A shape-persistent D,L-dipeptide building block for the assembly of rigidified oligopeptides

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Mannuronic acid (**5**) was transformed into the densely functionalised D,L-dipeptide mimic **1** and subsequently inserted into the cyclic hexapeptide **3**. The *gulo*-configurated seven-membered lactam **1** exhibits an inverted ring conformation compared to the previously described L,L-dipeptide mimic **2**. In spite of this considerable difference, both prefer the same *i* to *i* + 1 positions of a β-turn within a cyclic hexapeptide.

Introduction

Peptides are of outstanding interest in pharmaceutical research because they play an important role in many biologically relevant processes. Macrocyclisation[1] and short-range[2] cyclisation, respectively, are effective methods to restrain either globally or locally the flexibility of peptides in order to obtain selective protein ligands. Fused rings lock further torsions by eliminating the possible ring inversions of monocyclic systems. Various classes of bicyclic dipeptides were recently reviewed by Cluzeau and Lubell.[3] The published synthetic strategies which make use of two amino acid precursors are generally cumbersome because temporary protecting groups are needed, many bicyclic lactams are obtained as bridge-head epimers, and above all, the bicyclic ring formation consumes the amino acid side chain functionalities. The chemistry of sparsely functionalised oxa-[4] or thiazolidinlactams[5] is well developed.

Our research is focused on uronolactone precursors because sugars bear a hydroxyl group on every carbon, thus allowing the optional attachment of amino acid side chains on any ring position of the bicyclic dipeptide.[6,7] The hybrid dipeptides **1** and **2** combine the over-functionalised character of sugars with the rigidity of fused dipeptides. Their synthesis is straightforward because the lactam forms without the need of a coupling reagent and the bridge-head stereocenter is fully controlled by the stereochemistry of the neighbouring hydroxyl. In relation to the well studied conformational preferences of *gluco*-configured **2** (a L,L-dipeptide), the bicyclic lactam **1** has an inverted chair-conformation and an L-*gulo*-configuration, and thus represents a D,L-dipeptide (Fig. 1). In order to emphasise the side chain variability of our approach, **1** was decorated with apolar functional groups combining different amino acid side chains, which are the geminal methyl group (Val or Leu mimetic), a benzyl group (Phe mimetic), and a fused thiaproline (*trans*-Pro mimetic). Three different side chains on a dipeptide backbone make **1** a locked mimic of tripeptide sequences like Val-Phe-Pro (found in, for example, ergopeptine) or Leu-D-Phe-Pro (found in, for example, gramicidin S). Conformational preferences of potentially turn-inducing dipeptide isosteres are identified in model peptides with Gly, which fits any turn position. The C_2-symmetric cyclic hexapeptide **3** was synthesised in order to experimentally characterise the conformational behaviour of **1** in an oligopeptide context, and to study the compatibility of the side chain modifications with peptide chemistry.

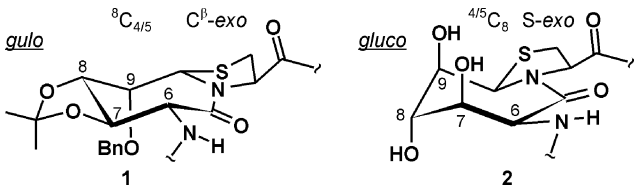

Fig. 1 The seven-membered ring of the *gulo*-configurated bicycle **1** exhibits the inverted chair-conformation ($^{8}C_{4,5}$) of the *gluco*-configured bicycle **2** ($^{4,5}C_{8}$). A C$^{\beta}$-*exo*-conformation is observed in the thiaproline ring of **1**. In the thiaproline of **2** the S-atom experiences the largest out-of-plane displacement in the twisted ring (S-*exo*).

Results and discussion

According to the observation that D-amino acids are likely to be found in the *i* + 1 position of a βII′-turn and that Pro fits into the *i* + 2 position of a β-turn, **1** is expected to occupy the *i* + 1 to *i* + 2 positions of a β-turn.[8] Previous studies have already shown the limitations of such general rules,[9] and the first X-ray structures of cyclic hexapeptides with varying amino acid composition have been recently described.[10] Independently of the chirality of other amino acids, the fused dipeptide **2** is found in the long side of the cyclic hexapeptide, equivalent to the *i* to *i* + 1 positions of each of the two β-turns, in the solid state as well as in solution, with the hydrogen-bonding pattern indicated in Fig. 2. In spite of the severe change of incorporating two stereocenters with D-configuration in the backbone of hexapeptide **3**, there are obvious similarities between the NMR data of hexapeptides **3** and **4**. The ^{1}H NMR resonance signals of the amide protons of **3** in DMSO[d_6] show a strongly deshielded GlyNH, which exhibits a sizable temperature dependence ($\Delta\delta/\Delta T = -4.7$ ppb K^{-1}) as opposed to 6NH, which is shielded and exhibits a weak temperature dependence ($\Delta\delta/\Delta T = +0.2$ ppb K^{-1}). It follows from the above that GlyNH is solvent-accessible while 6NH participates in an intramolecular hydrogen bond. The strong NOE contacts GlyNH–6NH and GlyNH–H3 identify the βII-turn (Fig. 3). The weak NOE contact GlyNH–9aH

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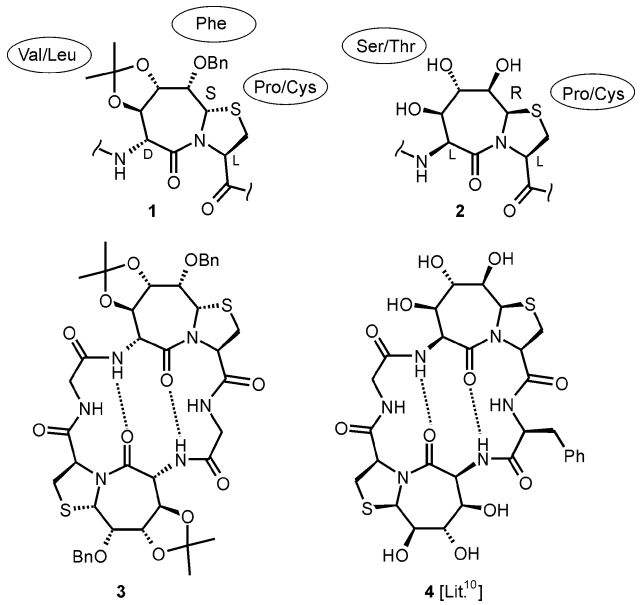

Fig. 2 The seven-membered lactam ring of D,L-dipeptide **1** is functionalised to mimic amino acids with hydrophobic and aromatic side chains. Intramolecular hydrogen-bonds of **3** equal those of **4** in spite of the inverted stereochemistry of the C^{α} between the two antiparallel hydrogen-bonds. Further distinctions are the 7-membered ring conformations ($^{8}C_{4,5}$ chair *vs.* $^{4,5}C_{8}$ chair) and the 5-membered ring puckering (C^{β}-*exo vs.* S-*exo*).

cannot arise from this main conformation, but can result from the contribution of a βI–βII flip, which is fast on the NMR time scale but which can be observed for many turns,[11] even in rigid heptacyclic fused ring systems like **3** and **4**, respectively. The 5-membered thiazolidine ring of **1** assumes the C^{β}-*exo* conformation with a $^{3}J_{3\text{-H},2\text{-H}^{proS}}$ coupling too small to be resolved. All derivatives of **1** exhibit this ring-puckering in the solid state[12] as well as in solution. Epimerisation can be excluded by the observation of a tripeptide signal set in the NMR spectra of C_2-symmetric hexapeptide **3**. *Cis*-amide bonds are excluded by the absence of α,α-NOEs. An energy-minimised average structure, obtained using molecular modelling that included torsional and NOE restraints is shown in Fig. 4. The calculated backbone torsions of the dipeptide building block in **3** are shown in Table 1.

Table 1 Backbone torsions of the dipeptide mimetics in **3** and **4**; large ϕ_1 and ψ_1 values are found in the β-sheet region *i.e.* in the long side of the hexapeptide; thiaproline (ϕ_2, ψ_2) fits the $i + 1$ position of a βII-turn

	ϕ_1/°	ψ_1/°	ϕ_2/°	ψ_2/°
3	+149	−178	−65	+121
4 (βII-turn)[10]	−155	−177	−53	+134
4 (βI-turn)[10]	−152	−179	−63	−34

Synthesis

Uronic acids condense with vicinal aminothiols by forming thiazolidinlactams of variable ring-size and stereochemistry. In all cases studied, the stereochemistry of the new bridge-head was controlled by the stereochemistry of the neighbouring hydroxyl group.[7] Likewise, mannurono-3,6-lactone (**5**) and the methyl ester of L-cysteine formed the expected fused ring system **6** in MeOH–pyridine (10 : 1). A single acetonide **7** was obtained upon reaction with dimethoxypropane (DMP). The isopropylidene group spans the bisequatorial *trans*-diol moiety, leaving the α-hydroxyl accessible for the introduction of the azido group. Acetonide **7** was mesylated in the more activated α-position using mesyl chloride (MsCl) in the presence of triethylamine to give lactam **8**, and subsequently benzylated at the single remaining hydroxyl to yield **9**.[12] The axial mesylate was exchanged in an S_N2-type fashion with an equatorial azide, yielding exclusively the D,L-dipeptide precursor **10**. Hydrolysis of the methyl ester and subsequent peptide-coupling with glycine methyl ester formed the tripeptide **11**. Separate N-terminal hydrogenation and C-terminal saponification yielded the semiprotected tripeptides **12**

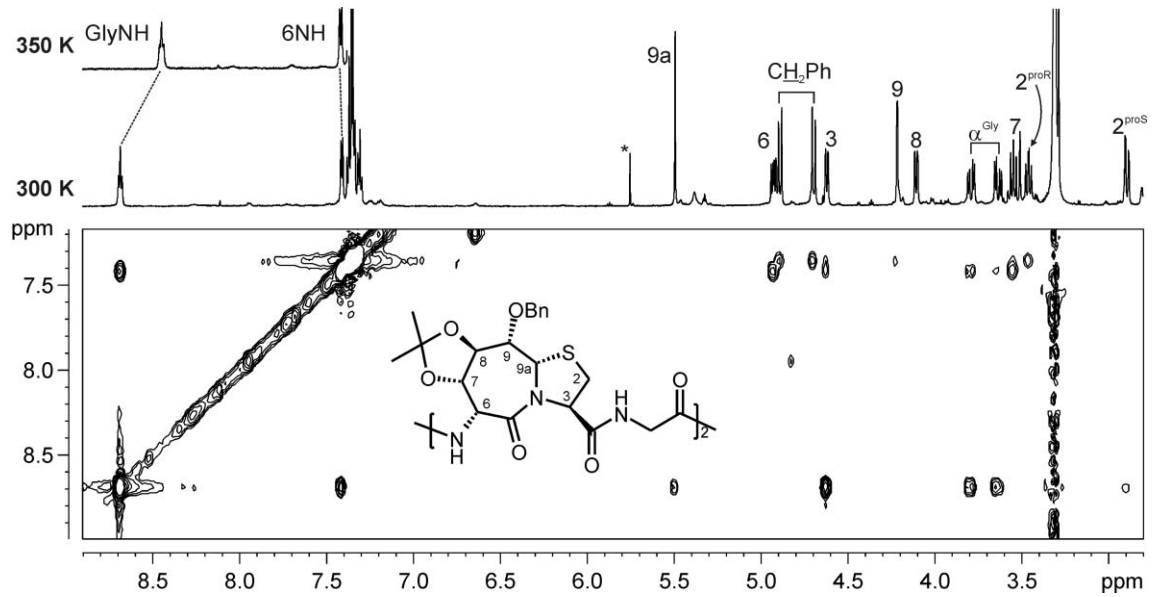

Fig. 3 ^{1}H NMR and ROESY spectra of the cyclopeptide **3** (600 MHz, solvent: DMSO[d_6], * = DCM). The expansion from the ^{1}H NMR at 350 K shows the strong temperature dependence of GlyNH. The high-field resonance at 3.6 ppm shows an NOE contact to 6NH and was assigned as Gly$H\alpha^{proR}$. The NOE contact between the aromatic protons (7.3–7.4 ppm) and $2H^{proR}$ possibly indicates that the benzylic group is in the vicinity of the thiaproline ring.

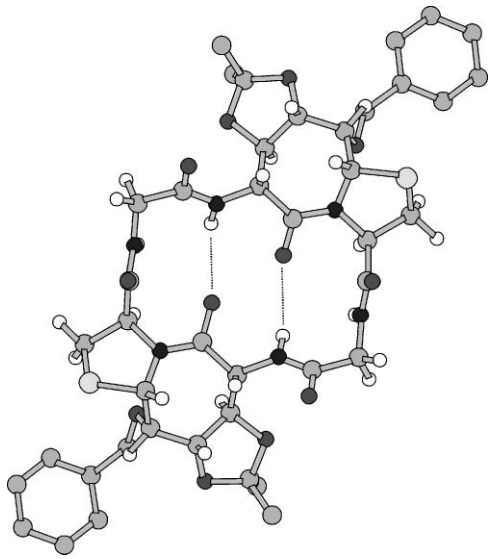

Fig. 4 Energy-minimised average conformation of hexapeptide **3**. The direction of view is perpendicular to the macrocyclic 18-membered ring. The two hydrogen-bonds of the antiparallel mini-β-sheet are indicated as dashed lines. The benzylic and the acetonide protons are not shown for clarity. The backbone torsions are $\phi_{Gly} = 90°$, $\psi_{Gly} = 32°$.

and **13**, respectively. Peptide-coupling to give **14** was followed by another hydrogenation (yielding **15**) and saponification, leading to hexapeptide **16**, which was cyclised under dilute conditions with diphenylphosphorylazide (DPPA) and solid base to give compound **3** (Fig. 5). The *O*-benzyl group in ring-position 9 was not affected by the two hydrogenation steps.

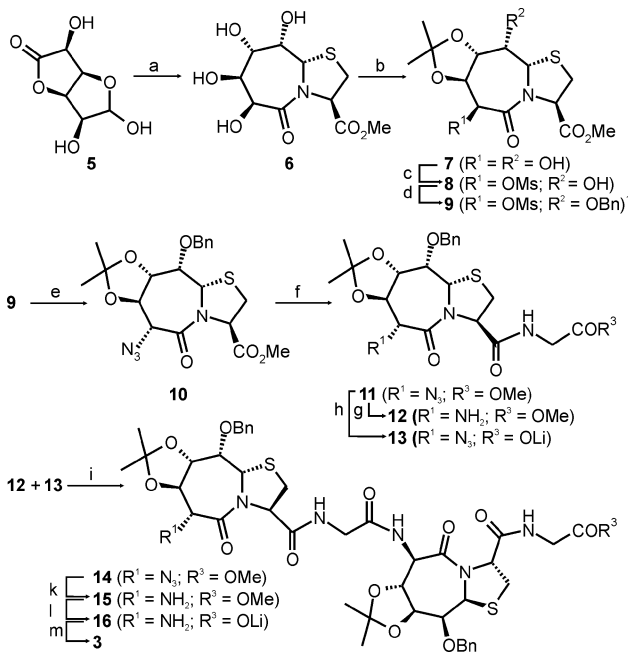

Fig. 5 *Reagents and conditions*: a) L-CysOMe·HCl, MeOH–Py (10 : 1), 60 °C, 80%; b) DMP, *p*TsOH, DMF, 93%; c) MsCl, Et_3N, DCM–Py (5 : 1), −78 °C, 82%; d) BnBr, NaH, DMF, 0 °C, 75%; e) NaN_3, DMF, 80 °C, 95%; f) 1. 1 N LiOH, MeOH, 2. HCl, 3. HCl·H_2N-Gly-OMe, PyBOP, NMM, DMF, 89%; g) H_2, Pd/C, MeOH, 99%; h) 1 N LiOH, MeOH, quant.; i) PyBOP, NMM, DMF, 45%; k) H_2, $Pd(OH)_2$, MeOH, 54%; l) 1. 1 N LiOH, MeOH, 2. HCl, quant.; m) DPPA, $NaHCO_3$, DMF, 4 °C, 43%.

Conclusion

An α-amino acid with D-stereochemistry in the *i* position of a β-turn in a cyclic hexapeptide has not previously been observed, but this fits into the general picture that bicyclic dipeptides prefer extended conformations. Dipeptide **1** is not a so-called β-turn mimic (as these are expected to occupy the $i + 1$ to $i + 2$ positions of reverse turns), even in the context of a cyclic hexapeptide like **3** which is forced to form two turns. Dipeptide **1** and other 6,5- and 7,5-bicyclic lactams are more suited to terminate β-sheets by restraining an extended backbone conformation with their lactam ring, and inducing a kink with their 5-membered ring.

Experimental

General methods and materials

Solvents were purified and dried according to standard procedures. Dry solvents were kept over molecular sieves. Dry DMF and DMF for peptide synthesis were bought. Chromatography was performed on Merck silica 60 (0.040–0.063 nm) and the solvent was eluted under pressure.

Characterisation

Measurements of optical rotations were performed on a Perkin–Elmer 241 polarimeter in a 1 dm cell at the temperature and concentration (*c*/g per 100 mL) noted. HRMS spectra were recorded on a Finnigan MAT 95 spectrometer. IR spectra were recorded on a Bruker IFS 88 spectrometer. NMR spectra were recorded with a Bruker DRX-500 and a Bruker Avance-600 spectrometer at 300 K unless otherwise noted. The residual peak of the solvent was used as the internal standard. Chemical shifts are shown in ppm. Splitting patterns are designated as s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, p = pseudo. For better comparability, the atom numbering of each compound in the NMR analysis is referenced to the numbering of compound **6**, not according to IUPAC nomenclature. In the case of ^{13}C-shifts only being detectable in the 2D-spectra, only one decimal place is specified. Diastereomeric groups which are not assigned are marked with t (low-field shift) and h (high-field shift).

(3*R*,6*S*,7*S*,8*S*,9*S*,9a*S*)-Methyl 6,7,8,9-tetrahydroxy-5-oxo-octahydrothiazolo[3,2-*a*]azepine-3-carboxylate (6). A solution of mannurono-3,6-lactone (**5**) (11.0 g, 62.6 mmol) and L-cysteine methyl ester (16.2 g, 94.2 mmol) in 250 mL of MeOH–pyridine (10 : 1) was stirred for 5 days at 60 °C under a N_2 atmosphere. The solvent was removed to leave a brown resin, which was purified by column chromatography on silica using MeOH–DCM (5 : 1) to give the bicycle **6** (14.9 g, 50.7 mmol; 82%) as a colourless powder; ν_{max}(KBr)/cm^{-1} 3503, 2957, 2908, 2853, 1783, 1625, 1436, 1363, 1347, 1301, 1284, 1241, 1190, 1113, 1056, 1005, 742; δ_H(500 MHz, DMSO[d_6]) 6.09 (d, $^3J_{6\text{-OH},6\text{-H}} = 5.0$ Hz, 1H, 6-OH), 5.58 (s, 1H, 9a), 5.27 (d, $^3J_{9\text{-OH},9\text{-H}} = 4.9$ Hz, 1H, 9-OH), 4.90 (dd, $^3J_{3\text{-H},2}{}^{proR}{}_{\text{-H}} = 7.7$ Hz, $^3J_{3\text{-H},2}{}^{proS}{}_{\text{-H}} = 1.4$ Hz, 1H, 3-H), 4.80 (d, $^3J_{7\text{-OH},7\text{-H}} = 4.6$ Hz, 1H, 7-OH), 4.65 (d, $^3J_{8\text{-OH},8\text{-H}} = 5.3$ Hz, 1H, 8-OH), 4.18 (dd, $^3J_{6\text{-H},6\text{-OH}} = 5.0$ Hz, $^3J_{6\text{-H},7\text{-H}} = 1.6$ Hz, 1H, 6-H), 3.87 (dd, $^3J_{9\text{-H},8\text{-H}} = 3.0$ Hz, $^3J_{9\text{-H},9\text{-OH}} = 5.0$ Hz, 1H, 9-H), 3.72 (ddd, $^3J_{8\text{-H},7\text{-H}} = 9.4$ Hz, $^3J_{8\text{-H},9\text{-H}} = 3.0$ Hz, $^3J_{8\text{-H},8\text{-OH}} = 5.2$ Hz, 1H, 8-H), 3.61 (s, 3H, OCH_3), 3.52 (dd, $^2J_2{}^{proR}{}_{\text{-H},2}{}^{proS}{}_{\text{-H}} = 11.4$ Hz,

$^3J_{2^{proR}\text{-H},3\text{-H}}$ = 7.7 Hz, 1H, 2^{proR}-H), 3.47 (ddd, $^3J_{7\text{-H},8\text{-H}}$ = 9.4 Hz, $^3J_{7\text{-H},7\text{-OH}}$ = 4.6 Hz, $^3J_{7\text{-H},6\text{-H}}$ = 1.6 Hz, 1H, 7-H), 2.93 (dd, $^2J_{2^{proS}\text{-H},2^{proR}\text{-H}}$ = 11.4 Hz, $^3J_{2^{proS}\text{-H},3\text{-H}}$ = 1.4 Hz, 1H, 2^{proS}-H); δ_C(125 MHz, DMSO[d_6]) 170.1 (5), 169.1 (3-*C*O), 79.1 (9), 77.8 (6), 72.0 (8), 68.1 (7), 63.7 (3), 61.1 (9a), 52.0 (OCH_3), 31.0 (2); HRMS (ESI) Calcd for M + Na^+: 316.0458; found: 316.0461; optical rotation $[\alpha]_{589}^{26} = -146.4$, $[\alpha]_{578}^{26} = -152.7$, $[\alpha]_{546}^{26} = -173.9$, $[\alpha]_{365}^{26} = -230.2$, (*c* 0.8 in MeOH); mp 189–191 °C.

(3a*S*,4*S*,4a*S*,7*R*,9*S*,9a*S*)-Methyl 4,9-dihydroxy-2,2-dimethyl-8-oxo-octahydro-1,3-dioxa-5-thia-7a-azacyclopenta[*f*]azulene-7-carboxylate (7). Compound **6** (6.0 g, 20.4 mmol), 2,2-dimethoxypropane (5.0 mL, 40.8 mmol) and a catalytic amount of *p*TsOH were dissolved in 50 mL of DMF. The solution was stirred overnight. Brine (100 mL) was added and the mixture was extracted with ethyl acetate (7 × 50 mL). The combined organic phases were dried over $MgSO_4$ and the solvent was evaporated to yield the desired product (**7**) (5.81 g, 89%) as a colourless solid; ν_{max}(KBr)/cm^{-1} 3470, 3293, 2988, 2967, 2925, 1763, 1633, 1440, 1379, 1265, 1232, 1201, 1177, 1131, 1081, 724, 629; δ_H(500 MHz, DMSO[d_6]) 6.48 (brs, 1H, 6-OH), 5.63 (brs, 1H, 9-OH), 5.31 (s, 1H, 9a-H), 4.89 (dd, $^3J_{3\text{-H},2^{proR}\text{-H}}$ = 7.4 Hz, $^3J_{3\text{-H},2^{proS}\text{-H}}$ = 1.1 Hz, 1H, 3-H), 4.39 (brs, 1H, 6-H), 4.15–4.11 (m, 2H, 8-H, 9-H), 3.80 (d, $^3J_{7\text{-H},8\text{-H}}$ = 9.0 Hz, 1H, 7-H), 3.62 (s, 3H, OCH_3), 3.50 (dd, $^2J_{2^{proR}\text{-H},2^{proS}\text{-H}}$ = 11.5 Hz, $^3J_{2^{proR}\text{-H},3\text{-H}}$ = 7.5 Hz, 1H, 2^{proR}-H), 2.96 (dd, $^2J_{2^{proS}\text{-H},2^{proR}\text{-H}}$ = 11.5 Hz, $^3J_{2^{proS}\text{-H},3\text{-H}}$ = 1.1 Hz, 1H, 2^{proS}-H), 1.33 (s, 3H, isopr.-CH_3^t), 1.31 (s, 3H, isopr.-CH_3^h); δ_C(125 MHz, DMSO[d_6]) 169.89 (5), 168.77 (3-CO), 107.21 (isopr.$^{quat.}$), 75.66 (8), 73.67 (9), 72.02 (7), 71.81 (6), 64.83 (3), 61.55 (9a), 52.04 (OCH_3), 30.42 (2), 26.73 (isopr.-CH_3^t), 26.66 (isopr.-CH_3^h). HRMS (ESI) Calcd for M + Na^+: 356.0774; found: 356.0788; pyrolysis 238 °C.

(3a*S*,4*S*,4a*S*,7R,9*S*,9a*S*)-Methyl 4-hydroxy-9-methanesulfonyloxy-2,2-dimethyl-8-oxo-octahydro-1,3-dioxa-5-thia-7a-azacyclopenta[*f*]azulene-7-carboxylate (8). MsCl (1.4 mL, 18.0 mmol) was slowly added to a solution of **7** (5.0 g, 15.0 mmol) and Et_3N (2.5 mL, 18.0 mmol) in 100 mL of $DCM_{abs.}$–$pyridine_{abs.}$ (5 : 1) at 0 °C. After 40 min, the reaction mixture was poured onto ice and extracted with ethyl acetate (3 × 80 mL). The combined organic phases were dried ($MgSO_4$) and the solvent was removed to give a brown oil, which was purified by column chromatography on silica (ethyl acetate–toluene = 2 : 1) to give the activated alcohol **8** (5.41 g, 73%) as a yellow solid; ν_{max}(KBr)/cm^{-1} 3459, 3021, 2986, 2956, 2935, 2913, 1760, 1652, 1409, 1373, 1336, 1212, 1180, 1131, 1071, 945, 909, 841, 527; δ_H(600 MHz, DMSO[d_6]) 5.96 (d, $^3J_{9\text{-OH},9\text{-H}}$ = 6.2 Hz, 1H, 9-OH), 5.24 (s, 1H, 9a-H), 5.23 (d, $^3J_{6\text{-H},7\text{-H}}$ = 1.1 Hz, 1H, 6-H), 5.05 (dd, $^3J_{3\text{-H},2^{proR}\text{-H}}$ = 7.1 Hz, $^3J_{3\text{-H},2^{proS}\text{-H}}$ = 1.1 Hz, 1H, 3-H), 4.19 (dd, $^3J_{9\text{-H},9\text{-OH}}$ = 6.1 Hz, $^3J_{9\text{-H},8\text{-H}}$ = 2.5 Hz, 1H, 9-H), 4.10 (dd, $^3J_{8\text{-H},7\text{-H}}$ = 9.4 Hz, $^3J_{8\text{-H},9\text{-H}}$ = 2.5 Hz, 1H, 8-H), 4.06 (dd, $^3J_{7\text{-H},8\text{-H}}$ = 9.4 Hz, $^3J_{7\text{-H},6\text{-H}}$ = 1.1 Hz, 1H, 7-H), 3.65 (s, 3H, OCH_3), 3.51 (dd, $^2J_{2^{proR}\text{-H},2^{proS}\text{-H}}$ = 11.8 Hz, $^3J_{2^{proR}\text{-H},3\text{-H}}$ = 7.1 Hz, 1H, 2^{proR}-H), 3.29 (s, 3H, SCH_3), 3.07 (dd, $^2J_{2^{proS}\text{-H},2^{proR}\text{-H}}$ = 11.8 Hz, $^3J_{2^{proS}\text{-H},3\text{-H}}$ = 1.1 Hz, 1H, 2^{proS}-H), 1.36 (s, 3H, isopr.-CH_3^t), 1.35 (s, 3H, isopr.-CH_3^h); δ_C(150 MHz, DMSO[d_6]) 169.38 (5), 162.98 (3-*C*O), 108.22 (isopr.$^{quat.}$), 78.31 (6), 75.91 (8), 72.96 (9), 69.85 (7), 65.51 (3), 61.88 (9a), 52.37 (OCH_3), 38.57 (SCH_3), 30.44 (2), 26.50 (isopr.-CH_3^t), 26.37 (isopr.-CH_3^h); HRMS (ESI) Calcd for M + Na^+: 434.0550; found: 434.0569; mp 154 °C.

(3a*S*,4*S*,4a*S*,7*R*,9*S*,9a*S*)-Methyl 8-oxo-octahydro-1,3-dioxa-5-thia-7a-azacyclopenta[*f*]azulene-7-carboxylate (9). NaH (86.0 mg, 3.59 mmol) was carefully added to a solution of **8** (985 mg, 2.39 mmol) and BnBr (8.53 mL, 7.18 mmol) in 20 mL of $DMF_{abs.}$ at 0 °C. The reaction mixture was stirred for 2.5 h at room temperature, then poured onto ice and extracted with toluene (3 × 20 mL). The combined organic phases were dried over $MgSO_4$ and the solvent was evaporated. The crude product was purified by column chromatography on silica using ethyl acetate–toluene (1 : 1) to give compound (**9**) (863 mg, 72%) as a yellow powder; ν_{max}(KBr)/cm^{-1} 3049, 2995, 2955, 2907, 2109, 1749, 1649, 1391, 1370, 1353, 1219, 1178, 1158, 1087, 842; δ_H(600 MHz, DMSO[d_6]) 7.38–7.28 (m, 5H, Ph), 5.30 (s, 1H, 9a-H), 5.27 (s, 1H, 6-H), 5.06 (d, $^3J_{3\text{-H},2^{proR}\text{-H}}$ = 6.8 Hz, 1H, 3-H), 4.90 (d, $^2J_{Bn}$ = 11.2 Hz, 1H, Ph-CH_2^t), 4.73 (d, $^3J_{Bn}$ = 11.2 Hz, 1H, Ph-CH_2^h), 4.30 (d, $^3J_{9\text{-H},8\text{-H}}$ = 2.2 Hz, 1H, 9-H), 4.26 (dd, $^3J_{8\text{-H},7\text{-H}}$ = 9.5 Hz, $^3J_{8\text{-H}\text{-}9\text{-H}}$ = 2.2 Hz, 1H, 8-H), 4.15 (dd, $^3J_{7\text{-H},8\text{-H}}$ = 9.5 Hz, $^3J_{7\text{-H},6\text{-H}}$ < 1 Hz, 1H, 7-H), 3.64 (s, 3H, OCH_3), 3.37 (dd, $^2J_{2^{proR}\text{-H},2^{proS}\text{-H}}$ = 11.8 Hz, $^3J_{2^{proR}\text{-H},3\text{-H}}$ = 7.0 Hz, 1H, 2^{proR}-H), 3.29 (s, 3H, SCH_3), 3.09 (d, $^2J_{2^{proS}\text{-H},2^{proR}\text{-H}}$ = 11.8 Hz, 1H, 2^{proS}-H), 1.39 (ps, 6H, isopr.-CH_3); δ_C(150 MHz, DMSO[d_6]) 169.24 (5), 163.02 (3-*C*O), 137.93, 128.30, 127.82, 127.71 (Ar), 108.61 (isopr.$^{quat.}$), 80.51 (9), 78.10 (6), 76.23 (8), 75.11 (*C*H_2-Ph), 70.47 (7), 65.63 (3), 60.84 (9a), 52.43 (OCH_3), 38.59 (SCH_3), 30.47 (2), 26.33 (isopr.-CH_3^t), 26.24 (isopr.-CH_3^h); HRMS (ESI) Calcd for M + Na^+: 524.1019; found: 524.1041.

(3a*S*,4*S*,4a*S*,7*R*,9*R*,9a*S*)-Methyl 9-azido-4-methanesulfonyloxy-2,2-dimethyl-8-oxo-octahydro-1,3-dioxa-5-thia-7a-azacyclopenta[*f*]azulene-7-carboxylate (10). A solution of **9** (0.5 g, 1.00 mmol) and NaN_3 (195 mg, 3.00 mmol) in 80 mL of DMF was stirred for 5 days at 80 °C. The reaction mixture was diluted with H_2O (250 mL) and extracted with toluene (3 × 100 mL). The combined organic phases were washed with H_2O (1 × 50 mL), dried ($MgSO_4$) and the solvent was removed to give the dipeptide precursor **10** (415 mg, 93%) as a yellow solid; ν_{max}(KBr)/cm^{-1} 3033, 2985, 2952, 2891, 2113, 1753, 1664, 1454, 1437, 1380, 1307, 1210, 1176, 1151, 1082, 1043, 1027; δ_H(500 MHz, DMSO[d_6]) 7.38–7.28 (m, 5H, Ph), 5.32 (s, 1H, 9a-H), 4.94 (dd, $^3J_{3\text{-H},2^{proR}\text{-H}}$ = 7.4 Hz, $^3J_{3\text{-H},2^{proS}\text{-H}}$ < 1 Hz, 1H, 3-H), 4.88 (d, $^2J_{Bn}$ = 11.2 Hz, 1H, Ph-CH_2^t), 4.77 (d, $^3J_{6\text{-H},7\text{-H}}$ = 10.8 Hz, 1H, 6-H), 4.70 (d, $^2J_{Bn}$ = 11.2 Hz, 1H, Ph-CH_2^h), 4.25 (d, $^3J_{9\text{-H},8\text{-H}}$ = 2.4 Hz, 1H, 9-H), 4.03 (dd, $^3J_{8\text{-H},7\text{-H}}$ = 8.9 Hz, $^3J_{8\text{-H}\text{-}9\text{-H}}$ = 2.4 Hz, 1H, 8-H), 3.80 (dd, $^3J_{7\text{-H},8\text{-H}}$ = 8.9 Hz, $^3J_{7\text{-H},6\text{-H}}$ = 10.8 Hz, 1H, 7-H), 3.65 (s, 3H, OCH_3), 3.44 (dd, $^2J_{2^{proR}\text{-H},2^{proS}\text{-H}}$ = 11.8 Hz, $^3J_{2^{proR}\text{-H},3\text{-H}}$ = 7.4 Hz, 1H, 2^{proR}-H), 3.06 (d, $^2J_{2^{proS}\text{-H},2^{proR}\text{-H}}$ = 11.8 Hz, $^3J_{2^{proS}\text{-H},3\text{-H}}$ < 1 Hz, 1H, 2^{proS}-H), 1.42 (s, 3H, isopr.-CH_3^t), 1.37 (s, 3H, isopr.-CH_3^h); δ_C(125 MHz, DMSO[d_6]) 169.44 (5), 165.72 (3-*C*O), 137.98, 128.30, 127.76, 127.67 (Ar), 108.63 (isopr.$^{quat.}$), 82.01 (8), 79.89 (9), 75.03 (*C*H_2-Ph), 70.47 (7), 65.03 (3), 61.42 (6), 60.71 (9a), 52.27 (OCH_3), 30.83 (2), 26.55 (isopr.-CH_3^t), 26.21 (isopr.-CH_3^h); HRMS (ESI) Calcd for M + Na^+: 471.1309; found: 471.1327; mp 68 °C.

(3a*S*,4*S*,4a*S*,7*R*,9*R*,9a*S*)-Methyl [(9-azido-4-benzyloxy-2,2-dimethyl-8-oxo-octahydro-1,3-dioxa-5-thia-7a-azacyclopenta[*f*]-azulene-7-carbonyl)amino]acetate (11). A solution of **10** (300 mg, 0.69 mmol) in 10 mL of MeOH was treated with 1 N LiOH (1.37 mL, 1.37 mmol) for 4 h at room temperature, and the solution then neutralised with 1 N HCl. The solvent was removed and the desired free acid (332 mg, quant.) was used without

further purification. PyBOP (484 mg, 0.93 mmol) was added to a solution of the free acid (300 mg, 0.62 mmol) and glycine methyl ester hydrochloride (117 mg, 0.93 mmol) in 20 mL of DMF at 0 °C, and the pH of the solution adjusted to 7–8 with DIPEA. The mixture was stirred overnight at room temperature. The solvent was evaporated and the remaining brown oil was purified by column chromatography with ethyl acetate–toluene (3 : 1) as the eluant to yield product **11** (280 mg, 89%) as a colourless powder. ν_{max}(KBr)/cm^{-1} 3281, 3089, 3033, 2986, 2953, 2875, 2110, 1752, 1668, 1642, 1407, 1382, 1277, 1229, 1217, 1155, 1082; δ_H(500 MHz, DMSO[d_6]) 8.33 (t, $^3J_{\text{Gly-NH},\alpha\text{Gly-H}}$ = 5.8 Hz, 1H, Gly-NH), 7.42–7.27 (m, 5H, Ph), 5.26 (s, 1H, 9a-H), 4.90 (d, $^2J_{Bn}$ = 11.2 Hz, 1H, Ph-CH_2^t), 4.79 (d, $^3J_{3\text{-H},2^{proR}\text{-H}}$ = 7.4 Hz, 1H, 3-H), 4.69 (d, $^2J_{Bn}$ = 11.2 Hz, 1H, Ph-CH_2^h), 4.57 (d, $^3J_{6\text{-H},7\text{-H}}$ = 11.0 Hz, 1H, 6-H), 4.25 (d, $^3J_{9\text{-H},8\text{-H}}$ = 2.3 Hz, 1H, 9-H), 4.04 (dd, $^3J_{8\text{-H},7\text{-H}}$ = 8.8 Hz,$^3J_{8\text{-H},9\text{-H}}$ = 2.3 Hz, 1H, 8-H), 3.90–3.78 (m, 2H, Gly-H), 3.81 (dd, $^3J_{7\text{-H},6\text{-H}}$ = 11.0 Hz, $^3J_{7\text{-H},8\text{-H}}$ = 8.8 Hz, 1H, 7-H), 3.62 (s, 3H, OCH_3), 3.44 (dd, $^2J_2{}^{proR}{}_{\text{-H},2^{proS}\text{-H}}$ = 11.5 Hz, $^3J_2{}^{proR}{}_{\text{-H},3\text{-H}}$ = 7.4 Hz, 1H, 2^{proR}-H), 2.90 (d,$^2J_2{}^{proS}{}_{\text{-H},2^{proR}\text{-H}}$ = 11.5 Hz, 1H, 2^{proS}-H), 1.43 (s, 3H, isopr.-CH_3^t), 1.38 (s, 3H, isopr.-CH_3^h); δ_C(125 MHz, DMSO[d_6]) 170.28, 170.07, 165.27 (5, 3-*C*O, α^{Gly}-*C*O), 138.07, 128.33, 127.75, 127.70 (Ar), 108.66 (isopr.$^{quat.}$), 82.20 (8), 80.44 (9), 75.03 (*C*H_2-Ph), 70.37 (7), 66.60 (3), 61.53 (6), 61.15 (9a), 51.64 (OCH_3), 40.59 (α^{Gly}), 32.06 (2), 26.58 (isopr.-CH_3^t), 26.24 (isopr.-CH_3^h); HRMS (ESI) Calcd for M + Na^+: 528.1523; found: 528.1529.

(3a*S*,4*S*,4a*S*,7*R*,9*R*,9a*S*)-Methyl [(9-amino-4-benzyloxy-2,2-dimethyl-8-oxo-octahydro-1,3-dioxa-5-thia-7a-azacyclopenta[*f*]-azulene-7-carbonyl)amino]acetate (12). Compound **11** (120 mg, 0.240 mmol) was dissolved in MeOH (15 mL) and treated with Pd/C (25.0 mg) under an H_2 atmosphere. The suspension was stirred vigorously for 7 h. The reaction mixture was filtered through Celite and the solvent was removed to give product **12** as a colourless solid (110 mg, 97%). δ_H(600 MHz, DMSO[d_6]) 8.28 (t, $^3J_{\text{Gly-NH},\alpha\text{Gly-H}}$ = 5.9 Hz, 1H, Gly-NH), 7.40–7.27 (m, 5H, Ar), 5.26 (s, 1H, 9a-H), 4.90 (d, $^2J_{Bn}$ = 11.2 Hz, 1H, Ph-CH_2^t), 4.78 (d, $^3J_{3\text{-H},2^{proR}\text{H}}$ = 7.5 Hz, 1H, 3-H), 4.69 (d, $^2J_{Bn}$ = 11.2 Hz, 1H, Ph-CH_2^h), 4.18 (d, $^3J_{9\text{-H},8\text{-H}}$ = 2.4 Hz, 1H, 9-H), 3.91 (dd, $^3J_{8\text{-H},7\text{-H}}$ = 9.0 Hz,$^3J_{8\text{-H},9\text{-H}}$ = 2.4 Hz, 1H, 8-H), 3.86–3.77 (m, 3H, Gly-H, 6-H), 3.61 (s, 3H, OCH_3), 3.51 (dd, $^3J_{7\text{-H},6\text{-H}}$ = 10.6 Hz, $^3J_{7\text{-H},8\text{-H}}$ = 9.0 Hz, 1H, 7-H), 3.44 (dd, $^2J_2{}^{proR}{}_{\text{-H},2^{proS}\text{-H}}$ = 11.3 Hz, $^3J_2{}^{proR}{}_{\text{-H},3\text{-H}}$ = 7.5 Hz, 1H, 2^{proR}-H), 2.86 (d,$^2J_2{}^{proS}{}_{\text{-H},2^{proR}\text{-H}}$ = 11.3 Hz, 1H, 2^{proS}-H), 1.67 (brs, 2H, NH_2), 1.40 (s, 3H, isopr.-CH_3^t), 1.34 (s, 3H, isopr.-CH_3^h); δ_C(150 MHz, DMSO[d_6]) 170.10 (α^{Gly}-*C*O), 169.91 (3-*C*O), 169.52 (5), 138.27, 128.30, 127.61 (Ar), 107.74 (isopr.$^{quat.}$), 82.33 (8), 80.77 (9), 74.86 (*C*H_2-Ph), 73.37 (7), 66.50 (3), 61.03 (9a), 53.92 (6), 51.62 (OCH_3), 40.59 (α^{Gly}), 32.01 (2), 26.87 (isopr.-CH_3^t), 26.28 (isopr.-CH_3^h); HRMS (ESI) Calcd for M + H^+: 480.1799; found 480.1795.

(3a*S*,4*S*,4a*S*,7*R*,9*R*,9a*S*)-Lithium [(9-azido-4-benzyloxy-2,2-dimethyl-8-oxo-octahydro-1,3-dioxa-5-thia-7a-azacyclopenta[*f*]-azulene-7-carbonyl)amino]acetate·lithium chloride (13). A solution of **11** (120 mg, 0.24 mmol) in MeOH–THF (1 : 1) (15 mL) was treated with 1 N LiOH (0.48 mL, 0.48 mmol) for 4 h at room temperature, and the solution then neutralised with 1 N HCl. The solvent was removed and the desired product **13** (130 mg, quant.) was used without further purification. δ_H(600 MHz, DMSO[d_6]) 7.63 (brs, 1H, Gly-NH), 7.39–7.28 (m, 5H, Ph), 5.32 (s, 1H, 9a-H), 4.88 (d, $^2J_{Bn}$ = 11.1 Hz, 1H, Ph-CH_2^t), 4.84 (d, $^3J_{3\text{-H},2^{proR}\text{-H}}$ = 7.5 Hz, 1H, 3-H), 4.69 (d, $^2J_{Bn}$ = 11.1 Hz, 1H, Ph-CH_2^h), 4.65 (d, $^3J_{6\text{-H},7\text{-H}}$ = 10.8 Hz, 1H, 6-H), 4.23 (d, $^3J_{9\text{-H},8\text{-H}}$ = 2.3 Hz, 1H, 9-H), 4.06 (dd, $^3J_{8\text{-H},7\text{-H}}$ = 8.8 Hz,$^3J_{8\text{-H},9\text{-H}}$ = 2.3 Hz, 1H, 8-H), 3.80 (dd, $^3J_{7\text{-H},8\text{-H}}$ = 8.8 Hz,$^3J_{7\text{-H},6\text{-H}}$ = 10.8 Hz, 1H, 7-H), 3.47 (dd, $^2J_{\alpha\text{Gly–H}}$ = 17.0 Hz,$^3J_{\alpha\text{Gly-H}^t,\text{N-H}}$ = 5.4 Hz, 1H, Gly-H^t), 3.41 (dd, $^2J_2{}^{proR}{}_{\text{-H},2^{proS}\text{-H}}$ = 11.4 Hz, $^3J_2{}^{proR}{}_{\text{-H},3\text{-H}}$ = 7.5 Hz, 1H, 2^{proR}-H), 3.39 (dd, $^2J_{\alpha\text{Gly-H}}$ = 17.0 Hz,$^3J_{\alpha\text{Gly-H}^h,\text{N-H}}$ = 4.5 Hz, 1H, Gly-H^h), 2.94 (d,$^2J_2{}^{proS}{}_{\text{-H},2^{proR}\text{-H}}$ = 11.4 Hz, 1H, 2^{proS}-H), 1.41 (s, 3H, isopr.-CH_3^t), 1.38 (s, 3H, isopr.-CH_3^h); δ_C(150 MHz, DMSO[d_6]) 170.6 (α^{Gly}-*C*O), 168.2 (3-*C*O), 165.2 (5), 138.1, 128.33, 127.7, 127.6 (Ar), 108.5 (isopr.$^{quat.}$), 81.8 (8), 80.2 (9), 74.8 (*C*H_2-Ph), 70.2 (7), 66.6 (3), 61.5 (6), 60.8 (9a), 43.1 (α^{Gly}), 32.0 (2), 26.3 (isopr.-CH_3^t), 26.0 (isopr.-CH_3^h); HRMS (ESI) Calcd for M^-: 490.1391; found: 490.1389.

N_3-Hexapeptide-OMe 14. Compound **13** (110 mg, 0.20 mmol), **12** (97.0 mg, 0.20 mmol) and PyBOP (160 mg, 0.31 mmol) were dissolved in DMF (15 mL) at 0 °C, and the pH of the solution was adjusted to 7–8 with DIPEA. The mixture was stirred overnight at room temperature. The solvent was evaporated and the remaining yellow oil was purified by column chromatography with DCM–MeOH (25 : 1) as the eluant to yield product **14** as a colourless powder (105 mg, 54%); δ_H(600 MHz, DMSO[d_6]) δ = 8.31–8.25 (m, 2H, Gly-NH^A, Gly-NH^B), 7.82 (d, $^3J_6{}^B{}_{\text{-NH},6^B\text{-H}}$ = 8.8 Hz, 1H, 6^B-NH), 7.40–7.28 (m, 10H, Ar^A, Ar^B), 5.43 (s, 1H, 9a^B-H), 5.28 (s, 1H, 9a^A-H), 4.99–4.97 (m, 1H, 6^B-H), 4.90 (d, $^2J_{Bn}$ = 11.0 Hz, 1H, Ph-C$H_2{}^{B,t}$), 4.89 (d, $^2J_{Bn}$ = 11.1 Hz, 1H, Ph-C$H_2{}^{A,t}$), 4.78–4.73 (m, 2H, 3^B-H, 3^A-H), 4.71 (d, $^2J_{Bn}$ = 11.0 Hz, 1H, Ph-$CH_2{}^{B,h}$), 4.69 (d, $^2J_{Bn}$ = 11.1 Hz, 1H, Ph-C$H_2{}^{A,h}$), 4.55 (d, $^3J_6{}^A{}_{\text{-H},7^A\text{-H}}$ = 10.9 Hz, 1H, 6^A-H), 4.24 (d, $^3J_9{}^A{}_{\text{-H},8^A\text{-H}}$ = 2.4 Hz, 1H, 9^A-H), 4.20 (d, $^3J_9{}^B{}_{\text{-H},8^B\text{-H}}$ = 2.4 Hz, 1H, 9^B-H), 4.04 (dd, $^3J_8{}^A{}_{\text{-H},7^A\text{-H}}$ = 8.8 Hz, $^3J_8{}^A{}_{\text{-H},9^A\text{-H}}$ = 2.4 Hz, 1H, 8^A-H), 4.04 (dd, $^3J_8{}^B{}_{\text{-H},7^B\text{-H}}$ = 8.8 Hz, $^3J_8{}^B{}_{\text{-H},9^B\text{-H}}$ = 2.4 Hz, 1H, 8^B-H), 3.87–3.71 (m, 5H, $\alpha_{Gly}{}^A$-H, $\alpha_{Gly}{}^B$-H, 7^A-H), 3.71–3.61 (m, 1H, 7^B-H), 3.60 (s, 3H, OCH_3), 3.45 (dd,$^2J_2{}^{proR,A}{}_{\text{-H},2^{proS,A}\text{-H}}$ = 11.4 Hz, $^3J_2{}^{proR,A}{}_{\text{-H}/3^A\text{-H}}$ = 7.46 Hz, 1H, $2^{proR,A}$-H), 3.59 (dd,$^2J_2{}^{proR,B}{}_{\text{-H},2^{proS,B}\text{-H}}$ = 11.2 Hz, $^3J_2{}^{proR,B}{}_{\text{-H}/3^B\text{-H}}$ = 7.3 Hz, 1H, $2^{proR,B}$-H), 3.00 (d,$^2J_2{}^{proS,A}{}_{\text{-H},2^{proR,A}\text{-H}}$ = 11.4 Hz, 1H, $2^{proS,A}$-H), 2.86 (d,$^2J_2{}^{proS,B}{}_{\text{-H},2^{proR,B}\text{-H}}$ = 11.2 Hz, 1H, $2^{proS,B}$-H), 1.43 (s, 3H, isopr.-$CH_3{}^{A,t}$), 1.38 (s, 3H, isopr.-$CH_3{}^{A,h}$), 1.35 (s, 3H, isopr.-$CH_3{}^{B,t}$), 1.32 (s, 3H, isopr.-$CH_3{}^{B,h}$); δ_C(150 MHz, DMSO[d_6]) 170.11 (α^{Gly}-*C*O^B), 169.66 (3-*C*O^B), 169.35 (3-*C*O^A), 168.10 (α^{Gly}-*C*O^A), 166.61 (5^B), 165.41 (5^A), 138.22, 138.07, 128.30, 127.72, 127.62, (Ar), 108.64 (isopr.$^{quat.,A}$), 127.62 (isopr.$^{quat.,B}$), 82.67, 82.26 (8^A, 8^B), 80.73 (9^A), 80.43 (9^B), 74.97, (Ph-*C*$H_2{}^A$, Ph-*C*$H_2{}^B$), 70.79 (7^B), 70.38 (7^A), 66.84 (3^A), 66.58 (3^B), 61.57 (6^A), 61.21 ($9a^A$), 61.11 ($9a^B$), 52.08 (6^B), 51.60 (OCH_3), 40.55, 40.06 ($\alpha^{Gly,A}$, $\alpha^{Gly,B}$), 32.22 (2^A), 32.04 (2^B), 26.71 (isopr.-$CH_3{}^{B,t}$), 26.58 (isopr.-$CH_3{}^{A,t}$), 26.30 (isopr.-$CH_3{}^{B,h}$), 26.23 (isopr.-$CH_3{}^{A,h}$); HRMS (ESI) Calcd for M + Na^+: 975.2987; found 975.2976.

H_2N-Hexapeptide-OMe 15. Compound **14** (50.0 mg, 53.0 μmol) was dissolved in MeOH (7.00 mL) and treated with $Pd(OH)_2$ (5.0 mg) under H_2 atmosphere. The suspension was stirred for 20 h at room temperature, filtered through Celite and the solvent was removed to give a yellow crude product, which was purified by column chromatography (MeOH–DCM = 1 : 15). The desired product **15** (29.0 mg, 60%) was obtained as a colourless solid; δ_H(600 MHz, DMSO[d_6]) 8.28 (t, $^3J_{Gly}{}^A{}_{\text{-NH},Gly}{}^A{}_{\text{-H}}$ = 6.1 Hz, 1H, Gly-NH^A), 8.26 (t, $^3J_{Gly}{}^B{}_{\text{-NH},Gly}{}^B{}_{\text{-H}}$ = 6.1 Hz, 1H,

Gly-NH^{B}), 7.74 (d, $^{3}J_{6^{B}\text{-NH},6^{B}\text{-H}} = 8.8$ Hz, 1H, 6^{B}-NH), 7.39–7.28 (m, 10H, Ar^{A}, Ar^{B}), 5.43 (s, 1H, 9a^{B}-H), 5.29 (s, 1H, 9a^{A}-H), 4.98 (brs, 1H, 6^{B}-H), 4.90 (d, $^{2}J_{Bn} = 11.1$ Hz, 1H, Ph-C$H_2^{B,t}$), 4.89 (d, $^{2}J_{Bn} = 11.2$ Hz, 1H, Ph-C$H_2^{A,t}$), 4.75–4.73 (m, 2H, 3^{B}-H, 3^{A}-H), 4.71 (d, $^{2}J_{Bn} = 11.1$ Hz, 1H, Ph-C$H_2^{B,h}$), 4.69 (d, $^{2}J_{Bn} = 11.2$ Hz, 1H, Ph-C$H_2^{A,h}$), 4.20 (d, $^{3}J_{9^{B}\text{-H},8^{B}\text{-H}} = 2.3$ Hz, 1H, 9^{B}-H), 4.19 (d, $^{3}J_{9^{A}\text{-H},8^{A}\text{-H}} = 2.3$ Hz, 1H, 9^{A}-H), 4.04 (dd, $^{3}J_{8^{B}\text{-H},7^{B}\text{-H}} = 8.9$ Hz, $^{3}J_{8^{B}\text{-H},9^{B}\text{-H}} = 2.3$ Hz, 1H, 8^{B}-H), 3.92 (dd, $^{3}J_{8^{A}\text{-H},7^{A}\text{-H}} = 8.9$ Hz, $^{3}J_{8^{A}\text{-H},9^{A}\text{-H}} = 2.3$ Hz, 1H, 8^{A}-H), 3.91–3.64 (m, 6H, 6^{A}-H, $\alpha_{Gly}{}^{A}$-H, $\alpha_{Gly}{}^{B}$-H, 7^{B}-H), 3.60 (s, 3H, OCH$_3$), 3.54 (m, 1H, 7^{A}-H), 3.45 (dd, $^{3}J_{2^{proR,A}\text{-H},2^{proS,A}\text{-H}} = 11.4$ Hz, $^{3}J_{2^{proR,A}\text{-H}/3^{A}\text{-H}} = 7.6$ Hz, 1H, 2^{proR,A}-H), 3.44 (dd, $^{2}J_{2^{proR,}\text{-H},2^{proS,B}\text{-H}} = 11.3$ Hz, $^{3}J_{2^{proR,B}\text{-H}/3^{B}\text{-H}} = 7.3$ Hz, 1H, 2^{proR,B}-H), 2.97 (d, $^{2}J_{2^{proS,A}\text{-H},2^{proR,A}\text{-H}} = 11.4$ Hz, 1H, 2^{proS,A}-H), 2.86 (d, $^{2}J_{2^{proS,B}\text{-H},2^{proR,B}\text{-H}} = 11.3$ Hz, 1H, 2^{proS,B}-H), 1.41, 1.35, 1.34, 1.31 (s, 3H, isopr.-CH$_3$); δ_C(150 MHz, DMSO[d$_6$]) 170.4, 169.7, 169.6, 168.1 (3-*C*O^{B}, 3-*C*O^{A}, α^{Gly}-*C*O^{A}, α^{Gly}-*C*O^{B}, 5^{B}, 5^{A}), 138.2, 128.1, 127.6 (Ar), 107.9, 107.8 (isopr.$^{quat.}$), 82.4 (8^{B}), 82.1 (8^{A}), 80.4 (9^{A}, 9^{B}), 74.7, 74.6 (*C*H$_2$-Ph^{A}, *C*H$_2$-Ph^{B}), 72.6 (7^{A}), 70.6 (7^{B}), 66.6, 66.4 (3^{A}, 3^{B}), 60.9 (9a^{A}, 9a^{B}), 53.6 (6^{A}), 51.4 (OCH$_3$), 41.7 ($\alpha_{Gly}{}^{A}$), 40.3 ($\alpha_{Gly}{}^{B}$), 31.9 (2^{B}), 31.8 (2^{A}), 26.6 (isopr.-CH$_3$), 26.4 (isopr.-CH$_3$), 26.1 (isopr.-CH$_3$), 26.0 (isopr.-CH$_3$); HRMS (ESI) Calcd for M + Na^{+}: 949.3082; found 949.3075.

H$_2$N-Hexapeptide-OH 16. A solution of **15** (16 mg, 17.2 μmol) in MeOH (0.15 mL) was treated with 1 N LiOH (36 μL, 36 μmol) for 3 h at room temperature, and the solution then neutralised with 1 N HCl. The solvent was removed and the desired product **16** (17 mg, quant.) was used without further purification. δ_H(600 MHz, DMSO[d$_6$]) 8.31 (t, $^{3}J_{Gly^{A}\text{-NH},Gly^{A}\text{-H}} = 6.1$ Hz, 1H, Gly-NH^{A}), 8.03 (brs, 1H, Gly-NH^{B}), 7.70 (d, $^{3}J_{6^{B}\text{-NH},6^{B}\text{-H}} = 8.7$ Hz, 1H, 6^{B}-NH), 7.39–7.26 (m, 10H, Ar^{A}, Ar^{B}), 5.45 (s, 1H, 9a^{B}-H), 5.31 (s, 1H, 9a^{A}-H), 4.98 (brs, 1H, 6^{B}-H), 4.90 (d, $^{2}J_{Bn} = 11.1$ Hz, 1H, Ph-C$H_2^{B,t}$), 4.89 (d, $^{2}J_{Bn} = 11.2$ Hz, 1H, Ph-C$H_2^{A,t}$), 4.78–4.73 (m, 2H, 3^{B}-H, 3^{A}-H), 4.70 (d, $^{2}J_{Bn} = 11.1$ Hz, 1H, Ph-C$H_2^{B,h}$), 4.69 (d, $^{2}J_{Bn} = 11.2$ Hz, 1H, Ph-C$H_2^{A,h}$), 4.20 (d, $^{3}J_{9^{A}\text{-H},8^{A}\text{-H}} = 2.4$ Hz, 1H, 9^{A}-H), 4.19 (d, $^{3}J_{9^{B}\text{-H},8^{B}\text{-H}} = 2.4$ Hz, 1H, 9^{B}-H), 4.05 (dd, $^{3}J_{8^{B}\text{-H},7^{B}\text{-H}} = 9.0$ Hz, $^{3}J_{8^{B}\text{-H},9^{B}\text{-H}} = 2.5$ Hz, 1H, 8^{B}-H), 3.98 (dd, $^{3}J_{6^{A}\text{-H},7^{A}\text{-H}} = 10.8$ Hz, 1H, 6^{A}-H), 3.95 (dd, $^{3}J_{8^{A}\text{-H},7^{A}\text{-H}} = 8.9$ Hz, $^{3}J_{8^{A}\text{-H},9^{A}\text{-H}} = 2.4$ Hz, 1H, 8^{A}-H), 3.79 (dd, $^{2}J_{Gly^{A}} = 16.9$ Hz, $^{3}J_{Gly^{A}\text{-H}^{t},Gly^{A}\text{-NH}} = 6.4$ Hz, 1H, α_{Gly}-H^{A,t}), 3.70 (dd, $^{2}J_{Gly^{A}} = 16.9$ Hz, $^{3}J_{Gly^{A}\text{-H}^{h},Gly^{A}\text{-NH}} = 5.7$ Hz, 1H, α_{Gly}-H^{A,h}), 3.68–3.55 (m, 4H, $\alpha_{Gly}{}^{B}$-H, 7^{A}-H, 7^{B}-H), 3.50–3.40 (m, 2H, 2^{A}-H, 2^{B}-B), 2.97 (d, $^{2}J_{2^{proS,A}\text{-H},2^{proR,A}\text{-H}} = 11.3$ Hz, 1H, 2^{proS,A}-H), 2.88 (d, $^{2}J_{2^{proS,B}\text{-H},2^{proR,B}\text{-H}} = 11.3$ Hz, 1H, 2^{proS,B}-H), 1.41 (s, 3H, isopr.-CH$_3$), 1.35 (s, 3H, isopr.-CH$_3$), 1.34 (s, 3H, isopr.-CH$_3$), 1.31 (s, 3H, isopr.-CH$_3$); δ_C(150 MHz, DMSO[d$_6$]) 170.7 (α^{Gly}-*C*O^{B}), 169.9 (5^{A}), 169.4 (3-CO^{A}), 168.8 (3-CO^{B}), 167.9 (α^{Gly}-*C*O^{A}), 166.6 (5^{B}), 138.0, 128.1, 127.4, (Ar), 107.7 (isopr.$^{quat.,A}$, isopr.$^{quat.,B}$), 82.4 (8^{B}), 82.1 (8^{A}), 80.5 (9^{B}), 80.3 (9^{A}), 74.7, 74.5, (Ph-*C*H$_2{}^{A}$, Ph-*C*H$_2{}^{B}$), 72.0 (7^{A}), 70.6 (7^{B}), 66.7, 66.4 (3^{B}, 3^{A}), 60.9 (9a^{A}), 60.9 (9a^{B}), 53.5 (6^{A}), 41.7, 41.2 ($\alpha_{Gly}{}^{A}$, $\alpha_{Gly}{}^{B}$), 31.9 (2^{A}), 31.8 (2^{B}), 26.6 (isopr.-CH$_3$), 26.4 (isopr.-CH$_3$), 26.1 (isopr.-CH$_3$), 26.0 (isopr.-CH$_3$); HRMS (ESI) Calcd for M + Na^{+}: 935.2926; found 935.2913.

Cyclohexapeptide 3. DPPA (5.85 μL, 27.0 μmol) was added to a solution of **16** (9.00 mg, 9.42 μmol) and NaHCO$_3$ (3.80 mg, 45.0 μmol) in DMF (25 mL) at 0 °C. The solution was stirred for 5 days at 4 °C. The reaction was quenched with two drops of H$_2$O and the solvent was removed. The oily crude product was purified by column chromatography (DCM–MeOH = 15 : 1) to give the cyclohexapeptide **3** (3.5 mg, 42%) as a white powder. δ_H(600 MHz, DMSO[d$_6$]) 8.69 (t, $^{3}J_{Gly\text{-NH},\alpha Gly\text{-H}} = 6.3$ Hz, 2H, Gly-NH), 7.41 (d, $^{3}J_{6\text{-NH},6\text{-H}} = 6.3$ Hz, 2H, 6-NH), 7.39–7.29 (m, 10H, Ar), 5.49 (s, 2H, 9a-H), 4.93 (dd, $^{3}J_{6\text{-H},6\text{-NH}} = 6.3$ Hz, $^{3}J_{6\text{-H},7\text{-H}} = 10.$ Hz, 2H, 6-H), 4.89 (d, $^{2}J_{Bn} = 11.2$ Hz, 2H, Ph-CH_2^{t}), 4.70 (d, $^{2}J_{Bn} = 11.2$ Hz, 2H, Ph-CH_2^{h}), 4.62 (dd, $^{3}J_{3\text{-H},2^{proR}\text{-H}} = 8.2$ Hz, $^{3}J_{3\text{-H},2^{proS}\text{-H}} = 1.6$ Hz, 2H, 3-H), 4.22 (d, $^{3}J_{9\text{-H},8\text{-H}} = 2.1$ Hz, 2H, 9-H), 4.11 (dd, $^{3}J_{8\text{-H},7\text{-H}} = 9.0$ Hz, $^{3}J_{8\text{-H},9\text{-H}} = 2.1$ Hz, 2H, 8-H), 3.79 (dd, $^{2}J_{Gly} = 17.1$ Hz, $^{3}J_{Gly\text{-H}^{t},Gly\text{-NH}} = 6.2$ Hz, 2H, Gly-H^{t}), 3.63 (dd, $^{2}J_{Gly} = 17.1$ Hz, $^{3}J_{Gly\text{-H}^{h},Gly\text{-NH}} = 6.4$ Hz, 2H, Gly-H^{h}), 3.55 (dd, $^{3}J_{7\text{-H},6\text{-H}} = 10.5$ Hz, $^{3}J_{7\text{-H},8\text{-H}} = 9.0$ Hz, 2H, 7-H), 3.46 (dd, $^{2}J_{2^{proR}\text{-H},2^{proS}\text{-H}} = 11.3$ Hz, $^{3}J_{2^{proR}\text{-H},3\text{-H}} = 8.2$ Hz, 2H, 2^{proR}-H), 2.89 (dd, $^{2}J_{2^{proS}\text{-H},2^{proR}\text{-H}} = 11.3$ Hz, $^{3}J_{2^{proS}\text{-H},3\text{-H}} = 1.6$ Hz, 2H, 2^{proS}-H), 1.35, 1.30 (s, 3H, isopr.-CH$_3$); δ_C(150 MHz, DMSO[d$_6$]) 169.7 (3-*C*O), 168.5 (Gly-CO), 165.6 (5), 138.1, 128.0, 127.4, (Ar), 107.6 (isopr.$^{quat.}$), 82.5 (8), 80.5 (9), 74.6 (Ph-*C*H$_2$), 72.0 (7), 69.5 (3), 61.1 (9a), 51.7 (6), 42.6 (α_{Gly}), 31.3 (2), 26.3 (isopr.-CH$_3{}^{t}$), 26.0 (isopr.-CH$_3{}^{h}$); HRMS (ESI) Calcd for M + Na^{+}: 917.2820; found 917.2807.

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