Journal of Computer-Aided Molecular Design, **16:** 415–430, 2002.
KLUWER/ESCOM

Exploring privileged structures: the combinatorial synthesis of cyclic peptides

Douglas A. Horton[†], Gregory T. Bourne[†] & Mark L. Smythe[†#*]
[†]*Institute for Molecular Bioscience, The University of Queensland, St.Lucia, 4072, Qld., Australia;* [#]*Protagonist Pty. Ltd., Level 7 Gehrmann Laboratories, The University of Queensland, St.Lucia, 4072, Qld., Australia*

Key words: combinatorial chemistry; cyclic peptide; diketopiperazine; library synthesis; piperazine-2,5-dione; privileged structure; ring contraction; solid-phase

Summary

Head-to-tail cyclic peptides have been reported to bind to multiple, unrelated classes of receptor with high affinity. They may therefore be considered to be privileged structures. This review outlines the strategies by which both macrocyclic cyclic peptides and cyclic dipeptides or diketopiperazines have been synthesised in combinatorial libraries. It also briefly outlines some of the biological applications of these molecules, thereby justifying their inclusion as privileged structures.

Introduction

The post-genomic era will initiate major changes in the landscape of the pharmaceutical industry. It will change from an environment in which few known targets are carefully validated towards one in which many targets will exist with little validation. This will create a huge burden on target validation and lead compound identification, which will rely heavily on bioinformatics [1], structural genomics [2], proteomics [3], chemical genomics [4,5], high throughput screening [6] and combinatorial chemistry [7]. It is well documented that to-date, combinatorial chemistry and high throughput screening have not produced the results that were initially anticipated [8–10]. These technologies will need to keep in-step and in-pace with this changing target landscape.

The 'mechanics' of drug discovery (combinatorial chemistry and high throughput screening) are now sufficiently mature to meet their earlier expectations of synthesising and screening large arrays of compounds. Due to the immense size of the chemical universe, the selection of molecules to 'feed' the mechanics of drug discovery is rapidly becoming one of the most challenging issues facing medicinal chemists. A great source of inspiration for this problem may be obtained from nature, in which the relentless evolutionary selection of biologically active molecules has led to an enormous amount of natural diversity.

Antibodies are an excellent example of the manner in which nature has dealt with this problem. Antibodies are macromolecular proteins that can bind an infinite number of antigens. The available chemical diversity of these proteins is immense, as defined by the topographic arrangement of combinations of the 20 natural amino acids on the protein surface. However, binding is achieved exclusively through six-loops (β-hairpins) on the protein surface, known as complementarity determining regions (CDR's). Hence, it appears that a limited amount of the total protein diversity is biologically relevant. In comparison, the FC fragment of immunoglobulin G also binds numerous ligands using the same binding determinants [11–14]. This became clear when Wells and colleagues [15] used phage display to isolate peptides that bound to the FC fragment without selecting for biological function. The peptide that bound the tightest to the FC fragment did so at the functional binding site (the hinge

*Correspondence: m.smythe@imb.uq.edu.au

region), suggesting that the physiochemical features of this binding site enable it to bind multiple ligands.

This indicates that whilst the available diversity of the surfaces of proteins is almost infinite, only a subset of that space may be biologically relevant. In some respects this has been known for many years. For example, it is well known that β-turns are an important recognition element of peptides and proteins [16–19]. A great deal of effort has been focussed on clustering these motifs [20–29] and has been expended on generating synthetic methods towards synthesising organic equivalents [19,30–39]. Thus, the goal has been to harness the favourable binding characteristics of β-turns, or to exploit the biologically relevant regions of diversity space.

This concept has also been observed in small molecule drug development. Data from high throughput screening has suggested that some libraries of organic molecules have significantly higher hit rates then typical high-throughput screening results. In other words, these molecules have the ability to bind to a wide range of receptors. The term privileged structure has been used to describe this type of promiscuous scaffold, since it was first introduced by Evans *et al.* in 1988 in reference to the benzodiazepine scaffold [40]. The benzodiazepine scaffold is a core element of the natural product aspercilin, and is a prototypical privileged structure, as exemplified by the ability of 1,4-benzodiazepine-2-ones (such as **1**) to bind to cholecystokinin (CCK) (eg: **1**), gastrin and central benzodiazepine receptors (eg: **2**). In addition to these, the benzodiazepine scaffold is also found in neurokinin-1 antagonists (**3**), as enzyme inhibitors such as κ-secretase inhibitors (**4**) and farnesyl transferase inhibitors (**5**) and as ion channel ligands such as the delayed rectifier K^+ current modulator **6** (Figure 1) [41]. Since then, many other privileged structures have been identified, including diphenylmethane, biphenyltetrazole, spiropiperidine, indole and benzylpiperidine [42].

Interestingly, benzodiazepines have also been reported to be β-turn mimetics [43]. As many proteins and peptides use β-turns as a primary recognition element, it is interesting to speculate that natural products displaying wide-ranging biological activities have co-evolved to mimic the side-chain positions of these motifs. Obviously more data is required to derive more concrete conclusions.

One strategy to improve the efficiency of combinatorial chemistry and high throughput screening is to identify and exploit these privileged structures. [44–50]. A notable example of this was reported by Nicolaou *et al.*, in which the synthesis of a large combinatorial library of natural product like privileged structures was accomplished [51,52].

Cyclic peptides are another class of naturally occurring privileged structure. Once again, it is interesting to speculate that this maybe due to their ability to mimic biologically relevant regions of protein diversity, such as β-turns. In comparison to linear peptides, cyclic peptides are more stable to degradative peptidases, more bioavailable and also possess entropic advantages in molecular recognition. Despite this, they are not considered a source of drug-like molecules. However, several cyclic peptides are marketed drugs, such as the labour-inducing drug octreotide and the immunosuppressant cyclosporin A.

Libraries of cyclic peptides are exceedingly useful as 'molecular toolkits'. Constraining a single set of pharmacophoric groups into different conformational substates (as illustrated in Figure 2) would provide a valuable library for probing various receptors. Such a library is 'optimally diverse', since it explores both the conformational and chemical elements of diversity. Active and inactive analogues from such a library would allow one to deduce a pharmacophore for biological activity, which could be subsequently used to drive a small-molecule program. In this review we summarise some of the synthetic methods that are now available to synthesise such a library.

Cyclic Peptides

Head-to-tail cyclized peptides represent a large class of biologically-relevant molecules. For example, cyclosporin A is a cyclo undecapeptide that is clinically used as an immunosuppressant in the treatment of autoimmune disorders and for preventing organ transplant rejection [53]. Tyrocidine A and gramicidin S are cyclo decapeptide antibiotics [54,55]. Cyclic octapeptides can act as ion channel adapters, reducing unitary conductance and allowing organic molecules to act as channel blockers by binding to the cavity within the adapter [56]. Bicyclic octapeptides are also found in poisonous mushrooms (*Amanita phalloides*), as peptides that inhibit eukaryotic DNA-dependent RNA polymerases (mRNA synthesis) [57]. These mushrooms also contain mono- and bi-cyclic hexapeptides that bind to F-actin. Other cyclic hexapeptides are known as somatostatin agonists [58,59], neurokinin-1 and -2 receptor antagonists [58,60], δ-opioid recep-

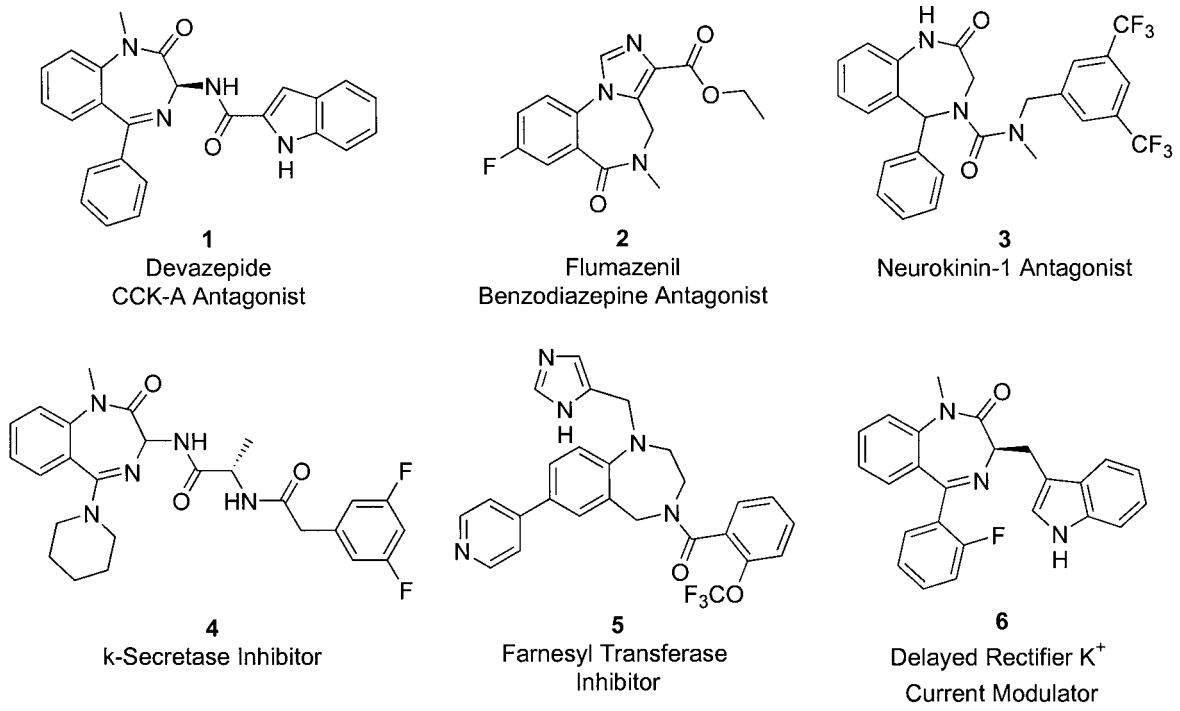


Figure 1. Representative biological activities of benzodiazepine scaffold

tor antagonists [61], $\alpha_4\beta_1$integrin receptor antagonists [62], as the Fe^{3+} siderophore ferrichrome (found in *E. coli*) [63,64] and as antifungals which act through the inhibition of glucan synthesis (the candins) [65]. Cyclic pentapeptides have been observed binding to the $\alpha_v\beta_3$integrin receptor [66] and as endothelin antagonists (BQ-123) [67]. Cyclic tetrapeptides also possess a range of biological activities, including histone deacetylase inhibitors (anti-tumour properties) [68,69] and a variety of very potent and highly selective phytotoxic and plant growth regulating peptides, including the potential herbicide tentoxin (from *Alternaria alternata* which acts on a variety of weeds of soybean and corn without affecting these crops) [70–72] and HC-toxin (a potent histone deacetylase inhibitor) [73–76].

There are five commonly observed classes of cyclic peptides (Figure 3). The most common of these is a head-to-tail cyclic product (A, Figure 3) in which lactamisation occurs between the carboxyl and amino end groups [77-80]. Alternatively, cyclisation can be affected between a side chain and the carboxyl terminus (B1) or amino terminus of the peptide (B2). Cyclisation may also be achieved between two side chains (C), which often involve the use of an additional chemical spacer. The final method by which cyclisation may be attained is through two backbone amide nitrogens (D).

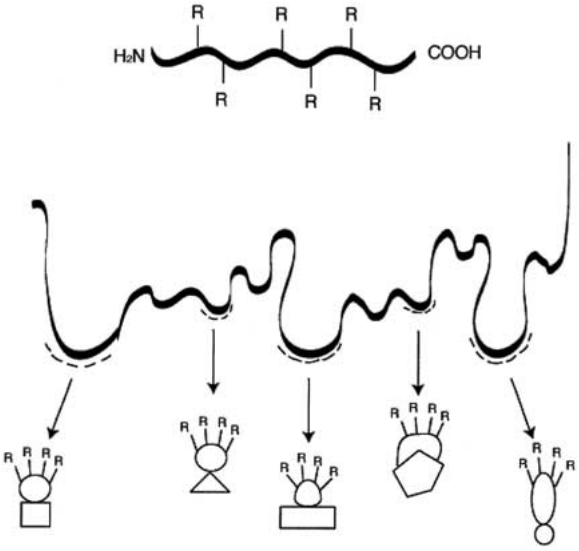


Figure 2. Schematic illustration of series of constrained cyclic peptides collectively sampling the broad conformational space of a linear analogue. The rectangles, triangles etc. represent organic scaffolds that force peptides into different conformational substates.

All of these strategies have different requirements for the orthogonal protecting groups employed. The

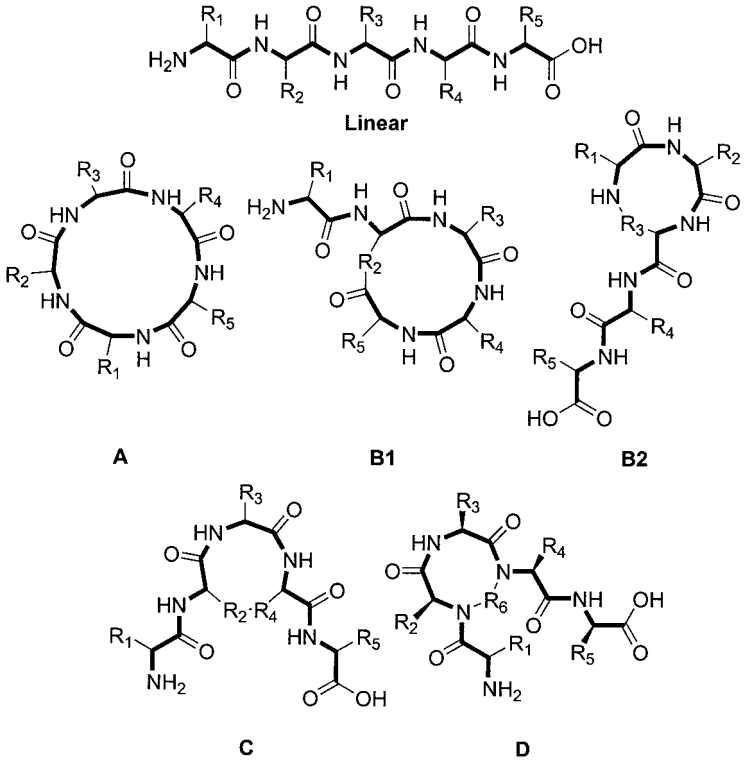


Figure 3. Strategies for cyclic peptide synthesis.

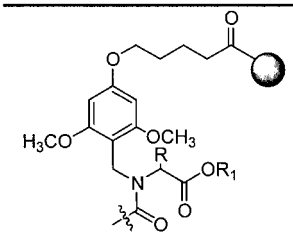
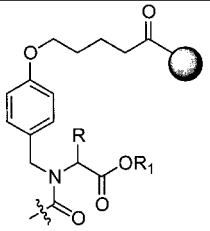
head-to-tail cyclic product (A) is generally the most conformationally constrained, due to steric restrictions imposed by multiple amide bonds in succession. The other strategies allow a greater number of saturated groups in the macrocyclic ring, increasing the number of conformations the macrocycle can adopt which leads to a corresponding decrease in the steric strain of the molecule. The placement of conformationally constraints on cyclic peptides is an important element of the library schematically illustrated in Figure 2. Due to the numerous number of reviews in the area [77–81] and that each strategy follows similar synthetic methods, only the most recent head-to-tail cyclisations will be discussed.

Four main strategies have been used to synthesise head-to-tail cyclic peptides on solid-support. Each of these requires a different type of linker. The solid support may be attached to the C-terminus of the peptide through a 'safety catch linker' or an activated linker, or alternatively, the solid support may be linked to the peptide through a side chain, or through an amide bond nitrogen.

The most common method for synthesising cyclic peptides on solid support is via attachment of the peptide through a side chain functionality [67,82–98]. This strategy requires orthogonal protection at the C-terminus, which is usually accomplished as an allyl protecting group and, requires an asparagine, glutamine or lysine (NH_2), histidine (imidazole), cystine (SH), tyrosine or serine (OH) or aspartic or glutamic acid (CO_2H) in the peptide sequence to link the peptide to the solid support. Cyclisation is achieved on resin, and side-chain deprotection and cleavage occurs in one step.

Spatola *et al.* utilised this strategy to synthesise a library of cyclic peptides, which led to the rediscovery of the endothelin antagonist BQ-123 [67]. The starting point for the synthesis was attachment of Boc-D-Asp-OFm to a *p*-hydroxymethylpolystyrene resin. Synthesis of the peptide chain then proceeded according to normal solid-phase peptide synthesis protocols. After the peptide chain was complete, the *N*-terminal *tert*-butyoloxycarbonyl (Boc) group and the C-terminal flurorenylmethyloxycarbonyl (Fm) group were removed and on-resin cyclisation was accomplished using benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) / 1-hydroxybenzotriazole (HOBt). Treatment

Table 1. Backbone amide linkers.

Linker	Cleaved in	Ref.
	TFA	[99-101]
	HF	[102-104]

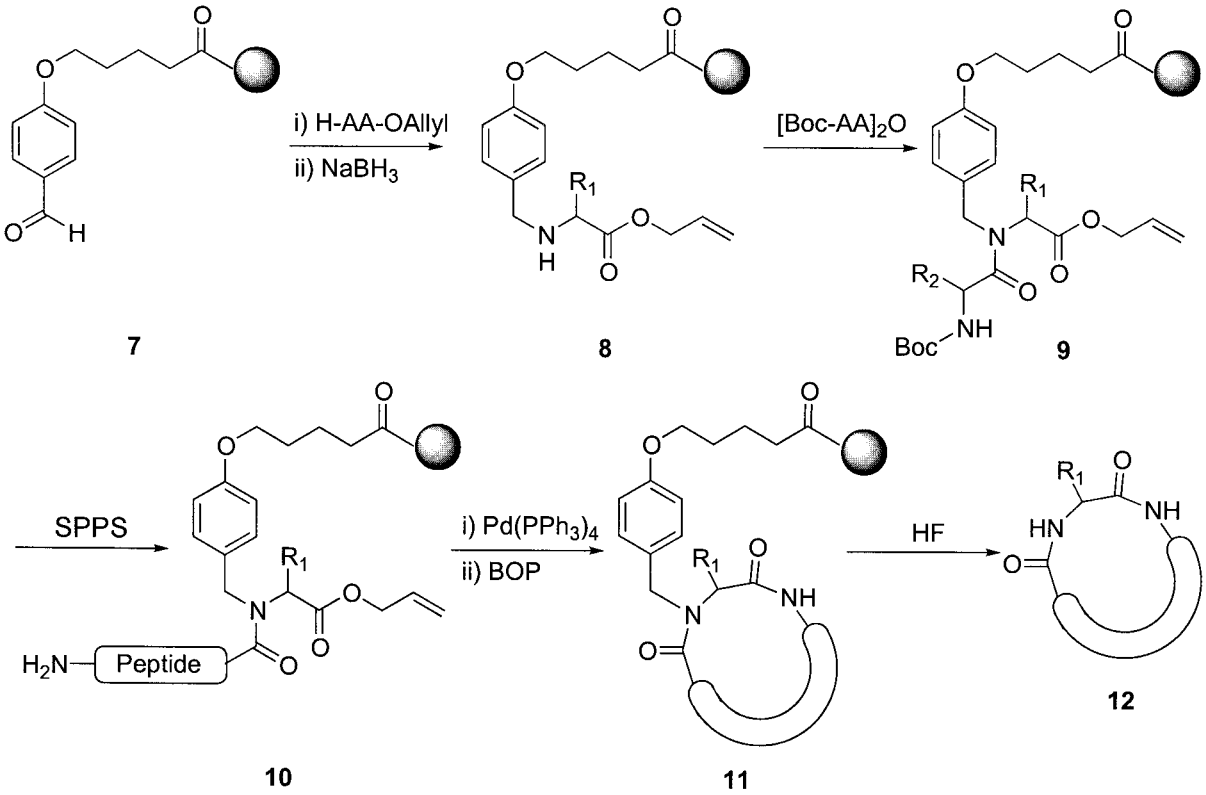
of the cyclised product on resin with hydrofluoric acid (HF) then cleaved the peptide from resin along with all of the side-chain protecting groups, affording the crude cyclic products.

A disadvantage of this method is that it necessitates the presence of an amino acid with appropriate side-chain functionality in the sequence. This can be circumvented through the use of a backbone amide linker (Table 1), with which any backbone amide nitrogen (except for proline) may be attached to resin [99-104]. In addition to this, backbone amide substitution is known to inhibit solid-phase aggregation [105] and can aid in the cyclisation of difficult sequences through promotion of the cis-amide bond conformation (due to reduction of the *cis-trans* amide bond rotational energy barrier) [106,107].

This strategy has been utilised a number of times in the synthesis of cyclic peptides. For example, Bourne *et al.* reported the synthesis of several cyclic peptides using this strategy (Scheme 1) [102,103]. After addition of the backbone amide linker **7** to the resin, an allyl C-terminally protected amino acid was added to the aldehyde, followed by sodium borohydride treatment to reductively aminate the amino acid to the linker (**8**). The next amino acid was then added as the symmetric anhydride due to the difficult coupling to the secondary amine (**9**). Following this, Boc-based solid-phase peptide synthesis could be used to synthesise the rest of the peptide chain (**10**). Once the desired peptide had been synthesised, the allyl group was removed through treatment with a catalytic amount of palladium tetrakis(triphenylphosphine). BOP mediated head-to-tail cyclisation could then affected yielding the cyclic peptide on resin (**11**), which could then be cleaved with hydrofluoric acid to yield the desired product (**12**).

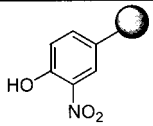
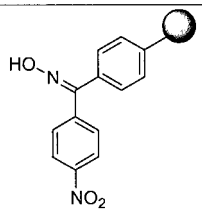
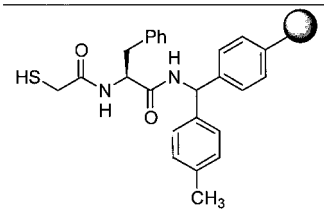
Despite the robustness of the above systems to a variety of conditions, both the existing backbone and side-chain attachment linker strategies require further purification of the product after cleavage to remove large excesses of scavengers, protecting groups and salts. The ideal type of linker would eliminate the need for further workup. The development and use of a "safety catch" style linker, in which deprotection occurs on resin prior to cyclisation, would be significantly more attractive. The evolution towards this type of linker was first initiated via activated linkers. Several examples of activated linkers are shown in Table 2 [108–123].

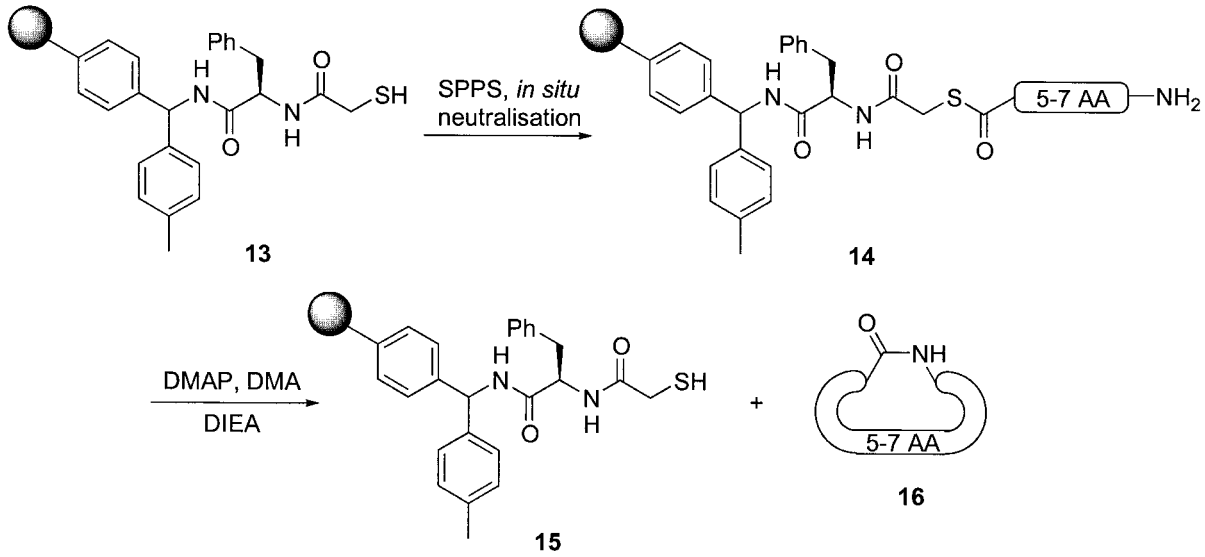
The critical feature of the activated linker strategy is the stability of the resin-to-peptide bond. In Boc-based peptide synthesis, this bond is designed to be relatively stable to repeated acidic cleavage of amino-protecting groups, to be resistant to nucleophilic attack during peptide assembly, and yet at the end of the synthesis be cleaved through head-to-tail cyclisation under acceptably mild conditions.



Scheme 1. Synthesis of cyclic peptides using the backbone linker approach [102–104]

Table 2. Activated linkers.

Linker	Ref.
	[108,109]
	[110-121]
	[123]



Scheme 2. Synthesis of cyclic peptides using an activated linker [123]

One example using this strategy has been reported by Richter *et al.* [123] for the synthesis of a small library of 8 cyclic peptides (Scheme 2). The synthesis began with MBHA resin with phenylalanine as a spacer joined to thioglycolic acid (**13**). The first amino acid was then added using N,N'-diisopropylcarbodiimide (DIC) with a tertiary amine base. Following this the linear peptide was built using the standard Boc-protocol. After the entire peptide had been assembled (**14**), the resin was treated with diisopropylethylamine (DIEA) and dimethylaminopyridine (DMAP) to accomplish cyclisation (**16**). Following this, the side-chain protecting groups were removed with HF.

Difficulties with the activated linker approach are often associated with the lability of the bond attaching the peptide to the resin. This can often result in small losses of peptide and also in possible oligomerisation during synthesis. In addition, current implementations of this strategy require cyclisation of fully-protected peptides. Thus, side-chain protecting group deprotection and further purification are required after cleavage.

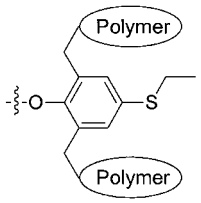
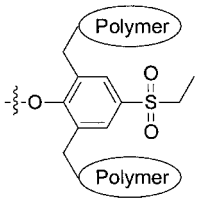
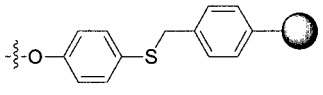
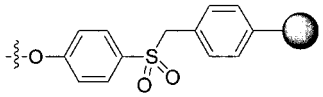
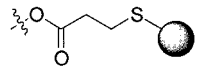
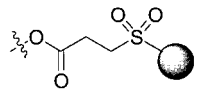
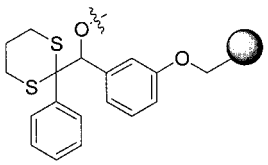
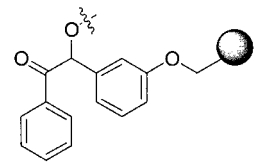
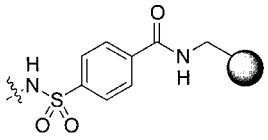
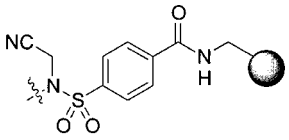
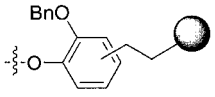
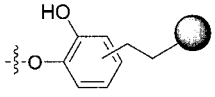
These weaknesses may be overcome through the use of a 'safety catch' linker. These linkers are attached to the peptide chain through a stable bond. Upon completion of peptide synthesis, treatment of the resin with an appropriate reagent transforms the bond linking the peptide to resin into a far more labile bond, which allows subsequent cyclisation to occur.

There are many examples in the literature describing 'safety catch' linkers [124–153]. The first 'safety catch' linker for cyclic peptide synthesis was developed by Marshall and Flannigan (linkers **17** and **18** Table 3) [126,127]. The major problem with this strategy was that unmasking the activated group from the sulphide to the sulphone required relatively harsh oxidative conditions. These conditions could potentially produce problems when either cysteine or methionine was present within the peptide sequence [154]. Likewise, Rothe *et al.* developed a similar linker (**19**), which also required oxidative activation (H_2O_2) [142]. Routledge *et al.* utilised a different strategy in the development of a dithiane 'safety catch' linker (**20**) [143], which upon oxidation with periodic acid breaks down to **20b**, and then to benzofuran, releasing the linear peptide. Once again, this 'safety catch' linker suffers from the harsh conditions required to activate the linker.

The best known 'safety catch' linker was developed by Kenner (**21**) [148]. This linker has been used many times in the laboratory for the synthesis of combinatorial libraries [149–153], including a library of cyclic peptides by Gough *et al* [155]. It should be noted that the conditions required for the alkylation of the sulphonamide bond could potentially cause alkylation of the amino acid side chains, especially for cysteine and tryptophan residues [154].

The basic principle behind this strategy is well illustrated by the final entry in Table 3 (**22**), which has been used with great success for cyclic peptide syn-

Table 3. 'Safety catch' linkers.

Linker		Activation	Ref.
Pre-activated	**Activated**		
 **17a**	 **17b**	m-CPBA/ dioxane	[126]
 **18a**	 **18b**	m-CPBA/ dioxane	[126,127]
 **19a**	 **19b**	H_2O_2/H_2O	[142]
 **20a**	 **20b**	$(CF_3CO)_2IC_6H_5$ or $Hg(ClO_4)_2$ or HIO_4	[143]
 **21a**	 **21b**	ICH_2CN	[153,155]
 **22a**	 **22b**	HF : p-cresol, 9:1 or HBr/TFA : p-cresol, 9:1	[124-126]

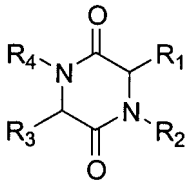
thesis by Bourne *et al.* (Scheme 3) [124,125]. The synthesis began with the addition of the amino acid as the symmetrical anhydride to the protected catechol on resin (**23**) using DIC to form the symmetrical anhydride and DIEA to deprotonate the catechol. After this, standard Boc-solid-phase peptide synthesis occurred until the entire peptide chain was assembled (**25**). Once this was complete, the protecting groups and the benzyl protecting group on the catechol was removed using standard peptide synthesis cleavage conditions, trifluoromethansulphonic acid (TFMSA) / trifluroroacetic acid (TFA) (**26**). Loss of the benzyl group on the catechol then provided a significant rate enhancement, so that upon treatment with diisopropylethylamine the peptide cyclised forming the cyclic product (**27**).

Yields from any of the processes outlined above are highly dependent on the size of the ring being formed and on the particular amino acid sequence of the linear precursor, giving rise to the well-known sequence-dependent effect of cyclisation [156]. Consequently, some cyclic peptides are inaccessible as they will not cyclise. To overcome this problem, a photolabile cyclisation auxiliary (6-nitro-2-hydroxy benzyl group) was developed to facilitate cyclisation of difficult sequences (Scheme 4) [156]. The strategy required the initial formation of a more accessible but reactive cyclic nitrophenylester intermediate (**29**), which spontaneously ring contracts through an *O*-to-*N* acyl transfer reaction to generate the cyclic lactam (**30**). Photolytic removal of the nitrobenzyl auxiliary then provided the target cyclic product (**31**). Unfortunately, this method has not yet been transferred to solid support.

Piperazine-2,5-diones

Piperazine-2,5-diones (**32**) (diketopiperazines or cyclic dipeptides) are small peptide derivatives found in nature [157]. Although some of the strategies used for the synthesis of these molecules are similar to those utilised for larger cyclic peptides, these scaffolds are much smaller and more organic in structure, and as a result different strategies are applicable that cannot be used for the larger cyclic peptides. Consequently, these molecules are discussed separately.

Piperazine-2,5-diones are a small, conformationally constrained heterocyclic scaffold that orientates its substituents in a spatially defined manner. In a similar manner to larger cyclic peptides, piperazine-

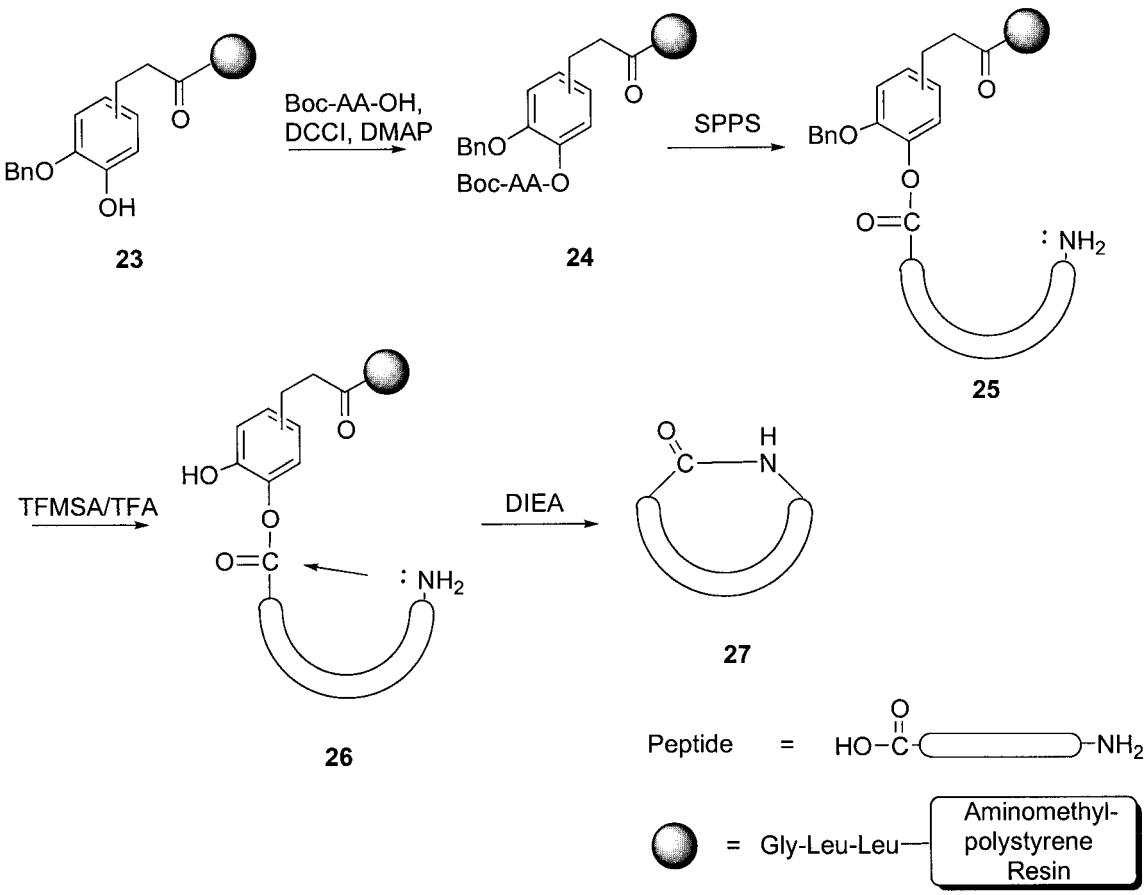


32

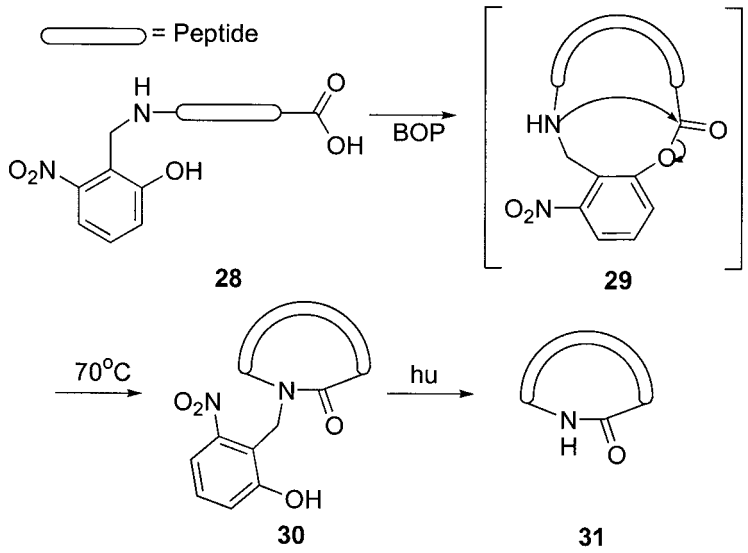
2,5-diones are typically stable to proteolysis [158]. These characteristics make them attractive scaffolds for medicinal chemistry.

The cyclic dipeptide heterocycle is often found alone or as part of larger and more complex molecules, and is also enzymatically synthesised in several members of the protist and plant kingdoms [157]. They are also found in mammals, and molecules such as cyclo(His-Pro) have been found throughout the mammalian brain and exhibit a wide variety of central nervous system, endocrine, electrophysiological and cardiovascular effects [157]. Other piperazine-2,5-diones have been shown to be opioid antagonists (cyclo(Tyr-Arg)), enterostatin analogues (cyclo(Asp-Pro)), and several piperazine-2,5-diones and derivatives are plant pathogens such as cyclo(Tyr-Pro) [157].

The medicinal properties of these heterocycles are also quite diverse. Piperazine-2,5-diones have been used in medicine as antibiotics, synthetic vaccines and in cancer chemotherapy [159-162]. Specifically, these activities include antagonism at the $\alpha_v\beta_3$ integrin receptor (possible application against osteoporosis) [163], at matrix metalloproteinases such as collagenase-1 and gelatinase-B (therapeutic targets with indications in cancer, arthritis, autoimmunity and cardiovascular disease) [158,164], at the bradykinin B_2 receptor (possible role against inflammation) [165], for breast cancer resistance protein transporter [166], at neurokinin-1 and -2 receptors (potential therapeutic use in a variety of neurological disorders) [167], inhibit thrombin (utility in the treatment of venous and arterial thrombosis, pulmonary embolism and restenosis following angioplasty, and atherosclerosis, inflammation and neurodegenerative diseases) [168], and increase the affinity of the dopamine D_2 receptor for agonists (possible application against Parkinson's Disease) [169]. The piperazine-2,5-dione scaffold has also been mixed with the arylpiperazine scaffold (many antagonists at serotonin receptors contain an arylpiperazine structure) to produce serotonin antagonists (which may be useful against cognition



Scheme 3. Synthesis of cyclic peptides using the safety approach [124–126]



Scheme 4. Ring contraction strategies for the synthesis of difficult cyclic peptides [156]

disorders) [170]. 3-Ylidenepiperazine-2,5-diones also exhibit antibacterial activity and inhibit tumour growth in mice [171]. Piperazine-2,5-diones also have agricultural applications, and have been reported to act as germination promoters for rice seeds under low-temperature stress, as well as resistance inducers in rice seedlings against water stress [172].

There are a great number of different strategies through which cyclic dipeptides may be synthesized. Perhaps the most obvious, and the most amenable to combinatorial chemistry, is through the cyclisation of dipeptide derivatives. Other strategies include intramolecular attack of amines on activated carbonyl groups, use of the Ugi reaction, cyclisation of *N*-pyruvoyl amino acid amides, intramolecular Diels-Alder reactions, and synthesis from α-haloacyl derivatives of amino acid esters [173]. However, in combinatorial synthesis, there are few methods that do not utilise amino acid building blocks [19].

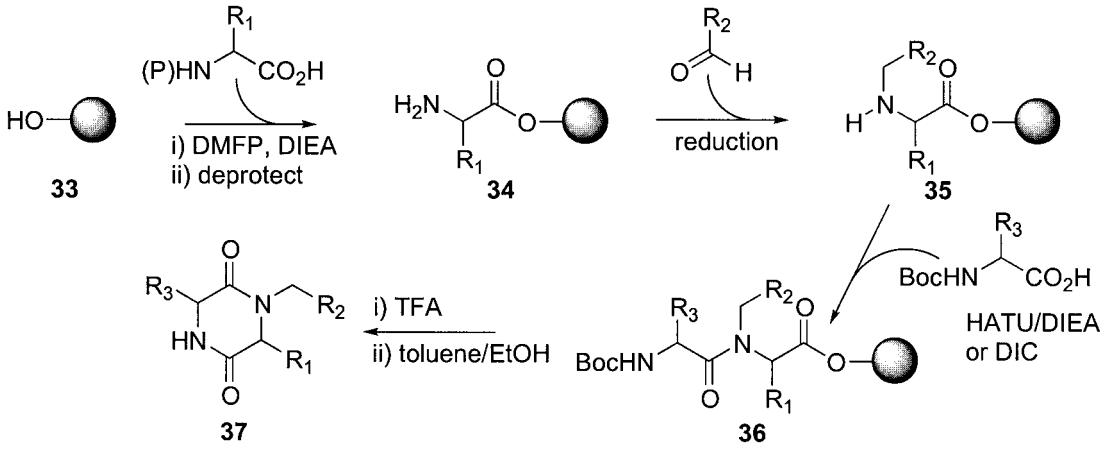
Many combinatorial libraries of piperazine-2,5-diones have been synthesised through solid-phase peptide synthesis of the linear peptide, followed by cyclisation. The cyclisation reaction has been established to be either acid or base catalysed and is strongly dependent on the sequence of the amino acids [101]. An example of this is shown below in Scheme 5. In this approach by Szardenings *et al.* [158,164,174], either Tentagel S-OH or PAM resin (**33**) was esterified using diisopropylethylamine or 1,3-dimethyl-2-fluoropyridinium-4-toluenesulphonate (DMFP) with either a Boc or Fmoc protected amino acid with acid labile protecting groups. Following deprotection of the *N*-terminus (**34**), the terminal amine was reductively alkylated with a primary aldehyde, producing the secondary amine (**35**). Acylation of the secondary amine with a Boc-protected amino acid was then accomplished, followed by treatment with TFA to remove the Boc group and all acid labile protecting groups without removing the dimer from the resin. Treatment of the solid support with toluene/ethanol mixtures under acidic or basic conditions then yielded the piperazine-2,5-dione (**37**) in very good purity. Many other combinatorial libraries have been synthesised on the solid-phase utilising a similar resin cleavage/cyclisation strategy [165,166,175–178]. In another case, a library was prepared by piperazine-2,5-dione formation on resin, followed by cleavage as a separate step [178]. Other groups have reported strategies of the latter type that are amenable for combinatorial synthesis [101,179]. A slightly different solid-phase approach to that employed in Scheme 5 was reported by Lin *et al.* [180]. In this approach, *N*-alkylated amino acids such as **35** are treated with bromoacetic acid, followed by substitution with primary amines to yield an *N*1 and *N*2 dialkylated dipeptide on-resin. Cleavage followed by acidic cyclisation yielded the piperazine-2,5-dione. Liquid phase syntheses, although less prevalent, have also been reported [168].

The Ugi reaction has also been utilised for piperazine-2,5-dione formation. An advantage of this approach is that it is a multi-component reaction, which reduces the number of reaction steps, and also circumvents the sometimes difficult secondary amine acylation (which is especially prevalent in sterically hindered amino acids such as valine) that is necessary in the process outlined in Scheme 5. An example of piperazine-2,5-dione synthesis using this reaction is displayed in Scheme 6 [158,174]. The efficiency of this reaction is most sensitive to the nature of the aldehyde component: aliphatic aldehydes react in minutes, whereas aromatic aldehydes and ketones require several hours [158]. Other combinatorial libraries have been synthesised utilising this reaction either on the solid [181] or liquid phases [182].

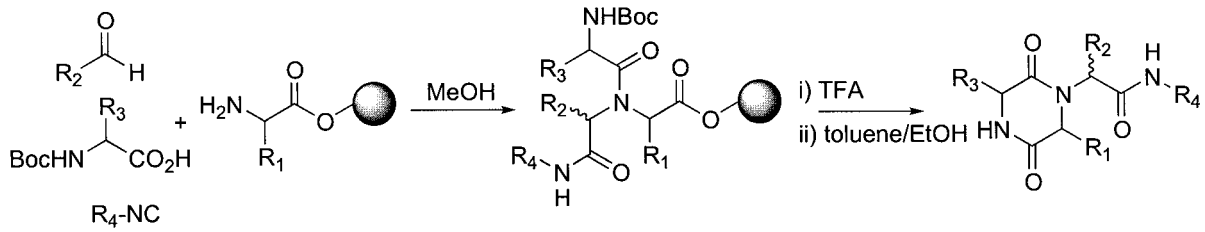
Although the two methods above are the most common synthetic strategies for piperazine-2,5-dione generation, other methods have also been reported [159,170,183,184]. One strategy reported by Falorni *et al.* involved the preparation of a protected piperazine-2,5-dione with C_2 symmetry in a single pot reaction (Scheme 7) [183,184]. These molecules could then be differentially deprotected for further derivatisation. This strategy is especially amenable to split and mix syntheses.

Conclusion

Cyclic peptides are attractive molecular targets for several reasons. Firstly, the chemistry of peptide synthesis is very mature; allowing the chemist to append a variety of chiral functional groups (from natural and unnatural amino acids) in a highly optimised and reproducible manner. Secondly, it is relatively straightforward to sample the conformational elements of diversity and force side chains into different topographical relationships using conformational constraints such as backbone cyclisation. Thirdly, a variety of active cyclic peptides have been observed to bind to a wide range of receptors. It is this range of biological activities, when coupled to their relative ease of synthesis, the wealth of chiral starting materi-



Scheme 5. Piperazine-2,5-dione synthesis through dipeptide formation [158, 164, 174]

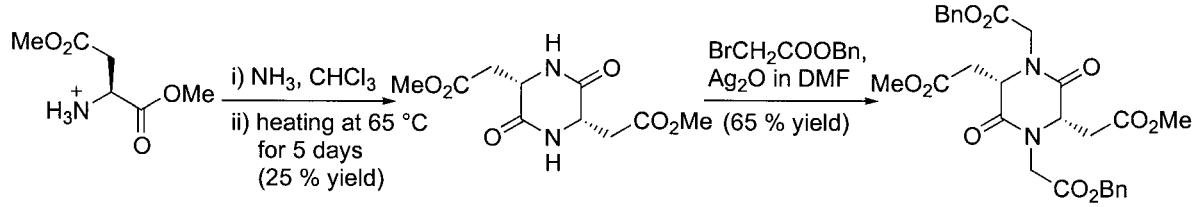


Scheme 6. Piperazine-2,5-dione synthesis utilising the Vgi reaction [158, 174]

als and the ability to easily sample the conformational and chemical elements of diversity that makes cyclic peptides appealing targets in drug development.

Additional drug-like characteristics may also be introduced into cyclic peptides. Such optimization may include either the introduction of amide bond isosteres or unnatural oligomers such as reduced amides or ureas. Alternatively, the macrocycle may be reduced to a smaller wholly organic scaffold. In this context, a library of molecules, such as cyclic peptides, that display a broad range of functional groups in a spatially controlled manner, represents a powerful tool through which a pharmacophore may be determined.

The broad range of biological activities that may be accessed from a cyclic peptide template makes the combinatorial synthesis of these molecules quite appealing. However, few syntheses of these compounds in large libraries have been reported. This is primarily due to difficult orthogonal deprotection strategies, which require a judicious choice of synthetic strategy (see Figure 4). For example, a solution phase cyclisation requires purification at each step of the synthesis (ie after synthesis of the linear, cyclised protected and after deprotection). In comparison, the activated linker allows cyclisation on resin, but this merely forms the protected cyclised peptide, which must be purified, deprotected and then purified again. The backbone amide and side chain attachment strategies are a significant improvement on this, as they allow synthesis on resin followed by a combined deprotection/cleavage step, leaving a mixture of cyclic peptide, scavengers and cleaved protecting groups. All of the above methods require a significant amount of time to be spent of purifying the cyclic peptide after synthesis. However, the "safety catch" linker allows the chemist to remove the protecting groups on resin before cleavage by cyclisation, alleviating the need for further workup. This is a very attractive process when producing large libraries. The only deficiency with this strategy is that the side-chain functionalities are deprotected on cyclisation, and thus side-chain cyclised products may be observed. This may be circumvented, at least in part, by careful selection of the order of the sequence. Large arrays of cyclic peptides have been synthesised for high throughput screening using the 'safety catch' linker of Bourne *et al.* [124,125]. The next evolution of linkers may entail cyclisation on solid support, followed by removal of protecting groups on resin, then mild cleavage conditions which would require very little work-up for purification.



Scheme 7. Symmetrical piperazine-2,5-dione formation [183, 184]

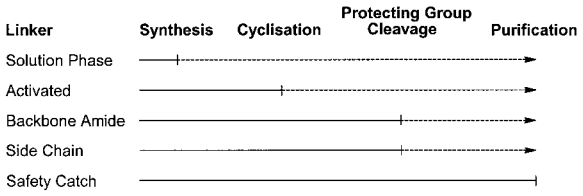


Figure 4. The extent to which cyclic peptide synthetic strategies may be accomplished on solid support.

Piperazine-2,5-diones are a simpler story. It is clear from the examples above that most strategies for piperazine-2,5-dione synthesis utilise a cyclisation/cleavage step. In effect, this is merely an activated linker. However, it must be noted that due to the size of the molecule, and the number of steps required for its synthesis, cyclisation or oligerimisation is less of a problem. This also allows removal of the side-chain protecting groups prior to cyclisation. Piperazine-2,5-diones have also been assembled by both side-chain attachment and backbone amide linker strategies. It does appear that although more advanced resin linking strategies can easily be applied to these small molecules, they are not as crucial as in the synthesis of larger cyclic peptides.

Head-to-tail cyclic peptides of many ring sizes may be considered to be privileged structures. The maturity of peptide chemistry has allowed medicinal chemists to devise a number of strategies for their synthesis. Nevertheless, very few of these strategies allow access to large arrays of cyclic peptides, with the exception of 'safety catch' linkers. Libraries of these molecules show great application as molecular probes to define binding determinants for new targets and through optimisation they may be used as the starting point to generate novel therapeutics. Cyclic peptides represent a promising means by which medicinal chemists may better understand and more rapidly identify biologically active molecules against an ever-increasing number of biological targets.

Acknowledgements

This review has attempted to be as comprehensive as possible. However, we apologise for any oversights in the literature that may have occurred and these emissions are the responsibility of the authors alone. The authors would like to thank present and past group members for their contributions to the concepts presented, particularly Tran Trung Tran, Stephen Love, Ross McGeary, Andrew McDevitt, Marc Campitelli, Simon Golding and Wim Meutermans. Various colleagues also contributed, particularly Mike Hann, Peter Seale, Alun Jones, Peter Cassidy, Paul Alewood, Mike Waters, Garland Marshall and Peter Andrews.

References

1. Goodman, N., Curr. Opin. Biotechnol., 13 (2002) 68–71.
2. Burley, S.K., Nat. Str. Biol., 7 (Suppl) (2000) 932–934.
3. Kreider, B.L., Annu. Rep. Med. Chem., 36 (2001) 227–235.
4. Caron, P.R., Mullican, M.D., Mashal, R.D., Wilson, K.P., Su, M.S., Murcko, M.A., Curr. Opin. Chem. Biol., 5 (2001) 464–470.
5. Schreiber, S.L., Bioorg. Med. Chem., 6 (1998) 1127–1152.
6. Wolcke, J., Ullmann, D., Drug Discov. Today, 6 (2001) 637–646.
7. Darvas, F., Dorman, G., Urge, L., Szabo, I., Ronai, Z., Sasvari-Szekely, M., Pure Appl. Chem., 73 (2001) 1487–1498.
8. Dean, P.M., Zanders, E.D., Bailey, D.S., Trends Biotechnol., 19 (2001) 288–292.
9. Hann, M.M., Leach, A.R., Harper, G., J. Chem. Inf. Comput. Sci., 41 (2001) 856–864.
10. Leach, A.R., Hann, M.M., Drug Discov. Today, 5 (2000) 326–336.
11. Deisenhofer, J., Biochemistry, 20 (1981) 2361–2370.
12. Sauer-Eriksson, A.E., Kleywegt, G.J., Uhlen, M., Jones, T.A., Structure, 3 (1995) 265–278.
13. Corper, A.L., Sohi, M.K., Bonagura, V.R., Steinitz, M., Jefferis, R., Feinstein, A., Beale, D., Taussig, M.J., Sutton, B.J., Nat. Struct. Biol., 4 (1997) 374–381.
14. Burmeister, W.P., Huber, A.H., Bjorkman, P.J., Nature, 372 (1994) 379–383.
15. DeLano, W.L., Ultsch, M.H., de Vos, A.M., Wells, J.A., Science, 287 (2000) 1279–1283.
16. Rose, G.D., Gierasch, L.M., Smith, J.A., Adv. Protein. Chem., 37 (1985) 1–109.

17. Andrianov, A.M., Mol. Biol., 33 (1999) 534–538.
18. Li, S.Z., Lee, J.H., Lee, W., Yoon, C.J., Baik, J.H., Lim.S.K., Eur. J. Biochem., 265 (1999) 430–440.
19. Freidinger, R.M., Veber, D.F., Perlow, D.S., Brooks, J.R., Science, 210 (1980) 656–658.
20. Wilmot, C.M., Thornton, J.M., J. Mol. Biol., 203 (1988) 221–232.
21. Wilmot, C.M., Thornton, J.M., Protein Eng., 3(6) (1990) 479–493.
22. Richardson, J.S., Adv. Protein. Chem., 34 (1981) 167–339.
23. Venkatachalam, C.M., Biopolymers, 6 (1968) 1425–1436.
24. Lewis, P.N., Momany, F.A., Scheraga, H.A., Biochim. Biophys. Acta, 303 (1973) 211–229.
25. Hutchinson, E.G., Thornton, J.M., Protein Sci., 3 (1994) 2207–2216.
26. Ball, J.B., Andrews, P.R., Alewood, P.F., Hughes, R.A., Fed. Eur. Biochem. Soc., 273 (1990) 15–18.
27. Ball, J.B., Hughes, R.A., Alewood, P.F., Andrews, P.R., Tetrahedron, 49 (1993) 3467–3478.
28. Garland, S.L., Dean, P.M., J. Comput. Aided Mol. Des., 13 (1999) 469–483.
29. Garland, S.L., Dean, P.M., J. Comput. Aided Mol. Des., 13 (1999) 485–498.
30. Ball, J.B., Alewood, P.F., J. Mol. Recognit., 3 (1990) 55–56.
31. Li, W., Burgess, K., Tetrahedron Lett., 40 (1999) 6527–6530.
32. Halab, L., Lubell, W.D., J. Org. Chem., 64 (1999) 3312–3321.
33. Terrett, N., Drug Discov. Today, 4 (1999) 141–141.
34. Gardner, R.R., Liang, G.B., Gellman, S.H., J. Am. Chem. Soc., 121 (1999) 1806–1816.
35. Lombardi, A., D'Auria, G., Maglio, O., Nastri, F., Quartara, L., Pedone, C., Pavone, V., J. Am. Chem. Soc., 120 (1998) 5879–5886.
36. Fink, B.E., Kym, P.R., Katzenellenbogen, J.A., J. Am. Chem. Soc., 120 (1998) 4334–4344.
37. Souers, A.J., Ellman, J.A., Tetrahedron, 57 (2001) 7431–7448.
38. Slomczynska, U., Chalmers, D.K., Cornille, F., Smythe, M.L., Beusen, D.D., Moeller, K.D., Marshall, G.R., J. Org. Chem., 61 (1996) 1198–1204.
39. Cornille, F., Slomczynska, U., Smythe, M.L., Beusen, D.D., Moeller, K.D., Marshall, G.R., J. Am. Chem. Soc., 117 (1995) 909–917.
40. Evans, B.E., Rittle, K.E., Bock, M.G., Dipardo, R.M., Freidinger, R.M., Whitter, W.L., Lundell, G.F., Veber, D.F., Anderson, P.S., Chang, R.S.L., Lotti, V.J., Cerino, D.J., Chen, T.B., Kling, P.J., Kunkel, K.A., Springer, J.P., Hirshfield, J., J. Med. Chem., 31 (1988) 2235–2246.
41. Patchett, A.A., Nargund, R.P., Annu. Rep. Med. Chem., 35 (2000) 289–298.
42. Mason, J.S., Morize, I., Menard, P.R., Cheney, D.L., Hulme, C., Labaudiniere, R.F., J. Med. Chem., 42 (1999) 3251–3264.
43. Fecik, R.A., Frank, K.E., Gentry, E.J., Menon, S.R., Mitscher, L.A., Telikepalli, H., Med. Res. Rev, 18 (1998) 149–185.
44. Yang, L., Berk, S.C., Rohrer, S.P., Mosley, R.T., Guo, L., Underwood, D.J., Arison, B.H., Birzin, E.T., Hayes, E.C., Mitra, S.W., Parmar, R.M., Cheng, K., Wu, T.J., Butler, B.S., Foor, F., Pasternak, A., Pan, Y., Silva, M., Freidinger, R.M., Smith, R.G., Chapman, K., Schaeffer, J.M., Patchett, A.A., Proc. Natl. Acad. Sci. U. S. A., 95 (1998) 10836–10841.
45. Patchett, A.A., Nargund, R.P., Tata, J.R., Chen, M.H., Barakat, K.J., Johnston, D.B.R., Cheng, K., Chan, W.W.S., Butler, B., Hickey, G., Jacks, T., Schleim, K., Pong, S.S., Chaung, L.Y.P., Chen, H.Y., Frazier, E., Leung, K.H., S.H.L., C., Smith, R.G., Proc. Natl. Acad. Sci. USA, 92 (1995) 7001–7005.
46. Smith, A.B.I., Cho, Y.S., Zawacki, L.E., Hirschmann, R., Pettit, G.R., Org. Lett., 3 (2001) 4063–4066.
47. Hirschmann, R., Hynes, J.J., Cichy-Knight, M.A., van Rijn, R.D., Sprengeler, P.A., Spoors, P.G., Shakespeare, W.C., Pietranico-Cole, S., Barbosa, J., Liu, J., Yao, W., Rohrer, S., Smith, A.B.I., J. Med. Chem., 41 (1998) 1382–1391.
48. Hirschmann, R., Ducry, L., Smith, A.B.I., J. Org. Chem., 65 (2000) 8307–8316.
49. Liu, J., Underwood, D.J., Cascieri, M.A., Rohrer, S.P., Cantin, L.-D., Chicchi, G., Smith, A.B.I., Hirschmann, R., J. Med. Chem., 43 (2000) 3827–3831.
50. Abrous, L., Hynes, J.J., Friedrich, S.R., Smith, A.B.I., Hirschmann, R. Org. Lett., 3 (2001) 1089–1092.
51. Nicolaou, K.C., Pfefferkorn, J.A., Mitchell, H.J., Roecker, A.J., Barluenga, S., Cao, G.Q., Affleck, R.L., Lillig, J.E., J. Am. Chem. Soc., 122 (2000) 9954–9967.
52. Nicolaou, K.C., Pfefferkorn, J.A., Roecker, A.J., Cao, G.Q., Barluenga, S., Mitchell, H.J., J. Am. Chem. Soc., 122 (2000) 9939–9953.
53. Emmel, E.A., Verweij, C.L., Durand, D.B., Higgins, K.M., Lacy, E., Crabtree, G.R., Science, 246 (1989) 1617–1620.
54. Mootz, H.D., Marahiel, M.A., J. Bacteriol., 179 (1997) 6843–6850.
55. Kratzschmar, J., Krause, M., Marahiel, M.A., J. Bacteriol., 171 (1989) 5422–5429.
56. Sanchez-Quesada, J., Ghadiri, M.R., Bayley, H., Braha, O., J. Am. Chem. Soc., 122 (2000) 11757–11766.
57. Wieland, T., Int. J. Peptide Protein Res., 22 (1983) 257–276.
58. Hirschmann, R., Yao, W.Q., Cascieri, M.A., Strader, C.D., Maechler, L., Cichy-Knight, M.A., Hynes, J., van Rijn, R.D., Sprengeler, P.A., Smith, A.B., J. Med. Chem., 39 (1996) 2441–2448.
59. Rohrer, S.P., Schaeffer, J.M., J. Physiol. Paris, 94 (2000) 211–215.
60. Quartara, L., Pavone, V., Pedone, C., Lombardi, A., Renzetti, A.R., Maggi, C.A., Regul. Pept., 65 (1996) 55–59.
61. Schiller, P.W., Berezowska, I., Nguyen, T.M.-D., Schmidt, R., Lemieux, C., Chung, N.N., Falcone-Hindley, M.L., Yao, W., Liu, J., Iwama, S., Smith, A.B., Hirschmann, R., J. Med. Chem., 43 (2000) 551–559.
62. Boer, J., Gottschling, D., Schuster, A., Semmrich, M., Holzmann, B., Kessler, H., J. Med. Chem., 44 (2001) 2586–2592.
63. Braun, V., Bös, C., Braun, M., Killmann, H., J. Infect. Dis., 183 Suppl 1 (2001) S12–S16.
64. Braun, V., Killmann, H., Benz, R., FEBS Lett., 346 (1994) 59–64.
65. Tkacz, J.S., DiDomenico, B., Curr. Opin. Microbiol., 4 (2001) 540–545.
66. Belvisi, L., Bernardi, A., Checchia, A., Manzoni, L., Potenza, D., Scolastico, C., Castorina, M., Cupelli, A., Giannini, G., Carminati, P., Pisano, C., Org. Lett., 3 (2001) 1001–1004.
67. Spatola, A.F., Crozet, Y., deWit, D., Yanagisawa, M., J. Med. Chem., 39 (1996) 3842–3846.
68. Yoshida, M., Furumai, R., Nishiyama, M., Komatsu, Y., Nishino, N., Horinouchi, S., Cancer Chemother. Pharmacol., 48 Suppl 1 (2001) S20–S26.
69. Yoshida, M., Horinouchi, S., Beppu, T., Bioessays, 17 (1995) 423–430.

70. Lax, A.R., Shepherd, H.S., ACS Symp. Ser., 380 (1988) 24–34.
71. Lax, A.R., Shepherd, H.S., Edwards, J.V., Weed Technol., 2 (1988) 540–544.
72. Edwards, J.V., Dailey, O.D., Bland, J.M., Cutler, H.G., ACS Symp. Ser., 380 (1998) 35–56.
73. Bernardi, E., Cros, S., Viallefont, P., Lazaro, R., Bull. Soc. Chim. Fr., 131 (1994) 944–948.
74. Shute, R.E., Kawai, M., Rich, D.H., Tetrahedron, 44 (1988) 685–695.
75. Kawai, M., Jasensky, R.D., Rich, D.H., J. Am. Chem. Soc., 105 (1983) 4456–4462.
76. Liesch, J.M., Sweeley, C.C., Staffeld, G.D., Anderson, M.S., Weber, D.J., Scheffer, R.P., Tetrahedron, 38 (1982) 45–48.
77. Hruby, V.J., Wilke, S., Al-Obeidi, F., Jiao, D., Lin, Y., React. Polym., (1994) 231–241.
78. Kates, S.A., Sole, N.A., Albericio, F., Barany, G., In Basava, C., Anantharamaiah, G.M. (Eds.), *In Peptides: Design, Synthesis, and Biological Activity*, Birkhäuser, Boston, USA, 1994, pp. 39–58.
79. Plaué, S., Int. J. Pept. Protein Res., 35 (1990) 510–517.
80. Backes, B.J., Ellman, J.A., Curr. Opin. Chem. Biol., 1 (1997) 86–93.
81. Alewood, P.F., Alewood, D., Miranda, L.P., Love, S., Meutermans, W.D.F., Wilson, D., *Methods in Enzymol.*, 289 (1997) 14–29.
82. Cabrele, C., Langer, M., Beck-Sickinger, A.G., J. Org. Chem., 64 (1999) 4353–4361.
83. Rovero, P., Quartara, L., Fabbri, G., Tetrahedron Lett., 32 (1991) 2639–2642.
84. Spatola, A.F., Darlak, K., Romanovskis, P., Tetrahedron Lett., 37 (1996) 591–594.
85. Sugiura, T., Hamada, Y., Shioiri, T., Tetrahedron Lett., 28 (1987) 2251–2254.
86. Valero, M.L., Giralt, E., Andreu, D., Tetrahedron Lett., 37 (1996) 4229–4232.
87. Lee, J.H., Griffin, J.H., Nicas, T.I., J. Org. Chem., 61 (1996) 3983–3986.
88. Tromelin, A., Fulachier, M.-H., Mourier, G., Ménez, A., Tetrahedron Lett., 33 (1992) 5197–5200.
89. Trzeciak, A., Bannwarth, W., Tetrahedron Lett., 33 (1992) 4557–4560.
90. Kates, S.A., Sole, N.A., Johnson, C.R., Hudson, D., Barany, G., Albericio, F., Tetrahedron Lett., 34 (1993) 1549–1552.
91. McMurray, J.S., Tetrahedron Lett., 32 (1991) 7679–7682.
92. McMurray, J.S., Lewis, C.A., Obeyesekere, N.U., Pept. Res., 7 (1994) 195–206.
93. Sabatino, G., Chelli, M., Mazzucco, S., Ginanneschi, M., Papini, A.M., Tetrahedron Lett., 40 (1999) 809–812.
94. Kobayashi, K., Hayashi, K., J. Am. Chem. Soc., 112 (1990) 6051–6053.
95. Alsina, J., Rabanal, F., Giralt, E., Albericio, F., Tetrahedron Lett., 35 (1994) 9633–9636.
96. Romanovskis, P., Spatola, A.F., J. Pept. Res., 52 (1998) 356–374.
97. Alsina, J., Rabanal, F., Chiva, C., Giralt, E., Albericio, F., Tetrahedron, 54 (1998) 10125–10152.
98. Lee, Y., Silverman, R.B., J. Am. Chem. Soc., 121 (1999) 8407–8408.
99. Jensen, K.L., Alsina, J., Songster, M.F., Vágner, J., Albericio, F., Barany, G., J. Am. Chem. Soc., 120 (1998) 5441–5452.
100. Jensen, K.J., Songster, M.F., Vágner, J., Alsina, J., Albericio, F., Barany, G., In Kaumaya, P.T.P., Hodges, R.S. (Eds.), *Peptides: Chemistry, Structure and Biology: Proceedings of the 14th American Peptide Symposium*, Mayflower Scientific, Kinswinford, U.K., 1996, pp. 30–32.
101. del Fresno, M., Alsina, J., Royo, M., Barany, G., Albericio, F., Tetrahedron Lett., 39 (1998) 2639–2642.
102. Bourne, G.T., Golding, S.W., Meutermans, W.D.F., Smythe, M.L., Lett. Pept. Sci., 7 (2001) 311–316.
103. Bourne, G.T., Meutermans, W.D.F., Alewood, P.F., McGeary, R.P., Scanlon, M., Watson, A.A., Smythe, M.L., J. Org. Chem., 64 (1999) 3095–3101.
104. Bourne, G.T., Meutermans, W.D.F., Smythe, M.L., Tetrahedron Lett., 40 (1999) 7271–7274.
105. Okayama, T., Burritt, A., Hruby, V.J., Lett., 2 (2000) 1787–1790.
106. Ehrlich, A., Heyne, H.-U., Winter, R., Beyermann, M., Haber, H., Carpino, L.A., Bienert, M., J. Org. Chem., 61 (1996) 8831–8838.
107. Ehrlich, A., Klose, J., Heyne, H.-U., Beyermann, M., Carpino, L.A., Bienert, M., In Kaumaya, P.T.P.H., Robert S. (Eds.), *Pept.: Chem., Struct. Biol., Proc. Am. Pept. Symp., 14th*, Mayflower Scientific, Kingswinford, UK, 1996, pp. 75–76.
108. Fridkin, M., Patchornik, A., Katchalski, E., J. Am. Chem. Soc., 90 (1968) 2953–2957.
109. Fridkin, M., Patchornik, A., Katchalski, E., J. Am. Chem. Soc., 87 (1965) 4646–4648.
110. DeGrado, W.F., Kaiser, E.T., J. Org. Chem., 45 (1980) 1295–1300.
111. DeGrado, W.F., Kaiser, E.T., J. Org. Chem., 47 (1982) 3258–3261.
112. Kaiser, E.T., Mihara, H., Laforet, G., Kelly, J.W., Walters, L., Findeis, M.A., Sasaki, T., Science, 243 (1989) 187–192.
113. Ösapay, G., Profit, A., Taylor, J.W., Tetrahedron Lett., 31 (1990) 6121–6124.
114. Ösapay, G., Taylor, J.W., J. Am. Chem. Soc., 112 (1990) 6046–6051.
115. Nishino, N., Xu, M., Mihara, H., Fujimoto, T., Ueno, Y., Kumagai, H., Tetrahedron Lett., 33 (1992) 1479–1482.
116. Nishino, N., Xu, M., Mihara, H., Fujimoto, T., Ohba, M., Ueno, Y., Kumagai, H., J. Chem. Soc., Chem. Commun., (1992) 180–181.
117. Kapurniotu, A., Taylor, J.W., Tetrahedron Lett., 34 (1993) 7031–7034.
118. Zhang, L.H., Ma, P., Tetrahedron Lett., 35 (1994) 5765–5768.
119. Mihara, H., Yamabe, S., Niidome, T., Aoyagi, H., Kumagai, H., Tetrahedron Lett., 36 (1995) 4837–4840.
120. Nishino, N., Hayashida, J., Arai, T., Mihara, H., Ueno, Y., Kumagai, H., J. Chem. Soc., Perkin Trans. 1, 9 (1996) 939–946.
121. Lee, B.H., Tetrahedron Lett., 38 (1997) 757–760.
122. Wu, X., Grathwohl, M., Schmidt, R.R., Org. Lett., 3 (2001) 747–750.
123. Richter, L.S., Tom, J.Y.K., Burnier, J.P., Tetrahedron Lett., 35 (1994) 5547–5550.
124. Bourne, G.T., Golding, S.W., McGeary, R.P., Meutermans, W.D.F., Jones, A., Marshall, G.R., Alewood, P.F., Smythe, M.L., J. Org. Chem., 66 (2001) 7706–7713.
125. Bourne, G.T., McGeary, R.P., Golding, S.W., Meutermans, W.D.F., Alewood, P.F., Smythe, M.L., In Fields, G.B., Tam, J.P., Barany, G. (Eds.), *Peptides for the New Millennium: Proceedings of the 16th American Peptide Symposium*, Kluwer Academic Publishers, Dordrecht, Netherlands, 2000, pp. 98–99.

126. Flanigan, E., PhD Thesis, Marshall, G.R., Tetrahedron Lett., 27 (1970) 2403–2406.
127. Flanigan, E., PhD Thesis, Washington University, St. Louis., 1971.
128. Marshall, D.L., Liener, I.E., J. Org. Chem., 35 (1970) 867–868.
129. Pátek, M., Int. J. Pept. Protein Res., 42 (1993) 97–117.
130. Kiso, Y., Fukui, T., Tanaka, S., Kimura, T., Akaji, K., Tetrahedron Lett., 35 (1994) 3571–3574.
131. Hoffmann, S., Frank, R., Tetrahedron Lett., 35 (1994) 7763–7766.
132. Chan, W.C., Bycroft, B.W., Evans, D.J., White, P.D., J. Chem. Soc., Chem. Commun., 21 (1995) 2209–2210.
133. Kimura, T., Fukui, T., Tanaka, S., Akaji, K., Kiso, Y., Chem. Pharm. Bull., 45 (1997) 18–26.
134. Hulme, C., Peng, J., Morton, G., Salvino, J.M., Herpin, T. Labaudiniere, R., Tetrahedron Lett., 39 (1998) 7227–7230.
135. Breitenbucher, J.G., Johnson, C.R., Haight, M., Phelan, J.C., Tetrahedron Lett., 39 (1998) 1295–1298.
136. Beech, C.L., Coope, J.F., Fairley, G., Gilbert, P.S., Main, B.G., Plé, K., J. Org. Chem., 66 (2001) 2240–2245.
137. Cano, M., Ladlow, M., Balasubramanian, S., J. Org. Chem., 67 (2002) 129–135.
138. Miller, M.W., Vice, S.F., McCombie, S.W., Tetrahedron Lett., 39 (1998) 3429–3432.
139. Panke, G., Frank, R., Tetrahedron Lett., 39 (1998) 17–18.
140. Pátek, M., Lebl, M., Tetrahedron Lett., 32 (1991) 3891–3894.
141. Pátek, M., Lebl, M., Biopolymers, 47 (1998) 353–363.
142. Rothe, M., Sander, A., Fischer, W., Mästle, W., Nelson, B., In Goodman, M., Meienhofer, J. (Eds.), *Peptides: Proceedings of the 5th American Peptide Symposium*, John Wiley and Sons, New York, USA, 1977, pp. 506–509.
143. Routledge, A., Abell, C., Balasubramanian, S., Tetrahedron Lett., 38 (1997) 1227–1230.
144. Sola, R., Saguer, P., David, M., -L., Pascal, R., J. Chem. Soc., Chem. Comm., 23 (1993) 1786–1788.
145. Sola, R., Mery, J., Pascal, R., Tetrahedron Lett., 37 (1996) 9195–9198.
146. Todd, M.H., Oliver, S.F., Abell, C., Org. Lett., 1 (1999) 1149–1151.
147. Wade, W.S., Yang, F., Sowin, T.J., J. Comb. Chem., 2 (2000) 266–275.
148. Kenner, G.W., McDermott, J.R., Sheppard, R.C., J. Chem. Soc., Chem. Comm., (1971) 636–637.
149. Fitzpatrick, L.J., Rivero, R.A., Tetrahedron Lett., 38 (1997) 7479–7482.
150. Backes, B.J., Ellman, J.A., J. Am. Chem. Soc., 116 (1994) 11171–11172.
151. Backes, B.J., Ellman, J.A., J. Org. Chem., 64 (1999) 2322–2330.
152. Maclean, D., Hale, R., Chen, M., Org. Lett., 3 (2001) 2977–2980.
153. Backes, B.J., Virgilio, A.A., Ellman, J.A., J. Am. Chem. Soc., 118 (1996) 3055–3056.
154. Bannwarth, W., Küng, E., Vorherr, T., Bioorg. Med. Chem. Lett., 6 (1996) 2141–2146.
155. Gough, S.L., Clemens, I.R., Seale, P.W., In Bajusz, S., Hudecz, F. (Eds.), *Peptides 1998, Proceedings of the 25th European Peptide Symposium*, Akademiai Kiado, Budapest, Hungary, 1999, pp. 770–771.
156. Meutermans, W.D.F., Golding, S.W., Bourne, G.T., Miranda, L.P., Dooley, M.J., Alewood, P.F., Smythe, M.L., J. Am. Chem. Soc., 121 (1999) 9790–9796.
157. Prasad, C., Peptides, 16 (1995) 151–164.
158. Szardenings, A.K., Burkoth, T.S., Lu, H.H., Tien, D.W., Campbell, D.A., Tetrahedron, 53 (1997) 6573–6593.
159. Loughlin, W.A., Marshall, R.L., Carreiro, A., Elson, K.E., Bioorg. Med. Chem. Lett., 10 (2000) 91–94.
160. Cui, C., Kakeya, H., Osada, H., Tetrahedron, 52 (1996) 12651–12666.
161. Wang, H., Ganesan, A., Org. Lett., 1 (1999) 1647–1649.
162. Funabashi, Y., Horiguchi, T., Iinuma, S., Tanida, S., Harada, S., J. Antibiot., 47 (1994) 1202–1218.
163. Royo, M., Van Den Nest, W., del Fresno, M., Frieden, A., Yahalom, D., Rosenblatt, M., Chorev, M., Albericio, F., Tetrahedron Lett., 42 (2001) 7387–7391.
164. Szardenings, A.K., Harris, D., Lam, S., Shi, L.H., Tien, D., Wang, Y.W., Patel, D.V., Navre, M., Campbell, D.A., J. Med. Chem., 41 (1998) 2194–2200.
165. Goodfellow, V.S., Laudeman, C.P., Gerrity, J.I., Burkard, M., Strobel, E., Zuzack, J.S., McLeod, D.A., Mol. Divers., 2 (1996) 97–102.
166. van Loevezijn, A., Allen, J.D., Schinkel, A.H., Koomen, G.J., Bioorg. Med. Chem. Lett., 11 (2000) 29–32.
167. Nefzi, A., Giulianotti, M.A., Houghten, R.A., Tetrahedron, 56 (2000) 3319–3326.
168. Cody, W.L., Cai, C., Doherty, A.M., Edmunds, J.J., He, J.X., Narasimhan, L.S., Plummer, J.S., Rapundalo, S.T., Rubin, J.R., Van Huis, C.A., St-Denis, Y., Winocour, P.D., Siddiqui, M.A., Bioorg. Med. Chem. Lett., 9 (1999) 2497–2502.
169. Baures, P.W., Ojala, W.H., Costain, W.J., Ott, M.C., Pradhan, A., Gleason, W.B., Mishra, R.K., Johnson, R.L., J. Med. Chem., 40 (1997) 3594–3600.
170. Lopez-Rodriguez, M.L., Morcillo, M.J., Fernandez, E., Porras, E., Orensanz, L., Beneytez, M.E., Manzanares, J., Fuentes, J.A., J. Med. Chem., 44 (2001) 186–197.
171. Liebscher, J., Jin, S.D., Chem. Soc. Rev., 28 (1999) 251–259.
172. Ienaga, K., Nakamura, K., Kurohashi, M., Nakanishi, T., Ichii, T., Phytochemistry, 29 (1990) 35–39.
173. Rajappa, S., Natekar, M.V., In Kratritzky, A.R. (Eds.), *Adv. Heterocycl. Chem.*, Academic Press, San Diego, 1993, Vol. 57, pp. 187–289.
174. Szardenings, A.K., Antonenko, V., Campbell, D.A., DeFrancisco, N., Ida, S., Shi, L., Sharkov, N., Tien, D., Wang, Y.W., Navre, M., J. Med. Chem., 42 (1999) 1348–1357.
175. Gordon, D.W., Steele, J., Bioorg. Med. Chem. Lett., 5 (1995) 47–50.
176. Smith, R.A., Bobko, M.A., Lee, W., Bioorg. Med. Chem. Lett., 8 (1998) 2369–2374.
177. Golebiowski, A., Klopfenstein, S.R., Chen, J.J., Shao, X., Tetrahedron Lett., 41 (2000) 4841–4844.
178. Bianco, A., Sonksen, C.P., Roepstorff, P., Briand, J.P., J. Org. Chem., 65 (2000) 2179–2187.
179. Groth, T., Meldal, M., J. Comb. Chem., 3 (2001) 45–63.
180. Lin, X.D., Dorr, H., Nuss, J.M., Tetrahedron Lett., 41 (2000) 3309–3313.
181. Chucholowski, A., Masquelin, T., Obrecht, D., Stadlwieser, J., Villalgordo, J.M., Chimia, 50 (1996) 525–530.
182. Hulme, C., Morrissette, M.M., Volz, F.A., Burns, C.J., Tetrahedron Lett., 39 (1998) 1113–1116.
183. Falorni, M., Giacomelli, G., Porcheddu, A., Taddei, M., Eur. J. Org. Chem., (2000) 1669–1675.
184. Falorni, M., Giacomelli, G., Nieddu, F., Taddei, M., Tetrahedron Lett., 38 (1997) 4663–4666.