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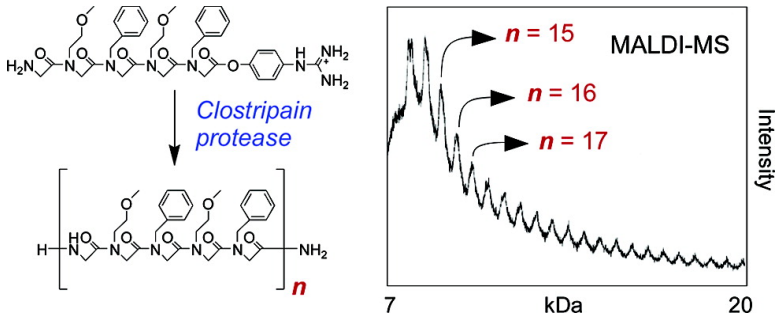

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Protease-Mediated Ligation of Abiotic Oligomers

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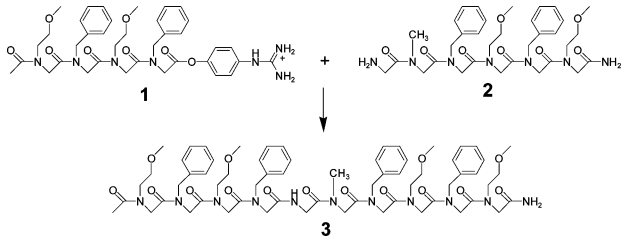
N-substituted glycine oligomers, or peptoids, are an important class of peptidomimetics that demonstrate a propensity to form stable secondary structures.[1] These "foldamers"[2] can display rudimentary biological activities[3] along with resistance to proteolysis.[4] Ongoing studies seek to discover peptoids that are capable of autonomous folding into compact tertiary structures.[1c,d] Such efforts have been hampered by the difficulty in synthesizing sequence-specific heteropolymers of the requisite chain lengths. Segment condensation or ligation is an approach that can address this challenge.[5] A number of studies have shown that proteases can be coerced to run in reverse, catalyzing the formation of peptide bonds.[6−8] One major impediment to the use of proteases for peptide synthesis is the sequence limitation imposed by the protease—the product itself can be a target for proteolysis. In comparison, protease-catalyzed ligation of peptoid segments is an attractive strategy due to their inherent resistance to proteolytic degradation. Here, we report enzyme-mediated ligation for the generation of biomimetic macromolecules through the condensation of peptoid fragments.

Our protocols utilize a protease in concert with its corresponding substrate mimetic,[9] a chemical moiety resembling the protease's natural substrate. Clostripain, from *Clostridium histolyticum*, is a cysteine protease that catalyzes the hydrolysis of the amide bond following an arginine residue.[10] This enzyme was selected for its broad amino acid sequence tolerance, particularly in the positions adjacent to the scissile bond.[11] *p*-Guanidinophenyl esters have been reported as substrate mimetics for trypsin and, more recently, for clostripain.[7] In this ligation approach, the *p*-guanidinophenyl ester serves as a recognition element for the protease and also acts as an efficient leaving group. Catalyzed formation of the amide bond is conducted in competition with the hydrolysis of the *p*-guanidinophenyl ester.

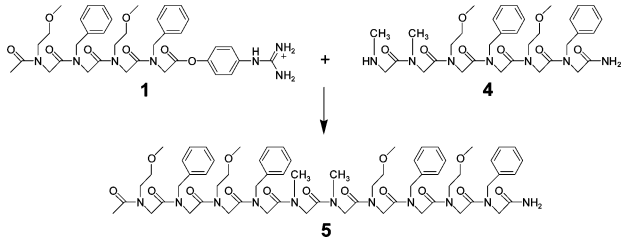
To determine the compatibility of the substrate mimetic approach with peptoid fragments, two model oligomers were evaluated for ligation (Scheme 1). A peptoid tetramer was synthesized and N-acetylated on chlorotrityl resin. The oligomer was then cleaved from the solid support and modified with *p*-guanidinophenol to form the acyl donor **1**. The acyl acceptor **2** was prepared using a combination of standard peptoid "submonomer" chemistry[12] and Fmoc chemistry on Rink amide resin. An unsubstituted glycine residue was positioned at the N-terminus of **2**, followed by an *N*-methylglycine (sarcosine) at the subsequent residue. The ligation of **1** and **2** progressed efficiently in the presence of clostripain, as monitored by analytical HPLC (Figure 1). Complete conversion of the acyl donor was observed within 4 h, yielding primarily decamer **3** along with some hydrolysis product. Identity of **3** was confirmed by electrospray mass spectrometry ([M + H$^+$], calcd *m*/*z* 1236.63; obs. *m*/*z* 1237.0). No ligation was observed in the absence of enzyme.

Experiments were undertaken to probe sequence tolerance and fragment length requirements for ligation. Oligopeptoid acyl acceptors bearing unprotected amino- or guanidino-functionalized

Scheme 1



Scheme 2 [a]



[a] Reaction conditions: 0.2 M HEPES, pH 8.0, 100 mM NaCl, 1 mM $CaCl_2$, 12% DMF, 5 mM **1**, 10 mM **2** or **4**, 1.6 μM clostripain, rt, 4 h (Scheme 1) or 8 h (Scheme 2).

side chains were used to generate peptoid products of 10, 16, and 32 residues (see Supporting Information). HPLC profiles and mass spectrometry of the reaction mixtures revealed the anticipated fragment condensations with minimal side product formation. Product hydrolysis or degradation was not observed, even following overnight incubation in the presence of clostripain. These results demonstrate that protecting groups may not be required for potentially reactive side chains and further highlight the resistance of peptoids to protease-catalyzed degradation.

The ligation of oligomers possessing N-substituent side chains at all residues was investigated (Scheme 2). Acyl donor **1** and acyl acceptor **4** were used for this reaction. Peptoid **4** incorporates two sarcosine monomer units at the N-terminal positions. The ligation reaction successfully generated oligomer **5** ([M + H$^+$], calcd *m*/*z* 1250.65; obs. *m*/*z* 1250.9), but with a marked decrease in yield. Steric constraints at or near the active site of clostripain may alter the efficiency of amide bond formation between the acyl donor and acceptor.[11] Overall, these reactions underscore the compatibility

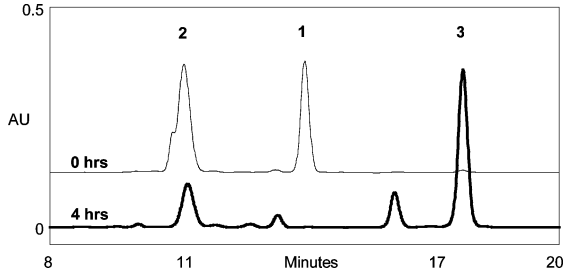


Figure 1. Peptoid ligation reaction monitored by reversed-phase HPLC. **1**, **2**, and **3** correspond to Scheme 1 entries.

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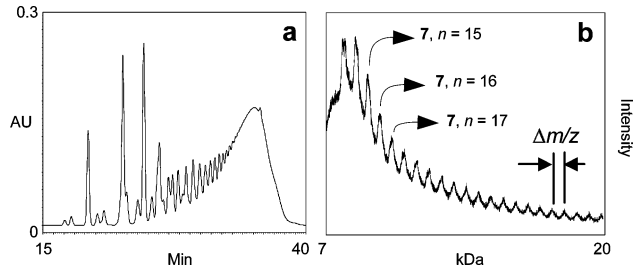
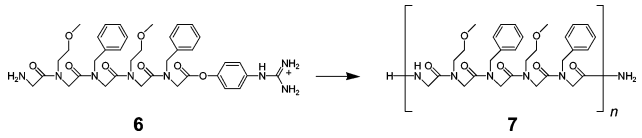


Figure 2. Peptoid concatenation reaction. (a) HPLC of peptoid concatenation products **7**. (b) MALDI mass spectrometry of concatemer products **7**. Average $\Delta\ m/z = 581$, corresponding to mass of pentamer repeat units.

Scheme 3 [a]



6 **7**

[a] Reaction conditions: 0.2 M HEPES, pH 8.0, 100 mM NaCl, 1 mM $CaCl_2$, 12% DMF, 5 mM **6**, 1.6 μM clostripain, rt, 12 h; $n = 1,2,3,...>30$.

of the ligation approach with a nonnatural peptidomimetic system. Broad sequence tolerance and efficient ligation of fragments resulting in long chain products up to 32 residues in length establish that this approach is compatible with a diverse range of peptoid substrates.

To examine the potential of generating macromolecular condensation products, reactions with a single peptoid pentamer **6** bearing a free N-terminus and C-terminal *p*-guanidinophenyl ester were performed with the expectation that the oligomer could function as both acyl donor and acceptor (Scheme 3). Remarkably, the clostripain-catalyzed reaction yielded peptoids that exhibited a range of molecular weights up to and beyond 20 kDa, as confirmed by MALDI mass spectrometry (Figure 2). Mass differences observed between the product molecules **7** correspond to one oligomer repeat unit (avg. $\Delta m/z = 581$). The HPLC and MALDI-MS data indicate that the highly polydisperse reaction products are the result of numerous iterative ligation events (>30), ultimately forming large concatenation products of the starting oligomer.

Our preliminary data suggest that protease-mediated ligation may allow the synthesis of more complex sequence-specific peptoid macromolecules exhibiting properties similar to those of proteins. The mild conditions for the ligation reactions should obviate the need for protecting groups on reactive chemical functionalities present on peptidomimetic side chains. This is especially important in the synthesis of long chain polymers, where large numbers of protecting groups can present deprotection and solubility difficulties. Additionally, our approach may be adapted to construct polymer mimics of protein biomaterials or chimeric products that blend polymer types.

Investigations to further elucidate the effect of peptoid chain length, sequence, and conformation on enzymatic efficiency are underway. Improvements in ligation efficiencies may be realized through different enzyme systems, alterations in reaction medium, and protease engineering to enhance the accommodation of nonnatural oligomers as substrates. Protease-catalyzed peptide synthesis has been well documented for both L- and D-amino acids as well as for small peptide isosteres.[7,9,13] Additionally, ribosome-based translation systems have been utilized for the incorporation of abiotic residues into short peptide sequences.[14] The study presented here further broadens the possibilities for appropriating biological catalysts in order to generate nonnatural products. The synthesis of peptoid concatenation products **7** suggests that the ligation approach will indeed prove to be generally suitable for assembling peptoid macromolecules. We believe the products reported here are among the largest sequence-specific peptidomimetics generated to date.

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Supporting Information Available: Experimental procedures; HPLC data; mass spectrometry data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Kirshenbaum, K.; Barron, A. E.; Goldsmith, R. A.; Armand, P.; Bradley, E. K.; Truong, K. T.; Dill, K. A.; Cohen, F. E.; Zuckermann, R. N. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 4303−4308. (b) Wu, C. W.; Kirshenbaum, K.; Sanborn, T. J.; Patch, J. A.; Huang, K.; Dill, K. A.; Zuckermann, R. N.; Barron, A. E. *J. Am. Chem. Soc.* **2003**, *125*, 13525−13530. (c) Burkoth, T. S.; Beausoleil, E.; Kaur, S.; Tang, D. Z.; Cohen, F. E.; Zuckermann, R. N. *Chem. Biol.* **2002**, *9*, 647−654. (d) Lee, Y. C.; Zuckermann, R. N.; Dill, K. A. *J. Am. Chem. Soc.* **2005**, *127*, 10999−11009.

(2) (a) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173−180. (b) Hill, D. J.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. *Chem. Rev.* **2001**, *101*, 3893−4012.

(3) (a) Patch, J. A.; Barron, A. E. *Curr. Opin. Chem. Biol.* **2002**, *6*, 872−877. (b) Patch, J. A.; Barron, A. E. *J. Am. Chem. Soc.* **2003**, *125*, 12092−12093. (c) Wender, P. A.; Mitchell, D. J.; Pattabiraman, K.; Pelkey, E. T.; Steinman, L.; Rothbard, J. B. *Proc. Natl. Acad. Sci. U.S.A.* **2000** *97* 13003−13008.

(4) Miller, S. M.; Simon, R. J.; Ng, S.; Zuckermann, R. N.; Kerr, J. M.; Moos, W. H. *Drug Dev. Res.* **1995**, *35*, 20−32.

(5) (a) Horn, T.; Lee, B. C.; Dill, K. A.; Zuckermann, R. N. *Bioconjugate Chem.* **2004**, *15*, 428−435. (b) Kimmerlin, T.; Seebach, D.; Hilvert, D. *Helv. Chim. Acta* **2002**, *85*, 1812−1826. (c) Jang, H.; Fafarman, A.; Holub, J. M.; Kirshenbaum, K. *Org. Lett.* **2005**, *7*, 1951−1954. (d) Cheng, R. P.; DeGrado, W. F. *J. Am. Chem. Soc.* **2002**, *124*, 11564−11565.

(6) (a) Chang, T. K.; Jackson, D. Y.; Burnier, J. P.; Wells, J. A. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 12544−12548. (b) Abrahmsen, L.; Tom, J.; Burnier, J.; Butcher, K. A.; Kossiakoff, A.; Wells, J. A. *Biochemistry* **1991**, *30*, 4151−4159. (c) Joe, K.; Borgford, T. J.; Bennet, A. J. *Biochemistry* **2004**, *43*, 7672−7677.

(7) (a) Sekizaki, H.; Itoh, K.; Toyota, E.; Tanizawa, K. *Tetrahedron Lett.* **1997**, *38*, 1777−1780. (b) Bordusa, F.; Ullmann, D.; Elsner, C.; Jakubke, H. D. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2473−2475. (c) Wehofsky, N.; Koglin, N.; Thust, S.; Bordusa, F. *J. Am. Chem. Soc.* **2003**, *125*, 6126−6133.

(8) Klibanov, A. M. *Nature* **2001**, *409*, 241−246.

(9) (a) Günther, R.; Bordusa, F. *Chem.—Eur. J.* **2000**, *6*, 463−467. (b) Günther, R.; Stein, A.; Bordusa, F. *J. Org. Chem.* **2000**, *65*, 1672−1679.

(10) Mitchell, W. M.; Harrington, W. F. *J. Biol. Chem.* **1968**, *243*, 4683−4692.

(11) Ullmann, D.; Jakubke, H. D. *FEBS Lett.* **1994**, *223*, 865−872.

(12) Zuckermann, R. N.; Kerr, J. M.; Kent, S. B. H.; Moos, W. H. *J. Am. Chem. Soc.* **1992**, *114*, 10646−10647.

(13) Wehofsky, N.; Thust, S.; Burmeister, J.; Klussmann, S.; Bordusa, F. *Angew. Chem., Int. Ed.* **2003**, *42*, 677−679.

(14) (a) Tan, Z.; Forster, A. C.; Blacklow, S. C.; Cornish, V. W. *J. Am. Chem. Soc.* **2004**, *126*, 12752−12753. (b) Frankel, A.; Millward, S. W.; Roberts, R. W. *Chem. Biol.* **2003**, *10*, 1043−1050.

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