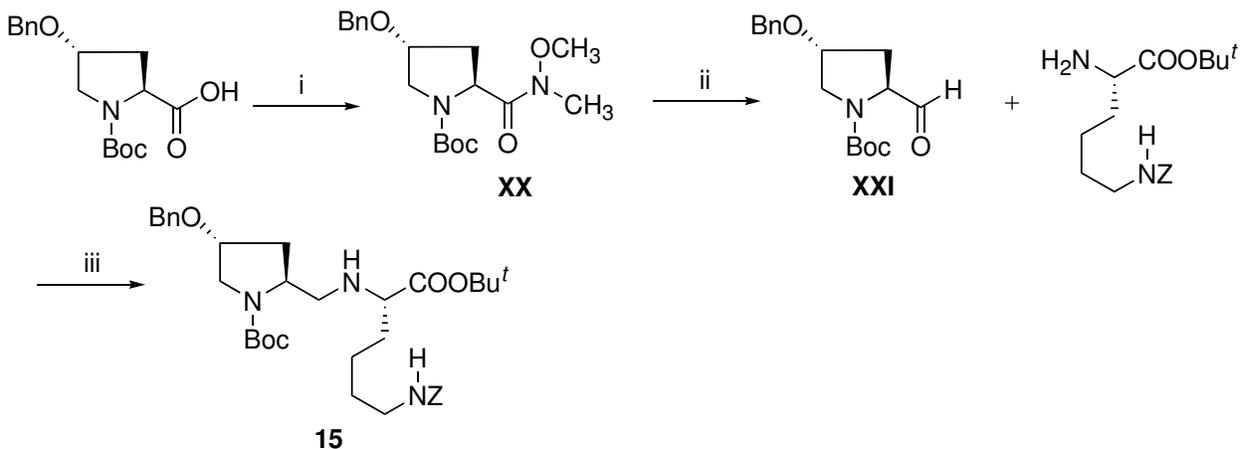
(4*R*)-Cyclohexylmethoxy-pyrrolidine-1,(2*S*)-dicarboxylic acid 1-*tert*-butyl ester (XIX). A mixture of commercial *N*^α-Boc-*trans*-4-Hyp(Bn)-OH (200 mg, 0.623 mmol), Rh-Al₂O₃ (5%, 35 mg, 0.018 mmol) and *i*-propylalcohol (4 mL) was stirred under hydrogen atmosphere at room temperature overnight. The resulting mixture was filtered to remove catalyst. The filtrate was concentrated under reduced pressure to give **XIX** (203 mg, 100% yield) as a colorless oil with no purification needed. IR (Film, cm⁻¹): 3418 (ν_{COOH}), 2974 2924 (ν_{CH}), 1705 1682 ($\nu_{\text{C=O}}$), 1368 (δ_{CH_3} *t*-Bu), 1257 ($\nu_{\text{C-C-O}}$ asym), 1163 ($\nu_{\text{O-C-C}}$ asym); ¹H NMR (300 MHz, CDCl₃, δ): 1.17-1.27 (m, 3CH₂ cHex, 6H), 1.43, 1.50 (s, C(CH₃)₃ Boc 1st and 2nd conf., 9H), 1.65-1.76 (m, 2CH₂ and CH cHex, 5H), 2.16 (m, CH _{β} , 1H), 2.39 (m,

CH_{β} , 1H), 3.21 (m, CH_2N , 2H), 3.54 (m, CH_2O , 2H), 4.05 (m, $CH-O$, 1H), 4.34, 4.46 (2t, $J = 7.7$ Hz and 7.3 Hz, CH_{α} , 1H), 5.00-5.90 (br, $COOH$, 1H).

N^{α} -Boc-L-(4R)Hyp(CH_2 cHex)-L-Lys(Z)-OtBu (12). According to the general procedure GP-A, **12** was obtained from **XIX** and commercial H-Lys(Z)-OtBu.HCl in 67% yield as a yellow oil after purification by flash chromatography on silica gel (petroleum ether:ethyl acetate 6:4). $[\alpha]_D^{26}$ -17.0 (c 1.00, CH_2Cl_2); IR (Film, cm^{-1}): 3325 (ν_{NH}), 3066 3034 ($\nu_{\phi CH}$), 2977 2925 (ν_{CH}), 1681 ($\nu_{C=O}$ amide), 1454 ($\nu_{\phi C=C}$), 1394 1368 (δ_{CH_3} *t*-Bu), 1248 (ν_{C-C-O} asym), 1159 (ν_{O-C-C} asym); 1H NMR (300 MHz, $CDCl_3$, δ): 1.44, 1.47 (2s, $C(CH_3)_3$ Boc 1st and 2nd conf., 9H), 1.12-1.89 (m, 3 CH_2 Lys, 5 CH_2 and CH cHex, 17H), 2.06 (m, CH_{β} , 1H), 2.34 (m, CH_{β} , 1H), 3.13-3.21 (m, 2 CH_2N , 4H), 3.46 (br, CH_2O Hyp, 2H), 3.95-4.04 (br, CH_{α} 1st and 2nd conf., 1H), 4.31 (m, CH_{α} , 1H), 4.43 (m, $CH-O$ Hyp, 1H), 5.10 (M+s, NH and CH_2Ph , 3H), 7.06 (d, $J = 7.9$ Hz, NH , 1H), 7.36 (s, ArH, 5H); ^{13}C NMR ($CDCl_3$, 75 MHz): 21.4, 22.3, 22.7, 29.4, 30.1, 30.3, 30.4, 32.4 (8C), 28.4, 28.7 (6C), 34.7 (C), 38.4 (C), 41.0 (C), 52.3 (C), 52.9 (C), 59.2 (C), 66.9 (C), 75.5 (C), 77.6 (C), 80.7 (C), 82.3 (C), 128.4, 128.8 (5C), 137.1 (C), 155.9, 156.8 (2C), 171.6, 172.8 (2C); MS-ESI (m/z): 1313.3 [2M + Na]⁺, 668.3 [M + Na]⁺, 646.1 [M + H]⁺; HRMS-ESI (m/z): [M + Na]⁺ calcd for $C_{35}H_{55}N_3O_8Na$, 668.3887; found, 668.38834.

N^{α} -Boc-L-Tyr(*t*Bu)-L-Lys(Z)-OtBu (13). According to the general procedure GP-A, **13** was obtained from commercial N^{α} -Boc-Tyr(*t*Bu)-OH and H-Lys(Z)-OtBu.HCl in 83% yield as a yellow solid after purification by flash chromatography on silica gel (petroleum ether:ethyl acetate 7:3). Mp 112-115°C; $[\alpha]_D^{27}$ +3.0 (c 1.00, CH_2Cl_2); IR (KBr, cm^{-1}): 3356 (ν_{NH}), 2980 2933 (ν_{CH}), 1725 1700 1683 1657 ($\nu_{C=O}$), 1455 ($\nu_{\phi C=C}$), 1389 1366 (δ_{CH_3} *t*-Bu), 1255 (ν_{C-C-O} asym), 1165 (ν_{O-C-C} asym); 1H NMR (300 MHz, $CDCl_3$, δ): 1.32 (s, $C(CH_3)_3$ *t*-Bu, 9H), 1.41, 1.45 (2s, 2 $C(CH_3)_3$ Boc and *t*-Bu, 18H), 1.21-1.90 (m, 3 CH_2 Lys, 6H), 3.00 (t, $J = 6.3$ Hz, $CH_{2\beta}$, 2H), 3.15 (m, CH_2NH , 2H), 4.30-4.45 (m, 2 CH_{α} , 2H), 5.11 (s+M, CH_2Ph Z and NH , 3H), 6.51 (d, $J = 7.9$ Hz, NH , 1H), 6.90 (d, $J = 8.2$ Hz, ArH Tyr, 2H), 7.09 (d, $J = 8.2$ Hz, ArH Tyr, 2H), 7.36 (s, ArH Z, 5H); ^{13}C NMR ($CDCl_3$, 75 MHz): 22.3 (C), 28.6, 29.2, 29.3 (9C), 29.6, 32.4 (2C), 37.9 (C), 41.0 (C), 52.8 (C), 56.1 (C), 66.9 (C), 78.8 (C), 80.5 (C), 82.5 (C), 124.7, 128.4, 128.5, 128.8, 130.1 (9C), 131.8 (C), 137.0 (C), 154.5 (C), 155.9, 156.9 (2C), 171.3, 171.6 (2C); MS-ESI (m/z): 1333.1 [2M + Na]⁺, 678.2 [M + Na]⁺, 556.2 [M + H - isobutene - CO_2]⁺, 500.2 [M + H - isobutene - 2 CO_2]⁺; HRMS-ESI (m/z): [M + Na]⁺ calcd for $C_{36}H_{53}N_3O_8Na$, 678.3730; found, 678.37266.

N^{α} -Boc-L-Tyr(*t*Bu)-Gly-OtBu (14). Commercial N^{α} -Boc-Tyr(OtBu)-OH (2.87 mmol, 970 mg) was dissolved in DMF (57.4 mL). HOBt (3.39 mmol, 460 mg), EDCI (3.39 mmol, 650 mg), DIPEA (5.48 mmol, 0.96 mL) and commercial H-Gly-OtBu (2.61 mmol) were added successively. After stirring of the mixture overnight at room temperature, DMF was removed in vacuo and the resulting solid diluted with ethyl acetate (50 mL). The organic layer was washed successively with water (30 mL), saturated $NaHCO_3$ (2 × 30 mL), water (30 mL) and finally HCl 1 M (2 × 30 mL), dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by flash chromatography on silica gel (dichloromethane:methanol 9:1 then petroleum ether:ethyl acetate 1:1) to afford 574 mg of **14** (48.9% yield) as a foamy solid. IR (KBr, cm^{-1}): 3321 (ν_{NH}), 2979 (ν_{CH}), 2933 (ν_{CH}), 1745 ($\nu_{C=O}$), 1664 ($\nu_{C=O}$), 1508 ($\nu_{\phi C=C}$), 1392 (δ_{CH_3} *t*-Bu), 1367 (δ_{CH_3} *t*-Bu), 1237 (ν_{C-C-O} asym), 1161 (ν_{O-C-C} asym); 1H NMR (300 MHz, $CDCl_3$, δ): 1.25 (s, $C(CH_3)_3$ *t*-Bu, 9H), 1.28, (s, $C(CH_3)_3$ *t*-Bu, 9H), 1.41 (1s, $C(CH_3)_3$ *t*-Bu, 9H), 2.66 (dd, $J = 10.8$ Hz and 13.8 Hz, CH Gly, 1H), 2.96 (dd, $J = 3.6$ Hz and 14. Hz, CH Gly, 1H), 3.70 (dd, $J = 5.7$ Hz and 17.4 Hz, $CH_{2\beta}$, 1H), 3.79 (dd, $J = 6.0$ Hz and 17.4 Hz, $CH_{2\beta}$, 1H), 4.20 (m, CH_{α} , 1H), 6.85 (d, $J = 8.4$ Hz, ArH Tyr, 2H), 6.92 (d, $J = 8.4$ Hz, NH Tyr, 1H), 7.17 (d, $J = 8.4$ Hz, ArH Tyr, 2H), 8.27 (t, $J = 5.7$ Hz NH Gly, 1H); MS-ESI (m/z): 922.8 [2M + Na]⁺, 473.1 [M + Na]⁺, 451 [M + H]⁺; HRMS-ESI (m/z): [M + Na]⁺ calcd for $C_{24}H_{38}N_2O_6Na$ 473.2628; found, 473.26306.

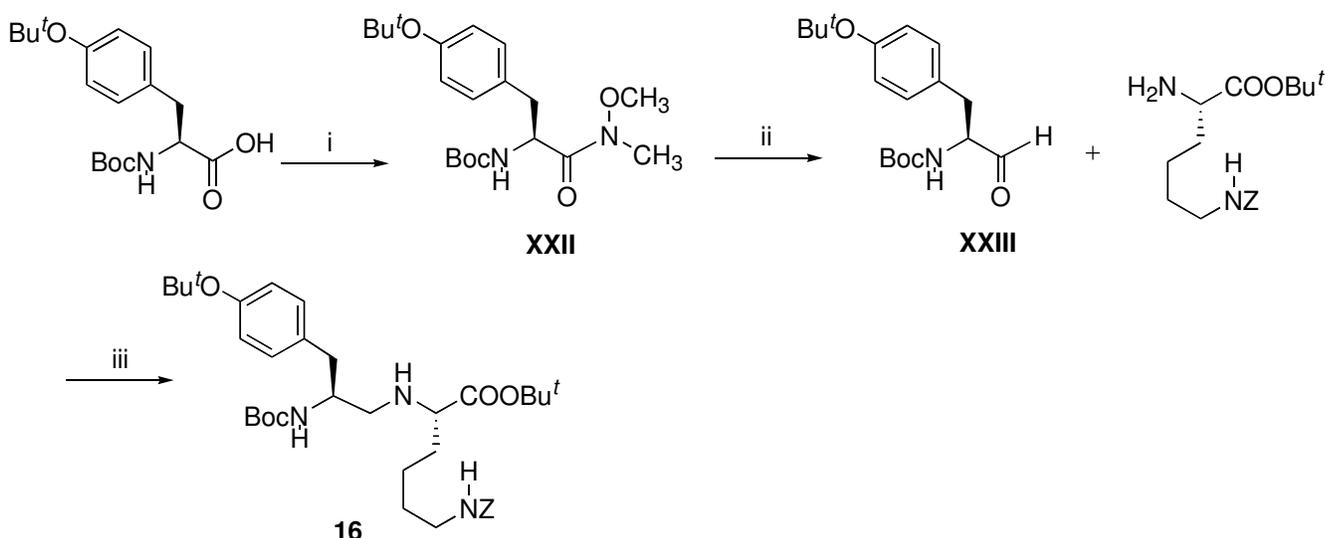


Scheme 7. Synthesis of compound 15. (i) TBTU, *N,O*-dimethylhydroxylamine.HCl, Et₃N, CH₂Cl₂, 1 h, RT, 66%. (ii) LiAlH₄, 30 min, 0 °C, 69%. (iii) NaBH₃CN, MeOH, CH₃COOH, 1 h, RT, 52%.

(4*R*)-Benzyloxy-(2*S*)-(methoxy-methyl-carbamoyl)-pyrrolidine-1-carboxylic acid *tert*-butyl ester (XX). Triethylamine (225 μ L, 1.6 mmol) was added to a stirred solution of commercial *N* ^{α} -Boc-*trans*-4-Hyp(Bn)-OH (515.7 mg, 1.6 mmol) in CH₂Cl₂ (10 mL). Then, TBTU (0.514 g, 1.6 mmol) was added, followed after few minutes by *N,O*-dimethylhydroxylamine hydrochloride (0.19 g, 1.92 mmol) and triethylamine (270 μ L, 1.92 mmol). The reaction was stirred at room temperature overnight. The mixture was then diluted with CH₂Cl₂ (10 mL) and washed three times successively with 10% HCl solution, saturated sodium bicarbonate solution and brine. The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. The colorless oily residue was purified by flash chromatography on silica gel (petroleum ether:ethyl acetate 5:5) to afford 387 mg of **XX** (66% yield) as a yellow oil. IR (Film, cm⁻¹): 3064 3031 (ν_{CH}), 2975 2935 (ν_{CH}), 1736 1695 1683 ($\nu_{\text{C=O}}$), 1455 ($\nu_{\text{C=C}}$), 1403 1367 (δ_{CH_3} *t*-Bu), 1250 ($\nu_{\text{C-C-O}}$ asym), 1164 ($\nu_{\text{O-C-C}}$ asym); ¹H NMR (300 MHz, CDCl₃, δ): 1.43, 1.47 (2s, C(CH₃)₃ Boc 1st and 2nd conf., 9H), 2.04 (m, CH _{β} , 1H), 2.38 (m, CH _{β} , 1H), 3.21 (s, CH₃N, 3H), 3.56-3.80 (m+2s, CH₂N and CH₃O 1st and 2nd conf., 5H), 4.19, 4.26 (2m, CH _{α} 1st and 2nd conf., 1H), 4.53 (m, CH₂Ph, 2H), 4.78, 4.86 (2t, *J* = 7.5 Hz and 7.3 Hz, CHOBn 1st and 2nd conf., 1H), 7.34 (m, ArH, 5H).

(4*R*)-Benzyloxy-(2*S*)-formyl-pyrrolidine-1-carboxylic acid *tert*-butyl ester (XXI). According to the general procedure GP-E, **XXI** was obtained from **XX** in 69% yield as a yellow oil after purification by flash chromatography on silica gel (petroleum ether:ethyl acetate 6:4). IR (Film, cm⁻¹): 3065 3032 (ν_{CH}), 2978 2931 (ν_{CH}), 2715 (ν_{CHO}), 1738 1704 1694 ($\nu_{\text{C=O}}$), 1455 ($\nu_{\text{C=C}}$), 1398 1367 (δ_{CH_3} *t*-Bu), 1256 ($\nu_{\text{C-C-O}}$ asym), 1163 ($\nu_{\text{O-C-C}}$ asym); ¹H NMR (300 MHz, CDCl₃, δ): 1.43, 1.49 (2s, C(CH₃)₃ Boc 1st and 2nd conf., 9H), 1.98 (m, CH _{β} , 1H), 2.28 (m, CH _{β} , 1H), 3.55, 3.81 (2m, CH₂N 1st and 2nd conf., 2H), 4.15 (m, CH _{α} , 1H), 4.25, 4.36 (2m, CHOBn 1st and 2nd conf., 1H), 4.54 (AB, *J* = 11.6 Hz, CH₂Ph, 2H), 7.34 (m, ArH, 5H), 9.45, 9.57 (2d, *J* = 3.7 Hz and 2.6 Hz, CHO 1st and 2nd conf., 1H).

***N* ^{α} -Boc-*trans*-L-(4*R*)Hyp(Bn)- ψ (CH₂-NH)-L-Lys(Z)-OtBu (15).** According to the general procedure GP-F, **15** was obtained from **XXI** and commercial H-Lys(Z)-OtBu.HCl in 52% yield as a yellow oil after purification by flash chromatography on silica gel (petroleum ether:ethyl acetate 6:4). [α]_D²⁶ -15.0 (*c* 1.00, CH₂Cl₂); IR (Film, cm⁻¹): 3343 (ν_{NH}), 3065 3033 (ν_{CH}), 2976 2933 (ν_{CH}), 1728 1716 1704 1694 1682 ($\nu_{\text{C=O}}$), 1455 ($\nu_{\text{C=C}}$), 1394 1367 (δ_{CH_3} *t*-Bu), 1247 ($\nu_{\text{C-C-O}}$ asym), 1162 ($\nu_{\text{O-C-C}}$ asym); ¹H NMR (300 MHz, CDCl₃, δ): 1.46 (2s, C(CH₃)₃ Boc, 9H), 1.35-1.68 (m, 3CH₂ Lys, 6H), 2.11 (m, CH_{2 β} , 2H), 2.65 (m, CH₂NH, 2H), 3.19 (q, *J* = 6.4 Hz, CH₂NHZ, 2H), 3.45 (m, CH₂N, 2H), 3.91-4.14 (m, 2CH _{α} , 2H), 4.49 (AB, *J* = 13.3 Hz, CH₂Ph Hyp, 2H), 4.80 (br, CHOBn, 1H), 5.10 (s, CH₂Ph Z, 2H), 7.31-7.38 (m, 2ArH, 10H); ¹³C NMR (CDCl₃, 75 MHz): 23.4 (C), 28.5, 28.8 (6C), 30.1, 33.6 (2C), 41.3 (C), 51.1, 51.3, 51.9 (3C), 56.6 (C), 62.6 (C), 66.8 (C), 71.2 (C), 76.6 (C), 79.9 (C), 81.4 (C), 128.0, 128.5, 128.8, 128.9 (10C), 137.0 (C), 138.5 (C), 155.4, 156.8 (2C), 175.2 (C); MS-ESI (*m/z*): 626.2 [M + H]⁺, 570.2 [M + H - isobutene]⁺, 514.1 [M + H - 2 isobutene]⁺, 470.3 [M + H - 2 isobutene - CO₂]⁺; HRMS-ESI (*m/z*): [M + H]⁺ calcd for C₃₅H₅₁N₃O₇, 626.3805; found, 626.38092.



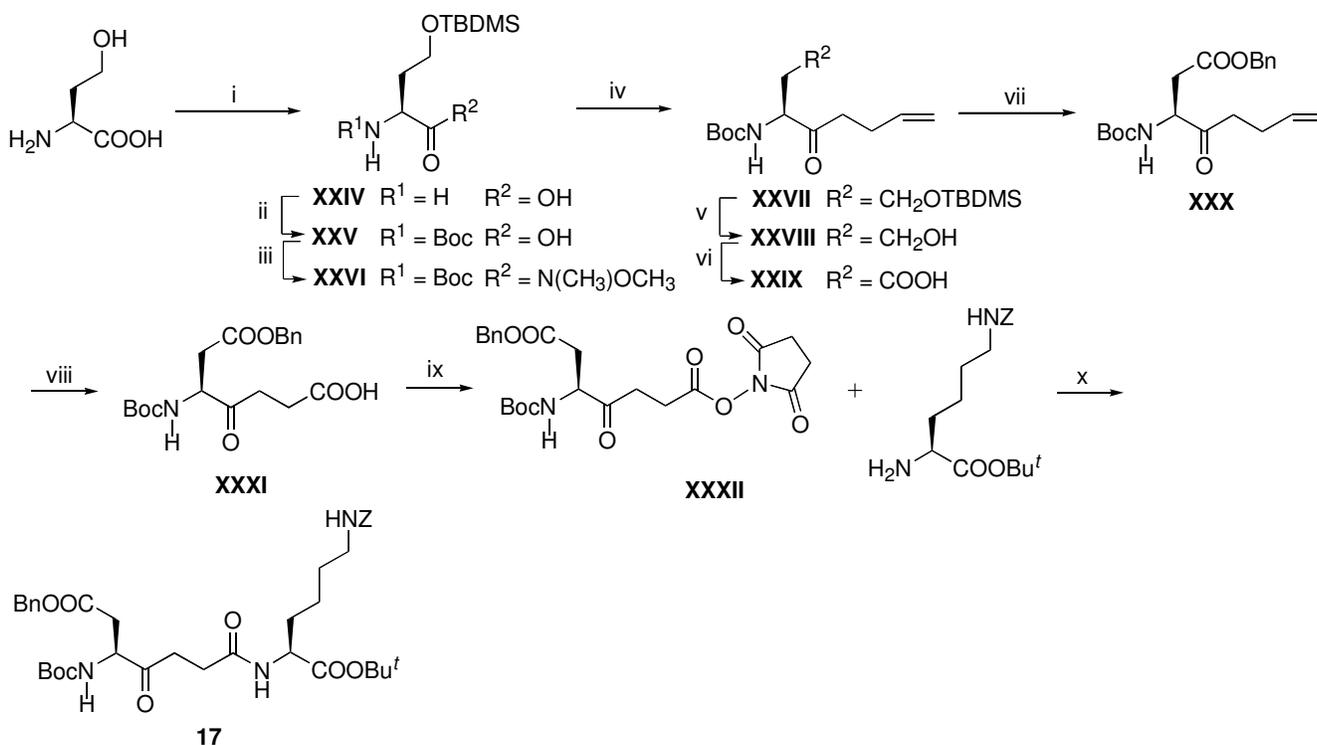
Scheme 8. Synthesis of compound 16. (i) TBTU, *N,O*-dimethylhydroxylamine.HCl, Et₃N, CH₂Cl₂, 1 h, RT, 93%. (ii) LiAlH₄, 30 min, 0 °C, 85%. (iii) NaBH₃CN, MeOH, CH₃COOH, 1 h, RT, 55%.

(2S)-tert-Butoxycarbonylamino-3-(tert-butoxyphenyl)-*N*-methoxy-*N*-methyl-propanamide (XXII). To a solution of commercial *N*^α-Boc-Tyr(*t*Bu)-OH in CH₂Cl₂ (10 mL) was added triethylamine (261 μL, 1.86 mmol) followed by TBTU (600 mg, 1.86 mmol). After 5 min of stirring at room temperature, *N,O*-dimethylhydroxylamine hydrochloride (220 mg, 2.23 mmol) and again triethylamine (314 μL, 2.23 mmol) were added. The reaction mixture was stirred at room temperature for 24 h. Dichloromethane (10 mL) was added and the organic layer was washed three times successively with 10% HCl solution, saturated sodium bicarbonate solution and brine, dried over anhydrous sodium sulfate, filtered and concentrated. The product **XXII** (533 mg, 93% yield) was obtained as a colorless oil with no purification needed. IR (Film, cm⁻¹): 3324 (ν_{NH}), 2977 2934 (ν_{CH}), 1713 (ν_{C=O} Boc), 1661 (ν_{C=O} amide), 1391 1366 (δ_{CH₃} *t*-Bu), 1237 (ν_{C-C-O} asym), 1164 (ν_{O-C-C} asym); ¹H NMR (300 MHz, CDCl₃, δ): 1.32, 1.40 (2s, 2C(CH₃)₃ Boc and *t*-Bu, 18H), 2.83 (m, CH_{2β}, 2H), 3.15 (s, NCH₃, 3H), 2.62 (s, OCH₃, 3H), 4.90 (m, CH_α, 1H), 5.18 (d, *J* = 7.9 Hz, NH, 1H), 6.91 (d, *J* = 8.2 Hz, ArH, 2H), 7.08 (d, *J* = 8.2 Hz, ArH, 2H).

(2S)-tert-Butyloxycarbonylamino-3-(tert-butoxyphenyl)-propanal (XXIII). According to the general procedure GP-E, **XXIII** was obtained from **XXII** in 85% yield as a colorless oil without purification needed. IR (Film, cm⁻¹): 3354 (ν_{NH}), 3029 (ν_{φCH}), 2978 2933 (ν_{CH}), 2820 2725 (ν_{CH} aldehyde), 1695 (ν_{C=O}), 1456 (ν_{φC=C}), 1392 1367 (δ_{CH₃} *t*-Bu), 1238 (ν_{C-C-O} asym), 1163 (ν_{O-C-C} asym); ¹H NMR (300 MHz, CDCl₃, δ): 1.26, 1.36 (2s, 2C(CH₃)₃ Boc and *t*-Bu, 18H), 2.99 (d, *J* = 6.7 Hz, CH_{2β}, 2H), 4.33 (q, *J* = 6.7 Hz, CH_α, 1H), 4.97 (d, *J* = 6.0 Hz, NH, 1H), 6.85 (d, *J* = 8.2 Hz, ArH, 2H), 6.99 (d, *J* = 8.2 Hz, ArH, 2H), 9.55 (s, CHO, 1H).

***N*^α-Boc-L-Tyr(*t*Bu)-ψ(CH₂-NH)-L-Lys(Z)-OtBu (16).** According to the general procedure GP-F, **16** was obtained from **XXIII** and commercial H-Lys(Z)-OtBu.HCl in 55% as a yellow oil after purification by flash chromatography on silica gel (petroleum ether:ethyl acetate 6:4). [α]_D²⁷ +2.0 (*c* 1.00, CH₂Cl₂); IR (Film, cm⁻¹): 3347 (ν_{NH}), 3064 3032 (ν_{φCH}), 2977 2933 (ν_{CH}), 1715 (ν_{C=O}), 1455 (ν_{φC=C}), 1391 1366 (δ_{CH₃} *t*-Bu), 1247 (ν_{C-C-O} asym), 1162 (ν_{O-C-C} asym); ¹H NMR (300 MHz, CDCl₃, δ): 1.32 (s, C(CH₃)₃ *t*-Bu, 9H), 1.42, 1.43 (2s, 2C(CH₃)₃ Boc and *t*-Bu, 18H), 1.21-1.80 (m, 3CH₂ Lys, 6H), 2.66-3.06 (m, CH₂NH and CH_{2β}, 4H), 3.20 (q, *J* = 6.5 Hz, CH₂NHZ, 2H), 3.80 (br, CH_α, 1H), 4.70 (br, CH_α, 1H), 4.90 (br, NH, 1H), 5.10 (s, CH₂Ph Z, 2H), 6.90 (d, *J* = 8.2 Hz, ArH Tyr, 2H), 7.08 (d, *J* = 8.2 Hz, ArH Tyr, 2H), 7.36 (s, ArH Z, 5H); ¹³C NMR (CDCl₃, 75 MHz): 23.3 (C), 28.4, 28.8, 29.1 (9C), 30.1, 33.1 (2C), 38.5 (C), 41.2 (C), 50.3 (C), 52.2 (C), 62.5 (C), 66.9 (C), 78.6 (C), 79.9 (C), 81.5 (C), 124.5, 124.6, 128.4, 128.5, 128.9, 130.1 (9C), 133.4 (C), 137.0 (C), 154.1 (C), 155.5, 156.0 (2C), 174.8 (C); MS-ESI (*m/z*):

664.3 [M + Na]⁺, 642.2 [M + H]⁺; HRMS-ESI (*m/z*): [M + Na]⁺ calcd for C₃₆H₅₅N₃O₇Na, 664.3938; found, 664.39401.



Scheme 9. Synthesis of compound 17. (i) TBDMS, CH₃CN, DBU, 24 h, RT, 82%. (ii) (Boc)₂O, Et₃N, acetone/H₂O, 24 h, RT, quant. yield. (iii) TBTU, HOBt, DIEA, *N,O*-dimethylhydroxylamine.HCl, 18 h, RT, 61%. (iv) 4-bromobut-1-ene, Mg, THF, -78 °C – RT, 4 h, 55%. (v) TBAF 1M in THF, RT, 1 h, 87%. (vi) PDC, DMF, RT, 1 h, 71%. (vii) Cs₂CO₃, MeOH, H₂O, benzyl bromide, RT, 4 h, 86%. (viii) NaIO₄, H₂O, RuCl₃.xH₂O, CH₃CN, RT, 1 h, 96%. (ix) *N*-hydroxysuccinimide, DMAP, DCC, THF, 10 min, 0° C – RT, 48 h, 49%. (x) DIEA, DMF, RT, 24 h 94%.

(2*S*)-Amino-4-(*tert*-butyl-dimethyl-silyloxy)-butanoic acid (XXIV). To a solution of *tert*-butyldimethylsilyl chloride (9 g, 58.8 mmol) in acetonitrile (20 mL) was added commercial L-homoserine (2 g, 16.8 mmol). The suspension was cooled to 0 °C and 1,8-diazabicyclo[5.4.0]undec-7-ene (8.8 mL, 57.12 mmol) was added. The reaction mixture was stirred for 24 h at room temperature under a nitrogen atmosphere. The solvent was evaporated and the solid residue was washed several times with diethyl ether. The diethyl ether layer was washed with water, dried over anhydrous sodium sulfate, filtered and concentrated. The oily residue was dried under a pump for two days. The resulted white solid was washed with diethyl ether to eliminate silanol. The product **XXIV** (3.2 g, 82% yield) was obtained as a white solid in a suitable state for the next reaction. Mp 203-205 °C; IR (KBr, cm⁻¹): 3434 3079 (ν_{NH2} and ν_{COOH}), 2930 (ν_{CH}), 1638 (ν_{C=O}), 1407 (δ_{OH acid}), 1257 (ν_{C-C-O asym}), 1100 (ν_{C-O} and ν_{C-N}), 1006 838 (ν_{Si-O-C}), 776 (ν_{Si-CH₃}); ¹H NMR (300 MHz, CD₃OD, δ): -0.002 (s, Si(CH₃)₂, 6H), 0.81 (s, Si-C(CH₃)₃, 9H), 1.84 (m, CH_β, 1H), 2.04 (m, CH_β, 1H), 3.56 (dd, *J* = 4.5 Hz and 7.5 Hz, CH_α, 1H), 3.75 (t, *J* = 6.2 Hz, CH₂O, 2H), 4.78 (s, NH₂, 2H); ¹³C NMR (CD₃OD, 75 MHz): -5.9 (2C), 18.0 (C), 25.4 (3C), 33.5 (C), 53.7 (C), 60.9 (C), 173.2 (C).

(2*S*)-*tert*-Butoxycarbonylamino-4-(*tert*-butyl-dimethyl-silyloxy)-butanoic acid (XXV). To a suspension of H-Hse(TBDMS)-OH **XXIV** (8.58 mmol) in 50% aqueous acetone (48 mL) were added, with stirring, triethylamine (1.8 mL, 12.87 mmol) and (Boc)₂O (9.44 mmol). After stirring overnight at room temperature, the solvent was removed under reduced pressure, and the oily residue was dissolved in water (40 mL). This solution was acidified to pH 3-4 with 5% citric acid and extracted with ethyl acetate (3 × 15 mL). The combined organic extracts were washed with saturated sodium chloride, dried over anhydrous sodium sulfate, filtered and concentrated to give *N*^α-Boc-Hse(TBDMS)-OH **XXV** in quantitative yield (2.3 g) as a light yellow oil which was used without purification. IR (Film, cm⁻¹): 3335

(ν_{NH} and ν_{COOH}), 2931 (ν_{CH}), 1715 ($\nu_{\text{C=O}}$), 1393 1369 (δ_{CH_3} *t*-Bu), 1256 ($\nu_{\text{C-C-O}}$ *asym*), 1165 ($\nu_{\text{O-C-C}}$ *asym*), 1006 838 ($\nu_{\text{Si-O-C}}$), 778 ($\nu_{\text{Si-CH}_3}$); ^1H NMR (300 MHz, CDCl_3 , δ): 0.10 (s, $\text{Si}(\text{CH}_3)_2$, 6H), 0.92 (s, $\text{Si-C}(\text{CH}_3)_3$, 9H), 1.45 (s, $-\text{C}(\text{CH}_3)_3$ Boc, 9H), 2.09 (m, $\text{CH}_{2\beta}$, 2H), 3.82 (m, CH_2O , 2H), 4.35 (m, CH_α , 1H), 5.93 (d, $J = 6.0$ Hz, *NH*, 1H).

[3-(*tert*-Butyl-dimethyl-silyloxy)-(1*S*)-(methoxy-methyl-carbamoyl)-propyl]-carbamic acid *tert*-butyl ester (XXVI). To a mixture of *N* $^\alpha$ -Boc-Hse(TBDMS)-OH **XXIV** (2.2 g, 6.6 mmol), TBTU (2.12 g, 6.6 mmol), HOBT (0.9 g, 6.6 mmol) and DIEA (1.2 mL, 6.6 mmol) in CH_2Cl_2 (80 mL) was added at 0 °C a mixture of *N,O*-dimethylhydroxylamine hydrochloride (0.72 g, 7.26 mmol) and DIEA (1.3 mL, 7.26 mmol) in CH_2Cl_2 (40 mL). After stirring overnight at room temperature, the solvent was evaporated to dryness and the residue was taken up in EtOAc (200 mL) and 5% aqueous KHSO_4 (80 mL). The organic phase was separated, washed with 5% aqueous KHSO_4 (80 mL), 5% aqueous NaHCO_3 (80 mL) and brine (80 mL), dried over anhydrous sodium sulfate, filtered and concentrated. The yellow oily residue was purified by flash chromatography on silica gel (petroleum ether:ethyl acetate 7:3) to afford 460 mg of **XXVI** (61% yield) as a white solid. Mp 38-40 °C; IR (KBr, cm^{-1}): 3325 (ν_{NH}), 2931 (ν_{CH}), 1715 ($\nu_{\text{C=O}}$ Boc), 1668 ($\nu_{\text{C=O}}$ amide), 1391 1366 (δ_{CH_3} *t*-Bu), 1254 ($\nu_{\text{C-C-O}}$ *asym*), 1005 836 ($\nu_{\text{Si-O-C}}$), 777 ($\nu_{\text{Si-CH}_3}$); ^1H NMR (300 MHz, CDCl_3 , δ): 0.01 (s, $\text{Si}(\text{CH}_3)_2$, 6H), 0.85 (s, $\text{Si-C}(\text{CH}_3)_3$, 9H), 1.37 (s, $-\text{C}(\text{CH}_3)_3$ Boc, 9H), 1.66 (m, CH_β , 1H), 1.91 (m, CH_β , 1H), 3.15 (s, NCH_3 , 3H), 3.63 (m, CH_2O , 2H), 3.73 (s, OCH_3 , 3H), 4.69 (m, CH_α , 1H), 5.40 (d, $J = 7.5$ Hz, *NH*, 1H).

{(1*S*)-[2-(*tert*-Butyl-dimethyl-silyloxy)-ethyl]-2-oxo-hex-5-enyl} carbamic acid *tert*-butyl ester (XXVII). The Weinreb amide **XXVI** (440 mg, 1.17 mmol) was dissolved in distilled THF (25 mL), cooled to -78 °C under argon atmosphere. A solution of 4-bromo-but-1-ene (0.61 mL, 5.85 mmol) in distilled THF (9 mL) was added dropwise to magnesium turnings (0.14 g, 5.85 mmol) in distilled THF (1 mL) in order to obtain a solution of but-3-enyl-magnesium bromide. The resulting but-3-enyl-magnesium bromide was added dropwise at -78 °C to the Weinreb amide solution. The cooling bath was removed and the resulting mixture was stirred for further 4 h. Et_2O (75 mL) and 5% aqueous NH_4Cl (40 mL) were added, the organic phase separated, washed with water, dried over anhydrous sodium sulfate, filtered and concentrated. The yellow oily residue was purified by flash chromatography on silica gel (petroleum ether:ethyl acetate 9:1) to afford 238 mg of **XXVII** (55% yield) as a colorless oil. $[\alpha]_{\text{D}}^{23} +21.0$ (*c* 1, CH_2Cl_2); IR (Film, cm^{-1}): 3413 (ν_{NH}), 3080 (ν_{CH} vinyl), 2931 (ν_{CH}), 1714 ($\nu_{\text{C=O}}$ ketone), 1643 ($\nu_{\text{C=C}}$), 1392 1367 (δ_{CH_3} *t*-Bu), 1254 ($\nu_{\text{C-C-O}}$ *asym*), 1005 837 ($\nu_{\text{Si-O-C}}$), 913 (δ_{CH} vinyl), 777 ($\nu_{\text{Si-CH}_3}$); ^1H NMR (300 MHz, CDCl_3 , δ): 0.05 (s, $\text{Si}(\text{CH}_3)_2$, 6H), 0.90 (s, $\text{Si-C}(\text{CH}_3)_3$, 9H), 1.45 (s, $-\text{C}(\text{CH}_3)_3$ Boc, 9H), 1.97 (m, $\text{CH}_{2\beta}$, 2H), 2.34 (m, $\text{CH}_2\text{CH}=\text{CH}_2$, 2H), 2.66 (m, CH_2CO , 2H), 3.69 (t, $J = 5.8$ Hz, CH_2O , 2H), 4.30 (q, $J = 5.6$ Hz, CH_α , 1H), 5.02 (m, $\text{CH}=\text{CH}_2$, 2H), 5.74-5.88 (br, $\text{CH}=\text{CH}_2$ and *NH*, 2H).

[(1*S*)-(2-Hydroxy-ethyl)-2-oxo-hex-5-enyl]-carbamic acid *tert*-butyl ester (XXVIII). Ketone **XXVII** (1.124 g, 3 mmol) was dissolved in THF (60 mL) and 1 M tetrabutylammonium fluoride solution in THF was introduced dropwise (3.6 mL, 3.6 mmol). After the mixture was stirred at room temperature for 1h, the reaction mixture was diluted with diethyl ether (100 mL), washed successively with water (100 mL) and brine (80 mL) dried over anhydrous sodium sulfate, filtered and concentrated. The yellow oily residue was purified by flash chromatography on silica gel (petroleum ether:ethyl acetate 4:6) to afford 670 mg of **XXVIII** (87% yield) as a yellow oil. $[\alpha]_{\text{D}}^{24} +14.0$ (*c* 1, CH_2Cl_2); IR (Film, cm^{-1}): 3391 (ν_{NH} and ν_{OH}), 3080 (ν_{CH} vinyl), 2931 (ν_{CH}), 1694 ($\nu_{\text{C=O}}$), 1643 ($\nu_{\text{C=C}}$), 1392 1367 (δ_{CH_3} *t*-Bu), 1253 ($\nu_{\text{C-C-O}}$ *asym*), 912 (δ_{CH} vinyl); ^1H NMR (300 MHz, $\text{DMSO} + \text{D}_2\text{O}$, δ): 1.42 (s, $-\text{C}(\text{CH}_3)_3$ Boc, 9H), 1.74 (m, $\text{CH}_{2\beta}$, 2H), 2.22 (m, $\text{CH}_2\text{CH}=\text{CH}_2$, 2H), 2.57 (m, CH_2CO , 2H), 3.43 (m, CH_2OH , 2H), 4.05 (dd, $J = 4.5$ and 9.4 Hz, CH_α , 1H), 5.01 (m, $\text{CH}=\text{CH}_2$, 2H), 5.81 (m, $\text{CH}=\text{CH}_2$, 1H); ^{13}C NMR (DMSO , 75 MHz): 27.9 (C), 29.0 (3C), 34.8 (C), 37.8 (C), 57.5 (C), 58.1 (C), 78.7 (C), 115.8 (C), 138.4 (C), 156.0 (C), 210.7 (C).

(3*S*)-*tert*-Butoxycarbonylamino-4-oxo-oct-7-enoic acid (XXIX). Alcohol **XXVIII** (0.78 mmol) was dissolved in freshly distilled DMF (3.6 mL) and solid pyridinium dichromate (1.5 g, 3.9 mmol) was added in one portion. After 3h the reaction was quenched by addition of saturated aqueous NaHCO_3 (26 mL). The mixture was extracted once with petroleum ether and the aqueous phase was acidified to pH 4

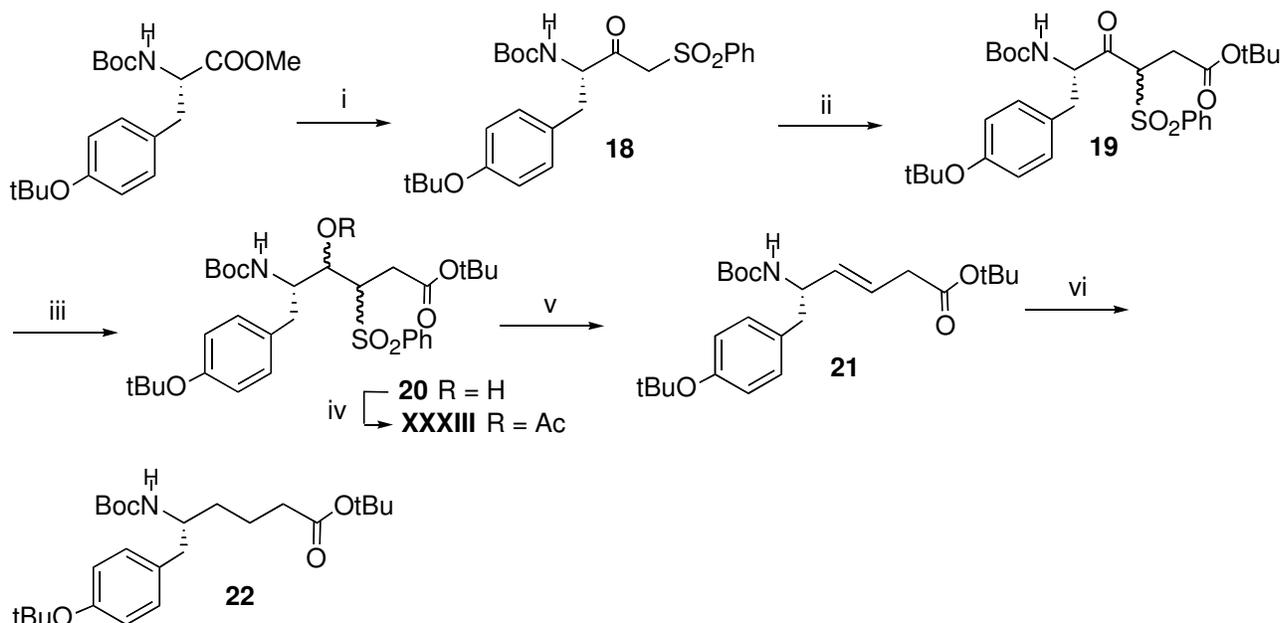
by addition of 1 M HCl solution. The solution was extracted twice with diethyl ether, dried over anhydrous sodium sulfate, filtered and concentrated. The yellow oily residue was purified by flash chromatography on silica gel (first with ethyl acetate:petroleum ether 6:4 then with the same eluent + 0.1% formic acid) to afford 150 mg of **XXIX** (71%) as a yellow oil. $[\alpha]_D^{23} +2.0$ (*c* 1, CH₂Cl₂); IR (Film, cm⁻¹): 3351 (ν_{NH} and ν_{COOH}), 2934 (ν_{CH}), 1714 (ν_{C=O}), 1394 1369 (δ_{CH₃ *t*-Bu), 916 (δ_{CH} vinyl); ¹H NMR (300 MHz, CDCl₃, δ): 1.39 (s, -C(CH₃)₃ Boc, 9H), 2.26 (m, CH₂CH=CH₂, 2H), 2.82-3.00 (m, 2CH₂CO, 4H), 4.37 (m, CH_α, 1H), 4.94 (m, CH=CH₂, 2H), 5.52 (d, *J* = 8.6 Hz, NH, 1H), 5.72 (m, CH=CH₂, 1H).}

(3S)-tert-Butoxycarbonylamino-4-oxo-oct-7-enoic acid benzyl ester (XXX). According to the general procedure GP-J, **XXX** was obtained from acid **XXIX** in 86% yield as a colorless oil after purification by flash chromatography on silica gel (petroleum ether:ethyl acetate 8:2). $[\alpha]_D^{25} +5.0$ (*c* 1, CH₂Cl₂); IR (Film, cm⁻¹): 3369 (ν_{NH}), 3035 (ν_{φCH}), 2933 (ν_{CH}), 1715 (ν_{C=O}), 1642 (ν_{C=C}), 1456 (ν_{φC=C}), 1392 1367 (δ_{CH₃ *t*-Bu), 1252 (ν_{C-C-O} asym), 914 (δ_{CH} vinyl); ¹H NMR (300 MHz, CDCl₃, δ): 1.47 (s, -C(CH₃)₃ Boc, 9H), 2.33 (m, CH₂CH=CH₂, 2H), 2.67 (m, CH₂CH₂CO, 2H), 2.80 (dd, *J* = 4.8 Hz and 16.9 Hz, CH_β, 1H), 3.05 (dd, *J* = 4.9 Hz and 17.3 Hz, CH_β, 1H), 4.46 (m, CH_α, 1H), 5.02 (m, CH=CH₂, 2H), 5.13 (AB, *J* = 12.4 Hz, CH₂Ph, 2H), 5.60 (d, *J* = 8.2 Hz, NH, 1H), 5.80 (m, CH=CH₂, 1H), 7.37 (m, ArH, 5H).}

(3S)-tert-Butoxycarbonylamino-4-oxo-heptanedioic acid 1-benzyl ester (XXXI). According to the general procedure (GP-F), **XXXI** was obtained from **XXX** in 96% yield as a viscous oil with no purification needed. $[\alpha]_D^{23} +9.0$ (*c* 1, CH₂Cl₂); IR (Film, cm⁻¹): 3355 (ν_{NH} and ν_{COOH}), 3035 (ν_{φCH}), 2931 (ν_{CH}), 1715 (ν_{C=O}), 1456 (ν_{φC=C}), 1393 1368 (δ_{CH₃ *t*-Bu), 1252 (ν_{C-C-O} asym), 1165 (ν_{O-C-C} asym); ¹H NMR (300 MHz, CDCl₃, δ): 1.47 (s, -C(CH₃)₃ Boc, 9H), 2.65 (t, *J* = 6.7 Hz, CH₂CO, 2H), 2.80-3.08 (m, CH₂CO₂H and CH₂CO₂Bn, 4H), 4.52 (m, CH_α, 1H), 5.13 (AB, *J* = 12.8 Hz, CH₂Ph, 2H), 5.62 (d, *J* = 9.0 Hz, NH, 1H), 7.36 (m, ArH, 5H).}

(3S)-tert-Butoxycarbonylamino-4-oxo-heptanedioic acid 7-benzyl ester 1-(2,5-dioxo-pyrrolidin-1-yl) ester (XXXII). According to the general procedure GP-H, **XXXII** was obtained in 49% yield as a white solid after purification by flash chromatography on silica gel (petroleum ether:ethyl acetate 5:5). IR (KBr, cm⁻¹): 3327 (ν_{NH}), 2930 (ν_{CH}), 1817 1785 1740 (ν_{C=O}), 1456 (ν_{φC=C}), 1368 (δ_{CH₃ *t*-Bu), 1207 1070 (ν_{NO}); ¹H NMR (300 MHz, CDCl₃, δ): 1.47 (s, -C(CH₃)₃ Boc, 9H), 2.83 (s, 2CH₂CON, 4H), 2.87-3.16 (m, CH₂CO, CH₂CO₂N and CH₂CO₂Bn, 6H), 4.51 (m, CH_α, 1H), 5.12 (s, CH₂Ph, 2H), 5.60 (d, *J* = 10.5 Hz, NH, 1H), 7.37 (m, ArH, 5H).}

N^α-Boc-L-Asp(OBn)-ψ(CO-CH₂)-Gly-L-Lys(Z)-OtBu (17). According to the general procedure GP-I, **17** was obtained from **XXXII** and commercial H-Lys(Z)-OtBu.HCl in 94% yield as a viscous yellow oil after purification by flash chromatography on silica gel (petroleum ether:ethyl acetate 4:6). $[\alpha]_D^{24} +14.0$ (*c* 1, CH₂Cl₂); IR (Film, cm⁻¹): 3326 (ν_{NH}), 2931 (ν_{CH}), 1716 (ν_{C=O}), 1456 (ν_{φC=C}), 1368 (δ_{CH₃ *t*-Bu), 1248 (ν_{C-C-O} asym), 1159 (ν_{O-C-C} asym); ¹H NMR (300 MHz, DMSO, δ): 1.38 (s, 2C(CH₃)₃ Boc and *t*-Bu, 18H), 1.23-1.82 (m, 3CH₂ Lys, 6H), 2.26-2.82 (s, CH₂CO, CH₂CONH and CH₂CO₂Bn, 6H), 2.97 (q, *J* = 6.5 Hz, CH₂N, 2H), 4.02 (m, CH_α, 1H), 4.33 (m, CH_α, 1H), 4.99 (s, CH₂Ph, 2H), 5.08 (s, CH₂Ph, 2H), 5.57 (d, *J* = 8.6 Hz, NH, 1H), 7.34 (m, 2ArH, 10H), 7.45 (d, *J* = 8.2 Hz, NH, 1H), 8.10 (d, *J* = 9.0 Hz, NH, 1H); ¹³C NMR (CDCl₃, 75 MHz): 28.4, 28.7 (6C), 25.3, 29.7, 30.2 (3C), 32.5, 34.3, 36.1, 41.0 (4C), 52.8 (C), 56.4 (C), 66.9, 67.2 (2C), 80.7, 82.5 (2C), 128.4, 128.6, 128.8, 128.9, 129.0 (10C), 135.8, 137.1 (2C), 155.8, 156.9, 157.1 (3C), 171.9, 172.0 (2C), 208.0 (C); MS-ESI (*m/z*): 1416.7 [2M + Na]⁺, 720.2 [M + Na]⁺, 698 [M + H]⁺; HRMS-ESI (*m/z*): [M + Na]⁺ calcd for C₃₇H₅₁N₃O₁₀, Na 720.3472; found, 720.34692.}



Scheme 10. Synthesis of compound 19 to 22. Reagents and conditions: (i) $\text{PhSO}_2\text{CHLi}_2$, THF, 0°C 30 min then -30°C 3 h, 71%. (ii) $\text{BrCH}_2\text{COOtBu}$, K_2CO_3 , DMF, RT, 6 h, 78%. (iii) NaBH_4 , MeOH, -10°C 3 h, 83%. (iv) Ac_2O , DMAP, pyridine, RT, 3 h, 93%. (v) Na/Hg , MeOH, Na_2HPO_4 , -10°C , 2 h, 70%. (vi) H_2 , Pd/C, EtOH 95° , RT, 20 h, 90%.

2-oxo-3-[(*tert*-butyloxy)carbonylamino]-4-(4*S*)-(4-*tert*-butyloxy)phenyl)-butyl phenylsulfone (18). Methylphenylsulfone (1.75 g, 11.1 mmol) was dissolved in 10 ml of anhydrous THF. At 0°C , a solution of *n*-butyllithium 1.6 M in hexane (29.9 mmol, 18.76 mL) was added dropwise and the solution stirred for 30 min. The temperature was lowered to -30°C and a solution of N^α -Boc-Tyr(*t*Bu)-OMe (2.07 g, 5.9 mmol) in distilled THF (5 mL) was then added dropwise. The reaction mixture was stirring for 3 h and then hydrolyzed with 1 M HCl (1 mL). The aqueous phase was extracted three times with 10 ml ethyl acetate. The organic phases were collected, washed three times with brine (10 mL) and then dried over anhydrous sodium sulfate. The yellow oily residue was purified by flash chromatography on silica gel (petroleum ether:ethyl acetate 7:3) to yield **18** (2 g, 71% yield) as a white solid. $[\alpha]_D^{20} +16.2$ (c 0.1235, CHCl_3); IR (Film, cm^{-1}): 3395 (ν_{NH}), 3070 (ν_{CH}), 2977 2928 (ν_{CH}), 1715 ($\nu_{\text{C=O}}$), 1540 1507 1448 ($\nu_{\text{C=C}}$), 1367 (δ_{CH_3} *t*-Bu), 1307 (ν_{SO_2}), 1153 ($\nu_{\text{O-C-C asy}}$, ν_{SO_2}); $^1\text{H NMR}$ (300 MHz, CDCl_3 , δ): 1.34 (s, $\text{C}(\text{CH}_3)_3$ *t*-Bu, 9H), 1.38 (s, $\text{C}(\text{CH}_3)_3$ Boc, 9H), 2.88 (dd, $J = 8.5$ and 14.0 Hz, CH_β , 1H), 3.11 (dd, $J = 5.5$ and 14.3 Hz, CH_β , 1H), 4.19 (d, $J = 14$ Hz, CHSO_2Ph), 4.34 (d, $J = 14$ Hz, CHSO_2Ph), 4.44 (sext, $J = 5.7$, 8.0 and 8.0 Hz, CH_α , 1H), 5.09 (d, $J = 7.0$ Hz, NH, 1H), 6.92 (d, $J = 8.5$ Hz, ArH, 2H), 7.04 (d, $J = 8.5$ Hz, ArH, 2H), 7.57 (m, SO_2ArH , 2H), 7.68 (m, SO_2ArH , 1H), 7.87 (m, SO_2ArH , 2H); MS-ESI (m/z): 476.3 [$\text{M} + \text{H}$] $^+$, 420.2 [$\text{M} + \text{H} - t\text{-Bu}$] $^+$, 364.1 [$\text{M} + \text{H} - 2 t\text{-Bu}$] $^+$; HRMS-ESI (m/z): [$\text{M} + \text{Na}$] $^+$ calcd for $\text{C}_{31}\text{H}_{43}\text{NSO}_8\text{Na}$, 612.2607; found, 612.26031.

(3*S*)-Phenylsulfonyl-4-oxo-(5*S*)-[(*tert*-butyloxy)carbonylamino]-6-(4-*tert*-butyloxy)phenyl)hexanoic acid *tert*-butyl ester (19). Potassium carbonate (387.6 mg, 2.8 mmol) and *tert*-butyl bromoacetate (716 μL , 2.8 mmol) were added to a solution of **18** (2.8 mmol, 1.34 g) in DMF (10 ml). The solution was stirred for 5 h at room temperature. The reaction mixture was then dissolved in water (10 mL) and extracted 3 times with ethyl acetate (10 mL). The organic phase was collected, washed three times with diluted HCl (10 ml) and then dried over anhydrous sodium sulfate. The oily residue was purified by flash chromatography on silica gel (petroleum ether:ethyl acetate 8:2) to afford **19** as a white solid (1.29 g, 78% yield). IR (Film, cm^{-1}): 3418 (ν_{NH}), 3061 (ν_{CH}), 2979 2933 (ν_{CH}), 1722 ($\nu_{\text{C=O}}$), 1608 1507 1448 ($\nu_{\text{C=C}}$), 1393 1367 (δ_{CH_3} *t*-Bu), 1324 (ν_{SO_2}), 1160 ($\nu_{\text{O-C-C asy}}$, ν_{SO_2}); $^1\text{H NMR}$ (300 MHz, CDCl_3 , δ): 1.32 (s, $\text{C}(\text{CH}_3)_3$ *t*-Bu, 9H), 1.37 (s, $\text{C}(\text{CH}_3)_3$ Boc or *t*-Bu, 9H), 1.39 (s, $\text{C}(\text{CH}_3)_3$ Boc or *t*-Bu, 9H), 2.73 to 3.39 (m, $\text{CH}_2\beta$ and $\text{CH}_2\text{CO}_2t\text{Bu}$, 4H), 4.98 to 5.10 (m, CHSO_2Ph and CH_α 2H), 5.23 and 5.39 (d, $J =$

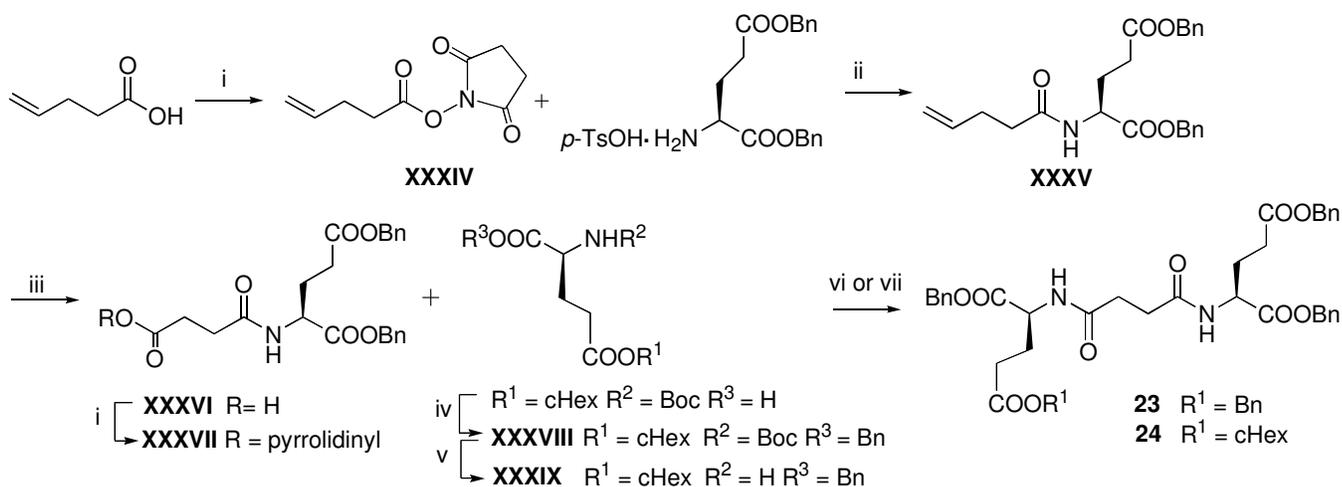
9.1 Hz, NH, 1H), 6.91 (m, ArH, 2H), 7.13 (m, ArH, 2H), 7.48 to 7.76 (m, SO₂ArH, 5H); MS-ESI (*m/z*): 590.5 [M + H]⁺, 534.3 [M + H - *t*-Bu]⁺, 478.2 [M + H - 2 *t*-Bu]⁺, 422.1 [M + H - 3 *t*-Bu]⁺; HRMS-ESI (*m/z*): [M + Na]⁺ calcd for C₃₁H₄₃NSO₈Na, 612.2607; found, 612.26031.

(3*RS*)-Phenylsulfonyl-(4*RS*)-hydroxy-(5*S*)-[(*tert*-butyloxy)carbonylamino]-6-(4-*tert*-butyloxy)-phenyl)hexanoic acid *tert*-butyl ester (20**).** Sodium borohydride (387.6 mg, 2.8 mmol) was added at -10 °C to a solution of **19** (328mg, 0.5 mmol) in 2 mL of methanol. The reaction mixture was stirred for 3 h and then hydrolyzed with 1M HCl (2 mL). The solution was diluted with water (5 mL) and the aqueous phase was extracted three times with ethyl acetate (5 mL). The organic layers were collected, washed three times with brine (5 mL) and then dried over anhydrous sodium sulfate. The oily residue obtained after evaporation was purified by flash chromatography on silica gel (petroleum ether:ethyl acetate 2:8) to afford the title product as a colorless oil (275 mg, 83% yield). IR (Film, cm⁻¹): 3500 (ν_{OH}), 3378 (ν_{NH}), 3061 (ν_{φCH}), 2979 2933 (ν_{CH}), 1729 1711 (ν_{C=O}), 1609 1505 1448 (ν_{φC=C}), 1367 (δ_{CH₃ *t*-Bu}), 1304 (ν_{SO₂}), 1161 (ν_{O-C-C} _{asym}, ν_{SO₂}); ¹H NMR (300 MHz, CDCl₃, δ): 1.30 (s, C(CH₃)₃ *t*-Bu, 9H), 1.32 (s, C(CH₃)₃ Boc or *t*-Bu, 9H), 1.36 (s, C(CH₃)₃ Boc or *t*-Bu, 9H), 2.60 to 2.92 (m, CH_{2β} and CH₂CO₂*t*Bu, 4H), 3.81 to 4.13 (m, CHSO₂Ph CH_α and CHOH 3H), 4.31 (s, OH, 1H), 4.80 4.89 (d, *J* = 9.3 Hz, NH, 1H), 6.87 (m, ArH, 2H), 7.07 (m, ArH, 2H), 7.52 to 7.95 (m, SO₂ArH, 5H); MS-ESI (*m/z*): 492.5 [M + H]⁺, 536.3 [M + H - *t*-Bu]⁺, 480.2 [M + H - 2 *t*-Bu]⁺; HRMS-ESI (*m/z*): [M + Na]⁺ calcd for C₃₁H₄₅NaNSO₈, 614.2764; found, 614.27594.

(3*RS*)-Phenylsulfonyl-(4*RS*)-acetoxy-(5*S*)-[(*tert*-butyloxy)carbonylamino]-6-(4-*tert*-butyloxy)-phenyl)hexanoic acid *tert*-butyl ester (XXXIII**).** DMAP (22.9 mg, 0.19 mmol) and acetic anhydride (55 mL, 0.93 mmol) were added at room temperature to a solution of **20** (110 mg, 0.19 mmol) in 2 mL of pyridine. The reaction mixture was left under stirring for 3 h and then diluted with ethyl acetate before being hydrolyzed with 1 M HCl to neutrality. The aqueous phase is extracted three times with ethyl acetate (5mL). The organic layers were collected, washed three times with brine (5 mL) then dried over anhydrous sodium sulfate. The solvent is evaporated and the remaining pyridine is eliminated by coevaporation with toluene. The title product was obtained as an oil (110 mg, 93% yield). IR (Film, cm⁻¹): 3376 (ν_{NH}), 3060 (ν_{φCH}), 2978 2932 (ν_{CH}), 1732 1714 (ν_{C=O}), 1609 1506 1448 (ν_{φC=C}), 1367 (δ_{CH₃ *t*-Bu}), 1309 (ν_{SO₂}), 1161 (ν_{O-C-C} _{asym}, ν_{SO₂}); ¹H NMR (300 MHz, CDCl₃, δ): 1.32 (s, C(CH₃)₃ *t*-Bu, 9H), 1.37 (s, C(CH₃)₃ Boc or *t*-Bu, 9H), 1.38 (s, C(CH₃)₃ Boc or *t*-Bu, 9H), 2.50 to 2.95 (m, CH_{2β} and CH₂CO₂*t*Bu, 4H), 4.02 to 4.08 (m, CHSO₂Ph or CH_α 1H), 4.65 to (m, CHSO₂Ph or CH_α, 1H), 4.64 and 4.99 (d, *J* = 9.6 Hz, NH, 1H), 5.23 to 5.38 (m, CHOAc, 1H) 6.90 (m, ArH, 2H), 7.06 (m, ArH, 2H), 7.50 to 7.95 (m, SO₂ArH, 5H); MS-ESI (*m/z*): 634.6 [M + H]⁺, 592.5 [M + H - CH₃CO]⁺, 578.4 [M + H - *t*-Bu]⁺, 522.3 [M + H - *t*-Bu - CH₃CO]⁺; HRMS-ESI (*m/z*): [M + Na]⁺ calcd for C₃₃H₄₇NSO₉Na, 656.2869; found, 656.28633.

N^α-Boc-L-Tyr(*t*Bu)-ψ(CH=CH)-Gly-O*t*Bu (21**).** K₂HPO₄ (470 mg, 3 mmol) and Na / Hg amalgam (495 mg, 0.19 mmol Na) were added at -10 °C to a solution of **XXXIII** (190 mg, 0.3 mmol) in methanol (5 mL). The reaction mixture was stirred for 3 h at -10 °C. The suspension was decanted and diluted with water. The aqueous phase was then extracted three times with ethyl acetate (5 mL) and the organic layers were collected and washed three times with brine (5 mL) before being dried over anhydrous sodium sulfate. The solvent was finally evaporated to yield a crude solid purified by flash chromatography on silica gel (petroleum ether:ethyl acetate 2:8). The title product (90 mg, 70% yield) was obtained as a glassy solid. [α]_D²⁰ +2.6 (c 0.57, CHCl₃); IR (Film, cm⁻¹): 3360 (ν_{NH}), 2932 (ν_{CH}), 1713 (ν_{C=O}), 1503 (ν_{φC=C}), 1391 (δ_{CH₃ *t*-Bu}), 1237 (ν_{C-C-O} _{asym}), 1162 (ν_{O-C-C} _{asym}); ¹H NMR (300 MHz, CDCl₃, δ): 1.32 (s, C(CH₃)₃ *t*-Bu, 9H), 1.39 (s, C(CH₃)₃ Boc or *t*-Bu, 9H), 1.43 (s, C(CH₃)₃ Boc or *t*-Bu, 9H), 2.78 (d, *J* = 6.2 Hz, CH_{2β} or CH₂CO₂*t*Bu, 2H), 2.94 (d, *J* = 6.6 Hz, CH₂CO₂*t*Bu or CH_{2β}, 2H), 4.37 (m, CH_α or NH, 1H), 4.46 (m, NH or CH_α, 1H), 5.50 (dd, *J* = 15.8 Hz and 5.1 Hz, =CH, 1H), 5.62 (m, =CH, 1H), 6.90 (d, *J* = 8.3 Hz, ArH, 2H), 7.05 (d, *J* = 8.3 Hz, ArH, 2H); MS-ESI (*m/z*): 456.1 [M + Na]⁺; HRMS-ESI (*m/z*): [M + Na]⁺ calcd for C₂₅H₃₉NaNO₅, 456.2726; found, 456.27302.

***N*^α-Boc-L-Tyr(*t*Bu)-ψ(CH₂-CH₂)-Gly-OBu (22).** **21** (180 mg, 0.41 mmol) was dissolved in 5 mL of methanol. The solution was placed in a Parr device with a catalytic amount of palladium on activated charcoal (10% Pd) under H₂ (10 bars) for 20 h. After opening, the solution was filtered and the solvent removed under reduced pressure to yield **22** as a glassy solid (180.5 mg, quantitative yield). [α]_D²⁰ +25.6 (c 0.195, CHCl₃); IR (Film, cm⁻¹): 3364 (ν_{NH}), 3000 (ν_{CH}), 2977 2930 (ν_{CH}), 1714 (ν_{C=O}), 1609 1506 1456 (ν_{φC=C}), 1391 1366 (δ_{CH₃ *t*-Bu}), 1237 (ν_{C-C-O asym}), 1162 (ν_{O-C-C asym}); ¹H NMR (300 MHz, CDCl₃, δ): 1.32 (s, C(CH₃)₃ *t*-Bu, 9H), 1.40 1.41 (2s, 2C(CH₃)₃ Boc and *t*-Bu, 18H), 1.64 (m, 2CH₂, 4H), 2.19 (m, CH₂CO₂*t*Bu, 2H), 2.70 (d, *J* = 5.8 Hz, CH_{2β}, 2H), 3.78 (m, CH_α or NH, 1H), 4.34 (m, NH or CH_α, 1H), 7.05 (d, *J* = 8.1 Hz, ArH, 2H); MS-ESI (*m/z*): 436 [M + H]⁺, 380 [M + H - *t*-Bu]⁺, 324 [M + H - 2 *t*-Bu]⁺; HRMS-ESI (*m/z*): [M + Na]⁺ calcd for C₂₅H₄₁NaNO₅, 458.2882; found, 458.28789.



Scheme 11. Synthesis of compounds **23** and **24**. (i) *N*-hydroxysuccinimide, DMAP, DCC, THF, 10 min, 0° C – RT, 48 h, 94% for **XXXIV**, 73% for **XXXVII**. (ii) DIEA, DMF, 24 h, RT, quant. yield. (iii) NaIO₄, H₂O, RuCl₃.xH₂O, CH₃CN, RT, 1 h, 73%. (iv) Cs₂CO₃, MeOH, H₂O, benzyl bromide, RT, 4 h, 73%. (v) ethyl acetate, H₂SO₄, RT, 1 h, 96%. (vi) a. ethyl chloroformate, Et₃N, CH₂Cl₂, 30 min, 0° C. b. H-Glu(OBn)-OBn.*p*TsOH, Et₃N, CH₂Cl₂, 0° C, 2 h – RT, 24 h, 35 %. (vii) H-Glu(OcHex)-OBn, Et₃N, CH₂Cl₂, 0° C, 2 h – RT, 24 h, 45 %.

Pent-4-enoic acid 2,5-dioxo-pyrrolidin-1-yl ester (XXXIV). According to the general procedure GP-H, **XXXIV** was obtained from pent-4-enoic acid in 94% yield as a white solid after purification by flash chromatography on silica gel (0.02% MeOH in CH₂Cl₂). Mp 122-124 °C; IR (KBr; cm⁻¹): 3083 3070 3009 (ν_{CH₂=CH}), 2979 2952 2925 (ν_{CH}), 1818 1788 1726 (ν_{C=O}), 1643 (ν_{C=C}), 1204 1070 (ν_{NO}); ¹H NMR (300 MHz, CDCl₃, δ): 2.40-2.60 (q, CH₂COO, 2H), 2.70 (t, CH₂-CH=CH₂, 2H), 2.80-3.00 (s, CH₂CH₂CON, 4H), 5.00-5.20 (t, CH=CH *cis*, 2H), 5.60-5.85 (m, CH=CH *trans*, 1H).

(2S)-Pent-4-enoylamino-pentanedioic acid dibenzyl ester (XXXV). According to the general procedure GP-I, **XXXV** was obtained from **XXXIV** and commercial H-Glu(OBn)-OBn.*p*-TsOH in quantitative yield as an orange oil with no purification needed. IR (Film; cm⁻¹): 3298 (ν_{NH}), 3066 (ν_{φCH}), 2931 (ν_{CH}), 1738 (ν_{C=O} ester), 1662 (ν_{C=O} amide and ν_{C=C}), 1537 1498 (ν_{φC=C}), 1168 (ν_{O-C-C asym}); ¹H NMR (200 MHz, CDCl₃, δ): 2.09 (m, CH_β, 1H), 2.21-2.47 (m, CH_β, CH₂CONH, CH₂CO₂Bn and CH₂CH=CH₂, 7H), 4.72 (m, CH_α, 1H), 5.03 (m, CH₂=CH, 2H), 5.16 (s, CH₂Ph, 2H), 5.20 (2H, s, CH₂Ph), 5.80 (m, CH₂=CH, 1H), 6.23 (d, *J* = 7.6 Hz, NH, 1H), 7.31 (s, 2ArH, 10H); ¹³C NMR (CDCl₃, 75 MHz): 27.6 (C), 29.7 (C), 30.6 (C), 35.9 (C), 53.0 (C), 66.9 (C), 67.7 (C), 116.1 (C), 128.6, 128.7, 128.9, 129.0 (10C), 135.5 (C), 136.0 (C), 137.2 (C), 172.2, 172.7, 173.0 (3C).

(2S)-(3-Carboxy-propionylamino)-pentanedioic acid dibenzyl ester (XXXVI). According to the general procedure GP-G, **XXXVI** was obtained from **XXXV** in 73% yield as a yellow oil after purification by flash chromatography on silica gel (initially with petroleum ether:ethyl acetate 4:6 then with the same eluent + 0.1% formic acid). IR (Film, cm⁻¹): 3354 (ν_{NH} and ν_{COOH}), 3066 3035 (ν_{φCH}), 2955 (ν_{CH}), 1732 (ν_{C=O} ester), 1659 (ν_{C=O} amide), 1547 1455 (ν_{φC=C}), 1171 (ν_{O-C-C asym}); ¹H NMR (300

MHz, CDCl₃, δ): 1.85 (m, CH₂CH₂CO₂Bn, 2H), 2.22-2.58 (m, CH₂CO₂Bn, CH₂CONH, 4H), 2.67 (m, CH₂COOH, 2H), 4.68 (m, CH_α, 1H), 5.10 (s, CH₂Ph, 2H), 5.16 (s, CH₂Ph, 2H), 6.60 (d, *J* = 8.2 Hz, NH, 1H), 7.35 (s, 2ArH, 10H).

(2S)-[3-((1S),3-Bis-benzyloxycarbonyl-propylcarbamoyl)-propionylamino]-pentanedioic acid dibenzyl ester (23). According to the general procedure GP-A, **23** was obtained from **XXXVI** in 35% yield as a white solid after recrystallization from petroleum ether:ethyl acetate 4:6. Mp 108-109°C (lit. 106-107°C); [α]_D²⁵ +3.0 (*c* 0.29, CH₂Cl₂); IR (KBr, cm⁻¹): 3448 (ν_{NH}), 3065 3035 (ν_{φCH}), 2925 (ν_{CH}), 1736 (ν_{C=O} ester), 1637 (ν_{C=O} amide), 1540 1498 (ν_{φC=C}), 1167 (ν_{O-C-C} asym); ¹H NMR (300 MHz, CDCl₃, δ): 1.90-2.29 (m, 2CH₂CH₂CO₂Bn, 4H), 2.35-2.61 (m, 4CH₂CO, 8H), 4.60-4.67 (m, 2CH_α, 2H), 5.09 (s, 2CH₂Ph, 4H), 5.15 (s, 2CH₂Ph, 4H), 6.57 (d, *J* = 8.7 Hz, 2NH, 2H), 7.34 (m, 4ArH, 20H); ¹³C NMR (CDCl₃, 75 MHz): 27.4 (2C), 30.6 (2C), 31.8 (2C), 52.2 (2C), 66.9 (2C), 67.7 (2C), 128.6, 128.7, 128.9, 129.0 (20C), 135.5 (2C), 136.1 (2C), 172.2, 172.4 172.9 (6C); MS-ESI (*m/z*): 1495.1 [2M + Na]⁺, 1473.1 [2M + H]⁺, 759.4 [M + Na]⁺, 737.3 [M + H]⁺; HRMS-ESI (*m/z*): [M + Na]⁺ calcd for C₄₂H₄₄N₂O₁₀Na, 759.2894; found, 759.28890.

(2S)-[3-(2,5-Dioxo-pyrrolidin-1-yloxycarbonyl)-propionylamino]-pentane-dioic acid dibenzyl ester (XXXVII). According to the general procedure GP-H, **XXXVII** was obtained from **XXXVI** in 73% yield as a white solid after purification by flash chromatography on silica gel (petroleum ether:ethyl acetate 4:6). IR (KBr, cm⁻¹): 3326 (ν_{NH}), 3065 3035 (ν_{φCH}), 2930 (ν_{CH}), 1816 1784 1739 1732 1682 (ν_{C=O}), 1455 (ν_{φC=C}), 1207 1071 (ν_{NO}); ¹H NMR (300 MHz, CDCl₃, δ): 1.85 (m, CH₂CH₂CO₂Bn, 2H), 2.43 (m, CH₂CONH, 2H), 2.61 (m, CH₂CO₂Bn, 2H), 2.73 (s, 2CH₂CONO, 4H), 2.97 (m, CH₂CO₂N, 2H), 4.69 (m, CH_α, 1H), 5.11 (s, CH₂Ph, 2H), 5.17 (s, CH₂Ph, 2H), 6.38 (d, *J* = 7.5 Hz, NH, 1H), 7.35 (m, 2ArH, 10H).

(2S)-tert-Butoxycarbonylamino-pentanedioic acid 1-benzyl ester 5-cyclohexyl ester (XXXVIII). According to the general procedure GP-J, **XXXVIII** was obtained from commercial *N*^α-Boc-Glu(OcHex)-OH in 70% yield as white crystals after recrystallization from warm petroleum ether (45 °C). Mp 76 °C (petroleum ether); IR (KBr, cm⁻¹): 3379 (ν_{NH}), 3035 (ν_{φCH}), 2958 2930 (ν_{CH}), 1758 1717 1687 (ν_{C=O}), 1450 (ν_{φC=C}), 1392 1366 (δ_{CH₃} *t*-Bu), 1150 (ν_{O-C-C} asym); ¹H NMR (300 MHz, CDCl₃, δ): 1.34-1.39 (m, 2CH₂ cHex, 4H), 1.41 (s, -C(CH₃)₃ Boc, 9H), 1.60-1.88 (m, 3CH₂ cHex, 6H), 2.10 (m, CH_{2β}, 2H), 2.44 (m, CH_{2γ}, 2H), 4.42 (m, CH_α, 1H), 4.81 (m, CH-O cHex, 1H), 5.16 (br, NH, 1H), 5.21 (d, *J* = 4.0 Hz, CH₂Ph, 2H), 7.40 (s, ArH, 5H).

(2S)-Amino-pentanedioic acid 1-benzyl ester 5-cyclohexyl ester (XXXIX). Compound **XXXVIII** (400 mg, 0.954 mmol) was dissolved in ethyl acetate. Concentrated sulfuric acid (3 mmol) was then added. The reaction mixture was stirred for one hour at room temperature then neutralized with saturated NaHCO₃ solution. The resulted solution was finally extracted with ethyl acetate. The free α-amine **XXXIX** was obtained as yellow oil with no purification needed (291mg, 96% yield). IR (Film, cm⁻¹): 3385 3322 (ν_{NH2}), 3034 (ν_{φCH}), 2937 (ν_{CH}), 1732 (ν_{C=O}), 1455 (ν_{φC=C}), 1177 (ν_{O-C-C} asym); ¹H NMR (300 MHz, CDCl₃, δ): 1.21-1.62 (m, 3CH₂ cHex, 6H), 1.71 (m, CH₂ cHex, 2H), 1.80-2.00 (m, CH_β, CH₂ cHex, 3H), 2.11 (m, CH_β, 1H), 2.47 (t, *J* = 7.4 Hz, CH_{2γ}, 2H), 3.53 (dd, *J* = 5.2 Hz and 8.2 Hz, CH_α, 1H), 4.76 (m, CH-O cHex, 1H), 5.19 (s, CH₂Ph, 2H), 7.36 (s, ArH, 5H).

(2S)-[3-((1S)-Benzyloxycarbonyl-3-cyclohexyloxycarbonyl-propylcarbamoyl)-propionylamino]-pentanedioic acid dibenzyl ester (24). According to the general procedure GP-I, **24** was obtained from **XXXVII** and **XXXIX** in 45% yield as a white powder after recrystallisation from petroleum ether:ethyl acetate 4:6. Mp 125-127°C; [α]_D²⁷ +9.0 (*c* 1.00, CH₂Cl₂); IR (KBr, cm⁻¹): 3438 (ν_{NH}), 3065 3034 (ν_{φCH}), 2935 (ν_{CH}), 1756 1731 (ν_{C=O} ester), 1638 (ν_{C=O} amide), 1539 1499 (ν_{φC=C}), 1171 (ν_{O-C-C} asym); ¹H NMR (300 MHz, CDCl₃, δ): 1.90-2.29 (m, CH₂CH₂CO₂Bn, CH₂CH₂CO₂cHex and 5CH₂ cHex, 14H), 2.34-2.52 (m, 4CH₂CO, 8H), 4.58-4.71 (m, 2CH_α et CH-O, 3H), 5.08 (s, CH₂Ph, 2H), 5.13 (s, 2CH₂Ph, 4H), 6.60 (d, *J* = 2.6 Hz, NH, 1H), 6.62 (d, *J* = 3.4 Hz, NH, 1H), 7.32 (m, 3ArH, 15H); ¹³C NMR (CDCl₃, 75 MHz): 24.1, 25.3, 25.7, 25.9, 27.4, 27.5, 30.6 (7C), 31.0, 31.8 (2C), 31.9 (C), 34.2 (C), 52.2, 52.3 (2C), 66.9 (C), 67.7 (2C), 73.5 (C), 128.7, 128.8, 128.9, 129.0 (15C), 135.5, 135.6 (2C), 136.1 (C), 172.2,

172.3, 172.4, 172.5, 172.6, 172.9 (6C); MS-ESI (m/z): 1479.2 $[2M + Na]^+$, 1457.1 $[2M + H]^+$, 751.4 $[M + Na]^+$, 729.3 $[M + H]^+$; HRMS-ESI (m/z): $[M + Na]^+$ calcd for $C_{41}H_{48}N_2O_{10}Na$, 751.3207; found, 751.32037.

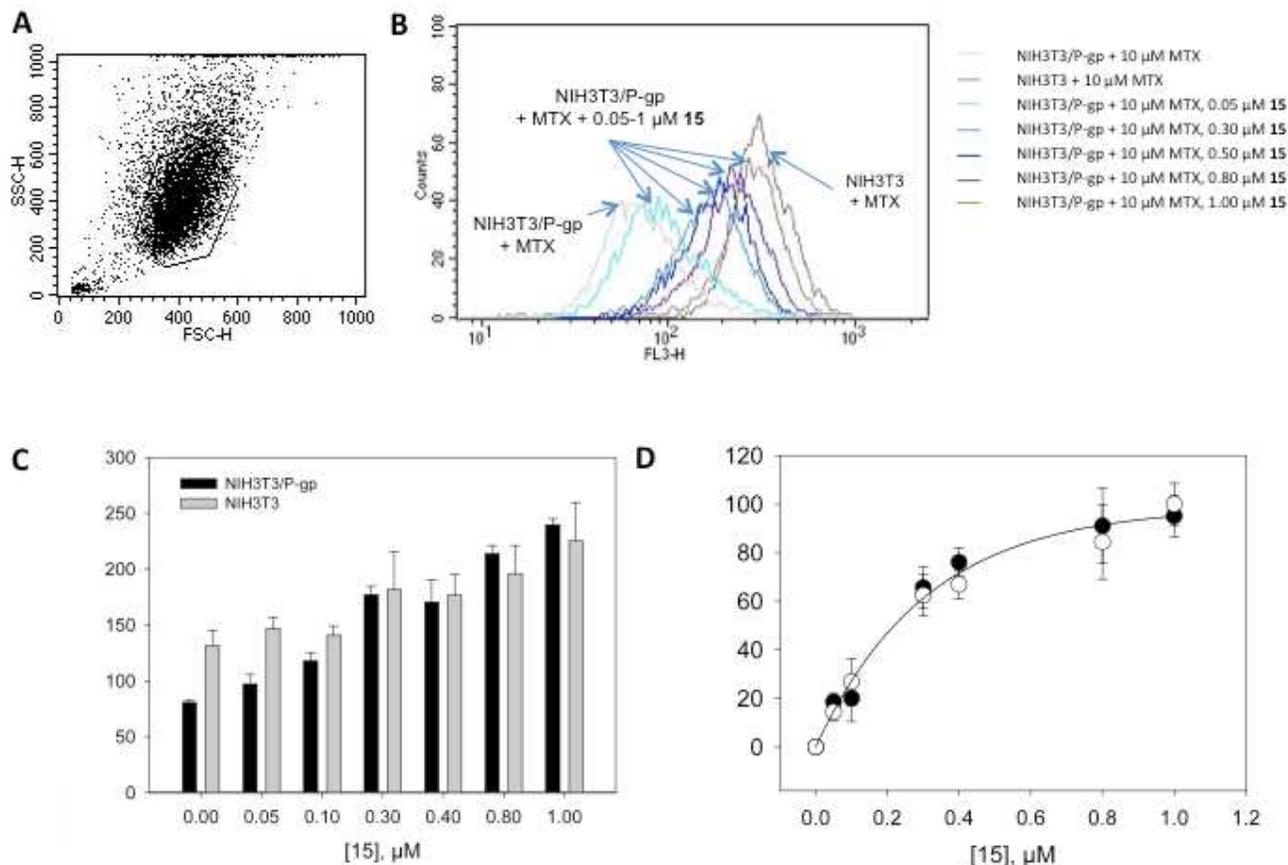


Figure SI 1. Typical experiment of fluorescence coupled to flux cytometry for quantifying the intracellular amount of mitoxantrone. The experiment has been carried out as described in the experimental section, with NIH3T3 cells expressing P-gp and control NIH3T3 cells, in the presence of 10 μ M mitoxantrone (MTX) and 0 -1 μ M of **15**. Panel A display the population of cells selected by the flow cytometer for the quantification of MTX. Panel B displays the fluorescence measured inside cells (x axis) in the presence of MTX and modulator (only one curve for control cells is shown for clarity), and reported in panels C and D to determine the IC₅₀ of **15**.