

Lawrence Berkeley National Laboratory

LBL Publications

Title

Submonomer synthesis of sequence defined peptoids with diverse side-chains

Permalink

<https://escholarship.org/uc/item/4zn0m4nd>

ISBN

9780128212530

Authors

Connolly, Michael D

Xuan, Sunting

Molchanova, Natalia

et al.

Publication Date

2021

DOI

10.1016/bs.mie.2021.04.022

Peer reviewed

Submonomer synthesis of sequence defined peptoids with diverse side-chains

Michael D. Connolly, Sunting Xuan^a, Natalia Molchanova and Ronald N. Zuckermann*
Molecular Foundry, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, United States.

^acurrent address: College of Chemistry, Chemical Engineering and Materials Science, Soochow University, Suzhou, Jiangsu, China, 215123

Contents

1. Introduction

2. Materials

2.1 Reagents

2.2 Equipment

3. Solid-Phase Submonomer Peptoid Synthesis

3.1 Manual Submonomer Peptoid Synthesis

3.2 Automated Peptoid Synthesis

4. Peptoid Submonomers

4.1 Free-basing methods

4.2. Submonomers with Amino-acid like Functionality

4.3 Unprotected Heterocyclic Submonomers

4.4 Structure-inducing Submonomers

4.5 Ether Containing Submonomers

4.6 Lipid-like Submonomers

4.7 Peptide/Peptoid Hybrid Sequences

4.8 Peptoid Conjugation and *N*-terminal Modification

5. Cleavage and Side-Chain Deprotection

5.1 Standard Cleavage/Deprotection Method

6. Characterization and Purification

6.1 Basic Characterization Guidelines

6.2 Peptoid Purification Guidelines

7. Acknowledgements

References

Abstract

Peptoids are a diverse family of sequence-defined oligomers of *N*-substituted glycine monomers, that can be readily accessed by the solid-phase submonomer synthesis method. Due to the versatility and efficiency of this chemistry, and the easy access to hundreds of potential monomers, there is an enormous potential sequence space that can be explored. This has enabled researchers from many different fields to custom-design peptoid sequences tailored to a wide variety of problems in biomedicine, nanoscience and polymer science. Here we provide detailed protocols for the synthesis of peptoids, using optimized protocols that can be performed by non-chemists. The submonomer method is fully compatible with Fmoc-peptide synthesis conditions, so the method is readily automated on existing automated peptide synthesizers using protocols provided here. Although the submonomer synthesis for peptoids is well established, there are special considerations required in order to access many of the most useful and desirable

sidechains. Here we provide methods to include most of the amino-acid-like side chains, some of the most important non-natural monomer classes, as well as the creation of peptoid conjugates and peptide-peptoid hybrids.

1. INTRODUCTION

Oligomeric *N*-substituted glycines (a.k.a. peptoids) are a family of sequence-defined biomimetic synthetic polymers which have a history of nearly three decades.(Simon et al., 1992) Peptoids, with the side chains appended to the nitrogen rather than the α -carbon as in peptides, lack both hydrogen-bond donors and chiral centers along their backbones (Figure 1).(Sun & Zuckermann, 2013) Compared to peptides, peptoids have shown excellent properties including enhanced chemical and enzymatic stability, thermal processability, and good solubility in common solvents.(Chan et al.; Secker, Brosnan, Luxenhofer, & Schlaad, 2015; Xuan & Zuckermann, 2020a, 2020b) Also, studies have shown that peptoids exhibit good cellular permeability(Wender et al., 2000), low cytotoxicity(Xuan et al., 2017; Xuan et al., 2016), low immunogenicity(Meister, Taimoory, & Trant, 2019), and can bind to biological targets with high affinity.(Alluri, Reddy, Bachhawat-Sikder, Olivos, & Kodadek, 2003; Cai, Lee, Chiang, & Kodadek, 2011; Simon et al., 1992; R. N. Zuckermann et al., 1994) More generally, peptoids offer a versatile platform where a tremendous variety of chemically distinct side chains can be efficiently arranged into precisely controlled, information-rich sequences. The widely adopted iterative submonomer solid-phase synthesis method provides a low-cost and practical route to build peptoids from hundreds of amine synthons up to greater than 50 monomers in length. (Ronald N. Zuckermann, Kerr, Kent, & Moos, 1992) Thus, polypeptoids provide a general approach to custom design molecular attributes, properties and functions, by control of their

side-chain chemistry and monomer sequence. For these reasons, peptoids have received considerable attention from many areas of exploration spanning material science and biomedicine (Figure2).

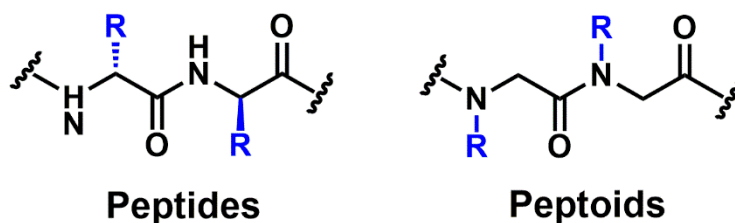


Figure 1. Peptoids share the same glycine backbone with peptides, but have their side chains appended to the nitrogen atom instead of the alpha carbon.

Since their discovery, peptoid oligomers (< 10mers) have gained considerable interest as promising drug leads. Peptoids, unlike most small molecules, can be easily synthesized using an endless pool of readily available amines, and are ideally suited for combinatorial approaches to drug discovery.(Alluri et al., 2003; R. N. Zuckermann & Kodadek, 2009) Multiple short peptoids demonstrated potency against a range of molecular and cellular targets including multi-resistant bacteria(Czyzewski et al., 2016; Khara et al., 2020; Molchanova, Hansen, & Franzyk, 2017), fungi(Spicer et al., 2019), cancer(Huang et al., 2014; Schneider et al., 2018), leishmaniasis(Eggimann, Bolt, Denny, & Cobb, 2015) and Huntington’s disease.(Chen et al., 2011) Additionally, short peptoids have demonstrated utility as diagnostics in the detection of cancer, (Hooks, Matharage, & Udugamasooriya, 2011; Matharage, Minna, Brekken, & Udugamasooriya, 2015) amyloidogenic misfolded protein and prions(Hornemann et al., 2019; Yam et al., 2011), light(Kang et al., 2017), cyanide(Lim & Lee, 2016), mercury(Knight et al.,

2017), pH(Shin & Kirshenbaum, 2007), cations(Schettini et al., 2018)^(Culf, 2019), and can serve as antifouling materials themselves(Barry, 2019). Peptoid-peptide hybrids created *via* incorporation of peptoid residues into a peptide backbone have also been reported to demonstrate high therapeutic potency(Molchanova et al., 2017; Olsen, 2010).

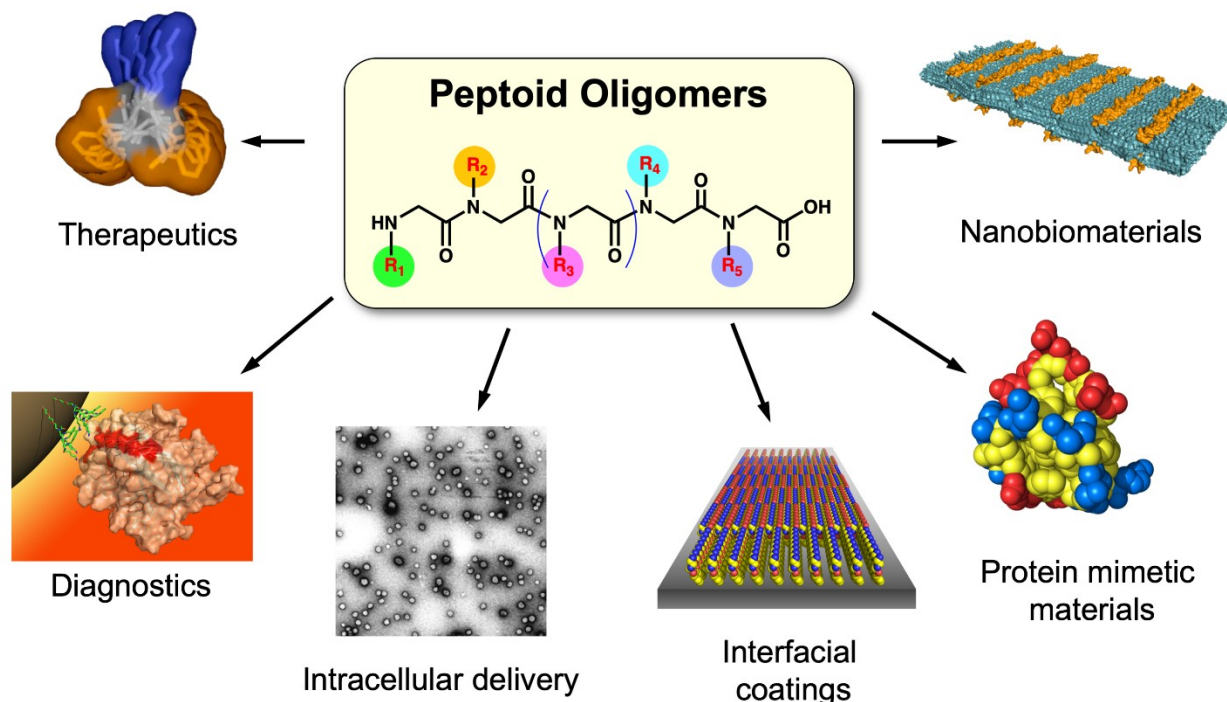


Figure 2. Due to their facile synthesis, sidechain diversity, and robustness, peptoids have been used in a wide variety of biomedical and material science applications.

Peptoids have also emerged as attractive building blocks to construct a wide array of biomimetic ordered nanostructures which are beginning to rival the structural and functional complexity found in nature.(Xuan & Zuckermann, 2020a, 2020b) Peptoids, though lacking both hydrogen-bond donors and chiral centers on the backbones, have the capacity to form single-chain folded structures, assemble into different supramolecular structures, and phase-separate/crystallize into various bulk morphologies depending on their monomer sequence and side-chain chemistry. Studies have shown that certain individual peptoid chains can fold into an array of secondary

structures including helices(Cindy W. Wu, Tracy J. Sanborn, Ronald N. Zuckermann, & Annelise E. Barron, 2001), ribbons(Crapster, Guzei, & Blackwell, 2013), and square-helices(Benjamin C. Gorske, Mumford, Gerrity, & Ko, 2017). Amphiphilic diblock copolypeptoids are well-known to phase-separate into lamellar morphologies in bulk and self-assemble in solution into a variety of well-defined nanostructures including helices(Murnen, Rosales, Jaworski, Segalman, & Zuckermann, 2010), spherical micelles(Sternhagen et al., 2018), fibers(Lee, Smart, Guo, Epps, & Zhang, 2011), vesicles(Sun, Jiang, Siegmund, et al., 2016), nanotubes(Jin et al., 2018; Sun, Jiang, Lund, et al., 2016), and nanosheets(Jin et al., 2016; Robertson et al., 2016; Xuan et al., 2019) through intra- and inter-molecular interactions. Because the peptoid backbone is comprised of tertiary amides, they can readily adopt both the *cis* and *trans* conformations in solution. However, the choice of side chain can greatly influence which isomer is preferred.(B. C. Gorske, Stringer, Bastian, Fowler, & Blackwell, 2009) For example, cationic alkyl ammonium ethyl side chains have been identified as a potent inducer of the *cis*-backbone conformation of peptoids in solution.(Wijaya et al., 2019) Interestingly, crystalline peptoids, regardless of their different side chains, almost always adopt a nearly identical rectangular crystalline lattice with extended, *cis*-backbone conformation.(Greer, Stolberg, Kundu, et al., 2018) Peptoids also have well-defined intrinsic conformational preferences that are distinct from peptides. Only two regions of the peptoid Ramachandran plots (derived from both *cis* and *trans* amide conformations) were shown to be populated by an analysis of backbone dihedral angle pairs (ϕ and ψ) in 46 high quality, experimentally determined peptoid structures reported in literatures (Figure 3).(Spencer et al., 2019)

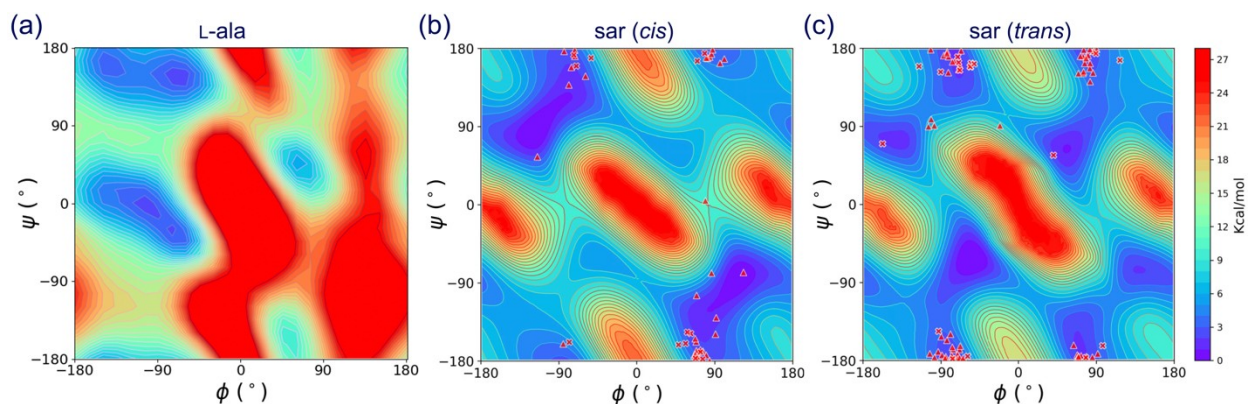


Figure 3. Comparison of the calculated conformational free energy landscape (Ramachandran plots) for (a) a model peptide Ac-L-Ala-N(Me)₂ vs. a model peptoid Ac-Sar-Sar-N(Me)₂ in both the (b) *cis* conformation, and (c) in the *trans* conformation. The red triangles in (b) and (c) indicate conformers observed in known peptoid x-ray crystal structures, and the red X's indicate conformers observed in known peptoid NMR structures. Adapted with permission (Spencer et al. 2019). Copyright 2019, Wiley.

With all the developments and discoveries in the structure, property and application of peptoids, as well as the emerging universal design rules that govern peptoid folding and assembly, it is important to present clear synthesis protocols to enable chemists and non-chemists alike, to construct peptoids tailored to any problem of interest.

2. MATERIALS

2.1 Reagents

1. *N,N'*-Dimethylformamide (DMF)
2. *N*-methylpyrrolidinone (NMP)
3. 1,2-Dichloromethane (DCM)
4. Tetrahydrofuran (THF)
5. Dichloroethane (DCE)
6. 20% (v/v) 4-methylpiperidine in DMF for Fmoc group removal

7. *N,N'*-diisopropylcarbodiimide (DIC) in DMF
8. 1-2 M amine solutions in NMP (See Section 4 Submonomer Amines below)
9. 95% trifluoroacetic acid (TFA)/2.5% triisopropylsilane (TIS)/2.5% water for deprotection and cleavage from resin.
10. Rink Amide-MBHA resin (~0.5 to 0.75 mmol/gram loading) 100-200 mesh

Note: The methods outlined here use Rink Amide-MBHA resin which results in *C*-terminal amides after cleavage. A wide variety of other resins commonly used in peptide synthesis can be employed for peptoid synthesis as well. (Culf & Ouellette, 2010) For most automated synthesis systems, 100-200 mesh resin is used so the fritted vessels are not clogged.

11. 0.25 M solution of AgClO₄ in THF
12. 1,4-diaminobutane (CAS # 110-60-1)
13. 1*H*-2-acetyldimedone pyrazole-1-carboxamidinium hydrochloride (CAS #4023-02-3)-acetyldimedone (CAS #1755-15-3)
14. O-(1*H*-6-Chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (*HCTU*) (CAS #330645-87-9)
15. 2 M formic acid in NMP
16. MALDI matrix such as α -cyano-4-hydroxycinnamic acid (CHCA)
17. Potassium hydroxide solution (KOH) in Water

2.2 Equipment

1. Fritted polypropylene syringes and caps for manual synthesis (Torviq, Tucson AZ, Part #SF-1000-LL, PC-LL) (Manual reaction vessel see section 3.1)
2. 6 mL polypropylene cartridge with 20 μ m PE frit (Applied Separations Allentown, PA part #2416) (See section 5.1 cleavage/deprotection method)

3. 20 mL glass scintillation vials with polypropylene lined caps
4. 3 mL Polypropylene disposable pipettes
5. Rocking or orbital shaker
6. Solvent evaporators (Biotage V10, Genevac HT-3)

Note: A standard rotary evaporator can be used but will not be as efficient.
7. Lyophilizer
8. Analytical HPLC or UPLC
9. C4 Analytical UPLC column such as Waters Acquity UPLC BEH300 C4, 300Å Pore Size, 1.7 µm particle size, 2.1 x 100 mm, part # 186003686
10. C18 Analytical UPLC column such as Waters Acquity UPLC Peptide BEH C18 Column, 300Å Pore Size, 1.7 µm particle size, 2.1 mm X 100 mm, part # 186004496
11. Preparative HPLC system such as Waters Prep 150 HPLC
12. C4 Preparative HPLC column such as Waters Symmetry300™ 5 µm, 19 x 100 mm
13. C18 Preparative HPLC column such as Waters Xbridge™ BEH300 5 µm, 19 x 100 mm
14. Cyano Functionalized Preparative HPLC column such as Waters XSelect™ HSS CN (Cyano) 5 µm, 19 x 150 mm.
15. 0.2 µm syringe filter PVDF (hydrophilic) or PTFE (hydrophobic) membrane, 13 mm
16. 0.45 µm syringe filter PVDF or PTFE membrane, 25 mm
17. Electrospray and/or MALDI mass spectrometer systems

18. Automated Peptide Synthesizer (such as the Gyros Protein Technologies Prelude X or Symphony X, CEM Liberty Blue, or similar)

3. SOLID-PHASE PEPTOID SYNTHESIS

Precise control over the sequence of chemically distinct monomers along the peptoid chain is a prerequisite to precisely control the peptoid structures and their associated properties. It has been nearly three decades since the first report of the submonomer solid-phase synthesis of peptoids. This efficient and robust synthetic method involves a two-step monomer addition cycle, without using any main chain protecting groups. The first step is an acylation reaction of a resin-bound amine with bromoacetic acid, and the second step is a displacement reaction with a primary amine submonomer, to iteratively and sequentially incorporate chemically distinct monomers along the chain. This synthetic strategy is greatly simplified compared to the use of fully-protected monomer units. (Simon et al., 1992) Owing to the cheap, readily accessible and diverse family of primary amine synthons, a vast variety of peptoids with diverse side chains and precisely controlled sequences can be easily approached. The beauty of this method is the designability, robustness and simplicity, as well as the accessibility to a wide array of diverse peptoid sequences with nearly monodisperse molecular weight distribution. The standard submonomer method requires certain reactive side chains to be protected with common acid-cleavable protecting groups (e.g., boc and *tert*-butyl) and is thus fully compatible with Fmoc solid-phase peptide synthesis to build peptoid-peptide hybrids. With the advances in peptoid science and technology, a myriad of peptoid sequences, including the ones with anionic and cationic monomers, chiral monomers, heterocyclic monomers, functional monomers and superhydrophobic monomers, have been synthesized using the submonomer solid-phase

synthesis method with modification and optimization accordingly. Since some of these monomers require modifications to the standard submonomer synthesis conditions, we present here detailed synthesis protocols to enable chemists and non-chemists alike to readily incorporate these diverse monomers into sequences of their own design.

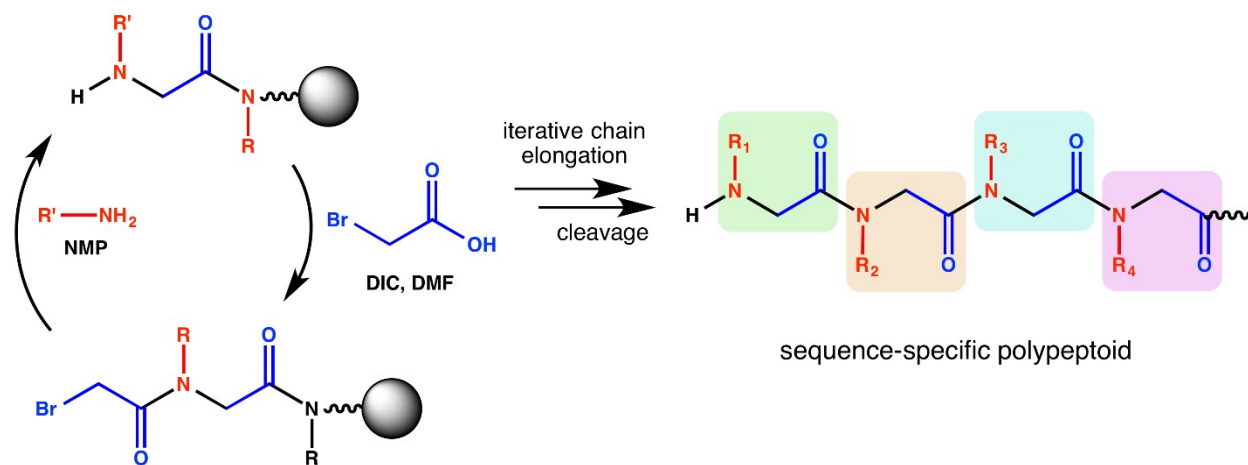


Figure 4. Sequence-defined peptoids are made by the solid-phase submonomer method, using a simple two-step monomer addition cycle from cheap, stable precursors, at room temperature in open vessels. The first step is an acylation of a resin-bound amine with a haloacetic acid, and the second step introduces the sidechain via a halogen displacement reaction with a primary amine. The method is general for hundreds of amines, yet subtle modifications are required for certain monomer classes. Reprinted with permission from Sun & Zuckermann, 2013. Copyright 2013 American Chemical Society.

3.1 Manual Submonomer Peptoid Synthesis

Note: All work with hazardous chemicals should be performed in a chemical fume hood with appropriate personal protective equipment. DMF and DCE are reasonably suspected carcinogens. *N,N'*-diisopropylcarbodiimide (DIC), 4-methylpiperidine, and bromoacetic acid are hazardous to the skin, eyes and respiratory tract. DIC may be toxic if inhaled or absorbed through the skin and may result in sensitization.

The method below is appropriate for a 50 μmol scale and is conducted at room temperature in air. All reagents were used without further purification. Once the resin-bound Fmoc group is removed, peptoid synthesis proceeds from the initial *C*-terminal residue to the final *N*-terminal residue by repeating the acylation and displacement steps.

1. Add 83 mg of Rink amide-MBHA resin (0.6 mmol/g loading) to a fritted syringe reaction vessel. Swell the resin by adding 2 mL of DMF. Agitate by rocking or shaking for 30 minutes. Drain the DMF from the syringe to isolate the swelled resin.
2. Fmoc removal: Add 1 mL of 20% 4-methylpiperidine in DMF (v/v) to deprotect the resin bound Fmoc group. Agitate for 2 minutes and drain. Repeat with an additional 1 mL of 20% 4-methylpiperidine in DMF (v/v) with a 12 minute incubation.
3. Wash the resin by adding 2 mL of DMF, agitating for 1 minute, draining. Repeat for a total of 3x.
4. Acetylation: Add 1 mL of 0.8 M bromoacetic acid in DMF followed by 1 mL of 0.8 M *N,N'*-diisopropylcarbodiimide (DIC). Cap the syringe and incubate with rocking for 20 minutes, then drain and wash with DMF (5 x 2 mL).
5. Displacement: Add 1 mL of 1-2 M amine in NMP. Incubate with rocking for 30-120 minutes, then drain and wash with DMF (4 x 2 mL).
6. Repeat acetylation step 4 and displacement step 5 in turn to elongate peptoid sequence. To pause during peptoid synthesis finish a displacement step wash with 3 x 2 mL dichloromethane, cap and store at 4°C. It is recommended that the synthesis is not paused after the second displacement due to a competing diketopiperazine side product reaction.(Figliozi, Goldsmith, Ng, Banville, & Zuckermann, 1996) To continue growing the peptoid chain, first re-initiate the synthesis by re-swelling the dried resin in 2 mL of DMF for 10 min.

7. After the final displacement is complete, wash with DMF (5 x 2 mL), then DCM (3 x 2 mL).
Cap and store reaction vessel until ready for cleavage.

3.2 Automated Peptoid Synthesis

Due to the good solubility and stability of the reagent solutions used, and their tolerance to air exposure, the submonomer method can be readily adapted to commercially available peptide synthesis instrumentation. In practice, the chemistry can be implemented on these machines such that there is a very close match to the manual synthesis conditions. One difference is that in an automated run, all the reagents must be prepared ahead of time and be stable for hours to potentially days. Since, like peptide synthesis, the submonomer method uses a repeating two-step monomer addition cycle, any existing peptide synthesizer software can be slightly modified to accommodate the peptoid chemistry. Researchers have adapted the submonomer method to many standard commercial peptide synthesizers, such as the Aapptec Apex 396, CEM Liberty Blue microwave synthesizer, and Gyros Peptide Technologies Prelude X and Symphony X systems.

In preparation for an automated synthesis, the instruments are setup with submonomer amine solutions placed in the containers typically used for amino acid solutions. Most submonomer solutions and reagents are stable for several days at room temperature over the duration of an extended synthesis provided they stay soluble in solution. All needed reagents, bromoacetic acid, DIC, deprotection agent 4-methylpiperidine, and wash solvents DMF and DCM are loaded into the synthesizer reagent containers. Extra reagent may be consumed due to priming and dead volumes in the system, so enough reagent should be prepared initially. Syntheses are typically performed at room temperature, but the submonomer chemistry can be performed with significantly shorter times at elevated temperatures.(Burkoth, Fafarman, Charych, Connolly, &

Zuckermann, 2003; Wijaya et al., 2019) Table 1 provides a generic method sequence for an automated peptoid synthesizer appropriate for a 50-100 μmol scale. The start cycle is performed to swell the resin and remove the resin-bound Fmoc group. The peptoid coupling cycle is repeated for each peptoid monomer addition in the sequence starting at the *C*-terminus and proceeding to the *N*-terminus of the sequence. The end sequence includes final washes and resin drying steps performed at the conclusion of the synthesis. Additional synthesizer specific steps may be required for system initialization and setup.

Table 1. Submonomer synthesis methods adapted for use on commercial automated peptide synthesizers.

Automated Synthesis - Synthesis Start Cycle:

STEP	OPERATION	REAGENT	REACTION TIME (HH:MM:SS)	VOLUME (ML)	DRAIN	REPETITION
1	Swell Resin	DMF	00:10:00	3.0	Yes	3
2	Fmoc Deprotection 1	20% 4-Methylpiperidine in DMF	00:00:30	2.0	Yes	1
3	Fmoc Deprotection 2	20% 4-Methylpiperidine in DMF	00:12:00	2.0	Yes	1
4	Wash	DMF	00:00:30	2.5	Yes	6

Automated Synthesis - Peptoid Coupling Cycle:

STEP	OPERATION	REAGENT	REACTION TIME (HH:MM:SS)	VOLUME (ML)	DRAIN	REPETITION
1	Bromoacetylation	0.8 M Bromoacetic Acid in DMF	00:00:00	1.0	No	1
2		0.8 M DIC in DMF	00:20:00	1.0	Yes	1
3	Wash	DMF	00:00:30	2.5	Yes	6
4	Displacement	1.0 M Submonomer Amine	01:00:00	1.0	Yes	1
5	Wash	DMF	00:00:30	2.5	Yes	6

Automated Synthesis - Synthesis End Cycle:

STEP	OPERATION	REAGENT	REACTION TIME (HH:MM:SS)	VOLUME (ML)	DRAIN	REPETITION
1	Base Wash	20% 4-Methylpiperidine in DMF	00:15:00	2.0	Yes	1
2	Wash	DMF	00:00:30	2.5	Yes	6
3	Wash	Dichloromethane	00:00:30	2.5	Yes	6
4	Drain to Dry Resin		00:10:00		Yes	1

4. SUBMONOMER AMINES

A wide diversity of amines have been incorporated into peptoids through the submonomer synthesis method.(Culf & Ouellette, 2010) The majority of amines routinely used in peptoid synthesis are commercially available in quantities required at a reasonable cost. Most amines can be used as provided without any additional purification. Some submonomer amines that have reactive side-chain groups are not commercially available in their protected form, and their preparation requires the addition of a protecting group through one or two-step methods available in the literature.(Wuts, Greene, & Greene, 2014)

The standard peptoid synthesis conditions described above have been successfully used with the great majority of submonomers, however, there are several important classes of side chains that require distinct synthesis conditions to incorporate them efficiently. These modified protocols are noted and described below.

4.1 Free-basing Methods

Some amines are provided by the supplier in their protonated salt form (e.g. hydrochloride). These salts are often solids and are more stable for long-term storage. However, submonomer amine solutions need to be in the free base form. This requires a free-basing step involving the addition of base and removal of the resulting salt. This can be done by a liquid-liquid extraction process followed by solvent evaporation, or by an *in situ* neutralization/decanting process. The extraction method is more controlled and versatile since the purified free-base is isolated. The *in situ* method does not allow isolation of the free base, but it is a faster and simpler method and is sufficient for many amine hydrochlorides.

4.1.1 Free-basing of Amine Salts by Liquid Extraction:

1. Prepare KOH solution by dissolving KOH (0.071 mol) in 20 mL of water.

2. Dissolve the amine salt (0.071 mol) in 20 mL of the KOH solution.
3. Transfer the solution into an appropriately-sized separatory funnel.
4. Wash the aqueous phase with DCM (3 x 60 mL). Collect DCM fractions.
5. Dry combined fractions over Na_2SO_4 (5-10 min).
6. Filter DCM solution from Na_2SO_4 (into a pre-tared round flask and evaporate on a rotary evaporator).

Note: Losses of ~30% are typical so sufficient excess material should be used in this process.

4.1.2 *In situ* free-basing of amine salts:

1. Prepare a 1M suspension of desired amine salt in 13.5 mL DMF in a 50 mL Falcon tube.
2. Prepare a 50% (w/w) KOH solution in 0.72 mL water.
3. Quickly add KOH solution to the amine suspension, cap and vortex vigorously. A precipitate typically forms.
4. Optionally, sonicate the suspension in a warm water bath for 10 min.
5. Centrifuge the solution to pellet the precipitated KCl/water.
6. Carefully decant the supernatant to provide a ~1M free based amine solution in DMF. This solution can be used directly as the submonomer solution in the displacement step.

Note: If the solution is cloudy after centrifugation, filter the solution using a syringe and 0.45 μm PVDF filter before use.

4.2 Submonomers with Amino-acid like Functionality

Fundamental to the field of peptoid science is biomimicry. Thus, there is great interest in incorporating peptide monomers that recapitulate the functional groups found in the natural amino acids. Fortunately, many amino acid side chains can be directly incorporated as amines,

whereas others require using a slightly analogous structure to readily availability or stability.

Mimicry of the side chain functionality found in the amino acids is possible using the submonomers summarized in Table 2. Other choices are possible, but these are among the most commonly implemented in the field.

Table 2. Amino acid-like submonomer amines.

Sidechain (R)	Protection	Submonomer Amine	CAS #	Amino Acid Mimic	Method Note
-CH ₃		Methylamine	74-89-5	Ala	3.1*
-CH ₂ CH ₂ CH ₂ N=C(NH ₂) ₂				Arg	4.2.1
-CH ₂ CO ₂ H	<i>t</i> -butyl	Glycine <i>tert</i> -butyl ester hydrochloride	27532-96-3	Asp	4.1
-CH ₂ CH ₂ CO ₂ H	<i>t</i> -butyl	β-Alanine <i>tert</i> -butyl ester hydrochloride	58620-93-2	Glu	4.1
-CH ₂ CONH ₂		Glycinamide Hydrochloride	1668-10-6	Asn	4.1
-CH ₂ CH ₂ -(4-imidazolyl)		Histamine	51-45-6	His	4.3
-CH ₂ CH(CH ₂) ₂		Isobutylamine	78-81-9	Leu	3.1
-CH ₂ CH ₂ NH ₂	Boc	<i>tert</i> -Butyl <i>N</i> -(2-aminoethyl)carbamate	57260-73-8	Lys	3.1
-CH ₂ CH ₂ CH ₂ CH ₂ NH ₂	Boc	<i>tert</i> -Butyl <i>N</i> -(4-aminobutyl)carbamate	68076-36-8	Lys	3.1
-CH ₂ C ₆ H ₅		Benzylamine	100-46-9	Phe	3.1
-CH ₂ CH ₂ OH	TBDMS	2-(<i>tert</i> -Butyldimethylsilyloxy)ethanamine	101711-55-1	Ser	3.1
-CH ₂ CH ₂ -(3-indolyl)		Tryptamine	61-54-1	Trp	4.3
-CH ₂ CH ₂ - <i>p</i> -C ₆ H ₄ OH	<i>t</i> -butyl	2-(4-(<i>t</i> -Butoxy)phenyl)ethan-1-amine	157981-64-1	Tyr	3.1
-CH ₂ CH ₂ SH	Trityl	S-Tritylcysteamine hydrochloride	15297-43-5	Cys	4.1
-CH ₂ CH ₂ SCH ₃		2-(Methylthio)ethylamine	18542-42-2	Met	3.1
-CH(CH ₃) ₂		2-Aminopropane	75-31-0	Val	3.1
-CH(CH ₃)CH ₂ CH ₃		<i>sec</i> -butylamine	13952-84-6	Ile	3.1

*Used in standard method as a 40% Methylamine solution in water for displacement reaction.

4.2.1 Arginine-like Monomer Addition: Guanidino Group Incorporation

Protected guanidinopropyl amine submonomer have been used to incorporate arginine like residues into peptoids.(Uno, Beausoleil, Goldsmith, Levine, & Zuckermann, 1999) However, poor solubility and low coupling efficiency coupled with the lack of commercial availability have

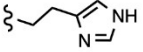
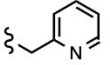
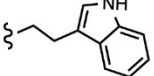
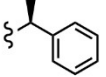
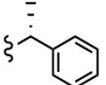
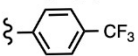
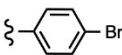
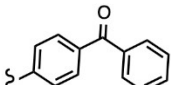
limited this approach. More recent methods have been developed where amino groups are introduced into the growing peptoid, which are then guanidinylated on-resin just prior to cleavage. The method below was developed to selectively add the guanidino group on resin using an orthogonal deprotection strategy with Dde-OH (2-acetyldimedone). (Bolt & Cobb, 2016)

Note: This method is at 100 μ mol resin scale.

1. To include a guanidine functionalized monomer, following a standard acetylation reaction with bromoacetic acid, perform a displacement step by adding 1 mL of 1.5 M unprotected diamine such as 1,4-diaminobutane (CAS # 110-60-1). Incubate with rocking for 60 minutes, then drain and wash with DMF (3 x 2 mL).
2. Protect the newly added diamine by adding 0.5 mL of 2-acetyldimedone (0.2g, 1 mmol) in DMF. Incubate with rocking for 1 hour at room temperature.
3. Drain and wash the resin with DMF (3 x 2 mL).
4. Perform additional couplings to complete the sequence following standard methods.
5. Deprotect the Dde by adding 2 mL of a 2% hydrazine solution in DMF (v/v) incubate with rocking for 3 min. Repeat for a total of 4 times.
6. Guanidinylate the free amines, on resin, by adding 6 equivalents of 1*H*-pyrazole-1-carboxamide hydrochloride per free amine and 6 equivalents of DIPEA per free amine in the minimum volume of DMF. Incubate with rocking for 1 hour at room temperature.

7. After the final displacement is complete, wash with DMF (5 x 2 mL), then dichloromethane (DCM) (3 x 2 mL). Cap and store reaction vessel until ready for cleavage.

Table 3: Heterocyclic, chiral and *N*-aryl peptoid submonomers.

Sidechain (R)	Protection	Submonomer Amine	CAS #	Method Note
Heterocyclic 	--	Histamine	51-45-6	4.3
	--	2-Picolylamine	3731-51-9	4.3
	--	Tryptamine	61-54-1	4.3
Chiral 	--	(<i>S</i>)-(-)-alpha-Methylbenzylamine	2627-86-3	4.4.1
	--	(<i>R</i>)-(+)-alpha-Methylbenzylamine	3886-69-9	4.4.1
<i>N</i>-Aryl glycines 	--	4-trifluoromethylaniline	455-14-1	4.4.2
	--	4-bromoaniline	106-40-1	4.4.2
	--	4-aminobenzophenone	1137-41-3	4.4.2

4.3 Unprotected Heterocyclic Submonomers

Most amines that contain heterocyclic nitrogen in the side chain do not incorporate efficiently. This is thought to be due to irreversible alkylation of these nitrogens during each acetylation step. However, this alkylation can be dramatically reduced in many cases by using chloroacetic acid instead of bromoacetic acid in the acylation step. The method below can be used to

efficiently incorporate unprotected imidazoles, pyridines, pyrazines, indoles, and quinolines (see Table 3). However, this alkylation can be dramatically reduced in many cases by using chloroacetic acid instead of bromoacetic acid in the acylation step. The method below can be used to efficiently incorporate unprotected imidazoles, pyridines, pyrazines, indoles, and quinolines (see Table 3). (Burkoth et al., 2003)

Note: This method is at 50 μ mol resin scale. The standard submonomer synthesis method with bromoacetic acid can be used for couplings prior to the first unprotected heterocycle addition. However, chloroacetic acid conditions should be used for the first heterocycle and all couplings that follow.

1. Acetylation: Add 850 μ L of 0.4 M chloroacetic acid (0.34 mmol) in DMF and 200 μ L of 2 M DIC (0.4 mmol) in DMF. Cap the syringe and incubate with rocking for 5 minutes, then drain and wash with DMF (5 x 2 mL).
2. Displacement: Add 850 μ L of 2 M amine in NMP. Incubate with rocking for 60 minutes, then drain and wash with DMF (4 x 2 mL).

Note: If the C-terminal position in the sequence is a monomer requiring a chloroacetic acid acetylation extend the displacement time to 180 min to ensure completion.

2. Repeat acetylation step 1 and displacement step 2 to elongate the peptoid sequence.
3. After the final displacement is complete, wash DMF (5 x 2 mL), then DCM (3 x 2 mL). Cap and store reaction vessel until ready for cleavage.

4.4 Structure-inducing Submonomers

4.4.1 Chirality

Though peptoids lack both hydrogen bonding donors and chiral centers along the backbone, they have the capacity to adopt stable helices in organic and aqueous solution, where all the backbone

amides are in a *cis* conformation. Monomers with α -chiral aromatic side chains, such as (*S*)- or (*R*)-*N*-1-phenylethyl and (*S*)- or (*R*)-*N*-1-naphthylethyl are common helix-inducing units. (Sanborn, Wu, Zuckerman, & Barron, 2002; Stringer, Crapster, Guzei, & Blackwell, 2011; C. W. Wu, T. J. Sanborn, R. N. Zuckermann, & A. E. Barron, 2001) Chiral aliphatic side chains, such as (*S*)-*N*-1-*tert*-butylethyl, (*S*)-*N*-(1-cyclohexylethyl and (*S*)-*N*-*sec*-butyl, have also been shown to induce and stabilize α -helices of peptoids driven by the steric influence of the bulky, chiral side chains.(Rzeigui et al., 2020; C. W. Wu et al., 2001)

Chirality is often introduced at the alpha position of a sidechain (Table 3). This introduces some steric hinderance and in some cases acid sensitivity. The (*S*)- or (*R*)-*N*-1-phenylethyl side-chain have been well utilized because they incorporate in excellent yield. The incorporation of these submonomers in this class may be sluggish (due to the alpha branching) and may benefit from elevated reaction temperatures.

Note: *N*-1-phenylethyl and *N*-1-naphthylethyl side chains are sensitive to extended acid exposure. TFA cleavage and deprotection times should be minimized to prevent loss of these side-chains. Rapid removal of TFA using an evaporator like the Biotage V10 is helpful.

4.4.2 *N*-Aryl Glycines

N-aryl glycines, where the phenyl ring is directly attached to the peptoid backbone nitrogen, have been shown to strongly induce a *trans* backbone amide bond geometry.(Crapster et al., 2013; Paul et al., 2012) The standard submonomer synthesis methods work for electron rich aniline submonomers such as 4-ethylaniline and 4-methoxyaniline with displacement times of 1 to 3 hours. However, standard methods often fail due to the sluggish reactivity of weakly

nucleophilic electron poor aniline submonomers such as 4-trifluoromethylaniline, 4-bromoaniline, and 4-aminobenzophenone (Table 3). In those cases, a simple method that is often effective is to co-dissolve potassium iodide in the amine solution. (Burkoth et al., 2003; Wijaya et al., 2019) A modified method that adds halophilic silver salts to the displacement reaction facilitates bromide abstraction and AgBr precipitation dramatically accelerating the displacement reaction for these electron poor aniline submonomers. (Proulx, Yoo, Connolly, & Zuckermann, 2015)

Note: Some automated peptide synthesizers may not be chemically compatible with THF. Check with the instrument manufacturer.

Modified method for the addition of electron poor aniline submonomers

Note: This method is at 60 μmol resin scale.

1. Following the standard method acetylation step, wash the resin with DMF (3 x 2 mL) followed by THF (2 x 2 mL).
2. Add 2.5 mL of a 1.5 M of the aniline submonomer in THF
3. Add 710 μL of a 0.25M solution of AgClO_4 in THF (3 equivalents).
4. Incubate the reaction for 1 hour with rocking.
5. Wash with DMF (3 x 2 mL)
6. If the next residue is a peptoid add 1 mL of 0.8 M bromoacetic acid in DMF and 1 mL of 0.8 M DIC. Cap the syringe and incubate with rocking for 20 minutes. Repeat this step.
7. If the next residue is a standard submonomer use standard displacement conditions and proceed with the synthesis detailed in the standard submonomer method.

4.4.3 Submonomers that Induce *cis* Amide Conformation

A number of side chains have been incorporated into peptoids to induce the *cis* amide backbone conformation. A common approach to favor the *cis* amide conformation is to use bulky, α -branched side-chains that disfavor steric interactions present in the *trans* conformation. Side-chains in this category include 1-naphthylethyl, *tert*-butyl, (benzyltriazolyl)ethyl.(Caumes, Roy, Faure, & Taillefumier, 2012; B. C. Gorske et al., 2009; Roy et al., 2013) Another class of side chains leverages attractive intramolecular interactions between the amide carbonyl oxygen and the side-chain moiety to stabilize *cis*-amide isomers, such as the 4-methylpyridinium side chain. (B. C. Gorske et al., 2009) Cationic alkyl ammonium ethyl side-chains exhibit potent enforcement of the *cis*-amide backbone using an ensemble of weak intramolecular CH-O and/or NH-O hydrogen bonds between the side-chain and the backbone carbonyl moieties.(Wijaya et al., 2019)

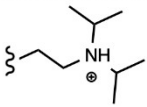
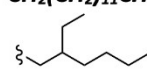
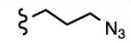

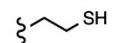
Cationic alkyl ammonium ethyl amine submonomer (*N,N'*-diisopropylethylene diamine) coupling

Note: Chloroacetic acid with a short reaction time is used for the acetylation step for the residue following the incorporation of the *N,N'*-diisopropylethylene diamine submonomer to avoid the formation of a cyclized ketopiperazine product. Bromoacetic or chloroacetic acid acetylation can be used for the incorporation of the initial *N,N'*-diisopropylethylene diamine residue in the sequence.

1. Wash/swell the resin by rocking with 2 mL of NMP for 15 min. Repeat.

2. Acetylation: Add 0.5 mL of 2 M DIC in NMP and 0.5 mL of 2M chloroacetic acid in NMP. Cap the syringe and incubate with rocking for 2 minutes, then drain. Wash with NMP (3 x 2 mL).
3. Displacement: Add 1 mL of 1 M *N,N'*-diisopropylethylene diamine in NMP. Incubate with rocking for 60 minutes, then drain and wash with NMP (3 x 2 mL).
4. Acetylation: Add 0.5 mL of 2M chloroacetic acid in NMP, and 0.5 mL of 2 M DIC in NMP. Cap the syringe and incubate with rocking for 2 minutes, then drain. Wash with NMP (3 x 2 mL).
5. Displacement step appropriate for next residue.

Table 4. Submonomers for *cis* induction, ether and lipid incorporation, and conjugation.

Sidechain (R)	Protection	Submonomer Amine	CAS #	Method Note
Cationic alkyl ammonium ethyl 	--	<i>N,N'</i> -Diisopropylethylenediamine	121-05-1	4.4.3
Ether containing				
-CH ₂ CH ₂ OCH ₃	--	Methoxyethylamine	109-85-3	4.5
-(CH ₂ CH ₂ O) ₂ CH ₃	--	2-(2-Methoxyethoxy)ethylamine	31576-51-9	4.5
-(CH ₂ CH ₂ O) ₃ CH ₃	--	2-(2-(2-Methoxyethoxy)ethoxy)ethylamine	-	4.5
Lipid-like				
-CH ₂ (CH ₂) ₅ CH ₃	--	Heptylamine	111-68-2	4.6
-CH ₂ (CH ₂) ₆ CH ₃	--	Octylamine	111-86-4	4.6
-CH ₂ (CH ₂) ₈ CH ₃	--	Decylamine	2016-57-1	4.6
-CH ₂ (CH ₂) ₁₀ CH ₃	--	Dodecylamine	124-22-1	4.6
-CH ₂ (CH ₂) ₁₁ CH	--	Tridecylamine	2869-34-3	4.6
	--	2-Ethyl-1-hexylamine	104-75-6	4.6
Peptod conjugation				
	--	3-azido-1-aminopropane	88192-19-2	4.8
	--	Propargyl amine	2450-71-7	4.8
	--	S-tritylcysteamine hydrochloride	15297-43-5	4.1,4.8

4.5 Ether-containing Submonomers

Ether-containing monomers (Table 4) have been successfully incorporated into homo- and copolypeptoids. Homopolypeptoids bearing different lengths of ether-like side chains are hydrophilic and are amorphous with low glass transition temperature (T_g) which facilitates their rapid chain motion in bulk. (Sun, Stone, Balsara, & Zuckermann, 2012) The low- T_g feature and the ability to complex with lithium cations make them potential polyelectrolytes for lithium-ion transport in batteries. Ether-containing side chains are highly hydrophilic and non-charged, which enables polyethylene glycol like behavior to be introduced. These have been incorporated to increase the aqueous solubility of hydrophobic peptoids, as antifouling surface coatings, and

for the passivation of DNA and carbon-based nanostructures.(Chio et al., 2019; Wang et al., 2020) The high hydrophilicity of these side chains are also good building blocks for constructing amphiphilic diblock copolypeptoids to induce phase separation in bulk and self-assembly in aqueous solution.(Greer, Stolberg, Kundu, et al., 2018; Xuan et al., 2019) The either-containing monomers can be readily incorporated into peptoid chains by standard peptoid synthesis methods.

4.6 Lipid-like Submonomers

Polypeptoids bearing lipid-like monomers (Table 4) are highly hydrophobic and crystalline. (Greer, Stolberg, Xuan, et al., 2018) Diblock copolypeptoids containing a hydrophobic block with lipid-like side chains and a hydrophilic block have been shown to phase-separate into lamellar morphology in bulk and self-assemble into nanosheets and nanotubes in aqueous solution, driven by the crystallization of the lipid-like block.(Greer, Stolberg, Xuan, et al., 2018; Jiang et al., 2018; Sun, Jiang, Lund, et al., 2016) Some antimicrobial peptoids also use these lipid-like monomers.(Chongsiriwatana et al., 2011) Polypeptoids bearing lipid-like side chains are often achievable by standard peptoid synthesis methods. However, due to the high hydrophobicity of the resulting peptoids, the purification and characterization of the final product can be challenging. Due to limitations of common C4 and C18 columns, this class of peptoid often requires use of less hydrophobic cyano columns (e.g. Waters XSelect HSS CN (Cyano) 5 um 19 x 150 mm). A linear, binary elution gradient (solvent A and B) was used from 50 to 95% B in 20 min at a flow rate of 15 mL/min. Here solvent A was 10% isopropanol in water with 0.1% TFA, and solvent B was 10% isopropanol in acetonitrile with 0.1% TFA.

Note:

1. Some very hydrophobic amines (e.g. tridecylamine) are insoluble in DMF and require a mixture of 50:50 (v/v) DMF:DCE in order to dissolve and react efficiently.
2. Reaction with poorly soluble lipid amines requires prolonged times, usually between 2-3 hours, but if left for longer times (e.g. overnight) the lipid amine can start to precipitate, thus requiring more rigorous washes.

4.7 Peptide/Peptoid Hybrid Sequences

Since peptide and peptoid chemistries use the same acid-deprotection strategy, and many of the reagents are the same, their chemistries are readily interchangeable. Peptide residues can be added to peptoid sequences to form hybrid peptide/peptoid backbones, or peptomers, using standard peptide coupling methods.(J. H. Kim et al., 2020; Olsen, 2010; Ostergaard & Holm, 1997; Park, Wetzler, Jardetzky, & Barron, 2013) The coupling of peptide residues to the secondary amine at the *N*-terminus of the growing peptoid chain can be somewhat sterically-hindered, and is thus a more challenging peptide coupling, similar to a peptide coupling to a proline residue, and may require double couplings. The coupling of a peptoid to the primary amine of an amino acid *N*-terminus, on the other hand, is relatively facile and proceeds with standard peptoid coupling methods.

4.7.1 Peptide Coupling to a Peptoid *N*-terminus

Note: This method is at a 50 μ mol resin scale.

1. Add 1 mL of 0.4 M Fmoc-protected amino acid in NMP. Add 1 mL of 0.4 M HCTU in DMF and 1 mL of 0.4 M *N*-methylmorpholine in DMF. Incubate with rocking for 1 hour.
Drain and repeat.

2. Remove the Fmoc protecting group by adding 1 mL of 20% 4-methylpiperidine in DMF (v/v) to deprotect the resin bound Fmoc group. Agitate for 2 minutes and drain. Repeat with an additional 1 mL of 20% 4-methylpiperidine in DMF (v/v) with a 20 minute incubation.
3. Wash the resin with DMF (4 x 2 mL).
4. Proceed to the next coupling in the sequence.

4.8 Peptoid Conjugation and *N*-terminal Modification

N-terminal modification and the incorporation of appropriate amine submonomers facilitate conjugation of the resulting peptoids to fluorescent dyes, nanoparticles, or surfaces. Fmoc-aminohexanoic acid and Fmoc-beta alanine can be coupled to the *N*-terminus using standard amino acid coupling methods.(see section 4.7) to provide an *N*-terminal primary amine for conjugation. Thiol functionality can be incorporated at the *N*-terminus by capping with *S*-trityl mercaptopropionic acid, and at various sequence positions using the *S*-tritylcysteamine as a submonomer. Azide-alkyne “click chemistry” can be conveniently performed using the submonomers 3-azido-1-aminopropane (CAS #88192-19-2) and propargyl amine (CAS #2450-71-7), which can be incorporated as side-chains anywhere in the sequence with standard peptoid coupling methods.(Holub, Jang, & Kirshenbaum, 2006)

***N*-formylation to avoid *N*-terminal degradation with *N*-acetylated peptoids**

N-terminal acetylated peptoids are often unstable for extended periods in the TFA cleavage cocktail and acid solutions including TFA buffered HPLC solvents.(S. Kim et al., 2014) *N*-Formylated peptoids do not suffer this instability.(Wijaya et al., 2019)

Note: This method is at a 128 μ mol resin scale.

1. Swell the resin by incubating with rocking in 2 mL of DMF for 30 min. Drain the DMF.

2. Add 0.5 mL of 2 M DIC in NMP to the resin quickly followed by 0.5 mL of 2 M formic acid in NMP. Incubate for 10 min with rocking, drain, and repeat once.
3. Wash with DMF (3 x 2 mL).

5. CLEAVAGE AND SIDE-CHAIN DEPROTECTION

5.1 Standard Cleavage/deprotection Method

Notes: Trifluoroacetic acid (TFA) is a highly corrosive acid that is extremely destructive to the upper respiratory tract, eyes, and skin. TFA is also volatile. All TFA handling steps should be conducted wearing appropriate PPE and in a chemical fume hood.

1. Transfer all of the dried resin to a 20 mL scintillation glass vial with polypropylene lined cap. Do not use vials with foil lined caps as the adhesive will contaminate the sample.
2. Add 4 mL of trifluoroacetic acid (TFA) cleavage cocktail (King, Fields, & Fields, 1990) (e.g. 95% TFA, 2.5% triisopropyl silane, 2.5% water) to the scintillation glass vial and cap tightly. Shake for 10 minutes to 2 hours at room temperature.

Note: The cleavage cocktail used here works for most sequences. However, additional additives may be required depending on the protecting groups used in the sequence.

3. Collect the TFA cleavage solution by filtering the resin through a disposable, polypropylene fritted cartridge into a new, pre-weighed 20 mL scintillation glass vial. A disposable, polypropylene pipette is convenient to transfer the cleavage cocktail solutions.
4. Add 1 mL of fresh cleavage cocktail to rinse the resin and collect any residual peptoid. Repeat 2X.
5. Evaporate the TFA rapidly using a Biotage V10 evaporator.

Note: A standard laboratory rotary evaporator with dry ice trap can be used instead of the Biotage V10 evaporator but may not be as fast or efficient.

6. Redissolve the crude oil/solid in 6 mL of acetonitrile/water. Freeze and lyophilize. Repeat.
7. Record the weight of the crude product. Although most peptoids are stable at room temperature for extended periods, new compounds should be stored as a dry powder at -20 °C.

6. CHARACTERIZATION AND PURIFICATION

Analytical chromatography (HPLC, or UPLC), electrospray LC-MS, and/or MALDI-TOF mass spectrometry can be used to determine the purity and identity of the crude and purified peptoid product. Mass spectrometry can also be used to sequence peptoids to assist in identification. (Ren et al., 2020; Thakkar, Cohen, Connolly, Zuckermann, & Pei, 2009) This is particularly useful for hit identification from combinatorial libraries.

6.1 Basic Characterization Guidelines

1. Prepare analytical HPLC/UPLC or electrospray LC-MS samples by dissolving the peptoid in HPLC grade water with the minimal acetonitrile needed for solubility to give a ~20 µg/mL solution. The sample should be centrifuged to pellet any particulates or filtered through a 0.2 mm filter.
2. Analytical chromatography is conducted using a C4 or C18 column based on the hydrophobicity of the polypeptoid. C18 is the default column for most peptoids with C4 used for more hydrophobic sequences. For basic analysis an acetonitrile/water with 0.1% TFA solvent system with a gradient from 5 to 95% acetonitrile is typical.

3. To prepare a standard MALDI sample mix 1 μL of ~ 20 mg/mL peptoid with 1 μL MALDI matrix such as α -cyano-4-hydroxycinnamic acid (CHCA). Spot 1 μL on the MALDI plate and allow to air dry.

6.2 Peptoid Purification Guidelines

Crude peptoids are purified by reverse-phase preparative HPLC. The sample should be dissolved in water with a minimum amount of acetonitrile to achieve full solubility. If necessary, it is better to add more volume decreasing the sample concentration than to add more acetonitrile since this will negatively impact the resolving power of the chromatography. The sample should be centrifuged to pellet any particulates, or filtered through a 0.45 μm syringe filter. Note that the filter membrane type depends on the hydrophobicity of the peptoid. Select PVDF or PTFE membrane that will not adsorb the peptoid. The solvent gradient and column (C4 or C18) are selected based on the hydrophobicity of the polypeptoid, with the gradient starting at or above the percent of acetonitrile used to dissolve the sample to prevent the sample crashing out on the column. After HPLC the purified fractions are combined, evaporated, re-solubilized in water with minimal acetonitrile, frozen, and lyophilized resulting in a fluffy white powder. The resulting peptoids are the trifluoroacetic acid salt form.

Formation of HCl salt form

1. Dissolve the lyophilized powder in 100 mM HCl(aq) with minimal acetonitrile. Transfer to pre-weighed glass vial. Freeze and re-lyophilize. Repeat 2x.

7. ACKNOWLEDGEMENTS

Work at the Molecular Foundry was supported by the Office of Science, Office of Basic Energy Sciences, of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231. The authors thank R. Spencer and J. Edison for their help creating the Ramachandran plots.

REFERENCES

- Alluri, P. G., Reddy, M. M., Bachhawat-Sikder, K., Olivos, H. J., & Kodadek, T. (2003). Isolation of protein ligands from large peptoid libraries. *J Am Chem Soc*, *125*(46), 13995-14004. doi:10.1021/ja036417x
- Barry, M. E. D., E. C.; Zhang, C.; Patterson, A. L.; Yu, B.; Leonardi, A. K.; Duzen, N.; Malaviya, K.; Clarke, J. L.; Finlay, J. A.; Clare, A. S.; Chen, Z.; Ober, C. K.; Segalman, R. A. (2019). The Role of Hydrogen Bonding in Peptoid-Based Marine Antifouling Coatings. *Macromolecules*, *52*(3), 1287-1295.
- Bolt, H. L., & Cobb, S. L. (2016). A practical method for the synthesis of peptoids containing both lysine-type and arginine-type monomers. *Org Biomol Chem*, *14*(4), 1211-1215. doi:10.1039/c5ob02279g
- Burkoth, T. S., Fafarman, A. T., Charych, D. H., Connolly, M. D., & Zuckermann, R. N. (2003). Incorporation of unprotected heterocyclic side chains into peptoid oligomers via solid-phase submonomer synthesis. *J Am Chem Soc*, *125*(29), 8841-8845. doi:10.1021/ja0352101
- Cai, D., Lee, A. Y., Chiang, C. M., & Kodadek, T. (2011). Peptoid ligands that bind selectively to phosphoproteins. *Bioorg Med Chem Lett*, *21*(17), 4960-4964. doi:10.1016/j.bmcl.2011.06.011
- Caumes, C., Roy, O., Faure, S., & Taillefumier, C. (2012). The click triazolium peptoid side chain: A strong *cis*-amide inducer enabling chemical diversity. *J Am Chem Soc*, *134*(23), 9553-9556. doi:10.1021/ja302342h
- Chan, B. A., Xuan, S., Li, A., Simpson, J. M., Sternhagen, G. L., Yu, T., . . . Zhang, D. Polypeptoid polymers: Synthesis, characterization, and properties. *Biopolymers*, e23070-n/a. doi:10.1002/bip.23070
- Chen, X., Wu, J., Luo, Y., Liang, X., Supnet, C., Kim, M. W., . . . Bezprozvanny, I. (2011). Expanded polyglutamine-binding peptoid as a novel therapeutic agent for treatment of Huntington's disease. *Chem Biol*, *18*(9), 1113-1125. doi:10.1016/j.chembiol.2011.06.010
- Chio, L., Del Bonis-O'Donnell, J. T., Kline, M. A., Kim, J. H., McFarlane, I. R., Zuckermann, R. N., & Landry, M. P. (2019). Electrostatic assemblies of single-walled carbon nanotubes and sequence-tunable peptoid polymers detect a lectin protein and its target sugars. *Nano Lett*, *19*(11), 7563-7572. doi:10.1021/acs.nanolett.8b04955
- Chongsiriwatana, N. P., Miller, T. M., Wetzler, M., Vakulenko, S., Karlsson, A. J., Palecek, S. P., . . . Barron, A. E. (2011). Short alkylated peptoid mimics of antimicrobial lipopeptides. *Antimicrob Agents Chemother*, *55*(1), 417-420. doi:10.1128/aac.01080-10
- Crapster, J. A., Guzei, I. A., & Blackwell, H. E. (2013). A peptoid ribbon secondary structure. *Angew Chem Int Ed*, *52*(19), 5079-5084. doi:10.1002/anie.201208630
- Culf, A. S. (2019). Peptoids as tools and sensors. *Biopolymers*, *110*(6). doi:10.1002/bip.23285
- Culf, A. S., & Ouellette, R. J. (2010). Solid-phase synthesis of N-substituted glycine oligomers (alpha-peptoids) and derivatives. *Molecules*, *15*(8), 5282-5335. doi:10.3390/molecules15085282
- Czyzewski, A. M., Jenssen, H., Fjell, C. D., Waldbrook, M., Chongsiriwatana, N. P., Yuen, E., . . . Barron, A. E. (2016). In vivo, in vitro, and in silico

- characterization of peptoids as antimicrobial agents. *PLoS One*, 11(2), e0135961. doi:10.1371/journal.pone.0135961
- Edison, J. R., Spencer, R. K., Butterfoss, G. L., Hudson, B. C., Hochbaum, A. I., Paravastu, A. K., . . . Whitelam, S. (2018). Conformations of peptoids in nanosheets result from the interplay of backbone energetics and intermolecular interactions. *Proceedings of the National Academy of Sciences of the United States of America*, 115(22), 5647-5651. doi:10.1073/pnas.1800397115
- Eggimann, G. A., Bolt, H. L., Denny, P. W., & Cobb, S. L. (2015). Investigating the anti-leishmanial effects of linear peptoids. *ChemMedChem*, 10(2), 233-237. doi:10.1002/cmdc.201402416
- Figliozzi, G. M., Goldsmith, R., Ng, S. C., Banville, S. C., & Zuckermann, R. N. (1996). Synthesis of N-substituted glycine peptoid libraries. *Methods Enzymol*, 267, 437-447. doi:10.1016/s0076-6879(96)67027-x
- Gorske, B. C., Mumford, E. M., Gerrity, C. G., & Ko, I. (2017). A Peptoid square helix via synergistic control of backbone dihedral angles. *J Am Chem Soc*, 139(24), 8070-8073. doi:10.1021/jacs.7b02319
- Gorske, B. C., Stringer, J. R., Bastian, B. L., Fowler, S. A., & Blackwell, H. E. (2009). New strategies for the design of folded peptoids revealed by a survey of noncovalent interactions in model systems. *J Am Chem Soc*, 131(45), 16555-16567. doi:10.1021/ja907184g
- Greer, D. R., Stolberg, M. A., Kundu, J., Spencer, R. K., Pascal, T., Prendergast, D., . . . Zuckermann, R. N. (2018). Universal relationship between molecular structure and crystal structure in peptoid polymers and prevalence of the *cis* backbone conformation. *J Am Chem Soc*, 140(2), 827-833. doi:10.1021/jacs.7b11891
- Greer, D. R., Stolberg, M. A., Xuan, S., Jiang, X., Balsara, N. P., & Zuckermann, R. N. (2018). Liquid-crystalline phase behavior in polypeptoid diblock copolymers. *Macromolecules*, 51(23), 9519-9525. doi:10.1021/acs.macromol.8b01952
- Holub, J. M., Jang, H. J., & Kirshenbaum, K. (2006). Clickity-click: highly functionalized peptoid oligomers generated by sequential conjugation reactions on solid-phase support. *Org Biomol Chem*, 4(8), 1497-1502. doi:10.1039/b518247f
- Hooks, J. C., Matharage, J. P., & Udugamasooriya, D. G. (2011). Development of homomultimers and heteromultimers of lung cancer-specific peptoids. *Biopolymers*, 96(5), 567-577. doi:10.1002/bip.21596
- Hornemann, S., Schwarz, P., Rushing, E. J., Connolly, M. D., Zuckermann, R. N., Yam, A. Y., & Aguzzi, A. (2019). Enhanced detection of prion infectivity from blood by preanalytical enrichment with peptoid-conjugated beads. *PLoS One*, 14(9), e0216013. doi:10.1371/journal.pone.0216013
- Huang, W., Seo, J., Willingham, S. B., Czyzewski, A. M., Gonzalgo, M. L., Weissman, I. L., & Barron, A. E. (2014). Learning from host-defense peptides: cationic, amphipathic peptoids with potent anticancer activity. *PLoS One*, 9(2), e90397. doi:10.1371/journal.pone.0090397
- Jiang, X., Greer, D. R., Kundu, J., Ophus, C., Minor, A. M., Prendergast, D., . . . Downing, K. H. (2018). Imaging unstained synthetic polymer crystals and defects on atomic length scales using cryogenic electron microscopy. *Macromolecules*, 51(19), 7794-7799. doi:10.1021/acs.macromol.8b01508
- Jin, H., Ding, Y.-H., Wang, M., Song, Y., Liao, Z., Newcomb, C. J., . . . Chen, C.-L. (2018). Designable and dynamic single-walled stiff nanotubes assembled

- from sequence-defined peptoids. *Nat Commun*, 9(1), 270. doi:10.1038/s41467-017-02059-1
- Jin, H., Jiao, F., Daily, M. D., Chen, Y., Yan, F., Ding, Y.-H., . . . Chen, C.-L. (2016). Highly stable and self-repairing membrane-mimetic 2D nanomaterials assembled from lipid-like peptoids. *Nat Commun* 7, 12252. doi:10.1038/ncomms12252
- Kang, B., Yang, W., Lee, S., Mukherjee, S., Forstater, J., Kim, H., . . . Seo, J. (2017). Precisely tuneable energy transfer system using peptoid helix-based molecular scaffold. *Sci Rep*, 7(1), 4786. doi:10.1038/s41598-017-04727-0
- Khara, J. S., Mojsoska, B., Mukherjee, D., Langford, P. R., Robertson, B. D., Jenssen, H., . . . Newton, S. M. (2020). Ultra-short antimicrobial peptoids show propensity for membrane activity against multi-drug resistant mycobacterium tuberculosis. *Front Microbiol*, 11, 417. doi:10.3389/fmicb.2020.00417
- Kim, J. H., Kim, S. C., Kline, M. A., Grzincic, E. M., Tresca, B. W., Cardiel, J., . . . Zuckermann, R. N. (2020). Discovery of stable and selective antibody mimetics from combinatorial libraries of polyvalent, loop-functionalized peptoid nanosheets. *ACS Nano*, 14(1), 185-195. doi:10.1021/acsnano.9b07498
- Kim, S., Biswas, G., Park, S., Kim, A., Park, H., Park, E., . . . Kwon, Y. U. (2014). Unusual truncation of N-acylated peptoids under acidic conditions. *Org Biomol Chem*, 12(28), 5222-5226. doi:10.1039/c3ob42572j
- King, D. S., Fields, C. G., & Fields, G. B. (1990). A cleavage method which minimizes side reactions following Fmoc solid-phase peptide synthesis. *Intl J Pept Protein Res*, 36(3), 255-266. doi: 10.1111/j.1399-3011.1990.tb00976.x
- Knight, A. S., Kulkarni, R. U., Zhou, E. Y., Franke, J. M., Miller, E. W., & Francis, M. B. (2017). A modular platform to develop peptoid-based selective fluorescent metal sensors. *Chem Commun (Camb)*, 53(24), 3477-3480. doi:10.1039/c7cc00931c
- Lee, C.-U., Smart, T. P., Guo, L., Epps, T. H., & Zhang, D. (2011). Synthesis and characterization of amphiphilic cyclic diblock copolypeptoids from N-heterocyclic carbene-mediated zwitterionic polymerization of N-substituted N-carboxyanhydride. *Macromolecules*, 44(24), 9574-9585. doi:10.1021/ma2020936
- Lim, B., & Lee, J. (2016). A peptoid-based fluorescent sensor for cyanide detection. *Molecules*, 21(3), 339. doi:10.3390/molecules21030339
- Matharage, J. M., Minna, J. D., Brekken, R. A., & Udugamasooriya, D. G. (2015). Unbiased selection of peptide-peptoid hybrids specific for lung cancer compared to normal lung epithelial cells. *ACS Chem Biol*, 10(12), 2891-2899. doi:10.1021/acscchembio.5b00592
- Meister, D., Taimoory, S. M., & Trant, J. F. (2019). Unnatural amino acids improve affinity and modulate immunogenicity: Developing peptides to treat MHC type II autoimmune disorders. *Pept Sci*, 111(1), e24058. doi:10.1002/pep2.24058
- Molchanova, N., Hansen, P. R., & Franzyk, H. (2017). Advances in development of antimicrobial peptidomimetics as potential drugs. *Molecules*, 22(9). doi:10.3390/molecules22091430
- Murnen, H. K., Rosales, A. M., Jaworski, J. N., Segalman, R. A., & Zuckermann, R. N. (2010). Hierarchical self-assembly of a biomimetic diblock copolypeptoid into homochiral superhelices. *J Am Chem Soc*, 132(45), 16112-16119. doi:10.1021/ja106340f

- Olsen, C. A. (2010). Peptoid-peptide hybrid backbone architectures. *ChemBioChem*, *11*(2), 152-160. doi:10.1002/cbic.200900618
- Ostergaard, S., & Holm, A. (1997). Peptomers: A versatile approach for the preparation of diverse combinatorial peptidomimetic bead libraries. *Molecular Diversity*, *3*(1), 17-27. doi:10.1023/A:1009698507588
- Park, M., Wetzler, M., Jardetzky, T. S., & Barron, A. E. (2013). A readily applicable strategy to convert peptides to peptoid-based therapeutics. *PLoS One*, *8*(3), e58874. doi:10.1371/journal.pone.0058874
- Paul, B., Butterfoss, G. L., Boswell, M. G., Huang, M. L., Bonneau, R., Wolf, C., & Kirshenbaum, K. (2012). N-Naphthyl peptoid foldamers exhibiting atropisomerism. *Org Letters*, *14*(3), 926-929. doi:10.1021/ol203452f
- Proulx, C., Yoo, S., Connolly, M. D., & Zuckermann, R. N. (2015). Accelerated submonomer solid-phase synthesis of peptoids incorporating multiple substituted N-aryl glycine monomers. *J Org Chem*, *80*(21), 10490-10497. doi:10.1021/acs.joc.5b01449
- Ren, J. H., Tian, Y., Hossain, E., Ho, J. S., Mann, Y. S., Zhang, Y. T., . . . Zuckermann, R. N. (2020). Mass spectrometry studies of the fragmentation patterns and mechanisms of protonated peptoids. *Biopolymers*, *111*(7), e23358. doi:10.1002/bip.23358
- Robertson, E. J., Battigelli, A., Proulx, C., Mannige, R. V., Haxton, T. K., Yun, L., . . . Zuckermann, R. N. (2016). Design, synthesis, assembly, and engineering of peptoid nanosheets. *Acc Chem Res*, *49*(3), 379-389. doi:10.1021/acs.accounts.5b00439
- Roy, O., Caumes, C., Esvan, Y., Didierjean, C., Faure, S., & Taillefumier, C. (2013). The tert-butyl side chain: A powerful means to lock peptoid amide bonds in the *cis* conformation. *Org Lett*, *15*(9), 2246-2249. doi:10.1021/ol400820y
- Rzeigui, M., Traikia, M., Jouffret, L., Kriznik, A., Khiari, J., Roy, O., & Taillefumier, C. (2020). Strengthening peptoid helicity through sequence site-specific positioning of amide *cis*-inducing NtBu monomers. *J Org Chem*, *85*(4), 2190-2201. doi:10.1021/acs.joc.9b02916
- Sanborn, T. J., Wu, C. W., Zuckerman, R. N., & Barron, A. E. (2002). Extreme stability of helices formed by water-soluble poly-N-substituted glycines (polypeptoids) with alpha-chiral side chains. *Biopolymers*, *63*(1), 12-20. doi:10.1002/bip.1058
- Schettini, R., Costabile, C., Della Sala, G., Iuliano, V., Tedesco, C., Izzo, I., & De Riccardis, F. (2018). Cation-induced molecular switching based on reversible modulation of peptoid conformational states. *J Org Chem*, *83*(20), 12648-12663. doi:10.1021/acs.joc.8b01990
- Schneider, J. A., Craven, T. W., Kasper, A. C., Yun, C., Haugbro, M., Briggs, E. M., . . . Logan, S. K. (2018). Design of peptoid-peptide macrocycles to inhibit the beta-catenin TCF interaction in prostate cancer. *Nat Commun*, *9*(1), 4396. doi:10.1038/s41467-018-06845-3
- Secker, C., Brosnan, S. M., Luxenhofer, R., & Schlaad, H. (2015). Poly(α -peptoid)s revisited: Synthesis, properties, and use as biomaterial. *Macromol Biosci*, *15*(7), 881-891. doi:10.1002/mabi.201500023
- Shin, S. B., & Kirshenbaum, K. (2007). Conformational rearrangements by water-soluble peptoid foldamers. *Org Lett*, *9*(24), 5003-5006. doi:10.1021/ol702207n

- Simon, R. J., Kania, R. S., Zuckermann, R. N., Huebner, V. D., Jewell, D. A., Banville, S., . . . Marlowe, C. K. (1992). Peptoids: a modular approach to drug discovery. *P N A S*, *89*(20), 9367-9371. doi:10.1073/pnas.89.20.9367
- Spencer, R. K., Butterfoss, G. L., Edison, J. R., Eastwood, J. R., Whitelam, S., Kirshenbaum, K., & Zuckermann, R. N. (2019). Stereochemistry of polypeptoid chain configurations. *Biopolymers*, *110*(6), e23266. doi:10.1002/bip.23266
- Spicer, S. K., Subramani, A., Aguila, A. L., Green, R. M., McClelland, E. E., & Bicker, K. L. (2019). Toward a clinical antifungal peptoid: Investigations into the therapeutic potential of AEC5. *Biopolymers*, *110*(6), e23276. doi:10.1002/bip.23276
- Sternhagen, G. L., Gupta, S., Zhang, Y., John, V., Schneider, G. J., & Zhang, D. (2018). Solution self-assemblies of sequence-defined ionic peptoid block copolymers. *J Am Chem Soc*, *140*(11), 4100-4109. doi:10.1021/jacs.8b00461
- Stringer, J. R., Crapster, J. A., Guzei, I. A., & Blackwell, H. E. (2011). Extraordinarily robust polyproline type I peptoid helices generated via the incorporation of alpha-chiral aromatic N-1-naphthylethyl side chains. *J Am Chem Soc*, *133*(39), 15559-15567. doi:10.1021/ja204755p
- Sun, J., Jiang, X., Lund, R., Downing, K. H., Balsara, N. P., & Zuckermann, R. N. (2016). Self-assembly of crystalline nanotubes from monodisperse amphiphilic diblock copolypeptoid tiles. *P N A S*, *113*(15), 3954-3959. doi:10.1073/pnas.1517169113
- Sun, J., Jiang, X., Siegmund, A., Connolly, M. D., Downing, K. H., Balsara, N. P., & Zuckermann, R. N. (2016). Morphology and proton transport in humidified phosphonated peptoid block copolymers. *Macromolecules*, *49*(8), 3083-3090. doi:10.1021/acs.macromol.6b00353
- Sun, J., Stone, G. M., Balsara, N. P., & Zuckermann, R. N. (2012). Structure-conductivity relationship for peptoid-based PEO-mimetic polymer electrolytes. *Macromolecules*, *45*(12), 5151-5156. doi:10.1021/ma300775b
- Sun, J., & Zuckermann, R. N. (2013). Peptoid polymers: A highly designable bioinspired material. *ACS Nano*, *7*(6), 4715-4732. doi:10.1021/nn4015714
- Thakkar, A., Cohen, A. S., Connolly, M. D., Zuckermann, R. N., & Pei, D. (2009). High-throughput sequencing of peptoids and peptide-peptoid hybrids by partial Edman degradation and mass spectrometry. *J Comb Chem*, *11*(2), 294-302. doi:10.1021/cc8001734
- Uno, T., Beausoleil, E., Goldsmith, R. A., Levine, B. H., & Zuckermann, R. N. (1999). New submonomers for poly N-substituted glycines (peptoids). *Tet Lett*, *40*(8), 1475-1478. doi:10.1016/S0040-4039(98)02696-3
- Wang, S.-T., Gray, M. A., Xuan, S., Lin, Y., Byrnes, J., Nguyen, A. I., . . . Gang, O. (2020). DNA origami protection and molecular interfacing through engineered sequence-defined peptoids. *P N A S*, *117*(12), 6339-6348. doi:10.1073/pnas.1919749117
- Wender, P. A., Mitchell, D. J., Pattabiraman, K., Pelkey, E. T., Steinman, L., & Rothbard, J. B. (2000). The design, synthesis, and evaluation of molecules that enable or enhance cellular uptake: Peptoid molecular transporters. *P N A S*, *97*(24), 13003-13008. doi:10.1073/pnas.97.24.13003
- Wijaya, A. W., Nguyen, A. I., Roe, L. T., Butterfoss, G. L., Spencer, R. K., Li, N. K., & Zuckermann, R. N. (2019). Cooperative intramolecular hydrogen bonding strongly enforces *cis*-peptoid folding. *J Am Chem Soc*, *141*(49), 19436-19447. doi:10.1021/jacs.9b10497

- Wu, C. W., Sanborn, T. J., Zuckermann, R. N., & Barron, A. E. (2001). Peptoid oligomers with alpha-chiral, aromatic side chains: Effects of chain length on secondary structure. *J Am Chem Soc*, *123*(13), 2958-2963. doi:10.1021/ja003153v
- Wu, C. W., Sanborn, T. J., Zuckermann, R. N., & Barron, A. E. (2001). Peptoid oligomers with α -chiral, aromatic side chains: Effects of chain length on secondary structure. *J Am Chem Soc*, *123*(13), 2958-2963. doi:10.1021/ja003153v
- Wuts, P. G. M., Greene, T. W., & Greene, T. W. (2014). *Greene's protective groups in organic synthesis* (Fifth edition / ed., pp. 1 online resource).
- Xuan, S., Gupta, S., Li, X., Bleuel, M., Schneider, G. J., & Zhang, D. (2017). Synthesis and characterization of well-defined PEGylated polypeptoids as protein-resistant polymers. *Biomacromolecules*, *18*(3), 951-964. doi:10.1021/acs.biomac.6b01824
- Xuan, S., Jiang, X., Spencer, R. K., Li, N. K., Prendergast, D., Balsara, N. P., & Zuckermann, R. N. (2019). Atomic-level engineering and imaging of polypeptoid crystal lattices. *P N A S*, *116*(45), 22491-22499. doi:10.1073/pnas.1909992116
- Xuan, S., Lee, C.-U., Chen, C., Doyle, A. B., Zhang, Y., Guo, L., . . . Zhang, D. (2016). Thermoreversible and injectable ABC polypeptoid hydrogels: controlling the hydrogel properties through molecular design. *Chem Mater*, *28*(3), 727-737. doi:10.1021/acs.chemmater.5b03528
- Xuan, S., & Zuckermann, R. N. (2020a). Diblock copolypeptoids: a review of phase separation, crystallization, self-assembly and biological applications. *J. Mater. Chem. B*, *8*, 5380-5394. doi:10.1039/D0TB00477D
- Xuan, S., & Zuckermann, R. N. (2020b). Engineering the atomic structure of sequence-defined peptoid polymers and their assemblies. *Polymer*, *202*, 122691. doi: 10.1016/j.polymer.2020.122691
- Yam, A. Y., Wang, X. M., Gao, C. M., Connolly, M. D., Zuckermann, R. N., Bleu, T., . . . Salisbury, C. M. (2011). A universal method for detection of amyloidogenic misfolded proteins. *Biochemistry*, *50*(20), 4322-4329. doi:10.1021/bi200215j
- Zuckermann, R. N., Kerr, J. M., Kent, S. B. H., & Moos, W. H. (1992). Efficient method for the preparation of peptoids [oligo(N-substituted glycines)] by submonomer solid-phase synthesis. *J Am Chem Soc*, *114*(26), 10646-10647. doi:10.1021/ja00052a076
- Zuckermann, R. N., & Kodadek, T. (2009). Peptoids as potential therapeutics. *Curr Opin Mol Ther*, *11*(3), 299-307. PMID: 19479663
- Zuckermann, R. N., Martin, E. J., Spellmeyer, D. C., Stauber, G. B., Shoemaker, K. R., Kerr, J. M., . . . Moos, W. H. (1994). Discovery of Nanomolar Ligands for 7-Transmembrane G-Protein-Coupled Receptors from a Diverse N-(Substituted)Glycine Peptoid Library. *J Med Chem*, *37*(17), 2678-2685. doi:10.1021/jm00043a007
- Alluri, P. G., Reddy, M. M., Bachhawat-Sikder, K., Olivos, H. J., & Kodadek, T. (2003). Isolation of protein ligands from large peptoid libraries. *J Am Chem Soc*, *125*(46), 13995-14004. doi:10.1021/ja036417x
- Barry, M. E. D., E. C.; Zhang, C.; Patterson, A. L.; Yu, B.; Leonardi, A. K.; Duzen, N.; Malaviya, K.; Clarke, J. L.; Finlay, J. A.; Clare, A. S.; Chen, Z.; Ober, C. K.; Segalman, R. A. (2019). The Role of Hydrogen Bonding in Peptoid-Based Marine Antifouling Coatings. *Macromolecules*, *52*

(3), 1287-1295.

- Bolt, H. L., & Cobb, S. L. (2016). A practical method for the synthesis of peptoids containing both lysine-type and arginine-type monomers. *Organic & Biomolecular Chemistry*, 14(4), 1211-1215. doi:10.1039/c5ob02279g
- Burkoth, T. S., Fafarman, A. T., Charych, D. H., Connolly, M. D., & Zuckermann, R. N. (2003). Incorporation of unprotected heterocyclic side chains into peptoid oligomers via solid-phase submonomer synthesis. *Journal of the American Chemical Society*, 125(29), 8841-8845. doi:10.1021/ja0352101
- Cai, D., Lee, A. Y., Chiang, C. M., & Kodadek, T. (2011). Peptoid ligands that bind selectively to phosphoproteins. *Bioorganic & Medicinal Chemistry Letters*, 21(17), 4960-4964. doi:10.1016/j.bmcl.2011.06.011
- Caumes, C., Roy, O., Faure, S., & Taillefumier, C. (2012). The Click Triazolium Peptoid Side Chain: A Strong cis-Amide Inducer Enabling Chemical Diversity. *Journal of the American Chemical Society*, 134(23), 9553-9556. doi:10.1021/ja302342h
- Chan, B. A., Xuan, S., Li, A., Simpson, J. M., Sternhagen, G. L., Yu, T., . . . Zhang, D. Polypeptoid polymers: Synthesis, characterization, and properties. *Biopolymers*, e23070-n/a. doi:10.1002/bip.23070
- Chen, X., Wu, J., Luo, Y., Liang, X., Supnet, C., Kim, M. W., . . . Bezprozvanny, I. (2011). Expanded polyglutamine-binding peptoid as a novel therapeutic agent for treatment of Huntington's disease. *Chem Biol*, 18(9), 1113-1125. doi:10.1016/j.chembiol.2011.06.010
- Chio, L., Del Bonis-O'Donnell, J. T., Kline, M. A., Kim, J. H., McFarlane, I. R., Zuckermann, R. N., & Landry, M. P. (2019). Electrostatic Assemblies of Single-Walled Carbon Nanotubes and Sequence-Tunable Peptoid Polymers Detect a Lectin Protein and Its Target Sugars. *Nano Letters*, 19(11), 7563-7572. doi:10.1021/acs.nanolett.8b04955
- Chongsiriwatana, N. P., Miller, T. M., Wetzler, M., Vakulenko, S., Karlsson, A. J., Palecek, S. P., . . . Barron, A. E. (2011). Short Alkylated Peptoid Mimics of Antimicrobial Lipopeptides. *Antimicrobial Agents and Chemotherapy*, 55(1), 417-420. doi:10.1128/aac.01080-10
- Crapster, J. A., Guzei, I. A., & Blackwell, H. E. (2013). A Peptoid Ribbon Secondary Structure. *Angewandte Chemie International Edition*, 52(19), 5079-5084. doi:10.1002/anie.201208630
- Culf, A. S. (2019). Peptoids as tools and sensors. *Biopolymers*, 110(6). doi:ARTN e23285
- 10.1002/bip.23285
- Culf, A. S., & Ouellette, R. J. (2010). Solid-Phase Synthesis of N-Substituted Glycine Oligomers (alpha-Peptoids) and Derivatives. *Molecules*, 15(8), 5282-5335. doi:10.3390/molecules15085282
- Czyzewski, A. M., Jenssen, H., Fjell, C. D., Waldbrook, M., Chongsiriwatana, N. P., Yuen, E., . . . Barron, A. E. (2016). In Vivo, In Vitro, and In Silico Characterization of Peptoids as Antimicrobial Agents. *PLoS One*, 11(2), e0135961. doi:10.1371/journal.pone.0135961
- Eggimann, G. A., Bolt, H. L., Denny, P. W., & Cobb, S. L. (2015). Investigating the anti-leishmanial effects of linear peptoids. *ChemMedChem*, 10(2), 233-237. doi:10.1002/cmdc.201402416
- Figliozzi, G. M., Goldsmith, R., Ng, S. C., Banville, S. C., & Zuckermann, R. N. (1996). Synthesis of N-substituted glycine peptoid libraries. *Methods Enzymol*, 267, 437-447. doi:10.1016/s0076-6879(96)67027-x

- Gorske, B. C., Mumford, E. M., Gerrity, C. G., & Ko, I. (2017). A Peptoid Square Helix via Synergistic Control of Backbone Dihedral Angles. *Journal of the American Chemical Society*, 139(24), 8070-8073. doi:10.1021/jacs.7b02319
- Gorske, B. C., Stringer, J. R., Bastian, B. L., Fowler, S. A., & Blackwell, H. E. (2009). New Strategies for the Design of Folded Peptoids Revealed by a Survey of Noncovalent Interactions in Model Systems. *Journal of the American Chemical Society*, 131(45), 16555-16567. doi:10.1021/ja907184g
- Greer, D. R., Stolberg, M. A., Kundu, J., Spencer, R. K., Pascal, T., Prendergast, D., . . . Zuckermann, R. N. (2018). Universal Relationship between Molecular Structure and Crystal Structure in Peptoid Polymers and Prevalence of the cis Backbone Conformation. *Journal of the American Chemical Society*, 140(2), 827-833. doi:10.1021/jacs.7b11891
- Greer, D. R., Stolberg, M. A., Xuan, S., Jiang, X., Balsara, N. P., & Zuckermann, R. N. (2018). Liquid-Crystalline Phase Behavior in Polypeptoid Diblock Copolymers. *Macromolecules*, 51(23), 9519-9525. doi:10.1021/acs.macromol.8b01952
- Holub, J. M., Jang, H. J., & Kirshenbaum, K. (2006). Clickity-click: highly functionalized peptoid oligomers generated by sequential conjugation reactions on solid-phase support. *Organic & Biomolecular Chemistry*, 4(8), 1497-1502. doi:10.1039/b518247f
- Hooks, J. C., Matharage, J. P., & Udugamasooriya, D. G. (2011). Development of homomultimers and heteromultimers of lung cancer-specific peptoids. *Biopolymers*, 96(5), 567-577. doi:10.1002/bip.21596
- Hornemann, S., Schwarz, P., Rushing, E. J., Connolly, M. D., Zuckermann, R. N., Yam, A. Y., & Aguzzi, A. (2019). Enhanced detection of prion infectivity from blood by preanalytical enrichment with peptoid-conjugated beads. *PLoS One*, 14(9), e0216013. doi:10.1371/journal.pone.0216013
- Huang, W., Seo, J., Willingham, S. B., Czyzewski, A. M., Gonzalgo, M. L., Weissman, I. L., & Barron, A. E. (2014). Learning from host-defense peptides: cationic, amphipathic peptoids with potent anticancer activity. *PLoS One*, 9(2), e90397. doi:10.1371/journal.pone.0090397
- Jiang, X., Greer, D. R., Kundu, J., Ophus, C., Minor, A. M., Prendergast, D., . . . Downing, K. H. (2018). Imaging Unstained Synthetic Polymer Crystals and Defects on Atomic Length Scales Using Cryogenic Electron Microscopy. *Macromolecules*, 51(19), 7794-7799. doi:10.1021/acs.macromol.8b01508
- Jin, H., Ding, Y.-H., Wang, M., Song, Y., Liao, Z., Newcomb, C. J., . . . Chen, C.-L. (2018). Designable and dynamic single-walled stiff nanotubes assembled from sequence-defined peptoids. *Nature Communications*, 9(1), 270. doi:10.1038/s41467-017-02059-1
- Jin, H., Jiao, F., Daily, M. D., Chen, Y., Yan, F., Ding, Y.-H., . . . Chen, C.-L. (2016). Highly stable and self-repairing membrane-mimetic 2D nanomaterials assembled from lipid-like peptoids. *7*, 12252. doi:10.1038/ncomms12252
- <https://www.nature.com/articles/ncomms12252#supplementary-information>
- Kang, B., Yang, W., Lee, S., Mukherjee, S., Forstater, J., Kim, H., . . . Seo, J. (2017). Precisely tuneable energy transfer system using peptoid helix-based molecular scaffold. *Sci Rep*, 7(1), 4786. doi:10.1038/s41598-017-04727-0
- Khara, J. S., Mojsoska, B., Mukherjee, D., Langford, P. R., Robertson, B. D., Jenssen, H., . . . Newton, S. M. (2020). Ultra-Short Antimicrobial Peptoids Show Propensity for Membrane Activity Against Multi-Drug Resistant Mycobacterium tuberculosis. *Front Microbiol*, 11, 417. doi:10.3389/fmicb.2020.00417

- Kim, J. H., Kim, S. C., Kline, M. A., Grzincic, E. M., Tresca, B. W., Cardiel, J., . . . Zuckermann, R. N. (2020). Discovery of Stable and Selective Antibody Mimetics from Combinatorial Libraries of Polyvalent, Loop-Functionalized Peptoid Nanosheets. *ACS Nano*, *14*(1), 185-195. doi:10.1021/acsnano.9b07498
- Kim, S., Biswas, G., Park, S., Kim, A., Park, H., Park, E., . . . Kwon, Y. U. (2014). Unusual truncation of N-acylated peptoids under acidic conditions. *Organic & Biomolecular Chemistry*, *12*(28), 5222-5226. doi:10.1039/c3ob42572j
- King, D. S., Fields, C. G., & Fields, G. B. (1990). A Cleavage Method Which Minimizes Side Reactions Following Fmoc Solid-Phase Peptide-Synthesis. *International Journal of Peptide and Protein Research*, *36*(3), 255-266. Retrieved from <Go to ISI>://WOS:A1990DX90500008
- Knight, A. S., Kulkarni, R. U., Zhou, E. Y., Franke, J. M., Miller, E. W., & Francis, M. B. (2017). A modular platform to develop peptoid-based selective fluorescent metal sensors. *Chem Commun (Camb)*, *53*(24), 3477-3480. doi:10.1039/c7cc00931c
- Lee, C.-U., Smart, T. P., Guo, L., Epps, T. H., & Zhang, D. (2011). Synthesis and Characterization of Amphiphilic Cyclic Diblock Copolypeptoids from N-Heterocyclic Carbene-Mediated Zwitterionic Polymerization of N-Substituted N-Carboxyanhydride. *Macromolecules*, *44*(24), 9574-9585. doi:10.1021/ma2020936
- Lim, B., & Lee, J. (2016). A Peptoid-Based Fluorescent Sensor for Cyanide Detection. *Molecules*, *21*(3), 339. doi:10.3390/molecules21030339
- Matharage, J. M., Minna, J. D., Brekken, R. A., & Udugamasooriya, D. G. (2015). Unbiased Selection of Peptide-Peptoid Hybrids Specific for Lung Cancer Compared to Normal Lung Epithelial Cells. *ACS Chem Biol*, *10*(12), 2891-2899. doi:10.1021/acscchembio.5b00592
- Meister, D., Taimoory, S. M., & Trant, J. F. (2019). Unnatural amino acids improve affinity and modulate immunogenicity: Developing peptides to treat MHC type II autoimmune disorders. *Peptide Science*, *111*(1), e24058. doi:10.1002/pep2.24058
- Molchanova, N., Hansen, P. R., & Franzyk, H. (2017). Advances in Development of Antimicrobial Peptidomimetics as Potential Drugs. *Molecules*, *22*(9). doi:10.3390/molecules22091430
- Murnen, H. K., Rosales, A. M., Jaworski, J. N., Segalman, R. A., & Zuckermann, R. N. (2010). Hierarchical Self-Assembly of a Biomimetic Diblock Copolypeptoid into Homochiral Superhelices. *Journal of the American Chemical Society*, *132*(45), 16112-16119. doi:10.1021/ja106340f
- Olsen, C. A. (2010). Peptoid-Peptide Hybrid Backbone Architectures. *ChemBiochem*, *11*(2), 152-160. doi:10.1002/cbic.200900618
- Ostergaard, S., & Holm, A. (1997). Peptomers: A versatile approach for the preparation of diverse combinatorial peptidomimetic bead libraries. *Molecular Diversity*, *3*(1), 17-27. doi:Doi 10.1023/A:1009698507588
- Park, M., Wetzler, M., Jardetzky, T. S., & Barron, A. E. (2013). A Readily Applicable Strategy to Convert Peptides to Peptoid-based Therapeutics. *PLoS One*, *8*(3). doi:ARTN e58874
10.1371/journal.pone.0058874
- Paul, B., Butterfoss, G. L., Boswell, M. G., Huang, M. L., Bonneau, R., Wolf, C., & Kirshenbaum, K. (2012). N-Naphthyl Peptoid Foldamers Exhibiting Atropisomerism. *Organic Letters*, *14*(3), 926-929. doi:10.1021/ol203452f

- Proulx, C., Yoo, S., Connolly, M. D., & Zuckermann, R. N. (2015). Accelerated Submonomer Solid-Phase Synthesis of Peptoids Incorporating Multiple Substituted N-Aryl Glycine Monomers. *Journal of Organic Chemistry*, 80(21), 10490-10497. doi:10.1021/acs.joc.5b01449
- Ren, J. H., Tian, Y., Hossain, E., Ho, J. S., Mann, Y. S., Zhang, Y. T., . . . Zuckermann, R. N. (2020). Mass spectrometry studies of the fragmentation patterns and mechanisms of protonated peptoids. *Biopolymers*, 111(7). doi:ARTN e23358
10.1002/bip.23358
- Robertson, E. J., Battigelli, A., Proulx, C., Mannige, R. V., Haxton, T. K., Yun, L., . . . Zuckermann, R. N. (2016). Design, Synthesis, Assembly, and Engineering of Peptoid Nanosheets. *Accounts of Chemical Research*, 49(3), 379-389. doi:10.1021/acs.accounts.5b00439
- Roy, O., Caumes, C., Esvan, Y., Didierjean, C., Faure, S., & Taillefumier, C. (2013). The tert-Butyl Side Chain: A Powerful Means to Lock Peptoid Amide Bonds in the Cis Conformation. *Organic Letters*, 15(9), 2246-2249. doi:10.1021/ol400820y
- Rzeigui, M., Traikia, M., Jouffret, L., Kriznik, A., Khiari, J., Roy, O., & Taillefumier, C. (2020). Strengthening Peptoid Helicity through Sequence Site-Specific Positioning of Amide cis-Inducing NtBu Monomers. *Journal of Organic Chemistry*, 85(4), 2190-2201. doi:10.1021/acs.joc.9b02916
- Sanborn, T. J., Wu, C. W., Zuckerman, R. N., & Barron, A. E. (2002). Extreme stability of helices formed by water-soluble poly-N-substituted glycines (polypeptoids) with alpha-chiral side chains. *Biopolymers*, 63(1), 12-20. doi:DOI 10.1002/bip.1058
- Schettini, R., Costabile, C., Della Sala, G., Iuliano, V., Tedesco, C., Izzo, I., & De Riccardis, F. (2018). Cation-Induced Molecular Switching Based on Reversible Modulation of Peptoid Conformational States. *Journal of Organic Chemistry*, 83(20), 12648-12663. doi:10.1021/acs.joc.8b01990
- Schneider, J. A., Craven, T. W., Kasper, A. C., Yun, C., Haugbro, M., Briggs, E. M., . . . Logan, S. K. (2018). Design of Peptoid-peptide Macrocycles to Inhibit the beta-catenin TCF Interaction in Prostate Cancer. *Nat Commun*, 9(1), 4396. doi:10.1038/s41467-018-06845-3
- Secker, C., Brosnan, S. M., Luxenhofer, R., & Schlaad, H. (2015). Poly(α -Peptoid)s Revisited: Synthesis, Properties, and Use as Biomaterial. *Macromolecular Bioscience*, 15(7), 881-891. doi:10.1002/mabi.201500023
- Shin, S. B., & Kirshenbaum, K. (2007). Conformational rearrangements by water-soluble peptoid foldamers. *Organic Letters*, 9(24), 5003-5006. doi:10.1021/ol702207n
- Simon, R. J., Kania, R. S., Zuckermann, R. N., Huebner, V. D., Jewell, D. A., Banville, S., . . . Marlowe, C. K. (1992). Peptoids: a modular approach to drug discovery. *Proceedings of the National Academy of Sciences*, 89(20), 9367-9371. doi:10.1073/pnas.89.20.9367
- Spencer, R. K., Butterfoss, G. L., Edison, J. R., Eastwood, J. R., Whitelam, S., Kirshenbaum, K., & Zuckermann, R. N. (2019). Stereochemistry of polypeptoid chain configurations. *Biopolymers*, 110(6), e23266. doi:10.1002/bip.23266
- Spicer, S. K., Subramani, A., Aguila, A. L., Green, R. M., McClelland, E. E., & Bicker, K. L. (2019). Toward a clinical antifungal peptoid: Investigations into the therapeutic potential of AEC5. *Biopolymers*, 110(6), e23276. doi:10.1002/bip.23276

- Sternhagen, G. L., Gupta, S., Zhang, Y., John, V., Schneider, G. J., & Zhang, D. (2018). Solution Self-Assemblies of Sequence-Defined Ionic Peptoid Block Copolymers. *Journal of the American Chemical Society*, *140*(11), 4100-4109. doi:10.1021/jacs.8b00461
- Stringer, J. R., Crapster, J. A., Guzei, I. A., & Blackwell, H. E. (2011). Extraordinarily Robust Polyproline Type I Peptoid Helices Generated via the Incorporation of alpha-Chiral Aromatic N-1-Naphthylethyl Side Chains. *Journal of the American Chemical Society*, *133*(39), 15559-15567. doi:10.1021/ja204755p
- Sun, J., Jiang, X., Lund, R., Downing, K. H., Balsara, N. P., & Zuckermann, R. N. (2016). Self-assembly of crystalline nanotubes from monodisperse amphiphilic diblock copolypeptoid tiles. *Proceedings of the National Academy of Sciences*, *113*(15), 3954-3959. doi:10.1073/pnas.1517169113
- Sun, J., Jiang, X., Siegmund, A., Connolly, M. D., Downing, K. H., Balsara, N. P., & Zuckermann, R. N. (2016). Morphology and Proton Transport in Humidified Phosphonated Peptoid Block Copolymers. *Macromolecules*, *49*(8), 3083-3090. doi:10.1021/acs.macromol.6b00353
- Sun, J., Stone, G. M., Balsara, N. P., & Zuckermann, R. N. (2012). Structure-Conductivity Relationship for Peptoid-Based PEO-Mimetic Polymer Electrolytes. *Macromolecules*, *45*(12), 5151-5156. doi:10.1021/ma300775b
- Sun, J., & Zuckermann, R. N. (2013). Peptoid Polymers: A Highly Designable Bioinspired Material. *ACS Nano*, *7*(6), 4715-4732. doi:10.1021/nn4015714
- Thakkar, A., Cohen, A. S., Connolly, M. D., Zuckermann, R. N., & Pei, D. (2009). High-Throughput Sequencing of Peptoids and Peptide-Peptoid Hybrids by Partial Edman Degradation and Mass Spectrometry. *Journal of Combinatorial Chemistry*, *11*(2), 294-302. doi:10.1021/cc8001734
- Uno, T., Beausoleil, E., Goldsmith, R. A., Levine, B. H., & Zuckermann, R. N. (1999). New submonomers for poly N-substituted glycines (peptoids). *Tetrahedron Letters*, *40*(8), 1475-1478. doi:10.1016/S0040-4039(98)02696-3
- Wang, S.-T., Gray, M. A., Xuan, S., Lin, Y., Byrnes, J., Nguyen, A. I., . . . Gang, O. (2020). DNA origami protection and molecular interfacing through engineered sequence-defined peptoids. *Proceedings of the National Academy of Sciences*, *117*(12), 6339-6348. doi:10.1073/pnas.1919749117
- Wender, P. A., Mitchell, D. J., Pattabiraman, K., Pelkey, E. T., Steinman, L., & Rothbard, J. B. (2000). The design, synthesis, and evaluation of molecules that enable or enhance cellular uptake: Peptoid molecular transporters. *Proceedings of the National Academy of Sciences*, *97*(24), 13003-13008. doi:10.1073/pnas.97.24.13003
- Wijaya, A. W., Nguyen, A. I., Roe, L. T., Butterfoss, G. L., Spencer, R. K., Li, N. K., & Zuckermann, R. N. (2019). Cooperative Intramolecular Hydrogen Bonding Strongly Enforces cis-Peptoid Folding. *Journal of the American Chemical Society*, *141*(49), 19436-19447. doi:10.1021/jacs.9b10497
- Wu, C. W., Sanborn, T. J., Zuckermann, R. N., & Barron, A. E. (2001). Peptoid oligomers with alpha-chiral, aromatic side chains: Effects of chain length on secondary structure. *Journal of the American Chemical Society*, *123*(13), 2958-2963. doi:10.1021/ja003153v
- Wu, C. W., Sanborn, T. J., Zuckermann, R. N., & Barron, A. E. (2001). Peptoid Oligomers with α -Chiral, Aromatic Side Chains: Effects of Chain Length on Secondary Structure. *Journal of the American Chemical Society*, *123*(13), 2958-2963. doi:10.1021/ja003153v

- Wuts, P. G. M., Greene, T. W., & Greene, T. W. (2014). *Greene's protective groups in organic synthesis* (Fifth edition / ed., pp. 1 online resource).
- Xuan, S., Gupta, S., Li, X., Bleuel, M., Schneider, G. J., & Zhang, D. (2017). Synthesis and Characterization of Well-Defined PEGylated Polypeptoids as Protein-Resistant Polymers. *Biomacromolecules*, *18*(3), 951-964. doi:10.1021/acs.biomac.6b01824
- Xuan, S., Jiang, X., Spencer, R. K., Li, N. K., Prendergast, D., Balsara, N. P., & Zuckermann, R. N. (2019). Atomic-level engineering and imaging of polypeptoid crystal lattices. *Proceedings of the National Academy of Sciences*, *116*(45), 22491-22499. doi:10.1073/pnas.1909992116
- Xuan, S., Lee, C.-U., Chen, C., Doyle, A. B., Zhang, Y., Guo, L., . . . Zhang, D. (2016). Thermoreversible and Injectable ABC Polypeptoid Hydrogels: Controlling the Hydrogel Properties through Molecular Design. *Chemistry of Materials*, *28*(3), 727-737. doi:10.1021/acs.chemmater.5b03528
- Xuan, S., & Zuckermann, R. N. (2020a). Diblock copolypeptoids: a review of phase separation, crystallization, self-assembly and biological applications. *Journal of Materials Chemistry B*. doi:10.1039/D0TB00477D
- Xuan, S., & Zuckermann, R. N. (2020b). Engineering the atomic structure of sequence-defined peptoid polymers and their assemblies. *Polymer*, *202*, 122691. doi:<https://doi.org/10.1016/j.polymer.2020.122691>
- Yam, A. Y., Wang, X. M., Gao, C. M., Connolly, M. D., Zuckermann, R. N., Bleu, T., . . . Salisbury, C. M. (2011). A Universal Method for Detection of Amyloidogenic Misfolded Proteins. *Biochemistry*, *50*(20), 4322-4329. doi:10.1021/bi200215j
- Zuckermann, R. N., Kerr, J. M., Kent, S. B. H., & Moos, W. H. (1992). Efficient method for the preparation of peptoids [oligo(N-substituted glycines)] by submonomer solid-phase synthesis. *Journal of the American Chemical Society*, *114*(26), 10646-10647. doi:10.1021/ja00052a076
- Zuckermann, R. N., & Kodadek, T. (2009). Peptoids as potential therapeutics. *Curr Opin Mol Ther*, *11*(3), 299-307. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/19479663>
- Zuckermann, R. N., Martin, E. J., Spellmeyer, D. C., Stauber, G. B., Shoemaker, K. R., Kerr, J. M., . . . Moos, W. H. (1994). Discovery of Nanomolar Ligands for 7-Transmembrane G-Protein-Coupled Receptors from a Diverse N-(Substituted)Glycine Peptoid Library. *Journal of Medicinal Chemistry*, *37*(17), 2678-2685. doi:DOI 10.1021/jm00043a007