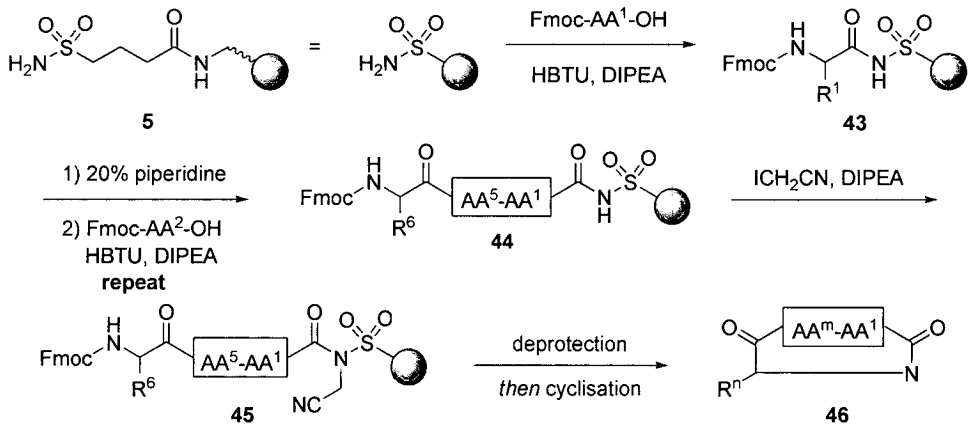
3 Experimental Design and Methodology

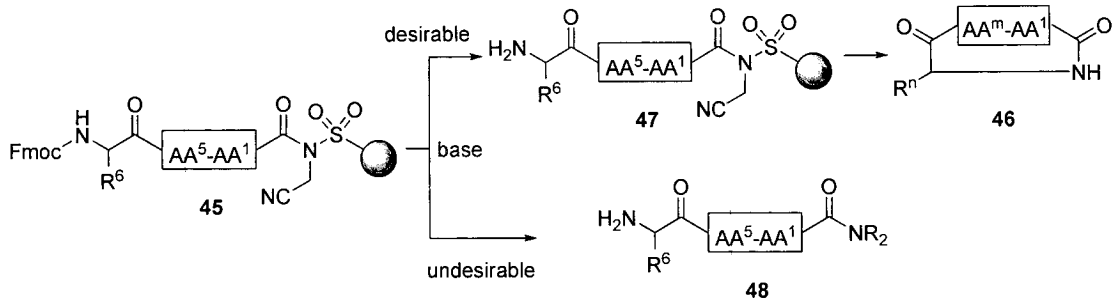
The project was divided into three phases:

Phase I was intended to centre on preliminary studies of macrocyclisation on a simple cyclic peptide. This was deemed important in order to gain understanding of the deprotection and cyclisation, which would proceed concurrently with cleavage from the resin by the intramolecular nucleophilic displacement. In the case of cyclic peptides, the proposed method would be investigated as illustrated in Scheme 10. Direct cyclisation would be attempted as soon as the Fmoc group was removed without it being switched to an acid-labile protecting group.



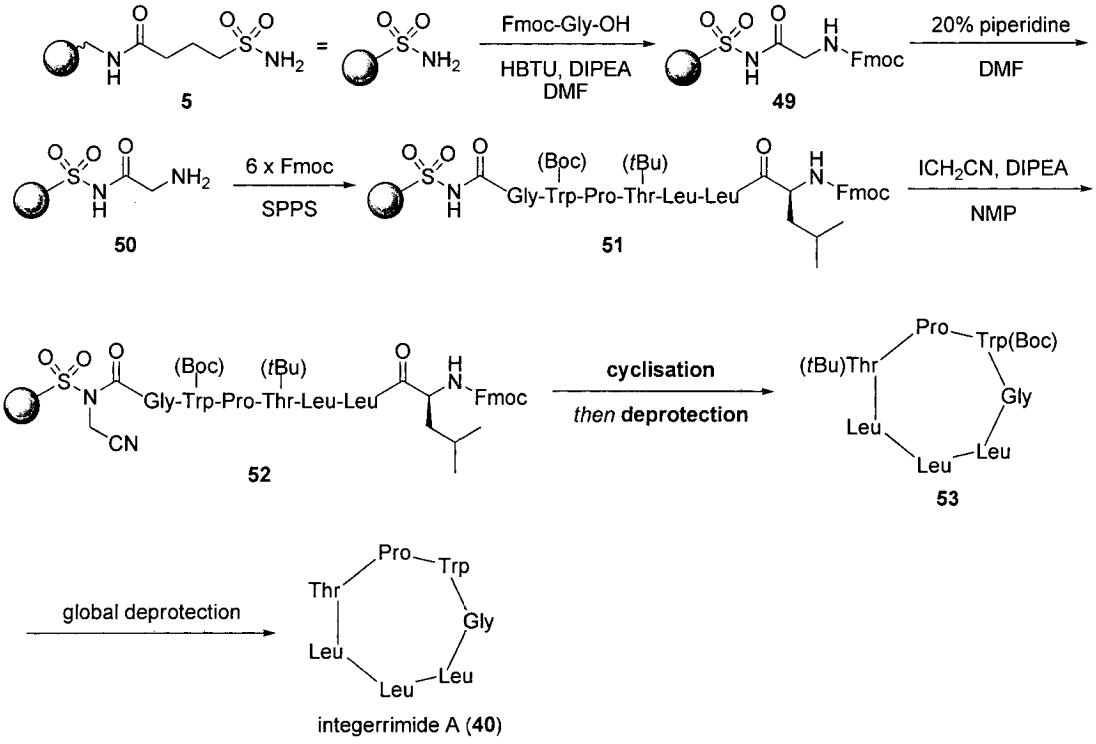
Scheme 10 Projected synthesis of cyclic peptide employing Ellman's sulfonamide safety-catch linker

The main studies involved development of reaction conditions to remove the Fmoc group efficiently and to advance the intramolecular amide bond formation without competitive attack at the activated acylsulfonamide. Instead of the usual 20% piperidine in dimethylformamide (DMF), non-nucleophilic bases, such as diaza(1,3)bicyclo[5.4.0]undecane (DBU), would be probed. DBU is a sterically hindered amidine base which is commonly used where side reactions, due to the inherent nucleophilicity of a basic nitrogen, are a problem. The base of choice should selectively cleave the Fmoc protecting group liberating the corresponding free amine **47**, which then undergoes intramolecular nucleophilic displacement to afford the desired cyclic peptide **46** (Scheme 11).



Scheme 11 The treatment of base strategy for the development of Fmoc deprotection conditions to promote cyclisation

Among peptidic natural products, integerrimide A[73] (**40**) was chosen as a suitable target compound to investigate as a model system due to its simplicity. To date, no synthesis of the natural product has been reported. All seven residues of **40** are the natural form of five amino acids (glycine, tryptophan, proline, threonine and leucine) and the cyclisation step would involve only another peptide bond formation (Scheme 12).



Scheme 12 Proposed synthesis of integerrimide A (**40**)

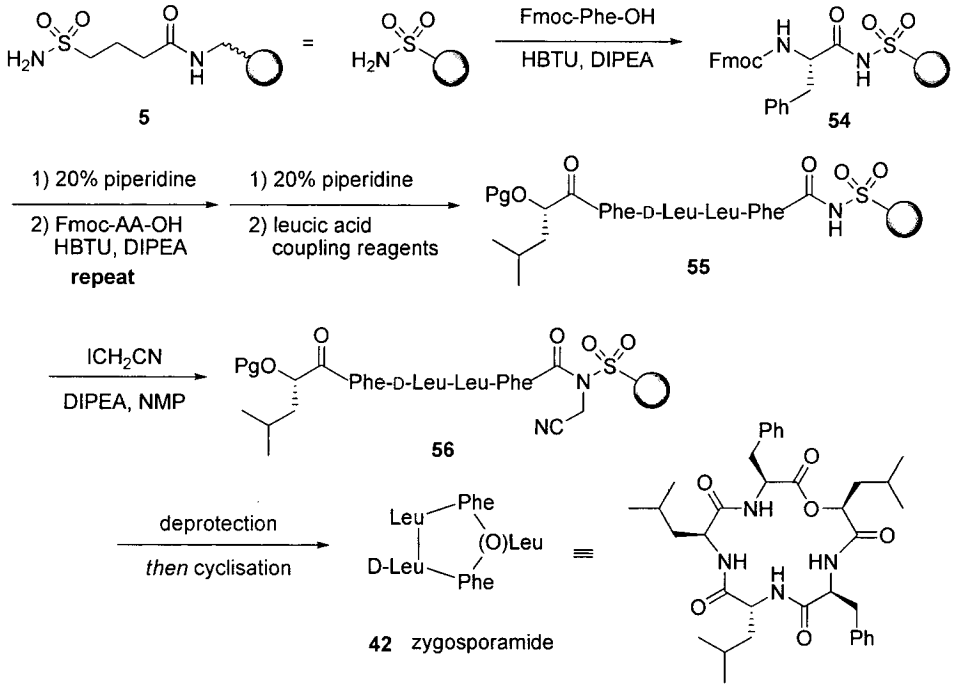
It normally requires coupling of the first amino acid onto a solid support repeated twice to ensure its loading efficiency. Glycine was chosen as the first residue, which was directly attached to the resin-bound acylsulfonamide linker, in order to avoid any potential racemisation caused during the first coupling. Standard Fmoc synthesis would be applied and careful cyclisation studies

conducted. Once released into solution, the peptide would be analysed and identified using liquid chromatography (LC) and mass spectroscopy (MS) techniques combined. Precisely, the crude products would be liquid chromatographed and then their components analysed and quantified by high performance liquid chromatography (HPLC) and electrospray ionisation mass spectroscopy, respectively.

However, instead of going into optimising reactions of the synthesis of integerrimide A, the actual macrolactonisation studies would be concentrated and optimised in Phase II.

Phase II focused on on-resin cyclisation studies of a peptide with a hydroxyl terminus prepared using an α-hydroxy acid as the final residue. The synthetic strategy would be as shown in Scheme 13. The product cleaved from the solid support after cyclisation would be the required cyclic depsipeptide.

Although the synthesis of zygosporamide has been reported,[75] it was selected as an initial target depsipeptide. This was because it was composed of only five simple residues, namely L- and D-leucine, two of L-phenylalanine and (*S*)-leucic acid (Scheme 13).



Scheme 13 Plan for the synthesis of zygosporamide

Standard Fmoc protocol would be applied to the studies towards the synthesis of zygosporamide using solid-supported Ellman's sulfonamide linker, just as in Phase I. After the last residue, leucic acid, was coupled, the linker would be activated to give **56**. Focuses would be directed towards

deprotection and cyclisation studies to achieve depsipeptide **42**. The necessity of protection of the hydroxyl group would also be determined. Optimisation studies would be carried out in this phase.

Once the proposed methodology has been successfully developed to generate cyclic depsipeptides in an efficient manner, the method would be incorporated into studies directed towards the synthesis of a biologically active cyclic depsipeptide natural product in Phase III.

Phase III was intended to involve application of the developed method to the synthesis of a natural product of choice. The main aim of this part was to select target compounds for synthesis. There were important aspects to consider. These included when the chosen compounds were isolated, whether their total syntheses have been completed, commercial availability of amino acid residues and their biological activities. No decision towards any specific targets was yet made until the methodology has been developed. This was because the choice of natural product candidates should still be pertinent to selection criteria at the time the methodology has been developed. Examples of possible target cyclic depsipeptides are shown in Figure 4.

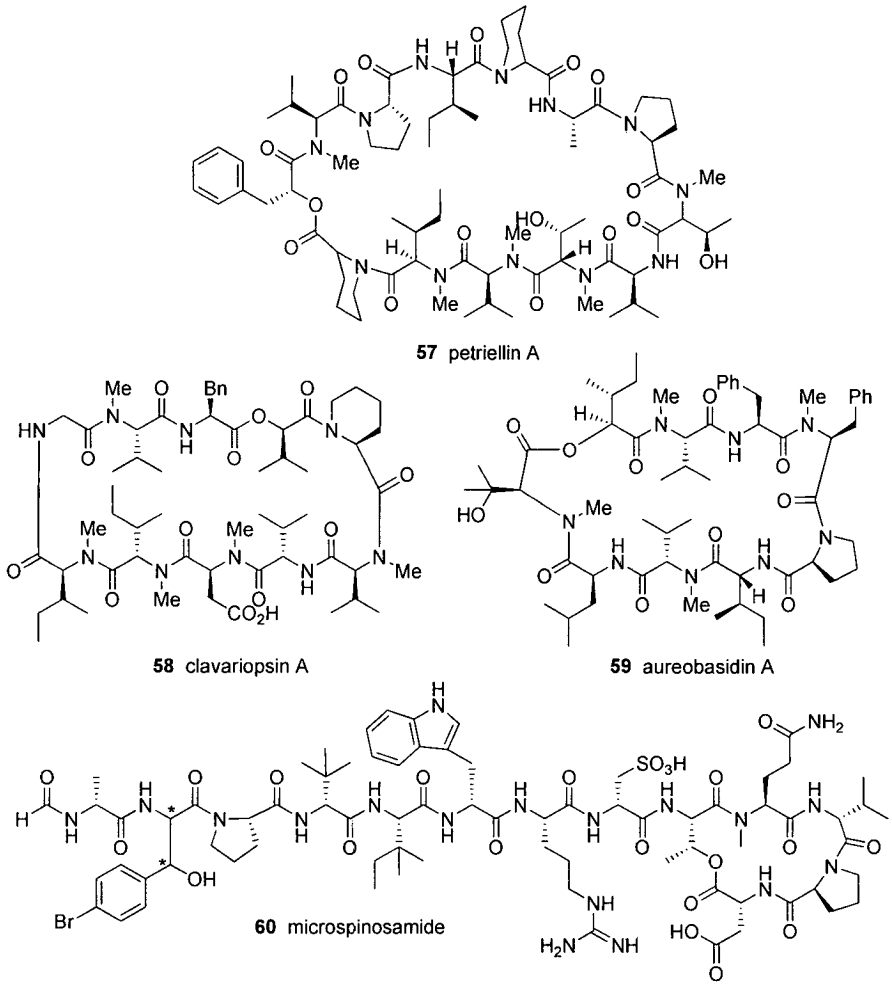


Figure 4 Possible cyclic depsipeptide natural product candidates

Once the natural product of choice could be selected, its synthesis would be conducted. If not commercially available, it should only require known and simple chemistry to prepare the amino acid or α-hydroxy acid residues necessary. As optimisation studies would have been accomplished since Phase II, the experimental work for this part should be straightforward. Liquid chromatography and mass spectroscopy techniques as delineated in Phase II would be used to analyse, identify, and quantify the synthesised products. Nuclear magnetic resonance spectroscopy would be utilised to confirm the structure of the target natural product.