Rapid Multistep Synthesis of a Bioactive Peptidomimetic Oligomer for the Undergraduate Laboratory

Yeliz Utku, Abhinav Rohatgi, Barney Yoo, and Kent Kirshenbaum*
Department of Chemistry, New York University, New York, New York 10003
*kent@nyu.edu

Ronald N. Zuckermann
Biological Nanostructures Facility at the Molecular Foundry, Lawrence Berkeley National Laboratory, Berkeley, California 94720

Nicola L. Pohl
Department of Chemistry, Iowa State University, Ames, Iowa 50011

Multistep synthesis is a critical skill in organic chemistry *(1)*. Nevertheless, it is infrequently conducted in lower-level undergraduate laboratory classes owing to time and resource constraints. Modern innovations in chemical synthesis can expedite multistep reaction strategies. Performing syntheses on solid-phase resins, for example, can enhance yields and speed synthesis times by minimizing the need for purification of intermediates *(2−5)*. We present a solid-support-based protocol to introductory organic chemistry students, allowing the synthesis of a peptidomimetic oligomer. The end product (Figure 1), "peptoid" trimer (**I**), is generated through six bond-forming reactions that can be performed within one laboratory session. The trimer peptoid was previously identified within a small library and found to possess antiproliferative activity on human cancer cells in cell culture *(6)*, demonstrating the ability of small peptide mimetic oligomers to exhibit biological activity. The laboratory experience described here thus illustrates a "real-world" relationship between synthetic organic chemistry and the biomedical sciences *(6)*. The synthesis is conceptually and technically straightforward, rapid, and inexpensive. Analytical methods employed for characterizing the product can be varied from basic to advanced, making this experiment applicable to a variety of undergraduate lab settings.

Peptoids are oligomers composed of N-substituted glycine units. They were introduced as a versatile platform for combinatorial drug discovery by Zuckermann and colleagues in 1992 *(7)*. Their ease of synthesis stems from their rapid assembly using simple and inexpensive haloacetic acid and primary amine reagents. The vast repertoire of oligomer side chains that can be incorporated into peptoids makes them attractive compounds for basic research in biomimetic chemistry as well as for a variety of biomedical applications *(8, 9)*. The structures of peptoids (Figure 2) are sufficiently similar to peptides to enable peptide-like functions *(10, 11)*, yet peptoids exhibit additional desirable characteristics, such as resistance to proteolysis *(12)*.

Peptoids are routinely synthesized through iteration of a two-step monomer addition cycle: an acylation of a resin-bound amine by a haloacetic acid (typically bromoacetic acid), followed by nucleophilic displacement of the halogen by a primary amine. The peptoid oligomer can be synthesized in the desired length by

simple iteration of these two steps (Scheme 1). The speed, conditions, and the insensitivity of these reactions to atmospheric O_2 and H_2O render this lab suitable for undergraduates. Although biological assays of the product are beyond the scope of this experiment, students are introduced to multistep synthesis, biomimetic chemistry, and biologically active compounds, demonstrating the relationship between organic synthesis and drug discovery.

Experimental Procedure

Materials

All chemicals were purchased from Sigma-Aldrich. Rink amide resin was purchased from NovaBiochem. Fritted polypropylene syringes (5 mL) and corresponding caps were obtained from Torviq (Niles, MI). Silica gel plates were purchased from Sorbtech (Atlanta, GA).

Synthesizing the Tripeptoid

Students start by swelling 50 mg of the resin in *N,N*-dimethylformamide (DMF) for 10 min in a capped syringe. Deprotection of the Fmoc (9*H*-fluoren-9-ylmethoxycarbonyl) group from the resin to expose the amine functionality is carried out with washes of 20% piperidine in DMF. The washes are collected to enable calculation of theoretical yield by determining concentration of the Fmoc group via UV−vis spectroscopy (details in the supporting information). Peptoid synthesis is then initiated by performing the initial acylation via the addition of 1 M *N,N'*-diisopropylcarbodiimide (DIC) in DMF and 1.2 M bromoacetic acid (BrAA) in DMF for 2 min (Scheme 1). The nucleophilic displacement is carried out by adding 1 M amine solution in DMF and reacting for 5 min at room temperature with occasional agitation. The cycle is repeated twice to generate the trimer peptoid. 3,3-Diphenylpropylamine, 3,3-diphenylpropylamine, and tetrahydrofurfurylamine are used in succession to generate the desired product.

Monitoring the Reaction by Chloranil Test

Chloranil tests can be performed to visually monitor the quantity of amine present and thus the progress of each step. A darker blue color indicates presence of unreacted secondary

amines. Accordingly, the color of the resin beads is expected to be yellow after acylation and dark blue after amine addition. The tests are performed by transferring a few beads of the resin into a mixture of 2% chloranil and 2% acetaldehyde in DMF and observing the color of the beads.

Cleaving the Peptoid from Solid Support

After the last amine addition, the resin is rinsed first with DMF, followed by a dichloromethane (DCM) wash. The peptoid is cleaved with 10% trifluoroacetic acid (TFA) in H_2O for 10 min with occasional agitation (Scheme 1). The cleavage cocktail is then collected and prepared for characterization.

Product Characterization

Various methods routinely employed for oligopeptide characterization can be used for the characterization of the peptoid product. Techniques such as thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), electrospray ionization mass spectrometry (ESI-MS) (13), nuclear magnetic resonance spectroscopy (NMR), mass spectrometry (MS), or Fourier transform infrared spectroscopy (FTIR) have been implemented routinely for peptide characterization and are similarly applicable to peptoid species (14 and references therein).

TLC is a simple and convenient technique for characterizing the final product. Small-scale cleavages can be performed
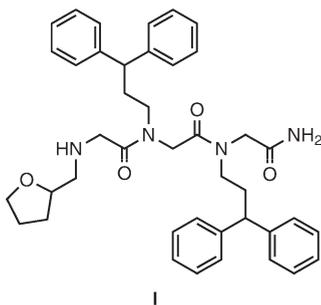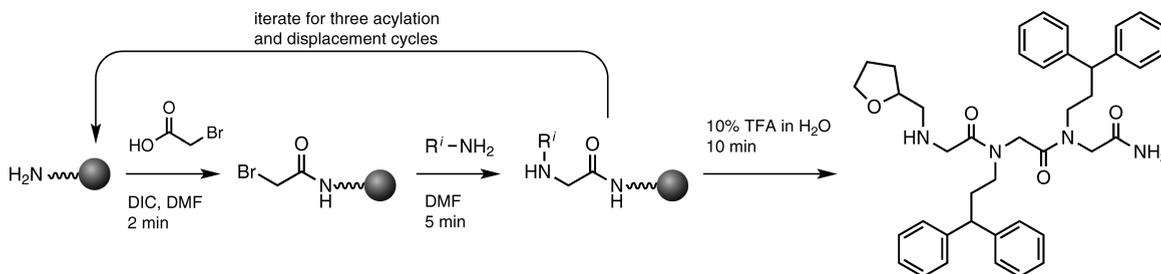

Figure 1. An N-substituted glycine "peptoid" oligomer reported to exhibit biological activity (12).

Figure 2. Structures of peptides (left) versus peptoid oligomers (right) (R = side chain). The two structures differ in the location of the R group.

after each two-step monomer addition cycle to monitor the progress of the synthesis. The peptoid is diluted in 10% methanol in DCM and TLC is performed on a silica gel plate in 10% methanol in DCM. An R_f value of 0.65 is observed for the trimer product. The product can also be characterized by HPLC and MS (Figure 3). (See the supporting information for detailed information.)

Hazards

Chemicals used in this lab are harmful to inhale, ingest, or contact directly. Piperidine, trifluoroacetic acid (TFA), and bromoacetic acid are corrosive to the eyes, skin, and mucous membranes. Students should refrain from handling TFA. The TFA solution should be prepared by an instructor. N,N-dimethylformamide and dichloromethane are reasonably expected to be carcinogenic. Acetaldehyde,
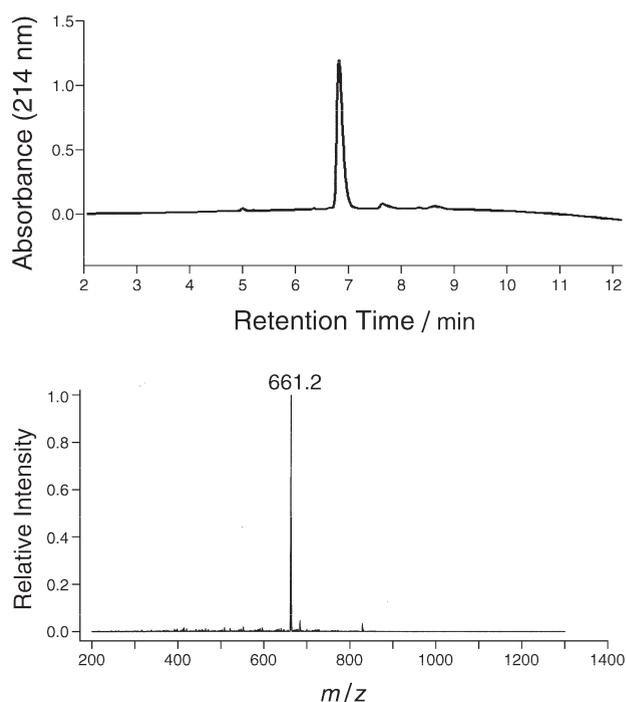

Figure 3. The peptoid product was characterized by HPLC and MS. (Top) The HPLC chromatogram indicates >90% purity of the crude product. (Bottom) The MS shows the targeted mass peak at $[M + H]^+$ of 661.2 Da (calculated value 661.4 Da).

Scheme 1.^{a}

^{a} Peptoid synthesis is achieved through a "submonomer" addition scheme consisting of two iterated steps. This cycle is conducted three times to generate the desired peptoid trimer where R^1 and R^2 are 3,3-diphenylpropylamine and R^3 is tetrahydrofurfurylamine. The product is obtained by cleaving the peptoid chain from the solid support with trifluoroacetic acid (TFA). DMF is N,N-dimethylformamide and DIC is N,N'-diisopropylcarbodiimide.

tetrahydrofurfurylamine, and acetonitrile are flammable. Diisopropyl-carbodiimide (DIC) is highly toxic, as alkylcarbodiimides are contact allergens and skin sensitizing agents. Students should be cautioned regarding the use of syringes as reaction vessels, as there is a potential for unintentional spray of reagents. The toxicity of the peptoid trimer product has not been thoroughly investigated, apart from its anti-proliferative activity in cell culture *(6)*. Therefore, contact with the product should be avoided. Students must wear personal protective equipment throughout the experiment. All reactions should be performed in a ventilated fume hood.

Results and Discussion

The chemical reactions performed in this experiment are straightforward and rapid. Students are presented with reactions of fundamental importance for introductory organic chemistry including an acylation step (DIC-activated addition of bromo-acetic acid to form an amide bond) and a nucleophilic displace-ment (the amine addition to displace a halide, forming a nitrogen–carbon bond). The students thus strengthen their knowledge about these reactions by gaining a practical experience through this peptoid synthesis.

A wide variety of peptoid sequences are accessible because a large number of primary amines can be utilized as the amine submonomer. This allows the instructor to modify this procedure to create a wide variety of oligomers from inexpensive and com-mercially available primary amine reagents. Owing to the rapid, high-yielding nature of these reactions, they can be conducted for brief periods (i.e., 2 min for acylation and 5 min for displacement). Typically, yields are maximized by performing reaction steps for 20 min at 35 °C or longer for displacements using deactivated pri-mary amines *(15)*. Reaction times for more difficult amines can also be enhanced through the use of microwave irradiation *(16, 17)*.

The bioactive peptoid trimer was successfully synthesized by the large majority of second-year students within a 4-h laboratory period. A small portion of students obtained deletion products, possibly because they performed an additional acylation step instead of amine addition. The chloranil tests performed to monitor the reactions were successful in almost all cases.

The synthesis module may be followed by an optional subsequent lab period for additional characterization of the com-pound. The resultant trimer peptoid was obtained at >90% purity (crude product) as determined by HPLC. The theoretical yield can be calculated by collecting the Fmoc washes following Fmoc deprotection and recording the absorbance of the Fmoc group at 304 nm (see the supporting information). Students can determine actual yield by concentrating the cleaved peptoid to dryness and weighing the product.

Conclusion

A straightforward undergraduate teaching lab experiment is described. Students use their knowledge of common chemical reactions in organic chemistry to perform a multistep synthesis to generate a molecule of a real-world biomedical importance. The synthesis is an inexpensive multistep protocol conducted on solid support and can be completed in a 4-h lab period. If desired, a second lab period can be dedicated to characterizing the peptoid product.

Acknowledgment

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Supporting Information Available

List of chemicals and equipment; instructor notes; student in-structions; details about the theoretical yield calculation by determining concentration of the Fmoc group via UV–vis spectrophotometry and product characterization by HPLC and MS. This material is available via the Internet at http://pubs.acs.org.