Organic & Biomolecular Chemistry

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Cite this: *Org. Biomol. Chem.*, 2011, **9**, 3471

www.rsc.org/obc

PAPER

Synthesis of a family of cyclic peptide-based anion receptors†

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Received 24th November 2010, Accepted 16th February 2011
DOI: 10.1039/c0ob01072c

We report here the design and synthesis of a family of novel backbone modified cyclic peptides, bearing dipicolylamine side chains for metal complexation and subsequent anion binding studies. Two approaches to the cyclic peptides were investigated. Initially, a stepwise approach was employed, involving solid-phase assembly of oxazole-based building blocks, followed by solution-phase macrolactamisation of the resulting linear precursor. The alternative strategy involved the formation of linear bisoxazole fragments in solution-phase, followed by a cyclodimerisation reaction. The zinc(II) complexes of these receptors bind selectively to di- and tri-phosphate ions over hydrogenphosphate.

Introduction

In recent years, the *Lissoclinum* family of natural heterocycle-containing cyclic peptides has been identified as suitable scaffolds for molecular recognition.[1–6] The cyclic hexa- and octapeptides are biologically modified to contain aromatic oxazole and thiazole heterocycles derived from serine, threonine and cysteine moieties, which alternate with standard amino acid residues. The heterocycles are linked by *trans*-amide bonds to form a rigid macrocyclic structure which is almost planar, a virtue which results from a network of bifurcated hydrogen bonds between the heterocyclic nitrogen atoms and the amide hydrogen atoms which line the interior of the macrocycle. Furthermore, if all amino acid side chains are of the same stereochemical configuration, they are presented from the same face of the cyclic peptide. The side chains can be readily elaborated to a range of functional groups. These attractive features have recently been utilised in the development of molecular receptors, chiral ligands and combinatorial libraries.[4,6–11]

We are particularly interested in using these scaffolds for anion recognition. Anions such as pyrophosphate ($P_2O_7^{4-}$) and adenosine triphosphate (ATP) are of importance in numerous biological processes (*e.g.* as enzyme substrates or the products of enzymatic reactions). Therefore, sensors capable of the selective detection of these anions under physiological conditions have the potential for real-time monitoring of such reactions. We recently reported the first example of an anion receptor based on a *Lissoclinum*-type cyclic octapeptide scaffold, bearing amino acid side chains functionalised with two Zn(II)-dipicolylamine ligands (**1·Zn$_2$**, Fig. 1).[9] We showed that receptor **1·Zn$_2$** binds to pyrophosphate ($P_2O_7^{4-}$) with high affinity (log K_a = 8.0 ± 0.1) and selectivity of two orders of magnitude over ATP and ADP

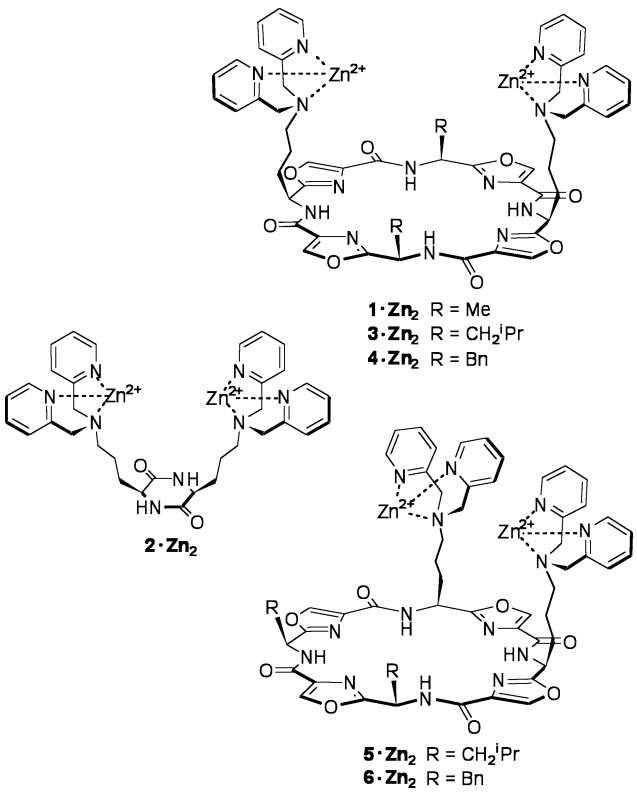

Fig. 1 Structures of cyclic peptide-based anion receptors **1·Zn$_2$**–**6·Zn$_2$**.

under mimicked physiological conditions (aqueous solution, pH 7.4, high salt concentrations), as demonstrated by a fluorescent indicator displacement assay. Such high binding affinity for pyrophosphate can be ascribed to the large size of the receptor cavity and the large distance between the binding sites, which complement the size and shape of the large pyrophosphate anion. This was confirmed by the synthesis of a comparatively smaller receptor **2·Zn$_2$**,[12] based on a diketopiperazine scaffold, which displayed lower affinity (log K_a = 6.0 ± 0.2) and selectivity between

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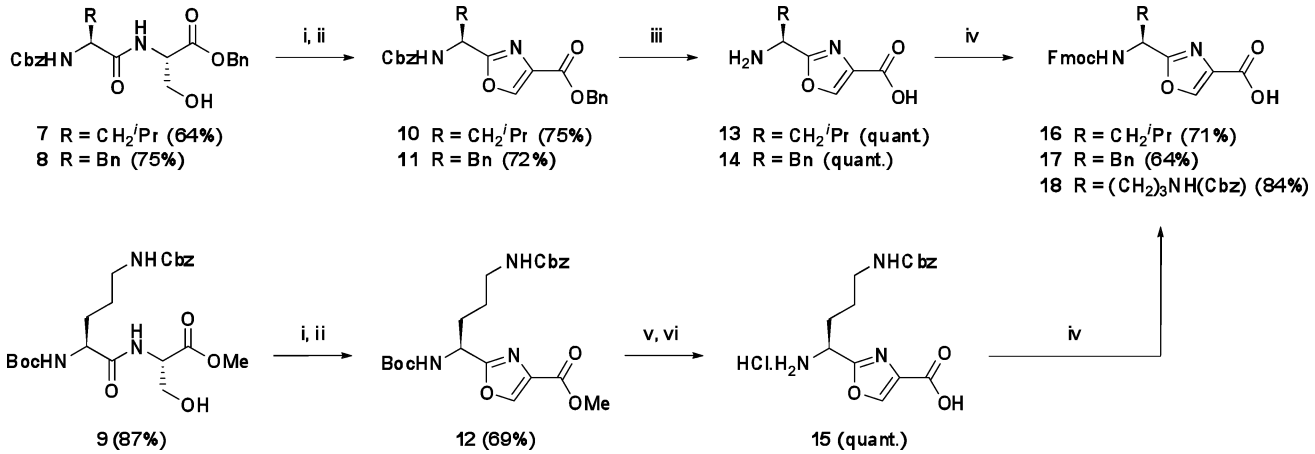
† Electronic supplementary information (ESI) available: ^{1}H and ^{13}C NMR spectroscopic data for new compounds. See DOI: 10.1039/c0ob01072c



Scheme 1 Synthesis of oxazole-based amino acids **16–18**. Conditions: (*i*) Deoxo-Fluor, CH_2Cl_2, –20 °C then $NaHCO_3$, H_2O; (*ii*) DBU, $BrCCl_3$, CH_2Cl_2, 0 °C; (*iii*) H_2 (1 atm.), Pd/C, MeOH, THF; (*iv*) Fmoc-OSu, $NaHCO_3$, H_2O, 1,4-dioxane; (*v*) NaOH, MeOH–H_2O (7 : 3 v/v); (*vi*) HCl, 1,4-dioxane.

di- and triphosphate oxoanions compared to the larger receptor **1**·**Zn**$_2$. Given that these receptors differ only in the size of the cyclic peptide scaffold, it is clear that a larger cyclic peptide is required to effectively accommodate pyrophosphate. Even so, a higher degree of selectivity is required if receptor **1**·**Zn**$_2$ is to be used as a bioanalytical sensing tool. For example, an effective sensor for the real-time monitoring of the enzymatic action of DNA polymerase must detect low concentrations of pyrophosphate byproduct, in the presence of a large excess of the structurally similar enzyme substrate ATP.[13,14]

To this end, we have now synthesised a second generation of cyclic peptide-based anion receptors **3**·**Zn**$_2$–**6**·**Zn**$_2$ (Fig. 1). The structures of these new compounds vary in the spacing between the two Zn(II)-Dpa binding sites and the steric bulk and functionality of the 'non-binding' side chains. It was anticipated that these structural modifications would have significant effects on the binding affinity and selectivity for di- and triphosphate oxoanions, allowing us to determine the optimal structure of a pyrophosphate selective receptor.

We required sufficient quantities of the cyclic-peptide based receptors to allow a comprehensive investigation of their anion binding capabilities. Therefore, several synthetic approaches to these compounds were explored. The first route involves the macrolactamisation of a linear precursor, which is prepared in a traditional stepwise manner by coupling together oxazole-based building blocks. The second approach expands on the success of cyclooligomerisation procedures,[10,15–17] and involves the cyclodimerisation of a linear peptide, which is prepared by coupling together two oxazole-based amino acids. We report here the synthesis of receptor family **1** and **3–6** *via* these two approaches.

Results and discussion

Macrolactamisation approach

The unsymmetrical cyclic peptide scaffolds of receptors **5** and **6** were synthesised by the macrolactamisation route, as this enabled the side chain functional groups to be selectively addressed, thereby defining the sequence and position of the binding sites relative to the 'non-binding' amino-acid side chains. This stepwise approach was also utilised for the synthesis of the C_2-symmetric receptor **3**; however, this resulted in an unacceptable overall yield. Therefore, we investigated alternative routes for the preparation of C_2-symmetric cyclic peptides **1**, **3** and **4**, which involved fewer synthetic steps (see below).

We chose to prepare receptors **5** and **6** using solid-phase peptide synthesis (SPPS), due to its operational simplicity. Kelly and coworkers have previously utilised the solid-phase strategy in their total syntheses of several naturally occurring azole-modified cyclic peptides.[18,19] We proposed that similar methodology would be applicable in the solid-phase assembly of the linear precursors to receptors **5** and **6**.

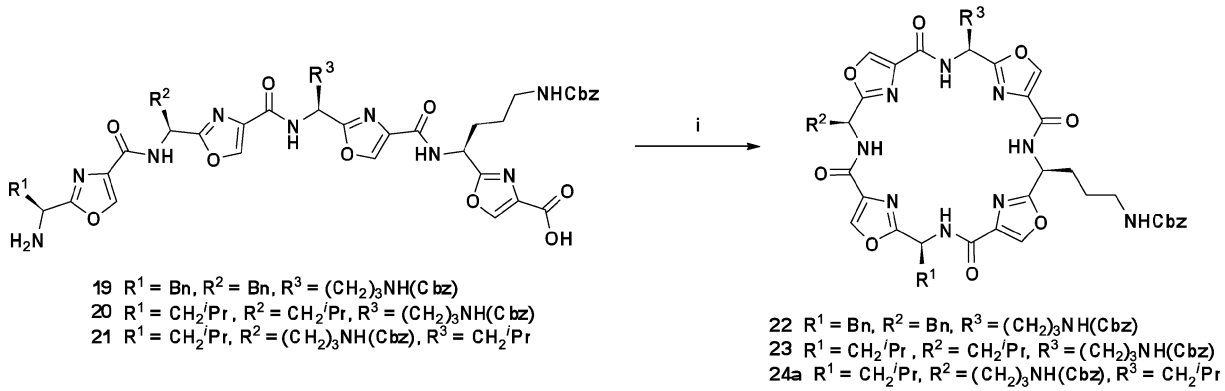
We first prepared dipeptides **7** and **8** (Scheme 1) in good yields by coupling Cbz-leucine or Cbz-phenylalanine with serine benzyl ester utilising 1-hydroxybenzotriazole (HOBt) and *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) as the coupling reagents. Likewise, dipeptide **9** was synthesised by coupling the orthogonally protected Boc-Orn(Cbz)-OH with serine methyl ester under standard peptide coupling conditions. The dipeptides were then converted to the corresponding oxazoles **10–12** in high yields using a well-established two-step procedure,[20] involving treatment with Deoxo-Fluor reagent followed by oxidation of the intermediate oxazolines using bromotrichloromethane and 1,8-diazabicyclol[5.4.0]undec-7-ene (DBU).

With oxazoles **10–12** in hand, we turned our attention to installing the appropriate functionalisation for use in Fmoc-strategy SPPS. Thus, the *N*- and *C*-terminal protecting groups of **10** and **11** were removed in a single synthetic step by catalytic hydrogenolysis, to provide the fully-deprotected oxazole **13** and the known oxazole **14**[21] in quantitative yield. Saponification of the *C*-terminal methyl ester of **12** using sodium hydroxide, followed by removal of the *N*-terminal Boc protecting group using 4 M hydrochloric acid in dioxane, gave the hydrochloride salt **15** in excellent yield. Subsequent treatment of amines **13–15** with Fmoc-succinimide afforded the desired Fmoc-protected oxazoles **16–18** in good yields, ready for incorporation into SPPS.

Table 1 Conditions for the macrolactamisation of linear peptides **19–21**

Linear peptide	Coupling reagent[a]	Concentration (mol L^{-1})	Solvent	Product	Yield (%)
19	FDPP	0.005	DMF	**22**	34
19	FDPP	0.01	DMF	**22**	35
19	FDPP	0.05	DMF	**22**	48
19	FDPP	0.05	CH_3CN	**22**	23
19	PyBOP	0.05	DMF	**22**	40
20	DMTMM	0.05	DMF	**23**	51
21	FDPP	0.05	DMF	**24a**	38[b]

[a] Abbreviations for coupling reagent names: FDPP: pentafluorophenyl diphenylphosphinate; PyBOP: benztriazol-1-yl-oxytrispyrrolidino-phosphonium hexafluorophosphate; DMTMM: 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium tetrafluoroborate. [b] Linear precursor was cyclised without purification.



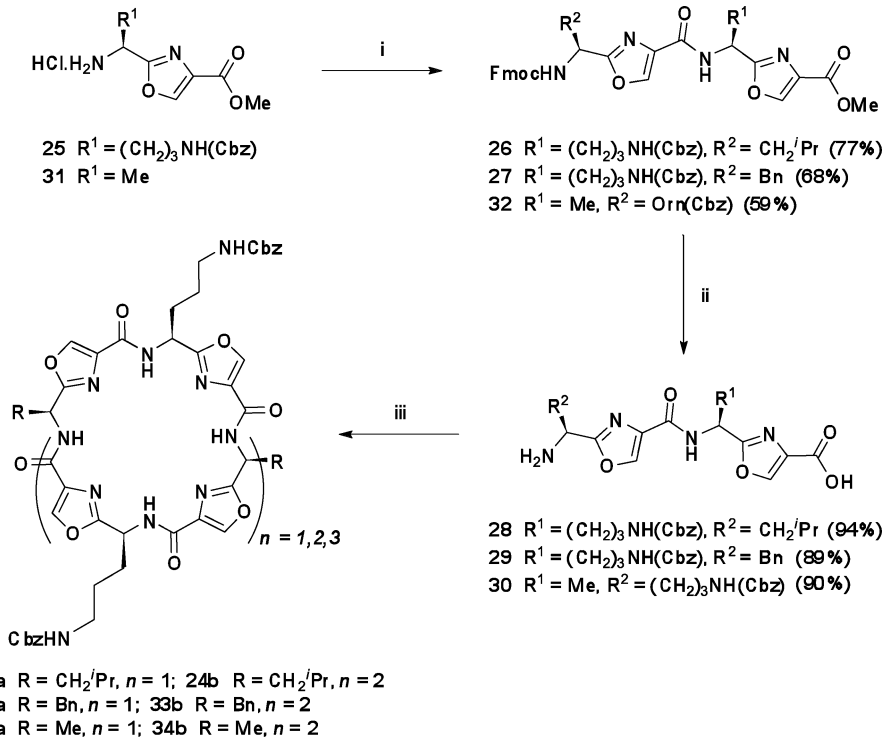
Scheme 2 Macrolactamisation of linear precursors **19–21**. General conditions: (*i*) Coupling reagent (3 equiv.), Hünig's base, DMF, 72 h. See Table 1 for cyclisation yields.

The linear precursors to cyclic peptides **3**, **5** and **6** were constructed using Fmoc-strategy SPPS, utilising 2-(1*H*-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate methanaminium (HATU) as the coupling reagent. The acid-labile 2-chlorotrityl chloride resin was chosen as the solid support to enable cleavage of the linear peptides from the resin with the side chain protecting groups intact. Due to the limited supply of the oxazole-based amino acids **16–18**, a manual SPPS procedure was devised that enabled complete couplings with the use of just 1.5 equivalents of each amino acid building block. This was achieved by using two couplings (1 equiv. of building block followed by a further 0.5 equiv.) and extended reaction times of 24 h. The side chain-protected peptides were then cleaved from the resin using a mixture of hexafluoroisopropanol and dichloromethane (1 : 4 v/v). LCMS analyses of the final cleaved materials indicated formation of the desired linear precursors **19–21** (Scheme 2) in approximately 70–80% yield. However, purification of linear precursors **19** and **20** by preparative RP-HPLC was problematic due to the insolubility of the crude materials in the required solvent system (water–acetonitrile). This resulted in precipitation of the crude linear peptides on the column and subsequent loss of material. As such, the linear peptides **19** and **20** were isolated in much lower yields (51% and 27% respectively) than expected from the LCMS analyses of the crude peptides. To avoid further loss of material, **21** was cyclised without further purification. A range of conditions for the macrolactamisation of linear precursors **19–21** was explored (Table 1) and the optimum conditions were found to be stirring a 0.05 M solution of linear peptide in DMF in the presence of either FDPP or DMTMM coupling reagent for 3 days at room temperature, with PyBOP giving slightly lower cyclisation yields than either of these reagents under identical conditions. Under these conditions the desired cyclic peptides **22**, **23** and **24a** were formed as the major products in 38–51% yields. ^{1}H and ^{13}C NMR spectroscopy of cyclic peptide **24a** indicated C_2-symmetry as expected, whereas the non-symmetrical nature of cyclic peptides **22** and **23** resulted in more complex spectra (see supplementary information†).

Cyclodimerisation approach

The solid-phase assembly of cyclic peptides **22**, **23** and **24a** was successful, enabling the synthesis of scaffolds with different arrangements of side chain functional groups. However, the cyclisation yields were lower than expected, and because of the small reaction scales permitted by SPPS, we were unable to prepare the cyclic peptide scaffolds in large quantities. Therefore, we investigated a more convergent approach to the C_2-symmetric receptors **1**, **3** and **4**, which involved cyclodimerisation of a fully-deprotected linear peptide, which is in turn prepared by coupling two oxazole-based building blocks in solution-phase. This approach has previously been shown to afford azole-modified cyclic octapeptides in significantly improved yields and higher purity compared to the macrolactamisation approach.[15,22,23]

Synthesis of the required linear precursors to cyclodimerisation commenced with acidic removal of the *N*-terminal Boc protecting group of oxazole **12** to afford the hydrochloride salt **25**, which was then coupled to the free acid building blocks **16** or **17** in solution using HBTU/HOBt as the coupling reagents to afford



Scheme 3 Solution-phase synthesis of C_2-symmetric cyclic peptides **24a**, **33a** and **34a**. Conditions: (*i*) ***For 26*****: 16**, **25**, HBTU (1.2 equiv), HOBt (1.2 equiv), Hünig's base, DMF; ***For 27*****: 17**, **25**, HBTU (1.2 equiv), HOBt (1.2 equiv), Hünig's base, DMF; ***For 32*****: 18**, **31**, HBTU (1.2 equiv), HOBt (1.2 equiv), Hünig's base, DMF; (*ii*) NaOH, MeOH, THF, H_2O; (*iii*) FDPP, Hünig's base, DMF (see Table 2 for cyclisation yields).

the protected linear peptides **26** and **27** in good yields (Scheme 3). Simultaneous removal of the *N*-terminal Fmoc and *C*-terminal methyl ester protecting groups of **26** and **27** using 1 M NaOH provided the linear precursors **28** and **29** in 94% and 89% yields, respectively.

Using the same methodology, the alanine-derived linear precursor **30** was synthesised to enable the construction of the previously reported receptor **1** *via* the cyclodimerisation approach, thereby providing a comparison to the original stepwise synthesis.[24] The previously prepared hydrochloride salt **31**[24] was coupled to the free acid **18** in solution to provide linear peptide **32** in moderate yield. Subsequent removal of the *N*- and *C*-terminal protecting groups under the basic conditions described above gave the linear precursor **30** in 90% yield.

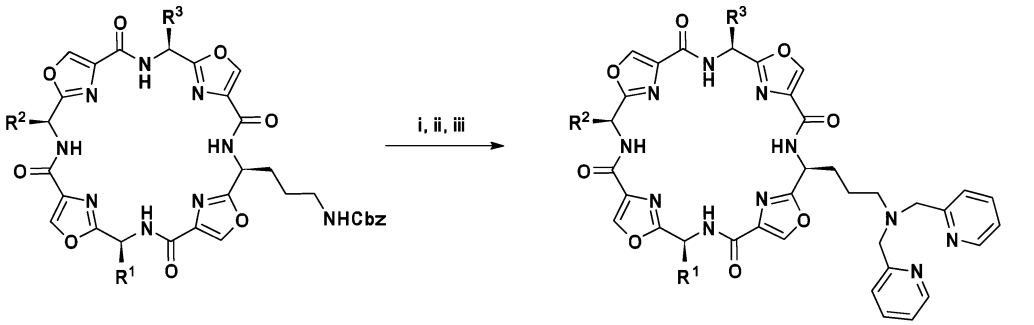
Treatment of linear peptides **28–30** with FDPP coupling reagent in the presence of Hünig's base under high dilution (0.01–0.05 M) in DMF resulted in the formation of the desired cyclic dimers **24a**, **33a** and **34a** ($n = 1$) as the major products (26–50%) (Scheme 3). ^{1}H and ^{13}C spectra of the cyclic dimers **24a**, **33a** and **34a** exhibit the expected number of signals for compounds with C_2-symmetry. As anticipated, the cyclic trimers **24b**, **33b** and **34b** ($n = 2$) were also observed as minor products in comparatively lower yields (5–12%), and trace amounts (< 3%) of higher cyclic oligomers were also obtained as evidenced by mass spectra of the crude reaction mixture.

We explored the effect of different concentrations and reaction times on the yields of cyclic peptides **24a**, **33a** and **34a**. Table 2 summarises the reaction conditions and corresponding yields of

Table 2 Conditions for the cyclodimerisation of linear peptides **28–30**

			Cyclooligomerisation products			
Linear peptide	Concentration (mol L^{-1})	Reaction time (h)	Dimer	Yield (%)	Trimer	Yield (%)
28	0.01	72	**24a**	26	**24b**	8
28	0.025	72	**24a**	33	**24b**	8
28	0.05	72	**24a**	44	**24b**	5
28	0.05	48	**24a**	42–48	**24b**	5
28	0.05	24	**24a**	39	**24b**	5
29	0.025	48	**33a**	36	**33b**	n/i[a]
29	0.05	48	**33a**	40–50	**33b**	n/i[a]
30	0.025	48	**34a**	28	**34b**	n/i[a]
30	0.05	48	**34a**	36	**34b**	12

[a] n/i: not isolated



34a R^1 = Me, R^2 = $(CH_2)_3NH(Cbz)$, R^3 = Me
24a R^1 = $CH_2{}^iPr$, R^2 = $(CH_2)_3NH(Cbz)$, R^3 = $CH_2{}^iPr$
33a R^1 = Bn, R^2 = $(CH_2)_3NH(Cbz)$, R^3 = Bn
23 R^1 = $CH_2{}^iPr$, R^2 = $CH_2{}^iPr$, R^3 = $(CH_2)_3NH(Cbz)$
22 R^1 = Bn, R^2 = Bn, R^3 = $(CH_2)_3NH(Cbz)$

1 R^1 = Me, R^2 = $(CH_2)_3NH(Dpa)$, R^3 = Me (55%)
3 R^1 = $CH_2{}^iPr$, R^2 = $(CH_2)_3NH(Dpa)$, R^3 = $CH_2{}^iPr$ (67%)
4 R^1 = Bn, R^2 = $(CH_2)_3NH(Dpa)$, R^3 = Bn (76%)
5 R^1 = $CH_2{}^iPr$, R^2 = $CH_2{}^iPr$, R^3 = $(CH_2)_3NH(Dpa)$ (87%)
6 R^1 = Bn, R^2 = Bn, R^3 = $(CH_2)_3NH(Dpa)$ (77%)

Scheme 4 Side chain functionalisation of cyclic peptides **22**, **23**, **24a**, **33a** and **34a**. Yields are reported over 3 steps. Conditions: (*i*) 45% HBr in AcOH; (*ii*) 0.3 M NaOH; (*iii*) 2-pyridinecarboxaldehyde, $NaHB(OAc)_3$, DMF.

the desired cyclic dimers and undesired cyclic trimers obtained after purification by preparative reverse-phase HPLC.

Initially, a 0.01 M solution of linear peptide **28** was treated with three equivalents of FDPP in the presence of Hünig's base and the reaction mixture was stirred at room temperature for 72 h, affording the cyclic dimer **24a** and cyclic trimer **24b** in 26% and 8% yields, respectively. Significant improvement in the yield of **24a** was achieved using a higher concentration of 0.05 M. Additionally, it was found that a greater ratio of the desired dimer to the undesired trimer (9 : 1) was obtained using a concentration of 0.05 M, compared to when 0.025 M (4 : 1) and 0.01 M (3 : 1) solutions of the linear peptide **28** were used. A reduced reaction time of 48 h resulted in formation of **24a** in the highest yields obtained (42–48%). A shorter reaction time of 24 h resulted in a slightly lower yield of the cyclic dimer (39%), suggesting that the cyclodimerisation had not reached completion.

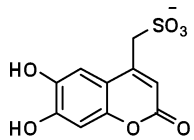
The optimum conditions for cyclodimerisation were found to be stirring a 0.05 M solution of linear peptide in DMF using FDPP activation at room temperature for 48 h. Using these conditions, cyclic dimers **33a** and **34a** were formed in 50% and 36% yields, respectively. The cyclodimerisation of linear peptide **29** afforded cyclic products in the ratio 8 : 1 (dimer/trimer), as determined by LCMS analysis. As such, insufficient amounts of the cyclic trimer **33b** were isolated for its characterisation. In contrast, the cyclisation of linear fragment **30** gave a lower ratio of the desired cyclic dimer **34a** to the cyclic trimer **34b** (3 : 1). Therefore, the isolation of the cyclic trimer was possible. In both cases, the use of higher dilution (0.025 M) resulted in slightly diminished yields of the desired cyclic dimers **33a** and **34a**.

Thus, an efficient route to the C_2-symmetric cyclic peptides **24a**, **33a** and **34a** has been developed. The cyclodimerisation reaction was optimised to provide cyclic peptides **24a**, **33a** and **34a** in 32%, 30% and 16% overall yields, starting from amino acid building block **25**, and in the case of scaffold **34a**, building block **31**. In comparison, the overall yield of cyclic peptide **24a** *via* the macrolactamisation route amounts to only 17%, indicating that cyclooligomerisation with a lower number of steps in the linear sequence is the preferred route.

Having prepared sufficient quantities of the cyclic peptide scaffolds **22**, **23**, **24a**, **33a** and **34a**, our attention turned to their functionalisation with the dipicolylamino groups required for anion binding studies. Cleavage of the Cbz protecting groups of cyclic peptides **22**, **23**, **24a**, **33a** and **34a** using a solution of 45% hydrogen bromide in acetic acid gave the corresponding amines as the dihydrobromide salts in quantitative yields (Scheme 4). The hydrobromide salts were neutralised by a basic work-up procedure using 0.3 M sodium hydroxide solution to give the free amines, which were then subjected to reductive amination with 2-pyridinecarboxaldehyde using our previously established conditions,[9] to provide the desired Dpa-functionalised cyclic peptides **1** and **3–6** in good to excellent yields.

Preliminary anion binding studies for the bis-Zn(II) complexes of compounds **1** and **3–6** [prepared by addition of 2 equiv. of $Zn(NO_3)_2$ to each cyclic peptide][9] were undertaken in aqueous solution (HEPES buffer, 5 mM, pH 7.4) at 25 °C, using our previously reported indicator displacement assay[26] with the fluorescent coumarin derivative **35**.[9,12,27,28] Apparent stability constants of **1**·$\mathbf{Zn_2}$ and **3–6**·$\mathbf{Zn_2}$ for **35** were of similar magnitude, ranging from log K_{in} 6.2–6.9 (± 0.1), with the leucine derivatives **3**·$\mathbf{Zn_2}$ and **5**·$\mathbf{Zn_2}$ having the lowest and highest affinity for **35**, respectively. Titration of 1 : 1 solutions of the indicator: receptor complexes (10 μM) with aliquots of the sodium salts of pyrophosphate, ATP and ADP resulted in increases in fluorescence indicating displacement of the indicator, while hydrogenphosphate was not able to displace the indicator from any of the receptors to an appreciable extent (<10% fluorescence recovery upon addition of 10 equiv hydrogenphosphate) indicating the selectivity of these receptors for di- and tri-phosphate anions over monophosphate anions. The receptors showed some differences in their selectivity towards PPi, ATP and ADP. Notably, while **1**·$\mathbf{Zn_2}$ displayed selectivity for PPi (log K_a 7.2 ± 0.1) over ATP and ADP (log K_a 6.5 ± 0.1 and 6.3 ± 0.1, respectively), increasing the steric bulk of the non-binding side chains resulted in loss of this selectivity, with the leucine derived receptor **3**·$\mathbf{Zn_2}$ displaying similar affinity for all three anions [log K_a 7.4–7.6 (± 0.2)]. Increased discrimination between PPi and ATP or ADP was observed when the binding sites were moved closer together. For example, the phenylalanine

derived receptor **4·Zn$_2$** binds PPi, ATP and ADP with log K_as of 8.4, 8.6 and 7.5 (± 0.2), respectively, whereas **6·Zn$_2$**, which bears the same side chains but with the two DPA functionalised sidechains at proximal rather than distal positions as in **4·Zn$_2$**, binds PPi (log K_a 7.9 ± 0.1) with greater affinity than either ATP (log K_a 7.2 ± 0.1) or ADP (log K_a 7.0 ± 0.1).



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Conclusions

In summary, two viable approaches to the synthesis of *Lissoclinum*-type cyclic peptide-based anion receptors **1** and **3–6** were implemented: the stepwise macrolactamisation pathway was useful for the preparation of the non-C_2 symmetric cyclic peptides **5** and **6** bearing different side chain functionalities, whereas the more convergent cyclodimerisation pathway was preferreed to provide access to substantial quantities of C_2-symmetric cyclic peptides **1**, **3** and **4**. Through the combined use of these approaches, we have synthesised sufficient amounts of compounds **1** and **3–6** to allow a comprehensive investigation of the anion binding capabilities of their bis-zinc(II) complexes **1·Zn$_2$** and **3·Zn$_2$–6·Zn$_2$** (Fig. 1) in aqueous solution. Preliminary studies indicated that all of the receptors bind di- and tri-phosphate anions with selectivity over hydrogenphosphate and that the 'non-binding' side chains have a significant influence on the ability of the receptors to discriminate between di- and tri-phosphate derivatives. The results of more extensive anion binding studies will be reported in due course.

Experimental

General procedures

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 or a Bruker Avance DPX 300 spectrometer at a frequency of 300.13 MHz, and are reported in parts per million (ppm) relative to the residual isotopomer with one less deuterium than 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 F254). 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. Preparative RP-HPLC was performed on a Waters 600E multisolvent delivery system with a Waters U6K injector, Waters 490E programmable multiwavelength detector, Waters busSAT/IN module and Waters Empower 2 software. Separation was achieved on a Sunfire™ PrepC_{18} OBD™ column (5 µm, 150 × 19 mm ID). Compounds were eluted at a flow rate maintained at 7.0 mL min^{-1} over the stated linear gradient, using solvent A: 100 : 0.05 v/v Milli-Q water/TFA, and solvent B: 100 : 0.05 v/v acetonitrile/TFA, 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. All other reagents were commercially available and were used as supplied.

General procedures for solid-phase peptide synthesis

1. Loading of amino acid onto 2-chlorotrityl chloride resin. Under an atmosphere of nitrogen, 2-chlorotrityl chloride resin (resin capacity 1.01–1.4 mmol g^{-1}) was swollen in a sinter-fitted syringe in anhydrous dichloromethane for 1 h. The resin was drained and treated with a solution of Fmoc-protected amino acid (1.5 equiv. relative to resin capacity) and Hünig's base (5 equiv. relative to resin capacity) in anhydrous dichloromethane–DMF (9 : 1 v/v, 0.1 M) and the resulting suspension was gently agitated at rt for 24 h under an atmosphere of nitrogen. At this time the resin was drained and then treated with a mixture of methanol-Hünig's base-dichloromethane (2 : 1 : 17 v/v/v, 2 × 5 mL × 15 min). The resin was then washed sequentially with DMF (2 × 10 mL), dichloromethane (3 × 10 mL) and diethyl ether (3 × 10 mL). The residual solvent was removed under reduced pressure.

2. *N*-terminal Fmoc deprotection. The resin-bound peptide was agitated in a solution of 10% piperidine in DMF (2 × 5 mL × 15 min) and then drained and washed sequentially with DMF (2 × 5 mL), dichloromethane (3 × 5 mL) and DMF (3 × 5 mL). The resulting resin-bound amine was used immediately in the next peptide coupling step.

3. Estimation of resin loading. The drained Fmoc deprotection solution was diluted with a solution of 10% piperidine in DMF so that the maximum concentration of the fulvene-piperidine adduct was in the range of 2.5–7.5 × 10^{-5} M. A sample of this solution (2 × 3 mL) was transferred to two matched

1 cm quartz glass cuvettes and the UV-Vis absorbance at 301 nm was measured, using the solution of 10% piperidine in DMF as a reference. An average of the two absorbance values was used to calculate the loading, using $\varepsilon = 7800\ M^{-1}\ cm^{-1}$.

4. Manual SPPS peptide coupling. Under an atmosphere of nitrogen, a solution of Fmoc-protected oxazole (1.5 equiv. relative to loading), HATU (2 equiv. relative to peptide) and Hünig's base (3 equiv. relative to peptide) in anhydrous DMF (0.1 M relative to dipeptide) was added to the resin and the resulting suspension was agitated at rt for 24 h. The resin was then washed sequentially with DMF (2 × 5 mL), dichloromethane (3 × 10 mL) and DMF (3 × 5 mL).

5. Cleavage of peptides from the resin. A mixture of hexafluoroisopropanol-dichloromethane (1 : 4 v/v, 3 × 5 mL) was added to the resin and the resulting suspension was agitated at rt for 15 min. The solutions were drained and combined and the solvent was removed under reduced pressure to afford the crude linear peptide.

6. Macrolactamisation. To a solution of the linear tetraoxazole (1 equiv.) in anhydrous DMF (0.05 M) was added coupling reagent (3 equiv.) (see Table 1 for details) and Hünig's base (5 equiv.) and the resulting mixture was stirred at rt for 72 h under an atmosphere of nitrogen. The mixture was acidified by the addition of hydrochloric acid (0.3 M, pH 5) and then partitioned between water (20 mL) and chloroform-isopropanol (3 : 1 v/v, 20 mL). The aqueous phase was extracted with chloroform-isopropanol (3 : 1 v/v, 3 × 20 mL) and the combined organic fractions were dried ($MgSO_4$) and concentrated under reduced pressure.

General procedures for solution-phase synthesis

7. Peptide coupling. To a stirred solution of the acid (1 equiv.) and the amine hydrochloride salt (1 equiv.) in anhydrous DMF (0.15 M) was added HBTU (1.2 equiv.), HOBt (1.2 equiv.), then Hünig's base (3.2 equiv.) under an atmosphere of nitrogen. The resulting mixture was stirred at rt for 22 h. At this time, the mixture was concentrated under reduced pressure to half the initial volume and then partitioned between sat. aq. NH_4Cl solution (5 mL per mmol of starting material) and chloroform-isopropanol (5 mL per mmol of starting material). The organic phase was extracted with water (5 mL per mmol of starting material × 3) and the combined aqueous fractions were back-extracted with chloroform-isopropanol (3 : 1 v/v, 10 mL per mmol of starting material × 2). The combined organic fractions were washed with half-strength brine solution (10 mL per mmol of starting material), then dried ($MgSO_4$) and the solvent was removed under reduced pressure.

8. Oxazole formation. Under an atmosphere of nitrogen, Deoxo-fluor (1.2 equiv.) was added dropwise to a stirred solution of the dipeptide (1 equiv.) in anhydrous dichloromethane (0.10 M) cooled to –20 °C (EtOH/ice/CO_2 chips). The resulting mixture was stirred at –20 °C for 1 h, then slowly warmed to rt and stirred for a further 6 h. At this time the mixture was cooled to –5 °C and quenched with sat. aq. $NaHCO_3$ solution (5 mL per mmol of starting material) and then extracted with chloroform (5 mL per mmol of starting material × 3). The combined organic fractions were washed with half-strength brine solution (10 mL per mmol of starting material), then dried ($MgSO_4$) and concentrated under reduced pressure to give the crude oxazoline. The crude oxazoline (1 equiv.) was dissolved in anhydrous dichloromethane (0.15 M) and cooled to 0 °C under an atmosphere of nitrogen. The solution was treated with $BrCCl_3$ (2 equiv.) and DBU (2 equiv.) and the resulting mixture was stirred at rt for 7 h. The mixture was quenched by the addition of hydrochloric acid (0.3 M, pH 6) and extracted with chloroform-isopropanol (3 : 1 v/v, 5 mL per mmol of starting material × 3). The combined organic fractions were washed with half-strength brine solution (10 mL per mmol of starting material), then dried ($MgSO_4$) and concentrated under reduced pressure.

9. *N*-terminal Fmoc protection. To a stirred solution of the amine (1 equiv.) and sodium carbonate (3 equiv.) in distilled water (0.3 M) cooled to 0 °C, was added a solution of Fmoc succinimide (1.5 equiv.) in 1,4-dioxane (0.3 M). The resulting mixture was slowly warmed to rt and stirred for 24 h. At this time the mixture was quenched with hydrochloric acid (0.3 M, pH 5) and the organic solvent was removed under reduced pressure. The remaining solution was extracted with chloroform-isopropanol (3 : 1 v/v, 5 mL per mmol of starting material × 3) and the combined organic fractions were washed with half-strength brine solution (10 mL per mmol of starting material), then dried ($MgSO_4$) and the solvent was removed under reduced pressure.

10. *N*-terminal Fmoc and *C*-terminal methyl ester deprotection. To a solution of the bisoxazole (1 equiv.) in methanol–THF (1 : 1 v/v, 0.15 M) was added a solution of NaOH (10 equiv.) in water (0.15 M) and the resulting mixture was stirred at rt for 5 h. The mixture was then acidified by the addition of hydrochloric acid (0.3 M, pH 6) and the organic solvent was removed under reduced pressure. The residue thus obtained was partitioned between chloroform-isopropanol (3 : 1 v/v, 5 mL per mmol of starting material) and water (5 mL per mmol of starting material) and the aqueous phase was extracted with chloroform-isopropanol (3 : 1 v/v, 5 mL per mmol of starting material × 3). The combined organic fractions were washed with half-strength brine solution (10 mL per mmol of starting material), then dried ($MgSO_4$) and concentrated under reduced pressure.

11. Cyclodimerisation. To a stirred solution of the bisoxazole (1 equiv.) in anhydrous DMF (0.05 M) was added FDPP (3 equiv.) and Hünig's base (5 equiv.) under an atmosphere of nitrogen. The resulting mixture was stirred at rt for 48 h after which time analysis by LCMS revealed complete conversion of starting material. The mixture was concentrated under reduced pressure to a quarter of the initial volume and then partitioned between chloroform-isopropanol (3 : 1 v/v, 5 mL per mmol of starting material) and hydrochloric acid (0.3 M, pH 3). The aqueous phase was extracted with chloroform-isopropanol (3 : 1 v/v, 5 mL per mmol of starting material × 3) and the combined organic fractions were washed with half-strength brine solution (10 mL per mmol of starting material), then dried ($MgSO_4$) and concentrated under reduced pressure.

12. Functionalisation of cyclic peptide side chains.

Step 1. Under an atmosphere of nitrogen, a solution of hydrogen bromide in acetic acid (33% v/v, 2.0 mL) was added to the carbamate (1 equiv.) and the resulting mixture was stirred at rt for 24 h. Anhydrous ether (25 mL) was added to give a cream coloured precipitate which was condensed by centrifuge.

Subsequent trituration of the precipitate with anhydrous ether (10 × 25 mL) and removal of the final clear ethereal layer under reduced pressure, gave the dihydrobromide salt. This material was partitioned between chloroform-isopropanol (3 : 1 v/v, 20 mL) and NaOH (0.3 M, 20 mL) and the aqueous phase was extracted with chloroform-isopropanol (3 : 1 v/v, 4 × 20 mL). The combined organic fractions were dried ($MgSO_4$) and the solvent was removed under reduced pressure to give the free diamine.

Step 2. To a stirred solution of the free diamine in anhydrous DMF (2.0 mL) was added 2-pyridinecarboxaldehyde (20 equiv.) and sodium triacetoxyborohydride (25 equiv.) under an atmosphere of nitrogen. The resulting mixture was deoxygenated and refilled with nitrogen three times, then slowly warmed to 35 °C and stirred for 72 h. The mixture was concentrated to almost dryness and then partitioned between chloroform-isopropanol (3 : 1 v/v, 20 mL) and NaOH (0.3 M, pH 8). The aqueous phase was extracted with chloroform-isopropanol (3 : 1 v/v, 4 × 20 mL) and the combined organic fractions were washed with half-strength brine solution (40 mL), then dried ($MgSO_4$) and the solvent was removed under reduced pressure.

Synthesis

Cbz-Leu-Ser-OBn (7). The acid Cbz-Leu-OH (15.0 g, 56.5 mmol) and the hydrochloride salt of H-Ser-OBn (13.1 g, 56.5 mmol) were coupled in solution according to general procedure 7. The crude material was purified by flash chromatography (dry loaded onto silica gel; eluting first with EtOAc–hexane, 3 : 1 v/v, then neat EtOAc) to give the desired dipeptide **7** (16.0 g, 64%) as a colourless solid. m.p. 122–124 °C; $[\alpha]_D^{20} = -1.50$ (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.91 (d, J = 6.4 Hz, 6H), 1.52 (m, 1H), 1.59–1.72 (m, 2H), 2.48 (br s, 1H), 3.93 (m, 2H), 4.24 (m, 1H), 4.68 (m, 1H), 5.02 (d, J = 12.2 Hz, 1H), 5.10 (d, J = 12.2 Hz, 1H), 5.19 (s, 2H), 5.42 (d, J = 8.1 Hz, 1H), 7.07 (d, J = 7.8 Hz, 1H), 7.29–7.37 (m, 10H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 23.9, 24.8, 26.6, 43.3, 55.7, 56.8, 64.7, 69.2, 69.5, 130.0, 130.1(8), 130.2(1), 130.5(0), 130.5(2), 130.6, 137.1, 138.0, 158.5, 172.1, 174.6; HRMS (ESI) calcd. for $C_{24}H_{30}N_2O_6Na$ $[M + Na]^+$ 465.1996, found 465.1989.

Cbz-Phe-Ser-OBn (8). The acid Cbz-Phe-OH (10.0 g, 33.4 mmol) and the hydrochloride salt of H-Ser-OBn (7.74 g, 33.4 mmol) were coupled in solution according to general procedure 7. The crude material was triturated with ethyl acetate (3 × 200 mL) to give the desired dipeptide **8** (12.0 g, 75%) as a colourless solid. m.p. 115–117 °C [lit.[25] m.p. 115–118 °C]; $[\alpha]_D^{20}$ = +3.20 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, CD_3OD): δ 2.81 (dd, J = 9.5, 13.9 Hz, 1H), 3.12 (dd, J = 4.8, 13.9 Hz, 1H), 3.82 (dd, J = 4.4, 11.3 Hz, 1H), 3.93 (dd, J = 4.4, 11.3 Hz, 1H), 4.47 (dd, J = 4.8, 9.5 Hz, 1H), 4.57 (m, 1H), 4.99 (AB quartet, 2H), 5.18 (s, 2H), 7.13–7.42 (m, 15H), N–H and O–H signals not observed; ^{13}C NMR (75.5 MHz, CD_3OD): δ 39.1, 56.3, 57.6, 62.8, 67.6, 68.1, 127.7, 128.6, 128.9, 129.2, 129.3, 129.4(0), 129.4(2), 129.6, 130.3, 137.1, 138.1, 138.5, 158.3, 171.4, 174.3; HRMS (ESI) calcd. for $C_{27}H_{28}N_2O_6Na$ $[M + Na]^+$ 499.1844, found 499.1840.

Boc-Orn(Cbz)-Ser-OMe (9). The acid Boc-Orn(Cbz)-OH (10.0 g, 27.3 mmol) and the hydrochloride salt of H-Ser-OMe (4.25 g, 27.3 mmol) were coupled in solution according to general procedure 7. Subjection of the crude material to flash chromatography (silica gel: $CHCl_3$–MeOH, 95 : 5 v/v) gave the desired dipeptide **9** (11.1 g, 87%) as a colourless solid. m.p. 67–68 °C; $[\alpha]_D^{20} = -2.09$ (*c* 1.1, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.42 (s, 9H), 1.62 (m, 3H), 1.86 (m, 1H), 3.16 (m, 1H), 3.34 (m, 1H), 3.42 (br s, 1H), 3.74 (s, 3H), 3.93 (m, 2H), 4.27 (br m, 1H), 4.64 (m, 1H), 5.08 (AB quartet, 2H), 5.15 (t, J = 6.6 Hz, 1H), 5.39 (d, J = 8.1 Hz, 1H), 7.33 (m, 5H), O–H not observed; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 26.0, 28.4, 29.9, 40.1, 52.7, 53.7, 54.9, 62.8, 66.9, 80.4, 128.2, 128.6, 136.6, 156.1, 157.2, 171.0, 172.7, one signal obscured or overlapping; HRMS (ESI) calcd. for $C_{22}H_{33}N_3O_8Na$ $[M + Na]^+$ 490.2160, found 490.2157.

Cbz-Leu-Oxz(Ser)-OBn (10). The dipeptide **7** (9.73 g, 22.0 mmol) was converted to the crude oxazole **10** according to general procedure 8. The crude material was purified by flash chromatography (silica gel; hexane–EtOAc, 4 : 1 v/v) to give the desired oxazole **10** (6.94 g, 75% over two steps) as a colourless solid. m.p. 75–77 °C; $[\alpha]_D^{20} = -4.35$ (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD): δ 0.93 (d, J = 6.6 Hz, 3H), 0.96 (d, J = 6.6 Hz, 3H), 1.58–1.84 (m, 3H), 4.92 (dd, J = 6.6, 8.8 Hz, 1H), 5.08 (AB quartet, 2H), 5.34 (s, 2H), 7.19–7.48 (m, 10H), 8.48 (s, 1H), N–H signal not observed; ^{13}C NMR (100.6 MHz, CD_3OD): δ 22.0, 23.1, 25.8, 42.9, 67.8, 128.8, 129.0, 129.4(5), 129.4(7), 129.6, 134.0, 137.1, 138.1, 146.2, 158.3, 162.3, 167.4, three signals obscured or overlapping; HRMS (ESI) calcd. for $C_{24}H_{26}N_2O_5Na$ $[M + Na]^+$ 445.1734, found 445.1733.

Cbz-Phe-Oxz(Ser)-OBn (11). The dipeptide **8** (4.26 g, 8.94 mmol) was converted to the crude oxazole **11** according to general procedure 8. Subjection of the crude material to flash chromatography (dissolved in $CHCl_3$ and loaded to silica gel; hexane–EtOAc, 2 : 1 v/v) gave the desired oxazole **11** (2.95 g, 72% over two steps) as a colourless solid. m.p. 117–119 °C; $[\alpha]_D^{20}$ = –2.75 (*c* 0.8, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.25 (m, 2H), 5.09 (AB quartet, 2H), 5.28 (m, 1H), 5.36 (s, 2H), 5.55 (d, J = 8.8 Hz, 1H), 7.03 (m, 2H), 7.23 (m, 2H), 7.28–7.46 (m, 11H), 8.11 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 40.3, 50.8, 67.0, 67.3, 127.3, 128.2, 128.3, 128.6(5), 128.6(9), 128.8(0), 128.8(1), 129.4, 133.4, 135.4, 135.5, 136.2, 144.2, 155.6, 160.9, 164.5, one signal obscured or overlapping; HRMS (ESI) calcd. for $C_{27}H_{28}N_2O_6Na$ $[M + Na]^+$ 479.1583, found 479.1578.

Boc-Orn(Cbz)-Oxz(Ser)-OMe (12). The dipeptide **9** (12.5 g, 26.7 mmol) was converted to the crude oxazole **12** according to general procedure 8. The crude material was purified by flash chromatography (silica gel; eluting first with EtOAc–hexane, 1 : 1 v/v, then EtOAc–hexane, 2 : 3 v/v) to give the desired oxazole **12** (8.63 g, 69% over two steps) as a colourless solid. m.p. 83–84 °C; $[\alpha]_D^{20} = -9.40$ (*c* 1.0, MeOH); ^{1}H NMR (300 MHz, CD_3OD): δ 1.43 (s, 9H), 1.47–1.67 (m, 2H), 1.75–2.02 (m, 2H), 3.15 (m, 2H), 3.87 (s, 3H), 4.79 (m, 1H), 5.05 (s, 2H), 7.02 (t, J = 6.6 Hz, 1H), 7.27–7.34 (m, 4H), 8.46 (s, 1H), N–H signal not observed; ^{13}C NMR (75.5 MHz, CD_3OD): δ 27.2, 28.7, 31.4, 41.2, 41.3, 52.5, 67.4, 80.8, 128.8, 128.9, 129.4, 133.9, 138.4, 146.0, 157.7, 158.9, 163.0, 167.2; HRMS (ESI) calcd. for $C_{22}H_{29}N_3O_7Na$ $[M + Na]^+$ 470.4713, found 470.1897.

H_2N-Leu-Oxz(Ser)-OH (13). To a solution of the oxazole **10** (6.12 g, 14.5 mmol) in anhydrous methanol (145 mL) was added Pd/C catalyst (10 wt%) and the mixture was stirred under an atmosphere of hydrogen for 20 h, after which time a colourless

precipitate had formed. The reaction mixture was filtered through a pad of celite and washed with hot methanol (3 × 100 mL) followed by hot water-aq. ammonia (95 : 5 v/v, 3 × 200 mL). The filtrates were collected and the solvent was removed under reduced pressure to give the fully-deprotected oxazole **13** (2.85 g, quant.) as a colourless solid. m.p. 184–185 °C; ^{1}H NMR (300 MHz, CF_3CO_2D): δ 0.91 (d, J = 6.5 Hz, 3H), 0.95 (d, J = 6.5 Hz, 3H), 1.53 (m, 1H), 2.01–2.15 (m, 2H), 5.03 (dd, J = 6.5, 9.9 Hz, 1H), 8.46, (s, 1H), N–H and O–H signals not observed; ^{13}C NMR (75.5 MHz, CF_3CO_2D): δ 22.2, 23.2, 27.1, 42.9, 51.4, 135.0, 149.7, 166.9, one signal obscured or overlapping; HRMS (ESI) calcd. for $C_9H_{13}N_2O_3$ $[M - H]^-$ 197.0932, found 197.0928.

H_2N-Phe-Oxz(Ser)-OH (14). To a solution of the oxazole **11** (5.36 g, 11.7 mmol) in anhydrous methanol–THF (2 : 1 v/v, 90 mL) was added Pd/C catalyst (10 wt%) and the mixture was stirred under an atmosphere of hydrogen for 20 h, after which time a colourless precipitate had formed. The reaction mixture was filtered through a pad of celite and washed with hot methanol (3 × 100 mL) followed by hot water-aq. ammonia (98 : 2 v/v, 3 × 100 mL). The filtrates were collected and the solvent was removed under reduced pressure to give the fully-deprotected oxazole **14** (2.72 g, quant.) as a colourless solid. m.p. 245–246 °C; ^{1}H NMR (400 MHz, CD_3OD): δ 3.34 (dd, J = 6.9, 13.9 Hz, 1H), 3.39 (dd, J = 7.8, 13.9 Hz, 1H), 4.87 (dd, J = 6.9, 7.8 Hz, 1H), 7.15 (m, 2H), 7.24–7.33 (m, 3H), 8.49 (s, 1H), N–H and O–H signals not observed; ^{13}C NMR (75.5 MHz, CF_3CO_2D): δ 39.5, 53.5, 130.9, 131.0, 131.6, 133.1, 134.5, 149.4, 162.6, 166.5. NMR data is in good agreement with that previously reported.[21] HRMS (ESI) calcd. for $C_{12}H_{12}N_2O_3Na$ $[M + Na]^+$ 255.0740, found 255.0743.

HCl.H_2N-Orn(Cbz)-Oxz(Ser)-OH (15). To a solution of the oxazole **12** (1.00 g, 2.24 mmol) in methanol (16 mL) was added a solution of NaOH (0.894 g, 22.4 mmol) in water (6 mL) and the resulting mixture was stirred at rt for 18 h under an atmosphere of nitrogen. The mixture was acidified (pH 6) by the addition of hydrochloric acid (30 mL) and the organic solvent was removed under reduced pressure. The residue thus obtained was partitioned between water (60 mL) and chloroform-isopropanol (3 : 1 v/v, 100 mL). The aqueous phase was extracted with chloroform-isopropanol (3 : 1 v/v, 3 × 100 mL) and the combined organic fractions were dried ($MgSO_4$) and concentrated under reduced pressure to afford the carboxylic acid (0.973 g, quant.) as a colourless foam. The carboxylic acid (0.973 g, 2.24 mmol) was dissolved in anhydrous dichloromethane (7.5 mL) and treated with a solution of hydrochloric acid in dioxane (4 M, 8.4 mL, 33.6 mmol) under an atmosphere of nitrogen. The solution was stirred at rt for 4 h, after which time analysis by TLC revealed complete conversion of starting material. The reaction mixture was concentrated under reduced pressure to give the desired hydrochloride salt **15** (0.793 g, quant.) as a colourless solid. ^{1}H NMR (400 MHz, CD_3OD): δ 1.60 (m, 2H), 2.07 (m, 2H), 3.16 (t, J = 6.6 Hz, 2H), 4.69 (t, J = 6.6 Hz, 1H), 5.07 (s, 2H), 7.33 (m, 5H), 8.60 (s, 1H), N–H and O–H signals not observed; ^{13}C NMR (75.5 MHz, CD_3OD): δ 18.6, 26.5, 30.2, 40.8, 58.3, 128.8, 129.0, 129.5, 135.2, 138.3, 147.3, 159.0, 161.8, 163.6.

Fmoc-Leu-Oxz(Ser)-OH (16). Compound **13** (4.66 g, 23.5 mmol) was Fmoc-protected according to general procedure 9. The crude material thus obtained was purified by flash chromatography (silica gel; eluting first with hexane–EtOAc, 5 : 1 v/v, then hexane–EtOAc, 2 : 1 v/v) to give the desired Fmoc-protected oxazole **16** (7.04 g, 71%) as a colourless solid. m.p. 134–136 °C; $[\alpha]_D^{20}$ = –11.2 (c 1.0, MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ 0.93 (d, J = 6.6 Hz, 3H), 0.96 (d, J = 6.6 Hz, 3H), 1.50–1.83 (m, 3H), 4.19 (t, J = 6.9 Hz, 1H), 4.36 (dd, J = 6.9, 10.5 Hz, 1H), 4.41 (dd, J = 6.9, 10.5 Hz 1H), 4.90 (partially obscured m, 1H), 7.28 (t, J = 7.3 Hz, 2H), 7.37 (t, J = 7.6 Hz, 2H), 7.64 (dd, J = 3.4, 7.6 Hz, 2H), 7.77 (d, J = 7.3 Hz, 2H), 8.41 (s, 1H), N–H and O–H signals not observed; ^{13}C NMR (100.6 MHz, CD_3OD): δ 22.0, 23.1, 25.7, 42.8, 48.9, 67.7, 120.8, 126.1, 128.0, 128.7, 134.5, 142.5, 145.0, 145.7, 158.2, 163.8, 167.1, one signal obscured or overlapping; HRMS (ESI) calcd. for $C_{24}H_{24}N_2O_5Na$ $[M + Na]^+$ 443.1583, found 443.1584.

Fmoc-Phe-Oxz(Ser)-OH (17). Compound **14** (2.72 g, 11.7 mmol) was Fmoc-protected according to general procedure 9. Subjection of the crude material to flash chromatography (silica gel; $CHCl_3$–MeOH, 98 : 2 v/v) gave the desired dipeptide (R_f 0.2) which had co-eluted with an impurity (R_f 0.3). The appropriate fractions were concentrated under reduced pressure and triturated with diethyl ether (3 × 30 mL) to afford the desired Fmoc-protected oxazole **17** (3.40 g, 64%) as a colourless solid. m.p. 174–176 °C; $[\alpha]_D^{20}$ = –11.8 (c 0.8, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.15 (dd, J = 7.2, 13.9 Hz, 1H), 3.21 (dd, J = 6.9, 13.9 Hz, 1H), 4.12 (t, J = 6.9 Hz, 1H), 4.26 (dd, J = 6.9, 10.8 Hz, 1H), 4.36 (dd, J = 6.9, 10.8 Hz, 1H), 5.16 (dd, J = 6.9 Hz, 1H), 7.00 (d, J = 6.9 Hz, 2H), 7.14–7.28 (m, 5H), 7.34 (t, J = 7.3 Hz, 2H), 7.51 (t, J = 7.6 Hz, 2H), 7.70 (d, J = 7.3 Hz, 2H), 8.10 (s, 1H), N–H and O–H signals not observed; ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 39.9, 47.1, 50.6, 67.0, 120.0, 125.1(0), 125.1(3), 127.1, 127.7, 128.6, 129.2, 133.6, 135.6, 141.3, 143.8, 144.1, 156.0, 162.8, 164.5; HRMS (ESI) calcd. for $C_{27}H_{22}N_2O_5Na$ $[M + Na]^+$ 477.1421, found 477.1420.

Fmoc-Orn(Cbz)-Oxz(Ser)-OH (18). Compound **15** (0.793 g, 2.24 mmol) was Fmoc-protected according to general procedure 9. The crude material thus obtained was triturated in anhydrous diethyl ether (3 × 40 mL) to afford the desired Fmoc-protected oxazole **18** (1.05 g, 84%) as colourless solid. m.p. 131–133 °C; $[\alpha]_D^{20}$ = –5.30 (c 1.1, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.25–1.54 (m, 2H), 1.65–1.91 (m, 2H), 3.00 (m, 2H), 4.15–4.44 (m, 3H), 4.72 (m, 1H) 5.00 (m, 2H), 7.24–7.45 (m, 10H), 7.69 (m, 2H), 7.88 (m, 2H), 8.05 (m, 2H), O–H signal not observed; ^{13}C NMR (100.6 MHz, DMSO-d_6): δ 25.9, 30.5, 46.7, 48.7, 65.2, 65.6, 120.1, 125.2, 125.3, 127.1, 127.6, 127.7, 128.4, 137.2, 140.1, 140.7, 143.7, 143.8, 155.9, 156.2, 163.1, 164.4, one signal obscured or overlapping; HRMS (ESI) calcd. for $C_{31}H_{29}N_3O_7Na$ $[M + Na]^+$ 578.1898, found 578.1914.

$CF_3CO_2H.H_2$N-[Phe-Oxz(Ser)]$_2$-[Orn(Cbz)-Oxz(Ser)]$_2$-OH (19). The oxazole **18** was loaded onto 2-chlorotrityl chloride resin (0.275 g, resin capacity 1.01 mmol g^{-1}) according to general procedure 1 (loading 0.98 mmol g^{-1}). The appropriate Fmoc-protected oxazole building blocks **18** and **17** were coupled to the resin-bound amine according to the manual SPPS procedure 4 to give the resin-bound tetraoxazole. The *N*-terminal Fmoc protecting group was then removed (general procedure 2) and the resulting amine was cleaved from the resin (general procedure 5) to give a colourless solid, which was purified by preparative RP-HPLC [gradient of 20% to 70% acetonitrile (0.05% TFA) in

water (0.05% TFA) over 60 min] to afford the TFA salt of the desired linear tetraoxazole **19** (t_R = 38.8 min 0.150 g, 51%) as a colourless solid. m.p. 119–122 °C; $[\alpha]_D^{20}$ = +16.4 (*c* 0.8, MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ 1.58 (m, 4H), 1.95–2.19 (m, 4H), 3.16 (m, 4H), 3.27 (partially obscured m, 2H), 3.43 (m, 2H), 4.70 (dd, *J* = 6.2, 8.2 Hz, 1H), 5.03 (s, 2H), 5.04 (s, 2H), 5.34 (dd, *J* = 5.9, 8.8 Hz, 1H), 5.54 (m, 2H), 7.15–7.33 (m, 20H), 8.26 (s 1H), 8.30 (s, 1H), 8.42 (s, 1H), 8.52 (s, 1H), N–H and O–H signals not observed; ^{13}C NMR (100.6 MHz, CD_3OD): δ 26.6, 27.3, 30.1, 30.8, 39.6, 40.7, 41.1, 49.6, 49.9, 50.0, 67.4, 67.5, 122.4, 128.0, 128.7, 128.8, 128.9(7), 129.0(3), 129.4(6), 129.4(7), 129.6, 129.7, 130.3, 134.7, 136.7, 137.2, 137.7(0), 137.7(1), 138.3, 138.4, 143.5, 143.8, 144.8, 146.1, 158.9(6), 159.0(0), 161.3, 162.0, 162.2, 162.3, 162.5, 163.9, 164.7, 165.1, 165.5, three signals obscured or overlapping; HRMS (ESI) calcd. for $C_{56}H_{57}N_{10}O_{13}$ $[M + H]^+$ 1077.4101, found 1077.4093.

$CF_3CO_2H.H_2N$-[Leu-Oxz(Ser)]$_2$-[Orn(Cbz)-Oxz(Ser)]$_2$-OH (20). The oxazole **18** was loaded onto 2-chlorotrityl chloride resin (0.300 g, resin capacity 1.01 mmol g^{-1}) according to general procedure 1 (loading 1.00 mmol g^{-1}). The appropriate Fmoc-protected oxazole building blocks **18** and **16** were coupled to the resin-bound amine according to the manual SPPS procedure 4 to give the resin-bound tetraoxazole. The *N*-terminal Fmoc protecting group was deprotected (general procedure 2) and the resulting amine was cleaved from the resin (general procedure 5) to give a colourless solid, which was purified by preparative RP-HPLC [gradient of 20% to 80% acetonitrile (0.05% TFA) in water (0.05% TFA) over 60 min] to afford the TFA salt of the desired linear tetraoxazole **20** (t_R = 29.2 min, 81 mg, 27%) as a colourless solid. m.p. 124–125 °C; ^{1}H NMR (400 MHz, CD_3OD): δ 0.92–1.03 (m, 12H), 1.51–1.70 (m, 6H), 1.81–2.17 (m, 8H), 3.11–3.22 (m, 4H), 4.71 (dd, *J* = 6.5, 8.8 Hz, 1H), 5.04 (s, 4H), 5.28–5.35 (m, 2H), 5.42 (dd, *J* = 5.9, 9.5 Hz, 1H), 7.23–7.34 (m, 10H), 8.35 (s 1H), 8.36(9) (s, 1H), 8.37(4) (s, 1H), 8.53 (s, 1H), N–H and O–H signals not observed; ^{13}C NMR (100.6 MHz, CD_3OD): δ 22.0, 22.1, 22.7, 23.2, 25.7, 25.9, 27.3, 30.9, 41.1, 41.9, 42.4, 46.8, 67.4, 128.8, 128.9, 129.5, 135.6, 136.8, 137.3, 138.4, 143.6, 144.7, 145.6, 158.9, 161.8, 162.0, 162.5, 162.6, 164.7, 165.3, 165.6, 165.7, twelve signals obscured or overlapping; HRMS (ESI) calcd. for $C_{50}H_{61}N_{10}O_{13}$ $[M + H]^+$ 1010.0785, found 1010.0751.

H_2N-[Leu-Oxz(Ser)-Orn(Cbz)-Oxz(Ser)]$_2$-OH (21). The oxazole building block **18** was loaded onto 2-chlorotrityl chloride resin (80 mg, resin capacity 1.4 mmol g^{-1}) according to general procedure 1 (loading 0.73 mmol g^{-1}). The appropriate Fmoc-protected oxazoles **16** and **18** were coupled to the resin-bound amine according to the manual SPPS procedure 4 to give the resin-bound tetraoxazole. The *N*-terminal Fmoc protecting group was then removed (general procedure 2) and the resulting amine was cleaved from the resin according to general procedure 5 to afford the desired linear tetraoxazole **21** (74 mg, quant.) as a colourless solid, which was used in the next step without further purification. ^{1}H NMR (300 MHz, CD_3OD): δ 0.96 (m, 12H), 1.46–2.16 (complex m, 12H), 3.15 (m, 4H), 5.04 (s, 4H), 5.37 (m, 4H), 7.30 (s, 10 H), 8.14 (s, 1H), 8.33 (s, 1H), 8.37 (s, 1H) 8.44 (s, 1H), N–H and O–H signals not observed; LRMS (ESI) *m*/*z* = 1009 $[M + H]^+$.

Cyclo[Phe-Oxz(Ser)]$_2$-[Orn(Cbz)-Oxz(Ser)]$_2$ (22). The linear tetraoxazole **19** (65 mg, 0.060 mmol) was cyclised according to general procedure 6. Subjection of the crude material to preparative RP-HPLC [gradient of 30% to 80% acetonitrile (0.05% TFA) in water (0.05% TFA) over 60 min] gave the desired cyclic tetraoxazole **22** (t_R = 45.7 min, 31 mg, 48%) as a brown solid, m.p. 107–109 °C; $[\alpha]_D^{20}$ = +22.3 (*c* 0.6, MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ 1.52–1.71 (m, 4H), 1.95–2.12 (m, 4H), 3.25–3.45 (partially obscured m, 8H), 5.06 (s, 4H), 5.43 (m, 2H), 5.62 (t, *J* = 7.3 Hz, 1H), 5.67 (dd, *J* = 6.4, 8.1 Hz, 1H), 7.16–7.34 (m, 20H), 8.24 (s 1H), 8.32 (s, 1H), 8.36 (s, 2H), N–H signals not observed; ^{13}C NMR (100.6 MHz, CD_3OD): δ 27.3, 27.4, 31.4, 31.5, 40.1, 40.2, 41.2, 47.5, 47.6, 67.4, 128.2, 128.8, 129.0, 129.5, 129.6, 130.4(0), 130.4(4), 136.7, 136.8, 137.4, 137.5, 138.4, 143.8, 143.9, 144.0, 159.0, 161.7, 161.8, 162.0, 162.1, 164.9, 165.0, 165.5, 165.6, fourteen signals obscured or overlapping; HRMS (ESI) calcd. for $C_{56}H_{54}N_{10}O_{12}Na$ $[M + Na]^+$ 1081.3815, found 1081.3817.

Cyclo[Leu-Oxz(Ser)]$_2$-[Orn(Cbz)-Oxz(Ser)]$_2$ (23). The linear peptide **20** (81 mg, 0.080 mmol) was cyclised according to general procedure 6. Subjection of the crude material to preparative RP-HPLC [gradient of 30% to 80% acetonitrile (0.05% TFA) in water (0.05% TFA) over 60 min] gave the desired cyclic tetraoxazole **23** (t_R = 43.5 min, 40 mg, 51%) as a colourless oil. $[\alpha]_D^{20}$ = –32.8 (*c* 1.0, MeOH); ^{1}H NMR (400 MHz, CD_3OD); δ 0.99 (d, *J* = 6.4 Hz, 6H), 1.00 (d, *J* = 6.4 Hz, 6H), 1.50–1.74 (m, 7H), 1.84–2.17 (m, 7H), 3.19 (m, 4H), 4.99–5.07 (m, 5H), 5.25–5.45 (m, 2H), 5.49 (dd, *J* = 5.8, 9.5 Hz, 1H), 7.21–7.34 (m, 10H), 8.35 (s, 2H), 8.36 (s, 2H), N–H signals not observed; ^{13}C NMR (100.6 MHz, CD_3OD): δ 22.1, 23.1, 26.0, 27.3, 31.4, 31.5, 41.2, 43.1, 43.2, 46.0, 47.5, 67.4, 128.7, 128.9, 129.4, 136.8, 138.4, 143.8, 143.9(0), 143.9(2), 144.0, 158.9, 162.0, 162.1, 165.5(6), 165.6(2), 166.0(5), 166.0(8), sixteen signals obscured or overlapping; HRMS (ESI) calcd. for $C_{50}H_{58}N_{10}O_{12}Na$ $[M + Na]^+$ 1013.4128, found 1013.4117.

Cyclo[Leu-Oxz(Ser)-Orn(Cbz)-Oxz(Ser)]$_2$ (24a).

Method A. The linear tetraoxazole **21** (34 mg, 0.033 mmol) was cyclised according to general procedure 6. The crude material was purified by preparative RP-HPLC [gradient of 10% to 80% acetonitrile (0.05% TFA) in water (0.05% TFA) over 60 min] to give the desired cyclic tetraoxazole **24a** (t_R = 45.3 min, 12 mg, 38%) as a colourless solid. Characterisation data was identical to that obtained below (fraction A).

Method B. Bisoxazole **28** (0.264 g, 0.514 mmol) was cyclodimerised according to general procedure 11. The crude material was subjected to preparative RP-HPLC [gradient of 45% to 70% acetonitrile (0.1% TFA) in water (0.1% TFA) over 50 min] to give two fractions A and B.

Concentration of fraction A (t_R = 37.4 min) gave the desired cyclic dimer **24a** (0.122 g, 48%) as a colourless solid. m.p. 124–126 °C; $[\alpha]_D^{20}$ = –26.9 (*c* 1.0, MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ 0.99 (d, *J* = 6.6 Hz, 6H), 1.01 (d, *J* = 6.6 Hz, 6H), 1.53–1.75 (m, 6H), 1.89 (m, 2H), 2.02–2.14 (m, 6H), 3.20 (m, 4H), 5.06 (s, 4H), 5.46 (m, 2H), 5.49 (dd, *J* = 6.0, 9.6 Hz, 2H), 7.31 (m, 10H), 8.36 (s, 2H), 8.37 (s, 2H), N–H signals not observed; ^{13}C NMR (100.6 MHz, CD_3OD): δ 22.1, 23.1, 25.9, 27.3, 31.4, 41.1, 43.1, 46.0, 47.4, 67.4, 128.7, 128.9, 129.4, 136.8, 138.4, 143.8, 144.0, 159.0, 162.1, 165.5, 166.2, two signals obscured or overlapping; HRMS (ESI) calcd. for $C_{50}H_{58}N_{10}O_{12}Na$ $[M + Na]^+$ 1013.4128, found 1013.4131.

Concentration of fraction B (t_R = 42.0 min) gave the cyclic trimer **24b** (13 mg, 5%) as a colourless oil. ^{1}H NMR (400 MHz, CD_3OD): δ 0.97 (m, 18H), 1.49–1.73 (m, 10H), 1.86–2.21 (m, 11H), 3.17 (m, 6H), 5.03 (s, 6H), 5.25–5.43 (m, 6H), 7.23–7.36 (m, 15H), 8.3 (m, 6H), N–H signals not observed; ^{13}C NMR (100 MHz, CD_3OD): δ 22.9, 23.4, 25.9, 27.3, 30.8, 41.2, 42.5, 46.7, 47.4, 67.4, 128.8, 129.0, 129.5, 136.8, 136.9, 138.4, 143.6(9), 143.7(4), 158.9, 162.6, 162.7, 165.5, 166.0; HRMS (ESI) calcd. for $C_{75}H_{87}N_{15}O_{18}Na$ [M + Na]$^+$ 1508.6246, found 1508.6250.

Fmoc-Leu-Oxz(Ser)-Orn(Cbz)-Oxz(Ser)-OMe (26). Oxazole **12** (0.973 g, 2.24 mmol) was dissolved in anhydrous dichloromethane (7.5 mL) and treated with a solution of hydrochloric acid in dioxane (4 M, 8.4 mL, 33.6 mmol) under an atmosphere of nitrogen. The solution was stirred at rt for 4 h, after which time the mixture was concentrated under reduced pressure to give the hydrochloride salt **25** (0.973 g, quant.). The acid **16** (0.291 g, 0.693 mmol) and the hydrochloride salt **25** (0.256 g, 0.693 mmol) were then coupled in solution according to general procedure 6. The resulting crude material was purified by flash chromatography (silica gel; EtOAc–hexane, 2 : 1 v/v) to give the desired bisoxazole **26** (0.402 g, 77%) as a colourless foam. $[\alpha]_D^{20}$ = –12.4 (*c* 0.8, MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ 0.94 (d, *J* = 6.4 Hz, 3H), 0.97 (d, *J* = 6.6 Hz, 3H), 1.61 (m, 4H), 1.77 (s, 2H), 1.96 (m, 1H), 2.10 (m, 1H), 3.17 (t, *J* = 6.6 Hz, 2H), 3.85 (s, 3H), 4.20 (t, *J* = 6.9 Hz, 1H), 4.37–4.45 (m, 2H), 4.90 (m, 1H), 5.04 (s, 2H), 5.29 (dd, *J* = 6.5, 9.1 Hz, 1H), 7.23–7.34 (m, 7H), 7.37 (t, *J* = 7.3 Hz, 2H), 7.62 (d, *J* = 7.6 Hz, 1H), 7.66 (d, *J* = 7.6 Hz, 1H), 7.78 (d, *J* = 7.8 Hz, 2H), 8.33 (s, 1H), 8.41 (s, 1H), N–H signals not observed; ^{13}C NMR (75.5 MHz, CD_3OD): δ 22.0, 23.2, 25.7, 27.1, 30.9, 38.9, 41.1, 42.7, 48.2, 52.5, 67.3, 67.7, 120.9, 126.1, 128.1, 128.7, 128.9, 129.4, 134.0, 136.6, 138.4, 142.6, 143.4, 145.0, 145.2, 146.1, 158.3, 158.8, 162.6, 162.8, 165.9, 166.5; HRMS (ESI) calcd. for $C_{41}H_{43}N_5O_9Na$ [M + Na]$^+$ 772.2953, found 772.2958.

Fmoc-Phe-Oxz(Ser)-Orn(Cbz)-Oxz(Ser)-OMe (27). The acid **17** (1.52 g, 3.35 mmol) and the amine hydrochloride salt **25** (1.29 g, 3.35 mmol) were coupled in solution according to general procedure 7. Subjection of the crude material to flash chromatography (silica gel; EtOAc–hexane, 3 : 2 v/v) afforded the desired bisoxazole **27** (1.78 g, 68%) as a colourless solid. m.p. 83–85 °C; $[\alpha]_D^{20}$ = –9.86 (*c* 0.7, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.53–1.90 (m, 4H), 1.91–2.16 (m, 2H), 3.22 (m, 3H), 3.86 (s, 3H), 4.20 (m, 1H), 4.33–4.49 (m, 2H), 4.92 (br m, 1H), 5.07 (s, 2H), 5.23 (dd, *J* = 7.0, 14.3 Hz, 1H), 5.40 (dd, *J* = 8.4, 15.4 Hz, 1H), 5.53 (br d, *J* = 7.7 Hz, 1H), 7.01 (m, 2H), 7.21–7.42 (m, 12H), 7.52 (m, 2H), 7.76 (d, *J* = 7.3 Hz, 2H), 8.09 (s, 1H) 8.10 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 26.3, 31.2, 39.8, 40.5, 46.9, 47.3, 51.0, 52.4, 66.9, 67.2, 120.2, 125.2, 127.2, 127.4, 127.9, 128.2(3), 128.2(5), 128.7, 128.8, 129.4, 133.5, 135.5, 136.6, 141.5, 141.9, 143.8, 143.9, 144.2, 155.7, 156.6, 160.2, 161.5, 163.4, 164.4; HRMS (ESI) calcd. for $C_{44}H_{41}N_5O_9Na$ [M + Na]$^+$ 806.2797, found 806.2784.

H_2N-Leu-Oxz(Ser)-Orn(Cbz)-Oxz(Ser)-OH (28). The *N*-terminal Fmoc and *C*-terminal methyl ester protecting groups of bisoxazole **26** (1.14 g, 1.52 mmol) were removed according to general procedure 10. The crude material was purified by flash chromatography (silica gel; eluting first with neat $CHCl_3$, then $CHCl_3$–MeOH, 80 : 20 v/v) to give the fully-deprotected bisoxazole **28** (0.73 g, 94%) as a colourless solid. m.p. 135–136 °C; $[\alpha]_D^{20}$ = –4.75 (*c* 0.8, MeOH); ^{1}H NMR (300 MHz, CD_3OD): δ 0.96 (d, *J* = 6.6 Hz, 3H), 1.00 (d, *J* = 6.6 Hz, 3H), 1.52–1.73 (m, 3H), 1.85 (m, 1H), 1.96–2.20 (m, 3H), 3.18 (t, *J* = 6.6 Hz, 2H), 4.72 (dd, *J* = 6.2, 6.6 Hz, 1H), 5.05 (s, 2H), 5.34 (m, 1H), 7.32 (m, 5H), 8.36 (s, 1H), 8.53 (s, 1H), N–H and O–H signals not observed; ^{13}C NMR (75.5 MHz, CD_3OD): δ 22.2, 22.7, 25.7, 27.2, 31.0, 41.1, 42.0, 67.4, 128.7, 128.9, 129.5, 136.0, 137.3, 138.4, 144.6, 145.3, 158.9, 161.8, 162.0, 165.0, 165.5, two signals obscured or overlapping; HRMS (ESI) calcd. for $C_{25}H_{31}N_5O_7Na$ [M + Na]$^+$ 536.2116, found 536.2119.

H_2N-Phe-Oxz(Ser)-Orn(Cbz)-Oxz(Ser)-OH (29). The *N*-terminal Fmoc and *C*-terminal methyl ester protecting groups of bisoxazole **27** (1.66 g, 2.12 mmol) were removed according to general procedure 10. The crude material was purified by flash chromatography (silica gel; eluting first with neat $CHCl_3$, then $CHCl_3$–MeOH, 80 : 20 v/v) to afford the bisoxazole with a free *C*- and *N*-termini **29** (1.04 g, 89%) as a colourless solid. m.p. 134–136 °C; $[\alpha]_D^{20}$ = –0.25 (*c* 0.8, MeOH); ^{1}H NMR (300 MHz, CD_3OD): δ 1.58 (m, 2H), 1.94–2.19 (m, 2H), 3.17 (t, *J* = 5.9 Hz, 2H), 3.39 (m, 2H), 4.91 (partially obscured m, 2H), 5.05 (m, 2H), 7.17 (m, 2H), 7.25–7.34 (m, 8H), 8.35 (s, 1H), 8.45 (s, 1H), N–H and O–H signals not observed; ^{13}C NMR (75.5 MHz, CD_3OD): δ 27.2, 31.0, 39.2, 41.1, 51.2, 67.4, 128.7, 128.8(8), 128.9(4), 129.5, 130.1, 130.4, 135.2, 136.2, 137.2, 138.4, 144.5, 145.2, 158.9, 161.4, 162.0, 165.2, 165.4, one signal obscured or overlapping; HRMS (ESI) calcd. for $C_{28}H_{29}N_5O_7Na$ [M + Na]$^+$ 570.1959, found 570.1963.

H_2N-Orn(Cbz)-Oxz(Ser)-Ala-Oxz(Ser)-OH (30). The *N*-terminal Fmoc and *C*-terminal methyl ester protecting groups of bisoxazole **32** (0.216 g, 0.305 mmol) were removed according to general procedure 10. Subjection of the crude material to flash chromatography (silica gel; eluting first with neat $CHCl_3$, then $CHCl_3$–MeOH, 8 : 2 v/v) gave the fully-deprotected bisoxazole **30** (0.133 g, 90%) as a colourless solid. m.p. 152–155 °C; ^{1}H NMR (400 MHz, CD_3OD): δ 1.56 (m, 2H), 1.63 (d, *J* = 6.6 Hz, 3H), 2.03 (m, 2H), 3.15 (t, *J* = 6.6 Hz, 2H), 4.57 (t, *J* = 6.6 Hz, 1H), 5.04 (s, 2H), 5.36 (m, 1H), 7.25–7.33 (m, 5H), 8.14 (s, 1H), 8.44 (s, 1H), N–H and O–H signals not observed; ^{13}C NMR (100.6 MHz, CD_3OD): δ 19.0, 26.7, 31.0, 40.9, 44.4, 67.4, 128.8, 129.0, 129.5, 137.1, 138.4, 143.4, 144.3, 159.0, 159.5, 161.9, 163.1, 165.4, 167.9, one signal obscured or overlapping; HRMS (ESI) calcd. for $C_{22}H_{25}N_5O_7Na$ [M + Na]$^+$ 494.1652, found 494.1644.

Fmoc-Orn(Cbz)-Oxz(Ser)-Ala-Oxz(Ser)-OMe (32). The acid **18** (0.784 g, 1.41 mmol) and the previously prepared amine hydrochloride salt **31** (0.269 g, 1.41 mmol) were coupled in solution according to general procedure 7. Subjection of the crude material to flash chromatography (silica gel; hexane–EtOAc, 3 : 1 v/v) gave the desired bisoxazole **32** (0.589 g, 59%) as a colourless foam. $[\alpha]_D^{20}$ = –11.0 (*c* 0.7, MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ 1.56 (br m, 2H), 1.63 (d, *J* = 7.1 Hz, 3H), 1.79–2.03 (m, 2H), 3.15 (td, *J* = 2.7, 6.9 Hz, 2H), 3.85 (s, 3H), 4.21 (t, *J* = 6.6 Hz, 1H), 4.41 (d, *J* = 6.6 Hz, 2H), 4.84 (m, 1H), 5.05 (s, 2H), 5.35 (q, *J* = 7.1 Hz, 1H), 7.24–7.34 (m, 7H), 7.37 (t, *J* = 7.3 Hz, 2H), 7.64 (d, *J* = 7.6 Hz, 1H), 7.66 (d, *J* = 7.6 Hz, 1H), 7.82 (d, *J* = 7.6 Hz, 2H), 8.33 (s, 1H), 8.42 (s, 1H), N–H signals not observed; ^{13}C NMR (100.6 MHz, CD_3OD): δ 18.6, 27.2, 31.1, 41.1, 44.3, 50.5, 52.5, 67.4, 67.8, 120.9, 126.2, 128.2, 128.8, 129.0, 129.5, 134.0, 136.7, 138.4, 142.6, 143.5, 145.1, 145.2, 146.2, 158.4, 159.0, 162.4, 162.9,

166.0, 166.7, one signal obscured or overlapping; HRMS (ESI) calcd. for $C_{38}H_{37}N_5O_9Na$ $[M + Na]^+$ 730.2489, found 730.2479.

Cyclo[Phe-Oxz(Ser)-Orn(Cbz)-Oxz(Ser)]$_2$ (33a). The bisoxazole **29** (0.250 g, 0.456 mmol) was cyclodimerised according to general procedure 11. Subjection of the crude material to preparative RP-HPLC [gradient of 45% to 70% acetonitrile (0.1% TFA) in water (0.1% TFA) over 50 min] gave the desired cyclic dimer **33a** (t_R = 37.0 min, 0.112 g, 46%) as a colourless solid. m.p. 125–127 °C; ^{1}H NMR (400 MHz, CD_3OD); δ 1.55–1.70 (m, 4H), 1.97–2.18 (m, 4H), 3.22 (t, J = 6.9 Hz, 4H), 3.29 (partially obscured m, 2H), 3.38 (dd, J = 7.0, 13.9 Hz, 2H), 5.06 (s, 4H), 5.42 (dd, J = 6.4, 8.6 Hz, 2H), 5.64 (dd, J = 7.0, 7.8 Hz, 2H), 7.15–7.32 (m, 20H), 8.25 (s, 2H), 8.30 (s, 2H), N–H signals not observed; ^{13}C NMR (100.6 MHz, CD_3OD): δ 27.4, 31.3, 40.2, 41.2, 47.4, 67.4, 128.2, 128.8, 129.0, 129.5, 129.6, 130.4, 136.7, 136.8, 137.4, 138.4, 143.8, 143.9, 159.0, 161.8, 162.0, 165.1, 165.4, one signal obscured or overlapping; HRMS (ESI) calcd. for $C_{56}H_{54}N_{10}O_{12}Na$ $[M + Na]^+$ 1081.3815, found 1081.3819.

Cyclo[Ala-Oxz(Ser)-Orn(Cbz)-Oxz(Ser)]$_2$ (34a). The bisoxazole **30** (70 mg, 0.144 mmol) was cyclodimerised according to general procedure 11. Subjection of the crude material to preparative RP-HPLC [gradient of 20% to 70% acetonitrile (0.05% TFA) in water (0.05% TFA) over 60 min] gave two fractions A and B.

Concentration of fraction A (t_R = 44.8 min) gave the desired cyclic dimer **34a** (23 mg, 36%) as a colourless powder. m.p. 115–118 °C [lit.[24] m.p. 116–118 °C]; $[\alpha]_D^{25} = -101$ (c 0.5, $CHCl_3$) [lit.[24] $[\alpha]_D^{25} = -108$ (c 0.51, $CHCl_3$)].

Concentration of fraction B (t_R = 51.0 min) gave the cyclic trimer **34b** (11 mg, 12%) as a colourless powder. ^{1}H NMR (400 MHz, CD_3OD): δ 1.51–1.67 (m, 15H), 1.99 (m, 4H), 2.16 (m, 2H), 3.17 (m, 6H), 5.03 (s, 6H), 5.29–5.40 (m, 6H), 7.23–7.34 (m, 15H), 8.36 (m, 6H), N–H signals not observed; HRMS (ESI) calcd. for $C_{66}H_{69}N_{15}O_{18}Na$ $[M + Na]^+$ 1382.4837, found 1382.4839.

Cyclo[Ala-Oxz(Ser)-Orn(Dpa)-Oxz(Ser)]$_2$ (1). The Cbz-protected side chains of cyclic peptide **34a** (18 mg, 0.020 mmol) were functionalised with Dpa groups according to general procedure 12. Subjection of the crude material to preparative RP-HPLC [gradient of 5% to 40% acetonitrile (0.1% TFA) in water (0.1% TFA) over 50 min] gave the desired Dpa-functionalised cyclic peptide **1** (t_R = 26.9 min, 11 mg, 55%) as a colourless oil. $[\alpha]_D^{20} = -74.1$ (c 0.60, MeOH) [lit.[9] $[\alpha]_D^{20} = -86.8$ (c 0.65, MeOH)].

Cyclo[Leu-Oxz(Ser)-Orn(Dpa)-Oxz(Ser)]$_2$ (3). The Cbz-protected side chains of cyclic peptide **24a** (51 mg, 0.050 mmol) were functionalised with Dpa groups according to general procedure 12. The crude material was purified by preparative RP-HPLC [gradient of 2% to 40% acetonitrile (0.1% TFA) in water (0.1% TFA) over 50 min] to give the desired Dpa-functionalised cyclic peptide **3** (t_R = 40.3 min, 41 mg, 75%) as a colourless solid. m.p. 81–83 °C; $[\alpha]_D^{20} = -17.7$ (c 0.7, MeOH); ^{1}H NMR (400 MHz, CD_3OD); δ 1.00 (d, J = 6.7 Hz, 6H), 1.02 (d, J = 6.9 Hz, 6H), 1.70 (m, 2H), 1.83–2.20 (m, 12H), 3.30 (partially obscured m, 4H), 4.54 (s, 8H), 5.38 (dd, J = 5.6, 9.1 Hz, 2H), 5.49 (dd, J = 5.9, 9.7 Hz, 2H), 7.52 (ddd, J = 1.2, 5.1, 7.8 Hz, 4H), 7.57 (ddd, J = 0.9, 1.2, 7.8 Hz, 4H), 7.98 (ddd, J = 1.7, 7.8, 7.8 Hz, 4H), 8.38 (s, 2H), 8.39 (s, 2H), 8.66 (ddd, J = 0.9, 1.7, 5.1 Hz, 4H), N–H signals not observed; ^{13}C NMR (100.6 MHz, CD_3OD): δ 22.1, 22.2, 23.1, 25.9, 31.2, 42.8, 46.0, 46.9, 55.6, 58.5, 125.8(0), 125.8(4), 136.7, 136.8, 140.7, 144.0, 144.2, 149.3, 152.6, 162.0, 162.2, 165.0, 166.1; HRMS (ESI) calcd. for $C_{58}H_{67}N_{14}O_8$ $[M + H]^+$ 1087.5261, found 1087.5276.

Cyclo[Phe-Oxz(Ser)-Orn(Dpa)-Oxz(Ser)]$_2$ (4). The Cbz-protected side chains of cyclic peptide **33a** (0.112 g, 0.106 mmol) were functionalised with Dpa groups according to general procedure 12. Subjection of the crude material to preparative RP-HPLC [gradient of 5% to 40% acetonitrile (0.1% TFA) in water (0.1% TFA) over 50 min] gave the desired Dpa-functionalised cyclic peptide **4** (t_R = 39.5 min, 93 mg, 89%) as a colourless solid. m.p. 76–78 °C; $[\alpha]_D^{20} = -19.9$ (c 0.7, MeOH); ^{1}H NMR (400 MHz, CD_3OD); δ 1.83–2.21 (m, 8H), 3.31 (m, 4H), 3.36 (dd, J = 8.5, 13.9 Hz, 2H), 3.46 (dd, J = 6.9, 13.9 Hz, 2H), 4.56 (s, 8H), 5.36 (dd, J = 6.1, 9.0 Hz, 2H), 5.67 (dd, J = 6.9, 8.5 Hz, 2H), 7.16–7.27 (m, 10H), 7.49 (dd, J = 5.4, 7.6 Hz, 4H), 7.54 (d, J = 7.6 Hz, 4H), 7.94 (ddd, J = 1.6, 7.6, 7.6 Hz, 4H), 8.31 (s, 2H), 8.35 (s, 2H), 8.65 (d, J = 5.4 Hz, 4H), N–H signals not observed; ^{13}C NMR (100.6 MHz, CD_3OD): δ 22.2, 31.1, 39.9, 46.9, 58.6, 125.7, 128.1, 129.6, 130.3, 136.7, 136.8, 137.6, 140.4, 144.1, 149.5, 152.5, 161.8, 162.1, 164.9, 165.2, four signals obscured or overlapping; HRMS (ESI) calcd. for $C_{64}H_{63}N_{14}O_8$ $[M + H]^+$ 1155.4948, found 1155.4931.

Cyclo[Leu-Oxz(Ser)]$_2$-[Orn(Dpa)-Oxz(Ser)]$_2$ (5). The Cbz-protected side chains of cyclic peptide **23** (40 mg, 0.040 mmol) were functionalised with Dpa groups according to general procedure 12. Subjection of the crude material to preparative RP-HPLC [gradient of 5% to 50% acetonitrile (0.05% TFA) in water (0.05% TFA) over 50 min] gave the desired Dpa-functionalised cyclic peptide **5** (t_R = 35.0 min, 38 mg, 87%) as a colourless oil. $[\alpha]_D^{20} = -34.0$ (c 0.8, MeOH); ^{1}H NMR (400 MHz, CD_3OD); δ 0.98–1.03 (m, 12H), 1.64–1.74 (m, 2H), 1.83–2.22 (m, 12H), 3.23–3.33 (partially obscured m, 4H), 4.53 (s, 8H), 5.33–5.39 (m, 2H), 5.46–5.53 (m, 2H), 7.54 (m, 4H), 7.60 (d, J = 7.8 Hz, 4H), 8.01 (m, 4H), 8.36 (m, 2H), 8.38 (m, 2H), 8.68 (d, J = 5.1 Hz, 4H), N–H signals not observed; ^{13}C NMR (100.6 MHz, CD_3OD): δ 22.0(6), 22.1(1), 22.3, 23.1, 25.9, 31.0, 31.1, 43.0, 45.9, 46.1, 46.9, 55.5, 55.6, 58.4, 125.9, 126.0, 136.7, 136.8, 136.9, 141.1, 143.8, 144.1, 144.3, 149.0, 152.7, 161.3, 161.7, 162.0, 162.2, 162.3, 165.0, 165.1, 166.0, 166.1, ten signals obscured or overlapping; HRMS (ESI) calcd. for $C_{58}H_{67}N_{14}O_8$ $[M + H]^+$ 1087.5261, found 1087.5272

Cyclo[Phe-Oxz(Ser)]$_2$-[Orn(Dpa)-Oxz(Ser)]$_2$ (6). The Cbz-protected side chains of cyclic peptide **22** (38 mg, 0.035 mmol) were functionalised with Dpa groups according to general procedure 12. Subjection of the crude material to preparative RP-HPLC [gradient of 5% to 50% acetonitrile (0.05% TFA) in water (0.05% TFA) over 50 min] gave the desired Dpa-functionalised cyclic peptide **6** (t_R = 33.8 min, 31 mg, 77%) as a colourless solid. m.p. 62–64 °C; ^{1}H NMR (400 MHz, CD_3OD); δ 1.83–2.23 (m, 8H), 3.32–3.48 (partially obscured m, 8H), 4.56 (s, 4H), 4.58 (s, 4H), 5.36 (dd, J = 6.1, 8.8 Hz, 1H), 5.41 (dd, J = 5.9, 9.1 Hz, 1H), 5.61 (m, 1H), 5.69 (m, 1H), 7.18–7.29 (m, 10H), 7.46 (m, 4H), 7.52 (d, J = 7.6 Hz, 4H), 7.87–7.94 (m, 4H), 8.23 (s 1H), 8.33 (s, 1H), 8.39 (m, 2H), 8.64 (m, 4H), N–H signals not observed; ^{13}C NMR (100.6 MHz, CD_3OD): δ 22.1(9), 22.2(1), 30.9(6), 31.0(1), 40.0, 40.1, 46.8, 47.0, 55.6, 58.7, 125.6(1), 125.6(2), 128.2, 129.6, 130.3, 130.4, 136.6(8), 136.7(3), 136.8, 137.5, 137.6, 140.0(8), 140.1(0), 143.8, 144.1,

144.2, 144.3, 149.7(9), 149.8(3), 152.4, 152.5, 161.6(7), 161.7(4), 162.1(7), 162.2(0), 164.9, 164.9(6), 165.0(0), ten signals obscured or overlapping; HRMS (ESI) calcd. for $C_{64}H_{63}N_{14}O_8$ $[M + H]^+$ 1155.4948, found 1155.4939.

Acknowledgements

The authors thank the Australian Research Council for financial support (DP0877726) and Dr Kelvin Picker (The University of Sydney) for technical support with HPLC purification.

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28 A less competitive solvent was used in the present studies (previous studies employed 5 mM HEPES buffer and 145 mM NaCl) because the phenylalanine derivatives **4**·$\mathbf{Zn_2}$ and **6**·$\mathbf{Zn_2}$ were not sufficiently soluble in the NaCl solution to allow binding constants to be determined .