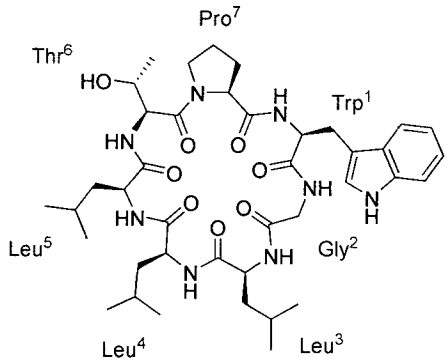
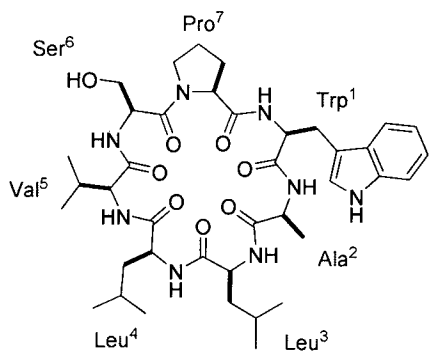
Executive Summary

Although the project was initially aimed at developing a method to synthesise cyclic depsipeptides using zygosporamide as a model study, the direction has been diverted to focus on a total synthesis of a cyclic peptide natural product, namely integerrimide A, instead due to unforeseen difficulties in protecting the last residue of the peptide sequence before coupling. The problem was intrinsic in nature and, therefore, made it difficult to solve and would also add many more steps to synthesise a cyclic depsipeptide natural product of interest later. The project was hence diverted to a small, constrained cyclic peptide natural product, which would be difficult to cyclise otherwise.

Integerrimide A is a cyclic heptapeptide natural product isolated in 2006 together with its other family member, integerrimide B, from the latex of *Jatropha integerrima* obtained from freshly cut leaf stalks collected in Thailand. It was found to significantly inhibit neurite outgrowth in neuronal cell culture at 50 μM and partially block proliferation of human IPC-298 melanoma cells and migration of human Capan II pancreatic carcinoma cells.



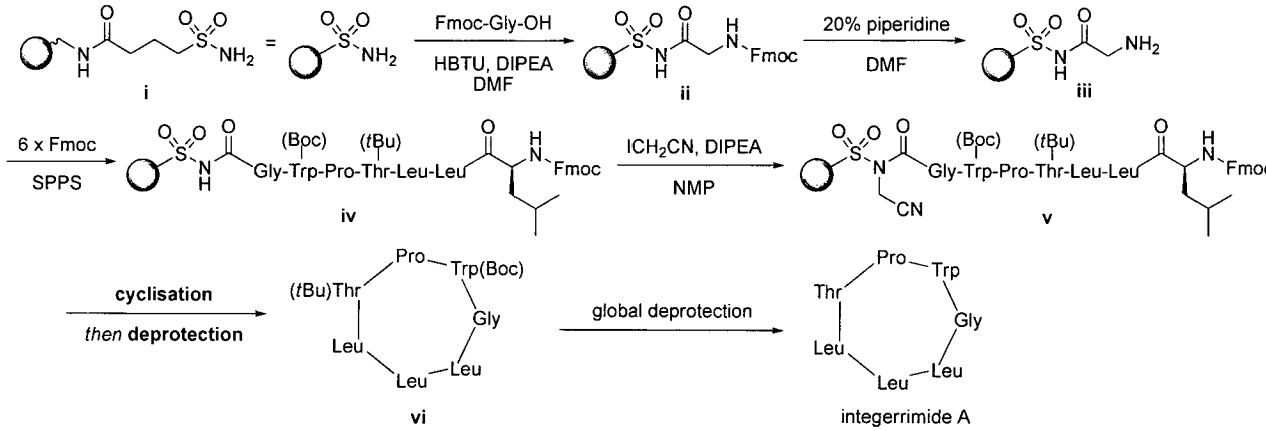
integerrimide A



integerrimide B

Although it is known in literature that the presence of proline, which is considered a turn-inducer, in a given linear peptide can encourage macrocyclisation to take place by pre-organising the linear peptide in solution so that both ends are in close proximity, cyclisation of such a peptide with only three to eight amino acids is still usually very difficult, especially on-resin. Apart from being generally low yielding, such difficulty may possibly lead to epimerisation at the *C*-terminal amino acid and, particularly in solid-phase synthesis, formation of cyclic dimers and oligomers. Moreover, proline-containing cyclic peptides often exist as mixtures of conformers because the *cis*- and *trans*-peptide bonds of a proline residue are restricted to interconversion by conformational constraints. Together with its reported biological activity and potential in further structure-activity relationship studies, these make the synthesis of integerrimide A a challenge in synthetic chemistry.

A sulfonamide safety-catch linker, namely 4-sulfamylbutyryl resin (**3**), which was first developed by Kenner and later modified to its current form by Ellman, was chosen to perform the synthesis.



Projected synthesis of integerrimide A

The synthesis was planned to involve macrocyclisation between glycine (Gly) and leucine (Leu) so that Gly, which lacks stereogenic centres, would be the first residue and proline (Pro) in the middle of the sequence in order to provide a strong turn-inducing element to facilitate effective cyclisation. Although standard solid-phase peptide synthesis from *C*- to *N*-termini is reported to offer minimal racemisation, Gly was still chosen as the first amino acid for coupling with the solid-supported safety-catch linker. This was to avoid any chance of racemisation the first coupling might cause as it is usually carried out twice to ensure maximum loading. Furthermore, it was envisaged that the terminal free amine would more readily attack a less sterically hindered electrophilic carbonyl during the crucial macrocyclisation step. The linear peptide sequence was constructed using a standard Fmoc protocol. A new macrocyclisation approach to perform terminal Fmoc deprotection and intramolecular cyclisation in a tandem fashion was investigated.

Optimisation studies were carried out to find the most suitable reaction conditions to cyclise the activated peptide intermediate (**v**) on-resin in a tandem manner where intramolecular macrocyclisation could take place as a method of cleaving the peptide from the solid support once the terminal protecting group was removed. Triethylamine was found to be the base of choice for the reaction of 7 days at room temperature in DMF in the presence of 4-Å molecular sieves. The reaction was successfully scaled up to prepare sufficient material for use as a calibration standard in quantitative LCMS analysis. The cyclic intermediate was then subjected to orthogonal deprotection for 1 h using a 95:2.5:2.5 TFA/water/TIPS mixture to afford integerrimide A in 19% overall isolated yield, based on the manufacturer's stated resin substitution, over 16 steps. Pleasingly, the spectral characteristics of the resulting cyclic product was identical in all

respects to those of natural integerrimide A, except the ratio of conformers exhibiting *cis*- and *trans*-Pro amide bonds in ^{1}H NMR spectra recorded in DMSO-d_6 which was believed to be due to the difference in concentration (2.4:1, *cf.* 4:1 in lit.). Particularly, the specific optical rotation of synthetic integerrimide A was found to match that of the natural isolate, suggesting that all amino acids constituting the natural product are of their natural L-form indicating the absolute stereochemistry of integerrimide A as drawn and that this synthesis caused no epimerisation. The route described herein to synthesise integerrimide A may also be used to allow access to a library of its analogues for structure-activity relationship studies.