1 Introduction

1.1 Literature review

A large number of cyclic peptides and depsipeptides have been found in nature[1, 2] particularly in fungi,[3] bacteria[4] and marine organisms.[5] They often exhibit promising biological activities, including anticancer, antibacterial, antiviral, antifungal and anti-inflammatory properties, and therefore are attractive candidates for drug development in many disease indications.[6] Moreover, their enhanced metabolic stabilities, biological specificities, bioavailability and conformational constrained structural feature over their linear counterparts make them important leads for drug discovery, and as actual drugs.[7]

Cyclic peptides are widely applied in many areas of biomedical research to study the conformational requirements of peptide and protein recognition and as diagnostically and therapeutically useful tools.[8-15] Peptide cyclisation becomes an effective and commonly employed strategy for peptide modifications. During the past several decades, great efforts have been made to develop more efficient methods for synthesis of cyclic peptides and peptidomimetics.[7] However, lactamisation (peptide bond formation) is usually chosen as the macrocyclisation endgame even in the case of cyclic depsipeptides where ester bond formation may seem more obvious. A much-strained conformation is also imposed on a linear peptide through cyclisation. Direct macrolactonisation as a ring closing strategy for this system thus offers a synthetic challenge in organic chemistry and particularly in peptide synthesis.

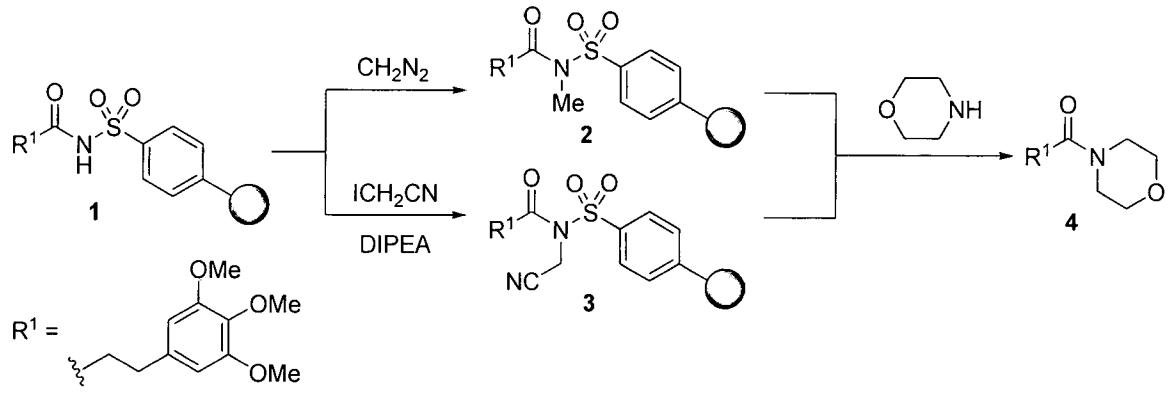
The synthesis of cyclic peptides and depsipeptides is generally hampered by low yields in the cyclisation step, which requires high dilution conditions and is accompanied by dimerisation and oligomerisation side reactions.[16] Conventionally, the Yamaguchi esterification[17, 18] and the Mitsunobu reaction[19] are exploited, in solution-phase, to undergo intramolecular esterification in order to minimise the formation of side products. Nevertheless, prolonged reaction times, low yields and the number of isolation/purification steps still pose significant disadvantages.

The chemistry used in the solid- and solution-phase synthesis of peptides is highly precedented and often taken for granted.[20-22] Solid-phase synthesis also allows rapid access to substantial quantities of target peptides and is amenable to the generation of libraries of structurally related analogues which greatly assists the studies of their mechanism of biological activity and enhanced activity development.

In solid-phase synthesis, linkers are molecules which keep intermediates bound to the support. They should also enable the easy attachment of the starting material to the support, be stable under a broad variety of reaction conditions, and yet facilitate selective cleavage at the end of a synthesis without causing damages to the required product.[23] In general, the assembly of cyclic

peptides can be achieved by head-to-tail, head-to-side-chain, side-chain-to-tail or side-chain-to-side-chain macrocyclisation after cleaving a required linear peptide from the resin.[24] Such cyclisation is, therefore, solution-phase. However, there, actually, are a number of approaches, including on-resin cyclisation, followed by separate cleavage; a peptide fragment cyclising after being cleaved from the solid support; or cyclisation taking place as the peptide being cleaved from the resin. The latter, as known as *on-resin* cyclisation, has been utilised using two main types of linking strategies. One involves a side-chain functional group of peptides or their backbone covalently anchored onto a solid support.[16, 25-29] The other is a cyclisation–cleavage approach, where the *C*-terminal of the linear peptide is attached to an electrophilic linker that is labile to free amines.[30-33] One major advantage of the latter is its generality as a specific side-chain does not need to be anchored.

The term "safety catch" was introduced in the solid-phase peptide synthesis community by Kenner in 1971 to describe a strategy that allows a linker, which separates a peptide to be synthesised from its solid support, to remain stable under reaction conditions to construct the peptide sequence until it is subsequently activated for cleavage by a chemical modification to release the peptide from the support.[34] Kenner's acylsulfonamide linker (**1**) was stable to basic and strongly nucleophilic conditions. However, it suffered from poor loading efficiencies, racemisation and poor reactivity of the activated group towards nucleophilic displacement.[35] Backes and Ellman were able to increase the reactivity of the activation step by using a haloacetonitrile instead of diazomethane (Scheme 1).[36]



Scheme 1 Activation of Kenner's safety-catch linker

In order to improve matters, Ellman and co-workers introduced a modified alkylsulfonamide linker (**5**) which was slightly more nucleophilic than **1** and a better spacer that placed the resin further from the reactive site (Figure 1).[37] It was found that **5** was able to load all twenty amino acids with minimal racemisation and high loading efficiencies (>80% with <1% racemisation).[37]

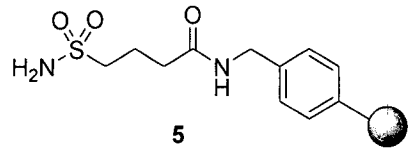
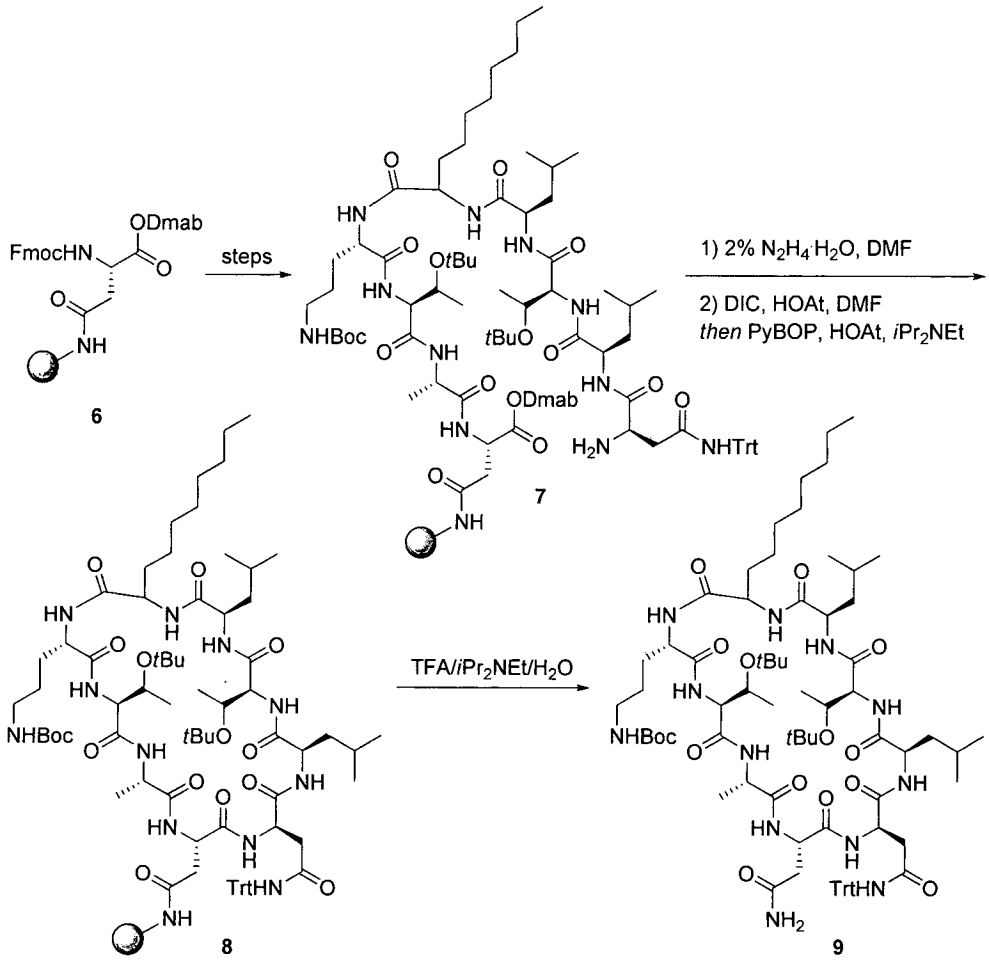


Figure 1 Ellman's modified alkanesulfonamide safety-catch linker

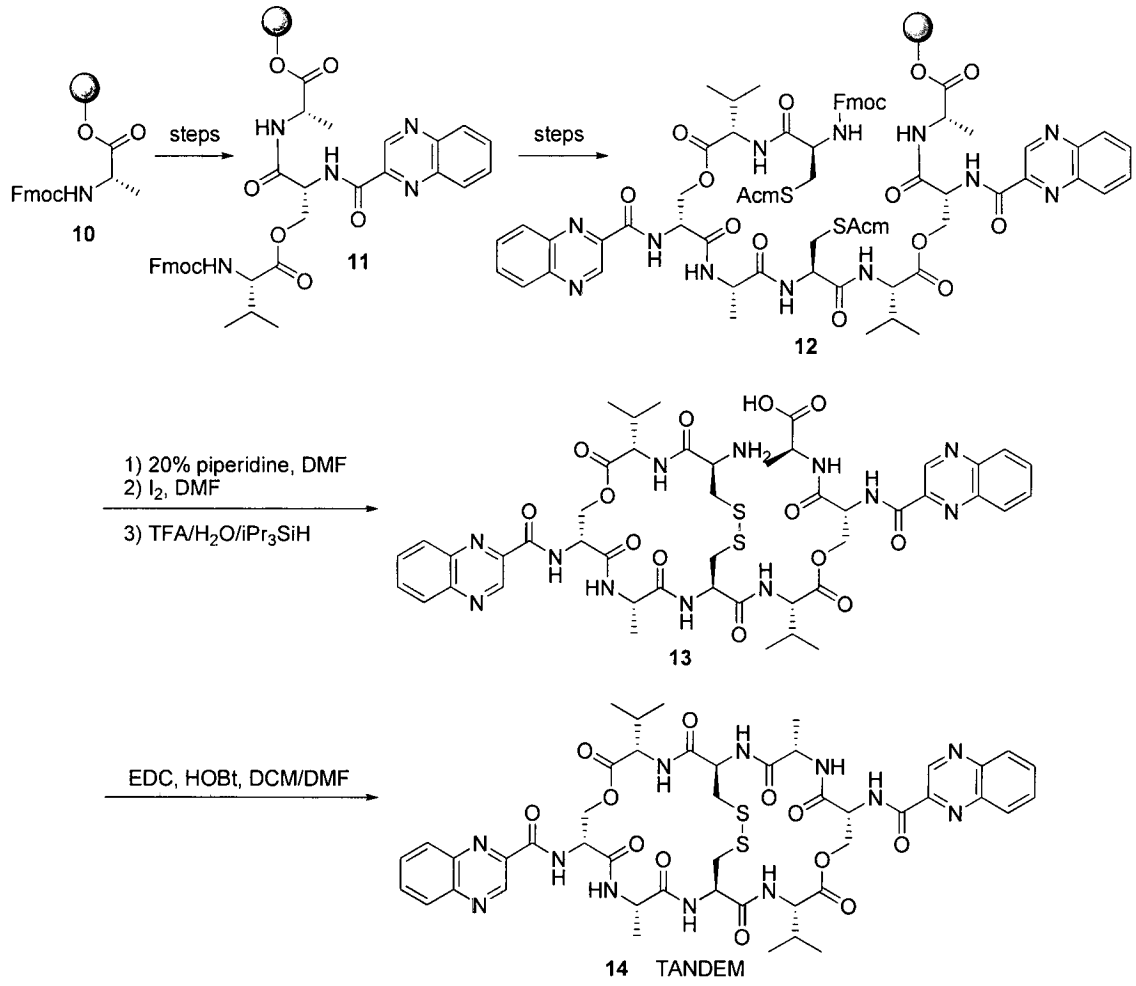
Generally, safety-catch linkers play an important role as a protecting group to provide libraries of carbonyl compounds with varied terminal groups through nucleophilic displacement.[38-40]

Two distinct solid-phase protocols for the synthesis of cyclic peptides are employed. One extensively studied approach involves on-resin cyclisation of a peptide anchored through a side-chain functional group such as imidazole,[41] acid,[25, 26, 42-44] amine[45, 46] or alcohol.[47] The orthogonally protected peptide is assembled, using regular Boc or Fmoc synthesis, with an ester side-chain at the *C*-terminus. After macrocyclisation *via* intramolecular nucleophilic displacement. The resulting cyclic peptide is cleaved from the support, followed by orthogonal deprotection. This strategy was also applied in the synthesis of chlorofusin and its analogues (Scheme 2).[29, 48]

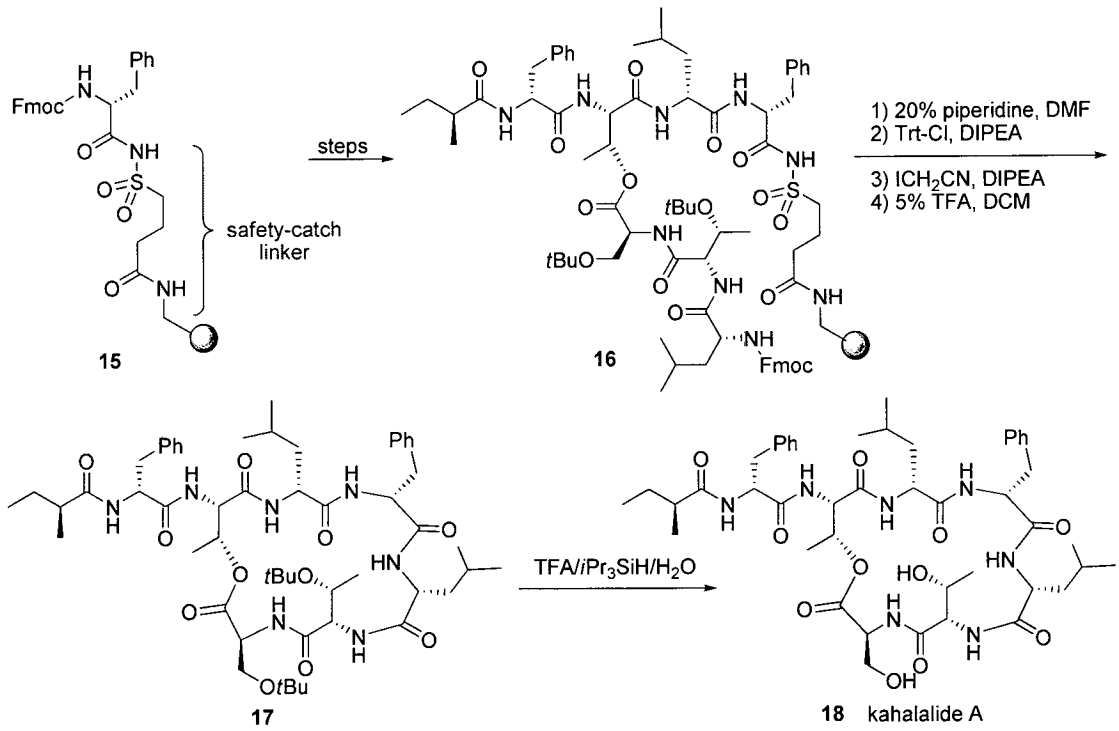


Scheme 2 Solid-phase synthesis of the cyclic peptide portion of chlorofusin with a side-chain anchored onto a solid support[48]

In the case of a cyclic depsipeptide, macrolactamisation is often chosen as the cyclisation endgame with the ester bond already installed during the synthesis of the corresponding linear peptide. The solid support can be covalently attached onto a side-chain functional group of the depsipeptide intermediates[16, 49, 50] (for example, see Scheme 3) or onto their backbone[51, 52] (for example, see Scheme 4).

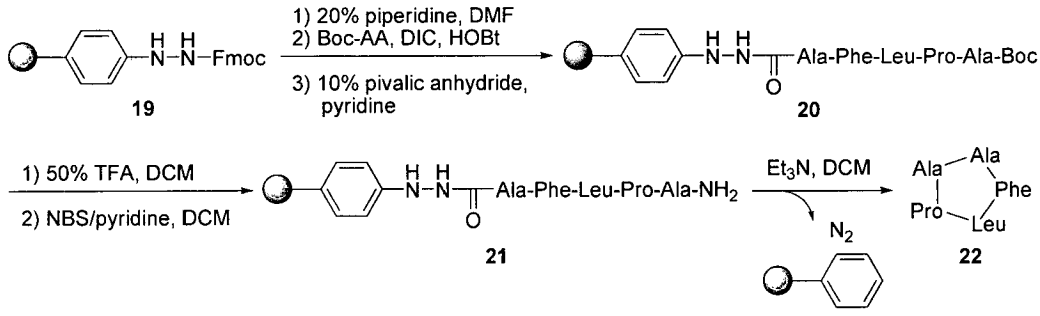


Scheme 3 A solid-phase synthesis of the bisintercalator TANDEM (**14**) using a side-chain-tethered approach[49]



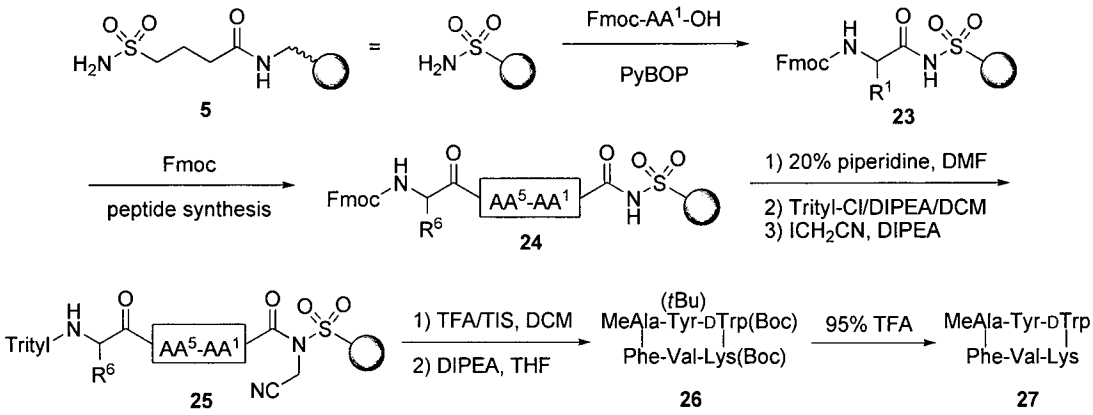
Scheme 4 A solid-phase synthesis of kahalalide A (**18**) employing the backbone cyclisation strategy[52]

The other is the cyclisation–cleavage approach where cyclisation takes place concurrently with cleavage after stepwise synthesis of a corresponding linear peptide.[30-33, 53-58] Different types of resin such as the Kaiser oxime,[31, 53, 54] thioester[30] and aryl hydrazine linkers on a solid support[33, 59] have successfully been utilised in synthesis of various cyclic peptides.



Scheme 5 Cyclic peptide synthesis using an aryl hydrazine linker developed by Rosenbaum and Waldmann *via* the cyclisation–cleavage strategy[33]

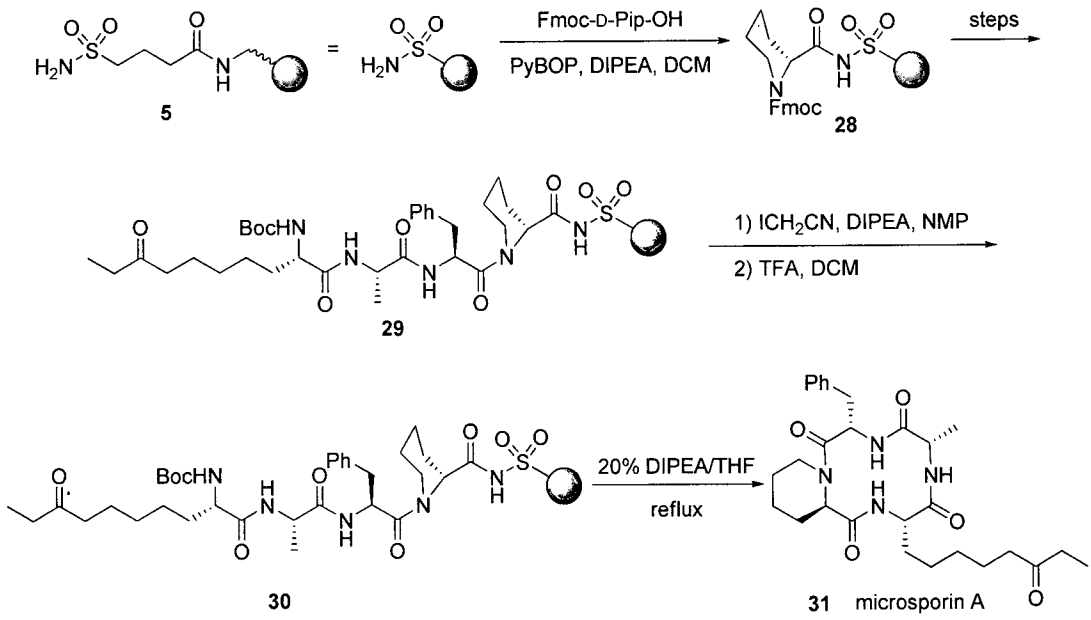
While Fmoc chemistry is appropriate with the aryl hydrazide linker, the Kaiser and thioester resins require Boc. Alternatively, Ellman's sulfonamide linker, which is compatible with Boc and Fmoc strategies, has been employed in the cyclisation–cleavage synthesis of cyclic peptides using the Fmoc protocol (Scheme 6).[32, 60]



Scheme 6 A method developed by Yang and Morriello for the synthesis of cyclic peptides using Ellman's sulfonamide safety-catch linker[32]

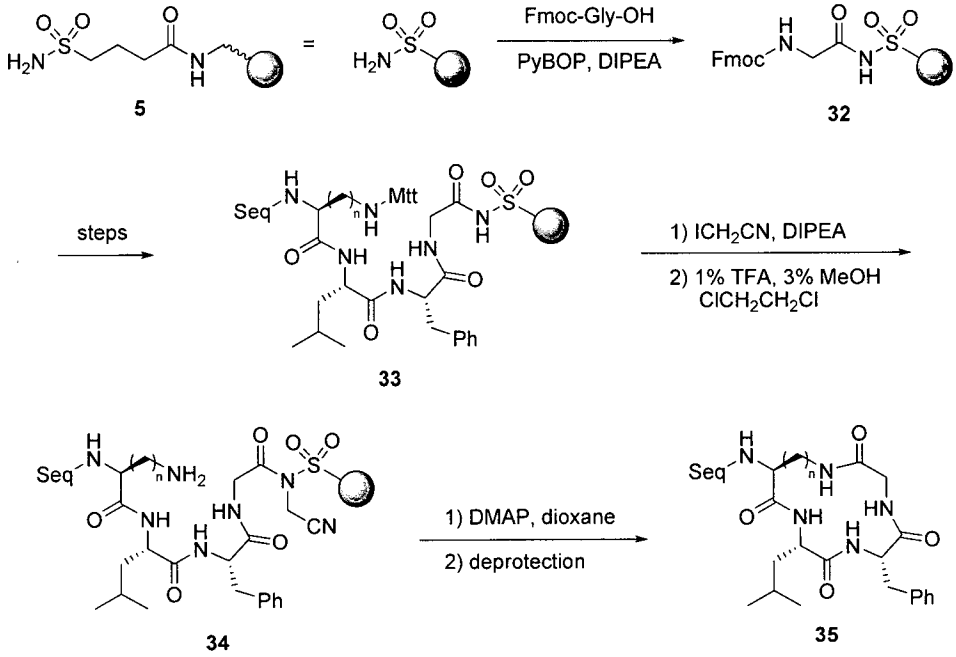
This method by Yang and Morriello involved preparation of desired linear peptides using Fmoc chemistry onto Ellman's sulfonamide safety-catch linker (**5**) where the final base-labile Fmoc group was replaced by trityl, which was acid-labile, prior to linker activation.[32] The trityl group was then removed and then cyclisation encouraged under basic conditions (DIPEA, THF). The method has also been applied successfully in a total synthesis of phakellistatin 12.[60] It was thought that the use of strongly basic and nucleophilic piperidine during the final Fmoc deprotection would competitively attack the carbonyl of the activated linker; otherwise Fmoc would not withstand the conditions employing iodoacetonitrile to activate the safety-catch linker. To overcome this potential problem, Fmoc was replaced by trityl, a bulky and acid-labile protecting group. By this approach, three extra steps were hence added to remove Fmoc, introduce trityl and then remove it.

Another alternative yet similar approach was reported by Silverman and co-workers using Fmoc chemistry in the solid-phase synthesis of microsporin A.[61] However, the final amino acid residue was acid-labile Boc protected (Scheme 7). This was to avoid the potentially problematic Fmoc deprotection conditions without three additional steps. It is worth noting that each amino acid unit of the natural product required no side-chain protection, which would normally be acid-labile to accommodate the Fmoc protocol, making the approach inappropriate for those requiring side-chain protecting groups and, hence, less general.



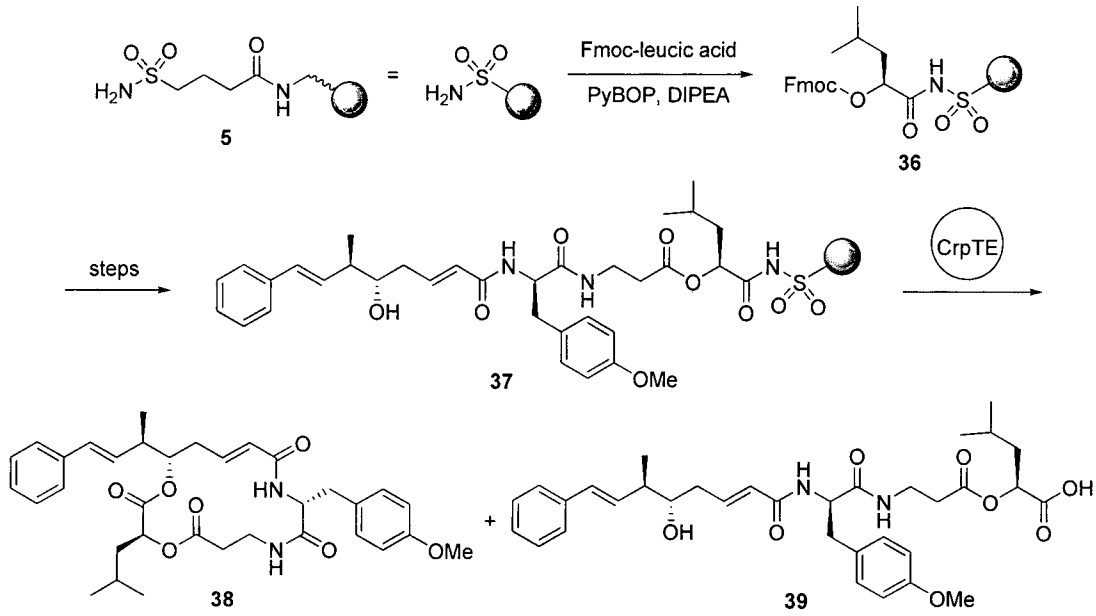
Scheme 7 The solid-phase synthesis of microsporin A (**31**)[61]

Otherwise, linear peptides can be prepared using solid-phase synthesis techniques and subsequently liberated from the resin prior to cyclisation to obtain both cyclic peptides[46, 62, 63] and cyclic depsipeptides.[64, 65] Essentially, the cyclisation step takes place in solution. Scheme 8 shows an example of the side-chain-to-tail macrocyclisation approach. However, the use of DMAP suggested that the linear peptide had actually been cleaved from the resin before cyclisation actually took place.[66]



Scheme 8 Solid-phase synthesis of cyclic peptide inhibitors employing Ellman's sulfonamide safety-catch linker by Andrews *et al.*[66]

Recently, cryptophycin thioesterase (CrpTE) was used as an enzymatic catalyst for macrolactonisation of cryptophycins-1, 21 and 24 (Scheme 9).[51] It was reported as the first enzyme-catalysed macrolactone formation using solid-phase techniques. CrpTE, in fact, cleaved the linear substrate from the solid support prior to cyclisation. This was because the nucleophilic serine residue of CrpTE directly displaced the activated acylsulfonamide releasing seco-cryptophycin (**39**) into solution.



Scheme 9 Solid-phase synthesis of cryptophycin 1 with the macrolactonisation step catalysed by CrpTE[51]

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,[67-70] cyclisation of such a peptide with only three to eight amino acids is still usually very difficult, especially on-resin.[24, 71, 72] Apart from being generally low yielding,[66] 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.[67, 68] 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.

1.2 Natural products of interest

Integerrimide A (**40**) is a cyclic heptapeptide natural product isolated in 2006 together with its other family member, integerrimide B (**41**), from the latex of *Jatropha integerrima* obtained from freshly cut leaf stalks collected in Thailand.[73] 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.[73]

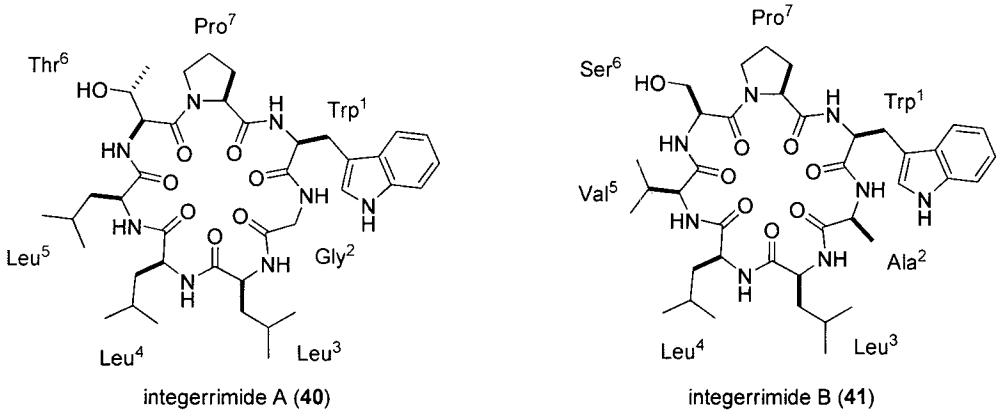


Figure 2 The integerrimide family of natural products

Zygosporamide (**42**) is is a cyclic depsipeptide isolated from the seawater-based fermentation broth of a marine-derived fungus identified as *Zygosporium masonii* (Figure 3).[74] There are four hydrophobic amino acids (D-Leu, L-Leu, and two L-Phe) and one hydrophobic hydroxy acid ((*S*)-2-hydroxy-4-methylpentanoicacid; *O*-Leu) constituting the cyclic structure of zygosporamide. In the National Cancer Institute 60-cancer cell line screen, the molecule displayed highly selective cytotoxicity against central nervous system (CNS) cancer cell line SF-268 and renal cancer cell line RXF 393, with GI_{50} values of 6.5 nM and less than 5.0 nM, respectively.

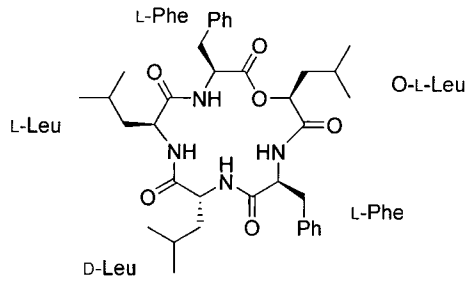


Figure 3 Zygosporamide (**42**)