The Synthesis of Novel Thiazole Containing Cyclic Peptides *via* Cyclooligomerisation Reactions

Anna Bertram, Jeffery S. Hannam, Katrina A. Jolliffe, Felix González-López de Turiso, Gerald Pattenden*

School of Chemistry, University of Nottingham, Nottingham, UK, NG7 2RD

Fax +44 (0)115 951 3564; E-mail: GP@nottingham.ac.uk

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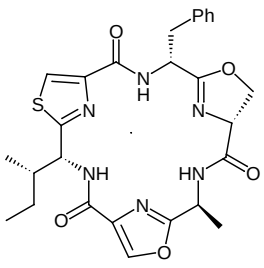
Abstract: Thiazole containing cyclic peptides have been synthesised in high yields by cyclooligomerisation reactions of amino acid substituted thiazole monomers.

Key words: cyclic peptides, macrocycles, cyclizations, heterocycles

A large number of cyclic peptides containing 'unnatural' D-amino acids, together with modified amino acids in the form of azole heterocycles have been isolated from marine organisms and algae in recent years. The *Lissoclinum* class of cyclic peptides *e.g.* raocyclamide **1**,[1] lissoclinamide 4 **2**,[2] ascidiacyclamide **3**,[3] is one such group, characterised by the presence of oxazoline/oxazole/thiazoline/thiazole heterocycles alternating with amino acid residues.[4] The size and conformations of these macrocycles and the functional groups they possess have suggested that they have potential for metal ion chelation and transport *in vivo*.[5] This, together with the cytotoxic and antineoplastic properties observed for a number of these compounds,[4] has inspired work towards their synthesis, usually *via* a linear approach followed by a macrocyclisation step.[1-4] We now wish to report the preparation of some novel thiazole-based analogues of natural cyclic peptides using a concise high-yielding cyclooligomerisation procedure from appropriate amino acid substituted thiazole precursors.



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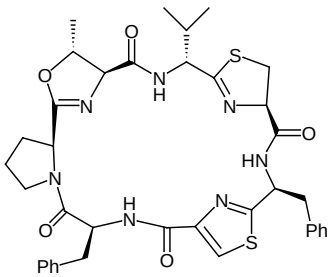

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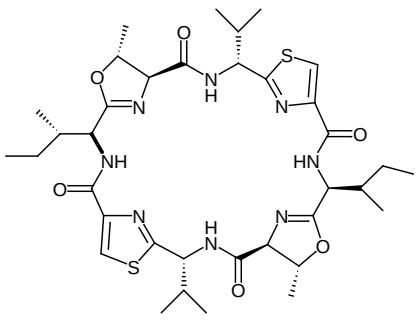

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Thus, the fully protected amino acid thiazoles **4** were first prepared using a modified Hantzsch synthesis.[6,7] Saponification of the thiazole esters, **4** with NaOH next gave the corresponding carboxylic acids **5**, which were then immediately subjected to amine deprotection using a 4 M solution of HCl in dioxane, leading to the amino acids **6** as their hydrochloride salts in essentially quantitative yield.[8]

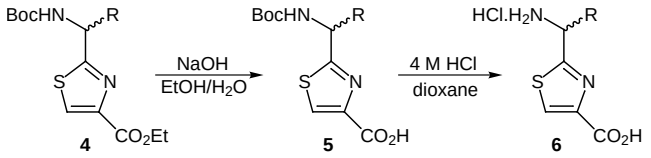
Cyclooligomerisation experiments with **6** were performed initially using the D-valine derived thiazole **6a**. Cyclooligomerisation of **6a** was attempted using a number of peptide coupling reagents (Table 1) and pentafluorophenyl diphenylphosphinate (FDPP) was found to give remarkably consistent high yields (80 - 96 %) of cyclic products.[9] The optimum concentration for this cyclooligomerisation reaction was found to be in the range 10 - 50 mM (Table 1). At lower concentrations (1 mM) the yield was markedly lower (60 %) whilst at higher concentrations (0.5 M) the formation of insoluble polymeric products was found to increase.

Table 1 Cyclooligomerisation reactions of **6a**.

Coupling Reagent	Concentration	Yield of Cyclic Products
DPPA[a]	0.05 M	47 %
DPPCl[b]	0.05 M	55 %
EDC[c]	0.05 M	43 %
FDPP[d]	0.5 M	60 %
FDPP	0.1 M	62 %
FDPP	0.05 M	91 %
FDPP	0.01 M	93 %
FDPP	0.005 M	60 %

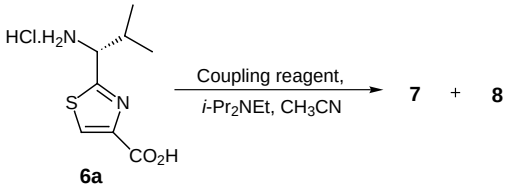
a) diphenyl phosphorazidate; b) diphenylphosphinyl chloride;
c) 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride;
d) pentafluorophenyl diphenylphosphinate.

Under the optimum conditions, *i.e.* yields > 80 %, the major isolated cyclic products resulting from the cyclooligomerisation of **6a** were found to be the cyclic trimer **7** and the cyclic tetramer **8** which were produced in a 5:2 ratio



a, R = D-CH(CH$_3$)$_2$; **b**, R = D,L-CH(CH$_3$)$_2$; **c**, R = D-CH$_2$Ph; **d**, R = D,L-CH$_2$Ph.

(measured by ^{1}H nmr and HPLC).[10] Trace amounts of higher oligomers (pentamer, hexamer, heptamer, octamer and nonamer) were also observed by FAB mass spectrometry, although these compounds were not isolated. The ratio of the trimer **7** and the tetramer **8** did not vary significantly when the reaction was carried out at different concentrations. The ^{1}H nmr spectra of the separated cyclic peptides **7** and **8** in $CDCl_3$ at 22 °C indicated that they were C_3 and C_4 symmetric, respectively. The ^{1}H nmr spectra of **7** and **8** were similar, although the signal attributable to the amide protons in **7** is 0.6 ppm further downfield from that in **8**, suggesting that intramolecular hydrogen bonding may be stronger in **7** than in **8**. The vicinal $^3J_{NHCH}$ values of 9.3 Hz and 9.1 Hz for **7** and **8**, respectively correspond to dihedral angles of 150° < θ < 180° in both the macrocycles.[11]

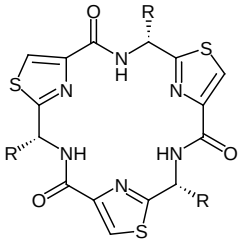


X-ray quality crystals of **7** were obtained by slow diffusion of diethyl ether into a dichloromethane solution of the cyclic peptide. The x-ray structure indicates that **7** is a rigid molecule in which all of the nitrogen atoms point towards the centre of the macrocycle and the valine side chains all lie on the same face of the molecule and adopt axial positions.[12] The NHαCH dihedral angles of the three amide linkages in **7** were found to be between 159 and 167° which is consistent with the values determined by ^{1}H nmr spectroscopy. This correlation suggests that the cyclic tetramer **8** is also a flat molecule in which the thiazole units form the corners of a square with all side chains on the same face of the molecule.[13]

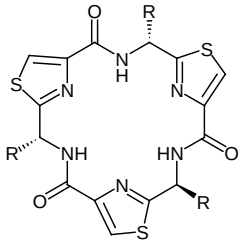
The successful outcome of the cyclooligomerisation of **6a** prompted us to investigate further reactions with other thiazole amino acid based monomers. When the racemic thiazole **6b** was subjected to the same cyclooligomerisation reaction, a statistical mixture of diastereomeric trimers, **7** and **9**, and tetramers, **8**, **10**, **11** and **12** was obtained. This mixture was separated by preparative HPLC and the relative stereochemistry of each of the pure compounds was assigned on the basis of their symmetry as determined by ^{1}H nmr. The overall yield of the cyclic products **7** - **12** was 75 % and the trimer:tetramer ratio was found to be 1:1, which is substantially different to that observed using the homochiral thiazole precursor **6a**. This difference may reflect the relative ease of cyclisation of linear precursors containing a mixture of D- and L-amino acid residues.[14]

The cyclooligomerisations of the phenylalanine derived thiazoles **6c** and **6d** were also studied. Interestingly, when the homochiral precursor **6c** was subjected to the cyclooligomerisation conditions, none of the expected cyclic trimer **13** was produced, although a small amount of its diastereomer **14** (21 %) together with the expected tetramer **15** (10 %) and a diastereomeric tetramer **16** (23 %) were obtained. The formation of both **14** and **16** can be attributed to the presence of the opposite enantiomer (enantiomeric excess as determined by the formation of a Mosher's amide derivative = 60 %)[15] in the starting material. When the racemic phenylalanine derived thiazole **6d** was subjected to the cyclooligomerisation reaction a non-statistical mixture of the trimer **14** and the diastereomeric tetramers **15** - **18** was isolated. The major product isolated was the tetramer **16** where alternating phenylmethyl side chains were positioned on opposite faces of the macrocycle. The overall trimer:tetramer ratio (1:1) observed in this cyclooligomerisation was substantially different to that obtained for the enantiomerically enriched thiazole **6c** (1:3), suggesting that the unsymmetrical trimer **14**, in which one of the side chains lies on the opposite face of the macrocycle is formed more readily than its C_3 symmetrical analogue **13**, where all of the side chains must lie on the same face of the macrocycle. This outcome may be a result of the steric congestion imposed upon **13** by the rigid backbone of the cyclic trimer. We are currently investigating the trimer:tetramer ratio obtained when thiazole monomers bearing different side chains are subjected to the cyclooligomerisation reaction, to determine whether steric congestion is responsible for this difference.

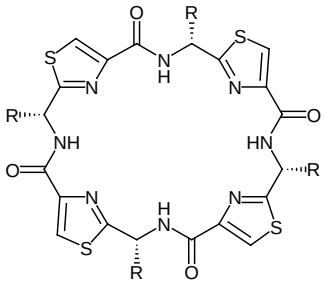
The cyclooligomerisation of an oxazoline based amino acid monomer has previously been employed in the formation of the C_3 symmetric natural product westelliamide (or cyclooxazoline) together with a C_4 symmetric analogue,[13] and in contemporaneous studies the synthesis of a number of thiazole containing symmetrical macrocycles *via* the cyclooligomerisation of a thiazole containing tetrapeptide has been reported.[16] However, in both of these examples the yields of cyclic oligomers obtained were found to be less than 50 %. We have now shown that the cyclooligomerisations of amino acid substituted thiazole monomers can be achieved in high yields (typically >80 %), thereby providing rapid access to large amounts of novel and unusual cyclic peptide analogues. Applications of this reaction to other heterocyclic monomers for the purpose of preparing libraries of novel modified cyclic peptides are now in progress in our laboratories. These macrocycles will then be examined to determine their conformations, biological activity and metal chelating properties together with their potential applications as templates for synthetic receptors or scaffolds for the development of macromolecular devices.



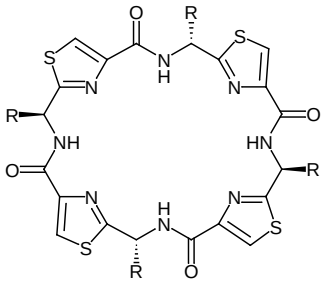
7 R = *i*-Pr
13 R = CH_2Ph



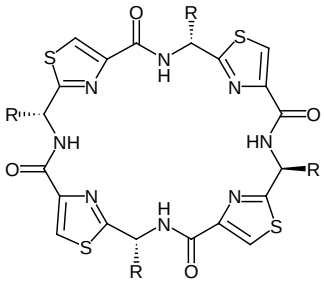
9 R = *i*-Pr
14 R = CH_2Ph



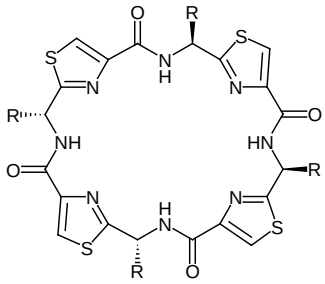
8 R = *i*-Pr
15 R = CH_2Ph



10 R = *i*-Pr
16 R = CH_2Ph



11 R = *i*-Pr
17 R = CH_2Ph



12 R = *i*-Pr
18 R = CH_2Ph

Acknowledgement

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References and Notes

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(8) All new compounds gave satisfactory spectroscopic data together with high resolution mass spectrometric and/or microanalytical data.

(9) The following general cyclooligomerisation procedure is described: Diisopropylethylamine (3 eq.) and FDPP (1.5 eq.) were added to a suspension of **6** (2 mmol) in anhydrous acetonitrile (42 mL) and the solution was stirred at ambient temperature for 18 h before evaporating to dryness *in vacuo*. The residue was partitioned between ethyl acetate (50 mL) and aq. HCl (2 M, 50 mL) and the separated organic layer was washed with aq. HCl (2 M, 50 mL). The combined aqueous solutions were back extracted with ethyl acetate (50 mL) and then the organic solutions were combined and washed successively with aq. NaOH (1 M, 2 x 50 mL), H_2O (50 mL) and brine (50 mL). The solution was dried ($MgSO_4$) and the solvent was then removed under reduced pressure to leave a mixture of cyclic peptide products which were separated by column chromotography (silica gel) or by preparative HPLC (Dynamax Silica Gel Cartridge Column, 30 cm x 10 mm internal diameter).

(10) Spectroscopic data for **7**: mp 258 - 260 °C (from Et_2O); $[\alpha]^{298}_D$ +126.8 (c = 0.53, $CHCl_3$); δ_H (360 MHz, $CDCl_3$) 8.45 (3H, d, *J* 9.3, 3 x N*H*), 8.1 (3H, s, 3 x C*H*=C), 5.4 (3H, dd, *J* 9.3 and 5.8, 3 x NHC*H*), 2.3 (3H, m, 3 x C*H*$(CH_3)_2$), 1.1 (9H, d, *J* 6.8, 3 x CH_3), 1.0 (9H, d, *J* 6.8, 3 x CH_3); δ_C (90 MHz, $CDCl_3$) 168.6 (C=O), 159.7 (Cq), 149.1 (Cq), 123.4 (CH), 55.4 (CH), 35.3 (CH), 18.8 (CH_3), 18.3 (CH_3); *m/z* (ES) 569 $(M^+ Na)^+$; Found C, 52.5; H, 5.6; N, 15.0 %. $C_{24}H_{30}N_6O_3S_3$ requires C, 52.7; H, 5.5; N, 15.0 %.
Spectroscopic data for **8**: mp 152 - 154 °C (from Et_2O); $[\alpha]^{298}_D$ +204.6 (c = 0.57, $CHCl_3$); δ_H (360 MHz, $CDCl_3$) 8.0 (4H, s, 4 x C*H*=C), 7.85 (4H, d, *J* 9.1, 4 x N*H*), 5.2 (4H, dd, *J* 9.1 and 8.2, 4 x NHC*H*), 2.6 (4H, m, 4 x C*H*$(CH_3)_2$), 1.2 (12H, d, *J* 6.7, 4 x CH_3), 1.0 (12H, d, *J* 6.6, 4 x CH_3); δ_C (90 MHz, $CDCl_3$) 169.3 (C=O), 160.3 (Cq), 148.9 (Cq), 124.2 (CH), 55.3 (CH), 32.6 (CH), 19.6 (CH_3), 18.9 (CH_3); *m/z* (ES) 751 $(M + Na)^+$, 1479 $(2M + Na)^+$; Found C, 50.9; H, 5.5; N, 14.6 %. $C_{32}H_{40}N_8O_4S_4.0.5\ H_2O$ requires C, 50.9; H, 5.7; N, 14.8 %.

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