A General Approach for the Nonstop Solid Phase Synthesis of TAC-Scaffolded Loops towards Protein Mimics containing Discontinuous Epitopes

Cristina Chamorro, John A. W. Kruijtzer, Marina Farsaraki, Jan Balzarini and Rob M. J. Liskamp

Abbreviations

Aloc: allyloxycarbonyl; BOP: benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate; Boc: *tert*-butyloxycarbonyl; ^{t}Bu: *tert*-butyl; Bzl: benzyl; CDR: complementary determining region; CH_3CN: acetonitrile; DCC: *N,N'*-dicyclohexylcarbodiimide; DCM: dichloromethane; DiPEA: *N,N*-diisopropyl-*N*-ethylamine; DMAP: 4-dimetylaminopyridine; DMB: dimethoxybenzyl; DMF: dimethylformamide; Et_2O: diethyl ether; Fmoc: 9-fluorenylmethoxycarbonyl; FmocOSu: 9-fluorenylmethyloxycarbonyl-*N*-hydroxysuccinimide; HBTU: 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; $HOBt{\cdot}H_2O$: *N*-hydroxybenzotriazol hydrate; MALDI-TOF-MS: Matrix assisted laser desorption ionization time of flight mass spectrometry; MS (ESI): mass spectrometry (electrospray ionization); MTBE: methyl-*tert*-butyl ether; NMP: *N*-methylpyrrolidone; *o*NBS: *o*-nitrobenzenesulfonyl; Pbf: 2,2,4,6,7-pentamethyl-dihydrobenzofuran-5-sulfonyl; $Pd^0(Ph_3P)_4$: tetrakis triphenylphosphine palladium (0); PTMSE: (2-phenyl-2-trimethylsilyl)ethyl; Rt: retention time; TAC: triazacyclophane scaffold; $TBAF{\cdot}3H_2O$: tetrabutylammonium fluoride trihydrate; TDM: *N,N,N',N'*-tetramethyl-4,4'-diaminodiphenylmethane; TEA: trietylamine; TFA: trifluoroacetic acid. TIS: triisopropyl silane; Trt: triphenylmethyl or trityl.

General

Peptide grade DCM, NMP, TFA, and HPLC grade solvents were purchased from Biosolve B. V. (Varkenswaard, The Netherlands). DiPEA was obtained from Acros Organics ('s-Hertogenbosch, The Netherlands). The rest of the solvents and reagents were obtained from commercial sources and used without further purification. Analytical thin layer chromatography (TLC) was performed on Merck pre-coated silica gel 60 F_{254} (0.25mm) plates. Spots were visualized with UV light, ninhydrine, or Cl_2-TDM.[1] Column chromatography was performed using Merck Kieselgel 60 (40-63 μm). ^{1}H NMR, ^{13}C NMR and bidimensional HSQC and HSBC spectra were obtained on a Varian 300 MHz and 500 MHz spectrometers. Chemical shifts are given in ppm with respect to internal standard TMS for ^{1}H NMR. ^{13}C NMR spectra were recorded using the attached proton test (APT) pulse sequence. [$Pd^0(Ph_3P)_4$] was prepared and stored at 4 ºC under argon atmosphere and exclusion of light[2]. The Fmoc-protected amino

acids were purchased from GL Biochem (Shanghai) Ltd. The side chain protecting groups were chosen as: Boc for lysine and tryptophan, ^{t}Bu for aspartic acid, glutamic acid, serine and threonine, Trt for asparagine and glutamine, Pbf for arginine. To prevent aspartimide formation in the sequence of the third arm, the dipeptide Asp-Gly was introduced by coupling of Fmoc-Asp(O^{t}Bu)-(Dmb)-Gly-OH. TentaGel® S RAM resin functionalized with a modified Rink Amide linker,[3] (low crosslinked polystyrene grafted with polyethylene glycol, 0.20-0.27 mmol.g^{-1}, particle size 90 μm) was purchased from Rapp Polymere GmbH, Germany. Manual syntheses on the solid phase were performed in polyethylene glycol (PE) syringes with PE frits. The linear peptides were synthesized automatically on an Applied Biosystems 433A peptide synthesizer with UV-monitoring system[4] was used to monitor the Fmoc removal step *i.e.* formation of the dibenzofulvene-piperidine adduct absorbing at 301 nm. An additional deprotection cycle was carried out if the deprotection was slow. Kaiser and chloranil tests[5,6] were used for detection on the solid phase of primary and secondary amines, respectively, while free carboxylic acids were detected by malachite test.[7] The capping solution used was a mixture of 0.5M acetic anhydride (21.3 mL), 0.125M DiPEA (0.98 mL), 0.015M HOBt·H_2O (1.03 g), and NMP (450 mL). Loading of a resin sample was assessed by Fmoc-photometric quantification[8] of the absorbance of the dibenzofulvene-piperidine adduct at 301 nm in a Perkin Elmer Lambda 2 UV/VIS spectrometer. ESI-MS spectra were measured on a Shimadzu LCMS-QP8000 single quadrupole bench-top mass spectrometer operating in a positive ionization mode. Analytical LC-MS was preformed on Thermo-Finnigan Deca XP Max. MALDI-TOF-MS spectra were recorded on a Kratos Analytical (Shimadzu) AXIMA CFR mass spectrometer using α-cyano-4-hydroxycinnamic acid (CHCA) as a matrix and oxidized insulin as internal standard. Microcleavage of a small fraction of resin (~5 mg) was performed to check the completion of the reactions. The purity was assessed by analytical HPLC on a Shimadzu-10Avp (Class VP) using UV-detector operating at 214 and 254 nm and/or PL-ELS-100 detector. Either Phenomenex Luna C8 column (100 Å, 5 μm, 250×4.60 mm) or Alltech Adsorbosphere C8 column (90 Å, 5 μm, 250×4.6 mm) were used at a flow rate 1 mL min^{-1} with a standard protocol: 100% buffer A for 2 min then a linear gradient of buffer B (100% in 48 min) from 100% buffer A. Either TFA buffers (buffer A: H_2O:CH_3CN, 95:5, v:v; buffer B: H_2O:CH_3CN, 5:95, v:v, both containing 0.1% TFA (Alltech) or buffer A: H_2O:CH_3CN, 95:5, v:v; buffer B: H_2O:CH_3CN, 40:60, v:v, both containing 0.1% TFA (Phenomenex)) or NH_4OAc buffers (buffer A: H_2O:CH_3CN, 95:5, v:v; buffer B: H_2O:CH_3CN, 40:60, v:v, both containing 10 mmol of NH_4OAc) were used. Purification of the final compound was performed by a Prep LCMS-QP8000α HPLC system (Shimadzu) using a Phenomenex Luna C8 column (10 μm, C8, 100 Å, 250×21.2 mm) at a flow rate of 12.5 mL min^{-1} with standard protocol: 100% buffer A for 5 min then a linear gradient of buffer B (100% in 100 min) from 100% buffer A using NH_4OAc buffers.

Fmoc-removal. The resin (1 equiv) was swollen in NMP (20 mL $mmol^{-1}$ for 2 min). After draining the solvent the resin was shaken with 20% piperidine in NMP (3 × 40 mL $mmol^{-1}$, each 10 min) and washed with NMP (4 × 20 mL $mmol^{-1}$, each 2 min) and DCM (4 × 20 mL $mmol^{-1}$, each 2 min). Positive Kaiser test indicated Fmoc removal.

Capping of the resin. The resin (1 equiv) was swollen in NMP (20 mL $mmol^{-1}$ for 2 min) and the solvent was drained. Capping solution (20 mL $mmol^{-1}$) was added and the resin was shaken for 1 h. The resin was washed with NMP (4 × 20 mL $mmol^{-1}$, each 2 min) and DCM (4 × 20 mL $mmol^{-1}$, each 2 min).

Automatic synthesis of linear peptide on the TAC scaffold. The linear peptides were synthesized using 'double coupling and capping' protocol on a 0.25 mmol scale. Either Fmoc-amino acid containing acid labile side chain protecting groups or Fmoc-Glu(OPTMSE)-OH were used. Each synthetic cycle consisted on N^{α}-Fmoc removal by a 10 min treatment with 20% piperidine in NMP, a 6 min NMP wash, a 45 min coupling step with 1.0 mmol of preactivated Fmoc-amino acid building blocks in the presence of DiPEA (2 equiv), and a 6 min NMP wash. N^{α}-Fmoc amino acids were activated in situ with 0.9 mmol HBTU[9]/ HOBt·H_2O (0.36M in NMP) in the presence of DiPEA (2.0 mmol). The synthetic cycle was repeated until the required peptide sequence was completed.

PTMSE-removal. The resin (1 equiv) was swollen in DCM (2 × 20 mL $mmol^{-1}$, each 2 min) and the solvent was drained. TBAF·$3H_2O$ (4 equiv) and DCM (40 mL $mmol^{-1}$) were added and the mixture was shaken for 20 min. The resin was washed with DCM (4 × 20 mL $mmol^{-1}$, each 2 min). A positive malachite test indicated removal of the PTMSE-group.

Solid-phase cyclization. The resin (1 equiv) was swollen in DMF (2 × 20 mL $mmol^{-1}$, each 2 min) and the solvent was drained. BOP (3 equiv), HOBt (3 equiv) and DMF (40 mL $mmol^{-1}$) were added and the mixture was shaken until complete dissolution, then DiPEA (9 equiv) was added and the reaction was shaken overnight. The resin was washed with DMF (4 × 20 mL $mmol^{-1}$, each 2 min) and DCM (4 × 20 mL $mmol^{-1}$, each 2 min). Negative malachite and Kaiser tests indicated successful cyclization.

Cleavage from the resin and side-chain deprotection. The resin was treated with TFA:H_2O:TIS (95:2.5:2.5, v:v:v) (20 mL $mmol^{-1}$) for 2 h, filtered and washed with TFA:H_2O (95:5, v:v). The filtrates were combined and a solution of MTBE:hexane (1:1, v:v) at -20 °C was added. After centrifugation (3500 rpm, 5 min) the supernatant was decanted and the pellet was resuspended in cold MTBE:hexane (1:1, v:v) and centrifuged again. Finally, the pellet was washed twice with MTBE:hexane (1:1, v/v), dissolved in CH_3CN:H_2O: (1:1, v:v) and lyophilized. For microcleavage of small fraction of resin (~5 mg) identical procedure was followed and direct analysis without lyophilization was performed.

Synthesis of TAC-scaffolded protein mimic 9.

*(Fmoc, Aloc, oNBS)-TAC scaffold-rink resin (**2**).* TentaGel® S RAM resin (1.20 g, 0.28 mmol) was Fmoc-deprotected following the general procedure. Then, the resin was swollen in NMP (1 × 15 mL mmol^{-1}, each 2 min) and the solvent was drained. TAC scaffold (**1**) (0.42 g, 0.56 mmol), BOP[22] (244 mg, 0.56 mmol) and NMP (4.2 mL, 15 mL mmol^{-1}) were added and shaken until complete dissolution, then DiPEA (192 μL, 1.12 mmol) was added. After shaking overnight, the resin was washed with NMP (4 × 15 mL mmol^{-1}, each 2 min) and, DCM (4 × 15 mL mmol^{-1}, each 2 min). Loading of the resin was measured after drying *in vacuo* overnight (0.22 mmol g^{-1}) and corrected by the added mass (0.24 mmol g^{-1}, 99%). The resin **2** was capped following the general procedure.

*Side-chain protected (SSGGDPEIVEGG, Aloc, oNBS)-TAC scaffold-rink resin (**3**).* The resin **3** was obtained after automatic synthesis of the linear peptide arm 1 including one last automatic N^{α}-Fmoc removal, starting with resin **2** (1.14 g, 0.25 mmol). The resin **3** was washed with DCM (15 mL mmol^{-1} for 2 min).

*Side-chain protected ($c^{1,10}$-S^{1}SGGDPEIVE^{10}GG, Aloc, oNBS)-TAC scaffold-rink resin (**4**).* PTMSE-removal of resin **3** (~0.25 mmol), solid-phase cyclization and N^{α}-capping were performed following the general procedures to give resin **4**. Analysis after microcleavage, MALDI-TOF monoisotopic mass [*M*+H]$^{+}$ calcd for $C_{69}H_{98}N_{17}O_{26}S$: 1612.66; found, 1612.68 [*M*+H]$^{+}$, 1634.68 [*M*+Na]$^{+}$, 1650.64 [*M*+K]$^{+}$ and monoisotopic mass [*M*-H]$^{-}$ calcd for $C_{69}H_{96}N_{17}O_{26}S$: 1610.64; found, 1610.67.

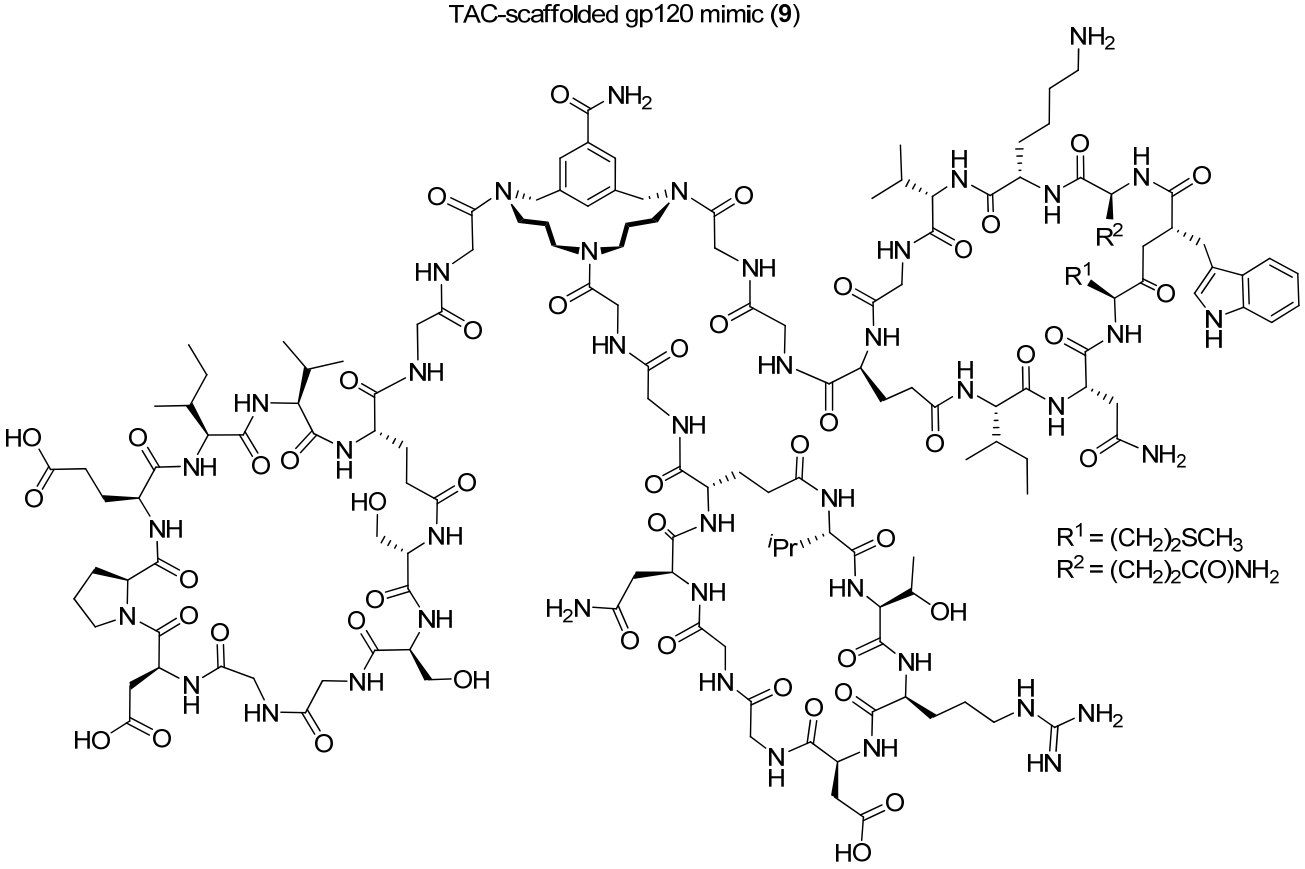
*Side-chain protected ($c^{1,10}$-S^{1}SGGDPEIVE^{10}GG, Aloc, NH)-TAC scaffold-rink resin (**5**).* The resin **4** (~0.25 mmol) was swollen in DMF (2 × 20 mL mmol^{-1}, each 2 min) and, after draining the solvent, DMF (5 mL, 20 mL mmol^{-1}), DBU (187 μL, 1.25 mmol) and β-mercaptoethanol (176 μL, 2.50 mmol) were added. N_2 was bubbled through the mixture for 30 min and the solution was replaced by an identical fresh mixture maintaining the N_2 bubbling for 30 min. The resin was washed with DMF (4 × 20 mL mmol^{-1}, each 2 min), and DCM (4 × 20 mL mmol^{-1}, each 2 min) to obtain resin **5**. Analysis after microcleavage, MS (ESI) *m/z* (monoisotopic mass) [*M*+H]$^{+}$ calcd for $C_{63}H_{95}N_{16}O_{22}$: 1427.68; found, 1427.79 [*M*+H]$^{+}$, 714.98 [*M*+2H]$^{2+}$. MALDI-TOF found: 1427.64 [*M*+H]$^{+}$ and, monoisotopic mass [M-H]$^{-}$ calcd for $C_{63}H_{93}N_{16}O_{22}$: 1425.67; found, 1425.75.

*Side-chain protected ($c^{1,10}$-S^{1}SGGDPEIVE^{10}GG, Aloc, $c^{1,10}$-I^{1}NMWQKVGE^{9}GG)-TAC scaffold-rink resin (**6**).* Automatic synthesis of the linear peptide arm 2 was performed on resin **5** (~0.25 mmol). The first cycle started with preactivation and coupling. A last automatic N^{α}-Fmoc removal was included in the automatic synthesis. After that, PTMSE-removal, solid-phase cyclization and N^{α}-capping using the general procedures gave resin **6**. Analysis after microcleavage, MALDI-TOF monoisotopic mass [*M*+H]$^{+}$ calcd for $C_{116}H_{174}N_{31}O_{36}S$: 2609.25; found, 2609.22 [*M*+H]$^{+}$, 2631.25 [*M*+Na]$^{+}$, 2647.21 [*M*+K]$^{+}$ and monoisotopic mass [*M*-H]$^{-}$ calcd for $C_{116}H_{172}N_{31}O_{36}S$: 2607.23; found, 2607.36.

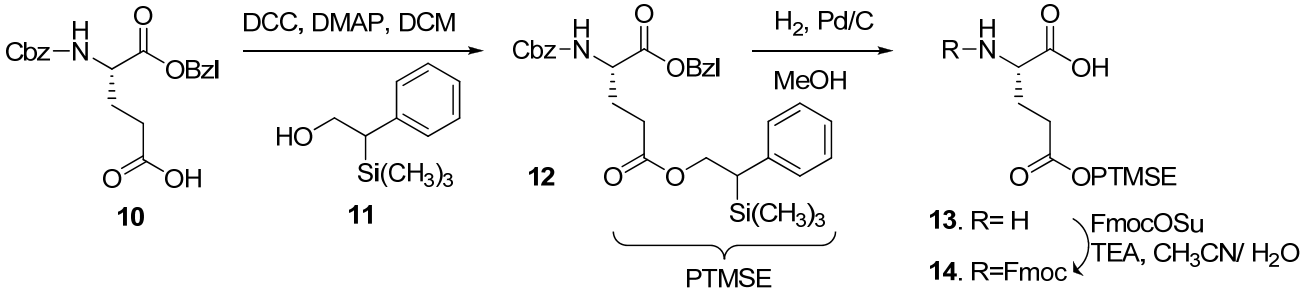
*Side-chain protected ($c^{1,10}$-S^{1}SGGDPEIVE^{10}GG, NH, $c^{1,9}$-I^{1}NMWQKVGE^{9}GG)-TAC scaffold-rink resin (**7**).* The resin **6** (~0.25 mmol) was swollen in NMP (2 × 20 mL mmol^{-1}, each 2 min) and, after draining the solvent, anilinium *p*-toluensulfinate[10] (1.24 g, 5 mmol) and NMP (4.2 mL, 15 mL mmol^{-1}) were added. A gentle stream of argon was bubbled through the mixture for 10 min and $Pd^{0}(Ph_3P)_4$ (0.09 g, 0.075 mmol) was added. Argon bubbling was continued through the reaction for 45 min with exclusion of light. The resin was washed with NMP (3 × 20 mL mmol^{-1}, each 2 min), 0.1% sodium diethyldithiocarbamate trihydrate in NMP (20 mL mmol^{-1} for 2 min), 20% DiPEA in NMP (20 mL mmol^{-1} for 2 min), NMP (3 × 20 mL mmol^{-1}, each 2 min), and DCM (3 × 20 mL mmol^{-1}, each 2 min). The procedure was repeated three times, including washing steps to obtain resin **7**. Analysis after microcleavage, MALDI-TOF monoisotopic mass $[M+H]^+$ calcd for $C_{112}H_{170}N_{31}O_{34}S$: 2525.22; found, 2524.85 $[M+H]^+$, 2546.78 $[M+Na]^+$.

*($c^{1,10}$-S^{1}SGGDPEIVE^{10}GG, $c^{1,8}$-L^{1}TRDGGNE^{8}GG, $c^{1,9}$-I^{1}NMWQKVGE^{9}GG)-TAC scaffold-amide (**9**).* The third linear peptide arm was constructed in resin **7** (~0.25 mmol) by automatic synthesis following the same procedure as used for arm 2. PTMSE-removal, solid-phase cyclization, and N^{α}-capping were performed. Final cleavage from the resin gave the TAC protein mimic **9** as a crude trifluoroacetate salt fluffy solid (55 mg). The crude lyophilized **9** was dissolved in a minimum amount of CH_3CN: H_2O: (1:1, v:v) and loaded onto the preparative HPLC column and purified by prep LCMS. Fractions corresponding to **9** were pooled and lyophilized to give 0.43 µmol (ca. 1.5 mg, 0.17% overall yield, 97% average per step). HPLC (Luna C8, NH_4OAc buffers): Rt= 24.65 min, purity >99%. MALDI-TOF monoisotopic mass $[M+H]^+$ calcd for $C_{149}H_{228}N_{45}O_{49}S$: 3463.65; found: 3463.58 $[M+H]^+$. MS (ESI) *m/z* (average mass) $[M+H]^+$ calcd for $C_{149}H_{228}N_{45}O_{49}S$: 3465.80; found: 1733.08 $[M+2H]^{2+}$ and 1156.31, $[M+3H]^{3+}$ giving an average $[M+H]^+$: 3466.05.

TAC-scaffolded gp120 mimic (**9**)



Synthesis of Fmoc-Glu-(OPTMSE)-OH (14)



*Cbz-Glu(OPTMSE)-OH (**12**).* To a cool solution (0ºC) of the commercially available Cbz-Glu-OBzl (**10**) (5.56 g, 15 mmol) and 2-(methyltrimethylsilyl) ethanol (**11**)[11] (3.20 g, 16.5 mmol) in DCM (90 mL), a solution of DCC (3.09 g, 15 mmol) in DCM (5 mL) was added dropwise. After stirring for 10 min at 0ºC, catalytic amount of DMAP (0.18 g, 1.5 mmol) was added and the suspension was stirred at room temperature overnight. The reaction was filtered, the solvent was evaporated and redissolved in EtOAc (100 mL). The mixture was washed with $KHSO_4$ (1M) (2 × 75 mL), H_2O (75 mL), and brine (75 mL). After drying (Na_2SO_4) and evaporating the solvent, the crude product was purified by column chromatography using hexanes: Et_2O, (7:3, v:v) to obtain **12** (7.51 g, 91%) as a colorless oil. R_f = 0.84 (DCM: methanol: AcOH, 95:5:0.5, v:v:v). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.26 (m, 10H, Ar-Cbz, Bzl),

7.19 (t, 3H, Ar-PTMSE, J= 7.7 Hz), 7.06, 7.00 (2d, 2H, Ar-PTMSE), 5.59 (bt, 1H, NH, J= 10.5 Hz), 5.06, 5.02 (2s, 4H, CH_2Ph), 4.56 (t, 1H, CH_{2a}-PTMSE, $J_{a,b}$ = 11.2 Hz), 4.46-4.39 (m, 1H, CH_{2b}-PTMSE), 4.32-4.30 (m, 1H, CH-α), 2.52, 2.48 (2d, 1H, CH-PTMSE, $J_{CH,CH2a}$= 4.7 Hz, $J_{CH,CH2b}$= 4.4 Hz), 2.18-2.16 (m, 2H, CH_2-γ), 2.10-2.00 (m, 1H, CH_{2a}-β), 1.84-1.79 (m, 1H, CH_{2b}-β), -0.04 (s, 9H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 172.57 (CO_2-PTMSE), 171.40 (CO_2-Bzl), 155.69 (CONH), 140.23 (Ar-*C*-PTMSE), 135.98, 135.00 (Ar-*C*-Cbz, Bzl), 128.26, 128.17, 127.98, 127.90, 127.76, 127.14, 124.79 (Ar-*H*), 66.81, 66.59 (OCH_2-Cbz, Bzl), 65.34 (OCH_2-PTMSE), 53.07 (C-α), 36.90 (CH-PTMSE), 29.84 (C-γ), 26.94 (C-β), -2.92 (CH_3).

*H-Glu-(OPTMSE)-OH (**13**).* To a cool solution (0ºC) of **12** (7.39 g, 13.49 mmol) in methanol (200 mL), Pd charcoal (10%) (0.74 g, 10% w/w) was added and the suspension was stirred under H_2 pressure from a balloon at room temperature overnight. The reaction was filtrated and the solvent was evaporated. The crude was purified by column chromatography using a gradient of DCM: methanol (85:15, v:v) to DCM: methanol (70:30, v:v) to give **13** (4.23 g, 97%) as white foam. ^{1}H NMR (CD_3OD, 300 MHz) δ: 7.24 (t, 2H, Ar-PTMSE, J= 7.3 Hz), 7.13-7.07 (m, 3H, Ar-PTMSE), 4.65 (bt, 1H, CH_{2a}-PTMSE, $J_{a,b}$= 11.3 Hz), 4.55-4.48 (m, 1H, CH_{2b}-PTMSE), 3.52-3.48 (m, 1H, CH-α), 2.62, 2.58 (2d, 1H, CH-PTMSE, $J_{CH,CH2a}$= $J_{CH,CH2b}$= 4.4 Hz), 2.45-2.37 (m, 2H, CH_2-γ), 2.04-1.99 (m, 1H, CH_2-β), 0.00 (s, 9H, CH_3). ^{13}C NMR (CD_3OD, 75 MHz) δ: 175.37 (COOH), 174.78 (CO_2-PTMSE), 142.22 (Ar-*C*-PTMSE), 129.51, 128.89, 126.39 (Ar-*H*), 67.03 (OCH_2), 55.47 (C-α), 38.71 (CH-PTMSE), 31.26 (C-γ), 27.52 (C-β), -2.37 (CH_3).

*Fmoc-Glu-(OPTMSE)-OH (**14**).* Compound **13** (4.16 g, 12.87 mmol) was dissolved in water (30 mL) and the pH adjusted to 9.0-9.5 with TEA. To this mixture, a solution of Fmoc-OSu (4.34 g, 12.87 mmol) in CH_3CN (45 mL) was added in one portion. The mixture was stirred at room temperature for 1.5 h and the pH maintained at 8.5>pH>9.0 by addition of TEA. The reaction mixture was concentrated *in vacuo* to remove CH_3CN and acidify at pH~2 with $KHSO_4$ (1M). The aqueous layer was extracted with EtOAc (3 × 100 mL) and the combined organic layers were washed with $KHSO_4$ (1M) (100 mL), H_2O (100 mL), and brine (100 mL). After drying (Na_2SO_4) and evaporating the solvent, the crude product was purified by column chromatography using a gradient of DCM to DCM: methanol (80:20, v:v) to give a colorless oil. Coevaporation with Et_2O led to **14** as a white foam (1.45 g, 89 %) as white foam. R_f = 0.36 (DCM: methanol: AcOH, 90:10:0.5, v:v:v). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.71-8.51 (bs, 1H, COOH), 7.63, 7.46 (2d, 4H, Ar-Fmoc, J= 6.0 Hz), 7.27, 7.20 (2t, 4H, Ar-Fmoc), 7.13, 7.00 (2t, 3H, Ar-PTMSE, J= 7.4 Hz), 6.94 (d, 2H, Ar-PTMSE), 5.49, 5.44 (2d, 1H, NH, J= 8.2 Hz, J= 7.7 Hz), 4.58-4.44 (m, 1H, CH_{2a}-PTMSE), 4.39-4.34 (m, 1H, CH_{2b}-PTMSE), 4.28 (d, 2H, OCH_2-Fmoc, J= 6.8 Hz), 4.21, 3.93 (2m, 1H, CH-α), 4.09 (t, 2H, CH-Fmoc), 2.47, 2.43 (2d, 1H, CH-PTMSE, $J_{CH,CH2a}$= 3.6 Hz, $J_{CH,CH2b}$= 3.0

Hz), 2.20, 2.10 (2m, 2H, CH_2-γ), 2.06-1.96 (m, 1H, CH_{2a}-β), 1.95-1.78 (m, 1H, CH_{2b}-β), -0.08 (s, 9H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 175.54 (COOH), 173.33 (CO_2-PTMSE), 156.12 (CONH), 143.70, 143.51, 141.16 (Ar-*C*-Fmoc), 140.32 (Ar-*C*-PTMSE), 128.20, 127.61, 127.33, 126.98, 125.00, 119.95 (Ar-*H*), 66.99 (OCH_2-Fmoc), 65.71 (OCH_2-PTMSE), 53.07 (C-α), 46.98 (CH-Fmoc), 37.12 (CH-PTMSE), 30.20 (C-γ), 26.94 (C-β), -2.76 (CH_3). MS (ESI) *m/z* (monoisotopic mass) $[M+H]^+$ calcd for $C_{31}H_{36}NO_6Si$: 546.2, found: 546.9. HPLC (Adsorbosphere C8, TFA buffers): Rt= 24.04 min, purity >99%.

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10. After dissolving *p*-toluensulfinic acid sodium salt trihydrate (15 g) in boiling water (250 mL) HCl (1N, 85 mL), was added and the reaction was cold down at room temperature. The *p*-toluensulfinic acid crystals were filtrated, washed with ice-water and dried. The *p*-toluensulfinic acid (4 g) was dissolved in DCM (25 mL) and aniline (2.33 mL) was added. Anilinium *p*-toluensulfinate was obtained as white crystals by slow addition of hexanes.
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