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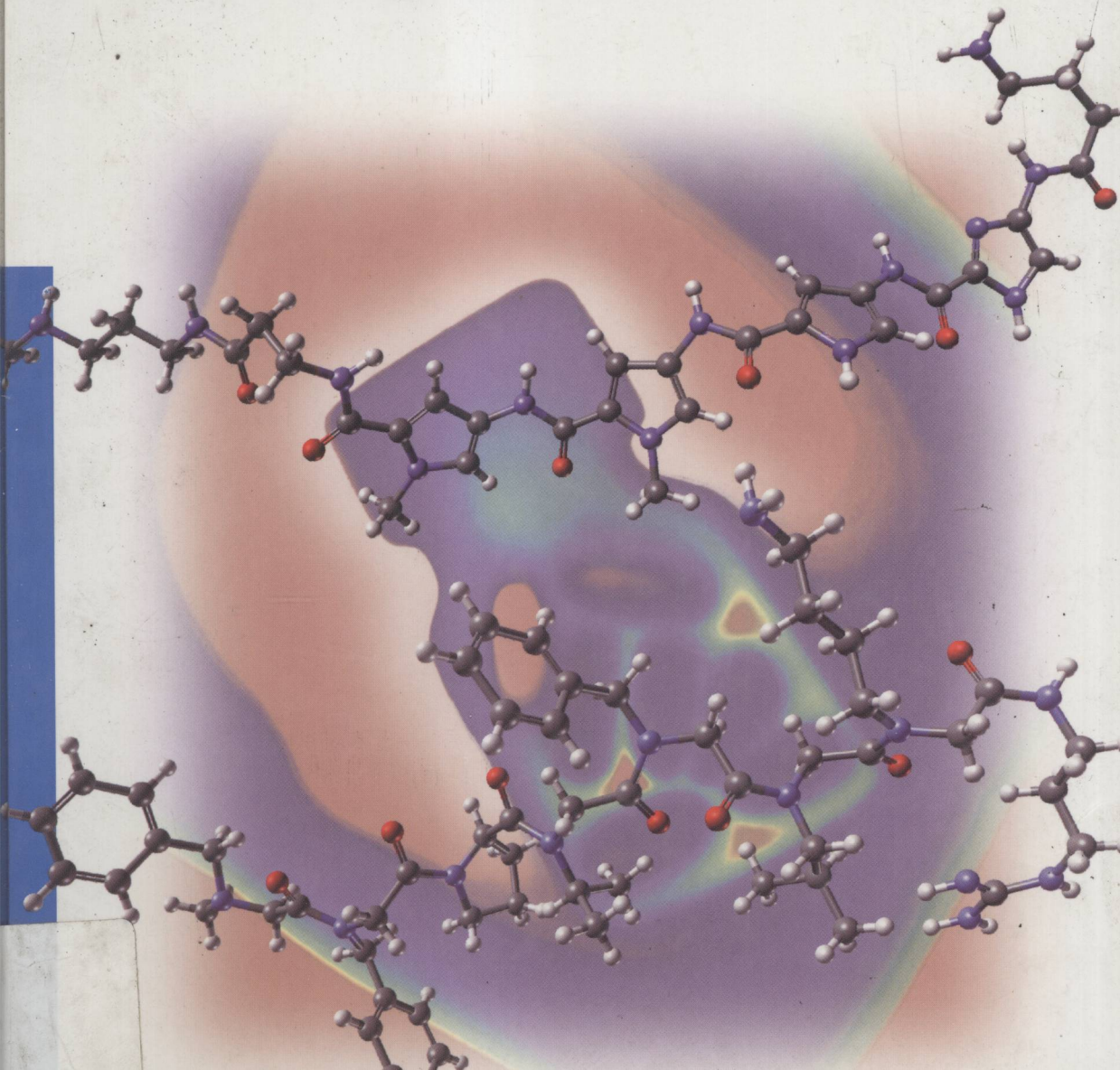
Pseudo-Peptides in Drug Discovery



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Pseudo-peptides in Drug Discovery

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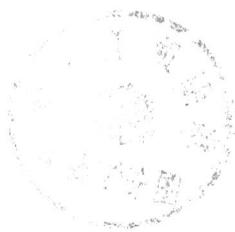

Preface

Peptides are used extensively by Nature for a variety of signalling functions in both unicellular and multicellular organisms including man. For example, many peptide hormones and analogous short peptides exert their action by binding to membrane receptors. Peptides may also show biological activities in the form of nerve toxins, antibacterial agents or general cell toxins. Therefore, it is no wonder that medical drug discovery has extensively exploited peptides as lead compounds. This development was further accelerated by the development of very effective methods for solid phase synthesis of peptides, and in particular the development of combinatorial methods for synthesizing and screening peptide libraries.

However, most natural peptides are composed of L-form α-amino acids and because of the ubiquitous prevalence of peptidases they have limited biostability, and consequently low bioavailability. Thus, a novel field of peptidomimetics has emerged in drug discovery, in attempts to design non-peptide compounds mimicking the pharmacophore and thus the activity of the original peptide.

This field has also inspired the development of a range of pseudo-peptides, that is polyamides composed of amino acids other than α-amino acids. These include for instance peptoids, β-amino acid oligomers and also compounds such as peptide nucleic acids and DNA binding polyamides, all of which share the amide (peptide) chemistry with natural peptides.

The present book attempts to present the state of the art in the rapidly expanding field of pseudo-peptides, in particular relating to (long term aims of) drug discovery. Many chemists are realizing the power and versatility of "peptide" chemistry, and the large structural and functional space attainable using this technology. Hopefully the book will inspire new developments.

I am extremely grateful to the friends and colleagues who have made this project possible by investing their time and expertise.

Copenhagen, October 2003 *Peter E. Nielsen*

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Contents

1
Versatile Oligo(*N*-Substituted) Glycines: The Many Roles of Peptoids in Drug Discovery

James A. Patch, Kent Kirshenbaum, Shannon L. Seurynck, Ronald N. Zuckermann, and Annelise E. Barron

1.1 Introduction

Despite their wide range of important bioactivities, polypeptides are generally poor drugs. Typically, they are rapidly degraded by proteases *in vivo*, and are frequently immunogenic. This fact has stimulated widespread efforts to develop peptide mimics for biomedical applications, a task that presents formidable challenges in molecular design. Chemists seek efficient routes to peptidomimetic compounds with enhanced pharmacological properties, which retain the activities of their biological counterparts. Since peptides play myriad roles in living systems, it is likely that no individual strategy will suffice. Indeed, a wide variety of different peptidomimetic oligomer scaffolds have been explored [1]. In order to address multiple design criteria for applications ranging from medicinal chemistry to materials science, researchers have worked to identify a non-natural chemical scaffold that recapitulates the desirable attributes of polypeptides. These include good solubility in aqueous solution, access to facile sequence-specific assembly of monomers containing chemically diverse side chains, and the capacity to form stable, biomimetic folded structures.

Among the first reports of chemically diverse peptide mimics were those of (*N*-substituted) glycine oligomers (peptoids) [2]. Sequence-specific oligopeptoids have now been studied for over a decade, and have provided illustrative examples of both the potential of peptidomimetics and the obstacles faced in translating this potential into clinically useful compounds. We begin this chapter with a summary of the desirable attributes of peptoids as peptide mimics, along with a description of strategies for their chemical synthesis. Throughout the remainder of the chapter, we present an overview of biomedically relevant studies of peptoids with an emphasis on recently reported results. The chapter includes discussion of peptoid combinatorial libraries, folded peptoid structures, and biomimetic peptoid sequences. Finally, we conclude by suggesting promising avenues for future investigations.

Peptoids are an archetypal and relatively conservative example of a peptidomimetic oligomer (Tab. 1.1). In fact, the sequence of atoms along the peptoid backbone is identical to that of peptides. However, peptoids differ from peptides in the manner of side chain appendage. Specifically, the side chains of peptoid oligo-

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Tab. 1.1 Comparison of key characteristics of peptides and peptoids

	Peptides	***Peptoids***
Identity	Sequence-specific polymers of amino acids	Sequence-specific polymers of *N*-substituted glycines
Synthesis	Solid-phase. Polymerization of *N*-protected α-amino acids (Fmoc or Boc).	Solid-phase sub-monomer synthesis
Number of side chains?	20+	Derived from hundreds of available primary amines
Secondary structures	Helices (3^{10}, α), β-sheets	Helices
Structural stabilization	Intra-chain hydrogen bonds	Steric and electronic repulsions
Thermal stability of structures?	Up to ~40 °C	>75 °C
Structural stability to solvent environment?	May denature in high salt, organic solvent, or at pH extremes	Generally stable to salt, pH, and organic solvent
In vivo stability	Rapidly degraded (proteolysis)	Stable to proteolysis, excreted whole in urine

mers are shifted to become pendant groups of the main-chain nitrogen atoms (Fig. 1.1). The presentation of peptide and peptoid side chains is roughly isosteric, potentially allowing for suitable mimicry of the spacing between the critical chemical functionalities of bioactive peptides. Peptoid monomers are linked through polyimide bonds, in contrast to the amide bonds of peptides (with the sole exception of proline residues, which are also -imino acids). Peptoids lack the hydrogen of the peptide secondary amide, and are thus incapable of forming the same types of hydrogen bond networks that stabilize peptide helices and β-sheets, respectively. The peptoid oligomer backbone is achiral; however, chiral centers can be included in the side chains to obtain secondary structures with a preferred handedness [3, 4]. In addition, peptoids carrying *N*-substituted versions of the proteinogenic side chains are highly resistant to degradation by proteases [5], which is an important attribute of a pharmacologically useful peptide mimic.

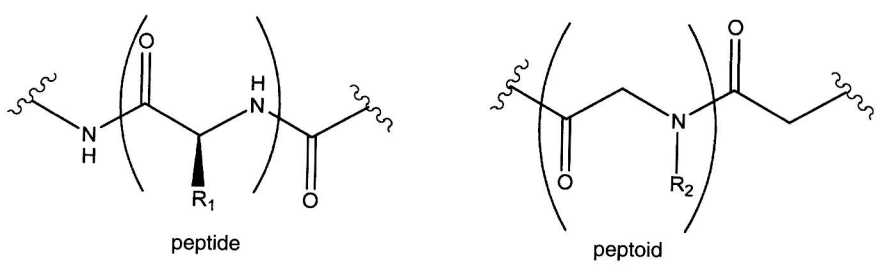

Fig. 1.1 Comparison of the primary structure of peptide and peptoid oligomers

The efficient solid-phase synthesis of peptoids (Section 1.2) enables facile combinatorial library generation. In the "sub-monomer" synthetic strategy, peptoid monomers are synthesized by a two-step process from a haloacetic acid and a primary amine. A wide variety of amines are commercially available, which facilitates the incorporation of chemically diverse side chains. High synthetic yields typically attained at each synthetic step permit the propagation of peptoid chains to substantial lengths. For instance, peptoids up to 48 residues long have been synthesized with reasonable yields of the full-length target sequence [6].

1.2
Peptoid Synthesis

1.2.1
Solid-Phase Synthesis

Sequence-specific heteropolymers, as a class of synthetic molecules, are unique in that they must be made by chemical steps that add one monomer unit at a time. Moreover, to create truly protein-like structures, which typically have chain lengths of at least 100 monomers and a diverse set of 20 side chains (or more), extremely efficient and rapid couplings under general reaction conditions are necessary. For these reasons, solid-phase synthesis is typically used, so that excess reagents can be used to drive reactions to completion, and subsequent reaction work-ups are quite rapid.

A common feature of most solid-phase oligomer syntheses (e.g. peptide, oligonucleotide, peptide nucleic acid, β-peptide, etc.) is that they are made by a two-step monomer addition cycle. First, a protected monomer unit is coupled to a terminus of the resin-bound growing chain, and then the protecting group is removed to regenerate the active terminus. Each side chain group requires a separate N^{α}-protected monomer. The first oligopeptoids reported were synthesized by this method, for which a set of Fmoc-protected peptoid monomers was made [2].

Specifically, the carboxylates of N^{α}-Fmoc-protected (and side chain-protected) *N*-substituted glycines were activated and then coupled to the secondary amino

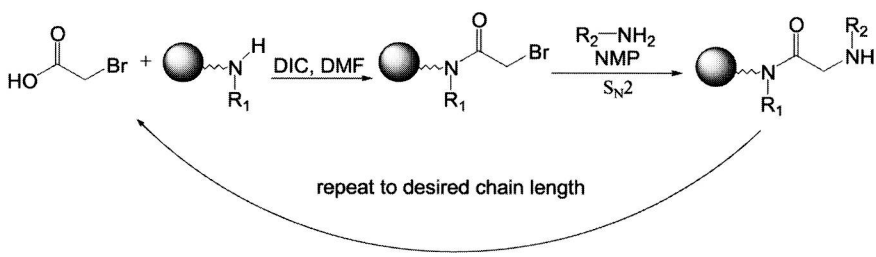

Fig. 1.2 Solid-phase sub-monomer peptoid synthesis

group of a resin-bound peptoid chain. Removal of the Fmoc group was then followed by addition of the next monomer. Thus, peptoid oligomers can be thought of as condensation homopolymers of *N*-substituted glycine. There are several advantages to this method [7], but the extensive synthetic effort required to prepare a suitable set of chemically diverse monomers is a significant disadvantage of this approach. Additionally, the secondary *N*-terminal amine in peptoid oligomers is more sterically hindered than the primary amine of an amino acid, which slows coupling reactions.

1.2.2 Sub-monomer Solid-Phase Method

A major breakthrough came in 1992 when a much more efficient method of peptoid synthesis was invented [8]. In this method, each *N*-substituted glycine (NSG) monomer is assembled from two readily available "sub-monomers" in the course of extending the NSG oligomer [9]. This method is known as the sub-monomer method, in which each cycle of monomer addition consists of two steps, an acylation step and a nucleophilic displacement step (Fig. 1.2). Thus, peptoid oligomers can also be considered to be alternating condensation copolymers of a haloacetic acid and a primary amine. This method is unique among solid-phase oligomer syntheses in that there are no protecting groups used in elongating the main chain. As in the original method, the direction of oligomer synthesis utilizing these sub-monomers occurs in the carboxy to amino direction.

In the first step, a resin-bound secondary amine is acylated with bromoacetic acid, in the presence of *N,N*-diisopropylcarbodiimide. Acylation of secondary amines is difficult, especially when coupling an amino acid with a bulky side chain. The sub-monomer method, on the other hand, is facilitated by the use of bromoacetic acid, which is a very reactive acylating agent. Activated bromoacetic acid is bis-reactive, in that it acylates by reacting with a nucleophile at the carbonyl carbon, or it can alkylate by reacting with a nucleophile at the neighboring aliphatic carbon. Because acylation is approximately 1000 times faster than alkylation, acylation is exclusively observed.

The second step introduces the side chain group by nucleophilic displacement of the bromide (as a resin-bound α-bromoacetamide) with an excess of primary amine. Because there is such diversity in reactivity among candidate amine sub-monomers, high concentrations of the amine are typically used ($\sim$1–2 M) in a polar aprotic solvent (e.g. DMSO, NMP or DMF). This S_N2 reaction is really a mono-alkylation of a primary amine, a reaction that is typically complicated by over-alkylation when amines are alkylated with halides in solution. However, since the reactive bromoacetamide is immobilized to the solid support, any over-alkylation side-products would be the result of a cross-reaction with another immobilized oligomer (slow) in preference to reaction with an amine in solution at high concentration (fast). Thus, in the sub-monomer method, the solid phase serves not only to enable a rapid reaction work-up, but also to isolate reactive sites from

one another. However, the primary advantage of the method is that each primary amine is much simpler in structure than a protected full monomer. The fact that several hundred primary amines are commercially available greatly facilitates peptoid synthesis.

Most primary amines that are neither sterically hindered nor very weak nucleophiles will incorporate into the peptoid chain in high yield. However, protection of reactive side-chain functionalities such as carboxyl, thiol, amino, hydroxy and other groups may be required to minimize undesired side reactions [10]. Acid-labile protecting groups are preferred, as they may be removed during peptoid cleavage from solid support with trifluoroacetic acid (TFA). The mild reactivity of some side-chain moieties toward displacement or acylation allows their use without protection in some cases (e.g. indole, phenol). In these cases, the side chain may become transiently acylated during the acylation step and will subsequently revert back to the free side chain upon treatment with the amine in the displacement step. Heterocyclic side-chain moieties such as imidazole, pyrazine, quinolines and pyridines may also become transiently acylated. However, since these groups are more nucleophilic they are susceptible to alkyation by the activated bromoacetic acid. In these cases, clean incorporation can be achieved by replacing bromoacetic acid with chloroacetic acid [11].

1.2.3 Side Reactions

There are a few competing side reactions that are unique to the synthesis of peptoid oligomers. For example, peptoid dimer synthesis often leads to formation of the cyclic diketopiperazines instead of the linear molecule [12]. Sub-monomers whose side chains bear a nucleophile three or four atoms from the amino nitrogen, are also prone to cyclizations after bromoacetylation. We have found the sub-monomers trityl-histamine, 2-(aminomethyl)benzimidazole, and 2-aminoethylmorpholine fall into this category. Electron-rich benzylic side chains, such as those derived from *p*-methoxy-1-phenylethylamine or 2,4,6-trimethoxybenzylamine, will fall off the main chain during the post-synthetic acidolytic cleavage with TFA.

1.2.4 Post-Synthetic Analysis

Like peptide oligomers, peptoids can be analyzed by HPLC and by mass spectrometry. They can be sequenced by Edman degradation [13] or by tandem mass spectrometry [14] since, like polypeptides, they conveniently fragment along the main chain amides [15, 16].