Peptidomimetics in Organic and Medicinal Chemistry
Peptidomimetics in Organic and Medicinal Chemistry

The Art of Transforming Peptides in Drugs

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WILEY
Dedicated to our families:
Nicoletta and Tommaso
Lory, Francesco, Sara and Tommaso


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Preface

Peptidomimetic design and synthesis are powerful and well-established tools for the generation of small-molecule-based drugs acting as enzyme inhibitors or receptor ligands. Several decades since the introduction of the concept of peptidomimetics, this approach is still timely in drug discovery, owing to the never-ending interest in developing novel drugs derived from bioactive peptides and protein fragments. In fact, the field of small molecules encompassing the panorama of peptide drugs is covered by the generation of peptidomimetics with the aim of reducing the conformational flexibility and the peptide character, so as to improve the potency and selectivity, and to achieve hit compounds possessing optimal bioactivity and improved pharmacokinetics profile. Over the years the basic concepts and approaches to peptidomimetic compounds have evolved to diverse compounds and synthetic strategies, spanning from combinatorial chemistry to solid-phase synthesis and heterocyclic chemistry. In this respect, research efforts within organic and medicinal chemistry have produced novel peptidomimetic entries of improved technology with the aim of generating diverse arrays of enzyme inhibitors and receptor ligands. Specifically, 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 scaffold-based peptidomimetics possessing reduced or absent peptide character, though maintaining the structural features identified by the pharmacophore. Moreover, interest in larger peptidomimetics, such as foldamers and macrocycles, is growing, especially with a view to developing novel antimicrobial therapeutics, and to identifying bioactive compounds addressing protein–protein interactions.

This book is intended to give a comprehensive view of peptidomimetics and their classification based on common structural features, and to discuss the most successful synthetic approaches underlying the building of bioactive compounds of ‘peptidomimetic nature’ based on the structure of natural bioactive peptides. Moreover, selected case studies in relevant biomedical areas are presented to illustrate the relevance of peptidomimetics in the hit-to-lead process towards drug development. The book has been organized into three main parts: the first one describes the basics of peptidomimetics, followed by a comprehensive overview of synthetic methods and molecules and, finally, selected applications in medicinal chemistry are presented.

Part I encompasses the basic concepts underlying the development of peptidomimetic compounds, including their classification, and describes diverse strategic approaches to peptidomimetic design, such as the modification of amino acids, and the introduction of global restrictions to a target bioactive peptide, and also discusses successful examples of
peptidomimetic drugs, such as ACE inhibitors and thrombin inhibitors. These concepts are covered in more detail in the subsequent chapter. In particular, Chapter 2 reports the approaches to local and global modifications, the classification of single amino acid modifications, including the presentation of key peptide isosteres, and the principles of peptidomimetic scaffold design.

In Part II, synthetic methods and molecules of peptidomimetic character are reported in detail. Chapter 3 presents synthetic approaches to peptidomimetic bioisosteres, including peptide bond isosteres and transition-state isosteres, which have found wide application in drug discovery as protease inhibitors. This chapter discusses both side-chain and dipeptide isosteres. Chapter 4 reports the relevance of solid-phase synthesis and combinatorial chemistry as straightforward approaches towards the generation of libraries of peptidomimetics. Click chemistry is presented in Chapter 5 as a powerful concept addressing modern peptidomimetic compounds, taking advantage of the triazole ring as a privileged peptidomimetic scaffold, in terms of developing novel peptidomimetic inhibitors in drug discovery. The special case of peptoids as peptidomimetics containing peptide bond isosteres is described in Chapter 6. The last chapters of this part (Chapters 7–10) discuss the major area of peptidomimetic scaffolds, which is connected to the generation of unnatural amino acids. Specifically, Chapter 7 explores the family of sugar amino acids, which belongs to an important class of scaffolds with high density of stereocentres and functional groups, while Chapter 8 gives a picture of cyclic amino acids as proline surrogates, particularly addressing diverse ring size and tethers in the building of cyclic chemotypes. Some of these compounds are also discussed in Chapter 9, which deals with the subclass of β-turn peptidomimetics that play a crucial role in many biological recognition systems and protein–protein interactions. Chapter 10 concludes Part II by addressing the field of foldamers, where peptidomimetics occupy a prominent role given their similar profile to that of peptides, which fold into helices, sheets and strands. This timely issue is relevant in addressing ‘difficult-to-target’ proteins, which are of major concern in therapeutic areas where target proteins interacting with large surface contacts are involved.

Finally, Part III presents significant applications in medicinal chemistry, specifically reporting research in the fields of HIV protease inhibitors and integrin ligands as key studies aiming to give a picture of the important role of peptidomimetic chemistry in past, present and future biomedical research. The topic of peptidomimetic HIV protease inhibitors is reported in Chapter 11, describing the principles underlying the selection of HIV protease as a key therapeutic target, and discussing both peptidomimetic drugs out in the market and novel peptidomimetic scaffolds as promising lead compound addressing drug-resistant strains. Chapter 12 reports on the concepts essential to peptidomimetic ligands for interacting with αvβ3 integrin, which is a relevant target for cancer research, and discusses both peptide-based and scaffold-based as two diverse approaches to peptidomimetic integrin ligands.

These presentations have been conceived for a broad readership, and should interest not only those readers who currently work in the field of organic and medicinal chemistry addressing drug discovery but also those who are considering this approach in the field of chemical biology, taking advantage of peptidomimetic compounds as small-molecule chemical probes to provide important tools for dynamically interrogating biological
systems and for investigating potential drug targets. We hope that these chapters will stimulate further advances in the ever-developing field of peptidomimetics.

Andrea Trabocchi
Antonio Guarna
_Florence, July 2013_
Abbreviations

AC6C  \(\alpha\)-aminocyclohexane carboxylic acid
ACE  angiotensin-converting enzyme
ACHC  aminocyclohexane carboxylic acid
AChE  acetylcholinesterase
ACPC  aminocyclopentane carboxylic acid
Acpca  \(\gamma\)-aminocyclopentane carboxylic acid
Aib  2-amino-isobutyric acid
Aic  2-aminoindan-2-carboxylic acid
AIDS  acquired immunodeficiency syndrome
AIP  autoinducing peptides
AMPS  antimicrobial peptides
APV  Amprenavir
Ate  2-aminotetralin-2-carboxylic acid
ATV  Atazanavir
azPro  azaproline
BAL  backbone amide linker
BBI  Bowman–Birk inhibitor
Bcl-x\(_L\)  B-cell lymphoma-extra large
BGS  bicycle from glyceraldehyde and serine
Boc  \(t\)-butoxycarbonyl
BTAa  bicycle from tartaric acid and amino acid
BTG  bicyclic from tartaric acid and glycine
BTPP  \(t\)-butylimino-tri(pyrrolidino)-phosphorane
Cbz  benzylxycarbonyl
CCR5  CC chemokine receptor 5
CD  circular dichroism
\(m\)CPBA  \(m\)-chloroperbenzoic acid
cGMP  cyclic guanine monophosphate
CNS  central nervous system
CuAAC  Cu-catalysed azide alkyne cycloaddition
CXCR4  CXC chemokine receptor type 4
DCC  \(N,N'\)-dicyclohexylcarbodiimide
DCM  dichloromethane
de  diastereomeric excess
DFT  density functional theory
DIC  \(N,N'\)-diisopropylcarbodiimide
<table>
<thead>
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<th>Abbreviation</th>
<th>Full Form</th>
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<td>DIEA</td>
<td>$N,N$-diisopropylethylamine</td>
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<tr>
<td>DKP</td>
<td>2,5-diketopiperazine</td>
</tr>
<tr>
<td>DMF</td>
<td>$N,N$-dimethylformamide</td>
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<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<td>DOPA</td>
<td>3,4-dihydroxyphenylalanine</td>
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<tr>
<td>DOS</td>
<td>diversity-oriented synthesis</td>
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<td>DPPA</td>
<td>diphenylphosphoryl azide</td>
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<td>DRV</td>
<td>Darunavir</td>
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<tr>
<td>Dtc</td>
<td>5,5-dimethylthiazolidine-4-carboxylic acid</td>
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<td>EGF</td>
<td>epidermal growth factor</td>
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<td>Fmoc</td>
<td>fluorenylmethyloxycarbonyl</td>
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<td>FPV</td>
<td>fosamprenavir</td>
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<tr>
<td>FRET</td>
<td>fluorescence resonance energy transfer</td>
</tr>
<tr>
<td>GABA</td>
<td>$\gamma$-aminobutyric acid</td>
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<td>GPCR</td>
<td>G protein-coupled receptors</td>
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<td>HATU</td>
<td>$N,N,N',N'$-tetramethyl-$O$-(7-azabenzotriazol-1-yl)uronium hexafluorophosphate</td>
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<td>HCV</td>
<td>hepatitis C virus</td>
</tr>
<tr>
<td>HIV</td>
<td>human immunodeficiency virus</td>
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<tr>
<td>HPLC</td>
<td>high-performance liquid chromatography</td>
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<td>HSP</td>
<td>heat shock protein</td>
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<tr>
<td>HSQC</td>
<td>heteronuclear single quantum coherence spectroscopy</td>
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<td>HUVEC</td>
<td>human umbilical cord vein endothelial cell</td>
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<td>IDV</td>
<td>Indinavir</td>
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<td>IR</td>
<td>infrared spectroscopy</td>
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<td>Lac</td>
<td>lactic acid</td>
</tr>
<tr>
<td>LC-MS</td>
<td>liquid chromatography–mass spectrometry</td>
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<td>LF</td>
<td>lethal factor</td>
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<tr>
<td>LHMDS</td>
<td>lithium bis(trimethylsilyl)amide</td>
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<td>LHRH</td>
<td>luteinizing hormone releasing hormone</td>
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<td>LPV</td>
<td>Lopinavir</td>
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<tr>
<td>MC/SD</td>
<td>Monte Carlo/stochastic dynamics</td>
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<td>mCPBA</td>
<td>3-chloroperoxybenzoic acid</td>
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<tr>
<td>MCR</td>
<td>melanocortin receptors</td>
</tr>
<tr>
<td>MD</td>
<td>molecular dynamics</td>
</tr>
<tr>
<td>MIDAS</td>
<td>metal ion-dependent adhesion site</td>
</tr>
<tr>
<td>miniAMP</td>
<td>mini atrial natriuretic polypeptide</td>
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<tr>
<td>MMP</td>
<td>matrix metalloproteases</td>
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<tr>
<td>Mor</td>
<td>morpholine</td>
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<td>MTT</td>
<td>3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide</td>
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<td>Nal</td>
<td>naphthylalanine</td>
</tr>
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<td>NFV</td>
<td>Nelfinavir</td>
</tr>
<tr>
<td>NGF</td>
<td>nerve growth factor</td>
</tr>
<tr>
<td>NMR</td>
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<td>NOESY</td>
<td>nuclear Overhauser effect spectroscopy</td>
</tr>
<tr>
<td>NPR-A</td>
<td>natriuretic peptide receptor A</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
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</tr>
<tr>
<td>NT1</td>
<td>neurotensin 1</td>
</tr>
<tr>
<td>PAI-1</td>
<td>plasminogen activator inhibitor-1</td>
</tr>
<tr>
<td>PAM</td>
<td>(4-hydroxymethyl)phenylacetamide</td>
</tr>
<tr>
<td>PCC</td>
<td>pyridinium chlorochromate</td>
</tr>
<tr>
<td>PDB</td>
<td>Protein Data Bank</td>
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<tr>
<td>PI</td>
<td>protease inhibitors</td>
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<td>PIP</td>
<td>piperidine</td>
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<td>PK/PD</td>
<td>pharmacokinetics/pharmacodynamics</td>
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<td>plasmepsins</td>
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<td>PPI</td>
<td>peptidyl-prolyl isomerase</td>
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<td>PyBOP</td>
<td>benzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate</td>
</tr>
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<td>PyBrOP</td>
<td>bromotripyrrolidinophosphonium hexafluorophosphate</td>
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<td>RCM</td>
<td>ring-closing metathesis</td>
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<td>RGD</td>
<td>Arg-Gly-Asp peptide</td>
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<td>RNA</td>
<td>ribonucleic acid</td>
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<td>SAA</td>
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<td>San A</td>
<td>sansalvamide A</td>
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<td>structure–activity relationship</td>
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<td>$S_N^2$</td>
<td>bimolecular nucleophilic substitution</td>
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<td>SPAV3</td>
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<td>SPECT</td>
<td>single-photon emission computed tomography</td>
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<td>SPPS</td>
<td>solid-phase peptide synthesis</td>
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<td>STAT3</td>
<td>signal transducers and activators of transcription 3</td>
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<td>t-PA</td>
<td>tissue-type plasminogen activator</td>
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<td>$t$-butylsilyl</td>
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<td>TBAF</td>
<td>tetrabutylammonium fluoride</td>
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<td>TEA</td>
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<td>TEMPO</td>
<td>2,2,6,6-tetramethyl-1-piperidinyloxy, free radical</td>
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<td>TFA</td>
<td>trifluoroacetic acid</td>
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<td>TGF</td>
<td>transforming growth factor</td>
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<td>THF</td>
<td>tetrahydrofuran</td>
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<td>Tic</td>
<td>tetrahydroisoquinoline-3-carboxylic acid</td>
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<td>TRH</td>
<td>thyrotropin-releasing hormone</td>
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<td>US Center for Diseases Control</td>
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<tr>
<td>US FDA</td>
<td>US Food and Drug Administration</td>
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<td>VEGF</td>
<td>vascular endothelial growth factor</td>
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Part I

The Basics of Peptidomimetics
1
The Basics of Peptidomimetics

1.1 Introduction

During the last three decades an important number of biologically active peptides has been discovered and characterized, including hormones, vasoactive peptides and neuropeptides. As a consequence of interaction with their membrane-bound receptors, these bioactive peptides influence cell–cell communication and control a series of vital functions. Thus, they are of great interest in the biomedical field, and the number of native and modified peptides used as therapeutics is ever increasing. Many bioactive peptides have been prepared on a large scale and tested both in pharmacology and the clinic, thus allowing for the development of new therapies for various pathologies.

However, the use of peptides as therapeutics is limited due to several factors, including low metabolic stability towards proteolysis in the gastrointestinal tract, poor absorption after oral ingestion, low diffusion in particular tissue organs (i.e. the central nervous system, CNS), rapid excretion through liver and kidneys and undesired effects due to interaction of flexible peptides with several receptors (Figure 1.1) [1]. In particular, the flexibility of medium-sized polypeptides (<30 amino acids) is due to the multiple conformations that are energetically accessible for each residue constituting the peptide. The flexibility of each residue constituting a peptide is due to two degrees of conformational freedom addressed by $N-C_\alpha$ and $C_\alpha-CO$ rotational bonds and described by $\phi$ and $\psi$ dihedral angles, respectively, which result in a population of local conformations, all contributing to the overall flexibility of the peptide in dynamically interconverting equilibria in aqueous solution.

Besides all these drawbacks, biomedical research is constantly oriented towards the development of new therapeutics based on peptides and proteins, by introducing both structural and functional specific modifications and maintaining the features responsible for biological activity.

These requirements are all matched in the development of peptidomimetics [2, 3]. In this approach, peptides and proteins are considered as tools for the discovery of other classes of compounds.
Figure 1.1 Conformational flexibility of peptides and their affinity with proteases cause off-target interactions and degradation, respectively, resulting in undesired biological effects, and inactive fragments from proteolytic events. (Reproduced with permission from Reference [1]. Copyright 1993 Wiley-VCH Verlag GmbH & Co. KGaA.) (See plate section for colour version)
1.2 Definition and Classification

A peptidomimetic compound may be defined as a substance having a secondary structure, besides other structural features, similar to native peptide, such that it binds to enzymes or receptors with higher affinity than the starting peptide. As an overall result, the native peptide effects are inhibited (antagonist or inhibitor) or increased (agonist). Since their introduction as a new concept for developing drug candidates, peptidomimetics have shown great promise both in organic and medicinal chemistry. Apart from being much more selective and efficient than native peptides, thus resulting in fewer side effects, peptidomimetics show greater oral bioavailability and the biological activity is prolonged due to lowered enzymatic degradation [4, 5]. The generation of peptidomimetics is basically focussed on knowledge of the electronic and conformational features of the native peptide and its receptor or active site of an enzyme. Thus, the development of peptidomimetics as compounds with potential biological activity must take account of some basic principles [6], including:

- Replacement of peptide backbone with a non-peptide framework: if an amide bond substitution does not change the biological activity or amide bonds are not exposed to the active site, then the template may be designed to eliminate peptide bonds.
- Preservation of side-chains involved in biological activity, as they constitute the pharmacophore. In the development of second-generation mimetics, several modifications may be introduced to improve biological activity, including chain length modification, introduction of constraints, cyclopeptide bond replacement with a covalent one and introduction of isosteric replacements [7].
- Maintenance of flexibility in first-generation peptidomimetics: if biological activity is observed for a flexible mimetic, then the introduction of elements of rigidity to side-chains is a rational approach to improve the preliminary activity observed.
- Selection of proper targets based on a pharmacophore hypothesis. In other words, knowledge of the structure–activity relationship or the three-dimensional structure of bioactive conformation is a promising route to rapidly achieve the best compound, without generating a huge number of compounds with poor biological activity.

Peptidomimetics may be divided into three classes depending on their structural and functional characteristics [8]:

- Type I mimetics, or structural mimetics, show an analogy of a local topography with the native substrate, and they carry all the functionalities responsible for the interaction with an enzyme or a receptor in a well-defined spatial orientation.
- Type II mimetics, or functional mimetics: here the analogy with the native compound is based on the interaction with the target receptor or enzyme, without apparent structural analogies.
- Type III mimetics, or functional-structural mimetics, are generally conceived as possessing a scaffold with a structure different from that of the substrate, in which all the functional groups needed for biological interactions are mounted in a well-defined spatial orientation. Many examples have been reported in the literature in which an unnatural framework substitutes the peptide backbone and carries the required functional groups for biological activity.
An elegant example of a peptidomimetic scaffold is given by a thyrotropin-releasing hormone (TRH) mimic based on a cyclohexane scaffold (1, Figure 1.2), which replaces the peptide backbone, and the three functional groups that constitute the pharmacophore are placed on the scaffold with the same spatial orientation of amino acid side-chains found in TRH hormone [5]. Other examples include replacement of peptidic fragment in somatostatin receptor binding cyclopeptide with a D-glucose scaffold (2) [9], and steroidal scaffold 3 to mimic the type II' β-turn structure of RGDfV cyclopeptide (Figure 1.2) [10].

The workflow towards the development of peptidomimetics has been proposed within the drug discovery process in the case of peptide molecules as hit compounds towards an identified target [11].

Figure 1.2  Peptidomimetic compounds consisting of cyclohexane (1), glucose (2) or steroid (3) scaffolds. (Reproduced with permission from Reference [2]. Copyright 1994 Wiley-VCH Verlag GmbH & Co. KGaA.) (See plate section for colour version)
Accordingly, the first step in a drug discovery process is hit identification; this is generally carried out by scanning peptide libraries for binding affinity (i.e. by phage display or combinatorial chemistry of synthetic peptide libraries). Molecular biology techniques, such as sequencing, cloning and site-directed mutagenesis experiments, are essential to achieve structural information regarding receptor residues responsible for peptide recognition in combination with molecular modelling calculations. Such information is very important in selecting a bioactive peptide to be successively processed in a hierarchical way, also taking advantage of structural data. The hierarchical approach takes advantage of several steps, which are important in giving insight into structure–activity relationships with respect to the starting bioactive peptide hit compound to be converted into a peptidomimetic lead:

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

The so-obtained first-generation peptidomimetics are then subjected to further conformational studies aimed at defining the rationale for ligand–receptor (or enzyme–inhibitor) key interactions. The results are then applied for the optimization of hit peptidomimetics towards improved compounds possessing a non-peptide framework.

Chapter 2 presents a detailed overview of the design principles and applications to peptidomimetics.

1.3 Strategic Approaches to Peptidomimetic Design

A major effort in peptidomimetic chemistry is connected to the development of compounds capable of replacing one or more amino acids in a peptide sequence without altering the biological activity of the native peptide. The overall result of this structural intervention is to stabilize the molecule with respect to metabolic processes that occur in vivo, thus giving access to orally available drugs and compounds with improved pharmacokinetics/pharmacodynamics (PK/PD) properties.

The development of peptidomimetics has generally been approached by synthesizing novel amino acids possessing several features, including synthetic accessibility from commercially available enantiopure reagents, such as amino acid and sugar derivatives, or straightforward synthetic methods for asymmetric synthesis, to access a wide array of novel compounds. Moreover, the need to achieve partially rigid compounds has been pursued to probe a limited number of conformations with the aim of understanding the bioactive topology and giving insight into requisites to design improved bioactive compounds. Access to novel amino acids as peptide isosteres has been pursued by either modifying the atoms involved in backbone formation of a peptide or in manipulating the side-chain moiety, for example by introducing chemical tethers as rigidifying elements. Moreover, peptidomimetic chemistry has been oriented to the development of higher isosteres, taking into account di-, tri- or tetrapeptides motifs to be replaced by more complex molecular architectures. Finally, the approach to intervening in terms of the overall peptide structure has been accessed by working on global restrictions of the native peptide conformation.
1.3.1 Modification of Amino Acids

Manipulation of the peptide structure with aim of reducing molecular recognition by proteases and of introducing conformational restrictions is achieved locally by intervening on either backbone or side-chains by introduction of modified amino acids. Accordingly, a well-established approach is to replace proteinogenic amino acids locally and systematically with their corresponding d-variants, Cβ-alkylated, Cα-alkylated or Nα-alkylated amino acids. For example, substitution of α-aminocycloalkanecarboxylic acids varying in ring size (Figure 1.3) into various positions of enkephalin (H-Tyr-Gly-Gly-Phe-Leu-OH), a peptide responsible for modulating pain response, resulted in a peptidomimetic with greater in vivo activity [12].

β-Methylamino acids have been reported for restricting the conformations of a bioactive peptide through the insertion of a stereocenter at the β-position. Indeed, four configurations are accessible by varying the two stereocenters; as an exemplificative entry to this approach, the systematic incorporation of β-MePhe into somatostatin peptidomimetics has resulted in a model for the ligand–receptor interaction, based on the changes in activity induced by different configurations at the β centre [13]. Proline analogues are important for introducing strong conformational bias to the peptide, as the φ angle corresponding to the rotation of the N-Cα bond is constrained to $-65 \pm 15^\circ$, preventing α-helix formation and encouraging the formation of β-turns. Moreover, as the barrier to proline cis/trans isomerism is $\sim 2 \text{ kcal mol}^{-1}$, compared to that of secondary amide (10 kcal mol$^{-1}$), proline analogues have been proposed with the aim of orienting the equilibrium towards a preferred geometry, generally the cis form owing to its importance in peptide folding. This has been approached by varying the ring size, the substitution pattern around the cyclic backbone and introducing heteroatoms. For example, the substitution of 5,5-dimethylthiazolidine-4-carboxylic acid (Dtc) for Pro (Figure 1.4) in angiotensin II, a key peptide in blood pressure regulation, resulted in a peptidomimetic with 39% greater agonist activity than the natural peptide [14].

Moreover, many applications consisting of other unnatural amino acids have been proposed, including unsaturated, cyclic and β-amino acids, which allowed for the addition of conformational bias to the overall structure. For example, proline analogues and

![Figure 1.3 α-Aminocycloalkane carboxylic acids varying in ring size](image)

![Figure 1.4 5,5-Dimethylthiazolidine-4-carboxylic acid (Dtc) as a proline analogue in angiotensin II peptidomimetics](image)