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2.204. Peptoids: Synthesis, Characterization, and Nanostructures

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Glossary

Antifouling agent An antifouling agent prevents undesirable accumulation of microorganisms on wetted surfaces.

Antimicrobial peptoids Antimicrobial peptoids are designed to mimic antimicrobial peptides. Typical antimicrobial peptoids are cationic and facially amphipathic.

Chemoselectivity Chemoselectivity refers to the selective reactivity of one functional group in the presence of others.

Foldamer A polymer or an oligomer that adopts a secondary structure stabilized by noncovalent interactions.

Glycopeptoids Glycopeptoids are peptoids that contain carbohydrate moieties covalently attached to the side chains of the peptoid residues.

Heteropolymers A polymer comprising two or more monomers that are different from one another is called heteropolymer.

Lipitoids Conjugates of cationic lipids and peptoids are called lipitoids.

Peptidomimetics A peptidomimetic is a compound that is designed to mimic a biologically active peptide, but has structural differences that give advantages for its function.

Peptoid A class of peptidomimetic polymers, whose side chains are appended to the nitrogen atom of the peptide backbone rather than to the α -carbon. They are also called poly(*N*-substituted glycines).

Peptoid helix Peptoids fold into helical secondary structures by the incorporation of bulky chiral side chains. Unlike peptide helices, peptoid helices result from the repeating local steric influence of the side chain which restricts backbone rotation.

Peptoid nanosheet Amphiphilic periodic peptoids self-assemble to form well-defined 2D sheet architecture. The free-floating sheets have a thickness of 2.7 nm.

Submonomer synthesis An efficient solid-phase synthesis method to generate peptoids from simple starting materials. Instead of using monomers, two 'submonomers' are successively incorporated to form a monomer unit.

Abbreviation

AMP	Antimicrobial peptide	MS	Mass spectrometry
Apaf	Apoptotic protease activating factor	NaOAc	Sodium acetate
Boc	<i>N</i> - <i>tert</i> -butoxycarbonyl	NCA	<i>N</i> -substituted carboxyanhydrides
BSL-2	Biosafety level 2	NHC	<i>N</i> -heterocyclic carbenes
CD	Circular dichroism	NLeu	<i>N</i> -isobutylglycine
CE	Capillary electrophoresis	NLys	<i>N</i> -(4-aminobutyl)glycine
CPP	Cell-penetrating peptides	NMDA	<i>N</i> -methyl- <i>D</i> -aspartate
DIC	<i>N,N'</i> -diisopropylcarbodiimide	Nme (or Nmeg)	<i>N</i> -(2-methoxyethyl)glycine
DMF	<i>N,N</i> -dimethylformamide	NMR	Nuclear magnetic resonance spectroscopy
DMPE	Dimyristoyl phosphatidyl-ethanolamine	Nspe	(<i>S</i>)- <i>N</i> -(1-phenylethyl)glycine
DOPA	3,4-Dihydroxyphenylalanine	OBOC	One-bead-one-compound
EPM	Equimolar peptide mixtures	PITC	Phenylisothiocyanate
Fmoc	Fluorenylmethoxycarbonyl	PMC	2,2,5,7,8-Pentamethylchromane-6-sulfonyl
GlcNAc	<i>N</i> -acetylglucosamine	PTC	Phenylisocyanate
GPCR	G-protein coupled receptor	PyBOP	Benzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate
HDM	Human double minute	PyBrOP	Bromotris(pyrrolidino)phosphonium hexafluorophosphate
HPLC	High performance liquid chromatography	QD	Quantum dot
HTS	High-throughput screening	ROP	Ring opening polymerization
Hyp	Hydroxyproline	SDS	Sodium dodecyl sulfate
IC ₅₀	Half maximal inhibitory concentration	SH3	SRC (sarcoma) homology 3
K _d	Dissociation constant	SP	Surfactant proteins
LC	Liquid chromatography	TEMPO	2,2,6,6-Tetramethylpiperidine-1-oxyl
MAOS	Microwave-assisted organic synthesis	TM	Transmembrane
MBNL	Muscleblind protein	UPLC	Ultra performance liquid chromatography
MeNPOC	(<i>R,S</i>)-1-[3,4-[methylene-dioxy]-6-nitrophenyl]ethyl chloroformate	VEGFR	Vascular endothelial growth factor receptor
MHC	Major histocompatibility complex	VR	Vanilloid receptor
MIC	Minimal inhibitory concentration		

2.204.1. Introduction

2.204.1.1. Bioinspired Polymers

Sequence-specific heteropolymers are growing in importance as useful tools in chemical biology, drug discovery/delivery, and materials science. Recent advances in synthetic chemistry have made it possible to generate relatively simple protein-like

structures and functions from nonbiological synthetic heteropolymers. Many of these synthetic heteropolymers are stable to biological proteases and extreme environments such as high temperatures and harsh chemical conditions. Further, they can be assembled in high yields from relatively cheap building blocks. Proteins are marginally stable; they are easily degraded by biological proteases and denatured by various chemicals

and high temperatures. Thus, there is a great opportunity to develop a new class of protein-mimetic polymers that are capable of molecular recognition and catalysis and yet are highly robust to the environment. The unusual stability properties and synthetic versatility of synthetic sequence-specific heteropolymers provide great promise for such materials and will have great impact in therapeutic, diagnostic, and materials science applications.

Researchers are making progress toward creating synthetic polymers that mimic the sophisticated structures and functions of proteins, although no synthetic polymer currently has the capacity to perform complex biological functions such as selective molecular recognition, catalysis, transport, energy conversion, etc. It has been difficult to find a single polymer system in which one can achieve stable secondary structures, sequence diversity, and long main chain lengths. It has been only the past decade since researchers have begun to mimic protein-like tertiary structures and functions.

The creation of new synthetic heteropolymers has been inspired and guided by protein folding and design principles. Advances in synthetic, combinatorial, and physical chemistry have provided a variety of useful tools to create folded protein-mimetic polymers where a variety of functionalized monomers can be arranged in a particular sequence. However, despite decades of study, the rules that govern the kinetics and thermodynamics of folding polymer chains into stable tertiary structures are not yet fully understood. Energetically, the dominant forces that drive folding of polymer chains in aqueous solution are well known,¹ and this has been useful for the design of new foldable polymer sequences. Still it has been difficult to understand the atomic structural details of the molecular interactions that drive unique and stable tertiary structures. Synthetic heteropolymers that allow detailed control of molecular interactions would help to understand folding principles on an entirely new level.

This chapter will focus on peptoids (*N*-substituted glycine polymers), one of several in the class of sequence-specific synthetic heteropolymers. Readers can find many comprehensive reviews regarding other synthetic heteropolymers.^{2–4} Ever since peptoids first found utility in drug discovery,⁵ many new areas of peptoid research have recently emerged as a result of their many useful properties. New structural motifs and design principles have emerged. Peptoids offer great new opportunities to create protein-like tertiary structures and functions. Peptoid polymers that spontaneously organize into well-defined supramolecular nanostructures are a promising new platform in materials science.

2.204.1.2. Properties of Peptoids as Useful Biomaterials

Peptoids are *N*-substituted glycine polymers in which the side chains are appended to the backbone nitrogen (Figure 1). This *N*-substitution in peptoids precludes the intra-backbone hydrogen bonding that is found in proteins, providing us the opportunity to explore polymer properties and chain folding in the absence of backbone hydrogen bonding.

Peptoids are efficiently synthesized using a solid-phase synthesis. Using the solid-phase submonomer method that we developed, 48-mer polypeptoids have been synthesized in excellent yields.⁶ In terms of sequence diversity, over 300

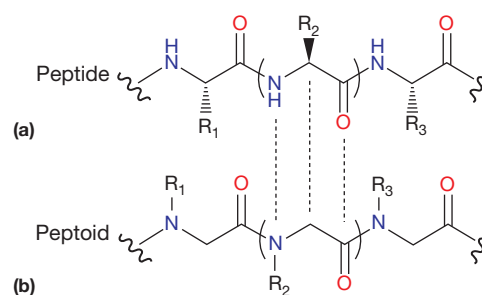


Figure 1 The comparison of (a) peptide and (b) peptoid.

primary amines are currently available that can be incorporated into peptoids as side chains. Thus, the diversity of peptoid side chains is much greater than that found in proteins.

Peptoids as short as 5-mers have been shown to adopt helical conformations when they contain chiral side chains adjacent to the main chain nitrogen.^{7,8} These peptoid helical structures showed extreme stability to chemical denaturants and temperature.⁹ The peptoids have been also shown to be stable to proteolysis,^{10,11} opening the door to therapeutic, diagnostic, and biomaterials applications. Numerous short peptoid oligomers have been found that bind to therapeutically relevant proteins, acting as antagonists, inhibitors, or activators.¹² In this chapter, many of these useful features and examples will be discussed.

2.204.2. Synthesis

2.204.2.1. Solid-Phase Synthesis

Originally standard peptide solid-phase synthesis techniques were utilized for peptoid synthesis using Fmoc-protected *N*-substituted glycine monomers (Figure 2(b)).⁵ This monomeric method involves tertiary amide bond formation between the existing secondary amine on the resin and the incoming monomer by the activation of carboxyl group in the monomer using benzotriazol-1-yloxytris(pyrrolidino) phosphonium hexafluorophosphate (PyBOP) or bromotris(pyrrolidino)phosphonium hexafluorophosphate (PyBroP). Then, the Fmoc group is deprotected with piperidine, generating a secondary amine for next amide coupling. This monomeric peptoid synthesis requires labor-intensive synthesis of Fmoc-protected *N*-substituted glycine monomers.

The invention of the submonomer solid-phase synthesis method for peptoids in 1992 was a major breakthrough because it greatly increased the synthetic efficiency, synthesis yields, and available side chain diversity, while also dramatically reducing time and costs.⁶ As shown in Figure 2(a), a secondary amine on the resin is first acylated by an activated haloacetic acid, such as bromoacetic acid, with *N,N*-diisopropylcarbodiimide (DIC). Then the bromine is displaced by a primary amine. In this S_N2 reaction, ~300 commercially available primary amines can be added, expanding the diversity and convenience of introducing side chains. Moreover, many new submonomers have been synthesized and incorporated into peptoids. These include *N*-BOC-tryptamine, *O*-*t*-butyl tyramine, and PMC-guanidino-propylamine to mimic natural amino acids tryptophan, tyrosine, and arginine.¹³ In addition, carboxamide, carboxylic acid, and thiol derivatives of (*S*)-1-phenylethylamine

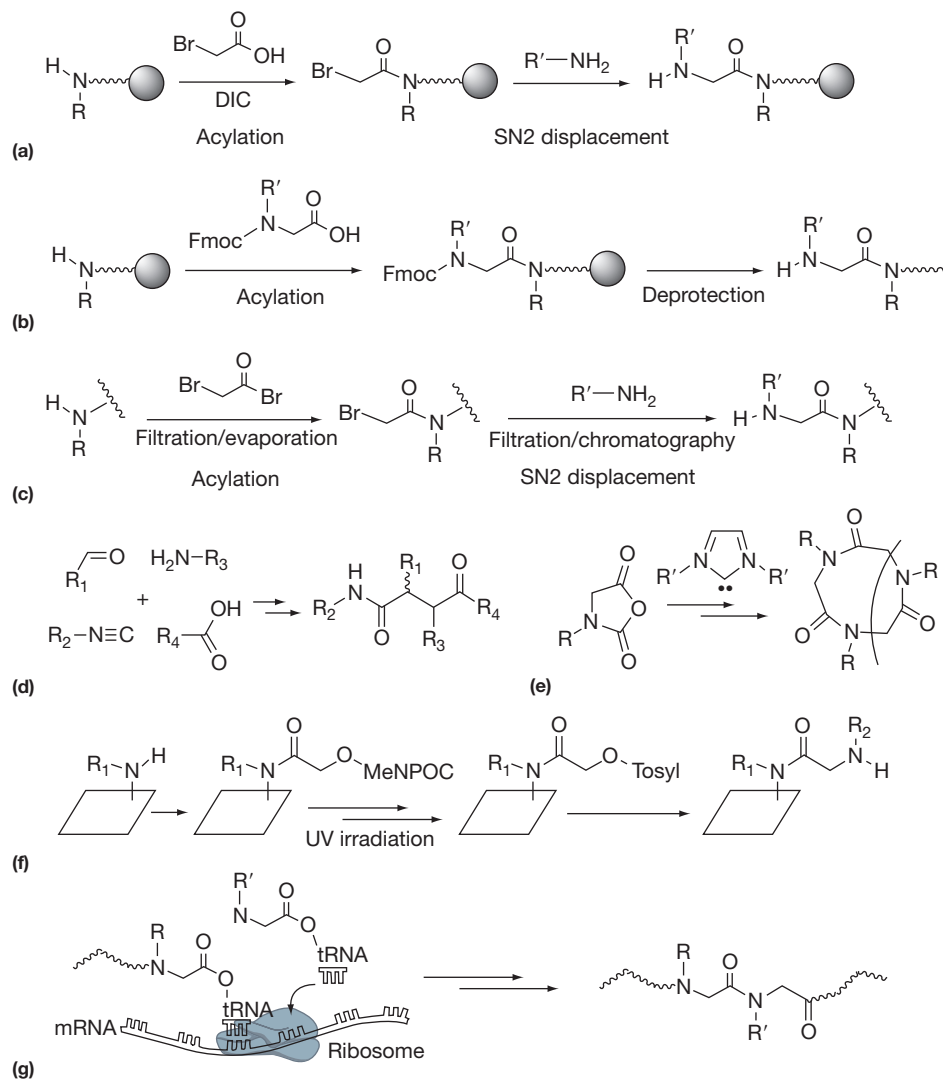


Figure 2 Methods for peptoid synthesis. (a) Solid-phase synthesis: submonomer method. (b) Solid-phase synthesis: Fmoc-method. (c) Solution-phase synthesis: bromoacetyl bromide-method. (d) Solution-phase synthesis: Ugi four-component reaction. (e) Solution-phase synthesis: amine-initiated ring-opening polymerization. (f) Photolithographic synthesis. (g) *In vitro* ribosomal synthesis.

were synthesized to introduce a helical structure decorated with chemical functionalities.¹⁴ A variety of chemoselective conjugation groups such as thiol, activated disulfides, aldehyde, aminoxy, and hydrazine groups have been also incorporated into peptoids via N- and C-terminal modification or the submonomer method that allowed an efficient ligation between peptoids.¹⁵ Recently, amine derivatives of heterocycles such as histamine, pyridine and tryptamine were successfully incorporated into peptoids using chloroacetic acid in the acylation step.¹⁶ Conformationally constrained cyclic peptoids can also be synthesized using the submonomer method, followed by solution-phase cyclization.¹⁷ The submonomer synthesis is still widely used for peptoid synthesis.

2.204.2.2. Solution-Phase Synthesis

There are a couple of methods for making peptoids completely in solution as shown in **Figure 2(c)**. So far, the lengths of

sequences made by this method are limited to <10 residues. One of the solution-phase peptoid syntheses is a submonomeric method using bromoacetyl bromide.¹⁸ The first step is the acylation reaction of the N-terminus of the existing peptoid chain with bromoacetyl bromide. After filtration and evaporation, the crude bromoacetamide intermediate is then reacted with a primary amine. The newly formed peptoid is then isolated by flash chromatography before the next cycle of submonomer addition. Thus, this method requires labor-intensive filtration, evaporation, and chromatography, although it generates reasonably large quantities.

Another method for solution-phase synthesis is the Ugi four component reaction (4-CR) as shown in **Figure 2(d)**.¹⁹ The Ugi-4CR is an efficient process in which a primary amine, an oxo compound, a carboxylic acid, and an isocyanide react in one pot to form a dipeptoid backbone. The Ugi-4CR has been combined with macrocyclization, generating many useful compounds such as functional macrocycles, cryptands, cages, etc.^{20,21}

2.204.2.3. Other Methods for Peptoid Synthesis

Amine-initiated ring-opening polymerization (ROP) has been utilized to synthesize high molecular weight peptoid macrocycles (Figure 2(e)).²² *N*-heterocyclic carbenes (NHCs) mediate ROP of cyclic substrates *N*-substituted carboxyanhydrides (*N*^R-NCA) through a zwitterionic propagating species and yield cyclic peptoid polymers in a controlled and efficient manner. This method is limited to single type of side chains in the peptoid polymers. In addition to this ROP, poly β -peptoids had been produced by the polymerization reaction of aziridines and carbon monoxide.^{23,24}

The submonomer method of peptoid synthesis had been adapted for photolithographic application (Figure 2(f)).²⁵ A four-step monomer addition cycle was developed for the photolithographic synthesis of peptoids. Glycolic acid protected with a light-sensitive protecting group (MeNPOC) was first coupled to an amine-modified surface. The hydroxyl group was unmasked by UV irradiation and then activated with tosyl chloride. Finally, the tosylate was displaced with a primary amine to complete the construction of a monomer unit. This chemistry allows the spatially addressable synthesis of peptoids on an array by photolithography, since hydroxyl group unmasking, activation, and amine displacement will occur only at addresses that have been irradiated with UV light.

To our surprise, peptoid polymers have also been synthesized on a small scale using biosynthetic machinery. An *E. coli* cell-free translation system was used with genetically coded mRNA and artificial tRNAs charged with peptoid monomers as shown in Figure 2(g).²⁶ In this translation system, certain proteinogenic amino acids and/or cognate aminoacyl-tRNA synthetases are withdrawn (*withdrawn* PURE system; *wPURE*), diminishing the competing background incorporation of the proteinogenic amino acids in the translation elongation event. They prepared a wide variety of tRNAs charged with nonproteinogenic peptoid monomers using artificial tRNA acylation ribozymes. Using this method, six peptoid monomers were translated from the genetic code and purified.

2.204.3. Peptoid Structure and Characterization

2.204.3.1. Peptoid Secondary Structures: Helices and Turns

As we learned from biological polymers, efficient functions are derived from well-defined structures. One of the key strategies to forming well-defined, atomically ordered folded polypeptides is to control the local secondary structure. Control over local and global molecular interactions can reduce the conformational freedom of the polymer chain resulting in ordered states in aqueous environment. Borrowing from nature's design principles found in proteins, many synthetic polymer systems have been developed to create protein-mimetic materials. However, most synthetic sequence-specific polymers are relatively short in main chain length, due to synthetic inefficiencies. Still, with relatively short chain lengths, many specific protein ligands have been developed. Longer sequences have been used for antifouling materials, antimicrobial agents, lung surfactant mimetics, drug delivery vehicles and protein mimicry. However, it is still in infancy to create protein-like structures and functions in purely nonbiological polymers.

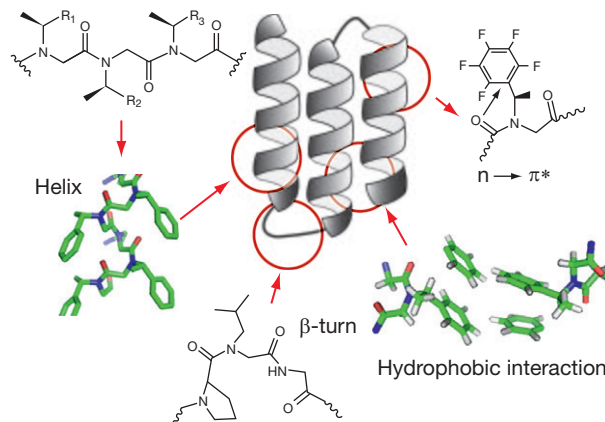


Figure 3 Schematic diagram for a hierarchical approach toward creating a tertiary structure in peptoids from local to global interactions. The local interactions include the formation of secondary structures such as helices and turns, and the tunable interactions such as $n \rightarrow \pi^*$ interaction. The hydrophobic interaction has been utilized as a folding core of peptoid helix bundles.

A number of proteins fold up sequentially from initial secondary structures to well-defined tertiary structures. Thus, one of the methods toward unique tertiary structures in nonbiological polymers would be first to create secondary structures and then, engineer them further to assemble in three-dimension (3D). In peptoids, we have been using this sequential strategy, aiming toward unique 3D peptoid structures (Figure 3).

Like the secondary structures in proteins, helices and turns have been generated in peptoids. Peptoid helical structures are well established and exhibit extreme chemical and thermal stability.⁹ Intrinsically, the peptoid backbone is more flexible than the peptide backbone due to the lack of hydrogen bond donors and main chain chirality. However, a bulky chiral side chain appended to the $N\alpha$ position in peptoids significantly reduces the conformational freedom of the chain backbone and induced well-defined helical structures with three residues per turn (Figure 3).⁷ The peptoid helical structures have been characterized by a range of high-resolution techniques, such as 2D-NMR, X-ray crystallography, and computational modeling.^{27–29} The structural parameters including backbone ϕ and ψ angles and the distance between two helical pitches showed that the peptoid helical structures given by α -chiral steric bulk resemble the polyproline type-I helical structure in peptides. Because of the steric hindrance given by the bulky chirality at the $N\alpha$ position, the backbone ϕ angle is limited to a certain range of angles to prevent the steric clash with nearby backbone carbonyl groups. This molecular steric hindrance in peptoids allows even small pentamer peptoids to form stable helical structures with extreme chemical and thermal stability. The peptoid helical structures were stable to 8 M urea and to 75 °C.⁹

The turn structures in peptoids are recently generated, but not well established comparing to the helices. A series of tripeptides containing either *N*-methylglycine or *N*-isobutyglycine in position $i + 1/i + 2$ were synthesized and tested for intramolecularly H-bonded β -turn formation in chloroform solution.³⁰ The population of the type-II β -turn increased when they incorporated the achiral peptoids at the position $i + 2$. Toward the β -turn mimic, geometrically constrained triazole ring had

been introduced into peptoids as a monomer unit, stabilizing a turn in the middle of linear peptoids. This triazole motif mimicked the β -hairpin structures in peptides.³¹

2.204.3.2. Noncovalent Local Interactions in Peptoids

Several types of noncovalent interactions have been found,^{32–35} expanding significantly the scope of peptoid design and the utility of peptoids for a broad range of applications. These interactions stabilize peptoid secondary structures further and can even switch the conformation, depending on the environment such as pH and solvent.

Hydrophobic interactions and $n \rightarrow \pi^*$ interactions have been shown to play a role in peptoid folding.^{32,36,37} The conformation of the secondary structures could be tunable by these noncovalent interactions. For example, the rotameric *cis* to *trans* equilibria of the backbone amides could be controlled by $n \rightarrow \pi^*$, steric, and hydrogen bonding interactions (Figure 3).^{32,34} Especially, *cis*-amides in the peptoid backbone could be exclusively formed by *N*-*R*-chiral acetanilide and *N*-1-naphthylethyl side chains, by $n \rightarrow \pi^*_{C=O}$ /hydrogen bonding interactions and $n \rightarrow \pi^*_{Ar}$ /steric interactions, respectively.³⁴ The pair interaction of fluoroaromatic and other electron-deficient aromatic side chain in peptoids could be an effective strategy for controlling peptoid structures.³³

The rotameric *cis* to *trans* equilibrium of the backbone amides could be also dependent on the nearby amides in the backbone. Using NMR, the kinetics of *cis* to *trans* equilibrium of the backbone amides could be measured in monomers, di- and tri-peptoids³⁸ with a range of rate constants from 0.03 to 0.37 s⁻¹. These findings would be useful for understanding the transition between different conformations and developing an accurate force field in peptoids.

Previously, a threaded loop structure was found in peptoids by well-defined local interactions.³⁹ This structure is unique to peptoid nonamers with achiral side chains and was first identified in a homonamer of Nspe. The threaded loop structure is stabilized by three intramolecular hydrogen bonds from backbone carbonyl groups (residues 5, 7, and 9) to the *N*-terminal secondary ammonium, and one intramolecular hydrogen bond from a backbone carbonyl (residue 2) to the *C*-terminal primary amide. The peptoid threaded loop contains four *cis* and four *trans* amide bonds. Interestingly, methanol was able to disrupt the set of intramolecular hydrogen bonds, converting the threaded loop to helical structure.

L-phenylalanine *tert*-butyl ester had been utilized for the synthesis of (*S*)-*N*-(1-carboxy-2-phenylethyl)glycine oligomers.³⁵ These peptoids formed stable secondary structures in aqueous solution in which the conformation is dramatically responsive to variations in pH and solvent composition. The electrostatic interaction between monomer units was responsible for this pH-dependent conformational switch.

2.204.3.3. Cyclic Peptoids

Cyclization of the linear polymer chains has been developed over the past few decades as an effort to find an efficient method for conformational ordering.⁴⁰ This type of confined structures via cyclization provides a useful platform for high-affinity molecular recognition due to the gain of the entropic term in

thermodynamic binding free energy. Head-to-tail macrocyclization was established in peptoids, allowing the enhancement of the conformational ordering and the crystallization of cyclic peptoid hexamer and octamer.¹⁷ The high-resolution crystal structures of these cyclic peptoids revealed that the peptoid backbone could accommodate tight turns via distorted *cis* and *trans* amide conformers in the solid state.¹⁷ This head-to-tail macrocyclization have been utilized recently for useful alkali metal ion transporters.⁴¹

Another macrocyclization have been also established in peptoids via chemical linkages of side chains. Especially, the azide-alkyne cycloaddition was used widely in peptoids for macrocyclization.^{42–44} Amide coupling through side chains was also developed^{45,46} and employed further for cyclic peptoid arrays.⁴⁵

2.204.3.4. Computational Modeling

Computational modeling of biological molecules has been advanced significantly in the past decade, providing a very useful tool for predicting secondary and tertiary structures in proteins and nucleic acids. This success was made possible due to the development of super-computing power and more accurate force fields in amino acids and nucleotides than ever, and the database of protein and nucleic acid structures. The computational tools are now being applied to many other nonbiological synthetic heteropolymers as an aid for the design of new molecules. However, accurate force fields are required for the success of design and understanding of synthetic heteropolymers. There are a number of computational studies in peptoids to guide us toward more accurate models of peptoid structures.^{27,28,47–50} The recent high-level quantum mechanics simulation had a good agreement with available peptoid atomic-detail structures as shown in the Ramachandran plot of peptoid backbone ϕ versus ψ angle (Figure 4).⁴⁸ This simulation revealed that local energetics dictates the conformational preference in peptoids.⁴⁸

Distance geometry and ensemble calculations have been carried out using the distance information from NMR data in order to refine the peptoid helical structures and understand the amide backbone *trans* to *cis* isomerism.^{28,50} The *trans* conformation of the backbone amide is generally not preferred in peptoids due to the *N*-substitution.³⁸ Quantum mechanical *ab initio* calculations have been applied for this problem.^{47,48} Although the angle of the amide backbone depends on the side chains, it is not fully understood yet about the determining factors of this amide backbone *trans* to *cis* isomerism. The accurate force field of peptoids is necessary for understanding this problem.

In order to understand the folding cooperativity in peptoid helix bundles, a dynamic programming approach was carried out using statistical mechanical partition functions of foldamer chain molecules.⁵¹ This model showed that the peptoid three-helix bundle fold anticooperatively and predicts that two-helix bundles are unstable in proteins but stable in peptoids.

2.204.3.5. Protein-Like Structures and Functions

One of the ultimate goals in the area of bioinspired heteropolymers is to create precisely folded nanostructures with protein-like functions. There have been recent efforts to construct

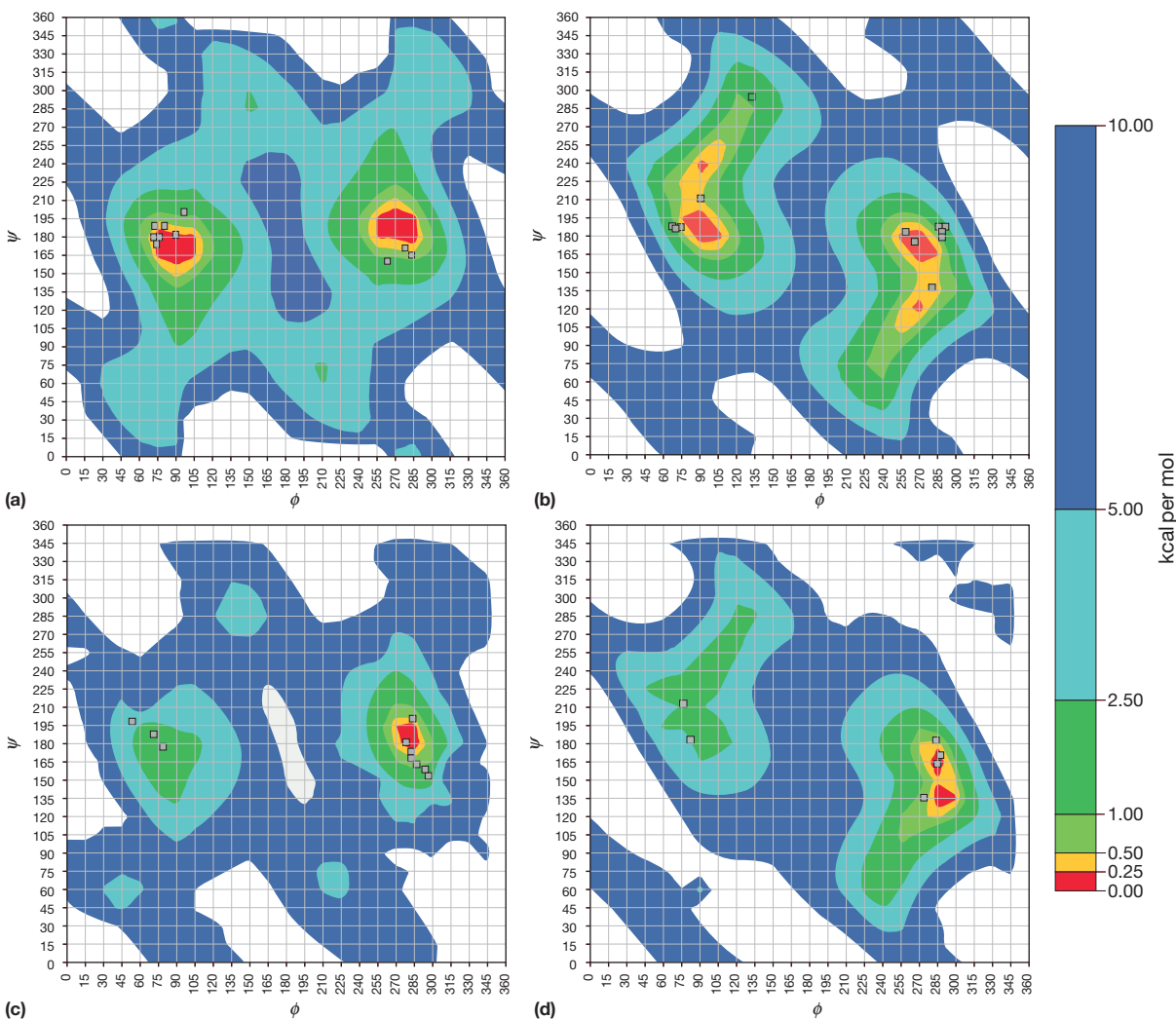


Figure 4 Ramachandran plot of peptoid backbone phi vs. psi angles revealed by a recent high-level quantum mechanical simulation. Monomeric peptoids, Ac-Nme-N(CH₃)₂ (a and b) and Ac-Nspe-N(CH₃)₂ (c and d) were calculated by the quantum mechanical calculation at the B3LYP/6-311+G(2d,p)//HF/6-31G* level. The backbone ω is *cis* at the left plots (a and c), and *trans* at the right plots (b, d). Squares are corresponding experimental residues from crystal structures. Nme and Nspe stand for *N*-(methoxyethyl)glycine and (*S*)-*N*-(1-phenylethyl)glycine, respectively. Adapted from Butterfoss, G. L.; Renfrew, P. D.; Kuhlman, B.; Kirshenbaum, K.; Bonneau, R. *J. Am. Chem. Soc.* **2009**, *131*, 16798–16807, with permission from American Chemical Society.

synthetic polymers that mimic protein properties. Although it has been difficult to achieve stable secondary structures, a multiletter alphabet, and long chain lengths within a single type of polymer, our group has been able to create helix bundle structures with hydrophobic folding cores and introduce one of the simplest biological functions such as high-affinity zinc binding into peptoid two-helix bundles.^{36,37} The advancement of synthetic technologies and design principles would enable us to create more complicated protein-like structures and functions.

The helix bundle was the first target toward tertiary structures in peptoids because the helical secondary structures are well established in peptoids. One of the key strategies for generating protein-like helix bundles was utilizing amphiphilic self-assembled peptoid helical structures.⁵² The hydrophobic groups at every third position allow the amphiphilicity of peptoid helices and provide a stable hydrophobic folding core inside

self-assembled helix bundles. The combinatorial chemistry had been carried out to identify amphiphilic peptoid helices that have a well-defined hydrophobic core.⁵² Using these peptoid helices, single-chain folded helix bundles had been created by orthogonal chemoselective conjugations between peptoid helices. By three consecutive conjugations, a 12 kDa peptoid four-helix bundle had been created previously.³⁷

To mimic protein-like functions, our group was able to introduce high-affinity zinc-binding site into the peptoid two-helix bundles.³⁶ Borrowing from well-understood zinc-binding motifs in proteins, thiol and imidazole moieties were positioned within the peptoid two-helix bundles such that both helices must align in close proximity to form a binding site. Certain peptoid two-helix bundles bind zinc with nanomolar affinities and high selectivity compared to other divalent metal ions. This work is a significant step toward generating protein-like structures and functions.

2.204.3.6. Peptoid Self-Assembly: Nanostructures

Polypeptoids are a unique material that allows not only the mimicry of proteins, but the precise engineering of polymers. Sequence level control and the ability to synthesize relatively high molecular weights have resulted in a number of interesting applications. The nanoscale self-assembly of polymeric peptoid materials is an area of tremendous potential, as the fields of protein folding and polymer self-assembly become ever closer. Peptoid polymers have been studied as bulk solids and shown to have improved process ability over polypeptides. Recently, certain sequences of peptoids showed their ability to form nanoribbon/tapes and 2D floating sheets in aqueous solution,^{53–55} gaining their potentials for useful nanostructured materials.

A series of peptoid homopolymers were studied with respect to their thermal properties as bulk solids.⁵⁶ Many of these sequences form crystalline phases with discrete melting temperatures. Interestingly, a comparison was made between a polypeptide and a perfectly analogous polypeptoid (i.e., everything in the structure was identical except for the point of side chain attachment). The peptoid was found to exhibit a reversible melting transition at modest temperatures, whereas the corresponding peptide did not melt all the way up to its decomposition temperature. This suggests that the lack of hydrogen bonding in polypeptoids is an important feature that may contribute to improved processability in the bulk.

During the development of a novel class of peptoid β -sheet breaker as amyloid inhibitors, a retro-peptoid sequence corresponding to the amyloidogenic peptide sequences amylin, SNNFGAILSS, was found to form the supramolecular nanoribbon/tape structures.⁵³ Due to the formation of this self-assembled peptoid nanostructures, this retro-peptoid was not efficient to inhibit the amyloid formation of amylin, but provided a useful nanostructure.

Recently, our group systematically explored the impact of amphiphilic sequence patterns in the self-assembly of peptoid polymers, and we discovered one of the largest 2D organic crystals known. Remarkably well-defined 2D free-floating peptoid nanosheets formed spontaneously in aqueous solution after mixing a 1:1 ratio of two oppositely charged peptoid 36-mers of a specific sequence.⁵⁴ A repeating, alternating sequence pattern of a polar ionic monomer followed by a nonpolar aromatic monomer was key to formation of the sheet architecture. These giant free-floating sheets were shown to have a bilayer structure (i.e., two molecules thick) and have a thickness of only 2.7 nm, yet a width and length of tens of microns (Figure 5). The unprecedented direct imaging of individual peptoid chains was made possible by the recent development of aberration-corrected transmission electron microscopy (TEAM 0.5) at the National Center for Electron Microscopy, Lawrence Berkeley National Laboratory. Direct observation of individual polymer chains revealed that the peptoid chains are fully extended in structure and run parallel to one of the sheet edges. Further, the chain-chain spacing of 4.5 Å could be directly imaged, which corresponded well to the spacings observed by X-ray and electron diffraction experiments. It was further demonstrated that the peptoid nanosheets could be functionalized with biologically active ligands and that they could specifically bind their protein target with high specificity.

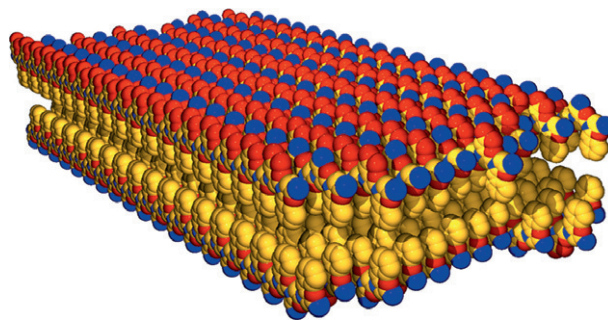


Figure 5 Free-floating nanosheets can be formed by the self-assembly of two oppositely charged peptoid 36-mers of a specific sequence pattern. The peptoid sequence is a repeat of an alternating sequence of a polar ionic monomer and a nonpolar aromatic monomer. The peptoid nanosheets consist of a bilayer, which is <3 nm in thickness, yet many tens of microns in length and width. Adapted from Nam, K. T.; Shelby, S. A.; Marciel, A. B.; *et al. Nat. Mater.* **2010**, *9*, 454–460.

Importantly, this is the first demonstration that biological sequence information (a β -sheet-like alternating sequence) can be introduced into a synthetic polymer to generate an atomically defined biomimetic structure. Interestingly, because the peptoids are achiral, these peptoid nanosheets are completely flat, in contrast to peptidic β sheets, which have an inherent twist in their structure that is a result of the amino acid chirality. Thus, the synthetic flexibility and biocompatibility of peptoids provide a flexible and robust platform for integrating functionality into defined nanostructures.

2.204.4. Combinatorial Discovery of Peptoid Ligands

Molecular diversity as a source of potential drug candidates, protein-mimetics, and various other functional materials has been of enormous interest. Combinatorial library synthesis provides an efficient route to achieve great molecular diversity and has been extensively used both in academic and industrial research.¹² Inspired by nature's intrinsic molecular diversity provided by biopolymers (i.e., proteins), researchers at Chiron Corp, now part of Novartis AG, pursued new drug discovery technologies employing a peptoid combinatorial library platform.⁵ The invention of the solid-phase submonomer synthesis methods facilitated the library synthesis and enabled peptoids to be an ideal class of molecules for combinatorial library synthesis. First, the solid-phase peptoid synthesis allows the resin-splitting methods, which are crucial for mix-and-split type combinatorial library synthesis where equimolar compound mixtures can be synthesized. Second, each residue incorporation step is composed of highly efficient bromoacetylation and amine displacement reactions. These reactions provide high yields and are not sensitive to air and moisture; therefore, the chemistry is automatable. Third, due to the availability of a number of peptoid submonomers as primary amines, a wide variety of chemical functionalities can be incorporated. These favorable aspects of peptoids for combinatorial library synthesis along with the availability of state-of-the-art lab automation instruments, the synthesis of millions of peptoids now became a straightforward process. In addition, the ability to screen and analyze peptoids

rapidly and inexpensively from the libraries has been a primary tool in modern peptoid research.

2.204.4.1. Synthesis Technologies

2.204.4.1.1. Automated synthesis

Solid-phase synthesis techniques have been introduced in the early 1960s by Bruce Merrifield, a Nobel laureate in 1984, and have been applied to biopolymer synthesis including peptides, oligonucleotides, nonnatural peptides, and oligosaccharides. In the case of peptide synthesis, the key steps of the synthetic cycle, namely deprotection and coupling, are repeated until the complete sequence is assembled on the solid matrix. Afterward, the crude product is cleaved from the support, purified, and characterized.⁵⁷ Nowadays the synthesis of peptides is fully automated and routinely performed on automated synthesizers. Because the peptoid monomer addition cycle is also two steps, peptoid synthesis can be carried out by most commercial peptide synthesizers with only a few minor programming changes.⁵⁸ In the automated synthesis of peptoids, amine solutions are placed in the 'amino acid' reservoirs. Depending on the synthesizer's specification, (1) many samples are handled in parallel (up to ~30 wells) on a typical scale of 0.1 mmol per well; or (2) one-bead-one-compound (OBOC) peptoid libraries of tens to hundreds of thousands of compounds can be synthesized using an automated resin mix-and-split step.^{59,60}

2.204.4.1.2. Parallel synthesis

In parallel synthesis, each library member is synthesized in a separate reaction vessel. For the synthesis of 16 peptoid dimers, 16×2 cycles of peptoid monomer unit incorporation is required. For the same number of peptoid dimer synthesis, a mix-and-split method needs only 8 cycles and is well suited for a large size library synthesis to cover larger chemical space.⁶¹ However, the advantages of the parallel synthesis are that the entities in the library are known and that a large amount of peptoid can be prepared in each reaction vessel. A library prepared by the mix-and-split method often requires additional deconvolution or encoding steps to identify peptoid on a specific resin.

Microwave-assisted organic synthesis (MAOS) was originally developed for simple organic transformations such as hydrolysis. Then the technique has been successfully applied to the synthesis of peptides, peptidomimetics, and carbohydrates.⁶² The microwave irradiation for peptoid library synthesis has been reported and has shown advantages in terms of both higher purity and dramatically shorter reaction time.^{63,64} The optimal conditions for the bromoacetylation step involved irradiation for 30 s and heating to 35 °C using temperature control probe, and for the amine displacement, irradiation for 90 s and heating to 95 °C again using the temperature control (at room temperature, typical reaction time for bromoacetylation is 20 min and for amine displacement is 60 min). The MAOS is being used actively in the preparation of peptoid library synthesis either in the parallel synthesis or in the mix-and-split library synthesis.

2.204.4.1.3. SPOT synthesis

The SPOT synthesis technique employs the parallel synthesis method mentioned above, but the difference is that the

synthesis is carried out directly on a cellulose membrane rather than on resin beads in a reaction vessel.⁶⁵ The SPOT method follows the standard peptoid submonomer synthesis protocol: preparation and functionalization of the cellulose membrane, stepwise bromoacetylation and amine displacement, and cleavage of the side chain protecting groups. If necessary, peptoids can be cleaved from the cellulose membrane by the incorporation of appropriate linkers between the membrane and the peptoid. Peptoid synthesis on cellulose allows the parallel synthesis of large numbers of positionally addressable peptoids in small quantities, and the cost of SPOT synthesis is far cheaper than that of conventional resin-based synthesis.

Wenschuh *et al.* first reported the SPOT synthesis of peptomers (peptide-peptoid hybrids) and peptoids.^{66–68} They used Whatman 50 cellulose membranes and modified by treatment with epibromohydrin and 4,7,10-trioxo-1,13-tridecanediamine to provide homogeneous terminal primary amines on the cellulose membrane. Slight modification on conventional peptoid submonomer protocol was employed by using bromoacetic acid 2,4-dinitrophenyl ester instead of commonly used bromoacetic acid/DIC, and selective bromoacetylation on the terminal secondary amines over residual hydroxyl groups on cellulose was achieved. For the dry state cleavage of peptoids, the authors used a photo-labile linker system to cleave membrane-bound peptoids via UV irradiation. The peptoid SPOT array consisting of 8000 peptoids and peptomers are screened for the monoclonal antibody Tab-2, and micromolar affinity ligands were identified demonstrating the potential utility of this method for the rapid identification of novel nonpeptidic protein ligands.

2.204.4.1.4. Split/mix synthesis

In the early 1990s, the OBOC combinatorial library method was first introduced by Lam and coworkers.^{69,70} With the OBOC library technique, tens of thousands to millions of compound beads could be rapidly prepared and then screened for a specific biological activity. For the synthesis of OBOC libraries, a mix-and-split synthesis method is utilized such that each bead displays only one compound.⁷¹ Originally, Lam *et al.* developed the OBOC library technique for the synthesis of short linear peptides. Afterwards, led by Zuckermann and Kodadek, the application of the technique was expanded to the preparation of the peptoid libraries.^{58,72}

Prior to the library synthesis, the size of the library including the length of the oligomer and the number of amine submonomers must be considered. If nothing is known about the target protein, diverse sets of amines are used. However, the information about the target-binding site can help design the library and significantly reduce the number of amine submonomers to be used. Typically, 6–30 amine submonomers are used to conveniently prepare an OBOC library. The scale of the library synthesis depends on the amount of material needed for screening. Generally, for the synthesis of peptoid trimer library, 2.4 mmol of resin is used, yielding about 1 ml of each of 24 pools, each at a concentration of 100 μ M per compound. This quantity is sufficient for hundreds of screens when screened at 1 μ M per compound.

In 1992, Zuckermann and coworkers introduced a fully automated peptoid synthesis protocol that is capable of synthesizing equimolar peptide mixtures (EPM).⁵⁹ Equimolarity

was achieved by employing the resin-splitting method, namely isopycnic slurry method, where the resin was suspended as slurry in a solvent system that had a similar density to resin, causing the resin particles to settle very slowly. The authors suspended a polystyrene resin (100–200 mesh and 1% cross-link with divinylbenzene) as a 3% w/v free-flowing slurry in 65% 1,2-dichloroethane/DMF. The resin slurry was then transferred by a robotic pipet hand into separate reaction vessels (up to 36). When short peptides/peptoids were synthesized, the equimolarity of the library was well maintained after repeated split-and-pool processes. With the automated EPM synthesizer on hand, the authors prepared ~5000 peptoid dimers and trimers to identify high-affinity ligands for 7TM/GPCR (see Section 2.204.5.1 for further discussions).⁷³ In this work, iterative deconvolution method was successfully employed for the structural determination of the peptoid hits. This method is based on an iterative process of screening and resynthesis of smaller sublibraries in an attempt to fractionate a mixture into its most active constituents. Popularized by Houghten *et al.*,⁷¹ iterative deconvolution has been used as a screening method to identify hits from non-OBOC libraries.

2.204.4.2. Analytical Methods

2.204.4.2.1. Separations

In combinatorial chemistry, a vast number of diverse compounds are generated by robotic instruments; for example, thousands of compounds can be synthesized daily. Due to the increase in the rate of compounds synthesized, the demand for rapid analysis and characterization of the library is high. For separation purposes, widely used analytical instruments are LC/MS (liquid chromatography/mass spectrometry), HPLC (high performance liquid chromatography), and more recently UPLC (ultra performance liquid chromatography). Typically, liquid chromatography separations are carried out on a reverse phase mode; C18 or C4 supports are used as stationary phase, and water/acetonitrile gradients are eluted as mobile phase. Technical advances in this field have enabled high-resolution separations of mixtures and high sensitivity detection of compounds in small quantity.

One active area of investigation is in the development of capillary electrophoresis (CE). CE is designed to separate species based on their size to charge ratio in the interior of a small capillary filled with a matrix. The advantages of CE are the requirement of small sample quantity and the wide variety of separation mechanisms modulated by types of matrices and by mobile phase solutions. The superior separation efficiency of CE makes it a powerful tool for many of the analytical challenges in biopharmaceutical research.

Lunte and coworkers evaluated the utility of the CE technique for the separation of a combinatorially synthesized peptoid mixture.^{74,75} The mixture consisted of 24 trimeric peptoids that ranged in molar mass from 392 to 699 with pK_a values ranging from 3 to 10. With reverse phase HPLC, separation of this mixture posed a significant challenge. Hence, the authors used a fused-silica capillary electrophoresis and surveyed different types of mobile phase buffers and additives for efficient separation. Significantly enhanced separation was achieved when they used a combination of heptane sulfonic acid (25 mM) and methyl- β -cyclodextrin (40 mg ml⁻¹) in

250 mM sodium phosphate buffer at pH = 2.0. As for the additives, heptane sulfonic acid was used as an ion-pairing agent to reduce hydrophobic intramolecular interactions and to disrupt electrostatic interactions with the capillary wall, and methyl- β -cyclodextrin was used to provide host-guest interactions and resolve very hydrophobic components of the peptoid mixture. Later the authors reported the use of SDS micelles in conjunction with methyl- β -cyclodextrin, and the additives in the same sodium phosphate buffer (pH = 2.0) provided dramatic improvement in the separation of the peptoid mixture.

Lunte and coworkers demonstrated the potential advantages of CE over conventional HPLC for the analysis of complex peptoid mixtures. Only nanoliters of sample are injected for each analysis, and this offers tremendous benefit when extremely small amounts of sample are available as is typical with OBOC combinatorial chemistry.

2.204.4.2.2. Characterization

Numerous techniques have been applied for the characterization of combinatorial libraries. For libraries prepared by parallel synthesis, all compounds in the library are addressed individually, and characterization process is relatively straightforward. However, evaluation of libraries prepared by mix-and-split synthesis is much more challenging. Taking into account that an OBOC library is composed of individual beads containing unique chemical entities, the first approach to characterize the mix-and-split library is to analyze the individual beads. This approach is often limited because each bead contains only a picomolar quantity of a compound; therefore, highly sensitive analytical methods have to be used. Generally, the methods of choice are mass spectrometry, microsequencing and amino acid analysis. Sensitivity of modern automatic microsequencers is greatly improved and allows the characterization of the small quantity of compound on a single bead. Sequencing based on Edman degradation can be combined with mass spectrometry, and evaluation of mixtures generated by the degradation using mixture of phenylisothiocyanate (PITC) and phenylisocyanate (PTC) can be performed. The use of PTC does not cleave the N-terminal amino acid: this amino acid is capped and the resulting phenylcarbamoyl peptide (or peptoid) resists further degradation. Repeated cycles of this procedure provide a mixture of peptide (or peptoid) fragments differing by a single peptoid monomer unit, which can be characterized by mass spectrometry. For peptoid Edman degradation, Kodadek *et al.* noted the importance of a strong denaturing wash (with hot 1% SDS buffer) of peptoid beads prior to sequencing; this step eliminated any undesirable interference of bound proteins on sequencing reactions. With minor modifications, peptoid Edman sequencing protocol was well established using a standard peptide sequencer.^{72,76}

Using the Edman degradation, peptide/peptoid structures can be determined directly on resin without being cleaved; however, only 3–4 peptides/peptoids can be sequenced each day with an automated sequencer.^{69,77,78} Analysis of unnatural amino acids by Edman degradation is especially slow because it requires the synthesis and analysis of standards for each unnatural residue.⁷⁸ Mass spectrometry (MS) offers both high sensitivity and speed for characterizing peptoid combinatorial library. Tandem MS (or MS/MS) has been routinely used for

the sequence determination of peptides, and this technique was shown to be readily applicable to analyze peptoids.^{79,80}

Zuckermann and coworkers developed a method for the rapid identification of sequence of hit compounds from OBOC peptoid libraries.⁸¹ They used a cleavable hydrophilic linker to reduce nonspecific binding to biological samples and allows for the attachment of a halogen tag, which facilitates postscreening sequencing by tandem mass spectrometry (MS/MS). The linker is based on a tartaric acid unit and produces a C-terminal aldehyde upon cleavage from resin. Then the aldehyde can be derivatized with a bromine-containing aminoxy compound that serves as an isotope tag for subsequent MS/MS analysis. The authors showed a number of peptoids can be synthesized using the linker and demonstrated very low levels of nonspecific binding to proteins in a bead screening and sequencing step. Another strategy for rapid and robust sequencing of peptoids and peptomers from OBOC libraries was developed by Pei and coworkers.⁸² In this strategy, beads were subjected to multiple cycles of partial Edman degradation by treatment with a 1:3 molar mixture of phenyl isothiocyanate (PITC) and 9-fluorenylmethyl chloroformate (Fmoc-Cl) to generate a series of N-terminal truncation products for each resin-bound peptoid. After the partial Edman degradation step, the Fmoc groups were removed by piperidine treatment. The resulting mixture of the full-length peptoid and its truncation products was analyzed by mass spectrometry, and the full-length peptoid was sequenced. The authors showed this method was also readily applicable to peptomers (peptide-peptoid hybrids).

2.204.4.3. Screening Methods

A reliable high-throughput assay is essential to successfully screen a combinatorial library. A systematic screening of diverse compound collections has proved to be a fruitful source of a number of pharmaceutical lead compounds.⁸³ For combinatorial library screening, solid-phase and solution-phase assays have been developed. In the solid-phase assays, the ligands are still attached to the resin, and the assay involves either (1) direct binding of molecular target to the bead-attached ligand or (2) detection of functional properties of the bead-attached ligand such as identifying the activity of the ligand as a substrate to a protein. Solution-phase assays require cleavage of ligands from the beads, and the ligands have to be spatially separated such as in 96-well plates.

In peptoid research, major advances in screening technology have enabled combinatorial peptoid libraries to be mined quickly and inexpensively for specific protein binders.⁷⁶ Kodadek and colleagues focused on the discovery of peptoid binders for proteins, with the purpose of using the high-affinity binders for the construction of protein-detecting microarrays. The primary approach they used was the OBOC peptoid library screening. The screening was conducted by binding a fluorescently-labeled target protein to the OBOC peptoid library beads. After trial and error to find an optimal system for peptoid library screening, they found a Texas Red-labeled protein to a library of tens to hundreds of thousands of peptoids displayed on TentaGel beads worked well for their purpose.⁷⁷ A visual examination and identification of the beads that contained peptoids and the Texas Red-labeled protein were performed under a fluorescent microscope, and the fluorescent

beads were isolated manually using a micropipette. Technical improvement was made to increase the visual contrast between hits and nonhits by employing a biotinylated protein and detecting its binding to the bead by subsequent hybridization with a streptavidin-coated quantum dot. The intense signal from the quantum dot made it easier to distinguish hits from nonhits (autofluorescent beads), and the sequence of the peptoid on the bead was determined by Edman degradation and mass spectrometry. For the sequencing step after this on-bead screening, bound proteins were stripped from the bead by the treatment of 1% SDS solution, and the bead was subjected to an automated Edman sequencer. If peptoids are not attached on beads, the sequence can be determined by tandem mass spectrometry⁸¹ or partial Edman degradation and mass spectrometry.⁸² Recently, Kodadek *et al.* reported a new bead screening technique for the identification of specific peptoid ligands for cell surface receptors.⁸⁴ Because of the difficulty of handling membrane receptors (i.e., poor solubility and/or lack of stability when isolated), the standard screening methods developed for soluble proteins could not be easily employed for membrane receptors. Therefore, cell-based assay techniques have been developed based on the use of live cells carrying target receptor.⁸⁵ Kodadek *et al.* used a two-color, cell-based screening method to isolate peptoid hits to exhibit specificity for a membrane protein, VEGFR-2 (vascular endothelial growth factor receptor-2). They labeled cells lacking VEGFR-2 with a green quantum dot (QD), and cells carrying VEGFR-2, but were otherwise identical, were labeled with a red QD. The QDs did not contaminate the cell surface because they were taken up into cells by endocytosis. The cells were then incubated with ~300 000 OBOC beads displaying nonameric peptoids under carefully controlled conditions to minimize nonspecific adhesion of cells to the beads. The binding of cells on beads were then inspected under fluorescent microscope. Hundreds of beads were observed to bind both red and green cells implying that the peptoids were not specific ligands for VEGFR-2. Five beads bound only red cells, meaning that the peptoids were likely to be specific VEGFR-2 ligands. Subsequent *in vitro* assay confirmed the five peptoids were specific ligands for VEGFR-2 with binding affinity of low-micromolar range. Later, the authors used the hits to develop peptoid 'antibody surrogate' with extremely high specificity toward VEGFR-2 (see Section 2.204.5.1). Solution-phase assays, usually in the 96-well plate format, have been used for synthetic compounds or natural products that are added as a soluble form into each individual well for biological testing. In principle, all solution-phase biological assays including competitive receptor-binding assays, antibacterial assays, and anticancer assays can be adapted to combinatorial library screening. Because the numbers of compounds are enormous, the current screening strategy is to use miniaturized and automated solution-phase assay set-ups. Ligands are attached to the beads via cleavable linkers; then the ligands are released from the each bead into solution phase, and the biological assays are carried out. The released compounds are subsequently identified (or sequenced).

Researchers have mimicked a biological event where the genetic material encoding molecules with superior functions survives natural selection and propagates to next generations. Harbury and colleagues utilized the concept of evolutionary mechanism and DNA-programmed combinatorial chemistry to

prepare a large library of peptoids individually associated with specific DNA sequences, enabling amplification of compound hits.⁸⁶ The authors adopted the bioinspired but completely abiotic approach to prepare a collection of 100 million distinct peptoids. The library was then subjected to a selection process for binding to the N-terminal SH3 domain of the proto-oncogene Crk, and novel ligands were discovered after six amplification steps. The hits bind to the protein tightly with affinities similar to those of peptide SH3 ligands discovered from phage display libraries.

2.204.5. Drug Discovery

In general, peptides possess distinctive advantages including (1) relatively straightforward synthesis and modification; (2) high affinity and specificity of peptides for different molecular targets such as receptors, antigens, and enzymes; and (3) low toxicity relative to synthetic small molecules. However, peptide drugs often exhibit poor oral bioavailability, a short plasma half-life, a potential for immunogenicity, and unfavorable pharmacokinetic profile; therefore, considerable effort has been directed to the design and development of nonnatural surrogates for peptides. Peptoids exhibit enhanced stability toward proteolysis relative to peptides,¹⁰ significantly different lipophilicity and physicochemical properties, better cell penetration,⁸⁷ and low immunogenicity. Hence peptoids find interesting applications in biological probe development as well as in drug discovery.

2.204.5.1. Protein Receptor Ligands

2.204.5.1.1. Protein–protein interaction inhibitors

Protein–protein interactions play a crucial role in cellular and biological processes. Consequently, the chemical intervention of protein–protein interactions would enable new therapeutic possibilities. Targeting protein–protein interactions often can be a challenging task for small molecules that satisfy Lipinski's rules because of the large protein surface area to cover. High-throughput screening (HTS) programs can sometimes lead to the discovery of small molecules that are capable of disrupting certain protein–protein interactions; however, the eventual success of these compounds as drugs remains uncertain. Peptides and peptidomimetics, which are larger molecules than the small molecules, have been identified as high-affinity binders to protein–protein interaction surfaces and are currently being developed as drug candidates.

Apoptosis, a critical biological process that is strongly regulated by protein–protein complex formation, is implicated in human disease states such as cancer, ischemic injuries, and neurological disorders. Development of an apoptosis modulator through inhibition of apoptosome assembly formation has been reported by Pérez-Payá and coworkers.^{88,89} Apoptosome, a multi-protein complex consisting of cytochrome *c*, apoptotic protease activating factor-1 (Apaf-1), procaspase 9, and dATP, mediate the caspase cascade and apoptosis. From the screening of a positional scanning diversity-oriented library (~5000 peptoids) and further chemical optimization process, Pérez-Payá and coworkers identified peptoid oligomers (Figure 6, (1) and (2)) that directly bind Apaf-1 and showed the decrease in the apoptotic phenotype in mitochondrial-mediated models of

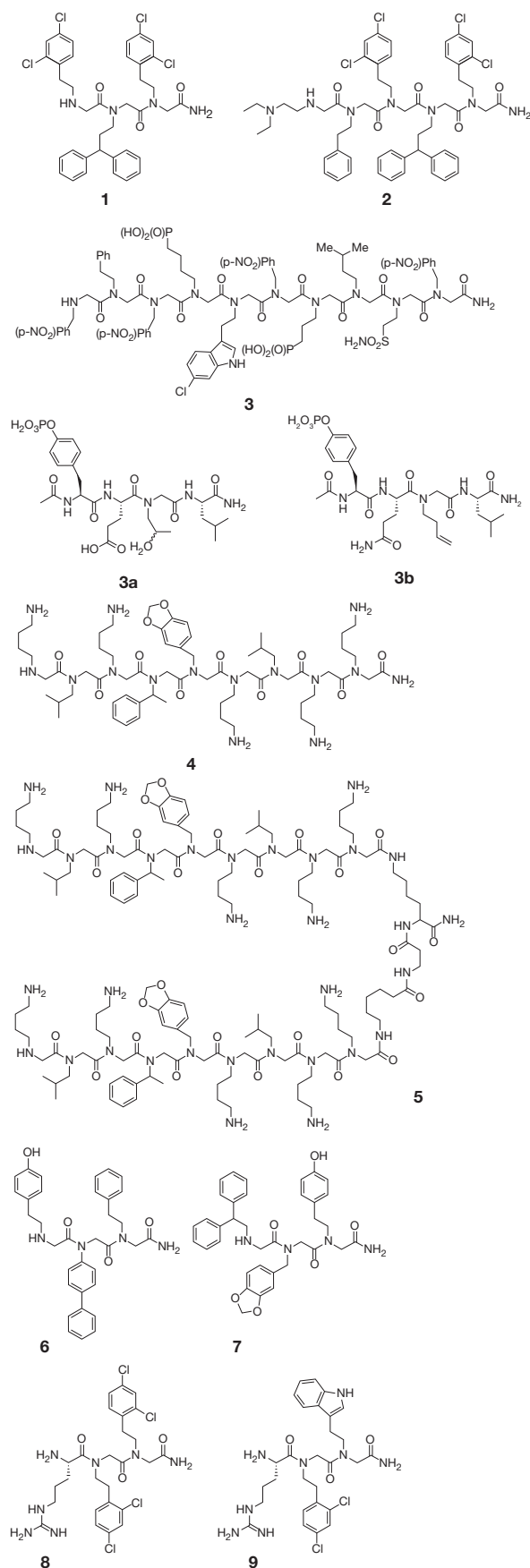


Figure 6 Peptoid ligands that bind to protein receptors.

cellular apoptosis. To increase antiapoptotic activity of **1**, the authors conjugated the peptoid trimer to poly-L-glutamic acid (pL-Glu), and the resulting peptoid-polymer conjugate not only enhanced the antiapoptotic activity but also lowered cytotoxicity of **1** in various cell lines. Further studies include conjugation of **1** to cell-penetrating peptides (i.e., penetratin and HIV-tat) and cyclization of **1**. Along with the pL-Glu-**1** conjugate, penetratin-**1** and cyclo-**1** indicated better cell permeability and efficacy in an *ex vivo* assay, and the authors are moving forward to examine the effectiveness of Apaf-1 inhibitors for *in vivo* myocardial infarction model.⁹⁰

Connections between cancer and protein 53 (or p53) have been well established.⁹¹ Protein 53 is a tumor suppressor protein that regulates the cell cycle and prevents cancer; therefore, when p53 is inactive, cells are able to grow and proliferate. Human double minute 2 (HDM2), which has emerged as an independent anticancer drug target, downregulates p53 function and inactivates the protein. Overexpression of HDM2 has been linked to tumor aggressiveness, and inhibition of HDM2 can restore p53 function and prevent tumor growth. The crystal structure of a p53 peptide bound to HDM2 provides useful information for the design of HDM2 inhibitors. Using structure-guided design, Appella and coworkers designed peptoid-based HDM2 inhibitors.⁹² To mimic the p53 peptide fragment bound to HDM2, the authors initially prepared peptoid helices as HDM2 binders. However, their best inhibitor (Figure 6, (3)) lacked a helical structure but still maintained good binding affinity against HDM2 ($IC_{50} \approx 6.6 \mu\text{M}$). Dissociation constants measured by isothermal titration calorimetry indicated that the binding affinity of **3** ($K_d \approx 1.23 \mu\text{M}$) was about a half of the p53 (15–29) peptide fragment ($K_d \approx 0.62 \mu\text{M}$). From their experience, the authors concluded that starting with rigid peptoid scaffold may not always be optimal to develop new inhibitors.

Alternatively, Kodadek and coworkers used peptoid combinatorial library screening to identify an inhibitor of HDM2 protein.⁷⁷ Constructed by standard mix-and-split synthesis on TentaGel, their peptoid libraries were composed of one- to five-hundred thousand peptoid hexamers. They used 13 peptoid submonomers with distinct functionalities, so their peptoid library was chemically diverse. On-bead screening using Texas Red-labeled HDM2 protein provided a peptoid octamer with $K_d \approx 37 \mu\text{M}$.

Src homology 3 (SH3) and WW protein interaction domains participate in diverse signaling pathways, and their functional roles in cancer, osteoporosis, and inflammation are implicated. The SH3 protein domains recognize a specific proline-rich sequence motif, PXXP, where P is proline and X is any amino acid. In addition, it is known that these domains broadly accept amide N-substituted residues instead of recognizing only the critical prolines. In comparison to natural peptides which have proline as the only N-substituted amino acid, peptoids provide enormous number of N-substituted nonnatural amino acids. Lim *et al.* exploited the diversity offered by peptoid residues, screened a series of ligands in which key prolines were replaced by nonnatural N-substituted residues, and discovered a ligand that selectively bound the Grb2 SH3 domain with 100 times greater affinity than natural peptide.⁹³ The authors carried out further variation of the peptoid residues and created highly domain-specific peptide-peptoid hybrids.⁹⁴ Members of the Src homology 2 (SH2) family have also been targeted with peptide-peptoid hybrids. The structures of the peptomer

ligands for Syk⁹⁵ and Shc⁹⁶ domains are shown in Figure 6 ((3a) and (3b), respectively). The peptomer ligands were discovered by a peptoid scan which provided a useful method to examine to what extent a peptide sequence can be transformed into a peptoid (or a peptomer) while maintaining its binding affinity.

The binding of vascular endothelial growth factor (VEGF) to the receptor VEGFR is an important signaling event in angiogenesis, and inhibition of the hormone-receptor binding can lead to a possible treatment of cancer. Monoclonal antibodies such as Avastin[®] and Erbitux[®] have shown to block the VEGF signaling pathway; however, more effective VEGFR antagonists will help increase patients survival time significantly.

Employing mix-and-split combinatorial peptoid library as a source of receptor-binding ligands, Kodadek *et al.* screened on-bead peptoid oligomers for VEGFR2 using a two-color, cell-based screening technique.⁸⁴ From the screening of $\sim 300\,000$ size peptoid library, they discovered peptoid nonamer **4** (Figure 6) with low-micromolar-binding affinity to VEGFR2 *in vitro* ($K_d = 2 \mu\text{M}$). Because VEGFR2 functions as a homodimer, the authors envisioned that homodimer of **4** would increase the binding affinity and, indeed, found homodimer **5** had a binding affinity of $K_d = 30 \text{ nM}$ for VEGFR2. The peptoid antibody surrogate was proven to be active in a mouse model and inhibited tumor growth *in vivo*. In the following study, the authors identified three side chains in the peptoid nonamer as minimally required pharmacophore and found backbone amide atoms participated in the binding with VEGFR-2.⁹⁷ Interestingly, the peptoid ligand did not bind to the anticipated site and did not compete with VEGF for binding to VEGFR-2. The authors proposed an allosteric mechanism of the peptoid antagonist that might prevent the receptor from acquiring the proper conformation to propagate downstream signals.⁹⁸

2.204.5.1.2. Receptor ligands

Highly potent peptoid ligands for 7-transmembrane G-protein-coupled receptors (7TM/GPCR) were identified from a peptoid combinatorial library.⁷³ Peptoid timers **6** and **7** (Figure 6) showed low-nanomolar-binding affinities for α_1 -adrenergic receptor ($K_i = 5 \text{ nM}$) and μ -opiate receptor ($K_i = 6 \text{ nM}$), respectively. This study was the first demonstration of the generation of high-affinity ligands for a pharmaceutically relevant receptor from a synthetic combinatorial library. The peptoid library design was based on an analysis of known GPCR ligands, which revealed that each peptoid should contain at least one aromatic hydrophobic side chain and one hydrogen-bond donating side chain. This rational design strategy lowered total number of peptoids in the library (~ 5000 peptoids), and successfully provided tight binders to GPCR receptors.

Following the Zuckermann's discovery of the peptoid GPCR ligands, a vast number of peptoids or peptomers were reported as ligands for various receptors. Adan and coworkers reported discovery of peptomers targeting the melanocortin receptors.^{99,100} The melanocortin receptors are G-protein-coupled receptors with five different isoforms, MC1R–MC5R. Particularly, MC4R are known to be an excellent drug target for the treatment of obesity. The authors used a peptoid scan to transform an MC4R selective peptide heptameric ligand into peptomers, assayed the 31 peptomers for binding toward melanocortin subtypes, and found that the peptomers generally

retained selectivity for MC4R. Although introduction of peptoid moieties resulted in a decrease of affinity, many of the peptomers were still active at submicromolar concentrations. In addition, the authors observed increased bioavailability of the peptomers when three or more of the seven peptide residues were substituted with peptoid residues.

The vanilloid receptor subunit 1 (VR1), also known as the capsaicin receptor, plays essential role in inflammatory pain in the peripheral nervous system. Ferrer-Montiel *et al.* synthesized and screened trimeric peptoid library to discover novel VR1 antagonists.¹⁰¹ Compound 8 in Figure 6 showed submicromolar potency and *in vivo* analgesic and anti-inflammatory activities. Using the scaffold for further optimization, Albericio *et al.* synthesized 20 indole-containing peptoids and evaluated the biological activity of the peptoids as novel VR1 antagonists.¹⁰² In this series, compound 9 (Figure 6) showed the best potency and selectivity: ~4-fold stronger potency and 10-fold higher selectivity than the parent peptoid 8 was obtained.

Other than the peptoid or peptomer ligands introduced here, there are a vast number of peptoids or peptomers discovered as ligands for various receptors and proteins. Cholecystokinin receptor antagonists,¹⁰³ hsst2 receptor-binding somatostatin mimics,¹⁰⁴ Tachykinin NK₃ receptor antagonists,¹⁰⁵ concanavalin A-binding oligomannopeptoids,^{106,107} P-glycoprotein-binding multi-drug resistance reversal peptoids,¹⁰⁸ NMDA (*N*-methyl-D-aspartate) receptor antagonists,¹⁰⁹ VLA-4 inhibitors,¹¹⁰ MHC-II ligands,¹¹¹ maltose-binding protein ligands,¹¹² and urokinase plasminogen activator receptor ligands¹¹³ are all included in this category. All these works demonstrated that peptoids were an excellent source of biologically active receptor ligands. Most of the compounds isolated in these efforts had potency in the low-micromolar range. But there are some lessons learned from the earlier works regarding peptoid ligand discovery: (1) Simple transformation of a known peptide ligand to a peptoid (or to a peptomer) may not always retain binding affinity of the parent peptide. Often a significant conformational change occurs upon single mutation of a peptide residue to a peptoid residue. In addition, the unavailability of backbone-NH proton in peptoid can lead to a loss of an existing hydrogen bonding interaction. (2) Peptoid ligands identified in library screening do not always bind to expected binding sites and may have different mechanism of action from that of natural ligands as was shown by Kodadek's VEGFR-2 ligand.⁹⁸ (3) Generally, peptoid library that is highly diverse, but modestly sized (i.e., 5000~10 000 compounds from 20 different amines), was sufficient to provide high-quality ligands.¹²

2.204.5.2. Nucleic Acid Binders

Antisense oligonucleotides have been used in biological studies for the determination of gene function and in medicine for the suppression of disease-related genes.¹¹⁴ More recently, synthetic molecules are being used to target RNAs. Most of the RNA targeting synthetic molecules have been discovered by high-throughput screening of a library for binding therapeutically relevant RNAs. Disney *et al.* prepared peptoid microarrays that were composed of peptoid scaffolds with moieties known to bind RNA.¹¹⁵ They screened the library for inhibiting the group I intron RNA from *Canadida albicans*, an opportunistic pathogen that kills immunocompromised host. Each peptoid

ligand identified from the screening inhibited self-splicing in the presence of 1 mM nucleotide concentration of bulk yeast tRNA with IC₅₀ values between 150 and 2200 μM. Based on the structural features of the peptoid binders, second generations of peptoids were designed and synthesized; all second generation peptoid inhibitors showed enhanced potencies with IC₅₀ values lower than 100 μM. The best peptoid in this study had an IC₅₀ of 31 μM and contained one phenylguanidino- and three indole-functionalities (1 in Figure 7). Peptoid 1 was sixfold more tightly binding to the RNA than pentamidine, a clinically used drug that inhibits self-splicing. This study is a successful example of rational library design and screening to identify a synthetic RNA binder.

The same group reported the discovery of RNA binders through rational design of peptoid RNA binders solely based on the information about the ligand-binding site in RNA. They employed modularly assembled ligands targeting the RNA that causes myotonic dystrophy (DM), an inherited disease that is characterized by wasting of muscles.^{116,117} 6'-*N*-5-Hexynoate kanamycin A is known to bind 2 × 2 nucleotide, pyrimidine-rich RNA internal loops. Multiple copies of such loops are found in the RNA hairpin that causes DM2. The authors displayed 6'-*N*-5-hexynoate kanamycin A on a peptoid scaffold with various degrees of multivalency and spacing to target several internal loops simultaneously. The multivalent display of 6'-*N*-5-hexynoate kanamycin A on a peptoid scaffold yielded a peptoid that inhibited the interaction of DM2 RNA and muscleblind protein (MBNL-1). The most potent binder displays three 6'-*N*-5-hexynoate kanamycin A modules, each separated by four spacer peptoid monomers (2, Figure 7). The peptoid showed an IC₅₀ value of 25 nM for the inhibition of the RNA-protein complex formation, and binds the DM2 RNA at least 30 times more tightly than related RNAs and 15 times more tightly than MBNL-1. In addition, they showed peptoid 2 could penetrate cell membrane by an uptake study into a mouse myoblast cell line.

2.204.5.3. Antimicrobial Agents

Over the past decades, a broad class of antimicrobial peptides (AMPs) has been identified as an intrinsic defense in animals, plants, and in a wide variety of organisms. AMPs have been of great interest to researchers not only because AMPs belong to the innate host-defense immune system, but also because they are promising candidates for the development of antibiotics. The antimicrobial activity of AMPs encompasses diverse species including Gram-positive and Gram-negative bacteria, fungi, and virus. The mechanism of action for most AMPs is permeabilization of the bacterial cytoplasmic membrane, which is facilitated by their cationic amphipathic structure.^{118,119} Another possible mechanism of AMPs is that the peptides penetrate cell membrane and target intracellular substances such as nucleic acids.¹²⁰ Both mechanisms do not necessarily involve specific receptors, so the chances for bacteria to develop resistance for AMPs are thought to be slim.

AMPs are typically short (10–50 amino acids) sequences with α-helical, β-hairpin, extended or loop structures and usually have a cationic amphipathicity. Bacterial membranes are composed of an excess of phosphatidylserine over phosphatidylcholine and are negatively charged; hence, the cationic

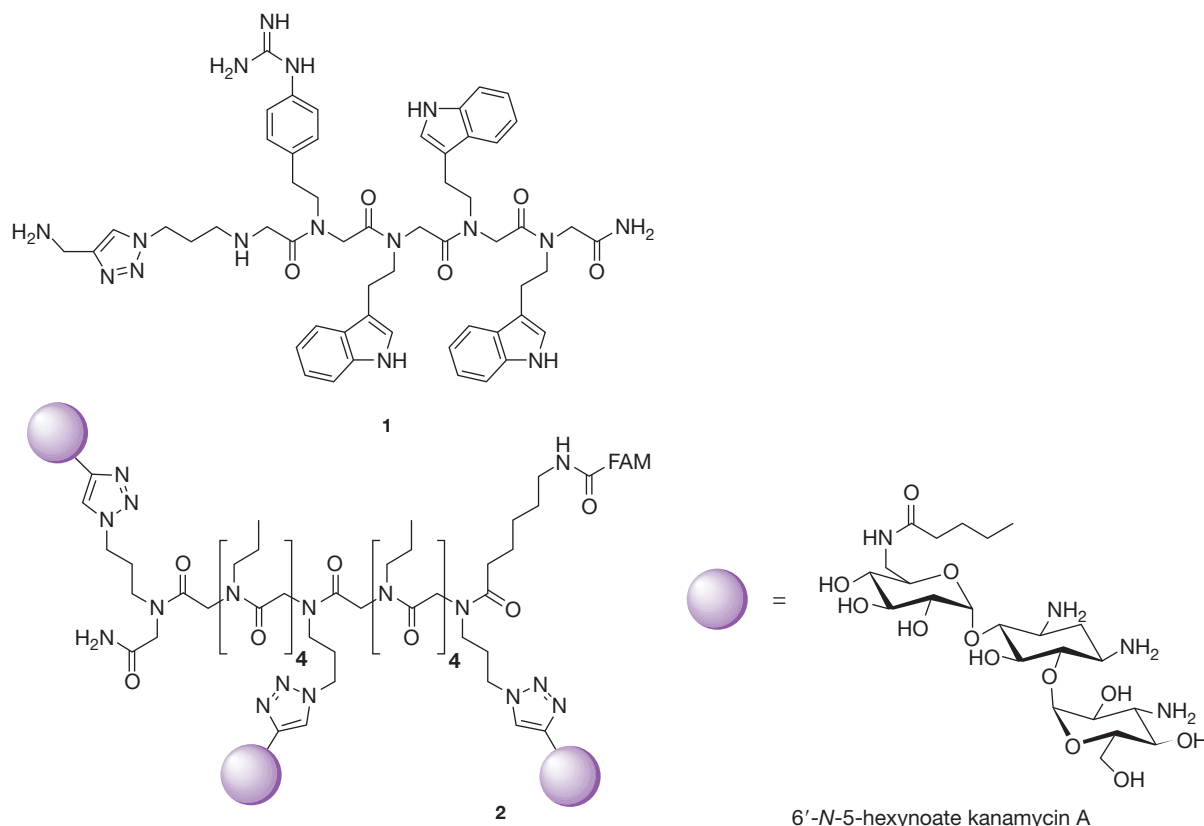


Figure 7 Peptoids that target nucleic acids.

region of AMPs provides a degree of selectivity toward the bacterial membranes over mammalian membranes. The hydrophobic portions of AMPs are believed to play a role for the insertion or penetration into the bacterial cell membrane.

Discovery of antimicrobial agents has been a very active area in peptoid research. Although AMPs possess a number of positive aspects, they have not been developed as drugs due to the poor pharmacokinetics. This problem motivates the effort to develop peptoid mimics of AMPs as antibiotics.

Magainin-2, a linear, cationic, facially amphipathic helical AMP, was successfully mimicked by Barron and coworkers using peptoid helices.^{121–124} Several members of antimicrobial peptoids or ampetoids were discovered with low-micromolar MIC (minimal inhibitory concentration) value and relatively low toxicity (1 and 2, Figure 8). Peptoid 1 is composed of a helix-inducing and hydrophobic residue, Nspe, and a cationic residue Nlys. The latter was incorporated every third position to create a cationic face on the helix (peptoid helix has a periodicity of three residues per turn). Peptoid 2, a variant of peptoid 1, was created by replacing the Nspe at position 6 with L-proline. Circular dichroism (CD) study indicated that L-proline is well accommodated in right-handed type-I polyproline-like peptoid helices, and 1 and 2 showed similar helical intensity. Compared to the parent peptoid, 2 exhibited similar antimicrobial activity while less hemolysis and less cytotoxicity were observed. Broad spectrum activities of these ampetoids against clinically relevant BSL-2 pathogens including *Staphylococcus aureus* were also shown in their study. Carefully designed structure–activity

relationship study by Barron and coworkers provides some important information: (1) excessive hydrophobicity and helicity in a peptoid leads to greater hemolysis and toxicity; (2) number of cationic charges needs to be higher than +3; and (3) selective peptoids appear to have induced helicity increase upon interaction with negatively charged membrane. These design strategy will help the development of more selective antimicrobial peptoids in the future.

Earlier than Barron's work, Winter and coworkers used combinatorial library screening to discover peptoid trimers that inhibit bacterial growth.^{125,126} The MIC of the representative peptoid (3, Figure 8) showed 5–40 μM . Excessive hydrophobicity, however, caused the peptoid to be hemolytic.

Mutations of AMPs with various peptoid residues generated selective AMP analogs.¹²⁷ Melittin (4), an active component of bee venom, is a highly potent AMP, but at the same time, it is nonselective. Melittin contains leucine zipper motif, two leucine residues and an isoleucine are positioned at 7-residue intervals, and the motif promotes dimerization of melittin. Shin and coworkers generated a series of melittin mutants by replacing Leu-6, Leu-13, and Ile-20 with Nala, Nleu, Nphe, or Nlys (5, 6, 7, 8, respectively), and investigated their secondary structure, dimerization capability, antimicrobial activity, and cell selectivity.¹²⁸ CD study demonstrated that the substitutions disrupted the helical integrity of melittin, and Nleu, Nphe, or Nlys substitution disturbed the dimerization of melittin in an aqueous media. Compounds 5–8 maintained the interaction with negatively charged bacterial membrane but lost the interaction with

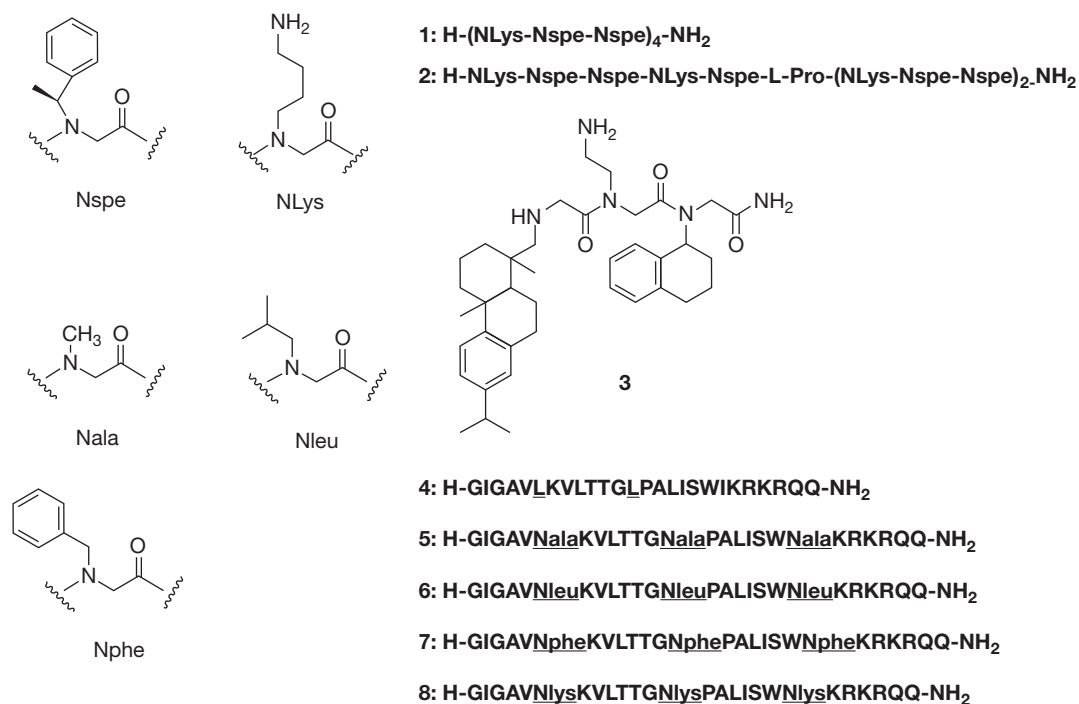


Figure 8 Peptoids with antimicrobial activity.

zwitterionic mammalian cell membrane providing significant selectivity increase. In their study, melittin analogs 7 and 8 showed the best selectivity toward bacterial cells. The authors concluded that the peptoid substitution changed melittin's mode of action from membrane-targeting mechanism to possible intracellular component targeting mechanism.

2.204.5.4. Lung Surfactants

Surfactant proteins (SP) play an important role for innate host defense and are essential for physiological lung function. Four pulmonary SP have been identified so far, known as SP-A, -B, -C, and -D. SP-B and -C are small, hydrophobic proteins that function in conjunction with lipids to reduce surface tension at the air-liquid interface of the lung.¹²⁹ A deficiency of SP-B and -C, frequently observed in premature infants, results in impaired lung function and gas exchange known as infant respiratory distress syndrome (IRDS). SP-A and -D are hydrophilic collagen-like lectins that bind oligosaccharides on the surface of microorganisms.¹³⁰ As members of innate immune system, these two proteins opsonize and aggregate bacteria and viruses; therefore, SP-A and -D enhance uptake of pathogens by immune cells such as alveolar macrophages and neutrophils. Reduced levels of SP-A and -D make the host susceptible to bacterial and viral infections.

Surfactant replacement therapy (SRT) using exogenous lung surfactant (SP-B or SP-C) has been an effective treatment for IRDS, and premature infant survival has improved significantly. However, there are drawbacks regarding the usage of the current animal-derived surfactant proteins (SP-B and SP-C) such as high production costs, batch-to-batch variability, and possible transfer of cross-species infectious agents, which

has prompted investigation into the discovery of synthetic SP preparations.

Development of synthetic and biomimetic surfactants utilizing peptoids has been actively investigated by Barron and colleagues.¹²⁹ First, sequence-specific peptoid analogs of SP-C (5-32) have been reported.¹³¹⁻¹³³ The sequences of the peptoid-based SP-C mimics were designed to adopt key structural features of the natural protein; (1) a helical and hydrophobic region and (2) an N-terminal amphipathic achiral region were incorporated. The peptoid comprising 22 monomers acted as an excellent mimic of SP-C protein (1 and 2, Figure 9). When integrated into a lipid film, the amphipathic helical peptoid SP-C mimic captures the essential biophysical surface-activity of the natural protein. The authors carried out a systematic structure-function relationship study and characterized the peptoid mimics using various biophysical characterizations including CD, Langmuir-Willhelmy surface balance, fluorescence microscopy, and pulsating bubble surfactometry. They concluded that (1) the aromatic-based SP-C peptoids have superior surface activity and film morphology than the aliphatic-based peptoids and (2) increased activity was observed upon increasing helical length.

Along with the biomimetic SP-C, Barron group actively developed peptoid-based SP-B mimics.¹³⁴ Initially, they designed and synthesized simple amphipathic peptoid helices (3 and 4, Figure 9). Surface pressure-area isotherms, surfactant film phase morphology, and dynamic adsorption behavior indicated that the peptoids were promising mimics of SP-B₁₋₂₅. The helicity and lipophilicity of the peptoids were shown to be important in the activities of the SP-B mimics. Later, the authors attempted to mimic natural SP-B protein in a more precise way: (1) the incorporation of a hydrophobic, helical insertion region with aromatic side chains showed significantly improved *in vitro*

in a dose-dependent manner. The rates of systemic clearance of 6 following intravenous administration were 60 and 104 ml min⁻¹ kg⁻¹ in rats and guinea pigs, respectively. Another peptoid trimer, CHIR-5585, which was discovered as a potent inhibitor of the urokinase plasminogen activator receptor, was also shown to be an active antagonist *in vivo*. Intranasal administration of the peptoid to rats and subsequent tissue distribution study demonstrated the significant delivery of the peptoid throughout the central nervous system and deep cervical lymph nodes.¹¹³ The results suggest that intranasal administration of peptoids may provide a way to bypass the blood–brain barrier.

2.204.6. Cellular Delivery/Uptake Vectors

2.204.6.1. Cell-Penetrating Peptoids

In recent years, methods to enhance or control selective passage of therapeutics or diagnostics through cell membrane have been actively investigated. Research in this area introduced a variety of delivery vectors and enabled the intracellular uptake of various molecular cargoes (e.g., antisense oligonucleotides, plasmid DNA, siRNA, prodrugs, peptides, proteins, imaging agents, and nanomaterials). Cell-penetrating peptides (CPPs) have become widely used vectors for such delivery and hold great potential in basic and applied biomedical research.¹³⁹ Among many CPPs, HIV-tat is the most popular CPP; the sequence responsible for the cellular uptake of the HIV-tat peptide consists of the highly basic region, amino acid residues 49–57 (RKKRRQRRR). Poly-lysine, another effective cellular transporter, is also composed of highly basic amino acids.

Using the structure–function relationships obtained with CPPs, Wender and coworkers designed a series of polyguanidine peptoid derivatives.¹⁴⁰ Significantly enhanced cellular uptake was observed for guanidine peptoid 9-mer over R9 (L-Arg 9-mer) and r9 (D-Arg 9-mer), and the stability of peptoid could explain this result (peptoid > D-peptide > L-peptide). The authors also found both the number of guanidine residues and

the length of side chain spacer affected the function of cell-penetrating peptoids; more number of guanidino groups and longer side chain spacer appeared to be beneficial for higher cellular uptake. The best cell-penetrating peptoid discovered in this study is shown in Figure 10 (compound 1).

Instead of using guanidine-containing side chains, Bradley and coworkers focused on amine-containing side chains and evaluated the polylysine-like peptoids for cellular uptake.^{141,142}

A series of fluorescein-labeled polylysine-like peptoids were synthesized, and their uptakes into HeLa, L929, and K562 cell lines were examined via flow cytometry. As was observed in guanidino-peptoids, longer cationic peptoids showed greater cellular penetration.

Bräse and coworkers compared the two types of peptoids, one with guanidino-side chains and the other with amino-side chains, and compared their cellular uptake (2 and 3, Figure 10).^{143,144} In this study, the authors found that amino-peptoid 2 required longer times to complete translocation into the cell, while the uptake rate for guanidino-peptoid 3 was much faster, and reasoned that different translocation mechanisms were involved for the two types of peptoids. Intracellular localization of the two peptoids was also different; amino-peptoid 2 resides in the cytosol, but guanidino-peptoid 3 accumulated preferentially in the nucleus. Hence, they showed the interesting possibility that the uptake rate and cellular localization of the peptoid transporter can be fine-tuned by simple modification on the peptoid side chain. Notably, no significant cytotoxicities of 2 and 3 were observed.

Membrane permeability of peptoids has led the design of peptoid-based transcription factor mimics, allowing for up-regulation of targeted genes. Kodadek and coworkers screened a peptoid library to identify oligomers capable of binding a transcriptional co-activator. A peptoid hit sequence was conjugated to a polyamide to provide specific binding to a target region of DNA.^{145,146} The biological activity of the conjugate was facilitated by the membrane permeability of cationic peptoid.

An elegant method to measure the relative cell permeability of synthetic compounds was reported by the Kodadek group.

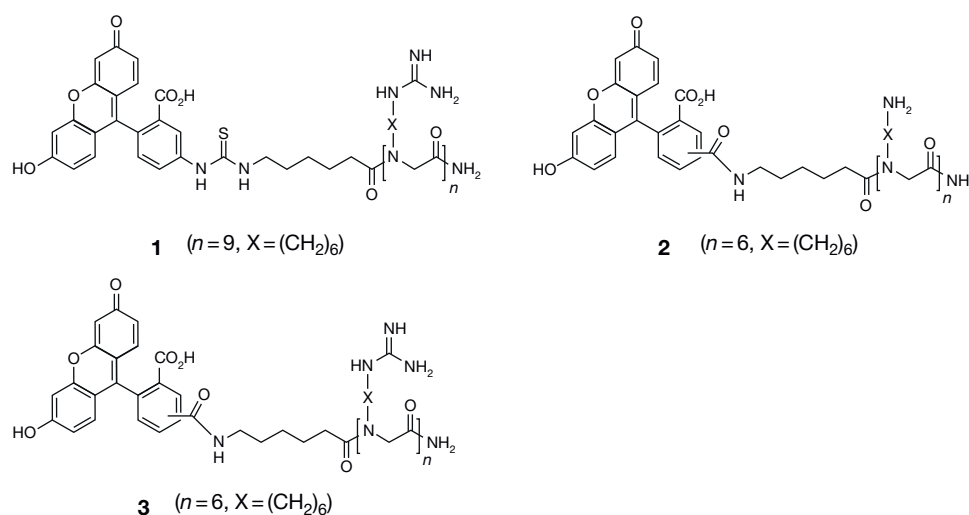


Figure 10 Peptoids with cell-penetrating activity.

The compound of interest was conjugated to a dexamethasone derivative. Upon entry of the conjugate into living cells, the Gal4-responsive luciferase gene was activated. The level of luciferase expression was therefore quantitatively proportional to the cell permeability of the conjugate. Using this reporter gene-based assay, the cell permeability of peptide and peptoid was compared,⁸⁷ and general trend showed superior cell permeability of peptoid over peptides. The authors reasoned the lack of backbone-NH protons in peptoids resulted in the increased lipophilicity and therefore membrane permeability. The authors also compared relative cell permeability of linear and cyclic peptides. Generally, cyclic peptides are thought to be more cell permeable than their linear counterparts due to the conformational rigidity. However, the authors proved that cyclic peptides were not generally more cell permeable than linear peptides.¹⁴⁷

2.204.6.2. Lipitoids for Cellular Delivery of Nucleic Acids

Although synthetic nonviral vectors have shown promise for the delivery of plasmid DNA, their efficiencies in gene transfer have not matched those of viral vectors. Therefore, the need for developing a new class of synthetic nonviral vectors is high. Zuckermann and coworkers first showed the ability of peptoids to serve as nucleic acid delivery vector.¹⁴⁸ Simple mimicry of peptide transporters (e.g., poly-lysine) did not directly lead to active gene delivery peptoids. Employing a library screening method, they found 36-mer peptoids with a specific triplet motif was the most active transfection agent. The triplet motif was composed of two hydrophobic residues and a cationic (i.e., *N*-2-aminoethyl glycine) residue. The cationic peptoids were able to form complexes with DNA and facilitated cell transfection with efficiencies similar to that of commercial cationic lipids. After the discovery, Zuckermann *et al.* systematically investigated the structure–activity relationship of cationic peptoid–lipid conjugates (or lipitoids); they prepared a small library of lipitoids and evaluated their ability as gene transfer agents.¹⁴⁹ The authors showed that several lipitoids condensed plasmid DNA into very discrete and uniform 100-nm particles and protected DNA from nuclease digestion. The best lipitoid in the study was DMPE-(Nae-Nmpe-Nmpe)₃ (**1**, Figure 11). Lipitoid **1** was active in the presence of serum and exhibited remarkably low cellular toxicity, indicating its potential for

in vivo studies. Further, **1** showed better efficiency than lipofectin or DMRIE-C, two commercial cationic lipid transfection reagents. Later, Kirshenbaum *et al.* demonstrated this reagent's effectiveness as an siRNA (short interfering RNA) transfection reagent.¹⁵⁰ The lipitoid proved to have remarkably low toxicity and be highly effective in specific siRNA-mediated gene silencing across several cell lines, including primary cells.

2.204.7. Biomimetic Materials

2.204.7.1. Collagen Mimicry

Collagen is the most abundant fibrous protein in the body. It is responsible for providing the scaffolding matrix upon which complex biological structures are supported. So mimicry of collagen and pursuit of novel collagen-like materials has been a major area of biomimetic materials research. The collagen mimics have diverse potential applications in drug delivery, biomedical devices, and tissue engineering (i.e., wound healing); therefore, major research has been performed to understand how its conformation is controlled by its typical Gly-X-Y repeats.

Goodman *et al.* first reported the incorporation of peptoid residues into the collagen-like triple helical structures. Initially, they focused on a bulky hydrophobic peptoid residue *N*-isobutylglycine (or Nleu) and introduced the submonomer as a proline surrogate.^{49,151,152} A series of peptide–peptoid hybrids were synthesized based on (Gly-Pro-Nleu), (Gly-Nleu-Pro) or (Gly-Nleu-Nleu) sequences, which were then coupled to KTA scaffold (cis, cis-1,3,5-trimethylcyclohexane-1,3,5-tricarboxylic acid, also known as the Kemp triacid).¹⁵³ Biophysical analysis revealed that the sequences (Gly-Pro-Nleu)_n and (Gly-Nleu-Pro)_n ($n \geq 9$ and $n \geq 6$, respectively) formed stable triple helices. Interestingly, (Gly-Nleu-Pro)_n formed more stable triple helices than (Gly-Pro-Nleu)_n did, and the authors explained with molecular modeling that the isobutyl side chain of Nleu could have more hydrophobic contact with Pro in triple helices composed of (Gly-Nleu-Pro)_n than in those composed of (Gly-Pro-Nleu)_n.

Unlike the other two sequences, (Gly-Nleu-Nleu)_n did not form a triple helical conformation; it had to be included in a host–guest fashion within sequences such as (Gly-Pro-Hyp)_n to adopt the triple helix conformation.¹⁵⁴ An example of the sequence is Ac-(Gly-Pro-Hyp)₃-(Gly-Nleu-Nleu)₃-(Gly-Pro-Hyp)₃-NH₂, and the guest–host structure retained triple helicity. Another sequence that behaved like Gly-Nleu-Nleu was Gly-N_x-Pro sequences where N_x was composed of a variety of alkyl peptoid residues. Using these guest–host collagen mimetic structures as model systems, the authors elucidated the contributions of steric and hydrophobic effects that are important for the triple helix formation.

2.204.7.2. Antifouling Agents

Exposure of medical devices to biological fluids is often accompanied by undesirable accumulation of proteins, cells, and microorganisms on the surface. Biofouling of surfaces can result in compromised device performance and in some cases may be life threatening to the patient. Various antifouling polymers have been used as coating materials for such medical devices and have

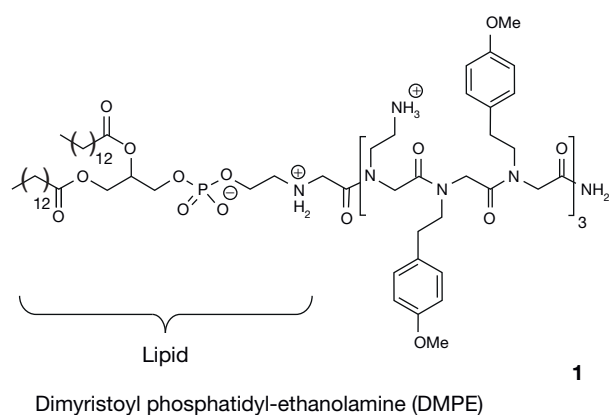


Figure 11 Lipitoid reagent for intracellular delivery of nucleic acids.

proven to be efficient in preventing protein and cell adsorption; however, few are ideal for providing long-term biofouling resistance. In this regard, Messersmith and Barron introduced a novel strategy to develop an efficient biomimetic antifouling system that was composed of a water-soluble inert peptoid and an anchor derived from mussel adhesive proteins.¹⁵⁵ The methoxyethyl side chain (Nmeg) of the peptoid portion was chosen for its chemical resemblance to the repeat unit of the known antifouling polymer poly(ethylene glycol) (PEG), and the 5-mer anchoring peptide was chosen to mimic the DOPA- and Lys-rich sequence of a known mussel adhesive protein (1 in Figure 12).

Peptide-peptoid conjugate 1 was found to be highly soluble in aqueous solution and adsorbed strongly onto TiO₂ surfaces by simple immersion process. Both modified and unmodified TiO₂ surfaces were then exposed to whole human serum, and the modified surface showed dramatic reduction of protein adsorption and resistance to mammalian cell attachment for over 5 months. Hence, these new antifouling polymers showed potential as long-term control of surface biofouling in physiological environments.

Numerous pathogenic microorganisms are capable of attaching and aggregating themselves on a surface and forming

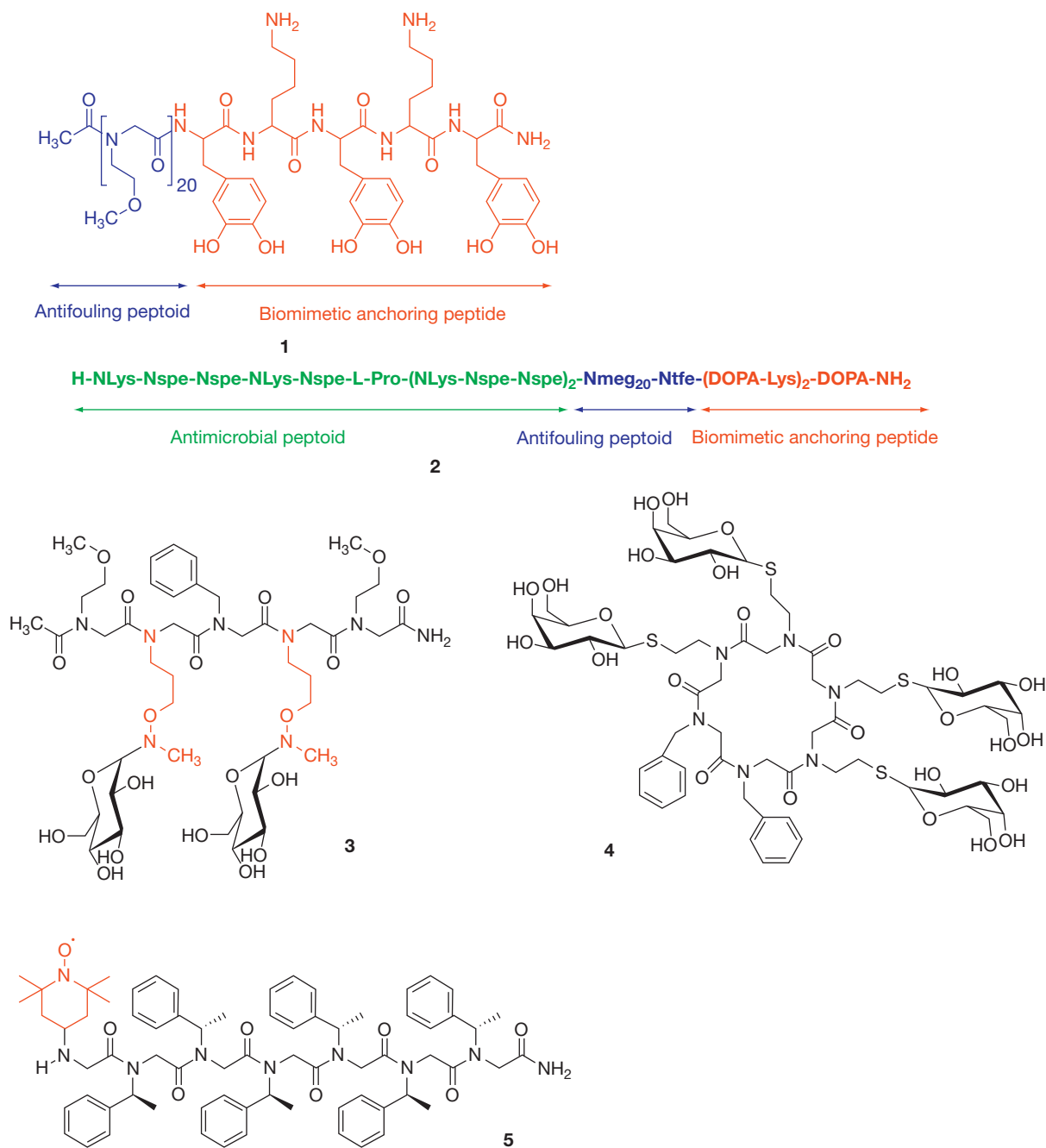


Figure 12 Peptoid-based biomaterials.

biofilm. Biofilms are known to be extremely difficult to eradicate and have been found to be involved in a wide variety of microbial infections in the body. Messersmith, Barron, and coworkers advanced their original antifouling peptide-peptoid hybrids by attaching antimicrobial peptoids at the N-terminus (2 in Figure 12).^{156,157} Surface modifications with this peptide-peptoid hybrid created a surface that was both antimicrobial (active) and antifouling (passive), and this material provided a promising solution to infections associated with implantable medical devices.

2.204.7.3. Glycopeptoids

Glycosylation, a ubiquitous posttranslational modification in proteins, plays critical roles in protein folding, stabilization, trafficking, and recognition. Owing to the inherent complexity of carbohydrates, glycosylation can produce enormous structural diversity in proteins and induce a variety of functional changes. In an attempt to decipher these structure-function relationships, protein and peptide chemists have developed various chemical and enzymatic methods for the synthesis of homogeneous and well-defined glycoconjugates. Among the glycosylation methods, the *N*-alkylaminooxy-strategy is attractive because it employs native, completely unprotected sugars to glycosylate *N*-alkylaminooxy-containing peptides.¹⁵⁸ Carrasco and coworkers synthesized an *N*-methylaminooxy-containing primary amine as a peptoid submonomer, incorporated the submonomer into various peptoid chains, and demonstrated glycosylation of the peptoids using native sugars (Scheme 1).¹⁵⁹ The authors optimized the glycosylation conditions: a mildly acidic aqueous solution (0.1 M NaOAc, pH = 4.0) and a gentle microwave heating at 40 °C for a short time (~10 min) worked well for *D*-glucose, *D*-maltose, *D*-melibiose, *D*-lactose, maltotriose, and GlcNAc. Carbohydrates that contain an axial hydroxyl group such as galactose (C4 position) or *D*-mannose (C2 position) did not provide pure glycopeptoid products; they contained a portion of furanose by-products. Next the authors showed a divalent glycopeptoid was readily prepared using microwave conditions (3 in Figure 12). The chemoselectivity of the glycosylation was also demonstrated using a peptoid that contained hydroxyl, amino, sulfhydryl, and carboxamido functionalities in addition to the *N*-methylaminooxy group. This *N*-methoxyaminooxy-strategy provides an efficient way to

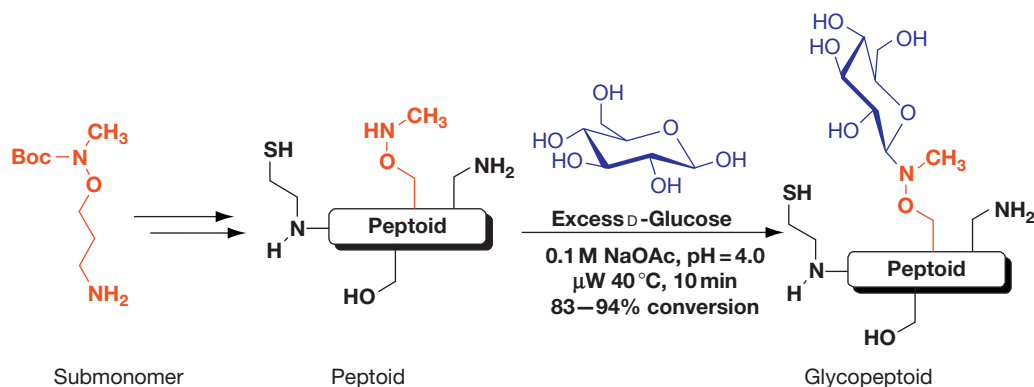
generate an extensive range of biologically functional glycopeptoids and multivalent glycopeptoids.

Another strategy of preparing glycopeptoids is to use protected carbohydrate-containing monomer units^{160,161} or submonomeric units^{106,162} as building blocks. This strategy is amenable for the synthesis of glycopeptoids that contain more than two different types of sugars in a single peptoid chain. But the potential drawback of this strategy is that a large-scale preparation of the peptoid monomers (or submonomers) can sometimes be cumbersome. Several groups reported *O*-linked, *N*-linked or *C*-linked glycopeptoid synthesis using this strategy.^{107,163–165} Also Comegna *et al.* recently reported a method for an inexpensive and rapid synthesis of linear and cyclic *S*-linked glycopeptoids (4 in Figure 12).¹⁶² These glycopeptoids provide well-defined glycopeptide or glycoprotein mimics and are useful in many applications such as lectin-binding ligands, glycopeptide antigen mimics, multivalent carbohydrate display, and antifreezing protein mimics.

2.204.7.4. Other Applications

2.204.7.4.1. Enantioselective catalysts

Proteins form tertiary structures and provide chiral microenvironments required for asymmetric catalysis. Since Whitesides pioneered the conversion of a protein to a homogeneous enantioselective hydrogenation catalyst, artificial enzymes have been developed for various asymmetric reactions.^{166,167} Mimicry of this effect with peptoid polymers has been achieved by Kirshenbaum and coworkers by positioning a catalytic center in a chiral microenvironment using peptoid helices.¹⁶⁸ Substrates that meet the spatial arrangement can access the active site and are converted to desired products. They used TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) as the catalytic center for the oxidation of secondary alcohols to ketones and incorporated this active site into either middle position or *N*-terminal position of various peptoid sequences. Interestingly, best result was obtained when TEMPO was positioned at the *N*-terminus of peptoid helix (5 in Figure 12); 84% conversion with >99% ee was achieved when 1% peptoid catalyst 5 was loaded. The reaction was carried out at 0 °C for 2 h. When TEMPO was incorporated into peptoid helix with the opposite handedness, the catalyst exhibited the opposite enantioselectivity with similar reaction conversion. This work



Scheme 1 General synthesis of glycopeptoids using *N*-methylaminooxy functionality.

provides a nice proof-of-concept study: a well-defined conformational ordering created by synthetic foldamers can mimic the asymmetric microenvironment produced by protein secondary or tertiary structures. This is an important step toward emulating protein function.

2.204.8. Summary and Future Directions

Peptoids are a bioinspired material whose properties lie in-between natural biopolymers and nonnatural synthetic polymers. Like biopolymers, they are information-rich polymers, offering precise control of main chain length, side chain functionality, and monomer sequence. Because peptoids are synthesized one monomer at a time from readily available building blocks, an almost infinite sequence-space awaits to be explored. The structural similarity to polypeptides has been responsible for the impressive diversity of biological activity observed for peptoids. The ability to precisely engineer polypeptoid structure is unprecedented for a synthetic polymer and will open up many fundamental studies in polymer physics and polymer self-assembly. The lack of a hydrogen-bond donor in the backbone holds great promise for polypeptoid-based self-assembly.

An impressive body of work on peptoids has been achieved in the past 20 years. Advances in the chemistry of peptoid synthesis have enabled a better understanding and control of peptoid secondary and tertiary structure. Computational methods to model and predict polypeptoid conformation are at an early stage, and improvements in these tools are greatly needed. Combinatorial synthesis and screening methods of peptoid libraries have been developed for a variety of functions, ranging from drug discovery and drug delivery to materials science.

Ultimately, as the understanding of peptoid conformational control, chain folding, and self-assembly continues to grow, we expect to be able to generate a new family of advanced materials that rival the structure and function of proteins. Such protein-mimetic materials would be capable of sophisticated functions like molecular recognition and catalysis, and yet would have enhanced stability to biological, chemical, and environmental stresses.

The accelerating efforts in peptoid research described in this chapter are a result of the practical nature of their synthesis, as well as in their intriguing properties. Yet the field is actually in an unusual situation; synthesis of polypeptoids is not limiting the field. These tools are well developed and easily adopted. The challenges are now centered on how to design molecules with the desired properties and activities. Combinatorial screening methods will continue to be important while the tools for prediction of peptoid structure and function catch up. It is a very exciting time for the field, as we see the immense gap between biopolymers and synthetic polymers begin to close.

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