Pyridone Dipeptides – Synthesis of a Novel Peptide Chromophore

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Dedicated to Horst Kessler on the occasion of his 65th birthday

Keywords: Peptidomimetics / Peptides / Heterocycles / Lactams / Bicyclic dipeptides / Amino acids

Sequential elimination of two water molecules transfers the diols **2** and **3** into the pyridone dipeptide **1**. Both dehydration steps become part of the peptide synthesis under appropriate reaction conditions. A straightforward strategy for the incorporation of **1** in oligopeptide sequences is presented here. As part of the oligopeptide backbone, **1** has a local rigidifying effect which was studied in the cyclic octapeptides **16** and **25**. Both cyclopeptides exhibit conformational averaging although a fixed conformation was identified for the **1**-Phe tripeptide fragment of octapeptide **25**. The pyridone dipeptide **1** serves also as a fluorescence label and the UV/Vis-spectroscopic properties of tetrapeptide **13** are investigated here. The peptide backbones of the two cyclic octapeptides **16** and **25** arrange a pair of chromophores **1** in close vicinity of each other.

Introduction

Dipeptide lactams impose conformational constraints on peptides by restricting the peptide bond (ω) to the *trans* conformation and ψ_1 to about –120°.[1] Therefore, bicyclic dipeptide lactams like the β-turn dipeptide (BTD, n = 1) were proposed as surrogates of the type II′ β-turn motif.[2] Yet, the overall constraint fixed by a bicyclic dipeptide is closer to a right angle (90°) than to a hairpin turn (180°) and the concept of β-turn mimetics was challenged by the observation that 7,5-bicyclic thiazolidine lactams (n = 2) occupy the *i* to *i+1* positions of a β-turn, independently of the chirality of neighbouring amino acids.[3] Smaller lactam rings (n = 0, 1) are flattened and minima of –140° or 60° are expected for φ_1.[4] The pyridone dipeptide **1** combines an NH donor and CO acceptor of parallel orientation (φ_1 = 180°) and a thiaproline ring which induces a close to right angle in the peptide structure (φ_2 = –60° to –90°). The synthetic strategy presented here extends our earlier approach towards bicyclic thiazolidine lactams. Sequential elimination of two water molecules transfers the sugar precursor into an aromatic ring. Only few protocols convert sugars into hydrocarbons by repeated dehydration.[5] Pyridone rings find application as peptide mimetics.[6] To the best of our knowledge, the synthesis of a pyridine ring from a sugar precursor has never been described before.

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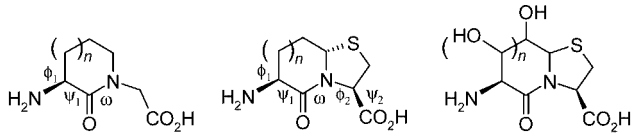

Results and Discussion

Synthesis

The diastereomeric thiazolidine lactam dipeptides **2** (6*R*) and **3** (6*S*) are obtained in only four steps from D-alduruno-2,5-lactone (**4**) and L-cysteine methyl ester (Scheme 1).[7] Under standard peptide coupling conditions, the 6,5-bicyclic thiazolidine lactams yield unsaturated products more readily than the polyhydroxylated 7,5-bicyclic dipeptides which do not show elimination reactions.[8] Dehydrated products like the dehydro tripeptide **5** were initially obtained as side products (10% yield) from the ester hydrolysis of **2** followed by peptide coupling with glycine methyl ester. Tosylate **6** forms the elimination product **7** in 69% yield. The elimination reactions are favoured by the pseudo-axial orientations of 7-OH and 8-OH (Scheme 2).[7] Both eliminations can be combined in one molecule without the necessity of activating 8-OH as shown in Scheme 3. Longer reaction times of the methyl ester hydrolysis of **2** favours the elimination leading to dehydro dipeptide **8** as the only product after several days. The C-6 epimers **2** and **3** are in slow equilibrium under these reaction conditions. Higher reaction temperatures accelerate the transformation, yet with less than quantitative yields. Acidolysis of the Boc

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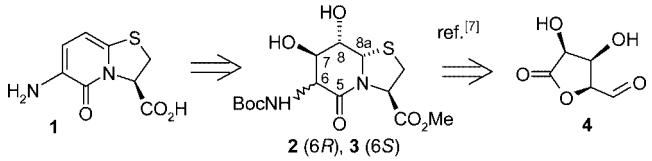
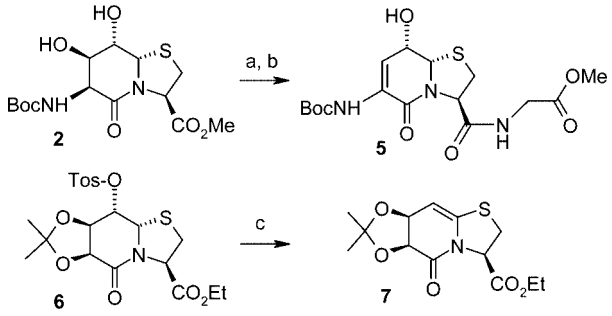
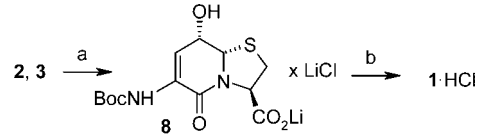
group is accompanied by the elimination of the second water molecule leading to the hydrochloride of unprotected dipeptide **1** in excellent overall yield. Fmoc-protected **1** was used as a building block in solid-phase peptide synthesis of several bioactive oligopeptides.[9]



Scheme 1.

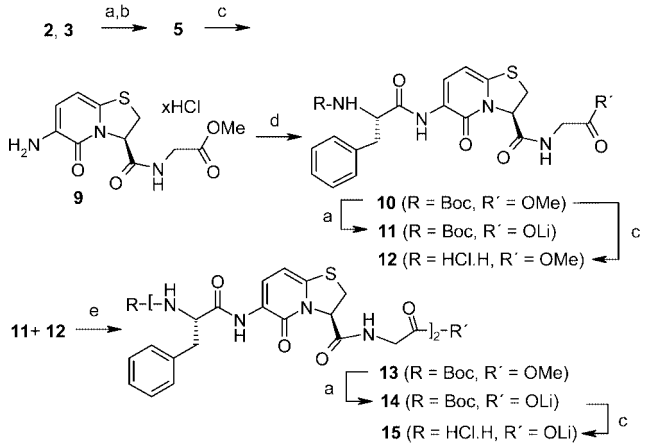


Scheme 2. a) 1 N LiOH, MeOH; b) HCl·H-Gly-OMe, EDC, HOBT, DIPEA (10% **5**, 46% tripeptide without elimination); c) NaN_3, DMF 80° (69%).

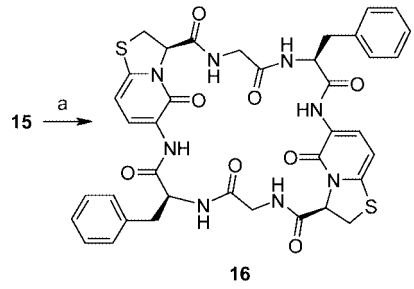


Scheme 3. a) 1 N LiOH/MeOH, room temp., 12 d, then adjust pH to 7 with 1 N HCl (quantitative); b) Et_2O/HCl (96%).

Scheme 4 shows how both elimination reactions are implemented in the solution peptide synthesis without the necessity of isolating unprotected dipeptide **1**. Methyl ester hydrolysis of the diastereomers **2** and **3** together with the first elimination step is followed by peptide coupling to tripeptide **5** (87%) without the necessity of isolating the intermediate **8**. Freeing of the amino terminus of **5** with HCl/Et_2O yields the salt **9** which is subsequently acylated by Boc-Phe-OH. The reduced nucleophilicity of the aromatic amino group does not cause problems. This condensation is mediated by PyBOP [benzotriazol-1-yl(oxy)tripyrrolidinylphosphonium hexafluorophosphate] as a coupling reagent yielding the tetrapeptide **10** in 88% yield. Deprotection of either the amino terminus or the carboxy terminus yields the monoprotected tetrapeptides **11** and **12**, respectively, which are combined in the next coupling step to the protected octapeptide **13**. After saponification (→ **14**) and acidolysis (→ **15**) the cyclisation to **16** (Scheme 5) is accomplished under dilution conditions in DMF with HATU [*O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate] in 76% isolated yield.

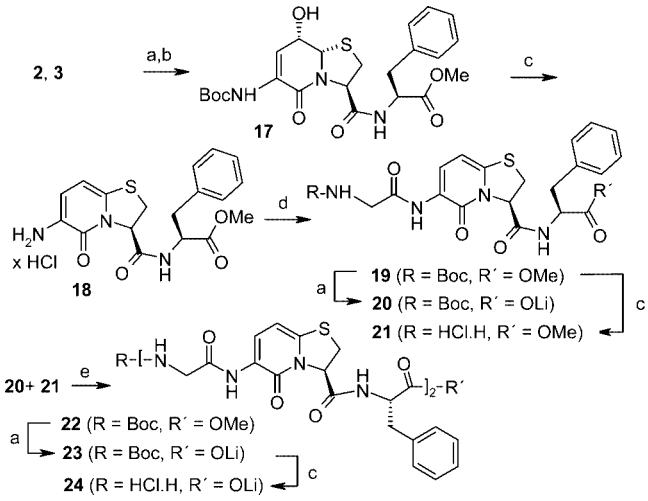


Scheme 4. a) 1 N LiOH, MeOH; b) HCl·H-Gly-OMe, EDC, HOBT, DIPEA, DMF (87% both steps); c) HCl in Et_2O (93%); d) Boc-Phe, PyBOP, NMM, DMF (88%); e) PyBOP, NMM, DMF (95% three steps).



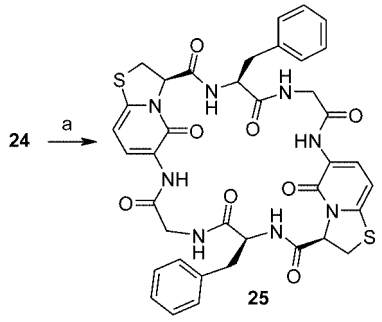
Scheme 5. a) HATU, HOAT, collidine, DMF, room temp. (76%).

Synthesis of an octapeptide with a phenylalanine at the C-terminal position of **1** is shown in Schemes 6 and 7. The tripeptide **17** (Figure 1) is obtained in 94% yield by coupling of HCl·H-Phe-OMe with the dehydro amino acid received from the saponification of **2** and **3**. Acidolysis (→ **18**) and condensation with Boc-Gly-OH leads to the



Scheme 6. a) 1 N LiOH MeOH; b) HCl·H-Gly-OMe, EDC, HOBT, DIPEA, DMF (94% both steps); c) HCl in Et2O (87%); d) Boc-Gly, PyBOP, NMM, DMF (96%); e) PyBOP, NMM, DMF (68% three steps).

tetrapeptide **19**, which is selectively deprotected to **20** or **21**, respectively. The octapeptide **22** obtained from the fragment condensation of tetrapeptides **20** and **21** is deprotected by saponification (→ **23**) and acidolysis (→ **24**). The cyclisation of octapeptide **24** yields the desired product **25** in only 35% yield, although the reaction conditions were the same as described for **16**.



Scheme 7. a) HATU, HOAT, collidine, DMF, room temp. (35%).

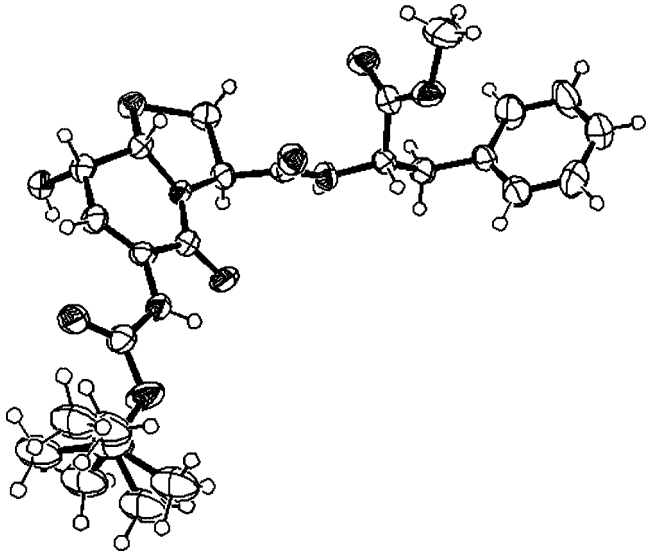


Figure 1. Crystal structure of **17** with disordered *t*Bu group.

Structural Analysis

The amino group (6-NH) and the pyridinone carbonyl group assume a close to parallel orientation (φ_1 = –166°) in the crystal structure of tripeptide **17** (Figure 1). By this, the pyridinone ring mimics an extended peptide conformation which is terminated by the thiazolidine ring (φ_2 = –60°; φ_2 = –156°). The close to right angle is mainly due to the thiazolidine ring mimicking an L-proline in the *i+1* position of a hairpin turn. A fully extended conformation for φ_1 and ψ_1 is expected for **1**, stabilized by the antiparallel alignment of the 5-CO and 6-NH dipoles. The benzylic side chain of **17** is found in an extended conformation (χ_1 = Nα–Cα–Cβ–Cγ = 180°) in the crystal structure.

Natural cyclic octapeptides exhibit a complex conformational behaviour[10] and the two cyclic octapeptide models **16** and **25** were assembled around pyridone dipeptide **1** to study its rigidifying influences. Both C_2-symmetric cyclic octapeptides contain two of the rigid dipeptides **1** together with two Phe-Gly or Gly-Phe dipeptide units, respectively. Cyclic octapeptides cannot assume antiparallel β-sheet conformations like cyclic hexapeptides which have an even number of antiparallel hydrogen bonds.[3] Therefore, only kinked structures are possible for cyclic octapeptides and the glycine residues are expected to form flexible joints between turn structures stabilized by **1**. Indeed, conformationally averaged Gly $^3J_{NH,H\alpha}$ coupling constants of 5.0 and 6.8 Hz and Phe $^3J_{H\alpha,H\beta}$ coupling constants of 5.5 Hz and 9.5 Hz are observed for **16**. A fixed side-chain orientation is found for the two phenylalanines of the cyclic octapeptide **25** in DMSO solution. The $^3J_{H\alpha,H\beta}$ coupling constants ($^3J_{H\alpha,H\mathit{proS}}$ = 3.8, $^3J_{H\alpha,H\mathit{proR}}$ = 12.6 Hz) together with the NOE pattern identify a χ_1 = –60° orientation as the main rotamer (> 80%). NOE and coupling data indicate a type-I β-turn motif with Phe occupying the *i*+2 positions. None of the linear precursors (**17**, **19**, and **22**) of the cyclopeptide **25** shows preferred side-chain rotamers for Phe. Thus, the conformational dynamics of octapeptide **25** is restricted to the glycine residues. The overall C_2-symmetry of both cyclic octapeptides together with the proton-poor heterocyclic ring structure yield only few reliable NOEs which are exclusively sequential.

Figure 2 shows the spectroscopic data of tetrapeptide **10** which exhibits an intense UV absorption (λ_{max} = 346 nm, ε = 17000). This absorption is by far more intense than that of Phe (λ_{max} = 256 nm, ε = 267) and is exclusively caused by the pyridine dipeptide **1**. The peptide backbone chromophore **1** shows fluorescencense at 408 nm.[11] These spectroscopic data of **1** do not vary significantly between the dif-

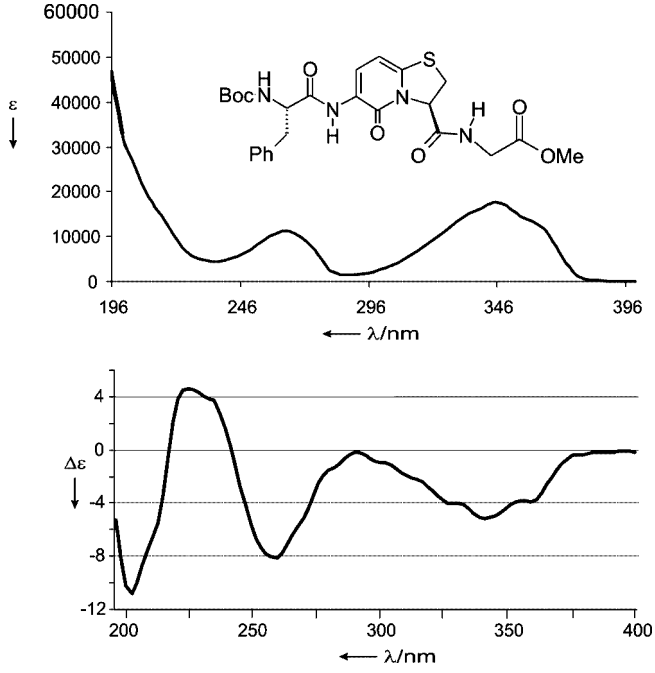


Figure 2. UV and CD spectra of tetrapeptide **10** (*c* = 0,452 mmol/L in MeOH; 0,1 cm cell).

ferent peptides investigated here, thus indicating that the two pyridone dipeptides in each of the linear or cyclic octapeptides behave like independent chromophores and do not interact with each other due to their close spatial proximity.

Conclusions

Two molecules of water are eliminated from a bicyclic thiazolidine lactam to obtain the pyridone dipeptide **1**, thus transferring a sugar precursor into an amino acid chromophore of the peptide backbone. Two cyclic octapeptides are described here which prove the feasibility of the synthetic concept and to study their conformational preferences. Cyclic octapeptides are known to form parallel β-sheets together with kinked and double-helical "Figure eight" conformations.[10] Even the pentacyclic octapeptides **16** and **25** exhibit conformational averaging although the bicyclic dipeptide **1** stabilizes two reverse turns in **16**. The glycines serve as joints where the backbone bends from an antiparallel β-sheet structure to kinked conformations. Within a peptide sequence, **1** can lock an extended dipeptide conformation. In the context of bioactive peptides, **1** can serve simultaneously as a scaffold and as a fluorescence marker to monitor the fate of the peptide by fluorescence spectroscopy in biological testing systems.

Experimental Section

General Remarks: Solvents were purified according to standard procedures. Flash chromatography was performed on J. T. Baker silica gel 60 (0.040–0.063 mm) at a pressure of 0.4 bar. Thin layer chromatography was performed on Merck silica gel plastic plates, $60F_{254}$; compounds were visualized by treatment with a solution of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (20 g) and $Ce(SO_4)_2$ (0.4 g) in 10% sulfuric acid (400 mL) and heating at 150 °C. NMR spectra were recorded with Bruker Avance 400 or 600 spectrometers. TMS, or the resonance of the residual solvent ([D$_6$]DMSO: δ = 2.49 ppm), was used as internal standard. Diasterotopic proton pairs that could not be assigned stereochemically are characterized as highfield (h) and lowfield (l) in the ^{1}H NMR spectroscopic data. The NMR assignements of the amino-terminal tetrapeptide fragments of the octapeptides are labeled "A" and the carboxy-terminal fragments "B". FAB mass spectra were acquired with a Finnigan MAT 312 in the positive mode, using NBA as the matrix. CCDC-263833 (**17**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; Fax: + 44-1223/336-033; E-mail: deposit@ccdc.cam.ac.uk. Abbreviations: DIPEA = *N*,*N*′-diisopropylethylamine, HATU = *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, HOAT = 1-hydroxy-7-azabenzotriazole, PyBOP = benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate.

Ethyl (3*R*,6*S*,7*S*)-6,7-Isopropylidene-5-oxo-5*H*-[1,3]thiazolo[3,2-*a*]-pyridine-3-carboxylate (7): 100 mg (1.5 mmol) of NaN_3 was added to 250 mg (0.55 mmol) of compound **6**[7] in 15 mL of DMF. The solution was stirred at room temp. overnight and then the solvent was removed. The residue was purified by flash chromatography (ethyl acetate/toluene, 5:4, R_f = 0.55). 112 mg (0.38 mmol, 69%) of compound **7** was obtained as a colorless solid. ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 5.20 (d, $^3J_{8,7}$ = 5.6 Hz, 1 H, 8-H), 5.16 (dd, $^3J_{3,2proS}$ = 1.5, $^3J_{3,2proR}$ = 7.6 Hz, 1 H, 3-H), 4.68 (t, $^3J_{7,6/8}$ = 6.5 Hz, 1 H, 7-H), 4.64 (d, $^3J_{6,7}$ = 6.8 Hz, 1 H, 6-H), 4.13 (m, 2 H, OCH_2CH_3), 3.53 (dd, $^3J_{2proR,3}$ = 7.6, $^2J_{gem}$ = 11.4 Hz, 1 H, 2-H^{proR}), 3.37 (dd, $^3J_{2proS,3}$ = 1.5, $^2J_{gem}$ = 11.4 Hz, 1 H, 2-H^h), 1.32 (s, 3 H, $CH_3^{Isopr,proR}$), 1.28 (s, 3 H, $CH_3^{Isopr,proS}$), 1.18 (t, $^3J_{CH2CH3,CH2CH3}$ = 7.0 Hz, 3 H, OCH_2CH_3) ppm. ^{13}C NMR: δ = 168.36 (CO_2), 166.08 (C-5), 140.41 (C-8a), 108.38 (C_q^{Isopr}), 90.77 (C-8), 72.14 (C-6), 71.36 (C-7), 61.51 (OCH_2CH_3), 59.97 (C-3), 30.52 (C-2), 27.28 ($CH_3^{Isopr,proS}$), 25.89 ($CH_3^{Isopr,proR}$), 13.89 (OCH_2CH_3) ppm. FAB-MS: *m*/*z* = 300 [M + H]$^+$. $C_{13}H_{17}NO_5S$ (299.35): calcd. C 52.16, H 5.72, N 4.68; found C 52.36, H 5.78, N 4.42.

Lithium (3*R*,8*S*,8a*S*)-6,7-Dihydroxy-5-oxo-5*H*-[1,3]thiazolo[3,2-*a*]-pyridine-3-carboxylate (8): 3 mL of 1 N LiOH was added to 700 mg of **2/3** (1.93 mmol) in 15 mL of MeOH. The reaction mixture was brought to pH = 6 with 1 N HCl at room temp. after 12 d. The solvent was removed and the residue dried in high vacuum to obtain analytically pure **8** as a white solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.43 (s, 1 H, 6-NH), 6.75 (d, $^3J_{7,8}$ = 6.5 Hz, 1 H, 7-H), 5.10 (d, $^3J_{8OH,8}$ = 7.0 Hz, 1 H, 8-OH), 5.01 (d, $^3J_{8a,8}$ = 3.4 Hz, 1 H, 8a-H), 4.71 (dd, $^3J_{3,2h}$ = 6.7, $^3J_{3,2t}$ = 2.0 Hz, 1 H, 3-H), 4.03 (m, 1 H, 8-H), 3.17 (dd, $^3J_{2t,3}$ = 2.0, $^2J_{gem}$ = 9.9 Hz, 1 H, 2-H^t), 3.10 (dd, $^3J_{2h,3}$ = 6.7, $^2J_{gem}$ = 9.9 Hz, 1 H, 2-H^h), 1.43 (s, 9 H, CH_3 *t*Bu) ppm. ^{13}C NMR δ = 171.46, 157.80, 151.95 (CO_2, C-5, CO *t*Bu), 129.12 (C-6), 113.48 (C-7), 80.13 (C_q *t*Bu), 65.02 (C-8a), 63.95 (C-3), 61.24 (C-8), 33.23 (C-2), 27.92 (CH_3 *t*Bu) ppm. ESI-MS: *m*/*z* = 328.9 [M – H]$^-$, 659.2 [2 M – H]$^-$, 665.3 [2 M – 2 H + Li]$^-$.

(3*R*)-6-Amino-5-oxo-5*H*-[1,3]thiazolo[3,2-*a*]pyridine-3-carboxylic Acid Hydrochloride (1·HCl): 950 mg (2.6 mmol) of the protected dipeptides **2/3** were dissolved in 20 mL of MeOH. 4 mL of 1 N LiOH was added and the solution was kept at room temp. for two weeks. A pH of 6 was adjusted by addition of 1 N HCl. The solvent was removed and 15 mL of Et_2O/HCl was added to the dried residue cooled by an ice bath. The mixture was sonicated for 1 h and stirred at room temp. 2 d. The precipitate was removed by filtration and dried in high vacuum over KOH. 790 mg (2.5 mmol, 96%) of hydrochloride **1** was obtained as a black powder. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.11 (d, $^3J_{7,8}$ = 7.7 Hz, 1 H, 7-H), 6.21 (d, $^3J_{8,7}$ = 7.5 Hz, 1 H, 8-H), 5.49 (dd, $^3J_{3,2h}$ = 1.6, $^3J_{3,2t}$ = 8.6 Hz, 1 H, 3-H), 3.89 (dd, $^3J_{2t,3}$ = 8.7, $^2J_{gem}$ = 11.9 Hz, 1 H, 2-H^t), 3.57 (dd, $^3J_{2h,3}$ = 1.6, $^2J_{gem}$ = 11.8 Hz, 1 H, 2-H^h) ppm. ^{13}C NMR: δ = 169.12 (CO_2), 156.90 (C-5), 125, 123 (br. s, C-6, C-7), 99.18 (C-8), 62.75 (C-3), 32.18 (C-2) ppm. IR (KBr): $\tilde{\nu}$ = 3414, 2870, 2597, 1738, 1629, 1536, 1404, 1376, 1233, 760 cm^{-1}. ESI-MS: *m*/*z* = 212.7 [M + H]$^+$, 218.7 [M + Li]$^+$, 250.8 [M + K]$^+$.

Tripeptide 5: 4 mL of 1 N LiOH was added to 850 mg (2.35 mmol) of **2/3** in 30 mL of MeOH. The pH was adjusted to 6 with 1 N HCl after 20 d and the solvent was removed under high vacuum. The residue was dissolved in 10 mL of DMF together with 400 mg of H-GlyOMe·HCl (3.2 mmol, ca. 1.3 equiv.). Then 400 mg of HOBT (2.96 mmol, ca. 1.2 equiv.), 500 μL of EDCI (442 mg, 2.8 mmol, ca. 1.2 equiv.) and 550 μL of DIPEA (3.2 mmol) were added. The reaction mixture was stirred overnight. The solvent was removed and the tripeptide separated by flash chromatography (ethyl acetate/toluene, 4:1, R_f = 0.55). 818 mg (2.04 mmol, 87%) of compound **5** was obtained as a colourless solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.47 (t, $^3J_{NH,H\alpha/H\alpha'}$ = 6.0 Hz, 1 H, Gly-NH), 7.51 (s, 1 H, 6-NH), 6.80 (d, $^3J_{7,8}$ = 6.7 Hz, 1 H, 7-H), 5.29 (d, $^3J_{8OH,8}$ = 7.5 Hz, 1 H, 8-OH), 5.06 (d, $^3J_{8a,8}$ = 3.2 Hz, 1 H, 8a-H), 4.97 (dd, $^3J_{3,2h}$ = 2.7, $^3J_{3,2t}$ = 6.7 Hz, 1 H, 3-H), 4.08 (m, 1 H, 8-H),

3.83 (m, 2 H, Gly-CH_2) 3.62 (s, 3 H, OCH_3), 3.23 (dd, $^3J_{2,3}$ = 6.7, $^2J_{gem}$ = 11.0 Hz, 1 H, 2-H^t), 3.07 (dd, $^3J_{2h,3}$ = 2.7, $^2J_{gem}$ = 11.1 Hz, 1 H, 2-H^h), 1.43 (s, 9 H, CH_3 *t*Bu) ppm. ^{13}C NMR: δ = 169.86, 169.33 (3-CO, Gly-CO), 158.52, 151.94 (C-5, CO*t*Bu), 129.33 (C-6), 114.14 (C-7), 80.06 (C_q, *t*Bu), 65.61 (C-8a), 62.69 (C-3), 61.89 (C-8), 51.67 (OCH_3), 40.73 (Gly-CH_2), 32.56 (C-2), 27.80 (CH_3, *t*Bu) ppm. ESI-MS: *m*/*z* = 402.0 [M + H]$^+$, 424.0 [M + Na]$^+$, 825.3 [2 M + Na]$^+$. $C_{16}H_{23}N_3O_7S$ (401.44): calcd. C 47.87, H 5.78, N 10.47; found C 51.19, H 6.17, N 7.68.

Tripeptide Hydrochloride 9: 25 mL of Et_2O/HCl was added to 550 mg (1.37 mmol) of compound **5**. The resulting suspension was stirred at room temp. overnight and the colorless precipitate was separated and dried over KOH in high vacuum. 405 mg (1.27 mmol, 93%) of tripeptide **9** was obtained. ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 9.00 (t, $^3J_{NH,H\alpha/H\alpha'}$ = 5.9 Hz, 1 H, Gly-NH), 7.52 (d, $^3J_{78}$ = 7.6 Hz, 1 H, 7-H), 6.30 (d, $^3J_{8,7}$ = 7.6 Hz, 1 H, 8-H), 5.58 (dd, $^3J_{3,2h}$ = 1.8, $^3J_{3,2t}$ = 9.1 Hz, 1 H, 3-H), 3.96 (dd, $^3J_{2t,3}$ = 9.1, $^2J_{gem}$ = 11.7 Hz, 1 H, 2-H^t), 3.93 (dd, $^3J_{H\alpha t,NH}$ = 6.2, $^2J_{gem}$ = 17.3 Hz, 1 H, Gly-CH^t), 3.87 (dd, $^3J_{H\alpha h,NH}$ = 5.6, $^2J_{gem}$ = 17.3 Hz, 1 H, Gly-CH^h), 3.63 (s, 3 H, OCH_3), 3.49 (dd, $^3J_{2h,3}$ = 2.0, $^2J_{gem}$ = 11.7 Hz, 1 H, 2-H^h) ppm. ^{13}C NMR: δ = 169.89, 167.57 (Gly-CO, CO_2), 156.87 (C-5), 147 (br. s, C-8a), 130 (br. s, C-7), 119 (br. s, C-6), 98.27 (C-8), 63.44 (C-3), 51.79 (OMe), 40.66 (Gly-CH_2), 32.82 (C-2). IR (KBr): $\tilde{\nu}$ = 3297, 3027, 2795, 2525, 1753, 1661, 1512, 1376, 1203, 760 cm^{-1}. ESI-MS: *m*/*z* = 283.8 [M + H]$^+$.

Tetrapeptide 10: 320 mg (1.0 mmol) of hydrochloride **9** and 400 mg (1.5 mmol) BocPheOH were dissolved in 8 mL of DMF. 1 g (1.9 mmol) of PyBOP and 1 mL of NMM were added and the reaction mixture was stirred overnight. The solvent was evaporated and the product purified by flash chromatography (ethyl acetate/toluene, 5:1, R_f = 0.55). The combined fractions which contained product were concentrated and hexane was added until a white precipitate was observed. The suspension was kept at 4 °C overnight, the precipitate separated and washed with Et_2O to obtain 469 mg (0.88 mmol, 88%) of tetrapeptide **10** as a yellowish powder. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 9.18 (s, 1 H, 6-NH), 8.76 (t, $^3J_{NH,H\alpha/H\alpha'}$ = 5.9 Hz, 1 H, Gly-NH), 8.18 (d, $^3J_{7,8}$ = 7.8 Hz, 1 H, 7-H), 7.3 [m, 6 H, PheH/Boc-NH, (DQF-COSY: 7.35)], 6.26 (d, $^3J_{8,7}$ = 7.8 Hz, 1 H, 8-H), 5.54 (dd, $^3J_{3,2proS}$ = 2.1, $^3J_{3,2proR}$ = 8.6 Hz, 1 H, 3-H), 4.35 (dd, $^3J_{H\alpha,H\beta proR}$ = 11.0, $^3J_{H\alpha,H\beta proS}$ = 4.1, $^3J_{H\alpha,NH}$ = 8.4 Hz, 1 H, Pheα-H), 3.9 (m, 3 H, GlyCH$_2$/ 2-H^{proR}), 3.64 (s, 3 H, OCH_3), 3.46 (dd, $^3J_{2proS,3}$ = 2.1, $^2J_{gem}$ = 11.8 Hz, 1 H, 2-H^{proS}), 3.10 (dd, $^3J_{H\beta proS,H\alpha}$ = 4.1, $^2J_{gem}$ = 13.8, 1 H, Pheβ-H^{proS}), 2.77 (dd, $^3J_{H\beta proR,H\alpha}$ = 11.0, $^2J_{gem}$ = 13.8, 1 H, Pheβ-H^{proR}), 1.28 (s, 9 H, CH_3, *t*Bu) ppm. ^{13}C NMR: δ = 170.78, 169.93, 167.70 (CO_2, Phe-CO, Gly-CO), 156.18, 155.39, (C-5, CO, *t*Bu) 140.92 (C-8a), 138.04 (Phe-C_q), 129.13, 128.02, 126.20 (Phe-$CH_{arom.}$), 124.03, 123.88 (C-7/C-6), 98.75 (C-8), 78.42 (C_q, *t*Bu), 63.56 (C-3), 56.68 (Phe-Cα), 51.78 (OCH_3), 40.68 (Gly-CH_2), 36.83 (Phe-Cβ), 32.41 (C-2), 28.05 (3C, CH_3 *t*Bu) ppm. IR (KBr): $\tilde{\nu}$ = 3339, 2977, 1753, 1678, 1649, 1512, 1239, 1170, 767, 702 cm^{-1}. ESI-ESI: *m*/*z* = 531.2 [M + H]$^+$, 548.2 [M + NH_4]$^+$, 553.2 [M + Na]$^+$, 1078.5 [2 M + NH_4]$^+$. $C_{25}H_{30}N_4O_7S$ (530.60) calcd. C 56.59, H 5.70, N 10.56; found C 55.84, H 5.62, N 10.37.

Octapeptide 13. a) Saponification of 10: Within 5 min, 400 µL of 1 N LiOH was added to 100 mg (0.19 mmol) of tetrapeptide **10**, suspended in 5 mL of MeOH. Another 5 mL of MeOH was added, until the peptide was fully dissolved. After 2 h 40 min, an additional 1 mL of 0.1 N LiOH was added. After 3 h 15 min, the reaction mixture was adjusted to pH = 6 with 1 N HCl and then the solvent was removed. **b) Acidolysis of 10:** 100 mg of **10** was suspended in 6 mL of Et_2O/HCl and stirred overnight. Then the solvent was removed and the residue dried over KOH in high vacuum. **c) Peptide Coupling:** The selectively deprotected tetrapeptides **11** and **12** were dissolved in 3 mL of DMF together with 150 mg (0.29 mmol) of PyBOP. The pH was adjusted to 8 with NMM and the reaction mixture was stirred overnight. Then the solvent was removed and the octapeptide purified by flash chromatography (ethyl acetate/methanol, 7:1 R_f = 0.4) to obtain 164 mg (0.18 mmol 95%) of **13**. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 9.32 (s, 1 H, 6B-NH), 9.17 (s, 1 H, 6A-NH), 8.78 (t, $^3J_{NH,H\alpha/H\alpha'}$ = 5.9 Hz, 1 H, GlyB-NH), 8.54 (t, $^3J_{NH,H\alpha/H\alpha'}$ = 5.7 Hz, 1 H, GlyA-NH), 8.43 (d, $^3J_{NH,H\alpha}$ = 8.0 Hz, 1 H, PheB-NH), 8.17 (d, $^3J_{7,8}$ = 7.8 Hz, 1 H, 7A-H), 8.11 (d, $^3J_{7,8}$ = 7.8 Hz, 1 H, 7B-H), 7.33 (d, $^3J_{NH,H\alpha}$ = 8.6 Hz, 1 H, Boc-NH), 7.25 (m, 10 H, PheA/B-H_{arom}), 6.25 (d, $^3J_{8,7}$ = 7.8 Hz, 1 H, 8A-H), 6.23 (d, $^3J_{8,7}$ = 7.8 Hz, 1 H, 8B-H), 5.52 [m, 2 H, DQF-COSY: 5.53 (3B), 5.51 (3A)], 4.80 (ddd, $^3J_{H\alpha,H\beta h}$ = 9.4, $^3J_{H\alpha,H\beta t}$ = 4.9, $^3J_{H\alpha,NH}$ = 8.0 Hz, 1 H, PheB-αH), 4.33 (ddd, $^3J_{H\alpha,H\beta h}$ = 11.0, $^3J_{H\alpha,H\beta t}$ = 4.1, $^3J_{H\alpha,NH}$ = 8.4 Hz, 1 H, PheA-αH), 4.0–3.8 [m, 4 H, DQF-COSY: 3.91 (GlyB-CH_2), 3.91 (2B-H^t), 3.85 (2A-H^t)], 3.75 (m, 2 H, GlyA-CH_2), 3.63 (s, 3 H, OCH_3), 3.4 [m, 2 H, DQF-COSY: 3.45 (2B-H^h), 3.44 (2A-H^h)], 3.1 (m, 2 H DQF-COSY: 3.09 (PheA-βH^t), 3.08 (PheB-βH^t)], 2.83 (dd, $^3J_{H\beta h,H\alpha}$ = 9.7, $^2J_{gem}$ = 13.8 Hz, 1 H, PheB-βH^h), 2.76 (dd, $^3J_{H\beta h,H\alpha}$ = 11.1, $^2J_{gem}$ = 14.0 Hz, 1 H, PheA-βH^h), 1.27 (s, 9 H, CH_3 *t*Bu) ppm. ^{13}C NMR: δ = 170.76, 170.14, 169.93, 168.39, 167.76, 167.34 (PheA/B-CO, GlyA/B-CO, 3A/B-CO), 156.21, 156.18 (CO-5A/B), 155.38 (CO, *t*Bu), 141.27, 141.09 (C-8aA/B), 138.01, 137.46 (PheA/B-C_q), 129.20, 129.11, 128.05, 128.01, 126.33, 126.19 (PheA/B-$CH_{arom.}$), 124.88 (C-7B), 123.95 (C-7A) 123.89 (br. s, C-6), 98.69, 98.63 (C-8A/B), 78.42 (C_q, *t*Bu), 63.72, 63.53 (C-3A/B), 56.70 (PheA-Cα), 54.75 (PheB-Cα), 51.79 (OCH_3), 41.69 (GlyA-CH_2), 40.67 (GlyB-CH_2), 37.15 (PheB-Cβ), 36.86 (PheA-Cβ), 32.44 32.37 (C-2A/B), 28.04 (3 C, CH_3, *t*Bu) ppm. ESI-MS: *m*/*z* = 929.4 [M + H]$^+$, 946.5 [M + NH_4]$^+$, 951.3 [M + Na]$^+$.

Cyclic Octapeptide 16: 4 equiv. of 1 N LiOH were added over 5 h to a solution of 50 mg (0.054 mmol) of octapeptide **13** in 15 mL of MeOH and 5 mL of DCM. The solution was neutralized with 1 N HCl and the solvent was removed. The dry residue was taken up in 8 mL of Et_2O/HCl and sonicated for 30 min. The Suspension was stirred overnight. The solvent was removend and the reaction mixture dried over KOH in high vacuum. The deprotected peptide was dissolved in 500 mL of DMF. 25 mg (0.066 mmol) of HATU, 10 mg (0.073 mmol) of HOAT, and 75 mL of collidine (0.69 mmol) were added and the solution was stirred overnight. The solvent was removed and the residue was purified by flash chromatography [$CHCl_3$/MeOH, 5:1, R_f($CHCl_3$/MeOH, 7:1) = 0.15]. 33 mg (0.041 mmol, 76%) of the cyclic octapeptide **16** was obtained as a white solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 9.18 (s, 2 H, 6-NH), 8.59 (br. s, 2 H, Gly-NH), 8.36 (d, $^3J_{NH,H\alpha}$ = 9.5 Hz, 2 H, Phe-NH), 8.10 (d, $^3J_{7,8}$ = 7.9 Hz, 2 H, 7-H), 7.1–7.2 (m, 10 H, Phe-$H_{arom.}$), 6.28 (d, $^3J_{8,7}$ = 7.9 Hz, 2 H, 8-H), 5.49 (dd, $^3J_{3,2t}$ = 8.4, $^3J_{3,2h}$ = 2.2 Hz, 2 H, 3-H), 4.86 (m, 2 H, Pheα-H), 3.85–3.75 (m, 4 H, Gly-CH^t, 2-H^t), 3.54–3.44 (m, 4 H, GlyCH^h, 2-H^h), 2.99 (dd, $^3J_{H\beta t,H\alpha}$ = 5.5, $^2J_{gem}$ = 13.8 Hz, 2 H, Pheβ-H^t), 2.81 (dd, $^3J_{H\beta h,H\alpha}$ = 9.5, $^2J_{gem}$ = 13.6 Hz, 2 H, Pheβ-H^h) ppm. ^{13}C NMR: δ = 128.2, 127.6, 125.9 (Phe-$CH_{arom.}$), 125.4 (C-7), 99.3 (C-8), 63.9 (C-3), 54.5 (Phe-Cα), 41.4 (Gly-CH_2), 37.1 (Phe-Cβ), 32.3 (C-2) ppm. ESI-MS: *m*/*z* = 797.4 [M + H]$^+$, 803.4 [M + Li]$^+$.

Tripeptide 17 (CCDC-263833)**:** 2.5 mL of 1 N LiOH was added to a solution of 620 mg (1.7 mmol) of **2/3** in 15 mL of MeOH. The reaction mixture was stirred for 17 d at room temp. and neutralized with 1 N HCl. The solvent was removed and the residue dried in high vacuum and dissolved in 10 mL of DMF together with 500 mg (2.3 mmol) of LPhe-OMe, 400 mg (2.1 mmol) of EDCI·HCl, and

300 mg of HOBT. The solution was brought to pH = 7 with DIPEA and stirred at room temp. overnight. After removal of the solvent, the residue was purified by flash chromatography (ethyl acetate/toluene, 9:1, R_f = 0.7) to obtain 780 mg (1.6 mmol, 94%) of **17** as a white solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.38 (d, $^3J_{NH,H\alpha}$ = 7.7 Hz, 1 H, Phe-NH), 7.50 (s, 1 H, 6-NH), 7.17–7.3 (m, 5 H, Phe-H$_{arom.}$), 6.81 (d, $^3J_{7,8}$ = 6.8 Hz, 1 H, 7-H), 5.26 (d, $^3J_{8-OH,8}$ = 7.3 Hz, 1 H, 8-OH), 5.00, (d, $^3J_{8a,8}$ = 3.3 Hz, 1 H, 8a-H), 4.94 (dd, $^3J_{3,2h}$ = 3.1, $^3J_{3,2t}$ = 6.8 Hz, 1 H, 3-H), 4.5 (m, 1 H, Pheα-H), 4.09 (m, 1 H, 8-H), 3.59 (s, 3 H, OCH$_3$), 3.21 (dd, 1 H, $^3J_{2t,3}$ = 6.8, $^2J_{gem}$ = 11.0 Hz, 1 H, 2-H^t), 3.04 (dd, 1 H, $^3J_{H\beta t,H\alpha}$ = 5.9, $^2J_{gem}$ = 13.9 Hz, 1 H, Pheβ-H^t), 2.96 (m, 2 H, 2-H^h, Pheβ-H^h), 1.44 (s, 9 H, CH$_3$ *t*Bu) ppm. ^{13}C NMR: δ = 171.45, 168.92 (C3-*C*O, Phe-CO), 158.40, 151.89 (CO-5, CO *t*Bu) 136.93 (Phe-C$_q$), 129.08 (C-6), 129.05, 128.18, 126.49 (Phe-CH$_{arom.}$), 114.35 (C-7), 80.06 (C$_q$, *t*Bu), 65.91 (C-8a), 62.49 (C-3), 61.64 (C-8), 53,67 (Phe-Cα), 51.83 (OCH$_3$), 36.40 (Phe-Cβ), 32.43 (C-2), 27.79 (3C, CH$_3$ *t*Bu) ppm. IR (KBr): $\tilde{\nu}$ = 3442, 3387, 3278, 2988, 1743, 1701, 1662, 1633, 1518, 1434, 1157, 697 cm^{-1}. ESI-MS: *m*/*z* = 492.2 [M + H]$^+$, 509.2 [M + NH$_4$]$^+$. C$_{23}$H$_{29}$N$_3$O$_7$S (491.57): calcd. C 56.20, H 5.95, N 8.55; found C 56.05, H 5.77, N 8.40.

Tripeptide Hydrochloride 18: 1.5 g (3.1 mmol) of **17** was dissolved in 3 mL of MeOH and cooled with an ice bath. Then, 20 mL of Et$_2$O/HCl was added and a colorless precipitate formed after 1 min. The suspension was sonicated for 2 h and then stirred at room temp. overnight. The precipitate was separated by filtration and dried over KOH to obtain 1.1 g (2.7 mmol, 87%) of **18**. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.94 (d, $^3J_{NH,H\alpha}$ = 7.7 Hz, 1 H, Phe-NH), 7.5 (m, 1 H, 7-H), 7.3–7.2 (m, 5 H, Phe-H$_{arom.}$), 6.30 (d, $^3J_{8,7}$ = 7.7 Hz, 1 H, 8-H), 5.56 (dd, $^3J_{3,2h}$ = 2.2, $^3J_{3,2t}$ = 9.2 Hz, 1 H, 3-H), 4.49 (m, 1 H, Pheα-H), 3.93 (dd, $^3J_{2t,3}$ = 9.2, $^2J_{gem}$ = 11.9 Hz, 1 H, 2-H^t), 3.60 (s, 3 H, OCH$_3$), 3.42 (dd, $^3J_{2h,3}$ = 2.0, $^2J_{gem}$ = 11.9 Hz, 1 H, 2-H^h), 3.01 (m, 2 H, Pheβ-H) ppm. ^{13}C NMR: δ = 171.36 (Phe-CO), 167.09 (3-CO), 156.85 (CO-5), 148 (br. s, C-8a), 136.65 (Phe-C$_q$), 131 (br. s, C-7), 129.07, 128.27, 126.63 (Phe-CH$_{arom.}$), 119 (br. s, C-6), 98.18 (C-8), 63.42 (C-3), 53.94 (Phe-Cα), 51.94 (OCH$_3$), 36.43 (Phe-Cβ), 32.63 (C-2) ppm. CI-MS (NH$_3$): *m*/*z* = 374.1 [MH]$^+$, 391.2 [M + NH$_4$]$^+$. C$_{18}$H$_{20}$ClN$_3$O$_4$S (409.89): calcd. C 52.77, H 4.92, N 10.25; found C 52.44, H 5.14, N 9.94.

Tetrapeptide 19: 850 mg (2.07 mmol) of hydrochloride **18** and 500 mg (2.85 mmol) of BocNH-Gly-OH were dissolved in 20 mL of DMF. 1.25 g (2.54 mmol) of PyBOP and 2 mL of NMM were added and the reaction misture was stirred at room temp. overnight. The solvent was removed and the residue purified by flash chromatography [ethyl acetate/toluene, 5:1 → 9:1, R_f(EtOAc/toluene, 9:1) = 0.55] to obtain 1.05 g (1.98 mmol, 96%) of tetrapeptide **19**. ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 9.00 (s, 1 H, 6-NH), 8.75 (d, $^3J_{NH,H\alpha}$ = 7.5 Hz, 1 H, Phe-NH), 8.15 (d, $^3J_{7,8}$ = 7.8 Hz, 1 H, 7-H), 7.2 (m, 6 H, Phe-H$_{arom.}$/Boc-NH, DQF-COSY: 7.27), 6.23 (d, $^3J_{8,7}$ = 7.8 Hz, 1 H, 8-H), 5.53 (dd, $^3J_{3,2proS}$ = 2.0, $^3J_{3,2proR}$ = 9.0 Hz, 1 H, 3-H), 4.51 (m, 1 H, Pheα-H), 3.87 (dd, $^3J_{2proR,3}$ = 9.0, $^2J_{gem}$ = 11.9 Hz, 1 H, 2-H^{proR}), 3.71 (m, 2 H, Gly-CH$_2$), 3.60 (s, 3 H, OCH$_3$), 3.38 (dd, $^3J_{2proS,3}$ = 2.0, $^2J_{gem}$ = 11.7 Hz, 1 H, 2-H^{proS}), 3.03 (dd, $^3J_{H\beta t,H\alpha}$ = 5.9, $^2J_{gem}$ = 13.9 Hz, 1 H, Pheβ-H^t), 2.96 (dd, $^3J_{H\beta h,H\alpha}$ = 7.9, $^2J_{gem}$ = 13.9, 1 H, Pheβ-H^h), 1.38 (s, 9 H, CH$_3$, *t*Bu) ppm. ^{13}C NMR: δ = 171.37 (Phe-CO), 168.51 (Gly-CO), 167.21 (3-CO), 156.12 (CO-5), 155.86 (CO, *t*Bu), 140.72 (C-8a), 136.61 (Phe-C$_q$), 129.05, 128.25, 126.59 (Phe-CH$_{arom}$), 124.02 (C-6), 123.69 (C-7), 98.67 (C-8), 78.38 (C$_q$, *t*Bu), 63.45 (C-3), 53.83 (Phe-Cα), 51.90 (OCH$_3$), 44.23 (Gly-CH$_2$), 36.50 (Phe-Cβ), 32.17 (C-2), 28.08 (CH$_3$ *t*Bu) ppm. IR (KBr): $\tilde{\nu}$ ≙ = 3277, 2979, 1752, 1672, 1636, 1501, 1240, 1164, 768, 738 cm^{-1}. ESI-MS: *m*/*z* = 531.3 [M + H]$^+$, 548.3 [M + NH$_4$]$^+$, 553.3 [M + Na]$^+$. C$_{25}$H$_{30}$N$_4$O$_7$S (530.60) calcd. C 56.59, H 5.70, N 10.56; found C 55.96, H 5.59, N 10.43.

Octapeptide 22. a) Saponification of 19: 60 mg (0.11 mmol) of tetrapeptide **19** was dissolved in 2 mL of MeOH. Aliquots of 1 N LiOH were added starting with 250 µL, 50 µL after 1 h, 50 µL after 1 h 30 min. The reaction mixture was brought to pH = 6 with 1 N HCl after 2 h and then the solvent was removed and dried. **b) Acidolysis of 19:** 60 mg (0.11 mmol) of **19** was dissolved in 3 mL of MeOH. 1 mL of TFA was added and another 3 mL after 15 min. The solution was stirred at room temp. for 3 h and another 2 h at 45 °C. The solvent was removed by coevaporation. c) Peptide coupling: Both selectively deprotected tetrapeptides **20** and **21** were dissolved in 4 mL of DMF. Then, 100 mg (0.19 mmol) of PyBOP was added and the pH adjusted to 8 with NMM. After 16 h of stirring at room temp., the solvent was removed and the raw product purified by flash chromatography (CHCl$_3$/MeOH, 10:1, R_f = 0.25) and dried in high vacuum to obtain 70 mg (0.075 mmol, 68%) of octapeptide **22** as a colourless foam. ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 9.13 (s, 1 H, 6B-NH), 9.03 (s, 1 H, 6A-NH), 8.78 (d, $^3J_{NH,H\alpha}$ = 7.1 Hz, 1 H, PheB-NH), 8.56 [m, 2 H, DQF-COSY: 8.56 (PheA-NH), 8.57 (GlyB-NH)], 8.16 (d, $^3J_{7,8}$ = 7.9 Hz, 1 H, 7A-H), 8.15 (d, $^3J_{7,8}$ = 7.9 Hz, 1 H, 7B-H), 7.29 (t, $^3J_{NH,H\alpha/H\alpha'}$ = 6.0 Hz, 1 H, Boc-NH), 7.3–7.1 (m, 10 H, PheA/B-H$_{arom.}$), 6.24 (d, $^3J_{8,7}$ = 7.9 Hz, 1 H, 8B-H), 6.22 (d, $^3J_{8,7}$ = 7.9 Hz, 1 H, 8A-H), 5.52 (dd, $^3J_{3,2proR}$ = 8.5, $^3J_{3,2proS}$ = 1.9 Hz, 1 H, 3B-H), 5.51 (dd, $^3J_{3,2proR}$ = 8.5, $^3J_{3,2proS}$ = 1.9 Hz, 1 H, 3A-H), 4.55 (m, 1 H, PheA-αH), 4.49 (m, 1 H, PheB-αH), 3.9–3.85 [m, 3 H, DQF-COSY: 3.92 (GlyB-CH$_2$), 3.88 (2 B-H^{proR})], 3.75 (dd, $^3J_{2proR,3}$ = 8.8, $^2J_{gem}$ = 11.8 Hz, 1 H, 2A-H^{proR}), 3.71 (m, 2 H, GlyA-CH$_2$), 3.59 (s, 3 H, OCH$_3$), 3.53 (dd, $^3J_{2proS,3}$ = 1.9, $^2J_{gem}$ = 11.8 Hz, 1 H, 2A-H^{proS}), 3.40 (dd, $^3J_{2proS,3}$ = 1.9, $^2J_{gem}$ = 11.8 Hz, 1 H, 2 B-H^{proS}), 3.07 (dd, $^3J_{H\beta t,H\alpha}$ = 5.2, $^2J_{gem}$ = 14.0 Hz, 1 H, PheA-βH^t), 3.02 (dd, $^3J_{H\beta t,H\alpha}$ = 5.8, $^2J_{gem}$ = 14.0 Hz, 1 H, PheB-βH^t), 2.95 (dd, $^3J_{H\beta h,H\alpha}$ = 8.2, $^2J_{gem}$ = 14 Hz, 1 H, PheB-βH^h), 2.83 (dd, $^3J_{H\beta h,H\alpha}$ = 8.5, $^2J_{gem}$ = 14.0 Hz, 1 H, PheA-βH^h), 1.38 (s, 9 H, CH$_3$, *t*Bu) ppm. ^{13}C NMR: δ = 171.44 (PheB-CO), 171.20 (PheA-CO), 168.53 (GlyA-CO) 167.81 (GlyB-CO), 167.29 (3B-CO), 167.00 (3A-CO), 156.19, 156.16 (C-5A, C-5B), 155.88 (CO *t*Bu), 140.92, 140.84 (C-8aA, C-8aB), 137.28 (PheA-C$_q$), 136.61 (PheB-C$_q$), 129.15, 129.01, 128.25, 128.05, 126.60, 126.23 (PheA/B-CH$_{arom.}$),124.26 (C-7B), 124.06 (6A/B-C), 123.70 (C-7A), 98.75, 98.68 (C-8A, C-8B), 78.39 (C$_q$, *t*Bu), 63.64 (C-3B), 63.43 (C-3A), 54.15 (PheA-Cα), 53.97 (PheB-Cα), 51.92 (OCH$_3$), 44.25 (GlyB-CH$_2$), 43.01 (GlyA-CH$_2$), 37.59 (PheA-Cβ), 36.51 (PheB-Cβ), 32.27 (C-2B), 32.22 (C-2A), 28.11 (CH$_3$, *t*Bu) ppm. ESI-MS: *m*/*z* = 929.5 [M + H]$^+$, 946.5 [M + NH$_4$]$^+$. C$_{44}$H$_{48}$N$_8$O$_{11}$S$_2$ (929.05): calcd. C 56.89, H 5.21, N 12.06; found C 56.06, H 5.29, N 11.73.

Cyclic Octapeptide 25: 100 mg (0.11 mmol) of **22** was dissolved in 3 mL of CH$_2$Cl$_2$/MeOH, 1:1. 0.22 mL, 0.1 mL, and 0.1 mL of 1 N LiOH were added at room temp. within 5 h. The solution was neutralized with 1 N HCl. The solvent was removed and the dry residue was suspended in 10 mL of Et$_2$O/HCl and sonicated in an ice bath for 3 h and then stirred overnight at room temp. **24** was separated by filtration and dried over KOH in high vacuum. The deprotected peptide was dissolved in 600 mL of DMF. Then, 50 mg (0.13 mmol) of HATU, and 15 mg (0.11 mmol) of HOAT, and 0.2 mL (1.5 mmol) of collidine were added. After 3 d at room temp., the solvent was removed and the raw product purified by flash chromatography [CHCl$_3$/MeOH, 7:1 → 4:1, R_f(CHCl$_3$/MeOH, 4:1) = 0.7] to obtain 31 mg (0.39 mmol, 35%) of cyclic octapeptide **25** as a white solid. ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 9.12 (s, 2 H, 6-NH), 8.58 (br. s, 2 H, Gly-NH), 8.55 (d, $^3J_{NH,H\alpha}$ = 9.1 Hz, 2 H, Phe-NH), 8.10 (d, $^3J_{7,8}$ = 7.9 Hz, 2 H, 7-H), 7.1–

6.9 (m, 10 H, Phe-$H_{arom.}$), 6.34 (d, $^3J_{8,7}$ = 7.9 Hz, 1 H, 8-H), 5.47 (dd, $^3J_{3,2t}$ = 8.2, $^3J_{3,2h}$ = 3.6 Hz, 2 H, 3-H), 4.54 (m, 2 H, Pheα-H), 3.98 (m, 4 H, Gly-CH_2), 3.68 (dd, $^3J_{2t,3}$ = 8.5, $^2J_{gem}$ = 11.3 Hz, 2 H, 2-H^t), 3.46 (m, 2 H, 2-H^h), 3.26 (dd, $^3J_{H\beta t,H\alpha}$ = 3.8, $^2J_{gem}$ = 13.7 Hz, 2 H, Pheβ-H^t), 2.67 (t, $^3J_{H\beta h,H\alpha/H\beta t}$ = 12.6 Hz, 2 H, Pheβ-H^h) ppm. ^{13}C NMR (HMQC): δ = 128.1, 127.6, 125.9 (Phe-$CH_{arom.}$), 125.0 (C-7), 100.2 (C-8), 64.8 (C-3), 53.8 (Phe-Cα), 43.4 (Gly-CH_2), 36.9 (Phe-Cβ), 29.9 (C-2) ppm. ESI-MS: *m*/*z* = 797.3 $[M + H]^+$, 814.2 $[M + NH_4]^+$.

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