On-Resin *N*-Terminal Peptoid Degradation: Toward Mild Sequencing Conditions

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ABSTRACT:

A novel approach to sequentially degrade peptoid N-terminal N-(substituted)glycine residues on the solid-phase using very mild conditions is reported. This method relies on the treatment of resin-bound, bromoacetylated peptoids with silver perchlorate in THF, leading to an intramolecular cyclization reaction to liberate the terminal residue as a N-substituted morpholine-2,5-dione, resulting in a truncated peptoid upon hydrolysis and a silver bromide byproduct. Side-chain functional group tolerance is explored and reaction kinetics are determined. In a series of pentapeptoids possessing variable, non-nucleophilic side-chains at the second position (R^2), we demonstrate that sequential N-terminal degradation of the first two residues proceeds in 87% and 74% conversions on average, respectively. We further demonstrate that the degradation reaction is selective for peptoids, and represents substantial progress toward a mild, iterative sequencing method for peptoid oligomers. © *2016 Wiley Periodicals, Inc. Biopolymers (Pept Sci) 106: 726–736, 2016.*

Additional Supporting Information may be found in the online version of this article.
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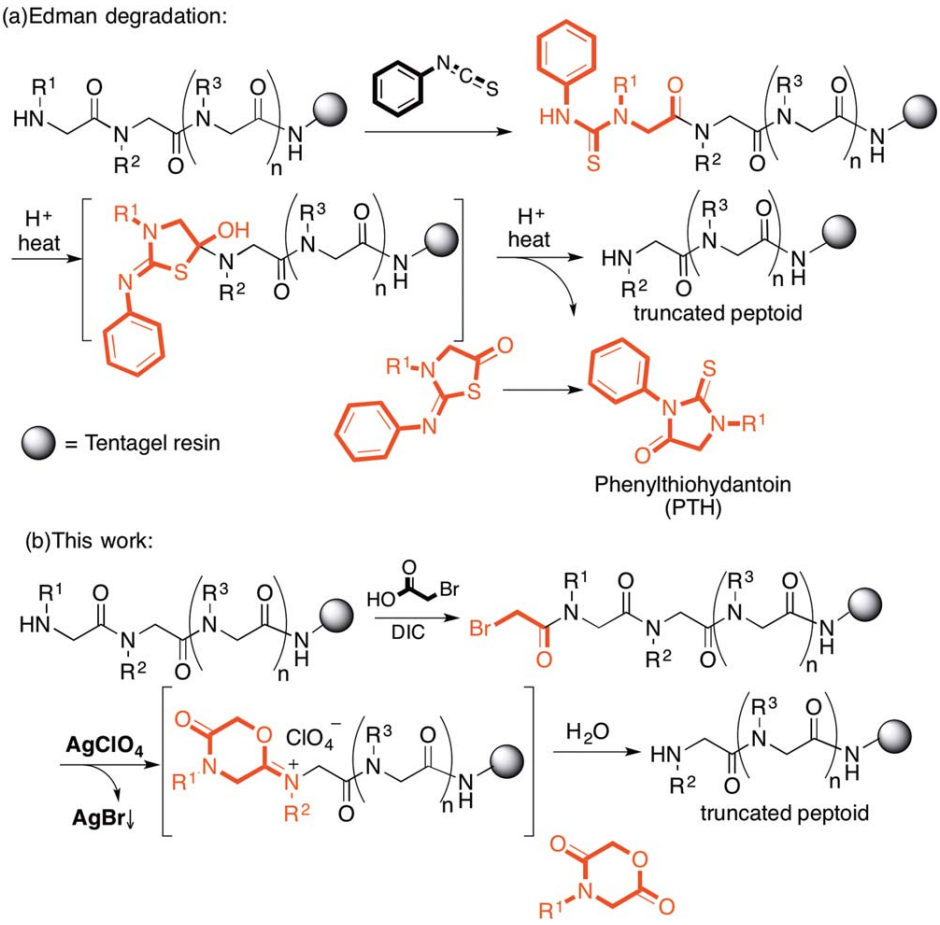
INTRODUCTION

Sequential degradation of polypeptides is an essential analytical tool to determine the linear sequence of proteins of unknown structure. This sequencing process is typically achieved using the iterative Edman degradation cycle, which consists of two steps: 1) reaction of the *N*-terminal amino group with phenylisothiocyanate (PITC), followed by 2) treatment with a strong acid (trifluoroacetic acid, TFA), to liberate the *N*-terminal amino acid fragment as a phenylthiohydantoin (PTH) (Scheme 1a).[1] Partial Edman degradation has also been used to generate molecular weight ladders that can be analyzed by mass spectrometry for sequence determination.[2] Although the Edman degradation was developed for the sequencing of polypeptides, it has been applied with good success to the sequencing of *N*-(substituted)glycine peptoid oligomers as well.[3,4] The method has found great utility in the identification of hits from combinatorial peptoid libraries.[5,6] However, the Edman degradation conditions utilize relatively harsh, acidic conditions, as well as being limited to peptides $\leq 50-60$ residues in length and requiring extended reaction times. As we seek to develop sequencing methods for peptidomimetic polymer systems, we aim to develop efficient, rapid, and mild reaction conditions that can be applied generally and with a large diversity of sidechain functionalities. Here, we report alternate, mild conditions to degrade



SCHEME 1 (a) Edman degradation conditions[1] vs. (b) a mild approach for *N*-terminal degradation using silver salts.

peptoid polymers, one monomer at a time starting from the *N*-terminus (Scheme 1b).

N-terminal peptoid degradation reactions have in fact been previously reported, although they use harsher conditions and have not been applied iteratively.[7] For example, *N*-acylated peptoids have been shown to cleave off the *N*-terminal residue under acidic cleavage conditions (TFA), leading to mixtures of the desired *N*-acylated peptoid and truncated products.[7] This reaction proceeds *via* an intramolecular cyclization reaction, liberating an oxazolinium ion and the deletion sequence, which can be suppressed by using electron-poor acyl groups at the *N*-terminus (*e.g.* 4-nitrobenzoyl or trifluoroacetyl groups). In analogy to the degradation of *N*-acylated peptoids, *N*-terminal Ac-*N*-methyl amino acid residues and *N*-benzoyl amino acid residues have also been shown to undergo acid-promoted degradation reactions.[8,9] However, in all the above-mentioned cases, the cyclization reactions used harsh conditions, similar to the Edman degradation conditions (TFA), and were dependent on both the side-chain chemistry of the *N*-terminal residue and the nature of the acyl group. In addition, none of these approaches have been explored iteratively for sequencing purposes, with efforts focusing mainly on suppression of this truncation reaction using electron poor acyl groups to create libraries with diversity at the *N*-acylated terminal residue and isolate the desired *N*-acylated peptide or peptoid.

We recently reported a way to accelerate displacement reactions in submonomer peptoid synthesis using aniline weak nucleophiles, where halophilic silver salts were employed to promote halide abstraction and formation of an AgBr precipitate.[10] In the course of that work, we found that the treatment of resin-bound, bromoacetylated peptoids with $AgClO_4$ solutions in the absence of an amine submonomer leads to on-resin truncation of the *N*-terminal residue via an intramolecular cyclization reaction, generating an *N*-substituted morpholine-2,5-dione byproduct (Scheme 1b). These mild reaction conditions allow the *N*-terminal degradation of peptoid

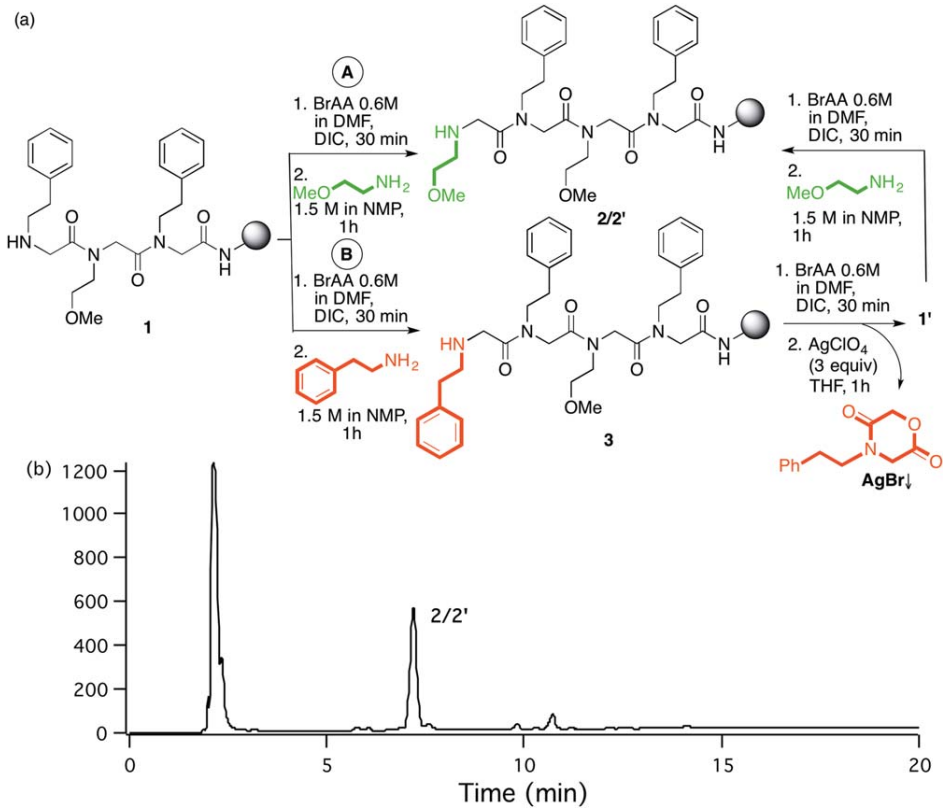


FIGURE 1 (a) $AgClO_4$-promoted *N*-terminal deletion of a *N*-(2-phenylethyl)glycine residue followed by peptoid elongation. (b) HPLC trace of tetrapeptoid (**2**) and corrected tetrapeptoid (**2'**) co-injected.

residues to be performed on acid-labile Rink amide polystyrene resin, leaving the truncated peptoid bound to solid-phase. To evaluate the potential of this method for sequencing of one-bead-one-compound (OBOC)[11] combinatorial libraries, we further performed this degradation reaction on Tentagel resin, and report here on the side-chain tolerance using a variety of *N*-substituted glycine monomers in a series of chemically diverse pentapeptoids.

RESULTS AND DISCUSSION

To establish that deletion of an *N*-terminal residue was occuring, we first demonstrated that we could remove one *N*-terminal residue and replace it with another. Thus, tetrapeptoids **2** and **3** were synthesized in parallel, where the *N*-terminal residue was either a *N*-(2-methoxyethyl)glycine (*N*me) or a *N*-(2-phenylethyl)glycine (*N*pe) residue, respectively (Figure 1a). *N*-terminal truncation of the *N*pe residue (**3**) was accomplished by sequentially treating the resin with bromoacetic acid and *N*,*N*'-diisopropylcarbodiimide (DIC), followed by $AgClO_4$ in tetrahydrofuran (THF) for 1 h at room temperature. The resulting truncated peptoid (**1'**) was bromoacetylated and treated with a solution of 2-methoxyethylamine in *N*-methylpyrrolidinone (NMP), and the HPLC retention times of the obtained (corrected) tetrapeptoid (**2'**) was compared with that of synthesized tetrapeptoid **2** (Figure 1b). The molar mass (as determined by electrospray mass spectrometry) and HPLC retention times of both tetramers proved to be identical, confirming the occurence of a mild and efficient silver-mediated, on-resin *N*-terminal truncation reaction. Interestingly, the resin does not have to be treated with any additional reagents to hydrolyze the cyclic iminium intermediate (Scheme 1b), which occurs readily during the washing step with (wet) *N*,*N*-dimethylformamide (DMF). Monitoring of the reaction kinetics by HPLC revealed that complete disappearance of the bromoacetylated peptoid occured after 1 h, with subsequent DMF wash affording the truncated tripeptoid (**1'**) (Figure 2). Of note, the aforementioned reaction was performed on Rink amide polystyrene resin without any cleavage of the peptoid from the resin. Because the peptoid

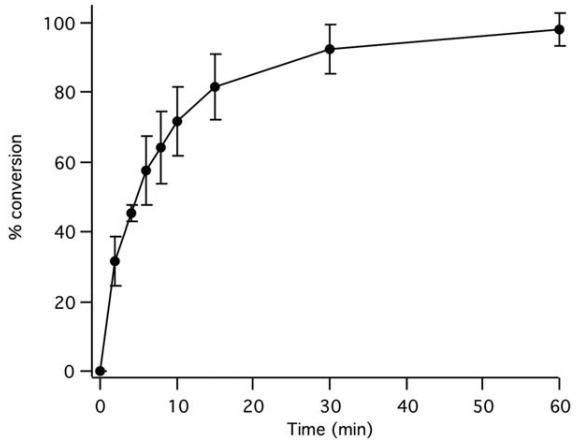


FIGURE 2 Progress of the *N*-terminal degradation reaction (% conversion) vs time of peptoid **3** to yield **1'**.

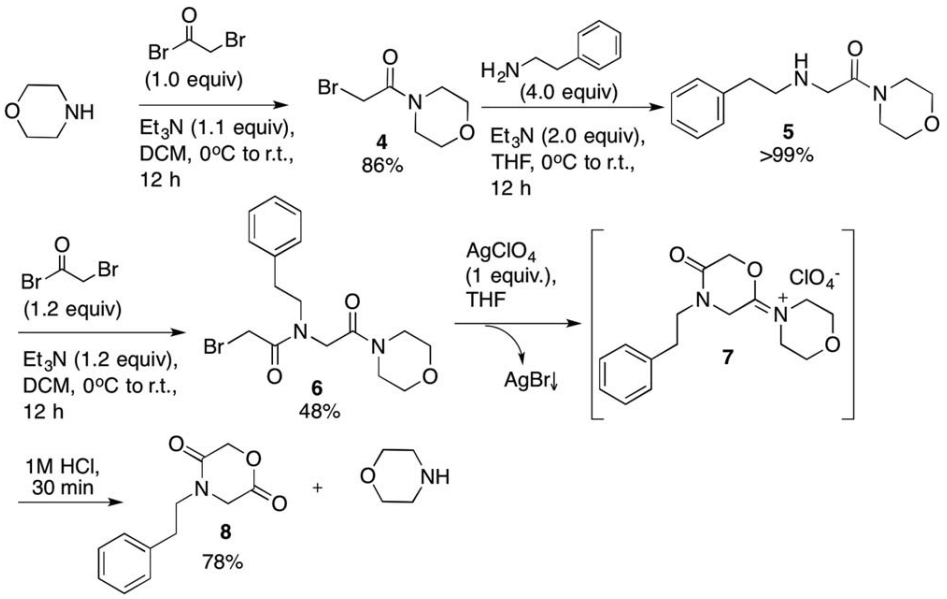
degradation conditions do not involve treatment with trifluoroacetic acid (TFA), premature cleavage of the peptoid from solid support was not observed.

The mechanism of the degradation reaction was confirmed by synthesizing model peptoid monomer **5**. This compound was bromoacetylated to give **6** and treated with silver perchlorate to afford cyclic product **8** in 78% yield, following the acid-catalyzed hydrolysis of intermediate **7** (Scheme 2). Compound **8** was verified to be the *N*-substituted morpholine 2,5-dione byproduct by ^{1}H and ^{13}C NMR. Moreover, collection of the filtrate following the on-resin truncation of tetrapeptoid **3** to give tripeptoid **1'** revealed *N*-substituted morpholine 2,5-dione **8** as the major product by NMR, following THF evaporation and extraction with 1M HCl/EtOAc (see supporting information).

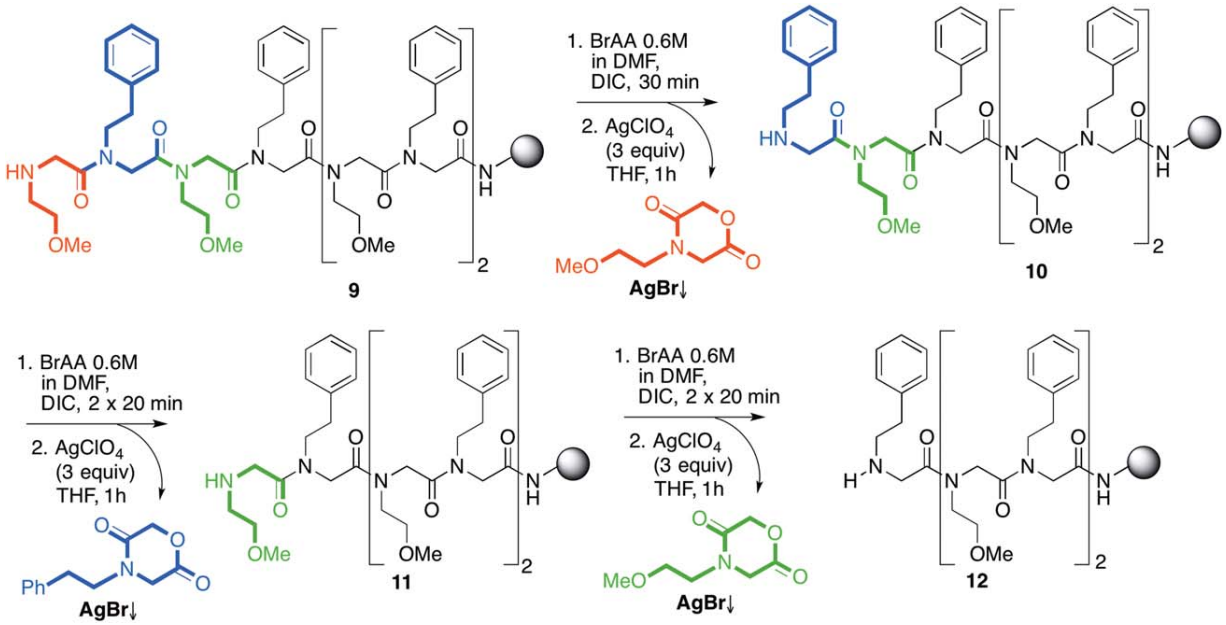
Using $(NmeNpe)_4$ octapeptoid **9** as starting material, we further demonstrated that the degradation reaction could be performed iteratively three cycles in a row, affording the desired truncated peptoids **10-12** as the major products (Scheme 3 and Figure 3). The reaction conversions were estimated by measuring the ratio between the crude purities of the truncated product and starting peptoid, monitored by HPLC at 214 nm, yielding conversions of 86%, 69%, and 76% for cycles 1, 2, and 3, respectively.

It is noteworthy that in contrast to the Edman degradation procedure, which liberates a phenylthiohydantoin byproduct that can be easily detected by HPLC, the lack of a persistent chromophore on the *N*-substituted morpholine-2,5-dione product released prevents its use in HPLC analysis for sequencing purposes. However, partial degradations to generate molecular weight ladders analyzed by mass spectrometry would likely be achievable, similarly to known partial Edman degradation procedures that have demonstrated utility in sequencing.[2]

With the desire to employ these newfound mild conditions as an alternative to Edman degradation conditions for peptoid and peptide sequencing, we synthesized a series of hexamers with side-chain diversity on Tentagel M-NH_2 resin. We designed our test peptoids **13a-m** to contain diverse side-chain chemistry at the first two positions (R^1 and R^2), followed by



SCHEME 2 Synthesis of model bromoacetylated peptoid **6** and silver-promoted formation of *N*-substituted morpholine 2,5-dione product **8**.



SCHEME 3 Iterative silver-promoted *N*-terminal truncations of octapeptoid **9** to afford pentapeptoid **12** after three cycles.

two *N*pe monomers and one *N*-(2-aminoethyl)glycine (*N*ae) residue, to facilitate HPLC detection and mass spectrometry analyses, respectively (Scheme 4 and Table I). A *C*-terminal methionine residue was incorporated to enable CNBr-mediated cleavage from solid-support for analysis. The *N*-terminal degradation reactions were monitored by HPLC, where the purity of the obtained truncated products were compared to the purities of the starting peptoids (Table I). To enable accurate reaction monitoring by HPLC and mass spectrometry, quenching of unreacted bromoacetylated peptoid was achieved by treating the resin with a solution of 1.5 M 2-phenylethylamine in DMF prior to cleavage from the solid support. The ratios between the crude purities of the desired truncated product and starting peptoids are reported in Table II (conversions).

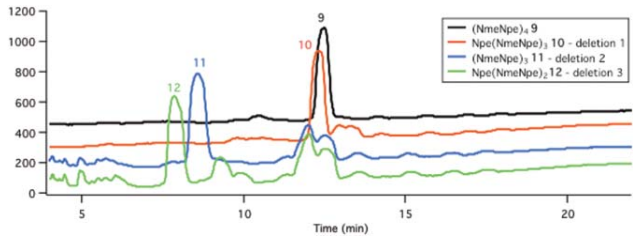
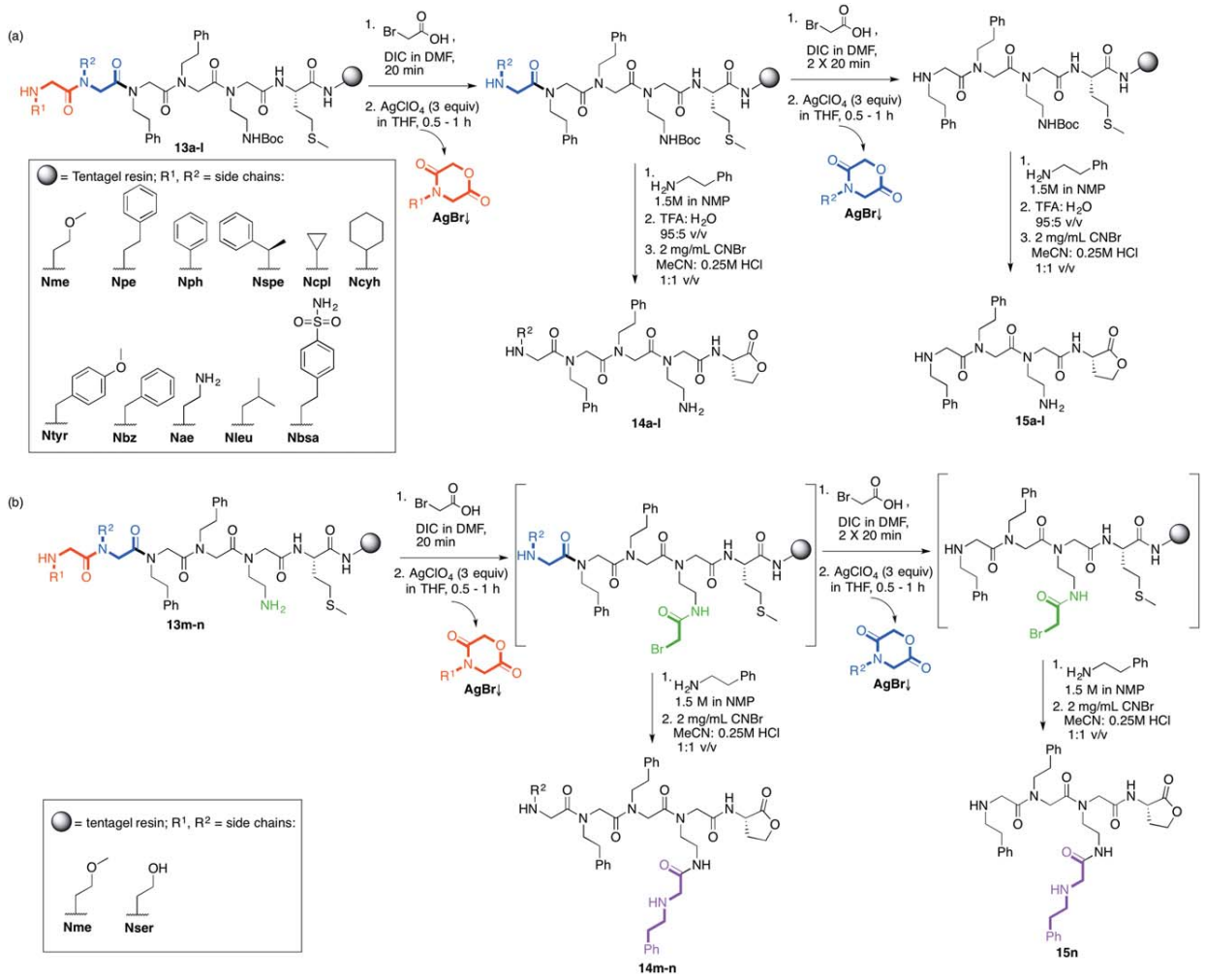


FIGURE 3 HPLC chromatograms of starting peptoid **9** and truncated peptoids **10-12** following one, two, and three silver-promoted truncation reactions, respectively.

We first attempted the *N*-terminal deletion of *N*pe and *N*me residues (**13a-b**), in order to determine the efficiency of the reaction using simple, non-nucleophilic side-chains. In these cases, completion of the degradation reaction was observed after treatment of bromoacetylated peptoid with $AgClO_4$ for 30 minutes, with the crude purities dropping by only 1-2% (Table I, 13a-b), indicating a near quantitative *N*-terminal degradation reaction (Table II, 13a-b). We next examined the effect of conformationally restricted monomers at both the *N*-terminal and second position (R^2), such as *trans*-inducing *N*-phenyl glycine (*N*ph)[12] and *cis*-inducing (*S*)-*N*-(1-phenylethyl)glycine (*N*spe)[13] residues (**13c-f**). The reaction was inhibited (65% conversion) when the *N*ph monomer was located at the second position (R^2) (Tables I and II, **13c**), likely due to the reduced nucleophilicity of the electron-deficient *N*-phenyl glycine residue. Of note, analysis of *N*ph-terminated peptoid was complicated by air oxidation and degradation of the peptoid over time.[10] As expected, deletion of the *trans*-inducing *N*ph residue was sluggish, providing the desired truncated tetrapeptoid in only 17% conversion (Tables I and II, **13d**). A 24% drop in crude purity was observed for the peptoid containing a *N*spe residue at the second position (R^2) (Tables I and II, **13e**), where the sterically encumbered *cis*-inducing *N*spe residue might be preventing efficient formation of the cyclic intermediate. In contrast, deletion of an *N*spe monomer at the *N*-terminal position (R^1) proceeded smoothly, affording the desired tetramer in 95% conversion (Table II, **13f**).



SCHEME 4 Sequential $AgClO_4$-promoted degradation reactions conducted with peptoids **13a-n**, where (a) the amino nucleophilic side-chain of the *N*ae residue is Boc-protected, and (b) the nucleophilic side-chains (*N*ae, *N*ser) are deprotected prior to degradation cycle.

In order to study the side-chain functional group tolerance of the silver-promoted deletion reaction more in depth, peptoids **13g-l** were prepared (Scheme 4a and Table I), where the side-chain chemistry at the second position (R^2) was varied, and an *N*-terminal *N*me residue was incorporated (R^1). Performing two consecutive deletion reactions provided insight on the ability of each *N*-substituted glycine to be deleted, as well as their effect on the degradation of the preceeding *N*me residue. On average, the *N*-terminal *N*me residue of peptoids **13g-l** was degraded with only 12% loss in crude purity (Table I) to give tetrapeptoids **14g-l** in 87% conversion (Table II). This indicates that the nature of the preceeding residue (R^2), unless it is a electron-poor residue (e.g., *N*ph) or sterically encumbered monomer (e.g., *N*spe), has little effect on the efficiency of the on-resin truncation. In most cases, the drop in purity was due to the appearance of a combination of many minor side products, whose structures could not be easily inferred from mass spectrometry analysis (see supporting information for representative HPLC traces).

We next tried to perform a second consecutive *N*-terminal degradation reaction cycle. In this case, the bromoacetylation step was performed 2 x 20 min in order to ensure that the AgBr precipitate from the first degradation cycle was fully dissolved and washed away.[10] Following treatment of the resin-bound bromoacetylated tetrapeptoids with silver perchlorate to give tripeptoid **15**, a drop of ~23% in purity was observed on average (Table I). Nonetheless, in most cases, the desired truncated tripeptoid **15** was obtained as the major product in >50% crude purity, with an average conversion of 74% to the desired truncated peptoid (Table II). As a notable exception, degradation of *N*cpl residue in tetrapeptoid **14g** only resulted in 27% conversion to **15** after treatment of bromoacetylated

Table I Crude Purity, Mass, and Retention Time of Pentamers, Tetramers, and Trimers Following 1 or 2 Subsequent *N*-Terminal Silver-Promoted Degradation Reactions of the Pentamers

Peptoid	Crude purity of 5mers (%)	RT of 5mers (min)[a]	*m/z* obsd (calcd) of 5mers $[M+1]^+$	Crude purity of 4mers **14a-n** (%)	RT of 4mers **14a-n** (min)[a]	*m/z* obsd (calcd) of 4mers 11a-n $[M+1]^+$	Crude purity of 3mer **15** (%)
13a: R^1 = *N*me, R^2 = *N*me	92	7.71	755.0 (753.4)	92	6.60	639.9 (639.3)	n/a
13b: R^1 = *N*pe, R^2 = *N*me	88	10.55	800.8 (799.4)	86	6.63	640.3 (639.3)	n/a
13c: R^1 = *N*me, R^2 = *N*ph	95	24.88 [b]	773.1 (772.4)	62	28.07 [b]	-[c](657.3)	n/a
13d: R^1 = *N*ph, R^2 = *N*me	71	27.64[b]	774.2 (772.4)	12	19.84 [b]	663.0[d] (639.3)	n/a
13e: R^1 = *N*me, R^2 = *N*spe	97	11.03	801.2 (800.4)	73	9.79	686.1 (685.4)	n/a
13f: R^1 = *N*spe, R^2 = *N*me	96	10.05	801.2 (800.4)	91	6.67	640.1 (639.3)	n/a
13g: R^1 = *N*me, R^2 = *N*cpl	97	7.79	737.4 (736.4)	88	6.44	622.1 (621.3)	24
13h: R^1 = *N*me, R^2 = *N*cyh	96	9.93	779.5 (778.4)	84	9.07	664.6 (663.4)	77
13i: R^1 = *N*me, R^2 = *N*tyr	92	10.28	818.0 (816.4)	81	9.19	702.6 (701.4)	63
13j: R^1 = *N*me, R^2 = *N*bz	96	10.33	787.3 (786.4)	72	8.88	674.6 (671.4)	56
13k: R^1 = *N*me, R^2 = *N*leu	90	9.28	753.4 (752.4)	82	8.00	638.2 (637.4)	64
13l: R^1 = *N*me, R^2 = *N*bsa	81	7.55	880.7 (879.4)	74	6.75	784.0 (764.3)	66
13m[e]: R^1 = *N*me, R^2 = *N*me	92	7.71	754.7 (754.4)	60	9.77	802.1 (800.4)	n/a
13n[e]: R^1 = *N*me, R^2 = *N*ser	86	5.65	741.4(740.4)	<67	7.39	788.3(786.4)	<38

[a] Analytical HPLC analyses were performed on a Vydac column (4.6 mm x 150 mm, 5 μm, C18) at 60°C with a flow rate of 1.0 mL/min using a 20−80% gradient of CH_3CN (0.1% TFA) in water (0.1% TFA) over 20 min. HPLC traces were monitored at 214 nm.

[b] Analytical HPLC analyses were performed as above, using a 5−40% gradient of CH_3CN (0.1% TFA) in water (0.1% TFA) over 20 min.

[c] Mass was not observed due to degradation of Npe-terminated peptoids.

[d] Observed mass corresponds to sodium adduct, $[M+Na]^+$.

[e] Resin was treated with TFA for side-chain protecting group removal, followed by bromoacetylation and Ag-mediated degradation reaction.

peptoid with $AgClO_4$ for 1 h, indicative of slower reaction kinetics with an *N*-cyclopropyl side-chain.

We next attempted to perform *N*-terminal degradation reactions on more challenging substrates, containing a nucleophilic heteroatom in the side-chain (Scheme 4b, **13m-n**). In these cases, the resin-bound peptoids were first treated with TFA for 10 minutes to remove of the *tert*-butyloxycarbonyl (Boc) and tetrahydropyranyl acetal (THP) protecting groups, respectively, followed by exposure to the degradation cycle conditions as above. Peptoid **13m** is structurally similar to peptoid **13a**, where the Boc protecting group on the *N*ae residue has been removed. The first step presumably leads to bromoacetylation at both the *N*-terminal *N*-substituted glycine and the amino side-chain functional group, which raises the question of how a bromoacetylated side-chain might react upon treatment with the halophilic silver salt. In attempting to degrade the *N*-terminal *N*me residue in peptoid **13m**, tetrapeptoid **14m** was obtained as the major product in 60% purity after quenching with 2-phenylethylamine (Scheme 4b), corresponding to a 32% drop in purity compared to the starting peptoid (Table I). Despite obtaining the expected truncated peptoid as the major product, a significant increase in the number of side products formed was observed compared to peptoid analog **13a**, where less than 1% drop in purity was observed when the *N*ae side-chain was Boc-protected. The

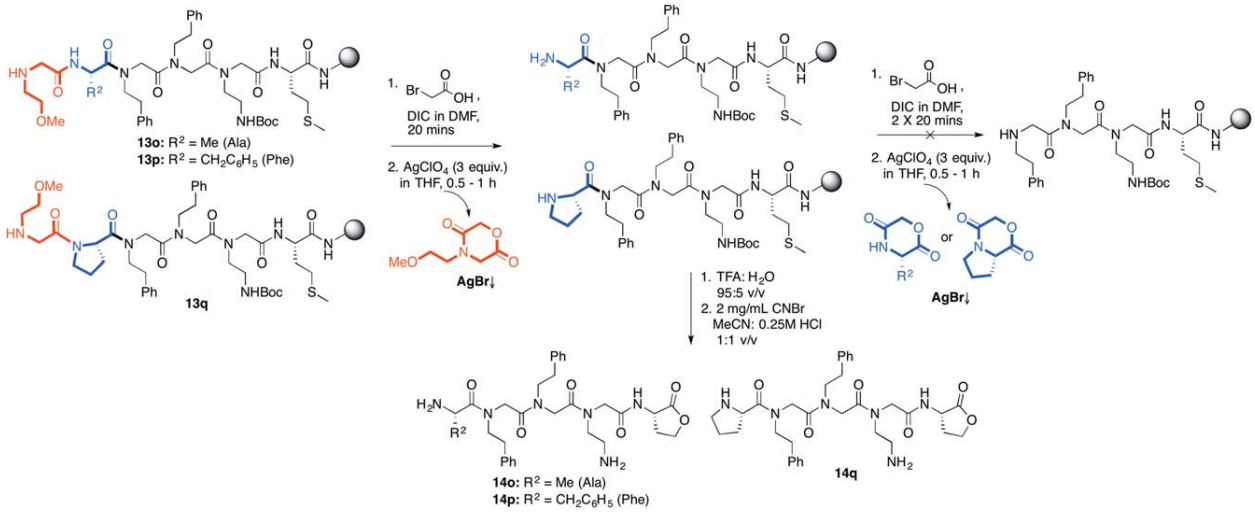
Table II % Conversions for the First and Second Degradation Reactions

Peptoid	% Conversion[a] for degradation 1	% Conversion[a] for degradation 2
13a: R^1 = *N*me, R^2 = *N*me	>99	n/a
13b: R^1 = *N*pe, R^2 = *N*me	98	n/a
13c: R^1 = *N*me, R^2 = *N*ph	65	n/a
13d: R^1 = *N*ph, R^2 = *N*me	17	n/a
13e: R^1 = *N*me, R^2 = *N*spe	75	n/a
13f: R^1 = *N*spe, R^2 = *N*me	95	n/a
13g: R^1 = *N*me, R^2 = *N*cpl	91	27
13h: R^1 = *N*me, R^2 = *N*cyh	88	92
13i: R^1 = *N*me, R^2 = *N*tyr	88	78
13j: R^1 = *N*me, R^2 = *N*bz	75	78
13k: R^1 = *N*me, R^2 = *N*leu	91	78
13l: R^1 = *N*me, R^2 = *N*bsa	91	89
13m[b]: R^1 = *N*me, R^2 = *N*me	65	n/a
13n[b]: R^1 = *N*me, R^2 = *N*ser	<78[c]	<57[c]

[a] % conversion = [(% purity of degraded peptoid)/(% purity of starting peptoid)] x 100.

[b] Resin was treated with TFA for side-chain protecting group removal, followed by bromoacetylation and Ag-mediated degradation reaction.

[c] HPLC analysis was hindered by the presence of multiple peaks near the injection peak.



SCHEME 5 Sequential $AgClO_4$-promoted degradation reactions conducted with peptoid-peptide hybrids **13o-q**.

structure of the multiple minor side products could not be easily determined by mass spectrometry analysis. Similarly, performing two sequential degradation reactions on peptoid **13n** possessing both an unprotected *N*ser residue at the second position (R^2) and unprotected *N*ae residue yielded multiple side products and afforded the desired truncated trimer in less than 40% purity (Table I).

In addition to the aforementioned challenging substrates, we attempted to perform the degradation reaction in peptoid-peptide hybrids **13o-q**, where the second residue was either an alanine, phenylalanine, or proline amino acid (Scheme 5, Table III). Degradation of the *N*-terminal *N*me residue proceeded in 76 − 84% conversions (Table IV, **13o-q**) to give peptide-peptoid hybrids **14o-q**, revealing that amino acid residues do not inhibit formation of the six-membered cyclic intermediate when located at the second position (R^2). However, degradation of the amino acids proved to be unsuccessful in all three cases, giving unreacted bromoacetylated *N*-terminal amino acids exclusively, as detected by mass spectrometry following quenching with an amine submonomer. In order to rule out the possibility that steric interactions prevented amino acids residues to be deleted in peptide-peptoid hybrids, a test peptide sequence was synthesized to evaluate its ability to be degraded using our conditions. Specifically, tetrapeptide Phe-Phe-Lys-Met was subjected to our degradation conditions following Fmoc removal. Once again, deletion of the *N*-terminal amino acid (Phe) proved to be unsuccessful, reiterating that amino acid residues cannot be degraded by this mechanism, and that the degradation reaction is selective for peptoids.

CONCLUSION

In summary, we demonstrated that *N*-substituted glycine peptoid monomers can be degraded sequentially from the *N*-

Table III Crude Purity, Mass, and Retention Time of Peptoid-Peptide Hybrid Pentamers and Tetramers Following *N*-Terminal Silver-Promoted Degradation Reactions of *N*me Residue

Peptoid	Crude purity of 5mers (%)	RT of 5mers (min)[a]	*m/z* obsd (calcd) of 5mers $[M + 1]^+$	Crude purity of 4mers **14n-p** (%)	RT of 4mers **14n-p** (min)[a]	*m/z* obsd (calcd) of 4mers 14a-n $[M + 1]^+$
13o: R^1 = *N*me, R^2 = Me (Ala)	90	6.12	711.1 (710.9)	71	5.60	596.6 (595.7)
13p: R^1 = *N*me, R^2 = $CH_2C_6H_5$ (Phe)	87	8.77	787.2 (786.9)	67	8.40	672.0 (671.8)
13q: R^1 = *N*me, R^2 = Pro	81	6.39	737.1 (736.9)	68	5.89	622.0(621.8)

[a] Analytical HPLC analyses were performed on a Vydac column (4.6 mm x 150 mm, 5 μm, C18) at 60°C with a flow rate of 1.0 mL/min using a 20−80% gradient of CH_3CN (0.1% TFA) in water (0.1% TFA) over 20 min. HPLC traces were monitored at 214 nm.

Table IV % Conversions for the First Degradation Reactions of Amino Acid-Containing Peptide-Peptoid Hybrid Pentamers

Peptoid	% Conversion[a] for degradation 1
13o: R^1 = *N*me, R^2 = Me (Ala)	79
13p: R^1 = *N*me, R^2 = Ph (Phe)	76
13q: R^1 = *N*me, R^2 = Pro	84

[a] % conversion = [(% purity of degraded peptoid)/(% purity of starting peptoid)] x 100.

terminus using novel, mild conditions that avoid treatment with strong acids. Specifically, resin-bound peptoids are (1) bromoacetylated and (2) treated with a silver salt, thereby promoting an intramolecular cyclization reaction that leads to removal of the *N*-terminal residue as an *N*-substituted morpholine-2,5-dione and a free *N*-terminus. The identity of the morpholine-2,5-dione byproduct was confirmed via solution-phase synthesis, isolation and characterization of a model 4-(2-phenylethyl)morpholine-2,5-dione compound (**8**). Using a series of pentapeptoids with variable, non-nucleophilic side-chains at the second position (R^2), we demonstrated that two sequential degradation reactions could be performed in 87% and 74% conversions on average, respectively. Amino acids, however, could not be truncated using these conditions, indicating that this reaction is selective for peptoids. For resin-bound peptoids with protected side-chains, the mild conditions for *N*-terminal degradation described here may find utility in the activity-based editing of peptoids, where inactive compounds identified from OBOC library screening may be subjected to degradation of the *N*-terminal residue, followed by addition of a new monomer. Further developments will aim to improve the yields of each degradation cycle to allow sequencing of longer peptoid oligomers.

EXPERIMENTAL SECTION

General

Solid-phase chemistry was performed using polystyrene Rink Amide resin (0.57 mmol/g) or Tentagel M-NH_2 resin (0.3 mmol/g) in filtration tubes equipped with caps and stopcocks. Analytical HPLC analyses were performed on a 5 μm, 150 mm x 4.6 mm C18 Vydac column™ at 60°C with a flow rate of 1.0 mL/min using a 20 − 80% gradient, where solvent A = water [0.1% trifluoroacetic acid (TFA)] and solvent B = MeCN (0.1% TFA). LCMS analyses were performed on a 5 μm, 50 mm x 2.1 mm C18 Vydac column with a flow rate of 0.35 mL/min using 3-100% gradient. HPLC traces were monitored at 214 nm. Analytical thin-layer chromatography (TLC) was performed on glass-backed silica gel plates. Visualization of the developed chromatogram was performed by UV absorbance. Silica gel chromatography was performed using an SP1 Flash Chromatography instrument from Biotage. Nuclear magnetic resonance spectra (^{1}H, ^{13}C) were recorded on a Bruker AV300.

Reagents

Bromoacetic acid, *N,N*'-diisopropylcarbodiimide (DIC), trifluoroacetic acid (TFA), triisopropylsilane (TIPS), O-(Benzotriazol-1-yl)-*N,N,N'N'*-tetramethyluronium hexafluorophosphate (HBTU), *N,N*-diisopropylethylamine (DIEA), cyanogen bromide, all amine and amino acid reagents were purchased from commercial sources and used without further purification. Silver perchlorate was dried by azeotrope evaporation with benzene and placed in a desiccator under vacuum with P_2O_5 to give a white solid that was stored in a desiccator wrapped with foil. Solvents were purchased from commercial sources.

Synthetic Experimental Procedures

Peptoids and peptoid-peptide hybrids **13a-q** were synthesized according to standard solid-phase procedures.[14]

2-Bromo-1-morpholinoethan-1-One (4)

Morpholine (0.43 mL, 4.95 mmol) was dissolved in 25 mL of dry DCM in a dry 50 mL flask, treated with triethylamine (0.76 mL, 5.45 mmol), and cooled to 0°C. Bromoacetyl bromide was added drop-wise at 0°C, and the reaction mixture was allowed to warm to room temperature overnight. The solution was placed in a separatory funnel and the organic phase was washed sequentially with H_2O (25 mL), 5% citric acid (25 mL), followed by saturated sodium bicarbonate (25 mL). The organic phase was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude oil was purified by silica gel chromatography using an SP1 Flash Chromatography instrument from Biotage with a gradient from 60 to 70% ethyl acetate in hexanes. Evaporation of the collected fractions afforded 2-bromo-1-morpholinoethan-1-one **4** as a yellow oil (886 mg, 86% yield): R_f 0.34 (1:1 EtOAc: hexanes). ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.40 (2H, t, J = 5.0 Hz), 3.47 (2H, t, J = 5.0 Hz), 3.55 (2H, t, J = 4.5 Hz), 3.60 (2H, t, J = 5.0 Hz), 3.76 (2H, s). ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.3, 66.4, 66.3, 47.0, 42.3, 25.7. MS *m/z* 208, $(M + H)^+$ calcd for $[C_6H_{11}BrNO_2]^+$: 208.0.

1-Morpholino-2-(Phenethylamino)ethan-1-One (5)

2-Bromo-1-morpholinoethan-1-one **4** (1 g, 4.83 mmol) was dissolved in 25 mL of dry THF in a dry 50 mL flask, cooled to

0°C, and treated sequentially with triethylamine (1.35 mL, 9.66 mmol) and phenylethylamine (2.43 mL, 19.32 mmol) at 0°C. The solution was allowed to warm to room temperature and stirred overnight. Reaction mixture was filtered, THF was removed *in vacuo*, and the crude oil was purified using an SP1 Flash Chromatography instrument from Biotage with a gradient from 1 to 6% methanol in dichloromethane. Evaporation of the collected fractions afforded 1-morpholino-2-(phenethylamino)ethan-1-one **5** as a dark yellow oil (1.2 g, >99% yield): R_f 0.22 (10% MeOH in DCM). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.83-2.86 (2H, m), 2.89-2.93 (2H, m), 3.40 (2H, t, $J = 5.0$ Hz), 3.44 (2H, s), 3.62-3.70 (6H, m), 7.21-7.33 (5H, m). ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.7, 139.9, 128.7, 128.5, 126.2, 66.9, 66.5, 51.4, 50.5, 45.0, 42.1, 36.7. MS *m/z* 249, $(M + H)^+$ calcd for $[C_{14}H_{21}N_2O_2]^+$: 249.2.

2-Bromo-*N*-(2-morpholino-2-Oxoethyl)-*N*-Phenethylacetamide (6)

1-Morpholino-2-(phenethylamino)ethan-1-one **5** (1.2 g, 4.83 mmol) was dissolved in 25 mL of dry THF in a dry 50 mL flask, cooled to 0°C, and treated sequentially with triethylamine (808 μL, 5.80 mmol) and bromoacetyl bromide (505 μL, 5.80 mmol), and stirred at 0°C for 1 h. The volatiles were removed on a rotary evaporator under reduced pressure to give a residue, which was purified using an SP1 Flash Chromatography instrument from Biotage with a gradient from 60 to 100% ethylacetate in hexanes. Evaporation of the collected fractions afforded 2-bromo-*N*-(2-morpholino-2-oxoethyl)-*N*-phenethylacetamide **6** as an orange solid (840 mg, 48%): R_f 0.16 (8:2 EtOAc: hexanes). ^{1}H NMR (500 MHz, $CDCl_3$) Major rotamer: δ 2.99 (2H, t, $J = 7.0$ Hz), 3.46 (2H, t, $J = 4.8$ Hz), 3.59-3.64 (2H, m), 3.66 (2H, s), 3.70-3.75 (6H, m), 4.15 (2H, s), 7.22-7.36 (5H, m). Minor rotamer: 2.91 (2H, t, $J = 7.0$ Hz), 3.22 (2H, t, $J = 4.9$ Hz), 3.62 (2H, 2), 3.59-3.64 (2H, m), 3.70-3.75 (6H, m), 3.87 (2H, s), 7.22-7.36 (5H, m). ^{13}C NMR (125 MHz, $CDCl_3$) (mixture of rotamers) δ 167.6, 167.3, 166.1, 165.9, 139.2, 137.9, 129.0, 128.8, 128.7, 128.6, 127.0, 126.5, 66.8, 66.5,66.2, 51.2, 50.5, 50.4, 46.9, 45.4, 45.0, 42.5, 42.3, 34.7, 33.9, 26.7, 25.6. MS *m/z* 369, $(M + H)^+$ calcd for $[C_{16}H_{22}BrN_2O_3]^+$: 369.1.

4-(2-Phenylethyl)morpholine-2,5-Dione (8)

2-Bromo-*N*-(2-morpholino-2-oxoethyl)-*N*-phenethylacetamide **6** (54 mg, 0.145 mmol) was dissolved in 2 mL of dry THF in a dry 5 mL flask, treated with $AgClO_4$ (38 mg, 0.183 mmol) and stirred for 1 h. 1 mL of a 1M HCl solution was added to the flask, and the reaction mixture was left stirring for 30 minutes. The solution was filtered and the volatiles were removed under reduced pressure. EtOAc was added and the organic phase was washed with brine (3 x 5 mL), dried over Na_2SO_4, filtered, and evaporated under reduced pressure to give 4-phenethylmorpholine-2,5-dione **8** as a white solid (23 mg, 78%): R_f 0.36 (8:2 EtOAc: hexanes). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.94 (2H, t, $J = 7.0$ Hz), 3.68 (2H, t, $J = 7.0$ Hz), 3.89 (2H, s), 4.74 (2H, s), 7.22-7.37 (5H, m). ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.7, 163.3, 137.7, 129.0, 128.7, 127.1, 67.7, 48.6, 48.2, 33.4. MS *m/z* 220, $(M + H)^+$ calcd for $[C_{12}H_{14}NO_3]^+$: 220.1.

Representative Procedure for Silver perchlorate-Mediated Degradation Reaction of Peptoid Monomers

To 20 mg of resin-bound peptoid **13a-q** in a 1 mL fritted cartridge was added 500 μL of DMF. Resin was allowed to swell for 20 minutes before draining. 500 μL of a 0.6 M solution of bromoacetic acid and 43 μL (0.92 equiv, 0.28 mmol) of DIC was added and allowed to react for 20 minutes. The resin was washed with DMF (3 x 1 mL) and THF (2 x 1 mL) before adding 500 μL of a 36 m*M* stock solution (3 equivalents) of $AgClO_4$ in THF. The reaction was allowed to proceed for 0.5 to 1 h, after which the reagents were drained and the resin was washed with DMF (3 x 1 mL) and DCM (1 x 1 mL). For reaction monitoring, a small amount of resin was treated with 500 μL 1.5 M solution of 2-phenylethylamine in DMF for 10 minutes to quench any unreacted bromoacetylated peptoids. The resin was drained and washed with DMF (3 x 1 mL) and DCM (1 x 1 mL), followed by treatment with a 95:5 TFA:H_2O v/v mixture for 10 minutes to ensure complete deprotection of Boc-protected side-chains. The resin was washed with DCM (3 x 1 mL), treated with 100 μL of 2 mg/mL of CNBr in MeCN:0.25 N HCl 1:1, covered with aluminum foil, and allowed to react for 12-16 h. The cleavage solution was collected by filtering the resin through a disposable, polypropylene fritted cartridge into a 1.5 mL Eppendorf tube. The filtrate was evaporated to dryness under vacuum using a SpeedVac concentrator, and re-dissolved in 1:1 MeCN:H_2O prior to analysis by HPLC and LC/MS.

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