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Synthesis of backbone modified cyclic peptides bearing dipicolylamino sidearms

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ABSTRACT

Three analogues of the *Lissoclinum* class of cyclic peptides, bearing dipicolylamino functionalised side chains, have been synthesised using a stepwise approach followed by macrocyclisation. Attempts to incorporate dipicolylamino functionalised side chains prior to peptide synthesis resulted in epimerisation, but this was overcome by functionalising the ornithine side chains with dipicolylamino groups after the macrocyclisation reaction.

1. Introduction

Macrocyclic molecular scaffolds that present multiple functional groups in a preorganised fashion, e.g., calixarenes, resorcinarenes, crown and aza-crown ethers and porphyrins are widely used in molecular recognition research.[1,2] While such scaffolds are often prepared by a cyclooligomerisation reaction, it is generally difficult to access unsymmetrical or highly functionalised scaffolds using this approach. An alternative approach to the preparation of macrocyclic scaffolds is to cyclise a linear precursor that has been prepared in a stepwise manner. This allows the sequential introduction of individual subunits bearing different functional groups and can be used to prepare both symmetrical and unsymmetrical macrocycles. Cyclic peptides are ideal candidates for synthesis via this approach. The large number of amino acid derivatives available for synthesis allows ready incorporation of a wide variety of functionalised pendant arms into these molecules and the stepwise nature of standard peptide synthesis protocols means that the sequence of functional groups in the macrocycle can be systematically varied.

In recent years, numerous oxazole and thiazole-containing cyclic peptides have been isolated from ascidians, cyanobacteria and other sources. In particular, the *Lissoclinum* family of cyclic peptides contains cyclic hexa-, hepta- and octa-peptides characterised by the presence of oxazoline/oxazole/thiazoline/thiazole heterocycles alternating with proteinogenic amino acid residues.[3] The azole heterocycles present in these natural products are derived from the condensation of cysteine, serine and threonine side chains with the adjacent amino acids in a peptide sequence. The presence of the modified amino acids influences the three dimensional structures and bioactivity of these natural products and provides added rigidity to the macrocycles. In addition, the alternating hydrogen bond donor and acceptor sites that line the interior of the molecules form a network of bifurcated hydrogen bonds that further rigidifies the macrocycle. This, together with the observation that in all-*syn* substituted compounds the side chains are presented on the same face of the macrocycle, has led to the recent use of analogues of these natural products as molecular scaffolds for the development of molecular receptors, chiral ligands, artificial proteins and combinatorial libraries.[4–6]

Our interest in the recognition of large, biologically interesting anions [e.g., pyrophosphate ($P_2O_7^{4-}$, PPi) and adenosine triphosphate (ATP)] led us to explore the use of this class of cyclic peptide scaffold for the development of anion receptors.[5] The cyclic hexapeptide and octapeptide scaffolds have diameters of approximately 6.6 and 9 Å, respectively,[6] allowing binding sites to be positioned at suitable distances to complex these large anions. We report here the use of a stepwise synthetic approach to one such cyclic octapeptide **1**,[5] with pendant dipicolylamino (Dpa) side chains suitable for complexation of Zn(II) ions to in turn bind phosphate oxoanions, together with the related cyclic hexapeptides **2** and **3** (Fig. 1). Compounds **1** and **2** were designed to investigate the effect that changing the size of the scaffold, and hence the distance between the two Dpa side chains, would have on the ability of these compounds to bind pyrophosphate ions, while scaffold **3**, which bears only a single Dpa side chain, was prepared as a reference compound. The ornithine side chains were chosen to provide some flexibility in the binding sites, thereby allowing 'induced fit' of a target anion.

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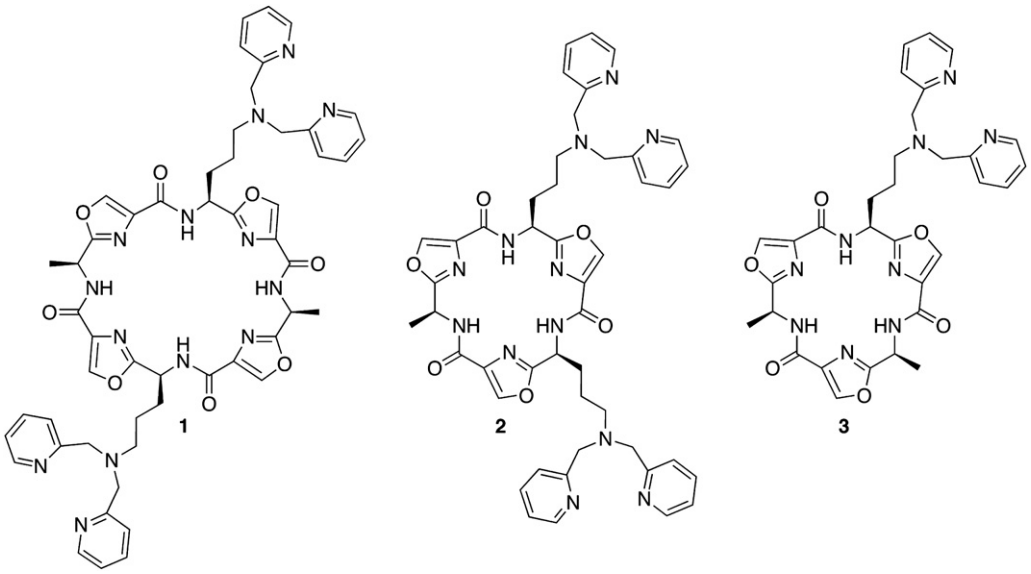


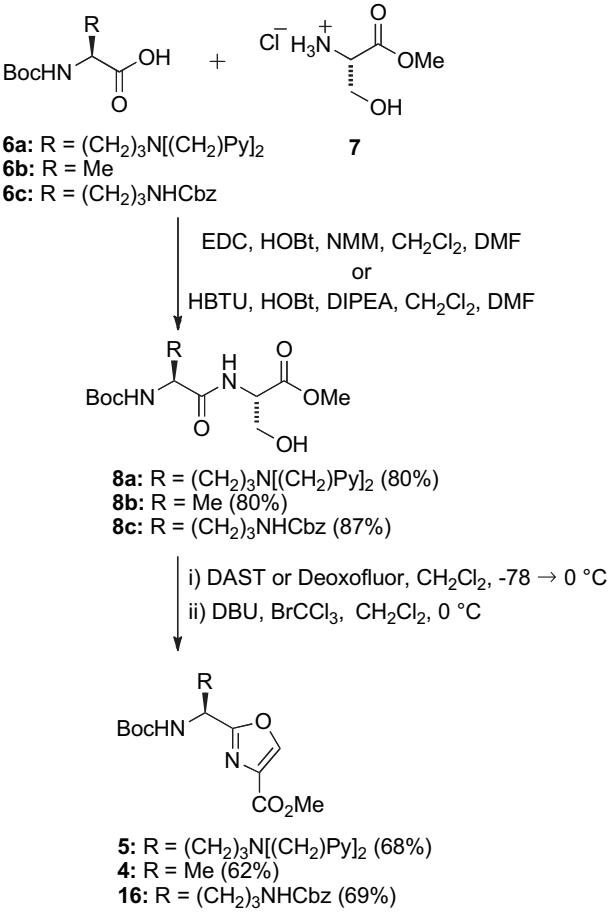
Fig. 1. Structures of compounds **1**–**3**.

2. Results and discussion

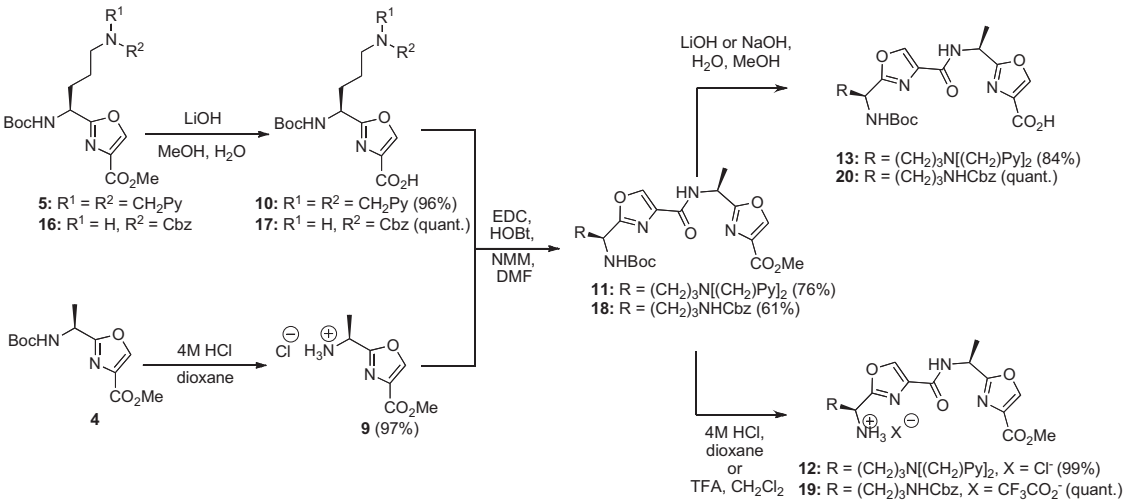
There are a number of possible approaches to the synthesis of **1**–**3**, the most attractive of which involves the coupling of oxazole-containing amino acid building blocks with appropriately functionalised side chains. We chose to prepare **1**–**3** using a stepwise approach followed by macrocyclisation, rather than via a cyclodimerisation approach in the case of **1**,[7] as this would provide a route to allow us to synthesise similar scaffolds with different sizes and arrangements of functional groups. In our initial approach we chose to functionalise the amino acid side chains with the required Dpa groups prior to oxazole formation. This provided us with two target oxazole building blocks; the known alanine-derived oxazole **4**[8] and an oxazole bearing a Dpa pendant sidearm, **5** (Scheme 1). We envisaged that coupling of the suitably protected oxazoles **4** and **5**, followed by macrocyclisation would provide the required anion receptors. Our initial synthetic target was therefore the Dpa-substituted oxazole **5**. We first prepared Dpa-substituted Boc-ornithine **6a** via our recently reported reductive amination method.[9] This was subsequently coupled to serine methyl ester **7** using *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC)/hydroxybenzotriazole (HOBt) as the coupling reagents to give the dipeptide **8a** in 80% yield. Treatment of **8a** with diethylaminosulfur trifluoride (DAST), followed by oxidation of the intermediate oxazoline with 1,8-diazabicyclol[5.4.0]undec-7-ene (DBU) and bromotrichloromethane,[10] gave the oxazole **5** in 68% yield. The alanine-derived oxazole **4** was prepared from Boc-alanine **6b** and **7** using the same procedure. Treatment of oxazole **4** with 4 M HCl in dioxane resulted in cleavage of the Boc group to give the amine **9** as the hydrochloride salt. Hydrolysis of the methyl ester group of **5** was achieved upon treatment with lithium hydroxide to yield the carboxylic acid **10**. Acid **10** and amine **9** were then coupled to give the bisoxazole **11** using EDC/HOBt as the coupling reagents (Scheme 2).

For the synthesis of **1**, we envisaged that two bisoxazole units could be coupled to provide the required tetraoxazole linear precursor. Hence, N-deprotection of **11** under identical conditions to those described above gave the amine **12** as the hydrochloride salt. Alternatively, treatment of **11** with lithium hydroxide resulted in C-deprotection to give the carboxylic acid **13**. These two bisoxazole fragments (**12** and **13**) were then coupled under standard conditions to yield the protected linear tetraoxazole **14** (Scheme 3).

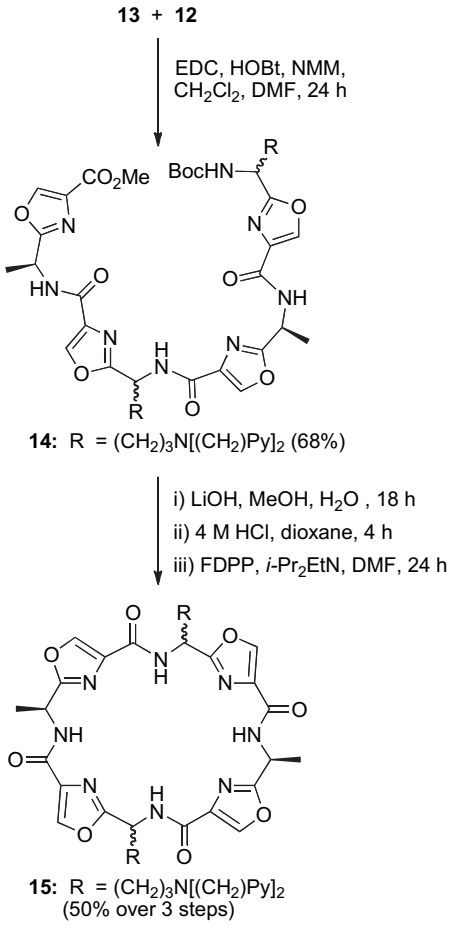
At this point, careful inspection of the ^{1}H and ^{13}C NMR spectra of **14** suggested that either a mixture of diastereoisomers or rotamers was present (no evidence for the presence of diastereomers or rotamers was observed for bisoxazole **11**, whose ^{1}H and ^{13}C spectra contained only a single set of signals). To confirm which was the case, the *C*- and *N*-termini of **14** were deprotected under the



Scheme 1. Synthesis of the oxazole building blocks.

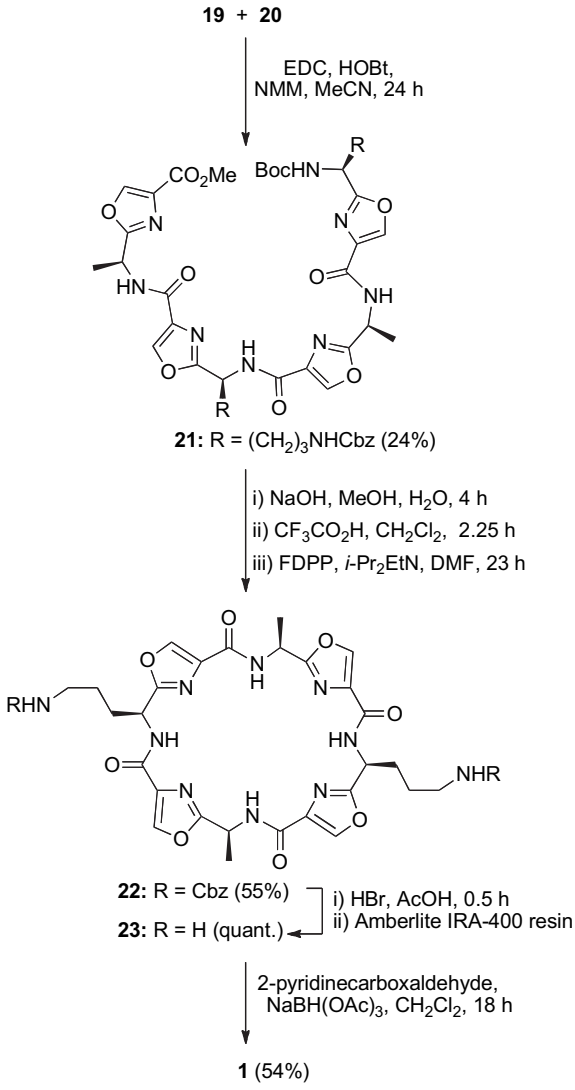


Scheme 2. Synthesis of bisoxazoles.



Scheme 3. Synthesis of **15** as a mixture of diastereoisomers.

standard conditions described above, followed by macrocyclisation of the resulting peptide upon treatment with pentafluorophenyl diphenylphosphinate (FDPP)[11] to give the cyclic peptide **15** in 50% yield (Scheme 3). The ^{1}H NMR spectra of **15** in either CD_3OD or DMSO-d_6 contained broad signals and notably, when DMSO-d_6 was used as the solvent, multiple signals attributable to the amide protons were observed rather than the expected two signals. Multiple sets of signals were also observed in the ^{13}C NMR spectrum of **15**. These could not be attributed to conformers and it was determined that a mixture of diastereomeric peptides, including the desired isomer **1** had been obtained. Re-examination of each step of the synthetic route enabled us to ascertain that substantial epimerisation was occurring during the synthesis of the oxazole **5**



Scheme 4. Synthesis of dipicolylamino functionalised cyclic peptide **1**.

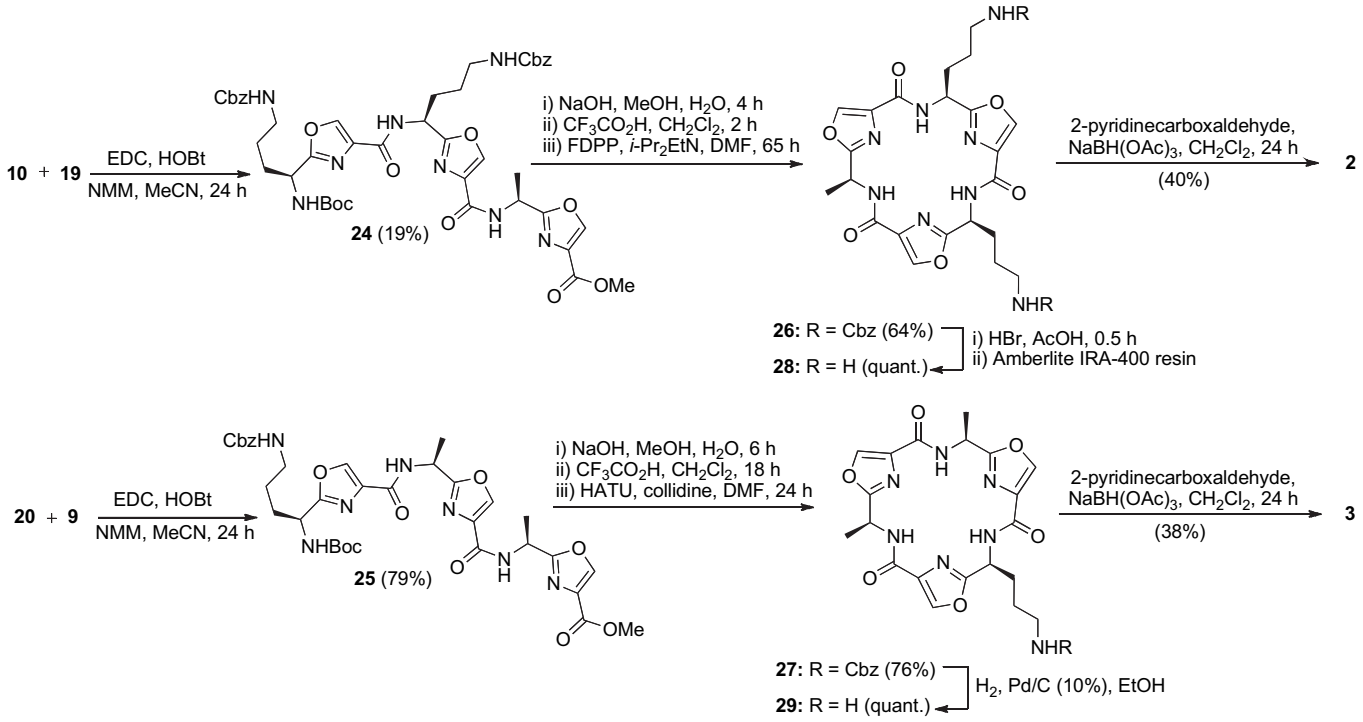
from dipeptide **8a**. This was surprising since our synthesis of **4** from **8b** under identical conditions proceeded without any detectable epimerisation of the α-carbon (as examined by the preparation of Mosher's amides[12] and comparison of the optical rotation with reported values[8]), which is in agreement with results reported in the literature for similar compounds.[10] However, it is known that the α-carbon of dipeptide derived oxazolines is prone to epimerisation under both acidic and basic catalysis[13] and has been observed to occur during oxidation of an oxazoline to the corresponding oxazole.[14] We postulate that the epimerisation occurring in **5** is a result of facile abstraction of the acidic proton at the stereocentre of the oxazoline by the tethered dipicolylamino group, which is six atoms distant from this proton. All attempts to optimise the synthesis of **5** to minimise epimerisation were unsuccessful.

The loss of stereochemical integrity of the oxazole **5** required us to revise our synthetic route. We did this by delaying functionalisation of the ornithine side chains until after the cyclic peptide was formed. Hence, Boc-Orn(Cbz)-OH **6c** was coupled with serine methyl ester **7** and the resulting dipeptide **8c** was converted to the corresponding oxazole **16** using the two-step procedure described above, employing the more stable Deoxofluor instead of DAST (Scheme 1). As expected,[15] no detectable epimerisation occurred during the synthesis of **16**, (as examined by the preparation of Mosher's amides[12]) further suggesting that it is the basic nitrogen atoms in **5** that are responsible for the epimerisation observed for this compound. Hydrolysis of the methyl ester of **16** under standard conditions gave the free acid **17** (Scheme 2), which was coupled with amine **9** to give the tetrapeptide **18** in good yield. Selective removal of the Boc group was achieved upon treatment of **18** with trifluoroacetic acid to give the amine **19**.[16] Alternatively, treatment of **18** with sodium hydroxide resulted in C-deprotection to give the acid **20**, which was coupled with **19** to give the octapeptide **21** (Scheme 4). Sequential C- and N-deprotection of **21** was followed by macrocyclisation upon treatment with FDPP to give the cyclic peptide **22** in 58% yield over three steps. Cbz deprotection occurred upon treatment of **22** with HBr in acetic acid and, following neutralisation, the corresponding diamine **23** was subjected to reductive amination with 2-pyridinecarboxaldehyde in the presence of sodium triacetoxyborohydride to install the Dpa ligands, yielding **1**. The ^{1}H and ^{13}C NMR spectra of both **22** and **1** exhibit the expected number of signals for compounds with C_2 symmetry, confirming that only a single diastereoisomer was obtained from the macrocyclisation.

With the synthesis of **1** as a single diastereomer confirmed, we proceeded to prepare compounds **2** and **3** using a similar approach. Thus, acid **10** was coupled to bisoxazole **19** under standard conditions to provide linear trioxazole **24**, while bisoxazole **20** was coupled to oxazole **9** to provide linear trioxazole **25** (Scheme 5). Both **24** and **25** were subject to N- and C-deprotection using the same procedures as described above for the deprotection of tetraoxazole **21**, and macrocyclisation of the resulting free peptides gave cyclic peptides **26** and **27** in 64% and 76% yields, respectively. Removal of the Cbz groups from **26** was achieved by using HBr in acetic acid, followed by neutralisation to give amine **28**, while compound **27** was subjected to hydrogenolysis in the presence of Pd/C to give the corresponding free amine **29** (Pd catalysed hydrogenolysis was sluggish when more than one Cbz-protected amine was present). Both **28** and **29** were then subject to reductive amination with 2-pyridinecarboxaldehyde to give the cyclic trioxazoles **2** and **3** as single diastereoisomers, respectively. Compounds **1**–**3** were found to be stable when stored for up to 2 years at −15 °C and were not observed to undergo epimerisation during this time, or when stood in $CDCl_3$ solution at rt for up to 7 days.

3. Conclusions

In summary, cyclic peptide scaffolds **1**–**3** bearing dipicolylamino side chains were synthesised using a stepwise approach to couple the oxazole units, followed by a macrocyclisation step. Installation of the dipicolylamino group prior to oxazole formation was found to result in epimerisation of the carbon α- to the azole, presumably through deprotonation during oxidation of the oxazoline to the oxazole **5**. Given that the similar oxazoles, **4** and **16**, were prepared under identical conditions with minimal epimerisation and that



Scheme 5. Synthesis of dipicolylamino functionalised cyclic peptides **2** and **3**.

numerous other chiral oxazoles have been prepared by this method,[10] we postulate that epimerisation of **5** is facilitated by the tethered dipicolylamino group. This loss of stereochemical integrity was overcome by altering the synthetic route such that the dipicolylamino groups were installed in the final step of the synthetic sequence. The compounds prepared in this manner were obtained as single stereoisomers, and appear stable under a range of conditions. The bis-zinc(II) complexes of **1** and **2** are under evaluation for their ability to bind selectively to large phosphate oxoanions under physiological conditions; some of the results of these studies have already been published,[5] the remainder will be published elsewhere.

4. Experimental

4.1. General methods

Melting points were obtained using a Stanford Research Systems Optimelt melting point apparatus and are uncorrected. Optical rotations were obtained using a Perkin–Elmer model 341 polarimeter at 589 nm and 20 °C, using the indicated spectroscopic grade solvent. ^{1}H nuclear magnetic resonance spectra were recorded using a Bruker Avance DPX 400 spectrometer at a frequency of 400.13 MHz, a Bruker Avance DPX 300 spectrometer at a frequency of 300.13 MHz, or a Bruker Avance DPX 200 spectrometer at a frequency of 200.13 MHz, and are reported in parts per million (ppm) relative to the residual isotopomer with one less deuterium then the quoted perdeuterated solvent. The data is reported as chemical shift (δ), multiplicity (br=broad, s=singlet, d=doublet, t=triplet, q=quartet, dd=doublet of doublets, ddd=doublet of doublet of doublets, m=multiplet), coupling constant (J Hz) and relative integral. ^{13}C nuclear magnetic resonance spectra were recorded on a Bruker Avance DPX 400 spectrometer at a frequency of 100.61 MHz, or a Bruker Avance DPX 300 spectrometer at a frequency of 75.47 MHz and are reported in parts per million (ppm) relative to the internal perdeuterated solvent resonance, unless otherwise stated. Low resolution mass spectra were recorded on a Thermo Finnigan LCQ Deca Ion Trap mass spectrometer using electrospray ionisation (ESI). High resolution mass spectra were recorded on a Bruker BioApex Fourier Transform Ion Cyclotron Resonance mass spectrometer (FT-ICR) with an analytical ESI source, operating at 4.7 T. Analytical thin layer chromatography (TLC) was performed using pre-coated silica gel plates (Merck Kieselgel 60 F_{254}). Preparative flash chromatography was carried out using Merck Kieselgel Silica Gel 60 (particle size 0.040–0.065 mm) with the indicated ratio of solvents (volume/volume), which were mixed as specified. Liquid chromatography mass spectrometry (LCMS) was performed on a Thermo Separation Products: spectra System using a P400 Pump and a UV6000LP photodiode array detector. Separation was achieved using a Phenomenex Jupiter column (5 μm, 150×2.1 mm ID), which was coupled to a Thermoquest Finnigan LCQ Deca mass spectrometer (ESI). Flow rate was maintained at 0.2 mL min^{-1} over a linear gradient from 0% to 100% solvent B (solvent A: 100:0.1 v/v Milli-Q water/formic acid, solvent B: 100:0.1 v/v acetonitrile/formic acid) over 30 min. Reactions were performed under an inert atmosphere of anhydrous nitrogen unless otherwise stated. Dichloromethane, methanol and acetonitrile were distilled from calcium hydride. Tetrahydrofuran (THF) was dried over sodium wire. HPLC grade *N*,*N*-dimethylformamide was obtained from LabScan and used without further purification. Boc-Orn(Dpa)-OH was prepared according to a previously reported procedure.[9] All other reagents were commercially available and were used as supplied.

*4.1.1. Boc-Orn(Dpa)-Ser-OMe (**8a**).* Under an atmosphere of nitrogen, Boc-Orn(Dpa)-OH **6a** (1.57 g, 3.79 mmol) and HCl·NH_2-Ser-OMe **7** (0.589 g, 3.79 mmol) were dissolved in anhydrous dichloromethane/DMF (3:1 v/v, 40 mL). EDC (0.799 g, 4.17 mmol), HOBt (0.638 g, 4.17 mmol) and then *N*-methylmorpholine (1.25 mL, 11.4 mmol) were added, and the resulting mixture was stirred at rt for 24 h. The mixture was partitioned between satd aq $NaHCO_3$ solution (100 mL) and ethyl acetate (100 mL). The organic phase was isolated and the aqueous fraction was further extracted with ethyl acetate (3×100 mL). The combined organic fractions were washed first with water (100 mL) and then brine (100 mL), dried ($MgSO_4$) and concentrated under reduced pressure to give a yellow oil. Subjection of the crude material to flash chromatography (silica gel; $CHCl_3$/MeOH/aq ammonia, 190:9:1 v/v/v) gave the desired Dpa-functionalized dipeptide **8a** (1.56 g, 80%) as a pale yellow solid. Mp 54–57 °C; $[\alpha]_D^{20}$ +12.1 (*c* 1.0, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.55 (d, J=4.9 Hz, 2H), 8.10 (br s, 1H), 7.65 (t, J=7.6 Hz, 2H), 7.41 (d, J=7.7 Hz, 2H), 7.19 (t, J=7.0 Hz, 2H), 5.95 (br d, 1H), 4.60 (m, 1H), 3.99 (m, 3H), 3.77 (s, 4H), 3.75 (s, 3H), 2.55 (s, 2H), 1.76–1.25 (m, 4H), 1.39 (s, 9H), O–H signal not observed; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 173.1, 171.1, 159.3, 156.0, 149.1, 136.9, 123.8, 122.4, 79.8, 62.5, 60.1, 55.1, 54.4, 53.8, 52.6, 30.1, 28.6, 22.5; IR ν_{max} (NaCl)/cm^{-1} 3296, 2952, 1660, 1519, 1434, 1168, 1049, 734; MS (ESI) *m*/*z* 538 [(M+Na)$^+$, 35%], 516 (100), 438 (16), 416 (26); HRMS (ESI) calcd for $C_{26}H_{37}N_5O_6Na$ (M+Na)$^+$ 538.2636, found 538.2634.

*4.1.2. Boc-Orn(Dpa)-Oxz(Ser)-OMe (**5**).* Under an atmosphere of nitrogen, (diethylamino)sulfur trifluoride (1.61 mL, 12.1 mmol) was added dropwise to a solution of dipeptide **8a** (1.56 g, 3.03 mmol) in anhydrous dichloromethane (50 mL) at −78 °C. Stirring of the resulting mixture was continued at −78 °C for 1 h and then at rt for a further 24 h. After cooling to 0 °C, the reaction mixture was quenched by the addition of anhydrous K_2CO_3 (2.09 g, 15.2 mmol) in one portion. The reaction mixture was poured into satd aq $NaHCO_3$ solution (50 mL) and the biphasic mixture was extracted with ethyl acetate (3×50 mL). The combined organic fractions were dried (Na_2SO_4) and the solvent was removed under reduced pressure to give the corresponding oxazoline as a brown oil.

A stirred solution of oxazoline (1.47 g, 2.96 mmol) in anhydrous dichloromethane (40 mL) at 0 °C under an atmosphere of nitrogen was treated dropwise with bromotrichloromethane (0.583 mL, 5.92 mmol), followed by 1,8-diazabicyclo[5.4.0]undec-7-ene (0.885 mL, 5.92 mmol). The reaction mixture was warmed to rt and stirred for 24 h prior to quenching with satd aq $NaHCO_3$ solution (40 mL) and extracted with ethyl acetate (3×40 mL). The combined organic fractions were dried (Na_2SO_4) and the solvent was removed under reduced pressure. The crude material thus obtained was subjected to flash chromatography (silica gel; $CHCl_3$/MeOH/aq ammonia, 190:9:1 v/v/v) to afford the desired Dpa-functionalized oxazole **5** (1.01 g, 68%) as a yellow oil. $[\alpha]_D^{20}$ −4.24 (*c* 0.7, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.55 (d, J=4.8 Hz, 2H), 8.13 (s, 1H), 7.63 (dt, J=7.6 and 1.7 Hz, 2H), 7.45 (d, J=7.7 Hz, 2H), 7.14 (t, J=6.5 Hz, 2H), 5.43 (d, J=7.2 Hz, 1H), 4.86 (d, J=5.6 Hz, 1H), 3.90 (s, 3H), 3.77 (s, 4H), 2.56 (t, J=6.8 Hz, 2H), 1.87–1.21 (m, 4H), 1.41 (s, 9H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 165.9, 161.9, 159.4, 155.5, 149.4, 144.2, 136.9, 133.6, 123.6, 122.5, 80.5, 60.6, 54.0, 52.6, 49.3, 32.3, 28.7, 23.2; IR ν_{max} (NaCl)/cm^{-1} 3300, 2950, 1737, 1699, 1589, 1434, 1169, 999, 760; MS (ESI) *m*/*z* 518 [(M+Na)$^+$, 70%], 496 (100), 418 (27), 396 (87); HRMS (ESI) calcd for $C_{26}H_{34}N_5O_5Na$ (M+H)$^+$ 518.2379, found 518.2373.

*4.1.3. Boc-Ala-Ser-OMe[17] (**8b**).* To a solution of the amino acid Boc-Ala-OH **6b** (6.00 g, 31.7 mmol) and HCl·NH_2-Ser-OMe **7** (4.93 g, 31.7 mmol) in anhydrous dichloromethane/DMF (3:1 v/v, 100 mL) under an atmosphere of nitrogen were added EDC (6.68 g, 34.9 mmol), HOBt (5.34 g, 34.9 mmol) and *N*-methylmorpholine (10.5 mL, 95.1 mmol). The reaction mixture was stirred at rt for 4 h

after which time it was partitioned between hydrochloric acid (0.50 M, 100 mL) and ethyl acetate (100 mL). The aqueous phase was isolated and extracted with ethyl acetate (3×100 mL). The combined organic fractions were washed first with water (2×100 mL) and then brine (100 mL), dried ($MgSO_4$) and concentrated under reduced pressure to give a colourless oil, which was purified by flash chromatography (silica gel; $CHCl_3$/MeOH, 9:1 v/v) to afford the desired dipeptide **8b** (7.36 g, 80%) as a colourless oil. $[\alpha]_D^{20}$ +11.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.98 (d, *J*=4.9 Hz, 1H), 5.11 (d, *J*=7.0 Hz, 1H), 4.64 (m, 1H), 4.14 (m, 1H), 3.95 (m, 2H), 3.79 (s, 3H), 1.43 (s, 9H), 1.40 (d, *J*=7.0 Hz, 3H), OH not observed. Compound **8b** had spectroscopic data identical to that reported in the literature.[17]

*4.1.4. Boc-Ala-Oxz(Ser)-OMe[8] (**4**).* (Diethylamino)sulfur trifluoride (1.93 mL, 14.5 mmol) was added dropwise to a solution of **8b** (2.11 g, 7.27 mmol) in anhydrous dichloromethane (40 mL) at −78 °C under an atmosphere of nitrogen. The resulting mixture was stirred at −78 °C for 3 h after which time it was quenched by the addition of anhydrous K_2CO_3 (2.51 g, 18.2 mmol). After warming to rt the mixture was poured into satd aq $NaHCO_3$ solution (40 mL) and the biphasic mixture was extracted with dichloromethane (3×40 mL). The combined organic fractions were dried (Na_2SO_4) and the solvent was removed under reduced pressure to give the corresponding oxazoline as a yellow oil.

A stirred solution of oxazoline (1.74 g, 6.39 mmol) in anhydrous dichloromethane (35 mL) at 0 °C under an atmosphere of nitrogen was treated dropwise with bromotrichloromethane (1.26 mL, 12.8 mmol), followed by 1,8-diazabicyclo[5.4.0]undec-7-ene (1.91 mL, 12.8 mmol). The reaction mixture was warmed to rt and stirred for 24 h prior to quenching with satd aq $NaHCO_3$ solution (40 mL) and extracted with ethyl acetate (3×40 mL). The combined organic fractions were dried (Na_2SO_4) and the solvent was removed under reduced pressure. The crude material thus obtained was subjected to flash chromatography (silica gel; EtOAc/hexane, 2:3 v/v) to afford the desired oxazole **4** (1.20 g, 62%) as a white solid. Mp 108–110 °C; $[\alpha]_D^{20}$ −53.2 (*c* 0.5, $CHCl_3$) {lit.[8b] $[\alpha]_D^{20}$ −50.0 (*c* 0.5, $CHCl_3$)}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.18 (s, 1H), 5.30 (br s, 1H), 5.02 (d, *J*=8.2 Hz, 1H), 3.91 (s, 3H), 1.54 (d, *J*=5.9 Hz, 3H), 1.43 (s, 9H). Compound **4** had spectroscopic data identical to that reported in the literature.[8]

*4.1.5. HCl·H$_2$N-Ala-Oxz(Ser)-OMe (**9**).* Oxazole **4** (0.289 g, 1.07 mmol) was dissolved in a solution of hydrochloric acid in dioxane (4.0 M, 2.80 mL) and stirred under an atmosphere of nitrogen for 4 h. The reaction mixture was concentrated under reduced pressure to give the desired hydrochloride salt **9** (0.214 g, 97%) as a colourless foam, which was used without further purification. ^{1}H NMR (200 MHz, $CDCl_3$): δ 9.41 (d, 3H), 8.25 (s, 1H), 4.96 (d, 1H), 3.89 (s, 3H), 1.93 (d, *J*=6.6 Hz, 3H); MS (ESI) *m*/*z* 171 (M^+, 100%), 363 (18), 341 (86).

*4.1.6. Boc-Orn(Dpa)-Oxz(Ser)-OH (**10**).* To a solution of the oxazole **5** (0.459 g, 0.926 mmol) in methanol (9 mL) was added a solution of LiOH (66 mg, 2.8 mmol) in water (3 mL) and the resulting mixture was stirred at rt for 18 h. The mixture was neutralized (pH 7) and the organic solvent was removed under reduced pressure. The residue thus obtained was partitioned between water (30 mL) and $CHCl_3$/^{i}PrOH (3:1 v/v, 30 mL), and the isolated aqueous phase was further extracted with $CHCl_3$/^{i}PrOH (3:1 v/v, 3×30 mL). The combined organic fractions were dried (Na_2SO_4) and concentrated under reduced pressure to afford the carboxylic acid **10** (0.428 g, 96%), which was used without further purification; $[\alpha]_D^{20}$ −58.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.46 (d, *J*=4.8 Hz, 2H), 7.86 (s, 1H), 7.74 (t, *J*=6.1 Hz, 2H), 7.48 (d, *J*=7.9 Hz, 2H), 7.23 (t, *J*=6.1 Hz, 2H), 4.67 (br s, 1H), 4.33 (br s, 1H), 3.69 (s, 4H), 2.42 (m, 2H), 1.90–1.23 (m, 4H), 1.35 (s, 9H), O–H signal not observed; IR ν_{max} (NaCl)/cm^{-1} 3296, 2932, 1616, 1435, 1217, 754; MS (ESI) *m*/*z* 481 (M^+, 21%), 504 (100), 404 (24); HRMS (ESI) calcd for $C_{25}H_{31}N_5O_5Na$ $(M+Na)^+$ 504.2218, found 504.2223.

*4.1.7. Boc-Orn(Dpa)-Oxz(Ser)-Ala-Oxz(Ser)-OMe (**11**).* Under an atmosphere of nitrogen, carboxylic acid **10** (0.463 g, 0.962 mmol) and the hydrochloride salt **9** (0.198 g, 0.962 mmol) were dissolved in anhydrous dichloromethane/DMF (3:1 v/v, 20 mL). EDC (0.203 g, 1.06 mmol), HOBt (0.162 g, 1.06 mmol) and *N*-methylmorpholine (0.317 mL, 2.89 mmol) were added and the resulting solution was stirred at rt for 7 h. The mixture was partitioned between hydrochloric acid (0.50 M, 50 mL) and ethyl acetate (50 mL), the aqueous phase was isolated and further extracted with ethyl acetate (3×50 mL). The combined organic fractions were washed first with water (2×50 mL) and then brine (50 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The crude material thus obtained was subjected to flash chromatography (silica gel; $CHCl_3$/MeOH/aq ammonia, 93:6:1 v/v/v) to give the desired bisoxazole **11** (0.465 g, 76%) as a yellow solid. Mp 59–60 °C; found: C, 57.2; H, 6.3; N, 14.2% ($C_{32}H_{39}N_7O_7\cdot 2H_2O$ requires C, 57.4; H, 6.5; N, 14.6%); $[\alpha]_D^{20}$ −11.5 (*c* 0.8, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.52 (d, *J*=4.3 Hz, 2H), 8.19 (s, 1H), 8.11 (s, 1H), 7.62 (t, *J*=7.5 Hz, 2H), 7.45 (d, *J*=8.2 Hz, 2H), 7.16 (t, *J*=5.4 Hz, 2H), 5.48 (br d, 2H), 4.81 (br s, 2H), 3.91 (s, 3H), 3.78 (s, 4H), 2.57 (t, *J*=6.6 Hz, 2H), 1.84–1.25 (m, 7H), 1.44 (s, 9H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 165.5, 161.8, 160.3, 159.7, 149.4, 144.5, 141.8, 136.8, 135.8, 133.7, 123.5, 122.4, 80.5, 60.7, 53.9, 52.6, 49.3, 43.2, 32.3, 29.7, 28.7, 23.1; IR ν_{max} (NaCl)/cm^{-1} 3402, 2929, 1708, 1670, 1593, 1506, 1112, 730; MS (ESI) *m*/*z* 634 [$(M+H)^+$, 49%], 828 (21), 656 (100), 556 (47), 534 (32); HRMS (ESI) calcd for $C_{32}H_{39}N_7O_7Na$ $(M+Na)^+$ 656.2801, found 656.2809.

*4.1.8. HCl.H$_2$N-Orn(Dpa)-Oxz(Ser)-Ala-Oxz(Ser)-OMe (**12**).* The bisoxazole **11** (0.465 g, 0.734 mmol) was dissolved in a solution of hydrochloric acid in dioxane (4.0 M, 1.92 mL) and stirred under an atmosphere of nitrogen for 4 h. The reaction mixture was concentrated under reduced pressure to give the desired hydrochloride salt **12** (0.414 g, 99%) as a colourless foam, which was used without further purification. ^{1}H NMR (200 MHz, CD_3OD): δ 8.83 (d, *J*=5.9 Hz, 2H), 8.53 (d, *J*=2.1 Hz, 2H), 8.44 (t, *J*=7.2 Hz, 2H), 7.99 (d, *J*=7.5 Hz, 2H), 7.90 (t, *J*=6.8 Hz, 2H), 5.41 (m, 1H), 4.68 (m, 1H), 4.38 (s, 2H), 3.86 (s, 3H), 3.66 (s, 4H), 2.86 (t, *J*=7.6 Hz, 2H), 1.71–1.30 (m, 7H), one N–H signal not observed; MS (ESI) *m*/*z* 534 (M^+, 100%), 556 (18), 535 (40).

*4.1.9. Boc-Orn(Dpa)-Oxz(Ser)-Ala-Oxz(Ser)-OH (**13**).* To a solution of the bisoxazole **11** (0.169 g, 0.266 mmol) in methanol (3 mL) was added a solution of LiOH (19 mg, 0.798 mmol) in water (1 mL) and the resulting mixture was stirred at rt for 18 h. The mixture was neutralized (pH 7) and the organic solvent was removed under reduced pressure. The residue thus obtained was partitioned between water (30 mL) and $CHCl_3$/^{i}PrOH (3:1 v/v, 30 mL). The organic phase was isolated and the aqueous phase was further extracted with $CHCl_3$/^{i}PrOH (3:1 v/v, 3×30 mL). The combined organic fractions were dried (Na_2SO_4) and concentrated under reduced pressure to afford the carboxylic acid **13** (0.140 g, 84%), which was used without further purification. ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.76 (br d, *J*=8.0 Hz, 1H), 8.68 (s, 1H), 8.57 (s, 1H), 8.47 (d, *J*=4.0 Hz, 2H), 7.75 (t, *J*=7.5 Hz, 2H), 7.51 (t, *J*=9.3 Hz, 2H), 7.24 (t, *J*=5.8 Hz, 2H), 5.26 (m, 1H), 4.35 (d, *J*=3.5 Hz, 6H), 3.78 (t, *J*=5.9 Hz, 2H), 1.90–1.22 (m, 7H), 1.36 (s, 9H), O–H signal not observed; MS (ESI) *m*/*z* 620 [$(M+H)^+$, 100%], 642 (25), 520 (34); HRMS (ESI) calcd for $C_{31}H_{38}N_7O_7$ $(M+H)^+$ 620.2831, found 620.2791.

*4.1.10. Boc-[Orn(Dpa)-Oxz(Ser)-Ala-Oxz(Ser)]$_2$-OMe (**14**).* Under an atmosphere of nitrogen, compound **13** (0.140 g, 0.226 mmol) and compound **12** (0.129 g, 0.226 mmol) were dissolved in anhydrous dichloromethane/DMF (3:1 v/v, 2.4 mL). EDC (48 mg, 0.249 mmol),

HOBt (38 mg, 0.249 mmol) and then *N*-methylmorpholine (75 μL, 0.680 mmol) were added, and the resulting mixture was stirred at rt for 24 h. The mixture was partitioned between satd aq $NaHCO_3$ solution (10 mL) and ethyl acetate (10 mL). The organic phase was isolated and the aqueous fraction was further extracted with ethyl acetate (3×10 mL). The combined organic fractions were washed sequentially with water (10 mL) and brine (10 mL), then dried ($MgSO_4$) and concentrated under reduced pressure to give a yellow oil. Subjection of the crude material to flash chromatography (silica gel; $CHCl_3$/MeOH, 9:1 v/v) afforded the tetraoxazole **14** (0.174 g, 68%) as a white solid. Mp 68–71 °C; $[\alpha]_D^{20}$ −12.9 (*c* 0.7, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.52 (s, 4H), 8.13 (m, 3H), 8.11 (s, 1H), 7.63 (t, *J*=6.7 Hz, 4H), 7.44 (d, *J*=5.7 Hz, 4H), 7.19 (t, *J*=7.0 Hz, 4H), 5.44 (m, 4H), 4.79 (br s, 2H), 3.90 (s, 3H), 3.78 (m, 8H), 2.58 (s, 4H), 2.35 (s, 2H), 1.79–1.27 (m, 14H), 1.45 (s, 9H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 165.5–162.5, 161.4, 160.0, 149.4, 144.5, 142.0, 136.8, 135.5, 133.2, 123.1, 122.0, 80.1, 60.3, 53.9, 52.1, 48.9, 43.2, 31.5, 28.2, 23.3, 19.6; MS (ESI) *m*/*z* 1135 [$(M+H)^+$, 100%], 1157 (25), 1069 (98), 968 (35), 770 (51), 619 (34); HRMS (ESI) calcd for $C_{58}H_{67}N_{14}O_{11}$ $(M+H)^+$ 1135.5116, found 1135.5068.

*4.1.11. HCl·H₂N-[Orn(Dpa)-Oxz(Ser)-Ala-Oxz(Ser)]₂-OH (**30**).* To a solution of the tetraoxazole **14** (0.151 g, 0.133 mmol) in methanol (1.5 mL) was added a solution of LiOH (9.5 mg, 0.399 mmol) in water (0.5 mL) and the resulting mixture was stirred at rt for 18 h. The mixture was neutralized (pH 7) and the organic solvent was removed under reduced pressure. The residue thus obtained was partitioned between water (15 mL) and $CHCl_3$/^{i}PrOH (3:1 v/v, 15 mL). The organic phase was isolated and the aqueous phase was further extracted with $CHCl_3$/^{i}PrOH (3:1 v/v, 3×15 mL). The combined organic fractions were dried (Na_2SO_4) and concentrated under reduced pressure to afford the desired carboxylic acid. This material was immediately dissolved in a solution of hydrochloric acid in dioxane (4.0 M, 0.322 mL) and stirred under an atmosphere of nitrogen for 4 h. The reaction mixture was concentrated under reduced pressure to give the desired hydrochloride salt **30** (0.128 g, 99%) as a colourless foam, which was used without further purification. ^{1}H NMR (200 MHz, CD_3OD): δ 8.87 (d, *J*=5.1 Hz, 4H), 8.54–8.25 (m, 8H), 8.15–7.99 (m, 8H), 4.36 (d, *J*=4.9 Hz, 4H), 3.69 (s, 8H), 2.84 (m, 4H), 1.74–1.31 (m, 14H), N–H and O–H signals not observed; MS (ESI) *m*/*z* 1022 [$(M+H)^+$, 73%], 1044 (24), 511 (100).

*4.1.12. Cyclo[Orn(Dpa)-Oxz(Ser)-Ala-Oxz(Ser)]₂ (**15**).* To a stirred solution of the hydrochloride salt **30** (0.140 g, 0.132 mmol) in anhydrous DMF (26 mL) under an atmosphere of nitrogen was added pentafluorophenyl diphenylphosphinate (70 mg, 0.198 mmol) and Hünig's base (69 μL, 0.396 mmol). The reaction mixture was stirred at rt for 24 h after which time the solvent was removed under reduced pressure to give a crude orange coloured oil, which was partitioned between water (10 mL) and $CHCl_3$/^{i}PrOH (3:1 v/v, 20 mL). The organic phase was isolated and the aqueous phase was further extracted with $CHCl_3$/^{i}PrOH (3:1 v/v, 2×20 mL). The combined organic fractions were washed sequentially with 2 M aq NaOH solution (30 mL), water (30 mL), and brine (30 mL), then dried ($MgSO_4$) and concentrated under reduced pressure to give an orange oil. Purification by flash chromatography (silica gel; $CHCl_3$/MeOH/aq ammonia, 90:9:1 v/v/v) afforded the cyclic peptide **15** (66 mg, 50%) as a yellow solid and an inseparable mixture of diastereoisomers. $[\alpha]_D^{20}$ −36.0 (*c* 0.7, MeOH); ^{1}H NMR (200 MHz, CD_3OD): δ 8.40 (br m, 8H), 7.74 (br m, 4H), 7.59 (br m, 4H), 7.26 (br m, 4H), 5.36 (br m, 4H), 3.77 (br m, 8H), 2.58 (br m, 4H), 1.69–1.29 (br m, 14H), N–H signals not observed; ^{13}C NMR (75.47 MHz, MeOD) δ 166.3,# 166.0,# 165.9,# 165.8,# 165.6, 165.5, 164.8,# 162.4,# 162.2,# 162.0,# 161.9, 161.8,# 161.7,# 161.6,# 160.6, 160.5,# 149.4, 149.3,# 144.2,# 144.1,# 143.9,# 143.8,# 143.7,# 143.6,# 143.5, 138.6, 138.5,# 137.2,# 137.0,# 136.9, 136.8,# 136.7,# 133.6,# 132.7,# 132.6,# 132.4, 130.1,# 129.9, 124.9, 123.8, 61.2, 54.9, 44.4, 43.9,# 43.8,# 43.5,# 40.2, 33.0,# 32.5,# 32.0,# 31.8,# 31.6, 24.9,# 24.3, 24.2,# 24.0,# 23.7,# 19.4,# 19.2,# 19.1, 18.7,# 18.4,# (#minor diastereomer); (numerous signals obscured or overlapping); MS (ESI) *m*/*z* 1003 [$(M+H)^+$, 100%], 1026 (26); HRMS (ESI) calcd for $C_{52}H_{55}N_{14}O_8$ $(M+H)^+$ 1003.4329, found 1003.4322.

*4.1.13. Boc-Orn(Cbz)-Ser-OMe (**8c**).* Under an atmosphere of nitrogen, Boc-Orn(Cbz)-OH **6c** (10.0 g, 27.3 mmol) and the hydrochloride salt of Ser-OMe **7** (4.25 g, 27.3 mmol) were dissolved in anhydrous dichloromethane/DMF (1:4 v/v, 110 mL). The solution was stirred and cooled to 0 °C and then HBTU (12.4 g, 32.8 mmol), HOBt (4.43 g, 32.8 mmol) and Hünig's base (14.3 mL, 81.9 mmol) were added. The resulting mixture was slowly warmed to rt and stirred for 18 h. The mixture was concentrated under reduced pressure to half of the initial volume and then partitioned between water (200 mL) and $CHCl_3$/^{i}PrOH (3:1 v/v, 200 mL). The organic phase was extracted with water (3×200 mL) and the combined aqueous fractions were back-extracted with $CHCl_3$/^{i}PrOH (3:1 v/v, 2×300 mL). The organic fractions were combined and washed with satd aq $(NH_4)_2CO_3$ solution (400 mL), half-strength brine solution (400 mL) and then dried ($MgSO_4$). The solvent was removed under reduced pressure to give a yellow oil, which was purified by flash chromatography (silica gel: $CHCl_3$/MeOH, 95:5 v/v) to afford the desired dipeptide **8c** (11.1 g, 87%) as a colourless solid. IR ν_{max} (NaCl)/cm^{-1} 3319, 2972, 2881, 2359; mp 67–68 °C; $[\alpha]_D^{20}$ −2.09 (*c* 1.1, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.33 (m, 5H), 5.39 (d, *J*=8.1 Hz, 1H), 5.15 (t, *J*=6.6 Hz, 1H), 5.08 (m, 2H), 4.64 (m, 1H), 4.27 (t, *J*=7.3 Hz, 1H), 3.93 (m, 2H), 3.74 (s, 3H), 3.42 (br s, 1H), 3.34 (m, 1H), 3.16 (m, 1H), 1.86 (m, 1H), 1.62 (m, 3H), 1.42 (s, 9H), O–H signal not observed; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 172.7, 171.0, 157.2, 156.1, 136.6, 128.6, 128.2, 80.4, 66.9, 62.8, 54.9, 53.7, 52.7, 40.1, 29.9, 28.4, 26.0, one signal obscured or overlapping; HRMS (ESI) calcd for $C_{22}H_{33}N_3O_8Na$ $(M+Na)^+$ 490.2160, found 490.2157.

*4.1.14. Boc-Orn(Cbz)-Oxz(Ser)-OMe (**16**).* Under an atmosphere of nitrogen, Deoxo-Fluor® (5.90 mL, 32.0 mmol) was added dropwise to a stirred solution of the dipeptide **8c** (12.5 g, 26.7 mmol) in anhydrous dichloromethane (270 mL) at −20 °C (EtOH/ice/CO_2 chips). The reaction mixture was stirred at −20 °C for 1 h, then slowly warmed to rt and stirred for a further 6 h. Analysis by TLC at this time revealed complete conversion of starting material. The solution was quenched with satd aq $(NH_4)_2CO_3$ solution (250 mL) at −20 °C, and then warmed to rt and extracted with chloroform (3×250 mL). The combined organic fractions were washed with half-strength brine solution (400 mL), dried ($MgSO_4$) and concentrated under reduced pressure to afford the intermediate oxazoline as a yellow oil.

Under a nitrogen atmosphere, the crude oxazoline (12.0 g, 26.7 mmol) was dissolved in anhydrous dichloromethane (270 mL) and cooled to 0 °C. The solution was then treated with bromotrichloromethane (5.27 mL, 53.4 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (8.00 mL, 53.4 mmol) and the resulting mixture was stirred at rt for 4 h. The mixture was quenched by the addition of hydrochloric acid (0.30 M, 400 mL) and extracted with $CHCl_3$/^{i}PrOH (3:1 v/v, 3×300 mL). The combined organic fractions were washed with half-strength brine solution (600 mL), dried ($MgSO_4$) and concentrated under reduced pressure to give a brown oil. Purification of the crude material by flash chromatography (silica gel; eluting first with hexane/EtOAc, 1:1 v/v, then hexane/EtOAc, 2:3 v/v) afforded the desired oxazole **16** (8.63 g, 69% over two steps) as a colourless solid. Mp 83–84 °C; $[\alpha]_D^{20}$ −9.40 (*c* 1.0, MeOH); ^{1}H NMR (300 MHz, CD_3OD): δ 8.46 (s, 1H), 7.27–7.34 (m, 5H), 7.02 (t, *J*=6.6 Hz, 1H), 5.05 (s, 2H), 4.79 (m, 1H), 3.87 (s, 3H), 3.15 (m, 2H), 1.75–2.02 (m, 2H), 1.56 (m, 2H), 1.43 (s, 9H), one N–H signal not observed; ^{13}C NMR (75.5 MHz, CD_3OD): δ 167.2, 163.0, 159.0, 157.7,

146.0, 138.4, 133.9, 129.4, 128.9, 128.8, 67.4, 52.5, 50.0, 41.3, 41.2, 31.4, 28.7, 27.2; MS (ESI) *m/z* 470 [(M+Na)$^+$, 100%], 414 (28); HRMS (ESI) calcd for $C_{22}H_{29}N_3O_7Na$ (M+Na)$^+$ 470.1898, found 470.1908.

*4.1.15. Boc-Orn(Cbz)-Oxz(Ser)-OH (**17**).* To a stirred solution of the oxazole **16** (5.88 g, 13.1 mmol) in methanol (167 mL) and water (55 mL) was added NaOH (1.58 g, 39.3 mmol). Stirring was continued at rt for 2 h after which time analysis by TLC revealed complete conversion of the starting material. The reaction mixture was acidified (ca. pH 5) with 2 M HCl and extracted with $CHCl_3$/^{i}PrOH (3:1 v/v, 3×100 mL). The combined organic fractions were dried ($MgSO_4$) and concentrated under reduced pressure to give the desired carboxylic acid **17** (5.70 g, 100%) as a colourless foam. ^{1}H NMR (400 MHz, CD_3OD): δ 8.41 (s, 1H), 7.34–7.27 (m, 4H), 5.06 (s, 2H), 4.80 (br m, 1H), 3.14 (t, *J*=6.7 Hz, 2H), 1.99–1.82 (m, 2H), 1.63–1.48 (m, 4H), 1.43 (s, 9H), N–H and O–H signals not observed; ^{13}C NMR (100.6 MHz, CD_3OD): δ 165.6, 162.6, 157.5, 156.3, 144.4, 137.0, 133.2, 128.0, 127.5, 127.4, 79.4, 66.3, 39.8, 30.0, 27.3, 25.8, 23.9; MS (ESI) *m/z* 456 [(M+Na)$^+$, 100%], 889 [(2M+Na)$^+$, 55%]; HRMS (ESI) calcd for $C_{21}H_{27}N_3O_7Na$ (M+Na)$^+$ 456.1742, found 456.1737.

*4.1.16. Boc-Orn(Cbz)-Oxz(Ser)-Ala-Oxz(Ser)-OMe (**18**).* To a stirred solution of the carboxylic acid **17** (3.53g, 6.04 mmol) and the hydrochloride salt **9** (1.37 g, 6.61 mmol) in anhydrous acetonitrile/DMF (7:1 v/v, 125 mL) was added HOBt (1.11 g, 7.27 mmol), EDC (1.39 g, 7.27 mmol) and *N*-methylmorpholine (3.30 mL, 29.7 mmol). The reaction mixture was stirred at rt for 22 h after which time the solvent was removed under reduced pressure to give an orange coloured oil, which was partitioned between satd aq $NaHCO_3$ solution (70 mL) and $CHCl_3$/^{i}PrOH (3:1 v/v, 70 mL). The organic phase was isolated and the aqueous phase was further extracted with $CHCl_3$/^{i}PrOH (3:1 v/v, 2×50 mL). The combined organic fractions were washed first with water (70 mL) and then brine (70 mL), dried ($MgSO_4$) and concentrated under reduced pressure to give an orange coloured oil, which was purified by flash chromatography on silica gel to afford the desired bisoxazole **18** as a colourless foam (2.18 g, 61%). $[\alpha]_D^{25}$ −25.0 (*c* 0.80, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD): δ 8.47 (s, 1H), 8.33 (s, 1H), 7.33–7.27 (m, 5H), 5.36 (q, *J*=7.2 Hz, 1H), 5.05 (s, 2H), 4.79 (br m, 1H), 3.86 (s, 3H), 3.18–3.14 (m, 2H), 2.01–1.92 (m, 2H), 1.66 (d, *J*=7.2 Hz, 3H), 1.63–1.47 (m, 2H), 1.43 (s, 9H), N–H signals not observed; ^{13}C NMR (100.6 MHz, CD_3OD): δ 165.4, 164.8, 161.5, 161.1, 157.5, 156.3, 144.8, 141.9, 137.0, 135.3, 132.7, 128.0, 127.5, 127.4, 79.4, 66.0, 51.9, 43.0, 40.0, 30.0, 27.3, 25.8, 17.3, one signal obscured or overlapping; MS (ESI) *m/z* 608 [(M+Na)$^+$, 100%], 552 (42), 530 (35), 486 (48); HRMS (ESI) calcd for $C_{28}H_{35}N_5O_9Na$ (M+Na)$^+$ 608.2327, found 608.2331.

*4.1.17. $CF_3CO_2H\cdot H_2N$-Orn(Cbz)-Oxz(Ser)-Ala-Oxz(Ser)-OMe (**19**).* Under an atmosphere of nitrogen, trifluoroacetic acid (10.7 mL, 0.138 mmol) was added to a solution of the bisoxazole **18** (404 mg, 0.691 mmol) in anhydrous dichloromethane (10.8 mL). The reaction mixture was stirred at rt for 1.5 h after which time the solvent was removed under reduced pressure to give a crude oil, which was azeotropically dried with toluene to afford the desired TFA salt **19** (485 mg, 100%) as a pale yellow foam, which was used immediately in the subsequent peptide coupling reaction; MS (ESI) *m/z* 486 (M$^+$, 100%); HRMS (ESI) calcd for $C_{23}H_{28}N_5O_7$ (M$^+$) 486.1984, found 486.1971.

*4.1.18. Boc-Orn(Cbz)-Oxz(Ser)-Ala-Oxz(Ser)-OH (**20**).* To a stirred solution of the bisoxazole **18** (328 mg, 0.561 mmol) in methanol (7.1 mL) and water (2.4 mL) was added NaOH (67 mg, 1.68 mmol). Stirring was continued at rt for 1 h 35 min after which time analysis by TLC revealed complete conversion of the starting material. The solution was acidified (ca. pH 5) using 2 M HCl before extracting with $CHCl_3$/^{i}PrOH (3:1 v/v, 3×25 mL). The combined organic fractions were washed with brine (50 mL), dried ($MgSO_4$) and concentrated under reduced pressure to give the desired carboxylic acid **20** (342 mg, 100%) as a colourless foam. ^{1}H NMR (400 MHz, CD_3OD): δ 8.42 (s, 1H), 8.33 (s, 1H), 7.33–7.27 (m, 5H), 5.40–5.34 (m, 1H), 5.05 (s, 2H), 4.79 (br m, 1H), 3.17–3.14 (m, 2H), 1.99–1.81 (m, 2H), 1.66 (d, *J*=6.9 Hz, 3H), 1.61–1.48 (m, 2H), 1.43 (s, 9H), N–H and O–H signals not observed; ^{13}C NMR (100.6 MHz, CD_3OD): δ 165.2, 164.8, 162.6, 161.0, 157.5, 156.3, 144.6, 141.9, 137.0, 135.4, 133.5, 128.0, 127.5, 127.4, 79.4, 66.0, 43.0, 39.8, 30.0, 27.3, 25.8, 23.9, 17.3; MS (ESI) *m/z* 594 [(M+Na)$^+$, 100%]; HRMS (ESI) calcd for $C_{27}H_{33}N_5O_9Na$ (M+Na)$^+$ 594.2171, found 594.2188.

*4.1.19. Boc-[Orn(Cbz)-Oxz(Ser)-Ala-Oxz(Ser)]$_2$-OMe (**21**).* To a stirred solution of the bisoxazole carboxylic acid **20** (663 mg, 1.16 mmol) and the bisoxazole amine TFA salt **19** (1.16 mmol) in anhydrous acetonitrile (30 mL) was added HOBt (196 mg, 1.28 mmol), EDC (245 mg, 1.28 mmol) and *N*-methylmorpholine (383 μL, 3.48 mmol). The reaction mixture was stirred at rt for 23.5 h after which time the solvent was removed under reduced pressure to give an orange coloured oil, which was partitioned between satd aq $NaHCO_3$ solution (25 mL) and $CHCl_3$/^{i}PrOH (3:1 v/v, 25 mL). The organic phase was isolated and the aqueous phase was further extracted with $CHCl_3$/^{i}PrOH (3:1 v/v, 2×25 mL). The combined organic fractions were washed first with water (25 mL) and then brine (30 mL), dried ($MgSO_4$) and concentrated under reduced pressure to give an orange coloured oil, which was purified by flash chromatography on silica gel to afford the desired tetraoxazole **21** (290 mg, 24%) as a colourless foam. $[\alpha]_D^{25}$ −37.8 (*c* 0.91, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD): δ 8.46 (s, 1H), 8.35 (s, 1H), 8.32 (s, 2H), 7.31–7.26 (m, 10H), 5.40–5.30 (m, 3H), 5.04 (s, 4H), 4.79 (br m, 1H), 3.85 (s, 3H), 3.19–3.14 (m, 4H), 2.13–1.46 (m, 14H), 1.42 (s, 9H), N–H signals not observed; ^{13}C NMR (100.6 MHz, CD_3OD): δ 163.8, 163.2, 163.0, 162.2, 159.9, 159.7, 159.4(2), 158.9(5), 155.9, 154.7, 143.2, 140.7, 140.6, 140.5, 135.4, 133.9, 133.8, 131.1, 126.5, 126.0, 125.8, 77.8, 64.4, 49.5, 47.1, 41.4, 38.2, 28.4, 27.9, 25.7, 24.3, 15.7(2), 15.6(9), 11 signals obscured or overlapping; MS (ESI) *m/z* 1061 [(M+Na)$^+$, 100%], 939 (80); HRMS (ESI) calcd for $C_{50}H_{58}N_{10}O_{15}Na$ (M+Na)$^+$ 1061.3976, found 1061.3973.

*4.1.20. Boc-[Orn(Cbz)-Oxz(Ser)-Ala-Oxz(Ser)]$_2$-OH (**31**).* To a stirred solution of the tetraoxazole **21** (287 mg, 0.276 mmol) in methanol (3.5 mL) and water (1.2 mL) was added NaOH (33 mg, 0.828 mmol). Stirring was continued at rt for 4 h after which time analysis by TLC revealed complete conversion of the starting material. The solution was acidified (ca. pH 5) with 2 M HCl before extracting with $CHCl_3$/^{i}PrOH (3:1 v/v, 3×25 mL). The combined organic fractions were washed with brine solution, dried ($MgSO_4$) and concentrated under reduced pressure to give the desired carboxylic acid **31** (268 mg, 95%) as a colourless foam. ^{1}H NMR (400 MHz, CD_3OD): δ 8.41 (s, 1H), 8.34 (s, 1H), 8.31 (s, 2H), 7.31–7.24 (m, 10H), 5.39–5.28 (m, 3H), 5.03 (s, 4H), 4.78 (br m, 1H), 3.18–3.11 (m, 4H), 2.10–1.48 (m, 8H), 1.63 (d, *J*=9.2 Hz, 6H), 1.41 (s, 9H), N–H and O–H signals not observed; ^{13}C NMR (100.6 MHz, CD_3OD): δ 166.6, 166.2, 165.9, 165.1, 163.9, 162.6, 162.4, 162.3, 158.9, 157.7, 146.0, 143.7, 143.5, 143.4, 138.4, 136.9, 136.8, 136.7, 134.8, 80.8, 67.4, 44.3, 41.2, 31.4, 30.8, 28.7, 27.2, 18.7(4), 18.6(8), 14 signals obscured or overlapping; MS (ESI) *m/z* 1047 [(M+Na)$^+$, 100%], 925 (47), 663 (31); HRMS (ESI) calcd for $C_{49}H_{56}N_{10}O_{15}Na$ (M+Na)$^+$ 1047.3819, found 1047.3803.

*4.1.21. $CF_3CO_2H\cdot H_2N$-[Orn(Cbz)-Oxz(Ser)-Ala-Oxz(Ser)]$_2$-OH (**32**).* Under an atmosphere of nitrogen, a stirred solution of the carboxylic acid **31** (269 mg, 0.262 mmol) in anhydrous dichloromethane (4.0 mL) was treated with trifluoroacetic acid (4.0 mL). Stirring was continued at rt for 2 h 15 min after which time analysis by TLC revealed complete conversion of the starting material. The

reaction mixture was concentrated under reduced pressure to give the desired TFA salt **32** (251 mg, 100%) as a colourless foam. ^{1}H NMR (300 MHz, CD_3OD): δ 8.51 (s, 1H), 8.43 (s, 1H), 8.36 (s, 1H), 8.34 (s, 1H), 7.32–7.28 (m, 10H), 5.41–5.30 (m, 3H), 5.04 (s, 4H), 4.72–4.68 (m, 1H), 3.17 (br m, 4H), 2.16–1.97 (m, 4H), 1.68–1.49 (m, 10H), N–H and O–H signals not observed; ^{13}C NMR (75.5 MHz, CD_3OD): δ 167.1, 166.3, 165.6, 164.4, 163.0, 162.7, 162.1, 161.7, 159.3, 146.5, 145.1, 144.1, 143.9, 137.7, 137.3, 135.2, 129.9, 129.44, 129.37, 129.2, 67.9, 44.9, 41.5, 41.2, 31.3, 30.6, 27.7, 27.0, 19.1, 11 signals obscured or overlapping; MS (ESI) *m/z* 925 (M^+, 100%); HRMS (ESI) calcd for $C_{44}H_{49}N_{10}O_{13}$ $(M+H)^+$ 925.3476, found 925.3458.

*4.1.22. Cyclo[Orn(Cbz)-Oxz(Ser)-Ala-Oxz(Ser)]$_2$ (**22**).* To a stirred solution of the TFA salt **32** (243.9 mg, 0.235 mmol) in anhydrous DMF (47 mL) under an atmosphere of nitrogen was added pentafluorophenyl diphenylphosphinate (124 mg, 0.353 mmol) and Hünig's base (123 μL, 0.705 mmol). The reaction mixture was stirred at rt for 23 h after which time the solvent was removed under reduced pressure to give a crude orange coloured oil, which was partitioned between water (20 mL) and $CHCl_3$/^{i}PrOH (3:1 v/v, 20 mL). The organic phase was isolated and the aqueous phase was further extracted with $CHCl_3$/^{i}PrOH (20 mL, 3:1 v/v). The combined organic fractions were washed sequentially with 2 M aq NaOH solution (30 mL), water (30 mL) and brine (30 mL), dried ($MgSO_4$) and concentrated under reduced pressure to give an orange oil. Purification by flash chromatography on silica gel afforded the desired cyclic peptide **22** (123 mg, 58%) as a colourless foam. Mp 116–118 °C; $[\alpha]_D^{25}$ −108 (*c* 0.51, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD): δ 8.37 (s, 2H), 8.36 (s, 2H), 7.24–7.33 (m, 10H), 5.48 (m, 2H), 5.41 (m, 2H), 5.10 (s, 4H), 3.20 (dt, *J*=2.9, 6.9 Hz, 4H), 2.13 (m, 2H), 2.03 (m, 2H), 1.66 (d, *J*=6.9 Hz, 6H), 1.60 (m, 4H), N–H signals not observed; ^{13}C NMR (100.6 MHz, CD_3OD): δ 166.2, 165.5, 162.2, 161.8, 159.0, 144.0, 143.9, 138.4, 136.9, 136.7, 129.4, 128.9, 128.8, 67.4, 47.5, 43.6, 41.1, 31.4, 27.3, 19.1; MS (ESI) *m/z* 929 [$(M+Na)^+$, 100%], 824 (58); HRMS (ESI) calcd for $C_{44}H_{46}N_{10}O_{12}Na$ $(M+Na)^+$ 929.3189, found 929.3184.

*4.1.23. Cyclo[Orn(Dpa)-Oxz(Ser)-Ala-Oxz(Ser)]$_2$ (**1**).* Under an atmosphere of nitrogen, a solution of hydrogen bromide in acetic acid (33% v/v, 4.0 mL) was added to the cyclic tetraoxazole **22** (100 mg, 0.110 mmol) and the resulting mixture was stirred at rt for 30 min. The orange solution that resulted was combined with anhydrous ether (30 mL) to give a cream coloured precipitate. The precipitate was centrifuged and the ethereal layer was removed. Subsequent trituration of the precipitate with anhydrous ether (8×30 mL) and removal of the final clear ethereal layer under reduced pressure, gave the dihydrobromide salt as a cream solid. The free amine was liberated from the dihydrobromide salt by treatment of the methanolic solution with basic resin (Amberlite lRA-400). Removal of the resin by filtration, followed by concentration of the filtrate, gave the crude amine **23**.

A suspension of **23** in dichloromethane (10 mL) was treated with 2-pyridinecarboxyaldehyde (150 μL, 1.57 mmol) and sodium triacetoxyborohydride (510 mg, 2.4 mmol) under an atmosphere of nitrogen for 4 h. More 2-pyridinecarboxyaldehyde (75 μL, 0.78 mmol) and sodium triacetoxyborohydride (255 mg, 1.2 mmol) were then added and the mixture was stirred overnight. The mixture was filtered through a plug of silica (EtOAc) and the solvent was removed under reduced pressure. The residue thus obtained was purified by flash chromatography (silica gel; eluting first with EtOAc/toluene/Et_3N, 50:50:1 v/v/v, then MeOH/EtOAc/Et_3N, 10:90:1 v/v/v) to afford the Dpa-functionalized cyclic peptide **1** (66 mg, 54%) as a pale yellow oil. $[\alpha]_D^{25}$ −21.7 (*c* 0.65, MeOH); ^{1}H NMR (300 MHz, CD_3OD): δ 8.45 (br d, *J*=4.4 Hz, 4H), 8.42 (s, 2H), 8.41 (s, 2H), 7.74 (m, 4H), 7.62 (br d, *J*=7.8 Hz), 7.25 (m, 4H), 5.48 (ABquartet, 4H), 5.28 (dd, *J*=6.4, 8.7 Hz, 4H), 3.74 (s, 8H), 2.60 (ABquartet, 4H), 2.06 (m, 4H), 1.92 (m, 4H), 1.67 (d, *J*=5.4 Hz, 6H), 1.60 (m, 4H); ^{13}C NMR (75.5 MHz, CD_3OD): δ 165.1, 164.7, 161.0, 160.7, 159.6, 148.4, 143.0, 142.8, 137.6, 135.8, 123.9, 122.8, 60.3, 53.9, 46.1, 42.5, 30.8, 23.4, 18.1; MS (ESI) *m/z* 1026 [$(M+Na)^+$, 100%]; HRMS (ESI) calcd for $C_{52}H_{54}N_{14}O_8Na$ $(M+Na)^+$ 1025.4147, found 1025.4142.

*4.1.24. Boc-Orn(Cbz)-Oxz(Ser)-Orn(Cbz)-Oxz(Ser)-Ala-Oxz(Ser)-OMe (**24**).* To a stirred solution of compound **10** (503 mg, 1.16 mmol) and compound **19** (696 mg, 1.16 mmol) in acetonitrile (30 mL) was added HOBt (196 mg, 1.28 mmol), EDC (245 mg, 1.28 mmol) and *N*-methylmorpholine (383 μL, 3.48 mmol). The reaction mixture was stirred at rt for 23.5 h after which time the solvent was removed under reduced pressure to give an orange coloured oil, which was partitioned between satd aq $NaHCO_3$ solution (25 mL) and $CHCl_3$/^{i}PrOH (3:1 v/v, 25 mL). The organic phase was isolated and the aqueous phase was further extracted with $CHCl_3$/^{i}PrOH (3:1 v/v, 2×25 mL). The combined organic fractions were washed with water (25 mL) and saturated brine (30 mL), then dried ($MgSO_4$) and concentrated under reduced pressure to give an orange coloured oil, which was purified by flash chromatography on silica gel to afford the desired trisoxazole **24** (234 mg, 19%) as a colourless foam. $[\alpha]_D^{25}$ −23.0 (*c* 1.2, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD): δ 8.45 (s, 1H), 8.33 (s, 2H), 7.31–7.25 (m, 10H), 5.38–5.30 (m, 2H), 5.03 (s, 4H), 4.78 (br s, 1H), 3.84 (s, 3H), 3.21–3.15 (m, 4H), 2.15–1.83 (m, 4H), 1.64 (d, *J*=7.0 Hz, 3H), 1.65–1.55 (m, 8H), 1.42 (s, 9H); ^{13}C NMR (100.6 MHz, CD_3OD): δ 165.4, 164.8, 163.8, 161.5, 161.3, 161.0, 157.5, 156.3, 144.8, 142.2, 137.0, 135.5, 135.3, 132.7, 79.4, 66.0, 51.1, 43.0, 40.0, 30.0, 29.5, 27.3, 25.8, 13.1, 14 signals obscured or overlapping; MS (ESI) *m/z* 923 (M+Na, 100%), 801 (79); HRMS (ESI) calcd for $C_{44}H_{52}N_8O_{13}Na$ $(M+Na)^+$ 923.3547, found 923.3531.

*4.1.25. $CF_3CO_2H\cdot H_2N$-Orn(Cbz)-Oxz(Ser)-Orn(Cbz)-Oxz(Ser)-Ala-Oxz(Ser)-OH (**33**).* To a stirred solution of the trisoxazole **24** (210.8 mg, 0.234 mmol) in methanol (3.0 mL) and water (1.0 mL) was added NaOH (28 mg, 0.702 mmol). Stirring was continued at rt for 4 h after which time analysis by thin layer chromatography revealed complete conversion of the starting material. The solution was acidified (ca. pH 5) with 2 M hydrochloric acid before extracting with $CHCl_3$/^{i}PrOH (3:1 v/v, 3×25 mL). The combined organic fractions were washed with saturated brine (25 mL), then dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give the carboxylic acid (194 mg, 93%) as a colourless foam. The acid was immediately dissolved in anhydrous dichloromethane (3.3 mL) under an atmosphere of nitrogen and treated with trifluoroacetic acid (3.3 mL). Stirring was continued at rt for 2 h 10 min after which time analysis by thin layer chromatography revealed complete conversion of the starting material. The reaction mixture was concentrated under reduced pressure to give the desired TFA salt **33** (208 mg, quant.) as a colourless foam, which was used immediately in the next step. ^{1}H NMR (300 MHz, CD_3OD): δ 8.52 (s, 1H), 8.43 (s, 1H), 8.36 (s, 1H), 7.31–7.28 (m, 10H), 5.40–5.33 (m, 2H), 5.05 (s, 4H), 4.72–4.67 (br m, 1H), 3.19–3.15 (m, 4H), 2.23–2.00 (m, 4H), 1.65 (d, *J*=7.0 Hz, 3H), 1.66–1.57 (m, 4H), N–H and O–H signals not observed; MS (ESI) *m/z* 787 (M^+, 100%); HRMS (ESI) calcd for $C_{38}H_{43}N_8O_{11}$ $(M+H)^+$ 787.3046, found 787.3058.

*4.1.26. Cyclo[Orn(Cbz)-Oxz(Ser)-Orn(Cbz)-Oxz(Ser)-Ala-Oxz(Ser)] (**26**).* To a stirred solution of the TFA salt **33** (208 mg, 0.231 mmol) in anhydrous DMF (46 mL) under an atmosphere of nitrogen was added pentafluorophenyl diphenylphosphinate (122 mg, 0.347 mmol) and Hünig's base (121 μL, 0.693 mmol). The reaction mixture was stirred at rt for 65 h after which time the solvent was removed under reduced pressure to give a crude orange coloured oil, which was partitioned between water and $CHCl_3$/^{i}PrOH (3:1 v/v, 20 mL). The organic phase was isolated and the aqueous phase was further extracted with $CHCl_3$/^{i}PrOH (3:1 v/v, 3×20 mL). The combined

organic extracts were washed sequentially with 2 M aqueous NaOH solution (30 mL), water (30 mL) and brine (30 mL), then dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give a dark orange oil. Purification by flash chromatography on silica gel afforded the desired cyclic peptide **26** (113 mg, 64%) as a colourless foam. $[\alpha]_D^{25}$ −41.2 (*c* 0.5, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.50 (d, *J*=8.3 Hz, 1H), 8.39 (dd, *J*=12.2, 9.2 Hz, 2H), 8.19 (s, 2H), 8.17 (s, 1H), 7.30–7.25 (m, 10H), 5.26–5.13 (m, 5H), 5.04 (s, 4H), 3.23–3.21 (br m, 4H), 2.17–2.09 (m, 2H), 2.00–1.91 (m, 2H), 1.68 (d, *J*=9.0 Hz, 5H), 1.54–1.50 (m, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 164.8, 163.9, 163.7, 159.7, 159.6, 159.3, 156.7, 141.9, 136.8, 135.5(3), 135.4(7), 128.7, 128.3, 68.8, 48.2(5), 48.1(5), 44.7, 40.7, 32.3, 25.6, 20.9, 13 signals obscured or overlapping; MS (ESI) *m*/*z* 792 [(M+Na)$^+$, 100%]; HRMS (ESI) calcd for $C_{38}H_{40}N_8O_{10}Na$ (M+Na)$^+$ 791.2760, found 791.2749.

*4.1.27. Cyclo[Orn(Dpa)-Oxz(Ser)-Orn(Dpa)-Oxz(Ser)-Ala-Oxz(Ser)] (**2**).* The cyclic peptide **26** (50 mg, 0.063 mmol) was treated with hydrogen bromide in acetic acid (33% v/v, 3 mL) for 30 min. The solution was then slowly added to a stirred solution of anhydrous ether (20 mL), resulting in the formation of a colourless solid, presumably the hydrobromide salt. This solid was collected by filtration and then subsequently dissolved in MeOH (20 mL). The free amine was liberated from the hydrobromide salt by treatment of the methanolic solution with base resin (Amberlite lRA-400). Removal of the resin by filtration, followed by concentration of the filtrate, gave the desired crude amine **34**.

A suspension of the amine **34** in dichloromethane (5 mL) was treated with 2-pyridinecarboxyaldehyde (50 μL, 0.520 mmol) and sodium triacetoxyborohydride (102 mg, 0.624 mmol) for 4 h. More 2-pyridinecarboxyaldehyde (20 μL, 0.780 mmol) and sodium triacetoxyborohydride (102 mg, 0.624 mmol) were then added and the mixture was left to stir overnight. The mixture was filtered through a plug of silica (EtOAc) and the filtrate concentrated under reduced pressure. The residue was then purified by flash chromatography (silica gel; eluting first with EtOAc/toluene/Et_3N, 50:50:1 v/v/v, then EtOAc/toluene/Et_3N, 10:90:1 v/v/v) to give the Dpa-functionalized cyclic peptide **2** (34 mg, 40%) as a pale yellow oil. $[\alpha]_D^{25}$ −18.8 (*c* 0.68, MeOH); ^{1}H NMR (300 MHz, CD_3OD): δ 8.47 (s, 1H), 8.46 (s, 1H), 8.44 (s, 1H), 8.41 (br d, *J*=4.4 Hz, 4H), 7.75 (m, 4H), 7.56 (br d, *J*=7.8 Hz, 4H), 7.22 (m, 4H), 5.12–5.23 (m, 3H), 4.83 (br s, 8H), 2.52–2.56 (br m, 4H), 1.82–2.23 (m, 5H), 1.34–1.72 (m, 5H), 1.28–1.49 (d, *J*=9.0 Hz, 4H); ^{13}C NMR (75.5 MHz, CD_3OD): δ 164.8, 164.1, 164.0, 160.3, 160.2, 159.7, 148.4, 148.3, 142.7, 137.6, 135.2, 123.9, 122.7, 60.3, 54.2, 44.8, 31.8, 22.3, 19.6, 15 signals obscured or overlapping; MS (ESI) *m*/*z* 887 [(M+Na)$^+$, 100%]; HRMS (ESI) calcd for $C_{46}H_{49}N_{12}O_6$ (M+H)$^+$ 865.3898, found 865.3893.

*4.1.28. Boc-Orn(Cbz)-Oxz(Ser)-Ala-Oxz(Ser)-Ala-Oxz(Ser)-OMe (**25**).* To a stirred solution of the compound **20** (1.80 g, 3.15 mmol) and the compound **9** (1.7 g, 8.22 mmol) in acetonitrile (100 mL) was added HOBt (1.39 g, 9.10 mmol), EDC (1.69 g, 8.80 mmol) and *N*-methylmorpholine (4.12 mL, 37.6 mmol). The reaction mixture was stirred overnight after which time the solvent was removed under reduced pressure to give an orange coloured oil, which was partitioned between satd aq $NaHCO_3$ solution (50 mL) and ethyl acetate (50 mL). The organic phase was isolated and the aqueous phase was further extracted with ethyl acetate (3×50 mL). The combined organic fractions were washed with water (50 mL) and saturated brine (50 mL), then dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give an orange coloured oil, which was purified by flash chromatography (silica gel; eluting first with EtOAc/hexane, 1:1 v/v, then 6:4 v/v) to afford the desired trisoxazole **25** (1.80 g, 79%) as a colourless foam. $[\alpha]_D^{25}$ −31.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.23 (m, 2H), 8.10 (s, 1H), 8.07 (s, 1H), 8.04 (s, 1H), 7.29–7.25 (m, 5H), 5.58 (m, 1H), 5.42 (m, 2H), 5.18 (m, 1H), 5.04 (s, 2H), 4.83 (m, 1H), 3.83 (s, 3H), 3.17 (m, 2H), 2.23 (m, 2H), 1.43–1.72 (m, 8H), 1.40 (br s, 9H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 165.7, 164.9, 164.6, 161.7, 160.5, 156.9, 155.6, 144.4, 142.2, 142.0, 136.9, 135.8, 135.7, 128.9, 128.4, 80.7, 67.0, 52.5, 48.6, 43.3, 43.2, 40.8, 31.0, 28.7, 26.6, 19.8, 19.2, three signals obscured or overlapping; MS (ESI) *m*/*z* 741 [(M+NH_4)$^+$, 100%]; HRMS (ESI) calcd for $C_{34}H_{41}N_7O_{11}Na$ (M+Na)$^+$ 746.2764, found 746.2756.

*4.1.29. $CF_3CO_2H\cdot H_2N$-Orn(Cbz)-Oxz(Ser)-Ala-Oxz(Ser)-Ala-Oxz(Ser)-OH (**35**).* To a stirred solution of the trisoxazole **25** (458 mg, 0.632 mmol) in methanol (3.0 mL) and water (1.0 mL) was added NaOH (76 mg, 1.90 mmol). Stirring was continued at rt for 6 h after which time analysis by thin layer chromatography revealed complete conversion of the starting material. The solution was acidified (ca. pH 5) with 2 M HCl before extracting with dichloromethane (3×25 mL). The combined organic fractions were washed with saturated brine (25 mL), dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give the desired carboxylic acid (445 mg, quant.) as a colourless foam. This material was immediately treated with trifluoroacetic acid (2 mL) in dichloromethane (10 mL) overnight. The solution was concentrated under reduced pressure to give the desired TFA salt **35** (340 mg) as an orange oil, which was used without further purification. ^{1}H NMR (300 MHz, CD_3OD): δ 8.49 (s, 1H), 8.42 (s, 1H), 8.34 (s, 1H), 7.29–7.25 (m, 5H), 5.36 (m, 2H), 5.03 (br s, 2H), 4.71 (m, 1H), 3.16 (m, 2H), 2.09 (m, 2H) 1.53–1.65 (m, 8H), N–H and O–H signals not observed; MS (ESI) *m*/*z* 610.1 [(M+H)$^+$, 100%]; HRMS (ESI) calcd for $C_{28}H_{32}N_7O_9Na$ (M+Na)$^+$ 610.2262, found 610.2256.

*4.1.30. Cyclo[Orn(Cbz)-Oxz(Ser)-Ala-Oxz(Ser)-Ala-Oxz(Ser)] (**27**).* The TFA salt **35** (173 mg, 0.284 mmol) was dissolved in methanol and treated with weak base resin. Removal of the resin by filtration, followed by concentration of the filtrate (with co-evaporation with toluene), gave the crude amine. The amine was dissolved in a solution of DMF and dichloromethane (1:2 v/v, 180 mL), to which was added collidine (250 μL, 1.87 mmol) and HATU (237 mg, 0.620 mmol). After stirring for 24 h the solution was concentrated under reduced pressure. The residue was partitioned between ethyl acetate (25 mL) and 1 M hydrochloric acid (25 mL). The organic phase was isolated and washed sequentially with satd aq $NaHCO_3$ solution (20 mL), water (20 mL) and brine (20 mL), then dried ($MgSO_4$) and concentrated under reduced pressure. Purification by flash chromatography (silica gel; EtOAc/hexane 1:1 v/v, then 8:2 v/v) gave the desired cyclic peptide **27** (128 mg, 76%) as a colourless oil. $[\alpha]_D^{25}$ −28.6 (*c* 1.09, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.60–8.53, (m, 2H), 8.21 (br d, *J*=15 Hz, 1H), 8.23 (s, 1H), 8.21 (s, 2H), 7.32–7.29 (m, 5H), 5.19 (m, 3H), 5.05 (s, 2H), 4.83 (m, 1H), 3.24 (m, 2H), 2.23 (m, 2H), 1.96 (m, 1H), 1.70 (d, *J*=6.6 Hz, 6H), 1.51 (m, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 164.9, 164.8, 163.8, 159.9, 159.6, 156.8, 141.9, 135.6, 128.8, 128.4, 67.0, 48.4, 45.0, 32.2, 21.0, 20.9, 10 signals obscured or overlapping; MS (ESI) *m*/*z* 592.3 [(M+H)$^+$, 100%]; HRMS (ESI) calcd for $C_{28}H_{30}N_7O_8$ (M+H)$^+$ 592.2156, found 592.2151.

*4.1.31. Cyclo[Orn(Dpa)-Oxz(Ser)-Ala-Oxz(Ser)-Ala-Oxz(Ser)] (**3**).* The cyclic peptide **27** (78 mg, 0.13 mmol) was dissolved in ethanol (10 mL) and treated with hydrogen in the presence of palladium on charcoal (20 mg, 10%) overnight. The mixture was subsequently filtered through Celite and concentrated under reduced pressure to afford the crude amine **29**. A suspension of the amine in dichloromethane (10 mL) was treated with 2-pyridinecarboxaldehyde (50 μL, 0.520 mmol) and sodium triacetoxyborohydride (180 mg, 0.850 mmol) for 4 h. More 2-pyridinecarboxyaldehyde (50 μL, 0.520 mmol) and sodium triacetoxyborohydride (180 mg, 0.850 mmol) were then added and the mixture was left to stir overnight. The mixture was filtered through a plug of silica (EtOAc) and the filtrate concentrated under reduced pressure. The residue

was then purified by flash chromatography (silica gel; MeOH/$CHCl_3$, 5:95 v/v) to give the Dpa-functionalized cyclic peptide **3** (32 mg, 38%) as a pale yellow oil. $[\alpha]_D^{25}$ −15.0 (*c* 0.8, MeOH); ^{1}H NMR (300 MHz, CD_3OD): δ 8.47 (s, 1H), 8.45 (s, 1H), 8.43 (s, 1H), 8.39 (br d, *J*=4.1 Hz, 2H), 7.75 (m, 2H), 7.58 (br d, *J*=7.9 Hz, 2H), 7.25 (m, 2H), 5.19 (m, 3H), 3.74 (s, 4H), 2.55 (m, 2H), 2.23–1.87 (m, 10H), N–H signals not observed; ^{13}C NMR (75.5 MHz, CD_3OD): δ 164.9, 164.8, 164.0, 160.2, 159.7, 148.3, 142.7, 137.8, 137.6, 135.3, 123.9, 122.7, 64.5, 60.5, 54.2, 44.9, 31.6, 22.1, 19.5, 19.4, six signals obscured or overlapping; MS (ESI) *m*/*z* 662.3 [$(M+Na)^+$, 100%]; HRMS (ESI) calcd for $C_{32}H_{34}N_9O_6$ $(M+H)^+$ 640.2632, found 640.2627.

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Supplementary data

Supplementary data associated with this article can be found in online version at doi:10.1016/j.tet.2010.11.10. These data include MOL files and InChIKeys of the most important compounds described in this article.

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