REVIEW

# The synthesis of cyclic peptides

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Covering: 1997 to mid-2000.



## 1 Introduction

Cyclic peptides and their derivatives continue to hold the attention of synthetic chemists and biologists alike. Apart from the occurrence of a variety of naturally occurring bioactive metabolites, cyclic peptides are often more stable *in vivo* than their linear counterparts and therefore often represent promising drug candidates. Another feature that contributes to the appeal of cyclic peptides is their reduced conformational mobility which allows them to be used in the study and mimicry of protein folding and to present diverse functionality in a defined and predictable manner. Given the continuing and growing interest in cyclic peptides and their chemistry, a review of the area seems timely. This review is intended to serve as a distillation of common and recently reported methods for the synthesis of cyclic peptides and their analogues. In collecting and presenting the literature, our aim has been to develop an overview of available methods, possibly for those unfamiliar with the area, with a focus on the key chemistries used for the closure of linear precursors to generate a target macrocycle. The literature covered by this review is primarily from the period of 1997 to mid-2000 and it is our belief that the many articles published during this period comprehensively represent the range of methods available. The field of peptide chemistry abounds with an intimidating array of acronyms and abbreviations. We have used this Journal's common acronyms where possible but many more are defined later in this review. In collecting these articles, we have tried to focus on novel and chemoselective cyclisation reactions. One notable and deliberate omission is cyclisation *via* the formation of disulfides. The peptide literature abounds with references to this reaction and the reader is directed to a number of recent key references.[1–3]

## 2 Diketopiperazines and their derivatives

Diketopiperazines (DKPs), head-to-tail cyclic dipeptides, are often formed as unwanted side products in solution and solid phase synthesis of linear peptides. However, there has been a great deal of interest in the synthesis of DKPs as scaffolds for the combinatorial generation of compound libraries for drug discovery[4–6] and as scaffolds to constrain cyclic peptides within biologically active conformations.[7,8] Although DKPs are formed *via* head-to-tail cyclisation, their constrained nature requires a strategically different approach to their synthesis from that used in the formation of head-to-tail macrocyclic peptides.

Szardenings *et al*. have established two impressive methods, A and B, for the generation of compound libraries using DKPs.[5] These are outlined in Scheme 1. In Method A a resin-bound amino acid is reductively alkylated using an appropriate aldehyde, the resulting secondary amine is then acylated to yield an *N*-alkylated dipeptide. Deprotection followed by cleavage using toluene–ethanol solvent mixtures under acidic or basic conditions induces cyclisation to yield a DKP. An attractive feature of this cleavage-mediated cyclisation is that potential side-products which might be formed during the synthesis, such as non-alkylated or non-acylated products, remain tethered to the solid support. Acylation of the secondary amine, often a difficult task, was found to be best performed using DIC–HOBT with symmetrical anhydride couplings being performed at high concentrations (0.5 M).

Method B makes use of a multicomponent Ugi reaction, wherein a resin-bound amino acid is reacted with an appropriate aldehyde, isocyanide and second amino acid in a one-pot reaction to form the *N*-alkylated cyclic precursor dimer. The advantage of this approach is that the potentially problematic acylation of the secondary amine employed in Method A is circumvented. The second method also generates another point of diversity in the DKP. Following the Ugi condensation, cyclisation to give a DKP is effected by cleavage of the dimer from the solid support using a toluene–ethanol mixture. DKPs were generally obtained in higher yields using Method B (22–98%) as compared to method A (11–37%).

In a conceptually different approach Albericio and co-workers have used the backbone amide linker strategy to generate DKPs in near quantitative yields (95%).[4] In this approach (Scheme 2) an appropriate amino acid methyl ester was attached to a 5-(4-formyl-3,5-dimethoxyphenoxy)valeric acid functionalised solid support by reductive amination. The secondary α-amino group was then acylated with an appropriate Fmoc-protected amino acid to form dipeptide **1**. Cyclisation was effected by removal of the Fmoc group with piperidine in DMF and the resulting DKP was then cleaved from the solid support under acidic conditions.

Robinson and co-workers have reported the solution phase synthesis of bicyclic (**2**)[8] and tricyclic (**3**)[7] DKPs. The dipeptide precursors of these compounds were synthesised in solution and cyclisation was triggered by removal of an α-amino Z

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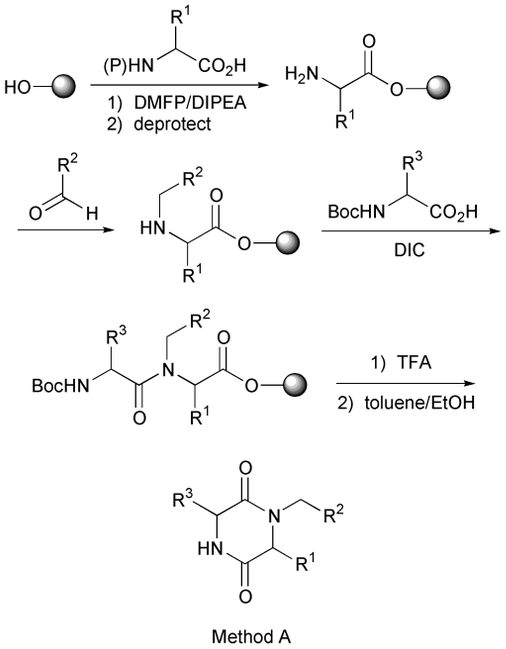
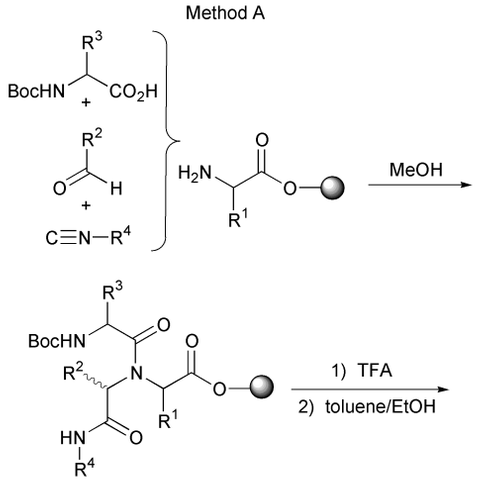
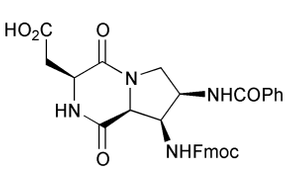



**Scheme 1**

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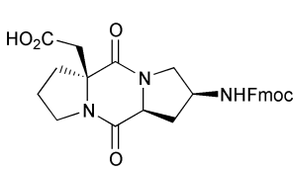
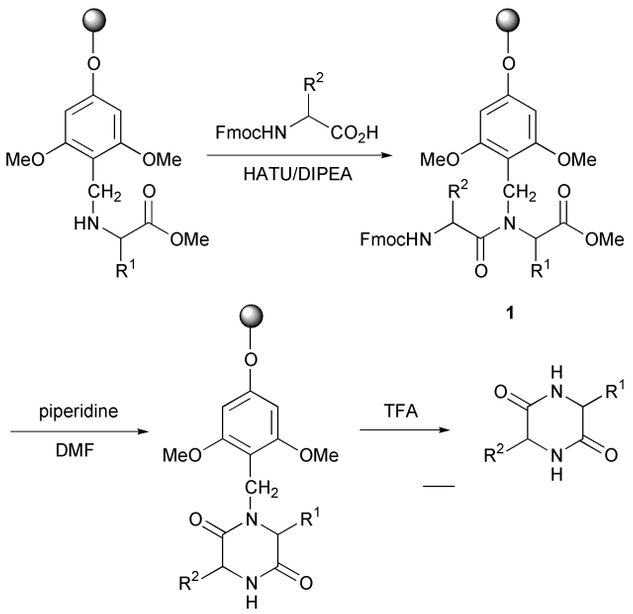



**3**

protecting group. These two compounds contain a free acid and a protected amino group making them suitable substrates for standard Fmoc peptide synthesis. To this end the same workers have incorporated compounds **2** and **3** into cyclic peptide mimetics which serve as templates that stabilise a β-hairpin conformation.

A number of reports have been published recently describing the synthesis of DKP derivatives. Like DKPs, these derivatives provide interesting scaffolds for the synthesis of biologically active compounds. Szardenings *et al.*[5] have used the multi-component Ugi reaction (Method B, Scheme 1) discussed




**Scheme 2**

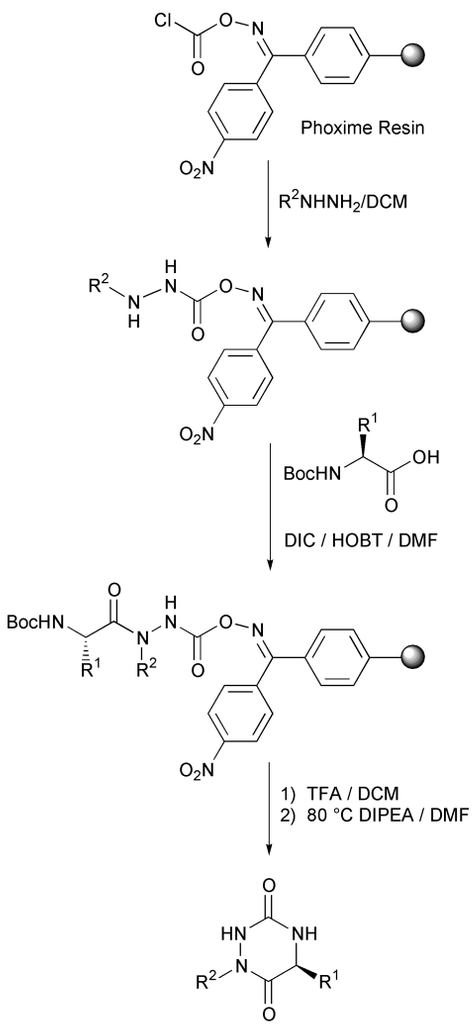
earlier to synthesise diketomorpholines (DKMs). This was achieved using an α-hydroxy acid in place of the soluble amino acid, to form the *N*-alkylated dimer. Scialdone and co-workers have reported novel methodology using Phoxime Resin™ for the synthesis of 1,2,4-triazine-3,6-diones (**4**)[6] (Scheme 3). The Phoxime Resin™ was reacted with a monosubstituted hydrazine to form a carbazate derivative to which a Boc-protected amino acid was coupled. Deprotection followed by thermolysis under basic conditions yielded the target triazine **4**. Finally, the synthesis of functionalised seven-membered ring cyclic dipeptides from suitably protected β-carboxyaspartic acid and *N*-substituted α-amino residues has been reported by Lavergne and co-workers.[9]

## 3 Solution phase head-to-tail cyclisation

While many ingenious approaches have been developed to enable the efficient head-to-tail cyclisation of linear peptides and their analogues, the great majority of published reports still employ 'traditional' macrocyclisations of activated precursors in the solution phase. Indeed, there are many examples where the convenience of adopting this straightforward strategy is accompanied by highly acceptable and high yielding outcomes. When faced with the problem of ensuring both effective carboxy activation and efficient interception by a nucleophilic amine, the chemist is presented with a bewildering array of reagents and their accompanying acronyms. Fig. 1 attempts to summarise a number of the most commonly encountered activation reagents and the following sections describe areas of cyclic peptide chemistry where head-to-tail cyclisations have been employed using these reagents.

### 3.1 Natural product cyclic peptides

A major concern in the area of the synthesis of cyclic peptides has been the total synthesis of natural products and their analogues. Indeed, a significant number of naturally occurring cyclic peptides possess powerful and useful biological activities. Recently reported examples involving head-to-tail cyclisation include a comparative study of the merits of various activation reagents for the synthesis of cyclosporin O,[10] the DPPA-mediated cyclisation of a precursor to eurystatin A,[11] various bradykinin analogues,[12] the marine natural product phakellistatin 5,[13] and the didemnin-like depsipeptide (−)-tamandarin A.[14]




**4**

**Scheme 3**

### 3.2 Peptidomimetics and pseudopeptides

Solution phase cyclisations have been successfully applied to the synthesis of numerous peptidomimetics and pseudopeptides. Indeed, many of the relevant substrates cyclise more efficiently than those leading to their 'natural' counterparts. A particularly impressive example of efficient cyclisation of a pseudoproline containing peptide **5** to produce **6** has been reported by the Mutter group (Scheme 4). In this case, oligomerisation was not observed even at concentrations of linear peptide in the vicinity of 0.1 M.[15] This example serves to illustrate the powerful effect of preorganisation of linear precursors into conformations approximating the cyclic target. Other peptide mimics which demonstrate remarkably efficient cyclisation incorporate aromatic groups as part of their backbone[16–18] or contain β-peptide derivatives such as homoalanines[19] and homoserine/homoaspartate/homoglutamate.[20] Each of these β-peptide systems was cyclised by the formation of pentafluorophenyl esters, acidolytic *N*-terminal deprotection and neutralisation of the so-formed trifluoroacetate salt in dilute solution. An example is provided by conversion of homoaspartate β-peptide **7** into macrocycle **8** (Scheme 5).

### 3.3 Clooligomerisations

A number of reports have described cyclooligomerisation reactions where, when presented with appropriate reaction conditions, simple peptides undergo spontaneous assembly to form cyclic products. One such example uses various metal ions to dimerise linear dipeptide methyl esters and thereby assist

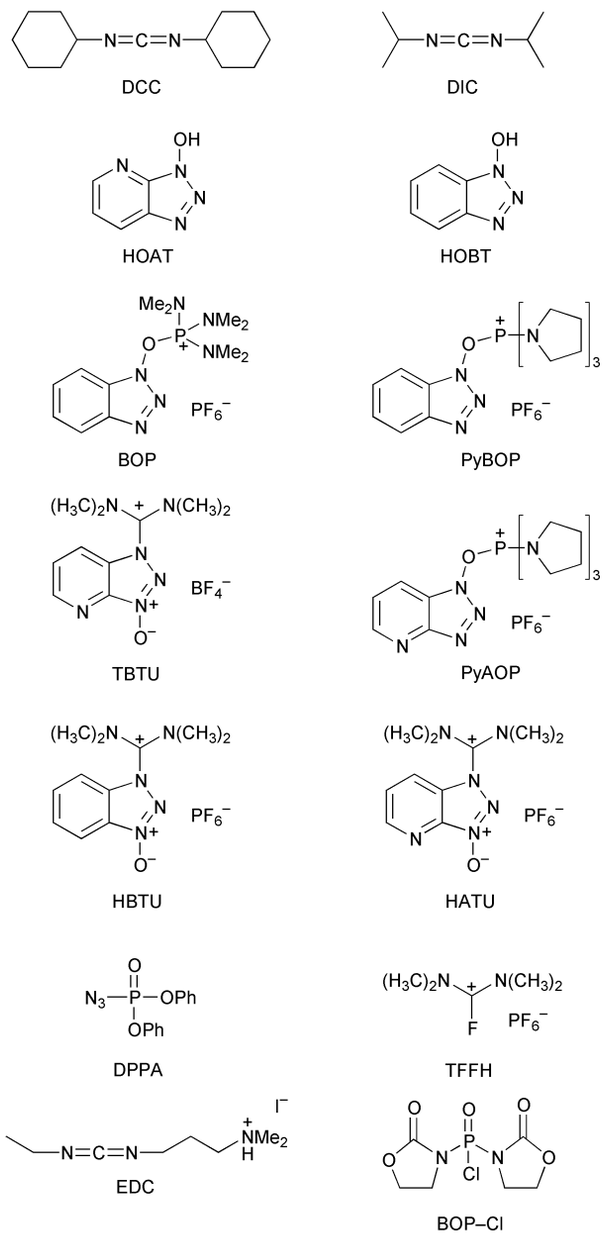
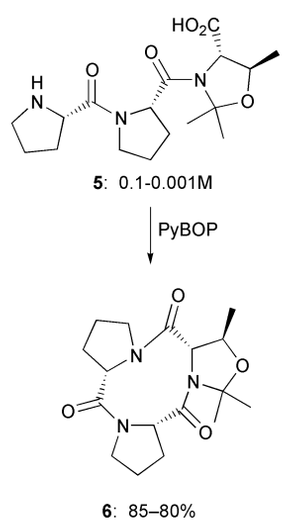
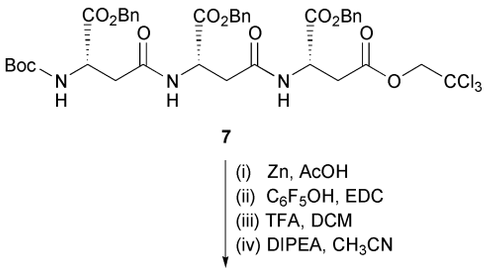



**Fig. 1**



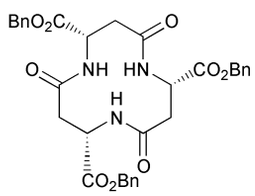

**Scheme 4**

formation of $C_2$-symmetric cyclic tetrapeptides (**9**). Acidolytic metal ion removal them provided macrocyclic peptides with varying ring sizes (Scheme 6).[21] In other recent reports, linear



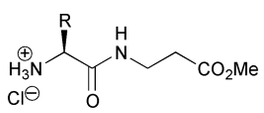

**7**

(i) Zn, AcOH
(ii) $C_6F_5OH$, EDC
(iii) TFA, DCM
(iv) DIPEA, $CH_3CN$

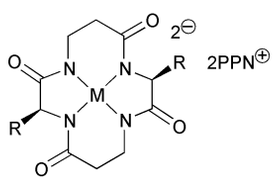



**8** 72%

**Scheme 5**

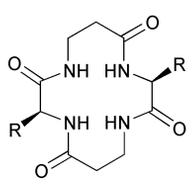



(i) $Na_2(PdCl_4)$, $NiCl_2$ or $CuCl_2$, NaOMe
(ii [PPN]Cl, PPN = $(Ph_3PNPPh_3)^+$




**9** 17–96%

HCl

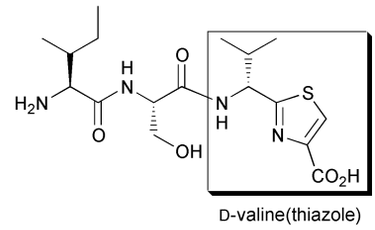


**Scheme 6**

tetrapeptides[22] and monomers[23] containing thiazole units have been found to undergo concentration-dependent cyclooligomerisation (Scheme 7). A noteworthy feature of both of these efficient cyclisation processes is that the linear precursors to both classes of cyclooligomers contain the β-turn inducing dipeptide derivative D-valine(thiazole).

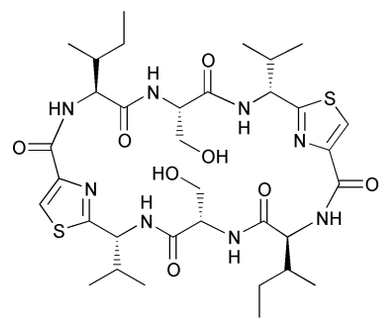
### 3.4 Cyclic peptides as models of protein motifs

The conformational constraints of cyclic peptides have allowed them to serve as useful and predictable models of various protein structural motifs. Head-to-tail cyclisation methods leading to the formation of β-sheet mimics (*e.g.* **10**) have included the use of diphenoxyphosphoryl azide (DPPA) for the cyclisation of acetylenic precursors[24] (Scheme 8) and the extensive studies from the Ghadiri laboratory on the synthesis and properties of homochiral[25] and syndiotactic cyclic peptides.[26,27] These remarkable materials self-assemble into tubular arrays and are held together by either parallel or antiparallel β-sheet interactions. Peptide synthesis of these materials can be performed using Boc or Fmoc[28] chemistries and the final cyclisation is commonly accomplished in solution using HATU–HOAT activation.



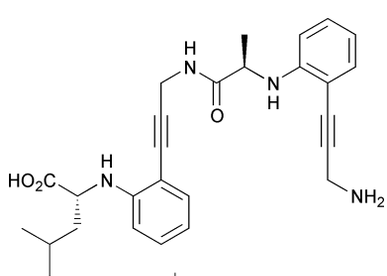

BOP/DIPEA in DMF

Cyclic oligomers: $n$ = 2–19

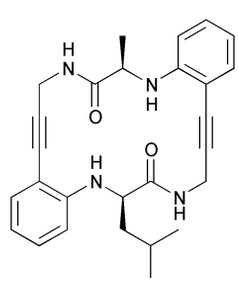


$n$ = 2

**Scheme 7**



DPPA, $NaHCO_3$, DMF



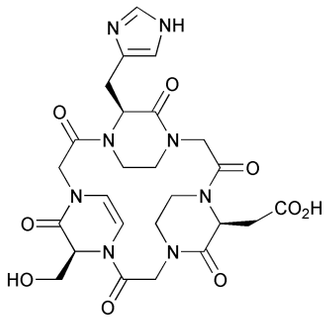
**10** 28%

**Scheme 8**

Other methods for structural motif stabilisation include steroid derivatives able to promote formation of β- and γ-loops,[29] the use of side chain bridges for α-helix stabilisation[30] and a novel cyclic tripeptidomimetic of the helix–turn–helix DNA-binding motif.[31] An interesting extension of the concept of protein fold stabilisation is the quest to generate enzyme active site mimics using cyclic peptide scaffolds. To this end cyclic peptides containing piperazin-2-one units (**11**) have been prepared as lipase active site mimics and were reported to accelerate the hydrolysis of 4-nitrophenyl acetate.[32] Similarly, a serine protease active site model has been prepared that is reported to be able to accelerate the hydrolysis of peptide and amino acid 4-nitroanilides.[33]

### 3.5 Peptide libraries

Given the explosion of interest in the synthesis of compound libraries and the fact that much of the earlier work in combina-



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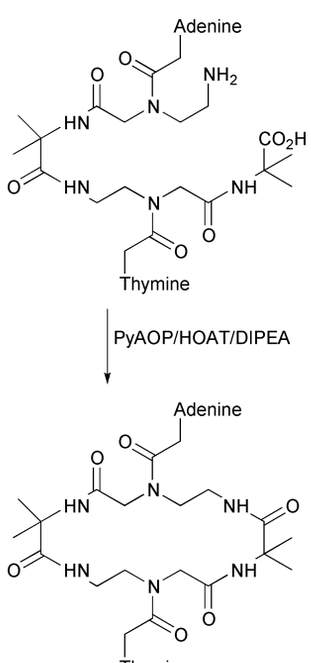
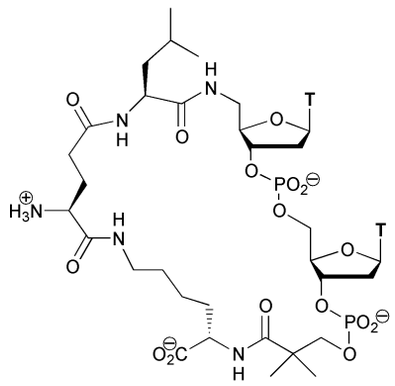
torial chemistry was originally performed using amino acids as diversity elements, it is not surprising that the synthesis of cyclic peptide libraries is a well explored area. A number of reports have appeared where peptide analogues are used as scaffolds for library synthesis and these include the investigation of stereochemical diversity in cyclic RGD-containing cyclic peptides,[34] the use of cyclic peptide natural product analogues as library templates[35] and a pseudopeptide template with potential as a source of new receptor libraries.[36] Other head-to-tail cyclic peptide libraries include those containing ψ[$CH_2NH$] amide bond surrogates[37] and where certain members were found to be novel strepavidin-binding compounds.[38]

### 3.6 Cyclic peptide–nucleic acid hybrids

The emergence of peptide nucleic acids (PNAs)[39] as novel oligonucleotide analogues has led to the discovery that certain PNAs can be cyclised to form cyclic PNAs. In these systems, nucleobases are attached *via* methylenecarbonyl linkages to the peptide backbone nitrogens and cyclisation is performed using standard Pfp ester chemistry[40] or PyAOP activation (Scheme 9).[41] Others have applied EDC, a water soluble diimide, to the cyclisation of 3′-*N*-aminoacyl-5′-nucleotides to form cyclic phosphoramidates. Possibly the most original and impressive synthesis of cyclic peptide–nucleic acids lies in a report from the Richert group.[42] In this study, cyclic hybrids (*e.g.* **12**), consisting of two or more nucleotides and a varying number of amino acid units, have been efficiently prepared. The peptide unit of these compounds was shown to confer nuclease stability on these macrocycles: thus exposure of **12** to nuclease S1 and




Scheme 9



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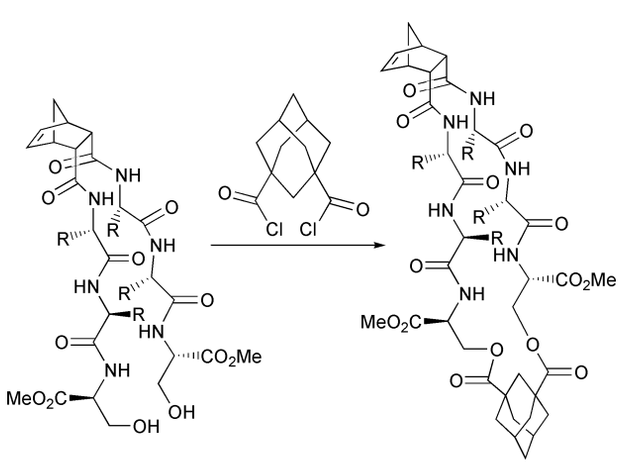
snake venom phosphodiesterase failed to produce any nucleolytic cleavage products. The same report details the synthesis of small libraries of such hybrids.

### 3.7 Cyclic depsipeptides

Cyclic depsipeptides represent an important class of cyclic peptides and are particularly well represented in the form of marine natural products. Because of their marine origins, many cyclic depsipeptides bear unusual amino acid side chains which adds to their interest and synthetic complexity. Methods for the synthesis of cyclic depsipeptides vary but, for the present purpose of describing the synthesis of cyclic peptides, these can be classified into either one of two categories: those where the critical cyclisation step involves formation of an ester (macrolactonisation) and those where ring closure involves formation of an amide (macrolactamisation).

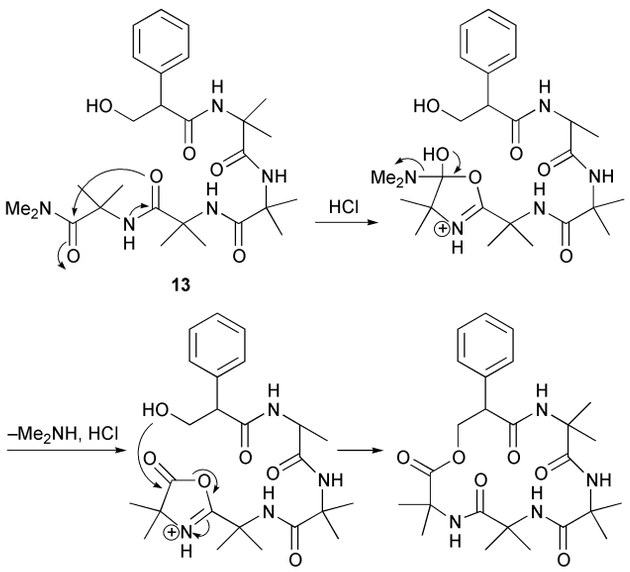
In recent reports where macrolactamisation is the key step *en route* to the cyclic depsipeptide, the ionophoric fungal metabolite valinomycin[43] and the marine natural product (−)-tamandarin A[14] have been prepared. In both these examples, cyclisation was performed in solution using HATU as the activation reagent.

As examples of macrolactonisation ring closures, the work of Ranganathan *et al.* provides an interesting example of both appropriate methodology and the use of organic frameworks as conformational constraints. In these cases, linear precursors are generally reacted with appropriate dicarbonyl dichlorides to produce cyclic depsipeptides in reasonable yields (Scheme 10). In these now extensive studies,[44,45] cyclic depsipeptides containing a variety of metal-coordination sites have now been prepared. Also of interest is the production of a peptide catenane (albeit in 5% yield) using this methodology.[46] Analogous systems that incorporate a cysteine bridge have also been reported by this group[47,48] and others.[49]



Scheme 10

Another highly innovative cyclisation method involves the so-called 'azirine/oxazolone' cyclisation process for the formation of cyclic depsipeptides.[50,51] One example of this method is shown in Scheme 11. Here, a 2,2-disubstituted-3-aminoazirine is used to prepare a linear precursor (**13**) bearing both a *C*-terminal dialkylamide and an *N*-terminal nucleophile. The *C*-terminal dialkylamide can then be either subjected to acid hydrolysis (to produce another carboxylic acid) or exposed to HCl in DMF. The latter reaction leads to formation of an oxazolone which is intercepted by the tethered nucleophile to produce, depending on the nature of the nucleophile, either the peptide or depsipeptide product. This ingenious approach, while apparently limited to the synthesis of α,α-disubstituted peptides, is nevertheless highly efficient and potentially of great value.

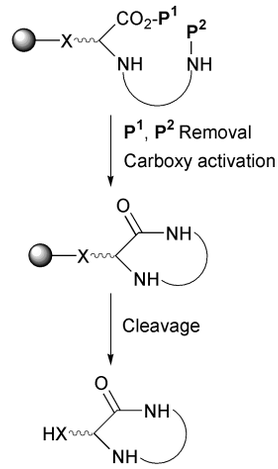



**Scheme 11**

## 4 On-support cyclisation using backbone/sidechain attachment

Many macrocyclisations require overcoming a considerable entropic barrier that may prevent the successful alignment of chain termini in a reactive conformation. While performing such macrocyclisations in dilute solution may, in many cases, increase the probability of intramolecular reactions between chain termini, an alternative approach is to limit intermolecular reactions and favour intramolecular reaction by performing cyclisations on immobilised linear precursors where resin loadings are deliberately kept low. This strategy, sometimes referred to as 'pseudo dilution' has been successfully applied by peptide chemists to the synthesis of head-to-tail cyclic peptides.

One approach to the formation of immobilised cyclisation precursors is to commence peptide synthesis with an amino acid that has been anchored to a resin *via* its side chain (Scheme 12). In this regard, orthogonally protected versions of Asp and Glu have been particularly useful. Orthogonal α-carboxy protection has been accomplished using Pd[0]-labile allyl esters,[52] and hydrazine-labile 4-{*N*-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl]amino}benzyl (DMAB) esters.[53] One comprehensive report has appeared where a variety of methods have been compared for the head-to-tail cyclisation of anchored peptides using orthogonally protected versions of Asp, Glu, Asn, Gln, Lys, Orn, diaminobutyric acid (DAB) and Ser.[54] The phenolic hydroxy group of tyrosine has also served as a useful point of attachment for certain cyclic peptides and initial immobilisation of orthogonally protected tyrosine has been accomplished using Mitsunobu methodology.[54,55] Selected data concerning some head-to-tail cyclisations of anchored peptides are presented in Table 1.

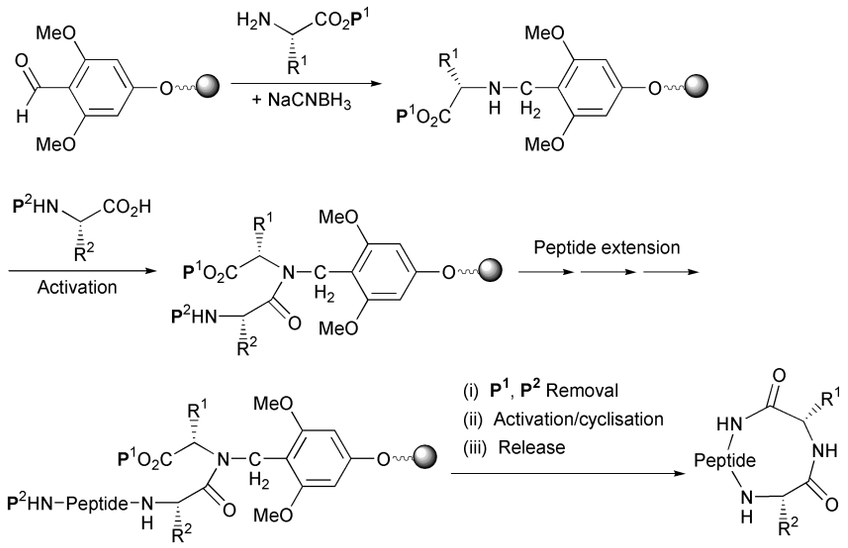



**Scheme 12**

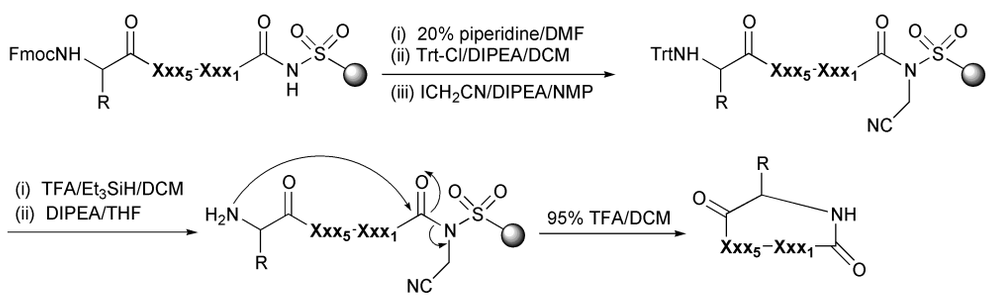
The preceding examples demonstrate the immobilisation of cyclic peptide precursors *via* suitably reactive side chain functionality. Clearly, this approach is limited to those amino acids with reactive side chains and an alternative approach that allowed for expansion of the repertoire of immobilisable amino acids would be desirable. Such approaches have been developed for both Boc and Fmoc chemistries by way of backbone amide linker (BAL) attachment (Scheme 13).[56] In this process, the first amino acid (usually as a methyl or allyl ester) is introduced by reductive amination of an aromatic aldehyde. This reaction can be performed either in solution or on a resin-bound aldehyde, although the latter is preferable as it avoids the need for *N*-protection prior to resin anchoring. Once installed on the resin, the secondary benzylic amine is acylated with the next amino acid and so on until the linear precursor is assembled. Exposure of the *C*-terminal carboxylate is then followed by cyclisation and cleavage to deliver the target macrocycle. While the flexibility of the BAL strategy theoretically allows any amino acid of a cyclic target to serve as a point of attachment to a resin support, this method does suffer from a propensity for the formation of diketopiperazine by-products upon deprotection of the second amino acid. Formation of such by-products can be avoided either by the use of Boc chemistry[57] or by the use of a highly acid labile $N^{\alpha}$-amino protecting group for the penultimate amino acid.[58]

**Table 1** Selected strategies for head-to-tail cyclisation of anchored peptides

| Anchored amino acid(s) | α-Amino protection | Resin | Orthogonal protecting group | Ref. |
|---|---|---|---|---|
| Asp | Fmoc | PAC/PAL | All | 52 |
| Asp, Glu | Fmoc | Pepsyn-K® | DMAB | 53 |
| Asp, Glu, Lys, Orn, DAB | Boc | HM-polystyrene | Fmoc | 54 |
| Asp, Glu | Boc | MBHA | Fmoc | 54 |
| Ser | Boc | AM-polystyrene | NB | 54 |
| Tyr | Fmoc | HMPB-MHBA/Wang/Sasrin | OMe | 55 |
| Tyr | Boc | HM-polystyrene | NB | 54 |

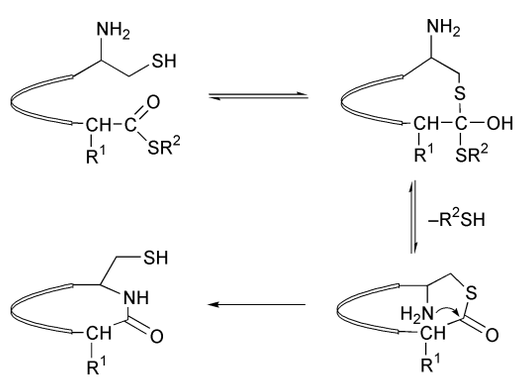



**Scheme 13**




**Scheme 14**

A conceptually different approach to 'on-resin' head-to-tail cyclisation is one where cyclisation and cleavage are intimately connected. Such strategies usually involve immobilisation of the first amino acid on a specialist resin *via* its $C^{\alpha}$-carboxy group in the 'traditional' manner. Once the linear precursor is assembled, head-to-tail cyclisation/release is triggered. In a recent report using this strategy, Kenner's 'safety-catch' linker was used for the synthesis of cyclic hexapeptides. Following assembly of the linear precursors, intramolecular aminolysis of *C*-terminal cyanomethylsulfonamides delivered cyclic peptide products in variable (17–52%) yields (Scheme 14).[59] Related methods where cyclisation and resin release are connected have employed intramolecular aminolysis of Kaiser oxime resin attachments[60,61] and thioester linkages.[62]

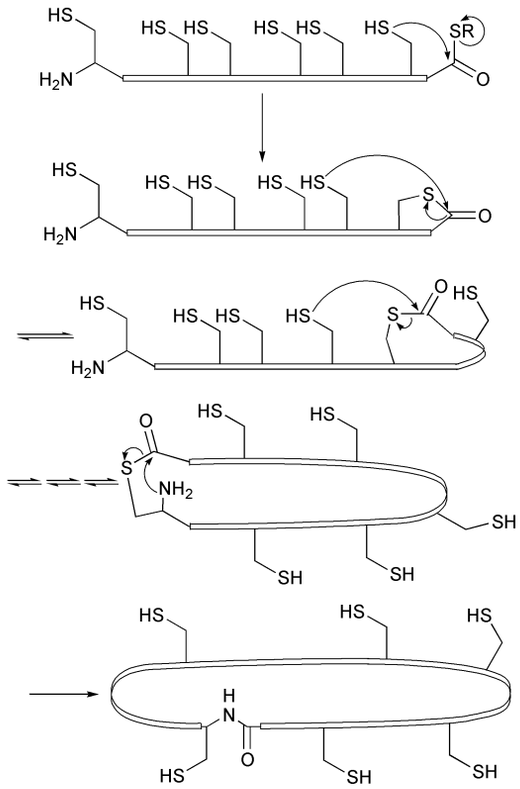



**Scheme 15**

## 5 Thiol-mediated native cyclisation

Thiol-mediated intermolecular native ligation of peptide segments was first reported by Kent and co-workers[63] and since its successful demonstration, modified versions of this methodology have enjoyed increasing popularity as means of peptide cyclisation. The feasibility of applying this technique to the preparation of head-to-tail cyclic peptides, *via* regioselective intramolecular transthioesterification and ring contraction, was first documented by Tam and Zhang in 1997.[64] This adaptation of Kent's native ligation methodology exploits the ring-chain tautomeric equilibrium that exists for linear peptide precursors containing an *N*-terminal cysteine and a *C*-terminal thioester (Scheme 15). The thiolactone intermediate generated in this process subsequently undergoes an irreversible proximity-driven ring contraction through *S*- to *N*-acyl migration, resulting in the formation of an amide-linked cyclic peptide.

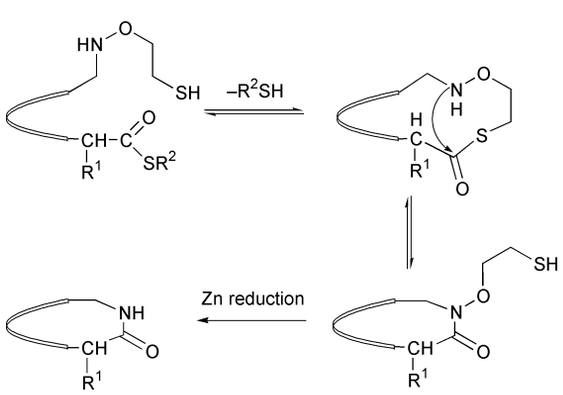
The generation of a native peptide bond provides the same outcome as standard head-to-tail cyclisation, but this ring contraction technique offers significant advantages over conventional lactamisation methods which usually require strong acyl activation of protected peptides in organic solvents at high dilution. Tam's studies, on the other hand, were performed using unprotected peptides in relatively concentrated aqueous solutions.[64] At pH 6.0–7.5 cyclisation proved to be highly selective, with no significant by-products observed even in the presence of competing side-chain groups such as lysinyl amines and cysteinyl thiols at various internal positions. The reaction was found to be essentially concentration-independent and was performed at peptide concentrations of up to 20 mM without affecting the yield or inducing oligomerisation. Tam's synthesis of the naturally occurring 31 residue peptide cyclopsychotride well illustrates the utility of the native ligation approach,[65] but this cysteine-rich molecule has also been synthesised recently *via* an elegant extension of the ring contraction chemistry in the form of the "thiazip" cyclisation (Scheme 16).[66] Requirements for the zip reaction include an *N*-terminal cysteine, a *C*-terminal thioester and one or more internal free thiols. Transthioesterification between an internal thiol and the terminal thioester gives rise to a thiolactone, and successive thiol–thiolactone exchanges lead to the α-amino thiolactone,



Scheme 16

which finally undergoes irreversible *S*- to *N*-acyl migration to yield the lactam. This successive ring-expansion method substantially lowers the entropic barrier and results in cyclisation rates more than 100 times faster than those obtained through one-step thiolactone formation and *S*,*N*-acyl migration. This acceleration in rate is beneficial for the sake of convenience, but also efficiency: slow cyclisations frequently lead to side reactions such as oligomerisation and epimerisation. It is envisaged that this method might enable the generation of very large peptide macrocycles, the formation of which has previously been considered too entropically unfavourable due to the difficulty in aligning the two termini in a reactive conformation.

Despite the obvious value of these thiol-mediated procedures, the necessity of a cysteine residue at the site of cyclisation imposes an obvious limitation on the method and consequently several research efforts have focussed on circumventing this requirement. To this end, Kent's group has described the use of a removable auxiliary group, oxyethanethiol, attached to the α-amine of an *N*-terminal glycine[67] (Scheme 17). This auxiliary, in effect, mimics the role previously played by the *N*-terminal cysteine. Cyclisation of the necessary $C^{\alpha}$-carboxythioester peptide proceeds *via* a thiolactone intermediate which then undergoes *S*–*N* acyl rearrangement to yield the



Scheme 17

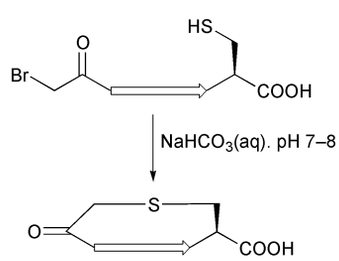
desired end-to-end lactam. The oxyethanethiol group may then be removed reductively with zinc in dilute acid. As with Tam's method, no appreciable oligomerisation or racemisation was detected.

Another novel lactam-forming approach which avoids the incorporation of a cysteine residue is silver ion-assisted cyclisation.[68] This method has also been established by Tam's group, and uses the thiophilic silver ion to coordinate the termini of a linear peptide thioester, forming a cyclic intermediate and facilitating intramolecular ligation through both entropic and enthalpic activation. This technique has now been investigated in considerable detail, and cyclisation of unprotected flexible peptides may be effected selectively under pH control through the *N*-terminal α-amine, a lysinyl amine or a tyrosinyl oxygen.[69] The efficiency of the silver-assisted cyclisation method also appears to be independent of peptide concentration.

Although the model peptides included in the studies of Tam and Kent have been cyclised with high efficiency, the contribution of inherent structural features of the linear peptides precursors to this outcome has not yet been determined. Inability of chain termini to align in a conformation conducive to ligation is a known cause of cyclisation failure, and it is noteworthy that the peptides selected for these studies all contain residues or sequences favourable to the adoption of stabilised turn structures. The efficacy of this methodology for cyclising peptides with hindered cyclisation sites or difficult rigid sequences has yet to be properly investigated. Attempts by Meutermans *et al*. to cyclise a difficult 5 residue sequence using an ethanethiol auxiliary installed on an *N*-terminal alanine—similar to the cysteinyl mimetic employed by Kent—failed to yield the desired lactam due to the inability of the thiolactone intermediate to undergo the necessary *S*- to *N*-acyl transfer.[70] It is worth pointing out, however, that the use of a nitrohydroxybenzyl, rather than a thiol auxiliary has done much to overcome this difficulty, thus potentially opening a new field of entropic activation methods.[70]

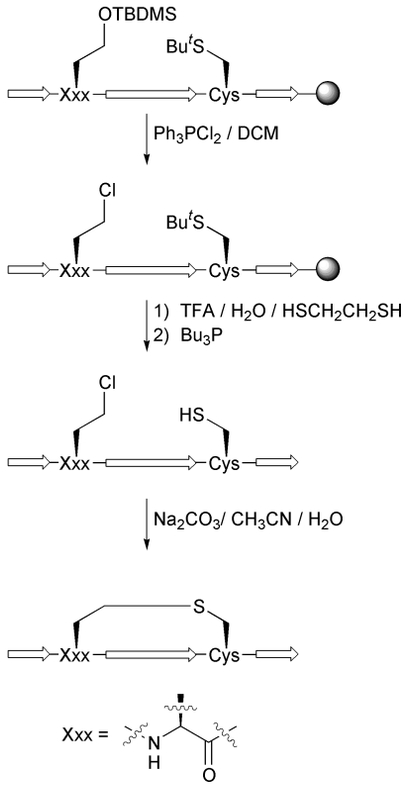
## 6 Approaches employing thioalkylation

Thioalkylation reactions offer a facile and versatile approach to the synthesis of cyclic peptides. Their use in this context was first established by Robey and Fields,[71] where the thiol of a *C*-terminal cysteine was used to displace an *N*-terminal acetyl bromide to form a cyclic thioether (Scheme 18). Akamatsu *et al*. have used a variation of Robey's methodology, wherein linear precursors bearing an *N*-terminal chloroacetyl function were reacted with *C*-terminal cysteine sulfanyl groups to form two 8 residue cyclic peptides.[72]



Scheme 18

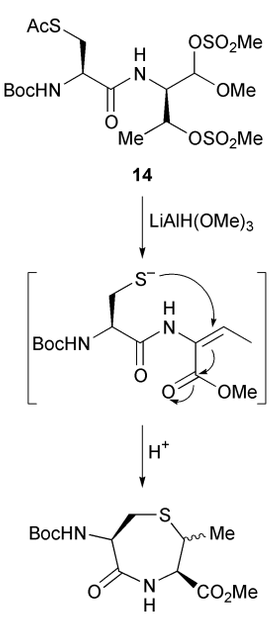
Yu *et al*. have reported novel methodology where an incipient leaving group is incorporated into one of the side-chains of the linear peptide precursor (Scheme 19).[73] Thus, chlorination of a homoserine residue on the solid support was performed using triphenylphosphine dichloride. The chlorinated linear precursor was then cleaved from the solid support and cyclised under basic conditions. This methodology augments the thioalkylation approach as homoserine and cysteine residues can be placed anywhere in the peptide sequence, allowing for extension of the peptide beyond the cyclic link. A similar approach has




**Scheme 19**

been used by Fotouhi *et al*. for incorporating a bromide leaving group into linear precursors for the synthesis of 13-membered thioether spirocyclic peptidomimetics.[74]

Efficient methodology has been reported where cyclisation *via* thioalkylation and cleavage of the linear precursor from the solid support are carried out in a single step.[75] This methodology is novel as cyclisation of peptides using thioalkylation is generally carried under mildly basic conditions.[71–74] Using this methodology a library of 12 cyclic pentapeptides was prepared with yields of generally greater than 95%. A conceptually different approach, wherein thioalkylation proceeds *via* Michael addition of a thiolate anion to an α,β-unsaturated ester, has been used by Botta and co-workers to prepare cyclic thioether dipeptides with a 1,4-thiazepinone scaffold (Scheme 20).[76] Preparation of the dipeptide **14** is followed by treatment with

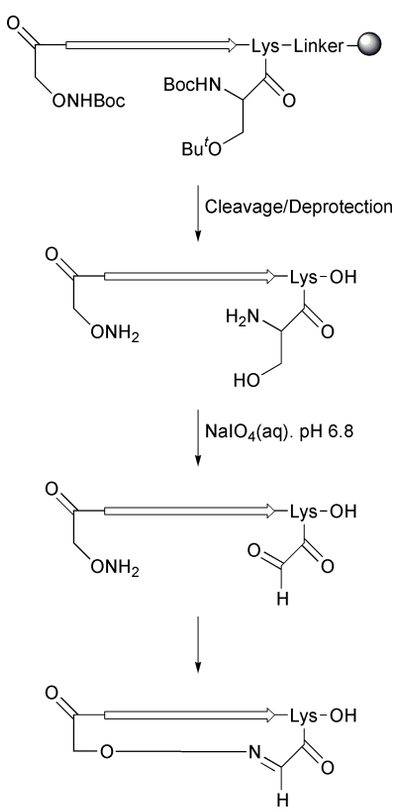



**Scheme 20**

lithium trimethoxyaluminium hydride and results in concomitant generation of both a Michael donor and acceptor which then undergo intramolecular conjugate addition to form a thioether cyclic dipeptide.

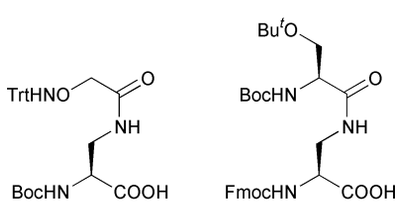
## 7 Oxime and thiazolidine formation

A versatile approach to the cyclisation of linear peptides employing intramolecular oxime formation has been established by Tam and Pallin (Scheme 21).[77] In this work, a linear peptide precursor was generated bearing an *N*-terminal *O*-alkylhydroxylamine and an $N^{\varepsilon}$-lysyl glyoxaldehyde obtained by the oxidative cleavage of an $N^{\varepsilon}$-lysyl serine. *In situ* condensation of the hydroxylamine with the glyoxaldehyde delivered the target cyclic oxime, presumably as a mixture of (*E*)- and (*Z*)-isomers although data supporting this were not recorded. This methodology is highly flexible as the hydroxylamine–aldehyde pair can be placed in a number of different configurations. For example Tam and Pallin have also positioned the *O*-alkylhydroxylamine internally through the use of an additional lysine to afford a sidechain-to-sidechain cyclic product.[78]




**Scheme 21**

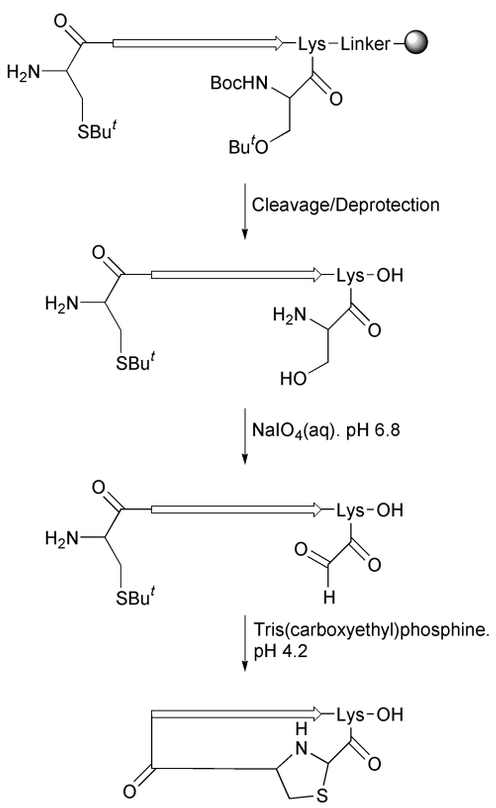
Mutter and Wahl have devised an innovative approach to incorporating a reactive hydroxylamine–aldehyde pair into a linear peptide precursor.[79] Two novel, orthogonally protected amino acid derivatives of diaminopropionic acid were synthesised, one containing a protected hydroxylamine (**15**) and the other a masked aldehyde (serine) (**16**). To initiate cyclisation, the masked aldehyde was exposed by oxidative cleavage




**15** **16**

of the serine residue. These amino acids are compatible with standard Fmoc synthesis and have been used effectively in the synthesis of cyclic oxime analogues of oxytocin.

A conceptually similar approach also established by Tam's group is the use of intramolecular thiazolidine formation (Scheme 22).[80] In this approach a linear peptide precursor is prepared containing an *N*-terminal cysteine and an $N^{\varepsilon}$-lysyl glyoxaldehyde. Deprotection of the cysteinyl thiol with a trialkylphosphine effects cyclisation wherein the 1,2-aminothiol of the *N*-terminal cysteine reacts with the glyoxaldehyde to form a thermodynamically stable thiazolidine ring. Cyclisation using thiazolidine formation has been efficiently accomplished at high concentrations (up to 7 mM) without any detectable polymerization.




**Scheme 22**

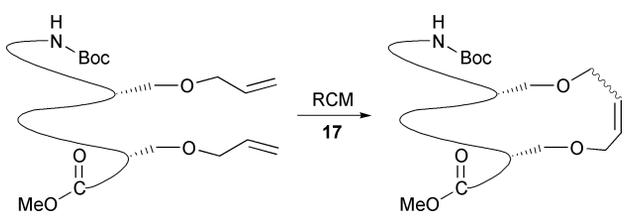
An impressive feature common to these methodologies is that cyclisation is carried out on unprotected linear peptide precursors.[77,78,80] This is a direct result of the highly chemoselective nature of intramolecular oxime and thiazolidine formation. Under the mildly acidic cyclisation conditions, the *O*-alkylhydroxylamine (oxime formation) and 1,2-aminothiol (thiazolidine formation) are the most potent nucleophiles present, with any other potentially nucleophilic functional groups on the linear peptide being protonated. The use of unprotected linear peptides in cyclisation reactions has a number of distinct advantages over the use of their protected counterparts: unprotected peptides are more likely to display secondary structures, such as β-turns, that may assist (or impede) cyclisation and the cyclic peptide, once formed, does not require further deprotection, thus simplifying the synthesis and often allowing the cyclic product to be used directly in biological assays.

## 8 Ring-closing olefin metathesis

Secondary structure can exert a profound influence on a peptide's affinity and selectivity for a biological receptor and consequently the imposition of conformational constraints is a well-recognised principle in drug design. The metabolic stability of C–C bonds makes ring-closing olefin metathesis (RCM) a particularly attractive method for the synthesis of constrained cyclic peptide structures. Recently, the remarkable functional group tolerance of Grubbs' catalyst $(PCy_3)_2Cl_2Ru$=CHPh (**17**)[81] has facilitated numerous efforts aimed at stabilising bioactive conformations of peptides and proteins.

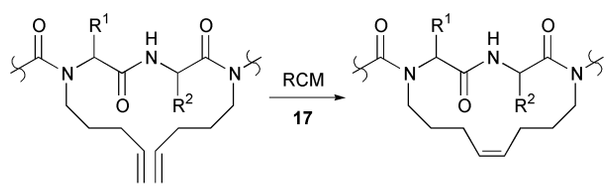
A conceptually simple methodology has been adopted by Rich's group,[82] wherein 2-aminohept-6-enoic acid (for synthesis, see ref. 83) is incorporated during a standard Boc peptide synthesis. When two such residues are included in a peptide sequence at $i$ and $i + 2$ positions, their respective alkene side-chains are positioned in appropriate proximity to undergo RCM upon treatment with carbene complex **17**. This pathway leads to a 15-membered macrocyclic tripeptide architecture which has previously been shown to stabilise enzyme-bound β-structures. Ring size is a critical determinant of the success of the ring-closure step, and attempts to generate 12- and 13-membered macrocyclic β-turn mimics by RCM have failed.[84]

The Grubbs group have reported an impressively facile procedure wherein RCM is used to introduce a link between the $i$ and $i + 4$ amino acid side chains[85] (Scheme 23). L-Serine and L-homoserine residues are derivatised as *O*-allyl ethers, then treated with ruthenium complex **17** to yield 21- or 23-membered olefinic macrocycles respectively, which subsequently may be reduced by catalytic hydrogenation. Linkers bridging the $i$ and $i + 4$ positions have previously delivered substantial stabilisation of helical motifs.[86–88]




**Scheme 23**

Methodology for the RCM of peptides without need for compromising the $C^{\alpha}$-side chains has been demonstrated recently by Liskamp and co-workers.[89] This approach is exemplified in Scheme 24. Of particular note is the fact that the introduced "loops" connect two amide nitrogens, leaving the amino acid sequence unaffected. Importantly, these macrocycles may be formed by connecting any two amide nitrogens, so long as the length of the alkene substituents is adjusted appropriately. It was established during the course of this work that RCM can be conducted using *N*-allylamides for a loop bridging two amides, *N*-pentenylamides for a loop spanning three amides, and *N*-homoallylamides when four or more amides are involved in the ring. The alkene substituents were introduced *via* Mitsonobu reaction of an olefinic alcohol with the activated *N*-terminus of a growing peptide chain,[90] which was synthesised using a solid phase Fmoc strategy. After cleavage from the resin, RCM was effected with alkylidene catalyst **17** in satisfactory to good (50–70%) yields. This technique offers the substantial benefit that the cyclisation is apparently sequence-independent, and because the introduced loops may span any number of amide bonds, it is envisaged that this methodology will enable the use of combinatorial approaches to generate libraries for the systematic probing of alternative conformations.



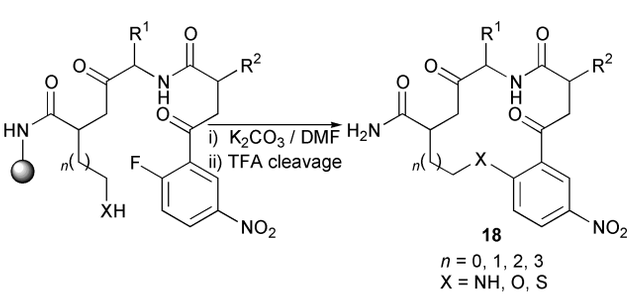

**Scheme 24**

## 9 $S_NAr$ cyclisation

$S_NAr$ displacement reactions involving haloaryl electrophiles have been employed with considerable success toward the synthesis of cyclopeptide alkaloids[91] and macrocyclic biaryl ether peptides,[92–95] as well as in the field of β-turn mimetics.[96–98] Extensive study of this reaction by Burgess, Zhu and co-workers[92] has led to the development of accessible conditions enabling $S_NAr$ macrocyclisation of peptides on the solid phase. It is predicted that this technology will lend itself to the rapid generation of compound libraries.

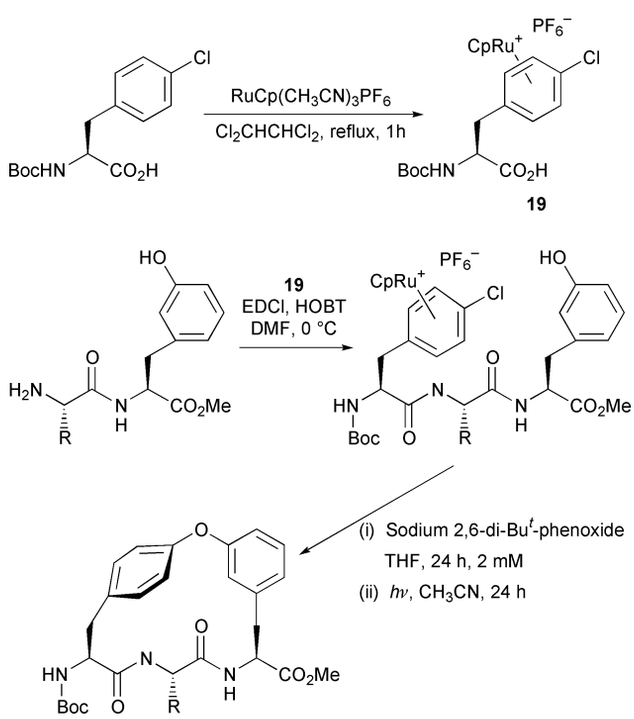
The utility of macrocyclisation *via* $S_NAr$ biaryl ether formation has long been recognised and has been amply demonstrated in the recent literature by several groups including those of Burgess,[92] Kiselyov,[95] Zhu[92,94] and Rich.[93] Biaryl ether groups are important motifs in antibiotics such as vancomycin and teicoplanin as well as in various other families of biologically active peptidic macrocycles. Notably, the development of solid phase techniques has enabled the rapid purification of some highly complex synthetic targets.[92,95] The choice of base used during the cyclisation step frequently proves critical, and while certain generalisations have been made when dealing with libraries of closely related targets, $S_NAr$ methodology very often entails the need to engage in optimisation studies with a variety of bases.

Recent studies and optimisation by Burgess and co-workers have seen the production of β-turn mimics under conditions freely amenable to high throughput parallel synthesis[96,97] (Scheme 25). Molecules of the general structure **18** have been generated, featuring a two amino acid β-turn region held in reactive conformation by a rigidifying cyclic scaffold. This method has been tested for *S*-, *O*- and *N*-nucleophiles, with ring sizes ranging between 13 and 17 members. In each case the cyclisation step is complete within a matter of hours, but formation of 13-membered rings proved the most demanding and it is likely that ring strain is an important determinant of cyclisation efficiency. A major attraction of this approach is that the cyclisation precursors are prepared *via* a conventional solid phase Fmoc method, using standard acid-labile protecting groups for the $R^1$ and $R^2$ side chains.




**Scheme 25**

A key requirement of this and other related methods is the need to selectively deprotect the amino acid side chain that serves as the nucleophile in the $S_NAr$ macrocyclisation. These nucleophilic side chains must remain protected during synthesis of the peptide sequence, although the chosen deprotection conditions must be sufficiently selective as to avoid cleavage from the solid support. Appropriate choice of protecting group strategy is consequently fundamental to the success of this approach. It is suggested by Burgess that optimal protection of *S*-nucleophiles is achieved with methoxytrityl, *N*-nucleophiles with methyltrityl and *O*-nucleophiles using unmodified trityl groups. In each case, deprotection may be effected using 1% $CF_3CO_2H$ and 4% $HSiPr^i_3$ in $CH_2Cl_2$. For the cyclisation step, $K_2CO_3$ in DMF proved the most broadly effective base of those examined. For *O*-nucleophiles, higher conversion was achieved using $Bu_4NF$ in DMF, although extensive epimerisation was observed in some cases. Significant amounts of cyclic dimers form as by-products in these reactions, and failure of this methodology is most often attributable to this competing process. It has been established, however, that the extent of formation of dimeric products can be substantially reduced by decreasing the resin loading. Using solution phase chemistry, Rich and Janetka have demonstrated the use of ruthenium-activated complexes to facilitate cycloetherification under mild reaction conditions without the need for strong electron-withdrawing substituents on the electrophilic arene.[93] This work is an adaptation of earlier efforts by Pearson *et al*. which involved intermolecular ether formation under similar conditions.[99] The method used by Rich entails initial $\eta^6$-complexation of Boc-3-chlorophenylalanine to cyclopentadienylruthenium hexafluorophosphate to form complex **19** (Scheme 26). Subsequent peptide couplings are performed using standard solution phase conditions, then displacement of chlorine by intramolecular aryl nucleophiles at high dilution followed by photolytic removal of the ruthenium complex leads to the target cyclic ethers in up to 75% yield. Comparison of cyclisation precursors with conventionally prepared linear peptides have confirmed that the necessary complexation–decomplexation processes do not cause epimerisation of the chiral peptide framework. This ruthenium activation method has now also been extended to aryl alkyl ethers and to *O*-, *N*- and *S*-based nucleophiles.[98] Optimisation of the nucleophile protection–deprotection strategy again proved vital, and during Rich's work with cysteine and lysine nucleophiles the fluoride-labile 2-(trimethylsilyl)ethoxycarbonyl (TEOC) group was found to provide results superior to those obtained using acid-labile trityl groups.



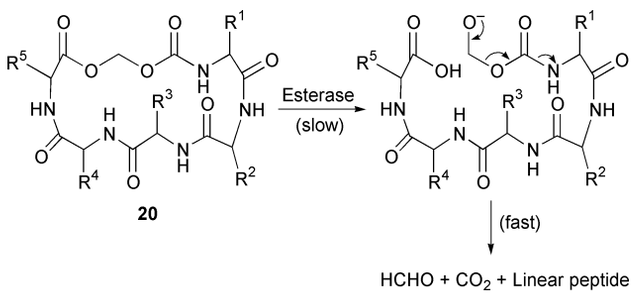

**Scheme 26**

## 10 Cyclic peptide prodrugs

A problematic aspect of the development of peptide drugs is that many of the essential features that impart a peptide's capacity for pharmacological activity can simultaneously restrict its ability to gain access to the targeted site of action. Frequently important functional characteristics such as side-chain and terminal amino and carboxy groups can severely limit a peptide's ability to permeate biological barriers. The prodrug approach, whereby unfavourable physicochemical

characteristics of a molecule are transiently altered, is one strategy proposed to aid the bioavailability of peptide drugs.

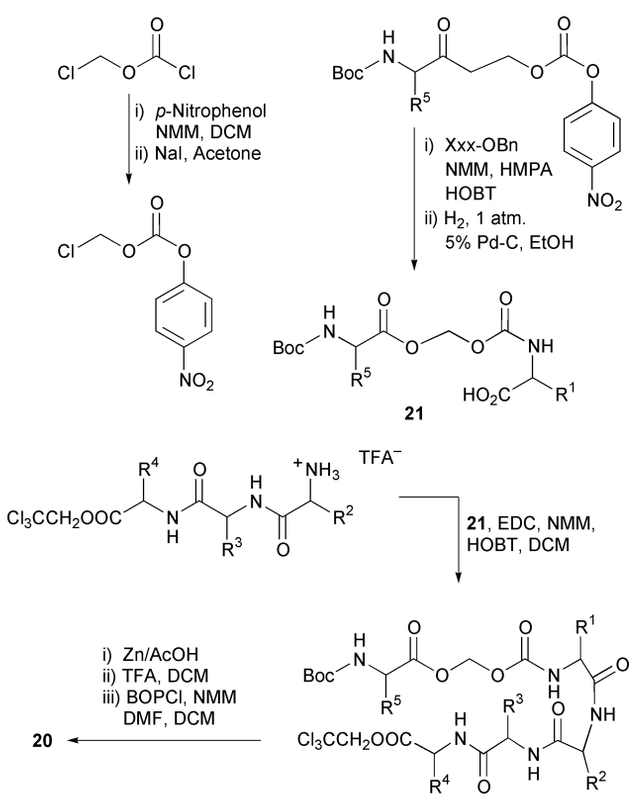
A methodology for the synthesis of esterase-sensitive cyclic prodrugs of linear peptides has recently been developed by Borchardt's group.[100,101] These molecules contain an (acyloxy)-alkoxy promoiety which is susceptible to slow esterase metabolism, and this step is followed by rapid chemical conversion to the linear peptide (Scheme 27).




20

**Scheme 27**

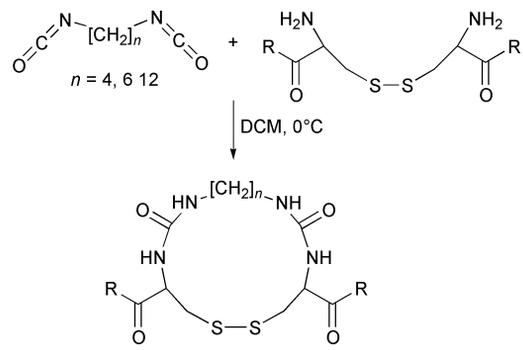
Initial attempts to generate prodrug **20** using standard solid phase Fmoc chemistry failed. The amino terminal of the linear peptide was coupled with 1-chloromethyl chloroformate before cleavage from the solid support, but cyclisation of this precursor to form the (acyloxy)alkoxy group could not be effected under various basic conditions. Preparation of these compounds was ultimately accomplished *via* a convergent solution phase method which involved the formation of the key (acyloxy)-alkoxy promoiety (**21**) prior to the cyclisation step (Scheme 28).[100] This procedure was initially demonstrated for the model hexapeptide H-Trp-Ala-Gly-Gly-Asp-Ala-OH utilising Boc and Bn *N*- and *C*-terminus protection strategies. The stability of this prodrug (**20**) was evaluated in human blood and was as expected found to degrade to the desired linear peptide. Transport studies also indicated significantly improved permeation characteristics for the cyclic prodrug as compared to its linear degradation product.




**Scheme 28**

## 11 Miscellaneous methodologies

This section of the review covers those reports that cannot be easily categorised into any of the sections discussed so far, but nonetheless describe novel and facile approaches to the synthesis of cyclic peptides that are worthy of discussion. In their continuing work on the preparation of self-assembled peptide nanotubes, Ranganathan *et al.* have prepared 16-, 18-, and 24-membered macrocyclic bisureas[102] (Scheme 29). Cyclisation and construction of the macrocycles were carried out in a one step process by the condensation of an ω-alkyl diisocyanate with either L-cysteine dimethyl ester or its extended *C*,*C*′- or *N*,*N*′-dipeptides in a 1:1 ratio under high dilution conditions.




**Scheme 29**

Le Grel and co-workers have reported the first synthesis of cyclic hydrazino pseudo-peptides where the backbone amide nitrogens are replaced with an *N*-methylhydrazine moiety.[103]

Erlanson and Wells have established methodology for the synthesis of cyclic di-, tri-, tetra- and pentasulfide peptides, using the reagent bis(tetrabutylammonium) hexasulfide (BTH).[104] Cyclisation proceeds *via* reaction of the BTH with two cysteinyl thiols of a linear precursor in an aqueous buffer. The number of sulfur atoms introduced into the sulfide cyclic link cannot be controlled effectively, so a mixture of cyclic di-, tri-, tetra- and pentasulfide peptides is obtained. The authors did note, however, that the number of sulfur atoms introduced into the sulfide cyclic link is influenced by the ratio of peptide to BTH.

## 12 Conclusion

The diverse functionality presented by unprotected linear peptides presents a unique challenge to those wishing to make selective intramolecular connections between different points of the peptide chain. In response, peptide chemists have developed many ingenious applications of appropriately chemoselective transformations and immobilised partially protected precursors. Just as the approach to many of these compounds is triggered by a desire to better understand the chemical processes at the heart of many biological systems, future work in this area will rely upon the application of selective and novel chemistry in the presence of the diverse functionality embodied within peptide backbones.

## 13 Abbreviations

| | |
|---|---|
| AM | aminomethyl |
| BAL | backbone amide linker |
| BOP | benzotriazolyloxytris(dimethylamino)-phosphonium hexafluorophosphate |
| BOPCl | bis(2-oxooxazolidin-3-yl)phosphinic chloride |
| BTH | bis(tetrabutylammonium) hexasulfide |
| DIC | diisopropylcarbodiimide |
| DKM | diketomorpholine |
| DKP | diketopiperazine |

| | |
|---|---|
| DMAB | 4-{*N*-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl]amino}benzyl |
| DMFP | 1,3-dimethyl-2-fluoropyridinium toluene-4-sulfonate |
| DPPA | diphenoxyphosphoryl azide |
| EDC | 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride |
| EDT | ethane-1,2-dithiol |
| HATU | *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]-pyridin-1-ylmethylene]-*N*-methylmethaminium hexafluorophosphate *N*-oxide |
| HBTU | *N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)-methylene]-*N*-methylmethaminium hexafluoro-phosphate *N*-oxide |
| HM | hydroxymethyl |
| HMPB | 4-(4-hydroxymethyl-3-methoxyphenoxy)butyric acid |
| HOAT | 1-hydroxy-7-azabenzotriazole |
| MBHA | 4-methylbenzhydrylamine |
| NB | 4-nitrobenzyl |
| NMM | *N*-methylmorpholine |
| NMP | *N*-methylpyrrolidone |
| PAC | phenacyl |
| PAL | 5-(4-aminomethyl-3,5-dimethoxyphenoxy)valeric acid |
| Pfp | pentafluorophenyl |
| PNA | peptide nucleic acid |
| PyAOP | 7-azabenzotriazol-1-yloxytris(pyrrolidino)-phosphonium hexafluorophosphate |
| PyBOP | 1*H*-benzotriazol-1-yloxytris(pyrrolidino)-phosphonium hexafluorophosphate |
| RCM | ring-closing metathesis |
| TBTU | *O*-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate |
| Teoc | 2-(trimethylsilyl)ethoxycarbonyl |
| TFFH | tetramethylfluoroformamidinium hexafluoro-phosphate |
| Xxx | amino acid |

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