Incorporation of Chemoselective Functionalities into Peptoids via Solid-Phase Submonomer Synthesis

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A simple route to the introduction of a number of chemoselective functional groups into peptoids (oligo-(N-substituted glycines)) by an extension of the standard solid-phase submonomer method is reported. The following groups were introduced: aminooxyacetamide, *N*-(carbamoylmethyl)acetohydrazide, mercaptoacetamide, 2-pyridinesulfenylmercaptoacetamide, and aldehyde-terminated peptoids. The method uses commercially available reagents, is fully compatible with standard peptoid submonomer synthesis conditions, is easily automated, and generates the desired functionalized peptoid in high yield and purity. Peptoids with suitable pairs of chemoselective ligation groups were joined in high yield.

INTRODUCTION

Biomolecules conjugated to particles, surfaces, or reporter groups are widely used to measure molecular interactions in rapid screening procedures (*1*−*5*) and in diagnostics and therapeutics (*6*). These bioconjugates are expensive to prepare and are often of limited stability. With the advent of combinatorial libraries, scientists now want to attach synthetic compounds to surfaces, biomolecules, and reporter groups to perform similar analyses.

Advances in combinatorial chemistry have made huge numbers of synthetic molecules available; and small organic molecules, shorter peptide oligomers (*7*), and nucleic acids (*8*, *9*), as well as nonnatural oligomers (peptoids (*10*) and other peptomimetics), have found numerous practical applications. The ability to attach synthetic ligands to surfaces has led to discoveries in chemical genomics (*11*), proteomics (*12*, *13*), and drug discovery (*7*).

Screening procedures that require the synthetic molecules to be attached to a solid phase, such as a bead, or a planar surface, can be prepared by chemical synthesis of the synthetic compound library directly onto the surface, or by synthesis, cleavage, and reattachment of the compound. Some methods involve synthesis of peptides and DNA oligomers directly on glass (*9*), cellulose membranes (*14*), and hydrophilic beads (*15*). The main disadvantage of these approaches, however, is that it is not possible to analyze or purify the immobilized compounds prior to screening.

We were interested in approaches where individual compounds of a library are assembled by chemical synthesis on a solid support in a form suitable for subsequent specific conjugation to a surface or a reporter group. This route facilitates compound analysis and purification, as well as conjugation of the compound library to the type of surface that is best suited for the actual screening procedures. Alternatively, synthetic soluble ligands with appropriate functional groups can be conjugated to another molecule to add useful properties (such as a drug, toxin, fluorophore, photoprobe, inhibitor, enzyme, hapten, reporter group, and (poly-(ethylene glycol)) spacer). Synthetic proteins have been assembled from short functionalized peptides using conjugation techniques that rely on unique chemical moieties (native protein ligation (*16*), protein analogues (*17*), cyclic peptides (*18*), template-assembled synthetic proteins (*19*)).

Many chemoselective groups are conveniently introduced into peptides and other ligands by direct chemical solid-phase synthesis, but the necessary reagents are seldom commercially available and have in the past required the elaborate synthesis of protected amino acid compounds (*20*, *21*). It is therefore highly desirable to simplify the introduction of chemoselective groups into molecules, especially in the parallel synthesis of large numbers of potential ligands.

Peptoids (N-substituted glycines) are an important class of biomimetic oligomers that in recent years have made a significant impact in the areas of combinatorial drug discovery (*22*), gene therapy (*23*), drug delivery (*24*), and biopolymer folding (*25*, *26*). Sequence-specific peptoid oligomers are easily assembled from primary amines by a solid-phase submonomer method (*27*, *28*).

Here we describe a simple direct chemical route to the introduction of a number of chemoselective groups into peptoids by an extension of the standard solid-phase submonomer method: aminooxyacetamide, *N*-(carbamoylmethyl)acetohydrazide, mercaptoacetamide, *S*-acetylmercaptoacetamide, 2-pyridinesulfenylmercaptoacetamide, and aldehyde-terminated peptoids. The method uses commercially available reagents, is fully compatible with standard peptoid submonomer synthesis conditions, is easily automated, and generates the desired functionalized peptoid in high yield and purity. We also demonstrate the conjugation of these functionalized peptoids with themselves to generate peptoid dimers, a necessary step toward the synthesis of artificial proteins (*26*). These methods should also be applicable to the synthesis of

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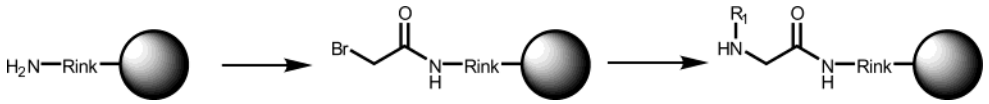


Figure 1.

Table 1. Chemoselective Pairs Commonly Used in Bioconjugation

reactant 1	reactant 2	type of bond formed
aldehyde/ketone	hydrazide	hydrazone
aldehyde/ketone	aminooxy	oxime
thiol	maleimide	thioether
thiol	iodoacetyl	thioether
thiol	activated disulfide	disulfide

many other molecules (heterocycles, peptides, β-peptides, and others (*29*)) assembled on a solid support. These features should greatly expand the utility of synthetic combinatorial libraries, since a diverse set of compounds can be simultaneously functionalized and conjugated to another molecule or surface of interest.

RESULTS AND DISCUSSION

Formation of chemically defined conjugates requires the introduction of uniquely reactive chemical moieties into each ligation partner, preferably at a specific location that does not interfere with the desired activities. These conjugation methods rely on chemoselective pairs of functionalities that are orthogonal to other groups in the molecules to be conjugated. Additionally, the conjugation reaction conditions should be conducted under mild conditions and be compatible with physiological conditions to preserve biological activity. A number of electrophile−nucleophile pairs, that fulfill these requirements, are listed in Table 1.

Peptoids can be efficiently prepared by submonomer solid-phase synthesis (Figure 1). Each monomer addition cycle consists of two steps: (1) acylation of an N-terminal amino group with bromoacetic acid in the presence of a condensing agent, and (2) nucleophilic displacement of the bromide with excess primary amine (*27*). These two steps are repeated with the appropriate amines to build the desired N-substituted glycine sequence.

Chemoselective ligation groups are introduced at the N-terminus with one additional submonomer cycle. The N-terminus, typically a secondary amine, is bromoacetylated and the desired ligation group is then introduced by displacement of the bromide by an excess of a nucleophilic reagent. In a final step, any protecting group on the newly introduced functionality is removed with an appropriate reagent, or further treatment converts it into the desired ligation group prior to cleavage of the product peptoid from the solid support. In some cases the protecting group comes off during trifluoroacetic acid (TFA[1]) cleavage. If desired, a spacer can be added prior to introduction of the ligation group to increase the distance between the conjugated molecules.

To demonstrate the utility of this method, a simple model peptoid (Figure 2) was synthesized and functionalized with several useful ligation groups. The product peptoids were characterized by reverse-phase HPLC and electrospray mass spectrometry (Table 2).

[1]Abbreviations: FMOC, 9-fluorenylmethoxycarbonyl; Boc, *tert*-butoxycarbonyl; Tr = trityl, triphenylmethyl; TFA, trifluoroacetic acid; DBU, diazabicyclo[5.4.0]undecane; DTT, dithiothreitol; DTNB, 5, 5′-dithio-bis(2-nitrobenzoic acid) (Ellman's reagent); TCEP, tris(2-carboxyethyl)phosphine; DCE, 1,2-dichloroethane.

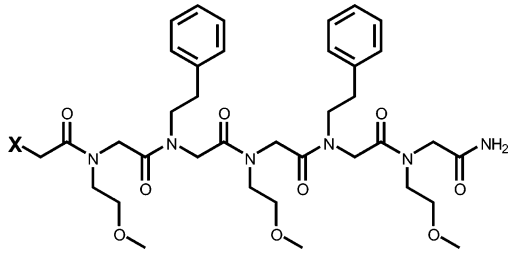


Figure 2. 2. Model peptoid pentamer. X denotes chemoselective functionality.

Table 2. Analytical Data

compound	synthesis yield, %	purity, %	mass spec (calcd/exp)
1	70	95	757.9/758.4
2	60	95	798.9/799.4
3	65	85	852.8/853.3
4	55	85	826.0/827.3
5	95	95	801.0/801.3
7a[a]	79	95	868.1/868.3
7b[a]	77	90	956.1/956.3
7c[a]	75	85	913.1/913.3
6[b]	80	85	758.9/759.3
7d[b]	90	95	912.1/912.3
8[b]	60	95	1515.9/1516.6

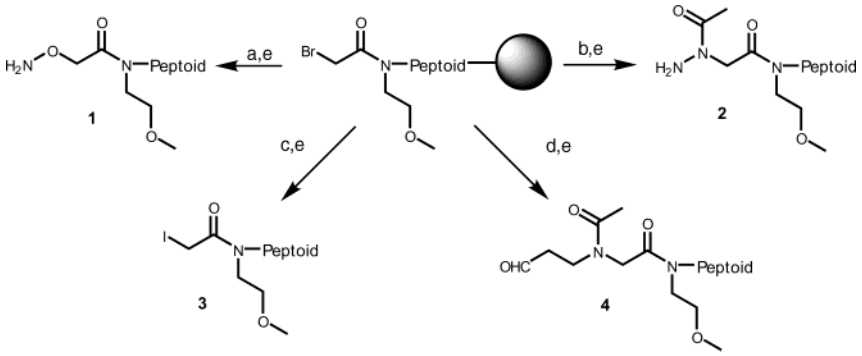
[a] Derived from *S*-acetyl intermediate, **5**. [b] Derived from *S*-trityl intermediate, **10**.

Aminooxyacetamide (aldehyde-specific). Oxime bond formation has been utilized extensively for the introduction of aminooxyacetamide-containing ligands into biomolecules. An aminooxy linker was added to a DNA oligomer during solid-phase synthesis, and conjugation with an aldehyde-containing reporter group was efficient and rapid (*30*). Site-specific modification of peptides and proteins with aminooxy groups was used to prepare well-defined biosensors and probes assembled via oxime linkages (*31*). A new strategy for the assembly of triple-function branched peptides through stepwise chemoselective ligations via oxime bonds has been reported (*32*). Oxime linkages have also been used in the assembly of peptide-based conjugates, such as peptide vaccine candidates (*33*) and in protein conjugates of defined structure (*34*). In those reports an N-Boc-protected aminooxyacetic acid was coupled to the N-terminus of peptides during solid-phase synthesis in the presence of a condensing agent (*34*−*37*). The N-protected aminooxyacetic acid reagent is, however, a rather expensive intermediate.

We introduced the same conjugation moiety by a much simpler submonomer route. The aminooxyacetamide functionality was generated in high yield using *N*-hydroxysuccinimide in the displacement of a solid supported N-terminal bromoacetamide−peptoid (Scheme 1A). Removal of the *N*-succinimide protection with hydrazine hydrate gave the free aminooxyacetamide−peptoid, **1**. *N*-Aminooxyacetamide−peptoid reacted rapidly with benzaldehyde in dilute aqueous solution at pH 4.5 to give the oxime in nearly quantitative yield (data not shown). An aminooxyacetamide−peptoid was ligated to an aldehyde−peptoid (see Peptoid-to-Peptoid Ligation).

Carboxyhydrazide (aldehyde-specific). Carboxyhydrazides form hydrazones with aldehydes and ketones that in general are fairly stable (*38*). They have been used

Scheme 1. Ligation Groups from Bromoacetylated Peptoids[a]



[a] Reagents: (a) (i) *N*-hydroxysuccinimide/DBU, (ii) hydrazine; (b) (i) Boc-$NHNH_2$, (ii) Ac_2O/pyridine; (c) KI; (d) (i) $(CH_3O)_2CHCH_2CH_2NH_2$, (ii) Ac_2O/pyridine; (e) 95% aq TFA.

extensively for conjugation of ligands with biomolecules that contain saccharides (glycoproteins such as antibodies, glycoconjugates, and enzymes (like horseradish peroxidase)) because aldehyde groups are readily introduced into such molecules by enzymatic or chemical (periodate) oxidation (*39*).

Many reporter groups, such as hydrazide derivatives of biotin and fluorescein dyes, are commercially available. Peptides containing a C-terminal hydrazide have been prepared by using an ester linkage to the solid support and cleaving the completed peptide chain by hydrazinolysis (*40*). Reagents have also been developed for converting lysine residues in peptides and proteins into hydrazide functionalities in aqueous solution (*41*), and in a scheme for the chemoselective acylation of 2-hydrazinylacetamide peptides a tris-Boc derivative of 2-hydrazinylacetic acid had to be prepared in order to avoid polymerization of 2-(2-*tert*-butoxycarbonylhydrazinyl)-acetic acid during coupling (*21*).

Using the new modified submonomer procedure, a hydrazide group is readily introduced at N-terminal and internal positions of a peptoid oligomer. Displacement of the bromoacetamide with N-Boc-protected hydrazine (Boc-$NHNH_2$), followed by acetylation with acetic anhydride (Ac_2O) in pyridine produced the N-terminal *N*-(carbamoylmethyl)acetohydrazide−peptoid, **2**. Displacement with N-Boc protected hydrazine (Boc-$NHNH_2$) and continuation of peptoid synthesis produced a peptoid with an internal hydrazide function. Cleavage of the product peptoid with TFA simultaneously removed the Boc group. The resulting hydrazide−peptoid undergoes rapid hydrazone formation with benzaldehyde in dilute aqueous solution at pH 4.5 (data not shown). The N-terminal 2-hydrazinylacetamide peptoid is the product if the acetylation step is omitted. A hydrazide group, incorporated at internal position of a peptoid, reacted with an intramolecular aldehyde to generate a cyclic peptoid (E. Beausoleil, personal communication). A *N*-(carbamoylmethyl)acetohydrazide peptoid was ligated to an aldehyde−peptoid (see Peptoid-to-Peptoid Ligation).

Iodoacetyl (thiol-specific). The iodoacetyl and maleimido groups are thiol-specific ligation groups that generate a stable thioether linkage. Numerous iodoacetyl reagents are commercially available (such as fluorescent derivatives from Molecular Probes). We have also routinely coupled iodoacetic acid and 3-maleimidopropionic acid (*42*) to the N-terminus of peptides and peptoids during solid-phase synthesis, and they have been used successfully for ligations.

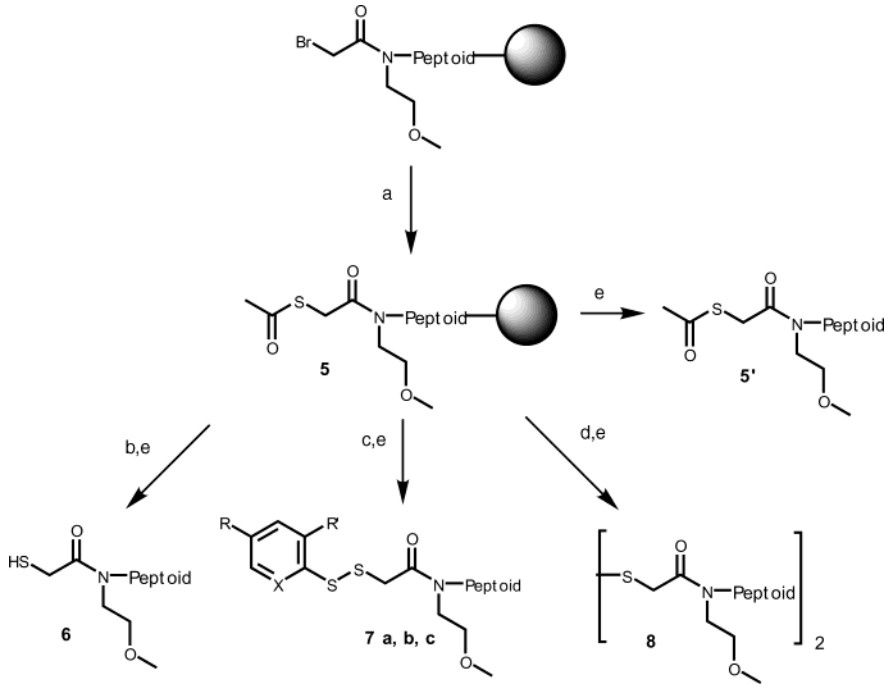
To simplify the synthesis of a thiol-specific group, we first investigated the N-terminal bromoacetamide−peptoid but found that the bromoacetamide group is not completely stable to the acid cleavage and deprotection conditions (using 95% aqueous TFA), resulting in the formation of 5−10% 2-hydroxyacetamide−peptoid byproduct. Instead, we used a displacement reaction on-resin (as an alternative to direct coupling of iodoacetic acid to the peptoid) to generate the more stable iodoacetamide−peptoid, **3**, by a Finkelstein reaction with potassium iodide in DMF (*43*), a reaction that proceeded in quantitative yield. The iodoacetamide−peptoid reacts readily with a thiol compound in aqueous solution at pH 8 to form a thioether (data not shown).

Aldehyde Groups. Peptides with aldehyde moieties have often been synthesized by incorporation of an N-terminal serine. After synthesis, the serine is oxidized to a terminal aldehyde with aqueous sodium periodate. Others have synthesized peptides as the C-terminal ester on a PAM support and released the C-terminal masked aldehyde by aminolysis with aminoacetaldehyde dimethyl acetal followed by TFA treatment to give the unprotected peptide aldehyde. This could then be coupled to aminooxyacetamide peptide derivatives by chemoselective ligation in aqueous solution (*44*). Recently, DNA probes that incorporate a modified nucleoside containing a ketone functionality allowed postsynthetic introduction of several aminooxyacetyl ligands under physiological conditions (*45*). The synthesis of oxime-linked mucin mimics was accomplished via the incorporation of multiple ketone residues into a peptide followed by reaction with aminooxy sugars corresponding to the tumor-related TN and sialyl TN (STN) antigens (*46*).

Peptoids containing aldehyde groups are readily synthesized. An aldehyde is incorporated at any position of the peptoid using commercially available amino aldehydes where the aldehyde function is protected (1-amino-2,2-dimethoxyethane and 1-amino-3,3-diethoxypropane were used in this study) as a submonomer. The CHO−peptoid, **9**, was synthesized in high yield. It reacted with aminooxyacetic acid in aqueous solution at pH 4 to give the oxime in high yield (data not shown). An aldehyde−peptoid was ligated to aminooxyacetamide− and hydrazide−peptoids (see Peptoid-to-Peptoid Ligation).

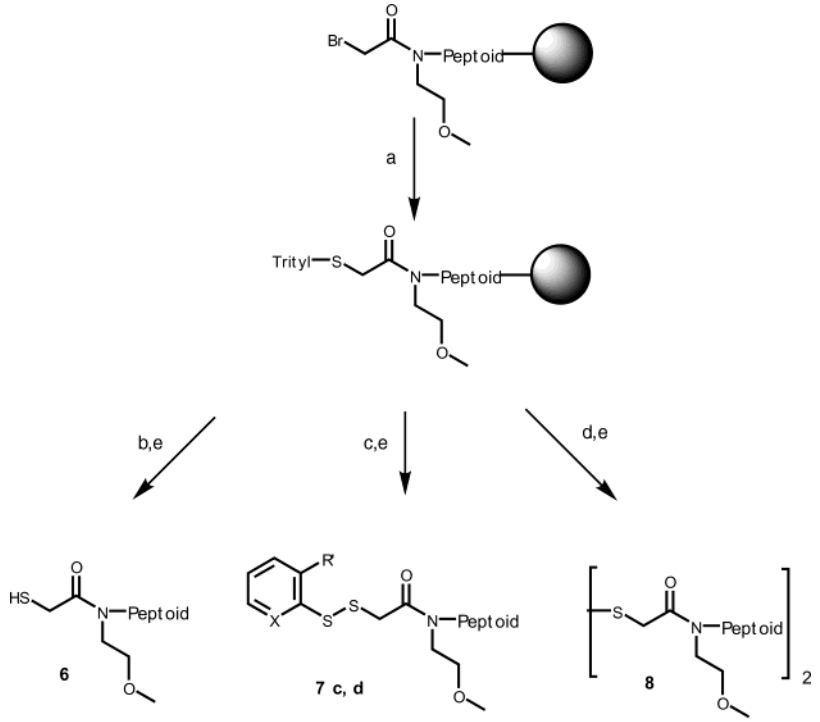
Thiol/Activated Disulfide/Homodisulfide Peptoids. Properly protected cysteine (Cys) residues incorporated during peptide synthesis have been used extensively to modify peptides for conjugation with proteins, labels, surfaces, etc., and typically results in a cleavable disulfide or thioether linkage (*47*) in the conjugate. To introduce a thiol functionality by a simple submonomer protocol we used potassium thioacetate (1 M in DMF/water (95:5 v/v)) and triphenylmethylmercaptan (0.25 M in DMF containing 1 equiv of DBU) to displace the bromide in the bromoacetamide−peptoid to generate key

Scheme 2. Thiol Peptoids via 2-(*S*-Acetylthio)acetamide Intermediate[a]



[a] Reagents: (a) Ac-S$^-$K$^+$; (b) NH_2OH/pyridine; (c) (i) NH_2OH/pyridine, (ii) 2,2′-dithiodipyridine (for **7a**), 5,5′-dithiobis(2-nitrobenzoic acid) (for **7b**), or 2,2′-dithiobis(5-nitropyridine) (for **7c**); (d) (i) NH_2OH/pyridine, (ii) iodine; (e) 95% aq TFA.

Scheme 3. Thiol Peptoids via 2-(*S*-Tritylthio)acetamide−Peptoid Intermediate[a]



[a] Reagents: (a) triphenylmethylmercaptan/DBU; (b) TFA/Et_3SiH; (c) 3-nitro-2-pyridinesulfenyl chloride (for **7c**) or 2-nitrophenylsulfenyl chloride (for **7d**); (d) iodine; (e) 95% aq TFA.

intermediate S-protected mercaptoacetamide−peptoids, 2-(S-acetylthio)acetamide−peptoid, **5** (Scheme 2), and 2-(S-tritylthio)acetamide−peptoid, **10** (Scheme 3). Both can be directly converted into the free thiol or into a number of useful thiol derivatives as described below.

Free Thiol. The free mercaptoacetamide peptoid, **6**, can be obtained in high yield from the *S*-acetyl derivative, **5**, by treatment with hydroxylamine (1 M hydroxylamine hydrochloride in pyridine) (*48*) followed by cleavage with TFA. The 2-mercaptoacetamide peptoid can also be obtained from the *S*-trityl derivative by a modified TFA cleavage mixture that contains a scavenger (TFA/H_2O/triethylsilane (Et_3SiH) (92.5:5:2.5 v/v)) (*49*). A mercaptoacetamide−peptoid forms the mixed disulfide with 5,5′-dithio-bis(2-nitrobenzoic acid) (Ellman's reagent; DTNB) in sodium phosphate buffer at pH 8 (data not shown).

We have successfully reacted the solid-supported mercaptoacetamide−peptoid, derived from the *S*-acetylmercaptoacetamide derivative, **5**, by treatment with hydroxylamine, with maleimide compounds, and reaction with

fluorescein-5-malemide (25 mM in pyridine for 1 h) resulted in the formation of the acid-stable thioether-linked chromophore adduct in 90% yield (data not shown).

A thiol is readily incorporated into peptoids at internal positions using the standard 2-(*S*-tritylthio)ethanamine monomer unit (*50*). The intermediate 2-(*S*-tritylthio)-ethyl−peptoid can be manipulated on the support as described for the terminal 2-(*S*-tritylthio)acetamide−peptoid. A mercaptoacetamide−peptoid was ligated to disulfide-activated peptoids (see Peptoid-to-Peptoid Ligation).

Activated Disulfides. The resin-bound *N*-mercaptoacetamide peptoid, **6**, is readily converted into activated disulfides. Treatment with 2,2′-dipyridyl disulfide (1 M in DMF/acetic acid (95:5 v/v)) resulted in the formation of 2-pyridinesulfenylmercaptoacetamide−peptoid, **7a**, in high yield. Other activated disulfides are also readily prepared by disulfide exchange: 4-nitro-3-carboxybenzenesulfenylmercaptoacetamide, **7b**, by treatment with 5,5′-dithiobis(2-nitrobenzoic acid) (1 M in pyridine), and 3-nitro-2-pyridinesulfenylmercaptoacetamide, **7c**, with 2,2′-dithiobis(5-nitropyridine) (1 M in DMF/acetic acid (95:5 v/v)). The activated disulfides are stable to TFA deprotection and underwent complete exchange with excess thiol compounds under mildly acidic conditions at pH 6 (for **7a** and **7c**) (*47*) and mildly basic conditions at pH 8 (for **7b**) (data not shown).

Treatment of resin-bound 2-(*S*-tritylthio)acetamide−peptoid, **10**, with 2-nitrophenylsulfenyl chloride (0.25 M in dichloroethane/acetic acid (95:5 v/v)) converts it directly into 2-nitrophenylsulfenylmercaptoacetamide−peptoid, **7d**. (*47*, *51*) This activated disulfide is completely stable to TFA deprotection, and it underwent complete exchange with excess thiol compounds under mildly basic conditions at pH 8 (*47*) (data not shown). 3-Nitro-2-pyridinesulfenyl chloride can also be used, and the resulting 3-nitro-2-pyridinesulfenylmercaptoacetamide−peptoid, **7b**, is stable to acid deprotection. It underwent complete exchange with excess thiol compounds under mildly acidic conditions at pH 6 (*47*) (data not shown). Treatment on the support or in solution with tris(2-carboxyethyl)phosphine (TCEP) (*52*) or dithiothreitol (DTT) (*53*) completely reduced the activated disulfides to the mercaptoacetamide−peptoid. Disulfide activated peptoids were ligated to a mercaptoacetamide−peptoid (see Peptoid-to-Peptoid Ligation).

Peptoid Disulfides. Exposure of resin-bound mercaptoacetamide peptoid, **6**, or 2-(*S*-tritylthio)acetamide−peptoid, **10**, to dilute iodine in DCE resulted in complete conversion to the homo-peptoid disulfide which was cleaved from the support and deprotected with TFA to give free homo-peptoid disulfide, $(SAc\text{-}N\text{−}peptoid\text{-}NH_2)_2$, **8**, in high yield. We have successfully used this route to dimerize the model pentamer peptoid at the N-terminus, and a hexamer peptoid that contains a 2-(*S*-tritylthio)-ethyl side group near the C-terminus. Mercaptoacetamide−peptoids were also readily oxidized with iodine in aqueous solution to the corresponding homo-peptoid disulfides, $(SAc\text{-}N\text{−}peptoid\text{-}NH_2)_2$ and $(Ac\text{-}N\text{−}peptoid\text{-}N\text{-}(ethyl\text{-}S\text{-})CH_2CONH_2)_2$, respectively, in high yield (data not shown).

The acid-stable 2-(*S*-acetylthio)acetamide−peptoid, **9**, was also cleaved from the support with TFA. The *S*-acetyl protection group could conveniently be removed in situ with hydroxylamine (0.05 M) in aqueous solution (phosphate buffer at pH 7.5), and the liberated mercaptoacetamide reacted completely with 3-maleimidopropionic acid or Ellman's reagent in a reaction sequence similar to bioconjugations using *N*-succinimidyl *S*-acetylthioacetate (SATA) (*48*).

Peptoid-to-Peptoid Ligations. To demonstrate the utility of some of the ligation groups, we chose to synthesize peptoid dimers. We joined peptoids using several of the most common ligation pairs (Table 1, entries a−c). Reactions were performed with the two types of peptoids in 1:1 stoichometry in dilute solution (0.1 and 0.5 mM of each component) for 30 min. Higher yields could be expected if higher concentrations and longer reaction times were used. Hydrazone formation by reaction of an aldehyde and a hydrazide peptoid proceeded in 45% yield (a result that is comparable to yields recorded in the literature (*38*)). Oxime formation between aldehyde and aminooxyacetamide peptoids resulted in dimer formation in 83% yield (Rose et al. (*36*) observed near-quantitative yields after 20 h). Disulfide formation between mercaptoacetamide peptoid and 2-nitrophenylsulfenylmercaptoacetamide and 2-pyridylsulfenylmercaptoacetamide peptoids proceeded in 88% and 92% yield, respectively. HPLC chromatograms of peptoid-to peptoid ligations are available in the Supporting Information.

CONCLUSION

We have shown that several chemoselective functionalities can readily be introduced into peptoids (and presumably into peptides and many other solid-phase organic chemistries) using a simple extension of the standard solid-phase peptoid submonomer method. The method was implemented primarily for N-terminal modification. Several of the functionalities can also be incorporated into peptoids at internal positions using standard monomers: thiol, aldehyde, and hydrazide functions are added using 2-(*S*-tritylthio)ethanamine (*50*), 1-amino-3,3-diethoxypropane, and $Boc\text{−}NHNH_2$, respectively. Alternatively, this method can be adapted so the desired functional group can be placed at other positions by using orthogonal protection of a lysine residue (for peptides) or an alkyl diamine monomer (for peptoids and peptides (*54*)). Some of the steps in this method may not be fully compatible with all functionalities in peptides, or non-standard protection schemes may be required.

The method uses commercially available reagents, is fully compatible with standard peptoid submonomer synthesis conditions, is easily automated, and generates the desired peptoid in high yield and purity. The method should simplify the introduction of chemoselective functionalities into molecules assembled by a solid-phase synthesis strategy (combinatorial libraries of ligands) for further screening for compounds with useful properties.

EXPERIMENTAL PROCEDURES

All chemicals were purchased from Aldrich, except for fluorescein-5-malemide (Molecular Probes, Eugene, OR), hydroxylamine hydrochloride, and tris(2-carboxyethyl)-phosphine (TCEP) (Pierce). The 2-(S-tritylthio)ethanamine monomer unit was prepared as described (*50*).

General Procedures. *Peptoid Synthesis.* Peptoid oligomer syntheses were performed on a robotic synthesizer (*55*). The syntheses were conducted with Rink amide polystyrene resin (50 μmol; 65 mg; substitution level 0.75 mmol/g). Acylation reactions were performed by the addition of bromoacetic acid (0.83 mL of 1.2 M in DMF; 1 mmol), followed by *N*,*N*′-diisopropylcarbodiimide (200 μL, 1.25 mmol). Reaction mixtures were agitated at 35 °C for 20 min. Displacement reactions were performed by addition of primary amine as 1 M solutions in DMF (1.0 mL; 1.0 mmol), followed by agitation for 20 min at

35 °C. These two steps are repeated with the appropriate amines to build the desired N-substituted glycine sequence.

Chemoselective Ligation Group Synthesis. The chemoselective ligation group was next introduced at the N-terminus with one additional submonomer cycle. The N-terminus, typically a secondary amine, was bromoacetylated, as described above, and the desired ligation group was introduced by displacement of the bromide by an excess of a nucleophilic reagent. In each reaction 1.25 mL of reagent was used, and the reaction mixture was agitated at 35 °C for 20 min. A common wash cycle consisting of 5 × 2 mL of DMF was used after each reagent addition with agitation at 35 °C for 1 min. All nucleophilic displacement reagents and auxiliary solutions were prepared immediately before use.

Aminooxyacetamide, 1. Bromoacetylated peptoid was treated with (a) *N*-hydroxysuccinimide/DBU (1.25 mL; 1 M/1 M in DMF) for 20 min, followed by (b) hydrazine hydrate (1.25 mL; 1 M in DMF) for 20 min.

***N*-(Carbamoylmethyl)acetohydrazide, 2.** Bromoacetylated peptoid was treated with Boc-$NHNH_2$ (1.25 mL; 1 M in DMF) for 20 min, followed by acetic anhydride (1.25 mL; 1 M in pyridine) for 20 min.

Iodoacetamide, 3. Bromoacetylated peptoid was treated with KI (1.25 mL; 1 M in DMF) for 20 min.

Aldehyde, 4. Bromoacetylated peptoid was treated with (a) 1-amino-3,3-diethoxypropane or 1-amino-2,2-dimethoxyethane (1.25 mL; 1 M in DMF) for 20 min, followed by (b) acetic anhydride (1.25 mL; 1 M in pyridine) for 20 min.

2-(*S*-Acetylthio)acetamide, 5. Bromoacetylated peptoid was treated with potassium thioacetate (1.25 mL; 1 M in DMF/water (95:5 v/v)) for 20 min.

2-Mercaptoacetamide, 6. *S*-Acetylmercaptoacetamide, **5**, was treated with hydroxylamine-HCl (1.25 mL; 1 M in pyridine) for 20 min.

2-Pyridinesulfenylmercaptoacetamide, 7a. *S*-Acetylmercaptoacetamide, **5**, was treated with a) hydroxylamine·HCl (1.25 mL; 1 M in pyridine) for 20 min, followed by b) 2,2′-dithiodipyridine (1.25 mL; 0.25M in DMF/acetic acid (95:5 v/v)) for 20 min.

4-Nitro-3-carboxyphenylsulfenylmercaptoacetamide, 7b. Acetylmercaptoacetamide, **5**, was treated with (a) hydroxylamine·HCl (1.25 mL; 1 M in pyridine) for 20 min, followed by (b) 5,5′-dithiobis(2-nitrobenzoic acid) (1.25 mL; 0.25 M in pyridine) for 20 min.

3-Nitro-2-pyridinesulfenylmercaptoacetamide, 7c. Acetylmercaptoacetamide, **5**, was treated with (a) hydroxylamine-HCl (1.25 mL; 1 M in pyridine) for 20 min, followed by (b) 2,2′-dithiobis(5-nitropyridine) (1.25 mL; 0.25M in DMF/acetic acid (95:5 v/v)) for 20 min.

Homo-disulfide-acetamide (SAc-N−peptoid-NH_2)$_2$, 8. Acetylmercaptoacetamide, **5**, was treated with (a) hydroxylamine-HCl (1.25 mL; 1 M in pyridine) for 20 min, followed by (b) iodine (1.25 mL; 0.05 M in DCE) for 20 min.

2-(*S*-Tritylthio)acetamide, 10. Bromoacetylated peptoid was treated with triphenylmethanethiol/DBU (1.25 mL; 0.25 M/0.25 M in DMF) for 40 min. The triphenylmethanethiol/DBU reagent is prepared by first dissolving triphenylmethanethiol in DMF followed by addition of the equimolar amount of DBU.

2-Mercaptoacetamide, 6. 2-(*S*-Tritylthio)acetamide, **10**, was treated with TFA/H_2O/triethylsilane (92.5:5:2.5 v/v; 2.5 mL) for 60 min.

3-Nitro-2-pyridinesulfenylmercaptoacetamide, 7c. 2-(*S*-Tritylthio)acetamide, **10**, was treated with 3-nitro-2-pyridinesulfenyl chloride (1.25 mL; 0.25 M in DCE/acetic acid (95:5 v/v)) for 20 min.

2-Nitrophenylsulfenylmercaptoacetamide, 7d. 2-(*S*-Tritylthio)acetamide, **10**, was treated with 2-nitrophenylsulfenyl chloride (1.25 mL; 0.25 M in DCE/acetic acid (95:5 v/v)) for 20 min.

Homo-disulfide-acetamide (SAc-N−peptoid-NH_2)$_2$, 8. 2-(*S*-Tritylthio)acetamide, **10**, was treated with iodine (1.25 mL; 0.05 M in DCE) for 20 min.

After conclusion of the synthesis, side-chain protecting groups were removed, and the oligomer was released from the resin support by treatment of the oligomer-resin with 95% trifluoroacetic acid in water (2.5 mL) for 60 min at room temperature, followed by filtration, dilution, and lyophilization. The crude products were analyzed by reversed-phase HPLC using the following conditions: Duragel G C18 (5 mμ; 50 × 2 mm) column; 5−95% gradient at 1 mL/min over 10 min at 60 °C; solvent A: water + 0.1% (v/v) TFA, solvent B: acetonitrile + 0.095% (v/v) TFA.

Ligation of Peptoids. The crude functionalized peptoids were purified by reversed-phase HPLC prior to ligation using the following conditions: Duragel G C4 HS (5 mμ; 50 × 20 mm) column; 20−65% gradient at either 20 or 40 mL/minute over 30 min at 60 °C; solvent A: water + 0.1% (v/v) TFA, solvent B: acetonitrile + 0.1% (v/v) TFA, using a Rainin dynamics SD-200 solvent delivery system.

Aldehyde/Aminooxyacetamide and Aldehyde/Hydrazide Ligations. Aminooxyacetamide−peptoid, **1**, or *N*-(carbamoylmethyl)acetohydrazide−peptoid, **2** (2 μL of a 5 mM stock solution in water containing 20% acetonitrile) and aldehyde−peptoid **4** (88 μL of a 100 μM stock solution in water containing 30% acetonitrile and 0.1% TFA) were added to 10 μL of a 1 M sodium acetate (pH 4.7) buffer. The reaction mixture was incubated at 20 °C for 30 min prior to HPLC analysis.

Mercaptan and Activated Disulfide Ligations. Mercaptoacetamide−peptoid, **6** (5 μL of a 5 mM stock solution in water containing 20% acetonitrile) and activated disulfide−peptoid, **7a** or **7d** (5 μL of a 5 mM stock solution in water containing 45% acetonitrile), were added to 40 μL 20mM tris-HCl buffer (pH 8.0) containing 0.4 mM EDTA/acetonitrile (1:1 v/v). The reaction mixtures were incubated at 20 °C for 30 min prior to hplc analysis.

Ligation mixtures were analyzed by reversed-phase HPLC using the following conditions: Duragel G C4 (5 micron; 50 × 2 mm) column; 5−95% gradient at 0.8 mL/minute over 10 min at 60 °C; solvent A: water + 0.1% (v/v) TFA, solvent B: acetonitrile + 0.095% (v/v) TFA. HPLC chromatograms of peptoid-to peptoid ligations are available in the Supporting Information.

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Supporting Information Available: HPLC chromatograms of functionalized peptoids and of peptoid dimer ligations. This material is available free of charge via the Internet at http://pubs.acs.org.

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