

# A Field Guide to Optimizing Peptoid Synthesis

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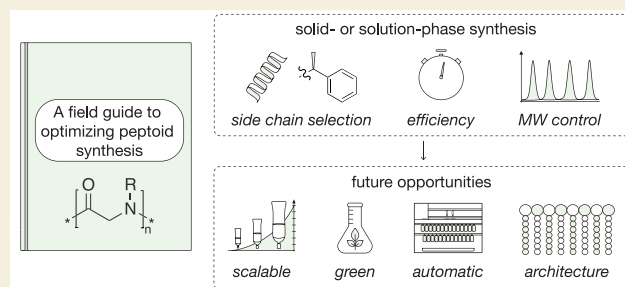
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**ABSTRACT:** N-Substituted glycines (peptoids) are a class of peptidomimetic molecules used as materials for health, environmental, and drug delivery applications. Automated solid-phase synthesis is the most widely used approach for preparing polypeptoids, with a range of published protocols and modifications for selected synthetic targets. Simultaneously, emerging solution-phase syntheses are being leveraged to overcome limitations in solid-phase synthesis and access high-molecular weight polypeptoids. This Perspective aims to outline strategies for the optimization of both solid- and solution-phase synthesis, provide technical considerations for robotic synthesizers, and offer an outlook on advances in synthetic methodologies. The solid-phase synthesis sections explore steps for protocol optimization, accessing complex side chains, and adaptation to robotic synthesizers; the sections on solution-phase synthesis cover the selection of initiators, side chain compatibility, and strategies for controlling polymerization efficiency and scale. This text acts as a “field guide” for researchers aiming to leverage the flexibility and adaptability of peptoids in their research.

**KEYWORDS:** *peptoids, solid-phase synthesis, solution-phase synthesis, robotic synthesis, sequence specific, green chemistry*



## 1. INTRODUCTION

Peptoids, poly(N-substituted glycines), are expanding the materials landscape for therapeutics, diagnostics, carbon capture, and antifreeze agents.<sup>1–5</sup> The ability for a diverse array of side chains to be readily incorporated into peptoids gives rise to tunable physicochemical properties and secondary structures, allowing for the rational design of peptoid-based materials. Similar to peptides, peptoids are biocompatible but have distinct advantages, including increased protease stability, decreased immunogenicity, and improved cellular permeability.<sup>6–8</sup> With recent synthetic advances, a range of chain lengths can be achieved, allowing for the exploration of their potential utility as both small-molecules and polymers. For instance, sequence-controlled peptoid oligomers exhibit antifreeze activity, which has relevance in the food and biomedical industries, and have also been demonstrated to sequester carbon dioxide as carbon capture agents.<sup>4</sup> Higher molecular weight peptoids that self-assemble into nanosheets have been explored as diagnostics for Alzheimer's disease and H<sub>2</sub>S detection in cells.<sup>9,10</sup> However, in order to translate peptoid research industrially, there is a need for reliable, scalable, and sequence-controllable synthetic methods.

Current strategies for accessing polypeptoids are not one-size-fits-all, resulting in a trade-off between sequence specificity and scalability. Solid-phase synthesis of peptoids offers precise sequence control and can be automated on robotic synthesizers. This is typically limited to milligram scales and oligomers as minor yield loss from individual reactions is

compounded over many steps, resulting in low effective reaction yields for longer chain lengths. Nonetheless, all reagents needed to synthesize peptoids are commercially available and amines synthesized in-house can also be incorporated. Compared to solid-phase synthesis, solution-phase techniques provide an opportunity to synthesize polypeptoids with higher degrees of polymerization (DP) and on larger scales. While these methodologies currently only offer statistical sequence-control and limited monomer diversity, chain lengths are well-defined with minimal dispersity. As peptoids are translated from lab to industrial scale synthesis, it will be imperative to develop versatile synthetic approaches that can close the gap between scalability and sequence specificity.

In this Perspective, we discuss strategies for optimizing the solid- and solution-phase synthesis of peptoids to provide an accessible guide for the modern peptoid scientist (Figure 1). Although peptoid synthesis is usually straightforward, we highlight recent synthetic advances that address challenges unique to the peptoid field, including the incorporation of difficult side chains, solubility issues, and robotic synthesis. We

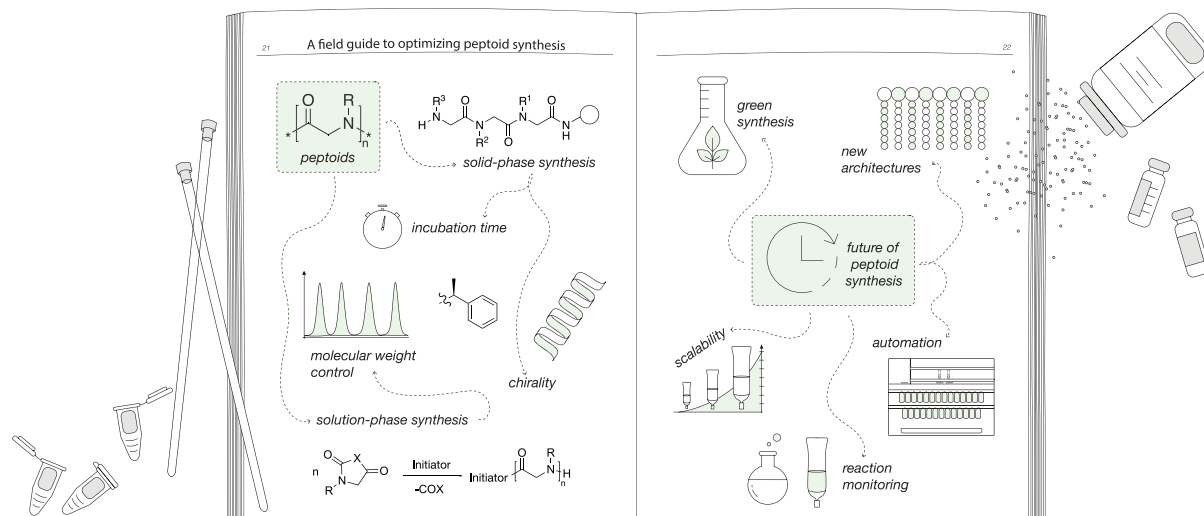
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**Figure 1.** Field guide and outlook of peptoid synthesis. Left: Present approaches for peptoid synthesis, including solid-phase synthesis on resin and solution-phase synthesis via ring-opening polymerizations of *N*-substituted *N*-carboxyanhydrides and *N*-thiocarboxyanhydrides. Right: Future directions for peptoid synthesis including improvements in scalability, green solvents, new architectures, and advances in reaction monitoring for automated synthesis.

hope that this Perspective will enable others to adopt peptoids into their research easily to further advance the field. We would encourage readers to also access other published reviews on polypeptoids which focus on their synthesis (e.g., solid-phase,<sup>11–14</sup> ring-opening polymerization,<sup>15</sup> Ugi polymerization,<sup>16–18</sup> polypeptoid containing copolymers,<sup>19,20</sup> macrocyclization<sup>21–23</sup>), properties (e.g., self-assembly,<sup>24,25</sup> stimuli-responsiveness<sup>26</sup>), and applications.<sup>27,28</sup> We would also like to highlight more comprehensive reviews.<sup>29–31</sup> Here, each section outlines methodologies currently employed to optimize peptoid synthesis, such as in improving efficiency, incorporating desired functionalities, and increasing polymerization control. The Perspective concludes with our outlook on opportunities in the peptoid field that have yet to be explored fully, including interesting polymer architectures as well as synthetic methods that are more efficient, green, sequence-controlled, and scalable.

## 2. OPTIMIZING THE SOLID-PHASE SYNTHESIS OF PEPTOIDS

The submonomer strategy is the most common approach to the solid-phase synthesis of peptoids, and while standard protocols can be used in most instances, modifications can be made to meet specific research needs. Here, we aim to highlight the optimization of the submonomer protocols to increase reaction efficiency as well as key considerations for the incorporation of different classes of side chains in solid-phase synthesis.

### 2.1. Submonomer Protocols

The standard submonomer approach (Figure 2A) uses a repeated two-step cycle involving the acylation of a terminal amine bound to a solid resin support (e.g., Rink Amide, Wang, 2-chlorotrityl chloride)<sup>32</sup> with bromoacetic acid (BrAc) activated by *N,N'*-diisopropylcarbodiimide (DIC) followed by displacement with a primary amine. A significant benefit of the submonomer strategy is that primary amines with diverse side chains are more widely available and synthetically accessible compared to the protected monomer units used in the monomer method. The modularity and stepwise nature of

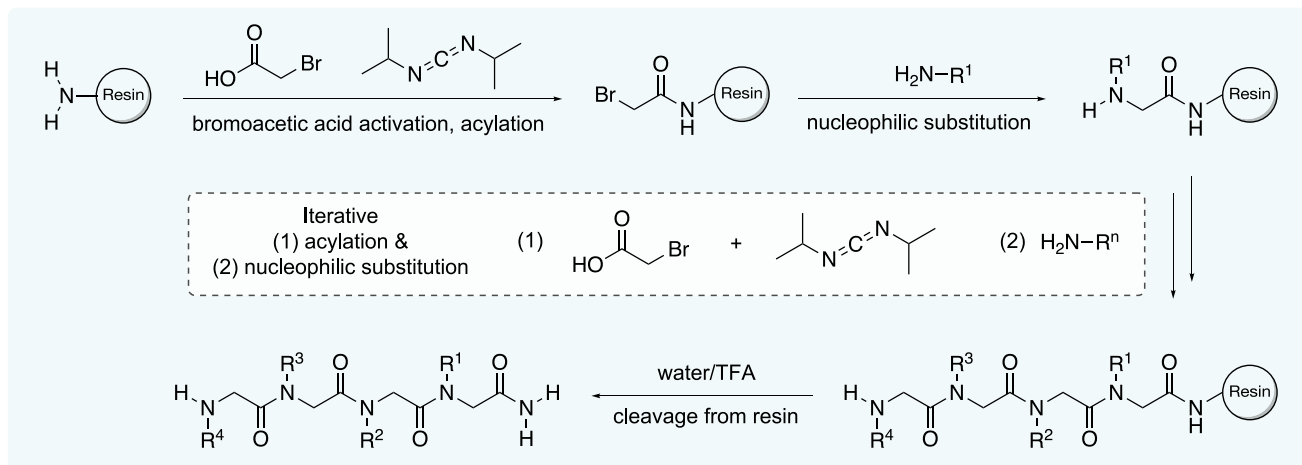
the submonomer method allows for the incorporation of a wide range of side chains (Figure 2B) with precise control over their position in the peptoid backbone.

Although the acylation and displacement steps are robust and high-yielding, the number of steps (two reactions per monomer unit) typically limits chain lengths to a maximum of 60 monomer units, as small losses in yield accumulate during synthesis. Elevated temperatures have been shown to increase yields during acylation and can be achieved using heat blocks and microwave<sup>33,34</sup> or UV heating<sup>35,36</sup> up to 35 °C. The optimization of the acylation conditions has also been conducted by Zuckermann and co-workers, who identified an ideal molar ratio of 1:0.93 for BrAc:DIC.<sup>37</sup> In addition, the incubation times for each step requires consideration, especially at higher chain lengths. For example, Zuckermann and co-workers increased the length of displacement from 60 to 90 min after the addition of the first ten monomers in the synthesis of 30–60-mer peptoids, as reactivity decreases with longer chain lengths. In solid-phase synthesis, resin cleavage as well as side chain deprotection are often performed simultaneously with one cleavage cocktail. However, peptoid sequences containing multiple protecting groups may require additional optimization to maximize final yields following cleavage. Due to the similarities with solid-phase peptide synthesis, successful peptide cleavage cocktails can be readily adopted to the synthesis of peptoids with various sequences.<sup>38–40</sup> The selection of the resin can be used to tune the peptoid end groups for postmodifications. For example, 2-chlorotrityl resin yields peptoids with a C-terminal carboxylic acid functionality, allowing for head-to-tail cyclization in the synthesis of peptoid macrocycles.<sup>21</sup>

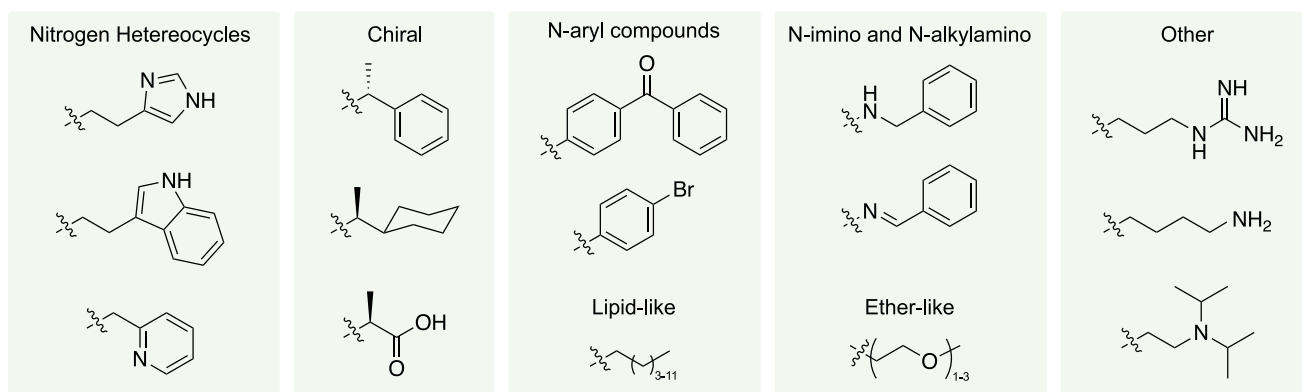
### 2.2. Side Chain Diversity

The variety of side chains accessible to solid-phase techniques provides opportunities to explore the influence of functional groups on different application-relevant properties such as self-assembly, biological activity, and biorecognition.<sup>41–45</sup> While there are well-established protocols for automated and manual solid-phase synthesis, optimization is required for accessing select side chains, such as those that are heterocyclic, chiral,

## A SOLID-PHASE SYNTHESIS



## B SIDE CHAINS REQUIRING CONSIDERATION



**Figure 2.** Solid-phase synthesis of peptoids. (A) Solid-phase peptoid synthesis proceeds with iterative steps of acylation of a terminal amine followed by displacement by a primary amine. These steps are repeated to grow longer polypeptoids, which are then cleaved from the resin. (B) A variety of side chains which have been incorporated onto the peptoid backbone.

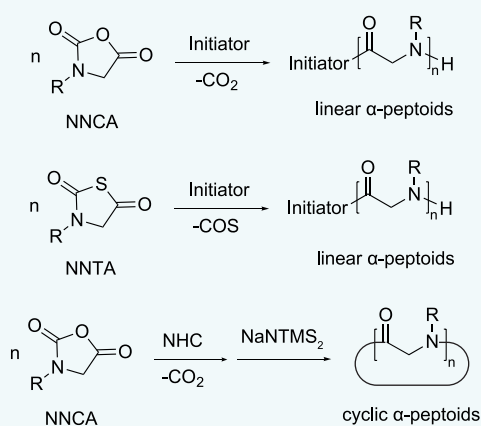
bulky, hydrophobic, and charged, and are not accessible through post-modifications.<sup>46–49</sup> Side chains containing functionalities such as amino or carboxyl groups require protection to prevent cross-reaction during synthesis and the formation of undesired linkages. Side chain protecting groups such as *tert*-butoxycarbonyl (Boc) and *tert*-butyl esters are acid-labile and can be simultaneously deprotected during resin cleavage conditions, avoiding additional deprotection reactions. Furthermore, some of these amines can be purchased and stored as a salt for long-term stability and require an extra free-basing step which can be performed via liquid–liquid extraction prior to use.<sup>13</sup> In the synthesis of water-soluble peptoids, two-thirds of the sequence is required to contain hydrophilic pendant groups, which must be spread uniformly along the peptoid backbone to ensure solubility.<sup>50</sup> However, hydrophobic groups are required to maintain therapeutic activity, thus limiting the practical applications of hydrophilic peptoids. Maayan and co-workers found that incorporating piperazine or homopiperazine units into the backbone of hydrophobic peptoids allowed for water solubility without the modification of their sequence or structure. The incorporation of heterocyclic side chains (Figure 2B) has also necessitated alterations to the standard submonomer protocol as BrAc causes unwanted alkylation of the aromatic nitrogen. Zuckermann and co-workers found that using chloroacetic acid during acylation reduced unwanted alkylations due to the

weaker leaving group quality of chloride over bromide.<sup>51</sup> Amine concentrations were increased from 1 to 2 mol/L with incubation times of 1 h to account for the reduced reactivity of chloroacetic acid. The lower reactivity of chloroacetic acid has also been exploited to avoid cyclization byproducts in the incorporation of *cis* backbone-inducing *N,N'*-diisopropylethylenediamine (Figure 2B).<sup>52</sup>

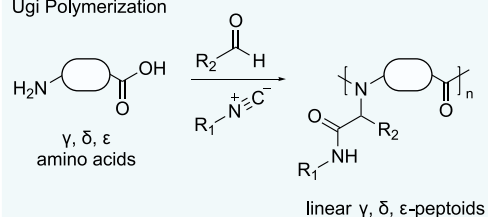
As the chemical structure of the peptoid backbone is inherently achiral, the selection of appropriate side chains can be used to induce chirality, allowing access to chiral secondary structures. The high steric hindrance of chiral amines (Figure 2B) often contributes to their slow reactivity, requiring increased reaction times and temperatures during acylation and displacement.<sup>53</sup> In order to improve yields when incorporating bulky chiral side chains, Segalman and co-workers performed two sequential bromoacetylation steps during each cycle to ensure acylation went to completion.<sup>54</sup> The lack of chirality and hydrogen bonding in peptoids increases backbone flexibility, which can be controlled by the selection of side chains that induce either *cis*- or *trans*-amide bond configurations. For example, *N*-aryl side chains favor the *trans*-conformation while 1,2,3-triazolium side chains favor the *cis*-conformation.<sup>55,56</sup> In exploring new *trans*-directing monomer structures, Proulx and co-workers have applied hydrazones as submonomers to generate *trans*-inducing *N*-imino and *N*-alkylamino glycines (Figure 2B) with hydrogen bonding

## A SOLUTION-PHASE APPROACHES

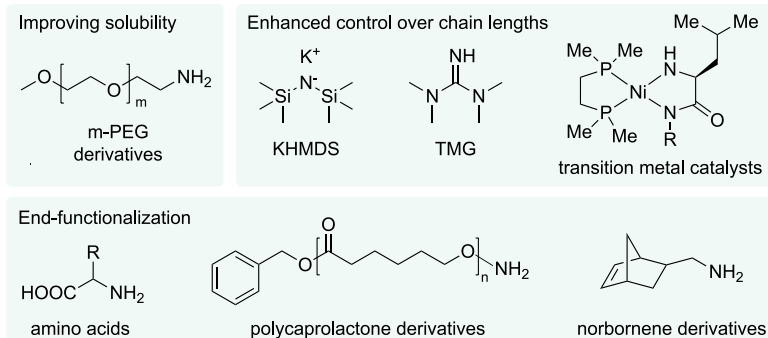
## Ring Opening Polymerizations



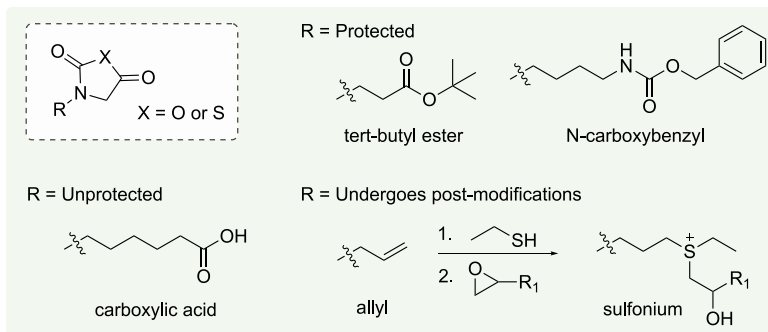
## Ugi Polymerization



## B INITIATOR SELECTION



## C SIDE CHAIN DIVERSITY



**Figure 3.** Solution-phase peptoid synthesis. (A) Solution-phase synthetic approaches include the ring-opening polymerization of *N*-substituted *N*-carboxyanhydride (NNCA) and *N*-substituted *N*-thiocarboxyanhydride (NNTA) as well as the Ugi polymerization of amino acids. (B) The choice of initiator will affect the solubility, chain lengths, and potential end groups of the synthesized polypeptoids. (C) Side chains accessible via solution-phase techniques can be protected, unprotected, or modified postpolymerization.

capabilities.<sup>57</sup> Control over the *cis/trans* backbone geometry can give access to a range of secondary structures such as helices, sheets, ribbons, turns, and loops.<sup>58</sup> However, some of these structure-inducing side chains are weak nucleophiles, such as 4-bromoaniline and 4-aminobenzophenone, resulting in poor reactivity (Figure 2B). The addition of salts such as potassium iodide and silver perchlorate allows for the rapid abstraction of the bromide during displacement and the acceleration of reaction rates up to 76-fold.<sup>59,60</sup> Self-assembly into sheets has also been observed with polypeptoids bearing a long hydrophilic block and complementary lipid-like block.<sup>61</sup> However, the high hydrophobicity of these lipid-like precursor amines, such as tridecylamine, causes poor solubility in dimethylformamide (DMF) and lowers overall reaction rates. Higher yields can be obtained by preparing the amine solutions in *N*-methylpyrrolidone (NMP) or 1,2-dichloroethane (DCE) to avoid precipitation and increasing the length of the displacement reaction (2–3 h).

### 3. OPTIMIZING THE SOLUTION-PHASE SYNTHESIS OF PEPTOIDS

Solution-phase approaches such as the ring-opening polymerization (ROP) of *N*-substituted *N*-carboxyanhydrides (NNCAs), ROP of *N*-substituted *N*-thiocarboxyanhydrides (NNTAs), and the Ugi reaction have become more amenable to the preparation of linear high molecular-weight polypeptoids with advances made toward increasing side chain diversity and improving control over chain lengths (Figure 3A).<sup>16,29</sup> The ROP of NNCA and NNTAs relies on nucleophilic initiation, commonly by primary amines, and has been shown to proceed in a living and controlled

manner.<sup>29,62</sup> However, the polymerization of monomers with diverse and application-relevant side chains (e.g., bulky or hydrophilic), control over chain lengths above a DP of 100, and improving the speed of polymerization (e.g., days to hours) are some of the challenges that recent synthetic approaches have begun to address.<sup>15</sup> There are several recently published reviews that discuss the solution-phase synthesis of polypeptoids.<sup>11,15,19,20,29,63</sup> Here, we aim to highlight recent techniques used to optimize solution-phase peptoid synthesis to improve polymerization, solubility, and synthetic versatility.

#### 3.1. Initiator Selection

The selection of initiators for the ROP of NNCA and NNTA can influence the time required for polymerization as well as the resulting solubility, molecular weight, and chain-end functionalization of the synthesized polypeptoids.<sup>64–66</sup> Primary amines are the most well-explored initiators, producing polypeptoids with secondary amine groups at their chain ends with a range of linear and cyclic side chains.<sup>65</sup> While the ROP of NNCA yields polypeptoids with longer chain lengths than solid-phase techniques, polymer solubility limits the efficiency of polymerization and chain propagation. The selection of a soluble initiator for ROP can help to overcome this limitation and prevent the precipitation of lower molecular-weight polypeptoids. Li and co-workers found that initiation of their NNCA monomers by a water-soluble methoxypolyethylene glycol amine (mPEG-NH<sub>2</sub>) yielded similar peptoid chain lengths when compared to initiation by benzylamine, a common small-molecule ROP initiator.<sup>66</sup> Tong and co-workers were able to achieve peptoid chain lengths up to 35 units using mPEG-NH<sub>2</sub> initiator in DMF ([M]:[I] of 40) while initiation with hexylamine ([M]:[I] of 40) yielded

oligomers of only 21 units.<sup>67</sup> Due to the solubility of PEG in a variety of polar solvents, PEG-based initiators have been shown to increase the solubility of the resulting polypeptoids, allowing for access to higher molecular weights, improved yields, and characterization techniques (Figure 3B).

Initiators can also eliminate the need for modifications postpolymerization by introducing functionality to the peptoid chain ends. For example, Ling and co-workers used L-methionine, L-tryptophane, and L-phenylalanine as amino acid initiators for the ROP of NNTAs to produce end-functionalized polypeptoids (Figure 3B).<sup>68</sup> This pathway required the addition of acetic acid to increase the solubility of the amino acids, preventing the formation of salts which would impede initiation. Polycaprolactone functionalized with oxyamine was used as an initiator for the polymerization of NNTAs by Li and co-workers (Figure 3B).<sup>69</sup> The synthesized block copolymers self-assembled into polymersomes for potential use in biomedical applications as drug carriers or contrast agent carriers, due to their low cytotoxicity. Similarly, initiators can also expand the potential architectures of polypeptoids, which may lead to emergent properties. For example, cyclic peptoids can be synthesized using a N-heterocyclic carbene initiator followed by treatment with dithranol or sodium bis(trimethylsilyl)amide (NaN(TMS)<sub>2</sub>).<sup>70,71</sup> Ling and co-workers developed heterograft molecular polymer brushes with polypeptoids initiated by an amine functionalized norbornene handle (Figure 3B).<sup>72</sup> These brushes contained heterografts of polylactone, polytetrahydrofuran, and self-assembled into micelles, which are valuable structures in drug delivery. Star-like polypeptoid architectures have also been achieved by Bonduelle and co-workers through the use of a dendritic macroinitiator like poly(amidoamine).<sup>73</sup> Star polymers have been shown to have enhanced antimicrobial potency compared to their linear counterparts, making synthetic strategies to access these architectures highly desirable.

Initiators can influence the degree of control over the molecular weight of the synthesized polypeptoids. One of the central challenges in the ROP of NNCA and NNTAs is the loss of control over chain lengths at high monomer to initiator ratios (>100:1) where a plateau in molecular weight is observed.<sup>74</sup> This is likely due to the presence of impurities in the synthesized monomers, unwanted initiation, or other competing reactions.<sup>29,75</sup> Excellent precision over molecular weight can be obtained at DP below 100 using primary amines, disilazides, alcohols, and N-heterocyclic carbenes.<sup>76–79</sup> For example, Liu and co-workers demonstrated good correlation between the observed and expected number-average molecular weights based on the monomer to initiator feed ratios when using alkali-metal hexamethyldisilazides (KHMDs) with an upper DP limit of 40 (Figure 3B).<sup>80</sup> However, using the same initiator system and a different NNCA monomer, Bonduelle and co-workers demonstrate the loss of control at high molecular weights as a monomer to initiator ratio of 400:1 yielded polypeptoids with DP only up to 115.<sup>77</sup> Ling and co-workers addressed this concern in the ROP of NNTAs using initiators with high catalytic activity, rare earth borohydrides.<sup>81</sup> Monomer feed ratios up to 516 yielded unimodal products with predictable molecular weights and chain lengths up to 390 units. However, there is minimal control over the chain ends of the polypeptoids using this strategy and molecular weight plateauing was still observed at high DPs. 1,1,3,3-Tetramethylguanidine (TMG) was used to initiate the ROP of methyl-

NNTAs by Zhang and co-workers, producing well-defined polypeptoids with no plateauing observed for monomer to initiator ratios up to 400:1 and predictable chain ends (Figure 3B).<sup>82</sup> A novel peptoid synthetic method inspired by the controlled, living polymerization of NCAs with transition-metal catalysts was created by Kramer and Clauss to produce chain-length controlled polypeptoids with functionalized end groups (Figure 3B).<sup>75</sup> Using nickel amido-amidate preformed catalysts, the ROP of NNCA yielded polypeptoids with excellent control over chain lengths for a variety of hydrophilic and hydrophobic side chains. Additionally, this polymerization was characterized as living, allowing for the production of block copolymers composed of polypeptoids and polypeptides. Since the catalyst/initiator system is preformed, it allows for precise control over the incorporation of functional end groups relevant for biological targeting or postmodifications.

### 3.2. Side Chain Diversity

Designing peptoids for various biomaterials applications requires synthetic versatility, which has remained a challenge in solution-phase peptoid synthesis. Increasing the variety of accessible side chains provides opportunities to explore the influence of functional groups on different application-relevant properties such as self-assembly, biological activity, and biorecognition.<sup>83–85</sup> Most solution-phase peptoid research has focused on linear alkyl side chains because the polymerization rate has been shown to be affected by side chain length and bulkiness. Density functional theory (DFT) calculations have shown that steric hindrance of beta-branched alkyl side chains contributes to reduced rates of polymerization and van der Waals interactions lead to aggregation for linear and gamma-branched side chains.<sup>86</sup> While the polymerization of NNCA with bulky functional groups is still possible, additives and alternative initiators should be explored to help decrease the reaction time.

The incorporation of nonalkyl side chains in solution-phase approaches can also be challenging as it must be compatible with the synthesis of NNCA monomers and should not form undesired linkages during the polymerization. To address the limited side chain functionalities able to be incorporated during NNCA monomer synthesis, Li and co-workers presented the conversion of natural  $\alpha$ -amino acids to NNCA monomers using Schiff base and reductive amination reactions.<sup>66</sup> The reaction conditions are mild and tolerant to various functional groups, which provides opportunities to incorporate side chains that may be inaccessible via alternative methods for NNCA preparation. Protecting groups can be useful for exploring interesting functionalities in peptoid synthesis. For example, carboxyl groups can be protected as *tert*-butyl esters during NNCA monomer synthesis and cleaved under acidic conditions following polymerization (Figure 3C).<sup>87</sup> These carboxyl-functionalized polypeptoids are attractive as stimuli-responsive materials capable of self-assembly. The carboxybenzyl protecting group has also been used for NNCA with amine terminated side chains (Figure 3C).<sup>73</sup> The deprotection of these polypeptoids requires additional purification and can result in significant yield loss, motivating the polymerization of monomers with unprotected groups. NNTA polymerization has been shown to be tolerant to both unprotected hydroxyl and carboxyl functional groups but is dependent on the length of the carbon linker.<sup>88</sup> Sun and co-workers reported successful NNTA polymerization with side chains containing a carboxyl group separated by a five-carbon

linker, producing polypeptoids with DPs up to 58 and low dispersities (Figure 3C). However, polymerization was not observed for shorter linker lengths, producing cyclization products during monomer synthesis or early propagation. The postfunctionalization of polypeptoids also offers access to a wider range of functional groups unable to be introduced into the monomers. For example, Chen and co-workers polymerized NNCA substituted with an allyl group and converted the side chains to polysulfoniums, which showed potent antibacterial activity (Figure 3C).<sup>89</sup>

### 3.3. Polymerization Efficiency and Scale

NNCA monomers and their polymerizations are highly sensitive to air, moisture, and heat, and often show slow reactivity when using primary amines as initiators. Increasing the speed of the solution-phase synthesis of polypeptoids has been explored using commercially available organocatalysts. Liu and co-workers demonstrated that urea derivatives can accelerate the polymerization of bulky NNCA monomers through hydrogen-bonding interactions, reducing polymerization time from 7 days to 5 h.<sup>76</sup> In addition to the time required for NNCA polymerization, polypeptoid synthesis is also limited by the instability of the NNCA monomers. Schlaad and co-workers address this limitation through the in situ preparation and polymerization of NNCA monomers using activated urethane derivatives in the presence of a tertiary amine base and a primary amine initiator. This method involves mild reaction conditions and uses air and moisture stable starting reagents, increasing the synthetic accessibility of NNCA monomers.<sup>90</sup> NNTAs are more stable than their NNCA counterparts and do not require the complete elimination of moisture for successful polymerization.<sup>81</sup> However, the presence of water at concentrations greater than 10 000 ppm inhibits NNTA polymerization due to the formation of hydrogen sulfide, which reacts with the monomers.<sup>91</sup> However, by bubbling the polymerization solution with an inert gas, Ling and co-workers found that the hydrogen sulfide was dispelled and obtained polypeptoids of high molar masses, good yields, and low dispersities.

Despite the various strategies developed for optimizing the solution-phase synthesis of peptoids, relatively little has been reported on increasing the scale of the polymerization as this likely has more industrial and less academic relevance. The instability of NNCA monomers and high purity requirements limits the large-scale preparation of polypeptoids using this polymerization technique. However, peptoid oligomers have been synthesized on a gram-scale using alternative solution-phase methods that rely on the iterative addition of stable monomers. For example, Taillefumier and co-workers developed an iterative submonomer approach involving an aza-Michael addition between a protected acrylate and an amine, followed by acylation.<sup>92</sup> This strategy was scaled up to produce peptoid tetramers in large quantities (2.5 g) yet was limited to shorter chain lengths (DP of four). Faure and co-workers also investigated submonomer synthesis in solution via alternating substitution and acylation of a protected bromoacetate derivative.<sup>93</sup> Peptoid trimers containing bulky *tert*-butyl side chains were successfully synthesized on a gram scale (2.2 g) using these conditions, but the efficiency of this approach declined significantly for chain-lengths longer than three units. While a [3 + 3] blockwise coupling of the synthesized trimers was used to generate the desired hexamers, these reactions were performed on a milligram scale. The Ugi

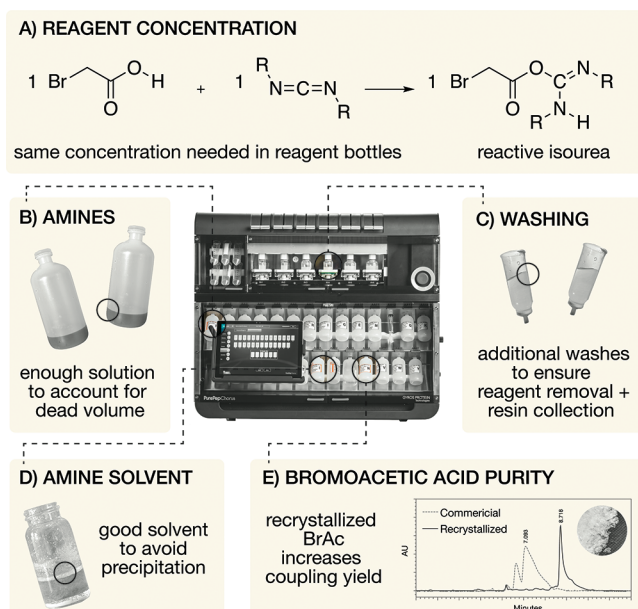
reaction for polypeptoids also offers scalability but is not amenable to the synthesis of alpha-polypeptoids due to undesired cyclization.<sup>16,17</sup> This Ugi strategy involves a four-component reaction between an aldehyde, amine, isocyanide, and a carboxylic acid, uses mild conditions, and does not require an inert atmosphere. A Boc-protected lysine monomer was successfully polymerized by Wang and co-workers on a multigram scale with high yields using the Ugi method.<sup>18</sup> The Ugi reaction produces polypeptoids with shorter chain lengths (DP up to 30), which can also be generated via solid-phase synthesis. However, access to the large-scale synthesis of higher molecular weight polypeptoids (DP > 30) remains a persistent challenge in the optimization of solution-phase techniques.

## 4. ADAPTATION OF PEPTOID SYNTHESIS ONTO ROBOTIC SYNTHESIZERS

The solid-phase submonomer approach to peptoid synthesis is amenable to automation as the reactions are generally air- and moisture-stable and require simple techniques (e.g., pipetting, mixing, filtration). Thus, peptoid synthesis has been readily adapted to most commercially available peptide synthesizers.<sup>94</sup> However, there are a wide range of protocols reported for automation with differing reaction times, temperatures, concentrations, and reagent delivery ratios.<sup>13,95–97</sup> Variations in the type of robotic synthesizer used as well as the side chain being incorporated can account for these modifications, with researchers optimizing protocols to meet their unique needs. The following section notes some intricacies which should be taken into consideration for scientists seeking to adopt a program reported in literature to their instrument.

Solid-phase submonomer protocols for automated peptoid synthesis are not necessarily reproducible between instruments due to differences in volume calibration and delivery. Fluid transfers can vary between differently calibrated instruments as well as between experiments on the same instrument if using reagents or solvents with different viscosities than those used during calibration. For example, in the operation of our own peptoid synthesizer, a Gyros PurePep Chorus, we noted that several reagents consistently deliver lower volumes than expected and cannot be recalibrated. In the small volumetric scale of automated peptoid synthesis (mg), even relatively small variations in reagent volume (250  $\mu$ L) can significantly affect yields during coupling, given its sensitivity to the ratio of BrAc and DIC. To ensure that our desired ratio (Figure 4A) is maintained within the reaction vessel, even in the case of volume underdelivery, we prepare monoconcentrated reagents (0.8 M) that are programmed to be delivered in equal volumes, and program an additional 250  $\mu$ L in the reagent delivery for the coupling step to correct for underdelivery. We recommend users measure the delivery of reagents to their reaction vessels and account for discrepancies in programmed volumes for future protocols.

Compared with manual solid-phase synthesis, automated synthesis involves a different set of concerns and potential drawbacks. Users must prepare additional amine submonomer solution in excess of the volume required for synthesis to account for priming and dead volumes in delivery (Figure 4B). This often amounts to an additional 5 mL of solution, which is undesirable for expensive or synthetically complex amines. Unlike manual peptoid synthesis where users can visually inspect their glassware after successive washes, robotic synthesizers require more washes to ensure that the entirety of the resin is washed to the bottom of the vessel (Figure 4C).



**Figure 4.** Considerations for solid-phase peptoid synthesis. (A) Preparing BrAc and DIC in equal concentration and delivering the reagents in equal volume maintains the desired ratio in the reaction vessel. (B) Additional primary amine solution is required for priming and accounting for dead volumes. (C) Reaction vessels must be thoroughly washed with solvent between coupling and displacement reactions to ensure reagents are removed and the resin is washed to the bottom of the vessel. (D) Solvents selected for preparing the amine solution must thoroughly dissolve the amine to avoid precipitation in the lines. (E) Recrystallized BrAc improves the yield of the coupling reaction and avoids formation of unwanted side products.

Moreover, the significant amount of tubing in robotic synthesizers represents increased surface area which must be washed between steps, contributing to a greater waste volume than manual syntheses. While manual synthetic protocols can easily be translated between different research groups, additional considerations are required for translating protocols between different automated synthesizers. For example, tubing connections may differ between automated synthesizers, necessitating different numbers of washes between each step. As well, the heating capabilities, volume delivery, scale, UV monitoring, and mixing type (e.g., oscillation, vortex, magnetic stirring, nitrogen) may differ between automated instruments, requiring modifications to synthetic protocols.

For solid-phase synthesizers that are open to the air (often adapted from parallel-format synthesizers), the evaporation of the amine solution and precipitation of unstable amines in nonideal solvents (Figure 4D) is a concern. Amine crystallization decreases the concentration of the prepared solution and can clog smaller tubing, resulting in decreased yields or even damage to the instrument. This issue is exacerbated in the case of multiday syntheses required for longer peptoids, which increase the likelihood of amine precipitation. In our experiences with the Gyros Purepep Chorus, we noted an improvement in crude purity and yield (Figure 4E) when using BrAc recrystallized in hexanes compared to the commercial as-received sample.

## 5. OUTLOOK

The various routes for the synthesis of polypeptoids have been well optimized, with each capable of yielding materials tuned for desired applications. Solid-phase techniques allow for sequence and molecular-weight definition at small chain lengths, ideal for the synthesis of peptoid oligomers for drug-delivery platforms, therapeutics, and nanomaterials. Solution-phase strategies offer access to higher molecular weights and with new initiator and catalyst systems, good control over chain lengths. However, we anticipate several opportunities for the future of peptoid synthesis, namely the development of strategies to access novel polymer architectures, increase the efficiency of solid- and solution-phase methodologies, introduce greener solvents and reagents, as well as improve sequence control and scalability. As these are emerging areas of interest, this section highlights potential methods to address them in peptoid synthesis.

The polymerization techniques developed for peptoid synthesis are amenable to different polymer architectures, such as block copolymers, bottlebrush polymers, and star polymers.<sup>72,73,77,98,99</sup> Access to these structures increases the versatility of peptoids, enabling their exploration in various biomaterials applications. Block copolymers offer tunable chemistries and mechanical properties, and polypeptoid containing block copolymers have been used in many areas of research, including self-assembly, crystallization, biosensing, antifouling, and drug delivery.<sup>9,100–104</sup> A recent review by Ling and co-workers summarized the various synthetic approaches for polypeptoid block copolymers which included sequential ROP, macroinitiation, polypeptoid initiation, and chemical ligation.<sup>19</sup> The inclusion of polymers with novel properties in future polypeptoid containing block copolymers could allow for opportunities to explore peptoids in materials with shape-memory behavior, self-healing capacities, or new forms of self-assembly. While these properties have yet to be explored for peptoids, one area they could be applied is in shape memory polymers which require flexible soft segments and physically cross-linked “netpoints” to exhibit shape memory behaviors.<sup>105</sup> Peptides have been explored as the soft segments in shape memory polymers because their self-assembly and capacity for hydrogen-bonding allow for good shape recovery and modulation of the shape memory response.<sup>106</sup> It is conceivable that peptoids could also be successfully incorporated as blocks in shape memory polymers. Peptoids exhibiting ordered and predictable self-assembly and hydrogen-bonding motifs can be incorporated in side chains to design shape memory materials with tunable functions.

Bottlebrush polymers are also used in a wide range of applications such as coatings and drug carriers, due to their unique architecture and mechanical properties.<sup>107</sup> While there has not been extensive investigation of peptoid-based bottlebrushes, Zhang and co-workers synthesized polypeptoid brush copolymers using a grafting-through approach and analyzed their thermoresponsive properties.<sup>99</sup> Ling and co-workers also prepared heterograft peptoid-based bottlebrush polymers which self-assemble into micellar aggregates, useful for drug delivery.<sup>72</sup> Further incorporation of peptoids in bottlebrush architectures could open avenues to explore novel peptoid-based materials with useful properties. For example, Kramer and co-workers have synthesized glycopolypeptide bottlebrush polymers comprised of sugars and amino acids as synthetic mimics of natural glycan bottlebrush polymers.<sup>108</sup> As

well-defined materials capable of self-assembly, peptoid-based bottlebrushes could provide new opportunities for the synthesis of protease-resistant biomimetic materials.

This Perspective explored the various strategies that have been used to optimize peptoid synthesis, yet there are still remaining challenges related to efficiency that require creative solutions. For example, techniques for monitoring reaction completion in solid-phase synthesis have been challenging to develop because the growing chain is covalently attached to an insoluble resin bead. As a result, analysis can only be done on the final polypeptoid, where it is difficult to identify steps requiring troubleshooting, or after isolation and cleavage of the resin midpolymerization, which is irreversible. All solid-phase protocols use excess reagents to ensure reaction completion, so the ability to monitor reaction progress could help to reduce the amount of waste produced and conserve expensive or synthetically challenging amines. On-resin reversible stain protocols capable of detecting remaining amino groups following acylation or primary amines following the amination step could help to increase efficiency by minimizing the length of steps in the protocol and the amounts of reagents consumed.<sup>109</sup> Additionally, the incorporation of photolabile linkers onto the resin can allow for the direct analysis of the growing resin-bound peptoid chain via matrix-assisted laser desorption/ionization (MALDI). This technique has been applied in peptide chemistry but, to the best of our knowledge, has not been adapted to peptoid synthesis. This method allows for fast and efficient monitoring of reaction progress, similar to thin-layer chromatography.<sup>110</sup> More efficient techniques in solid-phase peptide synthesis have also allowed for reduced reaction times and reagent consumption and can potentially be applied to the synthesis of peptoids.<sup>111,112</sup> For example, in the synthesis of pentapeptides, ultrasonication during coupling decreased reaction times from 30 to 5 min, requiring only 2 equiv of reagents and reaction temperatures below 30 °C.<sup>111</sup> Many peptoid synthesis reactors use gentle bubbling or mechanical shaking for mixing and rely on the addition of excess reagents to compensate for any inefficiencies in diffusion. Hurevich and co-workers developed a peptide synthesis setup relying on high temperatures (90 °C) and overhead stirring to produce a 9-mer peptide in under 30 min.<sup>112</sup> Coupling took place in 30 s using 2 equiv of the coupling agent and desired amino acid. In most peptoid synthesis protocols, the acylation step is completed in 20 min with approximately 16 equiv of BrAc and DIC.<sup>13</sup>

Inspiration can also be drawn from the peptide field when exploring green peptoid synthesis. DMF is the most used solvent in solid-phase peptoid synthesis but its manufacture and use are being restricted by the European Commission due to the risks it poses to human health. Dichloromethane (DCM) and NMP have also been declared hazardous to human and environmental health. Greener solvents such as gamma-valerolactone, 2-methyltetrahydrofuran, ethyl acetate, and *N*-butyl pyrrolidinone have already been explored as alternatives for the Fmoc deprotection and amino acid coupling steps in solid-phase peptide synthesis.<sup>113–118</sup> We would also like to highlight several recent reviews on green peptide synthesis.<sup>119–122</sup> To the best of our knowledge, there are no reports of the investigation of green solvents in peptoid synthesis. Optimization of the solvent selection and composition of binary solvent mixtures will be required for the acylation and amination steps in submonomer peptoid synthesis as the solubility and reactivity of the reagents and

byproducts differ from those seen in peptide synthesis. Solid-phase synthesis also generates a significant amount of waste with most protocols calling for repeated washing following acylation and amination, requiring approximately 20 mL of DMF washes with each monomer addition.<sup>13</sup> Cárdenas and co-workers addressed waste generation in peptide synthesis by implementing a circular economy strategy that recycled the final two DMF washes from a coupling cycle as the first two washes in the next cycle.<sup>123</sup> This protocol allowed for a 25–30% reduction in DMF consumption, minimizing costs and waste production without compromising the purity of the synthesized peptides. While the recycling of washes can be easily adapted to manual peptoid synthesis, this concept should also be compatible with automated protocols. Programming for robotic synthesizers could be altered to allow for the collection of specific washes in secondary containers, allowing them to be reused for additional steps as opposed to being sent to waste. In solution-phase peptoid synthesis, polypeptoids are often purified by precipitation in diethyl ether, which is considered a hazardous solvent due to its low flash point and its capacity to form peroxides. Albericio and co-workers found that the less hazardous cyclopentyl methyl ether is a suitable replacement as a precipitation solvent for peptides and we predict that similar results can be obtained for peptoids.<sup>124</sup> The incorporation of sustainable and bioderived materials into peptoid synthesis is another opportunity to apply the principles of green chemistry to this field. Tao and co-workers used furfural, an aldehyde produced from renewable agricultural residues, in a Ugi polymerization of polypeptoids carried out in water.<sup>16</sup> Looking forward, incorporating biobased amines into the design of peptoids could help to increase the sustainability of their synthesis.<sup>125</sup> The thoughtful incorporation of green chemistry principles and sustainable practices into peptoid synthesis can help to reduce waste generation and reliance on restricted and hazardous chemicals.

The trade-off between sequence specificity and scalability remains a persistent challenge in the optimization of peptoid synthesis. In solid-phase techniques, absolute sequence control is possible as washing removes any excess reactants following each addition. However, its shortcomings include low resin loading capacities as well as the low level of incomplete reaction (e.g., 1% loss) with each step that accumulates to significantly reduced yields at high chain lengths (e.g., 13% yield for 100-mer). Larger scales can be obtained with solution-phase methods, but these lack the sequence control and side chain diversity accessible to solid-phase submonomer synthesis. Scalable synthetic methods will increase the industrial relevance of peptoid research while control over the peptoid sequence is highly desirable for their exploration in new applications such as the development of highly sensitive sensors, synthetic receptors, or data storage.<sup>9,10,126,127</sup> Peptoid research would benefit from techniques able to maintain the sequence control seen in solid-phase synthesis while allowing for higher loading performances. In peptide synthesis, small molecule supports with unique solubilities allow for rapid purification through precipitation and have higher loading values (3.8–4.7 mmol/g) compared to solid-phase resins (0.3–0.8 mmol/g).<sup>128</sup> For example, Qin and co-workers developed several phosphate and phosphonate derivatives as reusable small molecule supports in the resin-free synthesis of peptides up to 10 residues long without chromatographic purification.<sup>128,129</sup> This technique required 3.6 equiv of the desired amino acid and coupling reagents which Chiba and co-



workers improved upon with hydrophobic benzyl alcohol tags, using only 1.5 equiv during coupling.<sup>130</sup> The final 10-mer peptides were successfully synthesized on a 100 g scale in 65% yield and with >99% purity. These tag-assisted chemistries may be applicable to peptoid synthesis and could help to prevent compromising scalability for sequence control.

## 6. CONCLUSION

Peptoids, a class of synthetically accessible and sequence programmable materials with diverse structures, can be synthesized using both solid-phase and solution-phase approaches. However, a universal method for their optimized synthesis is impractical given their diversity of applications, each with unique requirements (e.g., chain length, sequence definition and control, and side chain selection). In this Perspective, we discuss tailoring various synthetic strategies to meet the specific needs of researchers, such as the incorporation of side chains that induce self-assembly or the use of catalysts to access higher degrees of polymerization. Looking forward, we anticipate that new peptoid architectures and enhanced synthetic efficiency will broaden the potential applications of peptoids. As new chemical regulations are implemented to address risks to human and environmental safety, we will need to find alternative solvents and more sustainable methods for peptoid synthesis. A key challenge will involve bridging the gap between sequence control and scalability to increase the industrial relevance of peptoid research. We hope that the synthetic insights offered within this Perspective will encourage scientists from a wide range of disciplines to use peptoids to address both new and long-standing research questions, allowing for new developments in the field of peptoid science.

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### Notes

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