A Cyclooligomerisation Approach to Backbone-Modified Cyclic Peptides Bearing Guanidinium Arms

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Dedicated to Professor Gerry Pattenden on the occasion of his 70th birthday.

Abstract: Cyclooligomerisation of the pentafluorophenyl ester derivatives of oxazoles, derived from dipeptides containing protected ornithine, diaminobutanoic acid and diaminopropionic acid residues, gives the cyclic trimers as the major products. Deprotection and treatment with guanidinylating agents provides efficient access to backbone rigidified cyclic peptides with guanidinium functionalised side chains.

Key words: peptides, macrocycles, cyclisation, heterocycles, amides

The Lissoclinum class of natural products (e.g., westelliamide[1] and ascidiacyclamide[2]) consists of cyclic hexa-, hepta- and octapeptides that are characterised by the presence of azole heterocycles derived from serine, threonine or cysteine residues, alternating with amino acid residues.[3] These molecules often exhibit interesting biological activities (e.g., cytotoxic, antibiotic or immunoregulatory activity) and this has led to great interest in their synthesis.[3,4] It is well established that the alternating pattern of heterocycles and amide bonds in the backbone of these naturally occurring macrocycles results in a network of bifurcated hydrogen bonds between the azole nitrogen atoms and the amide protons. This hydrogen-bonding network is strongest in the hexapeptide derivatives[5] and holds the macrocyclic backbone of the compounds relatively flat. If all side chains are of the same configuration, they are presented on the same face of the macrocycle in a convergent manner. This, together with the development of efficient syntheses of analogues of the natural products,[1,5,6] has led to recent interest in the use of such molecules as molecular scaffolds for the development of molecular receptors, chiral ligands, artificial proteins and combinatorial libraries.[7] Whereas syntheses of cyclic peptide scaffolds incorporating oxazoles, thiazoles and/or imidazoles with a variety of side chains have been reported, there are relatively few reports in the literature of their functionalisation with moieties capable of molecular recognition.[7b,m,8] We report here the efficient synthesis of Lissoclinum analogues bearing guanidinium arms (**1**–**4**; Figure 1). Guanidinium groups offer both hydrogen-bonding and electrostatic interactions over a wide pH range, as a result of their high pK_a (typically between 12 and 13) and this has led to their incorporation in numerous anion receptors and enzyme mimics.[9] Cyclic peptide scaffolds bearing guanidinium arms should find application as anion receptors, enzyme mimics[9] and, given their cationic peptide structure, may have antibiotic or other biological activity.

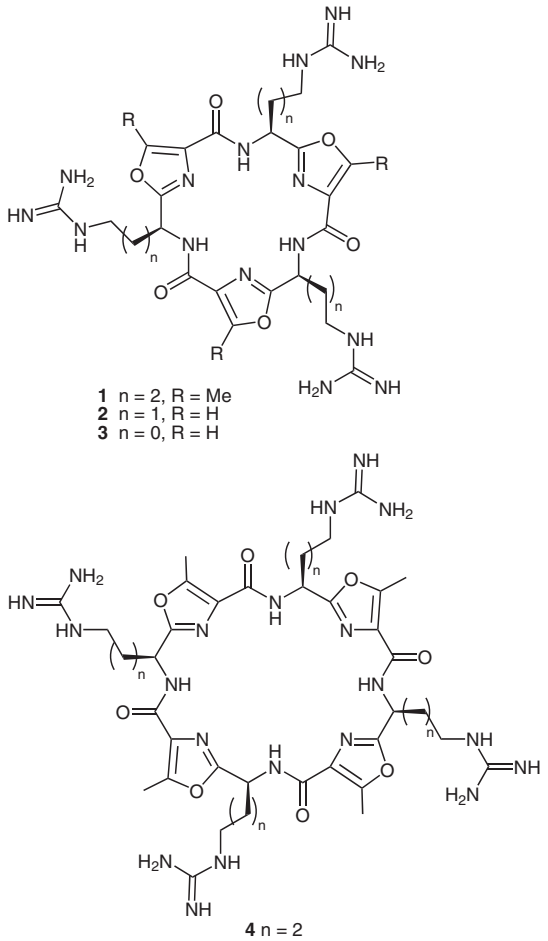

Figure 1 Structures of the guanidinium functionalised cyclic peptides **1**–**4**

There are two synthetic approaches to guanidinium-functionalised lissoclinamide analogues. The first of these involves synthesising the oxazole building blocks with a protected guanidinium group in place, followed by cyclooligomerisation to give the required macrocycles; in cyclooligomerisations of this type, the cyclic trimer and

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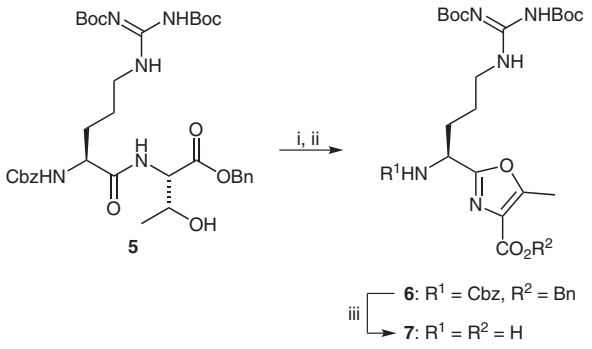
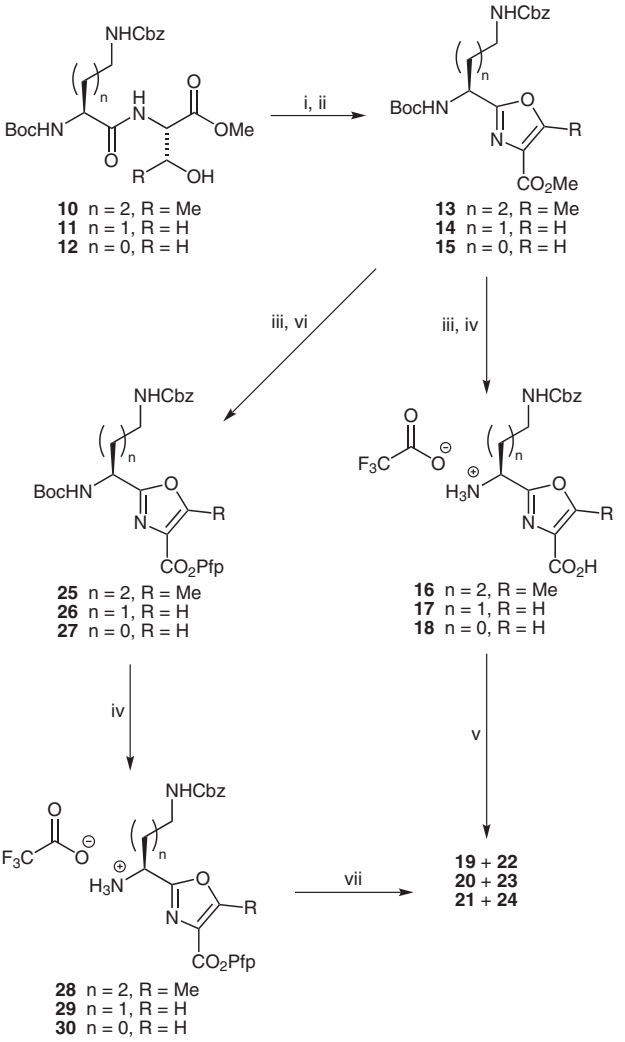
cyclic tetramer are normally the major products, with the trimer/tetramer ratio dependent on the side chain structure and normally favouring the cyclic trimer.[5,6,7a] The second approach involves post-functionalisation of the side arms attached to a prepared cyclic peptide scaffold. Our initial approach to the target guanidinium compounds used the former strategy, that is, via cyclooligomerisation of an oxazole amino acid building block derived from arginine. Dipeptide **5**, which was prepared by coupling Cbz-Arg(Boc)$_2$-OH with NH$_2$-Thr-OBn under standard solution-phase peptide coupling conditions, was converted into the corresponding oxazole **6**, using a well-established two-step (cyclodehydration followed by oxidation) procedure (Scheme 1).[10]



Scheme 1 Synthesis of an arginine-derived oxazole. *Reagents and conditions:* (i) DAST, CH_2Cl_2, –78 °C, then K_2CO_3, H_2O; (ii) DBU, $BrCCl_3$, CH_2Cl_2, 0 °C, 60% over 2 steps; (iii) H_2, Pd/C, MeOH, 90%.

The N- and C-terminal protecting groups were then removed in a single hydrogenolysis step to provide oxazole **7** in 90% yield. Unfortunately, all attempts to cyclooligomerise **7** gave only traces of the required cyclic trimer **8** or cyclic tetramer **9** (see Scheme 3 for structures of **8** and **9**). We therefore decided to synthesise these compounds using the alternative strategy, commencing from the corresponding amino-functionalised cyclic peptides derived from ornithine (Orn). In addition, we prepared amino-functionalised cyclic peptides derived from diaminobutanoic acid (Dab) and diaminopropionic acid (Dpr), in which the number of methylene units between the oxazole and the amine group was varied, to provide molecular scaffolds with functional groups at different distances from the peptide scaffold. This was undertaken to investigate the effect this distance has on anion binding of the corresponding guanidinium derivatives, since it has previously been shown that similar macrocycles can bind anions through the amide protons.[11] Hence, the orthogonally protected Boc-Orn(Cbz)-OH was coupled with threonine methyl ester, while the similarly protected amino acids with shortened side chains, Boc-Dab(Cbz)-OH and Boc-Dpr(Cbz)-OH, were each coupled with serine methyl ester, under standard peptide coupling conditions to give the dipeptides **10**–**12**, respectively (Scheme 2).

The dipeptides were then treated with DAST to give the intermediate oxazolines, which were not isolated but immediately treated with DBU/$BrCCl_3$ to give the corresponding oxazoles **13**–**15**. Hydrolysis of the methyl esters, followed by treatment with trifluoroacetic acid to remove the Boc protecting groups, furnished the amino acids **16**–**18** as the trifluoroacetate salts. Cyclooligomerisation of **16**–**18** was attempted using a variety of peptide coupling reagents (DPPA, FDPP, HBTU), with FDPP giving the best yields of cyclic trimers **19**–**21** and cyclic tetramers **22**–**24** in each case. However, in all cases, purification of these compounds from the coupling reagent by-products (in particular, diphenylphosphinic acid) was problematic. Therefore, we investigated an alternative method of cyclooligomerisation in which the oxazoles **13**–**15** were converted into the corresponding pentafluorophenyl esters **25**–**27** after ester hydrolysis and treatment with pentafluorophenyl acetate.[12] Selective removal of the Boc protecting groups from **25**–**27** was achieved by treatment with trifluoroacetic acid to give the amines **28**–**30**, which were immediately subjected to cyclooligomerisation in DMF, in the presence of diisopropylethylamine



Scheme 2 Synthesis of cyclic peptides **19**–**24**. *Reagents and conditions*: (i) DAST, CH_2Cl_2, –78 °C then K_2CO_3, H_2O; (ii) DBU, $BrCCl_3$, CH_2Cl_2, 0 °C, 59–90% over 2 steps; (iii) NaOH, MeOH, H_2O, 66–100%; (iv) TFA, CH_2Cl_2, 95–100%; (v) FDPP, DIPEA, DMF, 0.05 M, 11–21%; (vi) pentafluorophenyl 2,2,2-trifluoroacetate, CH_2Cl_2, DMF, 51–98%; (vii) DIPEA, DMF, 0.05 M, 24–51%.

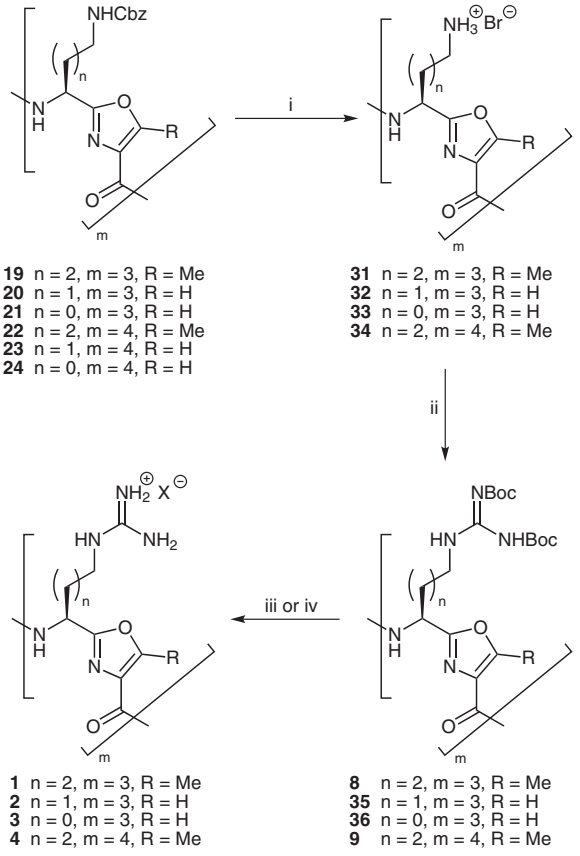
(DIPEA). In all cases, this procedure gave significantly higher yields of the cyclic trimers **19**–**21** and cyclic tetramers **22**–**24** than were obtained from the acids **16**–**18**, respectively, using any of the coupling reagents; furthermore, the pentafluorophenol by-product was easily removed by a base washing.[13] Notably, cyclooligomerisation of the pentafluorophenyl ester **30** gave cyclic trimer **21** in 41% yield, which is more than twice the yield (19%) previously obtained when DPPA was used as a coupling agent to cyclise **18** in its free-base form.[6k] The cyclic oligomers obtained from the pentafluorophenyl esters were readily separated by either flash column chromatography or preparative reverse-phase HPLC. In all cases, the cyclic trimers were the major products (24–42%), with only trace amounts of the cyclic tetramers isolated in most cases.

The cyclic trimers **19**–**21** and the cyclic tetramer **22** were treated with a solution of HBr in acetic acid to provide the amines **31**–**34**, respectively, as their hydrobromide salts (Scheme 3). Several reagents were then investigated for the conversion of the amines **31**–**34** into the corresponding guanidine derivatives **8**, **35**, **36** and **9**, respectively. We found that this was best achieved in a two-step procedure by guanidinylation with either *N*,*N*′-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine[14] or the more active *N*,*N*′-bis(*tert*-butoxycarbonyl)-*N*″-triflylguanidine[15] (with the latter generally giving higher yields),[16] followed by removal of the Boc protecting groups upon treatment with either trifluoroacetic acid or tin(IV) chloride in ethyl acetate to provide the guanidinium derivatives **1**–**4** as the trifluoroacetate or chloride salts, respectively. Yields for the guanidinylation reaction were found to decrease as the length of spacer between the cyclic peptide scaffold and the amine to be guanidinylated was shortened. Given the bulky nature of the Boc-protected guanidinylation reagents, this is most likely a result of an increase in steric crowding about the macrocycles as the side chains become shorter.

The tris- and tetra-guanidinium compounds **1**–**4** were tested for their activity against three fungal strains (*C. albicans*, *A. fumigatus* and *C. neoformans*) and four bacteria (*E. coli*, *P. aeruginosa*, *S. aureus* and MRSA) but none showed any significant antifungal or antibacterial activity against any of the pathogens tested. This is in striking contrast to antibacterial activity observed for similar compounds in which four guanidinium groups are attached to calixarene scaffolds[17] and is most likely a result of the lack of amphipathicity in the cyclic peptide structures.[18]

In summary, the backbone-modified cyclic peptides **1**–**4** have been efficiently synthesised via the cyclooligomerisation of Orn, Dab and Dpr derived oxazoles, followed by deprotection and guanidinylation. We are currently investigating the ability of **1**–**4** to bind anions and the results of these studies will be reported in due course.

Supporting Information for this article containing all experimental prodedures is available online at http://www.thieme-connect.com/ejournals/toc/synlett.



Scheme 3 Synthesis of cyclic peptides **1**–**4**. *Reagents and conditions*: (i) HBr, AcOH, quant.; (ii) *N*,*N*′-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine, DIPEA, DMF, 23–57% or *N*,*N*′-bis(*tert*-butoxycarbonyl)-*N*″-triflylguanidine, DIPEA, CH_2Cl_2, 42–82%; (iii) TFA, CH_2Cl_2, 90–100%, $X^- = CF_3CO_2^-$; (iv) $SnCl_4$, EtOAc, 81–100%, $X^- = Cl^-$.

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(13) Representative cyclooligomerisation procedure: Compound **25** (1.19 g, 1.95 mmol) was dissolved in CH_2Cl_2 (5 mL) and TFA (15 mL) was added in one portion. The reaction mixture was stirred at r.t. for 2 h and the solvents were removed to give the trifluoroacetate salt **28** as a yellow foam. This was dissolved in DMF (39 mL) and DIPEA (1.5 mL, 8.78 mmol) was added in one portion. The pale-orange solution was stirred at r.t. for 3 d, then the DMF was removed in vacuo to give an orange oil. This was redissolved in EtOAc (50 mL) and washed with 1 M NaOH (2 × 3 mL), brine (2 × 7 mL) and H_2O (2 × 7 mL), dried (Na_2SO_4) and concentrated under reduced pressure to give a dark-orange foam. This was dissolved in EtOAc–hexane (1:2) and passed down a silica plug (EtOAc–hexane, 1:2→2:1) to give a mixture of cyclic oligomers. These were then separated by preparative HPLC {gradient 50→100% B [MeCN–TFA (100:0.1)] in A [H_2O–MeCN–TFA (95:5:0.1)] over 60 mins} to give the cyclic trimer **19**, t_R = 28.0 min (225 mg, 42%) and cyclic tetramer **22**, t_R = 30.1 min (58 mg, 9%) as colourless foams. Data for **19**: $[\alpha]_D^{20}$ –21.6 (*c* 1.0, $CHCl_3$). ^1H NMR (200 MHz, $CDCl_3$): δ = 8.33 (d, *J* = 4.4 Hz, 3 H), 7.42–7.24 (m, 15 H), 5.22 (m, 3 H), 5.14 (m, 3 H), 5.06 (s, 6 H), 3.24 (m, 6 H), 2.63 (s, 9 H), 2.12–1.90 (m, 12 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 160.79, 160.75, 156.4, 153.9, 136.5, 128.3, 128.2, 127.9, 127.8, 66.4, 47.5, 40.5, 32.0, 25.2, 11.5. MS (ESI): *m*/*z* = 1010 $[M + Na]^+$. HRMS (ESI): *m*/*z* $[M + Na]^+$ calcd. for $C_{51}H_{57}N_9O_{12}Na$: 1010.4019; found: 1010.3999. Data for **22**: $[\alpha]_D^{20}$ –73.3 (*c* 0.9, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.40 (d, *J* = 9.0 Hz, 4 H), 7.31–7.27 (m, 20 H), 5.36 (app dt, *J* = 9.0, 7.5 Hz, 4 H), 5.12 (m, 4 H), 5.04 (s, 8 H), 3.23 (m, 8 H), 2.60 (s, 12 H), 2.08 (m, 4 H), 1.95 (m, 4 H), 1.62–1.60 (m, 8 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 161.1, 161.0, 156.7, 154.3, 136.5, 128.6, 128.4, 128.2, 128.1, 66.7, 45.7, 40.6, 31.2, 26.3, 11.8. MS (ESI): *m*/*z* (%) = 1339 (100) $[M + Na]^+$. HRMS: *m*/*z* $[M + Na]^+$ calcd for $C_{68}H_{76}N_{12}NaO_{16}$: 1339.5395; found: 1339.5407.
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(16) Representative guanidinylation procedure: To a suspension of **31** (0.12 g, 0.15 mmol) in CH_2Cl_2 (5 mL), DIPEA (0.14 mL, 0.75 mmol) was added and the solution was stirred at r.t. for 15 min. *N*,*N*′-di-Boc-*N*″-triflylguanidine (0.32 g, 0.83 mmol) in CH_2Cl_2 (2 mL) was added via cannula and the reaction mixture was stirred at r.t. for 21 h. The mixture was diluted with CH_2Cl_2 (10 mL) and washed with 2 M $NaHSO_4$ (10 mL), $NaHCO_3$ (10 mL), brine (10 mL), dried ($MgSO_4$) and concentrated under reduced pressure to give a pale-brown foam. The crude material was purified by flash chromatography (hexane–EtOAc, 2:3) to give **9** as a colourless foam (0.16 g, 82%); $[\alpha]_D^{20}$ –1.0 (*c* 3.5, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 11.49 (s, 3 H), 8.32 (s, 3 H), 8.31 (s, 3 H), 5.14 (m, 3 H), 3.47–3.45 (m, 6 H), 2.66 (s, 9 H), 2.15 (m, 3 H), 1.98 (m, 3 H), 1.81 (m, 3 H), 1.55 (m, 3 H), 1.48 (s, 54 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 161.0, 160.8, 156.1, 154.2, 153.3, 128.5, 83.3, 79.5, 47.8, 40.6, 32.1, 28.3, 28.1, 24.7, 11.7. MS (ESI): *m*/*z* (%) = 1312 (100) $[M + H]^+$, 1334 (20) $[M + Na]^+$. HRMS: *m*/*z* $[M + H]^+$ calcd for $C_{60}H_{94}N_{15}O_{18}$: 1312.6896; found: 1312.6900.
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