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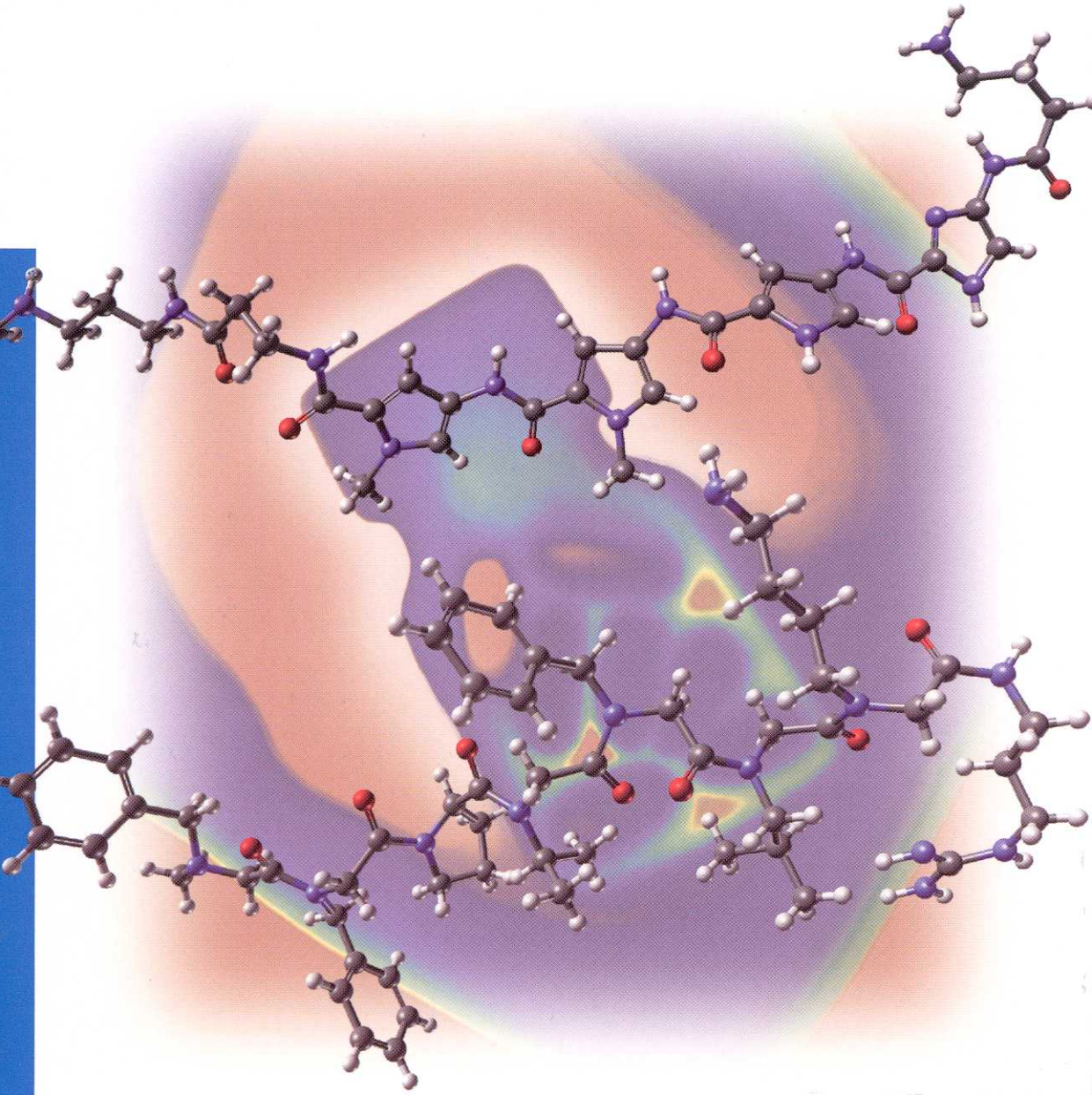
Pseudo-Peptides in Drug Discovery



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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*

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.

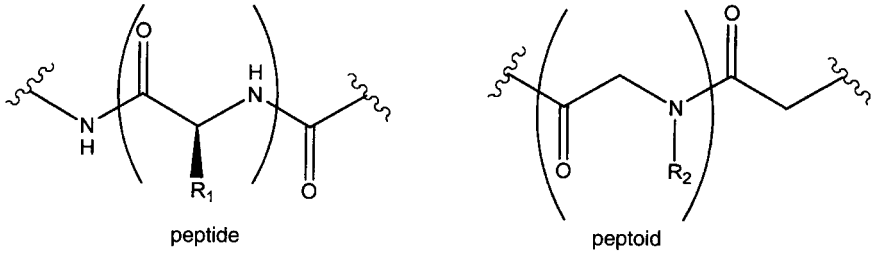


Fig. 1.1 Comparison of the primary structure of peptide and peptoid oligomers

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

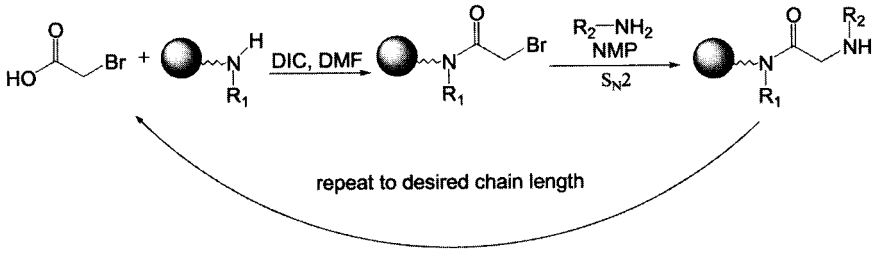


Fig. 1.2 Solid-phase sub-monomer peptoid synthesis

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].

1.3 Drug Discovery via Small-Molecule Peptoid Libraries

Because of their ease of synthesis and their structural similarity to peptides, many laboratories have used peptoids as the basis for combinatorial drug discovery. Peptoids were among the first non-natural compounds used to establish the basic principles and practical methods of combinatorial discovery [17]. Typically, diverse libraries of relatively short peptoids (<10 residues) are synthesized by the mix-and-split method and then screened for biological activity. Individual active compounds can then be identified by iterative re-synthesis, sequencing of compounds on individual beads, or indirect deduction by the preparation of positional scanning libraries.

1.3.1 Peptoid Drugs from Combinatorial Libraries

Early on, peptoid trimers that were nanomolar ligands for the opiate and α_1-adrenergic receptors were identified by *in vitro* receptor binding experiments [17] (Fig. 1.3). These ligands were discovered from a library of 3500 trimers by iterative re-synthesis, where activity was followed through successive rounds of re-synthesis of smaller pools. These compounds showed some *in vivo* activity, despite their poor pharmacokinetic properties [18]. Trimeric peptoid libraries were also screened for antimicrobial activity in whole cell assays, which yielded compounds with modest activities against *S. aureus* and *E. coli* [19]. Very large libraries of peptoid trimers (~350,000 compounds) were screened against a variety of agrochemical targets to provide a sub-micromolar antagonist of the nicotinic acetylcholine receptor, following iterative deconvolution [20]. Positional scanning libraries of peptoid trimers were used to discover noncompetitive antagonists of the vanilloid receptor subunit 1 (VR1) [21]. Another research group prepared a library of 328,000 peptoid trimers, and used iterative deconvolution to discover micromolar ligands for both the melanocortin type 1 (MC1) and gastrin-releasing peptide/bombesin receptors [22]. Finally, a small family of peptoid trimers was generated in order to mimic the Agouti-related protein, including one member that showed micromolar affinity for the melanocortin type 4 receptor [23].

1.3.2 Peptoid Inhibitors of RNA-Protein Interactions

Peptoid libraries have also yielded compounds active in the disruption of RNA-protein interactions. Compounds not derived from library syntheses are discussed in Section 1.4.1. A peptoid 9-mer with a number of cationic groups was discovered (Fig. 1.4) after several rounds of mixture deconvolution, that was able to block the interaction of HIV-1 Tat protein with TAR RNA at nanomolar concentra-

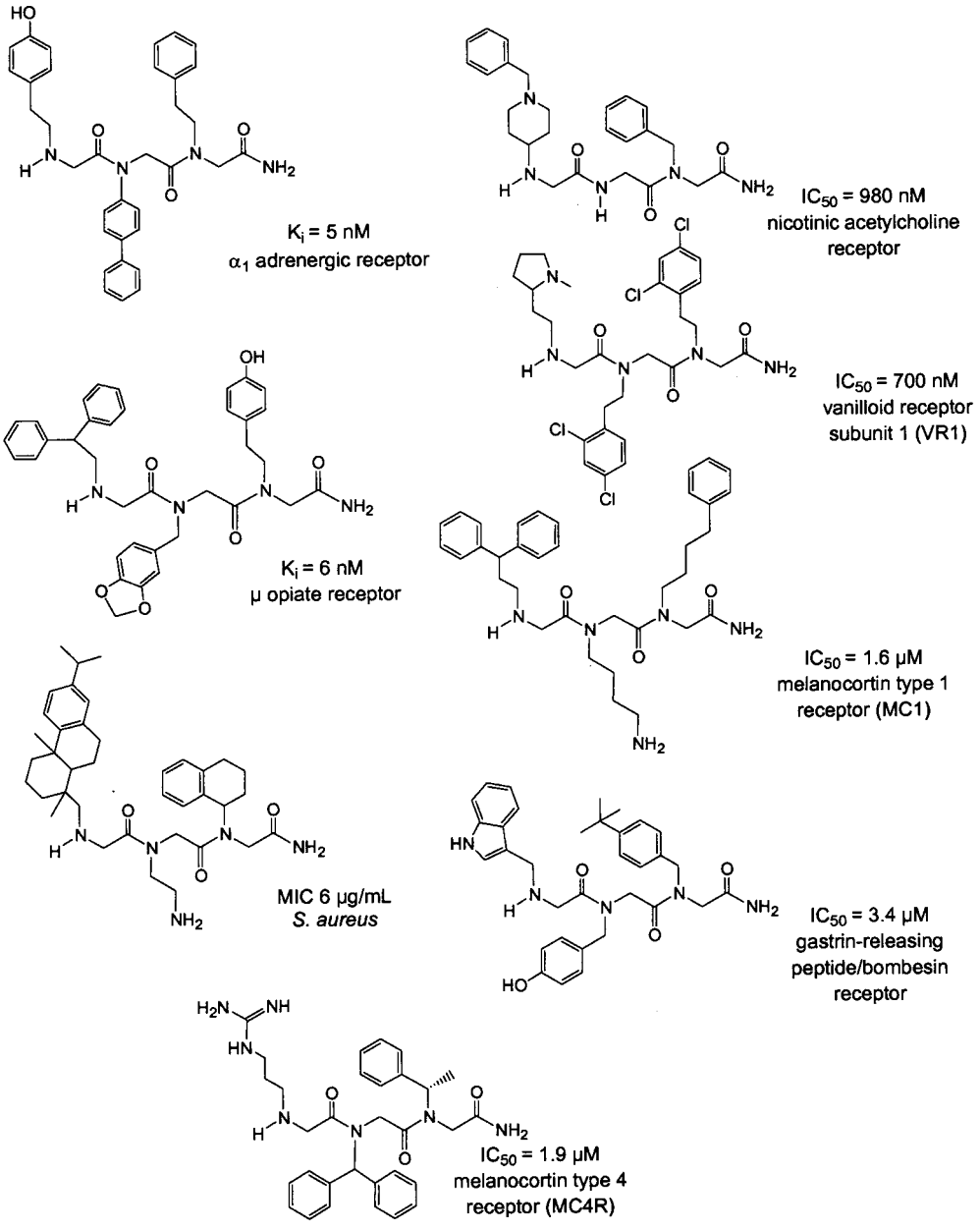


Fig. 1.3 Various small-molecule peptoid ligands derived from combinatorial libraries

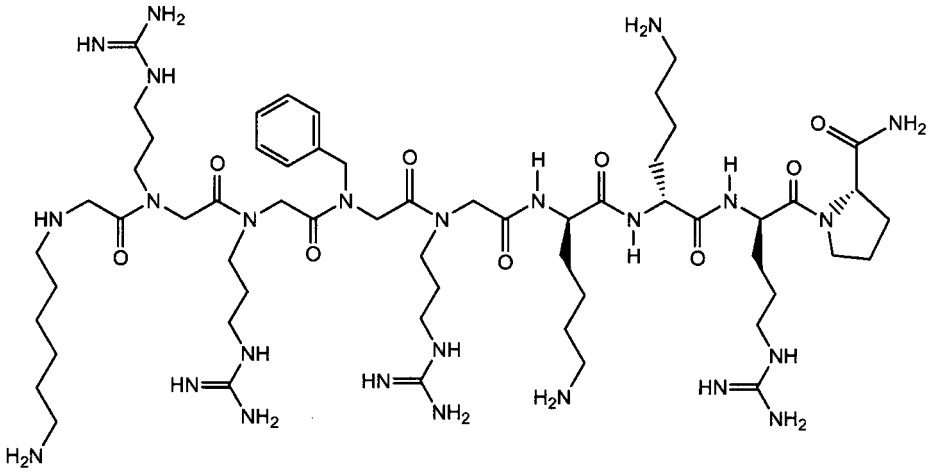


Fig. 1.4 Structure of a peptoid/peptide hybrid that is a submicromolar inhibitor of the HIV-1 Tat/Tar interaction

tions, as well as block HIV-1 replication *in vivo* [24]. Other cationic peptoids with similar side chains, which also inhibit this interaction have been discovered [25].

1.4
Peptoid-Based Drug Delivery and Molecular Transporters: Cellular Uptake

The cellular membrane presents a formidable barrier to drug uptake. In order to exhibit passive diffusion into the cell, drugs must be polar so as to facilitate distribution into the aqueous cellular environment, yet not so polar as to prevent diffusion across the hydrophobic interior of the cellular membrane. In addition, other physical drug characteristics (e.g. molecular weight > ~700 Da) can limit bioavailability. Promising drugs that do not possess the requisite characteristics for passive cellular entry can instead be delivered by novel techniques. For instance, a highly lipophilic drug might be delivered by packaging in liposomes, or a very polar drug might be functionalized with a lipophilic moiety. Similarly, certain large polycationic homopolymers of lysine [26], ornithine [27], and arginine [27] (between 4 and 200 kDa in weight) have been shown to facilitate membrane translocation in cells, and can be covalently ligated to biomolecules to promote cellular entry. However, these large polycations can be toxic *in vivo*, difficult to produce, and expensive.

1.4.1 Peptoid Mimics of HIV-Tat Protein

Alternatively, one interesting drug delivery technique exploits the active transport of certain naturally-occurring and relatively small biomacromolecules across the cellular membrane. For instance, the nuclear transcription activator protein (Tat) from HIV type 1 (HIV-1) is a 101-amino acid protein that must interact with a 59-base RNA stem–loop structure, called the *trans*-activation region (Tar) at the 5′ end of all nascent HIV-1 mRNA molecules, in order for the virus to replicate. HIV-Tat is actively transported across the cell membrane, and localizes to the nucleus [28]. It has been found that the arginine-rich Tar-binding region of the Tat protein, residues 49–57 (Tat_{49-57}), is primarily responsible for this translocation activity [29].

Recently, there has been significant interest in peptidomimetic forms of Tat_{49-57}, not only because of its membrane translocation activity, but as a means of treating HIV infection [1]. Several peptoids, similar in sequence to Tat_{49-57}, have been synthesized with the intention of preventing the HIV-Tat/Tar interaction, and thus preventing HIV replication [24, 25, 30, 31]. However, only recently has this class of peptoids been applied to membrane translocation and drug delivery applications.

Short peptoid-based Tat_{49-57} analogs are more advantageous drug delivery vehicles than large polycationic homopolymers of lysine, ornithine, and arginine. Not only are peptoids more readily synthesized and potentially bioavailable than such large polymers (Section 1.2), they are less likely to be proteolytically degraded [5] or to cause an immunological response than are peptide-based analogs.

Wender and colleagues investigated various truncated and alanine-substituted fluorescently labeled peptoid analogs of Tat_{49-57} in order to determine the requisite structural features for membrane translocation activity in Jurkat cells [32]. They determined that the presence of at least six arginine residues (guanidino moieties) was critical for rapid cell entry, while no specific charge or structural element was important. Correspondingly, they synthesized a series of fluorescently-labeled oligoguanidine peptoids, and compared their capacity for cellular uptake in Jurkat cells with peptide D-arginine oligomers between five and nine residues in length. They identified one compound, *N*-hxg9, which was superior to a D-arginine nonamer in cellular uptake, which in turn was about 100-fold more rapidly translocated across the cell membrane than a fluorescently-labeled Tat_{49-57}.

1.4.2 Cellular Delivery of Nucleic Acids

Peptoids have also shown great utility in their ability to complex with and deliver nucleic acids to cells, a critical step toward the development of antisense drugs, DNA vaccines, or gene-based therapeutics. Most non-viral nucleic acid delivery systems are based on cationic molecules that can form complexes with the polyan-

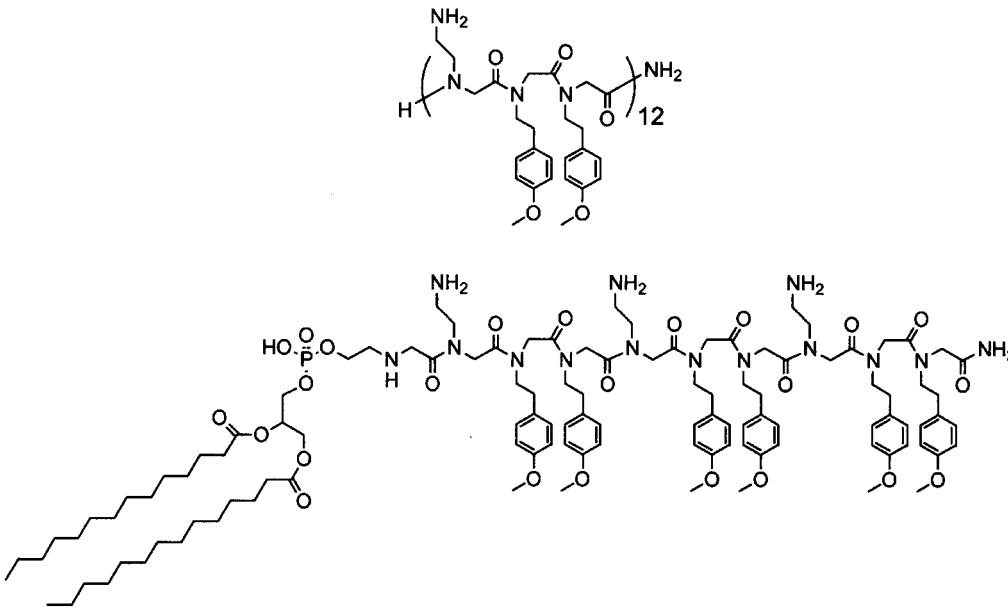


Fig. 1.5 Structure of a 36mer peptoid and the corresponding "lipitoid"

ionic nucleic acid [33]. These cationic materials are typically either polymeric or lipid-based structures, whose performance depends on a balance of factors including overall size, type of cation, density of charge, hydrophobicity, and solubility.

Peptoids are ideally suited to this task because their primary and secondary structures can be precisely predicted and controlled through their sequence. Combinatorial libraries of cationic peptoids were synthesized and evaluated for their ability to condense, protect, and deliver plasmid DNA to cells in culture [6]. An effective, 36mer compound was discovered that contained a repeating cationic trimer motif: cationic-hydrophobic-hydrophobic (Fig. 1.5). In an effort to refine the activity of this compound, solid-phase chemistry was developed to rapidly conjugate lipid moieties to the *N*-termini of peptoids. These cationic peptoid-lipid conjugates (called "lipitoids") were substantially more active in delivering plasmid DNA to cells, and also showed reduced cellular toxicity relative to the lead compound [34]. The most active lipitoids are composed of a natural phosphatidyl ethanolamine lipid conjugated to a 9mer with the same trimeric motif.

Although *in vivo* delivery studies using these reagents are still an early stage, high *in vitro* activity and low toxicity make lipitoids ideal transfection reagents. Recent work has shown that very small structural changes in the lipitoid can result in molecules that efficiently deliver antisense oligonucleotides and RNA.

1.5
Peptoid Mimics of Peptide Ligands

One straightforward approach to the design of biologically-active peptoid sequences is the systematic modification of an active peptide target sequence. For example, constituent amino acids of a target peptide may be substituted by peptoid residues with identical side chains. This modification results in a peptoid oligomer with side chains that are shifted relative to their original positions in the peptide template. An alternative approach is to generate a library of compounds in which site-specific substitutions are made using a diverse set of peptoid monomers, followed by screening to identify the most active compounds. In these methods, the partial substitution of amino acids by peptoid residues generates peptide/peptoid hybrids, which should ideally possess improved pharmokinetic and/or binding properties.

Characterization of peptoid-containing analogs of peptide ligands can provide valuable information. For example, this process can help to elucidate the position of critical residues in the protein target that provide important binding determinants. It can identify molecules with enhanced activity relative to the starting structure and/or with enhanced specificity to a protein target. Finally, it can identify active species with peptoid monomer substitution levels sufficient to grant significantly improved protease stability relative to the original peptide of interest.

The utility of peptoid/peptide hybrids was exemplified by a study of hybrid ligands to proteins containing the Src homology 3 (SH3) domain [35, 36]. As part of their signal transduction activity, these proteins recognize peptides with a PxxP motif (where P = proline, and x = any other amino acid). In general, peptide ligand binding to SH3 domains occurs with low affinity and low specificity. Peptide sequences derived from wild-type protein partners exhibit significant binding to the large family of proteins with SH3 domains. Nguyen *et al.* conducted studies aimed at an improved understanding of the role of proline residues in the core PxxP motif at the binding interface. Previous structural studies had suggested that recognition requires the presence of an *N*-substituted amino acid at proline positions, but that proline itself was not specifically required. Peptide/peptoid hybrids containing a variety of different peptoid substitutions for proline in the core PxxP region were synthesized and evaluated for binding to various proteins containing SH3 domains. It was found that single-site substitutions can have a strong effect on binding affinity. For instance, a hybrid oligomer bearing a dimethoxybenyzl *N*-substitution showed a 100-fold increase in binding affinity over the wild-type peptide (to a K_D of 30 nm), accompanied by a dramatic gain in specificity for binding to the SH3 domain of the protein *N*-Grb2 relative to those of Src and Crk. Multiple substitutions were also well tolerated. For example, an analog of a peptide with partial specificity for Crk was synthesized, in which both proline residues in the PxxP motif were replaced by peptoid monomers. The new hybrid retained strong binding to Crk, but no longer recognized Src or *N*-Grb2 [35]. The Goodman group has similarly investigated the effect of a variety of peptoid substitutions at the proline position in a cyclic hexapeptide analog of somatostatin, cyclo-

[Phe-Pro-Phe-D-Trp-Lys-Thr]. This study also yielded compounds with an enhanced binding selectivity, in this case, for the hsst2 receptor [37].

An alternative approach involved the use of a "peptoid scan" strategy to identify peptoid–peptide hybrids that bind to the Src homology 2 (SH2) domain of Syk tyrosine kinase. Ruitjenbeek *et al.* investigated hybrid oligomers in which conserved side chains of the Syk ligand Ac-pTyr-Glu-Thr-Leu-NH_2 were shifted sequentially to the corresponding *N*-pendant peptoid positions [38]. They found that peptoid substituents were accommodated at one or both of the Thr and Leu positions with less than 10-fold loss of inhibitory activity and binding affinity but that substitutions at other positions eliminated activity. These results were ascribed to the alteration of distances between side chains and/or the loss of hydrogen-bond contacts between the *N*-terminal region of the ligand and the protein target.

The Liskamp group also examined the ability of peptoid–peptide hybrids to be bound by the MHC Class II receptor, an important component of the human immune system [39]. Two of three peptoid substitutions in the 14-residue peptide caused substantial decreases in binding affinity, despite the fact that these were solvent-exposed residues. These results were attributed to a loss of hydrogen-bond contacts as well as to steric clashes caused by unfavorable positioning of the new side chain groups.

The Kessler group has generated side chain-shifted peptoid substitutions within peptidic antagonists of the $\alpha 4\beta 7$ integrin interaction with the mucosal addressin cell adhesion molecule 1 (MAdCAM-1) [40]. Peptoid substitutions within the core Leu-Asp-Thr sequence of the peptide abolished activity in a cell adhesion assay. However, it was possible to substitute the phenylalanine in the cyclo(Phe-Leu-Asp-Thr-Asp-D-Pro) peptide with analogous peptoid and azapeptide monomers, with some retention of activity. The extreme sensitivity of activity to substitutions in the core sequence was attributed to a need to maintain a precise spacing between side chains, and to the importance of the peptide backbone, in this case, in providing productive binding interactions.

An evaluation of the several attempts to find peptoid analogs for wild-type peptide ligands suggests that it is possible, in some cases, to identify specific positions amenable to peptoid substitution. In particular, sites at which binding occurs through recognition of proline residues and in which hydrogen bonding does not play a strong role may be particularly suitable for substitution by peptoid residues. However, it may be challenging to fully replace protein-binding peptide sequences entirely by peptoids. Difficulties with the "translation" of peptides into peptoid sequences may arise due to some of the following reasons:

1. In cases where the presentation of side-chain functionalities and backbone hydrogen bonding partners with a precise spacing and geometry are essential for optimal binding interactions in the wild-type peptide, peptoids will exhibit reduced binding efficacy due to the lack of amide NH groups and the modified distances between side chains and adjacent carbonyls. Some attempts have been made to address this issue by the synthesis of retropeptoid sequences, with mixed results [7].

2. If peptide residues are converted to peptoid residues, the conformational heterogeneity of the polymer backbone is likely to increase due to *cis/trans* isomerization at amide bonds. This will lead to an enhanced loss of conformational entropy upon peptoid/protein association, which could adversely affect binding thermodynamics. A potential solution is the judicious placement of bulky peptoid side chains that constrain backbone dihedral angles.
3. If the individual residues of a peptide are transformed into the corresponding peptoid monomers to make hybrid oligomers, there will be a perturbation in the distance between side chains at the boundary between oligomer types. That is, spacing of side chains at a peptoid-peptide linkage will be different from that between either two peptide or peptoid residues.

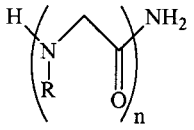
1.6 Peptoids with Folded Structure

One of the most intriguing features of peptoids is their capacity to adopt stable, helical conformations in a variety of solvent media. The helix is an important component of folded protein structure. Additionally, helical polypeptides have important roles *in vivo* (e.g. in certain ligand-binding interactions and in the transmembrane regions of membrane-bound proteins). As such, similar peptoid structures may be valuable tools for drug development. Due to their ability to adopt folded structure, peptoids are among a select class of sequence-specific peptidomimetic materials, referred to as "foldamers" [41], which can be designed to adopt specific backbone conformations. This section describes early work in the discovery and characterization of helical peptoid structures. The following section (Section 1.7) then details some recent applications of these peptoid helices.

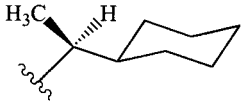
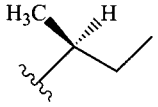
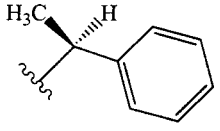
1.6.1 Restricting Conformational Space

The inherently achiral nature of the oligo-(*N*-substituted) glycine backbone enables peptoid oligomers with achiral, unbranched side chains to sample a large conformational space. However, experimental work has shown that the incorporation of side chains with branched carbons immediately adjacent to the main-chain nitrogen to which they are appended (α-branched) allows the formation of helices with *cis*-amide bonds [4]. Modeling of a peptoid octamer with this type of side chain confirmed that substitution with bulky, chiral groups significantly reduces the energetically accessible conformational space [3]. As *N*-substituted glycines are incapable of backbone hydrogen bonding, structures in these conformationally restricted peptoids were instead predicted to be stabilized by a combination of side chain–backbone steric repulsions and dipole–dipole repulsions between main

Tab. 1.2 Examples of chiral α-methyl *N*-substituted glycine side chains



N-substituted glycine oligomer, or *peptoid*

R=Side chain	*Designator*
	*N*sch=(S)-*N*-(1-cyclohexylethyl)glycine
	*N*ssb=(S)-*N*-(2-butyl)glycine)
	*N*spe=(S)-*N*-(1-phenylethyl)glycine

chain amide carbonyl electrons and, for specific peptoid sequences, aromatic side chains [3]. It was later determined that the incorporation of bulky, chiral, α-methyl side chains can provide significant conformational restriction without compromising synthetic yields (Tab. 1.2) [4].

1.6.2 Peptoid Helices

1.6.2.1 CD and NMR Studies of a Helical Peptoid Pentamer with α-Chiral Aromatic Side Chains

There have been several studies that investigated, by a variety of techniques, the folding behavior of peptoids with diverse chiral, α-branched side chains (Tab. 1.2) [4, 42]. Peptoids as short as five monomers in length, substituted with chiral aromatic residues, exhibit circular dichroism (CD) spectra indicative of chiral secondary structure, with spectral features similar to those of peptide α-helices [42]. Structural handedness is dictated by the choice of side-chain enantiomer, such that peptoids containing side chains of opposing handedness exhibit mirror-image CD (Fig. 1.6) [4, 43]. The CD spectra of homooligomeric peptoid sequences based on the α-chiral, aromatic monomer (S)-*N*-(1-phenylethyl)glycine are particularly intense, with a maximum near 190 nm and double minima near 204 and 218 nm

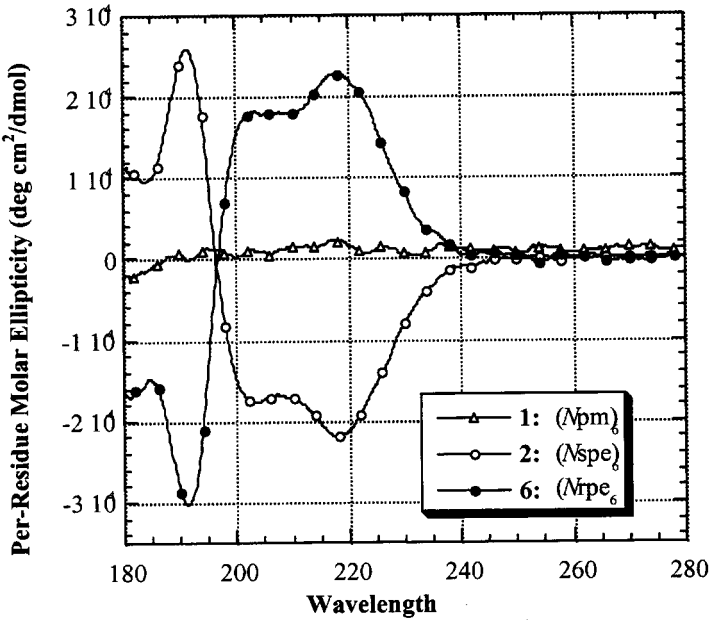


Fig. 1.6 A comparison of the CD spectra of oligopeptoids with achiral *N*pm side chains (1) and with α-chiral, aromatic sidechains of S and R chirality (2 and 6, respectively). Sample concentration was ~60 μM in acetonitrile. Spectra were acquired at room temperature. *N*pm = (*N*-[1-phenylmethyl]glycine); *N*spe = (S)-*N*-(1-phenylethyl)glycine; *N*rpe = (R)-*N*-(1-phenylethyl)glycine

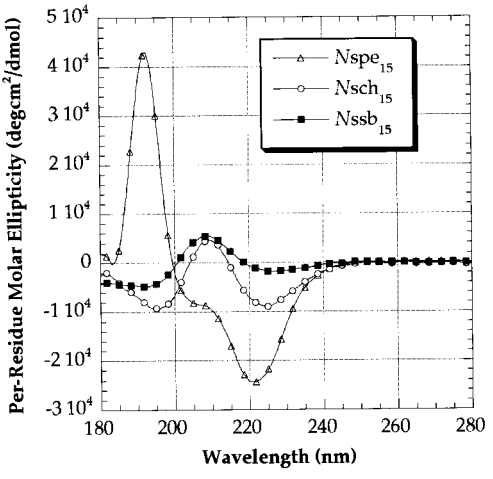


Fig. 1.7 Illustration of the distinct CD exhibited by peptoid helices containing solely aromatic or solely aliphatic residues. Sample concentrations were ~60 μM in acetonitrile. Spectra were acquired at room temperature. *N*spe = (S)-*N*-(1-phenylethyl)glycine; *N*sch = (S)-*N*-(1-cyclohexylethyl)glycine; *N*ssb = (S)-*N*-(*sec*-butyl)glycine

[4, 42, 44] (Figs. 1.6 and 1.7). This type of CD spectrum is observed for certain heterooligomeric peptoid sequences with as few as 33% chiral aromatic residues, in both aqueous and polar organic solvent (acetonitrile, methanol).

A peptoid pentamer of five *para*-substituted (S)-*N*-(1-phenylethyl)glycine monomers, which exhibits the characteristic α-helix-like CD spectrum described above, was further analyzed by 2D-NMR [42]. Although this pentamer has a dynamic structure and adopts a family of conformations in methanol solution, 50–60% of the population exists as a right-handed helical conformer, containing all *cis*-amide bonds (in agreement with modeling studies [3]), with about three residues per turn and a pitch of $\sim$6 Å. Minor families of conformational isomers arise from *cis*/*trans*-amide bond isomerization. Since many peptoid sequences with chiral aromatic side chains share similar CD characteristics with this helical pentamer, the type of CD spectrum described above can be considered to be indicative of the formation of this class of peptoid helix in general.

1.6.2.2 CD Studies of Longer Peptoid Helices Containing α-Chiral Aromatic Side Chains

A more comprehensive folding study by CD and NMR that investigated aromatic side chain-containing peptoids in acetonitrile solution was carried out subsequent to the NMR structural studies described above. In particular, the effect of chain length on structure in homooligomers of (R)-*N*-(1-phenylethyl)glycine (*N*rpe) [44] and the effect of sequence on structure in a variety of more than 30 heterooligomeric peptoids were studied in acetonitrile solution [45]. CD spectra of homooligomers of *N*rpe display helical characteristics at lengths as short as five residues, in agreement with the modeling results of Armand and co-workers [3] and prior observations described above [4]. The CD spectral intensity initially diminishes as peptoid chain length increases from five to about eight residues, with spectra passing through an isodichroic point at 196 nm. At a chain length of nine monomers, a unique, non-helical CD spectrum is observed (in acetonitrile solution), which we have since found corresponds to a novel planar cyclic peptoid structure, the details of which are described elsewhere [46]. At the 10-monomer length, the CD spectrum is again characteristically helical, and gradually intensifies with increasing chain length. At a length of 13 residues and longer, the spectrum is fully developed, and exhibits no further intensification or shape change as the chain is further lengthened through 20 monomers [44].

General sequence requirements for helix formation in heterooligomeric peptoids [45] have also been determined. Peptoids ranging in length from six to 24 residues and containing mixed chiral and non-chiral aromatic and aliphatic side chains were synthesized and studied by CD and 2D-NMR. Although the prior studies of Kirshenbaum and colleagues [4] suggested that helical sequences contained at least two-thirds α-chiral residues, with at least one-third also being α-chiral aromatic, Wu *et al.* endeavored to determine sequence requirements for helix formation in peptoids with non-periodic sequences. They found peptoids which include achiral residues form stable helices in sequences as short as six re-

sidues if at least 50% α-chiral aromatic residues are incorporated. In peptoids shorter than about 12–15 residues, helix formation is also promoted by incorporation of an α-chiral aromatic residue at the peptoid C-terminus, as well as by inclusion of an aromatic helical face (patterned via three-fold sequence periodicity) and a carboxyamide C-terminal moiety. In longer peptoids (>12–15 residues), residue placement effects seem less important, although the percentage of α-chiral aromatic side chains required for helicity remains unchanged.

In general, peptoid helical structure is thermally stable in both organic solvent and in aqueous solution [4, 44, 47]. Complete thermal unfolding of peptoid helices has so far not been observed [44, 47]. However, studies of several different peptoid oligomers have shown a diminishing CD signal with increasing temperature, with spectra passing through one or more isodichroic points. These observations indicate that temperature can be used to induce partial melting of repeating peptoid secondary structure. Although it was reported that a water-soluble peptoid 30mer underwent a cooperative and reversible unfolding transition due to combined thermal and pH effects [4], we found with further studies of this molecule that the observed phase transition was complicated by a solubility-to-insolubility, LCST-type phase transition (unpublished results).

The thermal and solvent stability of aromatic side chain-containing peptoid helices was investigated in more detail by Sanborn and colleagues using CD in a heterooligomeric 36mer sequence, containing one-third (S)-*N*-(1-phenylethyl)glycine (*N*spe) residues and two-thirds chiral, anionic aliphatic (S)-*N*-(1-carboxyethyl)-glycine (*N*sce) residues [47]. Aromatic side chains were patterned with 3-fold periodicity in this sequence. This peptoid displayed the concentration-independent α-helix-like CD spectrum that is characteristic of the aromatic side chain-containing peptoid helices previously described by Kirshenbaum *et al.* [4] and Wu *et al.* [44, 45]. Sanborn *et al.* found that the folded structure of this peptoid 36mer, as reported by CD, was remarkably robust. The helical CD signal is invariant over an increase of three orders of magnitude in buffer concentration, and is stable to treatment with 8 M urea and to heating through 75 °C (separately, and in combination). The extraordinary resilience of this peptoid helix to unfolding agrees with the dominance of steric forces in helix stabilization, consistent with prior hypotheses [3]. Correspondingly, the CD signal of this 36mer peptoid is also largely invariant with solvent (water, acetonitrile, and 20 mol% aqueous 2,2,2-trifluoroethanol), showing modest intensification of the CD signal in aqueous TFE (in a manner analogous to polypeptides [48]).

1.6.2.3 Structural Studies of Peptoids with Aliphatic Side Chains by CD, NMR, and X-ray Crystallography

Peptoids based on α-chiral aliphatic side chains can form stable helices as well [43]. A crystal of a pentameric peptoid homooligomer composed of homochiral *N*-(1-cyclohexylethyl)glycine residues was grown by slow evaporation from methanol solution, and its structure determined by X-ray crystallographic methods. In the crystalline state, this pentamer adopts a helical conformation with repeating *cis*-

amide bonds, a periodicity of ~3 residues per turn, and a pitch of ~6.7 Å. Longer peptoid homooligomers of *N*-(1-cyclohexylethyl)glycine (12–15 residues) give rise to fully developed and intense CD signals, distinct from those obtained from aromatic side chain-containing peptoids (Fig. 1.7). In contrast, the CD spectrum of this aliphatic peptoid is strongly reminiscent of the spectrum of a peptide polyproline type-I helix, and contains a distinct maximum at 210 nm and two shallow minima at 200 and 225 nm. 2D-NMR studies of 6, 9, 12, and 15mer peptoid homo-oligomers of *N*-(1-cyclohexylethyl)glycine were also undertaken [43]. When considered jointly, the highly degenerate NMR spectra (which are increasingly degenerate at longer chain lengths), CD spectra that intensify as peptoid chain length increases, along with the helical crystal structure described above, corroborate the adoption of a repeating helical structure similar to that observed in the *N*-(1-phenylethyl)glycine pentamer previously studied by 2D-NMR [42]. These NMR spectra indicate that there are two major families of conformers present in solution, specifically *cis–trans* isomers of the backbone amide bonds present in a 2.6 : 1 ratio respectively, in acetonitrile solution.

1.6.2.4 Summary

Appropriately substituted peptoids longer than about five residues can form highly stable helical structures. Sequences that contain at least 50% *α*-chiral aromatic residues can form stable helices, the handedness of which may be controlled by choice of side chain enantiomer. The extent of helical structure increases as chain length grows, and for these oligomers becomes fully developed at a length of approximately 13 residues. Aromatic side chain-containing peptoid helices generally give rise to CD spectra that are strongly reminiscent of that of a peptide *α*-helix, while peptoid helices based on aliphatic groups give rise to a CD spectrum that resembles the polyproline type-I helical CD. Despite the differences in CD spectra, both peptoid types form the same type of helix, which is structurally similar to the type-I polyproline helix. As peptoid helices are stabilized predominantly by steric repulsions, their structure is remarkably stable to thermal, ionic, and chaotropic destabilizing influences.

1.6.3 Protein-mimetic Structures

Proteins derive their powerful and diverse capacity for molecular recognition and catalysis from their ability to fold into defined secondary and tertiary structures and display specific functional groups at precise locations in space. Functional protein domains are typically 50–200 residues in length and utilize a specific sequence of side chains to encode folded structures that have a compact hydrophobic core and a hydrophilic surface. Mimicry of protein structure and function by non-natural oligomers such as peptoids will not only require the synthesis of >50mers with a variety of side chains, but will also require these non-natural sequences to adopt, in water, tertiary structures that are rich in secondary structure.

In order for folded helices to assemble into tertiary structures in water, they need to be amphipathic (e.g. where one helical face is hydrophobic and the other is hydrophilic). Because the first helical peptoids contained very hydrophobic chiral residues, ways to increase the water solubility and side-chain diversity of the helix-inducing residues were investigated [49]. It was found that a series of side chains with chiral-substituted carboxamides in place of the aromatic group could still favor helix formation, while dramatically increasing water solubility.

Because there is little precedent for the *de novo* design and synthesis of a completely synthetic macromolecule of defined structure [50], a combinatorial synthesis and screening process was used to identify sequences that could adopt tertiary structures [14]. Rather than attempt to synthesize a continuous single chain, discrete single amphipathic 15mer oligomers were made and tested for their ability to assemble into defined multimers. This was accomplished in a high-throughput mode by adding a dye molecule that only fluoresces when bound in a hydrophobic environment – such as a folded protein core. A library of 3400 water-soluble amphiphilic helices was screened for dye binding and a small number of 15mers were discovered that formed defined helical assemblies as judged by size-exclusion chromatography, circular dichroism and analytical ultracentrifugation [14]. This is a significant initial step toward the synthesis of an artificial protein, but in itself does not yet represent true tertiary structure. Future work will investigate the formation of such bundles in longer, contiguous peptoid oligomers.

1.7
Biomimetic Peptoid Structures for Therapeutic Applications

The well-defined helical structure associated with appropriately substituted peptoid oligomers (Section 1.6) can be employed to fashion compounds that closely mimic the structure and function of certain bioactive peptides. There are many examples of small helical peptides (<100 residues) whose mimicry by non-natural oligomers could potentially yield valuable therapeutic and bioactive compounds. This section describes peptoids that have been rationally designed as mimics of antibacterial peptides, lung surfactant proteins, and collagen proteins. Mimics of HIV-Tat protein, although relevant to this discussion, were described previously in this chapter (Sections 1.3.2 and 1.4.1).

1.7.1
Peptoid Mimics of Antibacterial Peptides

The magainins are a class of linear, cationic, facially amphipathic and helical antibacterial peptides derived from frog skin [51]. The magainins exhibit highly selective and potent antimicrobial activity against a broad spectrum of organisms [52, 53]. As these peptides are facially amphipathic, the magainins have a cationic heli-

cal face (at physiological pH), composed of mostly lysine residues, as well as hydrophobic aromatic (phenylalanine) and hydrophobic aliphatic (mostly valine, leucine, and isoleucine) helical faces. It has been shown that the structure and physicochemical properties of the magainins, rather than any specific receptor–ligand interactions, are responsible for their activity [54].

As such, the magainins provide a useful initial target for peptoid-based peptidomimetic efforts. Since the helical structure and sequence patterning of these peptides seem primarily responsible for their antibacterial activity and specificity, it is conceivable that an appropriately designed, non-peptide helix should be capable of these same activities. As previously described (Section 1.6.2), peptoids have been shown to form remarkably stable helices, with physical characteristics similar to those of peptide polyproline type-I helices (e.g. *cis*-amide bonds, three residues per helical turn, and ~6 Å pitch). A facially amphipathic peptoid helix design, based on the magainin structural motif, would therefore incorporate cationic residues, hydrophobic aromatic residues, and hydrophobic aliphatic residues with three-fold sequence periodicity.

A series of peptoid magainin mimics with this type of three-residue periodic sequence, has been synthesized [55]. Each was purified by reversed-phase HPLC to >97%, and their minimum inhibitory concentrations (MIC) determined (using broth-dilution techniques) against both *E. coli* JM109 and *B. subtilis* BR151 in Luria Broth medium (Tab. 1.3). The peptoids in Tab. 1.3 have been arranged in order of increasing hydrophobicity, as determined from reversed-phase HPLC retention times. The T2 and T3 sequence motifs, at lengths between 12 and 17 residues, are the most effective antibacterial compounds, with low micromolar and even submicromolar MIC values, similar to those of magainin-2 amide. In all cases, peptoids are individually more active against the Gram-positive species.

Tab. 1.3 Magainin-mimetic peptoid sequences, and antibacterial and hemolytic activities

Peptoid	*Sequence*	*MIC (μM)*		*% hemolysis*
		E. coli JM109	*B. subtilis BR151*	*At E. coli JM109 MIC*
T1-17	H-*N*ssb-*N*ssb-(*N*Lys-*N*ssb-*N*ssb)$_5$-NH_2	>100	>100	0%
T2-6	H-(*N*Lys-*N*ssb-*N*spe)$_2$-NH_2	>487	>730	0%
T2-9	H-(*N*Lys-*N*ssb-*N*spe)$_3$-NH_2	218	55–82	0%
T2-12	H-(*N*Lys-*N*ssb-*N*spe)$_4$-NH_2	49	7.8	0%
T2-15	H-(*N*Lys-*N*ssb-*N*spe)$_5$-NH_2	9.9	4.4	0%
T2-17	H-*N*spe-*N*spe-(*N*Lys-*N*ssb-*N*spe)$_5$-NH_2	19	1.4	1.2%
T3-12	H-(*N*Lys-*N*spe-*N*spe)$_4$-NH_2	9.9	0.82	1.4%
T3-17	H-*N*spe-*N*spe-(*N*Lys-*N*spe-*N*spe)$_5$-NH_2	7.7	1.2	51%
T4-17	H-*N*sch-*N*sch-(*N*Lys-*N*sch-*N*sch)$_5$-NH_2	>75	>75	100%

*N*ssb = (S)-*N*-(sec-butyl)glycine; *N*spe = (S)-*N*-(1-phenylethyl)glycine; *N*sch = (S)-*N*-(1-cyclohexylethyl)glycine; *N*Lys = *N*-(4-aminobutyl)glycine.

Certain of these peptoid antibiotics are also selective for bacterial, rather than mammalian, cells. The selectivity of these peptoids has been measured in terms of their capacity to cause hemolysis of human erythrocytes at or near their MIC (Tab. 1.3). Interestingly, the amount of hemolysis induced by these peptoids correlates well with their hydrophobicity, as there is an increasing extent of hemolysis as molecular hydrophobicity increases. These results suggest that highly hydrophobic compounds of this class are poorly selective antibiotics. The most active antibacterial peptoids, T2-15 and T3-12, have quite low hemolytic activity near their MICs. Although highly antibacterial *in vitro*, T3-17 is also very hemolytic at its MIC value.

All of these water-soluble peptoids were designed to be helical, based on the loose guidelines provided by previous studies (Section 1.6.2). In some cases, though, these were novel sequences whose helical extent could not be predicted. Therefore, circular dichroism spectroscopy was used to characterize the structure of these oligomers in solution. In aqueous Tris-HCl buffer (pH 7.0), the most effective peptoids (T2 and T3 between 12 and 17 residues long) exhibit a spectrum characteristic of the peptoid helix, with a single ellipticity maximum near 190 nm, and double ellipticity minima near 202 and 220 nm. Interestingly, ineffective antibacterial compounds T1-17 and T4-17 both exhibit a random coil-like CD in both aqueous buffer and hydrophobic solvent environments [55]. We also find that selective (non-hemolytic) and effective antibacterial peptoids exhibit intense, helical CD in bacterial-mimetic vesicles (composed of 70 mol% POPE : 30 mol% POPG), but show relatively weak CD in mammalian red blood cell-mimetic DMPC vesicles, nearly indistinguishable from CD in aqueous buffer. On the other hand, non-selective (hemolytic) antibacterial peptoids exhibit strong CD intensification in both types of vesicles, as compared to CD in buffer [55].

In summary, these recently obtained results demonstrate that certain amphipathic peptoid sequences designed to mimic both the helical structure and approximate length of magainin helices are also capable of selective and biomimetic antibacterial activity. These antibacterial peptoids are helical in both aqueous buffer and in the presence of lipid vesicles. Ineffective (non-antibacterial) peptoids exhibit weak, random coil-like CD, with no spectral intensification in the presence of lipid vesicles. Selective peptoids exhibit stronger CD signals in bacterial-mimetic vesicles than in mammalian-mimetic vesicles. Non-selective peptoids exhibit intensely helical CD in both types of vesicles.

1.7.2
Peptoid-Based Mimics of Lung Surfactant Proteins

There is a clinical need for non-natural, functional mimics of the lung surfactant (LS) proteins B and C (SP-B and SP-C), which could be used in a biomimetic LS replacement to treat respiratory distress syndrome (RDS) in premature infants [56]. An effective surfactant replacement must meet the following performance requirements: (i) rapid adsorption to the air–liquid interface, (ii) re-spreadability

upon multiple, successive surface compressions, and (iii) attainment of near-zero surface tension upon compression [57]. Currently, neonates suffering from RDS are treated with bovine-derived surfactant. However, these surfactant preparations are expensive and may contain viruses or other hazards, which raises concerns regarding the safety of their clinical administration. Entirely synthetic surfactants, which do not contain SP-B and SP-C, currently do not meet the performance requirements outlined above [58]. As an alternative, in recent work peptoids have been used to create functional mimics of both SP-B and SP-C with the eventual goal of creating better synthetic LS replacements [59].

SP-C is a helical, cationic, amphipathic protein just 35 amino acids in length. Its structure is relatively simple, containing a hydrophobic α-helix 37 Å long, capped by two adjacent, positively-charged residues, and a relatively unstructured amino terminus [60, 61]. The valyl-rich helix of SP-C inserts into lipid monolayers and bilayers and interacts with hydrophobic alkyl tails, while cationic side chains interact with polar lipid head groups. This ionic interaction is thought to bind SP-C to the lipid monolayer [60, 62]. In contrast, SP-B has a more complex structure, which is also predominantly helical, but with numerous intra-chain disulfide bonds. *In vivo*, the SP-B protein exists as a homodimer via an additional disulfide linkage. However, a short, helical segment from the *N*-terminus of one monomer ($SP\text{-}B_{1-25}$) seems to retain much of the biologically relevant surface activity of the full-length synthetic peptide [63, 64]. CD studies show that $SP\text{-}B_{1-25}$ is α-helical in structure. NMR studies, as well as modeling and sequence analysis, predict that this helix has both cationic and non-polar helical faces [65, 66].

Recently, peptoid-based mimics of both SP-C and SP-B have been designed to adopt helical secondary structures, and also mimic (to varying degrees) the sequence patterning of hydrophobic and polar residues found in the natural surfactant proteins. Peptoid-based SP-C mimics of up to 22 monomers in length, were synthesized and characterized by *in vitro* experimental methods [67, 68] (Fig. 1.8). The secondary structure of all molecules was assessed by circular dichroism and found to be helical. The surface activities of these peptoids, in comparison to the actual SP peptides described above, were characterized by surfactometry using

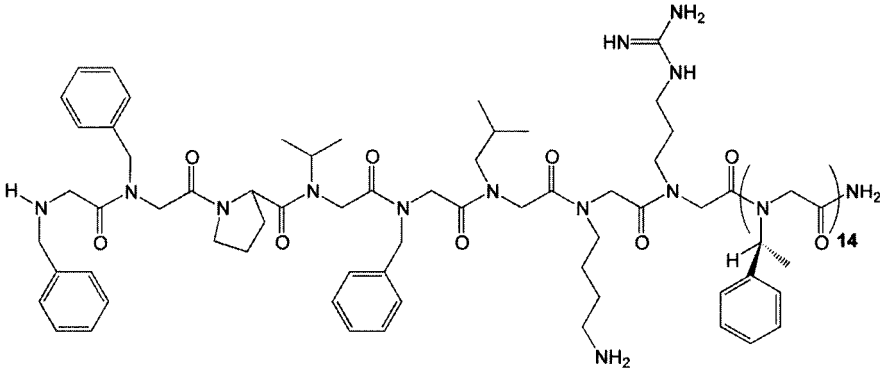


Fig. 1.8 Structure of a peptoid mimic of $SP\text{-}C_{5-32}$

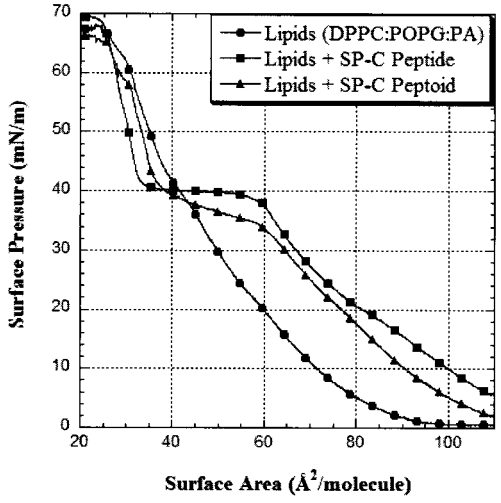


Fig. 1.9 Surface pressure (π)–area (A) isotherms obtained for a lipid mixture (DPPC:POPG:PA, 68:22:9 (by weight)), alone and with 10% (w/w) of either SP-C peptide or SP-C peptoid added. Results indicate that the addition of the SP-C mimics engenders biomimetic surface activity, as indicated by lift-off at a higher molecular area and the introduction of a plateau

both a Langmuir–Wilhelmy surface balance (LWSB) in conjunction with fluorescence microscopy (FM), and a pulsating bubble surfactometer (PBS).

When added to a biomimetic lipid mixture (DPPC:POPG:PA, 68:22:9, which behaves similarly to the natural lipid mixture found in pulmonary surfactant), a variety of peptoid SP analogs show biomimetic surface activity and a substantial improvement over that which is observed for a lipid film without SP-mimics. Surfactometry results in general, show peptoid SP-mimics behave similarly to the natural SP-C peptide and the SP-B_{1-25} mimic. Specifically, LWSB experiments reveal high lift-off surface areas (i.e. rapid surface adsorption) and high film-collapse surface pressures, as well as a plateau in the π-A isotherm that is characteristic of natural LS (Fig. 1.9). Surface phase morphology, observed by LWSB in conjunction with FM, shows that the SP-mimics cause biomimetic fluidization of the surface film at high compression. PBS studies reveal dynamic surface activity similar to that of the analogous α-peptides, specifically an increased rate of surface adsorption and decreased minimum and maximum surface tensions at the minimum and maximum bubble surface areas, respectively. Hence, peptoid mimics of the surfactant proteins seem to be promising as biostable replacements for SP-B and SP-C, and may prove useful in the development of novel therapies for the treatment of respiratory distress. They also provide an example, similar to the magainin-like peptoids, of structural mimicry enabling good recapitulation of function in a membrane-interactive oligomer.

1.7.3
Collagen-based Structures Containing Peptoid Residues

Collagen is the major protein component of connective tissue and constitutes approximately 25% of the total protein content in humans. There are more than 19

different types of collagen known to exist. The most abundant type, Type I, is composed of ~300 repeats of a three-amino acid sequence, (Gly-Xaa-Yaa), in which Xaa and Yaa are most commonly proline (Pro) and hydroxyproline (Hyp) respectively. In the native conformation, three of these long peptide chains adopt left-handed polyproline type-II-like helices, which are in turn twisted about one another into a right-handed helical superstructure. This helix, created by entwining three individual helices together, is called the collagen triple helix. These triple helices are further organized into fibrils, which provide collagen with its mechanical strength [69].

Collagen-like molecules have been synthesized which incorporate peptoid residues and which are capable of forming a triple helix structure. These peptoid-peptide chimerae are hypothesized to be more biostable and more resistant to enzymatic proteolytic degradation than natural collagen. Such resilient and durable collagen constructs would conceivably find numerous applications as biomaterials. Toward this end, Goodman and co-workers have published several reports in which they have investigated substitution of either or both Xaa and Yaa positions within the repetitive trimeric collagen sequence by various peptoid residues.

Initially, Goodman's group studied structures with the repetitive trimeric sequence (Gly-Pro-*N*leu)$_n$ (n=1, 3, 6, 9) in which the *C*-terminus was amidated and the *N*-terminus was either acetylated or covalently ligated to Kemp triacid (KTA), a rigid trivalent template that promotes triple helix formation [70]. Here, *N*leu signifies the *N*-isobutylglycine peptoid residue. Through CD, NMR, and molecular modeling experiments, Goodman and colleagues found that KTA-[Gly-(Gly-Pro-*N*leu)$_6$-NH_2]$_3$ forms a collagen-like triple helix structure [71]. Moreover, this substitution of *N*leu into the collagen sequence did not seem to significantly reduce helical stability. In fact, KTA-[Gly-(Gly-Pro-*N*leu)$_6$-NH_2]$_3$ has a melting temperature somewhat higher than Ac-(Gly-Pro-Pro)$_{10}$ [72].

Other collagen-like structures that incorporate the repetitive trimeric sequence (Gly-*N*leu-Pro) were found to be even more stable in the triple-helix conformation than analogous (Gly-Pro-*N*leu) structures [73, 74]. Certain "host–guest" structures, in which an achiral (Gly-*N*leu-*N*leu) sequence was incorporated in between collagen-like repeats of (Gly-Pro-Hyp), were synthesized and retained a triple-helix conformation [75]. Additional "host–guest" constructs were created in which the (Gly-*N*leu-*N*leu) sequence was located in between repeats of (Gly-*N*leu-Pro) [76] and were also found to adopt the triple helix conformation. However, these host–guest structures were significantly less stable than analogous constructs composed solely of (Gly-Pro-Hyp) or (Gly-*N*leu-Pro) repeats.

The ability of these peptidomimetic collagen-structures to adopt triple helices portends the development of highly stable biocompatible materials with collagen-like properties. For instance, it has been found that surface-immobilized (Gly-Pro-*N*leu)$_{10}$-Gly-Pro-NH_2 in its triple-helix conformation stimulated attachment and growth of epithelial cells and fibroblasts *in vitro* [77]. As a result, one can easily foresee future implementations of biostable collagen mimics such as these, in tissue engineering and for the fabrication of biomedical devices.

1.8
Obstacles to the Development of Biomedically-useful Peptoids

Numerous barriers remain to be overcome before the promise of biomedically-useful peptoids can be more completely fulfilled. The following sections detail some examples.

1.8.1
Enhance the Diversity of Secondary Structure in Peptoid Foldamers

Although high-resolution structures have been obtained of the polyproline type-I helix formed by certain oligopeptoids, circular dichroism spectra suggest that distinct sequence motifs can form alternative types of secondary structure (Section 1.6). Ongoing studies seek new side chain types that will generate novel, stable folded species. One outstanding challenge in particular is the quest for monomers that will provide for side-chain/main-chain and side-chain/side-chain hydrogen bonding [49].

1.8.2
Improve Understanding of Peptoid Sequence/Structure Relationships

Secondary structure prediction from the primary sequence of polypeptides has improved continuously over the course of more than 30 years. This approach has allowed the *a priori* design of peptides that present chemical functionalities in a predetermined orientation. Structure prediction tools have been pivotal for guiding molecular recognition strategies in peptides, and as we gain a more detailed knowledge of structure types formed by polypeptoids, these tools may also prove to be of similar utility in these non-natural systems. Peptoids have been shown to be amenable to structural prediction techniques, since the polyproline type-I helix formed by peptoid oligomers containing chiral *N*-(1-phenylethyl)glycine residues was predicted by a combined molecular mechanics/quantum mechanical approach [3].

1.8.3
Translate Bioactive Peptide Sequences into Bioactive Peptoid Sequences

There have been notable successes in the replacement of individual peptide residues by peptoid monomers with retention of *in vitro* activity and enhancement of specificity. Unfortunately, attempts to completely transform those bioactive peptides that function via specific peptide–protein binding events into entirely peptoid-based oligomers have so far proven successful only at short chain lengths (e.g. [23]). It remains to be seen whether any general strategy can be developed in

order to address this difficulty, either for longer peptoid oligomers or in other peptidomimetic oligomers. Again, computational tools may prove essential. On the other hand, substantial success has been achieved in the mimicry of membrane-active peptides using peptoid sequences, including peptoid molecular transporters, antimicrobial peptoids, and SP-mimetic peptoids.

1.8.4
Develop Peptoids with Stable Tertiary Structure

The "foldamer" field is still in a juvenile stage regarding the mimicry of diverse peptide secondary structures [78]. Nevertheless, it is not too soon to address challenges in the mimicry of stable protein folds. The ability to design true proteomimetics that incorporate tertiary folds will eventually enable a more sophisticated approach to molecular recognition problems and may ultimately lead to non-natural enzyme-like molecules capable of catalysis and regulation. Preliminary approaches toward non-natural tertiary structure are likely to focus on identifying and exploiting folding forces driven by the solvophobic sequestration of polymer side chains [14]. An important prerequisite will be improving synthetic yields so that polypeptoids of sufficient length to form a solvent-shielded core can be made readily. Although high yields are a characteristic of the solid-phase sub-monomer peptoid synthetic strategy, the method will require further refinement in order to further improve the yield of long chains. Alternatively, employing techniques for concatenating individual peptoid oligomers to form macromolecular species may provide a solution.

1.8.5
Develop Peptoid Shuttles for Intracellular Import of Xenobiotic Agents

Certain polycationic peptoid sequences have been shown to be capable of crossing the cell membrane (Section 1.4). The use of these "lipitoids" as transfection agents that drive the intracellular delivery of DNA and RNA [34] may be extended to other intracellular targets. The efficacy of peptides or xenobiotics that target intracellular species may be greatly enhanced upon conjugation to similar membrane-penetrating polycationic peptoids through covalent or non-covalent linkages.

1.8.6
Optimize Pharmacological Profile of Oligopeptoids

Initial studies on short peptoid oligomers have revealed relatively poor pharmacokinetic properties [18, 79]. Despite the numerous advantageous attributes of peptoids *in vitro*, there are currently no peptoid-based therapeutics. However, a more thorough exploration of peptoid sequences may reveal species with more appropri-

ate characteristics, such as improved oral availability and slower excretion rates. Moreover, peptoid–peptide chimerae may exhibit improved behavior. As the delivery of protein therapeutics by injection has become more common, it may soon be possible to explore similar strategies for the delivery of peptoid therapeutics, mitigating some pharmacokinetic limitations. Helical peptoids for antimicrobial and lung surfactant replacement applications will also be explored for *in vivo* use, which will yield more useful information on the fate of peptoids in biological systems.

1.9 Conclusion

Efforts to investigate the questions posed here will lead to more useful peptoid designs while simultaneously leading to a better fundamental understanding of molecular recognition and sequence/structure/function relationships in non-natural, sequence-specific peptidomimetic oligomers.

1.10 References

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