2
Synthetic Approaches towards Peptidomimetic Design

2.1 Introduction

Peptide derivatives are the result of modifications of the original sequence in various degrees, and a classification based on the importance of the chemical modifications, from conservative to drastic, has been proposed accordingly [1]:

- **Modified peptides**: these molecules contain relatively small modifications that do not modify the peptide bond, and thus they still possess a chemical structure of peptide nature.
- **Pseudopeptides**: in these compounds partial modifications of either peptide bonds or side-chains are introduced, contributing to the generation of molecules possessing a chemical structure of only partial peptide nature.
- **Peptidomimetics**: these compounds do not possess any amide bond of the original peptide, and the structural resemblance is related to the pharmacophore and to the pharmacological activity of the bioactive peptide conformation.

As introduced in Section 1.3, the first steps in developing a lead peptidomimetic compound starting from a bioactive peptide are connected to comprehension of the key residues of the parent peptide for bioactivity, and also of potential weak sites for guiding the rational design of chemical modifications to improve enzymatic stability [1]. This is particularly important when no structural data corresponding to the interaction with the target receptor are available, and currently it is a relevant issue in the panorama of protein–protein interactions. Potential cleavage sites have been identified experimentally by subjecting peptides to tissue homogenates, plasma and serum and cocktails of purified proteases, followed by characterization of the corresponding fragments by high-performance liquid chromatography (HPLC) or liquid chromatography–mass spectrometry (LC-MS) techniques.
The introduction of chemical modifications, along with the comprehension of structure–activity data, are achieved according to a hierarchical approach, which consists of several structural modifications of the original bioactive peptide, including:

- size reduction
- alanine scanning
- d-amino acid scanning
- N-methylation
- introduction of local and global constraints to define the bioactive conformation.

Reduction of the size of the original peptide sequence is carried out with aim of identifying the key peptide sequence interacting directly with the active site of the target enzyme or receptor. The result of this process is important in terms of developing small molecule peptidomimetic derivatives, and this step is approached by assaying an array of peptides generated by the systematic removal of amino acids from either N- or C-termini, ultimately resulting in the minimal peptide sequence carrier of bioactivity. This is the hit peptide sequence encompassing the pharmacophore that is taken into account for subsequent modifications of the peptide structure.

Alanine scanning consists of the synthesis and biological evaluation of an array of peptides that contain an alanine residue in the place a different amino acid of the original bioactive peptide sequence. The peptides containing an alanine residue in place of key amino acids in the original sequence lose bioactivity as a consequence of the lack of side-chains interacting with the target receptor. This evidence is important for identifying the amino acids of the reference peptide that play an active role in the interaction with the target enzyme or receptor.

The introduction of d-amino acids systematically in the parent peptide sequence is a similar approach to alanine scanning in selecting the key residues that carry bioactivity. Moreover, as the shift of configuration results in a different arrangement of the side-chains, this approach gives insight into the structural organization of the bioactive conformation.

The generation and assay of N-methylated peptides is another well-established approach for understanding the role of each amino acid constituting the bioactive peptide. Specifically, the alkylation of amide bonds is a strategy for identifying which amino acids act as hydrogen-bonding donors in the interaction with the target enzyme/receptor. Additionally, the N-methylation generates a tertiary amide bond, which is prone to establish cis/trans equilibrium at the amide bond, ultimately contributing to provide insight into the relationship between the conformational preferences of the peptide and bioactivity.

Once the minimal peptide sequence carrier of bioactivity is identified together with the role of each amino acid constituting the parent peptide, structural modifications are introduced hierarchically to develop a compound with reduced peptide character, and possibly improving both the intrinsic binding affinity to the target receptor or enzyme and the pharmacokinetics/pharmacodynamics (PK/PD) profile from a pharmacological point of view.

### 2.2 Local Modifications

The most conservative approach to dealing with modifications of the peptide is the introduction of local structural changes. These modifications are restricted to single amino acids and, thus, are local alterations of the peptide structure, and can be grouped into side-chain
and backbone modifications, with the aim of introducing conformational restrictions and to stabilize the molecule towards protease-mediated degradation.

Extensive work has been undertaken with the aim of replacing peptide bonds with suitable moieties, to improve the resistance of the peptide; such modification of the peptide backbone generally refers to the isosteric or isostructural exchange of units in the peptide chain, and to the introduction of additional fragments. This goal is generally achieved by single amino acid modifications or by introducing dipeptide analogues. Both approaches are conceived to constrain the backbone rotational freedom, resulting in an organized conformation that may also resemble secondary structure, such as helices or turns, or to limit the rotational freedom of side-chains and to allow for the topological orientation required by the pharmacophore.

Modifications at every part of a single amino acid have been reported [2]. Specifically, (i) the amino group can be replaced with isosteric atoms or groups, such as oxygen, keto-methylene or N-hydroxyl moieties; (ii) the alpha carbon with nitrogen atoms, C-alkyl to achieve quaternary amino acids, or boron atoms; (iii) the carbonyl group has been replaced with thiol, methylene, phosphinic and boronic groups (Figure 2.1). Retro-inverso peptides have been also proposed, which consist of an amino acid moiety in which the relative positions of the original amino and carboxylic groups have been reversed.

As backbone modification is mainly addressed by introducing amide bond surrogates with the aim of improving the stability of the peptide in vivo, several amide bond isosteres have been proposed (Figure 2.2) that mimic the structural features of the peptide bond and in some circumstances modify the conformational profile and the hydrogen-bonding capability, too [3]. For example, the introduction of aliphatic moieties augments the conformational flexibility locally, whereas the application of olefin isosteres does not alter such topology. Moreover, the hydrogen-bonding capabilities are modulated by applying

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**Figure 2.1** Isosteric local modification of the amino acid structure. (Reproduced with permission from Reference [2]. Copyright 1994 Wiley-VCH Verlag GmbH & Co. KGaA.)
Figure 2.2 Panel of isosteres of the peptide bond

diverse amide bond isosteres, such as sulfonamide, phosphinic or peptoids, depending on the accessibility of donors or acceptors for such interactions.

The introduction of local modifications around side-chains is aimed mainly at modulating the conformational profile of the peptide, thus intervening on all the rotatable bonds present in the amino acid unit. Accordingly, several tethering approaches have been proposed to restrict the conformational freedom of selected dihedrals (Figure 2.3). Moreover, side-chain modifications have been introduced to explore pharmacophoric steric and electronic interactions, such as modulation of the hydrophobic content by adding aromatic moieties or the introduction of polar appendages to address any polar or hydrogen-bonding interactions with the target receptor.

Figure 2.3 Dihedral descriptors for conformational flexibility around the peptide main chain
2.2.1 Single Amino Acid Modifications

The approach of modifying a single amino acid unit within a peptide sequence is generally achieved by introducing constraining elements so as to reduce the conformational flexibility. Backbone alkylation causes the angles $\phi$, $\psi$, $\chi$ to be constrained, and $N$-alkylation facilitates $cis/trans$ amide bond isomerism, whereas in the backbone $C_\alpha$-alkylation $\phi$, $\psi$ are constrained to a helical or extended linear structure (Figure 2.4).

$\alpha$-Substituted amino acids are a common approach to reduce the torsional freedom around the backbone of such amino acids. $\alpha$-Methyl $\alpha$-amino acids possess a methyl at the $C_\alpha$, which greatly reduces the rotational freedom around $N$-$C_\alpha$ and $C_\alpha$-$CO$ bonds. As an example, the introduction of $\alpha$-Me-alanine in a peptide reduces the rotational freedom around its backbone bonds by about 90%. This is the most widely studied $\alpha$-alkylated amino acid, which is able to restrict the dihedrals $\phi$, $\psi$ to angles present in $\alpha$ or $\beta$ helices [4]. Moreover, for these quaternary amino acids the referred conformation is in an extended structure with dihedral angles $\phi$, $\psi$ being about $180^\circ$. Other entries to $\alpha$-substituted $\alpha$-amino acids are represented by both glycine derivatives and cyclic amino acids. $\alpha,\alpha$-Dialkyl glycines are characterized by an extended conformation (Figure 2.5), whereas cyclic amino acids contribute to constrain the folded conformation to various degrees as a function of the ring size, as well as improving the potency of the bioactive peptide compound.

In particular, substitution of $\alpha$-aminocyclohexane carboxylic acid (AC6C) into various positions of enkephalin, a peptide responsible for modulating pain response, resulted in a peptidomimetic with greater $in$ $vivo$ activity [5].

2.2.1.1 $N_\alpha$-$C_\alpha$ Cyclized Amino Acids

The amino acids belonging to this class are generally considered as proline mimetics, which is unique among proteinogenic amino acids in having a cyclic nature and possessing the

![Figure 2.4](image-url)
capability of giving cis/trans isomerization due to a reduced energetic barrier of interconversion (about 2 kcal mol$^{-1}$).

These amino acids have been widely taken into account in the peptidomimetic area as tools for generating new drugs from peptide hits, and much research has been devoted to the development of a wide array of proline mimetics with high chemical diversity (see Chapter 8 for a more detailed overview of this class of compounds).

Major structural features of these cyclic molecules are:

- the presence of a bond connecting N$_\alpha$ to C$_\alpha$ that forms a cycle responsible for the reduced conformational freedom around this amino acid;
- rotation around the C$_\alpha$–C=O bond is partially impaired for the nonbonded interaction between the carbonyl group and the ring;
- the steric hindrance between the proline mimetic and vicinal residues affects the overall conformation around this peptide sequence.

Specifically, proline and all the corresponding peptidomimetics are able to constrain the conformational freedom related to the torsional angle $\phi$; some approaches generally followed are:

- modulation of the ring size, ranging from aziridines to omoproline;
- inclusion of heteroatoms, such as azaproline or silaproline;
- introduction of substituents at positions 3, 4, 5 to improve the conformational restriction.

The structural diversity of proline mimetics is given by different ring size, the inclusion of heteroatoms in the ring, the presence of substituents on the ring and the polycyclic nature as found in some scaffolds (Figure 2.6).

Two cyclic amino acid derivatives found in nature and possessing the pyrrolidine ring, in addition to proline, are pyroglutamic acid, derived from the lactamization of glutamic acid, and 4-hydroxyproline, which is key in stabilizing the helical structures of collagen (Figure 2.7).
2.2.1.2 Constraining the Side-Chain Rotational Freedom

The inclusion of rigidifying elements in amino acids and peptidomimetics with the aim of reducing the conformational freedom of side-chains may be addressed by taking advantage of double bonds or cycles (Figure 2.8). The application of \( \alpha, \beta \)-unsaturated \( \alpha \)-amino acids, also called dehydro-amino acids, allows blocking the \( C_\alpha-C_\beta \) rotation, as defined by the \( \chi \) angle, and selecting of an \((E)\) or \((Z)\) isomer depending on the desired position of the \( R \)
Figure 2.9  Conformationally restricted Phe and Tyr analogues that limit the conformational range of the $\chi$ angles

side-chain. This chemical bias locks the $\chi$ angle to 0 or 180° depending on the stereochemistry of the olefin moiety. In addition, dehydro-amino acids favour the formation of $\beta$- or $\gamma$-turns when placed in the $(i + 2)$ position of the putative $\beta$-turn sequence.

The tethering approach has also been popular in blocking the conformational freedom of side-chains by including cyclopropane rings at $C_{\alpha}-C_\beta$ positions (Figure 2.8), similarly to the conformational result for dehydro-amino acids, or by developing bicyclic $\alpha$-amino acids to embed such a bond within a ring, as shown in Figure 2.9 for phenylalanine. Major attention has been directed toward the incorporation of conformationally restricted Phe and Tyr analogues in $\delta$-opioid receptor tetrapeptide agonists, as these amino acids are critical for opioid receptor binding [6]. In this respect, for modifications that limit the conformational range of $\chi'$, which is the dihedral angle describing the rotation about the $C_{\alpha}-C_\beta$ bond, tethered amino acids such as tetrahydroisoquinoline-3-carboxylic acid (Tic), 2-aminotetralin-2-carboxylic acid (Ate) and 2-aminoindolin-2-carboxylic acid (Aic), as phenylalanine replacements, and their appropriate aryl ring hydroxylated counterparts, HO-Tic, Hat and Hai, respectively, as tyrosine replacements have been taken into account. The capability in limiting conformational freedom about the $C_{\alpha}-C_\beta$ bond due to the inclusion of this bond in a five- or six-membered ring was thus demonstrated. In addition, limitations to the allowed values of $\chi^2$, the dihedral angle about $C_\beta-C_\gamma$, due to their bicyclic structures were observed.

### 2.2.2 Dipeptide Isosteres

Special interest in the development of peptidomimetic compounds has been oriented towards the generation of novel amino acid structures and of molecular scaffolds acting as dipeptide isosteres [7]. This approach found many applications that aim to improve the stability of peptidomimetics towards proteolysis by replacing the amide moiety with alternative molecular fragments often embedded within cyclic structures. Moreover, the scaffold approach in generating dipeptide isosteres resulted in a remarkable strategy to
constrain the conformational freedom of a specific region of a peptide compound by blocking \( \phi \), \( \psi \) or \( \omega \) rotations around backbone covalent bonds.

A major contribution to this field has been made by the work of Freidinger, who conceived the idea of utilizing dipeptide lactams as conformational constraints in peptides to restrict the peptide bond to the trans conformation, and the application of tethers to limit the \( \psi \) backbone dihedral angle [8]. The effect of the ring was found to influence the conformation of the peptide locally, as the proximity of the lactam ring would restrict conformations of dihedral angles \( \phi \), and the size of the lactam ring could potentially bias the peptide backbone conformation in different ways. The first dipeptide lactam was prepared using protected ornithine as the starting material by way of an intramolecular acylation approach (Scheme 2.1). Catalytic hydrogenolysis was employed to remove the side-chain Cbz protecting group, followed by addition of glyoxylic acid to the reaction mixture to attain the newly formed amine moiety in 2 by means of reductive alkylation in the same pot. This carboxy-methylated compound cyclized smoothly to give, upon warming in dimethylformamide (DMF), the \( \delta \)-lactam dipeptide 3 in an overall yield of 51% while maintaining the stereogenic centre intact. Similarly, the protected lysine precursor was taken into account to achieve the corresponding \( \epsilon \)-lactam dipeptide isostere.

Intramolecular alkylation was employed in the synthesis of five-membered ring dipeptide lactams using methionine as the starting material (Scheme 2.2). Specifically, Boc-L-methionine (4) was coupled with an amino acid methyl ester of choice (5) using a carboxylic acid activator as the coupling agent, such as diphenylphosphoryl azide (DPPA) or dicyclohexylcarbodiimide (DCC). The resulting dipeptide 6 was manipulated by converting the side chain of methionine into a leaving group through methylation of the methylthio group with methyl iodide to produce the methyl sulfonium salt 7. Successively, treatment with sodium hydride resulted in cyclization with expulsion of dimethyl sulfide to form the desired lactam 8. The synthetic process preserved the stereogenic centres from the starting amino acids and allows for various side chains to be incorporated in the second amino acid of the lactam dipeptide.

![Scheme 2.1](image)

\textit{Scheme 2.1} Six-membered ring lactam-based dipeptide isostere synthesized from ornithine
Results from the research groups of Freidinger and others indicated that dipeptide lactams are useful for potency enhancement, greater receptor selectivity, insight into the biologically active conformation of the parent peptide and in increasing the stability towards protease degradation [9]. As a noteworthy example, Thorsett pioneered the application of dipeptide lactams in the context of angiotensin converting enzyme inhibitors [10]. Following this concept, diverse molecules, such as lactams, piperazinones and imidazolinones, have been employed as molecular scaffolds capable of constraining the conformation around a dipeptide unit (Figure 2.10).

**Figure 2.10** Molecular scaffolds developed as constrained dipeptide isosteres
The application of such lactams in peptidomimetic structures has been generally taken into account with the aim of constraining the $\psi$ and $\omega$ torsional angles. Pyrrolidinone moieties, such as those referring to Freidinger’s lactams, proved to function as dipeptide analogues capable of constraining the torsional freedom of a dipeptide backbone and of improving the stability of the central peptide bond by including it in the lactam moiety.

In addition, the combination of two cyclic moieties, such as those found in spiro compounds, allowed simultaneous blocking of both $\phi$ and $\psi$, $\omega$ torsional angles, thus resulting in a blocked conformation. Significant examples of dipeptide isosteres that have been developed during last decades will be presented in Chapter 3 within the panorama of peptidomimetic isosteres.

### 2.2.3 Retro-inverso Peptides

A common approach for improving the resistance of a peptide compound towards protease degradation is to develop a peptidomimetic structure possessing chemical modifications around amide bonds that are subjected to hydrolysis. Retro-inverso isomerization is a method for modifying the structure of the backbone so as to prevent the protease recognizing the peptide-based inhibitor as a substrate. This can be achieved by replacing one or more l-amino acids with the parent enantiomer, and at the same time inverting the backbone direction from N $\rightarrow$ C to C $\rightarrow$ N. The retro-inverso modification does not lead to a more constrained polypeptide, but rather the major advantage over the corresponding peptide lies in the higher \textit{in vivo} stability due to the modification of amide bonds, which are recognized by proteases for their hydrolysis. This approach is well-represented by the retro-inverso peptidomimetic of the key tetrapeptide sequence found in gastrin (Figure 2.11) [11].

![gastrin tetrapeptide fragment](image1)

![retro-inverso peptidomimetic antagonist of gastrin](image2)

\textit{Figure 2.11} Retro-inverso peptidomimetic of the key tetrapeptide sequence found in gastrin
Another elegant example of a retro-inverso peptidomimetic is the generation of a peptidomimetic of tuftsin (Figure 2.12), which is an immune system stimulator that is completely degraded in vivo in about 8 min [12]. The retro-inverso peptidomimetic, consisting of switching the amide moiety around the Thr-Lys dipeptide unit, showed less than 2% hydrolysis after 50 min and retention of bioactivity.

### 2.2.4 N-Methylation of Peptides

As introduced at the beginning of this chapter, N-methylated peptides are a well-established approach for understanding the role of each amino acid constituting the bioactive peptide. This is strategic in identifying which amino acids act as hydrogen-bonding donors in the interaction with the target enzyme/receptor, and in providing insight into the relationship between the conformational preferences of the peptide and bioactivity.

In the process of transforming a peptide into a peptidomimetic drug, after the simplification step, where the minimal bioactive sequence is identified, the conformational freedom is reduced to achieve the desired activity and selectivity. Reduction of the conformational space is generally achieved by cyclization, although increased selectivity and potency is attained provided that the correct conformation is addressed. To explore the conformational space more accurately and lessen the risk of failure, the group of Kessler envisaged a simple approach of N-methylation to overcome various obstacles of peptides as a ‘rational’ way toward drug development [13]. Taking advantage of the role of the natural cyclic amino acid proline in the modulation of the conformational profile of proteins, the strategy of systematically applying N-methylation to bioactive peptides was conceived, thus considering proline as a ‘mimic for N-methylation’ in proteins [13b]. Mono- and multiple-N-methylations of cyclic peptides were investigated to elucidate their remarkable conformational modulation ability by imparting steric constraints in the peptidic backbone and in improving the pharmacokinetic profile of the peptides to be used as drug leads. In this respect, N-methylation introduces another dimension to this ‘spatial screening’ [14] due to the remarkable property of conformational modulation. In fact, N-methylation
facilitates the occurrence of a cis-peptide bond and allows us to study the role of amide protons in establishing potential hydrogen bonds, resulting in optimization of the conformational and structural profile of a peptidomimetic. Efficient methods for the site-selective N-methylation of peptides have been developed by Fukuyama et al. [15] and Miller and Scanlan [16]. This is a three-step procedure involving amine activation by protection with an o-nitrobenzenesulfonyl group (o-NBS), followed by alkylation and deprotection of the o-NBS group on a solid support.

Elucidation of the conformational impact of N-methylation on cyclic peptides greatly facilitates the design of bioactive peptides by ‘spatial screening’, wherein the side chains in the template structures are functionalized by appropriate pharmacophores. As reported by Kessler:

> the improvement of oral bioavailability by multiple N-methylation is a significant advance toward the development of peptide-based therapeutics, which has hampered over the years due to poor pharmacokinetic properties. Multiple N-methylation resulted in enhancement in the activity and selectivity of receptor subtypes using either library or designed approaches and helps in understanding finer details of the bioactive conformation [13a].

### 2.2.5 Azapeptides

Azapeptides are an interesting and synthetically easy to approach class of peptidomimetics in which the Cα atom of the backbone is replaced isoelectronically by a nitrogen atom (Figure 2.13). Azapeptides can be synthesized very easily from substituted hydrazines or hydrazides [17], such as through the acylation of hydrazines [18] and the incorporation ofaza-amino acid esters into a peptide chain. They have been shown to be therapeutically relevant, such as in the case of serine and cysteine proteases inhibitors [19].

### 2.2.6 Peptoids

Peptoids can be described as mimetics of α-peptides in which the side chain is attached to the backbone amide nitrogen instead of the α-carbon (Figure 2.14). This modification results in the formal shift of the position of the side chain with respect to the parent peptide backbone.

Oligomers of N-substituted glycine or peptoids were first reported by Bartlett and coworkers in 1992 [20]. They were initially proposed as an accessible class of molecules from which lead compounds could be identified for drug discovery. Sequence-specific

![Figure 2.13](image-url) Structural features of azapeptides as compared to the peptide moiety

\[X = O, \text{NH, CH}_2\]
peptoid oligomers are easily assembled from primary amines by the solid-phase submonomer method. Of particular interest are their applications to the exploration of peptoid secondary structures and drug design. Major advantages of peptoids as research and pharmaceutical tools include the ease and economy of synthesis, highly variable backbone and side-chain chemistry possibilities.

Chapter 6 presents a more detailed description of peptoids, including an overview of the most relevant applications in medicinal chemistry.

2.3 **Global Restrictions through Cyclic Peptidomimetics**

The generation of cyclic peptidomimetics is very attractive in terms of constraining a native peptide structure into a conformationally-reduced molecule. This approach is different from the introduction of cyclic scaffolds, as it does not modify the parent peptide locally but involves modification of the overall conformational profile of the target peptide compound. The macrocyclic peptide has several advantages in improving the quality of the bioactive compound in terms of bioavailability and potency, as the high proportion of *cis* amide bonds and the absence of free *C*- and *N*-termini confer higher metabolic resistance, In addition, the limited conformational freedom results in higher receptor selectivity and binding affinity, by reducing unfavourable entropic effects.

The cyclization strategies can be classified with respect to backbone and side-chains as the chemical moieties used to introduce the constraint [21].

Cyclization between backbone elements is approached in several ways:

- by tethering two amide nitrogen atoms with a linker (backbone to backbone);
- by introducing a chemical junction between a *C*<sub>α</sub> and a nitrogen atom (backbone to backbone);
- by linking an *N*-terminal amino group with an amide nitrogen atom with a spacer (head to backbone);
- by cyclizing the two *N* - and *C* -terminal ends of a peptidomimetic structure with an amide bond (head-to-tail).

Backbone cyclization combines cyclization with *N*-alkylation to enhance the stability of peptides [22]. This is generally carried out in solution, and the most popular approach is the
head-to-tail generation of cyclic peptide via amide bond formation according to standard peptide chemistry [23]. Macrolactamization is carried out in high dilution to promote the intramolecular reaction; relevant examples can be found in the generation of cyclic peptidomimetics containing the Arg-Gly-Asp (RGD) sequence (see Chapter 12 for a detailed overview on RGD cyclopeptides). This approach has been extensively studied, and several carboxylic activators and the role of the solvent towards the outcome of the cyclization have been studied in detail. Solid-phases approaches have been also taken into account by linking on resin the side-chains of selected amino acids, such as Asp, Glu, Lys, Ser and Thr, and performing the macrolactamization before cleavage from the solid support, thus taking advantage of the ‘pseudo-dilution effect’ of the solid support. Finally, cyclative cleavage approaches have been pursued by developing suitable linkers to attain the macrolactamization with concomitant release from the solid support. Relevant examples include Kaiser’s oxime resin [24], Kenner’s safety catch linker [25] and the backbone amide linker (BAL) developed by Barany and Albericio [26].

Another popular approach is the generation of cyclic peptidomimetics through a chemical bond involving two side-chains. Generally, cyclization is achieved by exploiting basic and amino acid residues to form an amide bond or by taking advantage of cysteine amino acids for the development of cyclic peptidomimetics through disulfide bridges between the two side-chains.

Other cyclization strategies that have been undertaken involve the development of mimetics of the side-chain to side-chain disulfide bond between cysteines. Although the introduction of disulfide bond to peptides confers high stability towards proteases, the sensitivity to reduction is often an issue. Consequently, other linkages have been developed to improve the metabolic stability. For example, the thioetherification method consists of replacing one of the two sulfur atoms with a methylene unit [27]; this linkage is also found in the natural compounds lantibiotics possessing the lanthionine building block, where two alanine residues are linked at their β-carbons with a thioether bond [28]. The well-established ring-closing metathesis allows for the generation of macrocyclic constructs by means of high metabolically stable olefin linkages, taking advantage of alkenyl moieties that are generally introduced in the peptide compound by means of allylglycines [29].

Finally, ether-bridged peptidomimetics have been developed for the construction of macrocyclic compounds. These have been approached by introducing bis-aryl ether bonds using S_NAr [30], copper-catalysed Ullman reaction [31] or aryl boronic acid-mediated cyclization [32], or by developing alkyl-aryl ether bonds by means of S_NAr [33] and intramolecular Mitsunobu reactions [34]. This approach has been applied to achieve a tether between backbone moieties linked to side-chain functional groups, resulting in the corresponding cyclic compound. Generally, lysine and ornithine are taken into account as amine counterparts if the C-terminal backbone is used for the cyclization, whereas glutamic and aspartic acids are the side-chain functional groups used for amide bond formation with the N-terminal moiety. Other approaches have been proposed using linkers and other side-chain groups, such as human immunodeficiency virus (HIV)-protease inhibitors exploiting the tyrosine phenolic moiety to cyclize the C-terminal backbone with the aid of a spacer [21a, 35]. As shown in Scheme 2.3, by treatment of Cs_2CO_3 in DMF the phenoxide ion belonging to the Tyr side-chain is cyclized to the backbone alkyl group possessing bromine as the leaving group.
2.4 Peptidomimetic Scaffolds

A peptidomimetic compound is thought of as a small molecule mimicking the biological activity of a peptide although being no longer a peptide in chemical nature [1]. Generally, peptidomimetic molecules do not contain any peptide bonds and possess a modular structure deriving from amino acids, carbohydrates or other types of building blocks. The ideal peptidomimetic molecules are developed with the aim of possessing favourable PK properties for oral administration, and with improved stability and specificity with respect to the parent bioactive peptide.

Peptidomimetics are basically developed according to rational design or random screening. The first route generally follows a hierarchical approach as discussed above, together with structural information about the target or conformational models of the parent peptide to ascertain the rationale for molecular recognition.

The most successful route, however, combines both approaches, starting from the random screening of wide arrays of small molecule mimetics of the parent bioactive peptide, followed by structural elaboration of hit compounds according to a rational approach based on available structural data. A remarkable example of such a combined approach has been reported for the development of peptidomimetic ligands of the bradykinin receptor [36].

Part II of this book will encompass several approaches that have been undertaken during recent decades with the aim of developing small molecule and oligomeric peptidomimetic scaffolds, taking advantage of reminiscent biological architectures as found in peptide/proteins and oligo/polysaccharides, such as amino acids (peptoids, β-turn mimetics, proline mimetics and peptidomimetic foldamers) and sugar derivatives (sugar amino acid scaffolds), respectively.
2.5 Conclusions

The development of peptidomimetic compounds from a bioactive peptide is mainly addressed with aim of reducing the conformational flexibility and of reducing the peptide character, so as to improve the potency and selectivity and to achieve better bioavailability and PK/PD profile, respectively. In this respect, several synthetic approaches have been proposed, which can be broadly divided into local and global modifications of the parent peptide, together with the generation of a scaffold-based peptidomimetic possessing poor peptide character, though maintaining the structural features identified by the pharmacophore. The systematic modification of a bioactive peptide towards a non-peptide peptidomimetic compound possessing a central scaffold, going from local to global modifications, is a straightforward route to understanding the structure–activity profile of a peptide/peptidomimetic compound, in terms of obtaining a peptidomimetic lead compound. Some of these synthetic approaches have found little popularity over the years, and have been limited to special cases in the development of peptidomimetic drugs; in contrast, in others areas such as the field of unnatural cyclic amino acids and dipeptide isosteres, macrocycle formation and N-methylation are still strategic for peptidomimetic design. In addition, interest in peptoids and the corresponding cyclic analogues is growing, especially with a view to developing novel antimicrobial therapeutics and identifying bioactive compounds addressing protein–protein interactions.

References