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Click to Fit: Versatile Polyvalent Display on a Peptidomimetic Scaffold

Hangjun Jang, Aaron Fafarman, Justin M. Holub, and Kent Kirshenbaum*

Department of Chemistry, New York University, 100 Washington Square East, New York, New York 10003-6688

kent@nyu.edu

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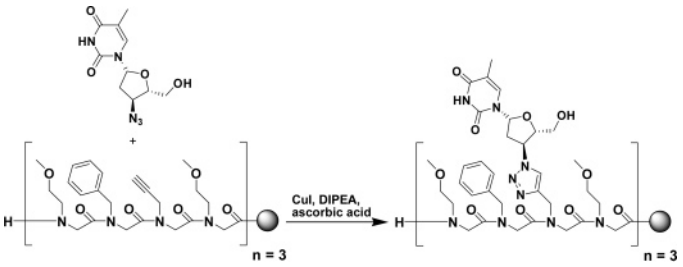
ABSTRACT



We describe an efficient protocol to effect multisite conjugation reactions to oligomers on solid-phase support. Sequence-specific *N*-substituted glycine "oligopeptoids" were utilized as substrates for azide–alkyne cycloaddition reactions. Diverse groups, including nucleobases and fluorophores, were conjugated at up to six positions on peptoid side chains with yields ranging from 88 to 96%. This strategy will be broadly applicable for generating polyvalent displays on peptides and other scaffolds, allowing precise control of spacing between the displayed groups.

N-Substituted glycine oligomers, or "peptoids", are an important class of peptidomimetics that recapitulate many desirable attributes of natural peptides.[1] For example, they can form stable secondary structures and exhibit a range of biological activities.[2] Furthermore, peptoids show marked resistance to proteolysis.[3] As part of an effort to enhance the functional capabilities of biomimetic heteropolymers, we seek to further elaborate the chemical and structural diversity of peptoid sequences.[4] Here we report an efficient synthetic strategy for the multisite modification of peptoids on solid phase using "click chemistry", implemented by Cu(I)-catalyzed azide−alkyne [3 + 2] cycloaddition reactions.

Inspired by Nature's strategy of posttranslational modifications, we performed postoligomerization modifications (Scheme 1) of peptoids exploiting the intrinsic advantages of click chemistry.[5] These reactions have broad orthogonality to many biologically relevant chemical functionalities, thus obviating the requirement for introduction of protecting groups.[6] Furthermore, triazole products are generated with high regioselectivity and near-quantitative yields.[7] We adapted click chemistry protocols for application to peptoid sequences and coupling partners on solid-phase support, achieving highly efficient multivalent conjugation.

* To whom correspondence should be addressed.

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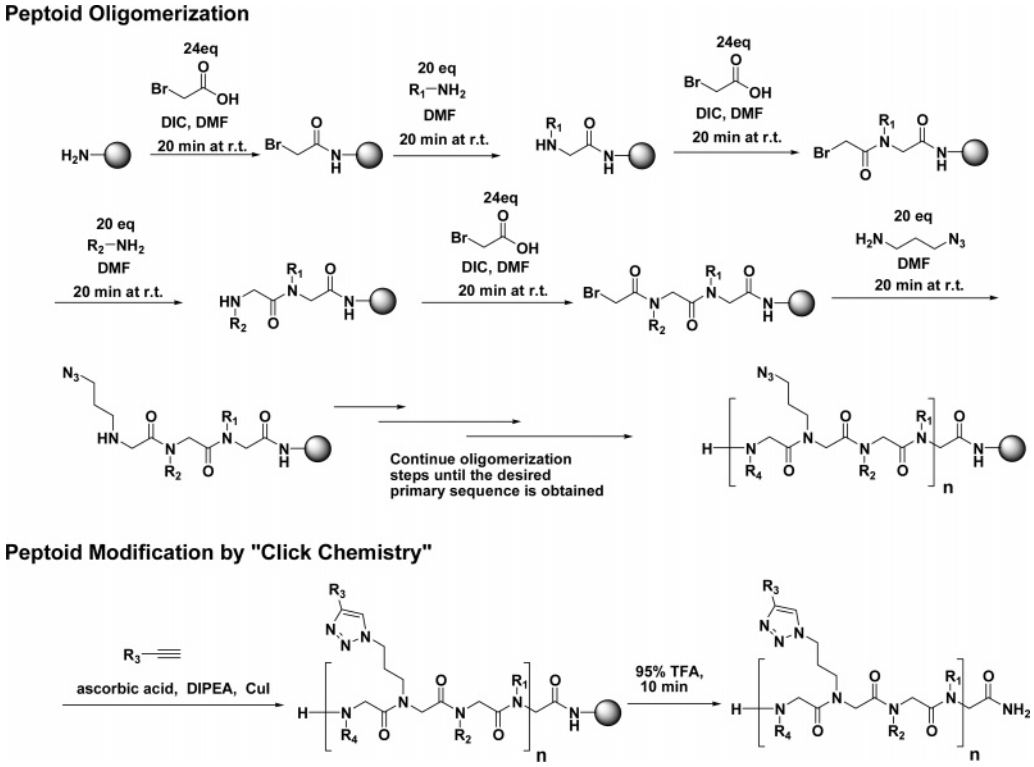
Scheme 1. Postoligomerization Modification Protocol[a]



[a] Propargylamine can be used as a submonomer to replace 1-azido-3-aminopropane. In this implementation, azide coupling partners are used to effect modification.

Our strategy entailed the synthesis of linear peptoid sequences including multiple reactive groups at specific side chain positions (Scheme 1). Peptoids were conveniently synthesized by standard "submonomer" automated protocols.[4c] Azide or alkyne functional groups were readily incorporated using 1-azido-3-aminopropane or propargylamine as submonomer reagents, affording oligomers **1a**−**2c** on solid-phase support. We then conjugated a corresponding alkyne or azide partner, respectively, to these selectively reactive sites on the peptoid scaffolds (Scheme 2).

Both azide and alkyne groups within the oligomer sequence were modified with equal efficiency through click chemistry (Table 1; entries 1 and 5, entries 9 and 10). In the absence of Cu(I) catalyst, no desired product is observed (entries 2 and 6). In the presence of copper iodide, addition of ascorbic acid dramatically improves yields, suggesting that this reducing agent is capable of stabilizing Cu in its +1 oxidation state[7a] (see entries 4 and 8). The tertiary hindered base, DIPEA, is also essential to the reaction (see entries 3 and 7), possibly through the promotion of Cu(I) insertion into terminal alkynes.[8] Up to six positions on the 24mer peptoid oligomer scaffolds **1b** and **2b** can be modified in very high overall yield (up to 94%, entries 9−12). The

Scheme 2. Postoligomerization Modifications to Peptoid Side Chains by Regiospecific Azide−Alkyne [3 + 2] Cycloaddition

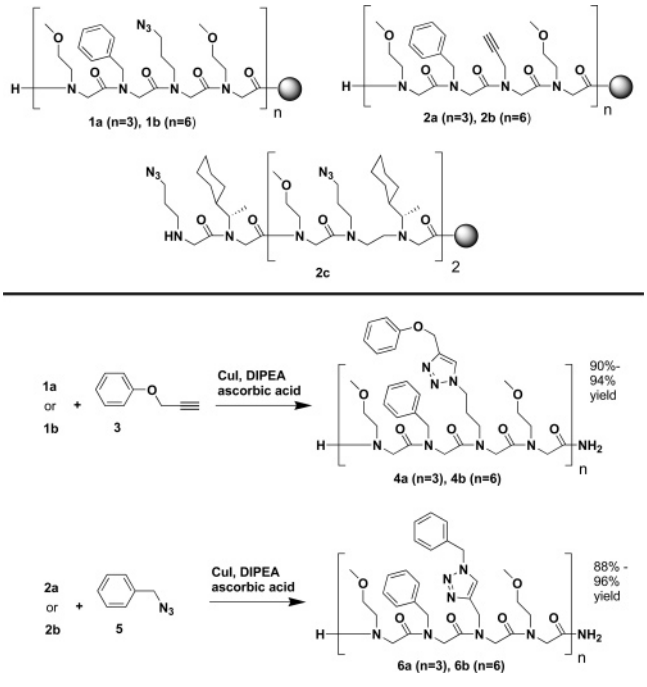


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Table 1. [3 + 2] Cycloaddition Reactions of Peptoid **1** and **2** with Phenylpropargyl Ether **3** or Benzyl Azide **5**

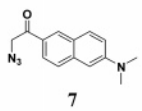
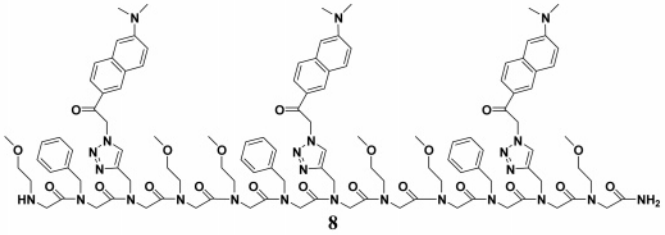
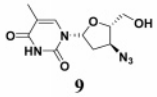
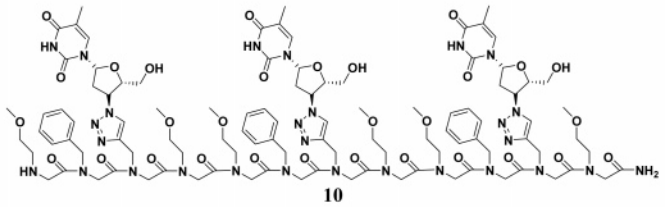
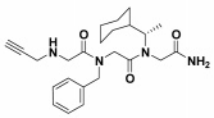
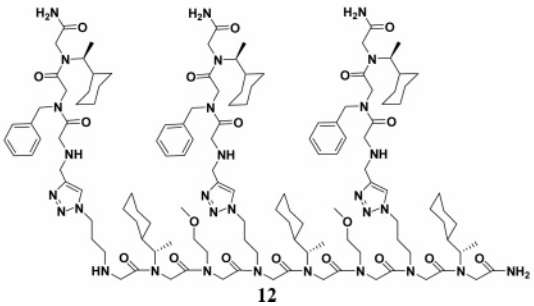
entry	reagents	CuI[a,d]	DIPEA[b,d]	**3** or **5** (equiv)[d]	Vit-C[c,d]	peptoid purity[e]	appoximate yield[f]
1	**1a** + **3**	+	+	7	+	58%	94%
2	**1a** + **3**	−	+	7	+	58%	0%
3	**1a** + **3**	+	−	7	+	58%	0%
4	**1a** + **3**	+	+	7	−	58%	12%
5	**2a** + **5**	+	+	7	+	55%	96%
6	**2a** + **5**	−	+	7	+	55%	0%
7	**2a** + **5**	+	−	7	+	55%	0%
8	**2a** + **5**	+	+	7	−	55%	29%
9	**1b** + **3**	+	+	7	+	39%	90%
10	**2b** + **5**	+	+	7	+	32%	88%
11	**1b** + **3**	+	+	20	+	39%	94%
12	**2b** + **5**	+	+	20	+	32%	94%

[a] Performed with addition of 13 equiv of CuI where indicated (+). [b] Performed with addition of 17 equiv of DIPEA where indicated (+). [c] Performed with addition of 7 equiv of ascorbic acid where indicated (+). [d] Equivalents based on total moles of azide or alkyne functional groups on peptoid scaffold. Peptoids were allowed to react without further purification after synthesis on Rink amide resin. [e] Portion of starting material was analyzed to report crude purity prior to conjugation. [f] Average cycloaddition yields were based upon averages from triplicate reactions. Yields were approximated by RP-HPLC and calculated using the ratios of % area of reactant species to % area of product species, as confirmed by MS (see Supporting Information for details). Reactions were conducted in 2-ethoxyethanol/pyridine 7:3 for 15 h at rt, followed by 10 min of treatment with 95% TFA in water for cleavage of products. All products were confirmed by high-resolution LC/MS-TOF and MS/MS sequencing.

addition of 7 equiv of coupling partners is sufficient to obtain these yields (compare entries 9 and 11 and entries 10 and 12).

To demonstrate the broad utility of this reaction, we sought to employ diverse coupling partners (Table 2). PRODAN, or 6-propionyl-2-(dimethylamino)naphthalene, is a solvato-

Table 2. Synthesis of Trivalent Conjugates on Peptoid Scaffolds

entry	coupling partners	scaffolds	product	conditions[a]
1	 **7**	**2a**	 **8**	A
2	 **9**	**2a**	 **10**	A
3	 **11**	**2c**	 **12**	B

[a] Conditions: (A) 7 equiv of **7** or **9**, 13 equiv of CuI, 17 equiv of DIPEA, 7 equiv of ascorbic acid in DMF/pyridine 7:3, rt, 15 h; (B) 7 equiv of **11**, 13 equiv of CuI, 17 equiv of DIPEA, 7 equiv of ascorbic acid in 2-butanol/DMF/pyridine, 5:3:2, rt, 72 h. All products were cleaved from solid support by 95% TFA in water, 10 min, rt. Based upon RP-HPLC analysis, these reactions exhibited conversion efficiencies similar to those found in Table 1.

chromic fluorophore that has proven to be useful as a probe in biological systems.[9] We conjugated the 6-azido-acetyl analogue[10] **7** to three sites on the peptoid scaffold **2a** and synthesized a trivalent peptoid fluorophore **8** (Table 2, entry 1; Figure 1).

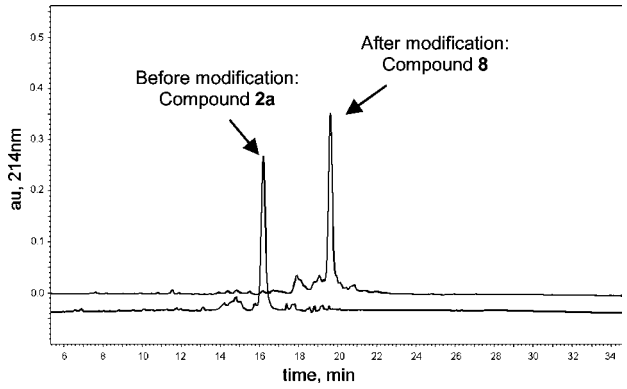


Figure 1. Representative analytical RP-HPLC showing modification of **2a** to give **8**. (Trace of **8** displaced in *y* direction only.)

We similarly synthesized a trivalent peptoid−nucleoside conjugate **10** using azidothymidine **9** (Table 2, entry 2). Nucleobase conjugation to peptoid scaffolds at multiple residues may offer a new type of synthetic oligomer similar to peptide nucleic acids for use as novel probes and diagnostic agents.

In addition, we conjugated peptoid trimers **11** to three positions on a helical peptoid[2b] octamer **2c**, constructing a trivalent display **12** (Table 2, entry 3). This complex molecule was generated by a convergent "one-step" reaction, demonstrating the capability of click chemistry to efficiently generate elaborate branched architectures.[11]

We investigated whether the triazole linkages generated upon click chemistry cycloadditions are compatible with efficient peptoid chain extension by standard submonomer chemistry (see Scheme S1 and Figure S5, Supporting Information). This would enable us to perform sequential cycles of modifications during the course of oligomer chain elongation on solid-phase support. First, phenyl propargyl ether was coupled to the azido-functionalized side chain of a resin-bound peptoid trimer. The click chemistry reagents were then washed from the solid-phase, and three complete peptoid monomer addition cycles were executed, resulting in a peptoid hexamer with an *N*-terminal propargyl side chain. This site was used to conjugate benzyl azide in a second cycle of click chemistry modification. The capability for performing multiple cycles of conjugation reactions will allow the generation of complex modular structures in which multiple heterogeneous groups are site-specifically positioned along the peptoid scaffold.

Previous reports have typically utilized sodium ascorbate or tris(carboxyethyl)phosphine for in situ reduction of Cu(II) salts in aqueous systems.[6a,7a] Here we demonstrate that Cu(I) salts can be directly used without complications in organic solvent systems, when stabilized by ascorbate in the presence of DIPEA. Reactions are conducted without the formation of undesired byproducts, the necessity for prior preparation of Cu(I) ligands,[12] or the need for rigorous exclusion of oxygen. We have utilized ascorbic acid in a variety of organic solvent systems, including pyridine, DMF, and alcohols, suggesting the compatibility of this method with diverse solid-phase resins. Thus, at low cost and with remarkable convenience, these procedures can be incorporated into common solid-phase organic synthesis protocols, potentially allowing seamless utilization in automated synthesizers.

This report demonstrates the facility of click chemistry for conjugating complex and biologically relevant ligands to heteropolymer side chains site-specifically and in the presence of other unprotected chemical functionalities on solid phase. The reaction is sufficiently robust to permit the generation of complex branched constructs. We anticipate that this approach will be broadly applicable for enhancing the efficiency of conjugation reactions to peptides and other oligomer systems on solid phase. Potential applications include new routes to antigen-presenting compounds and the generation of sensors bearing multivalent probes. Our future studies will investigate how the precise ligation of bioactive ligands onto helical peptoid scaffolds may facilitate testing principles of polyvalent interactions[7b,13] and provide peptidomimetics with enhanced avidities for biomolecular targets.

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Supporting Information Available: Further information concerning peptoid syntheses, postoligomerization modification methods, peptoid sequencing, and sequential click chemistry. This material is available free of charge via the Internet at http://pubs.acs.org.

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