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Total Synthesis of Lissoclinamide 5, a Cytotoxic Cyclic Peptide from the Tunicate *Lissoclinum patella*

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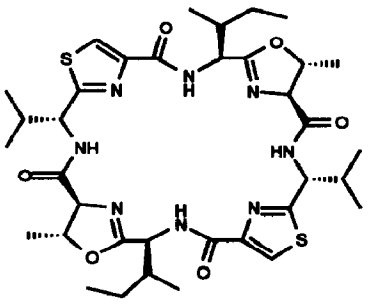
Abstract: A total synthesis of lissoclinamide 5, and some of its stereoisomers, shows that its stereostructure should be revised to **13**.

The tunicate *Lissoclinum patella* produces a prodigious range of novel and unusual cyclic peptides,[1] which are distinguished from other natural cyclic peptides by the presence of thiazole, thiazoline, and oxazoline rings in the macrocyclic skeleton, e.g. ascidiacyclamide **1**[2], lissoclinamide 3 **2**,[3]



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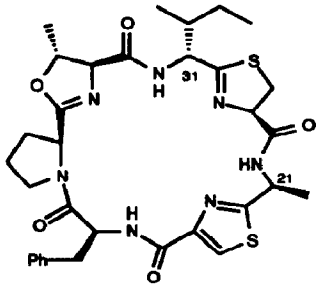

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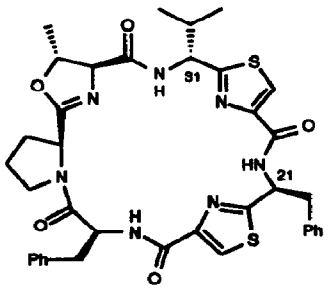

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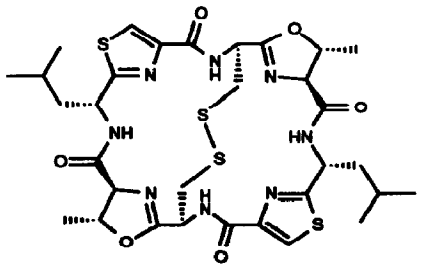

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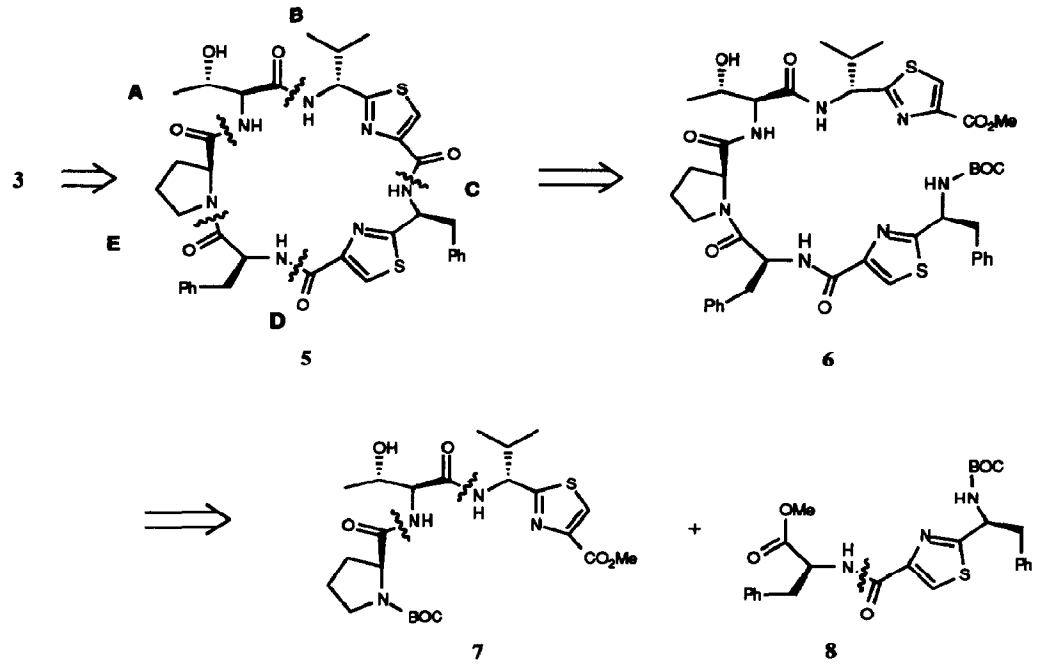
lissoclinamide 5 **3**,[4-6] and ulithiacyclamide A **4**.[7] In common with better known ascidian metabolites like didemnin B, cyclic peptides from *L. patella* are showing considerable promise as potential

antineoplastic agents. It is not surprising, therefore, that synthetic investigations within these families of natural products have been reasonably intensive. Spectroscopic methods, together with an X-ray study in the case of ascidiacyclamide **1**, have played a crucial role in the assignment of the structures of cyclic peptides from *L. patella*, but in several instances these structures have needed significant revision following synthetic investigations.[8] The correct assignment of chiral centres adjacent to the thiazoline and thiazole rings in these structures is particularly problematical, since these are not amenable to assignment following the usual methods of acid catalysed hydrolytic degradation. Given the importance of these chiral centres in determining cytotoxicity,[9] unambiguous assignment of chirality is a prerequisite to establishing a meaningful structure-activity correlation within these classes of natural product..

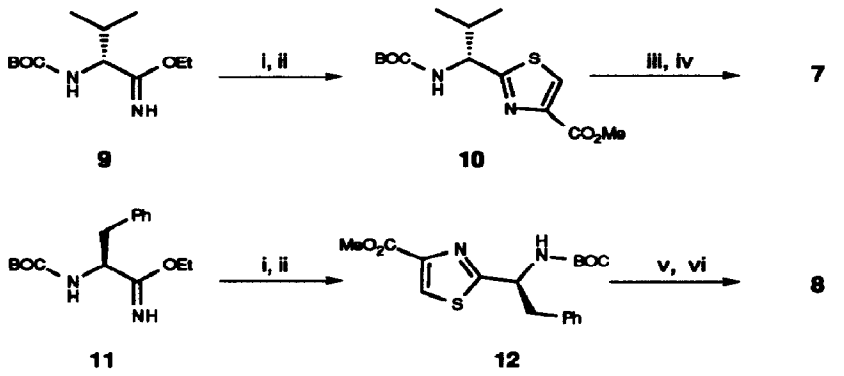
Cyclic peptides can assume a range of conformational structures, each of which can have different biological properties. Many marine organisms accumulate inorganic salts in considerable quantities, and in a recent review article, we have intimated that lissoclinamide - metal complexation could play a significant role in the pronounced biological activity of these metabolites.[10] Indeed, molecular modelling studies on lissoclinamides 3 and 5, and on other related cyclic peptides based on conformational data from nmr studies and X-ray structure data,[2,6] suggest that these peptides could be involved in a significant way in metal chelation and transport *in vivo*. With the aim of investigating the capacity and the extent to which the lissoclinamides bind to particular metal ions, and correlating these data with the biological profiles of the metabolites, we have examined synthetic routes to specific lissoclinamides. In the *Letter* we describe a total synthesis of lissoclinamide 5 and some of its stereoisomers.



Scheme 1

The design we adopted for the synthesis of the structure **3** proposed for lissoclinamide 5 is shown in Scheme 1. Thus, we decided to first elaborate the thiazoline containing tetra- and tri-peptides **7** and **8** respectively, then to condense these two fragments at amide bond E, leading to **6**, in readiness for macrolactamisation at position C producing "pre-lissoclinamide 5" **5**. Cyclodehydration of **5**, using precedent established from the work of Shioiri and of Schmidt,[7,8] was then expected to lead to **3**.

In earlier reported model studies we have described concise and efficient syntheses of optically pure thiazoline and thiazole containing amino acids, based on straightforward condensation reactions

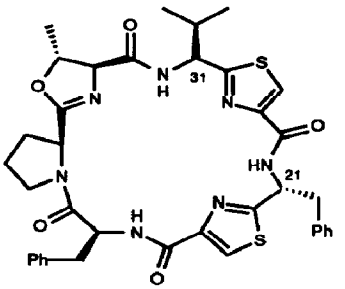


Reagents: i , Cys-OMe hydrochloride, EtOH, 25°C; ii, MnO_2, CH_2Cl_2, 25°C; iii, CF_3CO_2H, CH_2Cl_2, 25°C; iv, BOC-Pro-aThr-OH, DCC, HOBt, N-methylmorpholine, CH_2Cl_2, 0-5°C; v, LiOH, THF-H_2O; vi, Phe-OMe hydrochloride, pentafluorophenyl diphenyl phosphinate, N-methylmorpholine, MeCN, 25°C.

Scheme 2

between cysteine esters and N-protected imino ethers derived from chiral amino acids.[11] When this sequence was extended to the N-BOC protected imino ethers **9** and **11** derived from *D* - valine and from *L*- phenylalanine respectively, the chiral thiazoles **10** and **12** were obtained in satisfactory overall yields (*ca* 30-40%) (Scheme 2). Deprotection of **10** and coupling to BOC-Pro-aThr-OH then produced the tetrapeptide **7** in 84% yield; and saponification of **12** and coupling to Phe-OMe (FDPP, 81%) led to the corresponding tripeptide unit **8**.

Saponification of **8** (LiOH, THF-H_2O; 95%) and condensation, using DCC-HOBt, of the resulting carboxylic acid with the amine produced by BOC-removal from **7** next led to the protected heptapeptide (**6**, 70-75%) as a foam. Saponification of **6**, followed by removal of the BOC protection and treatment of the resulting amino-acid with diphenyl phosphorazide in the presence of N-methylmorpholine then afforded **5** as a solid, in 35-40% yield. The synthesis of **3** was completed by treatment of **5** with thionyl chloride (CH_2Cl_2, 0°C, 48hr)[7,8], which gave the cyclic peptide (73%) as a foam. Comparison of the pmr and cmr data for the synthetic material with those obtained for natural lissoclinamide 5 showed that the two compounds had different stereochemistries, most probably at the two chiral centres C21 and C31, adjacent to the thiazole rings in their structures. Accordingly, based on our synthetic work, and on analysis and correlation of nmr data for natural lissoclinamide 5, the isomer **3**, and other lissoclinamides, we reassigned the stereochemistry shown in structure **13** to the natural product. Furthermore, we effected a total synthesis of this stereostructure, starting from *L*-valine and from



13

D-phenylalanine, and proceeding by the same route summarised above for the isomer **3**. This gave lissoclinamide 5 which exhibited nmr and cmr spectroscopic data[12] identical with those found for the natural product isolated from *L patella*.[4] The stereostructure assigned to lissoclinamide 5 must therefore be modified to **13**, which differs from that proposed earlier by having an (β-) L-valine (C31) residue and an (α-) D-phenylalanine (C21) orientation. Further synthetic studies are now in progress, to complement other investigations designed to examine and to rationalise the metal-chelating capabilities of lissoclinamides and related cyclic peptides through direct studies, modelling, and biological profiling.

Acknowledgements

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12. Satisfactory spectroscopic data and microanalytical or mass spectrometry data were obtained for all new compounds. Spectroscopic data for synthetic (**13**): δ_H (400 MHz, $CDCl_3$) , 9.23 (1H, d, J = 5.8), 8.71 (1H, d, J = 7.4), 8.08 (1H, s), 7.92 (1H, d, J = 10.0), 7.90 (1H, s), 7.20-7.35 (10H, m), 7.13 (2H, d, J = 6.43), 5.44 (1H, ddd, J = 10.2, 5.8, 4.1), 5.19 (1H, dd, J = 11.7, 10.0), 4.88 (2H, m), 4.58 (1H, t, J = 7.8), 4.30 (1H, d, J = 3.9), 3.89 (1H, dd, J = 13.2, 4.1), 3.27 (1H, dd, J = 12.6, 4.5), 3.26 (1H, m), 2.91 (1H, dd, J = 12.7, 10.4), 2.79 (1H, m), 2.77 (1H, dd, J = 13.2, 10.2), 2.12 (2H, m), 1.89 (1H, m), 1.75 (2H, m), 1.46 (3H, d, J = 6.3), 1.09 (3H, d, J = 6.7), 0.79 (3H, d, J = 6.6); δ_C (100 MHz, $CDCl_3$): 171.5 (s), 171.1 (s), 169.8 (s), 168.8 (s), 167.7 (s), 160.8 (s), 159.9 (s); 150.6 (s), 148.1 (s), 136.3 (d), 136.1 (d), 129.8 (d), 129.6 (d), 128.75 (2C, d), 127.4 (d), 127.2 (d), 123.1 (d), 123.0 (d), 82.7 (d), 75.3 (d), 56.7 (d), 55.3 (d), 54.6 (d), 54.0 (d), 47.2 (t), 42.9 (t), 40.9 (t), 33.0 (d), 28.9 (t), 25.2 (t), 21.9 (q), 20.4 (q), 20.0 (q). MS (FAB), *m/z* (%): 762 (M + Na, 81), 740 (M + H, 100), 648 (18), 490 (13), 413 (9), 330 (24), 308 (17), 290 (14). HRMS *m/z* for $C_{38}H_{42}N_7O_5S_2$ (M^+ + H) , calc: 740.2689; found: 740.2691.

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